DNA Cleavage, Antioxidant and Antibacterial Activity studies of new Schiff base Co(II), Ni(II), Cu(II) and Mn(II) complexes derived from ophthalaldehyde

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Abstract

A new series of binuclear Schiff base Cu(II), Co(II), Ni(II) and Mn(II) complexes have been synthesized with Schiff base derived from o-phthalaldehyde and pchloroaniline. The chelation of the metal complexes has been proposed in the light of analytical, spectral (IR, UV–Vis), magnetic, ¹H NMR and ESR studies. These data reveals that the Schiff base complexes exhibit the hexa-coordinated geometry. The Schiff base and their metal complexes have been screened for their antibacterial and antioxidant activities. The results of these studies show the metal complexes to be more antibacterial and more significant antioxidant as compared to free Schiff base ligand. All the metal complexes cleave the pUC18DNA in presence of H2O2.

Keywords: Symmetric Schiff Base, Mononuclear Metal Complexes, Antibacterial Activity, Antioxidant, DNA Cleavage

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]. DNA cleavage activity of transition metal complexes under physiological conditions has gained considerable current interest for varied applications of such complexes in nucleic acids chemistry [2]. Some kind of metal complexes interacted with DNA could induce the breakage of DNA strands by covalent binding, electrostatic interactions, groove binding or by intercalation methods. In the case of cancer genes, after DNA strands are cleaved, the DNA double strands break. The replication ability of cancer gene is destroyed. The Compounds showing the properties of effective binding as well as cleaving double stranded DNA under physiological conditions are of great importance since these could be used as diagnostic agents in medicinal and genomic research. Their ability of the individual metal ions to have quite distinct roles in the functioning of the metalloenzymes concerned has led to a search for symmetrical ligands, and also gaining insight into the pathways of electron transfers in biological systems.

In continuation of our series of investigations, we attempted to widen the scope of derivatization by providing more flexibility through Schiff base formation with o-phthalaldehyde and p-chloroaniline and finally complexation with Cu(II), Co(II), Ni(II) and Mn(II) metal ions to form potentially active Mononuclear Schiff base complexes. The Schiff base structure affords a greater choice and flexibility, and complexation with a metal ion adds to the stability and versatility of the compounds.

Experimental section

All the chemicals were of reagent grade and the solvents were distilled before use according to the standard procedure.

Physical measurements

Carbon, hydrogen and nitrogen analyses of the complexes were carried out on a CHN analyzer. Carlo Erba Model 1108. The infrared spectra (4000–200 cm⁻¹ KBr disks) of the

samples were recorded on a Perkin–Elmer FT-IR-8300 spectrophotometer. The electronic absorbance spectra in the 200–800 nm were recorded on Double beam spectrometer Cyber lab between 200 - 800 nm. ¹H NMR spectra of ligand were recorded on Bruker Spectrometer at 400 MHZ in DMSO. The chemical shifts are given in PPM, using Tetramethylsilane (TMS) as the internal reference. Magnetic susceptibility measurements were carried out by employ the Gouy method at room temperature on powder sample of the complexes using CuSO₄.5H₂O as calibrant. The metal contents of the complexes were determined according to the literature method [3].

Synthesis of Schiff base ligand

The Schiff base ligand was synthesized by adding o-phthalaldehyde (1 mM) in 20 ml of ethanol, p-chloroaniline (2 mM) in 20 ml of ethanol. The mixture was refluxed for 2-3 hrs. Then solution of the ligand was kept for slow evaporation and dark yellow colored precipitate was collected and dried in air [4].

Synthesis of Schiff base metal complexes

The ethanolic solution of synthesized ligand (2 mM) was added dropwise stirring to an ethanolic solution of the metal chloride (1 mM) (Metal = Cu(II), Co(II), Ni(II) and Mn(II)) with constant stirring 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 vacuuo [5].



Fig.1. Synthetic route of Schiff base ligand



M=Cu(II), Ni(II), Co(II) and Mn(II)

Fig.2. Synthetic route of Mononuclear Schiff base Metal(II) complexes

RESULTS AND DISCUSSION

Elemental Composition

All the reported mononuclear Schiff base metal (II) complexes are extremely insoluble in water and most of organic solvents, but easily soluble in DMF and DMSO. All the reported complexes show deep in colour and stable at room temperature. The elemental analysis results were in good agreement with the proposed formulae as illustrated in Table 1. **Molar Conductivity Studies**

The molar conductivity measurements have demonstrated to be a extremely helpful tool in the examination of geometrical structures of inorganic metal complexes. The molar conductivity for the complexes was measured at the room temperature in 10^{-3} M solution DMF as solvent. The molar conductance was applied to examine the being of anion outer the coordination sphere for all the complexes showed a important molar conductance values range of 12-15 ohm⁻¹cm²mol⁻¹ where indict them non electolytic in nature [6].

difference in number of protons in mononuclear Co(II), Ni(II), Cu(II), and Mn(II) metal complexes perhaps points out the difference in sizes of the +2 oxidation state of reported metal ions, with various polarizing because of its different sizes.

Table 1. Analytical data of newly synthesized	Schiff base ligand and its mononuclear
metal complexes	

Molecular Formula	color	Yield %	Melti ng	% of Nitrogen		% M	of etal	Molar conductance
			Point (°)	Cal	Ехр	Cal	Ехр	$\begin{array}{c} \Lambda_{m} \left(ohm^{-} \right. \\ \left. {}^{1}cm^{2}mol^{-1} \right. \right) \end{array}$
$C_{20}H_{14}N_2Cl_2$	Dark yellow	85	150	8.81	8.79	-	-	-
Cu(C40H28N4Cl6)	Deep green	80	>200	8.56	8.52	9.71	9.70	12
Ni(C40H28N4Cl6)	Yellowi sh green	75	>200	8.63	8.62	9.08	9.05	15
Co(C ₄₀ H ₂₈ N ₄ Cl ₆)	Dark pink	83	>200	8.62	8.61	9.07	9.06	13
Mn(C ₄₀ H ₂₈ N ₄ Cl ₆)	Dark red	77	>200	8.68	8.67	8.51	8.50	12

Where L = Ligand, Y = 2, 2'- bipyridyl and Z = Chloride ion.

Infrared Spectra

The IR Spectral analysis of the Schiff base ligands and its derived complexes absorption bands characteristics of different bond types gives an information regarding the creation of the ligand and its mode of coordination in the complexes. The IR spectral data of all synthesized complexes are listed in Table 2.

A comparison of spectra of metal complexes and its Schiff bases which indicates that here all the complexes the metal ions are coordinated to Schiff bases through the imino nitrogen(s) which is suitable to the downfield shift in the stretching band of the C=N group from ligands (1622 cm⁻¹) to complexes (1610 ±1601 cm⁻¹). This be capable of explained based on a drift of the lone pair density of azomethine nitrogen in direction of the metal atom [7]. New vibrations at 448-468 cm⁻¹ which are not present in the free Schiff base are related to the subsistence of v (M-N). The appearance of new bands at 270-280 cm⁻¹ was due to formation of M-Cl. This report predicts that chloride ions were coordinated inside in the coordination sphere [8].

Table 2. Infrared Spectroscopic Data of Schiff Base Ligand and its mononuclear metal complexes

	(C=N)	(M-N)	(M-Cl)
Compounds	(cm ⁻¹)	(cm ⁻¹)	(cm ⁻¹)
$C_{20}H_{14}N_2Cl_2$	1622		
Cu(C ₄₀ H ₂₈ N ₄ Cl ₆)	1610	456	279
Ni(C ₄₀ H ₂₈ N ₄ Cl ₆)	1605	461	279
Co(C ₄₀ H ₂₈ N ₄ Cl ₆)	1601	468	278
$Mn(C_{40}H_{28}N_4Cl_6)$	1608	448	280

ELECTRONIC SPECTRA

The electronic spectra are frequently more useful in the assessment of results which is furnished by other methods such as structural investigation. Electronic spectral data of all reported complexes were demonstrated in DMF solution medium are shown in Table 3.

The absoption peaks which are obtained at the range of 250- 272 cm^{-1} was accredited to the intra ligand charge transfer transition $(\pi \rightarrow \pi^*)$. These high intensity absoption peaks comes from $\pi \rightarrow \pi^*$ transitions exactly localized at the aromatic groups of the complex. Intense peaks obtained here at the range of 350-376 cm⁻¹ were assigned to $n \rightarrow \pi^*$ and ligandto-metal charge transfer transition, correspondingly. The octahedral Cu(II) (d⁹) split beneath the influence of the distortion and the distortion can be such as to reason the three transitions ${}^{2}B_{1}g \rightarrow {}^{2}B_{2}g$; ${}^{2}B_{1}g \rightarrow {}^{2}Eg$ and ${}^{2}B_{1}g \rightarrow {}^{2}A_{1}g$ arises at the range of 625, 560 and 525 nm [9]. The electronic spectrum of Ni(II) complex showed the three bands at 630, 560 and 520 nm attributed to ${}^{3}A_{2}g \rightarrow {}^{3}T_{2}g$ (F) (v_{1}) , ${}^{3}A_{2}g \rightarrow {}^{3}T_{1}g$ (F) (v_{2}) and ${}^{3}A_{2}g \rightarrow {}^{3}T_{1}g$ (P) (v_{3}) transitions, correspondingly [10]. The UV spectrum of Co(II) complex exhibited three bands at the region of 625,540 and 585 nm which were assigned tentatively to ${}^{4}T_{1}g \rightarrow 4T_{2}g(F)$ (v₁), ${}^{4}T_{1}g \rightarrow {}^{4}A_{2}g(F)(v_{2}) \text{ and } {}^{4}T_{1}g \rightarrow {}^{4}T_{1}g(P)(v_{3}) \text{ transitions ,respectively [6]. The electronic spectra$ of Mn(II) complexes show the absorption peaks in the range 620, 536 and 520 nm. These absorption peaks due to the ${}^{6}A_{1}g \rightarrow {}^{4}A_{1}g$, ${}^{6}A_{1}g \rightarrow {}^{4}A_{2}g$, and ${}^{6}A_{1}g \rightarrow {}^{4}Eg$, ${}^{4}A_{1}g$ transitions, respectively [11]. These bands recommend that the complexes which possess a distorted octahedral geometry.

S.	Compound		Geometry of the			
No	F	$\pi \rightarrow \pi^*$	n→π*	L→M	d-d	complex
1	$C_{20}H_{14}N_2Cl_2$	272	376	-	-	-
2	$Cu(C_{40}H_{28}N_4Cl_6)$	265	350	460	625,560,525	
3	Ni(C40H28N4Cl6)	250	364	442	630,560,520	Distorted

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

4	$Co(C_{40}H_{28}N_4Cl_6)$	270	372	455	625,540,585	Octahedral
5	Mn(C40H28N4Cl6)	272	350	420	620,536,520	

¹H NMR Spectral Analysis

¹H NMR spectra of all Schiff base $C_{20}H_{14}N_2Cl_2$ ligand were clearly recorded in DMSO d₆ at room temperature as given in Figure 3. The ligand was prepared by dissolution in DMSO d₆ and chemical shifts were recorded with respect to TMS. Here in this case, two types of different of protons were identified i) characteristic resonance which is attributed to azomethine proton in Schiff base ligand $C_{20}H_{14}N_2Cl_2$ appears at 8.103 ppm was observed and ii) the other signals in the region 6.56–7.82 ppm exhibits due to aromatic protons. All these observations exactly support the infrared conclusions [12].



Fig. 3. ¹H- NMR spectra of C₂₀H₁₄N₂Cl₂ ligand

Magnetic Studies

The magnetic moment results are useful in the assessment of structural investigations. The magnetic moments of solid complexes were measured at the room temperature. The magnetic moment value of 1.89 B.M. falls at the range which is usually obtained for distorted octahedral Cu(II) complex. The value in the range of magnetic moment was 5.22 B.M. which clearly illustrates the presence of Co(II) complex of distorted octahedral geometry. The value of magnetic moment was 3.77 B.M; consequently distorted octahedral geometry is suggested for this Ni(C₄₀H₂₈N₄Cl₆) complex. The Mn(II) complex gives the value of magnetic moments is 5.55 B.M. at room temperature which is equivalent to five unpaired electrons that suggest distorted octahedral geometry.

EPR Spectral Studies

The EPR-spectral analysis of $Cu(C_{40}H_{28}N_4Cl_6)$ complex were measured at X-band frequencies in the particular range of 9.1 GHz. The EPR-spectra of $Cu(C_{40}H_{28}N_4Cl_6)$ complex

exactly provides valuable information about the coordination environment which is around Cu(II) ion [13]. The EPR-spectra of the Cu(C₄₀H₂₈N₄Cl₆) complex showed a very sharp band exactly in the range $g_{iso}=2.0012$ which shows Fig. 4. Therefore, the results proposed that Cu(II) complex possesses distorted octahedral geometry.



Fig. 4. EPR spectra of Cu(C₄₀H₂₈N₄Cl₆) complex DNA Cleavage Activity

Deoxyribonucleic acid (DNA) is major target molecule for the main part of the antiviral and anticancer therapies. Gel electrophoresis precisely works on the movement of DNA molecule which will comes under the control of an electric potential [14]. The $Cu(C_{40}H_{28}N_4Cl_6)$, $Ni(C_{40}H_{28}N_4Cl_6)$, $Co(C_{40}H_{28}N_4Cl_6)$ and $Mn(C_{40}H_{28}N_4Cl_6)$ complexes were examined for their DNA cleavage activity by a method called agarose gel electrophoresis against pUC18DNA and the gel picture clearly showing DNA cleavage in Fig. 5.

The gel electrophoresis studies clearly explained that, all the reported complexes have acted efficiently on DNA as that was molecular weight differentiation which shows among treated DNA samples and the control. As the prepared complexes were observed to cleave DNA effectively, it concluded that, the metal complexes efficiently which inhibit the growth of certain pathogenic by cleaving same genome.

From Figure it is evident that $Cu(C_{40}H_{28}N_4Cl_6)$ complex cleave DNA more capably in the presence of an oxidant hydroxyl radical (OH) reaction with DNA. This is reliable with the increased production of OH radicals by cuprous ions similar to the well known Fenton reaction. The distinction in the amount of protons in the mononuclear Co(II), Ni(II), Cu(II), and Mn(II) metal complexes approximately undoubtedly points the difference in sizes of the +2 oxidation state of these metal ions, through the diverse DNA-cleavage effectiveness due to its various sizes. From these results, we conclude that the Cu(C₄₀H₂₈N₄Cl₆), Ni(C₄₀H₂₈N₄Cl₆), Co(C₄₀H₂₈N₄Cl₆) and Mn(C₄₀H₂₈N₄Cl₆) complexes act as a potent nuclease agents.



Fig. 5. Changes in agarose gel electrophoretic pattern of pUC18DNA which is induced by H₂O₂ and metal complexes. Lane 1-DNA alone; Lane 2- DNA alone + H₂O₂; Lane 3-DNA + Cu(C₄₀H₂₈N₄Cl₆) + H₂O₂; Lane 4-DNA + Co(C₄₀H₂₈N₄Cl₆) + H₂O₂; Lane 5-DNA + Ni(C₄₀H₂₈N₄Cl₆); Lane 6-DNA+ Mn(C₄₀H₂₈N₄Cl₆) + H₂O₂. Antibacterial Studies

The prepared Schiff base ligand and metal complexes $C_{20}H_{14}N_2Cl_2$, $Cu(C_{40}H_{28}N_4Cl_6)$, $Ni(C_{40}H_{28}N_4Cl_6)$, $Co(C_{40}H_{28}N_4Cl_6)$ and $Mn(C_{40}H_{28}N_4Cl_6)$ were screened for antibacterial activity against a variety of pathogenic bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* as gram positive bacteria and *Klebsiella pneumoniae* and *Escherichia coli* as gramnegative bacteria by a certain method called disc diffusion method. From table 4, the Gram +ve bacteria of synthesized metal complexes were found to hinder efficiently all the tested bacteria at various rates and activity shown as following order of $Cu(C_{40}H_{28}N_4Cl_6) > Co(C_{40}H_{28}N_4Cl_6) > Ni(C_{40}H_{28}N_4Cl_6) > Mn(C_{40}H_{28}N_4Cl_6)$. While the Gram –Ve *bacteria* too go behind the same order and all the complexes which show higher bacterial activity than reported ligand.

The activities of synthesized complexes may be explained on basis of chelation theory, chelation actually which actually reduces the polarity of metal atom and mainly due to partial sharing of its positive charge with donor groups and probable π - electron delocalisation within the whole chelate ring. Also chelation which then favours its permeation from side to side the lipid layer of cell membrane [15]. Antibacterial activity of the reported ligands and metal complexes was compared with the standard drug as *Streptomycin*.

In the current study the higher antibacterial activity of the some prepared metal complexes were might be because of the electron withdrawing groups i.e., Cl group substituted present in the ligand environment, the electron withdrawing groups always ready to pull electron towards itself so that the positive charge of metal ion gets increased automatically , there of penetration of the complex during the lipid membrane was effortlessness and therefore they could reduce the growth of the microorganism effectively [16]. The antibacterial activity of Cu(II) complex are shows higher than the ligand and Ni(II), Co(II) and Mn(II) complexes. It might be probably due to the Cu(II) ion is small in size with that of high electronegativity when compare to other metal ions.

				Zoi	Zone of inhibition (mm)											
	Gram positive bacteria							Gram negative bacteria								
Compounds	Staphylococcus aureus			Bacillus subtilis				Escherichia coli				Klebsiella pneumoniae				
	Concentration (µg/mL)															
	25	25 50 75 100 25 50 75 100					25	50	75	100	25	50	75	100		
$C_{20}H_{14}N_2Cl_2$	9	9	10	12	8	9	10	13	8	9	9	10	8	8	9	9
$Cu(C_{40}H_{28}N_4Cl_6)$	12	15	17	20	13	15	19	21	10	11	12	16	10	12	15	17
Ni(C40H28N4Cl6)	10	13	15	17	11	13	17	18	8	10	10	11	7	8	9	10
Co(C40H28N4Cl6)	11	14	16	19	12	14	18	20	11	12	15	15	10	11	13	15
$Mn(C_{40}H_{28}N_4Cl_6)$	9	10	14	16	10	11	16	17	8	8	8	10	7	8	9	10
Streptomycin	15	16	19	22	16	17	20	22	16	16	19	19	14	15	18	20

Table 4. Antibacterial activity for Schiff base ligands and mononuclear metal complexes



Fig. 6. Antibacterial activity of Schiff base ligand and mononuclear Schiff base metal complexes against *Staphylococcus aureus*



Fig. 7. Antibacterial activity of Schiff base ligand and mononuclear Schiff base metal complexes against *Bacillus subtilis*



Fig. 8. Antibacterial activity of Schiff base ligand and mononuclear Schiff base metal complexes against *Escherichia coli*



Fig. 9. Antibacterial activity of Schiff base ligand and mononuclear Schiff base metal complexes against *Klebsiella pneumoniae*

DPPH Radical Scavenging Assay

Figure 10 & 11 depicts $Cu(C_{40}H_{28}N_4Cl_6)$ complex are significantly more proficient in quenching DPPH radical than the reported free ligand $C_{20}H_{14}N_2Cl_2$ and other synthsized complexes. From the results it was clearly found that IC_{50} values of $Cu(C_{40}H_{28}N_4Cl_6)$ complex is 5.82 mg/ml show very good activity compared to other complexes and reported ligand. Ascorbic acid is used as standard here.



Fig. 10. DPPH Radical Scavenging Activity Spectrophotometric Assay of various of Metal Complexes



Fig. 11. DPPH Scavenging Capacities (IC50) of Metal Complexes

Conclusion

The synthesis and spectral analysis of mononuclear $C_{20}H_{14}N_2Cl_2$, $Cu(C_{40}H_{28}N_4Cl_6)$, Ni(C₄₀H₂₈N₄Cl₆), Co(C₄₀H₂₈N₄Cl₆) and Mn(C₄₀H₂₈N₄Cl₆) Schiff base complexes have been demonstrated in this report. All the reported complexes synthesized from o-pthalaldehyde and 4-chloroaniline containing N₄ donors set in diverse environments. The reported compounds characterized by spectral and analytical data. The molar conductance values of synthesized complexes were indicated that complexes which show non- electrolytic in nature. FT-IR spectra clearly reveal that the azomethine stretching frequency was observed for all the reported complexes and thereby suggesting the complex formation and far IR spectra indicates that chloride ion bonded to metal ion. The magnetic moment studies, UV-Vis, and EPR spectral data of complexes suggested a distorted octahedral geometry for the reported The DNA cleavage studies reveals that the $Cu(C_{40}H_{28}N_4Cl_6)$ complexes. and Co(C₄₀H₂₈N₄Cl₆) complexes cleaved DNA was more efficiently when compared to other complexes and ligand. The antibacterial activity of prepared Cu(C₄₀H₂₈N₄Cl₆) complexes are higher than the ligand, $Ni(C_{40}H_{28}N_4Cl_6)$, $Co(C_{40}H_{28}N_4Cl_6)$) and $Mn(C_{40}H_{28}N_4Cl_6)$ complexes. The IC₅₀ values of Cu(C₄₀H₂₈N₄Cl₆) complex clearly indicates that the Cu(II) shows graet DPPH Scavenging activity compared to other synthesized compounds.

DECLARATIONS

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Conflict of interests

The authors declare that they have no conflict of interest

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