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## Synthesis, Characterization and Antimicrobial Studies of Ni(II), Cu(II) and Mn(II) Complexes with Schiff Base derived from Ciprofloxacin and 2-Amino Pyridine

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**Abstract** A novel Schiff base ligand (HL) was successfully synthesized by the condensation of ciprofloxacin with 2-aminopyridine and *in situ* complexed with Ni(II), Cu(II) and Mn(II) chloride. All the complexes are coloured and found to be stable at room temperature and soluble in most polar solvent such as distilled water, ethanol and methanol and slightly soluble in non-polar solvent: chloroform, benzene, n-hexane and acetone. The ligand and its metal(II) complexes were characterized by physicochemical analysis: molar conductance, Infrared spectra and electronic spectra. The hydrogen bonding of the ligand was probed with <sup>1</sup>H NMR spectra. The *in vitro* antimicrobial activity of all the compounds at their different concentrations were screened against four bacterial pathogens, namely: *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* and also on fungal strain; *Aspergillus niger* and *Aspergillus fumigatus*. They showed better activity of antibacterial strain compared with the parent and control drugs but no activity on fungal strain.

**Keywords** Metal complexes, Schiff base ligand, Ciprofloxacin imine, 2-aminopyridine, antimicrobial activity

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### Introduction

The knowledge of complexation is very relevant in chemistry and pharmacy as a means of modifying the pharmacological, toxicological and physicochemical properties of drugs. Some properties such as solubility, energy absorption, conductance, partitioning behaviour and chemical reactivity may be altered in the course of complex formation [1]. Metal complexes with Schiff base as a ligand play an important role in the development of coordination chemistry [2]. Schiff bases are formed typically by the condensation of a primary amine and an aldehyde/ketone. The resultant compound,  $R_1R_2C=NR_3$  is called a Schiff base, where  $R_1$  is an aryl group,  $R_2$  is a hydrogen atom and  $R_3$  is either an alkyl or aryl group. They contain an azomethine or imine group. Schiff base of aliphatic aldehyde are relatively unstable and are readily polymerizable while those of aromatic aldehyde having an effective conjugation system are more stable. Imine or azomethine groups are present in various natural and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities [3, 4]. Schiff, a German Chemist, Nobel Prize winner, was the first Scientist who discovered Schiff bases in 1864, hence the ligand was named after him [5, 6]. Schiff base ligands with aldehydes are formed more readily than with ketone (carbonyl carbon). Schiff bases are generally bi, tri, or tetra-dentate chelate ligands and form very stable complexes with metal ions.

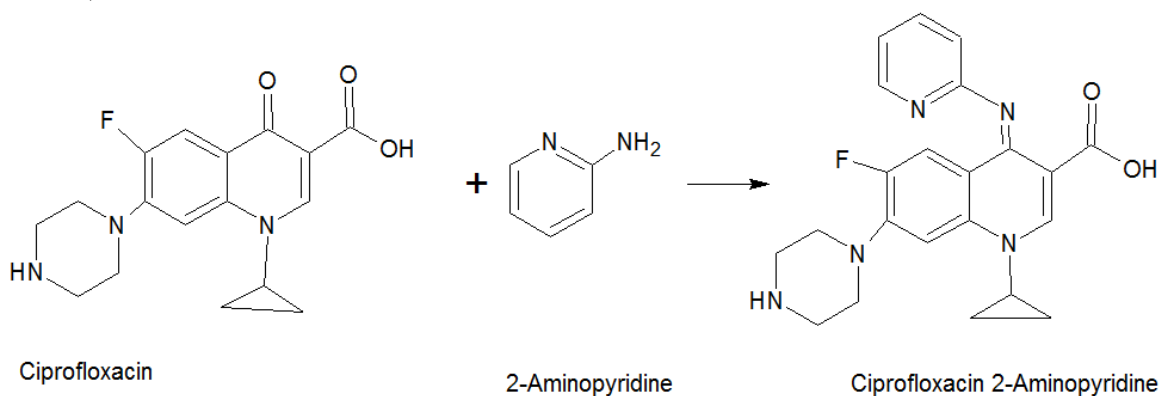


## Materials and Methods

All chemicals used in the synthesis were of analytical reagent grade (AR) and were used without further purification. Ciprofloxacin antibiotics in pure (generic) form, 2-aminopyridine and ethanol were purchased from Sigma- Aldrich through Bristol Scientific Company, Lagos, Nigeria. The metal(II) salts used were nickel(II) chloride hexahydrate, copper(II) chloride dihydrate manganese(II) chloride tetrahydrate, Other reagents and solvents used were acetic acid, methanol, ethanol, chloroform, n-hexane, benzene and acetone were of BDH and Merck products which were of absolute purity. Distilled water was used for all solution preparations.

### Synthesis of the Ligand (Ciprofloxacin-Imines)

The Schiff base ligand (HL) was synthesized with reference to a modification of literature procedures [7-9]. Ciprofloxacin-imine (HL) was prepared by condensation of pure ciprofloxacin with 2-aminopyridine in ethanol. A 20 ml ethanolic solution of ciprofloxacin (1mmol, 0.331g) and a 20 ml ethanolic solution of 2-aminopyridine (1mmol, 0.094g) were slowly mixed with constant stirring. The mixture was refluxed for 4 hours. The resulting solution was concentrated on a water bath and allowed to cool at 0 °C (on ice chips). The whitish solid (HL) formed was filtered, washed with cold ethanol and dried in vacuo over calcium chloride.



### Synthesis of Metal Complexes

A 20 ml hot ethanolic solution of the required metal(II) salt, (1mmol) was mixed with 20ml of the hot ethanolic solution of ciprofloxacin imine ligand (2mmol). The reaction mixture was continuously stirred and refluxed for 4 hours. The resulting complex was cooled, filtered, washed with ethanol and dried in vacuo over calcium chloride.

### Results and Discussion

The physical properties and the analytical data of the ligands and metal complexes are summarized in Table 1. The ligand on interaction with Ni(II),Cu(II) and Mn(II)chlorides yielded complexes corresponding to the general formula  $ML_2$  [9-12]. The low molar conductance values of range (0.006-0.182) of the complexes reveal their non-electrolytic nature. These complexes are more soluble in polar solvent than non-polar solvent. They are coloured and are stable at room temperature. Analysis shows that metal to ligand ratio is 1:2 [10, 13, 14]

### Infrared

The infrared spectra of the free ciprofloxacin imine and the metal complexes were measured as KBr discs (Table 2).In order to study the binding mode of both ligand and metal complexes, the IR of the Schiff base ligand was compared with the spectra of metal complexes. It was observed that the position or intensities of the peaks changed upon complexation. The infrared spectrum of ciprofloxacin 2-aminopyridine shows the absence of the bands attributable to  $\nu(NH_2)$  group of 2-aminopyridine and  $\nu(C=O)$  of ciprofloxacin. Instead, newly formed very strong bands at  $1622\text{ cm}^{-1}$  and  $1541\text{ cm}^{-1}$  which are attributed to  $\nu(C=N)$  stretching vibration were obtained. This suggests the complete condensation of the amino groups with keto group [15-17]. The IR spectra of the complexes revealed



characteristics changes as compared with the spectrum of the ligand confirming complexation. Its complexes showed shifts to higher frequencies between 1626-1638  $\text{cm}^{-1}$  (4-16  $\text{cm}^{-1}$  shift) except in Mn(II) which showed a lower frequency of 1612  $\text{cm}^{-1}$ . The shift in the frequencies revealed coordination of the Schiff base to metal(II) ions through the azomethine nitrogen [13,15, 19] and azine ring of the pyridine [18]. The higher  $\nu(\text{C}=\text{N})$  values observed in the metal(II) complexes indicate the possibility of  $\text{M} \rightarrow \text{L} \pi$  bonding; which increases the bond order and consequently leads to a higher frequency of absorption. The involvement of the pyridine-N in metal coordination depends largely on its position relative to the imine-N [18].

The IR spectra of the ligand (HL) showed bands in the region 1725-1730  $\text{cm}^{-1}$  assignable to the COOH group. The absence of these bands in the metal complexes reveals the deprotonation of the -COOH group on complexation [9]. The IR spectra of the ligand (HL) showed a broad band at 3438  $\text{cm}^{-1}$  due to the stretching vibration of carboxylic hydroxyl, the broadness is due to intermolecular hydrogen bonding between the -COOH groups and the azomethine group [20]. In the complexes, the broad band at 3438  $\text{cm}^{-1}$  shifted to lower frequencies between 3401-3428  $\text{cm}^{-1}$  except in the Cu(II) complex which shifted to higher frequency of 3544  $\text{cm}^{-1}$ , which is an indication of coordination of the hydroxyl group with the metal ion.

The bands at 542  $\text{cm}^{-1}$  - 624  $\text{cm}^{-1}$  which is assignable to  $\nu(\text{M}-\text{N})$  are absent in the spectra of the (HL) ligand. The band in the range of 420-506  $\text{cm}^{-1}$  are also absent in the spectra of the (HL) ligand, assignable to  $\nu(\text{M}-\text{O})$ . These non-ligand spectra bands are indicators to the possible coordination of the azomethine nitrogen, azine nitrogen and the carboxylate oxygen to the metal ions [14,20].

From the spectroscopic data (HL) behaves as tridentate ligand in complex formation through the azomethine -N, pyridinium (azine) -N and O-atom of the hydroxyl group [14,18].

### Electronic Spectra

The electronic absorption spectra of the Schiff base ligand (HL) in methanol are shown in Table 3. In (HL), two bands appeared at 282 nm (35461  $\text{cm}^{-1}$ ) and 320 nm (31250  $\text{cm}^{-1}$ ). The bands are assigned to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions respectively. However, comparing the electronic absorption spectra of the metal(II) complexes with the Schiff base ligand, there are observed shifts in the spectra. These shifts indicate coordination of the metal(II) ion to the Schiff base ligand. The shift to longer wavelength (bathochromic shift) together with colour change authenticates complex formation [9].

The Ni(II) complex showed two bands in the UV region at higher frequencies at 284 and 330 nm (30303 and 35461  $\text{cm}^{-1}$ ) These transitions in the UV region correspond to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  [18]. Comparing the electronic spectra of the Ni(II) complexes with those of the Schiff base ligands, there are observed shifts in the spectra. Ni(HL)<sub>2</sub> made a shift from 320 nm to 330 nm. The shift indicates coordination of the Ni(II) to the Schiff base ligand [9]. The electronic spectra of Cu(HL)<sub>2</sub> has two outstanding intense bands at 284 and 329 nm (35211 and 30395  $\text{cm}^{-1}$ ). Cu(II) complex indicated shifts to longer wavelength; a bathochromic shift. These shifts are indications of complex formation and hence the transitions are assigned;  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  respectively [16]. Mn(HL)<sub>2</sub> showed bands at 280 and 330 nm (35714 and 30303  $\text{cm}^{-1}$ ), hence there are shifts which are indications of coordination. The transitions are assigned to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$ .

The <sup>1</sup>H NMR spectra of the Schiff base ligand (HL) recorded in MeOD against tetramethylsilane (TMS) as internal reference are presented in Table 4. The <sup>1</sup>H NMR spectrum of the ligands (HL) revealed a singlet signal at  $\delta$  3.15-3.50 ppm which is assigned to the methyl group (CH<sub>3</sub>) of the MeOD. The signal due to cyclo propane -CH<sub>2</sub>CH<sub>2</sub>-cyclo was observed at 1.15 ppm as multiplet while signals appearing in the range  $\delta$  7.7-8.0 ppm are attributable to aromatic proton (Ar-H) [16]. The singlet signal appearing in the range  $\delta$  8.88 ppm is attributable to amine proton which are implicated in hydrogen bond interaction [21].

Based on the above results, the absence of sharp signals between 2.2-2.9 ppm in HL show that there is a deprotonation of the amine group in the formation of the azomethine ring hence a confirmation of the presence of Schiff base.



### Antimicrobial Studies

The *in vitro* biological screening effects of the investigated compound were tested against four bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* and also on two fungal strains; *Aspergillus niger* and *Aspergillus fumigatu* (Table 5). The minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC) value were also investigated. The results suggest an enhanced antibacterial activity of the ligand (HL) and metal complexes. The ligand bears activity even greater than the parent drug; ciprofloxacin and control drug; ketoconazole at higher concentrations. This is due to interference in the normal cell process of organism caused by the formation of hydrogen bond through the azomethine group with the active center of cell constituents [22]. On the fungal strain assay no activity was shown on the tested ligand and its metal(II) complexes. The metal complexes have increasing activity when compared with the free ligand HL. This may be attributed to the basis of oxidation state, overtone concept and chelation theory [23] which reduces the polarity of the metal ion by partial sharing of the positive charge with donor atom of the ligand (imine and oxygen). According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antimicrobial activity. On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of  $\pi$ -electrons over the whole chelate ring and enhances the lipophilicity of complexes. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [9, 24].

**Table 1:** Physico-analytical Data for Ciprofloxacin Schiff base and its Metal(II) complexes

Compound	Proposed formula/ (F. Weight)	Colour	Conductivity ( $\text{Scm}^2\text{mol}^{-1}$ )	M.P ( $^{\circ}\text{C}$ )	Yield (%)	Metal (%) Found (Cald)
HL	$\text{C}_{22}\text{H}_{22}\text{FN}_5\text{O}_2$ 407.45	White	0.018	149-150	88	-
Ni(HL) <sub>2</sub>	$\text{Ni}(\text{C}_{22}\text{H}_{22}\text{FN}_5\text{O}_2)_2$ 873.61	Light green	0.148	178-180	71	6.75 (6.71)
Cu(HL) <sub>2</sub>	$\text{Cu}(\text{C}_{22}\text{H}_{22}\text{FN}_5\text{O}_2)_2$ 878.61	Light green	0.140	168-170	62	7.10 (7.23)
Mn(HL) <sub>2</sub>	$\text{Mn}(\text{C}_{22}\text{H}_{22}\text{FN}_5\text{O}_2)_2$ 869.84	Dark yellow	0.182	184-185	61	6.10 (6.31)

**Table 2:** Relevant Infrared Spectra of the HL Ligand and its Metal(II) Complexes ( $\text{cm}^{-1}$ )

Compounds	$\nu(\text{OH})$	$\nu(\text{C}=\text{N})_{\text{azo}}$	$\nu(\text{C}=\text{N})_{\text{azi}}$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$
HL	3438b	1626sh	1541sh		
Ni(HL) <sub>2</sub>	3428b	1622	1566sh	552w	420w
Cu(HL) <sub>2</sub>	3544sh	1638m	1554sh	548w	491w
Mn(HL) <sub>2</sub>	3401b	1612sh	1575sh	542w	503m

Where: sh=sharp, m=medium, b=broad, w=weak, s=strong.

**Table 3:** Electronic Spectra of the Ligand HL and its Metal(II) Complexes

Compound	$\lambda_{\text{max}}$ (nm)	Wave Number ( $\text{cm}^{-1}$ )	Assignment
HL	320	31250	$n \rightarrow \pi^*$
	282	35461	$\pi \rightarrow \pi^*$
Ni(HL) <sub>2</sub>	330	30303	$n \rightarrow \pi^*$
	282	35461	$\pi \rightarrow \pi^*$
Cu(HL) <sub>2</sub>	329	30395	$n \rightarrow \pi^*$
	284	35211	$\pi \rightarrow \pi^*$
Mn(HL) <sub>2</sub>	330	30303	$n \rightarrow \pi^*$
	280	35714	$\pi \rightarrow \pi^*$



**Table 4:**  $^1\text{H}$  NMR of the Ligand (Chemical Shift in ppm)

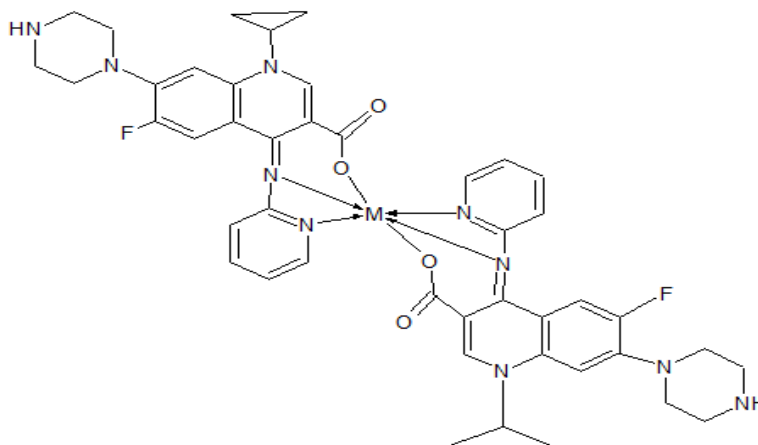
Ligand	Aryl-H $\delta$	Cyclopropane $\delta$ -H	Amine $\delta$ N-H	Solvent MeOD $\delta$ CH <sub>3</sub>
HL	7.7–8.0 (m,5H)	1.2-1.5 (m,5H)	8.85 (s,1H)	3.15-3.50 (s,4H)

Where HL is Ciprofloxacin 2-Aminopyridine, s = singlet, m = multiplet

**Table 5:** The *in vitro* Antimicrobial Activities of the Schiff base Ligand HL and its Metal(II) Complexes

Compound	Conc. ( $\mu\text{g/ml}$ )	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. typhi</i>	<i>E. coli</i>	<i>A. niger</i>	<i>A. Fumigatus</i>
HL	30	36.66 $\pm$ 0.33 <sup>a</sup>	30.00 $\pm$ 0.00 <sup>a,a'</sup>	37.33 $\pm$ 0.33 <sup>a,a'</sup>	30.00 $\pm$ 0.00 <sup>a</sup>	R	R
	20	29.33 $\pm$ 0.33 <sup>b</sup>	24.33 $\pm$ 0.33 <sup>b,b'</sup>	31.33 $\pm$ 0.33 <sup>b</sup>	24.33 $\pm$ 0.33 <sup>b,b'</sup>	R	R
Ni(HL) <sub>2</sub>	30	29.00 $\pm$ 0.00 <sup>b,b'</sup>	31.00 $\pm$ 0.00 <sup>c,a'</sup>	34.66 $\pm$ 0.33 <sup>c,c'</sup>	35.00 $\pm$ 0.00 <sup>c,c'</sup>	R	R
	20	22.66 $\pm$ 0.33 <sup>c,c'</sup>	25.33 $\pm$ 0.00 <sup>d,b'</sup>	28.66 $\pm$ 0.33 <sup>d,d'</sup>	23.33 $\pm$ 0.33 <sup>d</sup>	R	R
Cu(HL) <sub>2</sub>	30	32.00 $\pm$ 0.00 <sup>d</sup>	35.00 $\pm$ 0.00 <sup>e</sup>	36.33 $\pm$ 0.33 <sup>e,a'</sup>	33.00 $\pm$ 0.00 <sup>e,b'</sup>	R	R
	20	25.66 $\pm$ 0.33 <sup>e,c'</sup>	28.33 $\pm$ 0.33 <sup>f</sup>	29.33 $\pm$ 0.33 <sup>f,d'</sup>	25.33 $\pm$ 0.33 <sup>f,b'</sup>	R	R
Mn(HL) <sub>2</sub>	30	29.33 $\pm$ 0.33 <sup>b</sup>	29.66 $\pm$ 0.33 <sup>a,a'</sup>	37.66 $\pm$ 0.33 <sup>a,a'</sup>	39.33 $\pm$ 0.33 <sup>g</sup>	R	R
	20	24.33 $\pm$ 0.33 <sup>f,e'</sup>	24.33 $\pm$ 0.33 <sup>b,b'</sup>	22.66 $\pm$ 0.33 <sup>g</sup>	24.33 $\pm$ 0.33 <sup>h,b'</sup>	R	R
Ciprofloxacin	30	30.00 $\pm$ 0.00 <sup>b</sup>	30.00 $\pm$ 0.33 <sup>a,b',a'</sup>	35.00 $\pm$ 0.00 <sup>k,c',a'</sup>	37.66 $\pm$ 0.33 <sup>h,c'</sup>	-	-
	20	24.33 $\pm$ 0.33 <sup>f,c',e'</sup>	24.33 $\pm$ 0.33 <sup>b</sup>	29.00 $\pm$ 0.00 <sup>f,d'</sup>	30.66 $\pm$ 0.33 <sup>a</sup>	-	-
Ketoconazole	30	-	-	-	-	30.33 $\pm$ 0.00	30.33 $\pm$ 0.00
	20	-	-	-	-	-	-

Different superscripted letters along the same column are significantly (P<0.05) different.



Metal(II) Complex of Ciprofloxacin -2-Aminopyridine

Proposed Structure for the metal(II) complex

Where M is Ni(II), Cu(II) or Mn(II)

## Conclusion

Ciprofloxacin 2-Aminopyridine(HL) ligand and its metal complexes were successfully synthesized. The new compounds were characterized using molar conductance, IR and electronic spectra.  $^1\text{H}$  NMR was used to probe the hydrogen bonding of the ligand. The conductivity measurement suggested that the synthesized compounds are non-electrolytes and are soluble in most polar solvents. The sharp melting point of the ligand and the metal(II) complexes gave an indication that they are probably pure. The analytical data with some physical properties show that the ligand on interaction with Ni(II), Cu(II), and Mn(II) chlorides formed complexes corresponding to the



general formula  $[ML_2]$ . The IR analysis revealed that the Schiff base ligand (HL) behaves as tridentate. The  $^1H$  NMR of the ligand suggests the formation of the azomethine bond. The electronic spectra of the Schiff base ligand (HL) also showed evidences of the non-bonding electrons present on the nitrogen of the azomethine group. Furthermore the Schiff base ligand and the metal(II) complexes were screened *in vitro* for antimicrobial assay. Based on the result, the ligand bears greater potency than the parent drug ciprofloxacin and the control drug ketonazole. In the same vein, the metal(II) complexes were even highly active against all the selected antibacterial pathogen at their higher concentration than the Schiff base. This greater activity is attributed to azomethine linkage and heteroatoms present in these metal(II) complexes. Hence, the prepared metal(II) complexes are clinically relevant for new drug antibiotic agent development.

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