



Synthesis, characterization and biological studies of transition metal complexes derived from 2-hydroxynaphthoic acid derivatives bearing isatin moiety

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ABSTRACT

Metal (II) (Co(II), Ni(II), Cu(II) and Zn(II)) complexes have been synthesized from the Schiff base ligand derived from 2-hydroxynaphthoic acid derivatives (obtained through esterification reaction followed by the condensation with hydrazine) and 4-nitroisatin. They were characterized using molar conductance, elemental analysis, magnetic susceptibility, FT-IR spectroscopy, electronic spectra, ESR, mass spectra, and powder XRD techniques. All the metal (II) complexes exhibited square planar geometry. The ESR spectra of the copper complex in DMSO solution at 300 and 77 K were recorded and their salient features are reported. The *in vitro* biological screening effects of the investigated compounds were tested against the bacterial species, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species, *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by serial dilution method. A comparative study of inhibition values of the Schiff base ligands and their complexes indicated that the complexes showed higher antimicrobial activity than the Schiff base ligands. Superoxide dismutase and antioxidant activities of the metal complexes have also been studied. The electrochemical behavior and “*f*” factor values indicated that the higher biological activity.

Keywords: Isatin, Biological activity, superoxide, electrochemical behavior

1. INTRODUCTION

The emergence of multidrug-resistant bacteria and fungi as human pathogens warrants a continued focus on the development of new pharmacophores for the treatment of these devastating and often fatal

infections. There is a growing interest in the ligand system containing isatin derivatives and their coordination compounds exhibited their biological activity [1,2]. Many clinically successful antibiotic drugs were either naturally occurring molecules or have been developed from their synthetic analogs

containing isatin moiety. Metal complexes have unique properties enhancing their role as antitumor agents. It is the ability of metals to form positively charged ions in an aqueous solution that can bind to negatively charged biological molecules [3, 4]. The high electron affinity of metal ions can significantly polarize groups that are coordinated to them, leading to the generation of hydrolysis reactions [4]. Furthermore, metal ions also has the ability to coordinate ligands in a three dimensional configuration, thus allowing functionalization of groups that can be tailored to defined molecular targets [5, 6]. Recently, considerable attention has been drawn to isatin derivatives. The isatin molecule (1H-indole-2, 3-Dione) is a versatile moiety that displays diverse biological activities. The isatin derivatives and their metal complexes are reported for their remarkable biological activities [7-9]. Significant antitumor, antifungal, herbicidal, antibacterial and anti-convulsant activities were reported by several researchers [10]. Isatin-thiosemicarbazone copper (II) complexes found to have antiviral effect [11].

In the literature evidences, the metal – isatin binary complexes were advantageous over simple isatin in chemotherapy and found to act as anticancer agents, especially Schiff base transition metal complexes derived from isatin [12]. This created a great interest in researchers to synthesize variety of isatin derivatives and screened them for their diverse biological activities such as anticancer, anti-HIV, anthelmintic, antimycobacterial, anti-inflammatory, antidiabetic, antimicrobial, trypanocidal as well antimalarial activities [13]. Information obtained from this study will be helpful to understand the mechanism of isatin derivatives interaction with DNA, and should be useful to develop excellent anticancer activity and new therapeutic reagents for some diseases. The free ligand and its complexes have been tested for in vitro antimicrobial activity against seven different bacteria and four different fungi by minimum inhibitory concentration (MIC). In continuation of our research work, in the present investigations focused on the synthesis, characterization and biological studies of metal complexes containing Schiff base ligands.

2. MATERIALS AND METHODS

2.1 Material

All chemicals and solvents were reagent grade and were purchased from Merck. The supporting electrolyte solution was prepared using analytical

grade reagents and doubly distilled water. Calf thymus DNA purchased from Genei Biolab, Bangalore, India.

2.2 Instrumentation

Elemental analysis of ligand and its metal complexes were carried out using Perkin-Elmer elemental analyzer. Molar conductance of the complexes was measured using a coronation digital conductivity meter. The ^1H NMR spectra of the ligands were recorded using TMS as internal standard. The chemical shifts are expressed in units of parts per million relative to TMS. The IR spectra of the ligands and their copper complexes were recorded on a Perkin-Elmer 783 spectrophotometer in 4000-200 cm^{-1} range using KBr disc. Electronic spectra were recorded in a Systronics 2201 Double beam UV-Vis., spectrophotometer within the range of 200-800 nm region. Magnetic moments were measured by Guoy method and corrected for diamagnetism of the component using Pascal's constants. Cyclic voltammetry was performed on a CHI 604D electrochemical analyzer with three electrode system of glassy carbon as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Tetrabutylammoniumperchlorate (TBAP) was used as the supporting electrolyte. Solutions were deoxygenated by eradication with N_2 previous to measurements. The interactions between metal complexes and DNA were studied using electrochemical and electronic absorption techniques.

2.3 Preparation ligand and metal complexes

2.3.1 Stage : I Sulphonation of 2-hydroxy 3-naphthoic acid

2-Hydroxy 3-naphthoic acid (0.02 M) and catalytic amount of H_2SO_4 were taken in ethanolic medium under refluxing conditions for 8 h. It was filtered and washed with warm ethanol to afford yellow colour product (I).

2.3.2 Stage 2: Synthesis of hydrazide derivative

The ester (0.01 M) was then transformed to the hydrazine (0.01 M) at refluxing conditions in ethanol for 8 h. The hydrazide was then condensed with a previously synthesized ketone to form the ketonehydrazide derivatives (II).

2.3.3 Stage 3: Synthesis of Schiff base ligand of product (III)

A mixture of hydrazide derivative (0.01 M) and 4-Nitroisatin (0.01 M) were refluxed for 20 h in ethanol

under nitrogen atmosphere. The reaction mixture was filtered and washed with warm ethanol to afford light yellow color solid product.

2.3.4 Stage 4: Synthesis of metal complexes

A mixture of hot ethanolic solutions of Schiff base ligand (0.01 M) and metal salts (0.01 M) were refluxed for 2 h. The reaction mixture was filtered and washed with warm ethanol to afford coloured products.

2.4 DNA Binding Studies

In these studies the complexes were dissolved in DMSO and then diluted to the desired concentration with Tris-HCl buffer. The complexes remained dissolved after dilution. The spectroscopic titrations were carried out by adding increasing amounts of CT DNA to a solution of the complex at a fixed concentration contained in a quartz cell, and recording the UV-Vis spectra after each addition. Solutions of CT DNA (calf-thymus DNA) in 50 mM NaCl/50 mM tris-HCl (pH = 7.2) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of ~1.8–1.9, indicating that DNA was sufficiently free of protein contamination [14]. DNA concentration was determined by UV absorbance at 260 nm after 1 : 100 dilutions. The molar absorption coefficient was taken as $6600 \text{ M}^{-1} \text{ cm}^{-1}$.

2.4.1 Absorption titration experiment

Absorption titration experiment was performed by maintaining a constant concentration of the complex while varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the copper complex stock solution and by mixing various amounts of DNA stock solutions while maintaining the total volume constant [14]. This resulted in a series of solutions with varying concentrations of DNA but with a constant concentration of the complex. The absorbance (A) of the most red-shifted band of complex was recorded after each successive additions of CT DNA. The intrinsic binding constant, K_b , was determined from the plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs $[\text{DNA}]$, where $[\text{DNA}]$ is the concentration of DNA in base pairs, ϵ_a , the apparent extinction coefficient which is obtained by calculating $A_{\text{obs}}/[\text{complex}]$ and ϵ_f corresponds to the extinction coefficient of the complex in its free form. The data were fitted to the following equation where ϵ_b refers to the extinction coefficient of the complex in the fully bound form.

$$[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f) \quad (4)$$

Each set of data, when fitted to the above equation, gave a straight line with a slope of $1/(\epsilon_b - \epsilon_f)$ and a y-intercept of $1/K_b(\epsilon_b - \epsilon_f)$. K_b was determined from the ratio of the slope to intercept.

2.4.2 Viscosity experiments

Viscosity experiments were conducted on an Ubbelohde viscometer, immersed in a water bath maintained at $25.0 \pm 0.1^\circ\text{C}$. Titrations were performed for the compound (10–90 μl) and each compound was introduced into CT-DNA solution (50 μl) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound to CT-DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA containing solutions corrected from the flow time of buffer alone (t_0), $\eta = (t - t_0)$ [15].

2.5 Antioxidant Assay

2.5.1 Superoxide dismutase activity (SOD)

The superoxide dismutase activity (SOD) of the copper(II) complexes were evaluated using alkaline DMSO as source of superoxide radicals (O_2^-) generating system in association with nitro blue tetrazolium chloride (NBT) as a scavenger of superoxide. Add 2.1 ml of 0.2 M potassium phosphate buffer (8.6 pH) and 1 ml of 56 μl of NBT solutions to the different concentration of copper complex solution. The mixtures were kept in ice for 15 min and then 1.5 ml of alkaline DMSO solution was added while stirring. The absorbance was monitored at 540 nm against a sample prepared under similar condition except NaOH was absent in DMSO [16].

2.6 Antimicrobial activities

The *in vitro* antimicrobial activities of the investigated compounds were tested against the bacterial species (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* *Escherichia coli*) were evaluated by the disc diffusion method. The 5mm diameter and 1mm thickness of the disc was filled with the test solutions of synthesized copper complexes using a micropipette and the plates were incubated at 37°C for 24 h. During this period, the test solution was diffused and affected the growth of the inoculated bacteria. The zone of inhibition, developed on the plate was measured.

3. RESULTS AND DISCUSSION

The prepared ligands and metal complexes were structurally confirmed using analytical and spectroscopy techniques. The elemental analytical data for the ligands and their complexes were in good agreement with the values calculated, indicating that the composition of the complex was confirmed. They are sparingly soluble in common organic solvents but soluble in DMF and DMSO. The ligands were isolated in the solid form. All the complexes are stable at room temperature, insoluble in water but soluble in completely soluble solvents. The purity of the Schiff base ligands and their complexes are checked by TLC.

The metal complexes were dissolved in DMSO and the molar conductivities of 10^{-3} M of their solution at room temperature were measured. The lower conductance values ($5-15 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$) of the complexes support their non-electrolytic nature. Thus, the present complexes have non-electrolytic nature as evidenced by the involvement of acetate ions in coordination. This result was further confirmed from the chemical analysis of acetate ions, not precipitated by addition of FeCl_3 [17] (Scheme 1).

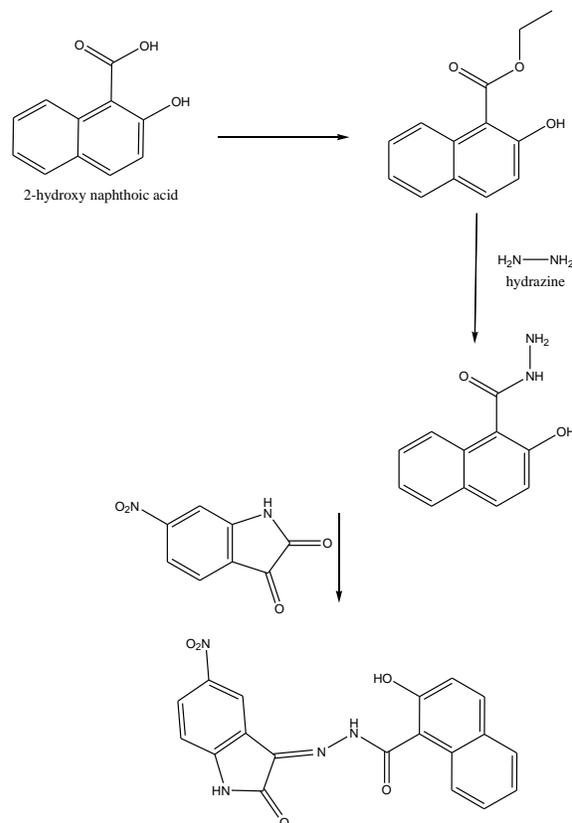
3.1 Magnetic moment

Magnetic studies have been used for the confirmation of the geometry of complexes. The magnetic moment of CoL complex is 2.22 B.M indicating a square planar environment around the metal ion. In the case of NiL complex showed magnetic moment value is 0 B.M. Cu (II) complex showed μ_{eff} 1.77 B.M suggesting an octahedral structure with one unpaired electron. ZnL complex was found to be diamagnetic as expected.

3.2 IR Spectra

In IR Spectrum of the Schiff base ligand system of L, the disappearance of C=O group, which is compared with the position in the free ligand appeared indicates the formation of the Schiff base ligand system of 2-hydroxynaphthoic acid derivatives, which is further confirmed by the appearance of $\nu(\text{C}=\text{N})$ bands in the region 1656 cm^{-1} assigned to the C=N stretching of imine system. In the complex, this band is shifted to 1631 cm^{-1} (lower wave number) upon complexation with the metal, which may be attributed to the coordination of the imine nitrogens to the metal centre [20–23]. The ligand coordination to the metal centre is substantiated by a band appearing at 482 and 450 cm^{-1} which is mainly attributed to $\nu_{\text{Cu-N}}$ in the complexes, respectively. The IR spectra of all the

complexes exhibit two new additional bands, which are not present in the spectrum of the ligands are at $438-474 \text{ cm}^{-1}$ and $520-512 \text{ cm}^{-1}$ assignable to $\nu(\text{M}-\text{N})$ and $\nu(\text{M}-\text{O})$ modes, respectively [18]. The presence of these bands confirms the coordination of metal formation.



Scheme-1: Schematic outline for the synthesis of ligand

3.3 Electronic Spectroscopy

Electronic spectrum of synthesized ligand system shows the absorption bands at 286 and 339 nm attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions within the ligand. In the spectrum of the complex, the CT band at 329 nm remains intact, in agreement with the $\pi \rightarrow \pi^*$ transition of the isatin moiety and imine group. Further, it interact with metal ion, it shifts into 242 nm, 264 nm regions, respectively. A broad intense band at ca. 540 nm in the electronic spectra of the complexes is reasonably assignable to a combination of ligand to metal charge transfer and metal d-d band transitions corresponds to ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$, suggests a distorted square planar geometry metal ion (Figure 1).

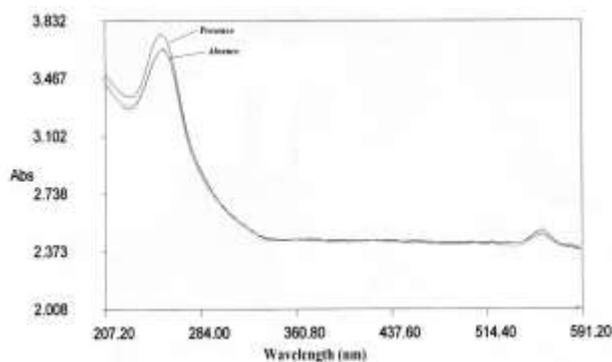


Figure.1. Electronic absorption spectrum of [CuL(OAc)] complex in various concentration of DNA

In the metal complexes this band is shifted to a longer wavelength with increasing intensity. This shift may be attributed to the donation of the lone pairs of electron of the nitrogen atoms of the Schiff base to the metal ion ($M \leftarrow N$) [19]. We have observed that decreased absorbance (hypochromism successive addition of CT- DNA to the complex solution along with bathochromic shift. The hypochromism and bathochromic shift are observed for the complexes suggesting that binding is intercalative mode. The absorption spectra of complexes in the absence and presence of CT-DNA of complexes are given in Fig.1. The observed K_b values for metal complexes are equal to the classical intercalators bound to CT-DNA. The metal complexes of L are 1.2×10^6 (Ni), 1.9×10^6 (Cu), 1.6×10^6 (Co), 1.7×10^6 (Zn), respectively and compared with classical intercalator (ethidium bromide-DNA) was found to be $1.4 \times 10^6 M^{-1}$. The results show that the present complexes are involved in intercalative interactions with CT-DNA.

3.4 Cyclic voltammetry

Cyclic voltammetry is useful technique for investigating the interaction of the metal complexes with DNA. In the cyclic voltammetric (CV) study, metal complexes in the copper complexes and absence of CT DNA are shown in Figure 2. In the absence of DNA, the cyclic voltammogram of the [CuL(OAc)] complex in DMSO solution at 300 K in the potential range 1.2 to -0.2 V. It shows a well-defined redox process corresponding to the formation of the quasi-reversible Cu(II)/Cu(I) couple. In the absence of CT DNA, it shows two redox peaks shown in Fig.2. The first redox appeared at $E_{pc} = -0.123$ and $E_{pa} = 0.514$ mV, ($\Delta E_p = -0.391$ mV and $E_{1/2} = -0.318$ mV) is corresponding to $Cu(II) \rightarrow Cu(I)$, whereas the second peak shows at $E_{pc} = -1.234$ mV and $E_{pa} = -0.945$ mV, $\Delta E_p = -0.291$ mV and $E_{1/2} = -$

1.178 mV. The i_{pa}/i_{pc} ratios of these redox peaks 1.15 are 1.11 respectively, which indicate that the reaction of the Cu(II) complex exhibited quasi-reversible redox process [20].

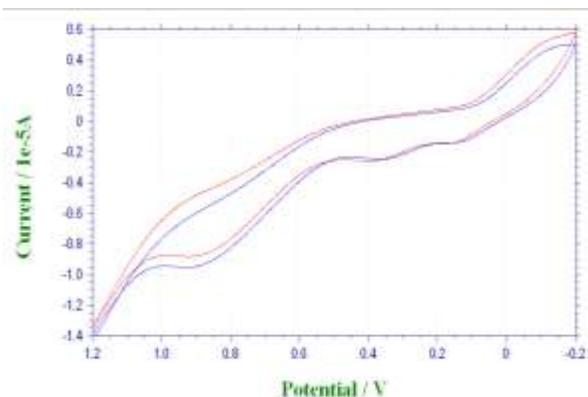


Figure 2. Cyclic voltammogram of [CuL³(OAc)₂] in the presence (blue line) and absence CT-DNA (red line)

3.5 Thermal Denaturation Studies

The double- stranded DNA tends to gradually dissociate to single strands on increase in the solution temperature and generates a hyperchromic effect on the absorption spectra of DNA bases (at 268 nm). In the present study melting temperature (T_m) of DNA in the absence of copper complexes was found to be $58 \pm 1^\circ C$. Under the same set of experimental conditions, addition of complexes increased the melting temperature T_m ($\pm 1^\circ C$) from $10^\circ C$ to $10.3^\circ C$, for all copper complexes respectively [21]. This experimental data indicates that the Cu(II) complex has interacted with double helix CT-DNA. The intercalation of small molecules into the double helix has as a result an increase of melting temperature at which the double helix denaturates into single helix DNA, The significant increase of T_m ($\Delta T_m = 10.3^\circ C$) suggests that the interaction of the all copper complexes with DNA is performed through intercalation binding mode.

3.6 Antioxidant assay

Cu(II) complexes with their redox potentials are in the suitable range for superoxide scavenging. Literature studies indicated that the bioactive ligands of N, S or O donor atoms of Cu-complexes showed wide spectrum of activities. Accordingly, reactivity of these copper complexes toward $O_2^{\bullet-}$ and H_2O_2 was systematically studied and redox behavior of the complexes responsible for its antioxidant activity. Electrochemical properties have best correlations with antioxidant properties due to their redox

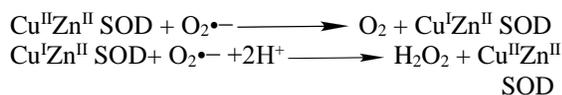
potentials. It is found that compounds with strong scavenging capabilities are oxidized at relatively low potentials. The redox potential of almost all the complexes fall between 1.2 V to -0.2 V, which is shown in the Fig.2. The results reveal that the redox potential values of these complexes fall into the redox potential range that resembles the SOD enzyme [22].

The IC₅₀ of present metal complexes was found at the range of 20-46 μmol dm⁻³ which are higher than the value exhibited by the native enzyme (IC₅₀ = 0.04 μmol dm⁻³). All the tested compounds show SOD activity. Similar values obtained for all compounds. The SOD values of metal (II) complexes is in the order of

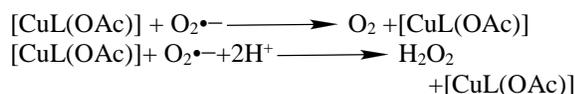


The mechanism of SOD is,

Native Enzyme



Synthesized copper complex

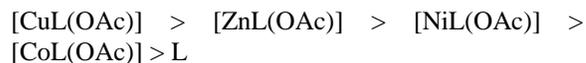


3.7 Antimicrobial activities

The *in vitro* antimicrobial activities of the investigated compounds were tested against the bacterial species, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* by Disk Diffusion method. The inhibitions around the antibiotic discs were measured after incubation and Streptomycin was used as standard drug. It was stated that the synthesized copper complexes of 2-hydroxynaphthoic acid derivatives showed more activity than its free ligands. The enhanced activity of the complexes can be explained on the basis of Overtone's concept [23] and Tweedy's Chelation theory [24]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid soluble materials makes which liposolubility as an important factor, which controls the antimicrobial activity.

All the compounds tested revealed moderate to strong antimicrobial activity. Among the test compounds at tempted, [CuL(OAc)] complex showed slightly higher activities when compared with standard drug Streptomycin. The antibacterial results evidently

show that the activity of the Schiff base compounds becomes more pronounced when coordinated to the metal ions. The MIC values indicated that all the compounds tested exhibit moderate to strong antimicrobial activity on the tested microorganisms. It was observed that increased activity was found in the order of



4. CONCLUSION

The synthesized copper complexes with distorted square planar geometry capable of protecting cells against O₂⁻ attack with ability of generating or scavenging reactive species by interaction with specific biological targets. We promising that the stable nontoxic copper complexes which catalase the superoxide anion show considerable promise as SOD mimics for Alzheimer's Disease.

Conflicts of Interest

There are no conflicts of interest.

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