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A New Platinum(II) complex for Bioimaging Applications

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Abstract

Two platinum(II) complexes containing imidazolyl terpyridine (**1**) and benzimidazolyl terpyridine (**2**) were synthesized and characterized by ESI-MS, ^1H NMR, UV-Visible and fluorescence spectroscopy. Pt(II) complex **1** emit weakly in aqueous solution but not complex **2**. On addition of DNA, a 21-fold increase in the emission intensity of complex **1** was observed. Both complexes did not exhibit any change in emission maxima with serum albumin. No uptake of the complexes by live cells was observed. In dead cells, complex **1** stains the nuclear DNA specifically without addition of any external fluorophore. The apoptotic pathway of the cells induced by plumbagin was monitored using complex **1**. Application of complex **1** as DNA staining dye was tested in agarose gel electrophoresis. Taken together, the results from this study presents the new platinum(II) complex as DNA staining agent an alternative to highly mutagenic ethidium bromide in gel electrophoresis as well as in dead cells at a non-toxic concentration.

Introduction

Fluorescent/phosphorescent imaging of biological samples using soft or non-invasive probes has been widely used to study the morphological changes in the cells. In this context, vast majority of the commercially available cell staining dyes are based on organic molecules. However, limitations such as solubility issue, shorter excited state lifetime and smaller Stokes shift of these organic molecules have prompted researchers to find alternatives for organic dyes as cell staining molecules.¹⁻³ To overcome these shortcomings, researchers have turned their attention to transition metal complexes due to their (i) longer excited state lifetime and large Stokes shift, (ii) biocompatibility, (iii) sensitivity of emission properties with respect to changes in local environment and (iv) relatively less complexity in synthesis.⁴⁻⁶ The facile synthesis of transition metal complexes bearing different functional groups on the ancillary ligands has been explored for specific applications such as chemosensors for solvents and different metal ions as well as biological applications like radiotherapy, protease inhibitor, antitumor agents and protein probes.⁷⁻¹⁵ For example, the classical ruthenium complex, SYPRO Ruby dye exhibit high sensitivity towards protein and has been applied in peptide mass-fingerprint analysis as well as for protein staining.¹⁶

To study the application of transition metal complexes as cell staining dyes, researchers have used the metal-peptide conjugates for rapid nuclear localization inside the HeLa cells.^{17, 18} Though the polyarginine or molecular transport agents like PEG facilitates the distribution of Ru(II) polypyridyl complexes throughout the cytoplasm and organelles, poor uptake by the nucleus has encouraged the researchers to devise a substitute by varying either the metal center or changing the ligand moiety.^{1, 19, 20} Very recently, dinuclear Ru(II) complex has been reported as two-photon absorbing luminescence lifetime probes for cellular DNA.²¹ Amoroso et al., have studied the distribution of Re(I) complexes containing lipophilic esters of 3-

hydroxymethylpyridine within the cytoplasm of live cells.²² Similarly, other metal complexes such as cyclometallated Ir(III) complex has been studied for its phosphorescent imaging properties of live cell as well as dead cells but with specificity towards cytoplasm.²³ Furthermore, an octahedral iridium(III) complex has been shown to stain the histidine-rich proteins but with limitation of downstream proteomic applications.²⁴

In contrast to Ru(II) and Ir(III), which are octahedral complexes, platinum (II) complex possess square planar structure with strong emissive behavior when it coordinated with cyclometallated ligands.²⁵ Recent studies have shown that the high photostability of PtLCl (HL: 1,3-di(2-pyridyl)benzene and derivatives) under prolonged irradiation has benefited the researchers in the development of tissue friendly Near IR two photon excitation for live cell imaging.^{26, 27} In dead cells, these complexes stain both cytoplasm and nucleus without any specificity. In addition, few other pyridyl and cyclometallated Pt(II) complexes are also known to exhibit high selectivity towards serum albumin.^{28, 29} Further, cyclometallated platinum(II) complex has shown to exhibit selective optical switch property for quadruplex DNA.^{30, 31}

In this study, we have demonstrated that by tuning ligand environment around the Pt(II) metal center one can modify the specificity of the target. In addition, we have shown that Pt(II) complexes at non-toxic concentration can be used as DNA staining agent in gel electrophoresis, a plausible replacement for mutagenic ethidium bromide.

Results and Discussion

Synthesis and characterization of Pt(II) complexes. Two platinum(II) complexes comprised of terpyridyl derivatives (Fig. 1) were synthesized and characterized using ESI-MS, UV-Visible, NMR and fluorescence spectroscopy. The electrospray ionization studies confirmed the

formation of desired complexes with molecular ion peaks observed at m/z 530.2 and 580.1, for complexes **1** and **2**, respectively (Fig. S1 and S2). The UV- region (200-350 nm) of the absorption spectra of complexes **1** and **2** was dominated by intraligand (π - π^*) transitions, while the 3 MLCT band was observed at around 410-450 nm. Similar characteristic spectral bands have also been observed with other reported platinum(II) complexes containing terpyridyl derivatives.³² The complex formation was further confirmed by 1 H NMR studies with downfield chemical shift in the ligand peaks compared to ligand alone (data not shown).

Absorption spectral titration studies. The interaction of Pt(II) complexes with DNA was monitored by using UV-visible absorption titration. The absorption titration of DNA was carried out by varying the concentration of DNA while keeping the concentration of Pt(II) complex constant. Generally, intercalation results in hypochromism with red shift in the spectral band, due to stacking ability of the chromophore between the DNA base pairs. Upon addition of incremental amounts of DNA, the absorption intensity at 410 nm of complex **1** increased with red shift in the spectral band (Fig. 2). However, in the case of complex **2**, the intensity of the spectral band at 450 nm increased but no red shift in the position of the absorption band was observed upon addition of incremental amount of DNA. The spectral titration results reveal that both the complexes bind to DNA with similar affinity in the range of 10^4 M⁻¹. The binding constants reported here are similar to previously reported groove binders/partial intercalators, such as ruthenium(II) and chromium(III) complexes.^{33, 34} The partial intercalative mode of binding of the two complexes to DNA is not surprising, as the itpy and bzitpy ligands might adopt non-planar structure due to free rotation of C-C bond of imidazole moiety and terpyridine unit. A similar hyperchromic effect was reported earlier in the case of [Pt(DIP)Cl₂] in which complex binds with DNA in a partial intercalative mode and resulted in deformation of DNA

structure.³⁵ Earlier reports also shown that partial intercalative mode along with hyperchromic effect for other transition metal complexes.^{36, 37}

Luminescence studies of Pt(II) complexes with DNA and serum albumin. The luminescence properties of the two Pt(II) complexes were examined by exciting Pt(II) complexes in the range of 350-380 nm. Complex **1** exhibited weak emission at 460 nm as well as at 533 nm. The band at 460nm has been assigned as ³IL charge transfer transition. However, complex **2** which possess a benzimidazolyl in the tpy ligand did not exhibit any emission (Fig. S3). The photoluminescence property of complex **1** was further tested with biomolecules such as CT DNA and BSA. The emission intensity of complex **1** was enhanced upto 21-fold upon addition of DNA (Fig. 3). However, the increase in emission intensity of **1** with DNA is much lower than that observed with the mononuclear cyclometallated Pt(II) complex (271-fold) and binuclear Pt(II) complex (117-fold) containing planar aromatic ligand moiety.³⁸ The quantum yield of complex **1** with and without DNA was measured using quinine sulfate as standard reference. The quantum yield of complex **1** was found to increase from 0.004 to 0.21 in a fully bound state with DNA. We further tested the emission property of complex **1** with different artificial DNA systems like poly(dA-dT) as well as poly(dG-dC) nucleotides. In both systems, the emission profile did not exhibit any quenching, in turn, it does not show any specificity towards particular base pairs. This is in contrast to previous studies in which most of the platinum(II) complex containing terpyridine system exhibit specificity towards G-C nucleotides followed by A-T base pairs.^{39, 40}

From the fluorescence titration data, binding isotherm was constructed to determine the binding constant as well as binding site size. A plot of r/C_F vs. binding coefficient (r) provided the binding isotherm and best fit of the data to eqn. 3 give rise to a binding constant of 1.75 ± 0.2

$\times 10^4 \text{ M}^{-1}$ and site size (n) as 2.1 ± 0.3 (Fig. S4). The binding constant obtained from the fluorescence titration data is consistent with the absorption titration data and within the experimental error of the two different methods. The binding site size for complex **1** with CT DNA was estimated to be 2.1 ± 0.3 for complex and found to be less than that of other cyclometallated platinum(II) complex as well as $[\text{Ru}(\text{bpy})_2(\text{phi})]^{2+}$ with binding site size of 4 in base pairs⁴¹. The results obtained from this study further indicates that the complex **1** binds weakly compared to other Pt(II) cyclometallated complexes.

The molecular light switch effect observed here can be attributed to the proximity of the Pt(II) center to the hydrophobic region of the DNA thereby preventing solvent-induced quenching of emission of metal complex. However, the lower enhancement in emission intensity of **1** compared to other Pt(II) complexes can be explained in terms of the non-planar structure of the ligand that may result in partial intercalation of the complex between the DNA base pairs. A similar DNA molecular switch effect was previously observed for other ruthenium(II) dipyrrophenazine complex as well as cyclometallated Pt(II) complexes.⁴² The molecular switch effect of complex **1** was further explored for proteins such as serum albumin. However, no increase in emission intensity of complex **1** was observed with addition of serum albumin (data not shown). This is in contrast to $\text{Pt}(\text{bzimpy})\text{Cl}]^+$ reported previously, in which the emission intensity increased drastically with blue shift of 72 nm from 625 to 553 nm.²⁸ The lifetime measurements were also performed in air-saturated aqueous solution and found to be 4 ns which is from ³IL charge transfer transition. Upon addition of DNA, the lifetime of complex **1** doubled to 8 ns. In contrast, previous reports have shown that cyclometallated Pt(II) complex exhibit longer lifetime and it was explained in terms of rigid chelate framework that restricts the motion of Pt-C center and also the efficiency of vibrationally assisted decay.⁴³ The observed results

clearly revealed that by tuning the ligand environment, the luminescent property of the complexes vary dramatically and thus can be utilized for bio-imaging application.

Cytotoxicity of complexes 1 and 2. We have tested the cytotoxicity of the two Pt(II) complexes towards A431 and HaCat cells by treating both these cells with varying concentration of the two complexes. The cytotoxic effect of complexes **1** and **2** was analyzed using the MTT assay. The results of MTT assay are shown in Fig. 4. It can be seen from the Fig. 4 that in the presence of 20 μM of the complex **1**, viability of the HaCaT cells decreased to 41% from 100% observed in the presence of 10 μM of the complex and any further increase in the concentration of this complex had only marginal influence on the viability of HaCaT cells. On the other hand, viability of this cell in the presence of 20 μM complex **2** was only marginally different from that observed at 10 μM .; the viability remained same. Any subsequent increase in the concentration of complex **2** however led to drastic decrease in the viability of the cells. In the case of A431 cells the cell viability gradually decreased with increase in the concentration of the complexes. Both the complexes had similar effect on A431 cells. Complex **1** exhibited IC_{50} value of 20 μM for both HaCat and A431 cell lines. However, in the case of complex **2**, IC_{50} value for HaCat and A431 cell lines were between 20- 40 μM . The results indicates that complex **2** is 2-fold less toxic compared to complex **1** in the case of A431 cell line. We observed that the complexes showed better anti cancerous activity when compared to cis-platin. The IC_{50} values for cisplatin in HaCaT and A431 cells were observed to be 50 and 100 μM respectively. The results are provided in the supplementary information (Fig. S5).

Cell Imaging by complexes 1 and 2. The synthesized Pt(II) complexes were tested for their staining ability of the cells. Both the Pt(II) complexes at various concentrations were treated with HaCat cells. We observed no fluorescence from cells at concentrations below toxicity level i.e.

10 μM whereas significant fluorescence was observed at concentrations above IC_{50} of these complexes (Fig. 5). The results suggest that when the cell integrity and viability is challenged, the complexes gave fluorescence from the cells. It shows that the complexes are impermeable in viable cells and when the cell integrity is lost the cell membrane become permeable for these complexes (Fig. 5 and 6). Complex **1** stains the nuclear region in the cells selectively (Fig. 5 and S6) while no fluorescence was observed for complex **2** (data not shown). The results indicated that the fluorescence of complex **1** was observed only when the cell viability is lost. Hence the complex **1** was further explored for its application in imaging cells undergoing apoptotic events by inducing apoptosis in the cells with a well-known apoptosis inducer plumbagin. The cells were categorized into four groups (i) cells treated with plumbagin for 12h followed by treatment with complex **1**, (ii) cells treated with both plumbagin and complex **1** simultaneously, (iii) cells treated with complex **1** alone and (iv) control cells. The results indicated that the complex **1** can be used to image the various apoptotic features of the cells such as cell blebbing, nuclear condensation and fragmentation (Fig. S7).

The groups (i) and (ii) (Fig. 7) showed significant fluorescence whereas no fluorescence was observed from groups (iii) and (iv) cells indicating that complex concentration as low as 10 μM can detect dead cells and the concentration of complex **1** used was nontoxic to cells.

DNA staining by complex 1. To explore the DNA staining ability by complex **1**, gel electrophoresis was performed by treating complex **1** with DNA and compared with ethidium bromide (a known DNA staining agent). The results showed that complex **1** gave fluorescence when bound with DNA extracted from the cells. The fluorescence intensity was compared with ethidium bromide (10 μg). The fluorescence intensity increased with increase in concentration of complex **1** (Fig. 8a). We observed fluorescence even at concentration as low as 10 μM although

the intensity was less compared with ethidium bromide (Fig. 8b). However, the toxicity of complex **1** was significantly low in comparison to ethidium bromide.

To demonstrate that complex **1** can be used as a plausible alternative to ethidium bromide for detecting circular DNA, plasmid pUC18 DNA was incubated with complex **1** with different concentrations and compared with ethidium bromide. In contrast to genomic DNA staining, increasing concentration of complex **1** did not enhance the fluorescence intensity upon binding with plasmid DNA and as low as 10 μM concentration of complex **1** exhibit the similar property as of mutagenic ethidium bromide but with less toxicity (Fig. 9). We assume that the complex display emission enhancement with cellular DNA but not plasmid DNA due the fact that the plasmid DNA are circular and supercoiled whereas genomic DNA are linear. Hence in case of genomic DNA more flexibility is available there for the Pt complexes to interact with DNA when compared to the circular supercoiled Plasmid DNA which may hinder the accessibility of Pt complexes to interact with plasmid DNA. The results implies that the Pt(II) center in the complex binds to DNA effectively and specifically. In contrast to previously reported Pt(II)-thiol side chain complexes in which the complexes involves in the cell lysis but could not reach the cell nucleus to intercalate into DNA¹², the results observed from this cell based study is consistent with luminescence studies in which enhancement in the emission intensity upon addition of CT DNA while no change in emission intensity was observed with serum albumin.

Conclusions

In summary, our data reveals that (i) Pt(II) complex with imidazolyl derivative exhibit enhancement in emission intensity upon addition of CT DNA but not with serum albumin. In contrast, no emission was observed for complex **2** containing benzimidazolyl terpyridine moiety

with DNA; (ii) uptake of both complexes in the live cells are absent; (iii) in dead cells, complex **1** stains the nuclear DNA specifically; (iv) complex **1** stains the apoptotic events induced by plumbagin; (v) complex **1** can be used for staining DNA in gel electrophoresis as effectively as highly mutagenic ethidium bromide at a concentration much lower than IC₅₀ value of complex **1** indicating that it can be a better staining agent than ethidium bromide. The present study demonstrates the versatility of Pt(II) complex with five-membered imidazolyl linked terpyridyl ligand system in the development of new staining probes for biological applications.

Experimental Section

Materials and Methods. BSA (fraction V powder, lipid free, 66 kDa) and potassium tetrachloroplatinate (II) (K₂PtCl₄) were purchased from Sigma (St. Louis, MO, USA) and used as such without any further purification. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and calf thymus DNA (CT DNA) was purchased from SRL Chemicals. All the DNA related experiments were carried out at pH 7.0 at 25 °C. The solvents were purchased from Sigma-Aldrich Chemicals and used as received unless otherwise specified. Plasmid pUC18 was purchased from Bangalore Genei, India.

Synthesis and characterization of Pt(II) complexes. Ligands Itpy and bzitpy were synthesized as per the reported literature.⁴⁴ Briefly, the two Pt(II) complexes were synthesized by mixing the corresponding ligand and K₂PtCl₄ (1:1 molar ratio) in 10 mL dimethyl sulfoxide and refluxing for 6 hrs. The reddish orange compound was precipitated by adding water to the reaction mixture. The obtained product was washed with hot methanol and dried under vacuo. The product was recrystallized from methanol.

[Pt(itpy)Cl]Cl (1). ESI-MS: *m/z* 530.20 ([M-Cl]⁺); ¹H NMR in dms_o-d₆(δ , ppm): 13.1 (s, 2H), 8.8 (d, 2H), 8.4(d,2H), 7.8 (d, 2H); 7.5(2H,d). Elemental analysis: calcd: C, 39.39; H, 2.61; N,

12.09. Found: C, 38.98; H, 2.8; N, 11.89. **[Pt(bzitypy)Cl]Cl (2)**. ESI-MS: m/z 580.13 ($[M-Cl]^+$); 1H NMR in dms o - d_6 (δ , ppm): 13 (s, 2H), 8.8 (d, 2H), 8.5(d,2H), 7.9 (d, 2H); 7.7 (d, 2H); 7.3 (m, 2H). Elemental analysis: calcd: C, 43.89; H, 2.72; N, 11.13. Found: C, 43.41; H, 2.65; N, 10.87.

Interaction of Pt(II) complexes with biomolecules. Absorption titration studies were carried out using Shimadzu UV-1800 spectrophotometer. Thermal denaturation studies of DNA with Pt(II) complexes were carried out using JASCO-815 spectropolarimeter equipped with Peltier temperature controller. Luminescence studies of the interaction of Pt(II) complexes with DNA were performed using Cary Eclipse fluorescence spectrophotometer. ESI-MS analysis of the metal complexes was performed using Thermo-Finnigan LCQ Advantage max ion trap mass spectrometer. 1H NMR spectra of metal complexes in dms o - d_6 were recorded with Bruker Topspin 400 MHz spectrometer.

UV titration of Pt(II) complexes with DNA. A solution of calf thymus DNA was prepared by dissolving in the Tris buffer. The purity of DNA was confirmed by monitoring UV absorbance ratio at 260 and 280 nm of about 1.8–1.9:1, indicating that the DNA was sufficiently free from protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($6600 M^{-1} cm^{-1}$) at 260 nm.⁴⁵ Absorption titration experiments involving the interaction of the complex with DNA were carried out in Tris buffer (10 mM Tris, pH 7.2). Titration experiments were performed by maintaining the metal complex concentration constant (20 μM) and varying the concentration of nucleic acid (20–260 μM). While measuring the absorption spectra, equal amount of DNA was added to both complex solution and the reference solution to eliminate the absorbance of DNA itself. From the absorption data, the intrinsic binding constant K_b was determined using the following eq. (1) through a plot of $[DNA]/(\epsilon_a - \epsilon_f)$ vs. $[DNA]$

$$[\text{DNA}]/(\varepsilon_a - \varepsilon_f) = 1/K_b (\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) \dots \dots \text{eqn (1)}$$

where $[\text{DNA}]$ is the concentration of DNA, the apparent absorption coefficient ε_a , ε_f and ε_b correspond to $A_{\text{obsd}}/[\text{Pt}]$, the extinction coefficient for free Pt complex and extinction coefficient for platinum complex in the fully bound form, respectively. Binding constant K_b was determined from slope/intercept of the linear plot.

Luminescence studies of Pt(II) complexes with DNA/Protein. The luminescence studies of Pt(II) complexes with DNA or protein were performed in 3mL quartz cuvette containing 40 μM metal complex solution. The CT DNA concentration was varied from 50 to 800 μM at an interval of 50 μM concentration. A similar concentration of protein was used for luminescence assay. The samples were excited between 350-410 nm and the emission spectra were recorded between 430-700 nm. From the fluorescence titration experiments, the binding isotherm were constructed to determine the intrinsic binding constants and binding site size by using the following equations (2) and (3)

$$C_F = C_T(I_0/I - P)/(1 - P) \dots \dots \dots (2)$$

where C_T is the total concentration of the probe added while C_F is the concentration of the free probe. The fluorescence intensity of the probe in the absence and presence of DNA were represented as I_0 and I , respectively. The ratio of observed fluorescence quantum yield of the bound probe to that of free probe was represented by P and obtained by plotting I_0/I vs. $1/[\text{DNA}]$ with limiting fluorescence quantum yield given by the y-intercept. The amount of bound probe (C_B) at any concentration was equal to $C_T - C_F$. A plot of r vs. r/C_F , where r is binding coefficient and equal to $C_B/[\text{DNA}]$, was constructed according to the modified Scatchard equation (2) given

$$r/C_F = K_i(1 - nr)[(1 - nr)/(1 - (n-1)r)]^{n-1} \dots \dots \dots (3)$$

by McGhee and Von Hippel⁴⁶⁻⁴⁸. In equation (3), K_i is the intrinsic binding constant and n is the binding site size in base pairs. The binding data were fitted to eqn. (3) using non-regression analysis in Origin 9.0 software. From the best fit of the data to the equation, K_i and n were obtained.

Time-resolved emission studies for the complex in the absence and presence of DNA was performed using a picosecond-laser-excited TCSPC spectrometer. The excitation source was a tunable Ti-Sapphire laser (Tsunami Spectrophysics, USA) with a pulse width of less than 2 ps and a repetition rate of 82 MHz. Samples were excited at 405 nm and the emission of the complex with and without DNA was monitored with an MCP-PMT (Hamamatsu-C 4878) detector. The decay traces were deconvoluted by using a non-linear least-squares analysis with IBH software.

The quantum yield measurements for the complex **1** with and without DNA were determined using the equation $\Phi = \Phi_{\text{ref}} \times (\nabla / \nabla_{\text{ref}}) \times (\eta^2 / \eta_{\text{ref}}^2)$, where Φ and Φ_{ref} are the quantum yields of the sample and reference, ∇ and ∇_{ref} are the slopes of linear plots of the absorbance at the excitation wavelength vs. Integrated emission intensity, and η and η_{ref} are the refractive indices of the solvents used. Absorbance values were kept below 0.1 AU at the excitation wavelength. Both the samples and reference were excited at 377 nm. The reference used for this study was quinine sulfate in air-equilibrated water ($\Phi:0.546$)

Cell Cytotoxicity and biocompatibility of Pt(II) complexes. The biocompatibility of platinum complexes **1** and **2** was evaluated to understand the IC_{50} value of this molecule. The Pt(II) complexes **1** and **2** at various concentrations ranging were treated with HaCaT (immortalized human normal keratinocytes) and A431 (Human Epidermoid carcinoma). Briefly, 12-15 K A431 and HaCaT cells/well were seeded onto 24 well tissue culture plates and allowed to grow it for

24 hours in DMEM medium at 37°C (5% CO₂ and 95% air) in a CO₂ incubator. The medium was removed and washed with 1X PBS then fresh medium containing various concentration of Pt complex **1** and complex **2** namely 10µM, 20 µM, 40 µM, 60 µM, 80 µM and 100 µM were treated to the cells. Cells were also treated with various concentrations of cisplatin (as positive control) to compared the toxic effect of Pt complexes with cisplatin. All the samples were tested in triplicates. After 24 hours the medium was removed and the cells were treated with 0.5mg/mL of MTT (Thiazolyl Blue Tetrazolium Bromide salt) in 1X PBS (250 µL/well) and kept it for 4 hours in a CO₂ incubator. The MTT solution was then removed and the blue coloured formazan crystal formed was solubilized in 100 µL DMSO. The absorbance was measured at 570 nm.

Fluorescent studies of Pt(II) complexes in cells. The two Pt(II) complexes were treated with HaCaT (immortalized keratinocytes) and A431 (Epidermoid carcinoma) cells. A431 and HaCaT cells approximately 12-15 K cells/well were seeded onto 24 well tissue culture plates and allowed attach and spread by incubating for 24 hours at 37°C in a 5% CO₂ and 95% air atmosphere in a CO₂ incubator. After 24 hours of incubation, the cells were examined under phase contrast microscope and observed that the cells had good morphology and attained 60-70% confluence. The medium was removed and the cells were washed with 1X PBS. Fresh DMEM medium containing varying concentrations of Pt(II) complexes namely 10µM, 20 µM, 40µM, and 100µM were treated with the cells. All experiments were carried out in duplicates. The cells were maintained in the culture for 24 hours at 37°C in a CO₂ incubator. Cells were viewed and imaged using Leica fluorescence microscope.

Differential staining of dead and viable cells by complex 1. A431 and HaCaT cells were seeded onto 48 well tissue culture plates (approximately 12-15 K cells/well) and allowed to grow for 24 hours at 37°C in a CO₂ incubator. The cells were grouped (Group i to iv) into four, based

on the treatment. Group i) 12 wells were treated with plumbagin (3 μ M) alone and incubated for 12 hours. After incubation, the cells were treated with complex **1**(10 μ M)for 30 minutes. Group ii) 12 wells were treated with plumbagin (3 μ M) along with complex **1**(10 μ M) for 12 hours. Group iii) 12 wells were treated with complex (10 μ M) alone. Group iv) 12 wells were maintained as untreated control. After 12 hours incubation the cells were viewed under the Leica fluorescent microscope against blue/violet filter.

***In vitro* application of complex 1 for DNA visualization.** DNA was extracted from plumbagin treated HaCaT cells. The purity and concentration of extracted DNA was quantified by absorbance at 260/280. Equivalent amount of DNA in loading buffer containing complex **1**(25 μ M, 50 μ M, 100 μ M, 150 μ M and 200 μ M) was loaded into the well of 1% agarose gel. The electrophoresis was done using 1X TBE buffer at 100V. After electrophoresis, the gel was visualized and subjected to documentation using BioRad Gel Doc system.

Circular DNA visualization by complex 1 in vitro. Equivalent amount of circular DNA (plasmid pUC18) in loading buffer containing complex **1** with different concentrations was incubated and loaded into the well of 1% agarose gel. The control sets were prepared with equivalent amount of ethidium bromide. The electrophoresis was done using 1X TBE buffer at 100V. After electrophoresis the gel was visualized and subjected to documentation using BioRad Gel Doc system.

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Figure Legends

Figure 1. Structures of Pt(II) complexes

Figure 2. Absorption titration of Pt(II) complexes with DNA (a) complex **1** and (b) complex **2**.

Inset: Plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ vs. $[\text{DNA}]$

Figure 3 Emission spectra of complex **1** (40 μM) in the absence and presence of CT DNA (50-800 μM) in Tris buffer (10 mM, pH 7.2). λ_{ex} : 400 nm

Figure 4. Biocompatibility of Pt(II) complexes on (a) HaCaT and (b) A431 cell lines.

Figure 5. *In vivo* imaging of cells using complex **1** at different concentrations (a,e) 10 μM (b,f) 20 μM , (c,g) 40 μM and (d, h) 100 μM . Top: Fluorescence image and bottom: Phase contrast image. (Scale bar represents 10 μm)

Figure 6. Differentiation of live and dead cells by complex **1**. (A) Phase contrast (B) fluorescence and (C) Overlay image. (Scale bar represents 10 μm)

Figure 7. Differential staining of Pt complex **1** against apoptotic and non apoptotic cells *in vivo*. Figures a,e,i,m represents the untreated HaCaT and A431 cells respectively. b,f,j,n - HaCaT and A431 cells treated with complex **1** (10 μM) alone. c,g, represents the HaCaT and k,o represents A431 cells treated with plumbagin (3 μM) and complex **1** (10 μM) simultaneously. d,h,l,p represents the HaCaT and A431 cells treated with plumbagin (3 μM) and incubated for 24 hours to induce apoptosis followed by treatment with complex **1** (10 μM). Scale bar represents 10 μm .

Figure 8. *In vitro* DNA fragment visualization by using (a) complex **1** and (b) ethidium bromide

Figure 9. Circular DNA visualization by using complex **1**

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Figure 1.

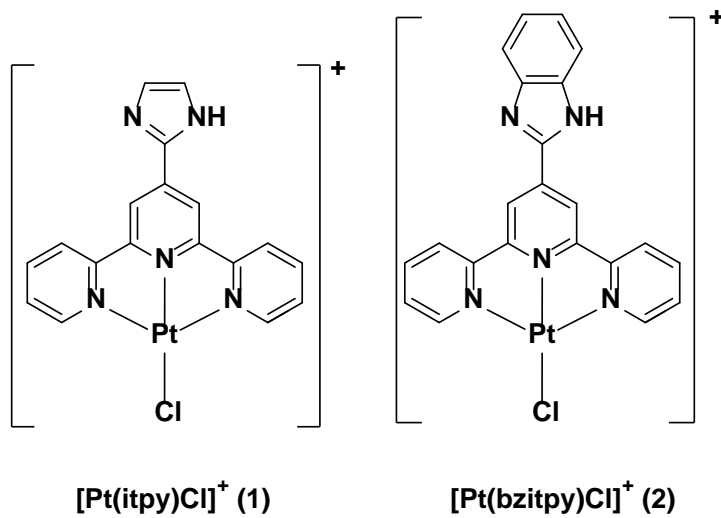


Figure 2.

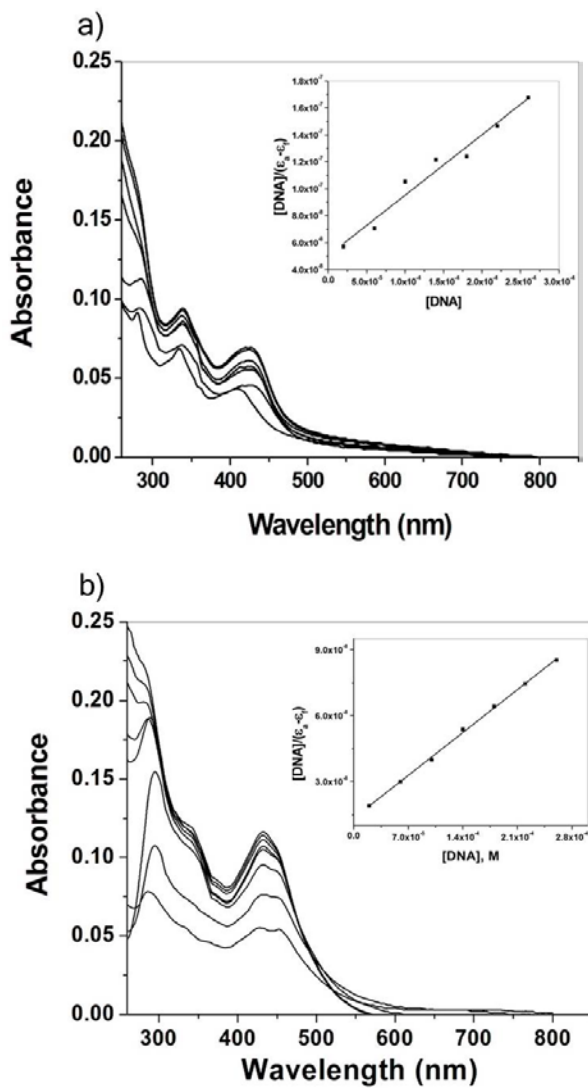


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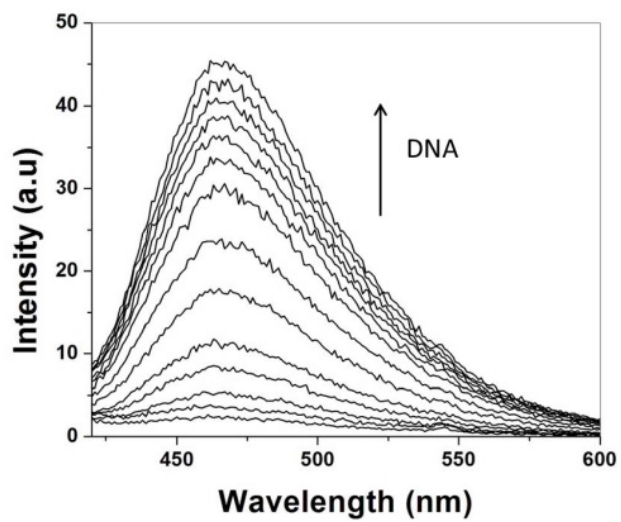


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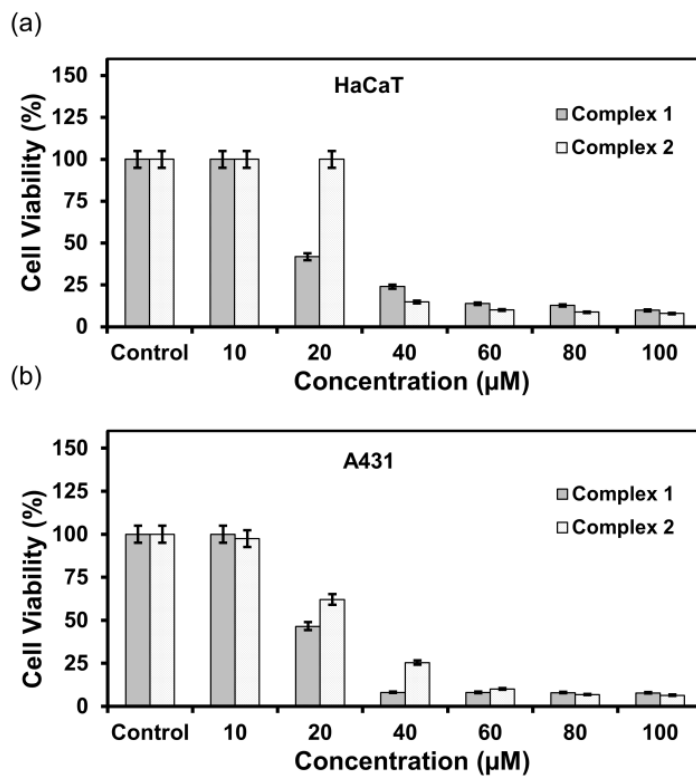


Figure 5.

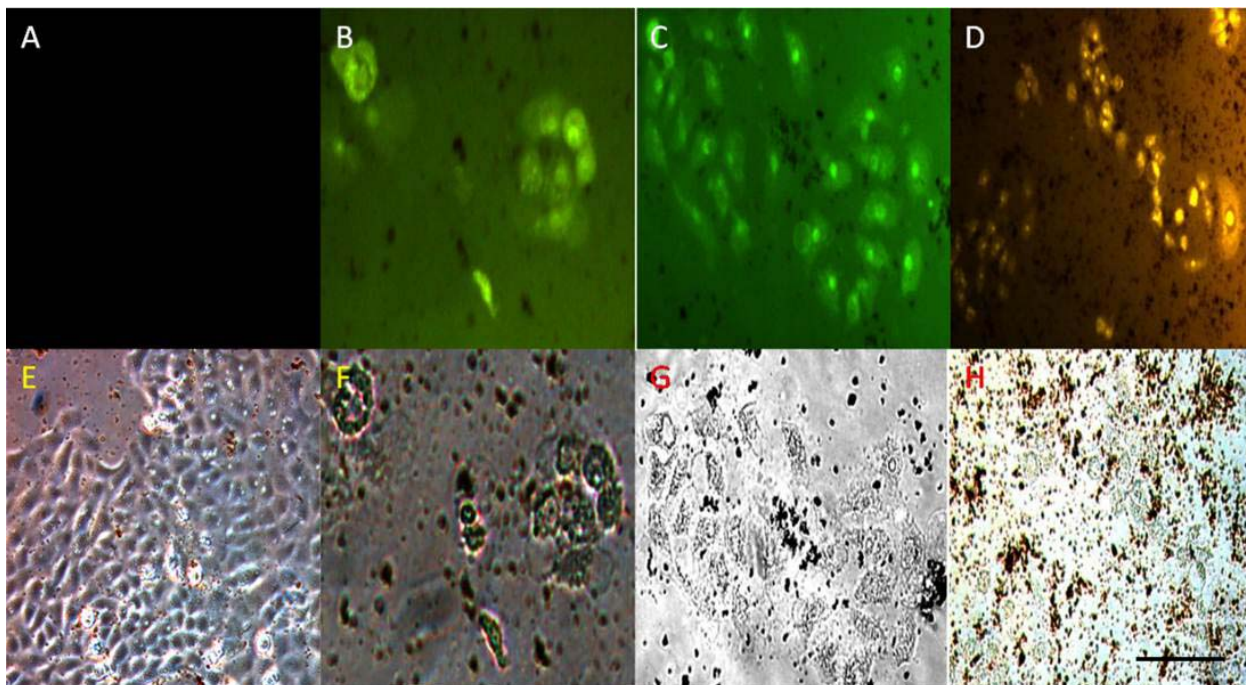


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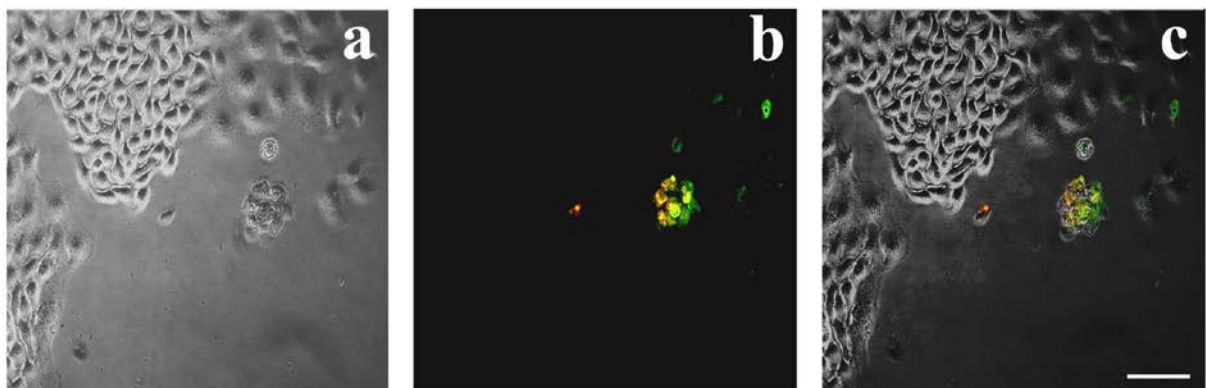


Figure 7.

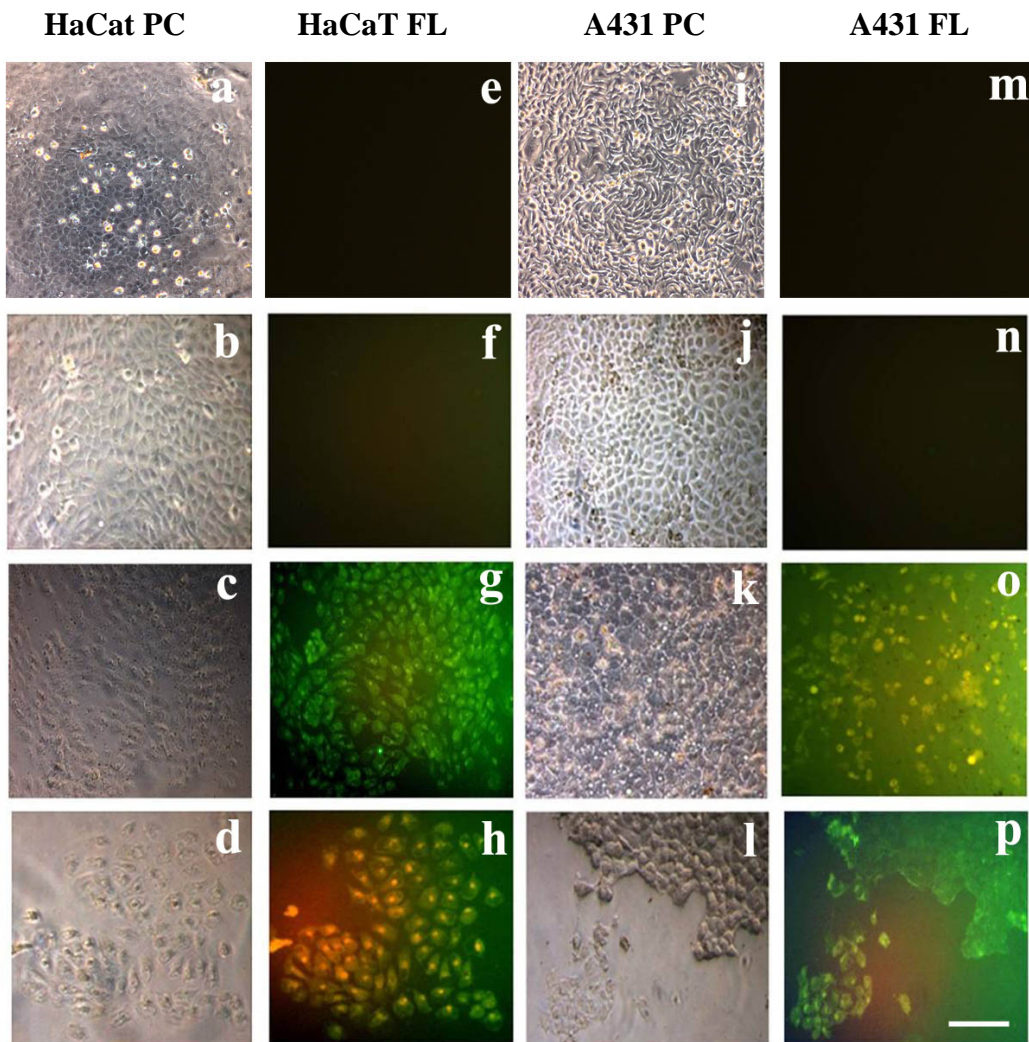


Figure 8.

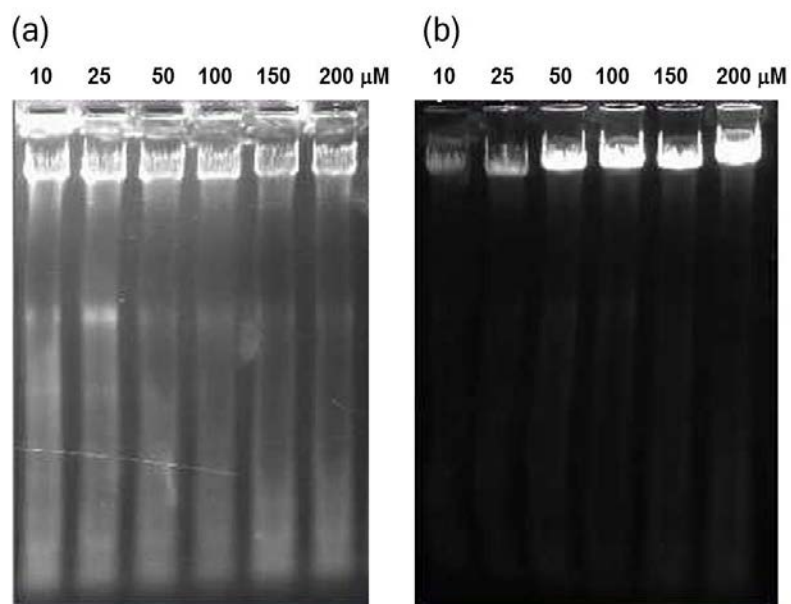
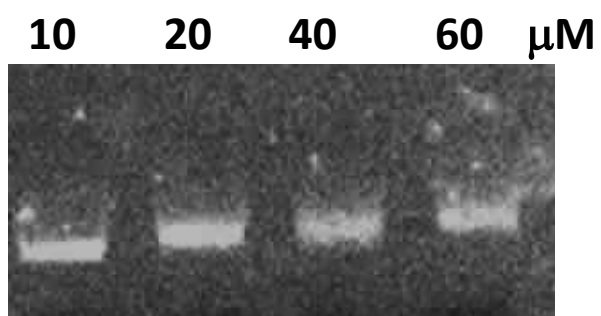
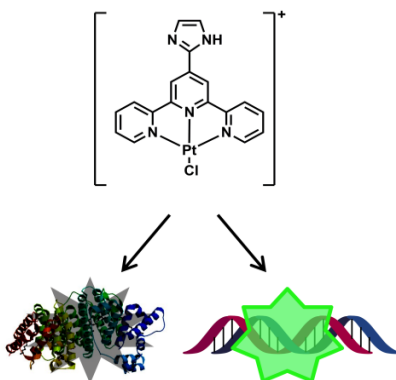


Figure 9.

Graphical Abstract

Eco-friendly Pt(II) complex as DNA staining agent: A new Pt(II) complex bearing terpyridine derivative exhibit specificity towards nuclear DNA. The staining ability has been explored in cell imaging as well as in gel electrophoresis an alternative to highly mutagenic ethidium bromide



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A New Platinum(II) complex for Bioimaging Applications