

The mechanism of action of praziquantel: six hypotheses.

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Abstract

Despite being one of the most commonly used drugs, the molecular mechanism of action of the anthelmintic praziquantel remains unknown. There are some unusual features of this drug.

Critically, widespread resistance to praziquantel has not developed despite decades of use. Here we set out some challenges in praziquantel research and propose six provocative hypotheses to address these. We suggest that praziquantel may have multiple pharmacologically relevant targets and the effects on these may synergise to produce an overall, detrimental effect on the parasite.

Praziquantel also acts on a number of host proteins and we propose that these actions are important in the drug's overall mechanism. Although the drug is largely used in the treatment of human and domestic animal worm infections, there is a considerable "grey literature" along with some academic studies which may have been overlooked. It appears that praziquantel may be effective against *Hydra spp.* It may also be effective against some unicellular parasites such as *Giardia spp.* Further scientific work on these understudied areas may be useful in understanding the molecular mechanism in *Trematoda*. The lack of widespread resistance suggests that praziquantel may act, at least in part, on a protein-protein interaction. Altered drug metabolism or enhanced drug efflux are the most likely ways resistance may arise. There is a critical need to understand the biochemical pharmacology of this drug in order to inform the discovery of the next generation of anthelmintic drugs.

Keywords: Neglected tropical disease, drug mechanism, schistosomiasis, *Schistosoma spp.*, calcium homeostasis, praziquantel

Introduction

Praziquantel (PZQ) is one of the world's most successful drugs and is taken by millions of people every year (Figure 1). It is listed as one of the World Health Organisation's (WHO) essential medicines [1]. The drug is used to treat infections with parasitic worms from the genus *Schistosoma* [2]. However, it can also be used to treat a number of other helminth infections. It is effective against trematodes such as *Clonorchis sinensis*, *Opisthorchis viverrini* and *Paragonimus westermani* [3-6]. In addition, some tapeworms, notably *Taenia solium*, can be eliminated by PZQ [7]. The drug is generally considered ineffective against liver flukes from the genus *Fasciola* [8]. The drug is cheap to manufacture and the cost of a course treatment in the developing world is generally less than US\$1 per patient [9]. In the absence of a vaccine, PZQ has been used in mass drug administration programmes in order to break the cycle of infection and provide protection to communities [10]. In most patients, the side-effects are negligible or mild; however, some do suffer from abdominal pain, gastrointestinal problems, nausea, joint pains, muscle pains and headaches [11, 12]. Some of these may result from the death and lysis of worms within the host, rather than from a pharmacological effect of the drug on the patient.

This remarkable success, however, hides a problem. PZQ is about the only drug available for the treatment of schistosomiasis. Another drug, oxamniquine (OXA), is less widely used since it is not effective against two of the main causes of schistosomiasis, *S. japonicum* and *S. haematobium* although it is effective against *S. mansoni* [13]. Prior to the discovery of these two drugs, a range of trivalent antimony compounds were used to treat the disease. These work by targeting the glycolytic enzyme phosphofructokinase [14-16]. However, they have a very low therapeutic index, and result in severe, debilitating and long-lasting side effects [17]. Resistance has been documented to almost all known anti-infective drugs once they have been in clinical use for a number of years. The emergence of resistance is an evolutionary near-certainty. Anti-infectives place severe selective pressures on the organisms they target. Therefore, even genotypes which result in a substantial loss

of fitness can be tolerated if they enable the organism to survive the drug. Poor prescribing practices and poor patient compliance can contribute the increased speed of emergence of resistance. To date, there are no definitive reports of resistance to PZQ in a clinical setting. However, *when* it does emerge, there is a significant risk that there will be no alternative drugs which can treat the infection as effectively as PZQ.

There are number of detailed, comprehensive reviews on the unknown mechanism of action of PZQ and its use as a therapeutic (e.g. [18-26]). Our aim here is not to reproduce that work. Here, we raise a number of questions and oddities about PZQ and suggest some hypotheses which may explain the observations (Table 1). The hypotheses are intended to be provocative and we hope that they will stimulate further thinking, debate and experimentation. They are not intended to present a comprehensive view of how PZQ works. However, they are responses to some of the unusual features of the biochemical pharmacology of this fascinating and globally important drug.

The mechanism of action of PZQ is unknown

It has been known since the 1970s that PZQ disrupts calcium homeostasis in schistosomes [27]. While there is some strong evidence that the drug interferes with the action of voltage-gated calcium channels, no binding site on one of these proteins has yet been identified. Furthermore, extensive influx of calcium ions and subsequent effects like irreversible contraction of the muscles might be an indirect effect of PZQ's actions on other targets. To date, the only schistosome protein which has a well-characterised PZQ binding site is glutathione S-transferase (GST; Figure 2). In this protein, the drug binds in a groove between the two subunits of this homodimeric enzyme. Selectivity for the parasite over mammalian GSTs is achieved through interaction with a tyrosine residue (Tyr104 in *S. japonicum* GST) which is not present in the host enzyme [28]. The biochemical effects of PZQ on GST are not known. To date, no detailed study has been carried out to determine whether, or not, the drug acts as an inhibitor of the enzyme. However, one report suggests that PZQ

treatment does not reduce the activity of GST in *S. mansoni* [29]. Thus, a link between this binding event and the pharmacological consequences of the drug has not been made.

The cytoskeletal protein actin has also been suggested as a target for PZQ, but this hypothesis has been largely discounted (Figure 3a) [30, 31]. The drug has also been shown to reduce adenosine uptake. This may be particularly significant since adenosine can modulate calcium channels in mammals, and may also do so in schistosomes [32]. A component of the molecular motor protein myosin, the regulatory light chain has also been shown to bind to PZQ (Figure 3b). However, as with GST, any link between this biochemical activity and the fatal effects on the worm have yet to be established.

Recently several members of the tegumental allergen (TAL) family of proteins have been shown to interact with PZQ (Figure 3c). These proteins, which appear to be unique to helminths, combine an N-terminal EF-hand binding domain with a C-terminal dynein light chain-like domain [33, 34]. The *S. mansoni* proteins SmTAL1, SmTAL4, SmTAL5 and SmTAL8 all interact with PZQ, but the binding site has yet to be mapped [35, 36]. The physiological functions of these proteins are currently unknown. Of the four which bind PZQ, all except SmTAL5 also interact with calcium ions [35, 36]. It is therefore reasonable to speculate that they mostly play a role in calcium signalling processes and the presence of the dynein light chain-like domain suggests some possible involvement with cytoskeletal processes.

The most straightforward mechanism of action for PZQ would be the direct antagonism of one or more of the worm's calcium channels or pumps. This antagonism would result in the uncontrolled influx of calcium ions into the cytoplasm of the organism's cells. This influx would result in a number of deleterious consequences: inappropriate activation of proteases, uncontrolled muscle contraction and the promotion of apoptosis. This mechanism would require the drug to interact directly with the target channels. Since the drug is not known to dysregulate host calcium homeostasis, it would be reasonable to assume that PZQ binds to a calcium transport protein which

does not occur in mammals or a protein which contains at least one region of significant difference to the mammalian enzyme. There is nothing in the current scientific literature to disprove this hypothesis conclusively. However, there is also not much significant biochemical data to support it. No calcium transport component has been shown to interact with PZQ and there is no clear evidence of inhibition of efflux pumps or unregulated opening of influx pumps. In light of this, we propose two hypotheses:

Hypothesis 1: PZQ does not work through direct action on a calcium transport protein.

Hypothesis 2: PZQ has multiple, pharmacologically relevant targets in schistosomes. The biochemical effects on these targets synergise to produce the overall pharmacological outcome.

Hypothesis 1 would be hard to address directly. One of its corollaries is that direct, specific interaction with a calcium transport protein is unlikely or, if it did occur, it would not result in a pharmacologically relevant outcome. However, proving absence of interaction is experimentally challenging. This hypothesis would be supported by strong evidence for an alternative mechanism involving another molecular target. There is already some evidence in support of Hypothesis 2. A diverse range of possible targets has been proposed and a few proteins have been identified as interaction partners. However, as noted above few of these have been definitively linked to a pharmacologically relevant outcome.

PZQ exists as two enantiomers, the *R*(-) isomer which is the pharmacologically active form and the *S*(+) form which is inactive. The *S*(+) form is also responsible for the bitter taste of the drug and some side effects [37-40]. Therefore any pharmacologically relevant target for PZQ should distinguish between the two isomers and respond only to the *R*(-)-enantiomer. The simplest way that this could be achieved would be by the target only binding to this enantiomer at physiologically relevant concentrations of the drug. It is, however, possible that both isomers might bind, but only the *R*(-)-enantiomer elicits the conformational or other changes in the protein to bring about the pharmacological effect.

PZQ has pharmacological targets in the host

It is now clear that PZQ also has effects on the mammalian host. Recent work has established that PZQ is a partial agonist of the human serotonergic 5-hydroxytryptamine_{2B} (5HT_{2B}) receptor [41]. This agonism, which occurs at therapeutically relevant concentrations is sufficient to elicit physiological responses. Activation of the 5HT_{2B} receptor by PZQ results in calcium ion release from intracellular stores. This, in turn, causes constriction of the mesenteric vasculature of the host. The constriction of these muscles may assist in the displacement of the worms from the blood vessels to the liver [41]. PZQ can also induce vasodilation through activation of several members of the mammalian transient receptor potential (TRP) channels, including TRPA1, TRPC3, TRPC7 and TRPM8 [42, 43]. Interestingly, TRPA1 is also a pharmacologically relevant target of the mild analgesic paracetamol (acetaminophen) and TRPM8 is a likely target in menthol's action as an analgesic [44].

Hypothesis 3: The effects of PZQ on host proteins and systems are important in its activity as an anthelmintic.

A 5HT_{2B} receptor knock-out mouse is viable, albeit with significant abnormalities of cardiac function [45]. In theory this would provide a model in which the ability of PZQ to clear schistosome infections could be compared. However, there would be difficulties in comparing the results since the knock-out mice suffer from a number of serious abnormalities and may, thus, be more susceptible to infections. A TRPM8 knockout mouse is also available. The phenotype appears to be less severe than the 5HT_{2B} receptor knock-out [46]. However, the existence of multiple TRP channel targets of PZQ may complicate any analysis. Antagonists of the 5HT_{2B} receptor might be expected to reduce the efficacy of PZQ in a wild-type animal model since they would compete for access to the receptor without inducing the physiological response.

There is a considerable “grey literature” on PZQ

In most countries, PZQ is only available for human use on prescription or through clinics and hospitals. However, there is widespread and less strictly regulated use of the drug in veterinary medicine. The broad spectrum of PZQ’s anthelmintic activity beyond *Schistosoma spp.* means that the drug is useful in treating helminth infections in companion animals and livestock. PZQ is included in a number of combination therapies sold to farmers to treat helminth infections or as prophylactics. In addition, PZQ is commonly used to treat tanks used for fish and other aquatic animals, such as shrimps. The drug appears to be effective at killing planarian flatworms. This is reasonably well-documented in the scientific literature [18]. However, some other uses of PZQ have not been well-studied under controlled, scientific conditions. A considerable “grey literature” has been developed in magazines, internet discussion forums and blogs etc which cater for aquarium hobbyists and there is also some anecdotal evidence of the efficacy of the drug on internet message boards run by amateur and professional shrimp farmers. Considerable care needs to be taken when assessing these data. Often the formulations recommended in these forums include other anti-parasitics commonly benzimidazoles such as fenbendazole. In general, these users are obtaining drugs intended for use in companion animals and then diluting tablets or liquid formulations into aquariums. So it is not always possible to be certain that the reported effects are solely due to PZQ. Rigorous scientific method is rarely applied to these studies. Consequently, the users are not necessarily reporting other changes made to the tanks at the time of administration. However, there is some consistency in the findings reported in these sources suggesting that it is likely that the phenomena reported are worthy of further, more rigorous investigation.

Nevertheless, if approached cautiously, this grey literature provides some information about the species in which PZQ is effective (Table 2). There is a strong consensus that PZQ kills most flatworms. It also appears to be effective against hydra. Given its widespread use in fish and shrimp tanks without reported side-effects, it might be assumed that there are no immediately obvious

effects on these organisms. However, some forums report that PZQ kills shrimp (Table 2). The drug does not appear to cause significant harm to mammals, birds, fish, amphibians and reptiles [47-50]. In addition there is no evidence of any antibacterial or antifungal activities; however, there are few detailed studies on this and the majority of experiments in which microbes have been exposed to PZQ had the aim of measuring any mutagenic effects of the drug (e.g. [51]). Plants do not appear to be affected, but detailed studies have not been carried out on most plant phyla. Interestingly, the single-celled parasite *Giardia lamblia* can be effectively treated by PZQ [52, 53]. However, these findings do not appear to have followed up.

Hypothesis 4: PZQ's molecular mechanism of action in hydra is broadly similar to that in parasitic helminths.

No detailed studies have been carried out on the mechanisms of PZQ toxicity in hydra or other non-helminth invertebrates. Assuming that the drug causes death through similar mechanisms, it seems probable that there would be common molecular targets in these other groups of animals.

Therefore, any molecular target proposed in schistosomes and other helminths should also occur with high sequence and structural similarity in hydra.

Resistance to PZQ is rare

PZQ has been used to treat helminth infections since the 1970s. Currently millions of people are treated every year. In addition, there is widespread veterinary use and also "unofficial" and experimental uses such as those described above in aquariums. Yet, despite this extensive use, there are relatively few reports of resistance. A number of reports describe treatment "failure" or reduced efficacy of the drug but it is often unclear if this results from the emergence of genuine resistance [54-63]. In the cases of travellers from areas of low incidence of schistosomiasis becoming infected the lower efficacy of the drug may be due to a lack of partial immunity which is

acquired by many residents of countries with higher incidences [54, 64]. In areas with high levels of the disease, the high worm burden and the chances of rapid reinfection may account for some treatment failures [65]. Mass drug administration programmes may be associated with subsequent reduced efficacy, although more work is required to confirm this [66].

Even if some reports do represent evidence of genuine resistance, the level of resistance appears to be surprisingly low. This is particularly the case when compared with antimicrobials where resistance typically develops within 10 years of widespread use. Exposure of the pathogen to sub-lethal doses of the antimicrobial is generally considered a key factor in accelerating the emergence of resistance to these drugs. This may occur if patients fail to comply with the treatment regime, for example by missing doses. It can also occur if low concentrations of the drug are released into the environment. This is often unavoidable since, in some cases, the drug is excreted unchanged by metabolism or is metabolised into derivatives which retain some antimicrobial activity. It can be exacerbated by the indiscriminate or less regulated uses in agriculture and in the *ad hoc* treatment of companion animals. Similar causes have been attributed to the development of resistance to other antiparasitic drugs, such as triclabendazole [67]. All these factors which increase the risk of resistance occur with PZQ. However, PZQ is efficiently metabolised by mammals and probably less than 1% is excreted unchanged [68]. It should also be noted that the majority of the less regulated uses are likely to occur in the developed world and are often targeted against organisms which are not pathogens of humans, such as planarian worms and hydra. In contrast, the vast majority of human helminth infections occur in the developing world. These factors might partly mitigate the selective pressures for the development of resistance in pathogenic helminths.

Nevertheless, it is hard to escape the conclusion that there is something special about PZQ compared to many other anti-infective agents. This may reflect the molecular mechanism of action of the drug. Resistance would be expected to develop relatively rapidly if there was a straightforward, single target mechanism. If this was the case, it would be expected that drug

treatment would select for alleles of the gene which encode variants of the target which retain biological activity, but have reduced affinity for PZQ. In general terms, this selection would act mainly on pre-existing polymorphisms in the gene encoding the target. However, it is possible that novel, random mutations in the target which have the same effect may also arise and be selected for. Selection for resistance-conferring alleles would be expected to be greatest in areas where the amount of PZQ use is sustained at high levels. The absence of such resistance suggests that the drug's mechanism of action is more complex.

If there are multiple, pharmacologically relevant targets (see above) then selection of an allele encoding a variant of one with lower PZQ affinity may not be sufficient to result in a significantly resistant phenotype. While PZQ action at that target would be mitigated, the remaining targets would still be affected. Furthermore, selection of the lower affinity variant is likely to reduce the biological activity of the protein concerned and, consequently, the evolutionary fitness of the individual worm. If the host targets are important in the mechanism of action of PZQ, then it is difficult to see how selection acting on the corresponding genes which result in loss of PZQ activity would confer an evolutionary advantage to the host. Indeed, it would be likely to do the opposite in areas with high levels of schistosomiasis.

Hypothesis 5: At least one of the targets of PZQ may be a protein-protein interaction.

Relatively few drugs are known to target protein-protein interactions. However, they do offer some advantages as targets, perhaps especially in the treatment of infectious disease. Disrupting a vital protein-protein interaction will prevent any downstream events, resulting in a pharmacological outcome. However, resistance may require selection for alleles which affect both proteins, such that the drug no longer blocks interaction but retaining sufficient affinity between the two proteins. This is much less likely than selection affecting a single target. Therefore, resistance is much less likely to occur. It is possible that PZQ disrupts the interaction between a calcium transport protein and one of its regulatory subunits. It is well-established that mammalian voltage-gated calcium channels are

regulated by calmodulin [69, 70]. *S. mansoni* has two calmodulin isotypes [71]. Experiments with recombinant *S. mansoni* calmodulins and peptides from the organism's voltage-gated ion channels identified one sequence, an IQ-motif, which interacts with both calmodulin isotypes. However PZQ does not inhibit this interaction [72]. Another possibility is that one or more of the SmTAL proteins can also act as a regulatory subunit of the voltage-gated calcium channels. Given that some of these proteins interact with PZQ, it is possible that the drug inhibits this interaction. No experimental tests of this have yet been reported.

Some trematode species may not be susceptible

PZQ is generally considered to be ineffective against liver flukes from the genus *Fasciola* [8, 73-79]. The drug has no effect on the ultrastructure of the tegument, in contrast to drugs which do affect this parasite [80-84]. However, there are some reports to the contrary suggesting that PZQ can be effective in the treatment of fascioliasis [85, 86]. It is possible that this discrepancy may reflect the treatment of people in endemic versus non-endemic countries. The effectiveness of PZQ in the treatment of schistosomiasis appears to vary between these two groups - an effect which is thought to result from partial immunity of many people who have repeatedly exposed and which may also occur in *Fasciola spp.* [64]. If PZQ is not as effective against *Fasciola spp.*, it is not clear why this is the case. It has been postulated that the tegument of these organisms prevents the effective uptake of PZQ. However, radiolabelling studies provided evidence for some uptake of PZQ [87]. It is therefore more likely that subtle difference in one or more of the drug's targets results in a lack of activity. Further work is required to resolve whether, or not, PZQ is effective against *Fasciola spp.* If it is confirmed that the drug has no, or substantially lower, efficacy against these species, the investigation of the sequences and structures of suspected targets would be desirable. Differences between these structures and sequences in susceptible and non-susceptible organisms would provide clues about the mechanism of action and the location of PZQ binding sites.

Resistance to PZQ could arise

Despite the lack of clear evidence for widespread resistance to PZQ it seems likely that it will, eventually, arise. If we assume that the molecular mechanism is sufficiently complex to make selection acting on a single target gene(s) an unlikely cause of resistance, there remain other possibilities. In microbes, two common causes of resistance are metabolism of the drug to a harmless compound and enhanced removal of the drug from the pathogen's cells. Either mechanism would be theoretically possible in parasitic trematodes.

Hypothesis 6: Resistance will arise, but not through selection acting on the gene(s) encoding the target(s)

Resistant isolates of *Schistosoma spp.* have been generated in the laboratory by several laboratories [88-94]. This is often achieved through the repeated, sub-lethal dosing with PZQ. The resistant phenotype can be inherited, suggesting that it results from selection of resistant alleles in one or more genes [95, 96]. In some of these isolates, increased activity of p-glycoprotein, a relatively non-specific active transport protein family which is responsible for the efflux of many drug-like molecules, has been observed [89, 97, 98]. *S. mansoni* is not known to metabolise PZQ [87]. However, there is evidence for the existence of cytochrome P450 systems in *Schistosoma spp* [99]. These systems are known to metabolise PZQ in the host [100, 101]. Therefore, it seems possible that selection for genes encoding variants of parasite cytochrome P450 systems which can detoxify PZQ would be a potential route to a resistant phenotype.

Conclusions

Despite decades of use, PZQ remains something of a biochemical enigma. While it seems likely that its mechanism involves the disruption of calcium ion homeostasis, the molecular target remains

unknown. The lack of resistance suggests something special, or unusual, about this mechanism. Further biochemical investigations are urgently required to address some of these issues. These studies might include work on animals not previously considered. Hydra, for example, have the advantage of being relatively easy to grow. Since these animals are not endoparasites, it would not be necessary to maintain a complex life cycle involving one or more hosts species. Understanding the mechanism of action of this drug will be critical once resistance arises. It will identify genuinely druggable molecules and systems in the parasites against which novel compounds could be designed.

There is also a pressing need to understand the biochemistry, cell biology and physiology of Trematoda. While much of this will be similar to the much better understood systems in mammals, even subtle differences may provide opportunities which can be exploited in drug discovery. This will require the ability to think creatively and to advance realistic hypotheses. We hope that the hypotheses proposed here will stimulate further thinking.

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Figure legends

Figure 1: The chemical structure of PZQ. The pharmacologically active *R*-(-) enantiomer is shown.

Figure 2: The interaction between PZQ and *S. japonicum* glutathione *S*-transferase. The images were made using PDB 1GTB and PyMol [28]. The right hand image is rotated 90° around the y-axis compared to the left hand one. Only one subunit of the dimeric GST is shown (ribbon format) and PZQ (stick format) binds at the interface between the two subunits.

Figure 3: Structures of some other proteins which may interact with PZQ. (a) *S. mansoni* actin (GenBank: AAC46966). (b) *S. mansoni* myosin regulatory light chain (Genbank: AAR99584). (c) *S. mansoni* tegumental allergen protein 1 (SwissProt: P14202; SmTAL1) with calcium ions (spheres) bound at its two EF-hand motifs. In all cases, the protein was modelled using Phyre2 [102] and displayed using PyMol. The SmTAL1 model has been reported previously [36].

Tables

Table 1: A summary of the hypotheses advanced in this review

Number	Hypothesis	Significance	Potential tests
1	PZQ does not work through direct action on a calcium transport protein.	The search for binding sites in voltage-gated calcium channels etc, may be futile; however, efforts to identify new molecules which target these proteins may still be a viable route to novel anti-schistosomal drugs	It is hard to “prove a negative” through experiment. However, if an alternative target was identified, this would support this hypothesis.
2	PZQ has multiple, pharmacologically relevant targets in schistosomes. The biochemical effects on these targets synergise to produce the overall pharmacological outcome.	Some, or all, of the currently proposed targets may be pharmacologically relevant. It is possible that the failure to demonstrate definitive effects at these targets may result from the need for the drug to hit multiple targets for a synergistic effect.	Identification of PZQ-binding proteins (e.g. through proteomic methods, affinity chromatography etc). Further work to verify binding to the currently proposed targets and to map binding sites biochemically and/or by molecular modelling. Investigation of the biochemical consequences of binding to the target (e.g. does PZQ inhibit GST?)
3	The effects of PZQ on host proteins and systems are important in its activity as an anthelmintic.	It may be necessary to look at the host as well as the parasite when trying to put together an overall picture of how PZQ works.	Clear demonstration that some of the effects on the host vasculature are important in clearing worms. Studies on knock-out/knock-down animal models. Hosts lacking these targets should be more susceptible to infection by schistosomes.
4	PZQ’s molecular mechanism of action in hydra is broadly similar to that in parasitic helminths.	Hydra, and other animals affected by PZQ, may make good experimental models for understanding the mechanism(s) of action.	Definitive scientific, experimental investigation of PZQ’ effect on hydra. This would include tests on overall morphology and physiology following administration of the drug. If PZQ does kill hydra then identification of binding partners would be valuable as would comparative genomics/proteomics to determine

5	At least one of the targets of PZQ may be a protein-protein interaction.	This may help explain the lack of evidence for widespread, clinically relevant resistance. The interaction may be a viable target for new drugs.	<p>common proteins between Hydra and schistosomes.</p> <p>Mapping of protein-protein interactions in schistosomes, perhaps focussing on those involved in calcium ion homeostasis. Clear demonstration that PZQ antagonises at least one of these. This may require model systems, e.g. yeast models in which the parasite proteins are expressed with some form of “read-out” for lack of interaction.</p> <p>Monitoring of cytochrome P450 sequences and biochemical testing to determine if any have activity against PZQ. Measurement of p-glycoprotein activity in resistant isolates generated in the laboratory.</p>
6	Resistance will arise, but not through selection acting on the gene(s) encoding the target(s)	It may be possible to detect resistance even without knowing the targets since we might expect alterations in cytochrome P450 diversity or increased p-glycoprotein activity to accompany resistance.	

Table 2: Some effects of PZQ on non-helminth groups.

Species or group	Reported Effect or Consequence	Source
Bacteria	No mutagenicity	[51]
Yeasts	No mutagenicity	[51]
Hydra	Clears hydra from fish tanks etc	www.plantedtank.net/forums
Insects	No mutagenicity	[51]
Shrimps	May kill shrimp "Probably" safe	www.shrimponow.com/forum www.fishforums.net
Fish	No harm linked to the drug reported Safe to use as anthelmintic	Various aquarium forums [50]
Plants	Safe	www.fishforums.net
Snails	Minimal effects. May reduce fecundity, but this is probably related to worm burden not the drug Safe for apple snails; possibly toxic to nerite snails	[103] applesnail.net/forum3
Plasmodium	May potentiate the effects of chloroquine and partially reverse chloroquine resistance	[104]
Reptiles	Safe to use as anthelmintic	[49, 105]
Amphibians	Safe to use as anthelmintic	[48]
Birds	Safe to use as anthelmintic	[47, 106] [107]

	May be toxic to finches if injected	
<i>Giardia spp.</i>	Effective treatment. Clears parasite from host in >80% of cases	[52, 53]
<i>Blastocystis hominis</i>	No effect on the parasites in host	[108]
Toxoplasma	No effect	[109]
Cryptosporidium	Cures ~50% of patients	[110]

Some of these data were obtained from websites and internet discussion forums aimed at hobbyists and small-scale producers. To date, many of these findings have not been confirmed in the peer-reviewed scientific literature. Therefore, considerable caution should be used when interpreting the data (see text). The effects on shrimp appear to be particularly controversial with some forums describing PZQ as “safe” and others reporting the results of small scale experiments which suggest that it is harmful to shrimps.

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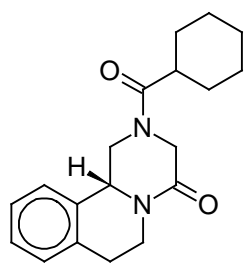


Figure 1

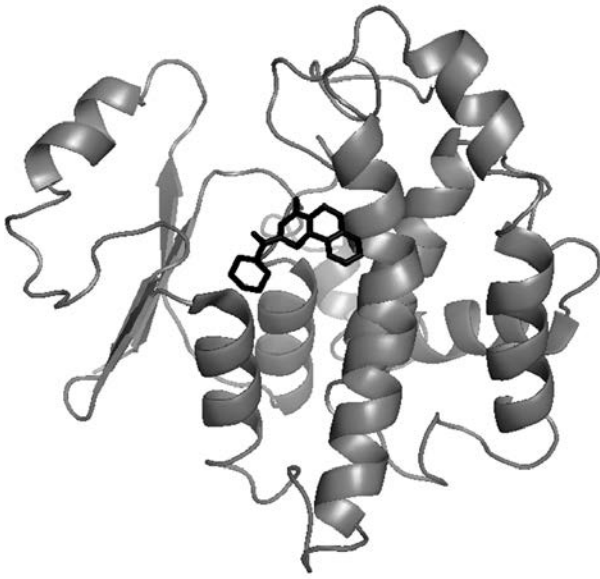


Figure 2

(a)



(b)



(c)

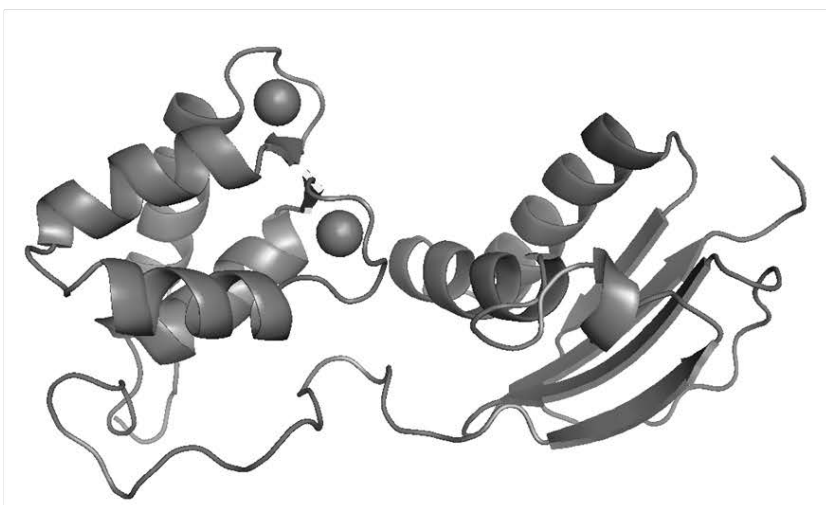


Figure 3