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Inspired research on the DNA binding ability of 4-aminoantiprine derived mixed ligand complexes

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ABSTRACT

A novel 4-aminoantipyrine derived Schiff base and its four mixed ligand complexes have been synthesized and characterized. The binding properties of metal complexes with DNA have been investigated by electronic absorption spectra and viscosity measurements showing that the complexes have the ability of interaction with DNA by intercalative mode. The effect of the metal complexes on DNA was carried out by pUC19 DNA agarose gel electrophoresis at 50 V for 2 h. The damage effect of the added ascorbic acid into the medium is dependent on the free radicals produced from oxidation of ascorbic acid by molecular oxygen and this damage is considered to be reduced by the metal complexes.

Transition metals have an important place within medicinal biochemistry. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases like carcinomas, lymphomas, infection control, anti-inflammatory, diabetes, and neurological disorders [1]. Among them, binding of small molecules to DNA would be invaluable in the rational design of sequence specific DNA binding molecules for application in chemotherapy and in the development of tools for biotechnology [2]. During several years, tremendous interest has been aroused to explore the potential applications of metal complexes as non-radioactive probes of nucleic acid structure and as possible DNA cleaving agents [3].

In recent years, 4-aminoantipyrine transition metal complexes and their derivatives have been extensively examined due to their wide applications in various fields like biological, analytical and therapeutical. Further, they have been investigated due to their diverse biological properties as antifungal, antibacterial, analgesic, sedative, antipyretic, anti-inflammatory agents [4] and DNA binding properties [5]. However, to the best of our knowledge no attention was paid on the interaction of DNA and Schiff base and its mixed ligand metal complexes derived from 2-hydroxy benzilidene 4-aminoantipyrine and alanine (Scheme 1). In this communication we describe the synthesis, DNA binding and cleavage abilities of a novel series of 4-aminoantipyrine based Schiff base and its mixed ligand complexes with various metal ions.

The Schiff base ligand and its mixed ligand complexes were prepared by a typical procedure [6–13]. We got the likely composition of complexes, [ML(phen)] where phen = phenanthroline, through elemental analyses, magnetic susceptibility, ^{1}H NMR [The ^{1}H NMR spectra of the ligand and its [ZnL(phen)] complex are given in Fig. S1 and Fig. S2 (Supplementary data)], IR, Mass, UV spectra and molar conductivity measurements since no single crystals suitable for X-ray determination could be isolated. All the instrumental details are given in Supplementary data.

The mass spectrum of Schiff base ligand showed the molecular ion peak at m/z 416 [M + 1] (91%) abundance corresponding to $[C_{21}H_{20}N_4O_3K]$ ion. Also the spectrum exhibited peaks for the fragments at m/z 323, 199, 93 and 77 corresponding to $[C_{15}H_{15}N_4O_2K]$ [M + 1], $[C_{11}H_{11}N_4]$ [M+], $[C_6H_5O]$ [M+] and $[C_6H_5]$ [M+] with 0.5%, 0.9%, 0.4% and 0.3% abundances respectively. The spectra of Cu(II), Ni(II), Co(II), and Zn(II) complexes showed molecular ion peaks at m/z 619.34 [M+], 615.53 [M + 1], 614.33 [M+] and 621.20 [M+] with 96%, 100%, 40% and 69% abundances respectively that are equivalent to their molecular weights. The Cu(II) complex gave a fragment ion peak with loss of metal and 1,10 phenanthroline m/z 376. The m/z of all the fragments of ligand and its complexes confirm the stoichiometry of the complexes as [ML(phen)]. It was further supported by the mass spectra of all the complexes. The observed peaks were in good agreement with their formulae as expressed from microanalytical data. The ESI–MS spectra of the ligand and its [CuL(phen)] complex are given in Fig. S3 and Fig. S4 (Supplementary data).

The binding behavior of the metal complexes to DNA helix is often investigated using absorption spectral titration, followed by the changes in absorbance and shift in wavelength. With increasing concentration of CT-DNA, the absorption bands of the complexes were affected, resulting in the tendency of hypochromism and a slight red shift was observed in all the complexes due to the intercalative binding between DNA and metal complexes. Hyperchromic and hypochromic

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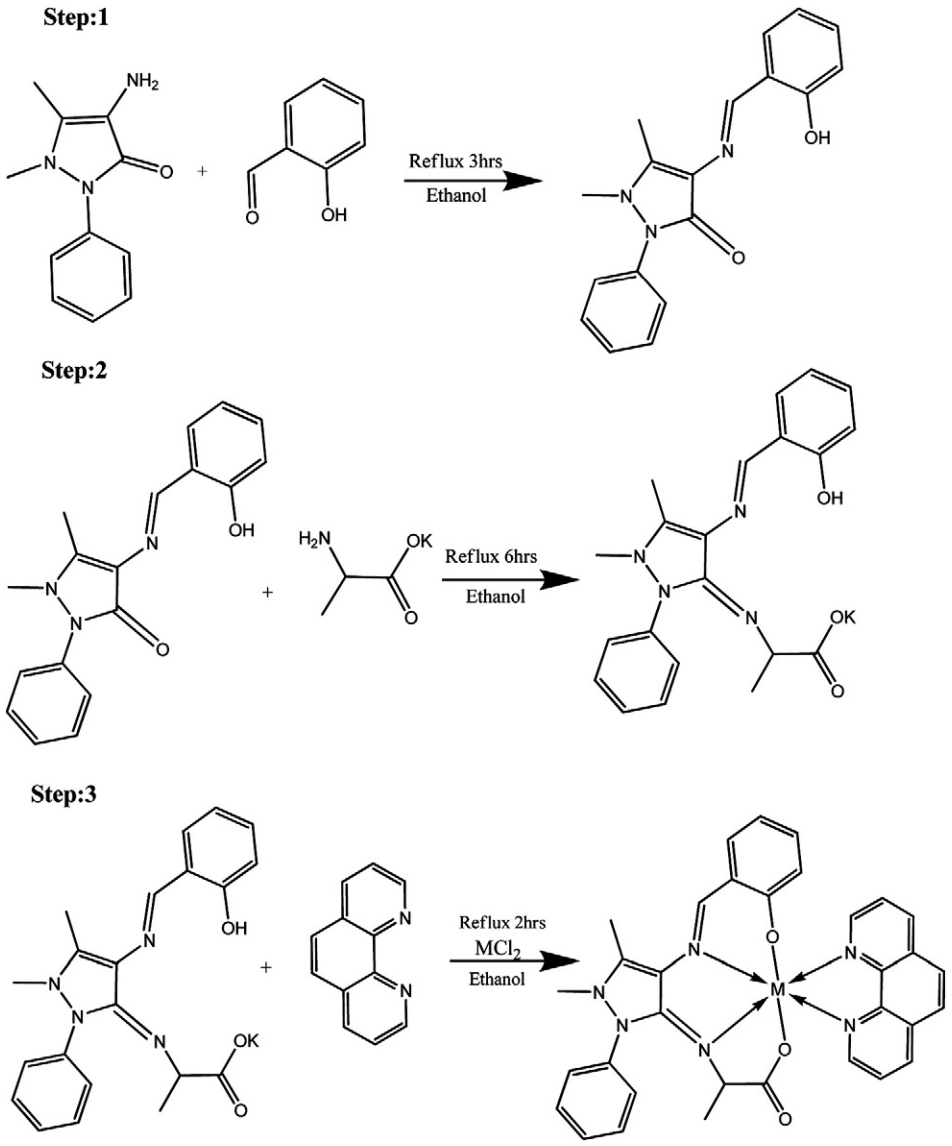

Scheme 1. Schematic route for the synthesis of Schiff base ligand and its metal complexes.

effects are the spectral features of DNA concerning its double helix structure. This spectral change showed that the change of DNA in its conformation and structure after the complex bound to DNA. Hypochromism results from the contraction of DNA in the helix axis, as well as from the change in conformation on DNA, while hyperchromism results from the damage of the DNA double helix structure [14]. The absorption spectra of DNA in the absence and presence of copper(II) and nickel(II) complexes are given in Figs. 1 and 2 respectively.

The absorption spectra of the four metal(II) complexes have been characterized by metal-to-ligand charge transfer (MLCT) transition in the visible region. The absorption spectra of complexes exhibit lowest energy bands at 428.5 nm for copper, 402.3 nm for nickel, 420.6 nm for cobalt and 404.5 for zinc which are assigned to the metal-to-ligand charge transfer (MLCT) transition. All the metal complexes showed decrease in absorption intensity (hypochromism) with a slight red shift which is due to the intercalative binding between DNA and metal complexes.

The nature of binding of the complexes to the CT-DNA was further investigated by viscometric studies. The relative specific viscosity of DNA was determined by varying the concentration of the added metal complexes. Measuring the viscosity of DNA is a classical technique used to analyze the DNA binding mode in solution. In the

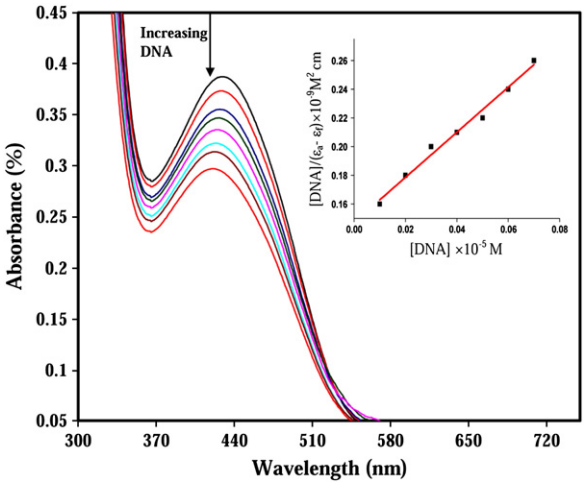

Fig. 1. Absorption spectral changes on addition of CT DNA to the solution of [CuL(phen)] (20 μM) in buffer pH = 7.2 at 25 C in the presence of increasing amount of DNA (0–140 μM). Arrow indicates the changes in absorbance upon increasing the DNA concentration. Inset: plot of $[DNA]/(\varepsilon_a - \varepsilon_f) \times 10^{-9}$ M^2 cm *versus* $[DNA] \times 10^{-5}$ M.

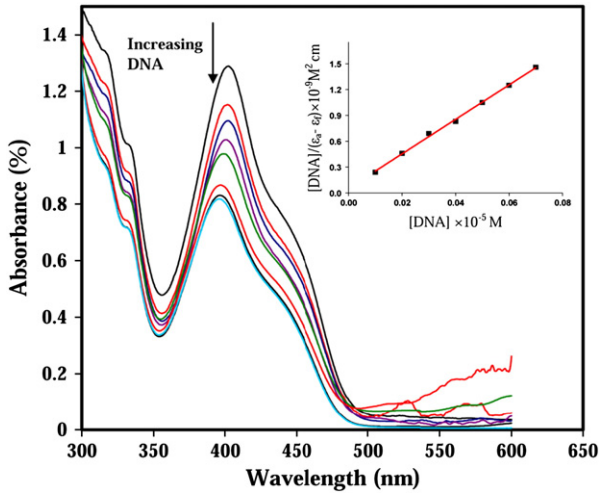

Fig. 2. Absorption spectral changes on addition of CT DNA to the solution of [NiL(phen)] (20 μM) in buffer pH = 7.2 at 25 C in the presence of increasing amount of DNA (0–140 μM). Arrow indicates the changes in absorbance upon increasing the DNA concentration. Inset: plot of $[DNA]/(\varepsilon_a - \varepsilon_f) \times 10^{-9}\ M^2$ cm *versus* $[DNA] \times 10^{-5}$ M.

absence of crystallographic structural data, hydrodynamic methods that are sensitive to DNA length change are regarded as the least ambiguous and the most critical tests of binding in solution. A classical intercalation model results in the lengthening of the DNA helix as the base pairs are separated to accommodate the binding molecule, leading to an increase in the DNA viscosity. However, a partial and/or non-classical intercalation of ligand may bend (or kink) DNA helix, resulting in the decrease of its effective length and concomitantly its viscosity [15]. The plots of $(\eta/\eta o)^{1/3}$ *vs.* [Complex]/[DNA] = R (where η and ηo are the relative viscosities of DNA in the presence and absence of complex respectively) give a measure of the viscosity changes. The effects of all the complexes on the viscosity of CT DNA are shown in Fig. 3.

A significant increase in the viscosity of DNA on addition of complex results due to the intercalation which leads to the separation among the DNA bases to the increase in the effective size in DNA which could be the reason for the increase in the viscosity [16].

Gel retardation assay is a useful tool for identifying metal complexes which interact with DNA and mediate cell functions such as gene expression, DNA repair and DNA packaging. DNA cleavage is

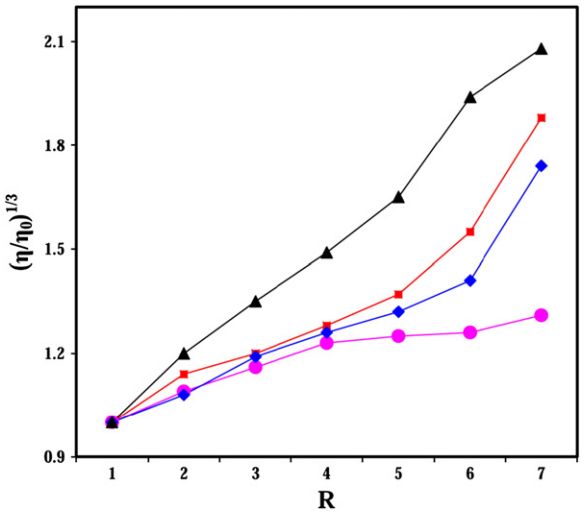

Fig. 3. Plot of relative viscosity $(\eta/\eta o)^{1/3}$ *vs.* [Complex]/[DNA] = R. Effect of increasing amounts of [CuL(phen)] (▲), [NiL(phen)](■), [ZnL(phen)] (♦), and [ZnL(phen)] (•), on the viscosity of DNA. [Complex] = 0–350 μM, [DNA] = 50 μM.

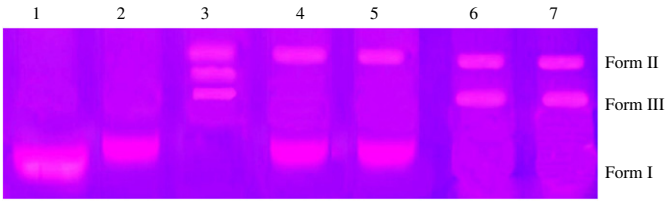

Fig. 4. Gel electrophoresis diagram showing the cleavage of pUC19 DNA (10 μM) by Cu(II), Ni(II) and Zn(II) complexes (30 μM) in a buffer containing 50 mM Tris–HCl and 50 mM NaCl in the presence of ascorbic acid (AH_2, 10 μM) at 37 °C. Lane 1, DNA control; lane 2, DNA + AH_2; lane 3, DNA + [ligand(L)] + AH_2; lane 4, DNA + AH_2 + [CuL(phen)]; lane 5, DNA + AH_2 + [NiL(phen)]; lane 6, DNA + AH_2 + [CoL(phen)]; lane 7, DNA + AH_2 + [ZnL(phen)].

controlled by relaxation of super coiled circular form of pUC19 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slowly moving open circular from (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between [17,18]. The ability of the complexes in affecting DNA cleavage has been investigated by gel electrophoresis using super coiled pUC19 DNA in 5 mM Tris–HCl/50 mM NaCl buffer solution (pH 7.2). All the complexes are found to exhibit nuclease activity. Fig. 4 shows the result of gel electrophoretic separations of plasmid pUC19 DNA induced by an addition of metal(II) complexes in the presence of AH_2 (ascorbic acid). Under the same conditions, free AH_2 produced no cleavage of pUC19 DNA. When pUC19 DNA was allowed to interact with mixture of metal complexes and in presence of ascorbic acid, the mobility of the band was found to increase slightly as shown in Fig. 4. These phenomena imply that Cu(II), Co(II), Ni(II) and Zn(II) complexes damage more to plasmid pUC19 DNA in the presence of AH_2.

In summary, a series of the mixed ligand metal complexes with Schiff base ligand derived from 2-hydroxy-benzylidene-4-aminoantipyrine and alanine was synthesized and characterized. The binding behavior of metal complexes with DNA was studied by UV spectra, viscosity and gel retardation assay under physiological conditions. All the experimental evidences indicate that these four complexes can strongly bind to CT DNA *via* an intercalation mechanism. All the metal complexes show enhanced DNA cleavage activity of pUC19 DNA in the presence of ascorbic acid. The results show that mixed ligand metal complexes exhibit more damage to pUC19 DNA in presence of ascorbic acid. Results obtained from our present work would be very useful to understand the mechanism of interactions of the small molecules binding to DNA and helpful in the development of their potential applications in biological, pharmaceutical and physiological fields in future.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.inoche.2011.12.029.

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[5] N. Raman, S. Sobha, A. Thamaraichelvan, A novel bioactive tyramine derived Schiff base and its transition metal complexes as selective DNA binding agents, Spectrochim. Acta A 78 (2011) 888–898.
[6] An ethanolic solution of (40 mL) 4-aminoantipyrine (0.02 mol) was added to an ethanolic solution of 2-hydroxybenzaldehyde (0.02 mol). The resultant mixture was refluxed for *ca.* 3 h. The solid product formed (2-hydroxy-benzylidene-4-aminoantipyrine) was filtered and recrystallized from ethanol.
[7] The potassium salt of alanine was prepared by the following general procedure. The alanine (0.01 mol) dissolved in 1:1 water–ethanol (40 mL) was added to a hot ethanolic solution (30 mL) of KOH (0.01 mol) and the resulting solution was stirred to obtain a homogeneous solution. Then, to this solution was added drop-wise an ethanolic solution of 2-hydroxy-benzylidene-4-aminoantipyrine (0.01 mol) and the resultant mixture was refluxed for *ca.* 6 h. The dark yellow colored crystalline product (Schiff base, L) formed was filtered and recrystallized from ethanol. Yield: 75%.
[8] Anal.Calc. for $C_{21}H_{20}N_4O_3K$: C, 60.7; H, 4.8; N, 13.4%. Found: C, 60.2; H, 4.7; N, 13.1%. FT-IR (KBr), cm^{-1}: υ(OH): 3480, υ(C N): 1653, υ(HC N): 1614, υ_{asy} $(COO)^-$: 1549, υ_{sy} $(COO)^-$: 1447, ^1H NMR ($CDCl_3$, 300 MHz, δ, ppm): 6.91–7.46 (m, 9H, Ar–H), 9.80 (s, 1H, –HC N), 5.42 (s, 1H, –OH), 2.40 (s, 3H, Ar–C–CH_3), 2.18 (s, 3H, Ar–N–CH_3), 3.69 (d, 3H, alanine–CH_3) 1.24 (q, 1H, alanine–CH). λ_{max} in (DMSO), cm^{-1}: 37,878, 36,363 and 31,250 cm^{-1}. ESI MS: m/z 416(91%) [M+1].
[9] A solution of metal(II) chloride in ethanol (2 mmol) was stirred with an ethanolic solution of the Schiff base (2 mmol), for 30 min on a magnetic stirrer at room temperature. To the above stirring solution about (2 mmol) mol of 1, 10 phenanthroline in the ethanolic solution was added and refluxed for *ca.* 2 h. The resultant solid product was washed and recrystallized from ethanol. Yield: 82–79%.
[10] Anal.Calc. for $C_{33}H_{27}N_6O_3Cu$: C, 64.0; H, 4.4; N, 13.5; Cu, 10.2%. Found: C, 63.7; H, 4.3; N, 13.1; Cu, 9.8%. FT-IR (KBr), cm^{-1}: υ(C N): 1604, υ(HC N): 1564, υ_{asy} $(COO)^-$: 1442, υ_{sy} $(COO)^-$: 1317, υ(M O): 510, υ(M N): 425. Molar conductance × 10^{-3}, (S cm^2 mol^{-1}): 13.75; μ_{eff} (BM):1.73. λ_{max} in (DMSO), cm^{-1}: 43,478, 37,593 and 13,568 cm^{-1}. ESI MS: m/z 619.34 (96%) [M+].
[11] Anal.Calc. For $C_{33}H_{27}N_6O_3Ni$: C, 64.5; H, 4.4; N, 13.6; Ni, 9.5%. Found: C, 64.1; H, 4.3; N, 13.3; Ni, 9. 2%. FT-IR (KBr), cm^{-1}: υ(C N): 1635, υ(HC N): 1606, υ_{asy} $(COO)^-$: 1460, υ_{sy} $(COO)^-$: 1323, υ(M O): 520, υ(M N): 420; Molar conductance × 10^{-3}, (S cm^2 mol^{-1}): 15.60. μ_{eff} (BM): 3.15. λ_{max} in (DMSO), cm^{-1}: 14,453, 16,735 and 22,201 cm^{-1}. ESI MS: m/z 615.53 (100%) [M+1].
[12] Anal.Calc. for $C_{33}H_{27}N_6O_3Co$: C, 64.4; H, 4.4; N, 13.6; Co, 9.5%. Found: C, 64.2; H, 4.4; N, 13.1; Co, 9.1%. FT-IR (KBr), cm^{-1}: υ(C N): 1636, υ(HC N): 1599, υ_{asy} $(COO)^-$: 1444, υ_{sy} $(COO)^-$: 1317, υ(M O): 486, υ(M N): 414, Molar conductance × 10^{-3}, (S cm^2 mol^{-1}): 21.83; μ_{eff} (BM): 4.72. λ_{max} in (DMSO), cm^{-1}: 14,070, 16,738 and 21,147 cm^{-1}. ESI MS: m/z 614.33 (40%) [M+].
[13] Anal.Calc. for $C_{33}H_{27}N_6O_3Zn$: C, 63.8; H, 4.3; N, 13.5; Zn, 10.5%. Found: C, 63.7; H, 4.3; N, 13.1; Zn, 10.2%. FT-IR (KBr), cm^{-1}: υ(C N):1604, υ(HC N): 1564, υ_{asy} $(COO)^-$: 1442, υ_{sy} $(COO)^-$: 1317, υ(M O): 510, υ(M N): 420. ($CDCl_3$, 300 MHz, δ, ppm): 6.91–7.03 (m, 9H, Ar–H), 7.26–7.58. (m, 8H, (phen) Ar–H), 8.61 (s, 1H, –HC N), 2.20 (s, 3H, Ar–C–CH_3), 1.80 (s, 3H, Ar–N–CH_3), 2.62 (d, 3H, alanine–CH_3) 1.64 (q, 1H, alanine–CH). Molar conductance × 10^{-3}, (S cm^2 mol^{-1}): 19.40. μ_{eff} (BM): 1.84. λ_{max} in (DMSO), cm^{-1}: 32,679, 35,842 and 37,878 cm^{-1}. ESI MS: m/z 621.2 (6 9%) [M+].
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