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Synthesis and Characterisation of Some Mixed Artemisinin-Thiamin Chloride and Artemisinin-Pyridoxine- Metal Complexes and Their Antimicrobial Activity Studies

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Abstract

Metal complexes of Zn^{2+}, Mn^{2+}, Cu^{2+} and Fe^{3+} with mixed Artemisinin-thiamin chloride and Artemisinin-pyridoxine have been synthesised and characterised using infrared, electronic and ^{1}H-NMR spectroscopy, melting point and conductivity measurements. Artemisinin-thiamin chloride complexes coordinate through the hydroxyl and amide groups, while Artemisinin-pyridoxine complexes coordinate through the hydroxyl and cyanide groups, both in bidentate coordination geometry. Antibacterial screening of the complexes against five bacteria species- *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas auriginosa* and *Proteus mirabilis*, was also investigated. The metal complexes were found to have varied degrees of inhibitory effect against the bacteria species under study.

Keywords: Artemisinin, Thiamin Chloride, Pyridoxine, Metal complexes, Antimicrobial activities.

Introduction

Broadly speaking, a drug is any substance that, when absorbed into the body of a living organism, alters its normal bodily functions (Combs, 2006). It may be any compound taken to correct any abnormality in the normal physiological activities of the cell.

Vitamins are non-energy producing complex organic compounds occurring naturally in foods, required as nutrients in tiny amounts and essential to the normal physiological functioning of living bodies (Puetter, 2000). Nutritionists categorise vitamins by the materials that a vitamin

will dissolve in. The B-group vitamins are said to dissolve in water, therefore thiamin chloride and pyridoxine are water-soluble vitamins. These vitamins dissolve easily in water, and in general are readily excreted from the body to the degree that urinary output is a strong predictor of vitamin consumption (Fukuwatari and Shibata, 2008). Because they are not readily stored, consistent daily intake is important as they travel through the bloodstream. Whenever water-soluble vitamins are not used by the body, they are eliminated in the urine which means there is need for a continuous supply of the vitamins in the food (Fukuwatari and Shibata, 2008).

On the other hand, antimalarial drugs are drugs that combat malaria caused by a parasite known as *Plasmodium falciparum* usually carried by a female anopheles mosquito. As the name implies, antimalarials attack the different stages of the parasite growth and development. An example is Artemisinin which has a strong blood schizonticidal action and reduces gametocyte transmission (Peters, *et al*. 1993).

Previous research has shown that complexing drugs to metal ions could enhance their activities (El Guindi, *et al*. 1988). It is also known that some drugs act via chelation or by inhibiting metalloenzymes but for most of the drugs that act as potential ligands, a lot of studies are being carried out to ascertain how metal binding influences the activities of the drugs (Behrens, *et al*. 1986). Metal complexes are gaining increasing importance in the design of drugs on coordination with metals. Therefore, it is considered necessary to have some more antimalarial-vitamin- drug-metal complexes in order to contribute to the effort being made to produce drugs that can be used against drug-resistant diseases.

Materials and Experimental

Materials

The ligands- artemisinin and vitamins (thiamin chloride and pyridoxine), metal salts and the solvents used for this research were of analytical grade and were used as received without further purification. The ligands – Artemisinin and Vitamins (thiamin chloride and pyridoxine) were gifts respectively from Bond Chemical industries, Oyo State and Peace Pharmaceuticals, Kwara State, Nigeria. The metal salts - zinc(II) chloride dihydrate ($ZnCl_2.2H_2O$), manganese(II) chloride tetrahydrate

($MnCl_2.4H_2O$), copper(II) chloride dihydrate ($CuCl_2.2H_2O$), and iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) and various solvents used were obtained from the Department of Chemistry University of Ilorin. The test organisms- *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas auriginosa* and *Proteus mirabilis* were obtained from University of Ilorin Teaching Hospital, Ilorin, Nigeria. The melting points were determined using the Gallenkamp melting point apparatus and the open capillary method. The solubility of the complexes was studied in various solvents such as distilled water, acetone, ethanol, methanol, toluene, ethyl acetate and benzene. The conductivity measurements for the ligands and complexes were also carried out using the WTW conductometer Bridge with cell constant $0.82cm^{-1}$. The infrared spectra of the samples were recorded on Nicolet iS5 Fourier Transform Infrared spectrophotometer. The electronic spectra of the complexes in solution run in the range 200-1000 nm on Beckman Coulter DU 730 life Science Ultraviolet-visible spectrophotometer. The Nuclear Magnetic Resonance analysis for some of the compounds was also carried out.

Synthesis of the metal complexes of Artemisinin-thiamine chloride

A 5 mmol of the metal salts ($ZnCl_2.2H_2O$ (0.034 g), $MnCl_2.4H_2O$ (0.031 g) and $CuCl_2.2H_2O$ (0.043 g)) each was weighed accurately and dissolved in 20 ml methanol in a beaker. Also artemisinin (0.141 g, 10 mmol) and thiamine hydrochloride (0.169 g, 10 mmol) were weighed and dissolved separately with 30 ml methanol. The solution of the metal salts and the ligands were then mixed together in a 250 ml conical flask. The mole ratio of the metal salt to ligand was maintained at 1:2:2. The solution was then heated to reflux for about two hours at 70^oC. Thereafter the solution was allowed to cool to room temperature. The crystals formed were washed in distilled water and dried in desiccator.

Synthesis of the metal complexes of Artemisinin-pyridoxine

A 5 mmol of the metal salts ($ZnCl_2.2H_2O$ (0.034 g), $MnCl_2.4H_2O$ (0.031 g), $CuCl_2.2H_2O$ (0.043 g) and $FeCl_3.6H_2O$ (0.068 g)) each was weighed accurately and dissolved in 20 ml ethanol in a beaker. Also 10 mmole of artemisinin (0.141 g, 10 mmol) and Pyridoxine (0.085 g, 10 mmol) were weighed and dissolved separately with 30ml Ethanol. The solution of the metal salts and the ligands were then mixed together in a 250ml conical flask. The mole ratio of the metal salt to ligand was maintained at 1:2:2. The solution was then refluxed for about two hours at 70^oC. Thereafter the

solution was allowed to cool to room temperature. The crystals formed were washed in distilled water and dried in a desiccator.

Antimicrobial screening of the ligands and metal complexes

The antimicrobial screening of the ligands and complexes was investigated using Punched hole method. The test organisms are- *E. coli*, *S. aureus, K. pneumonia*, *P. aureginosa*, and *P. mirabilis*. Nutrient agar was prepared and poured on some petridishes. The petridishes were allowed to dry in the oven. Each organism was dissolved in normal saline water and innoculated separately on the nutrient agar. Six holes were then punched on each plate and the dissolved complexes and ligands were introduced into the holes. The plates were incubated for 24hrs after which some zones of inhibition were seen for some the organisms. These zones of inhibition were then measured. The antimicrobial screening of the ligands and complexes was carried out at the Department of Serology, University of Ilorin Teaching Hospital, Ilorin.

Results and discussion

Table 1 Analytical test data and some physical properties of the ligands and metal complexes

Sample analyzed	Melting point (^{0}C)	Colour	Conductivity µS	Electronic transition ʎmax(nm)	% Yield
Artemisinin	152 – 156	White	-	293	-
ThCl	224 – 228	Cream yellow	-	300	-
Zn(Art)(Th)Cl_2	236 – 240	Cream yellow	2	327	74.42
Mn(Art)(Th)Cl_2	232 -236	Cream yellow	25	294	66.28
Cu(Art)(Th)Cl_2	234-238	Yellow	105	-	80.45
Pyr	118 – 122	White	-	298	-
Zn(Art)(Pyr)Cl_2	246 – 249	Brown	12	291	84.62
Mn(Art)(Pyr)Cl_2	204 – 207	White	13	306	77.04
Cu(Art)(Pyr)Cl_2	203 – 205	Cream white	457	311	85.13
Cu(Pyr)Cl_2	210 – 212	Yellow	683	305	92.97
Fe(Art)(Pyr)Cl_3	203 – 205	Reddish brown	47	312	93.88

Table 2 Selected Infrared spectra data of the ligands and metal complexes

Ligand /Complex	Tentative assignment				
	$\bar{\upsilon}$(O-H)cm^{-1}	$\bar{\upsilon}$(C=O)cm^{-1}	$\bar{\upsilon}$ (N-H)cm^{-1}	$\bar{\upsilon}$ (C=N)cm^{-1}	$\bar{\upsilon}$ (C=C)cm^{-1}
Artemisinin	3456.55 vw,sh	1737.92 vs,sh	-	-	-
ThCl	3508.63 s,sh	-	3235.73 vs,b	1664.62 vs,b	1614.47 vs,sh
Zn(Art)(Th)Cl_2	3456.55 m,sh	1735.99 vs,sh	-	1647.26 m,sh	-
Mn(Art)(Th)Cl_2	3506.7 s,sh	-	3230.87 vs,b	1654.98 vs,b	1614.47 vs,sh
Cu(Art)(Th)Cl_2	3352.39 m,sh	-	3281.02 m,sh	1656.91 vs,sh	1604.83 vs,sh
Pyr	3354.32 vs,b	-	-	1647.26 w,sh	1626.05 m,sh
Zn(Art)(Pyr)Cl_2	3331.18 vs,b	1830.51 vw,sh	-	-	1624.12 m,sh
Mn(Art)(Pyr)Cl_2	3329.25 vs,sh	1732.13 m,sh	-	-	1626.05 m,sh
Cu(Art)(Pyr)Cl_2	3327.32 vs,sh	1732.13 vw,sh	-	-	1626.05 m,sh
Cu(Pyr)Cl_2	3331.18 vs,b	-	-	1647.26 w,sh	1626.05 m,sh
Fe(Art)(Pyr)Cl_3	3354.32 vs,b	1732.13 m,sh	-	-	1626.05 vs,sh

Table 3 NMR spectra data of the ligands and metal complexes

Ligand/ Complex	^{1}HNMR peak (ppm)
Artemisinin	1.5
ThCl	4.6
Zn(Art)(Th)Cl_2	1.3
Mn(Art)(Th)Cl_2	2.6
Pyr	2.6
Zn(Art)(Pyr)Cl_2	2.6
Cu(Pyr)Cl_2	4.6

Table 4 Result of the Percentage zone of inhibition of the ligands and their metal complexes at the concentration of 0.1%w/v on the microbial population.

Ligand/ Complex	*E. coli*	*S. aureus*	*K. pneumonia*	*P. mirabilis*	*P. auriginosa*
Artemisinin	18	0	10	0	15
ThCl	15	8	0	0	0
$Zn(Art)(Th)Cl_2$	23	8	22	17	22
$Mn(Art)(Th)Cl_2$	20	20	17	8	21
$Cu(Art)(Th)Cl_2$	23	8	8	17	22
Pyr	0	10	8	0	0
$Zn(Art)(Pyr)Cl_2$	22	8	17	22	17
$Mn(Art)(Pyr)Cl_2$	20	20	20	18	15
$Cu(Art)(Pyr)Cl_2$	22	17	17	8	17
$Cu(Pyr)Cl_2$	23	22	17	8	17
$Fe(Art)(Pyr)Cl_3$	17	22	15	22	17

Discussion

It was observed from Table 1 that the complexes had higher melting points than the ligands. This might be attributed to the large carbon atoms in them. The colors exhibited by the complexes might be the result of charge transfer from the ligands to the metal ions in the complex molecules. The conductivity values also showed that there were electronic transitions from one d-orbital to another in the complex molecules. However, the copper complexes showed extremely high conductivity values as compared with the other metal-complexes.

The electronic spectra data (Table 1) had been interpreted in terms of electron transfer from the metal to the antibonding orbital of the ligands (Williams and Flemming, 1980). Higher bands than that of the ligands were assigned to n-π* transitions (i.e bathochromic shift) while the lower bands were assigned to π-π* transitions (i.e hypsochromic shift). Therefore, the band at 300nm in free thiamin chloride underwent bathochromic shift in its complex with Zinc metal salt and hypochromic shift in its other metal salts. On the other hand, the band at 298nm in free pyridoxine underwent a hypochromic shift in its complex with zinc metal salt and bathochromic shift in the other metal salts.

In essence electrons moved from the non-bonding orbital of zinc metal to the antibonding orbital of each ligand and from the bonding orbital of the other metals to the antibonding orbital of each ligand. These shifts in bands could be a result of complexation.

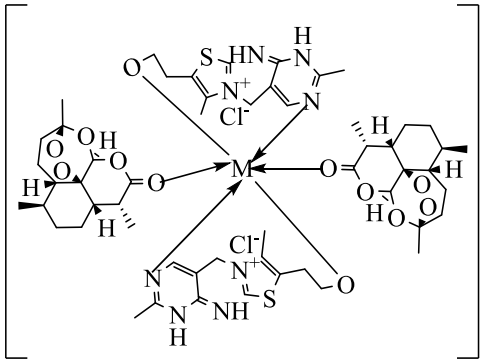
The very strong and sharp absorption band of artemisinin in the infrared region (Table 2) at a wavelength of 1737cm^{-1} might be due to υ(C=O) vibrational stretching of an ester or lactone group (Coartes, 2000). This band experienced a slight shift to a lower frequency in its complexes showing that there was coordination at the (C=O) group of artemisinin. The bands at 3508cm^{-1} and 3234cm^{-1} in free thiamin chloride spectrum were due to υ(O-H) and υ(N-H) stretching frequencies respectively.

These bands were shifted to lower frequencies in its metal complexes. Only the zinc metal complex of thiamin chloride showed no υ(N-H) frequency. These shifts in bands might be the effect of coordination of thiamin chloride through the (O-H) and (N-H) groups. The very strong but broad band of free pyridoxine at 3354cm^{-1} which might be attributed to υ(O-H) stretching frequency was seen to undergo a shift to a lower frequency in all its corresponding metal complexes except iron. Also, the weak but sharp band at 1647cm^{-1} which might be due to υ(C=N) bending frequency in free pyridoxine were not observed in all its metal complexes except in copper-pyridoxine complex. This in essence might indicate coordination of pyridoxine at the (O-H) group.

The ^{1}HNMR spectra of artemisinin and thiamin chloride (Table 3) showed strong signals at 1.5 ppm and 4.6 ppm respectively. In all their complexes, these signals were of lower intensities which might have resulted from

complexation. On the other hand, only the zinc metal complex of pyridoxine showed the same intensity as that of pyridoxine. Therefore, it was observed that zinc did not form a complex with the artemisinin-pyridoxine while copper did.

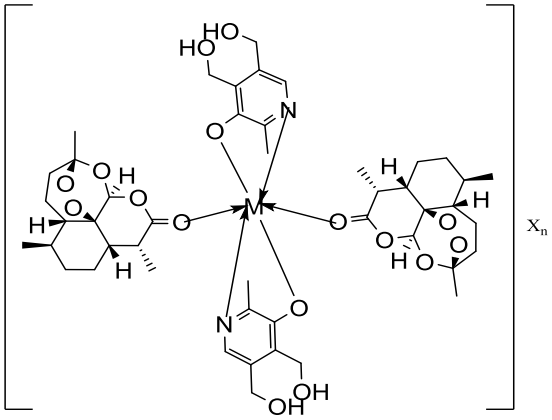
On the basis of the analytical and spectroscopic data, the tentatively proposed structure for the metal complexes of mixed artemisinin-thiamin chloride and artemisinin-pyridoxine are shown below:



Proposed structure for $M(Art)_2(Th)_2X_n$

Where M = Zn^{2+}, Mn^{2+} and Cu^{2+}; X = Cl and n = 2

Art = Artemisinin and Th = Thiamin chloride



Proposed structure for $M(Art)_2(Pyr)_2X_n$

Where M = Zn^{2+}, Mn^{2+} and Cu^{2+}; X = Cl and n =2 while if M = Fe^{3+} , n = 3

Art = Artemisinin and Pyr = Pyridoxine

Antibacterial screening

The determination of various properties of the complexes and their antimicrobial screening could be used to ascertain whether or not the complexes were better than their parent antimalarial drug. The diameter of the zone of inhibition is a measure of antibacterial activity. The increase in the percentage zone of inhibition for metal complexes (Table 4) when compared with artemisinin and the vitamins used was an indication that the metal complexes were able to decrease the population of the bacteria species. It was also an indication that the metal complexes were more effective than their parent drugs.

References

Behrens, N. B., Diaz, G. M. and Goodgame, D.M.L (1986). "Metal complexes of the antibiotics Nalidixic acid", *Inorg. Chim. Acta*. 125:21-264.

Coartes J (2000). "Interpretation of Infrared Spectra, A Practical Approach", *Encyclopedia of Analytical Chemistry, R.A. Meyers (Ed.),* p 10815-10837.

Combs G.F (2006). "The Vitamins: Fundamental Aspects in Nutrition and Health", 3rd Ed. Elsevier Academic Press, USA, p 7-33.

El Guindi, N.M., Abdel Geward, F.M. (1988). "Chelating tendencies of some sulfa drugs", *Egypt.J. Pharm. Sci*., 29 (1-4): 457-463.

Fukuwatari T and Shibata K (2008). ''Urinary water-soluble vitamins and their metabolite contents as nutritional markers for evaluating vitamin intakes in young Japanese women'' *J. Nutr.Sci*. Vitaminol. 54 (3): 223-229.

Peters W, Robinson B.L, Milhous W.K (1993). "The chemotherapy of rodent malaria (LI): Studies on a new 8-aminoquinoline", WR 238,605. *Ann. Trop. Med. Parasitol*. 87:547–552.

Puetter R (2000). "Vitamins, Supplements and Their Uses", *Personal vitamins/supplement use and practice*, p 1-12.

Williams H.D and Flemming I (1980). "Spectroscopic Methods in Organic Chemistry", 4th ed. McGraw-Hill Book Ltd, London.