

**Discovery of A Dual-Targeting Organometallic Ruthenium  
Complex With High Activity Inducing Early Stage Apoptosis of  
Cancer Cells**

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## Experimental details

### Inhibition of DNA Replication

To examine if the ruthenium complexes bound DNA leads to the inhibition of the DNA replication by polymerase, the Homo Sapiens High Mobility Group Box 1 sequence (HMGB1, 648bp, NM\_002128.4) was used as a template to carry out PCR and to detect the inhibition of elongation function of the DNA polymerase as a result of the binding of ruthenium complex **3** with DNA. 50 ng HMGB1 DNA was incubated with *ca.*  $10^4$ -fold complex **3** (*w/w*) for 48 hours at 37 °C. Then, 10 ng HMGB1 DNA or complex **3** bound DNA were added to forward primer and reverse primer (each final concentration is 0.125  $\mu$ M) in 20  $\mu$ L PCR reaction systems respectively. The forward primer sequence is 5'-ATGGCAAAGGAGATCCT-3' and the reverse sequence is 5'-TTATTCATCATCATCATCTTC-3'. Reactions were cycled 20 times for 30 s at 94 °C and 60 s at 72 °C. All of the PCR products were loaded to electrophoresis for product analysis.

### **Cellular uptake**

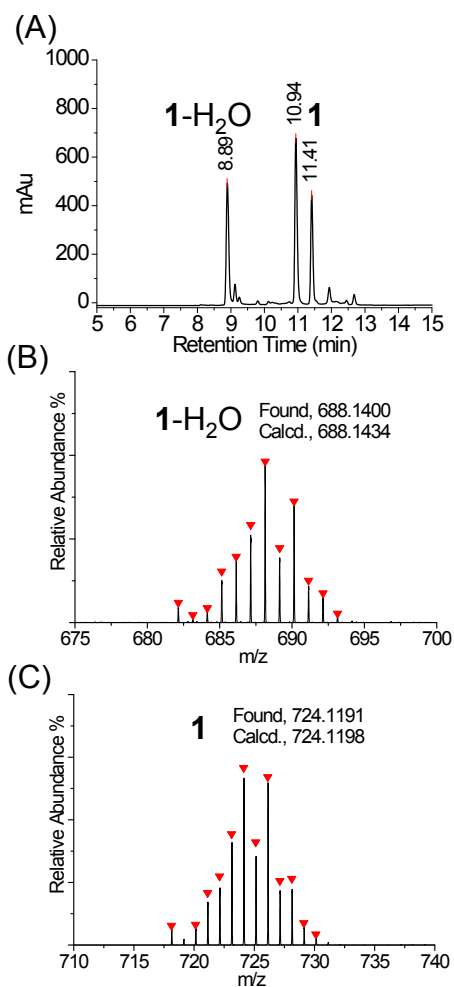
Ru compound was dissolved in DMSO to yield a 6 mM stock solution. A549 cells were seeded in a Corning cellular culture dish containing 8 mL of growth medium, when coverage up to 90%, cells were treated with 50  $\mu$ M (0.5% DMSO) of the Ru compound at 310K for 48 h. The control cells were incubated in DMSO medium (0.5% DMSO). Then the media were removed, the cells were washed with PBS solution three times. PBS containing 0.04% EDTA (3 mL) was used to detach the cells. The combined cells were centrifuged (500 g) for 2 min at 4 °C, and the cells were washed three times with 1 mL of ice-cold PBS. The cell suspension was divided into two parts, in which the metal located in the membrane protein or nucleus DNA were analyzed respectively. The Bestbio-Membrane Protein Extraction kit was used to extract the membrane protein, and TIANamp Genomic DNA Kit RNase A (TIANGEN Biotech (Beijing) Co., Ltd.) was used to extract the nucleus DNA. The protein concentration was determined using BCA Protein Assay Kit (TIANGEN Biotech (Beijing) Co., Ltd.). The DNA concentration was determined by UV-Visible spectroscopy (260 nm). 150  $\mu$ L of the remaining extracting solutions were transferred to PFA crucible, adding 4 ml 20% HNO<sub>3</sub> and digested at 200 °C until completely dried. The solid residues were re-dissolved in 1% HNO<sub>3</sub> and the ruthenium was determined by ICP-MS. Levels of Ru were expressed as ng Ru per mg protein or DNA. Results are presented as the mean of seven determinations for each experiment and expressed as mean  $\pm$  SD (standard deviation).

**Table S1.** Crystallographic data and structure refinement of complex **4**.

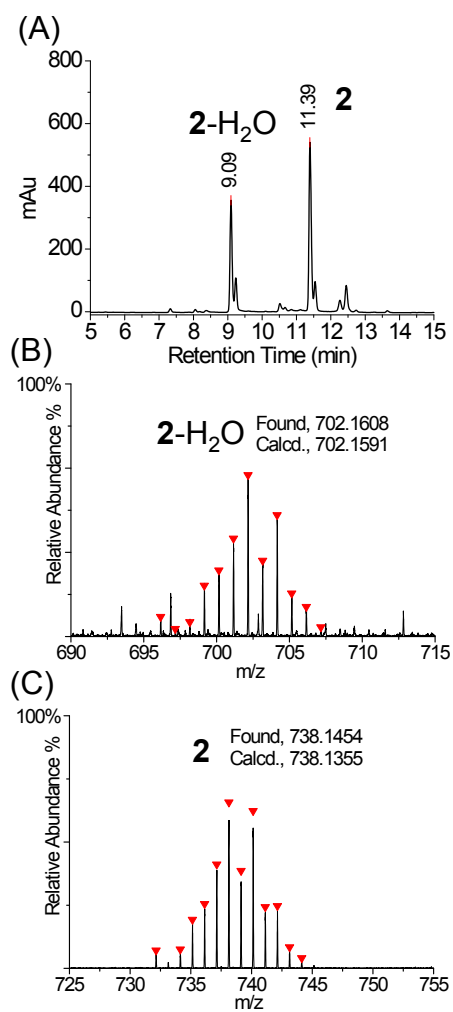
Formula	$C_{32}H_{39}ClF_{12}N_7O_2P_2Ru \cdot C_2H_5OC_2H_5 \cdot 2CH_3OH \cdot PF_6$
Molecular weight	1282.33
$T$ (K)	173.1500
Wavelength ( $\text{\AA}$ )	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
$a$ ( $\text{\AA}$ )	10.036(2)
$\alpha$ ( $^\circ$ )	73.62(3)
$b$ ( $\text{\AA}$ )	14.855(3)
$\beta$ ( $^\circ$ )	76.36(3)
$c$ ( $\text{\AA}$ )	18.895(4)
$\gamma$ ( $^\circ$ )	87.09(3)
$V$ ( $\text{\AA}^3$ )	2626.0(11)
$Z$	2
$D_x$ (g / $\text{cm}^3$ )	1.622
$\mu$ ( $\text{mm}^{-1}$ )	0.556
$F(000)$	1302
Crystal size (mm)	$0.428 \times 0.224 \times 0.118$
$\theta$ range for data collection ( $^\circ$ )	2.072 to 27.486
Index ranges	$-13 \leq h \leq 13, -18 \leq k \leq 19, -24 \leq l \leq 24$
Reflections collected	22286
Independent reflections	11740 [R(int) = 0.0519]
Completeness to $\theta = 25.242^\circ$	97.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.0000 and 0.8081
Refinement method	Full-matrix least-squares on $F^2$
Data / restraints / parameters	11740 / 103 / 713
Goodness-of-fit on $F^2$	1.120
Final R indices [ $I > 2\sigma(I)$ ]	$R_1 = 0.0840, wR_2 = 0.1707$
$\Delta\rho_{\text{max}}, \Delta\rho_{\text{min}}$ ( $e.\text{\AA}^{-3}$ )	0.970, -1.017

**Table S2.** Selected bond lengths (Å), angles (°) and torsion (°) for complex **4**.

Ru1-N5	2.100(4)	Ru1-C23	2.183(6)
Ru1-N6	2.140(4)	Ru1-C24	2.176(5)
Ru1-N7	2.129(5)	Ru1-C25	2.217(5)
Ru1-C26	2.175(6)	Ru1-C27	2.184(6)
Ru1-C28	2.216(6)	N5-Ru1-N6	86.12(16)
N5-Ru1-N7	86.67(17)	N5-Ru1-C23	158.02(19)
N5-Ru1-C24	119.92(19)	N5-Ru1-C25	91.59(19)
N5-Ru1-C26	89.58(19)	N5-Ru1-C27	114.2(2)
N5-Ru1-C28	151.6(2)	N6-Ru1-C23	115.8(2)
N6-Ru1-C24	152.9(2)	N6-Ru1-C25	162.1(2)
N6-Ru1-C26	124.6(2)	N6-Ru1-C27	96.4(2)
N6-Ru1-C28	93.0(2)	N7-Ru1-N6	79.31(17)
N7-Ru1-C23	95.2(2)	N7-Ru1-C24	94.0(2)
N7-Ru1-C25	118.3(2)	N7-Ru1-C26	155.5(2)
N7-Ru1-C27	158.5(2)	N7-Ru1-C28	121.1(2)
C22-N6-Ru1	109.7(3)	C21-N7-Ru1	111.2(3)
Ru1-N5-C19-C18	-166.6(4)	Ru1-N5-C20-N4	167.6(3)
Ru1-N6-C22-C21	-44.4(5)	Ru1-N7-C21-C22	-39.8(5)



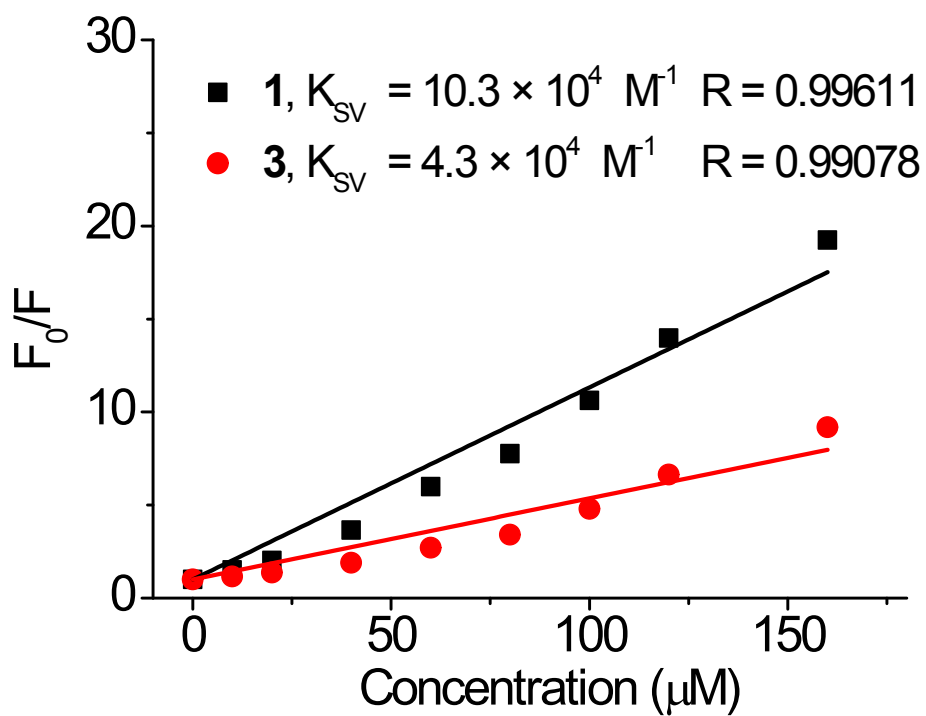
**Fig. S1.** (A) The HPLC chromatogram with UV detection at 254 nm for the hydrolysis of complex **1** for 1 h at 37 °C. The peak with retention time of 8.89 min corresponds to the aqua species of complex **1** (**1**-H<sub>2</sub>O). The peaks with retention time of 10.94 min and 11.41 min correspond to complex **1**. (B) and (C) show the mass spectra of **1**-H<sub>2</sub>O (a) and complex **1** (b). Reversed red triangles indicate the theoretical isotope distributions.



**Fig. S2.** (A) The HPLC chromatogram with UV detection at 254 nm for the hydrolysis of complex **2** for 1 h at 37 °C. The peak with retention time of 9.23 min corresponds to the aqua species of complex **2** (**2**-H<sub>2</sub>O). The peak with retention time of 11.39 min corresponds to complex **2**. (B) and (C) show the mass spectra of **2**-H<sub>2</sub>O and complex **2**. Reversed red triangles indicate the theoretical isotope distributions.

**Fig. S3.** (A) The HPLC chromatogram with UV detection at 254 nm for the hydrolysis of complex **3** for 1 h at 37 °C. The peak with retention time of 11.10 min corresponds to the di-aqua species of complex **3** (**3**-2H<sub>2</sub>O). The peak with retention time of 9.07 min corresponds to the mono-aqua species of complex **3** (**3**-H<sub>2</sub>O). No peaks corresponding to the free ligands were found. (B) and (C) show the mass spectra of the **3**-2H<sub>2</sub>O and **3**-H<sub>2</sub>O. Reversed red triangles indicate the theoretical isotope distributions.

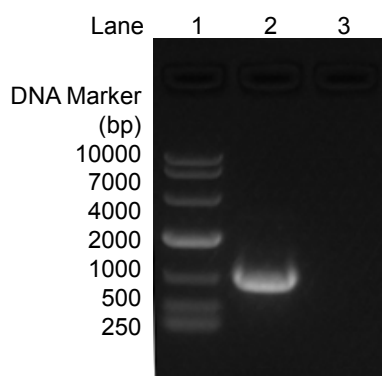




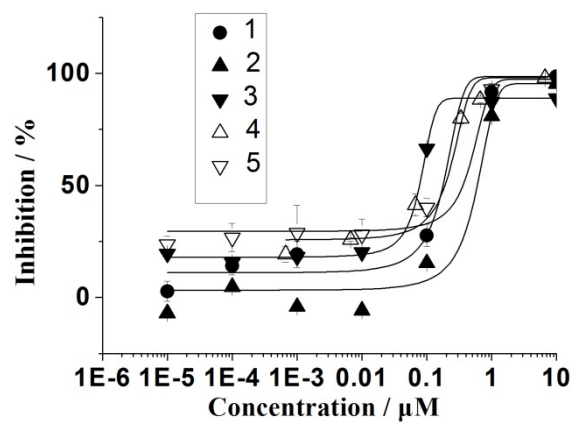
**Fig. S4.** Stern–Volmer plots for competitive reactions of the Hoechst 33342 : CT DNA (20 : 200  $\mu\text{M}$ ) complex with complex **1** or **3** based on the fluorescence titration results shown in Fig. 3 in the main text.

### Inhibition of DNA replication

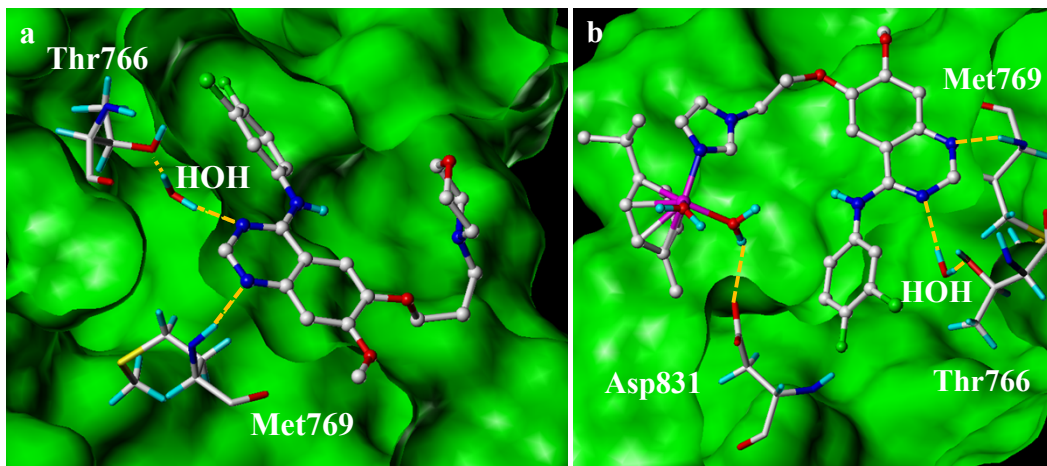
As shown in the result of electrophoresis (Fig. S5), the ruthenated HMGB1 DNA template cannot be amplified by PCR, whereas the intact HMGB1 DNA in the control group was amplified and the band was found between the 500 bp and 1000 bp of the DNA markers, which is in line with the template DNA used (648 bp). It can be concluded that the binding of the ruthenium complex **3** to the DNA can inhibit its replication.



**Fig. S5.** Electrophoresis of the PCR products. The lane 1 is the DNA marker. Lane 2 and 3 are the PCR products using HMGB1 DNA and complex **3** bound HMGB1 DNA as template, respectively.



**Fig. S6.** Dose-dependent inhibitory efficiency curves of the complexes 1 – 5 towards EGFR. Points: mean  $\pm$  SD of triplicate determinations.



**Fig. S7.** The docked conformations of gefitinib (a) and di-aqua complex **3** (b) at the ATP binding pocket of EGFR kinase. The yellow broken lines illustrate the possible H-bond interactions.

The three dimensional molecular models of di-aquated **3** or gefitinib docked in EGFR ATP binding pocket were constructed by Sybyl-X 1.1 program based on the structure of EGFR-erlotinib complex from PDB. The docked conformation of gefitinib (Fig. S7a) shows two H-bonds: one is between the N1 of quinazoline group and N-H of Met769, and the other between N3 of quinazoline and Thr766 through a water molecule as an “H-bond bridge”. These two key H-bonds stabilize the binding conformation, and are similar to that of erlotinib binding to EGFR. The di-aquated **3** can form similar H-bonds with EGFR (Fig. S7b) to those of gefitinib and erlotinib. Moreover, the aquated **3** formed an additional H-bond between the aqua ligand and the C=O group of Asp831, which appears to compromise the steric hindrance, thus increase the binding affinity of the complex to EGFR.