ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(10): 957-971
www.allresearchjournal.com
Received: 24-07-2015
Accepted: 25-08-2015

Maha Z Rizk
Therapeutic Chemistry Dept., National Research Centre (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt, P.O.12622

Hanan F Aly
Therapeutic Chemistry Dept., National Research Centre (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt, P.O.12622

Correspondence
Maha Z Rizk
Therapeutic Chemistry Dept., National Research Centre (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt, P.O.12622

Recent therapeutic approaches in control of parasitic diseases with special reference to schistosomiasis

Maha Z Rizk, Hanan F Aly

Abstract
Control of schistosomiasis relies on a single drug, praziquantel (PZQ). Given the rising concerns about the potential emergence of PZQ-resistant strains, it has now become necessary to search for novel therapeutics. Efforts have been made for developing new schistosomicidal drugs, or testing existing drugs originally used for non-related diseases. However, the current pace for antischistosomal drug discovery is slow; hence, repositioning of existing approved drugs can offer a safe, rapid and cost effective solution. The literature regarding antischistosomal compounds contains a large number of natural products screened for their schistosomicidal properties. However, only a few of these may be promising drug leads in the development of a therapeutic reserve for schistosomiasis. Therefore, it is important to continue to identify new drugs and to explore alternative strategies to improve screening efficacy. Most of the extracts or natural compounds were only evaluated with *In vitro* studies; it is expected that they will be evaluated using *in vivo* experimental models. Further, it must be mentioned that the results of *In vitro* assays with many drugs do not correspond to what is observed *in vivo*; however, *In vitro* screening could identify novel anthelmintics that could eventually translate into practical applications. Thus, while *In vitro* tests are recommended initially, the assessment of therapeutic activity using *in vivo* models should be performed. The analysis of the *S. mansoni* genome and transcriptome offers great possibilities for identifying possible new drug targets and will facilitate further exploration of differences between host and parasite metabolic pathways. In addition to the isolation and structural determination of new drugs from natural products and information from the originating plant, the integration of the pharmacological properties of natural products with the functional genomics and proteomic studies in schistosome and *In vitro* screening methods with improved automatic high-content screening will be important tools to identify possible new drugs in the future and shed light on the approaches of helminth chemotherapy.

Keywords: Therapeutic approaches, parasitic diseases, schistosomiasis

Introduction
Schistosomiasis, or bilharzias, is a neglected disease that remains a considerable public health problem in tropical and subtropical regions. This parasitic disease is the most important human helminth infection in terms of morbidity and mortality and is a growing concern worldwide. It is estimated that more than 200 million people have been infected and that 779 million are at risk of infection, resulting in 280,000 deaths annually [1,2]. Schistosomiasis is caused by blood-dwelling fluke worms of the genus schistosoma and is endemic in African, Asian and South American countries. The main disease-causing species are *S. mansoni*, *S. haematobium*, and *S. japonicum. S. mansoni* is the most widely distributed, affecting people in Africa, the Middle East, South America, and the Caribbean, while *S. japonicum* is confined to China, Indonesia, and the Philippines. *S. haematobium* is found in Africa and the Middle East. The adult worm's colonies the veins of either the portal system (*S. mansoni* and *S. japonicum*) or the urinary bladder plexus (*S. haematobium*) and can live for years or even decades in human hosts; thus, the disease runs a chronic and debilitating course. Egg production is responsible for both the transmission of the parasite and the etiology of the disease. Schistosomal species are distinguished by differences in their morphology, both in their parasite stages and in their eggs; further species distinction is made by the species of intermediate host snails that support transmission of the parasite [3].The global strategy for the control of schistosomiasis is by chemotherapy. Systematic searching for chemotherapeutic drugs began almost a century ago, and the development of praziquantel

(PZQ) in 1970 was essential for a reduction in morbidity and mortality due to schistosomiasis. Currently, treatment is still based on the use of PZQ, but the long-term application of PZQ results in decreased efficiency and serious concerns regarding the onset of resistance. In addition, PZQ has no prophylactic properties and is ineffective against larval stages of parasites (schistosomula), meaning that for effective treatment and sustainable control, PZQ must be given on a regular basis. Thus, it is prudent to search for novel therapeutics, and recent discussions have focused on reawakening the need to search for alternatives to PZQ [4, 5]. Given the rising concerns about the potential emergence of PZQ-resistant strains, it has now become necessary to search for novel therapeutics. However, the current pace for anti-schistosomal drug discovery is slow; hence, repositioning of existing approved drugs can offer a safe, rapid and cost-effective solution.

Schistosomiasis, epidemiology, prevalence and incidence

One of the most chronic and debilitating parasitic disease is schistosomiasis. Schistosomiasis disease is caused by the parasitic trematode worms and it continues to threaten millions of people, particularly the rural in the developing world [6]. Of the estimated 200 million infected people, more, than half of them have symptoms and 20 million exhibit severe disease manifestations [6].

A large number of schistosomes are known; however, only five appear to be primarily responsible for human infections. These include *Schistosoma mansoni, S. japonicum*, *S. intercalatum*, S. *mekonji*, and *S. haematobium* [7]. Infection with the former 4 species is associated with chronic hepatic and intestinal fibrosis, ascites and oesophagogastric varices could be demonstrated in advanced cases of *schistosoma* fibrosis with portal hypertension, while infection with *S. hamatobium* can lead to ureteric and bladder fibrosis and calcification of the urinary tract [7]. Schistosomiasis is now showing increasing prevalence and incidence because its occurrence in domestic animals such as cows, pigs, goats and mice [8].

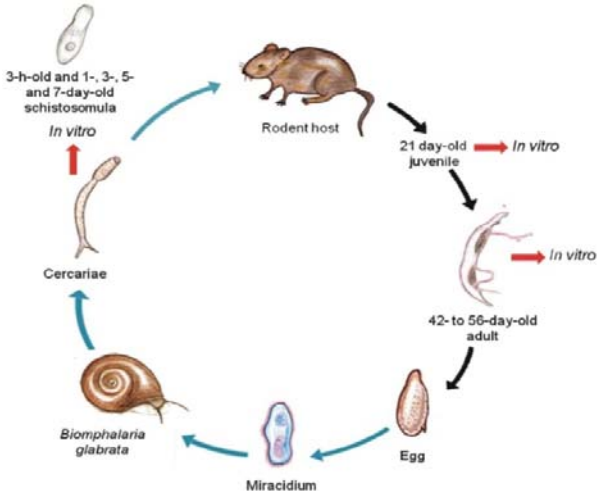
Life cycle of Schistosomiasis

Schistosomiasis is characterized by complex life cycle involving a phase of sexual reproduction by adult worms in human (definitive host) and asexual phase in specific aquatic snails (intermediate host). Infection occurs when human come into contact with fresh water containing free swimming larval forms of the parasite (cercariae). Cercariae penetrate the intact human skin and transformed into schistosomula. They spend a few days in the skin, enter the venous circulation, migrate to the lung (5-7 days post infection), and travel through the blood stream for several days (> 15 days) before they differentiate into male and female worms and unite. Adult worm pairs residue in the mesenteric veins *(S. mansoni, S. japonicum, S. intercalatum, S. mekonji)* or the vesical plexus and veins that drain the ureter *(S. haematobium)* [9].

Depending on the species, oviposition commences 4-9 weeks post infection and continues until the worms die. Some eggs are passed through the bladder or intestinal mucosa and are finally excreted through urine or stool, while others are trapped in the tissues surrounding the worms. This gives rise to acute granulomatous responses and, in the long term, is the primary cause of morbidity. Finally, the life cycle is completed when the eggs reach a fresh water body, hatch and release tiny miracidia that infect specific[10].

Fresh water snails (*S. mansoni* infects *Biomphalaria spp, S. japonicum* infects *Oncomelania spp*, *S. haematobium* infects *Bulinus spp*) [10].

For detection of Schistosoma *mansoni* infection, Raid *et al*. [11] Demonstrate sensitive various methods for early detection of infection.



Life Cycle of *Schistosoma* species

Overview of Current Efforts

A. Biological feasibility for vaccine development

Evidence that vaccine development is biologically feasible including from development of naturally acquired immunity, from vaccine development for related pathogens, from animal models or *In vitro* data Immunity as a result of natural exposure to a pathogen is often taken as evidence of the biological feasibility for vaccine development. In the case of human schistosomiasis, rates and intensity of infection have been found to diminish with age, especially after puberty. However it is apparently unclear if acquired immunity is solely responsible for this observation, and furthermore, the likelihood that such immunity is related to an IgE-mediated mechanism, complicates an approach mimicking this natural immunity. An alternative vaccinology approach, inducing immunity with attenuated parasites has provided the strongest animal proof of concept that vaccines against schistosomiasis are feasible. Vaccination studies with radiation -attenuated cercariae have demonstrated protection in mice (> 90%) and primates (86%) [12]. In mice, both cell - mediated and humoral echanisms appear to operate consecutively (against lung-stage parasites) and immunity can be augmented to very high levels by co-administration of radiation-attenuated cercariae with interleukin 12 (IL-12) as an adjuvant[13]

Although, the radiation -attenuated approach is unlikely to be feasible from a human vaccine standpoint, it represents a tool to mine for protective antigens delivered via the recombinant approach. General approaches to vaccine development for this disease for low and middle income country markets. Vaccine development strategies against schistosomes currently target the prevention of infection and/or the reduction of parasite reproduction. Schistosomes do not multiply in the human host and pathology of the disease results from the eggs deposited by the reproducing worms. The worm burden is also thought to affect the human hosts' immune system in a deleterious manner, compromising

immune responses too their infections [14].Among the major disease targets for vaccination are the migrating schistosomulum stages, as well as adult female worms that release tissue destructive eggs. In addition, vaccines that would specifically reduce parasite reproduction and egg viability may also be a desirable goal.

Most current approaches have focused on exploiting molecular and recombinant technology to identify possible protective antigens from stage-specific parasites and deliver these purified extracts or recombinant constructs in various formulations. An independent preclinical evaluation of sixpotential candidates against *S. mansoni* in mice was sponsored by WHO/TDR in the 1990s; none of the candidates reached to 40% or better worm load reduction target. Although knowledge on immunity to the disease is limited and limits the ability to ascertain protective antigens with assurance, completion of schistosome "omics" projects for high throughput identification of candidates ,coupled with studies in animal vaccination experiments have identified a couple of promising target that are being developed as vaccine candidates. For urogenital schistosomiasis, vaccination experiments in primates identified an antigen shared between the schistosomula and adult schistosome stages of *S. haematobium*, Sh28GST, which been shown in human studies to induce IgG3 that is associated with decrease in egg -production in *S. haematobium* infection, an effect that if successfully reproduced, could lead to decrease urinary tract pathology and transmission [15]. Early phase 1 and 2 clinical trials have shown an acceptable safety profile and induction of high titers of antibodies that neutralizedSh28GSTactivity and the vaccine will undergo phase 3 testing soon(16).For intestinal schistosomiasis, there are two vaccines in early stage clinical testing, including an integral membrane *S. mansoni* surface protein, Sm-TSP-2, has also been identified, and has shown to reduce worm burden in mice by 60-70%.The antigen is also recognized by IgG1 and IgG3 from naturally resistant human subjects, who are not chronically infected (Tran,2006) Sm14 is another *S. mansoni* antigen that is undergoing clinical testing, while Smp80 (calpain) has shown great promise in non-human primates. For Asian schistosomiasis caused by *S. japonicum,* there is interest in developing a veterinary vaccine for water buffalo, cattle, and pigs as a potential means towards a transmission blocking approach for humans [16].

Because several of these antigens are highly conserved among different species, there is interest in developing Schistosoma vaccine. In addition, because schistosomiasis geographically overlaps with hookworm, where they are co-endemic there is additional interest in developing a multivalent vaccine that targets both helminth parasites [17].

The general approach for vaccine development is to target school aged children in endemic areas in Africa and Brazil. The development of a vaccine would require a relatively low cost and efficacious vaccine with a suitable shelf -life for the low to middle income countries. An additional and important potential benefit would be through targeting urogenital schistosomiasis caused by *S. haematobium,* a vaccine could reduce HIV/AIDS transmission in Africa [17].

B. Technical and Regulatory Assessment

The pathway for WHO endorsement for these candidate vaccines would be to generate a 40% or better reduction in challenge derived worm burdens relative to non-immunized controls. The available Schistosoma antigens and prototype vaccine formulations induce 40 to 50% protection in animal models with the standard readouts looking at reduced worm burden or egg production and viability. The challenge is translating these antigens with similar or superior protection in humans. The development of a vaccine with suitable efficacy is the greatest challenge for schistosomiasis vaccines. The immune correlates of protection for a schistosomiasis vaccine are thought to be observed in IgG1 and IgG3 antibody recognition of the antigens. The standard animal model for screening potential antigens is the mouse model used to test the efficacy of schistosomiasis vaccines [17].

Recombinant vaccines are mostly being expressed in low-cost bacteria or eukaryotic expression systems. The selected antigens require processing through the endoplasmic reticulum due to their expression sites in the parasite (i.e., secreted or anchored in the tegument), which can be difficult. Antigen identification and successful protective results will not be sufficient if GMP scale-up manufacture cannot be performed on the antigen [18].Selecting the appropriate adjuvant and delivery platform to stimulate the correct immune response is important to ultimately produce an efficacious antischistosome vaccine. The factors listed above must be taken into consideration for low to middle income countries when selecting antigen strain, production feasibility and cost per dose [18].

C. Status of vaccine R&D activities

Although there remains no current commercially available vaccines for schistosomiasis, the various antigens and candidates in clinical trials are listed in Table (1) and Table (2). The combination of public databases that provide the *S.mansoni* and *S. japonicum* transcriptomes and such techniques as DNA microarray profiling, proteomics, glycomics, and immunomics provide the opportunity to provide new vaccine target molecules with increased potency/efficacy than current schistosome antigens. A significant advancementis the successful application of RNA interference (RNAi) to schistosomes. This technique has been able to determine the functions of schistosome genes/proteins and which ones are essential for survival and reproduction. If antigens were able to silence the expression of numerous *S. mansoni* genes, it would validate their importance as targets for vaccines and new drugs [16].

Elrigal *et al*. (2011) declare that nucleoprotein from susceptible or resistant *Biomphalaria alexandrina* snails produce protection against oxidative damage induced by *Schistosoma mansoni.* More recently, the role of DNA from susceptible or resistant *Biomphalaria alexandrina* snails was evaluated as a vaccine against *Schistosoma mansoni* infected mice. The results revealed significant amelioration in different metabolic pathways and significant eradication in worm burden and oogram and ova count [20].

Table 1: Species, Vaccines and antigenic targets in Clinical Trials

Parasite species targeted	Vaccine	Major antigens/adjuvants	Sponsor	Primary Endpoint	Status
Schistosoma haematobium	Bilhvax	Sh28GST (28-kDa recombinant glutathione -S-transferase) Alum formulation	Institut Pasteur and Inserm	Looking for safety/efficacy of the Vaccine. Immunogenicity was evaluated by antibody production, capacity of sera to inhibit enzymatic activity of the antigen	Completed Phase 2, initiating Phase 3
Schistosoma mansoni	Sm-TSP-2 schistosomiasis vaccine	Sm-TSP-2 (9-kDa recombinant tetraspanin) Alhydrogel® ±GL	Sabin Vaccine Institute Product Development Partnership/ Division of Microbiology and Infectious Diseases/ National Institutes of Health /Baylor College of Medicine Vaccine and Treatment Evaluation Unit	Looking for safety/efficacy of the Vaccine. The immunology aspect will look at the IgG Level by ELISA.	Initiating Phase 1 trial in USA in 2014 at Baylor College of Medicine
S. mansoni	Sm-14 schistosomiasis vaccine	Sm-14 (14-kDa recombinant fatty acid binding protein) with the adjuvant GLA	Oswaldo Cruz Foundation (Fiocruz)	The primary target of the study is to determine the safety and tolerability of the vaccine in healthy adults. The immunological properties monitored are serocon version and cellular immune responses	Active status, the study is ongoing but not recruiting patients[

Table 2: Development Status of Current Vaccine Candidates

Candidate Name/Identifier	Preclinical	Phase I	Phase II	Phase II
SmTSP-2c (tetraspanin D		x		
SmTSP-1	x			
Sm29	x			
Sm23	x			
Sm-p80	x			
Sh-GST28			x	
Sm14e		x		
Sm28-GSTe		x		
Sm28-TPIe		x		
Sm97paramyosineCT-SOD	x	x		

Antischistosomal drugs

For the control of schistosomiasis, which at present is dependent on chemotherapy, it is not satisfactory to have only one single effective treatment [4, 5]. Ideally, other antischistosomal drugs would be available so that the classical strategy of alternating treatments to avoid the development of resistance could be used. Unfortunately, the other drugs used before the advent of PZQ, oxamniquine and metrifonate, are restricted in their use. Metrifonate, a drug that exhibits activity against *S.haematobium*, has recently been withdrawn from the market because of medical, operational, and economic criteria [8]. Oxamniquine is the only alternative antischistosomal drug, but it is effective only against *S. mansoni*. In the1970s, oxamniquine was used for individual and mass treatment of schistosomiasis, with satisfactory results regarding efficacy and tolerance. However, its use is currently declining and is being replaced by praziquantel [21]. As there is currently no available vaccine for this disease in people [22], chemotherapy may now be at a crucial point. Chemotherapy against schistosomiasis was reviewed extensively by Cioli *et al*. [23], with an emphasis on compounds that were used in the past. Additionally, Cioli [24], summarized some interesting laboratory studies on potential antischistosomal compounds and the possible emergence of praziquantel-resistant schistosomes. More recently, Rodrigues *et al*. [25] reviewed results from a comprehensive search of the scientific literature for substances and compounds tested for schistosomiasis therapy over the past century. The authors gathered information on the therapeutic action in humans or animal models and the mechanisms of action of over 40 drugs. Briefly, antimonial compounds were introduced in 1918, and this group of drugs has been the

major point of schistosome chemotherapy for approximately 50 years. However, they cause numerous side effects, such as nausea, vomiting, diarrhoea, anorexia, and cardiovascular, hepatic, and dermatological disturbances. Lethality from cardiac syncope and anaphylactic shock was also reported. Emetine, a drug used to treat amoebiasis, was employed in the second decade of the past century, but the doses required against schistosomiasis were at the very limit of toxicity. The introduction of 2, 3-dehydroemetine reduced the toxicity of the parent compound, but patients had to be hospitalized over a month for treatment. Thus, the use of 2, 3-dehydroemetine as an antischistosomal agent was abandoned [23]. Only in the 1960s was there a breakthrough in the treatment of schistosomiasis, with the rise of metrifonate, nitrofurans, lucanthone, niridazole, hycanthone, and, finally, oxamniquine. In the 1970s, several schistosomicidal drugs emerged, such as tubercidin, amoscanate, PZQ and its benzodiazepine derivative Ro11- 3128, and oltipraz. Nevertheless, the therapeutic doses of most of these drugs were found to cause major side effects. PZQ, an isoquinoline-pyrazine derivative, immediately proved to be superior to any other schistosomicidal drug and quickly became the drug of choice in most endemic areas Because of the reliance on a single drug for the treatment and control of schistosomiasis and the considerable concern regarding the development of PZQ resistance, it is timely to review potential alternatives, with an emphasis on natural products [25].

Since no vaccine exists against schistosomiasis and the molluscs acting as intermediate hosts are not easy to attack, chemotherapy is the main approach for schistosomiasis control. Praziquantel is currently the only available antischistosomal drug and it is distributed mainly through mass administration programs to millions of people every year. A number of positive features make praziquantel an excellent drug, especially with regard to safety, efficacy, cost and ease of distribution. A major flaw is its lack of efficacy against the immature stages of the parasite. In view of its massive and repeated use on large numbers of individuals, the development of drug resistance is a much feared possibility. The mechanism of action of praziquantel is still unclear, a fact that does not favor the development of derivatives or alternatives. A large number of compounds have been tested as potential antischistosomal agents. Some of them are promising, but none so far represents a suitable substitute or adjunct to praziquantel. The research of new antischistosomal compounds is an imperative and urgent matter [26].

Praziquantel is the drug used for treatment of schistosomiasis; nevertheless failure of treatment has been reported. Consequently, the identification of new effective schistosomicidal compounds is essential to ensure the effective control of schistosomiasis in the future. In this work we investigated the immune-modulatory and anti-parasitic effects of the crude leaves extract of *Mentha x piperita* L. (pep-permint) on murine *Schistosomiasis mansoni*. Female Balb/c mice were infected each with 50 *S. mansoni* cercariae and divided into three experimental groups: (I) untreated; (II) treated daily with *M. x piperita* L. (100 mg/kg) and III) treated on 1/42/43 days post-infection with praziquantel (500 mg/kg). Another group with uninfected and untreated was used as a control. Subsequently, seven weeks post-infection, *S. mansoni* eggs were counted in the feces, liver and intestine. Worms were recovered by perfusion of the hepatic portal system and counted. Sera levels of IL-10, IL-5, IL-13, IFN- _, IgG1, IgE and IgG2a were assayed by ELISA. Animals treated with a daily dose of *M. x piperita* L. showed increased sera levels ofIL-10, IFN-α, IgG2a and IgE. Besides, *M. x piperita* L. treatment promoted reduction in parasite burden by35.2% and significant decrease in egg counts in the feces and intestine [26].

The potential emergence of PZQ-resistant strains, it has now become necessary to search for novel therapeutics. However, the current pace for anti-schistosomal drug discovery is slow; hence, repositioning of existing approved drugs can offer a safe, rapid and cost-effective solution. The anti-malarial synthetic artemisin in-derivatives trioxolanes demonstrated anti-schistosomal efficacies against the three major species infecting humans and, unlike PZQ, showed activities against both juvenile and adult worm stages. The 1,2,4-trioxolane/OZ277 (arterolane maleate) in combination with a partner drug: piperaquine phosphate was recently developed as an anti-malarial drug and manufactured by Ranbaxy (India) as SynriamTM (SYN). Herein, the *in vivo* activities of SYN were investigated in a mouse model of *Schistosoma mansoni*, compared to PZQ. We show that a single fixed dose of 240 mg/kg SYN (40 mg/kg arterolane and 200 mg/kg piperaqine) induced significant protective effects in mice, in terms of reduction in worm and tissue egg burdens, which were evident against all schistosome developmental stages. Extensive alterations in the tegument and subtegumental tissues of SYN-exposed worms were revealed by both scanning and transmission electron microscopes. Progressive decrease in worm activity and occurrence of death were noticed *In vitro* upon exposure to the drug − more pronounced in the presence of haemin. This report provides the first evidence of the efficacy of a combination of 1, 2, 4-trioxolane and piperaquine against *S. mansoni* in mice. Being effective against young stages, SYN could be used to prevent early schistosoma infection [25].

Malaria and schistosomiasis are the two most important parasitic diseases in the tropics and sub-tropics region. Efforts have been made for developing news chistosomicidal drugs, or testing existing drugs originally used for non-related diseases. The antimalarial artemisin in–naphthoquine phosphate combination (CO-ArNp) was recently reported to be a promising novel antischistosomal therapy with potent in vivo activity against *Schistosoma mansoni*. The reported *In vitro* dose-and time-response effect of CO-ArNp against the Egyptian strain of *S. mansoni*, and its snail host, *Biomphalaria alexandrina*. Incubation of adult *S. mansoni* with CO-ArNpat40or 20 mg/ml for48 or72h killed all worms. Exposure of *S. mansoni* miracidia and cercariae to the molluscicidal LC50ofCO-ArNp (16.8 mg/ml) resulted in 100% mortality of the free larval stages within 90and 15min, respectively. Moreover, incubation of adult *B. alexandrina* snails with this drug combination killed allsnailsat40 mg/ml within24h.Scanning electron microscope revealed marked morphological and tegumental alterations on the different stages of the parasite and its snail soft issue. No doubt more studies are needed to clarify its potential value to control schistosomiasis [28].

Molluscicides

Until the 1970s, molluscicides were at the forefront of schistosomiasis control, to be later displaced by the newly available drugs for human use [29]. In spite of the adoption of a reasonably good chemical, niclosamide, the practice of

molluscicide has always faced serious problems. Local communities are understandably reluctant to accept that their water bodies turn yellowish while fish and other aquatic organisms undergo death and put refaction [29]. The molluscicidal effects are short-lived and a few surviving snails are sufficient to subsequently re-populate treated sites. In addition, the cost of chemicals is far from negligible, especially for large water bodies. Today, the consensus seems to be that only under special circumstances focal molluscicide may be recommended as an adjunct to chemotherapy and other measures. In spite of a substantial standstill in the practice of chemical snail control, a flourishing of reports has appeared over the years in the literature, regarding plant-derived molluscicides that could be potentially developed at the local level [29]. None of the proposed products, however, has been able, so far, to overcome the challenges of high efficacy and mass production. On a related topic, snail control has been attempted using predatory or competing organisms like fish, prawns or different snail species [30], but practical applications of this interesting approach are as yet unavailable.

Enter praziquante

The early events in the development of PZQ have been repeatedly reviewed[31].A series of compounds synthesized at Merck, Germany, in a project designed to find new tranquillizers, were passed on to Bayer to be screened for anthelmintic activity. The astonishing fact is that the screening for antischistosomal activity of the initial compounds and of over 400 subsequently tested derivatives was carried out using mice infected with *S. mansoni*, complemented with *In vitro* observation of whole parasites [31].Yet, the selected product, PZQ, is such a highly optimized com-pound that it is still unsurpassed for safety and anti-parasitic efficacy among countless chemicals (analogs and otherwise) that have been tested up to this day. The reasons for PZQ success can be classified under four main headings: efficacy, safety, operational convenience and price. When measured by parasite egg excretion about four weeks after treatment with 40 mg/kg, the effects of PZQ can be very broadly summarized as 60–90% cure (no eggs in feces) and 80–95%average reduction in the number of excreted eggs in non-cured patients. This can be regarded as a very good result, but it was pointed out [32], that 100% cure is seldom achieved and that these figures are probably overestimated due to the relative insensitivity of diagnostic methods. The standard dose of 40 mg/kg may be a sub-curative one, but increasing the dose to 60 mg/kg does not seem to improve results [33].Alternative explanations are thus necessary. An important fact in the mode of action of PZQ is that schistosomes are susceptible for the first few days after infection, but then susceptibility decreases to a minimum around day 28, to resume again gradually to a maximum after weeks 6-7 [31].If an individual harbors immature parasites at the time of treatment – a situation most likely to occur in areas of intense transmission of infection –cure will not be achieved. This is probably an explanation for a few instances where unusually low cure was obtained[31].To obviate the problem of low susceptibility of immature stages, it was pro-posed to administer a second dose of PZQ two weeks later [34],when immature forms have progressed to maturity, a procedure that actually resulted in higher cure rates[34].As currently used, PZQ is a racemic mixture of two stereoisomers, only one of which is endowed with antischistosomal properties [31], while the other one contributes a portion of side effects, is responsible for the unpleasant taste of the medication [35] and represents 50% of the bulk of tablets that are often difficult to swallow for children. Current efforts to devise an economically viable production of PZQ as a single enantiomer [36] will hopefully result in a much improved drug. In addition to its schistosomicidal activity, PZQ exerts remark-able effects on a number of other trematodes (Opisthorchis, Paragonimus, Fasciolopsis, Heterophyes, Metagonimus spp.), with the notable exception of Fasciola spp. [37]. PZQ is also effective against most cestodes (Hymenolepis, Echinococcus, Diphyllobothrium, Taenia spp.), with the exception of some larval cestode infections, like hydatid disease and sparganosis [37]. It may be mentioned that, even before its introduction into human therapy, PZQ had been marketed as a dog cestocide under the name Droncit. The activity of PZQ against these additional parasites clearly adds to its attractiveness in many areas where poly parasitism is often the rule. Safety, a massive amount of data has been collected over the years on the subject of PZQ safety, with regard to both immediate and delayed effects, and the overwhelming evidence points to the conclusion that PZQ may be considered the safest of all anthelmintic drugs. The same conclusion applies to different geographical settings, different parasite species, different patient ages and conditions [38]. Reversing previous practice, an informal WHO consultation concluded that pregnant and lactating women should also be treated, since the benefits of treatment clearly exceed hypothetical risks [39].Short-term adverse reactions do occur in a significant number of cases, but they are usually mild and of short duration. The frequency and the severity of side effects is directly correlated with the pre-treatment intensity of infection, suggesting that a proportion of the reactions are likely to be due to dying schistosomes and to the release of their products. Very rare instances of allergic reactions have been reported, but only in one case allergy could be directly attributed to PZQ on the basis of specific desensitization[40].Operational convenience over 42 million people were treated with PZQ in 2012, an impressive figure, although it represents only 14.4% of the population estimated to be in need of treatment[39]. Such a large scale distribution occurred largely through the school system and was made possible by the fact that PZQ is given as a single oral dose, does not require direct medical supervision, does not produce serious side effects and can be easily dosed on the basis of children's height [41].On these premises, PZQ is generally administered by mass treatment without previous individual diagnosis. In high risk areas (50% prevalence of infection) all school-age children and all adults are targeted for treatment. The current strategy of preventive chemotherapy envisages –where co-endemicity exists – the simultaneous administration of medication against lymphatic filariasis, on chocerciasis and soil-transmitted helminthiasis, a practice that represents a formid able boost to the cost efficiency of chemotherapy campaigns [41].

Nowadays the average cost of PZQ is around US$ 0.20 per treatment [17], freely available up to 250 million tablets PZQ/year and other menu factures and partner organizations will make additional con-attributions, but, as stated in a recent WHO document, 'the gap in- availability of praziquantel is huge and pledged amounts will not fill it in the near future' [39].The cost efficiency of chemotherapy campaigns.

PZQ resistance

The massive and exclusive use for many decades of a single drug has obviously raised legitimate fears that PZQ-resistant schistosomes may sooner or later appear. While, the experience with other anti-infective agents justifies such fears on theoretical grounds, another theoretical consideration points to the opposite direction. As previously mentioned, only a minor proportion of people at risk actually receive treatment, thereby leaving ample 'refugia'[42], for sensitive parasites. Thus, it is sadly ironic that the very inability to provide complete drug coverage may prevent further disasters. Leaving aside theoretical considerations; one should ask whether any evidence for the development of PZQ resistance has appeared so far in the field or in the laboratory. Extremely low cure rates (18%) were reported in Senegal, but this occurred in a special focus of very intense transmission, suggesting that low cure may have been largely due to the presence of many immature parasites. Eggs obtained from treated and uncured Egyptian patients gave rise to schistosomes that showed decreased susceptibility when tested in the laboratory. However, such insensitivity was only of moderate degree, was often unstable and investigations carried out ten years later in the same area failed to show any hint of PZQ resistance [43].A number of travelers returning with schistosomiasis from endemic areas had to be repeatedly treated (sometimes unsuccessfully) to clear the infection. However, most of these were infections caused by *S. haematobium* whose eggs are retained for a long time in tissues and diagnosis was rarely obtained on the basis of egg excretion. In any event, no highly resistant schistosome isolate was obtained from these patients. Also, it is possible that people coming from non-endemic areas may lack immunological component that has been shown to contribute to PZQ activity in experimental animals [26]

Mechanism of action of PZQ

It is remarkable that after so many years of use and so many million people treated, the mechanism of action of PZQ is still unsettled. However, the early effects exerted by the drug on these shistosome have been quite well described and can be summarized under three main headings: (i) calcium influx into whole parasites, (ii) muscle contraction and (iii) surface modifications [26].It is tempting to link these three phenomena into a single thread, assuming that the key event is calcium influx, which in turn causes muscle contraction and tegument alterations. Evidence collected in recent years gives strong but not definitive support to this hypothesis [26].It was initially observed that schistosomes possess two regulatory _ subunits of voltage-activated calcium channels, one of which can be defined 'variant' since it has an unusual structure and lacks two serine residues that constitute putative phosphoryation sites in the 'conventional' subunit. When the variant subunit was co-expressed in Xenopus oocytes together with a mammalian _1subunit, the resulting channel exhibited a novel PZQ sensitivity, consisting in increased Ca2+currents in the presence of the drug. A mutagenized variant subunit where the two candidate phosphorylation sites had been reconstituted, no longer exhibited this functional peculiarity. Conversely, a conventional mammalian _ subunit mutagenized to lose the two phosphorylation sites behaved functionally like the variant schistosome subunit. The idea that Ca2+channels containing the variant_ subunit could be the tar-get of PZQ action was reinforced by the finding that other organisms that are susceptible to PZQ (*Taenia solium*, *Clonorchis sinensis*) also possess the variant subunit. An apparently unrelated observation was made in the planarian *Dugesia japonica*, which is able to regenerate both its head and its tail when amputated at the two ends. If these planarians were exposed to PZQ soon after a double truncation, the resulting regenerated worms invariably presented two heads instead of a head and tail [44]. Suppression of planarian calcium channel subunits by RNAi inhibited the double head phenomenon, although – contrary to expectation – inhibition was more pronounced when the conventional subunit, rather than the variant subunit, was suppressed. Thus, in spite of some conflicting details, even in this system the biological activity exerted by PZQ appears to be broadly dependent on the activity of calcium channels. The analogy between the schistosome and planarian systems has been recently extended to show that compounds inducing regenerative bipolarity are often endowed with antischistosomal properties and vice versa, implying possible new research avenues to uncover antischistosomal drugs. The schistosomicidal activity of PZQ can be partially inhibited by some classical calcium channel inhibitors (nicardipine, nifedipine) and is completely abolished if schistosomes are pre-incubated with the actin depolymerizing agent cytochalas in D. This was initially interpreted as an effect of cytochalas in D on calcium channels (as documented in other mammalian systems), but it was later shown that PZQ-mediated calcium influx into the schistosomes is not at all inhibited by cytochalas in D, rather it is largely increased. This presents us with the puzzling situation in which schistosomes inundated with a large amount of calcium fail to exhibit the expected sequence of events leading to tegument disruption and death. A completely analogous coexistence of high calcium levels and of undisturbed survival is presented by immature stages of *S. mansoni* exposed to PZQ, to which they are largely insensitive. These phenomena seem to contradict the basic assumption thatcalcium is the key agent of PZQ schistosomicidal effects, but it must be admitted that our knowledge of the detailed molecular events connected with PZQ activity are still rather crude. A number of alternative hypotheses on PZQ mechanism of action have been put forward and are detailed in previous reviews [26].

Other drugs

The number of compounds that have been tested as possible antischistosomal agents is so large that it would be difficult to acknowledge them all. What follows is an incomplete mention of those compounds that, as of now, appear to hold some promise for the development of new antischistosomal drugs. PZQ derivatives; a relatively small number of PZQ derivatives have been synthesized and tested after the introduction of the parent drug into human use. No compound promised better performance than PZQ and scanty information could be derived from structure–activity relationships. Modifications of the aromatic ring generally led to decreased activity; moderate activity against juvenile worms was found in some compounds, but was not accompanied by satisfactory performance against adults; substitutions in the cyclohexyl group gave compounds with decreased activity [26].

Oxamniquine certainly not a new drug, oxamniquine (OXA) was used long before the introduction of PZQ, to treat many millions of people infected with *S. mansoni*. The main

limitation of OXA is that it is no active against *S. haematobium* or *S. japonicum*, a fact that discouraged its use outside of South America, where only *S. mansoni* exists. The restricted market of OXA prevented its competitive production and the expected price reduction, so that today PZQ is cheaper than OXA and has replaced it even in countries, like Brazil, where OXA has been for many years the successful cornerstone of control programs [26].

Antimalarial drugs derivatives of artemisinin are known for their antimalarial activity, but have also been found to possess antischistosomal properties. In general, these types of compounds have the notable characteristic of being more active against the immature schistosome stages than against the adults, just the opposite of PZQ, a feature suggesting combined treatments as their ideal utilization. Results from clinical trials show that artesunate alone gives lower cure rates than PZQ, while a combination of an artemisinin derivatives plus praziquantel is more effective than PZQ alone[45].

The antimalarial synthetic artemisinin-derivatives trioxolanes demonstrated antischistosomal efficacies against the three major species infecting humans and, unlike PZQ, showed activities against both juvenile and adult worm stages. The 1, 2, 4-trioxolane/OZ277 (arterolane maleate) in combination with a partner drug: piperaquine phosphate was recently developed as an anti-malarial drug and manufactured by Ranbaxy (India) as Synriam TM (SYN). Herein, the *in vivo* activities of SYN were investigated in a mouse model of *Schistosoma mansoni*, compared to PZQ. We show that a single fixed dose of 240 mg/kg SYN (40 mg/kg arterolane and 200 mg/kg piperaqine) induced significant protective effects in mice, in terms of reduction in worm and tissue egg burdens, which were evident against all schistosome developmental stages. Extensive alterations in the tegument and subtegumental tissues of SYN-exposed worms were revealed by both scanning and transmission electron microscopes. Progressive decrease in worm activity and occurrence of death were noticed *In vitro* upon exposure to the drug − more pronounced in the presence of haemin. This report provides the first evidence of the efficacy of a combination of 1,2,4-trioxolane and piperaquine against *S. mansoni* in mice. Being effective against young stages, SYN could be used to prevent early schistosoma infection [27].

Furoxan

While the antioxidant defenses of vertebrates are largely dependent on two enzymes, glutathione reductase and thioredoxin reductase, schistosomes rely on a single multifunctional selenocysteine-containing enzyme, thioredoxin–glutathione reductase (TGR) [26].

Antischistosomal: Natural product and natural product-derived compounds

The use of natural products for curative and therapeutic purposes has a long history, and compounds derived from natural products have made a big impact on the pharmaceutical industry [46]. In addition to microbes and plants, there has been growing interest in other living organisms, such as arthropods and amphibians, as important sources of biologically active compounds [47]. However, the potential for using living beings as sources of new antischistosomal drugs is still poorly explored. In recent decades, there has been a growing interest in the scientific community to search for extracts and pure compounds, especially those derived from plants that exhibit potential schistosomicidal properties, as one alternative method to the conventional chemical control. Plants have been traditionally used in the treatment of different diseases, including schistosomiasis, especially in Africa and Asia [48].In general, medicinal plants are prepared by traditional healers, who have empirical knowledge and cultural communities throughout the world. For example, in Zimbabwe, Ndamba *et al.* [48] investigated the herbal remedies used in the treatment of schistosomiasis. Based on interviews with 286 traditional healers, they composed a list of 47 plant species most widely used to treat urinary schistosomiasis. Based on this survey, the seven most commonly used plants, *Abrus precatorius* (Leguminosae) *Ozoroa insignis* (Anacardiaceae),*Dicoma anomala* (Cornpositae), *Ximenia caffra* (Oleaceae), *Lannea edulis* (Anacardiaceae),*Elephantorrhiza goetzei* (Leguminosae) and *Pterocarpus angolensis* (Leguminosae), were collected, prepared as described by the traditional healers, their efficacy was evaluated using laboratory animals previously exposed to *S. haematobium* cercariae, and the activity from the extract of *P. angolensis* bark was almost comparable to that of praziquantel. Later, Mølgaard *et al*. [49] screened extracts of 23 plant species, popularly used against schistosomiasis in Zimbabwe, for their anthelmintic effect against schistosomula of *S. mansoni*, and the best results against larval forms were obtained with stem and root extracts from *Abrus precatorius* (Fabaceae) and stem bark from *Elephantorrhiza goetzei* (Mimosaceae). All families and names of the plants that are used by traditional healers to treat urinary schistosomiasis in Zimbabwe are described by Ndamba *et al*. [48]. Some of the most interesting antischistosomal compounds are derivatives of artemisinin, such as artemether and artesunate [50]. They are highly effective in the treatment of malaria and have also been shown to exhibit antischistosomal properties. Artemisinin is a sesquiterpene lactone with an endoperoxide group, which was isolated from the leaves of *Artemisia annua* L. This plant has been used for centuries in Chinese traditional medicine as antidote to many different ailments [51]. Artemisinin has been used as an antimalarial since the early 1970s, and its antischistosomal activity was discovered in 1980 by a group of Chinese scientists. In 1982, antischistosomal properties were confirmed for artemether, the methyl ether derivative of artemisinin. Interestingly, artemether has been shown to be active against immature schistosome in experimentally infected animals, but it is less effective against adult worms [8].Significant progress has been made with artemether and its potential for the control of schistosomiasis, which has been reviewed by Utzinger *et al*. [8]. The mechanism of action of artemisinin and its derivatives appears to involve an interaction with heme, which cleaves the Endoperoxide Bridge of the drug to produce carbon-centered free radicals that then alkylate parasite proteins [52]. In addition, scanning electron microscopy showed that artemether caused extensive and severe damage to the tegument in 21-day-old *S. mansoni* harboured in mice [50]. Considering that artemether and praziquantel exhibit the highest activity against schistosomula and adult worms, respectively, combined treatment has been proposed to enhance the reduction in worm burden [8]Currently, new trials to use artemisinin and its synthetic derivatives as lead molecules for drug discovery against schistosomiasis and various other diseases are rapidly growing, and the studies are ongoing [50, 51]. Likewise,

research on other natural products and natural product-derived compounds against schistosome has been performed by many groups. Accordingly, several plants with antischistosomal properties have been described in the literature [53, 54].

Nature product

Natural products, mainly plants, have been the source of medicines for thousands of years. The discovery of pure compounds as active principles in plants was first described at the beginning of the 19th century, and the art of exploiting natural products has become part ofthe molecular sciences [47]. Several extracts or bioactive constituents fromliving organisms have been used in many communities worldwide against parasitic diseases, including schistosomiasis, and in the past decades, natural products have attracted renewed interest [47, 49]. *In vitro* screening systems are useful and affordable ways to discover potential anthelmintic candidates for *in vivo* tests [55]. Because amolecular-target approach is still rarely employed in schistosomicidal drug discovery, a more common strategy has been the complementary approach of whole-organism phenotypic screening *In vitro* to measure compound efficacy [55]. In this context, screening for natural products that are active against schistosome is important in the establishment of future strategies for new antischistosomal drug discovery to control schistosomiasis [54]. Considerable efforts are ongoing to develop novel schistosomicidal agents. As a result, many natural compounds with promising antischistosomal properties have been identified [53]. The efficacy of these new compounds against schistosome is defined using three strategies: a) curative, by killing the adult worm; b) prophylactic, by killing schistosomula; and c) suppressive, by inhibiting worm egg-laying. Thus, several parameters, such as motor activity, tegumental changes, and oviposition, are often evaluated as indicators of biological activity and toxicity in studies with schistosome species. The *In vitro* drug screening strategies used to discover new compounds active against *S. mansoni*, the most important species infecting humans, with an emphasis on natural products. Also highlighted are the best practices and challenges for drug screenings. Furthermore, information is provided about toxicity, susceptible schistosoma stages, and other interesting laboratory studies on potential antischistosomal compounds, both natural products and natural product-derived compounds [54].

The natural products and natural product derived compounds mostly come from plants. Extensive phytochemical investigations of many species have revealed the presence of a large number of novel compounds belonging to different classes [47]. For example, various secondary metabolites have been isolated from the family Piperaceae, and these plants have generated great interest as a result of their biologically active metabolites, such as pyrones, terpenes, lactones, chromenes, chalcones, lignoids, amides, and alkaloids [56]. Regarding the variety of biological properties in particular, de Moraes *et al*. [53], demonstrated the *In vitro* schistosomicidal activity of piplartine, an amide found in several piperspecies. It was found that, at low concentrations (9.5 μM) this amide can kill *S. mansoni* adult worms (male and female coupled) and that the sub-lethal concentration of piplartine (6.3 μM) caused a 75% reduction in egg production. Additionally, piplartine was not cytotoxic against mammalian cells when given at concentrations up to three times higher than what is needed for a schistosomicidal effect (31.5 μM). Furthermore, piper species are widely distributed in tropical and subtropical regions of the world, and they are among the most important medicinal plants used in various systems of medicine [54]. In addition to the wide geographical distribution and their use in folk medicine, the interest in these compounds and plant extracts is based on the fact that it is easy to isolate secondary metabolites and to propagate the plant, which has a short reproductive cycle. Thus, considering the *In vitro* schistosomicidal activity of the amide piplartine, the importance of more research on the biological activity of the natural compounds isolated from the family piperaceae and other plants is apparent in Table 3 [54].

Table 3: *In vitro* and *in vivo* antischistosomal characteristics of natural products.

Date*	Extract/ Compound and Biological Source	Relevant notes
1980	Artemisinin, active principle from the plant *Artemisia annua* L. Asteraceae)	Artemisinin derivatives: artemether (1982) and artenusate (1983); effective against immature schistosome in experimentally infected animals; morphological alteration on the tegument [8]
1989	Extracts from the plant *Pavetta owariensis P. Beauv* (Rubiaceae)contain proanthocyanins	Effective in mice infected with *S. mansoni* [57].
1997	Goyazensolide isolated from the plant *Eremanthus goyazensis*(Gardner) Sch. Bip.(Compositae)	*In vitro* activity on *Schistosoma* adult worms; inhibitory effect on egg-laying; female more susceptible than male; not tested on schistosomula[58]
1999	Extract of *Amm majus*L. fruit	Disturbancein different metabolic parameters and increase mortality rate of *Biomphalaria alexandrina* snail[59]
2000	Extract of leaf from the plant *Vernonia amygdalina*Del (Compositae)	Active against *S. mansoni* in mice[60]
2000	Extract of *Curcuma longa*	Active against *S. mansoni* in mice [61].
2001	Mirazidmyrrh, an oleogumres inform the stem of the plant *Commiphora molmol*(Burseraceae	There is a great debate about the efficacy and effectiveness of myrrh in the treatment of schistosome infections, both in laboratory and clinical settings [43]
2002	Oil from the plant *Nigellasativa* L. (Ranunculaceae	Active on *S. mansoni*-infected mice; crushed seed also has *In vitro* effects against *S. mansoni* miracidia,cercariae, and adult worms, and an inhibitory effect on egg-laying; not tested on schistosomula [62]
2002	Extract of the seeds and isoflavonoids from the plant *Millettia thonningii*	*In vitro* against *S. mansoni* adult worms; no egg production was observed for experimental worms; not tested on schistosomula. Schistosomicidal activity against *S. mansoni* cercariae and miracidiahas been previously described [63]

	(Schum. et Thonn.) Baker (Leguminosae)	
2005	Extract of rhizomes from the plant *Zingiber officinale* Roscoe (Zingiberaceae)	*In vitro* male worms seemed more susceptible than female; reduction in egg output; activity against *S.mansoni* in mice was conflicting between Mostafa *et al*. [64] and Sanderson *et al*. [65]morphological alteration on the tegument; not tested on schistosomula
2006	*Ailanthus altissima* and *Ziziphus spina*	Ameliorate hepatic marker enzymes and antioxidant level in liver of infected mice with *Schistosoma mansoni* [66, 67].
2007	Extract from plant *Curcuma longa* L. (Zingiberaceae)	Effective on *S. mansoni*-infected mice [67]the *In vitro* schistosomicidal activity of curcumin, the major constituent in the rhizome, and reduction in egg production has been reported[68]
2007	Extract from garlic *Allium sativum* L. (Liliaceae)	Active against *S. mansoni* in mice (50 mg/kg) and not effective in high dose (100 mg/kg); affects the development and maturity of *S. mansoni* eggs in mice and seems to be an agent in protecting hepatic tissue against oxidative damage due to *S.mansoni* infection. *In vitro* allicin (2011), the main constituent of garlic, causes alterations on the tegument of male worm in high doses (10 to 20µg/ml), but toxicity not assessed; not tested on schistosomula [69].
2009	Extract from the plant *Clerodendrum umbellatum* Poir (Verbenaceae)	Effective in *S. mansoni* mice model [70].
2009	Extract from the plant *Zanthoxylum naranjillo* Griseb (Rutaceae) and its isolated compounds	*In vitro* against *S. mansoni* adult worms; reduction in egg-laying; not tested on schistosomula; not toxic in mammalian cells [71]
2010	Phloroglucinol compounds from plants of the *Dryopteris* genus (Dryopteridaceae)	*In vitro* against *S. mansoni* adult worms; reduction in egg-laying; not tested on schistosomula; not toxic in mammalian cells [72]
2010	Essential oil from the plant of *Baccharis dracunculifolia* DC. (Asteraceae)	*In vitro* against *S. mansoni* adult worms; reduction in egg-laying; not tested on schistosomula; not toxic in mammalian cell [73].
2011	Essential oil from plant *Ageratum conyzoides* L. (Asteraceae)	*In vitro* against *S. mansoni* adult worms; reduction in egg-laying; not tested on schistosomula; not toxic in mammalian cells [53]
2011	Sulfated polysaccharide α-D-glucan extracted from lichen *Ramalina celastri*(Spreng.) Krog. & Swinsc	Effective in *S. mansoni*-infected mice [53].
2011	Piplartine, an amide isolated from plant *Piper tuberculatum* Jacq. (Piperaceae)	*In vitro* against *S. mansoni* adult worms; reduction in egg-laying; causes alterations on the tegument of worms; not tested on schistosomula; not toxic in mammalian cells [74]
2011	Epiisopiloturin, an alkaloid isolated from plant *Pilocarpus microphyllus* Stapf ex Holm (Rutaceae)	*In vitro* against *S. mansoni* adult worms; causes alterations on the tegument of worm; not tested on schistosomula; not toxic in mammalian cells [74]
2011	Extract from plants of the *Artemisia* genus (Asteraceae)	*In vitro* against *S. mansoni* adult worms; not tested on schistosomula[75]
2012	Essential oil of *Melaleukaarmillaris*leaves	Active against *S. mansoni* infected mice [76].
2013	*Zingiber official* and*Glycyrrhiza uralensis*	*Zingiber officinal* and *Glycyrrhiza uralensis* were used as total extract against *Schistosoma mansoni* different stages ; both extracts produce disturbances in amino acid metabolic pathways of parasite resulting in parasite eradication[77]
2014	blue green algae	The diameter and number of egg granuloma were significantly reduced after treatment of *S. mansoni* -infected mice with BGA, PZQ and their combination.
2015	*Moringa oleifera Lam*	Aqueous extract from *Moringa oleifera* Lam. flowers on *Biomphalaria glabrata* embryos and adults and on *Schistosoma mansoni* adult worms. The extract contains tannins, saponins, flavones, flavonols, xanthones, and trypsin inhibitor activity

As shown in Table 3, several *In vitro* studies have been conducted to search for new natural substances with schistosomicidal activity. Aqueous extract from Moringa oleifera Lam. flowers on *Biomphalaria glabrata* embryos and adults and on *Schistosoma mansoni* adult worms. The extract contains tannins, saponins, flavones, flavonols, xanthones, and trypsin inhibitor activity. The toxicity of the extract on *Artemia salina* larvae was also investigated to determine the safety of its use for schistosomiasis control. After incubation for 24 h, the flower extract significantly ($p < 0.05$) delayed the development of *B. glabrata* embryos and promoted mortality of adult snails (LC50: 2.37 ± 0.5 mg /ml). Furthermore, treatment with the extract disrupted the development of embryos generated by snails, with most of

them remaining in the blastula stage while control embryos were already in the gastrula stage. Flower extract killed *A. salina* larvae with a LC50 value (0.2 ± 0.015 mg/ ml) lower than that determined for snails. A small reduction (17%) in molluscicidal activity was detected when flower extract (2.37 mg/ ml) was exposed to tropical environmental conditions (UVI index ranging from 1 to 14, temperature from 25 to 30C, and 65% relative humidity). Toxicity to *A. salina* was also reduced (LC50 value of 0.28 ± 0.01 mg/ ml). Thus, *M. oleifera* flower extract had deleterious effects on *B. glabrata* adults and embryos. However, unrestricted use to control schistosomiasis should be avoided due to the toxicity of this extract on *A. salina* [78]

The immune-modulatory effects of a natural product, blue green algae (BGA) (100 mg/kg BW), alone or combined with praziquantel PZQ (250 mg/kg BW) on granulomatous inflammation, liver histopathology, some biochemical and immunological parameters in mice infected with *Schistosoma mansoni*. Results showed that the diameter and number of egg granuloma were significantly reduced after treatment of *S. mansoni* -infected mice with BGA, PZQ and their combination. The histopathological alterations observed in the liver of *S. mansoni* -infected mice were remarkably inhibited after BGA treatments. BGA decreased the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as well as the level of total protein (TP) while the level of albumin was increased. Treatment of infected mice with BGA, PZQ as well as their combination led to significant elevation in the activities of hepatic antioxidant enzymes glutathione peroxidase (GPX) and glutathione-S-transferase (GST) as compared with control group. Combination of BGA and PZQ resulted in significant reduction in the level of intercellular adhesion molecules-1 (ICAM-1), vascular adhesion molecules-1 (VCAM-1) and tumor necrosis factor-alpha (TNF- a) when compared to those of the *S. mansoni* -infected group. Overall, BGA significantly inhibited the liver damage accompanied with schistosomiasis, exhibited a potent antioxidant and immunoprotective activities. This study suggested that BGA can be considered as promising for development a complementary and/or alternative medicine against schistosomiasis [79].

The effects of both garlic (*Allium sativum*) and onion (*Allium cepa*) on some biochemical parameters in *Schistosoma mansoni* infected mice individually and mixed either with or without the currently used drug, praziquantel (PZQ) were investigated. These involved some immunological parameters, namely IgM, IgG, interleukins 2 and 6 (IL-2 and 6) and tumor necrosis factor (TNF-α), some antioxidant enzymes [catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPX)]. In addition, parasitological and histopathological investigations were performed. No changes were observed in the normal control mice treated with dry extract of onion or garlic, individually or mixed, with or without PZQ, compared to the normal healthy control group. Infection with *S. mansoni* showed an increase in IgG, IgM, IL-2, IL-6, TNF-α and catalase enzyme, accompanied with a decrease in GPX and SOD antioxidant enzyme activities. Remarkable amelioration was noticed in the levels of all the measured parameters in *S. mansoni* infected mice after administration of the studied extracts. Moreover a significant reduction in worm burden, hepatic and intestinal eggs and oogram count was noticed which was reflected in normalization of liver architecture [80].

Plant extracts is continuously investigated for their extensive inclusion of biologically active constituents that exert therapeutic activities against many diseases. The antioxidant/anti-schistosomal activities of the essential oil of the fresh leaves of *Melaleuca armillaris* (*M. armillaris*) compared to praziquantel on normal and *Schistosoma mansoni* infected mice. The oil was isolated by hydro-distillation and analyzed by gas chromatography/mass spectrometry (GC/MS). The oil was rich in 1,8-cineole (33.93%), terpinen-4-ol (18.79%), limonene (10.37%) and B-pinene (6.59%). *M. armillaris* oil (150 mg kg^{-1}, orally) was administered from the second week post infection twice per week for six weeks. PZQ (500 mg kg^{-1}, orally) was administered for two successive days 8 weeks post infection and mice sacrificed one week later. Total protein, Malondialdehyde (MDA), Glutathione (GSH), vitamins C and E, the antioxidant enzymes catalase and superoxide dismutase, as well as liver weights and liver/body weight were determined in the liver tissues. Results showed that, both treatments significantly ameliorated the disturbed levels of GSH and MDA in infected mice. Both vitamins were significantly elevated after treatment with the oil while a significant increase in catalase accompanied by a pronounced decrease in SOD were obtained after treatment with PZQ. Both treatments markedly improved liver and body weights in infected mice compared to the infected-untreated ones. Thus, natural plant sources may be used as promising alternative agents to chemical drugs for schistosomiasis treatment, since the latter may result in drug-induced resistance arising from repeated use [76].

Artemether (ART), the methylated derivative of artemisinin, is an efficacious antimalarial drug that also displays antischistosomal properties. This study was designed to evaluate the immunomodulatory action of a single intramuscular dose (50 mg/kg body weight) of ART in comparison with PZQ treatment (42 days PI). ART administration was 7, 14, 21 and 45 days PI. ART effect was studied parasitologically, histopathologically and immunologically. It was found that maximum effect was reached when ART treatment interfered with 14 or 21 days old schistosomula. ART treatment 14 or 21 days PI was associated with shift from Th2 to Th1 predominancy (decrease in IL-4 and upgrading of serum IFN-c levels). In conclusion, ART is a promising drug in control of *schistosomiasis mansoni* due to its reductive effect on worm burden and its role in improvement of hepatic granulomatous lesions [81].

Praziquantel is the drug used for treatment of schistosomiasis; nevertheless failure of treatment has been reported. Consequently, the identification of new effective schistosomicidal compounds is essential to ensure the effective control of schistosomiasis in the future. In this work we investigated the immunomodulatory and antiparasitic effects of the crude leaves extract of *Mentha x piperita* L. (pep-permint) on murine *Schistosomiasis mansoni*. Treatment of infected mice promoted reduction in parasite burden by35.2% and significant decrease in egg counts in the feces and intestine [79].

Garcinielliptone FC (GFC) is a natural prenylated benzophenone found in the seeds of *Platonia insignis* Mart. (Clusiaceae), a native Brazilian plant. It has been chemically characterized and it is known that GFC has several biological activities such as antioxidant and vaso relaxant properties. In this study, we report the *In vitro* effect of GFC against the

blood fluke *Schistosoma mansoni,* the parasite responsible for *schistosomiasis mansoni*. The anti-*S. mansoni* activity and cytotoxicity toward mammalian cells were determined for the compound. GFCP6.25 lM showed antischistosomal activity and confocal laser scanning microscopy analysis demonstrated several morphological alterations on the tegument of worms, and a correlation between viability and tegumental damage was observed. In addition, at sub-lethal concentrations of GFC (63.125 lM), the number of *S. mansoni* eggs was reduced. More importantly, GFC exhibited no activity toward mammalian cells and, therefore, there is an appreciable selectivity of this compound against the helminths. Thus, these findings indicate the potential of GFC as an antiparasitic agent [82].

The antischistosomal activity of betulin, betulinic acid and its triphenylphosphonium derivatives characterized by a covalently linkage of the hydrophobic fragment of triterpenoid at C (2) - or C (30)-position with the triphenylphosphonium moiety *via* a hydrocarbon bridge. The triphenylphosphonium salts showed *In vitro* antischistosomal activity against newly transformed schistosomula (NTS) and adult worms of *Schistosoma mansoni* at low micromolar concentrations. In contrast betulin and betulinic acid were inactive against NTS and adult *S. mansoni.* Of the triphenylphosphonium derivatives tested, the allyl salts 10 (IC50 of 0.76 lg/ml) and 11 (IC50 of 0.64 lg/ml) demonstrated the highest antischistosomal activity against adult *S. mansoni.* Low worm burden reductions of 22% were observed *in vivo* for these two compounds. So, triphenyl phosphonium derivatives were obtained from available natural betulin by simple transformations, rendering it practical and useful for large scale application. However, further structural modifications are necessary to translate the promising antischistosomal *In vitro* activities into *in vivo* [83]

The protective effect of *Aristolochia gehrtii* (*A. gehrtii*) leaves to inhibit liver toxicity and apoptosis in *Schistosoma malayensis* (*S. malayensis*) infection [84].

Conclusion

Schistosomiasis is a neglected disease that is one of the most common chronic infections among the poorest people in the world. Most chemotherapeutic-based programs attempting to eradicate schistosomiasis in the developing world rely on the effectiveness of a single drug, praziquantel; therefore, there is an urgent need to identify new parasite targets and effective antischistosomal compounds. Secondary plant metabolites have attracted the attention of many researchers over the years as a result of the variety of their chemical structures and their broad range of biological activities that may provide lead structures for the development of new drugs. Recently, marine organisms have also been recognized as an attractive source of antiparasitic compounds, and it can be expected that other living organisms, such as insects and amphibians, will emerge as additional sources in the future.

Discovering untapped natural sources of new anthelmintic compounds remains a major challenge and a source of novelty in the era of combinatorial chemistry and genomics. To find new anthelmintics, all sources of natural, synthetic and semi-synthetic lead compounds must be investigated. *In vitro* bioassays using parasitic worms have played a central role in the early pre-clinical stages of most research on potential natural anthelmintics. The identification of the antiplasmodial and antischistosomal activity of the sesquiterpene lactone artemisinin has stimulated interest in natural products, and soon, promising leads will be identified with new chemical types and active agents against schistosomiasis. Therefore, bioprospecting programmes related to the isolation of bioactive compounds must be rewarded, and the screening *In vitro* of chemical constituents belonging to different classes must be evaluated on the blood fluke *S. mansoni.* Attempting new combinations of natural or synthetic drugs will be also important in discovering alternative drugs to replace the use of praziquantel.

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