Centrepoint Journal (Science Edition) 2141-3819/2017 $5.00 + 0.00
Volume 25, No.2, pages 21-32
http//www.unilorin.edu.ng/centrepoin
CPJ 2019014/25202

Mixed metal complexes of Artemether, Lumefantrine and Ascorbic acid: Synthesis, characterization and biological activity studies

H.F. Babamale*[1], J.A. Obaleye[2]., A. Lawal, [2]., A.O. Rajee, [2]., M.O. Bamigboye[1], M. Lawal[3], and H.O. Sa′ad [2]

[1]*Department of Industrial Chemistry, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.*

[2]*Department of Chemistry, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.*

[3]*Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, P.M.B 1144 Aliero, Kebbi State.*

**Correspondence author: haliemashehu@gmail.com*

Abstract

Some transition metal complexes of mixed artemether, ascorbic acid and lumefantrine have been synthesized and characterized using UV/Vis, FTIR, Atomic Absorption Spectroscopy (AAS) and Gas chromatography-Mass Spectroscopy (GC-MS). The results revealed that the artemether and ascorbic acid are chelating ligands and coordinates through the oxygen of the carbonyl groups while lumefantrine coordinates through the hydroxyl group in a monodentate fashion. =The metal complexes were investigated for biological activity using five bacterial species - *Staphylococcus aureus, Escherichia coli, Bacillus aureus and Pseudomonas aeruginosa*. It was observed that the synthesized complexes were more effective towards the five bacteria species than the free drugs.

Keywords: Artemether, lumefantrine, ascorbic acid, metal complexes, biological activity.

Introduction

Artemether is an aliphatic tetra heterocyclic compound synthesized from artemisinin [1]. It is an important antimalarial drug that is highly effective against multi-strain resistant plasmodium species. It is on the World's Health Organization list of essential medicine in basic medical system [2]. Lumefantrine is also an antimalarial drug which belongs to the chemical family named "Aryl amino alcohol". It is always administered in combination with artemether. Ascorbic acid, also known as vitamin C and has two isomers; the L- and D- isomer. Only L-ascorbic acid is found in nature as well as being useful in the human body. Ascorbic acid is an essential

vitamin in the body which helps to repair and maintain growth of tissues. Its deficiency in the body leads to the various diseases such as scurvy. However, overdose of this essential vitamin may lead to nausea and diarrhea [3]. The study of metal complexes is gaining increasing importance in the design of drugs [4]. It is known that some drugs act via chelation [5]. The combination of the metal with the drugs may improve the activity of the drug [6]. In addition, the combination therapy of two antimalarial drugs haves been proved to provide the benefits for rapid cleaning of parasites from the blood stream. The most effective treatment against malaria is a combination of drugs using derivatives of artemisinin [7]. The combination of artesunate and mefloquine appear with a high prevalence of multidrug resistant parasites [8,9]. The combination of artesunate with pyrimethamine/sulphadoxine gave diverse results [10,11]. Artemisinin has also been combined with an antibiotic, clindamycin, but the resultant application produced a delayed activity in malaria parasite death [12].

As part of our research on mixed drug metal complexes, this paper report the synthesis, characterization and antibacterial activity study of mixed metal complexes of artemether, lumefantrine and ascorbic acid.

MATERIALS AND METHODS

Materials

The solvents (which include distilled water, methanol, ethanol, ethylacetate and acetone) and metal salts ($FeCl_3 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$**,** $CuCl_2 \cdot 6H_2O$and $ZnSO_4$) were from BDH Chemicals. Artemether, ascorbic acid and lumefantrine were obtained from Emzor Pharmaceutical Company, Lagos, Nigeria. The melting point and conductivity of the complexes were determined using Electrothermal IAg100 apparatus and HANNA instruments EC214 conductivity meter respectively. Infrared spectroscopic analysis of the complexes were done at LAUTECH, UV/Visible spectroscopy were carried out at University of Ibadan using Perkin Elmer UV/Visible spectrometer, Atomic Absorption Spectroscopy was done using AAS BUCK SCIENTIFIC 210 VGPand GC-MS analysis of the complexes were carried at University of Ilorin using MSD machine adopting ACQ method.

Biological activity study of the complexes was investigated on the five bacterial species mentioned above at Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

Methodology

Synthesis of $[M(ART)(ASC)Cl_2]$, $[M(ASC)(LUM)Cl_2]$ and $[M(LUM)(ART)Cl_2]$ complexes.

The procedure described by Obaleye *et al*. (13) was adopted for the synthesis of the complexes. Gramme equivalent of 1mmol each of the metal salts M, were weighed and dissolved in methanol. Similarly, gramme equivalent of 1mmol of the ligands were also weighed and dissolved in appropriate solvent. Two of the ligand solutions were added together and then mixed with one of the dissolved metal salt. The resulting solution was refluxed for about three hours. The refluxed solution was allowed to cool at room temperature, kept for five days after which there was crystal formation, which was filtered and stored in a desiccator over $CaCl_2$.

Biological activity study

The biological activity of the complexes and ligands were determined using agar diffusion method as prescribed by Matangi *et al*(14). The organisms used are S*taphylococcus aureus, Pseudononas aeruginosa, Bacillus aereus and Escherichia coli.* Nutrient agar was prepared by dissolving 4 g of nutrient agar into 500 ml of sterile distilled water. This was autoclaved for 15 minutes and transferred into sterilized Petri dish. One of the organisms was then streaked all over the agar. A hole is then bored on the agar using cork borer after which 0.5 ml of the standard solution of the drug was poured into the hole. It was then incubated for 24 hours after which the reading was taken by observing the inhibition zone. The diameter of this zone was then measured. This was done using each of the ten complexes and the three ligands at 10 mg/ml and 5 mg/ml concentration.

RESULTS AND DISCUSSION

The physical property tests which include melting point, conductivity and solubility test yielded result suggesting the formation of these complexes. It was found that the melting points of the complexes are generally higher those of their constituting ligands. The conductivities of the complexes were also different from those of the ligands. The solubility test result indicated some changes in the physical properties of the ligands as the resulting complexes have solubilities different from those of the ligands.

Table 1: Analytical data of mixed artemether, lumefantrine and ascorbic acid - metal complexes

Compounds	**Melting point (^{o}C)**	**Conductivity (µS)**
Ascorbic acid	190 – 192	5
Artemether	85 – 89	4
Lumefantrine	125 – 130	4
[Cu(Lum)(Asc)]Cl_2	220 – 223	10
[Co(Lum)(Asc)]Cl_2	262	5
[Zn(Lum)(Asc)]SO_4	155 – 157	5
[Cu(Art)(Asc)]Cl_2	222-224	5
[Zn(Asc)(Art)]SO_4	238(decompose)	4
[Co(Art)(Asc)]Cl_2	226-228	4
[Cu(Lum)(Art)]Cl_2	200 – 205	5
[Co(Lum)(Art)]Cl_2	300 (decompose)	10
[Zn(Lum)(Art)]SO_4	268-270	6
[Fe(Art)(Asc)]Cl_2	231-234	15

Table 2: UV-Visible spectral of mixed artemether, ascorbic acid and lumefantrine metal drug complexes

Compounds	Wavelength(nm)	Absorbance	Energy (cm^{-1})	Transition
Artemether	208.99	3.5792	47849.18	$\pi \rightarrow \pi^*$
Ascorbic acid	212.46	3.7041	47067.68	$\pi \rightarrow \pi^*$
	260.12	3.1352	38443.80	$\pi \rightarrow \pi^*$
Lumefantrine	232.03	5.7729	43097.88	$\pi \rightarrow \pi^*$
	359.67	3.4057	27803.26	$n \rightarrow \pi^*$
[Cu(Lum)(Asc)]Cl_2	247.32	3.7048	40485.83	$n \rightarrow \pi^*$
	386.42	2.7475	25878.58	$d \rightarrow d$
[Co(Lum)(Asc)]Cl_2	252.01	0.3578	39682.54	$\pi \rightarrow \pi^*$
	290.12	0.2569	34468.50	$n \rightarrow \pi^*$
[Zn(Lum)(Asc)]SO_4	215.98	6.1006	46511.63	$\pi \rightarrow \pi^*$
	295.47	3.8297	33898.31	$n \rightarrow \pi^*$
	375.57	1.1417	26666.67	$d \rightarrow d$
[Cu(Art)(Asc)]Cl_2	199.50	0.6327	50125.31	$\pi \rightarrow \pi^*$
	378.00	0.3531	2455.03	$n \rightarrow \pi^*$
	871.72	0.08612	11471.57	$d \rightarrow d$
[Zn(Asc)(Art)]SO_4	244.79	0.1039	40851.34	$\pi \rightarrow \pi^*$
[Co(Art)(Asc)]Cl_2	244.10	0.1874	40983.61	$\pi \rightarrow \pi^*$
[Cu(Lum)(Art)]Cl_2	280.18	3.4116	35691.34	$\pi \rightarrow \pi^*$
	311.52	3.3462	32100.67	$n \rightarrow \pi^*$
	384.89	0.4363	25981.45	$d \rightarrow d$
[Co(Lum)(Art)]Cl_2	272.35	3.3171	36717.46	$\pi \rightarrow \pi^*$
	295.00	3.3665	33898.31	$n \rightarrow \pi^*$
	384.89	0.5258	25981.45	$d \rightarrow d$
[Zn(Lum)(Art)]SO_4	245.37	3.076	40816.33	$\pi \rightarrow \pi^*$
	288.10	3.3384	34710.17	$n \rightarrow \pi^*$
	383.94	0.3815	26045.74	$d \rightarrow d$
[Fe(Art)(Asc)]Cl_2	247.32	3.2105	40433.45	$\pi \rightarrow \pi^*$
	311.52	3.4001	32100.67	$n \rightarrow \pi^*$
	387.18	2.1594	25827.78	$d \rightarrow d$

Table 3: Infrared spectral of mixed artemether, ascorbic acid, lumefantrine metal drug complexes

Complexes	-OH	C=O	C-O	C-N	C-H	C=C
Ascorbic acid	3526 – 3233	1676 – 1755	1024	2372	2947	1452
Artemether	3428	-	1034 – 1103	2369	2947	1452
Lumefantrine	3401	-	1074	2371	2953	1400
[Cu(Lum)(Asc)]Cl_2	3429	-	1109	-	2964	1456
[Co(Lum)(Asc)]Cl_2	3394	1628	1053-1113	2372	-	1450
[Zn(Lum)(Asc)]SO_4	3397	1638	1078	2365	2949	1473
[Cu(Art)(Asc)]Cl_2	3435	-	1060 – 1109	2372	2961	1456
[Zn(Asc)(Art)]SO_4	-	1656	1034 – 1105	2371	2876 – 2947	1454
[Co(Art)(Asc)]Cl_2	-	1710	1020 – 1105	2372	2949	1454
[Cu(Lum)(Art)]Cl_2	-	1709	1034 – 1099	2371	2949	1460
[Co(Lum)(Art)]Cl_2	3510	1633	1055 – 1090	2371	2950	1452
[Zn(Lum)(Art)]SO_4	3503	1632	1096	2371	2953	1400
[Fe(Art)(Asc)]Cl_2	3277	1632	1086	2372	2958	1404

Table 4: The results of AAS analysis of mixed artemether, ascorbic acid, lumefantrine metal drug complexes

Compounds	Weight of Sample Measured (g)	Amount of Metal Present (ppm)	Percentage Metal	
			Expected value (%)	Analyzed value (%)
[Cu(Lum)(Asc)]Cl_2	0.0310	1227.50	7.58	7.00
[Co(Lum)(Asc)]Cl_2	0.0065	134.80	7.07	7.07
[Zn(Lum)(Asc)]SO_4	0.0192	3.67	7.55	7.45
[Cu(Art)(Asc)]Cl_2	0.0029	144.00	10.48	10.40
[Zn(Asc)(Art)]SO_4	0.0595	1.86	10.28	10.22
[Co(Art)(Asc)]Cl_2	0.0330	0.65	9.77	9.50
[Cu(Lum)(Art)]Cl_2	0.0141	4.59	6.61	6.61
[Co(Lum)(Art)]Cl_2	0.0493	4.03	6.16	6.06
[Zn(Lum)(Art)]SO_4	0.0715	34.30	6.61	6.51
[Fe(Art)(Asc)]Cl_2	0.0561	203.5	9.16	9.16

The results of the spectroscopic studies confirmed the formation of coordination complexes. The UV-Vis spectra data indicate that the ligands majorly absorbed energy in the electromagnetic spectrum corresponding to $\pi \rightarrow \pi^*$ transition while the complexes absorbed lower energy corresponding to $n \rightarrow \pi^*$ transition which is because of coordination of metals to these ligands. The $\pi \rightarrow \pi^*$ transition was observed for lumefantrine ligand while its complexes absorbed lower energy corresponding to $d \rightarrow d$ transition which further confirmed the formation of these complexes[15].

Infrared spectra data of the ligands were compared with those of the complexes. The O-H vibrations observed between 3526 to 3400 cm^{-1}in the ligands were shifted to between 3435 to 3300 cm^{-1} in the mixed complexes due to coordination [13]. The C=O stretching vibrations at 1676 cm^{-1}in the ascorbic acid has been shifted to 1710 cm^{-1} and 1628 cm^{-1} in the mixed complexes [5,13].

The metal content of the prepared complexes was determined by atomic absorption spectroscopy and the results are presented in Table 4. The percentage metal analyzed was calculated from these concentrations and the values were then compared to the theoretical/percentage metal calculated for each of the complexes. The comparison showed that there was no significant difference between them. This further established the formation of the expected metal complexes.

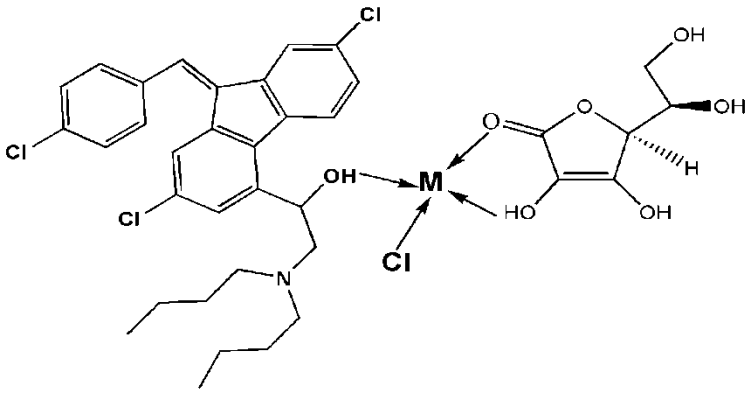
Table 5: Results of the biological activity study of the complexes

S/N	Complexes	Zone of inhibition (mm)									
		MRSA		*Staph*		*Bacillus*		*E. coli*		*Pseudo*	
		A	B	A	B	A	B	A	B	A	B
1.	$[Cu(Lum)(Asc)]Cl_2$	10	10	15	15	18	16	7	0	15	18
2.	$[Co(Lum)(Asc)]Cl_2$	15	10	16	12	20	18	0	0	0	0
3.	$[Zn(Lum)(Asc)]SO_4$	8	7	16	12	10	8	7	6	0	10
4.	$[Cu(Art)(Asc)]Cl_2$	10	8	11	10	16	12	8	6	0	0
5.	$[Zn(Asc)(Art)]SO_4$	18	14	10	8	15	13	10	7	0	0
6.	$[Co(Art)(Asc)]Cl_2$	10	10	12	9	12	8	8	7	0	12
7.	$[Cu(Lum)(Art)]Cl_2$	18	15	10	8	15	9	23	15	0	13
8.	$[Co(Lum)(Art)]Cl_2$	0	0	0	0	16	10	10	0	0	13
9.	$[Zn(Lum)(Art)]SO_4$	8	7	9	7	10	10	10	7	0	15
10.	$[Fe(Art)(Asc)]Cl_2$	0	0	10	8	17	10	0	0	8	14
11.	Ascorbic acid	0	0	20	18	21	18	10	0	11	15
12.	Artemether	15	12	14	12	13	8	10	9	0	0
13.	Lumefantrine	0	0	13	10	20	12	0	0	0	0

A = 5mg/ml complex concentration. **B** = 10mg/ml complex concentration

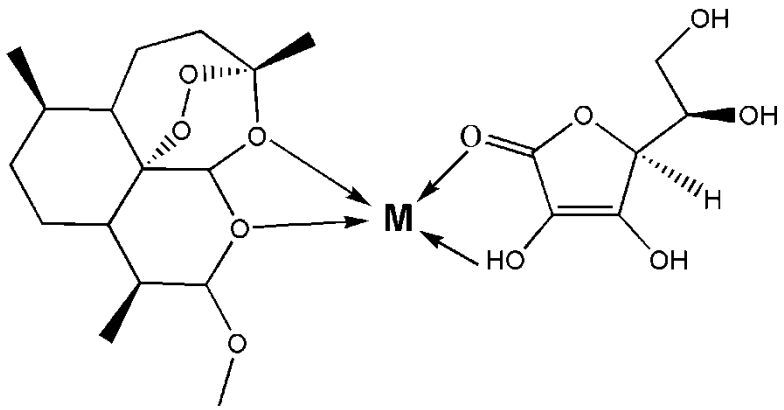
The biological activity study carried out with the complexes and the ligands revealed that both were effective. However, it was observed that the complexes are generally more effective on the bacteria used than the ligands. Also, if any of the complexes would be active on the bacteria at low concentration, increasing the concentration to a very high concentration would rather lower the effectiveness or activity of this complex/drug.

Proposed structure for lumefantrine-ascorbic acid metal complex



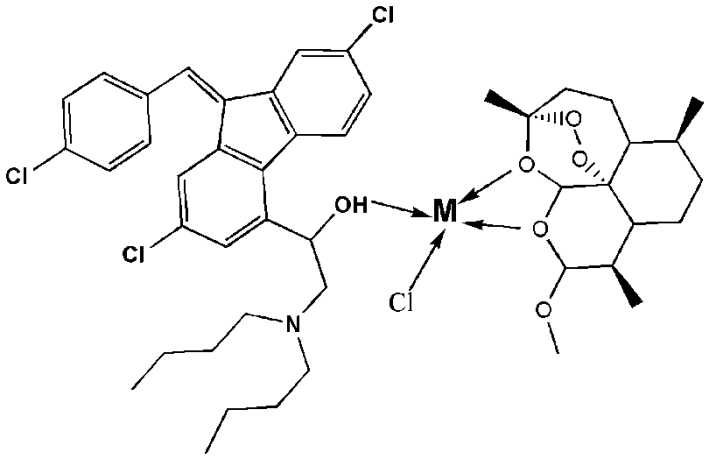
M = Zn, Cu, Co, Fe

Proposed structure for ascorbic acid-artemether metal complexes



M = Zn, Cu, Co, Fe

Proposed srtucure for lumefantrine-artemether metal complexes



M = Zn, Cu, Co, Fe

Conclusion

The Co^{2+}, Cu^{2+}, $Zn^{2+}$and Fe^{2+}complexes of mixed lumefantrine, ascorbic acid and artemether have been synthesized and characterized using different spectroscopic techniques and physico-chemical methods. The spectroscopic techniques revealed the coordination of the ligand to the metal. The ascorbic acid coordinated in a bidentate fashion, while the coordination modes of lumefantrine and artemether is monodentate and bidentate respectively. The biological activity sttudy revealed that the synthesized complexes were more effective against the five bacterial species than the free drugs.

Acknowledgement

The authors thank STEP B, TETFUND and University of Ilorin Senate Research for their kind support of this project.

References

Ho W. 2004, Studies on molecular mechanism of action and synthesis of new derivatives of the antimalarial drug artemisinin, *Hong Kong University of Science & Technology, (Thesis - DPhil)* pp. 1-38, 309-337.

"WHO Model List of EssentialMedicines". *World Health Organization*. October 2013. Retrieved 22 April 2014.

Lachapelle, M. Y.; Drouin, G. (2010). "Inactivation dates of the human and guinea pig vitamin C genes". *Genetica*Volume 139, Issue2, 199–207.

Abu-El- Wafa, S.M., El-Rifs, M.A and Ahmed, F.H (1987) Formation of Metformin complexes with some transition metal ions and their biological activity. inorg. Chim. Acta., 136, 127- 131

Behren, N.B., Diaz, G.M and Goodgame, D.M.L (1986) Metal complexes of the antibiotics, Nalixidic acid, Inorg.Chim., Acta, 125,2(1), 264

Hariprasath K., Deepthi B., Sudheer B. I., Venkatesh P., Sharfudeen S., Soumya V., (2010) Metal Complexes in Drug Research - A Review.*Journal of Pharmaceutical Chemistry Research.* Volume 2, Issue 4:496-499

Henri, V (2002) Synthesis of 1,6hexadecamethylenebis (N- methylpyrrolidinum) dibromide as antimalarial agent. Science, 295,1311.

Ezzet, F., Mull, R and Karbwang, J (1998) Population pharmacokinetics and therapeutic response of CGP56697 (artemether + benflumetol) In malaria patients. Br.J.Pharmacol, 46: 553-561

Van Vugl, M., Looareesuwan,S., Wilairatana, P., McGready, R., Villeagas,L., Gathman,

J., Mull, R., Brockman, A., White, N.J and Nosten, F (2000) Artemether-lumefantrine for the treatment of multidrug-resistant falciparum malaria . Trans. R.Soc. Trop. Med. Hyg., 94 (5): 545-548

Martinelli,A., Moreira, R and Cravo, P.V.L (2008) Malaria combination therapies advantages and shortcomings. Mini. Reviews in Medicinal Chemistry,8, 201-212

Elamin, S.B., Malik, E.M., Abdelgadir, T., Khamiss, A.H., Mohammed, M.M ., Ahmed,

E.S and Adam, I (2005) Artesunate plus sulfadoxine – pyrimethamine for treatment of uncomplicated plasmodium falciparum malaria in Sudan Malaria, J. 4:41

Goodman, C.D., Su,V and Mcfadden, G.T (2007) The effect of antibacterials on the malaria parasite plasmodium falciparum . Mol.Biochem. Parasitol, 15(2) :181-191

Obaleye, J.A and Orjiekwe, C.L.(1993). Synthesis and characterization of Cu(II) and Zn(II) complexes of ascorbic acid. Int. Chem., Vol 4, pg 37-51.

Matangi S., Pragathi J., Bathini U., and Gyana K. (2012) Synthesis, Characterization and Antimicrobial Activity of Transition Metal Complexes of Schiff Base Ligand Derived from 3-Ethoxy Salicylaldehyde and 2-(2-Aminophenyl) 1-H-benzimidazole E-Journal of Chemistry Volume 9 (2012), Issue 4, Pages 2516-2523.

Obaleye, J.A., Lawal, A**.** Rajee, A.O, Babamale, H.F and Shittu, F.B. (2014).Synthesis, Characterization and Antimicrobial Activity of Amodiaquine and Sulphadoxine Ligand-Metal Complexes. Nigerian Journal of Biochemistry and Molecular Biology**,** .29(2): 170- 178.