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RESEARCH ARTICLE

Synthesis, DNA binding, cleavage, antibacterial and cytotoxic activity of Novel Schiff base Co(II) Complexes of substituted isatin

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Abstract

Isatin (1H-indole-2, 3-dione) is a synthetically versatile substrate used for the synthesis of heterocyclic compounds and as a raw material for drug synthesis. Isatin and its derivatives exhibit anticonvulsant, antibacterial, antifungal, antiviral, and anticancer properties. Here, three novel cobalt(II) complexes with similar ligands were isolated and characterized by spectroscopic techniques, having their reactivity compared to the so far much active complex in this class. Elemental analysis and molar conductance values indicate that the complexes are non-electrolytes. All the complexes adopt octahedral geometry around the metal ions. In-vitro biological activities of the free ligands and its Co(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 cytotoxic property. DNA binding studies were then performed in the presence of these complexes, in order to verify the influence of ligand structural features in its nuclease activity.

Introduction

During recent years coordination compounds of biologically active ligands [1–3] have received much attention. Chelation causes drastic change in the biological properties of the ligands and also the metal moiety. It has been reported that chelation is the cause and cure of many diseases including cancer. A number of Schiff base complexes [4–7] have been tested for antibacterial activities and they have been found antibacterial [8–11], antifungal [10–12], anticancer [13, 14], and herbicidal [15] activities. Cancer is one of the major health issues in humanity and one of the primary targets in therapeutic chemistry [1–3]. Metal complexes can easily bind to DNA via non-covalent interactions, such as electrostatic, groove and intercalative binding. Among these, the intercalative mode of binding with metal complexes to DNA possesses superior applications in pharmaceutical industries and has been proven to be an effective binding in anticancer activities [4]. Cisplatin (cis-diamminedichloroplatinum(II)) is a well known metal based drug for cancer therapy, but it has its own limitations due to its resistance in tumor cells and serious side effects such as sickness, kidney and liver failure, typical of heavy metal toxicity [5–8]. Recently less toxic and target-specific non-covalent DNA binding anticancer drugs have been developed as an important area of research. Some new non-platinum anticancer metallodrugs also bind to DNA by intercalation [9–13]. In this context, the biological chemistry of copper has been rapidly expanded due to its increasing number of cobaltr complexes with potential impact in medicinal applications. Owing to cobalt (II) complexes having octahedral geometries, they show a remarkable intercalative binding affinity as well as DNA cleavage properties [14, 15]. These Schiff base compounds give not only new complexes with important pharmacological properties but also exhibit interesting and varying coordination modes in their complexes [16-20].

On the other hand, cobalt is an element of biological interest because its biological role is mainly focused on its presence in the active center of vitamin B_{12}, which regulates indirectly the synthesis of DNA. Additionally, there are at least eight cobalt-dependent proteins [21]. Cobalt is also involved in the co-enzyme of vitamin B_{12} and is used as a supplement of the vitamin [22]. Since the first reported studies into the biological activity of Co complexes in

1952, many cobalt complexes of biological interest have been reported with the most structurally characterized showing antitumor, antiproliferative, antimicrobial and antifungal activity [23-30].

To the best of our knowledge until date there is no report on the metal chelating ability and the antitumor activity of this class of ligands. Since our earlier work had revealed that copper complexation leads to enhancement of the antitumor activity of Schiff base ligands, we were motivated to explore similar trend in case of the Schiff base and its cobalt complexes.

All the above facts aroused our interest in the synthesis of the new ligands, (E)-3-(2,2-diphenylethylimino)indolin-2-one (HL1), (E)-3-(2,2-diphenylethylimino)-5-fluoroindolin-2-one (HL2), (E)-5-chloro-3-(2,2-diphenylethylimino)indolin-2-one (HL3), (E)-5-bromo-3-(2,2-diphenylethylimino)indolin-2-one (HL4), (E)-3-(2,2-diphenylethylimino)-5-methylindolin-2-one (HL5) and (E)-3-(2,2-diphenylethylimino)-5-nitroindolin-2-one (HL6) and its cobalt complexes with a view towards evaluating their structural and pharmacological properties, such as DNA binding, antibacterial and cytotoxic activity.

Experimental

Materials and Methods

All chemicals were purchased from Sigma-Aldrich, E-Merk and used as received withoutpurification. 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.

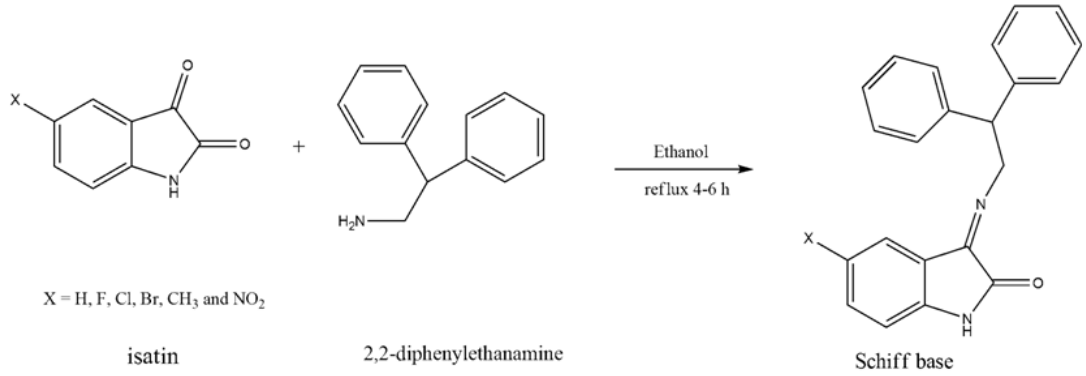
C, H and N analyses of the free Schiff base ligands and their complexes were performed in CHN analyzer Elementar Vario EL III. Metal contents were analyzed by the standard procedures. Hand-Held Meter LF330 was used to measure the molar conductance of the free Schiff base ligands and metal complexes in 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. ^1H 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.

Synthesis of Schiff base ligands and its Co(II) complexes

Synthesis of Schiff base ligands: (HL1-HL6)

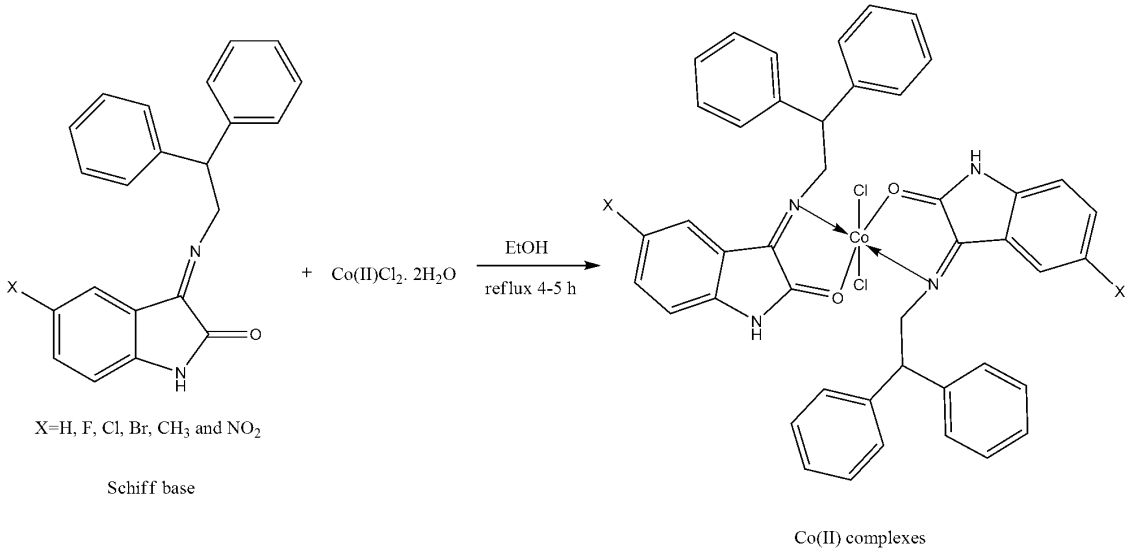
5-substituted isatin (1 mmol) with 2, 2-diphenylethanamine (1mmol) were dissolved in 50mL of absolute EtOH, three drops of glacial acetic acid was added and the resulting solution was refluxed for 4-6 hr. The compound precipitated upon cooling to room temperature, was collected by filtration and recrystallized from EtOH. All the ligands appeared at yellow colour powder, compound was obtained from the same solvent .Yield: 8595 %, m.pt. 130-150℃ (Scheme1).

Scheme 1. Synthesis of Schiff base ligands



Synthesis of complexes (HL1-Co to HL6-Co)
To 20 mL of methanolic solution of Schiff base ligands (1mmol) was added drop wise to the methanolic solution (10mL) of cobalt(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 1. Synthesis of Schiff base Co(II) complexes



Antibacterial studies

In vitro biological screening effects of the synthesized free ligands and their Co(II) complexes. The antimicrobial tests were performed by the standard disc diffusion method [31]. 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 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℃ 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

Nuclease studies

The concentration of CT-DNA was determined by UV absorbance at 260 nm (ε = 6600 M^{-1} cm^{-1}). CT DNA free from protein contamination was confirmed from its absorbance values at 260 nm, 280 nm and ratio A_{260}/A_{280} was found to be 1.87 [32]. Stock solutions were kept at 4℃ and used after not more than four days. To prepare buffer and other solutions redistilled water free from CO_2 have been used.

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 absorbance 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 [33].

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

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.

Cytotoxic activity evaluation

MTT assay

Cytotoxic effect of the six new complexes on human liver cancer cells (HepG2) were assayed by the 3-(4, 5-dimethylthiazol-2- yl)- 2,5- diphenyltetrazolium bromide (MTT) assay[34]. 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 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.

Tunel assay

Chemicon/Millipore ApopTag Plus Fluorescein In Situ Kit(S7111).

Fix cells in 1% PFA in Phosphate-buffered saline (PBS) (pH 7.4) for 10 minutes at RT and wash in PBS twice for 5 minutes each. Change to fresh PBS, parafilm and store at 4℃ until ready to proceed with straining. Post-fix in cooled ethanol was prepared by acetic acid (2:1) for 5 minutes at -20℃ (permeabilization). Wash in PBS twice for 5 minutes each and apply Equilibration buffer (13µl cm^{-2}). Immediately add working Strength TdT enzyme (77µl reaction buffer with 33µl TdT, vortex, store on ice) and incubate in humidified chamber at 37℃ for 1 h. Agitate for 15 seconds in working Strength Stop/Wash Buffer (1ml Stop/Wash Buffer, 34 ml dH_2O) and then incubate 10 minutes. Further, remove an aliquot of antioxigenin conjugate and warm to RT in Dark. Wash in PBS 3 times for 1 minute each and apply warmed Anti-Digoxigenin conjugate (13 µl cm^{-2}) and dark humidified chamber at RT for 30 minutes. Finally, wash in PBS 4 times for 2 minutes each and mount with DAPI, seal coverslip store at -20℃. [35, 36]

Results and Discussion

The bidentate NO type of Schiff base ligands (HL1-HL6) and its Co(II) complexes with 5-substituted isatin and 2,2-diphenylethanamine were synthesized and characterized by various spectral techniques. The synthesized Co(II) complexes were found to be air stable, amorphous nature, moisture free and soluble only in DMF and DMSO.

3.1. Elemental analysis and conductivity measurements

The synthesized Schiff base Co(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 complexes are well agreed with their calculated values, showing that 2:1 (ligand : metal) stoichiometry ratio. The observed low conductivity values (16.22 – 37.29 $\Omega^{-1} cm^2 mol^{-1}$) were accounted for the dissociation and hence the complexes are found as non-electrolytes [37].

Table1. Composition and physical characteristics of Co(II) complexes

complexes	Molecular Formula	Color	Found (Calculated) %			M.P (^{0}C)	Yield (%)	Ω (Ohm^{-1} cm^2 M^{-1})
			C	H	N			
HL1-Co	$C_{44}H_{36}Cl_2CoN_4O_2$	Dark brown	66.81 (67.53)	4.05 (4.64)	6.48 (7.16)	162	85	16.22
HL2-Co	$C_{44}H_{34}Cl_2CoF_2N_4O_2$	Dark brown	64.08 (64.56)	4.52 (4.19)	6.51 (6.84)	174	80	21.53
HL3-Co	$C_{44}H_{34}Cl_4CoN_4O_2$	Dark green	61.57 (62.06)	4.25 (4.02)	6.22 (6.58)	157	82	37.29
HL4-Co	$C_{44}H_{34}Br_2Cl_2CoN_4O_2$	Dark brown	65.75 (56.20)	3.01 (3.64)	5.37 (5.96)	1.25 (1.63)	70	24.16
HL5-Co	$C_{46}H_{40}Cl_2CoN_4O_2$	brown	67.82 (68.15)	4.27 (4.97)	6.03 (6.91)	1.62 (1.73)	70	14.09
HL6-Co	$C_{44}H_{34}Cl_2CoN_6O_6$	brown	60.29 (60.56)	3.41 (3.93)	9.24 (9.63)	1.22 (1.69)	65	23.62

Vibrational spectral studies

Vibrational spectra of free Schiff base ligands (HL1-HL6) were compared to investigate the mode of binding present in the synthesized Co(II) complexes. The FT-IR spectral data are summarized in Table 2. The IR spectrum of the free ligand (HL1 – HL6) 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 1610-1552 cm^{-1}. This suggested that coordination of the metal is through the azomethine nitrogen atom [38]. 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 1682-1659 cm^{-1} the spectra indicating the involvement of υ(C=O) with metal centre during complexation. The ligands bind with the Co(II) ions in a bidentate manner through azomethine N and carbonyl O atoms respectively. Further, the two new bands appeared in the infrared region at 445-462 cm^{-1} and 537-566 cm^{-1} were assigned to υ(M-N) and υ(M-O) respectively [39]. Thus, the IR spectral results provide evidence for bidentate complexation of Schiff bases with metals.

Fig.1.IR spectrum of HL1-Co complex

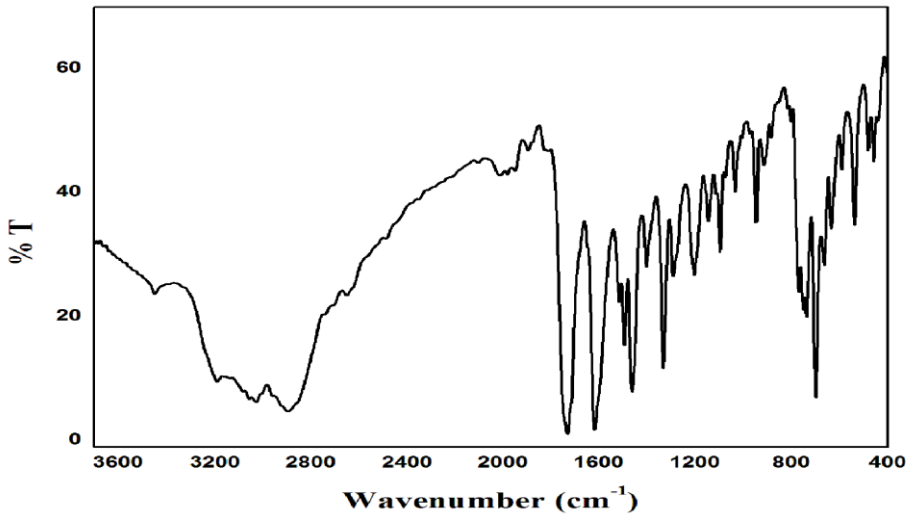


Table 2. Vibration spectral data for the Co(II) complexes and in KBr disc (cm^{-1})

Compounds	ν(NH) of indole ring	Lactonyl, ν(C=O) of indole ring	ν(C=N)	ν(M-N)	ν(M-O)
HL1-Co	3191	1659	1610	457	538
HL2-Co	3042	1656	1590	462	547
HL3-Co	3205	1664	1552	447	555
HL4-Co	3214	1671	1572	445	566
HL5-Co	3085	1682	1584	462	537
HL6-Co	3105	1673	1563	447	548

NMR Spectra

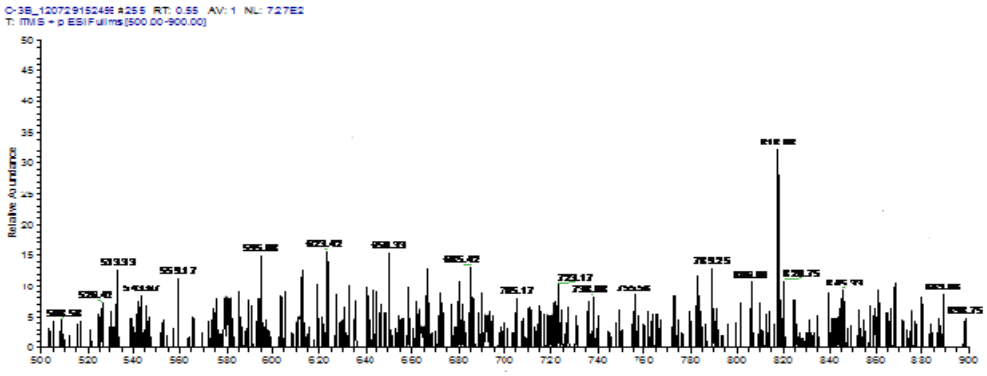
The ^{1}H NMR spectrum of ligands were recorded in DMSO-d_6 . The sharp signals around at δ = 9.13-10.23 ppm in the downfield region of the spectrum indicate the presence of –NH form of the isatin. Aromatic protons occur as multiplets in the region of 6.27-8.21 ppm. The signals around at δ = 5.0-4.10 and 4.13-4.81 ppm in the upfield region of the spectrum indicate the presence of aliphatic protons like CH_2 and CH.

The ^{13}C NMR spectra provide further support for the structural characterization of the Schiff bases. The signals around at δ = 163.02-165.14 ppm and δ = 158.95-161.01 ppm indicate the precence of carbonyl group of isatin and imine group of Schiff base. The signals around at and δ = 110.36-148.32 ppm indicate the aromatic region. The signals around at δ = 59.32-59.57 ppm and 52.41-52.88 ppm were due to methine carbon and methane carbon also a singlet at 2.3 ppm corresponds to the methyl carbon for Schiff base ligands.

Mass spectra

The mass spectrum of ligands and metal complexes is recorded under liquid secondary ion mass spectral conditions. The ligands HL1 – HL6 gave the peaks at m/z (M+1) = 327, 345, 361, 406, 341 and 372.The mass spectrum of HL2-Co complex was exhibited m/z peaks at 818 (M+1) adduct. These values confirm the molecular weight of the ligands and complexes (Fig. 1).

Fig.2. Mass spectrum of HL2-Co



Electronic spectra and magnetic moment values

The electronic spectra of the ligands and its cobalt(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 HL1; The Electronic spectra of HL1-Co(II) complex display two prominent bands. The electronic spectrum of the cobalt(II) complex shows two bands at 11627-16129 cm^{-1} which are assigned to $^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$, $^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}$, transitions, respectively, as expected for an octahedral cobalt(II) complex. Another high intensity band around 23584 - 25000 cm^{-1} is due to symmetry forbidden ligand → metal charge transfer. On the basis of electronic spectra distorted octahedral geometry around Co(II) ion is suggested [40, 41]. The Co(II) complex showed magnetic moment 3.96 BM. The spectrum of the Co(II) complexes HL1-HL6 exhibited bands and magnetic moment values are given in Table.3.

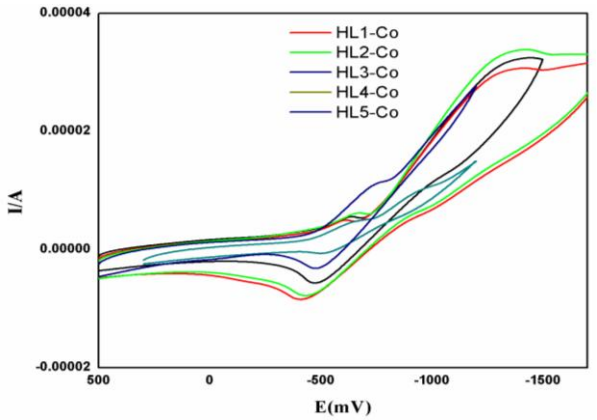
Table 3. Electronic spectral data (cm^{-1}) of Co(II) complexes and magnetic moment values

Compound	π→ π* (cm^{-1})	n→π* (cm^{-1})	LMCT	d-d	Assignment	Suggested Structure	μeff (B.M)
HL1-Co	37735	33898	24752	16806, 16155	$^4T_{1g}(F)\rightarrow{}^4T_{1g}$ (P)	Octahedral	1.95
HL2-Co	37593	33783	23584	15432, 14947	$^4T_{1g}(F)\rightarrow{}^4T_{1g}$ (P)	Octahedral	1.76
HL3-Co	37593	33557	24630	11792, 11627	$^4T_{1g}(F)\rightarrow{}^4A_{2g}$	Octahedral	2.34
HL4-Co	37735	33670	25000	15082, 12870	$^4T_{1g}(F)\rightarrow{}^4A_{2g}$	Octahedral	1.87
HL5-Co	37634	33650	24583	13087, 12770	$^4T_{1g}(F)\rightarrow{}^4A_{2g}$	Octahedral	2.10
HL6-Co	37674	33655	24530	13484, 12688	$^4T_{1g}(F)\rightarrow{}^4A_{2g}$	Octahedral	2.13

Cyclic voltammetry

The Co(II) complexes exhibits one electron quasi reversible transfer process with a reduction peak at Epc= 0.655 to -1.397 mV with a corresponding oxidation peak at Epa= -0.341 to -0.673 mV at a scan rate of 100mV/s (Figure2). The peak separation (ΔEp) of this couple is -0.442 to 0.966 mV. With the increasing scan rates, ΔEp value also increases giving further evidence for the quasi-reversible Co(II)/Co(I) couple. The difference between forward and backward peak potentials can offer a rough evaluation of the degree of the reversibility. 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 [41, 42].

Fig.3. Cyclic voltammogram of Co(II) complexes



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 [46, 47]. Hence, a complete electronic spectral study was conducted with the new complexes and CT-DNA. The absorption spectra of HL1-Co to HL6-Co complexes in the absence and presence of CT-DNA are given in Fig.4. With increasing CT-DNA concentration for the HL1-Co complex, the hypochromism in the band at the found 297 and 445 nm reaches as high as 46.17% and 52.24% respectively. Other Co(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 K_b values are shown in table 4. Hence the above phenomenon is indicative of most probable binding mode of Co(II) complexes for HL1 to HL6 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 [43, 44].

Fig.4. Absorption spectra of Co(II) complex for HL1, 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.

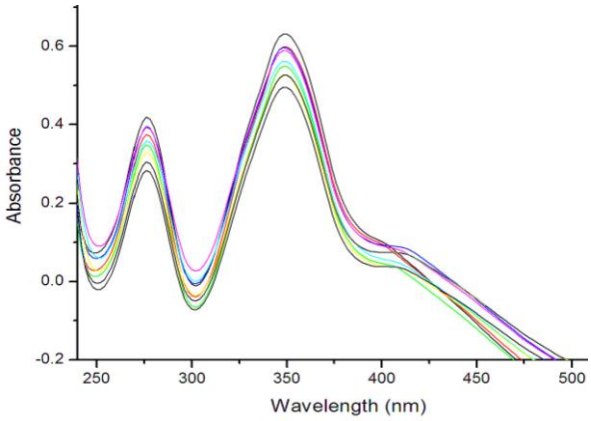


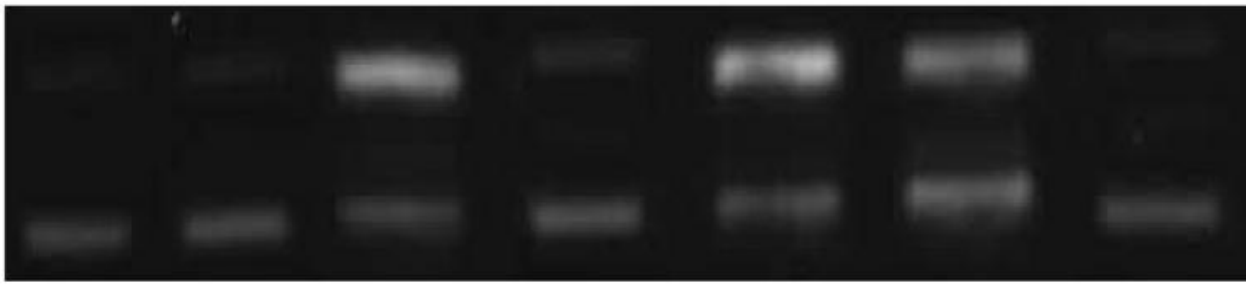
Table 4. Absorption properties of Co(II) complexes with CT-DNA.

Complex	λ max (nm)	Δλ (nm)	Hypochromicity (%)	Kbx104 ((mol L-1)-1)
HL1-Co	264, 445	3	46.19, 53.24	4.61
HL2-Co	263, 446	4	42.91, 49.03	5.42
HL3-Co	267, 442	2	50.21, 48.72	5.07
HL4-Co	268, 447	5	56.74, 62.93	7.84
HL5-Co	265, 443	3	43.97, 52.34	5.80
HL6-Co	266, 445	2	51.25, 48.60	5.92

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. 5. 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 [45].

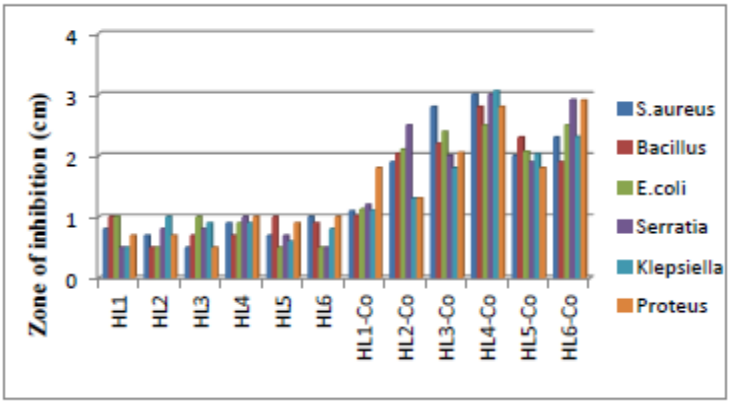
Fig.5. Cleavage of supercoiled pUC19 (10µM) by the Co(II) complexes in the presence of Tri Acetate EDTA (TEA) buffer at 37℃. Lane 1; DNA+H_2O_2, Lane 2; HL1-Co, Lane 3; HL_2-Co, Lane 4; HL3-Co, Lane 5, HL5-Co, Lane 7; HL6-Co



In-vitro antimicrobial assay

The antimicrobial results are shown in Fig. 6. 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. HL4-Co complex was excellent antibacterial acvivity against all the Gram +ve and Gram –ve bacteria.

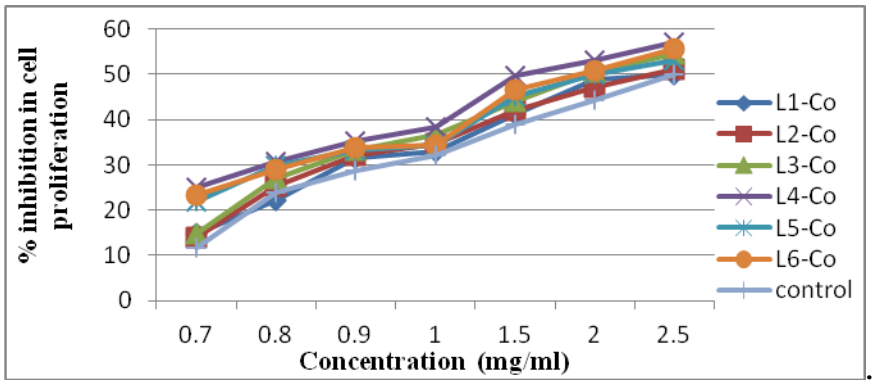
Fig.6. Antibacterial activities of Schiff base ligands and their C0(II) complexes with control and compounds at 40µl in different microorganism species.



Study of cytotoxicity by MTT assay

The cytotoxicity assays for the new HL1-Co to HL6-Co 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. 7). 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 (HL4-Co) and (HL6-Co) 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., [46-47]. Moreover, the percentage inhibition values of our complexes are comparable with the reported values of standard anticancer drugs such as cisplatin.

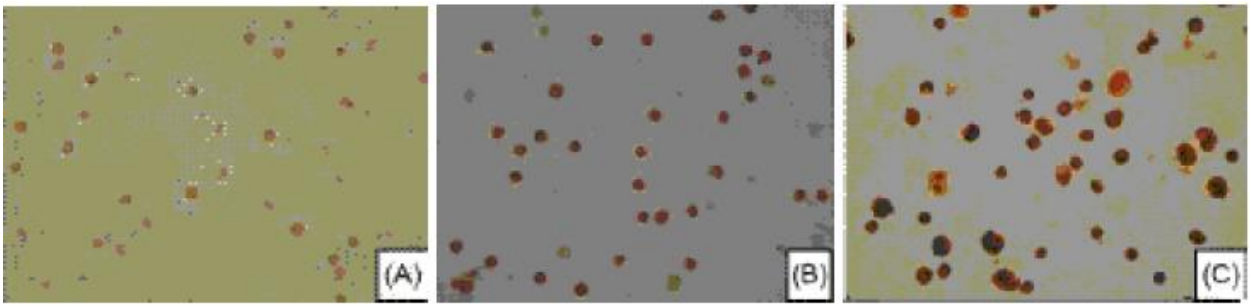
Fig.7. Plots of Percentage inhibition in cell proliferation against various percentage of complexes



Induction of apoptosis by MAIT on cells by Tunnel Assay

This study carried out only HL4-Co(II) complex because significant reactivity compared to other complexes (Fig. 8). The cells were treated with 0.1% DMSO (A, negative control), MAIT (B, 25 µg/ml) or DNase I (C, positive control) for h and subjected to TUNEL assay. Nuclei stained dark brown of the cells were observed after cell treatment with MAIT and Dnase I, where as no stained nucleus of control cells treated with DMSO was detected. (A-C) Magnification x100. Hence our data, HL4-Co complex was excellent cytotoxic activity comparing other complexes.

Fig.6. Induction of apoptosis by MAIT on cells by Tunnel Assay for HL4-Co(II) complexes



CONCLUSION

Hence in conclusion, a series of noval Co(II) complexes of 5-substituted isatin with 2,2-diphenylethanamine have been prepared and characterized by various spectral techniques. The UV-vis, IR, ESI-Mass data showed that Co(II) complexes adopt octahedral geometry. The antimicrobial actions of Co(II) complexes, the zone of inhibition for 4 and 6 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, the present work represents a good overall correlation between DNA binding and DNA cleavage activity for all the complexes. The data clearly suggest that the Co(II) complexes, especially complexes 4 and 6, are more effective than cis-platin on Hep G2 cancerous cells. From the MTT assay it is apparent that the complexes are able to inhibit the proliferation of the Hep G2 cell since they inhibit the proliferation of liver carcinoma cells by more than 50% at 2.5 µM concentration. Thus complex 4 and 6 looks like a promising candidate to further design and develop new cobalt (II) based complexes may be dur to Bromine attached 5^{th} position of isatin compounds, for a systematic assessment of their DNA binding and cleavage activity, and their potential application as therapeutic agents.

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