

Photoactive Ru(II) Complexes With Dioxinophenanthroline Ligands Are Potent Cytotoxic Agents

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S Supporting Information

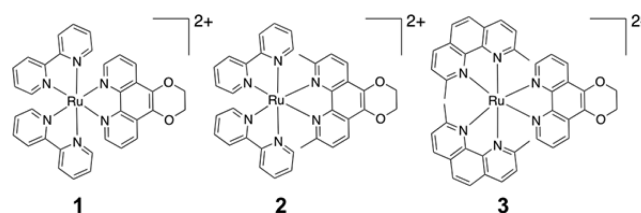
ABSTRACT: Two novel strained ruthenium(II) polypyridyl complexes containing a 2,3-dihydro-1,4-dioxino[2,3-*f*]-1,10-phenanthroline (dop) ligand selectively ejected a methylated ligand when irradiated with >400 nm light. The best compound exhibited a 1880-fold increase in cytotoxicity in human cancer cells upon light-activation and was 19-fold more potent than the well-known chemotherapeutic, cisplatin.

Photodynamic therapy (PDT) utilizes light-activated molecules to spatially limit nonspecific toxicity.¹ Most PDT agents induce damage through the production of singlet oxygen (¹O₂), causing single strand DNA breaks,² and ruthenium(II) polypyridyl complexes that can produce ¹O₂ when irradiated with visible light have been explored for this application. However, the design of light-activated Ru(II) molecules that produce active species capable of forming covalent adducts with DNA could greatly improve the damage caused upon light activation since adducts can be more difficult to repair than single strand breaks.³

Most Ru(II) polypyridyl complexes are photostable, but with the introduction of distortion into the octahedral geometry of the Ru(II) complex activates photochemical pathways that induce ligand ejection. Strain lowers the triplet metal-centered (³MC) state, allowing for thermal population from the triplet metal to ligand charge transfer (³MLCT) state,^{4,5} causing the loss of one or more ligands. The resulting ligand-deficient Ru(II) center can covalently modify DNA or other biomolecules, and induce cytotoxicity. Ideally, these molecules would have a large phototoxicity index (PI), the ratio of the toxicity in the dark and in the light. To optimize the PI, either the toxicity of the complex in the dark must be reduced or the light-triggered toxicity increased.

Reducing noncovalent interactions with essential biomolecules such as DNA should alleviate toxicity in the dark, while increasing the degree of distortion may increase the production of the active compound when exposed to light. Accordingly, nonplanar ligands analogous to the planar dipyrido[3,2-*f*:2',3'-*h*]-quinoxaline (dpq) ligand, a known DNA intercalator,⁶ were explored. Three chiral Ru(II) complexes containing a 2,3-dihydro-1,4-dioxino[2,3-*f*]-1,10-phenanthroline (dop) or 2,3-dihydro-1,4-dioxino[2,3-*f*]-2,9-dimethyl-1,10-phenanthroline (dmdop) ligand (Chart 1) were synthesized and characterized as racemic mixtures of the PF₆⁻ salts and were converted to the Cl⁻ salt prior to photochemical and biological testing. The

Chart 1. Structures of Compounds in This Study



series of Ru(II) complexes incorporated increasing structural distortion with each methylated ligand.

The ligands were synthesized from 5,6-dihydroxy-1,10-phenanthroline or its methylated analogue using an established procedure.⁷ Complexation of dop and dmdop with Ru-(bpy)₂Cl₂ yielded 1 and 2. The crystal structure of unstrained complex 1 shows typical Ru–N bond lengths (2.064 Å average) and little deviation of any ligand from the normal plane (see Table 1, Figure S1). The addition of two methyl groups in 2 resulted in the Ru–N bonds lengthening, with the greatest distance for the dmdop (Table 1, Figure S2). An 8.9° deviation from the normal plane is also seen for one of the 2,2'-bipyridine (bpy) ligands. To increase strain, 3 was synthesized through the complexation of dop with Ru(dmphen)₂Cl₂. Incorporation of two dmphen (2,9-dimethyl-1,10-phenanthroline) ligands re-

Table 1. Selected Bond Lengths (Å) and Torsion Angles (deg) for Crystal Structures of 1, 2, and 3

	1	2	3 ^a
Ru–N1	2.065(4)	2.086(11)	2.123(5)
Ru–N2	2.057(4)	2.089(11)	2.102(5)
Ru–N3	2.069(4)	2.065(11)	2.122(5)
Ru–N4	2.059(4)	2.072(11)	2.118(5)
Ru–N5	2.069(4)	2.131(10)	2.071(6)
Ru–N6	2.067(4)	2.124(10)	2.103(5)
L ₁ Bend ^b	0.1(5)	0.2(15)	−17.4(7)
L ₂ Bend ^b	−2.1(5)	8.9(15)	12.6(7)
L ₃ Bend ^b	−4.7(5)	−2.0(14)	0.5(8)
L ₃ Twist ^c	68.1(11)	63.3(15)	−59.9(19)

^aThe crystal structure of 3 contains two similar cations in the asymmetric unit, but only one was chosen for simplification. ^bL = ligand where L₁ is N1 and N2; L₂ is N3 and N4; L₃ is N5 and N6. The “bend” torsion angle represents the deviation from the normal plane. ^cThe “twist” torsion angle represents the rotation from planar of the O–C–C–O of the dop and dmdop ligands.

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sulted in a further Ru–N bond lengthening to 2.107 Å (average value) and significant deviation from the normal plane of both dmphen ligands by 12–17° (Table 1, Figure 1). Each structure shows the dop/dmdop ligand with a 60–68° twist in the dioxane ring.

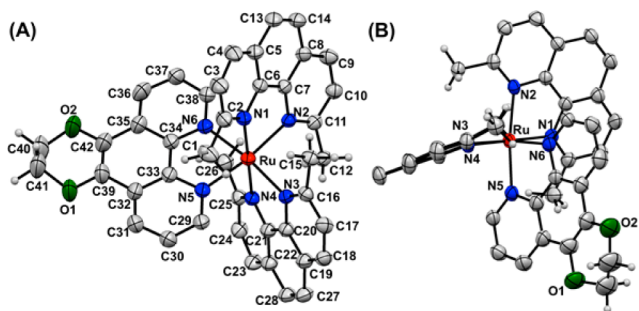


Figure 1. Ellipsoid plot of **3** at 25% probability with H atoms omitted for clarity. (A) Clear labeling of atoms. (B) Side view highlighting the distortion of the dmphen ligand. Note: only one cation of the asymmetric unit is shown.⁸

The strained Ru(II) complexes exhibited selective photoejection of the methylated ligand when irradiated with >400 nm light, as shown in Figure S3 and S4. The photochemical reactions were monitored by absorption spectroscopy, and the presence of an isosbestic point indicated the direct conversion to a single product (Figure 2). The half-life ($t_{1/2}$) of ligand

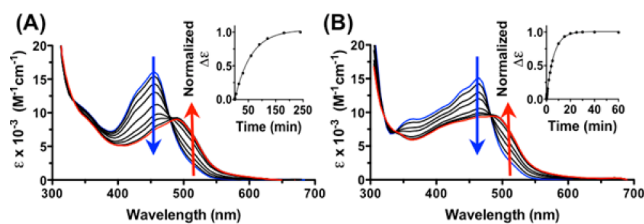


Figure 2. Photoejection of (A) **2** and (B) **3** in dH₂O monitored by UV/vis absorption spectroscopy. The monoexponential kinetic fit for the change in extinction coefficient vs time is shown in the inset.

ejection for **3** (4.1 ± 0.1 min) is 10× faster than for **2** (42 ± 2 min), which was attributed to the greater degree of distortion induced by the incorporation of two methylated ligands compared to one, as reflected in the crystal structures.

The addition of the nonplanar ligand (dop or dmdop) was anticipated to reduce DNA affinity in the dark if the interaction was through intercalation. Binding titrations were conducted with calf thymus DNA (CT DNA) to determine the binding constant (K_b) for each Ru(II) complex and compared to the DNA intercalator [Ru(bpy)₂dpq]²⁺ ($K_b = 2.2 \times 10^4$ M⁻¹). Contrary to expectations, the K_b values only decreased slightly (1.0×10^4 M⁻¹ for **1** and **3** and 2.0×10^4 M⁻¹ for **2**), indicating that the DNA affinity was not significantly altered by the incorporation of the 1,4-dioxane ring (Figure S5).⁹

DNA damage was assessed by gel electrophoresis using pUC19 plasmid DNA (Figure 3). Incubation of each Ru(II) complex with plasmid DNA in the dark showed no interactions, in contrast to the two types of DNA damage observed upon irradiation with >400 nm light. Unstrained complex **1** creates single strand breaks, forming relaxed circle DNA, likely through sensitization of singlet oxygen (¹O₂). The strained complex **3** undergoes ligand loss and covalent attachment to DNA. This

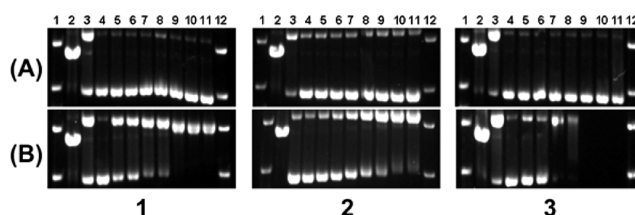


Figure 3. Agarose gel electrophoresis showing the dose response of **1**, **2**, and **3** incubated with 40 μg/mL pUC19 DNA (A) without and (B) with irradiation (>400 nm light). Lanes 1 and 12, DNA ladder; lane 2, *EcoRI*; lane 3, Cu(OP)₂; lane 4–11, 0–500 μM. *EcoRI* and Cu(OP)₂ are controls for linear and relaxed circle DNA.

was observed with the reduced mobility and loss of ethidium bromide (EtBr) staining of the DNA.^{5b} Compound **2** shows a combination of effects, with some single strand breaks, though to a lesser degree than with **1**, and less covalent damage than **3**. This difference may be due to competing excited state relaxation pathways for **2**. Little distortion is seen in the crystal structure, and the complex undergoes only slow photoejection; therefore, when **2** is irradiated, relaxation likely occurs via either sensitization of ¹O₂ or ligand loss.

Cytotoxicity studies of the Ru(II) complexes in HL60 (human promyelocytic leukemia) cells are shown in Figure 4.

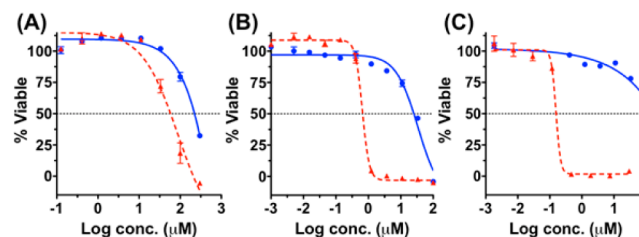


Figure 4. Cytotoxicity of Ru(II) complexes in HL60 cells: (A) **1**, (B) **2**, (C) **3**. Dark control (solid blue circles); irradiated with >400 nm hv (dashed red triangles).

The PI increased drastically with increasing distortion of the Ru(II) complex for this series. Compound **1** (dark IC₅₀ = 200 ± 17 μM, light IC₅₀ = 52 ± 1 μM) had the smallest PI (3.8), despite the DNA damage observed. Compound **2** was more effective, with PI = 52 (IC₅₀ = 34 ± 1 μM, 0.65 ± 0.1 μM). The most distorted complex, **3**, showed the highest PI, 1880, with dark toxicity >300 μM, while the IC₅₀ = 0.16 ± 0.01 μM in the light. This is 19-fold more potent than cisplatin, a benchmark DNA damaging metal complex, and **3** has one of the largest PI values of any previously reported PDT agents.^{5a,10}

In conclusion, the addition of the dop or dmdop ligand had little effect on the DNA affinity of Ru(II) complexes compared to analogous dpq complexes, and similar dark toxicities were observed.^{5a} The incorporation of methylated ligands (either dmdop or dmphen) resulted in increased distortion of the Ru(II) center and elevated the degree of distortion, which increased the rate of ligand ejection as well as light-induced cytotoxicity. Both **2** and **3** exhibited nM IC₅₀ values when irradiated; however, compound **3** was 4-fold more potent in the light and less toxic in the dark, suggesting DNA affinity is a poor predictor of dark toxicity or the PI. This study demonstrates that creating minor three-dimensional structural changes to photoreactive Ru(II) complexes has a major impact on their biological activities and suggests that ether-containing

ligands may be used to create a promising class of light-activated cytotoxic agents.

■ ASSOCIATED CONTENT

● Supporting Information

Supporting Information contains experimental details, full crystal structure analysis, and additional figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

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