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Light-activated ruthenium complexes photobind DNA and are cytotoxic in the photodynamic therapy window†

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Incorporation of biquinoline ligands into Ru(II) polypyridyl complexes produces light-activated systems that eject a ligand and photobind DNA after irradiation with visible and near-IR light. Structural analysis shows that distortion facilitates the photochemistry, and gel shift and cytotoxicity studies prove the compounds act as anti-cancer photodynamic therapy (PDT) agents in the tissue penetrant region.

Photodynamic therapy (PDT) is an approved cancer treatment that utilizes visible light as the trigger for the generation of singlet oxygen by organic sensitizers.[1] However, PDT efficacy is limited due to both this oxygen dependent mechanism and the poor chemical characteristics of the currently available photosensitizers. The reliance on oxygen precludes activity in hypoxic tumors, and the porphyrin-type organic photosensitizers used in PDT suffer from photobleaching, poor solubility, and retention in tissues, causing protracted photosensitivity. In light of these drawbacks, several groups are investigating photoactive metal complexes as alternative PDT agents.[2] Ruthenium polypyridyl complexes have tunable absorption properties,[3] and are known to induce $^1O_2$-mediated DNA photocleavage when exposed to UV or visible light.[4] Some ruthenium agents have been reported to act *via* O_2-independent mechanisms, allowing for activity in hypoxic tissues.[5] However, organic PDT sensitizers have retained one key advantage: the ability to be activated in the "therapeutic window" for PDT, using red and near IR light from 600–1100 nm. Very recently, metal complexes were reported that were able to damage DNA when activated with low energy light.[6] Here we report the next step towards functional PDT agents: simple Ru(II) polypyridyl complexes that can be activated in the therapeutic window and demonstrate both photo-activated DNA binding and potent cytotoxicity in cancer cells.

The photoactivity of the Ru(II) complexes is regulated by a single key feature: the induction of distortion into the octahedral geometry around the metal. Distortion is known to lower the energy of a dissociative ^{3}MC (metal centred) state. This allows for its thermal population following photoexcitation to the ^{3}MLCT (metal to ligand charge transfer) state, causing ejection of a ligand.[7] The structure of the Ru(II) complex is conveniently deformed by the incorporation of ligands that clash with one another when assembled around the metal centre such as the 2,2′-biquinoline ligand. Thus, it was anticipated that Ru(II) biq complexes would combine light-activated photochemical reactivity[8] with a sufficiently red-shifted absorption to allow for activity in the PDT therapeutic window.

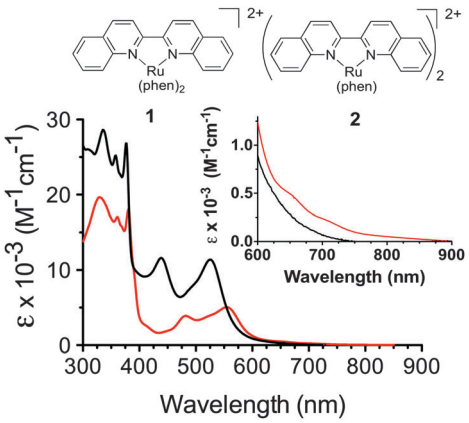

Fig. 1 Structures of complexes and UV/Vis absorption spectra of **1** (black) and **2** (red). Inset shows the absorption in the PDT window.

Two complexes were synthesized containing one and two biq ligands, and one or two smaller 1,10-phenanthroline (phen) ligands, as shown in Fig. 1. The addition of the biq ligands resulted in bathochromic shifts in the absorption spectra, as compared to $Ru(phen)_3$, a prototypical Ru(II) polypyridyl complex with λ_{max} = 450 nm for the low energy MLCT absorption. Addition of one biq ligand in **1** shifts the MLCT to λ_{max} = 525 nm, and incorporation of two biq ligands shifts the λ_{max} to 550 nm for **2**. There is some absorption at 700 nm for **1**, while **2** is able to absorb light up to 800 nm.[9]

Crystal structures for the biq complexes show the extent of distortion in the ground state structures. The deformation of the octahedral geometry is manifest in lengthening of the bonds between the metal and the ligand as well as twisting of the ligands (see Fig. 2 and Table 1). In comparison with the undistorted $Ru(phen)_3$, with average Ru–N bond lengths of 2.064 Å,[10] **2** is slightly distorted with the Ru–N bonds lengthened to 2.08–2.10 Å. In compound **1** (see Fig. S8, ESI†) two of

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ligands' Ru–N bonds are lengthened to 2.09 and 2.11 Å.[8b] The biq is twisted about the C–C bond between the two quinoline systems; a 5° twist is observed in **1**, while the two biq ligands show different distortions in compound **2**, with a 12.1(4)° twist in biq(2), and 2.7(4)° in biq(1). The biq ligands are bent by ~20° out of the normal plane in both complexes. The biq ligands maximize the space around them, which induces the distortion in **1** and **2**.

The complexes all undergo photo-substitution reactions upon exposure to visible light. The kinetics of the ejection process were followed by absorption spectroscopy, as shown in Fig. 3, Fig. S2 and S3 (ESI†). The number of sterically hindered ligands and the corresponding distortion in the complex affects the rate of the photosubstitution reaction, with an order of magnitude variation in $t_{1/2}$ for the different structures. The wavelength dependence of the photochemical process also correlated with the absorption spectra, with rapid reaction observed with blue and green light, while slower ejection was observed with red and near-IR light (see Table S1, ESI†). The presence of isosbestic points in the UV/Vis spectra indicated the direct conversion from starting material to a single product. Samples of **1** and **2** after light activation were subjected to analytical HPLC as shown in Fig. S4 (ESI†). The tris-polypyridyl compounds selectively ejected a biq ligand in all cases; the phen ligand was not observed in the chromatograms or in the mass

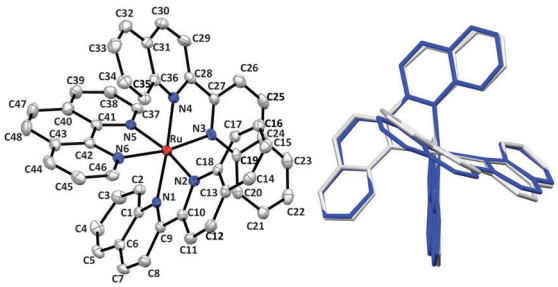

Fig. 2 Left: Ellipsoid plot (at 50% probability) of **2** (H atoms omitted for clarity). Right: capped stick overlay of **2**. In white the N(3)–N(4) biq is horizontal and in blue the N(1)–N(2) biq is horizontal. Both biq ligands have a 20° bend from the octahedral plane, but the N(3)–N(4) ligand has a larger twist about the C–C bond between the two quinolines than the N(1)–N(2) ligand.

Table 1 Selected bond lengths (Å), bond angles (°), and torsion angles (°) for compounds **1** and **2**

Compound **1**		Compound **2**	
$Ru–N_{1\text{-}biq}$	2.112(3)	$Ru–N_{1\text{-}biq(1)}$	2.084(2)
$Ru–N_{2\text{-}biq}$	2.095(3)	$Ru–N_{2\text{-}biq(1)}$	2.079(2)
$Ru–N_{3\text{-}phen(1)}$	2.063(3)	$Ru–N_{3\text{-}biq(2)}$	2.088(2)
$Ru–N_{4\text{-}phen(1)}$	2.056(3)	$Ru–N_{4\text{-}biq(2)}$	2.093(2)
$Ru–N_{5\text{-}phen(2)}$	2.091(3)	$Ru–N_{5\text{-}phen}$	2.104(3)
$Ru–N_{6\text{-}phen(2)}$	2.091(3)	$Ru–N_{6\text{-}phen}$	2.098(2)
$N_{2\text{-}biq}–Ru–N_{3\text{-}phen(1)}$	99.15(12)	$N_{2\text{-}biq(1)}–Ru–N_{3\text{-}biq(2)}$	97.14(10)
$N_{4\text{-}phen(1)}–Ru–N_{5\text{-}phen(2)}$	82.75(12)	$N_{4\text{-}biq(2)}–Ru–N_{5\text{-}phen}$	80.14(9)
$N_{1\text{-}biq}–Ru–N_{2\text{-}biq}–C_{10\text{-}biq}$	−20.72(4)	$N_{1\text{-}biq(1)}–Ru–N_{2\text{-}biq(1)}–C_{10\text{-}biq(1)}$	−20.41(19)
$N_{1\text{-}biq}–C_{9\text{-}biq}–C_{10\text{-}biq}–N_{2\text{-}biq}$	−5.62(5)	$N_{1\text{-}biq(1)}–C_{9\text{-}biq(1)}–C_{10\text{-}biq(1)}–N_{2\text{-}biq(1)}$	2.7(4)
		$N_{4\text{-}biq(2)}–Ru–N_{3\text{-}biq(2)}–C_{27\text{-}biq(2)}$	−19.5(2)
		$N_{3\text{-}biq(2)}–C_{27\text{-}biq(2)}–C_{28\text{-}biq(2)}–N_{4\text{-}biq(2)}$	12.1(4)

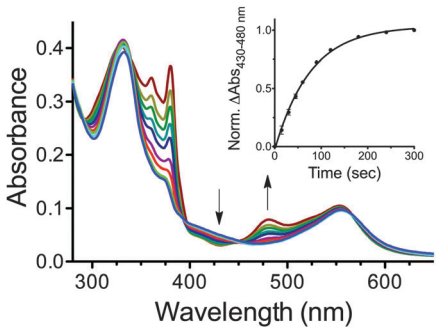

Fig. 3 UV/Vis absorption spectra at different time points for the photo-substitution reaction of **2** in H_2O with blue light. The inset shows the kinetic fit for the reaction.

spectra. The elongated Ru–N phen bonds in the crystal structure suggested that the phen would be released, but the selective labilization of the biq indicates that the distortion due to the twisting of the ligand drives the ejection process.[11]

The ability of the Ru(II) complexes to damage DNA upon light activation was determined by gel electrophoresis with supercoiled pUC19 plasmid, as shown in Fig. 4. All complexes induced a dose-dependent effect on the DNA mobility, with increasing retention indicating photobinding of the complex to the plasmid DNA. The decreased migration with increasing concentration of complex is consistent with a DNA cross-linking effect, similar to that observed for cisplatin.[12] Intercalation also causes decreased migration in agarose gels, but no effect was observed for **1** and **2** in the absence of light, making intercalation unlikely. Also, very similar results were obtained for the gel electrophoresis of the chemically synthesized $Ru(phen)_2(H_2O)_2$, which covalently modifies DNA[13] (see Fig. S6, ESI†). The compounds do not appear to photocleave DNA, as no increase in the linear or relaxed circle forms of DNA were observed.[14] A significant loss of the ethidium bromide (EtBr) signal was observed for the light activated **1**, **2**, and $Ru(phen)_2(H_2O)_2$ at concentrations >15 μM. To determine if this was due to degradation of the DNA, bands

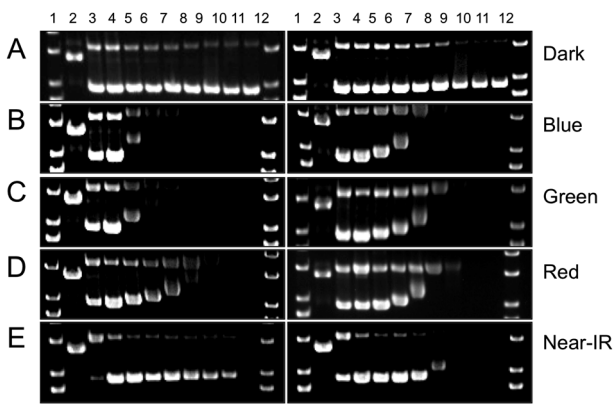

Fig. 4 Agarose gels showing the dose response of **1** (left) and **2** (right) with 40 μg mL^{-1} pUC19 plasmid with and without irradiation. A: Dark, B: Blue (>400 nm), C: Green (>450 nm), D: Red (>600 nm), E: near-IR (>650 nm). Lane 1 and 12: DNA ladder; Lane 2: EcoRI; Lane 3: $Cu(OP)_2$; Lanes 4–11: 0, 15, 30, 60, 125, 250, 500, 1000 μM. EcoRI and $Cu(OP)_2$ are used as controls for linear and relaxed circular DNA, respectively. EtBr was used to visualize the DNA.

Table 2 Photobiological activity in HL-60 cells

Compound	λ_{abs} (nm) (ε (M^{-1} cm^{-1}))	IC_{50} [μM]					Phototoxicity index (PI)	
		Dark	Blue (3 min)	Red (3 min)	Red (6 min)	IR (25 min)	Blue	IR
1	525 (8300)	52.5	1.2	13.8	7.6	15.8	43.8	3.32
2	550 (4950)	47.3	2.4	4.5	2.3	5.1	19.7	9.2
Cisplatin		3.1	3.1	N.D.	N.D.	N.D.	1	

were excised and purified (see ESI†, Fig. S6 and S7). The majority of the DNA was recovered, suggesting that the Ru(II) adducts interfere with EtBr–DNA binding or EtBr emission. The purified DNA exhibited the same decreased gel mobility on a second agarose gel, supporting a covalent, photobinding mechanism for **1** and **2**. This may be advantageous compared to the DNA single strand breaks induced by most PDT agents, which may be readily repaired.[15]

The photoreactivity of **1** and **2** was dependent on the wavelength of light used, with blue light producing the greatest potency. Significant activity was retained using red and near-IR light, but decreased approximately 4-fold using a >600 nm cut-off filter (red), and was further diminished with near-IR irradiation due to reduced light intensity and compound absorptivity, negligible reactivity with the DNA was observed in the dark.

Cytotoxicity studies were performed in the HL-60 human leukemia cell line to determine if the light-induced DNA damage translated to biological activity in cancer cells. The activity of the compounds was dependent on the light dose and wavelength, indicating that light-activation is directly correlated with cytotoxicity. As shown in Table 2, IC_{50} values of 1–2 μM were observed for **1** and **2** with blue light, comparable to cisplatin (IC_{50} = 3.1 μM). The activity decreased as red- and near-IR cut-off filters were used, but increasing the light dose increased the potency. The enhanced activity of **2** compared to **1** with red and near-IR light irradiation is consistent with its absorption profile. A phototoxicity index (PI) value (the toxicity in the dark *vs.* the light) of 43 was found with blue light for **1**. This is superior to the PDT drug ALA (aminolevulinic acid) which has an IC_{50} of 16.2 μM and a PI of >18. Compound **2** gave PI values of 20 and 9.2 with red- and near-IR light. To the best of our knowledge, this is the first report of light-activated metal complexes that are cytotoxic in cancer cells using irradiation with red and near-IR light.

While transition metal complexes have long been studied due to their combined DNA binding and photocleavage properties, very few metal compounds have been identified that show promise as PDT agents in cell studies. This study shows that strained Ru(II) 2,2′-biquinoline complexes act as DNA photobinding agents for PDT.[16] While previous reports of metal complexes that are capable of DNA covalent modification require UV or higher energy blue light,[17] the compounds described here are the first examples of light-activated metal complexes that kill cancer cells upon activation in the PDT therapeutic window. Future work is focused on increasing potency upon photoactivation while reducing dark toxicity.

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