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Synthesis, characterization and antimicrobial analysis of Schiff bases of o-phenylenediamine and 2-aminopyridine-3-carboxylic acid with ofloxacin and their metal (II) complexes

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ABSTRACT

The increasing multi-drug resistance of microbes to the existing anti-biotic drugs has been of great concern to medical scientists and challenge has directly concerned pharmacologists, inorganic chemists, molecular microbiologists etc. This work attempted modifying a second generation fluoroquinolone (ofloxacin) toward attaining more improved activity. 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 Ni (II), Mn (II) and Zn (II) to afford their metal complexes. The new compounds were characterized on the basis of physicochemical properties, IR spectroscopy, UV-visible spectroscopy, Molar conductance and metal analysis. The IR spectra revealed that the metal ions coordinated with the ligands through azomethine nitrogen and carboxylato oxygen depicting tetradentate character with the formular 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 *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli, Aspergellus niger and Aspergellus 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, ofloxacin, o-phenylenediamine, 2-aminopyridine-3-carboxylic acid, antimicrobial activity.

INTRODUCTION

Schiff bases named after Hugo Schiff (1864), a German Scientist, are formed when primary amine reacts with ketones or aldehydes under specified mole ratio and conditions. These are usually compounds carrying imine or azomethine (-C=N-) functional group, referred to as products of condensation of primary amines with carbonyl compounds (Ndahi and Nasiru, 2012). Schiff base forms an important class of the most widely used organic compounds and has a

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wide variety of applications in many fields including analytical, biological, medicinal, inorganic chemistry and as fine chemicals (Gangani and Parsania, 2014). The important and interesting roles of Schiff bases are as intermediates in the biologically important transmutation reactions. In azomethine derivatives, the C=N linkage is essential for molecular interactions, as several azomethine have been reported to possess remarkable antibacterial, antifungal, anticancer and antimalarial activity (Sridhar et al., 2001; Raman et al., 2003). Heterocyclic Schiff bases have been reported as antibacterial and antifungal activities. Schiff base compounds have also been used as fine chemicals and medical substrates (Shrikrishna et al., 2012).

Quinolones are synthetic antibacterial compounds based on a 4-quinolone skeleton and are present in numerous natural products, especially in alkaloids. Many quinolones display interesting pharmacological activities and have found applications as pharmaceuticals, e.g antimalarial drugs, such as quinine or chloroquin (Jayashree et al., 2012). The present therapeutic arsenal notwithstanding, the efficacy of existing antimicrobial molecules is limited by the increasing multi-drug resistant microbes (Akpa et al., 2016) which is among the prime factors that ensures success of adaptive somatic modifications for enhanced living of these pathogens (Ngassam et al., 2017).

Metal complexed antibiotics have been reported to show either equal or increased antibacterial profile in comparison to the original drugs. Ofloxacin has been discreetly reported to possess the potential of interacting with the metal ions as a mono anionic bidentate ligand bound to the metal through the pyridone and carboxylate oxygen atoms having [M $(Oflo)_2$ $(H_2O)_2$]. nH_2O formula, where M = Metal ion (Sultana et al., 2013). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there have been an alarming increase in the incidence of new and re-emerging infectious disease (Anand et al., 2011).

However, extensive works have been reported on modifications of other fluoroquinolones especially ciprofloxacin, gatifloxacin, norfloxacin and levofloxacin both in Schiff base synthesis and their metal complexes (Sultana et al., 2013; Verma et al., 2013; Sadeek et al., 2014), but not much has been explored on ofloxacin toward enhancing its activity especially with respect to its imine Schiff bases and their metal complexes.

In view of the challenges of checking the menace of multi-drug resistant pathogens, this work was aimed at synthesizing some Schiff bases containing ofloxacin drug with o-phenylenediamine and 2-aminopyridine-3-carboxylic acid and complex them with Ni (II), Mn(II) and Zn(II) Metal ions. The expected newly synthesized ofloxacin derivatives were characterized using various methods and their antimicrobial potency evaluated with reference to the parent/referenced drugs.

MATERIALS AND METHODS

Chemicals and Reagents

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; $NiCl_2.6H_2O$, $MnCl_2.4H_2O$ and Zn $(NO_3)_2.6H_2O$. 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 were characterized on the basis of conductance measurement using DDS-307 Conductometer bridge. The melting point temperatures were determined using Gallenkamp melting apparatus and are uncorrected. The metal content of the complexes was determined using complexometric titration with EDTA. The electronic spectral data were recorded with UV-Vis spectrophotometer at 800-200

nm and the FTIR analysis of the compounds was carried out in the range of 500-4000 cm^{-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 fungi like *Aspergillus niger* and *Aspergillus fumigatus*.

Synthesis of the ligands (ofloxacin imines)

The Schiff base ligands (ofloxacin-imines) were synthesized using a literature procedure (Imran et al., 2007; Sadeek et al., 2014), by the condensation of ofloxacin antibiotic with respective substituted amines in the appropriate molar ratio in ethanol. Ofloxacin powder (2 mmol, 0.7227 g) dissolved in 25 ml methanol was mixed with o-phenylenediamine (1 mmol, 0.108 g) dissolved in 12.5 ml methanol, (2:1) in a round bottom flask. The mixture was subjected to reflux in the presence of 2 drops of glacial acetic acid for 3 h. The resulting solution was concentrated on a water bath and allowed to cool at 0 ^{0}C. The yellowish white solid formed (HL^1) was filtered, washed with ethanol and dried in a desiccator containing $CaCl_2$. The same method 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.

Synthesis of metal complexes

The prepared Schiff base ligands (0.02 mol) were dissolved in 25 ml methanol and mixed with respective transition metal salts (0.01 mol) e.g $NiCl_2.6H_2O$ 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, after which it was concentrated and cooled at room temperature which gave coloured precipitates. The precipitates were filtered, washed with methanol and dried in a 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. (2007) and Al-Bayati et al. (2010) as adopted by Usman et al. (2007); Ndahi et al. (2012); Waziri et al. (2013) against different bacteria strains such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli,* and also on some fungi strains like *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 (30 µg, 20 µg, 10 µg) 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 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 (30 µg/ml) concentration (standard antibacterial) and Ketoconazole (30 µg/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 h at 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 Graphpad Instat, 2000 and expressed as ± SEM. P values (≤ 0.05) were considered significant in this study to test the extent of potency of the new compounds among themselves across the columns as relative to the reference drugs.

RESULTS

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 presumed formula weights of the synthesized compounds were found to be; 794.90 [HL^1], 889.59 [$Ni(HL^1).2H_2O$], 885.84 [$Mn(HL^1).2H_2O$] and 896.28 [$Zn(HL^1).2H_2O$]. The common colours of the ofloxacin derivatives were yellow and green crystals with various intensity. The conductivity measurement (Scm^2mol^{-1}) were found as; 5.3×10^{-5} [HL^1], 5.2×10^{-4} [$Ni(HL^1).2H_2O$], 5.4×10^{-4} [$Mn(HL^1).2H_2O$], and 4.0×10^{-4} (Scm^2mol^{-1}) for $Zn(HL^1).2H_2O$ which are relatively low values indicating possibly the compounds are weak or non-electrolytes. Their melting points were determined as; 156-158 ^oC [HL^1], 168-170 ^oC [$Ni(HL^1).2H_2O$], 162-164 ^oC [$Mn(HL^1).2H_2O$] and 178-180 ^oC [$Zn(HL^1).2H_2O$]. The percentage yield was appreciable as it ranges from 61.18% [$Zn(HL^1).2H_2O$] to 70.41% [$Mn(HL^1).2H_2O$]. The metal analysis presented the percentage of metal in the complexes for both experimental (found) and theoretical (cal); 6.60 (6.85) [$Ni(HL^1).2H_2O$], 6.20 (5.86) [$Mn(HL^1).2H_2O$] and 7.29 (6.97) % for [$Zn(HL^1).2H_2O$] which are both in close agreement.

Similarly, Table 2 shows the physical properties of HL^2 ligand and its metal(II) complexes as follows; Formula weights were proposed as; 481.50 [HL^2], 1057.69 [$Ni(HL^2)_2.2H_2O$], 1053.94 [$Mn(HL^2)_2.2H_2O$] and 1064.38 [$Zn(HL^2)_2.2H_2O$] complex. Similar colours but with different intensities were observed for HL^2 compounds which include pale yellow, whitish green, yellow and pale yellow for all the compounds respectively. Conductivity (Scm^2mol^{-1}); 6.1×10^{-5} [HL^2], 5.8×10^{-4} [$Ni(HL^2)_2.2H_2O$], 7×10^{-4} [$Mn(HL^2)_2.2H_2O$] and 6×10^{-5} [$Zn(HL^2)_2.2H_2O$]. Melting points are presented as; 132-134 ^oC [HL^2], 186-188 ^oC [$Ni(HL^2)_2.2H_2O$], 185-187 ^oC [$Mn(HL^2)_2.2H_2O$] and 186-189 ^oC [$Zn(HL^2)_2.2H_2O$]. Percentage (%) yields; 70.41% [HL2], 69.60% [$Ni(HL^2)_2.2H_2O$], 67.89% [$Mn(HL^2)_2.2H_2O$] and 68.29% [$Zn(HL^2)_2.2H_2O$]. The percentage (%) metal content of the complexes were found to be; 5.55 (5.28)% [$Ni(HL^2)_2.2H_2O$], 5.21 (5.49)% [$Mn(HL^2)_2.2H_2O$] and 6.14 (6.54)% [$Zn(HL^2)_2.2H_2O$]. Table 3 presented relevant infrared (IR) spectra of the parent drug (ofloxacin), amine used, synthesized ligands and their metal (II) complexes (cm^{-1}). The important spectra for the drug (ofloxacin) as obtained in Table 3 include; $\nu(NH_2)$- 2924 cm^{-1}, ν(OH)- 3803 cm^{-1}, 3749 cm^{-1}, ν(C=N)- 1519 cm^{-1} and ν(C-N)- 1458 cm^{-1}. The amine (o-phenylenediamine) showed the following spectra; $\nu(NH_2)$- 3363 cm^{-1}, 2924 cm^{-1}, ν(OH)- 3865 cm^{-1}, ν(C=N)- 1627 cm^{-1}, 1535 cm^{-1} and ν(C-N)- 1496 cm^{-1}. 2-aminopyridene-3-carboxylic acid had the following guide spectra; $\nu(NH_2)$-3255 cm^{-1}, ν(OH)- 3865 cm^{-1}, 3749 cm^{-1}, ν(C=N)- 1697 cm^{-1}, 1627 cm^{-1} and ν(C-N)- 1458 cm^{-1}. HL^1 ligand spectra; ν(OH)- 3406 cm^{-1}, 3043 cm^{-1} ν(C=N)- 1620 cm^{-1}, 1535 cm^{-1}, ν(H2O)- 995 cm^{-1}, 960 cm^{-1} and ν(C-N)- 1462 cm^{-1}. HL^2 ligand showed the same spectra as; ν(OH)- 3402 cm^{-1}, 3263 cm-1, ν(C=N)- 1631 cm^{-1}, 1535 cm^{-1}, $\nu(H_2O)$- 964 cm^{-1}, 868 cm^{-1} and ν(C-N)- 1465 cm^{-1}. The metal complexes of the ligand presented more spectra such as; $Ni(HL^1).2H_2O$ showed absorption at 3406 cm^{-1} assigned for ν(OH), 1643 cm^{-1}, 1535 cm^{-1} ν(C=N), 987 cm^{-1} attributed to $\nu(H_2O)$ products vibration, 1489 cm^{-1} assigned to ν(C-N), 516 cm^{-1} due to ν(M-N) and 615 cm^{-1} which is a non-ligand spectra of ν(M-O). $Mn(HL^1).2H_2O$ presented; 3398 cm^{-1} ν(OH), 1631 cm^{-1}, 1550 cm^{-1} for ν(C=N), 983 cm^{-1} $\nu(H_2O)$ vibration, 1473 cm^{-1} assigned to ν(C-N), 586 cm^{-1} for ν(M-N) and 451 cm^{-1} attributed to ν(M-O). $Zn(HL^1).2H_2O$ showed these spectra as follows; 3402 cm^{-1} ν(OH), 1635 cm^{-1}, 1535 cm^{-1} ν(C=N), 960 cm^{-1} for $\nu(H_2O)$, 1465 cm^{-1} assigned to ν(C-N), 682 cm^{-1} due to ν(M-N) and 405 cm^{-1} for ν(M-O). $Ni(HL^2)_2.2H_2O$ complexes showed peaks at; 3363 cm^{-1}, 3255 cm^{-1} assigned to ν(OH) vibration, 1639 cm^{-1}, 1546 cm^{-1} for ν(C=N), 879 cm^{-1}, 790 cm^{-1} due to $\nu(H_2O)$, 1419 cm^{-1} ν(C-N), 659 cm^{-1}, 578 cm^{-1} for ν(M-N) and 405 cm^{-1} attributed to ν(M-O). $Mn(HL^2)_2.2H_2O$ presented these vibration

spectra at; 3402 cm^{-1}, 3255 cm^{-1} assigned to ν(OH), 1543 cm^{-1}, 1639 cm^{-1} due to ν(C=N), 875 cm^{-1}, 790 cm^{-1} for ν(OH), 1481 cm^{-1} ν(C-N), 663 cm^{-1}, 582 cm^{-1} for ν(M-N) and 478 cm^{-1} ν(M-O). $Zn(HL^2)_2.2H_2O$ had its absorption spectra at; 3398 cm^{-1} for ν(OH), 1693 cm^{-1}, 1635 cm^{-1} assigned to ν(C=N), 864 cm^{-1}, 779 cm^{-1} due to ν(H_2O), 1462 cm^{-1} for ν(C-N), 644 cm^{-1}, 597 cm^{-1} for ν(M-N) and 405 cm^{-1} due to non-ligand ν(M-O) vibration. Table 4 showed electronic absorption spectra of the ligands and their metal complexes; The HL^1- (oflox-o-phdn) showed electronic spectra at λ_{max} 308 nm or 32468cm^{-1} wave number assigned to n→π*. HL^2- (oflox-2-Apydn) presented its spectra at λ_{max} 362 nm or 27624 cm^{-1} wave number also assigned to n→π* transition. $Ni(HL^1).2H_2O$ showed the electronic spectra at λ_{max} 295 nm or 33898 cm^{-1} wave number which is suggested for n→π* transition. The electronic spectra for Mn $(HL^1).2H_2O$ were observed at λ_{max} 343 nm or 29155 cm^{-1} assumed to be for metal to ligand charge transfer (MLCT). Zn $(HL^1).2H_2O$ electronic spectra was observed at λ_{max} 349 nm or 28653 cm^{-1} wave number also attributed to MLCT.

The HL^2 complexes also showed irregular absorption spectra for $Ni(HL^2)_2.2H_2O$ at λ_{max} 238 nm or 42017 cm^{-1} assigned to π→π* transition. Mn $(HL^2)_2.2H_2O$ showed its absorption at λ_{max} 221 nm or 45249 cm^{-1} attributed to π→π* electronic transition. $Zn(HL^2)_2.2H_2O$ had its absorption spectra at λ_{max} 353 nm or 28329 cm^{-1} assumed to be due to MLCT.

Tables 5 and 6 presented results for the *in vitro* antimicrobial activity evaluation for the Schiff base ligands and their metal (II) complexes. The synthesized compounds were tested against four medically important bacterial strains such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli* and, two fungal strains *Aspergellus niger* and *Aspergellus fumigatus*. The results of the activity of the ligands and their complexes on the bacterial strains are presented in millimeter zone of inhibition at three serial dilutions of 30 µg/ml, 20 µg/ml & 10 µg/ml. The observed results showed promising to excellent activity of the novel compounds on the test microbes (Table 5) while the fungal strains resisted all the compounds of HL^2 ligands as shown in Table 6.

The statistical analysis results are also presented in Tables 5 and 6 as superscripts along columns. Different superscripts along the same column are significantly ($P<0.05$) different and are referenced to the parent/reference drugs at the highest concentration of 30 µg/ml.

Table 1: Physical Characteristics of HL^1 Ligand and its Metal (II) Complexes.

Compound/Proposed Formula	F. Weight	Colour	Conductivity (Scm^2mol^{-1})	M.P (^0C)	Yield (%)	%M Found(Cal)
$(HL^1)/C_{42}H_{44}F_2N_8O_6$	794.90	Yellowish white	5.3×10^{-5}	156-158	66.44	-
$Ni(HL^1).2H_2O$	889.59	Green	5.2×10^{-4}	168-170	66.44	6.60 (6.85)
$Mn(HL^1).2H_2O$	885.838	Yellow	5.4×10^{-4}	162-164	70.41	6.20 (5.86)
$Zn(HL^1).2H_2O$	896.28	Lemon Yellow	4.0×10^{-4}	178-180	61.18	7.29 (6.97)

Table 2: Physical Characteristics of HL^2 Ligand and its Metal (II) Complexes.

Proposed Formula	F. Weight	Colour	Conductivity (Scm^2mol^{-2})	M.P (^0C)	Yield (%)	%M Found(Cal)
$(HL^2)/C_{24}H_{24}FN_5O_5$	481.50	Pale yellow	6.1×10^{-5}	132-134	70.41	-
$Ni(HL^2)_2.2H_2O$	1057.69	Whitish Green	5.8×10^{-4}	186-188	69.60	5.55 (5.28)
$Mn(HL^2)_2.2H_2O$	1053.938	Yellow	7×10^{-4}	185-187	67.85	5.21 (5.49)
$Zn(HL^2)_2.2H_2O$	1064.38	Pale Yellow	6×10^{-5}	186-189	68.29	6.14 (6.54)

Table 3: Relevant Infrared Spectra of the Drug, Amines, Ligands and their Metal (II) Complexes (cm^{-1}).

Compds.	v(NH_2)	v(OH)	v (C=N)	v(H_2O)	v(C-N)	v (M-N)	v (M-O)
Oflox.	2924sh	3803w 3749w	1519w	-	1458s	-	-
o-phdn	3363w 2924m	3865w	1627sh 1535m	-	1496s	-	-
2-Apdn	3255sh	3865sh 3749w	1697sh 1627m	-	1458sh	-	-
HL^1	-	3406sh 3043w	1620b 1535m	995w 960m	1462sh	-	-
HL^2	-	3402b 3263m	1631sh 1535m	964w 868w	1465m	-	-
$Ni(HL^1).2H_2O$	-	3406s	1643sh 1535m	987w	1489w	516w	613w
$Mn(HL^1).2H_2O$	-	3398sh	1631b 1550m	983m	1473m	586w	451w
$Zn(HL^1).2H_2O$	-	3402sh	1635m 1535m	960w	1465m	682w	405m
$Ni(HL^2)_2.2H_2O$	-	3363w 3255sh	1639m 1546m	879w 790s	1419m	659s 578b	405sh
$Mn(HL^2)_2.2H_2O$	-	3402sh 3255w	1543m 1639m	875w 790m	1481w	663sh 582b	478w
$Zn(HL^2)_2.2H_2O$	-	3398sh	1693w 1635m	864w 779m	1462w	644w 597w	405sh

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

Table 4: Electronic Absorption Spectra of the Ligands and their Metal 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	$n\rightarrow\pi^*$
$Ni(HL^1).2H_2O$	295	33898	$n\rightarrow\pi^*$
$Mn(HL^1).2H_2O$	343	29155	MLCT
$Zn(HL^1).2H_2O$	349	28653	MLCT
$Ni(HL^2)_2.2H_2O$	238	42017	$\pi\rightarrow\pi^*$
$Mn(HL^2)_2.2H_2O$	221	45249	$\pi\rightarrow\pi^*$
$Zn(HL^2)_2.2H_2O$	353	28329	MLCT

Table 5: The *in vitro* Antimicrobial Activity of the Schiff base Ligand (HL^1) and its 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	0.00 ± 0.00^{a}
	20	24.67 ± 0.33^{b}	26.00 ± 0.00^{f}	37.33 ± 0.33^{b}	14.33 ± 0.33^{b}	R	0.00 ± 0.00^{a}
	10	19.67 ± 0.33^{c}	20.00 ± 0.00^{g}	31.00 ± 0.00^{c}	10.000 ± 0.00^{c}	R	0.00 ± 0.00^{a}
$Ni(HL^1).2H_2O$	30	40.00 ± 0.00^{g}	36.33 ± 0.33^{a}	50.00 ± 0.00^{f}	25.00 ± 0.00^{e}	R	20.00 ± 0.00^{b}
	20	33.33 ± 0.33^{d}	30.00 ± 0.00^{b}	42.33 ± 0.33^{a}	19.33 ± 0.33^{a}	R	13.33 ± 0.33^{c}
	10	$26.00\pm0.00^{b'}$	23.67 ± 0.33^{c}	34.33 ± 0.33^{d}	13.00 ± 0.00^{d}	R	9.33 ± 0.33^{d}
$Mn(HL^1).2H_2O$	30	30.00 ± 0.00^{a}	43.00 ± 0.00^{h}	48.00 ± 0.00^{g}	28.00 ± 0.00^{f}	R	0.00 ± 0.00^{a}
	20	24.00 ± 0.00^{b}	36.33 ± 0.33^{a}	40.67 ± 0.33^{h}	21.67 ± 0.33^{g}	R	0.00 ± 0.00^{a}
	10	18.00 ± 0.00^{e}	28.00 ± 0.00^{i}	33.33 ± 0.33^{d}	15.33 ± 0.33^{b}	R	0.00 ± 0.00^{a}
$Zn(HL^1).2H_2O$	30	32.00 ± 0.00^{h}	30.00 ± 0.00^{b}	$45.00\pm0.00^{a'}$	17.67 ± 0.33^{h}	R	0.00 ± 0.00^{a}
	20	25.00 ± 0.00^{bb}	23.00 ± 0.00^{c}	37.67 ± 0.33^{b}	13.00 ± 0.00^{d}	R	0.00 ± 0.00^{a}
	10	18.00 ± 0.00^{e}	16.67 ± 0.33^{d}	30.33 ± 0.33^{c}	9.00 ± 0.00^{c}	R	0.00 ± 0.00^{a}
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.

Table 6: The *in vitro* Antimicrobial Activity of the Schiff base Ligand (HL^2) and its Metal (II) Complexes.

Compds.	Conc. (µg/ml)	*S.aureus*	*B.subtilis*	*S.typhi*	*E.coli*	*A. niger*	*A. fumigatus*
	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	10.00±0.00^{e}	15.33±0.33^{a}	20.00±0.00^{a}	0.00±0.00^{a}	R	R
$Ni(HL^2)_2.2H_2$	20	0.00±0.00^{d}	10.00±0.00^{b}	13.33±0.33$^{bb'}$	0.00±0.00^{a}	R	R
O	10	0.00±0.00^{d}	0.00±0.00^{c}	9.00±0.00^{c}	0.00±0.00^{a}	R	R
	30	18.67±0.33^{c}	15.00±0.00^{a}	20.00±0.00^{a}	0.00±0.00^{a}	R	R
$Mn(HL^2)_2.2H_2$	20	13.00±0.00^{f}	10.33±0.33^{b}	14.00±0.00^{b}	0.00±0.00^{a}	R	R
O	10	8.00±00.00^{g}	7.00±0.00^{i}	9.00±0.00^{c}	0.00±0.00^{a}	R	R
	30	30.00±0.00^{a}	30.00±0.00^{d}	35.33±0.33^{d}	15.33±0.33^{b}	R	R
$Zn(HL^2)_2.2H_2$	20	23.67±0.33^{b}	24.00±0.00^{e}	28.67±0.33^{e}	10.33±0.33^{c}	R	R
O	10	18.00±0.00^{c}	18.67±0.33^{j}	21.67±0.33^{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

NB. Different superscripts along the same column are significantly ($P<0.05$) different.

DISCUSSION

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 Ni(II) and Mn(II); and Zn(II)Nitrate 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 61% - 83%. The ligands and their metal (II) complexes were observed to be stable under normal conditions with various colours characteristics of the transition metals (Aliyu and Ado, 2011) and mainly attributed to the d-d electron transition (Oladipo et al., 2005), except for zinc(II) complexes which showed yellowish and pale yellow colours which may be due to MLCT transition (Vogler and Kunkely, 2000). 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 (Ogunniran et al., 2008). The melting points of the ligands and their complexes range from 134 ^{o}C (HL^2) – 189 ^{o}C Zn-$(HL^2)_2.2H_2O$. The consistent range difference of +2 ^{o}C melting points observed indicates that the synthesized compounds are presumed pure Table 1. 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 (Sadeek et al., 2014). The molar conductance measurements of the ligands and the complexes in 10^{-3}M DMSO range from 0.053 – 0.70 Scm^2 mol^{-1} for HL^1 and Mn-$(HL^2)_2.2H_2O$ complex which are relatively low, indicating that the new compounds are non-electrolytes in nature (Ndahi and Nasiru, 2012).

FT-Infrared

The IR absorption spectra of the ofloxacin drug, amines used and, Schiff base ligands (HL^1 & HL^2) and their complexes with Ni (II), Mn (II) and Zn (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 (George, 2008). 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 (Patel et al., 2011a). The Schiff base ligands (HL^1 & HL^2) showed a typical characteristic azomethine ν(C=N) vibration band at 1620 and 1631 cm^{-1} 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 (Nakamoto, 2006) at the following regions; 1643 and 1639 cm^{-1} [Ni(II)], 1631 and 1639 cm^{-1} [Mn(II)], 1635 and 1635 cm^{-1} [Zn(II)] complexes respectively (Table 3). 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 (Reddy et al., 2008). Also, the IR spectra of the drug and the amine, ofloxacin, o-phenylenediamine (o-phdn) and 2-aminopyridine (2-Apdn) showed the ν(C-N) stretching vibration band at 1458, 1496 and 1458 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 spectral band at a range of 1462-1489 cm^{-1}. The HL^2 and its metal complexes displayed the ν(C-N) vibration band at a higher range of 1419-1481 cm^{-1} than its amine (2-Apdn) which is displayed at 1458 cm^{-1}.

The IR spectra of the complexes displayed a discrete non ligand band with low intensity at 516-682 cm^{-1} for HL^1-complexes and 578-663 cm^{-1} for HL^2-complexes corresponding to ν(M-N) stretching vibrations. The band at 405-613 cm^{-1} for HL^1-complexes and at 405-478 cm^{-1} for HL^2-complexes is attributed to ν(M-O) stretching vibration mode which are similar to the reported works of Sultana et al. (2013); Verma et al. (2013); Patel et al. (2012); Ndahi et al. (2012) and Imran et al. (2007). These non-ligand spectral bands are indicators to the possible coordination of the azomethine nitrogen and the carboxylato oxygen to the metal ions (Sadeek et al., 2005). A broad diffuse bands of medium and sharp intensity in the regions (3255-3406 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 790-995 cm^{-1} corresponded with the stretching vibration of the product water molecules (Anacona and Toledo, 2001). 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 (Patel et al., 2011b).

Electronic Spectra of the Schiff Base Ligands and their Metal Complexes

The electronic spectral band of the free ligands and the metal complexes studied in methanol (Table 5) 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 (Nagajothi et al., 2013). In the metal complexes, only broad bands were seen in the visible region which are probably due to d-d transition and MLCT (Ogunniran et al., 2008).

The Ni(II) complexes exhibited electronic spectral band in the UV at higher intensities than the free ligands at 33898 and 42017 cm^{-1} for $Ni(HL^1).2H_2O$ and $Ni(HL^2)_2.2H_2O$ complexes respectively attributed to $n \rightarrow \pi^*$ and $\pi\rightarrow\pi^*$ transitions of the non-bonding and Pi electrons (Patel et al., 2012). These transitions occur in case of unsaturated hydrocarbons which contain ketone or azomethine group (Chohan, 2001; Sadeek et al., 2014). Mn(II) complexes showed bands at 29155 cm^{-1} and 45249 cm^{-1} respectively, which may be assigned to MLCT and $^6A_{1g}\rightarrow{}^4T_{2g}$ or $\pi\rightarrow\pi^*$ transitios (Ndahi et al., 2012; Sadeek and EL-Shwiniy, 2010). The electronic spectral bands for Zn (II) complexes were exhibited at 28653 cm^{-1} and 28329 cm^{-1} respectively which corresponded to MLCT transitions. However, these electronic absorption spectra results couldn't provide adequate information as determine the geometry of the novel compounds because absorptions were not observed in the visible region above 400 nm in the UV which may be due to other factors unchecked.

Based on the data obtained from the physicochemical studies, infrared spectral analysis and UV/Visible electronic absorption spectra, proposed structures were elucidated for HL^1 and HL^2 Schiff base ligands and their metal (II) complexes as shown in Figures 1-4.

Ligand (HL^1) and its metal complexes

Table 5: presented the antimicrobial activity of HL^1 Schiff base and its metal complexes. The derivatives synthesized showed a good to excellent activity towards all the mentioned panel of bacterial strains across the range of the serial concentrations (30 µg/ml, 20 µg/ml and 10 µg/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 activity was observed for $Ni(HL^1).2H_2O$ complex in *A. fumigatus* strain compared to the reference drug ketoconazole; while the rest of the new compounds showed no activity. However, *A. niger* strain of the fungi didn't show activity in all the tested compounds. The increasing activity of the metal complexes against the free ligands HL^1 may be explained on the basis of oxidation state, overtone concept and chelation theory (Osowole et al., 2008) 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 further growth of the organism (Obalaye et al., 2011). However, distinctively, HL^1 free ligand showed good activity on *S. typhi* at all dilutions (Table 5), followed by *B. subtilis*, *S. aureus* and least on *E. coli* (43.00±0.00, 32.67±0.33, 30.00±0.00 and 20.00±0.00) mm respectively 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 was sustained by its metal complexes in which Ni $(HL^1).2H_2O$ exhibited excellent activity on *S. typhi* (50.00±0.00, 42.33±0.33 and 34.33±0.33) mm inhibitions at 30, 20 & 10 µg/ml concentrations compared to 20.00±0.00 mm at 30 µg/ml for parent drug (ofloxacin). Very good activity were also observed in *S. aureus* (40.00±0.00, 33.33±0.33 and 26.00±0.00) mm *B. subtilis* (36.33±0.33, 30.00±0.00 and 23.63±0.33) mm and *E. coli* (25.00±0.00, 19.33±0.00 and 13.00±0.00) mm compared with the parent drug ofloxacin with (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 Ni>Mn>Zn>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 presented in Table 6. These set of 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 representative bacterial strains used with the highest inhibition diameter measured in *S. typhi* followed by *B. subtilis*, *E. coli* and *S. aureus*, showing more potency on Gram negative at all levels of the concentrations. 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 the ligand; with the $Zn(HL^2)_2.2H_2O$ complex showing higher activity in all the probed bacterial strains with the highest inhibition zone marked in *S. typhi* followed by *B. subtilis*, *S. aureus* and *E. coli* with inhibitory diameter of 35.33±0.33, 30.00±0.00, 30.00±0.00 and 15.33±0.33 mm (SEM) respectively at 30 µg/ml dilution (Table 6). Ni $(HL^2)_2.2H_2O$ complexes showed activity only at 30 µg dilution in *S. aureus* and no activity at 10 µg/ml in *B. subtilis*. This primary investigation also revealed that Ni $(HL^2)_2.2H_2O$ and $Mn(HL^2)_2.2H_2O$ did not show activity in *E. coli*. In cases where activity were observed, the extent of the *in vitro* inhibitions range from moderate to good potency compared to the parent drug. Furthermore, the antimicrobial assay of the HL^2 ligand and the complexes revealed no activity in the two fungal strains (*A. niger* and *A. fumigatus*) used.

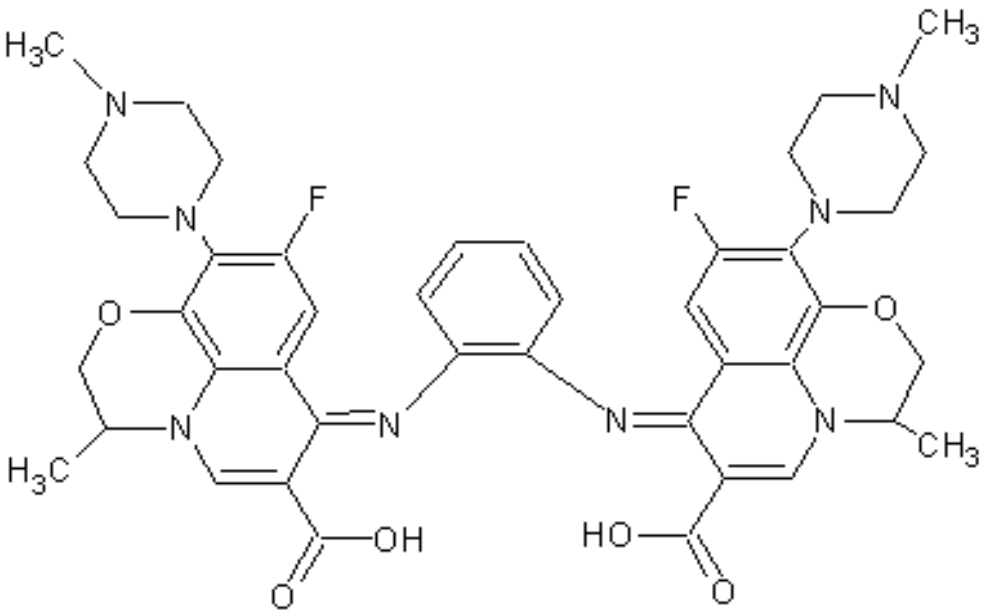

Figure 1: Proposed Structure for Ofloxo-o-phenylenediamine Ligand (HL^1).

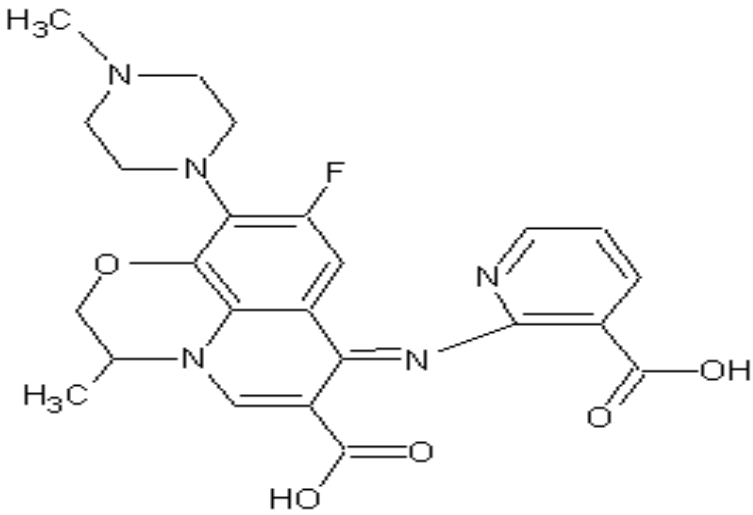

Figure 2: Proposed Structure for Ofloxo-2-Apydn Ligand (HL^2)

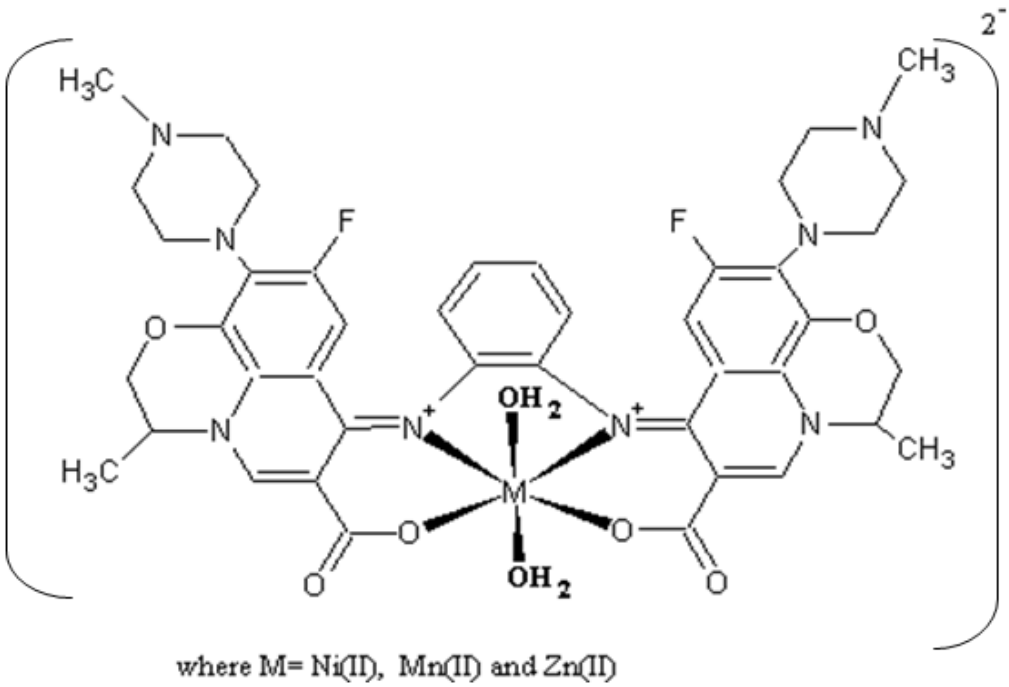

Figure 3: Proposed Structure for HL^1 Metal Complexes.

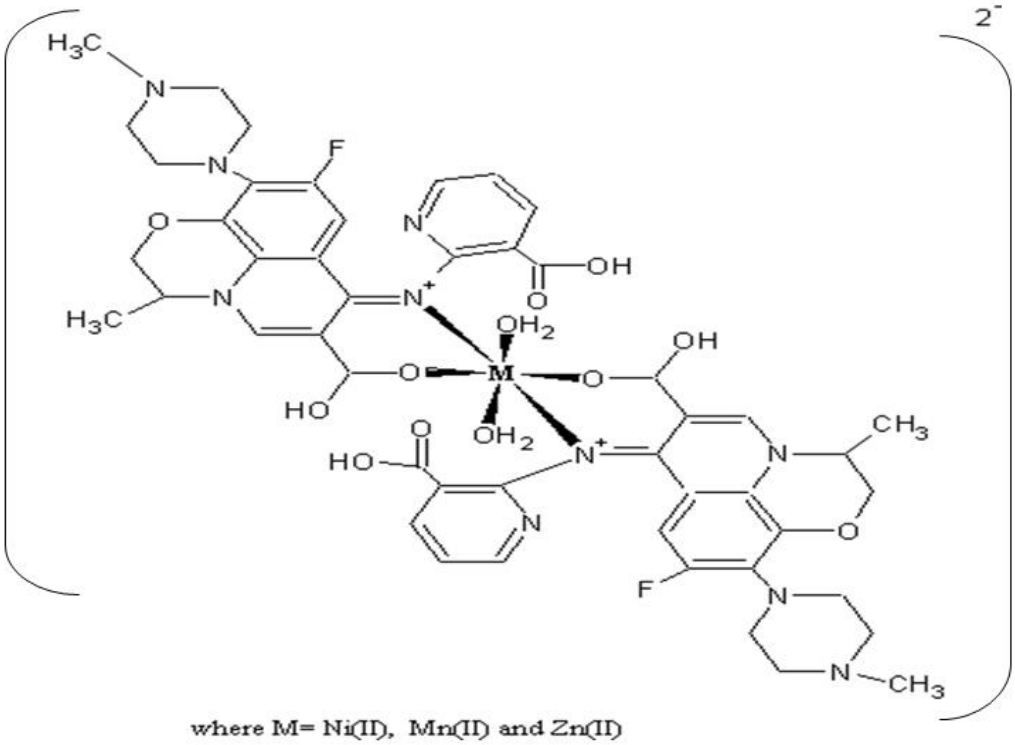

Figure 4: Proposed Structure for HL^2 Metal Complexes.

Conclusion

In continuous search for better activity drugs ahead of the existing antimicrobial agents which have been severally reported to show a declining action against todays' pathogens due to many human and behavioural factors; this work was designed to obtain or otherwise an improved antimicrobial agents through modification of the existing drugs (ofloxacin); using some Schiff bases containing ofloxacin drug with amines. These ligands were complexed with some transition metals and the newly synthesized compounds characterized and their antimicrobial potency evaluated. At the end of the analysis, the work afforded stable ofloxacin derivatives from Schiff bases derived from o-phenylenediamine and 2-aminopyridine-3-carboxylic acid and their metal complexes. The characterization results suggested 2:1 and 1:1 mole ratio for ofloxacin:amine condensation while 1:1 and 1:2 ratio was found for metal:ligand combinations. The novel compounds presented promising to excellent antimicrobial activity on the tested panel of microbes which is a positive breakthrough toward providing a lead to attainment of more improved antimicrobial agents.

COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

HANP designed, collected, processed data and wrote the article; NPN was instrumental in the conception and design of the research and supervised the whole work to its success; HSB supervised the antimicrobial aspect of the research; GM contributed to the design, participated in analysis of the noble products obtained and immensely facilitated the publication in this reputable journal; AAO read the manuscript several times and made relevant inputs as Co-Supervisor; and HG was

active part of the laboratory works and also participated in article writing.

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REFERENCES

Al-Bayati RIH, Mahdi FR, Ahmed AHA. 2010. Synthesis, Spectroscopic and Antimicrobial Studies of Transition Metal Complexes of N-amino Quinolone Derivatives. 14th International Electronic Conference of Synthetic Organic Chemistry, (ELSOC-14): pp. 1-30.

Aliyu HN, Ado I. 2010. Studies of Mn (II) and Ni(II) Complexes with Schiff base derived from 2-amino benzoic acid and Salicylaldehyde. *Bayero J. Pure Appl. Sci.,* **3**(1): 9-16. DOI: https://doi.org/10.4314/bajopas.v3i1.58803

Akpa SJ, Zoakouma RSP, Say VM, Ouattara M, Kone MW, Sissouma D, Adjou A. 2016. Synthesis and in vitro antifungal evaluation of 2-thioalkylaryl-benzimidazoles derivatives against Candida albicans. *Int. J. Biol. Chem. Sci.,* **10**(3): 1403-1412. DOI: https://dx.doi.org/10.4314/ijbcs.v10i3.38

Anacona JR, Toledo C. 2001. Synthesis and Antibacterial Activity of Metal Complexes of Ciprofloxacin. *J. Transit. Met. Chem.,* **26**(1): 228-231. DOI: https://doi.org/10.1023/a:1007154817081

Anand SP, Doss A, Nandagopalan V. 2011. Antibacterial Studies of *Clitoria ternatea Linn.* A high Potential Medicinal Plant. *Int. J. Appl. Biol. Pharm. Tech.,***2***:* 453456. DOI: http://www.ijabpt.com/pdf/15063Doss%20andro%5B1%5D.pdf.

Chohan ZH. 2001. Synthesis, Characterization and Biological Properties of Bivalent Transition Metal Complexes of Co (II), Cu (II), Ni (II) and Zn (II) with some Acylhydrazine Derived from Furanyl and Thienyl ONO and SNO donor Schiff base Ligands. *Synth. React. Inorg. Met-Org. Chem.,* **31**(1): 1-16. DOI: https://doi.org/10.1081/sim-100001928

Gangani BJ, Parsania PH. 2014. Ultrasonic Speed and Related Acoustical Parameters of Symmetric Double Schiff Bases Solutions at 308.15K. *J. Chem. Pharm. Res.,* **6**(11): 243-247.

George P. 2008. Mononuclear Metal Complexes with Ciprofloxacin: Synthesis, Characterization and DNA-Binding Properties. *J. Inorg. Biochem.,* **102**(12): 1798-1811. DOI: https://doi.org/10.1016/j.jinorgbio.2008.05.012

Imran M, Igbal J, Iqbal S, Ijaz N. 2007. *In vitro* Antibacterial Studies of Ciprofloxacin-imines and their Complexes with Cu (II), Ni (II) Co (II) and Zn (II). *Turk. J. Biol.,* **31**(2): 67-72. DOI: http://journals.tubitak.gov.tr/biology/issues/biy-07-31-2/biy-31-2-2-0610

Jayashree BS, Kaur M, Pai A. 2012. Synthesis, Characterization, Antioxidant and Anticancer Evaluation of Novel Schiff bases of 2-quinolones. *Elixier Org. Chem.,* **52**: 11317-11322. DOI: http://www.elixirpublishers.com/articles/1352272748_52%20%282012%29%2011317-11322.pdf

Nagajothi A, Kiruthika A, Chitra S, Parameswari K. 2013. Fe (III) Complexes with Schiff base Ligands: Synthesis, Characterization and Antimicrobial Studies. *Res. J. Chem. Sci.,* **3**(2): 35-43. DOI: https://scholar.google.com/scholar?hl

Nakamoto K. 2006. *"Infrared and Raman Spectra of Inorganic and Coordination Compounds:* Handbook of Vibrational Spectroscopy, 2006 - Wiley Online Library Print ISBN: 9780471988472| Online

ISBN: 9780470027325| DOI: 10.1002/0 470027320.

Ndahi NP, Nasiru YP. 2012. Complexes of Cobalt (II), Nickel (II) and Zinc (II) with Schiff bases Derived from 4-anisaldehyde. *Int. J. Pharm. Sci. Res.*, **3**(12): 5116-5120. DOI: http://dx.doi.org/10.13040/IJPSR.0975-8232.3(12).5116-20

Ndahi NP, Pindiga YN, Sandabe UK. 2012. Synthesis, Characterization and Antibacterial Studies of some Schiff base Complexes of Cobalt (II), Nickel (II) and Zinc (II). *Asian J. Biochem. Pharm. Res.*, **1**(2): 2012. https://scholar.google.com/scholar?lookup

Ngassam RFT, Tantse M, Kwetche PRF, Noumi DPN, Kouamouo J, Louodom JS, Kweyang BPT, Kourouma K. 2017. Multicenter study on antibiotic susceptibility/resistance trends in the western region of Cameroon. *Int. J. Biol. Chem. Sci.*, **11**(1): 131-143. DOI: http//dx.doi.org/10.4314/ijbcs.v11i1.11

Obalaye JA, Adediji JF, Adebayo MA. 2011. Synthesis and Biological Activities of Metal Complexes of 2, 5-diamino-1,3,4-thiadiazole Derived from Semicarbazide Hydrochloride. *Molecules.*, **16**(7): 5861-5874. DOI: https://doi.org/10.3390/molecules16075861

Ogunniran KO, Ajanaku KO, James OO, Ajani OO, Nwinyi CO, Allensela MA. 2008. Fe (III) and Co (II) Complexes of Mixed Antibiotics: Synthesis, Characterization Antimicrobial Potential and their effect on Alkaline Phosphatase Activities of Selected Rat Tissues. *Int. J. Physic. Sci.*, **3**(8): 177-182. http://eprints.covenantuniversity.edu.ng/219/

Oladipo MA, Woods JAO, Odunola OA. 2005. Synthesis, Vibrational Spectra and Magnetic Properties of Cobalt (II), Nickel (II) and Copper (II) Complexes of Barbituric Acid. *Sci. Foc.*, **10**(1): 49-52. https://scholar.google.com/scholar?hl

Osowole AA, Kolawole GA, Fagade OJ. 2008. Synthesis, Characterization and Biological Studies on Unsymmetrical Schiff base Complexes of Nickel(II), Copper(II) and Zinc(II) and Adducts with 2,2'- dipyridine and 1, 10 phenanthroline. *J. Coord. Chem.*, **61**: 1046-1049. DOI: https://doi.org/10.1080/00958970701482446

Patel KS, Patel JC, Dholariya HR, Patel VK, Patel KD. 2012. Synthesis of Cu(II), Ni(II), Co(II), and Mn(II) Complexes with Ciprofloxacin and their Evaluation of Antimicrobial, Antioxidant and Anti-tuberclular Activity; *Open J. Met. Sci. Res.*, **2**: 49-59. DOI: https://doi.org/10.4236/ojmetal.2012.23008

Patel NB, Agravate SN, Shaikh FM. 2011a. Synthesis and Antimicrobial Activity of New Pyridine Derivatives. *J. Med. Chem. Res.*, **20**(7): 1033-1041. DOI: https://doi.org/10.1007/s00044-010-9440-0

Patel VK, Patel PR, Nagar AA, Chugh NN, Bendale AR. 2011b. Sonochemical Synthesis and Antibacterial Screening of Ciproflaxacin-imines Metal ion Complexes. *Asian J. Pharm. Sci. Res.*, **1**(2): 91-99. DOI: https://www.researchgate.net/profile/Dr_Nagar2/publication/215485196

Raman N, Kulandaisamy A, Thangaraja C. 2003. Redox and antimicrobial studies of transition metal (II) tetradendate Schiff base complexes. *J. Trans. Met. Chem.*, **28**: 29-36.

Reddy V, Patil N, Angadi SD. 2008. Synthesis, Characterization and Antimicrobial Activity of Cu (II), Co (II) and Ni (II) Complexes with O,N and S donor Ligands. *Europ. J. Chem.*, **5**(3): 577-583. DOI: https://doi.org/10.1155/2008/170631

Sadeek AS, Mohammed SE, Sherif MA. 2014. Spectroscopic Investigation of New Schiff base Metal Complexes. *Int. J. Advan. Res.*, **2**(7): 90-107. http://journalijar.com/uploads/802_IJAR-3239.pdf.

Sadeek SA, EL-Shwiniy WH. 2010. Metal Complexes of the Third generation

Quinolone Antibacterial Drug Sparfloxacin: Preparation, Structure and Microbial Evaluation. *J. Coord. Chem.*, **63**(19): 3471-3482. DOI: https://doi.org/10.1080/00958972.2010.514049

Sadeek SA, Teleb SM, Refat MS, Elmosallamy MAF. 2005. Preparation, Thermal and Vibrational Studies of UO_2 (acac-o-phdn) (L) (L=H_2O, Py, DMF and Et_3N). *J. Coord. Chem.*, **58**(12): 1077-1085. DOI: https://doi.org/10.1080/00958970500122 79

Schiff H. 1864. Mittheilungen aus dem Universitätslaboratorium in Pisa: Eine neue 119. DOI: https://doi.org/10.1002/jlac.1864131011 3 Shrikrishna DT, Dinesh VB, Satish AD, Rajendra PP. 2012. Facile and Efficient Method for Preparation of Schiff bases Catalyzed by Ni $(NO_3)_2.6H_2O$ under room Temperature. *Int. J. Chem. Sci.,* **10**(4):1837-1843. https://scholar.google.com/scholar?lookup

Sridha SK, Saravan M, Ramesh A. 2001. Synthesis and antibacterial screening of hydrazones Schiff and Mannich bases of isatin derivatives. *Eur. J. Med. Chem.*, 36: 615-623.

Sultana N, Arayne MS, Rizvi SBS, Haroon U, Mesaik MA. 2013. Synthesis, Spectroscopic and Biological Evaluation of some Levofloxacin Metal Complexes. *J. Med. Chem.,* **22**(3): 1371-1377. DOI: https://doi.org/10.1007/s00044-012-0132-9

Usman H, Osuji JC. 2008. Phytochemical and *in vitro* Antimicrobial Assay of the Leaf Extract of *Newbouldia Laevis*. *Afri. J. Trad. Compl. Alt. Med.,* **4**(4): 476-480. DOI: https://doi.org/10.4314/ajtcam.v4i4.31240

Verma S, Sirbaiya A, Pandeya S. 2013. Antimicrobial Activity of Schiff base of Ofloxacin. *Asian Journal of Pharmaceutical Research and Development*, **1**(2): 56-64. DOI: http://www.ajprd.com/index.php/journal/article/view/26

Vogler A, Kunkely H. 2000. Photochemistry Induced by Metal-to-Ligand Charge Transfer excitation. *Coord. Chem. Rev.*, **208**(1): 321-329. DOI: https://doi.org/10.1016/s0010-8545(99)00246-5

Waziri I, Ndahi NP, Paul BB. 2013. Synthesis, Physicochemical and Antimicrobial Studies of Co (II), Zn (II) and Fe (III) Mixed Antibiotics Metal Complexes. *J. Chem. Pharm. Res.,* **5**(9): 84-89. https://scholar.google.com/scholar.