Synthesis, structure and bonding modes of pyrazine based ligands of Cp*Rh and Cp*Ir
complexes: The study of *in-vitro* cytotoxicity against human cell lines

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14 **Graphical Abstract**

15

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Abstract

The reaction of multidentate pyrazine based ligands was explored towards Cp*rhodium and Cp*iridium precursors. Mononuclear and dinuclear complexes formed by the ratio-based reaction between ligand and metal precursor. The representative complexes have been determined by single crystal X-ray diffraction studies. Cytotoxicity study of the ligands and their complexes are evaluated against human colorectal cancer cell lines HT-29, HCT-116 p53^{+/+} and HCT-116 p53^{-/-} and ARPE-19 (non-cancer retinal epithelium) cells. Complexes **2-5** and **7-8** were cytotoxic to cells and although the potency of these complexes was less than cisplatin, selectivity towards cancer cell lines as opposed to non-cancer ARPE-19 cells was comparable to cisplatin. From *in-vitro* cytotoxicity studies complexes **4** and **5** demonstrated good selectivity towards HCT116 p53^{-/-} cells suggesting that these complexes are promising leads for the treatment of p53 deficient cancers.

Keyword: rhodium, iridium, pyrazine, *in vitro* cytotoxicity

29 Introduction

30 Metal-based drugs such as cisplatin, oxaliplatin, and carboplatin compounds have a huge
31 potential in cancer chemotherapy over many years [1–3]. The clinical utility of these compounds
32 is limited to a relatively narrow range of tumors (sarcomas, small cell lung cancer, ovarian cancer,
33 lymphomas and germ cell tumors) [4], as it has high toxicity leading to side effects which limit the
34 administered dose and easily acquired drug resistance. In order to overcome these severe side
35 effects caused by platinum-based drugs intense research efforts have been devoted toward the
36 design of other metal-based drugs that have low toxicity [5] and recently there has been resurgence
37 in interest in organometallics as potential anti-cancer drugs, as they have different mechanisms of
38 action to the platinum compounds [6]. In one such approach, ruthenium-based compounds turned
39 out to show the most promising anticancer activity. In particular, ruthenium-arene half-sandwich
40 complexes have gained increasing interest in recent years due to favorable *in vivo* anticancer
41 properties [7–9]. Rhodium and iridium complexes like acetylacetonato-1,5-
42 cyclooctadienerhodium(I), and acetylacetonato-1,5-cyclooctadiene iridium(I) displayed promising
43 percentage of antitumor activity when tested in mice bearing Ehrlich ascites carcinoma, more over
44 they shows high selective toxicity for tumor cells and marginally nephrotoxic [10]. The half-
45 sandwich rhodium and iridium complexes have recently been explored, which have been less
46 investigated as metallo drugs [11-13]. Piano-stool pentamethylcyclopentadienyl group 9
47 complexes acts as chemotherapeutic agent [14–17], and in some cases, they exhibit a superior
48 activity to that of related ruthenium-arene complexes [18, 19]. Multinuclear complexes that
49 comprised more than one metal centers pay attracted interest in the field of medicinal chemistry.
50 The anticancer activity of these compounds can be enhanced because they might possess different
51 properties compared to their mononuclear derivatives in redox activities and selectivity towards

various biomolecules [20]. Rh and Ir complexes recently shown promising anticancer activity *via* intercalating target DNA in the cancer cells [21-23]. On the other hand, pyrazine based ligands and their complexes are well known for their pronounced biological activities. They are actively associated with antibacterial and anticancer activities including activity against hypoxic cells [24-27].

Recently, our group has reported many half-sandwich platinum group metal complexes containing pyridyl azine ligands [28-31]. In continuation of our previous work, we report the synthesis of half-sandwich Cp*rhodium and Cp*iridium complexes containing pyrazine based ligands. Herein, we describe the results of a preliminary study focusing on the preparation of two new Schiff bases ligands along with mononuclear and dinuclear Rh and Ir complexes. Azine ligands possess rotational flexibility around N-N bond this form of ligands could give rise to compounds with different binding modes and therefore explored this versatility in this present study. The products were analyzed for their cytotoxicity against cancer cells. The ligands used in present study are presented in Chart 1.

Chart 1: Ligands used in present study

2. Experimental Section

2.1. Physical methods and materials

All reagents used in this work were purchased commercially and used without further purification. Pyrazinecarbonitrile, 2-acetylpyridine, 2-pyridinecarbaldehyde were purchased from

Alfa Aesar and hydrazine hydrate was purchased from Qualigens. Starting compounds $[\text{Cp}^*\text{MCl}_2]_2$ ($\text{M} = \text{Rh}/\text{Ir}$) were synthesized using an Anton Paar monowave 50 synthesizer in 10 ml microwave vials equipped with magnetic stirring bars as previously reported [32]. The syntheses of all the metal complexes were performed at room temperature. Infrared spectra were recorded on a Perkin-Elmer 983 spectrophotometer as KBr pellets in the range 4000 to 400 cm^{-1} . ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer using CDCl_3 and DMSO-d_6 as solvents, with SiMe_4 as internal references at room temperature. Mass spectra were obtained from Waters ZQ 4000 mass spectrometer by ESI method using acetonitrile as solvent. UV–Vis absorption spectra were obtained on a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer in acetonitrile solution at room temperature. Elemental analyses of the complexes were carried out on a Perkin-Elmer 2400 CHN analyzer.

2.2. Single-crystal X-ray structures analyses

The crystals of complexes suitable for X ray crystal structure analyses were obtained by slow diffusion of hexane over a mixture of acetone and dichloromethane solution of the respective complexes. Single crystal X-ray diffraction measurements were collected on an Oxford Diffraction Xcalibur Eos Gemini diffractometer at 293 K equipped with graphite monochromated $\text{Mo-K}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). The strategy for the data collection was evaluated using the CrysAlisPro CCD software. Crystal data were collected by standard “phi–omega scan” techniques and were scaled and reduced using CrysAlisPro RED software. The structures were solved by direct methods using SHELXS-97 and refined by full-matrix least squares with SHELXL-97 refining on F^2 [33]. The positions of all the atoms were obtained by direct methods. Metal atoms in the complex were located from the E-maps and non-hydrogen atoms were refined anisotropically. The hydrogen atoms bound to the carbon were placed in geometrically constrained positions and

refined with isotropic temperature factors, generally 1.2 Ueq of their parent atoms. Crystallographic and structure refinement parameters for the complexes are summarized in Table S1 and selected bond lengths and bond angles are presented in Table 1. Figures 1-3, Figures S27 and S28 were drawn with ORTEP3 [34].

2.3. Cell line testing

The cytotoxic activity of the pyrazine based ligands and its rhodium and iridium complexes were evaluated against the human colorectal carcinoma cell lines HT 29, HCT116 p53^{+/+}, HCT116 p53^{-/-} and the non-cancer ARPE-19 (human epithelial cell line derived from the retina) cell line. HT-29 and ARPE-19 cells were originally purchased from ATCC and HCT116 cells (containing wild type p53 or deleted p53) were obtained from Professor Bert Vogelsteins laboratory [35]. Reagents used were purchased from Sigma Aldrich Co. Ltd (Dorset, UK). Cytotoxicity of pyrazine ligands and compounds were evaluated using the standard MTT (3-(4, 5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) cellular viability assay as follows. Cells were inoculated into 96 well plates at 1.5×10^3 cells per well and incubated for 24 hours at 37°C in an atmosphere of 5% CO₂ prior to drug exposure. The pyrazine based ligands and complexes **1-8** were all dissolved in Dimethyl sulfoxide (DMSO) at a concentration of 100 mM and diluted further with medium to obtain drug solutions ranging from 0.5 to 100 μ M. The final DMSO concentration was 0.1% (v/v), which is nontoxic to cells. Cisplatin was dissolved in phosphate buffered saline at a stock concentration of 25 mM. Cells were exposed to drug for 96 hours and cell survival was determined using the MTT assay [36]. Briefly, 20 μ L of MTT (0.5 mg/ml) in phosphate buffered saline was added to each well and it was further incubated at 37 °C for 4 hours in an atmosphere containing 5% CO₂. The solution was then removed and the formazan crystals formed were dissolved in 150 μ M DMSO. The absorbance of the solution was recorded at 550 nm using an ELISA

spectrophotometer. Percentage cell survival was calculated by dividing the true absorbance of treated cell by the true absorbance for controls (exposed to 0.1% DMSO). The IC_{50} values were determined from plots of % survival against drug concentration. Each experiment was repeated three times and a mean value obtained and stated as IC_{50} (μM) $\pm$ SD. To compare the response of non-cancer cells to cancer cells, the selectivity index (SI) was calculated as the IC_{50} for ARPE-19 cells divided by the IC_{50} of cancer cells. Values >1 shows that complexes have selective activity against cancer compared to non-cancer cells *in vitro*.

2.4. General procedure for synthesis of ligands (L1 and L2)

The ligands were synthesized following the reported procedure [37]. Reaction of pyrazine 2-carbonitrile (2 mmol) and hydrazine hydrate (3.5 mmol) under stirring for 24 hours resulted to the formation of yellow crystalline solid of pyrazine 2-carbohydrazonamide, the compound was washed with diethyl ether and dried. Pyrazine 2-carbohydrazonamide was further reacted in 1:1 molar ratio with the desired pyridine in ethanol and the mixture was reflux at 60 $^{\circ}C$ for about 4 hours. The yellow solid, which formed, was washed with diethyl ether and dried at room temperature (Scheme 1).

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Scheme 1. Synthesis of ligands

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2.5. Synthesis of mononuclear complexes (1-4)

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A mixture of starting metal precursor (0.1 mmol), ligands (0.2 mmol) and (0.25 mmol) of

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NH_4PF_6 were dissolved in dry methanol (10 ml) and stirred at room temperature for 8 hours

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(Scheme-2). A yellow colored compound precipitated out from the reaction mixture. The

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precipitate was filtered, washed with cold methanol (2 x 5 ml) and diethyl ether (2 x 5 ml) and air-

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dried.

Scheme 2. Synthesis of mononuclear complexes

2.5.1. [Cp*RhL1Cl]PF₆ [1]

Yield: 79%; IR (KBr, cm⁻¹): 3415, 3228 ν(N-H) (stretching), 3184 ν(C-H), 1639 ν(N-H) (bending), 1611 ν(C=N), 840 ν(P-F) (stretching), 557 ν(P-F) (bending); ¹H NMR (400 MHz, CDCl₃, δ ppm) : 9.58 (s, 1H), 9.14 (s, 1H), 8.79-8.89 (m, 2H), 8.35 (d, 1H), 8.22 (d, 1H, J = 8 Hz), 7.94 (s, 1H, J = 4 Hz), 7.80 (s, 2H), 7.13 (s, 1H), 1.77 (s, 15H); ¹³C NMR (100 MHz, CDCl₃ + DMSO-d₆, ppm): 157.76, 152.23, 151.58, 151.48, 146.50, 143.04, 142.87, 142.77, 139.65, 128.29, 127.27, 97.35, 8.24; ESI-MS (m/z): 464.00 [M-Cl-PF₆]⁺; Anal. Calc. for C₂₁H₂₅ClN₆RhPF₆ (644.79): C, 39.12; H, 3.91; N, 13.03. Found: C, 39.21; H, 3.94; N, 13.16.

2.5.2. [Cp*IrL1Cl]PF₆ [2]

Yield: 82%; IR (KBr, cm⁻¹): 3428, 3280 ν(N-H) (stretching), 3150 ν(C-H), 1632 ν(N-H) (bending), 1602 ν(C=N), 843 ν(P-F) (stretching), 560 ν(P-F) (bending); ¹H NMR (400 MHz, CDCl₃, δ ppm) : 9.50 (s, 1H), 8.72 (s, 2H), 8.57 (s, 1H), 8.09 (t, 2H, J = 8 Hz), 7.91 (d, 1H, J = 8 Hz), 7.75 (d, 1H, J = 4 Hz), 7.33 (s, 1H), 7.02 (s, 1H), 1.55 (s, 15H); ESI-MS (m/z): 554.13 [M-Cl-PF₆]⁺; Anal. Calc. for C₂₁H₂₅ClN₆IrPF₆ (734.10): C, 34.36; H, 3.43; N, 11.45. Found: C, 34.22; H, 3.40; N, 11.54

2.5.3. [Cp*RhL2Cl]PF₆ [3]

158 Yield: 65%; IR (KBr, cm^{-1}): 3426, 3268 $\nu(\text{N-H})$ (stretching), 3162 $\nu(\text{C-H})$, 1641 $\nu(\text{N-H})$ (bending), 1598 $\nu(\text{C=N})$,
159 843 $\nu(\text{P-F})$ (stretching), 559 $\nu(\text{P-F})$ (bending); ^1H NMR (400 MHz, CDCl_3 , δ ppm): 9.53 (s, 1H), 8.99 (s,
160 1H), 8.55 (s, 2H), 8.71 (s, 1H), 8.21 (t, 2H, $J = 8\text{Hz}$), 7.87 (t, 1H, $J = 4\text{Hz}$), 7.41 (s, 1H), 1.69 (s,
161 15H), 1.25 (s, 3H); ESI-MS (m/z): 478.17 $[\text{M-Cl-PF}_6]^+$; Anal. Calc. for $\text{C}_{22}\text{H}_{27}\text{ClN}_6\text{RhPF}_6$
162 (658.81): C, 40.11; H, 4.13; N, 12.76. Found: C, 40.23; H, 4.19; N, 12.88.

163 2.5.4. $[\text{Cp}^*\text{IrL}_2\text{Cl}]\text{PF}_6$ [4]

164 Yield: 71%; IR (KBr, cm^{-1}): 3405, 3276 $\nu(\text{N-H})$ (stretching), 3128 $\nu(\text{C-H})$, 1642 $\nu(\text{N-H})$ (bending), 1608 $\nu(\text{C=N})$,
165 841 $\nu(\text{P-F})$ (stretching), 557 $\nu(\text{P-F})$ (bending); ^1H NMR (400 MHz, DMSO-d_6 , δ ppm): 9.60 (s, 1H), 8.86
166 (s, 2H), 8.72 (s, 1H), 8.23 (t, 1H, $J = 8\text{Hz}$), 8.14 (d, 1H, $J = 4\text{Hz}$), 7.89 (t, 1H, $J = 8\text{Hz}$), 7.44 (s,
167 1H), 7.21-7.24 (d, 1H, $J = 12\text{ Hz}$), 2.70 (s, 3H), 1.68 (s, 15H); ^{13}C NMR (100 MHz, CDCl_3 +
168 DMSO-d_6 , ppm): 166.89, 154.75, 151.53, 150.94, 147.06, 143.40, 143.33, 143.16, 140.28, 129.30,
169 126.58, 90.31, 15.44, 8.49; ESI-MS (m/z): 567.94 $[\text{M-Cl-PF}_6]^+$; Anal. Calc. for $\text{C}_{22}\text{H}_{27}\text{ClN}_6\text{IrPF}_6$
170 (748.13): C, 35.32; H, 3.64; N, 11.23. Found: C, 35.58; H, 3.91; N, 11.36

171 2.6. Synthesis of dinuclear complexes (5-8)

172 A mixture of starting metal precursor (0.1 mmol), ligands (0.1 mmol) and (0.25 mmol) of
173 NH_4PF_6 were dissolved in dry methanol (10 ml) and stirred at room temperature for 8 hours
174 (Scheme-3). A bright orange colored compound precipitated out from the reaction mixture. The
175 precipitate was filtered, washed with cold methanol (2 x 5 ml) and diethyl ether (2 x 5 ml) and air-
176 dried.

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Scheme 3. Synthesis of dinuclear complexes

180 **2.6.1. $[(Cp^*RhCl)_2(\mu-L1)]PF_6$ (5)**

181 Yield: 64%; IR (KBr, cm^{-1}): 3422 $\nu(N-H)$ (stretching), 3149 $\nu(C-H)$, 1645 $\nu(N-H)$ (bending), 1602 $\nu(C=N)$, 850
 182 $\nu(P-F)$ (stretching), 558 $\nu(P-F)$ (bending); 1H NMR (400 MHz, $CDCl_3$, δ ppm) :9.57 (s, 1H), 8.91-8.93 (d,
 183 1H, $J = 8Hz$), 8.85 (d, 1H, $J = 8Hz$), 8.69 (d, 1H, $J = 4Hz$), 8.17 (d, 2H, $J = 4Hz$), 7.87 (t, 1H, J
 184 = 4 Hz), 7.46 (s, 1H), 7.26 (s, 1H), 1.77 (s, 15H), 1.69 (s, 15H); ^{13}C NMR (100 MHz, $CDCl_3$ +
 185 DMSO- d_6 , ppm): 166.33, 158.47, 151.68, 150.61, 144.00, 143.88, 140.20, 137.43, 134.06, 126.09,
 186 123.49, 92.92, 8.25. ESI-MS (m/z): 700.97 $[M-2Cl-PF_6]^+$; Anal. Calc. for $C_{31}H_{39}Cl_2N_6Rh_2PF_6$
 187 (917.36): C, 40.59; H, 4.29; N, 9.16. Found: C, 40.50; H, 4.35; N, 9.47.

188 **2.6.2.** $[[Cp^*RhCl]_2(\mu-L2)]PF_6$ (6)

189 Yield: 67%; IR (KBr, cm^{-1}): 3418 $\nu_{(N-H)}$ (stretching), 3142 $\nu_{(C-H)}$, 1623 $\nu_{(N-H)}$ (bending), 1597 $\nu_{(C=N)}$, 844
190 $\nu_{(P-F)}$ (stretching), 554 $\nu_{(P-F)}$ (bending); 1H NMR (400 MHz, $CDCl_3$ + DMSO- d_6 , δ ppm) : 8.74 (d, 1H, J
191 = 8Hz), 8.64 (m, 2H), 8.09 (t, 1H, J = 8Hz), 7.93 (d, 1H, J = 8 Hz), 7.76 (t, 1H, J = 8Hz), 7.42
192 (s, 1H), 6.78 (s, 1H) 1.61 (s, 30H), 1.18 (s, 3H). Anal. Calc. for $C_{32}H_{41}Cl_2N_6Rh_2PF_6$ (931.39): C,
193 41.27; H, 4.44; N, 9.02. Found: C, 41.62; H, 4.73; N, 8.94.

194 **2.6.3.** $[[Cp^*IrCl](\mu-L1)\{Cp^*IrCl_2\}]PF_6$ (7)

195 Yield: 56%; IR (KBr, cm^{-1}): 3435, 3249 $\nu_{(N-H)}$ (stretching), 2995 $\nu_{(C-H)}$, 1627 $\nu_{(N-H)}$ (bending), 1592 $\nu_{(C=N)}$,
196 850 $\nu_{(P-F)}$ (stretching), 558 $\nu_{(P-F)}$ (bending); 1H NMR (400 MHz, $CDCl_3$, δ ppm) :9.72 (s, 1H), 8.88 (d, 2H,
197 J = 8Hz), 8.73 (s, 2H), 8.23 (t, 1H, J = 8Hz), 8.14 (d, 1H, J = 4Hz), 7.92 (t, 1H, J = 8Hz), 7.37
198 (d, 1H, J = 4 Hz), 6.97 (s, 1H), 1.78 (s, 15H), 1.75 (s, 15H). ESI-MS (m/z): 880.56 $[M-3Cl-PF_6]^+$;
199 Anal. Calc. for $C_{31}H_{40}Cl_3N_6Ir_2PF_6$ (1132.45): C, 32.88; H, 3.56; N, 7.42. Found: C, 33.03; H, 3.69;
200 N, 7.49.

201 **2.6.4.** $[[Cp^*IrCl](\mu-L2)\{Cp^*IrCl_2\}]PF_6$ (8)

202 Yield: 52%; IR (KBr, cm^{-1}): 3431, 3262 $\nu_{(N-H)}$ (stretching), 3153 $\nu_{(C-H)}$, 1645 $\nu_{(N-H)}$ (bending), 1604 $\nu_{(C=N)}$,
203 843 $\nu_{(P-F)}$ (stretching), 559 $\nu_{(P-F)}$ (bending); 1H NMR (400 MHz, $CDCl_3$, δ ppm) :9.45 (s, 1H), 8.94 (s,
204 1H), 8.88 (t, 2H, J = 8Hz), 8.20 (d, 1H, J = 8Hz), 8.04 (d, 1H, J = 8Hz), 7.92 (s, 1H), 7.75 (t, 1H,
205 J = 8Hz), 7.21 (s, 1H), 1.71 (s, 15H) 1.63 (s, 15H), 1.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ +
206 DMSO- d_6 , ppm): 153.82, 151.34, 147.22, 146.83, 143.76, 143.65, 140.60, 135.39, 129.01, 126.54,
207 98.01, 16.12, 9.06; Anal. Calc. for $C_{32}H_{42}Cl_3N_6Ir_2PF_6$ (1146): C, 33.52; H, 3.69; N, 7.33. Found:
208 C, 33.71; H, 3.54; N, 7.76.

209 **3. Results and Discussion**

3.1. *Synthesis of complexes*

Over the years, we have synthesized several Cp* rhodium and Cp* iridium complexes with various ligands and we found that they are structurally analogous to the arene ruthenium complexes and follow the similar chemistry in many occasions. But in our present work we found that our effort to synthesize arene ruthenium complexes with these ligands was unsuccessful irrespective of the solvent used for the reaction. The mononuclear complexes **1-4** and dinuclear complexes **5-8** are synthesized and represented in Schemes 2 and 3. All these complexes were isolated as cationic salts with PF₆ counter ion. Metal complexes were obtained in good yields, non-hygroscopic and are yellow, orange or red in color. These complexes are soluble in dichloromethane, chloroform, acetonitrile and acetone but insoluble in diethyl ether and hexane. All the synthesized ligands and complexes were fully characterized by various spectroscopic techniques. Further the cytotoxic activity of the ligands and its metal complexes were evaluated against cancer cell lines HT-29, HCT-116 p53+/+ and HCT-116 p53-/- and one non-cancer cell line ARPE-19 (non-cancer retinal epithelium).

3.2. *Spectroscopic characterization of complexes*

The diagnostic infrared spectral peaks of the complexes **1-8** are depicted in experimental part. The ligand **L1** shows peaks at 3424 and 3298 cm⁻¹ and **L2** show peak at 3393 and 3276 cm⁻¹, the bands assignable to the stretching frequency of amine groups. The spectra also exhibit bands at 1599-1577, 1523-1531 cm⁻¹, which is due to $\nu_{(C=N)}$ of pyrazine and pyridine ring respectively. The IR spectra of metal complexes **1-4**, reveal two bands corresponding to $\nu_{(N-H)}$ bands of amine group. Whereas complexes **5** and **6** show only one $\nu_{(N-H)}$ band in which one band disappear upon complexation suggest that the ligands in these complexes undergo deprotonation of amine group and form a chelation to the metal center. Complex **7** and **8** show two bands corresponding to $\nu_{(N-}$

¹H) bands of amine group which clearly indicate that there is no deprotonation and the amine groups did not involve in coordination to the metal. IR spectra of all complexes showed that the C=N bond vibrated at higher frequency region as compared to the free ligand around 1597-1613 cm⁻¹ indicating the co-ordination of ligand to the metal. All complexes exhibits a band at 840-850 cm⁻¹ correspond to counter ion P-F stretching frequency.

The ¹H NMR spectra of the complexes **1-8** are presented in the supporting information Figures S1-S8. The ¹H NMR spectra of these complexes exhibit signals in the up field associated to Cp* ring protons. The aromatic proton signals associated with the ligands were observed in the downfield region around 9.72- 6.97 ppm. The formation of the mononuclear and dinuclear rhodium and iridium complexes was confirmed by their ¹H NMR spectra. The mononuclear rhodium and iridium complexes **1-4** displayed only one singlet for the methyl protons of the Cp* group around 1.77-1.55 ppm whereas its dinuclear complexes **5-8** possessed two singlets for the methyl proton of Cp* around 1.77-1.63 ppm except in the case of complex **6** where it shows only one singlet for two Cp* with 30 protons. For example, the ¹H NMR spectrum of the mononuclear rhodium complex (**1**) revealed only one singlet at 1.77 ppm for the 15 protons of Cp*, whereas its dinuclear complex (**5**) revealed two singlets with 15 protons each at 1.77 and 1.69 ppm. Similarly the mononuclear iridium complex (**2**) exhibited only one singlet for the Cp* protons at 1.55 ppm whereas the dinuclear iridium complex (**7**) exhibited two singlets at 1.78 and 1.75 ppm. Thus the ¹H NMR spectra of the complexes strongly confirmed the formation of mononuclear and dinuclear complexes.

The ¹³C NMR spectra of the representative complexes further validate the formation of complexes are provided in the supplementary materials (Figures S9-S12). The ¹³C NMR spectra of the complexes showed signals associated with the carbon of the ligand, ring carbon of Cp* and

methyl carbon of Cp*. The resonance of the azomethine carbon (C=N) group appeared in the lower frequency region around 151.73 to 158.47 ppm. The aromatic carbons signals for the ligands were observed in the range of 123.49 to 151.68 ppm. The signals associated with the ring carbons of the Cp* ligand was observed in the region 90.31 to 98.01 ppm and the methyl carbon resonances was observed as a sharp peak at 8.24 to 9.06 ppm. The methyl group attached to the azomethine carbon in complex **4** and complex **8** was observed at 15.44 and 16.12 ppm respectively. Overall results from the NMR spectral studies intensely support the formation of the metal complexes.

UV-Visible of the ligands and complexes **1-8** were recorded at room temperature at 20 μ M concentration in the range 200-600 nm. The electronic absorption spectra of the ligands and complexes were depicted in Figure S19- S26. The stock solutions used to determine the IC₅₀ value were prepared with DMSO there is a possibility that chloride ligand of metal complexes could undergo a ligand exchange with the DMSO molecule and that would affect the IC₅₀ value determination. So, we explored the stability of all the complexes in DMSO-d₆ solution using UV-visible spectroscopy. UV-visible spectra were recorded for a freshly prepared solution, and after 4, 10, 24 and 36 h. it has been found that there is no significant changes in the UV-vis spectra even after 36 h which indicated that the synthesized complexes is stable in DMSO solution.

The m/z values of all the complexes are listed in the experimental section and are presented in the supporting information figures S13-S18. Mononuclear complexes **1-4** display their predominant peaks at m/z: 464.00, 554.13, 478.17, and 567.94 respectively correspond to [M-Cl-PF₆]⁺ ion peak. The rhodium complex **5** displays the predominant peak at m/z: 700.97 correspond to [M-2Cl-PF₆]⁺. Similarly iridium complexes **7** displayed their prominent peaks at m/z 880.86, which correspond to [M-3Cl-PF₆]. The mass spectra of all complexes displayed the prominent peaks corresponding to the loss of chloride ion and counter ion. The peaks corresponding to the

loss of ligands as well as the Cp* ring are not observed which indicates the strong binding of metal to ligand and metal to arene. The appearance of these peaks in its mass spectra strongly supports the formation of complexes.

3.3. Description of the crystal structures of complexes

In addition to various spectroscopic analyses, the coordination of the ligand to the metal was further established by single crystal X-ray diffraction analysis. The solid-state structures of the mononuclear and dinuclear complexes were established and the ball and stick representation of the complexes along with crystallographic numbering schemes is depicted in the Figures 1-3 and Figures S27-S28. The summary of the crystal data, data collection and structure refinement parameters, are summarized in Table S1, selected bond lengths, bond angles values are listed in Table 1. The crystals of the complexes **1**, **2**, **3**, **4**, **5**, **6**, and **8** suitable for single crystal X-ray diffraction study were obtained by slow diffusion of hexane to the concentrated mixture of acetone and dichloromethane of the compound. Crystal structure of complexes **1**, **3** and **4** contains disordered CH₂Cl₂ molecule in their solved structure. A solvent disordered was observed in the crystal structure of complex **6** and it has been removed by SQUEEZE method. Crystal structures of complexes **5** and **8** have low theta value the structures are presented here to only confirm the composition and binding modes of molecule.

Single crystal analysis further established that the mononuclear complexes of both rhodium and iridium displayed same binding mode whereas, dinuclear complexes displayed different binding modes towards formation of complexes. These half sandwich complexes adopts a typical three legged “piano-stool” geometry in which the metal center are coordinated through two nitrogen atoms from chelating ligand, a terminal chloride and the Cp* moiety. Complex **1**, crystallized in monoclinic with space group *P21/n*, complexes **2**, **3**, and **4** crystallized in

302 monoclinic with space group $P2_1/c$ whereas complex **6** crystallized in triclinic system with space
 303 group $P\bar{1}$. The molecular structure of the mononuclear complexes showed that the coordination
 304 of the ligands occurs through one pyridine nitrogen N(1) and one azine nitrogen N(2) in a bidentate
 305 fashion forming a five membered metallacycle. In all these complexes, Cp* ligands are basically
 306 planar and distance between the metal to centroid of the Cp* ring are in the range of 1.782 – 1.821
 307 Å respectively. The M-N1 bond distances of complexes **1**, **2**, **3**, and **4** were found to be 2.093(5),
 308 2.078(5), 2.085(3), and 2.043(7) and the M-N2 bond distances were found to be 2.145(6), 2.113(4),
 309 2.125(3), and 2.105(7) respectively. Whereas the M-Cl(1) bond distances of complexes **1**, **2**, **3**,
 310 and **4** were found to be 2.420 (2), 2.390 (2), 2.445 (1) and 2.408 (2) respectively. The bond angle
 311 values the N1-M-N2, N1-M-Cl1 and N2-M-Cl1 angles are close to 90° which is consistent to
 312 pseudo octahedral arrangement about the metal center. These bond distances and the bond angle
 313 values are comparable with our previously reported complexes [31, 37]. The single crystal X ray
 314 study indicated that molecular structures have the dinuclear rhodium and iridium complexes shown
 315 different coordination behavior. In rhodium complexes **5** and **6**, ligand (L1) and (L2) functioned
 316 as uninegative tetradentate bridging ligand. In both these complexes the ligand coordinates to first
 317 metal center through pyridyl nitrogen and azine nitrogen and the second metal center through
 318 deprotonated amino and pyrazine nitrogen atom forming a five membered ring with both the metal
 319 center. Whereas, in the case of iridium complexes the ligand acted as tridentate bridging ligand,
 320 the ligand coordinate to the first metal center in a bidentate fashion through pyridyl nitrogen and
 321 one azine nitrogen forming five membered ring and in the second metal center the ligand
 322 coordinate through one pyrazine nitrogen atom. For complex **6** the distance between the Rh(1) to
 323 Cp* ring centroid is 1.818 Å while Rh(2) to Cp* ring centroid distance is 1.792 Å. The M(1)-N
 324 bond distance are 2.091(8) and 2.11(1) Å and the M1-Cl1 distance is 2.412(3) Å while the M(2)-

N bond distance are 2.088(9) and 2.160(9) Å and the M2-Cl2 distance is 2.408(3) Å respectively. The N(2)-N(3) bond distances in dinuclear complexes with deprotonated amino hydrogen is appreciably smaller (1.35 Å) which indicates the delocalization of the negative charge over the N-N bond.

Figure 1. (a) Ball and stick representation of complex **1** (b) Ball and stick representation of complex **2** (c) Ball and stick representation of complex **4**. Hydrogen atoms (except on N4) and counter ion (PF_6) are omitted for clarity.

Figure 2. Ball and stick representation of complex **6**. Hydrogen atoms (except on N4) and counter ion (PF₆) are omitted for clarity.

Figure 3. Ball and stick representation of complex **8**. Hydrogen atoms (except on N4) and counter ion (PF₆) are omitted for clarity.

3.4. Cytotoxicity studies

The cytotoxicity of the ligands and complexes **1-8** against HT 29, HCT-116 p53^{+/+}, HCT-116 p53^{-/-} cancer cell lines and the non-cancer cell line ARPE-19 was investigated compared to the activity of the established anticancer drug cisplatin. The results of chemosensitivity studies and analysis of selectivity indices are presented in Table 2 and figure 4 respectively. Ligands **1-2** and complexes **1** and **6** were inactive against all cell lines tested with IC₅₀ values >100 μM (the highest dose tested). The remaining complexes were cytotoxic to cells *in vitro* but their potency was less than that of cisplatin (figure S29). The lack of activity of the ligands indicates that the activity is solely attributed to the metal-ligand complexes. Out of the compounds evaluated complexes **4** and **5** displays better activity compared to the other complexes, but activity did not superseded the cytotoxicity of standard cisplatin. Despite this lack of potency, complexes **4-5** and **8** selectivity for cancer as opposed to non-cancer cell lines *in vitro* was comparable to that exhibited by cisplatin

(figure 4). Here, complexes **4-5** (and to a lesser extent complexes **7-8**) remain active against the HCT116 p53^{-/-} cells and this is reflected in a good selectivity index for this cell line (figure 4).

Figure 4. Selectivity ratios for complexes **2-5**, **7-8** and cisplatin. The selectivity ratio is defined as the ratio of IC₅₀ values for ARPE-19 divided by the IC₅₀ for each cancer cell line. Values greater than 1 (as indicated by the broken line) represent compounds that are preferentially active against cancer cells compared to non-cancer ARPE-19 cells. As the selectivity ratio is calculated using the mean IC₅₀ values presented in figure S29, there are no error bars on this figure.

Conclusion

In this work, we are unable to isolate arene ruthenium complexes with these ligands however we have successfully synthesized a series of eight Cp* rhodium and Cp* iridium complexes bearing pyrazine base ligands. These complexes were isolated as mononuclear and dinuclear cationic complexes with PF₆ as counter ion. We have examined the coordination behavior of the ligands towards Cp*Rh and Cp*Ir for both dinuclear and mononuclear complexes. In the mononuclear complexes, the ligand act as bidentate in which two-nitrogen atom coordinates

to the metal atom to form a five membered ring. The dinuclear rhodium and iridium complexes exhibited different coordination modes towards the metal atom, in rhodium complexes the ligands coordinate to both rhodium centers as a bidentate fashion, where as in iridium case ligands coordinate to one iridium center in a bidentate fashion and the other in mono-dentate fashion. The formation of dinuclear complexes is due to the coordinative flexibility of the azine ligand around N-N bond. Further the N-N bond distances in dinuclear complex with deprotonated amino hydrogen is smaller which indicates the delocalization of the negative charge over the N-N bond. Of the complexes evaluated complex **4** and **5** were promising in that selectivity towards HT-29 and HCT116 p53^{+/+} cancer cells was as good as that observed for cisplatin. Despite the relative lack of potency compared to cisplatin, the better selectivity of complex **4** and **5** to HCT116 where p53 has been knocked out suggests that these complexes are worth further investigation in cells that have lost p53 tumor suppressor functionality.

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Appendix A. Supplementary data

CCDC **1908856** (**1**), **1908857** (**2**), **1908858** (**3**), **1908859** (**4**), **1908860** (**6**) contains 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 336033

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Table 1. Selected bond lengths (Å) and bond angles (°) of complexes.

Complex	1	2	3	4	6
M(1)-CNT	1.782	1.821	1.790	1.788	1.818
M(2)-CNT	---	---	---	---	1.792
M(1)-N(1)	2.093(5)	2.078(5)	2.085(3)	2.043(7)	---
M(1)-N(2)	2.145(6)	2.113(4)	2.125(3)	2.105(7)	---
M(1)-N(4)	---	---	---	---	2.091(8)
M(1)-N(5)	---	---	---	---	2.11(1)
M(2)-N(1)	---	---	---	---	2.088(9)
M(2)-N(2)	---	---	---	---	2.160(9)
M(1)-Cl(1)	2.420(2)	2.390(2)	2.445(1)	2.408(2)	2.412(3)
M(2)-Cl(2)	---	---	---	---	2.408(3)
N(2)-N(3)	1.397(7)	1.391(6)	1.387(4)	1.38(1)	1.35(1)
N(1)-M(1)-N(2)	76.6(2)	75.9(2)	76.2(1)	75.8(3)	---
N(1)-M(1)-Cl(1)	85.9(1)	84.0(1)	84.34(9)	83.2(2)	---
N(2)-M(1)-Cl(1)	91.3(2)	89.7(1)	92.60(8)	91.1(2)	---
N(4)-M(1)-N(5)	---	---	---	---	75.4(3)
N(4)-M(1)-Cl(1)	---	---	---	---	92.9(2)
N(5)-M(1)-Cl(1)	---	---	---	---	83.7(1)
N(1)-M(2)-N(2)	---	---	---	---	77.0(4)
N(1)-M(2)-Cl(2)	---	---	---	---	85.2(3)
N(2)-M(2)-Cl(12)	---	---	---	---	90.6(2)

CNT represents the centroid of the arene/Cp* ring.

Table 2. Responses of cancer cell lines and ARPE-19 for complexes **1-8** and cisplatin. Each IC₅₀ value represents the mean $\pm$ standard deviation from three independent experiments. The IC₅₀ selectivity index is defined as the mean IC₅₀ for ARPE-19 cells divided by the mean IC₅₀ for cancer cell lines with values greater than 1 representing selective cell kill in cancer cells compared to non-cancer cells.

Compounds	HT-29	Selectivity index	HCT-116 p53+/+	Selectivity index	HCT-116 p53-/-	Selectivity index	ARPE-19
Ligand 1	>100		>100		>100		>100
Ligand 2	>100		>100		>100		>100
Complex 1	>100		>100		>100		>100
Complex 2	52.17 (± 4.78)	1.162545524	41.08 (± 17.74)	1.476387537	46.88 (± 2.15)	1.293728669	60.65 (± 4.78)
Complex 3	>100	0.5401	57.58 (± 5.393)	0.937999305	47.30 (± 7.94)	1.141860465	54.01 (± 9.99)
Complex 4	18.03 (± 3.26)	2.153078203	19.26 (± 0.81)	2.015576324	18.43 (± 0.24)	2.106348345	38.82 (± 13.68)
Complex 5	16.75 (± 2.02)	2.461492537	18.77 (± 1.94)	2.196590304	17.84 (± 0.89)	2.311098655	41.23 (± 15.40)
Complex 6	>100		>100		>100		>100
Complex 7	53.51 (± 2.79)	1.868809568	57.84 (± 2.89)	1.728907331	58.00 (± 5.56)	1.724137931	>100
Complex 8	37.32 (± 2.54)	2.679528403	52.63 (± 4.96)	1.900057002	57.19 (± 0.50)	1.74855744	>100
Cisplatin	2.58(± 0.72)	2.484496124	2.78(± 1.4)	2.305755396	7.52(± 0.64)	0.852393617	6.41(± 0.95)