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Research Article

Synthesis, characterization and Biological studies on some metal complexes incorporating Imidazole-2-carboxaldehyde, 4- aminoantipyrine and 2-aminophenol

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ABSTRACT

Metal complexes of Schiff base (L) ligand, prepared via condensation of imidazole 2 –carboxaldehyde 4-aminoantipyrine, and 2- aminophenol are prepared. The ligand is characterized based on elemental analysis, Mass, IR, and NMR spectra. Metal complexes are reported and characterized based on elemental analyses, IR, electronic spectra, magnetic moment, molar conductance and cyclic voltammetry (cv). From the elemental analyses, 1:1[M]:[Ligand] complexes are prepared with the general formulae [M(L CL2) (M=Co(II), Ni(II), Cu(II), Zn(II) and Cd(II)). The IR results demonstrate that co-ordination sites are the two azomethine nitrogen,imidazole nitrogen and carbonyl oxygen atoms. The electronic spectral and magnetic measurement data indicate that the complexes exhibit tetrahedral geometry and Cu(II) exhibits distorted square planar around the metal centre. The in vitro biological screening effects of the synthesized compounds were tested against various microbial species and the results show that the metal complexes are more biological active than the ligand. The DNA cleavage activity of the ligand and its complexes were assayed on pUC18 DNA using gel electrophoresis. The result shows all the complexes have completely cleaved the DNA.

Keywords: Schiffs base, Electronic spectra, Antimicrobial, DNA, Ligand, Cyclic voltammetry.

INTRODUCTION

Schiff bases of azomethine nitrogen donor heterocyclic ligand are well known due to their wide range of applications in pharmaceutical and industrial fields and have been found to act as antibacterial, antifungal, anticancer and herbicidal agents[1-7]. Schiff base can accommodate different metal centers involving various coordination modes thereby allowing successful synthesis of homo and hetro metallic complexes with varied stereochemistry[8,9]. This feature is employed for modeling active sites in biological systems. Results from these studies have also shown that complexation of metals to Schiff base ligands serves to improve the antimicrobial and anticancer activities of the ligands[10,11]. Metal complexes of nitrogen- oxygen chelating agents derived from 4- aminoantipyrine Schiff bases have been studied extensively due to their pronounced applications in biological, clinical, analytical and pharmacological areas[12-16]. Y.X. sun *et al* have studied the crystal structure of 4-(4-chlorobenzylideneaminno)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one[17]. The present study deals

with the synthesis, characterization and biological studies of the Schiff base derived from imidazole -2-carboxaldehyde 4-aminoantipyrine and 2-aminophenol and its Co(II),Ni(II) Cu(II),Zn(II) and Cd(II) complexes.

EXPERIMENTAL SECTION

4- Aminoantipyrine and imidazole 2-carboxaldehyde- were obtained from Sigma. Metal (II) chlorides were purchased from Merck. All other chemicals used were of AnalaR grade. Solvents were purified and distilled before use. The metal content in the complexes was determined by EDTA titration[18]. Elemental analysis was obtained using a Perkin-Elmer elemental analyzer. Conductivity measurements were made on freshly prepared 10^{-3} M solutions in DMSO at room temperature with a coronation digital conductivity meter. The IR spectra were recorded in KBr pellet on a JASCO FT/IR-410 spectrometer in the range 4000-400 cm^{-1}. Electronic spectra were recorded on a perkin Elmer Lambda-25 UV/VIS spectrometer. The room temperature magnetic measurements were carried out using Guoy balance and the diamagnetic corrections were made using Pascal's constant. Cyclic voltammetric measurements were carried out in a Bio-Analytical system (BAS) model CV-50W electrochemical analyzer. The three electrode cell comprised of a reference Ag/AgCl, auxiliary platinum and working glassy electrodes. Tetrabutylammonium perchlorate was used as supporting electrolyte.

Synthesis of Schiff base ligand

Imidazaldimine – 4 – amino antipyrine was prepared by the condensation of imidazole – 2 carboxaldehyde and 4 aminoantipyrine. Imidazaldimine 4 amino antipyrine (2 mmol) and 2 – aminophenol (2 mmol) were taken in methanol (50 ml) solvent. To this mixturel 1 g of anhydrous potassium carbonate was added and then refluxed for 42 hrs. The resutting solution was concentrated on a water bath and allowed to cool at 0^{o}c for around 38h. the red colour solid product formed was separated by filtration and washed thoroughly with cold EtoH and then dried in vacuum. The yield of the isolated ligand was found to be 35 %.

Synthesis of metal Schiff base complexes

A solution of imal – 4 – AAP – 2 AP (2mmol) in MeoH (20ml) was added to a solution of metal (II) Chloride (2mmol) in 20 ml of aqueous MeoH and the reaction mixture was stirred and then refluxed for 2 hrs. The resulting solution was cooled to room temperature and the volume was reduced to half of the initial volume under reduced pressure. The precipitate was filtered, washed several times with cold EtoH, ether and then dried in vacuum over anhydrous $cacl_2$

In vitro antimicrobial activity

Antibacterial and antifungal activities of the ligand and its complexes were tested in vitro against the bacterial species Escherichia coli,Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus;fungal species,Aspergillus niger,Aspergillus flavus and Candida albicans by the disc diffusion method[19]. Amikacin was used as the standard antibacterial agent whereas Nystatin was used as the standard antifungal agent. The test organisms were grown on nutrient agar medium in petri plates. The compounds were prepared in DMF and soaked in filter paper disc of 5 mm diameter and 1 mm thickness. The discs were placed on the previously seeded plates and incubated at 37 ^{O} C and the diameter of inhibition zone around each disc was measured after 24 h for bacterial and 72 h for fungal species.

DNA cleavage analysis

The compounds were added separately to the pUC18 DNA sample. The samples mixtures were incubated at 37 ^{O}C for 2h. The electrophoresis of the samples was done according to the following procedure. Weigh 300 mg of agarose and dissolve it in 25 ml of TAE buffer (4.84 g Tris base,pH 8.0,0.5 M EDTA/1 ltr) by boiling. When the gel attains ~55 ^{O} C,pour it into the gel cassette fitted with comb. Let the gel to solidify. Carefully remove the comb,place the gel in the electrophoresis chamber flooded with TAE buffer. Load DNA sample (mixed with bromophenol blue dye @ 1:1 ratio),carefully into the wells, along with standard DNA marker and pass the constant 100 V of electricity till the dye front reaches the end of gel. Remove the gel and carefully stain with ETBR solution (10ug/ml) for 10-15 min and observe the bands under UV transilluminator.

RESULTS AND DISCUSSION

Characterization of Schiff base ligand

The results of elemental analysis (Table 1) of the Schiff base ligand are in good agreement with those calculated for the suggested formula. The Schiff base is soluble in all common organic solvents. The DART mass spectrum of the ligand shows a well-defined molecular ion peak at m/z=372.89(Relative Intensity=100%)which coincides with formula weight of the Schiff base.in the ^{1}H-NMR spectrum (Fig.1) the singnal for azomethine proton (-CH=N-) in the ligand appears as a singlet at 9.45 ppm. The multiplet signals obtained in the δ 7.0-8.0 ppm range

are due to the aromatic protons of Schiff base ligand. The signal for pyrazolone ring carbon attached methyl protons (-CH_3) appear as a singlet at δ 2.42 ppm while pyrazolone ring nitrogen attached methyl protons (>N-CH_3) appear as a singlet at δ 3.09 ppm. In the ^{13}C-NMR spectrum (Fig. 2), the azomethine carbon signal has appeared at 171ppm. The pyrazolone ring carbon attached methyl carbon (-CH_3) and pyrazolone ring nitrogen attached methyl carbon (>N-CH_3)peaks have been observed in the expected range at 12and 36 ppm. The aromatic and indole ring carbon signals are seen at 106-171 ppm range depending on their electronic environment. The IR spectrum of the ligand displays a sharp band at 1630 which can be assigned to > C=N stretching frequency. The electronic spectrum of the ligand shows a broad band at 344 nm, due to π - π * transition of the azomethine(>C=N) chromophore. In addition, the other intense absorption band at higher energy,200-300 nm,is due to theπ-π*transition of the benzene ring other characteristic transition of the Schiff base .

Characterization of metal Schff base complexes

The analytical data and physical properties of the metal Schiff base complexes are listed in table 1. The Schiff base complexes are soluble in CH_3CN,DMF and DMSO and insoluble in other common organic solvents. The analytical data indicate that the metal to ligand ratio1:1 for all the complex systems. The high conductivity values of the metal complexes (Table 1)suggest their -electrolytic nature[20]. The DART mass spectrum of Co(II),Ni(II),Cu(II),Zn(II) and Cd(II) complexes shows a peak at m/z 465.38 (10%), 465. 74(8 %) 471.07 (11%) 472.65 (14 %) and 520.02 (12%) respectively, corresponding to their molecular weight. The mass spectrum of all the complexes indicates that the complexes are monomeric confirming the metal to ligand ratio to be 1:1 in the complexes.

IR Spectra

The important IR spectral data are given in Table 2. On complexation the band at 1595 cm-1 and 1630 for the two azomethine groups in the free ligand was shifted to lower frequency in the range 1588– 1614 indicating the coordination of the azomethine nitrogen atom to the metal ion.

In the Schiff base ligand imidazole nitrogen band appeared at 1290cm.This band wqas shifted to lower frequency indicating that the imidazole nitrogen is co-ordinated to the matal ion.Further the disappearance of (OH)peaks indicating the linkage between the deprotonatedhydroxyl oxygen atom to the metal ion.. Further the spectrum of all the metal complexes show new bands in the 552-473 cm -1 and 545 – 472 cm-1 regions, which may probably be due to the formation of M-O and M-N bonds, respectively[21].

Electronic Spectra

The electronic spectrum of co (II) complex exhibits transition at 550nm. The transitions of the co (II) complex can be assigned to $^4A_2$(f) -> $^4T_1$ (P) which demonstrates its Tetrahedral. The electronic spectrum of the Ni(II) complex shows band in the region 640nm attributable to $^3T_1$(F) -> $^3T_1$(P) transition suggesting tetrahedral geometry around the Ni(II)[22]. The Cu(ii) complexes in their spectra display a broad band at 620nm region due to $^2B_{1g}$ -> $^2A_{1g}$ indicating the complex to have distorted square planer geometry[22].

Magnetic measurements

The Co (II) complexes has a magnetic moment of 4.33 B.M(Table 1), which is in agreement with the reported value for Tetrahedral Co (II) complexes [23,24]. The present Ni (II) complexe shows magnetic moment value of 3.59 within the range of 3.2-4.1 [23,24]. Suggesting tetra hedral environment. The Cu(II) complex shows magnetic moment value 1.73 BM, higher than the spin-only value 1.79 BM expected for one unpaired electron, monomeric and consistent with a distorted square planar geometry[23,24]. The complexes of Zn(II) and Cd(II) are diamagnetic and according to the empirical formulae of these complexes, and tetrahedral geometry is proposed. Based on the above results, one can deduce the probable structures of the complexes as shown Fig.3.

Cyclic voltammetric studies

The cyclic voltammogram of the Co(II) complex shows a well defined redox process corresponding to the formation of the quasi-reversible Co(II)/Co(I) couple. The cathodic peak at -0.840 V versus Ag/AgCl and the associated anodic peak at -0.637 corresponds to the Co(II)/Co(I) couple. The peak to peak separation (ΔEp) is 0.203 indicating quasi-reversible one electron transfer process. The redox property of Ni (II) complex displayed quasi – reversible one electron transfer process cathodic peak at -0.991, anodic peak, -0.543 and the peak to peak separation (ΔEp) of 0.448. The cyclic voltammogram of the Cu(II) complex displayed two reduction couples at + 0.059 and -0.675v versusAg|Agcl with the corresponding anodic wave for the first reduction and without the corresponding anodic wave(+0.02) for the second reduction on the reverse scan. The former has alower peak separation value of 0.032

indicating totally reversible character for the two electron transfer reaction of metal based Cu(II)/Cu(0) couples and the later one can be assigned to Cu(II)/Cu(I) ir-reversible reduction process. . The cyclic voltammogram of the Zn(II) and Cd(II) complexes didn't show any characteristic peak potential indicating that the complexes stabilize the ligand in +2 oxidation state.

Biological studies

In vitro antimicrobial activity

The in vitro biological screening results are given in table 3. The standard error for the experiment is =0.001 cm and the experiment is repeated three times under similar conditions. DMF is used as negative control and Amikacin is used as positive standard for antibacterial and Nystatin for antifungal activities.

From the result, it has been observed that the metal complexes showed better activity than the free ligand under identical experimental condition. The enhanced activity of the complexes can be explained on the basis of Overtone`s concept[25] and Tweedy`s chelation theory[26]. According to Overtone`s concept of cell permeability,the lipid membrane that surrounds the cell favours the passage of only the lipid –soluble materials makes 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 the positive charge of the metal ion with donor group s. Further ,it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the permeation of the complexes in to 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 the proteins that restricts further growth of the organisam and as a result microorganisms die. On comparing the biological activity of the Schiff base and its metal complexes with the standard,it is seen that the biological activity follows the order, Cu(II)> Co(II)> Zn(II)>Ni(II)>Cd(II)>L.

DNA cleavage studies

Gel electrophoresis experiments using pUC18 DNA were performed with ligand and its metal complexes in the presence of H2o2 as an oxidant. From Fig 4,it is evident that all the complexes cleave DNAcompletely in the presence of H_2O_2. The general oxidative mechanisms proposed account for DNA cleavage by hydroxyl radicals via abstraction of a hydrogen 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. The capacity of metal complexes to active dioxygen, or its reduced form hydrogen peroxide, will lead to the functionalization of an inert C-H bond of DNA to a C-O bond. DNA oxidation by metal complexes occurs by C-H bond activation at the deoxyriboses[27, 28].

CONCLUSION

Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes with the schiff base ligand derived from imidazole 2-carboxaldehyde , 4- aminoantipyrine and 2-aminophenol were synthesized and characterized by various physico – chemical method. The analyses confirmed the composition and structures of the newly obtained complex combinations. The coordination of the Schiff base to the metal atom was found to be through the azomethine nitrogen, imidazole Nitrogen and the carbonyl oxygen atoms. The geometry of the complexes is assigned as tetrahedral. The Cu(II) complex shows better activity against most of the microbial species compared to that of ligand and other complexes. The DNA cleavage studies show that all the complexes have completely cleaved the DNA.

Table. 1
Analytical and physical data of Imal-4-AAP-2-AP and its complexes

Compounds	Empirical formula	Colour	Mol. Wt	Found (Calc.) (%)				Molar conductance ($\Omega^{-1}cm^2$ mol^{-1})
				C	H	N	M	
Imal-4-AAP-2AP	$C_{21}H_{20}N_6O$	Brown	372.89	67.86 (67.73)	5.36 (5.41)	22.93 (22.57)	-	-
Co(II)-Imal-4-AAP-2AP	$C_{21}H_{19}N_6OClCo$	Dark green	465.38	54.65 (54.15)	3.94 (4.11)	18.33 (18.04)	12.38 (12.65)	93
Ni(II)-Imal-4-AAP-2AP	$C_{21}H_{19}N_6OClNi$	Light green	465.74	54.49 (54.18)	4.02 (4.11)	18.31 (18.05)	11.09 (11.61)	95
Cu(II)-Imal-4-AAP-2AP	$C_{21}H_{19}N_6OClCu$	Brown	471.07	53.18 (53.62)	3.91 (4.07)	17.28 (17.87)	13.76 (13.51)	102
Zn(II)-Imal-4-AAP-2AP	$C_{21}H_{19}N_6OClZn$	Light yellow	472.65	53.21 (53.41)	3.89 (4.06)	17.54 (17.80)	13.36 13.85)	90
Cd(II)-Imal-4-AAP-2AP	$C_{21}H_{19}N_6OClCd$	Light yellow	520.02	48.07 (48.57)	3.54 (3.69)	16.34 (16.18)	21.47 (21.65)	95

Table 2
IR spectral data of Imal-4-AAP-2-AP and its complexes (cm^{-1}).

Compounds	υ(C=N)	υ_{ring}(C=N)	υ_{ring}(C-N)	υ(OH)	υ(M-O)	υ(M-N)
Imal-4-AAP-2-AP	1595	1630	1290	3180	-	-
Co(II)-Imal-4-AAP-2-AP	1590	1620	1290	-	552	473
Ni(II)-Imal-4-AAP-2-AP	1592	1622	1291	-	548	474
Cu(II)-Imal-4-AAP-2-AP	1590	1614	1291	-	548	477
Zn(II)-Imal-4-AAP-2-AP	1593	1615	1290	-	541	471
Cd(II)-Imal-4-AAP-2-AP	1588	1620	1296	-	545	472

Table . 3
Antimicrobial activity results of Imal-4-AAP-2-AP and its complexes

Compounds	Inhibition zone (mm)						
	Bacteria species				Fungi species		
	S.aures	*E.coli*	*P. aeruginosa*	*B. subtilis*	*C. albicans*	*A. niger*	*A. flavus*
Imal-4-AAP-2-AP	_	10.2	10.2	12.5	_	10.2	_
Co(II)-Imal-4-AAP-2-AP	12.3	12.5	10.2	12.5	_	_	8.5
Ni(II)-Imal-4-AAP-2-AP	_	_	12.5	-	10.5	12.5	9.8
Cu(II)-Imal-4-AAP-2-AP	14.3	11.2	10.5	10.5	11.5	12.5	_
Zn(II)-Imal-4-AAP-2-AP	12.5	12.5	12.2	-	10.5	9.5	9.5
Cd(II)-Imal-4-AAP-2-AP	8.5	10.5	11.4	-	-	-	-
*Amikacin**	19.2	20.5	20.5	18.5	-	-	-
*Nystain**	-	-	-	-	19.5	20.0	19.5

*standard

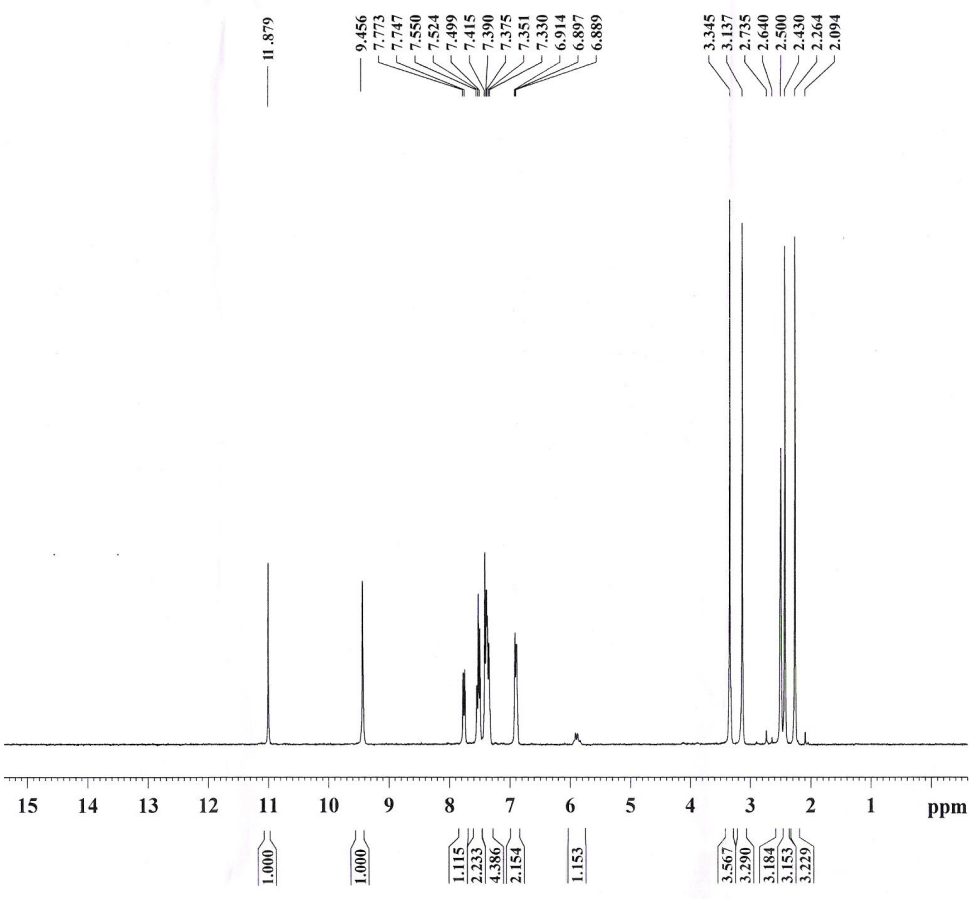


Fig. 1[1]H-NMR spectrum of Imal-4-AAP-2-AP

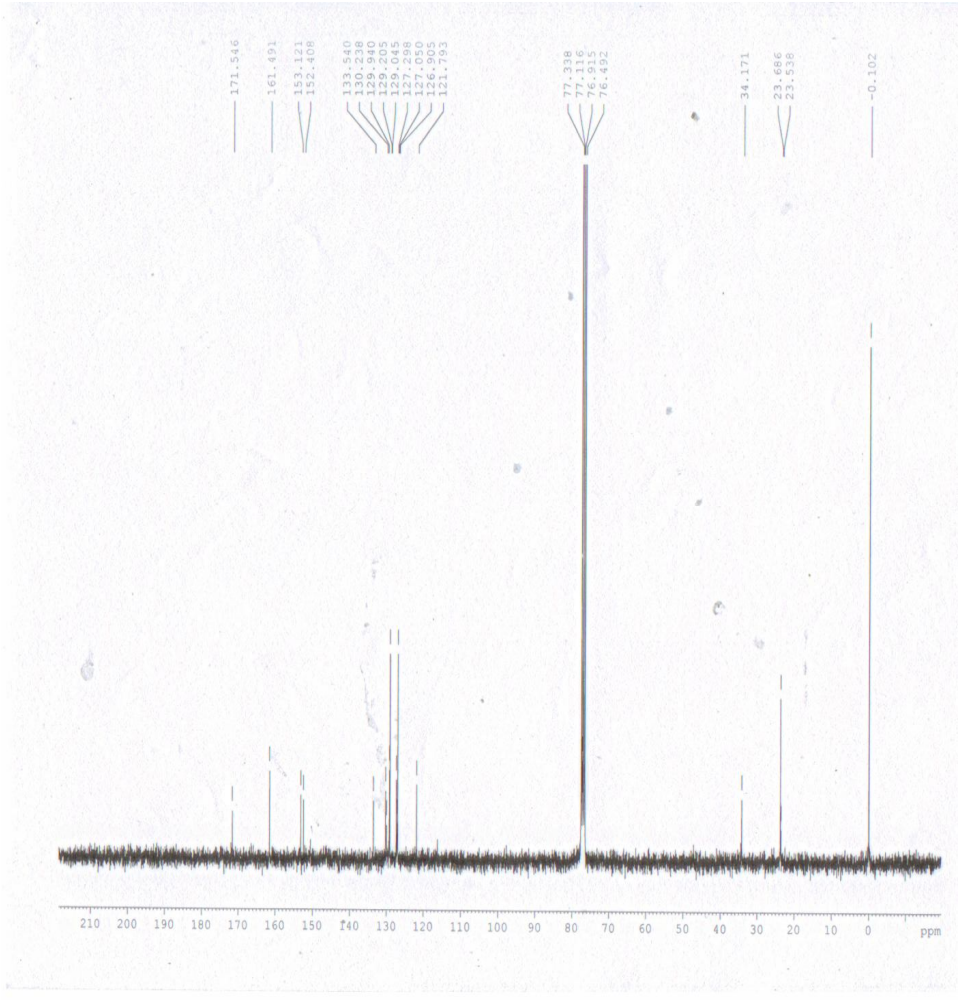
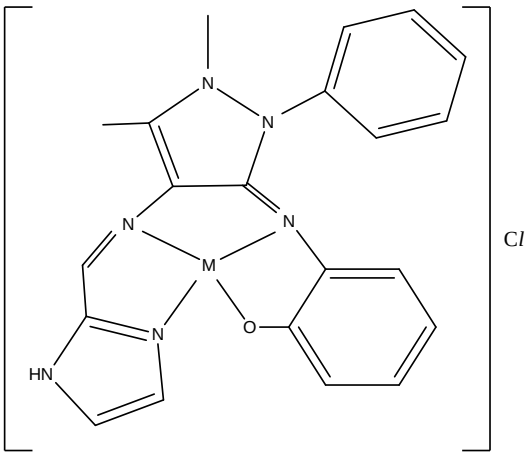


Fig. 2. ^{13}C-NMR spectrum of Imal-4-AAP-2-AP



M = Co, Ni, Cu, Zn and Cd

Fig.3. Proposed structure of M(II)-Imal-4-AAP-2-AP complexes

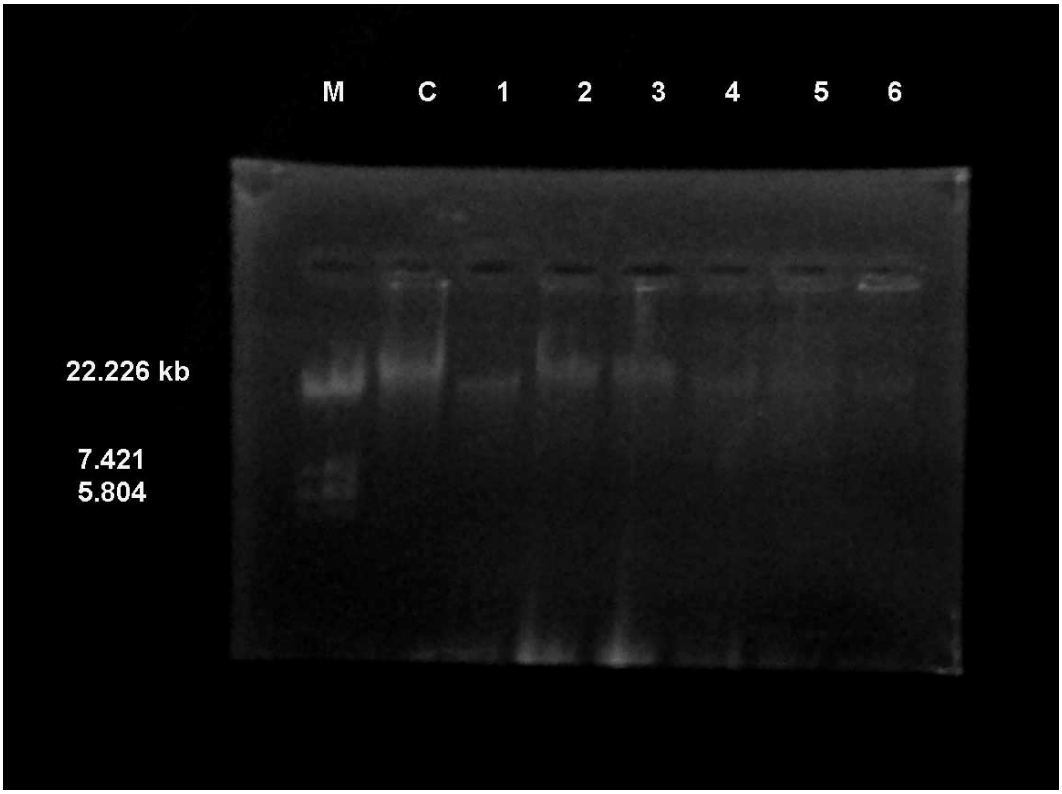


Fig.4 DNA cleavage studies of Imal-4-AAP-2-AP and its complexes

M- Marker,

C- Control pUC 18 DNA (untreated sample),

1- [CuL]Cl + DNA + H_2O_2,

2- [CoL]Cl + DNA + H_2O_2,

3- [CdL]Cl + DNA + H_2O_2,

4- [NiL]Cl + DNA + H_2O_2,

5-[ZnL]Cl + DNA + H_2O_2,

6- ligand + DNA + H_2O_2

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