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MINIREVIEW

Praziquantel for Schistosomiasis: Single-Drug Metabolism Revisited, Mode of Action, and Resistance

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ABSTRACT Schistosomiasis, a major neglected tropical disease, affects more than 250 million people worldwide. Treatment of schistosomiasis has relied on the anthelmintic drug praziquantel (PZQ) for more than a generation. PZQ is the drug of choice for the treatment of schistosomiasis; it is effective against all major forms of schistosomiasis, although it is less active against juvenile than mature parasites. A pyrazino-isoquinoline derivative, PZQ is not considered to be toxic and generally causes few or transient, mild side effects. Increasingly, mass drug administration targeting populations in sub-Saharan Africa where schistosomiasis is endemic has led to the appearance of reduced efficacy of PZQ, which portends the selection of drug-resistant forms of these pathogens. The synthesis of improved derivatives of PZQ is attracting attention, e.g., in the (i) synthesis of drug analogues, (ii) rational design of pharmacophores, and (iii) discovery of new compounds from large-scale screening programs. This article reviews reports from the 1970s to the present on the metabolism and mechanism of action of PZQ and its derivatives against schistosomes.

KEYWORDS cytochromes P450, enantiomers, metabolism, praziquantel, schistosomiasis

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Schistosomiasis, a major neglected tropical disease, is considered the most important helminthic disease of humanity in terms of morbidity and mortality rates. More than 200 million people are infected worldwide, and 600 million are at risk of infection (1, 2). Control strategies have been employed to block transmission and reduce the disease burden, including mass and targeted chemotherapy, improvements to sanitation, modification of the environment, and the use of molluscicides (3, 4). However, schistosomiasis remains a major public health problem, especially in rural regions of sub-Saharan Africa (2). The infection is caused by three main species of blood flukes, *Schistosoma haematobium* in Africa and the Middle East, *S. mansoni* in Africa and South America, and *S. japonicum* in China and the Philippines, and two less common ones, *S. intercalatum* in Africa and *S. mekongi* in Southeast Asia (5). Moreover, recent outbreaks reveal the reemergence of urogenital schistosomiasis in southern Europe (6). Additionally, infection with *S. haematobium* is classified as a group I biological carcinogen by the International Agency for Research in Cancer of the World Health Organization (WHO) (7). Table 1 summarizes the species that commonly infect humans, the geographical ranges of endemicity, and the major disease symptoms (5, 7, 8).

Male and female schistosomes dwell in copula within the mesenteric veins (*S. mansoni*, *S. japonicum*) or the venous plexus (*S. haematobium*) of the human host,

TABLE 1 *Schistosoma* species, regions of prevalence, and major signs and symptoms of schistosomiasis

Species	Regions of prevalence	Pathology, symptoms, signs
S. mansoni	Africa, Middle East, Caribbean, South America	Liver/periportal fibrosis, hepatomegaly, intestinal fibrosis, diarrhea
S. japonicum	China, Southeast Asia (Philippines, Indonesia)	Blood in stool, portal hypertension, hepatomegaly, intestinal fibrosis, diarrhea, blood in stool, CNS[a] complications
S. mekongi	Cambodia, Lao People's Democratic Republic	Same as for *S. japonicum*
S. hematobium	Africa, Middle East, southern Europe (Corsica, France)	Urogenital tract fibrosis, female genital schistosomiasis, bladder cancer, renal failure, infertility

[a]CNS, central nervous system.

laying hundreds to thousands of fertilized eggs per day, depending on the species. The eggs traverse the intestinal wall (e.g., *S. mansoni*) or the bladder wall (*S. haematobium*) and exit the host to the external environment in feces or urine, respectively. However, many eggs are retained in host tissues, where they induce inflammation, granuloma, and fibrosis. In the external environment, the eggs hatch when they reach freshwater, releasing a free-living larva, the miracidium, that is ciliated and seeks to infect the obligate intermediate host, a snail. Within the snail, the parasite undergoes cycles of asexual reproduction through mother and daughter sporocyst stages, eventually shedding thousands of cercariae into the water. The cycles of asexual reproduction of the parasite within the snail require from 4 to 6 weeks before cercariae are released. The cercaria is the infectious developmental stage for humans and other mammals. After penetrating the skin, the cercariae shed the tail and the juvenile larvae, termed schistosomula, migrate within the circulatory system, reaching the lungs, the liver, and finally the portal venous system or the venous system that drains the pelvic organs, depending on the species, where the parasite fully matures. Adult *S. mansoni* worms migrate to the superior mesenteric veins, *S. japonicum* worms migrate to the inferior mesenteric and superior hemorrhoidal veins, and *S. haematobium* worms migrate to the vesical plexus and veins draining the ureters, bladder, and other pelvic organs. Male and female schistosomes mate, produce eggs, and thus complete the developmental cycle (Fig. 1) (6).

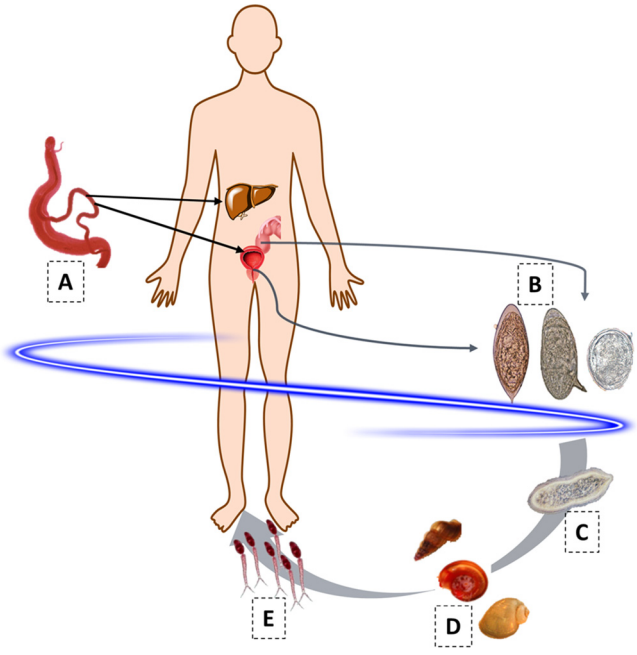

FIG 1 The developmental cycle of *S. mansoni*, *S. haematobium*, and *S. japonicum*. Stages: A, paired adult worms (larger male enfolding slender female); B, eggs (left to right, *S. haematobium*, *S. mansoni*, and *S. japonicum*); C, ciliated miracidium; D, intermediate host snails (left to right, genera *Oncomelania*, *Biomphalaria*, and *Bulinus*); E, cercariae (infective stage).

TABLE 2 Clinical phases of schistosomiasis and its associated symptoms[a]

Clinical phase	Symptoms
Immediate	Acute, pruritic, maculopapular eruption at site of cercarial skin penetration within 1 day following exposure
Acute	Systematic hypersensitivity reaction against migrating schistosomula, fever, fatigue, myalgia, malaise, nonproductive cough, eosinophilia, patchy infiltrates, weight loss, dyspnea, diarrhea, diffuse abdominal pain, toxemia, hepatosplenomegaly, widespread rash
Chronic	Affects gastrointestinal and urogenital tracts, leading to hepatosplenic and pelvic organ diseases, portal and pulmonary hypertension, abdominal ascites, upper gastrointestinal varices and hemorrhage, female genital schistosomiasis, infertility, increased risk of HIV-1 transmission, and squamous cell carcinoma of the bladder

[a]See references 7 and 9 to 13.

The infection clinically progresses from an immediate phase to acute and chronic stages (7, 9–13). The initial phase is typically characterized by an acute, pruritic, maculopapular eruption at the site of cercarial skin penetration within the first 24 h after exposure. This may last several days, may occur even with zoonotic schistosome species that do not usually mature in humans, and is also known as cercarial dermatitis or swimmer's itch. Acute schistosomiasis (Katayama fever) is a systemic hypersensitivity reaction to the migrating schistosomula that occurs a few weeks to months after a primary infection. The disease starts suddenly with fever, fatigue, myalgia, malaise, nonproductive cough, eosinophilia, and patchy infiltrates on chest radiography. Abdominal symptoms develop later, following the migration and residence of the mature worms in the blood vessels of the intestines and bladder. Most persons recover spontaneously from the acute stage after 2 to 10 weeks, but some develop a persistent and more serious disease with weight loss, dyspnea, diarrhea, diffuse abdominal pain, toxemia, hepatosplenomegaly, and a widespread rash (7, 9, 10). During chronic or advanced schistosomiasis, which can persist for decades in the absence of treatment, the gastrointestinal and urogenital tracts are affected, leading to hepatosplenic and pelvic organ diseases and other complications, including portal and pulmonary hypertension, abdominal ascites, upper gastrointestinal varices and hemorrhage, infertility, and increased risk of HIV-1 transmission (Table 2) (10–13).

The paucity of information on new derivatives of praziquantel (PZQ1) is curious, especially since not only is schistosomiasis one of the major neglected tropical diseases but infection with *S. haematobium* is a biological carcinogen (14). Neglect of the latter undoubtedly relates to the lack of reliable rodent models of urogenital schistosomiasis. Nonetheless, the design of novel, rational compounds with potential antischistosomal activity is hindered by the absence of the definitive mode of the antischistosomal action of PZQ1. Although investigation of novel PZQ1 derivatives apparently continues, there is not a wealth of information available on the mode of drug action. Here, we review recent developments on derivatives of PZQ1, including activity and metabolites, as well as modes of action and drug resistance. We believe that review of this information will be beneficial for the identification of novel antischistosomal drugs and new drug targets.

A SINGLE DRUG FOR TREATMENT AND CONTROL OF SCHISTOSOMIASIS

The pyrazino-isoquinoline derivative PZQ1 (Fig. 2) was developed by Bayer in the 1970s and shown to be active against parasitic flatworms, including schistosomes. Remarkably, treatment and control of schistosomiasis have relied only on this drug for over 40 years (15–17). In animal tests, PZQ1 showed minimal toxicity (18) and no genotoxic risks (19) were detected in assays for mutagenicity (20). The few observations that suggested accumulation of potentially mutagenic metabolites may have been abnormalities among otherwise overwhelming evidence indicating that PZQ1 is a safe drug (21). Generally, PZQ1 induces only mild and transient side effects, if any. The

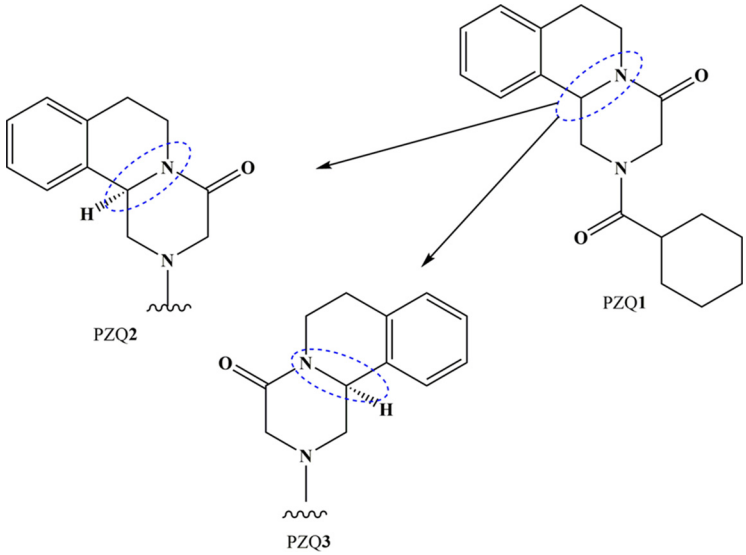

FIG 2 Enantiomers of PZQ1 and biologically active (*R*-PZQ, PZQ2) and inactive (*S*-PZQ, PZQ3) isomers.

frequency and intensity of these effects are correlated with the intensity of infection, and the most severe side effects of bloody diarrhea or edematous urticaria observed in areas with high intensities of infection may be due to the release of antigens and other metabolites by dying worms (22, 23).

During the past few years, the renewed acknowledgment of the burden imposed by schistosomiasis has led to the implementation of mass drug administration (MDA) programs for the control and possible elimination of the this major human helminthiasis, yet the WHO recently reported that less than one-third of individuals who required "preventive chemotherapies" received treatment (24). PZQ1 has been widely used since 2006 through "preventive chemotherapy" programs distributing the drug to school age children or at-risk populations, depending on prevalence rates. In 2010, 34 million people received PZQ1, mostly in sub-Saharan Africa (16). It has been estimated that by 2018, as many as 235 million people will have been treated with PZQ1, a projected use of 645 million tablets of PZQ1 (25). Also, PZQ1 is effective in the treatment of hypertension due to chronic schistosomiasis (10). This continues to be a key drug in the treatment of schistosomiasis and, indeed, most other fluke and cestode infections (17).

According to the Biopharmaceutics Classification System and the Biopharmaceutics Drug Disposition Classification System, PZQ1 is a class II drug that displays a high ability to permeate tissues and low solubility (0.4 mg/ml) and proceeds through extensive metabolism (discussed below) (26, 27) via hydroxylation of the absorbed drug to inactive metabolites, such that only minimal concentrations contact the parasites within the blood system. Currently, PZQ1 is distributed as a racemate that includes equivalent proportions of the biologically active *R*-PZQ (PZQ2, Fig. 2) and inactive *S*-PZQ (PZQ3, Fig. 2) (28) enantiomers, the consequence of which is that half the PZQ1 dose is pharmacologically inactive. This requires the use of a 600-mg tablet to provide a final dose of 40 mg/kg. Moreover, PZQ3 probably contributes to the unpleasant taste of PZQ1. These disadvantages contribute to inefficient treatment of school age children, since children frequently avoid swallowing the medicine because of its less-than-pleasant taste (28). Meyer et al. (29) investigated the bitterness value of enantiomers in regard to additional incentives for low-cost production of pure active PZQ1 (29). Indeed, the pure enantiomer of PZQ2 can probably be synthesized economically (30, 31). Among these variants, however, PZQ1 presents other disadvantages, such as decreased or complete absence of activity against juvenile schistosomes (32, 33). Accordingly, a complete cure is not reliably achieved with a single dose of PZQ1, particularly given that reinfection is routine (8, 34).

Despite decades of extensive use, much remains unknown about PZQ1, in particular, its exact mode of action, its *in vivo* metabolism, and its molecular target(s). Herein, these aspects are reviewed along with prospective derivatives of PZQ1.

PZQ PHARMACOKINETICS

Although PZQ1 has been employed for decades, few pharmacokinetic studies have been performed with humans (26), although a study carried out with healthy volunteers demonstrated that absorption of PZQ1 is relatively fast (time to maximum concentration of drug in serum [T_{max}], 2.0 to 2.6 h) and nearly complete (>80%), which demonstrates an extensive first-pass effect (35). The systemic bioavailability of PZQ1 is low and varies considerably among individuals. After the administration of 40 mg/kg to a healthy adult, the half-life ($t_{1/2}$) was reported to range from 2.2 to 8.9 h and the area under the curve (AUC) was reported to range from 2,100 to 5,400 ng h/ml. Oral drugs display higher pharmacokinetic variability than drugs administered by the parenteral route, which is explained by the blood flow at the absorption site, the absorptive surface area, the transit time, and the gastric pH (36). These factors are also influenced by concurrent food intake; the bioavailability of PZQ1 increases with concomitant food administration. Following the administration of 1,800 mg (~25 mg/kg for a 70-kg body weight) to healthy adults, the AUC from 0 to 8 h was 2.7-fold higher with a fatty diet and ~4 times as high with a high-carbohydrate diet (37). The effect of food on bioavailability may also be due to changes in hepatic flow, altered cytochrome P450 (CYP) expression in response to the diet, or changes in the first-pass metabolism of PZQ1 (38, 39). The bioavailability of PZQ1 has also been analyzed during schistosomiasis. Comparing the bioavailability of PZQ1 in healthy volunteers and infected people after the administration of 40 mg/kg, the C_{max} (the maximum or peak concentration) and AUC were 1.7- and 4.2-fold higher in patients, the T_{max} was 0.6 times shorter, and the $t_{1/2}$ was 5.2 times longer (40). PZQ1 is mainly concentrated in the liver and kidneys. Concentrations higher than those in plasma occur in the lungs, pancreas, adrenal glands, pituitary gland, and salivary glands (41). However, the volume of distribution is not known (41). In addition, PZQ1 binds to proteins (~80% exclusively to albumin). Hence, nutritional status and other factors, including chronic inflammation, influence the levels of the free drug (35, 42).

INSIGHTS INTO METABOLISM OF PZQ

As noted, PZQ1 is mainly metabolized to PZQ2 and PZQ3, which in turn breaks down into various mono- or dihidroxy metabolites and *S-trans*- and *S-cis*-4-OH-PZQ, while PZQ2 is metabolized to *R-trans*-4-OH-PZQ or *R-cis*-4-OH-PZQ (Fig. 3) (43–45). Since higher drug concentrations in plasma and slightly longer half-lives are achieved with metabolites than with PZQ1, the metabolites likely contribute to the drug's antischistosomal activity (46). In fact, *in vitro* studies using PZQ2 and PZQ3 and its major metabolites against *S. mansoni* developmental stages (newly transformed schistosomula and adult worms) demonstrated that PZQ2 and its metabolites exhibit 100- and 1,000-fold higher activities than their *S* counterparts. These findings confirm that PZQ2 is the main effector, whereas PZQ3 and its metabolites do not contribute significantly to the drug's antischistosomal activity (15). Nonetheless, metabolites of PZQ1 are less active than the parent drug (47). Although the enzymes that metabolize PZQ1 are not fully known, PZQ1 is primarily metabolized by CYP 3A and to a lesser extent by CYP 2D6 (35). Several studies have been performed to clarify the metabolic profile of PZQ1, as well as the enzymes involved and the identities of the phase II metabolites (48–53).

Development of new PZQ derivatives might be a good strategy to circumvent the major drawbacks of current PZQ1 therapy. Substantial investigation has been directed to the design of different types of PZQ derivatives. Through the years, several PZQ derivatives have been developed and assessed via *in vitro* and *in vivo* studies mainly against *S. mansoni* and *S. japonicum*. Design of urea and amide derivatives (Fig. 4) led to a moderate reduction of worm motility *in vitro*, but generally, this activity was not observed *in vivo*. However, one derivative of these series, PZQ7, stood out in regard to

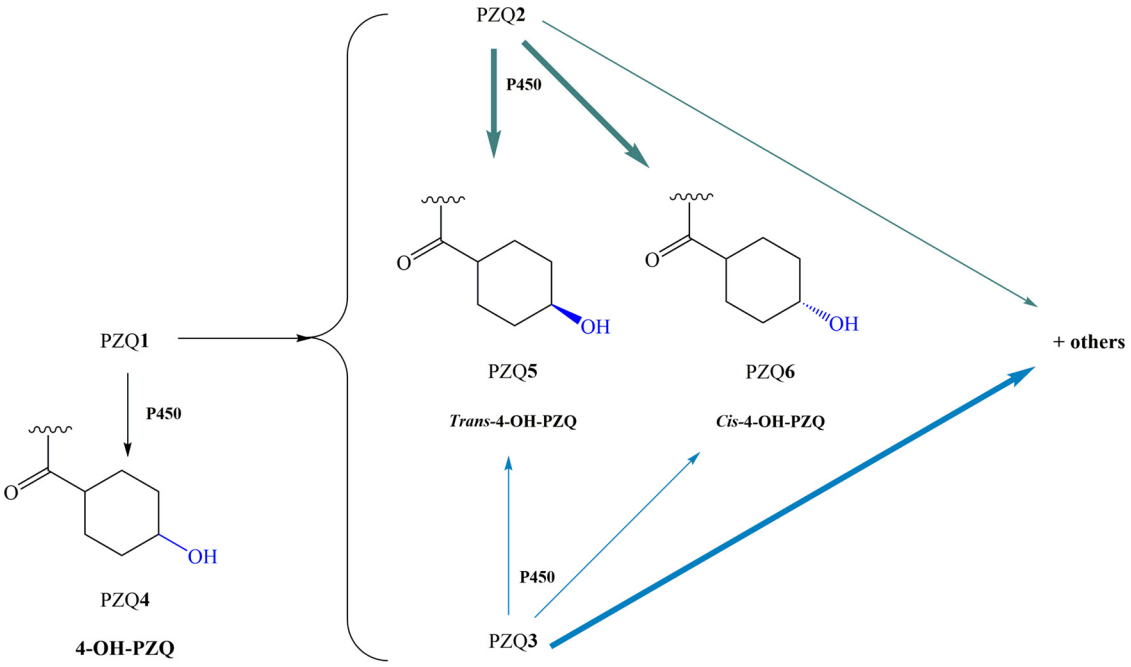

FIG 3 PZQ1 is metabolized by CYP 450, resulting in PZQ4 as the main product and other minor enantiomers such as PZQ5 and PZQ6. In addition, enantiomers of PZQ1 also undergo metabolism. Bold green and blue arrows indicate major metabolites of PZQ enantiomers PZQ2 and PZQ3, respectively. PZQ2 is mainly metabolized into *trans*- and *cis*-4-OH-PZQ (PZQ6), whereas PZQ3 is mainly metabolized into other mono- or dihydroxylated forms of PZQ1 and, to a less extent, into *trans*- and *cis*-4-OH-PZQ (PZQ5, PZQ6). P450 enzymes perform these transformations.

significant activity *in vivo*, which may derive from its *in vivo* reduction to a *trans*-cyclohexanol metabolite, PZQ4 (54). With this structure in mind, chiral PZQ derivatives (PZQ10 and PZQ11) were developed, as well as chiral PZQ7. Racemic PZQ10 and PZQ11 had modest activity against *S. japonicum in vitro*, while their enantiomers (*S* and *R*) failed to display any activity, with the exception of racemic PZQ7 and its enantiomers. Overall, all chiral PZQ derivatives display modest activity *in vivo* compared to that of PZQ1. Intriguingly, the size of the ring with a carbonyl group in these derivatives had an appreciable impact on *R* isomers, increasing their activity, but had almost no effect on *S* isomers (55). Using organometallic moieties such as ferrocenyl (already present in anticancer, antibacterial, and antimalarial drugs [56–58]) Fc-PZQ derivatives (types A and B, Fig. 4) displayed anthelmintic activity in the micromolar range but were considerably less active than PZQ1 (59). Upon alteration of the organometallic moiety to $Cr(CO)_3$ (CrPZQ16, CrPZQ17, Fig. 4), the derivatives exhibited marked activity against *S. mansoni in vitro*; however, they exerted low activity *in vivo* (60). This fact might be related to the metabolite liability of these derivatives, resulting in less-active metabolites (Fig. 5), and due to protein binding or distribution (61). Following a different design strategy, a combination of artesunate (AS) and PZQ1 led to new derivatives that incorporate these two moieties, DW-3-15. Because of the complementary effect of these two drugs (AS is more effective against schistosomula and juvenile worms, whereas PZQ is effective against adult worms), it was expected that this derivative would demonstrate potential broad-spectrum antischistosomal activity (62, 63). In fact, biological evaluation of DW-3-15 (Fig. 4) proved that the complementary functions of AS and PZQ1 were more effective than PZQ1 alone. Therefore, this might encourage rational drug design by combining pharmacophore moieties of discrete bioactive compounds with dual modes of action (62, 63). In comparison to DW-3-15, it was expected that endoperoxide-PZQ derivatives would have increased bioavailability (since their molecular weight was less than that of DW-3-15) and thereby improved antischistosomal activity. Although these derivatives presented good efficacy against adult *S. mansoni* worms *in vitro*, their activity was lower than that of DW-3-15.

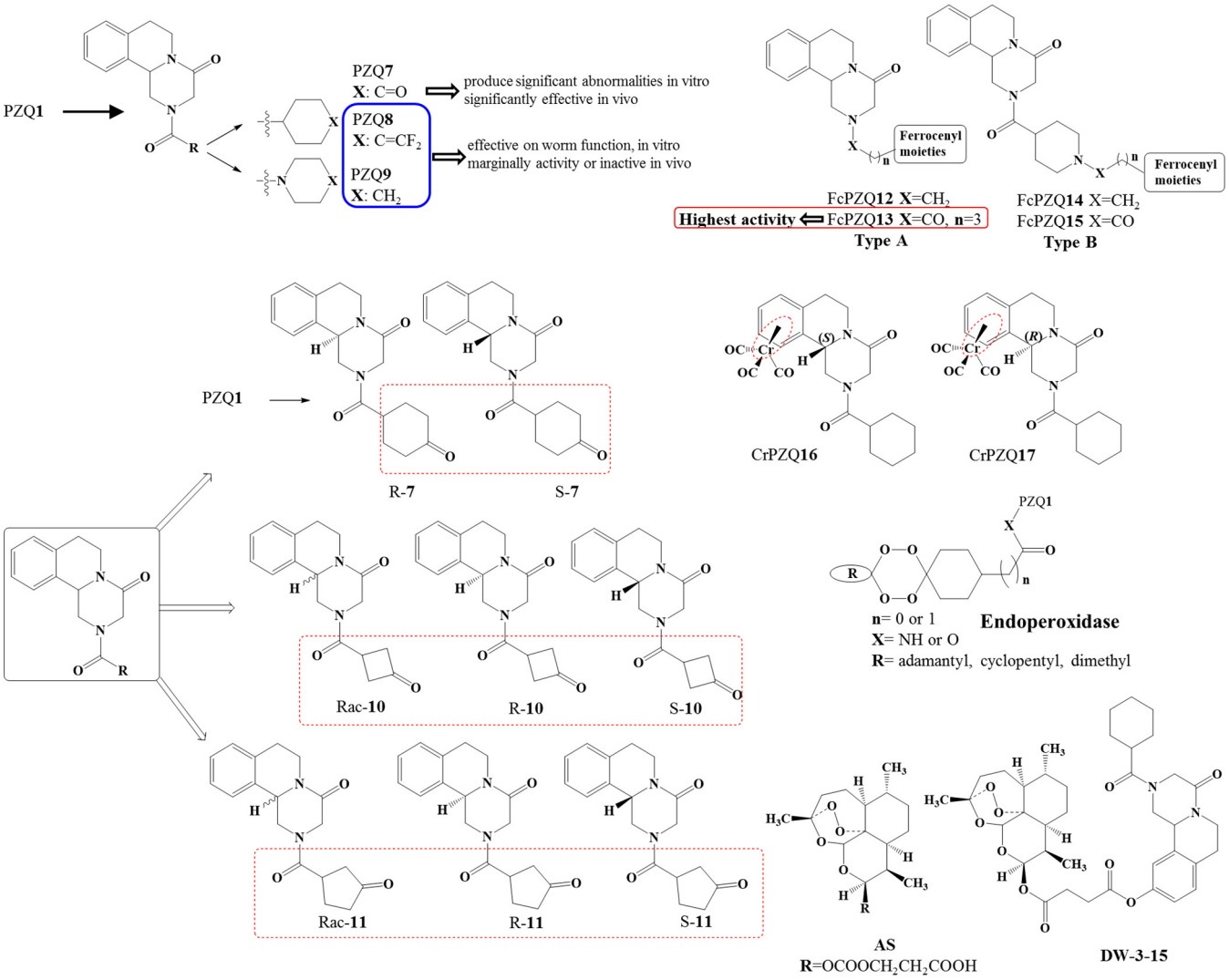

FIG 4 Structures of diverse PZQ derivatives developed and assessed for activity against schistosomes.

Moreover, potential action *in vitro* did not translate to impressive killing *in vivo* (64). The diminished activity of endoperoxidase derivatives might be associated with intrinsic aspects of their *in vivo* metabolism.

Apparently, the positions of chemical modifications played an important role in the compounds' activity. It seems that linkage through the metabolically liable cyclohexyl might not afford active derivatives, e.g., organometallic moieties. Moreover, PZQ derivatives have generally not achieved improved activity compared to that of the parent drug. Furthermore, in most cases, the promising *in vitro* activity of candidate drugs cannot be extrapolated to good *in vivo* activity since their pharmacokinetics and metabolic profiles are key determinants of their *in vivo* efficacy (53). Much remains to be done to develop an improved and effective derivative of PZQ1.

HOW DOES PZQ KILL SCHISTOSOMES?

Despite many years of use and the treatment of many millions of people, the mechanism(s) of action of PZQ1 has not been established yet. However, the early effects exerted by PZQ on the schistosome have been summarized under three main headings, (i) calcium influx into the whole parasite, (ii) muscle contraction, and (iii) surface modifications (65). It is tempting to link these phenomena into a single thread, assuming that calcium influx is the key event, which in turn induces muscle contraction and alterations to the tegument (65, 66). The correlation between increased intracel-

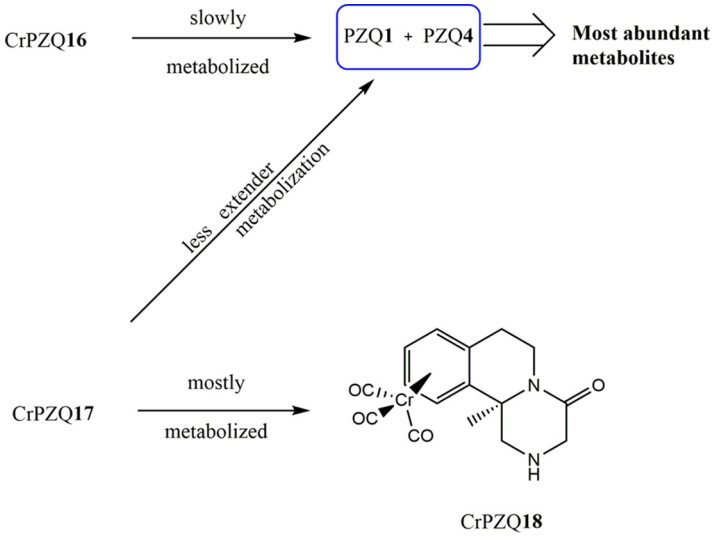

FIG 5 Metabolism of Cr-PZQ derivatives *in vitro* by human liver microsomes.

lular Ca^{2+} and muscular contractions in schistosomes exposed to PZQ has been known for decades. However, how PZQ1 disrupts homeostasis in schistosomes remains largely unknown. Diverse studies have focused on the phenomenon (Table 3). Initially, it was hypothesized that PZQ1 affects Ca^{2+} influx through voltage-operated calcium channels (67–73). However, in subsequent studies, it was shown that calcium accumulation by itself, as measured in parasites maintained *in vitro*, may not explain the schistosomicidal activity of PZQ1 (71, 72).

High-throughput transcriptomic approaches have been employed to address the refractory/susceptible nature of the developmental stages of schistosomes in terms of PZQ1 activity (73–77). These studies revealing genes that might be evolved in aerobic metabolism and cytosolic calcium regulation, suggesting that schistosomes undergo a transcriptomic response similar to that seen during oxidative stress (74). Moreover, it was demonstrated that CamKII (calcium/calmodulin-dependent protein kinase type II) appears to play a key role in the mode of action of PZQ1 and hence might be considered a promising novel drug target (76, 77). The use of mass spectrometry

TABLE 3 Key reports and findings focused on the mechanism of action of PZQ

Study design	Finding(s)	Reference(s)
Exposure of *S. mansoni* males *in vitro*	Muscular paralysis and rapid Ca^{2+} influx, removal of Ca^{2+} inhibited PZQ effect, tegument blebbing and disruption	67, 68
VOCC[a] β subunits expressed in *Xenopus oocytes*	Schistosome β subunits associated with drug sensitivity	69
Block VOCC, exposing schistosome to cytochalasin D	Inhibition of Ca^{2+} channels suppressed schistosomal activity	70, 71
Suppression of *Dugesia japonica* Ca^{2+} channel subunits by RNA interference	Suppression of Ca^{2+} channels of amputated parasite in two heads leads to inhibition of regeneration of two heads and tail	72, 73
Transcriptional response of *S. mansoni* to heat shock	>600 genes upregulated as possible targets of PZQ; schistosomes undergo oxidative-stress-like transcriptomic response	74
Gene expression in adult and juvenile *S. mansoni* cultured in PZQ	Juvenile schistosomes show enhanced transcriptomic elasticity	75
RNA interference-based silencing of CamKII	CamKII mitigated effect of PZQ by stabilizing Ca^{2+} fluxes within parasite muscles and tegument and might play role in mode of action	76, 77
Mass spectrometric characterization of surface lipids of schistosomes	Distinct chemical markers in female vs male responses to PZQ; PZQ may inhibit sphingomyelinase activity, impairing reproduction in females, whereas PZQ may impair activity of Na^+/K^+-ATPase in males	78, 79

[a]VOCC, voltage-operated calcium channels.

techniques revealed the existence of chemical markers that are distinct according to sex after drug exposure. Apparently, PZQ1 alters the conformation of the usual surface double lipid bilayer that surrounds schistosomes (78). Perhaps PZQ1 inhibits sphingomyelinase activity and thereby impairs reproduction by impeding the continuous release of eggs (79, 80).

Despite these efforts to understand how PZQ1 acts, the molecular targets remain elusive. Although from a medical point of view, how the drug acts might not be important as long as the drug is efficacious, the mechanism of action is relevant to improvement of the efficacy of new PZQ1 derivatives.

IS PZQ RESISTANCE IMMINENT?

Reliance on PZQ1 raises legitimate concerns about selection for PZQ resistance (65). MDA never reaches all of the infected people in a community, and so, the worm population remaining after treatment is not composed solely of resistant worms; there will still be a susceptible population that, in turn, reduces the likelihood of resistance (81–83). Whereas widespread drug resistance has not been proved, researchers have identified field and experimental isolates that exhibit significantly reduced susceptibility. These findings could portend the emergence of resistance to PZQ1 in schistosomes. Over the years, evidence of resistance to PZQ1 has been widely reviewed and remains controversial (81, 83–87). Moreover, the criteria used to classify a schistosome strain PZQ resistant are also controversial (81, 82). Here, we present an overview and synthesis of findings on this topic and also highlight potential mechanisms of drug resistance.

EXPERIMENTALLY INDUCED PZQ RESISTANCE

Attempts to induce resistance to PZQ1 in the laboratory were reported as early as the 1970s and have continued until the present, mostly focused on *S. mansoni* (88–90). Table 4 highlights some of the key studies (from our viewpoint) that attempt to demonstrate the appearance of resistance to PZQ. A hallmark study by Fallon and Doenhoff (91) published in 1994 demonstrated that *S. mansoni* developed resistance to PZQ1 over the course of several subcurative multiple doses of PZQ1 in mice; by the seventh generation of PZQ1 pressure, the population of schistosomes was 93% resistant to three PZQ1 doses of 300 mg/kg, a dose that killed 89% of the control schistosomes. Ismael and colleagues (92) studied the effect of PZQ1 at 300 and 500 mg/kg on successive generations of *S. mansoni* worms in mice and observed that at low subcurative doses, resistance to therapeutic doses of the drug appeared after several generations of the treatment regimen (92). More recently, Couto et al. (93) reported a novel method to induce resistance to PZQ1 in *S. mansoni*. Snails infected with schistosomes were treated successively with PZQ1 at 100 mg/kg for 5 consecutive days. Subsequently, mice were infected with cercariae released from the snails and treated with PZQ1 at 200, 400, or 800 mg/kg. This method is effective for inducing resistance of *S. mansoni* to PZQ1 in the laboratory and is far less costly and labor intensive than some other approaches mentioned above (93). Other studies have reported the generation of resistance to PZQ1 in *S. japonicum*, assayed in adult worms, cercariae, and miracidia (94). In contrast, we are not aware of reports describing experimental induction of resistance to PZQ1 in *S. haematobium*. Finally, it is also worth noting that the mode of action of the drug would be expected to be altered in strains that are insensitive to PZQ1 (81).

PZQ RESISTANCE IN THE FIELD

Reports of field resistance or therapeutic failure of PZQ are listed in Table 5. Most field surveys of resistance to PZQ1 focus on *S. mansoni*. Reduced susceptibility to PZQ1 has been widely found in foci of endemicity, notably in Africa, including Egypt and Senegal. An extremely low cure rate (18%) was reported in Senegal (95); however, it was suggested that failure of PZQ1 therapy occurs because of factors other than drug resistance, including very intense transmission and the presence of PZQ-refractory juvenile worms (immature parasites) (96). In Egypt, eggs obtained from treated and

TABLE 4 Key studies and findings focused on experimentally induced PZQ resistance in different *Schistosoma* species

Strain(s)	Study design	Treatment	Findings/outcomes	Reference(s)
S. mansoni WW and LE	Miracidia from feces of infected patients were used to infect *B. glabrata* to obtain cercaria (strain WW), which were used to infect mice; infected mice were treated with antischistosomal drugs; sensitivities of strains WW and LE to drugs were compared	Hycanthone, 80 and 20 mg/kg; niridazole, 100 and 50 mg/kg/day for 5 days; oxamniquine, 100 and 50 mg/kg, 1 dose	Hycanthone altered oogram pattern of 100% of mice infected with strain LE; hycanthone did not affect oogram pattern of mice infected with strain WW; strain WW was more resistant to niridazole and oxamniquine	88, 89
S. mansoni Brazilian	Mice were infected with *S. mansoni* obtained from infected individual, they were treated with different antischistosomal drugs during the time of embryological development of genital organs of schistosomula of both sexes	Oxamniquine, 50 mg/kg, 1 dose; oltipraz, 60 mg/kg daily for 5 days; PZQ, 50 mg/kg for 5 days	All treated groups had larger percentages of worms in liver and portal vein and significantly lighter parasite loads than control group, high rates of worm reduction, low rates of surviving worms, and 100% had changed oogram pattern; failure to induce resistance in Brazilian strain	90
S. mansoni Egyptian	*S. mansoni* infected mice were treated with subcurative different doses of PZQ after 6 wk p.i.;[a] eggs produced by worms that survived to treatment were used to infect snails	PZQ, 3 × 300 mg/kg	*S. mansoni* subjected to drug pressure may develop resistance to schistosomicidal drugs after relatively few passages; first demonstration of resistance to PZQ	91
	Miracidia obtained from eggs from infected patient were used to infected mice; eggs produced by worms that survive to treatment 6 wk p.i. were used to infect snails and mice of the following generations	PZQ, 300 or 500 mg/kg	Subcurative dose of PZQ led to development of resistance to therapeutic dose of PZQ in following generations	92
S. mansoni LE	Infected *B. glabrata* snails were treated with PZQ; after treatment, cercariae obtained from these snails (LE-PZQ isolate) and susceptible LE strains were used to infect mice that were treated p.i.	*B. glabrata*, 3 × 100 mg/kg, 5 consecutive days; infected mice, 45 days p.i., 200, 400, 800 mg/kg	Experimental model of development of resistance to *S. mansoni* using infected snails; mean no. of worms recovered from group of mice infected with LE-PZQ isolate treated with 200 and 400 mg/kg was significantly higher than that from mice infected with LE strain with same treatment; *in vitro*, worms of LE-PZQ isolates were also less susceptible to PZQ	93
S. japonicum	Mice were infected with isolates from two distinct regions, PZQ-susceptible isolates and PZQ-induced isolates, and then treated with PZQ; cercariae and miracidia of different isolates were exposed to PZQ solution, and morphological alterations were observed	Infected mice, 35 days p.i., 0,37.5,75, 150, 300, and 600 mg/kg; cercariae and miracidia, 10^{-5}, 5×10^{-6}, 5×10^{-7}, and 10^{-} M	PZQ-resistant isolates of *S. japonicum* were established in mice with subcurative doses of PZQ by artificial selection in laboratory; drug resistance might be exhibited by different developmental stages (miracidia, cercaria, adult worms); established PZQ ED_{50}s for different developmental stages	94

[a]p.i., postinfection.

uncured patients gave rise to schistosomes (*S. mansoni*) that showed 3- to 5-fold lower sensitivity to PZQ1 (97). In fact, *in vitro* measurements of PZQ1 susceptibility correlated well, in some cases, with the drug dose producing 50% of the maximal effect (ED_{50}), as determined in murine infections, further indicating that factors in the worms them-

TABLE 5 Key reports of field resistance or therapeutic failure of PZQ

Species	Country	Yr	Sample size	Treatment(s)	Outcome measure	Reference
S. mansoni	Senegal	1991	422	40 mg/kg	12 wk after treatment, cure rate only 18%, antigen detection positive in 90% of individuals; low cure rates may be due to intense transmission and/or development immune responses	95
	Egypt	1994	1,607	1 dose of 40 mg/kg, 3 successive doses of 40, 40 and 60 mg/kg	1–2.4% of villagers treated could not be completely cured of infection, and 3 of every 1,000 treated villagers may harbor parasites that can tolerate high doses of PZQ; extensive use of PZQ has not resulted in dramatic change in its efficacy	97
		2005	1,405		Compared with results obtained in 1994 in same villages, decreased prevalence and intensity of infections; first treatment resulted in normal cure rate (73–92%); after 3 successive doses, no uncured patients; drug failure did not increase over 10 years of therapeutic pressure in these villages	99
S. hematobium	Malawi[a]	1995	1	~40 mg/kg (30% was spit up)	3 doses of PZQ necessary to cure infection; concomitant *Giardia lamblia* infection might have caused malabsorption of drug; repeated courses of therapy may be necessary to cure infection, and both parasite and host factors should be considered if infection persists; 7 treatments necessary to eliminate eggs from parasites	100
	Senegal[a]	2006	2	40 mg/kg	Repeated standard treatment failed to clear infection	102
S. japonicum	Sichuan Province, China	1985	185	2 × 40 mg/kg	Low no. of treatment failures (only 1 remained infected); good compliance with treatment; PZQ remains effective against this schistosome	106
		2010	584		6 wk after treatment, 95% had no detectable eggs, 3% were still excreting eggs, and 2nd dose of drug was given; no detectable eggs were observed 6 wk after 2nd dose; efficacy of PZQ still high; no evidence of resistance detected	108
		2012	43	Single oral dose of 40 mg/kg, 30 mg/kg for 2 days	6 wk after second treatment, eggs not found were found in infected villagers; no evidence of resistance	110

[a]Travelers from this area of endemicity.

selves were responsible for the reduced susceptibility of these isolates to PZQ1 (98). Studies carried out 10 years later in the same area failed to show any hint of resistance to PZQ1 (99).

As noted, there is no evidence of *S. haematobium* resistance to PZQ1. However, some studies have reported failures of treatment to cure infections with this species (100–102). For example, Alonso et al. (102) described the case of two Spanish travelers with urogenital schistosomiasis in whom repeated standard treatment (a single 40-mg/kg dose of PZQ1) failed to clear the infection. Sabah et al. (103) hypothesized that people coming from areas where schistosomiasis is not endemic may lack an immunological component that has been shown to contribute to the activity of PZQ1 in experimental animals. Emergence of resistance of *S. japonicum* to PZQ1 has also received attention (101–105). However, despite large-scale and repeated use, the current efficacy of PZQ1 remains unchanged and it is highly effective at a curative dosage (a single dose of 40 mg/kg) in the main areas of China where schistosomiasis is endemic (106–108). Seto et al. (106) conducted a cross-sectional survey, in which the efficacy of PZQ1 was evaluated in 33 villages in Sichuan Province, where the prevalence of infection was found to be 5.7%. Of 3,269 persons tested, 185 were infected. The infected persons were treated two times with a 40-mg/kg dose of PZQ1, and only one remained infected, findings that support the notion that PZQ1 remains effective for the treatment of infection with *S. japonicum* in China.

Insensitive measurement of infection burdens may lead to overestimation of PZQ1

efficacy and thereby confound attempts to discriminate between reduced PZQ1 susceptibility and drug resistance. Diagnostic techniques for schistosomiasis are time-consuming, and many epidemiological assessments rely on microscopic observation of viable eggs in urine (*S. haematobium*) and feces (*S. mansoni*, *S. japonicum*) (109, 110). However, fluctuation of egg output in urine or stool occurs routinely, negatively influencing the sensitivity of the assay in the absence of repeated sampling (111). New diagnostic techniques such as egg detection by PCR aim to improve sensitivity, but the sampling limitations persist (112, 113). Despite the development of new tools for diagnosis (reviewed in reference 114), there remains a need for better diagnostics, both in the field and in the clinic. In addition to the importance of improvements for clinical diagnosis, advances in diagnostic tools are also critical in programs targeting elimination by MDA and for the development and assessment of new drugs and vaccines (3).

MECHANISM OF PZQ RESISTANCE

In the absence of the exact mechanism of action of PZQ1, the mechanism of drug resistance in schistosomes also remains unclear (115). However, the likely nature of the mechanism of PZQ resistance has been described, such as induction of ATP-binding cassette (ABC) transporters (ABC transporters are proteins involved in the transport of toxins and xenobiotics). Several members of this family, like P-glycoprotein (Pgp) and multidrug resistance (MDR)-associated proteins (MRPs) represent two classes of these MDR transporters (116, 117). ABC transport protein homologues from *S. mansoni* are known, i.e., SmMRP1 (orthologue of MRP1) and SMDR2 (orthologue of Pgp) (106). Juvenile schistosomes express ~2.5-fold higher basal levels of SMDR2 and SmMRP1 than adults, higher levels of SMRD2 RNA are seen in females than in males, and higher SmMRP1 levels are seen in males than in females (118). Furthermore, SMRD2 is modulated by PZQ1, suggesting that PZQ1 is also a substrate for SMRD2 (119). Transcriptomic analysis reveals increasing levels of transcripts encoding the ABC transporters SMDR1, SmMRP1, SmMRD2, and SMDR3 in juveniles exposed to PZQ1 *in vitro*, supporting the notion that ABC transporters participate in resistance to PZQ1 in schistosomes (75). Guglielmo et al. (120) developed a series of PZQ NO-donors furoxans that are worthy of investigation in view of their potential activity against PZQ-resistant schistosomes. Involvement of Ca^{2+} channel changes in resistance to PZQ has been widely described (121). Nonetheless, whether these phenomena are responsible for drug action or represent downstream consequences has not been established (83, 122).

CONCLUDING REMARKS

Because of its efficacy, safety, cost, and indeed the lack of alternatives, PZQ1 has remained the drug of choice for schistosomiasis treatment and transmission control for >40 years (15, 16). Yet PZQ1 has drawbacks, including inactivity against juvenile schistosomes. Moreover, reliance on a single drug for the treatment of a disease with the global public significance of schistosomiasis risks facilitating the development and spread of drug resistance, especially since reduced susceptibility has occurred frequently both in the field and in the laboratory. A pressing need for new interventions has arisen, including novel compounds with modes of action discrete from those of PZQ1 and methods to detect the appearance and spread of resistance to PZQ1 (123). Despite the novel structures of several derivatives of PZQ1, most are sufficiently efficacious to warrant closer investigation in clinical trials. In addition, understanding the mechanism of action of PZQ1 and its metabolism is critical since this information would facilitate the elucidation of novel targets and/or lead to improvements in the efficacy of this essential and singular medicine.

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