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Synthesis, Characterization and Antimicrobial Assessment of Ofloxacin Derivatives and Their Complexes with Cobalt(II) and Copper(II) Metals

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Abstract Schiff base Ligands HL^1 and HL^2 were synthesized by separate condensation of o-phenylenediamine and 2-aminopyridine-3-carboxylic acid with ofloxacin in 2:1 and 1:1 mole ratio respectively. These ligands were reacted with chloride salts of Co(II) and Cu(II) to obtain (HL^1) and (HL^2)-Metal complexes respectively. The novel compounds were characterized using physicochemical properties, IR spectroscopy, UV-visible spectroscopy, Molar conductance and metal analysis. The IR and UV/vis spectra revealed that the metal ions coordinated with the ligands through azomethine nitrogen and carboxylato oxygen presumed to exhibit tetradentate character with the possible formula type ML and ML_2 for HL^1 and HL^2 metal complexes respectively. The antimicrobial evaluation of the ligands and their respective complexes in *S. aureus*, *B. subtilis*, *S. typhi*, *A. niger* and *A. fumigatus* presented a promising to excellent activity except for *A. niger* which showed no activity in all the tested compounds.

Keywords Schiff base, Metal Complexes, o-phenylenediamine, 2-Aminopyridine-3-carboxylic acid, activity

Introduction

There is increasing interest in the chemistry of Schiff base due to its pharmacological and physiological activity [1]. Schiff base ligands and their metal complexes have been reported to show improved biological activities such antibacterial, antifungal, anticancer, antitumor and anti-tubercular activity [2]. The tridentate and tetradentate Schiff bases with heterocyclic amines containing O and N donor series have been used for coordination with transition metals [3] and have been used as fine chemicals and medical substrates [4].

Quinolones are synthetic antibacterial compounds based on a 4-quinolone skeleton andare present in numerous natural products, especially in alkaloids. Many quinolones display interesting pharmacological activities and have found applications as pharmaceuticals, e.g anti-malarial drugs, such as quinine or chloroquin [5]. Increasing multi-drug resistant microorganisms have continued to pose serious challenges especially in recent times of population growth. Metal complexed antibiotics haveseverally been reported to exhibit either similar or improved antibacterial profile in comparison to the original drugs [6-8]. [9] reported ofloxacin, a second generation fluoroquinolone to be inherent with an active terminalsfor interacting with the metal ions as a mono anionic bidentate ligand coordinated to the metal through the pyridone and carboxylate oxygen atoms showing $[M(Oflo)_2 (H_2O)_2]. nH_2O$ formula, where M = Metal ion. The alarming and striking rate of emerging multi-drug resistant microbes has called for more synergy in exploring more improved and effective novel chemotherapeutic agents to curb with menace of such pathogens [10].

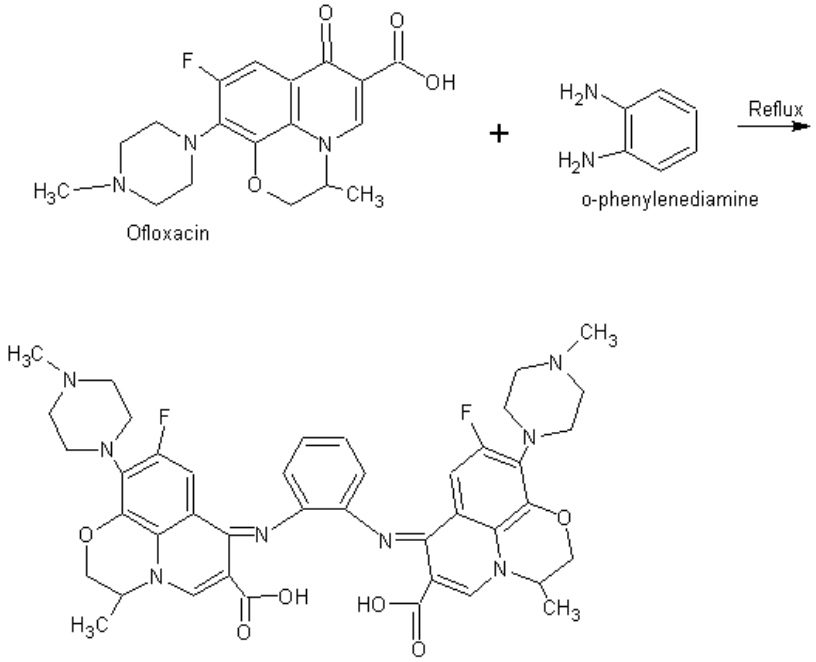
A lot of works on structural modifications of other fluoroquinolones especially ciprofloxacin, gatifloxacin, norfloxacin and levofloxacin have been reported both in Schiff base synthesis and their metal complexes [6, 9, 11-14], but little has been reported on ofloxacin toward enhancing its activity especially with respect to its Schiff base metal complexes with o-phenylenediamine and 2-aminopyridine-3-carboxylic acid.

In view of the challenges of containing the menace of multi-drug resistant microorganisms, this study intends to synthesize and characterize some polynuclear Schiff bases containing ofloxacin drug with o-phenylenediamine and 2-Aminopyridine-3-carboxylic acid and will be further complexed with Co(II) and Cu(II) Metal ions. The synthesized ofloxacin derivatives will be assayed for their antimicrobial activity.

Experimental

Materials and Methods

All chemicals used in the synthesis were of analytical grade (AR). Ofloxacin antibiotic in pure (generic) form, o-phenylenediamine and 2-Aminopyridine-3-Carboxylic Acid were all purchased from Sigma Aldrich through Bristol Scientific Company, Lagos, Nigeria. The metal(II) salts used were chloride; $CoCl_2.6H_2O$ and $CuCl_2.5H_2O$. Solvents used for the synthesis and other probes were of absolute purity which include; distilled water, methanol, ethanol, chloroform, n-hexane, benzene and acetone. All new compounds (Ligands and their metal complexes) were characterized on the basis of expected molecular formula, electrical and conductance measurement using DDS-307 Conductometer bridge. The melting point temperatures were determined using Gallenkamp melting point apparatus and are uncorrected. The metal content of the metal complexes was determined using complexometric titration with EDTA. The electronic data were recorded from UV-Vis spectrophotometer at 800-200 nm and the FTIR analysis of the new compounds was carried out in the range of 500-4000cm^{-1} on SHIMADZU Corporation FTIR-8400S spectrophotometer. Disc diffusion method was used to evaluate the antimicrobial activities of the synthesized ligands and their metal(II) complexes against some strains of bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* and fungal strain like *Aspergillus niger* and *Aspergillus fumigatus*.

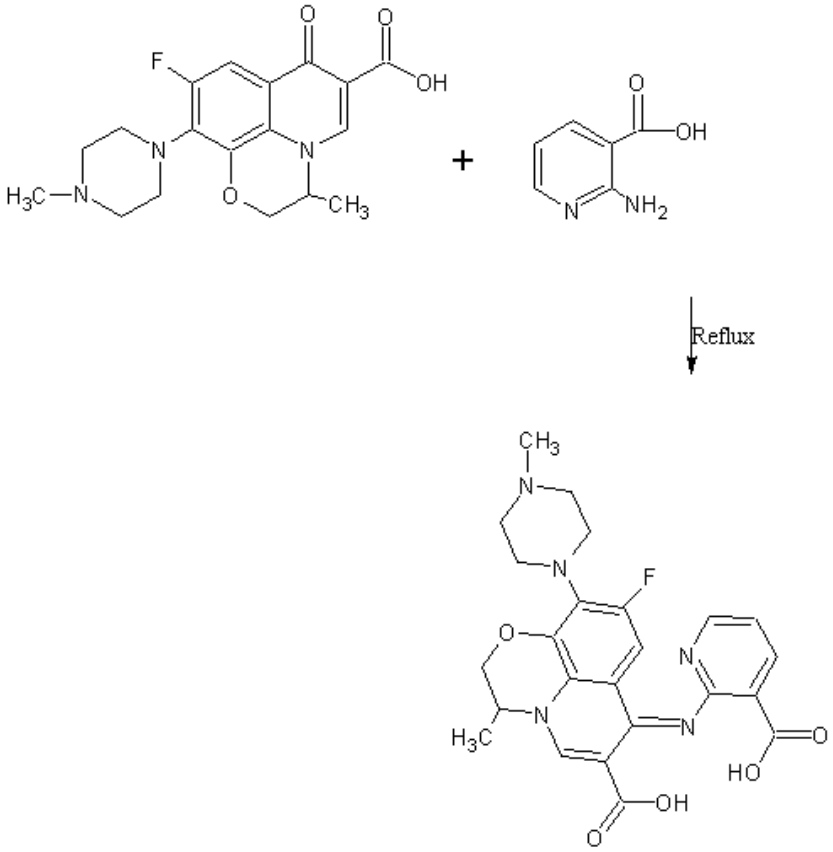


Scheme 1: Ofloxacin-o-phenylenediamine (HL^1) Ligand Condensation

Synthesis of the ligands (ofloxacin-imines)

The Schiff base ligands (ofloxacin-imines) were synthesized using a literature procedure [6, 15] by the condensation of ofloxacin antibiotic with respective substituted amines in the appropriate molar ratio in ethanol. Ofloxacin

powder 2mmol, 0.7227g dissolved in 25ml methanol was mixed with o-phenylenediamine 1mmol, 0.108 dissolved in 12.5ml methanol, (2:1) in a round bottom flask. The mixture was subjected to reflux in the presence of 2 drops of glacial acetic acid separately for 3 h. The resulting solution was concentrated on a water bath and allowed to cool at 0 ºC. The yellowish white solid formed (HL^1) was filtered, washed with ethanol and dried in a desiccator containing $CaCl_2$. The samemethod was employed to prepare ofloxacin-2-aminopyridine-3-carboxylic acid (HL^2) in 1:1 ratio combination and the whitish lemon yellow solid was obtained.



Scheme 2: Ofloxacin-2-aminopyridine-3-carboxylic acid (HL^2) Ligand Condensation

Synthesis of metal(II) complexes

The prepared Schiff base ligands above (0.02 mol) was dissolved in 25 ml methanol and mixed with respective transition metal salt (0.01 mol) e.g of Co(II) in 20 ml methanol (1:1 or 1:2) ratio for HL^1 and HL^2 ligands respectively. The reaction mixture was refluxed for 3 h, concentrated and cooled over ice chips which afforded coloured precipitates. The precipitates were filtered, washed with methanol and dried in desiccator containing $CaCl_2$.

Antimicrobial Assay

Antimicrobial activity of the complexes, Amines used and ligands were evaluated by Disc diffusion method as reported by Imran et al [15] and Al-Bayati et al [16] as adopted by Usman *et al*. [17] and Ndahi *et al.* [18] against different bacteria strains such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli,* and also on some fungi strains viz; *Aspergillus niger* and *Aspergillus fumigatus.*

The nutrient *agar* medium (Peptone, Beef extract, NaCl and *Agar-Agar*) and 5 mm diameter paper disks (Whatman No. 1) were used. The investigated test compounds i.e. ligands, amines used and the complexes were dissolved (30µg, 20µg, 10µg) in methanol. 25 ml nutrient *agar* media was poured into each Petri plates. After solidification, 0.1 ml of test bacteria was spread over the medium. The disks of the Whatman No.1 filter paper was placed at twelve equidistant places at a distance of 2 cm from the center in the *inoculated* petri plates. Another filter paper

disks treated with methanol served as control whereas media with Ofloxacin (30µg/ml) concentration (standard antibacterial) and Ketoconazole (30µg/ml) concentration (standard antifungal) were used as positive controls or referenced drugs. The plates were then incubated for 24 h at 37 ^{0}C for bacteria and 48 hat room temperature for fungi, and the zone of inhibition around each disk were measured and recorded (mm) as the degree of activity on the target microorganisms.

Statistical Analysis

The raw data obtained from *in vitro* assay for minimum inhibition zone (mm) activity of the test agents on the selected panel of microorganism strains were subjected to one way ANOVA (Turkey-Kramer Multiple Comparisons Test) statistical analysis on pyramid GraphpadInstat, 2000 and expressed as ± SEM. P values ($\leq$ 0.05) were considered significant in this study.

Results and Discussion

The analytical data of the Schiff base ligands (HL^1& HL^2) and their metal(II) complexes along with some physical properties are summarized in Table 1. The Schiff bases were afforded as crystalline light yellowish derivatives of ofloxacin condensed with some amines (o-phenylenediamine and 2-aminopyridine-3-carboxylic acid) in a ratio 1:2 or 1:1 (amine:oflox) to obtain HL^1 and HL^2 Schiff bases respectively. The ligands on interaction with chlorides of Co(II) and Cu(II) yielded complexes corresponding to the general formula [ML] and $[M(L)_2]$ for HL^1 and HL^2 Schiff bases respectively. An appreciable percentage yield of all the new compounds were obtained which ranged from 66% - 82%. The ligands and their metal(II) complexes were observed to be stable under normal conditions with colours characteristics of the transition metals [19, 20] and mainly attributed to the d-d electron transition [21, 22]. The complexes showed a steady trend of higher melting points than those of the ligands which may be due to the inter-molecular bonding as a result of metallic lattice and increase in molecular weight [23]. The melting points of the ligands and their complexes range from 134 ^{o}C (HL^2) – 189 ^{o}C Cu-$(HL^2)_2.2H_2O$. The consistent range difference of +2 ^{o}C melting points observed may indicate that the synthesized compounds are presumed pure. The structures of the Schiff base ligands and their metal(II) complexes suggested from the theoretical elemental analysis and metal estimates agree to some extent with their proposed formulae [6]. The molar conductance measurements of the ligands and the complexes in 10^{-3}M DMSO range from 0.053 – 0.60 Scm^2 $mol^{-1}$for HL^1 and Co-$(HL^1).2H_2O$ complex which are relatively low, indicating that the new compounds are non-electrolytes in nature [24, 25, 3].

FT-Infrared: The IR absorption spectra of the ofloxacin drug, amines used and, Schiff base ligands (HL^1& HL^2) and their complexes with Co(II) and Cu(II) are presented in Table 3. The infrared spectra of fluoroquinolone compounds are observed to be complex as a result of several functional moieties in their structures and hence, only two linkage sites are feasible in condensation and coordination [26]. The most common vibrations considered as important region in the ofloxacin IR spectra appeared at 1519 and 2924 cm^{-1} for ν(C=N) and ν(NH_2) vibrations respectively. The spectra of the two amines, o-phenylenediamine (o-phdn) and 2-aminopyridine-3-carboxylic acid (2-Apdn) showed ν(C=N) vibration bands at higher values than the ligands at 1627 and 1697 cm^{-1} respectively as against 1620 and 1631 cm^{-1} in HL^1 and HL^2 ligands spectra. The amines also showed a distinctive ν(NH_2/N-H) vibration band at 3363 and 3255 cm^{-1} respectively which is absent in the ligands and their metal complexes due to coordination [27]. The Schiff base ligands (HL^1& HL^2) showed a typical characteristic azomethine ν(C=N) vibration band at 1620 and 1631 cm^{-1}[28] and the absence of ν(N-H) vibrations of the amines between 2785-3363 cm^{-1} in the ligands further established the condensation of the drug with the amines which afforded the two ligands (HL^1& HL^2).

On complexation with metal(II), the ν(C=N) band shifted to higher frequency [28] at the following regions; 1647 cm^{-1} [Co(II)] and 1639, 1635 cm^{-1} [Cu(II)] complexes respectively. The shift from the azomethine stretching vibrations of the ligands observed in the spectral bands of the complexes may be ascribed to the coordination of the azomethine nitrogen to the metal(II) ions [2, 3]. Also, the IR spectra of the drug and the amines(ofloxacin, o-phdn and 2-Apdn) showed the ν(C-N) stretching vibration band at 1458_{strong}, 1496_{strong} and $1458_{sharp}cm^{-1}$ respectively.

The o-phdn IR presented this band at higher frequency of 1496 cm^{-1} than the HL^1 and its metal complexes which exhibited this weak spectral band at 1492cm^{-1} for both $Co(HL^1)$ and $Cu(HL^1)$ complexes. The HL^2 and its metal complexes displayed the ν(C-N) vibration band at a higher range of 1465 and 1543 cm^{-1}than its amine (2-Apdn) which displayed same at 1458 cm^{-1}(Table 3).

The IR spectra of the complexes displayed a discrete non ligand band with low intensity at 621 and 586 cm^{-1} for $Co(HL^1)$ and $Cu(HL^1)$ complexes and medium and broad bands at 651, 590 and 648, 582 cm^{-1} for $Co(HL^2)$ and $Cu(HL^2)$ complexes corresponding to ν(M-N) stretching vibrations. The band at 470 and 451 cm^{-1} for HL^1-complexes and at 478 and 405 cm^{-1} for HL^2-complexes is attributed to ν(M-O) stretching vibration mode.

These non-ligand spectral bands are indicators to the possible coordination of the azomethine nitrogen and the carboxylato oxygen to the metal ions [12, 29] which further establishes the evolution of the novel compounds. A broad diffuse bands of medium, sharp and weak intensity in the regions (3263-3865 cm^{-1}) is assigned to the OH stretching vibrations of the COOH group of Ofloxacin and 2-amino- pyridine-3-carboxylic acid in both the Schiff base ligands and the complexes. Similarly, the weak and medium bands appearing at 786-995 cm^{-1}in all the compounds corresponded with the stretching vibration of the product water molecules [13]. However, worth noting also is the absence of a pair of band expected at 3245 and 3309 cm^{-1}corresponding to ν(NH_2) of the amine moieties used which further confirmed the coordination of the drug Ofloxacin to the two amines forming azomethine linkage [27].

Electronic spectra of the Schiff base ligands and their metal(II) complexes

The electronic spectral band of the free ligands and the metal complexes studied in methanol (Table 4) revealed that there is $n\rightarrow\pi^*$transition of the ethylenic double bond or non-bonding electrons on nitrogen atom of the azomethine bond at 32468 cm^{-1} and 27624 cm^{-1} for HL^1 and HL^2 ligands respectively assigned to ν(C=O), ν(C=N) and ν(OH) vibration groups [30-32]. In the metal complexes, only broad bands were seen which are probably due to d-d transition and MLCT effects [23, 33].

The Co(II) complexes exhibited electronic spectral band in the UV at lower intensities than the free ligand (HL^1) but higher than that of (HL^2) free ligand at 28329 and 28653 cm^{-1} for $Co(HL^1).2H_2O$ and $Co(HL^2)_2.2H_2O$ complexes respectively (Table 4) attributed to $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions of the non-bonding electrons in ligands and Pi electrons MLCT in the metal complexes [34, 35]. These transitions occur in case of unsaturated hydrocarbons which contain ketone or azomethine group [6, 36]. Cu(II) complexes presented similar band at 29240 cm^{-1} and 44444 cm^{-1} for $Cu(HL^1)$ and $Cu(HL^2)$ complexes respectively, which may be assigned to MLCT and $^6A_{1g}\rightarrow{}^4T_{2g}$ or $\pi\rightarrow\pi^*$ transitions in ligands [18, 37].

Table 1: Physical characteristics of HL^1 and HL^2 ligands and their metal(II) complexes

Compds.	Proposed Formula /(F. Weight)	Colour	Conductivity (Scm^2mol^{-1}) 10^{-3}	M.P (^oC)	Yield (%)	Metal Cal/ (Found)
HL^1	$C_{42}H_{44}F_2N_8O_6$ (794.9)	Yellowish white	0.05	156-158	66.44	-
$Co(HL^1)$	$Co[C_{42}H_{44}F_2N_8O_6].2H_2O$ (889.8)	Pink	0.60	170-172	68.07	6.62 (6.09)
$Cu(HL^1)$	$Cu[C_{42}H_{44}F_2N_8O_6].2H_2O$ (894.4)	Dark Green	0.19	182-184	82.67	7.11 (7.20)
HL^2	$C_{24}H_{24}FN_5O_5$ (481.5)	Pale yellow	0.06	132-134	70.41	-
$Co(HL^2)$	$Co[C_{24}H_{24}FN_5O_5]_2.2H_2O$ (1057.9)	Pitch Red	0.13	186-188	80.76	5.57 (5.50)
$Cu(HL^2)$	$Cu[C_{24}H_{24}FN_5O_5]_2.2H_2O$ (1062.6)	Light Green	0.15	187-189	75.93	5.98 (5.93)

Key: HL^1= Ofloxacin-o-phenylenediamine Ligand, HL^2=Ofloxacin-2-aminopyridine-3-carboxylic acid Ligand

Table 2: Solubility profiles of the ligands and their metal(II) complexes in some polar and non-polar solvents

Compds.	Water		Methanol		Ethanol		Acetone		Chloroform		Benzene		n-Hexane	
	c	h	c	h	c	h	c	h	c	h	c	h	c	h
HL^1	ss	s	s	vs	is	s	ss	vs	s	vs	ss	s	ss	ss
HL^2	is	vs	ss	ss	ss	s	ss	s	is	ss	ss	ss	ss	ss
$Co(HL^1).2H_2O$	vs	vs	vs	vs	s	vs	ss	ss	s	s	is	is	is	is
$Cu(HL^1).2H_2O$	vs	vs	is	is	is	is	is	is	is	is	is	is	is	is
$Co(HL^2)2.2H_2O$	s	vs	s	vs	ss	ss	ss	ss	ss	ss	ss	ss	ss	ss
$Cu(HL^2)2.2H_2O$	s	vs	ss	ss	is	is	ss	ss	is	is	is	is	is	is

Key:-s= soluble, vs= very soluble, ss=slightly soluble, is= insoluble, c= cold, h= hot

Table 3: Relevant infrared spectra of the drug, amines, ligands and their metal(II) complexes (cm^{-1})

Compds.	F.Weight	ν(NH_2)	ν(OH)	ν(C=N)	ν(H_2O)	ν(C-N)	ν(M-N)	ν(M-O)
Oflox.	361.38	2924sh	3803w 3749w	1519w	-	1458s	-	-
o-phdn	108.00	3363w 2924m	3865w	1627sh 1535m	-	1496s	-	-
2-Apdn	138.12	3255sh	3865sh 3749w	1697sh 1627m	-	1458sh	-	-
HL^1	794.90	-	3406sh	1620b 1535m	995w 960m	1462sh	-	-
HL^2	481.50	-	3402b 3263m	1631sh 1535m	964w 868w	1465m	-	-
$Co(HL^1).2H_2O$	889.80	-	3402b	1635sh	840w	1492w	621w	470w
$Cu(HL^1).2H_2O$	894.40	-	3406b	1639m	1037w 995w	1492w	586w	451m
$Co(HL^2)_2.2H_2O$	1057.90	-	3402sh 3263m	1697m 1647m	868w 786m	1543m	651m 590b	478w
$Cu(HL^2)_2.2H_2O$	1062.60	-	3398sh 3263m	1697w 1635m	879w 790sh	1543m	648m 582b	405sh

Key: sh=sharp, m=medium, b=broad, w=weak, s= strong, oflox=ofloxacin, o-phdn= o-phenylenediamine, 2-Apdn=2-aminopyridine-3-carboxilic acid

Table 4: Electronic absorption spectra and structural assignments of the ligands and their metal(II) complexes

Compds.	λ_{max}(nm)	Wave number (cm^{-1})	Assignment
HL^1(oflox-ophdn)	308	32468	$n\rightarrow\pi^*$
HL^2(oflox-2-Apydn)	362	27624	$n\rightarrow\pi^*$
$Co(HL^1).2H_2O$	353	28329	MLCT
$Cu(HL^1).2H_2O$	342	29240	MLCT
$Co(HL^2)_2.2H_2O$	349	28653	MLCT

The data presented from the physicochemical studies, infrared spectral analysis and UV/Visible electronic absorption spectra, suggested the following structures for the HL^1 and HL^2 Schiff base ligands and their metal(II) complexes.

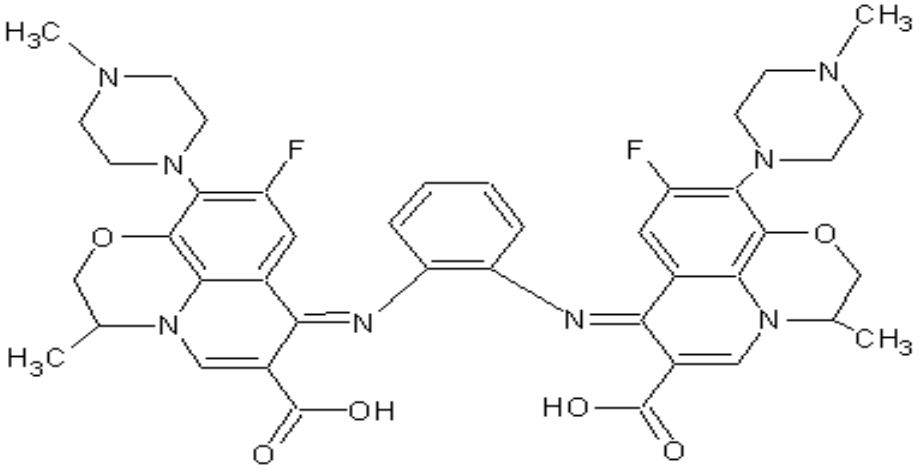


Figure 1: Proposed structure for ofloxo-o-phenylenediamine Ligand (HL^1)

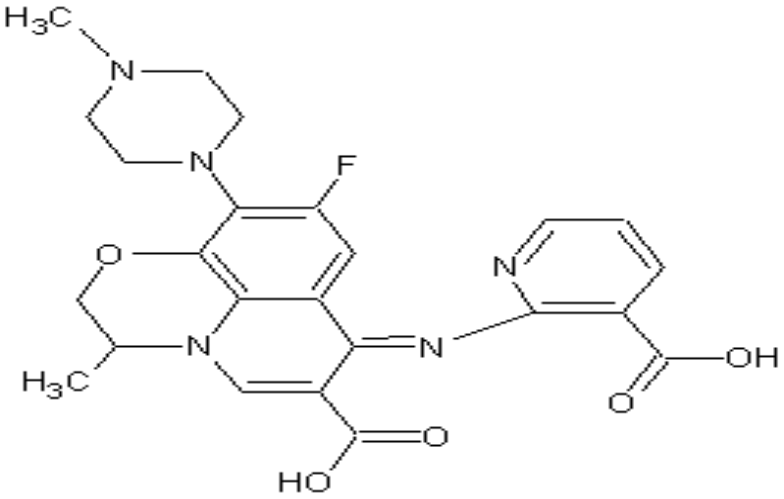
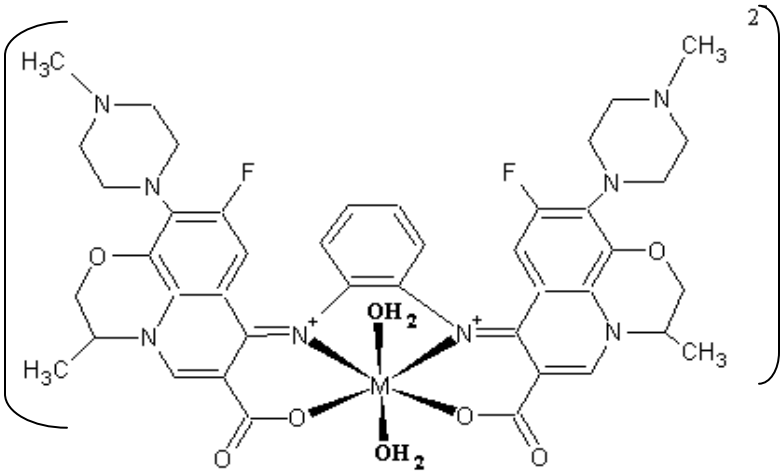


Figure 2: Proposed structure for ofloxo-2-Apydn Ligand (HL^2)



where M= Ni(II), Mn(II) and Zn(II)

Figure 3: Proposed structure for HL^1metal(II) complexes

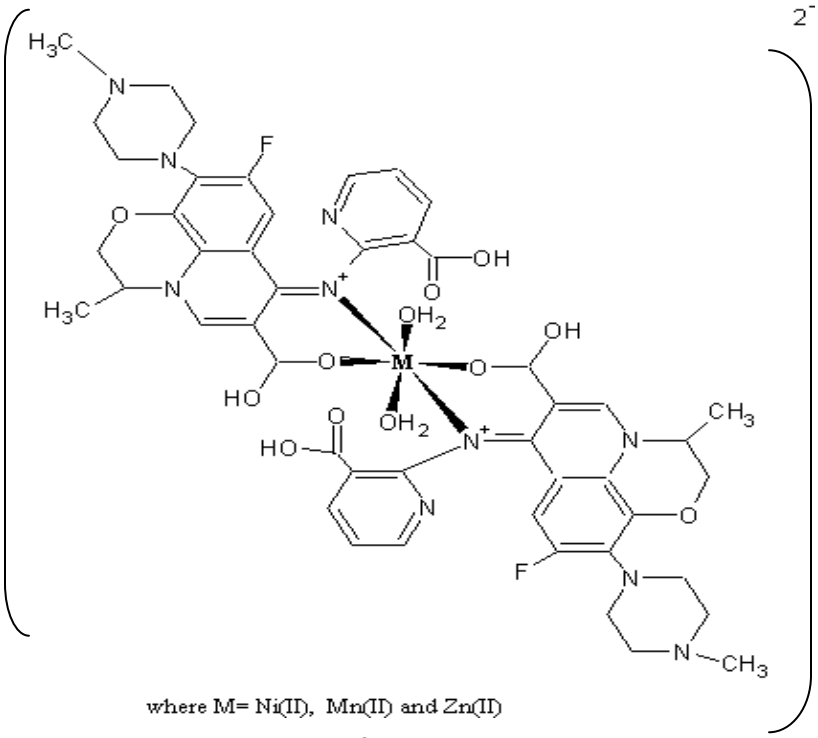


Figure 4: Proposed structure for HL^2 metal(II) complexes

Table 5: The *in vitro* antimicrobial activity of the Schiff base ligands (HL^1) and (HL^2) their metal(II) complexes

Compds.	**Conc. (µg/ml)**	***S. aureus***	***B. subtilis***	***S. typhi***	***E. coli***	***A. niger***	***A. fumigatus***
HL^1	30	30.00 ± 0.00^{a}	32.67 ± 0.33^{e}	43.00 ± 0.00^{a}	20.00 ± 0.00^{a}	R	R
	20	24.67 ± 0.33^{b}	26.00 ± 0.00^{f}	37.33 ± 0.33^{b}	14.33 ± 0.33^{b}	R	R
	10	19.67 ± 0.33^{c}	20.00 ± 0.00^{g}	31.00 ± 0.00^{c}	10.000 ± 0.00^{c}	R	R
	30	34.33 ± 0.33^{d}	30.00 ± 0.00^{b}	42.67 ± 0.33^{a}	20.00 ± 0.00^{a}	R	20.00 ± 0.00^{b}
$Co(HL^1).2H_2O$	20	27.33 ± 0.33^{f}	23.00 ± 0.00^{c}	35.33 ± 0.33^{d}	13.00 ± 0.00^{d}	R	13.67 ± 0.33^{c}
	10	$21.00\pm0.00^{c'}$	16.00 ± 0.00^{d}	28.00 ± 0.00^{i}	9.00 ± 0.00^{c}	R	10.00 ± 0.00^{d}
	30	34.33 ± 0.33^{d}	30.00 ± 0.00^{b}	$44.33\pm0.33^{aa'}$	20.33 ± 0.33^{a}	R	25.00 ± 0.00^{e}
$Cu(HL^1).2H_2O$	20	27.67 ± 0.33^{f}	23.67 ± 0.33^{c}	37.67 ± 0.33^{b}	$13.33\pm0.33^{bd'}$	R	19.33 ± 0.33^{b}
	10	$20.67\pm0.33^{cc'}$	18.00±0.00j	20.67 ± 0.33^{e}	9.33 ± 0.33^{c}	R	13.00 ± 0.00^{c}
	30	29.67 ± 0.33^{a}	35.00 ± 0.33^{f}	37.33 ± 0.33^{g}	30.00 ± 0.00^{d}	R	R
HL^2	20	24.0 ± 0.00^{b}	26.33 ± 0.33^{g}	31.33 ± 0.33^{h}	24.00 ± 0.00^{e}	R	R
	10	18.67 ± 0.33^{c}	20.00 ± 0.00^{h}	25.00 ± 0.00^{i}	18.33 ± 0.33^{f}	R	R
	30	0.00 ± 0.00^{d}	15.00 ± 0.00^{a}	18.00 ± 0.00^{j}	0.00 ± 0.00^{a}	R	R
$Co(HL^2)_2.2H_2O$	20	0.00 ± 0.00^{d}	10.00 ± 0.00^{b}	$12.67\pm0.00^{b'}$	0.00 ± 0.00^{a}	R	R
	10	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	9.00 ± 0.00^{c}	0.00 ± 0.00^{a}	R	R
	30	29.33 ± 0.33^{a}	30.0 ± 0.00^{d}	34.67 ± 0.33^{d}	10.33 ± 0.33^{c}	R	R
$Cu(HL^2)_2.2H_2O$	20	23.00 ± 0.00^{b}	23.33 ± 0.33^{e}	28.00 ± 0.00^{e}	0.00 ± 0.00^{a}	R	R
	10	16.67 ± 0.33^{h}	17.00 ± 0.00^{k}	21.33 ± 0.00^{f}	0.00 ± 0.00^{a}	R	R
Ofloxacin	30	18.00 ± 0.00^{e}	16.00 ± 0.00^{d}	20.00 ± 0.00^{e}	15.00 ± 0.00^{b}	-	-
Ketoconazole	30	-	-	-	-	30.00±0.00	30.00 ± 0.00^{f}

NB. Different superscripts along the same column are significantly (P<0.05) different, **R***= Resistant.

Antimicrobial Evaluation

Ligand (HL^1) and its metal(II) complexes

Table 5 presented the antimicrobial activity of $HL^1$and HL^2Schiff bases and their Co(II) and Cu(II) metal complexes. These final scaffolds of ofloxacin derivatives synthesized showed a good to excellent activity toward all the mentioned panel of bacterial strains across the range of the serial concentrations (30µg/ml, 20µg/ml and 10µg/ml) compared with the parent drug ofloxacin and most were significant ($P<0.05$) on one way ANOVA analysis. On the fungal strains bioassay, moderate and promising activity was observed for $Co(HL^1).2H_2O$ complex in *A. fumigatus* strain compared to the referenced drug ketoconazole; while $Cu(HL^1).2H_2O$ presented good activity in the same strain (*A. fumigatus*). HL^2 ligand and its metal complexes showed no activity in *A. fumigatus*. However, *A. niger* strain of the fungi resisted all the tested novel compounds.

The increasing activity of the metal complexes against the free ligands HL^1as demonstrated by HL^1-complexes in *A.fumigatus* fungal strain may be explained on the basis of oxidation state, overtone concept and chelation theory [38] which reduces polarity of the metal ion by partial sharing of the positive charge with donor atoms of the ligand (imine & oxygen). This atomic shake-up increases the lipophilic character, favouring the permeation through lipid layers of the bacterial membrane and consequently restricting the further growth of the organism [39]. However, distinctively, HL^1 free ligand showed good to excellent activity on *E. coli* and, *S. aureus*, *B. subtilis* & *S. typhi* respectively at all dilutions such as; (43.00±0.00, 32.67±0.33, 30.00±0.00 and 20.00±0.00) mm at 30µg/ml respectively (Table 5) compared to the parent drug ofloxacin values. No activity was observed in the fungal strains (*A. niger* and *A. fumigatus*) by the free ligand HL^1. This potency of the HL^1 ligand on bacterial strains was sustained by its metal complexes in which $Cu(HL^1).2H_2O$ exhibited excellent activity on *S. typhi* (44.33±0.33, 37.67±0.33 and 20.67±0.33) mm inhibitions at 30, 20 & 10µg/ml concentrations compared to 20.00±0.00 mm at 30µg/ml for parent drug (ofloxacin). Both metal complexes presented very good activity in all the test microorganisms with highest activity recorded in *S. typhi* followed by *S. aureus* and *B. subtilis* while the least but very good effect was observed in *E. coli* compared to the reference drug ofloxacin with 20.00±0.00, 18.00±0.00 16.00±0.00 and 15.00±0.00 mm respectively. The activity of the ligand and its complexes on the test organisms followed the trend $Cu>Co>HL^1$ and these variations were tested to be significantly ($P<0.05$) different on ANOVA statistical tool.

Ligand(HL^2) and its Metal Complexes

The preliminary antibacterial screening results for HL^2 and its metal complexes are also presented in Table 5 above. These novel ofloxacin derivatives presented a divergent activity from the common and popular higher activity by the complexes against the free ligands. In this study, the free ligand HL^2 exhibited higher and good *in vitro* activity than the complexes in the entire test bacterial strains with the highest inhibitory diameter observed in *S. typhi* (37.33±0.33) followed by *B. subtilis* (35.00±0.33), *E. coli* (30.00±0.00) and *S. aureus* (29.67±0.33), at 30µg/ml and showing more potency on gram negative at all levels of the concentrations compared to the referenced drug. The HL^2 metal complexes showed moderate and good activity on some of the test organisms. However, to the contrary from the previous experience with HL^1 ligand and its complexes, the activity of the $M(HL^2)_2.2H_2O$ complexes appeared to be lower than the values observed for their ligand; with $Cu(HL^2)_2.2H_2O$ complex showing higher activity in all the probed bacterial strains than $Co(HL^2)_2.2H_2O$ with the highest inhibition zone recorded in *S. typhi* followed by *B. subtilis*, *S. aureus* and *E. coli* with inhibitory diameter of 34.67±0.33, 30.00±0.00, 29.33±0.33 and 10.33±0.33 mm (SEM) respectively at 30µg/ml concentration (Table 5). Furthermore, the antifungal assay of the HL^2 ligand and its complexes revealed no activity in the two fungal strains *A. niger* and *A. fumigatus* used.

Summary

The study on "synthesis, characterization and antimicrobial evaluation of metal(II) complexes of ofloxacin derivatives" have been successfully carried out. Schiff base Ligands HL^1 and HL^2 were obtained from separate condensation of o-phenylenediamine and 2-Aminopyridine-3-carboxylic acid with ofloxacin in 2:1 and 1:1 mole ratio respectively. These ligands were complexed with chloride salts of Co(II) and Cu(II) to obtain (HL^1) and (HL^2)-Metal complexes respectively. The novel compounds were characterized on the basis of physicochemical properties, IR

spectroscopy, UV-visible spectroscopy, Molar conductance and metal analysis. The IR and UV/vis spectra revealed that the metal ions coordinated with the ligands through azomethine nitrogen and carboxylato oxygen of the drug moieties exhibiting tetradentate character with the formular type ML and $ML_2$for HL^1 and HL^2 metal complexes respectively. The geometry of the new compounds were not established due to instrumental errors in UV/vis spectra but however, the previous reported data on similar re-arrangements suggested d-d transition to favour octahedral geometry and MLCT. The antimicrobial evaluation of the ligands and their respective complexes in *S. aureus, B. subtilis, S. typhi, A. niger* and *A. fumigatus* presented a promising to excellent activity on the scale of the referenced standard drugs (ofloxacin and ketoconazole) except for *A. niger* which showed no activity in all the tested compounds.

Conclusion

This work afforded stable ofloxacin derivatives from Schiff bases derived from o-phenylenediamine and 2-aminopyridine-3-carboxylic acid and their metal complexes with promising to excellent antimicrobial activity on some microbes compared to the referenced drug modified.

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