RESEARCH ARTICLE

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of organometallic derivatives of the antimalarial
drug mefloquine as new antischistosomal drug
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We present the design, synthesis, characterization and biological evaluation of new ferrocenyl and ruthenocenyl derivatives of the organic antimalarial mefloquine, a drug also known for its antischistosomal activity. The two metallocenyl derivatives prepared (**3** and **4**) demonstrated comparable activity to mefloquine against adult-stage *Schistosoma mansoni* *in vitro*. Importantly, both compounds were found to have lower toxicity in all cell lines than mefloquine itself. Administration of a 200 mg kg⁻¹ oral dose of **3** and **4** to *S. mansoni*-infected mice did not significantly reduce worm burden, contrary to mefloquine.

Introduction

Schistosomiasis is a neglected tropical disease that affects an estimated 190 million individuals worldwide, mostly children in rural areas of Sub-Saharan Africa.¹ It is caused by an infection of parasitic worms from the *Schistosoma* genus, where *Schistosoma haematobium* (which causes urinary schistosomiasis) as well as *S. mansoni* and *S. japonicum* (which cause intestinal schistosomiasis) are the most prevalent species that infect humans.² Individuals become infected when they come into contact with freshwater contaminated by free-swimming cercarial forms of the parasite, which enter the host *via* skin penetration. They migrate *via* blood circulation first to the lungs and then to the liver, where they mature and sexually pair. They finally move to reside in the veins of the mesenteries (intestinal schistosomiasis) or the urogenital plexus (urinary schistosomiasis) of the human host, where they can survive 3 to 5 years on average.³ The female produces tens to thousands of eggs a day, half of which become trapped in tissues such as the liver, the intestine or the bladder where they induce immune reactions and damage the organs.³ This results in symptoms such as portal hypertension, bloody diar-

rhoea or anaemia, which can lead to developmental stunting in children.²

The exclusive treatment to date for schistosomiasis is praziquantel (PZQ). The drug is effective against all main schistosome species and is administered to affected populations in mass drug administration campaigns to prevent morbidity.⁴ However, there are several drawbacks associated with the use of PZQ, such as its lack of activity against juvenile worms or its low metabolic stability *in vivo*.^{2,5} Moreover, the use of only PZQ to treat and control millions of people raises the risk of PZQ-resistant schistosome strains. Reduced susceptibility of *S. mansoni* to PZQ was first reported in 1973 by Katz *et al.* in Brazil and sporadic treatment

Scheme 1 Schematic representation of newly designed organometallic derivatives of mefloquine, [2,8-bis(trifluoromethyl)quinolin-4-yl] ferrocenemethanol (**3**) and [2,8-bis(trifluoromethyl)quinolin-4-yl] ruthenocenemethanol (**4**).

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failures have been reported since then, although it is not known if they are due to drug resistance.^{6,7} Therefore, there is an urgent need to develop novel antischistosomal drug candidates with different modes of action.⁸

As *de novo* drug discovery is often a long and expensive process, a commonly employed strategy, especially for neglected tropical diseases like schistosomiasis, is drug repurposing.⁹ This approach investigates already existing drugs as novel antischistosomal drug candidates.⁹ As schistosomiasis and malaria are often co-endemic, antimalarials have been explored for their antischistosomal activity.^{10,11} Moreover, many antimalarials are thought to target the heme detoxification pathway from hemoglobin digestion by the malaria-causing *Plasmodium* parasites, a pathway that also exists in the blood-feeding *Schistosoma* spp.¹² In previous studies, mefloquine (Scheme 1), a well-known antimalarial drug,¹³ was identified as a potential antischistosomal drug candidate.¹⁴ Keiser *et al.* reported that mefloquine administered to mice infected with *S. mansoni* and *S. japonicum* showed promising antischistosomal properties. In the adult infection model, total and female worm burden reductions of 72–100% were achieved with a single dose of 200 mg kg⁻¹ to 400 mg kg⁻¹. A single dose of 100 mg kg⁻¹ to 400 mg kg⁻¹ of mefloquine given to mice infected with juvenile stages of *S. mansoni* resulted in a high total and female worm burden reduction of 87% to 100% – an important result considering PZQ's lack of activity against the juvenile stage. The promising *in vivo* results obtained with mefloquine were further investigated in clinical phase II trials, alone and in combination with PZQ.^{15–17} The authors, however, noted that antimalarial parent compounds should not be used in treatment campaigns, as they might increase the risk of resistant *Plasmodium* spp., which are faster mutating and have already demonstrated resistance to previous antimalarials. The investigation of potential derivatives has therefore been employed.¹⁸

With this in mind, our research groups have decided to derivatize mefloquine with various organometallic moieties. Organometallic compounds are defined as metal complexes bearing at least one direct metal–carbon bond.¹⁹ The derivatization of a known organic drug with organometallic moieties has already proven to be successful in various areas of medicinal chemistry.^{19–28} In 1997, Biot *et al.* synthesized ferroquine, a ferrocenyl derivative of the antimalarial drug chloroquine, which was active against both chloroquine-sensitive and chloroquine resistant *Plasmodium falciparum*, the most virulent *Plasmodium* spp.^{29,30} Interestingly, the incorporation of the ferrocene fragment brought an additional metal-specific mode of action: the favourable redox properties of the Fe(III)/Fe(II) core of ferrocene produced reactive oxygen species, which can increase toxicity to the parasite.^{31–33} In addition, the attachment of ferrocene enhanced the lipophilicity of the compound, as has been observed for previous ferrocene derivatives.³⁴ In phase II trials, ferroquine was effective against chloroquine resistant *P. falciparum*, both as a monotherapy and in conjunction with artesunate.³⁵

The promising results obtained with ferroquine drew interest in the development of other organometallic drug candidates. Especially, there has been a growing interest in the design of organometallic drug candidates as antischistosomal agents.^{36–44} To incorporate the drug repurposing strategy, the research focused mainly on structure-based optimization of already established drugs. The antimalarial agent chloroquine and its organometallic derivatives ferroquine, ruthenoquine and hydroxyl-ferroquine were analysed as potential antischistosomal agents by the groups of Biot, Keiser and Gasser.^{11,14} The organometallic compounds evaluated did not, however, increase the weak antischistosomal properties of the parent drug chloroquine *in vivo* (worm burden reduction of 11% for chloroquine and 19% for ferroquine at a dose of 200 mg kg⁻¹).^{11,14}

Herein, we report two organometallic mefloquine derivatives, in which the piperidinyl group of the organic drug is replaced with a ferrocenyl or a ruthenocenyl moiety (Scheme 1). We describe their synthesis, characterization and antischistosomal activities against *S. mansoni* *in vitro* and *in vivo* and compare them to the parent drug mefloquine. We note that similar organometallic compounds were evaluated against malaria by Biot *et al.*⁴⁵ Biot replaced the piperidinyl unit of mefloquine with a ferrocenyl moiety bearing an amine. He intended to mimic intramolecular hydrogen bonding of mefloquine, trying to adapt the pharmacokinetics of the organic drug. But the ferrocenyl compounds exhibited a lower antimalarial activity than the mefloquine itself. Also, these compounds have never been tested on schistosomiasis. As the precise mode of action of mefloquine for treating schistosomiasis is not yet understood, it is uncertain if this particular intramolecular hydrogen bonding is crucial. Therefore, in this study, we intended to try a simple and efficient approach to repurpose an already known drug and thus test metallocenyl derivatives of mefloquine for the first time on adult-stage *S. mansoni*.

Results and discussion

Synthesis and characterization

The ferrocenyl and ruthenocenyl analogues of mefloquine were prepared in a one-step reaction procedure, starting from *in situ* generated **2** reacting directly with the respective metallocenecarboxaldehyde, as presented in Scheme 2. Both organometallic derivatives **3** and **4** were isolated as racemates (see ESI† Fig. S9 and S10). We have decided to evaluate the

Scheme 2 Reagents and conditions: (i) nBuLi, dry Et₂O, 20 min, –78 °C; (ii) ferrocenecarboxaldehyde (**3a**), dry Et₂O, 1 h, –78 °C, 94%; (iii) ruthenocenecarboxaldehyde (**4a**), dry Et₂O, 1 h, –78 °C, 78%.

Table 1 *In vitro* activity of mefloquine derivatives **3** and **4** as compared to mefloquine against *S. mansoni* adult worms and cytotoxicity against HeLa, RPE-1 and TIB-75 cells

Compound	IC ₅₀ value [μ M]			
	<i>S. mansoni</i>	HeLa	RPE-1	TIB-75
3	3.3 $\pm$ 0.0	37 $\pm$ 1.0	81 $\pm$ 3.5	22 $\pm$ 1.0
4	4.0 $\pm$ 0.1	32 $\pm$ 0.8	50 $\pm$ 4.8	6.2 $\pm$ 0.4
Mefloquine	3.5 $\pm$ 0.0	4.7 $\pm$ 0.2	11 $\pm$ 0.4	4.1 $\pm$ 0.1

racemic mixtures first in our *in vitro* and *in vivo* studies in order to obtain initial insights into their potential antischistosomal properties.

The first step in the synthesis of the desired organometallic analogues is the lithiation of 2,8-bis-(trifluoromethyl)-4-bromoquinoline **1** with ⁿBuLi. The resulting activated quinoline **2** reacted directly *in situ* with the ferrocenecarboxaldehyde **3a** to obtain [2,8-bis(trifluoromethyl)quinolin-4-yl] ferrocenemethanol **3** in 94% yield. In order to improve our understanding of the possible redox-based mode of action of the ferrocene moiety, we also synthesized the corresponding ruthenocenyl analogue (**4**) of **3**. Although both ferrocene and ruthenocene are isoelectronic and have a similar geometry and steric demand, they have a different electrochemical behaviour and redox properties. The ruthenocenecarboxaldehyde was synthesized following the literature procedure of Sanders *et al.*⁴⁶ Compound **4** was prepared in a similar fashion as **3**, where the *in situ* generated **2** was reacted directly with ruthenocenecarboxaldehyde (Scheme 2). [2,8-Bis-(trifluoromethyl)quinolin-4-yl] ruthenocenemethanol **4** was obtained in 78% yield. The ferrocenyl- and ruthenocenyl-analogues of mefloquine (**3** and **4**) were characterized by ¹H, ¹³C, ¹⁹F NMR spectroscopies, high-resolution ESI-mass spectrometry and their purity was confirmed by elemental analysis.

Biological evaluation

***In vitro* activity against adult *S. mansoni* and cytotoxicity against mammalian cell lines.** The biological efficacy of the metallocene derivatives **3** and **4** were evaluated against *S. mansoni*, together with mefloquine, which was used as a reference compound. All drugs showed similar activity, with IC₅₀ values of 3.3, 4.0 and 3.5 μ M for **3**, **4** and mefloquine, respectively, after 72 h incubation (Table 1).

With the demonstrated *in vitro* antischistosomal activity of **3** and **4**, we were eager to investigate the selectivity of the compounds toward parasites. Therefore, we evaluated the cytotoxicity of **3** and **4** using three mammalian cell lines: a cervical cancer cell line (HeLa), a noncancerous human retinal pigmented epithelial cell line (RPE-1) and a noncancerous mouse liver cell line (TIB-75), to determine the selectivity of our compounds. The results obtained are summarized in Table 1. The two compounds **3** and **4** were moderately cytotoxic to HeLa cells with IC₅₀ values of 36.6 $\pm$ 1.0 and 31.6 $\pm$ 0.8 μ M, respectively. Similarly, moderate toxicity was observed for **3** and **4** in RPE-1 cells (IC₅₀ = 81.4 $\pm$ 3.5, 49.7 $\pm$ 4.8 μ M, respectively). However, TIB-75 cells were more susceptible to **3** and **4** than noncancerous RPE-1 and cancerous HeLa cell lines with IC₅₀ values of 22.1 $\pm$ 1.0 and 6.2 $\pm$ 0.4 μ M, respectively. Importantly, our two compounds were found to have lower toxicity in all cell lines than mefloquine itself (IC₅₀ = 4.7 $\pm$ 0.2, 11.1 $\pm$ 0.4, and 4.1 $\pm$ 0.1 μ M, respectively).

***In vivo* activity against adult *S. mansoni*.** With the encouraging *in vitro* results, we progressed compounds **3** and **4** to *in vivo* studies, with mefloquine as a reference, in a patent *S. mansoni*-mouse model (Table 2).

The ferrocene derivative **3** indicated no activity with a single oral 200 mg kg⁻¹ dose while the total worm burden reduction for the ruthenocene derivative **4** was 27.4%. For comparison, at 200 mg kg⁻¹, mefloquine achieved total worm clearance in *S. mansoni*-infected mice.

Thus, a single oral 200 mg kg⁻¹ dose (curative dose for mefloquine) yielded weak or no antischistosomal activity from compounds **3** and **4** *in vivo*. The good antischistosomal activity *in vitro* but the lack of activity *in vivo* might be explained by any number of host processes. We subsequently tested if protein binding might reduce drug efficacy by repeating the adult *S. mansoni* *in vitro* assay with an added physiological (45 g L⁻¹) concentration of albumin. The efficacy was lowered for all 3 compounds: IC₅₀ values increased to 25 μ M for mefloquine, 65 μ M for compound **3** and >>100 μ M for compound **4**. The higher values of **3** and **4** as compared to mefloquine might provide a hint to their lowered efficacy, though likely there are additional reasons, which may include poor absorption, inactivation of the drug along the metabolic route or a very fast clearance of the drug *in vivo*. We note also that since the doses are in mg kg⁻¹, the actual amount of organometallic compound given

Table 2 Effect on worm burden of single oral doses (200 mg kg⁻¹) of mefloquine derivatives **3** and **4** and mefloquine in adult stage *S. mansoni*-infected mice

Compound	Dose (mg kg ⁻¹)	No. of mice	Worm burden		Worm burden reduction (%)	
			Female	Total	Female	Total
Control	Untreated	8	23	46	—	—
3	200	3 ^a	41	81	0	0
4	200	4	17	34	28	27
Mefloquine	200	4	0	0	100	100

^a One mouse died prematurely.

to the mice is inferior by 28% compared to the organic compound.

Additionally, one mouse treated with compound 3 died a few days after treatment revealing severe damage to the liver at dissection. Surprisingly, this occurred even though derivative 3 was shown to be the least toxic against the TIB-75 liver cell line. Further structure–activity relationship and pharmacokinetic data would be required to fully understand the *in vivo* results.

Conclusions

Facing the urgent need to develop new drug candidates against schistosomiasis, two organometallic compounds, [2,8-bis(trifluoromethyl)quinolin-4-yl] ferrocenemethanol (3) and [2,8-bis(trifluoromethyl)quinolin-4-yl] ruthenocenemethanol (4) were synthesized. These candidates are derivatives of mefloquine, a well-known antimalarial agent also known for its anti-schistosomal activity. These new compounds were shown to be moderately cytotoxic to mammalian cell lines, although less cytotoxic than mefloquine itself, and thereby show selective activity on parasites. Their activities against *S. mansoni* were compared to mefloquine. The *in vitro* activities against adult *S. mansoni* were equivalent to mefloquine. However, the organometallic compounds showed only weak antischistosomal properties *in vivo*. The results obtained suggest that derivatization of mefloquine may be a possible avenue for drug repositioning against schistosomiasis, however studies with additional derivatives would be required to optimize *in vivo* activity.

Conflicts of interest

There are no conflicts to declare.

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