Activity of Praziquantel Enantiomers and Main Metabolites Against Schistosoma mansoni

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Abstract: A racemic mixture of R and S enantiomers of praziquantel (PZQ) is currently the treatment of choice for schistosomiasis. Though the S enantiomer and the metabolites are presumed to contribute only a little to the activity of the drug, in-depth side-by-side studies are lacking. The aim of this study was to investigate the in vitro activities of PZQ and its main metabolites, namely, R- and S-cis- and R- and S-trans-4'-hydroxypraziquantel, against adult worms and newly transformed schistosomula (NTS). Additionally, we explored the in vivo activity and hepatic shift (i.e., the migration of the worms to the liver) produced by each PZQ enantiomer in mice. Fifty percent inhibitory concentrations of R-PZQ, S-PZQ, and R-trans- and R-cis-4'-hydroxypraziquantel of 0.02, 5.85, 4.08, and 2.42 g/ml, respectively, for adult S. mansoni were determined in vitro. S-trans- and S-cis-4'-hydroxypraziquantel were not active at 100 g/ml. These results are consistent with microcalorimetry data and studies with NTS. In vivo, single 400-mg/kg oral doses of R-PZQ and S-PZQ achieved worm burden reductions of 100 and 19%, respectively. Moreover, worms treated in vivo with S-PZQ displayed an only transient hepatic shift and returned to the mesenteric veins within 24 h. Our data confirm that R-PZQ is the main effector molecule, while S-PZQ and the metabolites do not play a significant role in the antischistosomal properties of PZQ.

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Activity of praziquantel enantiomers and main metabolites against *Schistosoma mansoni*

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Abstract

A racemic mixture of *R* and *S* enantiomers of praziquantel (PZQ) is currently the treatment of choice against schistosomiasis. Though the *S* enantiomer and the metabolites are presumed to contribute only little to the drug activity, in depth side-by-side studies are lacking. The aim of this study was to investigate the *in vitro* activities of PZQ and its main metabolites, namely *cis*- and *trans*-4'-*R*/*S* hydroxypraziquantel, on adult worms and newly transformed schistosomula (NTS). Additionally, we explored the *in vivo* activity and hepatic shift (i.e. the migration of the worms to the liver) produced by each PZQ enantiomer in mice. IC_{50}s of 0.02, 5.85, 4.08 and 2.42 µg/mL on adult *S. mansoni* were determined *in vitro* for *R*-PZQ, *S*-PZQ, *R-trans* and *R-cis* hydroxypraziquantel, respectively. *S-trans* and *S-cis* were not active at 100 µg/mL. These results are consistent with microcalorimetry data and studies on NTS. *In vivo*, single 400 mg/kg oral doses of *R*-PZQ and *S*-PZQ, achieved worm burden reductions of 100 and 19%, respectively. Moreover, worms treated *in vivo* with *S*-PZQ displayed only a transient hepatic shift and returned to the mesenteric veins within 24 h. Our data confirm that *R*-PZQ is the main effector molecule, while *S*-PZQ and the metabolites do not play a significant role in the antischistosomal properties of PZQ.

Keywords: schistosomiasis, chemotherapy, praziquantel, *cis*-4'-hydroxypraziquantel, *trans*-4'-hydroxypraziquantel

Introduction

Schistosomiasis or bilharzia is caused by blood flukes from the genus *Schistosoma*, and is part of the group of Neglected Tropical Diseases (NTDs) affecting more than 207 million people in tropical areas (1-3).

The exclusive treatment to date against schistosomiasis is praziquantel (PZQ), discovered in the 1970s by Merck and Bayer. PZQ is administered as a racemic mixture of *R* and *S* enantiomers in tablets of 600 mg. The recommended dosage to treat schistosomiasis is 20 mg/kg three times in one day and since PZQ does not act on juvenile worms a follow-up treatment 4-6 weeks later is strongly advised (4). In "preventive chemotherapy" programs, PZQ is administered at a single 40 mg/kg dose to at-risk populations (5). PZQ undergoes a significant first-pass metabolism through the liver enzyme cytochrome P_{450} (CYP) 3A4 and to a smaller extent through 1A2 and 2C19 (6). *R*-PZQ is metabolized at a much faster rate than *S*-PZQ. *R*-PZQ is mainly transformed into *cis* and *trans* hydroxypraziquantel (4-OH-PZQ), while *S*-PZQ is converted to other monohydroxylated metabolites. In rat liver microsomes, the main metabolite is *cis*-4-OH-PZQ (7, 8), while in humans it is *trans*-4-OH-PZQ (9).

The difference in antischistosomal activity of each PZQ enantiomer is known since 1983 (10) and several studies observed a higher activity of *R*-PZQ over *S*-PZQ *in vitro* and *in vivo* (11-13). A clinical trial in *S. japonicum* patients also recorded a higher efficacy of *R*-PZQ over racemic PZQ at the same dosage (14, 15). Additionally, treatment with *R*-PZQ resulted in fewer adverse events than the standard treatment (14). However, since higher plasma concentrations and slightly longer half-lives are achieved with the metabolites compared to PZQ (16), it is possible that the metabolites contribute to the antischistosomal activity of PZQ. Efficacy of racemic *trans*-4-OH-PZQ was evaluated *in*

vitro by Staudt et al. (11), who observed similar antischistosomal properties against adult worms of the *trans* metabolite and *R*-PZQ.

In this study, we comparatively assessed the *in vitro* activities of *R*-PZQ and *S*-PZQ, and the metabolites *cis*- and *trans*-4-OH-PZQ against adults and newly transformed schistosomula (NTS). Drug effects were evaluated using both microscopic readout and isothermal microcalorimetry. Since the metabolites are also chiral molecules, we evaluated for the first time the *in vitro* efficacy of the respective *R* and *S* enantiomers. We also studied the *in vivo* activity of each parent enantiomer in mice and estimate the hepatic shift of the worms after each treatment.

Materials and Methods

Mice and infection

All *in vivo* experiments were performed at the Swiss Tropical and Public Health Institute (Basel, Switzerland) and followed Swiss and cantonal animal welfare regulations (license no. 2070). Female NMRI mice (age 3 weeks, weight ca. 14 g) were purchased from Charles River (Sulzfeld, Germany) or Harlan Laboratories (Blackthorn, United Kingdom). The animals were allowed to adapt for 1 week under controlled conditions (22°C, 50% humidity, 12 h light, and free access to water and rodent diet) before experimental handling.

NMRI mice were infected subcutaneously with 80 to 100 cercariae, as previously described (17).

Drugs and media

RPMI 1640 medium (Life technologies, Carlsbad, CA USA) supplemented with 5% heat-inactivated foetal calf serum (iFCS), penicillin (Life technologies, 100 U/mL) and streptomycin (Life technologies, 100 µg/mL) was used for adult schistosome *in vitro* and microcalorimetry experiments. For NTS *in vitro* culture medium, Medium 199 (Life technologies) was supplemented with iFCS and antibiotics.

Racemic (rac) PZQ was purchased from Sigma-Aldrich (Buchs, Switzerland). Enantiomers of PZQ and *cis*- and *trans*-4-OH-PZQ were acquired from Merck Serono (Darmstadt, Germany), and synthesized by Prof. Matthew Todd (University of Sydney, Australia) (18). Racemic *cis*- and *trans*-4-OH-PZQ were obtained from Prof. Gilles Gasser (University of Zurich, Switzerland) (19). For *in vitro* studies, each compound was dissolved in dimethyl sulfoxide (DMSO) (Fluka, Buchs, Switzerland) at a concentration of 10 mg/mL. For *in vivo* studies, the drugs were dissolved in 7% (v/v) Tween 80 and 3% (v/v) ethanol before oral treatment.

In vitro *studies*

NTS were obtained from cercariae by mechanical transformation (17). Six to twelve hours later the schistosomula (100 NTS/well) were incubated in flat-bottom 96-well plates (BD Falcon) containing the drug solution in medium at 1.2, 3.7, 11.1, 33.3 and 100 µg/mL. Control NTS were incubated with the highest concentration of drug solvent used in the assays (2% DMSO). The plates were incubated at 37°C and 5% CO_2 for 72 h and compound activity was microscopically assessed using a motility scale ranging from 3 (normal activity) to 0 (no activity and granularity present) (20).

To test the effect of each compound on the adult worms, drugs were diluted in medium in flat-bottom 24-well plates (BD Falcon) at concentrations ranging from 0.01 to 10 µg/mL for rac PZQ and *R*-PZQ, and 0.4 to 100 µg/mL for *S*-PZQ and the metabolites. Control wells consisted of drug-free medium with 2% DMSO. Seven to 8 weeks post-infection *S. mansoni*-infected mice were euthanized with CO_2, dissected and adult worms collected from the hepatic portal and mesenteric veins. Four to 6 worms of both sexes were deposited in each well and incubated at 37°C. After 4 and 72 h, the worm condition was microscopically evaluated using a scale from 3 (normal activity and no tegumental alteration) to 0 (dead, highly granulated) (20). To test the recovery of adult worms following a short exposure to *S*-PZQ, we incubated adult worms in medium with 100, 200, 300 or 400 µg/mL *S*-PZQ for 1 or 2 h, and next transferred them to a drug-free medium for up to 72 h. Motility values at 72 h were compared to values of worms incubated in *S*-PZQ for 72 h and control worms incubated in drug-free medium. IC_{50} and IC_{90} values were determined with CompuSyn® software using the motility values obtained from different dosages. The eudysmic ratio (21) was calculated as follows:

$$\text{Eudysmic ratio} = IC_{50}\text{distomer} / IC_{50}\text{eutomer}$$

where the eutomer, the active enantiomer, is represented by *R*-PZQ, and the distomer by *S*-PZQ.

Isothermal microcalorimetry

The microcalorimetry experiments were performed in triplicate on a 48-channel isothermal microcalorimeter (TAM48, TA Instruments, New Castle, DE USA). First, glass ampoules were filled with 2900 µl medium and 4 worms of both sexes were added to each vial. Ampoules were then placed in the channels for the equilibration phase. Twelve hours later, 100 µl of prewarmed drug solution prepared in medium were

injected with 1-mL syringes (BD Plastipak, Becton, Dickinson S.A., Madrid, Spain). End concentrations reached 0.04, 0.2, and 1 µg/mL for rac and *R*-PZQ and 1, 5 and 50 µg/mL for *S*-PZQ and the metabolites. Ampoules containing schistosomes in the presence of DMSO alone (final concentration of 2%) served as negative controls, while ampoules containing dead worms, obtained by dipping them in ethanol 70% for 5 min and rinsing in medium solution, served as positive controls. Schistosome motility data derived from noise amplitudes were recorded for 5 days and analyzed using R software and Excel (22). The noise amplitudes produced by worm movements and metabolism decay exponentially as the worms die, until reaching the background noise level recorded in the dead worm positive controls. The intersection of both curves determines the endpoint of worm motility (22).

In vivo *studies*

Forty-nine days post-infection (chronic *S. mansoni* infection), groups of 3-6 mice were treated orally with 400 mg/kg for racemic PZQ, 400 or 800 mg/kg for *S*-PZQ or 100, 200 or 400 mg/kg for *R*-PZQ. At 14 days post-treatment, the mice were euthanized and dissected. The worms in the veins and liver were sexed and counted (23).

Mean worm burdens of treated mice were compared to untreated mice and worm burden reductions (WBR) were determined. IC_{50}s and eudysmic ratio were calculated as described above.

The hepatic shift was investigated as follows. Groups of 5 mice infected with adult schistosomes were treated with 400 mg/kg of *S*-PZQ, 400 mg/kg rac PZQ, or 200 mg/kg *R*-PZQ. After 30 min, 1 h, 4 h, 24 h and 7 days, one mouse of each group was euthanized, dissected, and worm burden in the veins and liver evaluated.

Statistical tests were performed with Stata (version 12.1, StataCorp, TX USA). Differences in worm burden were assessed using unpaired t-test allowing for unequal variances by comparing the control groups with the treated groups. The significance threshold was set at 0.05.

Results

In vitro *studies*

Table 1 summarizes the *in vitro* IC_{50} and IC_{90} of racemic and optically pure PZQ and 4-OH-PZQ metabolites against adult *S. mansoni* after 4 and 72 h of incubation. *R*-PZQ displayed the highest activity with an IC_{50} of 0.04 µg/mL after 4 h of incubation. The IC_{50} of *R*-PZQ after 72 h was half of the value for the racemic mixture, while the IC_{50} of *S*-PZQ was higher by a factor 100. The IC_{50} values of the metabolites at 72 h showed the same pattern: the *R* conformation was twice as active as the racemic form, while no activity was detected for the *S* metabolites at 100 µg/mL. When comparing the activities of the *cis* and the *trans* configurations, *cis* metabolites displayed a slightly better activity than *trans* metabolites but the IC_{50}s of the metabolites were nevertheless much higher than racemic PZQ. The eudysmic ratio for PZQ *in vitro* determined 72 h post exposure was estimated at 292.5. The antischistosomal activity of *S*-PZQ following short-term incubation is depicted in Figure 1. Worms incubated 1 or 2 h in high concentrations of *S*-PZQ recovered almost completely and displayed high motility values after 3 days (1.25 to 2.5), compared to worms fully incubated 72 h in *S*-PZQ that did not score above 0.5.

The results of the *in vitro* assays against NTS are displayed in Table 2. The IC_{50} of *R*-PZQ was estimated at 0.03 µg/mL. *S*-PZQ showed a markedly lower activity, with an IC_{50} of 39.97 µg/mL. The eudysmic ratio calculated against NTS was 1196. The IC_{50s} of the *trans* metabolites were determined as 133.12 µg/mL and 28.54 µg/mL for rac and *R* derivatives respectively, while *cis* showed a moderate activity for the *R* enantiomer (IC_{50}=34.3 µg/mL).

Isothermal microcalorimetry

Results of worm motility endpoints after PZQ enantiomer and metabolite treatment are summarized in Table 3. For *R*-PZQ, worm motility ceased in the first 3 h post-injection at concentrations as low as 0.04 μg/mL, while the same effect was observed for racemic PZQ only at 0.2 μg/mL. The racemic and *R* metabolites did not display a decrease in worm motility when exposed to a concentration of 1 μg/mL. For racemic and *R-cis*-4-OH-PZQ, the motility endpoint at 5 μg/mL was estimated, as depicted in Figure 2, at 96.7 h and < 3 h post-injection, respectively. The racemic *trans*-4-OH-PZQ was not active at 5 μg/mL, but the *R-trans*-4-OH-PZQ displayed a motility endpoint at 75 h post-injection. At a very high concentration of 50 μg/mL of the racemic and *R* metabolites motility of worms stopped within 3 h. None of the *S* derivatives interfered with worm motility after incubation for 5 days at 50 μg/mL.

In vivo *studies*

In Table 4, the WBRs after different single oral doses of *R*- and *S*-PZQ are presented. Racemic PZQ produced a WBR of 94.1% at 400 mg/kg, while at 100 mg/kg no significant effect was observed. *R*-PZQ showed a WBR of 52% at 100 mg/kg, and WBRs greater than 98% at 200 and 400 mg/kg doses. *S*-PZQ displayed a low WBR of 19.6% at 800 mg/kg. When comparing the worm burdens at 400 mg/kg, there were significant differences between rac PZQ and *R*-PZQ and the control group (p-values < 0.001). There was no statistically difference between worm burdens of controls and *S*-PZQ-treated mice (p= 0.68). The ED_{50}s were 95.4 and > 1000 mg/kg for *R*- and *S*-PZQ, respectively, and the corresponding eudysmic ratio was higher than 10.

The hepatic shift obtained with a single mouse per time point observed for PZQ enantiomers is illustrated in Figure 3. Racemic PZQ acted rapidly: at 30 minutes post-

220 treatment, only a few living worms were still observed in the mesenteric veins, while
221 from 1 h onwards all the worms were found dead in the liver. Treatment with *R*-PZQ at
222 half the dose of rac PZQ produced fairly similar effects. Living worms in veins, however,
223 were observed until 4 h post-treatment. In contrast, treatment with *S*-PZQ resulted in a
224 high number of dead worms in the liver 30 minutes post-treatment, after which the
225 number of worms killed decreased over time, and after 4 h post-treatment only a small
226 amount of worms were found dead. At the 4 h examination point all worms had
227 migrated to the liver following treatment with *S*-PZQ. Twenty-four hours post-treatment
228 the majority of worms had returned to the mesenteric veins.

Discussion

In the framework of a public-private-partnership (PPP) including Merck Serono, Astellas Pharma, the Swiss TPH and TI Pharma, efforts are ongoing to develop a pediatric formulation of PZQ. The project is currently in the pre-clinical phase and within this work we have for the first time conducted thorough side-by-side *in vitro* and *in vivo* studies with PZQ enantiomers and metabolites, which will aid the development process.

Our data show that the antischistosomal activity is mainly driven by the *R* configuration. We observed that *R*-PZQ and the *R*- hydroxylated metabolites reveal a 100 and 1000-fold higher activity than the *S* counterparts *in vitro*. The racemic compounds display IC_{50} values twice as high as their respective *R* configurations. Note that the IC_{50}s observed against NTS were much higher than the ones against adults, which is in line with previous findings (24, 25). Nevertheless, *R* enantiomers are again more active than *S* conformations against NTS.

Microcalorimetry findings are consistent with our IC_{50} values determined microscopically against adults *in vitro*. The loss of motility noticed for *R*-PZQ at 0.04 and 0.2 µg/mL and between 0.04 and 0.2 µg/mL for racemic PZQ correlate nicely with IC_{50} data (0.02 and 0.05 µg/mL, respectively). As observed in the *in vitro* microscopic assays, *S*-PZQ is not active at 1 µg/mL. Microcalorimetric measurements confirmed that *R-cis* and *R-trans* are the active metabolites (e.g. at 5 µg/mL a loss of motility was observed 96.7 and 75 h post-injection for *R-cis* and *R-trans*, respectively). These observations are in agreement with our IC_{50} data (2.42 and 4.08 µg/mL for *R-cis* and *R-trans*, respectively) based on a microscopic viability score.

A similar pattern was observed *in vivo*: A single oral dose of 400 mg/kg of racemic PZQ shows a similar activity as 200 mg/kg *R*-PZQ. In contrast, treatment with 800 mg/kg *S*-PZQ did not result in a significant WBR and none of the treated mice were cured.

Dissecting mice at different time points after treatment allowed us to investigate the hepatic shift caused by PZQ and its enantiomers. The hepatic shift of worms into the liver had been earlier characterized for PZQ as well as for several other drugs, including mefloquine (26), artemether (27), oxamniquine (28) or older antischistosomal drugs (29). Treatment with the racemate and *R*-PZQ efficiently immobilized or killed the majority of worms, which were carried by blood flow to the liver, where they disintegrate over time. In contrast, treatment with *S*-PZQ killed only a few worms. Worms migrated to the liver and 24 h post-treatment returned back to the mesenteric veins. The typical translocation of the worms into the liver might be explained by a loss of grip on the mesenteric vein wall due to the chemical action of the compound and when the therapeutic effect ceases, they migrate back to the mesenteric veins (29). The return of worms into the mesenteric veins has been described for sub-therapeutic doses or inefficient compounds (30). The transient hepatic shift observed in *S*-PZQ treated mice is therefore a strong additional evidence of its inefficacy.

In order to place our *in vitro* findings in context we have summarized pharmacokinetic (PK) parameters of *R*-PZQ, *S*-PZQ, *R-trans* and *S-trans* obtained in humans (16) in Table 5. The maximal concentration (C_{max}) of *R*-PZQ (0.16 µg/mL) is a factor 8 and 4 higher than its IC_{50} (0.02 µg/mL) and IC_{90} (0.04 µg/mL) values at 72 h, and still a factor 4 higher than its IC_{50} (0.04 µg/mL) at 4 h. Besides, the high ratio of the area under the curve (AUC)/ IC_{50} of 43.5 of *R*-PZQ might also describe its excellent antischistosomal activity. On the other hand, for *S*-PZQ and the *R-trans* enantiomer plasma concentrations

do not exceed the calculated IC_{50} calculated in our work at any time (IC_{50}s approximately 11 and 3 times higher than the C_{max}, respectively). Though the AUC is much higher for the *R-trans* metabolite than the ones observed for *R*-PZQ and *S*-PZQ the AUC/IC_{50} ratio is only 2.1. Furthermore, our *in vitro* recovery experiments with *S*-PZQ, even using up to 700-fold higher concentrations than its C_{max} (16), demonstrated that the worms are still alive and recover from a 2 h exposure. As mentioned before, the *S-trans* metabolite is not active at 100 μg/mL.

Published PK data for the *cis* metabolite are not yet available, but in light of their high IC_{50} values compared to *R*-PZQ, it is also unlikely that it significantly contributes to the antischistosomal activity of PZQ.

Changes in the activity of CYP450 enzymes can dramatically change the PK parameters of PZQ and thereby its therapeutic activity. For example, co-administration of CYP3A4 inducers such as dexamethasone dramatically reduces PZQ plasma levels in patients with neurocysticercosis (6, 31, 32). Albendazole is an inhibitor of CYP enzymes and when it is administered concomitantly with PZQ, plasma levels of *R*-PZQ are increased (16). The expression of CYP450 is as well modulated during chronic schistosomiasis, with a marked lower activity in infected mice, probably resulting from the immune response towards the infection (33). Interestingly, resistant isolates of *S. mansoni* do not inhibit host CYP450 as much as the susceptible do. This mechanism of resistance produces a faster first-pass metabolism, hence a shorter exposure time to the parent drug (34). These results support the evidence that *R*-PZQ is the active molecule and metabolites do not have a major role in PZQ activity.

We conclude that the activity of PZQ is almost exclusively based on *R*-PZQ and that neither *S*-PZQ nor the metabolites significantly contribute to the therapeutic effect. Our results favor the development of a child friendly formulation of *R*-PZQ, since an enantiopure formulation displays two major advantages: first, it would allow clinicians to reduce the dosage by half, and second it would ease the administration to children, bothered by the bitter taste of *S*-PZQ (35).

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Tables

Table 1: IC_{50} and IC_{90} values of racemic and enantiomeric PZQ and 4-OH metabolites against adult *S. mansoni* calculated 4 and 72 h post-incubation *in vitro*

	IC_{50} at 4 h (µg/mL)	IC_{50} at 72 h (µg/mL)	IC_{90} at 72 h (µg/mL)	Eudysmic ratio
Rac-PZQ	0.1	0.05	0.4	
R-PZQ	0.04	0.02	0.04	293
S-PZQ	5.7	5.9	17.9	
Rac-*trans*-4-OH-PZQ	16.7	7.9	3694.1[a)]	
R-trans-4-OH-PZQ	13.4	4.1	58.4	
S-trans-4-OH-PZQ	Not active at 100	Not active at 100	Not active at 100	
Rac-*cis*-4-OH-PZQ	15.8	4.8	81.4	
R-cis-4-OH-PZQ	4.5	2.4	48.7	
S-cis-4-OH-PZQ	Not active at 100	Not active at 100	Not active at 100	

[a)] Extrapolated value determined by CompuSyn

Table 2: IC_{50} and IC_{90} values of racemic and enantiomeric PZQ and 4-OH metabolites against NTS 72 h post-incubation *in vitro*

	IC_{50} at 72 h (µg/mL)	IC_{90} at 72 h (µg/mL)	Eudysmic ratio
Rac-PZQ	1.5	34.5	
R-PZQ	0.03	18.8	1196
S-PZQ	40.0	522.5[a)]	
Rac-*trans*-4-OH-PZQ	133.1[a)]	5852.2[a)]	
R-trans-4-OH-PZQ	28.5	747.4[a)]	
S-trans-4-OH-PZQ	Not active at 100	Not active at 100	
Rac-*cis*-4-OH-PZQ	911.1[a)]	2448837[a)]	
R-cis-4-OH-PZQ	34.3	1161.8[a)]	
S-cis-4-OH-PZQ	Not active at 100	Not active at 100	

[a)] Extrapolated value determined by CompuSyn

Table 3: Endpoints of worm motility in hours (SD) determined by noise amplitudes for different concentrations of racemic and enantiomeric PZQ and 4-OH metabolites

	0.04 µg/mL	0.2 µg/mL	1 µg/mL
Rac-PZQ	> 120	< 3	< 3
R-PZQ	< 3	< 3	< 3
S-PZQ	> 120	> 120	> 120
	1 µg/mL	5 µg/mL	50 µg/mL
Rac-*trans-4*-OH-PZQ	> 120	> 120	< 3
R-trans-4-OH-PZQ	> 120	75 (5)	< 3
S-trans-4-OH-PZQ	> 120	> 120	> 120
Rac-*cis*-4-OH-PZQ	> 120	96.7 (16.1)	< 3
R-cis-4-OH-PZQ	> 120	< 3	< 3
S-cis-4-OH-PZQ	Not tested	Not tested	> 120

Table 4: Total and female worm burden reduction (WBR) obtained with racemic PZQ, *R*-PZQ and *S*-PZQ at different dosages in mice harboring adult *S. mansoni*

Rac PZQ	Number mice	WBR [%] (SD)	ED_{50} (mg/kg)	
400 mg/kg	4	94.1 (8.6)	246.5	
100 mg/kg[b)]	6	15 (9.5)		
R-PZQ			ED_{50} (mg/kg)	
400 mg/kg	3	100.0 (0)	95.4	
200 mg/kg	6	98.1 (2.3)		
100 mg/kg	6	52.0 (30.8)		
S-PZQ			ED_{50} (mg/kg)	Eudysmic ratio
800 mg/kg	6	19.6 (22.2)	3066777[a)]	32136
400 mg/kg	4	18.0 (21.4)		

[a)] Extrapolated value determined by CompuSyn
[b)] Data from (36)

Table 5: PK parameters after an oral dose of 23.3 mg/kg in human volunteers*: maximal concentration (C_{max}), time to maximal concentration (t_{max}), half-life ($t_{1/2}$), area under the curve (AUC), and ratio C_{max}/ IC_{50} and AUC/IC_{50}****

	C_{max} (µg/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC (µg ml^{-1} h)	C_{max} /IC_{50}	AUC/IC_{50}
R-PZQ	0.16	2.67	1.55	0.87	8	43.5
S-PZQ	0.52	2.55	1.46	2.99	0.09	0.5
R-trans-4-OH-PZQ	1.31	2.72	1.70	8.80	0.31	2.1
S-trans-4-OH-PZQ	0.78	3.05	1.91	5.60	No IC_{50}	No IC_{50}

*adapted from (16)
**IC_{50} values from adults after 72 h

Figures (legends)

Figure 1: Motility values of adult worms (n=4-6, in triplicate) after 1 h (∗) or 2 h (▲) incubation in *S*-PZQ followed by incubation in drug-free medium until 72 h, compared with adults incubated 72 h in *S*-PZQ (●) and controls 72 h in drug-free medium (dashed line).

Figure 2: Example of heat production recorded by microcalorimetry: rac *cis*-4-OH and *R*-*cis*-4-OH-PZQ at 5 µg/mL and *S*-*cis*-4-OH-PZQ at 50 µg/mL

Figure 3: *In vivo* hepatic shift after treatment with rac PZQ 400 mg/kg, *R*-PZQ 200 mg/kg or *S*-PZQ 400 mg/kg: number of worms alive in the mesenteric veins (white), alive in the liver (dashed) and dead in the liver (black)