

NOVEL TRANSITION METAL CHELATORS ACTS AS AN ANTIBIOTICS: SYNTHESIS, CHARACTERIZATION AND ANTI-BIOGRAM ASSAY

M. Selvaganapathy^{a*}, M. Samuel^b, V. Muniyandi^c, G. Robin Wilson^d

^{a*}Department of Chemistry, Mount Carmel College, Bengaluru – 560052, India

^bResearch Department of Chemistry, VHNSN College, Virudhunagar -626001, India

^cDepartment of Chemistry, P.S.R. Engineering College (Autonomous), Sivakasi – 626140, India

^dDepartment of Chemistry, Indian Academy Degree College, Bengaluru – 560043, India

Corresponding Author: selva.chemist5@gmail.com; Tel.: +91-8072973947

Abstract

In the present research work, a series of novel thiazole derivatives of Schiff base metal complexes [Cu(II), Co(II), Ni(II) and Zn(II)] has been synthesized and characterized by various physicochemical and spectroscopic techniques. The synthesized complexes showed significant antibacterial activity against the organisms *Bacillus subtilis* and *Escherichia coli* when compared with the standard antibiotic Streptomycin. The antimicrobial screening emphasize the higher activity exhibited by these complexes which have a highly conjugative planar ligand, 2-amino-6-methylbenzothiazole in its natural environment. The results indicated that Cu(II) complex could be responsible for the potential contender eliciting antimicrobial activity. It can be attributed to the combined effect of the substituents and thiazole structural core present in the ligands. The results showed Cu(II) and Co(II) compounds had absolute specificity for these organisms, which implied a good application prospect in pharmaceutical probes.

Keywords: thiazole derivatives; Streptomycin; Cu(II) and Co(II) compounds; antibacterial activity.

1. INTRODUCTION

Thiazole and benzothiazole derivatives are well-known sulfur and nitrogen atom containing heterocycles. They have fascinated much attention by researchers who are working in various fundamental and applied research fields such as biological and non-biological applications and apart from that they have been used extensively used as solar cell materials and other chemo sensors. During recent years much attention has been devoted to the synthesis of new benzothiazole derivatives due to their diverse chemical reactivity and broad spectrum of biological activity such as anticancer agents^{1,2}, antimicrobial^{3,4}, analgesics⁵, anthelmintic⁶, anticancer^{7,8}, antibacterial⁹⁻¹¹, antifungal¹² and anti-HIV¹³ activity. By cogitating all these facts, the ultimate objective of this article is to synthesize and characterize some transition metal derivatives with 2-amino-6-methylbenzothiazole Schiff base moiety. The newly prepared complexes were confirmed by investigating various spectroscopic and analytical techniques like UV-Vis, FT-IR, ¹H NMR, ¹³C NMR Mass, ESR. The biopotency of azo ligand and its metal [Cu(II), Co(II), Ni(II) and Zn(II)] complexes have been tested against various microbial and fungal strains and also free radical scavenging activity have been screened. It is estimated that this ligand system coordinates to the metal ion through the cyclic carbonyl group of the pyrazolone ring and the enolisable carbonyl group of 2-amino-6-methylbenzothiazole and the azomethine nitrogen atom of the Schiff base.

2. Materials and Methods

All the reagents, Mercaptoacetaldehyde, Acetylacetone, 4-bromobenzaldehyde, 2-amino-6-benzothiazole, and Metal chloride salts were of Merck products and they were used as supplied. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. Solvents used for the various spectroscopic studies were purified by standard procedures¹⁴. Using CarboErba 1108 analyzer, the microanalyses (C, H and N) were performed at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow (India). Molar conductivities in DMSO (10^{-3} M) at room temperature were measured by using Systronic model-304 digital conductivity meter. Magnetic susceptibility measurement of the complexes was carried out by Gouy balance using copper sulphate pentahydrate as the calibrant. Infrared spectra ($4000\text{--}350\text{ cm}^{-1}$ KBr disc) of the samples were recorded on an IR Affinity-1 FT-IR Shimadzu spectrophotometer. The absorption spectra were recorded by using Shimadzu model UV-1601 spectrophotometer at room temperature. NMR spectra were recorded on a Bruker Avance Dry 300 FT-NMR spectrometer in DMSO- d_6 , using TMS as the internal reference. Mass spectrometry experiments were performed on a JEOL-AccuTOF JMS-T100LC mass spectrometer equipped with a custom-made electrospray interface (ESI). EPR spectra were recorded on a Varian E 112 EPR spectrometer in DMSO solution both at room temperature (300 K) and liquid nitrogen temperature (77 K) using TCNE (tetracyanoethylene) as the g-marker.

2.1. Experimental protocols

2.1.1. Microbial analyses

Antibacterial screening of the complexes against three (*Escherichia coli* and *Bacillus subtilis*) bacterial strains in DMSO were carried out employing disc diffusion method^{15,16}. The discs measuring 5 mm in diameter were prepared from Whatman No.1 filter paper sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in concentration (10 µg/mL) of the test compounds were placed in a nutrient agar medium. The plates were inverted and kept in an incubator at 30 ± 1 °C. The inhibition zone thus formed was measured (in mm) after 24 h and amikacin was used as the standard. The nutrient broth, which contained logarithmic serially twofold diluted amount of test compounds and controls, was inoculated within approximately 5×10^5 c.f.u. of actively dividing bacteria cells. The number of c.f.u. was counted after 18-24 h of incubation at 35 °C.

The newly prepared complexes were also screened for their antifungal activity against *Aspergillus niger*, *Rhizoctonia bataticola* in DMSO by agar diffusion method^{17,18}. Agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL). Normal saline water was used to make suspension spore of fungal strain lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get suspension of corresponding species. Twenty millilitres of agar media were poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h using an agar punch. A control was also prepared in triplicate and maintained at 37 °C for 3-4 days. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with controls. The cultures were incubated for 48 h at 35 °C and the growth was monitored.

3. Synthesis of Schiff base (L)

The synthesis of Schiff base is schematically presented in Scheme 1. The Schiff base was synthesized by the condensation of 2-amino-6-methylbenzothiazole and 4-bromobenzaldehyde (1:1 molar ratio), dissolved in methanol. The resulting reaction mixture was stirred for 5 h. The yellow precipitate of Schiff base condensate obtained was filtered, washed with distilled water dried, recrystallized from ethanol, dried and finally preserved in a desiccator.

[C₁₅H₁₁BrN₂S](L): Yield (%): 74, M.Wt: 331.23, Anal. Calc. (%): C, 54.39; H, 3.35; N, 8.46; S, 9.68 Found (%): C, 53.25; H, 3.27; N, 8.12; S, 9.27. ¹H NMR (DMSO-d₆, δ/ppm): 6.94-7.98 (Ph-H), 9.70 (CH=N), IR (KBr, ν/cm⁻¹): 1643 ν[(CH=N)(azomethine)]; UV-Vis (DMF, λ_{max}/cm⁻¹): 27,778.

Table 3.1. Physical and analytical data of the synthesized Schiff base and its complexes

Compound	Found (Calc)%					Formula weight	Molar conductance Ω ⁻¹ cm ⁻² mol ⁻¹	μ _{eff} (BM)
	M	C	H	N	S			
Ligand	-	54.39 (53.25)	3.35 (3.27)	8.46 (8.12)	9.68 (9.27)	329	-	-
[CuL ₂ Cl ₂]	7.68 (7.52)	46.48 (46.39)	3.41 (3.56)	6.77 (6.53)	9.68 (9.7)	826	14.1	1.93
[NiL ₂ Cl ₂]	7.41 (7.11)	46.75 (46.67)	3.43 (3.32)	6.81 (6.74)	7.80 (7.53)	822	16.2	3.56
[CoL ₂ Cl ₂]	7.17 (6.89)	46.74 (46.39)	3.43 (3.36)	6.81 (6.53)	7.80 (7.57)	821	17.9	4.31
[ZnL ₂ Cl ₂]	7.89 (6.72)	46.37 (46.32)	3.41 (3.35)	6.76 (6.64)	7.74 (7.67)	828	19.3	Dia

3.1. Synthesis of Cu(II), Co(II), Ni(II) and Zn(II) complexes

An methanolic solution (25 mL) of Schiff base (0.512 g, 2 mmol) was mixed with methanolic solution (25 mL) of CoCl₂·6H₂O (0.237 g, 1 mmol)/NiCl₂·6H₂O (0.237 g, 1 mmol)/ CuCl₂·2H₂O (0.170g, mmol)/ZnCl₂ (0.136 g, 1 mmol) and refluxed for 3 h. The separated complex was filtered, washed thoroughly and dried in vacuum over fused CaCl₂ (Scheme 1).

[CuC₃₂H₂₈Br₂N₄S₂Cl₂](1): Yield (%): 72, M.Wt: 826.98, Anal. Calc. (%): Cu, 7.68; C, 46.48; H, 3.41; N, 6.77; S, 9.68; Cl, 8.57 Found (%): Cu, 7.52; C, 46.39; H, 3.56; N, 6.53; S, 9.71; Cl, 8.46. IR (KBr, ν/cm⁻¹): 1653

$\nu[(\text{CH}=\text{N})(\text{azomethine})]$, 435 $\nu(\text{Cu}-\text{O})$, 428 $\nu(\text{Cu}-\text{N})$; UV-Vis (DMF, $\lambda_{\text{max}}/\text{cm}^{-1}$): 30,303 ; molar conductance (DMF, $\text{ohm}^{-1}\text{cm}^2 \text{mol}^{-1}$): 14.1; μ_{eff} (B.M.): 1.93

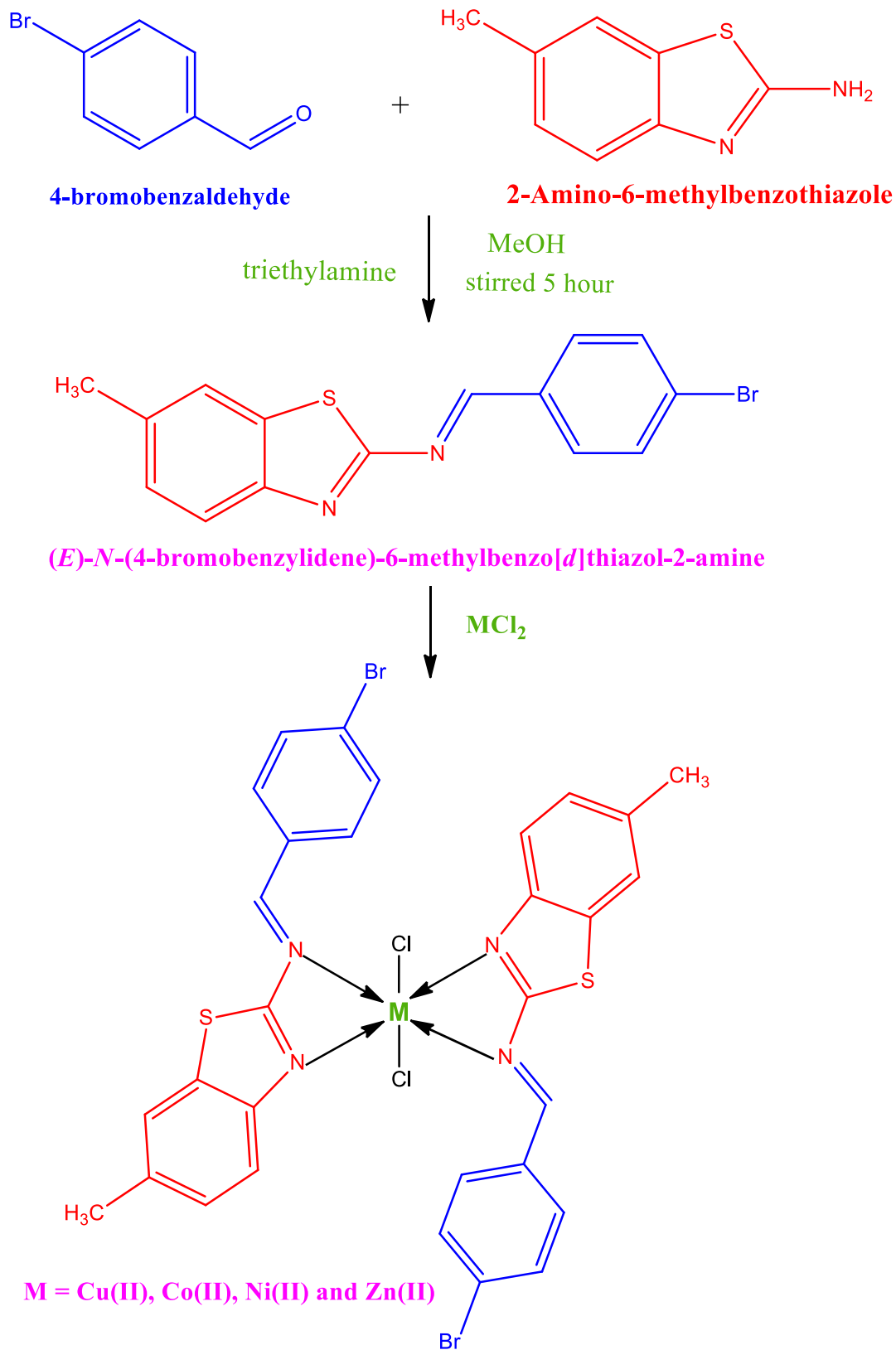
[ZnC₃₂H₂₈Br₂N₄S₂Cl₂](2): Yield (%): 68, M.Wt: 828.8 , Anal. Calc. (%): Zn, 7.89; C, 46.37; H, 3.41; N, 6.76; S, 7.74; Cl, 8.56. Found (%): Zn, 6.72; C, 46.23; H, 3.35; N, 6.64; S, 7.67; Cl, 8.12. ¹H NMR (DMSO-d₆, δ/ppm): 6.9-8.1 (Ar-H), 8.72 (CH=N), IR (KBr, ν/cm^{-1}): 1641 $\nu[(\text{CH}=\text{N})(\text{azomethine})]$, 432 $\nu(\text{Zn}-\text{Cl})$, 452 $\nu(\text{Zn}-\text{N})$; UV-Vis (DMF, $\lambda_{\text{max}}/\text{cm}^{-1}$): 29,940; molar conductance (DMF, $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$): 19.3; μ_{eff} (B.M.): diamagnetic.

[NiC₃₂H₂₈Br₂N₄S₂Cl₂](3): Yield (%): 65, M.Wt: 822.1, Anal. Calc. (%): Ni, 7.41; C, 46.75; H, 3.43; N, 6.81; S, 7.80; Cl, 8.62 Found (%): Ni, 7.11; C, 46.67; H, 3.32; N, 6.74; S, 7.53; Cl, 8.38 . IR (KBr, ν/cm^{-1}): 1637 $\nu[(\text{CH}=\text{N})(\text{azomethine})]$, 439 $\nu(\text{Ni}-\text{O})$, 426 $\nu(\text{Ni}-\text{N})$; UV-Vis (DMF, $\lambda_{\text{max}}/\text{cm}^{-1}$): 28,705; molar conductance (DMF, $\text{ohm}^{-1}\text{cm}^2 \text{mol}^{-1}$): 16.2; μ_{eff} (B.M.): 3.56

[CoC₃₂H₂₈Br₂N₄S₂Cl₂](4): Yield (%): 69, M.Wt: 822 , Anal. Calc. (%): Co, 7.17; C, 46.74; H, 3.43; N, 6.81; S, 7.80; Cl, 8.62 Found (%): Co, 6.89; C, 46.39; H, 3.36; N, 6.53; S, 7.57; Cl, 8.46. IR (KBr, ν/cm^{-1}): 1643 $\nu[(\text{CH}=\text{N})(\text{azomethine})]$, 431 $\nu(\text{Co}-\text{O})$, 437 $\nu(\text{Co}-\text{N})$; UV-Vis (DMF, $\lambda_{\text{max}}/\text{cm}^{-1}$): 29,301 ; molar conductance (DMF, $\text{ohm}^{-1}\text{cm}^2 \text{mol}^{-1}$): 17.9; μ_{eff} (B.M.): 4.31.

4. RESULTS AND DISCUSSION

The Schiff base ligand and its Cu(II), Ni(II), Co(II) and Zn(II) complexes have been synthesized and they are found to be air stable. The ligand is soluble in CHCl_3 , DMF and DMSO but the complexes are soluble only in DMSO. The ligand and its complexes have been characterized by analytical and spectral techniques. The analytical data of the complexes correspond to general formula ML_2 (**Scheme 1**) where, M= Cu(II), Ni(II), Co(II) and Zn(II). The magnetic susceptibility values of the complexes at room temperature are reliable with octahedral geometry around the central metal ion (Table 3.1). The low molar conductance of the chelates supports their electrolytic nature.



Scheme 1. Schematic illustration for the synthesis of benzothiazole derived metal complexes

4.1 IR Spectroscopy

The IR spectra afford valuable information regarding the coordinating sites of ligands. A relative study of the IR spectra of ligand and their metal complexes reveals that certain peaks are common (Fig. 4.1). Therefore, only important peaks which have either shifted/newly appeared and are discussed. The band in the region 1653cm^{-1} due to $\nu(\text{C}=\text{N})$ vibration of thiazole ring and azomethine group in the ligands respectively. These values are increased (thiazole ring) and shifted to lower frequencies (azomethine group) after complexation¹⁹. The band due to $\nu(\text{C}-\text{S}-\text{C})$ in region $761\text{-}725\text{ cm}^{-1}$ remains in the same region both in free ligands and after complexation that means the sulfur atom in thiazole group doesn't coordinate with metal in complexes²⁰. New weak bands in the region $420\text{-}435\text{cm}^{-1}$ were observed in the spectra of metal complexes which are due to $\nu(\text{M}-\text{O})$ ²¹ and the band at 400 cm^{-1} is due to $\nu(\text{M}-\text{Cl})$.

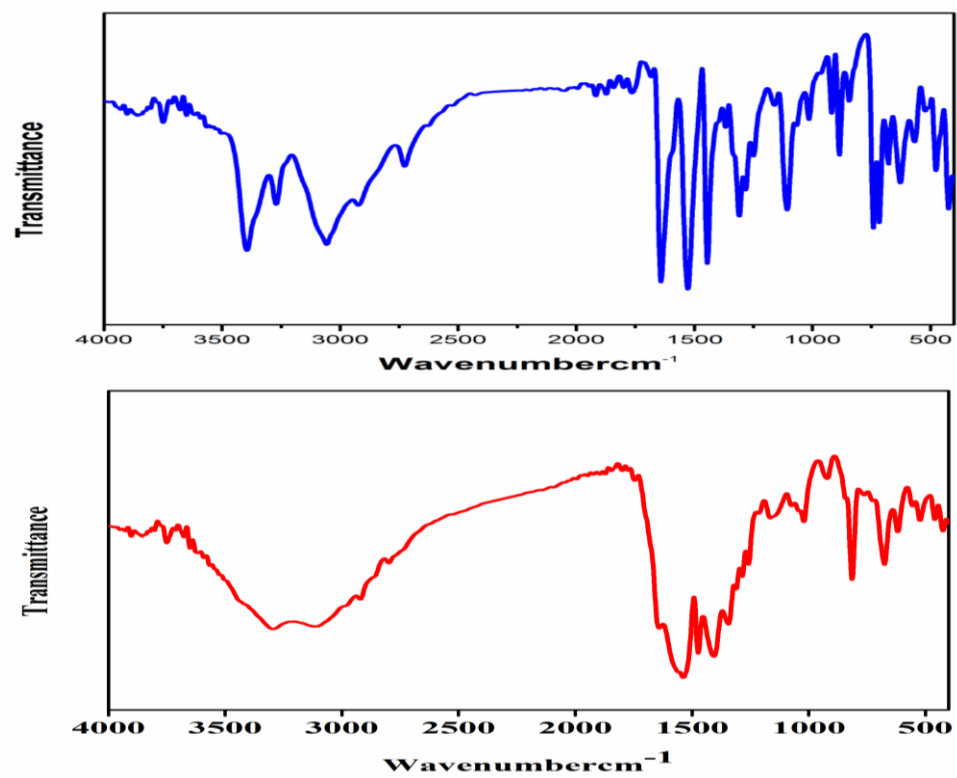


Fig. 4.1. IR spectra of the Schiff base (top) and its copper complex (bottom)

4.2 Electronic absorption spectroscopy (UV)

The electronic absorption spectra of the ligand and its complexes are shown in Figure 4.2. The electronic spectrum of ligand shows an absorption band at $27,778\text{ cm}^{-1}$ which is attributed to $n \rightarrow \pi^*$ transition respectively. The electronic spectrum of copper(II) complex shows d-d transition at $14,705\text{ cm}^{-1}$ which can be assigned as ${}^1A_{1g} \rightarrow {}^1B_{1g}$ transition respectively. The magnetic moment value of copper(II) complex (1.86 B.M), reveals that the copper(II) complex exists in distorted octahedral geometry. The magnetic moment of cobalt(II) has been found to be 4.79 B.M. This value of magnetic moment is higher than the spin only value (3.87 B.M) for three unpaired electrons and may be ascribed to substantial orbital contribution to the moment which is applicable to high spin octahedral cobalt(II)

complex and shows d-d band at $13,793\text{ cm}^{-1}$ which may have existence of ${}^2E_g \rightarrow {}^2T_{2g}$ transition. The magnetic moment for nickel(II) complex is 3.43 B.M and the spectrum of this complex shows band at $14,084\text{ cm}^{-1}$ which may be due to ${}^1A_{1g} \rightarrow {}^1A_{2g}$ transition and therefore, an octahedral configuration is suggested²². Electronic spectral data and magnetic moment values of Schiff base ligand and its complexes are given in Table 4.1.

Table 4.1. Electronic spectral data and magnetic moment values

Compound	$\pi\text{-}\pi^*$ (nm)	$n\text{-}\pi^*$ (nm)	d-d (nm)	μ_{eff} (BM)
Ligand	246	325	-	-
CuL_2Cl_2	298	380	710	1.93
NiL_2Cl_2	287	410	724	3.56
CoL_2Cl_2	293	395	780	4.31
ZnL_2Cl_2	286	402	---	dia

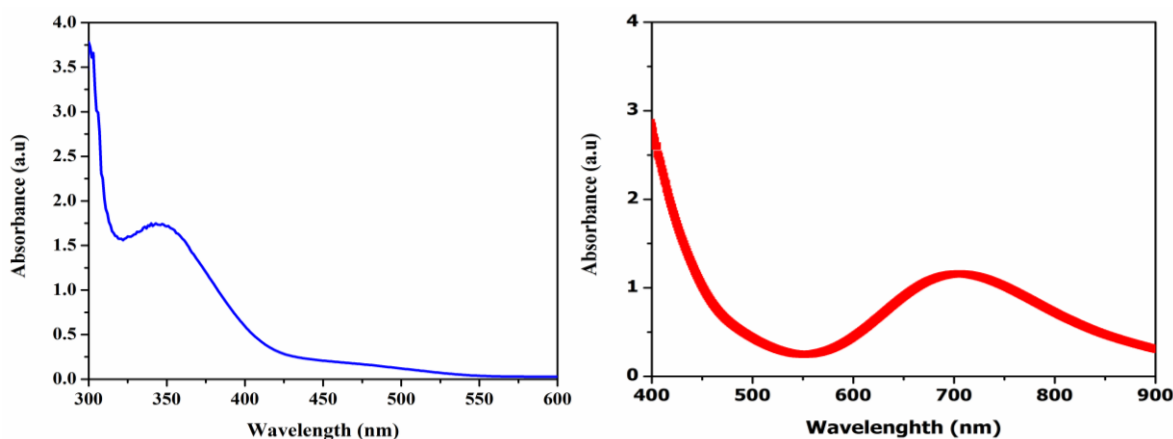


Fig. 4.2. Electronic absorption spectrum of the (i) Schiff base ligand and (ii) copper(II) complex

4.3 ${}^1\text{H}$ NMR and ${}^{13}\text{C}$ NMR Spectroscopy

${}^1\text{H}$ NMR spectra of the ligand (L) and its $[\text{ZnL}_2\text{Cl}_2]$ complex have been recorded in DMSO-d_6 using TMS as the internal standard. The obtained ${}^1\text{H}$ NMR and ${}^{13}\text{C}$ NMR spectra of [L] and $[\text{ZnL}_2\text{Cl}_2]$ are illustrated in following Figure 4.3. The ${}^1\text{H}$ NMR spectrum of ligand (L) exhibits phenyl multiplet at 6.94 – 7.98 ppm due to aromatic protons of phenyl ring. The ligand (L) shows one singlet at 9.70 ppm due to azomethine ($-\text{CH}=\text{N}$) proton, 3.86 ppm due to (Ph-Br). In the ${}^1\text{H}$ NMR spectrum of complex the protons of Schiff base ligand is shifted to down field due to the coordination with metal ion²³.

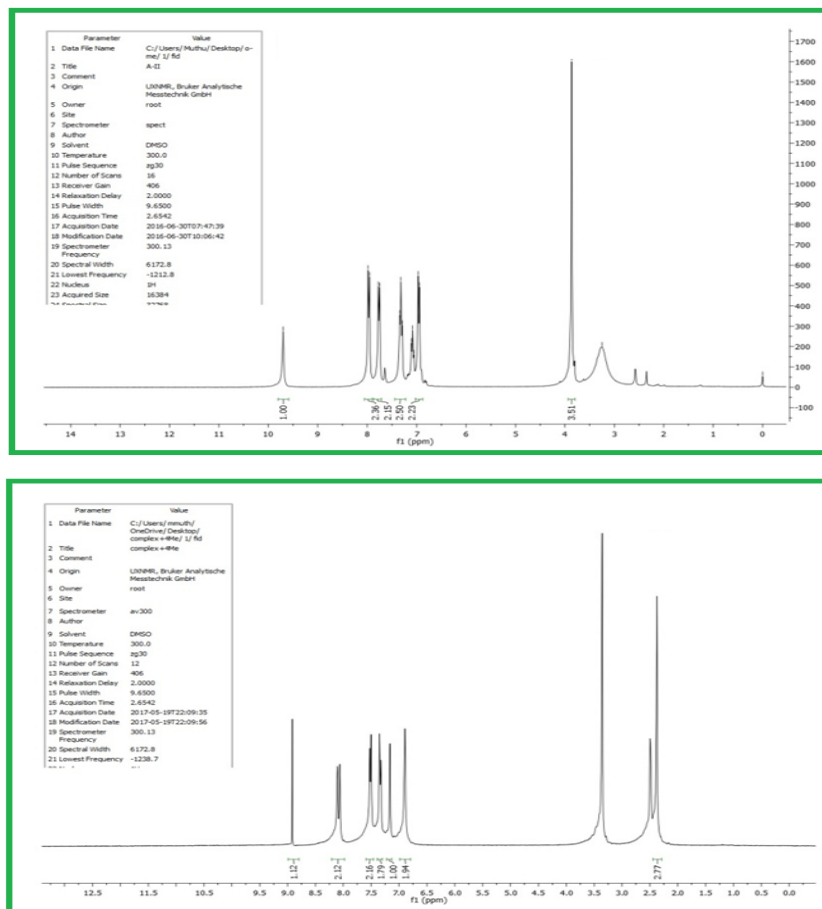


Fig. 4.3 ¹H-NMR spectrum of the Ligand and its Zn(II) complex

The ¹³C NMR spectra of the ligand showed aromatic carbons in the range of 112.71–128.76 ppm and 39.69 ppm become (CH₃) group. The ligand also showed 161.38 ppm due to CH=N carbon which was shifted to upfield at 164.79 ppm upon coordination indicating the participation of -CH=N group in metal complex formation. There was no appreciable change in all other peaks²⁴ as shown in Figure 4.4.

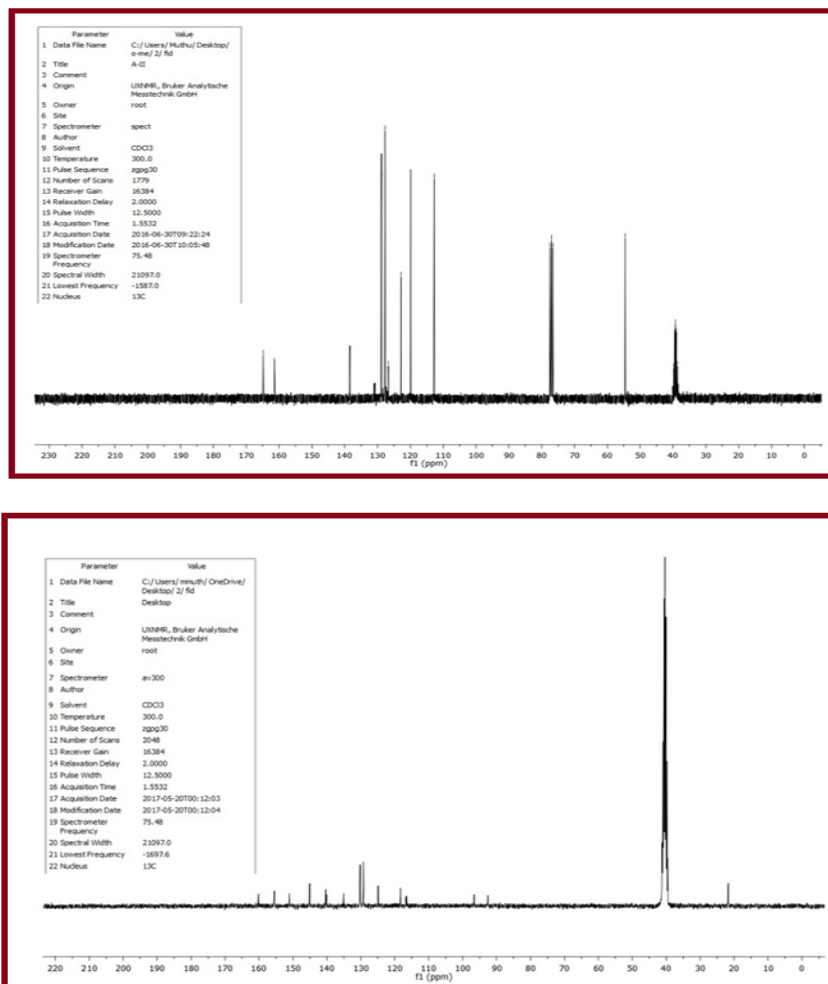


Fig. 4.4 ^{13}C -NMR spectrum of the Ligand and its Zn(II) complex

4.4 Mass spectroscopy

The mass spectra of the ligand and complexes were recorded and compared for their stoichiometric composition. The high-resolution mass spectra of the ligand and its copper complex are shown in Figures 4.5. The molecular ion peak for the ligand was observed at 331 m/z which is also supported by the “Nitrogen rule” since, the compound possesses four nitrogen atoms. For copper(II) complex, the molecular ion peak appeared at 826 m/z which confirms the stoichiometry of metal complexes as being of the ML_2 type. It is also supported by the mass spectra of other complexes²⁵. The mass spectra $[\text{CoL}_2\text{Cl}_2]$, $[\text{NiL}_2\text{Cl}_2]$ and $[\text{ZnL}_2\text{Cl}_2]$ complexes showed the peaks at m/z 821, 822 and 828 corresponding to the molecular ion peak of the respective complexes.

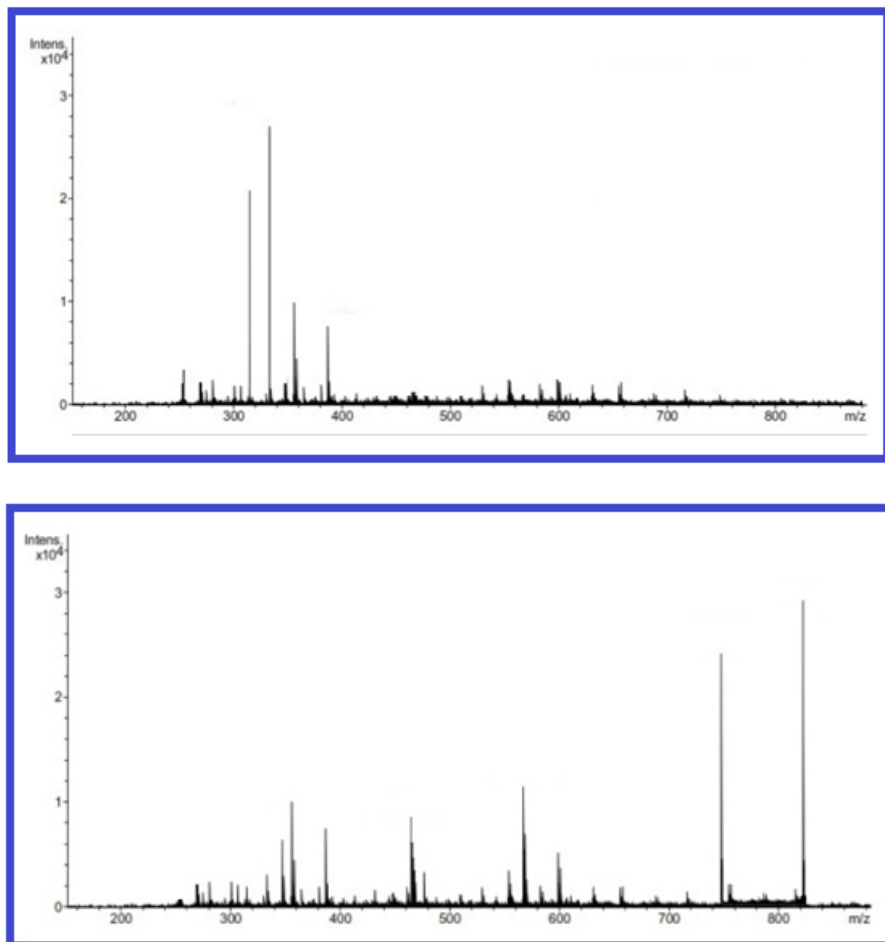


Fig.4.5. HR-Mass spectrum of the Ligand and its Cu(II) complex

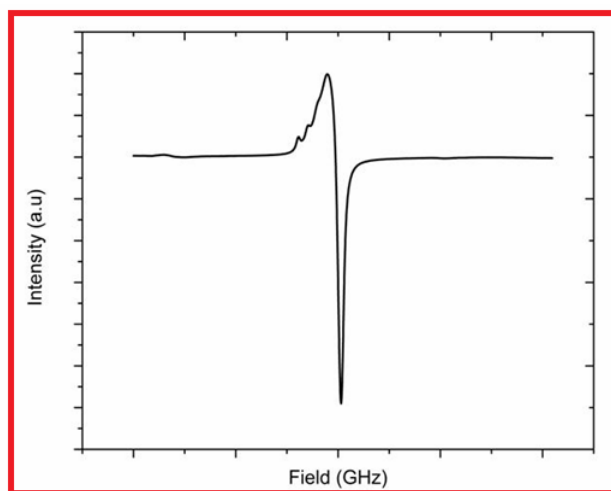
4.5 Electron paramagnetic resonance spectra

EPR spectroscopy has been recognized as the conventional technique to investigate the geometry of metal complexes and the nature of the bonding between the metal ion and its ligands, in particular Cu(II) complexes. The X-band EPR spectrum of the Cu(II) complex was recorded both at liquid nitrogen temperature (LNT) and at room temperature (RT). This spectrum shows four resolved peaks in low field region and one intense peak in the high field region. Because of the tumbling motion of the molecules, an isotropic intense absorption was found in the high field region in the spectra. The EPR spectrum was displayed in Figure.4.6. The spin Hamiltonian parameters of Cu(II) complex was calculated and displayed in Table 4.2. It is calculated that A_{\parallel} (180) > A_{\perp} (93.5). The axially symmetric g -tensor values are g_{\parallel} (2.226) and g_{\perp} (2.024). g_{\parallel} value is greater than g_{\perp} which proves the existence of $d_{x^2-y^2}$ as ground state in the copper complex. This is due to the octahedral geometrical nature of [CuL] complex²⁶.

Table 4.2. The spin Hamiltonian parameters of the $[\text{CuL}_2\text{Cl}_2]$ complex in DMSO solution at LNT

Complex	g-tensor			$A \times 10^{-4} (\text{cm}^{-1})$			$g_{\parallel}/A_{\parallel}$	G
	g_{\parallel}	g_{\perp}	g_{iso}	A_{\parallel}	A_{\perp}	A_{iso}		
$[\text{CuL}_2\text{Cl}_2]$	2.226	2.024	2.072	180	93.5	128.5	124	4.70

In accordance with the literature study, if $G > 4.0$, the local tetragonal axes are in parallel alignment or slightly misaligned or if the value of $G < 4.0$, there is a significant exchange coupling occurs between the multiple copper centers and the distortion is more pronounced. The G value for the copper complex is 4.70 which stoutly supports the local tetragonal axes which are parallelly aligned or slightly distorted and the unpaired electron is present in the $d_{x^2-y^2}$ orbital. This also shows that, there is no exchange coupling present between the Cu(II) centers in solid state²⁷. $g_{\parallel}/A_{\parallel}$ value is 124 cm^{-1} which also implies that Cu(II) complex exhibits the distorted octahedral geometry²⁸.

**Fig.4.6.** EPR spectrum of Cu(II) complex

5. Biological Screenings

5.1. *In vitro* antimicrobial studies

Antimicrobial activity of the Schiff base ligand and its complexes on *E.coli* and *B.subtilis* have shown in Figure 4.7. The zone of inhibition of the Schiff base ligand and its metal complexes against Gram-positive and Gram-negative bacterial strains was determined by using disc diffusion method. The inhibition values are observed that all the metal complexes have shown greater activity than free ligand (Figure. 4.8). This is explained on the basis of chelation theory and overtones concept²⁹.

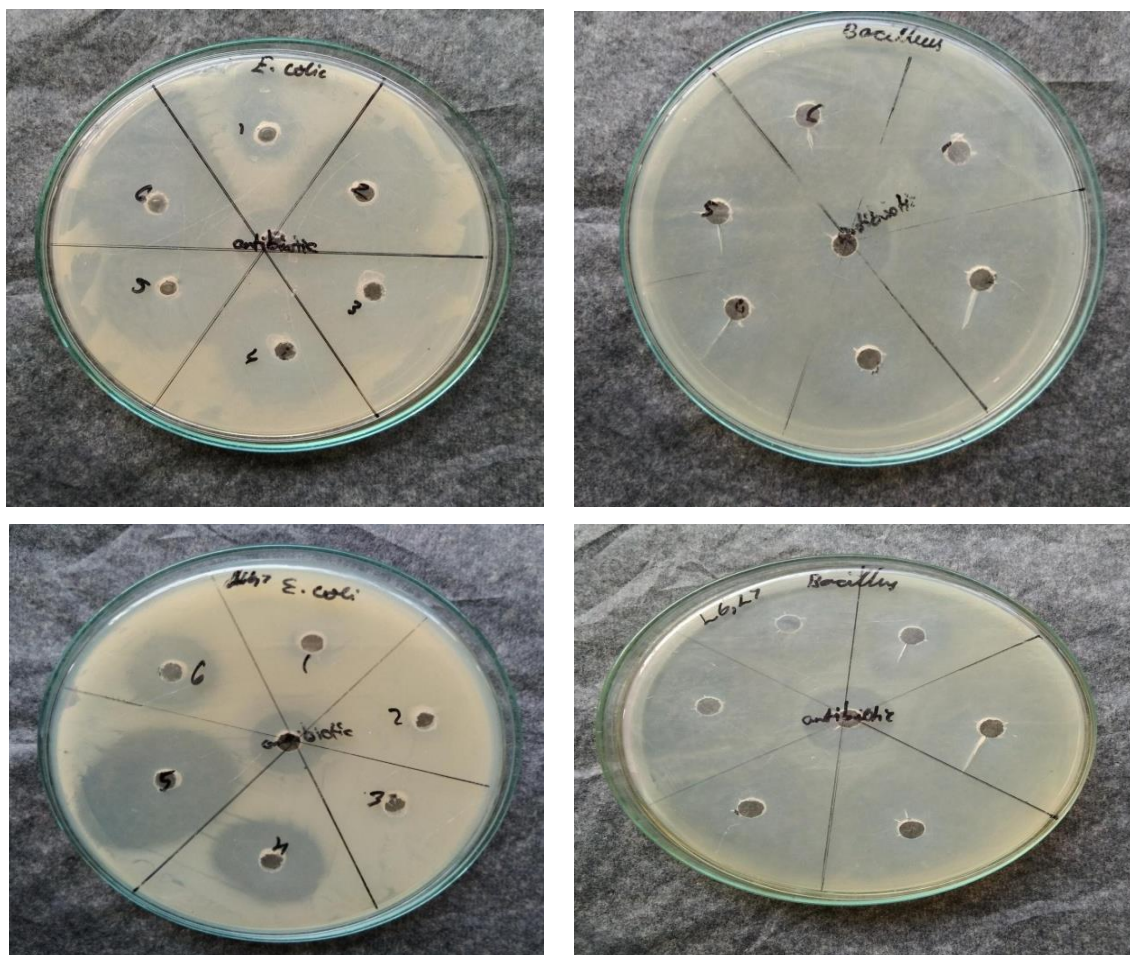


Fig.4.7. Zone formation against growth of bacteria (*B.subtilis* and *E.coli*)

The Cu(II) and Ni(II) complexes have shown improved activity against the *B.subtilis.*, *E.coli* and sensible activity of Co(II) complex with *B.subtilis* and *E.coli*. The Zn(II) complex has shown moderate activity against *B.subtilis* and ligand has shown a lesser amount of activity against *B.subtilis* and *E.coli* bacterial strains. The order of activity is Cu>Ni>Zn>Co. Streptomycin is used as standard drug for Gram-positive and Gram-negative strains respectively.

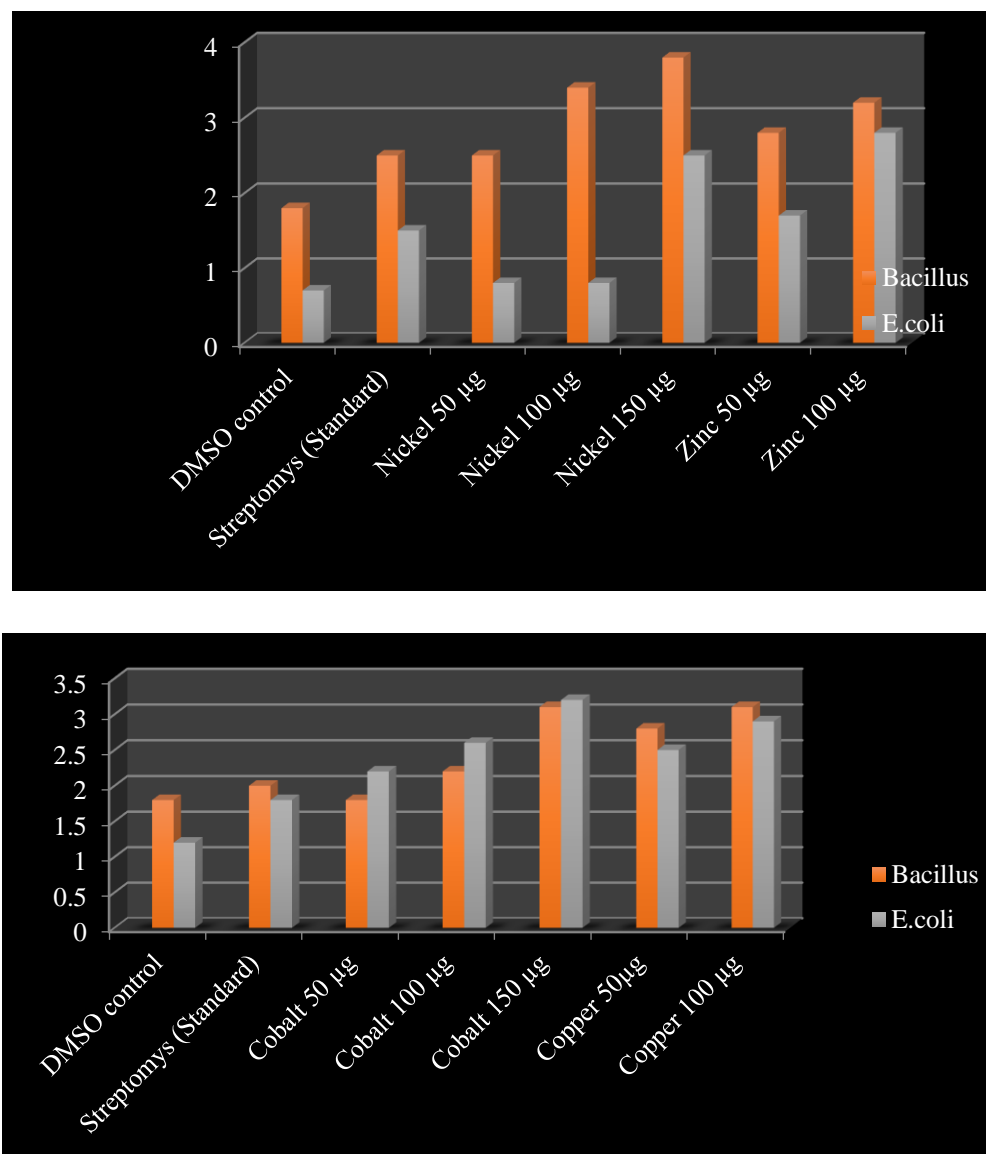


Fig. 4.8. Antimicrobial activity of the synthesized metal complexes using different microbial strains

6. Conclusion

Owing to the existence of nitrogen donors, mononuclear octahedral metal complexes show diverse biological potencies. A novel bidentate Schiff base ligand was prepared by the condensation of 4-bromobenzaldehyde and 2-amino-6-methylbenzothiazole. The ligand has been characterized by microanalytical data, IR, UV-Vis, $^1\text{H NMR}$, EPR and Mass spectra. Its complexes of Cu(II), Ni(II), Co(II), and Zn(II) were synthesized and characterized by spectral and analytical techniques. The other investigations like antimicrobial screening emphasize the higher activity exhibited by the Cu(II) and Co(II) complexes which has a highly conjugative planar ligand, 2-amino-6-methylbenzothiazole in its natural environment. These novel complexes show profound activity with antimicrobial agents and also can be fine-tuned to make them into better antimicrobial agents in future.

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