Supplementary Information

Synthesis, DNA binding, and dual topoisomerase I/II inhibitory activities of $[Ru(tpy)(adtpy)]^{2+}$ and $[Ru(dtp)(adtpy)]^{2+}$

Jun-Feng Kou[a,b]

[a] College of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming, Yunnan, 650500, P. R. China

[b] MOE Laboratory of Bioinorganic and Synthetic Chemistry, State Key Laboratory of Optoelectronic Materials and Technologies,
School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P. R. China

E-mail: kjf416@163.com

Received 29 March 2024; accepted (revised) 22 January 2025

Materials and Instrumentation

The compounds 2-formyl-9,10-anthraquinone, [1] Ru(tpy)$Cl_3$and Ru(dtp)Cl_3 [2] were synthesized according to the literature methods. Doubly distilled water was used to prepare buffers. Calf thymus DNA (CT-DNA) was obtained from the Shanghai Sangon Biological Engineering Technology & Services Co. Ltd., Other materials were commercially available and of reagent grade. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.8-1.9:1, indicating that the DNA was sufficiently free of protein [3]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [4].

Microanalysis (C, H, and N) was carried out with a Vario EL elemental analyzer. ^{1}H-NMR spectra were recorded on a Varian INOVA 500NB NMR spectrometer with $(CD_3)_2SO$ as solvent at room temperature and TMS as the internal standard. Fast atom bombardment (FAB) mass spectra were acquired on a VG ZAB-HS spectrometer in a 3-nitrobenzyl alcohol matrix. Electrospray mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA). The spray voltage, tube lens offset, capillary voltage and capillary temperature were set at 4.50 KV, 30.00 V, 23.00 V and 200 ^{o}C, respectively, and the quoted *m/z* values are for the major peaks in the isotope distribution. UV-Vis spectra were recorded on a Perkin-Elmer Lambda 850 spectrophotometer. Emission spectra were recorded on a Perkin-Elmer LS 55 spectrofluorophotometer at room temperature.

Syntheses

1. Synthesis of 4’-(2-Anthryl)-2,2’:6’,2”-terpyridine (adtpy)

2-Acetylpyridine (0.61 g, 5.0 mmol) was added into a solution of 2-formyl-9,10-anthraquinone (0.52 g, 2.5 mmol) in EtOH (25 mL). KOH pellets (0.39 g, 85%, 5.0 mmol) and aq NH_3 (7.5 mL, 29.3%, 6.3 mmol) were then added to the solution. The solution was heated at reflux for 24 h. After cooling down to ambient temperature, The off-yellow solid was collected by filtration and washed with EtOH (3 × 10 mL). The yellow-green solid obtained was dried *in vacuo*. Yield: 0.33 g, 30%. Anal. Anal. Calcd for $C_{29}H_{17}N_3O_2$: C, 79.26; H, 3.90; N, 9.56. Found: C, 76.30; H, 3.85; N, 9.58. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.10 (s, 2H), 8.96 (d, J = 8 Hz, 2H), 8.60 (d, J = 8 Hz, 2H), 8.46(s, 1H), 8.44 (t, J = 6 Hz, 2H), 8.27 (d, J = 6 Hz, 1H), 8.14(m, J = 8 Hz, 1H), 8.09(d, 1H)7.77 (d, 2H),7.29 (d, J = 8 Hz, 2H).FAB-MS: m/z = 440 ($[M+H]^+$).

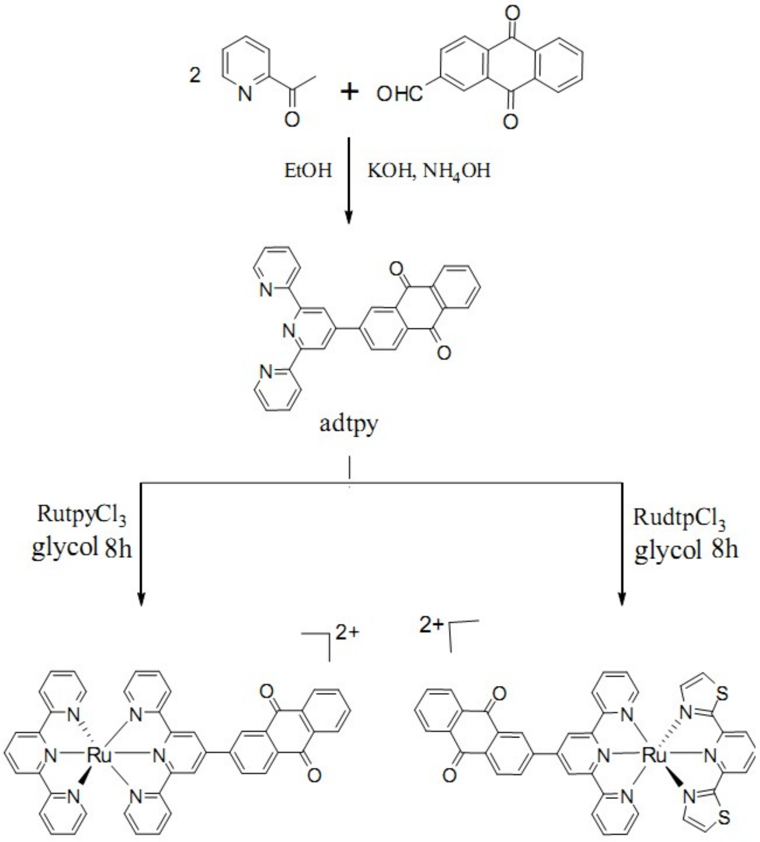
2. Synthesis of [Ru(tpy)(adtpy)]$(ClO_4)_2$ (1)

A mixture of Ru(tpy)Cl_3 (0.104 g, 0.2 mmol), adtpy (0.074 g, 0.22 mmol), 10 cm^3 glycol, 1 cm^3 triethylamine was heated at 120 °C under argon for 8 h to give a red-brown solution. The solution was cooled and diluted with 50 cm^3 of water. After filtration, the solution was treated with a saturated aqueous solution of $NaClO_4$ to obtain a red-brown precipitate. The crude product was purified by column chromatography on alumina using toluene-acetonitrile (1:5, v/v) as eluent. The solvent was removed under reduced pressure and black red microcrystals were obtained. Yield: 0.16 g, 72%. Anal. Calcd for $C_{44}H_{28}N_6Cl_2O_{10}Ru$: C, 54.30; H, 2.92; N, 8.61. Found: C, 54.33; H, 2.90; N, 8.64%. ^1H NMR (ppm, DMSO-d_6， 400 MHz): 9.65 (s, 2H),9.19 (m, 3H), 9.02(d, 2H), 8.90 (d, 1H), 8.85 (d, 2H), 8.36(m, 2H), 8.11 (d, 1H), 8.07(d, 2H), 8.05 (d, 2H), 8.03 (d, 1H), 7.60 (d, 2H),7.48(d, 2H), 7.32 (t, 2H), 7.27 (d, 2H), ES-MS [CH_3CN, m/z]: 386.6($[M - 2ClO_4]^{2+}$)。

3. Synthesis of $[Ru(dtp)(adtpy)](ClO_4)_2$ (2)

This red-brown complex was synthesized in a manner identical to that described for complex **1,** with $Ru(dtp)Cl_3$ (0.1 g, 0.177 mmol) in place of $Ru(tpy)Cl_3$ Yield: 0.15 g, 88%. Anal. Calcd for $C_{40}H_{24}N_6Cl_2O_{10}RuS_2$: C, 48.81; H, 2.48; N, 8.51%.Found: C, 48.79; H, 2.46; N, 8.53. ^1H NMR (ppm, DMSO-d_6, 400 MHz): 9.60 (s, 2H), 9.16 (d, 2H), 9.14 (s, 1H), 8.93 (d, 2H), 8.87 (d, 2H), 8.59 (d, 2H), 8.48 (t, 1H),8.35(m, 2H), 8.11 (d, 1H), 8.09 (d, 1H), 8.07 (d, 2H),8.05 (d, 1H), 8.03 (d, 1H), 7.42(d, 2H), 7.33 (t, 2H), 7.27 (d, 2H). ES-MS [CH_3CN, m/z]: 392.6($[M - 2ClO_4]^{2+}$).

The complexes **1** and **2** were converted to the chloride salt by dissolving in a minimum amount of acetone and then slow addition of a saturated solution of tetrabutylammonium chloride in acetone. The chloride salt cleanly precipitated and was filtered, washed with acetone and dried [5].



Scheme S1 Chemical structures of Ru (II) complexes **1** and **2**.

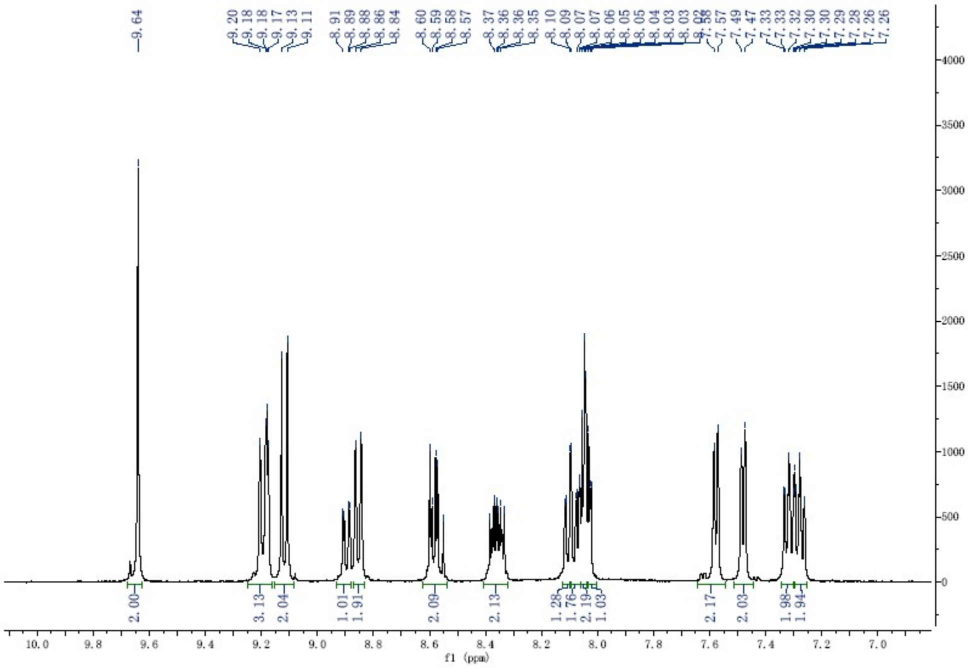


Fig. S1. ^{1}HNMR of Ru (II) complexes **1**

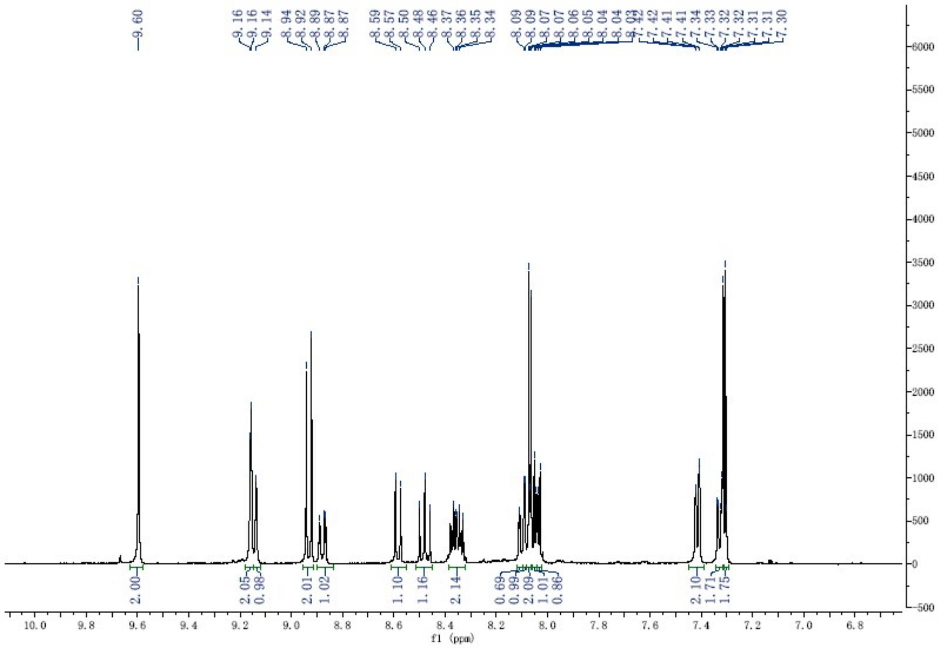


Fig. S2. ^{1}HNMR of Ru (II) complexes **2**.

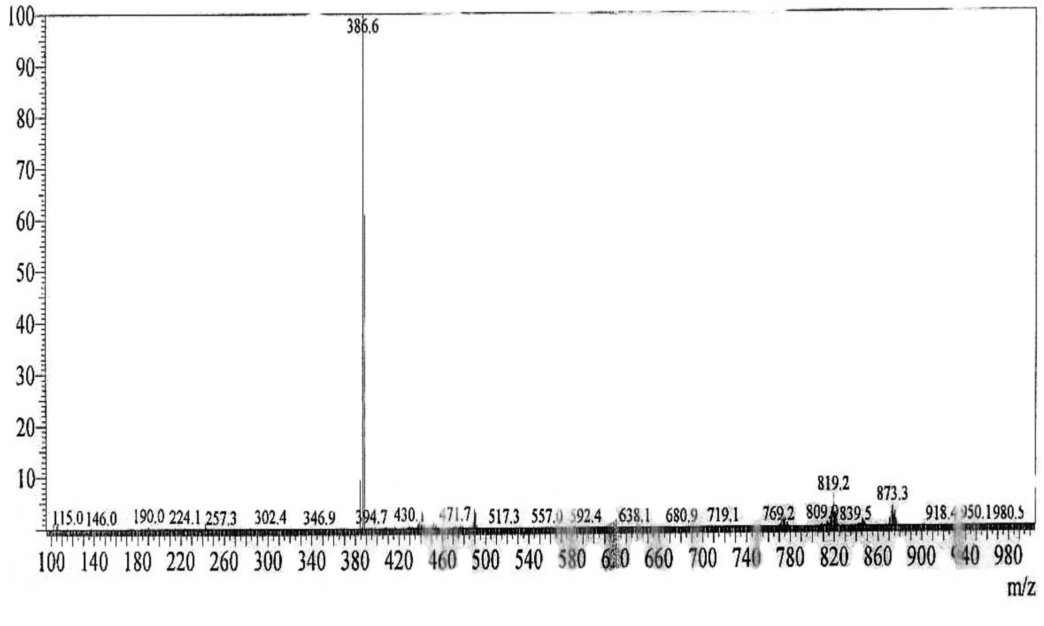


Fig. S3. LC-MS of [Ru(tpy)(adtpy)]$(ClO_4)_2$

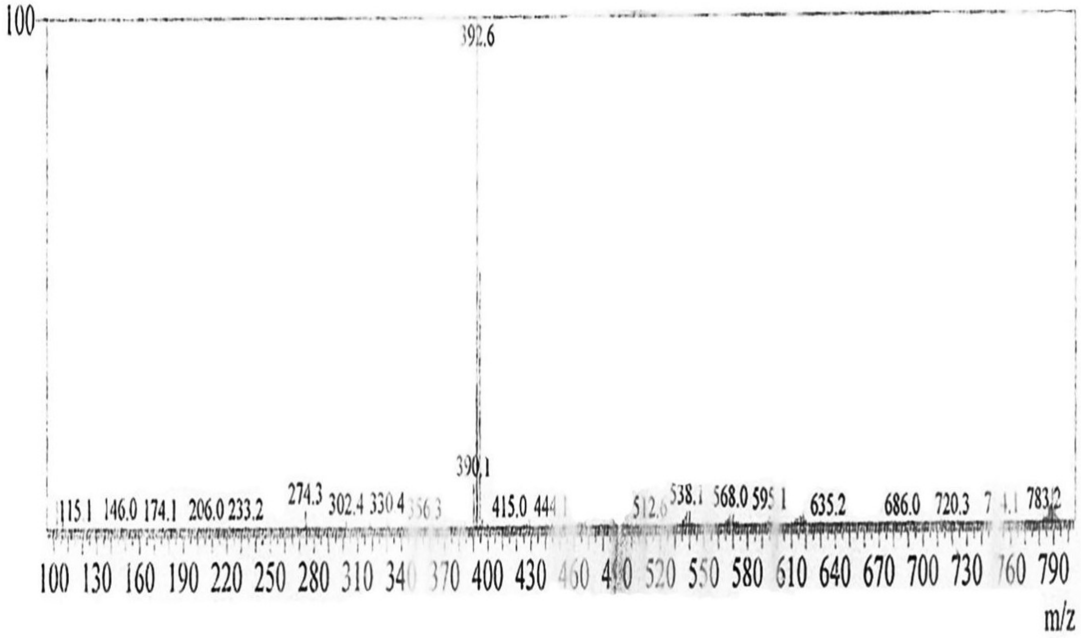


Fig. S4. LC-MS of $[Ru(dtp)(adtpy)](ClO_4)_2$

1.Mart, N. ; Perez's, S. L. And Seoane, C. Synthesis and Properties of the First Highly Conjugated Tetrathiafulvalene Analogues Covalently Attached to [60] Fullerene. *J. Org. Chem*. 1997, 62, 5690-5695. DOI: 10.1021/jo970320.

2.(a) Sullivan's, P. ; Calvert's, M. and Meyer. T. J. Cis-trans Isomerism in $(trpy)(PPh_3)$ $RuC1_2$. Comparisons Between the Chemical and Physical Properties of a Cis-trans Isomeric Pair. *Inorg. Chem*, 1980, 19, 1404-1407. DOI : 10.1021/ic50207a066; (b) Zeng, L. L. ; Chen, Y. ; Huang, H. Y. ; Wang, J. J. ; Zhao, D. L. ; Ji, L. N. and Chao, H. Cyclometalated Ruthenium(II) Anthraquinone Complexes Exhibit Strong Anticancer Activity in Hypoxic Tumor Cells. *Chem. Eur. J*, 2015, 21, 15308-15319. DOI: 10.1002/chem.201502154.

3.Marmur, J. a Procedure for the Isolation of Deoxyribonucleic Acid from Micro-Organisms. *J. Mol. Biol*. 1961, 3, 208-218. DOI: 10.1016/S0022-2836(61)80047-8.

4. Reichmann, M. E.; Rice, S.A.; Thomas, C. A. and Doty, P. a Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid. *J. Am. Chem. Soc*. 1954, 76, 3047–3053. DOI: 10.1021/ja01640a067.

5. Hiort, C. Lincoln, P. and Nordén, B. DNA Binding of Δ - andΛ-Ru(phen)$_2$DPPZ]$^{2+}$. *J. Am. Chem. Soc.*1993*,* 115, 3448-3454. DOI: 10.1021/ja00062a007.