NISEB JOURNAL Vol. 11, No. 3 , July 31, 2011
Printed in Nigeria

1595-6938/2011 $5.00 + 0.00

http://www.nisebjournal.org

NISEB 2011075/11308

Synthesis, characterisation and antimicrobial studies of some copper (II) complexes of mixed fluoroquinolone ligands

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(Received March 22, 2011; Accepted July 10, 2011)

ABSTRACT: Copper (II) complexes of mixed fluoroquinolone (ciprofloxacin, norfloxacin and ofloxacin) ligands (Cip-Nor, Cip-Ofl and Nor-Ofl) were synthesized. The products were characterized by elemental analysis, conductivity measurements, UV-Vis and IR spectroscopy. The result of the analyses was compared with the single ligand-metal complexes. The result of the analyses supports the formation of metal-ligands compounds of mole ratio 1:1:1. The antimicrobial activity showed that the complexes have comparable activity with their parent compounds and single ligand-metal complexes.

Keywords: Ciprofloxacin, fluoroquinolone, metal complexes, norfloxacin, ofloxacin.

Introduction

Fluoroquinolones are new derivatives from the quinolone antibiotic family, due to modification on the older quinolones. Examples are ciprofloxacin, norfloxacin and ofloxacin (Figure 1) [1]. Their broad spectrum of antimicrobial activity, bioavailablility, penetration into tissues, long serum half-life and safety have made the new fluoroquinolones very attractive agents for treating numerous infectious diseases [2]. The site of action of fluoroquinolones has been pinpointed to a subunit of that remarkable enzyme, DNA gyrase which unwind the supercoiled DNA helix prior to replication and transcription [3].

A lot of work has been carried out on synthesis of metal-fluoroquinolone complexes [4,5]. Simply changing the condition of reaction e.g. pH, solvent etc, has been known to yield different new complexes. Fluoroquinolone antibiotics can participate in the formation of complexes in a number of ways. This is because they have the relevant ionisable functional groups, that is, the 3-carboxyl group and the N4 at the piperazine substituent. Therefore they can exist as FQH_2^+, an acidic cation; FQH, a neutral nonionised specie: $FQH_2^{\pm}$, an intermediate zwitterions and FQ^-, a basic anion, all depending on the pH [4].

Mixed ligand complexes of some fluoroquinolones have also been reported [4,6,7]. However in this study, the possible products of the reaction of two different fluoroquinolones in the presence of Copper(II) metal salts were investigated.

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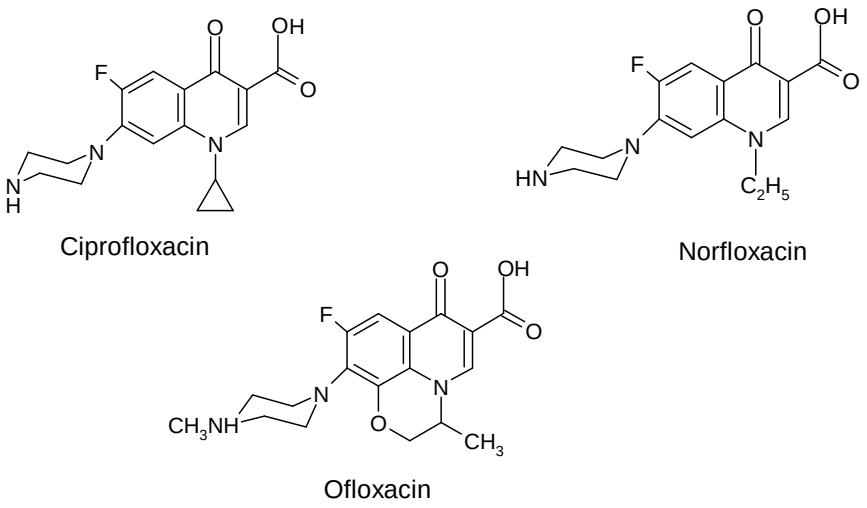


Figure 1: Examples of fluoroquinolone antibiotics.

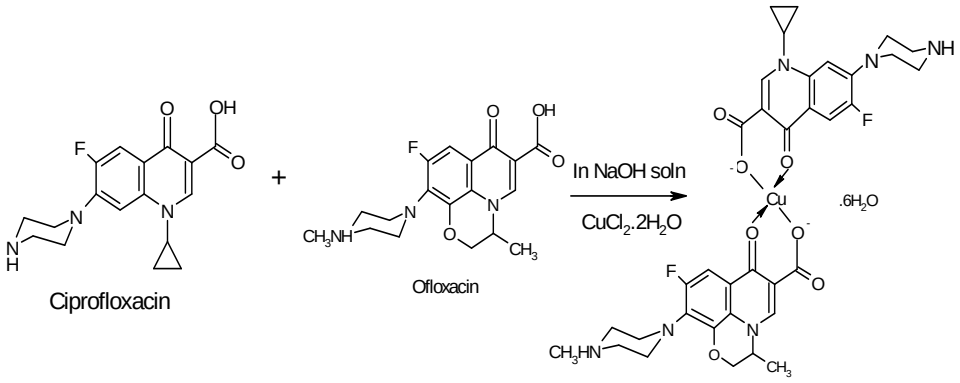
Materials and Methods

Experimental

All reagents were received from commercial sources. Ciprofloxacin, Norfloxacin and Ofloxacin were purchased from Sigma-Aldrich Chemie, Germany. The pH was measured by using the Crison model pH meter. Melting points and decomposition temperatures were determined using the Gallenkamp melting point apparatus in open capillary tubes. The conductivity was measured by using the Hanna instrument conductivity meter. The instrument was calibrated with 0.01 M standard solution of potassium chloride. The analysis of chemical elements (C, H, and N) was carried out at the laboratory of Desert Analytics, Tucson, Arizona, USA. The metal concentration was determined on an alpha 4 Atomic Absorption Spectrophotometer. The samples were digested in concentrated HCl and diluted with a known volume of water. The UV-Vis spectra between 190-800 nm were obtained on Aquamate UV-Vis spectrometer V.4.60. The sample solutions were prepared by using distilled water. The I.R. spectra were recorded using KBr pellets with Buck scientific infrared spectrometer M500 at the range of 4000-600 cm^{-1}.

Synthesis

The method described by Mendoza-Diaz *et al.*[6] and Wu *et al.*[7] was adopted here. Equimolar amount (1.0 mmole) of two of the ligands [Cip-Nor, Cip-Ofl, Ofl-Nor] was added to 100 mL solution of distilled water containing 2.0 mmole of NaOH. The ligand mixture was heated slightly above room temperature and stirred to give a homogeneous mixture. To this was added 10 mL of aqueous solution of $CuCl_2.2H_2O$ (1.0 mmole). The pH of the resulting solution was adjusted to about 8.0. The solution was stirred with a magnetic stirrer for 30 min. (The Ofl-Nor ligand-metal mixture was stirred for 8 h and at 46 ^oC). The precipitate formed was filtered and washed with water. The Ofl-Nor M-L mixture was filtered cold (over ice).



Scheme 1: Schematic illustration of metal complex formation

Antimicrobial Activity Study

The antimicrobial assays were carried out on the ligands, some of the metal complexes and the metal salt used. Sensitivity tests, MIC and MBC were determined using standard methods as obtained in literature. Test sample stock solution. of 100 μg/mL were prepared for each metal complex and ligands.

Clinical cultures of *Staph. aureus, Klebsiella sp, E.coli, Pseudomonas aeruginosa, N.gonorrhoea, S.typhi, Shigella, Penicillin sp, Aspergillus sp.* were used. The microorganism isolates were obtained from the University Teaching Hospital, Ilorin. The test organisms were individually grown overnight on nutrient agar slant at 35 ^{o}C. The suspension was then prepared by washing the growth off with the normal saline solution and further serially diluting with sterile 20 mM phosphate buffer to give counts of factor of 1x 10^6.

Sensitivity Test was carried out using Media plates of sensitivity test agar (STA). Antifungal activity test were also performed on plates filled with the SDA agar (see result in Table 3). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined as described by Obaleye *et al*. [5]. The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentration (MBC) are presented in Table 4.

Results and Discussion

Some Physical properties of the compounds

From the microanalysis data, the mole ratio, anion present and water molecules (coordinated or not coordinated) were determined. Qualitative tests for chloride carried out confirmed the presence of the anion outside the coordination sphere. The terminal nitrogen on the 7-piperazinyl group may be protonated where simple inorganic anions are present to balance the charge on the complex. However the molar conductivities of the FQ-metal complexes were much lower than those of known strong electrolytes, for example H_2SO_4 which has a limiting molar conductivity of 509.8 Scm^2mol^{-1} and weak electrolyte, for example CH_3COONa which has a limiting molar conductivity of 91.0 Scm^2mol^{-1} [8,9]. With this it can be concluded that the FQ- metal complexes are very poor electrolytes or non-electrolytes.

The metal complexes showed bands at similar regions since they have similar chromophores. The intense UV bands at 260- 340 nm were assigned to intraligand π- π* transitions. The strong absorption peak corresponds to the chromophore involving the nitrogen atom at position 1 to carboxyl group. The weak absorption peak at the long wavelength side corresponds to the chromophore involving the nitrogen of the piperazinyl group attached to the 7-carbon to the keto group [10].

Table 1: Physico-analytical data of the mixed metal complexes

Products	Molar mass	Microanalysis: Found(Calcd.)%				Yield %	Melting point °C	Solubility	Λ Scm^2mol^{-1}
		C	H	N	Me				
$[Cu(Cip)_2Cl_2].2H_2O$	833.35	49.43	5.18	9.73		60.7	224-226	Soluble in MeOH	0.21
$(C_{34}H_{40}N_6F_2O_8CuCl_2)$		(49.00)	(4.84)	(10.09)	(7.63)				
$Cu(Nor)_2Cl_2.H_2O$	791.46	49.02	4.81	10.55		88.4	219-220	Sparingly soluble in MeOH	-
$(C_{32}H_{38}N_6F_2O_7CuCl_2)$		(48.58)	(4.84)	(10.62)	(8.03)				
$[Cu(Ofl)_2Cl_2].3H_2O$	911.35	47.93	5.08	9.21		84.6	255-256	Sparingly soluble in EtOH	0.25
$(C_{36}H_{46}N_6F_2O_{11}Cu\ Cl_2)$		(47.45)	(5.09)	(9.22)	(6.97)				
$[Cu(Cip)(Ofl)].6H_2O$	862.35	48.96	5.60	9.74	6.82	72.9	214-215	Sparingly soluble in MeOH	0.23
$(C_{33}H_{48}N_6F_2O_{13}Cu)$		(48.75)	(5.61)	(9.75)	(7.37)				
$[Cu(Cip)(Nor)Cl_2].3H_2O$	837.25	47.94	5.09	10.15	7.05	72.9	216-217	Sparingly soluble in MeOH	0.26
$(C_{33}H_{42}N_6F_2O_9Cu\ Cl_2)$		(47.34)	(5.06)	(10.04)	(7.59)				
$[Cu(Nor)(Ofl)Cl_2].H_2O$	853.25	49.19	4.95	10.03	6.66	81.8	266-267	Sparingly soluble in MeOH	0.23
$(C_{34}H_{40}N_6F_2O_8Cu\ Cl_2)$		(49.01)	(4.84)	(10.08)	(7.63)				

Table 2: Relevant I.R. frequencies (cm^{-1}) (in KBr) and UV (nm) data of Ligands and Mixed fluoroquinolone metal complexes

Tentative Assignment	I.R. frequencies (cm^{-1})						UV (nm) [intraligand π- π* transitions]	
	υ(OH)for H_2O molecule υ(N-H)*	$\upsilon(C=O)_c$	$\upsilon(C=O)_P$	$\upsilon_{as}(OCO)$	υ(C-C,C-N)	$\upsilon_s(OCO)$	Band 1	Band 2
Ciprofloxacin	3048-2852	-	1626	1597	1504	1373	276.5	327.5
Norfloxacin	3500-2500	-	1616	1581	1490	1393	281.5	328.0
Ofloxacin	3421-3046	1714	1626	-	1461	-	296.0	325.0
.$[Cu(Cip)_2Cl_2].2H_2O$	3500-3000	-	1626	1573.5	1475	1407	297.5	329.0
. $Cu(Nor)_2Cl_2.H_2O$	3500-3300	-	1631	1602.9	1490.	1402	277.0	329.0
.$[Cu(Ofl)_2Cl_2]..3H_2O$	3435	-	1626	1587.5	1485	1402	293.0	326.0
$[Cu(Cip)(Ofl)].6H_2O$	3416	-	1621	1581	1475	1388	284.5	326.0
.$[Cu(Cip)(Nor)Cl_2].3H_2O$	3421	-	1626	1582	1480	1393	283.5	325.5
.$[Cu(Nor)(Ofl)Cl_2].H_2O$	3445	-	1621	1581	1475	1402	289.5	326.5

Table 3. Result of Sensitivity Tests Using Sensitivity disks (10 µg/mL)

Test Samples	*S.typhi*	*Shigella Spp*	*E.coli*	*Klebs. spp*	*S. aureus*	*Pseud spp.*	*N. gonor.*
Ciprofloxacin	30	24	22	28	30	30	36
$[Cu(Cip)_2Cl_2].2H_2O$	23	30	20	22	23	25	31
Norfloxacin	26	21	16	16	21	25	30
$Cu(Nor)_2Cl_2.H_2O$	20	24	10	22	18	20	22
Ofloxacin	23	23	16	21	26	21	28
$Cu(Ofl)_2Cl_2.6H_2O$	15	22	18	16	18	15	17
CIP-NOR	22	20	23	21	13	17	22
CIP-OFL	20	22	21	22	19	20	23
NOR-OFL	20	22	21	22	19	20	23
$[Cu(Cip)(Ofl)].6H_2O$	29	35	24	18	23	30	36
$[Cu(Cip)(Nor)Cl_2].3H_2O$	31	26	19	21	26	38	40
$[Cu(Nor)(Ofl)Cl_2].H_2O$	19	19	19	16	31	28	30

Table 4 Minimum inhibitory concentration (μg/mL) and Minimum bactericidal concentration (μg/mL) of some fluoroquinolone metal complexes

Test Samples	Minimum inhibitory concentration (μg/mL) of the ligands, FQs-metal s and metal salts dilution.							Minimum bactericidal concentration (μg/mL) of the ligands, FQs-metal s and metal salts dilution.						
	S.typhi	*Shigell. spp*	*E.coli*	*Klebs spp*	*S.aureus*	*Pseud spp.*	*N.gonor*	*S.typhi*	*Shigell. spp*	*E.coli*	*Kleb. spp*	*S.aureus*	*Pseud. spp*	*N.gonor.*
Ciprofloxacin	60	150	450	150	150	150	60	80	180	500	200	200	180	80
$[Cu(Cip)_2Cl_2].2H_2O$	250	100	500	350	350	350	450	300	150	550	400	400	400	500
Norfloxacin	200	100	500	500	500	100	200	300	200	600	500	600	200	300
$Cu(Nor)_2Cl_2\ H_2O$	300	400	550	300	450	350	200	400	500	650	400	550	450	300
Ofloxacin	300	200	500	300	350	400	350	400	300	600	400	450	500	450
$Cu(Ofl)_2Cl_2.3H_2O$	500	350	500	500	500	500	600	600	450	650	600	600	600	700
CIP-NOR	500	400	400	300	550	500	550	500	500	500	400	650	650	700
CIP-OFL	400	300	200	200	450	400	300	550	450	300	300	550	500	400
NOR-OFL	300	350	150	300	500	500	350	400	450	250	400	600	650	500
$[Cu(Cip)(Nor)Cl_2].3H_2O$	80	30	450	350	200	15	15	100	50	500	400	250	30	30
$[Cu(Cip)(Ofl)].6H_2O$	120	30	350	500	250	120	80	150	50	400	550	300	150	100
$[Cu(Nor)(Ofl)Cl_2].H_2O$	400	400	450	500	100	100	300	500	500	550	600	200	200	400

Infrared Spectroscopy

Tentative assignments of the IR bands were made based on reports of similar fluoroquinolone metal complexes and also comparing the spectra of the ligands to that of the metal complexes. The shift in absorption bands of the complexes as compared to those of the ligands shows the changes in the nature of the functional groups of each ligand upon complexation to the metal ion.

The infrared spectra of Ciprofloxacin (Cip) and Norfloxacin (Nor) shows they exist as zwitterions since no band exists for $\upsilon(C=O)_c$ [11,12]. However, peaks appear for $\upsilon_{as}(OCO)$ and $\upsilon_s(OCO)$. The IR spectrum of ofloxacin showed bands at 1714.1 cm^{-1} assigned to $\upsilon(C=O)_{carboxyl}$ showing that the carboxyl group in the ligand is protonated, therefore the ligand exists in its nonionised neutral form. The ligands all show peaks at 1600 cm^{-1} assigned to $\upsilon(C=O)_{pyridone.}$ Strong peak also appears around 1490 cm^{-1} assigned to vibrations for C-C, and C-N, which appears at a lower frequency on complexation. The presence of water (lattice or coordinated water) is shown by the broad peak at 3600-3000 cm^{-1} of the IR spectra.

Metal complexes

$\upsilon(C=O)_{carboxyl}$ and $\upsilon(C=O)_{pyridone}$

No bands appeared for $\upsilon(C=O)_c$ of the carboxylic group for the single ligand complexes at 1700-1750 cm^{-1} indicating that the carboxyl group is deprotonated and coordinated to the metal ion. The band assigned to $\upsilon(C=O)_p$ of the pyridone group appeared at slightly lower frequency for most of the complexes, as compared with the free ligands.

The IR spectra of $[Cu(Cip)(Ofl)].6H_2O$, $[Cu(Cip)(Nor)Cl_2].3H_2O$, and $[Cu(Nor)(Ofl)Cl_2].H_2O$ are similar to those of the single ligand-metal complexes ($[Cu(Cip)_2Cl_2].2H_2O$, $Cu(Nor)_2Cl_2.H_2O$ and $.[Cu(Ofl)_2Cl_2]..3H_2O$) of their respective ligands [4,5]. There is no evidence of $\upsilon(C=O)_c$ absorption band which is due to deprotonation of the carboxyl group and its involvement in metal ion interaction. Strong bands at 1600 cm^{-1} were assigned to $\upsilon(C=O)_p$.

$\upsilon_{as}(OCO)$ and $\upsilon_s(OCO)$

The presence of bands at 1390-1410 cm^{-1} and 1580-1610 cm^{-1} were assigned to asymmetric and symmetric vibrations ($\upsilon_{as}(OCO)$ and $\upsilon_s(OCO)$) in the single ligand-metal complexes confirmed that coordination was through the carboxyl group. The $\upsilon_{as}(OCO)$ and $\upsilon_s(OCO)$ vibrations appeared as rather weak or medium absorption bands as for ionic carboxylates and metal- FQs showing that the carboxyl moiety is coordinated to the metal ion.

Based on these results the following structure was proposed for the single and mixed fluoroquinolone compounds [see Figures 2].

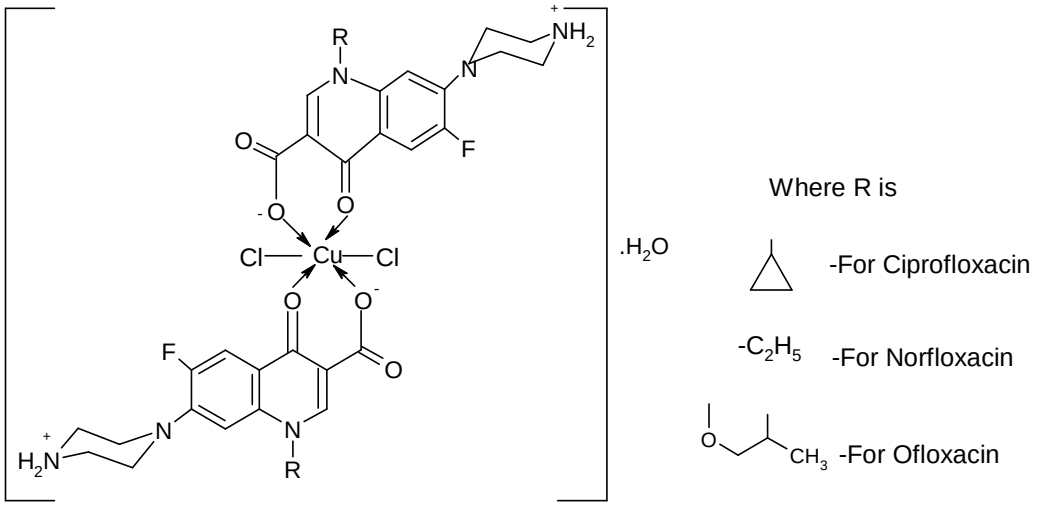


Figure 2: Proposed Structure of Mixed Fluoroquinolone Metal Complexes

Antimicrobial Assay

All the ligands and metal complexes showed antimicrobial effect against the tested organism species except against the molds of *Penicillim* and *Aspergillus* as presented in table 3-5. *Neissera gonorrhoea* was the most sensitive organism to the fluoroquinolones and their complexes. Some of the metal complexes showed comparable activity or greater activity against some of the microorganisms in comparison to the parent compounds. The MIC of the samples against the various isolates ranged from 15 µg/mL to 600 µg/mL of the antimicrobial dilutions, while that of the MBC ranged from 25 µg/mL to 700 µg/mL. These concentrations in comparison to previously reported MIC_{90} of the ligands are seemingly high. This could be due to the different conditions under which the studies were carried out. These are reflections of the fact of possible interference from the media broth and some other materials and chemicals used during the test, which are not absolutely compatible with conditions present in the cells [1,13,14].

In general Ciprofloxacin and its metal complexes showed greater activity than the other fluoroquinolones and their metal complexes which agree with the studies carried out on antimicrobial activity of Ciprofloxacin in comparison to other fluoroquinolones [15,16]. It was also observed that Ciprofloxacin containing metal complexes, most especially $[Cu(Cip)_2Cl_2].2H_2O$, $[Cu(Cip)(Nor)Cl_2].3H_2O$ and $[Cu(Cip)(Ofl)].6H_2O$ (and others to a lesser extent) are usually active at low concentration. Increasing the concentrations of the samples led initially to increased bactericidal effect to a certain point, after which the reverse effect occurs, that is increasing concentration leading to decreasing sensitivity.

For a particular antimicrobial, the organism involved is an important factor. *Salmonella typhi, Shigella, Pseudomonas aeruginosa, Neisseria gonorrhoea* are relatively more sensitive to the Ciprofloxacin complexes than the organisms, *klebsiella, Escherichia coli* and *Staphylococcus aureus*.

The mixed fluoroquinolone – metal complexes (especially Cip-Nor and Cip-Ofl) showed increased activity in terms of MIC or MBC in comparison to the mixed ligands. In the mixed FQ- metal complexes, however arenot as active as the single ligands and their metal complexes. From the sensitivity tests, MIC and MBC, the Norfloxacin and Ofloxacin containing metal complexes also show comparable activity to their ligands depending on the microorganism.

Conclusion

Structures were proposed for the products obtained based on the microanalytical and spectroscopic data with coordination occurring through the ring carbonyl oxygen and one of the oxygens of the carboxylate group for all the fluoroquinolones. Some of metal complexes studied for their antimicrobial activities showed comparable and even higher activity for some species than their parent drugs but they were not active against fungal species. Ciprofloxacin metal complexes were most active. The mixed fluoroquinolone metal complexes did not show as much activity as their single ligand complexes and parent drugs.

ACKNOWLEDGEMENT: We are grateful for financial support from the Science and Technology Education Post Basic Project (Step B), University of Ilorin and University of Agriculture, Abeokuta.

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