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Synthesis, Spectroscopic Characterization, Biological Screening, and DNA Nuclease Activity of Schiff base Metal Complexes Derived from o-phenylenediamine

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Abstract

Novel series of mononuclear Schiff base Cu(II), Co(II), Ni(II) and Mn(II) complexes have been synthesized with Schiff base derived from 2- thiophenecarboxaldehyde, o- phenylenediamine, furfuraldehyde and 2,2' bipyridyl. The chelation of the metal complexes has been proposed in the light of analytical, spectral (IR, UV, ¹H NMR, ESR) and magnetic studies. These data reveals that the Schiff base complexes exhibit the octahedral geometry. The Schiff base and their metal complexes have been screened for their antibacterial activities. The complexes show significant growth inhibitory activity against the bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia* than the free ligands. The nucleolytic cleavage activities of the complexes were assayed on pUC18 plasmid DNA using gel electrophoresis in the presence of H₂O₂ and the complexes show promising nuclease activity.

Keywords: 2- thiophenecarboxaldehyde, nuclease activity, 2, 2' bipyridyl

Introduction

The developments in field of bioinorganic chemistry have increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species ^[1]. A rational control of the nuclearity of transition metal complexes is important to design systems with the desired properties, as some of these applications require the presence of O, S and N donor atoms in the particular complex ^[2]. Ligands bearing variable donor functionalities such as O, S and N in their structural framework have been studied for several years and continue to be an area of active research for understanding the properties of donors that offer the opportunity to design metal complexes for specific purposes and applications ^[3]. The coordination chemistry of Schiff base ligands containing 'hard' donor atoms (such as N and O) along with a 'soft' donor (S) is interesting because such a ligand framework can provide insight into competitive coordination behaviour between the 'hard' and 'soft' donors towards the metal center ^[4] and also stabilize low as well as high oxidation states of a metal atom. Such molecular systems may be important in transition metal catalyzed asymmetric synthesis ^[5]. In fact, a number of polydentate ligands bearing N₄N₂, N₄O₂, N₄OS and NS₂O₂ are well documented and among them, a few of the systems have shown promising catalytic, biological, environmental and material properties ^[6]. We report herein the design and synthesis of a new (N₄OS) molecular system, having both 'hard' and 'soft' donors in the ligand framework, and its reactivity towards Cu(II), Co(II), Ni(II) and Mn(II) ions. We also report our findings on a DNA cleavage and antibacterial activities with the synthesized complexes.

Materials and Methods

Measurements

Elemental analyses for C, H and N were carried out using a Perkin-Elmer 2400-II elemental analyzer. FT-IR data were recorded as KBr disc using Thermo Nicolet, Avatar 370 model spectrometer in the range 4000-400 cm⁻¹. UV-Vis spectra were obtained in DMF on a Perkin-Elmer Lambda 40(UV-Vis) spectrometer in the range 200-800 nm. Molar conductance of the complexes in DMF was measured using an Elico model conductivity meter. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature. NMR signals were obtained from Bruker Avance III, 400MHz model spectrometer. EPR spectra of compounds were recorded on an E-112 ESR Spectrometer with Xband microwave frequency (9.5 GHz).

Synthesis of Schiff base

The Schiff base ligand was synthesized by adding 2- thiophenecarboxaldehyde (1 mM) in 20 ml of ethanol, o-phenylenediamine(1 mM) in 20 ml of ethanol and furfuraldehyde (1 mM) in 20 ml ethanol. The mixture was refluxed for 2-3 hrs. Then solution of the ligand was kept for slow evaporation and coloured precipitate was collected and dried in air.

Synthesis of mononuclear Schiff base Metal complexes

The ethanolic solution of synthesized ligand (1 mM) was added dropwise stirring to an ethanolic solution of the metal salt (1 mM) with constant stirring, followed by the addition of 2, 2' bipyridyl (1 mM) and the mixture was boiled under reflux for 3-5 hrs. Then, the volume of the reaction mixture was reduced by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried in *vacuo*.

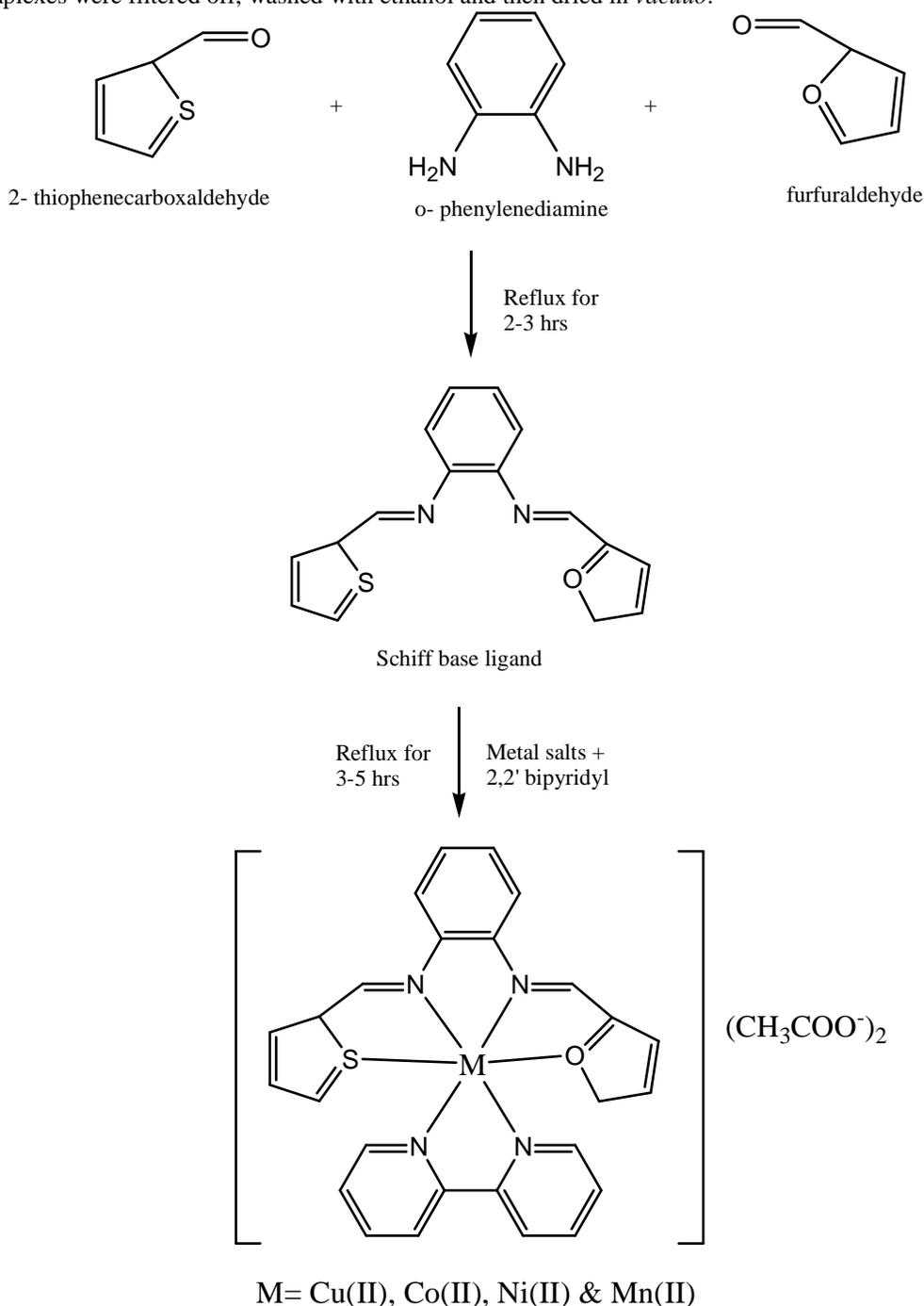


Figure1. Proposed structure of Schiff base metal complexes

Anti-bacterial activity

The anti-bacterial activity was carried out at Progen Lab at Salem, Tamilnadu (India). The standard disc-agar diffusion method ^[7] was followed to determine the activity of the synthesized compounds against the sensitive organisms. *Streptomycin* was used as a standard. The tested compounds were dissolved in DMF (which have no

inhibition activity), to get concentration of 100 µg/mL. The test was performed on medium potato dextrose agar contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar. Uniform size filter paper disks (three disks per compound) were impregnated by equal volume from the specific concentration of dissolved tested compounds and carefully placed on incubated agar surface. After incubation for 36 hrs at 27°C inhibition of the organism which evidenced by clear zone surround each disk was measured and used to calculate mean of inhibition zones.

Agarose Gel electrophoresis

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis. The gel electrophoresis experiments were performed by incubation of the samples containing 40 µM pUC18 DNA, 50 µM metal complexes and 50 µM H₂O₂ in tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 µg cm⁻³ ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature^[8].

Results and Discussion

Analytical data of the Schiff base ligands and their mononuclear metal complexes are given in Table.1 and are in well agreement with the expected values. All the Cu(II), Co(II), Ni(II) and Mn(II) complexes are colored, non hygroscopic solids, stable in air. They are sparingly soluble in common organic solvents, but soluble in DMF and DMSO. The analytic data show that the complexes may be formulated as [MLX], where L = Schiff base X= 2, 2' bipyridyl and M = Cu(II), Co(II), Ni(II) and Mn(II). The solubility of the complexes in DMF permitted calculation of the molar conductivity (Λ_m) of 10⁻³ M solutions at 25°C shows that all the complexes are found to be 1:2 type electrolytes in DMF solution.

Table 1: Analytical data of the Schiff base ligand and its mononuclear metal complexes

Compound	Molecular Formula	color	Yield %	Melting Point (°)	% of Nitrogen		% of Metal		Molar conductance Λ_m (scm ² mol ⁻¹)
					Cal	Exp	Cal	Exp	
L	C ₁₆ H ₁₅ N ₂ OS	Yellow	80	140	9.88	9.86	-	-	-
[CuLX]	[Cu(C ₂₆ H ₂₂ N ₄ OS)]	Dark Green	70	>200	11.15	11.14	12.64	12.63	132
[NiLX]	[Ni(C ₂₆ H ₂₂ N ₄ OS)]	Brown Black	75	>200	11.26	11.23	11.80	11.79	145
[CoLX]	[Co(C ₂₆ H ₂₂ N ₄ OS)]	Brown	70	>200	11.25	11.23	11.83	11.82	148
[MnLX]	[Mn(C ₂₆ H ₂₂ N ₄ OS)]	Black	75	>200	11.34	11.33	11.12	11.10	151

IR spectra

The IR spectra provide valuable information regarding the coordinating sites of Schiff base ligand which has been already discussed by Raman et al^[9]. The IR spectra of the complexes were compared with that of the free ligand to determine the changes that might have taken place during the complexation. A comparative study of the IR spectra of ligand and its metal complexes reveals that certain peaks are common and therefore, only important peaks, which have either shifted or have newly appeared, are discussed. Table 2 shows that ν (C-O) and ν (C=N) modes occur at 1224-1332 cm⁻¹ and 1595-1620 cm⁻¹ respectively. The shifting of (C-O) towards higher frequency as compared to the ligand (1224 cm⁻¹) is due to the conversion of hydrogen bonded structure into a covalent metal bonded structure. Lowering of (C=N) in the complexes as compared to the ligand (1620 cm⁻¹) is due to reduction of double bond character of carbon-nitrogen bond of the azomethine group. The band at 1440 cm⁻¹ and 1522 cm⁻¹ were due to symmetric stretching frequency and asymmetric frequency of acetate ion. This result predicts that the acetate ions were coordinated outside the coordination sphere. Metal-ligand bond is further confirmed by the appearance of a medium intensity band in the range 444-468, 402-410 and 530-560 cm⁻¹ in the spectra of the complexes assigned to stretching frequencies of (M-N) bond, (M-S) bond and metal-oxygen bond formation respectively^[10].

Table 2: Infrared Spectroscopic Data of the Schiff Base Ligand and its mononuclear metal complex

Compounds	(C=N) (cm ⁻¹)	(C-S) (cm ⁻¹)	(C-O) (cm ⁻¹)	(M-N) (cm ⁻¹)	(M-O) (cm ⁻¹)	(M-S) (cm ⁻¹)
C ₁₆ H ₁₅ N ₂ OS	1620	730	1244	--	--	--
[Cu(C ₂₆ H ₂₂ N ₄ OS)]	1605	710	1288	460	530	406
[Ni(C ₂₆ H ₂₂ N ₄ OS)]	1609	744	1320	454	544	410
[Co(C ₂₆ H ₂₂ N ₄ OS)]	1595	752	1332	468	560	408
[Mn(C ₂₆ H ₂₂ N ₄ OS)]	1600	718	1324	444	553	402

Electronic spectra and magnetic moment

The electronic spectral data of the metal complexes in DMF solution are displayed in Table 3. The nature of the ligand field around the metal ion was deduced from the electronic spectra. The electronic spectrum of Co(II) complex exhibited three bands in the region of 660, 570 and 530 nm which were tentatively assigned to ${}^4T_{1g} \rightarrow {}^4T_{2g}(F)$ (ν_1), ${}^4T_{1g} \rightarrow {}^4A_{2g}(F)$ (ν_2) and ${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$ (ν_3) transitions, respectively. The value of magnetic moment was 5.12 B.M. which indicates the presence of Co(II) complex in octahedral geometry^[11]. The electronic spectrum of the Ni(II) complex showed three bands at 695, 542 and 575 nm assignable to ${}^3A_{2g} \rightarrow {}^3T_{2g}(F)$ (ν_1), ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (ν_2) and ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$ (ν_3) transitions, respectively. The value of magnetic moment was 3.42 B.M; therefore octahedral geometry is suggested for this complex^[12]. The 2E_g and ${}^2T_{2g}$ states of the octahedral Cu(II) (d^9) split under the influence of the tetragonal distortion and the distortion can be such as to cause the three transitions ${}^2B_{1g} \rightarrow {}^2B_{2g}$; ${}^2B_{1g} \rightarrow {}^2E_g$ and ${}^2B_{1g} \rightarrow {}^2A_{1g}$ to remain unresolved in the spectra. It is concluded that, all three transitions 690, 570 and 535 nm lie within the single broad envelope centered at the same range previously mentioned. This assignment is in agreement with the general observation that Cu(II) d-d transitions are normally close in energy. The magnetic moment of 1.97 B.M. falls within the range normally observed for octahedral Cu(II) complexes^[13].

The electronic spectra of Mn(II) complexes show the absorption bands in the range 694, 555 and 522 nm. These absorption bands may be assigned to the ${}^6A_{1g} \rightarrow {}^4A_{1g}$, ${}^6A_{1g} \rightarrow {}^4A_{2g}$, and ${}^6A_{1g} \rightarrow {}^4E_g$, ${}^4A_{1g}$ transitions, respectively. These bands suggest that the complexes possess an octahedral geometry. The Mn(II) complex show magnetic moments is 5.92 B.M. at room temperature corresponding to five unpaired electrons which suggest octahedral geometry^[14]. In the spectra of the Schiff base ligand, the absorption band observed at 281-294 nm were assigned to intra-ligand $\pi \rightarrow \pi^*$ transition and the band at 342-390 nm were assigned due to $n \rightarrow \pi^*$ transition associated with the azomethine chromophore ($-C=N$).

Table 3: Electronic Spectral data of Schiff base ligand and its complexes.

Compound	Electronic spectra (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L → M	d-d	
C ₁₆ H ₁₅ N ₂ OS	294	386	-	-	-
[Cu(C ₂₆ H ₂₂ N ₄ OS)]	285	374	480	690,570,535	Octahedral
[Ni(C ₂₆ H ₂₂ N ₄ OS)]	292	390	440	695,542,575	
[Co(C ₂₆ H ₂₂ N ₄ OS)]	281	342	445	660,570,530	
[Mn(C ₂₆ H ₂₂ N ₄ OS)]	293	346	430	694,555,522	

¹H NMR spectra

The ¹H NMR Schiff base was recorded in DMSO- d₆ at room temperature. Three different type of protons were identified i) resonance exhibits due to phenolic -OH protons around 10.26 ppm, ii) characteristic resonance due to azomethine proton in the Schiff base appears at 8.227 ppm was observed and iii) the other signals in the region 6.24–7.72 ppm exhibits due to aromatic protons. All these observations support the infrared conclusions.

EPR spectra

The EPR spectra of complexes provide information of importance in studying the metal ion environment. The EPR spectra of the [Cu(C₂₆H₂₂N₄OS)] Schiff base complexes recorded on powder samples with room temperature, on X-band at frequency 9.3 GHz under the magnetic field strength 4000 G. The EPR spectrum of the [Cu(C₂₆H₂₂N₄OS)] (Figure 2) complexes show a broad signal with g_{iso} at 1.9998 which is consistent with an octahedral geometry^[15].



Figure.2. EPR spectra of Cu (C₂₆H₂₂N₄OS) complex

Antibacterial activity

Biological activity of the ligand and a series of its metal complexes [Cu(II), Ni(II), Co(II) and Mn(II)] were screened for anti-bacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia* by using disc-agar diffusion method (Figure 3). The results indicate that the complexes show more activity and the ligand have less activity against same microorganisms under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane. The copper(II) complex shows higher anti-fungal activity than other complexes. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells ^[16].

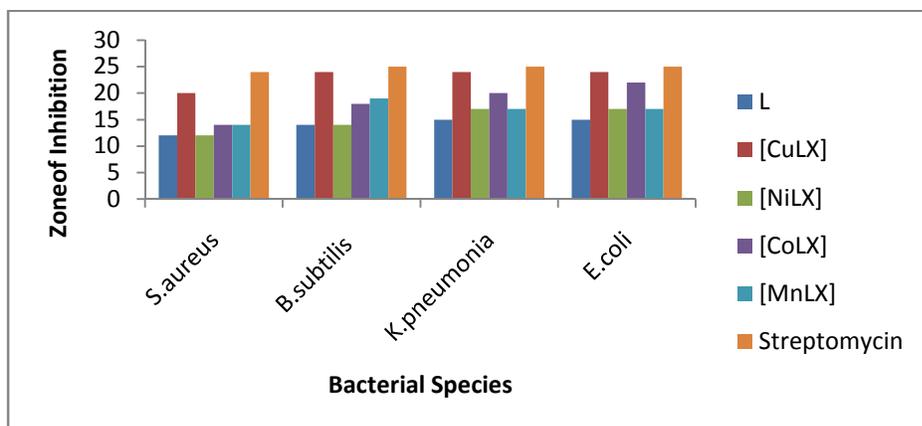


Figure 3: Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against Bacterial Strains

DNA cleavage

Gel electrophoresis experiments were performed using pUC18DNA with ligand, complexes in presence and absence of H_2O_2 . Complexes exhibit cleavage ability at low concentration (40 μM). When calf-thymus DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated ^[17, 18]. From Figure 4 the complexes show more activity in the presence of oxidant which may be due to the reaction of hydroxyl radical with DNA. The results of DNA cleavage studies have been shown in Figure 4. All metal complexes were able to convert DNA (Form I) into open circular (Form II). The mononuclear Cu(II) complex was found to be highly active in cleaving DNA in the presence of hydrogen peroxide.

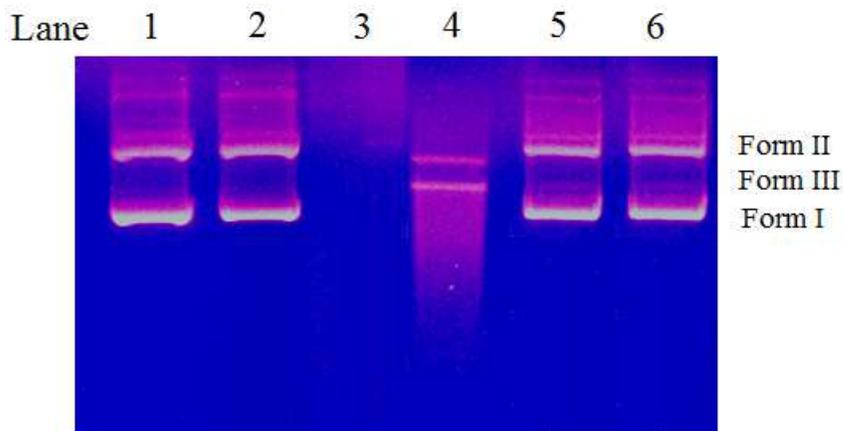


Figure.4 Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H_2O_2 and metal complexes. Lane 1-DNA alone; Lane 2- DNA alone + H_2O_2 ; Lane 3-DNA + $[Cu(C_{26}H_{22}N_4OS)] + H_2O_2$; Lane 4-DNA + $[Ni(C_{26}H_{22}N_4OS)] + H_2O_2$; Lane5-DNA + $[Co(C_{26}H_{22}N_4OS)] + H_2O_2$; Lane 6-DNA+ $[Mn(C_{26}H_{22}N_4OS)] + H_2O_2$.

Conclusion

The present study revealed octahedral geometry around Cu(II), Co(II), Ni(II) and Mn(II) complexes, in which the coordinating through nitrogen and oxygen atoms. The results of antimicrobial activity show that the compounds exhibit antimicrobial properties and it is important to note that the metal chelates show more inhibitory effects than the parent ligand. DNA cleavage studies show that all the complexes significantly cleaves DNA molecule.

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