Artemether-Lumefantrine Mixed Ligand Complexes: Synthesis and *in vitro* AntiplasmodialActivity Studies

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Abstract
Malaria is one of the main causes of mortality and morbidity in the world, endangering billions and affecting millions of people each year. Resistance to common antimalarial drugs has proven to be a challenging problem in malaria control. In an attempt to develop efficacious compounds against sensitive and multidrug resistant falciparum malaria, four new complexes of mixed ligands (artemether and lumefantrine) were synthesized using template method. Their antiplasmodial activity potential was evaluated and compared with chloroquine and the parent ligands. The compounds were active against the tested parasite strains. The presence of the metal ions significantly improved the antiplasmodial action against the drug-resistant parasite strains when compared to chloroquine. Additionally, their biological activity was assessed using several in vitro assays. Biological and physical properties were correlated to the antimalarial activity.

Keywords: Malaria, Metal Complexes, Antiplasmodial, Biological activity

1.0 Introduction

Despite decades of research advanced in its prevention and treatment, malaria remains one of the main causes of mortality and morbidity in the world. As reported by the WHO in 2015, 3.3 billion people were at risk of malaria, mainly in the 106 malaria-endemic countries located in the tropical and subtropical zones of the globe [1]. Nearly half of the world's population lives under the constant threat of malaria, with the heaviest toll borne by the poorest and most vulnerable [2].

The use of combination chemotherapy is the current innovative strategy in controlling malaria. It involves the use of a short half-life acting anti-malarial agent of artemisinin drug in combination with long half-life conventional drug, example being artemether-lumefantrine and artesunate-melfoquine drugs [3]. Artemether-lumefantrine (AL) is one of the artemisisnin-based combination therapies recommended for treatment of malaria. The drug combination is highly efficacious against sensitive and multidrug resistant *falciparum* malaria. The artemisinin drug (short half-life) kills the parasites and it is excreted rapidly, resulting in re-emerging of the parasites after a short period. Therefore, its use in combination with longer half-life antimalarial drugs results in achieving full eradication of the parasites preventing the recrudescence that occur with the use of artemisinin mono-therapy [4].

Currently, the drugs being used in combination with artemisinin drugs are the conventional drugs (lumefantrine, mefloquine and amodiaquine), which have developed resistance to P. *falciparum* parasites when used in monotherapy. The use of the conventional drugs in combination therapy might not solve the problem of multidrug resistance of the parasites in the near future. There is an urgent need to discover and develop new antimalarial drugs from incorporation of transition metal moiety into these compounds.

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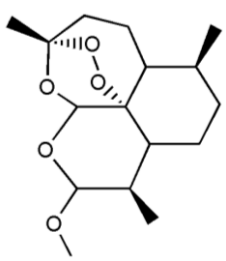
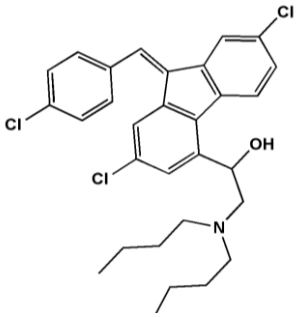
The attraction between opposite charges of metal ions which are electron deficient and most biological molecules such as proteins, DNA and drugs which are electron rich, leads to a general tendency for metal ions to bind to and interact with biological molecules [5,6]. Metals such as copper, zinc, iron and manganese are incorporated into catalytic proteins (metalloenzymes) which mediate a multitude of biochemical reactions [7,8]. The intentional introduction of a metal ion into biological systems will be for either therapeutic or diagnostic purpose. The efficacy of the therapeutic agent is known to be enhanced upon coordination to a metal ion [9-11]. In the search for novel drugs against chloroquine–resistant malaria parasite, the modification of existing antimalarial drugs by coordination to a metal centre has attracted considerable attention in recent years [12-18].

In continuation of previous efforts to search for novel chemotherapeutic drugs against the resistant strains of *Plasmodium falciparum*, the present study reports the synthesis, characterization and *in vitro* antimalarial activity study of Cu(II), Fe(II), Co(II) and Mn(II) of mixed ligands metal complexes of artemether and lumefantrine.



(a) Artemether



(b) Lumefantrine

Figure 1: Structures of the ligands

2.0 Materials and Methods

The ligands artemether and lumefantrine were obtained from Emzor Pharmaceuticals Company Limited, Lagos, Nigeria. All solvents and other reagents were of high purity (Aldrich and Sigma) and were used without further purification. $FeSO_4.7H_2O$, $MnSO_4.H_2O$, $CoSO_4.7H_2O$ and $CuSO_4.5H_2O$ were used as metal ion sources. The Perkin-Elmer 2400 Series II Analyzer was used to determine the percentage of carbon, hydrogen and nitrogen (C, H and N) in the synthesised compounds. The electrospray ionization mass spectra were recorded on Micromass Autoseptic Premier/Agilent HP6890GC. Standard methods were used to determine the metal contents in the complexes.

2.1 Synthesis of the Metal Complexes

The complexes were prepared based on previously reported procedures with slight modifications [19,20]. They were prepared by the reactions of artemether and lumefantrine with metal salts in the ratio 1:1:1.

2.1.1 Complex A: Cu-ART-LUF Mixed Ligand Complexes

To a mixed solution of 0.50 g $CuSO_4.5H_2O$ (2 mmol) and 0.597 g (2 mmol) artemether (ART) in 20 mL methanol, 1.058g (2 mmol) lumefantrine (LUF) was added dropwise with constant stirring at 60°C mild reflux for 6 hours. The reaction mixture was cooled, and the solid product was collected by filtration, washed with diethyl ether and dried in vacuo. A light-green precipitate was obtained with a yield of 66.8%. [Cu(ART)(LUM)(H_2O)]: **Yield**. 0.924 g, 51 %; **Anal. Calc**. for $C_{46}H_{60}Cl_3CuNO_7$; C, 60.79; H, 6.65; N, 1.54; **Found**: C, 60.10; H, 6.36; N, 1.81; **UV** (CH_3OH) λ (nm): 290,360, 665.

2.1.2 Complex B: Fe-ART-LUF Mixed Ligand Complexes

To a mixed solution of 0.556 g $FeSO_4.7H_2O$ (2 mmol) and 0.597 g (2 mmol) artemether (ART) in 20 mL methanol, 1.058 g (2 mmol) lumefantrine (LUF) was added drop wise with constant stirring at 60 °C mild reflux for 6 hours. The reaction mixture was cooled, and the solid product was collected by filtration, washed with diethyl ether and dried in vacuo. A brown precipitate was obtained with a yield of 66.8%. [Fe(ART)(LUM)Cl_2]: **Yield**. 0.866 g, 46 %, **Anal. Calc**. for $C_{46}H_{60}Cl_3FeNO_7$; C, 61.31; H, 6.71; N, 1.55; **Found**: C, 61.54; H, 6.35; N, 1.41; **UV** (CH_3OH) λ (nm): 260, 344, 485.

2.1.3 Complex C: Co-ART-LUF Mixed Ligand Complexes

To a mixed solution of 0.562 g $CoSO_4.7H_2O$ (2 mmol) and 0.597 g (2 mmol) artemether (ART) in 20 mL methanol, 1.058 g (2 mmol) lumefantrine (LUF) was added drop wise with constant stirring at 60°C mild reflux for 6 hours. The reaction mixture was cooled, and the solid product was collected by filtration, washed with diethyl ether and dried in vacuo. A reddish-brown precipitate was obtained with a yield of 66.8%. [Co(ART)(LUM)Cl_2]: **Yield**. 0.746 g, 40%, **Anal. Calc.** for $C_{46}H_{60}Cl_3CoNO_7$; C, 61.10; H, 6.69; N, 1.55; **Found:** C, 60.32; H, 6.21; N, 1.46; **UV** (CH_3OH) λ (nm): 295, 485, 550, 680.

2.1.4 Complex D: Mn-ATM-LUF Mixed Ligand Complexes

To a mixed solution of 0.338 g $MnSO_4.H_2O$ (2 mmol) and 0.597 g (2 mmol) artemether (ART) in 20 mL methanol, 1.058g (2 mmol) lumefantrine (LUF) was added dropwise with constant stirring at 60 °C mild reflux for 6 hours. The reaction mixture was cooled, and the solid product was collected by filtration, washed with diethyl ether and dried in vacuo. A pale-yellow precipitate was obtained with a yield of 66.8%. [Mn(ART)(LUM)Cl_2]: **Yield**: 0.828 g, 44 %, **Anal. Calc**. for $C_{46}H_{60}Cl_3MnNO_7$; C, 61.37; H, 6.72; N, 1.56; **Found**: C, 40.10; H, 4.36; N, 7.81; **UV** (CH_3OH) λ (nm): 290, 360, 415.

2.2 Evaluation ofAntiplasmodial Activity

The test samples were carried out in triplicate on two occasions against three (3) strains; the chloroquine-sensitive (CQ^S) - D10 strain, and the chloroquine-resistant (CQ^R) - RSA11 and Dd2 strains of Plasmodium *falciparum*. Continuous *in vitro* cultures of asexual 30 erythrocyte stages of P. *falciparum* were maintained using a modified method of Trager and Jensen [21]. Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler. The test samples were prepared as a 2 mg/mL stock solution in 10% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20^{o}C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as reference drug in all experiments as well as the parent ligands. Test samples were initially tested at three concentrations (10 µg/mL, 5 µg/mL and 2.5 µg/mL). A full dose-response was performed on all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value).

For D10, samples were tested at a starting concentration of 100 µg/mL, which was then serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 0.2 µg/mL. The same dilution technique was used for all samples. CQ and the ligands used were tested at a starting concentration of 100 ng/mL.

For RSA11 and Dd2, samples were tested at a starting concentration of 1000 ng/mL, which was then serially diluted 2-fold in a complete medium to give 10 concentrations, with the lowest concentration being 2 ng/mL. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 1000 ng/mL. The highest concentration of solvent to which the parasites were exposed had no measurable effect on the parasite viability.

3.0 Results

3.1 Physical Properties of the Ligands/Metal Complexes

The melting points of the complexes are higher than their respective ligands with different melting point ranging from 156- 212^{o}C (Table 1). The complexes showed various colours as characteristics of transition compounds (Table 1). Cu (II) Complex was light green in colour while Complexes of Fe (II), Co (II) and Mn (II) showed dark brown, reddish brown and pale-yellow colours respectively. The yields (%) of the complexes are averagely commendable. Cu (II) complex has the highest yield of 51 % while Co (II) complex has the lowest yield of 40 %.Co (II) complex has the highest conductivity followed by Mn (II) complex and Fe (II) and Cu (II) complexes respectively. As expected, the conductivities of the complexes are all higher than those of their ligands.

3.2 Elemental Analysis of Ligands and Metal Complexes

The results of the elemental analysis of the complexes are presented in Table 2. Theoretical CHN and metal content (%) obtained were found to compete favourably to experimental values obtained (Table 2).

3.3 Solubility of Ligands and Metal Complexes in Selected Solvents

The results of the solubility test determination of the ligands and their complexes in selected solvents namely distilled water, ethanol, methanol, acetone, chloroform and ethyl acetate are presented in Table 3.

Table 1: Some Physical Properties of the Ligands/Metal Complexes

Compound	Melting point (°C)	% Yield	Colour	Rf	Conductivity ($\Omega^{-1}cm^{-1}dm^{-3}$)
Artemether	88	-	White	-	1.65×10^{-6}
Lumefantrine	130	-	Yellow	-	1.80×10^{-6}
Cu(II) Complex	190-192	51	Light green	0.56	5.20×10^{-4}
Fe(II) Complex	168-170	46	Brown	0.62	2.50×10^{-3}
Co(II) Complex	210-212	40	Reddish brown	0.70	6.13×10^{-3}
Mn(II) Complex	156-158	44	Pale yellow	0.68	4.10×10^{-3}

Table 2: Molecular Formula and Elemental Composition of Ligands and Metal Complexes

Compound	Molecular Formula	Molecular Weight (g/mol)	Elemental Analysis (Calc) (%)Found			
			C	H	N	M
Artemether	$C_{16}H_{26}O_5$	298.37	-	-	-	-
Lumefantrine	$C_{30}H_{32}Cl_3NO$	528.94	-	-	-	-
Cu(II) Complex	$C_{46}H_{60}Cl_3CuNO_7$	908.88	(60.79) 60.10	(6.65) 6.36	(1.54) 1.81	6.99 (6.81)
Fe(II) Complex	$C_{46}H_{60}Cl_3FeNO_7$	901.18	(61.31) 61.54	(6.71) 6.35	(1.55) 1.41	6.20 (6.64)
Co(II) Complex	$C_{46}H_{60}Cl_3CoNO_7$	904.26	(61.10) 60.32	(6.69) 6.321	(1.55) 1.46	6.52 (6.50)
Mn(II) Complex	$C_{46}H_{60}Cl_3MnNO_7$	900.27	(61.37) 61.44	(6.72) 6.10	(1.56) 1.68	6.10 (6.22)

Table 3: Solubility of the Ligands and Metal Complexes in Selected Solvents

Compound	Distilled Water	Ethanol	Methanol	Acetone	Chloroform	Ethyl acetate
Artemether	SS	SS	S	SS	SS	S
Lumefantrine	NS	NS	SS	NS	NS	SS
Cu(II) Complex	NS	SS	S	NS	NS	SS
Fe(II) Complex	NS	SS	S	NS	NS	SS
Co(II) Complex	NS	SS	S	NS	NS	SS
Mn(II) Complex	NS	SS	S	NS	NS	SS

S = Soluble, SS = Strongly soluble, NS = Not soluble

3.4 In vitro Antiplasmodial Activity

The antiplasmodial activity of the synthesized complexes (Figure 2) was evaluated *in vitro* against the chloroquine-sensitive D10 (Table 4) and the chloroquine-resistant RSA11 and Dd2 strains of *P. falciparum* (Table 5). A full dose-response was performed on the compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Chloroquine diphosphate (CQDP) was tested as the positive control and the resistance index (RI) values for each compound ranged from 0.3 to 26.0 (Table 5).

Table 4: *In vitro* antiplasmodial activity against *P. falciparum* (CQS) D10 strain

Compound	Parasite survival (%)		
	10 µg/mL	5 µg/mL	2.5 µg/mL
CQDP	15.78	39.06	90.53
Artemether	12.72	12.68	14.41
Lumefantrine	13.90	11.74	10.60
Cu(II) Complex	5.660	5.90	5.62
Fe(II) Complex	24.68	23.77	20.21
Co(II) Complex	24.15	18.27	12.35
Mn(II) Complex	18.85	18.16	12.89

Table 5: *In vitro* antiplasmodial activity and resistance indices against *P. falciparum* CQ^SD10, CQ^R RSA11 and CQ^R Dd2 strains

Compound	IC_{50} - D10 (CQ^S) (nM)	IC_{50}– RSA11 (CQ^R) (nM)	RI^a	IC_{50} – Dd2 (CQ^R) (nM)	RI^b
CQDP	29.1	180.3	6.2	758.0	26.0
Artemether	368.1	1136.8	3.0	1500	24.7
Lumefantrine	329.0	126.5	2.6	114.1	2.8
Cu(II) Complex	323.0	224.3	0.7	260.5	5.9
Fe(II) Complex	84.4	146.2	1.7	90.9	0.3
Co(II) Complex	444.2	269.2	0.6	307.3	0.9
Mn(II) Complex	176.0	129.7	1.4	291.6	0.7

[a] Resistance index (RI^a) = IC50RSA11/ IC50 D10
[b] Resistance index (RI^b) = IC50 Dd2/ IC50 D10

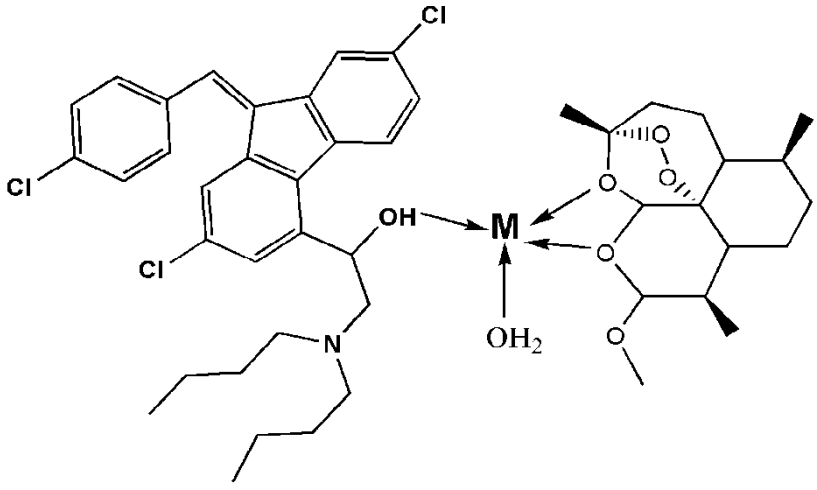


Figure 2: Proposed Structure of synthesized complexes

4.0 Discussion

Results recorded from the physical tests conducted on the complexes support the claim of complexation of the ligands with the metals. The melting point obtained for each of the complexes is higher than the melting point of the ligands ranging from 156-212^{o}C. This indicates that there is coordination between the ligands and their metal salts, thereby resulting to complexation [17]. The complexes are non-hygroscopic; air stable crystalline powders with different melting point ranging from 168-212^{o}C. As expected, colours being a characteristic of transition compounds, all the complexes are coloured. This further indicates complexation of the ligands with the metals. The results of the conductivity measurements in methanol revealed that the complexes are non-electrolyte. The conductivity values of the complexes are higher than those of the ligands, suggesting increase in the ionic content in solutions of the complexes due to the presence of metal ions in them. The solubility of the metal-complexes in various solvents confirmed the diversity of the complexes as the ligands. It is established from our results of the physicochemical analysis that the ligands (artemether and lumefantrine) employed in this work coordinate with the metals. The proposed structure (Fig. 2) shows the coordination of the ligands to the central metal ions. The artemether ligand acting as a bidentate ligand whilst the lumefantrine acting as a monodentate ligand, with a water molecule coordinated to the central metal atom to give a tetrahedral geometry of the complexes. The results obtained support this tentative structure.

On the biological (antiplasmodial) activity of the compounds, excellent results are obtained. An initial three-point concentration screen showed that the compounds have very good activity with less than 10% parasite survival at 2.5µg/mL (Table 4). The activities of these compounds were compared to the reference drug chloroquine diphosphate (CQDP) along with the starting ligands. All the mixed ligand complexes demonstrated activity against all the tested parasite strains. The most active compound of this group was Fe (II) complex with IC_{50} values of 84.4 nM and 146.2 nM in the parasite strains D10 and RSA11, respectively. In general, the complexes were more active in the RSA11 (CQ^{R}) strain than in the D10 (CQ^{S}) and Dd2 (CQ^{R}) strains, with the exception of Fe (II) complex. When compared against control, the complexes were less active than chloroquine against D10 (CQ^{S}), as expected. However, Fe (II) and Mn (II) complexes demonstrated more activity than chloroquine against RSA11 (CQ^{R}), as seen for the complexes against Dd2 (CQ^{R}), overcoming the parasitic resistance associated to the quinoline fragment.

The RI, defined as the IC_{50} value in the CQ^{R} strain divided by the IC_{50} value in the CQ^{S} strain, provides a quantitative relationship on how the compound behaves against chloroquine-sensitive and chloroquine-resistant strains. If this variation is small, it shows that the compound is active irrespective of the susceptibility of the parasitic strain. On the contrary, if the difference is large (the compound is much more active in a sensitive strain than in a resistant strain, most likely) it is an indication of loss of activity due to resistance development or prospect of resistance development. A promising drug would have a RI since it is an indication that the drug candidate is not being detected by whatever resistance mechanism. As anticipated, the synthesized complexes showed very small tendency to develop resistance due to their structural disparity to chloroquine indicated by the low values of RI. Some of these compounds are equally or more potent in drug-resistant parasite strains than in drug-sensitive strains. This indicates that they follow a different mechanism which allows them to evade detection by the transmembrane proteins contrary to the mechanism of accumulation of chloroquine. This may be responsible for lower accumulation of the drug (origin of resistance) [22,23].

5.0 Conclusion

This study has shown the feasibility and justification for the synthesis of mixed antimalarial metal complexes. The complexes possessed interesting physical properties and have much more improved antiplasmodial activity than chloroquine and their parent ligands. The low RI values of the synthesized complexes showed very small tendency to develop resistance due to their structural disparity to chloroquine. The evaluation test from this research showed that the mixed ligand complexes are more effective antimalarial agent than the parent ligands.

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