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# DNA cleavage efficiency of transition metal(II) schiff base complexes of 4-aminoantipyrine derivative by synthesis, characterization and antimicrobial study

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**Abstract**
A new bidentate Schiff base ligand derived from 4-aminoantipyrine and O-acetoacetotoluidide and its $Cu^{II}$, $Co^{II}$, $Ni^{II}$ and $Zn^{II}$ metal complexes have been synthesized and characterized by microanalytical data, IR, UV-Vis, 'I NMR and mass spectra. The conductance measurements indicate that all the complexes are electrolytes. The IR spectra indicate the coordination of azomethine nitrogen atom of the Schiff base and cyclic carbonyl group of the pyrazolone ring. The UV-Vis spectral data demonstrate that the complexes have square-planar geometry. The FAB mass spectra confirm the composition of the complexes of the type ML where M $Cu^{II}$, $Co^{II}$, $Ni^{II}$ and $Zn^{II}$. Their magnetic sus- ceptibility values provide evidence for the monomeric nature. The gel electrophoresis experiment has been carried out on the interaction of the complexes with CT-DNA. The nuclease activity of the above metal complexes shows that only the copper complex cleaves DNA in the presence of oxidant. Antimicrobial activities of the compounds are tested *in vitro* against four bacteria and three fungi by the disc diffusion method. The MIC value against the growth of micro-organisms is much larger for metal chelates than the ligand.

**Keywords:** DNA cleavage, 4-aminoantipyrine, schiff base, transition metals

## Introduction
Metal complexes of Schiff bases have played a central role in the development of coordination chemistry. Schiff bases offer a versatile and flexible series of ligands capable to bind with various metal ions to give complexes with suitable properties for theoretical and/or practical applications. During the past two decades, considerable attention has been paid to the chemistry of the metal complexes of Schiff bases containing nitrogen and other donors [1]. This may be attributed to their stability, biological activity [2] and potential applications in many fields. The interaction of transition metal complexes with DNA is a significant area of research which has attracted much attention of both inorganic and biochemists, because biochemical studies have shown that they are related to the development of new DNA reagents for biotechnology and medicine [3]. Even though a lot of work has been done on the synthesis, characterization and antimicrobial activities of transition metal complexes of Schiff bases, the work on DNA interaction with such type of complexes is very less [3]. Hence, in continuation of the work [4], we report herein the synthesis of a new type bidentate ligand formed by the condensation of 4-aminoantipyrine and O-acetoacetotoluidide and its $Cu^{II}$, $Co^{II}$. $Ni^{II}$ and $Zn^{II}$ complexes. The interaction of these complexes with DNA and antimicrobial activities are also investigated.

## Materials and Methods
All the chemicals used were of A.R. grade. Anhydrous grade ethanol and DMSO were purified according to standard procedures. Micro analytical data of the compounds was recorded at Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow. The $^1H$ NMR spectra of the compounds were recorded at Indian Institute of Chemical Technology, Hyderabad using tetramethylsilane as internal standard. The FAB mass spectra of the samples were performed at the Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow (RSIC, CDRI). The FAB-mass spectrum of the complex was recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10 mA) as the FAB-gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzylalcohol (NBA) as the matrix. The IR spectra of the samples were obtained as KBr discs in the range 400-4000 $cm^{-1}$ on a Shimadzu spectrophotometer. The UV-Vis spectra of the samples in the range 200-1100 nm in DMSO were recorded on a Shimadzu UV-1601 spectrophotometer. ESR spectra of the paramagnetic complexes were recorded at room temperature and liquid nitrogen temperature as a corresponding glass in the X-band region on a Varian 112 at RSIC, IIT, Chennai, X-band spectrometer equipped with 100 kHz field modulation. Tetracyanoethylene (TCNE) was used as the standard. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature on powder sample of the complex. Copper sulphate was used as calibrant. The molar conductivity of the complex was measured on a Systronic Model-304 digital direct reading conductivity meter using DMSO as solvent. 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, $4_{260}/A_{280}$ of ca. 1.8-1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1: 100 dilutions. The molar absorption coefficient was taken as 6600 $M^{-1}cm^{-1}$. Stock solutions were kept at 4 °C and used after not more than 4 days. Doubly distilled $H_2O$ was used to prepare the buffer.

**Preparation of Schiff base**: An ethanolic solution (20 mL) of 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one (4-aminoantipyrine) (2.03 g, 0.01 M) and an ethanolic solution (20 mL) of o-acetoacetotoluidide (1.91 g, 0.01 M) was boiled under reflux for ca. 5 h. On cooling colourless solid, o-acetoacetotoluidido-4-aminoantipyrine was obtained. It was filtered and recrystallised from ethanol and dried in vacuo (m.p. 165 °C; yield 75%).

**Preparation of metal complexes:** The Schiff base (0.01 M) dissolved in hot ethanol (50 mL) was added to a hot ethanolic solution (25 m) of the metal salts (0.005 M). The mixture was boiled under reflux for ca. 3 h. The volume of the solution was reduced to one third and then cooled to 0 °C. The pasty mass obtained was treated with ether. The solid separated was filtered, washed with ether and dried in vacuo. Yield: 55-60%.

**Antimicrobial activity:** The *in vitro* biological screening effects of the investigated compounds were tested against the bacteria: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* by the well diffusion method, using agar nutrient as the medium. The antifungal activities of the compounds were evaluated by the well diffusion method against the fungi *viz*. *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola* cultured on potato dextrose agar as medium. The stock solution (10-2 M) was prepared by dissolving the compounds in DMSO and the solutions were serially diluted in order to find the MIC values. In a typical procedure [5], a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette 5 and the plate was incubated, 24 h for bacteria and 72 h for fungi at 35 °C. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted.

**Gel electrophoresis:** The gel electrophoresis experiments were performed by incubation at 35 °C for 2 h as follows: CT-DNA 30 µM, 50 µM each complex, 50 uM $H_2O_2$ in 50 mM tris-HCl buffer (pH = 7.2). The samples were electrophoresed for 2 h at 50 V on 1% agarose gel using tris-aceticacid-EDTA buffer, pH = 8.3. After electrophoresis, the gel was stained using 1 µg/cm³ ethidiumbromide (EB) and photographed under UV light.

**Results and Discussion**

The Schiff base ligand and its complexes are found to be air stable. The ligand is soluble in chloroform, DMF and DMSO but the complexes are soluble only in DMF and DMSO. The physical characterization. microanalytical, molar conductance and magnetic susceptibility data of the ligand and its complexes are given in Table-1. The analytical data of the complexes correspond well with the general formula $ML_2$ where M $Cu^{II}$, $Ni^{II}$. $Co^{II}$ and $Zn^{II}$. Their magnetic susceptibility values of the complexes at room temperature are consistent with square-planar geometry around the central metal ion, the high conductance of the chelates supports their electrolytic nature.

**Table 1:** Physical characterization, analytical, molar conductance and magnetic susceptibility data of the ligand and the complexes

| Compd. | Molecular formula | Colour | M.p. (°C) | Analysis (%): Found (Calcd.) | | | | Molar Conductance $A_m$ ($\Omega^{-1}cm^2mpl^{-1}$) | $\mu_{eff}$ (B.M.) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | C | H | N | | |
| Ligand | $C_{22}H_{24}N_4O_2$ | White | 165 | - | 70.2 (70.2) | 6.3 (6.4) | 14.7 (14.9) | - | - |
| $[CuL_2]Cl_2$ | $CuC_{44}H_{48}N_8O_4Cl_4$ | Brown | 180 | 7.1 (7.2) | 59.3 (59.6) | 5.2 (5.4) | 12.5 (12.6) | 30.6 | - |
| $[NiL_2]Cl_2$ | $NiC_{44}H_{48}N_8O_4Cl_4$ | Green | 270 | 6.5 (6.7) | 59.7 (59.9) | 5.4 (5.5) | 12.5 (12.7) | 32.4 | 1.74 |
| $[CoL_2]Cl_2$ | $CoC_{44}H_{48}N_8O_4Cl_4$ | Pink | 275 | 6.4 (6.7) | 59.7 (59.9) | 5.3 (5.5) | 12.6 (12.7) | 35.7 | 3.58 |
| $[ZnL_2]Cl_2$ | $ZnC_{44}H_{48}N_8O_4Cl_4$ | White | 280 | 7.2 (7.4) | 59.3 (59.4) | 5.3 (5.4) | 12.3 (12.6) | 33.8 | - |

The FAB mass spectra of the ligand and its complexes were recorded and compared for their stoichiometric composition. The molecular ion peak for the ligand observed at 376 m/z is supported by the "Nitrogen Rule", since the compound possesses four nitrogen atoms. For copper complex, the molecular ion peak appeared at 887 m/z confirms the stoichiometry of the metal complexes as being of the $ML_2$ type. It is also supported by the mass spectra of other complexes. This is further supported by the elemental analyses of the complexes.

The IR-spectra of the complexes clearly indicate the bonding association of the ligand with the metal ion. From IR-spectral data, it is evident that even though the ligand is having three donor sites, only two are involved in coordination with the metal ion, i.e. the ligand acts as a bidentate, bonded to metal ion through azomethine nitrogen atom and cyclic carbonyl group of the pyrazolone ring. The broad band for the OH group of the ligand at ca. 3400-3500 cm is also seen in all the spectra of the complexes which indicates that the chelation does not take place via the OH group. The strong band at 1590 $cm^{-1}$, characteristic of the azomethine group in the free Schiff base, is shifted to lower frequency of ca. 1570- 1580 $cm^{-1}$ in the complexes indicates that the azomethine nitrogen is one of the coordinating atoms in the Schiff base. The band observed at 1656 cm-¹, characteristic of the cyclic carbonyl group of the pyrazolone ring of free Schiff base, is shifted to lower frequency of ca. 1610- 1620 $cm^{-1}$ in the complexes indicates the coordination of the above group with metal ion. This is further supported by the formation of new bands in the regions 460-510 $cm^{-1}$ and 410-460 $cm^{-1}$ which are due to $^{V}$M-O and $^{V}$M-N bands respectively [6].

The $^1$H NMR spectrum of the Schiff base in $CDCl_3$ shows the following signals: phenyl multiplets at $\delta$7.2- 7.4 range, C-CH, at $\delta$ 2.8, -NHPhMe at $\delta$ 2.2. -N- CH, at $\delta$ 3.0, C-CH at $\delta$ 6.6 and -$PhCH_3$ at $\delta$ 2.3. The peak at $\delta$ 10.2 is attributed to the enolic -OH group present in the o-acetoacetotoluidide moiety. Presence of this peak in the zinc complex shows that the -OH proton is not involved in complexation. This observation reinforces the IR spectral evidence of the non-involvement of -OH group in metal coordination. There is no appreciable change in the spectra of both the ligand and its zinc complex.

**Table 2:** Electric observation spectral data of the compounds

| Compd. | Solvent | Absorption ($cm^{-1}$) | Band Assignment | Geometry |
|---|---|---|---|---|
| Ligand | DMSO | 42372 33277 | INCT INCT | - |
| $[CuL_2]\ Cl_2$ | DMSO | 29070 24390 18238 | INCT INCT $^2B_{1g} \rightarrow {}^2A_{1g}$ | Square planer |
| $[NiL_2]\ Cl_2$ | DMSO | 29325 24038 14327 | INCT $^1A_{1g} \rightarrow {}^1A_{2g}$ $^1A_{1g} \rightarrow {}^1B_{2g}$ | Square planer |
| $[CoL_2]\ Cl_2$ | DMSO | 31153 14970 | INCT $^1A_{1g} \rightarrow {}^1B_{1g}$ | Square planer |

The electronic spectra can often provide quick and reliable information about the ligand arrangement in transition metal complexes. The electronic absorption spectra of the ligand and its complexes were recorded at 300 K in DMSO. The absorption regions, assignments and the proposed geometry of the complexes are given in Table-2. This table clearly indicates that all the complexes are having square-planar geometry around the metal ions [7]. The observed magnetic moment (0 B.M. for the nickel complex and 3.58 B.M. for cobalt complex) at room temperature is also consistent with the square-planar geometry around the central metal ion and monomeric nature of the complexes. The magnetic susceptibility measurement of copper complex (1.74 B.M) corresponding to one electron, indicating that the complex is mononuclear. This fact is also supported by the ESR spectrum.

The X-band ESR spectra of the copper complex, recorded in DMSO solution at 300 K and 77 K. The frozen solution spectrum shows a well-resolved fourline spectrum and no features characteristic for a dinuclear complex i.e. the absence of a half-field signal observed in the spectrum at 1600 G due to the $m_S = \pm 2$ transitions ruling.out Cu-Cu interaction [8]. The spin Hamiltonian parameters for the copper complex are calculated from the spectra. The observed order ($A_{\parallel} = 180 > A_{\perp} = 30$; $g_{\parallel} = 2.16 > g_{\perp} = 2.04$) indicates that the complex is akin to square planar geometry. Further, it is also supported from the fact that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital. Recalling the complexes with the $d_{x^2-y^2}$ ground state strong interaction along the z-axis is accompanied by an increase in the value of $g_{\parallel}$. Strong axial bonding leads to an increase in the length of the bond in the x-y plane, which results in a decrease of both in-plane covalency and the energy of $d_{x^2-y^2}$ transition. Both these effects tend to increase the value of $g_{\parallel}$. If the value of G is larger than four, exchange interaction is negligible while the value of G is less than four, exchange interaction is considerable and the local tetragonal axes are misaligned. For this system, the G value is 4.22, indicating that the exchange coupling effects are aligned parallel or slightly misaligned and consistent with $d_{x^2-y^2}$ ground state [9]. Based on the above spectral data, structure of the complex is given in Figure -1.

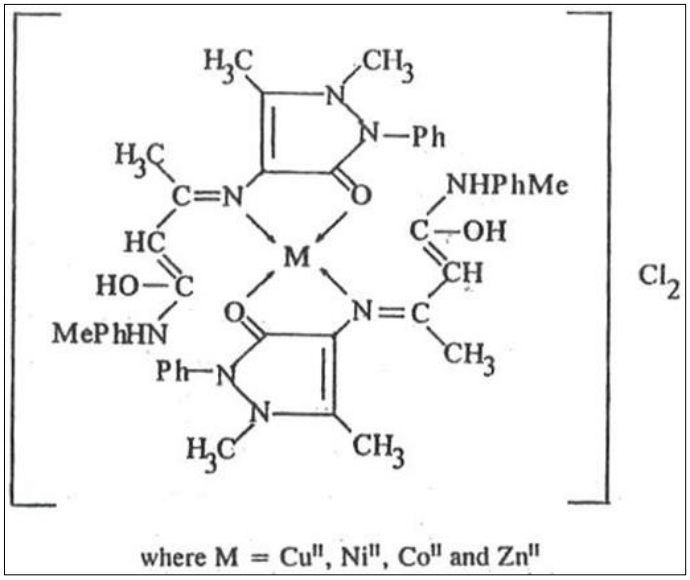


**Fig 1:** The proposed structure of the Schiff base complexes

**Table 3:** Antimicrobial activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration x 10-2 M)

| Compd. | *S. typhi* | *S. aureus* | *E. coli* | *B. subtilis* |
|---|---|---|---|---|
| L | 6.2 | 6.1 | 6.3 | 5.9 |
| $[CuL_2]\ Cl_2$ | 4.9 | 4.8 | 4.9 | 4.7 |
| $[CoL_2]\ Cl_2$ | 5.1 | 4.9 | 4.7 | 4.9 |
| $[NiL_2]\ Cl_2$ | 4.9 | 4.8 | 4.7 | 4.8 |
| $[ZnL_2]\ Cl_2$ | 4.8 | 5.0 | 4.9 | 4.7 |
| | *A. niger* | *A. flovus* | *R. bataicola* | |
| L | 7.9 | 7.6 | 7.8 | |
| $[CuL_2]\ Cl_2$ | 5.6 | 6.1 | 6.3 | |
| $[CoL_2]\ Cl_2$ | 5.9 | 6.3 | 6.5 | |
| $[NiL_2]\ Cl_2$ | 5.8 | 6.2 | 6.3 | ` |
| $[ZnL_2]\ Cl_2$ | 5.9 | 6.4 | 6.6 | |

For *in vitro* antimicrobial activity, the investigated compounds were tested against the bacteria *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* and fungi *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola.* The minimum inhibitory concentration (MIC) values of the investigated compounds are summarized in Table-3 From the table, the observed MIC values indicate that most of the complexes have higher antimicrobial activity than the free ligand. Such increased activity of the metal chelates can be explained on the basis of chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the

delocalization of π-electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [10].

The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA-binding ability. The metal complexes were able to convert super coiled DNA into open circular DNA. The general oxidative mechanisms proposed account of DNA cleavage by hydroxyl radicals via. abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [11]. The cleavage is inhibited by the free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by metallocomplexes bound hydroxyl radical or a peroxo species generated from the co-reactant $H_2O_2$. In the present study, the CT-DNA gel electrophoresis experiment was conducted at 35°C using our synthesized complexes in the presence of $H_2O$, as an oxidant. As can be seen from the results in Figure-2, at very low concentration, only copper complex exhibits nuclease activity in the presence of $H_2O_2$. Control experiment using DNA alone (lane 1) does not show any significant cleavage of CT-DNA even on longer exposure time. From the observed results, we conclude that the copper complex (lane 2), cleaves DNA as compared to control DNA while other complexes (lane 3-5) do not cleave DNA in the presence of $H_2O_2$. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage [12].

Lanes left to right →
Lane 1, DNA Alone
Lane 2, DNA + $[CuL_2]$ $Cl_2$ + $H_2O_2$
Lane 3, DNA + $[NiL_2]$ $Cl_2$ + $H_2O_2$
Lane 4, DNA + $[CoL_2]$ $Cl_2$ + $H_2O_2$
Lane 5, DNA + $[ZnL_2]$ $Cl_2$ + $H_2O_2$

## Conclusions

In this paper, a new Schiff base ligand, obtained from the reaction of 4-aminoantipyrine and o-acetoacetotoluidide and its $Cu^{11}$. $Co^{11}$. $Ni^{11}$ and $Zn^{11}$ complexes have been synthesized and characterized by spectral and analytical data. Based on these data, a square planar geometry has been assigned to the complexes. The metal complexes have higher antimicrobial activity than the ligand. The interaction of these complexes with CT-DNA was investigated by gel electrophoresis. From the observation. copper complex cleaves DNA as compared to control DNA and other complexes in the presence of $H_2$

## References


1. Djebbar SS, Benali BO, Deloume JP. *J. Chem. Soc*,1997:16:2175-2178.
2. Liu CM, Xiong RG, You XZ, Liu YJ, Cheung KK. *Polyhedron*,1996:15:4565-4567.
3. Long NJ. *Angew. Chem. Int. Ed. Engl*,1995:34:21-24.
4. Raman N, Rajasekaran K, Sakthivel A. *J. Indian Chem. Soc*,2007:84:117-120.
5. Pelczar MJ, Chan ECS, Krieg NR. *"Micro- biology", 5th ed., New York*, 1998.
6. Thankamony M, Mohanan K. *Indian J. Chem., Sect. A*,2007:*46*:247-250.
7. Lever ABJ, Mantovani E. *Inorg. Chem*,1971:10:817-820.
8. Ramakrishna Reddy K, Madhusudan Reddy K, Mahendra KN. *Indian J. Chem., Sect. A*,2006:45:377-380.
9. Benial AMF, Ramakrishnan V, Murugesan R. *Spectrochim. Acta, Part A*,2000:56:2775-2778.
10. Dharmaraj N, Viswanathamurthi P, Natarajan K. *Trans. Met. Chem*,2001:26:26-28.
11. Prativel G, Pitie M, Bemadou J, Meunier B. *Angew. Chem. Int. Ed. Eng*,1991:30:702-704.
12. Thomos AM, Naik AD, Nethaji M, Chakravarty AR. *Indian J. Chem., Sect. A*,2004:43:691-694.