

The mechanism of action of praziquantel: can new drugs exploit similar mechanisms?

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Abstract

Praziquantel (PZQ) is the drug of choice for treating infection with worms from the genus *Schistosoma*. The drug is effective, cheap and has few side-effects. However, despite its use in millions of patients for over 40 years its molecular mechanism of action remains elusive. Early studies demonstrated that PZQ disrupts calcium ion homeostasis in the worm and the current consensus is that it antagonises voltage-gated calcium channels. It is hypothesised that disruption of these channels results in uncontrolled calcium ion influx leading to uncontrolled muscle contraction and paralysis. However, other experimental studies have suggested a role for myosin regulatory light chains and adenosine uptake in the drug's mechanism of action. Assuming voltage-gated calcium channels do represent the main molecular target of PZQ, the precise binding site for the drug remains to be identified. Unlike other commonly used anti-parasitic drugs, there are few definitive reports of resistance to PZQ in the literature. The lack of knowledge about PZQ's molecular mechanism(s) undermines our ability to predict how resistance might arise and also hinder our attempts to develop alternative antischistosomal drugs which exploit the same target(s). Some PZQ derivatives have been identified which also kill or paralyse schistosomes in culture. However, none of these are in widespread clinical use. There is a pressing need for fundamental research into the molecular mechanism(s) of action of PZQ. Such research would enable new avenues for antischistosomal drug discovery.

Keywords: praziquantel; schistosomiasis; voltage-gated calcium channels; neglected tropical disease; calcium signalling; drug mechanism

Introduction: Schistosomiasis

Schistosomes (blood-dwelling parasites of the *Schistosoma* genus), are the causative agents of schistosomiasis (bilharzia), a devastating parasitic disease, second only to malaria with respect to its detriment to global health and socio-economic impact [1]. Most cases of schistosomiasis are attributable to one of three schistosome species, *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium*, whose habitats are predominantly confined to tropical and sub-tropical regions.

Concerted treatment strategies have resulted in almost total elimination of this disease in Japan, and substantial progress has been made in other endemic regions, such as the Caribbean islands, Brazil and China [2]. However, despite this progress, schistosome infections are becoming increasingly prevalent in previously non-endemic regions. Moreover, the disease burden, morbidity and mortality rates in sub-Saharan Africa are still high [3, 4]. Indeed, on a global scale, it is estimated that almost 800 million people are at risk of schistosome infections, and over 200 million individuals are already infected [4]. Of those already infected, a staggering 85 % are located in Africa, resulting in approximately 200,000 deaths *per annum* in sub-Saharan Africa alone [1, 5].

Despite these alarming figures, schistosomiasis is still considered a “neglected tropical disease” (NTD). This is primarily due to the socio-economic standing of the individuals affected by this disease, as infections tend to be confined to poor, rural (and often marginalised) communities [6-9]. Furthermore, as schistosomiasis is a chronic disease, causing long-term (and often subtle) pathologies, its eradication is perhaps not as high on the priority list as global health initiatives directed against other infectious diseases, such as malaria and HIV [2]. Nevertheless, with the advent of large-scale treatment interventions in Africa, and new health initiatives in the endemic regions of Egypt, it seems that these paradigms may be shifting.

Schistosomes

Platyhelminthes (flatworms), are a diverse phylogenetic lineage comprising both free-living (turbellarian), and parasitic (trematode, monogenean and cestode) clades [10]. Schistosomes belong to the trematode (flake) lineage (Phylum: *Platyhelminthes*; class; *Trematoda*; sub-class: *Digenea*; order: *Strigeidada*; family: *Schistosomatidae*; genus: *Schistosoma*), a large sub-group of parasitic platyhelminths, which require both a molluscan (intermediate), and vertebrate (definitive) host to complete their lifecycle [11, 12].

Schistosomes have a complex lifecycle, comprising both free-living and parasitic stages, and encompassing a remarkable series of morphological and physiological changes [12] (Fig. 1). Briefly, schistosome eggs are either shed in the urine (*S. haematobium*) or faeces (*S. mansoni* and *S. japonicum*) into freshwater lakes and rivers, where they hatch, releasing miracidia. Miracidia invade intermediate freshwater snail hosts, where they multiply, undergoing several rounds of asexual reproduction to transition from the larval sporocyst stage, to the infective cercarial stage (a process which takes approximately 4-6 weeks) [13]. Free-swimming cercariae are then released from the snail, back into the water where they encounter their definitive mammalian host, typically humans bathing in freshwater lakes, pools or rivers.

Infective cercariae penetrate the skin of their human host (using digestive enzymes), and upon doing so, shed their tails to become schistosomula (Fig. 1). Schistosomula reside within the skin for over 24 hours (a period often referred to as the skin stage) [14], before making their way through the dermal

tissue, to the veins. Migratory juveniles then travel to the lungs *via* the pulmonary artery (5-6 days post-infection), where they are now referred to as “lung schistosomula” [15]. At this point, schistosomula re-enter the circulatory system and travel to the liver, where they begin to feed on blood, and grow rapidly for 8-10 days [16].

From here, immature worms migrate to the venous plexus of the bladder (*S. haematobium*), or the superior mesenteric veins of the intestine (*S. mansoni* and *S. japonicum*), where they mature into adults (approximately 5-7 weeks post-infection) [13]. Upon reaching sexual maturity, male and female worms couple and reproduce (Fig. 1), allowing the female to produce hundreds to thousands of eggs per day [13]. Eggs are then excreted *via* the bladder (*S. haematobium*), or the lumen of the intestine (*S. mansoni* and *S. japonicum*), so that the infection cycle can start anew.

However, such prolific egg production means that eggs frequently become trapped in host tissues. Indeed, the pathologies associated with schistosomiasis are predominantly caused by eggs lodged in the intestines or liver (*S. mansoni* and *S. japonicum*) or in the bladder/urogenital system (*S. haematobium*) [17]. The host pro-inflammatory immune response to egg antigens (and those of migratory juveniles) results in acute schistosomiasis, or ‘Katayama syndrome’, which manifests as fever, myalgia, headache, fatigue, and abdominal pain [18]. Whereas the granulomas that form around these eggs can result in a broad spectrum of chronic pathologies, ranging from anaemia and stunted growth, to infertility, tissue fibrosis, portal hypertension, haemorrhaging and squamous cell carcinoma [13]. In certain instances, egg granulomas can also be found in the brain and spinal cord of infected individuals, leading to severe neurological complications [19].

Although *S. mansoni*, *S. japonicum* and *S. haematobium* share considerable similarity in terms of their lifecycle, development and transmission, there exist some subtle (yet notable) differences between the three schistosome species, particularly in terms of their geographical distribution and intermediate host availability. For example, *S. mansoni* and *S. haematobium* share an overlapping habitat in sub-Saharan Africa and the Middle East; whereby *S. haematobium* parasitizes freshwater snails of the *Bulinus* genus, and *S. mansoni* parasitizes snails of the *Biomphalaria* genus. Indeed, the broad geographical distribution of *Biomphalaria* snails means that *S. mansoni* isolates can be found further afield, with habitats in the Caribbean islands and South America [13]. Conversely, *S. japonicum* isolates are localised to Asia (including Indonesia, mainland China and the Philippines), where they parasitize freshwater snails of the *Oncomelania* genus [13].

Due to their differential localisation in host tissues, *Schistosoma* spp. also display distinct diagnostic traits and pathologies. For example, urinary tract schistosomiasis (caused by infection with *S. haematobium*), is often diagnosed by the presence of blood in the urine, and is associated with a higher risk of squamous-cell bladder carcinomas [20], and infertility [13]. Whereas intestinal schistosomiasis (caused by infection with *S. mansoni* or *S. japonicum*), can often go undiagnosed due to an absence of consistent diagnostic symptoms, ranging from the presence of blood in the stools, abdominal pain and cramps, to hepatomegaly (enlarging of the liver), and/or splenomegaly (enlarging of the spleen) [6].

Treatment of schistosomiasis

In the absence of an effective vaccine against schistosomiasis, treatment strategies centre on the administration of the anthelmintic drug, praziquantel (PZQ) (Fig. 2), a pyrazino-isoquinoline derivative [21, 22]. PZQ is an effective, and relatively inexpensive drug with few side effects, with activity against a broad spectrum of parasitic flukes and tapeworms [23-26]. Indeed, due to its efficacy against all

forms of human schistosomiasis [27], PZQ has largely supplanted other antischistosomal compounds, such as oxamniquine (OXA) (Fig. 2), a tetrahydroquinoline derivative with activity against *S. mansoni*, but not against the other members of the *Schistosoma* genus [28]. This drug was shown to inhibit the synthesis of DNA, RNA and proteins in *S. mansoni* suggesting that its target may be one (or more) of the proteins involved in nucleic acid synthesis [29]. Later work demonstrated that it binds directly to DNA, probably following activation by a parasite-specific sulphotransferase enzyme (SmSULT-OR) [30, 31]. The enzyme from *S. mansoni* processes the drug more efficiently than the equivalent enzyme from other *Schistosoma* species, thus explaining why OXA is more effective in *S. mansoni* [32]. Mutations which result in loss of function in *S. mansoni* SmSULT-OR can result in resistance to OXA [33]. The availability of structures of the sulphotransferase enzyme from *S. japonicum* and *S. haematobium* mean that rational, structure-based, medicinal chemistry can now be applied to the development of OXA derivatives which are effective against all these species in addition to *S. mansoni* [32].

The recommended dose of PZQ is approximately 40-60 mg kg⁻¹ body weight, with the lower dose generally sufficient for treatment of *S. mansoni* and *S. haematobium* infections, and the higher dose recommended for treatment of *S. japonicum*. Upon administration of this drug, cure rates range between 83 – 89 % for *S. haematobium*, 79 – 87 % for *S. mansoni* and are approximately 86 % for *S. japonicum* [34].

In addition to its antischistosomal activity, praziquantel can be used to treat diseases caused by other trematode infections, such as clonorchiasis (caused by infection with the Chinese liver fluke, *Clonorchis sinensis*), and opisthorchiasis (caused by infection with the Southeast Asian (or carcinogenic) liver fluke, *Opisthorchis viverrini*). Cestodes, such as the beef tapeworm, *Taenia saginata*, and the pork tapeworm, *Taenia solium*, are also susceptible to PZQ [34]. There are also veterinary uses of the drug – for example in fish farming, companion animals and agriculture [35-38]. Interestingly, there is also a considerable “grey literature” on the use of PZQ to clear ornamental fish tanks etc of infestation by planarians and other worms. In general, this has only been subject to limited rigorous scientific investigation, but it does represent a significant use of the drug in the more developed parts of the world [39].

Thus, due to its efficacy and versatility, praziquantel has become the “treatment of choice” against schistosomiasis, and numerous other trematode/ cestode infections. However, this drug is not without its limitations. For example, PZQ is of limited use in the treatment of hydatid disease (a zoonotic disease caused by infection with *Echinococcus* spp.) [40], and is largely ineffective against fasciolosis, a parasitic disease caused by liver flukes of the *Fasciola* genus, including *Fasciola gigantica* and *Fasciola hepatica* [41-43]. Moreover, a major shortcoming of PZQ lies with its biphasic efficacy against schistosomes [44]. Cercariae and “skin stage” schistosomula are highly susceptible to PZQ treatment, as are adult worms. Conversely, juveniles are less sensitive to PZQ, and largely refractory to this drug around 3-4 weeks post-infection, gradually regaining full susceptibility 6–7 weeks after infection [24, 45, 46]. This drawback has serious implications for the treatment and control of schistosomiasis, as, depending on the stage of infection, chemotherapy against the juvenile worm can lead to apparent drug failure, lower cure-rates, prolonged infective periods, and the requirement for multiple rounds of treatment [25].

In the absence of an effective vaccine to protect against schistosome infection, some countries and regions have established mass drug administration programmes (MDA) [47]. In a typical MDA programme, the large groups of people (often vulnerable groups such as children) are offered prophylactic doses of PZQ. This strategy works in an analogous way to “herd immunity” resulting from wide-spread vaccination for other infectious diseases. In areas where schistosomiasis is

endemic, the release of urine or faeces into communal water ensures that infected individuals expose others to the risk of the disease. In an MDA programme, the majority of individuals suffering from schistosomiasis will be cured. This limits the release of schistosomes into the environment. Furthermore, non-infected individuals will have PZQ in their system and so are less likely to become infected if exposed to the parasite. Thus, the cycle of infection can be broken. There is some evidence that individuals treated with PZQ as part of a MDA programme are more likely to develop some limited immunity towards future infections. Treatment with PZQ results in a reduction in the immunomodulatory effects of the parasite and the release of tegumental antigens [48]. However, there is a risk that over a longer time period reducing the number of people infected will result in a lower fraction of the population having partial immunity [49]. MDA programmes can be highly successful [50]. This success rests on the relatively low cost of the drug (which enables very large numbers of people to be offered it cost-effectively), the generally limited side-effects (which removes one possible cause of non-compliance in the targeted populations) and the lack of widespread resistance to PZQ (see below). Concerns have been raised that such wide-spread use of the only effective drug to treat schistosomiasis will increase the selective pressure for the development of resistance (in a manner analogous to the emergence of antibiotic resistance in bacteria) [47, 51].

Praziquantel: pharmacology

Praziquantel, marketed as “Biltricide” (Bayer HealthCare Pharmaceuticals Inc., Germany), is the generic name for 2-(cyclohexylcarbonyl)-1,2,3,6,7, 11b-hexahydro-4H-pyrazino [2, 1-a] isoquinolin-4-one (Fig. 1.3). PZQ was originally developed as a novel tranquiliser. However, it proved to be less effective than existing compounds and was passed from Merck to Bayer for testing as a veterinary medicine which showed it to be highly effective in the treatment of schistosomiasis in a range of mammals [52, 53]. Following successful trials in animals it was re-considered as a human medicine and entered clinical and field trials which demonstrated the drug’s efficacy and tolerability [54, 55]. In its commercial form, PZQ exists as a racemic mixture of *laevo* R(-), and *dextro* S(+) enantiomers, of which only the R-enantiomer exhibits antischistosomal activity [56-59]. **In general, the drug is formulated and marketed in the racemic mixture. This is largely due to the reduced cost of producing the drug in this form [60]. However, there is some evidence that side-effects would be reduced by using the purified R-enantiomer [61]. The bitter taste of the drug is also largely ascribed to the S-enantiomer [62]. Consequently, efforts have been made to synthesise enantiomerically pure R-praziquantel or to design effective purification of this isomer from the racemic mixture [63-67]. The reasons for the greater effectiveness of the R-enantiomer are not clear. The most obvious explanation is that only this isomer is able to bind to the target, a situation which is common for chiral drugs. This arises because the binding sites for molecules in proteins are themselves generally chiral and will thus have different affinities for the two enantiomers. In the absence of clear identification of the target biomolecule and the location of the specific binding site, it is currently impossible to verify this hypothesis. It is also possible that the two molecules are processed differently (or with different efficiencies) by the host and/or parasite metabolic systems. Again, this would arise from the binding of the drug to chiral active sites in the enzymes which catalyse these metabolic processes.**

PZQ is classed as a “Class II” drug in the Biopharmaceutics Classification Systems (BCS), signifying a low solubility in water, and a high permeability in the gastrointestinal (GI) tract [68]. Hence, the dissolution rate of this drug represents a limiting step in its absorption [69], requiring higher doses than may otherwise be necessary [26].

Upon first pass metabolism in the liver, PZQ undergoes extensive metabolism by cytochrome P-450 enzymes (2B1 and 3A), to form numerous derivatives and metabolites [70-72], with rapid disappearance from the circulatory system (plasma half-life \approx 1-3 h), and clearance from the body within approximately 24 hours [24]. One of the main metabolites resulting from this biotransformation, a polar *trans*-cyclohexanol derivative (*trans* 4-OH-PZQ), has an approximate 4 to 10-fold lower efficacy than PZQ on *S. mansoni* [26]. Thus, the bioavailability of PZQ can be increased when administered with inhibitors of cytochrome P-450, such as cimetidine [73, 74]. Bioavailability can also be increased when administered with food (especially carbohydrates) [75, 76], but is substantially reduced upon simultaneous administration of antiepileptic drugs, such as carbamazepine or phenytoin, or upon treatment with corticosteroids, such as dexamethasone [77, 78]. Side-effects are generally considered to be minimal, but some caution may be required if the patient has other serious conditions such as heart failure [79].

Praziquantel: mechanism of action

Despite the widespread use of PZQ for over 40 years, its cellular target and mechanism of action remain elusive. However, evidence suggests that PZQ acts to disrupt calcium-mediated processes in adult schistosomes, potentially through antagonism (or partial agonism which results in dysregulation) of the parasite's voltage-gated calcium (Ca_v) channels and/or molecules which interact with and regulate these channels [25, 80, 81]. These hypotheses are largely based on the observed effects of this drug.

Early studies, conducted *in vitro*, demonstrated that PZQ altered the membrane permeability of adult *S. mansoni* and *S. japonicum* worms, causing rapid calcium influx, sustained muscle contractions and profound disruption to the tegument [21, 82, 83], a syncytial structure which forms the outer layer of the parasite, acting as both a protective shield, and dynamic interface with the host environment [84]. These effects were dose dependent, and were inhibited in the absence of calcium ions, implying a mechanistic link between PZQ activity and calcium-mediated processes [21, 85]. Thus, in physiological terms, PZQ-induced vacuolation and blebbing of the tegument is thought to lead to the increased exposure of surface antigens that can be recognized, and attacked by the host immune system. In addition, sustained contraction of the worm musculature results in the paralysis of the parasite, detachment from host tissues, and subsequent expulsion from the host [56, 86]. These early experiments were replicated with other PZQ-susceptible flatworms, such as the trematodes *C. sinensis* and *O. viverrini* [87], and numerous cestode species, including members of the *Hymenolepis* and *Taenia* genera [88], but not with the PZQ-refractory trematode, *F. hepatica*, whose unusually thick and robust tegument may (in part) negate the effects of this drug [83].

Indeed, subsequent studies revealed that the tegument was likely to play an integral role in the PZQ mechanism of action, as evidenced by comparative analyses between intact and detegumented *S. mansoni* adult male parasites. For example, Blair and co-workers demonstrated that PZQ produced a dose-dependent tonic contraction in intact *S. mansoni* worms, when the parasites were bathed in standard RPMI media. However, when the medium was supplemented with a high magnesium: calcium ion ($[Mg^{2+}]:[Ca^{2+}]$) ratio, tonic contractions were inhibited, resulting in a PZQ-dependent biphasic contraction and flaccid paralysis (no response to subsequent stimuli which would be expected to cause muscle contraction), indicating that an extracellular Ca^{2+} source was required for the full effects of this drug, *in vitro* [89]. In contrast, detegumented worms, whilst still responding to PZQ, exhibited a single phasic contraction when incubated in elevated $[Mg^{2+}]$ media [89]. Furthermore, when calcium ions were removed from the medium, PZQ evoked a transient response in intact worms,

but did not evoke a response in detegumented worms until the medium was reconstituted with calcium ions. This implied that the tegument and sarcoplasmic membranes both contained PZQ-sensitive sites, but the tegument was required for full response to this drug [89]. As such, the authors proposed that PZQ altered the activity of calcium channels, located in the tegument and muscles of schistosomes.

Ca_v channels are typically large, multi-domain complexes, consisting of a pore-forming α_1 subunit (which defines the functionality and pharmacology of the channel), and a subset of smaller auxiliary subunits, including the β and $\alpha_2\delta$ subunits (Fig. 1.6), which act to modulate the activity of the channel, whilst directing expression to the plasma membrane [90, 91]. Calcium channels are categorised according to the current gated by the α_1 subunit, and can be classed as either high voltage activated (HVA) channels, which open in response to strong membrane depolarization (including Ca_v1 and Ca_v2 channel sub-types); or low voltage activated (LVA) channels, which respond to weak depolarization (including the Ca_v3 channel sub-type) [91-93]. HVA channels can be further defined by their localisation to certain tissue types (Table 1.1), and their sensitivity to various drugs. L-type channels are sensitive to dihydropyridines, such as nifedipine, verapamil, nicardipine and nemadipine. Whereas non-L-type channels are refractory to dihydropyridines, but can be further divided into N, R, P/Q and T-types based on their differing sensitivities to certain toxins [91, 94].

Furthermore, as the C-terminal region of the α_1 subunit is also believed to function as a binding site for (calcium-free) apo-calmodulin. Evidence suggests that calmodulin is constitutively tethered to the Ca_v channel, allowing for a rapid response to changes in cellular calcium ion concentration [95-97]. However, the location of the tethering site remains controversial, with some studies implicating the IQ motif [98], and others implicating the pre-IQ domain [97], or a C-terminal site in proximity to and/ or encompassing the two domains [96].

The hypothesis that PZQ acts (at least in part) through voltage-gated calcium channels was supported by a series of studies by Kohn and co-workers [99-101]. In these experiments, schistosome voltage-gated calcium (Ca_v) channels were shown to contain an unusual Ca_v channel β subunit (termed the variant β subunit, or " β_{var} "), which was structurally distinct from its mammalian homologues, and unique to *Platyhelminthes* [81]. The authors demonstrated that this variant subunit could confer PZQ-sensitivity to an otherwise insensitive mammalian Ca_v channel α_1 subunit when heterologously expressed in *Xenopus* oocytes, a characteristic attributed to the absence of two serine residues within a conserved region of the protein, termed the β interaction domain (BID), which typically function as protein kinase C (PKC) phosphorylation sites [101]. At the time, the BID was believed to contact the α_1 subunit (the pore-forming component of the channel), playing an integral role in channel regulation, thereby providing a putative mechanism by which PZQ could elicit its effects on calcium homeostasis [102]. However, the X-ray crystal structure of a mammalian Ca_v channel complex revealed that the BID was buried within the matrix of the protein, and did not contact the α_1 subunit [103]. Thus, the method by which the variant β subunit mediated its effects on the α_1 subunit remained unresolved [81]. Moreover, as subsequent studies revealed that the sequence and expression levels of the β variant were seemingly unaltered between PZQ-susceptible adult *S. mansoni* worms and PZQ-refractory juveniles [81, 104, 105], this cast further doubt on the role of the variant β subunit in the PZQ mode of action.

Nevertheless, these observations do not eliminate the possibility that schistosome Ca_v channels serve as a molecular target for PZQ. It should be noted that although juvenile worms can withstand drug concentrations that are lethal to adult parasites, they still respond to PZQ treatment with

increased calcium influx and the onset of muscular contractions [106]. Thus, whereas Ca_v channels may serve as an initial receptor for this drug, its antischistosomal activity may be mediated by downstream components, which are differentially expressed between PZQ-susceptible and PZQ-refractory parasites [81].

The notion that Ca_v channels function as determinants of PZQ-susceptibility has been further explored in other species of *Platyhelminthes*; most notably in the planarian, *Dugesia japonica*. Planarians (free-living turbellarian flatworms), have been extensively studied due to their regenerative properties [107]. For example, upon amputation of the head and tail, *D. japonica* trunk fragments can regenerate a complete body plan within 5 to 7 days, retaining the anterior–posterior polarity of the worm. However, when this organism is exposed to PZQ, the regeneration process takes a rather unexpected turn: the anterior-posterior axis of the worm is duplicated, leading to a two-headed organism with a duplicated (and integrated) central nervous system (CNS), and organ systems [108]. As with schistosome species, *D. japonica* encodes two Ca_v β subunits; a “conventional” β subunit (with homology to mammalian β subunits), and a variant sub-type lacking two conserved serine residues in the BID domain, which shares approximately 34 % sequence identity with “Ca_v β_{var}” (the variant subunit previously implicated as a putative molecular target of PZQ in *S. mansoni* worms). Thus, Nogi and co-workers set out to test whether these Ca_v channel subunits were involved in the observed effects of PZQ on the *D. japonica* regeneration process. Using RNA interference (RNAi) technology, they demonstrated that silencing of the genes encoding either Ca_v β subunit antagonised the ability of PZQ to produce bipolar (two-headed) worms. Moreover, at higher doses of PZQ, RNAi-silenced worms displayed increased resistance to this drug in lethality assays, providing further indication that the components of platyhelminth Ca_v channels played a role in the PZQ mechanism of action [108].

Interestingly, subsequent RNAi experiments directed against the pore-forming, α₁ subunits of the *D. japonica* Ca_v channel complex [109], demonstrated that RNAi-silencing of one such subunit (Ca_v1A), resulted in a loss of the PZQ-mediated bipolar phenotype, prevented PZQ-mediated calcium uptake in a neuronally enriched cell fraction, and prevented PZQ-mediated inhibition of *wnt-1* and *wnt11-5* (the genes regulated by hedgehog signalling) [110]. Whereas RNAi-mediated knockdown of another Ca_v α₁ subunit (Ca_v1B), significantly increased the ability of PZQ to miscue tissue regeneration [109]. These analyses highlighted two intriguing observations. First, the authors reasoned that PZQ was able to selectively activate the Ca_v1A α₁ subunit, thereby inhibiting neuronal hedgehog signals and dysregulating tissue regeneration [109]. Second, as PZQ action was seemingly antagonised by calcium influx through Ca_v1B, this suggested that PZQ-sensitivity was modulated by a complex interplay between two opposing regulatory mechanisms [109].

Similar phenotype assays have not yet been conducted in schistosomes. However, if these findings are broadly applicable to parasitic platyhelminths, then (in terms of future drug-development studies), agonists of the Ca_v1A channel sub-type may be expected to enhance PZQ activity, or convey a PZQ-like phenotype in the worm, whereas antagonists of the Ca_v1B channel sub-type may be expected to sensitise the worm to PZQ [109, 111].

Interestingly, there is little structural similarity between PZQ and other drugs which are hypothesised to target mammalian voltage-gated calcium channels. A number of these drugs are used in humans, primarily to control hypertension [112]. These include nifedipine, verapamil, nicardipine and nemedipine. In general, these are described as calcium channel “blockers” because they inhibit or antagonise the uptake of calcium ions by the cell. This is in contrast to well-

documented effect of PZQ, namely the uncontrolled influx of the ion. Nifedipine targets mammalian L-type voltage-gated calcium channels, but also has activity against some other sub-types [113, 114]. This drug does have anti-schistosomal activity resulting in similar anatomical and physiological dysfunction to the administration of PZQ [115]. Verapamil mildly inhibits the hatching of *S. mansoni* eggs and disrupts the regulation of muscle contraction by neuropeptides [116, 117]. Nicardipine has the same effect on the regulation of muscle contraction [116]. Verapamil also disrupts the worm's tegument in a similar manner to PZQ, but has a different overall effect on the worm: while PZQ causes rapid muscle contraction resulting in the worm forming a tight coil, verapamil causes the worm to adopt a long, gentle spiral form [118]. Taken together these studies demonstrate that voltage gated calcium channels represent a viable target for antischistosomal drugs. While it is unlikely that any current anti-hypertensive drugs will be suitable for repurposing as anthelmintics, these pharmacological experiments demonstrate that calcium channel blocking in schistosomes results in morphological damage, physiological disruption and often death of the worm. Thus, further characterisation of the biochemistry and pharmacology of schistosome voltage-gated calcium channels may well be useful, even if they ultimately prove not to be the main target of PZQ. While these calcium channel blockers generally had similar effects on schistosomes to PZQ, there were key differences, suggesting that PZQ has a different molecular mechanism of action. This may be related to its ability to cause rapid calcium ion influx rather than to "block" calcium channels. It is entirely possible that PZQ binds at a different site to the channel blockers, or even to a different subunit.

Ca²⁺ signalling in schistosomes

Despite the wealth of information available on the intricacies of mammalian Ca²⁺ signalling pathways, relatively little is known of the processes that govern these pathways in parasitic flatworms. Two calmodulin proteins have been identified in *S. mansoni*, designated SmCaM1 and SmCaM2, which share considerable sequence identity to mammalian calmodulin [119]. These proteins display distinct, yet overlapping expression profiles in the early larval stages of the parasite's lifecycle. RNAi-mediated knockdown of either gene suggests that they play an important role in larval development [119]. Antagonism of *S. mansoni* calmodulin results in substantial disruption to the organism's tegument and muscles [120] and egg hatching is also blocked by calmodulin antagonists [117]. In mammals, the interaction between calmodulin and voltage-gated calcium channels has been extensively investigated and this protein is known to modulate channel activity in response to cellular calcium ion concentrations [121]. However, comparatively little is known about the interaction of calmodulin with targets such as schistosome Ca_v channels or the functional consequences thereof.

Given that *S. mansoni* encodes an atypical complement of Ca_v channel subunits (including four pore-forming α₁ subunits, two auxiliary α₂ δ subunits and two modulatory β subunits) [122], which each display distinct structural/ sequence motifs, distinguishing them from mammalian Ca_v channels (or those of some other invertebrates) [81, 123], mapping their interaction with CaM would be an invaluable first step in elucidating the mechanisms by which schistosomes regulate calcium homeostasis. Moreover, schistosomes contain an unusual family of calcium binding proteins, which combine an EF-hand domain at their N-terminus and a dynein light chain-like (DLC-like) domain at their C-terminus [124-126]. The biological function of these "EF-hand/ DLC-like domain" proteins is currently unknown, however, their structural similarity to CaM (at the N-terminus) is consistent with a role in calcium signalling, or calcium-mediated processes, whereas their structural similarity to dynein (at the C-terminus), suggests that some of these proteins could be involved in cytoskeletal

transport. Indeed, as one family member (SmTAL3) was isolated in a large, multi-protein complex at the *S. mansoni* tegument, which was also shown to contain dynein [127], it is possible that some of these proteins are also required for a structural role at the tegument. Intriguingly, at several members of the SmTAL family (SmTAL1 or Sm22.6, SmTAL4, SmTAL5 and SmTAL8) interact with PZQ, whereas there is no evidence for the drug interacting with calmodulin or antagonising any processes mediated by this protein [120, 126, 128].

Alternative hypotheses for PZQ action

Ca_v channels serve as important entry sites for extracellular calcium, mediating the processes that define electrical excitability in cells and regulating neurotransmission [129]. Thus, given that the observed effects of PZQ on adult schistosomes, *in vitro*, are consistent with the disruption of calcium homeostasis and subsequent dysregulation of the neuromuscular system [22], the body of (albeit, indirect) evidence points towards a role for these membrane-bound protein complexes in the PZQ mechanism of action; a theorem coined the “Ca²⁺ hypothesis” of PZQ action [111]. However, due to difficulties in the functional expression of schistosome Ca_v channel α₁ subunits, these hypotheses have yet to be explicitly tested [81]. As such, there remains a possibility that any disruption of calcium homeostasis is merely a secondary effect of PZQ binding to an unrelated molecular target [102]. Indeed, a study by Pica-Mattocchia and co-workers demonstrated that when adult, male *S. mansoni* worms were incubated in a medium containing radioactive carbon, PZQ treatment resulted in rapid calcium uptake from the medium (an observation which is consistent with previous studies) [21, 106]. However, when these worms were pre-treated with cytochalasin D, a compound previously shown to suppress the antischistosomal effects of PZQ [130], parasites not only survived exposure to PZQ, but also underwent an increase in PZQ-induced calcium uptake [106] thereby implying that increased calcium influx was not correlated with the antischistosomal effects of this drug *in vitro*.

Thus, several alternatives to the so-called “Ca²⁺ hypothesis” of PZQ action have been proposed. For example, given that cytochalasin D acts to depolymerise the actin cytoskeleton in mammalian systems [106, 130], one might reason that its inhibitory effect on the antischistosomal activity of PZQ could signify an overlapping binding site between the two compounds. Interestingly, actin has been implicated as a possible molecular target for PZQ in adult *S. mansoni* worms. Pull-down assays (whereby PZQ was immobilised on a cellulose acetate membrane), demonstrated that this drug bound a ~45 kDa protein species consisting predominantly of schistosome actin, when a Triton-insoluble extract of *S. mansoni* surface membrane antigens was applied [131]. However, that actin functions as a potential target for PZQ remains controversial; subsequent studies, using affinity chromatography, indicated that actin bound not only to immobilised PZQ, but also the unconjugated support used to affix PZQ to the column [132], implying that binding was non-specific, and merely an artefact of the high abundance of actin in *S. mansoni* extracts.

Nevertheless, several studies have provided viable alternatives to the “Ca²⁺ hypothesis” of PZQ action. It has been proposed that PZQ may inhibit the uptake of adenosine, an essential nucleoside which cannot be synthesised by the parasite [133]. Whereas peptide-mapping showed that PZQ bound to the N-terminal region of *S. mansoni* myosin light chain (SmMLC), triggering its phosphorylation [134]. (Note, myosin light chains have similar structures to calmodulin, although the majority are not regulated by direct binding to calcium ions [135].) Given that MLC proteins play an essential role in smooth muscle contraction [136], and that their phosphorylation is associated with increased calcium mobilisation [137], the authors proposed that PZQ-induced phosphorylation of SmMLC may account

for the observed effects of this drug on schistosomes, potentially playing an important role in the PZQ mechanism of action [134].

Early structural work demonstrated that PZQ could complex with *S. japonicum* glutathione S-transferase (SjGST), an essential detoxification enzyme in the parasite and putative vaccine target. As PZQ was shown to bind in proximity to the catalytic site of SjGST, the authors proposed that this interaction could have an inhibitory effect on enzyme function [138]. However, subsequent kinetic studies utilising a variety of model substrates found little evidence to support this hypothesis [139].

PZQ also has at least one molecular target in the host. Recent work has established that the drug acts as a partial agonist of the serotonergic 5HT_{2B} receptor [140]. This receptor helps control vascular tone resulting in vasoconstriction [141]. These actions may promote the movement of worms paralysed by PZQ to the liver where they are eliminated before they can lay eggs [140].

Interestingly, the efficacy of PZQ has also been linked to various antigenic proteins. Given that PZQ acts to disrupt the tegument, thereby exposing parasite antigens to the host immune system [86], one may reason that antigens on (or near) the tegumental surface could aid in immune system recognition, amplifying the effects of this drug [25]. Accordingly, several antigenic targets have been identified in schistosomes, which appear to have a synergistic effect on the antischistosomal activity of PZQ; including a 27 kDa esterase-like protein in *S. mansoni* [142], a 200 kDa *S. mansoni* glycoprotein [143], and a 24 kDa tegument antigen in *S. japonicum* (SjTP22.4) [144], which shares homology with the SmTAL protein family in *S. mansoni* [125].

Finally, one must also consider the possibility that the drug has multiple targets. Evidence suggests that PZQ is likely to have multiple receptors [111], which may explain why its mechanism of action has been so difficult to delineate. Ironically, whilst the existence of multiple molecular targets for this drug may be problematic for the rational design of novel anthelmintics, this phenomenon may explain why schistosomes have yet to develop clinically-relevant resistance to PZQ. A variant to the multiple targets hypothesis is that PZQ targets and disrupts an interaction between two or more proteins. In the simplest form, the drug might bind at the interface between two proteins and sterically hinder the interaction. Alternatively, binding of PZQ to one protein might result in allosteric changes to the structure which reduces the affinity for a specific protein partner. Given the well-documented effects of PZQ on calcium homeostasis, an obvious potential protein-protein interaction target would be one of the complexes involved in calcium uptake or storage. An attractive possibility would be the calmodulin-voltage-gated ion channel interaction (which, under certain circumstances in mammals, acts to down-regulate calcium ion uptake). Assuming that a similar mechanism operates in schistosomes, antagonism of this interaction would result in dysregulation of calcium uptake causing increased cellular concentrations of the ion. However, the lack of evidence that PZQ affects calmodulin-mediated processes in schistosomes [120], suggests that this (relatively simple) mechanism is unlikely to operate. One, as yet unexplored, possibility is that some SmTAL proteins can function analogously to calmodulin in the regulation of voltage-gated calcium channels. If so, the binding of PZQ to SmTAL1 may be a pharmacologically relevant event which results in the disruption of protein complexes involved in the regulation of calcium uptake.

Drug resistance

Widespread resistance to PZQ has not yet emerged. However, resistance has been generated in the laboratory [145], and there are numerous examples of other parasitic worms that have evolved resistance to commonly used anthelmintics. Certain field isolates of the liver fluke, *F. hepatica*, for

instance, possess reduced susceptibility to the drug, triclabendazole (TCBZ) [146], which may be the result of a single amino acid substitution in the ABC multidrug transporter, P-glycoprotein (Pgp) [147], and several species of gastrointestinal nematodes have developed resistance to the broad-spectrum anthelmintic, ivermectin [148]. Thus, the emergence of clinically-significant resistance to PZQ appears to be inevitable in the medium to long term.

In support of this prediction, reports of lower cure rates, and/or resistance to PZQ have been recorded in Senegal, Kenya, China, Egypt and Brazil [25, 149]. For example, after an outbreak of schistosomiasis in Northern Senegal [150], infected individuals were treated with the recommended dose of PZQ (~ 40 mg kg⁻¹ body weight); cure rates of 18-36 % were recorded, compared to a 79 % cure rate with OXA [151, 152]. A subsequent laboratory (murine) model based on the Senegal *S. mansoni* field isolate demonstrated that the parasite had a longer prepatent phase than Puerto Rican or Kenyan lab strains, which implied that the observed resistance to PZQ could be an artefact of high-intensity infection/re-infection, or drug failure, due to chemotherapy against the juvenile fluke [149]. However, when 8-week-old infections with this isolate were treated with PZQ or OXA under controlled laboratory conditions, chemotherapy resulted in a 50 % or 98 % reduction in worm burden, respectively [153], demonstrating that the Senegal strain was fully susceptible to treatment with OXA, but had an increased tolerance to PZQ. Given that both drugs are ineffective against juvenile *S. mansoni* parasites, this implied that increased resistance to PZQ could not be solely attributed to the developmental stage of the parasite at the time of treatment [153].

A similar murine model was developed in Egypt, based on *S. mansoni* isolates collected from individuals who had failed three successive rounds of chemotherapy with PZQ. After propagation in mice, isolates exhibited a 3 to 5-fold lower susceptibility to drug treatment [154, 155]. Moreover, subsequent studies revealed that some of the isolates retained this trait after multiple passages in the absence of PZQ, suggesting that sustained selective pressure was not required for the maintenance of the PZQ-resistant phenotype [156, 157]. Interestingly, as isolates that retained reduced susceptibility to PZQ exhibited a reduction in reproductive fitness (as measured by a decrease in cercariae production, *in vitro*) [156], this suggested that the biological “cost” of resistance may limit the spread of the resistant phenotype [149].

Without a definitive molecular target, it is difficult to speculate how resistance to PZQ might occur. However, one might start by examining the mechanism by which *S. mansoni* worms develop resistance to oxamniquine (OXA) [158]. OXA is highly effective against (adult) *S. mansoni* worms, but not against *S. haematobium* or *S. japonicum* [27]. This is because the drug is activated by a sulfotransferase enzyme which is absent in most schistosome species [31]. The active form of the drug acts as an alkylating agent of parasite DNA, inhibiting nucleic acid synthesis [30]. As resistance to this drug is carried on a single autosomal recessive allele, organisms that inherit this gene do not express the sulfotransferase enzyme and are therefore unable to activate the drug [27]. However, as reduced sensitivity to PZQ appears to be a dominant trait [159, 160], the mechanism of resistance to PZQ is unlikely to follow a similar pattern of inheritance. Moreover, *S. mansoni* parasites subjected to selective drug pressure in the laboratory, exhibited experimentally-induced resistance to PZQ, but no reduction in sensitivity to OXA (and *vice versa*), thereby indicating that drug resistance was induced *via* different mechanisms [145]. Alternatively, resistance might arise through enhanced removal of PZQ in a mechanism analogous to the likely resistance mechanism to TCBZ in *F. hepatica* [147].

New treatment strategies

Despite the fact that clinically-relevant resistance to PZQ has not yet emerged, over-reliance on a single drug to treat schistosomiasis (with no alternatives in the pipeline), places us in a rather precarious position. Analysis of the global changes that occur following PZQ treatment (*via* transcriptomics, proteomics or DNA sequencing methodologies), may help with this endeavour, by identifying molecules or pathways involved in the PZQ mechanism of action [149]. Moreover, a similar rationale could be applied to study PZQ-susceptible *versus* PZQ-resistant isolates, or changes in the gene expression profiles of different lifecycle stages with variable susceptibility to this drug [105]. For example, a microarray study by Hines-Kay *et al.* which detailed changes to the adult and schistosomulum *S. mansoni* transcriptome following exposure to PZQ, identified several molecular pathways which may be differentially regulated upon treatment with this drug [161]. Based on these data, the authors proposed a possible role for multidrug transporters in the PZQ mechanism of action, as well as implicating various molecular components of calcium-mediated processes (such as calmodulin and Ca_v channel subunits), apoptosis and stress-related protein [161]. Furthermore, a comparison of gene expression patterns between PZQ-refractory juvenile flukes and PZQ-sensitive adults indicated that immature worms exhibited greater transcriptomic flexibility, potentially providing a means by which schistosomula could tolerate and survive exposure to this drug [161].

We suggest that there are three main drug-discovery strategies which could be employed: (i) the development of more effective derivatives of PZQ; (ii) the identification of novel compounds which act on the same target(s) as PZQ; or (iii) the identification of completely new targets and compounds which act on them.

The design of novel anthelmintics, based on the structure of the PZQ molecule has been attempted by a number of groups, with varying success. Several analogues of PZQ were synthesised *via* modification of the aromatic ring (Fig. 3), but each of these analogues exhibited reduced potency compared to the parent drug [162]. Conversely, a 10-hydroxy (hydroxylation at position 10 of the aromatic ring) PZQ-artesunate hybrid (Fig. 3) has activity not only against *S. japonicum* adults, but also against PZQ-refractory juveniles [163]. (Note that artemisinin and related compounds alone also have anti-schistosomal activity [164, 165].) There have been other attempts to vary the PZQ structure to produce novel antischistosomal drugs and to understand structure-activity relationships (reviewed recently by da Silva *et al.* [26]). To date, none of these are in widespread clinical use. This approach may be limited, however. When widespread resistance to PZQ emerges, it may also result in resistance to many PZQ derivatives. By analogy with bacterial resistance to antibiotics, it may be assumed that there are three main ways in which resistance may arise: modification of the target which reduces the affinity for the drug, breakdown or modification of the drug to an inactive form, or enhanced transport of the drug out of the organism. All of these involve interaction between the drug and biomolecules from the parasite. All are likely to be effective with molecules which have a similar structure to PZQ. Even where this does not occur (or the effect is minimal), the evolutionary distance to more effective resistance is likely to be short. Again, by analogy with microbial antibiotic resistance, β -lactamase enzymes are effective not just against penicillin, but also a wide variety of its derivatives [166].

Knowledge of the PZQ mechanism of action would enable the design of small drug-like molecules which interfere with, or alter the function of the desired molecular target, serving to “phenocopy” the effects of PZQ [111]. The lack of definitive knowledge of the molecular mechanism of PZQ limits attempts to discover other compounds with similar mechanisms. However, the unusual subunit structure of schistosome voltage gated calcium channels combined with the critical role of calcium ions in signalling makes them an attractive target for novel drug discovery regardless. Targeting these channels would result in disruption of calcium ion homeostasis. Either a failure to transport enough

calcium into the cells or uncontrolled influx are likely to be detrimental to the parasite. Insufficient calcium would result in the failure of processes requiring the ion as a second messenger and the misfolding of proteins which require calcium to maintain their structure. Excessive influx would lead to inappropriate, uncontrolled stimulation of calcium-mediated pathways such as muscle contraction and apoptosis. Thus, developing biochemical systems to permit study of the schistosome voltage-gated calcium channels should be considered a priority in research. As membrane proteins, they are inevitably more difficult to work with and to express in recombinant systems than soluble, globular proteins. It may be necessary to work with them in model systems such as *Xenopus* oocytes or yeast [167, 168]. Once established, both systems would enable the relatively rapid screening for antagonists in multiwell formats.

While the preponderance of evidence favours a molecular mechanism in which PZQ disrupts calcium signalling, it should be noted that the other potential targets may also be interesting for future drug discovery. Antagonism of myosin light chain function would disrupt the muscles of the parasite, most likely resulting in loss of motility. Little is known about this protein in schistosomes. However, it appears to be distantly related to mammalian myosin regulatory light chains and its sequence shows greater similarity to a different myosin light chain isoform in mammals, the essential light chain (43% identity to the most similar human myosin essential light chain compared to approximately 30% for regulatory light chains) [134]. This low level of sequence similarity suggests structures which may be different enough to enable species selective antagonists to be discovered. It also suggests that there may be differences in the details of the regulation of muscle contraction between parasite and host which could also be exploited in drug discovery. Indeed, it has been shown that the ultrastructure of schistosome muscle filaments more closely resembles that seen in arthropods than mammals, suggesting significant differences in the regulatory mechanisms [169]. By a similar argument, targeting adenosine uptake may also be worth further investigation.

It may also be prudent to look for *de novo* drug targets which may, or may not be, related to the PZQ mechanism of action. For example, using the previously described comparative analyses, in combination with extensive drug screens and/or RNAi technology, it may be possible to identify novel target molecules which are unique to the parasite, essential for parasite survival and amenable to pharmacological intervention. In these respects, the components of parasite calcium signalling pathways represent putative candidates in their own right, regardless of their involvement in the PZQ mechanism of action. First, calcium signalling is an essential process in all eukaryotic cells [170], and second, anthelmintics that target the neuromuscular system of the parasite (and in particular, the ion channels that underlie the neuromuscular system) have a proven track record. Indeed, ion channels serve as the molecular targets for numerous anthelmintic drugs [129, 171]. Thus, molecules that play an important role in these pathways, yet are unique to the parasite (or sufficiently different from their counterparts/homologues in mammals), represent attractive targets for future drug development programs. It may also be worth revisiting the modes of action of less commonly used anti-schistosomal drugs. Oxamniquine appears to act through modification of parasite DNA following modification by a parasite-specific enzyme [30]. It may be possible to discover other compounds with similar mechanisms. Interrogation of the (extensive) literature on anti-cancer drug discovery may be fruitful here since a number of anti-cancer agents work by similar mechanisms [172]. There may be compounds which have failed as anti-cancer drugs, but which may be useful leads as anti-schistosomals. Prior to the use of OXA and PZQ, antimony compounds were used in the treatment of human schistosomiasis. These were discontinued largely due to their severe side-effects [173]. One target of these agents is believed to be the glycolytic enzyme phosphofructokinase [174, 175]. Clorsulon, a drug largely used to treat helminth infections in animals, also targets glycolytic enzymes, in this case phosphoglycerate mutase and phosphoglycerate kinase [176-178]. This compound has

some activity against *S. mansoni*, although it is not as effective as PZQ [179, 180]. Therefore, a renewed focus on studying and targeting metabolic enzymes and processes in schistosomes may also be a worthwhile strategy [181]. While the identification of novel targets provides the greatest scope for the discovery of new anti-schistosomal drugs, the variety and diversity of possible targets is considerable. It is likely that this approach would require the greatest investment of time (and money) to yield results.

Conclusions

PZQ has been a remarkably successful drug. It is taken by millions of people each year, has high cure rates and low reported side-effects. Despite being used for decades, there are no conclusive reports of resistance emerging in a clinical setting. However, PZQ is the only drug in common use to treat schistosomiasis, a disease which afflicts hundreds of millions of people globally. If (or when) resistance does emerge, there is no alternative with comparable efficacy to PZQ. Our ability to respond to that situation will be hampered by the significant unanswered questions about this drug:

1. What is PZQ's molecular target (or targets)?
2. Where, and how, does PZQ interact with its target(s)?
3. Why is R-praziquantel the active form? Why does S-praziquantel result in side-effects and a bitter taste?
4. Which of the observed consequences of PZQ administration represent direct results of the drug-target interaction and which result from the general dysregulation of the parasite's physiology?
5. How do the various subunits of schistosome voltage-gated calcium channels collaborate to function?
6. How does the regulation of these voltage-gated calcium channels differ from the situation in mammals?
7. Do the observations that PZQ binds to, or affects, myosin subunits, adenosine uptake and SmTAL1 have any significance for the mechanism of action?
8. Why has resistance been so slow to emerge to PZQ? Does this reflect something special or unusual about the molecular mechanism?
9. If resistance did occur, which molecular mechanisms are likely?
10. Why are some trematode species (e.g. *F. hepatica*) refractory to treatment by PZQ?

Addressing these questions will require a concerted effort to understand the fundamental biochemistry of the organism, particularly in regards to calcium signalling. A deeper understanding of the basic biology of schistosomes should facilitate both mechanistic studies on PZQ and the rational identification of further drug targets in the parasite. The highly successful use of PZQ suggests that alternative drugs with the same or related targets may also be effective. Thus, the need to understand PZQ's mechanism of action is not just a basic science need, but also has the potential to catalyse the development of the next generation of anti-schistosomal drugs.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Figure legends

Figure 1: The life cycle of schistosomes. The organism infects two animals, an intermediate snail host and the definitive human host. Other mammals can also act as definitive hosts. The life cycle is broadly similar for the three main species of schistosomes. However, the mode of exit of the eggs differs. *Schistosoma mansoni* and *Schistosoma japonicum* eggs are passed largely in the faeces whereas *Schistosoma haematobium* eggs are passed largely in the urine.

Figure 2: Current drugs for treating schistosomiasis. The structures of *R*-praziquantel (the pharmacologically active isomer) and oxaminquine are shown.

Figure 3: Some examples of new compounds with antischistosomal activity. (a) The praziquantel framework has been modified at the positions indicated by R¹ and R². R¹ variations included amino and nitro groups and R² variations included cyclohexanyl, 3-aminobenzyl, 4-aminobenzyl and 4-hydroxycyclohexanyl groups. None of the compounds had better efficacy than PZQ in worm killing or paralysis assays with *S. mansoni* [162]. (b) 10-hydroxypraziquantel has increased efficacy against both juvenile and adult *S. japonicum*. Furthermore, using the 10-hydroxyl group to conjugate the PZQ derivative to artemisinins also increased efficacy, presumably due to the combined effects of the two drugs [163].

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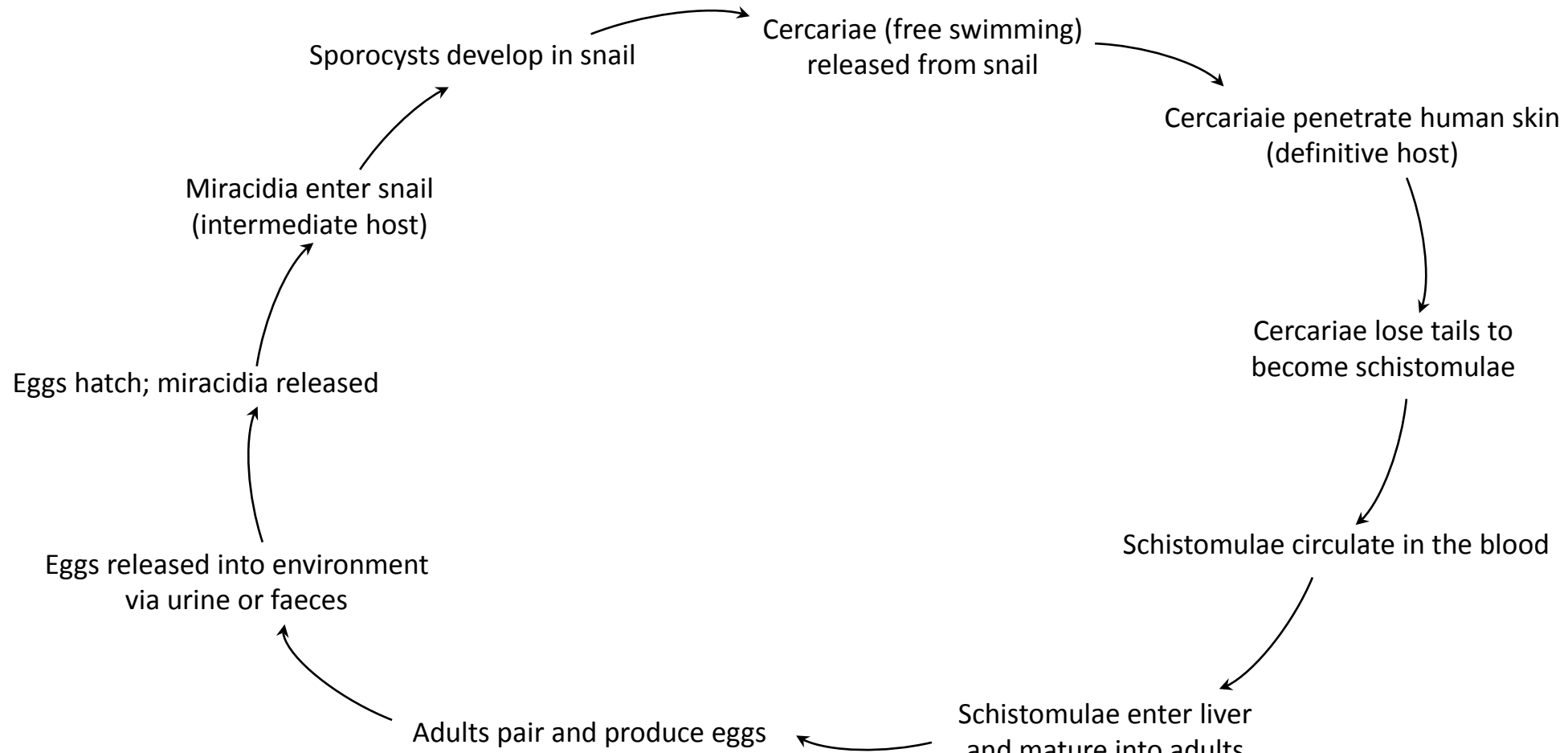
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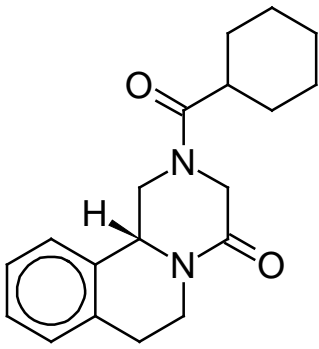
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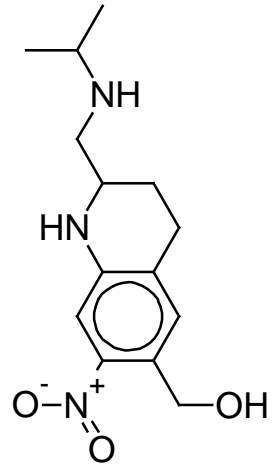
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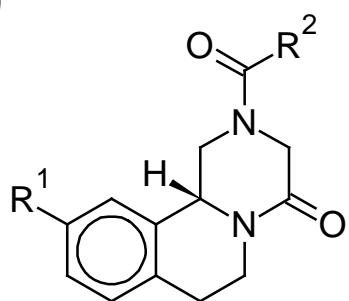


(R)-Praziquantel

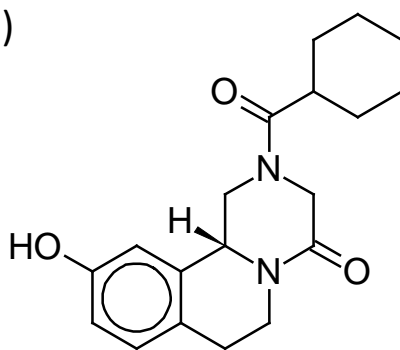


Oxamniquine

(a)



(b)



Gene	Channel sub-type	Type	Tissue location(s)
<i>CACNA1S</i>	Ca _v 1.1	L	Skeletal muscle
<i>CACNA1C</i>	Ca _v 1.2	L	Cardiac and smooth muscle
<i>CACNA1D</i>	Ca _v 1.3	L	CNS and endocrine cells
<i>CACNA1F</i>	Ca _v 1.4	L	Skeletal muscle
<i>CACNA1A</i>	Ca _v 2.1	P/Q	CNS, heart, pituitary, dendrites and presynaptic terminals
<i>CACNA1B</i>	Ca _v 2.2	N	CNS, cell bodies, dendrites and presynaptic terminals
<i>CACNA1E</i>	Ca _v 2.3	R	presynaptic terminals
<i>CACNA1G</i>	Ca _v 3.1	T	CNS, heart, placenta, lung, kidney
<i>CACNA1H</i>	Ca _v 3.2	T	CNS, heart, placenta, lung, kidney
<i>CACNA1I</i>	Ca _v 3.3	T	CNS, heart, placenta, lung, kidney

Table 1: Mammalian Ca_v channel sub-types. Channel types: L-type, long-lasting current; P/Q-type, Purkinje fibre located/cerebellar granule neuron-located; N-type, neural; R-type, residual current; T-type, transient current. L- P/Q- and N-types are high voltage activated (HVA) channels, R-type channels are intermediate voltage activated (IVA) and T-type are low voltage activated (LVA).