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ARTICLE TYPE

Rare Observation of 'Aggregation Induced Emission' in Cyclometalated Platinum(II) Complexes and their Biological Activities

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Strong solid state emissive three cyclometalated platinum(II) complexes [Pt(C^N)(CH^N)(Cl)] (1) [C^N/CH^N = 2-phenylpyridine, C^N = bidentate and CH^N= monodentate), [Pt(C^N)(P^P)]Cl [P^P = Bis(diphenylephosphino)ethane (2) and *cis*-1, 2-Bis(diphenylephosphino)ethene (3)] were reported. These were identified as 'Aggregation Induced Emission (AIE)' active complexes based on controlled experiments. Cytotoxicity and cell imaging have been studied for the complex 2.

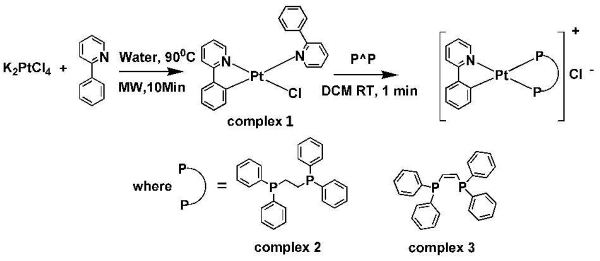
Phosphorescent heavy metal complexes like platinum(II) and iridium(III) are becoming increasingly important to scientists with respect to their applications in different fields such as, bioimaging[1], sensing[2] and organic light emitting devices (OLEDs)[3] due to their high luminescence quantum yields, color tunability, fair stability, excellent emission properties and straightforward synthetic routes[4]. In these cases, 100% internal quantum efficiency can be achieved due to strong spin orbit coupling[5]. The strong emission of the luminophores (organic as well as organometallic complexes) often quenched in their aggregate form called 'Aggregation caused Quenching (ACQ)' effect[6]. This is one of the major challenges to apply these materials in practical applications. In 2001 Tang and co workers[7] achieved tremendous success in developing the anti–ACQ flourophores called 'Aggregation Induced Emission (AIE)' compounds. This emission phenomenon is manifested by compounds exhibiting significant enhancement of their light-emission in solid state whereas weak emission in solution. To date, many AIE flourophores have been reported[8] but the development of heavy metal complexes with AIE properties is still limited[9]. Out of the heavy metal complexes, reports of platinum(II) complex with this unusual property is rare[10]. Yam and Che reported[11] AIE active Pt(II) complexes. The proposed mechanism of AIE in case of Pt(II) is restricted intramolecular rotation (RIR)[12], hydrogen bonding[10a] which can lead to suppression in molecular motion. Apart from these, significant contribution of MMLCT excited state to the lowest excited states lead to strong emission in solid state[12].

The normal luminophores suffer from basic problems like interference from background and scattered light[13]. The development of AIE active Pt(II) complexes is effective solution to overcome these problems. The AIE active Pt(II) compounds with such properties as low photo-bleaching, low light scattering and rich photophysical and strong emission in the solid state make these promising candidates for bioimaging[10b] .

Herein, we reported the syntheses of three AIE active cyclometalated complexes [Pt(C^N)(CH^N)Cl] (**1**), Pt(C^N)P^P]Cl where [C^N = 2-phenylpyridine; P^P is Bis(diphenylephasphino)ethane (**2**) and *cis*- 1,2-Bis (diphenylephasphino)ethene (**3**), studied their photophyscial properties. The computational studies of one of the complexes was performed and correlated with its spectroscopic observations. All these complexes are found to exhibit AIE activity and emit very strongly in the solid state. Complex **1** is used for cytotoxicity study against non-resistant and *cis*-platin- resistant cell line showing very good results[14]. This result encouraged us to go for MTT cytotoxicity study for the rest of the synthesised complexes. The MTT cytotoxicity study has been carried out for complex **2**. Utilizing its AIE and rich photophysical properties, we have used complex **2** in bio-imaging applications as in staining ability of cancer cells, human hepatocellular carcinoma cells, Hep3B.

The syntheses of all three complexes **1**, **2** and **3** are presented in **scheme 1**. The green synthetic approach has been applied for the syntheses of these complexes. Complex **1** has been synthesized by using K_2PtCl_4 as the platinum precursor along with four equivalents of 2-phenyl pyridine. The reaction was completed using water as the solvent in presence of microwave (MW) in 10 minutes .The greenish yellow product, **1** was isolated from water as a solid mass and characterized by ^{1}H and ^{13}C NMR (Fig S1-S2). The synthesized pendent complex **1** is one of the important precursors for synthesis of many luminescent Pt(II) complexes[15].

Scheme 1 Synthetic route and chemical structures of Complexes **1**, **2** and **3**



The synthesis of complex **2** was reported[16] by M. G. Haghighi *et al* using two step synthetic protocol with using of unusual platinum(II) precursors, (i) [PtMe(κ^{1}C-ppy)(dppe)] (ii) [Pt(ppy)(CF_3CO_2)(SMe_2)]. Herein, the syntheses of bis-chelate phosphine complexes **2** and **3** were carried out using complex **1** in a facile and in very short reaction time. Reaction between **1** and bis(diphenylphosphine)ethene (dppe) / bis (diphenylphosphine)ethylen (dppen) in (1:1) ratio resulted complexes **2** and **3** after stirring the reaction mixture for 1 minute at room temperature in dichloromethane (DCM). The complexes **2** and **3** were characterized by ^{1}H, ^{13}C and ^{31}P NMR.

^{1}H NMR spectra of complex **2** shows aromatic proton signals in the range δ= 6.8-8.3 ppm, the four protons of (CH_2-CH_2) of the dppe ligand were observed as multiplets at δ = 2.63 ppm (Fig. S3). The ^{13}C NMR spectra of the complexes **2** and **3** correspond to their structure (Fig. S4 and S5). The ^{31}P NMR of this complex shows two distinct singlets at δ = 41.39, for the P *trans* to N with $^1J_{(PtP)}$ = 3772 Hz, and δ = 51.16, for the P *trans* to C with a much lower value of $^1J_{(PtP)}$ = 1877 Hz due to the *trans* influence of C being much greater than that of N. The range of $^1J_{(PtP)}$ between 1877-3762 Hz indicates *cis* coordination of the phosphine ligand[17] (Fig.S6). The ethylene (CH=CH) proton signal for the dppen ligand appears at δ = 7.2 ppm as a multiplate[18] (Fig. S7). The ^{31}P spectrum of complex **3** shows two doublet of doublets, one at δ = 43.8 ppm with $^3J_{(PP\ b)}$= 17 Hz and $^1J_{(PtP\ a)}$ = 3782 Hz for P *trans* to N, and δ = 59.0 ppm with $^3J_{(PP\ a)}$= 17 Hz and $^1J_{(PtP\ b)}$= 1839 Hz for P *trans* to C (Fig. 1), the lower value of $^1J_{(PtP)}$ suggests *trans* effect of carbon with respect to nitrogen.

The complexes are soluble in solvents such as, dichloromethane (DCM), dimethyl formamide (DMF), 1,4 dioxane, methanol, acetonitrile etc, but are insoluble in water as well as in hexanes. These complexes show very week emission in all these solvents but they are intensely emissive in the solid state. These observations hint that all three complexes are expected to show AIE behaviour. In order to investigate this AIE property, water was used as poor solvent for complex **1** and hexane for complexes **2** and **3**.

Different amounts of water fraction (f_w = 0-90%) were added to the pure THF solution of **1** with keeping the same concentration of each solution to 1x 10^{-5} M. The emission intensity increased gradually with increasing f_w resulting in a cloudy solution indicating the formation of aggregates. The maximum emission intensity was observed with f_w= 90% which was 12.5 times higher than the intensity of its solution in pure THF (Fig. S8).

For complexes **2** and **3**, different amounts of hexane fraction (f_h= 0-90 %) [(Fig.2(i) - (vi)] were added to their solutions, (keeping the concentration of each solution remains same to 5 x 10^{-5}M). Maximum emission intensity was observed at f_h=90 %, for both the complexes. The emission intensity was increased by 19 times for **2** and 20 times for **3** as compared with their original respective solution intensities. The solid vs solution PL emission

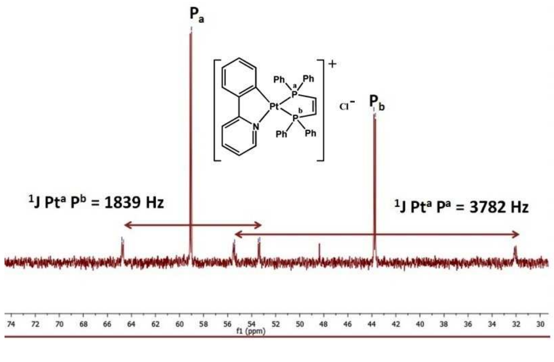
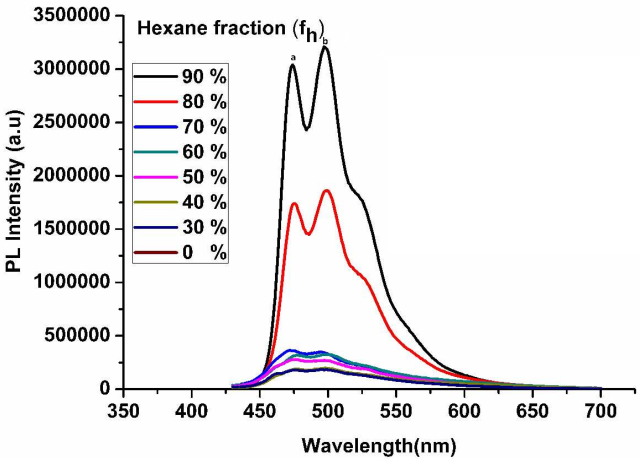


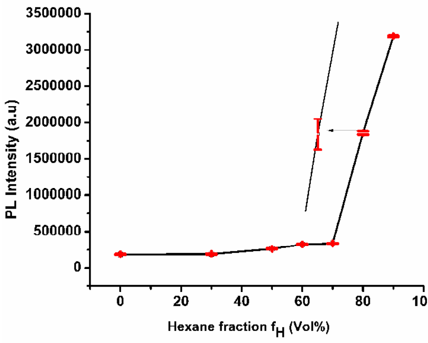
Fig. 1 ^{31}P NMR spectrum of [Pt(Pppy)(dppen)]Cl (**3**) in $CDCl_3$

spectra (in THF) for the complexes **1**, **2** and **3** are shown in Figs. S9-S11. The solid state absolute quantum efficiency (QE) for the complexes **2** and **3** were measured and found to 0.253 and 0.247, respectively. The solution quantum efficiency were found to be 0.001 and 0.0009, respectivley (Table S1). So, the solid state QE for the complexes **2** and **3** rises to ϕ_{solid}/ $\phi_{solution}$ > 250 (*i.e.*, **2** and **3** show 253 and 274 times higher QE than their respective solutions states). There was very significant rise of QE observed as far as the AIE property of the complexes are concerned.

To undestand the origin of the AIE property, the crystal-packing of **1** and **2** were examined (Fig. 3 , S12; Table S2, S3). The crystal structure of complex **1** shows short contacts, which are mainly C-H$\cdots\pi$ type of interactions and falls in the range of 2.64-2.85 **Å** [15d] (Fig.3a). This is shorter than the van der Waals radius of C-H. The crystal structure of **2** exhibits many C-H$\cdots\pi$ interactions in the range of 2.81-2.99 **Å** (Fig 3b). These interactions may be responsible for restricted rotation of the phenyl rotors present in these molecules in their solid states and hence the complexes exhibit AIE activity**.**

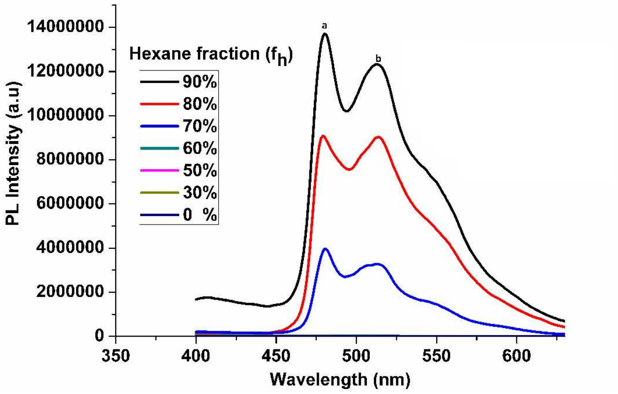


(i)

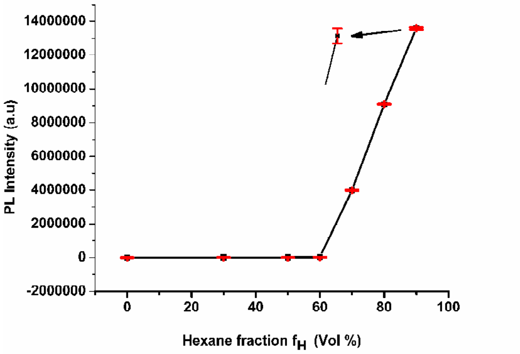


(ii)

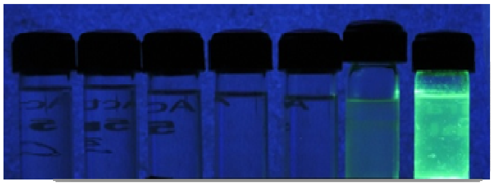
(iii)



(iv)

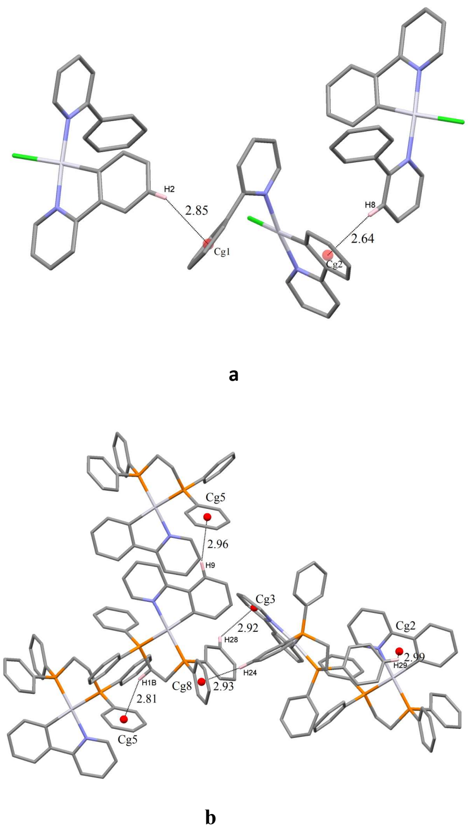


(v)



(vi)

Fig.2 PL spectra of **2** and **3** in DCM/hexane mixed solvents with different f_h with excitation at 385 nm [for (i) and (iv) for **2** and **3**, respectively], (λ_{max} : a=479 and b=512 nm for 1 and a=490 and b=517 nm for 2); Variation of PL intensity with respect to changes of wavelength with inclusion of error bar [(ii) and (v) for 2 and 3, respectively]; Luminescent images of **2** and **3** [(iii) and (vi) for **2** and **3**, respectively] (irradiated with an ultraviolet light at 365 nm) in hexane–DCM mixed solvents with the concentration kept at 2 × 10^{-5} mol·L^{-1}



a

b

Fig.3 (a) Crystal packing diagram of complex **1** showing C-H⋯π type short contacts, H2—Cg1 = 2.85 Å and H8—Cg2 2.64 Å (b) crystal packing diagram of complex **2** short contacts H1B—Cg5 = 2.81, H24—Cg8 = 2.93 H28—Cg3 = 2.92, H29—Cg2 = 2.99 and H9—Cg5 = 2.96 (the counter ion is omitted for clarity).

The computed energy gap between ground singlet state and first excited singlet state is 362.6 nm (Fig.4). This is in agreement with experimental absorption wavelength from spectroscopic study.

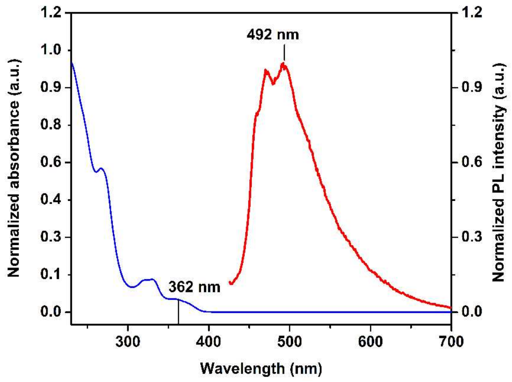
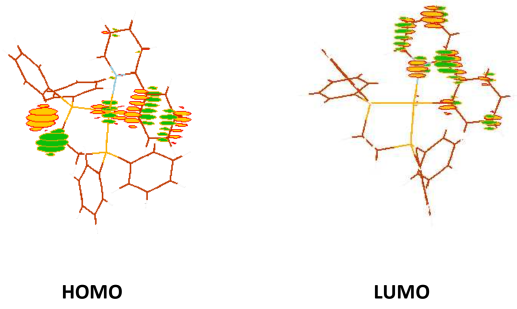


Fig. 4 UV–Visible absorption and photoluminescence spectra of complex **2** in 1x 10^{-5} M DCM.

The oscillator strength is high as compared to previously reported[19] iridium(III) complexes indicating stronger singlet to singlet absorption. The energy gap between ground singlet state to first excited triplet state (491.1 nm) (Fig. 4) is in accordance with emission wavelength obtained from spectroscopy. Although emission is an excited state property, results based on ground state optimization qualitatively describes the process[20]. Assignments of transitions (Table S4) show the relative involvement of different frontier orbitals in absorption and emission spectra. Natural Bond Orbital (NBO) analyses have revealed that chlorine atom makes major contribution towards HOMO, along with platinum(II). Whereas LUMO is exclusively distributed over the fused ring attached to platinum(II) (Fig. 5).



HOMO **LUMO**

Fig.5 Frontier orbitals for Platinum complex. Major contribution of HOMO comes from the counter ion, chlorine. LUMO is distributed over the fused ring. These orbitals are obtained from DFT calculations of the Platinum complex after ground state optimization. Calculations are performed by GAMESS US software. Visualization of the molecular orbital is through MOLDEN software

This data along with the assignments, indicate the strong possibility of metal to ligand charge transition (MLCT) in this complex (Table S4).

Biological applications: Human hepatocellular carcinoma cells, Hep3B were treated with increasing doses of platinum compound, **2** for varied time points and the cell viability was determined through several assays. As shown in Fig.6, the platinum compound showed increased cytotoxicity in Hep3B cells in a dose-dependent manner as determined by MTT assay. The IC_{50} of the compound was found to be around 5µM at 24h. Similar comparable results were obtained with WST-1 (Fig S13) and Trypan Blue assay (data not shown) performed to validate results from MTT assay. Time kinetic study was also performed by incubating the cells with increasing doses of platinum compound for 24, 48 and 72h. With increase in time of treatment with platinum compound, the cell viability was found to be significantly decreased (Fig. 6).

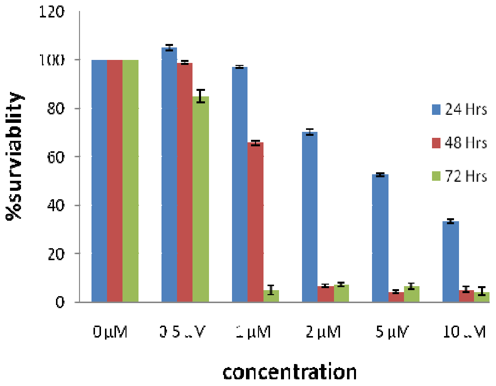
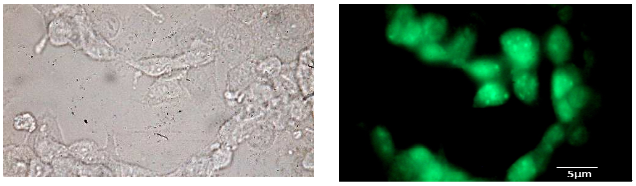


Fig.6 The time kinetic study of complex **2** as assayed by MTT

Further, this compound was successfully checked for cellular internalization potential through fluorescence imaging of live Hep3B cells (Fig. 7). An exclusive staining of the nucleus of live cells was observed; the platinum compounds are well known for their ability to form DNA-adducts. We speculate that the cytotoxicity that we observed in Hep3B cells can be attributed to the increased internalization potential of the drug and also to its property of binding to cellular DNA. Hence, the use of this compound provides one with dual option, not only for its use as an anti-cancer drug, but also as a cell-visualization or bio-imaging agent because of its fluorescence upon aggregation property and good cell membrane permeability.

A facile and greener synthetic methodology has been developed for the synthesis of these AIE active cyclo-metalated platinum(II) complexes. The synthesized complexes are also showing 'aggregation induced emission (AIE)' behaviour. In our laboratory, we are currently exploring the possible options to fabricate this compound for enhanced targeting of specifically cancer cells *in vivo*, compared to normal. Furthermore, we are also channelizing our future research to understand the molecular mechanism of anti-cancer activity of the compound so that they can be tagged with appropriate adjuvant to increase specificity and anti-cancer activity.



a **b**

Fig.7 Bright field image (**a**) and fluorescence image (b) (right, 100X) of Hep3B cells following treatment with complex **2.** Fluorescence image depicts successful internalization of the complex by Hep3B cells and its AIE characteristics. Scale bar (5µm).

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Notes and references

Crystal data for 2: the CIF file for 2 has been submitted to the Cambridge Crystallographic Data Centre (CCDC) CCDC number 1007679; $C_{37}H_{32}ClNP_2Pt$, space group C2/c , 100K, Z = 8, a = 18.0210(14) , b = 23.0522(17), c =16.6920(13) Å, β= ,V = 6927.3(9) $Å^3$.

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