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COMMUNICATION

Dinuclear[[{(p-cym)RuCl}₂(μ-ppy)](PF₆)₂ and heterodinuclear [(ppy)₂Ir(μ-ppy)Ru(p-cym)Cl](PF₆)₂ complexes: synthesis, structure and anticancer activity†

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Phpy bridged homodinuclear Ru-Ru (1) and heterodinuclear Ir-Ru complex (2) have been developed. The complex 2 induces autophagy towards cisplatin resistant human breast cancer (MCF7) cell line, whereas 1 is inactive.

Cisplatin and their analogues are most well-known anticancer agents used in cancer therapy. However, their high toxicity leading to several side effects and cancer cells resistance limit the use of platinum based drugs. To overcome such problems, efforts have been made to develop non-platinum based novel organometallic compounds of ruthenium, gold, copper, etc.¹ Among these, ruthenium complexes demonstrate very high *in vivo* and/or *in vitro* anticancer activity and two such complexes, namely, KP1019 and NAMI-A have entered clinical trials.^{1e,f} Conversely, no serious attention has been paid to explore the biological activity of cyclometallated Ir(III) complexes incorporating C[∞]N chelating ligands in spite of their widespread applications in chemistry and materials sciences.² Recent reports demonstrate that cyclometallated Ir(III) complexes could be very useful to design structurally diverse molecular scaffolds for potent and selective enzyme inhibitor.³

The alternative approach to compensate the limitations of cisplatin is to develop drugs which induce cell death *via* non-apoptotic mode of action.^{1a,4} The type II programmed cell death or autophagy has become one of the very promising routes for the suppression of tumour growth.⁵ Autophagy acts as a double-edged sword in oncology and involves either a cell survival mechanism during starvation or a cell death pathway when other cell death mechanisms are deficient.^{5d} It can also follow suicidal pathway by digesting essential cellular proteins under excessive stress condition.^{5d} Hence, drugs that induce autophagy can kill cancer cells resistant to apoptosis. Nevertheless, metal based drugs that trigger autophagy are relatively rare.^{1d,1g,11} Thus, development of metal complexes that

induce autophagy is extremely important in order to understand the mechanism of cell death and drug resistant cancer treatment.^{1d,1g,5a,5c}

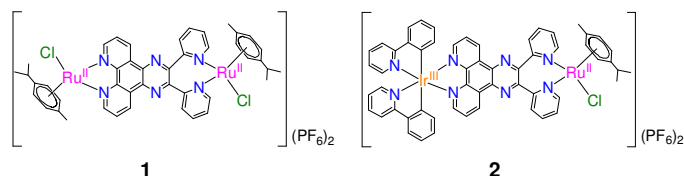


Fig. 1 Structures of complexes **1** and **2**.

Nevertheless, exploration of multimetallic molecular framework is a fascinating area since they combine the properties of the individual metals in the same system. Such systems may exhibit synergistic interaction, which could be useful in understanding enzymatic reactions and development of functional materials for catalysis, magnetic, optical and electronic applications.⁶

Inspired by these facts, we aim to utilise the advantages of η⁶-arene ruthenium(II) and cyclometallated iridium(III) fragment along with a potentially trinucleating polypyridyl based ligand 2,3-di(pyridin-2-yl)pyrazino[2,3-f][1,10]phenanthroline (ppy) for the development of efficient anticancer drug. Polypyridyl ligands are well known to enhance cellular uptake *via* intercalating with DNA.^{1j,7} It is to be noted that the ppy ligand is known; however, its metallation has not so far been reported.⁸ Thus, the communication presents the synthesis and characterisation of new dinuclear [(p-cym)RuCl]₂(μ-ppy)](PF₆)₂ (**1**) and heterodinuclear [(ppy)₂Ir](μ-ppy){Ru(p-cym)Cl}](PF₆)₂ (**2**) complexes with known but hitherto unexplored ppy ligand. The anticancer activity of the complexes towards breast (MCF7), ovarian (SKOV3), prostate (PC3) and endometrial (ishikawa) cancer cell lines has been tested. The mechanism of cell death has been investigated by flow cytometry, confocal microscopy and western blot analysis. It is noteworthy that this is the first example of heterodinuclear Ir-Ru complex which induces autophagic cell death against cisplatin resistant MCF7 cells.

The ppy ligand has been prepared *via* the condensation reaction between 5,6-diamino-1,10-phenanthroline with commercially available 2, 2'-bipyridyl. Complexation reaction of the ppy ligand has been done by refluxing with appropriate ratios of metal precursors in suitable solvents (ESI, †).

Both **1** and **2** are 1:2 electrolytic in acetonitrile and give satisfactory microanalytical data (ESI, †). The presence of PF₆⁻

^aSchool of Basic Sciences, Indian Institute of Technology Bhubaneswar, Bhubaneswar-751007, India. Tel: +91-674 2576053, E-mail: srikanta@iitbbs.ac.in,

^bDivision of Toxicology, School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do, Republic of Korea. Tel: +82-31-290-7789, E-mail: hkims@skku.edu.

ⁱInternational Institute of Information Technology, Bhubaneswar-751003, India

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counter ion has been confirmed by the characteristic IR vibration near 845 cm^{-1} .

The identities of the complexes are confirmed by +ve ion ESI mass spectra (Fig. S1). The presence of molecular ion peaks centred at 1072.95 (calcd. 1072.87) for $[1 - \text{PF}_6]^+$ and 1302.81 (calcd. 1302.73) for $[2 - \text{PF}_6]^+$ confirm the existence of entire molecular framework. Further, the ^1H NMR spectra of **1** and **2** in DMSO- d_6 exhibit 22 and 34 aromatic proton signals in the region 4.5–10.5 ppm confirming the presence of phpy ligand, $[(p\text{-cym})\text{Ru}]$ and $[(\text{ppy})_2\text{Ir}]$ fragments (Fig. S2). Intense singlets at 0–2.0 ppm due to CH_3 protons further support the existence of $[(p\text{-cym})\text{Ru}]$ fragment in the complexes.

The formation of the complex **2** is further confirmed by single crystal X-ray crystallography (Fig. 2). Important crystallographic parameters and selected bond lengths and bond angles are presented in Tables T1 and T2 (ESI, †). It is observed that $[(\text{ppy})_2\text{Ir}]$ fragment is coordinated to the phenanthroline end of the phpy ligand with slightly distorted octahedral geometry with N5–Ir1–N6, N7–Ir1–C45 or N8–Ir1–C46 bite angles of $77.3(3)^\circ$, $81.2(4)^\circ$ or $80.6(4)^\circ$, respectively. The $[(p\text{-cym})\text{RuCl}]$ fragment is coordinated with pyridyl N with the bite angle N1–Ru1–N2 of $86.8(3)^\circ$ in an expected piano stool geometry by forming a seven membered metallacycle with boat conformation. The dihedral angle between two pyridyl rings of phpy ligand is 72.08° . The Ru–C_{centroid} and Ru–Cl distances are 1.687 Å and 2.389(3) Å, respectively, are in good agreement

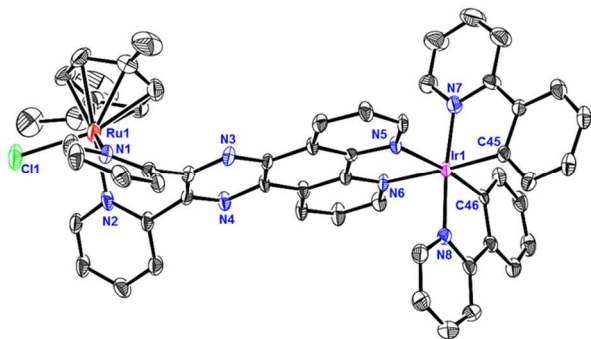


Fig. 2 ORTEP diagram of **2.4THF**. Ellipsoids are drawn at 30% probability level. Hydrogen atoms, counter anions (PF_6^-) and THF are eliminated for clarity.

with the reported literature values.⁹ The Ir–C (1.990–2.026 Å) and Ir–N (2.030–2.139 Å) bond distances of the coordinated ppy ligands are within the range with the previously reported complexes.^{3c,10} The intermetallic distance between Ru and Ir centres is 10.308 Å. Both dinuclear **1** and heterodinuclear **2** in acetonitrile show low energy weak spin forbidden MLCT band at 580 nm and 470 nm, respectively. In addition, intense ligand based charge transfer transitions appear in the UV region for both **1** and **2** (Fig. S3a).^{9,11} Further, enhancement of intensity in heterodinuclear **2** is observed as compared to that of homodinuclear **1** which may be attributed to the presence of mixed oxidation states of metals.¹² Complex **1** is found to be non-emissive upon excitation. However, complex **2** has shown a moderately strong $^3\text{MLCT}(\text{d}\pi)(\text{Ir}^{\text{III}}) \rightarrow \pi^*(\text{N}^{\wedge}\text{N})$ emission at 600 nm ($\lambda_{\text{ex}} = 470\text{ nm}$) with quantum yield 0.07 in acetonitrile at room temperature (Fig. S3b).¹¹

Complex **1** exhibits two irreversible oxidation processes, E_{pa} at 1.37 V and 1.60 V which may be attributed to $\text{Ru}^{\text{II}}/\text{Ru}^{\text{III}}$ oxidation of Ru^{II} centres at phenanthroline and pyridine end, respectively (Fig S4). The difference in oxidation potentials between two Ru^{II} centres may be due to the increased charge and different coordination environment of the complex.¹³ For heterodinuclear **2**, two irreversible oxidation peaks, E_{pa} at 1.03 V and 1.63 V corresponding to $\text{Ir}^{\text{III/IV}}$ and $\text{Ru}^{\text{II/III}}$, respectively, is observed.^{6a} In addition, both the

complexes have shown ligand based reductions at negative potentials in cyclic voltammetry (Table T3).

The cytotoxicity of the complexes **1** and **2**, phpy ligand, KPF_6 and reference cisplatin has been tested in MCF7, PC3, SKOV and Ishikawa cancer cell lines by standard MTT assay. The cell viability vs concentration of the complexes **1** and **2** after exposure for 24 h and 48 h is shown in Figs. S5 and S6, respectively. The IC_{50} values of the complexes are depicted in Table 1. No anticancer activity of complex **1**, phpy ligand and KPF_6 has been observed. However, complex **2** has shown dose dependent cell suppression towards all the cell lines with better anticancer activity than cisplatin (Table 1). Complex **2** is found to be more effective against MCF7 as compared to other cell lines. The excellent cytotoxic activity of **2** against the tested cell lines may be due to better cellular uptake inside the cells which is evident by fluorescence images (Fig. S7). To understand the molecular mechanism of repression of cell viability, fluorescence activated cell sorting (FACS) analysis of MCF7 cells have been

Table 1 In vitro growth inhibition of the complexes **1** and **2** against human breast (MCF7), ovarian (SKOV3), endometrial (Ishikawa), and prostate (PC3) cancer cell lines.

Complex	IC_{50} values (μM)							
	MCF7		SKOV3		Ishikawa		PC3	
	24h	48h	24h	48h	24h	48h	24h	48h
1	>50	>50	>50	>50	>50	>50	>50	>50
2	3.22	0.92	13.85	8.25	6.20	1.57	6.52	3.36
Cisplatin	>50	35.6	>50	>50	>50	>50	>50	>50

conducted (Fig. S8). The FACS results reveal that the cell population is negligible at sub G1 phase whereas it is significant at G1 phase and distribution of cells are not varied much on varying concentration of **2** which give an indication of non-apoptotic mode of cell death.¹⁴ Further, the expressions of proteins levels responsible for apoptosis such as PARP, bcl2, Bax have been assessed *via* western blot analysis (Fig S9). No significant change in the proteins levels and cleavage of PARP is observed, suggesting non-apoptotic mode of cell death.¹⁵ On the other hand, microscopic analysis of MCF7 cells treated with **2** reveals the formation of acidic vacuoles (Fig. 3b). These changes in morphological features of the cells indicate classical autophagic characteristics. To further confirm autophagy, western blot analysis has been conducted. Overexpression of LC3II is a hallmark and well accepted assessment of autophagosome formation.^{5b} The western blot analysis shows that significant increase of LC3II after the treatment of **2** (Fig. 3c). In addition, beclin-1 and Atg5 proteins levels, required for initiation of autophagosome formation, have also been increased after treatment of **2** (Fig. 3c). On the contrary, no notable change for Atg7 protein level is observed (Fig. 3c). It is notable that Atg7 independent autophagy is observed earlier.¹⁶ Thus, taken together the experimental results, it could be inferred that complex **2** induces Atg7 independent autophagy. Further studies are required to understand the exact mechanism of cell death and structural modification of this kind of potentially useful complex in order to

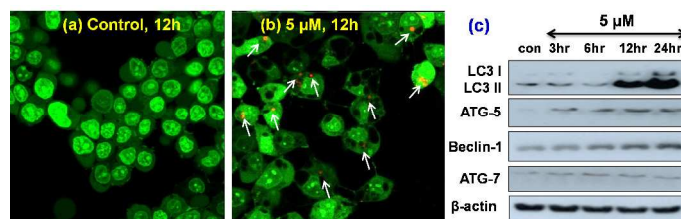


Fig. 3 Overlapped fluorescence microscopic images (a) and (b) (400x, acridine orange stained), and autophagy related proteins expression (c) of human breast (MCF7) cancer cells after the treatment of complex **2** at indicated concentrations. The arrows indicate the acidic vacuoles.

alleviate the cytotoxicity to develop efficient anticancer drugs.

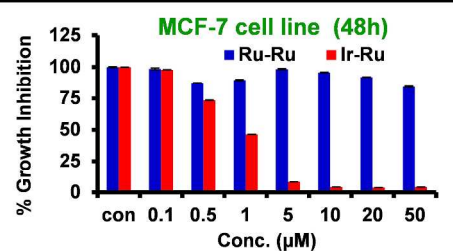
In summary, homodinuclear **1** and heterodinuclear **2** complexes with phpy ligand have been prepared and characterised. The crystal structure of **2** reveals the expected distorted octahedral and piano stool geometry of iridium and ruthenium, respectively. Complex **1** is inactive towards tested cancer cells, whereas **2** is highly cytotoxic towards cisplatin resistant MCF7 cancer cell (IC₅₀ value, 0.92 µM) and moderately active towards other cancer cells. Complex **2** is the first example of heterodinuclear Ir-Ru complex which induces autophagy. We believe that the present findings will furnish useful information for the development of effective anticancer drugs. Further investigations are underway to garner insight into the structure-activity relationship between the complex framework and autophagy mechanism.

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Heterodinuclear Ir-Ru (**2**) with 2,3-di(pyridin-2-yl)pyrazino [2,3-f][1,10]phenanthroline (phpy) ligand shows autophagy induced cell death, whereas homodinuclear Ru-Ru (**1**) is inactive.



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