Synthesis, spectral characterization and DNA/Protein binding studies on Cobalt (II) complex containing Mixed ligand Ethylenediamine with 2-Hydroxy-1-Naphthaldehyde

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Abstract

New cobalt (II) complex has been synthesized and characterized by elemental analysis, UV-Vis, FT-IR and thermal analysis. Binding of this Co (II) complex with calf thymus DNA was investigated by UV-Visible absorption, fluorescence spectroscopy techniques. The intrinsic binding constants $K_b$ of complex with CT-DNA obtained from UV-Vis absorption studies were $4.43 \times 10^5 \text{M}^{-1}$. Further, the in vitro cytotoxic effect of the complexes examined on cancerous cell line, such as human breast cancer cells (MCF-7).

Keywords: Co (II) complex, DNA interaction and cytotoxicity activity.

1. Introduction

Cobalt is an essential trace element in humans, exhibiting many useful biological functions. Numerous compounds, naturally occurring and man-made, contain the cobalt at two common oxidation state Co (II) and Co (III) There is growing interest in investigating cobalt and other transition metal complexes for their interaction with DNA [1-5]Deoxyribonucleic acid plays an important role in the life process, because it bears heritage information and instructs the biological synthesis of proteins and enzymes through the replication and transcription of genetic
information in living cells. DNA is a particularly good target for metal complexes as it offers a wide variety of potential metal binding sites [6,7]. Transition metal complexes with beta-diketones are known to possess bacteriocidal. Antiviral, antifungal and antialgal activities [8]. Metal complexes having N, O donor atoms are very important because of their significant biological properties such as antibacterial, antifungal, anticancer and herbicidal [9]. Cobalt is an essential trace element in humans, exhibiting many useful biological functions. Numerous compounds, naturally occurring and man-made, contain the cobalt at two common oxidation states Co(II) and Co(III). There is growing interest in investigating cobalt and other transition metal complexes for their interaction with DNA. This may be partly influenced by the results of extensive investigation into two areas of research, viz (i) the binding specificity of small organic molecules for their possible modulation and inhibition of DNA replication, transcription and recombination, and (ii) anticancer, antiviral and antibacterial drugs [10,11]. The interest on cobalt complexes in experimental cancer therapy research results from their role as systemic anticancer agents and their ability to redox-dependent targeting the tissue of solid tumours. The former role was firstly attributed to the fact that vitamin B12 (cobalamin) is substituted together with folic acid in chemotherapy regimens involving antimetabolites to reduce unwanted side effects, and, secondly, to the fact that fast proliferating cells require higher amounts of vitamins B12 than normal ones [12]. Since the first reported studies into the biological activity of Co complexes [13] in 1952, diverse structurally characterized cobalt complexes showing antitumor anti-proliferative [14,15], antimicrobial [16,17], antifungal [18,19], antiviral [20,21] and antioxidant [22] activities have been reported.

Accordingly, we intend to report herein, the synthesis of Co(II) complex with 2-hydroxy-1-naphthaldehyde and ethylenediamine ligand and characterization of complex was
carried out by elemental analysis, IR, UV-Vis and thermal studies. The binding properties of this complex to CT-DNA have been carried out using different physico-chemical methods and the binding modes are discussed.

2. Physical measurements

Elemental analyses of carbon, hydrogen, and nitrogen were performed on a Carlorerba-1106 microanalyzer. The electronic spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. FT-IR spectra were recorded in the 4000–400 cm\(^{-1}\) region using KBr pellets on a Bruker EQUINOX 55 spectrometer. The fluorescence study was carried out on an Elico SL174 spectrofluorometer. The molar conductivity of a freshly prepared solution of the complexes (10\(^{-3}\) M) in DMF was measured using an Elico Model SX80 conductivity bridge.

2.2. Absorption spectrophotometric studies

Absorption spectra titrations were performed at room temperature in Tris-HCl/NaCl buffer (50 mMTris-HCl/1 mMNaCl buffer, pH 7.5) to investigate the binding affinity between CT-DNA and complex. 2 mL solutions of the blank Tris–HCl/NaCl buffer and the Co(II) complex sample ([complex] = 5 \times 10^{-6} M) were placed into two 1 cm path cuvettes, respectively. Then one aliquot (10 μL) of buffered CT-DNA solution (0.01 M) was added to each cuvette in order to eliminate the absorbance of DNA itself. Before the absorption spectra were recorded, the Co(II)-DNA solutions were incubated at room temperature for 5 min in order to full reaction.

2.3. Competitive Binding Experiments

The relative binding of complexes to CT-DNA were determined with an EB-bound CT-DNA solution in Tris–HCl/NaCl buffer (pH=7.2, 5 mMTris–HCl, 50 mMNaCl). The experiments were carried out by adding a certain amount of a solution of complexes ([complex]
= 1.0 × 10^{-3} \text{ M})\ step \ by \ step \ into \ the \ EB–DNA \ solution \ (2.4 \mu \text{M} \ EB \ and \ 48 \mu \text{M} \ CT–DNA). \ The \ influence \ of \ the \ addition \ of \ complexes \ to \ the \ EB–DNA \ complex \ have \ been \ obtained \ by \ recording \ the \ variation \ of \ fluorescence \ emission \ spectra \ with \ excitation \ at \ 510 \text{ nm} \ and \ emission \ at \ 602 \text{ nm}. \ Before \ the \ emission \ spectra \ were \ recorded, \ the \ Co(II)–DNA \ solutions \ were \ incubated \ at \ room \ temperature \ for \ 5 \text{ min} \ in \ order \ to \ full \ reaction.

2.4. \ Cell \ culture

The \ MCF-7 \ human \ breast \ cancer \ cell \ line \ was \ obtained \ from \ National \ Center \ for \ Cell \ Sciences \ (NCCS), \ Pune, \ India. \ The \ cell \ line \ was \ cultured \ in \ the \ Dulbecco’s \ Modified \ Eagles \ medium \ (DMEM) \ supplemented \ with \ 10\% \ fetal \ bovine \ serum \ (FBS), \ 200 \text{ mM} \ L–glutamine, \ 100 \text{ U/mL} \ penicillin, \ and \ 10 \text{ mg/mL} \ streptomycin \ in \ a \ humidified \ atmosphere \ consisting \ of \ 5\% \ CO_2 \ at \ 37^\circ \text{ C}.

2.5. \ Evaluation \ of \ cytotoxicity

The \ cytotoxic \ effect \ of \ complexes \ (1-3) \ against \ MCF-7 \ cells \ was \ evaluated \ by \ MTT \ [3-(4, \ 5\text{-dimethylthiazol-2-yI})-2,5\text{-diphenyltetrazolium \ bromide]} \ assay. \ Briefly, \ MCF-7 \ cells \ were \ seeded \ (5 \times 10^4 \text{ cells/well}) \ in \ a \ 96\text{-well} \ plate \ and \ kept \ in \ CO_2 \ for \ attachment \ and \ growth \ for \ 24 \text{ h}. \ Then, \ the \ cells \ were \ treated \ with \ various \ concentrations \ of \ complexes \ dissolved \ in \ DMSO \ (0.25-100 \mu \text{M}) \ and \ incubated \ for \ 24 \text{ h}. \ After \ incubation, \ the \ culture \ medium \ was \ removed \ and \ 15 \mu \text{L} \ of \ the \ MTT \ solution \ (5 \text{ mg/mL} \ in \ PBS) \ was \ added \ to \ each \ well. \ Following \ 4 \text{ h} \ incubation \ in \ dark, \ MTT \ was \ discarded \ and \ DMSO \ (100 \mu \text{L/well}) \ was \ added \ to \ solubilize \ the \ purple \ formazan \ product. \ The \ experiment \ was \ carried \ out \ in \ triplicates \ and \ the \ medium \ without \ complex \ served \ as \ control. \ The \ absorbance \ was \ measured \ colorimetrically \ at \ 570 \text{ nm} \ using \ an \ ELISA \ microplate \ reader. \ The \ percentage \ of \ cell \ viability \ was \ calculated \ using \ the \ following \ formula \ and \ expressed \ as \ IC_{50};
% cell viability = (OD value of treated cells) / (OD value of untreated cells (control) ×100

The IC$_{50}$ value is calculated using linear regression from excel.

2.6. Synthesis of complex

2.6.1. [Co(L$_3$).NCS]

To a solution of 2-hydroxy-1-naphthaldehyde (0.144 g, 0.84 mmol) in methanol (10 ml), sodium hydroxide (0.03 g, 0.84 mmol) was added and the resulting solution was stirred for 15 min at room temperature. CoCl$_2$.6H$_2$O (0.20 g, 0.84 mmol) in methanol (10 ml) followed by ethylenediamine (0.05 g, 0.84 mmol) in methanol (10 ml) were added to the solution. To the reaction mixture ammonium thiocyanate (0.081 g, 0.84 mmol) are added, followed by stirring for 3 h. The green colored precipitate that formed. Yield: 59%; (0.17 g). m.p. 143 °C (dec). Anal. Calc. (%) for C$_{14}$H$_{13}$CoN$_3$OS: C, 50.21; H, 3.91; N, 12.55; S, 9.57. Found (%): C, 49.95; H, 4.09; N, 12.30; S, 9.36. FT-IR (KBr, $\nu$, cm$^{-1}$) selected peaks: 3550br, 3420s, 2068, 1720s, 1629s, 1602s, 1567s, 1525s, 1478s, 1447s, 1343s, 1295s, 1157s, 1157s, 1096s, 1064s, 1032s, 762s, 698s, 531s, 520s, 509s (br, broad; s, sharp). UV-Vis in DMF [$\lambda_{\text{max}}$/nm ($\varepsilon_{\text{max}}$/ mol$^{-1}$ cm$^{-1}$)]: 271(4999), 279(3610), 303(2875), 315(2730), 402(179). ESI-MS (CH$_3$OH) m/z (%): 330(10%) [M$^+$]. Conductivity ($\Lambda$M/S cm$^2$ mol$^{-1}$) in DMF: 14.

The complexes [Ni(L$_2$).NCS] (8) and [Cu(L$_2$).NCS] (9) were synthesized by following the above procedure using cobaltous chloride instead of using Nickel chloride (1.99 g, 0.84 mmol) and copper chloride (0.14 g), respectively.
3. Results and discussion

3.2. IR and UV/Vis spectroscopy

There are conspicuous differences between the complex and free ligand in IR spectrum [23]. In the free ligand a medium-intensity band at 3187.66 cm\(^{-1}\), assigned to \(\nu\)(OH) of diketone tautomer, and two strong bands around 1675.05 and 1017.66 cm\(^{-1}\), due to (C O) of the lateral chain and (C O) of pyrazolone ring respectively, are absent in the complex [24]. In the complex, there are four new bands at 2987.66 cm\(^{-1}\), 1985.81 cm\(^{-1}\) and 563.60 cm\(^{-1}\), respectively, assigned to \([\nu(C\cdots O)]\), \(\nu(C\ C\ C)\), water molecules [25] and Co–O, respectively (Fig. 1).
UV–vis absorption spectra of complex in ethanolic solution were recorded in the wavelength range 200–800 nm. Two absorptions of the complex at 269, 293 nm are attributed to $\pi-\pi^*$ transitions of aryl ring, carbonyl, and $n-\pi^*$ transition of carbonyl [26]. In the visible region, weaker absorptions near 607 and 672 nm for the Co(II) complex are assigned as $4T_{1g} \rightarrow 4A_{2g}$, $4T_{1g} \rightarrow 4T_{2g}$ (F) for d–d transitions, consistent with Co in an octahedral environment (Fig. 2).
3.3. Thermal studies

Thermogravimetric (TG) and differential thermogravimetric (DTA) analysis (Fig.3) were carried out for the complex 1 under nitrogen flow at the constant heating of 10°C per minute. The thermogram of this complex decomposition observed in the range 100-200°C (50% corresponds to the weight loss 2-hydroxy-1-naphthaldehyde and ethylenediamine moiety present in the complex 1. There are four DTA endotherm peak observed at 55.85, 86.08, 138.09 and 168.79°C, respectively, also confirmed the decomposition and weight loss of ligand moieties. The residue left in the crucible consists of corresponding metal oxide [27].

Fig.3. Thermal analysis of complex 1.
3.4. DNA binding studies

3.4.1. Study of the interaction of the complexes with CT DNA with UV spectroscopy

Transition metal complexes can bind to DNA via both covalent (via replacement of a labile ligand of the complex by a nitrogen base of DNA) and/or noncovalent (intercalation, electrostatic or groove binding) interactions [28]. In the literature, a metal complex has been found to bind to DNA via the intercalative mode [29]. The UV spectra have been recorded for a constant CT DNA concentration in different [complex]/[DNA] mixing ratios. UV spectra of CT DNA in the presence of a compound derived for diverse r values are shown representatively for Fig. 6. The band at $\lambda_{\text{max}} = 258$ nm exhibits a red-shift up to 2 nm for all compounds, indicating that the interaction with CT DNA results in the direct formation of a new complex with double-helical CT DNA [30] with a simultaneous stabilization of the CT DNA duplex [31]. In the UV spectra of the complex, the intense absorption bands observed are attributed to the intra-ligand transitions of the coordinated groups of acetylacetone ligands. Any interaction between complex and CT DNA that could perturb its intraligandcentred spectral transitions during the titration upon addition of CT DNA in diverse r values can be observed [32]. In general, the changes observed in the UV spectra upon titration may give evidence of the existing interaction mode, since a hypochromism due to $\pi-\pi^*$ stacking interactions may appear in the case of the intercalative binding mode, while red-shift (bathochromism) may be observed when the DNA duplex is stabilized [33]. According to linear Stern-Volmer equation: [34],
Fig. 4. Absorption spectra of complex 1 in 5 mMTris-HCl buffer upon addition of DNA. The inner plot of \([\text{DNA}/(\varepsilon_a - \varepsilon_t)]\) vs [DNA] for the titration of DNA with complex

\[ I_0/I = 1 + K_{sv}r, \]

where \(I_0\) and \(I\) are the fluorescence intensities in the absence and the presence of complex. \(K_{sv}\) is a linear Stern-Volmer quenching constant, \(r\) is the ratio of the total concentration of complex to that of DNA. The quenching plot illustrates that the quenching of EB bound to DNA by the complexes is in good agreement with the linear Stern-Volmer equation, which also indicated that the complexes binds to DNA. In the plot of \(I_0/I\) versus [Complex]/[DNA], \(K_{sv}\) is
given by the ratio of the slope to intercept. The $K_{sv}$ value for our complex thus obtained is $4.43 \times 10^5 \text{M}^{-1}$, respectively. This may suggest that the complex 1 has intercalative mode of binding that involves a stacking interaction between the complexes and the base pairs of DNA.

### 3.4.2. Competitive study with ethidium bromide

Ethidium bromide (EB) is a typical indicator of intercalation since it can form soluble complexes with nucleic acids resulting in the emission of intense fluorescence due to the intercalation of the planar phenanthridine ring between adjacent base pairs on the double helix of CT DNA. The changes observed in the spectra of EB on its binding to CT DNA are often used for the interaction study between DNA and metal complex [35]. Complex 1 shows no fluorescence at room temperature in solution or in the presence of CT DNA, and their binding to DNA cannot be directly predicted through the emission spectra (Fig. 5). Hence competitive EB binding studies may be undertaken in order to examine the binding of each compound with DNA since the fluorescence intensity is highly enhanced upon addition of CT DNA, due to its strong intercalation with DNA base pairs. Addition of a second molecule, which may bind to DNA more strongly than EB, results in a decrease the DNA-induced EB emission due to the replacement of EB, and/or electron transfer [36,37].
Fig. 5. Fluorescence emission spectra of the EB-DNA in presence of complex 1 in 5mM TrisHCl buffer (pH 7.2). The inner plot of [I₀/I] vs complex concentration.

The emission spectra of EB bound to CT DNA in the absence and presence of each compound have been recorded for [EB] = 20 mM, [DNA] = 26 mM for increasing amounts of each compound. The addition of complex 1 at diverse \( r \) values (Fig. 6) results in a significant decrease of the intensity of the emission band of the DNA-EB system at 592 nm indicating the competition of the complex with EB in binding to DNA. The observed significant quenching of DNA-EB fluorescence for complex 1 suggests that they displace EB from the DNA-EB complex.
I and they can probably interact with CT DNA by the intercalative mode [38]. The Stern-Volmer constant $K_{sv}$ may be used to evaluate the quenching efficiency for each compound according to the equation:

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

where $I_0$ and $I$ are the emission intensities in the absence and the presence of the quencher, respectively, $[Q]$ is the concentration of the quencher and $K_{sv}$ is obtained by the slope of the diagram $I_0/I$ vs $[Q]$. The $K_{sv}$ value for our complex thus obtained is $3.9 \times 10^4$ M$^{-1}$, respectively. The Stern-Volmer plots of DNA-EB for the compounds illustrate that the quenching of EB bound to DNA by the compounds is in good agreement ($R = 0.9983$) with the linear Stern-Volmer equation, which proves that the replacement of EB bound to DNA by each compound results in a decrease in the fluorescence intensity.

### 3.4.3. Circular dichroism

CD spectroscopic technique can be successfully utilized to detect the conformational changes of DNA induced by its interaction with metal complex. The CD spectrum of CT-DNA was recorded in the range 220–320 nm in 0.1 M phosphate buffer (pH 7.2) and it has been found that there were one positive band at 275 nm due to base stacking and one negative band at 245 nm due to helicity [39]. The examination of Fig. 7 indicates that the positive band slightly increased in intensity and the negative band decreased in intensity after the binding of complex with DNA however no considerable shift in $\lambda_{max}$ could be observed. A very small decrease in the intensity of positive band and a small increase in the intensity of negative band were observed when the DNA. Intercalation binding interaction of the complex with DNA have been shown to bring about only marginal changes in the intensity of negative band as well as the positive band of DNA and no significant shift observed positive and negative band.
3.4.4. Interaction of the complexes with serum albumin proteins

It is important to consider the interactions of drugs with blood plasma proteins particularly with serum albumin, which is the most abundant protein in plasma and is involved in the transport of metal ions and metal complex with drugs through the blood stream. Binding to these proteins may lead to loss or enhancement of the biological properties of the original drug, or provide paths for drug transportation [40]. Bovine serum albumin (BSA) is the most extensively studied serum albumin, due to its structural homology with human serum albumin (HSA). BSA (containing two tryptophans, Trp-134 and Trp-212) solutions exhibit a strong
fluorescence emission with a peak at 343 nm, respectively, due to the tryptophan residues, when excited at 295 nm [41]. The interaction of complex 1 with serum albumins has been studied from tryptophan emission-quenching experiments. The changes in the emission spectra of tryptophan in BSA are primarily due to change in protein conformation, subunit association, substrate binding or denaturation. Complexes 1 exhibited a maximum emission at 355 nm (Fig. 7) under the same experimental conditions and the SA fluorescence spectra have been corrected before the experimental data processing [42]. Addition of complex 1 to BSA results in relatively low fluorescence quenching (Fig.7), due to possible changes in protein secondary structure of BSA indicates the binding of the compounds to BSA [43]. The Stern–Volmer and Scatchard graphs may be used in order to study the interaction of a quencher with serum albumins. According to Stern–Volmer quenching equation:
Fig. 7. Fluorescence spectra of BSA in presence of various concentration of complex 1.
Fig. 8. Plot of % relative fluorescence intensity at $\lambda_{em} = 355$ nm ($I/I_0$ (%)) vs $(r = [\text{compound}]/[\text{BSA}])$ for complex 1 in buffer solution (pH 7.0).

\[ \frac{I_0}{I} = 1 + k_q \tau_0 [Q] = 1 + K_{sv}[Q] \]

where $I_0$ = the initial tryptophan fluorescence intensity of SA, $I$ = the tryptophan fluorescence intensity of SA after the addition of the quencher, $k_q$ = the quenching rate constants of SA, $K_{sv}$ = the dynamic quenching constant, $\tau_0$ = the average lifetime of SA without the quencher, [Q] = the concentration of the quencher respectively,

\[ K_{sv} = k_q \tau_0 \]
and, taking as fluorescence lifetime ($\tau_0$) of tryptophan in SA at around $10^{-8}$s, the dynamic quenching constant ($K_{sv}$, M$^{-1}$) can be obtained by the slope of the diagram $I_0/I$ vs $[Q]$ (Fig. 8), and subsequently the approximate quenching constant ($k_q$, M$^{-1}$ s$^{-1}$) may be calculated. The calculated values of $K_{sv}$ and $k_q$ for the interaction of the compounds with BSA are given in Table 3 and indicate a good BSA binding propensity of the complex exhibiting the highest BSA quenching ability. The $k_q$ values are higher than diverse kinds of quenchers the existence of a static quenching mechanism.

Using the Scatchard equation [44, 45]:

![Fluorescence spectra of BSA in emission intensity $I_0/I$ vs $[Q]$. $[Q = \text{complex}]$](image-url)

**Fig. 9.** Fluorescence spectra of BSA in emission intensity $I_0/I$ vs $[Q]$. $[Q = \text{complex}]$
\[
\frac{\Delta I/I_0}{[Q]} = nK - K\left(\frac{\Delta I}{I_0}\right)
\]

where \(n\) is the number of binding sites per albumin and \(K\) is the association binding constant, \(K\) (M\(^{-1}\)), may be calculated from the slope in plots \((\Delta I_0/I)/[Q]\) versus \((\Delta I/I_0)\) (Fig. 9) and \(n\) is given by the ratio of the y intercept to the slope. The \(K\) values of the complexes (1-3) for albumins, being in the value given Table 3. The Stern-Volmer equation applied for the interaction with BSA in Fig. 12 shows that the curves have fine linear relationships \((r = 0.9921)\). The calculated values of \(K_{sv}\), \(n\) and \(k_q\) are given in Table 3 and indicate their good BSA binding propensity with complexes. From the Scatchard graph (Fig. 10], the association binding constant to BSA of each compound has been calculated (Table 3) with complex 2 exhibiting higher \(K\) values than other complexes. Therefore, the study of the binding to albumins may reveal useful information concerning future application.

**Table 3.** The BSA binding constant and parameters \((K_{sv}, k_q, K, n\) and \(r\)) derived for complex 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(K_{sv}) (M(^{-1}))</th>
<th>(k_q) (M(^{-1})s(^{-1}))</th>
<th>(K) (M(^{-1}))</th>
<th>(N)</th>
<th>(R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>(5.5 \times 10^4)</td>
<td>(5.5 \times 10^{12})</td>
<td>0.0845</td>
<td>0.0932</td>
<td>0.9921</td>
</tr>
</tbody>
</table>
3.8. Cytotoxic activity

Cytotoxic potential of newly synthesized complex 1 was investigated on human breast cancer cell (MCF-7). The complex 1 were applied in range of concentration 0.25-100 µM for MCF-7 and left for 48 h. The activities of the complexes were determined by MTT test \textit{in vitro} and the results were expressed in terms of IC$_{50}$ values. The relations of inhibition rates and complexes concentrations against human breast cancer cell (MCF-7) were shown in Fig.11. The
inhibition effects were further enhanced by increasing the concentration of complexes. At the concentration of 100 µM, inhibition rates of the complex 1 against human breast cancer cells reached nearly same values. The values of IC₅₀ for the complex 1 were >100 µM. It is commonly believed that the biological activities of anticancer metal complex are dependent on their ability to bind DNA and damage its structure resulting in the impairment of its function, which is followed by inhibition of replication, transcription processes and eventually cell death, if the DNA lesions are not properly repaired. The type of metal ion may be another reason for their different anticancer activity [42]. This is due to the fact that cobalt complexes have the capacity to reduce the energy status in tumors, as well as to enhance tumor hypoxia, which also influences their antitumor activities.
Fig. 11. Cytotoxic effect of complexes (1-3) against MCF-7 at different concentration. Cell viability decreased with increasing concentration of complex 1.
3.9. Conclusions

The complex [Co] has been synthesized and characterized. Complex1 binds to CT DNA through intercalative binding mode. The complex shows good binding affinity to BSA proteins and to give relatively high binding constant. The in vitro cytotoxicity study on breast cancer cell line (MCF-7) indicates that complex has the potential to act as effective anticancer drug. These studies form an important rationale for drug design and warrant further in vivo experiments and pharmacological assays.

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Reference


[23] The infrared spectrum was recorded from a KBr flake by use of a Perkin Elmer FTIR-1730 spectrometer.


