PREPARATION, SPECTRAL CHARACTERIZATION, CYCLIC VOLTAMMETRIC AND BSA BINDING STUDIES OF NEW Ni(II) THIOSEMICARBAZONE COMPLEXES CONTAINING 2-CHLOROQUINOLINE-3-CARBOXALDEHYDE AND 2, 2’-BIPYRIDINE.

Gopeechandana G.B¹, Meghasurendran S.K², Kalaivani P³

¹Department of Chemistry, Nirmala College for Women, Coimbatore- 641 018, India
²Department of Chemistry, Nirmala College for Women, Coimbatore- 641 018, India
³Assistant Professor, Department of Chemistry Nirmala College for Women, Coimbatore- 641 018, India

Abstract- Four Ni(II) complexes were synthesized by the reaction of NiCl₂.6H₂O with 2-Chloro Quinoline-3-Carboxaldehyde-4(N)-substituted thiosemicarbazones and 2, 2’-bipyridine, where R= H, CH₃, C₂H₅ and C₆H₅. The new Ni(II) complexes were characterized by IR, UV-Vis and ¹H NMR Spectroscopy. The redox properties of the Ni(II) complexes were studied by Cyclic Voltammetry. Their binding ability were carried out using BSA by emission titration method. The observations indicated significant binding ability of the complexes 1-4 with BSA.

Key Words: Ni(II) thiosemicarbazones, Spectroscopy, BSA binding studies, Cyclic voltammetry

1.INTRODUCTION

Coordination chemistry, is the science deals with the interactions of organic and inorganic ligands with metal.[¹] Compounds are molecules that have one or multiple metal centers which is bound to ligands. The molecules or ions which surroundings the central metal ion is called ligands. These complexes can be neutral or charged.[²] Schiff bases are the compound that containing azomethine group (-HC=N-). They are formed through the condensation of ketones (or) aldehydes with primary amines. Common Schiff base are crystalline solids, that are insoluble in salts.[⁴] A Schiff base behaves as a flexi-dentate ligand and is it commonly coordinates through the O atom of the deprotonated phenolic group and the N atom of azomethine group.[⁷] Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications. Thiosemicarbazones (TSC) have been the subject of several studies because of their variable bonding modes, encouraging biological implications and structural variety.[⁸] Thiosemicarbazones, produced from the condensation of a thiosemicarbazide with aldehydes or ketones, are useful for attainment of coordination spheres with mixed N/S dentate.[⁹,¹⁰] They coordinate as a neutral molecule or after deprotonation as anionic ligands.[¹¹] Studies on thiosemicarbazamide complex help to knowing the metal-protein binding in the organism.[¹²] Thiosemicarbazone derivative found application in drug development, for treatment for CNS disorder, bacterial infection as well as analgesics.[¹³] Ni forms variety of coordination compounds. The interaction of Ni(II) with Schiff base offers an opportunity to understand various properties of Ni(II) complexes.[¹⁵] Nickel has a very important role in biological activities. Because there are so many Ni containing and nickel dependent enzymes are present. Some of them are urease, hydrogenase, methyl-S-coenzyme, M-reductance etc.[¹⁶] The biological activity of Ni(II) are anti-tumour, anti-biotic, anti-inflammatory, anti-bacterial, anti-oxidant etc.[¹⁷,¹⁸] A significant number of Ni(II) compounds acting as DNA intercalators, DNA cleaving agents.[¹⁹-²¹] The nickel complexes are commonly four coordinated ligands and showed good catalytic activities toward ethylene oligomerization.[²²] Quinolines are broadly distributed class of compounds in nature with potentially advantageous effects in the field of medicine, some derivatives of quinolines and 2-oxo-1,2-dihydroquinoline-3- carbaldehyde have been shown to have good biological activities such as antioxidant, antiproliferation, anti-inflammation and antimalarial activity. [²²-²⁴] With the above objectives in mind, herein we reporting the synthesis, spectral characterization, cyclic voltammetric and BSA binding studies of new
Ni(II) thiosemicarbazone complexes containing 2-chloroquinoline-3-carboxaldehyde and 2, 2′-bipyridine.

2. EXPERIMENTAL WORK

Bipyridine, metal salt (NiCl₂.6H₂O), 2-chloroquinoline-3-carboxaldehyde, thiosemicarbazide and 4(N)-substitutedthiosemicarbazide were purchased from TCI chemicals. All the reagents used were analytical grade, were purified and dried according to the standard procedure. The ligands (HL₁, and HL₂) were synthesized according to the literature procedures. For TLC technique, silica gel-G grade coated glass plates were used as stationary phase, for mobile phase were used as 75:25 petroleum ether; ethyl acetate and 95:5 Benzene; methanol. The spots in the stationary phase were visualized by using iodine chamber. Melting points were recorded using Sigma scientific apparatus. Nicolet Avatar Model FT-IR spectrophotometer was used to record IR spectra (4000 - 400 cm⁻¹) of the compounds using KBr pellets at Department of Chemistry, Bharathiar University Coimbatore. ¹H-NMR spectra were recorded in DMSO by using Bruker 400 MHz instrument at Gandhigram University, Dindigul. Emission studies carried out by Agilent spectrofluorometer at Department of Chemistry, Nirmala College for Women, Coimbatore. The Electronic absorption spectra of the compounds were done in DMSO by using Labman spectrofluorimeter and Cyclic Voltammetric studies were done in DMSO at room temperature by using CH-1660E electrochemical instrument at Department of Chemistry, Nirmala college for Women, Coimbatore.

2.1 Preparation of 2-chloroquinoline-3-carboxaldehyde thiosemicarbazone (HL₁)

To the methanolic solution (20cm³) of 2-chloroquinoline-3-carboxaldehyde (1 g; 5.2188 mmol), methanolic solution (20cm³) of thiosemicarbazide (0.4756g;5.2188 mmol) was added. The reaction mixture refluxed for 5 h. The resulted product was filtered, washed with cold methanol and dried under vacuum. The progress of the reaction monitored by Thin Layer Chromatography (TLC). The product was partially dissolved in warm methanol and acetonitrile and completely dissolved in DMF and DMSO. Yield: 84%, melting point : 226⁰C, colour : Yellow, Molecular Formula: C₁₁H₁₃N₃SCl, molecular weight (calculated): 264.7336

2.2 Preparation of 2-chloroquinoline-3-carboxaldehyde 4(N)-methylthiosemicarbazone(HL₂)

The ligand (HL₂) was prepared by the procedure as used for ligand (HL₁) with 2-chloroquinoline-3-carboxaldehyde (1 g ; 5.2188 mmol), and 4(N)-methylthiosemicarbazide (0.5488 g ; 5.2188 mmol). The product was sparingly dissolves in warm methanol and acetonitrile and completely dissolves in DMF and DMSO. Yield: 80%, Melting point : 220⁰C, Colour : Yellow, Molecular Formula: C₁₃H₁₅N₃SCl, molecular weight (calculated): 278.63

2.3 Preparation of 2-chloroquinoline-3-carboxaldehyde 4(N)-ethylthiosemicarbazone (HL₃)

The ligand (HL₃) was prepared by the procedure as used for ligand (HL₁) with 2-chloroquinoline-3-carboxaldehyde (1 g; 5.2188 mmol), and 4(N)-ethylthiosemicarbazide (0.5593 g - 5.2188 mmol). The product was partially dissolved in warm methanol and acetonitrile and completely dissolved in DMF and DMSO. Yield: 82 %, Melting point : 180 ℃, Colour : Yellow, Molecular Formula: C₁₃H₁₅N₃SCl, molecular weight (calculated): 293.7945

2.4 Preparation of 2-chloroquinoline-3-carboxaldehyde 4(N)-phenylthiosemicarbazone (HL₄)

The ligand (HL₄) was prepared by the procedure as used for ligand (HL₁) with 2-chloroquinoline-3-carboxaldehyde (1 g; 5.2188 mmol), and 4(N)-phenylthiosemicarbazide (0.8101 g ; 5.2188 mmol). The product was sparingly dissolves in warm methanol and acetonitrile and completely dissolves in DMF and DMSO. Yield: 80%, Melting point : 204⁰C, Colour : Yellow, Molecular Formula: C₁₅H₁₇N₃SCl, molecular weight (calculated): 341.8373

2.5 Preparation of Ni(II) complex 1

Acetonitrile solution (20cm³) of 2-chloroquinoline-3-carboxaldehyde thiosemicarbazone (HL₁) (0.1113 g ; 0.4206 mmol) and bipyridine (0.066g;0.4206 mmol) were added to the refluxing methanolic solution of NiCl₂.6H₂O (0.4206 mmol ; 0.1g). Further the reaction mixture was refluxing for 5h and the progress was monitored by Thin Layer Chromatography (TLC), until the completion of the reaction. The resultant light green product was filtered.
and dried under vacum. The product dissolves in organic solvents such as warm methanol, DMF and DMSO. Yield: 78%, Melting point: 198°C, Colour: Green, Molecular Formula: Ni(C$_2$H$_2$N$_6$SCl)$_3$, molecular weight (calculated): 550.4769

### 2.6 Preparation of Ni(II) complex 2

The complex 2 was prepared by the procedure as used for complex 1 with 2-chloroquinoline-3-carboxaldehyde 4(N)-methylthiosemicarbazone (HL)$^2$ (0.1171g; 0.4206 mmol), bipyridine (0.066g; 0.4206 mmol) and NiCl$_2$.6H$_2$O (0.1g; 0.4206 mmol). The product dissolves in organic solvents such as warm methanol, benzene, acetonitrile, DMF and DMSO. Yield: 81%, Melting point: 200°C, Colour : Reddish Brown, Molecular Formula: Ni(C$_2$H$_2$N$_6$SCl)$_3$, molecular weight (calculated): 564.5016

### 2.7 Preparation of Ni(II) complex 3

The complex 3 was prepared by the procedure as used for complex 1 with 2-chloroquinoline-3-carboxaldehyde 4(N)-ethylthiosemicarbazone (HL)$^3$ (0.1183g; 0.4206 mmol) bipyridine(0.066g; 0.4206 mmol) and NiCl$_2$.6H$_2$O (0.1g; 0.4206 mmol). The product dissolves in organic solvents such as warm methanol, DMF and DMSO. Yield: 77 %, Melting point: 182 °C, Colour :Light green, Molecular Formula: Ni(C$_2$H$_2$N$_6$SCl)$_3$, molecular weight (calculated): 578.5263

### 2.8 Preparation of Ni(II) complex 4

The complex 4 was prepared by the procedure as used for complex 1 with 2-chloroquinoline-3-carboxaldehyde 4(N)-phenylthiosemicarbazone (HL)$^4$ (0.1371g; 0.4206 mmol), bipyridine (0.066 g : 0.4206 mmol) and NiCl$_2$.6H$_2$O (0.1 g : 0.4206 mmol). The product dissolves in organic solvents such as warm methanol, DMF and DMSO. Yield : 83 %, Melting point : 212 °C, Colour: Brown, Molecular Formula: Ni(C$_2$H$_2$N$_6$SCl)$_3$, molecular weight (calculated): 626.5691.

### 3. RESULT AND DISCUSSION

#### 3.1 Synthesis of Ligands and new Ni(II) complexes

The ligands, 2-chloroquinoline-3-carboxaldehyde thiosemicarbazone (HL)$^3$ was prepared by the equimolar reaction between thiosemicarbazide with 2-chloroquinoline-3-carboxaldehyde using methanol as solvent. The same procedure was applied to the preparation of 2-chloroquinoline-3-carboxaldehyde 4(N)-substituted thiosemicarbazone (HL)$^4$. After confirming the ligand formation, the reactions of NiCl$_2$.6H$_2$O with an equimolar amount of 2-chloroquinoline-3-carboxaldehyde 4(N)-substituted thiosemicarbazone (HL)$^1$ and (HL)$^2$ and bipyridine were carried out in Methanol:CH$_2$CN medium resulted in the formation of new complexes (1-4) (Scheme.1). The complexes are soluble in methanol, dimethylformamide and dimethylsulphoxide.

#### 3.2 Spectroscopic studies

The IR spectra of ligands (HL)$^{1-4}$ and new complexes (1 - 4) provided the significant information about metal ligand bonding. The free Schiff base ligands (HL)$^{1-4}$ showed very strong absorption band at 1533 cm$^{-1}$, 1533 cm$^{-1}$, 1538 cm$^{-1}$, 1548 cm$^{-1}$ respectively, which corresponds to ν(C=N) vibration$^{[23]}$, the band was shifted to 1625 cm$^{-1}$, 1602 cm$^{-1}$ 1627 cm$^{-1}$, and 1538 cm$^{-1}$ in Ni(II) complexes (1 - 4) respectively indicating the coordination azomethine nitrogen atom to Ni ion. The ν(C=S) vibration showed band at 755 cm$^{-1}$, 752 cm$^{-1}$, 748 cm$^{-1}$ and 757 cm$^{-1}$ in ligands (HL)$^{1-4}$ respectively, which is shifted to 763cm$^{-1}$, 761cm$^{-1}$ 755 cm$^{-1}$ and 757 cm$^{-1}$ respectively in Ni(II) complexes (1-4) indicated the coordination thione sulphur to Ni ion$^{[26]}$. The ν(N-H) vibration showed band at 3426.89 cm$^{-1}$, 3376.75 cm$^{-1}$, 3145 cm$^{-1}$ and 3129 cm$^{-1}$ in ligands (HL)$^{1-4}$ respectively, which is shifted to 3424.96 cm$^{-1}$, 3411.46 cm$^{-1}$ 3386 cm$^{-1}$ and 3415 cm$^{-1}$ respectively in

---

**Scheme.1.** Synthesis of Ligands and new Ni(II) complexes
Ni(II) complexes (1-4). The electronic spectra of ligands (HL\(^{14}\)) and new complexes (1 - 4) displayed two and three bands in around 282 – 366 nm. The band appeared at 282- 290 nm assigned for (π-π*) and the band appeared at 332-345 nm assigned for (n-π*). The band appeared at 353 – 366 nm assigned for LMCT.\(^{[27]}\) The \(^1\)H NMR spectra of the ligands and corresponding complexes were recorded in DMSO, at the field strength of 400 MHz showed all the expected signals. In the spectra of ligands (HL\(^{14}\)), a sharp singlet corresponding to the HN-C=S has appeared at δ 11.817- 12.206 ppm. However, the singlet completely disappeared in complexes confirmed the involvement of thione sulphur in coordination. The spectra of ligands (HL\(^{14}\)) showed singlet at δ 8.809-9.404 ppm corresponding to the HC=N protons. In ligand (HL\(^{12}\)) showed singlet at δ 9.233-9.322 ppm corresponding to terminal NH protons. In all ligands (HL\(^{14}\)) showed aromatic proton region at δ 8.296- 10.320 ppm. In ligand (HL\(^2\)) showed doublet at δ 3.398-3.078 ppm which is assigned for methyl protons and in ligand (HL\(^3\)) showed triplet at δ 1.216-1.182 ppm which is corresponding to terminal ethyl protons. When come to the complexes, complex 2 and 3 showed sharp singlet at δ 14.896 ppm and δ 14.946 ppm respectively, complex 1 and 4 showed doublet in the region δ 14.956-14.853 ppm corresponding to the thione sulphur (HN-C=S). The spectra of complexes 1-4 showed singlet at the region δ 9.111-10.337 ppm corresponding to the azomethine (HC=N) protons.\(^{[28-30]}\) Complex 1 and 2 showed doublet in the region δ 10.167-11.572 ppm and the complexes 3 and 4 showed singlet in the region δ 9.156-12.215 ppm corresponding to the terminal NH protons. In complexes 1-4 showed aromatic proton region at δ 9.423-7.168 ppm. The complex 2 showed triplet at δ 1.363-1.074 ppm is corresponding to terminal methyl protons and complex 3 showed quadrat at 1.417-1.181 ppm is corresponding to terminal ethyl protons.

3.3 Protein Binding Studies

The crucial step in accessing a drug’s bioavailability is assigned to its interaction to plasma protein. In screening potential therapeutic agents, plasma protein binding is required.\(^{[31]}\) Bovine serum albumin (BSA) is the most extensively studied serum albumin, which is able to bind with variety of substrates including metal cations, hormones, and most therapeutic drugs. BSA possesses three fluorophores (tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe)). A solution of BSA (10 μM) was titrated with various concentrations of the compounds (0–100 μM) and fluorescence emission spectra were recorded in the range of 290–500 nm upon excitation at 280 nm. The changes observed on the emission spectra of BSA by the addition of increasing amounts of the compounds are shown in (Fig.1 and Fig.2) Upon the addition of ligand and complexes to BSA, a significant decrease in the fluorescence intensity was observed at 348 nm with hypochromism accompanied by 2 nm blue shift. The observed quenching may be attributed to the possible changes in the secondary structure of protein. According to Stern Volmer quenching equation,\(^{[32]}\)

\[
\frac{I}{I_{corr}} = 1 + K_{sv}[Q] \quad (1)
\]

The observed linearity in the plots (Fig 3-4 and Table 1.) indicates the ability of the complexes to quench the emission intensity of BSA. From \(K_{sv}\) values, the complexes exhibited better protein-binding ability than ligands. For the static quenching interaction, it is assumed that there are similar and independent binding sites in the biomolecule, the binding constant (Kb) and the number of binding sites (n) can be determined according to the method using the Scatchard equation (2).\(^{[33]}\)

\[
\log [(F_0−F)/F]= \log Kb + n \log [Q] \quad (2)
\]

Where, in the present case, \(K_b\) is the binding constant for the complex–protein interaction and ‘n’ is the number of binding sites per albumin molecule, which can be determined by the slope and the intercept of the double logarithm regression curve of \(\log [(F_0−F)/F]\) versus \(\log [Q]\).
The observed linearity in the plots indicated the ability of the complexes to quench the emission intensity of BSA. From $K_{SV}$ values, it is seen that the new complexes 1-4 exhibited protein-binding ability with enhanced hydrophobicity than ligand $HL_{1-4}$. Table 1 showed binding interaction of all the ligands and complexes with BSA. The complexes (1-4) showed good activity than corresponding ligands $HL_{1-4}$ and Complex 2 and 4 showed better activity than 1 and 3 due to presence of electron withdrawing group in the system. The value of $n$ at room temperature are approximately equal to 1 in complex 2 and 4, which indicates that there is just one single binding site in BSA in the complex.\cite{33}

Table 1: Quenching constant ($K_{SV}$), binding constant ($K_b$) and number of binding sites ($n$) for the interactions of ligands and complexes with BSA

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_{SV}$ (M$^{-1}$)</th>
<th>$K_b$ (M$^{-1}$)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$HL_1$</td>
<td>0.48X10$^4$</td>
<td>0.62X10$^3$</td>
<td>0.94</td>
</tr>
<tr>
<td>$HL_2$</td>
<td>0.91X10$^4$</td>
<td>0.15X10$^3$</td>
<td>0.80</td>
</tr>
<tr>
<td>$HL_3$</td>
<td>0.48X10$^4$</td>
<td>0.49X10$^3$</td>
<td>0.73</td>
</tr>
<tr>
<td>$HL_4$</td>
<td>0.36X10$^4$</td>
<td>0.02X10$^3$</td>
<td>0.72</td>
</tr>
<tr>
<td>1</td>
<td>0.45X10$^4$</td>
<td>0.78X10$^4$</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>1.18X10$^4$</td>
<td>0.69X10$^4$</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>0.46X10$^4$</td>
<td>1.40X10$^3$</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>0.48X10$^4$</td>
<td>2.78X10$^3$</td>
<td>0.94</td>
</tr>
</tbody>
</table>

3.4 Cyclic voltammetry

The oxidation and reduction potentials of the complexes (1 and 2) in DMSO were given in (Table 2.) Ni(II) complexes exhibited oxidation and reduction waves in the sweep range from -2.0 to +2.0 V (Fig 5.). Complexes (1 and 2) exhibited a quasi-reversible
oxidative response $E_{1/2}$ at 0.8008 V, 0.7377 V, 0.7714 V and 0.7885 V with the peak to peak separation of 347 mV, 333 mV, 34 mV and 44 mV respectively has been assigned to Ni(II)-Ni(III) oxidation. The complexes (1 and 2) exhibited quasi-reversible reduction at $E_{1/2}$ 0.3872 V, 0.3846 V, 0.3799 V and 0.4093 V with peak to peak separation of 74 mV, 103 mV, 44 mV and 44 mV respectively has been assigned to reduction of Ni(II)-Ni(I). The quasi-reversible ligand reduction of complexes (1, 2 and 4) with the potentials $E_{1/2}$ 1.358 V, 1.415 V and 1.553 V respectively with peak to peak separation of 311 mV, 167 mV and 47 mV.

Table 2: Electrochemical studies of new Ni(II) complexes (1 & 2)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Oxidation [Ni(II)]</th>
<th>Reduction [Ni(III)]</th>
<th>Ligand Oxidation</th>
<th>Ligand Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_a$ (V)</td>
<td>$E_c$ (V)</td>
<td>$E_x$ (V)</td>
<td>$i_R$ (mA)</td>
</tr>
<tr>
<td>1</td>
<td>0.8008</td>
<td>0.7377</td>
<td>0.7714</td>
<td>0.7885</td>
</tr>
<tr>
<td>2</td>
<td>0.8008</td>
<td>0.7377</td>
<td>0.7714</td>
<td>0.7885</td>
</tr>
<tr>
<td>3</td>
<td>0.8008</td>
<td>0.7377</td>
<td>0.7714</td>
<td>0.7885</td>
</tr>
<tr>
<td>4</td>
<td>0.8008</td>
<td>0.7377</td>
<td>0.7714</td>
<td>0.7885</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The new Ni(II) complexes were synthesized by the reaction of 2-chloroquinoline-3-carboxaldehyde thiosemicarbazone, 2-chloroquinoline-3-carboxaldehyde with 4(N)- substituted thiosemicarbazone and bipyridine with NiCl$_2$:6H$_2$O. The completion of reactions were monitored by Thin layer chromatographic technique (TLC). The resulted Ni(II) complexes were characterized by IR, UV-Vis and $^1$H NMR Spectroscopy. The redox properties of the new complexes were studied by Cyclic Voltammetry. Based on the spectral studies, a stable five membered chelate ring was formed by utilizing it’s the azomethine nitrogen, thione sulphur atoms from the Schiff base ligands and two nitrogen atoms from bipyridine, which resulted in a square pyramidal geometry. Further, the binding ability of the ligands and Ni(II) complexes were carried out using BSA by emission titration method. While comparing the binding affinity of compounds with BSA the complexes (1-4) exhibited better binding ability than ligands. The complex 2 and 4 showed good activity compared to complex 1, 3 and the ligands which could be attributed to the electrophobic nature of the compound.

ACKNOWLEDGEMENT

I greatly acknowledge DST-FIST (at zero level)-(SR/FST/college-254/2015), New Delhi, India for their financial support provided for the instrument like Electrochemical workstation for carrying out research work.

REFERENCES

6. El Sonbati, W. H. Mahmoud, G. Gehad Mohamed, M. A. Diab, M. Morgan,