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# Ferrocenyl Derivatives of the Anthelmintic Praziquantel: Design, Synthesis, and Biological Evaluation

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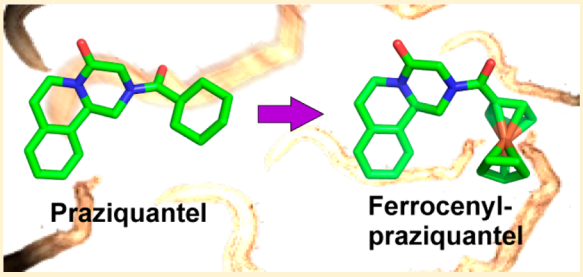
Supporting Information

**ABSTRACT:** The design, synthesis, and biological evaluation of 18 ferrocenyl derivatives (**4A**−**12A** and **4B**−**12B**) of the most well-known drug against schistosomiasis, namely praziquantel (PZQ), are reported. These compounds, which have been all isolated as racemates, were unambiguously characterized by $^{1}$H and $^{13}$C NMR spectroscopy, mass spectrometry, and elemental analysis as well as by X-ray crystallography for **4A**, **5A**, and **7A**. Cytotoxicity studies revealed that the complexes were moderately toxic toward a cervical cancer cell line (HeLa) and, importantly, significantly less active toward a noncancerous cell line (MRC-5). The *in vitro* anthelmintic activity of the 18 ferrocenyl PZQ derivatives was tested against adult *Schistosoma mansoni*, and values in the micromolar range (26−68 $\mu$M) were determined for the four most active compounds. It was also demonstrated using two compounds of the series as models (**8A** and **8B**) that the complexes were stable when incubated for 24 h at 37 °C in human plasma.




## INTRODUCTION

Schistosomiasis is a parasitic disease caused by trematodes of the genus *Schistosoma*. It is a major health problem worldwide, particularly in tropical regions where 280 000 deaths are reported annually.[1,2] In addition, more than 207 million people, mostly in Africa, are infected, and nearly 800 million are at risk of being infected.[3] Currently, praziquantel (PZQ, Figure 1) is widely used, as a racemic mixture, to control this infection in human as well as in animals. PZQ exhibits a broad spectrum anthelmintic activity against all five human *Schistosoma* species.

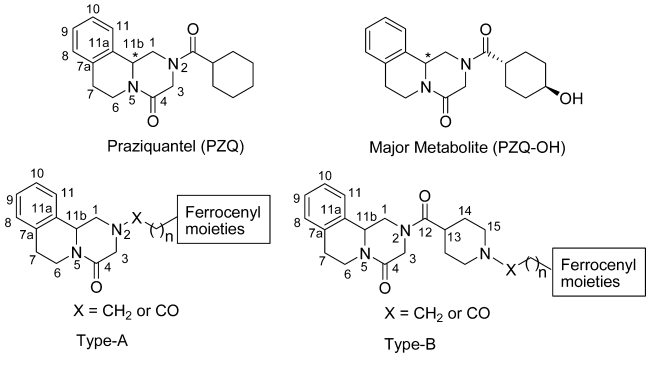



**Figure 1.** Structures including atom numbering of PZQ and of its major metabolite PZQ-OH as well as of the Fc-PZQ derivatives (type-A and -B) studied in this report.

Although no clear evidence exist to date, the voltage-gated $Ca^{2+}$ channels in the membrane are believed to be one of the possible targets for PZQ.[4,5] Despite its success, PZQ suffers from several drawbacks. For example, its metabolic stability is rather low. PZQ is indeed rapidly converted *in vivo* into the less active or inactive PZQ-OH (Figure 1) by hydroxylation of the cyclohexane ring.[6,7] Furthermore, PZQ is inactive against juvenile schistosomes; hence, it is necessary to retreat patients after a few weeks to remove those parasites that have since matured.[5] But potentially more worryingly, as millions of people are regularly treated with this drug, it is likely PZQ resistant parasites emerge in the near future.[4,5] Indeed, reduced susceptibility of *Schistosoma mansoni* to PZQ was already observed.[5,8]

In order to overcome these drawbacks, chemical modifications on the PZQ structure were undertaken. For example, Todd et al. reported several PZQ derivatives with a modification at the C10 position of the aromatic ring (see Figure 1 for the atom numbering in the PZQ structure).[9] They concluded that the aromatic part is not a suitable position for structural modification. Robert and Meunier et al. disclosed the synthesis and biological evaluation of trioxaquantels which are hybrid molecules made of the 1,2,4-trioxane unit of the antimalarial drug artemisinin and the pyrazinoisoquinoline

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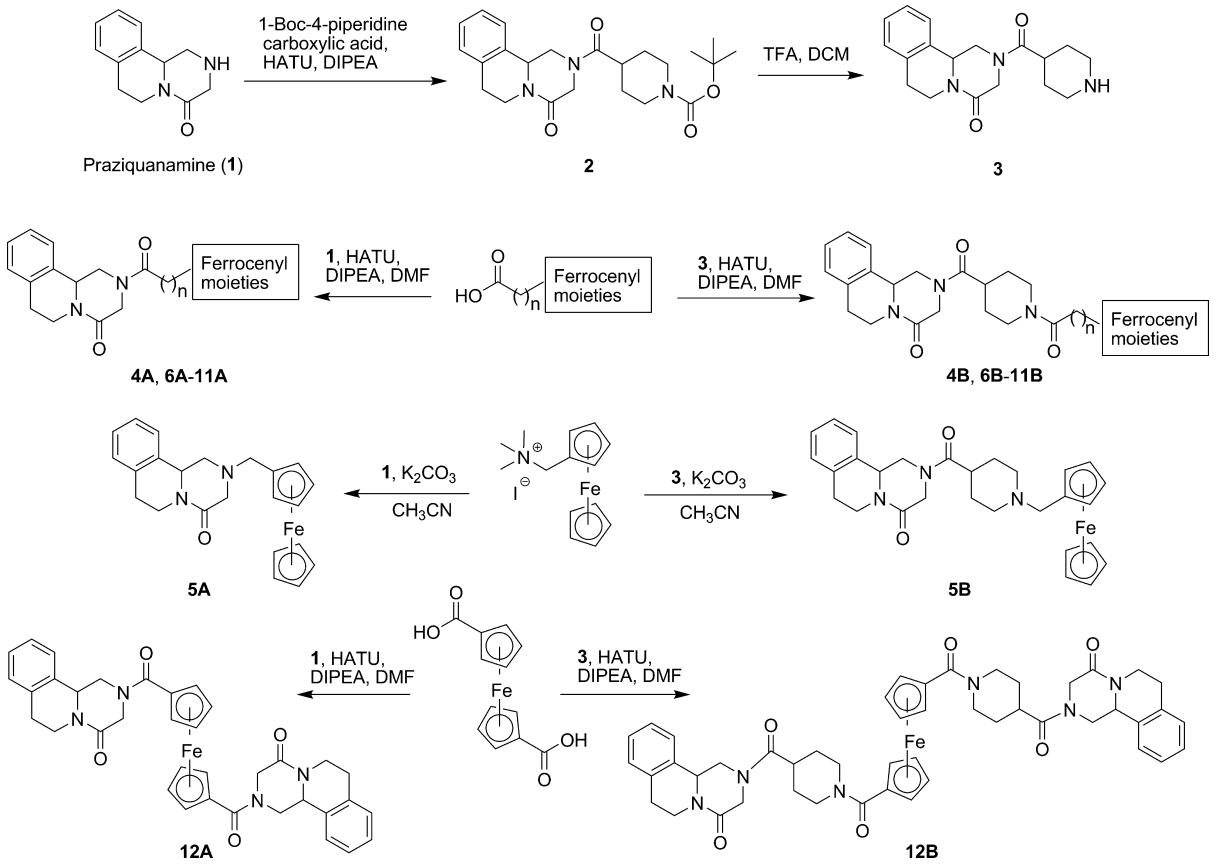
**Scheme 1. Synthesis of Fc-PZQ Derivatives**




moiety of PZQ.[10] Only one of their compounds was found to have a moderate antiparasitic activity. Recently, Domling et al. reported the synthesis of PZQ derivatives using a Ugi 4-component reaction,[11] and they observed that a few of their analogues were as active as the parent drug PZQ. Of high interest, Vennerstrom and co-workers recently prepared PZQ derivatives with activity against the juvenile stage of *S. mansoni*.[12] Nonetheless, to the best of our knowledge, no lead compound suitable for further preclinical testing was identified in these studies. This fact clearly emphasizes the need for a fundamentally different approach for the discovery of novel PZQ derivatives active against schistosomiasis.

With this in mind, we envisaged to derivatize PZQ with organometallic moieties as previously undertaken with anticancer, antibacterial, and antimalarial drugs.[13−26] This strategy was found to be very successful with ferroquine (FQ), a ferrocenyl analogue of the antimalarial drug chloroquine (CQ), as FQ is active on CQ-resistant *Plasmodium falciparum* strains. Furthermore, intensive chemical biology studies have recently allowed unveiling additional modes of action for FQ compared to the parent drug, which were attributed to the presence of the organometallic unit.[19,27] It is anticipated that these additional modes of action will help prolong the period of resistance development. Of note, Sánchez-Delgado and co-workers reported the preparation of ruthenium half-sandwich analogues of CQ which were found to be highly potent and to overcome the CQ resistance.[28] Organometallic compounds have also been investigated for their activity against other parasites such as the trypanosome *Trypanosoma cruzi* (*T. cruzi*) or *Echinococcus multilocularis* metacestodes.[29−31] For example, a ruthenium half-sandwich complex of the antifungal drug clotrimazole was found to be 58 times more potent than the parent drug in vitro against *T. cruzi*.[29]

Due to these promising results obtained with other parasitic drugs, we have embarked on a project to evaluate the potential of ferrocenyl derivatives of PZQ against *S. mansoni*. In analogy to what observed for FQ, we postulated that the derivatization of PZQ with a ferrocenyl moiety could potentially prevent future resistance as well as render the compound active against the juvenile stage of the parasite which is refractory to PZQ. In this article, we report our initial results on the synthesis and biological activity of the first organometallic derivatives of PZQ. As shown in Figure 1, our concept was to replace the cyclohexane ring of PZQ with a ferrocenyl unit as it has been reported that the replacement of the cyclohexane ring of PZQ by a benzene ring was not significantly altering the activity.[9,11,32] Moreover, we anticipated that the *in vivo* transformation of PZQ into PZQ-OH could be avoided due to the missing cyclohexane ring in our derivatives. In this study, two different structural classes (type-A and -B, Figure 1) of ferrocenyl-praziquantel (Fc-PZQ) derivatives were investigated. Ferrocenyl derivatives in type-A analogues are directly attached to the praziquanamine (**1**, Scheme 1) residue with different linkers. In contrast, type-B analogues are structurally closer to PZQ as the ferrocenyl moieties are linked to a piperidine unit replacing the cyclohexane ring attached to the praziquanamine via an amide bond (Figure 1).

## RESULTS AND DISCUSSION

**Synthesis.** The syntheses of the Fc-PZQ analogues are schematically presented in Scheme 1. For more details on the


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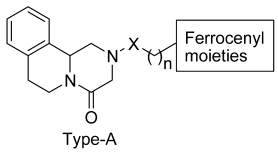
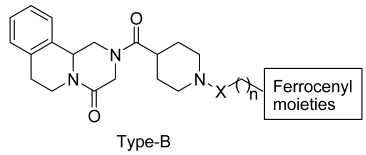
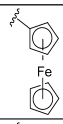
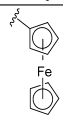
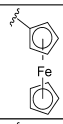
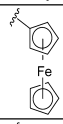
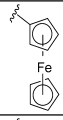
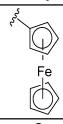
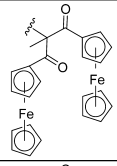
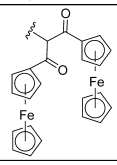
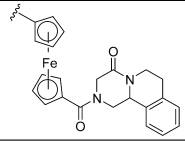
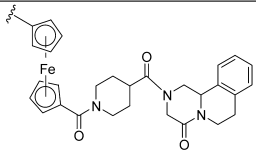
**Table 1. Structures and Yields of Fc-PZQ Derivatives**




| Fc-PZQ derivatives | | Ferrocenyl moieties | X | n | Yield | |
|---|---|---|---|---|---|---|
| Type-A | Type-B | | | | Type-A | Type-B |
| **4A** | **4B** |  | CO | 0 | 62% | 75% |
| **5A** | **5B** |  | $CH_2$ | 0 | 58% | 89% |
| **6A** | **6B** |  | CO | 1 | 36% | 25% |
| **7A** | **7B** |  | CO | 2 | 65% | 53% |
| **8A** | **8B** |  | CO | 3 | 73% | 65% |
| **9A** | **9B** |  | CO | 4 | 64% | 78% |
| **10A** | **10B** |  | CO | 2 | 78% | 55% |
| **11A** | **11B** |  | CO | 2 | 63% | 49% |
| **12A** | |  | CO | 0 | 44% | |
| | **12B** |  | CO | 0 | | 56% |

structure of the compounds, the readers are referred to Table 1. It should be also noted that all chiral compounds described in this study are racemates. Specifically, praziquanamine (**1**) was prepared following a previously reported literature procedure.[10] Compound **1** was then converted into **3** by treatment with 1-Boc-4-piperidinecarboxylic acid followed by the Boc group deprotection with TFA. All ferrocene-containing carboxylic acid derivatives[33−35] and trimethyl-(ferrocenylmethyl)ammonium iodide[36] are either commercially available or were prepared following standard literature procedures. The ferrocene-containing carboxylic acids were then attached to either **1** or **3** via a HATU mediated amide coupling reaction to provide **4A**, **6A**−**12A** and **4B**, **6B**−**12B**, respectively. Compounds **5A** and **5B** were prepared by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treatment of **1** and **3**, respectively, with trimethyl-(ferrocenylmethyl)ammonium iodide and $K_2CO_3$ in acetonitrile using a synthetic method similar to what employed by Spiccia et al.[37] All new compounds were unambiguously characterized using $^1H$ and $^{13}C$ NMR spectroscopy, ESI mass spectrometry, and elemental analysis (see Experimental Section and SI). The presence of rotamers in solution for all Fc-PZQ derivatives except for **4A**, **5A**, and **12A** was ascertained by analysis of their $^1H$ and $^{13}C$ NMR spectra. In ESI-mass spectrometry (positive detection mode), most of the compounds were identified as their $[M + Na]^+$ species or, in a few exceptions, as their $[M + H]^+$ or $[M + K]^+$ species. Of note, all ferrocenyl compounds were found to be soluble in aqueous solutions containing 0.5% of DMSO up to 100 $\mu$M concentration.

**X-ray Crystallography.** The molecular structures of **4A**, **5A**, and **7A** were confirmed by the determination of their respective X-ray single crystal structures. All the compounds crystallized as their racemates. ORTEP representations are shown in Figures 2, S1, and S2, respectively. Table S1 (in the SI) contains the relevant crystallographic data and parameters. For the three Fc-PZQ derivatives, the interatomic distances and angles are typical for such compounds.[38] For example, the average distances between the carbon atoms of the Cp rings of ferrocene and iron fluctuate between 2.026 and 2.059 Å for **4A**.

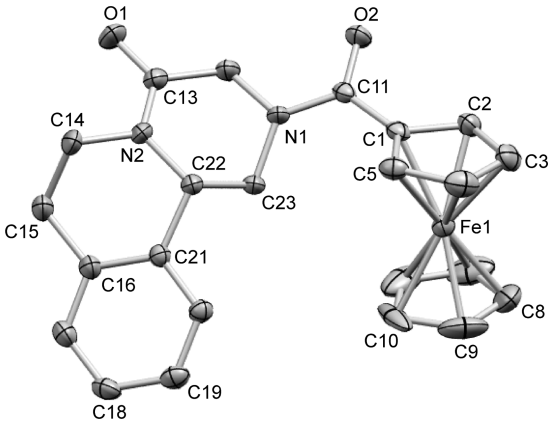


**Figure 2.** Molecular structure of **4A** (one enantiomer is shown), showing the numbering scheme and the displacement ellipsoids drawn at the 50% probability level. Hydrogen atoms are omitted for clarity.

**Biological Evaluation.** An ideal antiparasitic agent is supposed to kill selectively the parasites without, or to a significantly less extent, being harmful to the host. We therefore evaluated the toxic behavior of our Fc-PZQ derivatives toward two mammalian cell lines, namely a cervical cancer cell line (HeLa) and a noncancerous cell line (MRC-5). Cisplatin, a platinum based anticancer drug, was used as a reference compound. The resulting $IC_{50}$ values are summarized in Table 2. In general, the ferrocenyl compounds studied in this work were found to be moderately cytotoxic toward HeLa cells. Interestingly, a significant decrease in cytotoxicity was observed for all compounds except **11B** when their activity was analyzed on a nontransformed cell line. This shows an interesting selectivity toward cancer cells. Among all the compounds tested, **9A**, which has the longer $CH_2$-linker between the praziquanamine and the ferrocenyl moiety, inhibited HeLa cell viability with a half inhibitory concentration ($IC_{50}$) of 16.9 ± 1.0 $\mu$M, thus appearing to be the most cytotoxic compound of this study. The $IC_{50}$ value displayed by **9A** was comparable to that of cisplatin ($IC_{50}$ = 11.5 ± 2.9 $\mu$M). Interestingly, **9A** was much more selective toward the cancer cell line studied in this work compared to cisplatin. Similar results were obtained for **9B**. Shortening the length of the linker between the organic and the ferrocenyl moiety resulted in reduced anticancer activity (**9** > **8** > **7** > **6**, Table 2), a behavior that could be attributed to a decrease in lipophilicity. Such a trend was further confirmed with **12A**, which was more cytotoxic than **4A** despite the presence of a similar linker. However, the presence of two praziquanamine moieties attached to the ferrocenyl core in **12A** makes the compound certainly more lipophilic compared to **4A**.

We then tested the Fc-PZQ derivatives (**4A**/**B**−**12A**/**B**) against adult *S. mansoni* to assess their anthelmintic potential. PZQ was used as a reference compound, and the results obtained are listed in Table 2. Fourteen compounds did not show activity at the highest concentration tested (30 $\mu$g/mL). Four compounds (**5A**, **7**−**9A**), which displayed activity at 30 $\mu$g/mL, were further studied and $IC_{50}$ values of 25.6−68 $\mu$M determined. The highest activity was observed with **8A**, which displayed an $IC_{50}$ of 25.6 $\mu$M. All compounds were considerably less active than PZQ (0.1 $\mu$M).

**Table 2. Anthelmintic Activity against adult *S. mansoni* and Cytotoxicity against HeLa and MRC-5 Cells of Fc-PZQ Derivatives**

| | $IC_{50}$ values against *Schistosoma mansoni* ($\mu$M) | | $IC_{50}$ values for two different cell lines ($\mu$M) | | | |
|---|---|---|---|---|---|---|
| | | | HeLa | | MRC-5 | |
| compd | A | B | A | B | A | B |
| **4** | >72[b] | >70[b] | >100 | >100 | >100 | >100 |
| **5** | 68 | >59[b] | 57.9 ± 6.4 | 81.0 ± 0.2 | >100 | 93.9 ± 2.1 |
| **6** | >35[b] | >56[b] | 81.1 ± 2.2 | 78.0 ± 0.9 | >100 | >100 |
| **7** | 51.6 | >54[b] | 50.6 ± 4.8 | ND[a] | >100 | ND |
| **8** | 25.6 | >53[b] | 24.2 ± 3.5 | 28.9 ± 0.8 | 62.3 ± 1.3 | 70.6 ± 2.8 |
| **9** | 48.6 | >52[b] | 16.9 ± 1.0 | 18.9 ± 1.5 | 37.0 ± 1.9 | 36.1 ± 2.1 |
| **10** | >42[b] | >37[b] | >100 | ND | >100 | ND |
| **11** | >43[b] | >37[b] | 97.0 ± 4.2 | >100 | >100 | 42.4 ± 3.2 |
| **12** | >47[b] | >35[b] | 26.9 ± 1.6 | ND | 64.7 ± 5.4 | ND |
| **PZQ** | 0.1 | | >100 | | ND | |
| cisplatin | ND | | 11.5 ± 2.9 | | 7.9 ± 1.2 | |

[a]ND = not determined. [b]Compound did not show antischistosomal activity at the highest concentration tested (30 $\mu$g/mL).


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**Stability in Human Plasma.** In order to obtain preliminary insights into the behavior of our Fc-PZQ derivatives under physiological conditions, the stability of the most active compound **8A** and of its B-type analogue (**8B**) in human plasma was assessed. Specifically, **8A** and **8B** were incubated in human plasma for 24 h at 37 °C, and their stability was checked using an LC-MS technique (see Experimental Section for details).[39] The results were compared with that obtained for the parent drug PZQ in the same assay. As shown in Figures S3−S5 in SI, similar to PZQ, no significant changes were observed either in the UV traces or in the ratio of diazepam (internal standard) and **8A** or **8B** (see table S2 in SI) even after 24 h, suggesting that our Fc-PZQ derivatives are stable under physiological conditions.

## CONCLUSION

There is undoubtedly an urgent need for the discovery of novel drugs against schistosomiasis. Currently, this parasitic disease which affects millions of people worldwide is successfully treated with a single drug, namely PZQ. However, as the at risk population is regularly being treated with PZQ, there are indications that its widespread use could lead to emergence of PZQ resistant parasites in the near future. In this work, we envisaged an alternative method to enlarge the chemical space of potential drug candidates. Hence, we have successfully derivatized PZQ with different ferrocenyl moieties to give 18 new Fc-PZQ derivatives that were unambiguously characterized including by X-ray crystallography for three compounds. It was demonstrated using two compounds of the series as models (**8A** and **8B**) that the complexes were stable when incubated for 24 h at 37 °C in human plasma. Cytotoxic studies on cancerous (HeLa) and noncancerous (MRC-5) cell lines showed that the Fc-PZQs were significantly less active toward the healthy cell line than the cancer cell line studied in this work, except for one compound. An increase in the cytotoxicity against HeLa cells was correlated with an increase in the length of the linker between the organic and the ferrocenyl moieties, and this for both type-A and -B compounds. The Fc-PZQ compounds were found to have an anthelmintic activity in the micromolar range when tested against *S. mansoni in vitro*. Although the activity of the best compound is not sufficient to proceed into further *in vivo* testing, this study opens new avenues in the search for novel drug candidates against schistosomiasis. Further work with different types of organometallic compounds has been initiated in our laboratories, and our results will be published in due course.

## 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. Praziquanamine (**1**), 3-ferrocenylpropanoic acid, 4-ferrocenylbutyric acid, 5-ferrocenylpentanoic acid, 4,4-diferrocenoylbutanoic acid, 4,4-diferrocenoylpentanoic acid, and trimethyl(ferrocenylmethyl)ammonium iodide were prepared following standard literature procedures.[10,33−36] All new compounds whose biological activity was evaluated in this work have a purity ≥95% as confirmed by elemental microanalyses.

**Instrumentation and Methods.** $^{1}$H 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, $\delta$, 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). The $^{1}$H and $^{13}$C signals were assigned with the help of 2D NMR techniques and by comparison with previously reported NMR spectra of other PZQ derivatives.[11,40] $^{1}$H and $^{13}$C signal assignments follow the atom numbering presented in Figure 1. ESI mass spectra were recorded on a Bruker Esquire 6000 spectrometer. Elemental microanalyses were performed on a LecoCHNS-932 elemental analyzer.

**X-ray Crystallography.** Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo K$\alpha$ radiation ($\lambda$ = 0.7107 Å) that was graphite-monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), placed on a nylon loop that is mounted in a CrystalCap Magnetic from Hampton Research and immediately transferred to the diffractometer. The program suite CrysAlis$^{Pro}$ was used for data collection, multiscan absorption correction, and data reduction.[41] The structures were solved with direct methods using SIR97[42] and were refined by full-matrix least-squares methods on $F^2$ with SHELXL-97.[43] The structure of **7A** contains 2 molecules in the asymmetric unit; it is racemically twinned, and one of the ferrocene rings had to be refined with the help of DELU restraints. The structures were checked for higher symmetry with help of the program Platon.[44] CCDC 892689−892691 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

**Stability[39] of 8A, 8B, and PZQ in Human Plasma.** The human plasma was provided by the Blutspendezentrum, Zurich, Switzerland. Stock solutions in DMSO of 1.6 mM of **8A**, **8B** and PZQ and of 800 $\mu$M of diazepam (internal standard, obtained from Sigma-Aldrich) were first prepared. A 12.5 $\mu$L portion of the solution containing the compound to be studied and 12.5 $\mu$L of the diazepam solution were added to 975 $\mu$L of plasma. The resulting solution was shaken gently (ca. 300 rpm) at 37 °C for 24 h. Afterward, 6 mL of a 2/1 (v/v) methyl-tert-butyl ether/$CH_2Cl_2$ mixture was added to the plasma solution, and the mixture was shaken for 15 min at room temperature and finally centrifuged at 2000 × *g* at 4 °C for 10 min. Finally, the organic layer was separated from the water phase, and the solvent was evaporated with the help of a nitrogen flow. The resulting residue was dissolved in 130 $\mu$L of a 8/5 (v/v) $CH_3CN/H_2O$ mixture containing 0.02% TFA and 0.05% HCOOH. A 40 $\mu$L portion of this mixture was then injected into the HPLC (Acquity Ultra Performance LC, Waters) that was connected to a mass spectrometer (Bruker Esquire 6000) operated in ESI mode. The reverse phase column used was a Nucleosil 100-5 C18 column (250 mm × 3 mm) 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, 40% A; *t* = 6 min, 50% A; *t* = 16 min, 90% A; *t* = 20 min, 100% A; *t* = 23 min, 100% A; *t* = 25 min, 40% A.

**Cell Culture.** Human cervical carcinoma cells (HeLa) cells were cultured in DMEM (Gibco) supplemented with 5% fetal calf serum (FCS, Gibco), 100 U/mL penicillin, 100 $\mu$g/mL streptomycin at 37 °C and 5% $CO_2$. The normal human fetal lung fibroblast MRC-5 cell line was maintained in F-10 medium (Gibco) supplemented with 10% FCS (Gibco), penicillin (100 U/mL), and streptomycin (100 $\mu$g/mL).

**Cytotoxicity Studies.** Cytotoxicity studies were performed on two different cell lines, namely HeLa, and MRC-5, by a fluorometric cell viability assay using Resazurin (Promocell GmbH). Briefly, one day before treatment, cells were seeded in triplicates in 96-well plates at a density of 4 × 10$^3$ cells/well for HeLa and 7 × 10$^3$ for MRC-5 in 100 $\mu$L growth medium. Upon treating cells with increasing concentrations of Fc-PZQ derivatives for 48 h, the medium was removed, and 100 $\mu$L complete medium containing Resazurin (0.2 mg/mL final concentration) was added. After 4 h of incubation at 37 °C, fluorescence of the highly red fluorescent product Resorufin was quantified at 590 nm emission with 540 nm excitation wavelength in a SpectraMax M5 microplate reader.

**Determination of Antiparasitic Activity against Adult *S. mansoni*.** Studies were approved by the local veterinary agency (permit 2070). Female NMRI mice ($n$ = 5, obtained from Harlan Laboratories (Horst, The Netherlands)) were subcutaneously infected with ~100 cercarciae following standard procedures.[45] Seven weeks postinfection adult *S. mansoni* were removed from the hepatic portal system and mesenteric veins and cultured in RPMI 1640 culture medium (supplemented with 5% inactivated fetal calf serum (iFCS) and 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA)) at 37 °C in an atmosphere of 5% $CO_2$ until usage.

For the determination of activity against adult flukes all drugs were initially tested at a concentration of 30 μg/mL, using DMSO stock solutions (conc 10 mg/mL) diluted in supplemented RPMI 1640 medium within 24 flat bottom well plates (BD Falcon, USA) with a final volume of 2 mL per well. Three worms of both sexes were placed into each well. Wells with the highest concentration of DMSO in medium served as controls. PZQ served as positive control, and concentrations of 0.11, 0.33, 1.1, and 3.3 μg/mL were used to determine the $IC_{50}$ value. Phenotypes were monitored after 72 h using the motility scale described by Ramirez et al.[46] and an inverse microscope (Carl Zeiss, Germany, magnification 80×). Compounds presenting antischistosomal activity were characterized further. Therefore, three additional concentrations of selected test drugs were tested (1.1, 3.3, and 10 μg/mL) as described above. Each experiment was performed at least three times. $IC_{50}$ values of active compounds were calculated with CompuSyn software (Version 3.0.1, 2007; ComboSyn, Inc.) as described before.[47]

## SYNTHESIS

**General Procedure for Amide Coupling (GP-1).** To a stirred solution of the carboxylic acid in DMF were added HATU and DIPEA successively, and the mixture was allowed to stir for 30 min under a nitrogen atmosphere. The appropriate amine dissolved in DMF is then added and the mixture stirred at room temperature. The reaction mixture was then diluted with EtOAc and washed with 0.5 M HCl, $H_2O$, and brine. The organic phase was then dried over anhydrous $Na_2SO_4$, filtered, and concentrated. Compounds were purified either by washing with diethyl ether or by flash column chromatography on silica gel.

**Compound 4A.** Compound **4A** was synthesized following GP-1: ferrocene carboxylic acid (113 mg, 0.49 mmol), praziquanamine (100 mg, 0.49 mmol), HATU (279 mg, 0.73 mmol), DIPEA (128 mg, 0.99 mmol), DMF (3 mL), and reaction time (20 h). Flash column chromatography (silica gel, hexane/EtOAc 2/1→0/1) gave **4A** as an orange solid (yield: 127 mg, 62%). $R_f$ = 0.68 (silica gel, EtOAc). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 2.65−2.71 (m, 1H, H7), 2.77−2.82 (m, 1H, H6), 2.88−2.93 (m, 2H, H1 and H7), 4.01 (d, 1H, H3), 4.16 (s, 5H, $C_5H_5$), 4.30−4.32 (m, 2H, $C_5H_4$), 4.55 (s, br, 1H, $C_5H_4$), 4.59 (s, br, 1H, $C_5H_4$), 4.73−4.76 (m, 1H, H6), 4.84−4.86 (m, 1H, H11b), 4.93 (d, 1H, H3), 5.02 (m, 1H, H1), 7.12−7.28 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$): $\delta$ (ppm) 28.7 (C7), 38.8 (C6), 47.2 (C1), 50.6 (C3), 54.7 (C11), 69.3 ($C_5H_4$), 69.8 ($C_5H_4$), 70.0 ($C_5H_5$), 70.3 ($C_5H_4$), 71.7 ($C_5H_4$), 76.2 ($C_5H_4$), 125.4 ($C_6H_4$), 126.9 ($C_6H_4$), 127.5 ($C_6H_4$), 129.4 ($C_6H_4$), 132.7 ($C_6H_4$), 134.9 ($C_6H_4$), 164.8 (C4), 170.2 (N-CO−$C_5H_4$). ESI-MS (pos. detection mode) $m/z$ (%): 437.1 (80) $[M + Na]^+$, 851.1 (100) $[2M + Na]^+$. Anal. Calcd for $C_{23}H_{22}FeN_2O_2$: C 66.68, H 5.35, N 6.76. Found: C 66.92, H 5.17, N 6.66.

**Compound 5A.** A mixture of trimethyl(ferrocenylmethyl)-ammonium iodide (285 mg, 0.74 mmol), praziquanamine (100 mg, 0.49 mmol), and $K_2CO_3$ (126 mg, 0.91 mmol) in $CH_3CN$ (20 mL) was refluxed under $N_2$ atmosphere. After 16 h, the reaction mixture was cooled to room temperature, and $K_2CO_3$ was removed by filtration. The solvent was removed, and the resulting residue was subjected to flash column chromatography (silica gel, EtOAc/MeOH 15/1→10/1) to give **5A** as a yellow solid (yield: 115 mg, 58%). $R_f$ = 0.40 (silica gel, EtOAc). $^1$H NMR (401 MHz, $CDCl_3$): $\delta$ (ppm) 2.27−2.33 (m, 1H, H1), 2.72 (m, 1H, H7), 2.82−2.96 (m, 3H, H7, H6, H3), 3.51−3.55 (m, 4H, $C_5H_4$−$CH_2$, H1, H3), 4.16−4.22 (m, 9H, $C_5H_5$ and $C_5H_4$), 4.77−4.83 (m, 2H, H6, H11b), 7.10−7.29 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (100.6 MHz, $CDCl_3$): $\delta$ (ppm) 28.7 (C7), 38.6 (C6), 55.4 (C11b), 55.5 (C1), 56.4 (C3), 57 ($C_5H_4$-$CH_2$), 68.5 ($C_5H_4$), 68.6 ($C_5H_5$), 70.0 ($C_5H_4$), 70.3 ($C_5H_4$), 81.1 ($C_5H_4$), 124.6 ($C_6H_4$), 126.5 ($C_6H_4$), 126.9 ($C_6H_4$), 129.2 ($C_6H_4$), 134.4 ($C_6H_4$), 134.9 ($C_6H_4$), 166.4 (C4). ESI-MS (pos. detection mode) $m/z$ (%): 423.1 (70) $[M + Na]^+$, 439.0 (40) $[M + K]^+$, 823.2 (100) $[2M + Na]^+$. Anal. Calcd for $C_{23}H_{24}FeN_2O$: C 69.01, H 6.04, N 7.00. Found: C 69.21, H 6.11, N 6.95.

**Compound 6A.** Compound **6A** was synthesized following GP-1: ferrocenyl acetic acid (200 mg, 0.82 mmol), praziquanamine (234 mg, 1.16 mmol), HATU (440 mg, 1.16 mmol), DIPEA (199 mg, 1.54 mmol), 4 mL of DMF, and 7 h reaction time. Flash column chromatography (filter column on alumina, hexane/EtOAc 4/1→0/1) gave a brown solid. The solid was dissolved in 1:1 hexane/EtOAc mixture and kept at −20 °C for one week. The resulting precipitate was collected by filtration and washed with $Et_2O$ to give **6A** (yield: 128 mg, 36%). Note that the compound was found to be unstable on silica and slowly decomposes on alumina. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ (ppm) 2.74−3.10 (m, 4H, H1, H6, 2 × H7), 3.5 (s, 2H, $C_5H_4$−$CH_2$−CO), 3.61−3.72 (min) and 4.08−4.50 (maj) (rotamers, m, 11H, H3, $C_5H_5$ and $C_5H_4$), 4.61 (min) and 4.82−4.85 (maj) (rotamers, m, 2H, H6, H11b), 5.09 (m, 1H, H1), 7.10−7.29 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (100.6 MHz, $CDCl_3$): $\delta$ (ppm) 28.7 (maj) and 28.8 (min) (rotamers, C7), 35.1 (maj) and 35.5 (min) (rotamers, $C_5H_4$−$CH_2$−CO−N), 38.6 (min) and 39.1 (maj) (rotamers, C6), 45.2 (maj) and 46.2 (min) (rotamers, C1), 49.5 (maj) and 49.9 (min) (rotamers, C3), 54.8 (maj) and 55.3 (min) (rotamers, C11b), 67.8 (min) and 68.0 (maj) (rotamers, $C_5H_4$), 68.1 (maj) and 68.3 (min) (rotamers, $C_5H_4$), 68.8 (min) and 68.9 (maj) (rotamers, $C_5H_5$), 69.2 ($C_5H_4$), 80.7 (maj) and 81.3 (min) (rotamers, $C_5H_4$), 125.2 (min) and 125.5 (maj) (rotamers, $C_6H_4$), 126.9 (min) and 127.0 (maj) (rotamers, $C_6H_4$), 127.5 (maj) and 127.6 (min) (rotamers, $C_6H_4$), 129.3 (maj) and 129.6 (min) (rotamers, $C_6H_4$), 132.2 (min) and 132.6 (maj) (rotamers, $C_6H_4$), 134.7 (maj) and 135.4 (min) (rotamers, $C_6H_4$), 164.1 (maj) and 165.1 (min) (rotamers, C4), 169.2 (min) and 169.6 (maj) (rotamers, $C_5H_4$−$CH_2$−CO−N). ESI-MS (pos. detection mode) $m/z$ (%): 451.1 (100) $[M + Na]^+$. Anal. Calcd for $C_{24}H_{24}FeN_2O_2$: C 67.30, H 5.65, N 6.54. Found: C 67.02, H 5.49, N 6.61.

**Compound 7A.** Compound **7A** was synthesized following GP-1: 3-ferrocenylpropanoic acid (126 mg, 0.49 mmol), praziquanamine (99 mg, 0.49 mmol), HATU (279 mg, 0.74 mmol), DIPEA (126 mg, 0.98 mmol), 4 mL of DMF, and 7 h reaction time. Flash column chromatography (silica gel, hexane/EtOAc 1/2→0/1) gave **7A** as an orange sticky solid (yield: 141 mg, 65%). $R_f$ = 0.19 (silica gel, hexane/EtOAc 1:3). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 2.49−2.63 (m, 2H, $C_5H_4$−$CH_2$−$CH_2$−CO), 2.70−2.83 (m, 4H, $C_5H_4$−$CH_2$−$CH_2$−CO, H7, H1), 2.87−2.98 (m, 2H, H6, H7), 3.10−3.15 (min) and 3.93−3.97 (maj) (rotamers, m, 1H, H3), 4.09−4.30 (m, 10H, H3, $C_5H_4$ and $C_5H_5$), 4.69 (min) and 4.75−4.84 (maj) (rotamers, m, 2H, H6, H11b), 3.90 (min) and 5.12 (maj) (rotamers, m, 1H, H1), 7.05−7.21 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$): $\delta$ (ppm) 25.9 (maj) and 26.3 (min) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−CO), 29.2 (maj) and 29.3 (min) (rotamers, C7), 35.4 (maj) and 35.7 (min) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−CO), 39.2 (min) and 39.6 (maj) (rotamers, C6), 45.6 (maj) and 46.7 (min) (rotamers, C1), 49.6 (maj) and 50.3 (min) (rotamers, C3), 55.4 (maj) and 55.8 (min) (rotamers, C11b), 68.2 ($C_5H_4$), 68.4 ($C_5H_4$), 68.7 (min) and 68.9 (maj) (rotamers, $C_5H_4$), 69.1 ($C_5H_4$), 69.4 (maj) and 69.9 (min) (rotamers, $C_5H_5$), 88.1 ($C_5H_4$), 125.9 (min) and 126.1 (maj) (rotamers, $C_6H_4$), 127.4 (min) and 127.5 (maj) (rotamers, $C_6H_4$), 128.1 (maj) and 128.2 (min) (rotamers, $C_6H_4$), 129.8 (maj) and 130.1 (min) (rotamers, $C_6H_4$), 132.6 (min) and 133.2 (maj) (rotamers, $C_6H_4$), 135.3 (maj) and 135.9 (min) (rotamers, $C_6H_4$), 164.6 (maj) and 165.9 (min) (rotamers, C4), 171.3 (min) and 171.7 (maj) (rotamers, $CH_2$−$CH_2$−CO−N). ESI-MS (pos. detection mode) $m/z$ (%): 442.1 (70) $[M]^+$, 465.1 (100) $[M + Na]^+$, 481.1 (80) $[M + K]^+$. Anal. Calcd for $C_{25}H_{26}FeN_2O_2$: C 67.88, H 5.92, N 6.33. Found: C 68.09, H 5.78, N 6.19.

**Compound 8A.** Compound **8A** was synthesized following GP-1: 4-ferrocenylbutyric acid (200 mg, 0.73 mmol), praziquanamine (148

mg, 0.73 mmol), HATU (418 mg, 1.1 mmol), DIPEA (188 mg, 1.46 mmol), 5 mL DMF, and 7 h reaction time. Flash column chromatography (silica gel, hexane/EtOAc 1/2→1/3) gave **8A** as an orange sticky solid (yield: 245 mg, 73%). $R_f$ = 0.23 (silica gel, hexane/EtOAc 1/2). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 1.74−1.81 (m, 2H, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$), 2.19−2.37 (m, 4H, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$ and $C_5H_4$−$CH_2$−$CH_2$−$CH_2$), 2.67−2.91 (m, 4H, H1, H6, 2 × H7), 3.91−4.02 (m, 10H, H3, $C_5H_4$ and $C_5H_4$), 4.08 (min) and 4.22 (maj) (rotamers, d, 1H, H3), 4.71 (m, 2H, H6, H11b), 3.77 (min) and 5.05 (maj) (rotamers, m, 1H, H1), 7.07−7.24 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$): $\delta$ (ppm) 26.7 (maj) and 27.1 (min) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$), 29.3 (maj) and 29.4 (min) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$), 29.7 (C7), 33.1 (min) and 33.3 (maj) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$), 39.2 (min) and 39.7 (maj) (rotamers, C6), 45.6 (maj) and 46.7 (min) (rotamers, C1), 49.6 (maj) and 50.2 (min) (rotamers, C3), 55.5 (maj) and 55.6 (min) (rotamers, C11b), 67.9 (maj) and 68 (min) (rotamers, $C_5H_4$), 68.7 ($C_5H_4$), 68.1 ($C_5H_4$), 68.2 ($C_5H_4$), 68.7 (maj) and 68.9 (min) (rotamers, $C_5H_5$), 88.7 ($C_5H_4$), 125.9 (min) and 126.1 (maj) (rotamers, $C_6H_4$), 127.5 (min) and 127.6 (maj) (rotamers, $C_6H_4$), 128.1 (maj) and 128.2 (min) (rotamers, $C_6H_4$), 129.9 (maj) and 130.2 (min) (rotamers, $C_6H_4$), 132.6 (min) and 133.3 (maj) (rotamers, $C_6H_4$), 135.3 (maj) and 136 (min) (rotamers, $C_6H_4$), 164.8 (maj) and 165.9 (min) (rotamers, C4), 171.6 (min) and 172 (maj) (rotamers, $CH_2$−$CH_2$−CO−N). ESI-MS (pos. detection mode) $m/z$ (%): 479.1 (100) [M + Na]$^+$. Anal. Calcd for $C_{26}H_{28}FeN_2O_2$: C 68.43, H 6.18, N 6.14. Found: C 68.81, H 6.21, N 6.03.

**Compound 9A.** Compound **9A** was synthesized following GP-1: 5-ferrocenylpentanoic acid (200 mg, 0.69 mmol), praziquanamine (141 mg, 0.69 mmol), HATU (398 mg, 1.05 mmol), DIPEA (180 mg, 1.4 mmol), 5 mL DMF and 7 h reaction time. Flash column chromatography (silica gel, hexane/EtOAc 1/1→0/1) gave **9A** as an orange sticky solid (yield: 210 mg, 64%). $R_f$ = 0.7 (silica gel, EtOAc). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 1.48−1.52 (m, 2H, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 1.61−1.68 (m, 2H, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 2.24−2.34 (m, 4H, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$ and $C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 2.70−2.93 (m, 4H, H1, H6, 2 × H7), 3.97−4.07 (m, 10H, H3, $C_5H_4$ and $C_5H_5$), 4.18 (min) and 4.28 (maj) (rotamers, d, 1H, H3), 4.69−4.79 (m, 2H, H6, H11b), 3.91 (min) and 5.07 (maj) (rotamers, m, 1H, H1), 7.07−7.24 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$): $\delta$ (ppm) 25.4 ($C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 29.3 (maj) and 29.4 (min) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 30.0 (maj) and 30.1 (min) (rotamers, C7), 31.4 (maj) and 31.5 (min) (rotamers, $C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 33.7 ($C_5H_4$−$CH_2$−$CH_2$−$CH_2$−$CH_2$), 39.2 (min) and 39.7 (maj) (rotamers, C6), 45.6 (maj) and 46.7 (min) (rotamers, C1), 49.5 (maj) and 50.1 (min) (rotamers, C3), 55.5 (maj) and 55.9 (min) (rotamers, C11b), 67.8 (maj) and 67.9 (min) (rotamers, $C_5H_4$), 68.7 (maj) and 68.8 (min) (rotamers, $C_5H_4$), 69.1 (maj) and 69.2 (min) (rotamers, $C_5H_5$), 89.4 (maj) and 89.5 (min) (rotamers, $C_5H_4$), 125.9 (min) and 126.1 (maj) (rotamers, $C_6H_4$), 127.5 (min) and 127.6 (maj) (rotamers, $C_6H_4$), 128.1 (maj) and 128.3 (min) (rotamers, $C_6H_4$), 129.9 (maj) and 130.2 (min) (rotamers, $C_6H_4$), 132.6 (min) and 133.3 (maj) (rotamers, $C_6H_4$), 135.3 (maj) and 136 (min) (rotamers, $C_6H_4$), 164.8 (maj) and 166.1 (min) (rotamers, C4), 171.7 (min) and 172.2 (maj) (rotamers, $CH_2$−$CH_2$−CO−N). ESI-MS (pos. detection mode) $m/z$ (%): 493.1 (100) [M + Na]$^+$. Anal. Calcd for $C_{27}H_{30}FeN_2O_2$: C 68.94, H 6.43, N 5.96. Found: C 68.81, H 6.40, N 5.91.

**Compound 10A.** Compound **10A** was synthesized following GP-1: 4,4-diferrocenoylpentanoic acid (85 mg, 0.16 mmol), praziquanamine (33 mg, 0.16 mmol), HATU (92 mg, 0.24 mmol), DIPEA (42 mg, 0.32 mmol), 4 mL of DMF, and 7 h reaction time. Flash column chromatography (silica gel, hexane/EtOAc 1/2→1/3) gave **10A** as an orange solid (yield: 98 mg, 78%). $R_f$ = 0.33 (silica gel, EtOAc/hexane 3/1). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 1.59 (maj) and 1.63 (min) (rotamers, s, 3H, $CH_3$), 2.23−2.42 (m, 4H, N−CO−$CH_2$−$CH_2$−C and N−CO−$CH_2$-$CH_2$−C), 2.65−2.89 (m, 4H, H1, H6, 2 × H7), 3.10 (min) and 3.91 (maj) (rotamers, d, 1H, H3), 3.97 (min) and 3.99 (maj) (rotamers, s, 10H, $C_5H_5$), 4.23−4.32 (m, 5H, H3 and $C_5H_4$), 4.56 (m, 2H, $C_5H_4$), 4.66−4.75 (m, 4H, $C_5H_4$, H6 and H11b), 3.79 (min) and 5.03 (maj) (rotamers, m, 1H, H1), 7.06−7.23 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$): $\delta$ (ppm) 22.2 (maj) and 22.3 (min) (rotamers, $CH_3$), 29.2 (H7), 29.3 (maj) and 29.4 (min) (rotamers, N−CO−$CH_2$−$CH_2$−C), 33.3 (maj) and 33.8 (min) (rotamers, N−CO−$CH_2$−$CH_2$−C), 39.3 (min) and 39.6 (maj) (rotamers, C6), 45.7 (maj) and 46.8 (min) (rotamers, C1), 49.5 (maj) and 50.1 (min) (rotamers, C3), 55.5 (maj) and 56.1 (min) (rotamers, C11b), 64.7 (maj) and 64.9 (min) (rotamers, $C(CH_3)$), 70.7 (maj) and 70.8 (min) (rotamers, $C_5H_5$), 71.4, 71.5 (maj) and 71.6 (min) (rotamers, $C_5H_4$), 72.2 ($C_5H_4$), 72.3 ($C_5H_4$), 72.4 ($C_5H_4$), 72.5 (maj) and 72.6 (min) (rotamers, $C_5H_4$), 79.1 ($C_5H_4$), 125.3 (maj) and 125.4 (min) (rotamers, $C_6H_4$), 127.5 ($C_6H_4$), 128.1 (maj) and 128.3 (min) (rotamers, $C_6H_4$), 129.8 (maj) and 129.9 (min) (rotamers, $C_6H_4$), 132.6 (min) and 133.3 (maj) (rotamers, $C_6H_4$), 135.3 (maj) and 136 (min) (rotamers, $C_6H_4$), 164.6 (maj) and 165.8 (min) (rotamers, C4), 171.5 (min) and 171.9 (maj) (rotamers, N−CO−$CH_2$−$CH_2$−C), 200.5 (C−CO−$C_5H_4$). ESI-MS (pos. detection mode) $m/z$ (%): 733.2 (100) [M + Na]$^+$. Anal. Calcd for $C_{39}H_{38}Fe_2N_2O_4$: C 65.94, H 5.39, N 3.94. Found: C 66.08, H 5.31, N 3.89.

**Compound 11A.** Compound **11A** was synthesized following GP-1: 4,4-diferrocenoylbutanoic acid (150 mg, 0.29 mmol), praziquanamine (59 mg, 0.29 mmol), HATU (167 mg, 0.44 mmol), DIPEA (76 mg, 0.59 mmol), 4 mL of DMF, and 7 h reaction time. Flash column chromatography (silica gel, hexane/EtOAc 1/2→1/4) gave **11A** as an orange solid (yield: 128 mg, 63%). $R_f$ = 0.65 (silica gel, EtOAc). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 2.33−2.52 (m, 4H, N−CO−$CH_2$−$CH_2$ and N−CO−$CH_2$-$CH_2$), 2.64−2.87 (m, 4H, H1, H6, 2 × H7), 3.18 (min) and 3.91 (maj) (rotamers, d, 1H, H3), 4.02−4.05 (m, 10H, $C_5H_5$), 4.24 (m, 1H, H3), 4.45 (m, 4H, $C_5H_4$), 4.50 (m, 1H, $CH(CO)_2$), 4.65−4.73 (m, 2H, H6, H11b), 4.84−4.87 (m, 4H, $C_5H_4$), 3.80 (min) and 5.08 (maj) (rotamers, m, 1H, H1), 7.06−7.23 (m, 4H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$, mixture of tautomers): $\delta$ (ppm) 25.5 (maj) and 26.4 (min) (tautomers, N−CO−$CH_2$−$CH_2$), 29.2 (maj) and 29.3 (min) (tautomers, C7), 31.3 (N−CO−$CH_2$−$CH_2$), 39.3 (min) and 39.6 (maj) (tautomers, C6), 45.7 (maj) and 46.8 (min) (tautomers, C1), 49.3 (maj) and 50.1 (min) (tautomers, C3), 55.4 (maj) and 56.1 (min) (tautomers, C11b), 61.3 (min) and 61.5 (maj) (tautomers, $CH(CO)_2$), 70.5 (min) and 70.6 (maj) (tautomers, $C_5H_4$), 70.7 (maj) and 70.8, 70.9 (min) (tautomers, $C_5H_5$), 73.2, 73.3, 73.4, 73.5 (rotamers of tautomers, $C_5H_4$) 79.4 (maj) and 79.5 (min) (tautomers, $C_5H_4$), 126.1 ($C_6H_4$), 127.5 (maj) and 127.6 (min) (tautomers, $C_6H_4$), 128.1 (maj) and 128.2 (min) (tautomers, $C_6H_4$), 129.8 (maj) and 130.6 (min) (tautomers, $C_6H_4$), 132.6 (min) and 133.3 (maj) (tautomers, $C_6H_4$), 135.3 (maj) and 135.8 (min) (tautomers, $C_6H_4$), 164.6 (maj) and 165.2 (min) (tautomers, C4), 170.9 (min) and 171.2 (maj) (tautomers, N−CO−$CH_2$−$CH_2$), 200.4 (maj) and 200.5 (min) (tautomers, $CH(CO)_2$). ESI-MS (pos. detection mode) $m/z$ (%): 719.2 (100) [M + Na]$^+$, 735.2 (60) [M + K]$^+$. Anal. Calcd for $C_{38}H_{36}Fe_2N_2O_4$: C 65.54, H 5.21, N 4.02. Found: C 65.71, H 5.30, N 3.94.

**Compound 12A.** Compound **12A** was synthesized following GP-1: 1,1′-ferrocenedicarboxylic acid (200 mg, 0.73 mmol), praziquanamine (442 mg, 2.19 mmol), HATU (832 mg, 2.19 mmol), DIPEA (282 mg, 2.19 mmol), 10 mL of DMF, and 20 h reaction time. Flash column chromatography (silica gel, EtOAc/MeOH 15/1) gave **12A** as an orange solid (yield: 206 mg, 44%). $R_f$ = 0.13 (silica gel, EtOAc). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ (ppm) 2.64−2.98 (m, 8H, H1, H6, 2 × H7), 4.01 (m, 2H, H3), 4.34 (m, 4H, $C_5H_4$), 4.58 (m, 2H, $C_5H_4$), 4.66 (m, 6H, $C_5H_4$, H3 and H6), 4.84 (m, 2H, H11b), 4.92 (m, 2H, H1), 7.06−7.12 (m, 8H, $C_6H_4$). $^{13}$C{$^1$H} NMR (126 MHz, $CDCl_3$): $\delta$ (ppm) 29.3 (C7), 39.5 (C6), 47.3 (C1), 51.4(C3), 55.2 (C11b), 71.6 ($C_5H_4$), 71.8 ($C_5H_4$), 72.2 ($C_5H_4$), 72.5 ($C_5H_4$), 72.6 ($C_5H_4$), 74.1 ($C_5H_4$), 74.2 ($C_5H_4$), 79.4 ($C_5H_4$), 125.9 ($C_6H_4$), 127.5 ($C_6H_4$), 128.1 ($C_6H_4$), 129.9 ($C_6H_4$), 133.2 ($C_6H_4$), 135.3 ($C_6H_4$), 165.2 (C4), 169.7 (N−CO−$C_5H_4$). ESI-MS (pos. detection mode) $m/z$ (%): 665.3 (80) [M + Na]$^+$, 1307.4 (70) [2M + Na]$^+$. Anal. Calcd for $C_{36}H_{34}FeN_4O_4$: C 67.29, H 5.33, N 8.72. Found: C 67.08, H 5.18, N 8.54.

## ■ ASSOCIATED CONTENT

### Supporting Information

Synthesis and characterization data of compounds **2**, **3**, and **4B**-**12B**; $^{1}H$ and $^{13}C$ NMR spectra; molecular structures of **5A** and **7A**; crystallographic data and structure refinement for **4A**, **5A**, and **7A**; UV traces of the LC-MS analysis of PZQ; **8A** and **8B** in human plasma at $t$ = 0 min and 24 h; ratios of peak areas of **8A**/diazepam, **8B**/diazepam, and PZQ/diazepam in human plasma at $t$ = 0 min and $t$ = 24 h; CIF files for compounds **4A**, **5A**, and **7A**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

Fc, ferrocenyl; PZQ, praziquantel; Fc-PZQ, ferrocenyl praziquantel; HATU, 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; ESI-MS, electrospray ionization mass spectrometry; DIPEA, diisopropylethylamine; SAR, structure activity relationship

## ■ REFERENCES


(1) Sayed, A. A.; Simeonov, A.; Thomas, C. J.; Inglese, J.; Austin, C. P.; Williams, D. L. Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nat. Med.* **2008**, *14*, 407−412.

(2) Liu, R.; Dong, H.-F.; Guo, Y.; Zhao, Q.-P.; Jiang, M.-S. Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasites Vectors* **2011**, *4*, 201−218.

(3) Steinmann, P.; Keiser, J.; Bos, R.; Tanner, M.; Utzinger, J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect. Dis.* **2006**, *6*, 411−425.

(4) Beech, R. N.; Silvestre, A. Mutations associated with anthelmintic drug resistance. *Anti-Infect. Agents Med. Chem.* **2010**, *9*, 105−112.

(5) Doenhoff, M. J.; Cioli, D.; Utzinger, J. Praziquantel: Mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr. Opin. Infect. Dis.* **2008**, *21*, 659−667.

(6) Huang, J.; Bathena, S. P.; Alnouti, Y. Metabolite profiling of praziquantel and its analogs during the analysis of in vitro metabolic stability using information-dependent acquisition on a hybrid triple quadrupole linear ion trap mass spectrometer. *Drug Metab. Pharmacokinet.* **2010**, *25*, 487−499.

(7) Cioli, D.; Pica-Mattoccia, L.; Archer, S. Antischistosomal drugs: past, present . . . and future? *Pharmacol. Ther.* **1995**, *68*, 35−85.

(8) Ismail, M.; Botros, S.; Metwally, A.; William, S.; Farghally, A.; Tao, L. F.; Day, T. A.; Bennett, J. L. Resistance to praziquantel: direct evidance from *Schistosoma mansoni* isolated from Egyptian villagers. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 932−935.

(9) Ronketti, F.; Ramana, A. V.; Chao-Ming, X.; Pica-Mattoccia, L.; Ciolic, D.; Todd, M. H. Praziquantel derivatives I: modification of the aromatic ring. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4154−4157.

(10) Laurent, S. A.-L.; Boissier, J.; Coslédan, F.; Gornitzka, H.; Robert, A.; Meunier, B. Synthesis of "Trioxaquantel" derivatives as potential new antischistosomal drugs. *Eur. J. Inorg. Chem.* **2008**, 895−913.

(11) Liu, H.; William, S.; Herdtweck, E.; Botrosc, S.; Dömlinga, A. MCR synthesis of praziquantel derivatives. *Chem. Biol. Drug Des.* **2012**, *29*, 470−477.

(12) Dong, Y.; Chollet, J.; Vargas, M.; Mansour, N. R.; Bickle, Q.; Alnouti, Y.; Huang, J.; Keiser, J.; Vennerstrom, J. L. Praziquantel analogs with activity against juvenile *Schistosoma mansoni*. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2481−2484.

(13) Jaouen, G.; Top, S.; Vessieres, A.; Leclercq, G.; McGlinchey, M. J. The first organometallic selective estrogen receptor modulators (SERMs) and their relevance to breast cancer. *Curr. Med. Chem.* **2004**, *11*, 2505−2517.

(14) Hillard, E. A.; Vessières, A.; Jaouen, G. Ferrocene functionalized endocrine modulators as anticancer agents. In *Medicinal Organometallic Chemistry*; Jaouen, G., Metzler-Nolte, N., Eds.; Springer-Verlag: Heidelberg, 2010; Vol. *32*, pp 81−117.

(15) Dive, D.; Biot, C. Ferrocene conjugates of chloroquine and other antimalarials: The development of ferroquine, a new antimalarial. *ChemMedChem* **2008**, *3*, 383−391.

(16) Patra, M.; Gasser, G.; Pinto, A.; Merz, K.; Ott, I.; Bandow, J. E.; Metzler-Nolte, N. Synthesis and biological evaluation of chromium bioorganometallics based on the antibiotic platensimycin lead structure. *ChemMedChem* **2009**, *4*, 1930−1938.

(17) Gasser, G.; Ott, I.; Metzler-Nolte, N. Organometallic anticancer compounds. *J. Med. Chem.* **2011**, *54*, 3−25.

(18) Patra, M.; Gasser, G.; Metzler-Nolte, N. Small organometallic compounds as antibacterial agents. *Dalton Trans.* **2012**, *41*, 6350−6358.

(19) Biot, C.; Castro, W.; Botte, C. Y.; Navarro, M. The therapeutic potential of metal-based antimalarial agents: Implications for the mechanism of action. *Dalton Trans.* **2012**, *41*, 6335−6349.

(20) Herrmann, C.; Salas, P. F.; Cawthray, J. F.; deKock, C.; Patrick, B. O.; Smith, P. J.; Adam, M. J.; Orvig, C. 1,1′-Disubstituted ferrocenyl carbohydrate chloroquine conjugates as potential antimalarials. *Organometallics* **2012**, *31*, 5736−5747.

(21) Bruijnincx, P. C.; Sadler, P. J. New trends for metal complexes with anticancer activity. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197−206 and references therein..

(22) Hartinger, C. G.; Dyson, P. J. Bioorganometallic chemistry-from teaching paradigms to medicinal applications. *Chem. Soc. Rev.* **2009**, *38*, 391−401.

(23) Patra, M.; Gasser, G.; Wenzel, M.; Merz, K.; Bandow, J. E.; Metzler-Nolte, N. Synthesis of optically active ferrocene-containing platensimycin derivatives with a C6-C7 substitution pattern. *Eur. J. Inorg. Chem.* **2011**, 3295−3302.

(24) Patra, M.; Gasser, G.; Wenzel, M.; Merz, K.; Bandow, J. E.; Metzler-Nolte, N. Sandwich and half-sandwich derivatives of platensimycin: Synthesis and biological evaluation. *Organometallics* **2012**, *31*, 5760−5771.

(25) Glans, L.; Hu, W.; Jöst, C.; Kock, C. d.; Smith, P. J.; Haukka, M.; Bruhn, H.; Schatzschneider, U.; Nordlander, E. Synthesis and biological activity of cymantrene and cyrhetrene 4-aminoquinoline conjugates against malaria, leishmaniasis, and trypanosomiasis. *Dalton Trans.* **2012**, *41*, 6443−6450.

(26) Gasser, G.; Metzler-Nolte, N. The potential of organometallic complexes in medicinal chemistry. *Curr. Opin. Chem. Biol.* **2012**, *16*, 84−91.

(27) Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. Synthesis and antimalarial activity in vitro and in vivo of a new ferrocene−chloroquine analogue. *J. Med. Chem.* **1997**, *40*, 3715−3718.

(28) Rajapakse, C. S. K.; Martínez, A.; Naoulou, B.; Jarzecki, A. A.; Suárez, L.; Deregnaucourt, C.; Sinou, V.; Schrével, J.; Musi, E.; Ambrosini, G.; Schwartz, G. K.; Sánchez-Delgado, R. A. Synthesis, characterization, and in vitro antimalarial and antitumor activity of new ruthenium(II) complexes of chloroquine. *Inorg. Chem.* **2009**, *48*, 1122−1131.
(29) Martínez, A.; Carreon, T.; Iniguez, E.; Anzellotti, A.; Sánchez, A.; Tyan, M.; Sattler, A.; Herrera, L.; Maldonado, R. A.; Sánchez-Delgado, R. A. Searching for new chemotherapies for tropical diseases: ruthenium−clotrimazole complexes display high in vitro activity against *Leishmania major* and *Trypanosoma cruzi* and low toxicity toward normal mammalian cells. *J. Med. Chem.* **2012**, *55*, 3867−3877.
(30) Kuester, T.; Lense, N.; Barna, F.; Hemphill, A.; Kindermann, M. K.; Heinicke, J. W.; Vock, C. A. A new promising application for highly cytotoxic metal compounds: $\eta$6-arene ruthenium(II) phosphite complexes for the treatment of *Alveolar echinococcosis*. *J. Med. Chem.* **2012**, *55*, 4178−4188.
(31) Navarro, M.; Gabbiani, C.; Messori, L.; Gambino, D. Metal-based drugs for malaria, trypanosomiasis and leishmaniasis: Recent achievements and perspectives. *Drug Discovery Today* **2012**, *15*, 1070−1078.
(32) Brewer, M. D.; Burgess, M. N.; Dorgan, R. J. J.; Elliott, R. L.; Mamalis, P.; Manger, B. R.; Webster, R. A. B. Synthesis and anthelmintic activity of a series of of pyrazino[2,1-a][2]benzazepine derivatives. *J. Med. Chem.* **1989**, *32*, 2058−2062.
(33) Patra, M.; Gasser, G.; Wenzel, M.; Merz, K.; Bandow, J. E.; Metzler-Nolte, N. Synthesis and biological evaluation of ferrocene-containing bioorganometallics inspired by the antibiotic platensimycin lead structure. *Organometallics* **2010**, *29*, 4312−4319.
(34) Apreutesei, D.; Lisa, G.; Akutsu, H.; Hurduc, N.; Nakatsuji, S.; Scutaru, D. Thermotropic properties of ferrocene derivatives bearing a cholesteryl unit: Structure-properties correlations. *Appl. Organomet. Chem.* **2005**, *19*, 1022−1037.
(35) Plażuk, D.; Vessiéres, A.; Hillard, E. A.; Buriez, O.; Labbé, E.; Pigeon, P.; Plamont, M.-A.; Amatore, C.; Zakrzewski, J.; Jaouen, G. A [3]ferrocenophane polyphenol showing a remarkable antiproliferative activity on breast and prostate cancer cell lines. *J. Med. Chem.* **2009**, *52*, 4964−4967.
(36) Lindsay, J. K.; Hauser, C. R. Aminomethylation of ferrocene to form N,N-dimethylaminomethylferrocene and Its conversion to the corresponding alcohol and aldehyde. *J. Org. Chem.* **1957**, *22*, 355−358.
(37) Gasser, G.; Fischmann, A. J.; Forsyth, C. M.; Spiccia, L. Products of hydrolysis of (ferrocenylmethyl)trimethylammonium iodide: Synthesis of hydroxymethylferrocene and bis-(ferrocenylmethyl) ether. *J. Organomet. Chem.* **2007**, *692*, 3835−3840.
(38) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. J. Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds. *J. Chem. Soc., Perkin Trans. 2* **1987**, S1−S19.
(39) Lima, R. M.; Ferreira, M. A. D.; Ponte, T. M. d. J.; Marques, M. P.; Takayanagui, O. M.; Garcia, H. H.; Coelho, E. B.; Bonato, P. S.; Lanchote, V. L. Enantioselective analysis of praziquantel and trans-4-hydroxypraziquantel in human plasma by chiral LC−MS/MS: Application to pharmacokinetics. *J. Chromatogr., B* **2009**, *877*, 3083−3088.
(40) Kiec'-Kononowicz, K. Spectral and chemical properties of pyrazino-[2,1-a]-isoquinolin-4-one derivatives. *Arch. Pharm.* **1989**, *322*, 795−799.
(41) Oxford Diffraction Ltd. *CrysAlis$^{Pro}$ Software System, 171.32*; Oxford, U.K., 2007.
(42) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. SIR97: A new tool for crystal structure determination and refinement. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.
(43) Sheldrick, G. M. A short history of SHELX. *Acta Crystallogr.* **2008**, *A64*, 112−122.
(44) Spek, A. L. Single-crystal structure validation with the program PLATON. *J. Appl. Crystallogr.* **2003**, *36*, 7−13.
(45) Keiser, J. In vitro and in vivo trematode models for chemotherapeutic studies. *Parasitology* **2009**, *137*, 589−603.
(46) Ramirez, B.; Bickle, Q.; Yousif, F.; Fakorede, F.; Mouries, M.-A.; Nwaka, S. Schistosomes: Challenges in compound screening. *Expert Opin. Drug Discov.* **2007**, *2*, S53−S61.
(47) Ingram, K.; Ellis, W.; Keiser, J. Antischistosomal activities of mefloquine-related arylmethanols. *Antimicrob. Agents Chemother.* **2012**, *56*, 3207−3215.