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Enthused research on DNA-binding and DNA-cleavage aptitude of mixed ligand metal complexes

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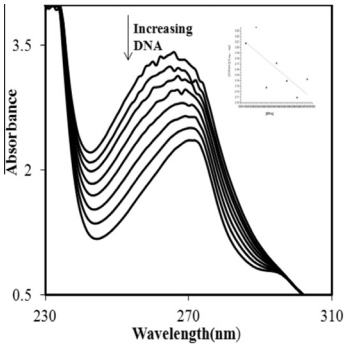
HIGHLIGHTS

- Synthesis of good DNA intercalators.
- Exploring efficient chemotherapeutic agents.
- Better antimicrobial active agents.
- Effective DNA cleavage activators.
- Excellent Cu(II) DNA binder.

GRAPHICAL ABSTRACT

The N-(4-aminophenyl)acetamide derived Schiff base mixed ligand complexes act as good DNA binding and DNA cleaving agents. The research has led to the discovery of new Schiff base mixed ligand compounds for further pharmacological investigation.



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ABSTRACT

Five new Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes have been synthesized using a Schiff base precursor (obtained by the condensation of N-(4-aminophenyl)acetamide and 4-chlorobenzaldehyde) as main ligand and 1,10-phenanthroline as co-ligand. They have been characterized by microanalytical data, IR, UV–Vis, magnetic moment values, conductivity and electrochemical measurements. The spectral data reveal that all the complexes exhibit octahedral geometry. The high electrical conductance of the complexes supports their electrolytic nature. The monomeric nature of the complexes has been assessed from their magnetic susceptibility values. These complexes are better antimicrobial active agents than the free ligands. DNA (CT) binding properties of these complexes have been explored by UV–Vis., viscosity measurements, cyclic voltammetry, and differential pulse voltammetry measurements. The oxidative cleavage activity of the complexes has been studied using supercoiled pUC19 DNA by gel electrophoresis. The experimental results show that the complexes are good intercalators.

Introduction

The interaction of metal complexes with DNA has been the subject of interest for the development of effective chemotherapeutic agents. The studies of transition metal complexes are becoming increasingly important to explore the possible new drug. Recent years have a great deal of interest in the synthesis and characterization of transition Schiff base metal complexes, containing an azomethine group (—C=N—). Transition metals are essential for the normal functioning of living organisms. Therefore, it is not surprising that transition metal compounds are of great interest as potential drugs. Schiff bases form an important class of organic compounds with a wide variety of biological properties [1].

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Development of a new chemotherapeutic Schiff base is now attracting the attention of medicinal chemist [2]. The heterocyclic rings containing nitrogen and oxygen import special biological activity to these Schiff bases and their metal complexes. 1,10-phenanthroline (phen) is a chelating bidentate ligand for transition metal ions that has played a significant role in the development of coordination chemistry [3–5]. It is a rigid planar, hydrophobic, electron-poor heteroaromatic system whose nitrogen atoms are beautifully placed to act cooperatively in cation binding.

Deoxyribonucleic acid (DNA) plays a significant role in the life process. It is considered as the primary target molecule for most anticancer and antiviral therapies according to cell biologists. The structural features determine its coordination ability toward metal ions. Transition metal complexes bind to DNA in a covalent or non-covalent fashion. The interaction between DNA and transition metal complexes is an important essential issue in life sciences [6]. Consequently, the design and synthesis of new transition metal complexes that interact with DNA are of considerable interest in nowadays [7–9]. Moreover, considerable attention has been focused on the use of phenanthroline complexes as intercalating agents of DNA and as artificial nucleases in coordination chemistry [10].

The use of mixed ligand complexes allows the development of methods with increased selectivity and has also great importance in the field of biological and environmental chemistry [11]. Bearing all the facts in our mind, we herein report the synthesis, structure, anti-biogram, DNA binding and cleavage studies of mixed ligand complexes having N-(4-aminophenyl)acetamide Schiff base and 1,10-phenanthroline.

Experimental

Reagents and instruments

All reagents N-(4-aminophenyl)acetamide, 4-chlorobenzaldehyde, 1,10-phenanthroline and metal(II) chlorides were of Merck products and used as supplied. Commercial solvents were distilled and then used for the preparation of ligand and its 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. Molar conductivities in DMSO (10^{-3} M) at room temperature were measured using Systronic-model-304 digital conductivity meter. Magnetic susceptibility measurements of the complexes were carried out by Gouy balance using copper sulfate pentahydrate as the calibrant. IR spectra were recorded with Perkin–Elmer 783 spectrophotometer in the 4000–400 cm^{-1} range using KBr pellets. NMR spectra were recorded on a Bruker Avance Dry 300 FT-NMR spectrometer in DMSO-d_6 with TMS as the internal reference. The absorption spectra were recorded using Shimadzu model UV-1601 spectrophotometer at room temperature. Electrochemical measurements were performed on a CHI620C electrochemical analyzer with three electrode system of a glassy electrode as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Solutions were deoxygenated by purging with N_2 prior to measurements.

Synthesis of Schiff base ligand and its metal complexes

Synthesis of Schiff base

The preparation of Schiff base ligand was already done by Raman and Johnson Raja [12]. It was prepared by the dropwise addition of an ethanolic solution (50 mL) of N-(4-aminophenyl)acetamide into a solution of 4-chlorobenzaldehyde (1:1 M ratio of 10 mM) in ethanol. After completion of the addition, the solution was refluxed on a water bath for 3 h and allowed to cool by standing at room temperature. The formed solid product was removed by filtration and recrystallized from ethanol, and dried in vacuum over fused $CaCl_2$.

Synthesis of metal complexes

A solution of Schiff base and 1,10-phenanthroline in ethanol was added to a solution of $MCl_2{\cdot}2H_2O$ (1:2:1 M ratio of 10 mM) in ethanol and the mixture was stirred for 1 h and the solution was refluxed on a water bath for 2 h. The solid product so formed was separated by filtration and washed thoroughly with ethanol and dried *in vacuo*.

DNA binding experiments

The interaction between metal complexes and DNA was studied using electronic absorption, viscosity and electrochemical methods. Disodium salt of calf thymus DNA was stored at 4 °C. All the experiments involving the interaction of the complexes with calf thymus (CT) DNA were carried out in Tris–HCl buffer (50 mM Tris–HCl, pH 7.2) containing 5% DMSO at room temperature. A solution of CT DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.89:1, indicating the CT DNA sufficiently free from protein [13]. The concentration of DNA was measured using its extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}) after 1:100 dilution. Stock solutions were stored at 4 °C and used not more than 4 days. Doubly distilled water was used to prepare solutions. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in DMSO and diluting properly with the corresponding buffer to the required concentration for all the experiments.

Absorption titration experiment was performed by keeping the concentration of the metal complex as constant at 50 μM while varying the concentration of the CT DNA within 40–400 μM. While measuring the absorption spectrum, equal quantity of CT DNA was added to both the complex solution and the reference solution to eliminate the absorbance of CT DNA itself. From the absorption data, the intrinsic binding constant (K_b) was determined from the plot of [DNA]/($\varepsilon_a-\varepsilon_f$) vs. [DNA] using the following equation:

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + [K_b(\varepsilon_b - \varepsilon_f)]^{-1} \quad (1)$$

where [DNA] is the concentration of CT DNA in base pairs. The apparent absorption coefficients ε_a, ε_f and ε_b correspond to $A_{obs.}$/[M], the extinction coefficient for the free metal(II) complex and extinction coefficient for the metal(II) complex in the fully bound form, respectively [14]. K_b is given by the ratio of slope to the intercept.

Cyclic voltammetry and differential pulse voltammogram studies were performed on a CHI620C 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. Solutions were deoxygenated by purging with N_2 prior to measurements.

Viscosity experiments were carried on an Ostwald viscometer, immersed in a thermostated water-bath maintained at a constant temperature at 30.0 ± 0.1 °C. CT DNA samples of approximately 0.5 mM were prepared by sonicating in order to minimize complexities arising from CT DNA flexibility [15]. Flow time was measured with a digital stopwatch three times for each sample and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the concentration of the metal(II) complexes, where η is the viscosity of CT DNA solution in the presence of complex, and η_0 is the viscosity of CT DNA solution in the absence of complex.

Viscosity values were calculated after correcting the flow time of buffer alone (t_0), $\eta = (t-t_0)/t_0$ [16].

DNA cleavage study

The extent of cleavage of super coiled (SC) pUC19 DNA (33.3 μM, 0.2 μg) to its nicked circular (NC) form was determined by agarose gel electrophoresis in 5 mM Tris–HCl buffer (pH 7.2) containing 50 mM NaCl. The samples after incubation for 2 h at 37 °C in a dark chamber were added to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 μL) and the solution was finally loaded on 0.8% agarose gel containing 1 μg/mL ethidium bromide [17]. Electrophoresis was carried out in a dark chamber for 3 h at 50 V in Tris–HCl–EDTA buffer. Bands were visualized by UV light and photographed. To enhance the DNA cleaving ability by the complexes, hydrogen peroxide (100 μmol L^{-1}) was added into each sample.

Antimicrobial studies

Antibacterial activity of the Schiff base ligand and its metal complexes was tested *in vitro* against the bacterial species viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis, Klebsiella pneumoniae* by the paper disk method using nutrient agar as the medium. Streptomycin was used as the standard antibacterial agent. The test organisms were grown on nutrient agar medium in petri plates. Disks were prepared and applied over the long culture. The compounds were prepared in DMSO and soaked in filter paper disk of 5 mm diameter and 1 mm thickness. The concentration of ligand and the complexes used in this study was 0.01 μg/mL. The disks were placed on the previously seeded plates and incubated at 37 °C and the diameter of inhibition zone around each disk was measured after 24 h for antibacterial activity. Growth inhibition was calculated according to Ref. [18].

Results and discussion

Synthetic routes for the preparation of different compounds are depicted in Scheme 1. The ligand and its complexes are found to be stable in air. The ligand (L) is soluble in common organic solvents but the complexes are soluble only in DMF and DMSO.

Elemental analysis and molar conductivity measurements

The results of elemental analysis for the metal complexes are in good agreement with the calculated values (Table 1) showing that the complexes of stoichiometry $[M(L)(phen)_2]Cl_2$ [19]. The metal(II) complexes were dissolved in DMSO and the molar conductivities of 10^{-3} M of their solution at room temperature were measured. The higher conductance values of the complexes support their electrolytic nature, implying the non-coordination of chloride anion to the central metal ion. The presence of counter chloride ion is confirmed from Volhard's test.

Magnetic susceptibility and ultraviolet spectral measurements

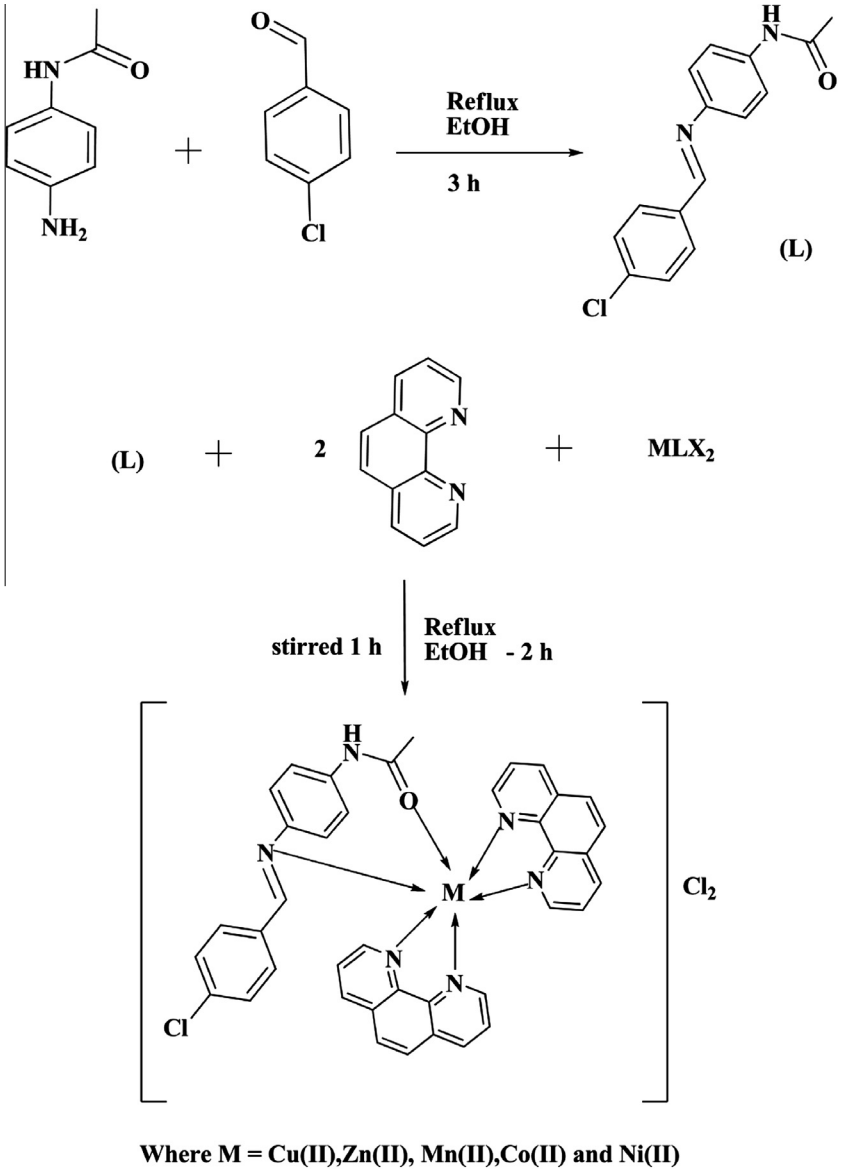
The free Schiff base ligand (L) exhibits two intense bands at 33,783 and 26,737 cm^{-1} due to π–π* and n–π* transitions respectively. The electronic absorption spectral data for the ligand and the complexes are given in Table 2. The electronic spectrum of $[Cu(L)(phen_{)2}]Cl_2$ complex exhibited intraligand charge transfer bands at ca. 33,989 and 37,037 cm^{-1} and a d–d band at 15,243 cm^{-1} which was due to $^2E_g \rightarrow {}^2T_{2g}$ transition [20–23]. This d–d band transition band strongly favored a distorted octahedral geometry around the metal ion. Its magnetic moment (1.79 BM) indicates that the complex exits in monomeric nature. The electronic spectrum of $[Co(L)(phen)_2]Cl_2$ complex showed three broad bands in the visible region at 14,705, 15,267 and 30,581 cm^{-1}, which are assigned to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ transitions respectively [24–26]. The electronic spectrum of $[Ni(L)(phen)_2]Cl_2$ complex exhibited three d–d bands at 10,695, 11,049 and 25,575 cm^{-1}, attributed to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transitions respectively, being characteristic of an octahedral geometry [27]. The magnetic measurement values of Co(II) complex (4.82 BM) and Ni(II) complex (3.41 BM) suggest steadiness with their octahedral environment [28,29]. The electronic spectrum of $[Mn(L)(phen)_2]Cl_2$ complex revealed four broad, low intensity bands in the visible region around 15,822, 23,980, 25,510, 28,089 cm^{-1} respectively which are assigned to $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow {}^4T_{2g}(G)$, $^6A_{1g} \rightarrow {}^4Eg$ $^6A_{1g} \rightarrow {}^4T_{2g}$ (D) transitions. The electronic spectral data suggest an octahedral geometry around Mn(II) ion. The magnetic measurement for Mn(II) complex showed magnetic moment value of 4.82 BM. This observed magnetic moment at room temperature indicates its monomeric nature and octahedral geometry [30]. The complex of Zn(II) is diamagnetic. According to the empirical formula, an octahedral geometry is proposed for this complex.

^{1}H NMR spectra

The ^{1}H NMR spectra of the Schiff base and its zinc complex were recorded at room temperature in DMSO-d_6. ^{1}H NMR spectrum of the Schiff base ligand showed peaks at 7.2–7.8 δ which are attributed to phenyl multiplet of Schiff base ligand. The Ph-NH- group gave singlet at 9.8 δ in the free ligand and remained unchanged in the zinc complex. It showed that the Ph-NH- group was not taking part in complexation. The azomethine proton (—CH=N) signal in the spectrum of the ligand showed a signal at 8.5 δ. The azomethine proton (—CH=N) signal in the spectrum of the zinc complex showed a downfield shift (8.1 δ) compared to the free ligand, suggesting deshielding of azomethine group due to the coordination with metal ion. The aromatic methyl proton showed (C—CH_3) signal at 3.4 δ. There was no appreciable change in all other signals of the complex [31].

IR spectra

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. In order to study the bonding mode of Schiff base to the metal complexes, the IR spectrum of the free ligand is compared with the spectra of the complexes. The main IR bands and their assignments are listed in Table 3. A sharp band observed at 1625 cm^{-1} in the IR spectrum of the Schiff base ligand (>C=N) was shifted downward by about 1602–1618 cm^{-1} in all the complexes indicating coordination through azomethine nitrogen [32]. The band in Schiff base ligand at 1662 cm^{-1}due to (C=O) vibration, appeared at 1647–1656 cm^{-1} in all the complexes. This shows chelation of (C=O) group with metal ion. The unaltered position of bands due to υ(NH) moiety of the N-(4-aminophenyl)acetamide in all the metal complexes indicated that these groups were not involved in coordination. The band at 3061 cm^{-1} (υ(NH)) did not change for ligand and complexes. The IR values of ν(C—H) observed at 860 cm^{-1} and 735 cm^{-1} for phenanthroline were red shifted to 852 cm^{-1} and 729 cm^{-1} respectively. These shifts can be explained by the fact that each of the two nitrogen atoms of phenanthroline ligands donates a pair of electrons to the central metal forming a coordinate covalent bond [33]. Ring stretching frequencies (υ(C=C) and (υ(C=N)) at 1503 and 1420 cm^{-1} for free phen were shifted to higher frequencies upon complexation (5–14 cm^{-1} and



Scheme 1. Schematic route for synthesis of Schiff base ligand and its metal complexes.

Table 1
Analytical and physical data of Schiff base ligand and its complexes.

Compound	Yield (%)	Color	Found (calc)%				Formula weight	(Λ_m)[a]	μ_{eff} (BM)
			M	C	H	N			
$[C_{15}H_{13}ON_2Cl]$	85	Yellow	–	65.3 (66.0)	4.5 (4.8)	10.0 (10.3)	272	–	–
$[CuC_{39}H_{29}N_6OCl]$	74	Dark green	8.9 (9.1)	69.8 (67.2)	3.9 (4.2)	12.2 (12.6)	696	140	1.79
$[NiC_{39}H_{29}N_6OCl]$	68	Pale green	8.2 (8.5)	67.1 (67.7)	3.7 (4.2)	11.8 (12.1)	691	90	3.41
$[CoC_{39}H_{29}N_6OCl]$	64	Pale brown	8.3 (8.5)	67.1 (67.6)	3.8 (4.2)	11.7 (12.1)	692	145	4.82
$[ZnC_{39}H_{29}N_6OCl]$	62	Yellow	9.0 (9.3)	66.7 (67.1)	3.9 (4.1)	11.8 (12.0)	698	138	Diamagnetic
$[MnC_{39}H_{29}N_6OCl]$	60	Dark yellow	7.6 (7.9)	67.8 (68.0)	3.6 (4.2)	11.6 (12.2)	688	146	5.75

[a] The unit of the molar conductance is Ω^{-1} mol^{-1} cm^2.

Table 2
Electronic absorption spectral data of the synthesized compounds at 300 K.

S. no	Compound	Solvent	Absorption (cm^{-1})	Band assignment	Geometry
1	L	DMSO	33,783	INCT	–
			26,737	INCT	
2	$[CuL(phen)_2]Cl_2$	DMSO	15,243	$^2E_g \rightarrow {}^2T_{2g}$	Distorted octahedral
			37,037	INCT	
			33,898	INCT	
3	$[CoL(phen)_2]Cl_2$	DMSO	14,705	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$,	Octahedral
			15,267	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$,	
			30,581	$^4T_1g(F) \rightarrow {}^4T_{2g}(P)$	
4	$[NiL(phen)_2]Cl_2$	DMSO	10,695	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$,	Octahedral
			11,049	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$,	
			25,575	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	
5	$[MnL(phen)_2]Cl_2$	DMSO	15,822	$^6A_{1g} \rightarrow {}^4T_{1g}$,	Octahedral
			23,980	$^6A_{1g} \rightarrow {}^4T_{2g}(G)$,	
			25,510	$^6A_{1g} \rightarrow {}^4E_g$	
			28,089	$^6A_{1g} \rightarrow {}^4T_{2g}(D)$	

Table 3
IR data of Schiff base ligand and its metal complexes.

Compound	υ_{N-H}	$\upsilon_{C=O}$	$\upsilon_{HC=N}$	υ_{M-N}	υ_{M-O}
$[C_{15}H_{13}ON_2Cl]$	3061	1662	1625	–	–
$[CuC_{39}H_{29}N_6OCl]$	3059	1647	1604	420	518
$[NiC_{39}H_{29}N_6OCl]$	3058	1656	1610	424	544
$[CoC_{39}H_{29}N_6OCl]$	3057	1651	1618	418	520
$[ZnC_{39}H_{29}N_6OCl]$	3055	1654	1616	422	520
$[MnC_{39}H_{29}N_6OCl]$	3056	1652	1609	416	524

3–10 cm^{-1} for all complexes) indicating coordination of the heterocyclic nitrogen. Absorptions at 516–544 and 415–424 cm^{-1} were ascribed to the formation of M—O and M—N bonds, respectively [34].

DNA binding of the metal complexes

Absorption spectral features of DNA binding

Titration with electronic absorption spectroscopy is an effective method to investigate the binding mode of DNA with metal complexes [35]. Transition metal complexes can bind to DNA *via* both covalent and/or non-covalent interactions. In the case of covalent binding, the labile ligand of the complexes can be replaced by a nitrogen base of DNA such as guanine N7, while the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of metal complexes outside of DNA helix, along major or minor groove. Generally, the binding of an intercalative molecule to DNA is accompanied by hypochromism and/or significant red shift (bathochromism) in the absorption spectra due to the strong stacking interaction between the aromatic chromophore of the ligand and DNA base pairs with the extent of hypochromism and red-shift commonly consistent with the strength of the intercalative interaction [36]. Therefore, in order to obtain evidence for the binding ability of each compound to CT DNA, spectroscopic titration of compound solutions with CT DNA should be performed [37]. The electronic absorption spectra of copper complex in the absence and presence of CT DNA are given in Fig. 1. In the UV region, the intense absorption bands observed in the metal complexes are attributed to the intra ligand π–π^* transition of the coordinated groups. With increasing concentration of CT DNA, for the Co(II), Ni(II), Cu(II) and Mn(II) complexes, the absorption bands at 327, 269, 270 and 264 nm respectively also show hypochromism of 21%, 16%, 32% and 11%, respectively. The hypochromisms observed for all the metal complexes are accompanied by small red shifts by about 2, 3, 1.5 and 2 nm respectively. The spectroscopic changes suggest that the intrinsic binding constants (K_b) of the Co(II), Ni(II) Cu(II) and Mn(II) complexes are $1.9 \times 10^5\ M^{-1}$, $4.2 \times 10^5\ M^{-1}$, $1.4 \times 10^6\ M^{-1}$ and $6.4 \times 10^4\ M^{-1}$, respectively (shown in Table 4). The results indicate that the binding strength of the complexes is decreased in the order of Cu(II) > Ni(II) > Co(II) > Mn(II). The higher values of Cu(II), Ni(II) and Co(II) complexes and lower value of Mn(II) complex are compared with that of the reported for classical intercalators (for ethidium bromide in 25 mM Tris–HCl/40 mM NaCl buffer, pH = 7.2 and [Ru(phen)(dppz)], the binding constants have been found to be in the order of 10^6–$10^7\ M^{-1}$) [38,39]. Especially, Cu(II), Ni(II) and Co(II) complexes have strong DNA binding ability as compared

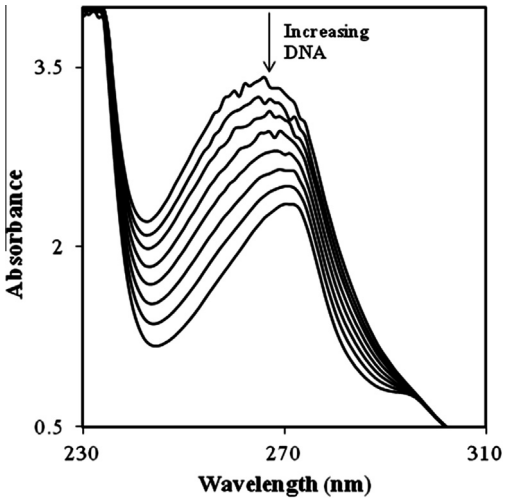


Fig. 1. Absorption spectrum of $[CuC_{39}H_{29}N_6OCl]$ complex in buffer pH = 7.2 at 25 °C in the presence of increasing amount of DNA. Arrow indicates the changes in absorbance upon increasing the DNA concentration.

Table 4
Electronic absorption spectral properties of Cu(II), Ni(II), Co(II) and Mn(II) complexes with DNA.

Compound	λ max		$\Delta\lambda$/nm	[a]H%	[b]K_b (M^{-1})
	Free	Bound			
$[CuC_{39}H_{29}N_6OCl]$	270	265.4	4.6	32	1.4×10^6
$[NiC_{39}H_{29}N_6OCl]$	269	266.2	2.8	16	4.2×10^5
$[CoC_{39}H_{29}N_6OCl]$	327	319.0	8.0	21	1.9×10^5
$[MnC_{39}H_{29}N_6OCl]$	264	261.6	2.4	11	6.4×10^4

[a] $H\% = [(A_{free} - A_{bound})/A_{free}] \times 100\%$.
[b] K_b = Intrinsic DNA binding constant determined from the UV–Vis absorption spectral titration.

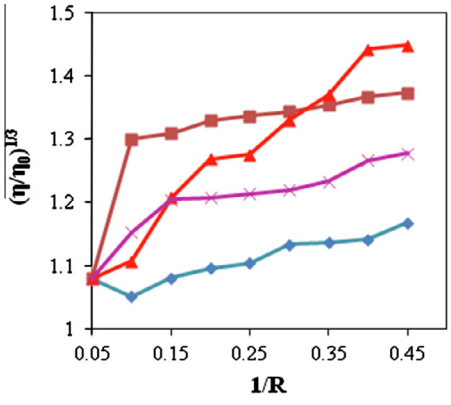


Fig. 2. Effect of increasing amounts of [$CuC_{39}H_{29}N_6OCl$] (■), [$NiC_{39}H_{29}N_6OCl$] (▲), [$CoC_{39}H_{29}N_6OCl$] (×) [$MnC_{39}H_{29}N_6OCl$] (♦) on the relative viscosity of CT DNA vs [complex]/[DNA] (1/*R*) ratio.

Table 5
Electrochemical parameters for the interaction of DNA with Cu(II), Ni(II), Co(II) and Mn(II) complexes.

Compound	[a]$E_{1/2}$ (V)		[b]ΔEp(V)		Ipc/Ipa	K[red]/K[oxd]
	Free	Bound	Free	Bound		
[$CuC_{39}H_{29}N_6OCl$]	0.309	0.323	0.601	0.646	1.48	1.85
[$NiC_{39}H_{29}N_6OCl$]	−0.682	−0.694	0.282	0.253	1.43	0.85
[$CoC_{39}H_{29}N_6OCl$]	−0.685	−0.669	0.193	0.237	1.42	0.86
[$MnC_{39}H_{29}N_6OCl$]	−0.677	−0.686	0.190	0.192	1.38	0.60

Data from cyclic voltammetric measurements.
[a] $E_{1/2}$(V) is calculated as average of anodic (E_{Pa}) and (Epc) peak potential $E_{1/2} = E_{Pa} + Epc/2$.
[b] $\Delta Ep(V) = E_{Pa} - Epc$.

with the other complex Mn(II). The electronic absorption spectra of cobalt and nickel complexes in the absence and presence of CT DNA are given in supplementary files (Fig. S1, Fig. S2). These results suggest an intimate association of the compounds with CT-DNA and it is also likely that compounds bind to the helix via intercalative mode. Hypochromism has been suggested for the interaction between the electronic state of the intercalating chromophore and that of the DNA bases [40–44].

Viscosity measurements

To know the nature of DNA binding of the mixed ligand complexes, viscosity measurements are carried out on CT DNA by varying the concentration of the added complexes. The values of changes in the relative specific viscosities of CT DNA in presence and absence of the complex respectively are plotted against 1/*R*, [complex]/[DNA]. A classical intercalation mode causes a significant increase in viscosity of DNA due to an increase in separation of base pairs at intercalation sites and hence an increase in overall DNA length. The results indicate that the presence of the metal complex increases the viscosity of the DNA solution, as illustrated in Fig. 2. As general rule metal complexes can increase the viscosity of DNA when they intercalate into the double-stranded DNA (or) bind to the phosphate group of DNA backbone [45]. The plots show that the relative viscosity increases with increase in concentration of complexes. The observed order of DNA-binding in viscosity measurements suggests that metal complexes bind DNA with a moderate intercalative mode.

DNA binding of electrochemical behavior

The application of cyclic voltammetry (CV) to the study of binding of metal complexes to DNA provides a useful complement to the method for the investigation of electronic absorption spectra

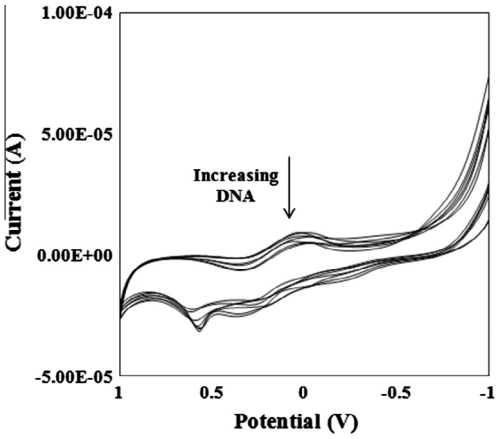


Fig. 3. Cyclic voltammogram of [$CuC_{39}H_{29}N_6OCl$] in buffer (pH = 7.2) at 25 °C in the presence of increasing amount of DNA. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

[46]. As further exploring the binding of the present complexes with DNA, cyclic and differential pulse voltammetric studies were carried out both in the presence and absence of DNA. The incremental addition of CT DNA to the complex causes decrease of anodic and cathodic peak current of the complex. This result shows that complex stabilizes the duplex (GC pairs) by intercalating way. The incremental addition of CT DNA to the complex causes shift in the potential of peak in cyclic voltammogram. Both the cathodic and anodic peaks show positive shifts which indicate intercalation of complex to DNA base pairs. Electrochemical parameters for the interaction of DNA with Cu(II), Ni(II), Co(II) and Mn(II) complexes are shown in Table 5.

The cyclic voltammogram of [$CuC_{39}H_{29}N_6OCl$] complex in the absence and in presence of varying amount of DNA is shown in Fig. 3. In the absence of CT DNA, the redox cathodic peak appeared at 0.0077 V for Cu-complex [ΔE_p = 0.601 V and $E_{1/2}$ = 0.309 V]. The ratio of ipc/ipa is approximately 1.48 which indicates that the reaction of the complex on the glassy carbon electrode surface is quasi-reversible redox process. The incremental addition of CT-DNA to the complex causes a positive shift in potential and a decrease in the current intensity. Among the three kinds of binding modes for small molecules to DNA, Bard has reported [47] that if $E_{1/2}$ is shifted to more negative value when small molecules interact with DNA, then the interaction mode is electrostatic binding. On the contrary, if $E_{1/2}$ is shifted to more positive value, the interaction mode is

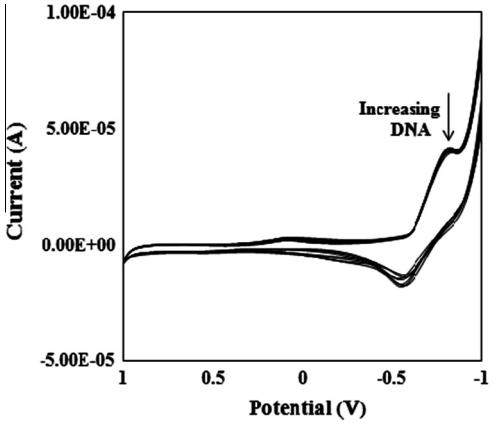


Fig. 4. Cyclic voltammogram of [$NiC_{39}H_{29}N_6OCl$] in buffer (pH = 7.2) at 25 °C in the presence of increasing amount of DNA. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

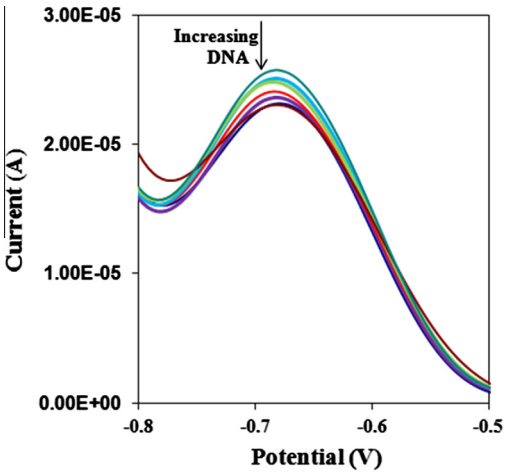


Fig. 5. Differential pulse voltammograms of $[MnC_{39}H_{29}N_6OCl]$ in buffer (pH = 7.2) at 25 °C in the presence of increasing amount of DNA. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

intercalative binding. Hence, it is concluded that the $[CuC_{39}H_{29}N_6OCl]$ complex could bind to DNA by intercalative mode.

For the CV behavior of $[NiC_{39}H_{29}N_6OCl]$ in the absence of DNA, the redox couple cathodic peak appeared at −0.824 V for Ni-complex (ΔEp = 0.282 V and $E_{1/2}$ = −0.682 V). Ip_c/Ip_a ratio of redox couple is approximately 1.43. This indicates that the reaction of the complex on the working electrode surface is quasi-reversible redox process. The results are similar to the above spectroscopic and viscosity data of the complexes in the presence of DNA. The cyclic voltammogram of $[NiC_{39}H_{29}N_6OCl]$ complex in the absence and in presence of varying amount of DNA is shown in Fig. 4.

For the CV behavior of $[CoC_{39}H_{29}N_6OCl]$ in the absence of DNA, the redox couple cathodic peak appeared at −0.781 V for Co-complex (ΔEp = 0.193 V and $E_{1/2}$ = −0.685 V). The ratio of ip_c/ip_a is approximately 1.42. It indicates that the reaction of the complex on the glassy carbon electrode surface is quasi-reversible redox process. The cyclic voltammogram of $[CoC_{39}H_{29}N_6OCl]$ complex in the absence and in presence of varying amount of DNA is shown in Supplementary file (Fig. S3).

For the CV behavior of $[MnC_{39}H_{29}N_6OCl]$ in the absence of DNA, the redox couple cathodic peak appeared at −0.783 V for Mn-complex (ΔEp = 0.190 V and $E_{1/2}$ = −0.677 V). The ratio of ip_c/ip_a is approximately 1.38. It indicates that the reaction of the complex on the glassy carbon electrode surface is quasi-reversible redox process. The cyclic voltammogram of $[MnC_{39}H_{29}N_6OCl]$ complex in the absence and in presence of varying amount of DNA is shown in Supplementary file (Fig. S4). From these data it is understood that all the synthesized complexes interact with DNA through intercalating way.

In differential pulse voltammogram of the complex, $[MnC_{39}H_{29}N_6OCl]$ in the absence and presence of varying amount of [DNA] with significant decrease of current intensity (Fig. 5), the shift in potential is related to the ratio of binding constant by the following equation:

$$E_b - E_f = 0.0591 \log(K_{[red]}/K_{[oxd]})$$

where E_b and E_f are peak potentials of the complex in the bound and free form respectively. All other complexes show considerable shift in both cathodic and anodic peak potentials in the presence of incremental addition of CT DNA. It is found that our synthesized complexes give both the anodic and cathodic peak potential shifts towards positive direction, i.e. positive (in copper complex) or from more negative region to less negative region (in other complexes). It indicates the intercalating mode of DNA binding with phen mixed ligand Schiff base complexes.

DNA cleavage study

DNA cleavage can be achieved by targeting its basic components like phosphodiester linkages, deoxyribose sugar or nucleobases. Transition metal complexes of polypyridyl ligands are known to cleave DNA under irradiation by UV or visible light. Gel electrophoresis experiments using pUC19 plasmid DNA were performed with free ligand and its complexes in the presence and absence of H_2O_2 as an oxidant. When super coiled DNA is conducted by electrophoresis, faster migration will be observed for DNA of closed circular confirmation (Form I). If one strand is cleaved, the supercoiled DNA will relax to produce a slower moving nicked circular form (Form II). If both strands are cleaved, a linear confirmation (Form III) that migrates between Form I and Form II will be generated [48]. This method using supercoiled pUC19 DNA in the presence of the ligand and its metal complexes was carried out in a medium of 50 μM Tris–HCl/NaCl buffer (pH 7.2). From figure (Fig. 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. 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 did not show any significant cleavage of pUC19 DNA even on longer exposure time. From the observed results, it is concluded that all the complexes effectively cleave the DNA as compared to control DNA.

Antimicrobial studies

The antibacterial activity of the Schiff base ligand, its metal complexes and Streptomycin (as a standard compound) were tested against bacteria. The organisms used in the present investigations included *S. aureus, P. aeruginosa, E. coli, S. epidermidis, K. pneumoniae*. The diffusion agar method was used to evaluate

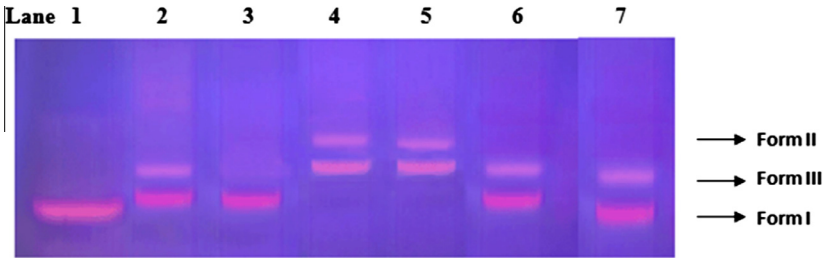


Fig. 6. The gel electrophoretic separation of plasmid pUC19 DNA treated with $[M(L)(phen)_2]Cl_2$ complexes. Lane 1; DNA control ; Lane 2: DNA + ligand + H_2O_2; Lane 3: DNA + $[Co(L)(phen)_2]Cl_2$; Lane 4: DNA + $[Cu(L)(phen)_2]Cl_2$ + H_2O_2; Lane 5: DNA + $[Zn(L)(phen)_2]Cl_2$ + H_2O_2; Lane 6: DNA + $[Ni(L)(phen)_2]Cl_2$ + H_2O_2; Lane 7: DNA + $[Mn(L)(phen)_2]Cl_2$ + H_2O_2.

Table 6
The *in vitro* antibacterial activity of Schiff base and its metal complexes (zone of inhibition in mm).

Compound	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. epidermidis*	*K. pneumoniae*
$[C_{15}H_{13}ON_2Cl]$	++	+	++	+	++
$[CuC_{39}H_{29}N_6OCl]$	++++	+++	+++	+++	++++
$[NiC_{39}H_{29}N_6OCl]$	++++	+++	+++	++	+++
$[CoC_{39}H_{29}N_6OCl]$	+++	++	++++	+++	++
$[MnC_{39}H_{29}N_6OCl]$	+++	++	+++	++	++++
Streptomycin	++	+++	+++	++	++
DMSO	–	–	–	–	–

Streptomycin is used as the standard.
DMSO was used as antimicrobial inert solvent.
+++++ = Excellent activity (100% inhibition).
+++ = Good activity (60–70% inhibition).
++ = Moderate activity (30–50% inhibition).
+ = Less activity (10–20% inhibition).

the antibacterial activity of the synthesized metal complexes. The results of the bactericidal study of the synthesized compounds are displayed in Table 6. All the metal complexes were found to have higher antibacterial activity than Schiff base ligand. The DMSO control showed no activity against any bacterial strain. Such increased activity of the complexes can be explained on the basis of the Overtone's concept [49] and Tweedy's chelation theory [50]. According to the Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid-soluble materials due to which liposolubility is an important factor that controls the antimicrobial activity.

Conclusion

In this paper, a novel N-(4-aminophenyl)acetamide derived Schiff base and its Cu(II), Ni(II), Co(II), Mn(II) and Zn(II) complexes have been synthesized and characterized by spectral and analytical data. Binding of these complexes to CT-DNA has been investigated in detail by electronic absorbance titrations, viscosity, cyclic voltammetry and differential pulse voltammogram analysis. The binding order is found to be in the following order Cu(II) > Ni(II) > Co(II) > Mn(II). In addition, all of these metal complexes are found to promote the photocleavage of pUC19 DNA. All the metal complexes are found to have higher antibacterial activity than Schiff base ligand.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.04.054.

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