# *In Vitro* Metabolic Profile and *In Vivo* Antischistosomal Activity Studies of ($\eta^6$-Praziquantel)$Cr(CO)_3$ Derivatives

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ABSTRACT. The *in vitro* metabolic behavior was investigated of two chromium tricarbonyl derivatives of the antischistosomal drug Praziquantel (PZQ) with the formula ($\eta^6$-PZQ)$Cr(CO)_3$ (**1** and **2**), using human liver microsomes. It could be demonstrated that the metabolic profiles of the derivatives differ significantly. The optically pure ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives, (*S*, *S*p)-**1**, (*R*, *R*p)-**1**, (*S*, *R*p)-**2** and (*R*, *S*p)-**2**, were also prepared to assess of the eudysmic ratios of **1** and **2** against *S. mansoni*. A strong enantioselective antischistosomal activity was observed. The *R*-enantiomers are highly active against adult schistosomes *in vitro* ($IC_{50}$: 0.08 - 0.13 μM) whereas both *S*-enantiomers lack activity. The *in vivo* activity of **1** and **2** was then studied in mice harboring a chronic *Schistosoma mansoni* (*S. mansoni*) infection. A single dose of **1** and **2** (400 mg/kg) resulted in low worm burden reductions of 24 and 29 % ($p>0.05$).

**Introduction.**

Schistosomiasis is the second most prevalent parasitic disease in the world after malaria with 207 million people infected each year. Surprisingly there is only one available drug on the market to treat the disease, Praziquantel (PZQ) (Figure 1).[1, 2] Despite the identification of various lead antischistosomal compounds, no new drugs have entered the market since the development of PZQ in 1970s.[3] Reliance on a single drug is dangerous and reduced susceptibility of *Schistosoma mansoni* (*S. mansoni*) to PZQ has already been noted in the field.[4-6] The important limitations of PZQ make the situation even more precarious. PZQ has low metabolic stability *in vivo*[7, 8] and lacks activity against the juvenile stage of *Schistosoma.*[9] Taken together, these facts are a strong indication that novel drugs to treat schistosomiasis are required. With this in mind, our groups have recently embarked on a project to study the potential of organometallic-containing PZQ derivatives as antischistosomal agents. This concept originates from the pioneering work of Jaouen, Biot and co-workers on organometallic derivatives of the anticancer agent Tamoxifen (Ferrocifens) and the antimalarial Chloroquine (Ferroquine).[10, 11] For both Ferrocifens and Ferroquine, the addition of a ferrocenyl moiety into the known organic drug allowed the addition of novel modes of action to the parent compounds.[12, 13] Encouraged by these results, we investigated the antischistosomal activity of 18 ferrocenyl derivatives of PZQ.[14] Unfortunately, only moderate *in vitro* activity could be determined.[14] On the other hand, it was found that two ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives (**1** and **2**, Figure 1) had *in vitro* activity against *S. mansoni* comparable to PZQ (nanomolar range).[15] These promising *in vitro* data persuaded us to study in more detail the *in vivo* behavior of **1** and **2**. Clearly a promising *in vitro* activity of a drug candidate cannot always be extrapolated into a good *in vivo* activity since the pharmacokinetics and metabolism profile of a drug are key determinants for its *in vivo* success. It is therefore

important to assess the metabolic fate of a drug candidate. As a continuation of our development on the ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives, we present herein the in-depth *in vitro* metabolic behavior of these chromium derivatives as well as their antischistosomal activity in mice infected with adult *S. mansoni*. Importantly, we could demonstrate, by preparing optically active (*S*, *S*p)-**1**, (*R*, *R*p)-**1**, (*S*, *R*p)-**2** and (*R*, *S*p)-**2** that, as for the parent drug PZQ, the ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives exhibit enantioselective *in vitro* antischistosomal activity.

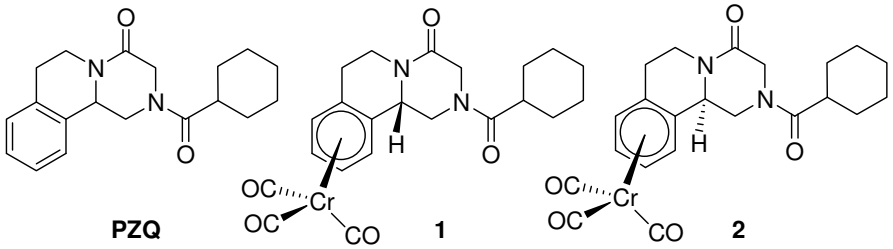



**Figure 1.** Structures of PZQ, ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives **1** and **2**. Note that all the compounds presented are racemic mixtures.

**Results and Discussion.**

***In Vitro* Metabolism Studies.** In order to gain insight into the metabolic fate of the racemic ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives **1** and **2**, we investigated their *in vitro* metabolic behavior using human liver microsomes. Accordingly **1**, **2** and PZQ were incubated with human liver microsomes in the presence of NADPH at 37 °C (see SI for details). Diazepam which is (known to be stable in human liver microsomes) was used as a positive control. After incubation, compounds were immediately extracted with dichloromethane which was evaporated to provide residues that were analyzed using LC-MS. The metabolites were identified by comparing the differences in the respective *m/z* values in MS spectra with those determined for the parent compound (**1** or **2**) and PZQ (see Table S1, S2 for details). The identities of the major metabolites were further

confirmed by comparing the retention time in the UV trace and the mass spectrum from the LC-MS analysis of authentic samples (*vide infra*). Semi-quantitative analysis of the ratio of parent compound and different metabolites present in the mixture after post incubation with human liver microsomes was achieved by comparing the areas under the respective peaks of different compounds visible in the UV traces of the LC analysis (see Table S3 for details).

In the case of **1**, formation of two major and three minor metabolites was observed (see Figure 2 and Table S1 and S3). After 2.5 h a considerable amount (*ca.* 50 %) of **1** remained unchanged in the mixture; the most abundant metabolites, **1.M1** and **1.M2** (22 and 15 %, respectively), were detected at retention times of 12.8 and 9.1 minutes, respectively, by LC-MS (positive ion electrospray mode). The corresponding MS spectrum for the **1.M1** peak provided base peaks at *m/z* = 313.2 and 335.2 which fit with the species $[PZQ+H]^+$ and $[PZQ+Na]^+$ (MW of PZQ 312.2), respectively. **1.M1** was therefore identified as PZQ that is formed by loss of the $Cr(CO)_3$ moiety of **1**. This identification was further confirmed by comparing the LC and MS traces of an authentic sample of PZQ (Figure S1 in SI). The metabolite **1.M2** was identified as a mono-hydroxylated species of PZQ (PZQ-OH) from the peaks at *m/z* = 329.1 and 311.2 in the MS spectrum which match with the species $[(PZQ\text{-}OH)+H]^+$ and $[(PZQ\text{-}OH)\text{-}H_2O+H]^+$, respectively. This metabolite **1.M2** was confirmed to be the *cis*-4-hydroxypraziquantel (*cis*-4-PZQ-OH, Scheme 1) by comparing the LC-MS traces of the synthetic standards *cis*-4-PZQ-OH and *trans*-4-hydroxypraziquantel (*trans*-4-PZQ-OH) (Figure S1 in SI).[16] The other three minor metabolites were detected at retention times of 10.1 (**1.M3**), 10.5 (**1.M4**) and 10.9 (**1.M5**) minutes (Figure 2 and Table S1). Metabolites **1.M3** and **1.M4** have a similar mass spectrum with an intense peak at *m/z* = 465.1 (Table S1) that matches well the protonated adduct of mono-hydroxylated species of **1** (Scheme 1). As they are present as trace metabolites in the mixture, further efforts were not

made to confirm the structure of these metabolites. The hydroxylation of **1**, most likely, occurs in the cyclohexane ring or at the tetrahydroisoquinoline part. The minor metabolite **1.M5** also has a similar mass spectrum pattern to PZQ-OH with a central peak at *m/z* = 329.2, which corresponds to another mono-hydroxylated derivative of PZQ (see Table S1). Although we could confirm, by comparing the UV traces from LC-MS analysis, that **1.M5** is not the *trans*-4-PZQ-OH (Figure S1 in SI), we could not structurally characterize **1.M5** due to a lack of any standard matching compound. However, as reported earlier, the hydroxylation in **1.M5** is expected to occur either at the tetrahydroquinoline part or at the aromatic ring of PZQ.[17] A plausible metabolic profile of **1** explaining the possible routes of formation of metabolites is presented in Scheme 1(a).

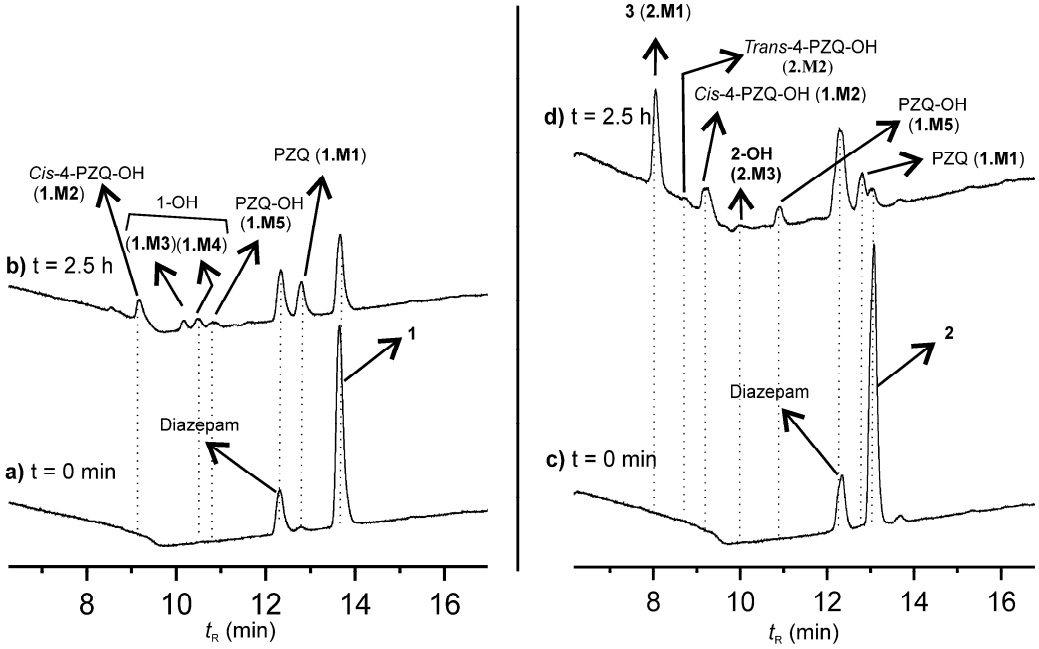
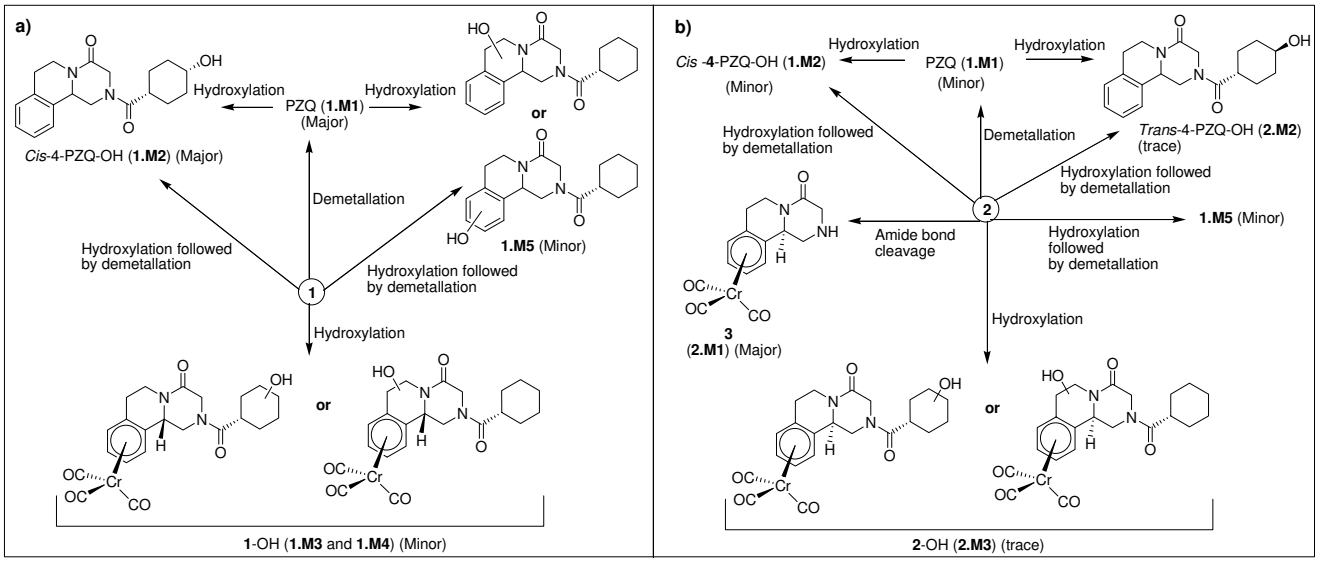



**Figure 2.** UV traces at 220 nm of LC analysis of **1** (left, a and b) and **2** (right, c and d) treated with human liver microsomes at t = 0 min and at t = 2.5 h.



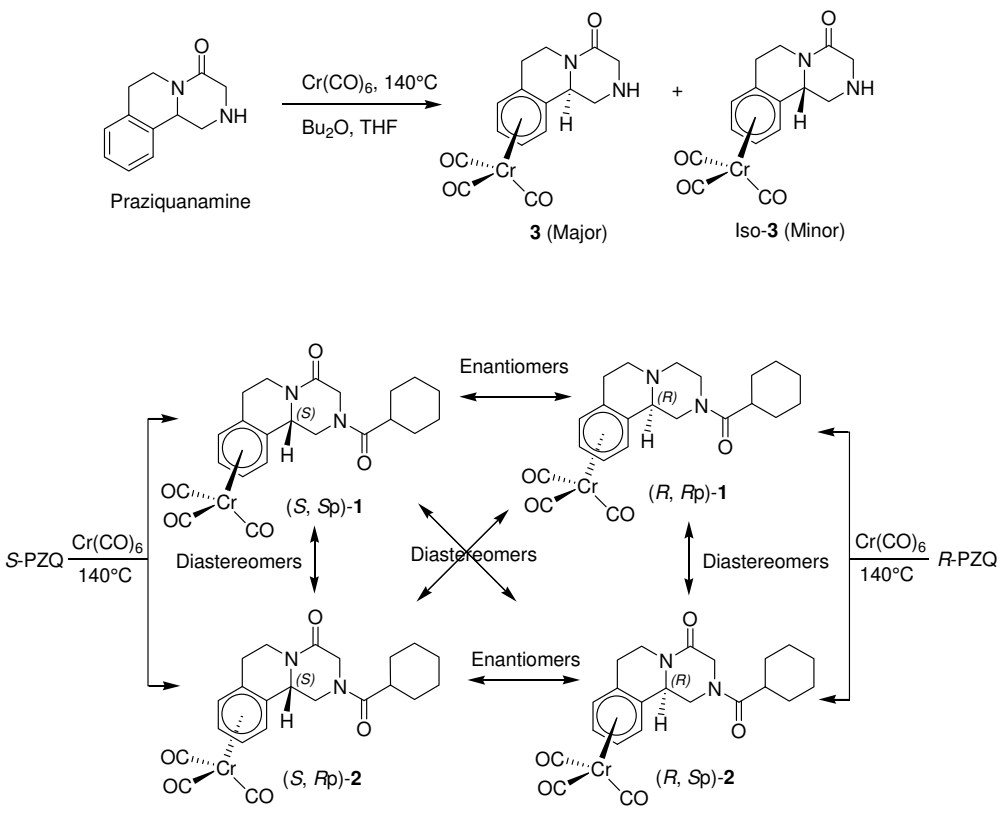

**Scheme 1.** Suggested metabolic profiles of **1** (a) and **2** (b) explaining possible routes to form the different metabolites.

Metabolism rate and profile data were obtained for compound **2** that were significantly different to those obtained for **1** (Figure 2, right, c) and d) and Table S2). A metabolic profile of **2** is schematically presented in Scheme 1. Only a trace amount of **2** was present in the microsomes/NADPH mixture after 2.5 h (Table S3). The most abundant metabolite **2.M1** (*ca.* 43%) was detected at a retention time of 7.9 minutes. The corresponding ESI-MS peak at *m/z* = 339.0 matches well the calculated molecular weight of the protonated species of **[**($\eta^6$-Praziquanamine)$Cr(CO)_3$ (**3**), i. e. $[\mathbf{3}+H]^+$ (calculated *m/z* = 339.01). Metabolite **3** can only be formed by the cleavage of the amide bond between Praziquanamine and the cyclohexanoyl moiety of **2**. This type of amide bond cleavage was not observed in the case of **1**. The formation of **3** was further confirmed by comparing the UV trace of the synthetic standard (See Figure S2 and Scheme 2 for synthetic details). Formation of six other less abundant metabolites, **2.M2** (*trans*-4-PZQ-OH), **1.M2** (*cis*-4-PZQ-OH), **2.M3** (**2**-OH), **1.M5** (PZQ-OH) and **1.M1** (PZQ)

was also observed and these were characterized from their corresponding UV traces and ESI-MS spectra (see Figure S2 and Table S2 in SI).

The metabolism study of **1** and **2** was authenticated by carrying out a metabolic study on PZQ itself using the same assay as for **1** and **2** and by further comparison with previously reported results.[17-22] As shown in Figure S3 in SI, PZQ was exclusively metabolized to its mono-hydroxylated species **1.M2** (*cis*-4-PZQ-OH) and **1.M5** (an unidentified mono-hydroxylated derivative). This observation is in good agreement with reports described earlier.[17, 22] Formation of a trace amount of *trans*-4-PZQ-OH was also observed.

Of note, using the experimental conditions and LC-MS setup reported in this study, we did not observe the presence of any dehydrogenated metabolites for PZQ, **1** and **2**, as previously reported for PZQ by Alnouti, Kiec-Kononowicz and co-workers using different analytical techniques and/or a slightly different *in vitro* model (human cytochrome P-450 3A4 expressed in *Escherichia coli* and *Saccharomyces cerevisiae* instead of microsomes).[8, 23] Although we were unable to detect any of these metabolites, the possibility of their formation cannot be completely ruled out. One can speculate that the dehydrogenated compounds could be formed as minor metabolites that co-elute with a major metabolite and that the MS only detects the more abundant component present in the mixture.




**Scheme 2.** Synthesis of **3** and optically pure ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives. Note that Praziquanamine, **3** and Iso-**3** are racemates.

***In Vitro* Antischistosomal Activity Studies of Optically pure ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives and selected metabolites of 1 and 2.** In order to assess if only one enantiomer of compounds **1** and **2** were active against *S. mansoni* (as is the case for PZQ) the optically pure ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives were prepared using a similar synthetic pathway as for *rac*-**1** and **2** (see Scheme 2 for details).[15] Optically pure PZQ (*S*-PZQ and *R*-PZQ),[24] was employed as starting material (see Figures S4-9 in the SI for chiral HPLC traces). The four new complexes, namely (*S*, *S*p)-**1**, (*R*, *R*p)-**1**, (*S*, *R*p)-**2** and (*R*, *S*p)-**2** were tested against adult schistosomes *in vitro* (Table 1). Enantioselective antischistosomal activity was observed for both derivatives. Only the (*R*)-enantiomers exhibited high levels of activity: (*R*, *R*p)-**1** with an $IC_{50}$ value of 0.08

μM and (*R*, *S*p)-**2** with 0.13 μM. Both (*S*)-enantiomers lacked activity ($IC_{50}$ > 66.9 μM). The *in vitro* activity of the major metabolites elucidated for **1** and **2** was likewise tested. As expected, high activity was observed for **1.M1** (PZQ) ($IC_{50}$: 0.1 μM). The second major metabolite of **1** (**1.M2**) showed good but 24-fold decreased antischistosomal activity ($IC_{50}$: 5.9 μM) when compared to the parent drug. The major chromium-containing metabolite of **2**, **2.M1**, presented only moderate activity with an $IC_{50}$ value of 11.6 μM. Taken together, the remarkably enantioselective antischistosomal activities observed during the *in vitro* experiments are a good indication that (*R*, *R*p)-**1** and (*R, S*p)-**2** act on the same target(s) as PZQ.[8, 25]

**Table 1.** *In vitro* activity of compounds **1** and **2**, the optically pure enantiomers of **1** and **2** and the main metabolites of **1** (**1.M1**, **1M.2**) and **2** (**2.M1**) against adult *S. mansoni*. 'R' represents the "Goodness of fit" which has to be ≥ 0.85)

| | Compound[a] | $IC_{50}$ [μM] | R |
|---|---|---|---|
| *Parent compounds* | **1**[b] | 0.25 | 1.0 |
| | **2**[b] | 0.27 | 1.0 |
| | PZQ | 0.10 | 1.0 |
| *Optically pure derivatives* | (*R*, *R*p)-**1** | 0.08 | 0.9 |
| | (*S*, *S*p)-**1** | > 66.9 | |
| | (*R*, *S*p)-**2** | 0.13 | 1.0 |
| | (*S*, *R*p)-**2** | > 66.9 | |
| *Major metabolites* | **1.M1** (PZQ) | 0.1 | 1.0 |
| | *Cis*-4-PZQ-OH (**1.M2**) | 5.9 | 0.9 |
| | **3** (**2.M1**) | 11.6 | 0.9 |

[a] Note that **1**, **2**, PZQ, *Cis*-4-PZQ-OH (**1.M2**) and **3** (**2.M1**) are racemates; [b] data published by *Patra et al.*[15]

***In Vivo* Antischistosomal Activity Studies.** To assess the *in vivo* potential of the chromium tricarbonyl derivatives, **1** and **2** were given orally to mice harboring a chronic *S. mansoni* infection. The racemate mixtures of **1** and **2** were given to mice for comparison purposes with

PZQ, which is itself given as a racemate. The results are summarized in Table 2. Treatment of mice at a single dose of 400 mg/kg of **1** and **2** resulted in low worm burden reductions of 24 and 29 % ($p>0.05$), respectively. In the treatment group of compound **1**, two mice died one day post-treatment whereas one of the mice treated with **2** died 4 days post-treatment. For comparison, at this dose (400 mg/kg) worm burden reductions of 96% are achieved with PZQ.[26] The toxicity observed was unexpected as **1** and **2** did not present any cytotoxicity on the non-cancerous cell line MRC-5 up to 100 μM compound concentration.[15] The low *in vivo* activity observed can be attributed to the metabolic lability of **1** and **2** resulting in less active metabolites (Table 2). It is interesting that both compounds exert a similar low *in vivo* activity despite compound **1** being metabolized to PZQ. However, since *S. mansoni* live in the mesenteric veins they might not be exposed to PZQ before it is metabolised in the liver through first-pass. It can also be hypothesized that the relatively low *in vivo* activity observed is due to protein binding or distribution problems. Further experiments outside the scope of this article should be undertaken to evaluate these assumptions.

**Table 2.** *In vivo* activity of two racemic ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives **1** and **2** administered at single oral doses of 400 mg/kg to mice harbouring adult *S. mansoni*.

| Group | No. of mice investigated | No. of mice cured | Mean number of worms (SD) | TWR [%] | p-value |
|---|---|---|---|---|---|
| Control | 9 | - | 36.7 (8.2) | - | - |
| **1** | 5[a] | 0 | 28 (5.9) | 24 | >0.05 |
| **2** | 5[b] | 0 | 26.3 (19.9) | 29 | >0.05 |
| PZQ | N/A | N/A | N/A | 96[c] | N/A |

[a] 2 mice died 24 h post-treatment; [b] 1 mouse died 96 h post-treatment; [c] taken from Dong *et al*.[31] N/A: Not available; SD: Standard Deviation; TWR: Total worm burden reduction.

## Conclusions

Taken together, the low *in vivo* antischistosomal activity as well as toxicity observed for **1** and **2** compared to PZQ contrast with the remarkable *in vitro* results previously described[15] and further emphasize the necessity for in *vivo* confirmation of *in vitro* hits. It is important to note that the toxicity observed cannot be at least fully attributed to chromium toxicity but might instead have been caused by the schistosome infection. The amount of chromium given to the mice (400 mg/kg of ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives), assuming a full release of chromium from **1** and **2** as well as the oxidation of Cr(0) to the more *in vivo* stable Cr(III), is insufficient to kill mice: the $LD_{50}$ values of Cr(III) salts are 3.2-15 g/kg, when administered orally in rats.[27] Of note, Cr(III) has even been used as an antidiabetic agent.[28]

In summary in this study we have demonstrated, using human liver microsomes, that two ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives, which were previously shown to have *in vitro* activity against *S. mansoni* comparable to PZQ, were metabolically labile. Clear differences in the rate of metabolism and identity of the metabolites produced were observed between **1** and **2**. Indeed, while **1** was metabolized slowly and produced PZQ and PZQ-OH as the most abundant metabolites, **2** was mostly metabolized to **3** and to a lesser extent to PZQ and PZQ-OH. Importantly, an enantioselective *in vitro* antischistosomal activity comparable to PZQ itself was determined for the organometallic compounds **1** and **2**. These results are a good indication that the active form of **1** and **2** bind to the same target(s) as PZQ. Finally, *in vivo* experiments demonstrated that low worm burden reductions of 24 and 29 % ($p>0.05$) were obtained when a single dose of **1** and **2** (400 mg/kg) were given to mice harboring a chronic *S. mansoni* infection. Overall the results presented herein will give valuable information to the rapidly growing field of medicinal organometallic chemistry field.[29] As highlighted by Hartinger, Metzler-Nolte and

Dyson in a recent review about organometallic anticancer compounds, there is an important lack of published *in vivo* studies on non-radioactive bioorganometallic compounds.[30] We therefore do believe that the work presented in this study will help to fill this important gap and hopefully allow more organometallic compounds to become part of drug research and development.

**Experimental section.**

**Materials.** All chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. Solvents were used as received or dried over molecular sieves. All preparations were carried out using standard Schlenk techniques. **1**, **2,** praziquanamine, *trans*-4-PZQ-OH and *cis*-4-PZQ-OH, *R*-PZQ and *S*-PZQ were prepared following standard literature procedures.[15, 16, 24, 31, 32] The optically pure four ($\eta^6$-PZQ)$Cr(CO)_3$ derivatives were prepared using *R*-PZQ and *S*-PZQ following a procedure reported for synthesis of **1** and **2**.[15] The purities of compounds (**1**, **2**, (*S*, *S*p)-**1**, (*R*, *R*p)-**1**, (*S*, *R*p)-**2** and (*R*, *S*p)-**2** and **3,** and *cis*-4-PZQ-OH) used for biological activity were checked by elemental analysis. The enantio-purities of (*S*, *S*p)-**1**, (*R*, *R*p)-**1**, (*S*, *R*p)-**2** and (*R*, *S*p)-**2** were checked using chiral HPLC technique and the enantiopurities was found to be ≥ 95%.

**Instrumentation and Methods.** *$^1H$* and *$^{13}C$ NMR spectra* were recorded in deuterated solvents on 400 ($^1$H: 400 MHz, $^{13}$C: 100.6 MHz) or 500 ($^1$H: 500 MHz, $^{13}$C: 126 MHz) MHz spectrometers at room temperature. The chemical shifts, δ, are reported in ppm (parts per million). The residual solvent peaks have been used as an internal reference. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). *Infrared spectra* were recorded on Perkin-Elmer ATR-FTIR spectrometer. *ESI mass spectra* were recorded using a Bruker ESQUIRE 6000

HCT quadrupole ion trap instrument (Bruker Daltonik GmbH, Leipzig, Germany), equipped with an electrospray ionization source. Waters ACQUITY ultra performance LC (Waters, Baden-Dätwill, Switzerland) with a diode array detector was directly interfaced to the ESI ion source. *LC-MS measurements* were run in the following conditions: alternating polarity, nebulizer gas ($N_2$) 35 psi, dry gas ($N_2$) 8 l/min, dry temperature 350°C, HV capillary 4000 V, HV EndPlate offset –500 V, capillary exit 166 V, skimmer 40 V, trap drive 80, mass range from m/z 100 to 2000. For metabolism studies, the Nucleosil 100-5 C18 (250 × 3 mm) reverse phase column was used with a flow rate of 0.5 mL $min^{-1}$ and UV-absorption was measured at 220 nm. The runs were performed with a linear gradient of A (acetonitrile (Sigma-Aldrich HPLC-grade) and B (distilled water containing 0.02% TFA and 0.05% HCOOH): t = 0-3 min, 20% A; t = 7 min, 50% A; t = 16 min, 80% A; t = 19 min, 100% A; t = 22 min, 100% A; t = 25 min, 20% A. The level of enantioenrichment of compounds was checked using a Chiralpak® IC (250 × 4.6 mm) column fitted to a HPLC apparatus (*HITACHI chromaster*). The runs were performed with a linear gradient of A (acetonitrile (Sigma-Aldrich HPLC-grade) and B (distilled water): t = 0-3 min, 70% A; t = 7 min, 90% A; t = 16 min, 90% A; t = 20 min, 100% A; t = 25 min, 100% A; t = 28 min, 70% A. A flow rate of 1 mL $min^{-1}$ was used and UV-absorption was recorded at 250 nm.

**Determination of *in vivo* activity against *Schistosoma mansoni.*** Studies were approved by the local veterinary agency (permission no. 2070). Female NMRI mice (n=19, obtained from Harlan Laboratories (Horst, the Netherlands)), were subcutaneously infected with ~ 100 cercarciae following standard procedures.[33] Groups of 5 infected NMRI mice characterized by a patent *S. mansoni* infection (49 days post-infection) were treated orally with the test drugs using single oral doses (400 mg/kg). Untreated mice (n=9) served as controls. Fourteen days post-treatment

animals were sacrificed by the $CO_2$ method and dissected. Worms were sexed and counted.[34] Worm burdens of treated mice were compared to control animals and reductions of worm burden calculated. To compare the medians of the responses between the treatment and control groups the Kruskal-Wallis test was utilized. A difference in median was considered to be significant at a significance level of 5%.

**Determination of *in vitro* activity against *Schistosoma mansoni.***

Schistosomes were cultured in RPMI 1640 culture medium (supplemented with 5% inactivated fetal calf serum (iFCS) and 100 U/mL penicillin as well as 100 µg/mL streptomycin (Invitrogen, Carlsbad, USA)) at 37 °C in an atmosphere of 5% $CO_2$. All compounds of interest were tested at a concentration range of 0.11 - 30 µg/ml (0.11, 0.33, 1.1, 3.3, 10 and 30 µg/ml), using DMSO stock solutions (conc. 10 mg/ml; final concentration of DMSO: 0.3 %) diluted in supplemented RPMI 1640 medium within 24 flat bottom well plates (BD Falcon, USA) with a final volume of 2 ml per well.[35] Three worms of both sexes, were placed into each well. Wells with the highest concentration of DMSO in medium served as controls. Praziquantel served as positive control. Phenotypes were monitored after 72 h using the motility scale described by Ramirez *et al.*[36] and an inverse microscope (Carl Zeiss, Germany, magnification 80x). Each experiment was performed three times. $IC_{50}$ values of active compounds and positive control were calculated using CompuSyn software (Version 3.0.1, 2007; ComboSyn, Inc) as described before.[35]

**Metabolic stability studies.** Human microsomes (Gibco) at a concentration of 20 mg/ml were slowly thawed on ice. 10 µl microsomes (0.46 mg/ml final concentration) were then incubated in a water bath with 382 µl of 100 mM phosphate buffer (pH 7.4), 4 µl of a 20 mM NADPH solution, 2.5 µl of the tested compound (stock solution: 5 mM in DMSO, final concentration 0.03 mM) and 2.5 µl of Diazepam (stock solution: 1 mM in DMSO:, final concentration 0.006 mM)

for 5 min at 37°C. Following this, the reaction was initiated by adding another 30 µl of a 20 mM NADPH solution (1.6 mM final concentration), the mixture was then incubated for required time at 37°C while shaking slowly (final DMSO concentration: 1.16 %). The reaction was then stopped by addition of 2 ml $CH_2Cl_2$. Sample was vortex and centrifuged for 5 min at 1000xg. The $CH_2Cl_2$ layer was taken up carefully and evaporated using a $N_2$-flow. The residue was dissolved in 100 µl of 8:5 ACN:$H_2O$ (containing 0.02% TFA + 0.05% HCOOH) and 40 µl was injected in LC-MS.

($\eta^6$-Praziquanamine)$Cr(CO)_3$ (**3**). A solution of praziquanamine (298 mg, 1.48 mmol) in 30 mL of $Bu_2O$/THF (8:1) was degassed by two freeze-thaw cycles. $Cr(CO)_6$ (488 mg, 2.2 mmol) was added to the solution and was degassed again by one more freeze-thaw cycle. The reaction mixture was heated at 140°C under a nitrogen atmosphere in dark for 30 h. The resulting yellow solution was then cooled to room temperature and solvent was removed, the residue was re-dissolved in $CH_2Cl_2$ and filtered through a celite pad and concentrated. The residue was subjected to silica column chromatography for two times (eluent: EtOAc:MeOH:$NEt_3$ 20:1:0 → 10:1:0.1→ 10:2:0.3) to remove the impurities and minor isomer Iso-**3**. Compound **3** was obtain as yellow solid (70 mg, 14%). Retention time = 7.9 min (RP-HPLC). $^1$H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.61-2.65 (m, 1H), 2.82-3.01 (m, 3H), 3.61-3.69 (m, 3H), 4.61-4.79 (m, 2H), 5.15-5.27 (m, 2H), 5.39 (m, 1H), 5.51 (m, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$): δ (ppm) 27.1, 38.9, 50.1, 50.4, 53.2, 88.2, 89.9, 90.1, 92.4, 108.1, 109.7, 157.1, 232.1. ESI-MS (pos. detection mode): *m*/*z* (%): 339.1 (100) $[M+H]^+$. IR bands(ν): 1940 and 1855 (Cr-*CO*) $cm^{-1}$. Anal. calcd. for $C_{15}H_{14}CrN_2O_4 \cdot MeOH$: C 52.54, H 4.55, N 7.91. Found: C 52.37, H 4.59, N 7.84.

ASSOCIATED CONTENT

**Supporting Information**. UV traces at 220 nm of the LC-MS analysis of **1**, **2** and PZQ after incubated with human liver microsome (Figures S1-S3). Tables showing the detection of the metabolites of **1** and **2** by LC-MS (Table S1-S2). Chiral HPLC traces of optically pure PZQ and ($\eta^6$-PZQ)Cr(CO)$_3$ (Figures S4-S9). This material is available free of charge via the Internet at http://pubs.acs.org

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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**Abbreviations.**

ACN: Acetonitrile; PZQ: Praziquantel; ($\eta^6$-PZQ)$Cr(CO)_3$: Chromium tricarbonyl derivative of Praziquantel; *cis*-4-PZQ-OH: *cis*-4-hydroxypraziquantel; *trans*-4-PZQ-OH: *trans*-4-hydroxypraziquantel; 1-OH: Monohydroxylated derivative of **1**; 2-OH: Monohydroxylated derivative of **2**; LC-MS: Liquid Chromatography-Mass Spectrometry; MW: Molecular Weight.

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**TOC Figure.**