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Synthesis, Characterization, Anti-Microbial and DNA Binding Studies on Mn(II) and Zn(II) Metal Complexes Containing Mixed Ligands

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Abstract: The DNA binding ability of the novel complexes $Mn(L_1)^2L_2(PF_6)_2$[Complex **(1)** and $Zn(L_1)^2L_2(PF_6)_2$ [Complex **(2)**] containing bioactive mixed ligand of typeL_1=8-Hydroxyquinoline and L_2=1,10-Phenanthroline have been isolated andcharacterized by analytical and spectral methods. The intrinsic binding constant K_b has been estimated at room temperature. The binding constant of the complexes in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2, are 13.10×10^6 $M^{-1}$and 17.41×10^6 $M^{-1}$for complex **(1)** and **(2)** respectively. The absorption spectra indicate that the complexes intercalate between the base pairs of the CT-DNA tightly. The synthesized compounds have been tested againstmicroorganisms such as Gram positive bacteria (*Staphylococcus aureus* and *Proteus vulgaris*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger*). A comparative study of theMinimum Inhibitory Concentration(MIC) values of the ligands andtheir complexes indicates that the complexes exhibit moderate antimicrobial activity than the freeligand and control.
Keywords: Manganese and Zinc complexes, DNA Binding and Antimicrobial studies.

INTRODUCTION

Organometallic chemistry has been developed, in the last four decades, to be thelargest and important branch as a link connecting the fields of organic and inorganic chemistry. One of the major applications of the transition metal complexes is its medical testing as antibacterial and antitumor agents aiming toward the discovery of an effective and safe therapeutic regimen for the treatment of bacterial infections and cancers. In addition, a great many Schiff' base complexes with metals have also provoked wide interest because they possess a diverse spectrum of biological and pharmaceutical activities, including antitumor and antioxidative activities. Moreover, mixed-ligand complexes are observed in biological systems or in the intermediate

chemical reactions with metal ions, which are important to understand the respective chemistry. Investigations concerning 1,10- phenanthroline mixed-ligand chelate systems would provide toward understanding the driving forces that led to the formation of such mixed ligand complexes[1].Moreover, Cu(II) and Zn(II) complexes with oxygen, sulphur and nitrogen containing ligands are the subject of intensive biological evaluation in the search for less toxic and more selective anticancer therapies [2,3].

The study of mixed ligand-complex formation is relevant in the field of analytical chemistry, where the use of mixed ligand complexes allows the development of methods with increased selectivity, sensitivity and has also great importance in the field of biological and environmental chemistry [4,5].

The study of mixed ligand-complex formation is relevant in the field of analytical chemistry, where the use of mixed ligand complexes allows the development of methods with increased selectivity, sensitivity and has also great importance in the field of biological and environmental chemistry [6,7].The effect of the presence of methyl substituent in the phenyl rings of aromatic Schiff bases on their antimicrobial activity has been reported [8].Although many Schiff bases are known to beactive against a wide range of micro-organisms, for example; *E.coli, B.subtilis*, and *S.aureus* and fungi *R.stolonifer* and *C.ablican* , antibacterial activity has been studied more than antifungal activity, since bacteria are usually more resistant to antibiotics through biochemical and morphological modifications. Moreover, the incorporation of transition metal into Schiff bases enhances the biological activity of the ligand and decreases the cytotoxic effects of both the metal ion and ligand on the host[9].

However the literature survey that the mixed ligands of 8-Hydroxyquinoline and 1-10 Phenanthroline derivatives with their transition metal complexes have not been reported and studied so far. Hence the present study aims for the Synthesis, Characterization, Antimicrobial and DNA binding studies of Manganese and Zinc complexes containing mixed ligands 8-hydroxyquinoline and 1-10 Phenanthroline.

EXPERIMENTAL

All reagents and solvents were of AR grade, solvents were purified and used.Metal salts such as Manganese Sulphate and Zinc Sulphate were purchased from Karnataka fine chemicals.Ligands such as 8-Hydroxyquinoline and 1,10-Phenanthroline was purchased from Himedia chemicals Ltd. (Bangalore).

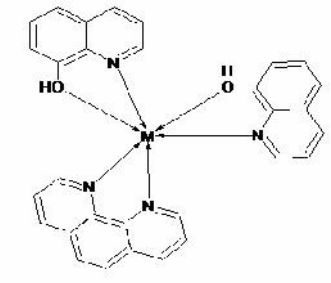
SYNTHESIS OF METAL COMPLEXES

Synthesis of Manganese complex ($Mn(L_1)^2L_2$] $(PF_6)_2$[Complex (1)]

Manganese sulphate (0.169 g, 1mmol) 8-hydroxyquinoline $\mathbf{L_1}$ (0.29 g, 2mmol) 1,10-phenanthroline $\mathbf{L_2}$ (0.234g,1mmol) were dissolved in ethanolic solution and refluxed on the water bath for 4 hours. The contents were cooled to obtain precipitate. The complex was filtered and dried under vacuum before being recrystallized in acetone.

Synthesis of Zinc complex ($Zn(L_1)^2L_2$] $(PF_6)_2$[Complex (2)]

Zinc sulphate (0.287 g, 1mmol) 8-hydroxyquinoline $\mathbf{L_1}$ (0.29 g, 2mmol) 1,10-phenanthroline $\mathbf{L_2}$ (0.234g,1mmol) were dissolved in ethanolic solution and refluxed on the water bath for 4 hours.The contents were cooled to obtain precipitate. The complex was filtered and dried under vacuum before being recrystallized in acetone.



Where M= Mn or Zn

Figure 1: Proposed structure of the metal complexes

SPECTRAL MEASUREMENTS

IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets. UV-visible absorption spectra were recorded using ELICO model SL-159 UV-Vis Spectrophotometerat room temperature.

DNA BINDING STUDIES

The concentration of CT-DNA per nucleotide [C(p)] was measured byusing its known extinction coefficient at 260 nm (6600 M−1 cm−1)[10]. TrisHCl-buffer [5mM Tris(hydroxymethyl) amino methane, pH 7.2, 50 mMNaCl] was used for the absorption, viscosity, and thermal denaturation experiments.

Absorption titration experiments were carried out by varying the DNAconcentration (0-100μM) and maintaining the metal complex concentrationconstant. Absorption spectra were recorded after each successiveaddition of DNA and equilibration (approximately 10 minutes). For both(**1**) and (**2**), the observed data were then fitted into Equation (1) to obtain theintrinsic binding constant K_b [11].

$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_a - \varepsilon_f)$,

where ε_a, ε_b, and ε_f are the apparent, bound, and free metal complex extinctioncoefficients, respectively, at 263 nm for (**1**) and (**2**). A plotof [DNA]/($\varepsilon_b - \varepsilon_f$) versus [DNA] gave a slope of 1/($\varepsilon_b - \varepsilon_f$) and a *y* interceptequal tol/K_b ($\varepsilon_b - \varepsilon_f$), where K_b is ratio of the slope to the *y* intercept.

ANTIMICROBIAL STUDIES

The ligands and its complexes were investigated for anti-bacterial and anti-fungal properties. Gram positive bacteria (*Staphylococcus aureus* and *Proteus vulgaris*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and one fungus (*Aspergillus niger*) were used in this study to assess their antimicrobial properties. The tested compounds were dissolved in DMSO and the solutions were serially diluted in order to find the MIC values. The antibiotic Chloramphenicol was used as standard reference in the case of Gram-negative bacteria, Amikacin was used as standard reference in case of Gram-positive bacteria and Clotrimazole was used as standard anti-fungal reference. The solvent DMSO was used as negative control. In a typical procedure, a well was made in the Muller Hinton agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plates were incubated at 37 ^{0}C at 72 h for fungi and 24 h for bacteria. During this period, the test solution diffused and affected the growth of the inoculated fungi and bacteria.Activity was determined by measuring the diameter of the zone of inhibition (mm).

RESULTS

CHARACTERIZATION OF COMPLEXES

The elemental analysis data are agreedwith the theoretical values within the limit of experimental error. These complexes are soluble in DMF, DMSO and in buffer(pH 7.2) solution.

The IR spectra of complexeswere recorded in the range of 4000-400 cm^{-1} on KBr pellets. The spectra of the ligands 8-Hydroxyquinoline and 1,10-Phenanthrolineshowed bands at 1577 cm^{-1}and 1597 cm^{-1}assigned to νC=N aromatic hydrocarbon, 3045 cm^{-1}and 3024cm^{-1}assigned to νC-H group and 3600cm^{-1}and 3800 cm^{-1}assigned to νO-H group. Thespectra of both the complexes showed a peak in the range 1510 cm^{-1}to 1630 cm^{-1}for νC=Ngroup are shifted slightlyindicating that the coordination taken place through nitrogen atom.

ABSORPTION SPECTRAL STUDIES

The absorption spectra of complexes **(1)** and **(2)** in the absence and presence of CT-DNA are given in Figures 2 and 3, respectively. Figure 2 and 3 depicts a well-resolved band at 263 nm for complex **(1)** and complex **(2)** with increasing DNA concentrations (0–100 μM). The result shows that the absorbance (hypochromism) decreased by the successive addition of CT-DNA to the complex solution. The hypochromism observed for the bands of complexes **(1)** and **(2)** is accompanied by a small bathochromic shift of 2 and 1 nm, as shown in Figures 2 and 3, respectively. The hypochromism and bathochromic shift observed for the complexes suggest that binding is

in intercalative mode. To compare quantitatively the DNA binding strengths of these complexes, the intrinsic DNA binding constants K_b are determined from the decay of the absorbance at 263 nm for complex **(1)** and complex **(2)** with increasing concentrations of DNA by using equation (1) given in our previous article[11,18]. The observed K_b values for complexes **(1)** and **(2)** are equal to the classical intercalators bound to CTDNA. The K_b values for complexes **(1)** and **(2)** are 13.10×10^6 $M^{-1}$and 17.41×10^6 M^{-1}, respectively. Thus, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

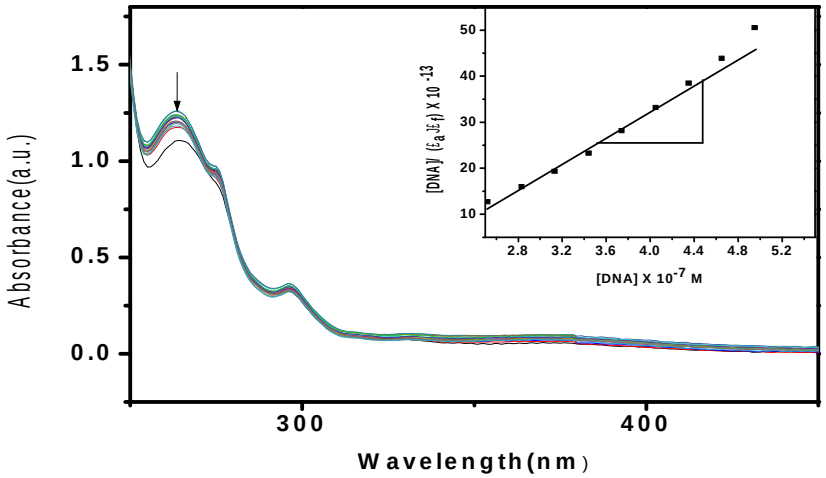


Figure 2: Absorption spectra of complex **(1)** in Tris-HCl buffer upon addition of DNA. [Mn] = 0.5 μM, [DNA] = 0–100 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.
Inset: The plot of [DNA]/(εa − εf) versus [DNA] for the titration of DNA with Mn(II) complex.

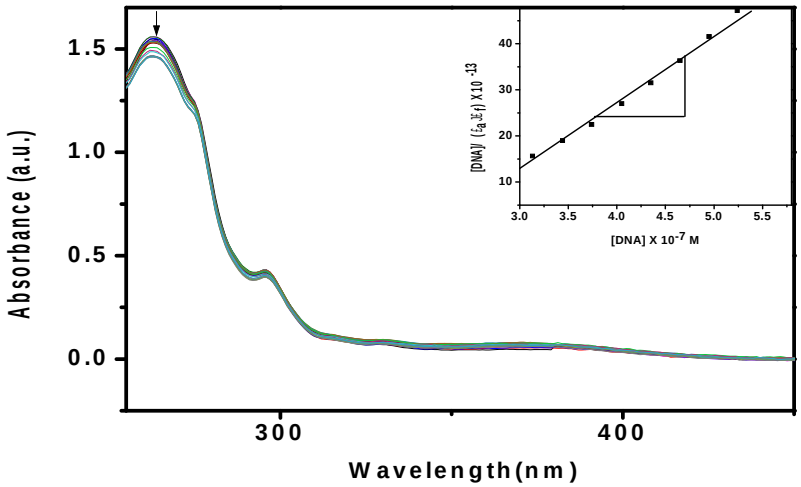


Figure 3: Absorption spectra of complex **(2)** in Tris-HCl buffer upon addition of DNA. [Zn] = 0.5 μM,[DNA] = 0–100 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.
Inset: The plot of [DNA]/(εa − εf) versus [DNA] for the titration of DNA with Zn(II) complex.

ANTIMICROBIAL STUDIES

The in vitro antimicrobial activity of the compounds was tested against the bacterial species including *Staphylococcus aureus,Proteus vulgaris, Escherichia coli* and *Pseudomonas aeruginosa* and the fungus *Aspergillus niger* by well diffusion method.These complexes are inhibiting Gram-positive and Gram negative bacterial strains. The importance of this unique property of the investigated Schiff base complexeslies in the fact that, it can be applied safely in the treatment of infections and some common diseases e.g. Septicaemia, Gastroenteritis, Urinary tract infections and hospital acquired infections[19]. The ligand and theircomplexes have been tested for in vitro growth inhibitory activity against gram-positive microbe *Staphylococcusaureus,Proteus*

*vulgaris*and gram-negative microbe's *Escherichia coli*, *Pseudomonas aeruginosa*by using well-diffusion method. Asthe test solution concentration increases, the biological activity also increases. The Minimum InhibitoryConcentration (MIC) values of the investigated compounds are summarized in Table 1. From the table, theobserved MIC values indicate that the complexes have higher antbacterial activity. But the complexes shows no inhibition against fungi. (*Aspergillus niger*).The metal complexes Mn(II) and Zn(II) have higher antbacterial activity than the ligand are shown in Figs. 4(a), 4(b) and 4(c). Theincrease in antimicrobial activity is due to the faster diffusion of metal complexes as a whole.

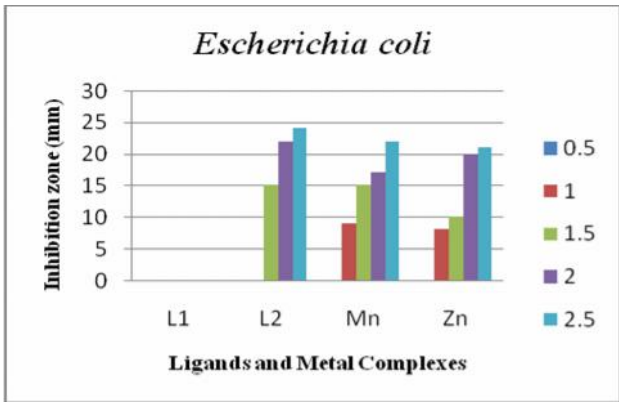


Fig4 (a): Activity of *E.coli* against Ligands and Complex (1) and (2)

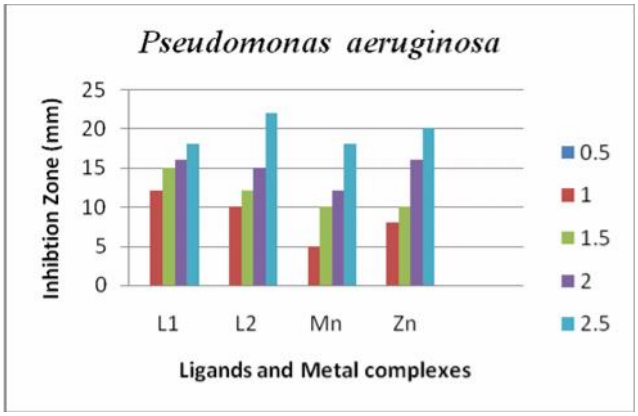


Fig4 (b): Activity of *P.aeruginosa*against Ligands and Complex (1) and (2)

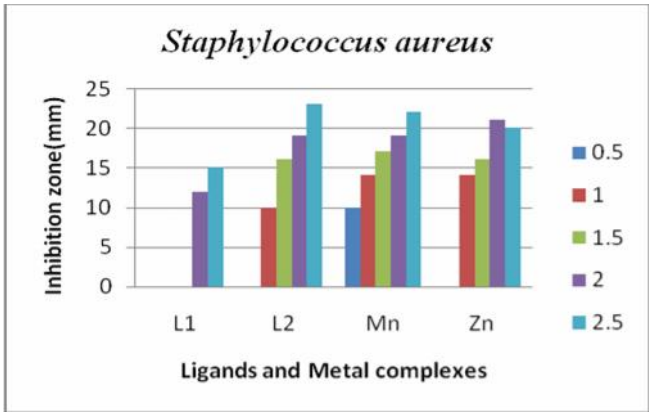


Fig4(c): Activity of *S.aureus*against Ligands and Complex (1) and (2)

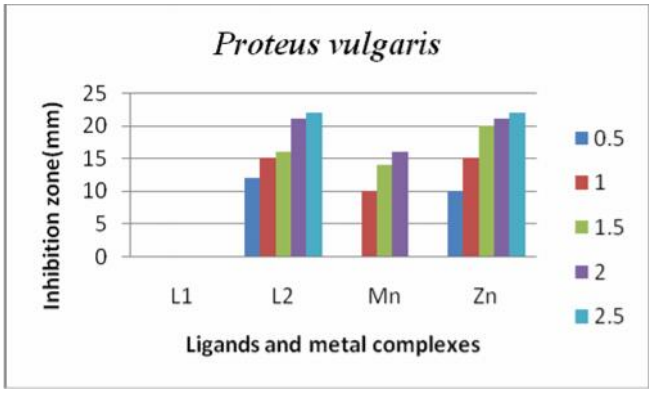


Fig4 (d): Activity of *Proteus vulgaris* against Ligands and Complex (1) and (2)

Table 1: Antimicrobial activity of the ligand and metal complexes

Sl.No	Extract	Conc (mg/ml)	*E.coli (mm)*	*P. aeruginosa (mm)*	*Staphylococcus aureus (mm)*	*Proteus vulgaris (mm)*	*A.niger (mm)*
1	L_1	0.5	0	8	0	0	0
		1	0	15	0	0	0
		1.5	0	16	0	0	0
		2	0	17	12	0	0
		2.5	0	19	15	0	0
2	L_2	0.5	0	0	0	12	0
		1	0	10	10	15	0
		1.5	15	12	16	16	0
		2	22	15	19	21	0
		2.5	24	22	23	22	0
3	$Mn(L_1)^2L_2$	0.5	0	0	10	0	0
		1	9	0	16	10	0
		1.5	13	9	17	14	0
		2	15	10	18	16	0
		2.5	20	16	22	20	0
4	$Zn(L_1)^2L_2$	0.5	0	0	0	0	0
		1	0	0	14	15	0
		1.5	9	10	18	20	0
		2	17	16	21	21	0
		2.5	21	20	20	22	0
5	**Antibiotic**	0.5	21	24	21	21	0
		1	21	22	20	22	0
		1.5	22	22	21	22	0
		2	24	24	24	24	0
		2.5	22	24	25	24	0
6	**DMSO**	0.5	0	0	0	0	0
		1	0	0	0	0	0
		1.5	0	0	0	0	0
		2	0	0	0	0	0
		2.5	0	0	0	0	0

Less than 10mm-- Inactive; Less than 10-15mm--Weakly active; Less than 15-20mm--Moderately active;More than 20mm—Highly active

Standard: Antibiotic Chloramphenicol for Gram negative bacteria, Amikacin for Gram-positive bacteria**,** Clotrimazole for fungi

Control: DMSO

CONCLUSION

In conclusion, we have synthesized and characterized two new complexes of the type $[Mn(L_1)^2L_2](PF_6)_2$**(1)** and $[Zn(L_1)^2L_2](PF_6)_2$**(2)**. Interactions of the new complexes with (double stranded) DNA were investigated by absorption spectra, viscosity and thermal denaturation studies. From the experimental results, it was confirmed that the complexes bound with the double stranded DNA with binding constantK_b=13.10 x 10^6 $M^{-1}$for (**1**) andK_b=17.41 x 10^4 $M^{-1}$for (**2**), respectively.The metal complexes have higher antimicrobial activity than the free ligand.

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