Metal Complexes as Chemotherapeutic Agents Against Tropical Diseases: Trypanosomiasis, Malaria and Leishmaniasis

Roberto A. Sánchez-Delgado* and Atilio Anzellotti

Centro de Química, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 21827 Caracas 1020-A, Venezuela

Abstract: Parasitic diseases represent a major world health problem with very limited therapeutic options, most of the available treatments being decades old and suffering from limited efficacy and/or undesirable collateral effects. The use of metal complexes as chemotherapeutic agents against these ailments appears as a very attractive alternative. Although the design of metal complexes with good therapeutic index is still rather empirical, a number of potential metal-based antiparasitic drugs have become available. In this review, advances in the use of metal complexes for the treatment of trypanosomiasis, malaria, and leishmaniasis as important representatives of the general area of tropical diseases is described.

Keywords: Trypanosomiasis, Malaria, Leishmaniasis, Metal complexes, Chemotherapeutic agents.

INTRODUCTION

There has been an increased interest in the last decades in the use of metal ions and complexes as chemotherapeutic agents. Emphasis has been placed mainly on cancer treatment, as a result of the great success of cisplatin and new generation analogous platinum compounds. More recently, titanium and ruthenium complexes have also entered clinical evaluation as alternatives to platinum-based drugs, aiming at novel antitumor agents exhibiting different clinical profiles and/or mechanisms of action. The development of medicinal inorganic chemistry is supported by the advances in bioinorganic chemistry, as can be evidenced in recent reviews where several applications of a variety of metal complexes in medicine, as well as the chemistry and biochemistry involved, are described with great detail [1-4].

Unfortunately, little attention has been paid so far to metal complexes in the treatment of tropical diseases like trypanosomiasis, malaria, or leishmaniasis, despite the fact that they are considered among the six diseases identified by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases as major world health problems [5]. This situation is partly due to the fact that these ailments do not constitute an important problem of the developed countries, where most of the funding for health issues originates. These large amounts of money are instead oriented to research on other important problems of the populations of those countries, such as cancer, AIDS, depression and ulcers. This dramatic situation is better appreciated in statistical terms: "of the 1233 new drugs identified as reaching the market between 1975 and 1997, only 13 were approved for tropical diseases" [6]. Since the vast majority of the millions of people affected by such diseases do not have the resources to cover the cost of a full treatment, and government policies in the affected countries have been far from adequate, there is little incentive for the pharmaceutical industry to invest large amounts of money in the development of new drugs that may not produce an economic return. Indeed many of the available treatments for parasitic diseases are 20 or more years old, and suffer from the fact that their efficacy is limited and frequently they cause undesirable collateral effects.

Thus there is an urgent need for research in this largely neglected area of medicinal chemistry and the use of metal complexes as possible chemotherapeutic agents appears as a very attractive alternative to tackle this immense problem. Such an approach makes use of the metal-drug synergism, which expresses two beneficial effects resulting from the coordination of an organic drug to a metal ion: The first is the enhancement of the biologic activity of the organic drug caused by complexation to the metal ion, possibly due to a longer time of residence of the drug in the organism allowing it to reach the biological targets more efficiently. The second effect is a decrease in the toxicity of the metal ion due to the fact that complexation with organic drugs carries the metal ion to the specific site of action and makes it less readily available for undesired reactions such as the inhibition of enzymes, or other damaging reactions leading to a malfunction in the organism. Early reports on the enhancement of activity of a therapeutic agent through metal complexation date from 1976, but it is not until the work of Williamson and Farrell where the first application of this concept is demonstrated for a tropical disease, namely trypanosomiasis [7]. Although the design of metal complexes with good therapeutic indices is still empirical, the metal-drug synergism is a powerful tool that has been used in efforts in that direction. In this review we will center our attention on the use of metal complexes for the treatment of trypanosomiasis, malaria, and leishmaniasis as important representatives of the general area of tropical diseases.

TRYPANOSOMIASIS

Trypanosomiases are a group of diseases caused by protozoa of the genus *Trypanosoma*. Two major forms affect humans: African Trypanosomiasis, also known as "sleeping sickness", is caused by *Trypanosoma brucei* and transmitted

*Address correspondence to this author at the Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 21827 Caracas 1020-A, Venezuela; Fax: (58)-212-504-1350; E-mail: rsanchez@ivic.ve

1389-5575/04 $45.00+.00

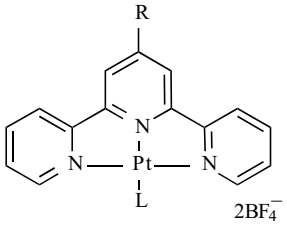
by the tse tse fly in a salivarian mode of transmission; American Trypanosomiasis, also known as "Chagas' disease", is caused by *Trypanosoma cruzi* and transmitted by reduviid bugs in a stercorarian mode. In Africa, vector control and other public health measures had a successful history of containing African Trypanosomiasis; however, war, civil unrest, and economic problems have resulted in a breakdown of these interventions, and the estimated annual incidence is now 300,000 to 500,000 cases with 66,000 deaths annually and 2.05 million "disability adjusted life years" (DALY) [8]. In Central and South America the situation is worse, with 16 to 18 million people infected and an estimated annual loss of 2.7 million DALY [9]; of particular importance is the spreading of this disease to North America due to infected immigrants which for 1987 were estimated between 25,000 and 100,000 in the U.S. [10].

The currently available chemotherapy for this type of infection is extremely limited. Pentamidine isethionate and suramin are the drugs of choice to treat the hemolymphatic stage of the west and the east African trypanosomiasis, respectively, while melarsoprol is the drug of choice for late disease with central nervous system involvement. Drugs for the treatment of Chagas disease include a nitroimidazole-based drug called benznidazole, which requires long treatments (60 days) and suffers from toxicity problems like peripheral neuropathy, central excitation, progressive dermatitis and digestive disorders. A nitrofuran-based drug, nifurtimox, was recently discontinued because of high toxicity and variable efficacy. Thus, the search for novel chemotherapeutic agents against American and African trypanosomiases is a topic of great importance and urgency. The use of metal compounds as agents against trypanosomiasis dates back to 1906, with the early observation of the activity of "ruthenrot" or ruthenium red against *T. brucei* made by Mesnil and Nicole, later confirmed by a study of Fischl and Schlossberger in 1932 [7].

Platinum Complexes

Due to the recognition of some biochemical similarities between trypanosomes and tumor cells, in terms of metabolism and lack of protecting enzymes like catalases and peroxydases, early efforts were directed to the use of antitumor agents as trypanocides, particularly cisplatin [11]. In a pioneering work by Farrell and Williamson [12, 13], a good correlation was found between antitumor and trypanostatic properties of standard platinum complexes such as cisplatin and carboplatin. In general Pt complexes were found to be active against *Trypanosoma rhodesiensis in vitro*, but not curative *in vivo* [11], unless it is administered in high doses concurrently with disulfiram or physiologic saline solution [14]. To circumvent toxicity problems related to the use of cisplatin, macromolecules such as polyglutamic acid have been employed successfully, showing a marked activity *in vivo* against *T. congolense*, responsible for Nagana cattle disease [7].

Complexes of Pt(II) with 2,2':6'2"-terpyridine ligands (Fig. (**1**)) are also known to inhibit the growth of the intracellular amastigote form of *T. cruzi* (the replicative form of Chagas disease in humans) up to 78% at a concentration of 1 μM, and to 100% for the trypomastigote form of *T. brucei* at a concentration of 0.03 μM [15]. The most effective complexes (4'chloro-2,2':6'2"-terpyridine)Pt(II) (ammine) and (4'-p-bromophenyl-2,2':6'2"-terpyridine)Pt(II) (4-picoline) were also shown to inhibit irreversibly and selectively the most important mechanism of detoxification of the trypanosoma parasite: trypanothione reductase [16].



L = NH_3, 4-CH_3-py
R = H, Cl, *p*-Br-C_6H_4

Fig. (1). Pt-terpy complexes active against Trypanosomes.

In an example of metal-drug synergism, Pt(II)(pentamidine) complexes (see Fig. (**9**)) were shown to be more effective than free pentamidine against *T. brucei in vitro* as well as *in vivo* [17].

Ruthenium Complexes

In the early work of Farrell and Williamson, the activity of a number of antitumor Ru-amine and Ru-dmso complexes (dmso = dimethylsulfoxide) was reported to be important, although lower than those achieved with Pt compounds [12]. A different approach undertaken in our laboratories, involves coordination of ruthenium to "Sterol Biosynthesis Inhibitors" (SBIs), such as clotrimazole (CTZ) and ketoconazole (KTZ), to produce remarkably effective compounds that make use of the metal-drug synergism. SBIs have been used in the rational design of new antiparasitic agents, since they can block specific lipid biosynthesis pathways by *e.g.* inhibiting the enzyme cytochrome P450 14α-demethylase involved in the production of ergosterol, which is essential for the parasite [18]. Coordination of clotrimazole to ruthenium in $RuCl_2(CTZ)_2$ (see Fig (**2**)) results in a 90% inhibition in the proliferation of the epimastigote form of *T. cruzi* at a concentration of 10^{-5} M, while the parental compound CTZ has only a modest effect on the growth rate at the same concentration. Also very importantly, whereas the parental drug produces a trypanostatic effect (lowers the rate of proliferation), the Ru complex is indeed a *trypanocide* (parasites are dead after treatment). In further experiments performed in the highly infective intracellular amastigotes of *T. cruzi* grown on mammalian (Vero) cells, eradication of the experimental infection was achieved at 10^{-8} M of the Ru complex, which represents an enhancement of the activity of free CTZ by a factor of about 10. Furthermore, no deleterious effects of $RuCl_2(CTZ)_2$ on the mammalian cells were evident at concentrations of the drug 10 times higher than the one required to eradicate the infection, whereas free CTZ did affect Vero cells at the concentration in which it was effective. This indicates that the Ru complex is not only much more active but also much less toxic than free CTZ [19].

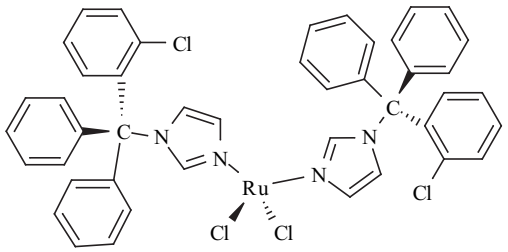


Fig. (2). Ru-clotrimazole complex with trypanocidal properties.

The mechanism of action of this complex has been investigated in some detail, and it is now thought that it involves hydrolysis of the chloride ligands to produce the bis(aquo) intermediate $[Ru(CTZ)_2(H_2O)_2]^{2+}$, which then interacts with DNA (through covalent binding). This results in the liberation of the CTZ ligand that then exerts its normal "SBI" action. The superimposition of these two modes of action (SBI + DNA-binding) determines the higher activity and lower toxicity observed [20, 21]. The Ru(III) analog, $RuCl_3(CTZ)_3$, also shows a good activity but the synergistic enhancement is not as good as for the Ru(II) compound [22].

Ketoconazole (KTZ) (Fig. (**3**)) is another "SBI" with improved pharmacokinetic and physicochemical properties that allows a better tissue penetration, higher biologic half-life and wider range of action. The activity of KTZ is also greatly enhanced upon coordination to ruthenium ions, displaying approximately 70% of inhibition in the proliferation of epimastigotes of *T. cruzi*, as compared to 20% exhibited by free KTZ, when administered as 10^{-6} M DMSO solutions; $RuCl_2(KTZ)_2$ and $RuCl_3(KTZ)_2(H_2O)$ also showed very similar activities [22]. Nevertheless, tests performed with $RuCl_3(KTZ)_2(H_2O)$ against amastigotes of *T. cruzi* grown on Vero cells revealed remarkable enhancement of activity with eradication of the experimental infection being achieved at concentrations as low as 10^{-10} M [23].

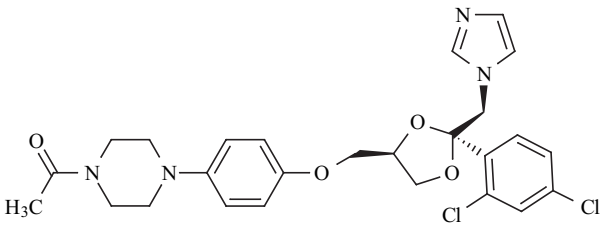


Fig. (3). Ketoconazole, an SBI that forms Ru(II) and Ru(III) complexes with trypanocidal properties.

Other Metal Complexes

The coordination of CTZ to other metal ions, as in RhCl(COD)(CTZ) (COD = 1,5-cyclooctadiene) and $AuCl_3$(CTZ), also resulted in the enhancement of the activity of the free organic drug, whereas in $[Cu(CTZ)_2]PF_6$ and in $K_2[PtCl_4(CTZ)_2]$ the effect on the proliferation of epimastigotes of *T. cruzi* was actually slightly lower than that of free CTZ at the same concentrations [20]. The higher efficacy of ruthenium compounds might be associated to a similarity in the metabolism of ruthenium and iron, which has also been claimed to be responsible for the low toxicity of ruthenium compounds as antitumor agents [24]. Further studies performed with $[Cu(CTZ)_4]Cl_2$, $[Cu(CTZ)Cl_2]_2$, $[Cu(KTZ)_3]Cl_2$, $[Cu(KTZ)Cl_2]_2$, $[Au(CTZ)PPh_3]PF_6$ and $[Au(KTZ)PPh_3]PF_6$, also on epimastigotes of *T. cruzi* showed that all these complexes were considerably more active than the free ligands, but the enhancement of the activity was not markedly influenced by the number of ligands attached to a single metal center or even to the identity of the metal ion [25].

The concept of metal-drug synergism against a parasite was introduced in the early work of Farrell and Willliamson who showed that the chemotherapeutic index of the trypanocides Berenil, Ethidium and Samorin (Fig. (**4**)) could be enhanced by complexation to Pt, Rh, and Ru, either through an increase in the activity, or a decrease in the toxicity, or both [12].

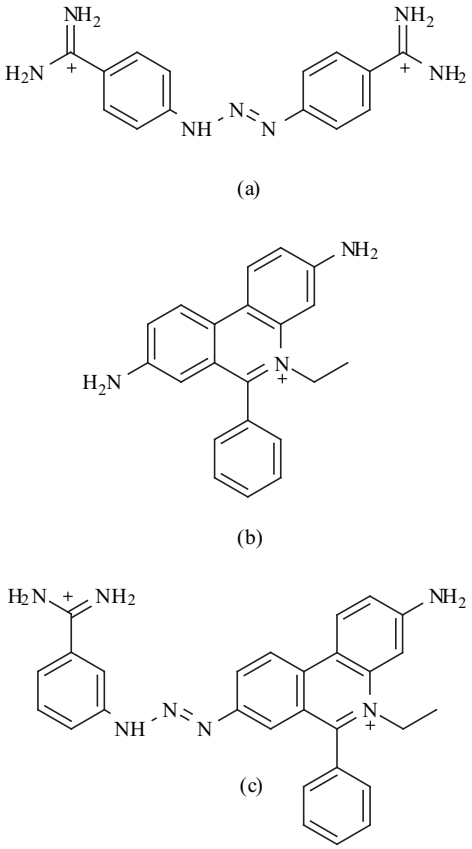


Fig. (4). Cations of (**a**) Berenil; (**b**) Ethidium; and (**c**) Samorin that form Pt, Rh, and Ru complexes with activity against Trypanosomes.

MALARIA

Malaria has been known since antiquity and the search for an appropriate chemotherapeutic agent has extended for more than 100 years. It is the most widespread tropical disease in the world affecting 300-500 million and killing 2 million people every year, including 200-300 children per hour. It is endemic in most of the tropical regions of the planet putting 2,000,000,000 persons at risk in 10 countries. This disease is transmitted by the female of a mosquito of the *Anopheles* genus and caused by protozoan of the genus *Plasmodium*. Early measures to eradicate this disease were aimed at controlling the insect vector, mainly through

widespread fumigation with DDT. Environmental concerns about the indiscriminate use of such toxic insecticides, together with the resistance developed by the insects to these chemicals, make them unacceptable today, and more efficacious and ecologically friendly insecticide alternatives are still lacking. This, coupled with a deficiency of adequate sanitary measures and social development programs in the affected countries, has caused a dramatic resurgence of this severe ailment in the last few years [5, 6].

Since the discovery of quinine (Q), several drugs have been used with success in the treatment of malaria, notably chloroquine (CQ), primaquine (PQ), and amodiaquine (ADQ) (Fig. (**5**)). Nevertheless, these drugs have lost efficacy due to the increased resistance developed by the parasites, which is particularly severe in parts of South East Asia and South America. Efforts toward the development of a vaccine against malaria have advanced considerably, but a preventive solution of this type does not seem to be an immediate possibility [26], and therefore efforts to develop novel antimalarials capable of overcoming resistance must be encouraged. Since molecules like the commonly employed aminoquinoline-type drugs provide ample coordination possibilities, they can be regarded as excellent ligands for metal ions and thus the use of metal complexes for this type of application offers ample and exciting new avenues for research and development.

The first attempt at producing metal complexes with antimalarial properties dates back to 1987 [27]. This work tried to take advantage of the metal-drug synergism through the binding of quinoline-based antimalarials such as amodiaquine and primaquine to several metal ions in different oxidation states. Nevertheless, in spite of the wide number of compounds tested, no increase in the activities of the organic drugs due to metal coordination was observed, nor was the effect of the parental drugs in any way dependent on the metal attached to it.

Metal-Chloroquine Complexes

More success on metal-based antimalarials has come from modifying the most widely used drug chloroquine (CQ) through coordination to metal-containing fragments. Although the mechanisms of action and of resistance of CQ have been extensively investigated, they are still not fully understood [28]. It is generally accepted that the mode of action of CQ targets the catabolism of the host's hemoglobin by the parasite, which takes place in an organelle called the "digestive vacuole", a very acidic site within the parasite cell. As a result of hemoglobin degradation, the heme group is released as a byproduct that is toxic for the parasite, which is capable of isolating it and subsequently polymerizing it into a non-toxic crystalline matrix called hemozoin. CQ and other related aminoquinolines can effectively block the polymerization reaction thereby result in the accumulation of toxic heme within the parasite. Nevertheless, a variety of *Plasmodium* parasites have gradually become resistant to the drug by becoming able to accumulate less CQ on its digestive vacuole. Attaching a large metal-containing fragment to this molecule results in a strong modification of the electronic properties of the parental compound, as well as of its transport properties. Furthermore, the almost unlimited variety of possible fragments that can be coordinated to CQ represents a very extensive area for exploring antimalarial activity.

The free (neutral) CQ base possesses three basic N-donor atoms well-adapted to binding to Lewis-acidic metal ions, thus offering excellent synthetic possibilities for new metal complexes with possible antimalarial activity. Ruthenium- and rhodium-CQ derivatives (Fig. (**6**)) have become available through relatively simple synthetic procedures [29]. Coordination of CQ to ruthenium in the complex $[RuCl_2(CQ)]_2$ (**1**) has proved effective to circumvent the resistance mechanism in the case of *P. falciparum*, since the

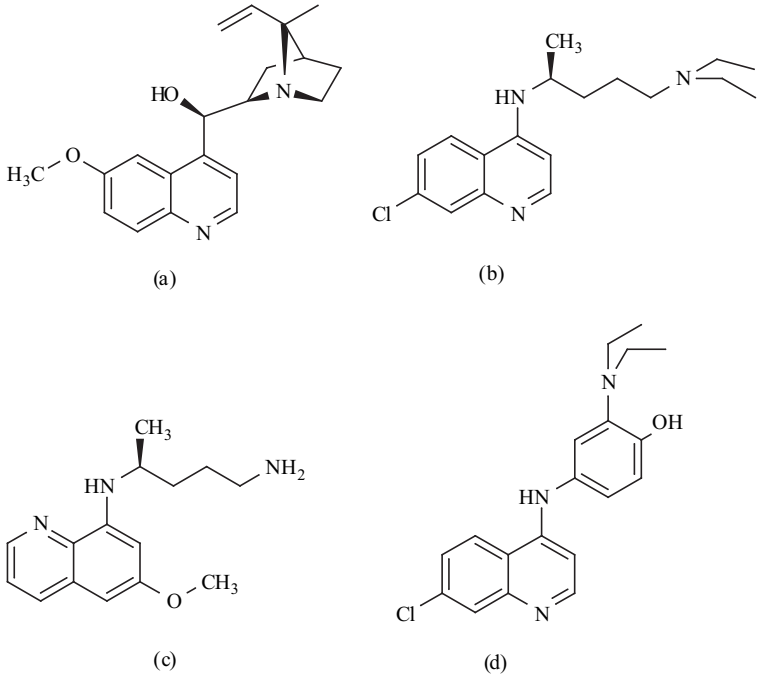


Fig. (5). Classical antimalarials (**a**) Quinine; (**b**) Chloroquine; (**c**) Primaquine; and (**d**) Amodiaquine.

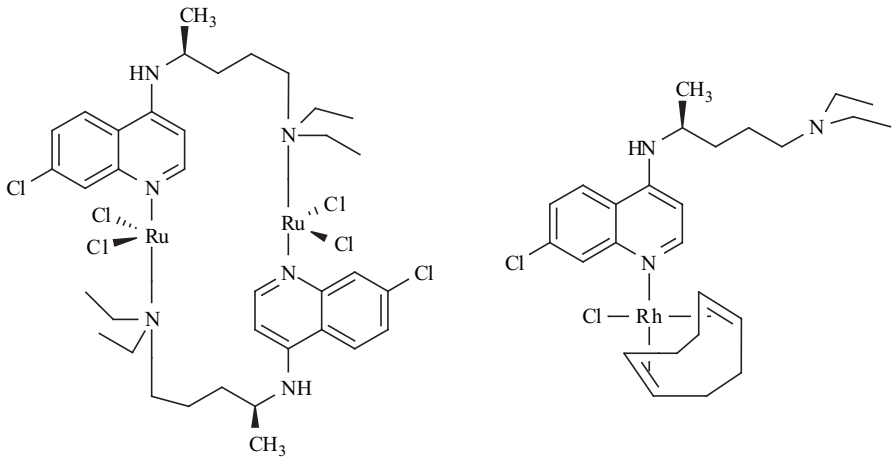


Fig. (6). Ru(II) and Rh(I) complexes with antimalarial properties.

activity of the parental drug is enhanced 4.5 times through the metal-drug synergism. Similarly, the *in vitro* activity against *P. berghei* was increased by a factor of 4, while the rhodium complex Rh(COD)(CQ)Cl (**2**) displayed essentially the same activity as CQDP. On the other hand, in *in vivo* tests (*P. berghei*) at equivalent CQ concentrations, CQDP reduced the parasitemia by 55% while for the Ru complex (**1**) the reduction reached 94% and for the Rh complex (**2**) 73%. No acute toxicity effects were observed in treated mice [29].

Gold complexes have also displayed good antimalarial activities. In *in vitro* experiments the complex $[Au(CQ)(PPh_3)][PF_6]$ was found to be 9 times more active than CQDP against the chloroquine-resistant FcB1 strain of *Plasmodium falciparum*, 5 times more active for the more aggressive FcB2 strain and 22 times more active against the rodent malarial parasite *P. berghei*. This high activity was also observed *in vivo*, as a concentration of CQ in **1** equivalent to 1 ED_{50} of the parental compound was sufficient to suppress parasitemia by 84% as compared to untreated controls. No acute toxicity was observed [30]. Further *in vitro* studies on the activity of gold compounds against several strains of *P. falciparum* have shown that in complexes $[Au(CQ)(PR_3)][X]$ ($X = PF_6$, NO_3) [31, 32] the nature of the anion is unimportant for the activity, which in these experiments [32] was lower than the one previously reported [30]. Varying the R substituent in the phosphine ligand did not indicate a clear trend. Au(III) derivatives, such as $[(CQ)_2Au(Cl)_2]Cl$ and $[(CQ)Au(Cl)(SR)(Et_2O)]Cl$ were also found active but again, no structure-activity correlations were evident.

Ferrocene-Chloroquine Derivatives

Another very interesting approach which has provided novel complexes of superior properties to those of CQDP takes advantage of the stability and non-toxicity of a ferrocenyl moiety, which is covalently linked to a side chain of a 4-aminoquinoline. This results in a potent CQ analog called ferroquine (FQ) where CQ is not coordinated to the iron atom, but rather it is bound through one of the cyclopentadienyl rings of the ferrocene (Fig. (**7**)) [33].

The *in vitro* activity of FQ against *P. falciparum* is similar to CQDP for chloroquine-sensitive strains, but up to 22 times more active against chloroquine-resistant strains. FQ also exhibited remarkably high antimalarial activity *in vivo* on mice infected with *Plasmodium berghei*, *Plasmodium yoeli* and *Plasmodium vinckei vinckei* [33, 34]. The activity of FQ was also evaluated against *Plasmodium falciparum* isolates from Senegal [35] and from Gabon [36] and it was found to be considerably more active than chloroquine diphosphate (35-times higher against CQ-resistant isolates) or a range of other antimalarial drugs. Although toxicological data on ferroquine do not seem to be available, ferrocene has been shown not to be toxic after oral administration in dogs [37]. Another interesting feature of ferroquine is that there is no difference in the activities associated with individual enantiomers, meaning that costly chiral resolution of the drug is not necessary [38].

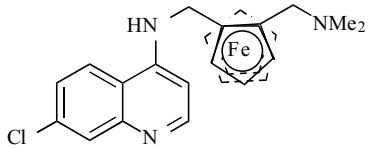


Fig. (7). The structure of ferroquine.

Further work on FQ derivatives aimed at finding some structure-activity correlations indicates that modifications at the terminal tertiary nitrogen residue with different alkyl groups resulted in lower activities [33]. A systematic study of the influence of the position of the ferrocene moiety in chloroquine revealed that although substitution in the side chain caused the activity to be greatly enhanced, tethering the ferrocenyl group to the quinoline nitrogen atom actually decreased the activity by a factor of 3 [39]. Neutral and cationic FQ-derived compounds exhibited similar IC_{50} values in *in vitro* tests, but the presence of a charge might increase the solubility and perhaps improve its *in vivo* activity [40]. In any case it has been stated that the ferrocenyl fragment does not have an intrinsic anti-malarial activity, but it enhances the effectiveness of the CQ when it is covalently bound to the molecule in the side chain. Amine and urea derivatives of FQ have also proved to be more active than CQ alone against both resistant and susceptible strains [41]. Bimetallic complexes where Rh- or Au-containing fragments are coordinated to the quinoline N-atom of FQ did not show any improvement in the behavior of FQ [32].

N_4O_2 Core Complexes

A different class of anti-malarials not based upon the quinoline moiety consists of an interesting group of metal complexes of the hexadentate ethylenediamine-*N,N'*-bis[propyl (2-hydroxy-(R)-benzylimino)] ligand ((R)-ENBPI), and the corresponding Schiff base reduced amino analog ((R)-ENBPA). Both ligands are capable of forming stable compounds with Al(III), Fe(III), Ga(III) and In(III) (Fig. (**8**)).

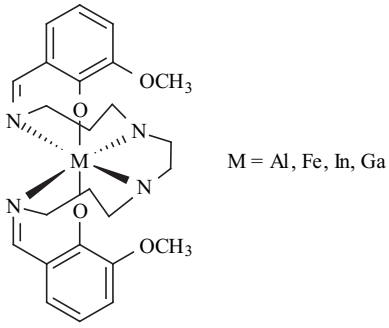


Fig. (8). Metal complexes of N_4O_2 ligands with antimalarial properties.

These compounds offer a modular nature and can be modified both by variation of the substituents on the aromatic rings and the hydrocarbon backbone independently, while retaining the biologically desirable lipophilic monocationic characteristics [42, 43]. The 4,6-dimethoxy-ENBPI Fe(III) and the 3-methoxy-ENBPA Ga(III) resulted the most potent among the series tested and generally antimalarial potency correlate well with the ability to inhibit heme polymerization [44]. The mechanism of action of these compounds has been related to the formation of a salt complex between the anionic propionate moiety of heme and the cationic drug complex, thus the overall charge of the complex is critical to the drug's ability to inhibit hemozoin formation [45]. Another complex of this type, namely [{1,12-bis-(2-hydroxy-3-methoxybenzyl)-1,5,8,12-tetraazadodecane}Ga(III)] or $[Ga(madd)]^+$ is able to inhibit heme polymerization and, very interestingly, displays selective antimalarial activity against chloroquine-resistant clones of *P. falciparum* [46]. The selective biological activity of this compound has been explained taking into account the spatial orientation of the peripheral regions of the aromatic moieties, including the methoxy functionalities, rather than the central metal core.

Porphyrins

Studies using metal-free porphyrins indicate inhibition of heme polymerization through an apparent π-π interaction with hematin. Ferrous-protoporphyrin IX [Fe(II)PPIX] also inhibited hemozoin polymerization [47]. The activity of the endoperoxide drug artemisinin was increased 11-fold by the complex [meso-tetrakis(4-sulfonatophenylporphyrin)Mn] (Mn-TPPS) [48]. Further *in vitro* and *in vivo* studies with Mn-TPPS show indeed synergistic effects of the complex with artemisinin, and other endoperoxide analogs like artemether and arteflene *in vitro*, but experimental results *in vivo* using infected mice with *P. vinckei petteri* showed good results only with artemisinin [49].

LEISHMANIASIS

Leishmaniases are a family of diseases that involve the parasitization of the reticuloendothelial system by haemoflagellates of the genus *Leishmania*. There are three main forms of the disease caused by different organisms, namely visceral leishmaniasis (*L. donovani*), muco-cutaneous leishmaniasis (*L. braziliensis*) and cutaneous leishmaniasis (*L. tropica*); the first being the most severe.

The available chemotherapy for leishmaniasis relies on pentavalent antimonial compounds *i.e.* sodium stibogluconate (Pentostam) or meglumine antimoniate (Glucantime) which are not totally safe and/or efficacious. For the treatment of unresponsive cases amphotericin B and pentamidine (introduced in the 1940s) can be used, although they are not fully effective either and also produce toxic side effects. Moreover drug-resistant strains of *Leishmania spp.* are beginning to appear, which determine an urgent need for the development of new drugs [50].

As in the cases of malaria and trypanosomiasis, the metal-drug synergism has been employed to try to produce new potent chemotherapeutic agents by complexation of pentamidine (PA) (Fig. **9**), a compound of known antileishmanial properties, to metal-containing fragments.

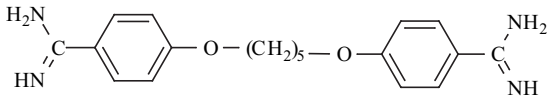


Fig. (9). Structure of pentamidine, an antileishmanial drug that forms active metal complexes.

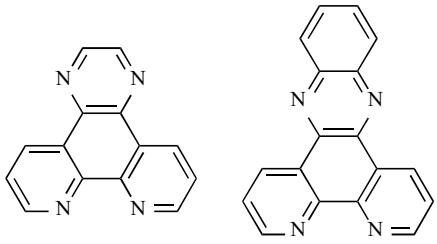
Several compounds of formula $[M(L_2)(PA)]_2$ 2[X] (where M = Rh, Ir; L_2 = 1,5-cyclooctadiene, 1,3,5,7-cyclooctatetraene, or $(CO)_2$; X = $B(C_6H_5)_4^-$, NO_3^-, ethylfumarate) were found to be active against *L. donovani* promastigotes, some of them considerably more active than pentamidine isethionate [51]. Although the complex [Ir(COD)(PA)][BPh_4] exhibits the same IC_{50} value as free pentamidine in *in vitro* experiments, its *in vivo* activity reaches 23% and 32% of parasite suppression for *L. donovani* and *L. major*, respectively, under conditions were pentamidine isethionate is inactive; the complex also displayed a lower toxicity than the parental compound [52, 53]. The related compound $[Ir_2(COT)_2(PA)]$[alizarin red]$_2$ was also shown to be at least twice as active as pentamidine isethionate against the amastigote form of *L. donovani* and a synergistic effect was noted when this complex was administered in combination with pentamidine, amphotericin B and paromomycin [54]. Pt(II)-pentamidine complexes appear to be less active than Rh(I) and Ir(I) analogs against amastigotes of *L. Donovani* [55].

Other complexes that have shown a slight activity against promastigotes and amastigotes of *L. donovani* are [cis-Pt(II)(dac)(2,5-dihydroxybenzenesulfonate)$_2$] (dac = 1,2-diaminocyclohexane), $[RhCl(CO)_2$(2-aminobenzothiazole)], and $[RhCl(CO)_2$(2-methylbenzothiazole)] [56], as well as a series of Os(III) complexes with nitroimidazole dithiocarbamates, benznidazole dithiocarbamates, and related ligands [57].

On the other hand, Pt-complexes of 2,2':6'2"-terpyridine of the type depicted in Fig. **1** are remarkably active and

exhibit 100% inhibition of the growth of the intracellular amastigote forms of *L. donovani* at a concentration of 1 μM [15]. These complexes also exploit the intercalative properties of the terpyridine ligand along with covalent binding of the Pt(II) center. The highest activity against *L. donovani* was found for the case of 4'-bromophenyl-terpyridine and a *trans*-NH_3 ligand.

Copper complexes with typical DNA-intercalating ligands, such as $[Cu(L)_n(NO_3)_{2-n}](NO_3)_n$ where L = dppz or dpq (Fig. (**10**)) have shown activity against *Leishmania braziliensis* (causative of the muco-cutaneous mode of the disease), and it has been demonstrated that their action is related to their ability to interact with DNA. $[Cu(dppz)_2](NO_3)_2$ was the most effective complex in this series, and the activity order was $[Cu(dppz)_2](NO_3)_2 > [Cu(dppz)(NO_3)](NO_3) > [Cu(dpq)_2](NO_3)_2 > [Cu(dpq)(NO_3)](NO_3)$ [58, 59].



dpq dppz

Fig. (10). DNA intercalating ligands used for Cu complexes with antileishmanial activity.

Other simple compounds such as $ZnSO_4$ have been tested clinically for the treatment of cutaneous leishmaniasis with very promising cure rates of 96.9% in a 45 days treatment with oral daily doses of 10 mg/kg [60].

FINAL COMMENTS

In this post-genomic era, the complete genomes of parasites are becoming completely sequenced, as was reported very recently for *Plasmodium falciparum* [61]. This certainly points to the future development of more efficient drugs when the potential targets are clearly identified. But it is also true that it could take several years to bridge the gap from gene function identification to a complete understanding of the whole organism. In view of the urgent need for new efficacious drugs to treat the millions of people suffering from parasitic diseases, the routes to improved drugs herein described, based on the rational design of metal-complexes appear as a very useful alternative possibly worth developing up to a commercial stage, if the biological activities can be made high enough and the corresponding toxicity is controlled. This is an exciting area of research that will certainly continue to produce important results and that needs to be encouraged worldwide.

ACKNOWLEDGEMENT

The Venezuelan Academy of Science is thanked for a Graduate Fellowship to Atilio Anzellotti.

ABBREVIATIONS

MIC = Minimum Inhibitory Concentration

COD = 1,5-Cyclooctadiene

COT = 1,3-1,5-Cyclooctatetraene

IC_{50} = Concentration of the Drug to achieve a 50% of inhibition of disease

IC_{90} = Concentration of the Drug to achieve a 90% of inhibition of disease

ED_{50} = Effective dose related to a reduction of parasitemia of 50% compared to untreated controls

ED_{90} = Effective dose related to a reduction of parasitemia of 90% compared to untreated controls

REFERENCES

[1] Guo, Z.; Sadler, P. J. *Advances in Inorg. Chem.* **2000**, *49*, 183.
[2] Guo, Z.; Sadler, P. J. *Angew.Chem. Int. Ed.* **1999**, *38*, 1512.
[3] Clarke, M. J.; Zhu F.; Frasca, D. R. *Chem. Rev.* **1999**, *99*, 2511.
[4] Reedijk, J. *Chimia* **1997**, *51*, 21.
[5] Brown, J. R. In *Chemotherapy of Tropical Diseases*; M. Hooper; Ed.; John Wiley & Sons: New York, **1987**; pp 72 –101.
[6] Pecoul, B., Chirac, P., Trouiller, P., Pinel, J. *J. Am. Med. Assoc.* **1999**, *281*, 361.
[7] Farrell, N. P. In Catalysis by Metal Complexes ; Ugo, R.; James, B. R., Eds.; Kluwer Academic Publishers; Dordrecht, **1989**; Vol. 11, Ch. 10, pp. 222-242.
[8] UNDP- World Bank –WHO. World Health Report **2001**.
[9] World Bank. World development report **1993**/**1994**: investing in health. Oxford University Press, Oxford, England.
[10] Kirchhoff, L.V., Gam, A.A., Gilliam, F. C. *Am. J. Med.* **1987**, *82*, 915.
[11] Kinnamon, K.E.; Steck, E.A.; Rane D.S. *Antimicrob. Agents. Chemother.* **1979**, *15*, 157.
[12] Farrell, N.P.; Williamson, J.; McLaren D.J. *Biochem. Pharmacol.* **1984**, *33*, 961.
[13] Williamson, J.; Farrell, N.; McLaren D.J. *Parasitology* **1982**, *85*, XIV.
[14] Wysor, M. S.; Zwelling, L. A.; Sanders, J. E.; Grenan, M. M. *Science* **1982**, *217*, 454.
[15] Lowe, G.; Droz, A.S.; Vilaivan, T.; Weaver G.W.; Tweedale, L.; Pratt, J.M., Rock, P.; Yardley, V.; Croft, S. L. *J. Med. Chem.* **1999**, *42*, 999.
[16] Bonse, S.; Richards, J.M.; Ross, S.A.; Lowe, G.; Krauth-Siegel, R.L. *J. Med. Chem.* **2000**, *43*, 4812.
[17] Dreyfuss, G; Pénicaut, B.; Nicolas, J. A.; Craciunescu, D.; Loiseau, P. *Trop. Med. Parasitol.* **1993**, *44*, 95.
[18] Urbina, J. A. *Parasitology*, **1997**, *114*, S91.
[19] Sánchez-Delgado, R. A.; Lazardi, K.; Rincon, L.; Urbina, J. A. *J. Med. Chem.* **1993**, *36*, 2041.
[20] Sánchez-Delgado, R. A.; Navarro, M.; Lazardi, K.; Atencio, R.; Capparelli, M.; Vargas, F.; Urbina, J. A., Bouillez, A.; Noels, A. F.; Masi, D. *Inorg. Chim. Acta* **1998**, *275-276*, 528.
[21] Sánchez-Delgado, R. A.; Anzellotti, A. unpublished results.
[22] Navarro, M.; Lehmann, T.; Cisneros-Fajardo, E. J.; Fuentes, A.; Sánchez-Delgado, R. A.; Silva, P.; Urbina, J. A. *Polyhedron.* **2000**, *19*, 2319.
[23] Sánchez-Delgado, R.A.; Lazardi, K.; Urbina, J. A. unpublished results.
[24] Frasca, D.; Ciampa, J.; Emerson, J.; Umans, R. S.; Clarke, M. J. *Met.-Based Drugs* **1996**, *3*, 197.
[25] Navarro, M.; Cisneros-Fajardo E. J.; Lehmann, T.; Sánchez-Delgado, R. A.; Atencio, R.; Silva, P.; Lira, R.; Urbina, J. A. *Inorg. Chem.* **2001**, *40*, 27, 6879.
[26] Lioy, E., Suarez, J., Guzman, F., Siegrist, S., Pluschke, G.; Patarroyo, M. *Angew. Chem. Int. Ed.* **2001**, *40*, 2631.
[27] Wash, N.; Singh, H. B.; Gajanana, A.; Raichowdhary, A. N. *Inorg. Chim. Acta* **1987**, *135*, 133.

[28] Emerson, L. R.; Nau, M. E.; Martin, R. K.; Kyle, D. E.; Vahey, M.; Wirth, D. F. *Antimicrob. Agents. Chemother*. **2002**, *46*, 3, 787.
[29] Sánchez-Delgado, R. A.; Navarro, M.; Pérez, H.; Urbina, J. A. *J. Med. Chem.* **1996**, *39*, 5, 1095.
[30] Navarro, M.; Pérez, H.; Sánchez-Delgado, R. A. *J. Med. Chem.* **1997**, *40*, 12, 1937.
[31] Navarro, M.; Vásquez, F.; Sánchez-Delgado, R. A.; Pérez, H.; Schrevel, J.; Sinou, V. (unpublished results).
[32] The complex [Au(CQ)(PPh_3)][NO_3] has been independently synthesized and evaluated in the group of Moss J. R. in South Africa: Blackie, M. A. L., New mono and bimetallic chloroquine derivatives: synthesis and evaluation as antiparasitic agents, Ph.D. Thesis, University Of Cape Town, **2002**.
[33] Biot, C.; Glorian, G.; Maciejewsky, L. A.; Brocard, J. S.; Domarle, O.; Blampain, G.; Millet, P.; Georges, A. G.; Abessolo, H.; Dive, D.; Lebibi, J. *J. Med. Chem.* **1997**, *40*, 3715.
[34] Delhaes, L.; Abessolo, H.; Biot, C.; Berry, L.; Delcourt, P.; Maciejewsky, L.; Brocard, J.; Camus, D.; Dive, D. *Parasitol. Res.* **2001**, *87*, 239.
[35] Pradines, B; Tall, A; Rogier, C. Spiegel, A.; Mosmier; J.; Marrama, L., Fusai, T.; Millet, P.; Panconi, E.; Trape, J. F.; Parzy, D. *TM & IH*, **2002**, *7* (3), 265.
[36] Pradines, B.; Fusai, T.; Daries, W.; Laloge, V.; Rogier, C.; Millet, P.; Panconi, E.; Kombila, M.; Parzy, D. *Antimicrob. Agents Chemother.* **2001**, *48*, 179.
[37] Yeary, R. A. *Toxicol. Appl. Pharmacol.* **1969**, *15*, 666.
[38] Delhaes, L.; Biot, Ch.; Berry, L.; Delcourt, Ph.; Maciejewski, L. A.; Camus, D.; Brocard, J. S.; Dive, D. *Chem. Bio. Chem.* **2002**, *3*, 418.
[39] Biot, C.; Delhaes, L.; Abessolo, H.; Domarle, O; Maciejewsky, L. A.; Mortuaire, M.; Delcourt, P.; Deloron, P.; Camus, D.; Dive, D.; Brocard, J. S. *J. Organomet. Chem.* **1999**, *589*, 59.
[40] Domarle, O; Blampain, G.; Agnaniet, H.; Nzadiyabi, T.; Lebibi, J.; Brocard, J.; Maciejewsky, L.; Biot, C.; Georges, A. J.; Millet, P. *Antimicrob. Agents. Chemother.* **1998**, *42*, 3, 540.
[41] Chibale, K.; Moss, J. R.; Blackie, M.; Van Schalkwyk, D.; Smith, P. J. *Tetrahedron Lett*. **2000**, *41*, 6231.
[42] Sharma, V.; Piwnica-Worms, D. *Chem. Rev.* **1999**, *99*, 2445.
[43] Sharma, V.; Crankshaw, C.; Piwnica-Worms, D. *J. Med. Chem.* **2000**, *39*, 3731.
[44] Goldberg, D. E.; Sharma, V.; Oksman, A.; Gluzman, I. Y.; Wellems, T. E.; Piwnica-Worms, D. *J. Biol. Chem.* **1997**, *272*, 10, 6567.
[45] Ziegler, J.; Schuerle, T.; Pasierb, L.; Kelly, C.; Elamin, A.; Cole, K. A.; Wrigth, D. *Inorg. Chem.* **2000**, *39*, 3731.
[46] Sharma, V.; Beatty, A.; Goldberg, D.; Piwnica-Worms, D. *Chem. Commun.* **1997**, *22*, 2223.
[47] Monti, D.; Vodopivec, B.; Basilico, N.; Olliaro, P.; Taramelli, D. *Biochemistry* **1999**, *38*, 8858.
[48] Benoit-Vical, F.; Robert, A.; Meunier, B. *Antimicrob. Agents Chemother*. **1999**, *43*, 10, 2555.
[49] Benoit-Vical, F.; Robert, A.; Meunier, B. *Antimicrob. Agents Chemother*. **2000**, *44*, 2836.
[50] Murray, H. W.; *Antimicrob. Agents Chemother*. **2001**, *45*, 2185.
[51] Mbongo, N.; Loiseau, Ph. M.; Lawrence, F.; Bories, C.; Craciunescu, D. G.; Robert-Gero, M. *Parasitol. Res.* **1997**, *83*, 515.
[52] Louiseau, Ph. M.; Mbongo, N.; Bories, C.; Boulard, Y.; Craciunescu D. G. *Parasite* **2000**, *7*, 103.
[53] Mbongo, N.; Loiseau, Ph. M.; Lawrence, F.; Craciunescu, D. G.; Robert-Gero, M. *Arzneim.-Forsc./Drug Res.* **1998**, *48*, 850.
[54] Mbongo, N.; Loiseau, P. M.; Craciunescu, D. G.; Robert-Gero, M. *Acta Tropica* **1998**, *70*, 239.
[55] Croft, S. L.; Neal, R. A.; Craciunescu, D. G.; Certad-Fombona, G. *Trop. Med. Parasitol.* **1992**, *43*, 24.
[56] Mesa-Valle, C. M.; Moraleda-Lindez, V.; Craciunescu, D.; Osuna, A. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro* **1996**, *91*, 625.
[57] Castilla, J. J.; Mesa-Valle, M. C.; Sánchez-Moreno, M.; Arnedo, T.; Rosales, M. J.; Mascaro, C.; Craciunescu, D.; Osuna, A. *Arzneim.-Forsch./Drug Res.* **1996**, *46*, 990.
[58] Cisneros-Fajardo, E. J.; Navarro, M.; Silva, P.; Marchan, E.; Arrieche, D. *J. Inorg. Biochem.* **2001**, *86*, 182.
[59] Navarro, M.; Cisneros-Fajardo, E. J.; Sierralta, A.; Fernández-Mestre, M.; Silva, P.; Arrieche, D.; Marchán, E. *J. Biol. Inorg. Chem.* **2003**, *8*, 401.
[60] Sharquie, K. E.; Najim, R. A.; Farjou, I. B.; Al-Timimi, D. J. *Clinical & Experimental Dermatology* **2001**, *26* (1), 21.
[61] Gardner, M. J.; Hall, N.; Fung, E.; White, O.; Berriman, M; Hyman, R. W.; Carlton, J. M.; Pain, A.; Nelson, K. E.; Bowman, S.; Paulsen, I. T.; James, K.; Eisen, J. A.; Rutherford, K.; Salzberg, S. L.; Craig, A.; Kyes, S.; Chan, M.-S.; Nene, V.; Shallom, S. J.; Suh, B.; Peterson, J.; Angiuoli, S.; Pertea, M.; Allen, J.; Selengut, J.; Haft, D.; Mather, M. W.; Vaidya, A. B.; Martin, D. M. A.; Fairlamb, A. H.; Fraunholz, M. J.; Roos, D. S.; Ralph, S. A.; McFadden, G. I.; Cummings, L. M.; Subramanian, G. M.; Mungall, C.; Venter, J. C.; Carucci, D. J.; Hoffman, S. L.; Newbold, C.; Davis, R. W.; Fraser, C. M.; Barrell B. *Nature* **2002**, *419*, 498.