

Received: 03rd May-2013

Revised: 09th June-2013

Accepted: 12th June-2013

Research article

**SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND DNA CLEAVAGE STUDIES OF
TRANSITION METAL COMPLEXES OF 4-(TRIFLUORO-4-YLIDENE) HYDRAZINE
CARBOTHIOAMIDE.**

Richa Kothari* and Brajraj Sharma¹

Department of Chemistry, ITM University, Gwalior India

Email: richa.kothari.chem@itmuniverse.in

ABSTRACT: New complexes of Co(II), Ni(II), Cu(II), Cd (II), Pt(II),and Pd(II) with 4-(trifluoro-4-ylidene) hydrazine carbothioamide have been synthesized. All the new compounds were characterized by elemental analysis, molar conductance measurements, magnetic susceptibility measurements, LC-MS. ¹H NMR, FTIR and electronic spectral studies. Based on the molar conductance measurements in DMF the complexes may be formulated as [Ni(L)₂Cl₂] and [M(L)₂]X₂ (where M = Co (II), Cu (II), Cd (II), Pt (II) and Pd(II) and X = Cl⁻, CH₃COO⁻ due to their non electrolytic nature respectively. The antimicrobial activities of the ligand and their complexes have been studied by screening the compounds against the bacteria *E. coli* and *S. aureus* and also the fungi *Asperillus niger* and candida albicans and results have been compared with standard drug streptomycin and fluconazole. The data indicate that the metal complexes have higher antimicrobial activity than the free ligand and the standard drug. The DNA cleavage experiments, performed using gel electrophoresis with the corresponding metal complexes in the presence of H₂O₂ showed that all the complexes afford a pronounced discernible DNA cleavage evidenced by the disappearance of form I (supercoiled) of DNA and the production of formII (Linear).

Keywords: Transition metal complexes, hydrazine carbothioamide, spectral studies, DNA cleavage, Antimicrobial studies.

INTRODUCTION

Interest in thiosemicarbazone chemistry has flourished for many years, largely as a result of its wide range of uses, for example, as antibacterial, antifungal, chemotherapeutic and bio- analytical reagents (Arion et al, 2002, Gracia et al, 2003, Hu et al, 2006, Jouad et al, 2001, Golcu et al, 2006, Balnz and French, 1968). Coordination complexes of thiosemicarbazone ligands are gaining increasing importance in recent years particularly in the design of repository; slow release or long acting drugs in nutrition and in the study of metabolism. The activity of these compounds is strongly dependent on the nature of the hetero atomic ring and the position of attachment to the ring as well as the form of thiosemicarbazone moiety (Singh et al, 2005). These are studied extensively due to their flexibility, selectivity and sensitivity towards the central metal atom and structural similarities with natural biological substances, and due to the presence of imine group (-N=CH-) which imparts the biological activity (Chandra et al, 2001, Raman et al, 2001, 2002, 2005, singh et al, 2004). Among the metal complexes of thiosemicarbazones, the Palladium (II) chelates have been especially studied regarding their antitumor potentials (Tosi, 1986, Hall et al 2000). Moreover, Palladium (II) complexes with nitrogen – containing ligands are the subject of intensive biological evaluation in the search for less toxic and more selective anticancer therapies (Jakupee et al, 2003, Giovagnini et al 2005). In addition to these, Ni (II) and Pt (II) complexes of thiosemicarbazones have been reported as compounds that present biological activity (Chandra et al 2007, Kovala et al 1997). In view of the above discussion, in the present paper “Synthesis, Characterization, Antimicrobial and DNA cleavage studies of transition metal complexes of 4-(trifluoro-4-ylidene) hydrazine carbothioamide” are discussed.

Experimental: - All reagents used for the preparation of the ligand and its complexes were of Merck products. All the employed solvents were of standard spectroscopic grade.

Synthesis of 4-(trifluoro-4-ylidene) hydrazine carbothioamide ligand.

A hot ethanolic solution (20ml) of thiosemicarbazide (2.5 gm, 0.020 mol) and an ethanolic solution (20 ml) of p-trifluorobenzaldehyde (2.1 g, 0.020 mol) were mixed slowly with constant stirring. This mixture was refluxed at 70-80°C for 4 hr. On cooling, a white colored compound precipitated out, which was filtered, washed with cold ethanol and dried under vacuum over P₄O₁₀. Yield: 64%; m.pt. 220°C Anal. Calcd. (%) for C₉H₈N₃F₃S (244); C(61.20); H,(3.42); N, (17.6)., Found (61.24); H, (3.44) N, (17.8).

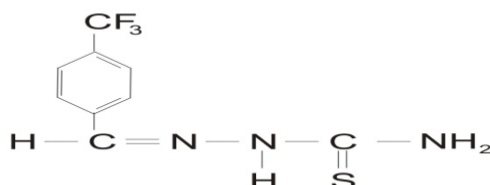


Fig 1. Structure of the 4-(trifluoro-4-ylidene) hydrazine carbothioamide ligand.

Synthesis of complexes

A hot ethanolic solution (20ml) of the required metal salts (1mmol) was mixed with a hot ethanolic solution 20ml of the required ligand (1mmol). This reaction mixture was continuously stirred and refluxed for 6 hr at 75°C. On cooling, a colored complex separated out, which was filtered, washed with cold ethanol and dried under vacuum over P₄O₁₀.

Physical measurements

Elemental analysis was performed at RSIC, CDRI, Lucknow and the nitrogen content of the complexes was determined using the Kjeldhal method (Vogels, 1989). The molar conductivity was measured on a Elico digital conductivity bridge. The magnetic moment was measured at room temperature on a Gouy balance using CuSO₄.5H₂O as the Calibrant. Electronic impact mass spectra were recorded on a JEOL – DX-303 mass spectrometer. The ¹H NMR spectra of the ligands and the metal complexes were of recorded at room temperature and a Bruker Advance DPX – 300 spectrometer using DMSO – d₆ as the solvent by employing TMS as internal standard. The IR spectra were recorded as KBr pellets on a FTIR spectrophotometer. The Electronic spectra were recorded in DMSO on a shimadzu-uv mini – 1240 spectrophotometer. Gel electrophoresis experiment was done by solutions of CT-DNA in 50 mM NaCl/5 mm tris – HCl (pH = 7.0). It gave a ratio of uv absorbance at 260 and 280 nm of Ca 1.8-1.9, indicating that the DNA was sufficiently free of protein contamination. The molar absorption coefficient was taken as 6600 m⁻¹cm⁻¹. Stock solutions were kept at 4° C and used within 4 days. Doubly distilled water was used to prepare the buffer.

Antibacterial screening:-

The invitro biological screening effects of the investigated compounds were tested against the bacterial strains Bacillus subtilis, E. coli and s.aureus by spread plate technique using nutrient agar medium. The stock solutions of compounds were prepared by dissolving the compounds in DMSO. Streptomycin was used as standard drug and DMSO served as control. Antibacterial potency of ligand and the metal (II) complexes were measured against all the tested bacteria according to the standard disc diffusion method where air dried sterile whatman filter paper discs (6 mm diameter) with centers of at least 24 mm apart were deposited on nutrient agar plate using aseptic technique. Bacterial inoculums containing approximately, 10⁴~10⁶ colony forming units CFU m⁻¹ were spread on the surface of nutrient agar. The test complex at dose 10 µg / disc was added into disc. The fourth disc was supplemented with reference drug streptomycin at dose 10 µg / disc serving as positive control. The plates were incubated immediately at 37° C for 24 hrs. Activity was determined by measuring the diameter of zones (mm) showing complete inhibition. Growth inhibition was calculated with respect to positive control.

Antifungal screening

One animal fungus (*Candida albicans*) and one plant fungi (*Aspergillus niger*) were selected for this study. Soubared dextrose agar (SDA) for animal fungi and potato dextrose agar (PDA) for plant fungi were used for fungal growth media.

Antifungal activities of ligands and the metal (II) complexes were observed against both the test microorganism according to the standard disc diffusion method to (Beur et al., 1966), where air dried sterile whatman filter paper discs (6 mm diameter) with centers of at least 24 mm apart were deposited on growth media in plate using aseptic technique (Patel et al., 1993). Fungal inoculum containing approximately 10^5 spores / ml was spread on the surface of growth media. The test compound at dose 10 $\mu\text{g}/\text{disc}$ and known drug Fluconazole INN at dose 10 $\mu\text{g}/\text{disc}$ serving as positive control were applied into disc respectively. Plates were kept at low temperature (4°C) for 24 hrs to allow maximum diffusion then incubated immediately at $18\text{--}27^\circ\text{C}$ for 5 days. Activity was confirmed by measuring the diameter of zone (mm) showing complete inhibition. Growth inhibition was calculated with respect to positive control.

Assay of Nuclease activity

The efficiency of DNA cleavage was measured by determining the ability of the complexes to form open circular (OC) or nicked circular (NC) DNA from its super coiled (SC) form. The DMF solution (1×10^{-3} M) containing metal complexes (5 μL , 250 μM) were taken in a clean eppendroff tube and 1 μg of CT-DNA was added. The contents were incubated for 30min at 37°C and loaded on 0.8% agarose gel after mixing 3 μL of loading buffer (0.25% bromophenol blue + 0.25% xylene cyanol + 30% glycerol sterilized distilled water). Electrophoresis was performed at constant voltage (75v) until the bromophenol blue reached upto $\frac{3}{4}$ length of the gel. Further the gel was stained for 10min by immersing it in ethidium bromide solution (5 $\mu\text{g}/\text{ml}$ of water) and then destained for 10 min by keeping it in sterile distilled water. The plasmid bands were visualized by photographing the gel under a Uv transilluminator. The reaction was carried out under oxidative or hydrolytic conditions [20].

RESULT AND DISCUSSION

The transition metal complexes of $[\text{NiL}_2\text{Cl}_2]$ and $[\text{M}(\text{L})_2\text{X}_2]$ (where M = Co(II), Cu(II), Cd(II), Pd(II) and Pt(II)) complexes were synthesized by the condensation of hydrazine carbothioamide ligand with metal salts in a 2:1 molar ratio. (Where X = Cl^- , CH_3COO^-). All complexes are stable to the atmosphere and had high melting points. Elemental analysis were within $\pm 0.5\%$ for C, H and N and the low molar conductance values of all the complexes in DMSO at room temperature indicated them to be non-electrolyte. However, we could not grow single crystals suitable for X-ray crystallographic studies. All complexes are freely soluble in DMF, DMSO, and Ethanol but insoluble in water. Physical properties and molecular weight calculated by cryoscopic method are given in Table 1.

Table-1 Physical and Analytical data of the ligand and their Transition metal complexes.

| S.No | Molecular formula & molecular weight | Color | Yield | M.P.($^\circ\text{C}$) | Molar Conductance ($\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) | % N | %C | % H | % S | % M |
|------|---|--------|-------|--------------------------|---|-----------------|------------------|----------------|------------------|--------------|
| 1. | $\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S}$ 244 | Yellow | 85 % | 150 | ---- | 17.6 (17.7) | 61.20 (61.21) | 3.42 (3.44) | 14.24 (14.26) | ---- |
| 2. | $[\text{Co}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ 617.93 | blue | 75 % | 238 | 40.1 | 17.4 (17.2) | 53.60 (53.61) | 3.34 (3.36) | 12.66 (12.68) | 6.5 (6.6) |
| 3. | $[\text{Ni}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ 617.70 | Green | 40 % | 310 | 36.4 | 32.8 (32.9) | 52.80 (52.81) | 3.42 (3.43) | 14.60 (14.61) | 7.2 (7.4) |
| 4. | $[\text{Cu}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2]$ $(\text{CH}_3\text{COO})_2$ 687.65 | Yellow | 75 % | 205 | 2.1 | 36.4 (36.6) | 52.43 (52.44) | 3.28 (3.29) | 15.08 (15.1) | 7.9 (8.0) |
| 5. | $[\text{Cd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ 671.91 | White | 95 % | 320 | 31.4 | 17.9 (17.10) | 53.94 (53.95) | 3.26 (3.27) | 12.27 (12.29) | 8.1 (8.3) |
| 6. | $[\text{Pd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ 665.4 | Red | 50% | 280 | 23.9 | 18.2 (18.4) | 60.88 (60.89) | 3.22 (3.24) | 12.26 (12.28) | 7.3 (7.4) |
| 7. | $[\text{Pt}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ 754.09 | Green | 65 % | 320 | 40.9 | 20.8 (20.9) | 60.88 (60.90) | 3.65 (3.66) | 30.16 (30.18) | 7.8 (8.0) |

IR spectra

The infrared spectra of the free Schiff base ligand (Table -2) showed a strong band in the region of 1532-1599 cm^{-1} , which is characteristic of the azomethine (C=N) group [21]. According to the literature (Mitra et al 2006, Kumar et al 2010, Rajendran et al, 2010, Pouralimardan et al, 2007, Imran et al, 2010, Mohamed and Sharaby, 2007, Nakamoto K, 1997), the participation of the nitrogen atom of the Schiff base ligands in the coordination sphere should reduce the bond order in the C=N group due to the strong donation of electron density toward the metal ion and result in a decrease in the C=N stretching frequency. In the IR spectra of the Schiff base complexes (Table-2), the band due to $\nu(\text{C}=\text{N})$ showed a negative shift and appeared at 1618-1636 cm^{-1} , indicating coordination of the azomethine nitrogen to metals (Roy, 2009, Naresh Kumar and Ramesh, 2004). This range of $\nu(\text{C}=\text{N})$ vibrations is in the region reported for the coordinated C=N group (Pawar et al 2004). The lower values of $\nu(\text{C}=\text{N})$ may be explained on the basis of a drift of the lone pair density of the azomethine nitrogen towards the metal atom. The medium intensity bands that appeared in the 440-471 cm^{-1} region are assignable (Singh et al 2010) to $\nu(\text{M}-\text{N})$ vibrations. They appear area of the sharp single band in the 3137-3443 cm^{-1} region assignable to the condensed N-H group (Prasad et al 2007). This is further confirmed by the appearance of a characteristics $\nu(\text{C}-\text{N})$ band at around 1163-1281 cm^{-1} . The bands in the region 697-762 cm^{-1} may be assigned to $\nu(\text{C}-\text{H})$ out of plane bending of aromatic ring.

Table 2: IR spectral data of the Schiff base ligand and their metal complexes.

| S.No. | Complex | $\nu(\text{NH}_2)$ | $\nu(\text{N-H})$ | $\nu(\text{Ar-H})$ | $\nu(\text{C}=\text{N})$ | $\nu(\text{C}=\text{S})$ |
|-------|---|--------------------|-------------------|--------------------|--------------------------|--------------------------|
| 1. | $\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S}$ | 3271 | 3443 | 3012 | 1599 | 1162 |
| 2. | $[\text{Co}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 3276 | 3434 | 3013 | 1609 | 1152 |
| 3. | $[\text{Ni}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 3273 | 3430 | 3006 | 1610 | 1165 |
| 4. | $[\text{Cu}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] (\text{CH}_3\text{COO})_2$ | 3354 | 3493 | 2926 | 1578 | 1168 |
| 5. | $[\text{Pd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 3251 | 3394 | 2991 | 1618 | 1163 |
| 6. | $[\text{Cd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 3289 | 3453 | 3004 | 1607 | 1187 |
| 7. | $[\text{Pt}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 3262 | 3454 | 3149 | 1613 | 1168 |

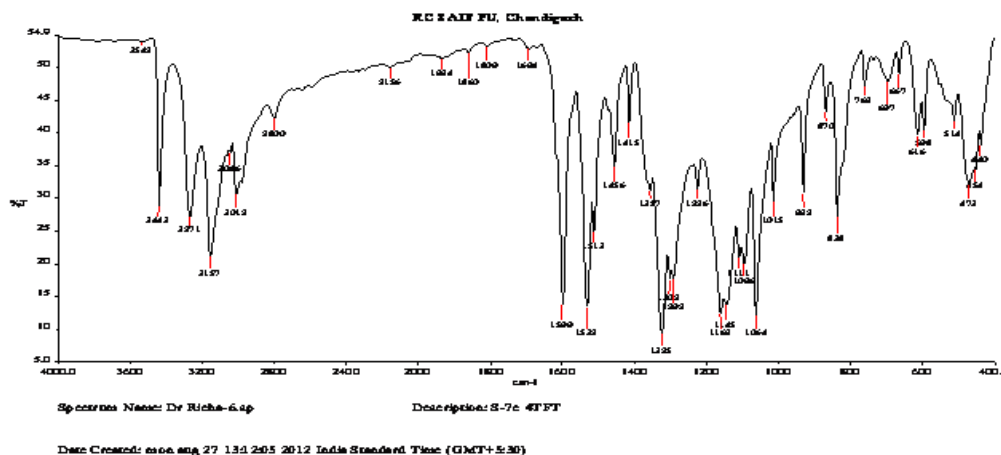


Fig-2. IR Spectra of 4-(trifluoro-4-ylidene) hydrazine carbothioamide ligand.

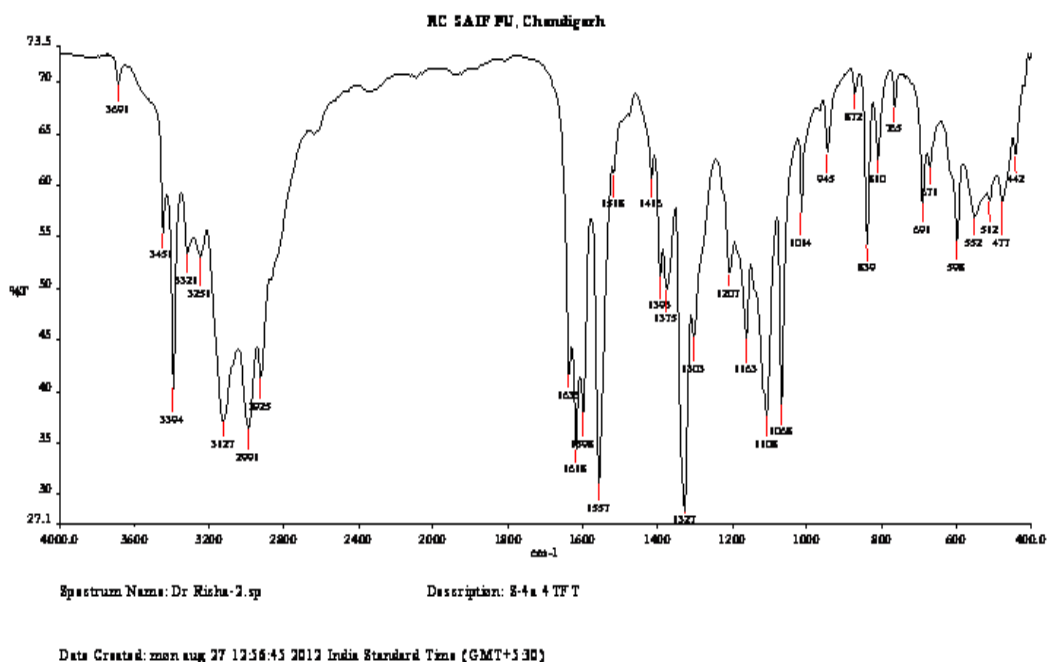


Fig-3. IR spectra of $[\text{Cd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ complex

NMR Spectra

The ^1H NMR spectra of all complexes were obtained in the CDCl_3 at room temperature using TMS as an internal standard. The aromatic region shows a sharp singlet at δ 7.63 ppm assigned to the phenyl protons and a quartet at δ 2.56 ppm due to NH_2 protons. The multiplets observed in the region 7.63-7.94 ppm may be assigned to the aromatic protons of benzene ring (Costamapna J, et al 2000). The ^1H NMR spectra of metal complexes shows signals corresponding to $-\text{NH}_2$, and aromatic (C-H) protons at 2.25 respectively.

Table 3 - ^1H NMR spectral data of Schiff base ligand and their metal (II) complexes.

| S.No. | Complexes | δNH_2 | δNH | $\delta \text{HC}=\text{N}$ | $\delta \text{Ar-C-H}$ |
|-------|---|----------------------|--------------------|-----------------------------|------------------------|
| 1. | $\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S}$ | 2.56 | 11.61 | 8.12 | 7.63 |
| 2. | $[\text{Co}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 2.67 | 11.30 | 7.98 | 7.62 |
| 3. | $[\text{Ni}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 2.56 | 11.65 | 7.86 | 7.47 |
| 4. | $[\text{Cu}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] (\text{CH}_3\text{COO})_2$ | 2.55 | 11.42 | 7.98 | 7.62 |
| 5. | $[\text{Pd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 2.12 | 10.79 | 8.6 | 7.63 |
| 6. | $[\text{Cd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 2.55 | 11.65 | 8.12 | 7.47 |
| 7. | $[\text{Pt}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 2.12 | 10.12 | 8.12 | 7.63 |

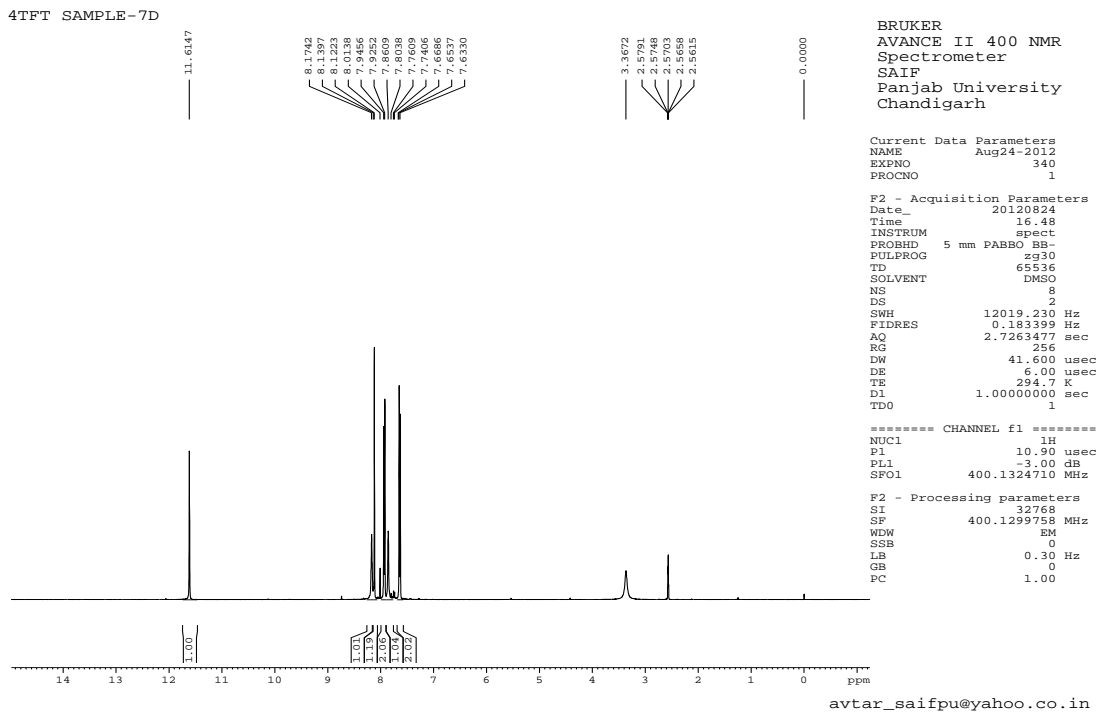


Fig-4 NMR spectra of 4-(trifluoro-4-ylidene) hydrazine carbothioamide ligand.

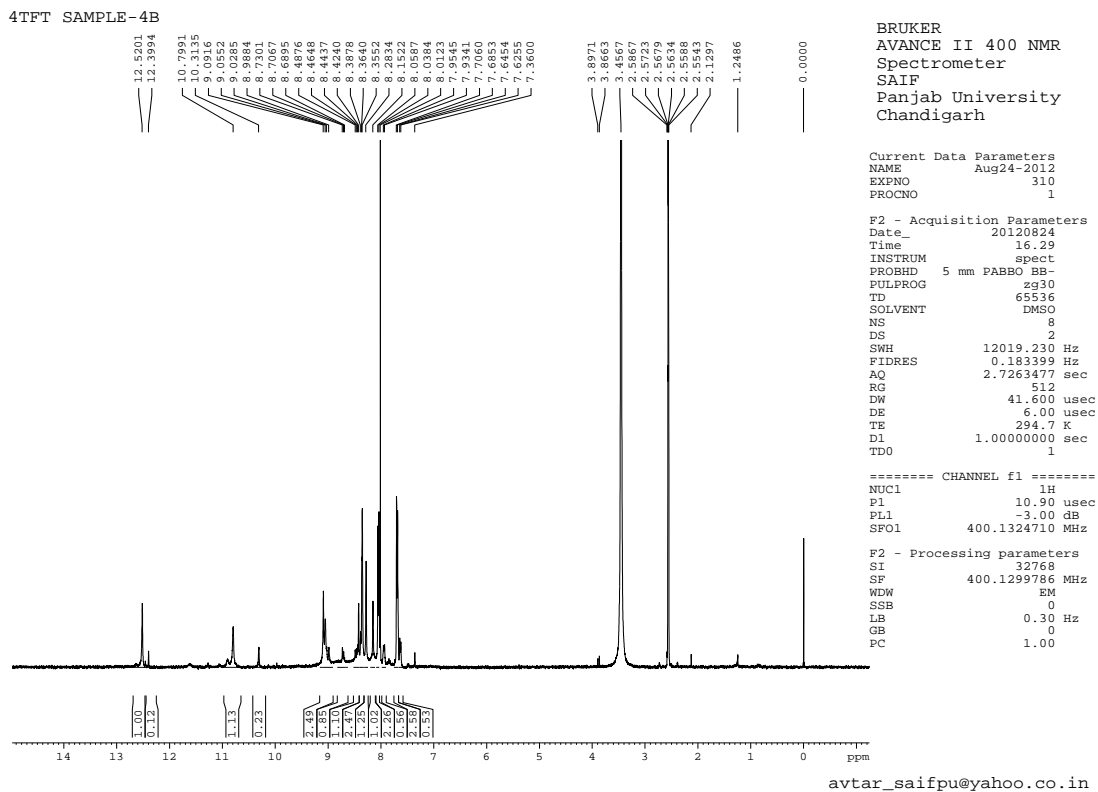


Fig-5 NMR spectra of [Pd(C₉H₈N₃F₃S)₂] Cl₂ complex

Mass spectra

The mass spectrum of ligand and their metal complexes of Co (II), Ni (II), Cu (II), Cd(II) Pt(II) and Pd(II) transition metal complexes were recorded under liquid secondary ion mass spectral condition (Lever, 1984, Serin 2001, Lever and Mantvani, 1971). All the spectra exhibited parent peaks due to molecular ions $[M]^+$. The mass spectra of ligand and their transition metal complexes showed abundant ions at m/z 248.1, 679, 588.1, 646.1, 448.0 and 760.2. The ions support the proposed composition and structure. The molecular ion peak occurs at 262 of ligand and 646.0 of their metal complexes. In addition to the molecular ion peaks, the spectra exhibited other peaks assignable to various fragments arising from the thermal cleavage of the complexes. The peak intensities given an idea of a stability of the fragments which showed at 231.1 for ligand and 248.1 for metal complexes.

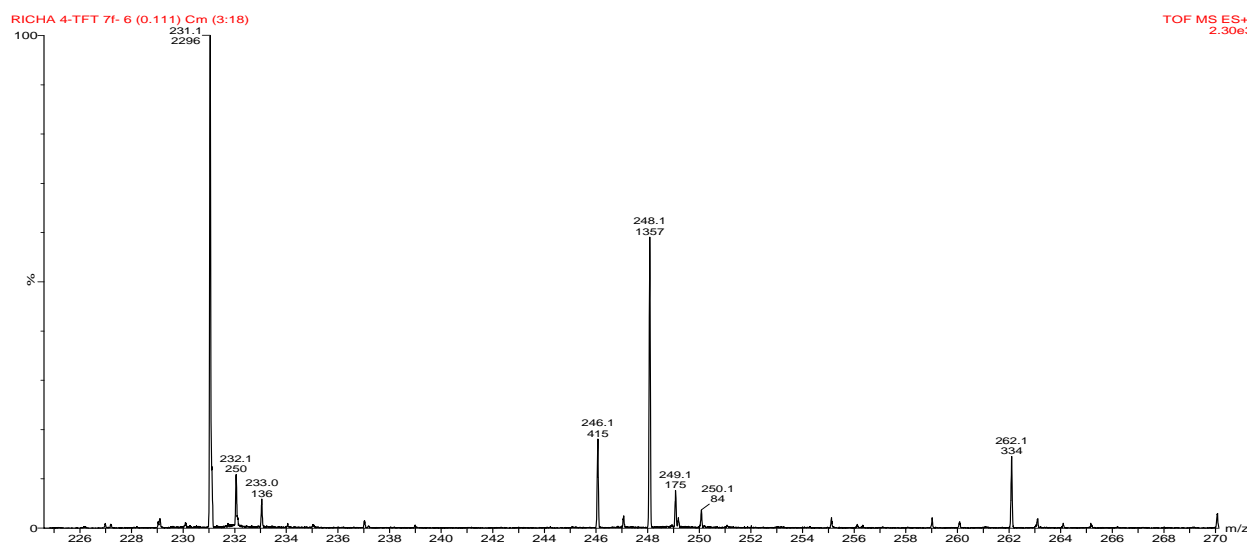


Fig-6. Mass Spectra of 4-(trifluoro-4-ylidene) hydrazine carbothioamide ligand.

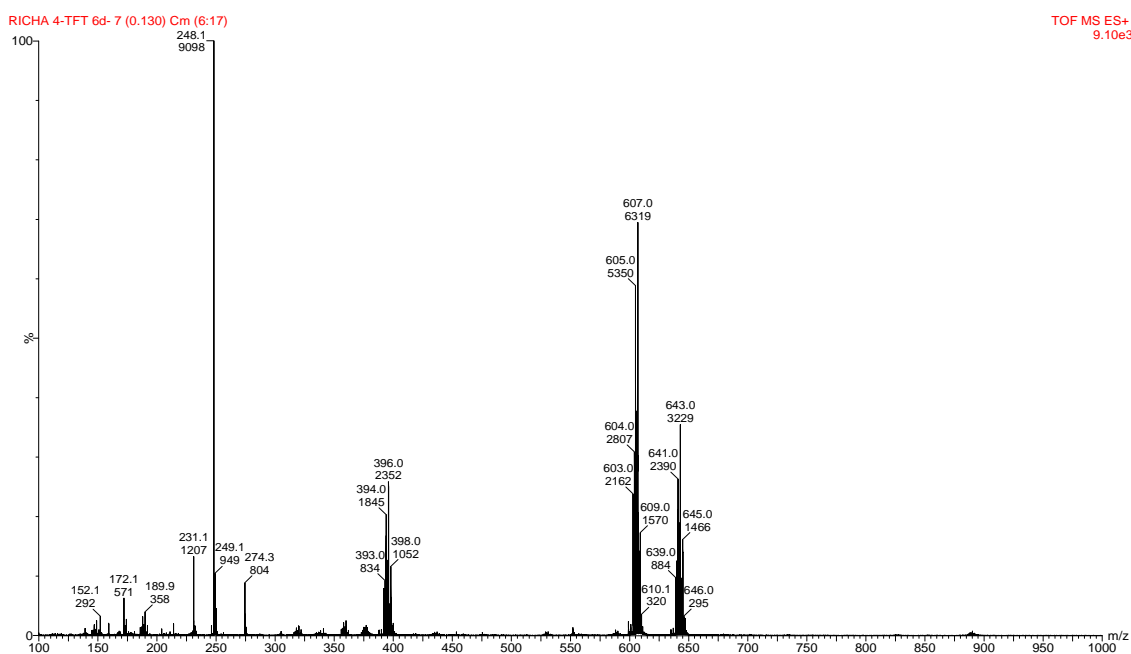


Fig-7. Mass spectra of $[Co (C_9H_8N_3F_3S)_2] Cl_2$ Complex

Magnetic measurements and Electronic spectra

Cobalt (II) Complex

The magnetic moments of the Co (II) complexes were measured at room temperature and were found in the range 3.87-3.97 B.M, which is consistent with 3 unpaired electrons. The solution spectra of the Co (II) complexes exhibited absorption in the region 26666.66 cm^{-1} . The spectra resemble to those reported for distorted octahedral Co (II) complexes (Singh et al, 1997, Krishnankutty et al 2007). The various bands may be assigned to ${}^4T_{1g} \rightarrow {}^4T_{2g}$ (F), (V_1); ${}^4T_{1g}$ (F) $\rightarrow {}^4A_{2g}$ (F) (V_2) and ${}^4T_{1g} \rightarrow {}^4T_{1g}$ (P) (V_3). This shows that these complexes have distorted octahedral geometry.

Nickel (II) Complex

The magnetic moments of the Ni (II) complexes lies in the range 2.94-2.98 B.M. corresponding to two unpaired electrons. These values are in tune with a high spin configuration and show the presence of an octahedral environment around the Ni (II) ion. The electronic spectra of the Ni(II) complexes show absorption bands at 8900, 12500 and 24000 cm^{-1} and may be assigned to the transition ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}$, ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$, and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, corresponding with an octahedral geometry.

Copper (II) complex

The Cu (II) complex under study display absorption bands 1300 and 16000 cm^{-1} [39]. These bands were assigned to the following transition from a distorted octahedral geometry [40]. ${}^2B_{1g} \rightarrow {}^2A_{1g}$, ${}^2B_{1g} \rightarrow {}^2B_{2g}$.

Pd (II) and Pt (II) complexes show diamagnetic behavior. The electronic spectra of the Pd (II) and Pt (II) complexes show three d-d spin-allowed transition. These correspond to the transition from the three lower lying d levels to the empty $d_{x^2 - y^2}$ orbital. The ground state is ${}^1A_{1g}$. The three d-d transitions were observed in the regions 473-530, 410-452 and 346-398 nm. These bands are attributed to ${}^1A_{1g} \rightarrow {}^1A_{2g}$ (v_1), ${}^1A_{1g} \rightarrow {}^1B_{1g}$ (v_2) and ${}^1A_{1g} \rightarrow {}^1E_g$ (v_3) transitions, respectively. The electronic spectra of these complexes indicate the square planar geometry around the Pd (II) and Pt (II) ion (West D.X et al 1979, Mahapatra and Panda, 1979). Based on the above spectral studies, the structures shown in Fig-8 may be suggested for the complexes.

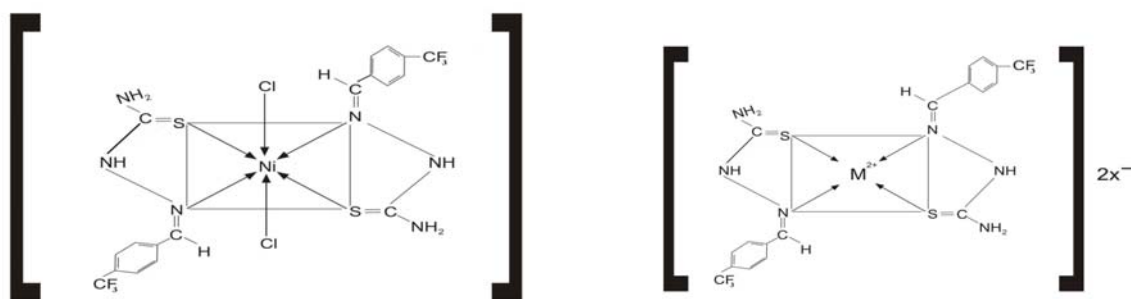


Fig-8. Suggested structure of the metal complexes (M = Co (II), Ni (II), Cu (II), Cd (II), Pt (II) and Pd (II))

Antimicrobial activities

Antibacterial activity

The test complex at dose 10 μg / disc was added into disc. The one disc was supplemented with reference drug streptomycin at dose 10 μg / disc serving as positive control. The plates were incubated immediately at 37° C for 24 hrs. Activity was determined by measuring the diameter of zones (mm) showing complete inhibition. Growth inhibition was calculated with respect to positive control. It was found that all the metal complexes were active against all the test bacteria but the metal complexes $[\text{Pd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2]\text{Cl}_2$, $[\text{Cu}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2](\text{CH}_3\text{COO})_2$ and $[\text{Pt}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2]\text{Cl}_2$ were most effective against all the pathogenic bacteria's as shown in Table 4.

Table 4 Minimum inhibitory concentration (MICs) shown by the ligand and the metal complexes against the test bacteria using the agar dilution assay

| Complexes | MIC $\mu\text{g/ml}$ | | |
|---|----------------------|--------------------------|------------------|
| | <i>E.Coli</i> | <i>Bacillus Subtilis</i> | <i>S. aureus</i> |
| $\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S}$ | 10 mm | 06 mm | 09 mm |
| $[\text{Co}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 12 mm | 15 mm | 12 mm |
| $[\text{Ni}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2\text{Cl}_2]$ | 10 mm | 20 mm | 12 mm |
| $[\text{Cu}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] (\text{CH}_3\text{COO})_2$ | 18 mm | 20 mm | 15 mm |
| $[\text{Pd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 22 mm | 20 mm | 21 mm |
| $[\text{Cd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 12 mm | 2 mm | 3 mm |
| $[\text{Pt}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 20 mm | 10 mm | 15 mm |
| Streptomycin (Standard Drug) | 15 mm | 18 mm | 12 mm |

Antifungal activities

The antifungal activities of the metal complexes were determined at concentration (10 $\mu\text{g}/\text{disc}$) against *Aspergillus niger* and *Candida albicans* in DMSO by serial plate dilution method using saboured agar media. Normal saline was used to make a suspension of corresponding species. 20 ml of agar media was poured in each Petri dish. Excess suspension was decanted and the plates were dried by using in an incubator at 37°C for 1 hrs. The fungal zone of inhibition values are given in table-5. The nutrient broth was incubated with approximately 1×10^5 cfu/ml. The cultures with ligand and metal complexes were incubated for 48 hr at 35°C and the growth was monitored.

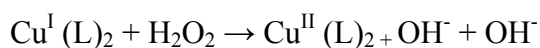
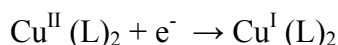
Table 5:- Minimum inhibitory concentration (MICs) showed by ligand and metal complexes against the test fungi using the agar dilution assay.

| Complexes | MIC $\mu\text{g/ml}$ | |
|---|----------------------|-------------------------|
| | <i>A.Niger</i> | <i>Candida albicans</i> |
| $\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S}$ | 10 mm | 10 mm |
| $[\text{Co}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 12 mm | 15 mm |
| $[\text{Ni}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2\text{Cl}_2]$ | 10 mm | 20 mm |
| $[\text{Cu}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] (\text{CH}_3\text{COO})_2$ | 11 mm | 3 mm |
| $[\text{Pd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 10 mm | 10 mm |
| $[\text{Cd}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 18 mm | 15 mm |
| $[\text{Pt}(\text{C}_9\text{H}_8\text{N}_3\text{F}_3\text{S})_2] \text{Cl}_2$ | 5 mm | 10 mm |
| Fluconazole (Standard Drug) | 12 mm | 15 mm |

It is known that chelation tends to make the ligand act as more powerful and potent bactericidal and fungicidal agents, thus killing more of the microorganism than the ligand. In a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands and there may be π – electrons delocalization over the whole chelate (Wissner.A et al 2000). The increased activity of the metal chelates can be explained based on the chelation theory (Sengupta et al 1998). On chelation, the polarity of the metal ion is reduced largely due to the overlap of the ligand orbital and the partial sharing of the positive charge of the metal ion with the donor groups. This increases the lipophilic character of these complexes seems to be the reason of their enhanced potent antimicrobial activity. There are some other factors which also increase the activity, such as solubility, conductivity and bond length between the metal and the ligand.

Nuclease activity studies

The nuclease activity of all metal complexes has been investigated on CT-DNA by agarose gel electrophoresis in the presence/absence of H_2O_2 . At micro-molar concentration for 30 min incubation periods the Schiff base ligand exhibits no significant activity in the absence and presence of the oxidant. The transition metal (II) complexes show enhanced nuclease activity due to the presence of the metal ions Co (II), Ni (II), Cu (II), Pd (II), Cd (II) and Pt (II). This is consistent with the increased production of hydroxyl radicals.



Where L = Ligand

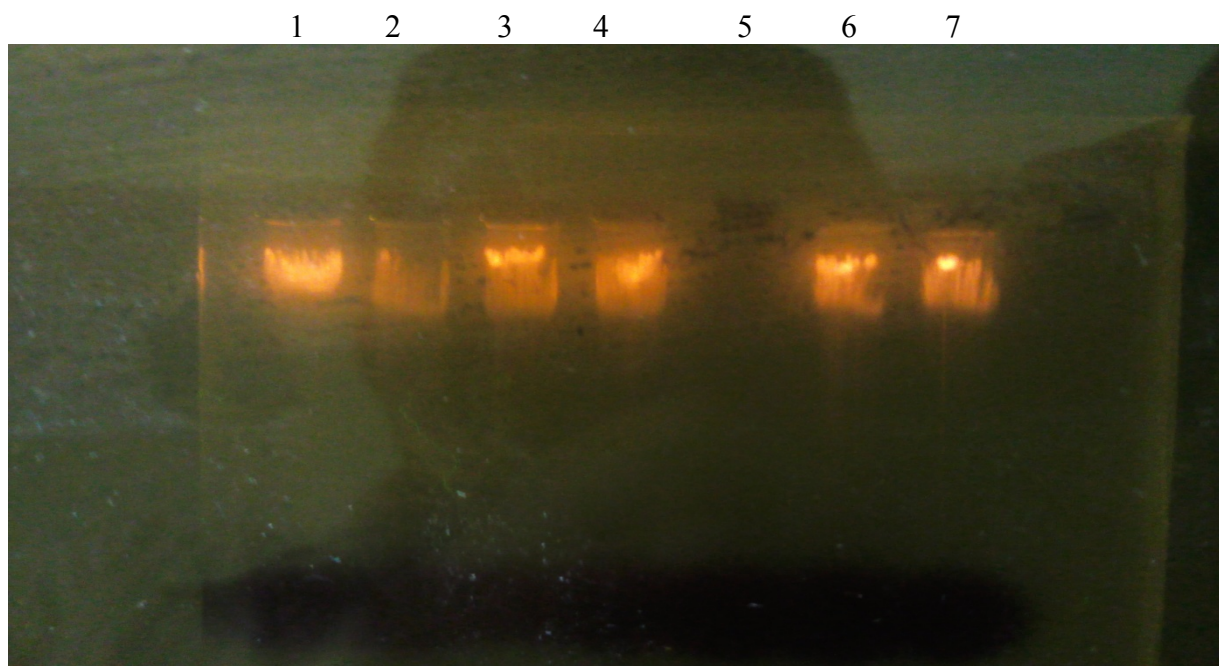


Fig. 13 Change in the agarose gel electrophoretic pattern of Genomic DNA.

Lanes left to right ->

Lane 1: DNA + H_2O_2

Lane 2: DNA + $[Co(C_9H_8N_3F_3S)_2] Cl_2 + H_2O_2$

Lane 3: DNA + $[Cd(C_9H_8N_3F_3S)_2] Cl_2 + H_2O_2$

Lane 4: DNA + $[Pt(C_9H_8N_3F_3S)_2] Cl_2 + H_2O_2$

Lane 5: DNA + $[Pd(C_9H_8N_3F_3S)_2] Cl_2 + H_2O_2$

Lane 6: DNA + $[Cu(C_9H_8N_3F_3S)_2] (CH_3COO)_2 + H_2O_2$

Lane 7: DNA + $[Ni(C_9H_8N_3F_3S)_2] Cl_2 + H_2O_2$

CONCLUSION

The present study revealed octahedral geometry around the Co(II), Ni(II), Cd(II) distorted octahedral geometry around Cu(II) and square planar geometry around the Pd(II) and Pt(II) complexes, in which the ligands act as bidentate chelating agents coordinating through the nitrogen and sulfur atom. The determined antimicrobial activities indicate that the metal chelates show a greater inhibitory effect than the parent ligand. It is also concluded that concentration plays a vital role in increasing the degree of inhibition, as the concentration increases, the activity increases. The determined nuclease activity indicates that the copper complex cleaves more effectively (supercoiled form to linear form). The other complexes are slightly giving rise to linear form compare to copper complex. All complexes plausible structure is supported by MS-ES mass spectral data along with physico-chemical and IR, NMR, Mass and electronic spectral data. The metal (II) complexes [M=Co (II), Ni (II), Cu (II), Cd (II), Pd (II) and Pt (II). And, X = Cl⁻, CH₃COO⁻] were prepared with the ligand and they were characterized with analytical and spectral techniques.

ACKNOWLEDGEMENT

The authors are thankful to the MPCST, Bhopal for financial support (No. 4566/cst/R&D/2010) and SAIF, Chandigarh, for providing spectral data. The authors also express their sincere thanks to Surendra singh parihaar, Dept. Of Biotechnology, ITM University Gwalior for assisting in studying the biological properties of the metal complexes.

REFERENCES

- Arion V. B., Jakupec, M. A., Galanski, M., Infried, P., and Keppler, B. K., (2002) "Synthesis, structure, spectroscopic and in vitro antitumour studies of a novel gallium(III) complex with 2-acetylpyridine ⁴N-dimethylthiosemicarbazone", *Journal of Inorganic chemistry*, vol. 91, no.1, pp. 298-305.
- Gracia, C. C., Brousse, B. N. and Carlucci et al., M. J., (2003) "Inhibitory effect of thiosemicarbazone derivatives on junin virus replication in vitro," *Antiviral Chemistry & Chemotherapy*, vol. 14, no. 2, pp. 99-105.
- Hu, W. X., Zhou, W., Xia, C. N. and Wen, X., (2006) "Synthesis and anticancer activity of thiosemicarbazones," *Bioorganic and Medicinal Chemistry Letters*, vol. 16, no. 8, pp. 2213-2218.
- Jouad, E. M., Larcher, G. and Allain et al., M., (2001) "Synthesis, structure and biological activity of nickel(II) complexes of 5-methyl 2-furfural thiosemicarbazone," *Journal of Inorganic Biochemistry*, vol. 86, no. 2-3, pp. 565-571.
- Golcu, A., Dolaz, M., Demirelli, H., Diorak, M. and Serin, S., (2006), "Spectroscopic and analytical properties of new copper (II) complex of antiviral drug valacyclovir," *Transition Metal Chemistry*, vol. 31, no. 5, pp. 658-665.
- Blanz Jr., E. J. and French, F. A., (1968), "The carcinostatic activity of 5-hydroxy-2-formylpyridine thiosemicarbazone." *Cancer Research*, vol. 28, no. 12, p.p. 2419-2422.
- Singh, R. V., Fahmi, N. and Biyala, M. K., (2005), "Coordination behavior and biopotency of N and S/O donor ligands with their Pd(II) and Pt(II) complexes," *Journal of the Indian Chemical Society*, vol.2, no.1, pp. 40-46.
- Chandra, S., Sangeetika and Rathi, A., (2001), "Magnetic and spectral studies on copper (II) complexes of N-O and N-S donor ligands," *Journal of Saudi Chemical Society*, vol. 5, no. 2, pp. 175-182.
- Raman, N., Kulandaisamy, A., Shunmugasundaram, A. and Jeyasubramanian, K., (2001), "Synthesis, spectral, redox and antimicrobial activities of Schiff base complexes derived from 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one and acetoacetanilide," *Transition Metal Chemistry*, vol. 26, no. 1-2, pp. 131-135.
- Raman, N., Kulandaisamy, A. and Jeyasubramanian, K., (2002), "Synthesis, spectral, redox and antimicrobial activity of Schiff base transition metal(II) complexes derived from 4-aminoantipyrine and benzil," *Reactivity in Synthesis and Inorganic and Metal-Organic Chemistry*, vol. 32, no.9, pp. 1583-1610.
- Singh, L., Sharma, D. K., Singh, U. and Kumar, A., (2004), "Synthesis and spectral studies of Cu(II) coordination compounds of 4[N-(cinnamalidene) amino] antipyrine semicarbazone," *Asian Journal of Chemistry*, vol. 16, no. 2, pp. 577-580.
- Raman, N., Thangaraja, C. and Johnsonraja, S., (2005), "Synthesis, spectral characterization, redox and antimicrobial activity of Schiff base transition metal(II) complexes derived from 4-aminoantipyrine and 3-salicylideneacetylacetone," *Central European Journal of Chemistry*, vol. 3, no. 3, pp. 537-555.
- Tosi, H. B., (1986), *Inorg. Chim. Acta* 125, 173.

- Hall, I. H., Lackey, C. B., Kistler, T. D., Durhan Jr., R. W., Jouad, E. M., Khan, M. A., Thanh, X. D., Djebbar-sid, S., Benali Baitich, O., Boret, G. M., (2000), *Pharmazie*, 55, 937.
- Jakupee, M. A., Galanski, M., Keppler, B. K., (2003), *Rev. Physiol. Biochem. Pharmacol.* 146, 53.
- Giovagnini, L., Ronconi, L., Aldinucci, D., Lorenzon, D., Sitran, S., Fregona, D., (2005), *J. Med. Chem.* 48, 1588.
- Chandra, S., Raizada, S., Tyagi, M., Gautam, A., (2007), *Bioinorg. Chem. Appl.* 2007 51483.
- Kovala-Demertzi, D., Domopoulou, A., Demertzis, M. A., Valle, G., Papageorgio, A., (1997), *J. Inorg. Biochem.* 68, 147.
- Vogel's Text Book Practical Organic Chemistry, Furnis, B. S., Hannaford, A. J., Smith, P. W. G., Tatchell, A. R., (1989), Eds., Longman, London.
- Kong, D. M.; Wang, J.; Zhu, L. N.; Jin, Y. W.; Li, X. Z.; She, H. X.; Mi, H. F., (2008), *J. Inorg. Biochem.*, 102, 824-832.
- Singh, D. P., Shishodia, N., Yadav, B. P., Rana, V. B., (1997), *Polyhedron* 16, 2229.
- Krishnankutty, K., Sayudevi, P., Ummathur, M. B., (2007), *J. Serb. Chem. Soc.* 72, 1075.
- Mitra, K., Biswas, S., Lucas, C. R., Adhikary, B., (2006), *Inorg. Chim. Acta* 359, 1997.
- Kumar, G., Kumar, D., Singh, C. P., Kumar, A., Rana, V. B., (2010), *J. Serb. Chem. Soc.* 75, 629.
- Rajendran, G., Amritha, C. S., Anto, R. J., Cheriyan, V. T., (2010), *J. Serb. Chem. Soc.* 75, 749.
- Pouralimardan, O., Chamayou, A., Janiak, C., Hosseini-Monfared, H., (2007), *Inorg. Chim. Acta* 360, 1599.
- Imran, M., Mitu, L., Latif, S., Mahmood, Z., Naimat, I., Zaman, S. S., Fatima, S., (2010), *J. Serb. Chem. Soc.* 75, 1075.
- Mohamed, G. G., Sharaby, C. M., (2007), *Spectrochim. Acta A* 66, 949.
- Nakamoto, K., (1997), *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, Wiley, Toronto, Canada.
- Roy, G. B., (2009), *Inorg. Chim. Acta* 362, 1709.
- Naresh Kumar, K., Ramesh, R., (2004), *Spectrochim. Acta*, A 60, 2913
- Pawar, N. S., Dalal, D. S.; Slimpi, S. R.; Mahulikar, P. P.; (2004), Studies of microbial activity of N-alkyl and N-thiazolyl-1H benzimidazoles. *Eur. J. Pharm. Sci.*, 21, 115.
- Singh, Dharmpal, Kumar, Krishan and Singh, Jitendra, (2010), *J. Serb. Chem. Soc.* 75(2) 217-228.
- Prasad, Prof. R. N.; Mathur, M., Upadhyay, A., (2007); *J. Indian Chem. Soc.* 84, 1202.
- Costamapna, J., Ferrandi, G., Villagran, M., Wolcan, E., (2000), *J. Chem. Soc. Dalton Trans.* 2631.
- Lever, A. B. P., (1984), *Inorganic Electronic Spectroscopy*, Elsevier Amsterdam.
- Serin, S., (2001), *Transition Met. Chem.*, 26, 300.
- Lever, A. B. V., Mantovani, E., 1971, *Inorg. Chem.* 10, 817.
- Shrivastava, Sharma, V. K., (1998), *Trans. Met. Chem.* 23, 349.
- Lever, A. B. P., (1984), *Inorganic electronic spectroscopy*, Elsevier, New York, NY, USA.
- West D. X., Salberg, M. M., Bair, G. A., liberta, A. E., Valdes-Martinez, J., Hernandez – Orteza S., *Transition Met. Chem.* 21, 206.
- Mahapatra, B. B., Panda, D., (1979), *Transition Met. Chem.* 41, 809.
- Wissner, A., Berge Berger, D. M., Boschelli et al D. H., (2000) "4 - anilins-6,7-dialkoxy quinoline-3- carbonitrile inhibitors of epidermal growth factor receptor kinase and their bio-iso steric relationship to the 4-anilino -6,7-dialkoxy quinazoline inhibitors", *Journal of Medicinal Chemistry*, vol. 43, no. 17, pp. 3244-3256,.
- Sengupta, S. K., Pandey, O. P., Shrivastava, B.K., . and Sharma, V. K., (1998), *Trans. Meta. Chem.* 23, 349.