Original Article

Synthesis, Characterization and Antimicrobial Activity of some Metal Complexes Derived from Thiazole Schiff Bases with *In-vitro* Cytotoxicity and DNA Cleavage Studies

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ABSTRACT

Five metal complexes with Schiff-base ligand, 3-((4-phenylthiazol-2-ylimino) methyl)-2-hydroxybenzoic acid were synthesized with metal ions such as Cu(II), Co(II), Ni(II), Cd(II) and Zn(II). The reaction likely proceeds via condensation of 2-amino-4-phenyl thiazole with 3-aldehydosalicylic acid and characterized by elemental analysis and various spectral studies like FT-IR, ^{1}H NMR, ESI mass, and TGA/TDA and molar conductance studies. The spectral results revealed bidentate O-O donor and forms the complexes having square planar geometry. The antibacterial and antifungal activity of the ligand and its metal complexes was found based on the determination of minimum inhibitory concentrations. The brine shrimp biological assay was also carry out to study the *In vitro* cytotoxicity properties for the ligand and its metal complexes against Artemia salina. Moreover, DNA cleavage experiments revealed that the Cu (II), Co (II) and Zn (II) complexes exhibited remarkable DNA cleavage activities via the generation of hydroxyl radical.

Key words: Thiazole, Schiff base, Antimicrobial, DNA cleavage, *In-vitro* cytotoxicity.

INTRODUCTION

The chemistry of the Schiff base ligands and their metal complexes evoke much current interest and encompasses a vast area of organometallic compounds and various aspects of bioinorganic chemistry.[1] Schiff bases are considered as privileged ligands because they are easily prepared by condensation of aldehydes or ketones with amines and are able to stabilize different metals in various oxidation states.[2,3] The importance of Schiff base complexes for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized and reviewed.[4] Large numbers of Schiff bases have shown to exhibit a wide range of biological activities, including antitumor[5] anti-bacterial[6,7] fungicidal [8] and anticarcinogenic.[9] properties. On the other hand, coordination compounds with heterocyclic Schiff base ligand has attracted much attention of the chemist in current years to find applications as potential drugs,[10,11] due to the presence of multifunctional groups.[12,13] The excessive attention of synthesizing determined broad range of N and S chelating ligands as thiazole molecule have attracted significant interest and gained special attention not only in structural chemistry of their multifunctional coordination modes but also showed importance in medicinal and

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pharmaceutical field.[14] This is because thiazoles have a great pharmacological activity. Many thaizole derivatives such as sulfathiazole, ritonvir, abafungi, blemycin and tiazofurin are well known as potent biologically active compounds.[15,16] Moreover, thiazoles are very important building blocks in medicinal chemistry and can be found in numerous natural products and biologically important compounds including anti-microbial, anti-inflammatory, anti-hypertensive, anti-HIV, anticancer and cytotoxic activity that can be well illustrated by the large number drugs in the market containing the moiety.[17,18] Thiazole ring also found applications in polymer, liquid crystals, photo-nucleases, fluorescent dyes, insecticides and antioxidants.[19,20] Their transition metal complexes have attracted a great deal of interest largely due to their ability to interact with DNA molecule.[21] These complexes that can bind or cleave DNA molecule at exact sites, plays an important role in genomic investigation and in photodynamic therapy against cancer.[22] It is well known that some coordination compounds can inhibit the growth of cancer cells by binding and damaging DNA.[23] Intrigued by the above observations and in continuation of our ongoing research work on synthesis and characterization of Schiff base ligands and their metal complexes, hereby we report the synthesis of a novel Schiff base ligand 3-((4-phenylthiazol-2-ylimino) methyl)-2-hydroxybenzoic acid and its metal complexes, their characterization by different spectroscopic techniques and their antibacterial, antifungal, DNA cleavage and *in vitro* cytotoxicity property.

MATERIALS AND METHODS

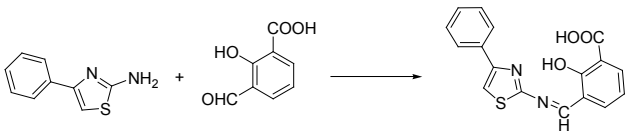
All chemicals employed in synthesis were used as analytical extra-pure grade and solvents are purified according to the literature methods.[24] The melting points of newly synthesized compounds are determined in open glass capillary tubes and are uncorrected. Purity of the compounds was checked by TLC and the spots observed in iodine vapours. The presence of metal and chlorides contents was determined according to standard procedure.[24] Compound, 2 amino- 4- phenyl thiazole were prepared according to reported method.[25]

Physical Measurements

IR spectra of the new synthesized Schiff base and its metal complexes were recorded in KBr pellets on a Perkin-Elmer FT-IR instrument in the range 4000-250 cm^{-1}. ^1H NMR spectra were recorded on Bruker Avance II 400 MHz NMR spectrometer in DMSO-d6 using TMS as an internal standard. ESI mass spectra were recorded by electrospary ionization (ESI) on a waters micro mass Q-TOF micro spectrometer. Thermal analysis of the complexes was carried out on a Perkin-Elmer STA 6000 thermal analyzer in static air with a heating rate of 20^0C/min. Molar conductance was measured on the ELICO (CM-185) conductivity bridge using 10^{-3} M solution in dry DMF by dip type conductivity cell fitted with a platinum electrode. Elemental analysis (C, H and N) were performed on a Vario EL III CHNS analyzer.

Synthesis of Schiff base ligand

Synthesis of Schiff base ligand was summarized in **Scheme 1**. An equimolar mixture of 2-amino-4-phenyl thiazole (0.01 mol) and 3- aldehydosalicylic acid (0.01 mol) with a catalytic amount of glacial acetic acid (1-2 drops) in ethanol (25 ml) was refluxed on a water bath for about 4-5 hrs. The reaction was monitored by thin-layer chromatography. The brown solid separates were filtered, washed with little ethanol, dried and recrystallized from alcohol. Melting point was (180-190^{0}C) yield 72%; molecular weight (324) g per mol $C_{17}H_{12}N_2O_3S$, Elemental analysis: found (calc.): C 63.20(63.15), H 3.36(3.73), and N 8.57 (8.60).



Scheme 1: Synthesis of Schiff base ligand

Syntheses of the Schiff base metal complexes

An ethanolic solution (25 ml, 0.01 mol) of the appropriate metal chloride MCl_2 (M=Co, 2.378 gm; M=Ni, 2.377 gm; M=Cu, 1.705gm, M=Zn, 1.363 gm, M=Cd, 2.013gm) was added to an ethanolic solution (30 ml) of the Schiff base. The reaction mixture was refluxed for about 4 hrs, during which no solid separated out. An aqueous alcoholic solution of sodium acetate (0.5gm) was added to the reaction mixture to maintain a pH about 6.0-7.0 and reflux was continued for about an hour. The reaction mixture was transferred into the distilled water. The separated solid complexes was collected by filtration, washed with minimum quantity of distilled water and dried in a vacuum over anhydrous calcium chloride in desiccators.

Pharmacology

Antimicrobial activities

The biological activities of the synthesize Schiff base and its metal complexes were studied under antibacterial and antifungal activities by disc and well diffusion method respectively.[26] The *In vitro* antibacterial activities of the compounds be tested against two Gram positive *Bacillus subtilis* (MTCC 736) and *Staphylococ-*

cus aureus (MTCC 3160) and two gram negative *Salmonella typhi* (MTCC 98) and *Escherichia coli* (MTCC 46) bacteria. The *In vitro* antifungal an activity was carried out against *Candida albicans* (MTCC 227), *Cladosporium oxysporum* (MTCC 1777) and *Aspergillus Niger* (MTCC 1881) fungi.[27,28] The stock solution of the test chemicals (1 mg mL^{-1}) was prepared by dissolving 10 mg of the each test compound in 15 ml of distilled DMSO solvent. The different concentration of the test compounds (100, 75, 50, 25 and 12.5 µg mL^{-1}) prepared by diluting the stock solution with the required amount of freshly distilled DMSO. In addition a controlled experiment was carried out by using freshly distilled DMSO solvent alone.

Antibacterial Screening

Muller-Hinton agar media were used for the antibacterial studies. The dehydrated Muller-Hinton agar (38gm) was dissolved in 1000 mL distilled water. The pure culture of the bacterial strains *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* was sub cultured by inoculating in the nutrient broth and they were incubated at 37^{0}C for 18 hrs. The agar plates were prepared by using the above Muller-Hinton agar media, and wells were dug with the help of 6 mm sterile metallic cork bore. Each plate was inoculated with 18 hrs old bacterial culture using a micropipette and spreaded regularly using bent glass rod on each plate. The drug Gentamycin is used as per standard. Different concentrations of the test compounds was incorporated into the wells using micropipette and the plates were kept for incubation period, the diameter of the inhibition zone generate by each test compound against bacterial growth was measured using anti biogram zone measuring scale.

Antifungal screening

Potato dextrose agar (PDA) media was used for the antifungal studies. The following ingredients were used prepare the media: potatoes (sliced washed unpeeled) 200 g, dextrose 20 g, and agar 20 g in 1000 mL distilled water. The pure cultures *Cladosporium oxysporum*, *Candida albicans*, and *Aspergillus Niger* were inoculated on PDA slants. These slants were incubated at 32^{0}C for 7 days. To these 7 days old slants of fungal strains, 10 mL of 0.1% tween-80 solution were added, and the culture were scraped with sterilized inoculating loop to get uniform spore suspension. The agar plates were prepared through using the above Potato dextrose agar media and wells were dug with the help of 6 mm sterile metallic cork bore. Each plate was inoculated with 7 days old spore suspension of each fungal culture using a micropipette and spreaded regularly using bent glass rod on each plate. Next each well was incorporated with the test compound solution of different concentrations. The drug *Flucanozole* is used as standard. All the inoculated plates were incubated at 32^{0}C for 48 hrs. Soon after the completion of incubation period the diameter of the inhibition zone generated by each test compound against fungal growth is measured using antibiogram zone measuring scale.

DNA cleavage Experiment

To extent to which the newly synthesized ligands and metal complexes could function as DNA cleavage agents they were examined using plasmid pBR322 DNA (Merck Genei, Bengaluru, Cat. No.105850) as target molecule according to the literature method.[29] The cleavage activity of the test compounds was analyzed by agarose gel electrophoresis method. The 600 mg of agarose was dissolved in 60 mL of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA) by boiling. When the gel attains approximately 55^{0}C, it was poured into the gel cassette fitted with comb. The gel was allowed to solidify and then carefully the combs were removed. The gel was placed in the electrophoresis chamber flooded with TAE buffer. A test compound was prepared in DMSO (1mg mL^{-1}). The test compounds were added separately to the isolated plasmid pBR322 DNA (225 mg) and incubated for 2 hrs at 37^{0}C. After the incubation period, the 20 μL of DNA sample (mixed with bromophenol blue dye at 1: 1 ratio) is loaded carefully into the electrophoresis chamber wells along with standard DNA marker and a constant electricity of 50V passed for about 30 min. The gel was removed carefully and stained with Ethidium bromide (EtBr) solution (10μg/mL) for 10-15 min. The bands were observed under UV transilluminator (UVP, Germany) and photographed to determine an extent of DNA cleavage, and the results were compared with those of a standard DNA marker.

In vitro cytotoxicity

The brine shrimp lethality biological assay has been chosen to evaluate the *in vitro* cytotoxicity effect of the newly synthesized Schiff base ligand and its metal complexes by using the protocol of Meyer *et.al.*[30] This is an efficient, rapid, inexpensive test and has been good correlation with cytotoxic activity. Brine shrimp (Artemia salina) eggs was hatch in a shallow rectangular plastic dish (22 x 32 cm) filled with artificial seawater, which was prepared with a mixture of commercial salt and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated apparatus. Approximately 50 mg of eggs are sprinkled into

the large compartment, which has darkened while the minor compartments were open to ordinary light. Two days after nauplii were collected by pipette by lighted side. Samples of the test compound were prepared by dissolving 20 mg of each compound in 2 ml of DMSO. From this stock solution 100, 50 and 25 μg mL^{-1} was transferred to nine vials (three for each dilution were used for each test sample and LD_{50} is the mean of three values and one vial were kept as control having 2 mL DMSO only. The solvent were allowed to evaporate overnight. After two days, when shrimp larvae was ready, 1 mL of seawater and 10 shrimps was added to each vial (30 shrimp/dilution) and the volume was adjusted with seawater to 10 mL per vial. After 24 hrs the number of survivors was counted. Data is analyzed by a Finney computer program to determine the LD_{50} values.[31]

RESULTS AND DISCUSSION

Chemistry

In our study, a new Schiff base based thiazole derivative has been synthesized by condensation of 2-amino-4-phenyl thiazole and 3-aldehydosalicylic acid in the presence of catalytic amount of glacial acetic acid with good yield of 80% as shown in **Scheme-1**. The purity of the current Schiff base ligand was checked by running TLC on a silica gel coated plate using Cyclohexane-Ethyl acetate (80:20%) as the eluent. The complexes were synthesized by the reaction of thiazole ligand with metal chloride in 1:1 M ratio in ethanol **Schemes-2**. The ligands and their complexes were found to be stable at room temperature and soluble in in DMSO and DMF. Molar conductance data of the metal complex was measured in DMF at 10^{-3} M and all the complexes showed conductance in the range of 40-62 ohm^{-1}cm^{2}mol^{-1} at ambient temperature indicating non-electrolytic in nature [24] and outside their coordination sphere there is no counter ion present. The thermal nature of the complexes has been obtained by TGA/DTA analysis. The analytical and physical data of thiazole ligand and their metal complexes are presented in Table 1. The formation of thiazole ligands frameworks and bidentate O-O donar nature of the Schiff base with metals for the formation of complexes were obtained from characteristic band positions in FT IR and resonance signals in ^{1}H NMR, Mass spectra and elemental analysis. These data of the metal complexes suggest that metal to ligand ratio of the metal complexes 1:1 stoichiometry of the type [(M) (L) $(Cl)_2$] where M is metal and L is ligand as shown in **Scheme-2**.

IR spectral studies

The identification for the formation of thiazole Schiff base were obtained from the absence of IR characteristic band for amino group attached to thaizole ring and the carbonyl group of aldehyde. The IR spectrum of the Schiff base ligand clearly indicates sharp band at 1697 cm^{-1} is due to presence of carbonyl group (C=O) of acid and displayed one sharp band at 1629 cm^{-1} is due to C=N of azomethine group of Schiff base. Two broad bands observed at 3152 cm^{-1} and 3326 cm^{-1} is attributed to the presence of phenolic -OH group and carboxylic -OH group in Schiff base compound respectively Figure 1. To study the binding mode of Schiff base to the central metal ion in the complex. IR spectrum of the metal complexes the disappearances of phenolic -OH group and carboxylic -OH stretching frequency was observed. This indicates that phenolic –OH and carboxylic -OH coordinates to the central metal ion. In all metal complexes the phenolic –OH and carboxylic -OH group stretching frequency disappeared at 3160 cm^{-1} and 3320 cm^{-1} region, this indicates that phenolic oxygen and carboxylic directly coordinating with metalions [25] Figure 2. The complexation of meal ion with ligand were further confirmed by the appearance of new weak intensity, non ligand bands in the region appears at 534 to 582 cm^{-1} due to presence of metal oxygen band (M-O) and another bond forms at region 354-379 cm^{-1} due to M-Cl bond Figure 3. The IR data of Ligand and its metal complexes were presented in Table 2.

^{1}H NMR spectrum

The ^{1}H NMR data of Schiff base and its Zn (II) complex are presented in Table 3. The proton spectrum of Schiff base display 8.0 ppm appears as singlet due to the presence of CH=N and 8 aromatic protons appears as multiplates in the region between 7.25 to 7.40 ppm. Carboxylic acid proton appears as singlet 12.80 ppm (s, 1H COOH) and a phenolic proton appears at 7.14 ppm (s, 1H OH). The ^{1}H NMR of zinc (II) complex displayed all aromatic protons as multiplates in region 7.30 to 8.09 (m, 8H Ar-H). ^{1}H NMR spectral data of Schiff base ligand and its zinc (II) complexes confirms the formation of Zn -complexes with ligand. One phenolic -OH proton and carboxylic -OH disappearance is observed in the Zn complex ^{1}H NMR spectra. Accordingly Figure 4 and 5 this clearly confirms about phenolic oxygen coordinate with metal ion in the complex.

Mass Analysis

The ESI mass spectra of Schiff base ligand and its Co (II) and Ni (II) complexes are performed to determine their molecular weight and study their fragmentation. The mass spectrum of ligand showed a peak recorded

at m/z 324 due to M^{+1} corresponding to the molecular weight of the ligand. Further, this molecular ion underwent fragmentation peak recorded at m/z 137 (38 %) followed by expulsion of $C_7H_5O_3$ molecule gave a fragment ion peak. The fragmentation of ligand may represent the C_3HN_2S gives at m/z 96.99 (12%) value and remaining fragmentations are in agreement with the molecular formula Figure 6. The ESI mass spectrum of Co (II) complex shown a peak due to M^{+1} at 450 m/z. this molecular ion underwent fragmentation at 292 and 162 loss of $C_8H_4Cl_2CoNO_3$ and C_9H_6NS respectively. Similarly, ESI mass spectrum of Ni (II) complex shows a peak due to M^{+1} at 449.9 m/z. This molecular underwent fragmentation at 291 and 162 due to loss of $C_8H_4Cl_2NNiO_3$ and C_9H_6NS respectively Figure 7.

Thermal studies

In the present investigation, heating rates were suitably controlled at 20^0C min^{-1} under inert atmosphere the weight loss was measured from the ambident temperature up to loss 700^0C. The results of the TGA of the metal complexes show that the metal complexes lost their lattice water in the rage show 100-180^0C. The presence water molecule in the lattice suggested by IR spectra is also confirmed by TGA. TGA and TDA curves Cu (II) complexes showed that the complex is stable up to 230^0C and no weight loss is observed before this temperature. The first degradation occurred at 231.88^0C, with the loss of the two chlorine atoms. The organic moiety decomposed further with increasing temperature. Although the decomposed fragments of ligand could not approximated owing to continuous weight loss as indicated by horizontal plateau on the

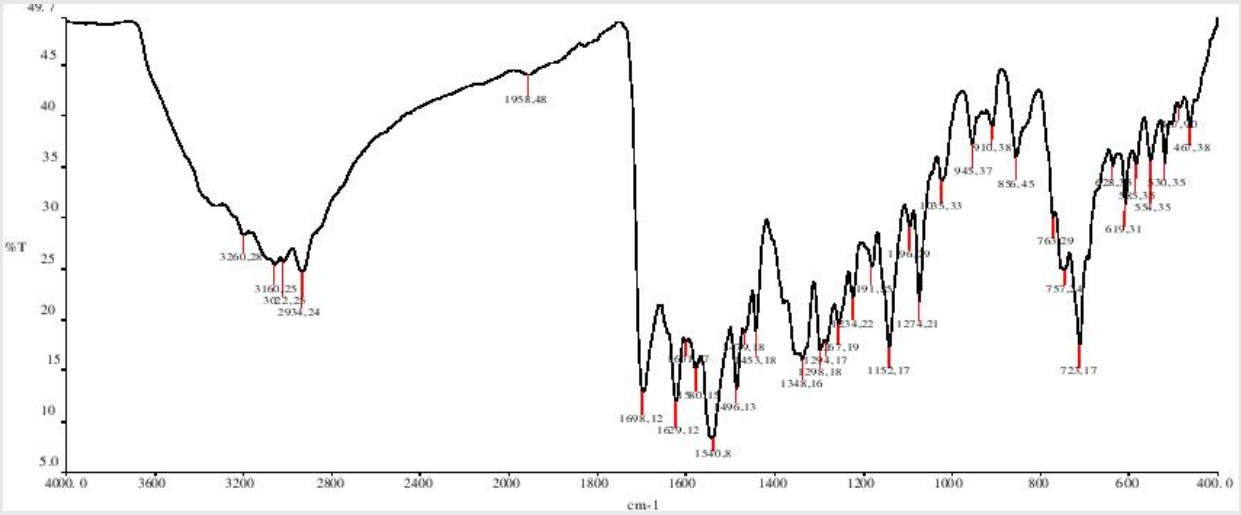


Figure 1: IR Spectra of Schiff base ligand.

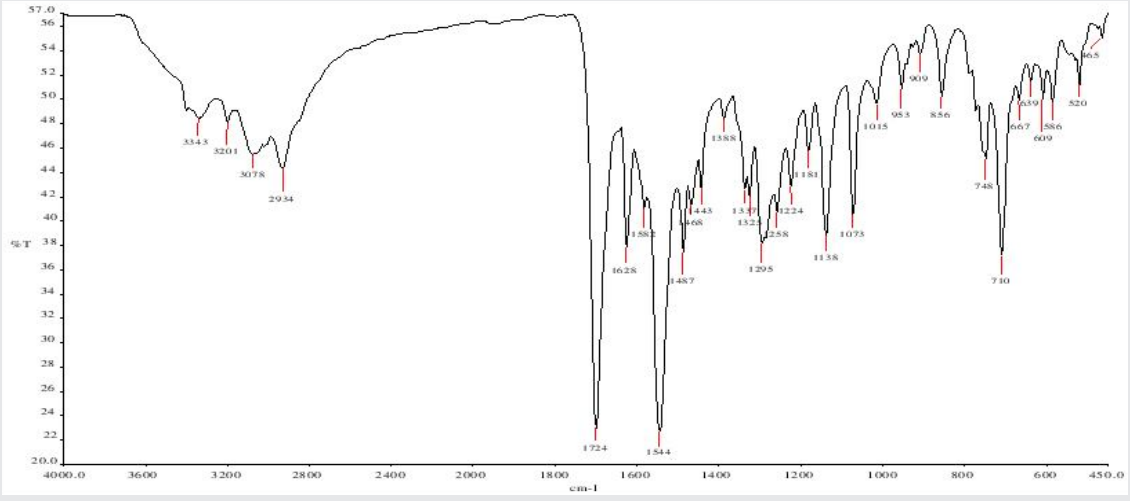


Figure 2: IR Spectra of metal complex Cu (II).

TG curves for Cu complex. The complete decomposition of ligand occurred at ≈620^{0}C for Cu complex. At the end final step i.e. 550^{0}C-700^{0}C stable metallic oxides were found.[26] The decomposition of complexes as higher than ligand indicates that the thermal stability of the complexes is increased due to the ligand coordinated with metal ion to form ring.

Antimicrobial Results

In vitro Antimicrobial Activity

The *In-vitro* antimicrobial activity of all newly synthesized compounds were screened against *E.coli*, *S.aureus*, *B.subtilis* and *S.typi* bacteria and *C.albicans*, *C.oxysporum*, and *A.niger* fungal strains by minimum inhibitory concentration (MIC) method. The MIC profile of the entire compound against bacteria and fungi are summarized in Table 4. A comparative study of the ligand and their metal complexes indicates that complexes exhibit higher antimicrobial activity than free ligand and this activity enhanced on coordination with metal ions. This enhancement in the activity may be rationalized on the basis that ligands mainly posses C=N bond. The enhanced activity of the complexes over the ligand can be explained on the basis of chelation theory.[27,28] It is observed that in complex, the

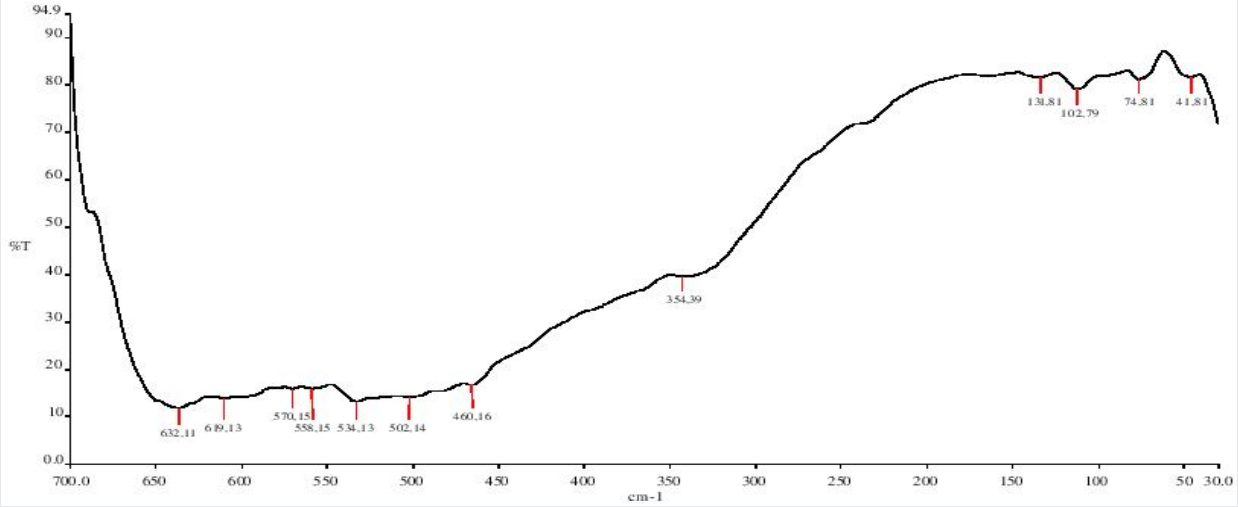


Figure 3: FAR- IR Spectra of metal complex Cu (II).

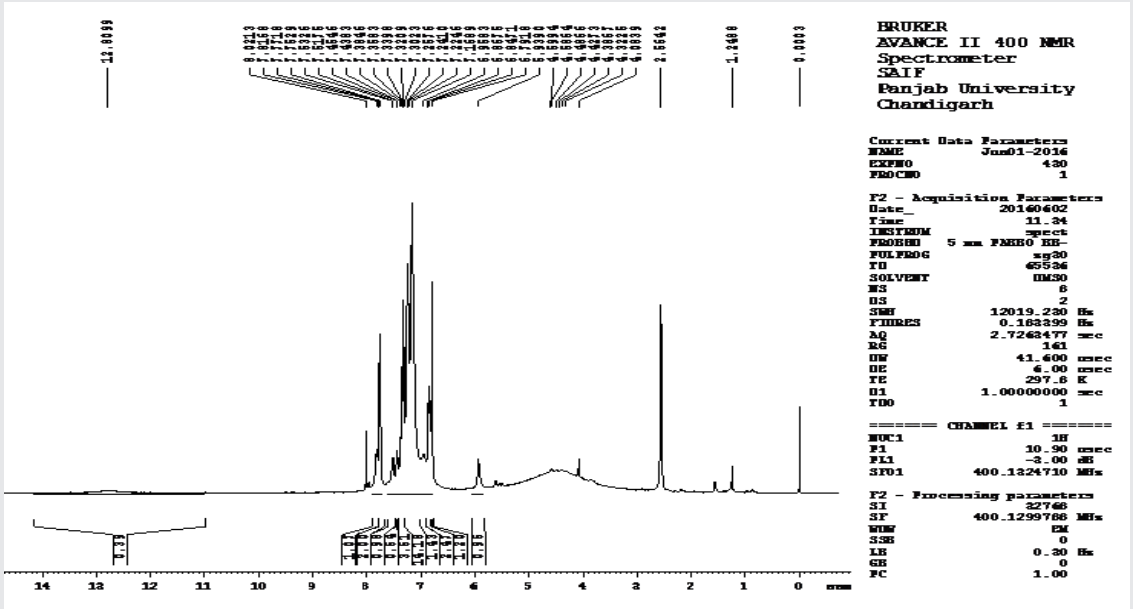


Figure 4: ^{1}H NMR of Schiff base ligand.

positive charge of the metal is moderately mutual with the donor atoms present in the ligand and there may be π-electron delocalization over the whole chelating.[29] This increases the lipophilic character of the metal chelates and favors its permeation through the lipoid layer of the bacterial membranes and also other factors which increase the activity, namely solubility, conductivity, and bond length between the metal and the ligand.

DNA Cleavage Activity

The interaction of plasmid pBR322 DNA with newly synthesized ligand and its metal complexes was studied using agarose gel electrophoresis method. The gel picture showing cleavage of plasmid pBR322 DNA is shown in Figure 8. The characterization of DNA recognition by transition metal complexes has been aided by the DNA cleavage chemistry associated with redox-active or photo activated metal complexes.[30,31] The electrophoresis analysis clearly revealed that the ligand and its metal complexes acted on DNA because of a difference in molecular weight between the control and treated DNA samples. The differences were observed in the bands of lanes of complexes compared with the

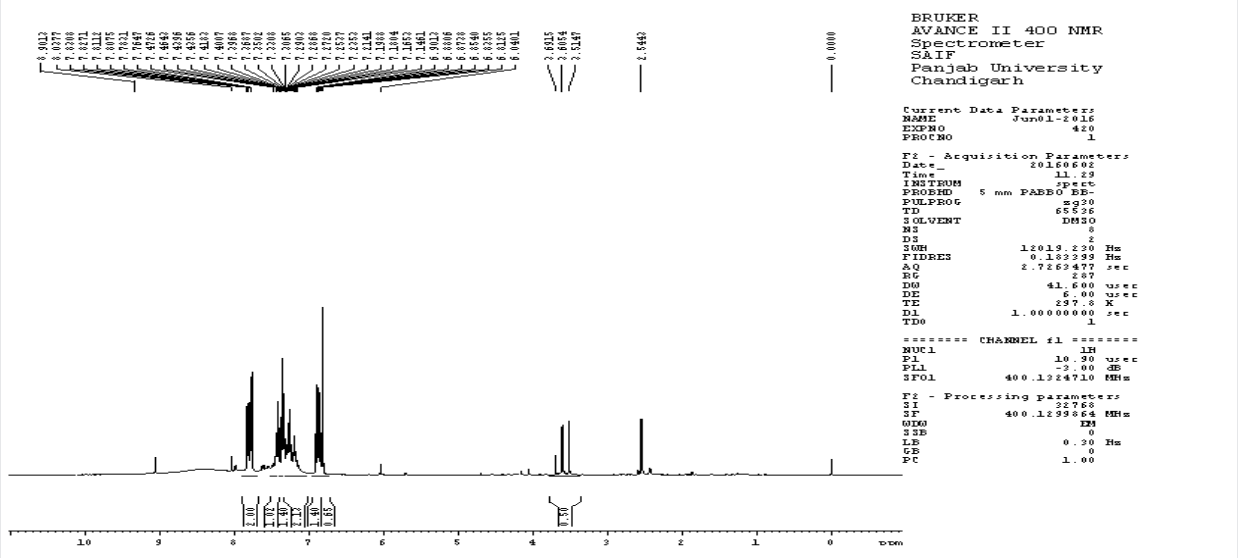


Figure 5: ^{1}H NMR of metal complex Zn (II).

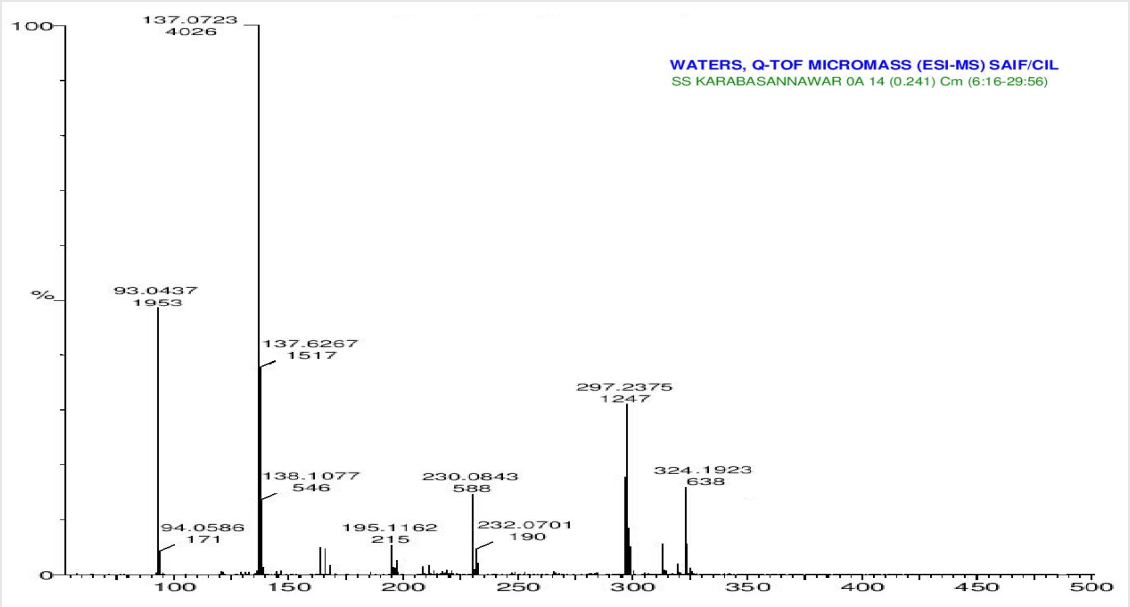


Figure 6: Mass spectrum of the Schiff base.

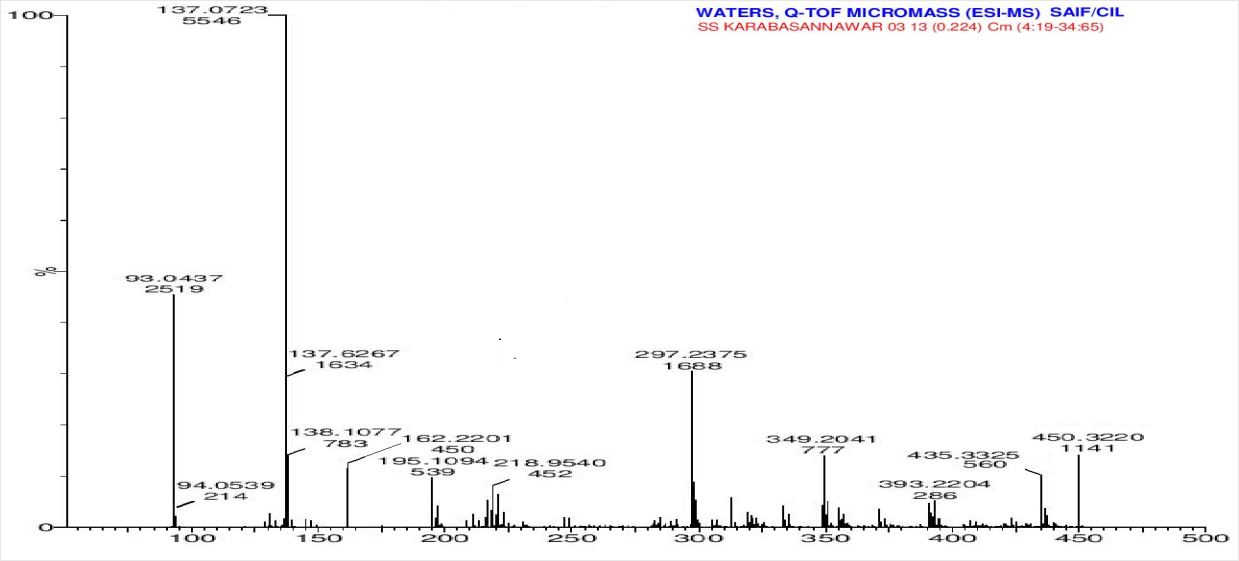


Figure 7: Mass spectrum of the metal complex Co (II).

Table 1: Physical, Analytical and Molar Conductance data of Ligand and its complexes

Compounds	Molecular formula	Mol. Wt	M .P	Elemental analysis [%] (calc.)					Molar Conductance	Color
				M	C	H	N	Cl		
Schiff base	$C_{17}H_{12}N_2O_3S$	324	180-190^{0}C	-	63.20 (63.15)	3.36 (3.73)	8.57 (8.60)	-	-	Brown
Cu complex	$[Cu(C_{17}H_{10}N_2O_3S)Cl_2]$	454.9	>250^{0}C	13.72 (13.89)	44.53 (44.59)	2.35 (2.40)	6.23 (6.14)	15.49 (15.52)	50	Green
Zn complex	$[Zn\ (C_{17}H_{10}N_2O_3S)Cl_2]$	458.6	>262^{0}C	14.18 (14.26)	44.82 (44.41)	2.23 (2.39)	5.59 (6.11)	15.43 (15.46)	48	Grey
Ni complex	$[Ni\ (C_{17}H_{10}N_2O_3S)Cl_2]$	459.9	>258^{0}C	6.10 (6.21)	44.19 (45.18)	2.13 (2.23)	5.09 (6.20)	15.39 (15.69)	56	Grey
Co complex	$[Co(C_{17}H_{10}N_2O_3S)Cl_2]$	452.1	>254^{0}C	12.90 (13.03)	45.06 (45.16)	2.03 (2.13)	5.19 (6.20)	15.78 (15.68)	60	Blue
Cd complex	$[Cd(C_{17}H_{10}N_2O_3S)Cl_2]$	505.6	>256^{0}C	22.09 (22.23)	44.07 (45.16)	2.09 (1.99)	6.02 (5.54)	15.07 (14.02)	55	Purple

Table 2: The IR data of Ligand and its metal complexes (cm^{-1})

Compounds	C=O	C=N	Phenolic -OH	Carboxylic -OH	M-Cl	M-O
Schiff base	1697	1629	3160	3260	---	---
Cu complex	1724	1628	----	----	534	368
Co complex	1708	1628	----	----	562	354
Ni complex	1719	1621	----	----	538	359
Zn complex	1721	1626	----	----	582	379
Cd complex	1716	1624	----	----	546	362

Table 3: The ^{1}H NMR data of ligand and its Zn (II) complex

Ligand/complex	^{1}H NMR ppm
Ligand	8.0 (s, 1H CH=N), 7.25 to 7.40 (m, 8H, Ar-H), 7.14(s,1H OH), 8.90 (s, 1H COOH),
Zn complex	8.15 (s, 1H CH=N), 7.30 to 7.45 (m, 8H, Ar-H),

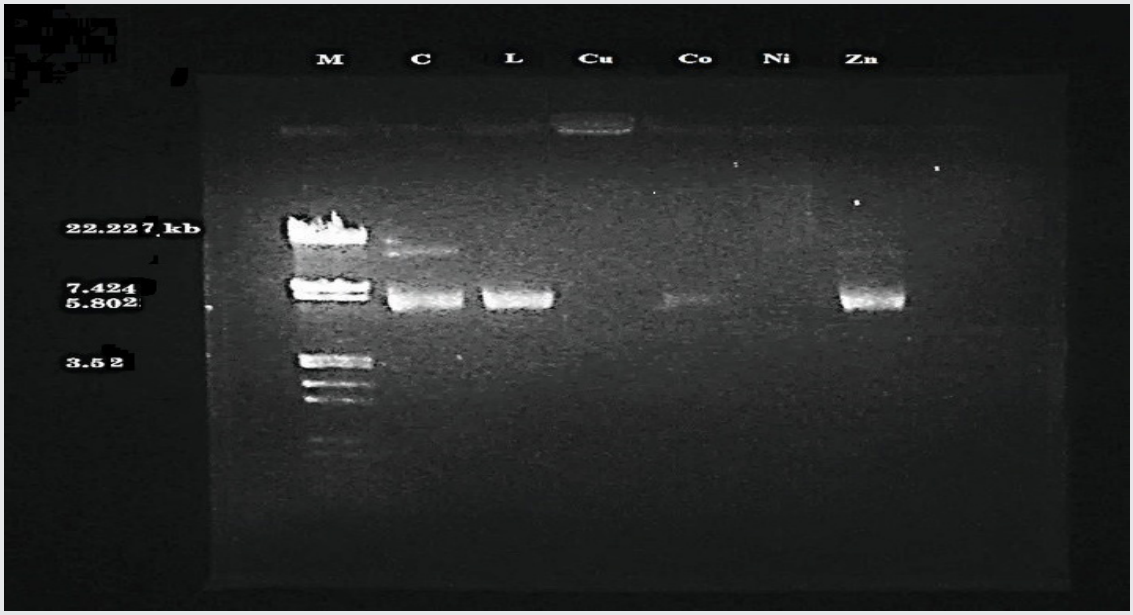


Figure 8: 8:DNA cleavage on plasmid pBR 322: M: standard DNA, C: control DNA, L: Schiff base ligand, Cu: Cu (II) complex, Co: Co(II) complex, Ni: Ni(II) complex and Zn: Zn(II) complex.

Table 4: Minimum inhibitory concentration (MIC μg mL^{-1}) of Schiff base ligand and its metal complexes.

	MIC Value in µg mL^{-1}						
Compounds	**Bacteria**				**Fungi**		
	E.coli	**S.aureus**	**B.subtilis**	**S.typhi**	**C.albicans**	**C.oxysporum**	**A.niger**
Schiff base	50	76	50	100	75	51	50
Cu complex	13	25	25	50	25	13	13
Co complex	25	50	13	50	13	13	25
Ni complex	13	25	25	50	25	13	25
Zn complex	25	50	25	75	50	25	25
Cd complex	13	13	13	25	13	25	13
Gentamycin	13	13	13	13	-	-	-
Fluconazole	-	-	--		13	13	13

control DNA of pBR322 due to the relaxation of circular DNA into linear form and this shows that the control DNA alone does not show any apparent cleavage, whereas the ligand and its metal complexes do show. In the current investigation, the Ethidium bromide (EtBr) stained banding pattern of plasmid pBR322 DNA were tested with newly synthesized ligand and its metal complexes. In the present case, the ligand and its Cu (II), Co (II) and Zn (II) complexes showed complete cleavage of super coiled DNA and the Ni (II) and Cd (II) complex showed incomplete cleavage of relaxed DNA and complete cleavage of super coiled DNA. This clearly revels the important role of coordination of O-O groups to the metal ion in these DNA cleavage activities. On the basis these information's, it has been concluded that all the newly synthesized compounds under present study are good pathogenic microorganisms inhibitor; as evident on the DNA cleavage of pBR322.

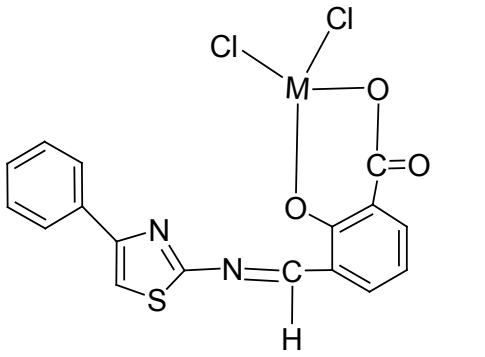
In vitro cytotoxicity

All the synthesized compounds was screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Mayer *et al*.[30] From the data recorded in table it is evident that all newly synthesized metal complexes exhibited potent activity when compared to the free ligand. The Cu (II) and Ni (II) complexes displayed significant potent activity as LD50=1.169 x 10^{-4} and 1.174 x 10^{-4} M/mL respectively against Artemia salina. The *In vitro* cytotoxicity results are summarized in Table 5.

Table 5: Brine shrimp bioassay data of the Schiff base (L) and its metal complexes	
Compound	LD 50(M/mL)
Ligand	2.5470×10^{-4}
Cu-complex	1.169×10^{-4}
Co-complex	1.287×10^{-4}
Ni-complex	1.174×10^{-4}
Cd-complex	1.316×10^{-4}
Zn-complex	2.309×10^{-4}

CONCLUSION

The newly synthesized Schiff base ligand 3-((4-phenylthiazol-2-ylimino) methyl)-2-hydroxybenzoic behaves as bidentate O-O donor and the complexes of square planar type [ML $(Cl)_2$]. The help of various physico-chemical and spectroscopic methods such as IR, ^{1}H NMR , the square planar geometries of Cu(II), Co(II), Ni(II), Cd(II) ad Zn(II) complexes have been proposed **scheme 2**. The newly synthesized metal complexes having good antimicrobial activity when compared to Schiff base ligand. The DNA cleavage activity of all the newly synthesized compounds showed the cleavage of plasmid DNA pBR 322 and cytotoxicities of Cu (II) and Ni (II) complexes indicate potent cytotoxic agents that might become potent anticancer agent in clinical trials.



Scheme 2: Proposed Structure of the complex

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CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATION USED

FT-IR: Fourier Trans- form Infrared; **1H NMR:** Proton Nuclear Magnetic Resonance; **ESI mass:** Electrospray Ionization Mass Spectrometry; **TGA/TDA:** Thermo gravimetric analysis/Differential thermal analysis; **DNA:** Deoxyribonucleic acid; **TLC:** Thin-layer chromatography; **DMSO:** Dimethyl sulfoxide; **TMS:** Tetramethylsilane; **DMF:** Dimethylformamide; **MTCC:** Microbial Type Culture Collection and Gene Bank; **PDA:** Potato dextrose agar; **EDTA:** Ethylene diamine tetraacetic acid; **MIC:** Minimum inhibitory concentration; **LD_{50}:** Lethal Dose; **ETBr:** Ethidium bromide.

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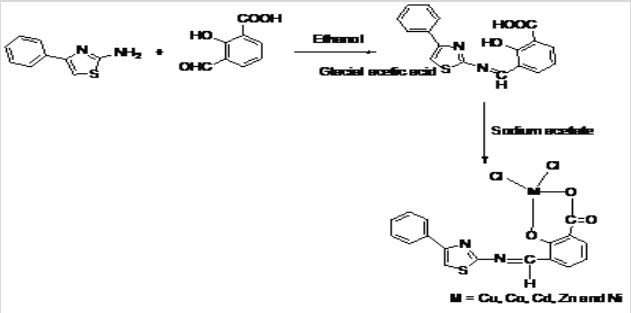
PICTORIAL ABSTRACT



SUMMARY

- The newly synthesized Schiff base ligand 3-((4-phenylthiazol-2-ylimino) methyl)-2-hydroxy-benzoic behaves as bidentate O-O donor and the complexes of square planar type [ML (Cl) 2]. The facilitate of various physicochemical and spectroscopic methods such as IR, 1H NMR , the square planar geometries of Cu (II), Co (II), Ni (II), Cd (II) ad Zn (II) complexes have been proposed. The currently synthesized metal complexes having good antimicrobial activity when compared to Schiff base ligand. The DNA cleavage activity of all the newly synthesized compounds showed the cleavage of plasmid pBR 322 DNA and cytotoxicities of Cu (II) and Ni (II) complexes indicate potent cytotoxic agents that might become potent anticancer agent in clinical trials.

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