CHAPTER 2

Arene ruthenium \{1, 3, 5-tris(di-2-pyridylaminomethyl)benzene\} complexes: Synthesis, structural and in vitro functional characterization

2.1 Introduction
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Graphical abstract

Mono-, di- and tri-nuclear half sandwich ruthenium complexes with 1,3,5-tris(di-2-pyridylaminomethyl) benzene have been synthesized, characterized and their activity over four pathogenic bacteria and four cancer lines have been assessed. Antibacterial activity against four bacteria viz *Staphylococcus aureus* MTCC96; *Escherichia coli* MTCC739; *Klebsiella pneumonia* MTCC2653 and *Pseudomonas aeruginosa* MTCC2453. Antiproliferative activity against four cancer cell lines viz *B16F10* (Mouse melanoma carcinoma), *THP-1* (Human acute monocytic leukemia), *PC3* (Human prostate carcinoma) and *SK-OV-3* (Human ovarian carcinoma).

Abstract

Ruthenium based complexes are growing as promising formulations for the cure of cancer with their distinct activity and less side effects. In the present study, a series of mono, di and trinuclear novel octahedral arene ruthenium complexes involving 1, 3, 5-tris(di-2-pyridylaminomethyl)-benzene (L) as a nitrogen donor ligand have been prepared by the reaction of [(arene)RuCl₂]₂ dimer and fully characterized by spectroscopic studies. The complexes 1-6 have the general compositions [{(benzene)RuCl₁₈L}ⁿ⁺ where n = 1 (1), n = 2 (2), n = 3 (3), [{(p-cymene)RuCl₁₈L}ⁿ⁺ where n = 1 (4), n = 2 (5) and n = 3 (6). The X-ray structure of [(p-cymene)RuLCl]⁺ (4) revealed a distorted octahedral coordination in the vicinity of the central metal atom. Antibacterial and the cytotoxicity characteristics of these complexes have been evaluated by the zone inhibition and anti-proliferative studies. Antibacterial studies evidenced highest zone of inhibition (17 nm) for Staphylococcus aureus and Escherichia coli and cytotoxicity experiments revealed more selectivity towards Carcinoma cell lines than the leukemia cell lines.

Key words: Arene ruthenium, antibacterial, Metal based drugs, cytotoxicity, MTT assay, Cancer
2.1. Introduction

World’s one of the major causes of death is cancer, which makes the body with an uncontrollable increment of diseased cells, those are distinctly different from healthy cells through their redox metabolism [1-3]. Such property was selectively controlled by one of the metal based drugs cis-platin in the 1960’s [4, 5]. Though huge number of metal complexes has been synthesized to fight against cancer, only few of them are in successful clinical trials. Among them, ruthenium based complexes are one of the metal based drugs, which are exhibiting distinct activity with less side effects than their platinum group (ruthenium, rhodium, palladium, osmium, iridium and platinum) counterparts [6-8].

Ruthenium, which belongs to early d-block iron group metal, which can mimic iron in its properties except existing in higher oxidation state of Ru(IV), where it is stabilized as RuO₂. The other oxidation states Ru(II) and Ru(III) are progressing their own activities under physiological conditions. One of the feasible coordination’s of ruthenium is six either in Ru(II) or in Ru(III), such a high coordination can tune the biological properties like binding to DNA, proteins and enzymes in human body [9-12]. The most transport proteins contain in the human blood are albumin at 40 mg per ml [13] and transferrin at 3.0 mg per ml [14], which are most welcoming partners for the metal based drugs when those are administered in human body [15, 16]. NAMI-A is the first ruthenium compound used in clinical practice (since 1999), which exhibits good amount of activity on lung carcinoma cell lines such activity makes the compound used as second line chemotherapy drug in lung metastases [17]. KP1019, is another ruthenium compound used in the clinical trials (since 2003) due to its low toxicity and high degree of tumor selectivity against cis-platin resistant tumors.

Besides this, half sandwich arene ruthenium complexes [Ru(arene)(X)(L-L)] another class of ruthenium complexes, which resemble like piano stool geometry. Among them RM 175 and series of RAPTA complexes are showing better antitumor and anti-metastatic properties and are standing at an advanced preclinical state [18, 19]. The activity of these complexes depends on three structural variations, those are arene, which occupies three coordination of metal as a seat of piano stool, X mostly chloride ion and lastly L-L either mono-dentate or bi-dentate as legs of the stool. Changing L-L can enhance the biological activity of the arene ruthenium complexes [20-24].

In this study, six new arene ruthenium complexes containing 1, 3, 5-tris (di-2-pyridyl aminomethyl) benzene ligand (L) were synthesized and assessed their biological properties, such as antibacterial on four human pathogenic bacteria by agar well diffusion method and
antiproliferative activity on four cancerous cell lines (three human and one mice cell lines) by MTT assay.

### 2.2. Experimental Section

#### 2.2.1. Materials and methods

Ligand L, was prepared according to the literature methods and ligand was purified by flash column chromatography [25].

#### 2.2.2. General procedure for synthesis of complexes 1-6

**Synthesis of complexes 1-3**

A mixture of [(benzene)RuCl₂]₂, ligand L and NH₄BF₄ with the corresponding ratio was stirred in dry DCM and methanol (1:1) (30 ml) at room temperature for 6 to 12 h during which yellow precipitate had formed, resulting precipitate was then filtered and washed with cold methanol followed by diethylether and dried in a vacuum. This precipitate is soluble in acetonitrile, DMSO, DMF and partially soluble in solvents such as DCM, acetone, methanol, but they are insoluble in non-polar solvents such as hexane and diethyl ether.

**2.2.2.1. [(benzene)RuClL]BF₄ (1)**

Ratio of metal precursor, ligand and ammonium tetrafluoroborate (1:2:2); for 6 h; Yield: 46%; Elemental Anal (%) Calc. for C₄₅H₃ₙBClF₄N₉Ru: C 58.19; H 4.23; N 13.57; found C 59.06; H 4.47; N 13.74; IR (KBr pellets, cm⁻¹): 1595 (νC=Ĉ), 1468 (νC=Ĉ), 1084 (νB–F); ¹H NMR (400 MHz, DMSO-d₆): δ 8.54 (d, J = 5.6 Hz, 2H), 8.24 (d, J = 4.4 Hz, 4H), 7.77 (t, J = 7.4 Hz, 2H), 7.44 (t, J = 7.4 Hz, 4H), 7.37 (s, 1H), 7.33 (s, 2H), 7.25 (t, J = 6.3 Hz, 2H), 7.17 (t, J = 6.3 Hz, 4H), 6.93 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.2 Hz, 4H), 6.25 (s, 6H), 5.38 (s, 4H), 5.31 (s, 2H); Molar conductivity ([^M, 10⁻³ M, CH₃CN]: 118 S cm² mol⁻¹.

**2.2.2.2. [{(benzene)RuCl₂L}₂(BF₄)₂ (2)**

(1:1:2); 10 h; Yield: 84%; Elemental Anal (%) Calc. for C₅₁H₅₂B₂Cl₂F₈N₉Ru₂: C 49.80; H 3.69; N 10.25; found C 49.06; H 3.47; N 10.74; IR (KBr pellets, cm⁻¹): 1596 (νC=Ĉ), 1467 (νC=Ĉ), 1084 (νB–F); ¹H NMR (400 MHz, DMSO-d₆): δ 8.69 (d, J = 4.2 Hz, 4H), 8.59 (d, J = 5.0 Hz, 2H), 7.95 (t, J = 7.5 Hz, 2H), 7.77 (t, J = 7.4 Hz, 4H), 7.36 (d, J = 8.3 Hz, 4H), 7.30 (s, 1H), 7.17 (d, J = 6.7 Hz, 2H), 7.15 (s, 2H), 6.77 (t, J = 6.6 Hz, 4H), 6.59 (t, J = 6.6 Hz, 2H), 6.35 (s, 12H), 5.48 (s, 4H), 5.31 (s, 2H); Molar conductivity ([^M, 10⁻³ M, CH₃CN]: 198 S cm² mol⁻¹.
2.2.2.3. \[\text{[(benzene)RuCl}_3L](BF_4)_3\] (3)

(1.5:1:2); overnight; Yield: 91%; Elemental Anal (%) Calc. for \(\text{C}_{57}\text{H}_{51}\text{B}_3\text{Cl}_3\text{F}_12\text{N}_9\text{Ru}_3\): C 44.71; H 3.36; N 8.23; found C 44.06; H 3.47; N 8.44; IR (KBr pellets, cm\(^{-1}\)): 1597 (\(\nu_{\text{C═C}}\)), 1465 (\(\nu_{\text{C═N}}\)), 1084 (\(\nu_{\text{B═F}}\)); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 8.64 (d, \(J = 5.7\) Hz, 6H), 7.89 (t, \(J = 7.3\) Hz, 6H), 7.33 (d, \(J = 8.5\) Hz, 6H), 7.21 (s, 3H), 7.04 (t, \(J = 6.6\) Hz, 6H), 6.25 (s, 18H), 5.46 (s, 6H); Molar conductivity (\(\lambda_M\), \(10^{-3}\) M, CH\(_3\)CN): 330 S cm\(^2\) mol\(^{-1}\).

Synthesis of complexes 4-6

A mixture of \([\text{(p-cymene)RuCl}_2]\), ligand L and NH\(_4\)BF\(_4\) with the corresponding ratio was stirred in dry DCM and methanol (1:1) (30 ml) at room temperature for 6 to 12 h during which the color of the solution changed from yellow to orange. The solvent was removed under vacuum and the residue was dissolved in dichloromethane (10 ml) and the solution was filtered through the bed of celite to remove ammonium chloride and excess salts. The orange solution was concentrated to ~ 2-3 ml and the addition of diethylether gave the complexes as orange-yellow precipitate, which was separated and dried under vacuum. They are soluble in polar organic solvents such as DCM, acetone, methanol and acetonitrile but they are insoluble in non-polar solvents such as hexane and diethyl ether.

2.2.2.4. \[\text{[(p-cymene)RuClL](BF_4)_2}\] (4)

(1:2:2); 6 h; Yield: 47%; Elemental Anal (%) Calc. for \(\text{C}_{49}\text{H}_{47}\text{BCl}_2\text{F}_8\text{N}_9\text{Ru}:\) C 59.75; H 4.81; N 12.80; found C 59.06; H 4.47; N 12.74; IR (KBr pellets, cm\(^{-1}\)): 1598 (\(\nu_{\text{C═C}}\)), 1468 (\(\nu_{\text{C═N}}\)), 1084 (\(\nu_{\text{B═F}}\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.66 (d, \(J = 5.7\) Hz, 2H), 8.63 (d, \(J = 4.6\) Hz, 4H), 7.75 (t, \(J = 7.8\) Hz, 2H), 7.46 (t, \(J = 7.8\) Hz, 4H), 7.39 (s, 1H), 7.31 (s, 2H), 7.15 (t, \(J = 6.5\) Hz, 2H), 7.07 (t, \(J = 6.5\) Hz, 4H), 6.98 (d, \(J = 8.6\) Hz, 2H), 6.87 (d, \(J = 8.4\) Hz, 4H), 5.61 (d, \(J = 5.9\) Hz, 2H), 5.38 (s, 4H), 5.34 (d, \(J = 4.8\) Hz, 2H), 5.31 (s, 2H), 2.68 (sep, 1H), 1.72 (s, 3H), 1.21 (d, \(J = 6.9\) Hz, 6H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 157.01, 156.76, 156.42, 156.12, 155.83, 153.81, 153.57, 148.09, 148.02, 147.86, 141.74, 141.09, 140.88, 140.17, 139.43, 137.56, 137.39, 137.13, 135.83, 134.01, 125.87, 125.43, 123.57, 120.85, 120.31, 117.25, 117.01, 116.19, 115.82, 114.61, 114.32, 114.06, 106.00, 105.89, 100.53, 100.11, 85.76, 85.59, 84.08, 83.81, 77.42, 77.11, 76.79, 56.02, 55.21, 51.25, 50.87, 30.61, 22.27, 17.69; Molar conductivity (\(\lambda_M\), \(10^{-3}\) M, CH\(_3\)CN): 125 S cm\(^2\) mol\(^{-1}\).

2.2.2.5. \[\text{[(p-cymene)RuCl}_2L](BF_4)_2\] (5)

(1:1:2); 10 h; Yield: 83%; Elemental Anal (%) Calc. for \(\text{C}_{59}\text{H}_{61}\text{B}_2\text{Cl}_2\text{F}_8\text{N}_9\text{Ru}_2\): C 52.60; H 4.58; N 9.39; found C 52.16; H 4.47; N 9.24; IR (KBr pellets, cm\(^{-1}\)): 1599 (\(\nu_{\text{C═C}}\)), 1467
\( ^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.67 (d, \( J = 4.4 \) Hz, 4H), 8.63 (d, \( J = 5.0 \) Hz, 2H), 7.92 (t, \( J = 7.8 \) Hz, 2H), 7.76 (t, \( J = 7.7 \) Hz, 4H), 7.37 (d, \( J = 8.6 \) Hz, 4H), 7.30 (s, 1H), 7.16 (d, \( J = 6.7 \) Hz, 2H), 7.14 (s, 2H), 6.75 (t, \( J = 6.8 \) Hz, 4H), 6.66 (t, \( J = 6.8 \) Hz, 2H), 5.67 (d, \( J = 5.6 \) Hz, 4H), 5.48 (s, 4H), 5.46 (d, \( J = 4.6 \) Hz, 4H), 5.31 (s, 2H), 2.72 (sep, 2H), 1.73 (s, 6H), 1.23 (d, \( J = 6.9 \) Hz, 12H); 13\(^{C}\) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 156.74, 156.40, 156.09, 155.74, 153.92, 153.78, 153.49, 148.07, 147.85, 141.76, 140.86, 140.13, 137.54, 137.37, 136.86, 135.83, 125.77, 123.54, 120.82, 120.26, 117.33, 117.23, 116.18, 115.84, 114.30, 114.03, 106.23, 105.87, 100.53, 100.31, 85.75, 85.44, 83.96, 83.79, 77.38, 77.06, 76.74, 55.23, 50.63, 30.53, 22.28, 17.87, 17.68; Molar conductivity (\( \Lambda_M \), 10\(^{-3} \) M, CH\(_3\)CN): 223 S cm\(^2\) mol\(^{-1}\).

2.2.2.6. \([(p\text{-}cymene)RuCl]_2L\)(BF\(_4\))\(_3\) (6)

(1.5:1:2); overnight; Yield: 92%; Elemental Anal (%) Calc. for C\(_{69}\)H\(_{75}\)B\(_3\)Cl\(_3\)F\(_{12}\)N\(_9\)Ru\(_3\): C 48.76; H 4.45; N 7.42; found C 49.06; H 4.47; N 7.74; IR (KBr pellets, cm\(^{-1}\)): 1600 (\( \nu \)C\(=\)C), 1466 (\( \nu \)C\(=\)N), 1084 (\( \nu \)B\(\cdots\)F); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.63 (d, \( J = 5.8 \) Hz, 6H), 7.91 (t, \( J = 7.2 \) Hz, 6H), 7.37 (d, \( J = 8.5 \) Hz, 6H), 7.20 (s, 3H), 7.16 (t, \( J = 6.6 \) Hz, 6H), 5.63 (d, \( J = 6.0 \) Hz, 6H), 5.46 (s, 6H), 5.42 (d, \( J = 6.1 \) Hz, 6H), 2.74 (sep, 3H), 1.79 (s, 9H), 1.24 (d, \( J = 6.9 \) Hz, 18H); 13\(^{C}\) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 160.35, 158.63, 146.66, 141.44, 128.27, 125.39, 120.92, 111.39, 104.73, 89.68, 89.09, 61.44, 44.77, 35.39, 26.94, 22.66; Molar conductivity (\( \Lambda_M \), 10\(^{-3} \) M, CH\(_3\)CN): 325 S cm\(^2\) mol\(^{-1}\).

2.3. RESULTS AND DISCUSSION

2.3.1. Synthesis

The di-nuclear arene ruthenium precursors [(arene)RuCl\(_2\)]\(_2\) (arene = benzene, \( p\)-cymene) react with two equivalents of ligand in 1:1 ratio of DCM and methanol, being stirred at room temperature in the presence of NH\(_4\)BF\(_4\) to form the mononuclear cationic complexes \([(\text{benzene})\text{RuCl}]L\)^+ (1) and \([(\text{p\text{-}cymene})\text{RuCl}]L\)^+ (4), similarly di-nuclear cationic complexes \([(\text{benzene})\text{RuCl}]_2L\)^2+ (2) and \([(\text{p\text{-}cymene})\text{RuCl}]_2L\)^2+ (5) and trinuclear cationic complexes \([(\text{benzene})\text{RuCl}]_3L\)^3+ (3) and \([(\text{p\text{-}cymene})\text{RuCl}]_3L\)^3+ (6) have been obtained with one and 0.5 equivalents of ligand respectively at room temperature (Scheme 2.1). Benzene contained complexes are yellow and \( p\)-cymene complexes are orange yellow color, non-hygrosopic and air stable solids. All complexes are soluble in acetonitrile, but complexes 1-3 are partially soluble in dichloromethane, chloroform and acetone whereas
complexes 4-6 are highly soluble in polar organic solvents like dichloromethane, chloroform and acetone. All these complexes are insoluble in water, diethyl ether and non-polar solvents.

2.3.2. Characterization of the cationic complexes 1-6

The infrared spectra of the complexes 1-6 exhibit a strong band in the region 1084 cm\(^{-1}\) for a typical \(\nu_{\text{B-F}}\) stretching frequency for the BF\(_4\) anion. Moreover, all complexes show peaks around \(\sim1600\) and \(1466\) cm\(^{-1}\) corresponding to \(\nu_{\text{C=C}}\) and \(\nu_{\text{C=N}}\) vibrations respectively. Molar conductivity measurements of these ionic complexes in acetonitrile displayed a typical 1:1 electrolyte for complexes 1 and 4, 1:2 electrolytes for complexes 2 and 5 and 1:3 electrolytes for complexes 3 and 6 [26].

The \(^1\)H NMR spectrum of free ligand exhibits a characteristic set of six resonances at 8.20 (d, 6H), 7.37 (t, 6H), 7.07 (s, 3H), 6.93 (d, 6H), 6.78 (t, 6H), 5.33(s, 6H)) ppm in the aromatic region for 33 protons in deuterated chloroform (CDCl\(_3\)) at room temperature. Upon coordination with metal atoms, the chemical shifts of aryl protons of the ligand (L) are \(\sim8.63\) (d), \(\sim7.91\) (t), \(\sim7.37\) (d), \(\sim7.20\) (s) and \(\sim7.16\) (t) ppm respectively. The \(^1\)H NMR spectra of the complexes (Figs. 2.1-2.2) protons exhibited downfield shift of aryl protons compared to the free ligand (L), such a shift suggests that metal precursors are bonded to the ligand (L). In

![Scheme 2.1: Schematic representation of the synthesis of complexes 1-6.](image)
addition to that arene ligand peaks also observed in downfield compared to their corresponding precursors (Figs. 2.1-2.2). Such a resonance shift is mainly due to the inductive effect produced by the ruthenium metal is regularly observed in those related half sandwich complexes [27, 28]. A proton, which gives doublet is adjacent to the pyridine nitrogen in the dipyridyl unit is the key proton, which also distinguishing the formation of monomer, dimer and trimer (Fig. 2.3). In the monomer (Fig. 2.3a) it exhibiting the two doublets ~ 8.60 ppm with an intensity of 1:2, which explains the ratio of bounded protons as two and unbounded protons as four, in the case of dimer (Fig. 2.3b) doublets intensity ratio is 2:1, where bounded protons are 4 and unbounded protons are 2 whereas in the case of trimer (Fig. 2.3c) there is only one doublet which explains all the three dipyridyl units are bounded to metal and is more downfield shifted compare to the free ligand. $^{13}$C NMR spectra

Fig. 2.1: $^1$H NMR spectrum of the complex 3

Fig. 2.2: $^1$H NMR spectrum of the complex 6
of the complexes are in good corroboration with the analytical formulations of the complexes. Peaks in the aliphatic region suggests that the methyl and methylene carbons of the p-cymene and methylene carbons of the ligand (L) of the complexes (Fig. 2.4).

**Fig. 2.3:** Splitting pattern of the $^1$H NMR spectrum of a proton (~ 8.63 ppm, Fig. 2.2), which is adjacent to the nitrogen in the pyridine ring of the ligand in monomer (a), dimer (b) and trimer (c) of the p-cymene contained complexes (4-6).

**Fig. 2.4:** $^{13}$C NMR spectrum of the complex 6. The multiplet peak (~ 82.5 ppm) belongs to the solvent

2.3.3. Molecular structure

The molecular structure of [{(p-cymene)RuCl}L]$^+$ (4) has been established by single crystal X-ray analysis as its tetrafluoroborate salt. Complex is crystallized by slow solvent diffusion method of hexane and acetone solutions. It crystalizes in the triclinic system with the P1 space group containing two molecules in each unit cell. The ORTEP diagram of the complex is shown as a rod model in Fig. 2.5, and the relevant crystallographic parameters are summarized in Table 2.1. The complex shows typical piano stool geometry around the metal
center, which is common for ruthenium half sandwich arene complexes. The metal is
coordinated by the spectator ligand \textit{i.e.}, \textit{p}-cymene, which occupies three coordinated sites, a
chloride and the chelating ligand (L). The geometry around the metal atom exhibited by the
complex is slightly distorted octahedral coordination arrangement with two cis nitrogen atoms
of the ligand acting as a bidentate chelating ligand through one of the dipyridyl moiety of the
1, 3, 5-tris(di-2-pyridylaminomethyl)benzene ligand. The ruthenium to nitrogen bond length
is close to 2.088 Å, while ruthenium to chloride bond length is 2.3929 Å. The greater bond
length of Ru-Cl suggests the lability of chloride ion than the nitrogen atoms of the ligand (L).
From the crystal structure, it was noticed that the isopropyl group of the \textit{p}-cymene ligand
opposite to the chloride ligand, where steric hindrance is playing much role than the hydrogen
bonding (Fig. 2.5). The distance between the ruthenium atom and centroid of the \textit{p}-cymene
ligand is 1.678 Å, which is almost identical with the arene ruthenium complexes [29]. The
N1-Ru-N2 and N1/N2-Ru-Cl bond angles of complex are found to be 80.1(1)°,
86.48(7)°/87.30(7)° respectively. All these bond lengths and angles are similar to those
observed dipyridyl complexes [29-31]. The solid state packing of the complex is exhibiting
two inter molecular hydrogen bonds in the unit cell as C-H(18)...Cl(1) and C-H(13)...N(5)
with bond lengths of 2.756 Å and 2.405 Å. The symmetric operations for the observed
hydrogen bonds is \(x, y, z\) and \(-x, -y, -z\), which are depicted in Fig. 2.6.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{complex.png}
\caption{A Schematic representation of X-ray crystal structure of complex 4, all hydrogens
and counter ions are omitted for clarity of the figure. Selected bond lengths (Å) and bond
angles (°). Bond lengths (Å): Ru1-N1 2.087(2), Ru1-N2 2.089(3), Ru1-Cl1 2.3929(9), Ru1-
centroid 1.678; Bond angles(°): N1-Ru1-N2 80.1(1), N1-Ru1-Cl1 86.48(7), N2-Ru1-Cl1
87.30(7).}
\end{figure}
Table 2.1: Data collection and structure refinement parameters for the ligand and complex 3.

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<th>Parameter</th>
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<td>Unique reflections</td>
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^a Structures were refined on $F_0^2$: $wR_2 = \left[ \Sigma \left( w(F_0^2 - F_c^2)^2 \right) \right]^{1/2} / \Sigma w(F_0^2)^2$, where $w^{-1} = \{ \Sigma(F_0^2) + (aP)^2 + bP \}$ and $P = \max(F_0^2, 0) + 2F_c^2)/3$.

Fig. 2.6: Packing diagram of complex 3, which is showing C-H (18)...Cl (1) and C-H (13)...N (5) hydrogen bonding interactions.
2.3.4. *UV-visible spectroscopy:*

UV-visible spectra of the complexes 1-6 were acquired in acetonitrile solution at 10 μM concentration in the range 200-800 nm. Electronic absorption spectra of the complexes are depicted in Fig. 2.7. Electronic absorption spectrum of ligand exhibits prominent and high intensity bands at around 303, 277 and 229 nm. The low energy bands at 303 and 277 nm and high energy band at 229 nm are tentatively assigned to n-π* and π-π* transitions respectively [32, 33]. All these ruthenium(II) complexes exist in low spin d<sup>6</sup> electronic configuration, such a symmetrically filled orbitals can interact with the low lying antibonding orbitals (π*) of the ligand, which leads the new band at around 396 nm, which has been tentatively assigned to metal to ligand (t<sub>2g</sub>-π*) charge transfer transition [34, 35]. In addition to this, an insignificant hypsochromic (Δλ, ~3 nm) shift is noted for the low energy band at 303 nm and a significant hypsochromic (Δλ, ~10 nm) shift is noted for the high-energy band at 229 nm of complexes 1-6 w.r.t ligand. Such changes are summarized in Table 2.2.

Table 2.2: UV-visible spectral data for complexes 1-6 in acetonitrile at 298K.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Complex</th>
<th>(\lambda_{max})(nm)/log ε 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[{(benzene)RuCl}L]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>396(2.1), 300(3.6), 253(3.6), 217(3.7)</td>
</tr>
<tr>
<td>2</td>
<td>[{(benzene)RuCl}&lt;sub&gt;2&lt;/sub&gt;L]&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>395(2.4), 301(3.6), 254(3.6), 217(3.7)</td>
</tr>
<tr>
<td>3</td>
<td>[{(benzene)RuCl}&lt;sub&gt;3&lt;/sub&gt;L]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>394(2.9), 302(3.8), 254(3.9), 220(4)</td>
</tr>
<tr>
<td>4</td>
<td>[{(p-cymene)RuCl}L]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>394(2.3), 301(3.6), 259(3.5), 218(3.7)</td>
</tr>
<tr>
<td>5</td>
<td>[{(p-cymene)RuCl}&lt;sub&gt;2&lt;/sub&gt;L]&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>395(2.8), 302(3.8), 259(3.8), 217(3.9)</td>
</tr>
<tr>
<td>6</td>
<td>[{(p-cymene)RuCl}&lt;sub&gt;3&lt;/sub&gt;L]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>396(2.9), 306(3.9), 258(4), 220(4.1)</td>
</tr>
<tr>
<td>7</td>
<td>Ligand-L</td>
<td>303(3.7), 277(3.8), 229(3.8)</td>
</tr>
</tbody>
</table>

Fig. 2.7: UV-Visible spectra of ligand and complexes 1-6.
2.3.5. Antibacterial activity

To explore the antibacterial potency of the synthesized complexes, *S. aureus*, *E. coli*, *K. pneumonia* and *P. aeruginosa* strains were used in the present study. The antibacterial properties of the complexes were examined using a size of the diameter of zones of inhibition against selected bacteria (Fig. 2.8), and the diameter of the zone is measured to the nearest millimeter. Data in Table 2.3 suggests that all the complexes shown different levels of inhibition against the tested human pathogenic bacteria. For comparison, the activity of gentamycin and amikacin recognized effective antibiotics against these bacteria, was also evaluated.

**Table 2.3**: Antibacterial activity of standards gentamycin, amikacin and complexes 1-6 against human pathogens *S. aureus*, *E. coli*, *K. pneumonia* and *P. aeruginosa*. Diameters of zone of inhibition are expressed in nearest millimeters with mean ±1 standard deviation from three independent experiments.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Gram-positive</th>
<th>Gram-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus a</td>
<td>E. coli b</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Amikacin</td>
<td>21</td>
<td>27</td>
</tr>
</tbody>
</table>

a *Staphylococcus aureus MTCC96*; b *Escherichia coli MTCC739*; c *Klebsiella pneumonia MTCC2653*; d *Pseudomonas aeruginosa MTCC2453*

The complexes are more active against the Gram-positive bacteria than the Gram-negative bacteria. Among the complexes, complex 2 (17 mm) on *S. aureus* and complex 5 (17 mm) on *E. coli* are noted as the highest zones of inhibition than the other strains. Interestingly, both these complexes are di-nuclear. The lowest zone of inhibition is noted on *P. aeruginosa* for
complex 6 (11 mm) (Fig. 2.9). In the present study, the selectivity of complexes on chosen bacterial strains is in following order

Complex 4 > Complex 5 > Complex 6 > Complex 3 > Complex 2 > Complex 1

**Fig. 2.8:** Zone inhibition obtained by the complexes 1-6 on *E. coli*

Complexes 4-6 are selective to the all-bacterial strains, which were used in the present study, whereas complexes 1-3 are resistant to the *P. aeruginosa*, where the benzene replaces *p*-cymene. Complex 4 has shown good activity on all the chosen bacterial strains among all the complexes, where neither highest nor least zone of inhibition was observed. *S. aureus* and *E. coli* are selectively inhibited by all the synthesized complexes. The selectivity of the strains to the complexes is found in the following order:

*S. aureus = E. coli > K. pneumonia > P. aeruginosa*

**Fig. 2.9:** Graphical representation of antibacterial activity (mm) of the two standards Gentamycin, amikacin and complexes 1-6 against pathogens used. Where, “*” represents the no zone of inhibition observed by the complexes.
2.3.6. Cytotoxicity in cancer cells

The cytotoxicity potency of the synthesized complexes was determined in three human cancer cell lines THP-1 (Acute monocytic leukemia), PC3 (Prostate carcinoma) and SK-OV-3 (Ovarian carcinoma) and one mice cancer cell line B16F10 (Mouse melanoma carcinoma) by means of the colorimetric MTT assay. Fluorouracil (5FU) and cisplatin, a potent anti-cancer drugs, was used as a standard comparative treatment. The IC$_{50}$ values (Table 2.4), the median cytotoxic concentrations, were determined after 24 h of drug exposure.

Cytotoxicity of the synthesized complexes was given by the IC$_{50}$ values, which can rationalize the structure-activity relationships of the synthesized complexes 1-6. In general, complex 4 was more sensitive to the all cell lines investigated in this study than the other complexes. The IC$_{50}$ value of the complexes varies within small ranges of micro molar concentrations in each cell line (Fig. 2.10). The lowest IC$_{50}$ value is noted in the PC3 cell line among the cell lines chosen. The PC3 cell line is more sensitive to the all complexes except in the case of complex 2 among the chosen cell lines, of course, all cell lines are resistant to complex 2, which are considered in the present study within the 100 micro molar range. For complexes 1 and 3, IC$_{50}$ values are found at ~ 50 micro molar concentrations on PC3 cell line, where cymene is replaced by benzene, is by less active than the cymene containing complexes 4 and 6 see Table 2.4. Among the tested complexes, complexes containing p-cymene i.e., 4-6 are more cytotoxic than the complexes containing benzene i.e., 1-3. Within the mono-, di-, and tri-nuclear complexes, mononuclear complexes have shown

Table 2.4: IC$_{50}$ values for fluorouracil and complexes 1-6 in B16F10, THP-1, PC3 and SK-OV-3 cancer cells after 24h drug exposure. Each value represents the mean ± standard deviation from three independent experiments.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>IC$_{50}$ value (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B16F10</td>
</tr>
<tr>
<td>1</td>
<td>75.2±3.7</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>25.1±2.5</td>
</tr>
<tr>
<td>5</td>
<td>48.3±2.9</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Positive control 5FU</td>
<td>79.0±1.6</td>
</tr>
<tr>
<td>cisplatin</td>
<td>7.39±0.31 $^a$</td>
</tr>
</tbody>
</table>

where IC$_{50}$ values correspond to the drug concentrations that inhibit growth of cells by 50%, for $^a$-$^d$ data taken from reference [36-39].
better cytotoxicity than the di and trinuclear complexes in the case of p-cymene, which was altered in the case of benzene contained complexes, which could be justified the phenomena of strong interaction by the glutathione, such a mechanism explained in the other half sandwich and platinum complexes [40, 41]. The complexes were exhibited lowest IC$_{50}$ values on the chosen carcinoma cell lines than the leukemia cell line, which concludes that complexes are more selective to the chosen carcinoma cell line and cell selectivity towards the p-cymene complexes are in the following order

$$\text{PC3} > \text{B16F10} > \text{SK-OV-3} > \text{THP-1}$$

**Fig. 2.10:** Cytotoxicity (IC$_{50}$) of the complexes 1-6 with the standard Fluorouracil against different cancer cell lines used. Where, “*” represents the complexes with no cytotoxicity at 100 μM.

### 2.4. Conclusions

In conclusion, the present chapter highlights the synthesis, characterization, antibacterial and antiproliferative activities of polynuclear arene ruthenium(II) complexes 1-6 derived from 1, 3, 5-tris(di-2-pyridylaminomethyl)benzene ligand. The complexes show moderately higher antibacterial activity against *S. aureus* (17 mm) and *E. coli* (17 mm) strains than *K. pneumonia* (14 mm) and *P. aeruginosa*. (11 mm). Among all the complexes, p-cymene contained complexes lead the in vitro activity, particularly complex 4. The complex shows reasonable in vitro anticancer activity and also shows moderate antibacterial activity against the pathogenic bacteria. Indeed, these complexes showed selectivity towards Carcinoma cell lines in comparison with the leukemia cell lines. Our results are of great help in order to understand the detailed mechanistic investigation and their activity against resistant strains, which extends the scope of further research.
2.5. References


