Activity of Praziquantel Enantiomers and Main Metabolites Against Schistosoma mansoni

Meister, Isabel; Ingram-Sieber, Katrin; Cowan, Noemi; Todd, Matthew H; Robertson, Murray N; Meli, Claudia; Patra, Malay; Gasser, Gilles; Keiser, Jennifer

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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 \textit{in vitro} activities of PZQ and its main metabolites, namely \textit{cis-} and \textit{trans-4'-R/S} hydroxypraziquantel, on adult worms and newly transformed schistosomula (NTS). Additionally, we explored the \textit{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 \( S. \) \textit{mansoni} were determined \textit{in vitro} for \( R \)-PZQ, \( S \)-PZQ, \( R \)-\textit{trans} and \( R \)-\textit{cis} hydroxypraziquantel, respectively. \( S \)-\textit{trans} and \( S \)-\textit{cis} were not active at 100 µg/mL. These results are consistent with microcalorimetry data and studies on NTS. \textit{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 \textit{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.

\textbf{Keywords:} schistosomiasis, chemotherapy, praziquantel, \textit{cis-4'}-hydroxypraziquantel, \textit{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% CO₂ 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 CO₂, 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. IC₅₀ and IC₉₀ values were determined with CompuSyn® software using the motility values obtained from different dosages. The eudysmic ratio (21) was calculated as follows:

\[
\text{Eudysmic ratio} = \frac{\text{IC}_{50}\text{distomer}}{\text{IC}_{50}\text{eutomer}}
\]

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$_{50}$s 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 ED50s 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-
treatment, only a few living worms were still observed in the mesenteric veins, while from 1 h onwards all the worms were found dead in the liver. Treatment with $R$-PZQ at half the dose of rac PZQ produced fairly similar effects. Living worms in veins, however, were observed until 4 h post-treatment. In contrast, treatment with $S$-PZQ resulted in a high number of dead worms in the liver 30 minutes post-treatment, after which the number of worms killed decreased over time, and after 4 h post-treatment only a small amount of worms were found dead. At the 4 h examination point all worms had migrated to the liver following treatment with $S$-PZQ. Twenty-four hours post-treatment 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 ($C_{\text{max}}$) 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).

Acknowledgements

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References


Tables

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>IC\textsubscript{50} at 4 h (µg/mL)</th>
<th>IC\textsubscript{50} at 72 h (µg/mL)</th>
<th>IC\textsubscript{90} at 72 h (µg/mL)</th>
<th>Eudysmic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rac-PZQ</td>
<td>0.1</td>
<td>0.05</td>
<td>0.4</td>
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<tr>
<td>R-PZQ</td>
<td>0.04</td>
<td>0.02</td>
<td>0.04</td>
<td>293</td>
</tr>
<tr>
<td>S-PZQ</td>
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<td>5.9</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>Rac-trans-4-OH-PZQ</td>
<td>16.7</td>
<td>7.9</td>
<td>3694.1\textsuperscript{a)}</td>
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<tr>
<td>R-trans-4-OH-PZQ</td>
<td>13.4</td>
<td>4.1</td>
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<td>S-trans-4-OH-PZQ</td>
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<td>Not active at 100</td>
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<tr>
<td>Rac-cis-4-OH-PZQ</td>
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\textsuperscript{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*

<table>
<thead>
<tr>
<th></th>
<th>IC$_{50}$ at 72 h (µg/mL)</th>
<th>IC$_{90}$ at 72 h (µg/mL)</th>
<th>Eudysmic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rac-PZQ</td>
<td>1.5</td>
<td>34.5</td>
<td></td>
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<tr>
<td>R-PZQ</td>
<td>0.03</td>
<td>18.8</td>
<td>1196</td>
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<tr>
<td>S-PZQ</td>
<td>40.0</td>
<td>522.5$^a$</td>
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<tr>
<td>Rac-trans-4-OH-PZQ</td>
<td>133.1$^a$</td>
<td>5852.2$^a$</td>
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<td>R-trans-4-OH-PZQ</td>
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<td>S-trans-4-OH-PZQ</td>
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<td>Not active at 100</td>
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<tr>
<td>Rac-cis-4-OH-PZQ</td>
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<td>R-cis-4-OH-PZQ</td>
<td>34.3</td>
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<td>S-cis-4-OH-PZQ</td>
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<td>Not active at 100</td>
<td></td>
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</table>

$^a$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

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<tr>
<th></th>
<th>0.04 µg/mL</th>
<th>0.2 µg/mL</th>
<th>1 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rac-PZQ</td>
<td>&gt; 120</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>R-PZQ</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>S-PZQ</td>
<td>&gt; 120</td>
<td>&gt; 120</td>
<td>&gt; 120</td>
</tr>
<tr>
<td></td>
<td>1 µg/mL</td>
<td>5 µg/mL</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Rac-trans-4-OH-PZQ</td>
<td>&gt; 120</td>
<td>&gt; 120</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>R-trans-4-OH-PZQ</td>
<td>&gt; 120</td>
<td>75 (5)</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>S-trans-4-OH-PZQ</td>
<td>&gt; 120</td>
<td>&gt; 120</td>
<td>&gt; 120</td>
</tr>
<tr>
<td>Rac-cis-4-OH-PZQ</td>
<td>&gt; 120</td>
<td>96.7 (16.1)</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>R-cis-4-OH-PZQ</td>
<td>&gt; 120</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>S-cis-4-OH-PZQ</td>
<td>Not tested</td>
<td>Not tested</td>
<td>&gt; 120</td>
</tr>
</tbody>
</table>
### 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*

<table>
<thead>
<tr>
<th></th>
<th>Number mice</th>
<th>WBR [%] (SD)</th>
<th>ED(_{50}) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rac PZQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>4</td>
<td>94.1 (8.6)</td>
<td>246.5</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>6</td>
<td>15 (9.5)</td>
<td></td>
</tr>
<tr>
<td><strong>R-PZQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>3</td>
<td>100.0 (0)</td>
<td>95.4</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>6</td>
<td>98.1 (2.3)</td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>6</td>
<td>52.0 (30.8)</td>
<td></td>
</tr>
<tr>
<td><strong>S-PZQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>6</td>
<td>19.6 (22.2)</td>
<td>3066777(^{a})</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>4</td>
<td>18.0 (21.4)</td>
<td>32136</td>
</tr>
</tbody>
</table>

\(^{a}\)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 ($C_{\text{max}}$), time to maximal concentration ($t_{\text{max}}$), half-life ($t_{1/2}$), area under the curve (AUC), and ratio $C_{\text{max}}$/IC$_{50}$** and AUC/IC$_{50}$**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC (µg ml$^{-1}$ h)</th>
<th>$C_{\text{max}}$/IC$_{50}$</th>
<th>AUC/IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-PZQ</td>
<td>0.16</td>
<td>2.67</td>
<td>1.55</td>
<td>0.87</td>
<td>8</td>
<td>43.5</td>
</tr>
<tr>
<td>S-PZQ</td>
<td>0.52</td>
<td>2.55</td>
<td>1.46</td>
<td>2.99</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>R-trans-4-OH-PZQ</td>
<td>1.31</td>
<td>2.72</td>
<td>1.70</td>
<td>8.80</td>
<td>0.31</td>
<td>2.1</td>
</tr>
<tr>
<td>S-trans-4-OH-PZQ</td>
<td>0.78</td>
<td>3.05</td>
<td>1.91</td>
<td>5.60</td>
<td>No IC$_{50}$</td>
<td>No IC$_{50}$</td>
</tr>
</tbody>
</table>

*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).