Pyriplatin-Boron-Dipyrromethene Conjugates for Imaging and Mitochondria-Targeted Photodynamic Therapy

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Supporting Information

ABSTRACT: Monofunctional pyriplatin analogues *cis*-[Pt(NH₃)₂(L)Cl](NO₃) (1–3) having boron-dipyrromethene (BODIPY) pendants (L) with 1,3,5,7-tetramethyl-8-(4-pyridyl)-4,4'-difluoroboradiazaindacene moieties were designed and synthesized, and their photocytotoxic properties were studied. The Pt-BODIPY conjugates displayed an absorption band within 505–550 nm and a green emissive band near 535 nm in 1% DMSO/DMEM (Dulbecco's modified Eagle's medium) buffer. Complex *cis*-[Pt(NH₃)₂(4-Me-py)Cl](NO₃) (4) was used as a control for determining the structural aspects by X-ray crystallography. The mono- and diiodinated BODIPY complexes 2 and 3 showed generation of singlet oxygen on light activation as evidenced from the 1,3-diphenylisobenzofuran (DPBF) titration experiments. The cytotoxicity of the BODIPY complexes was tested against A549 (human lung cancer), MCF-7 (human breast cancer), and HaCaT (human skin keratinocyte) cells in dark and visible light (400–700 nm, 10 J cm⁻²). While complexes 2 and 3 showed excellent photocytotoxicity (IC₅₀ ≈ 0.05 μM), they remained essentially nontoxic in the dark (IC₅₀ > 100 μM). The emissive bands of 1 and 2 were used for cellular imaging by confocal microscopy study, which showed their mitochondrial localization. This was further supported by platinum estimation from isolated mitochondria and mitochondrial depolarization through a JC-1 assay. The photomediated apoptotic cell death was evidenced from flow cytometric assays, annexin-V/FITC-PI (fluorescein isothiocyanate-propidium iodide) and cell cycle arrest in sub-G1 and G2/M phases. The complexes bind to 9-ethylguanine as a model nucleobase to form monoadducts. A mechanistic study on DNA photocleavage activity using pUC19 DNA showed singlet oxygen as the reactive oxygen species (ROS). The combination of photodynamic therapy with DNA cross-linking property enhanced the anticancer potential of the monofunctional BODIPY-conjugates of pyriplatins.

INTRODUCTION

Cisplatin and its analogues, namely, oxaliplatin and carboplatin, are the currently used platinum-based chemotherapeutic drugs.^{1–3} The efficacy of such bifunctional anticancer drugs is largely dependent on the rate of hydrolysis of the Pt–Cl or Pt–O bonds in the intracellular medium besides their cellular uptake properties. The ancillary ammine/amine ligand(s) play important roles in the bond dissociation kinetics. These platinum(II) drugs characteristically form intra- and/or interstrand DNA cross-links with nuclear DNA through covalent bonding at N7 positions of two guanosine residues and cause a variety of cellular responses including transcription inhibition and cell apoptosis. Despite the clinical success of these bifunctional drugs, they suffer from dose limiting and other side effects. To circumvent the inadequacies of such drug molecules, the chemistry of monofunctional platinum(II) complexes showing antineoplastic activities has received current interest (Figure 1).^{4–8} Lippard and co-workers have developed a series of monocationic platinum(II) complexes including pyriplatin, imidazoplatin, and phenanthriplatin and

tested their activity against several tumor cells.^{2,4,5,7a} Phenanthriplatin and pyriplatin have showed excellent cellular accumulation compared to cisplatin and oxaliplatin, thus having potential for tumor targeting. Although the transcription inhibition of the pyriplatin adducts are similar to that of cisplatin, their reduced efficacy is likely due to the nucleotide excision repair (NER) mechanism being functioning for nuclear DNA.⁹ The NER machinery can be circumvented by directing the complexes toward other cellular organelles such as mitochondria (mt) as the NER process is absent for mtDNA.¹⁰

Mitochondria being a regulator of the metabolic functions, targeting mitochondria for anticancer activity is a potential alternative strategy. Different types of nanoparticles, metal-chromophore, or organic dyes, namely, macrocyclic dyes like porphyrins that include the photodynamic therapy (PDT)

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Figure 1. Bifunctional platinum(II) complexes: cisplatin and CP-11.^{1,20a} Monofunctional platinum(II) complexes: phenanthriplatin,^{5b} trans-Pt-BA,^{8a} Pt-BODIPY,^{22a} and imidazoplatin-B.^{8b}

drug photofrin, and phthalocyanines, are developed to achieve this objective.^{11–14}

Boron-dipyrromethene (BODIPY) dyes are known to display localization in different cellular organelles, and the commercially available BODIPY dyes have been extensively used as trackers in cellular imaging studies.^{15–17} The photophysical and photochemical properties make them suitable for bioimaging and as therapeutic agents. Introducing a heavy atom (for example Br or I) on the BODIPY core increases its efficacy for singlet oxygen ($^1\text{O}_2$) generation with a high quantum yield and makes such BODIPY dyes as excellent photosensitizers for *in vitro* and *in vivo* photodynamic applications.¹⁸ Recent reports have shown that BODIPY-appended transition metal complexes are useful for real-time drug tracking inside the cells.^{19–23} Recently Guo and co-workers have shown that the monofunctional Pt-BODIPY conjugates sensitize the antitumor activity with the enhancement in the cellular accumulation on short photoirradiation time compared to cisplatin.^{8a} Xie and co-workers have demonstrated BODIPY-Pt conjugates as potential candidates for mitochondria targeted cytotoxicity with visualization of platinum distribution in the living cells.²¹ Furthermore, they confirmed from the *in vitro* and *in vivo* studies that simultaneously keeping with the fluorescent imaging properties, significant photocytotoxicity can be achieved even at a low concentration of the BODIPY-Pt complex in near-infrared region (NIR).^{21b} However, the complex exhibited undesirable dark cytotoxicity along with its PDT activity. Although PDT activity of macrocyclic organic dyes, namely, photofrin as hematoporphyrin species and its derivatives, has been investigated in detail, a similar study using platinum(II)-BODIPY conjugates remains virtually unexplored.^{22–25}

We have recently reported imidazoplatin-BODIPY conjugates as mitochondria-localizing photosensitizers.^{8b} DNA interaction studies have shown that the metal covalently binds to the plasmid pUC19 DNA, and in addition to their cellular PDT activity, the complexes show transcription inhibition property, possibly binding to the mt-DNA. Despite showing excellent photocytotoxic properties, the emissive imidazopla-

tin-B localized in different cellular organelles, namely, the mitochondria (PCC ≈ 0.7) and endoplasmic reticulum (ER), while the diiodo-imidazoplatin-B was used as a PDT agent.^{8b} Among different series of monofunctional platinum(II) compounds, pyriplatin was extensively studied due to its unique features in comparison to other cisplatin analogues. Pyriplatin was found to have higher anticancer activity in comparison to imidazoplatin as it forms the Pt adducts to impede the RNA II polymerase activity.^{2,4,5,7a} In continuation of our current efforts to develop the PDT chemistry of monofunctional platinum(II)-BODIPY conjugates, herein, we have designed a series of BODIPY-conjugated pyriplatin complexes, and the results are compared to that of the reported imidazoplatin-B analogues.^{8b,c} Herein, we present the synthesis, characterization, and photoinduced anticancer activity of a series of BODIPY-conjugated pyriplatin analogues, namely, *cis*-[Pt(NH₃)₂(L)Cl](NO₃) (1–3), where L is pyridyl moiety conjugated to 1,3,5,7-tetramethyl-8-(4-pyridyl)-4,4'-difluoroboradiazaindacene (BODIPY) (L¹ in 1), monoiodo-BODIPY (L² in 2) and diiodo-BODIPY (L³ in 3) (Scheme 1).

Scheme 1. Synthetic Pathways Involved and the Chemical Structures of the Ligands L¹–L³ and Their Platinum(II) Complexes 1–3: (i), HI/HIO₃ in Ethanol; (ii), *cis*-[Pt(NH₃)₂Cl₂] + AgNO₃ in DMF

The iodinated BODIPY moieties to different extents is to achieve both tracking by imaging and the PDT effect simultaneously, particularly for complex 2 having a monoiodo-BODIPY unit. Complex 4 of formulation *cis*-[Pt(NH₃)₂(L)Cl](NO₃) (4) with L as 4-methylpyridine (4-MePy) was prepared, structurally characterized by X-ray crystallography, and used as a control species. Involvement of singlet oxygen as the reactive oxygen species (ROS) is an essential requirement of PDT, and its generation by the complexes 1–3 was studied by titration experiments using 1,3-diphenylisobenzofuran (DPBF) as a $^1\text{O}_2$ quencher and from other cellular experiments. Complex 1 with a highly emissive noniodo BODIPY ligand is designed for cellular imaging, while the nonemissive complex 3 with a diiodo-BODIPY moiety is primarily designed as a photosensitizer to achieve high PDT activity in visible light. Complex 2 with a monoiodinated BODIPY unit is designed for dual activity, namely, for cellular imaging and PDT activity with a moderate effect.

The highlights of this work are (i) visible light-induced PDT activity of the complexes in the cancer cells by singlet oxygen as the ROS with nanomolar IC₅₀ values under light, while being less toxic when kept in the dark, (ii) observation of predominant mitochondrial localization as evidenced from the high Pearson's coefficient value, (iii) potential of DNA cross-link formation using 9-ethylguanine as a model nucleobase in noncellular experiments, and (iv) apoptotic cell death caused by the ROS. This work exemplifies monofunctional platinum-

(II) complexes, classified as “pyriplatin-B”, with the complexes showing their utility in photochemotherapy as a combination of PDT and chemotherapy toward enhancing the therapeutic potential of an anticancer agent.

EXPERIMENTAL SECTION

Materials and Instruments. The chemicals were purchased and used for reactions without further purification. Elemental analytical data (CHN) were obtained from the IISc analytical service facility using Thermo Finnigan FLASH EA 1112 equipment. The NMR and mass spectral data were obtained using a Bruker Avance NMR spectrometer and Agilent 6538 Ultra High Definition (UHD) Accurate Mass-Q-TOF (LC-HRMS) instrument. The electronic spectral studies were performed using PerkinElmer Spectrum 750 spectrophotometer and HORIBA Jobin Yvon IBH TCSPC fluorimeter (fitted with FluoroHub software analysis). The phototoxicity studies were conducted using a Luzchem photoreactor (model LZC-1, Ontario, Canada) fitted with eight fluorescent Sylvania white tubes ($\lambda = 400\text{--}700\text{ nm}$). The flow cytometric experiments were carried out using an FACS Verse instrument (BD Biosciences) fitted with a MoFlo XDP cell sorter and with three lasers ($\lambda = 488, 365, \text{ and } 640\text{ nm}$) containing 10-color parameters.

Synthesis. Synthetic steps involved in the formation of the BODIPY ligands and their respective complexes are given in [Scheme 1](#). Ligands L^1 , L^3 , and the complexes **1** and **4** were prepared according to modified literature procedures.^{5,8b,21a,26} The characterization data of the ligands are given as [Supporting Information](#).

Synthesis of *cis*-[Pt(NH₃)₂(L¹Cl)(NO₃)] (1–4**).** Cisplatin (150 mg, 0.5 mmol) was first dissolved in 5 mL *N,N*-dimethylformamide (DMF) followed by addition of AgNO₃ (76 mg, 0.45 mmol, 0.9 equiv) to the solution. The reaction mixture was protected from light and stirred at room temperature for 24 h. The precipitated silver chloride was removed by filtration, and the ligand (146 mg L^1 , 203 mg L^2 , 260 mg L^3 and 42 mg L^4 (0.45 mmol, 0.9 equiv)) was added to the solution. The reaction mixture was further stirred for another 24 h at room temperature. The solvent was removed by using rotary evaporator, and the residue was redissolved in 40 mL of methanol. The unreacted yellow colored cisplatin was separated by filtration. Diethyl ether (60 mL) was added into the filtrate under vigorous stirring condition to obtain the product as a solid precipitate. The desired product was obtained, treated twice with 50 mL of diethyl ether before vacuum drying, and kept in the dark protected from light.

cis-[Pt(NH₃)₂(L¹Cl)(NO₃)] (**1**).^{21a} Greenish-red solid. Yield: 200 mg (70%, 0.3 mmol). Anal. Calcd for C₁₈H₂₄BClF₂N₆O₃Pt (M_w : 651.7698): C, 33.17; H, 3.71; N, 12.89. Found: C, 32.85; H, 3.73; N, 12.67. HRMS m/z : Calcd for [M–NO₃]⁺, 589.1429; Found: 589.1429. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.91 (d, $J = 6.11\text{ Hz}$, 2H), 7.76 (d, $J = 8.03\text{ Hz}$, 2H), 6.25 (s, 2H), 4.75 (s, 3H), 4.37 (s, 3H), 2.46 (s, 6H), 1.40 (s, 6H) (s, singlet; d, doublet). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 157.12, 154.64, 151.48, 143.43, 130.16, 126.92, 124.11, 123.04, 15.44, 15.20, 15.03 ppm. ¹¹B NMR (100 MHz, DMSO-*d*₆): 0.54 (t, 1B) (t, triplet). ¹⁹F NMR (100 MHz, DMSO-*d*₆): –143.59 (quartet, 2F). UV–visible (1% DMSO/DMEM at pH 7.2): λ_{max} nm (ϵ , M^{–1} cm^{–1}) = 505 (41 100), 475 (11 790, shoulder), 371 (4045). Emission (1% DMSO/DPBS at pH 7.2): λ_{em} (λ_{ex} Φ_F) = 530 nm (490 nm, 0.07).

cis-[Pt(NH₃)₂(L²Cl)(NO₃)] (**2**). Red solid. Yield: 266 mg (76%, 0.34 mmol). Anal. Calcd for C₁₈H₂₃BClF₂IN₆O₃Pt (M_w : 777.6663): C, 27.80; H, 2.98; N, 10.81. Found: C, 27.99; H, 3.20; N, 10.57. HRMS m/z : Calcd for [M–NO₃]⁺, 715.0396; Found, 715.0303. ¹H NMR (400 MHz, CDCl₃): 7.86 (d, $J = 8.73\text{ Hz}$, 2H), 7.64 (d, $J = 9.1\text{ Hz}$, 2H), 5.95 (s, 1H), 4.20 (s, 3H), 3.82 (s, 3H), 2.52 (s, 6H), 1.41 (s, 3H), 1.31 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 160.85, 155.95, 155.86, 152.83, 148.06, 144.12, 137.65, 130.99, 122.57, 87.45, 39.67, 36.26, 17.43, 16.63 ppm. ¹¹B NMR (100 MHz, DMSO-*d*₆): 0.58 (t, 1B). ¹⁹F NMR (100 MHz, DMSO-*d*₆): –145.66 (quartet, 2F). UV–visible (1% DMSO/DMEM at pH 7.2) [λ_{max} nm (ϵ , M^{–1} cm^{–1})]: 525 (20 100), 490 (7450 sh), 374 (3060). Emission (1% DMSO/DMEM at pH 7.2): λ_{em} (λ_{ex} Φ_F) = 540 nm (505 nm, 0.02).

cis-[Pt(NH₃)₂(L³Cl)(NO₃)] (**3**). Red solid. Yield: 297 mg (73%, 0.32 mmol). Anal. Calcd for C₁₈H₂₂BClF₂I₂N₆O₃Pt (M_w : 903.5627): C, 23.99; H, 2.45; N, 9.30. Found: C, 24.22; H, 2.73; N, 8.97. HRMS m/z : Calcd for [M–NO₃]⁺, 840.9362; Found, 840.9495. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.93 (d, $J = 9.23\text{ Hz}$, 2H), 7.92 (d, $J = 7.29\text{ Hz}$, 2H), 4.76 (s, 3H), 4.55 (s, 3H), 2.55 (s, 6H), 1.39 (s, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 159.03, 157.07, 154.48, 149.69, 130.16, 128.02, 124.14, 87.64, 31.47, 16.71 ppm. ¹¹B NMR (100 MHz, DMSO-*d*₆): 0.59 (t, 1B). ¹⁹F NMR (100 MHz, DMSO-*d*₆): –145.96 (quartet, 2F). UV–visible (1% DMSO/DMEM at pH 7.2) [λ_{max} nm (ϵ , M^{–1} cm^{–1})]: 555 (11 000), 398 (4050).

cis-[Pt(NH₃)₂(L⁴Cl)(NO₃)] (**4**). White solid. Yield: 141.6 mg (75%, 0.34 mmol). Anal. Calcd for C₆H₁₃ClN₄O₃Pt (M_w : 419.7290): C, 17.17; H, 3.12; N, 13.35. Found: C, 16.87; H, 3.01; N, 13.17. HRMS m/z : Calcd for [M–NO₃]⁺, 357.0446; Found, 357.0461. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.56 (d, $J = 7.54\text{ Hz}$, 2H), 7.45 (d, $J = 8.49\text{ Hz}$, 2H), 4.60 (s, 3H), 4.25 (s, 3H), 2.39 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.89, 152.45, 128.40, 127.69, 31.60. UV–visible (1% DMSO/DMEM at pH 7.2) [λ_{max} nm (ϵ , M^{–1} cm^{–1})]: 565 (1400), 377 (300).

Crystal Structure and Theoretical Method. Complex **4** and the ligands L^1 , L^2 , and L^3 were structurally characterized by single-crystal X-ray diffraction. Complex **4** was crystallized as colorless rectangular blocks obtained from slow evaporation of saturated solutions of **4** in methanol, while L^1 – L^3 were crystallized as red colored rectangular blocks from their dichloromethane solution on slow evaporation. Paratone oil was used to mount the crystals on loops. All intensity and geometric data were collected using an automated Bruker SMART APEX (D8 QUEST) CMOS diffractometer with the SMART/SAINT software equipped with a Mo–K α X-ray source ($\lambda = 0.71073\text{ \AA}$) with increasing ω (width of 1° per frame) at a scan speed of 10 s frame^{–1}. Intensity data were collected using ω -2 θ scan mode and then corrected for Lorentz-polarization and absorption effects.²⁷ The structures were solved by direct methods and Fourier analysis and refined by the full-matrix least-squares method based on F^2 with all observed reflections using the SHELX-13 program incorporated into WinGX (2013).^{27b} Anisotropic displacement coefficients were used to refine the non-hydrogen atoms, and their coordinates were permitted to ride on their respective carbon atoms. Anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms were included in the final refinement. The perspective views were obtained using ORTEP.^{27c} Selected crystallographic parameters, bond distances and angles are given in Tables S1–S3 (see [Supporting Information](#)). The CCDC deposition numbers are 1821177–1821180. The geometries of the complexes were optimized using basis sets and B3LYP/LANL2DZ level of density functional theory (DFT) calculations.^{28,29}

Cellular Experiments. Experimental procedures used for the cellular studies are given in [Supporting Information](#). The MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, assay was done using three types of cells, namely, A549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), and HaCaT (immortalized human keratinocytes), in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (FBS) to obtain the half maximal inhibitory concentration (IC₅₀) values. Visible light source (1 h exposure, $\lambda = 400\text{--}700\text{ nm}$, light dose = 10 J cm^{–2}) using a Luzchem photoreactor (model LZC-1, Ontario, Canada) fitted with eight fluorescent white tubes of Sylvania make was used for photoexposure of one set of cells, whereas the other set was kept in the dark for 1 h.⁸ A TECAN microplate reader was used, and graphs were plotted by using GraphPad Prism 7 software. Intracellular ROS measurement was done from a 2,7-dichlorofluorescein diacetate (DCFDA) assay. The percentage population of cells generating ROS was determined by flow cytometric analysis. The cells (3.0×10^5 A549 cells) were incubated with the complexes **1–3** (1 μM) along with their respective free ligands L^1 – L^3 (1 μM) and control complex **4** (10 μM) for 4 h. One set of the cells was photoexposed to visible light (400–700 nm, 1 h), while the other set was kept in the dark. The cells were harvested by trypsinization, and a single cell suspension was prepared. The cells

were subsequently treated with 1 μM DCFDA solution prepared in DPBS (Dulbecco's phosphate-buffered saline) in the dark for 20 min at room temperature. The distribution of DCFDA stained A549 cells was obtained by flow cytometry in the FL-1 channel. Cellular localization was studied from confocal fluorescence imaging of the complexes **1** (1 μM), **2** (10 μM), and ligand **L**¹ (5 μM) in 1% DMSO/DMEM on a Carl Zeiss Microscopy and images were acquired with the help of ZEN lite software. Complex **3** was not used for this study for its nonemissive properties. Mitotracker Deep Red (MTR, 100 nM) and DAPI (300 nM, 4',6-diamidino-2-phenylindole) were used as mitochondria and nuclear staining agents, respectively.

Cellular apoptosis was studied from Annexin V-FITC/PI assay. Propidium iodide (PI) was used to stain necrotic cells with red fluorescence. A549 cells (3.0×10^5) and the complexes **2**, **3** (0.1 μM), and **4** (10 μM) were plated in six-well plates and cultured for 24 h. Photoirradiation (400–700 nm, 1 h) was done for one set in DPBS. The cells were resuspended in 1 $\times$ binding buffer. Annexin V-FITC and PI were added. The fluorescence of the cells was measured with a flow cytometer. Cells undergoing early apoptosis were stained with the annexin V-FITC alone. Cell cycle analysis experiment was performed to quantify the DNA content of the cell by flow cytometry. Propidium iodide (PI) was used as nuclear DNA binding agent. On the basis of the fluorescence intensity, the cells were distinguished into different phases of the cell cycle. A549 cells used for this experiment were treated with the complex (**2** and **3** of 0.1 μM and **4** of 10 μM) in dark or on 1 h of photoirradiation (400–700 nm). Hypotonic citrate stain in 0.10% trisodium citrate dihydrate containing 0.10% Triton X-100 was used. The cell suspension was centrifuged, and the nuclear pellet was resuspended in fresh hypotonic citrate stain supplemented to 50 $\mu\text{g mL}^{-1}$ PI for FACS analysis.

DNA Photocleavage Experiments. UV–visible spectral titration studies were made to obtain the calf thymus (ct) DNA binding constants of the complexes. The supercoiled (SC) plasmid pUC19 DNA photocleavage activity of the complexes was studied using a CW (continuous wave) diode laser (532 nm, 100 mW power, 1 h exposure time, 0.32 ± 0.02 mm beam diameter, Research Electro-Optics model no. EXLSR-532-100-CDRH, Colorado, U.S.A.) for photoirradiation followed by gel electrophoresis. Mechanistic studies were conducted to determine the nature of ROS such as hydroxyl radicals ($\cdot\text{OH}$), superoxide radicals and singlet oxygen ($^1\text{O}_2$) by using different external additives, namely, DMSO, KI, catalase as hydroxyl radical scavengers, TEMP (2,2,6,6-tetramethyl-4-piperidone), and sodium azide as singlet oxygen quenchers and superoxide dismutase (SOD) as superoxide scavenger.

RESULTS AND DISCUSSION

Synthesis and Characterization. The pyridyl ligands **L**¹–**L**³ having the BODIPY pendants were synthesized using literature procedures with modifications (Scheme 1).^{8b,21} The monofunctional platinum(II) complexes, *cis*-[Pt(NH₃)₂(L)-Cl](NO₃) (**1**–**4**), where L is BODIPY conjugated pyridyl ligands **L**¹–**L**³ (in **1**–**3**) and 4-methylpyridine (in **4**), were synthesized following a common procedure in which a solution of cisplatin in *N,N*-dimethylformamide (DMF) was initially treated with silver nitrate, and after removal of the silver chloride precipitate from the filtrate, respective ligand (L) was added to isolate the product as their nitrate salt in good yield. The complexes were characterized from the elemental analytical and spectral data (Figures S1–S32, Supporting Information). Selected physicochemical data are given in Table 1. The BODIPY derivatives **L**¹–**L**³ and the control complex **4** were structurally characterized by single-crystal X-ray crystallography (Figure 2, Tables S1–S3, Figures S33–S39, Supporting Information).²⁷ Density functional theory (DFT) calculations using B3LYP/LANL2DZ were done to optimize the structures of **1**–**4** (Figure 2, Table S4, Figure S40, Supporting Information).^{28,29} The IR spectra exhibited

Table 1. Selected Physicochemical Data for the Complexes **1**–**3** and Ligands **L**¹–**L**³

compound	$\Lambda_{\text{M}}^a/\text{S m}^2 \text{M}^{-1}$	$\lambda_{\text{max}}/\text{nm}$ ($\times 10^{-4} \text{ } \epsilon/\text{M}^{-1}$) ^b	$\lambda_{\text{em}}/\text{nm}$ (Φ_{F}) ^c	$\text{Log } P_{\text{O}_2}^d/\text{w}$	Φ_{Δ}^e ($^1\text{O}_2$)
1	74	505 (4.1), 475 (1.1)	530 (0.07)	0.85	0.26
2	73	525 (2.01), 490 (0.7 sh)	540 (0.02)	0.97	0.48
3	76	555 (1.1)		1.18	0.65
L ¹		505 (9.6)	515 (0.19)	2.51	0.21
L ²		525 (6.3)	535 (0.08)	2.69	0.37
L ³		550 (4.7)		2.76	0.49

^aMolar conductivity in DMF. ^bIn 1% DMSO/DMEM solution.

^cEmission wavelength and fluorescence quantum yield. Complex **3** and **L**³ are nonemissive. ^d*n*-Octanol/water partition coefficient.

^esinglet oxygen quantum yield in air-saturated DMSO/DMF.

characteristic bands of the free ligands and their respective complexes in accordance to their formulations. The N–H in Pt–NH₃ appeared as a broad peak near 3300–3100 cm^{-1} (Figures S29–S32, Supporting Information).^{8b}

NMR and Mass Spectral Studies. The purity and formulation of these Pt-BODIPY complexes **1**–**4** were ascertained from their ¹H, ¹¹B, ¹³C, and ¹⁹F NMR and HPLC spectral data (Figures S1–S21, Supporting Information). The aromatic hydrogen protons appearing in the range of 8.89–7.50 ppm are due to the pyridyl groups of the BODIPY ligands alone, while they appear downfield shifted within 8.90–8.76 ppm for the complexes due to coordination of the pyridyl group to platinum(II) (Figures S1–S7, Supporting Information). The absence of alpha proton signals in BODIPY ligand **L**³ within 6.25–6.10 ppm is due to diiodination of the BODIPY core when compared to its noniodo and monoiodo analogues showing the signals at 6.25 and 6.00 ppm in their respective complexes. The signals observed within 2.46–2.40 and 1.40–1.31 ppm are assignable to the pyrrolic methyl protons of BODIPY. Two additional signals for the ammine protons are in the range of 4.80–4.15 ppm for all the complexes. The ¹³C NMR spectra showed peaks in the range of 160–120 ppm for aromatic carbon atoms, and the peaks within 40–15 ppm are for aliphatic methyl carbons. The peaks corresponding to iodinated carbon atoms were observed at ~86 ppm in ¹³C NMR of complexes **3** and **4** (Figures S8–S14, Supporting Information). The ¹¹B NMR showed a characteristic triplet signal in the range of 0.84–0.29 ppm of the boron nuclei, resulting from coupling of two equivalent fluorine nuclei of the BF₂ moiety with a coupling constant of 29 Hz. The effect of iodination in **L**¹ on the boron atom is seen from the downfield shift from 0.03 and 0.07 ppm in the ¹¹B NMR of ligand **L**² (Figures S15–S17, Supporting Information). A small change in the symmetry of the BODIPY unit makes a notable change in the NMR spectra of the ¹⁹F signal. Both noniodo and monoiodo BODIPY ligands **L**¹ and **L**² showed a single quartet, whereas it is a quintet spectrum for the di-iodo ligand **L**³ (Figures S18–S20, Supporting Information).

The compositions of the ligands and their platinum complexes were investigated by HRMS. The ligands **L**¹–**L**³ displayed peaks corresponding to their respective molecular ion peaks in MeOH. Complexes **1**–**4** as nitrate salts exhibited primary peaks assignable to [M – NO₃]⁺. The isotopic distribution pattern in **1**–**4** indicated the charge carried by

Figure 2. ORTEP views of the ligands L^1 (a), L^2 (b), L^3 (c), and the monocationic complex of 4 (d) showing thermal ellipsoids at the 50% probability level and the numbering scheme for the hetero atoms and the metal. The energy minimized structures of complexes 1 (e), 2 (f), and 3 (g) using B3LYP/LANL2DZ level of theory. Color codes: Pt, red; I, pink; Cl, purple; F, sea green; O, blue; N, green; C, black; B, orange red.

platinum(II) complexes as unipositive (Figures S22–S28, Supporting Information). The molecular structures of the ligands L^1 – L^3 and complex 4 as a control species were obtained from X-ray crystallography.

Crystallography and Theoretical Study. Ligands L^1 – L^3 and complex 4 were structurally characterized by single crystal X-ray crystallography (Figure 2, Tables S1–S3, Figures S33–S39, Supporting Information).²⁷ Ligand L^1 crystallized in the orthorhombic space group $Pna2_1$, whereas L^2 and L^3 crystallized in the monoclinic and orthorhombic space group $P2_1/c$ and $Cmcm$, respectively. The asymmetric unit of the crystal lattice of L^1 has two molecules of the ligand, whereas ligand L^2 and L^3 has one molecule in the crystallographic asymmetric unit. Complex 4 crystallized in the monoclinic space group $P2_1/c$. The nitrate ion is present as counteranion in the cationic complex 4 (Figure 2a). The complex having platinum(II) coordinated to three N-donor atoms, has a square planar geometry with two ammine groups cis to each other, a pyridyl moiety, and a chloride ligand. The Pt–N bond distances in 4 are ~ 2.0 Å. The Pt–Cl distance is 2.299(4) Å, and the H_3N –Pt– NH_3 angle is $90.390(6)^\circ$. The Pt–Cl bonds of 2.308(19) and 2.313(2) Å in cisplatin are essentially similar to those observed in complex 4.^{8b} The unit cell packing diagram of 4 showed chemically significant hydrogen bonding interactions between the hydrogen atoms of the ammine group with the nitrate counteranion and the chloride ligand.

The structural data of ligands L^1 – L^3 and complex 4 from X-ray crystallography were used to generate the energy optimized structures of the complexes 1–3 by quantum calculations with B3LYP/LANL2DZ level of theory for all atoms (Figure 2e–g, Table S4, Figure S40, Supporting Information).^{27,28} The frontier orbitals were mapped on the atoms from the distribution of the electronic charge density. The HOMO of the BODIPY complexes is localized on the Pt–pyridyl core, while LUMO is localized on the BODIPY moiety.

Electronic Spectra. The Pt–BODIPY conjugates 1–3 and related free ligands L^1 – L^3 (5 and 10 μM) were studied by UV–visible absorption and fluorescence spectroscopy in 1%

DMSO/DMEM buffer of pH = 7.2 (Figure 3, Table 1, Figure S41, Supporting Information). The complexes displayed

Figure 3. (a) Absorption and (b) emission spectra ($\lambda_{ex} = 490$ nm) of the complexes 1–3 (5 μM) in 1% DMSO–DMEM solution (pH = 7.2) [color code: green, 1; red, 2; purple, 3].

intense absorption bands within 505–555 nm that are assignable to the electronic transition within the BODIPY core (Figure 3a).³⁰ Complex 1 showed an intense emission band at 530 nm ($\lambda_{ex} = 490$ nm) assignable to the noniodo BODIPY moiety (Figure 3b). A modest intensity emission band for monoiodo BODIPY complex 2 was observed at 540 nm ($\lambda_{ex} = 505$ nm). These emissive complexes were used for cellular imaging study. Incorporation of iodine atom to the noniodo BODIPY ligand (L^1) caused a red shift of ca. 25 and 45 nm of the absorption maximum for the monoiodo-BODIPY (L^2) and diiodo-BODIPY (L^3) ligands. This red shift is also observed for the complexes 2 and 3. Like the absorption spectra, iodination-induced red shift was detected in the emission spectra of the ligands and the complexes. The presence of the heavy atom iodine resulted in a decrease in the fluorescence quantum yield (Φ_F) values from 0.19 for noniodinated L^1 to 0.08 for monoiodinated L^2 and 0.04 for diiodinated L^3 .

Upon binding to the platinum(II) center, the fluorescence quantum yields of L^1 and L^2 get reduced further to 0.07 for 1 and 0.02 for 2. Complex 3 did not display any emission band, whereas a weak emissive band was observed for ligand L^3 . The

attachment of heavy iodine atoms in the BODIPY ligand and the platinum core together make the intersystem crossing (ISC) facile for **3**, thus resulting in reduced fluorescence intensity. ISC facilitates the type-II process resulting in the generation of singlet oxygen species by the energy transfer pathway from triplet complex **3** to triplet molecular oxygen.^{31,32} Complex **3** as a photosensitizer is found to be suitable for the PDT study in visible light.

Singlet Oxygen as the ROS. PDT causes cancer cell death by generating reactive oxygen species (ROS) in which singlet oxygen ($^1\text{O}_2$) is the preferred ROS in PDT as there is no apparent defense mechanism for this highly reactive species in our human body. Iodine atoms in the BODIPY core cause the heavy atom effect resulting in an enhancement of the singlet oxygen quantum yield. Singlet oxygen as the ROS was probed from a trap experiment in UV–visible spectral titration using 1,3-diphenylisobenzofuran (DPBF), which is a fluorescent dye used specifically for singlet oxygen detection. The noniodo BODIPY complex **1**, monoiodo-BODIPY complex **2**, diiodo-BODIPY complex **3**, and their free ligands L^1 – L^3 in 5 μM concentration were studied using DPBF (50 μM) in noncellular air saturated DMF medium (Figure 4a). A gradual

Figure 4. (a) Spectral changes of DPBF (50 μM) at 415 nm with time on treatment with complex **3** in air saturated DMF. (b) Plot showing the change in absorbance of DPBF at ~ 415 nm vs photoirradiation time in the presence of Rose Bengal (RB) (purple), ligands L^1 (black), L^2 (green), and L^3 (pink), complexes **1** (cyan), **2** (red), and **3** (blue). The observed order of the slope: $\text{RB} > \mathbf{3} > \text{L}^3 \geq \mathbf{2} > \text{L}^2 > \mathbf{1} > \text{L}^1$. (c) DCFDA assay showing shift in the band position of the complexes **1**–**4** in A549 cells in light (400–700 nm, 10 J cm^{-2}) with color code: black, cells only; green, cells + DCFDA; cells + DCFDA + complexes **1** (cyan), **2** (orange), **3** (blue), and **4** (gray) in dark and **1** (green), **2** (pink), **3** (purple), and **4** (greenish-blue) on 1 h exposure to visible light (400–700 nm).

decrease in the intensity of the band at 415 nm of DPBF at different time intervals upon photoexposure using a photo-reactor ($\lambda = 400$ – 700 nm, 10 J cm^{-2}) gave an estimate of the extent of singlet oxygen generation (Figure 4a, Figures S42, S43, Supporting Information). The plot revealed that the iodinated BODIPY complexes **2** and **3** with higher slopes are more efficient singlet oxygen generators than the noniodinated

BODIPY complex **1** and their respective free BODIPY ligands (Figure 4b). The singlet oxygen quantum yield (Φ_{Δ}) for the complexes and the ligands was measured using Rose Bengal as the standard having a Φ_{Δ} value of 0.76 (Table 1).³³ The efficacy of singlet oxygen generation of the complexes follows the order: $\mathbf{3} > \mathbf{2} > \mathbf{1} \gg \mathbf{4}$. The non-BODIPY complex **4** did not show any apparent change in the DPBF intensity on photoirradiation. The results highlight the importance of the BODIPY moieties in the complex design for generation of singlet oxygen in visible light. The Φ_{Δ} values of the pyriplatin-B complexes showed a higher trend in singlet oxygen generation than their imidazoplatin-B analogues possibly due to more efficient ISC in pyriplatin-B complexes in the presence of the pyridyl group directly attached to the BODIPY core without having any $-\text{CH}_2-$ spacer.

Intracellular ROS. 2,7-Dichlorofluorescein diacetate (DCFDA) is a nonpolar fluorescent dye used to measure the intracellular ROS.³⁴ H_2DCFDA on diffusion into the cell gets deacetylated by enzymatic reaction. The nonfluorescent compound converts to a fluorescent molecule 2,7-dichlorofluorescein (DCF) upon oxidation by ROS. To determine the percentage population of cells generating ROS, A549 lung cancer cells were incubated with the complexes **1**–**3** along with their free ligands L^1 – L^3 (1 μM) and control complex **4** (10 μM) for 4 h in dark and then exposed to visible light (400–700 nm) for 1 h. After 19 h post incubation, flow cytometry was done for quantification of the ROS in living cells by measuring the green fluorescence of DCF at λ_{em} of 525 nm. Complexes **1**–**3** showed enhancement in the fluorescence intensity of DCF upon light exposure compared to the identical set kept in the dark.

Control complex **4** showed no apparent change in dark and light indicating its inability to generate any ROS (Figure 4c, Figure S44, Supporting Information). The pyriplatin-B derivatives display higher cellular ROS formation than the Imidazoplatin-B derivatives (Figure S45, Supporting Information). These results showed the heavy atom effect of platinum metal on the pyridyl group that is directly attached to the BODIPY core as compared to imidazoplatin-B where Pt metal is bonded to the imidazole moiety linked to BODIPY core with a nonconjugating spacer.

Cell Viability Assay. As PDT is primarily effective for neck, lung, bladder, esophageal and skin cancers, we have chosen human lung cancer A549 cells, human breast cancer MCF-7 cells, and human skin keratinocytes HaCaT cells for this study.³⁵ The anticancer activity of the complexes **1**–**4** along with their respective free ligands L^1 – L^3 was investigated in both dark and light ($\lambda = 400$ – 700 nm) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Figure 5, Figures S46–S51, Supporting Information). The half maximal inhibitory concentrations (IC_{50}) are listed in Table 2 where the complexes **1**–**3** showed photocytotoxicity at nanomolar concentration in these cells with IC_{50} values ranging from 0.05 to 1.7 μM in visible light (1 h exposure, $\lambda = 400$ – 700 nm, light dose = 10 J cm^{-2}), while the ligands showed photocytotoxicity ranging from 0.11 to 29.8 μM . On the other hand, there was no appreciable cytotoxicity of these compounds in the dark (73 to $>100 \mu\text{M}$). The observed 25- and 40-fold increase in photocytotoxicity for the respective complexes **2** and **3** compared to the noniodinated complex **1** is attributed to the heavy atom effect of the mono- and diiodinated BODIPY core in generating significant quantity of singlet oxygen. The higher cytotoxicity of Pt-BODIPY

Figure 5. (a) Bar diagram for MTT assay data giving IC₅₀ values in light for the complexes 1–3 in A549 lung cancer, MCF-7 breast cancer, and HaCaT skin keratinocyte cells upon 1 h photoirradiation in visible light ($\lambda = 400\text{--}700\text{ nm}$, 10 J cm^{-2}) [color code: purple, 3; red, 2; green, 1]. The IC₅₀ values of complex 4, cisplatin and the ligands are given in Table 2. (b) Quantitative Cellular uptake analysis by flow cytometry upon incubating A549 cells with complexes 1–3 ($5\text{ }\mu\text{M}$) and their corresponding free ligands ($5\text{ }\mu\text{M}$) at $37\text{ }^{\circ}\text{C}$ for 4 h, with cells untreated as a control. The shift in the band position of the complexes 1–3 in A549 cancer cells clearly indicates that the complexes have a higher cellular uptake compared to their free ligands L¹–L³. BODIPY-A represents green emission for positive cells.

derivatives in comparison to the ligands is possibly because these platinum(II) complexes could potentially act as monofunctional DNA cross-linking agents besides their PDT activity as is known for pyriplatin and its analogues.^{5,8b} The potency of these complexes was compared in terms of photocytotoxicity with our previously reported Imidazoplatin-B analogues using same batch of cells of A549, and it was found that pyriplatin-B has a higher activity as evidenced from the singlet oxygen quantum yield and cellular ROS generation results besides the MTT assay data (Figure S52, Supporting Information).

Cellular Uptake and Mitochondrial Localization. The therapeutic effect of a drug often depends on their cellular uptake. Complexes 1–3 with monocationic charge showed a substantial increase in the cellular uptake compared to their charge neutral free ligands in A549 cancer cells within 4 h of incubation (Figure 5b).^{5,21,22a} This observation suggests a crucial role of the platinum metal in transporting the photosensitizer into the cells for better photoinduced cytotoxicity. These results are in good compliance with the oil/water partition coefficients, following the order: $L^1 \approx L^2 \approx L^3 \gg 3 > 2 > 1$ (Table 1). The enhanced aqueous solubility of 1–3 is possibly due to the cationic charge and the presence of two ammine ligands bound to platinum(II). Co-localization experiments were used to measure the intracellular presence of the BODIPY-platinum conjugates. A549 cells were used for cellular imaging study considering that the complexes are highly PDT active in this cancer cell. The intracellular distribution pattern of the emissive ligand L¹ and complexes 1 and 2 has been determined by confocal microscopy which displayed mitochondrial localization. Cytoplasmic localization was observed from the merged images of the ligand L¹ ($5\text{ }\mu\text{M}$) and complexes 1 ($1\text{ }\mu\text{M}$) and 2 ($10\text{ }\mu\text{M}$) when treated with 4',6-diamidino-2-phenylindole (DAPI) as the nuclear staining dye after 4 h of incubation in dark (Figure 6, Figure S53, Supporting Information). The overlay image of the complexes and Mito-Tracker Deep Red (MTDR, 100 nM) revealed selective localization of the complexes into the mitochondria. This was further evidenced from the high Pearson's correlation coefficient (PCC) value of ~ 0.8 .^{8b} Complex 3 being nonemissive was not used for any imaging study. Despite the colocalization of the complexes 1 and 2, the intracellular colocalization of ligand L² was not observed as its cellular uptake was comparatively lower in comparison to their respective monocationic Pt(II) complexes upon 4 h incubation (Figure 6, Figure S53, Supporting Information). The predominant localization of pyriplatin-B in mitochondria is a major advantage over distributed localization of imidazoplatin-B between mitochondria and endoplasmic reticulum (ER).

Mitochondrial membrane potential ($\Delta\Psi_m$) was measured to investigate the mitochondria targeting ability of these Pt-BODIPY conjugates. JC-1 dye was used for the experiment as a ratiometric probe giving a direct insight into the functioning of the complex via fluorescence microscopy.

Table 2. Photocytotoxicity (IC₅₀ in μM) and Mitochondrial Platinum Uptake Data for the Complexes 1–4 and Ligands L¹–L³

compounds	A549		MCF-7		HaCaT		Pt (ng) ^c
	(light) ^a	(dark) ^b	(light) ^a	(dark) ^b	(light) ^a	(dark) ^b	
1 ^{d,e}	1.70 ± 0.05	94.4 ± 1.2	0.40 ± 0.03	>100	1.0 ± 0.04	>100	1.07 ± 0.20
2	0.09 ± 0.03	>100	0.13 ± 0.02	>100	0.09 ± 0.03	>100	1.12 ± 0.13
3 ^f	0.05 ± 0.01	73.2 ± 1.1	0.07 ± 0.02	>100	0.06 ± 0.02	70.4 ± 1.2	1.14 ± 0.16
4	60.3 ± 1.4	>100	>100	>100	39.6 ± 1.3	>100	
L ¹	11.5 ± 0.1	>100	29.8 ± 0.9	>100	7.2 ± 0.1	>100	nd
L ²	3.5 ± 0.2	>100	3.7 ± 0.1	>100	2.3 ± 0.1	>100	nd
L ³	0.30 ± 0.06	>100	0.11 ± 0.03	>100	0.50 ± 0.01	87.8 ± 1.6	nd

^aAll the cells treated with the compounds for 4 h initial incubation in dark. ^bVisible light (1 h photoexposure, $400\text{--}700\text{ nm}$, 10 J cm^{-2}). Post photoexposure incubation time was 19 h in dark. ^cA549 cells treated with the complexes ($100\text{ }\mu\text{M}$) and the Pt content as Pt (ng/ng of mitochondria) determined by ICP-MS method. nd = not determined. ^dThe IC₅₀/μM values reported for complex 1 in ref 21a are 27.37 ± 1.14 in HeLa and 12.14 ± 2.44 in MCF-7 cancer cells in the dark with 48 h incubation. ^eThe IC₅₀ values [light ($400\text{--}700\text{ nm}$)/dark] of $[\text{Pt}(\text{NH}_3)_2(\text{L}_1)\text{Cl}]^+$ in A549, MCF-7 and HaCaT cells under identical conditions are 8.13 ± 1.1 />100, 2.8 ± 0.7 />100 and 0.76 ± 1.3 /100 μM (ref 8b). ^fThe IC₅₀ values [light ($400\text{--}700\text{ nm}$)/dark] of $[\text{Pt}(\text{NH}_3)_2(\text{L}_2)\text{Cl}]^+$ in A549, MCF-7 and HaCaT cells under identical conditions are 0.21 ± 0.04 />100, 0.16 ± 0.04 />50 and 0.12 ± 0.03 /83.54 $\pm 1.6\text{ }\mu\text{M}$ (ref 8b).

Figure 6. Confocal microscopic images of the noniodo BODIPY complex **1** ($5\ \mu\text{M}$) and monoiodo BODIPY complex **2** ($10\ \mu\text{M}$) showing green emission in A549 lung cancer cells on 4 h incubation in the dark in the left, the Mito-Tracker Deep Red (MTR) in the middle column, and the merged panels in the right column. The yellow/orange color in the right column indicates significant mitochondrial localization of the complexes. Scale bar = $10\ \mu\text{m}$. The fluorescence intensity being more for complex **1** than **2**, the merged panel for **1** gave the desired intense yellow color.

Figure 7. Confocal microscopic images from JC-1 assay showing the fluorescence images of JC-1 dye in both red and green channel in A549 lung cancer cells incubated with the PDT active complex **3** ($5\ \mu\text{M}$) for 4 h at $37\ ^\circ\text{C}$ in dark and on visible light irradiation ($\lambda = 400\text{--}700\ \text{nm}$, light dose = $10\ \text{J cm}^{-2}$). The last column shows the merged images of green and red channel. Scale bar is $20\ \mu\text{m}$.

The mitochondrial membrane potential is inversely proportional to the health of the cell. A low $\Delta\Psi_{\text{m}}$ is an indication of the healthy cells showing red fluorescence due to formation of J-aggregates. A high $\Delta\Psi_{\text{m}}$ leads to the formation of the monomer of JC-1 dye in cells leading to caspase-mediated cell death. ROS generation causes mitochondrial dysfunction that leads to variation in $\Delta\Psi_{\text{m}}$.

Complex **3** was chosen for this study based on its potent PDT activity in all the cells used, specifically in A549 cells along with valinomycin, a well-known mitochondrial depolarizing agent, as the positive control. After 4 h preincubation of complex **3** in A549 cells, the sample was exposed to visible light (1 h, $\lambda = 400\text{--}700\ \text{nm}$), and a gradual decrease in red fluorescence intensity was observed with subsequent increase

in the green signal of the JC-1 dye (Figure 7). Valinomycin as a positive control gave the expected green signal of the dye.

Mitochondrial platinum uptake measurements were made by inductively coupled plasma mass spectrometry (ICP-MS). Complexes 1–4 (100 μ M) were treated with A549 cells and incubated for 4 h in dark. The mitochondrial DNA (mtDNA) was purified and isolated by using an isolation kit. Agarose gel electrophoresis data indicated the confinement of mtDNA from the mitochondria of the A549 cells. The data, obtained as nanogram (ng) platinum per ng of mitochondria, are listed in Table 2. The substantial accumulation of platinum was observed using complexes 1–3 compared to control complex 4 implying the importance of BODIPY units for the mitochondria-targeted photodynamic therapy.

Apoptosis. Annexin-V-FITC/PI assay was carried out to determine the mode of cell death by flow cytometry. The green fluorescence of annexin V-FITC dye and red fluorescence of propidium iodide (PI) allowed us to examine the cellular apoptosis (early apoptosis- high annexin, low PI; late apoptosis- high annexin and high PI), viable cell (unstained, showing auto fluorescence), and necrosis (stained by PI). Complexes 2–4 (0.1 μ M for 2 and 3 and 10 μ M for 4) were first treated with A549 cells and preincubated in dark for 4 h and finally exposed to visible light irradiation (1 h exposure, λ = 400–700 nm). Assay was performed after 19 h of incubation to quantify the apoptotic cells by flow cytometry. The diiodinated BODIPY complex 3 on light exposure showed ~90% apoptosis compared to the monoiodinated BODIPY complex 2 (~51%) as viewed in the lower right quadrant of Figure 8 (Figures S54, S55, Supporting Information). Complexes 2 and 3 treated cells resulted in minimal (~10%) early apoptotic cell population, which is in accordance with the control set of cells that were left untreated or for complex 4 treated cells in dark and light. A comparative study showed the higher activity of the diiodo complex of pyriplatin-B over its Imidazoplatin-B analogue for higher efficacy of intracellular ROS generation (Figure S56, Supporting Information).

The cell cycle progression was used to investigate the mode of cell death. To examine the effect of visible light irradiation, the DNA content of the A549 cells treated with the complexes 2–4 (concentration: 0.1 μ M, 1–3; 10 μ M, 4) was measured using the PI dye. The statistical analysis of histogram showed an increase in the sub-G1 cell population of ~51% for 3, ~39% for 2, ~26% for 1, and 10% for 4 after 1 h of photoexposure (400–700 nm) as compared to those samples unexposed to light (Figure 8b, Figures S57, S58, Supporting Information).

Complex 4 due to its nontoxic nature showed similar behavior as that of the untreated controls. The cellular apoptosis and cell cycle arrest assay results suggest the photocytotoxic order: 3 > 2 > 1 $\gg$ 4. This order matches well with the singlet oxygen generation efficacy of the complexes.

9-Ethylguanine Binding. The monofunctional platinum(II) complexes are known to form single covalent adduct with the nuclear DNA by the loss of the chloride ligand.^{5,36–38} The binding ability of the complexes 1–4 with a model nucleobase, namely, 6-amino-9-ethyl-2-hydroxypurine (9-ethylguanine, 9-EtG), was studied by NMR and HRMS spectroscopy. The N7 atom of guanine is the binding position of the metal, and a downfield chemical shift of the H8 proton which is immediate next to the N7 atom is known to occur in the ¹H NMR spectrum. Addition of three molar equivalents of 9-EtG to the complexes 1–4 in D₂O: DMSO-*d*₆ resulted in the formation of

Figure 8. Dot plots obtained from the annexin V-FITC-PI assay using A549 lung cancer cells treated with the PDT active complex 3 and kept in the dark (a) or exposed to visible light (400–700 nm) (b). The respective % cell population is given in the respective quadrants (lower left = live cells, lower right = early apoptotic cells, upper right = late apoptotic/necrotic cells and upper left = dead cells). Panel (b) shows ~84% early apoptotic cell population. Panels (c) and (d) show the histogram diagram representing % cell population in different phases of cell cycle progression as denoted by M1-SubG1, M2-G1, M3-S, M4-G2/M indicated in the insets.

monoadducts as observed from the shift of the H8 proton signal of 9-EtG from 7.69 to 8.52 ppm (marked as $\ddagger$) within 12 h and HRMS spectra (Scheme S1, Figures S59–S66, Supporting Information). Substitution of the chloride ligand could result in the formation of the monoadduct $[\text{Pt}(\text{NH}_3)_2(\text{L})(9\text{-EtG})]^{2+}$ (L = pyridyl ligand) as evidenced from the ¹H NMR spectral data and from the prominent HRMS peak along with the isotopic distribution at 308.1560 *m/z* value. The reaction seems to proceed with monoaddition with the substitution of the chloride ligand as the first step followed by ligation of 9-EtG to platinum(II). The presence of two additional signals at 8.44 and 8.46 ppm in the ¹H NMR spectra in a 1:1 molar ratio suggests the possibility of both *cis* and *trans* adducts formation of $[\text{Pt}(\text{NH}_3)_2(\text{L})\text{Cl}(9\text{-EtG})]^+$ with the loss of one ammine group of *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{L})\text{Cl}]^+$ instead of the chloride due to greater *trans* effect of chloride than the ammine group in the noncellular medium.^{8b,36} Besides these, another peak was observed at 8.56 ppm which could possibly be due to the 9-EtG chelated adduct with the Pt(II) center with the loss of chloride and ammine ligands. This rather unusual chelation could occur through the N7 and O6 atoms of 9-EtG by displacing the ammine group of the pyriplatin core with the bidentate monoanionic 9-EtG giving a mass peak with *m/z* value of 715.2740 assignable to $[\text{Pt}^{\text{II}}(\text{NH}_3)(\text{L})(9\text{-EtG})]^+$ with characteristic isotopic distribution (Scheme S1, Figures S59–S66, Supporting Information).^{8b} A similar speciation of the related platinum complexes was observed in our earlier study and also reported in the literature.^{8b,36–38} A new peak at 8.25 ppm suggested the formation of the hydrolyzed intermediate

species (Scheme S1, Figures S59–S66, [Supporting Information](#)). The mechanistic data reveal the DNA cross-linking potential of the complexes, and this property could enhance their overall cytotoxicity when compared to that of the free ligands, thus highlighting the important role of platinum.

Photocleavage of Plasmid DNA. As the complexes showed predominant localization at the mitochondria (mt), the mtDNA could be a possible target for binding of these complexes. The ability of the Pt-BODIPY conjugates to damage DNA upon photo exposure was studied by gel electrophoresis using supercoiled (SC) pUC19 DNA in Tris-HCl buffer (pH = 7.2) and a monochromatic visible light of 532 nm using a CW (continuous wave) diode laser of 100 mW (Figure S67, [Supporting Information](#)). The samples were treated with the SC DNA in Tris-HCl buffer and incubated in dark for 1 h and finally exposed to light for 1 h. The complexes (20 μ M) did not show any significant DNA cleavage activity in the dark ($\sim$ 7% NC). However, the BODIPY complexes showed formation of a significant quantity of nicked circular (NC) DNA in the presence of light giving an order: $3 > 2 > 1 \gg 4$ (Figure S67, [Supporting Information](#)).

To ascertain the nature of ROS, complexes 1–4 (20 μ M) were treated with the pUC19 DNA and photoexposed in the presence of different singlet oxygen quenchers, hydroxyl radical scavengers, and SOD as superoxide radical scavenger. The observed DNA photocleavage activity is in agreement with the singlet oxygen generation ability of the complexes with the diiodo-BODY complex being the most active, followed by the monoiodo and noniodo BODIPY complexes. Complex 3 in D₂O showed enhancement in the DNA cleavage activity as singlet oxygen has longer lifetime in this solvent than in water. The photocleavage activity in the presence of NaN₃ and 2,2,6,6-tetra-methylpiperidine (TEMP) as singlet oxygen quenchers got significantly reduced. Radical scavengers like KI, DMSO, and SOD (superoxide dismutase) did not show any apparent inhibitory effect. The mechanistic data unequivocally established the formation of singlet oxygen as the only ROS for these BODIPY complexes. Complex 4 (20 μ M) being photoinactive did not show any apparent DNA cleavage in both light and dark.

CONCLUSIONS

Pyriplatin derivatives of formulation $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{L})\text{Cl}](\text{NO}_3)$ of the N-donor pyridyl ligand (L) having pendant highly fluorescent BODIPY (in L¹), moderately fluorescent monoiodo-BODIPY (in L²), and nonfluorescent diiodo-BODIPY (in L³) moieties were prepared and characterized as new monofunctional platinum(II)-based PDT agents. The fluorescent properties of the complexes 1 and 2 with an emissive L¹ and L² were used for cellular imaging study which showed their predominant mitochondrial localization as evidenced from the high Pearson's coefficient value. Designing complexes targeting mitochondria is of importance as this allows one to circumvent the drawbacks associated with the nuclear DNA binding bifunctional platinum(II) drugs. Complex 3 having a BODIPY moiety with two iodine atoms in L³ is an efficient photosensitizer, and the complex showed PDT activity in visible light with IC₅₀ values in nanomolar concentrations in different cells, while being essentially nontoxic in the dark. This highlights the utility of this class of platinum-based photosensitizers, named as "pyriplatin-B" (B stands for BODIPY), as potential substitutes for the organic macrocyclic PDT dyes, namely, photofrin and its porphyrin

and phthalocyanine analogues. The ROS involved in the PDT activity is singlet oxygen (¹O₂) with Φ_{Δ} values in the order: $3 > 2 > 1$. While complex 1 is primarily suitable for the cellular imaging study with its high Φ_{F} value, complex 3 is a potential PDT agent with a high Φ_{Δ} value. Interestingly, complex 2 with a monoiodo-BODY core fits in-between making the complex suitable for both cellular imaging and PDT activity with moderate Φ_{F} and Φ_{Δ} values. The mechanistic noncellular study established the formation of different Pt-DNA adducts involving the complexes and 9-ethylguanine (9-EtG) as a model substrate. The mechanistic pathway involves multiple adduct formation with a replacement of chloride along with the substitution of an ammine base instead of the chloride due to a higher *trans*-effect of the chloride than the ammine base. Further in-depth biological studies are needed to understand the probable reaction pathway(s) within the cells. This work, in summary, provides new insights and directions in the chemistry of monofunctional platinum(II) complexes. The complexes as photoanalogues of pyriplatin with excellent anticancer activity on light activation akin to PDT effect of hematoporphyrin could act as potential substitutes of the macrocyclic organic dyes in photodynamic therapy applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](#) at DOI: [10.1021/acs.inorgchem.8b02546](https://doi.org/10.1021/acs.inorgchem.8b02546).

Additional details of the experimental methods and measurements, NMR (Figures S1–S20), HPLC (Figure S21), HRMS (Figures S22–S28), IR spectra (Figures S29–S32), unit cell packing diagram of complex 4 and L¹–L³ (Figures S33–S39), optimized structure and frontier molecular orbitals (Figure S40), UV–visible data (Figure S41), singlet oxygen generation (Figures S42, S43), DCFDA (Figures S44, S45), MTT assay (Figures S46–S52), confocal (Figure S53), FACS analysis (Figures S54–S58), DNA binding study with 9-EtG (Figures S59–S66), chemical nuclease, DNA photocleavage and mechanistic aspects (Figure S67), Scheme S1, crystallographic parameters and bond distances/angles (Tables S1–S3) and Cartesian coordinates of the complexes 1–3 (Table S4) ([PDF](#)).

Accession Codes

CCDC [1821177–1821180](#) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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