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# Synthesis, Characterization of novel Cu(II) complexes of isatin derivatives as potential cytotoxicity, DNA binding, cleavage and antibacterial agents

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**Abstract:** 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. Some oxindole-Schiff base copper(II) complexes have shown potential antitumor activity towards deferent cells, inducing apoptosis in a process modulated by the ligand. Here, six novel copper(II) complexes with schiff base ligands were isolated and characterized by IR, $^1$H-NMR, UV-visible , CV, EPR and ESI-Mass. 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 $L_1$-Cu to $L_6$-Cu a spectroscopy are studied by UV-vis. and also cleavage studies of complexes have been done by agarose gel electrophoresis method. In-vitro biological activities of the free ligands and its Cu(II) complexes are screened against few Gram +ve and Gram –ve bacteria by disc diffusion technique. Cytotoxicity experiments carried out toward human Liver HepG2 cells confirmed its pro apoptosis property. Interestingly, compounds, $L_4$-Cu and $L_6$-Cu were found to be excellent anticancer activity against HepG2: liver cells and $L_4$-Cu was most significant cytotoxicity activity against MAIT cells .

**Keywords:** isatin, 2, 2-diphenylethanamine, Cu(II) complexes, antibacterial activity, DNA binding, cleavage and anticancer studies.

## I. INTRODUCTION

The synthesis and study of inorganic compounds containing biologically relevant ligands are encouraged by the importance of metal ions in a variety of biochemical processes [l-3]. The therapeutic application of metal complexes in modern medicine was arguably initiated by the discovery of the anticancer properties of cisplatin [4, 5]. In fact, cisplatin and the later compounds carboplatin, and oxaliplatin enjoy the status of the world's best-selling anticancer drugs. Copper is the greatest importance for life and essential for photosynthesis and mitochondrial respiration, for carbon and nitrogen metabolism, for oxidative stress protection, and is required for cell wall synthesis, to name only a many of its cellular tasks. Copper has long been used to control the growth of organisms in wood products as well as in aquaculture, agriculture and medicine. The multifaceted role of copper in biological systems is established by several studies. In particular the involvement of copper in human diseases has been described from a medicinal-chemical and a biochemical view focusing on the molecular physiology of Cu transport [6-8]. A number of Cu(II) chelate complexes that exhibit cytotoxic activity through cell apoptosis or enzyme inhibition have been reviewed. Such complexes containing bi-Schiff bases as ligands are effective in reducing tumor size, delaying of metastasis, and significantly increasing the survival of the hosts. Current interest in Cu complexes is stemming from their potential use as antimicrobial, antiviral, anti-inflammatory, antitumor agents, enzyme inhibitors, or chemical nucleases has been studied [9]. Moreover, several authors have brought to attention the antiviral and antibacterial activity of Cu(II) complexes. For instance, it was shown that the infectivity of influenza A virus is reduced after exposure on copper surfaces [10]. Copper(II) complexes are regarded as the most promising alternatives to cisplatin as anticancer drugs; an idea

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supported by a considerable number of research articles describing the synthesis, DNA binding and cytotoxic activities of numerous copper(II) complexes[11, 12]. In addition, the fundamental role of copper(II) complexes as important bioactive compounds *in vitro* and *in vivo* aroused an ever-increasing interest in these agents as potential drugs for therapeutic intervention in various diseases [13-17].

Isatin and its derivatives are unique members in the Schiff base family. The simple isatin based Schiff base compounds having, acyl, aroyl and heteroacroyl Schiff bases have additional donor sites >C=O, >C=N-, etc. These donor sites make them more flexible and versatile. This versatility has made them excellent chelating agents that can form a variety of complexes with various transition and inner transition metals and has attracted the attention of many researchers [18, 19]. Isatin, an endogenous indole and its derivatives exhibit a wide range of biological activities [20]. Isatin-based Schiff base copper(II) complex is related to the antiviral drug, methisazone. Significant interest in the design of metal compounds such as drugs and diagnostic agents is termed medicinal inorganic chemistry [21, 22]. Application of electroanalytical techniques includes determination of reaction mechanisms. Metal–isatin binary complexes were advantageous over simple isatin in chemotherapy and found to act as anticancer agents, especially Schiff-base transition metal complexes derived from isatin [23].

This created a great interest in researchers to synthesize variety of isatin derivatives and screened them for their diverse biological activities such as anticancer, anti-HIV, anthelmintic, antimycobacterial, anti-inflammatory, antidiabetic, antimicrobial, trypanocidal as well antimalarial activities [24]. Information obtained from this study will be helpful to understand the mechanism of isatin derivatives interaction with DNA, and should be useful to develop excellent anticeancer activity and new therapeutic reagents for some diseases. The free ligand and its complexes have been tested for in vitro antimicrobial activity against seven different bacteria and four different fungi by minimum inhibitory concentration (MIC).

## II. EXPERIMENTAL

### *A Materials and Methods*

All chemicals were purchased from Sigma-Aldrich, E-Merk and used as received without purification. isatin, 2,2-diphenylethanamine, DMSO, Calf Thymus (CT) DNA and pUC-19 plasmid DNA purchased from Sigma-Aldrich G.R grade, Bangalore. Metal chloride [$CuCl_2.2H_2O$] and solvents were purchased from E-Merk,A.R grade, Mumbai.

C, H and N analyses of the free Schiff base ligands and their complexes were performed in CHN analyzer Elementar Vario EL III. Metal contents were analyzed by the standard procedures. Hand-Held Meter LF330 was used to measure the molar conductance of the 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. $^1$H 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 100Hz modulation. Cyclic Voltammetric measurements were carried out in a Bio-Analytical System (BAS) model CV-50W electrochemical analyzer.

### *B Synthesis of Schiff base ligands and its Cu(II) complexes*

*Synthesis of Schiff base ligands: ($L_1$-$L_6$)*

$L_1$: (E)-3-(2,2-diphenylethylimino)indolin-2-one
$L_2$: (E)-3-(2,2-diphenylethylimino)-5-fluoroindolin-2-one
$L_3$: (E)-5-chloro-3-(2,2-diphenylethylimino)indolin-2-one
$L_4$: (E)-5-bromo-3-(2,2-diphenylethylimino)indolin-2-one
$L_5$: (E)-3-(2,2-diphenylethylimino)-5-methylindolin-2-one
$L_6$: (E)-3-(2,2-diphenylethylimino)-5-nitroindolin-2-one

5-substituted isatin (1mmol) with 2, 2-diphenylethanamine (1mmol) were dissolved in 50mL of absolute MeOH, three drops of glacial acetic acid was added and the resulting solution was refluxed for 4-6 hr. The compound precipitated

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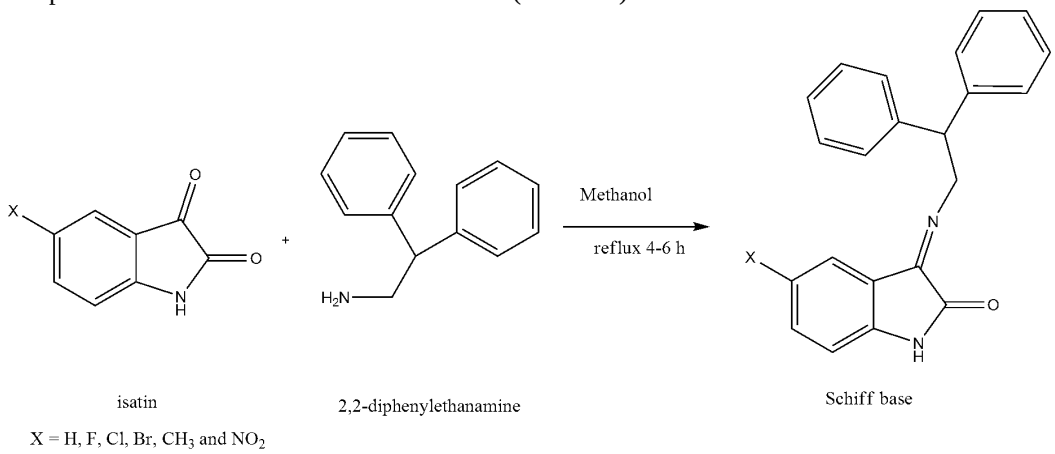
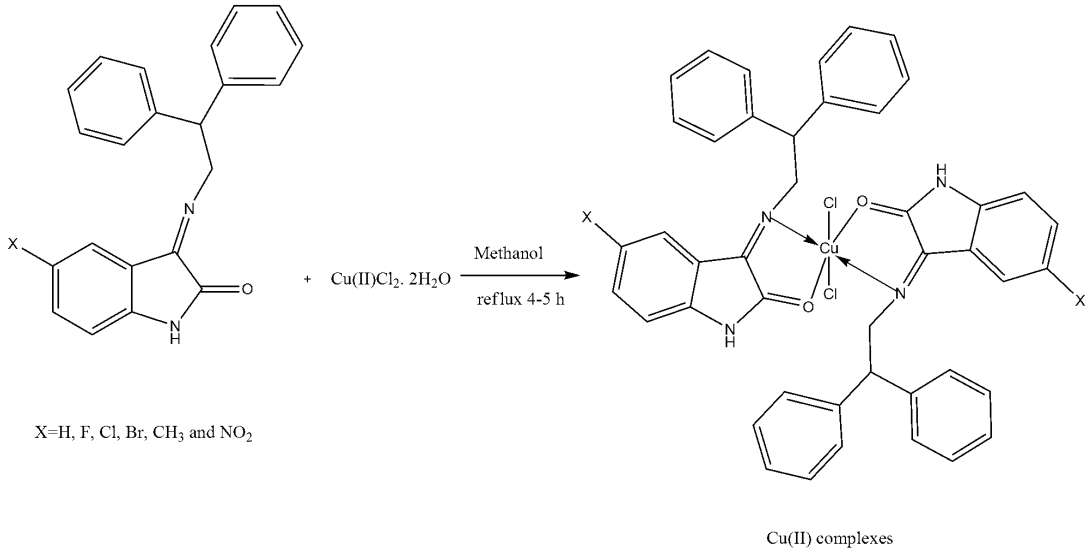
upon cooling to room temperature, was collected by filtration and recrystallized from MeOH. Yellow colour powder compound was obtained from the same solvent (Scheme1).




Scheme 1. Synthesis of Schiff base ligands

*Synthesis of complexes ($L_1$-Cu to $L_6$-Cu)*

To 20 mL of methanolic solution of Schiff base ligands (1 mmol) was added drop wise to the methanolic solution (10mL) of copper(II) chloride (0.5 mmol) and refluxed for 4-5 h. The resultant solution was reduced to one-third of its volume, filtered and evaporated to dryness. The solid product thus obtained was washed with water followed by cold methanol and dried in vacuo (Scheme 2).




Scheme 2. Synthesis of complexes

*C Antibacterial studies*

In vitro biological screening effects of the synthesized free ligands and their Cu(II) complexes. The antimicrobial tests were performed by the standard disc diffusion method [25]. The antibacterial activity of the complexes was studied against Gram-positive bacteria *Staphylococcus aureus, Bacillus* and Gram-negative bacteria *Escherichia coli, Serratia, Klepsiella and Proteus*. Each of the metal complex compounds dissolved in DMSO at a concentration of 1 mg/ml was prepared. Paper discs of Whatman filter paper no. 1 were cut and sterilized in an autoclave. The paper discs were saturated with 10 µl of the metal complex compounds dissolved in DMSO solution or DMSO as negative control and

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were placed aseptically in the Petri dishes containing Nutrient agar media inoculated with the above mentioned six bacteria separately. The petridishes were incubated at 37 0C and the inhibition zones were recorded after 24 h of incubation. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds.The mean value obtained for three individual replicates was used to calculate the zone of growth inhibition of each sample.

*D Nuclease studies*

The concentration of CT-DNA was determined by UV absorbance at 260 nm ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$). CT DNA free from protein contamination was confired from its absorbance values at 260 nm, 280 nm and ratio $A_{260}/A_{280}$ was found to be 1.87 [26].

*E Absorption studies*

The UV–Vis absorption spectroscopy studies and the DNA binding experiments were performed at room temperature. The purity of the CT-DNA was verified by taking the ratio of the obsorbance values at 260 and 280 nm in the respective buffer, which was found to be 1.8:1, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 $dm^3\ mol^{-1}\ cm^{-1}$ at 260 nm. The complexes were dissolved in a mixed solvent of 5% DMSO and 95% phosphate buffered saline for all the experiments. Absorption titration experiments were performed with a fixed concentration of the compounds (30 µM) while gradually increasing the concentration of DNA (5–50 µM). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorbance of DNA itself. For metal complexes, the intrinsic binding constant ($K_b$) was determined from the spectral titration data using the following equation [27].

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

Where, $\varepsilon_a$, $\varepsilon_b$ and $\varepsilon_f$ 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.

*F Cleavage studies*

pUC19 DNA at pH 7.2 in Tris-HCL buffered solution was used to perform agarose gel electrophoresis techniques. 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 mixtures were 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 [28].

*G Cytotoxic activity evaluation*

*3-(4, 5-dimethylthiazol-2- yl)- 2,5- diphenyltetrazolium bromide (MTT) assay*

Cytotoxic effect of the four new complexes on human liver cancer cells (HepG2) were assayed by the 3-(4, 5-dimethylthiazol-2- yl)- 2,5- diphenyltetrazolium bromide (MTT) assay[29]. The assay was carried out according to the instruction provided by the vendor. Briefly, cells were harvested from the logarithmic phase of cultures and re-suspended in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cell counts were adjusted and equal number of cells were plated into each well of 96-well cell culture plates and allowed to grow overnight at 37 $^oC$, in presence of 5% $CO_2$. The cells were treated with test substances at various concentrations ranging between 0.7 µM to 2.5 µM for 72h. In vehicle control culture wells, a maximum of 0.5% DMSO was added. Culture medium was renewed at every 24h with fresh culture medium supplemented with the test substances. Thereafter, 0.5 µM of MTT reagent was added to each well and the microplate was incubated further for 4h at 37 $^oC$ in presence of 5% $CO_2$. Finally, the cells were solubilized by adding solubilizing solution and allowed to incubate at 37 $^oC$ overnight. After complete solubilization of the formazan crystals the absorbance was read at 540 nm

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in a microplate reader (BioRad, USA). The results (mean OD ± SD) obtained from quadruplicate wells were used in calculation to determine the cytotoxicity (50% of inhibitory concentration, IC50) of the test compounds.

*Tryphan blue exclusion assay*

The trypan blue dye exclusion assay is the most commonly utilized test for cell viability [30]. The value of this procedure is limited since the number of blue-staining cells increases following addition of the dye, requiring that cells be counted within 3-5 min [30]. For cell growth cycle viability studies, a uniform suspension of cells was inoculated into triplicate 75 $cm^2$ tissue culture flasks and maintained in darkness in a standard $CO_2$ incubation chamber. Replicate samples from all flasks were counted each day for 7days using dye exclusion assays of cell viability. Comparative cell viability stain experiments were conducted three times. Following analysis of variance, data from all experiments were pooled for further statistical analysis For trypan blue staining, 200 µL of cells was aseptically transferred to a 1.5-mL clear Eppendorf tube and incubated for 3 min at room temperature with an equal volume of 0.4% (w/v) trypan blue solution prepared in 0.81 % NaCl and 0.06 % (w/v) dibasic potassium phosphate. Cells were counted using a dual-chamber hemocytometer and a light microscope. Viable and nonviable cells were recorded separately, and the means of three independent cell counts were pooled for analysis.

## III. RESULTS AND DISCUSSION

The bidentate NO type of Schiff base ligands ($L_1$-$L_6$) and its Cu(II) complexes with 5-substituted isatin and 2,2-diphenylethanamine were synthesized and characterized by various spectral techniques. The synthesized Cu(II) complexes were found to be air stable, amorphous nature, moisture free and soluble only in DMF and DMSO.

### *A Elemental analysis and conductivity measurements*

The synthesized schiff base ligands ($L_1$ to $L_6$) and their Cu(II) complexes were analyzed for their physico-chemical properties like melting point (m.p.), color, yield, elemental analysis and conductivity which are given in table.1. The elemental analytical data of ligands and their complexes are well agreed with their calculated values, showing that 2:1 (ligand : metal) stoichiometry ratio. The observed low conductivity values (15.81-36.55$\Omega^{-1}$ $cm^2$ $mol^{-1}$) were accounted for the dissociation and hence the complexes are found as non-electrolytes [31].

### *B Vibrational spectral studies*

Vibrational spectra of free Schiff base ligands ($L_1$-$L_6$) were compared to investigate the mode of binding present in the synthesized Cu(II) complexes. The FT-IR spectral data are summarized in Table 2. The IR spectrum of the free ligand ($L_1 - L_6$) showed broad band's 3151 – 3195 $cm^{-1}$, which can be attributed to υ(NH) stretching vibration of the isatin moiety The ligands showed strong bands around at 1614-1621 $cm^{-1}$ which assigned to azomethine moiety. In the spectra of the complexes, this peak is slightly shifted to lower frequency around 1600-1584 $cm^{-1}$. This suggested that coordination of the metal is through the azomethine nitrogen atom [32]. The strong intensity bands of ligands were observed at the region 1714-1735 $cm^{-1}$ of the spectra indicating carbonyl group. The positions of these bands were shifted to lower region 1658-1683 $cm^{-1}$ the spectra indicating the involvement of υ(C=O) with metal centre during complexation. The ligands bind with the Cu(II) ions in a bidentate manner through azomethine –N and carbonyl –O atoms respectively. Further, the two new bands appeared in the far infrared region at 455-462 $cm^{-1}$ and 536-570 $cm^{-1}$ were assigned to υ(M-N) and υ(M-O) respectively[33]. Thus, the IR spectral results provide evidence for bidentate complexation of Schiff bases with metals.

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**Table1**. Composition and physical characteristics of $L_1$ –$L_6$ and their Cu(II) complexes

| Ligands / complexes | Molecular Formula | Color | Found (Calculated) % | | | | M.P ($^0$C) | Yield (%) | Ω (Ohm$^{-1}$ cm$^2$ M$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| | | | M | C | H | N | | | |
| $L_1$ | $C_{22}H_{18}N_2O$ | Yellow | - | 81.62 (80.96) | 4.90 (5.56) | 8.01 (8.58) | 130 | 95 | - |
| $L_2$ | $C_{22}H_{17}FN_2O$ | yellow | - | 75.82 (76.73) | 5.41 (4.98) | 7.45 (8.13) | 124 | 90 | - |
| $L_3$ | $C_{22}H_{17}ClN_2O$ | yellow | - | 72.75 (73.23) | 4.20 (4.75) | 7.03 (7.76) | 120 | 90 | - |
| $L_4$ | $C_{22}H_{17}BrN_2O$ | yellow | - | 64.23 (65.20) | 4.70 (4.23) | 7.62 ( 6.91) | 118 | 85 | - |
| $L_5$ | $C_{23}H_20N_2O$ | Yellow | - | 80.30 (81.15) | 5.31 (5.92) | 8.01 (8.23) | 132 | 80 | - |
| $L_6$ | $C_{22}H_{17}N_3O_3$ | Yellow | - | 70.68 (71.15) | 4.21 (4.61) | 11.81 (11.31) | 148 | 80 | - |
| $L_1$-Cu | $C_{44}H_{36}Cl_2CuN_4O_2$ | Light brown | 8.51 (8.07) | 66.09 (67.13) | 4.21 (4.61) | 6.34 (7.12) | 258 | 85 | 26.43 |
| $L_2$-Cu | $C_{44}H_{34}Cl_2CuF_2N_4O_2$ | Dark green | 7.02 (7.72) | 63.04 (64.20) | 4.71 (4.16) | 7.05 (6.81) | 280 | 85 | 24.11 |
| $L_3$-Cu | $C_{44}H_{34}Cl_4CuN_4O_2$ | Dark green | 7.31 (7.42) | 60.74 (61.73) | 3.42 (4.00) | 5.90 (6.54) | 245 | 80 | 36.55 |
| $L_4$-Cu | $C_{44}H_{34}Br_2Cl_2CuN_4O_2$ | Dark brown | 5.36 (6.72) | 56.08 (55.92) | 3.07 (3.63) | 5.71 (5.93) | 260 | 85 | 15.81 |
| $L_5$-Cu | $C_{46}H_{40}Cl_2CuN_4O_2$ | brown | 7.81 (7.79) | 66.83 (67.77) | 4.31 (4.95) | 6.44 (6.87) | 250 | 75 | 18.03 |
| L6-Cu | $C_{44}H_{34}Cl_2CuN_6O_6$ | Dark geen | 7.22 (7.24) | 60.31 (60.24) | 3.58 (3.91) | 8.92 (9.58) | 256 | 70 | 22.54 |

**Table 2.** Vibration spectral data for the Cu(II) complexes and in KBr disc (cm$^{-1}$)

| Compounds | υ (NH) of indole ring | Lactonyl, υ(C=O) of indole ring | υ(C=N) | υ (M-N) | υ(M-O) |
|---|---|---|---|---|---|
| $L_1$-Cu | 3195 | 1686 | 1592 | 455 | 543 |
| $L_2$-Cu | 3192 | 1678 | 1600 | 458 | 536 |
| $L_3$-Cu | 3211 | 1691 | 1554 | 462 | 539 |
| $L_4$-Cu | 3180 | 1683 | 1598 | 460 | 570 |
| $L_5$-Cu | 3182 | 1697 | 1570 | 457 | 563 |
| $L_6$-Cu | 3179 | 1689 | 1583 | 458 | 543 |

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*C NMR Spectra*

The $^{1}$H-NMR (300 MHz, $CDCl_3$, δ/ppm) spectrum of the Schiff base exhibited the following signals around at 9.13-9.45 NH protons, 6.7-8.2 aromatic protons. In addition to these two singlet peaks observed at 4.03-4.8 (-CH) and 5.0-5.21 ($-CH_2$) for methine and methene protons of ligands $L_1$-$L_6$.

The $^{13}$C NMR (300 MHz, $CDCl_3$, δ/ppm) spectra provide further support for the structural characterization of the Schiff bases. The signals around at 164.03-164-91 (C=O, isatin), 158-95-159.01 (C=N, isoniazid), 110.36-144.82 (aromatic carbons), 59.32-59.57 (methine carbon) and 52.41-52.88 (methane carbon) for $L_1$-$L_6$.

*D Mass spectra*

The mass spectrum of ligands and metal complexes is recorded under liquid secondary ion mass spectral conditions. The ligands $L_1$ – $L_6$ gave the peaks at m/z (M+1) = 327, 345, 361, 406, 341 and 372.The mass spectrum of $L_6$-Cu complex was (Fig.1) exhibited m/z peaks at 878 (M+1) adduct. These values confirm the molecular weight of the ligands and complexes.

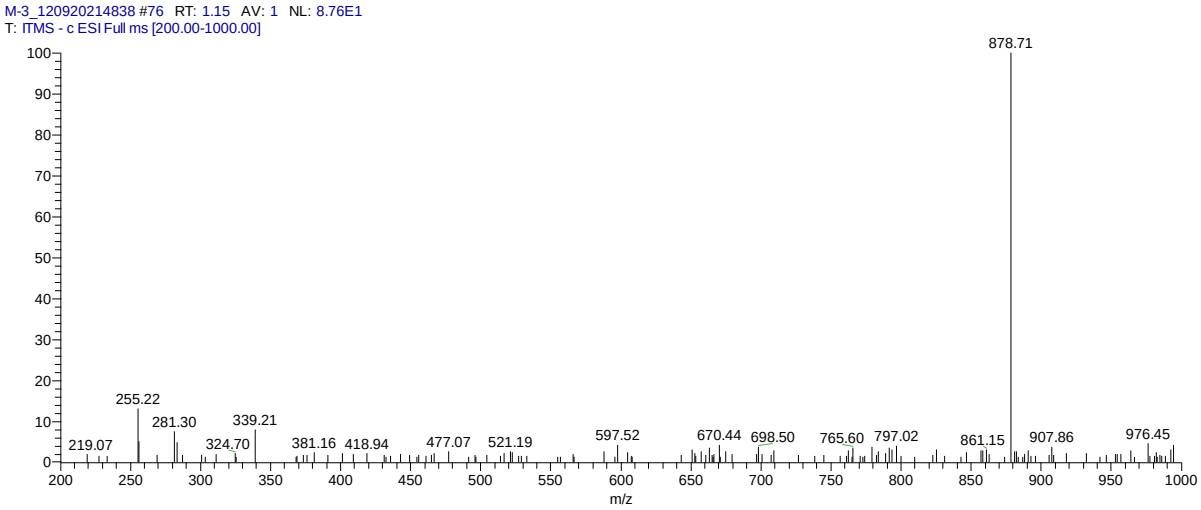


Fig.1. Mass spectrum of $L_6$-Cu

*E Electronic spectra and magnetic moment values*

The electronic spectra of the ligands and its metal(II) complexes were recorded in DMSO. The absorption bands at 38314 $cm^{-1}$ and 34364 $cm^{-1}$ attributed to $\pi \rightarrow \pi$ and n→π* transitions for $L_1$; The Electronic spectra of $L_1$-Cu(II) complex display two prominent bands. A low intensity broad band around 18867-16129 cm-1 is assignable to $^{2}T_{2g} \leftarrow {}^{2}E_g$ transition. Another high intensity band around 25641-22123 cm-1 is due to symmetry forbidden ligand → metal charge transfer. On the basis of electronic spectra distorted octahedral geometry around Cu(II) ion is suggested [34]. The Cu(II) complex showed magnetic moment 2.36 BM, is slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [35]. The spectrum of the Cu(II) complexes $L_1$-$L_6$ exhibited bands and magnetic moment values are given in Table 3.

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**Table 3**. Electronic spectra, spectral parameters and magnetic moment with suggested structures of ligands and their complexes

| Compound | π→ π* ($cm^{-1}$) | n→π* ($cm^{-1}$) | LMCT | d-d | Assignment | Suggested Structure | μeff (B.M) |
|---|---|---|---|---|---|---|---|
| $L_1$-Cu | 38610 | 34013 | 23752 | 17301 | $^2B_{1g} \rightarrow {}^2B_{2g}$ , $^2E_g$ | Octahedral | 1.95 |
| $L_2$-Cu | 37593 | 34013 | 23474 | 16808 | $^2B_{1g} \rightarrow {}^2B_{2g}$ , $^2E_g$ | Octahedral | 1.76 |
| $L_3$-Cu | 37174 | 34129 | 25380 | 18621 | $^2T_{2g} \leftarrow {}^2E_g$ | Octahedral | 2.34 |
| $L_4$-Cu | 37037 | 31250 | 22883 | 16129 | $^2B_{1g} \rightarrow {}^2B_{2g}$ , $^2E_g$ | Octahedral | 1.87 |
| $L_5$-Cu | 37313 | 31545 | 22883 | 16806 | $^2B_{1g} \rightarrow {}^2B_{2g}$ , $^2E_g$ | Octahedral | 2.10 |
| $L_6$-Cu | 37174 | 31250 | 22123 | 17482 | $^2T_{2g} \leftarrow {}^2E_g$ | Octahedral | 2.13 |

*F ESR spectra*

The X-band EPR spectrum of the copper(II) complexes were recorded in the solid state at room temperature. The complex has a well resolved $g_{II}$ and broadened g region and various Hamiltonian parameters have been calculated ($g_{II}$ =1.93; $g_{\perp}$=1.819; $A_{II}$ =102x$10^4$ for $L_1$-Cu, $g_{II}$ =2.392; $g_{\perp}$=2.031; $A_{II}$ =120x$10^4$ for $L_2$-Cu, $g_{II}$ =2.011; $g_{\perp}$=1.982; $A_{II}$ =108x$10^4$ for $L_3$-Cu, $g_{II}$ =2.221; $g_{\perp}$=2.103; $A_{II}$ =112x$10^4$ for $L_4$-Cu, $g_{II}$ =1.902; $g_{\perp}$=2.001; $A_{II}$ =107x$10^4$ for $L_5$-Cu and $g_{II}$ =2.131; g⊥=1.944; $A_{II}$ =116x$10^4$) the trend $g_{II} > g_{\perp}$ observed in this complex indicate that the unpaired electron is most likely to be in the $d_{x^2-y^2}$ orbital [36].

*G Cyclic voltammetry*

A cyclic voltammogram of Cu(II) complex is value presented in Table 4. Voltammogram displays a reduction peak at Epc= -2.8V with an associated oxidation peak at Epa= -0.4V at a scan rate of 50mV/s. The peak separation of this couple (ΔEp) is 0.7V and increases with scan rate. The ΔEp value increases at different scan scan rates respectively. Thus, the analyses of cyclic voltametric responses at different scan rate give the evidence for quasi-reversible one electron reduction. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) 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 [37].

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**Table. 4.** Electrochemical parameters for Cu(II), Co(II) and Ni(II) complexes

| Compound | Redox couple | Epa (V) | Epc(V) | ΔEp(V) | Ipa/Ipc |
|---|---|---|---|---|---|
| $L_1$-Cu | Cu(II)/Cu(I) | 0.8 | 1.4 | 0.6 | 1.02 |
| $L_2$-Cu | Cu(II)/Cu(I) | 0.7 | 1.6 | 0.9 | 0.85 |
| $L_3$-Cu | Cu(II)/Cu(I) | 0.7 | 1.5 | 0.8 | 0.81 |
| $L_4$-Cu | Cu(II)/Cu(I) | 0.9 | 1.9 | 1.0 | 0.97 |
| $L_5$-Cu | Cu(II)/Cu(I) | 0.8 | 1.7 | 0.9 | 1.03 |
| $L_6$-Cu | Cu(II)/Cu(I) | 0.6 | 1.4 | 0.8 | 0.73 |

*H DNA binding studies*

Of all the techniques used, electronic absorption spectroscopy is one of the most common techniques for the investigation of the mode of interaction of metal complexes with CT-DNA [38]. Hence, a complete electronic spectral study was conducted with the new complexes and CT-DNA. The absorption spectra of $L_1$-Cu to $L_6$-Cu complexes in the absence and presence of CT-DNA are given in Fig. 2. With increasing CT-DNA concentration for the $L_1$-Cu complexe, the hypochromism in the band at the found 435 and 445 nm reaches as high as 54.05% and 65.42% respectively. Other Cu(II) complexes also exhibit the similar results during the addition of increasing concentration of DNA, complexes showed hypochromicity and a red-shifted charge transfer peak maxima in the absorption spectra. The intrinsic binding constant $K_b$ is obtained from the ratio of slope to the intercept from the plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA]. The Kb values are shown in table 5. Hence the above phenomenon is indicative of most probable binding mode of Cu(II) complexes for $L_1$ to $L_6$ 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 [39].

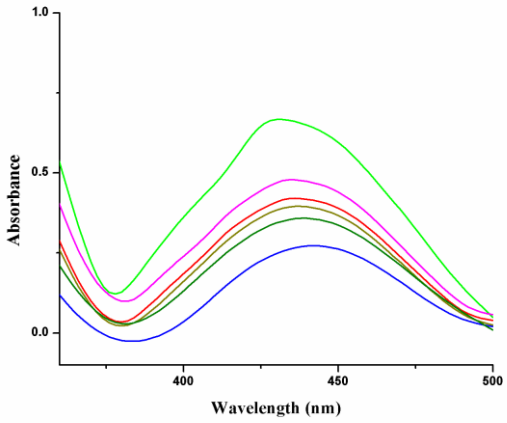


Fig.2. Absorption spectra of Cu(II) complex for $L_1$, in the absence and in the presence of the CT-DNA. [DNA]=30 µM, [complex] = 0 to 30 µM. The arrow indicates Absorption intensity decrease with increasing addition of the CT-DNA.

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Table 5. Absorption properties ofCu (II) complexes with CT-DNA.

| Complex | $\lambda_{max}$ (nm) | $\Delta\lambda$ (nm) | Hypochromicity (%) | $K_b x10^4$ ((mol $L^{-1}$)$^{-1}$) |
|---|---|---|---|---|
| $L_1$-Cu | 265, 298 | 4 | 56.25, 62.41 | 5.31 |
| $L_2$-Cu | 260, 293 | 3 | 43.13, 48.16 | 5.80 |
| $L_3$-Cu | 285, 297 | 2 | 47.53, 63.18 | 5.31 |
| $L_4$-Cu | 266, 298 | 5 | 62.15, 71.36 | 8.25 |
| $L_5$-Cu | 267,296 | 3 | 50.19, 55.16 | 5.04 |
| $L_6$-Cu | 268,287 | 4 | 41.71, 55.13 | 5.78 |

*I DNA cleavage studies*

The DNA cleavage activities of Cu(II) complexes have been studied by gel electrophoresis and a representative pictograph is shown in Fig. 3. The results showed that the supercoiled pUC19 DNA in buffer medium (pH=7.2; Tris-HCl/NaCl) was converted into open circular form due to the formation of metal chelation. During the cleavage process, the smallest fragments moved quickly towards anode than the larger fragments. Bromophenol blue was used as a photosesitizer that can be activated on irradiation by UV. The completion of gel electrophoresis experiment clearly indicated that the intensity of the treated DNA samples has diminished due to the cleavage of DNA. These results indicated that the metal ions played an important role in the cleavage of DNA [40].

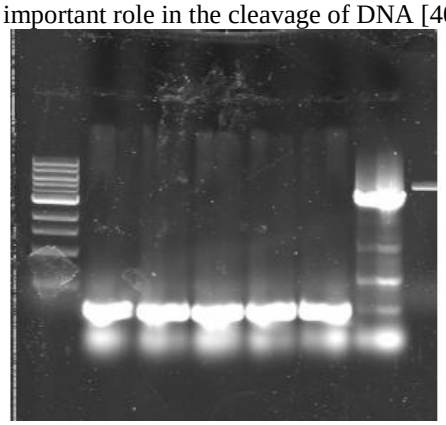

Fig.3. Cleavage of supercoiled pUC19 (10µM) by the Cu(II) complexes in the presence of Tri Acetate EDTA (TEA) buffer at 37℃. Lane 1; DNA+$H_2O_2$, Lane 2; $L_2$-Cu, Lane 3; $L_3$-Cu, Lane 4; $L_5$-Cu, Lane 5, $L_6$-Cu, Lane 7; DNA-control.

*J In-vitro antimicrobial assay*

The antimicrobial results are shown in Figure 4. From the antibacterial studies it is inferred that, the Schiff base was found to be potentially active against Gram-positive bacteria *Staphylococcus aureus, Bacillus* and Gram-negative bacteria *Escherichia coli, Serratia, Klepsiella and Proteus*. Some of the complexes were shown high antibacterial activity against *Escherichia coli* and B. subtilis. L4-Cu complex was excellent antibacterial acvivity against all the Gram +ve and Gram –ve bacteria.

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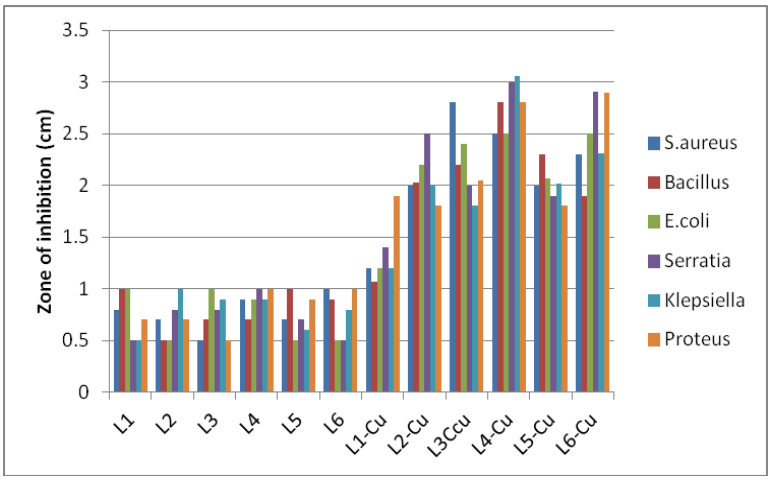


Fig.4. Antibacterial activities of Schiff base ligands and their Cu(II) complexes with control and compounds at 40µl in different microorganism species.

*K Study of cytotoxicity by MTT assay*

The cytotoxicity assays for the new $L_1$-Cu to $L_6$-cu complexes were assessed using the method of MTT reduction. Cisplatin was used as a positive control. All the complexes were found to be cytotoxic to liver cancer cell line (HepG2). All the complexes were significant activity even up to 2.5µM concentrations (Fig. 5). The complexes exhibited higher cytotoxic effects on liver cancer cells with lower percentage of inhibition in cell proliferation values indicating their efficiency in killing the cancer cells even at low concentrations. The cytotoxic effectiveness of these compounds with the percentage of inhibition of 0.7 µM ($L_4$-Cu) and ($L_6$-Cu ) were higher than that of control. When the concentrations of complexes were increased from 0.7µM to 2.5µM an increase in the percentage of cell inhibition was observed with six complexes on HepG2 cells. There are reports in the literature on the cytotoxic effects of the complexes with longer incubation time periods. The longer incubation period may result in the development of cellular resistance for that particular complex. Beckford et al have reported 50% inhibitory concentration of different complexes after an exposure for 72 h at µM concentrations. But, the data obtained for our complexes showed higher cytotoxicity with short incubation period (48 h). Hence, our data are highly significant when compared to the results of Beckford et al., [41-42]. Moreover, the percentage inhibition values of our complexes are comparable with the reported values of standard anticancer drugs such as cisplatin.

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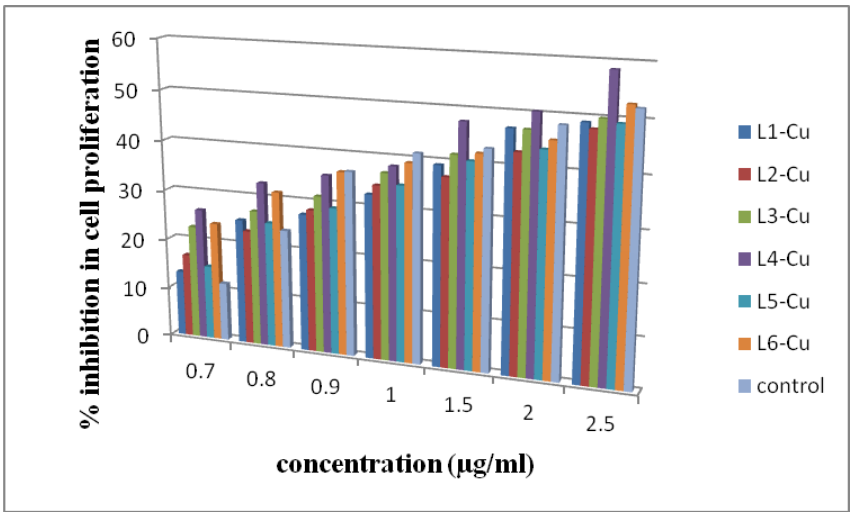


Fig. 5. Plots of Percentage inhibition in cell proliferation against various percentage of complexes

*L In vitro culture of MAIT cells and proliferation assay for L4-Cu complex*

Mucosal-associated invariant T (MAIT) cells are very abundant in humans and have antimicrobial specificity, but their functions remain unclear. Cytotoxic activity detected for $L_4$-Cu complex by MTT assay (Fig. 6). The cells were exposed to various concentrations of MAIT for 24 h. (B) Antiproliferative effects detected by Tryphan blue exclusion assay. Cells were treated with MAIT ranging in doses from 15 to 50'g/ml for 7 days. Control cells were treated with 0.1% DMSO. 0 'g/ml ( ), 15 'g/ml (*), 25 'g/ml (X) , 50 'g/ml (O). All the experiments were repeated three times, and the values and bars represent mean and S.D., respectively. Hence, our data, L4-Cu complex was excellent cytotoxic activity comparing other complexes.

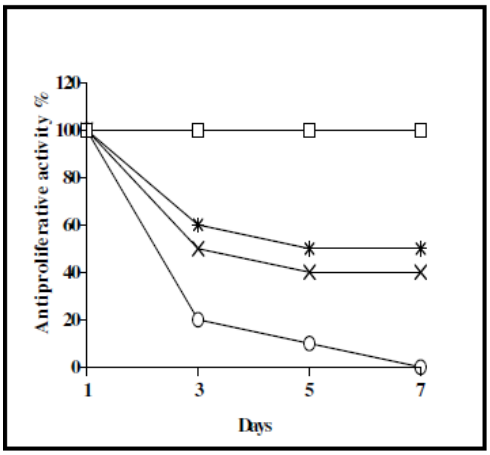


Fig. 6. Cytotoxic activity activity detected for $L_4$-Cu by MTT assay. The cells were exposed to various concentrations of MAIT for 24 h. (B) Antiproliferative effects detected by Tryphan blue exclusion assay. Cells were treated with MEIT ranging in doses from 15 to 50'g/ml for 7 days. Control cells were treated with 0.1% DMSO . 0 'µl ( ), 15 'µl (*), 25 'µl (X), 50 'µl (O). All the experiments were repeated three times, and the values and bars represent mean and S.D., respectively.

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## IV. CONCLUSION

A series of new Cu(II) complexes of 5-substituted isatin with 2,2-diphenylethanamine have been prepared and characterized by various spectral techniques. The UV-vis, IR, NMR ESI-Mass and EPR data showed that Cu(II) complexes adopt octahedral geometry. From DNA binding results showed that all the complexes are moderate intercalators. DNA cleavage indicated that the $L_4$-Cu(II) complex has better cleaver other than complexes. The antimicrobial actions of Cu(II) complexes, the zone of inhibition for $L_4$-Cu and $L_6$-Cu were excellent activity against *Staphylococcus aureus* and Serratia. This study can be extended to investigate the toxicity and pharmacokinetic aspects to get clear insight into the therapeutic utility of these compounds. Moreover, all the new complexes were screened for antitumor activity against Hep G2 cancer cell lines, and they were found to exhibit excellent cytotoxicity to cancer cell without affecting the normal cells. In all the above experimental results, we observed that complex $L_4$-Cu and $L_6$-Cu have the excellent activity, which may be due to the $5^{th}$ position attached to bromine and nitro compound of isatin.

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