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REVIEW

Medicinal Organometallic Chemistry – an Emerging Strategy for the Treatment of Neglected Tropical Diseases

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Organometallic moieties make excellent candidates for lead structures in drug design, due to their bioactivity, redox chemistry and structural diversity. Despite this, only a limited number of organometallic compounds have been recruited in combatting neglected tropical diseases, 17 of which are officially recognised by the World Health Organisation. N pharmaceuticals are needed to overcome the prominent side effects of existing treatments for these diseases, as well as to avoid emerging resistance genes in the causative parasites. This review focuses on the use of organometallic dr compounds in treating African Sleeping Sickness (Human African Trypanosomiasis), Chagas disease (American trypanosomiasis), leishmaniasis, schistosomiasis and echinococcosis. Studies from the past 5-10 years have generated promising lead compounds, most arising from the modification of an existing drug and all demonstrating the advantageous properties unique to organometallic chemistry. Most of the compounds introduced here remain in the le identification stage and require further *in vivo* and physiochemical studies

Introduction

While organometallic compounds have revolutionised synthetic chemistry as catalysts, their use as pharmaceuticals was not to be widely considered until relatively recently. The presence of a metal-carbon bond led to the initial consensus that organometallic compounds were toxic, unstable and therefore unfit for biological applications, but it has since been realised that this is not necessarily the case.

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Multiple endogenous structures have been characterised to reveal the presence of such bonds, such as the Co-C bond in Vitamin B_{12} , characterised in the 1950s. [1-3] Furthermore, it is believed that organoruthenium compounds are relatively safe, due to their ability to mimic endogenous iron in binding to serum albumin for non-toxic transport. [4,5] In general, organometallics are capable of redox reactions, ligand coordination and broad structural diversity, allowing structural and mechanistic possibilities beyond purely organic frameworks. The latter has been demonstrated by the group of Meggers *et al.* [6] in structures such as the kinase inhibitor Λ-FL411 (Fig. 1), in which a ruthenium centre can act as a 'virtual octahedral carbon,' allowing a vast range of finely-tunable stereoisomers unachievable using a purely organic structure. [6,7] Organometallic Pt(II) and Pd(II) compounds can similarly exhibit structural diversity, forming square planar complexes analogous to that of the inorganic Pt(II) anticancer compound, cisplatin (Fig. 1), making such structures promising candidates as DNA-intercalators. The prime example of a therapeutic organometallic compound is ferroquine (Fig. 1), an antimalarial, ferrocenyl analogue of chloroquine. The compound was first synthesised by Biot *et al.* [8] in the 1990s, exploiting the fact that parasites of the genus *Plasmodium* require iron for development inside red blood cells. Ferroquine is a combination of an iron-containing bait (ferrocene) and a poison (chloroquine) in the same molecule, allowing chloroquine to surreptitiously carry out its antiparasitic action. The biological advantages of ferroquine, as well as its low toxicity, are expected to carry it through the remainder of clinical trials in the near future. [9]

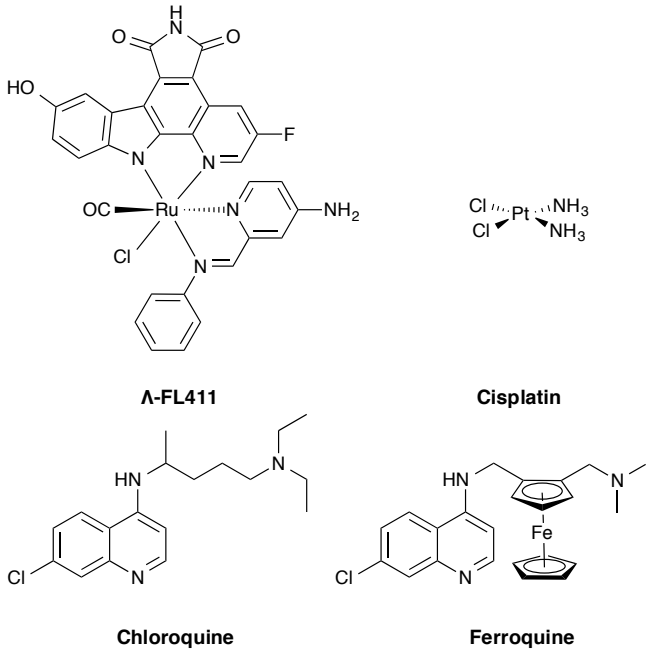


Fig. 1 Chemical structures of selected metal-containing drugs.

'Medicinal organometallic chemistry' has been defined [10] as 'the synthesis and biological evaluation of organometallic compounds for diagnostic or therapeutic applications, as well as investigations into their mode of action.' All of these facets have been encompassed in the success story of ferroquine and the area is also making an impact on the treatment cancer and HIV [11] however, a myriad of other diseases may benefit from medicinal organometallic chemistry. The World Health Organisation (WHO) has launched an initiative to address neglected tropical diseases, which affect a demographic lacking the financial resources to obtain medication, leaving little commercial incentive for new treatments to be developed. It is here that medicinal organometallic chemistry may make a significant impact. Previous reviews in this area have covered drug discovery in particular neglected diseases [12-15] as well as the use of metals specifically. [16] However, studies focusing on organometallics are of a much lower incidence, allowing for a detailed review of the current neglected tropical disease literature. This review will focus on the use of organometallics in combatting African sleeping sickness (human African trypanosomiasis), Chagas disease (American trypanosomiasis), leishmaniasis, schistosomiasis and echinococcosis, which have had the most attention from organometallic chemists of all the items on the WHO list of neglected tropical diseases. [17] Case studies have been chosen largely from the past 5 – 10 years and, though some groups may have reported additional compounds to those mentioned here, discussion has been limited to the organometallic compounds only.

Protozoan-Based Diseases

Introduction

Three of the items on the WHO list [17] are infections of unicellular protozoan parasites of the class Kinetoplastida. Two of these are of the genus *Trypanosoma*, the species *Trypanosoma brucei* being transmitted via the Tsetse fly to cause sleeping sickness and the species *Trypanosoma cruzi* being transmitted through the triatomine, or 'kissing' bug, to cause Chagas disease. The third disease, leishmaniasis, is an infection of one of 20 species of the genus *Leishmania,* transmitted via infected sandflies of the genus *Phlebotamus.* The prevalence and suboptimal treatment options for these protozoan infections call for the attention of chemists and biologists as, worldwide, an estimated 20 000 people are currently infected with sleeping sickness, [18] 7-8 million people with Chagas disease [19] and 1.3 million new cases of leishmaniasis are reported annually. [20]

The incidence of sleeping sickness is restricted to sub-Saharan Africa, being the habitat of the Tsetse fly vector. Infection can arise from either subspecies *T. brucei gambiense* or *T. brucei rhodesiense,* which affect central/western and southern/eastern sub-Saharan Africa, respectively. In contrast, the region affected by Chagas disease has crept up from Latin America to the US and Canada in recent years and cases have even been detected in parts of Europe. The life cycles of *T. brucei* and *T. cruzi* are

largely similar: both involve the transmission of the metacyclic trypomastigote form of the parasite to humans through either the bite of the Tsetse fly (sleeping sickness) or in the faeces of the triatomine bug (Chagas disease). Once transmitted, *T. brucei* cells multiply as bloodstream trypomastigotes to bring about itching, headaches, fever and the swelling of organs in Stage 1, before penetrating the central nervous system (CNS) to disrupt the sleep/wake cycle in Stage 2 and cause a characteristic sleeping sickness. [21] During Chagas disease, *T. cruzi* cells instead multiply as amastigotes, with clinical indications ranging from skin nodules, oedema, fever and myocarditis in the acute phase to heart failure and digestive legions in the chronic phase. [19] The biology of *Leishmania* parasites differs in that they are transmitted through the bite of infected sandflies in the promastigote form and multiply as amastigotes once engulfed by host macrophages. [22] The disease can manifest in a visceral, cutaneous or mucocutaneous form. Visceral leishmaniasis results exclusively from the infection of *Leishmania donovani* and *Leishmania infantum*, causing irregular fever, weight loss, anaemia and swelling of the liver and spleen. It is therefore the most fatal form of the disease, with 90% of cases occurring in Brazil, Bangladesh, Ethiopia, India, Sudan and South Sudan. [23] Infection by species such as *Leishmania major* is responsible for cutaneous leishmaniasis, a largely urban phenomenon affecting Brazil, Afghanistan, Algeria, Colombia, Iran, Pakistan, Peru, Saudi Arabia and the Syrian Arab Republic and leaving victims with legions and skin ulcers. [23] Mucocutaneous leishmaniasis, caused primarily by *Leishmania braziliensis,* manifests similarly on mucosal membranes of the nose, mouth and throat. This form has more concentrated distribution, with Bolivia, Brazil and Peru harboring almost 90% of cases. [23]

There are currently five main drugs recommended for the treatment of sleeping sickness. Recommended treatments for Stage 1 of the disease are pentamidine for infection by *T. b. gambiense* and suramin for *T. b. rhodesiense.* In the case of pentamidine (Fig. 2), an aromatic diamidine, a definitive cellular target has yet to be identified, though some information on its ability to cleave kinetoplast DNA is known. [24] In contrast, suramin (Fig. 2), a sulfonated napthylamine, has been shown to bind to low-density lipoprotein in human plasma, which is then taken up by bloodstream trypanosomes as a source of sterols. It is also known to inhibit multiple *T. brucei* enzymes, such as those involved in glycolysis and the pentose phosphate pathway. It is thought that the cumulative multi-target action of the drug is responsible for its activity. [24] Suramin is negatively charged *in vivo* and the higher isoelectric point of trypanosomal enzymes compared to those in humans is thought to favour suramin binding, allowing selectivity. [24] Stage 2 sleeping sickness requires drugs able to penetrate the blood-brain barrier due to the presence of trypanosomes in the CNS. Melarsoprol (Fig. 2) remains the most widely used for both subspecies, in the absence of the much preferred, but more expensive alternative eflornithine (Fig. 2). Trivalent arsenic compounds such as melarsoprol have a high affinity for sulfhydryl groups, which constitute the active sites of enzymes such as the kinases involved in glycolysis.

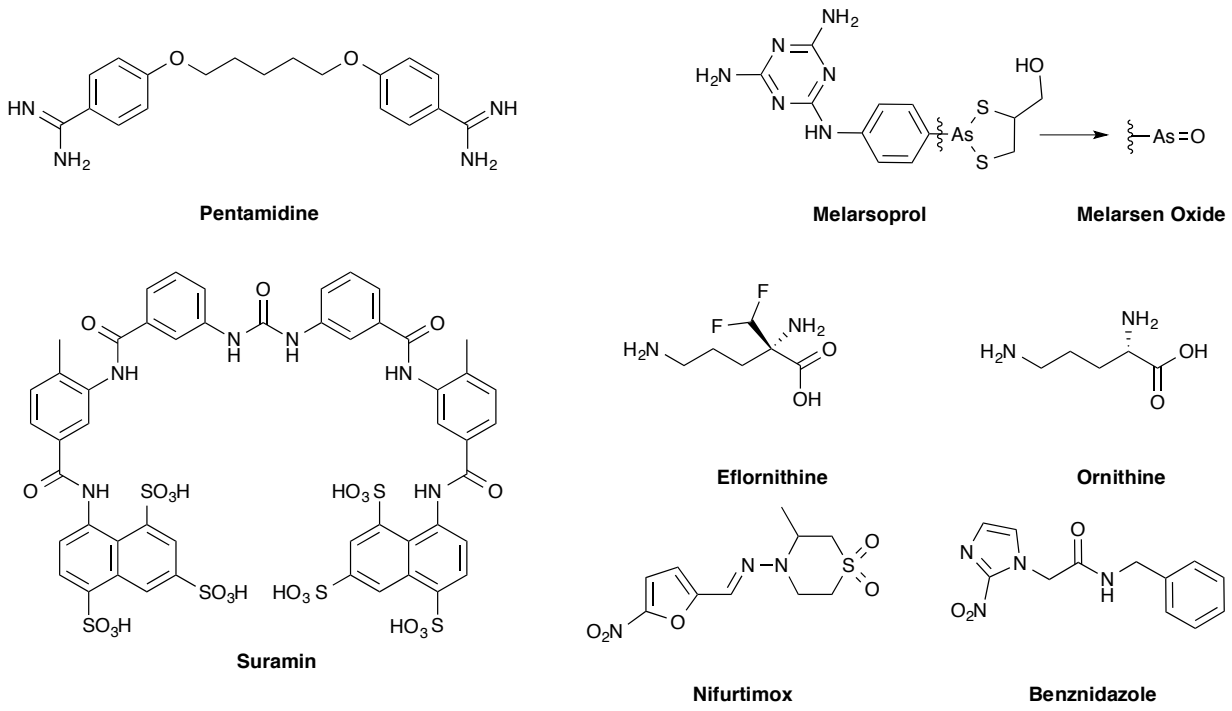


Fig. 2 Chemical structures of currently recommended drug treatments for sleeping sickness (pentamidine, suramin, melarsoprol, eflornithine and nifurtimox) and Chagas disease (nifurtimox and benznidazole).

Given that trypanosomes are highly reliant on glycolysis for energy synthesis, it is believed that, after conversion *in vivo* to the active metabolite melarsen oxide (Fig. 2), the drug may inhibit pyruvate kinase to disrupt energy production and cause cell lysis. [24,25] Eflornithine (Fig. 2) is the only new sleeping sickness drug introduced in the last fifty years [21] and is on the WHO List of Essential Medicines. [26] Effective mainly against *T. b. gambiense,* eflornithine is a suicide inhibitor, irreversibly binding to the enzyme ornithine decarboxylase (ODC) in place of the natural substrate and structural analogue, ornithine (Fig. 2). This ceases polyamine synthesis, eventually killing the cell. [27] ODC is also present in humans, though it is regenerated much more quickly here than in trypanosomes, allowing therapeutic selectivity. [27] In recent years, it has been recommended that, for the treatment of sleeping sickness, eflornithine should be used in combination with nifurtimox (Fig. 2), a nitro drug currently used to treat leishmaniasis and Chagas disease. The two currently recommended drug treatments for Chagas disease are nifurtimox and benznidazole (Fig. 2). Both drug compounds are activated via reduction of the nitro group, likely generating reactive oxygen species capable of damaging trypanosomal DNA and other membrane lipids. It was originally thought [28] that the enzyme responsible for this activation was trypanothione reductase, which catalyses the production of trypanothione, a protein essential to the organism, however it has since been found [29] that a specific class of nitro reductase enzymes are responsible for this. The drugs carry with them a myriad of side effects including nausea, anorexia, neuropathy and dermatitis, [25] as well as the inability to treat multiple stages of the disease. [30]

As for the three forms of leishmaniasis, a variety of treatments exist, though the side effects and limited efficacy of each leave them far from ideal. Amphotericin B (Fig. 3) is the primary treatment for visceral leishmaniasis, forming complexes with cell membrane sterols and altering ion balance to kill leishmanial cells. [15] Primary treatments for cutaneous and mucocutaneous leishmaniasis, however, are not as well understood. It is thought that the two antimony-based compounds, meglumine antimoniate and sodium stibogluconate (Fig. 3) are activated from the pentavalent (Sb^{5+}) to the lethal trivalent (Sb^{3+}) within the cell by an unknown mechanism. [15] The previously mentioned sleeping sickness drug pentamidine (Fig. 2) is the third major treatment and, though its antileishmanial effects include kinetoplast DNA binding, the primary mode of action has also not been elucidated. [15] Both the pentavalent antimonials and amphotericin B require prolonged intravenous administration and, despite the high efficacy of the latter, both drugs, as well as pentamidine, are limited by their toxicity. [15] The most recent antileishmanial drug was the anticancer drug, miltefosine (Fig. 3), approved by the US Food and Drug Administration in March 2014 for the treatment of all forms of leishmaniasis. Miltefosine is an alkylphosphocholine and, though its exact mechanism of antileishmanial action has not been elucidated, it is thought to act via the disturbance of lipid-dependent cell-signalling pathways and the induction of apoptosis. [31] Despite being the first orally-administered antileishmanial drug, the main concern with miltefosine is its teratogenicity, and so cannot be administered during pregnancy.

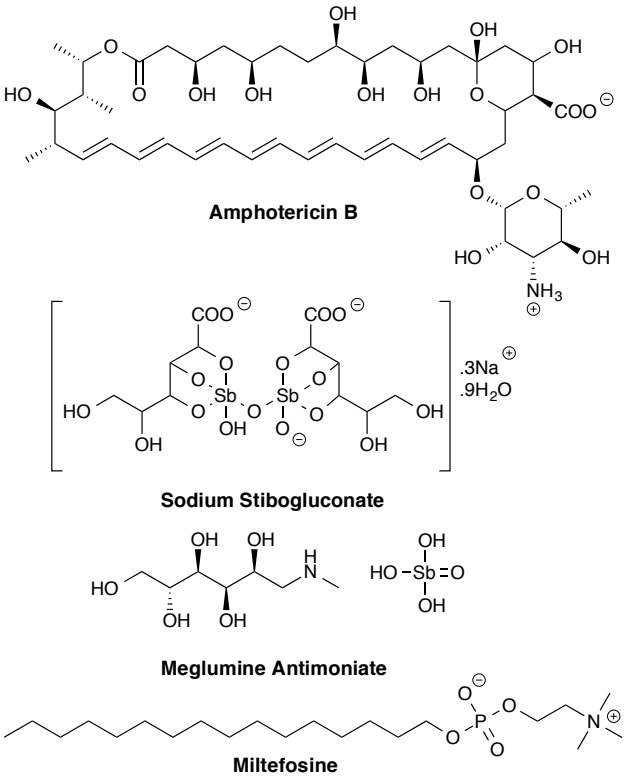


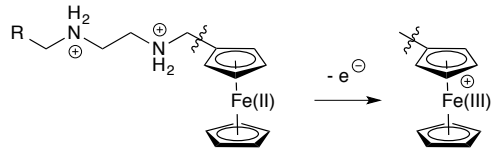
Fig. 3 Chemical structures of currently recommended drug treatments for leishmaniasis. Pentamidine (Fig. 2) is also a recommended treatment.

Most of the approaches to designing new treatments for protozoan neglected tropical diseases have involved the modification of an existing drug. Keeping the pharmacophore of a known compound can maintain a mode of action, with slight modifications improving activity and decreasing side effects, as well as avoiding resistance genes, which is a problem in sleeping sickness, Chagas disease and leishmaniasis. [16] Given the literature introduced here, it would appear that the low toxicity and unique, advantageous chemistry of organometallic moieties make them suitable additions to chemical structures of existing drug compounds.

Sleeping Sickness: Ferrocenyl Analogues of Pentamidine

Given that the method of modifying an existing drug, namely pentamadine, has progressed at least one diamidine compound into clinical trials, [27] further diamidine derivatives have continued to emerge. A recent study by Velásquez *et al.* [3] produced benzyl and ferrocenyl diamidines with promising *in vitro* activity against *T. brucei.* The compounds were produced as hydrochloride salts and tested *in vitro* against model strains such as *T. brucei brucei* 427, using pentamidine as a reference drug. Firstly, it was found that the ferrocenyl derivatives **(1** and **2**, Fig. 4) exhibited higher activity than the benzyl derivatives with many proving more potent than pentamidine. The most

promising results were obtained with compounds **1** (IC_{50} 0.36 μM) and **2** (IC_{50} 0.35 μM), which boasted an eighteen-fold improvement on pentamidine (IC_{50} 6.43 μM) in terms of anti-*T. brucei* activity. The compounds were also tested against human hepatoma cells and found to display comparable selectivity to pentamidine (Fig. 4). The greater activity of the ferrocenyl compounds was tentatively attributed to greater lipophilicity, redox activity and geometric structure. It was speculated that the oxidation of ferrocene to the ferrocinium ion (Fig. 4) may have facilitated the subsequent generation of cytotoxic radical species, foiling the trypanosomal defence mechanism against oxidative stress. It was also suggested that the ability of the ferrocenium ion to form charge transfer complexes with trypanosomal proteins may play a role in its trypanocidal action. [33]

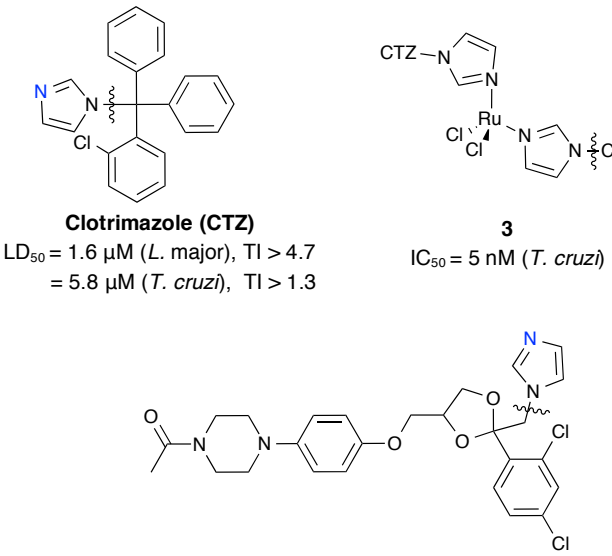


1: R = 4-OMeC$_6$H$_4$, IC_{50} = 0.36 μM, SI = 52
2: R = Ferrocene , IC_{50} = 0.35 μM, SI = 26
Pentamidine: IC_{50} = 6.43 μM, SI = 25

Fig. 4 Ferrocenyl derivatives of pentamidine synthesised by Velasquez *et al.* [32] with activity against *T. b. brucei* 427 (IC_{50}) and selectivity over human hepatoma cells (selectivity index, SI). The oxidation of the ferrocenyl fragment to the toxic ferrocenium ion has also been shown.

Chagas Disease and Leishmaniasis: Ruthenium Complexes of Clotrimazole and Ketoconazole

In a study by Demoro *et al.* [34] it was suggested that the cheaper and more effective approach to combating neglected tropical diseases would be to focus on broad-spectrum drugs. Fortunately, some of the new organometallic novel structures, such as 'piano stool' ruthenium complexes of the antifungals clotrimazole (CTZ) and ketoconazole (KTZ) (Fig. 5) have been proven active against both Chagas disease and leishmaniasis. Clotrimazole and ketoconazole are well-established sterol biosynthesis inhibitors and, considering the sterol biosynthesis of pathogenic fungi is similar to that in leishmanial and trypanosomal parasites, the antifungals can potentially treat both diseases. [35] The use of a transition metal is appealing due to the idea of a metal-drug synergy, where the activity of a metal-drug complex is greater than the sum of its individual components. [36] The investigation of clotrimazole complexes was initiated in 1993 by Sanchez-Delgado *et al.* [37] who found that the inorganic complex $RuCl_2[CTZ]_2$ (**3**, Fig. 5) displayed high anti-*T. cruzi* activity (IC_{50} 5 nM), greater than free clotrimazole (LD_{50} 5.8 μM) and ketoconazole (LD_{50} 1.5 μM), with no toxicity to mammalian cells. A later study [38] demonstrated a metal-drug synergy in that, while one clotrimazole fragment of **3** appeared to dissociate and exert its known sterol biosynthesis inhibition, the remaining Ru-CTZ fragment covalently bound to parasitic DNA to cause nuclear damage. Unfortunately, the highly insoluble nature of the drug prevented its effects *in vivo,* leaving the need for a more water-soluble compound.



Clotrimazole (CTZ)
LD_{50} = 1.6 μM (*L.* major), TI > 4.7
= 5.8 μM (*T. cruzi*), TI > 1.3

3
IC_{50} = 5 nM (*T. cruzi*)

Ketoconazole (KTZ)
LD_{50} = 1.9 μM (*L.* major), TI > 63
= 1.5 μM (*T. cruzi*), TI > 82

Fig. 5 Chemical structures of antifungals clotrimazole (CTZ) and ketoconazole (KTZ) with activity against *L. major* and *T. cruzi* (LD_{50}) and selectivity over human cells (therapeutic index, TI) as determined by Martinez *et al.* [35] and Iniguez *et al.* [36]. The coordination site of each has been shown in blue and the inorganic complex **3** synthesised by Sanchez-Delgado *et al.* [37] is shown, along with its activity against *T. cruzi* (IC_{50}).

The shift to organometallic complexes of these antifungals was commenced some time later. Over two studies by the groups of Martinez *et al.* [35] and Iniguez *et al.* [36] a set of eight organoruthenium complexes was synthesised and tested for antileishmanial and antichagasic activity. Each complex contained a ruthenium centre with four coordination sites, the first being occupied by a *p*-cymene ligand as the organometallic portion, providing stability and tunable physiochemical properties, the second by a clotrimazole [35] or ketoconazole [36] ligand and the remaining two sites by one of four ligands (**4-7**, Fig. 6). The complexes were tested *in vitro* against both *L. major* promastigotes and *T. cruzi* epimastigotes. Free clotrimazole and ketoconazole, as well as complexes lacking clotrimazole and ketoconazole, were used as controls to demonstrate that the activity of each organoruthenium complex was greater than the

sum of its parts, i.e. a metal-drug synergy. In terms of activity, it was found that both clotrimazole and ketoconazole were markedly improved when coordinated with ruthenium, with low toxicity to human fibroblasts and osteoblasts. Of the clotrimazole complexes, the most successful was the dichloride complex **7a** (Fig. 6), proving 110 and 58 times more effective than free clotrimazole against *L. major* and *T. cruzi* respectively (Fig. 6). This was also a six-fold improvement on the previously reported inorganic complex **3** (Fig. 5). Upon further testing of complex **7a** against the more therapeutically relevant, amastigote form of *L. major*, it was found that antileishmanial activity was retained (IC_{70} = 29 nM). [35]

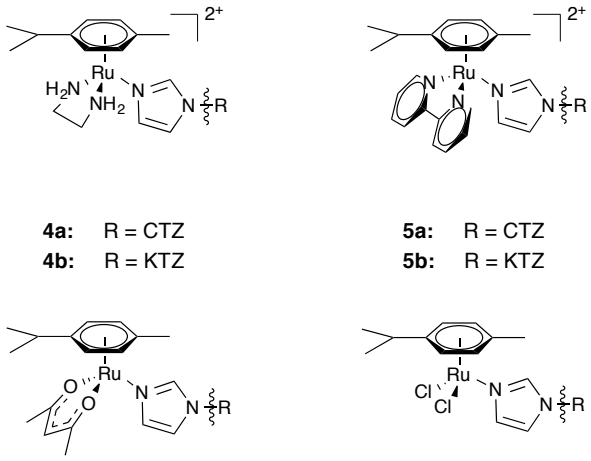


Fig. 6 Ruthenium 'piano stool' compounds reported by the group of Martinez *et al.* [35] (CTZ) and Iniguez *et al.* [36] (KTZ), with activity of **7a** against *L. major* and *T. cruzi* (LD_{50}) and selectivity over human cells (therapeutic/selectivity index, TI/SI).

In comparison to the initial inorganic complex **3**, the water solubility of the organoruthenium complexes was more favourable, and the dual mechanism of action originally proposed for this complex was investigated further. Conductivity and spectroscopic data revealed that the chloride ions of complex **7b** were labile at neutral pH and that ketoconazole did not undergo significant dissociation. From this, it was asserted that one or both chloride ligands were aquated to form a cationic species (**7c-f**, Fig. 7), which may then interact with biomolecules to liberate the antifungal ligand to carry out its known sterol biosynthesis inhibition, leaving the remains of the ruthenium complex to covalently bind to parasitic DNA and cause nuclear damage. Such insight into a plausible mode of action, as well as the demonstrated metal-drug synergy, leaves the complexes developed by the groups of Martinez *et al.* and Iniguez *et al.* excellent lead compounds for the treatment of both leishmaniasis and Chagas disease. Azole antifungals such as clotrimazole and ketoconazole, however, are known inhibitors of CYP450 enzymes such as CYP3A4, [39] interfering with the metabolism of both endogenous and drug compounds, likely resulting in drug-drug interactions. It is likely that this will be considered during further *in vivo* studies.

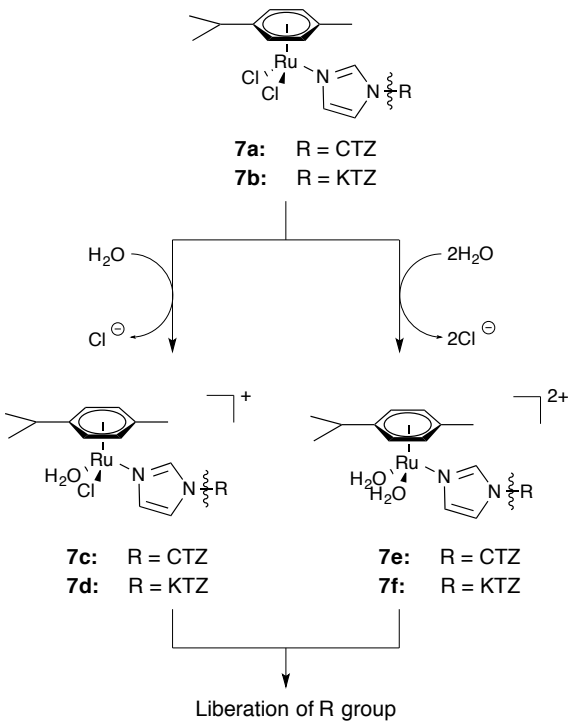


Fig. 7 The aquation of chloride ions to form active cationic species of complex **7** as proposed by the groups of Martinez *et al.* [35] and Iniguez *et al.* [36]

Chagas Disease and Sleeping Sickness: Ferrocene, Cyrhetrene and Ruthenium Analogues of Nifurtimox

Continuing with the approach of improving an existing drug, the next few studies have utilised organometallics to enhance the activity of the currently prescribed antichagasic drug nifurtimox. While Arancibia *et al.* [40,41] have discovered the importance of electronic considerations in promoting the bioreduction of nifurtimox, Demoro *et al.* [34,42] have elucidated that, when coordinated with an organoruthenium component, additional mechanisms of action present themselves.

The group of Arancibia *et al.* reported the first antichagasic organometallics in 2011. [40] All of these compounds retained the 5-nitrofuryl pharmacophore of nifurtimox, in the knowledge that its bioreduction from NO_2 to $NO_2^{\bullet-}$ would ensure a minimum antitrypanosomal activity. [43,44] It was the addition of a ferrocenyl or cyrhetrenyl fragment that served to enhance this mode of action and ultimately decrease the side effects observed with nifurtimox. Though the first set of compounds were less active than nifurtimox, all organometallic analogues exhibited higher activity against multiple strains of *T. cruzi* than the non-organometallic controls. Additionally, the inclusion of an electron-withdrawing cyrhetrenyl group yielded higher

activities than the electron-donating ferrocenyl group, suggesting that electronic communication through the conjugated system linking the organometallic fragment and 5-nitrofuryl pharmacophore was a dependent factor in antichagasic activity, as was the presence of an electron-withdrawing organometallic fragment to enhance the bioreduction of the nitro group. In light of these findings, the follow up study [41] included a second series of Schiff bases, retaining the pharmacophore as either a 5-nitrofuryl or 5-nitrothiophenyl group (**8a**-**9b**, Fig. 8). In order to confirm the importance of electronic communication, the analogues were synthesised with and without an alkyl group between the organometallic moiety and the imine bridge. A second assay revealed that when electronic communication was prevented by this alkyl group, the anti-*T. cruzi* activity suffered.

More interestingly, the cyrhetrenyl analogues (**8**, Fig. 8) demonstrated greater activity (IC_{50} 0.4-3.7 μM) compared to the ferrocenyl analogues (**9**, IC_{50} 12.3-18.3 μM, Fig. 8), particularly against the infective, trypomastigote form of *T. cruzi*. A cyclic voltametric analysis also suggested the highest incidence of nitro reduction occurred when the cyrhetrenyl moiety was present, suggesting that its electron-withdrawing effects left the 5-nitro pharmacophore electron deficient and therefore more likely to accept an electron during the bioreduction of NO_2 to $NO_2^{\bullet-}$ (Fig. 8). It was noted, however, that other possible explanations such as increased lipophilicity from the organometallic fragments could not be ruled out. The two cyrhetrenyl analogues **8a** and **8b** with electronic communication (Fig. 8) displayed activities comparable to nifurtimox.

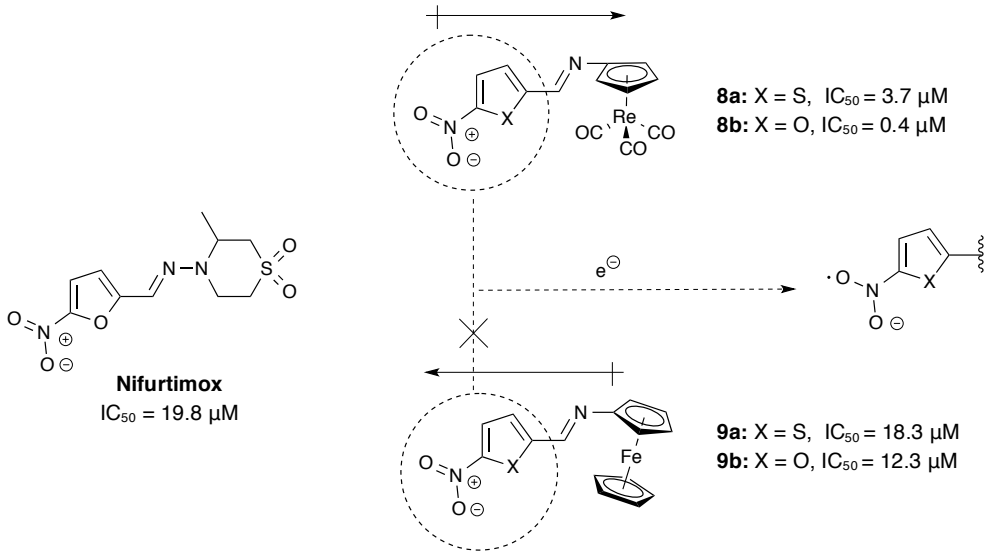
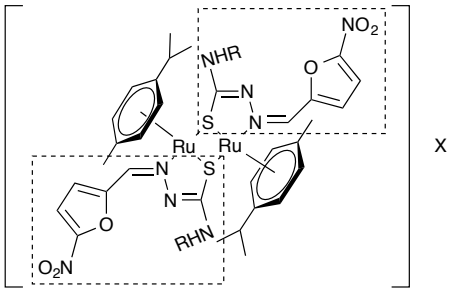


Fig. 8 Electronic effects of cyrhetrenyl (**8a** and **8b**) and ferrocenyl (**9a** and **9b**) analogues of nifurtimox on the bioreduction of NO_2 to $NO_2^{\bullet-}$ and therefore activity (IC_{50}) against *T. cruzi*, as proposed by Arancibia *et al.* [40,41]

The analogues synthesised by the group of Demoro *et al.* [34,42] were much more complex, containing two nifurtimox-like ligands bound to a larger structure (**10a-c**, Fig 9). The two organoruthenium components within this overall complex appeared to facilitate further modes of action, in addition to the bioreduction of NO_2 to $NO_2^{\bullet-}$. All complexes were found to be stable in DMSO and compound **10b** showed higher *in vitro* activity (IC_{50} 8.68-11.69 μM) than nifurtimox (IC_{50} 20.0-24.7 μM) against multiple strains of the infective, trypomastigote form of *T. cruzi*. Both **10a** and **10b** showed low toxicity to murine macrophages, though this was not evaluated for complex **10c**. While all organoruthenium compounds generated toxic free radicals (through chemical cascades initiated by the bioreduction of the 5-nitrofuryl ligands), this was not directly correlated to antichagasic activity, suggesting that other modes of action were also at play. These were suspected to be DNA binding and inhibition of cruzipain, the major cysteine protease in *T. cruzi*. Atomic force microscopy and viscosity measurements revealed that all complexes, particularly complex **10b**, interacted with DNA in a concentration-dependent manner, and a cruzipain screen revealed that complex **10b**, again, exhibited the highest anti-cruzipain activity. As observed previously, however, neither of these newly found capabilities directly correlated with overall antichagasic activity, suggesting that only a combination of these modes of action will allow the success of the new organoruthenium complexes. Demoro *et al.* had previously shown [42] that the same complexes were also active against *T. b. brucei* 427, a model strain of the causative parasite of sleeping sickness. It was found that complexes **10a** (IC_{50} 2.9 μM) and **10b** (IC_{50} 0.5 μM) were most active against *T. b. brucei,* when exposed to the parasites for a 24 hour period, though it was noted that exposure of **10b** for a further 24 hours resulted in significant cell

death, implying that **10b** may have been metabolized slowly to an active species, or irreversibly damaged the cells in an accumulative fashion. Importantly, the fact that the ruthenium-containing starting material was inactive against *T. b. brucei* demonstrated the importance of the nifurtimox-like ligand in the overall complex. A similar dual mechanism of action as discussed in the *T. cruzi* studies [34] was also proposed here, attributing cell damage to a combination of the reduction of the nitro group on the nifurtimox-like ligand to a cytotoxic free radical species, as well as the interaction of the Ru-arene moiety with trypanosomal DNA.



10a: R = H, X = Cl_2, IC_{50} = 2.9 μM (*T. brucei*)
= 59-117 μM (*T. cruzi*)
> 100 μM (mammalian cells)

10b: R = Ph, X = Cl_2, IC_{50} = 0.5 μM (*T. brucei*)
= 8.68-11.69 μM (*T. cruzi*)
= 26 μM (mammalian cells)

10c: R = Me, X = $(PF_6)_2$, IC_{50} = 10.6 μM (*T. brucei*)
= 193-231 μM (*T. cruzi*)

Nifurtimox: IC_{50} = 20.0-24.7 μM (*T. cruzi*)

Fig. 9 General structure of the antitrypanosomal complexes synthesised by Demoro *et al.* [34, 42] The nifurtimox-like ligands have been outlined. The activity (IC_{50}) of the complexes and the reference drug, nifurtimox, against *T. brucei*, multiple strains of *T. cruzi* and mammalian cells is given.

In building off the structure and mode of action of nifurtimox, the group of Arancibia *et al.* has demonstrated the importance of electronic considerations in organometallic derivatives, despite the lack of a definitive lead structure. On the other hand, the group of Demoro *et al.* has established a set of promising organoruthenium compounds with good selectivity and evaluated the previously discussed multiple-target mechanisms. Though, in terms of activity and selectivity, the drug compounds cannot be considered an official 'hit' under the guidelines set out by The Special Programme for Research and Training in Tropical Diseases (TDR), the studies have provided foundations for designing further antitrypanosomal drugs with a mechanism in mind. Perhaps more importantly, the studies can serve as a foundation for the development of broad-spectrum antitrypanosomal drugs, as the compounds were found to be active in treating both sleeping sickness and Chagas disease.

Leishmaniasis: Ferrocenyl Alkaloids and Palladium Complexes

Much of the research into novel organometallic antiprotozoal compounds has involved the modification of alkaloids, namely quinolone antibacterials. Some of these stem from the success of the 'bait-poison' concept employed in the ferrocenyl antimalarial, ferroquine, attempting to broaden the spectrum of its antiprotozoal activity with varying results. [45,46] Recently, the group of Sharma *et al.* [47] used the naturally occurring quinazolinone as an alkaloid building block for new compounds with some of the highest antileishmanial activity coming from a ferrocenyl derivative included in the study. Some of the studies involving quinolinic structures have been specifically devised with both ferrocene and leishmanasis in mind, including the work of Vale-Costa *et al.* [48] on ferrocenyl primaquine derivatives and, more recently, that of Quintal *et al.* [49] on a variety of ferrocenyl quinoline derivatives. Primaquine (Fig. 10) contains a quinolone heterocycle characteristic of the quinolone family and though its exact mechanism of action still eludes medicinal chemists, it is thought to generate reactive oxygen species that aid in its elimination of *Plasmodia* parasites responsible for Malaria. [50] As in the case for ferroquine, primaquine has shown activity against protozoan parasites, including *Leishmania,* [50] making it a suitable building block for the to combat visceral leishmaniasis. The organometallic primaquine derivatives synthesised by Vale-Costa *et al.* contained a ferrocenyl derivative linked to the aliphatic amine fragment of primaquine in order to prevent its premature metabolism via oxidative deamination (Fig. 10). Compound **11** (effective concentration 40 μM) was found to be more active *in vitro* than free primaquine (effective concentration > 80 μM) against the intracellular, amastigote form of *Leishmania infantum,* while keeping low toxicity against murine macrophages (Fig. 10). While, again, the exact mode of action was not elucidated, it was observed that the glycine spacer linking the ferrocenyl moiety to primaquine gave compound **11** an advantage over analogues containing different linkers, leaving compound **11** a potential lead structure in future antileishmanial research. It was noted, however, that compound **11** appeared to be stage-specific, and that any potential leads should be tested against multiple morphological stages of *Leishmania* parasites.

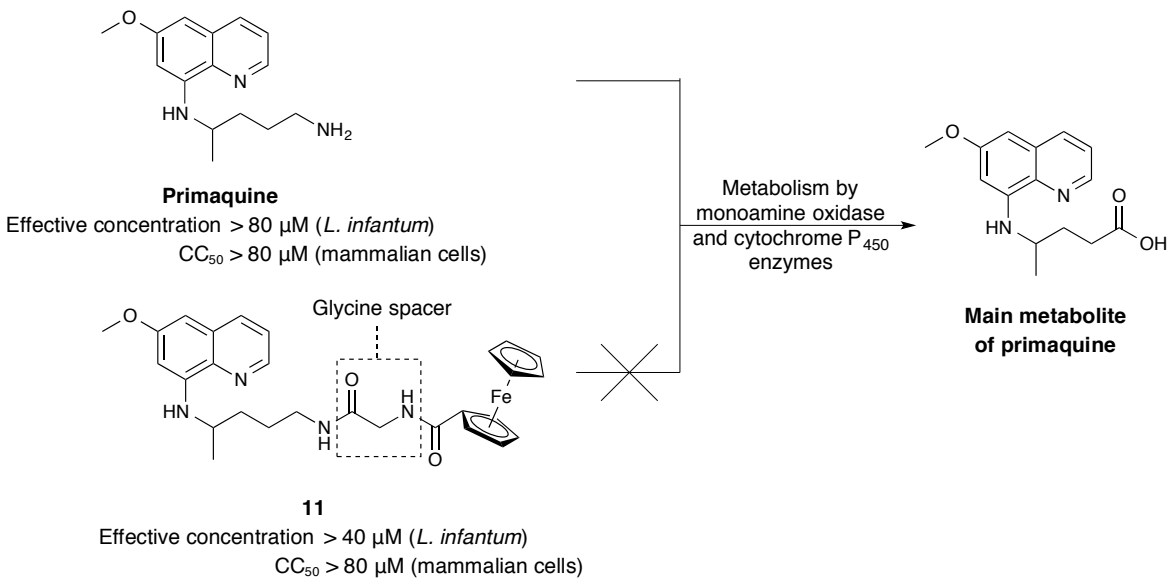


Fig. 10 Ferrocenyl analogue of primaquine synthesised by Vale-Costa *et al.* [48] with the purpose of overcoming premature oxidation of primaquine by monoamine oxidase and cytochrome P_{450} enzymes to its less active metabolite. The concentration required to eliminate *infantum in vitro* (effective concentration) and to induce 50% cytotoxicity in mammalian cells (CC_{50}) is also given.

The series of compounds investigated by the group of Quintal *et al.* [49] also contained various *N*-heterocycles such as quinoline, benznidazole, imidazole and pyridine components linked to ferrocene via either an amide or ester bridge. When tested *in vitro* on promastigotes of *Leishmania infantum,* none of the compounds surpassed the reference drug miltefosine in terms of potency. However, cyctotoxicity tests on human macrophages revealed that compounds **12-14** (Fig. 11) exhibited a greater therapeutic index than the reference drug, indicating good selectivity, and these were tested further on human macrophages infected with the amastigote form of the parasite. Here, it was found that the parasites were much more sensitive to the compounds (IC_{50} 5.19-5.73 μM), which proved more potent than the reference drug (IC_{50} 11.0 μM) this time around, and still exhibited a more favourable therapeutic index (Fig. 11). The cytotoxicity data suggested that compounds **12** and **14** were less toxic to host cells because they contained one, rather than two aromatic rings. On the other hand, electrochemical data suggested that the ease of oxidation from Fe(II) to Fe(III) increased antileishmanial activity, possibly due to the production of the toxic ferrocenium ion, as similarly suggested by Velásquez *et al.* [32] (see Fig. 4). It was also suggested that the complexes may have been more active against the parasite once inside the host cell, due to the production of ferrocenium only being facilitated in intracellular conditions.

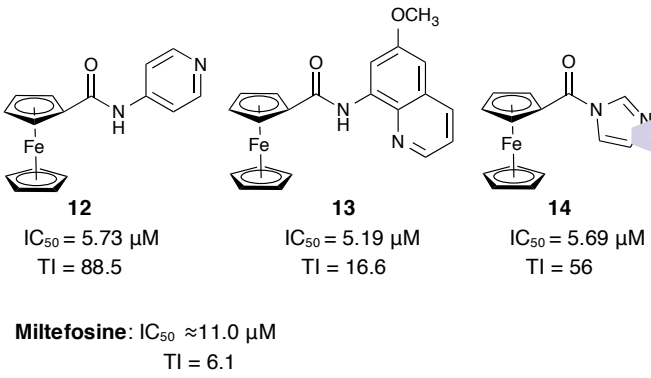
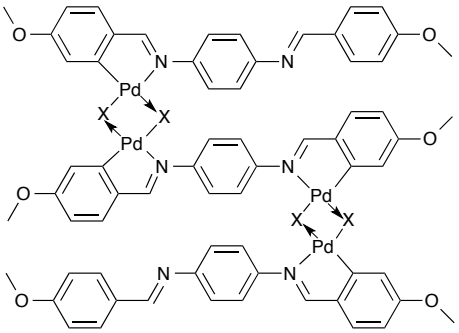
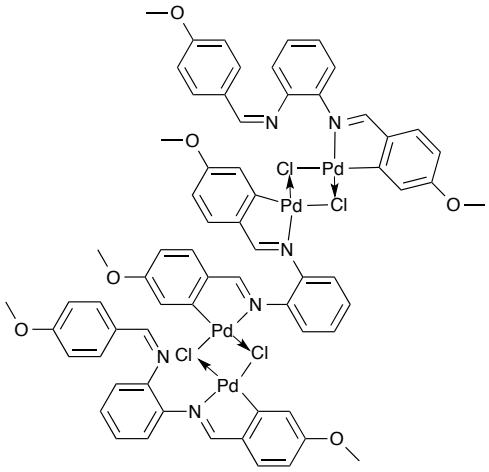


Fig. 11 Antileishmanial Compounds synthesised by Quintal *et al.* [49] with activity (IC_{50}) against *L. infantum* and selectivity over mammalian cells (therapeutic index, TI).

The final set of antiprotozoal compounds are also in relation to leishmaniasis and significantly differ in chemistry from the previous cases, encompassing all their organometallic character in a single carbon-palladium bond. The group of Franco *et al.* [5] have utilized two types of imine ligands, cyclometalating them with palladium to give the proposed structures **15a-16** (Fig. 12). Platinum and palladium are attractive candidates for drug compounds due to their ability to form square planar complexes in the +2 oxidation state and intercalate with DNA, as in the case of the anticancer drug cisplatin. Palladium was chosen over platinum due to its comparatively low toxicity. [52] Both the complexes and the corresponding free ligands were tested

against promastigotes and the more therapeutically relevant amastigotes of *Leishmania donovani,* with their selectivity being assessed using murine macrophages. Firstly, both imine ligands displayed higher activity when cyclometalated with palladium while keeping some selectivity, despite remaining less active than the reference drug, pentamidine (Fig. 12). However, as in the case of the previously discussed ferrocenyl derivatives from Quintal *et al.* [49] the selectivity indexes of the complexes (SI 0.71-1.71) were more favourable than the reference drug (SI 0.58). Furthermore, the antileishmanial activity was greater than organometallic Pt(II) and Rh(I) complexes reported almost twenty years earlier. [53] Though a mechanism of action is yet to be elucidated, it was recommended that further *in vitro* studies were commenced.





15a: X = SCN, IC_{50} = 14.84 μgmL^{-1}, SI = 1.71
15b: X = Cl, IC_{50} = 28.28 μgmL^{-1}, SI = 0.71
Free ligand: IC_{50} = 41.70 μgmL^{-1}, SI = 0.61

16: IC_{50} = 21.74 μgmL^{-1}, SI = 1.62
Free ligand: IC_{50} = 25.97 μgmL^{-1}, SI = 0.78
Pentamidine: IC_{50} = 6.62 μgmL^{-1}, SI = 0.58

Fig. 12: Proposed structures of cyclometalated organopalladium(II) complexes synthesised by Franco *et al.* [51] with activity (IC_{50}) against amastigote form of *L. donovani* and selectivity over mammalian cells (selectivity index, SI). The activity and selectivity of the free ligand and reference drug, pentamidine, are also given.

Helminth-Based Diseases

Introduction

Of the 17 items on the WHO list of neglected tropical diseases, [17] 8 are infections of the worm-like organisms known as helminths, or tapeworms. Despite constituting the largest subset on the list, the scope of research into organometallic pharmaceuticals for Helminth infections comes nowhere near that of protozoan infections. Two of these, schistosomiasis and echinococcosis, have garnered some attention within organometallic medicinal chemistry.

Schistosomiasis involves the transmission of blood flukes of the genus *Schistosoma*, while tapeworms of the genus *Echinococcus* and family Taeniidae are the basis of echinococcosis. The pathology of both diseases is of a disturbing nature. After transmission to the host, usually through infected freshwater, schistosomes dwell within the perivesical or mesenteric venous plexus. [54] Here, the 7-10 mm adult worms feed on blood and globulins through anaerobic glycolysis, regurgitating any debris back into the host's blood. [54] Sufferers are then left with internal lesions and inflammation, not from the worms themselves but their eggs, which become lodged in hepatic, intestinal, urinary and pulmonary tissues, proving potentially fatal if left untreated. [54] The pathology of echinococcosis is also dependent on the eggs of the parasite. In cystic echinococcosis, eggs of the species *Echinococcus granulosus* are transferred through contact with infected dogs and livestock, or indirectly through infected water and raw meat. The hatched embryos cluster together to form a cyst, which slowly accumulates several litres of fluid to the point of bursting, initiating allergic reactions and facilitating bacterial infections. [55] Alveolar echinococcosis, however, involves the eggs of *Echinococcus multilocularis*, which are commonly transmitted from infected cats, dogs and foxes to contaminate food sources. [56] The hatched tapeworm embryos, or oncospheres, proliferate primarily in the liver to vesicle-like metacestodes, which can exhibit tumour-like growth and metastasis as a larval mass. [55,56] Both forms of echinoccocosis are fatal if left untreated. In terms of distribution, both schistosomiasis and echinococcosis are dependent on the parasites and their mode of transmission, with

schistosomiasis mainly affecting regions of Africa, Asia and the Middle East depending on the species of schistosome, concentrated in areas lacking potable water. [54,57] Cases of cystic echinococcosis can be found across the globe, wherever livestock handling and slaughter is met with poor hygiene or where potable water is lacking. [58] Due to the prevalence of *E. multilocularis* in foxes, the alveolar form of echinoccocosis is generally restricted to the northern hemisphere. [56]

The comparative lack of research into new drug treatments for helminth infections, particularly echinococcosis, can be rationalised in terms of current treatment situations. For example, the first line of treatment for both forms of echinococcosis is not traditionally based in pharmaceuticals, but in surgery. The cysts resulting from cystic echinococcosis can be drained via a cycle of puncture, aspiration and injections. In the case of alveolar echinococcosis, immediate treatment involves the excision of the entire larval mass, usually involving the removal of the affected lobe of the liver. [55] Secondly, the chemotherapeutic options widely recommended for both forms of echinococcosis have proved generally successful. The benzimidazole compounds, albendazole and mebendazole (Fig. 13), are used in combination with cyst drainage in cystic echinococcosis and also as a primary method of preventing the growth and metastasis of larval masses in alveolar echinococcosis, both of these treatment options being long-term. [55] As a result of benzimidazole therapy, a third of cystic echinococcosis sufferers have been completely cured and the survival rate of alveolar echinococcosis dramatically increased. [55]

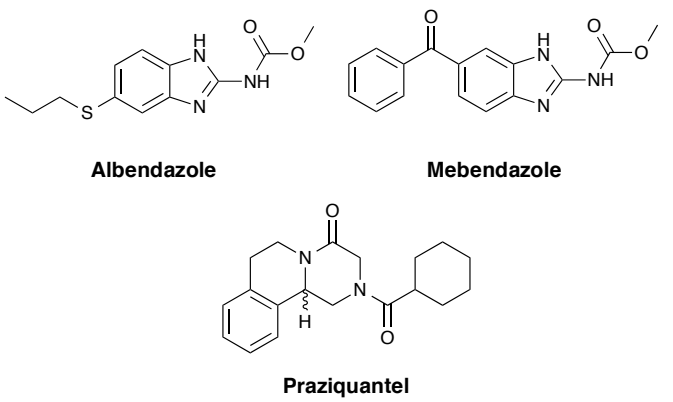


Fig. 13 Chemical structures of currently recommended drug treatments for echinococcosis (albendazole, mebendazole and praziquantel) and schistosomiasis (praziquantel).

Praziquantel (PZQ, Fig. 13), a broad-spectrum antihelmintic, can also be used during chemotherapy for echinococcosis but is widely recommended as the first line of action against schistosomiasis. Unlike treatments for protozoan infections, praziquantel is limited less by its toxicity and more by its activity and the fact that it is the only option available. [59] Despite a lack of clear evidence, it is suspected that praziquantel may act on voltage-gated calcium channels of the parasitic cell membrane but it is the rapid metabolism of the drug to the less active hydroxylated form that accounts for its limited activity. [59] In addition to this, praziquantel is inactive against juvenile schistosomes, calling for repeated administration. [59] Despite these limitations, praziquantel has proved generally successful and the main purpose of developing structural analogues is to avoid resistance genes, as well as providing alternatives to those who may not tolerate praziquantel. The latter is also a problem for sufferers of echinococcosis who cannot tolerate the existing benzimidazole chemotherapy. Bearing both issues in mind, a successful result in testing modified antihelmintic drugs would entail at least a retention of the same activity and toxicity of the existing drugs, only with a slightly different chemical structure.

Schistosomiasis: Ferrocenyl and Tricarbonylchromium Derivatives of Praziquantel

Considering it is the only pharmaceutical option available for the treatment of schistosomiasis, it is unsurprising that recent research into antischistosomal drugs has been in the modification of praziquantel. The group of Patra *et al.* [59,60] has investigated the effects of adding a ferrocenyl moiety to the *N* heterocyclic region of praziquantel [59] and, in a second study, [60] a tricarbonylchromium moiety to the aromatic region (Fig. 14). It had previously been reported [61] that replacement of the cyclohexane portion of praziquantel with a benzene fragment did not improve its activity and so the group replicated the same approach using organometallic moieties instead, acknowledging the success of similar approaches by Martinez *et al.* [35] and Iniguez *et al.* [36]. Unfortunately, the range of ferrocenyl derivatives synthesised (**17** and **18**) did not show comparable *in vitro* activity to praziquantel against adult *Schistosoma mansoni*. Turning to the aromatic part of praziquantel instead, the group opted for tricarbonylchromium moieties over ferrocenyl ones, due to their favourable drug-related properties, including ease of synthesis, increased lipophilicity and air and water stability. Praziquantel is administered as a racemate, though for the synthesized tricarbonylchromium complexes, the four possible stereoisomers were isolated and tested for antischistosomal activity, which was found to vary with each stereoisomer (Fig. 14). Only two of the four stereoisomers (**19a**, IC_{50} 0.13 μM and **19d**, IC_{50} 0.08 μM) displayed comparable *in vitro* activity to praziquantel (IC_{50} 0.10 μM) and all compounds were non-toxic to human fibroblast cells. Though possible modes of action were not investigated, the stability of the compounds in water and serum were assessed and it was confirmed that the activity was not simply due to the release of free praziquantel, but to the Cr-PZQ complex itself. Unfortunately, the promising results did not hold up in *in vivo,* with a final study [62] revealing low antischistosomal activity for all organochromium compounds. However, it was found that *in vivo* metabolism was enantioselective and it was concluded that the series of studies contributed to the understanding of novel organometallic drug design and stressed the importance of *in vivo* testing.

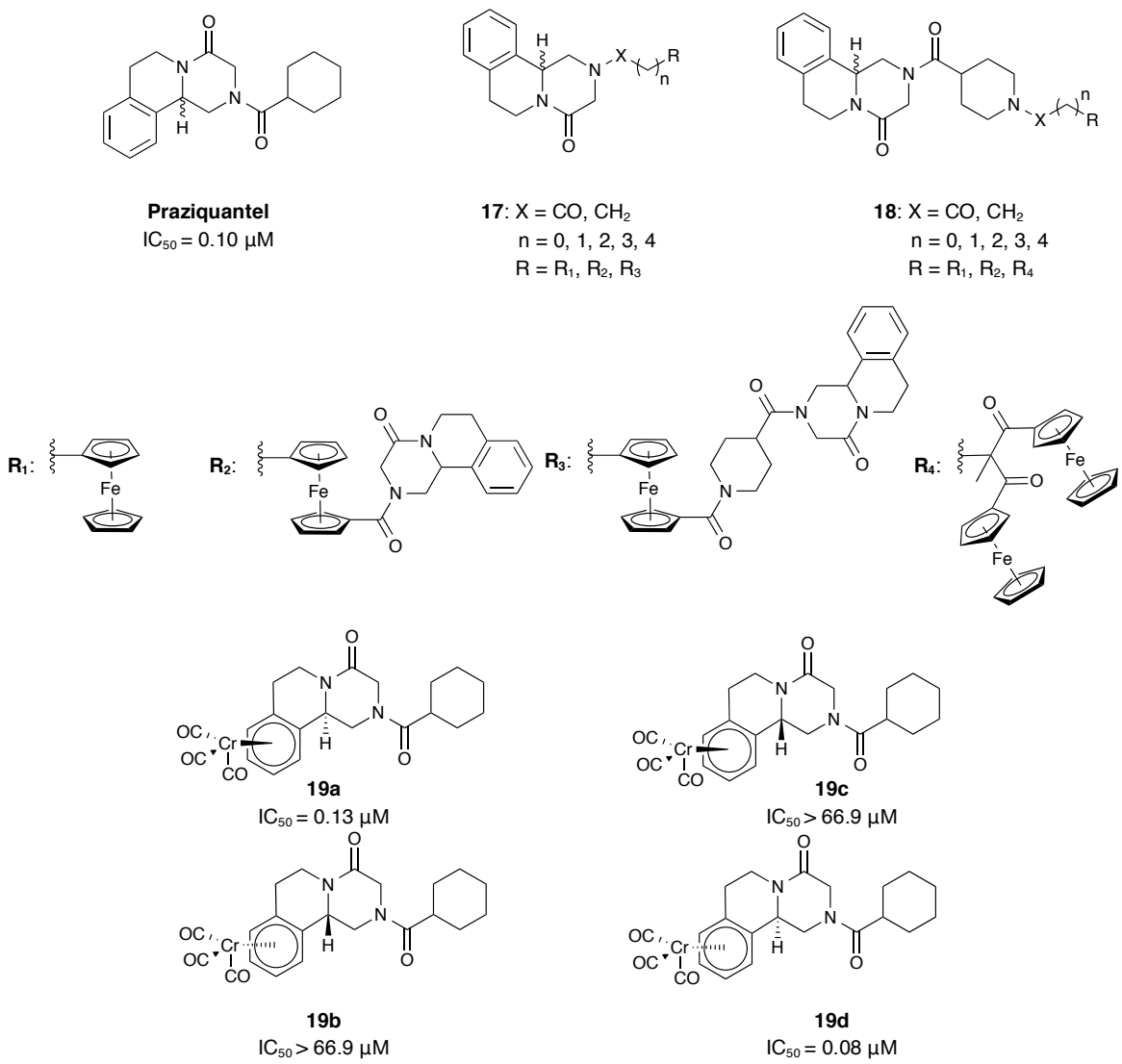
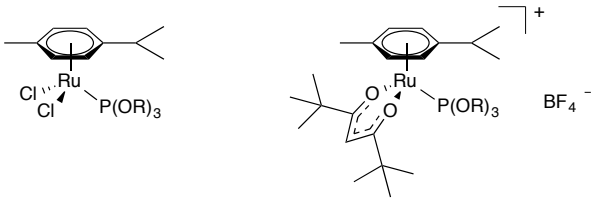


Fig. 14: Ferrocenyl and tricarbonylchromium analogues of praziquantel by Patra *et al.* [59,60] with *in vitro* activity (IC_{50}) against adul *S. mansoni.*

Alveolar Echinococcosis: Ruthenium Phosphite Complexes

Given the similarity that metacestode masses formed in alveolar echinococcosis have with tumours, it is only fitting that the major approach in finding new treatments has been in testing known anticancer compounds for antiechinococcal activity. This is precisely the direction that the group of Küster *et al.* [56] have chosen, using newly developed novel organoruthenium anticancer compounds, with additional compounds of their own (**20a**-**21c**, Fig. 15). The compounds took on the previously discussed 'piano stool' conformation, the purpose of the phosphite ligands being mainly to increase water solubility. The previously reported organoruthenium anticancer compounds [63,64] did not exhibit significant *in vitro* activity against *E. multilocularis*, nor did the first set of newly synthesis d compounds (**20a-c**, Fig. 15). However, the three variants of compound **21** did show significant activity, with **21a** (EC_{50} 1.4 μM) proving three times more active than **21b** and **21c** (EC_{5} 4.2-4.7 μM) and with comparable activity to the reference drug nitazoxanide (EC_{50} 1.2 μM). Unfortunately, the compounds were found to be toxic to human fibroblasts, though the group i hopeful that this may not translate in further *in vivo* studies. Though the mechanism of action for the complexes is stil undetermined, it was found that the activity of the compounds against *E. multilocularis* increased with hydrolytic stability, or the ability to resist hydrolysis. This likely leaves the compounds more lipophilic and therefore more readily taken up into parasitic cell membranes. Assertions were made about possible mechanisms of action, including the ability of the phosphite and

$^{t}Bu_2$acac ligands to form a 'lipophilic sphere' around the ruthenium centre, protecting the charged Ru^{2+} from forming aqua complexes. Bearing this in mind, it was proposed that the variants of complex **21** likely crossed the cell membrane and exerted their action on an intracellular target. Given that the ruthenium centre is able to donate π-electrons into both ligands, it was also asserted that the complex could lose its *p*-cymene ligand as a result, opening up sites at the ruthenium centre to possibly coordinate to the DNA of the parasite. Having somewhat anticipated the importance of ligand dissociation in the activity of the complexes, phosphite ligands were chosen over phosphine ligands due to their stronger π-accepting ability. The ruthenium centre can therefore donate π-electrons more readily to the phosphite ligands, weakening the Ru-*p*-cymene interaction. This, as well as the steric demand brought about by the phosphite ligands, would facilitate the necessary ligand dissociation.



20a: R = Et
20b: R = *i*-Pr
20c: R = Ph

21a: R = Et, EC_{50} = 1.4 μM
21b: R = *i*-Pr, EC_{50} = 4.2 μM
21c: R = Ph, EC_{50} = 4.7 μM
Nitazoxanide: EC_{50} = 1.2 μM

Fig. 15 'Piano stool' ruthenium phosphite compounds synthesised by Küster *et al.* [56] with concentrations required to induce 50% PGI release (EC_{50}) in a phosphoglucose isomerase (PGI) colorimetric assay against *E. multilocularis.* The activity of the reference drug, nitazoxanide, is also given.

Conclusion

It was asserted by W. E. Gutteridge in his 1985 review of existing chemotherapies for Chagas disease [25] that the ideal antichagasic drug would 'have minimal side effects and yet be active against all stages of the disease.' This criteria can be also be applied to ideal treatments for neglected tropical diseases alike, with the addition of structures overcoming resistance genes. Various approaches have been utilised in the search for such a compound, whether through retaining the pharmacophore of an existing drug such as nifurtimox, praziquantel, primaquine, clotrimazole or ketoconazole, or building entirely new structures with a mode of action in mind, as in the case of cyclometalated palladium(II) complexes. Most novel compounds detailed here still require further research, whether in testing against additional stages of the appropriate parasite, or elucidating a mode of action in more detail. Furthermore, it is difficult to avoid comparing any of these to the highly successful antimalarial compound, ferroquine. Whether new organometallic drugs are seen as emerging in ferroquine's shadow, or flowering in the path it has paved, one thing can be said with certainty: the redox activity, ligand coordination and structural diversity unique to organometallic compounds have shone through each case study introduced here. As more medicinal chemists harness this potential, what once was a niche is rapidly spanning a much larger field, securing organometallic medicinal chemistry as a truly credible and promising route to treating a wide variety of medical conditions, including the neglected tropical diseases of the world.

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