SUPPORTING INFORMATION

FOR

Ruthenium polypyridyl complexes as inducer of ROS-mediated apoptosis in cancer cells by targeting thioredoxin reductase

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

1. Methods.

1.1. Synthesis and characterization of the Ru(II) complexes.

The complexes Ru(diimine)$_3$(ClO_4)$_2$ were prepared by literature methods by slight changes and got the same results as the reported literature.[1-4] A typical procedure is described below. Hydrated rutrichloride (0.13 g, 0.5 mmol) and 1.5 mmol the diimine ligands (bpy,0.24 g ; phen, 0.27 g; ip, 0.33 g; pip, 0.47 g) were mixed and stirred under reflux in 20 cm^3 ethanol for 3 h at 85 ℃ until the solution colour no longer changed. After filtration, added a saturated aqueous $NaClO_4$ ethanolic solution to the filtrate by dropwise, we could obtain the precipitates and washed with ethanol. The products was purified by column chromatography on neutral alumina using MeCN and toluene as eluent and dried in vacuum oven P_4O_{10}.

1.1.1. Ru(bpy)$_3$(ClO_4)$_2$ (1)

The complex Ru(bpy)$_3$(ClO_4)$_2$ was prepared according to the above procedure and get the same results as the reported literature.[1] Yield 0.226 g, 58.9%. ESI-MS: *m*/*z* 285.4 [M-2(ClO_4^-)] $^{2+}$. Elemental analysis calcd (%) for $C_{30}H_{24}Cl_2N_6O_8Ru$: C, 46.88; H, 3.15; N, 10.94; found (%): C, 47.87; H, 3.16; N, 10.96.

1.1.2. Ru(phen)$_3$(ClO_4)$_2$ (2)

The complex Ru(phen)$_3$(ClO_4)$_2$ was prepared according to the above procedure and get the same results as the reported literature.[2] Yield 0.320 g, 76.2%. ESI-MS: *m*/*z* 322.4 [M-2(ClO_4^-)] $^{2+}$. Elemental analysis calcd (%) for $C_{36}H_{24}Cl_2N_6O_8Ru$: C, 51.44; H, 2.88; N, 10.00; found (%): C, 51.44; H, 2.89; N, 10.01.

1.1.3. Ru(ip)$_3$(ClO_4)$_2$ (3)

The complex $Ru(ip)_3(ClO_4)_2$ was prepared according to the above procedure and get the same results as the reported literature.[4] Yield 0.286 g, 59.6%. ESI-MS: *m*/*z* 381.1 $[M-2(ClO_4^-)]^{2+}$. Elemental analysis calcd (%) for $C_{39}H_{24}Cl_2N_{12}O_8Ru$: Elemental analysis: C, 48.76; H, 2.52; N, 17.50; found (%): C, 48.78; H, 2.51; N, 17.51.

1.1.4. $Ru(pip)_3(ClO_4)_2$ (4)

The complex $Ru(pip)_3(ClO_4)_2$ was prepared according to the above procedure and get the same results as the reported literature.[3] Yield 0.301 g, 50.7%. ESI-MS: *m*/*z* 495.2 $[M-2(ClO_4^-)]^{2+}$. Elemental analysis calcd (%) for $C_{57}H_{36}C_{l2}N_{12}O_8Ru$: C, 57.58; H, 3.05; N, 14.14; Ru, 8.50; found (%): C, 57.56; H, 3.06; N, 14.15.

2. Results

Table S1. The ESI-MS analysis of complexes **1-4**

Complexes	Theoretical Value (m/z)	Measured Value (m/z)	Belonging to (m/z)	Molecular Weight (MW)
1	285.1	285.4	$[M-2(ClO_4^-)]^{2+}$	768 .2
2	321.0	322.4	$[M-2(ClO_4^-)]^{2+}$	840.0
3	380.0	381.1	$[M-2(ClO_4^-)]^{2+}$	960.0
4	495.2	495.2	$[M-2(ClO_4^-)]^{2+}$	1188 .4

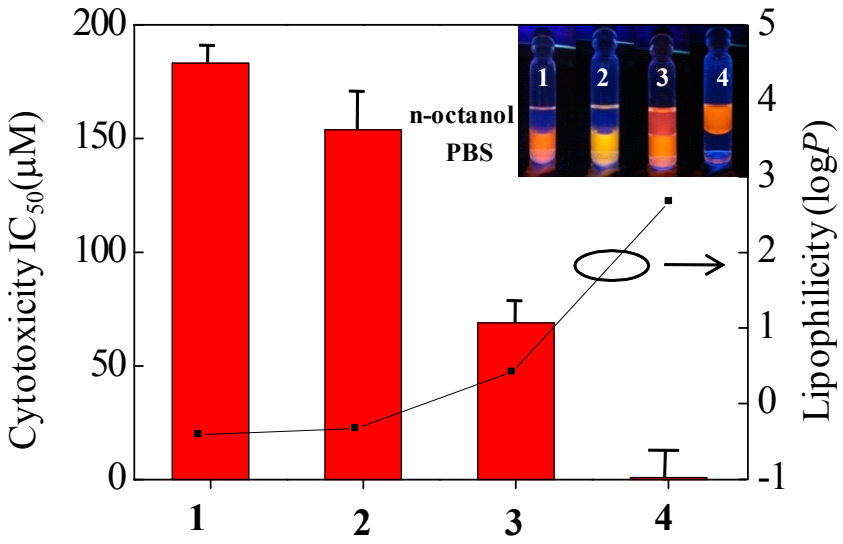

Figure S1. The relationship between cytotoxicity and lipophilicity of compounds 1-4. The bars represent complexes **1-4** IC_{50} values respectively.

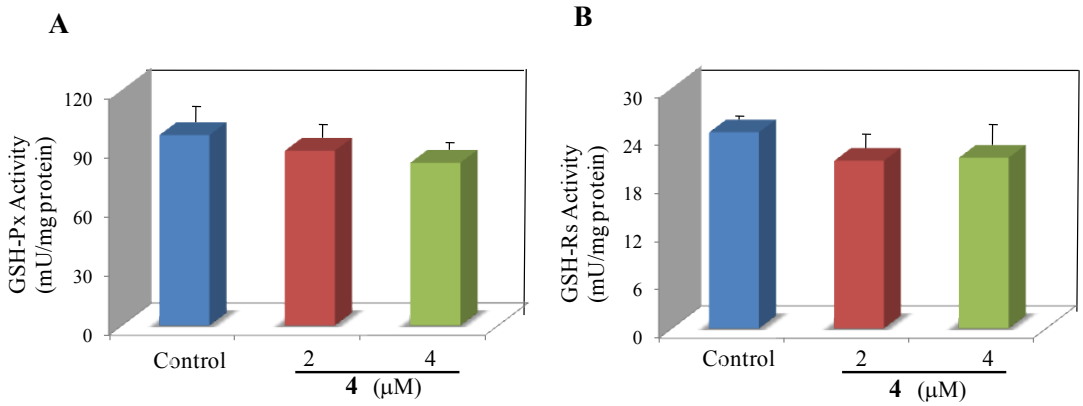

Figure S2. Enzymatic activities of GSH-Px (A) and GSH-Rs (B) in A375 cells after treatment with complex **4**.

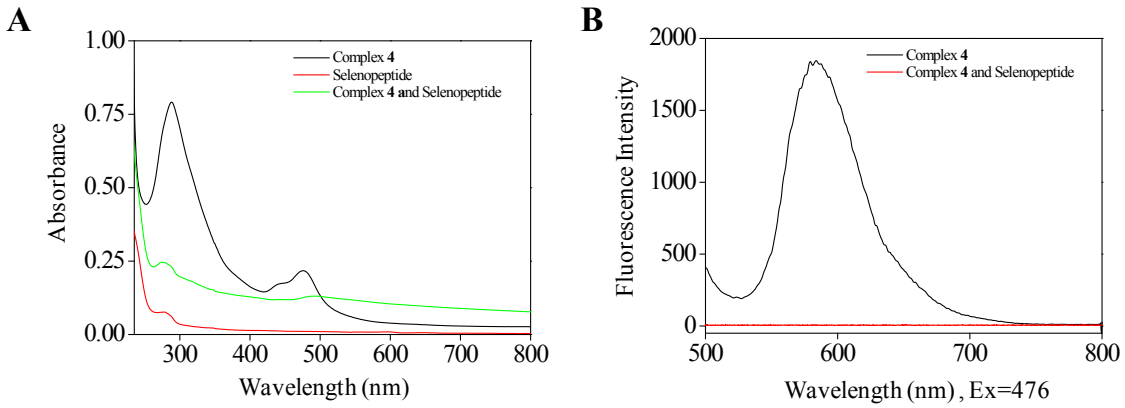

Figure S3. Photophysical changes of complex **4** during its reaction with selenopepetide (A) UV/vis absorption and (B) fluorescence emission spectra of complex **4** (20 μM) in the absence and presence of selenopeptide (20 μM). All spectra were acquired 12 h after selenopeptide addition at 37 ℃ in PBS buffer (pH 7.4).

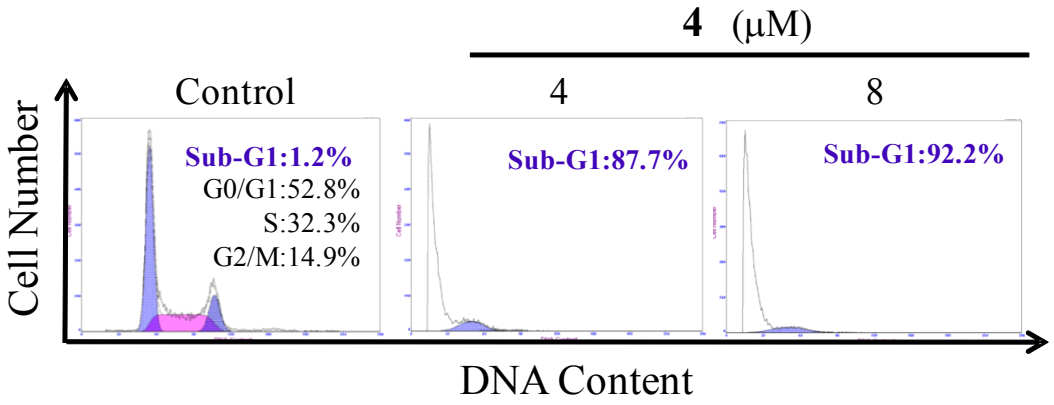

Figure S4. Complex 4 induces cancer cell apoptosis. Quantitative analysis of apoptotic cell death induced by **4** by flow cytometric analysis in A375 cells.

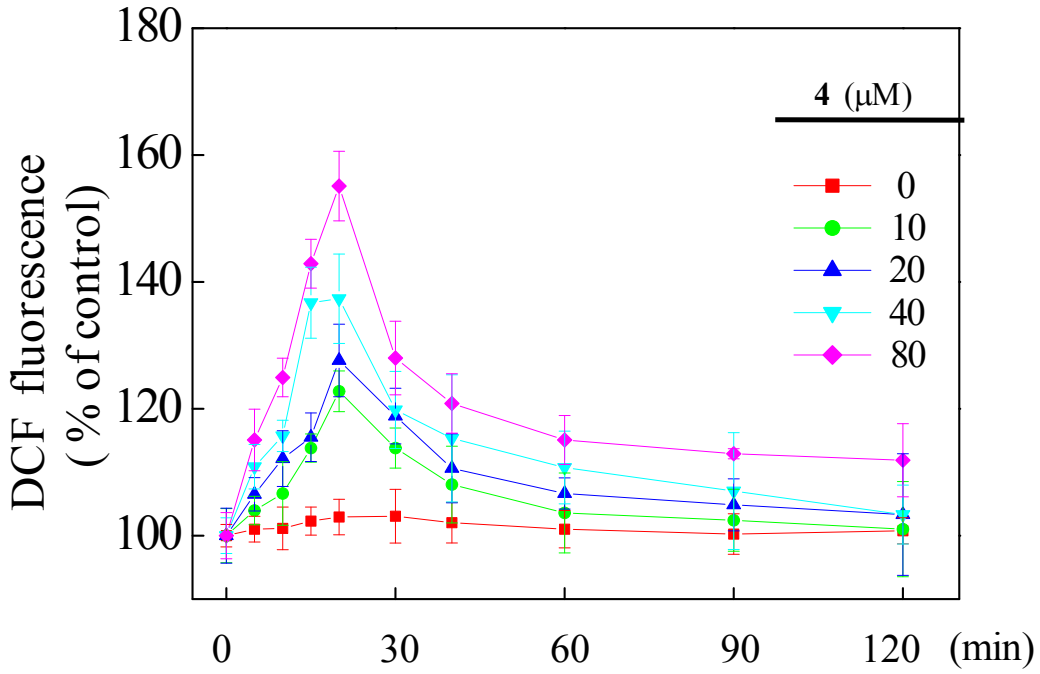

Figure S5. Important roles of ROS in cell apoptosis induced by complex 4. Effects of concentration on intracellular ROS generation after treatment with complex **4** in A375 cells. A375 cells were treated with 10 μM DCF-DA for 30 min.

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