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Synthesis, Spectral Characterization, Biological Screening and DNA Studies of 2-(5-Mercapto-1, 3, 4-Oxadiazol-2-yl) Phenol Transition Metal (II) Complexes

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Abstract: *A new series of transition metal (II) complexes of 2-(5-mercapto-1, 3, 4-oxadiazol-2-yl) phenol of a ligand,(where [M = Cu(II), Co(II), Ni(II) and Zn(II)] have been designed and synthesized by 2-hydroxybenzohydrazide in ethanol with carbandisulphide and KOH. All the synthesized complexes were characterized by analytical data, magnetic susceptibility measurements, IR, UV-Vis, ^{1}H-NMR, ^{13}C NMR and EPR spectral techniques. Spectral and magnetic studies of the metal (II) complexes suggest an octahedral geometry around the metal ion. The electrolytic behavior and monomeric nature of the complexes were confirmed from their conductance data. The redox behaviour of the copper (II) complex has been studied by cyclic voltammetry. The second harmonic generation (SHG) efficiency was measured by Kurtz and Perry method. The ligand and their metal complexes were screened by Well diffusion method. The interaction of complexes with CT- DNA was investigated by viscosity measurement. Results suggest that all the complexes bind to DNA via an intercalative mode. The DNA cleavage ability of all the complexes was examined on calf thymus (CT-DNA) plasmids using gel electrophoresis experiment in presence of H_2O_2. From the results it is concluded that all the complexes cleave DNA.*

Keywords:*1,3,4- oxadiazole, octahedral geometry ,SHG , biological activity, DNA cleavage*

I. INTRODUCTION

Ligand containing poly functional donors (S, O and N) also gained tremendous attention resulting from the interest of structural diversities as well as the biological activities (Fenton and Vigato, 1988; Devi and Singh, 2011; Singh et al., 2011). Moreover, ligand such as 1,3,4-oxadiazoles are a class of heterocyclic compound which also has received significant interest in medicinal chemistry and biological activities. The Five member heterocyclic compounds possess interesting biological activity. Among them the compounds bearing 1,3,4-oxadiazole nucleus have wide applications in medicinal chemistry. These compounds also have been reported to have significant anti-inflammatory [1], antifungal [2], antibacterial [3], antiviral [4], and anticancer [5] activities. Heterocyclic compounds like fluconazole [6, 7], itraconazole [8], ravuconazole [9], voriconazole [10, 11] and posaconazole [12], were used as therapeutically important medicines. Transition metal complexes of 1,3,4-Oxadiazoles have also been an active area of research [13]. 1,3,4-oxadiazole derivatives are also among the most widely employed electron conducting and hole blocking (ECHB) materials in organic light-emitting diodes (LEDS) [14]. NLO material capable of frequency conversion is generally composed of an electron donor (D), an acceptor (A) and a conjugated π- system as a bridge providing the electronic communication between the donor and acceptor [15]. Heterocyclic five-member ring polymers (furan, pyrrole, thiophene, triazole,etc) are the most extensively studied of the polyconjucated and conductive polymers [16, 17].

Experimental measurements suggest that organic molecules containing rings (furan, pyrrole, thiophene, triazole, etc) exhibit significant nonlinear optical properties. Theoretically and experimentally a new class of second order nonlinear optical materials which utilize the coupling of electron rich and electron deficient aromatic heterocyclic units to provide the charge asymmetry for nonlinear optical effect [18]. 1,3,4-Oxadiazole considered as an important five - member compound among the huge heterocyclic families has been studied as excellent candidate for material applications during the past years [19, 20]. The wide range of applications of the ligand and its metal complexes are used our interest to prepare a new series of some of those metal complexes. Prompted by the observed biological activities of the above mentioned derivatives and in continuation of our ongoing studies on novel biologically active molecules,

We have designed and synthesized and characterized new 1,3,4-Oxadiazoles derivative and its transition metals such as Cu(II), Co(II), Ni(II) and Zn(II) complexes. The results of this study are discussed in this paper.

II. EXPERIMENTAL

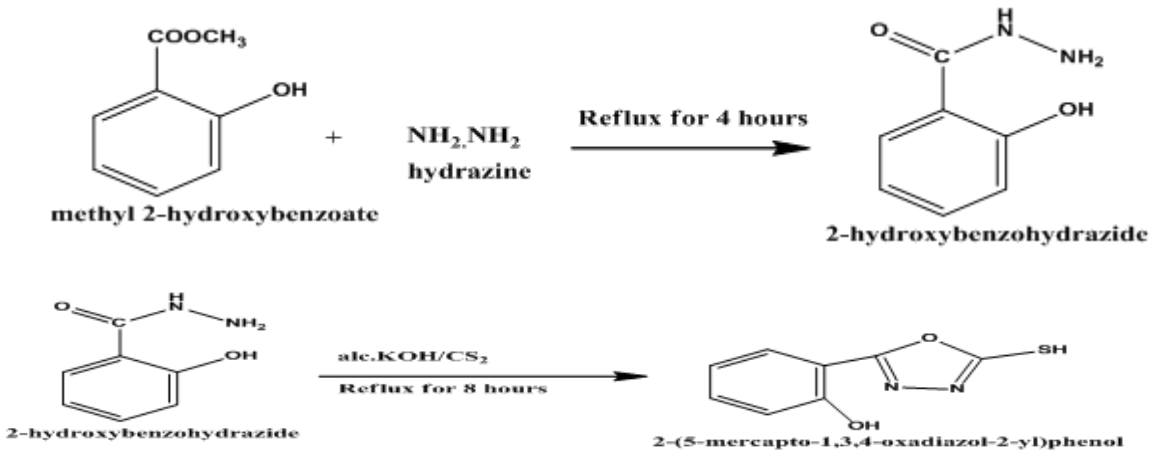
All chemicals were obtained from Aldrich Chemical & Co. and used without purification. The UV-Vis. spectra of the ligand and its metal complexes were recorded in DMSO using a JASCO V-530 spectrophotometer. IR spectra in KBr discs were recorded on a JASCO FT-IR 460 plus spectrophotometer at Thiagarajar College, Madurai. Cyclic voltammetry measurements were carried out at room temperature in DMSO (CH Instruments, USA, voltammograph) using a three-electrode cell containing a reference Ag/AgCl electrode, Pt wire auxiliary electrode, and glassy carbon working electrode with tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Elemental analyses were performed at SAIF, CDRI, Lucknow. ^{1}H-NMR, ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker DRX-300, 300 MHz NMR spectrometer. EI mass spectra were recorded at IIT, Madras. EPR spectrum was recorded at SAIF, IIT, Bombay. Magnetic moments of the complexes were measured on a Magnetic Susceptibility Balance Mark 1 Sherwood UK at Thiagarajar College, Madurai. Effective magnetic moments were calculated using the formula $\mu eff = 2.828 (\chi MT)^{1/2}$ where χM is the molar susceptibility. Molar conductance of the complexes (10^{-3} mol L^{-1}) was measured in DMF at room temperature using a Systronic conductivity bridge. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. DNA was purchased from Bangalore Genei (India).Microanalyses (C, H and N) were performed in Carlo Erba 1108 analyzer at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow, India. The second harmonic generation (SHG) activity was confirmed by the Kurtz and Perry powder technique at Indian Institute of Science, Bangalore.

2.1. Synthesis of 2-(5-mercapto-1, 3, 4-oxadiazol-2-yl) phenol (MODP) Preparation of 2-Hydroxybenzohydrazide

20 mmol of methyl 2-hydroxybenzoate and 20 mmol of hydrazine was taken in a round bottom flask. The above reaction mixture was reflux for 4 hours and kept aside overnight. The white precipitate 2-hydroxybenzohydrazide was filtered off and recrystallized from ethanol. The yield obtained was 78 %,(m.p. 150 ^{o}C)

Preparation of 2-(5-mercapto-1, 3, 4-oxadiazol-2-yl)phenol [MODP]

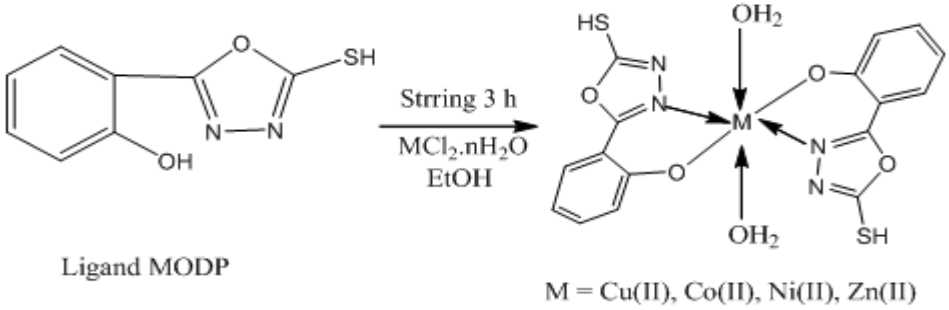
The 2-hydroxybenzohydrazide (10 mmole) in 30 ml ethanol were added with carbandisulphide (30 mmole) and KOH (12.5 mmole) and the mixture was refluxed for 8 hours. The solvent was evaporated. The residue was dissolved in water and acidified with dil. HCl and the precipited ligand was filtered and recrystallised from ethanol. The yield obtained was 80 %, m.p. 130 ºC. (Scheme .1)



Scheme .1. Synthesis of ligand MODP

2.2. Synthesis of metal(II) complexes:

The metal (II) complexes were obtained by stirring the MODP (0.388 g, 2 mmol) in ethanol (15 ml) with the metal chloride (1 mmol, M = Cu(II), Co(II), Ni(II) and Zn(II)). After all the metal(II) chloride had reacted in ethanol, the reaction mixture was stirred for 3 hours at room temperature, filtered and concentrated to 1-2 ml. Addition of petroleum ether yielded metal(II) complexes generally in high yield (75-85 %). It was recrystalised from ethanol. The yield obtained was 77 %, m.p. 215-230 ºC (Scheme. 2)



Scheme . 2. Synthesis of metal complexes from ligand (MODP)

III. RESULTS AND DISCUSSION

This study aims to synthesis of MODP and to investigate the bonding modes and geometry of the ligand with various transition metal (Cu(II), Co(II), Ni(II) and Zn(II)) which may act as a models to metalloenzymes. In this section the studies of novel new MODP ligand and their metal complexes have been investigated. The modern spectral techniques such as IR, UV-Vis, ^{1}H NMR ^{13}C NMR, CV, EPR and Mass are used for characterization. Elemental analysis data and physical characteristics of MODP ligand and complexes are summarized in table. 1. All these complexes are intensively colored, air and moisture firm amorphous solids. They are insoluble in common organic solvents and only soluble in DMF and DMSO. Molar conductance values of the soluble complexes in DMF (10^{-3}M solution at 25 °C) indicate that the complexes non electrolytic in nature. The elemental analyses data concur well with the planned formulae for the ligand and also recognized the $[ML_2].2H_2O$ composition of the metal(II) chelats.

Table.1.Physical characterization, analytical, molar conductance and magnetic susceptibility data of the ligand (MODP) and its metal(II)complexes

Compound	Formula weight	Color	Found (Calcd) (%)					m.p. (°C)	μ eff (B.M.)	Λ_M (Ω^{-1} cm^2 mol^{-1})
			M	C	H	N	S			
$C_8H_6N_2O_2S$(MODP)	194	Pale yellow	-	49.44 (49.47)	3.07 (3.11)	14.35 (14.42)	16.46 (16.51)	130	-	-
$[Cu(MODP)_2 2H_2O]$	501	Greenish brown	12.62 (12.68)	40.70 (40.75)	3.37 (3.42)	11.12 (11.18)	12.74 (12.80)	235	1.8	4.87
$[Co(MODP)_2 2H_2O]$	496	Dark green	11.82 (11.87)	41.08 (41.13)	3.40 (3.45)	11.24 (11.29)	12.87 (12.92)	230	4.82	8.7
$[Ni(MODP)_2 2H_2O]$	496	Yellowish green	11.78 (11.83)	41.08 (41.15)	3.40 (3.45)	11.78 (11.83)	12.88 (12.93)	215	3.02	15.47
$[Zn(MODP)_2 2H_2O]$	502	Yellow	12.96 (13.01)	40.55 (40.60)	3.37 (3.41)	11.10 (11.14)	12.70 (12.75)	220	---	12.42

3.1. IR spectral studies

Infrared spectroscopy is one of the most widely used tools for the detection of functional group in pure compounds and mixtures, nature of binding of atom in metal complexes. IR spectral data of the ligand [MODP] and its metal(II) complexes are shown in Table 4.3. The ligand do exhibit tautomerism [21] and one can expect both ν (S-H) and ν (C=S). A medium intensity band around 2523 cm^{-1} due to ν (S-H) indicates the thiol form of the ligand. The band due to ν (C=S) in the 780-740 cm^{-1} of the ligand has remained unperturbed in these complexes indicating that N or S of the thiamide group is not involved in the bond formation [22]. These observations suggest the non-involvement of sulfur atom in coordination. The IR spectrum of the ligand display at 1595 cm^{-1}, which may be assigned to the ν (C=N) oxadiazole ring. When compared to ligand this band is shift lower frequency (12-37 cm^{-1}) indicates the coordination of Nitrogen in the oxadiazole ring. The band around 3219 cm^{-1} due to phenolic OH, which is observed in ligand, but its disappears in all complexes, this indicates the ligand coordinate to the metal ion through phenolic oxygen atom of OH group via deprotonation [23]. A broad band appeared in the region 3443-3523 cm^{-1} in all complexes indicates the presence of coordinated water [24]. The participation of oxygen and nitrogen in coordination with the metal ion is further supported by the new band appearance of ν (M-N) and ν (M-O) at 550-520 cm^{-1} and 380-420 cm^{-1}, respectively in the far infrared region [25, 26].

Table. 2. IR spectral data (cm^{-1}) of the ligand MODP and its metal(II) complexes

Compounds	**Vibrational frequencies (cm^{-1})**						
	ν (OH)	**ν (C=N)**	**ν (S-H)**	**ν (C=S)**	**ν (H_2O)**	**ν (M-N)**	**ν (M-O)**
$C_8 H_6 N_2O_2S$ (MODP)	3219	1595	2523	780	-	-	-
[Cu(MODP)$_2$2H_2 O]	-	1534	2522	778	3425	560	424
[Co(MODP)$_2$2H_2 O]	-	1576	2522	779	3450	564	430
[Ni(MODP)$_2$2H_2 O]	-	1532	2524	778	3460	567	426
[Zn(MODP)$_2$2H_2 O]	-	1556	2525	778	3433	563	440

3.2. NMR Spectral studies

^{1}H NMR Spectra of ligand MODP

The ^{1}H NMR spectral data for the 2-(5-mercapto-1, 3, 4-oxadiazol-2-yl) phenol was recorded in $CDCl_3$. The ^{1}H NMR spectrums of ligand MODP shows multiplets were observed around δ 6.8-7.8 are due to phenyl protons. A proton due to -OH group at 2-position of Phenyl ring has resonated as a singlet at δ 10.4 and a signal at δ 7.02-7.6 due to (-CH_2) protons. The signal due to(S, 1H, HC=N) resonated at 8.4 δ and the Signal at 10.9 δ are due to SH proton. In addition to these signals, a sharp signal at 3.5 ppm is attributed to SH protons. These observations suggest that the ligand exist in thiol- thione tautomerism.

^{1}H NMR Spectra of Zn (II) complex

^{1}H NMR spectrum of the Zn(II)complex the aromatic protons have resonated in the region 6.8- 8.42 δ as a multiplet. The signal due to OH at 10.4 δ in ligand, disappears in case of complexes indicating the involvement of OH in the complexes formation via deprotonation and a sharp singlet at 10.92 δ due to NH, indicating the thione form of the ligand in the solution state. The signal due to(S, 1H, HC=N) resonated at 8.4 δ which is shifted slightly downfield at 8.0 δ which reveals the bonding of the azomethine nitrogen to Zn(II)ion. A new peak at 3.6 δ due to –SH is observed. This suggests the existence of ligand in the thione form in the complexes and the non-involvement of –SH in the coordination

^{13}C-NMR spectra

The ^{13}C-NMR spectrum shows peaks at δ ar C:[123.18 (CH), 124.57 (CH), 125.71 (2CH), 126.83 (CH), 129.54 (C)]. The peaks observed at δ 159.7 and 178.8 are due to C-2 and C-5 of the oxadiazole ring.

3.3. Mass spectra

The mass spectra of the ligand (MODP) and its cobalt (II) complex were recorded at room temperature and they are used to compare their stoichiometry composition. The ligand (MODP) shows a molecular ion peak at m/z = 194, which is also supported by the "Nitrogen Rule" since the compound possesses odd number of nitrogen atoms and the molecular ion peak for Co(II) complex at m/z = 496. The different competitive

fragmentation pathways of ligand give the peaks at different mass numbers at 69, 77, 92, 120, 139, 151, 179. The different competitive fragmentation pathways of metal complex give the peaks at different mass numbers at 90, 118, 147, 191, 250, 269, The intensity of these peaks reflects the stability and abundance of the ions. It is also supported by the mass spectra of the other complexes.

3.4. Electronic spectral studies and Magnetic susceptibility studies

The electronic spectral data of the MODP and its metal(II) complexes are presented in Table 4.2. The absorption spectrum of MODP, exhibits a strong peak in the UV range, centered at 37458 cm^{-1} due to charge transfer bands. On the other hand, the relatively strong peaks in the UV range of solutions of some transition metal(II) complexes confirmed the charge transfer between the ligands and the central ion. Generally, copper(II) complexes are blue or green. Very few are black and brown which are due to the strong charge transfer bands tailing off into the blue end of the visible spectrum. The Cu (II) complex gives absorption band at 16535 cm^{-1} which is assignable to $^2E_g \rightarrow {}^2T_{2g}$ having the Distorted octahedral structure [27]. The magnetic moment for Cu(II) complex is 1.8 BM [28].

In the electronic spectra of Co(II) complex the three absorption bands observed at 10112 cm^{-1}, 14494 cm^{-1} and 19159 cm^{-1}, due to $^4T_{1g}$ (F) → $^4T_{2g}$ (F) (ν_1), $^4T_{1g}$(F) → $^4A_{2g}$ (F) (ν_2) and $^4T_{1g}$(F) → $^4T_{2g}$ (P) (ν_3) transitions respectively. These transition suggest octahedral geometry for Co(II) complex [29] and the magnetic moment value of the cobalt(II) complex 4.82 B.M. confirms the octahedral geometry [30]. Ni(II)complexes exhibit three bands at 10082 cm^{-1}, 13420 cm^{-1} and 23230 cm^{-1} , due to $^3A_{2g}$(F)→$^3T_{2g}$ (F) (ν_1) $^3A_{2g}$(F) → $^3T_{1g}$(F) (ν_2) and $^3A_{2g}$(F) → $^3T_{1g}$(P) (ν_3) transitions respectively are in confirmatory with the octahedral geometry for the Ni(II) ion [31]. The Ni(II) complex reported has magnetic moment of 3.02 B.M. which indicates that the Ni(II) complex is six-coordinate and probably octahedral [32]. The electronic spectrum of the Zn(II) complex shows an absorption band at 23,455 cm^{-1} attributed to the LMCT transition, which is compatible with this complex having an octahedral structure.

Table 3. Electronic spectral data (cm^{-1}) of the ligand MODP and its metal(II)complexes

Compounds	Solvent	Frequency (cm^{-1})	Assignment	Geometry
$C_8 H_6 N_2O_2S$ (MODP)	DMSO	37458	INCT	-
$[Cu(MODP)_2 2H_2O]$	DMSO	16535	$^2E_g \rightarrow {}^2T_{2g}$	Distorted octahedral
$[Co(MODP)_2 2H_2O]$	DMSO	10112 14494 19159	$^4T_{1g}$ (F) → $^4T_{2g}$ (F)(ν_1) $^4T_{1g}$(F) → $^4A_{2g}$ (F) (ν_2) $^4T_{1g}$(F) → $^4T_{2g}$ (P) (ν_3)	octahedral
$[Ni(MODP)_2 2H_2O]$	DMSO	23230 13420 10082	$^3A_{2g}$(F)→$^3T_{2g}$(F), $^3A_{2g}$(F)→$^3T_{1g}$(F) $^3A_{2g}$(F) → $^3T_{1g}$(P)	octahedral

3.5. Electrochemical behavior

cyclic voltammetric studies of the copper(II) complex was performed in acetonitrile solution at room temperature with tetrabutylammonium perchlorate (TBAP) as supporting electrolyte; glassy carbon as working electrode; Pt wire as auxiliary electrode and Ag/AgCl as reference electrode; scan rate 100 mVs^{-1} (-0.2 to 1.4 V) shows a well-defined redox process. A cyclic voltammogram of Cu(II) displays two reduction peaks, first one at Epc = 0.275 V with an associated oxidation peak at Epa= -0.1 V and second reduction peak at Epc= 1.0 V with an associated oxidation peak at Epa = 0.9 V respectively at a scan rate of 0.2V/s. The value of ΔEp is 0.175 and 0.1 for first and second redox couples respectively and increases with scan rate giving evidence for quasi-reversible nature associated with one electron reduction. The ratio of anodic to cathodic peak currents (I_{pc}/I_{pa} = ~1) corresponding to a simple one-electron process was also reported by R. Klement *et al* [33]. The peak current for the complex varies with scan rate and the $\Delta E_p = (E_{pa} - E_{pc})$ values are greater than 200 mV which, indicates that the reduction process are quasi-reversible in nature and a chemical changes occurs with the electron transfer [34**]**. The cyclic voltammogram of the copper(II) complex is shown in Fig. 1.

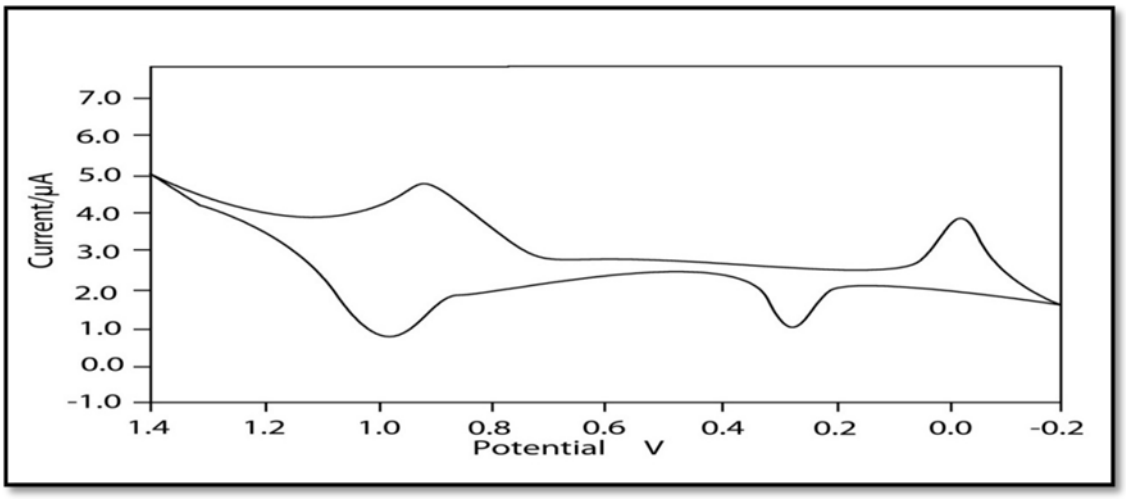


Fig. 1 cyclic voltammogram of the copper(II) complex

3.6. EPR spectral studies

The ESR spectrum of copper(II) complex are recorded in DMSO at 300 and 77 K and the spin Halmiltonian parameters of the complexes are listed in Table 4. The observed spectral parameters reveals that $g_{\parallel} > g_{\perp}$ characteristic of an axially elongated octahedral geometry [35]. The g_{iso} value is almost equal to 2.28 indicating the covalent character of the metal-ligand bond. Further, it is supported from the fact that the unpaired electrons lies predominantly in the $d_{x^2-y^2}$ orbital. The observed value of G for copper complex is 2.97, characteristic of mono nuclear configuration which also suggests that the exchange coupling is present and misalignment is appreciable. The magnetic moment of the copper(II) complex (1.8 BM) is calculated from the expression $\mu^2 = 3/4\ |g|^2$, which reflects the presence of an unpaired electron. The α^2 value (0.83) points out appreciable in-plane covalency.The calculated value of $g_{\parallel}/A_{\parallel}$ (122 cm) for the copper(II) complex reveals slightly distorted structure. The orbital reduction factors such as $K_{\parallel}$ and $K_{\perp}$ are estimated from the equation. $K_{\parallel}$ (0.916) > $K_{\perp}$ (0.55) indicates poor in-plane π bonding which is also reflected in β^2 values. The lower the value of α^2 compared to β^2 indicate that the in-plane σ bonding is more covalent than the in-plane π- bonding.

Table 4. EPR spectral data of the copper(II) complex

Compounds	**$g_{\parallel}$**	**$g_{\perp}$**	**g_{iso}**	**$A_{\parallel}$ 10^{-4}cm^{-1}**	**$A_{\perp}$ 10^{-4}cm^{-1}**	**A_{iso} 10^{-4}cm^{-1}**	**α^2**	**β^2**	**$g_{\parallel}/A_{\parallel}$ cm**
[Cu(MODP)$_2$]	2.27	2.09	2.28	179.8	96.9	161	0.83	0.75	122

On the basis of above discussion which is based upon elemental analysis, molar conductance, magnetic susceptibility measurement, IR, electronic and EPR spectral studies, the following structures, may be proposed for the complexes as shown in Fig, 2.

Fig. 2. Proposed structure of metal(II) complexes of MODP
M = Cu(II), Co(II), Ni(II), Zn(II)

3.7. Elemental analysis and molar conductivity measurements

The metal(II)complexes were dissolved DMF in and the molar conductivities of their solution at room temperature were measured. The lower conductance values of the complexes support their non-electrolytic nature of the compounds.

3.8. Nonlinear Optical Studies

NLO material capable of frequency conversion is generally composed of an electron donor (D), an acceptor (A) and a conjugated π- system as a bridge providing the electronic communication between the donor and acceptor [36]. The advantages offered by organic over inorganic systems include high Electronic susceptibility through high molecular polarizability ($\chi^{(2)}$),fast response time, facile modification through standard synthesis method and relative ease of device processing. Heterocyclic five-member ring polymers(furan, pyrrole, thiophene, triazole,etc) are the most extensively studied of the polyconjucated and conductive polymers [37]. Experimental measurements suggest that organic molecules containing rings (furan, pyrrole, thiophene, triazole, etc) exhibit significant nonlinear optical properties. Theoretically and experimentally a new class of second order nonlinear optical materials which utilize the coupling of electron rich and electron deficient aromatic heterocyclic units to provide the charge asymmetry for nonlinear optical effect [38].

3.8.1. Second Harmonic Generation (SHG) Test

The second harmonic generation efficiency of MODP sample was measured by using Kurtz–Perry powder SHG technique with potassium dihydrogen phosphate (KDP) crystal as reference material [39]. The finely grounded sample was packed between transparent glass slides and an input energy of 2.2 mJ/ pulse was used in this particular setup. The fundamental beam of 1064 nm from Qswitched Nd:YAG laser beam and 10 ns pulse width with an input rate of 10 Hz was used to test the NLO property. The fundamental beam was filtered by using an IR filter. A photomultiplier tube was used as detector. The output from the sample was monochromated to collect the intensity of 532 nm component and to eliminate the fundamental radiation. The generation of the second harmonic was confirmed by the emission of green light. The SHG output efficiency for MODP and KDP samples were found to be 195 mV and 55 mV respectively. This may be due to the presence of heterocyclic triazole nucleus. In general molecule with delocalized π-electron system can have large nonlinear polarizabilities [40] and it has been generally understood that the molecular nonlinearity can be enhanced by systems with strong donor and acceptor groups [41]. From the analysis of electronic transitions and molecular orbital involved, ligand orbital can improve the NLO properties. Thus, it is observed that SHG efficiencies of MODP sample were found to be 3.5 times higher than that of KDP.

3.9. Biological Study

The MODP and its metal (II) complexes were evaluated for antimicrobial activity against gram positive bacteria such as *Klebsillapneumonia, Staphylococcus aureus*, gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and and antifungal activity against *Candida albicans* by well diffusion method. The test solutions were prepared in DMSO, nutrient agar used as culture medium. The zone of inhibition was measured in mm and the values of the investigated compounds are summarized in Table.5. From the observed result it is clear that the metal(II) complexes showed enhanced antimicrobial activity than that of free ligand MODP. Probably this may be due to the greater lipophilic nature of the complexes. Such an enhanced activity of the metal (II) complexes can be explained on the basis of Overton's concept [42] and Chelation theory [43]. A possible explanation for the observed increased activity upon chelation is that the positive charge of the metal in chelated complex is partially shared with the ligand donor atoms so that there is an electron delocalization over the whole chelate ring. This, in turn, will increase the lipophilic character of the metal chelate and favors its permeation through the lipoid layers of the bacterial membranes.

Table .5. Antibacterial activity data of the ligand MODP and its metal(II) complexes

Compounds	**Zone of inhibition (mm)***				
	K.pneumonia,	*S.aureus*	*E.coli*	*P.aeruginosa*	*Candida albicans*
C_8 H_6 N_2O_2S (MODP)	09	10	12	10	12
[Cu(MODP)$_2$2H_2O]	11	14	15	15	15
[Co(MODP)$_2$2H_2O]	14	12	13	16	14
[Ni(MODP)$_2$2H_2O]	12	13	12	14	14
[Zn(MODP)$_2$2H_2O]	10	14	14	13	16
Amikacin	20	18	18	21	_
Ketokonazole	_	_	_	_	25

3.10. DNA Binding activity of metal(II) complexes

A large number of evidences indicate the mechanism of action of anticancer agents binds through distinctive binding modes to the DNA of cancer infected cell in such a way, that the cell cannot replicate further. This inhibition of replication finally leads to the death of the infected cell. The binding modes are responsible for activity and the mechanism by which DNA replication is totally inhibited in cancer cells [44]. Binding studies of small molecules to DNA are very important in the development of DNA molecular probes and new therapeutic reagents. Over the past few decades, DNA-binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron transfer probes, DNA foot printing and sequence-specific cleaving agents, and potential anticancer drugs [45].

3.11 Viscosity measurement

To further make clear the interaction between the complex and DNA, viscosity measurements were studied. Viscosity experiments were carried on an Ostwald viscometer, immersed in a thermo stated water-bath maintained at a constant temperature at 30.0±0.1 °C. CT- DNA samples of approximately 0.5mM, were prepared by sonication in order minimize complexities arising from CT DNA flexibility. Flow time was measured with a digital stopwatch three times for each sample and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of metal(II) complexes to DNA, where η is the viscosity of CT DNA solution in the presence of complex, and η_0 is the viscosity of CT DNA solution in the absence of complex. Viscosity values were calculated after correcting the flow time of buffer alone (t_0), $\eta = (t-t_0)/t_0$ .Optical photophysical studies are not enough to explain a binding between DNA and the complex. A classical intercalation model results in lengthening the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity [46, 47]. Intercalating agents are expected to elongate the double helix to accommodate the ligands in between the base leading to an increase in the viscosity of DNA. In contrast, complexes those bind exclusively in the DNA grooves by partial and/or non-classical intercalation, under the same conditions, typically cause less pronounced (positive or negative) or no change in DNA solution viscosity. The values of $(\eta/\eta_0)^{1/3}$ were plotted against [complex]/[DNA]. There is a marked effect of metal complexes on the viscosity of CT-DNA was observed.From the Fig. 3. The gradual increase in the relative viscosity was observed on addition of the metal complexes to DNA solution suggesting mainly intercalation mode of binding nature of the complexes. The results reveal that the complexes Cu(II), Co(II), Ni(II) and Zn(II) effect relatively in apparent increase in DNA viscosity. Not only does this result give sign of an intercalative binding mode of the complex, but it is also in agreement with the pronounced hypochromism and bathochromism of the complexes in the presence of DNA.

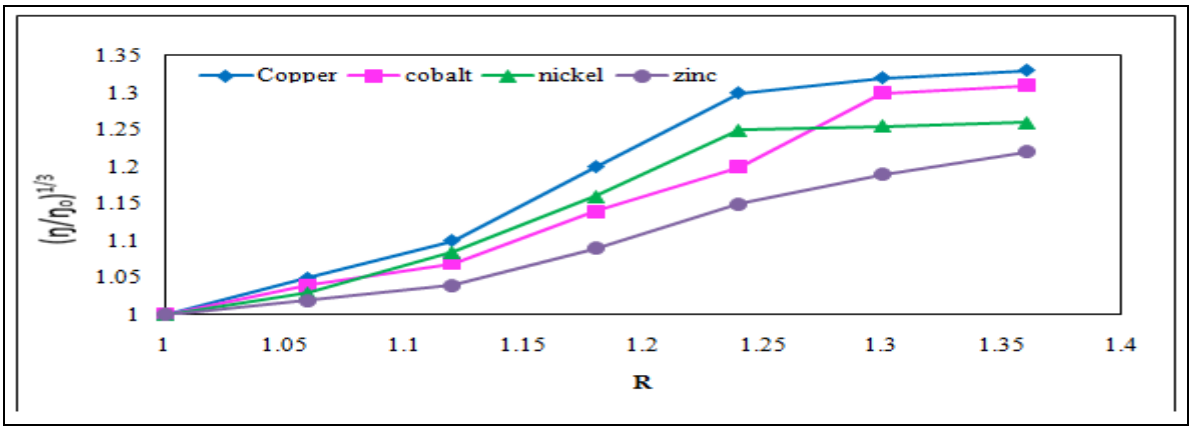


Fig. 3. Effect of increasing amounts of $[Cu(L3)_2.2H_2O]$ (♦),$[Co(L3)_2.2H_2O]$ (■), $[Ni(L3)_2.2H_2O]$(▼) $[Zn(L3)_2.2H_2O]$ (●), on the viscosity of DNA. R = [complex]/[DNA].

3.11. DNA cleavage activity of metal (II) complexes

The oxidative cleavage activity of the complexes of MODP was studied by gel electrophoresis using calf thymus DNA (15 µl) in Tris-HCl buffer (pH = 7.0). Selected CT- DNA cleavage activity of the gel diagram is shown in Fig. 8. The gel to conduct electrophoresis with such systems including DNA alone,

DNA+H_2O_2+M(II), (M = Cu(II), Co(II), Ni(II) and Zn(II) complexes, which were prepared under the same condition and kept at 2 h in order to eliminate the influence of the reaction speed. The cleavage activity of the complexes was carried out for 2 h exposure. The cleavage efficiency of the complexes compared with that of the control is due to their efficient DNA-binding ability. In the present study, the CT- DNA gel electrophoresis experiment was carried out at 35 °C using our synthesized complexes in presence of H_2O_2 as an oxidant. It was found that, all complexes exhibit nuclease activity in presence of H_2O_2.

The general oxidative mechanisms proposed to account for DNA cleavage by hydroxyl radicals *via.* abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [48]. The cleavage is inhibited by the free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. From Fig.4. it is evident that all the complexes cleave DNA more efficiently in the presence of an oxidant (H_2O_2). This may be attributed to the formation of hydroxyl free radicals. The production of a hydroxyl radical due to the reaction between the metal complex and oxidant may be explained as shown below:

$$(Ligand)Cu^{2+} + H_2O_2 \rightarrow (Ligand)Cu^{3+} + OH^{\bullet} + OH^{-}$$

The $OH^{\bullet}$ free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of a sugar phosphate backbone. All the complexes showed pronounced nuclease activity in the presence of the oxidant H_2O_2, which may be due to the increased production of hydroxyl radicals.

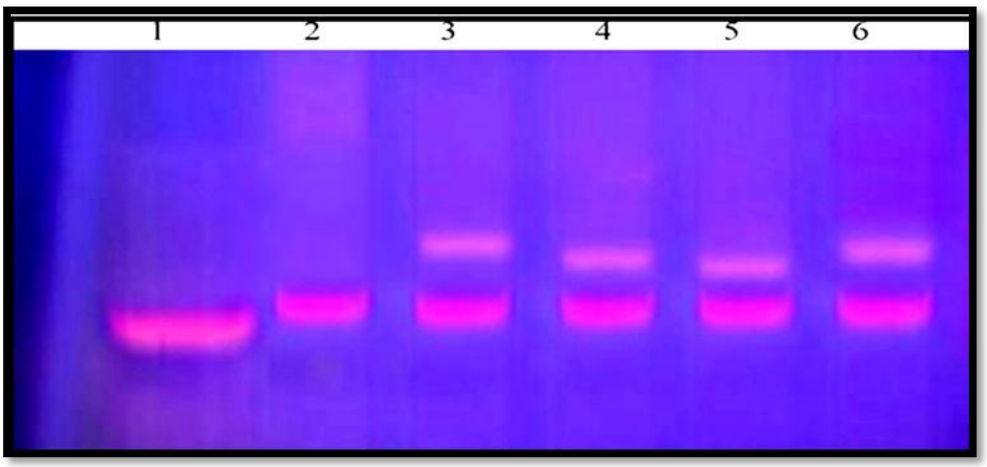


Fig. 4. Gel electrophoresis diagram for the metal(II) complexes

Lane 1: CT DNA alone	Lane 4: CT DNA + Co(II) complex
Lane 2: CT DNA + H_2O_2	Lane 5: CT DNA + Ni(II) complex
Lane 3: CT DNA + Cu(II) complex	Lane 6: CT DNA + Zn(II) complex

Control experiment using DNA alone does not show any significant cleavage of CT DNA even on longer exposure time. Hence, we conclude that the copper(II), cobalt(II), nickel(II) and zinc(II) complexes cleaves DNA as compared with control DNA in the presence of H_2O_2. Further, the gel diagram indicates the presence of radical cleavage [49].

IV. CONCLUSION

A new series of transition metal(II) complexes of 2-(5-mercapto-1, 3, 4-oxadiazol-2-yl) phenol of a ligand , (where M = Cu(II), Co(II), Ni(II) and Zn(II)) have been synthesized and characterized. Elemental, conductivity, and Mass spectrometry analyses revealed the stoichiometry and composition of the complexes. FT- IR, UV-Vis, ^{1}H NMR, ^{13}C NMR and EPR spectral data, as well as magnetic measurements, confirmed the bonding features of the above ligand complexes. From the results of cyclic voltammetry it is shown that copper(II) complex exhibit quasi-reversible cyclic voltammetric responses in DMSO solution corresponding to the Cu(II)/Cu(I) redox process.

The EPR parameters of copper(II) complex indicate that the complex has octahedral geometry. The SHG efficiency was measured using Kurtz and Perry method and is found to be about 1.2 times that of the standard KDP crystal. These results show that MODP was a NLO material with possible applications for

frequency conversion. The *in vitro* biological activity of the MODP and its metal(II) complexes indicate that the complexes have higher antimicrobial activity than the free ligand. The binding of these complexes to CT-DNA was investigated in detail via viscosity. The experimental results reveal that all of the complexes can interact with DNA through an intercalative binding mode.

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