Chapter-8

Section-A

Synthesis, Characterization and DNA Binding Studies of $S_2N_2$ Donor Bis-Mercaptoquinoline Co(II) and Ni(II) Metal Complexes: A new class of Antimicrobial Agent

Accepted: Phosphorus, Sulfur, and Silicon and the Related Elements 2009
Introduction

The accessibility of several oxidation states of transition metals is a recurrent theme in co-ordination chemistry, especially due to its importance in catalysis, either in bioinorganic systems or in organometallic chemistry. In particular, the redox chemistry of metal complexes has received considerable attention in the last few years due to its essential role in several enzymes, where the occurrence of different oxidation states for nickel during the catalytic cycle has been proposed [1]. This observation has spurred a great interest in the determination of the electronic and structural factors that contribute to stabilize a particular oxidation state for the nickel centre and several factors have been recognized to be particularly important in the stabilization of oxidation states, namely co-ordination number and geometry, type of donor atom and electronic characteristics of the ligand. Optimum co-ordination environments for nickel-(III) and -(I) are different, and while the high oxidation state prefers high co-ordination numbers coupled with hard donors, nickel(I) is known to be stabilized by low co-ordination numbers and soft p-acceptor ligands. In the last years, nickel complexes containing sulfur donors have received considerable attention due to the identification of a sulfur-rich co-ordination environment in biological nickel centres [2-12].
The nickel complexes containing sulfur donors have received considerable attention due to the identification of a sulfur-rich coordination environment in biological metal centres [13]. Several metal thiolate complexes have been proposed as simple model compounds, and considerable advances have been achieved in metal sulfur chemistry [14-19]. The recent crystal structure determination of the hydrogenase from Desulfovibrio gigas showed that the metal complex is co-ordinated by four sulfur donors and has revealed the hetero-bimetallic nature of the active site [20]. This finding has promoted the current investigation towards the synthesis on model compounds of bimetallic complexes [21]. Nevertheless, some important aspects of metal–sulfur chemistry have remained poorly understood, in particular the role of coordinated sulfur donors in the stabilization of unusual oxidation states for metal complexes.

The interaction of transition metal complexes with DNA has been extensively studied in the past few years. Barton and co-workers [22] have studied the interaction of nantiomers of Ru(phen)$_3$ with various DNA, the results lead them to the conclusion that
there were two modes of interaction, intercalative and electrostatic binding. Kharatishvili et al. also reported the effect on DNA binding in the presence of a planar intercalating ligand such as quinoline for both mononuclear and dinuclear Pt complexes. Mahadeven and Palaniandvavr [23] have studied copper(II) complexes of bis(pyrid-2-yl)-di/trithia ligands bound to calf thymus DNA and found that the coordination geometry and the ligand donor atom type play a key role in deciding the mode and extent of binding of complexes to DNA. On other hand, the interaction of transition metal complexes with CT-DNA has been extensively studied in the past few years. Among the first row transition metal ions, cobalt, nickel, manganese and copper offer the choice of biocompatibility in biological systems and have been recognized as having important biological effects. The study of antitumor activity and DNA binding properties of these metals complexes has been well documented in literature [24-27].

Present work

Due to the vital role and importance of metal complexes towards the biological application and bioinorganic chemistry we synthesized the ligand and its metal complexes of the type [CoMPQT] and [NiMPQT] and interaction with CT-DNA. The prepared ligand and its metal complexes are characterized by IR, $^1$H NMR, mass and elemental analyses agree with the proposed complex structure.

Experimental Section

The chemicals used for the synthesis of 3-\{[(2-\{[(2-mercaptoquinolin-3-yl) methylene]amino}phenyl]imino]methyl\}quinoline-2-thiol metal complexes were of analytical grade. The instruments used for structure elucidation is presented in chapter-2.
UV-visible absorption studies

The experimental details of DNA binding studies were discussed in the Chapter-2

Synthesis of ligand [MPQT]


A mixture of 2-mercaptoquinoline-3-carbaldehyde (5.67g, 0.03 mol) and o-phenylenediamine (3.24g, 0.03 mol) were stirred for 1h in DMF and then refluxed for 6hrs on a water bath. A greenish red solid precipitated on pouring into ice cold water. The resulting solid was collected by filtration dried, and recrystallized in ethanol. Yield, 78% m.p.=143-145°C; Anal. (%) Calcd for C_{26}H_{18}N_{4}S_{2} (450) Found: C, 69.27; H, 4.10; N, 12.48, S, 14.18 %.Calculated; C, 69.31; H, 4.03; N, 12.43; S. 14.23; IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$ 3134 cm$^{-1}$ (Ar-CH), 747 (C=S); 1623 cm$^{-1}$ (C=N) 1554, 1458, 1211, 1174, 1065 cm$^{-1}$. $^{1}$H-NMR (CDCl$_3$) $\delta$: 7.2–7.95 (m, 14H, Ar-H), 14.36 (s, 2H, SH), 13.25 (s, 2H, CH=N, D$_2$O exchangeable), mass spectra, m/z= 450 [M+H].

Preparation of complexes

A general method has been adopted for the preparation of the complexes. A hot ethanol solution of the ligand with the corresponding hydrated metal salt in 1:1 equimolar ratio was refluxed for about 3-4h, at 80±5 °C. The residue was recrystallized from ethanol /dichloromethane. Various attempts to develop the crystals suitable for X-ray diffraction studies such as slow diffusion, crystallization using mixtures of solvents and low temperature crystallization were unsuccessful.

The ligand $\text{L}$ (2.5g 0.005 mol) was dissolved in (25 mL) ethanol and added to the (25 mL) hot ethanolic solution of hydrated metal salt (1.34 g 0.0056 mol) cobalt(II) chloride in 1:1 molar ratio under boiling conditions and refluxed for 3-4 h. A dark blue colored precipitate formed, was collected by filtration and dried. Similarly, the same procedure was followed for Ni(II) complex. The observed experimental data are summarized in Table-8.1.

Result and Discussion

The complexes are crystalline in nature and found to be soluble in most of the organic solvents. The elemental analysis data in table 8.1 shows that the complexes have a composition of $[\text{Co(L)}]$ and $[\text{Cu(L)}]$.

The IR spectra of ligand show the absence of bands corresponding to the amino groups of o-phenylenediamine and carbonyl groups of aldehydic 2-mercaptoquinoline-3-carbaldehyde suggested the formation of the ligand (L). The confirmation regarding the formation of the ligand has been obtained from the appearance of intense bands at 3134 cm$^{-1}$ for (Ar-CH), 1623 cm$^{-1}$ for (C=N), and tautomeric form of (C=S) appears at 747 cm$^{-1}$[28], to the uncoordinated group respectively.

$^1$H-NMR spectra shows broad peaks at $\delta =14.36$, and 13.25 due to (s, 2H, SH, and 2H, CH=N, D$_2$O exchangeable), and 7.24–7.95 (m, 14H, Ar-H,) confirms the proposed structure of the ligand. Further the structure was confirmed by its mass spectra with molecular ion peak at m/z = 450 [M+H], corresponding to mass of the ligand. However, the IR spectra of complexes derived from the ligand(L) show a slight shift to the frequency in the region of 1651-1658 cm$^{-1}$ for (C=N), 746-754 cm$^{-1}$ for $\nu$(C=S) and 3152-3035 cm$^{-1}$ for (Ar-CH) respectively. The appearance of new medium–intensity band at 508-504 cm$^{-1}$ and 450-451 cm$^{-1}$ are assigned as $\nu$(M-N) and $\nu$(M-S) vibrations respectively.
Scheme-8.1. Synthesis of 3-\{[(2-\{[(2-mercaptoquinolin-3-yl) methylene] amino\}phenyl) imino]methyl\}quinoline-2-thiol metal complexes where M = Co(II) and Ni(II)
Fig. 8.1: IR spectra of the 3-\{[(2-\{(2-mercaptoquinolin-3-yl) methylene\} amino} phenyl) imino] methyl\} quinoline-2-thiol [MPQT]
Fig. 8.2: $^1$H-NMR spectra of the 3-[[2-[[2-mercaptoquinolin-3-yl] methylene] amino] phenyl]imino] methyl} quinoline-2-thiol [MPQT]
Fig. 8.3: Mass spectra of the 3-{(2-{{2-mercaptoquinolin-3-yl} methylene} amino} phenyl)imino] methyl} quinoline-2-thiol [MPQT]
Fig-8.4: IR spectra of 3-{{[(2-[(2-mercaptoquinolin-3-yl) methylene] amino} phenyl) imino]methyl} quinoline-2-thio cobalt complexes
Electronic spectra and magnetic moment

Electronic spectra of [CoMPQT] and [NiMPQT] complexes were recorded at room temperature in DMF. The electronic spectra of Co(II) complex (1) shows a weak band at 2066-2188 cm\(^{-1}\), \(^2\)B\(_{1g}\) \(\rightarrow\) \(^2\)A\(_{1g}\) attributed due to d-d transitions which are expected to be square planar geometry [28, 29]. The complex (2) has diamagnetic behavior and its electronic spectrum shows a shoulder at 1763-1854 cm\(^{-1}\) ascribed to \(^2\)A\(_{1g}\) \(\rightarrow\) \(^2\)B\(_{1g}\) transition supporting tetrahedral geometry around Ni(II) ion [22]. The observed magnetic moment value 3.26 BM for Co(II), greater than spin-only value 1.75 BM and hence paramagnetic in nature, whereas the Ni(II) complex is observed at 1.53 BM. The molar conductivity for both the complexes were in the range of 2.9-2.5 \(\Omega\)\(^{-1}\) cm\(^2\) mol\(^{-1}\), indicating that they are non-electrolytic in nature.
### Table-8.1. Analytical and physical properties of the metal complexes 3-\{((2-(2-mercaptoquinolin-3-yl) methylene] amino] phenyl) imino] methyl\} quinoline-2-thio metal complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Color</th>
<th>Molecular weight</th>
<th>Yield %</th>
<th>m.p °C</th>
<th>$\Delta m$ (\text{g/mol}^1)</th>
<th>$\mu_{\text{eff}}$ (B.M)</th>
<th>Elemental analysis</th>
</tr>
</thead>
</table>
| [MPQT] L C\(_{26}H_{18}N_4S_2\) | Greenish red | 450.58           | 78      | 143-145    | ---                           | ----                     | C: 69.31 (69.27)  
                  |             |                  |         |            |                               |                          | H: 4.03 (4.10)    
                  |             |                  |         |            |                               |                          | N: 12.43 (12.48)  
                  |             |                  |         |            |                               |                          | S: 14.23 (14.18)  |
| [Co(L)](1) C\(_{26}H_{16}S_2CoN_4\) | Dark Bluish | 509.45           | 63      | 226-228    | 2.7                           | 3.26                     | C: 70.11 (70.19)  
                  |             |                  |         |            |                               |                          | H: 4.07 (4.00)    
                  |             |                  |         |            |                               |                          | N: 12.58 (12.66)  
                  |             |                  |         |            |                               |                          | S: 12.64 (12.53)  
                  |             |                  |         |            |                               |                          | Co:13.23 (13.26)  |
| [Ni(L)](2) C\(_{26}H_{16}S_2NiN_4\) | Reddish brown | 509.15          | 67      | 242-243    | 2.9                           | 1.53                     | C: 70.15 (70.21)  
                  |             |                  |         |            |                               |                          | H: 4.08 (4.05)    
                  |             |                  |         |            |                               |                          | N: 12.59 (12.62)  
                  |             |                  |         |            |                               |                          | S: 12.64 (12.57)  
                  |             |                  |         |            |                               |                          | Ni:13.19 (13.26)  |

### Table-8.2. Characteristic IR absorption bands (in cm\(^{-1}\)) for ligand and metal complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>IR absorption bands (in cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L C(<em>{26}H</em>{18}N_4S_2)</td>
<td>3134m 1623s 15554w 1458m 1354w 1211s, 1137s, 747s 687m ---</td>
</tr>
<tr>
<td><a href="1">Co(L)</a> C(<em>{26}H</em>{16}CoN_4S_2)</td>
<td>3152m 2955w 1651s 1559m 1437m 1210s 1141s 754m 508w 450w</td>
</tr>
<tr>
<td><a href="2">Ni(L)</a> C(<em>{26}H</em>{16}NiN_4S_2)</td>
<td>3152m 2955w 1651s 1560m 1437m 1210s 1141s 756m 508w 451w</td>
</tr>
</tbody>
</table>

s = strong, m = medium w = weak.
DNA Binding Studies (Electronic absorption spectroscopy)

The absorption spectrum of complex (1) and (2) shows well resolved absorbance maxima at 214, 245 nm for complex (1) and 211, 240 nm for complex (2). The addition of increasing higher concentration of CT-DNA led to hypochromic and bathochromic changes in its visible absorption spectra as a result of formation of more stable complexes [30]. In the presence of increasing amounts of CT-DNA, complexes (1) and (2) showed a decrease in absorbance accompanied by a shift towards higher wavelengths hypochromicity:(about 7% for-(1) 10% for-(2)) and bathochromic shifts (maximum: 2±1 nm) for their most red-shift absorption peak maxima(Table-8.2). The change in the absorbance values with increasing amounts of CT-DNA were used to evaluate the intrinsic binding constants ($K_b$). The observed binding constant value for complexes (1) and (2) were $2.8 \times 10^4 \text{ M}^{-1}$ and $4.8 \times 10^4 \text{ M}^{-1}$, respectively suggesting that the complex (2) binds more strongly to CT-DNA. The obtained data are summarized in Table-8.3 [31].

**Table-8.3.** Absorption spectral properties and thermal denaturation mercaptoquinoline complexes of Co(II) and Ni(II) bound to CT-DNA

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$K_b$ (M$^{-1}$)</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex (1)</td>
<td>214</td>
<td>$2.8 \times 10^4$</td>
<td>65</td>
</tr>
<tr>
<td>Complex (2)</td>
<td>211</td>
<td>$4.8 \times 10^4$</td>
<td>68</td>
</tr>
</tbody>
</table>
Evaluation of antimicrobial activity

The *in vitro* antimicrobial activity was carried out against 24 hr old cultures of two bacteria and two fungi by cup-plate method [41]. Complexes have been tested for their antibacterial activity against *Pseudomonas aerugenosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Candida albicans*. Nutrient agar and potatodextrose agars were used to culture the bacteria and fungus respectively. The compounds were tested at a concentration of 0.005 mol / ml in DMSO solution. The solution of Chloramphenicol (2mg/ ml) and Flucanazole (2 mg/ ml) were prepared in sterilized water and used as standards for comparison of antibacterial and antifungal activities respectively. The compounds were tested at varied concentration. The minimum inhibition concentration was found to be 0.001mol/ ml in DMSO against all organisms. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria at 28 °C and 48 h for fungus at 35°C. Each experiment was repeated thrice and the average of the three independent determinations was recorded. The protocol shows Ni(II) complex exhibited significant activity compared to Co(II)complex and ligand. The resulted protocols were summarized in Table 8.4.
Fig-8.6. Absorption spectral traces of complex $[\text{Co}(L)]$ (2) in Tris HCl buffer (0.01M, pH 7.2) upon addition of CT-DNA=0.5μm, 5μm, 10μm, 20μm; 30μm; 40μm; 50μm; Arrow shows the absorbance changing upon increase of DNA concentration.
Fig-8.7. Absorption spectral traces of complex [Ni(L)] (2) in Tris HCl buffer (0.01M, pH 7.2) upon addition of CT-DNA=0.5μm, 5μm, 10μm, drug, 20μm; 30μm; 40μm; 50μm; Arrow shows the absorbance changing upon increase of DNA concentration.
Viscosity measurements

The binding modes of complexes with CT-DNA, was further confirmed by viscosity measurements. When complexes that binds exclusively in the DNA grooves by partial and /nonclassical intercalation, under the same conditions, typically cause negative or no change in DNA solution viscosity [32, 33]. The effects of the complex on the viscosity of rod like DNA are shown Figure-8.8. As expected, the viscosity of complex has obvious effect on relative viscosity of CT-DNA which increases with an increase in concentration of the added complex.

Fig-8.8. Effects of increasing amount of Complex relative viscosity of CT-DNA at 25 ± 0.1 °C.
Thermal denaturaration studies

The stability of the DNA helix with temperature indicates an interaction between DNA to metal complex in the concentration ratio of 25 and \( (T_m) \) values were determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. In the present study, when the complexes solutions are added to the solution of calf thymus-DNA the melting temperature is increased. This indicating that there is an interaction between DNA and metal complexes. The melting of DNA in absence of complex was found to be 60 ± 1 °C, under the same experimental conditions the presence of complexes (1) and (2) increased the melting temperature by about 5 to 8 °C, as shown in the Fig-8.9. [34-36].

![Fig-8.9. Melting curves of CT-DNA in the presence and absences of complex (1) and (2).](image)
## Table 8.4. Antimicrobial activity of ligand and its metal complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Antimicrobial activity</th>
<th></th>
<th>Antifungal activity</th>
<th>Zone of inhibition in mm</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Antibacterial activity</strong></td>
<td><strong>Antifungal activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zone of inhibition in mm</td>
<td>Zone of inhibition in mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>P. aerogenosa</strong></td>
<td><strong>S. Aureus</strong></td>
<td><strong>A. niger</strong></td>
<td><strong>C. albicans</strong></td>
<td><strong>P. aerogenosa</strong></td>
</tr>
<tr>
<td>MPQT(L)</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><a href="1">Co(L)</a> C_{26}H_{16}N_{2}S_{2}Co</td>
<td>21</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><a href="2">Ni(L)</a> C_{26}H_{16}N_{2}S_{2}Ni</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>22</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

![Image of petri dishes with antibiotic effects](image_url)
Conclusions

The new quinoline based ligand [MPQT] and its metal complexes of the type [CoMPQT] and [NiMPQT] was prepared and characterized using physical and spectroscopic methods IR, NMR, and mass date shows that the ligand has tetradeute nature. The electronic spectra and magnetic moment results suggested that square planner geometry for Co(II) tetrahedral for Ni(ii) complex. DNA binding studies of Co(II) and Ni(ii) complexes suggested, they have interaction with CT-DNA base pairs. The antimicrobial activity shows both the ligand and its complex have exhibited significant inhibitory activity.
Reference


251
Chapter-8

Section-B

Synthesis of Diquinolinine[1,3,7,9]tetraazacyclododecine-7, 15(14H, 16H)-Dibenzene, and DNA Binding Studies of Macrocyclic Co(II) and Cu(II) Complexes

Introduction

The design and preparation of tetraaza macrocyclic ligands and their transition metal complexes has long been a field of extensive investigation. The initial interest in these compounds derived from their potential as small-molecule analogues of the active sites of hem proteins and metalloenzymes. The class of tetraaza macrocycles was first reported by Jäger et al. [1] and well studied by changing the ring size, the peripheral substituents, and the central metals [2]. Metal complexes of these ligands were investigated in catalytic electrochemical carbon dioxide [3] and for the activation of dioxygen [4]. Further work by Busch provided a new series of lacunar cyclidene complexes that showed remarkable dioxygen affinity [5]. Rational design and finetuning led to successful non-porphyrin oxygen carriers. These complexes were also used as oxygenation catalysts with molecular dioxygen [6] and as hosts for the formation of inclusion complexes [7].

Research on diverse aspects of new macrocyclic compounds has attracted worldwide interest in recent years. Transition metal complexes of mixed donor macrocyclic ligands constitute a potentially important class of molecules for molecular electronics and catalytic reductions. Macrocyclic complexes are extensively studied from the viewpoint of molecular recognition, artificial catalyst and supramolecular structures [8]. Macrocyclic ligands form metal complexes, which in general are more stable than the complexes with analogous open chain ligands (Macrocyclic effect). Macrocyclic ligands have been employed as selective host for a wide variety of guest molecules and ions. The recognition of a metal ion by a macrocyclic ligand and modification of the properties of resulting complex is closely related to metal ion size compatibility with the ligand cavity [9-10].
Various macrocyclic ligands have been synthesized and their complexes were reported. [11] A variety of macrocyclic complexes derived from o-phenylenediamine including dinuclear macrocyclic complexes have been reported [12, 13]. Condensation between dicarbonyl and diamine species has played a vital role in the development of synthetic macrocyclic ligands, which have been proved to be a fruitful source of
tetraazamacrocycles [14]. In view of the fact that for specific dicarbonyl and diamine precursors, the structure of the condensation product can be controlled by the reaction condition, thus [1+1], and [1+2] condensation reactions lead to the formation of open chain and cyclic structures by selecting appropriate solvent, pH, temperature and the type of metal ion [15]. Macrocyclic complexes were prepared with the aid of metal ions as template to direct the steric course of the condensation reaction, which ultimately results in ring closure. Various macrocyclic ligands have been synthesized and their complexes have been reported. A variety of macrocyclic complexes derived from o-phenylenediamine including dinuclear macrocyclic complexes have been reported [16, 17].

The interaction of transition metal complexes with DNA has been extensively studied in the past few years. Among the first row transition metal ions, such as cobalt, nickel, manganese and copper offers the choice of biocompatibility in biological systems and have been recognized as important biological effects [18]. The study of DNA binding properties and anti-tumor activity of these metal complexes have been well documented in the literature [19, 20]. Barton and co-workers [21, 22] have studied the interaction of enantiomers of Ru(phen)₃ with various DNA; the results lead them to the conclusion that there were two modes of interaction, intercalative and electrostatic binding. Kharatishvili et. Al.; [20] reported the effect on DNA binding in the presence of a planar intercalating ligand such as quinoline for both mononuclear and dinuclear platinum complexes. Recently, the investigation based on DNA interaction with macrocyclic complexes have great importance in understanding the action mechanism of some anti-tumor and antiviral drugs, to design new DNA targeted drugs and to screen these drugs in vitro [26].
Present work

Due to the vital role and importance of macrocyclic complexes towards bioinorganic chemistry and the biological application authors we synthesized macrocyclic ligand and its metal complexes. And also studied their interaction with DNA by electronic, viscosity, and thermal denaturation methods.

Experimental Section

The chemicals used for the synthesis of diquinolineno[1,3,7,9]tetraazacyclododecine-7, 15 (14H, 16H)-dibenzene (L) and metal complexes were of analytical grade. The instruments used for structural elucidation is presented experimental part of chapter-2

Preparation of N,N'-bis[(2-chloroquinolin-3-yl) methylene] benzene-1,2-diamine(2a)

The ethanolic solution of 2-chloro-3-formyl-quinoline (7.64 g, 0.04 mol) and o-phenylenediamine (2.16 g, 0.02 mol) (25 ml each) in 2:1 molar ratio was refluxed for 3-4 h. The yellowish product separated out was washed with cold ethanol, dried under vacuum, and recrystallized from ethyl acetate/dichloromethane solvent system. Yield: 93%, m.p:120-122 °C.

Preparation of Diquinolineno[1,3,7,9] tetraazacyclododecine-7, 15 (14H, 16H)-dibenzene (L)

The compound 2a (4.55 g, 0.01 mol) was dissolved in (25 ml) DMF and added to the (25 ml) o-phenylenediamine (1.28 g, 0.01 mol) in 1:1 molar ratio. The solution was refluxed in the presence of potassium carbonate (1.61 g, 0.01 mol) as catalyst for 10-12 h. The reaction was monitored by TLC using petroleum ether and ethyl acetate (8:3) as eluent. A greenish white precipitate was separated in ice cold water. The resulting
product was collected by filtration, washed with cold water, dried under vacuum, and  
recrystallized from ethanol. Yield: 85% m.p:185-187 °C, FT-IR (cm⁻¹) 3430 (-NH-); 2924  
(Ar-CH); 1619 (C=N); (other peaks) 1451, 1277, 1032, 924, 648. ¹H-NMR (CDCl₃) δ:  
8.86 (s, 1H, NH); 7.6-8.0 (m, 8H, Ar-H, o-phenylendiamine); 8.93 (s, 1H, NH); 8.82 (d,  
1H, CHN, D₂O exchangeable proton); 7.2-7.5 (m, 10H, Ar-H, quinoline)  

General procedure for the preparation of complexes  

A simple method has been adopted for the preparation of the complexes. An hot  
ethanolic solution of ligand (L) and hydrated metal salt in 1:1 molar ratio were mixed.  
The mixture was refluxed for about 3-4 hr at 80 ± 5°C, the obtained residue was  
recrystallized from ethanol. Various attempts to develop the crystals suitable for X-ray  
diffraction studies such as slow diffusion, crystallization using mixtures of solvents and  
low temperature crystallization were unsuccessful.

Preparation of Cobalt(II) complex with ligand(L) diquinolineno[1,3,7,9]  
tetraazacyclododecine-7, 15 (14H, 16H)-dibenzene Co(L)Cl₂±H₂O  

The ligand(L) was dissolved in (25 ml) ethanol and added to the hot ethanolic  
solution of cobalt(II) chloride (25 ml) in 1:1 molar ratio under boiling conditions and  
refluxed for 3-4 hr. The blue colored precipitate formed was collected by filtration and  
dried. Similarly, the same procedure was followed for Cu(II) complex. The experimental  
results is summarized in the Table 1.
DNA binding studies

The experimental details of DNA binding studies were discussed in the Chapter-2.

Results and discussion

A novel diquinolineno[1,3,7,9]tetraazacyclododecine-7,15 (14H, 16H)-dibenzene, macrocyclic ligand (L) has been synthesized in two steps as per the scheme 1. In the first step the o-phenylenediamine reacts with 2-chloro-3-formyl-quinoline, in 1:2 molar ratio in ethanol, a yellowish colored product N-\[(2-chloroquinolin-3-yl) methylene]-N- (2-chloroquinolin-3-yl) methylene] benzene-1,2-diamine separated out, in a second step, it reacts with o-phenylenediamine in 1:1 molar ratio in DMF solvent, gave a greenish white colored solid. The TLC has established the purity of the compound by dissolving the ligand in ethanol using petroleum ether and ethyl acetate (8:3) as eluent. One spot was observed in the TLC plate after developed in an iodine chamber indicating that the compounds were pure. The formation of this macrocyclic molecule framework was confirmed based on the results of FT-IR and resonance peaks in the $^1$H-NMR and elemental analyses. By using this, new macrocyclic complexes of the type $[MLX_2]$, were synthesized by the reaction of the ligand(L) with the corresponding metal salts in 1:1 molar ratio in ethanol solution.
Sch-8.1.1  Synthesis of diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzenene(L)
The formation of the complex may be represented by the following reaction:

\[
\text{CoX}_2 \cdot \text{H}_2\text{O} + \text{L} \rightarrow \text{Co(L)}X_2 + n\text{H}_2\text{O}
\]

\[
\text{CuX}_2 \cdot \text{H}_2\text{O} + \text{L} \rightarrow \text{Cu(L)}X_2 + n\text{H}_2\text{O}
\]

The complexes are microcrystalline in nature and found to be soluble in most of the organic solvents. The elemental analysis data in table 8.1.1 shows that the complexes have a composition of [Co(L)Cl₂] and [Cu(L)Cl₂]. The magnetic moment value observed of 2.56 for Co(II) complex 1.99 for Cu(II) complexes which are greater than spin-only value is 1.75 (B.M) and hence, paramagnetic in nature, exhibits high-spin octahedral geometry. The coordination spheres of complexes, similar to those of nickel (II)-type macrocyclic complexes, have been reported to be six-coordinate octahedral geometry [23]. Hence, in the present studies, the experimental results suggest that the title complexes possess an octahedral geometry. Molar conductivity was studied in DMF, the range of 72-75 \( \Omega^{-1} \) cm\(^{-1} \) mol\(^{-1} \) indicating that both the complexes are 1:1 electrolytes and may be formulated as [MLX₂].

![Suggested structure of [M(L)X₂] where (M= Co(II), Cu(II) X=Cl)](image_url)

**Fig- 8.1.1.** Suggested structure of [M(L)X₂] where (M= Co(II), Cu(II) X=Cl)
Table 8.1.1.

Analytical and physical properties of the metal complexes diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzene(L)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Color</th>
<th>Molecular Wt (Yield %)</th>
<th>m.p. °C</th>
<th>μeff (B.M)</th>
<th>(Δm Ω^1 cm^2 mol^-1)</th>
<th>Elemental analysis Calcd. (Found %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L) (C_{32}H_{22}N_6)</td>
<td>Greenish white 490.55 (85)</td>
<td>180</td>
<td>----</td>
<td>----</td>
<td>C:78.35 (78.26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H:4.52 (4.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N:17.13 (17.05)</td>
</tr>
<tr>
<td>([\text{Co}(L)\text{Cl}_2]) (1)</td>
<td>Dark bluish 549.49 (83)</td>
<td>&gt;250</td>
<td>2.56</td>
<td>72</td>
<td>C:69.95 (69.88)</td>
<td></td>
</tr>
<tr>
<td>(C_{32}H_{22}N_6\text{Cl}_2\text{Co})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H: 4.04 (4.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N:15.29 (15.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Co: 10.7 (10.0)</td>
</tr>
<tr>
<td>([\text{Cu}(L)\text{Cl}_2]) (2)</td>
<td>Reddish 554.10 (71)</td>
<td>&gt;280</td>
<td>1.99</td>
<td>75</td>
<td>C:69.36 (69.28)</td>
<td></td>
</tr>
<tr>
<td>(C_{32}H_{22}N_6\text{Cl}_2\text{Cu})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H: 4.00 (3.97)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N:15.17 (15.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cu:11.47 (11.39)</td>
</tr>
</tbody>
</table>
Fig. 8.1.2. IR spectra of diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzene(L)
Fig. 8.1.3. $^1$H-NMR spectra of diquinolineno [1, 3, 7, 9] tetraazacyclododecine-7, 15 (14H, 6H)-dibenzene(L)
Fig-8.1.4. IR spectra of diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzene cobalt complex
Fig-8.1.5. IR spectra of diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzenene copper complex
Table 8.1.2. Important IR (cm\(^{-1}\)) bands of ligands and its metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>v(N-H)</th>
<th>v(Ar-CH)</th>
<th>v(C=N)</th>
<th>v(C=C)</th>
<th>v(C-H)</th>
<th>v(M-N)</th>
<th>v(M-Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L) C(<em>{32})H(</em>{22})N(_{6})</td>
<td>3430s</td>
<td>2924m</td>
<td>1619s</td>
<td>1488m</td>
<td>1451s</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>[Co(L)Cl(<em>{2})] (1) C(</em>{32})H(<em>{22})N(</em>{6})Cl(_{2})Co</td>
<td>3338b</td>
<td>3050m</td>
<td>1638s</td>
<td>1557m</td>
<td>1400s</td>
<td>761s</td>
<td>472m</td>
</tr>
<tr>
<td>[Cu(L)Cl(<em>{2})] (2) C(</em>{32})H(<em>{22})N(</em>{6})Cl(_{2})Cu</td>
<td>3371b</td>
<td>3057m</td>
<td>1663s</td>
<td>1494m</td>
<td>1405s</td>
<td>768s</td>
<td>473m</td>
</tr>
</tbody>
</table>

FT-IR Spectra

IR spectra of complexes were recorded in the matrix of KBr pellets with a Perkin-Elmer 1430 spectrometer the important IR spectral data presented in table 8.1.2. The absence of bands corresponding to the amino groups of o-phenylenediamine and carbonyl groups of aldehydic 2-chloro-3-formyl-quinoline, suggests the formation of the proposed macrocyclic ligand \(\text{(L)}\). Further, the two intensive bands at 1619 cm\(^{-1}\) and 3430 cm\(^{-1}\) assignable to uncoordinated v(C=N) and v(N-H) of amine group, respectively confirms the proposed structure \([24,25]\). In addition, the formation of macrocyclic structure was conformed by its \(^1\)H-NMR spectra. However, IR spectra of complexes derived from the ligand \(\text{(L)}\) shows a slight shift to the lower frequency in v(C=N) which appeared in the region 1638-1667 cm\(^{-1}\) suggesting its coordination with metal ion. In addition, a strong characteristic band of v(-NH-) appeared at 3371-3338 cm\(^{-1}\), and bands at 1557-1494 cm\(^{-1}\) for all the complexes correspond to C-H binding vibrations, respectively. The appearance of new medium–intensity bands in the region 755-750 cm\(^{-1}\) in the macrocyclic complexes.
may be assigned to $v$ (M-N) vibrations. The bands at 472-475 cm$^{-1}$ were assigned to $v$(M-Cl) vibrations, and the values are summarized in Table-8.1.2.

$^1$H-NMR Spectra

The $^1$H-NMR spectra were recorded on Jeol spectrometer (400 MHz), and chemical shifts ($\delta$) are given in ppm relative to the signal for TMS as the internal standard. The absence of proton resonance signals of free NH$_2$ and aldehydic (CHO) groups indicates the condensation between amine and carbonyl group of aldehydic 2-chloro-3-formyl-quinoline. The $^1$H-NMR spectra of the ligand recorded in CDCl$_3$ show a doublet at $\delta$: 8.82 ppm (d, 1H, CHN, D$_2$O exchangeable), due to hydrogen bonding and anisotropy effect of the adjacent and other aromatic resonated protons, and signal exhibits singlet at $\delta$: 8.86 ppm (s, 1H, NH) and 8.93 (s, 1H, NH) ascribed. A multiplet signal at 8.2-8.56 ppm (m, 8H, Ar-H$_x$) corresponds to the aromatic o-phenylenediamine. The multiplet signals attributed at 7.0-7.56 ppm are due to (m, 10H, Ar-H$_x$) of aromatic quinoline moiety.

Absorption spectral features of DNA binding

The interaction of DNA with the new complexes was studied by electronic absorption spectra the results are presented in figure 8.1.7, 8.1.8 respectively. The absorption spectra of complex (1), and (2) shows a well resolved absorption bond at 304 nm, 344 nm for (1) and 302 nm 347 (2), respectively. In the presence of increasing amounts of CT-DNA, both complexes showed a strong decrease in intensity (hypochromicity: 8% for Co(II) and 6% for Cu(II) complex) and bathochromic shifts (maximum: 3 ± 1 nm for cobalt(II) and 2 ± 1 nm for copper (II) complex) Table-8.1.3. The change in the absorbance values (at 304 nm and 344 nm for complex (1) and at 302
nm and 347 nm for complex (2)) with increasing amounts of CT-DNA were used to evaluate the intrinsic binding constants ($K_b$) for the complexes. The values of $K_b$ evaluated for ligand, complexes (1) and (2), using equation is $2.8 \times 10^3 \text{ M}^{-1}$ for ligand, $3.8 \times 10^4 \text{ M}^{-1}$ for (1) and $3.3 \times 10^4 \text{ M}^{-1}$ for (2), respectively. This value suggested that the complexes are bound more avidly to CT-DNA than the ligand. The observed $K_b$ values are comparable to those observed for typical classical intercalators [EthBr, $K_b$, $1.8 \times 10^6 \text{ M}^{-1}$ in 25 mM Tris-HCl/40 mM NaCl buffer, pH 7.9] and partial intercalating metal complexes [Ru(phen)$_2$(dpdz)$_2^+$, dpdz = dipyrido-[3,2-d: 2',3'-f]-phenazine, $K_b>10^6 \text{ M}^{-1}$] bound to CT-DNA.[26, 27].

**Table-8.1.3.** Absorption spectral properties and thermal denaturation of macrocyclic ligand and complexes of Co(II) and Cu(II) bound to calf thymus-DNA

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{max}$ (nm)</th>
<th>$K_b$ (M$^{-1}$)</th>
<th>$T_m$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand/[DNA]</td>
<td>355</td>
<td>$2.8 \times 10^3$</td>
<td>54</td>
</tr>
<tr>
<td>Complex (1)</td>
<td>304</td>
<td>$3.8 \times 10^4$</td>
<td>63</td>
</tr>
<tr>
<td>Complex (2)</td>
<td>302</td>
<td>$3.3 \times 10^4$</td>
<td>65</td>
</tr>
</tbody>
</table>
Fig. 8.1.6. Absorption spectral traces of complexes [Co(L)Cl₂] (I) in Tris-HCl buffer (0.01M, pH 7.2) upon addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; 50 μm; arrow shows the absorbance changing upon increase of DNA concentration. Inner plots are DNA con/ DNA [E₂₋₃].
Fig. 8.1.7. Absorption spectral traces of complexes [Cu(L)Cl_2] (2) in Tris-HCl buffer (0.01M, pH 7.2) upon addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; 50 μm; arrow shows the absorbance changing upon increase of DNA concentration. Inner plots are DNA con/ DNA [E_a-E_d].
5.4. Fluorescence studies

The complexes (1) and (2) can emit luminescence in Tris buffer (pH 7.0-7.2) at ambient temperature with maxima at 450, and 448 nm. Upon addition of CT DNA (= Calf thymus DNA), the emission intensities of both the complexes increases when compared to the intensity of complexes alone shown in Fig-8.1.8. It was previously reported that this enhanced fluorescence could be quenched, at least partly by the addition of second molecules [28, 29]. This implies that both the complexes are strongly interact with CT-DNA through intercalation mode, and be protected by DNA efficiently, since the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the duplex and the complexes mobility is restricted at the binding site, lead to decrease the vibrational modes of relaxation [30]. The increases extent of enhancement for both the complexes and which is consistent with the above absorption spectral results. The order of increase in emission intensity of two complexes is strengthened by absorption spectra viscosity measurements and thermal denaturaration studies.

This observation is further supported by the emission quenching experiments using $[\text{Fe(CN)}_6]^4-$ as quencher. The method essentially consists of titrating the amount of $[\text{Fe(CN)}_6]^{4+}$ (mmol/L) DNA binding-metal complexes with increasing the concentration of $[\text{Fe(CN)}_6]^{4+}$ and measuring the change in fluorescence intensity. The ion $[\text{Fe(CN)}_6]^{4+}$ has been shown to be able to distinguish differentially bound Cobalt and Nickel complexes. The positively charged free complex ions should be readily quenched by $[\text{Fe(CN)}_6]^{4+}$. When bound to DNA the complex can be protected from the quencher, because highly negatively charged $[\text{Fe(CN)}_6]^{4-}$ would be repelled by the negatively change DNA phosphate backbone. The slop can be taken as a measure of binding affinity. So we can conclude that both the complexes are bond through intercalation [31].
Fig. 8.1.8. Fluorescence emission of complexes: complex (1), complex (1) in Tri-HCl buffer. Fluorescence intensity increasing CT-DNA concentrations (5μl, 10μl, 15μl, 20μl,...). Inner: plots of relative emission intensity versus [DNA]/[complexes]
Fig- 8.1.9: Emission quenching of complexes with increasing the concentrations of [Fe (CN₆)₄]. Curves (A) for complex 1 + in presence of DNA and (B) for complex 2 + DNA.
Viscosity Measurements

In order to further elucidate the binding mode of the present complex, the viscosity measurements were carried out on CT-DNA by varying the concentration of added complex. The effects of the complex (1) and (2) on the viscosity of rod-like DNA were shown Fig 8.1.10. As expected for the complex (1) and (2), the viscosity of DNA increases with an increase in concentration of the added complex. The results revealed that the presence of the complex has an obvious effect on relative viscosity of CT-DNA [32, 33].

![Graph showing viscosity measurements](image)

**Fig. 8.1.10.** Effects of increasing amount of ligand and complex (1), (2) on the relative viscosity of CT-DNA at 25±0.1°C.
Thermal denaturation studies

Additional information on the DNA binding properties was obtained from melting studies. The stability of the DNA helix with temperature indicates an interaction between DNA to metal complex in the concentration ratio of 25 and \( T_m \) values were determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. In the present study, when the complex solutions are added to the DNA solution, the melting temperature was increased. This indicates that there is an interaction between DNA and metal complexes. The melting of DNA in the absence of any complex was found to be 60 ± 1°C, under the same experimental conditions, the presence of complexes (1) and (2) increased the melting temperature by about 3 to 6°C, as shown in Fig 8.1.11. [34].

![Fig. 8.1.11. Melting curves of CT-DNA in the presence and absence of ligand and its complexes.](image-url)
Conclusions

The synthetic route adopted for synthesis of macrocyclic ligand and its metal complexes of the type [M(L)X₂] was very simple and gave good yield. In DNA binding studies, the absorption spectral results indicate hypochromicity and bathochromic shifts (red shift) of the complex (1) and (2) when it binds with base pairs of calf thymus-DNA. The binding constant values of ligand (2.8×10³ M⁻¹), and complexes (3.8×10⁴ M⁻¹) for (1) and (2) (3.3×10⁴ M⁻¹) suggested that the complexes bind more avidly to CT-DNA than the ligand. In addition, increasing the viscosity of sonicated rod-like DNA fragments and the melting temperature of DNA, in the presence of complex solutions supports the binding mode.
Reference


