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Cyclometalated rhodium and iridium complexes with imidazole containing Schiff bases: Synthesis, structure and cellular imaging

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Abstract

Cyclometalated rhodium(III) and iridium(III) complexes (**1–4**) of two Schiff base ligands **L1** and **L2** with the general formula $[M(ppy)_2(Ln)]Cl$ {M = Rh, Ir; ppy = 2-phenylpyridine; $n = 1, 2$; L = Schiff base ligand} have been synthesized. The new ligands and the complexes have been characterized with spectroscopic techniques. Electrochemistry of the complexes revealed anodic behavior, corresponding to an M(III) to M(IV) oxidation. The X-ray crystal structures of complexes **2** and **4** have also been determined to interpret the coordination behavior of the complexes. Photophysical study shows that all the complexes display fluorescence at room temperature with quantum yield of about 3×10^{-2} to 5×10^{-2} . The electronic absorption spectra of all the complexes fit well with the computational studies. Cellular imaging studies were done with the newly synthesized complexes. To the best of our knowledge, this is the first report of organometallic complexes of rhodium(III) and iridium(III) with Schiff base ligands explored for cellular imaging. Emphasis of this work lies on the structural features, photophysical behavior, cellular uptake and imaging of the fluorescent transition metal complexes.

Keywords

Rhodium; Iridium; Schiff base; Fluorescence; Cellular imaging

1 Introduction

The photophysical and photochemical studies of organometallic d^6 transition metal complexes, having nitrogen donor ligands, has attracted a lot of attention mainly due to the significant emissive properties of these complexes. These excited state properties can be

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readily tuned by altering the metal center (Ru(II), Os(II), Ir(III), Rh(III), etc.) as well as the ligand framework [1–10]. While ruthenium and osmium complexes with nitrogen donor ligands in particular has received a wealth of attention in their applications as photosensitizer, production of solar fuels by artificial photosynthesis, light driven molecular machines, photoinduced intermolecular energy and electron-transfer processes and decomposition of water [10–23], the isoelectronic iridium(III) analogs started receiving serious attention only recently [24–34]. In particular, luminescent organometallic iridium(III) complexes has generated increasing interest because of their applications as phosphors in organic light emitting cells and diodes (OLEC and OLEDs) [35–40], sensors [41–43] and luminescent labels for biomolecules [44–52]. However rhodium complexes, apparently due to their lack of rich photophysical properties have not been sufficiently studied like their iridium analogs [53–56].

Ligand design is also crucial for the transition metal chelating core to maintain the physico-chemical properties of the core such as fluorescence, photoactivity and cytotoxicity [57–58]. Our work utilizes Schiff bases as ligands which are of great interest for their tolerance towards various organic functionalities and cellular environments. The Schiff bases can be easily synthesized and shows a wide range of complexing ability with transition metals widening their scope for use in various fields of biological chemistry [59–64]. Selective hydrolysis of Schiff base by biological systems can generate alkylating agents for nucleic acids and at the same time the generated amines can act as antimetabolites. The Schiff bases are also active intermediaries for various heterocyclic systems of biological importance [65].

Fluorescence imaging offers a unique approach for visualizing morphological details inside the cell. Polyaromatic organic chromophores are most widely used as probes in cellular imaging, as they are capable of passive cell delivery and are used across cell biology, molecular biology, microbiology, and flow cytometry applications. However, the usage of these compounds as chromophores in cellular imaging is often complicated by short lifetimes, resulting in photobleaching and loss of signal. Luminescent metal complexes are advantageous compared to the organic fluorophores as they are photostable having relatively long lifetimes, and show significant Stokes shifts for easy separation of excitation and emission wavelengths. These unique photophysical properties predominantly in heavy transition metal complexes make them potentially valuable as probes for cellular imaging.

It is interesting to note that, the organometallic iridium(III) complexes have been exploited recently as bioimaging probes [27,30,45–52], there has been a single report of rhodium complex in this field of study [53]. The present work introduces transition metal Schiff base complexes in the field of cellular imaging along with the application of rhodium complexes which is both a first of its kind. We have chosen imidazole fragment as part of the Schiff base ligand specifically in our studies, as imidazole has been part of several amino acid sequences in biological systems. The incorporation of the imidazole fragment, a biologically accepted pharmacophore bound to the periphery of the metal complexes can also influence the solubility of the compounds affecting the biological compatibility and in turn influences the activity and targeting of the metal complexes they are bound to [66–69]. The redox active rhodium and iridium have tremendous applicability in the catalysis [70–73] and its catalytic potential can be utilized within the cellular environment along with cellular

imaging. The biocompatibility was checked with *in vitro* cytotoxicity assay using MTT. This paper presents synthesis, characterization and TDDFT studies of four rhodium and iridium complexes with simple imidazole containing Schiff base ligands along with the cellular imaging studies in MCF7 human breast carcinoma cells.

2 Experimental

2.1 Materials

The starting materials $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$, $\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$, 2-phenylpyridine, 8-aminoquinoline, 1-naphthylamine and 1-methyl-2-imidazolecarboxaldehyde were purchased from Sigma–Aldrich and used without further purification. All the solvents were dried by usual methods prior to use. The cyclometalated iridium(III) and rhodium(III) chloro bridged dimer $[\text{Ir}(\text{ppy})_2\text{Cl}]_2$ and $[\text{Rh}(\text{ppy})_2\text{Cl}]_2$ was prepared according to the literature methods [74–75].

2.2 Synthesis of ligand

2.2.1 L1 —To a solution of 1-methyl-2-imidazolecarboxaldehyde (2.0 mmol) in methanol (20 mL) was added 8-aminoquinoline (2.0 mmol) in absolute methanol (20 mL). A red coloration was formed immediately. The mixture was heated to 60 °C and stirred for 0.5 h, the solvent was evaporated to dryness to obtain red solid which was washed with cold ethanol and dried in vacuum to obtain the desired compound. The product was characterized by ^1H NMR, ^{13}C NMR and mass spectrometry. Yield: 415.0 mg (88%). *Anal.* Calc. for $\text{C}_{14}\text{H}_{12}\text{N}_4$: C, 71.17; H, 5.12; N, 23.71. Found: C, 71.02; H, 5.04; N, 23.88%. ESI-MS: 236.32 (M^+). ^1H NMR (400 MHz, CDCl_3 , δ ppm): 4.27(3H, s), 7.05(1H, s), 7.29–7.31(1H, d, $J = 7.36$ Hz), 7.42–7.45(1H, q, $J = 4.28$ Hz), 7.53–7.56(1H, t, $J = 7.32$ Hz), 7.67–7.69(1H, dd, $J = 7.96$ Hz, 1.24 Hz), 8.16–8.19(1H, dd, $J = 8.56$ Hz, 1.84 Hz), 8.67(1H, s), 8.93–8.95(1H, q, $J = 1.84$ Hz), 9.82(1H, s). ^{13}C NMR (100 MHz, CDCl_3 , δ ppm): 35.41, 106.49, 109.61, 115.27, 115.44, 120.91, 121.07, 126.52, 127.03, 128.38, 135.58, 143.73, 146.96, 282.58. IR (cm^{-1} , KBr pellet): 748.31, 791.86, 820.86, 1125.80, 1379.31, 1479.54, 1514.40, 1576.44.

2.2.2 L2 —To a solution of 1-methyl-2-imidazolecarboxaldehyde (2.0 mmol) in methanol (20 mL) was added 1-naphthylamine (2.0 mmol) in absolute methanol (20 mL). A light red coloration was formed immediately. The mixture was heated to 60 °C and stirred for 0.5 h, the resulting light red liquid was washed with cold ethanol and dried in air to obtain the desired compound. The product was characterized by ^1H NMR, ^{13}C NMR and mass spectrometry. Yield: 432.0 mg (92%). *Anal.* Calc. for $\text{C}_{15}\text{H}_{13}\text{N}_3$: C, 76.57; H, 5.57; N, 17.86. Found: C, 76.64; H, 5.60; N, 17.94%. ESI-MS: 235.45(M^+). ^1H NMR (400 MHz, CDCl_3 , δ ppm): 4.29(3H, s), 7.09(1H, s), 7.10–7.12(1H, dd, $J = 7.32$ Hz, 0.92 Hz), 7.46–7.48(1H, d, $J = 7.8$ Hz), 7.49–7.56(3H, m), 7.74–7.76(1H, d, $J = 8.24$ Hz), 7.85–7.88(1H, m), 8.28–8.30(1H, m), 8.65(1H, s). ^{13}C NMR (100 MHz, CDCl_3 , δ ppm): 35.36, 112.03, 123.25, 125.32, 125.48, 125.74, 125.96, 126.00, 127.35, 128.43, 129.64, 133.53, 143.15, 147.83, 150.82. IR (cm^{-1} , KBr pellet): 774.01, 795.30, 836.15, 1150.60, 1386.52, 1429.85, 1477.87, 1570.54, 1622.00.

2.3 Synthesis of complexes

2.3.1 [Ir(ppy)₂L1]Cl(1)—A solution of [Ir(ppy)₂Cl]₂ (0.10 mmol) and **L1** (0.15 mmol) in dichloromethane-methanol (30 mL, 1:1 v/v) was stirred. After 4 h, the reddish-brown solution was evaporated to dryness under reduced pressure. The crude product was applied to a silica gel column and eluted with dichloromethane-methanol (9:1) to afford a reddish-brown solid. Yield: 65.2 mg (84%). *Anal.* Calc. for IrC₃₆H₂₈ClN₆: C, 55.99; H, 3.65; N, 10.88. Found: C, 56.03; H, 3.74; N, 10.72%. ESI-MS: 773.22 (M-Cl)⁺. ¹H NMR (500 MHz, CDCl₃, δ ppm): 4.43(3H, s), 5.96–5.97(1H, dd, *J* = 7.55 Hz, 0.95 Hz), 6.23–6.24(1H, d, *J* = 7.60 Hz), 6.37–6.40(1H, dt, *J* = 7.55 Hz, 1.25 Hz), 6.51–6.54(1H, dt, *J* = 7.55 Hz, 1.25 Hz), 6.65(1H, d, *J* = 0.95 Hz), 6.68–6.70(1H, dd, *J* = 7.55 Hz, 1.25 Hz), 6.76–6.79(1H, dt, *J* = 7.25 Hz, 1.25 Hz), 6.90–6.93(1H, dt, *J* = 7.90 Hz, 1.25 Hz), 6.96–6.99(1H, t, *J* = 7.55 Hz), 7.05–7.06(1H, dd, *J* = 7.90 Hz, 0.95 Hz), 7.16–7.22(2H, m), 7.27–7.30(1H, q, *J* = 4.40 Hz), 7.43–7.44(2H, d, *J* = 8.50 Hz), 7.48(1H, d, *J* = 1.25 Hz), 7.58–7.60(1H, dd, *J* = 7.85 Hz, 1.25 Hz), 7.62–7.65(1H, t, *J* = 7.73 Hz), 7.74–7.78(1H, dt, *J* = 8.20 Hz, 1.60 Hz), 7.83–7.85(1H, d, *J* = 7.90 Hz), 7.94–7.95(1H, d, *J* = 5.05 Hz), 7.96–7.98(1H, dd, *J* = 8.50 Hz, 1.55 Hz), 8.60–8.61(1H, dd, *J* = 4.05 Hz, 1.55 Hz), 9.33–9.34(1H, d, *J* = 5.05 Hz), 9.50(1H, s). ¹³C NMR (125 MHz, CDCl₃, δ ppm): 36.33, 118.47, 118.83, 119.78, 120.31, 121.39, 121.99, 122.80, 123.21, 123.55, 124.11, 124.58, 125.53, 125.83, 126.21, 126.84, 127.75, 127.95, 129.09, 129.59, 129.74, 131.56, 131.62, 133.33, 137.46, 137.63, 143.01, 143.13, 143.69, 146.36, 149.27, 149.71, 150.98, 151.32, 157.27, 167.19, 168.09. IR (cm⁻¹, KBr pellet): 760.36, 1036.36, 1163.52, 1317.12, 1419.90, 1478.70, 1583.95, 1606.47.

2.3.2 [Ir(ppy)₂L2]Cl (2)—A solution of [Ir(ppy)₂Cl]₂ (0.15 mmol) and **L2** (0.10 mmol) in dichloromethane-methanol (30 mL, 1:1 v/v) was stirred. After 4 h, the yellow solution was evaporated to dryness under reduced pressure. The crude product was applied to a silica gel column and eluted with dichloromethane-methanol (14:1) to afford a yellow solid. Yield: 67.4 mg (87%). *Anal.* Calc. for IrC₃₇H₃₀ClN₅: C, 57.54; H, 3.92; N, 9.07. Found: C, 57.66; H, 3.98; N, 9.14%. ESI-MS: 772.20 (M-Cl)⁺. ¹H NMR (500 MHz, CDCl₃, δ ppm): 4.43(3H, s), 6.09–6.10(1H, d, *J* = 6.60 Hz), 6.27–6.29(1H, d, *J* = 6.90 Hz), 6.49–6.52(1H, t, *J* = 7.25 Hz), 6.55–6.58(1H, t, *J* = 6.95 Hz), 6.72(1H, s), 6.77–6.80(1H, t, *J* = 7.55 Hz), 6.81–6.82(1H, d, *J* = 7.60 Hz), 6.91–6.94(1H, t, *J* = 7.25 Hz), 6.97–7.02(4H, m), 7.17–7.20(2H, m), 7.24–7.27(1H, m), 7.36–7.38(1H, t, *J* = 6.00 Hz), 7.42(1H, s), 7.43–7.45(1H, d, *J* = 8.80 Hz), 7.53–7.56(1H, t, *J* = 7.25 Hz), 7.61–7.62(2H, d, *J* = 7.85 Hz), 7.79–7.82(1H, t, *J* = 7.25 Hz), 7.87–7.89(1H, d, *J* = 7.90 Hz), 8.10–8.11(1H, d, *J* = 5.35 Hz), 8.96–8.97(1H, d, *J* = 5.65 Hz), 9.47(1H, s). ¹³C NMR (125 MHz, CDCl₃, δ ppm): 36.31, 118.61, 118.53, 121.23, 121.33, 121.99, 122.85, 122.97, 123.13, 123.28, 123.98, 125.56, 126.72, 127.52, 128.31, 129.01, 129.12, 129.79, 131.90, 136.03, 137.32, 137.43, 140.50, 143.08, 143.73, 144.04, 146.20, 149.57, 149.68, 150.20, 151.24, 152.55, 157.51, 167.34, 167.63. IR (cm⁻¹, KBr pellet): 759.41, 797.64, 1063.26, 1162.65, 1268.24, 1305.95, 1438.72, 1478.09, 1584.04, 1606.16.

2.3.3 [Rh(ppy)₂L1]Cl (3)—A solution of [Rh(ppy)₂Cl]₂ (0.10 mmol) and **L1** (0.15 mmol) in dichloromethane-methanol (30 mL, 2:1 v/v) was heated to reflux. After 4 h, the brown solution was cooled to room temperature, and then the solution was evaporated to dryness under reduced pressure. The crude product was applied to a silica gel column and

eluted with dichloromethane-methanol (9:1) to afford a brown solid. Yield: 48.2 mg (70%). *Anal.* Calc. for $C_{36}H_{29}ClN_6Rh$: C, 63.21; H, 4.27; N, 12.29. Found: C, 63.30; H, 4.45; N, 12.35%. ESI-MS: 684.20 (M-Cl)⁺. ¹H NMR (500 MHz, CDCl₃ δ ppm): 4.27(3H, s), 6.09–6.11(1H, d, *J* = 7.25 Hz), 6.25–6.27(1H, d *J* = 7.90 Hz), 6.52–6.56(1H, dt, *J* = 7.25 Hz, 1.25 Hz), 6.56–6.59(1H, dt, *J* = 7.25 Hz, 1.55 Hz), 6.72(1H, s), 6.79–6.82(1H, dt *J* = 7.60 Hz, 1.30 Hz), 6.85–6.86(1H, dd, *J* = 7.55 Hz, 1.55 Hz), 6.92–7.01(4H, m), 7.21–7.25(3H, m), 7.35–7.38(1H, m), 7.41–7.44(2H, m), 7.58–7.64(3H, m), 7.84–7.85(2H, m), 8.05–8.07(1H, d, *J* = 5.35 Hz), 8.86–8.87(1H, d, *J* = 5.65 Hz), 9.09(1H, s). ¹³C NMR (125 MHz, CDCl₃, δ ppm): 35.69, 118.46, 118.79, 121.24, 121.36, 122.29, 122.85, 122.88, 122.96, 123.08, 123.76, 125.55, 126.52, 127.29, 127.74, 128.37, 128.85, 129.17, 129.51, 131.38, 132.78, 135.92, 136.02, 137.56, 137.64, 143.02, 143.45, 146.20, 149.83, 151.70, 156.07, 164.27, 164.62, 167.67, 167.99, 182.04. IR (cm⁻¹, KBr pellet): 759.31, 792.12, 1026.37, 1163.24, 1314.90, 1419.27, 1481.24, 1604.40.

2.3.4 [Rh(ppy)₂L2]Cl (4)—A solution of [Ir(ppy)₂Cl]₂ (0.10 mmol) and **L2** (0.15 mmol) in dichloromethane-methanol (30 mL, 2:1 v/v) was heated to reflux. After 4 h, the yellow solution was cooled to room temperature and the solution was evaporated to dryness under reduced pressure. The crude product was applied to a silica gel column and eluted with dichloromethane-methanol (14:1) to afford a yellow solid. Yield: 46.1 mg (67%). *Anal.* Calc. for $C_{37}H_{30}ClN_5Rh$: C, 65.06; H, 4.43; N, 10.25. Found: C, 65.14; H, 4.49; N, 10.35%. ESI-MS: 683.78 (M-Cl)⁺. ¹H NMR (500 MHz, CDCl₃ δ ppm): 4.35(3H, s), 6.00–6.02(1H, d, *J* = 7.55 Hz), 6.25–6.26(1H, d, *J* = 7.55 Hz), 6.45–6.48(1H, dt, *J* = 7.25 Hz, 1.60 Hz), 6.59–6.63(1H, dt, *J* = 7.55 Hz, 0.95 Hz), 6.69–6.70(1H, d, *J* = 6.30 Hz), 6.72(1H, s), 6.81–6.85(1H, dt, *J* = 7.55 Hz, 1.25 Hz), 6.96–7.01(2H, q, *J* = 7.55 Hz), 7.12–7.13(1H, d, *J* = 6.35 Hz), 7.22–7.25(3H, m), 7.28–7.31(1H, q, *J* = 4.10 Hz), 7.62–7.64(1H, dd, *J* = 7.55 Hz, 1.25 Hz), 7.71–7.74(1H, dt, *J* = 7.60 Hz, 1.55 Hz), 7.84–7.85(2H, m), 7.93–7.94(1H, d, *J* = 5.65 Hz), 7.98–8.00(1H, dd, *J* = 8.20 Hz, 1.55 Hz), 8.62–8.63(1H, dd, *J* = 4.40 Hz, 1.55 Hz), 9.23(1H, s), 9.25–9.26(1H, d, *J* = 5.35 Hz). ¹³C NMR (125 MHz, CDCl₃, δ ppm): 35.96, 118.76, 119.11, 119.27, 120.75, 122.42, 122.93, 122.98, 123.07, 123.54, 123.90, 124.75, 125.61, 125.75, 126.15, 126.75, 127.78, 127.92, 129.28, 129.56, 132.58, 132.74, 133.48, 137.68, 137.83, 143.03, 143.58, 143.79, 146.19, 149.63, 150.83, 156.05, 164.33, 164.61, 165.16, 167.50, 167.76. IR (cm⁻¹, KBr pellet): 758.52, 797.40, 1025.94, 1162.58, 1269.82, 1314.04, 1417.89, 1487.98, 1486.44, 1604.12.

2.4 X-ray crystallography

Crystal Data were collected on a Bruker SMART APEXII CCD area-detector diffractometer using graphite monochromated Mo K α radiation (λ = 0.71073 Å). For both the crystals, X-ray data reduction was carried out using the Bruker SAINT program. The structures were solved by direct methods using the SHELXS-97 program and refinement using SHELXL-97 program. Selected crystal data and data collection parameters for all the complexes are given in Table 1. X-ray data reduction, structure solution and refinement were done using the SHELXL-97 program package [76].

[Ir(ppy)₂L2]Cl (2) and **[Rh(ppy)₂L2]Cl (4)**: Both the single crystals were obtained by slow evaporation of dichloromethane-methanol mixture solution.

2.5 Physical measurements

IR spectra were obtained on a Perkin-Elmer Spectrum RXI spectrophotometer with samples prepared as KBr pellets. Elemental analyses were performed on a Perkin-Elmer 2400 series II CHN series. Electronic spectra were recorded on a U-4100, HITACHI spectrometer. ^1H NMR spectra were obtained on a Bruker Avance III-500 NMR spectrometer using TMS as the internal standard. Electrochemical measurements were made using a PAR model 273 potentiostat. A platinum disk working electrode, a platinum wire auxiliary electrode and an aqueous saturated calomel reference electrode (SCE) were used in a three electrode configuration. Electrochemical measurements were made under a dinitrogen atmosphere. All electrochemical data were collected at 298 K and are uncorrected for junction potentials. Fluorescence spectra were taken on a HORIBA JOBINYVON spectrofluorimeter. Mass spectra were recorded on a Q-ToF Micromass spectrometer by positive ion mode electrospray ionization. Quantum yield data reported here were measured relative to Quinine sulfate in 0.1 M H_2SO_4 ($\lambda_{\text{ex}} = 350 \text{ nm}$, $\Phi = 0.577$). The integration of the emission spectra were obtained from the Fluoromax-3 instrument directly. All the computations are carried out using the GAUSSIAN 03 software [77]. The hybrid B3LYP functional [78] were employed along with LanL2DZ/6-31+g (d,p) as basis set for all the calculations. Geometry optimization carried out until global minima are achieved. TDDFT in solution were performed in dichloromethane solution using the conductor-like polarizable continuum model with basis set LanL2DZ/6-31+g (d,p). The accuracy and reliability of spin-restricted time-dependent density functional theory have a good match with experimental data.

2.6 Cellular imaging

2.6.1 Cellular imaging with confocal microscopy—Subconfluent MCF7 cells grow on glass coverslips in 24-well plates were treated with the complexes and precursors at 10 μM final concentration from 1 mM stock solutions in 30% DMSO for 1 h. Cells were also treated with DMSO as vehicle control. After treatment, cells were fixed with 4% paraformaldehyde in PBS and observed under a laser scanning confocal microscope (Zeiss LSM710) at 405 nm excitation wavelength and emission between 550 and 610 nm.

2.6.2 Cytotoxicity assay—*In vitro* cytotoxicity of the compounds was measured using methyl thiazolyl tetrazolium (MTT) assay. 0.5×10^4 MCF7 cells/well were seeded in a 96 well cell-culture plate in DMEM and kept for 24 h at 37 °C in 5% CO_2 . Complexes were added at concentrations of 1, 10, 25 and 50 μM in treatment groups and maximum concentration of DMSO in treatment group (0.005%) was added in vehicle control group. Cells were incubated for another 24 h. MTT was added to final concentration of 0.2 mg/ml to each well and incubated for additional 4 h. Media was removed and cells were ruptured and the MTT product solubilized by adding 100 μl of 0.1 N HCl in isopropanol to each well. OD was measured using an ELISA reader (BioTek ELx800) at 515 nm. Data were normalized against control group (no treatment) and represented as mean of three replicates with standard deviation.

3 Results and Discussion

3.1 Synthesis

The chloro-bridged dimeric complexes $[M(ppy)_2Cl]_2$ ($M = Rh$ or Ir ; $ppy = 2$ -phenylpyridine) are indispensable organometallic precursors to synthesize interesting luminescent complexes [59]. Reaction of **L1** and **L2** (Scheme 1) with $[M(ppy)_2Cl]_2$ [$M = Ir$, Rh] in dichloromethane-acetonitrile proceed with chloro-bridge cleavage reactions leading to the formation of four interesting cationic mononuclear complexes $[M(ppy)_2L]^+$ [$M = Rh$ or Ir ; $L = L1, L2$] (**1–4**) and separated as chloride salt for further study. The temperature during the synthesis is controlled to position the two nitrogen atoms of the C^N ligand in a mutually *trans* configuration. Prolonged reflux at elevated temperature will promote an isomer with a *cis*-N,N configuration [25,79]. The Schiff base ligands were synthesized by the condensation of 8-aminoquinoline and 1-naphthylamine with 1-methyl-2-imidazolecarboxaldehyde, respectively. These synthesized cationic complexes were purified by column chromatography and all the complexes were collected as chloride salt. They were characterized by a range of standard spectroscopic and spectrometric techniques like 1H NMR, ^{13}C NMR, ESI-MS, IR and elemental analysis. The molecular structures of **2** ($2 \cdot CH_3OH$) and **4** ($4 \cdot CHCl_2, CH_2Cl_2$) were confirmed by X-ray crystallographic study.

3.2 X-ray crystallography

The single crystal of **2** ($2 \cdot CH_3OH$) and **4** ($4 \cdot CHCl_2, CH_2Cl_2$), suitable for X-ray diffraction studies, were obtained from dichloromethane-methanol solution. The ORTEP diagrams and atom numbering schemes of **2** ($2 \cdot CH_3OH$) and **4** ($4 \cdot CHCl_2, CH_2Cl_2$) are presented in Fig. 1. Crystallographic data are summarized in Table 1 and selected bond lengths and bond angles are listed in Table 2. Computational studies have been carried out in the ground state. The optimized geometries for the complexes **1** and **3**, computed in solution phase are shown in Fig. 2 and the geometrical parameters are summarized in Table 2 together with the available X-ray data.

The molecular structures of **2** and **4** show that the metal center adopts distorted octahedral coordination geometry where, N atoms from 2-phenylpyridine (C^N) ligands are *trans* to each other. This is quite common in previously reported similar type of complexes. As we expected, the two nitrogen atoms on **L2** ligand coordinate well with the centered iridium(III) ion because of the strong affinity of iridium for nitrogen. The crystal structure of complex **2** and **4** consists of discrete $[M(ppy)_2(L2)]^+$ [$M = Rh$ or Ir] cations and chloride anions with no interionic contacts. The M^{III} center is coordinated in a slightly distorted octahedral fashion with the two N-donor groups of Schiff base ligands adopting a *cis* configuration and the metalated C atoms of the ppy ligands are in a mutually *cis*-arrangement. All bond lengths and bond angles are within normal ranges. The average value of the Rh–C distances is 1.9955 (5) Å, and the average Rh–N distances are 2.0375 (4) Å for the ppy, 2.196 (4) Å for Rh–N(imine) and 2.178 (4) Å for Rh–N(imidazole of L2 ligand), respectively. The significant difference between the Rh–N(ppy) and the Rh–N(Schiff N–N) distances indicates a strong *trans* influence of the Rh–C bonds on the Rh–N(**L2**) bonds. The strong *trans* influence of the phenyl groups also results in slightly longer Ir–N (N^N ligands) bond lengths than the distances of Ir–N(ppy). The average value of the Ir–C distances is 2.033 (4)

Å, and the average Ir–N distances are 2.049 (5) Å for the ppy, 2.167 (10) Å for Ir–N(imine) and 2.137 (5) Å for Ir–N(imidazole of **L2**), respectively. The bond angles and bond lengths (± 0.09 Å) of the geometrically optimized structure of **1** and **3** (Fig. 2) are almost in the same range of **2** and **4** (Table 2).

3.3 Electronic spectral properties

The electronic absorption and emission spectral data for complexes **1–4** are summarized in Table 3 and comparative absorption and emission spectra of complexes (**1–4**) (excitation at 330 nm) were presented in Fig. 3. The electronic absorption spectra of **1–4** show intense absorption bands and shoulders in the range of 252–292 nm, together with less intense low-energy absorption bands and shoulders at around 358–474 nm. The high-energy absorption bands with very high extinction coefficient observed around 252–259 and 292–294 nm assign ($\epsilon = 22000\text{--}32000\text{ M}^{-1}\text{ cm}^{-1}$) in the complexes have been assigned to spin-allowed intra-ligand (^1IL) ($\pi \rightarrow \pi^*$) transitions. The next higher wavelength absorptions occurring around 353–373 nm ($\epsilon = 7300\text{--}9500\text{ M}^{-1}\text{ cm}^{-1}$) have been assigned to spin allowed ^1IL transitions ($^1\pi \rightarrow \pi^*$), from both the ppy and Schiff base ligands. The complexes exhibit absorption peak in the range of 377–474 nm with low molar extinction coefficients ($\epsilon = 900\text{--}1300\text{ LM}^{-1}\text{ cm}^{-1}$ for iridium complexes; $7000\text{--}7500\text{ LM}^{-1}\text{ cm}^{-1}$ for rhodium complexes), can be attributed to charge transfer $d\pi$ ($\text{M} \rightarrow \pi^*$) ($^1\text{MLCT}$) transition. There may be contribution from spin-forbidden $^3\text{MLCT}$ and $^3\text{LLCT}$ transitions. The frontier orbitals of **1** and **2** (computation done in solution phase) are shown in Fig. 4, whereas the orbital energies and compositions in terms of atomic contributions are reported in Table 3.

A comparison of the experimental and calculated absorption spectral data for lowest energy absorption is given in Table 4. It shows that the computed data are in agreement with the experimental data for each of the four complexes. We observe a variation of 12–18 nm for all the absorption bands. Such differences are within the typical accuracy of TDDFT calculations for MLCT excitations in transition metal complexes. The key transition for the complexes with ligand **L1** with both rhodium and iridium is from HOMO/HOMO-1 $\rightarrow$ LUMO, whereas, the complexes with ligand **L2**, HOMO-2 $\rightarrow$ LUMO also have significant contribution. Further inspection shows that the three highest occupied MOs (HOMO, HOMO-1 and HOMO-2) in all the four complexes are mainly metal d-orbital (iridium and rhodium) (Table 5) in character but significant contributions come from the p-orbitals of the coligand 2-phenylpyridine, with very little contributions from Schiff base ligands **L1** and **L2**. So, the low energy band may be assigned to spin-forbidden $^3\text{MLCT}$ and $^3\text{LLCT}$ transitions along with $^1\text{MLCT}$ transition [80]. The strong covalency of the carbon–metal bonds attributes to the mixed character of HOMO being delocalized on the metal and on the cyclometalating ligand [65–67]. The LUMOs are mainly (77.21–82.65%) composed of Schiff base ligand **L1** and **L2** and contributions from metal d-orbitals are not insignificant. The highest metal contribution is 17.13 in **2** (LUMO), 14.45 for **4** (LUMO+1) and again 11.24 for **2** (LUMO+2). The emission spectra of the complexes have been studied at 300 K in acetonitrile solution. At room temperature, on excitation at low-energy band, all the complexes except **3** (only at 446 and 460 nm) show three maxima at around 445, 460 and 494–514 nm with quantum yields of 5.2×10^{-2} , 3.3×10^{-2} , 3.5×10^{-2} , 4.1×10^{-2} and lifetime for the complexes are ligand dependent. So, the broad and featureless emission with

short lifetimes and weak vibronic progression for the complexes can be attributed to ppy-based ^3LC emission [68].

Recent work on exploration of organometallic iridium complexes in the field of bioimaging coupled with the lifetime in the nanosecond range for all the complexes prompted us to investigate cellular uptake and localization of all the complexes within cellular environment along with the ligands. The detail regarding the imaging has been discussed in the latter part of the manuscript.

3.4 Electrochemical studies

Cyclic voltammetry (CV) was used to identify the electrochemical behavior of compound **1–4**. Electrochemical studies of compounds **1–4** were performed in dichloromethane solution and their redox potentials are summarized in Table 3. Complexes on oxidation exhibit an irreversible metal-based oxidation peak at 1.43 V for iridium and 1.52 V for rhodium. The oxidation peak around 1.15 V in the complexes **2** and **4** is ligand based and absent in the complexes containing 8-aminoquinoline (**L1**) containing Schiff base. The origin of reduction and oxidation are assigned from the HOMO and LUMO population. For all the complexes there occurs metal oxidation, as HOMO possesses metal character. Since the metal contribution is predominant in the HOMO of all four complexes, first oxidation is assigned to metal oxidation. The irreversible reductive response observed around $(-0.80) - (-0.99)$ V may be assigned to reduction of the imine fragment in the coordinated ligands as imine-ligands have predominant contribution in LUMO.

3.5 Cellular imaging and cytotoxicity assay

Cellular uptake and localization of each of the four complexes (**1–4**) was investigated by incubating MCF7 breast carcinoma cells with 10 μM final concentration of each complex dissolved in 30% DMSO in water for 1 h (Fig. 5). Cells were also incubated with the precursor Schiff base ligand and cyclometalated chlorobridged complex as control. The cells were observed by confocal microscopy under 405 nm excitation wavelength. The excitation wavelength corresponded to the minimum wavelength of the confocal system. While the Schiff bases failed to show any fluorescence the chlorobridged metal complex showed fluorescence and was distributed throughout the cell. All the four Schiff base-organometallic complexes showed cellular fluorescence of different intensities. However, interestingly all of them were localized in the cytoplasm and completely excluded from the nucleus. Especially, complex **2** showed high fluorescence intensity, and precise localization in the cytoplasm. This suggests that the association of the Schiff base ligand to the organometallic precursor restricts the resultant complex in the cytoplasm. This provides a very good tool for visualizing the cytoplasmic compartment specifically, and would also allow the delivery of bioactive groups to the cytoplasm to regulate cytoplasmic processes such as mRNA translation, vesicular trafficking or glycolysis. Further modifications in the side-chains might allow the generation of compounds with localization property in specific sub-cellular compartments, depending on the properties, such as pH, of the latter. Although this has been achieved for Ir complexes with polypyridyl ligands, it is yet to be explored for Schiff base ligands [44,81].

Any compound with a potential for use in bioimaging should have low cytotoxicity. Therefore, cytotoxicity was investigated by incubating cells with 1, 10, 20 and 50 μM of each compound. Cells were also treated with 0.005% DMSO as vehicle control. Cell survival was measured by MTT assay after 24 h of incubation with the complexes (Fig. 6). At 1 μM concentration none of the compounds exhibited any cytotoxic effect. At 10 μM concentration, at which the cellular imaging was performed, there was about 20% decrease in cell survival, which did not decrease beyond 40% for even the highest concentration (50 μM) of the compounds. This suggested that these compounds have very low cytotoxicity and are therefore appropriate for development as molecular tools for bioimaging.

4 Conclusions

The present study shows that four specially designed imidazole containing Schiff base ligands on formation of organometallic rhodium and iridium complexes capable of cellular imaging. The Schiff base ligands have been chosen to take the advantage of ease of synthesis as well as ease of hydrolysis of the ligands. It would be extremely useful if we could use the transition metal Schiff base complexes as bioimaging probe instead of polypyridyl based complexes. The implications are twofold, firstly, it is very easy to synthesize the Schiff base ligands and more importantly they hydrolyse quite easily to simple amines. Surprisingly, cellular imaging with organometallic rhodium complexes has not been explored. The introduction of rhodium Schiff base complexes would definitely be of importance, if we can exploit the catalytic properties with cellular imaging.

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Fig. 1.
Molecular structure of **2** and **4** with atoms labeled and thermal ellipsoids at the 30% probability level. All the hydrogen atoms and solvent molecules are omitted for clarity.

Fig. 3.
(a) absorption and (b) emission excited at 339 nm of complexes (1–4).

Fig. 4.
HOMO and LUMO distributions of complexes **1–2**.

Fig. 5. Confocal microscopy image of MCF7 human breast carcinoma cells treated with DMSO, ligand **L1** and complexes **1–4**. The left panels consist of the fluorescence images (Ex 405 nm, Em 550 to 610 nm), middle panel consists of bright field images and the right panel consists of superimposed images.

Fig. 6.

Cytotoxicity assay of MCF7 cells treated with complexes **1–4**. Cytotoxicity was estimated using MTT assay. Values are normalized to no-treatment control and represent mean of three replicates.

Scheme 1.

Molecular structure of ligands **L1**, **L2** and complexes **1-4**.

Table 1Crystallographic data for **2·CH₃OH** and **4·CHCl₂, CH₂Cl₂**.

	2·CH₃OH	4·CHCl₂, CH₂Cl₂
Empirical formula	C ₃₈ H ₃₃ ClIrN ₅ O	C ₃₉ H ₃₂ Cl ₅ N ₅ Rh
Formula weight	803.36	850.86
Space group	monoclinic, <i>P</i> 2 ₁	monoclinic, <i>P</i> 2 ₁ / <i>n</i>
<i>a</i> (Å)	10.9113(14)	10.8173(10)
<i>b</i> (Å)	10.4373(13)	10.2712(7)
<i>c</i> (Å)	17.508(2)	33.797(3)
β (°)	103.401(2)	94.670(2)
<i>V</i> (Å ³)	1939.6(4)	3742.6(5)
<i>Z</i>	2	4
Crystal size (mm ³)	0.35 × 0.24 × 0.11	0.28 × 0.24 × 0.15
Color	red	yellow
<i>T</i> (K)	296	100
μ (mm ⁻¹)	3.544	0.850
Absorption correction method	multi-scan	multi-scan
<i>T</i> _{min} / <i>T</i> _{max}	0.360/0.667	0.788/0.880
Data/parameters	7535/205	6570/461
θ Range (°)	1.20–27.00	1.94–25.08
ρ_{max} , ρ_{min}	1.211, –1.003	1.400, –1.427
Final <i>R</i> indices [<i>I</i> ² > 2σ(<i>I</i> ²)]	<i>R</i> ₁ = 0.0499 <i>wR</i> ₂ = 0.1410	<i>R</i> ₁ = 0.0456 <i>wR</i> ₂ = 0.0942
Final <i>R</i> indices (all data)	<i>R</i> ₁ = 0.0550 <i>wR</i> ₂ = 0.1482	<i>R</i> ₁ = 0.0718 <i>wR</i> ₂ = 0.1079
Goodness-of-fit (GOF)	1.110	1.027

Table 2

Key bond lengths [$\text{\AA}$] and angles [$^\circ$] of complexes **2**·CH₃OH and **4**·CHCl₂, CH₂Cl₂ (X-ray crystallographic data) and **1**, **3** derived from geometrically optimized structure.

Bond lengths	Bond angles	Bond angles
2·CH₃OH		
Ir1–N1 2.048(3)	N1–Ir1–N2 174.1(4)	N2–Ir1–C12 79.0(3)
Ir1–N2 2.050(6)	N1–Ir1–N3 86.0(3)	N3–Ir1–N5 76.7(4)
Ir1–N3 2.137(5)	N1–Ir1–N5 99.1(3)	N3–Ir1–C11 100.9(2)
Ir1–N5 2.167(10)	N1–Ir1–C11 80.92(12)	N3–Ir1–C12 173.4(2)
Ir1–C11 2.055(4)	N1–Ir1–C12 99.92(15)	N5–Ir1–C11 177.6(3)
Ir1–C12 2.011(4)	N2–Ir1–N3 95.4(4)	N5–Ir1–C12 99.2(3)
	N2–Ir1–N5 86.7(4)	C11–Ir1–C12 83.12(13)
	N2–Ir1–C11 93.2(4)	
4·CHCl₂, CH₂Cl₂		
Rh1–N1 2.039(4)	N1–Rh1–N2 174.96(16)	N2–Rh1–C12 80.97(17)
Rh1–N2 2.036(4)	N1–Rh1–N3 85.97(14)	N3–Rh1–N4 76.31(14)
Rh1–N3 2.196(4)	N1–Rh1–N4 96.80(15)	N3–Rh1–C1 94.86(16)
Rh1–N4 2.178(4)	N1–Rh1–C1 81.20(18)	N3–Rh1–C12 178.27(16)
Rh1–C1 1.993(5)	N1–Rh1–C12 95.17(17)	N4–Rh1–C1 171.09(16)
Rh1–C12 1.998(5)	N2–Rh1–N3 97.96(14)	N4–Rh1–C12 102.25(17)
	N2–Rh1–N4 87.22(15)	C1–Rh1–C12 86.60(18)
	N2–Rh1–C1 95.28(18)	
1		
Ir1–N1 1.9871	N1–Ir1–N2 91.01	N2–Ir1–C12 78.9
Ir1–N2 1.9873	N1–Ir1–N3 100.1	N3–Ir1–N5 77.2
Ir1–N3 1.9982	N1–Ir1–N5 177.1	N3–Ir1–C11 87.2
Ir1–N5 2.0132	N1–Ir1–C11 81.53	N3–Ir1–C12 172.1
Ir1–C11 2.0156	N1–Ir1–C12 83.26	N5–Ir1–C11 98.8
Ir1–C12 2.0158	N2–Ir1–N3 94.21	N5–Ir1–C12 98.7
	N2–Ir1–N5 87.2	C11–Ir1–C12 100.3
	N2–Ir1–C11 173.7	
3		
Rh1–N1 1.9981	N1–Rh1–N2 175.01	N2–Rh1–C12 81.1
Rh1–N2 2.019	N1–Rh1–N3 86.02	N3–Rh1–N4 76.48
Rh1–N3 2.167	N1–Rh1–N4 96.67	N3–Rh1–C1 95.18
Rh1–N4 2.156	N1–Rh1–C1 80.94	N3–Rh1–C12 178.43
Rh1–C1 1.9863	N1–Rh1–C12 95.28	N4–Rh1–C1 171.71
Rh1–C12 2.012	N2–Rh1–N3 98.05	N4–Rh1–C12 103.21
	N2–Rh1–N4 86.93	C1–Rh1–C12 87.34
	N2–Rh1–C1 95.33	

Table 3Electrochemical and electronic spectral data of complexes **1–4**.

Complex	Electronic spectral data λ , nm ($\epsilon \times 10^{-4}$, M ⁻¹ cm ⁻¹) ^a	Emission spectrum (excited at 330 nm)	Emission spectrum (excited at 405 nm)	τ (ns)	ϕ	Cyclic voltammetric data ^b , E, V vs. SCE ^{f,g}
L1	246(2.73), 281(0.88), 332(0.52)	411, 391		$\tau_1 = 2.100(79.5\%)$ $\tau_2 = 5.790(20.5\%)$		
L2	298(2.72), 337 (2.63)	413, 395		$\tau_1 = 7.644(100\%)$		
1	474(0.13), 363 ^c (0.95), 292(2.33), 252(3.21)	476, 411, 390	445, 460, 494	$\tau_1 = 1.390(72.5\%)$ $\tau_2 = 4.296(27.5\%)$	0.052	1.43, -0.92, -1.18 ^d (110) ^e
2	472(0.075), 373 ^c (0.73), 294(2.07), 257(2.37)	413, 390,	445, 460, 507	$\tau_1 = 7.906(94\%)$ $\tau_2 = 2.107(6\%)$	0.033	1.43, 1.16, -0.59, -1.07, -1.15 ^d (110) ^e
3	426(0.09), 363 ^c (0.95), 292(2.28), 253(3.12)	462, 413, 391	446, 460	$\tau_1 = 2.292(83\%)$ $\tau_2 = 4.517(17\%)$	0.035	1.52, -0.81, -1.11 ^d (100) ^e
4	377(0.70), 358(0.93), 294(2.42), 259(2.70)	413	445, 460, 514	$\tau_1 = 7.359(100\%)$	0.041	1.52, 1.15, -0.45, -0.85, -1.19 ^d (90) ^e

^a Acetonitrile solution.^b Dichloromethane, TBAP supporting electrolyte.^c Shoulder.^d $E_{1/2} = 0.5 (E_{pa} + E_{pc})$, where E_{pa} and E_{pc} are anodic and cathodic peak potentials respectively, scan rate 50 mV s⁻¹.^e $E_p = E_{pa} - E_{pc}$ in mV. s⁻¹^f E_{pa} value.^g E_{pc} value.

Table 4

Selected TDDFT data of the complexes.

Complex	Nature of transition ^a	Energy (eV)	Oscillator factor (<i>f</i>)	Computed λ_{max} (nm)	Observed λ_{max} (nm)
1	Ir(d π) $\rightarrow$ L1(π))	2.5643	0.015	487	474
2	Ir(d π) $\rightarrow$ L1(π)	2.5313	0.012	491	472
3	Rh(d π) $\rightarrow$ L2(π)	3.2850	0.009	379	363
4	Rh(d π) $\rightarrow$ L2(π)	3.1972	0.011	389	377

^aL means the coordinated Schiff base ligand.

Table 5

Composition of selected molecular orbital of the complexes.

Complex	Contributing fragments	% Contribution of fragments to					
		HOMO-2	HOMO-1	HOMO	LUMO	LUMO+1	LUMO+2
1	Ir	55.13	52.74	48.73	16.21	13.07	10.24
	ppy	40.09	41.92	43.65	4.23	6.74	8.43
	L1	4.78	5.34	7.62	79.56	80.19	81.33
2	Ir	69.12	66.93	63.88	17.13	14.42	11.54
	ppy	28.71	30.19	32.74	3.49	8.37	10.26
	L2	2.17	2.88	3.38	79.38	77.21	78.20
3	Rh	53.38	51.66	49.02	16.78	12.57	9.76
	ppy	38.57	43.89	42.28	4.23	5.23	7.82
	L1	8.05	4.45	8.71	78.99	82.2	82.42
	Rh	68.22	67.89	65.94	16.09	14.45	10.76
4	ppy	24.77	27.92	30.86	3.21	4.67	6.59
	L2	7.01	4.19	3.20	79.50	80.88	82.65

L means the coordinated Schiff base ligand.