CHAPTER-6

DNA interaction studies and Antimicrobial activity of new Palladium(II) Complexes with Quinolin-4(3H)-one derived Schiff bases
INTRODUCTION

Palladium (Pd), named after the asteroid Pallas, is arguably the most versatile and ubiquitous metal. Palladium has attracted much attention because it is a source of an increasing stream of new compounds of high intrinsic interest, particularly with respect to bonding and structure. The increase in the coordination chemistry of palladium has grown in recent years because of its electronic properties, the applications of those compounds in organometallic chemistry, analytical chemistry, catalysis and relevance to bioinorganic systems [1, 2].

Palladium is a soft silver-white metal that is quite similar to platinum in its chemical and physical properties. It has the lowest density and the lowest melting point among the platinum group metals. Palladium is chemically attacked by sulfuric, nitric and hydrochloric acids in which it dissolves slowly. Common oxidation states of palladium are 0, +1, +2 and +4. A key factor that might explain why platinum complexes can be active whereas the corresponding palladium complexes are generally inactive but toxic is the differences in the rates of their ligand exchange reactions. The hydrolysis of the leaving ligands in palladium complexes is about 105 times faster than their corresponding platinum analogues [3]. They dissociate readily in solution leading to very reactive species that are unable to reach their pharmacological targets [4] because of rapid binding with other ligands. In addition, some of them undergo conversion to inactive trans-conformation. Considerably higher reactivity of palladium complexes implies that if an antitumour palladium drug is to be developed, it must somehow be stabilized by a strongly coordinated nitrogen ligand and a suitable leaving group. If this group is reasonably non-labile, the drug may maintain its structural integrity in vivo long enough to show antitumour activity. A series of mononuclear and dinuclear palladium complexes has been investigated by different researchers including their cytotoxicity against a number of cancer cell lines. Some of the palladium compounds showed some anticancer activities but so far no promising candidate has emerged.

A large number of four and comparatively less number of five and six coordinated complexes have been reported [5]. Majority of divalent palladium complexes with coordination number four are all square planar or slightly distorted, which may be due to palladium(II) exhibits large crystal field splitting. Lott and Ramussen [6] have reported few palladium(II)
complexes which are having tetrahedral geometry. An excellent account of publications in the field of coordination compounds of palladium have been reviewed [7].

**LITERATURE REVIEW**

A thorough review of the literature relating to the chemistry of palladium particularly its complexes have been in the coordination chemistry review [8]. More recently reported palladium(II) complexes of different ligands and their structural studies are briefly summarized in the following paragraphs.

In this section a review of the palladium complexes that have been investigated for antitumour activity will be discussed. Palladium complexes based on 2-mercaptopyridines (MP) were synthesized and tested for their anticancer activity by Carrara et al. [9].

A general survey of chemical literature reveals that a large number of ligands containing nitrogen, oxygen or sulphur atoms have been reviewed. The review of some important ligands formed complexes with palladium are given in the following paragraphs. The ligands are dimethyl sulphoxide [10], ethyleneamine [11], 1,10-phenanthroline [12], salicylaldoxime [13], 2-(2’-thienyl)-pyridine [14], 4-benzylamide thiosemicarbazide [15], 2-hydroxy-1-naphthaldioxide [16], 1-(3-pyridyl)-1,3-butanedione [17]. The large number of Pd(II) complexes of various ligands have been reviewed [18, 19].

Delima et al. [20] reported the complexes of palladium(II) with 1, 1, 1-bis(diphenylphosphine) ferrocene. The crystal structure of these complexes is described. The complexes of O-vanillinsemicarbazone with Pd(II) have been reported by Hingorani et al. [21]. The isolation and structure of Pd(II) complex of 2-acetylpyridine semicarbazone has been investigated [22].

Naz Aghatabay et al. [23] prepared the novel Pd(II) complexes containing the ligand 1,6-bis(benzimidazole-2-yl)-3,4-dithiohexane and characterized by Raman, FT-IR and NMR spectroscopic studies. The prepared Pd(II) complexes were evaluated for antimicrobial activity. The spectroscopic results were in good agreement accordance with the square-planar geometry.

Adnan Abu-Surrah et al. [24] have been described the synthesis and crystal structure of enantiomerically pure chiral Pd(II) complex with trans-bis {endo-(1R)-1,7,7-
trimethylbicyclo[2,2,1]-heptan-2-amino} ligand. The solid state structure of the complex was determined by X-ray structure analysis. The compound crystallizes in the monoclinic space group.

Jinchao Zhang et al. [25] have reported the synthesis and cytotoxicity of mixed-ligand complexes of palladium(II) with aromatic diimine and 4-toluene sulfonyl-L-amino acid dianion. The complex was characterized by crystal studies. 1,10-phenanthroline and 2,2′-bipyridine were used as bidentate ligands in the mixed-ligand Pd(II) complexes.

Antitubercular activities of palladium and platinum complexes with fluoroquinolones as ligand have been discussed by Ligia Maria et al. [26]. They report synthesis, DNA binding and cytotoxicity of Pd(II) and Pt(II) complexes of 2,9-dimethyl-1,10-phenanthroline ligand. The novel Pd(II) complexes acts as potential antitumour agent due to its unique interaction mode with DNA.

Kakul Husain et al. [27] have been reported the synthesis of new Pd(II) complexes of 1-N-substituted thiosemicarbazones of 3-indolecarboxaldehyde. The Pd(II) complexes were evaluated for antiamoebic activity against E. histolytica. The IR spectral results show that the thionesulphur and the azomethine nitrogen atom of the ligand are bonded to the metal ion. The biological evaluation shows that the Pd(II) complexes endowed with important antiamoebic properties.

The above literature survey revealed that no attempt has been made for the synthesis of palladium(II) complexes with studied ligands, 3-(2-hydroxybenzylideneamino)-2-methylquinazolin-4(3H)-one (L₁), 2-methyl-3-(pyridine-2-ylmethyleneamino)quinazolin-4(3H)-one (L₂), 3-(2-hydroxy-3-methoxybenzylideneamino)-2-methylquinolin-4(3H)-one (L₃), 3-((5-ethylthiophene-2-yl)methyl-eneamino)-2-methylquinazolin-4(3H)-one (L₄). Therefore, in the present investigation the author has made an effort to synthesize and characterize Pd(II) complexes of the above cited Schiff base ligands (L₁-L₄). The synthesized complexes were characterized by analytical, physical and spectroscopic techniques. The antimicrobial activity of the synthesized Pd(II) complexes were evaluated against four bacteria namely Bacillus Subtilis, Escherichia coli and Staphylococcus aureus by disc diffusion method. The DNA binding studies
of the Pd(II) complexes were also performed with CT-DNA and nuclease activity with supercoiled DNA (pUC 19).

**EXPERIMENTAL**

**Materials and methods**

The solvents and chemicals used in this work were of Analar grade. Palladium chloride was obtained from Sigma-Aldrich (USA). All materials were of highest purity and used without further purification. Solvents employed were of 99% purity. The CT-DNA was purchased from Genie, Bangalore. Tris buffer, sodium chloride and hydrochloric acid (AR) were purchased from Merck.

**PROCEDURES**

**Synthesis of palladium(II) complexes [1-4]**

All Pd(II) complexes were prepared from chloride salts of palladium(II) in 1:1 ratio using synthesized Schiff base ligands (L<sub>1</sub>-L<sub>4</sub>).

A volume of 20 mL of methanolic solution of Schiff base ligand was added to a 20 mL hot methanolic solution of palladium chloride (0.340 g, 2 mmol) to obtain 1:1. The resulting mixture was stirred under reflux for 3 h where upon the complexes were precipitated. They were collected by filtration, washed with hot water, then diethyl ether and dried in air. The analytical and physical data were reported in Table 1.

**CHARACTERIZATION**

The synthesized Pd(II) complexes were characterized by various spectral techniques. Infrared spectra were recorded in the range 4000-200 cm<sup>-1</sup> on a JASCO FTIR-8400 spectrophotometer using Nujol mull. The electronic spectra of the complexes were recorded in DMSO using HITACHI-3900 spectrophotometer. The <sup>1</sup>H-NMR spectra of the Pd(II) complexes were recorded in DMSO-d<sub>6</sub> solution. The mass spectra of the Pd(II) complexes were determined on Varian 1200L model mass spectrometer. All these spectral techniques are discussed in results and discussion section.
BIOLOGY

Antimicrobial studies

Antibacterial and antifungal screening of the Pd(II)-Schiff base complexes (1-4) and free ligands (L₁–L₄) were carried out using disc diffusion technique. The detailed procedures are presented in Chapter II. In this study, the ligands and their Pd(II) complexes are subjected to antibacterial activity against B. subtilis, E.coli and S. aureus.

DNA binding and cleavage experiments

Electronic absorption spectroscopy is one of the most useful techniques for studying binding mode of metal complexes to DNA. The DNA binding experiments were carried out in Tris-HCl/NaCl buffer (pH 7.2). The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M⁻¹cm⁻¹) at 260 nm [28]. Absorption titration experiments were performed by varying the concentration of the DNA with the complex. All UV–Vis. spectra were recorded after equilibration.

The cleavage efficiency of the Pd(II) complexes compared to that of the control is due to their efficient DNA–binding ability. In the present study, the CT-DNA gel electrophoresis experiment was conducted at room temperature using the synthesized complex in the presence of H₂O₂ as an oxidant. The detailed procedures of DNA interaction experiments were discussed in Chapter III.

RESULTS AND DISCUSSION

The complexes [PdCl₂(L)] (where L= Schiff base ligands [L₁-L₄]) were obtained by reaction between the respective ligand and the palladium salt in methanolic medium. The desired product is immediately formed as an amorphous precipitate upon addition of the ligand to the palladium salt.

The elemental analysis data were in good agreement with the proposed formulae for the complexes (Table 1). The reactions involved in the formation of palladium complexes are well-
known substitution reactions of square-planar complexes which are favored by the trans-effect of the chloride ligands. The mass spectra of the complex (I) have molecular peaks; moreover, the peaks also provide information for the suggested composition and structure of the complex.

**Table 1: Elemental analysis of Pd(II) complexes.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular formula</th>
<th>Yield (%)</th>
<th>Found (calcd) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>C_{16}H_{13}N_{3}O_{2}PdCl_{2}</td>
<td>61</td>
<td>42.10 (42.31)</td>
</tr>
<tr>
<td>2</td>
<td>C_{17}H_{12}N_{4}O_{2}PdCl_{2}</td>
<td>67</td>
<td>40.90 (41.12)</td>
</tr>
<tr>
<td>3</td>
<td>C_{17}H_{15}N_{3}O_{2}PdCl_{2}</td>
<td>63</td>
<td>41.97 (42.09)</td>
</tr>
<tr>
<td>4</td>
<td>C_{16}H_{15}N_{3}OSPdCl_{2}</td>
<td>69</td>
<td>40.67 (40.86)</td>
</tr>
</tbody>
</table>

**Infrared spectroscopy**

The important IR bands of Pd(II) complexes and Schiff bases are listed in Table 2. The IR bands in the region 1675-1657 and 1598-1540 cm\(^{-1}\) in the Schiff bases are attributed to the \(\nu(C=O)\) and \(\nu(C=N)\), respectively. In the complexes 1-4, these bands are shifted to lower frequencies at around 1656-1647 and 1602-1595 cm\(^{-1}\), indicating the coordination of imine nitrogen and lactam oxygen atom to the metal ion, respectively [29]. In the IR spectra of complexes 1 and 3, a very broad band at ca. 3434 and 3414 cm\(^{-1}\) were observed which is due to the OH group present in the Schiff bases L\(_1\) and L\(_3\), respectively, revealing that the phenolic oxygen is not involved in the coordination [30]. The IR spectra of complexes 1 and 4 with their respective ligands are shown in Figures 1 and 2.

**Table 2: The important infrared frequencies (in cm\(^{-1}\)) of Pd(II) complexes.**

<table>
<thead>
<tr>
<th>compound</th>
<th>(\nu(C=O))</th>
<th>(\nu(C=N))</th>
<th>(\nu(M-N))</th>
<th>(\nu(M-O))</th>
</tr>
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<tbody>
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<td></td>
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<td></td>
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<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>L1</td>
<td>1603</td>
<td>1603</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L2</td>
<td>1597</td>
<td>1684</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L3</td>
<td>1586</td>
<td>1650</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L4</td>
<td>1574</td>
<td>1656</td>
<td>-</td>
<td>-</td>
</tr>
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<td>1</td>
<td>1647</td>
<td>1573</td>
<td>443</td>
<td>404</td>
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<td>2</td>
<td>1653</td>
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<td>3</td>
<td>1656</td>
<td>1540</td>
<td>423</td>
<td>411</td>
</tr>
<tr>
<td>4</td>
<td>1651</td>
<td>1565</td>
<td>438</td>
<td>406</td>
</tr>
</tbody>
</table>
Figure 1: IR spectra of (a) L₁ (b) its complex 1.
Figure 2: IR spectra of (a) \( L_4 \) (b) its complex 4.
**1H-NMR spectra**

The 1H-NMR spectra of Pd(II) complexes were recorded in DMSO-d$_6$ support the proposed structure of the compounds. Representative 1H-NMR spectrum of complex 3 is showed in Figure 3. The signal due to azomethine proton in ligands around 9.22-8.25 ppm shows a downfield shift ca. 9.74-8.39 ppm [32] in the spectra of complexes suggesting the coordination of the azomethine nitrogen to the metal ion. This downfield shift is due to desheilding of the =CH proton. The signal due to the phenolic OH group at 10.03 and 10.56 ppm in L$_1$ and L$_3$ was remained unchanged in the complexes 1 and 3, indicating the non-involvement of oxygen atom in the coordination. In ligands, the multiplet ca. 7.87-7.04 ppm is due to the aromatic protons and signals around 2.77-2.50 ppm due to the methyl protons and is unaffected in the case of 1H-NMR spectra of complexes 1-4.

**Figure 3: 1H-NMR spectrum of complex 3.**

**Mass spectra**

The characteristic peaks are observed within the mass spectrum of complex 3 is summarized in Scheme 1, and representative mass spectrum of complex 3 is depicted in Figure
3. The mass spectra exhibits molecular ion peaks and contains fragments that confirm the structures of all the complexes [33]. The mass spectra of the metal complexes show molecular ion peaks (M + 1) confirming their molecular weights and their fragmentation pathways can be initiated by loss of the two chloride ions giving (M - Cl₂).

![Mass spectrum of complex 3 (C₁₇H₁₅N₃O₃PdCl₂).](image)

**Figure 3:** Mass spectrum of complex 3 (C₁₇H₁₅N₃O₃PdCl₂).

**Thermal studies**

The thermal decomposition behavior of complexes along with the % weight loss at different temperatures is recorded under N₂ atmosphere. Figure 4 shows the TG and DTG curves of complex 3. From figure 4, it is clear that the complex do not lose weight up to 310 °C, indicating the absence of coordinated or lattice water molecules and the same result was confirmed by spectral studies. Further rise in the temperature causes decomposition of complex in two steps. The first decomposition spans from 290-320 °C through 380-400 °C corresponds to the liberation of chloride ion as hydrochloride [34]. The second decomposition step starts at 400-430 °C and terminates at about 490-520 °C, corresponding to the decomposition of Schiff base ligand leaving behind metal oxide as the end product [35].
**Figure 4:** TGA and DTG curves of complex 3 ($C_{17}H_{15}N_3O_3PdCl_2$).

**Table 3:** Stepwise Thermal Degradation Data obtained from TGA Curves and their Composition

<table>
<thead>
<tr>
<th>Complex</th>
<th>Process</th>
<th>Temp. range (°C)</th>
<th>Products</th>
<th>% Weight loss</th>
<th>% Residue</th>
<th>No. of moles</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{16}H_{13}N_3O_2PdCl_2$</td>
<td>I</td>
<td>300-360</td>
<td>2HCl</td>
<td>15.78</td>
<td>15.52</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>470-510</td>
<td>$C_{16}H_{15}N_3O_2$</td>
<td>61.14</td>
<td>60.86</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$C_{15}H_{12}N_4O_2PdCl_2$</td>
<td>I</td>
<td>290-350</td>
<td>2HCl</td>
<td>16.36</td>
<td>16.12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>460-520</td>
<td>$C_{15}H_{12}N_4O_2$</td>
<td>60.00</td>
<td>59.49</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$C_{17}H_{15}N_3O_3PdCl_2$</td>
<td>I</td>
<td>310-390</td>
<td>2HCl</td>
<td>14.40</td>
<td>13.91</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>450-500</td>
<td>$C_{17}H_{15}N_3O_3$</td>
<td>63.78</td>
<td>62.69</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$C_{16}H_{15}N_3OSPdCl_2$</td>
<td>I</td>
<td>280-370</td>
<td>2HCl</td>
<td>14.83</td>
<td>14.18</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>470-520</td>
<td>$C_{16}H_{15}N_3OS$</td>
<td>62.71</td>
<td>61.93</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
DNA interaction studies

UV absorption experiments

The absorption spectral traces of the complexes with increasing concentration of CT-DNA were performed. Figure 5a and 5b shows the variation of absorption spectra of complexes 2 and 3. As the concentration of DNA increased, the absorption band of complexes at 272-263 nm exhibits hypochromicity and red-shift. In general, hypochromism and red-shift are associated with the intercalative binding of the complex to the double helix, due to the strong stacking interactions between the aromatic chromophore of the complex and the base pair of DNA [36]. This causes the decreases of 45-50 % in the intensity of the charge transfer band [37]. The intrinsic binding constants ($K_b$) of complexes 2 and 3 were found to be $2.36\times10^3$ and $2.21\times10^3$ M$^{-1}$, respectively. The results indicate the binding obtained here are lower than that reported for classical intercalator [38]. Thus, complexes are weak binders and they bind in an intercalative stacking manner to the double helix [39].

![Figure 5: Absorption spectra of (a) complex 2 and (b) complex 3, in Tris-HCl buffer upon addition of DNA= 1x10^{-4} M,0-25 µl. Arrow shows the absorbance changing upon increasing the concentration of DNA.](image)

Viscosity measurements
In order to confirm the interactions between the prepared complexes and DNA, viscosity measurements were carried out in Tris-buffer solution. A stacking intercalation model results in the lengthening the DNA helix as base pairs were separated to accommodate the complex, leading to the increase of DNA viscosity. The effects of complexes 2 and 3 on the viscosity of CT-DNA at 25 °C are shown in Figure 6. Viscosity experimental results clearly show that Pd(II) complex can stack between adjacent DNA base pairs, causing an extension in the helix, and increase the viscosity of DNA.

![Graph](image)

**Figure 6:** Effect of increasing amount of complexes 2 and 3 on the relative viscosity of CT-DNA.

**Nuclease activity**

The degree to which the four complexes could function as DNA cleavage agents was examined using supercoiled pUC 19 plasmid DNA as the target. The efficiency of cleavage of these molecules was probed using agarose gel electrophoresis [40]. Complexes 2 and 3 were found to promote the cleavage of pUC 19 plasmid DNA from supercoiled Form (I) to the nicked Form (II) (Figure 7). A little DNA-cleavage was observed for the control in which metal complex was absent. The complexes can induce the obvious cleavage of the plasmid DNA at the concentration of $10^{-3}$M in the presence and absence of an oxidant ($H_2O_2$). The different DNA-cleavage efficiency of the complexes may be due to the different binding affinity of the
complexes to DNA. The similar observations have also been reported for the other complexes [41-43].

![Image of agarose gel electrophoresis](image)

**Figure 7**: Cleavage of supercoiled pUC19 DNA (0.5 µg) by the Pd(II) complexes 2 and 3 in a buffer containing 50 mMTris-HCl at 37 °C (30 min): lane M: marker; lane 1: DNA control; lane 2: complex 2 (10⁻³ M) + DNA; lane 3: complex 2 (10⁻³ M) + DNA + H₂O₂; lane 4: complex 3 (10⁻³ M) + DNA; lane 5: complex 3 (10⁻³ M) + DNA + H₂O₂.

**CONCLUSION**

In conclusion, the synthesis and characterization of four Pd(II) complexes are presented in this chapter. The molar conductance of the prepared Pd(II) complexes were in the range of 13-27 S cm² mol⁻¹ indicating the non-electrolytic nature of the complexes. The interaction of Pd(II) complexes with CT-DNA was confirmed by an electronic absorption and viscosity studies. The complexes have ability of interaction with DNA through stacking mode. The capability of cleavage of pUC 19 DNA by the complexes was investigated by agarose gel electrophoresis, indicating that the DNA cleaving ability of Pd(II) was weak in the absence of an oxidant, while a slightly stronger cleavage ability was found in the presence of an oxidant. Thus, DNA interaction experiments reveal that the Pd(II) complexes can be used as potential antitumor agent due its unique mode of interaction [44].
Based on the data obtained from the various physico-chemical technique, following structures (Figure 8) were proposed for the Pd(II) complexes (1-4).

Figure 8: Structures of Pd(II) complexes 1-4.
REFERENCES


