Published in final edited form as:

Coord Chem Rev. 2015 January 1; 282-283: 110–126. doi:10.1016/j.ccr.2014.05.018.

Control and utilization of ruthenium and rhodium metal complex excited states for photoactivated cancer therapy

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Abstract

The use of visible light to produce highly selective and potent drugs through photodynamic therapy (PDT) holds much potential in the treatment of cancer. PDT agents can be designed to follow an O₂-dependent mechanism by producing highly reactive species such as ¹O₂ and/or an O₂ independent mechanism through processes such as excited state electron transfer, covalent binding to DNA or photoinduced drug delivery. Ru(II)-polypyridyl and Rh₂(II,II) complexes represent an important class of compounds that can be tailored to exhibit desired photophysical properties and photochemical reactivity by judicious selection of the ligand set. Complexes with relatively long-lived excited states and planar, intercalating ligands localize on the DNA strand and photocleave DNA through ¹O₂ production or guanine oxidation by the excited state of the chromophore. Photoinduced ligand substitution occurs through the population of triplet metal centered (³MC) excited states and facilitates covalent binding of the metal complex to DNA in a mode similar to cisplatin. Ligand photodissociation also provides a route to selective drug delivery. The ability to construct metal complexes with desired light absorbing and excited state properties by ligand variation enables the design of PDT agents that can potentially provide combination therapy from a single metal complex.

Keywords

Photodynamic therapy; Excited state; Singlet oxygen; DNA photocleavage; DNA binding

1. Introduction

1.1. Cancer

The pursuit of anti-cancer therapies that are highly effective and exhibit low systemic toxicity is an important research area spanning many interdisciplinary fields. Cancer is defined as uncontrolled proliferation of abnormal cells that accumulate to form tumors or lesions that interfere with the functioning of normal tissues and organs [1]. When the cancerous cells from a primary tumor migrate through the body to affect a non-adjacent organ or tissue, metastatic cancer develops. The three current cancer treatments in clinical use are plagued by severe side effects: surgery or resection is invasive and increases the risk

of metastasis, radiation therapy can cause radiation poisoning and increase the probability of developing secondary tumors, and chemotherapy drugs suffer from systemic toxicity due to a lack of selectivity [2,3]. While cancer death rates have declined by >1% per year between 1999 and 2009 [2], a great need persists for better treatments that are minimally invasive and significantly less or non-toxic toward healthy cells.

1.1.1. Targeting DNA with metal complexes—The transcription and replication of DNA are central to cell proliferation, so targeting the material involved in this process is important in inhibiting the growth of cancerous tumors [1]. DNA codes genetic information through the sequence of hydrogen-bonded bases, adenine (A), cytosine (C), guanine (G), and thymine (T), with a double-helical secondary structure made up of two anionic deoxyribose phosphodiester backbones as the scaffold [4]. The DNA helical structure gives rise to minor and major grooves. Fig. 1(a) highlights the secondary structure of a B-DNA double helix and Fig. 1(b) shows the GC and AT base pairs.

The complexity of the DNA double helical structure provides various types of sites for a metal complex to bind through different modes [4–6]. The complex can form a covalent bond between the metal and the Lewis basic phosphodiester backbone or the nitrogen sites on the bases. Metal complexes with planar, aromatic ligands can bind via intercalation between adjacent base pairs, or through insertion of the molecule at mismatched or abasic sites, both of which are driven by π – π interactions between the ligand and DNA π -stack. The complex can also bind in the major or minor groove of the double helix based on size, shape, and their ability to hydrogen bond. Electrostatic attraction of metal complexes with cationic charges aid in binding to DNA.

1.1.2. Cisplatin and its derivatives—Cisplatin, *cis*-[Pt(NH₃)₂Cl₂] [7–9], and its derivatives carboplatin and oxaliplatin [10] are the only metal-based drugs approved in the United States for cancer therapy to date, and they are utilized in more than half of all cancer treatments, usually in combination with radiation or other drugs [6,11–13]. Cisplatin undergoes thermal ligand exchange in aqueous solution to produce the mono-aqua complex [Pt(NH₃)₂(OH₂)Cl]⁺, followed by a second ligand exchange to form the bis-aqua active species, *cis*-[Pt(NH₃)₂(OH₂)₂]²⁺. This species, by virtue of labile Pt–OH₂ bonds, binds primarily to the N7 on guanine bases and predominantly forms 1,2-GpG intrastrand crosslinks, resulting in a kinked DNA double helix [14]. This Pt-G covalent binding results in the inhibition of transcription and DNA replication which is the mode of action against tumors. Cisplatin is plagued by severe drawbacks, including its inability to distinguish between healthy and cancerous cells and causing aggressive systemic toxicity [11]. Drug resistance through enhanced or overexpression of nucleotide excision repair is another common limitation of cisplatin [15].

1.2. Photodynamic therapy

The use of visible light to activate a molecule and yield a potent drug with spatial and temporal selectivity can be achieved through photodynamic therapy (PDT) [16–18]. Ideal PDT candidates should be minimally toxic in the dark, absorb low energy visible light within the therapeutic window of 600–900 nm to penetrate tissue, selectively accumulate in

the tumor, and be amphiphilic to trans-verse the cellular membrane [19]. A Jablonski diagram depicting the photophysical processes involved in PDT is presented in Fig. 2. A ground state (^1GS) photosensitizer (PS) absorbs visible light to populate the singlet excited state (^1ES) which typically deactivates back to the ^1GS through fluorescence or through population of the triplet excited state (^3ES) via intersystem crossing (ISC). Deactivation of the ^3ES to the ^1GS by emission of a photon (radiative decay, phosphorescence) or by the release of heat (nonradiative decay, thermal) can then occur. If the ^3ES is relatively long-lived, it can undergo three types of reactions of interest in PDT. Type I is electron transfer to O_2 or other oxygen-containing species in solution which eventually result in the generation of reactive oxygen species (ROS). Type II reactivity is ascribed to energy transfer to ground state $^3\text{O}_2$ to produce the very reactive $^1\text{O}_2$, and Type III activity results from electron transfer from the excited state of the sensitizer to cellular targets. PDT has been utilized in the treatment of bladder, gastrointestinal, prostate, and gynecological lesions as well as early stage, inoperable esophageal cancer, early or late stage head and neck tumors, inoperable early central lung cancers, and dermatology [16,20–24]. Production of $^1\text{O}_2$ provides high selectivity and localization, as the typical lifetime of $^1\text{O}_2$ in a metabolically healthy cell is $\sim 3 \mu\text{s}$, resulting in an estimated intracellular diffusion distance of $2\text{--}4 \times 10^{-6} \text{ cm}^2/\text{s}$. [25]. Photofrin®, composed of hematoporphyrin and its oligomers, was approved by the FDA in 1995 to treat esophageal, head, and neck tumors and functions by the Type II mechanism [26,27]. The drug absorbs strongly at 400 nm due to the Soret band transition and in the 500–600 nm range arising from the Q band transitions. This PDT agent is hindered by its non-unity population of the ^3ES (quantum yield, Φ , of 0.83) which limits the quantum yield of $^1\text{O}_2$ production ($\Phi^1\text{O}_2$) to 0.65 [27]. The most aggressive and drug resistant tumors are hypoxic, which limits the efficacy of PDT drugs that function by sensitizing $^1\text{O}_2$ [26,28]. To overcome the limitations of $^1\text{O}_2$ -generating PDT agents, photochemotherapeutic agents that undergo photoinduced ligand dissociation to covalently bind DNA in a manner similar to cisplatin and/or deliver a drug with spatiotemporal selectivity are recently under ardent investigation [29,30].

Discussed herein are the applications of ruthenium and rhodium complexes in PDT, through both O_2 -dependent and O_2 -independent mechanisms. To date, all approved PDT agents are organic molecules that sensitize $^1\text{O}_2$ production, and cisplatin and its derivatives are the only approved metal-based cancer drugs for cancer therapy. Section 2 discusses Ru and Rh complexes that produce $^1\text{O}_2$ which ultimately results in DNA photocleavage. The relatively long-lived metal-to-ligand charge transfer ($^3\text{MLCT}$) excited states of Ru(II)-polypyridyl complexes and long-lived intraligand (^3IL) excited states of Ru(II) and Rh₂(II,II) complexes are well suited for $^1\text{O}_2$ production, and ligand set variation allows for more efficient association with DNA. Ru and Rh complexes that photodamage DNA in the absence of O_2 are discussed in Section 3. Excited state complexes with strong oxidizing power undergo photoinduced redox reactions with guanine bases to cleave DNA (Section 3.1). Photoinduced ligand dissociation can be exploited to produce a metal complex that covalently binds to DNA in a manner similar to cisplatin (Section 3.2), and if the photodissociated ligand is biologically active, light activated drug delivery can be achieved (Section 3.3). Comparing the DNA–metal complex interactions between studies performed in various laboratories is complicated by the factors that impact binding such as ionic

strength and pH of the buffer, concentration of metal complex and DNA, and the type of DNA selected for the study. Discussion is limited to Ru(II)-polypyridyl and Rh₂(II,II) complexes that function under visible light irradiation. With respect to light activated drug delivery via ligand dissociation, discussion will focus on the delivery of organic drugs featuring nitrile functional groups. Other transition metals, such as Pt, Ir, and Mo, have also been studied for photoactivated chemotherapy, and they are reviewed elsewhere [30]. A discussion of Ru and Rh metallo-intercalators and metalloinsertors for charge transport through DNA or mismatch targeting is not included as they are well reviewed elsewhere [31,32]. The substantial body of work on photoactivated delivery of NO [33–35], CO [36], and amino acids and neurotransmitters [37–41] from metal complexes is beyond the scope of the present review and is not discussed herein.

2. Oxygen-dependent DNA modification

Ru(II)-polypyridyl complexes are frequently utilized in the development of metal-based PDT agents as they exhibit rich visible light absorption and undergo population of relatively long-lived ³MLCT (metal-to-ligand charge transfer) excited states with unit quantum yields [42–44]. These ³MLCT states provide convenient probes into excited state reactivity, are typically emissive, can be strongly oxidizing and/or reducing, and are typically long-lived and efficiently generate ¹O₂ [45]. The cationic nature of many transition metal complexes aids in the pre-association of the complex with the polyanionic DNA through electrostatic attraction, and the ground and excited state properties are conveniently tunable as they are dictated by the ligand set [43,46]. The prototypical visible light absorber, [Ru(bpy)₃]²⁺ (bpy = 2,2'-bipyridine), Fig. 3, absorbs strongly between 400 and 500 nm due to Ru(dπ) → bpy(π*) ¹MLCT transitions [42]. The ³MLCT excited state of the complex is populated rapidly with unit efficiency ($\Phi = 1$), is relatively long-lived (0.61 μs in H₂O and 1.00 μs in D₂O) [47,48], and undergoes energy transfer to ³O₂ to form the highly reactive ¹O₂ with $\Phi = 0.22$ in air-saturated D₂O [49], thus photocleaving pBR322 plasmid ($\lambda_{\text{irr}} > 450$ nm) via the Type II PDT mechanism [46,50,51]. [Ru(bpy)₃]²⁺, a weak electrostatic DNA binder, is limited as a PDT agent by its inability to intercalate or covalently bind to DNA, resulting in less targeted ¹O₂ delivery. The 1,10-phenanthroline (phen) analog, [Ru(phen)₃]²⁺ (Fig. 3), has a longer excited state lifetime (0.962 μs in H₂O and 1.1 μs in D₂O) [52] and subsequently is a more efficient ¹O₂ producer in D₂O ($\Phi = 0.24$) [49]. While [Ru(phen)₃]²⁺ was initially believed to interact with DNA via intercalation of the phen ligand, the currently accepted binding mode is its semi-intercalation [53,54]. The incorporation of the tridentate 2,2':2',6'-terpyridine (tpy) ligand to provide the [Ru(tpy)₂]²⁺ analog results in a drastically shortened excited state lifetime of 0.12 ns due to the distorted octahedral geometry promoting population of the nonemissive ³MC state (Fig. 4). This excited state does not persist long enough to effectively sensitize ¹O₂ production or cause DNA photodamage. [Ru(tpy)₂]²⁺ electrostatically binds to the DNA polyanion in a manner similar to [Ru(bpy)₃]²⁺ [46]. The inability to absorb lower energy visible light in the therapeutic window is a limitation for these homoleptic chromophores.

A series of Ru(II) complexes incorporate 1,12-diazaperylene (DAP), a bpy analog with an extended π-system and larger surface area to facilitate more avid DNA binding via intercalation [55]. The series features one, two, or three DAP ligands coordinated around the

Ru(II) center resulting in $[\text{Ru}(\text{bpy})_2(\text{DAP})]^{2+}$, $[\text{Ru}(\text{bpy})(\text{DAP})_2]^{2+}$, and $[\text{Ru}(\text{DAP})_3]^{2+}$ (Fig. 3). The complexes are strong UV and visible light absorbers, with the $\text{Ru}(\text{d}\pi)\text{DAP}(\pi^*)$ $^1\text{MLCT}$ transitions centered at 552 nm, 576 nm, and $\rightarrow$ 588 nm in CH_3CN for complexes with one, two, and three DAP ligands, respectively. The red shift that results from additional DAP ligands is accompanied with an increase in molar absorptivity, owing to the strong absorption of transitions involving DAP in comparison to those of bpy. The absorption of $[\text{Ru}(\text{DAP})_3]^{2+}$ extends to 675 nm, an important characteristic in developing therapeutic agents for PDT. The complexes are nonemissive at room temperature (RT) in CH_3CN , and at 77 K in EtOH/MeOH glass weak emission is observed between 698 and 727 nm. The weak luminescence is attributed to the deactivation of the $^3\text{MLCT}$ state by a low-lying DAP dark $^3\pi\pi^*$ intraligand (IL) state. The interactions of these compounds with DNA were evaluated by electronic absorption titrations, thermal denaturation, and relative viscosity changes. The addition of calf thymus DNA to a solution of each of the DAP complexes reveals hypochromic and bathochromic shifts consistent with intercalation (binding constants, K_b , of $1.4 - 1.6 \times 10^6 \text{ M}^{-1}$). The poor solubility of $[\text{Ru}(\text{bpy})(\text{DAP})_2]^{2+}$ and $[\text{Ru}(\text{DAP})_3]^{2+}$ in H_2O and the high concentrations necessary for thermal denaturation and relative viscosity studies precluded their analysis by these methods. However, $[\text{Ru}(\text{bpy})_2(\text{DAP})]^{2+}$ increases the melting temperature (T_m) of calf thymus by $+6^\circ\text{C}$, a value similar to that of the known intercalator ethidium bromide (EtBr), with $T_m = +5^\circ\text{C}$ [56]. Moreover, changes in the relative viscosity of herring sperm DNA that parallels EtBr were measured in the presence of $[\text{Ru}(\text{bpy})_2(\text{DAP})]^{2+}$. These observations suggest a primary intercalative binding mode for the complex afforded by the presence of the DAP ligand. Photo-cleavage of 100 μM pUC18 supercoiled plasmid DNA occurs when 20 μM $[\text{Ru}(\text{bpy})_2(\text{DAP})]^{2+}$ is irradiated with $\lambda > 395 \text{ nm}$ for 30 min in the air, and no photocleavage occurs when the solution is deoxygenated. The involvement of $^1\text{O}_2$ production in the photoreactivity with DNA is supported by the increase in photocleavage in D_2O compared to H_2O .

The “DNA light-switch” complex $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ ($\text{dppz} = \text{dipyrido}[3,2\text{-}a:2',3'\text{-}c]\text{phenazine}$), Fig. 3, is an extensively studied molecule that intercalates between DNA base pairs via the rigid, planar dppz ligand [57–59]. The light switch effect refers to the enhancement of the luminescence of the excited state species in the presence of DNA, since the complex is non-emissive in aqueous media in the absence of DNA, but its luminescence “turns on” upon intercalating. $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ possesses two low-lying excited states: a dark (non-emissive) $^3\text{MLCT}$ state involving the phenazine moiety of dppz distal to the metal and a bright (emissive) $^3\text{MLCT}$ state involving the proximal bpy portion of the dppz ligand. In aqueous solution, the dark state is lower in energy than the bright state; the latter not thermally accessible at RT, such that the complex is non-emissive. In organic solvents or when intercalated into DNA, the dark state energy is raised in energy closer to the bright state energy, allowing thermal population and enhanced emission. The ability of this complex to participate in intercalative binding to DNA is useful in PDT applications. The impact of substituted dppz-type ligands on the intercalation and light-switch effect has been observed for a variety of complexes [60]. The ability of $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ to photocleave DNA in the presence of O_2 was compared to an analog, $[\text{Ru}(\text{bpy})_2(\text{dpqp})]^{2+}$ ($\text{dpqp} = \text{pyrazino}[2',3':5,6]\text{pyrazino}[2,3\text{-}f][1,10]\text{phenanthroline}$; Fig. 3), which exhibits unusually

strong emission in H₂O at RT [58]. Both complexes possess similar absorption characteristics, with the Ru → L ¹MLCT transition centered at 445 nm (16,300 M⁻¹ cm⁻¹) and 457 nm (12,300 M⁻¹ cm⁻¹) for L = dppz and dpqp, respectively. The dpqp complex is strongly emissive in aqueous solution at 617 nm ($\Phi_{\text{em}} = 0.039$, $\tau = 582$ ns). These values are quite similar to those of [Ru(bpy)₃]²⁺ ($\lambda_{\text{em}} = 626$ nm, $\Phi_{\text{em}} = 0.042$, 630 ns). Although the photophysical properties are not well understood, the emission of the dpqp complex is proposed to arise from a ³MLCT excited state localized on the bpy portion of the dpqp ligand. The binding constant for [Ru(bpy)₂(dpqp)]²⁺ with DNA was measured as 2.0 × 10⁶ M⁻¹ ($s = 1.62$), which is similar to those of [Ru(bpy)₂(dppz)]²⁺, 10⁶–10⁷ M⁻¹, indicating that the former likely undergoes intercalative binding in a manner similar to the latter. The DNA photocleavage ability of these complexes was probed with pUC18 plasmid DNA ($\lambda_{\text{irr}} > 455$ nm, 15 min) in the presence of O₂. A larger degree of photocleavage of the plasmid occurs with [Ru(bpy)₂(dppz)]²⁺ than with [Ru(bpy)₂(dpqp)]²⁺, consistent with the larger $\Phi^1\text{O}_2$ of [Ru(bpy)₂(dpqp)]²⁺ (0.76) compared to [Ru(bpy)₂(dppz)]²⁺ (0.16). An even larger $\Phi^1\text{O}_2$ of 0.81 was reported for [Ru(bpy)₃]²⁺, but very little photocleavage occurs under these conditions because it does not intercalate and it is only a weak electrostatic binder. In all cases, no photocleavage occurs in the absence of O₂.

The intercalating dppz moiety was incorporated into the [Ru(tpy)₂]²⁺ architecture to provide a means of DNA binding and extend the excited state lifetime to promote ¹O₂ production [57]. The heteroleptic [Ru(tpy)(pydppz)]²⁺ and homoleptic [Ru(tpy)(pydppz)]²⁺ (pydppz = 3-(pyrid-2'-yl)dipyrido[3,2-*a*:2',3'-*c*]phenazine), for which the MLCT absorption maxima are observed between 475 and 481 nm, are shown in Fig. 4. The visible light absorption features are quite similar to those of the parent [Ru(tpy)₂]²⁺ complex despite the presence of the dppz moiety in pydppz, attributed to the optical transition taking place from the metal to the proximal bpy or tpy portion of the pydppz ligand. Both complexes are weakly emissive at RT; [Ru(tpy)(pydppz)]²⁺ exhibits an emission maximum at 698 nm ($\Phi_{\text{em}} = 0.00021$) and [Ru(pydppz)₂]²⁺ at 678 nm ($\Phi_{\text{em}} = 0.00061$) in CH₃CN. The emission of each pydppz complex is red shifted and more efficient than that of [Ru(tpy)₂]²⁺ ($\lambda_{\text{em}} = 629$ nm, $\Phi_{\text{em}} < 5 \times 10^{-6}$), as the pydppz ligand is involved in the emissive ³MLCT state. The excited state lifetimes determined by transient absorption spectroscopy are 5 ns and 2.4 ns for [Ru(tpy)(pydppz)]²⁺ and [Ru(tpy)(pydppz)]²⁺, respectively. The short excited state lifetimes are due to thermal population of the low-lying ³MC state, typical of [Ru(tpy)₂]²⁺ - type complexes ($\tau = 126$ ps for [Ru(tpy)₂]²⁺). The intercalation of the pydppz ligand is supported by the typical hypochromic and bathochromic shifts in the electronic absorption spectra of the complexes upon addition of calf thymus DNA, a trend not observed for [Ru(tpy)₂]²⁺. The binding constants, 2.0 × 10⁶ M⁻¹ and 8.1 × 10⁶ M⁻¹ for [Ru(tpy)(pydppz)]²⁺ and [Ru(pydppz)₂]²⁺, respectively, are similar to those of [Ru(phen)₂(dppz)]²⁺ [61]. Intercalation was confirmed for [Ru(tpy)(pydppz)]²⁺ by relative viscosity measurements with herring sperm DNA, but the low solubility of [Ru(tpy)(pydppz)]²⁺ in buffer did not allow the same analysis, although it is expected to exhibit similar behavior as the heteroleptic analog. Additionally, the change in emission intensity upon addition of calf thymus DNA is quite different between the heteroleptic and homoleptic complexes, with enhancements of 8-fold and 1.2-fold, respectively. The difference can be attributed to the notion that only one pydppz ligand on [Ru(tpy)(pydppz)]²⁺ intercalates between the DNA

base pairs while the other pydppz remains unbound and exposed to solvent. It is expected that the lowest energy $^3\text{MLCT}$ excited is localized on the non-intercalated ligand, such that its surroundings are not affected significantly by the intercalation of the other pydppz ligand, such that the complex exhibits very little change in emission. When irradiated with $\lambda_{\text{irr}} > 395$ nm for 10 min in the presence of O_2 and pUC18 plasmid, only $[\text{Ru}(\text{tpy})(\text{pydppz})]^{2+}$ causes photocleavage. This photocleavage occurs only in the presence of O_2 despite the short 5 ns lifetime and a low $\Phi^1\text{O}_2$ of $1.9 \pm 0.3\%$. The shorter excited state lifetime of $[\text{Ru}(\text{pydppz})_2]^{2+}$ is consistent with its lower $\Phi^1\text{O}_2$ of $0.9 \pm 0.4\%$ and its lack of DNA photocleavage.

To extend the excited state lifetimes of the $[\text{Ru}(\text{tpy})(\text{pydppz})]^{2+}$ and $[\text{Ru}(\text{tpy})(\text{pydppz})]^{2+}$ complexes while maintaining the inter-calative binding mode, a series of analogous homoleptic and heteroleptic compounds with extended π -systems were constructed using the ligands pydppx (3-(pyrid-2'-yl)-11,12-dimethyldipyrido[3,2-*a*:2',3'-*c*]phenazine) and pydppn (3-(pyrid-2'-yl)-4,5,9,16-tetraaza-dibenzo[*a,c*]naphthacene) (structures shown in Fig. 4) [62]. The lowest energy $^1\text{MLCT}$ absorption bands are similar to those of the analogous pydppz complexes, however, the photophysical processes are greatly impacted when pydppn is employed. In the complexes containing dppn ligands, the lowest energy excited state is $^3\pi\pi^*$ in nature rather than $^3\text{MLCT}$, as is the case for the pydppz and pydppx complexes. This low-lying ligand-centered excited state is long lived with $\tau = 20.1$ and 24.3 μs for $[\text{Ru}(\text{tpy})(\text{pydppn})]^{2+}$ and $[\text{Ru}(\text{pydppn})_2]^{2+}$, respectively. These values are in stark contrast to the short $^3\text{MLCT}$ lifetimes of the aforementioned pydppz complex and those incorporating pydppx, with $\tau = 3.7$ for $[\text{Ru}(\text{tpy})(\text{pydppx})]^{2+}$ and 1.1 ns for $[\text{Ru}(\text{pydppx})_2]^{2+}$, as the $^3\pi\pi^*$ state is higher energy than the $^3\text{MLCT}$ state. The trend is consistent with the extended π -system and addition of electron donating substituents to the dppz motif. The long-lived excited states of the pydppn complexes result in large $\Phi^1\text{O}_2$ values of $0.92(2)$ and $1.07(7)$ for $[\text{Ru}(\text{tpy})(\text{pydppn})]^{2+}$ and $[\text{Ru}(\text{pydppn})_2]^{2+}$, respectively. The excited state lifetimes and $\Phi^1\text{O}_2$ values for the pydppx complexes, $0.014(3)$ and $0.010(4)$ for $[\text{Ru}(\text{tpy})(\text{pydppx})]^{2+}$ and $[\text{Ru}(\text{pydppx})_2]^{2+}$, respectively, are similar to those of the pydppz analogs. The remarkable $\Phi^1\text{O}_2$ values for the pydppn complexes are greater than that of hematoporphyrin and represent the most efficient $^1\text{O}_2$ production to date. The binding constants of the complexes with calf thymus DNA were determined to be $4.6 \times 10^6 \text{ M}^{-1}$ for $[\text{Ru}(\text{tpy})(\text{pydppn})]^{2+}$, $6.9 \times 10^5 \text{ M}^{-1}$ for $[\text{Ru}(\text{tpy})(\text{pydppx})]^{2+}$, and $3.5 \times 10^5 \text{ M}^{-1}$ for $[\text{Ru}(\text{pydppn})_2]^{2+}$. No binding constant was measured for $[\text{Ru}(\text{pydppx})_2]^{2+}$ due to aggregation of the complex. The lower K_b value for $[\text{Ru}(\text{tpy})(\text{pydppx})]^{2+}$ may be due to steric hindrance of intercalation by methyl substituents. The pydppn complexes efficiently photocleave pUC18 plasmid DNA by an O_2 mediated pathway when irradiated with $\lambda > 395$ nm for 10 min. Complete conversion to nicked, open circular plasmid occurs with $[\text{Ru}(\text{tpy})(\text{pydppn})]^{2+}$, consistent with single-strand breaks that result from the production of ROS and guanine oxidation derived from $^1\text{O}_2$. The photocleavage is not as efficient for $[\text{Ru}(\text{pydppn})_2]^{2+}$ due to its lower K_b value, and the short excited state lifetimes and inefficient $^1\text{O}_2$ generation of the pydppx complexes result in insignificant DNA photocleavage.

The efficient O₂ mediated DNA photocleavage by [Ru(tpy)(pydppn)]²⁺ and [Ru(pydppn)₂]²⁺ motivated the study of the impact of these complexes on photocrosslinking of Proliferating Cell Nuclear Antigen (PCNA) and p53 protein in mammalian cells and cell lysates [63]. The protein p53 maintains genetic stability and is involved in signaling pathways necessary for cell survival, aging, and cancer formation, prognosis, and treatment. More than half of all cancers exhibit mutations of the homotetrameric p53. PCNA, a circular homotrimer that encircles one ds-DNA, is vital in mammalian replication and DNA repair. These nuclear targets are important in the treatment of tumors. Photocrosslinking studies were performed with African green monkey kidney fibroblasts (CV-1) and human fibroblasts (GM639). While [Ru(tpy)(pydppn)]²⁺ and [Ru(pydppn)₂]²⁺ differ in their ability to photocrosslink p53 and PCNA in cells (white light, 3.15 J/cm², 7 min) with the heteroleptic complex more efficiently inducing crosslinks, the two complexes exhibit similar efficiencies in cell lysates. This result suggests a difference in ability of the complexes to enter the cells or to reach the nucleus. [Ru(tpy)(pydppn)]²⁺ also induces protein–DNA crosslinks and inhibits DNA replication when irradiated, suggesting that DNA damage signaling and cell cycle checkpoint pathways remain functional after damage to nuclear proteins.

A series of *cis*-[Rh₂(μ-O₂CCH₃)₂(dppn)(NN)]²⁺ complexes, where NN = bpy, phen, dpq = dipyrido[3,2-*f*:2',3'-h]quinoxaline, dppz, and dppn = benzo[i]dipyrido[3,2-*a*:2',3'-h]quinoxaline (Fig. 5), possess long-lived dppn ³ππ* states and to photocleave DNA with a dominant O₂-dependent mechanism; the complexes in the series exhibit varying degrees of toxicity toward HeLa and COLO-316 cancer cell lines [64]. The electronic absorption spectrum of each complex features ligand-centered ππ* transitions between 316 and 329 nm associated with both the NN and dppn ligands. The MLCT transitions are expected to occur with lower molar extinction coefficients between 400 and 430 nm, hidden beneath the broad ¹ππ* dppn transitions, and weak Rh₂(π*) → Rh₂(σ*) MC bands are observed in the 600–621 nm range. No ³MLCT emission is detected in H₂O for the *cis*-[Rh₂(μ-O₂CCH₃)₂(dppn)(NN)]²⁺ complexes, consistent with the transient absorption spectroscopy results which reveal that the long-lived, lowest energy excited state is dppn ³ππ* in nature, with lifetimes of 2.7, 2.4, 2.4, 3.5, and 4.1 μs for NN = bpy, phen, dpq, dppz, and dppn, respectively. However, the respective ¹O₂ quantum yields, Φ¹O₂, measured to be 0.7, 0.9, 0.8, 0.4, and 0.4, do not follow the trend expected based on the lifetimes. The lower ¹O₂ production efficiency for the dppz and dppn complexes, despite their longer-lived excited states, is attributed to the greater hydrophobicity of these two complexes relative to others in the series, which may cause aggregation in H₂O. The complexes were determined to either intercalate or aggregate in the presence of DNA (or both), the aggregation was established through the replacement of DNA with polyanionic polystyrene sulfonate (PSS) in optical titrations. The complexes with NN = bpy and phen intercalate between base pairs as NN is not long enough to block dppn intercalation (*K*_b = 1.2 × 10⁶ and 1.0 × 10⁵ M⁻¹, respectively). The dpq complex intercalates and aggregates on the DNA surface, while the dppz and dppn complexes interact with DNA solely through surface aggregation, enhanced by the polyanionic DNA or PSS structure. When irradiated in the presence of pUC18 plasmid DNA in an air saturated solution (λ > 375 nm, 20 min), the bpy, phen, dpq, and dppz complexes convert 64–68% of the supercoiled (SC) plasmid to the open circular (OC)

form, while dppn only converts 43%. The first four complexes undergo a dominant O₂-dependent and minor O₂-independent photocleavage mechanism, as removal of O₂ decreases activity but does not completely eliminate it. The removal of O₂ has no impact on the activity of the dppn complex. As the dppz and dppn complexes produce ¹O₂ with similar efficiencies, the difference in activity is unexpected; however, the complex with one dppz and one dppn ligand may be able to allow partial intercalation of dppn for more efficient ROS delivery, while that with two dppn ligands cannot intercalate.

The *cis*-[Rh₂(μ-O₂CCH₃)₂(dppn)(NN)]²⁺ (NN = bpy, phen, dpq, dppz, and dppn) complexes exhibit dark toxicity toward HeLa and COLO-316 cancer cell lines, with the bpy, phen, dpq, and dppz complexes transversing the cellular membrane to induce apoptosis, while the dppn complex is unable to enter the cell and induces necrosis [65]. The cytotoxicity studies of this series toward Hs-27 fibroblasts shows that the dppz and dppn complexes are less cytotoxic in the dark than the bpy, phen, and dpq complexes, with LC₅₀ values of 200 ± 20, 178 ± 17, 51 ± 5, 355 ± 18, and 384 ± 24 μM (LC₅₀ = median lethal concentration) for NN = bpy, phen, dpq, dppz, and dppn, respectively [64]. The enhanced hydrophobicity of the NN = dppz and dppn complexes (log *P* = 0.62 and 1.02, respectively) suggests that they may not cross through the cellular membrane as well as those with NN = bpy, phen, and dpq (log *P* = 0.3, 0.30, and 0.41, respectively). Upon irradiation, the complexes exhibit a greater potency toward the cells, with LC₅₀ values of 30 ± 5, 22 ± 4, 9 ± 3, 17 ± 3, and 16 ± 4 μM for NN = bpy, phen, dpq, dppz, and dppn, respectively. Whereas complexes with NN = bpy, phen, and dpq may enter into the cell and damage intracellular materials via ROS generation, the more hydrophobic complexes may remain bound within the cellular membrane and be photoreactive toward phospholipids to induce cell death. The increase in toxicity upon irradiation, between 5.7-fold and 8.4-fold, for NN = bpy, phen, and dpq are similar to the increase for hematoporphyrin, while L = dppz and dppn Exhibit 21-fold and 24-fold enhancements. Along with their lower dark toxicity than hematoporphyrin, these complexes represent potential improvements over current PDT drugs.

3. Oxygen-independent DNA modification

3.1. Redox reactions with DNA

A method for O₂-independent DNA photocleavage involves redox reactions with DNA, primarily guanine oxidation by an excited state species [66]. The [Ru(^tBu₂bpy)₂(Mebpy-V₁-V₂)]⁶⁺ complex (^tBu₂bpy = 4,4'-di-*tert*-butyl-2,2'-bipyridine) with two viologens tethered to the Ru(II)-polypyridyl chromophore (Fig. 6) was designed for charge separation (CS) between the spatially separated HOMO (localized on Ru) and LUMO (localized on V₂). The electronic absorption spectrum of this complex features a ¹MLCT absorption maximum at 449 nm (11,200 M⁻¹ cm⁻¹), typical of Ru(II)-polypyridyl complexes. Very weak emission is observed at 641 nm (Φ_{em} = 0.0050(5), N₂ purged H₂O), which is quenched by O₂. A long-lived CS state (τ = 1.7 μs) is produced upon excitation with λ = 532 nm as observed by transient absorption spectroscopy, which features peaks typical of reduced viologen at 395 and 605 nm. The [Ru(^tBu₂bpy)₂(Mebpy-V₁-V₂)]⁶⁺ complex (3 μM) photocleaves pUC18 plasmid DNA (100 μM) in the presence and absence of O₂ when irradiated with λ > 395 for 20 min. This O₂-independent cleavage occurs via guanine oxidation by the long-lived

photogenerated Ru(III) center in the excited state species. This effect is similar to Ru(II) tris-polypyridyl complexes in the presence of an electron acceptor [67,68].

$[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ was investigated as an analog to the O_2 -dependent DNA photocleavage agent $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ previously discussed. However, $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ cleaves plasmid DNA in the absence of O_2 [69]. The electronic absorption spectrum of $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ features dppn $^1\pi\pi^*$ transitions at 387 nm ($9900 \text{ M}^{-1} \text{ cm}^{-1}$) and 411 nm ($13,400 \text{ M}^{-1} \text{ cm}^{-1}$) and a $^1\text{MLCT}$ absorption centered at 444 ($13,500 \text{ M}^{-1} \text{ cm}^{-1}$). The spectral features of this complex in the visible light range are quite similar to those of $[\text{Ru}(\text{bpy})_3]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$. A weak emission is observed at 617 nm ($\Phi_{\text{em}} = 0.003$) from a $^3\text{MLCT}$ excited state that is relatively long-lived ($\tau = 803 \text{ ns}$). Transient absorption spectroscopy reveals population of a lowest energy, non-emissive excited state that is dppn-localized $^3\pi\pi^*$ in nature that is long lived ($\tau = 33 \mu\text{s}$). Ultrafast transient absorption studies indicate that intersystem crossing from the initially generated singlet state populates both the $^3\text{MLCT}$ and $^3\pi\pi^*$ states. As expected based its long-lived LC excited state, $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ more efficiently produces $^1\text{O}_2$ ($\Phi = 0.88 \pm 0.05$) than the dppz analog ($\Phi = 0.16 \pm 0.02$). The DNA binding constant of $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$, K_b , is on the order of 10^6 M^{-1} , similar to those of other known DNA intercalators, and the increased relative viscosity of herring sperm DNA upon addition of complex confirms intercalation as the primary binding mode of the complex. Complete and rapid photocleavage of pUC18 plasmid DNA (100 μM bases) occurs when irradiated with $\lambda > 455 \text{ nm}$ for 30 s, while no cleavage is observed for $[\text{Ru}(\text{bpy})_3]^{2+}$ or $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ under these conditions. Significant photocleavage occurs within 3 min of irradiation of the dppn complex with $\lambda > 550 \text{ nm}$. In D_2O only a slight enhancement of DNA photocleavage is observed compared to the system in H_2O , suggesting very little impact of $^1\text{O}_2$ on the reactivity with the duplex. A small amount of cleavage occurs when the sample is deoxygenated and in the presence of NaN_3 ($^1\text{O}_2$ or $\cdot\text{OH}$ scavenger) or superoxide dismutase (SOD), indicating that an additional, O_2 -independent mechanism plays a role in photocleavage. This mechanism involves guanine oxidation by the excited state complex. The excited state reduction potential (E_{red}^*) is approximately +1.64 V vs NHE, making it a strong oxidizing agent. Guanine oxidation in H_2O requires +1.29 V vs NHE at neutral pH, providing a thermodynamically favorable process ($G = -0.35 \text{ V}$). Stern-Volmer quenching of the excited state $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ upon addition of guanosine monophosphate (GMP) yields a quenching constant (k_q) of $2.1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, which represents a relatively efficient process. No quenching is observed for $[\text{Ru}(\text{bpy})_3]^{2+}$ by GMP, since guanine oxidation from its excited state is not thermodynamically favorable. While the Os(II) analog, $[\text{Os}(\text{bpy})_2(\text{dppn})]^{2+}$, absorbs in the therapeutic window and photocleaves DNA when irradiated with red light ($\lambda > 645 \text{ nm}$ for 1.5 h) in the presence of O_2 , it does not photocleave DNA under hypoxic conditions as the excited state species is too low in energy to oxidize guanine [70]. It is believed that the $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ complex undergoes interesting dual activity with DNA through a highly reactive MLCT state that oxidizes guanine and a long-lived $^3\pi\pi^*$ state that produces ROS.

Polypyridyl ligands structurally related to phen, such as tap (1,4,5,8-tetraazaphenanthrene) and hat (1,4,5,8,9,12-hexaazatriphenylene), were incorporated into Ru(II) complexes to

facilitate intercalation and photoinduced electron transfer to oxidize guanine bases [43,51,71]. The homoleptic and heteroleptic DNA cleaving agents in this series are $[\text{Ru}(\text{tap})_3]^{2+}$, $[\text{Ru}(\text{hat})_3]^{2+}$, $[\text{Ru}(\text{tap})_2(\text{NN})]^{2+}$, and $[\text{Ru}(\text{hat})_2(\text{NN})]^{2+}$ (NN = bpy or phen; Fig. 6). The complexes absorb efficiently with $^1\text{MLCT}$ transitions between 400 and 500 nm ($\epsilon = 12,000\text{--}19,000 \text{ M}^{-1} \text{ cm}^{-1}$) [71–75]. Thermal denaturation experiments and hypochromicity upon DNA addition are consistent with the planar ligands partially intercalating between the base pairs. Quenching of the $^3\text{MLCT}$ emission was observed upon addition of calf thymus DNA for all members of this series. This behavior is the opposite of that observed for DNA light-switch complexes, suggesting that the excited state reactivity is quite different. Intermolecular electron transfer from GMP ($E^\circ(\text{G}^{\bullet+}/\text{G}) = +0.92 \text{ V vs SCE}$) to the excited state of each complex is thermodynamically favorable ($E_{\text{red}}^* = +1.46 \text{ V}$ and $+1.32 \text{ V}$ for $[\text{Ru}(\text{hat})_3]^{2+}$ and $[\text{Ru}(\text{tap})_3]^{2+}$, respectively; $+1.23 \text{ V}$ and $+1.12 \text{ V}$ for $[\text{Ru}(\text{hat})_2(\text{phen})]^{2+}$ and $[\text{Ru}(\text{hat})_2(\text{bpy})]^{2+}$, respectively; and $+1.06 \text{ V}$ for both $[\text{Ru}(\text{tap})_2(\text{phen})]^{2+}$ and $[\text{Ru}(\text{tap})_2(\text{bpy})]^{2+}$), such that photocleavage of pBR322 plasmid ($\lambda_{\text{irr}} = 436 \text{ nm}$) via guanine oxidation is a likely mechanism. In contrast, $[\text{Ru}(\text{tap})(\text{bpy})_2]^{2+}$ and $[\text{Ru}(\text{tap})_2(\text{phen})]^{2+}$, with $E_{\text{red}}^* = +0.83 \text{ V}$ and $+0.87 \text{ V vs SCE}$, respectively, do not have sufficient excited state oxidizing power to participate in guanine oxidation, and consequently very little DNA photocleavage was observed.

A series of dyad complexes with Ru(II)-polypyridyl chromophores linked to a planar, rigid pyrene ligand through an ethynyl linker were studied for their ability to photo-cleave DNA. The photocytotoxicity of the complexes toward human leukemia cell lines (HL-60) and metastatic melanoma cell line Malme-3M was also investigated as a function of the placement of the pyrenylethynylene substituent on the phen ligand [76,77]. The $[\text{Ru}(\text{bpy})_2(3\text{-pyr-phen})]^{2+}$, $[\text{Ru}(\text{bpy})_2(4\text{-pyrphen})]^{2+}$, and $[\text{Ru}(\text{bpy})_2(5\text{-pyr-phen})]^{2+}$ complexes (Fig. 6) feature a $[\text{Ru}(\text{tap})_2(\text{phen})]^{2+}$ parent complex with the pyrenylethynylene substituent at the 3-, 4-, and 5-position on the phen ligand, respectively. The compounds exhibit strong IL and MLCT absorption in the ultraviolet and visible spectral regions, and the feature associated with pyrene at 382 nm is observed in each complex. $[\text{Ru}(\text{bpy})_2(3\text{-pyr-phen})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(5\text{-pyr-phen})]^{2+}$ exhibit similar absorption profiles with maxima near 412 nm and a lower energy shoulder at 450 nm, both corresponding to $^1\text{MLCT}$ transitions. The $^1\text{MLCT}$ absorption of $[\text{Ru}(\text{bpy})_2(4\text{-pyr-phen})]^{2+}$ is red shifted to 482 nm as substitution at the para position to the coordinated N atom of phen stabilizes the phen acceptor orbitals and increases the communication between the pyrene moiety and the Ru center. The complexes are weakly emissive ($\lambda_{\text{em}} = 609\text{--}625 \text{ nm}$, $\Phi_{\text{em}} < 0.005$) in air saturated CH_3CN , with a lowest-energy excited $^3\pi\pi^*$ state localized on the pyrene ligand.

$[\text{Ru}(\text{bpy})_2(3\text{-pyr-phen})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(5\text{-pyr-phen})]^{2+}$ have similar excited state lifetimes of 240 and 140 μs in deoxygenated CH_3CN , while $[\text{Ru}(\text{bpy})_2(4\text{-pyr-phen})]^{2+}$ has a shorter lifetime of 22 μs , but the three complexes produce $^1\text{O}_2$ with almost identical efficiency ($\Phi = 0.65, 0.68$, and 0.67 for the 3-, 4-, and 5-substituted complexes, respectively). The $^3\text{MLCT}$ excited state of each complex is also populated upon excitation but the lifetimes at RT could not be resolved with the $\sim 20 \text{ ns}$ instrument response. Binding titrations of $[\text{Ru}(\text{bpy}-d_8)_2(3\text{-pyr-phen})]^{2+}$ and $[\text{Ru}(\text{bpy}-d_8)_2(5\text{-pyrphen})]^{2+}$ with herring sperm DNA resulted in K_b values of 3.0×10^7 and $8.5 \times 10^6 \text{ M}^{-1}$, respectively, consistent with those of known intercalators

[78,79]. Thermal denaturation experiments with calf thymus DNA (pH 7.4) in the presence of the two complexes reveals a marked increase in the melting temperatures, with T_m values of +20 and +10 °C, respectively, relative to DNA alone. The difference in melting temperature is sensitive to the placement of the pyrene substituent around the phen ligand. Both complexes substantially increased the DNA viscosity, another confirmation of intercalation. The two bpy-*d*₈ complexes photocleave pUC19 plasmid DNA when irradiated with 420 nm light for 30 min with half maximal effective concentration (EC₅₀) values in the nanomolar range, and complete conversion to OC plasmid occurs with complex concentration as low as 2 μM. A plasmid with greater GC content, pDesR3, is linearized through double-strand photocleavage with concentrations as low as 3 μM for the 3-substituted complex, while no linearization occurs with the 5-substituted analog. The photocleavage occurs via an O₂-independent mechanism and it is proposed to take place through guanine oxidation by the excited state complex. This conclusion is supported by more avid photocleavage with increasing guanine content in the plasmid.

The cytotoxicity and photocytotoxicity of these complexes were first assayed with a human leukemia cell line, HL-60, which showed that they have high phototoxicity index (PI) values, defined as the ratio of the dark EC₅₀ to the irradiated EC₅₀. A species such as cisplatin, which is not photochemically active, has a PI equal to 1. The PI values for the 3-, 4-, and 5-substituted complexes are 104, 382, and 1747, respectively (photolyzed with 100 J/cm² white light for 1 h), with irradiated EC₅₀ values of 1.8, 0.22, and 0.15 μM, respectively. The 5-pyr-phen complex exhibits a very low toxicity in the dark, with EC₅₀ = 262 μM, which when combined with its low nanomolar irradiated EC₅₀ value, results in an unprecedented PI that is an order of magnitude greater than the largest reported to date (vide infra) [80]. A lower light dosage of 5 J/cm² still results in 0.5–1.5 μM EC₅₀ values, and the cell death occurs via apoptosis. It is important to note that the irradiated complexes are up to 170 times more potent than cisplatin and are 10–85 times less toxic in the dark than cisplatin, making these compounds potential PDT agents with low systemic toxicity in the dark and very high cytotoxicity when irradiated. The activity of the complexes against the metastatic melanoma cell line Malme-3M was also investigated in the dark and irradiated conditions. Malme-3M is an aggressive melanoma with a poor prognosis, no curative therapy, a relatively high recurrence rate of resected melanoma, and a median survival rate of only 9 months at stage IV. Because of its very low O₂ content, it is untreatable by compounds that produce ROS [81]. Irradiation of the cells in the presence of each of the three complexes with white light (7 J/cm², 15 min) resulted in cell death with nanomolar EC₅₀ values. The 5-pyr-phen complex exhibited an irradiated EC₅₀ value of 200 nM and those for the 3- and 4-substituted complexes were measured to be in the 600–700 nM range. The dark toxicity of the complexes toward the Malme-3M cell line is slightly greater than observed for the HL-60 cells, with EC₅₀ values of 62, 44, and 57 μM for the 3-, 4-, and 5-pyr-phen complexes, respectively, but the PI values are still quite large. The trends observed in the photo-cytotoxicity cannot be explained by the photophysical properties of the complexes. Factors such as subcellular localization, biological targets, cellular uptake, metabolism, and mechanism of action are all important in cytotoxicity, and differences in the size, shape, and hydrophobicity of the complexes are expected to impact toxicity. It is evident, however, that the combination of the low-lying ³ππ* state and the intercalating

ability and lipophilicity of the pyrene ligand is important for activity. This series of potent photocytotoxic agents with low dark cytotoxicities suggests that strong intercalation does not always induce dark cytotoxicity and this method of targeting DNA should not be overlooked.

In addition to the ruthenium-based systems, the acetate-bridged $\text{Rh}_2(\text{II,II})$ compounds depicted in Fig. 7 incorporate bidentate ligands with extended π -systems that impart interesting photophysical properties and intercalative abilities on the complexes [82,83]. The two complexes with one or two dppz ligands, *cis*- $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{dppz})(\eta^1\text{-O}_2\text{CCH}_3)(\text{CH}_3\text{OH})]^+$ (Rh_2 -mono-dppz) and *cis*- $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{dppz})_2]^{2+}$ (Rh_2 -bis-dppz) behave differently with DNA. Rh_2 -mono-dppz binds to DNA by intercalation of the dppz ligand and it also exhibits a small amount of surface aggregation. In contrast, Rh_2 -bis-dppz and the heteroleptic analog *cis*- $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{bpy})(\text{dppz})]^{2+}$ (Rh_2 -bpydppz) do not intercalate, as the binding constant for the latter of $2.8 \times 10^3 \text{ M}^{-1}$ determined by equilibrium dialysis is inconsistent with intercalation ($K_b \sim 10^5 - 10^6 \text{ M}^{-1}$). However, the presence of the DNA polyanion enhances intermolecular π -stacking of the complex. The three complexes photocleave pUC18 plasmid DNA upon irradiation with $\lambda > 395 \text{ nm}$ for 15 min, but the photocleavage by Rh_2 -mono-dppz is greater due to its ability to intercalate. In contrast, the homoleptic *cis*- $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{bpy})_2]^{2+}$ and *cis*- $(\mu\text{-O}_2\text{CCH}_3)_2(\text{phen})_2]^{2+}$ complexes do not photocleave DNA [84,85]. The DNA photocleavage of Rh_2 -mono-dppz slightly diminishes in the absence of O_2 , but the O_2 -independent mechanism is substantial. It is suggested that a lowest lying $^3\text{MLCT}$ excited state of Rh_2 -mono-dppz, Rh_2 -bis-dppz, and Rh_2 -bpy-dppz, in which electron density moves from the dirhodium core to the phenazine moiety of the dppz ligand, results a longer-lived charged-separated excited state relative to those of the bpy and phen analogs, allowing sufficient time for the partially oxidized dirhodium core to cleave DNA. These complexes were evaluated for their cytotoxicity and photocytotoxicity toward Hs-27 cells. When incubated with the cells in the dark, Rh_2 -mono-dppz, Rh_2 -bis-dppz, and Rh_2 -bpy-dppz have LC_{50} values of 27 ± 2 and 135 ± 8 , and $208 \pm 10 \text{ }\mu\text{M}$, respectively. It is believed that the intercalation of the Rh_2 -mono-dppz complex may contribute to its increased dark toxicity. When irradiated ($\lambda > 400\text{--}700 \text{ nm}$, 30 min), the LC_{50} values decreased to 21 ± 3 , 39 ± 1 , and $44 \pm 2 \text{ }\mu\text{M}$ for Rh_2 -mono-dppz, Rh_2 -bis-dppz, and Rh_2 -bpy-dppz, respectively. While the mono-dppz complex exhibits almost no difference between dark and irradiated cytotoxicity, the Rh_2 -bis-dppz and Rh_2 -bpy-dppz complexes represent good PDT candidates due to low dark toxicity and increased phototoxicity by factors that range from 3.4 to 4.7. In this motif, intercalation or strong DNA binding may not be desirable properties in developing successful PDT agents. The dirhodium species have promising potential when compared to the toxicities of these currently employed cancer therapeutics.

Recently, an unbridged $\text{Rh}_2(\text{II,II})$ complex with phen ligands that chelate to each Rh center, $[\text{Rh}_2(\text{phen})_2(\text{CH}_3\text{CN})_6]^{4+}$ (Fig. 7), was investigated for its ability to photocleave DNA when exposed to visible light [86]. Upon low energy visible light photolysis ($\lambda > 590 \text{ nm}$) in H_2O , the complex releases two equatorial CH_3CN ligands and undergoes homolytic Rh-Rh bond cleavage, resulting in the formation of two monometallic radical $\text{Rh}(\text{II})$ fragments, which then generate a mononuclear aqua complex ($\Phi_{\text{aq}} = 1.38$). The greater than unity quantum

yield can be indicative of an initial photoreaction followed by a dark reaction, or may be related to the formation of two mononuclear rhodium complexes from the bimetallic reactant. The two major products observed by ESI-MS are $[\text{Rh}(\text{phen})(\text{CH}_3\text{CN})(\text{OH})]^+$ and $[\text{Rh}(\text{phen})(\text{CH}_3\text{CN})(\text{OH}_2)_3(\text{BF}_4)]^+$. Formation of the reactive radical Rh(II) fragments under irradiation with $\lambda > 590$ nm photocleaves DNA in an O_2 -independent manner, while this activity does not occur in the dark. This complex represents a potentially useful phototherapeutic candidate owing to its reactivity with low energy light in the PDT window.

3.2. Covalent DNA binding

Dirhodium (II,II) and oxidized dirhodium(III,II) paddlewheel complexes with four acetate bridges, $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4]$ and $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4]^+$ (Fig. 8) exhibit anti-tumor activity against L1210 and Ehrlich ascites tumors in mice several decades ago [87–90]. It is believed that these complexes act by binding to DNA and inhibiting replication. Coordinate bond formation between the axial positions and ligands containing O, N, S, or P donor atoms (typically solvent molecules) occurs readily. The $\text{Rh}_2(\text{II,II})$ complexes have long-lived excited states (typically 3.5–5.0 μs), however no photocleavage of pUC18 plasmid occurs with visible light irradiation ($\lambda > 395$ nm). Upon addition of electron acceptors, such as 3-cyano-1-methylpyridinium or 1,8-anthraquinone disulfonate, oxidation of the complex occurs to produce the $\text{Rh}_2(\text{III,II})$ species which cleaves pUC18 plasmid with low energy excitation ($\lambda > 610$ nm), an important characteristic in development of PDT agents [85]. In the presence of O_2 , the activity is greatly decreased suggesting that the excited state is readily deactivated by O_2 . Addition of a non-labile ligand such as pyridine or PPh_3 which bind to the axial sites in place of the labile OH_2 prohibits photocleavage, as the ligand in the axial position must be labile in order to promote covalent interactions between one of the Rh atoms and DNA. Covalent binding of adenine and guanine bases to this complex has been observed by mass spectrometry. A major drawback of this system is that thermal axial ligand exchange occurs in the dark.

The first reported metal-metal bonded complex to covalently bind to ds-DNA under visible light irradiation is of the form $\text{cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{CH}_3\text{CN})_4(\text{OH}_2)_2]^{2+}$, where the water molecules are labile and occupy the axial positions [91]. The $\text{cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{CH}_3\text{CN})_6]^{2+}$ complex (Fig. 8), which exhibits relatively weak absorption centered at 363 and 525 nm (420 and $218 \text{ M}^{-1} \text{ cm}^{-1}$, respectively), undergoes rapid thermal axial ligand exchange in H_2O in the dark to form $\text{cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{CH}_3\text{CN})_4(\text{OH}_2)_2]^{2+}$. The lowest energy metal-centered $\text{Rh}_2(\pi^*) \rightarrow \text{Rh}_2(\sigma^*)$ absorption shifts from 525 to 555 nm ($160 \text{ M}^{-1} \text{ cm}^{-1}$), as expected for a transition that involves the $\text{Rh}_2(\sigma^*)$ orbital, such that its energy is sensitive to the nature of the axial ligands. Photolysis of the complex with $\lambda > 455$ nm for 5 h results in the exchange of two equatorial CH_3CN ligands with solvent H_2O molecules to yield $\text{cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{CH}_3\text{CN})_2(\text{OH}_2)_4]^{2+}$. This photoaquation causes a shift of the transitions formerly centered at 373 and 555 nm to 450 and 573 nm, as predicted by time-dependent density functional theory (TD-DFT) calculations. The two remaining CH_3CN ligands can occupy different equatorial positions, resulting in three possible isomers of the complex, and it is unclear which is formed. The quantum yield of photoaquation (Φ_{aq}) is dependent on irradiation wavelength with values of 0.37 and 0.09 for $\lambda_{\text{irr}} = 355$ and 509 nm, respectively.

The power dependence of product formation is consistent with a one photon process, such that the absorption of a single photon causes one CH₃CN substitution and the second CH₃CN substitution must occur by a dark reaction. Upon irradiation with $\lambda > 455$ nm, the complex covalently binds to bpy, 9-ethylguanine (9-EtG), and linearized pUC18 plasmid. This covalent binding does not occur in the dark. The complex's ability to kill Hs-27 human skin cells upon irradiation and in the dark was investigated. In the dark, the complex has an LC₅₀ value of 410 ± 9 μ M, which is significantly less potent than when the complex is irradiated LC₅₀ = 12 ± 2 μ M (400–700 nm for 30 min). The PI of 34 is a large improvement over hematoporphyrin, with a PI measured to be 5.5 under similar experimental conditions. The dark LC₅₀ value of hematoporphyrin (21 ± 1 μ M) makes it ~20 times more toxic *cis*-[Rh₂(-O₂CCH₃)₂(CH₃CN)₄(OH₂)₂]²⁺ in the dark. No photocytotoxicity is observed with *cis*-[Rh₂(μ -O₂CCH₃)₂(phen)₂(OH₂)₂]²⁺ as the phen ligands are not photolabile. Additionally, *cis*-[Rh₂(μ -O₂CCH₃)₂(CH₃CN)₄(OH₂)₂]²⁺ inhibits transcription by the T7-RNA polymerase enzyme with a C_{inh} 50 value of 2.6 μ M, showing that the complex is more effective than cisplatin under similar conditions (4.1 μ M) [92].

Ru(II)-polypyridyl complexes were designed that are inert in the dark and covalently bind to DNA in a manner similar to cisplatin only under visible light irradiation, whereby combining cisplatin's activity toward biological targets with the spatial and temporal selectivity of PDT. Photoaquation of *cis*-[Ru(bpy)₂(NH₃)₂]²⁺ upon visible light irradiation produces *cis*-[Ru(bpy)₂(OH₂)₂]²⁺ in acidic solution and *cis*-[Ru(bpy)₂(OH₂)(OH)₂]⁺ in aqueous solution at neutral pH ($\Phi = 0.024$ with $\lambda = 350$ nm; $\Phi = 0.018$ with $\lambda = 400$ nm). The photoinduced ligand exchange is believed to proceed via thermal population of the low-lying ³MC excited state that promotes monodentate ligand dissociation [93]. This photoactive cisplatin analog binds covalently to oligonucleotides via substitution of the thermally labile Ru OH₂ bonds. The electronic absorption spectrum of *cis*-[Ru(bpy)₂(NH₃)₂]²⁺ in H₂O exhibits Ru(d π) $\rightarrow$ bpy(π^*) ¹MLCT transitions at 345 nm (7340 M⁻¹ cm⁻¹) and 490 nm (8210 M⁻¹ cm⁻¹), and the complex is weakly emissive in EtOH/MeOH glass at 157 K ($\lambda_{em} = 741$ nm, $\Phi = 0.002$, $\tau = 52$ ns) and non-emissive in H₂O at RT. Sequential dissociation of the two NH₃ ligands is observed as evidenced by the biphasic changes to the electronic absorption spectrum as a function of irradiation time as the complex is photolyzed in H₂O under an argon atmosphere. The photoaquation product undergoes thermal reactions with 9-methylguanine (9-MeG) and 9-EtG to produce the corresponding [Ru(bpy)₂(9-MeG)]²⁺ and [Ru(bpy)₂(9-EtG)]²⁺ adducts. The bis-aqua product also covalently binds to a single-stranded 15-mer, and enhanced binding is observed with a strand containing a GpG step, one of the known preferential binding sites for cisplatin. Intrastrand covalent binding was demonstrated by the decrease in the melting temperature upon irradiation, $T_m = -5$ °C, of the double stranded 15-mer in the presence of the complex, as interstrand crosslinks destabilize the duplex and decrease the DNA melting temperature. For comparison, cisplatin causes an 8 °C decrease in the melting temperature of a double stranded 20-mer. Moreover, the decreased electrophoretic mobility of linearized pUC18 plasmid in agarose gels upon irradiation in the presence of the complex ($\lambda > 345$ nm, 15 min) is also consistent with covalent binding. The photoactive *cis*-[Ru(bpy)₂(NH₃)₂]²⁺ represents an improvement over the thermally activated analogs *cis*-[Ru(bpy)₂(OH₂)₂]²⁺ and

cis-[Ru(phen)₂Cl₂] species, since activation with light provides spatial and temporal control of reactivity [94,95].

A related series of photoactive [Ru(bpy)₂(LL)]²⁺ complexes with a variety of bidentate ligands, LL, featuring either N- or S-coordination to the metal were investigated to gain further understanding of the dominant factors associated with light driven ligand exchange from Ru(II) polypyridyl complexes [96]. The complexes [Ru(bpy)₂(LL)]²⁺, where LL = bete = 3,6-dithiaoctane, bpte = 1,2-bis(phenylthio)ethane, en = ethylenediamine, and dae = 1,2-dianilinoethane (Fig. 9), exhibit visible light absorption dictated by the nature of the ligand LL. In the S-coordinated complexes with LL = bete and bpte, the lowest energy ¹MLCT absorption maxima are observed at 422 and 404 nm, respectively, while these transitions in the N-containing complexes, LL = en and dae, are red-shifted to 485 and 469 nm, respectively. Analysis of the photoaquation efficiency ($\lambda_{\text{irr}} = 400$ nm) for the N-bound complexes was precluded by an overlap in spectra of the reactant and product, but [Ru(bpy)₂(bete)]²⁺ and [Ru(bpy)₂(bpte)]²⁺ undergo photoaquation with $\Phi_{\text{aq}} = 0.024(2)$ and 0.022(3), respectively. The complexes were photolyzed in CH₂Cl₂ with excess n-tetrabutylammonium chloride (TBACl) to compare the ligand substitution efficiencies for the formation of [Ru(bpy)₂Cl₂]. The N-containing ligands undergo photosubstitution almost an order of magnitude lower than the corresponding S-coordinated ligands, with $\Phi = 0.019(1)$, 0.016(3), 0.002(1), and 0.003(1) for LL = bete, bpte, en, and dae, respectively. The Ru-L bond elongation in the ³MLCT state was calculated to be greater with bete and bpte than with en and dae, a factor that likely plays a role in the enhanced ligand dissociation in the former. A thermal dark reaction between GMP and the photogenerated [Ru(bpy)₂(OH₂)₂]²⁺ complex from the bete complex produces a (bpy)₂Ru^{II}-GMP adduct. The complexes with phenyl-containing ligands (bpte and dae) covalently bind to linearized pUC18 upon irradiation ($\lambda > 395$ nm, 4 min) more efficiently than those containing bete and en, while the S-bound species are better DNA binders than the N-bound complexes, consistent with the trends in photoaquation yields.

The photoaquation of Ru(II)-polypyridyl complexes is enhanced through the distortion of the octahedral geometry with the introduction of sterically bulky bidentate ligands in the coordination sphere, which promotes more efficient 3MC state population. The two complexes [Ru(bpy)₂(Me₂bpy)]²⁺ and [Ru(bpy)₂(Me₂dpq)]²⁺ (Me₂bpy = 6,6'-dimethyl-2,2'-bipyridine; Me₂dpq = 7,10-dimethylpyrazino[2,3-f][1,10]phenanthroline; Fig. 9) were compared to [Ru(bpy)₃]²⁺, which lacks the sterically bulky ligand and binds to DNA through electrostatic interactions with low affinity [80]. Photoaquation of both complexes to produce *cis*-[Ru(bpy)₂(OH₂)₂]²⁺ occurs when irradiated with $\lambda > 450$ nm. This ligand dissociation, causing a red shift in the MLCT maximum from 450 to 480 nm, proceeds rapidly for the Me₂bpy complex with $t_{1/2} = 2$ min, which is enhanced by a factor of 30 compared to the analogous ligand exchange in the Me₂dpq complex. It is suggested that the rigidity of the planar dpq ligand facilitates its re-coordination after one Ru-N bond is cleaved, as a stepwise dissociative bond breaking process leads to ligand exchange. [Ru(bpy)₂(Me₂bpy)]²⁺ covalently binds to pUC19 upon irradiation, and the Me₂dpq complex both covalently binds and photocleaves the plasmid (white light, 200 W, 1 h). The

reactivity with DNA is unaffected by addition of glutathione (GSH), although GSH coordinates to and inhibits the activity of Pt drugs.

The cytotoxicity of $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{bpy})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{dpq})]^{2+}$ under dark and irradiated conditions was investigated with HL-60 leukemia cells and A549 lung cancer cells. The complexes are more potent upon irradiation than cisplatin and are virtually nontoxic in the dark, exhibiting PI values of 100–200. They also cause complete cell death when irradiated, a common difficulty for PDT agents. The dark IC_{50} values for $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{bpy})]^{2+}$ are $>300 \mu\text{M}$ (HL60) and $150(7) \mu\text{M}$ (A549) and those for $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{dpq})]^{2+}$ are $108(2) \mu\text{M}$ (HL60) and $250(5) \mu\text{M}$ (A549). Upon irradiation with $\lambda > 450 \text{ nm}$ (410 W, 3 min), the IC_{50} values decreased significantly for both complexes. Values of $1.6(2) \mu\text{M}$ (HL60) and $1.1(3) \mu\text{M}$ (A549) were reported for $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{bpy})]^{2+}$ and $2.6 \pm 1.0 \mu\text{M}$ (HL60) and $1.2 \pm 0.1 \mu\text{M}$ (A549) for $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{dpq})]^{2+}$. A549 3D tumor spheroids provide a more accurate model to approximate the complexity of in vivo tumors [97]. The complexes exhibit even lower dark toxicity with A549 spheroids, with LC_{50} values $>300 \mu\text{M}$ for both complexes. The irradiated IC_{50} values, 21.3 ± 2.3 and $64.6 \pm 4.7 \mu\text{M}$ for $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{dpq})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{bpy})]^{2+}$, respectively, are an order of magnitude larger than the monolayer models yet still effective. The multicellular resistance ratio (MCR) is a ratio of spheroid to monolayer IC_{50} values. It should also be noted that the complexes have MCR values of 19.4 and 54; the value for $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{bpy})]^{2+}$ is similar to the MCR value of 12.4 for cisplatin, which is lower than most chemotherapy drugs.

Following the principles of the methyl-substituted bpy and dpq ligands to distort the octahedral geometry in order to promote ligand dissociation, complexes featuring the sterically demanding ligand 2,2'-biquinoline (biq), $[\text{Ru}(\text{phen})_2(\text{biq})]^{2+}$ and $[\text{Ru}(\text{phen})(\text{biq})_2]^{2+}$ (Fig. 10), were investigated [98]. Employing the biq ligand in octahedral Ru(II)-polypyridyl complexes also serves to red-shift the visible light absorption into the therapeutic window because biq has lower-lying acceptor orbitals. The lowest energy absorption of $[\text{Ru}(\text{phen})_2(\text{biq})]^{2+}$ is centered at 525 nm with appreciable absorption extending to 700 nm. The absorption is even further red shifted for $[\text{Ru}(\text{phen})_2(\text{biq})]^{2+}$, with a lowest energy absorption centered at 550 nm and a tail extending to 800 nm. One biq ligand is released upon photolysis in H_2O in each complex, resulting in the formation of the corresponding bis-aqua species. The irradiation time required to generate the product is an order of magnitude longer for $[\text{Ru}(\text{phen})_2(\text{biq})]^{2+}$ than for $[\text{Ru}(\text{phen})(\text{biq})_2]^{2+}$ as a result of enhanced octahedral distortion from an additional bulky biq ligand in the latter. The complexes do not induce photocleavage of pUC19 plasmid, but the bis-aqua photolysis products covalently bind to DNA, reducing the mobility on agarose gel in a manner similar to cisplatin. The complexes are proposed to bind to the duplex via intrastrand crosslinking. No binding occurs in the dark, and the mobility is similar to the synthesized *cis*- $[\text{Ru}(\text{phen})_2(\text{OH}_2)_2]^{2+}$. An irradiation wavelength dependence shows the greatest activity with blue light, yet irradiation with red wavelengths still induces significant covalent binding to DNA. These complexes are also potent toward HL-60 human leukemia cells, with IC_{50} values of 1–2 μM under blue light for 3 min, similar to the toxicity of cisplatin with $\text{IC}_{50} = 3.1 \mu\text{M}$. The potency decreases under red light but longer irradiation times up to 6

min provides IC₅₀ values of 2.3–7.6 μM. The PI values under blue irradiation are 43.8 and 19.7 for [Ru(phen)₂(biq)]²⁺ and [Ru(phen)(biq)₂]²⁺, respectively.

A common disadvantage to using [Ru(bpy)₃]²⁺-type complexes for PDT applications is the need for high energy visible light excitation as these species typically do not absorb appreciably in the therapeutic window. To shift the visible light absorption of photoactive Ru(II)-polypyridyl cisplatin analogs into the PDT window, the deprotonated phenylpyridine (phpy⁻) ligand was incorporated to destabilize the Ru(dπ) orbitals in *cis*-[Ru(phpy)(phen)(CH₃CN)₂]⁺ (Fig. 11) [99]. Additionally, CH₃CN was employed as the photolabile ligand, as the photoaquation of nitriles is generally much more efficient than that observed for amines or pyridine [93,100]. The lowest energy electronic absorption peaks of the complex are centered at 461 nm (ε = 11,559 M⁻¹ cm⁻¹) and 486 nm (ε = 10,842 M⁻¹ cm⁻¹), corresponding to Ru(dπ) → phen(π*)¹MLCT. More importantly, the complex has an appreciable absorption tail extending beyond 600 nm into the therapeutic window. Upon irradiation with λ > 420 nm for 20 min, *cis*-[Ru(phpy)(phen)(CH₃CN)₂]⁺ covalently binds to linearized plasmid DNA, evidenced by slower migration in the gel mobility assay. The complex inhibits tumor growth in mice as well as cytotoxicity with light exposure, however detailed photo-physical or photochemical studies were not reported [101,102]. The complex exhibits dark and red-light induced cytotoxicity toward OVCAR-5 cells (human advanced ovarian epithelial cancer cell line), with a dark LC₅₀ value of 1.0 μM. This represents a fairly high dark toxicity and is attributed to the enhanced thermal ligand exchange in the presence of GSH, a molecule that is found at relatively high intracellular concentrations. The irradiated complex (λ = 690 nm, 100 s), however, is quite potent with an LC₅₀ value of 70 nM, representing a 14-fold increase in toxicity upon red light exposure well into the PDT window.

A series of photoactive piano-stool type complex, [(*p*-cym)Ru(NN)(L)]²⁺ (*p*-cym = *para*-cymene; NN = bpm = 2,2'-bipyrimidine or phen; L = py = pyridine or 4,4'-bpy = 4,4'-bipyridine; Fig. 12) [103,104] was designed to improve upon the previously reported [(*p*-cym)Ru(bpm)(X)]ⁿ⁺ complex (X = halide). The halide undergoes ligand exchange by a thermal process, which results in cytotoxicity toward several cancer cell lines, although it lacks spatial and temporal selectivity. The [(*p*-cym)Ru(NN)(L)]²⁺ series represent the first reported examples of Ru(II) arene complexes that selectively release a monodentate ligand and covalently bind to DNA upon irradiation with visible light. The electronic absorption spectrum of [(*p*-cym)Ru(bpm)(py)]²⁺ in H₂O features maxima at 254 and 383 nm, and these transitions red-shift upon photolysis, consistent with dissociation of py to form [(*p*-cym)Ru(bpm)(OH₂)]²⁺ in aqueous media. Similar shifts were observed for [(*p*-cym)Ru(phen)(py)]²⁺ and [(*p*-cym)Ru(bpm)(4,4'-bpy)]²⁺, for which the low energy absorption tail is composed of a mixture of transitions to ¹MC and ¹MLCT excited states. The lowest energy excited state has significant ³MC character, consistent with the lack of emission in solution and the observed photodissociation of the py ligand. Irradiation of [(*p*-cym)Ru(bpm)(py)]²⁺ in the presence of 9-EtG in aqueous solution forms [(*p*-cym)Ru(bpm)(9-EtG)]²⁺. This trend is consistent for the other two complexes of this motif, however, 9-ethyladenine (9-EtA) does not bind to the photolyzed complex, highlighting the selectivity toward guanine. While these complexes do not exhibit major differences in their

photochemistry, the reactivity with DNA differs upon structural changes. $[(p\text{-cym})\text{Ru}(\text{bpm})(4,4'\text{-bpy})]^{2+}$ undergoes 20% DNA binding in the dark after 48 h, potentially via intercalation of pendant 4,4'-bpy ligand, whereas <5% of $[(p\text{-cym})\text{Ru}(\text{bpm})(\text{py})]^{2+}$ and $[(p\text{-cym})\text{Ru}(\text{phen})(\text{py})]^{2+}$ bind to DNA over the same period of time. The binding is more rapid when the complex is irradiated prior to the addition of DNA as compared to the irradiation of solutions that are composed of the complex and DNA, but the overall degree of binding is not impacted. The DNA binding of the photoproduct is slow compared to that of cisplatin; this is an attractive feature for PDT drugs because low dark reactivity with biomolecules is desired to prevent unwanted side reactions. Upon irradiation in the presence of SC DNA, the complexes cause 6–7° unwinding, similar to that observed with monofunctional cisplatin adducts [105]. Additionally, the complexes inhibit DNA and RNA transcription upon irradiation, and mapping experiments determined that the stop sites are always guanine bases. Despite the lack of ligand dissociation in the dark, the complexes exhibit dark cytotoxicity toward the A2780 human ovarian cancer cell line, however, the mechanism remains unknown.

Two new complexes inspired by the photoreactive piano-stool Ru(II)-arene complexes were designed to incorporate receptor binding peptides for tumor-targeted drug delivery. The peptides are incorporated through the py ligand in $[(p\text{-cym})\text{Ru}(\text{bpm})(\text{py})]^{2+}$. The two selected peptides are the dicarba analog of octreotide, a potent endocrine hormone somatostatin agonist containing eight peptides, and the Arg-Gly-Asp (RGD) sequence, a moiety that selectively targets tumor endothelial cells over healthy cells. The receptors for each of the peptides are overexpressed in various tumors and have been employed in cytotoxic drug delivery [106]. The complexes do not undergo ligand substitution in the dark, but irradiation with blue light (420 nm) at 37 °C results in the exchange of py-substituted peptide with a solvent H₂O molecule, evident by the observation of the free substituted peptide and the aqua product $[(p\text{-cym})\text{Ru}(\text{bpm})(\text{OH}_2)]^{2+}$ by ¹H NMR and electronic absorption spectroscopy for each complex. Both complexes form adducts with 9-EtG when irradiated in H₂O in a manner similar to the parent $[(p\text{-cym})\text{Ru}(\text{bpm})(\text{py})]^{2+}$. When irradiated for 8 h followed by the addition of the single-stranded oligonucleotide 5' dCATGGCT-3' and overnight incubation, the octreotide complex covalently binds to either of the guanine bases. When irradiated in the presence of the oligonucleotide for 9 h, the two monofunctional adducts were observed in addition to a bifunctional adduct formed by dissociation of *p*-cym to form a more stable GG chelate. When a similar experiment was performed with an oligonucleotide containing two non-adjacent G bases, 5' -dAGCCATG, the two monofunctional adducts and a cyclic adduct by GG chelation following arene dissociation were formed. A peptide-oligonucleotide hybrid, Phac-His-Gly-Met-linker-p⁵ -dCATGGCT, was employed to investigate the competition between DNA and protein binding. This peptide was chosen because histidine and methionine are the two residues most likely to form complexes with Ru. Irradiation for 9 h results in no Met or His binding, but monofunctional G adducts were observed, indicative of the selectivity for DNA. The *p*-cym ligand only dissociated to enable GG chelation after extensive irradiation.

A supramolecular architecture coupling one or two Ru(II)-polypyridyl chromophores to a Rh^{III}Cl₂ DNA binding site exhibit photophysical properties that strongly influence their

ability to covalent bind and cleave DNA in the presence of light without O₂. A series of Ru(II),Rh(III),Ru(II) trimetallic complexes, $[(bpy)_2Ru(dpp)]_2RhCl_2]^{5+}$, $[(bpy)_2Os(dpp)]_2RhCl_2]^{5+}$, $[(tpy)RuCl(dpp)]_2RhCl_2]^{3+}$, and $[(bpy)_2Ru(bpm)]_2RhCl_2]^{5+}$ (dpp = 2,3-bis(2-pyridyl)pyrazine; Fig. 13), were studied to understand the impact of component variation on the biological activity [107–111]. The HOMO in each complex is localized on the Ru(II) or Os(II) center. The LUMO is localized on the Rh center for the dpp-bridged complexes as the Rh(σ^*) orbitals are lower energy than the dpp(π^*) orbitals; however, in the bpm-bridged complex it is centered on one of the bpm ligands, since the bpm(π^*) orbitals are lower lying than the Rh(σ^*) orbitals. The electronic absorption spectra feature Ru/Os($d\pi$) $\rightarrow$ dpp/bpm(π^*) 1MLCT transitions centered at 530 nm for the dpp complexes and 594 nm for the bpm complex, as bpm(π^*) orbitals are stabilized compared to dpp. The Os(II) complex also absorbs appreciably in the therapeutic window due to the direct spin-forbidden transition, $^1GS \rightarrow ^3MLCT$, afforded by the spin-orbit coupling induced by the third-row transition metal. Upon irradiation with $\lambda > 475$ nm, the dpp-bridged complexes covalently bind to and photocleave pUC18 and pBluescript DNA in the absence of O₂. The excited state complex rapidly populates a Ru/Os($d\pi$) $\rightarrow$ dpp(π^*) 3MLCT excited state followed by relaxation to the GS via radiative or nonradiative decay or population of a low-lying Ru/Os($d\pi$) $\rightarrow$ Rh(σ^*) 3MMCT (metal-to-metal charge transfer state) via intramolecular electron transfer. In the 3MMCT state the electron density localizes on the Rh center, causing labilization of the Rh(II) Cl bond and providing an open site for coordination of DNA bases. The bpm-bridged complex does not exhibit covalent DNA binding as the Ru($d\pi$) $\rightarrow$ bpm(π^*) 3MLCT state is lower in energy than the 3MMCT state, thus hindering electron transfer to the Rh center. The $[(bpy)_2Ru(dpp)]_2RhCl_2]^{5+}$ and $[(bpy)_2Os(dpp)]_2RhCl_2]^{5+}$ complexes, when irradiated with $\lambda > 460$ nm for 4 min, inhibit growth of African green monkey kidney epithelial cells [108]. Interestingly, the Os complex inhibits cell replication while the Ru complex kills cells in the presence of light.

Bimetallic Ru(II),Rh(III) analogs to the previously discussed Ru(II),Rh(III),Ru(II) trimetallic complexes were designed in an effort to provide a more sterically accessible Rh center, better cell permeability, a lower overall charge, fewer stereoisomers, and a more easily tunable Rh(III) bioactive site [112–114]. The three complexes, $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$, $[(bpy)_2Ru(dpp)RhCl_2(phen)]^{3+}$, and $[(bpy)_2Ru(bpm)RhCl_2(phen)]^{3+}$ exhibit similar photophysical properties to the trimetallic systems. The Ru/Os($d\pi$) dpp/bpm(π^*) $\rightarrow$ 1MLCT absorption occurs at 521, 515, and 592 nm, respectively. $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ absorbs broadly in the NIR with a maximum at 750 nm due to direct population of the 3MLCT state. Similar to the trimetallic complexes, the LUMO is localized on Rh in the dpp-bridged complexes and on bpm in the bpm-bridged complex. Slower halide dissociation upon Rh reduction is observed in electrochemical analysis of these systems compared to the trimetallic complexes. All three complexes bind to and photocleave pUC18 when irradiated with 455 nm light, and the activity with DNA is less efficient for the bpm complex. This is attributed to its shorter excited state lifetime (<10 ns) compared to that of $[(bpy)_2Ru(dpp)RhCl_2(phen)]^{3+}$ (30 ns). Further analysis of $[(bpy)_2Ru(dpp)RhCl_2(phen)]^{3+}$ demonstrates that when irradiated in NaH₂PO₄ buffer with 455 nm light for 30 min, chloride dissociation occurs as observed by a subtle blue shift in the 1MLCT absorption. Mass spectrometry reveals that OH₂, OH[−], and

HPO₄[−] bind to the open coordination site on Rh [114]. The Os complex is remarkably able to covalently bind to and fully photocleave DNA in the absence of O₂ with $\lambda > 645$ nm for 4 h [112]. The complex also inhibits DNA replication in PCR (polymerase chain reaction) assays using a 670 base pair fragment of pUC18. At a base pair-to-metal complex ratio as low as 50:1, DNA replication is disrupted with red light irradiation for 1 h in the absence of O₂ [113]. The ability of these mixed-metal systems to undergo 3MMCT-mediated halide dissociation to covalently bind to DNA, with some systems efficient under irradiation in the therapeutic window, and cleave DNA and inhibit replication makes these systems promising PDT agents.

3.3. Photoactivated drug release

The thermally accessible 3MC excited states of Ru(II)-polypyridyl complexes to induce ligand dissociation is employed for light-activated drug delivery. Cancer therapy drugs and cysteine protease inhibitors featuring nitrile substituents that form coordinate bonds with a Ru(II) chromophore and are photolabile upon visible light excitation are discussed herein.

The chemotherapy drug 5-fluorouracil (5FU) has been utilized for over 20 years and continues to be widely used in the treatment of malignancies, such as colorectal and breast cancers [115]. The related molecule 5-cyanouracil (5CNU) inhibits pyrimidine catabolism in vivo [116], and the nitrile functional group enables it to coordinate to a Ru(II) photoactive unit. Two Ru(II) complexes, *cis*-[Ru(bpy)₂(5CNU)₂]²⁺ and [Ru(tpy)(5CNU)₃]²⁺ (Fig. 12), are able to photochemically deliver both the 5CNU drug and the Ru(II)–OH₂ compounds that covalently bind to DNA with spatial and temporal control, making them potential dual activity chemotherapy agents [117,118]. The irradiation of *cis*-[Ru(bpy)₂(5CNU)₂]²⁺ with > 395 nm forms *cis*-[Ru(bpy)₂(OH₂)₂]²⁺ ($\Phi_{aq} = 0.16(4)$, 400 nm, for the first 5CNU exchange), evidenced by the red shift in the electronic absorption maximum from 410 nm to 450 nm at short irradiation times associated with the formation of the *cis*-[Ru(bpy)(5CNU)(OH₂)]²⁺ intermediate [117]. This step is followed by a shift from 450 nm to 490 nm with longer irradiation times, corresponding to formation of the bis-aqua product. The latter covalently binds to linearized pUC18 plasmid DNA with $\lambda > 395$ nm irradiation for 15 min, and no covalent binding occurs when the complex is incubated in the dark under similar experimental conditions; moreover the mono-aqua complex does not bind to DNA. *Cis*-[Ru(bpy)₂(5CNU)₂]²⁺ can deliver two equivalents of 5CNU and one equivalent of a Ru^{II}(bpy)₂-binding agent to provide biological activity that operates via two different mechanism, akin to the mixtures of drugs typically used for cancer treatment, commonly referred to as “drug cocktails.”

The tpy analog [Ru(tpy)(5CNU)₃]²⁺ was utilized to extend visible light absorption toward the therapeutic window relative to that of the *cis*-[Ru(bpy)₂(5CNU)₂]²⁺ complex II [118]. Additionally, Ru (tpy) complexes bind 9-EtG [119] and [Ru(tpy)Cl₃] is more cytotoxic than [Ru(bpy)₂Cl₂] [120]. The electronic absorption spectrum of [Ru(tpy)(5CNU)₃]²⁺ in H₂O exhibits an MLCT absorption centered at 420 nm and a broad, low energy tail that extends to almost 600 nm, compared to just beyond 500 nm for *cis*-[Ru(bpy)₂(5CNU)₂]²⁺. Photolysis with $\lambda > 395$ nm in H₂O results in a red shift of the absorption maximum from 420 to 450 nm, consistent with the exchange of one axial 5CNU ligand with a solvent H₂O

molecule. The substitution of the second axial 5CNU, evidenced by a further red shift of the absorption band to 475 nm, occurs with significantly longer irradiation times. The overall Φ from reactant to product was measured to be 0.022(2) ($\lambda_{\text{irr}} = 400$ nm), a process that results in covalent binding to linearized pUC19 plasmid ($\lambda > 395$ nm). The complex is not cytotoxic toward HeLa cells when incubated in the dark for 2 h, but irradiation for 1 h with $\lambda > 400$ nm kills HeLa cells with $\text{LC}_{50} = 156 \pm 18 \mu\text{M}$. This value is similar to that measured for the free 5CNU ligand, 151 ± 33 , suggesting only one 5CNU is released per complex under the photolysis conditions used for the cytotoxicity experiments. A control experiment with $[\text{Ru}(\text{tpy})(\text{CH}_3\text{CN})_3]^{2+}$, which releases axial CH_3CN ligands upon irradiation, does not exhibit photocytotoxicity. Since the mono-aqua species is expected to form under the same conditions of the cell studies, the lack of cytotoxicity is consistent because $[\text{Ru}(\text{tpy})(\text{CH}_3\text{CN})_2(\text{OH}_2)]^{2+}$ does not covalently bind to DNA. This result provides strong evidence that the 5CNU ligand is solely responsible for the photocytotoxicity as only the mono-aqua Ru complex is formed.

The $\text{Ru}^{\text{II}}(\text{bpy})_2$ moiety was also employed for light-activated delivery of a cysteine protease inhibitor [121]. Cysteine proteases play a contributing role in tumor growth, migration, angiogenesis, and metastasis, and they are overexpressed in many cancers, positioning them as important targets in cancer therapy [122]. Cysteine proteases are also critical for normal cell functioning, so controlled inhibition of these proteases is crucial for minimizing undesirable systemic toxicity. The coordination to a $\text{Ru}^{\text{II}}(\text{bpy})_2$ fragment by a peptidomimetic nitrile-based inhibitor serves to inactivate the inhibitor until it is released by visible light. This strategy provides high selectivity for cysteine protease inhibition, as the nitrile group is guarded from attack by a cysteine residue in the active site of the enzyme when it is coordinated to the $\text{Ru}^{\text{II}}(\text{bpy})_2$ fragment. *Cis*- $[\text{Ru}(\text{bpy})_2(\text{NC-peptide})_2]^{2+}$, depicted in Fig. 14, absorbs strongly in the visible region with a maximum at 414 nm in H_2O . The complex is stable in the dark but upon irradiation ($\lambda > 395$ nm) the MLCT band red shifts to 444 nm due to substitution of one NC-peptide to form the mono-aqua intermediate with $\Phi = 0.080(4)$. Further photolysis causes the substitution of the second NC-peptide to produce the bis-aqua $[\text{Ru}(\text{bpy})_2(\text{OH}_2)_2]^{2+}$ product with $\Phi = 0.011(1)$. An investigation into the in vitro activity of the complex with the cysteine protease papain shows that under irradiation ($\lambda > 395$ nm, 10 min) the concentration required to inhibit 50% of the enzyme activity, IC_{50} , has a value of 295 nM, which suggests that approximately two RCN ligands are released, since it is approximately half the IC_{50} value for the free RCN ligand, 638 nM. Cysteine protease inhibition is enhanced 32-fold when irradiated, based on a dark IC_{50} of 9.5 μM . This low level of dark toxicity may stem from a small amount of RCN dissociating under the reaction conditions or the complex may undergo nonbonding interactions with the enzyme that cause minor inhibition. The IC_{50} for the analogous $[\text{Ru}(\text{bpy})_2(\text{CH}_3\text{CN})_2]^{2+}$ complex is $>500 \mu\text{M}$ in the dark or irradiated, providing strong evidence that the released RCN ligand, and not the metal complex, is in fact the enzyme inhibitor. The complex is less potent for cathepsins B, K, and L, yet significant enhancement of inhibition is observed upon irradiation compared to the dark incubation.

4. Conclusions

PDT provides avenues for improving upon currently employed chemotherapy drugs by producing potent drugs with spatial and temporal selectivity determined by visible light irradiation. Ru(II) and Rh₂(II,II) complexes are attractive candidates for successful PDT agents and are under investigation due to the ability to tune the excited states toward more efficient low-energy visible light absorption, relatively long lifetimes, and control the type of reactivity to be achieved. Complexes with Ru^{II}(NN)₂ and Ru^{II}(NNN) fragments coordinated to planar ligands localize on DNA by intercalation between base pairs, and can produce ¹O₂ with quantum efficiency. The ¹O₂ producing Rh₂(II,II) motif, *cis*-[Rh₂(μ-O₂CCH₃)₂(dppn)(NN)]²⁺, exhibits a decreased dark toxicity and an increased phototoxicity toward Hs-27 fibroblasts compared to hematoporphyrin. Related complexes with intercalating ligands damage DNA via excited state electron transfer to oxidize gua-nine, and from this series, [Ru(bpy)₂(5-pyr-phen)]²⁺ exhibits an unprecedented PI of 1747 against human leukemia HL-60 cells and is quite active against Malme-3M aggressive melanoma cells that are untreatable by ROS. In addition to ¹O₂ production and excited state redox reactions, photoactive Ru and Rh complexes act as cisplatin analogs that covalently bind to DNA only under irradiation, such that they may overcome undesired effects on normal cells. The population of ³MC states facilitates ligand substitution to release monodentate or bidentate ligands from a Ru^{II}(NN)₂ fragment to form the cisplatin analog, [Ru(NN)₂(OH₂)₂]²⁺, which covalently binds to DNA. Members of this class of complexes are active against lung cancer and leukemia cell lines. Analogous complexes with a phpy⁻ ligand replacing one diimine NN ligand effectively red shifts the absorption into the therapeutic window and is active against an ovarian cancer cell line. A series of Ru(II) piano-stool complexes undergoes photoaquation from release of a pyridine-type ligand and subsequently bind to DNA covalently; appending the photoreleased pyridine with oligonucleotides and peptides affords a method for targeting tumors. Bimetallic and trimetallic complexes featuring one or two Ru(II) chromophores bridged to a Rh^{III}Cl₂ bioactive site covalently bind to DNA due to Cl⁻ dissociation resulting from population of an MMCT excited state, and DNA replication inhibition was observed under red light irradiation. Ligand substitution via ³MC state population is also exploited to release nitrile-containing drugs such as 5CNU and cysteine pro-tease inhibitors, while also producing Ru(II)-OH₂ complexes that covalently bind to DNA. In developing new Ru(II) and Rh₂(II,II) complexes to overcome the current challenges of PDT, complexes that require short irradiation times at long wavelengths are ideal. The need for low energy visible light absorption necessitates lowering the activation barrier for thermal population of the ³MC state from the low-lying ³MLCT state for complexes that undergo ligand dissociation for covalent binding or drug delivery. The ability to alter the excited states and biological activity of Ru(II) and Rh₂(II,II) complexes makes these systems versatile as they can function by more than one mechanism to enhance their effect on tumor cells while minimizing the impact on healthy cells. Additionally, exploration of the in vitro and in vivo activity of these complexes will be critical to their successes as PDT drugs. Collaborative, interdisciplinary efforts are essential in realizing the potential of PDT drug design to generate more effective and non-invasive cancer therapy.

Acknowledgments

The authors gratefully acknowledge support from the National Science Foundation (CHE-1213646) and the National Institutes of Health (RO1 EB016072).

Abbreviations

5CNU	5-cyanouracil
9-EtG	9-ethylguanine
9-MeG	9-methylguanine
bete	3,6-dithiaoctane
biq	2,2' -biquinoline
bpm	2,2' -bipyrimidine
bpte	1,2-bis(phenylthio)ethane
bpy	2,2' -bipyridine
CS	charge separated
dae	1,2-dianilinoethane
DAP	1,12-diazaperylene
dpp	2,3-bis(2-pyridyl)pyrazine
dppn	benzo[i]dipyrido[3,2- <i>a</i> :2',3' -h]quinoxaline
dppz	dipyrido[3,2- <i>a</i> :2',3' -c]phenazine
dpq	dipyrido[3,2- <i>f</i> :2',3' -h]quinoxaline
dpqp	pyrazino[2',3':5,6]pyrazino[2,3- <i>f</i>][1,10]phenanthroline
EC₅₀	half maximal effective concentration
en	ethylenediamine
EtBr	ethidium bromide
GMP	guanosine monophosphate
hat	1,4,5,8,9,12-hexaazatriphenylene
IL	intraligand
LC₅₀	median lethal concentration
MC	metal centered
MCR	multicellular resistance
Me₂bpy	6,6' -dimethyl-2,2' -bipyridine
Me₂dpq	7,10-dimethylpyrazino[2,3- <i>f</i>][1,10]phenanthroline
MLCT	metal-to-ligand charge transfer

MMCT	metal-to-metal charge transfer
OC	open circular
PDT	photodynamic therapy
phen	1,10-phenanthroline
phpy⁻	deprotonated phenylpyridine
PI	phototoxicity index
PS	photosensitizer
PSS	polystyrene sulfonate
pydppz	3-(pyrid-2'-yl)dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
pydppn	3-(pyrid-2'-yl)-4,5,9,16-tetraaza-dibenzo[<i>a,c</i>]naphthacene
pydppx	3-(pyrid-2'-yl)-11,12-dimethyldipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
pyr	pyrene
ROS	reactive oxygen species
SC	supercoiled
tap	1,4,5,8-tetraazaphenanthrene
^tBu₂bpy	4,4'-di-tert-butyl-2,2'-bipyridine
tpy	2,2':2',6'-terpyridine
Φ	quantum yield
τ	lifetime

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Fig. 1. Schematic representation of (a) the components of the B-DNA double helix and (b) guanine-cytosine and adenine-thymine base pairs formed by hydrogen bonding.

Fig. 2. Jablonski diagram representing the general scheme for PDT highlighting Type I, II, and III mechanisms.

Fig. 3. Structural representations of (a) the octahedral geometry of a tris-bidentate Ru(II) complex and (b) the bidentate NN ligands involved in the study of DNA photocleavage through $^1\text{O}_2$ production.

Fig. 4. Structural representations of (a) the octahedral geometry of a bis-tridentate Ru(II) complex and (b) the tridentate NNN ligands involved in the study of DNA photocleavage through $^1\text{O}_2$ production.

Fig. 5.
A series of Rh₂(II,II)-dppn complexes that photocleave DNA by a predominantly O₂-dependent mechanism.

Fig. 6. Structural representations of (a) the octahedral geometry of the Ru(II) complexes and (b) the ligands utilized in complexes that undergo photoinduced redox reactions with DNA.

Fig. 7.

$\text{Rh}_2(\text{II,II})$ -polypyridyl complexes that photocleave DNA in the absence of O_2 .

Fig. 8.
 $\text{Rh}_2(\text{II},\text{II})$ paddlewheel complexes that undergo photoinduced covalent binding with DNA.

Fig. 9.

Ru(II)-polypyridyl complexes that undergo photoinduced ligand release from a Ru^{II}(bpy)₂ fragment that covalently binds to DNA.

Fig. 10.

Ru(II)-polypyridyl complexes employing biq that covalently bind to DNA via photoinduced ligand release.

Fig. 11.

Ru(II)-polypyridyl complexes utilizing phpy^- that covalently bind to DNA via photoinduced ligand release.

Fig. 12.

Ru(II)-arene complexes that covalently bind DNA in the absence of O₂.

Fig. 13.

Ru(II),Rh(III) supramolecular complexes that undergo photoinduced covalent binding to DNA in the absence of O_2 .

Fig. 14.

Ru(II) complexes that deliver nitrile-containing drugs upon irradiation.