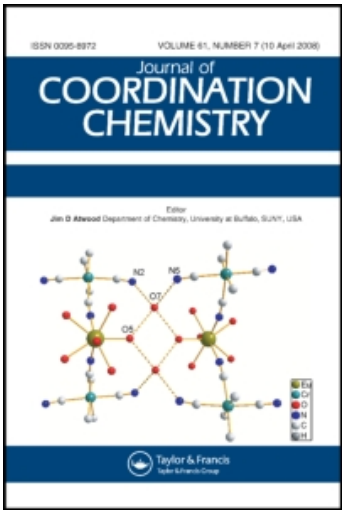

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Synthesis, characterization and *in vitro* antiprotozoal studies of iron(III) complexes of some antimalarial drugs

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We present the synthesis, characterization and *in vitro* antiplasmodial and antitrypanosomal studies of iron(III) complexes of amodiaquine, trimethoprim and pyrimethamine. The complexes have been characterized by elemental analyses, electronic and IR spectroscopy and room temperature magnetic susceptibility measurements. The spectra are consistent with octahedral geometry around the metal ions. The complexes were tested for *in vitro* activity against cultures of *Plasmodium falciparum*, *Tripanosoma brucei rhodosiense*, *L. donovani* and *Tripanosoma cruzi*. One of the complexes showed enhanced activity of about 8.4 times more than chloroquine against the resistant strain of *Plasmodium falciparum*.

Keywords: Malaria drugs; Iron; Complexes; Parasitic; Drug resistant

1. Introduction

Parasitic protozoa infect hundreds of millions of people every year and are collectively some of the most important causes of human misery, causing a number of diseases worldwide especially in developing countries, disproportionately affecting the poorest of the poor. The three that constituted the highest disease burden [1] are malaria caused by *Plasmodium* spp. [2], African trypanosomiasis caused by *Trypanosoma brucei rhodesiense* and *Tripanosoma brucei gambiense* [3] and Chagas disease caused by *Trypanosoma cruzi* [3]. Malaria is the most important parasitic disease that primarily affects people in the tropical and subtropical regions of the world and travelers to the endemic regions. At present, about 300–500 million people are infected and up to 2.7 million deaths occur annually [4]. The increase in the incidence of malaria is compounded by the development of drug-resistant strains over the last decades [5, 6].

In sub-Saharan Africa, sleeping sickness or human African trypanosomiasis [7], and in America, Chagas' disease [8] has been neglected to some extent. The WHO estimated that about 100 million people in Latin America are at risk of acquiring Chagas' disease, with a prevalence of human *T. cruzi* infection estimated at 18 million cases [9, 10] and

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about 5 million clinical cases annually. Human African trypanosomiasis and Chagas diseases are both chronic diseases that undergo distinct stages in their natural course and are potentially fatal [11]. Four drugs, suramin, pentamidine, melarsoprol and eflornithine, available for treatment are administered parenterally and are not effective against certain species of trypanosomes and can cause serious side effects [12, 13]. In contrast to other parasitic diseases and despite recent advances in the knowledge of the biochemistry of the parasites [14], no treatment is available for the long-term prevalent form of this illness [15]. The current drugs available for the treatment of parasitic diseases suffer from increasing problems with resistance. In the search for drugs against resistant parasites, the use of metal complexes has gained prominence [16–21]. In these studies, the metal complexes strongly inhibited the *in vitro* growth of chloroquine resistant strains of *Plasmodium berghei*. Although the mechanism of these metal-based agents is currently unknown, the presence of the metal ion results in enhanced activity. In our efforts [22, 23] to contribute to this search, we present the synthesis, characterization and *in vitro* antiplasmodial and antitrypanosomal activity of iron(III) complexes of amodiaquine, trimethoprim and pyrimethamine.

2. Experimental

2.1. *Materials and instrumentation*

All reagents, metal salts, trimethoprim, pyrimethamine and amodiaquine were used as obtained from Aldrich. Elemental analyses were performed at the Micro-analytical Laboratory of the School of Chemistry, The University of Manchester, UK. IR spectra were obtained as KBr discs on a Perkin-Elmer Paragon 1000 FTIR spectrophotometer equipped with CsI window (4000–250 cm^{-1}). UV-Vis spectra were obtained on a Perkin-Elmer Lambda 20 spectrophotometer. Magnetic susceptibility measurements were carried out at room temperature using a Sherwood scientific magnetic susceptibility balance. Diamagnetic corrections were made using Pascal's constants [24].

2.2. *Synthesis of [Fe(AQ)($C_3H_6NS_2$)Cl_3]*

$FeCl_3 \cdot 6H_2O$ 0.541 g (2.0 mmol) was dissolved in 20 mL of methanol followed by addition of amodiaquine 0.858 g (2.0 mmol) in 30 mL of methanol. The mixture was stirred at 50°C for 30 min after which N,N′-dimethydithiocarbamate, 0.143 g (1.0 mmol) in 20 mL of methanol was added. The mixture was refluxed for 3 h and the product was filtered under suction. The complex is formulated as [Fe(AQ)($C_3H_6NS_2$)Cl_3] (figure 1). Anal. Calcd for $C_{23}H_{34}Cl_4FeN_4O_4S_2$ (%): C, 39.90; H, 4.95; N, 8.09. Found: C, 40.57; H, 5.23; N, 7.93. Yield: 56%, m.p. 248°C.

2.3. *Synthesis of [Fe(pyrm)$_2$($C_6H_6NS_2$)Cl_2]*

$FeCl_3 \cdot 6H_2O$ 0.541 g (2.0 mmol) was dissolved in 20 mL of methanol followed by addition of 0.498 g (2.0 mmol) of pyrimethamine in 50 mL of methanol. The mixture was stirred at 50°C for 30 min after which 0.143 g (1.0 mmol) of

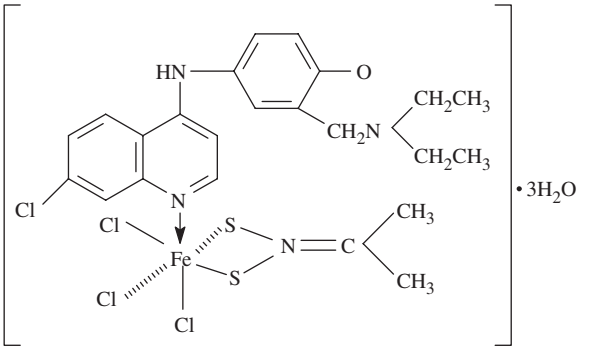

Figure 1. Proposed structure for $[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$.

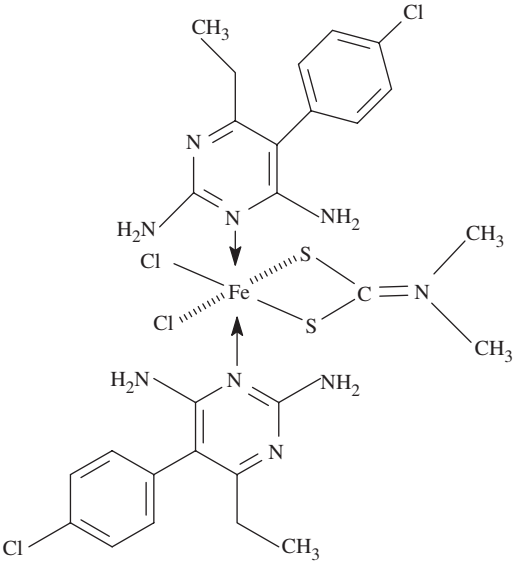

Figure 2. Proposed structure for $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$.

N,N′-dimethydithiocarbamate in 10 mL of methanol was added. This mixture was refluxed for 3 h and filtered. The filtrate was left to evaporate slowly at room temperature. Both the product from the filtrate and precipitate were analyzed to determine their composition. The precipitate analyzed as $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ (figure 2). Anal. Calcd for $C_{27}H_{32}Cl_4FeN_9S_2$ (%): C, 43.57; H, 4.33; N, 16.93. Found: C, 43.67; H, 4.33; N, 17.11. Yield: 57%, m.p. 223°C.

2.4. *Synthesis of $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$*

$FeCl_3\cdot 6H_2O$ 0.541 g (2.0 mmol) was dissolved in 20 mL of methanol followed by addition of 0.497 g (2.0 mmol) of pyrimethamine and 0.58 g (2.0 mmol) of trimethoprim in 60 mL of methanol. The mixture was refluxed for 3 h and filtered under suction.

The filtrate was left to evaporate slowly at room temperature. Both the product from the filtrate and precipitate were analyzed to determine their composition. The precipitate analyzed as $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$ (figure 3). Anal. Calcd for $C_{39}H_{48}Cl_4FeN_{12}O_6$ (%): C, 47.87; H, 4.94; N, 17.18. Found: C, 47.52; H, 4.39; N, 17.47. Yield: 64%, m.p. 237°C.

2.5. *Biological tests*

The tests were performed as micro plate assays using *T. b. rhodesiense* (STIB 900), *T. cruzi* (Tulahuenc C4), *L. donovani* (MHOM-ET-67/L82) and K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). *In vitro* activity against erythrocytic stages of *P. falciparum* was determined using the ^{3}H-hypoxanthine incorporation assay [25]. Cytotoxicity was assessed with rat skeletal myoblast (L-6 cells) with the same assay used for the determination of the antitrypanosomal activity. A description of these assays was recently reported [26]. The following substances were used as standards: melarsoprols (*T. b. rhodesiense*), *T. cruzi* (Benznidazole), *L. donovani* (Miltefosine) and *P. falciparum* (chloroquine).

3. Results and discussion

3.1. *Syntheses*

The iron(III) complexes formulated as $[Fe(AQ)(C_3H_6NS_2)Cl_3]$, $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ and $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$ have been synthesized by reaction of $FeCl_3\cdot 6H_2O$ and the antimalarial drugs in methanol. The complexes are air stable. The analytical and spectroscopic data are consistent with octahedral complexes.

3.2. *Infrared spectra for the complexes*

In order to clarify the mode of bonding and the effect of the metal ion on the ligands, the IR spectra of the free ligands and the metal complexes were studied and assigned on the basis of careful comparison of their spectra with that of the free ligands. The υ(N–H) stretching frequency of amodiaquine in $[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$ occurs as a broad medium band at 3429 cm^{-1}. The position of the υ(NH) was unaltered from that of the free ligand, indicating the non-bonding of N–H to iron. The ν(C=N) stretching vibration bands at 1587 and 1618 cm^{-1} in the free ligand shift to 1586–1652 cm^{-1} in the complex, supporting coordination of iron through the quinoline N(1). The N–S stretching frequency is assigned at 1096 cm^{-1} while Fe–N, Fe–S, and Fe–Cl stretching frequencies are assigned to bands at 420, 384 and 278 cm^{-1}, respectively.

Pyrimethamine possesses four potential donor sites while trimethoprim possess seven. The IR spectra of the free ligands and their metal complexes were compared and assigned. Bands in the region 3400–2900 cm^{-1} due to symmetrical and asymmetrical stretching modes of NH_2 in the spectra of the ligands undergo only slight changes in the spectra of the complexes. The multiple bands showed by the complexes in this region

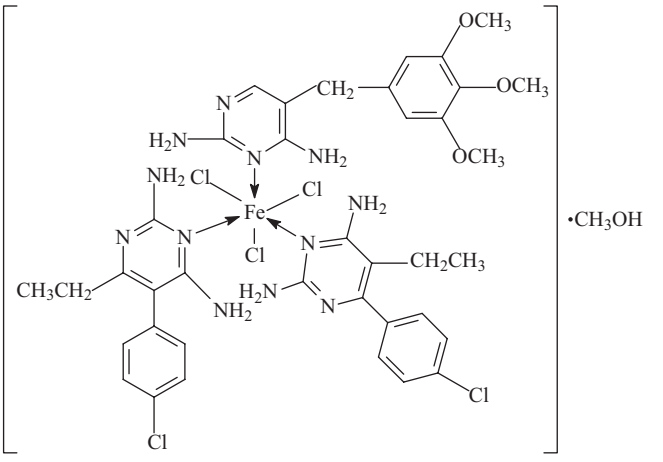

Figure 3. Proposed structure for $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$.

might be attributed to the presence of four free NH_2 group on $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ and six on $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$, confirming the iron preferentially coordinates through the N(1) atoms in pyrimethamine and trimethoprim. This is further confirmed by the substantial shift in the υ(C=N) stretching frequency which appears as multiple bands in the region 1664–1590 cm^{-1} in the ligands but as a single sharp band at 1676 cm^{-1} in $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ and at 1681 cm^{-1} in $[Fe(pyrm)_2(C_3H_6NS_2)Cl_3]\cdot CH_3OH$. The N–S stretching frequencies are assigned to bands at 1102 and 1109 cm^{-1}. The complexes showed medium to weak υ(Fe–N), υ(Fe–S) and υ(Fe–Cl) at 458, 364 and 272 cm^{-1}, respectively.

3.3. *Electronic spectra and magnetic properties*

Iron(III) is a moderately oxidizing ion and the electronic spectra of many of its complexes consist of forbidden transitions and hence weak bands often obscured by charge transfer bands [27–29]. In general, both $L \rightarrow {}^2t_{2g}$ and $L \rightarrow {}^2e_g$ transitions may be expected. The d–d absorption in octahedral complexes is rarely observed because the ligand to metal charge transfer (L→MCT) absorptions obscure such weak bands. Charge transfer transitions in Fe(III) complexes occur between 45,000–26,000 cm^{-1}. The intense bands in the regions 26,000 cm^{-1} in the electronic spectrum of $[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$ could be attributed to a π–π^* transition located largely on the ligand. The narrow band at 25,000–27,000 cm^{-1} could be assigned to $t_{2g}\rightarrow\pi^*$ charge transfer. The electronic spectrum of $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ exhibits charge transfer bands in the region 26,000–45,000 cm^{-1}. The absorption band at about 27,027 cm^{-1} is attributed to L→M charge transfer. The electronic spectrum of $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$ showed high intensity bands in the region 26,000–45,000 cm^{-1}. The absorption band at about 27,548 cm^{-1} is attributed to L→M charge transfer transitions. Other charge transfer transitions can be seen in the region 33300–36350 cm^{-1}.

High spin iron(III) complexes generally have room temperature magnetic moments very close to the spin only value of 5.9 BM, because of the absence of orbital angular

Table 1. *In vitro* antiprotozoal activities of the metal complexes.

Compounds	*P. falc.* K1 IC_{50} (μM)	*T. b. rhodesiense* IC_{50} (μM)	*T. Cruzi*	*L. donovani* IC_{50} (μM)	Cytotox. L6
$[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$	0.0088	0.5200	10.200	8.100	15.710
$[Fe(Prym)_2(C_3H_6NS_2)Cl_2]$	2.1935	0.1710	0.895	0.490	0.800
$[Fe(Prym)_2(TMP)Cl_3]\cdot CH_3OH$	>5.000	3.1885	3.750	15.600	2.681

momentum in the ground state $^6A_{1g}$ ($t^3{}_{2g}$, $e^2{}_g$). Thus, there is no effective mechanism for introducing coupling with the excited state. Low spin complexes with $^2T_{2g}$ ($t^5{}_{2g}$, $e^0{}_g$) ground state usually have orbital contributions to their magnetic moments and have room temperature magnetic moments more than the spin only value of 1.73 BM, sometimes up to 2.40 BM [30, 31]. In certain instances, some iron(III) complexes have magnetic moments between high and low spin values when the ligand field strength is comparable with the mean electronic pairing energy of the d^5 configuration in which the ground states $^6A_{1g}$ and $^2T_{2g}$ are in a thermal equilibrium leading to magnetic moments values in the range 5.92–2.0 BM. The effective magnetic moments of 5.3 BM for $[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$, 5.43 BM for $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ and 5.1 BM for $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$ confirm that the complexes are high spin octahedral complexes.

3.4. *Antiplasmodial and antitrypanosomal tests*

The complexes were evaluated against axenic *L. donovani* amastigotes, intracellular *T. cruzi* amastigotes and *T. b. rhodesiense* trypomastigotes. The exact IC_{50} values for the anti-parasitic assays and the cytotoxicity of the complexes are shown in table 1. The complexes showed the highest antitrypanosomal activity ($IC_{50} = 0.17$–$3.19\,\mu M$) against *T. b. rhodesiense*, compared to their activity against *T. Cruzi* ($IC_{50} = 0.89$–$10.2\,\mu M$) and *L. donovani* ($IC_{50} = 0.49$–$15.6\,\mu M$). Generally, $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ is the most active of all the complexes with IC_{50} in the range 0.17–0.89 μM while the other two complexes showed varied activities. $[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$ showed remarkable antiplasmodial activity ($IC_{50} = 0.0088\,\mu M$) with a relatively less cytotoxicity ($IC_{50} = 15.71\,\mu M$) compared to the widely used chloroquine ($IC_{50} = 188.5\,\mu M$) [32]. The complex is about 8.4 times more active than chloroquine ($IC_{50} = 0.0743\,\mu M$) against the resistant strain of *Plasmodium falciparum*. $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ showed decreased antiplasmodial activity ($IC_{50} = 2.1935\,\mu M$) but the cytotoxicity was dramatically improved ($IC_{50} = 0.8\,\mu M$). The complex is also more active than pyrimethamine ($IC_{50} = 2.65\,\mu M$) and trimethoprim ($IC_{50} = 4.733\,\mu M$) $[Fe(pyrm)_2(TMP)Cl_3]\cdot CH_3OH$ ($IC_{50} > 5\,\mu M$) exhibits the lowest activity but is less cytotoxic than the most active complex.

4. Conclusions

This study deals with the synthesis, characterization and *in vitro* studies of iron complexes of antimalarial drugs. The main objective is the development of metal-based

chemotherapies against malaria and other tropical diseases. The complexes were characterized by elemental analyses, electronic and IR spectra. The complexes are air stable with octahedral coordination around the metal ions. All the compounds were screened using *in vitro* assays for their antitrypanosomal and antiplasmodial activities as well as for their cytotoxicity. $[Fe(pyrm)_2(C_3H_6NS_2)Cl_2]$ is the most potent antitrypanosomal of all the complexes. $[Fe(AQ)(C_3H_6NS_2)Cl_3]\cdot 3H_2O$ exhibits excellent antiplasmodial activity. Its potency against the chloroquine resistant K1 strain of *P. falciparum* is more than for chloroquine against the sensitive strains. The complex can serve in the further optimization of future compounds. Although there is no correlation between the structure of the complexes and their activity, their coordination to iron changes their electronic structures and thus the pharmacological models of the antimalarial agents.

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