Electronic Supplementary Information

A Novel Azopyridine-based Ru(II) Complex with GSH-responsive DNA Photobinding Ability

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Experimental section

Materials. 4-(4-Diethylaminophenylazo)pyridine (L2) was purchased from TCI, 4-aminopyridine, 2,2'-bipyridine, NH₄PF₆, glutathione were purchased from Sigma-Aldrich. Supercoiled pBR322 plasmid DNA was purchased from TaKaRa Biotechnology Company.

Synthesis. Ru(bpy)₂Cl₂¹ and 4,4'-azopyridine (L1)² were synthesized according to the reported methods.

Synthesis of [Ru(bpy)₂(L)₂](PF₆)₂: 0.20 g Ru(bpy)₂Cl₂ and 0.21 g (1.5 equiv.) AgNO₃ were refluxed in 25 ml of methanol/water (1:1) for 3 h under N₂ atmosphere. Then the filtrate was refluxed again with L1 or L2 (1.5 equiv.) for 4 h under N₂ atmosphere. After removal of solvent and purification on silica gel using CH₃CN/H₂O/KNO₃ (40:4:1) as eluent, the solid obtained was dissolved in CH₃OH and precipitated by NH₄PF₆. The dark red solid was filtered, washed with water and vacuum dried. The yields are about 40% for the two complexes.

[Ru(bpy)₂(L1)₂](PF₆)₂ (complex 1): ¹H NMR (400 MHz, in CD₃CN): δ = 7.34-7.37 (t, 2H, J = 6.3 Hz), 7.61-7.62 (d, 4H, J = 5.6 Hz), 7.69-7.71(d, 4H, J = 4.4 Hz), 7.75-7.78 (t, 2H, J = 5.6 Hz), 7.88-7.93 (m, 4H), 8.10-8.14 (t, 2H, J = 7.6 Hz), 8.24-8.26 (d, 2H, J = 8.0 Hz), 8.33-8.35 (d, 2H, J = 8.0 Hz), 8.51-8.53 (d, 2H, J = 6.8 Hz), 8.79-8.80 (d, 4H, J = 4.2 Hz), 8.92-8.94 (d, 2H, J = 5.2 Hz). HR ESI-MS: m/z = 391.0949 (calcd value = 391.0958 for (M-2PF₆)²⁺).

[Ru(bpy)₂(L2)₂](PF₆)₂ (complex 2): ¹H NMR (400 MHz, in CD₃CN): δ = 1.18-1.22 (t, 12H, J = 7.6 Hz), 3.48-3.53 (q, 8 H, J = 7.2 Hz), 6.80-6.82 (d, 4H, J = 9.2 Hz), 7.36-7.40 (t, 2H, J = 7.2 Hz), 7.49-7.51 (d, 4H, J = 6.8 Hz), 7.77-7.82 (m, 6H), 7.92-7.95 (m, 4H), 8.12-8.16 (t, 2H, J = 8.8 Hz), 8.28-8.30 (d, 2H, J = 8.4 Hz), 8.34-8.38 (m, 6H), 8.99-9.01 (d, 2H, J = 5.6 Hz). ESI-MS: m/z = 461.1735 (calcd value = 461.1741 for (M-2PF₆)²⁺).

Computational Details

All calculations were performed with the Gaussian 03 (G03) program package³ employing the density functional theory (DFT) method with Becke’s three-parameter hybrid functional⁴ and Lee-Yang-Parr’s gradient corrected correlation functional (B3LYP).⁵ The LanL2DZ basis set and effective core potential were used for the Ru atom, and the 6-31 G* basis set was applied for H, C, and N. The ground-state geometry of 1 was optimized in CH₃CN using the conductive polarizable continuum model (CPCM), and frequency calculation was also performed to verify the optimized
structure to be at an energy minimum. Time-dependent density functional theory (TDDFT) calculation was used to characterize the properties of singlet and triplet excited states, and the CPCM model with CH$_3$CN as solvent was applied to the solvent effect.

Figure S1. $^1$H NMR spectra of [Ru(bpy)$_2$(py)$_2$]^{2+} in CD$_3$COCD$_3$/D$_2$O (4/1) before (a) and after (b) irradiation ($\lambda > 470$ nm) for 45 min.
Figure S2. ESI-MS spectrum of $[\text{Ru(bpy)}_2(\text{py})_2]^{2+}$ in CH$_3$CN before (top) and after (bottom) irradiation ($\lambda > 470$ nm) for 10 min.
Figure S3. $^1$H NMR spectra of 1 in CD$_3$COCD$_3$/D$_2$O (4/1) before (a) and after (b) irradiation ($\lambda > 470$ nm) for 45 min.

Figure S4. $^1$H NMR spectra of 2 in CD$_3$COCD$_3$/D$_2$O (4/1) before (a) and after (b) light irradiation ($\lambda > 470$ nm) for 45 min.
Figure S5. ESI-MS spectra of 1 in CH$_3$COCH$_2$/H$_2$O (4:1) before (top) and after (bottom) light irradiation (λ > 470 nm) for 45 min.
Figure S6. ESI-MS spectra of 2 in CH$_3$COCH$_3$/H$_2$O (4:1) before (top) and after (bottom) light irradiation ($\lambda > 470$ nm) for 45 min.
Figure S7. Selected molecular orbitals of 1 (trans configuration for azo-group, isovalue = 0.02).
Figure S8. ESI-MS spectrum of 1 (10 μM) in H₂O after addition of GSH (1 mM) for 20 min. 1-red = [Ru(bpy)₂(py-NH-NH-py)]²⁺, py-NH-NH-py = 1,2-di(pyridine-4-yl)hydrazine.

Figure S9. ¹H NMR spectra of 1 in CD₃COCD₂/D₂O (3/1) before (a) and after (b) GSH reduction.
Figure S10. $^1$H NMR spectra of L1 in CD$_3$COCD$_3$/D$_2$O (3/1) before (a) and after (b) GSH reduction.
Figure S11. EI-MS spectrum of L1 before (top) and after (bottom) GSH reduction.

Figure S12. ESI-MS spectrum of 1-red in CH₃CN after light irradiation (λ > 470 nm) for 10 min.
Figure S13. Agarose gel electrophoresis pattern of supercoiled pUC19 DNA (40 μg/ml) in Tris-CH$_3$COOH-EDTA buffer (pH = 7.4) in the presence of varied concentrations of 1 in the dark (Lane 1-4) or under light irradiation (λ > 470 nm) for 25 min (Lane 5-8). Lane 1: DNA alone; Lane 2: DNA + 60 μM 1; Lane 3: DNA + 100 μM 1; Lane 4: DNA + 160 μM 1; Lane 5: DNA + 60 μM 1; Lane 6: DNA + 100 μM 1; Lane 7: DNA + 160 μM 1; Lane 8: DNA alone. SC and NC denote supercoiled circular and nicked circular forms, respectively.

Figure S14. Agarose gel electrophoresis pattern of supercoiled pUC19 DNA (40 μg/ml) in Tris-CH$_3$COOH-EDTA buffer (pH = 7.4) in the presence of GSH (1 mM) and varied concentrations of 1. All samples were kept in the dark for 20 min. Lane 1: DNA alone; Lane 2-4: the concentration of 1 are, respectively, 60, 100, and 160 μM. SC and NC denote supercoiled circular and nicked circular forms, respectively.

Table S1. Selected TDDFT triplet transitions of 1 in the ground-state optimized geometry (trans configuration for azo-group).

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References