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Co (II), Ni (II) and Cu (II) Complexes with Schiff Base Ligand: Syntheses, Characterization, Antimicrobial Studies and Molecular Docking Studies

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Abstract

This paper is an analysis about the Schiff base ligands and its Co (II), Ni (II) and Cu (II) complexes. In the past few decades there has been an increased interest in this area. Synthesis of Schiff base can be done in many ways, but the most common method is the nucleophilic attack of primary amines on the carbon of aldehydes or ketones. The end result of this reaction is a compound in which C=O is replaced by a C=N. This study reported the synthesis of the new ligands, (E)-4-((5-benzoyl-2-((E)-(2-hydroxybenzylidine) amino) phenyl) amino) pentan-2-one. These Schiff base type ligands were respectively obtained by means of the reaction of Salicylaldehyde, 3, 4-diaminobenzophenone and acetyl acetone at molar ratio 1:1:1. The respective Co (II), Ni (II) and Cu (II) complexes were synthesized by the reaction of Ligand with metal nitrate hexa hydrate, at molar ratio 1:1. The chemical identification of ligands and respective complexes was established through spectroscopic data (FT-IR, UV-Vis, ^{1}H NMR and ^{13}C NMR). Macro cyclic complexes are non-electrolytic nature as their molar conductivities (ΛM) in DMSO of 10^{-3} M solution from the EPR study the complexes proposed to be octahedral geometry. The complexes have been tested against gram positive and gram negative bacteria using agar well diffusion method. It was found that compounds show different activity of inhibition on growth of the bacteria and finally the biological importance of the synthesized ligands are assessed by performing docking studies using Auto Dock VinaPyRx software.

Keywords: Schiff base; EPR study; Antimicrobial Activity; Docking study;

Introduction

Transition metals are metallic elements that have an incomplete d or f shells in the neutral or cationic states. These incomplete valance shell orbitals allow it to accept electrons from Lewis bases to form coordination complexes very easily compared to other group of elements. Ligands therefore must be a Lewis base. They must contain at least one pair of non-bonding electrons that can be donated to a metal ion. The Schiff base is named after Hugo Schiff and is a compound with a functional group that contains a C-N double bond with the nitrogen connected to an aryl or alkyl group. Schiff bases in a broad sense have the general formula $R^1R^2C=NR^3$, where R is an organic side chain. Schiff base is synonymous with azomethine and may also be referred to as imines.

Recently, Schiff bases are used as intermediates for the synthesis of amino acids or as ligands for preparation of metal complexes having a series of different structures [1]. Schiff base compounds and their metal complexes are very important as catalysts in various biological systems, polymers, dyes and medicinal and pharmaceutical fields [2,3] they comprise miscellaneous therapeutically potent applications in the field of medicinal chemistry [4].Their use in birth control, food packages and as an O_2 de-tector is also outlined [3]. They have been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, an-timalarial, anti-inflammatory, antiviral, and antipyretic properties [5,6].

A variety of Schiff's base and its complexes have been studied extensively. Several mo¬del systems, including those with bidentate, tridentate, tetradentate, multidentate Schiff base ligands, and their coordination chemistry of cobalt (II), Nickel (II) and copper (II) attracts much attention because of its biological relevance and its own interesting coordination chemistry such as geometry, flexible redox property. In this paper to discuss the synthesis, spectroscopic and antimicrobial studies of Schiff's base and its cobalt (II), nickel (II) and copper (II) complexes. The structures of the compounds are characterized by using IR, UV, NMR, ESI mass and EPR spectroscopic techniques.

Experimental

All the chemicals used were of analytic grade, and were purchased from Sigma-Aldrich. Metal salt were purchased from E. Merck and were used as received. All solvents used were of standard/spectroscopic grade.

Synthesis of Ligand (L)

Ligand (L) was synthesized by refluxing a hot ethanolic

__Corresponding author email:__ profelangoggactvm@gmail.com solution of Acetyl acetone, Salicylaldehyde. They were mixed slowly with constant stirring. To the above mixture was added an ethanolic solution of 3, 4-diaminobenzophenone. Their molar ratio is 1:1:1 temperature was maintained at 70° C for 2.30 h in the presence of Concentrated HCl. On cooling the content overnight at 0°C an off white crystalline compound was separated out. This was filtered, washed several times with ethanol and dried in a vacuum desiccator over anhydrous calcium chloride. Yield 62%, m.pt. 238°C.

Synthesis of complex

Hot ethanolic solution of ligand and hot ethanolic solution of a given metal salt (cobalt nitrate, nickel nitrate and copper nitrate hexa hydrate) in 1:1 molar ratio were mixed together with constant stirring. The reaction mixture was refluxed at 70° C for 2.30 h. The volume of the reaction mixture was reduced to 20-25%. The precipitate that formed was filtered off and washed with ethanol and dried under vacuum over anhydrous $CaCl_2$. These are insoluble in water, chloroform, carbon tetrachloride, acetonitrile and partially soluble in ether, alcohol but freely soluble in DMF and DMSO.

Physical methods

The infrared spectrum was recorded using KBr pellets in the range 4400-400 cm1. UV-Visible spectra of the ligand and the complexes were recorded on Perkin Elmer Lambda 3B UV-Visible Spectrophotometer in the range 200-900 nm. The molar conductance of the ligand and the complexes were measured using 10-3M solution of DMSO at 25°C using an Elico CM-180 Conductivity meter and Elico type CC-03 Conductivity cell of cell constant 1.05 cm^{-1}.The ^{1}H&^{13}C NMR spectra of the ligand and complex was recorded in Joel 500 MHz NMR spectrometer using $(CD_3)_2SO$. The mass spectra of the complexes were recorded by JEOL GC mate Mass Spectrophotometer. Magnetic susceptibility was measured at room temperature on a Gouy balance using $CuSO_4.5H_2O$ as a callibrant. The EPR spectra of the complex were recorded as crystalline sample and in the solution of DMF at room temperature on JEX-X3 Series of a system using the DPPH as the g-marker. Antimicrobial activity was tested by using agar well diffusion method and molecular docking studies were record using Auto Dock VinaPyRx software.

Result and discussion

Ligand

The purity of the ligand (L) 98.62% has been checked by HPLC. The ESI-mass spectrum (Figure 2) of the ligand (L) shows a parental ion peak (M+) m/z = 398. The spectrum exhibit a peak at m/z = 356 is due to $C_{23}H_{20}N_2O_2$ caution. A peak with high intensity is present at m/z = 315 (85%) is due to base peak. This peak is corresponding to cationic species with three aromatic rings. The other peaks at 271, 237, 220, 179, 157, 119, 96, 82 and 59 are corresponding to other fragments.

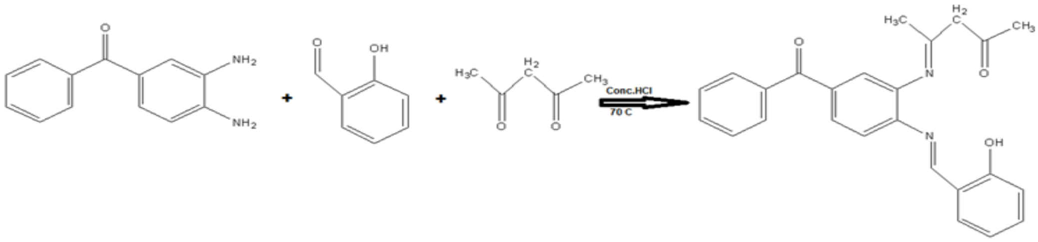

Figure 1: Proposed structure of Ligand

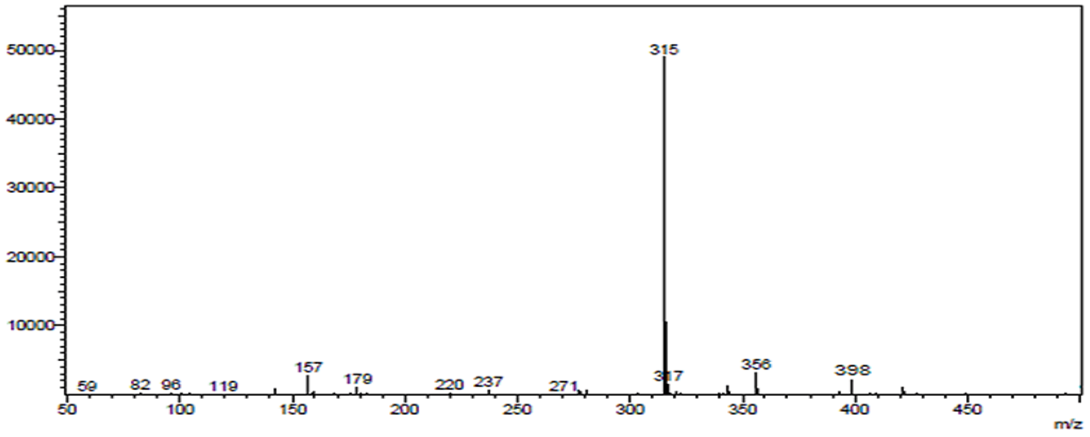

Figure 2: ESI Mass spectrum of Ligand (L)

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The FTIR spectrum of the ligand (Figure 3) shows at 1607 cm^{-1} corresponding to the ν(C=N) stretching vibration and 3291cm^{-1} phenolic hydroxyl group. IR spectra of the ligand showed band at 1287 cm^{-1} which is ascribed to the stretching vibration of the phenolic aromatic oxygen [7]. The bands at 2671 cm^{-1} and 3070 cm^{-1} are corresponding to C-H stretching of aromatic ring [8].

The electronic spectrum of the Schiff base ligand (Figure 4) shows mainly two absorption bands at 252 and 345 nm. The first band arise from π-π* transition with the azomethine chromospheres [7].The second band at 350 nm is due to the n-π* transition which is overlapping with the intermolecular CT from the phenyl ring to the azomethine group. On the complexation the absorption bands undergo a bathochromic shift compared to the free ligand as a result of coordination via the nitrogen atoms of the azomethine group.

In the 1H NMR spectrum of ligand (Figure 5) exhibit signals at 2.50 ppm due to CH_2-CN,3.39 ppm due to –CO-CH_2, 2.11 ppm due to CH_3-C,[8]8.10 ppm due to Ar-CH=N [9]A sharp multiplet signals7.03 – 7.81 ppm due to Ar-H. A singlet corresponding to one proton observed at 12.80 ppm is due to Ar-OH. As well as in the ^{13}C NMR spectrum of ligand (Figure 6) indicated new resonance are 19.05(C-CH_3), 56 (C-CH_2-CO), 113-138(C=C), 158.45(C=N), 195.95(Ph-CO-Ph) [14].

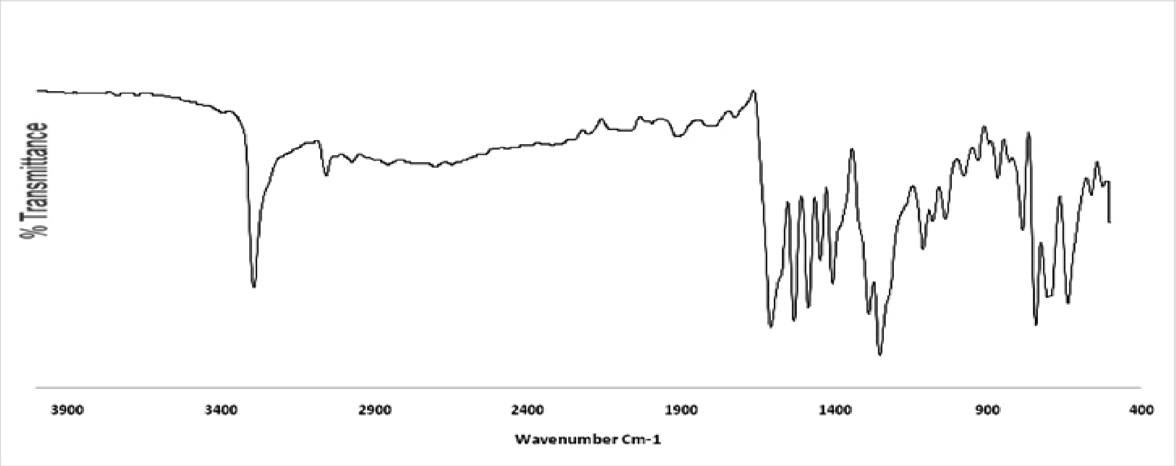

Figure 3: IR spectrum of Ligand (L)

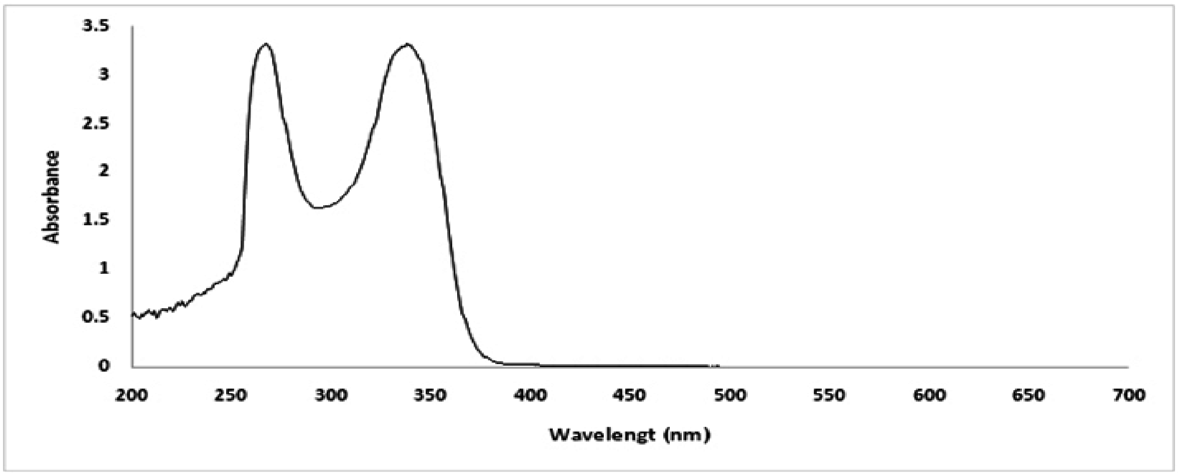

Figure 4: Electronic spectrum of Ligand (L)

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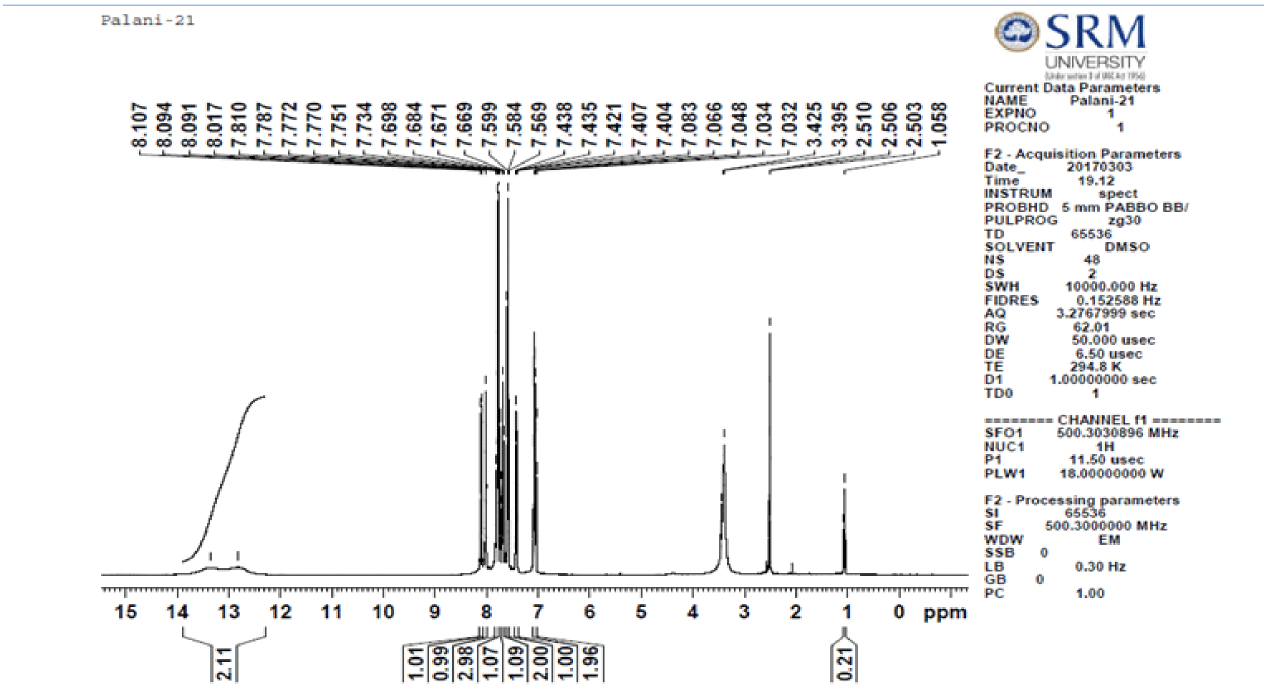

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FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 62.01
DW 50.000 usec
DE 6.50 usec
TE 294.8 K
D1 1.00000000 sec
TD0 1

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NUC1 1H
P1 11.50 usec
PLW1 18.00000000 W

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GB 0
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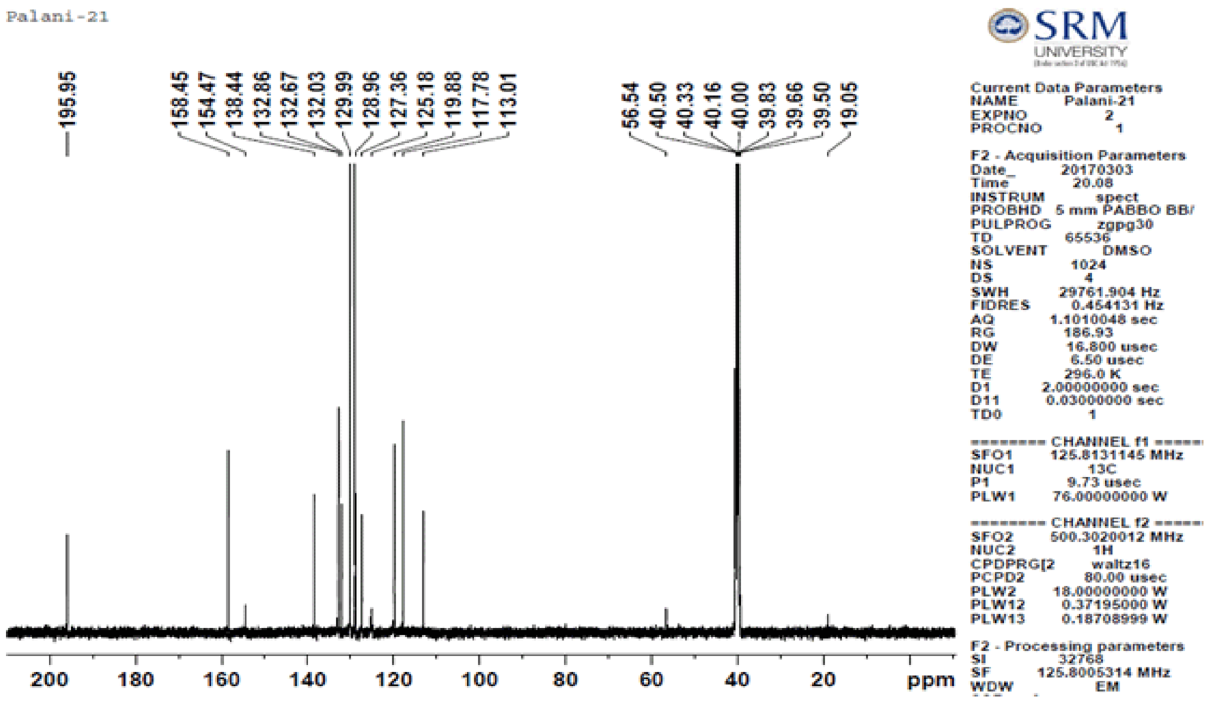
Figure 5: ^{1}H NMR spectrum of ligand (L)



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DE 6.50 usec
TE 296.0 K
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D11 0.03000000 sec
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PLW13 0.18708999 W

F2 - Processing parameters
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WDW EM

Figure 6: ^{13}C NMR spectrum of ligand (L)

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Metal Complexes

On the basis of molar conductance measurements (Table 1) of the complexes in DMSO corresponds to be non-electrolytic nature of the complexes. Thus the complexes may be formulated as [M (L)(NO$_3$)$_{-2}$].xH2O where M=Co(II), Ni(II) and Cu(II) and L is (E)-4-((5-benzoyl-2-((E)-(2-hydroxybenzylidine)amino)phenyl)imino) pentan-2-one.

Table 1: Molar conductance and Electronic Spectroscopic Data of the Schiff Base Ligand and its metal complex

Compound	**Molar conductance $\Omega^{-1}cm^2mol^{-1}$**	**Colour**	**M.P(°C)**	**Yield (%)**	Λmax **(nm)**
Ligand(L)	-	Off white	237	68	252,345
[Co(L)(NO$_3$)$_2$].xH$_2$O	16.23	Brown	> 300	48	268,345,437,514,776
[Ni(L)(NO$_3$)$_2$].xH$_2$O	9.80	Brown black	> 300	45	258,335,437,550,776
[Cu(L)(NO$_3$)$_2$].xH$_2$O	10.54	Dark brown	294	50	285,367,431,660,730

Infra-red spectral bands due to anion

A comparative study of the FTIR spectra of ligand and its metal complexes discloses that some peaks are common and therefore, only important peaks, which have either shifted or have newly appeared, are discussed. (Table 2) shows that ν(C-O) and ν(C=N) modes appear at 1287-1327cm^{-1} and 1607-1618cm^{-1} respectively. The shifting of (C-O) to higher frequency as compared to the ligand (1287cm^{-1}) is owing to the conversion of hydrogen bonded structure into a covalent metal bonded structure. Metal-ligand bond is further confirmed by the appearance of a medium intensity band in the range 474-478 and 522-536cm^{-1} in the spectra of the complexes allotted to stretching frequencies of (M-N) bond and (M-O) bond formation respectively [10]IR spectra of the nitrate complexes (Figure 7(a-c)), display three medium intensity bands due to (N-O) stretching in the region ~1382–1384 cm^{-1}, suggesting that both the nitrate groups are coordinated to the central metal ion [11].

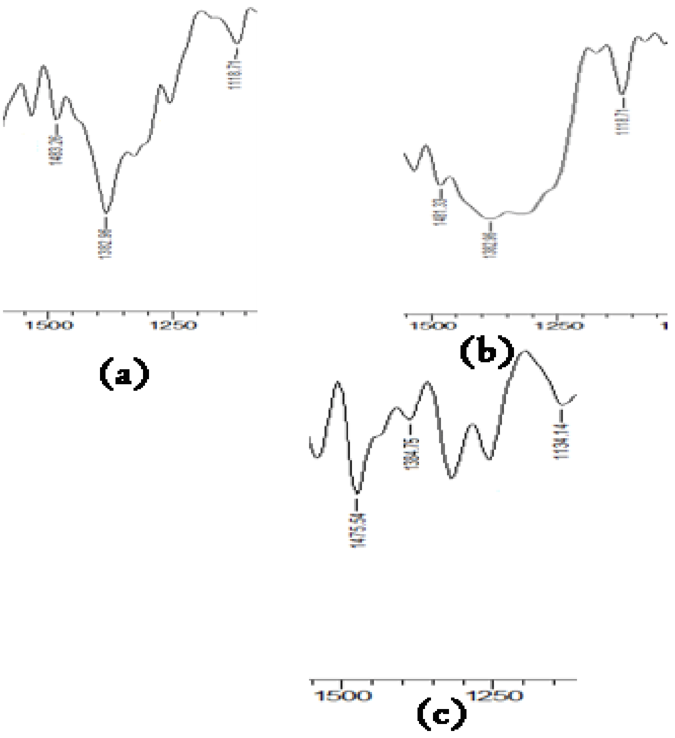

Figure 7: IR spectral bands of anions **(a)** [Co (L) (NO$_3$)$_2$].xH$_2$O; **(b)** [Ni (L) (NO$_3$)$_2$] .xH$_2$O; **(c)** [Cu (L)(NO$_3$)$_2$].xH$_2$O

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Table 2: Infrared Spectroscopic Data of the Schiff Base Ligand and its metal complex

Compound	*ν (C=N)*	*ν (C-O)*	*ν (M-N)*	*ν (M-O)*	**Ionic nitrate**
Ligand(L)	1607	1287	-	-	-
$[Co(L)(NO_3)_2].xH_2O$	1616	1327	476	536	1382
$[Ni(L)(NO_3)_2].xH_2O$	1618	1323	478	534	1382
$[Cu(L)(NO_3)_2].xH_2O$	1610	1317	474	522	1384

Cobalt (II) complex

The magnetic moment measurement of the cobalt (II) complex at room temperature lies in the range 4.98 –5.01 B.M. The electronic spectrum of Co (II) complex show three bands (Figure 8). These bands may be assigned to $^4T_1g \rightarrow {}^4T_2g$ (F) (ν_1), $^4T_1g \rightarrow {}^4A_2g$ (F) (ν_2) and $^4T_1g \rightarrow {}^4T_1g$ (P) (ν_3) transitions, respectively [12,13]. In the 1H NMR spectrum of cobalt (II) complex (Figure 9) exhibit A sharp multiplet signals 7.09 – 7.76 ppm due to Ar-H. A singlet corresponding to one proton observed at 12.00-13.00 ppm is due to Ar-OH. [8, 9] As well as the13C NMR spectrum (Figure10) agree with the expected absorptions indicated new resonance are 47.86 (C-CH_2-CO), 113-138(C=C), 159.00 (C=N), 195.64(Ph-CO-Ph) [14].

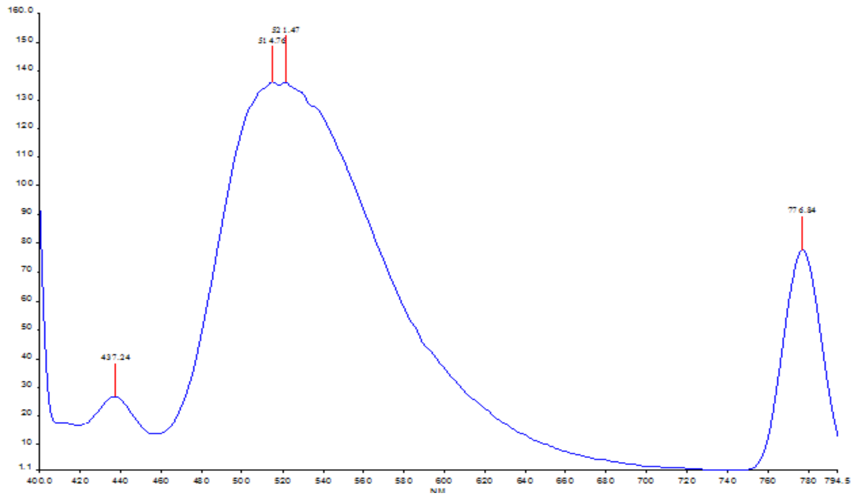

Figure 8: Electronic spectrum of Co (II) complex

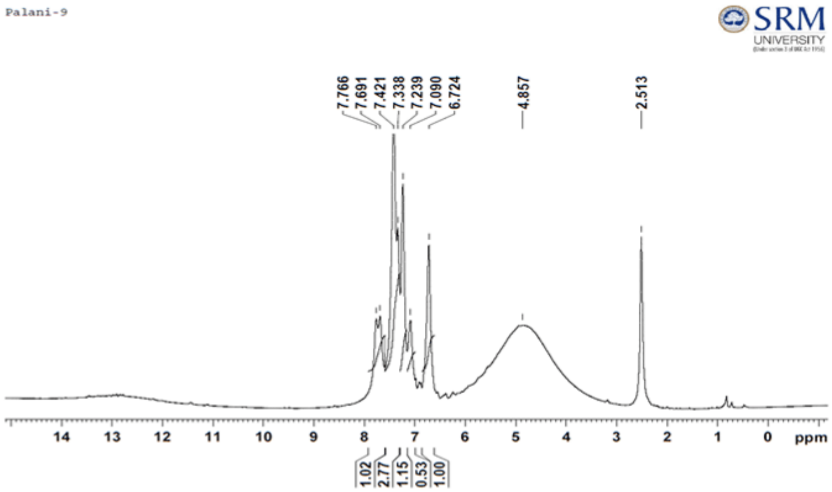

Figure 9: ^{1}H NMR spectrum of Cobalt (II) Complex

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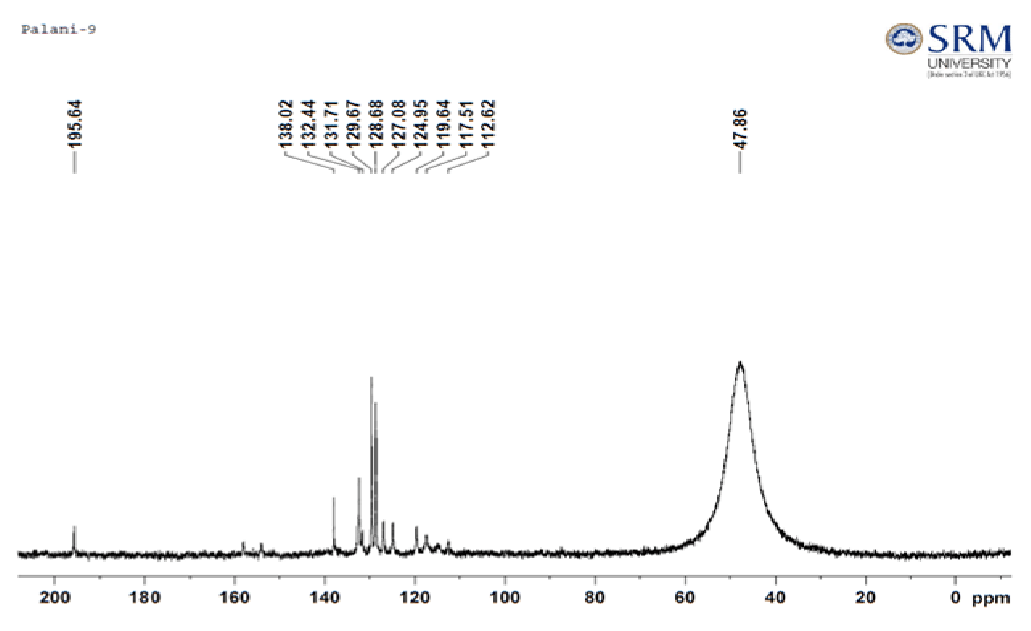

Figure 10:^{13}CNMR spectrum of Cobalt (II) Complex

Nickel (II) complex

The magnetic moment of the Ni(II) complex at room temperature lie in the range 2.96-2.98 B.M.[15]These values show the presence of octahedral configuration. These complex display three electronic spectral bands (Figure 11) at 776, 570 and 437nm assignable to $^3A_2g\rightarrow{}^3T_2g$ (F) (ν_1), $^3A_2g\rightarrow 3T_1g$ (F) (ν_2) and $^3A_2g\rightarrow{}^3T_1g$ (P) (ν_3) transitions, respectively. These bands indicate that the complex have also confirm an octahedral geometry and might possess D_{4h} symmetry [12].The 1H NMR and ^{13}C NMR spectrum shown in the (figure12, 13). These signals also confirm the hydrogen and the Carbon environment of the complex.

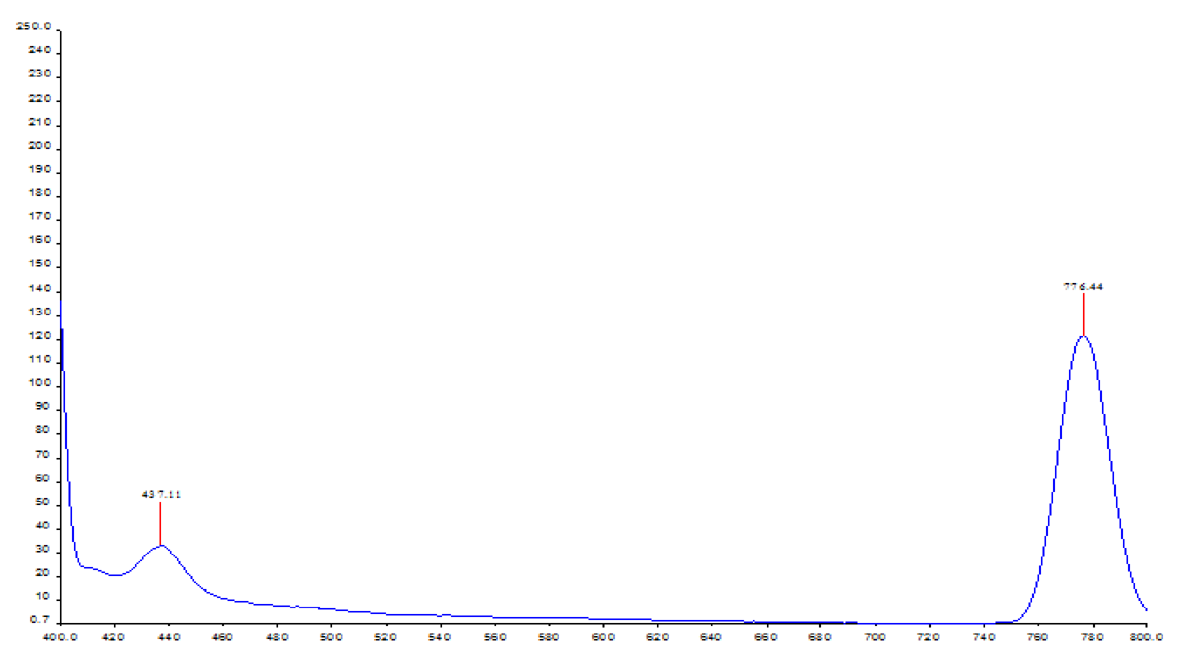

Figure 11: Electronic spectrum of Ni (II) complex

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Table 3: Diameter of zone inhibition (mm) for the Schiff base ligand and its complexes

Microorganism	Escherichia coli			Staphylococcus			Enterococci			Pseudomonas		
	25	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**
Ligand(L)	-	5mm	9mm	8mm	10mm	11mm	4mm	7mm	13mm	-	-	-
$[Co(L)(NO_3)_2].xH_2O$	-	8mm	10mm	-	-	-	5mm	7mm	9 mm	-	4mm	9mm
$[Ni(L)(NO_3)_2].xH_2O$	-	5mm	8mm	6mm	8mm	10mm	7mm	9mm	12mm	-	-	7mm
$[Cu(L)(NO_3)_2].xH_2O$	-	-	-	-	-	-	-	-	5 mm	-	-	4mm

Ligand/Complex	Binding Affinity (kcal/mol)
Ligand	-7.9
Copper (II) complex	-9.0
Nickel (II) complex	-9.5

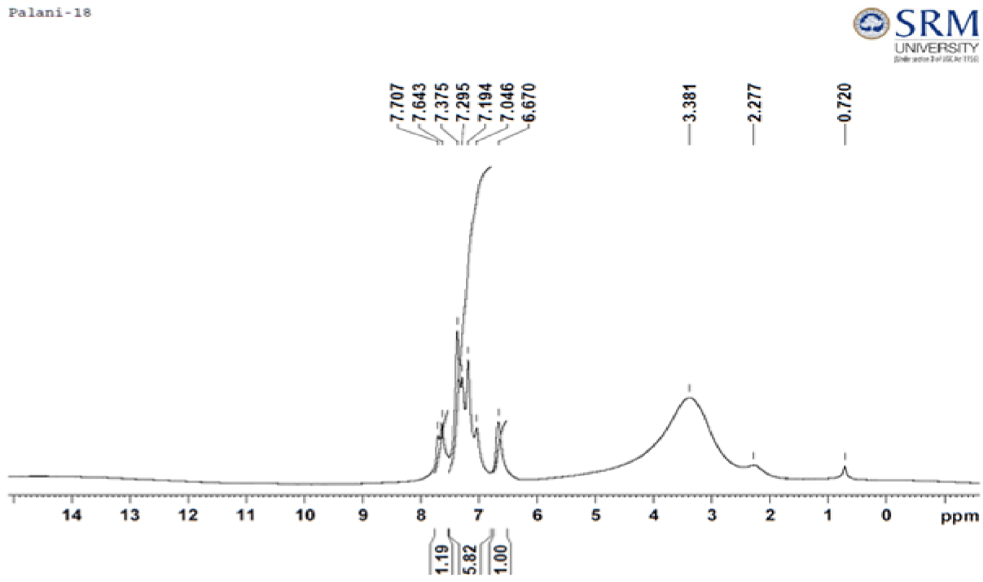

Figure 12: ^{1}H NMR spectrum of Cobalt (II) Complex

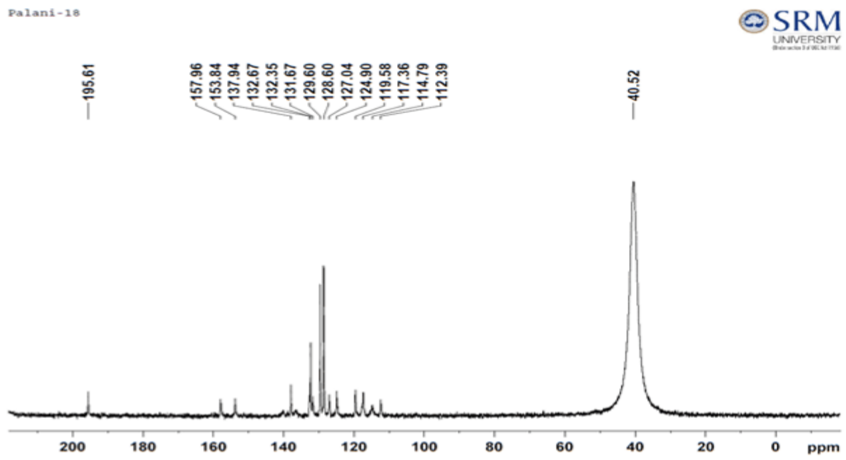

Figure 13: ^{13}C NMR spectrum of Cobalt (II) Complex

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Copper (II) complex

The magnetic moment measurement of the copper(II) complex at room temperature lie in the range 1.83 –1.85 B.M. [16,17] Three spin allowed transition may be expected in the visible region and the Eg and T_{2g} levels of 2D free ion will split into B_{1g}, A_{1g}, B_{2g} and E_g levels respectively. Electronic spectrum (Figure14) of six coordinated copper complex display band at 730,660 and 431nm corresponding to the following transitions $^2B_1g \rightarrow {}^2B_2g$; $^2B_1g \rightarrow {}^2Eg$ and $^2B_1g \rightarrow {}^2A_1g$. The EPR spectrum of Cu (II) complex (Figure15) is recorded at room temperature in DMF solution, lie in the range 2.0204-2.01142. The trend $g|| > g\perp > 2.0023$ observed for the complex indicates that the unpaired electron is localized in the dx dx^2-y^2 orbital of the Cu (II) ion and is characteristic for the axial symmetry. The shape of the ESR line indicates that the present complex has distorted octahedral complex

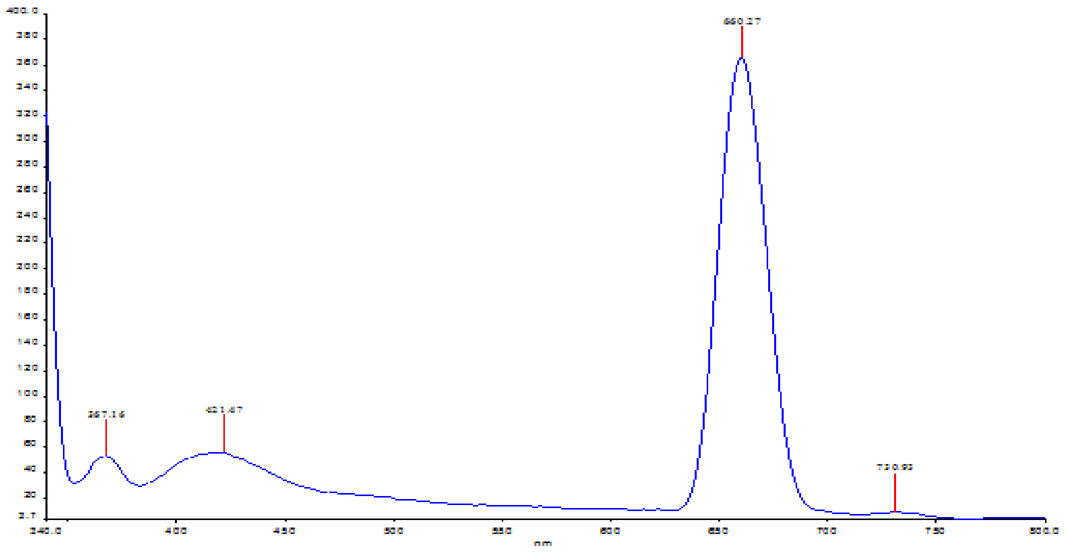

Figure 14: Electronic spectrum of Cu (II) complex

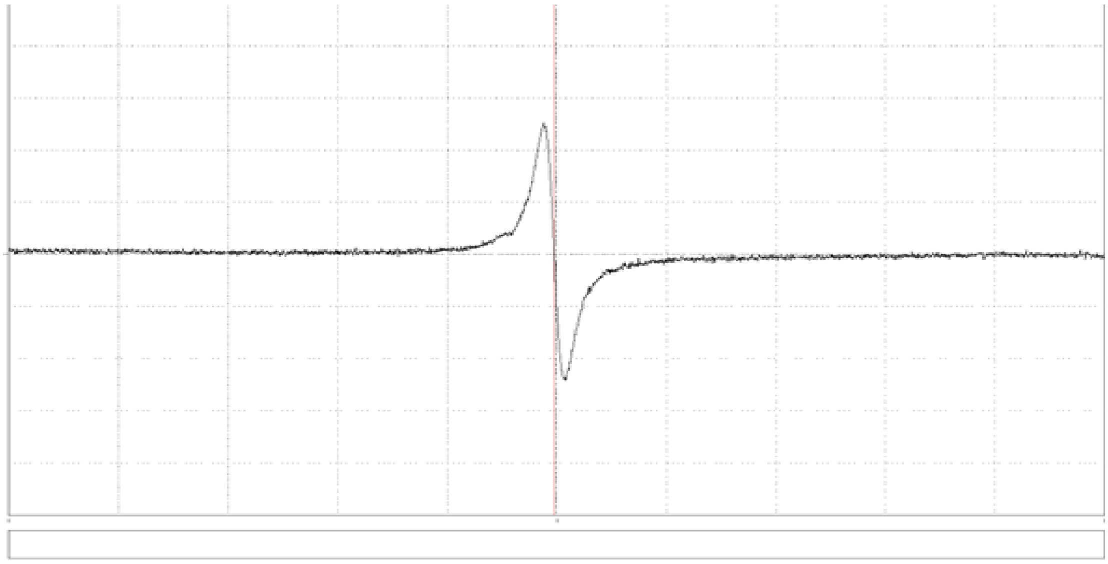

Figure 15: EPR spectrum of Cu (II) complex

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Antimicrobial activity

Antimicrobial activity was estimated as described by [18] Surface of the Mueller Hinton Agar (MHA) plates were inoculated by spreading a volume of the microbial inoculums (0.5 McFarland standards) over the entire agar surface. Then, a hole with a diameter of 6 to 8mm was punched aseptically with a sterile cork borer and a volume (20–100 mL) of the extract solution at desired concentration is introduced into the wells. Then, agar plates were incubated at 37°C for 24hours. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested the zone of inhibition was measured with a measuring scale. The antimicrobial activity of the ligand and its Co(II),Ni(II) and Cu(II) metal complexes were assayed against (i) Gram-positive bacteria: Staphylococcus aureus, Enterococci and (ii) Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa. The result of antimicrobial activity is summarized in (Table 3). From the data it is clear that the metal complex is effective against bacteria.

Molecular docking study

The biological importance of the synthesized ligands are assessed by performing docking studies using Auto Dock VinaPyRx software [19]. The retrieved pdb file (4s1y) is given as input in Auto Dock Vina and assigned as macromolecule that adds charges and hydrogen bonds to the atoms thus preparing the protein. Ligand preparation including the generation of various tautomers, assigning bond orders, ring conformations and stereo chemistries of the ligand were carried out. All the conformations generated were further used for docking study. A receptor grid was generated around the protein active site by selecting the active residues (His 288, Met 298, Met 329, and Met 548) and Run auto grid option. The docking calculations were performed using Run Vina and the Binding affinity was used to determine the best docked structure from the output. The predicted binding affinity is in kcal/mol. The pdb structure 4s1y [20] of human serum albumin is used for docking studies which plays a key role in increasing the growth and productivity of cells and increases overall cell health. The best docked complex selected has a binding score of -9.5 for Cu (II) complex which predicts a good inhibition. The pdb structure 4sy1 of human serum albumin is used for the docking studies with 2 different compounds containing the Copper and Nickel complexes. The one with best binding affinity is considered for analysis. The following table shows the binding affinity of ligand with 4sy1.

Docking score using Auto dock Vina with the macro molecule 4s1y.

The docked ligand interacts with the protein by forming three H bonds with the residues Ser192 and Glu292 with bond distances 3.33Å and 3.53Å respectively (Figure 16).

Similarly Copper (II) complex also forms 3 H-bonds with the protein in residues Glu292, Gln196, Lys 199 with bond distances 3.43 Å, 3.09Å and 3.21 Å respectively (Figure 17). Nickel (II) complex forms 2 H-bonds with Lys195Å and Ala191Å residue with bond distance 3.20Å and 3.35Å (Figure 18).

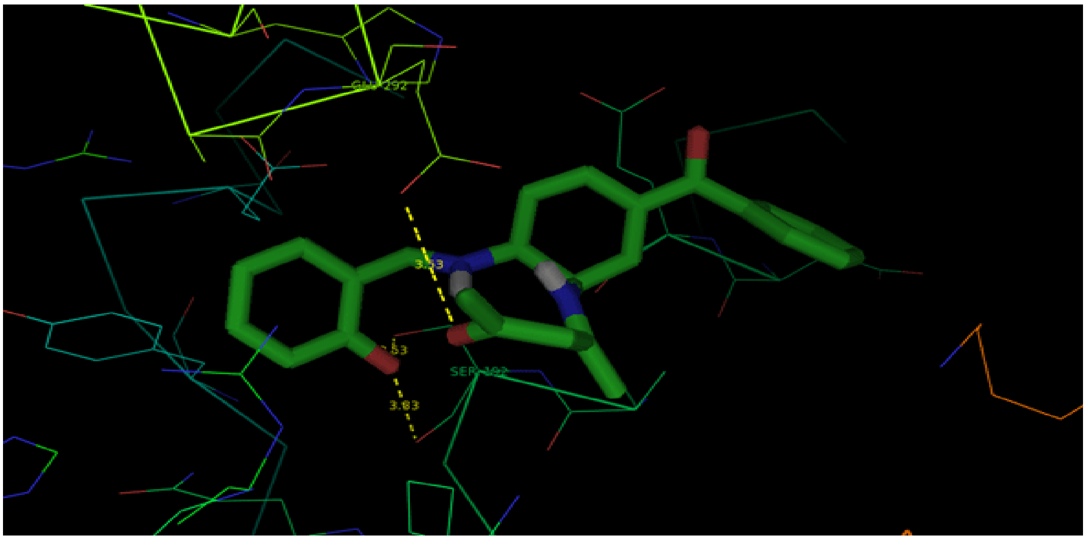

Figure 16: Ligand docked with 4s1y showing formation of hydrogen bond and distances

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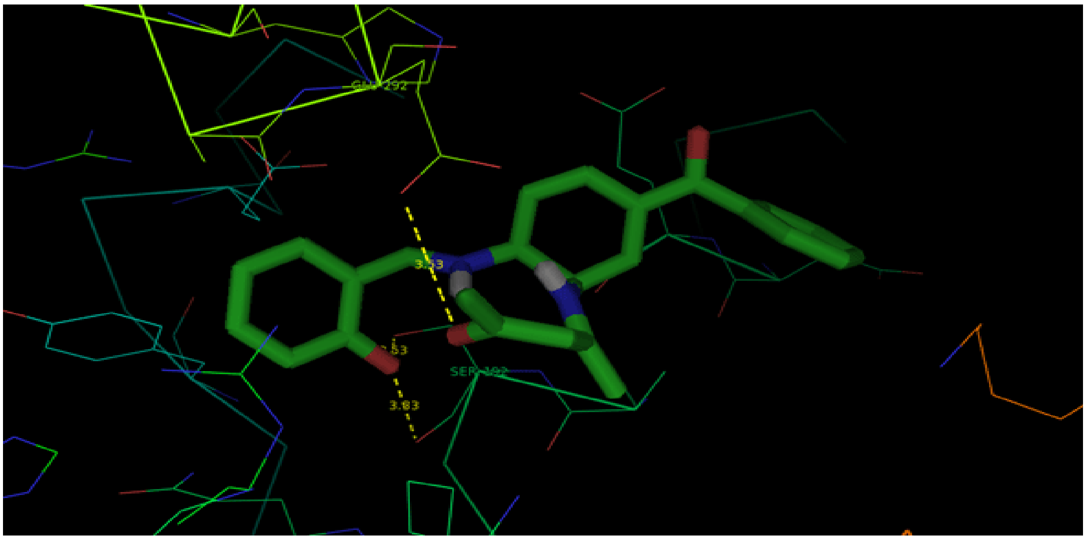

Figure 17: Copper (II) complex docked with 4s1y showing formation of hydrogen bond and distances

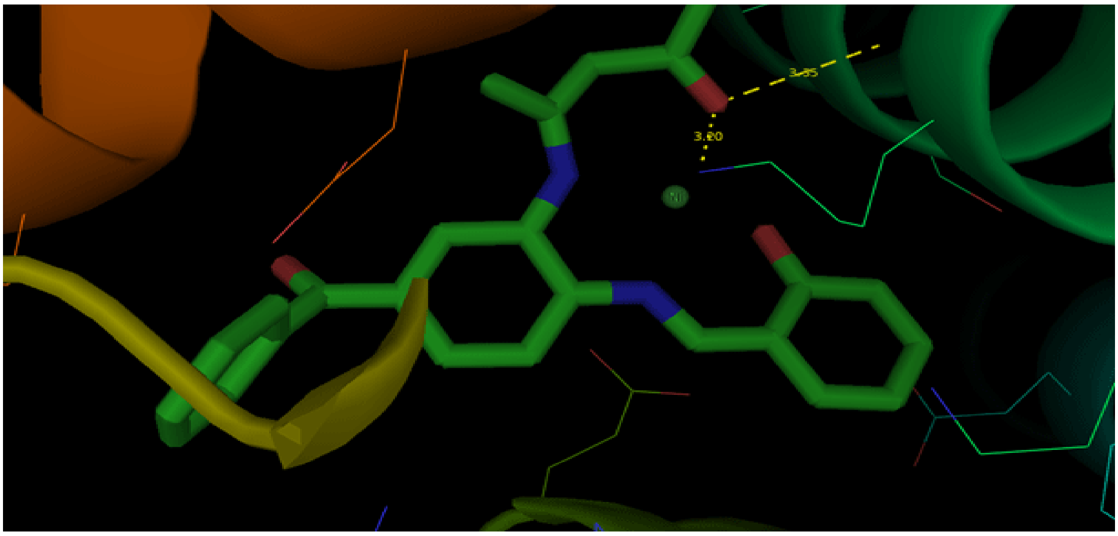

Figure 18: Nickel (II) complex docked with 4s1y showing formation of hydrogen bond and distances

Conclusions

Some new di-imino Schiff base ligands and their complexes with Co (II), Ni (II) and Cu (II) were synthesized and characterized. All the complexes were octahedral in nature. The binding affinity values were determined from docking studies using Auto Dock VinaPyRx software. The antimicrobial activity of the ligand and its metal complexes were assayed against Gram-positive and Gram-negative bacteria. The result of antimicrobial activity is shown that the metal complexes are effective against bacteria

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