

Synthesis, Spectral Characterization of Schiff base Transition Metal Complexes: DNA Cleavage and Antimicrobial Activity Study

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Abstract—Schiff base of 4-aminoantipyrine and its complexes have a variety of applications in biological, clinical, analytical and pharmacological areas^[1-3]. Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists^[4,5]. Earlier work reported that some drugs showed increased activity, when administered as metal complexes rather than as organic compounds^[6,7]. Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biologists. Some kind of metal complexes interacted with DNA could induce the breakage of DNA strands by appropriate 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. Copper complex could cleave DNA in the presence of ascorbate or hydroquinone. It was suggested that the reductive capability of reductants had a critical influence on DNA cleavage. The coordinating property of 4-aminoantipyrine ligand has been modified to give a flexible ligand system, formed by condensation with a variety of reagents like aldehydes, ketones thiosemicarbazides and carbazides, etc. Literature search reveals that no work has been done on the condensation process of 4-aminoantipyrine, 3-hydroxy-4-aminobenzaldehyde and 4-methyl-O-phenylenediamine.

1. INTRODUCTION

The coordinating property of 4-aminoantipyrine ligand has been modified to give a flexible ligand system, formed by condensation with a variety of reagents like aldehydes, ketones thiosemicarbazides and carbazides, etc.^[8] Literature search reveals that no work has been done on the condensation process of 4-aminoantipyrine, and 4-methyl-O-phenylenediamine. We report the synthesis, characterization, redox, antimicrobial and DNA cleavage studies of transition metal complexes containing Schiff base derived from 4-aminoantipyrine, 3-hydroxy-4-aminobenzaldehyde and 4-methyl-O-phenylenediamine.

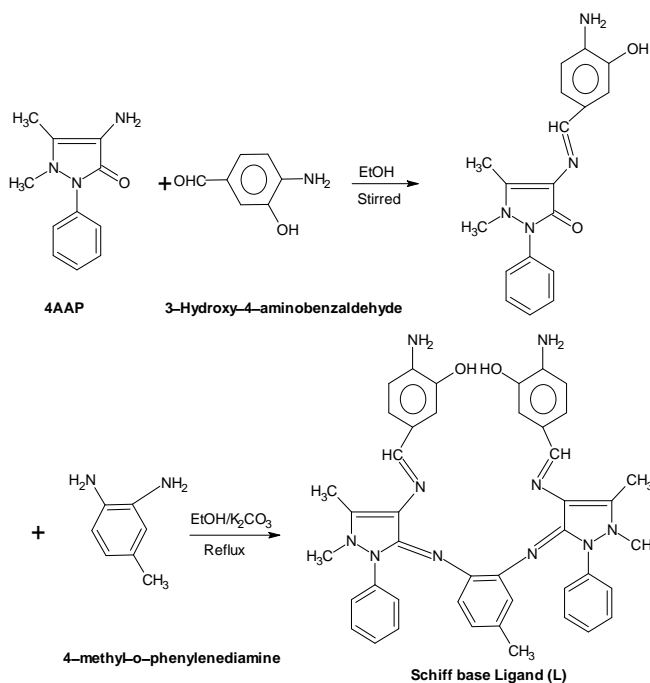


Figure 1: Formation of Schiff base ligand

2. EXPERIMENTAL PROCEDURE

2.1 IR Spectra:

IR spectra were obtained using KBr pellets on a Perkin-Elmer 783 spectrophotometer;

2.2 NMR Spectra:

NMR spectra were recorded in CDCl₃ and DMSO-d₆ on a Bruker DMX-300 spectrometer.

2.3 X-band ESR Spectra:

ESR spectra of the complexes were recorded at 300 and 77K.

2.4 UV-VIS spectra:

The UV-Vis spectra were recorded on a Shimadzu UV-1601 spectrophotometer using DMF as a solvent.

2.5 Magnetic susceptibility:

It was measured by Guoy balance using copper sulphate as calibrant.

3. MATERIAL USED

Reagents such as 4-aminoantipyrine, 3-hydroxy-4-aminobenzaldehyde, 4-methyl-o-phenylenediamine,

and various metal(II) chlorides were purchased from Merck and B.D.H. (India) chemicals.

CT DNA from A.I.I.M.S. were used as supplied.

For voltammetric experiments, tetrabutylammonium perchlorate (TBAP) (Sigma) was used as supporting electrolyte.

4. SOLVENT USED

Anhydrous grade ethanol, DMF and DMSO were purified according to standard procedures.

5. SYNTHESIS

5.1 Synthesis of Schiff base(L)

4-Aminoantipyrine (2.033 g, 10 mM) in 40 mL ethanol was stirred with 3-hydroxy-4-aminobenzaldehyde (1.671 g, 10 mM) for ~1h. The yellow solid (I) formed was filtered and recrystallized from ethanol. Compound (I) (7.04 g, 20mM) in 50 mL of ethanol was refluxed with 4-methyl-o-phenylenediamine (1.08 g, 10 mM) for ~36 h after adding anhydrous potassium carbonate. The potassium carbonate was filtered off from the reaction mixture and the solvent was evaporated. The red solid separated was filtered and recrystallized from ethanol.(Fig.1)

5.2 Synthesis of metal complexes

A solution of metal(II) chloride in ethanol (2 mM) was refluxed with an ethanolic solution of the Schiff base (2 mM) for ~5 h. The solution was then reduced to one-third on a water bath. The solid complex precipitated was filtered, washed thoroughly with ethanol and dried in vacuo. The oxovanadium(IV) complex was synthesized from the sulphate salt by the same procedure but in the presence of 5 mL of 5% aqueous sodium acetate solution.

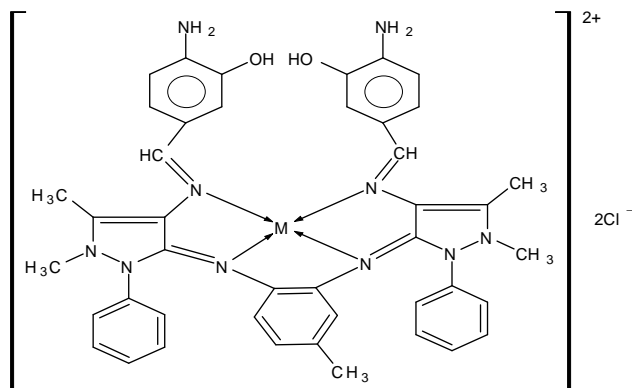


Figure 2: Structure of metal complex

6. RESULTS AND DISCUSSION

The analytical data for the ligand and complexes together with some physical properties are summarized in table 5. The data from complexes correspond well with the general formula ML_2 , where $M = \text{Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Cd(II), Hg(II) and VO(IV)}$; $L = \text{C}_{43}\text{H}_{42}\text{N}_{10}\text{O}_2$. The magnetic susceptibilities of the complexes at room temperature were consistent with square-planar geometry around the central metal ion, except for the VO(IV) complex which shows a square-pyramidal geometry. The higher conductance values of chelates support their electrolytic nature of metal complexes.

7. CHARACTERIZATION

7.1 Mass Spectral Studies

The ESI mass spectra of the ligand (L) and its copper complex $[\text{CuL}]\text{Cl}_2$ recorded at room temperature were used to compare their stoichiometry composition. The Schiff base showed a molecular ion peak at m/z 730 which was also supported by the 'nitrogen rule', since the compound possesses ten nitrogen atoms. The molecular ion peak for the copper complex, observed at m/z 864 confirms the stoichiometry of metal chelates as ML_2 type. It is also supported by the mass spectra of other complexes.

7.2 Infrared Spectral Studies

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. The IR spectra of the ligand showed a broad band in the region $3200\text{--}3600\text{ cm}^{-1}$ assignable to intramolecular hydrogen bonded -OH groups. The appearance of this peak in all the spectra of the complexes indicates that the -OH group is free from complexation. The spectrum of the ligand shows two different -C=N bands in the region $1590\text{--}1550\text{ cm}^{-1}$, which is shifted to lower frequencies in the spectra of all the complexes ($1570\text{--}1520\text{ cm}^{-1}$) indicating the involvement of -C=N nitrogen in coordination to the metal ion.^[9,10] Accordingly, the

ligand acts as a tetradentate chelating agent, bonded to the metal ion via the four nitrogen ($-C=N$) atoms of the Schiff base.

7.3 1H NMR Spectra

The 1H NMR spectrum of the compound I, recorded in $CDCl_3$ showed the following signals: $=C-CH_3$ proton at 2.5 ppm (s, 3H), $N-CH_3$ at 3.3 ppm (s, 3H), phenyl as multiplet at 7.2–7.8 ppm (m, 8H), $-CH=N-$ at 9.7 ppm (s, H). Furthermore, the peak obtained at 13.3 ppm (s, H) is attributable to phenolic $-OH$ group present in the 3-hydroxy-4-aminobenzaldehyde moiety. The 1H NMR spectra of the ligand (L) and its zinc complex were recorded in $DMSO-d_6$. The 1H -NMR spectrum of the ligand shows the following signals: $=C-CH_3$ proton at 2.4 ppm (s, 6H), $N-CH_3$ at 3.2 ppm (s, 6H), phenyl as multiplet at 6.9–7.5 ppm (m, 20H), $-CH=N-$ at 9.8 ppm (s, 2H) and the peak at 13.4 ppm (s, 2H) is attributable to the phenolic $-OH$ group present in the 3-hydroxy-4-aminobenzaldehyde moiety. (Table-1)

7.4 Electronic absorption spectral Studies:

The electronic absorption spectra of the Schiff base, Cu(II), Ni(II), Co(II) and VO(IV) complexes were recorded at 300 K. The absorption region, band assignment and the proposed geometry of the complexes are given in table 5.2. Based on these data, a square-planar geometry has been assigned to the complexes except VO(IV) complex which has square-pyramidal geometry (figure 2). These values are comparable with other reported complexes.^[11] (Table 2)

Table 1: Cyclic voltammetric data of copper and vanadium complexes in DMSO containing 0.1 M (TBAP). Scan rate 100 mVs⁻¹.

| Complex | Couple | Ep (V) | EP a(V) | Ipc (μA) | Ip a(μA) |
|----------------------|--------------------------------|---------------|---------------|-----------------|-----------------|
| [CuL]Cl ₂ | Cu(II)/Cu(I) | 0.24 | 0.53 | 11.05 | -10.26 |
| [VOL]SO ₄ | VO(IV)/VO(V) VO(IV)/VO(III) | 0.53 -1.03 | 0.62 -0.57 | 15.28 6.75 | -15.55 -7.44 |

Table 2: ESR spectral data of copper and vanadium complexes in DMSO at 300 and 77 K.

| Complex | A | A _⊥ | A _{iso} | g | g _⊥ | g _{iso} |
|----------------------|-----------------|----------------|------------------|-----------------|----------------|------------------|
| [CuL]Cl ₂ | 165 | 55 | 78 | 2.38 | 2.06 | 2.12 |
| [VOL]SO ₄ | 172 | 71 | 104 | 1.97 | 2.03 | 1.98 |

8. ANTIMICROBIAL ACTIVITY

For in vitro antimicrobial activity, the investigated compounds were tested against the bacteria *S. typhi*, *S. aureus*, *E. coli*, and *B. subtilis* and fungi *A. niger*, *A. flavus* and *R. bataicola*. The minimum inhibitory concentration (MIC) values of the investigated compounds are summarized in tables 3 and 4. The values indicate that most 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.^[12] (Table 3,4)

Table 3: Antibacterial activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration $\times 10^{-2}$ M).

| Compound | S. typhi | S. aureus | E. coli | B. subtilis |
|--|----------|-----------|---------|-------------|
| L(C ₄₃ H ₄₂ N ₁₀ O ₂) | 5.1 | 5.3 | 5.2 | 5.5 |
| [CuL]Cl ₂ | 4.0 | 3.8 | 4.1 | 3.9 |
| [NiL]Cl ₂ | 4.2 | 4.1 | 4.0 | 4.2 |
| [CoL]Cl ₂ | 4.0 | 4.5 | 4.1 | 4.4 |

9. DNA CLEAVAGE STUDIES

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 supercoiled DNA into open circular DNA. The proposed general oxidative mechanisms and account of DNA cleavage by hydroxyl radicals via abstraction of a hydrogen atom from sugar units that predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed. The cleavage is inhibited by free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metal complexes bound hydroxyl radical or a peroxy 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 synthesized complexes in the presence of H_2O_2 as an oxidant. As can be seen from the results, at very low concentration, few complexes exhibit 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 complexes, copper complex (lane 2), nickel complex (lane 3) and cobalt complex (lane 4) cleave DNA as compared to control DNA while other complexes (lane 5–9) do not cleave DNA in the presence of H_2O_2 . Probably this may be due to the formation of redox couple of the metal ions and its behaviour. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage.^(54–56)

Table 4: Antifungal activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration $\times 10^{-2}$)

| Compound | A.niger | A.flavus | R.bataicola |
|----------------------|---------|----------|-------------|
| L(C43H42N10O2) | 6.5 | 6.3 | 6.4 |
| [CuL]Cl ₂ | 4.6 | 5.0 | 4.8 |
| [NiL]Cl ₂ | 4.8 | 5.2 | 5.3 |
| [CoL]Cl ₂ | 5.1 | 4.9 | 4.8 |

Table 5: Physical characterization, analytical, molar conductance & magnetic susceptibility data of the ligand and the complexes.

| Compound | Mol. Formula | Colour | Yield (%) | Molar conductance Λ^M ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$) | μ_{eff} (BM) |
|----------------------|------------------------------|------------|-----------|---|-------------------------|
| L | (C43H42N10O2) | Red | 74 | – | – |
| [CuL]Cl ₂ | CuC43H42N10O2Cl ₂ | Dark brown | 56 | 42 | 1.77 |
| [NiL]Cl ₂ | NiC43H42N10O2Cl ₂ | Brown | 54 | 48 | – |
| [CoL]Cl ₂ | CoC43H42N10O2Cl ₂ | Red | 58 | 53 | 3.62 |
| [MnL]Cl ₂ | MnC43H42N10O2Cl ₂ | Pale brown | 60 | 44 | 5.31 |
| [ZnL]Cl ₂ | ZnC43H42N10O2Cl ₂ | Pale brown | 62 | 56 | – |

10. CONCLUSION

In this work coordination chemistry of a Schiff base ligand, obtained from the reaction of 4-aminoantipyrine, 3-hydroxy-aminobenzaldehyde and 4-methyl phenylenediamine, is described. Cu(II), Ni(II), Co(II), Mn(II), Zn(II), VO(IV), Hg(II) and Cd(II) complexes have been synthesized using the Schiff base ligand and characterized by spectral and analytical data. Based on these data, a square-planar geometry has been assigned to the complexes except VO(IV) complex which has square-pyramidal geometry. 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, Cu, Ni and Co complexes cleave DNA as compared to control DNA and other complexes in the presence of H₂O₂.

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