Antibacterial, *in vitro* antitumor activity and structural studies of rhodium and iridium complexes featuring the two positional isomers of pyridine carbaldehyde picolinic hydrazone ligand

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**Abstract** Half-sandwich organometallic rhodium and iridium complexes [1-6] have been synthesized with ligands L\textsubscript{1} (L\textsubscript{1} = Pyridin-2-ylmethylene picolinichydrazine) and L\textsubscript{2} (L\textsubscript{2} = Pyridin-3-ylmethylene picolinichydrazine). Treatment of [[Cp\textsuperscript{*}MCl\textsubscript{2}]]\textsubscript{2} (M = Rh/Ir) with L\textsubscript{1} in methanol has yielded mononuclear cationic complexes such as [[Cp\textsuperscript{*}M(L\textsubscript{1}N\textsubscript{1}N\textsubscript{2}Cl)]PF\textsubscript{6}] where \{M = Rh (1) and Ir (2)\} and dinuclear complexes such as [[(Cp\textsuperscript{*}MCl)\textsubscript{2}(L\textsubscript{1}N\textsubscript{1}N\textsubscript{2}N\textsubscript{3}N\textsubscript{4})]PF\textsubscript{6}] where \{M = Rh (3) and Ir (4)\} in 1:2 and 1:1 metal dimer to ligand ratios respectively. Reactions of [[Cp\textsuperscript{*}MCl\textsubscript{2}]]\textsubscript{2} with L\textsubscript{2} in both 1:2 and 1:1 metal dimer to ligand ratios have yielded two metalla-macrocyclic dinuclear and dicationic complexes such as [[Cp\textsuperscript{*}M(L\textsubscript{2}N\textsubscript{5}N\textsubscript{6}N\textsubscript{7}N\textsubscript{8}N\textsubscript{9})\textsubscript{2}]PF\textsubscript{6}] where \{M = Rh (5) and Ir (6)\}. Spectroscopic and crystallographic data were used to elucidate the structures of the synthesized complexes. The *in vitro* antitumor evaluation of the complexes 1 and 2 by fluorescence based
1. Introduction

There is an immense development in exploiting the medicinal properties of organometallic compounds in recent years (Hartinger et al., 2012). The study of the cytotoxic properties of pentamethylecyclopentadienyl (Cp*) rhodium and iridium complexes has captured the attention and is growing rapidly (Schmitt et al., 2008). The spectator Cp* ligand enhances the stability and cytotoxicity of the complexes, consequently making them to use as potent enzyme inhibitors (Amouri et al., 2010). Half-sandwich Rh(III) and Ir(III) tetranuclear metallacycles, complexes of trithiolato-bridged and thiaazole derived N≥N donor ligands have shown antiproliferative activity against human ovarian A2780 cancer cell lines and Dalton’s ascites lymphoma cells (Gupta et al., 2013a, 2013b).

The resistance acquired by bacteria especially toward the conventional and purely organic antibiotics attributes for the reluctance of the pharmaceutical companies to invest money into the development of new antibiotics which consequently declines the discovery of the antibiotics during the last two decades (Nicolaou et al., 2009; Saxena and Gomer, 2010; Silver, 2011). Metal specific modes of action of the metallo-drugs give a hope to overcome the development of antibiotic resistance and hence there is an urge for the focus on the utilization of organometallic compounds as antibacterial agents (Gasser et al., 2011; Hartinger and Dyson, 2009; Patra et al., 2012; Peacock and Sadler, 2008).

Aroyl hydrazone ligands are used as suitable chelators in the form of orally effective drugs for treating iron overload diseases like Friedreich’s ataxia (Subhendu et al., 2010). Various organic molecules containing hydrazine or hydrazone side chain exhibit good antibacterial activity (Sondhi et al., 2006). Hydrazine derived ligands are used as drugs for treating cancer, schizophrenia and leprosy, etc (Garkani-Nejad and Ahmadi-Roudi, 2002). The presence of hydrazine-hydrazone (–CO–NH–N–CH–) functional group plays a significant role as pharmaceutical agents, possessing anti-inflammatory, antimalarial, antimicrobial, anti-leishmanial, anticonvulsant, anti-tubercular and anti-tumor activities (Küçükgüzelt et al., 2002).

Hitherto, we have been synthesizing various organometallic complexes of Cp*Rh and Cp*Ir having nitrogen and oxygen donor ligands (Govindaswamy et al., 2005; Nongbri et al., 2009; Prasad et al., 2009; Singh et al., 2005). Recently, we have reported the complexes of rhodium and iridium with 2-pyridinecarbaldehyde nicotinichydrazone which is also a positional isomer of the ligands under study. In this ligand the pyridine of nicotinichydrazone acts as monodentate ligand leaving the two chlorides on the metal i.e., rhodium/iridium (Gupta et al., 2011). Keeping this in mind we have chosen the ligands under study by changing the position of the nitrogen atoms to study the effect of positional isomerism on mode of binding. Ligand L1 exhibited anticipated binding modes, whereas L2 exhibited serendipitous binding modes. The ligands under study were chosen not only based on their coordination chemistry but also relying on their biological activity. We have reported the complexes of Cp*Rh and Cp*Ir carrying salicylaldehyde-2-picolinichydrazone ligand exhibiting in vitro antitumor, antibacterial and fluorescence imaging properties (Rao et al., 2015). We have substituted salicylaldehyde by 2-pyridinecarbaldehyde and 3-pyridine carbaldehyde moieties to monitor the changes in binding modes as well as the biological activity in the corresponding rhodium/iridium complexes. According to our knowledge there are no reports available in the literature of these complexes with present ligands under study. Cyclooctadiene complexes of Rh and Ir with 2-picolyl amine derived Schiff base ligands were synthesized and their electronic properties were explored with DFT studies (Tejel et al., 2008a, 2008b). In this manuscript, we have described the synthesis, structural aspects, antibacterial and antitumor activities of rhodium and iridium complexes of the two positional isomers i.e., L1 and L2. Ligands and their binding modes to metal are presented in Chart 1.

2. Experimental section

2.1. Materials and methods

The synthesis and manipulation of the ligands and metal complexes were performed without using any inert atmosphere. Metal chlorides MC13+H2O (M = Rh and Ir) were purchased from Arora Matthey Limited. All the solvents used for synthesis were dried and distilled prior to use according to the standard procedures and stored over activated molecular sieves (Perrin and Armarego, 1996). Precursor compounds viz., [Cp*RhCl2]2 and [Cp*IrCl2]2 and the ligands L1 and L2 were prepared by following the literature method (Armstrong et al., 2003; Tönnessmann et al., 2013). Elemental analysis was performed on a Perkin-Elmer-2400 CHN analyzer. Infrared (IR) spectra were recorded on a Perkin-Elmer 983 spectrophotometer by dispersing compounds as KBr disks. 1H NMR spectra were recorded with Bruker Avance II 400 MHz spectrometer. UV–vis spectra were recorded using Perkin Elmer lambda 25 Spectrophotometer. Single crystals picked up from the samples were analyzed on a STOE IPDSII.
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2.2.2. Complex \([\text{Cp}^{*}\text{RhCl}_2]_2\) \(\text{L}_1\) \(\text{N},\text{N},\text{N}]/\text{PF}_6\) (3)

A mixture of \([\text{Cp}^{*}\text{RhCl}_2]_2\) (50 mg 0.08 mmol), ligand \(\text{L}_1\) (18.3 mg 0.08 mmol) and \(\text{NH}_4\text{PF}_6\) (26 mg 0.16 mmol) in methanol (20 ml) was stirred at room temperature for 6 h whereby yellow compound was precipitated out. The resulted yellow precipitate was filtered and washed with diethyl ether (2 x 15 ml) and dried in vacuum. Yield: 44 mg (73%). \(^{1}H\) NMR (400 MHz, \(\text{CDCl}_3\)): \(\delta = 11.19\) (s, 1H, \(\text{N} = \text{C} = \text{H}\) olefinic), 8.91 (d, 1H, \(\text{J}_{\text{HH}} = 4.8\) Hz, \(\text{H} = \text{Py}\)), 8.58 (m, 1H, \(\text{H} = \text{Py}\)), 8.58 (m, 1H, \(\text{H} = \text{Py}\)), 8.12 (d, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 8.05 (t, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 7.91 (t, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 7.59 (t, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 7.53 (t, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 1.73 (s, 15H, \(\text{Cp}^{*}\)). IR (KBr pellet): 3435 (b) \(\nu(\text{C} = \text{O})\), 2924 (m) \(\nu(\text{C} = \text{O})\), 1640 (s) \(\nu(\text{C} = \text{O})\), 843 \(\nu(\text{C} = \text{O})\) \(\text{cm}^{-1}\); UV/Vis (Water) \(\lambda_{\text{max}}\) nm (abs) = 231 (0.69), 274 (0.37), 370 (0.19), 440 (0.05). Anal. Calc. for \(\text{C}_{22}\text{H}_{25}\text{ClN}_4\text{O}\text{IrPF}_6\): C, 35.99; H, 3.43; N, 7.63; Found: C, 36.19; H, 3.58; N, 7.82.

2.2.2.3. Complex \([\text{Cp}^{*}\text{IrCl}_2]_2\) \(\text{L}_1\) \(\text{N},\text{N},\text{N}]/\text{PF}_6\) (4)

Complex 4 was prepared similar to complex 3 by taking \([\text{Cp}^{*}\text{IrCl}_2]_2\) (50 mg, 0.06 mmol), ligand \(\text{L}_1\) (15 mg 0.06 mmol) and \(\text{NH}_4\text{PF}_6\) (21 mg, 0.12 mmol). The complex was obtained as an orange solid. Yield: 40 mg (68%). \(^{1}H\) NMR (400 MHz, \(\text{CDCl}_3\) and \(\text{DMSO-d}_6\)): 8.79 (d, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 8.75 (s, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 8.07 (s, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 7.73 (t, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 7.69 (t, 1H, \(\text{J}_{\text{HH}} = 8.4\) Hz, \(\text{H} = \text{Py}\)), 1.76 (s, 15H, \(\text{Cp}^{*}\)). IR (KBr pellet): 3421 (b) \(\nu(\text{N} = \text{H})\), 2924 (m) \(\nu(\text{C} = \text{H})\), 1640 (s) \(\nu(\text{C} = \text{O})\), 843 \(\nu(\text{C} = \text{O})\) \(\text{cm}^{-1}\); UV/Vis (Water) \(\lambda_{\text{max}}\) nm (abs) = 231 (0.69), 274 (0.37), 370 (0.19), 440 (0.05). Anal. Calc. for \(\text{C}_{22}\text{H}_{25}\text{ClN}_4\text{O}\text{IrPF}_6\): C, 35.99; H, 3.43; N, 7.63; Found: C, 36.19; H, 3.58; N, 7.82.
2.2.5. Complex \{[Cp*RhL2_{\text{N,N,N}}]_{2}\}(PF_6)_{2} (5)

A mixture of \{[Cp*RhCl_2]_2\} (50 mg, 0.08 mmol), the ligand L2 (29 mg 0.16 mmol) and NH_4PF_6 (26 mg, 0.16 mmol) in methanol (20 ml) was stirred at room temperature for 6 h. The resulting orange precipitate was filtered and washed with diethyl ether (2 × 15 ml) and dried in vacuum. The complex was obtained as an orange solid. Yield: 52 mg (65%). ^1^H NMR (400 MHz, CDCl_3): δ = 9.19 (2H, H~Py~), 8.93 (d, 2H, H~NH~), 8.78 (s, 2H, =C=H olefinic), 8.65 (dd, 2H, J_{HH} = 4 Hz, H~Py~), 8.24 (d, 2H, J_{HH} = 8 Hz, H~Py~), 8.31 (d, 2H, J_{HH} = 4 Hz, H~Py~), 7.99 (t, 2H, J_{HH} = 4 Hz, H~Py~), 7.93 (t, 2H, J_{HH} = 4 Hz, H~Py~), 7.50 (m, 2H, H~Py~), 7.40 (t, 2H, H~Py~), 6.80 (m, 2H, H~Py~), 6.70 (m, 2H, H~Py~), 6.50 (m, 2H, H~Py~), 5.70 (m, 2H, H~Py~), 4.50 (m, 2H, H~Py~), 4.00 (m, 2H, H~Py~), 3.70 (m, 2H, H~Py~), 3.50 (m, 2H, H~Py~), 3.00 (m, 2H, H~Py~), 2.00 (m, 2H, H~Py~), 1.70 (m, 2H, H~Py~), 1.20 (s, 30H, Cp).


3. Results and discussion

3.1. Synthesis and characterization

Complexes 1–6 are obtained by treating ligands L1 and L2 with the corresponding precursor complexes in methanol (Scheme 1). All the complexes are isolated as their hexafluorophosphate salts and are obtained as yellowish to orange powders. They are soluble in polar organic solvents such as dichloromethane, methanol and acetonitrile. They are also soluble in water (1 mg/mL) but insoluble in nonpolar solvents such as diethyl ether and hexane. Reaction of rhodium and iridium metal precursors with L1 in 1:2 and 1:1 ratios yielded mononuclear complexes 1 and 2, and dinuclear complexes 3 and 4 respectively. Reaction of Rh and Ir metal precursors and ligand L2 in both 1:1 and 1:2 ratios yielded the dinuclear complexes 5 and 6 respectively.

3.2. Binding modes of ligands

The two ligands used in this study viz., L1 and L2 are obtained by condensing picolinic hydrazine with 2-pyridine-carbaldehyde and 3-pyridinecarbaldehyde respectively. The ligand L1 binds to the metal in chelating bidentate manner. There are two possibilities for the ligand to bind to the metal viz., the chelating site of picolinamide and pyridin-2-ylmethylene parts of the ligand. In case of complexes 1 and 2, the metal binds to the two nitrogen atoms of the pyridin-2-ylmethylene part of the ligand in mode 1 as shown in Chart 1 forming mononuclear complexes. The preferential binding of the metal at pyridin-2-ylmethylene part in mode 1 has led to the formation of cationic complexes since the two nitrogen atoms are neutral. If the metal binds to the two nitrogen atoms of the picolinamide part it would result in the formation of neutral complexes which was not observed. The dinuclear complexes 3 and 4 are resulting by the binding of metals at the two chelating sites of the ligand L1 as in mode 2 by deprotonation of N-H proton of the picolinamide part.

The ligand L2 acts as both chelating and bridging nitrogen donor (N=N=NH) as in the case of complexes 5 and 6. Metal can bind to the monodentate pyridine of pyridin-2-ylmethylene part of the ligand in mode 3 (Chart 1). The bridging nature of the ligand L2 is responsible for the displacement of a chloride from the metal center yielding the ionic complexes (5 and 6) with two PF_6 counteranions. In general, the monodentate pyridine binds to metal rhodium/iridium leaving the two chlorides on metal as reported previously by our group (Gupta et al., 2011). Contrastingly, in complexes 5 and 6 the monodentate pyridine has substituted the chloride/rhodium/iridium present at the chelating portion and has culminated to the formation of dinuclear complexes with three nitrogen atoms surrounding to each metal.

3.3. Spectral studies

3.3.1. Mononuclear complexes 1 and 2

The IR spectra of mononuclear complexes 1 and 2 show the stretching frequencies of N–H at 3435 cm\(^{-1}\) and 3512 cm\(^{-1}\) respectively. Carbonyl stretching frequencies are observed at 1623 cm\(^{-1}\) and 1640 cm\(^{-1}\) respectively. The \(^1^H\) NMR spectrum of complex 1 displays a singlet at \(\delta\) 1.73 corresponds to methyl protons of Cp\(^*\), a singlet at \(\delta\) 11.19 corresponds to N=H proton and a singlet at \(\delta\) 8.39 corresponds to olefin C=H proton which confirms the presence of both ligand and metal parts. Three doublets and one multiplet at \(\delta\) 8.65–7.58 and four triplets at \(\delta\) 8.02–7.53 corresponding to the pyridine rings are observed. The presence of signals corresponding to ten protons of the ligand and singlet corresponding N=H proton has suggested that the metal (Rh/Ir) is binding to the two nitrogen atoms of the pyridin-2-ylmethylene part of the ligand. The \(^1^H\) NMR spectrum of the complex 2 displays singlet resonances at \(\delta\) 1.69, \(\delta\) 10.02 and \(\delta\) 8.17 corresponding to the methyl protons of Cp\(^*\), N=H proton and olefin C=H proton which confirms the presence of both ligand and the metal parts. Two doublets and one multiplet at \(\delta\) 8.80, \(\delta\) 8.47 and \(\delta\) 8.25 respectively and four triplets at \(\delta\) 8.31–7.50 corresponding to pyridine rings confirm the formation of complex.

3.3.2. Dinuclear complexes 3 and 4

The IR spectra of the dinuclear complexes 3 and 4 show carbonyl stretching frequencies at 1665 cm\(^{-1}\) and 1611 cm\(^{-1}\) respectively and exhibit significant difference in carbonyl stretching frequencies compared to that of mononuclear complexes. In rhodium complex (complex 3) it has increased, whereas in iridium complex (complex 4) it has decreased compared to their corresponding mononuclear complexes. In
the $^1$H NMR spectra of complexes 3 and 4, the absence of characteristic N–H proton signal supports the binding of the second metal Rh/Ir at the picolinamide part of the ligand by deprotonating the N–H proton. Complex 3 exhibits olefinic singlet at $\delta$ 8.97, three doublets at $\delta$ 8.91–8.17, four triplets at $\delta$ 8.02–7.33 and a multiplet at $\delta$ 8.58 corresponding to the ligand. Complex 4 exhibits three doublets around $\delta$ 8.79, $\delta$ 8.74 and $\delta$ 8.19, two multiplets of three protons and two protons respectively at $\delta$ 8.07 and $\delta$ 7.67 correspond to ligand. The two singlet resonances of the two Cp rings in complex 3 are observed at $\delta$ 1.64 and $\delta$ 1.61 and those in complex 4 are observed at $\delta$ 1.62 and $\delta$ 1.58 which justifies the formation of the dinuclear complexes.

3.3.3. Dinuclear metalla-macrocyclic complexes 5 and 6

The IR spectra of complexes 5 and 6 display carbonyl stretching frequencies at 1643 cm$^{-1}$ and 1628 cm$^{-1}$ respectively. In complex 5, two singlets at $\delta$ 9.19 and $\delta$ 8.78 correspond to two protons of pyridine (proton on C(22) see Fig. 5) and two olefinic protons are observed. A doublet at $\delta$ 8.93, a doublet of doublet at $\delta$ 8.65, two doublets at $\delta$ 8.31 and $\delta$ 8.24, three triplets at $\delta$ 7.99, $\delta$ 7.93 and $\delta$ 7.40 and a multiplet at $\delta$ 7.50 corresponds to pyridine rings are also observed. Complex 6 exhibits two singlets at $\delta$ 9.07 and $\delta$ 8.53 correspond to two protons of pyridine (proton on C(22) see Fig. 6) and olefinic protons respectively. Four doublets at $\delta$ 8.81–8.02 and two triplets at $\delta$ 8.17 and $\delta$ 7.81 and a multiplet at $\delta$ 7.75 corresponds to pyridine rings are also observed. In complexes 5 and 6 the binding of the metal at picolinic part of the ligand by deprotonation is corroborated by the absence of N–H proton in $^1$H NMR spectra. The cationic nature of the complexes 1–6 with PF$_6$ counterion is supported by IR spectroscopy with the presence of characteristic P–F stretching frequencies at 842 cm$^{-1}$ to 845 cm$^{-1}$.

3.4. UV–visible spectral studies

UV–visible spectra of complexes 1–6 were recorded in water in 20 µM solution and the electronic spectra of these complexes are depicted in (Fig. 1). The electronic spectra display bands at 213 nm to 274 nm and 325 nm to 370 nm in ultraviolet region. The wavelengths below 300 nm are attributed to intraligand π–π* transitions. In complexes 2 and 6 wavelengths at 440 nm and 400 nm respectively observed in visible region are assigned to a mixture of metal-to-ligand charge transfer (MLCT) transition from metal t$_{2g}$ orbitals to π* orbitals of the ligand and intraligand charge transfer (ILCT) transitions (Table S4) (Chantzis et al., 2014).
3.5. Structural studies by X-ray crystallography

The molecular structures of complexes 1, 2, 3, 5 and 6 with various binding modes of the ligands were confirmed by X-ray structural analyses. Structure of complex 1 is found to have a disorder in the ellipsoids of Cp* ring and consequently has a high R value and its structure is presented only to illustrate the structure and connectivity of atoms. ORTEP drawings with an atom labeling scheme are shown in (Figs. 2–6). The summary of the crystallographic data for these complexes is presented in Table 1. Selected bond lengths and angles are presented in Table 2.

3.5.1. Molecular structures of mononuclear complexes 1 and 2

Yellow crystals of complex 1 suitable for single-crystal X-ray structure analysis were obtained by diffusing hexane into a chloroform solution of the complex. Orange crystals of complex 2 suitable for single-crystal X-ray structure analysis were obtained by diffusing hexane into an acetone solution of the complex. Selected bond lengths and angles are presented in Table 2.

Figure 2  Molecular structure of complex 1 with atom numbering scheme. Thermal ellipsoids are depicted with 50% probability level. Hydrogen atoms (except on N(3)), PF6 and chloroform are omitted for clarity.

Figure 3  Molecular structure of complex 2 with atom numbering scheme. Thermal ellipsoids are depicted with 50% probability level. Hydrogen atoms (except on N(3)), PF6 and acetone are omitted for clarity.

Figure 4  Molecular structure of complex 3 with atom numbering scheme. Thermal ellipsoids are depicted with 50% probability level. Hydrogen atoms and PF6 are omitted for clarity.
3.5.2. Molecular structure of dinuclear complex 3

Yellow crystals suitable for single-crystal X-ray structure analysis were obtained by diffusing hexane into a chloroform solution of the complex. In complex 3, C \(_{p^*}\) ring to Rh(1) centroid and C \(_{p^*}\) ring to Rh(2) centroid distances are 1.797 \(\text{Å}\) and 1.790 \(\text{Å}\) respectively. Metal to nitrogen bond distances viz., Rh(1)A\text{N}(1), Rh(1)A\text{N}(2), Rh(2)A\text{N}(3) and Rh(2)A\text{N}(4) are 2.119(3), 2.153(3), 2.090(4) and 2.131(3) respectively. Metal to chloride distances viz., Rh(1)A\text{Cl}(1) and Rh(2)A\text{Cl}(2) are 2.388(10) and 2.390(11) respectively. The bite angles around the metal Rh(1) viz., N(1)A\text{Rh}(1)A\text{Cl}(1), N(1)A\text{Rh}(1)A\text{Cl}(2), N(2)A\text{Rh}(1)A\text{Cl}(3) and N(2)A\text{Rh}(1)A\text{Cl}(4) are 76.57(13), 82.89(10) and 88.37(9) respectively. Metal to chloride distances viz., Rh(2)A\text{Cl}(1) and Rh(2)A\text{Cl}(2) are 76.34(14), 85.52(10) and 89.20(9) respectively. Bond angle around the carbonyl carbon i.e., C(15)A\text{C}(16)A\text{N}(2) is 116.9(4). In the crystal structure of complex 3 the electron density maps suggests that oxygen is present on both the sites i.e., on C(16) and C(17). The molecule flips 35% of the time one side and 65% of the time on the other side which results in oxygen and hydrogen appearing as if they are present on the same site, but they both are never at the same time. Only one of the two either oxygen or hydrogen is ever present on the same site at a given time. For instance, if oxygen is on C(16) this forces hydrogen atom to be attached to C(17) and if oxygen is on C(17) this forces hydrogen atom to be attached to C(16) (Fig. 4).

3.5.3. Molecular structures of dinuclear metalla-macrocyclic complexes 5 and 6

Orange crystals of complexes 5 and 6 suitable for single-crystal X-ray structure analysis were obtained by diffusing hexane into an acetone solution of the complex. In complex 5, C \(_{p^*}\) ring to Rh(1) centroid distance is 1.788 \(\text{Å}\).
Rh(I)−N(1), Rh(I)−N(2) and Rh(I)−N(4) is 2.096(4), 2.072 (4) and 2.114(4) respectively. The bite angles around the metal
viz., N(1)−Rh(1)−N(2), N(1)−Rh(1)−N(4) and N(2)−Rh(1)−N(4) are 76.85(18), 88.76(17) and 89.36(16) respectively.
In complex 6, C p* ring to Ir(1) centroid distance is 1.788 Å. Bond
lengths of Ir(1)−N(1), Ir(1)−N(2) and Ir(1)−N(4) are 2.068(7) Å, 2.088(5) Å and 2.135(5) Å respectively. The bite angles
around the metal viz., N(1)−Ir(1)−N(2), N(1)−Ir(1)−N(4) and N(2)−Ir(1)−N(4) are 76.6(2), 86.5(2) and 87.5(2) respectively.
In complex 6, the electron density of a disordered solven
t which is appeared to be acetone is removed through the
SQUEEZE command on PLATON. In complexes 5 and 6
the metal atom (Rh/Ir) is surrounded by three nitrogen atoms,
out of them two nitrogen atoms are in chelating fashion from
one ligand and third nitrogen atom from another ligand in
bridging fashion. The carbonyl bond lengths in complexes 1,
2, 3, 5 and 6 viz., C(17)−O(1), C(17)−O(1), C(16)−O(1), C
(16)−O(1) and C(16)−O(1) respectively are 1.166(16) Å, 1.277(10) Å, 1.205(6) Å, 1.212(6) Å and 1.221(10) Å support
the existence of the ligand in keto form. In complexes 1
and 2 the presence of N(3)−H(3) proton with a distance of 0.86 Å corroborates the presence of N−H bond which is not
deprotonated by metal. In contrast, the N−H bond is absent
in the crystal structures of complexes 3, 5 and 6 because it is
deprotonated while forming the complexes. Thus in complexes 1
and 2 the ligand is neutral whereas in complexes 3, 4, 5 and 6
the ligand is anionic which is attributed by the deprotonation
of ligand by metal in forming dinuclear complexes. Complexes 1,
2 and 5 have crystallized with chloroform, acetone and
hexane solvent molecules respectively. The hydrogen bonds
formed by solvent molecules and counterions with complexes
are depicted in (Fig. 7) and the corresponding bond lengths
and angles are given in Table 1. All the complexes possess a
“piano-stool” geometry with C p* ring on the top as seat. In
complexes 1–4 the positions of three legs are occupied by
two nitrogen atoms from the ligand and one chloride, whereas
in complexes 5 and 6, the three nitrogen atoms from the ligand,
two in chelating fashion and one as monodentate satisfy the
positions of the three legs. The metal chloride, P=O, the
C=C bond lengths within the C p* ring, C=Me distances and
metal to centroid distances are usual and are consistent with
the values reported previously (Prasad et al., 2008).

3.6. Apoptosis analysis in DL and normal PBMC cells

The nuclei of control DL cells are round in shape with uniform
green fluorescence without any membrane deformities prior
to the treatment with complexes 1 and 2. Cisplatin treatment

| Crystallographic and structure refinement parameters for complexes 1, 2, 3, 5 and 6. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                  | 1 [CHCl₃]       | 2 [CH₂COCH₂]    | 3               | 5 [C₃H₈]        | 6               |
| Empirical formula               | C₂H₃ClF₆N₄OPRh  | C₂H₃ClF₆Ir₂N₂O₂P₂| C₂H₃ClF₆N₄OPRh₂| C₂H₃Ir₂F₆N₂OPRh | C₂H₄Ir₂Cl₂F₆N₂O₂P₂|
| Formula weight                  | 764.16(10)      | 1526.24         | 917.36          | 651.42          | 1395.24         |
| Temperature (K)                 | 293(2)          | 293(2)          | 293(2)          | 293(2)          | 293(2)          |
| Wavelength (Å)                  | 0.71073         | 0.71073         | 0.71073         | 0.71073         | 0.71073         |
| Crystal system                  | Monoclinic      | Triclinic       | Monoclinic      | Orthonormic     | Orthonormic     |
| Space group                     | P(2)1/n         | P1              | P(2)1/c         | Fdd2            | Fdd2            |
| Unit cell dimensions (Å³)       |                   |                 |                 |                 |
| a                               | 8.4541(17)      | 11.2469(3)      | 8.3982(2)       | 55.595(11)      | 55.482(11)      |
| b                               | 16.316(3)       | 15.7675(5)      | 13.5154(4)      | 12.984(3)       | 12.950(3)       |
| c                               | 22.079(4)       | 18.1783(8)      | 32.0204(9)      | 15.109(3)       | 15.157(3)       |
| Volume (Å³)                     | 3007.8(10), 4   | 2786.12(17), 2  | 3613.76(17), 4  | 10906(4)        | 10890(4), 8     |
| Calculated density (mg m⁻³)     | 1.039           | 5.012           | 1.169           | 0.752           | 5.024           |
| Absorption coefficient (mm⁻¹)   | 0.2 x 0.15 x 0.1| 0.22 x 0.12 x 0.17| 0.11 x 0.18 x 0.29| 0.8 x 0.7 x 0.7| 0.35 x 0.32 x 0.22|
| Crystal size (mm³)              | 0.074           | 0.230           | 0.833           | 0.728           | 0.290           |
| Scan range                      | 3.078−3.67     | 2.1034          | 2.1749          | 2.1749          | 2.1749          |
| Reflections collected           | 784           | 705             | 737             | 737             | 876             |
| Independent reflections (Rmax) | 0.0278         | 0.0278          | 0.0278          | 0.0278          | 0.0278          |
| Refinement method               | Full-matrix least-squares on F² | Full-matrix least-squares on F² | Full-matrix least-squares on F² | Full-matrix least-squares on F² | Full-matrix least-squares on F² |
| Data/restraints/parameters      | 64120/0/357   | 113610/0/698    | 63880/0/459    | 46950/0/340    | 47000/0/322     |

* Structures were refined on F²r2 = |Σ[(F₄) − (F₄)]|/|Σ(F₄)|]¹/², where w⁻¹ = |Σ(F₄) + (aP)² + bP| and P = [max(F₄, 0) + 2F₀]/3.
cells for 12 h has shown many apoptotic features with membrane blebbing and fragmented nuclei. Complexes 1 and 2 have shown moderate to high apoptotic features which include membrane blebbing, swelling, chromatin condensation and cell membrane abnormality with fragmented nuclei. After treatment with the complex 1 at lower dose viz., 20 μg/mL (0.310 mM) and 40 μg/mL (0.620 mM) the appearance of membrane folding, blebbing and chromatin condensation is observed. At higher doses viz., 60 μg/mL (0.930 mM), 80 μg/mL (1.24 mM) and 100 μg/mL (1.55 mM) severe cell membrane abnormalities with fragmented nucleus and nucleolus, pyknosis and cytoplasmic vacuoles are noticed (Fig. 8 a). Similar changes in the cells are observed after treatment with the complex 2 at lower dose viz., 20 μg/mL (0.272 mM) and 40 μg/mL (0.544 mM) and higher doses viz., 60 μg/mL (0.816 mM), 80 μg/mL (1.08 mM) and 100 μg/mL (1.36 mM). Treated cells display a phenomenon of early apoptosis as shown by the emitted red fluorescence in both the cases. Complexes 1 and 2 show moderate apoptotic effect (Fig. 8b) at higher doses (60–100 μg/mL) with ~22% and ~30% apoptotic cell death. Rhodium and iridium complexes of salicylaldehyde 2-picolinichydrazone ligand have shown only 12% and 10% apoptotic cell death at this concentration (Rao et al., 2015). It suggests that complexes that are obtained by the substitution of salicylaldehyde by 2-pyridine carbaldehyde have shown increased apoptosis by ~100% and ~300% respectively.

After treatment with complexes 1 and 2, the apoptotic cell death in normal PBMC cells was found to be lesser as compared to cisplatin (reference drug) (Fig. 8c). At a concentration of 100 μg/mL complexes 1 and 2 have shown ~14% and ~10% apoptotic cell death on normal cells. The cytotoxicity of complexes 1 and 2 on normal cells has been observed as half of their activity on the DL tumor cells.

### 3.7. Antibacterial activity

The antibacterial potential of the four metal complexes 1, 2, 5 and 6 is evaluated according to their zone of inhibition against...
In the activity study, the concentration of every complex used is 10 µg/mL and 50 µL solution of the complex is taken in the bore of the agar. The results in terms of zone of inhibition are compared with the activity of the standards where Amoxicillin (10 µg/mL) was used as a positive control and sterilized Milli-Q water as a negative control. There are no zones of inhibition obtained around the negative control wells on agar, whereas in the wells containing complexes under investigation significant zones of inhibition are found. Complexes 1, 2, 5 and 6 have shown zones of inhibition 26 mm, 43 mm, 25 mm and 29 mm respectively against Proteus vulgaris (MTCC 426). Complexes 1, 2, 5 and 6 have shown zones of inhibition 35 mm, 34 mm, 30 mm and 35 mm respectively against Vibrio parahaemolyticus (MTCC 451) (Fig. 9a). These results reveal that all the four complexes are potent bactericidal against the two bacteria tested.

The two test organisms. The concentration of every complex used in the activity study is 10 µg/mL and 50 µL solution of the complex is taken in the bore of the agar. The results in terms of zone of inhibition are compared with the activity of the standards where Amoxicillin (10 µg/mL) was used as a positive control and sterilized Milli-Q water was a negative control. There are no zones of inhibition obtained around the negative control wells on agar, whereas in the wells containing complexes under investigation significant zones of inhibition are found. Complexes 1, 2, 5 and 6 have shown zones of inhibition 26 mm, 43 mm, 25 mm and 29 mm respectively against Proteus vulgaris (MTCC 426). Complexes 1, 2, 5 and 6 have shown zones of inhibition 35 mm, 34 mm, 30 mm and 35 mm respectively against Vibrio parahaemolyticus (MTCC 451) (Fig. 9a). These results reveal that all the four complexes are potent bactericidal against the two bacteria tested.
In the case of activity against *P. vulgaris* (MTCC 426) complexes 2 and 6 are more effective than the positive control Amocixillin. Complexes 1 and 5 are less effective than that of positive control. Among the four complexes, complex 2 has shown the highest activity with 43 mm and complex 5 is the least with 25 mm. The activity of the four complexes against *P. vulgaris* (MTCC 426) has followed the order complex 2 > complex 6 > complex 1 > complex 5 (Fig. 9b). Besides the enhancement in antitumor activity, there has been a tremendous improvement in antibacterial activity of the complexes of Rh and Ir obtained by substituting the salicylaldehyde by pyridine carbaldehyde. The Rh and Ir complexes of salicylaldehyde 2-picolinichydrazone (Rao et al., 2015) have shown no activity with *P. vulgaris* (MTCC 426) and *V. para-haemolyticus* (MTCC 451) but the corresponding Rh and Ir complexes obtained with ligands under study which were obtained by substituting salicylaldehyde by 2-pyridine carbaldehyde and 3-pyridine carbaldehyde have shown profound activity with zones of inhibition from 25 mm to 43 mm. This is probably because in the former case the complexes are neutral and in the latter they are ionic. The ionic nature of the complexes might have facilitated them for entering the cell crossing the membrane.

In case of activity against *V. para-haemolyticus* (MTCC 451), the complexes under study have shown significantly better activity than positive control. The positive control has shown only 1 mm zone of inhibition. Complexes 1 and 6 have shown the highest activity among the four complexes with a zone of inhibition 35 mm. The activity of four complexes against *V. para-haemolyticus* (MTCC 451) is found in the following order complex 1~ complex 6 > complex 2 > complex 5.

**Figure 9b** Antagonistic activity of complexes 1, 2, 5 and 6 against the test bacterial species.

**Figure 10a** Docking structure of thymidylate phosphorylase enzyme. Here, a and b show interactions with reference ligand i.e., 2’-Deoxyuridine 5’-monophosphate, c and d show interactions with complex 6. Hydrogen atoms are omitted for clarity. Dotted lines indicate the hydrogen bonds.
3.8. Theoretical studies

3.8.1. Docking analysis

Molecular docking study is carried out to understand the possible interaction(s) between proteins and five complexes viz., 1, 2, 3, 5 and 6. The protein structures considered in this study viz., ribonucleotide reductase, thymidylate synthase, thymidylate phosphorylase and topoisomerase II are associated with cancer progression. The mechanistic chemistry of these key enzymes is highly expressed and closely involved at various stages of cell multiplication and hence responsible for the propagation of cancer. Moreover, their inhibition by various chemotherapeutic agents is associated with the inhibition of cancer growth and invasion (Singh and Bhardwaj, 2008).

Therefore, in the present study these enzymes are used for molecular docking to investigate the possible binding with newly synthesized complexes. The docking studies have revealed that all the complexes tested interact with enzymes at their active pocket and their hydrogen bonding interactions are given in Table S1. Molecular docking study has shown that complex 1 could dock with ribonucleotide reductase and shows three hydrogen bond interactions with three different amino acids viz., Arg 251A, Arg 160A, Ser 213A and shows strong interaction with thymidine phosphorylase and renders seven hydrogen bonds with amino acids viz., Ser 117A, His 116A, Ser 217A, Arg 202A, Leu 148A, Lys 221A and four hydrogen bonds with thymidylate synthase with amino acids viz., Asn 229, Ser 232, Glu 60 and five hydrogen bonds with topoisomerase II with amino acids viz., Gln 17A, Tyr 28A, Thr 27B, Tyr 144A, Tyr 28B. Complex 6 shows strong interactions with thymidine phosphorylase with six hydrogen bonds with amino acids viz., Ser 117A, Tyr 199A, Thr 154A, Ser 144A (Figs. 10a, 10b, S1 and S2). The comparative binding results are shown in Table S2.

3.9. Density Functional Theory (DFT) calculations

3.9.1. Electronic properties

DFT computation of the complexes 1–6 has been performed with optimized geometry to correlate the electronic structure with the observed electronic spectra. HOMO and LUMO energy gaps in complexes 1–6 are found as 3.59 eV, 3.37 eV, 3.26 eV, 2.95 eV, 3.54 eV and 3.50 eV respectively. The energy gap of complex 4 is found lesser compared to the others. In complexes 1–6, HOMO and HOMO–1 energy gaps are found as 0.23 eV, 0.27 eV, 0.15 eV, 0.29 eV, 0.09 eV and 0.05 eV respectively (Table S3). The TDFFT results including the calculated absorption features, absorption wavelength (nm), and

Figure 10b Docking structure of thymidylate synthase enzyme. Here, a and b show interactions with reference ligand i.e., 2′-Deoxyuridine 5′-monophosphate, c and d show interactions with complex 1. Hydrogen atoms are omitted for clarity. Dotted lines indicate hydrogen bonds.
major transition and charge transfer assignments are given in Table S4. The contributions of the selected moieties of the model complexes 1–6 to the individual molecular orbitals were calculated by a Mulliken population analysis and are listed in the 1.3.2 of SI and Table S5. In complex 1, an absorption peak at \( \lambda = 270.2 \text{ nm} \) results from a major transition from HOMO to LUMO (71%) and an absorption peak at \( \lambda = 356.8 \text{ nm} \) results from a transition from HOMO–2 to LUMO (76%) and HOMO–3 to LUMO (15%) which attribute for ILCT transitions. In complex 2, an absorption peak at \( \lambda = 270.7 \text{ nm} \) results from a major transition from HOMO–3 to LUMO+1 (56%) which attribute for LMCT and ILCT transitions. An absorption peak at \( \lambda = 370.4 \text{ nm} \) results from a major transition from HOMO–2 to LUMO (53%), HOMO–1 to LUMO+1 (23%) which suggests the LMCT and ILCT transitions. An absorption peak at \( \lambda = 370.4 \text{ nm} \) results from a major transition from HOMO–3 to LUMO (76%) and HOMO–3 to LUMO (15%) which attribute for ILCT transitions. In complex 3, an absorption peak at \( \lambda = 300.0 \text{ nm} \) results from a major transition from HOMO–6 to LUMO+1 (31%), HOMO–5 to LUMO (12%) which contributes for XLCT (X=Cl) chloride to ligand charge transfer and MLCT transitions. In complex 4, an absorption peak at \( \lambda = 328 \text{ nm} \) results from a major transition from HOMO–1 to LUMO+3 (52%) which contributes for MLCT transition. In complex 5, an absorption peak at \( \lambda = 355 \text{ nm} \) results from a major transition from HOMO–2 to LUMO+5 (22%) and HOMO–3 to LUMO+4 (14%) which contributes for MLCT and ILCT transitions. In complex 6, an absorption peak at \( \lambda = 400.8 \text{ nm} \) results from a major transition from HOMO–1 to LUMO (67%) which suggests the ILCT transition.

3.9.2. Host guest chemistry of complexes 5 and 6

Complexes 5 and 6 are metalla-macrocycles which are formed by the substitution of the metal chloride by ligand (L2) and binding in chelating and bridging modes. Complexes 5 and 6 have formed a cycle including the metal atoms (Rh/Ir). The structure has got a resemblance with the porphyrin ring of course with metal atoms present in the ring. We have carried out a theoretical study by inserting various metal ions viz., Li+, Na+, Mg2+, Cu+, Ni2+ and Zn2+ inside the ring to check the feasibility of the insertion of metal ion inside the ring. Metal ion inserted derivatives of the complex 5 with Li+, Na+, Mg2+, Cu2+, Ni2+ and Zn2+ are represented as 5a–5f. Metal ion inserted derivatives of the complex 6 with Li+, Na+, Mg2+, Cu2+, Ni2+ and Zn2+ are represented as 6a–6f (Scheme 2). Complexes of rhodium with the optimized structures revealed that lithium, sodium and magnesium ions can bind to the two oxygen atoms of the two ligands so that it lies above the plane of the ring. In case of copper, nickel and zinc ions the metal enters inside the ring and binds to two nitrogen atoms present in the trans position (Figs. S4 and S5). We are investigating for the practical implementation of the theoretical results.

4. Conclusions

The preferential binding of the metal (Rh and Ir) toward pyridin-2-ylmethylene part of the ligand in L1 results to cationic complexes 1 and 2. In case of ligand L2, N=H of the picolinamide part of the ligand is deprotonated by metal (Rh and Ir). Ligand L1 is neutral and L2 is anionic in nature.

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while forming their respective complexes. In complexes 5 and 6 the pyridine of the pyridin-2-ylmethylene part of the ligand acts as bridging and is responsible for the substitution of the metal chloride and consequently forming dinuclear complexes. Complexes 1 and 2 have shown moderate apoptosis activity against Dalton’s ascites lymphoma cells with ~22% and ~30% apoptotic cell death. Though the cytotoxicity of complexes 1 and 2 against DL cells is comparatively lesser than that of cisplatin, their less cytotoxicity on normal cells (PBMC) could take them forward to implement as antimutagen agents. Complexes 1, 2, 5 and 6 are bactericidal against the two bacteria species studied. All the four complexes have shown high activity against V. para-haemolyticus (MTCC 451) where the positive control amoxicillin itself is inactive. P. vulgaris is more susceptible to iridium complexes (complexes 2 and 6). V. para-haemolyticus (MTCC 451) is equally susceptible to rhodium and iridium complexes (complexes 1 and 6). Complex 5 has shown the least activity among the four complexes against the two bacterial species.

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Appendix A. Supplementary material

CCDC 1061760 [1], CCDC 1061761 [2], CCDC 1061762 [3], CCDC 1061763 [5] and CCDC 1061764 [6] contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 366033. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2013.10.011.

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