



Preparation, Characterization study of 2-(2-hydroxy-4-(phenyldiazenyl)benzylidene)-N-(4-phenylthiazol-2-yl) hydrazinecarboxamide and its Metal Complexes

Mahadev D Udayagiri^{1,2}, Mahendra Raj K^{1,3}, Nagesh Gunavanthrao Yernale^{1,4}, Shivakumar K² and B.H.M. Mruthyunjayaswamy^{1*}

1. Department of Studies and Research in Chemistry, Gulbarga University
Kalburagi - 585 106, Karnataka, **INDIA**

2. Government College, Sedam Road, Kalburagi - 585 106, Karnataka, **INDIA**

3. BLDE Association's, PG Department of Chemistry, S.B. Arts & K.C.P. Science College,
Vijayapur - 586 103, Karnataka, **INDIA**

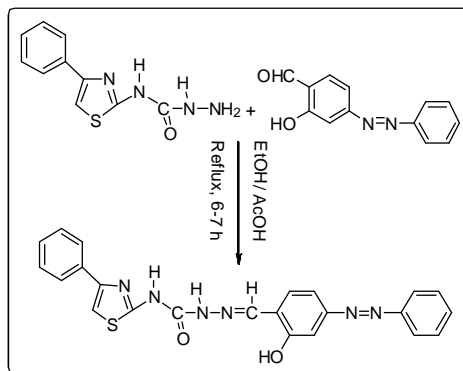
4. G.E. Society's, Aditya Women's Science and Commerce Degree College, Bhalki - 585 328
Karnataka, **INDIA**

E-mail: bhmmswamy53@rediffmail.com

Accepted on 2nd March, 2018

ABSTRACT

The Schiff base ligand (L) 2-(2-hydroxy-4-(phenyldiazenyl)benzylidene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide obtained by the condensation of N-(4-phenylthiazole-2-yl)hydrazinecarboxamide with 2-hydroxy-4-(phenyldiazenyl)benzaldehyde and its newly synthesized Cu(II), Co(II), Ni(II) and Zn(II) complexes have been characterized by elemental analysis, molar conductance, magnetic susceptibility, thermal analysis and various spectral studies like FT-IR, ¹H NMR, ESI mass, UV-Visible, ESR spectroscopy and powder X-ray diffraction data. The spectral studies confirmed tridentate ONO donor binding of the ligand involving oxygen atom of amide carbonyl, azomethine nitrogen and oxygen of hydroxyl via deprotonation. Spectral analysis indicates octahedral geometry for Cu(II), Co(II) and Ni(II) complexes and tetrahedral geometry for Zn(II) complex. Newly synthesized ligand and its metal complexes were screened for their antibacterial and antifungal activity by minimum inhibitory concentration (MIC) method. The DNA cleavage activities were studied using plasmid DNA pBR322 as a target molecule by agarose gel electrophoresis method. Furthermore, the antioxidant activity of the ligand (L) and its metal complexes were determined in vitro by reduction of 1,1-diphenyl-2-picryl hydrazyl (DPPH), the ligand exhibited more than potent in vitro-antioxidant activity than its metal complexes.

Graphical Abstract

Synthesis of 2-(2-hydroxy-4-(phenyldiazenyl)benzylidene)-N-(4-phenylthiazol-2-yl) hydrazinecarboxamide

Keywords: Transition metal complex, Thiazole Schiff base, 2-hydroxy-4-(phenyldiazenyl) benzaldehyde, Biological activity.

INTRODUCTION

Fundamental researches which are based on nucleus of the thiazole, azoaldehyde have a wide range of biological effects [1]. Derivatives of this heterocyclic system have well-known the world scientists due to its antifungal, antidepressant and anticancer properties [2]. The pharmacological activity of most organic compounds depends on several different factors, including bioavailability of the substance. Hence, it is very important to consider the results of the synthetic and biological researches and established dependence of structure on the biological action when scientists model new molecules or improve pharmacological properties of an existing structure. The transition metal complexes derived from Schiff base ligands have been among the most widely studied coordination compounds in recent years [3, 4]. The fact that copper, magnesium, calcium, molybdenum, zinc, chromium and vanadium are essential metallic elements and great biological activity when associated with certain metal-protein complexes, participating in oxygen transport, electronic transfer reaction or the storage of ions [5] has created enormous interest in the study of systems containing these metals [6]. Thiazoles are one of the most important classes of hetero-cycles that have attracted a great deal of interest owing to their wide range of biological properties such as antiprotozoal, antimicrobial, anti-inflammatory, CNS depressant, antitubercular, antidiabetics and herbicidal activities [7-9]. Thus, thiazole nucleus has been much studied in the field of medicinal chemistry. We intend to report here the synthesis, spectral, characterization, DNA cleavage, antimicrobial and antioxidant activities of novel Schiff base ligand 2-(2-hydroxy-4-(phenyldiazenyl)benzylidene)-N-(4-phenylthiazol-2-yl) hydrazinecarboxamide derived from N-(4-phenylthiazol-2-yl)hydrazinecarboxamide and 2-hydroxy-4-(phenyldiazenyl) benzaldehyde and its Cu(II), Co(II), Ni(II) and Zn(II) complexes.

MATERIALS AND METHODS

All the chemicals used were of high purity grade; solvents were dried and distilled before use. Melting points were determined by electro-thermal apparatus using open capillary tubes. Metal and chloride contents were determined as per standard procedures. The precursor N-(4-phenylthiazol-2-yl)hydrazinecarboxamide and 2-hydroxy-4-(phenyldiazenyl)benzaldehyde were prepared as per literature methods [10, 11].

Analysis and Physical measurement: The IR and Far-IR Spectra were recorded as KBr pellets on a Perkin Elmer - Spectrum RX-I FTIR instrument ($4000-400\text{ cm}^{-1}$ and $700-30\text{ cm}^{-1}$). The Elemental

analysis was obtained from Perkin Elmer 2400 CHN Elemental Analyser. ^1H NMR spectra of the ligand and its Zn(II) complex were recorded on FT NMR Spectrometer model Avance-II (Bruker), 400 MHz instrument using d_6 -DMSO as solvent. ESI-mass spectra were recorded on mass spectrometer equipped with electrospray ionization (ESI) source. UV-Visible spectra were recorded on Elico-SL 164 double beam UV-Visible spectrophotometer in the range 200-1000 nm in DMF solution at 1×10^{-3} M concentration. Powder XRD of the complexes was recorded in Bruker AXS D8 Advance (Cu, Wavelength 1.5406 Å sources). Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in dry DMF (10^{-3} M) solution using a dip-type conductivity cell fitted with a platinum electrode.

Synthesis of Schiff base ligand (L): An equimolar mixture of N-(4-phenylthiazol-2-yl)hydrazinecarboxamide and 2-hydroxy-4-(phenyldiazenyl)benzaldehyde (0.001 mol) in methanol (25 ml) with 1-2 drops of glacial acetic acid was refluxed on a water bath for about 6-7 h. Dark yellow colored solid separated in hot was filtered, washed with hot ethanol, dried and recrystallized (Fig-1)..

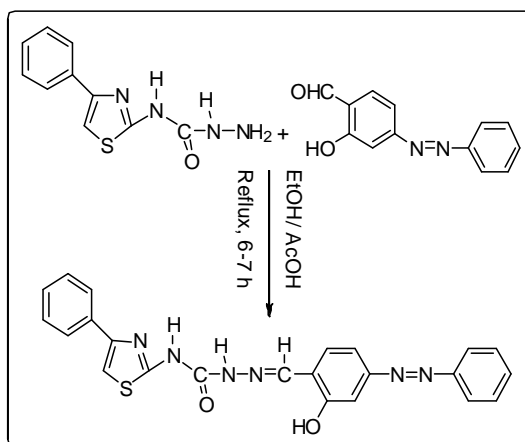


Figure 1. Synthesis of 2-(2-hydroxy-4-(phenyldiazenyl)benzylidene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide

General method for the preparation of metal (II) complexes: To the hot solution of 2-(2-hydroxy-4-(phenyldiazenyl)benzylidene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide (0.002 mol) in ethanol (30 mL) was added a hot ethanol solution (15 mL) of respective metal chlorides (0.002 mol). The reaction mixture was refluxed on a water bath for about 5h. Sodium acetate (0.5 g) was added to the reaction mixture to maintain a neutral pH and refluxing continued for 1h more. The reaction mixture was poured into distilled water. The colored solid complexes separated were collected by filtration, washed with sufficient quantity of distilled water, then with hot ethanol and dried in a vacuum over anhydrous calcium chloride in desiccators.

Antibacterial and antifungal assay: The newly synthesized ligand (L) and its metal (II) complexes were screened for their antibacterial and antifungal activities by using Muller-Hinton agar and potato dextrose agar (PDA) diffusion methods respectively [12, 13]. These activities were carried out in four different concentrations (100, 50, 25 and $12.5 \mu\text{g mL}^{-1}$ in DMSO solvent) against, *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*. The antifungal activities were carried out against *Candida albicans*, *Cladosporium oxysporum* and *Aspergillus niger* by minimum inhibitory concentration (MIC) method. The lowest concentration of each tested compound where the growth of bacteria/fungi was early inhibited is reported as MIC. The results were compared with the Gentamycin and Fluconazole, a broad-spectrum antibiotic for bacterial and fungal strains respectively.

DNA cleavage experiment: The electrophoresis method was employed to study the efficiency of cleavage by the synthesized compounds using Calf-thymus DNA (Cat. No. 105850) as target molecule. Each test compound (100 μ g) was added separately to the 225 μ g of DNA sample and these sample mixtures were incubated at 37 $^{\circ}$ C for 2 h. The electrophoresis of the test compounds was done according to the literature method [14].

Antioxidant assay (DPPH Free Radical Scavenging Activity): The Free radical scavenging activity of the test samples was determined by the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method [15]. Different concentrations of test compounds (12.5 μ g, 25 μ g, 50 μ g and 100 μ g mL $^{-1}$) and standard butylated hydroxyanisole (BHA) were taken in different test tubes and the volume of each test tube was adjusted to 100 μ L by adding distilled DMF. To the tubes containing sample solutions in DMF, 5ml methanolic solution of DPPH (0.1mM) was added to these tubes. The tubes were allowed to stand for 30 min. The control experiment was carried out as above without the test samples. The absorbance of test solutions was measured at 517 nm. The reduction of DPPH was calculated relative to the measured absorbance of the control. Radical scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging of DPPH} = [(Control OD - Sample OD) / Control OD] \times 100$$

RESULTS AND DISCUSSION

All the synthesized metal complexes are colored solids, amorphous and non-hygroscopic in nature and possess high melting points (>300 $^{\circ}$ C). The complexes are insoluble in water and common organic solvents, but soluble in DMF and DMSO. Elemental analysis and analytical data (Table 1) of the complexes suggest that the metal to ligand ratio of all the complexes were, 1:2 and 1:1 stoichiometry of the type [M(L) $_2$] for Cu(II), Co(II), Ni(II) complexes and [M(L)Cl] for Zn(II) complex respectively. The molar conductance values are too low to account for any dissociation of the complexes in DMF (18-33 ohm $^{-1}$ cm 2 mole $^{-1}$), indicating their non-electrolytic nature.

Table 1. Physical, Analytical and Molar conductance data.

Compounds	M.W.	M.P. ($^{\circ}$ C)	Colour (Yield %)	Elemental Analysis, Calc. (Found) [%]					λ_m	μ_{eff} (BM)
				C	H	N	M	Cl		
C $_{23}$ H $_{18}$ N $_6$ O $_2$ S (L)	442	268-270	Dark yellow (70)	62.44 (62.40)	4.07 (4.04)	19.00 (19.04)	--	--	--	--
[Cu(L) $_2$]	945.54	>320	Green (62)	58.37 (58.40)	3.59 (3.54)	17.76 (17.70)	6.71 (6.70)	--	26	1.86
[Co(L) $_2$]	940.93	>310	Brown (65)	58.66 (58.60)	3.61 (3.62)	17.85 (17.81)	6.26 (6.24)	--	20	4.78
[Ni(L) $_2$]	940.69	>312	Dark yellow (66)	58.68 (58.65)	3.61 (3.63)	17.85 (17.83)	6.23 (6.25)	--	19	2.93
[Zn(L)(Cl)]	541.39	>309	Yellow (59)	50.97 (50.99)	3.14 (3.11)	15.51 (15.53)	12.07 (12.05)	6.46 (6.43)	21	Dia.

IR Spectral studies: Infrared spectra help in getting an idea on how is the ligand coordinated to the metal ion. The important IR bands of the ligand and its metal complexes are represented in table 2. In the IR spectrum of ligand (L), showed a broad band at 3456 cm $^{-1}$ due to phenolic OH and two absorption bands due to amide NH and NH attached to the thiazole moiety of displayed at 3364 cm $^{-1}$, 3109 cm $^{-1}$ respectively. Sharp peaks observed at 1687 cm $^{-1}$, 1570 cm $^{-1}$ and 1276 cm $^{-1}$ are due to carbonyl, azomethine functions and phenolic C-O respectively. In the IR spectra of all the metal complexes exhibited bands with the appropriate shifts due to complex formation. In IR spectra of all the metal complexes, it was observed that the absence of absorption band due to phenolic OH at 3456 cm $^{-1}$ of ligand indicates the formation of coordination bond between the metal ion and phenolic oxygen atom via deprotonation. This is further confirmed by the increase in absorption frequency about 30-86 cm $^{-1}$ of phenolic C-O which appeared in the region 1306-1362 cm $^{-1}$ in all the complexes

indicating the participation of oxygen atom of phenolic OH in the coordination. Further the bands displayed in the region 3328-3348 cm^{-1} and 3058-3065 cm^{-1} were due to amide NH and NH attached to thiazole moiety respectively, which appeared almost at about the same position as in the case of ligand thus confirming their non-involvement in coordination. The shift of amide carbonyl C=O to lower frequency side about 52-85 cm^{-1} and which appeared in the region 1602-1635 cm^{-1} in all the complexes confirms the coordination of oxygen atom of amide C=O with the metal ions as such without undergoing enolization [16]. the absorption frequency due to azomethine C=N function also shifted to lower frequency side about 23-36 cm^{-1} and appeared in the region 1534-1547 cm^{-1} suggesting the involvement of nitrogen atom of azomethine function in complexation with metal ions [17]. Further the appearance of new non-ligand bands in the region 568-591 cm^{-1} and 463-502 cm^{-1} in the IR spectra of the complexes are assigned to frequencies of (M-O) and (M-N) stretching vibration respectively. Also, the appearance of new band at 356 cm^{-1} in [Zn(L)Cl] complex is due to (M-Cl) stretching vibration.

Table 2. IR spectral data (cm^{-1}) of Schiff base ligand (L) and its metal complexes

Ligand/ Complexes	ν_{OH} (phenolic)	ν_{NH} (amide)	ν_{NH} (thiazole)	$\nu_{\text{C=O}}$ (carbonyl)	$\nu_{\text{C=N}}$ (azomethine)	$\nu_{\text{C-O}}$ (phenolic)	$\nu_{\text{M-O}}$	$\nu_{\text{M-N}}$	$\nu_{\text{M-Cl}}$
L	3456	3364	3109	1687	1570	1276	--	--	--
[Cu(L) ₂]	--	3348	3058	1605	1534	1362	586	476	--
[Co(L) ₂]	--	3345	3064	1602	1542	1306	579	465	--
[Ni(L) ₂]	--	3331	3065	1635	1542	1340	568	463	--
[Zn(L)(Cl)]	--	3328	3058	1622	1547	1325	591	502	356

¹H NMR spectra: The ¹H NMR spectrum of Schiff base ligand (L) displayed three singlet's each at 11.804 ppm, 10.944 ppm, and 10.575 ppm which are due to the proton of phenolic OH, amide NH and NH attached to thiazole moiety respectively. The signal due to azomethine proton (CH=N) resonated at 8.912 ppm. The signals due to fourteen aromatic protons (ArH) have appeared as multiplets in the region 7.023-8.115 ppm. The Schiff base ligand (L) upon complexation with Zn(II) ion showed the disappearance of signal due to proton of phenolic OH confirms the involvement of bonding of phenolic oxygen to metal ion via deprotonation. The signals due to protons of amide NH and NH attached to thiazole have appeared at 10.981 ppm and 10.591 ppm respectively. The signal due to azomethine proton (CH=N) has resonated as a singlet at 9.223 ppm. The signals due to fourteen aromatic protons (ArH) have resonated as multiplets in the region 7.123-8.294 ppm. When compared to the ¹H NMR spectral data of the Schiff base ligand (L) and its [Zn(L)Cl] complex (Table 3), all the signals due to protons have been shifted towards down field strength confirming the complexation of with Zn(II) ion with the Schiff base ligand. Thus, the ¹H NMR spectral results further supports the IR spectral inferences and complexation of Zn(II) ion with Schiff base ligand.

Table 3. ¹H NMR spectral data

Ligand/ Complex	¹ H NMR data (ppm)
Ligand[L]	11.804 (s, 1H, phenolic OH), 10.944 (s, 1H, CONH), 10.575 (s, 1H, NH attached to thiazole), 8.912 (s, 1H, HC=N), 7.023-8.115 (m, 14H, ArH)
[Zn(L)Cl]	10.981 (s, 1H, CONH), 10.591 (s, 1H, NH attached to thiazole), 9.223 (s, 1H, HC=N), 7.123-8.294 (m, 14H, ArH)

ESI-mass spectral data: In the mass spectrum of ligand (L) the molecular ion was displayed at M⁺442 (34.55%) which is equal to its molecular weight, this on loss of a hydrogen radical gave fragment ions peak recorded as m/z 441 (100%) which also a base peak (Fig.2). This on simultaneous loss of a phenyl radical and nitrogen molecule followed by C₇H₄ON radical gave fragment ions peaks recorded as m/z 336 (3.67%) and 218 (2.20%) respectively. In another route, the molecular ion by

sequential expulsion 2-amino-4-phenylthiazole molecule, hydrogen radical, and NCO radical gave fragment ion.

peaks recorded at m/z 266 (4.41%), 265 (24.26%) and 223 (2.94%) respectively. Thus, the fragmentation pattern is in consistency with its structure (Fig. 3).

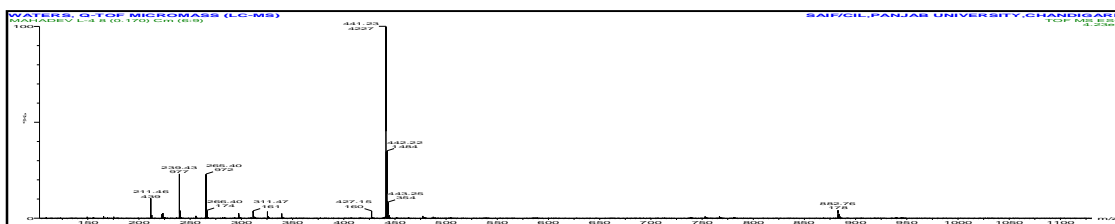


Figure 2 ESI-mass spectrum of Schiff base ligand (L)

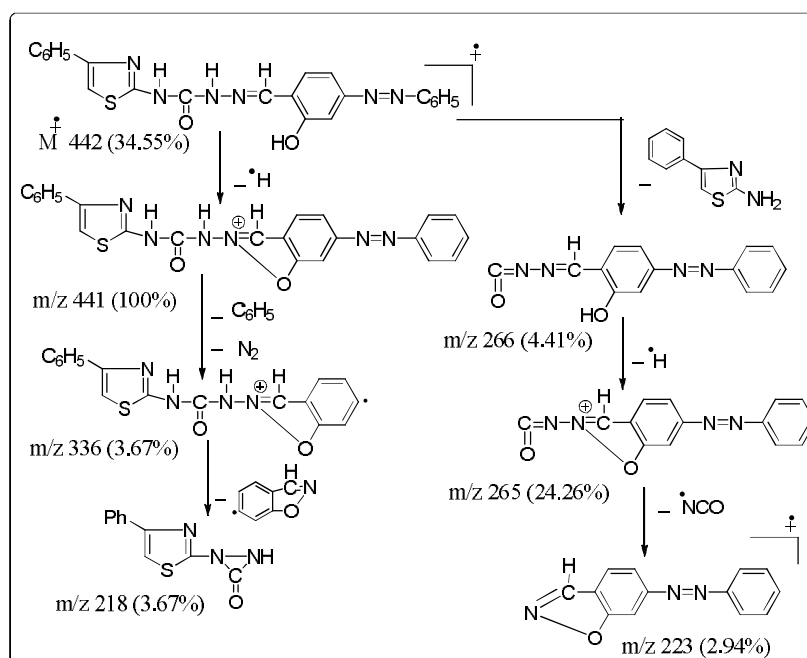


Figure 3. Mass fragmentation pattern of Schiff base ligand

The mass spectrum of $[\text{Cu}(\text{L})_2]$ complex displayed a peak at m/z 946 (12.40%) due to M^{+1} , This on loss of a hydrogen radical gave a fragment ions peak observed at M^{+1} 945 (8.02%) which is equal to its molecular weight. This on expulsion of a $\text{C}_{23}\text{H}_{17}\text{N}_6\text{O}_2\text{S}$ radical followed by a hydrogen molecule gave fragment ion peaks recorded at m/z 504 (8.75%) and 502 (16.05%) respectively. The molecular ion also underwent fragmentation by other two routes, in one route, by the simultaneous elimination of a $\text{C}_6\text{H}_4\text{N}_2$ molecule, $\text{C}_9\text{H}_6\text{N}_2\text{S}$ radical gave a fragment ion peak recorded at m/z 226 (16.78%). In another route the molecular ion on loss of a $\text{C}_6\text{H}_4\text{N}_2$ molecule and $\text{C}_9\text{H}_6\text{N}_2\text{S}$ molecule gave a fragment ion recorded at m/z 225 (100%). which is also a base peak. This on loss of a carbon monoxide molecule gave a fragment ion recorded at m/z 197 (39.41%). Thus, the fragmentation pattern of the Cu(II) complex is in consistency with its structure (Fig. 4).

In the mass spectrum of $[\text{Co}(\text{L})_2]$ complex, the molecular ion peak was observed at M^{+} 940 (12.31%) which is equal to its molecular weight. This on simultaneous expulsion a $\text{C}_9\text{H}_6\text{N}_2\text{S}$ radical, a benzene molecule, and $\text{C}_6\text{H}_4\text{N}_2$ molecule gave a fragment ion peak recorded at m/z 584 (4.34%) which on loss of C_3NS radical and hydrogen molecule gave a fragment ion recorded at m/z 500 (100%) which is

also base peak. This on loss of $C_{14}H_{11}N_5O_2$ molecule and hydrogen radical gave a fragment ion peak recorded at m/z 218 (49.27%) Thus the fragmentation pattern is in consistency with its structure. In the mass spectrum of $[Ni(L)_2]$ complex, the molecular ion peak was observed at M^+ 940 (12.40%) which is equal to its molecular weight. This on expulsion of a $C_{23}H_{17}N_6O_2S$ radical gave fragment ion peak recorded at m/z 499 (100%) which is also base peak. And this on loss of $C_9H_6N_2S$ radical, $C_6H_4N_2$ molecule, hydrogen molecule and hydrogen radical gave a fragment ion peak recorded at m/z 218 (5.83%). This fragmentation pattern of Ni(II) complex is in consistency with its structure. In mass spectrum of $[Zn(L)Cl]$ complex, the molecular ion peak was observed at M^+ 541, 543, (11.67%, 3.50%) which is equal to its molecular weight. This on simultaneous loss of a chlorine radical, $C_6H_4N_2$ molecule and a hydrogen radical gave fragment ion peak recorded m/z 401 (17.51%). This fragment was also obtained from the molecular ion by another route where in its losses $C_6H_4N_2$ molecule giving a fragment ion recorded at m/z 437 (100%) which also a base peak which on simultaneously loss of chloride radical and a hydrogen radical giving a fragment ion recorded at m/z 401 (17.51%) respectively. The molecular ion another route by the simultaneous loss of $C_6H_4N_2$ molecule, a chlorine radical and C_8H_4 molecule gave a fragment ion peak observed at m/z 302 (15.32%). This on loss of acetylene molecule and a hydrogen molecule gave a fragment ion recorded at m/z 274 (96.35%) which simultaneously losses a carbon sulphide molecule and nitrogen molecule giving a fragment ion peak recorded at m/z 202 (13.13%). The fragmentation pattern is in consistency with its structure.

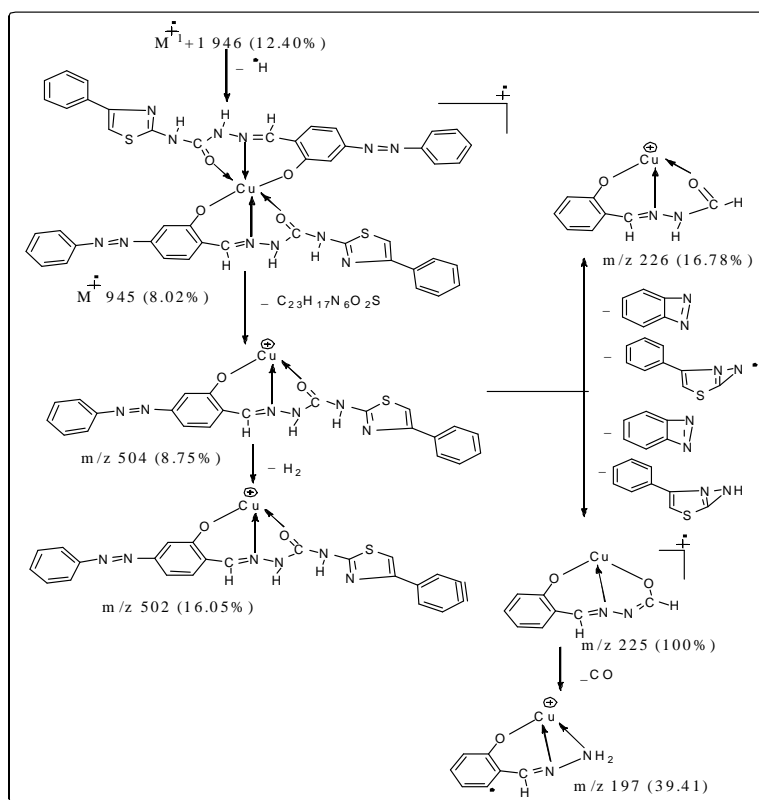


Figure 4. Mass fragmentation pattern of Cu(II) complex

Electronic spectral studies: The green colored $[Cu(L)_2]$ complex displayed a low intensity single broadband in the region $15527-17953\text{cm}^{-1}$. The broadness of the band designates the three transitions ${}^2B_{1g} \rightarrow {}^2A_{1g}(\nu_1)$, ${}^2B_{1g} \rightarrow {}^2B_{2g}(\nu_2)$ and ${}^2B_{1g} \rightarrow {}^2E_g(\nu_3)$, which are similar in energy and give rise to only one broad band and the broadness of the band may be due to dynamic Jahn-Teller distortion. The obtained data suggest the distorted octahedral geometry around the Cu(II) ion [24]. The $[Co(L)_2]$

complex under present study displayed two absorption bands at 16891 cm^{-1} and 20202 cm^{-1} . These bands are assigned to be ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ (ν_2) and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{P})$ (ν_3) transitions, respectively, which are in good agreement with the literature values for octahedral geometry [18, 19]. The lowest band, ν_1 could not be observed due to the limited range of the instrument used, but it could be calculated using the band fitting procedure suggested by Underhill and Billing [20]. The calculated ν_1 value is presented in table 4. These transition values suggest the octahedral geometry of the Co(II) complex. The $[\text{Ni}(\text{L})_2]$ complex under the present investigation exhibited two absorption bands in the region 15128 cm^{-1} and 25188 cm^{-1} , which are assigned to ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$ (ν_2) and ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$ (ν_3) transitions respectively suggesting an octahedral environment [21]. The transition value of band ν_1 was calculated by using a band fitting procedure. The proposed octahedral geometry for the complexes was further supported by the calculated values of ligand field parameters, such as Racah inter electronic repulsion parameter (B'), nephelauxetic parameter (β), ligand field splitting energy (10 Dq) and ligand field stabilization energy (LFSE). The calculated B' values for the $[\text{Co}(\text{L})_2]$ and $[\text{Ni}(\text{L})_2]$ complexes are lower than the free ion values, which is due to the orbital overlap and delocalization of d-orbitals. The β values are important in determining the covalency for the metal-ligand bond and they were found to be less than unity, suggesting a considerable amount of covalency for the metal-ligand bonds. The β value for the $[\text{Ni}(\text{L})_2]$ complexes were less than that of the $[\text{Co}(\text{L})_2]$ complexes, indicating the greater covalency of the metal-ligand (M-L) bond. Greater covalency of the metal-ligand (M-L) bond.

Table 4. Electronic spectral data

Complexes	Transitions in cm^{-1}			Dq (cm^{-1})	B' (cm^{-1})	β	$\beta\%$	ν_2/ν_1	LFSE (k cal.)
	ν_1^*	ν_2	ν_3						
$[\text{Cu}(\text{L})_2]$	15527-17953			--	--	--	--	--	28.69
$[\text{Co}(\text{L})_2]$	7874	16891	20202	901	891	0.917	8.238	2.14	15.44
$[\text{Ni}(\text{L})_2]$	8062	15128	25188	932	823	0.792	20.86	1.62	31.95

Magnetic susceptibility studies: The observed magnetic moment for Cu(II) complex is 1.86 BM which attributes to one unpaired electron with a slight orbital contribution to the spin only a value of 1.73 BM and the absence of spin-spin interactions in the complex accounting for the possibility of a distorted octahedral geometry [22]. In octahedral Co(II) complex, the ground state is ${}^4\text{T}_{1g}$ and the orbital contribution to the singlet state lowers the magnetic moment values for the various Co(II) complexes which are in the range 4.12-4.70 and 4.70-5.20 BM for tetrahedral and octahedral complexes respectively. In the present study the observed magnetic moment values for Co(II) complexes are 4.78 BM indicates octahedral geometry for Co(II) complex. In Ni(II) complex the observed magnetic moment value is 2.93 BM which is well within the expected range of Ni(II) complex with octahedral geometry, i.e. 2.83-3.50 BM [23].

Thermal studies: The thermal stabilities for Cu(II), Co(II), Ni(II) and Zn(II) complexes have been studied as a function of temperature. The proposed stepwise thermal degradation of the complexes with respect to temperature and the formation of respective metal oxides are depicted in table 5. TG-DTA curve of Cu(II) complex showed that the first stage of decomposition represents a weight loss due to a benzene molecule at 261°C with practical weight loss of 8.59% (Cal. 8.24%). The resultant complex underwent second stage of degradation and gave break at 355°C with a practical weight loss of 24.72% (Cal. 23.97%), which corresponds to the loss of two $\text{C}_6\text{H}_4\text{N}_2$ molecules. Thereafter, the compound showed decomposition in a gradual manner rather than with the sharp decomposition up to 719°C and onwards due to the loss of the remaining organic moiety. The weight of the residue corresponds to cupric oxide.

The thermo gram of Co(II) complex showed the first stage of decomposition at 280°C with practical weight loss of 9.12% (Cal. 8.28%), which corresponds to weight loss due to a benzene molecule. Further the complex underwent decomposition and gave a break at 360°C with a practical weight loss

of 11.20% (Cal. 12.05%) which corresponds to weight loss of $C_6H_4N_2$ molecule. And further, the complex underwent decomposition and gave a break at $465^\circ C$ with a practical weight loss of 36.85% (Cal. 36.63%), corresponds to weight loss of phenyl acetylene molecule and 2-amino-4-phenylthiazole molecule. Thereafter, the complex showed gradual decomposition up to $720^\circ C$ with a weight loss of the remaining organic moiety, the weight of the residue corresponds to cobalt oxide. In the thermo gram of the Ni(II) complex, the first stage of decomposition represents the weight loss due to an acetylene molecule. at $305^\circ C$ with a practical weight loss of 3.85% (Cal. 2.76%). The resultant complex underwent further degradation and gave break at $440^\circ C$ with a practical weight loss of 41.48% (Cal. 41.76%), which corresponds to the loss due to a $C_6H_4N_2$ molecule phenyl acetylene molecule and 2-amino-4-phenylthiazole molecule. Thereafter, the compound showed a gradual decomposition up to $720^\circ C$ with a weight loss of remaining organic moiety. The weight of the residue corresponds to nickel oxide.

In case of Zn(II) complex, the first stage of decomposition occurred at $300^\circ C$ with practical weight loss of 14.06% (Cal. 14.40%), which represents the loss due to a benzene molecule. Further the complex underwent second stage of decomposition and gave a break at $480^\circ C$ with practical weight loss of 50.35% (Cal. 51.14%), which corresponds to the loss due to a $C_6H_4N_2$ molecule, $C_3H_2N_2S$ molecule and a chlorine atom. Thereafter, the compound showed a gradual decomposition up to $718^\circ C$ with the weight loss of the remaining organic moiety. The weight of the residue corresponds to zinc oxide.

Table 5. Thermal degradation pattern of metal complexes

Metal complexes	Temp. °C	Weight loss (%)		Metal Oxide (%)		Inference
		Obs.	Cal.	Obs.	Cal.	
[Cu(L) ₂]	261	8.59	8.24	--	--	Loss due to a benzene molecule.
	355	24.72	23.97	--	--	Loss due to two $C_6H_4N_2$ molecules.
	Up to 719	--	--	8.43	8.41	Loss due to remaining organic moiety.
[Co(L) ₂]	280	9.12	8.28	--	--	Loss due to a benzene molecule.
	360	11.20	12.05	--	--	Loss due to a $C_6H_4N_2$ molecule.
	465	36.85	36.63	--	--	Loss due to phenyl acetylene and 2-amino-4-phenylthiazole molecules.
	Up to 720	--	--	7.95	7.96	Loss due to remaining organic moiety.
[Ni(L) ₂]	305	3.85	2.76	--	--	Loss due to acetylene molecule.
	440	41.48	41.76	--	--	Loss due to $C_6H_4N_2$, phenyl acetylene, and 2-amino-4-phenylthiazole molecules.
	Up to 720	--	--	7.90	7.93	Loss due to remaining organic moiety.
[Zn(L)Cl]	300	14.06	14.40	--	--	Loss due to a benzene molecule.
	480	50.35	51.14	--	--	Loss due to a C_6H_4N molecule, $C_3H_2N_2S$ molecule and a chlorine atom.
	Up to 718	--	--	15.07	15.03	Loss due to remaining organic moiety.

ESR Spectral Studies of Cu(II) complex: The ESR spectrum of the Cu(II) complex was recorded at room temperature. The spin Hamiltonian parameters for the Cu(II) complex is used to derive the ground state. In octahedral geometry the g-tensor parameter with $g_{\perp} > g_{\parallel} > 2.0023$, the unpaired electron lies in the d_z^2 orbital and $g_{\parallel} > g_{\perp} > 2.0023$, the unpaired electron lies in the $d_{x^2-y^2}$ orbital in the ground state [29]. In the present study the observed measurements of Cu(II) complex is $g_{\parallel} (2.162) > g_{\perp} (2.0420) > 2.0023$ indicating that the complex are axially symmetric and copper site has a $d_{x^2-y^2}$ ground state characteristic of octahedral geometry [24]. The g_{\parallel} value is an important function for indicating the metal-ligand bond character, for covalent character $g_{\parallel} < 2.3$ and for ionic $g_{\parallel} > 2.3$ respectively [25]. In the present case Cu(II) complex has the g_{\parallel} values were less than 2.3, indicating an appreciable covalent character of the metal-ligand bond. The geometric parameter (G), which is the measure of extent of exchange interaction and is calculated by using g-tensor values by the expression

$G = g_{\parallel} - 2.0023/g_{\perp} - 2.0023$. According to Hathaway [26], if the G value is less than 4, the exchange interaction between

the copper centres is noticed, where as if its value is greater than 4, the exchange interaction is negligible. The calculated G-value for the present Cu (II) complex is 4.257 indicating that the exchange coupling effects are not operating in the present complex [27].

Powder X-ray diffraction studies (Powder-XRD): Crystals that are suitable for single-crystal studies were not obtained since all the metal complexes are not soluble in common solvents, but soluble in some polar solvents like DMF and DMSO. Hence powder-XRD pattern of all the metal complexes has been studied in order to test the degree of crystallinity of the complexes. Powder X-ray diffraction pattern for Cu(II) complex (Fig.1) showed 8 reflections in the range of 6-28° (2 θ), which arise from diffraction of X-ray by the planes of the complex. The inter-planar spacing (d) has been calculated by using Bragg's equation, ($n\lambda = 2d \sin\theta$). The calculated inter-planar d-spacing together with relative intensities with respect to most intense peak have been recorded and depicted in table 6. The unit cell calculations have been calculated for cubic symmetry from the entire important peaks and $h^2 + k^2 + l^2$ values were determined. The observed inter-planar d-spacing values have been compared with the calculated ones and it was found to be in good agreement. The $h^2 + k^2 + l^2$ values are 1, 3, 6, 14, 16, 17, 18, and 20 for Cu(II) complex. It was observed that the absence of forbidden numbers (7, 15, 23, 71 etc.) indicates that the Cu(II) complex has cubic symmetry. Similar calculations were performed for Co(II), Ni(II) and Zn(II) complexes they showed reflections each in the range 3-80° respectively, which are arised from the diffraction of X-ray by the planes of these complexes. All the important peaks of the complexes have been indexed and observed values of inter-planar distances (d) have been compared with the calculated ones and it was found to be in good agreement. The unit cell calculations were performed for cubic system and the $h^2 + k^2 + l^2$ values were determined for the above complexes. The $h^2 + k^2 + l^2$ values were 1 for Co (II) complex It was observed that the absence of forbidden numbers (7, 15, 23, 71etc) indicates that Co (II) complex have cubic symmetry. The observed values were 1, 5, 7, 10, 24, 26, 30, 71, and 109 for Ni (II) complex. The presence of forbidden numbers 7, 15 and 71 (7, 15, 23, 71etc) indicates that the Ni (II) complex may belong to hexagonal or tetragonal systems. The observed value were 1, 2, 3, 3 and 13 for Zn(II) complex. It was observed that the absence of forbidden numbers (7, 15, 23, 71etc) indicates that Zn(II) complex has cubic symmetry.

Table 6: Powder X-ray data of [Cu(L)₂] complex

S. No	2 θ	θ	Sin θ	Sin ² θ	1000 Sin ² θ	1000 Sin ² θ /CF ($h^2+k^2+l^2$)	h k l	d		a in Å°
								Obs.	Calc.	
1	6.137	3.068	0.053	0.00286	2.865	1.00(1)	1 0 0	14.3895	14.3925	14.398
2	10.888	5.444	0.094	0.00900	9.000	3.14(3)	1 1 1	8.1190	8.1223	14.382
3	15.150	7.574	0.131	0.01737	17.377	6.06(6)	2 1 1	5.8858	5.8421	14.390
4	22.975	11.487	0.199	0.03966	39.662	13.84(14)	3 2 1	3.8678	3.8674	14.384
5	24.545	12.272	0.212	0.04518	45.182	15.77(16)	4 0 0	3.6238	3.6235	14.385
6	25.378	12.689	0.219	0.04825	48.250	16.84(17)	4 1 0	3.5068	3.5063	14.385
7	26.343	13.171	0.227	0.05192	51.923	18.12(18)	4 1 1	3.3804	3.3801	14.384
8	28.010	14.005	0.242	0.05850	58.567	20.44(20)	4 2 0	3.1829	3.1818	14.393

APPLICATIONS

Antimicrobial activity results: The antimicrobial screening results indicate that the newly synthesized metal complexes exhibited promising results greater than those of free Schiff base ligand. This activity was found to be enhanced on coordination with metal ions. This enhancement in the antimicrobial activity of the complexes over the free ligand can be explained on the basis of chelating theory [28, 29]. The enhancement in the activity may be rationalized on the basis that Schiff base ligands possess azomethine (C=N) bond. Moreover, in metal complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (N and O) present in the Schiff base ligand

and there may be π - electron delocalization over the whole chelating system [30]. Hence the increase in the lipophilic character of the metal chelates which favours its permeation through the lipid layer of the bacterial membranes and blocking of the metal binding sites in the enzymes of microorganisms. In general, metal complexes may serve as a vehicle for activation of Schiff base ligands as the principal cytotoxic species [31]. The minimum inhibitory concentration (MIC) values of the compounds against the respective bacterial and fungal strains are summarized in table 7.

Table 7. Minimum inhibitory concentration (MIC $\mu\text{g mL}^{-1}$) of Schiff base ligand (L) and its metal complexes

Compounds	Bacteria				Fungi			
	<i>S. aureus</i>	<i>B. Subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. oxysporum</i>	<i>A. Flavus</i>	<i>A. niger</i>
L	75	75	75	50	75	50	75	75
[Cu(L) ₂]	25	25	25	25	25	50	25	50
[Co(L) ₂]	25	25	25	25	50	25	25	50
[Ni(L) ₂]	25	50	2	50	50	25	50	25
[Zn(L) ₂]	50	50	25	50	50	25	25	50
Gentamicin	12.50	12.50	12.50	12.50	--	--	--	--
Fluconazole	--	--	--	--	12.50	12.50	12.50	12.50

Antioxidant assay (DPPH free radical scavenging activity): The free radical scavenging activity of the ligand and its metal complexes was done by DPPH method. The antioxidant activity of the test compounds was examined by measuring radical scavenging effect of DPPH radicals. The results of the free radical scavenging activity of the compounds at different concentrations are shown in (Fig.5). It was observed that the free radical scavenging activity of these compounds was concentration dependent. Among the examined compounds, ligand (L), Cu (II) and Co(II) complexes have exhibited good scavenging activity, whereas Ni(II) and Zn(II) complex showed moderate activity. The marked antioxidant activity of metal complexes is due to the coordination of metal with azomethine nitrogen and carbonyl oxygen of amide function. In case of metal complexes, the hydrogen of azomethine is more acidic hence, hydrogen of azomethine could be easily donated to the DPPH free radical and convert itself into the stable free radical. Moreover, the acidic nature of hydrogen atom attached to azomethine nitrogen increases on complexation with metal ions there by making that hydrogen atom more liable.

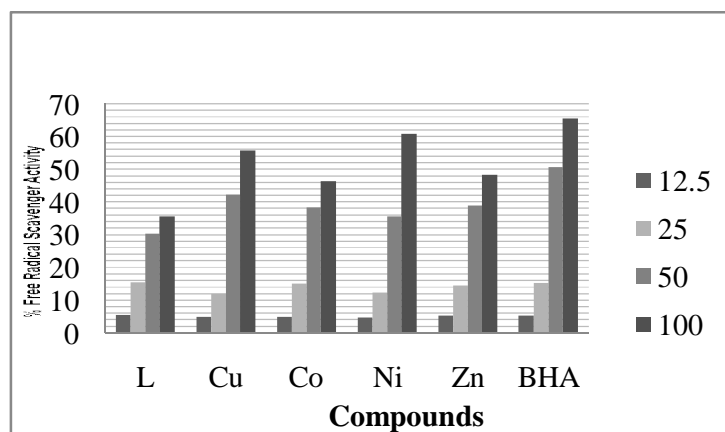


Figure 5. Antioxidant activity results.

DNA cleavage activity: The ligand and its Cu(II), Co(II), Ni(II) and Zn(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method against Calf-thymus DNA (Cat. No- 105850) as a target molecule and the gel picture showing cleavage is depicted in (Fig. 6.) Treatment of DNA on the ligand and complexes revealed that all the complexes have acted on DNA as there was molecular weight difference between the treated DNA samples and the control. The

difference was observed in bands of lanes compared to the control Calf-thymus DNA. The results indicate the important role of nitrogen and oxygen atoms to the metal ions in these isolated DNA cleavage reactions [32]. On the basis of the cleavage of DNA observed in case of ligand and its Cu(II), Co(II), Ni(II) and Zn(II) complexes, it can be concluded that all the compounds under present study inhibited the growth of pathogenic organism by DNA cleavage as has been observed on the DNA cleavage of Calf-thymus DNA.

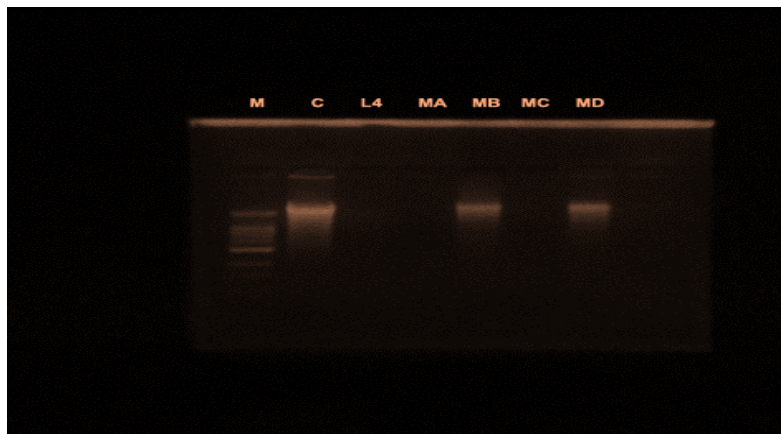


Figure 6. DNA cleavage on plasmid pBR 322: M: Standard DNA, C: control DNA (untreated pBR 322), L4: Schiff base ligand, MA: Cu(II) complex, MB: Co(II) complex, MC: Ni(II) complex and MD: Zn(II) complex

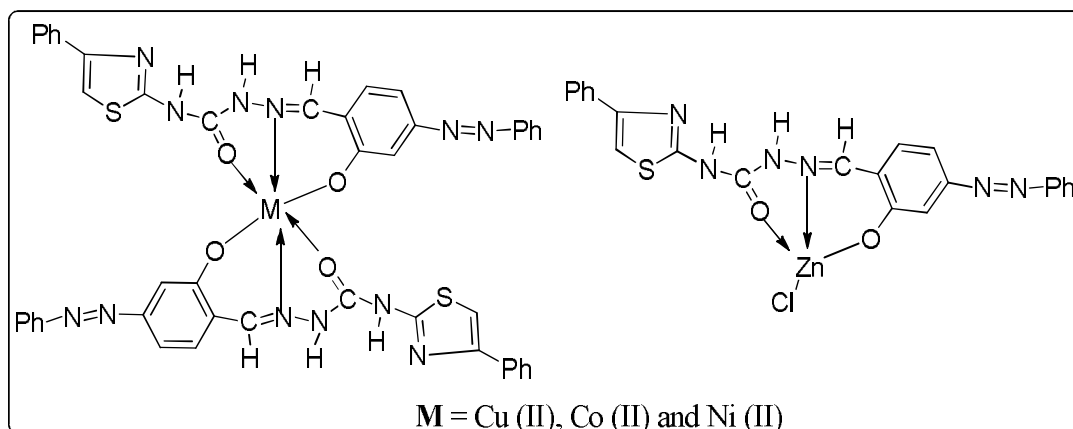


Figure 7. Proposed structures of metal complexes.

CONCLUSIONS

A series of Cu(II), Co(II), Ni(II) and Zn(II) complexes were prepared with tridentate ONO donor novel Schiff base ligand(L) 2-(2-hydroxy-4-(phenyldiazenyl) benzylidene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide by various physico-chemical techniques. The results demonstrate that Cu(II), Co(II) and Ni(II) complexes have octahedral geometry whereas Zn(II) complex has tetrahedral geometry. Based on physico-chemical evidence, the following structures were proposed for the complexes (Fig.7). The non-electrolytic nature of the complexes was confirmed on the basis of their molar conductance values. Also, the ligand (L) and its Cu(II) and Co(II) complexes showed good antioxidant activity and the electrophoretic studies indicated that Co(II) and Zn(II) complexes have good efficiency towards DNA cleavage.

REFERENCES

- [1]. G.Y. Nagesh, K. Mahendra Raj, B.H.M. Mruthyunjayaswamy, Synthesis, characterization, thermal study and biological evaluation of Cu(II), Co(II), Ni(II) and Zn(II) complexes of Schiff base ligand containing thiazole moiety, *J. Mol. Str.*, **2015**, 179, 423-432.
- [2]. M.S. Refat, M.Y. EL-Sayed, A.M.A. Adam, Cu(II), Co(II) and Ni(II) complexes of new Schiff base ligand: Synthesis, thermal and spectroscopic characterizations, *J. Mol. Struct.*, **2013**, 1038, 62-72.
- [3]. A.A. Nejo, G.A. Kolawole, A.O. Nejo, Synthesis, spectroscopic characterization and antibacterial activity of oxovanadium (iv) complexes of Schiff bases derived from substituted salicylaldehyde and diamines, *J. Coord. Chem.*, **2010**, 63, 4398-4410.
- [4]. P.A. Vigato, S. Tamburini, The challenge of cyclic and acyclic Schiff bases and related derivatives, *Coord. Chem. Rev.*, **2004**, 248, 1717-2128.
- [5]. N.K. Kaushik, A.K. Mishra, Synthesis, characterization and thermal studies of some new organotin (IV) complexes with aniline N-thiohydrazide and benzaldehyde aniline N-thiohydrazone, *Ind. J. Chem. Sec A.*, **2003**, 42, 2762-2766.
- [6]. M.S. Saraiva, S. Quintal, F.C.M. Porugal, Nitrogen donor ligands bearing N-H groups: effect on catalytic and cytotoxic activity of molybdenum η^3 -allyldicarbonyl complexes. *J. Organomet. Chem.*, **2008**, 693, 3411-3418.
- [7]. D.C. Crans, K.A. Wall, K. Prusinskas, M.D. Johnson, E. Norkus, Metal Speciation in Health and Medicine Represented by Iron and Vanadium, *Inorg. Chem.*, **2013**, 52, 12262-12275.
- [8]. G.Y. Nagesh, M.D. Udayagiri, B.H.M. Mruthyunjayaswamy, Synthesis, characterization, mass spectral fragmentation, thermal study and biological evaluation of new Schiff base ligand and its metal(II) complexes derived from 4-(diethylamino)salicylaldehyde and thiazole moiety, *Eur. J. Chem.*, **2016**, 7, 56-65.
- [9]. W. Reman, F. Saman, I. Ahmad, Synthesis, Characterization, and biological study of some biologically potent Schiff base transition metal complexes, *Russ. J. Coord. Chem.*, **2008**, 34, 678-682.
- [10]. B.H.M. Mruthyunjayaswamy, S.m. Basavarajaiah, Synthesis and Antimicrobial activity of novel ethyl-5 (Ethoxycarbonyl)-4 Methylthiazol-2-yl-Carbamate compounds, *Ind. J. Chem. Sec B*, **2009**, 48, 1274-1278.
- [11]. N. Yernale, M.Bennikallu Hire Mathada, Synthesis, Characterization, Antimicrobial, DNA cleavage, and In Vitro cytotoxic studies of some metal complexes of Schiff base ligand derived from thiazole and quinoline moiety, *Bioinorg. Chem. Appl.*, **2014**, 314963, 1-17.
- [12]. A.K. Sadana, Y. Miraza, K.R. Aneja, O. Prakash, Hypervalent iodine mediated synthesis of 1-aryl/hetryl-1,2,4-triazolo[4,3-a] pyridines and 1-aryl/hetryl 5-methyl-1,2,4-triazolo[4,3-a]quinolines as antibacterial agents, *Eur. J. Med. Chem.*, **2003**, 38, 533-536.
- [13]. N.G. Yernale, B.H.M. Mruthyunjayaswamy, Metal (II) Complexes of ONO donor Schiff base ligand as a new class of bioactive compounds containing indole core: Synthesis and characterization, *Int. J. Pharm. Pharm. Sci.*, **2016**, 8, 197-204.
- [14]. J. Sambrook, E.F. Fritsch, T. Maniatis, *Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor (NY)*, **1989**.
- [15]. R.P. Singh, K.N. Chidambara Murthy, G.K. Jayaprakash, Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models, *J. Agric. Food. Chem.*, **2002**, 50, 81-6.
- [16]. U.B. Gangadharmath, V.K. Revankar, V.B. Mahale, Synthesis and spectroscopic characterization of cationic mononuclear oxovanadium(IV) complexes with tetradentate Schiff bases as ligands, *Spectrochimica Acta A*, **2002**, 58, 2651- 2657.

- [17]. A.A. El-Bindary, A.Z. El-Sonbati, Synthesis and properties of complexes of copper(II), nickel(II), cobalt(II) and uranyl ions with 3-(p-tolylsulphonamido)rhodanine, *Polish J. Chem.*, **2000**, 74, 615-620.
- [18]. R. Kannappan, R. Mahalakshmy, T.M. Rajendiran, R. Venkatesan, P. Sambasiva Rao, Magnetic, catalytic, EPR and electrochemical studies on binuclear copper(II) complexes derived from 3,4-disubstituted phenol, *Proceedings of the Indian Academy of Sciences*, **2003**, 115, 1-14.
- [19]. D.N. Satyanarayana, Electronic Absorption Spectroscopy and Related Techniques, *Universities Press, India*, **2001**.
- [20]. A.E. Underhill, D.E. Billing DE, Calculations of the racah parameter b for nickel (II) and cobalt (II) compounds, *Nature*, **1966**, 210, 834-835.
- [21]. F.A. Cotton, G. Wilkinson, Advanced Inorganic Chemistry, John Wiley & Sons, New York, NY, USA, 3rd edition, **1972**.
- [22]. N.H. Pilkington, R. Robson, Corrigenda-complexes of binucleating ligands. III. Novel complexes of a macrocyclic binucleating ligand, *Aust. J. Chem.*, **1970**, 23, 2225-2236.
- [23]. N. Kavitha, P.V. Anantha Lakshmi, Synthesis, characterization and thermogravimetric analysis of Co(II), Ni(II), Cu(II) and Zn(II) complexes supported by ONNO tetradentate Schiff base ligand derived from hydrazino benzoxazine, *J. Soudi Chem. Soci.*, **2017**, 21, S457-S466.
- [24]. G.Y. Nagesh, B.H.M. Mruthyunjayaswamy Synthesis, characterization and biological relevance of some metal (II) complexes with oxygen, nitrogen and oxygen (ONO) donor Schiff base ligand derived from thiazole and 2-hydroxy-1-naphthaldehyde, *J. Mol. Str.*, **2015**, 1085, 198-206.
- [25]. A. Choudharya, R. Sharma, M. Nagar, Synthesis, characterization, and biological study of some biologically potent Schiff base transition metal complexes, *Int. Res. J. Pharm. Pharmacol.*, **2011**, 1, 172-187.
- [26]. K. Mahendra Raj, B. Vivekanand, G.Y. Nagesh, B.H.M. Mruthyunjayaswamy, Synthesis, spectroscopic characterization, electrochemistry and biological evaluation of some binuclear transition metal complexes of bicompartamental ONO donor ligands containing benzo[b]thiophene moiety, *J. Mol. Str.*, **2014**, 1059, 280-293.
- [27]. D. Branowska, A.A. Farahat, A. Kumar A. Synthesis and antiprotozoal activity of 2,5-bis[amidinoaryl]thiazoles, *Bioorg. Med. Chem.*, **2010**, 18, 3551-3558.
- [28]. P. Ikechukwu, Ejidike, A. Peter A. Ajibade, Synthesis, Characterization, Antioxidant, and Antibacterial Studies of Some Metal(II) Complexes of Tetradentate Schiff Base Ligand: (4E)-4-[(2-{(E)-[1-(2,4-Dihydroxyphenyl)ethylidene]amino}ethyl)imino], *Bioinorg. Chem. Appl.* **2015**, 2015, 890734.
- [29]. V.A. Shelke, S.M. Jadhav, V.R. Patharkar, S.G. Shankarwar, A.S. Munde, T.K. Chondhekar, Synthesis, spectroscopic characterization and thermal studies of some rare earth metal complexes of unsymmetrical tetradentate Schiff base ligand, *Arabian J. Chem.* **2012**, 5, 501-507.
- [30]. Z.H. Chohan, M. Arif, M.A. Akhtar, C.T. Supuran, Metal-based antibacterial and antifungal agents: synthesis, characterization, and in vitro biological evaluation of Co(II), Cu(II), Ni(II), and Zn(II) complexes with amino acid-derived compounds, *Bioinorg. Chem. Appl.*, **2006**, 1-13.
- [31]. B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini, N.S. Kumari, Synthesis of some new 2,4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents, *Eur. J. Med. Chem.*, **2003**, 38, 3131-3138.
- [32]. A. Kulkarni, S.A. Patil, P.S. Badami, DNA cleavage and in vitro antimicrobial studies of Co(II), Ni(II), and Cu(II) complexes with ONNO donor Schiff bases: Synthesis, spectral characterization, and electrochemical studies, *J. Enz. Inhib. Med. Chem.* **2010**, 25, 87-96.