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Synthesis, Spectral Characterization of N-benzyl isatin Schiff base Cu(II), Co(II) and Ni(II) complexes and their effect on Cancer Cell lines

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Abstract: The efficient antitumor drugs are ever demanding in the present scenario worldwide. The chemotherapeutic activity of isatin compounds can be achieved by chelation of transition metal ions with these bases and expanding in N-region of these compounds. In this present study, two series of Cu(II), Co(II) and Ni(II) complexes with N-benzyl isatin and isonicotinohydrazide Schiff base ligand (L_1) have been synthesized and characterized by using the modern analytical techniques such as IR, NMR, UV-visible, Mass, CV, and EPR *etc*. All the complexes are soluble in DMF and DMSO. Elemental analysis and molar conductance values indicate that the complexes are non-electrolytes. All the complexes adopt octahedral geometry around the metal ions. DNA binding activities of the complexes Cu(II), Co(II) and Ni(II) are studied by UV-vis and also cleavage studies of complexes have been done by agarose gel electrophoresis method. Cytotoxicity experiments carried out toward human AGS-gastric cancer cell line. Interestingly, compound, L_1-Ni was found to be excellent anticancer activity against AGS-gastric cancer cell line.

Keywords: N-benzyl isatin, isoniazid, transition metal complexes, DNA binding, cleavage, cytotoxicity studies

I. INTRODUCTION

Heterocyclic compounds bearing hydrazides and hydrazones are wide interest because of their specific biological and clinical applications [1, 2]. This has created the great interest among the chemist to design and synthesis of variety of hydrazide derivatives and screened for various biological activities such as antibacterial, antioxidant, antiproliferative, antimalarial, antitrypanosomal, anticonvulsant and antitumor *etc.,* [3, 4]. The interaction studies of hydrazones with DNA are important role for their pharmacological significance. These compounds are found to be known against the growth of that tumor cell and this functional mechanism may be the basis of new and more efficient antitumor drugs [5]. The isatin derivatives are proven antibacterial, antifungal, anticonvulsant, anti-inflammatory, anti-HIV, antitubercular and anticancer activities [6, 7]. In particular, N-substituted isatin with isonicotinohydrazide hase bearing used clinically as a antiviral agents and fined wide spread applications in the various fields for inhibiting DNA synthesis and induction of interferon secretion. Schiff-base ligands are potential anti-cancer, anti-bacterial and anti-viral agents and this activity tends to increase in metal(II) Schiff-base complexes [8]. The cleavage of plasmid DNA by octahedral Cu(II), Co(II) and Ni(II) complexes have been well-reported [9]. The development of metal complexes as diagnostic or therapeutic agents requires techniques that can fast and accurately provide information about the effects of structural alterations on DNA selectivity and affinity. The non-covalent bonding of metal complexes with DNA has resulted in a variety of applications such as synthetic restriction enzymes, DNA repair agents, development of selective probes of DNA structure and artificial regulators of gene expression [10, 11]. In this study the author report to design, synthesis and structurally characterizes a new potent N-benzylisatin with isonicotinohydrazide ligand and their Cu(II), Co(II) and Ni(II) complexes. Furthermore, in vitro biological, DNA and cytotoxicity activities of the synthesized ligands and their metal complexes were done and their results are discussed in details.

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Vol. 2, Issue 10, October 2013

II. EXPERIMENTAL

A Materials and Methods

All chemicals were purchased from Sigma-Aldrich, E-Merk and used as received without further purification. The isatin monohydrazone was prepared according to the literature procedure [12]. Isatin, Benzylbromide, isonicotinohydrazide, DMSO are GR grade, CT and *pUC-19* DNA were purchased from Genie, Bangalore. Metal chlorides [$CuCl_2.2H_2O$, $NiCl_2.6H_2O$, $CoCl_2.6H_2O$] and solvents were purchased from E-Merk,A.R grade, Mumbai.

C, H and N analyses of free Schiff base ligands and their metal complexes were performed in CHN analyzer Elemental Vario EL III. Metal contents were analyzed by the standard procedures. Hand-Held Meter LF330 was used to measure the molar conductance of free Schiff base ligands and metal complexes in DMSO ($1x10^{-3}$ M). The electronic spectra were recorded in DMSO solutions using Shimatzu Model 160 UV-visible spectrophotometer. The IR spectra of the complexes were recorded on a JASCO V-550 UV-Vis spectrophotometer in KBr pellets. NMR spectra were recorded on BRUKER DPX-300 High performance Digital FT-NMR spectrometer in DMSO-d^6 using TMS as internal standard. Electrospray ionisation mass spectrometry (ESI-MS) analysis was performed in the positive ion mode on a liquid chromatography-ion trap mass spectrometer (LCQ Fleet, Thermo Fisher Instruments Limited, US. Magnetic susceptibility measurement of the powdered samples was carried out by the Gouy balance. EPR measurements were carried out by using a Varian E4 X-band spectrometer equipped with 100 Hz modulation. Cyclic Voltammetric measurements were carried out in a Bio-Analytical System(BAS) model CV-50W electrochemical analyzer.

B DNA binding studies

B.a Electronic absorption studies

DNA-binding experiments were performed by UV-visible spectroscopy in Tris-HCl/NaCl buffer (5 mmol L^{-1} Tris–HCl / 50 mmol L^{-1} NaCl buffer, pH 7.2) and used DMSO (10%) solution of metal complexes. The concentration of CT-DNA was determined from the absorption intensity at 260nm with a value of 6600 (mol L^{-1}) $^{-1}cm^{-1}$ [13]. Absorption titration experiments were made using different concentrations of CT-DNA, while keeping the complex concentration constant. Correction was made for the absorbance of the CT-DNA itself. Samples were equilibrated before recording each spectrum. For metal complexes, the intrinsic binding constant (K_b) was determined from the spectral titration data using the following equation [14]:

$$[DNA] / (\varepsilon_a-\varepsilon_f) = [DNA]/ (\varepsilon_b-\varepsilon_f) + 1/K_b (\varepsilon_b-\varepsilon_f)$$

Where, ε_a, ε_b and ε_f are the molar extinction coefficients of the free complexes in solution, complex in the fully bound from with CT-DNA and complex bound to DNA at a definite concentration respectively. In the plot of [DNA] / ($\varepsilon_a-\varepsilon_f$) versus [DNA], K_b was calculated.

B.b Circular Dichroism (CD) Measurements

Circular dichroism spectra were registered in a JASCO J-810 spectropolarimeter, using a quartz cuvette of 0.2 cm path length, at room temperature, in the range 230–330 nm. The initial experimental DNA concentration was 800 µM, and the spectra were registered in the absence or in the presence of 10 to 50µM of each complex studied [15].

B.c Electrochemical studies

Cyclic voltammetry analysis was carried out in a Bio-Analytical System (BAS) model CV-50W electrochemical analyzer. All voltammetric experiments were performed in a single compartment cell of volume 10–15mL containing a three electrode system comprising a glassy carbon working electrode, Pt-wire as auxiliary electrode, and reference electrode as an Ag/AgCl.

C DNA Cleavage studies

pUC19 DNA at pH 7.5 in Tris-HCL buffered solution was used to perform agarose gel electrophoresis. Oxidative cleavage of DNA was examined by keeping the concentration of the 30µM of complexes and 2µL of pUC19 DNA and this was made up the volume to 16µL with 5mM Tris-HCl/5mM NaCl buffer solution. The resulting solutions were

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Vol. 2, Issue 10, October 2013

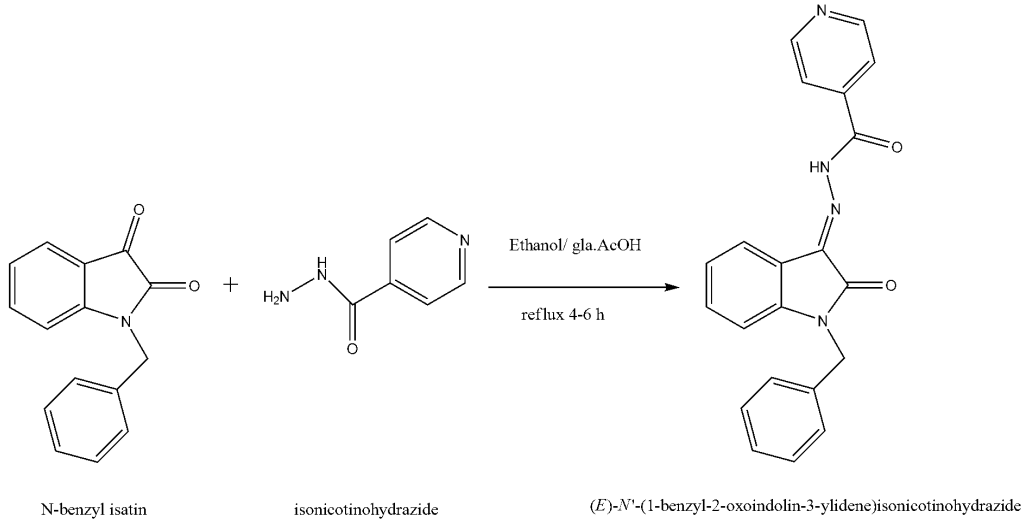
incubated at 37℃ for 2 h and followed by electrophoresed for 2 h at 50 V in Tris-aetate-EDTA (TAE) buffer using 1% agarose gel containing 1.0 µg/ml ethidium bromide (EB) and photographed under UV light [16].

D Cytotoxicity studies

Cytotoxicity studies were carried out using human gastric cancer cell line (designated AGS) which were obtained from National Centre for Cell Science (NCCS), Pune, India. Cell viability was carried out using the MTT assay method [17]. The AGS cells were grown in Dulbecco's Modified Eagle's Medium (DME) and Ham's F-12 Nutrient Mixture containing 10% fetal bovine serum (FBS), 1% Glutamine, 1% antibiotic, 1% sodium bicarbonate and 1% non-essential amino acids. For screening experiment, AGS cells were seeded into 96-well plates in 100 µL of respective medium containing 10% FBS, at plating density of 10,000 cells/well and incubated at 37 ºC, 5% CO2 for 24 h prior to addition of complexes. The complexes were dissolved in DMSO and diluted in the medium. After 24 h, the medium was replaced with respective medium containing the complexes at various concentration and incubated at 37 ºC, 5% CO2 for 48 h. Triplicate was maintained and the medium containing without the complexes were served as control. After 48 h, 10 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ºC for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were dissolved in 100 µL of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % of cell inhibition was determined using the following formula and chart was plotted between % of cell inhibition and concentration and from this IC50 value was calculated. % inhibition = [mean OD of untreated cells (control) / mean OD of treated cells (control)] x 100 [18].

E Preparation of Schiff-base ligand (E)-N'-(1-benzyl-2-oxoindolin-3-ylidene)isonicotinohydrazide(L_1).

1-Benzylisatin (0.223g, 1mmol) and isonicotinohydrazide (0.137g, 1 mmol) were dissolved in 50 ml of absolute EtOH, three drops of glacial acetic acid was added and the resulting solution was refluxed for 5 h. The results compounds were precipitated upon cooling to room temperature, isolated by filtration and recrystallized from EtOH. Yellow colored crystalline compounds were obtained (Scheme 1).L_1: Yield: 95%, m.p. 158℃, Elemental analysis: Found (calculated) (%) for L_2: C, 70.71 (70.77); H, 4.37 (4.53); N, 16.22 (15.72). IR (cm^{-1} in KBr pellets): 1604 (C=N), 1691(indole- C=O), 1689 (-NH-C=O), 3045 (NH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 14.19(s, 1H), δ 8.86 (d (doublet), pyridine protons, 4H), 7.93-6.81(m, aromatic protons); 4.97 (s, methyl protons) ^{13}C-NMR 162.01 (C=O, isatin), 161.21 (C=O, isoniazid), 150.86-110.48 (aromatic carbons), 30.84 (methine carbon), ESI-MS=357 (M+H).



Scheme 1. Synthesis of isatin Schiff base ligand

ISSN: 2319-8753

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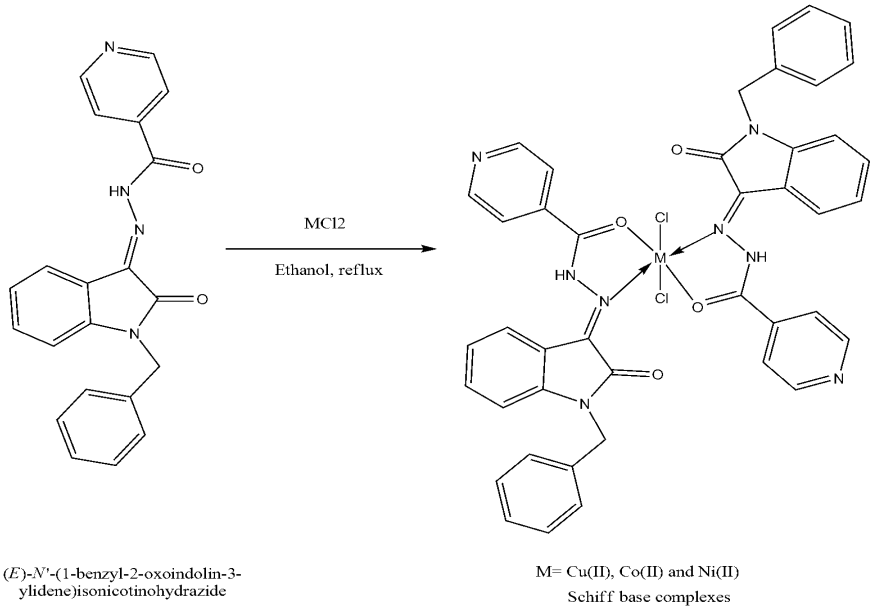
(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 10, October 2013

F Synthesis of Cu(II), Co(II) and Ni(II) complexes

The N-benzyl isatin based ligand was synthesized by condensation reaction of N-benzyisatin with isonicotinohydrazide followed by metallation with Cu(II)Cl_2, Co(II)Cl_2 and Ni(II)Cl_2 salt, according to general procedure in the literature [19] with suitable modification. The complexes were prepared by refluxing (6-8 h) an ethanol suspension of a metal(II) chloride and the corresponding ligand in the adequate molar ratio (1:2). Powdered solids obtained by cooling were filtrated, washed with ethanol and dried under vacuum (Scheme 2).The Novel Schiff base Cu(II), Co(II) and Ni(II) complexes were characterized by Elemental analysis, UV-visible, Infrared(IR), electron paramagnetic resonance (EPR) spectroscopy and Magnetic moment.



Scheme 2. Synthesis of complexes

Complex 1: Yield: 85%. m.p. 300-315 °C. Elemental analysis: Found (calculated) (%) for **L_1-Cu**: C, 59.81(59.90); H, 4.31 (4.09); N, 13.16 (13.00). UV-visible (in DMSO): λ_{max} (nm): 276(ILCT), 344(ILCT), 460 (MLCT), 810 (d-d). IR (cm^{-1}): 1614 (C=N), 1714(indole- C=O), 1683 (-NH-C=O), 3063(NH), 553(M-O), 445(M-N), $g_{||}$ =2.303; $g_{\perp}$=2.07; $A_{||}$ =116x10^4, μ_{eff} (300K): 3.10 μ_B.
Complex 2: Yield: 78%. m.p. 290-300 °C. Elemental analysis: Found (calculated) (%) for **L_1-Co**: C, 60.51 (60.22); H, 4.41 (4.11); N, 13.24 (13.07). UV-visible (in DMSO): λ_{max} (nm): 276(ILCT), 345(ILCT), 613,678 (d-d). IR (cm^{-1}): 1614 (C=N), 1701(indole- C=O), 1685 (-NH-C=O), 3057(NH), 554(M-O), 449 (M-N), μeff (300K): 4.13 μ_B.
Complex 3: Yield: 80%. m.p. 290-305 °C. Elemental analysis: Found (calculated) (%) for **L_1- Ni**: C, 59.91(60.24); H, 4.16 (4.11); N, 13.12 (13.05). UV-visible (in DMSO): λ_{max} (nm): 276(ILCT), 346(ILCT), 462 (MLCT), 808 (d-d). IR (cm^{-1}): 1612 (C=N), 1712 (indole- C=O), 1678 (-NH-C=O), 3061(NH), 554(M-O), 449(M-N), μ_{eff} (300K): 2.98 μ_B.

G Results and Discussion

The bidentate NO type of Schiff base ligand (L_1) and its complexes with N-benzyl isatin with isonicotinohydrazide were synthesized and characterized by various spectral techniques. These complexes were found to be air stable, amorphous, moisture free and soluble only in DMF and DMSO.

G.a Elemental analysis and Conductivity measurements

The synthesized schiff base ligand (L_1) and their complexes were analysed for their physico-chemical properties like melting point (m.p.), color, yield, elemental analysis and conductivity which are given in table.1. The elemental

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(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 10, October 2013

analytical data of ligands and their complexes are well agreed with their calculated values, showing that 1:2 stoichiometry ratio. The observed low conductivity values (21.10-26.10 Ω^{-1} cm^2 mol^{-1}) were accounted for the dissociation and hence the complexes are found as non-electrolytes [20].

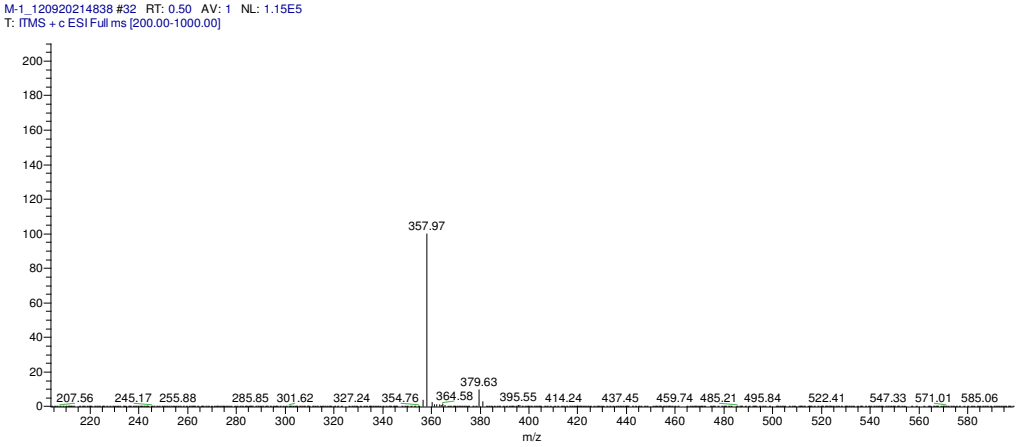

Fig.1. Mass spectrum of L_1

G.b Infrared Spectra

In order to study the bonding mode of ligand to metals in the complexes, IR spectra of the free ligands were compared with those of the metal complexes. The FT-IR spectral data are summarized in Table 2. The IR spectrum of the free ligand (L_1) showed broad band's 3230 and 3220cm^{-1}, which can be attributed to NH stretching vibration of the isoniazid moiety also the position of these bands remained same frequency in the spectra of the metal complexes suggesting that un-coordination of this group [21].

The ligand tested a sharp peak at 1604cm^{-1} was assigned to ν(C=N), which is characteristic of Schiff bases. In the spectra of the complexes, this peak is slightly shifted to higher frequency around 1612-1614 cm^{-1}. This suggested that one point of attachment of the metal is through the azomethine nitrogen atom [22]. The strong intensity bands of ligand was observed at the region 1691 cm^{-1} of the spectra indicating carbonyl group The positions of these band was shifted to lower region 1683 -1678 cm^{-1} the spectra indicating the involvement of υ(C=O) with metal centre during complexation. The bands at 16703 for ligand and 16701-1704 cm^{-1} in the spectrum of the free ligand and complexes, assigned to ν(C=O) of isatin moiety. The positions of these bands were founded at nearly the same frequency in spectra of the metal complexes, suggesting the un-coordination of this group. New bands observed in the 445–449 and 553–554 cm^{-1} represent for the complexes were assigned to stretching frequencies of M–N and M–O, respectively [23] Thus, the IR spectral results provide evidence for bidentate complexation of Schiff bases with metals.

G.c NMR Spectra

^{1}H-NMR Spectra

The ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm) spectrum of the Schiff base exhibited the following signal at 14.19 (s, 1H) was assigned to the NH proton of isonicotinohydrazide. The signals at 7.93-6.81(m, 15H) were assigned to aromatic protons of L_1. In addition to these, one singlet peak observed at 4.97 (s, 2H) for methine protons .

^{13}C NMR Spectra

The ^{13}C NMR (300 MHz, $CDCl_3$, δ/ppm) spectra provide further support for the structural characterization of the Schiff bases The signals at 162.01 (C=O, isatin), 161.21 (C=O, isoniazid), 150.86-110.48 (aromatic carbons), 30.84 (methine carbon) for L_1.

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International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 10, October 2013

G.d Electronic spectra and magnetic moment values

The electronic spectra of the ligand and its metal(II) complexes were recorded in DMSO. The absorption bands at 36363 cm^{-1} and 29154 cm^{-1} attributed to $\pi \rightarrow \pi$ and $n \rightarrow \pi^*$ transitions for L_1 are observed in the spectrum of the free ligand. The Cu(II) complex showed two bands at 12736, 12345 cm-1. These bands may be assigned to $^2B_{1g} \rightarrow {}^2B_{2g}$, ^2E_g transitions. Another high intensity band at 21739 cm^{-1} is due to symmetry forbidden ligand → metal charge transfer. The position of these bands is consistent with octahedral geometry around the Cu(II) ion. The observed magnetic moment of 3.10 BM for Cu(II) complex [44, 45]. The electronic spectra of Co(II) complexes exhibit absorption in the range of 16313, 14749 cm^{-1}. These bands may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, and $^4T_{1g}(F) \rightarrow {}^4A_{2g}$ transitions. The position of these bands is consistent with octahedral geometry around the Co(II) ion. The observed magnetic moment of 4.13 BM for Co(II) complex [24].

The electronic spectra of Ni(II) complexes exhibit absorption in the 12376 cm^{-1} and assigned to be $^3A_{2g} \rightarrow {}^3T_{2g}$ (υ_2) transitions. The position of these bands is consistent with octahedral geometry around the Ni(II) ion. The observed magnetic moment of 2.98 BM for Ni(II) complexes for L_1 and L_2 [25].

G.e EPR Spectra

The X-band EPR spectrum of the copper(II) complex was recorded in the solid state at room temperature and DMSO at liquid nitrogen temperature using the DPPH radical as the g marker. The complex has a well resolved $g_{||}$ and broadened g region and various Hamiltonian parameters have been calculated ($g_{||}$ =2.303; g⊥=2.07; $A_{||}$ =116x10^4) the trend $g_{||} > g_\perp$ observed in this complex indicate that the unpaired electron is most likely to be in the $d_{x^2-y^2}$ orbital [26].

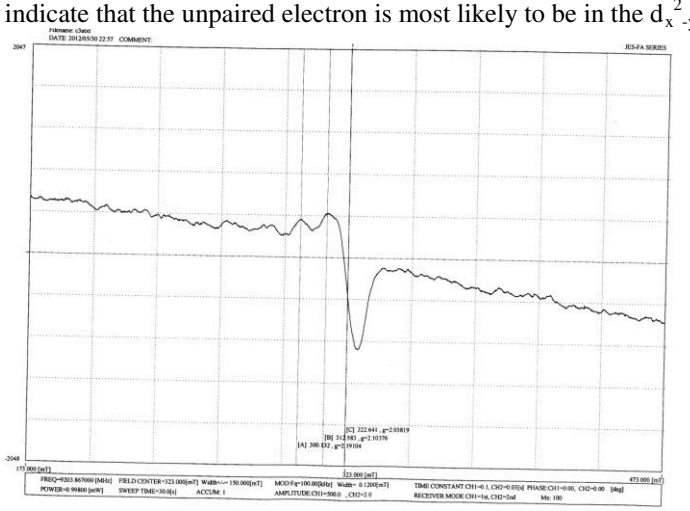

Fig.2. ESR spectrum of copper complex for L_1 presence of LNT

G.f Cyclic voltammetry

A cyclic voltammogram of Cu(II) complex presented in Fig.3. Voltammogram displays a reduction peak at Epc_1= 858mV, Epc_2= -121.9mV with an associated oxidation peak at Epa_1= 237.8mV, Epa_2= 725.6mV at a scan rate of 50mV/s. The peak separation of this couple (ΔEp) is 0.84V and increases with scan rate, I_{pa}/I_{pc} = 0.95. Thus, the analyses of cyclic voltammetric responses at different scan rate gave the evidence for quasi-reversible one electron reduction. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) and Cu(I)/Cu(O) couple. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates. This establishes the electrode process as diffusion controlled [24].

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Vol. 2, Issue 10, October 2013

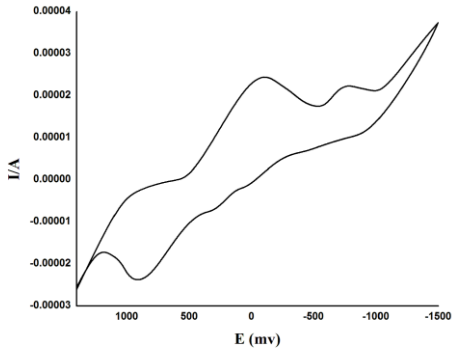

Fig.3. CV spectra of L_1-Cu

H Metal-DNA interaction

Transition metal complexes bind to DNA via both covalent and/or non-covalent interactions. In covalent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanine N7. On the other hand, the non-covalent DNA interactions include intercalation, electrostatic, and groove (surface) binding of metal complexes, outside of the DNA helix, along major or minor grooves.

H.a UV-Visible spectra

It is a well-known fact that DNA binding studies is the preliminary pharmacological target of many antitumor compounds and hence the interaction between DNA and metal complexes are important in understanding the mechanism of drug action. The binding mode of ligands and complexes with CT-DNA were studied by absorption spectroscopic methods at pH 7.2(in 5 mM Tris–HCl, 50 mM NaCl Buffer).

With increasing CT-DNA concentration for the L_1-Cu complex, the hypochromism in the band at the found 276 and 346 nm reaches as high as 64.25 % and 70.76 % respectively along with red shift. Other complexes also exhibit the similar results during the addition of increasing concentration of DNA. The intrinsic binding constant K_b is obtained from the ratio of slope to the intercept from the plots of [DNA]/ (ε_a-ε_f) versus [DNA]. The K_b values are shown in table 5. Hence the above phenomenon is indicative of most probable binding mode of Co(II) and Ni(II) complexes with calf thymus DNA. It should be noted that significant effect on the absorption bands of the molecule in the presence of double helical DNA, is characteristic of groove binder [27, 28].

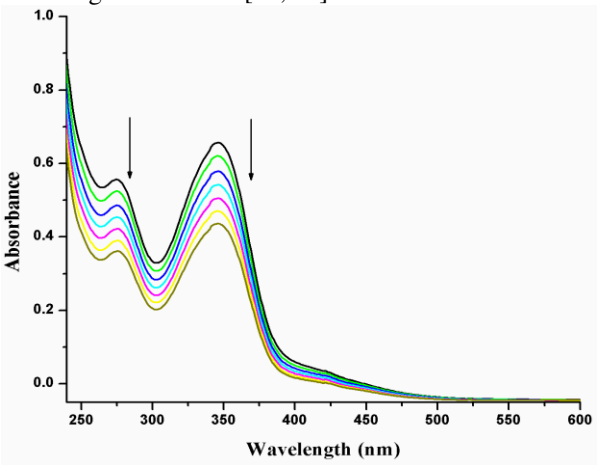

Fig.4. Absorption spectra of copper(II) complexes for L_1, in the absence and in the presence of the CT-DNA. [DNA]=30 µM, [CuL]=0 to 30 µM. The arrow indicates Absorption intensity decrease with increasing addition of the CT-DNA.

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Vol. 2, Issue 10, October 2013

H.b CD Spectra

CD spectra is a useful technique in diagnosing changes in DNA morphology during drug–DNA interactions, since CD signals are quite sensitive to the mode of DNA interactions with small molecules. In the case of CT-DNA interacting with metal complexes, the characteristic CD spectra consist of two bands: For L_1-Cu complex, a positive one at 275 nm due to the base stacking between the compounds and DNA bases, and a negative band at 243 nm due to the right-handed helicity B form of DNA [52]. Observed changes in those CD signals of DNA are usually assigned to corresponding changes in its structure. The simple groove binding or electrostatic interaction between of molecules and DNA causes less or no perturbation on the base stacking and helicity bands, whereas a classical intercalation enhances both CD bands, stabilizing the CT-DNA form B conformation, as observed for intercalative ligands [29]. The CD spectra of DNA taken after incubation of the complexes with CT-DNA are shown in Fig.14. In all three cases, the intensities of both the negative and positive bands decrease significantly. This suggests that the DNA binding of the complexes induces certain conformational changes, such as the conversion from a more B-like to a more A-like structure within the DNA molecule. These changes are indicative of a intercalation mode of binding of these complexes and offer support to their groove binding nature [30].

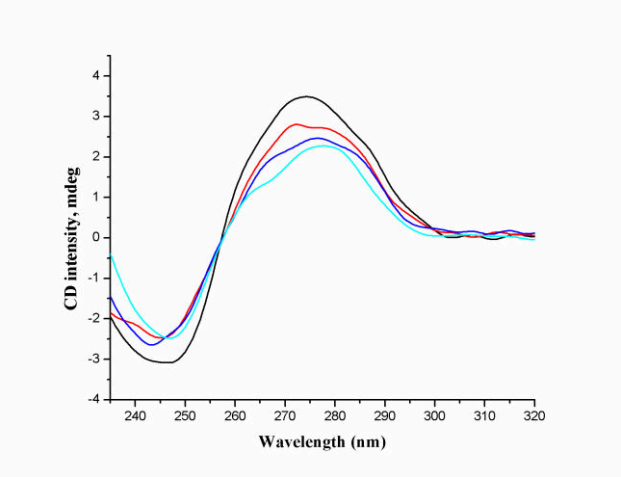

Fig. 5. CD spectra of CT-DNA (800 μM) in phosphate buffer 50 mM/NaCl 0.1 M, in the absence and presence of the copper(II) complex for L_1 at varied concentrations

H.c Cyclic voltammetry study of the DNA-binding

Electrochemical investigations of metal–DNA interactions provide a useful complement to spectroscopic methods. Cyclic voltammograms of copper complex in the presence of CT-DNA in various concentrations are shown in the Fig.16. CV data it explored that L_1-Cu exhibited a pair of redox peaks for two electron transfer couple of Cu(II)/Cu(I) at the scan rate of 50 mVs (curve). The ration of (Ipa/Ipc) value of 0.6 and the peak to peak separation (ΔEp) of 0.42 V, suggested the characteristic of the of the electro-transfer process and this was fairly common for Cu(II)/Cu(I) couple because of the reorganization of the coordination sphere. After interaction with CT-DNA, the value of ΔEp was decreased to 0.15V suggesting that the reversibility of the electron – transfer process of the copper complex was changed better. Moreover, both the oxidation and the reduction peak potentials underwent positive shifts accompanied by the decreases of the redox peak currents. It has pointed out that the electrochemical potential of the small molecules would shift positively when it interacted into DNA double helix, and if it bound to DNA by groove binding only takes place. So we thought that the greater affinity of L_1-Cu with CT-DNA is most likely caused by a specific binding mode [31]. The two quarsi-reversible redox couple for Cu(II) complex, other complexes are irreversible redox couple for Co(II) and Ni(II).

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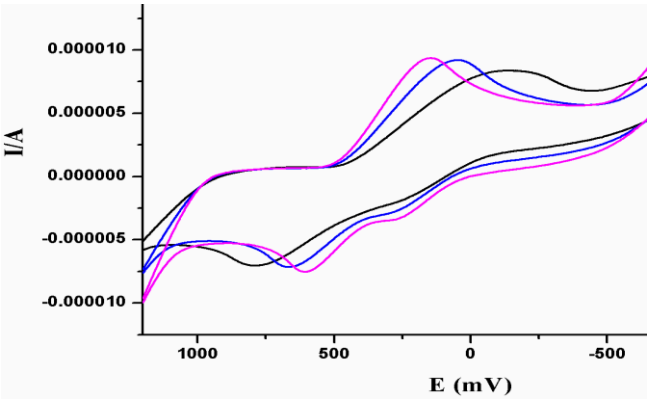

Fig.6. Cyclic voltammogram of copper(II) complex for L_1 in the absence and presence of increasing amounts CT-DNA at room temperature in DMSO : buffer (1:2) mixture (pH 7.2) (scan rate 0.1 Vs^{-1}). The arrows show the current changing upon increasing DNA concentration

I DNA Cleavage studies

The DNA Cleavage activities of complexes (Cu, Co and Ni) have been studied by gel electrophoresis and a representative picture shown in Fig.6. The interaction of plasmid pUC19 DNA with present complexes was studied in order to determine the efficiencies with which these complexes sensitize DNA cleavage. This objective was achieved by monitoring the transition from the naturally occurring, covalently closed circular form (Form I) to the nicked circular relaxed form (Form II) by means of gel electrophoresis of the plasmid. The cleavage of supercoiled (SC) DNA (Form I) to the nicked circular (NC) DNA (Form II) was observed for all six complexes regardless of different incubation periods and variation of the concentrations of the test solutions. The results of the experiments carried out in the concentration range 25 µM to 55 µM for complexes for Cu(II), Co(II) and Ni(II) after 1-2 h of incubation are displayed in Fig.18. Examination of these figures revealed that there observed no significant cleavage for controls; however, an increasing in the solution concentration of the complexes resulted in enhanced DNA cleavage [32].

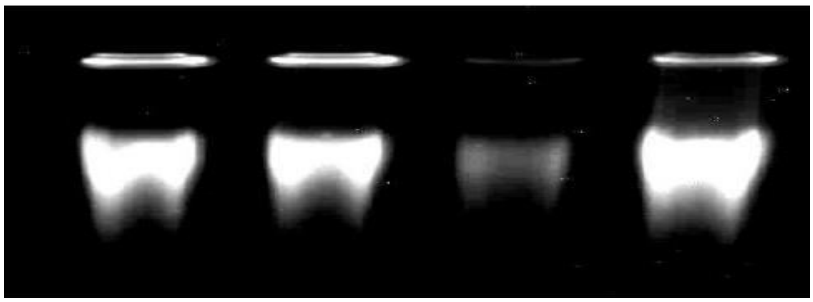

Fig.7. Cleavage of supercoiled pUC19 (10µM) by the Cu(II) complexes in the presence of Tri Acetate EDTA (TEA) buffer at 37℃. Lane 1; DNA+H_2O_2, Lane 2; L_1-Cu, Lane 3; L_1-Co, Lane 4; L_1-Ni.

J Study of cytotoxicity by MTT assay

MTT assay was performed on AGS (human gastric cancer cell line) to check the anticancer activity to study the toxicity of complexes Cu(II) and Co(II) did not show any significant activity even up to 100 µM of complexes on both the cells. However, Ni(II) complex showed excellent activity on the cancer cells. When the concentrations of complexes were increased from 0.1µM to 100µM, an increase in the percentage of cell inhibition was observed with Ni

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Vol. 2, Issue 10, October 2013

complex on gastric cells. An inhibition of 87.7%, was observed with 100 µM of Ni(II) complex (Fig. 19). However, complex Ni showed better activity though they posses octahedral geometry [33].

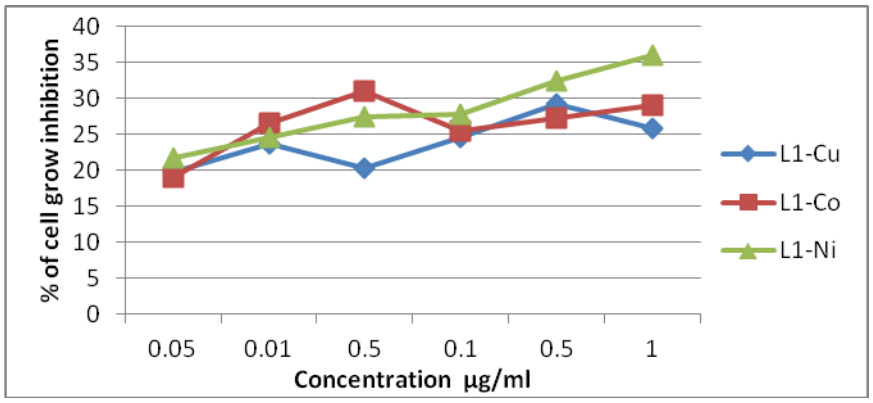

Fig. 8**.** Plots of Percentage of cell growth inhibition against various percentage of complexes on AGS cell lines.

IV. CONCLUSION

New bidentate Schiff base complexes adopt octahedral geometry. Experimental results indicate that the complexes bound to CT-DNA take the mode of groove binding, and complexes Co(II) and Ni(II) have stronger binding affinity than Cu(II).The results of cleavage studies using pUC19 DNA showed that the complexes had higher cleavage activities than the isatin-based ligands. The cytotoxic studies showed that the complexes Ni(II) exhibit good cytotoxic activity against AGS cell line. Furthermore, these complexes have potential practical applications to formulate into an efficient drug against Cancer.

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