



Studies on DNA Cleavage and Antimicrobial screening of Transition Metal complexes of 4-aminoantipyrene Schiff base

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Abstract

The coordination compounds using Co(II), Ni(II), Cu(II) and Zn(II) ions with Schiff base ligand derived from furfurylidene-4-aminoantipyrene with o-anisidine in a 1:1 molar ratio have been synthesized and characterised by elemental analysis, IR, NMR and electronic spectral studies. On the basis of spectral studies, octahedral geometry is suggested for Co(II), Ni(II), Cu(II) and tetrahedral geometry for Zn(II) complexes. The synthesized ligand and metal(II) complexes have been tested against bacterial species *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and fungal species *Aspergillus niger* (*A. niger*), *Aspergillus fumigatus* (*A. fumigatus*) and *Candida albicans* (*C. albicans*) in order to assess their antimicrobial properties. The results show that the metal complexes were more active than the ligand. The DNA cleavage activity of the ligand and its complexes were assayed using PUC18 DNA by gel electrophoresis. The result shows that complete cleavage of DNA was observed in Cu(II) and Zn(II) complexes and partial cleavage of DNA was observed in Co(II) and Ni(II) complexes.

Keywords: Schiff base ligand, transition metals, antimicrobial activity, DNA cleavage.

Introduction

When an aldehyde or a ketone is condensed with a primary amine a Schiff base is produced which is a compound containing azomethine group R-C=N-. Azomethine linkage is important for biological activity. Several azomethines were reported to possess important antibacterial¹⁻³, antifungal^{4,5} anti cancer⁶ and herbicidal⁷⁻⁹ activities. Schiff bases and their coordination compounds have been studied for their interesting and important properties e.g their ability to reversibly bind oxygen¹⁰, catalytic activity in hydrogenation of olefins¹¹ and transfer of an amino group¹² photochromic properties¹³ and complexing ability towards some toxic metals¹⁴. The high affinity for the chelation of the schiff bases towards the transition metal ions is utilized in preparing their solid complexes. Metal complexes of Schiff base have played a central role in the development of coordination chemistry and have many applications in various fields. Metal complexes of nitrogen-oxygen chelating agents derived from 4-aminoantipyrene Schiff base have studied extensively due to their pronounced applications in biological, clinical, analytical and pharmacological areas¹⁵⁻²³. DNA is the primary target for most anticancer and antiviral therapies according to cell biology^{24,25}. In Continuation of our research^{26,27} the aim of this work is to prepare and investigate some complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Schiff base ligand derived from furfurylidene-4-aminoantipyrene and o-anisidine. The ligand and complexes were characterised by physical and spectral data including IR, UV, and ¹H NMR.

Material and Methods

All the chemicals 4-aminoantipyrene, o-anisidine, furfuraldehyde and metal salts (acetates of Co(II) Ni(II) and Zn (II) are analar

grade and were used as supplied. The solvent like ethanol, methanol, DMSO etc were purified and dried by the standard procedures^{28,29}.

IR spectra were recorded on schimadzu FT IR 8400 s spectrometer in 4000-400 cm⁻¹ range using KBr pellet. The UV – Visible spectra were recorded by using schimadzu UV-spectrometer in the wavelength range 200-800 nm. The ¹H NMR was recorded on a Bruker DPX-300 spectrometer using DMSO as solvent and TMS as internal standard. The antimicrobial activity was determined by disc diffusion method. Stock solutions were prepared by dissolving the compounds in DMSO and serial dilutions of the compound were prepared in sterile distilled water to determine the minimum inhibition concentration (MIC). The DNA cleavage activity of the Schiff base and its Co(II), Ni(II), Cu(II) and Z(II) complexes was done by agarose gel electrophoresis method.

Synthesis of Schiff base ligand: Furfurylidene-4-amino antipyrene was prepared by the condensation of furfuraldehyde and 4-aminoantipyrene. A methanolic solution of furfurylidene-4-aminoantipyrene (0.01mol) and o-anisidine (0.01mol) was refluxed for 6 h. The solvent was then reduced to one third of its volume and resulting solution was cooled to room temperature. The solid product formed was removed by filtration and recrystallised from methanol.

Synthesis of metal complexes: A solution of metal (II) acetates and the Schiff bases (0.01 mol) in ethanol was stirred for 5 h using a magnetic stirrer. The solvent was then reduced to one third of its volume and the resulting solution was cooled to room temperature and kept undisturbed for 2-3 days. Then the precipitated complex was filtered washed with ether followed by ethanol and dried in vaccum. Scheme of the experiment given in figure-1.

Table-1
Physical and Analytical data of ligand and complexes

Compound	Mol. Formula (colour)	Mol. Wt (Yield %)	Found (Calcd.) %				
			C	H	O	N	M
C ₂₃ H ₂₂ O ₂ N ₃ (L)	C ₂₃ H ₂₂ O ₂ N ₃ (Yellow)	372 (85)	74.09 (74.19)	5.50 (5.91)	8.25 (8.60)	11.08 (11.29)	-
[Co L(OAc) ₂ (H ₂ O) ₂]	C ₂₇ H ₃₂ O ₆ N ₃ Co (Pink)	584.9 (53)	55.25 (55.39)	54.50 (54.70)	21.51 (21.88)	7.01 (7.28)	9.89 (10.17)
[Ni L(OAc) ₂ (H ₂ O) ₂]	C ₂₇ H ₃₂ O ₆ N ₃ Ni (Green)	584.7 (57)	55.01 (55.41)	54.20 (54.70)	21.59 (21.89)	7.28 (7.18)	10.10 (10.04)
[Cu L(OAc) ₂ (H ₂ O) ₂]	C ₂₇ H ₃₂ O ₆ N ₃ Cu (Brown)	589.5 (54)	54.56 (54.96)	54.03 (54.3)	21.28 (21.71)	7.03 (7.12)	10.28 (10.77)
[Zn L(OAc) ₂]	C ₂₇ H ₂₈ O ₄ N ₃ Zn (Brown)	519.4 (68)	54.29 (54.79)	15.20 (15.41)	21.15 (21.64)	6.90 (7.10)	10.85 (11.05)

Table-2
IR data for ligand and complexes (cm⁻¹)

Sl. No	Compound	OH in water molecule	C = N	M-N	M-O	Phenylring
						Vibration
1	C ₂₃ HO ₂₂ N ₄ = L	-	1592	-	-	1069 & 756
2	[Co L(H ₂ O) ₂ (OAc) ₂]	3470	1562	435	587	1014 & 758
3	[Ni L(H ₂ O) ₂ (OAc) ₂]	3111	1589	431	586	1071 & 757
4	[Cu L((H ₂ O) ₂ (OAc) ₂)]	3391	1560	430	598	1137 & 757
5	[Zn L(Ac) ₂]	-	1588	428	581	1061 & 756

Table-3
UV-Visible spectra data of ligand and complexes

Sl. No	Compound	d-d	$\pi-\pi^*$	n- π^*	L → MCT	Geometry
1	C ₂₃ HO ₂₂ N ₄ = L	-	271,260	343	-	-
2	[Co L(H ₂ O) ₂ (OAc) ₂]	667	279,242	346	426	Octahedral
3	[Ni L(H ₂ O) ₂ (OAc) ₂]	660	269,243	346	389	Octahedral
4	[Cu L((H ₂ O) ₂ (OAc) ₂)]	602	280,245	346	401	Octahedral
5	[Zn L(OAc) ₂]	-	270,250	346	360	Tetrahedral

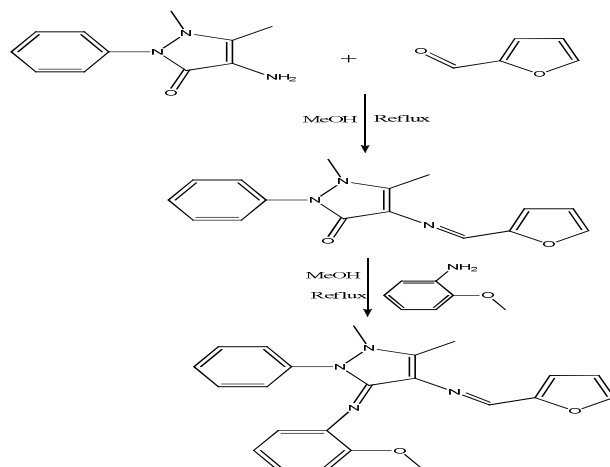


Figure-1
Synthetic route of ligand

Antibacterial activity: Test Organisms: The bacterial species *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* and fungal species *Candida albicans* were used as test organisms and they are maintained on Mueller Hinton Agar solid media (MHA) and Sabouraud's dextrose (SDA) media³⁰⁻³².

Assay of antimicrobial activity: Agar diffusion assay was carried out to evaluate the antimicrobial activity of some synthesized compounds. The plates were incubated at 37°C for 24 h during which activity was evidenced by the presence of a zone of inhibition surrounding the well and antibacterial and antifungal activity was expressed as mean of diameter of inhibition zones (mm) produced by the synthesized compounds when compared to controls.

Experimental Methods: MHA and SDA were prepared with lawn culture using desired test organisms. The inoculated plates were kept aside for few minutes. Using well cutter two wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipette, 20 μ L of compound extract was added into one well and to another well the same volume of corresponding control were added. After diffusion, the plates were incubated at 37°C for 24 h. After incubation, the inhibition of growth was analyzed and the results were recorded.

DNA cleavage: DNA cleavage experiments were done according to the literature³³. Nutrient broth [peptone: 10 g/L; yeast extract: 5 g/L; NaCl: 10 g/L] was used for the culture of *Staphylococcus aureus*. The 50 mL media was prepared, autoclaved for 15 min at 121°C under 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37°C for 24 h.

The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet which was then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 50 mM lysozyme). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min. After centrifugation at 10,000 rpm for 10 min, to the supernatant equal volume of chloroform:isoamyl alcohol (24:1) and 1/20 volume of 3 M sodium acetate (pH 4.8) was added. The solution was further centrifuged at 10,000 rpm for 10 min and to the supernatant, three volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in tris buffer (10 mM tris pH 8.0) and stored in cold condition. Cleavage products were analyzed by agarose gel electrophoresis method³⁴. Test samples (1 mg/mL) were prepared in DMSO. The samples (25 μ g) were added to the isolated DNA of *S. aureus*, and incubated for 2 h at 37 °C. Then 20 μ L of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, 0.5 M EDTA/L) and finally loaded on agarose gel and the constant 50 V of

electricity passed for around 30 min. The gel was removed and stained with 10 μ g/mL ethidium bromide for 10-15 min and the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage. The results are compared with standard DNA marker.

Results and Discussion

The analytical data for the ligand and complexes together with some physical properties like colour elemental analysis is summarized in table-1.

FT IR spectra: The IR spectra of the complexes are compared with that of the free ligand to determine the changes that have taken place during the complexation. All data are listed in table-2. IR spectrum exhibit a strong band at 1645 cm^{-1} which is characteristic of the azomethine group (C=N) in the free Schiff base. In IR spectra of complexes, this band shifts to lower frequency of 1588-1560 cm^{-1} which indicates that the azomethine nitrogen is one of the coordinating atoms in the Schiff base³⁵⁻³⁷. All complexes and ligand show band in the region 1014-1069 cm^{-1} and 756-758 cm^{-1} can be assigned to phenyl ring vibration. The IR broad band of metal complexes in the range of 3111-3470 cm^{-1} indicate the presence of coordinated water molecule^{38, 39} in the metal complexes. The spectra of the metal complexes also show some new bands in the region of 435-428 cm^{-1} and 581-598 cm^{-1} which are probably due to the formation of M-N and M-O bonds respectively^{40, 41}. Two bands were observed at 1644-1651 cm^{-1} and 1301-1371 cm^{-1} assigned to $\nu_{\text{assy}} \text{COO}^-$ and $\nu_{\text{sy}} \text{COO}^-$ in complex⁴².

¹H NMR spectra: The ¹H NMR Spectra of the ligand and its zinc complex were recorded in DMSO and shown in figure -2 and 3. The ¹H NMR spectrum of the ligand shows the following signals phenyl multiplets 7.2-7.4 δ range, C-CH₃ at 2.4 δ , N-CH₃ at 3.1 δ . The azomethine proton signal in the spectrum of the zinc complex is shifted down field compared to the free ligand suggesting deshielding of the azomethine group due to the coordination with metal ion. Thus the peak at 7.56 δ found in the ligand is shifted to 7.79 δ . There is no appreciable change in other signals of Zn(II) complex.

UV-Visible spectra: The UV-Visible spectra are often very useful in the evaluation of results furnished by other methods of structural investigation. The electronic spectral measurements were used for assigning the stereochemistry of metal ions in the complexes based on the positions and number of d-d transition peaks. The electronic absorption spectra of the Schiff base ligand and its complexes were recorded in DMF solution in the range of 200 to 800 nm regions and the data are presented in table-3. The absorption spectrum of free ligand consists of an intense band centered at 346 nm attributed to n- π^* transitions of the azomethine group.

Table-4
Antibacterial activity data of ligand and complexes

Compound	<i>S.aureus</i>	<i>E.coli</i>	<i>P. aeruginosa</i>
$C_{23}HO_{22}N_4 = L$	55	12	38
$[Co L(H_2O)_2(OAc)_2]$	45	60	45
$[Ni L(H_2O)_2(OAc)_2]$	35	40	65
$[Cu L((H_2O)_2(OAc)_2)]$	85	120	110
$[Zn L(OAc)_2]$	70	62	39

Table-5
Antifungal activity data of ligand and complexes

S. No.	Compound	<i>C. albicans</i>	<i>A. niger</i>	<i>A. fumigatus</i>
1.	$C_{23}HO_{22}N_4 = L$	25	20	15
2.	$[Co L(H_2O)_2(OAc)_2]$	85	72	68
3.	$[Ni L(H_2O)_2(OAc)_2]$	78	45	30
4.	$[Cu L((H_2O)_2(OAc)_2)]$	125	98	85
5.	$[Zn L(OAc)_2]$	60	53	48

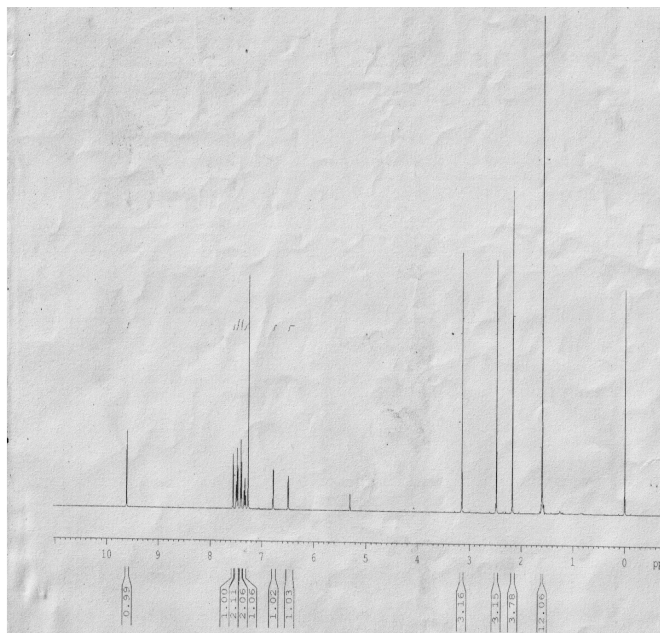


Figure-2
 1H NMR spectrum of ligand.

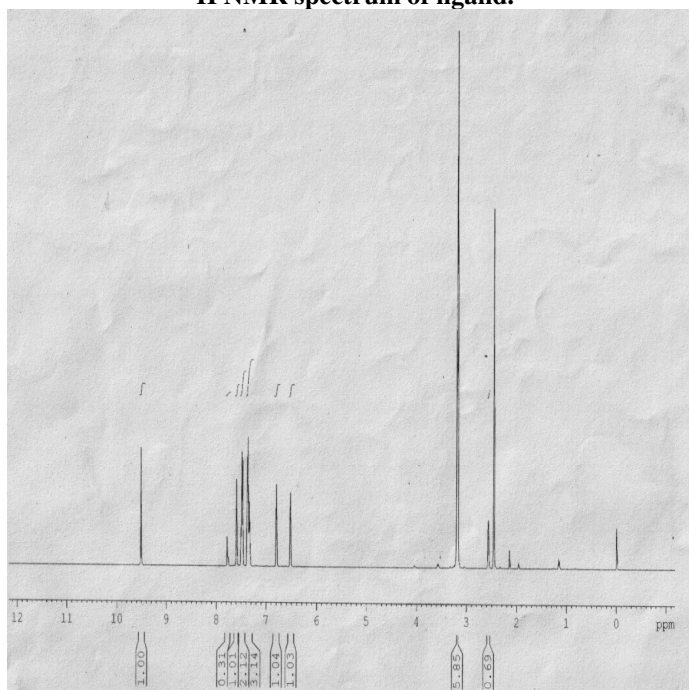


Figure-3
 1H NMR spectrum of Zn(II) complex

Another intense band in higher energy region of the spectrum of the free ligand was related to $\pi-\pi^*$ transitions of benzene rings. These transitions are also found in the spectra of the complexes, but they are shifted towards lower frequencies confirming the coordination of the ligand to the metal ions. Further the d-d transition of the complex showed a broad band centered at 667-602 nm for Co (II), Ni(II) and Cu(II) complexes. The spectrum of Co(II) complex shows band at 667 nm which can be attributed to $^4T_{1(g)}(F) \rightarrow ^4T_{2(g)}(F)$ transition for the octahedral geometry. The electronic spectrum of Ni(II) complex shows d-d transition at 660nm, due to $^3A_{2(g)}(F) \rightarrow ^3T_{1(g)}(P)$ transition for octahedral Ni(II) complex. The Cu(II) complex shows broad band at 602nm, due to $^2B_{1g} \rightarrow ^2E_g$ transitions^{43,44} suggesting octahedral geometry. The Zn(II) complex does not show any d-d transitions. In general four coordinate zn(ii) complexes would have tetrahedral geometry. the proposed structure of the complexes are shown in figure-4.

Antimicrobial Activity: The reactivity of the synthesized compounds towards the biological systems is important features of the current research and Schiff bases of transition metal complexes. The results of the antibacterial and antifungal activities are shown in table-4 and 5. all the synthesized compounds showed remarkable biological activity against all the three bacterial and one fungal species under investigation. Among the synthesized compounds Cu(II) complex showed very good antibacterial and antifungal activity against all the selected bacterial and fungal species under study. The toxicity of the complexes was found to be better than the ligand owing to the theory of Tweedy⁴⁵⁻⁵⁰. This is probably due to the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtone's concept of cell permeability. The lipid membrane that surrounds the cell favours the passage of lipid soluble materials due to which liposolubility is an important factor which controls the antimicrobial activity. 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 positive charge of the metal ion donor groups. Further, it increases the delocalization of π electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes

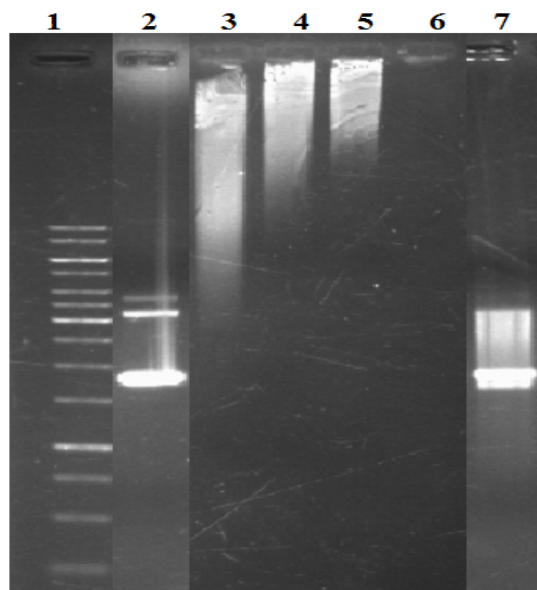


Figure-5

Gel electrophoresis showing the chemical nuclease activity of the pUC18 DNA incubated at 37 °C for a period of 1 h, of complex in the presence of H₂O₂ (100 mM) as an oxidizing agent: lane 1, Marker DNA

lane 2, Control DNA lane 3, pUC18 DNA +H₂O₂ + Ligand
 lane 4, pUC18 DNA+H₂O₂ + Co(II) complex lane 5, pUC18 DNA + H₂O₂ + Ni(II) complex
 lane 6, pUC18 DNA +H₂O₂ + Cu(II) complex lane 7, pUC18 DNA +H₂O₂ + Zn(II) complex .

into lipid membrane and blocks the metal binding sites on enzymes of microorganisms.

DNA Cleavage: The Schiff base Co(II), Ni(II), Cu(II) and Zn(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method figure-5. The gel after electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The complete cleavage of DNA was observed in Cu(II) and Zn(II) complexes of the Schiff base and partial cleavage of DNA was observed in Ni(II) and Co(II) complexes of the Schiff base. The difference was observed in the bands of the complexes compared to that of the control DNA. This shows that the control DNA alone does not show any apparent cleavage whereas the complexes show cleavage. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes is not clear. These results indicate that the metal ions play an important role in the cleavage of isolated DNA. As the compound was observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome^{51, 52}. The studies reveal that complete cleavage of DNA was observed by Cu(II) and Zn(II) complexes and partial cleavage of DNA was observed by Co(II) and Ni(II) complexes.

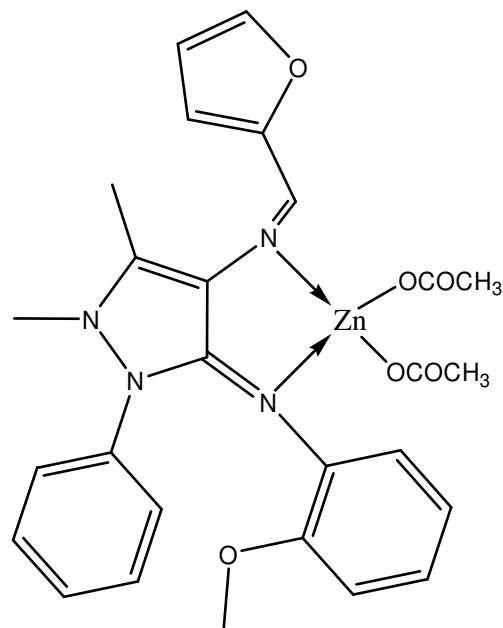
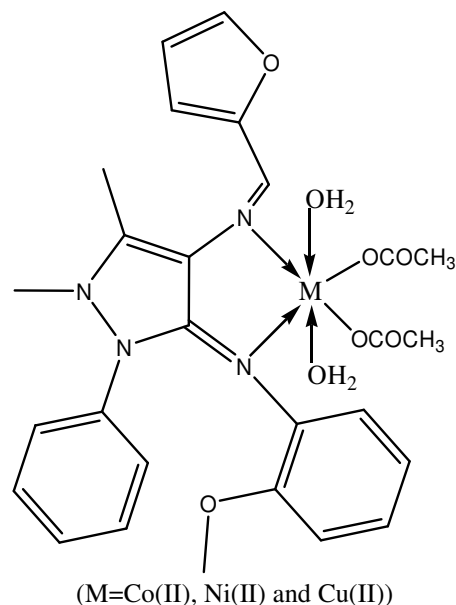


Figure-4

Proposed structure of the complexes

Conclusion

In this work a bidentate Schiff base ligand derived from 4-aminoantipyrine, furfuraldehyde and o-anisidine forms stable complexes with transition metals such as Cobalt (II), Nickel (II), Copper (II) and Zinc (II) are given. The ligand and its complexes are characterized using spectral and analytical data. These analytical and spectral data suggests octahedral geometry to Co(II), Ni(II), Cu(II) and tetrahedral geometry for Zn(II) complexes. The metal complexes have higher antibacterial and antifungal activity than the free ligand. The DNA cleavage studies reveal that complete cleavage of DNA was observed by

Zn(II) and Cu(II) complexes and partial cleavage of DNA was observed by Co(II) and Ni(II) complexes.

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