Cyclometallated imidazo-phenanthroline iridium complexes and their anticancer activity

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Abstract. Cyclometallated mononuclear iridium complexes (**1–3**) were synthesized using dimeric precursor [(ppy)₂Ir^{III}(μ-Cl)]₂ and imidazo-phenanthroline-based ligands (**L**₁–**L**₃). The complexes were characterised using various analytical techniques. The complexes exhibited spin forbidden metal-to-ligand charge transfer (MLCT) transitions at wavelengths >300 nm and high energy ligand-based transition below 300 nm. The complexes were found to be emissive and displayed emission bands at 580 nm upon excitation at 380 nm with quantum yields 0.07 with respect to standard [Ru(bpy)₃]Cl₂. The complexes **1–3** showed dose-dependent suppression of cell viability (IC₅₀ values ~2.5 μM) towards human breast (MCF7) cancer cell line. The hydrophobicity measurements and flow cytometry analysis suggest that cellular uptake is primarily responsible for observed cytotoxicity.

Keywords. Iridium; phenanthroline; imidazole; anticancer agent.

1. Introduction

Organometallic complexes of iridium have received considerable attention in the field of metal-based anti-cancer research. This is primarily due to their inertness leading to low toxicity and lesser side effects, bioavailability and cancer cell selectivity as compared to well-known cisplatin.^{1–3} In addition, iridium complexes are known to induce selective kinase inhibition property and often display non-apoptotic mode of cancer cell death which are useful for the treatment of resistant cancer.^{1,4–9} Moreover, the exciting luminescence properties of iridium complexes are often used as probes for understanding biological processes. In this aspect, a large number of cyclometallated iridium complexes have been developed and studied using various ligand frameworks.^{5,7,8,10–25} Among various ancillary ligands, pyridine-based N[^]N chelating ligands are most promising considering their excellent coordinating ability and stability under physiological condition.^{2,4,5,14,18,20,24–28}

Different substituents have been incorporated to N[^]N donor-based ligands in order to tune the property and activity of the complexes. On the contrary, the use of N[^]N donating ligands incorporating imidazo unit is limited.^{29–32} It is known that the imidazole unit efficiently interacts with biomolecules and display biological activity. In addition, imidazole unit provides synthetic accessibility to incorporate various donors and substituents which can help to control electronic and steric environment, required for hydrophobicity and solubility. Although there are C-substituted imidazole-based ligands and their corresponding complexes are known, N-substituted imidazole-based ligands and their corresponding complexes are limited. Considering the afore-mentioned observations, we plan to develop N-substituted imidazo-phenanthroline-based ligands (**L**₁–**L**₃) and their corresponding cyclometallated iridium complexes. Different substituents (pyridine, benzyl and cyclohexyl) are incorporated at imidazole unit to study their effect on the anticancer efficacy (Chart 1).

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Chart 1. Structure of ligands.

Herein, we report the development of cyclometallated iridium complexes [(ppy)₂Ir(L₁–L₃)] (**1–3**) using ligands: 1-(pyridin-2-ylmethyl)-1H-imidazo[4,5-f][1,10]phenanthroline (**L**₁); 1-benzyl-1H-imidazo[4,5-f][1,10]phenanthroline (**L**₂) and 1-(cyclohexylmethyl)-1H-imidazo[4,5-f][1,10]phenanthroline (**L**₃). The complexes were characterized by various analytical techniques. The anticancer activity of the complexes were tested against human breast cancer (MCF-7) cell line and their variation in anticancer activity were assayed using hydrophobicity measurement, flow cytometry analysis and fluorescence microscopy.

2. Experimental

2.1 Materials

1-H-imidazo [4,5-f][1,10]phenanthroline and the ligand **L**₂ were synthesized according to the reported procedure with slight modification.³³ The precursor complex [(ppy)₂Ir(μ-Cl)]₂ was synthesized by following the procedure as discussed in literature.³⁴ All the chemicals were purchased from commercial sources and used as received. Solvents were dried by conventional methods and distilled prior to use.

2.2 Instrumentation

Electrical conductivity of the complex solutions were checked using an OKATON PC 2700 Conductivity bridge. UV-Vis spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer. FTIR spectra were obtained using a Bruker Alpha FTIR spectrophotometer with samples prepared as KBr pellets. Electrospray ionisation (ESI) mass spectra were recorded on a Bruker microTOF QII high-resolution mass spectrometer. ¹H NMR spectra were acquired on a Bruker Avance III 400 spectrometer using DMSO-d₆ as the solvent. Electrochemical measurements were carried out under dinitrogen atmosphere using a CHI 760D electrochemical analyser with NEt₄ClO₄ as the supporting electrolyte (0.1 M), and the solute concentration was 10^{−3} M. For electrochemical measurements, a glassy carbon working electrode, Pt wire counter electrode and Ag/AgCl as the reference electrode were used. The half-wave potential *E*_{298K} was set equal to 0.5(*E*_{pa} + *E*_{pc}), where *E*_{pa} and *E*_{pc} are anodic and cathodic cyclic voltammetric peak potentials, respectively. In this cell, Fc/Fc⁺ couple had an *E*_{1/2} value of 0.26 V. Fluorescence measurements were carried out using a Fluoromax

4P spectrofluorimeter (Horiba Jobin Mayer, USA). The quantum yield of the complexes in acetonitrile were calculated by comparing the quantum yield value of [Ru(bpy)₃]Cl₂ in water using the following equations.³⁵

$$\Phi_{\text{complex in } H_2O} = \Phi_{[Ru(bpy)_3]Cl_2 \text{ in } H_2O} \times \frac{I_{\text{Complex}}}{I_{[Ru(bpy)_3]Cl_2}} \quad (1)$$

$$\Phi_{\text{complex in } CH_3CN} = \Phi_{\text{complex in } H_2O} \times \frac{I_{\text{Complex in } CH_3CN}}{I_{\text{Complex in } H_2O}} \times \frac{RI_{CH_3CN}^2}{RI_{H_2O}^2} \quad (2)$$

Where *RI* = refractive index of the solvent; *I* = area under the emission band of the complex in the solvent. The emission quantum yields of the complexes in water were evaluated from the standard value of [Ru(bpy)₃]Cl₂ in water (0.042) using equation 1.

2.3 Hydrophobicity measurement

The lipophilicity of the complexes was determined by a flask-shaking method using n-octanol/water solvent system by following the reported procedure.³⁶

2.4 In vitro cytotoxicity assay

Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 5 mg/mL). The cultures were initiated in 96-well plate after 24 h of pre-incubation with various concentrations of complexes (**1–3**) for 24 h and 48 h. After incubation, MTT reagent was added to each well and incubated for 4 h at 37 °C in dark. The supernatant was aspirated and formazan crystals were dissolved in 200 μL DMSO at room temperature for 15 min with gentle agitation. The absorbance per well was measured at 540 nm using the VERSA Max (Microplate Reader).

2.5 Flow cytometry assay

The MCF-7 cells were treated with complexes **1–3** (10 μM) for 24 h. The total numbers of cells were harvested separately, out of which 1 × 10⁶ cells were removed, washed with PBS. The fluorescence intensity of the complex treated cells was measured using Guava EasyCytePlus flow cytometer (Merck Millipore).

2.6 Synthesis of ligands

2.6a Synthesis of [1-(pyridin-2-ylmethyl)-1H-imidazo [4,5-f][1,10] phenanthroline] (L₁): To 180 mg (0.82 mmol) of 1-H-imidazo [4,5-f][1,10]phenanthroline 338.6 mg (2.5 mmol) K₂CO₃ was added in 10 mL dry DMF and reaction mixture was stirred at room temperature for 30 min. Then, to the stirred solution, 250 mg (0.98 mmol) 2-(bromomethyl)pyridine hydrobromide was added. After a reaction time of 3 h, the solvent was removed under

reduced pressure. The residue thus obtained was dissolved in chloroform and extracted with water. The organic phase was separated, treated with anhydrous Na_2SO_4 and dried to get pure yellow coloured ligand **L**₁. Yield: 220 mg (86%). ¹H NMR (400 MHz, CDCl_3 at 298K) δ 9.20 (dd, $J = 4$, 1.8 Hz, 1H), 9.10 (dd, $J = 4$, 1.6 Hz, 1H), 9.05 (dd, $J = 8$, 1.8 Hz, 1H), 8.71 (ddd, $J = 4$, 2, 1 Hz, 1H), 8.38 (dd, $J = 8$, 1.6 Hz, 1H), 8.20 (s, 1H), 7.77 (dd, $J = 8$, 4.4 Hz, 1H), 7.58 (td, $J = 8$, 1.8 Hz, 1H), 7.51 (dd, $J = 8$, 4 Hz, 1H), 7.26 (m, 1H), 6.81 (d, $J = 8$, 1H), 5.97 (s, 2H)

2.6b Synthesis of 1-(cyclohexylmethyl)-1H-imidazo[4,5-f][1,10]phenanthroline (L**₃):** The ligand **L**₃ was prepared by following the similar procedure as for **L**₁. Yield: 200 mg (71%). ¹H NMR (400 MHz, CDCl_3 at 298K) δ 9.19 (m, 2H), 9.00 (dd, $J = 8$, 2 Hz, 1H), 8.49 (dd, $J = 8$, 2 Hz, 1H), 7.95 (s, 1H), 7.74 (m, 2H), 4.42 (d, $J = 8$ Hz, 2H), 2.00 (m, 1H), 1.77 (m, 5H), 1.21 (m, 5H).

2.7 Synthesis of metal complexes

2.7a Synthesis of $[(\text{ppy})_2\text{Ir}]\text{L}_1(\text{PF}_6)(\mathbf{1})$: A mixture of $[(\text{ppy})_2\text{Ir}(\mu\text{-Cl})_2]$ (51.4 mg, 0.08 mmol) and **L**₁ (50 mg, 0.16 mmol) were dissolved in 20 mL 2-methoxyethanol. The mixture was refluxed under N_2 atmosphere for 12 h. The solvent was then reduced to 2 mL under reduced pressure. A saturated aqueous KPF_6 solution was added in resulted solution to yield the precipitate. The yellow coloured precipitate thus obtained was filtered off and washed thoroughly with distilled water. The crude product was purified by neutral alumina column using $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (50:1) as eluent to yield pure **1**. Yield: 59.6 mg (38.9%). Molar conductivity $[\Lambda_{\text{M}}/(\Omega^{-1}\text{cm}^2\text{M}^{-1})]$ in acetonitrile: 100. A positive ion ESI mass spectrum of **1** in CH_3OH exhibited signal at $m/z = 811$ corresponding to $[\mathbf{1} - \text{PF}_6]^+$ (calculated molecular mass 811.93). ¹H NMR (400 MHz, DMSO-d_6 at 298K): δ 9.20 (dd, $J = 8.3$, 1.5 Hz, 1H), 8.94 (dd, $J = 8.7$, 1.3 Hz, 1H), 8.87 (s, 1H), 8.45 (d, $J = 4.6$ Hz, 1H), 8.25 (t, $J = 8$ Hz, 2H), 8.17 (dd, $J = 5.1$, 1.5 Hz, 1H), 8.10 (m, 2H), 7.94 (m, 3H), 7.85 (m, 3H), 7.51 (d, $J = 8$ Hz, 1H), 7.46 (d, $J = 6$ Hz, 2H), 7.31 (dd, $J = 8$, 4, 1.1 Hz, 1H), 7.05 (tdd, $J = 7.6$, 6.4, 1.2 Hz, 2H), 6.96 (m, 4H), 6.26 (dt, $J = 8$, 1.5 Hz, 2H), 6.21 (s, 2H). FT-IR (KBr, ν): 845 cm^{-1} .

2.7b Synthesis of $[(\text{ppy})_2\text{Ir}]\text{L}_2(\text{PF}_6)(\mathbf{2})$: A similar procedure as followed for the synthesis of complex **1** was followed here. The crude product was purified by neutral alumina column using CH_2Cl_2 as eluent which led to pure **2**. Yield: 50.15 (33%). Molar conductivity $[\Lambda_{\text{M}}(\Omega^{-1}\text{cm}^2\text{M}^{-1})]$ in acetonitrile: 84. A positive ion ESI mass spectrum of **2** in CH_3OH exhibited signal at $m/z = 810$ corresponding to $[\mathbf{2} - \text{PF}_6]^+$ (calculated molecular mass: 810.94). ¹H NMR (400 MHz, DMSO-d_6 at 298K) δ 9.22 (d, $J = 8.2$ Hz, 1H), 8.90 (s, 1H), 8.85 (d, $J = 8.6$ Hz, 1H), 8.26 (dd, $J = 12.1$, 8.1 Hz, 2H), 8.18 (d, $J = 5.3$ Hz, 1H), 8.11 (m, 2H), 7.95 (m, 3H), 7.87 (m, 2H), 7.47 (t, $J = 7.22$ Hz), 7.36 (m, 2H), 7.28

(m, 3H), 7.06 (qd, $J = 7.5$, 1.3 Hz, 2H), 6.97 (m, 4H), 6.26 (d, $J = 7.5$ Hz, 2H), 6.14 (s, 2H). FT-IR (KBr, ν): 845 cm^{-1} .

2.7c Synthesis of $[(\text{ppy})_2\text{Ir}]\text{L}_3(\text{PF}_6)(\mathbf{3})$: Procedure followed was same as for the synthesis of complex **1**. The crude product was purified by neutral alumina column using $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (50:1) as eluent. Yield: 70.8 mg (46%). Molar conductivity $[\Lambda_{\text{M}}/(\Omega^{-1}\text{cm}^2\text{M}^{-1})]$ in acetonitrile: 82. A positive ion ESI mass spectrum of **3** in CH_3OH exhibited signal at $m/z = 816$ corresponding to $[\mathbf{3} - \text{PF}_6]^+$ (calculated molecular mass: 816.99). ¹H NMR (400 MHz, DMSO-d_6 at 298K) δ 9.17 (dd, $J = 8.3$, 1.3 Hz, 1H), 9.03 (d, $J = 9.5$ Hz, 1H), 8.68 (s, 1H), 8.27 (d, $J = 8.3$ Hz, 2H), 8.21 (d, $J = 5.4$ Hz, 1H), 8.17 (d, $J = 4.8$ Hz, 1H), 8.10 (ddd, $J = 18.6$, 8.3, 5.0 Hz, 2H), 7.96 (d, $J = 7.6$ Hz, 2H), 7.89 (m, 2H), 7.50 (dd, $J = 11.7$, 5.6 Hz, 2H), 7.07 (t, $J = 7.5$ Hz, 2H), 6.98 (m, 4H), 6.29 (t, $J = 7.36$ Hz, 2H), 4.65 (d, $J = 6.4$ Hz, 2H), 1.91 (m, 1H), 1.69 (d, $J = 6.8$ Hz, 3H), 1.62 (d, $J = 9.9$ Hz, 2H), 1.15 (m, 5H). FT-IR (KBr, ν): 845 cm^{-1} .

3. Results and Discussion

3.1 Synthesis and characterization

The ligands **L**₁–**L**₃ were prepared by following the procedure outlined in Scheme S1 (in Supplementary Information).³⁷ The pure ligands (**L**₁–**L**₃) were obtained by the reaction of 1H-imidazo[4,5-f][1,10]phenanthroline with corresponding halide (1:1.2 molar ratio) in the presence of K_2CO_3 in dry DMF under N_2 atmosphere at room temperature. The formation of pure ligands (**L**₁–**L**₃) was confirmed by ¹H NMR spectroscopy (Figure S1 in Supplementary Information). It is to be noted that the ligands **L**₁ and **L**₃ are new and their metallation is yet to be reported.

The mononuclear iridium complexes **1**–**3** were prepared by reacting the ligands (**L**₁–**L**₃) with dimeric precursor $[(\text{ppy})_2\text{Ir}(\mu\text{-Cl})_2]$ in an appropriate ratio in 2-methoxyethanol under a dinitrogen atmosphere for 12 h (Scheme 1). The pure complexes were isolated as their PF_6^- salts. All the complexes behaved as 1:1 electrolyte in CH_3CN . The presence of PF_6^- counter anion in the complexes was evidenced by observing characteristic IR vibration at 845 cm^{-1} .

The ¹H NMR spectra of the complexes (**1**–**3**) were recorded in DMSO-d_6 (Figure S2 in Supplementary Information). All the complexes exhibited expected the number of proton resonance within the chemical shift range 0–10 ppm indicating the presence of ppy and imidazole-based ligands (**L**₁–**L**₃) and their identities in solution.

Scheme 1. Synthetic outline for the preparation of complexes **1–3**.

Figure 1. Positive ion ESI mass spectra of the complexes **1–3** in CH_3OH .

The formation of complexes was confirmed by their positive ion electrospray mass spectrometry (Figure 1). In CH_3OH the complexes displayed molecular ion peaks centered at 812.0 for $\{\mathbf{1} - \text{PF}_6\}^+$ (Calcd. 811.94), 811 for $\{\mathbf{2} - \text{PF}_6\}^+$ (Calcd. 810.95), and 817.0 for $\{\mathbf{3} - \text{PF}_6\}^+$ (Calcd. 816.99) confirming the presence of their entire molecular frameworks.

Figure 2(a) displays the UV-Vis spectra of the complexes recorded in CH_3CN . The complexes exhibited ligand-based transitions ($\pi \rightarrow \pi^*$) in the high energy UV region ($<300\text{ nm}$) and low energy metal-to-ligand charge transfer transitions (MLCT) in the visible regions ($>350\text{ nm}$) (Table S1 in Supplementary Information). The shorter wavelength transitions with high extinction coefficient were assigned as spin-allowed ligand-based ($\pi \rightarrow \pi^*$) transitions from ppy and $\text{L}_1\text{–L}_3$ ligands.^{38–40} While the longer wavelength absorption shoulders above 400 nm might be assigned as an admixture of spin-allowed metal-to-ligand charge-transfer ($^1\text{MLCT} : d\pi \rightarrow \pi^*_{\text{phen}}$) and ligand-to-ligand charge transfer ($^1\text{LLCT} : \pi_{\text{ppy}} \rightarrow \pi^*_{\text{phen}}$) processes.^{3,41–45} The very low-intensity band observed in the range $400\text{–}500\text{ nm}$ (Figure 2(a)) might be assigned as spin-forbidden $^3\text{MLCT}$ and $^3\text{LLCT}$ transitions. This observation is not consistent with the similarly reported complexes.^{3,42–47} No noticeable changes in band position were observed for the complexes indicating that the substituents at the

Figure 2. (a) UV-Vis and (b) emission spectra of the complexes **1–3** recorded in CH_3CN .

imidazo end have a negligible or no effect on the metal centres.

The complexes **1–3** were found to be luminescent (Figure 2(b)). The emission behaviour of cyclometallated iridium complexes were studied in CH_3CN . It was observed that upon photoexcitation of the complexes at 390 nm exhibited emission band at 580 nm with a quantum yield of 0.07, with reference to the standard $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$.^{27,48} Such transitions generally appeared from the $^3\text{MLCT}$ ($d\pi(\text{Ir}) \rightarrow \pi^*(\text{imidazole})$) excited state.^{27,34}

The electrochemical behaviour of the complexes (**1–3**) was studied in CH_3CN at room temperature using Ag/AgCl reference electrode and glassy carbon working electrode. All the complexes showed metal-based quasi-reversible redox couple ($\text{Ir}^{\text{IV}}/\text{Ir}^{\text{III}}$) at $\sim 1.25\text{ V}$ (Table S1 in Supplementary Information). No significant shift in the oxidation potential of the metal centres was observed indicating no or negligible electronic contribution from the substituents present in the imidazole moiety, which is consistent with the results observed in UV-Vis spectroscopy. The ligand-based reductions were observed at the negative potential in the cyclic voltammograms.

3.2 Biological activity study

Cyclometallated iridium complexes are known to exhibit anticancer activity. The cytotoxicity of the complexes **1–3** were tested against human breast cancer cell line (MCF-7) by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The formation of formazan due to the reduction of tetrazole of MTT by mitochondrial dehydrogenases in the living cells was quantified at 595 nm . The complexes were found to show dose-dependent suppression of cell viability towards the tested cancer (MCF-7) cell line and better activity than standard cisplatin (Figure 3, Table 1). Results reveal that anticancer activity of the complexes **2/3** (IC_{50} values $1.5\text{ }\mu\text{M}$ for **2** and $2.5\text{ }\mu\text{M}$ for **3**) were better than for **1** ($\text{IC}_{50} \sim 20.7\text{ }\mu\text{M}$).

Figure 3. Dose-dependent suppression of cell viability of complexes **1–3** towards human breast (MCF-7) cancer cells.

Table 1. Lipophilicity of the complexes **1–3** and *in vitro* growth inhibition against human breast cancer (MCF-7) cells. $P_{o/w}$ is the dimensionless partition coefficient of the complexes in octanol/water solvent system.

Complex	$\log P_{o/w}$	IC_{50} (μM)	
		24 h	48 h
1	2.45	25.4	20.7
2	2.75	5.3	1.5
3	3.2	7.2	2.5
Cisplatin	—	—	35.6

To further understand the anticancer activity of **2/3** over **1**, we conducted a cellular uptake study using hydrophobicity measurement. Hydrophobicity value often provides indirect measure of cellular uptake. It was observed that the hydrophobicity values of complexes **2/3** were a little higher as compared to **1** which may contribute better uptake of former complexes than latter. To further understand the varying anticancer activity

Figure 4. Flow cytometry analysis results of MCF-7 cells incubated with blank medium (control) and complexes **1–3** ($10 \mu M$) at $37^\circ C$ for 3 h. (excitation, 530 nm; emission, 585 nm).

Figure 5. Fluorescence microscopic images (magnification 400x) of human breast cancer cells (MCF7) treated with or without treatment of $10 \mu M$ complexes **1–3**. The images were recorded 24 h after the treatment of the complexes.

of the complexes, we conducted flow cytometry analysis. It was observed that the cellular uptake of **2/3** was much higher than **1** (Figure 4), which corroborates the observed anticancer activity. We next recorded the fluorescence microscopy images of MCF-7 cells treated by complexes which are emissive (Figure 5). The fluorescence microscopy images of MCF-7 cells treated by complexes demonstrated good cellular accumulation inside the cells. It was also observed that the complexes were distributed well throughout the cells. Thus, the combined results suggest that the anticancer activity of the present set of complexes is mainly dependent on the cellular uptake. Although not significant, the distal

components (N-substituents of imidazole unit) of **L**₁–**L**₃ such as phenyl/pyridine/cyclohexyl also play an important role to control the hydrophobicity and thereby the anticancer activity.

4. Conclusions

Cyclometallated iridium complexes (**1**–**3**) using imidazo-phenanthroline based ligands (**L**₁–**L**₃) were synthesized. The complexes were characterized by various analytical techniques. The anticancer activity of the complexes against human breast cancer cell line (MCF7) was tested. The complexes were found to be highly active. The flow cytometry and hydrophobicity analyses result revealed that better cellular uptake is primarily responsible for the variation in anticancer activities. The iridium centre in a combination of ppy and ligand **L**₃ having cyclohexyl group at the imidazole end is found to be suitable to achieve desired anticancer activity. We believe that the present set of complexes would be useful for the design and development of efficient anticancer agents *via* suitable variation of substituents both at the ppy and pyrazine-based ligands.

Supplementary Information (SI)

Synthetic scheme for ligands, NMR spectra of ligands and complexes, spectra and electrochemical data are available as Electronic Supplementary Information at www.ias.ac.in/chemsci.

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