

## Synthesis, Characterization and Antibacterial Activity of a Tridentate Schiff Base Derived from Cephalexin and 1,6-Hexanediamine and its Transition Metal Complexes

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### Abstract

**Background:** The wide use of the antibiotics resulted in the serious medical problem of drugs resistance and public health concern. Preparation of new synthetic derivatives of antibiotics with novel mechanism of action has become an important task to cope with drug resistance problems.

**Objectives:** The goal of the present study is to prepare a family of new metal(II) coordination compounds of Mn, Co, Ni, Cu and Zn with a Schiff base (HL) derived from the condensation of cephalixin antibiotic with 1,6-hexanediamine and to investigate the antibacterial activity of the free cephalixin and the synthesized complexes against gram positive and gram negative strains.

**Methods:** Several physicochemical and spectroscopic methods including elemental and thermal analysis, molar conductance and magnetic susceptibility measurements, electronic, FT-IR, EPR and <sup>1</sup>H NMR spectral and modelling studies, were used to find out the stoichiometry and binding mode of these complexes. Free cephalixin and cephalixin Schiff base complexes have been screened for antibacterial activity by agar diffusion disc method.

**Results:** The Schiff base ligand HL behaves as a monoanionic tridentate NNO chelating agent. The mode of bonding and the geometry of the coordination compounds have been confirmed on the basis of spectral and magnetic data which suggested an octahedral geometry for all the complexes. Based on these studies, the general formulae  $[M(L)(H_2O)_3][PF_6]$  are proposed for the complexes. The characterization methods revealed that the investigated cephalixin exhibited modification in both solubility and antibacterial activity when they interacted with the metal ions under study. The solubility of cephalixin was remarkably decreased in most of the commonly used solvents on its complexation. The antibacterial activity results indicate that while some of the complexes had more bactericidal activity against some bacteria than the free cephalixin, other complexes exhibited less antibacterial activity.

**Conclusions:** The antibacterial activity of cephalixin was affected on complexation with metal ions. It was found that the synthesized novel metal complexes are more toxic than the free cephalixin and exhibited promising bactericidal activity against *E. coli*, *S. aureus* and *K. pneumonia*.

**Keywords:** Antibacterial activity; Antibiotic; Cephalexin; 1,6-hexanediamine; Schiff base; Transition metal complexes

### Introduction

The emergence of resistant human pathogens is a major problem in current antimicrobial therapy, encouraging efforts to develop novel drugs. Metal complexes were found to be particularly useful in this matter, extending the landscape of drug design and enabling novel mechanisms of action [1]. Through these years, Schiff bases have played a special role as coordinating ligands in main group and transition metal coordination chemistry, due to their stability under a wide variety of reductive and oxidative conditions, and to the fact that they are borderline between soft and hard Lewis bases [2,3].

Development of new therapeutic agent Schiff bases is now attracting the attention of medicinal chemist [4]. Several studies have been published regarding the biological activities of Schiff bases, including their genotoxicity [5], antimicrobial [6], antifungal, and herbicidal activities. Schiff bases derived from various carbonyl and amine derivatives were reported to possess mutagenicity [7,8], bactericidal [9], and antifungal activities [10].

The growing interest in transition metal complexes containing Schiff base antibiotics is derived from their functions and well-established chemical in biological systems as well as their pharmaceutical and catalytic applications [11,12]. In particular, the interaction between transition metals and  $\beta$ -lactamic antibiotics such as cephalixin has

been recently investigated [13-15]. Continuing with our metal-based antibiotics studies [16-19], we report herein the preparation and characterization of metal(II) coordination compounds containing a Schiff base ligand derived from the condensation of cephalixin antibiotic with 1,6-hexanediamine. The chemical structure of cephalixin is shown in Figure 1.

### Experimental

#### Materials and methods

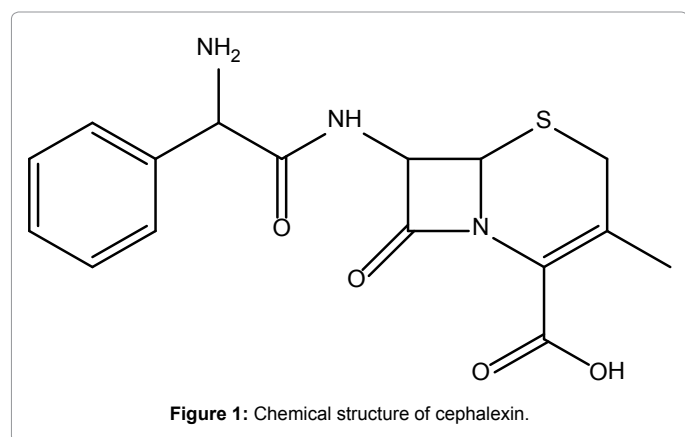
Analytical grade chemicals were used as received without further purification. All necessary precautions were taken to exclude moisture and oxygen during the synthesis and handling of the

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**Received** June 24, 2016; **Accepted** July 04, 2016; **Published** July 08, 2016

**Citation:** Anaconda J, Pineda Y, Bravo A, Camus J (2016) Synthesis, Characterization and Antibacterial Activity of a Tridentate Schiff Base Derived from Cephalexin and 1,6-Hexanediamine and its Transition Metal Complexes. Med chem (Los Angeles) 6: 467-473. doi:10.4172/2161-0444.1000385

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compounds. Fourier transform infrared (FTIR) spectra of the ligand and its coordination compounds as KBr pellets were recorded in the spectral range 4,000-400  $\text{cm}^{-1}$  with a Perkin-Elmer Series 2000 apparatus. Measurements of electronic transitions in the visible and UV regions were taken with a Perkin-Elmer spectrophotometer. The contents of C, H, N and S were analyzed on a LECO CHNS 932 model microanalytical instrument. The metal content of coordination compounds were analyzed with a Perkin-Elmer atomic absorption analyser, after decomposition with a mixture of  $\text{HNO}_3$  and HCl. Molecular weight determinations were carried out by the Rast camphor method. Magnetic susceptibilities were measured on a Johnson Matthey Magnetic Susceptibility balance at room temperature using  $\text{HgCo}(\text{NCS})_4$  as calibrant.  $^1\text{H}$  NMR spectrum was run at 80 MHz on a Varian spectrometer in DMSO against tetramethylsilane (TMS) as internal reference. EPR spectrum was recorded on a Bruker ECS 106 spectrometer operating in the X-band and DPPH free radical was used as the g marker. Thermograms were recorded on a simultaneous thermal analyzer, STA-6000 (Perkin Elmer) instrument at a heating rate of  $4^\circ\text{C min}^{-1}$  up to  $600^\circ\text{C}$ . X-ray powder diffraction patterns for the final solid product of thermal decomposition were recorded on a HZG 4 diffractometer. Measurements were taken over the range of  $2\theta = 2^\circ$ - $70^\circ$  using Ni filtered  $\text{CuK}\alpha$  radiation.

### Synthesis of Schiff base ligand HL

To 1 mmol of cephalaxin in 25 mL of hot ethanol was added 1 mmol of 1,6-hexanediamine. To this solution KOH (0.1% in methanol) was added to adjust the pH between 7.0-8.0 and the reaction mixture was refluxed at  $78^\circ\text{C}$  under nitrogen atmosphere for 2 h and left to stand overnight. The separated yellow Schiff base ligand was filtered off and washed with water, ethanol and ether, and dried under reduced pressure. The product was recrystallized from the same solvent. Yield 75%, mp= $175$ - $176^\circ\text{C}$ . Mol. wt. found (calcd.) 435.7 (444.6).  $^1\text{H}$  NMR (80 MHz, DMSO- $d_6$ ):  $\delta$ =10.21 (s, 1H, COOH), 9.01 (d, 1H, NH), 6.58-8.02 (m, 6H, benzene ring), 5.45 (d, 1H, N=C-CH), 4.97 (d, 2H,  $\text{NH}_2$ ), 4.80 (d, 1H, N-CH), 3.30 (q, 2H, dihydrothiazine- $\text{CH}_2$ ,  $J=16.9$  Hz), 2.46 (s, 1H, phenyl-CH), 1.95 (s, 3H,  $\text{CH}_3$ ), 1.20-3.52 (m, 12H,  $\text{CH}_2$ -) ppm; IR (KBr): 3300, 3200 (N-H), 3085, 3010 (phenyl CH), 2930 (aliph CH), 1710 (C=O, COOH), 1690 (C=O amide), 1635 (C=N imine), 1560, 1475 (benzene ring)  $\text{cm}^{-1}$ .

### General procedure for the synthesis of Schiff base metal complexes

Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes were prepared by the same general method and the synthesis for  $[\text{ZnL}(\text{H}_2\text{O})_3]\text{PF}_6$  complex is given as representative compound. To a hot solution of 1 mmol of the zinc(II) acetate salt in 20 mL of methanol was slowly added

with stirring a solution of 1 mmol of HL in 10 mL of methanol. To this solution KOH (0.1% in methanol) was added to adjust the pH between 7-8 and the mixture was refluxed under nitrogen atmosphere for 3 h. A clear colored solution was obtained. Addition of a concentrated MeOH solution of  $(\text{NH}_4)_2\text{PF}_6$  to the hot solution gave yellow precipitate of the pure product. The zinc(II) complex of HL was separated from the reaction mixture as amorphous solid after volume reduction by evaporation. The solid was filtered off and washed several times with water, methanol and ether and dried under reduced pressure at room temperature. The product was purified by recrystallization from the same solvent.

$[\text{ZnL}(\text{H}_2\text{O})_3][\text{PF}_6]$ : Yield 67%, m.p. (dec.)  $>250^\circ\text{C}$ . Mol. wt. found (calcd.) 731.4 (722.0).  $^1\text{H}$ -NMR (80 MHz, DMSO- $d_6$ ):  $\delta$ =9.01 (d, 1H, NH), 6.58-8.02 (m, 6H, benzene ring), 5.45 (d, 1H, N=C-CH), 5.08 (broad, 2H,  $\text{NH}_2$ ), 4.80 (d, 1H, NCH), 3.30 (q, 2H, dihydrothiazine- $\text{CH}_2$ ,  $J=16.9$  Hz), 2.46 (s, 1H, phenyl-CH), 1.95 (s, 3H,  $\text{CH}_3$ ), 1.31-3.72 (m, 12H,  $\text{CH}_2$ -) ppm; IR (KBr): 3260, 3180 (N-H), 3085, 3010 (phenyl CH), 2930 (aliph CH), 1690 (C=O amide), 1620 (C=N imine), 1590 (COO asym), 1560, 1475 (benzene ring), 1370 (COO sym)  $\text{cm}^{-1}$ .

$[\text{MnL}(\text{H}_2\text{O})_3][\text{PF}_6]$ : Yield 60%, m.p. (dec.)  $>250^\circ\text{C}$ . Mol. wt. found (calcd.) 723.2 (711.6). IR (KBr): 3270, 3185 (N-H), 3085, 3010 (phenyl CH), 2930 (aliph CH), 1690 (C=O amide), 1620 (C=N imine), 1560, 1475 (benzene ring), 1590 (COO asym), 1370 (COO sym)  $\text{cm}^{-1}$ .

$[\text{CoL}(\text{H}_2\text{O})_3][\text{PF}_6]$ : Yield 67%, m.p. (dec.)  $>250^\circ\text{C}$ . Mol. wt. found (calcd.) 703.2 (715.5). IR (KBr): 3270, 3180 (N-H), 3085, 3010 (phenyl CH), 2930 (aliph CH), 1690 (C=O amide), 1615 (C=N imine), 1595 (COO asym), 1560, 1475 (benzene ring), 1370 (COO sym)  $\text{cm}^{-1}$ .

$[\text{NiL}(\text{H}_2\text{O})_3][\text{PF}_6]$ : Yield 65%, m.p. (dec.)  $>250^\circ\text{C}$ . Mol. wt. found (calcd.) 726.1 (715.3). IR (KBr): 3275, 3190 (N-H), 3085, 3010 (phenyl CH), 2930 (aliph CH), 1690 (C=O amide), 1620 (C=N imine), 1590 (COO asym), 1560, 1475 (benzene ring), 1370 (COO sym)  $\text{cm}^{-1}$ .

$[\text{CuL}(\text{H}_2\text{O})_3][\text{PF}_6]$ : Yield 70%, m.p. (dec.)  $>250^\circ\text{C}$ . Mol. wt. found (calcd.) 711.7 (720.1). IR (KBr): 3260, 3180 (N-H), 3085, 3010 (phenyl CH), 2930 (aliph CH), 1690 (C=O amide), 1615 (C=N imine), 1595 (COO asym), 1560, 1475 (benzene ring), 1380 (COO sym)  $\text{cm}^{-1}$ .

### Molecular modeling

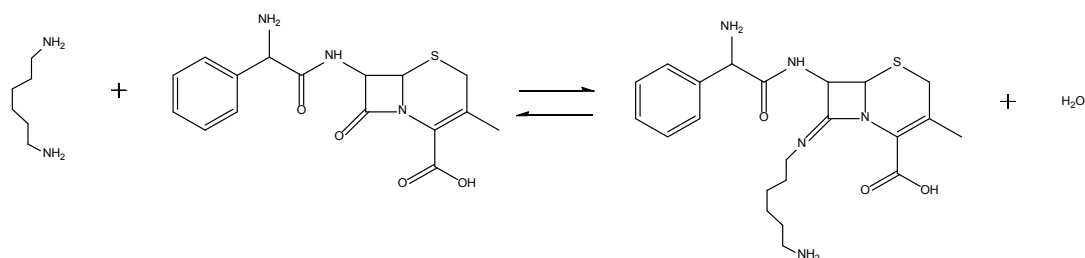
Molecular geometries of a representative compound were optimized with semi-empirical calculations, according to the parameterized number three model by using the GAUSSIAN 09 program package with the aid of the Gauss View visualization program [20].

### Antibacterial activity

Antibacterial activities of cephalosporins and their complexes were tested using the paper disc diffusion method [21]. The chosen strains were G(+) *Staphylococcus aureus* ATCC 25923 and G(-) *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 14990. The width of the growth inhibition zone around the disc was measured after 24 h incubation. Four replicate were made for each treatment and the values recorded are the mean average.

### Results and Discussion

The synthetic route of HL ligand is given in Scheme 1. The ligand and the metal(II) coordination compounds were isolated pure from methanol in very good yields and they are of various colors. The ligand, Zn(II) and Mn(II) solids are yellow while Co(II) product is red wine, and Ni(II) and copper(II) complexes are gray in color. The elemental analyses of the synthesized products are contained in Table 1 and they agree well with a 1:1:3 metal: ligand: coordinated water



**Scheme 1:** Transition coordination compounds with a Schiff base (HL) derived from the condensation of cephalixin antibiotic with 1,6-hexanediamine were synthesized, characterized and screened for antibacterial activity.

0Compound	Found (Calcd.) %						
	C	N	H	S	H <sub>2</sub> O	M <sup>1</sup>	M <sup>2</sup>
[(HL)]	59.6	16.1	6.4	7.2			
[(C <sub>22</sub> H <sub>30</sub> N <sub>5</sub> O <sub>3</sub> S)]	(59.4)	(15.8)	(6.8)	(7.2)			
[Mn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	38.7	10.3	4.9	4.4	7.8	7.5	7.7
[Mn(C <sub>22</sub> H <sub>35</sub> N <sub>6</sub> O <sub>6</sub> S)PF <sub>6</sub> ]	(38.9)	(10.0)	(5.0)	(4.5)	(7.6)	(7.7)	(7.7)
[Co(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	37.5	10.2	4.8	4.3	7.5	8.2	8.5
[Co(C <sub>22</sub> H <sub>35</sub> N <sub>6</sub> O <sub>6</sub> S)PF <sub>6</sub> ]	(37.7)	(10.0)	(5.0)	(4.6)	(7.7)	(8.4)	(8.4)
[Ni(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	37.4	10.3	5.2	4.5	7.9	8.7	8.1
[Ni(C <sub>22</sub> H <sub>35</sub> N <sub>6</sub> O <sub>6</sub> S)PF <sub>6</sub> ]	(37.7)	(10.0)	(5.0)	(4.6)	(7.7)	(8.4)	(8.4)
[Cu(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	37.6	10.2	4.7	5.4	7.5	9.6	9.4
[Cu(C <sub>22</sub> H <sub>35</sub> N <sub>6</sub> O <sub>6</sub> S)PF <sub>6</sub> ]	(37.4)	(9.9)	(5.0)	(4.5)	(7.6)	(9.0)	(9.0)
[Zn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	37.2	9.6	5.1	4.8	7.2	9.5	9.0
[Zn(C <sub>22</sub> H <sub>35</sub> N <sub>6</sub> O <sub>6</sub> S)PF <sub>6</sub> ]	(37.3)	(9.9)	(5.0)	(4.5)	(7.6)	(9.2)	(9.2)

M<sup>1</sup>=Complexometric analysis, M<sup>2</sup>=Thermal analysis

**Table 1:** Analytical and thermoanalytical (TG) results.

stoichiometry for all the coordination compounds. Thus, the general formulae [M(L)(H<sub>2</sub>O)<sub>3</sub>][PF<sub>6</sub>]<sub>n</sub> (M(II)=Mn, Co, Ni, Cu, Zn) have been assigned to the products. All the coordination compounds did not melt/decompose when heated up to 250°C and they are very air stable solids at room temperature without decomposition for a long time. The coordination compounds are insoluble in water and other common organic solvents such as methanol, ethanol, chloroform, acetone, benzene, dichloromethane, acetonitrile, DMF and ether but soluble in DMSO. The molar conductance values measured in DMSO at room temperature vary from 20.0 to 45.0 S cm<sup>2</sup> mol<sup>-1</sup>, revealing the 1:1 electrolytic nature of the coordination compounds [22]. This is supported by the IR spectra where two bands at 850 and 570 cm<sup>-1</sup> characteristic of the non-coordinated hexafluorophosphate anion are present.

### Thermal analysis

The mass loss was calculated from TGA curves for the different steps and compared with those theoretically calculated for the suggested formulae based on the results of elemental analyses and molar conductance measurements. TGA curves also indicated the formation of metal oxide as the end product from which the metal content could be calculated and compared with that obtained from complexometric analyses [23]. Thus, the overall thermogravimetric results are consistent with the formulation of these complexes. Thermograms of the hydrated metal complexes indicate endothermic decompositions in the 130-170°C temperature range assigned to loss of three coordinated water molecules (obs=7.2-7.9%, calc=7.6-7.7) and also reveal that the investigated complexes are stable with no hydration water and solvent molecules. Further heating of unstable anhydrous complexes in air atmosphere as well as in argon causes gradual decomposition and is not possible to distinguish intermediate solid products [23]. The final decomposition step above 200°C includes complete evaporation of the

ligand as well as formation of metal oxide as final product from which the metal content was found to be in very good agreement with the data obtained from EDTA titrations [24]. Their compositions have also been confirmed observing weak diffraction lines by X-ray diffraction measurements (2θ: NiO, 37.27 and 43.30; ZnO, 31, 37, 34.03 and 35.86). The diffraction patterns of obtained residues have been compared with reference patterns.

### <sup>1</sup>H NMR spectra

<sup>1</sup>H NMR spectrum of the Schiff base was recorded in DMSO-d<sub>6</sub>. The values of the chemical shifts obtained were found as to be in their expected region and similar to those of Schiff base ligands reported in the literature [25,26]. The conclusions drawn from this study lend further support to the mode of bonding discussed in its IR spectrum. The number of protons calculated from the integration curves and those obtained from the values of the expected elemental analyses agree well with each other. All the complexes are paramagnetic with the exception of zinc(II) complex, therefore the <sup>1</sup>H-NMR spectra of the complexes could not be obtained.

The <sup>1</sup>H-NMR spectra presented the persuasive confirmation of the coordination modes. Comparison of the <sup>1</sup>H-NMR spectrum of diamagnetic zinc(II) complex with that of the Schiff base, shows the absence of the signal assigned to the COOH proton of Schiff base ligand suggesting deprotonation and indicates the formation of a metal-COO bond [26]. It is also observed that the signals of methylene are broader with decreasing in their intensity and shifted to lower field, indicating that the coordination perturbs the electron density distribution through the imine and amino groups to some extent [26]. Thus, the Schiff base ligand acts as tridentate ligand through the imine, amino and carboxylic groups. Due to their low solubility it was not possible to record satisfactory <sup>13</sup>C-NMR spectrum for the diamagnetic complex.

### Infrared spectra

The assignments of the significant infrared spectral bands of the ligand and its metal complexes are presented in Table 2. The IR spectrum of the Schiff base ligand shows no absorption bands which can be assigned to lactam ν(C=O) vibrational mode coming from cephalixin at 1750 cm<sup>-1</sup>. The absence of such absorption together with the appearance of a new band at 1635 cm<sup>-1</sup> attributed to ν(C=N) vibration, is consistent with the product being the expected Schiff base ligand [27,28]. The infrared spectra of the metal complexes display absorption bands in the 1615–1620 cm<sup>-1</sup> range which can be assigned to the C=N stretching frequencies of the coordinated ligand (HL). The shift of this band on complexation towards lower wave numbers indicates coordination of the azomethine nitrogen to the metal centre [28]. The terminal NH<sub>2</sub> groups of the Schiff base give N–H vibrations at 3300 and 3200 cm<sup>-1</sup>. In the complexes the free amine groups are still present, coordination also occurs through the NH<sub>2</sub> groups, as is confirmed by the shift to lower frequencies in the N–H stretching and

Compound	$\nu\text{C=O}$ lactam	$\nu\text{C=O}$ amide	$\nu\text{C=N}$ imino	$\nu\text{COO}$ asymm	$\nu\text{COO}$ symm	$\Delta\nu$
Cephalaxin	1750	1690				
[HL]		1690	1635			
[Mn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]		1690	1620	1590	1370	220
[Co(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]		1690	1615	1595	1370	225
[Ni(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]		1690	1620	1590	1370	220
[Cu(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]		1690	1615	1595	1380	215
[Zn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]		1690	1620	1590	1370	220

**Table 2:** Main vibrational wavenumbers of the metal complexes (cm<sup>-1</sup>).

bending bands. Also, appearance in the complexes of new  $\nu_{as}$  and  $\nu_s$  modes of the (–COO<sup>-</sup>) group at 1600-1590 and 1390-1380 cm<sup>-1</sup> ranges, respectively, and disappearance of the stretching frequency at 1710 cm<sup>-1</sup> assigned to  $\nu(\text{COOH})$  in the ligand indicates that the Schiff base has reacted. These and the  $\Delta\nu$  value ( $\nu_{as} - \nu_s$ ) >200 cm<sup>-1</sup> are consistent with monodentate coordination for the carboxylate group with the metal atoms. The remaining carboxylate bands, namely  $\gamma(\text{COO})$ ,  $\omega(\text{COO})$  and  $\rho(\text{COO})$ , formerly at 785, 610 and 530 cm<sup>-1</sup>, respectively, also change as a result of coordination. The appearance of new bands attributed to  $\nu(\text{M–N})$  stretching vibrations in the 450-490 cm<sup>-1</sup> ranges, observed in the spectra of the complexes (absent in the Schiff base ligand) provide evidence that the C=N and NH<sub>2</sub> groups could be bonded to the metal ion through the nitrogen atom. The bands in the 350–400 cm<sup>-1</sup> region observed in the complexes, and absent in the free ligand, are tentatively assigned to  $\nu(\text{M–O})$  vibrations. The metal(II) complexes also show bands in the 1420-1460, 1070-1100 and 720-740 cm<sup>-1</sup> ranges which can be assigned to phenyl ring vibrations [27]. Furthermore, medium intensity band appearing in the 2930-2990 cm<sup>-1</sup> region corresponds to aliphatic  $\nu(\text{C–H})$ , while aromatic  $\nu(\text{C–H})$  stretches appear in the 3000-3100 cm<sup>-1</sup> region [28]. The [M(L)(H<sub>2</sub>O)<sub>3</sub>][PF<sub>6</sub>] compounds present a P-F stretching frequency quite broad, centred around 840 cm<sup>-1</sup>, and is typical of compounds having hexafluorophosphate counter-ions [29]. These overall data suggest that the azomethine-N, amino group and carboxylate-O group are involved in coordination and that the Schiff base behaves as a tridentate ligand monoanionic NNO chelating agent.

### Magnetic properties

From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants [30]. The magnitudes of the magnetic moments for the paramagnetic complexes fall within the ranges associated with spin-free high spin ions in octahedral fields, suggesting mononuclear species. The manganese(II) complex has a magnetic moment value of 5.80  $\mu_B$  which is typical of high spin d<sup>5</sup> systems with five unpaired electrons and S=5/2 ground state. The cobalt(II) complex has a magnetic moment of 4.60  $\mu_B$  which is a typical value of a d<sup>7</sup> system with three unpaired electrons indicating a quartet state in an octahedral arrangement around the metal, as compared with the reported values for octahedral complexes of cobalt(II) (4.7–5.2  $\mu_B$ ) [31]. The nickel(II) complex has a magnetic moment of 3.28  $\mu_B$  characteristic of two unpaired electrons and greater than the spin-only value, presumably due to the orbital contribution resulting from the transfer of an electron from the d<sub>x<sup>2</sup>-y<sup>2</sup></sub> orbital to the d<sub>xy</sub> orbital. The complex therefore probably has distorted octahedral geometry. At room temperature a magnetic moment of 1.9-2.2  $\mu_B$  is usually observed for mononuclear copper(II) complexes, regardless of stereochemistry [32]. A magnetic moment of 2.10  $\mu_B$  is observed for the copper(II) compound in the solid state. Zinc(II) complex is diamagnetic as expected for the d<sup>10</sup> configuration.

The room temperature X-band EPR spectrum of the powder sample of [Cu(L)(H<sub>2</sub>O)<sub>3</sub>][PF<sub>6</sub>] was recorded at room temperature. The spectrum exhibits no hyperfine splitting, which may be due to the fact that the paramagnetic center is not diluted. In the present Cu(II) complex, the observed g<sub>||</sub> and g values are 2.25 and 2.08, respectively, which suggest that

the unpaired electron resides in the d<sub>x<sup>2</sup>-y<sup>2</sup></sub> ground state. The observed g<sub>||</sub> value is less than 2.3 indicating considerable covalent character in the Cu–L bonds [33]. The absence of the half-field signal at 1600 G, corresponding to  $\Delta Ms = \pm 2$  transition, ruled out Cu–Cu interaction [34], consistent with mononuclear complexes. In axial symmetry, the g values are related by the expression  $G = (g_{||} - 2)/(g - 2)$ , which measures the exchange interaction between the copper centers in a polycrystalline solid state. The calculated G value for the present complex is 3.12 which suggests no exchange interaction in the solid complex [33-36].

### Electronic spectra

A long-term UV-Vis study was carried out to verify the stability of new complexes in DMSO solution. Compared with ligand, it is significant to note that the absorption wavelengths of new complexes hardly varied for up to 1 month, meaning that new complexes were stable in DMSO solution. The electronic spectra of the Schiff base ligand as well as the complexes in 10<sup>-3</sup> M DMSO solutions showed three broad bands at 310, 330 and 360 nm. The former two bands are assigned to the absorptions (<sup>1</sup>L<sub>b</sub> → <sup>1</sup>A<sub>1</sub>) of the aromatic ring and remain almost unchanged in the spectra of metal complexes, while the third band assigned to  $\pi-\pi^*$  (C=N–) transition of the azomethine group, is shifted to lower wavelength on coordination, confirming the formation of metal complexes and reflecting that azomethine nitrogen is involved in coordination [37].

The spectra of the metal complexes shows that the absorptions around 400-800 nm is due to ligand to metal charge transfer and d-d transition bands of the metal in the complexes [38]. The manganese(II) complex shows a very weak absorption at 360 nm probably due to the coincidence of charge transfer, d →  $\pi^*$ , L → M and intraligand n →  $\pi^*$  transitions [39,40]. The visible region spectrum of the cobalt(II) complex indicates additional two bands at 400 and 460 nm, ( $e_{max} < 100 \text{ M}^{-1} \text{ cm}^{-1}$ ), attributed to metal-ligand charge and <sup>4</sup>T<sub>1g</sub>(F) → <sup>4</sup>A<sub>2g</sub>(F) ( $\nu_2$ ) transition respectively, suggesting octahedral stereochemistry around the metal ion [41].

The UV-Vis spectrum of the nickel(II) complex presents two major absorptions maxima, at 19,230 and 26,315 cm<sup>-1</sup> due to d-d bands which may be assigned, considering that the immediate coordination sphere of the metal is O<sub>h</sub> symmetry, to the transitions <sup>3</sup>A<sub>2g</sub> → <sup>3</sup>T<sub>1g</sub>(F) ( $\nu_2$ ) and <sup>3</sup>A<sub>2g</sub> → <sup>3</sup>T<sub>1g</sub>(P) ( $\nu_3$ ) respectively. The <sup>3</sup>A<sub>2g</sub> → <sup>3</sup>T<sub>2g</sub> transition would be in the near IR region [42].

Ligand field parameters (10Dq, B,  $\beta$ ) and  $\nu_1$  have been calculated for Ni(II) complex by using  $\nu_2$ ,  $\nu_3$  and the procedure of Lever [38]. The results are satisfactory owing to the ratio  $\nu_2/\nu_1$  is found to be 1.60 as required for the octahedral Ni(II) complexes [43]. The values of  $\nu_1 = 12120 \text{ cm}^{-1}$ , the magnetic moment ( $\mu_{spin\ only} < \mu_{obs} \sim \mu_{S+L}$ ) and the calculated ligand field parameters of Dq=1212 cm<sup>-1</sup>, B=587 cm<sup>-1</sup> and  $\beta=0.556$  are also consistent with the octahedral geometry. The low value of Racah parameter (B) for the complexes compared to the free ion value indicates significant covalent character of the metal-ligand bond [44]. The nephelauxetic ratio  $\beta$  also supports covalent character in this complex. The optical absorption spectrum of copper(II) complex shows two bands at 25,640 and 13,890 cm<sup>-1</sup>. The strong band at 25,640 cm<sup>-1</sup> is assigned to charge transfer, mainly of the L → Cu type [38]. The other broad band at 13,890 cm<sup>-1</sup> is characteristic of Cu(II) ion in tetragonally distorted octahedral symmetry. Accordingly this broad band is assigned to <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>B<sub>2g</sub>, which corresponds to 10Dq=13,890 cm<sup>-1</sup>.

### Coordination sites

The Schiff base ligand containing cephalaxin has various potential donor atoms that might be involved in coordination with the transition metal ions. In the present case, the HL ligand containing cephalaxin

has a number of potential donor atoms in various positions which can bind to the metal ions forming multinuclear complexes. Molecular modeling was performed for  $[ZnL(H_2O)_3]^+$  unit as representative compound. The optimized structure of the complex is shown in Figure 2. The complex consists of one unit of ligand molecule with metal ion Zn(II). The complex is of six coordinates with distorted octahedral environment around the metal ion. The metal ion Zn(II) is coordinated to one amine nitrogen (1.95 Å), one azomethine nitrogen atom (1.91 Å) and one carboxylate oxygen atom (1.91 Å) of Schiff base ligand. The three water molecules in cis positions are equidistant from Zn(II) (1.94 Å) and complete the coordination sphere of metal. In most cases, the actual bond angles are close to an octahedral geometry predicting  $sp^3d^2$  hybridization and confirming the proposed structure of the  $[ZnL(H_2O)_3]^+$  ion. We have attempted to grow single crystals of the metal complexes but in no case we have had any success, due to their insolubility in common polar and nonpolar solvents. The complexes only form amorphous materials as revealed by their XRD patterns. Up to now no crystal structures of complexes containing cephalaxin have been reported. These studies represent a contribution to future crystallographic analyses, which are complicated by the difficulties in obtaining X-ray quality crystals of cephalosporins derived complexes.

### Antibacterial activity

Qualitative screening for antimicrobial activities was performed preliminarily using the disc diffusion assay. *In vitro* bactericidal activities were measured from the diameter of clear inhibition zones caused by samples against the same bacteria and under the same experimental conditions. To verify the stability of new complexes in DMSO solution a long-term UV-Vis study was performed. Compared with cephalaxin and Schiff base ligands, it is significant to note that the absorption wavelengths of new metal complexes hardly varied for up to six weeks, meaning that new complexes were stable in DMSO solution. As assessed by colour, the complexes remain intact during biological testing. In order to study the effects of DMSO and metal(II) acetate salts in the biological screening, separate studies were carried out with the solutions alone of DMSO, 1,6-hexanediamine and the free metal salt and they have been found that they have little or no effect on the growth of any microorganisms studied.

The antibacterial activity of Schiff base HL as well as its metal(II) complexes were tested on against G(+) and G(-) microorganisms and compared to free cephalaxin used as reference. Bactericidal activities were appreciated by measuring the growth inhibition zone against test organisms and Minimal Inhibition Concentration (MIC) [45], the values of which are illustrated in Tables 3 and 4 respectively. It was found that the synthesized novel metal complexes exhibited promising bactericidal activity against *E. coli*, *S. aureus* and *K. pneumonia*. Among the Schiff base coordination compounds synthesized, nickel(II) compound possess pronounced bactericidal activity against *S. aureus* and *E. coli* (MIC of 12.5 µg/ml) in comparison with the other compounds. All complexes were most active against *E. coli* with an MIC of 12.5-25 µg/ml which are lower than that of the reference drug cephalaxin. Generally, most of the coordination compounds have shown little or no activity against Gram-negative bacteria (*P. aeruginosa* and *S. epidermidis*) with the exception of cobalt(II) and copper(II) compounds which showed some activity, although without any significant MIC values.

It is known that the chelation tends to increase the antibacterial activity of the ligand. It is observed that, in a complex, coordination could augment the lipophilic character of the central metal atom, which favours further its permeability through the lipid layers of the cell membrane and disturbing the metal binding sites on enzymes of the organism [46]. However, our

results indicate that bactericidal activity must also be influenced by other factors beyond membrane permeability.

The targets for lactam carbonyl antibiotics and related derivatives, are cell wall-synthesizing enzymes (penicillin binding proteins, PBPs) which are found as both cytoplasmic and membrane-bound enzymes and are present in almost all bacteria. They vary from one bacterium to another differing in molecular weight, amount, affinity for antibiotic derivatives and enzymatic function (e.g., carboxypeptidase, transpeptidase, or endopeptidase) [47].

The obtained results shown in Tables 3 and 4 may highlight that the antibacterial activity of cephalaxin Schiff base metal complexes compared to free cephalaxin reflect a different mechanistic pathway by which they react with the PBP active sites to obtain formation of a stable PBP-inhibitor adduct. The level of affinity to metal-based antibiotics is determined by the nature and kinetic properties of the PBPs [47].

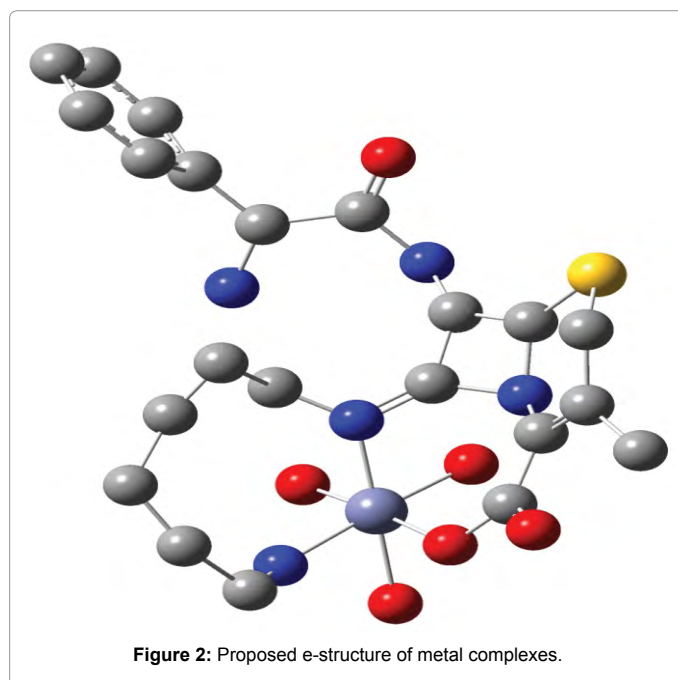
### Conclusions

A Schiff base derived from cephalaxin and 1,6-hexanediamine and its transition metal coordination compounds have been synthesized. The coordination to metal occurs through the carboxylate, C=N and  $NH_2$  moieties. The solubility of the Schiff base antibiotic and its metal

Compound	Zone of inhibition (mm)				
	E.C.	S.E.	S.A	K.P.	P.A.
Cephalaxin	25	22	30	10	6
[HL]	8	6	21	6	6
[Mn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	28	6	33	17	6
[Co(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	25	23	30	11	15
[Ni(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	30	26	32	10	6
[Cu(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	27	31	35	16	26
[Zn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	29	6	30	16	6
Control	8	6	6	6	6

E.Ch *Escherichia coli* ATCC 25922; S.E: *Staphylococcus epidermidis* ATCC 14990; S.A: *Staphylococcus aureus* ATCC 25923; K.P: *Klebsiella pneumoniae* ATCC 700603; P.A: *Pseudomonas aeruginosa* ATCC 27853

**Table 3:** Antibacterial Activity of Test Compounds.



**Figure 2:** Proposed e-structure of metal complexes.

Compound	<i>E. coli</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>
Cephalexin	100	25	100
[Mn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	25	25	100
[Co(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	25	25	100
[Ni(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	12.5	12.5	100
[Cu(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	25	25	100
[Zn(L)(H <sub>2</sub> O) <sub>3</sub> ][PF <sub>6</sub> ]	25	50	100

**Table 4:** Antibacterial Activity of Test Compounds (MIC µg/ml).

complexes in common organic solvents and water is reduced on complexation. The Schiff base metal complexes have higher bactericidal activity than the uncomplexed cephalixin against *E. coli* showing that they have a good activity as bactericides.

#### Conflict of Interest

None of the authors of the above manuscript has declared any conflict of interest which may arise from being named as an author on the manuscript.

#### Acknowledgements

The authors express their sincere thanks to Comision de Investigación from the Universidad de Oriente and Universidad de Playa Ancha for financial support.

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