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Synthesis, Spectral Characterization, Antimicrobial Screening And DNA Binding, Cleavage Studies Of Transition Metal Complexes Of Heterocyclic Ligand Derived From 4-Aminoantipyrine And 2-Mercaptobenzimdazole

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Abstract:
A new Mannich base 4-((2-mercapto-1H-benzoimidazol-1-yl)methylamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one was synthesized from 2-Mercapto benzimidazole, formaldehyde and 4-aminoantipyrine. Further the new ligand was made to react with metal(II) chlorides to obtain the metal complexes[Cu(II), Ni(II), Co(II) and Zn(II) complexes]. The structure of the complexes has been investigated by using elemental analysis, IR, UV-ViS, ^{1}H NMR, ^{13}C NMR, EPR, Mass spectra, magnetic susceptibility and conductivity measurements. The elemental analysis and spectral studies indicate octahedral geometry for all the complexes. The electrochemical property of the ligand and its metal complexes has been studied by cyclic voltammetry. Biological screening of the ligand and metal(II) complexes have been carried on Streptococci, Staphylococcus aureus, Pseudomonas, Klebsilla Pneumoniae, Chromo bacterium, Candida albicans by the well diffusion method which indicate that the metal(II) complexes possess higher activity than the free ligand. The binding properties of metal complexes with DNA were investigated by viscosity measurements. Detailed analysis reveals that the metal complexes intercalate into the DNA base stack as intercalators. 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: *Mannich base, 2-Mercaptobenzimidazole, 4-Aminoantipyrine, Biological activities, DNA cleavage.*

1.Introduction

Mannich base reaction is employed in the organic synthesis of natural compounds such as peptides, nucleotides, antibiotics and alkaloids. It is also used in the synthesis of medicinal compounds e.g. rolitetracycline (Mannich base of tetracycline), fluoxetine (antidepressant) and tolmetin (anti-inflammatory drug). In recent years, Mannich bases have gained importance because of their pharmaceutical importance [1]. A literature search reveals that some Mannich bases of substituted aminophenol and acetophenone possess broad spectrum biological activities, which include antineoplastic [2], antibacterial [3, 4], antifungal [5, 6], anti HIV [7, 8] and anticancer [9-11] activities. Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide range of biological activities.

Many effective antimicrobial agents show a heterocyclic moiety within their structure and, in particular, that substituted Benzimidazole, Benzoxazole (Sarhan et al.,2006) Similarly, 2-mercaptobenzimidazole derivatives possess varied biological activities such as anti-HIV [12], anthelmintic [13], antibacterial [14] and antifungal activity[15]. The transition metal complexes of 4-aminoantipyrine and its derivatives have been extensively examined due to their wide applications in various fields like biological, analytical and therapeutical agent [16−19]. In this view, we are interested in examining the biological activities of N, O, S donor Mannich bases and their transition metal complexes, in this article, we have studied the antifungal,antibacterial activities and DNA studies of Mannich base ligand 4-((2-mercapto-1H-benzo[d]imidazol-1-yl)methylamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (MBIDPP) and its metal (II)complexes.

2. 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.

2.1.Synthesis Of 4-((2-Mercapto-1H-Benzo[D]Imidazol-1-Yl)Methylamino)-1,5-Dimethyl-2-Phenyl-1H-Pyrazol-3(2H)-One. [MBIDPP] Ligand

A mixture of 2-Mercaptobenimdazole (10 mmol), Formaldehyde (10 mmol) and 4-aminoantipyrine (10 mmol) in 70 mL ethanol was refluxed for 8 hours. Subsequently ethanol was distilled off and the ligand was obtained by adding petroleum ether (40-60 ºC). The solid product was recrystallized from ethanol. The ligand designed as MBIDPP and dried in air the m.p.90 ºC. The Schematic representation of the ligand is given in figure. 1.

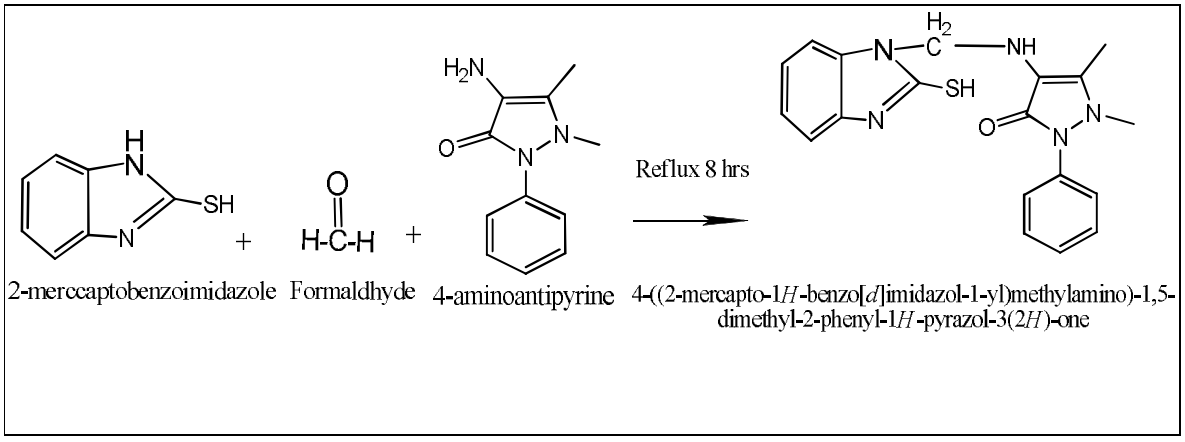

Figure 1: Synthesis Ligand MBIDPP

2.2. Synthesis Of Metal(II) Complexes

An ethanol solution of the above ligand (10 mmol) was added to a solution of metal (II) chloride (5 mmol) in ethanol and refluxed for 3 hours. After cooling the reaction mixture to an ambient temperature, the formed solid was filtered, washed with diethyl ether and finally dried in vacuum. The yield obtained was 60-70%. m.p. > 240 ºC.

3. Results And Discussion

The ligand MBIDPP forms stable complexes with Cu(II), Co(II), Ni(II)) and Zn(II). The analytical data of the ligand and the complexes together with their physical properties are given in Table 1. The molar conductance of the complexes implies that the complexes are non-electrolytes. The analytical data of the complexes are in good agreement with the general formula $[ML_2.2H_2O]$ where M = Cu(II), Co(II), Ni(II)) and Zn(II)]. The magnetic moments show paramagnetic nature of Cu(II), Co(II), and Ni(II) complexes and indicate the six-coordinate octahedral structure (Figure. 2)

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**			
Ligand (MBIDPP)	365	Brown	---	62.44 (62.41)	5.24 (5.21)	19.16 (19.13)	8.77 (8.73)	90	--	--
[Cu(MBIDPP) $2H_2O$]	829	Dark Brown	7.65 (7.61)	54.96 (54.93)	5.10 (5.07)	16.87 (16.83)	7.72 (7.70)	>240	1.83	16.9
[Co(MBIDPP) $2H_2O$]	825	Red	7.14 (7.12)	55.26 (55.22)	5.13 (5.10)	16.96 (16.93)	7.77 (7.74)	>240	4.8	18.0
[Ni(MBIDPP) $2H_2O$]	824	Green	7.11 (7.08)	55.28 (55.24)	5.13 (5.10)	16.96 (16.93)	7.77 (7.73)	>240	3.21	17.8
[Zn(MBIDPP) $2H_2O$]	830	Yellow	7.86 (7.83)	54.83 (54.80)	5.09 (5.04)	16.83 (16.80)	7.70 (7.64)	>240	--	10.9

Table 1: Physical characterization analytical and molar conductance data of the ligand (MBIDPP) and its metal(II) complexes

3.1.IR spectra

In order to study the binding mode of the ligand to metal in the complexes, the IR spectrum of the free ligand was compared with the corresponding metal complexes. Selected vibrational bands of the ligand and its metal complexes and their assignments are listed in Table 2. The IR spectrum the free ligand exhibited a strong band at 1647 cm^{-1} which could be assigned to ν(C=O) of the pyrazolone ring [20], a medium intensity band appeared at 1591 cm^{-1}, which could be attributed to the presence of ν(C=N) of the azomethine moiety [21]. A weak broad band around 3254 cm^{-1} could be attributed to stretching vibration of ν(N–H) bond[22]. Another strong band observed around 1136 cm^{-1}, can be assignable to ν (N–N) vibration mode [23]. In the metal complexes, the band corresponding to ν(C=O) of the pyrazolone ring was shifted to lower frequency around 1494 cm^{-1} suggesting its coordination with the metal ion. The C=N stretching frequency of the ligand was shifted towards lower values in all the complexes, indicating the involvement of the -C=N nitrogen in coordination to the central metal ion. The strong band observed around 1136 cm^{-1}, which could be assigned to ν(N-N) vibration modes was also affected on complexation. The bands due to ν(NH) of hydrazide remain same frequency in spectra of the metal complexes, suggesting the uncoordination of this group. A medium intensity band around 2729 cm^{-1} due to ν (S-H) of the ligand and it has remained unperturbed in these complexes indicating that sulphur of the thiol group is not involved in the bond formation[24]. These observations suggest the non-involvement of sulfur atom in coordination. A broad band appeared in the region 3414-3421cm^{-1} in all complexes indicates the presence of coordinated water or lattice water[25, 26]. 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) around 420-422cm^{-1} and 550-565cm^{-1}, respectively in the far infrared region[27-29].

compounds	Vibrational frequencies (cm^{-1})				
	ν(C=N)	**ν(C=O)**	**ν(H_2O)**	**ν(M−O)**	**ν(M−N)**
MBIDPP	1591	1647	-	-	-
[Cu(MBIDPP)$_2$.2H_2O]	1585	1630	3419	558	420
[Co(MBIDPP)$_2$.2H_2O]	1584	1632	3414	565	422
[Ni(MBIDPP)$_2$.2H_2O]	1586	1634	3416	556	420
[Zn(MBIDPP)$_2$.2H_2O]	1587	1635	3421	550	420

Table 2

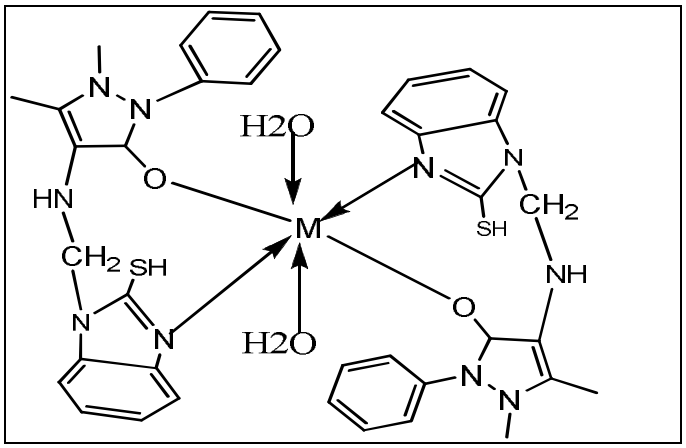

Figure. 2: Proposed structure of metal(II) complexes (M=Cu, Co, Ni, and Zn).

3.2.NMR spectroscopy

3.2.1.^{1}H NMR Spectrum Of The Ligand MBIDPP

The ^{1}H-NMR spectrum of the ligand was recorded in $CDCl_3$ solution. The peak observed as multiplets at 6.9-7.4 δ can be assigned to aromatic ring protons, the peaks at 4.2 δ for –N-CH_2

group, the SH proton at 3.4 δ, -C–NH 10.9 δ . The singlet at 3.1 for –N-CH_3 and 2.3 – 2.5 δ appeared due to the -C-CH_3 proton.

3.2.2.^{1}H NMR Spectrum Of Zn(II) Complex

The ^{1}H NMR spectrum, Zn (II) complex the aromatic protons have resonated in the region δ 6.9 - 7.4 as a multiplet. The signal due to SH proton appears at δ 3.4 unperturbed in the complexes indicates the non-involvement of sulfur atom of SH group.

3.2.3.^{13}C NMR Spectrum Of Ligand MBIDPP

The ^{13}C NMR spectra for the MBIDPP (L**)** was recorded in $CDCl_3$. The signals corresponding to the different non-equivalent carbon atoms at different values of δ as follows: at ca. δ 114.95, 116.45, 121.96 and 125.63 ppm due to the aromatic carbon atoms; at ca. δ 163.16 ppm (C=O) due to carbon atom of carbonyl group and at ca. δ 116.41 ppm (CH=CH) of pyrazoline at ca. δ 147.07 ppm and 154.14 ppm(H_3C-C) and (H_3C-N) due to carbon atoms of pyrazoline rings at ca. δ 162.24 ppm (C=N) due to carbon atoms for benzimdazole. at ca. δ 61.57 ppm (-CH_2)group.

3.3. Mass Spectra

Mass spectra of the complex provide a vital clue for elucidating the structure of compounds. The EI mass spectra of the ligand and its Nickel complex were recorded and used to confirm their stoichiometry composition. The ligand showed a molecular ion peak at m/z 365 corresponding to $[C_{19}H_{18}N_5OS]^+$ ion. Also, the spectrum exhibited the fragments at m/z 216, 150,

124 and 77. The mass spectrum of Ni(II) complex appeared at 824 m/z and the other fragments of the Ni(II)complex are at m/z 784, 284, 211 and 154. All the complexes underwent demetallation to form the species $[L]^+$, gave fragment ion peak at m/z 365 which is corresponding to the original molecular mass of the ligand under investigation.

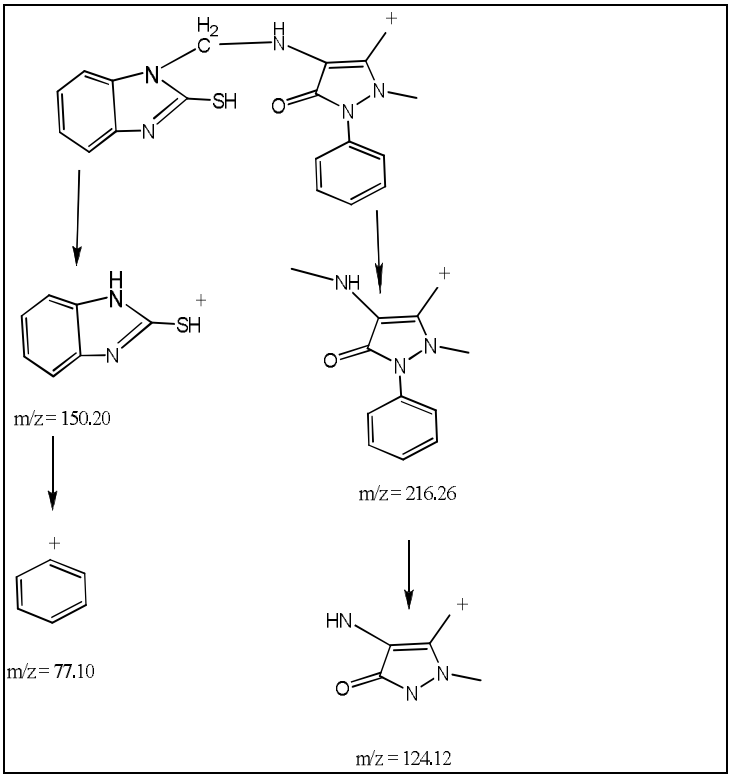

Figure 3: Mass fragmentation of the ligand MBIDPP

3.4. Electronic Spectral And Magnetic Moment Data Of The Metal Complexes

The electronic spectra of the metal complexes, recorded in DMSO solution are given in the Table-3. The absorption spectra of the ligand shows strong peaks at 33674 and 37740 cm^{-1}

which were assigned to the n - π* transition and π - π* transitions respectively. The electronic spectrum of Cu(II) complex displayed the d-d transition band in the region 15480 cm^{-1} which is due to $^2E_g \rightarrow {}^2T_{2g}$ transition. This band suggests a distorted octahedral geometry around the metal ion[30-33]. It is further supported by the magnetic moment value 1.83 B.M. Electronic spectrum of Co(II) complex displayed the bands in the region 9962, 14773 and 18906 cm^{-1} which are assigned to transitions, $^4T_{1g}$ (F) → $^4T_{2g}$ (F) (ν_1), $^4T_{1g}$(F) → $^4A_{2g}$ (F) (ν_2) and $^4T_{1g}$(F) → $^4T_{2g}$ (P) respectively. The transitions correspond to the octahedral geometry of the complex[34-38], which is also supported by its magnetic moment value (4.8 B.M). The absorption spectrum of Ni(II) complex displayed three bands at10102, 16894 and 21836 cm^{-1}. Assigned as $^3A_{2g}$ (F)→$^3T_{2g}$(F), $^3A_{2g}$ (F) → $^3T_{1g}$(F) and $^3A_{2g}$ (F) → $^3T_{1g}$(P) transitions, respectively, being characteristic of an octahedral geometry [39-41]. This geometry is further supported by its magnetic

susceptibility value (3.21 B.M). The complex of Zn(II) is diamagnetic. According to the empirical formula, an octahedral geometry is proposed for this complex

S.No.	Compound	Frequency(cm^{-1})	Assignment	Geometry
1	MBIDPP	37740 33674	INCT	---
2	$[Cu(MBIDPP)_2]2H_2O$	15480	$^2E_g \rightarrow {}^2T_{2g}$	Distorted Octahedral
3	$[Co(MBIDPP)_2]2H_2O$	9962 14773 18906	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(\nu_1)$ $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(\nu_2)$ $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)(\nu_3)$	Octahedral
4	$[Ni(MBIDPP)_2]2H_2O$	10102 16894 21836	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$	Octahedral

Table 3: Electronic spectral data of the ligand MBIDPP and its metal (II) complexes

3.5.Electrochemical Studies

In this work cyclic voltammetric studies of the copper(II) complex **(1)** 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} shows a well-defined redox process corresponding to the formation of Cu(II)/Cu(I) couple at E_{pa} = 0.525 V and E_{pc} = 0.825 V and is found to be quasi-reversible with ΔE_p = 0.3 V. 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 [42]. 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-irreversible in nature and a chemical changes occurs with the electron transfer [43]. The cyclic voltammogram of the copper(II) complex as shown in the fig. 4.

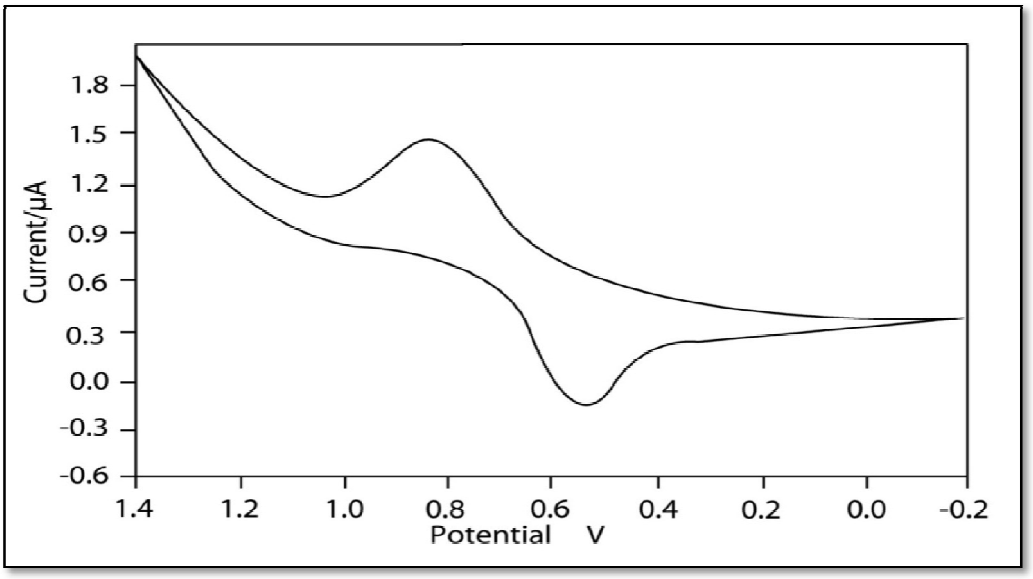

Figure 4: Cyclic voltammogram of the copper(II) complex

3.6.EPR Spectra

The EPR spectrum of Cu(II) complex provide information of importance in studying the metal ion environment. The EPR spectra of the Cu(II) complex, recorded in DMSO at liquid nitrogen temperature (77 K) and at room temperature (300 K). The spectrum of the Cu(II) complex at room temperature shows one intense absorption band in the high field and is isotropic due to the tumbling motion of the molecules. However, this complex in the frozen state shows four well resolved peaks with low field region. The copper complex exhibits the $g_{\|}$ value of 2.31 and $g_{\perp}$ value of 2.16. Which indicates that the ground state [44] of Cu(II) is predominantly in $dx^2–y^2$ orbital. The spin-orbit coupling constant, λ value (– 486 cm^{-1}) calculated using the relations, $g_{av} = 1/3[g\| + 2g_{\perp}]$ and $g_{av} = 2(1–2l/10Dq)$, is less than the free Cu(II) ion (–832 cm^{-1}) which also supports covalent character [45] of M–L bond in the complex. The G value of 3.82 indicates negligible exchange interaction of Cu–Cu in the complex. The covalence parameter α^2 is calculated (α^2= 0.82) using the following equation:

$$\alpha^2_{Cu} = –(A\|/0.036) + (g\| – 2.0023)+ 3/7(g_{\perp} – 2.0023) + 0.04.$$

If the value of α^2 = 0.5, it indicates complete covalent bonding, while the value of α^2= 1.0 suggests complete ionic bonding. The observed value of α^2 (0.82) of the complex is less than unity, which indicates that the complex has some covalent character in the ligand environment [46].

3.7.Molar Conductance

Values of molar conductance of the complexes are given in Table 1. The low values in DMF indicate that the complexes are non electrolytes.

3.8. Biological Activity

The antimicrobial activity (Table. 4) of the ligand and their metal complexes were tested against Streptococci, Staphylococcus aureus (gram +ve), Pseudomonas, Klebsilla Pneumoniae, Chromo bacterium (gram -ve) and the antifungal activity of Candida albicans by the diffusion

method [47]. Amikacin and ketoconazole were used as reference drugs for antibacterial and antifungal activities. Test solutions were prepared in acetonitrile, and nutrient agar was used as a culture medium. The zone of inhibition was measured in millimeter (Table. 4). From the observed result it is clear that the metal(II) complexes showed enhanced antimicrobial activity than that of free ligand MBIDPP 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 [48] and Chelation theory [49]. The values indicate that most complexes have higher antimicrobial activity than the free ligands.

Compound	**Zone of inhibition (in mm)**					
	Gram(+)ve		**Gram(-)ve**			**Antifungal**
	Streptococci	S. aureus	Pseudomonas	K.Pneumoniae	Chromo bacterium	Candida albicans
MBIDPP	8	10	8	12	17	10
$[Cu(MBIDPP)_2.2H_2O$	14	13	15	14	14	15
$[Co(MBIDPP)_2.2H_2O$	13	14	15	12	16	14
$[Ni(MBIDPP)_2.2H_2O$	12	13	12	13	17	13
$[Zn(MBIDPP)_2.2H_2O$	10	11	12	12	16	14
Amikacin	18	19	18	17	18	---
Ketokonazole	--	--	- -	--	--	18

Table 4 : Antimicrobial activity of the ligand and metal its metal complexes

3.9. DNA-binding Studies

DNA binding experiments were carried out in 0.5 mL Tris-HCl/NaCl buffer [50 mM Tris-HCl and 5 mM NaCl (pH 7.2)] using DMF solution (15 µL) of the complexes. Absorption titration measurements were done by varying the concentration of CT DNA but keeping the metal complex concentration as constant. The different modes of interaction of a metal complex with DNA can be studied not only by this technique but also cyclic voltammetry (CV) and differential pulse voltammetry (DPV). These were employed to probe the binding of metal complexes to DNA in solution. DPV data were used to obtain quantitative information about the interaction of these metal complexes with CT DNA. Due to its sensitivity to the change of length of DNA, viscosity measurement may be the most effective means to study the binding mode of complex to DNA, especially in the absence of crystallographic structure data. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/ \eta^0)^{1/3}$ versus binding ratio [50], where η is the viscosity of CT DNA in the presence of complex, and η^0 is the viscosity of CT DNA alone.

3.9.1. Viscosity Measurements

The application of an optical photophysical technique to investigate the interactions of DNA with metal complexes generally provides clues that are needed but not sufficient by themselves to support an intercalative binding model. Therefore, viscosity measurements were introduced to provide further support for this type of interaction between the complexes and DNA. In the absence of crystallographic structural data, hydrodynamic methods which are sensitive to the length of the DNA are known to be among the definitive and critical indicators of binding strength. A classical intercalation mode causes a significant increase in viscosity of CT DNA due to an increase in separation of base pairs at intercalation sites and hence an increase in overall CT DNA length [51, 52]. The significant increase in the viscosity of DNA that occurred upon the addition of a complex was due to intercalation, which caused the DNA bases to separate in order to the increase the effective size of the DNA, which could be the reason for the increase in the viscosity. A plot of $(\eta/ \eta^0)^{1/3}$ versus [complex]/[DNA] = (R). where η is the viscosity of CT DNA in the presence of complex, and η^0 is the viscosity of CT DNA alone.

The well-known DNA intercalator Ethidium bromide (EB) increases the viscosity of CT DNA with increments of the concentration. On the other hand, the viscosity of CT DNA increases dramatically upon addition of the metal (II) complexes. The changes in relative viscosity of CT DNA in the presence of complexes Cu(II),Co(II), Ni(II), Zn(II) and EB are shown in Fig.5. The increased degree of viscosity, which may depend on its affinity to DNA follows the order of EB > Cu >Co >Ni >Zn which is consistent with the above results a gradual increase in the relative viscosity was observed upon the addition of the metal complexes to the DNA solution, suggesting that the complexes mainly bind via an intercalation mode. [53, 54].

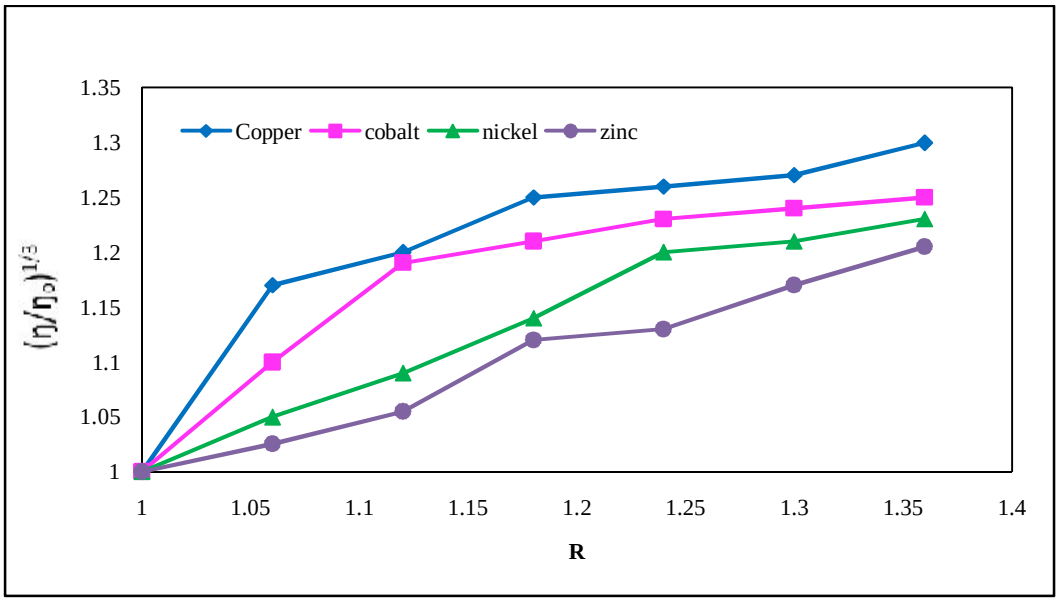

Figure 5: Effect of increasing amounts of [Cu(L3)$_2$.2H$_2$O] (♦),[Co(L3)$_2$.2H$_2$O] (■), [Ni(L3)$_2$.2H$_2$O](▼) [Zn(L3)$_2$.2H$_2$O] (●), on the viscosity of DNA. R = [complex]/[DNA].

3.10. DNA Cleavage Activity Of Metal(II) Complexes

The oxidative cleavage activity of the complexes of MBIDPP was studied by 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. 6. The agarose gel to conduct electrophoresis with such systems including DNA alone, DNA-H_2O_2 and DNA-H_2O_2-M(II), where (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.

In the CT DNA electrophoresis experiment for the metal (II) complexes exhibit nuclease activity in the presence of H_2O_2. Control experiment using DNA alone (Lane 1) does not show any significant cleavage of CT DNA against H_2O_2. The cleavage efficiency of the complexes compared with that of the control is due to their efficient DNA-binding ability. From Fig.6 (lanes 2-6), it is evident that 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.

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

The $OH^{\bullet}$ free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of a sugar phosphate back bone. All the complexes showed pronounced nuclease activity in the presence of oxidant H_2O_2 which may be due to the increased production of hydroxyl radicals. Control experiments using DNA alone (lane 1) and DNA+H_2O_2 (Lane 2) did not show any significant cleavage of CT-DNA even on longer exposure time. From the observed results, we conclude that the complexes, copper complex (lane 3), cobalt complex (lane 4), nickel complex (lane 5) and zinc complex (lane 6) cleave DNA as compared to control DNA in the presence of H_2O_2. Probably this may be due to the formation of redox couple of the metal ions and its behaviour. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage. [55]

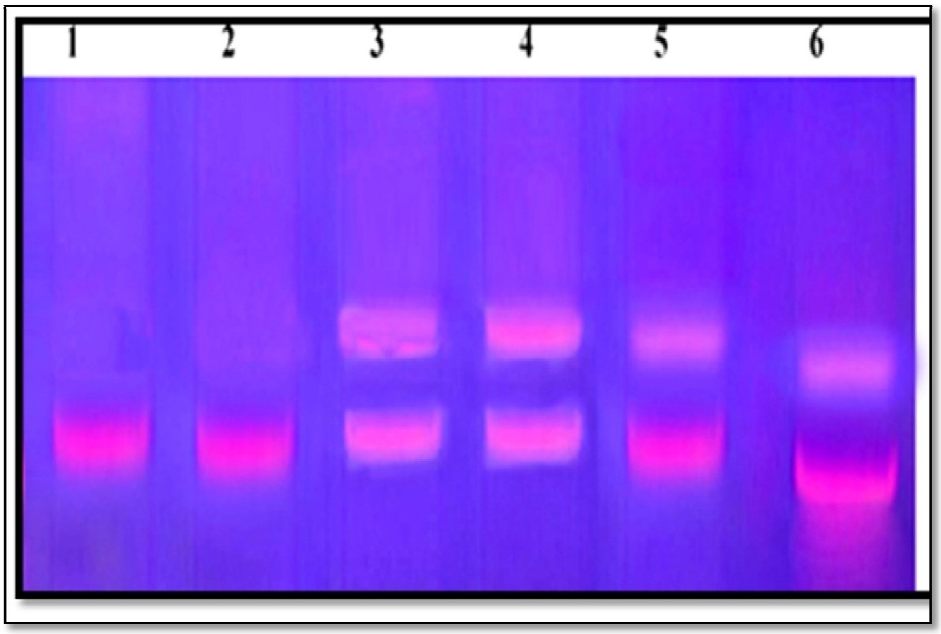

Figure 6 : Changes in the gel electrophoresis pattern of CT-DNA induced by H_2O_2 and Cu(II), Co(II), Ni(II) and Zn(II) complex,

Lane 1: DNA alone Lane 2: DNA+H_2O_2 Lane 3: DNA+[Cu(II)] + H_2O_2

Lane 4: DNA + [Co(II)]+ H_2O_2 Lane 5: DNA+[Ni(II)]+ H_2O_2 Lane 6: DNA+[Zn(II)]+ H_2O_2

4. Conclusion

A novel Mannich base has been designed and synthesized using the bioactive ligand obtained from 4-aminoantipyrine, 2-mercaptobenzimidozole. Its Cu(II), Co(II), Ni(II), Zn(II) complexes have also been synthesized in ethanol medium. The structural features have been arrived from their elemental analyses, magnetic susceptibility, molar conductance, Mass, IR, UV-Vis., ^{1}H NMR, ^{13}C NMR and EPR spectral studies. The data show that the complexes have composition of ML_2 type. The electronic absorption spectral data of the complexes suggest an octahedral geometry around the central metal ion. DNA cleavage activity of these metal complexes with CT-DNA was investigated by gel electrophoresis. From the results, it is found 20

that the metal complexes cleaved DNA efficiently in the presence of H_2O_2 as compared to the control DNA. Further, the promising results have been observed for the antimicrobial screening especially for the metal complexes against both the fungi and

bacteria and which may be attributed to the fact that the metal complexes are potentially active against bacterial cells than fungi cells.

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