



## Synthesis, Characterization, Antimicrobial, MTT assay, DFT study of Co(II), Ni(II), Cu(II) and Zn(II) Complexes with some New Schiff Base Ligands



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### Abstract

Novel Schiff base ligand HL was synthesized by the condensation of 3,5-dichlorosalicylaldehyde and 4-phenylsemicarbazide. Co(II), Ni(II), Cu(II) and Zn(II) complexes were synthesized in 1:1 molar ratio of metal to ligand. The structure of the synthesized ligand was established using <sup>1</sup>H NMR, IR, Mass spectrometry and the metal complexes were characterized using IR, Mass spectrometry, UV-Vis, powdered XRD and molar conductivity. The thermal stability of the complex was studied by TGA. In DFT studies, the geometries of Schiff bases and metal complexes were fully optimized with respect to the energy taking the 6-31+g (d,p) basis set using Gaussian 09w, several molecular properties were also calculated. The spectral data show that ligand HL behaves as uninegative tridentate. On the basis of spectral studies, an octahedral geometry has been assigned for Co(II), square planar for Cu(II) and tetrahedral for Ni(II) and Zn(II) complexes. The antimicrobial activities were examined against some human pathogenic strains. The MTT assay was screened against mouse fibroblast cell line. The result indicates that metal complexes show increase in cytotoxicity in proliferation to cell line as compared to the free ligand.

Key Words: Schiff base; metal complexes; powdered XRD; DFT; antimicrobial; MTT assay

### 1. Introduction

Schiff base ligands are the important class of compounds as they have azomethine [–C=N] group. Due to the presence of azomethine group in Schiff base transition metal complexes, they are responsible for various biological applications such as antibacterial, anticonvulsant, anti-HIV, anti-inflammatory, antifungal, antimicrobial, DNA linkage and anti-tumour activities [1-5]. Schiff base ligands are privileged ligands due to the ease of synthesis and stability under various conditions. The multidentate

Schiff base ligands are so versatile that they can easily form complex with any metal ion. Aromatic aldehyde forms stable Schiff bases as compared to aliphatic aldehydes due to the presence of effective conjugation in aromatic aldehydes [6]. Schiff base ligands having N or O donor atom are capable to bind with different metal ions [7]. From the literature survey, it is revealed that salicylaldehyde derivatives having one or more halogen atom in the aromatic ring, showed a broad range of biological activities such as antibacterial, antimicrobial and antifungal activities [8]. Amongst

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them, semicarbazone based Schiff base constitute a special class, which coordinate with various metal ions to form complexes [9].

We have synthesized Co(II), Ni(II), Cu(II) and Zn(II) complexes of novel Schiff base ligands which have been derived from 3,5-dichlorosalicylaldehyde and 4-phenylsemicarbazide. Due to the presence of -OH group at ortho position in 3,5-dichlorosalicylaldehyde, it easily gets deprotonated and has potential to coordinate with metal ion [10]. The synthesized ligand and its metal complexes have been characterized by IR, <sup>1</sup>H NMR, thermal behaviour, electronic data and powdered XRD. All the complexes are powdered in nature, so efforts to isolate single crystal were not successful. To better understand the molecular and electronic structures of the ligand and metal complexes, DFT calculation was performed.

## 2. Experimental

### 2.1. Materials and methods

All reagent were purchased from the chemical suppliers and used as supplied. 3,5-dichlorosalicylaldehyde and 4-phenylsemicarbazide used were of AR grade and obtained from Alfa Aesar and used without any further purification. All the Metal salts were purchased from Merck and used without any purification. Ethanol, Dimethyl sulfoxide and Dimethylformamide were of the spectroscopic grade.

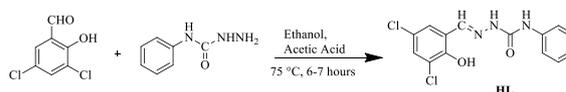
### 2.2. Instrumentation

The elemental analysis of the synthesized ligand and its metal complexes were recorded at Thermo Finnigan Flash EA 1112 series. IR spectra were recorded in the region 4000-400cm<sup>-1</sup> on FT-IR SPECTRUM-2000 using KBr pellets. Mass spectra were recorded on Agilent Technologies 6530 Accurate- Mass Q-TOF LC/MS. NMR spectra were recorded on JNM-EXCP400 at 400 MHz using Tetramethylsilane as an internal standard. The electronic spectra were recorded on Shimadzu UV mini-1240 spectrophotometer using Dimethyl sulfoxide as a solvent. Thermogravimetric Analysis (TGA) was carried out in a dynamic nitrogen atmosphere with a heating rate of 10°C/min using Pyris diamond TGA. Molar conductances of the metal complexes were measured in Dimethyl sulfoxide at room temperature on ELICO (CM82T) Conductivity Bridge. The magnetic susceptibility of the metal

complexes was recorded on a Guoy balance using CuSO<sub>4</sub>.5H<sub>2</sub>O. EPR spectra were recorded as a polycrystalline sample at room temperature on E4-EPR spectrometer using the DPPH as the g-marker. The ligand and their metal complexes were fully optimized by using Density Functional Theory (DFT) comprising 6-31+g(d,p) basis and B3LYP functional set method using the Gaussian 09W program [11,12].

### 2.3. Synthesis of Ligand HL

To an ethanolic solution of 3,5 dichlorosalicylaldehyde (0.01 mol, 1.91g), 2-3 drops of acetic acid was added and it was heated for 10 minutes. This solution was then added to hot ethanolic solution of 4-phenylsemicarbazide (0.01 mol, 1.51g) with continuous stirring and the reaction solution was refluxed at 78-80 °C for 6-7 hours. The reaction progress was monitored by Thin layer chromatography. It was allowed to stay at room temperature overnight. On cooling, the white coloured solid was separated out. It was filtered off, washed several times with distilled water and cold ethanol and then dried in vacuum over P<sub>4</sub>O<sub>10</sub>. Synthesis of ligand **HL** is given in **Scheme 1**. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.77 (s, 1H, -OH), 9.02 (s, 1H, -NH), 8.19 (s, 1H, -CH=N), 7.91 (s, 1H, -NH), 7.56-7.47 (m, 4H, -CH<sub>aromatic</sub>), 7.24-7.28 (m, 2H, -CH<sub>aromatic</sub>), 6.98 (t, 1H, -CH<sub>aromatic</sub>).



Scheme 1: Synthesis of Schiff base ligand HL.

### 2.4. Preparation of metal complexes of Ligand HL

Co(II), Ni(II), Cu(II) and Zn(II) metal complexes of ligand **HL** were synthesized in 1:1 molar ratio. A hot ethanolic solution (15 mL, 1mmol) of a Schiff base ligand **HL** was taken in a round bottom flask and heated for 15 minutes at 70 °C to make the clear solution. Then hot ethanolic solution of the corresponding metal salt (15 mL, 1mmol) was added with continuous stirring. The reaction was refluxed for 8-24 hours. On cooling the reaction mixture overnight at room temperature, the product was separated out, which was filtered, washed several times with cold ethanol and ether and dried in vacuum over P<sub>4</sub>O<sub>10</sub>.

## 2.5. Biological Study

### 2.5.1. Cytotoxicity Assessment (MTT test)

The cytotoxicity of compounds was evaluated on mouse fibroblast (ATCC L929) cell line. The cells were cultured in Dulbecco Modified Eagle Medium (DMEM) at 37 °C in 5% CO<sub>2</sub> atmosphere. A standardized quantity of cells ( $1 \times 10^4$ ) was inoculated in 100  $\mu$ L of DMEM in 96 well culture plates and incubated for stabilization for 24 hours before the treatment. The stock solution of compounds was prepared in Dimethyl sulfoxide (99.9%) at concentration of 10  $\mu$ g/ $\mu$ L. The final quantities of compounds 25 $\mu$ g, 12.5 $\mu$ g and 6.25 $\mu$ g was added and incubated for 24 hrs at 37 °C in 5% CO<sub>2</sub> atmosphere. After 24 hrs, 15  $\mu$ L dye solution from the Cell Titre 96® non-radioactivity cell proliferation assay kit (Promega, USA) was added in to the wells and kept for 4 hrs in incubation as per the recommendation. 100  $\mu$ L stopping solution was added in all the wells and incubated overnight to dissolve formazan product to get uniform readings. The absorbance was recorded at 570 nm in microplate spectrophotometer. The Dimethyl sulfoxide used as a solvent was taken as negative control in the assay (amount equal to prepare BS dilution). The viability of the cell line after treatment was used as criteria to select non-toxic material. Higher the percentage of the viability, lesser will be the toxicity of the sample.

### 2.5.2. Antimicrobial Activity

Minimum Inhibitory Concentration was calculated using serial dilution method. For this, various pathogens were used to examine the antimicrobial activity. Standard drugs used for the antifungal and antibacterial activities were Fluconazole and Ciprofloxacin, respectively. Colonies of microorganisms were chosen off a fresh isolation plate and immunised in corresponding tubes containing 5 mL of brain heart infusion broth. The broth was gestated for 6 hrs at 37 °C. The MC Farland No.5 standard was prepared by adding 0.05mL of 1% w/v BaCl<sub>2</sub>.2H<sub>2</sub>O in Phosphate Buffered Saline (PBS) to 9.95 mL of 1% v/v H<sub>2</sub>SO<sub>4</sub> in Phosphate Buffered

Saline. The growth of all the cultures was accustomed to Mc. Farland No.5 turbidity standard using sterile Phosphate Buffered Saline to obtained 10<sup>8</sup> cfu/mL suspension. The working inoculums of aforesaid different microorganisms containing 10<sup>5</sup> cfu/mL suspension was ready by diluting the 10<sup>8</sup> cfu/mL suspension 10<sup>3</sup> times in Brain heart infusion broth.

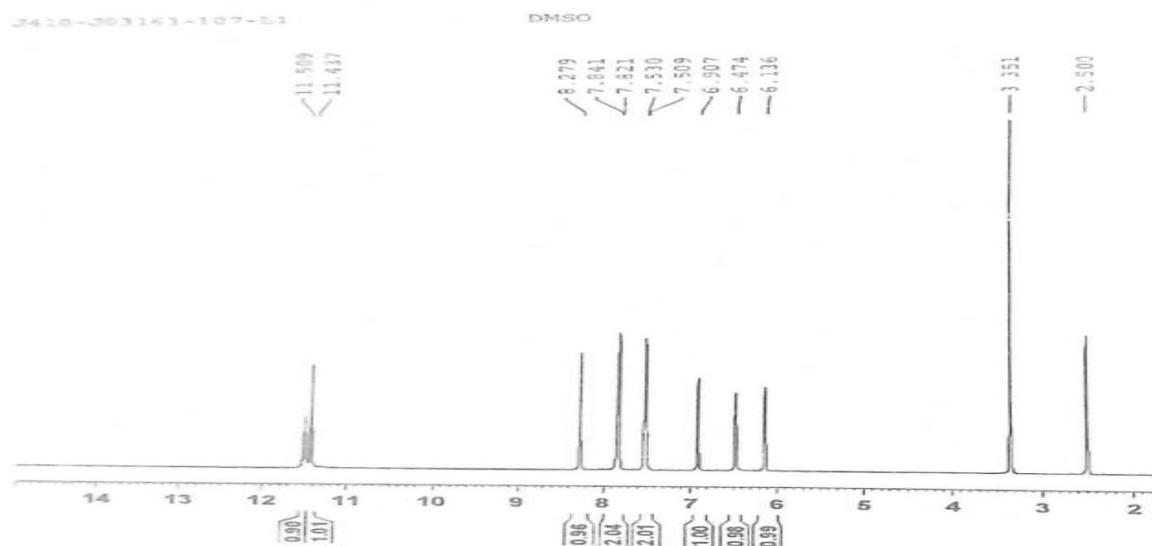
## 3. Result and Discussion

The synthesized metal complexes are non-hygroscopic in nature and are soluble in Dimethyl sulfoxide. All the metal complexes are stable at room temperature and decomposed above 280°C. The molar conductance of all metal complexes **1-8** was recorded in Dimethyl formamide at room temperature and lies in the range of 5.7-9.6  $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$  which consigned the non-electrolyte nature of metal complexes. Physicochemical and analytical data of ligand of ligand **HL** and its metal complexes have been listed in Table 1.

### 3.1. Mass spectrometry

In the ESI mass spectrum of the ligand **HL** peak corresponds to  $[\text{M}-\text{H}]^+$  ion appear as molecular ion peak at  $m/z=322.16$  which established the formation of Schiff base ligand **HL** having molecular formula C<sub>14</sub>H<sub>11</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>. The  $m/z$  value of the Schiff base ligand and its metal complexes **1-8** are given in Table 1. The mass spectrum of the Schiff base ligand **HL** is shown in Figure 1. The mass spectrum of the Schiff base ligand HL contains a non-covalent dimer at 645  $m/z$ . Non-covalent dimer ions,  $[2\text{M} + \text{H}]^+$  or  $[2\text{M}-\text{H}]^-$ , are commonly observed in either positive or negative electrospray ionisation (ESI) mass spectra, particularly for macromolecules [13]. The dimer may be formed through multi-hydrogen bonding over a proton bridge. The detection of the non-covalent dimer ion appears to be instrument-dependent, as the protonated molecule of the compound could be observed at less than 10% intensity in the mass spectrum.



Figure 2b: <sup>1</sup>H NMR spectrum of Zn(II) Complex

### 3.3. IR spectra

The main assignment of Infrared frequencies of Schiff base ligand and their metal complexes are given in the Table 2. The IR spectrum of Schiff base ligand shows azomethine peak at 1595  $\text{cm}^{-1}$ , however peak due to the carbonyl group is not present in the ligand which represents that no aldehyde is present in the ligand [15]. On complexation, peak due to the free azomethine group i.e. 1595  $\text{cm}^{-1}$  is shifted towards lower frequency in the range of 1548-1566  $\text{cm}^{-1}$ . It indicates that azomethine group is coordinated to the metal ion. The band in the region of 430-497  $\text{cm}^{-1}$  has been consigned to the M-N bond which further proposed the coordination of azomethine group. A significant decrease in  $\nu(\text{C-O})$  frequency in the complex formation (1284-1307) has been recommended that deprotonation of phenolic OH group takes place during complex formation. This has been further reinforced by the IR band at 509-563  $\text{cm}^{-1}$  assigned to  $\nu(\text{M-O})$  which specifies the coordination between metal ion and phenolic O atom of ligand. The peak due to carbonyl group of amide decrease from 1323  $\text{cm}^{-1}$  which proposed that amide [C=O] group binds to a metal ion.

A significant broad band appears at 3325-3549 in Co(II) complex which suggested that water molecule binds in the coordination sphere. However, there is no broad band seen in other complexes which suggested that there is no water molecule binded in the

coordination sphere. From the Infrared spectra, it has been established that ligand **HL** acts as a uninegative tridentate ligand.

**Table 2:** Important Infrared spectral bands ( $\text{cm}^{-1}$ ) of the synthesized compounds.

Comp.	$\nu(\text{OH})$	$\nu(\text{C=N})$	$\nu(\text{C=O})$	$\nu(\text{C-O})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
<b>HL</b>	3302	1595	1649	1323		
1	-	1548	1612	1292	491	561
2	-	1564	1593	1296	497	557
3	-	1562	1624	1284	449	563
4	-	1566	1593	1298	432	557
5	-	1560	1606	1307	441	509
6	-	1562	1620	1310	486	560
7	-	1562	1622	1284	497	561
8	-	1566	1620	1300	430	528

### 3.4. Electronic spectra of metal complexes and ligand field parameter

The UV-VIS spectra of Co(II), Ni(II) and Cu(II) were recorded in Dimethyl sulfoxide solution. The ligand field parameters have been intended by using

band fitting equations and electronic spectral data of all complexes are listed in Table 3. The electronic spectra of Co(II) complex display bands at 9,652-9,708  $\text{cm}^{-1}$ , 14,780-15,703  $\text{cm}^{-1}$  and 19,920-22,421  $\text{cm}^{-1}$  which may be assigned due to  ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ ,  ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}$  and  ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(p)$  transitions respectively suggested octahedral geometry of the complex. The magnetic moment of Co(II) are found in the range of 4.93-5.02 B.M. The Racah parameter B has been found to be 762  $\text{cm}^{-1}$  which is less than 971  $\text{cm}^{-1}$ . The Racah parameter of another cobalt complex is 934  $\text{cm}^{-1}$  which is also less than 971  $\text{cm}^{-1}$ . The nephelauxetic parameter  $\beta$  for Co(II) complexes have been found to be less than one which proposed that there is a covalent character between the metal and

ligand bond. The magnetic moment of Ni(II) complexes are found in the range of 3.01-3.05 B.M. which can be accredited due to the presence of two unpaired electrons. The electronic spectra of Ni(II) complexes shows band at 9,718-9,110, 10,416-13,290 and 19,960-22,727  $\text{cm}^{-1}$  suggested tetrahedral geometry of the complex.

Electronic absorption spectra of Cu(II) complexes display a transition in the range of 18,587-22,935  $\text{cm}^{-1}$ . These band assigned to  ${}^2B_{1g} \rightarrow {}^2A_{1g}$  transition suggested that Cu(II) complexes exist in the square planar geometry [16,17]. The magnetic moment of Cu(II) complexes are found in the range of 1.85-1.91.

**Table 3:** Electronic spectral data of Co(II), Ni(II) and Cu(II) complexes, ligand field parameters and conductivity.

Complex	$\lambda_{\text{max}} (\text{cm}^{-1})$	B ( $\text{cm}^{-1}$ )	B	LFSE (KJ/mol)	$\mu_{\text{eff}}$ (B.M)	Molar conductance ( $\text{Scm}^2\text{mol}^{-1}$ )
1	9652, 14780, 19920	762	0.784	103.60	5.02	7.4
2	9708, 15703, 22421	934	0.961	105.35	4.93	8.2
3	9110, 13290, 19960	436.66	0.419	87.177	3.01	5.7
4	9718, 10416, 22727	265.93	0.255	92.99	3.05	9.2
5	18587	-	-	-	1.91	8.7
6	22935	-	-	-	1.85	9.6

### 3.5. Electronic paramagnetic resonance spectra of Cu(II) and Co(II) complexes

The Electronic paramagnetic resonance spectra of Cu(II) complexes have been recorded at room temperatures and Electronic paramagnetic resonance spectra of Co(II) complexes at low temperatures as polycrystalline sample under the magnetic field strength of 3000G on X band at the frequency of 9.1 GHz. An Electronic paramagnetic resonance spectrum is used to study the presence of unpaired electrons and its interaction in the metal complexes. The analysis of Cu(II) complex 6 gives  $g_{\parallel} = 2.2420$ ,  $g_{\perp} = 2.0974$  and  $G = 2.5205$  (Table 4).

By using these  $g$  values, the ground state of the complex has been derived. In square planar complex, if electron lies in  $d_{x^2-y^2}$  orbital giving  ${}^2B_{1g}$  as the ground state with  $g_{\parallel} > g_{\perp} > 2$  and if it lies in  $d_{z^2}$  orbital giving  ${}^2A_{1g}$  as the ground state with  $g_{\perp} > g_{\parallel} > 2$  [18]. In this case, the observed order is  $g_{\parallel} > g_{\perp} > 2$  which indicates that unpaired electron lies in  $d_{x^2-y^2}$  orbital which suggested that there is a square planar geometry around the Cu(II) ion.  $G$  values represent the exchange interaction in solid complex. According to Hathaway, if  $G < 4$ , then there is an exchange interaction in solid complex and if  $G > 4$ , then there is no exchange

interaction. In present case, the value of  $G$  is 2.5205 which is less than 4; it means there is an exchange interaction in solid complex [19].

In complex 2, observed order is ( $g_{\parallel} = 2.0446 > g_{\perp} = 2.07 > 2.0023$ ) indicates that complex has octahedral geometry. This also suggests that unpaired electron lies in  $dx^2-y^2$  orbital. The value of  $g_{\parallel}$  is less than 2.3 representing that there is a covalent character between metal to ligand bond [20]. The value of  $g_{\text{iso}}$  is found to be 2.0193. Similarly, in complex 1,  $g_{\parallel} > g_{\perp} > 2.0023$  and the value of  $g_{\text{iso}}$  is found to be 2.1285.

**Table 4:** EPR spectra of Co(II) and Cu(II) complexes.

Complexes	$g_{\parallel}$	$g_{\perp}$	$g_{\text{iso}}$	$G$
1	2.3221	2.0318	2.1285	-
2	2.0446	2.0067	2.0193	-
6	2.2420	2.0974	-	2.5205

### 3.6. Thermal Analysis

The thermal stability of complex 2 was investigated using Thermogravimetric analysis (TGA). TGA was carried out in nitrogen atmosphere at heating rate of 10  $^{\circ}\text{C}/\text{min}$  over the temperature range of 40-900  $^{\circ}\text{C}$ .

Complex **2** shows two step decomposition within the temperature range of 40-500 °C (Figure 3).

First step corresponds to the loss of two water molecules and one acetic acid molecule (found 18.34 %, calcd. 20.12 %) in the temperature range of 40-250 °C. The second decomposition step in the temperature

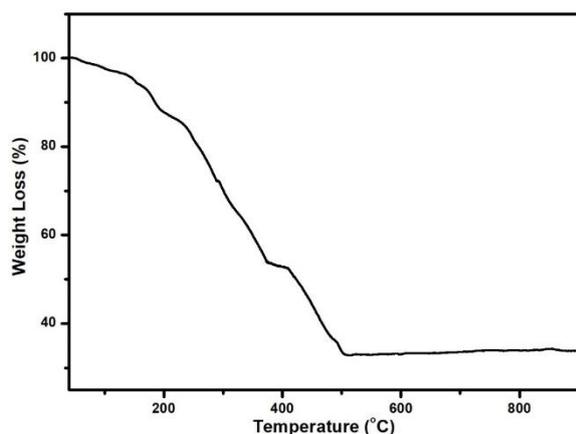
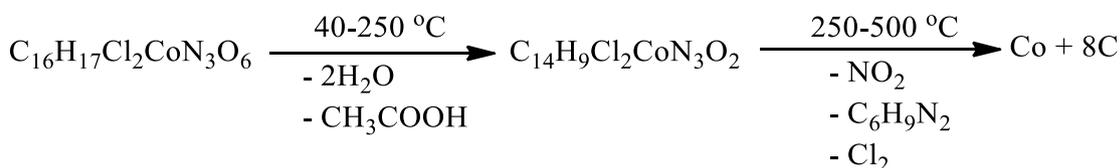


Figure 3: TGA curve of complex 2.

### 3.7. X-Ray Diffraction study

To isolate single crystal of these complexes, several efforts were made which became unsuccessful. The X-Ray diffraction pattern of these metal complexes displays well defined crystalline peaks signifying that these complexes were crystalline in phase [21]. The metal complexes display sharp crystalline X-Ray diffraction patterns which is different from that of the ligand. The advent of crystallinity in the metal-schiff base complexes is due to the intrinsic crystalline nature of the metallic compound. The grain size of the metal complexes  $d_{\text{XRD}}$  was obtained from Scherrer's formula [22]. By calculating the full width at half maximum of the XRD peaks (Figure 4).

$d_{\text{XRD}} = 0.9\lambda/\beta(\cos\theta)$ , where ' $\lambda$ ' is the wavelength, ' $\beta$ ' is the full width at half maximum and ' $\theta$ ' is the peak angle. The complex 2 has the average crystallite size of 10 nm, signifying the nanocrystalline.

range of 250-500 °C with mass loss of 48.35 % (calcd. 47.16 %) corresponding to the loss of  $\text{NO}_2$ ,  $\text{C}_6\text{H}_9\text{N}_2$  and  $\text{Cl}_2$  molecule and as a final product, it leaves Co and 8C as residue (found 33.35 %, calcd. 32.49 %). The thermal decomposition for this complex can be formulated as follows:

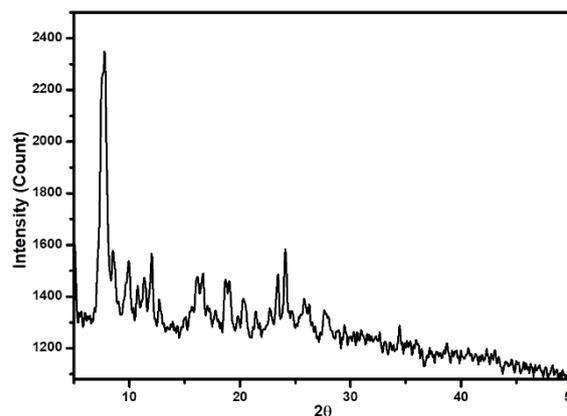


Figure 4 XRD pattern of Complex 2

### 3.8. Geometry optimization of Schiff base ligand HL and its metal complexes 1-8

Geometry optimization of the ligand **HL** and all the metal complexes has been done by Density Functional Theory (DFT) calculations using 6-31+g(d,p) basis and B3LYP functional set as integrated in the Gaussian 09W programme in gaseous phase.

The numbering scheme and fully optimized structures of the ligand **HL** and its metal complexes are demonstrated in Figure 5 and Figure 6. The optimized geometry of the ligand displays that the structure of the molecule is planar. However, the optimized geometries of metal complexes revealed that the planarity of the ligand is slightly lost after metal complexation. In Co(II) complex **1** of ligand **HL**, the metal ion is coordinated through oxygen atom of amide group in 4- phenylsemicarbazide as well as oxygen atom of 4,5-dichlorosalicylaldehyde and rest of the positions has been occupied by nitrogen atom of azomethine, water molecules and chloride ion, thus stabilizing it in an octahedral environment. The geometries are entirely stable and no bond breaking takes place during optimization.

Similarly, the Ni (II) and Zn(II) complexes were fully optimised in gas phase and the optimised molecular structure revealed that there is a tetrahedral environment around Ni(II) and Zn(II) ion. The ligand **HL** interact with metal ion in a tridentate manner through azomethine nitrogen and oxygen of hydroxyl group as well as oxygen atom of amide group present in 4-phenylsemicarbazide and the remaining one position are occupied by anions such as acetate/chlorides in complexes. However Cu(II) complexes shows square planar geometry, in this case ligand **HL** also interact in tridentate manner with metal ion through azomethine nitrogen, oxygen atom of phenolic group and oxygen atom of amide group. On equating the experimental and computed parameters, it was found that the computed bond lengths and bond angles are in covenant with the experimental value [23]. The computed parameters for ligand **HL** and metal complexes are given in Table 5. On the basis of overhead discussion and the result of various spectral

techniques, following structures of the complexes **1-8** have been established (Figure 7).

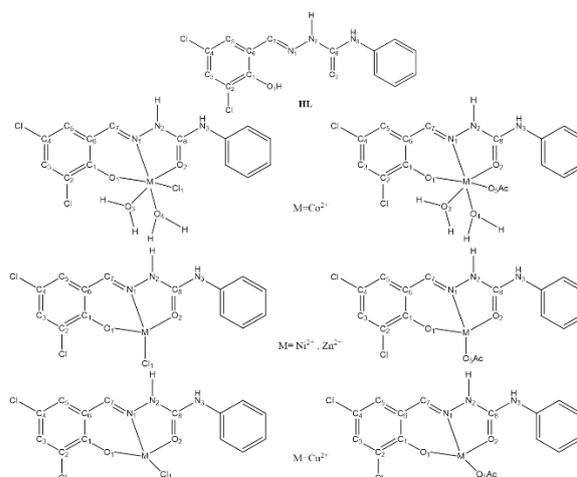


Figure 5: The numbering scheme of synthesized Schiff base ligand HL and its metal complexes 1-8.

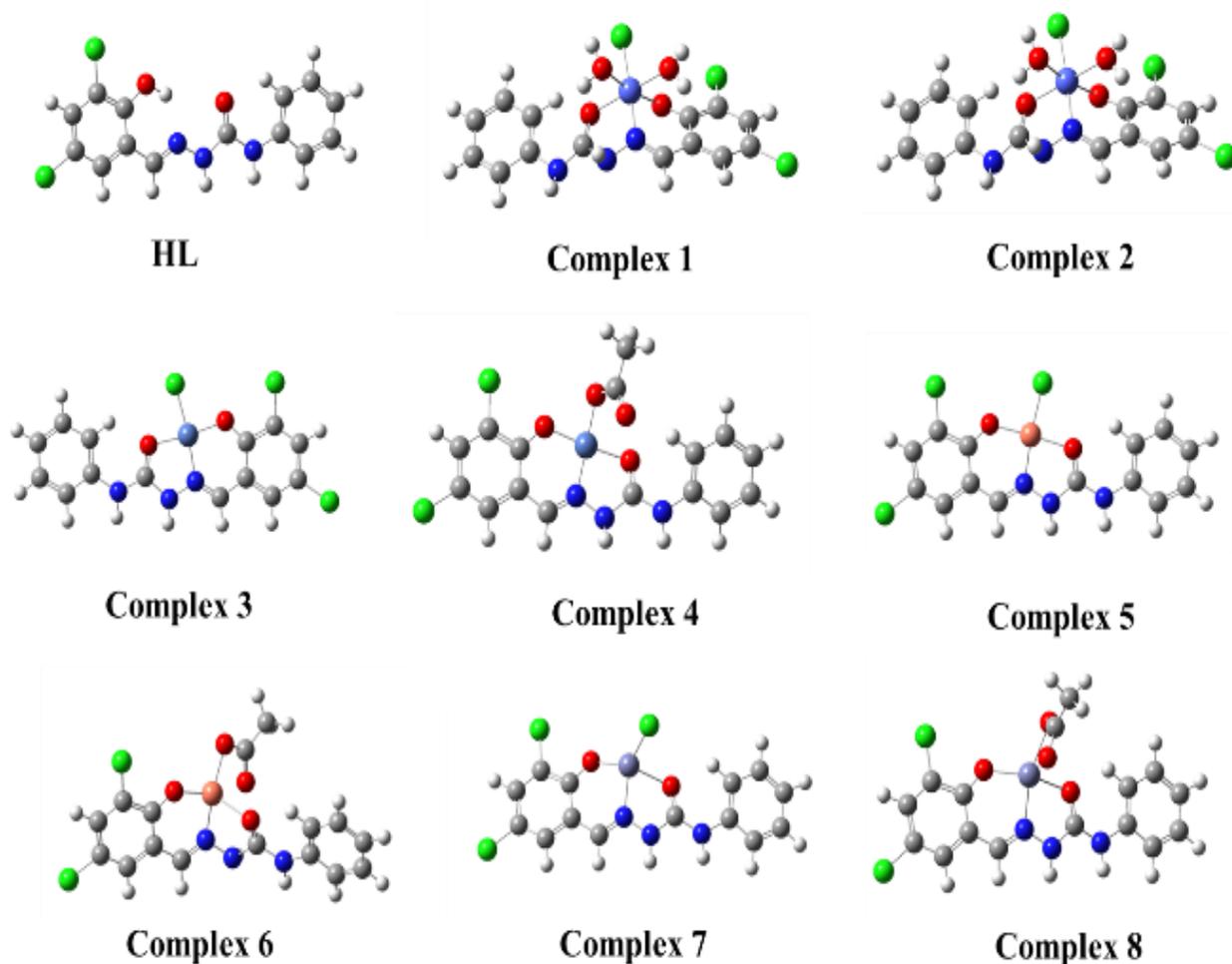


Figure 6: The optimized structures of synthesized Schiff base ligand **HL** and the metal complexes **1-8**.

Table 5: Optimized parameters of ligand HL and metal complexes 1-8 (bond lengths in Angstrom; bond angles in degree).

Parameters	HL	1	2	3	4	5	6	7	8
C <sub>1</sub> -O <sub>1</sub>	1.347	1.281	1.286	1.284	1.281	1.273	1.269	1.274	1.268
C <sub>1</sub> -C <sub>6</sub>	1.415	1.460	1.429	1.432	1.432	1.435	1.436	1.434	1.435
C <sub>6</sub> -C <sub>7</sub>	1.468	1.459	1.451	1.437	1.439	1.431	1.430	1.438	1.436
C <sub>7</sub> -N <sub>1</sub>	1.305	1.318	1.318	1.314	1.313	1.327	1.328	1.323	1.322
N <sub>1</sub> -N <sub>2</sub>	1.365	1.426	1.438	1.403	1.406	1.412	1.427	1.40	1.408
N <sub>2</sub> -C <sub>4</sub>	1.443	1.475	1.500	1.437	1.448	1.443	1.474	1.456	1.448
C <sub>8</sub> -O <sub>2</sub>	1.209	1.307	1.304	1.259	1.249	1.242	1.228	1.239	1.235
O <sub>2</sub> -M	-	1.947	1.969	1.883	1.926	1.977	2.142	2.216	2.25
O <sub>1</sub> -M	-	1.948	1.959	1.827	1.843	1.876	1.897	1.935	1.987
N <sub>1</sub> -M	-	1.850	1.834	1.866	1.851	1.892	1.865	2.009	2.047
Cl <sub>1</sub> -M	-	2.213	-	2.116	-	2.059	-	2.169	-
O <sub>3</sub> -M	-	2.0185	2.02	-	-	-	-	-	-
O <sub>4</sub> -M	-	2.037	2.034	-	-	-	-	-	-
O <sub>5</sub> -M	-	-	-	-	1.851	-	1.95	-	2.129
O <sub>1</sub> -M-N <sub>1</sub>	-	86.50	91.76	97.44	97.35	93.96	91.75	90.50	88.66
O <sub>2</sub> -M-N <sub>1</sub>	-	81.19	81.79	88.38	87.69	83.23	77.30	75.83	74.65
Cl <sub>1</sub> -M-O <sub>2</sub>	-	93.78	-	85.47	-	90.35	-	94.59	-
Cl <sub>1</sub> -M-O <sub>1</sub>	-	90.02	-	88.68	-	92.41	-	125.18	-
Cl <sub>1</sub> -M-N <sub>1</sub>	-	173.075	-	-	-	173.46	-	-	-
Cl <sub>1</sub> -M-O <sub>3</sub>	-	85.49	-	-	-	-	-	-	-
Cl <sub>1</sub> -M-O <sub>4</sub>	-	91.36	-	-	-	-	-	-	-
O <sub>2</sub> -M-O <sub>2</sub>	-	-	102.60	-	92.30	-	102.27	-	87.20
O <sub>2</sub> -M-O <sub>1</sub>	-	-	80.08	-	82.64	-	101.32	-	1.9.10
O <sub>2</sub> -M-O <sub>3</sub>	-	-	95.00	-	-	-	-	-	-
O <sub>2</sub> -M-O <sub>4</sub>	-	-	72.33	-	-	-	-	-	-

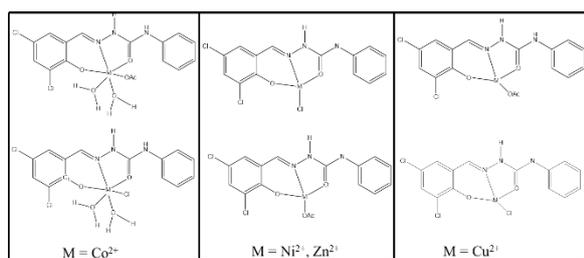


Figure 7: Proposed structures of metal complexes 1-8.

### 3.9. HOMO-LUMO energy gap and related molecular properties

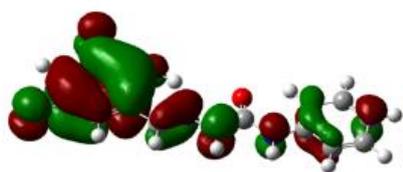
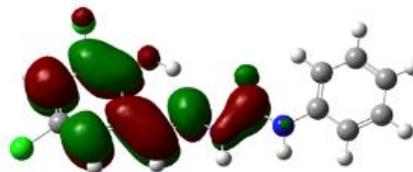
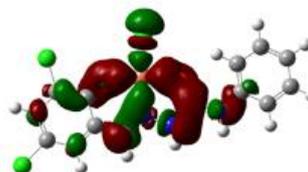
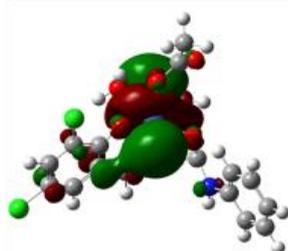
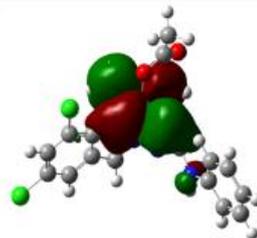
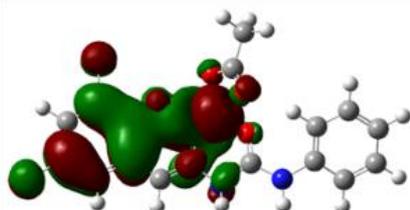
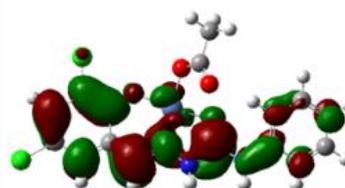
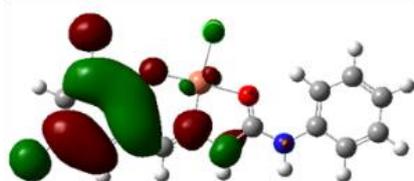
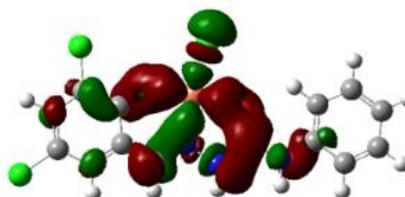
Frontier molecular orbital i.e. lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) plays a significant role in determination of chemical reactivity as well as electrical properties (Fig 8). By comparing HOMO-LUMO energy gap, the stability of the molecule can also be discussed. According to Koopman's theorem, the electron affinity 'A' and Ionisation energy 'I' can be communicated through HOMO and LUMO energies as  $I = -E_{\text{HOMO}}$  and  $A = -E_{\text{LUMO}}$ . [24].

The hardness of the molecule rest on the energy gap between the HOMO and LUMO. If the energy gap between the HOMO and LUMO is high, then the hardness in the molecule will be larger [25]. The global hardness  $\eta$  can be calculated as:  $\eta = \frac{1}{2} [E_{\text{HOMO}} - E_{\text{LUMO}}]$ . If the energy gap between the HOMO and LUMO is less, then this type of complex is accountable for the optoelectronic properties like light-emitting diodes, thin film transistors and photovoltaic cells, etc. and the physical properties of these type of compounds make them useful for building blocks for the progress of material for organic light emitting diodes. [26].

The electron donating power of the molecule is associated with energy of HOMO which suggested that a larger value of  $E_{\text{HOMO}}$  means the greater ease of electron donating power to the empty d-orbital of metal [27]. Molecule having low value of  $E_{\text{LUMO}}$  has shown the tendency of high electron accepting power [28].  $\Delta E$  value measures the stability of the compound. Larger the energy gap ( $\Delta E$ ) between the HOMO and LUMO indicates the high stability of the molecule [29]. The FMO energies of the complexes are registered in the Table 6.

### 3.10. Antimicrobial and MTT assay studies

The results of cytotoxicity valuation by MTT on mouse fibroblast (ATCC L929) signify that all the synthesized compounds show a toxicity in range of 15-35% while control and standard drugs (Ciprofloxacin and Fluconazole) show cytotoxicity of 15, 58 and 62%, respectively (Table 7). The ligands and the metal complexes show almost equal toxicity while some complexes are more toxic than the parent ligands. The results of antimicrobial activity by serial dilution method reveal that most of the synthesized molecules show significant activity when the results are compared with standard drugs (Figure 9). All the molecules are active against most of the pathogenic strains but the best activity is seen against *Listeria innocua* in the MIC value of most of the tested compounds lie in the range of 1.5625-3.125 while for the strains *Pseudomonas aeruginosa* and *Shigella flexneri*, most of the tested compounds are ineffective (Table 8).

**HL HOMO****HL LUMO****Complex 1 HOMO****Complex 1 LUMO****Complex 2 HOMO****Complex 2 LUMO****Complex 3 HOMO****Complex 3 LUMO****Complex 4 HOMO****Complex 4 LUMO****Complex 5 HOMO****Complex 5 LUMO**

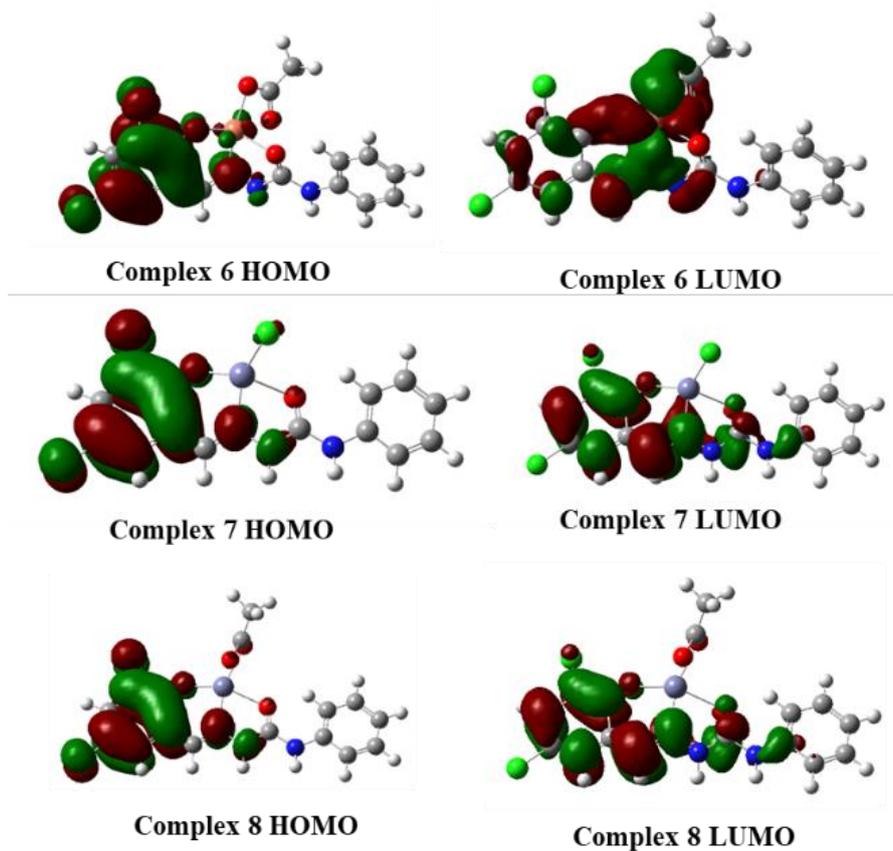


Figure 8: HOMO and LUMO orbitals of the synthesized compounds.

**Table 6:** Calculated frontier orbital energies, electronegativity, hardness and softness of ligand **HL** and complexes **1-8**.

Compound	$E_{\text{HOMO}}$ (ev)	$E_{\text{LUMO}}$ (ev)	$\Delta E_{\text{L-H}}$ (ev)	$I$ (ev)	$A$ (ev)	$\chi$ (ev)	$\eta$ (ev)
HL	-8.887	-1.050	7.837	8.887	1.050	4.969	3.918
1	-7.650	-3.196	4.454	7.650	3.196	5.423	2.227
2	-7.181	-2.483	4.698	7.181	2.483	4.832	2.349
3	-8.277	-1.335	6.942	8.277	1.335	4.806	3.471
4	-8.163	-1.078	7.085	8.163	1.078	4.621	3.542
5	-8.638	-1.500	7.138	8.638	1.500	5.069	3.569
6	-8.511	-1.350	7.161	8.511	1.350	4.931	3.580
7	-8.725	-1.493	7.232	8.725	1.493	5.109	3.616
8	-8.478	-1.212	7.266	8.478	1.212	4.845	3.633

Table 7: MTT assay of ligand **HL** and its complexes against mouse fibroblast cell.

Compound	HL	1	2	3	4	5	6	7	8	Ciprofloxacin	Flucanazole
Cell Viability(%)	72	58	65	67	62	70	71	67	67	42	38

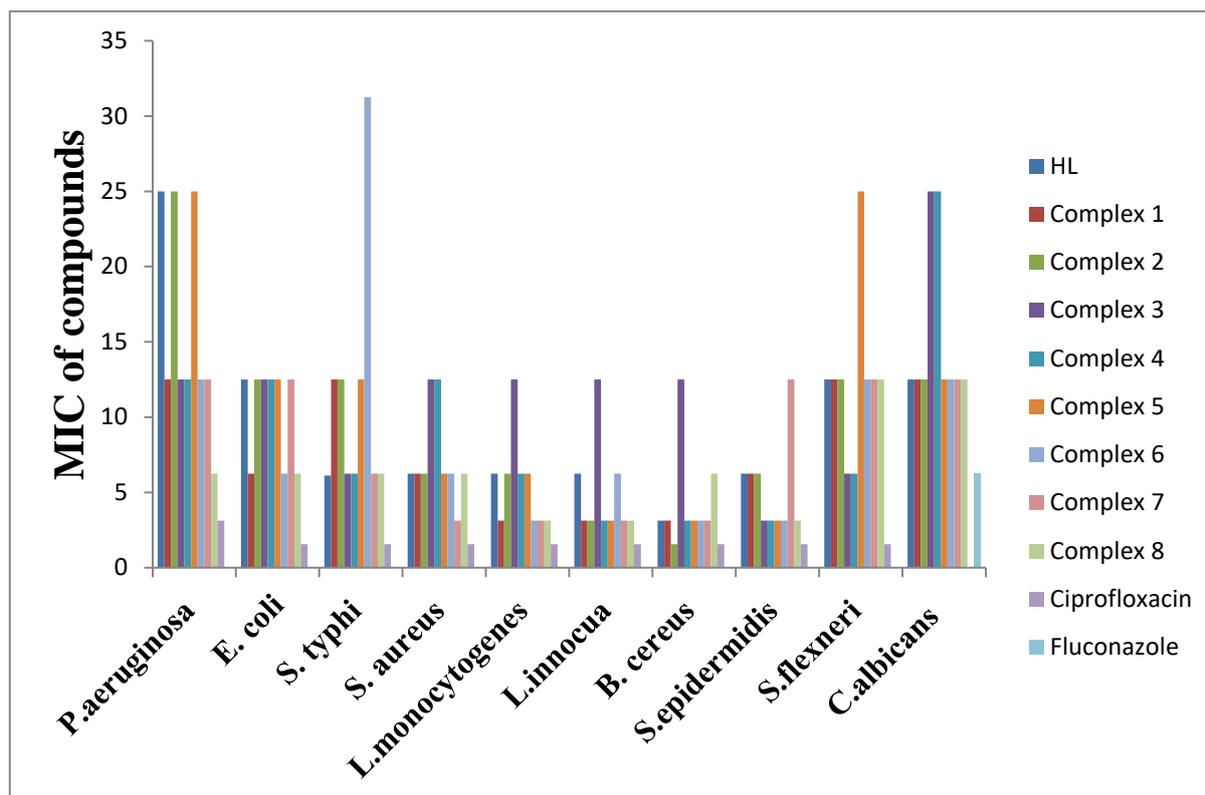


Figure 9: MIC (minimum inhibitory concentration) values of ligand **HL** and its metal complexes **1-8**

Table 8: MIC (minimum inhibitory concentration) values of ligand **HL** and its metal complexes **1-8**

Compounds	Pathogenic strains									
	P.aeruginosa	E.coli	S.typhi	S.aureus	L.monocytogenes	L.innocua	B.cereus	S.epidermidis	S.flexneri	C.albicans
HL	25	12.5	6.125	6.25	6.25	6.25	3.125	6.25	12.5	12.5
1	12.5	6.25	12.5	6.25	3.125	3.125	3.125	6.25	12.5	12.5
2	25	12.5	12.5	6.25	6.25	3.125	1.5625	6.25	12.5	12.5
3	12.5	12.5	6.25	12.5	12.5	12.5	12.5	3.125	6.25	25
4	12.5	12.5	6.25	12.5	6.25	3.125	3.125	3.125	6.25	25
5	25	12.5	12.5	6.25	6.25	3.125	3.125	3.125	25	12.5
6	12.5	6.25	31.25	6.25	3.125	6.25	3.125	3.125	12.5	12.5
7	12.5	12.5	6.25	3.125	3.125	3.125	3.125	12.5	12.5	12.5
8	6.25	6.25	6.25	6.25	3.125	3.125	6.25	3.125	12.5	12.5
Ciprofloxacin	3.125	1.5625	1.5625	1.5625	1.5625	1.5625	1.5625	1.5625	1.5625	-
Fluconazole	-	-	-	-	-	-	-	-	-	6.25

#### 4. Conclusion

The novel synthesized Schiff base ligand HL coordinates in tridentate fashion around the metal (II) centre. On the basis of various physicochemical techniques, Co(II) possess an octahedral geometry, Cu(II) possess square planar geometry and Ni(II), Zn(II) possess tetrahedral geometry. The presence of coordinated water molecule has been justified by IR and TGA data. The geometry of ligand HL and its metal complexes have been optimized by Density functional theory (DFT) confirms the structure of the complexes. Results of antimicrobial and MTT assay activities revealed that almost all the metal complexes are more effective as compared to the free ligand.

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