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SYNTHESIS, CHARACTERIZATION, ANTIPLASMODIAL AND ANTITRYPANOSOMAL ACTIVITY OF SOME METAL(III) COMPLEXES OF SULFADIAZINE

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ABSTRACT. The Fe(III), Ru(III), Rh(III), and Cr(III) complexes of 4-amino-N-(2-pyrimidinyl)benzene sulfonamide (sulfadiazine) have been synthesized and characterized by elemental analysis, electronic and IR spectroscopy, conductance and room temperature magnetic susceptibility measurements. Sulfadiazine acts as a bidentate ligand through the sulfonamido and the pyrimidinic N-atoms. The compounds are non-electrolytes and the electronic spectra are consistent with the proposed octahedral geometry around the metal ions. The complexes were tested for *in vitro* activity against cultures of the resistant strains of *Plasmodium falciparum*, tripamastigotes *T. b. rhodesiense* and amastigotes *L. donovani* to determine their antiprotozoal activities. The Fe(III) complex is more active than the other complexes against the parasitic protozoa.

KEY WORDS: Metal complexes, Sulfadiazine, Parasites, Protozoa, Drug resistance

INTRODUCTION

Malaria infections are caused by parasites of the genus Plasmodium. Malaria is one of the major infectious diseases ravaging the world. Despite more than a century of efforts to eradicate or control it, the disease remains a major and growing threat to public health and economic development of countries in the tropical and subtropical regions of the world, where 40 % of the world population live at the risk of infection. There are an estimated 300-500 million cases, up to 2.7 million deaths and 42 million disability adjusted life years (Daly's) annually. The mortality levels are greatest in sub-Saharan Africa, where children under 5 years of age account for 90 % of all deaths due to malaria [1]. In countries with endemic malaria, the annual economic growth rates over a 25-year period were 1.5 % lower than those of other countries [2]. Malaria was successfully reduced and it was erroneously believed that it could be eradicated after World War II because of the easy access to cheap insecticides such as DDT and readily available drugs such as chloroquine [3]. At present, malaria has re-emerged with a vengeance, killing one African child every 30 seconds [4]. The emergence and spreading of parasites resistant to anti-malarial drugs currently in use indicates that novel compounds need development by identification of chemotherapeutics targets [5].

Sleeping sickness is caused by the protozoan parasites *Trypanosoma brucei* and *T. b. gambiense*. About half a million people are infected annually, leading to the death of almost 100,000 people [6, 7]. At present, there are only four drugs for the treatment of trypanosomiasis. The current drugs suffer from a number of side effects, poor clinical efficacy, parenteral administration and increasing problems with resistance [8]. Eflornithine was introduced recently but was ineffective against *T. b. rhodesiense* [9]. Consequently, the need to develop new drugs against East African human trypanosomiasis is of paramount importance.

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The use of metal complexes as pharmaceuticals has shown promise in recent years particularly as anticancer agents [10]. Research is in fields such as cancer, arthritis and cardiovascular medicine. In the search for novel drugs against parasitic protozoa, the modification of existing drug by coordination to a metal centre has attracted considerable attention recently [11-14]. Interest in the metal complexes of sulfadiazine is due to its use as a pharmaceutical. Zinc sulfadiazine is used to prevent bacterial infection in burned animals and silvadene (2-sulfanilamidopyrimidine-silver(I)) is used commercially for the treatment of topical burn [15]. The crystal structures of silver sulfadiazine [16], zinc sulfadiazine [17] and cadmium sulfadiazine [18] have been reported. In our effort to contribute to metal-based drugs as alternative therapies in the management of chloroquine-resistant malaria, we have embarked on the synthesis, characterization and *in vitro* studies of metal complexes of antimalarial drugs [19]. Recently we reported the single crystal X-ray structures of the Co(II) complex of sulfadiazine [20]. In the present study, we present the synthesis, characterization and *in vitro* antiprotozoal studies of Fe(III), Ru(III), Rh(III), and Cr(III) complexes of sulfadiazine.

EXPERIMENTAL

General procedure

Solvents of analytical grade were used as obtained. Elemental analyses were performed at the micro-analytical laboratory, School of Chemistry, University of Manchester, UK. FTIR spectra were obtained on a Perkin-Elmer Paragon 1000 FTIR spectrophotometer in the range 4000-250 cm^{-1}. UV-Vis spectra were obtained on a Perkin-Elmer Lambda 20 spectrophotometer equipped with an integrating sphere for diffuse reflectance spectra. The solution spectra were in DMF solutions. Room temperature magnetic susceptibility measurements were carried out using Sherwood Scientific Magnetic Susceptibility balance and $Hg[Co(SCN)_4]$ as the calibrant. Diamagnetic corrections were estimated from Pascal's constants [21].

Synthesis of the complexes

Sodium sulfadiazine was dissolved in 50 mL of distilled/deionised water followed by drop wise addition of the corresponding metal salts in 30 mL of distilled/deionised water or methanol in a 2:1 or 3:1 mole ratios. The precipitate begins to form and the reaction was stirred continuously for 3 h. The precipitate is filtered off, washed with water and dried over $CaCl_2$. The complexes are formulated as $[Fe(SD)_3]\cdot 3H_2O$, $[M(SD)_2(H_2O)Cl]$ where M = Ru, Rh and Cr.

Biological tests

The tests were performed as micro plate assays using *T. b. rhodesiense* (STIB 900), *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 [22]. Cytotoxicity was assessed with rat skeletal myoblast (L-6 cells) with the same assay that was used for the determination of the antitrypanosomal activity. A description of these assays was reported recently [23]. The following substances were used as standards: melarsoprol (*T. b. rhodosiense*), *L. donovani* (Miltefosine) and *P. falciparum* (chloroquine).

RESULTS AND DISCUSSION

Synthesis of the complexes

Treatment of sodium sulfadiazine with the corresponding metal chloride in water or methanol afforded the complexes $[Fe(SD)_3]\cdot 3H_2O$ and $[M(SD)Cl(H_2O)]$, SD = sulfadiazine ($C_{10}H_9N_4O_2S$), M = Ru, Rh or Cr. The formation of the metal complexes may be rationalised by the general equation below:

$$FeCl_3\cdot 6H_2O + 3Na(C_{10}H_9N_4O_2S) \longrightarrow [Fe(C_{10}H_9N_4O_2S)_3]\cdot 3H_2O + 3NaCl + 3H_2O$$

$$MCl_3\cdot 3H_2O + 2Na(C_{10}H_9N_4O_2S) \longrightarrow [Fe(C_{10}H_9N_4O_2S)_2Cl(H_2O)] + 2NaCl + 2H_2O$$

(M = Ru, Rh, Cr)

The complexes are stable in air and insoluble in both polar and non-polar solvents but soluble in polar coordinating solvents such as DMF and DMSO. Their molar conductivities are in a range that indicates a non-electrolytic nature. The complexes have octahedral geometry and the metal ions are coordinated via sulfonamido and pyrimidinic N-atoms of the sulfadiazine. In the Fe(III) complex, the metal ion is coordinated to three molecules of sulfadiazine and crystallises with three lattice water. Ru, Rh and Cr coordinate to two molecules of sulfadiazine each and the octahedral arrangement around the metal ion is completed by a molecule of water and a chloride ion. The proposed structures for the complexes are given in Figures 1-2. The analytical data, melting point/decomposition temperature and room temperature magnetic moments for the complexes are listed in Table 1.

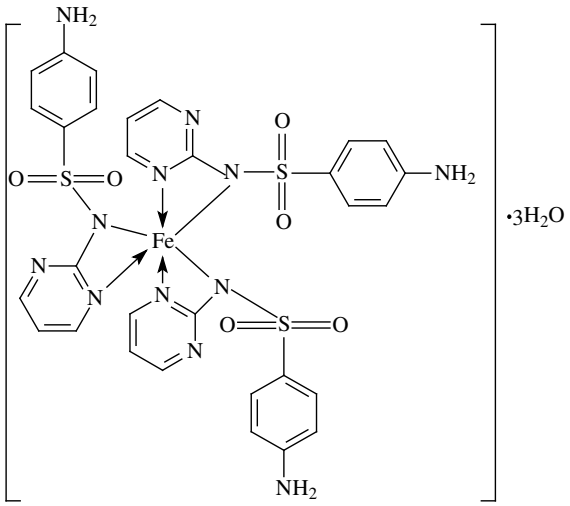

Figure 1. Proposed structure of $[Fe(SD)_3]\cdot 3H_2O$.

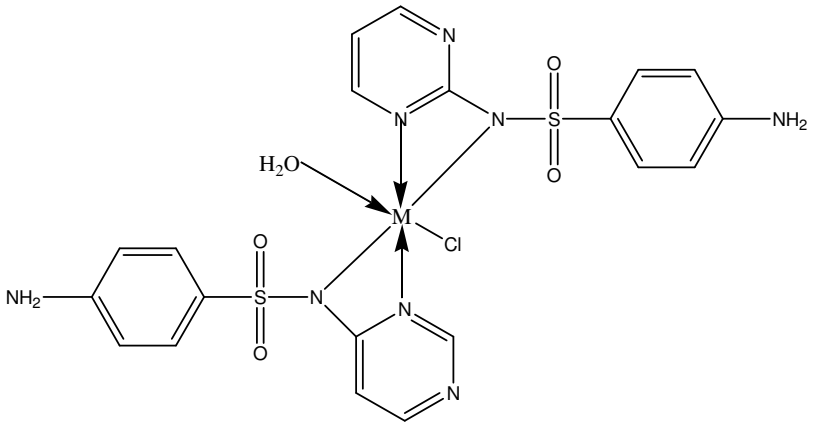

Figure 2. Proposed structure of $[M(SD)_2(H_2O)Cl]$ M = Ru, Rh, Cr.

Table 1. Analytical data for the compounds.

Compounds	Empirical formula	Formula mass	μ B.M	M.p. (°C)	Elemental analyses (expected) C	H	N	S
$[Fe(SD)_3].(H_2O)_3$	$FeC_{30}H_{33}N_{12}O_9S_3$	857.71	5.40	247	42.46	3.56	19.23	11.32
					(42.01)	(3.88)	(19.60)	(11.21)
$[Ru(SD)_2(H_2O)Cl]$	$RuC_{20}H_{20}N_8O_5S_2Cl$	653.07	1.89	224	37.14	3.59	17.21	9.63
					(36.78)	(3.09)	(17.16)	(9.82)
$[Rh(SD)_2(H_2O)Cl]$	$RhC_{20}H_{20}N_8O_5S_2Cl$	654.91	0.38	227	36.98	3.62	17.46	9.86
					(36.68)	(3.08)	(17.11)	(9.79)
$[Cr(SD)_2(H_2O)Cl]$	$CrC_{20}H_{20}N_8O_5S_2Cl$	604.00	3.87	232	39.95	3.18	18.62	10.54
					(39.77)	(3.34)	(18.55)	(10.62)

Infrared spectra of the complexes

In order to clarify the mode of bonding and the effect of the metal ion on the ligand, the IR spectra of the free ligand and the metal complexes were studied and assigned based on careful comparison of their spectra with that of the free ligand. Relevant IR bands for the ligand and metal complexes are presented in Table 2. The bands in the region 3450-3000 cm^{-1} due to symmetrical and asymmetrical stretching modes of NH_2 in the spectra of the ligand undergo some change in the spectra of the complexes [24, 25]. This might be attributed to the coordination of the metal ion through the sulfonamido N-atom and the possible interaction between the uncoordinated NH_2 groups on the complexes and the O–H stretching frequencies of coordinated water in the case of Ru(III), Rh(III) and Cr(III) complexes and lattice water in the Fe(III) complex. In addition to the three peaks observed in the sulfadiazine ligands that shifted to higher wavenumbers in the complexes, there were several other bands probably due to the presence of more than one molecule of sulfadiazine. The effect of the coordinated metal is also noticeable on the SO_2 symmetrical and asymmetrical stretching modes that are shifted to lower wavenumbers while the ν(S–N) is shifted to higher wavenumbers in all the complexes. These observations confirm the coordination of metal ions through the sulfonamido N atom [20]. Coordination through the pyrimidinic N(1) is confirmed by the shift in ν(C=N) stretching vibrations bands occur ring at 1652-1580 cm^{-1} in the free ligand, which showed appreciable changes in the metal complexes. The Fe–N is assigned to a band at 519 cm^{-1}. Two bands of

almost equal intensity at 575 and 550 cm^{-1} are assigned to Ru–N, while the bands at 370 and 280 cm^{-1} are assigned to Ru–O and Ru–Cl, respectively. The Cr–N and Cr–O are assigned to a band at 478 and 387 cm^{-1}, respectively. The multiple absorption bands in the regions 600-250 cm^{-1} are most probably "librational modes" that are due to rotational oscillations of the lattice water molecules, restricted by interactions with neighbouring atoms [24].

Table 2. IR data (cm^{-1}) for sulfadiazine and the complexes.

Formulation	$\nu(NH_2)$	$\nu(C=N)$	$\nu(SO_2)_{asym}$	$\nu(SO_2)_{sym.}$	$\nu(SN)$	νMN	νMO
Sulfadiazine	3422 vs 3355 vs	1652 vs 1580 s	1325 vs	1157 vs	942 s		
$[Fe(SD)_3].(H_2O)_3$	3423 s 3354 vs	1652 ms 1596 vs	1326 vs	1159 vs	946 vs	610 s	520 ms
$[Ru(SD)_2(H_2O)Cl]$	3423 s 3356 s	1624 ms 1594 vs	1326 vs	1155 s	945 vs	550 vs	370 ms
$[Rh(SD)_2(H_2O)Cl]$	3424 ms 3354 s	1626 ms 1594 vs	1326 vs	1159 vs	945 vs	572 s	341 ms
$[Cr(SD)_2(H_2O)Cl]$	3423 s 3354 ms	1625 ms 1596 vs	1327 vs	1158 vs	945 vs	561 vs	478 ms

Abbreviations: v = very strong; s = strong; m = medium.

Electronic spectra and magnetic properties of the complexes

Iron(III) is a moderately oxidizing ion and many of its complexes exhibit ligand to metal charge transfer transitions (L→MCT). In general, both L→ $^2t_{2g}$ and L→ ^2e_g transitions may be expected [26]. In most cases, the d—d absorption in octahedral iron(III) complexes is rarely observed because the L→MCT absorptions obscure it. Furthermore, high-spin iron(III) complexes are not stabilized by crystal field effects. Charge transfer transitions in Fe(III) complexes occur between 45,000-26,000 cm^{-1}. The electronic spectrum of $[Fe(SD)_3]\cdot 3H_2O$ showed absorption in the region 33330-28400 cm^{-1} that is attributed to L→MCT. Another band appears at 26,042 cm^{-1} and is assigned to a $^2t_{1u}$→ $^2t_{2g}$ charge-transfer transition. High-spin iron(III) complexes, in general, have magnetic moments at room temperature close to 5.9 B.M. and somewhat in excess of 2.0 B.M. due to orbital contribution if they are low-spin. A magnetic moment of 5.4 B.M. for $[Fe(SD)_3]\cdot 3H_2O$ indicates a high spin octahedral complex [27].

Ruthenium(III) complexes are almost invariably six coordinate unless doped in special lattices [26]. Charge-transfer absorption often occurs at low energies while the crystal field parameter is quite large; thus d—d bands are frequently obscured [26]. Though the spin-orbit coupling coefficient is greater than for iron(III), intra-configurational transitions within $^2T_{2g}$ still lie at too low an energy to be conveniently identified. The two lowest absorptions, the spin-forbidden $^4T_{1g} \rightarrow {}^2T_{2g}$ and $^4T_{2g} \rightarrow {}^2T_{2g}$, can frequently be observed but most often as shoulders. The electronic spectrum of $[Ru(SD)_2(H_2O)Cl]$ showed a very broad band at 20,000 – 18,000 cm^{-1} which is assigned to $^4T_{2g} \rightarrow {}^2T_{2g}$. The effective magnetic moments of 1.89 BM obtained for the complex show that it is a low-spin octahedral complex with one unpaired electron. This is typical of second and third row transition metal complexes with odd number of unpaired electrons. Rhodium is in the same group as iron and ruthenium. Apart from working on the metals in the same group to establish whether as one go down the group, the periodicity of properties have any effect on the activity of the metal complexes, rhodium(III) coordination complexes has shown considerable anti-tumor and antimicrobial activity. In the search for novel complexes against resistant strains of *Plasmodium falciparum*, chloroquine complexes of ruthenium and rhodium have been reported with enhanced activity [28]. The electronic spectra of Rh(III) complexes have been studied in detail [26, 29]. These complexes display long-

wavelength ligand field absorptions. Two absorption bands observed at 22,400 cm^{-1} and 31,000 cm^{-1} are due to the $(t_{2g})^5(e_g)^1$ state that are not obscured by charge transfer absorptions. These can be assigned to $^1A_{1g} \rightarrow {}^1T_{1g}(\sigma_1)$ and $^1A_{1g} \rightarrow {}^1T_{2g}(\sigma_2)$ transitions indicating octahedral stereochemistry around the Rh(III) ions [30]. A magnetic moment of 0.38 B. M indicated that the complex is diamagnetic. The splitting of the free–ion ground F term, along with the presence of the excited state P term of the same multiplicity, provides the possibility of three spin-allowed d—d transitions for Cr(III) complexes in an octahedral field [31]. The electronic spectrum of $[Cr(SD)_2(H_2O)Cl]$ exhibits two broad peaks in the spin-allowed absorption band regions. The bands at 17540 cm^{-1} and 27020 cm^{-1} are assigned to $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$ (F) respectively. The third band that occurs at about 37030-34210 cm^{-1} may be regarded as an overlap of $^4A_{2g} \rightarrow {}^4T_{1g}$ (P) and charge transfer transitions. The magnetic moment is expected to be very close to the spin-only value for three unpaired electrons, due to the absence of any orbital contribution. The room temperature magnetic moment for $[Cr(SD)_2(H_2O)Cl]$ is 3.87 B.M. It is lower than the spin-only value for a d^3 metal ion [32].

In vitro antiprotozoal test

The metal complexes were screened for their antiprotozoal activities against *P. falciparum* K_1, *T. b. rhodesience*, *L. donovani* and for cytotoxity activities. The concentration of the complexes and the ligand for 50 % inhibition (IC_{50}) was determined and are presented in Table 3. In general, $[Fe(SD)_3 \cdot 3H_2O$ exhibits higher antiplasmodial activities (IC_{50}: 4.5983 μM) than the other complexes and sulfadiazine itself. The complexes are more toxic than sulfadiazine but are less toxic than the widely used chloroquine (IC_{50} = 188.5 μM) [33]. The Fe(III) complex is a candidate for further investigation by varying the substituent's around the metal ion.

Table 3. *In vitro* activity of sulfadiazine and its metal complexes against *P. falciparum* strains.

Compounds	*P. falc.* K_1 IC_{50} (μM)	*T. b. rhodesiense* IC_{50} (μM)	*L. donovani* IC_{50} (μM)	Cytotoxicity L6 IC_{50} (μM)
Sulfadiazine	> 5.000			0.009
$[Fe(SD)_3].(H_2O)_3$	4.598	3.687	22.300	> 90.000
$[Ru(SD)_2(H_2O)Cl]$	> 5.000	56.108	17.900	> 90.000
$[Rh(SD)_2(H_2O)Cl]$	> 5.000	> 90.000	> 30.000	> 90.000
$[Cr(SD)_2(H_2O)Cl]$	> 5.000	> 90.000	8.500	> 90.000

The complexes were also evaluated for their antitrypanosomal activities. The studied stages are the most clinically relevant of the life cycle of the parasites. The Fe(III), Ru(III) and Cr(III) complexes showed inhibition of growth at the micromolar and $[Cr(SD)_2(H_2O)Cl]$, IC_{50} (8.5 μM) is the most active of all the complexes against *L. donovani*. The complexes of iron and ruthenium inhibit the growth of *T. b. rhodesiense* at the micromolar range. $[Fe(SD)_3] \cdot 3H_2O$, IC_{50} (3.687 μM) is the most active and it is 15 times more active than $[Ru(SD)_2(H_2O)Cl]$. Two compounds currently used to treat East African sleeping sickness are very toxic. The early stage of *T. b. rhodesiense* disease is treated with the nephrotoxic drug Suramin [34]. The other drug in use is melarsoprol, which has been restricted to the late-stage of sleeping sickness because of its toxicity. The iron(III) complex that is most active is less active than suramin (IC_{50} = 0.0075 μM), [35], and melarsoprol (IC_{50} = 0.0039 μM). The complexes from this work need to be optimized to make them more soluble in DMSO and, hopefully, water.

CONCLUSIONS

Fe(III), Ru(III), Rh(III) and Cr(III) complexes of sulfadiazine have been synthesised and characterised by elemental analyses, and from their electronic and IR spectra. All the complexes are air stable and insoluble in most common solvents. They have octahedral coordination in which the metal ions are coordinated to sulfadiazine molecules that act as bidentate ligands and some water molecules or chloride ion. *In vitro* antiprotozoal tests on the complexes showed that $[Fe(SD)_3]\cdot 3H_2O$ is generally more active against the resistant strain of *Plasmodium falciparum* than sulfadiazine and also against *T. b. rhodesiense,* while $[Cr(SD)_2(H_2O)Cl]$ is more active against *L. donovani* than the other complexes.

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