Synthesis, structural elucidation, biological investigation and docking analysis of bioactive Co(II), Cu(II), and Zn(II) complexes with Schiff base ligand derived from histidine and 1,3-indandione
4.1. **Introduction**

Compounds of the indan series with carbonyl group in the 1- and 3-positions of the five-membered ring are important class of ligands as they have strong electron acceptors, which can form, donor–acceptor systems through intramolecular charge transfer mechanism [1]. The preparation and investigation of these compounds are connected with the search for a new class of charge-transfer complexes and radical ion salts in view of their possible practical applications [2-6]. 1,3-Indandione complexes of Cu(II), Co(II) and Zn(II) ions are well-known for their ability to participate in the processes of blood coagulation and in the antispasmodic activity of amino derivatives [7].

It is interesting to understand that these ligands find potential applications in diverse fields such as optical materials [8], catalysis [9], chemical sensor [10] and biological probes [11]. Therefore, metal complexes of Schiff base ligands have been extensively studied because of their potency as active sites of metalloenzymes [12, 13], antibacterial, antivirus, antimicrobial and antifungal agent [14]. In addition the bioavailability and functionality of Cu(II), Co(II) and Zn(II) ions in nature draws interest due to their ability to interact with DNA.

Amino acids, the building blocks of proteins are indispensable because of their biological functions, as exemplified by the role of enzymes. Especially, histidine is a fundamental amino acid for living organisms which acts as a biological carrier for oxygen because of its complexes with transition metal ions that can reversibly bind oxygen [14]. The mixed ligand complexes derived from Schiff bases and amino acids containing N, O/S donor binding sites with metal(II) ions are used in a number of fields like biological, analytical, agricultural industrial and also in some therapeutic applications [2, 6]. A huge number of literature
reports are available for DNA binding studies of mixed ligand of Schiff bases and their metal complexes [15]. Knowledge of DNA binding parameters of these complexes help to explore their potency as anticancer drugs, since their binding mode could be associated with their ability to cause DNA damage [16]. This DNA damage can potentially lead to the inhibition of uncontrolled growth of cancerous cells.

Although there are enormous reports on DNA interaction studies, there is always a great demand in developing potent chemotherapeutic agents to target specific DNA sequences for biotechnological applications. Therefore, the present investigation is focused on physiochemical, analytical and biological studies of the complexes of the bio available metal ions Co(II), Cu(II) and Zn(II) of Schiff base ligand derived from 1,3-Indandione and L-Histidine. In addition, the \textit{in vitro} approach for the biological applications of these complexes was examined by DNA binding, DNA cleavage and molecular docking studies. The biological screening of free ligand and its metal complexes against different bacteria and fungi are reported here. The \textit{in vitro} cytotoxicity of the ligand and all the complexes against NIH/3T3 mouse fibroblast cells are also explored here.

4.2. Experimental Methods

4.2.1. Synthesis of Ligand (L2)

For the synthesis of ligand (L2), an ethanolic solution of 1, 3-Indandione (0.1 mM) was added to an ethanolic solution of histidine (0.1 mM) followed by the addition of a few drops of glacial acidic acid and the resultant mixture was refluxed for 6 h. The solid product formed was filtered, washed and recrystallized from ethanol and dried in vacuum. The structure of the ligand and its metal complexes is shown Figure 4.1.
Ligand [L2]

Yield: 0.25 g, 76%. M.p.: 180 °C. ESI–MS: m/z = 283 [C_{15}H_{13}N_{3}O_{3}]. Anal. Calc. for C_{15}H_{13}N_{3}O_{3}: C, 63.60; H, 4.63; N, 14.83; O, 16.94. Found: C, 63.58; H, 4.60; N, 14.82; O, 16.92 (%). FT-IR (ν, cm⁻¹): 1680 m, 2909 w, 1497 m, 1411 m.

¹H NMR [DMSO-d₆, ppm]: δ 8.6 (s, 3H), 7.2–7.3 (s, 6H), 9.8 (m, 1H), 3.18 (m, 1H).

¹³C NMR [DMSO-d₆, ppm]: δ 123.4–135.3 (m, Ar-C), 155.3 (m, -C=N), 189.2 (w, COOH), 190.9 (w, C=O). (vs, very strong; s, strong; m, medium; w, weak).

UV–Vis (DMSO, rt) [λ (nm), ε (M⁻¹ cm⁻¹)]: 252 (32,500), 368 (28,600). The formation of the Schiff base ligand and its metal complexes are given in Fig. 4.1.

![Figure 4.1. Schematic route for the synthesis of ligand (L2) and its M(II) complexes (where M(II) = Co(II), Cu(II) and Zn(II))](image-url)
4.2.2. Synthesis of Co(II), Cu(II) and Zn(II) Metal Complexes

To synthesize metal complexes 1-3, 2:1 molar mixture of ligand (L2) (0.2 M) and metal chloride salt (0.1 M) were mixed in a portion of ethanol (15 mL). This mixture was then refluxed for 5 h. The resultant product was washed and then recrystallized from ethanol. The solid product obtained was filtered, dried in vacuum and kept in the desiccator.

\[ \text{[Co(L2)\textsubscript{2}]} \]

Yield: 0.124 g, 65%. M.p.: 254 °C. ESI–MS: m/z = 623 [C\textsubscript{30}H\textsubscript{24}CoN\textsubscript{6}O\textsubscript{6}]. Anal. Calc. for C\textsubscript{30}H\textsubscript{24}CoN\textsubscript{6}O\textsubscript{6}: C, 57.79; H, 3.88; N, 13.48; O, 15.40; Co, 9.45. Found: C, 57.77; H, 3.86; N, 13.46; O 15.38; Co, 9.43 (%). FT-IR (\( \nu, \text{cm}\textsuperscript{-1} \)): 1619 vs, 1436 m, 1345 m, 626 w, 559 w. (vs, very strong; s, strong; m, medium; w, weak). UV–Vis (DMSO, rt) [\( \lambda \) (nm), \( \epsilon \) (M\textsuperscript{-1} cm\textsuperscript{-1})]: 345 (38,400), 518 (51,300). Am (\( \Omega\textsuperscript{-1} \text{cm}\textsuperscript{3} \text{mol}\textsuperscript{-1} )) 13.6. \( \mu_{\text{eff}} \) (BM) 2.51.

\[ \text{[Cu(L2)\textsubscript{2}]} \]

Yield: 0.25 g, 67%. M.p.: 240 °C. Anal. Calc. for C\textsubscript{30}H\textsubscript{24}CuN\textsubscript{6}O\textsubscript{6}: C, 57.37; H, 3.85; N, 13.38; O, 15.28; Cu, 10.12. Found: C, 53.34; H, 3.84; N, 13.37; O 15.26; Cu, 10.9 (%). FT-IR (\( \nu, \text{cm}\textsuperscript{-1} \)): 1613 vs, 1436 m, 1346 m, 560 w, 452 w. (vs, very strong; s, strong; m, medium; w, weak). UV–Vis (DMSO, rt) [\( \lambda \) (nm), \( \epsilon \) (M\textsuperscript{-1} cm\textsuperscript{-1})]: 265 (29,700), 543 (54,800). Am (\( \Omega\textsuperscript{-1} \text{cm}\textsuperscript{3} \text{mol}\textsuperscript{-1} )) 15.8. \( \mu_{\text{eff}} \) (BM) 1.57.

\[ \text{[Zn(L2)\textsubscript{2}]} \]

Yield: 0.29 g, 76%. M.p.: 294 °C. Anal. Calc. for C\textsubscript{30}H\textsubscript{24}ZnN\textsubscript{6}O\textsubscript{6}: C, 57.20; H, 3.84; N, 13.34; O, 15.24; Zn, 10.38. Found: C, 57.19; H, 3.81; N, 13.32; O, 15.22; Zn, 10.36 (%). FT-IR (\( \nu, \text{cm}\textsuperscript{-1} \)): 1609 m, 1471 vs, 1316 s, 568 m, 480 w. \( \textsuperscript{1}H \) NMR [DMSO-d\textsubscript{6}, ppm]: \( \delta \) 8.5 (m, 3H), 7.2–7.3 (s, 6H), 3.1 (s, 1H). \( \textsuperscript{13}C \) NMR [DMSO-d\textsubscript{6}, ppm]: \( \delta \) 123.5–135.3 (m, Ar-C), 155.4 (w, -C=N), 114
189.3 (w, COO⁻), 191.0 (w, C=O). (vs, very strong; s, strong; m, medium; w, weak).

UV–Vis (DMSO, rt) [λ (nm), ε (M⁻¹ cm⁻¹)]: 326 (44,200), 514 (56,400).

Λm (Ω⁻¹ cm³ mol⁻¹) 12.5. µeff (BM) diamagnetic.

4.3. **Result and Discussion**

4.3.1. **Structural Elucidation of the Ligand and its Metal(II) Complexes**

All the complexes were stable for extended periods and the complexes were remarkably soluble in DMSO. The elemental analysis data of the complexes were in good agreement with the calculated values and show that the complexes have the stoichiometry of [M(L)₂], wherein the Schiff base acts as a bidentate ligand. Molar conductance values of all the complexes in DMF (10⁻³ M solution at 25 °C) lies in the range of 12.5 - 15.8 Ω⁻¹ cm³ mol⁻¹ which indicates that the complexes are non-electrolytes having the molar ratio of metal: ligand as 1:2 [6].

4.3.1.1. **IR Spectral Studies**

FTIR spectral data provides adequate information to explain the coordination behaviour of the ligand and metal ions. The essential vibrational frequencies of the ligand and the complexes are compared and listed in the experimental section. In the FTIR spectrum of ligand (L₂) (Figure 4.2), the band at ~1680 cm⁻¹ could be attributed to ν(-C=N), which supports the formation of ligand (L₂) from 1,3-indandione and histidine [17].

Upon complexation with metal ions this band is shifted to lower frequencies (1604-1619 cm⁻¹) indicating the coordination of the azomethine nitrogen with metal ion [18]. It was further supported by M-N characteristic peaks observed at ~448-559 cm⁻¹ in all the complexes. In the same way, the band at ~1497 cm⁻¹ is assigned to ν(as)(COO⁻) of the ligand. After coordination of the ligand to the metal ions, this band shifted to lower frequencies (1436-1437 cm⁻¹),
signifying the involvement of carboxylate group in coordination with the metal ions [19]. The appearance of new band in the region of ~560-626 cm\(^{-1}\) accounts for M-O bond formation, which confirms the involvement of carboxylato anion in coordination with the metal centre of the complexes. From the above discussion, the formation of ligand and the complexes were confirmed and the ligand was found to be a bidentate donor in all the complexes.

![FTIR spectra of the ligand (L2) and its Co(II), Cu(II) and Zn(II) complexes](image)

**Figure 4.2.** FTIR spectra of the ligand (L2) and its Co(II), Cu(II) and Zn(II) complexes

### 4.3.1.2. Electronic Absorption Spectral and Magnetic Moment Studies

The absorption spectral data for Schiff base ligand (L2) and its metal complexes obtained in DMSO solution are given in Figure 4.3. The ligand (L2) exhibited an absorption band in UV region around 252 nm which is assigned to \(\pi\rightarrow\pi^*\) transition originating from the aromatic phenyl ring (C=C) and azomethine
group (\(-\text{C}=\text{N}\)). Another band observed at 368 nm is assigned to \(n\to\pi^*\) transition of non-bonded electrons available in imine groups.

In the UV spectra of the complexes, the bands of \(n\to\pi^*\) transitions shift to lower frequencies signifying the coordination of imine nitrogen atom with the metal ion. In absorption spectra, \(d\to d\) transition is highly beneficial to predict the geometry of the metal complexes [20]. However, the spectra of all complexes exhibit an absorption band in the region 514-543 nm which can be the characteristic of \(d\to d\) transition of metal ions. This suggests a square planar geometry for all the complexes [21]. The observed magnetic moment values of Zn(II) and Cu(II) complexes were 2.51 BM and 1.57 BM, respectively. These values are close to magnetic moments of square planar complexes reported earlier [12, 22], which suggests that all the complexes possess square planar geometry.

![Figure 4.3. UV-Visible absorption spectrum of the ligand (L2) and its Co(II), Cu(II) and Zn(II) complexes](image)

**Figure 4.3.** UV-Visible absorption spectrum of the ligand (L2) and its Co(II), Cu(II) and Zn(II) complexes
4.3.1.3. $^1$H NMR and $^{13}$C NMR Spectral Studies

![NMR Spectra](image)

Figure 4.4. $^1$H-NMR spectrum of the Schiff base ligand ($L_2$) and Zn(II) complex

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$^1$H NMR spectra of the ligand (L2) and the corresponding zinc complex were recorded in DMSO-d$_6$ using TMS as the internal standard and are illustrated in Figure 4.4. The free ligand (L2) shows a multiplet at 7.2-7.3 ppm, which is assigned to aromatic protons of the phenyl ring. In addition, it also exhibits signals at 8.6, 9.8 and 3.18 ppm corresponding to imidazole H (s), carboxylic –OH (m) and –CH (m) of histidine moiety. The absence of carboxylic –OH signal at 9.8 ppm in the spectra of the zinc complex suggested the coordination of carboxylic –OH group to the metal ion $via$ proton displacement. In the $^1$H NMR of the zinc complex, the signals corresponding to imidazole H, Ar-H, and –CH remain unchanged, which clearly indicates that these groups are not involved in the complex formation.

The $^{13}$C NMR spectra of the ligands and their zinc complexes were recorded in DMSO-d$_6$, using tetramethylsilane (TMS) as internal standard (Figure 4.5). $^{13}$C NMR spectrum of ligand displayed characteristic signals at $\delta$ 123.4-135.3 ppm, $\delta$ 155.3 ppm, $\delta$ 189.2 ppm and $\delta$ 190.9 ppm are due to the aromatic carbons, imine group, carboxylato carbon and carbonyl group (C=O) respectively.

In Zn(II) complex, the carboxylato anion and imine peaks were slightly shifted to an upfield region as compared with the free Schiff base ligand. These shifts greatly support the coordination of nitrogen of imine group and oxygen of carboxylate group to the metal centre. The signals due to other groups remain unchanged, which confirms their non-involvement in coordination. Both $^1$H and $^{13}$C NMR spectral results, evidently confirms the bidentate nature of the Schiff base ligand (L2). Further, it confirms the proposed structure of the ligand and its metal complexes as shown in Figure 4.1.
Figure 4.5. $^{13}$C-NMR spectrum of the Schiff base ligand (L2) and Zn(II) complex
4.3.1.4. Electron Paramagnetic Resonance Spectral Studies

![Graph](image)

**Figure 4.6.** X-band EPR spectra of Cu(II) complex at liquid nitrogen temperature.

EPR spectral studies of paramagnetic metal(II) complexes yield information about the distribution of unpaired electrons, geometry of metal complexes and the nature of bonding between the metal ion and its ligands. In the present study, X-band ESR spectrum of Cu(II) complex was studied at liquid nitrogen temperature (100 K) in DMSO using TNCE as g-marker (2.0027) which is illustrated in Figure 4.6. The values of \( g_\parallel \) (2.18) > \( g_\perp \) (2.05) support the fact that the unpaired electron is predominantly localized in \( d_{x^2-y^2} \) orbital, and also suggest a square planar geometry around Cu(II) ions as discussed in UV-visible spectroscopy [23, 29]. Hathway showed that for covalent environment \( g_\parallel \) is less than 2.3 [24]. In the present study, \( g_\parallel \) value of the Cu(II) complex is 2.18 which confirms the covalent environment around the metal center.

Further, in an axial symmetry the exchange coupling factor (G) can be expressed by the following equation:
\[ G = \frac{(g_g - 2)}{(g_e - 2)} \]  

\[ \text{......... (3)} \]

According to Hathaway [25], if the value of \( G \) is greater than 4, the exchange interaction between Cu(II) centres in the solid state is negligible. The \( G \) value obtained in the present Cu(II) complex is greater than 4, indicating the absence of exchange interaction between Cu(II) centres in the solid state.

4.3.1.6. Mass Spectrometry

Electrospray ionization (ESI) mass spectra of the Schiff base ligand and its Co(II) complex were recorded and are given in Figure 4.7. The mass spectrum of Schiff base ligand shows a well-defined molecular ion peak at \( m/z \) 283 \([C_{15}H_{13}N_3O_3]^+\), which matches with a formula weight of the Schiff base ligand.

Also, the spectrum showed the fragments at \( m/z \) 81, 109, 149, 161, 189, 217, 245 and 256 which can be assigned to \([C_5H_7N]^+, [C_7H_{11}N]^+, [C_{10}H_{15}N]^+, [C_{11}H_{15}N]^+, [C_{12}H_{15}NO]^+, [C_{13}H_{15}NO_2]^+, [C_{13}H_{15}N_3O_2]^+\) and \([C_{14}H_{14}N_3O_2]^+\) moieties, respectively. Similarly, the mass spectrum of Co(II) complex shows a molecular ion peak at \( m/z \) at 623 \([C_{30}H_{24}CoN_6O_6]^+\) which is equivalent to its molecular weight. The other molecular ion peaks appeared at \( m/z \) 81, 109, 123, 189, 248, 276, 414, 514, 541 and 571 in the mass spectrum is attributed to the fragments \([C_5H_7N]^+, [C_7H_{11}N]^+, [C_7H_9NO]^+, [C_{10}H_{11}N_3O]^+, [C_{10}H_{11}CoN_3O]^+, [C_{11}H_{11}CoN_3O_2]^+, [C_{18}H_{19}CoN_4O_4]^+, [C_{26}H_{17}CoN_4O_4]^+, [C_{26}H_{18}CoN_4O_6]^+\) and \([C_{28}H_{27}CoN_4O_6]^+\), respectively. The mass spectrum of the ligand and \([\text{Co(L2)}_2]\) complex confirmed the metal to ligand ratio to be 1:2 in the complexes. Thus, the mass spectral data reinforces the conclusion drawn from elemental analysis, magnetic and spectral studies.
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Figure 4.7. ESI-Mass spectrum of the ligand (L2) and Co(II) complex

4.3.2. DNA Binding Studies

In order to find the most probable binding mode of the synthesized complexes with CT-DNA, various techniques were adopted and they are discussed below.
4.3.2.1. Electronic Absorption Spectral Studies

Electronic absorption spectroscopy is a powerful and convenient technique to investigate binding behaviour of metal complexes with DNA. Figure 4.8 represents the absorption spectrum of the ligand and its Co(II), Cu(II) and Zn(II) complexes in buffer solution (pH~7.2) with increasing concentration of CT DNA. Upon increasing the CT-DNA concentration, Co(II), Cu(II) and Zn(II) complexes show a significant hypochromic effect accompanied by a moderate red shift of 1.4-5.8 nm, which indicates the stabilization of the DNA helix by metal complexes. Stabilization of DNA is commonly consistent with the strength of intercalative interaction between the metal complex and DNA [26]. From the above facts, it can be realized that the Co(II), Cu(II) and Zn(II) complexes have a strong binding to the DNA via intercalative mode.

This was further confirmed by the binding constant values presented in Table 4.1. The calculated binding constant ($K_b$) values for the association of Co(II), Cu(II) and Zn(II) complexes with CT-DNA are $2.89 \times 10^6$ M$^{-1}$, $2.64 \times 10^6$ M$^{-1}$, $2.41 \times 10^5$ M$^{-1}$ respectively. The observed $K_b$ values are comparable with the classical intercalator, ethidium bromide (EB-DNA $K_b= 1.4 \times 10^6$ M$^{-1}$) and higher than those of partial intercalating complexes such as [Cu(L$^1$)$_2$] (L$^1$-4-hydroxybenzohydrazide, 4-hydroxy-N’-[(1Z)-1-(naphthalen-2-yl) ethyldene] benzohydrazide) ($K_b = 1.16 \times 10^5$ M$^{-1}$) and [Cu(L$^2$)$_2$] (L$^2$- (Z)-ethyl 2- (4-(2-(1-(naphthalen-2-yl) ethyldene) hydrazinecarbonyl)phenoxy)acetate ($K_b= 8.00 \times 10^4$ M$^{-1}$), respectively [27]. Therefore, it is clear that the complexes act as intercalator which stacks between adjacent base pairs of DNA and they involve in intercalative mode of binding.

Furthermore, in the absorption titration curves of Co(II), Cu(II) and Zn(II) complexes with DNA an isobestic point appears which infers that there is
equilibrium between bound DNA and free form of the complex. It also ensures the occurrence of only one mode of binding of complexes with DNA that is intercalation.

Figure 4.8. Electronic absorption spectra of (a) Co(II) complex, (b) Cu(II) complex and (c) Zn(II) complex upon addition of CT-DNA. [complexes] = 5 × 10^{-5} M, and [DNA] = 0.0 - 9.1 × 10^{-4} M. Inset: Plot of [DNA]/\varepsilon_0 - \varepsilon_1 vs. [DNA].
**Table 4.1.** Electronic absorption spectral properties of Co(II), Cu(II) and Zn(II) complexes with DNA.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Δλ (nm)</th>
<th>$K_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>276.48</td>
<td>278.12</td>
<td>1.64</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>264.11</td>
<td>269.95</td>
<td>5.84</td>
</tr>
<tr>
<td>Zn(II) complex</td>
<td>276.34</td>
<td>277.83</td>
<td>1.49</td>
</tr>
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</table>

**4.3.2.2. Fluorescence Spectral Studies**

To further explore the nature of binding of complexes with DNA, the intrinsic binding constants ($K_b$) of the complexes were obtained by the luminescence titration method. Figure 4.9 shows the emission spectra of Co(II) and Zn(II) complex in the presence of increasing concentration of CT-DNA. The addition of the CT-DNA to the complexes resulted in an increase in fluorescence intensity which implies that the complexes can insert between base pairs of the CT-DNA. It has been proved that a significant increase in fluorescence intensity is normally observed for intercalative mode of binding [12]. Since, in the case of intercalation, the complexes were deeply stacked into the DNA helix and this will favour deactivation via fluorescence emission instead of radiationless deactivation. It also suggests that all the complexes were protected from water molecules by the hydrophobic environment inside the DNA helix [28].

According to the Scatchard equation [20], $K_b$ of Co(II) and Zn(II) complex are $4.527\times10^5$ M^{-1} and $7.715\times10^5$ M^{-1} respectively. The intrinsic binding constant ($K_b$) of these complexes are consistent with the value reported earlier [29] to the complex having similar kind of interaction with DNA (intercalative binding with $K_b= 10^6$ M^{-1}).
Figure 4.9. Emission spectra of (a) Co(II) complex and (b) Zn(II) complex in the presence of CT-DNA. The arrow shows the absorbance changes upon increasing amounts of CT-DNA. Inset: Plot of r vs. r/C, $1 \times 10^{-5}$ M, [DNA] = 0 - 5.24 $\times 10^{-4}$ M.
4.3.2.3. Competitive DNA Binding Studies

**Figure 4.10.** Fluorescence spectra of EB bound to CT-DNA in the presence of (a) Co(II) complex, (b) Cu(II) complex and (c) Zn(II) complex with different concentrations. Inset: Plot of [complex] vs. \(I_0/I\). \([EB]= 2.0 \text{ µM}, [DNA]= 24.0 \text{ µM} \) and \([\text{complex}]= 5\text{–}120 \text{ µM}\).

The mode of binding of the complexes to DNA was further examined by evaluating the fluorescence emission intensity of the EB-DNA system upon addition of the complex. The emission spectra of EB bound to DNA in the absence and in the presence of Co(II), Cu(II) and Zn(II) complexes are given in Figure 4.10.
The addition of the complex to DNA pre-treated with EB causes a gradual decrease in emission intensity. This is due to the interaction between the complex and EB-DNA, which leads to the quenching in the emission intensity of the EB-DNA system. This unveils the fact that the complex competes with EB in binding to DNA. The reason for the reduction in emission intensity is the displacement of EB from EB-DNA system by metal complexes [30]. Fluorescence quenching data were further analysed by means of the following Stern-Volmer equation [31]:

$$\frac{I_0}{I} = 1 + K_{sv} [Q]$$

........ (4)

where $I_0$ and $I$ are the fluorescence emission intensities in the absence and presence of quencher (complexes), respectively; $[Q]$ is the concentration of the quencher; $K_{sv}$ is the Stern-Volmer constant, which is obtained by the ratio of the slope to the intercept from the plot of $I_0/I$ vs $[Q]$. The Stern-Volmer constant ($K_{sv}$) values of Co(II), Cu(II) and Zn(II) complexes are 1.480, 1.448 and 4.446 respectively. The results prove that, all the complexes have a stronger affinity for DNA than the related complex [VO(o-Van-Val)(phen)]CH$_3$CN ($K_{sq} = 0.44$) (where o-Van= o-vanillin and Val= valine, phen= 1,10-phananthroline) [32]. This gives a clear evidence that all the complexes were bound to DNA via intercalation, which is in agreement with that derived from absorption spectra measurements.

4.3.2.4. Electrochemical Studies

The application of cyclic voltammetry (CV) to the study of binding of metal complexes to DNA provides useful information in probing the nature and mode of binding. If the metal complexes interact with DNA via intercalative mode, there is a positive shift in potential otherwise a negative shift will be observed for electrostatic interaction [33]. The typical cyclic voltammograms of Co(II), Cu(II)
and Zn(II) complexes in buffer (pH= 7.2) at 25 °C in the presence of increasing concentration of CT-DNA are shown in Figure 4.11. Electrochemical parameters of the synthesized complexes are shown in Table 4.2. The cyclic voltammogram of Co(II) complex in the absence of DNA featured the reduction of +2 to the +1 form at an anodic peak potential, \( E_{pa} = 1.355 \) V and a cathodic peak potential, \( E_{pc} = -0.031 \) V. Incremental addition of CT-DNA at the identical conditions, causes a considerable decrease in the peak potentials, both \( E_{pc} \) and \( E_{pa} \), as well as \( E_{1/2} \) as represented in Table 4.2 (\( E_{pa} = 1.213 \) V, \( E_{pc} = -0.011 \) V and \( E_{1/2} = 0.662 \)). The decrease in the peak potentials in the presence of CT-DNA can be attributed to the diffusion of the metal complex bound to the large, slowly diffusing DNA molecule. Similar results observed for Cu(II) and Zn(II) complexes, indicate that the synthesized complexes may stabilize the duplex DNA. It is also notable that, the redox couple of all the complexes were found to have approximately unity peak current ratio (\( I_{pa}/I_{pc} \)) which clearly indicating that the reaction of the complex on glassy carbon electrode surface is quasi-reversible redox processes. The combined results of voltammetric studies of the complexes support the fact that, the complexes were bound to DNA via intercalative mode of binding.

**Table 4.2.** Electrochemical parameters for interaction of DNA with Co(II), Cu(II) and Zn(II) complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>#( E_{1/2} ) (V) Free</th>
<th>#( E_{1/2} ) Bound</th>
<th>#( \Delta E_p ) (V) Free</th>
<th>#( \Delta E_p ) Bound</th>
<th>#( I_{pa}/I_{pc} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(II) complex</td>
<td>0.6481</td>
<td>0.6620</td>
<td>1.3240</td>
<td>1.2964</td>
<td>0.854</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>1.5888</td>
<td>1.594</td>
<td>-0.2222</td>
<td>0.1841</td>
<td>0.646</td>
</tr>
<tr>
<td>Zn(II) complex</td>
<td>0.1272</td>
<td>0.1623</td>
<td>0.3247</td>
<td>0.2544</td>
<td>0.670</td>
</tr>
</tbody>
</table>

\*\( E_{1/2} = E_{pa} + E_{pc} / 2; \#\Delta E_p = E_{pa} - E_{pc} \)
Figure 4.11. Cyclic voltammogram of (a) Co(II) complex, (b) Cu(II) complex and (c) Zn(II) complex in buffer (pH ~ 7.2) at 25 °C in the presence of increasing concentration of DNA.
4.3.2.5. Viscosity Measurements

To further validate the nature of the interaction between the complex and DNA, viscosity measurements were carried out since it is sensitive to changes in DNA chain length. In general, a classical intercalation between a ligand and DNA must lengthen the DNA helix as base pairs are separated to give space for the incoming ligand, leading to an increase in DNA viscosity. However, partial or non-classical intercalation of the ligand with DNA causes no obvious increase in DNA viscosity [34]. Figure 4.12. shows the effect of increasing concentration of ethidium bromide (EB) and Co(II), Cu(II) and Zn(II) complexes on the viscosity of CT-DNA. Addition of metal complexes to DNA causes a gradual increase in the viscosity of DNA, but the increase is less than that observed for the typical intercalator ethidium bromide which is consistent with the earlier literature [35].

This clearly shows that all the complexes have intercalative binding with DNA.

![Figure 4.12. Effect of concentration of EB and Co(II) (complex 1), Cu(II) (complex 2) and Zn(II) (complex 3) complexes on the relative viscosity of CT-DNA](image-url)
4.3.2.6. Thermal Denaturation Studies

In a typical thermal denaturation process, a double stranded DNA is denatured into single-stranded components by applying heat in the presence and absence of a compound. The DNA melting temperature ($T_m$) is firmly related to the stability of the double helix and therefore intercalation of any compound with DNA leads to a significant rise in thermal denaturation temperature due to the stabilization of the natural structure of DNA [36, 37]. Having this in mind, we have examined the dependence of melting temperature of DNA on the synthesized complexes by performing a typical thermal denaturation experiment.

![UV melting profiles for FS-DNA and Co(II) complex (complex 1), Cu(II) complex (complex 2) and Zn(II) complex (complex 3)](image)

**Figure 4.13.** UV melting profiles for FS-DNA and Co(II) complex (complex 1), Cu(II) complex (complex 2) and Zn(II) complex (complex 3)

Figure 4.13 shows the melting curves of FS-DNA in the absence and in the presence of Co(II), Cu(II) and Zn(II) complexes (complexes 1-3). In the present
work, it is found that thermal denaturation of FS-DNA in the absence of complexes is at 48.5 °C. As shown in Figure 4.13, addition of the complexes to the FS-DNA solution results in a significant rise in the melting temperature ($T_m$) of FS-DNA. The observed $T_m$ in the presence of Co(II), Cu(II) and Zn(II) complexes (complexes 1-3) are 56.9 °C, 53.5 °C and 52.3 °C, respectively. The moderate increase in $T_m$ ($\Delta T_m$) is 8.4 °C, 5.0 °C and 3.8 °C for Co(II), Cu(II) and Zn(II) complexes, respectively. It also appears that Co(II) complex (complex 1) is preferentially stabilizing the FS-DNA than Cu(II) complex (complex 2) and Zn(II) complex (complex 3). It is consistent with their binding abilities with CT-DNA. This provides strong support that Co(II), Cu(II) and Zn(II) complexes stabilize the FS-DNA conformation via intercalation mechanism.

4.3.3. Oxidative Cleavage of pUC19 DNA

![Figure 4.14](image-url)

**Figure 4.14.** Changes in the Agarose gel electrophoretic pattern of pUC19 plasmid DNA, induced by $\text{H}_2\text{O}_2$ and metal complexes. lane 1: Blank DNA (Control); lane 2: DNA+ ligand (L2) + $\text{H}_2\text{O}_2$; lane 3: DNA+ Co(II) complex + $\text{H}_2\text{O}_2$; lane 4: DNA+ Cu(II) complex + $\text{H}_2\text{O}_2$; lane 5: DNA + Zn(II) complex + $\text{H}_2\text{O}_2$. 
The ability of Co(II), Cu(II) and Zn(II) complexes to cleave the pUC19 DNA in the presence of H$_2$O$_2$ were monitored by agarose gel electrophoresis and the results were represented in Figure 4.14. The results revealed that, all the four complexes were able to cleave DNA from one form to another (i.e. supercoiled form to open circular form) at low concentrations (15 μM). This could possibly happen by the involvement of H$_2$O$_2$ by producing hydroxyl radical or a peroxo species. It is also to be noted that Co(II) complex (Lane 3) and Zn(II) complex (Lane 5) more effectively cleave the DNA than the ligand (L2) (Lane 2) Cu(II) complex (Lane 4) which in turn cleave the DNA more effectively than the control (Lane 1). The disparity in the nuclease activity of the complexes was due to their difference in their binding capacity to DNA.

4.3.4. Molecular Docking Studies

Molecular docking technique allows us to understand the interaction between a drug and DNA at the molecular level. In the present study, molecular docking of the ligand (L2) and Co(II), Cu(II) and Zn(II) complexes with DNA duplex of sequence d(CGCGAATTCCGCG)$_2$ dodecamer (PDB ID:1BNA) was performed in order to rationalize the mode of DNA binding and most favourable binding conformations of the molecules. Figure 4.15 shows the minimum energy docked pose of the ligand (L2) and Co(II), Cu(II) and Zn(II) complexes. From the results it is clear that, both the ligand (L2) and complexes interact with DNA via intercalation mode of binding. This could be explained by the fact that, stacking interaction of ligand and complexes with oxygen atom of the phosphate backbone leads to the formation of stable complex as reported in literature [38].

The resulting relative binding energy of ligand (L2) and Co(II), Cu(II) and Zn(II) complexes with DNA were found to be -206.89 KJ mol$^{-1}$, -307.48 KJ mol$^{-1}$,
-302.70 KJ mol\(^{-1}\) and -301.72 KJ mol\(^{-1}\), respectively. The results of the docking view revealed the fact that the complexes bind with DNA via intercalation and that the complexes stabilize the DNA by Van der Waal’s and hydrophobic interaction [39]. It is also to be noted that the complexes exhibit more binding affinity than ligand (L2). The binding energy of the complexes follows the order Co(II) complex > Cu(II) complex > Zn(II) complex, which is in good agreement with the binding constants obtained from absorption and emission spectral study.

![Molecular docked model](image)

**Figure 4.15.** Molecular docked model of (a) Ligand (L2), (b) Co(II) complex, (c) Cu(II) complex and (d) Zn(II) complex with BDNA.
4.3.5. *In Vitro* Biocidal Activity

The newly synthesized ligand and all its Co(II), Cu(II) and Zn(II) metal complexes were tested for their antimicrobial activity against human pathogenic bacteria (*Escherichia coli, Salmonella typhi, Salmonella sp., Bacillus subtilis* and *Vibrio cholera*) and anticandidal activity against (*Candida parapsilosis, Candida tropicalis, Candida kefyr* and *Candida albicans*) by agar well diffusion method. Ciprofloxacin and nystatin were used as the standard drugs for antibacterial and anticandidal studies, respectively.

![Graph showing bacterial growth inhibition](image)

**Figure 4.16.** Bacterial growth inhibition (in millimetres) of Schiff base ligand and its Co(II), Cu(II) and Zn(II) metal complexes at 200 µg/ml concentration

The results of antibacterial and anticandidal activity of the compounds were presented in Figure 4.16. and Figure 4.17., respectively. From the experimental results, it is found that *Bacillus subtilis* and *Vibrio cholera* are highly
susceptible to all the complexes while *Escherichia coli, Salmonella typhi, Salmonella sp.* are slightly susceptible. In case of fungi, all the complexes exhibited greater anticandidal activity against *Candida parapsilosis* and *Candida kefyr* and show a moderate effect on other species. Also, the values indicate that all the complexes have higher antimicrobial activity than the free ligand under identical experimental conditions. This may be due to the chelation of metal ion with ligand which reduces the polarity of the ligand to a greater extent. Further, the chelation of metal ion surges the delocalization of the π-electrons around the chelate ring, which results in an increase in the lipophilicity of the metal complexes, which in turn favours its penetration through the lipid layer of the membrane [40]. The results of antibacterial and anticandidal tests show that all the complexes are more active against bacteria than fungi.

![Figure 4.17. Fungal growth inhibition (in millimetres) of Schiff base ligand and its Co(II), Cu(II) and Zn(II) metal complexes at 200 µg/ml concentration.](image-url)
4.3.6. Cytotoxic Activity

The affirmative results from DNA binding and cleavage studies encouraged us to test the cytotoxicity of the ligand (L2) and the complexes against NIH/3T3 mouse fibroblast cells. Figure 4.18 shows the curves of dose-dependent effects of the ligand (L2) and the corresponding Co(II), Cu(II) and Zn(II) metal complexes on cell viability of NIH/3T3 cell lines. The ligand and complexes were dissolved in DMSO and diluted with culture medium. The blank samples containing the identical volume of DMSO were taken as controls.

![Graphs showing cell viability](image)

**Figure 4.18.** Cell viability of (a) ligand (L2), (b) Co(II) complex (c) Cu(II) complex and (d) Zn(II) complex against NIH/3T3 mouse fibroblast cells at different concentrations. Cell viability decreased with increasing concentrations of complexes.

The cytotoxic potential of the ligand (L2) and Co(II), Cu(II) and Zn(II) complexes were evaluated by determining the number of viable cells living after incubation with the ligand and complexes in the stipulated time period using CTB
analysis. It is evident from Figure 4.18 that the number of cells decrease with increasing concentration of the ligand and the complexes. Moreover, on comparison of the IC\textsubscript{50} values of the ligand and the Co(II), Cu(II) and Zn(II) complexes, Zn(II) complex exhibits the most potent inhibition against NIH/3T3 mouse fibroblast cell lines with IC\textsubscript{50} values of 2.01 ± 4.12 µmol/mL, while the ligand and the Co(II) and Cu(II) complexes possess the IC\textsubscript{50} values of 2.11 ± 3.01 µmol/mL, 2.47 ± 1.6 µmol/mL and 2.53 ± 2.5 µmol/mL, respectively. The cytotoxicity of the complexes against NIH/3T3 mouse fibroblast cell lines follows the order Zn(II) complex > ligand (L2) > Cu(II) complex > Co(II) complex.

4.4. Conclusion

Novel bioactive Schiff base ligand derived from 1,3-indandione and L-histidine and its metal complexes of Co(II), Cu(II) and Zn(II) ions were synthesized and characterized. Binding studies were carried out to understand the binding interaction of the complexes with CT-DNA through the complementary techniques UV-visible spectroscopy and fluorescence spectroscopy. Experimental results revealed that the nature of mode of interaction is intercalation. It was further confirmed by voltammetric studies, viscosity measurements and thermal denaturation behaviour. The large values of binding constants reflect that all the complexes have strong binding ability with DNA and the order of binding strength of the complexes follow the following trend Co(II) complex > Cu(II) complex > Zn(II) complex. The gel electrophoretic technique displayed profound efficiency of the complexes to cleave plasmid DNA which confirms intercalative binding of all the complexes. This was further confirmed by molecular docking studies. The ligand and its Co(II), Cu(II) and Zn(II) metal complexes were screened for antibacterial and antifungal activity. From the results, it is inferred that both the ligand and the complexes were more active against bacteria than fungi.
In vitro cytotoxicity studies revealed that all the complexes have potential inhibition effect against NIH/3T3 mouse fibroblast cells. Among the three complexes, Zn(II) complex showed higher cytotoxicity effect than Co(II) and Cu(II) complexes. It is noteworthy to mention that the binding affinity and biological potencies of the present complexes is greater than that of the complexes of 1,4-naphthoquinone-L-histidine ligand (L1). This increased binding and biological action is due to the fact that, the compounds of the indan series with amino acid groups in the 1- and 3-positions of the five-membered ring exhibits strong electron accepting properties, which form donor-acceptor systems with intramolecular charge transfer [41-43]. It is also reported that coordination of macroheterocyclic ligands will make significant changes in their physiochemical properties [44]. Comparing the binding property of M(II) complexes of newly synthesised ligand of 1,3-indandione with M(II) complexes of 1,4-naphthoquinone, the distinguished binding efficacy can be accounted for coordination of 1,3-indandione organic moiety with the metal ions which causes significant physiochemical properties [45]. The change in property leads to effective electron density redistribution on antibonding π-orbitals of the ligand upon coordination [41, 44]. This has been already proved by Gailis et al on his study in a number of polar 1,3-indandione derivatives [46, 47]. Earlier study on the derivatives of 1,3-indandione possess a high hyperpolarizability due to the extended conjugated π-electron system and dipolar character of the excited states. This study has brought out significant insights on this new class of complexes and it could be extended by varying the amino acid in the ligand framework, in such a way that, it enhances the binding affinity and bioavailability of the complexes considerably.
References


