Electronic Supplementary Information (ESI)

Ru(III) complexes with pyrazolopyrimidines as anticancer agents: bioactivities and the underlying mechanisms

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The detailed procedures of other experimental methods

1.1. X-ray crystallography

The X-ray single crystal diffraction data for complexes **1-3** were collected on an Agilent SuperNova with an EOS detector (Rigaku Corporation, Tokyo, Japan) equipped with graphite monochromatic Mo-Kα radiation (λ=0.71073 Å) at 293(2) K. The structures were solved with direct methods and refined using the OLEX 2 or SHELXL programs. The non-hydrogen atoms were refined in anisotropic thermal parameters.

1.2. Solubility and stability studies of the complexes 1-3 in PBS solution

The stability of the complexes **1-3** in 1×PBS buffer solution was assayed by UV-visible spectroscopy. The complexes **1-3** were freshly prepared by dissolving in DMSO (2.0×10^{-3} M) and then diluted with 1×PBS buffer solution to the final concentration of 20 μM (1% DMSO). The UV-visible spectroscopy of complexes **1-3** were recorded for three times (0 h, 12h, 24 h and 48 h, respectively) at 37℃.

1.3. Antiproliferative activity measurement

The human cell lines (SK-OV-3, A549, T-24, MGC-803, HeLa, NCI-H460 and HL-7702) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai). The cytotoxicity of synthesized compounds was checked by MTT assays and neutral red assay with several cancer cells. Cells were seeded in 96-well cell-culture plate in 180 μL of Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), and treated with test

compounds for 48 h at 37 °C under 5% CO_2, the final concentration of compounds ranged from 1.25 to 40 μM (1.25, 2.5, 5.0, 10.0, 20.0 and 40.0 μM).

MTT assay: The MTT solution [10 μL, 10 mg/mL in phosphate buffer saline (PBS)] was added to each well and the plates were incubated for another 4 h at 37 °C in a humidified atmosphere with 5% CO_2, to dissolve the formazan crystals DMSO (100 μL) were added to replace the MTT-containing medium. The absorbance was determined on a microplate reader and the cytotoxicity was evaluated based on the percentage of cell survival compared with the negative control. The Bliss method (n = 5) was used to calculated final IC_{50} values. All tests were repeated in at least three independent trials.

Neutral red assay: According to the instructions of neutral red cell proliferation and cytotoxicity assay kit, neutral red staining solution (20 μL) was added after the treatment with the complexes, then incubated for 2 h. The cell culture solution containing neutral red staining solution was removed and washed with PBS solution for 1-2 times, cell lysis solution (200 μL) was added and shacked for 10 min, then absorbance was measured at 540 nm and subtracted the value of 630 nm as a reference wavelength. All tests were repeated in at least three independent trials.

1.4. Cell viability analysis

Calcein/PI staining: Cells were plated onto 6 wells (2×10^5 cells/well) at 37℃ in a 5% CO_2 incubator overnight, the medium was aspirated, and 2 mL of fresh medium containing different concentrations of complex **1** was added to the well. After 8 h, living cells and dead cells were detected using Calcein/PI Cell Viability/Cytotoxicity Assay Kit (Beyotime; Cat: C2015M) in accordance with the manufacturer's instructions. Then, the cells were observed Automatic cell

imaging system (EVOS ™ FL Auto 2).

Colony formation assays: SK-OV-3 cells were inoculated in 12-well plates (2000 cells per well) overnight and treated with complex **1** at 37℃ for 24 h, then the non-drug DMEM medium should be replaced every 3 days. After that, the cells were fixed with paraformaldehyde for 30 min and stained with crystal violet for another 20 min. Finally, air dried and took photos.

1.4. Mitochondrial membrane potential (ΔΨ) analysis

Mitochondrial membrane potential was analyzed by a cell permeable cationic dye (JC-1 staining). The SK-OV-3 cells were seeded on a 6 wells plate, treated with complex **1** (5.0, 10.0 and 15.0 μM) for 24 h, then harvested and washed the cells for three times with PBS, resuspended with JC-1 from the Mitochondrial Membrane Potential Assay Kit (C2006; Beyotime, 5 mg/mL) for 30 min in dark, washed twice and measured by flow cytometry (λ_{ex}=488 nm).

1.5. Measurement of ROS generation

Determination of Intracellular Reactive Oxygen Species (ROS)： To determine the formation of intracellular ROS, especially, especially H_2O_2 and $O_2^{\bullet-}$, fluorescence microscopy technique was employed. Briefly, SK-OV-3 cells (2×10^4cells/mL) were seeded in a 6-well plate overnignt. The cells were then incubated with complex **1** (5.0, 10.0 and 15.0 μM) for another 8 h. After that, the cells were thoroughly washed with PBS and incubated with either 10 μM DCFH-DA for 30 min or 5 μM DHE for 15 min in dark conditions. The cells were again washed several times with PBS and the fluorescence images were captured using a fluorescence microscope (Cyration 5 confocal microscope). The green fluorescence images (DCFH-DA; λ_{Em}= 529 nm) were captured after

excitation at (λ_{Ex}) at 488 nm, while the red fluorescence images (DHE; λ_{Em}= 610 nm) were obtained after excitation (λ_{Ex}) at 535 nm by Automatic cell imaging system (EVOS ™ FL Auto 2).

Flow Cytometry: The complex **1** (10.0 μM) was treated for 8 h, and then the cells were harvested and incubated with DCFH-DA (10 μM) for 15 min, collected by centrifugation and washed with PBS buffer solution three times. The mean fluorescence intensity of the cells (1×10^4) was measured by flow cytometry immediately (λ_{ex}=488 nm).

Quantification of Intracellular Reactive Oxygen Species: The hydrogen peroxide assay kit (Beyotime) was used to determine the level of hydrogen peroxide in intracellular cells. The SK-OV-3cells (5×10^6 cells/mL) were seeded in a 100 mm were collected and the supernatant was discarded, washing with PBS for 3 times, then the lysate (200 μM) was added, followed by sufficient homogenization to break and lysate the cells (15 min). Centrifuged at 4℃ for about 12000 g for 5 min, supernatant was taken for subsequent determination. All the above operations must be performed on ice. The sample or standard (100 μL) were adding to test tube, and adding hydrogen peroxide detection reagent (100 μL) to each well. After gently shaking, the samples were left at room temperature for 30 min. The absorbance of 560nm wavelength was then determined immediately. The concentration of hydrogen peroxide in the sample was calculated according to the standard curve.

1.6. Intracellular free Ca^{2+} analysis

The fluorescent dye Fluo-3 AM was used to decide the level of intracellular free Ca^{2+}, which could across the membrane of the cell and be cut into Fluo-3 by intracellular esterase, then Fluo-3 combines with the Ca^{2+} and show a strong fluorescence (λ_{ex}=488 nm). The cells were exposed to **1**

(10.0 μM) for 24 h, harvested and washed three times with PBS, after adding Fluo-3 AM (5.0 μM) to resuspend the cells for 30 min in dark. Intracellular free Ca^{2+} analyst was carried by Flow cytometer (λ_{ex}=525 nm).

1.7. Caspase-3/9 activity assay

Caspase-3/9 activity were studied by CaspGlOW™ Fluorescein Active Caspase 3/8/9 Assay kit according to the instructions of manufacturer. 1×10^6 of SK-OV-3 cells were cultured for 24 h. After a treatment with complex **1** (5.0, 10.0 and 15.0 μM) for 24 h, the cells were harvested and washed three times with PBS and then were mixed with 300 μL serum medium. Subsequently cells were permissible to introduced in appropriate Caspase-3/9 reagents after that incubated for 0.5 h. The cells were investigated by Flow cytometer and the results were expressed as a percentage change on the activity comparing with the untreated control group.

1.8. Western blotting analysis

After treatment at various concentrations of complex **1** for 48 h, cells were harvested and washed three times with PBS and then lysed in ice-cold using the lysis buffer (150 mM NaCl, 100 mM Tris-HCl, pH 7.4, 1% Triton X-100, 10% glycerol, 10 mM NaF, 5 mM sodium orthovanadate, 5 mM sodium pyrophosphate, 0.1% SDS) with protease inhibitor. Lysates were cleared by centrifugation. Protein concentrations were determined using the BCA assay. The total protein samples (20 μg) were separated on 10% SDS-polyacrylamide gel. The gel was then transferred to vinylidene fluoride membranes, blocked with 5% BSA in TBST buffer and probed with a primary antibody at 4 ℃ overnight. It was then probed with the required secondary antibody. Finally, the

signal was detected with WEST-SAVE UP luminal-based ECL reagent (ABFrontier, Korea).

1.9. Topo I inhibition assay

The Topo I inhibition assay was studied on a commercialized DNA topoisomerase I kit (Takara) following the instruction. The reaction mixture (10 μL) contained Topo I buffur, pBR322 plasmid DNA (250 ng), Topo I (1 unit) and indicated complex concentrations (1% DMSO). The mixture were incubated for 30 min at 37℃, adding 2 μL 6× DNA loading buffer (bromophenol blue), then the samples were loaded to the 1% agarose gel. The gels was put into 1×TAE running buffer and were run at 70 V for 80 min. The gels were stained with EB (ethidium bromide, 0.5 μg/mL) for 20 min and destained in water for 10 min. Finally, the image is developed by irradiation under ultraviolet lamp.

1.10. Comet assay

The DNA damage induced by complex **1** was inspected by comet assay following the instructions of the manufacturer using DNA damage detection kit (SCGE). SK-OV-3 cells were incubated with complex **1**(5, 10 and 15 μM) for 24h, then the cells were collected and washed with PBS twice. The cell suspensions were mixed with 0.7% low-melting point agarose (v:v 1:5) and spread on slides precoated with normal agarose, then the low melting-point agarose was spread on slides. The slides were putted at 4 ℃ for 15 min and soaked in cold lysis solution for 90 min. After immersion in an alkaline electrophoresis buffer for 30 min, the slides was electrophoresed at 50 V for 25 min, then it was soaked in Tris-HCl buffer solution (0.4 mM, pH 7.35) for 20 min for three times. Finally, the slides were stained with PI dye for 10 min and photographed by confocal microscopy.

1.11. DNA binding studies

1.11.1. UV-Visible absorption assay

The UV–Visible absorption spectra of complex **1** (3 mL, 2×10^{-5} mol/L in Tris-HCl (pH = 7.4) by increasing the concentration of DNA from 0 to 3.36×10^{-5} mol/L were recorded in the wavelength range of 200–700 nm on a Cary 100 UV–Visible spectrophotometer (Agilent) at 25℃. The data were plotted using origin 8.0 software.

1.11.2. Competitive fluorescence measurements

Competitive fluorescence experiments were measured using a JASCO spectrofluorometer (FLS980). The aqueous solution of methylene blue (MB) was used to investigate the competitive ability of complex **1** for DNA binding sites. 3 mL solution of DNA-MB conjugate (ct-DNA=2×10^{-4} M and MB= 5×10^{-6} M in Tris-HCl pH = 7.4) was titrated by increasing concentration of the complex **1** from **0** to 8.3×10^{-6} M in the wavelength range of 640–750 nm with exciting wavelength at 630 nm. The fluorescence experiments were carried out at room temperatures.

1.11.3. Circular dichroism spectroscopy

DNA solution with a concentration of 5×10^{-5} M was prepared with buffer solution Tris-HCl (pH = 7.4). Then complex **1** was added into the ct-DNA solution ([DNA]/[complex] =10:1). After 10 min incubation, the changes of CD spectra were observed by a JASCO spectrofluorometer (J-1500) in a wavelength range of 200-350 nm at room temperature. The CD signals of the Tris-HCl buffer solution were subtracted as the background.

1.11.4. Viscosity experiments

LICHEN NDJ-9S Digital Viscometer was used in this experiment, and the temperature of the solution was kept constant at 25℃. A digital stopwatch was employed to measure the flow time at a

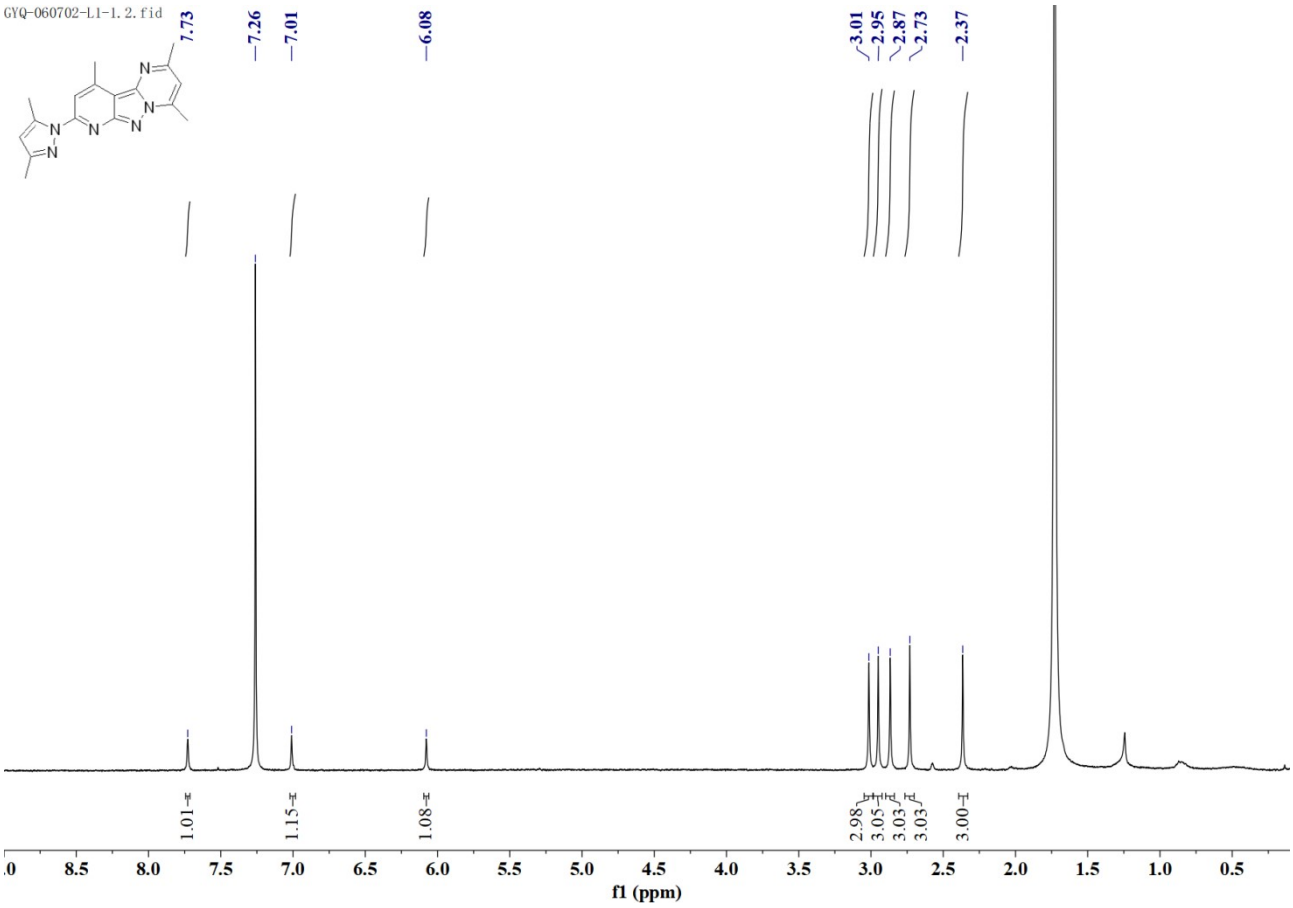
constant ct-DNA concentration (1×10^{-3} M). The viscosity (η) was determined from the mean value of three measurements. Results have been plotted as $(\eta/\eta_0)^{1/3}$ against the [**1**]/[DNA] ratio. In this model, η_0 and η refer to the relative viscosity contributions of DNA in the absence and the presence of complex **1**.

1.11.5. Agarose gel electrophoresis analysis

Effects of plasmid pBR322 on DNA: Complex **1** were prepared as 2×10^{-3} M stock solution of DMSO and diluted to 50 and 200 μM by 1×TBE buffer solution. Then, add 0.5 μL pBR322plasmid DNA (0.5 μg/ μL) to the sterilized microplastic centrifuge tube, different concentrations of complex **1** was added, respectively, the volume was fixed to 10 μL with Tris buffer solution, the mixture were reacted at 37 ℃ for 4 h, then added 2 μL bromophenol blue, which was used as an indicator for electrophoresis. The gels (1%) was performed at 80 V for 80 min in 1×TBE buffer solution, and finally imaged under UV lamp.

1.11.6. Molecular docking study

Molecular docking of complex **1** was simulated to three-dimensional X-ray structure of DNA (PDB code: 1D64). Docking processes were carried out using AutoDock 4.0. The DNA molecule was performed *via* AutoDock 4.0 to add polar hydrogen atoms, correct partial charge, and calculate gasteiger charges. Affinity grid maps of 50 × 50 × 50 Å in x, y, and z directions were generated using the AutoGrid program. Docking was performed *via* AutoDock 4.0. The best-docked structures for complex **1** were determined, based on the model energy score.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7.01
6.08
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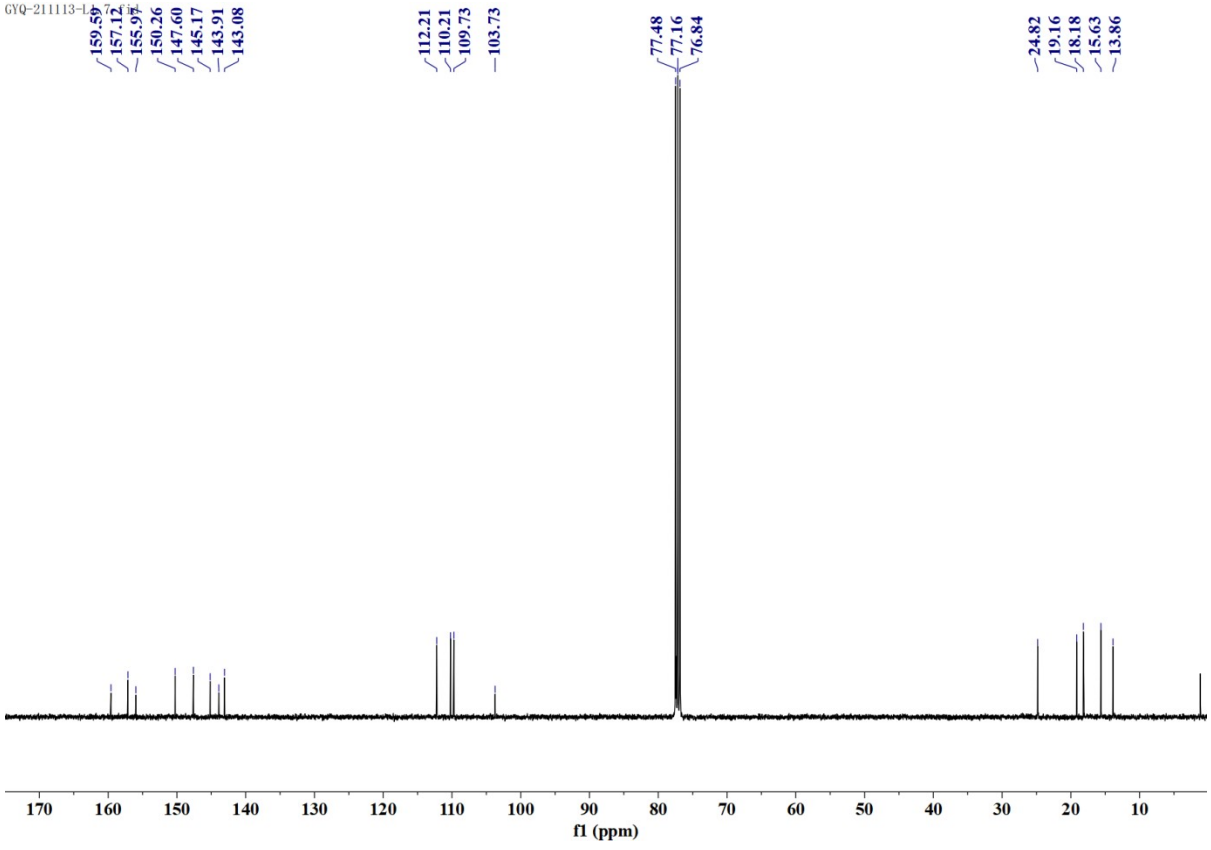
3.03
3.03
3.00
f1 (ppm)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24.82
19.16
18.18
15.63
13.86
f1 (ppm)

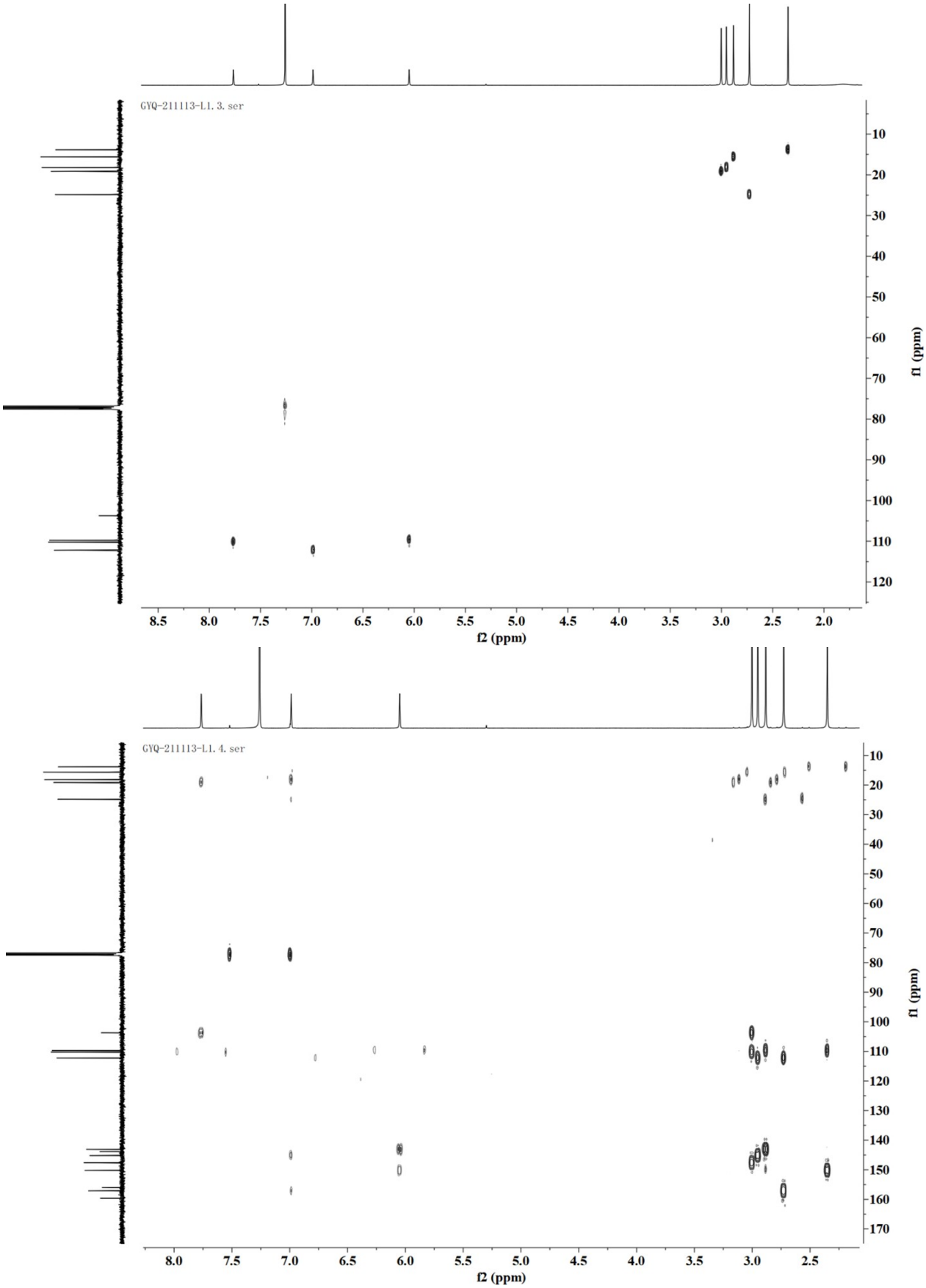
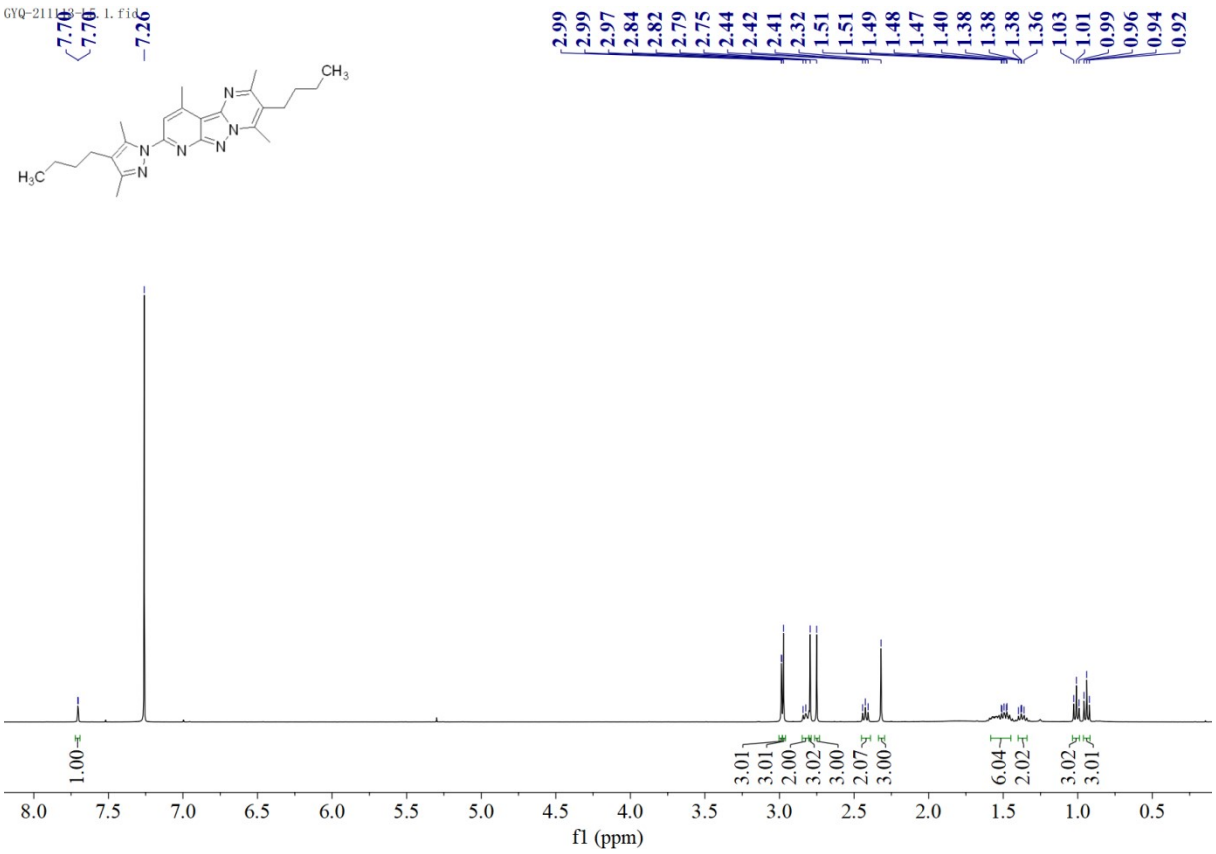


Fig. S1. The ^{1}H NMR (400 M, chloroform-*d*) and ^{13}C NMR (151 MHz, chloroform-*d*), HSQC and HMBC spectra of **L²**.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7.26
2.99
2.99
2.97
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2.82
2.79
2.75
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2.42
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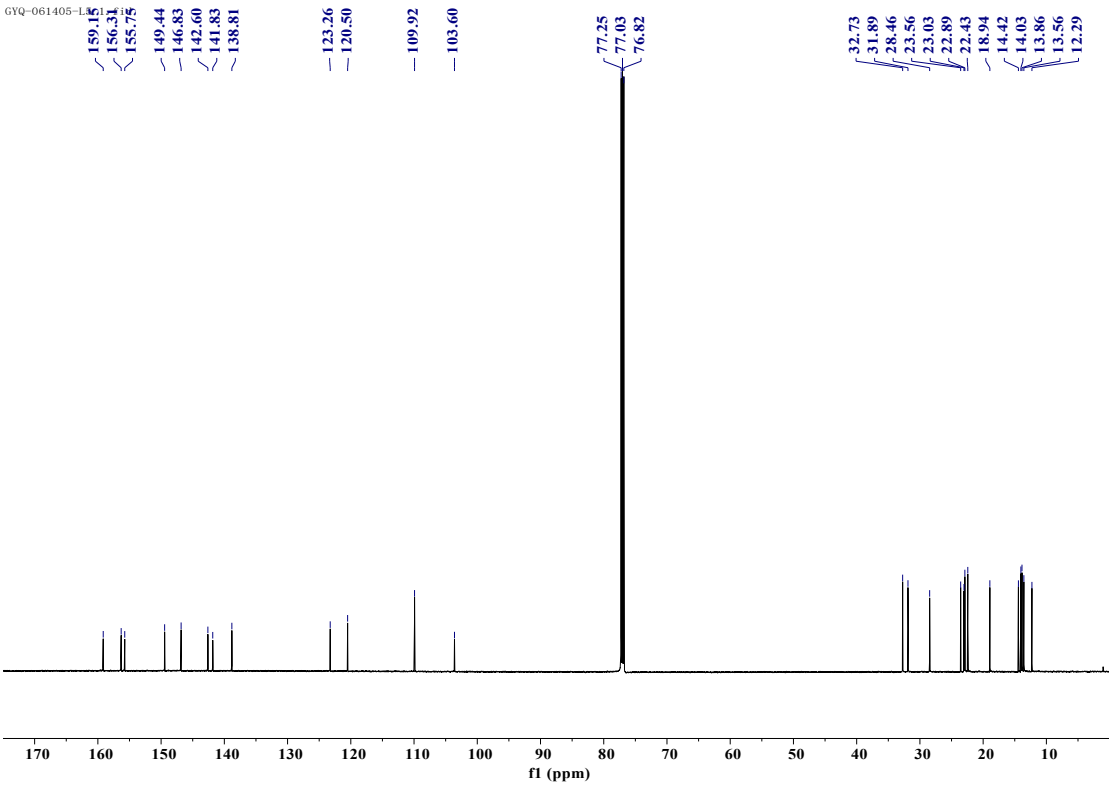
2.32
1.51
1.51
1.49
1.48
1.47
1.40
1.38
1.38
1.38
1.36
1.03
1.01
0.99
0.96
0.94
0.92
CH3
H3C
1.00
3.01
3.01
2.00
3.02
3.00
2.07
3.00
6.04
2.02
3.02
3.01
f1 (ppm)


159.15
156.31
155.75
149.44
146.83
142.60
141.83
138.81
123.26
120.50
109.92
103.60
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77.03
76.82
32.73
31.89
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14.03
13.86
13.56
12.29
f1 (ppm)

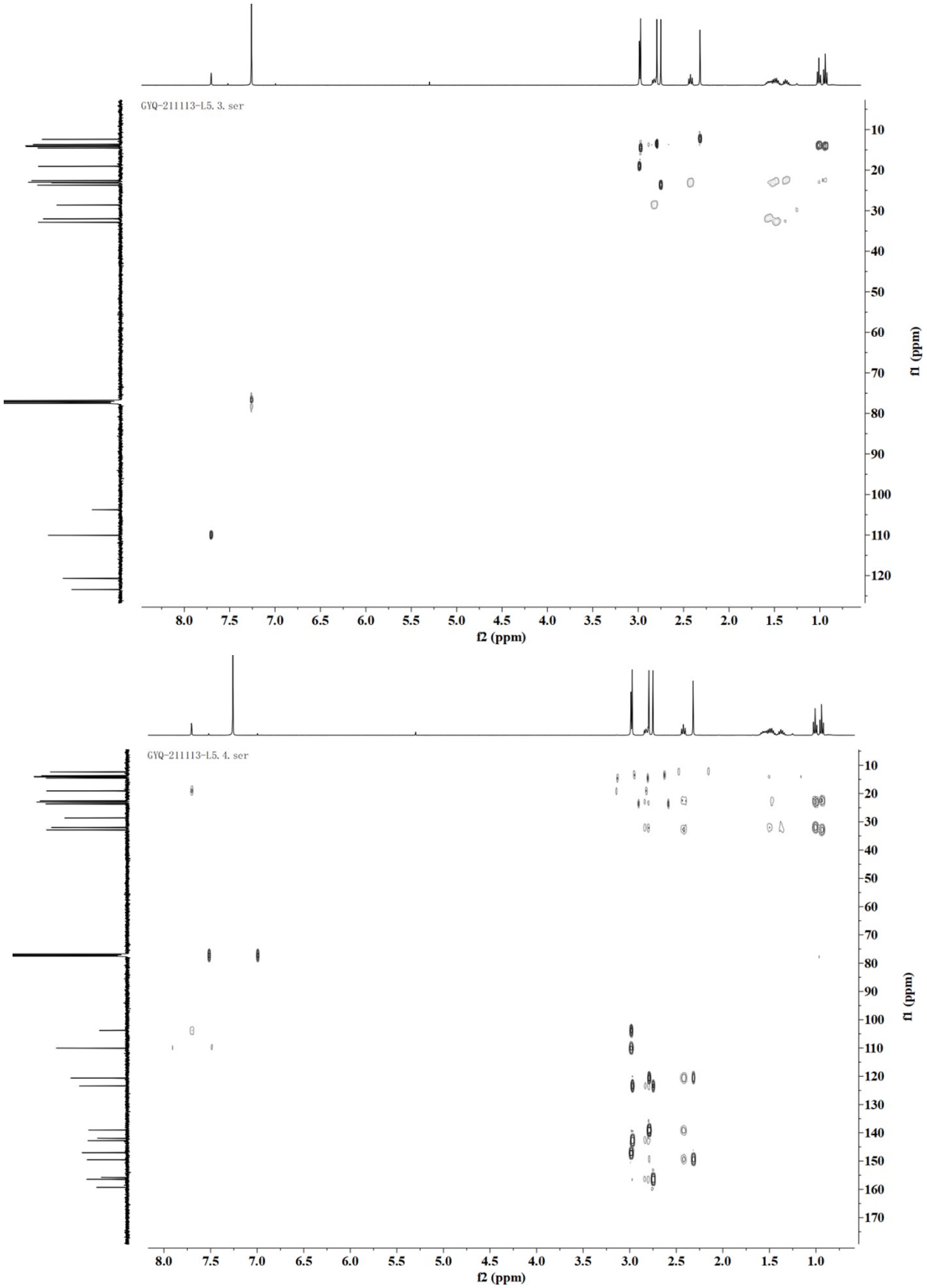

Fig. S2. The ^{1}H NMR (400 M, Chloroform-*d*) and ^{13}C NMR (6 00 M, Chloroform-*d*), HSQC and HMBC spectra of spectra of **L^{3}.**

Table S1 ^{1}H NMR and ^{13}C NMR spectroscopic data for **L²** and **L³**.

position	L^2		L^5	
	δ_C[a, c] mult	δ_C[a, c]	δ_C[a, c] mult	δ_C[a, b]
1		145.17		142.60
2	7.01 s	109.73		138.81
3		159.59		159.15
4		143.08		123.26
5		103.73		103.60
6		147.60		146.83
7	7.73 s	112.21	7.70 d	109.92
8		155.97		155.75
9		143.91		141.83
10	6.08 s	110.21		120.50
11		150.26		149.44
12		157.12		156.31
13	2.73 s	18.18	2.75 s	14.42
14	2.87 s	24.82	2.79 s	22.43
15	2.95 s	19.16	2.97 d	18.94
16	3.01 s	13.86	2.99 d	12.29
17	2.37 s	15.63	2.32 s	13.56
1’			2.83 d	28.46
2’			1.58-1.45 m	32.73
3’			1.58-1.45 m	23.03
4’			1.01 t	14.03
1’’			2.42 t	23.56
2’’			1.58-1.45 m	31.89
3’’			1.40-1.34 m	22.89
4’’			0.94 t	13.86

[a]Measured in 400 M, Chloroform-*d*, [b]600 MHz, [c]400 MHz. Assignments were made based on HSQC and HMBC experiments.

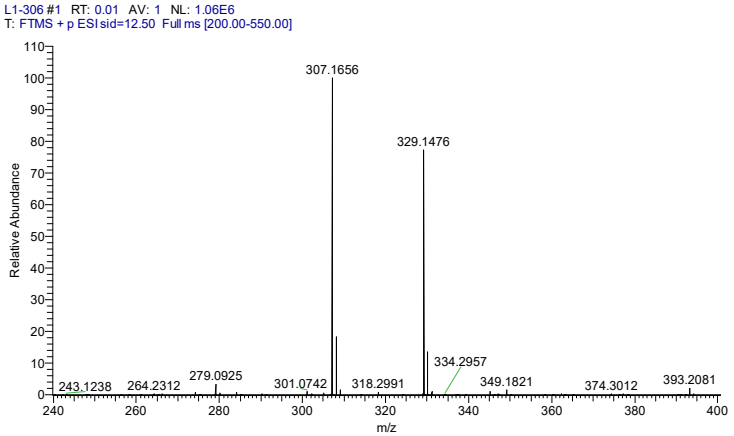

Fig. S3.The HRMS of the ligand **L2**.

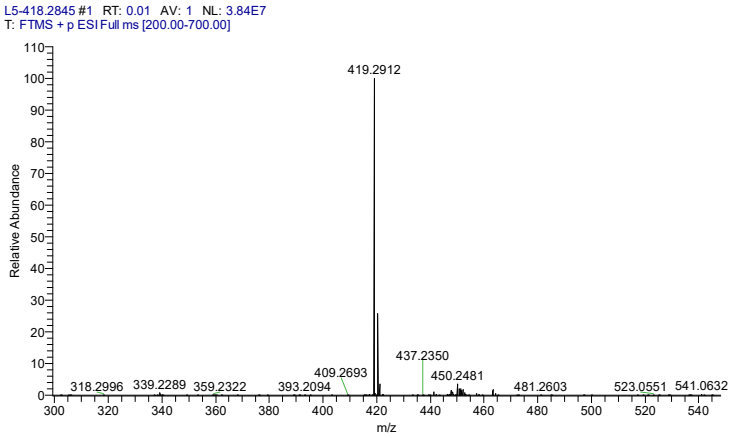

Fig. S4. The HRMS of the ligand **L3**.

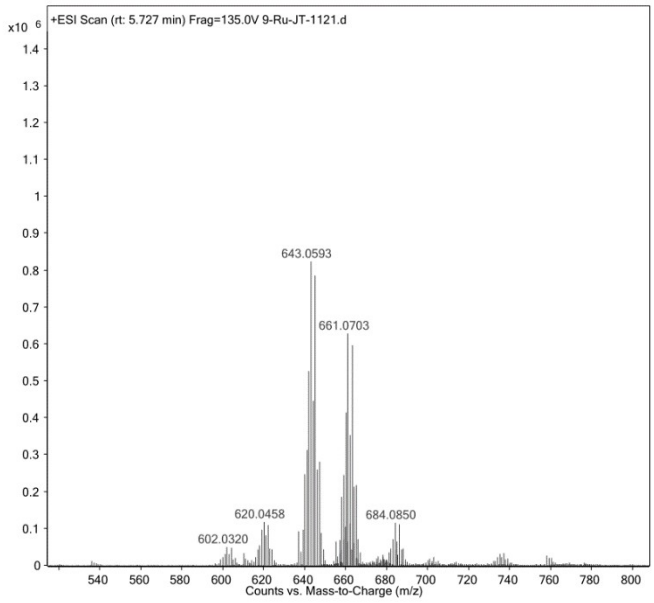

Fig. S5. The HRMS of the complex **1**.

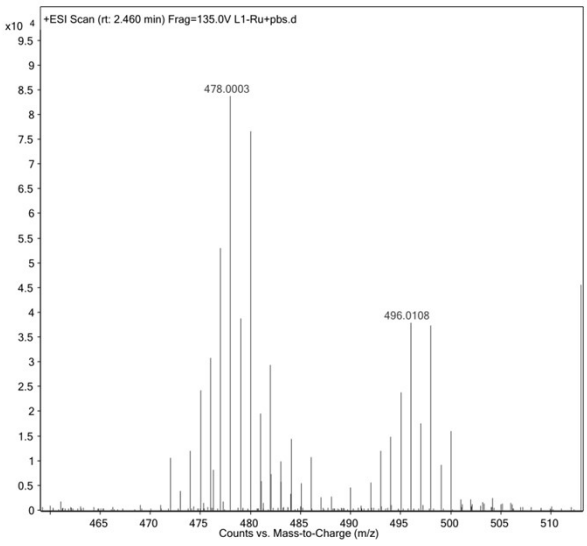

Fig. S6. The HRMS of the complex **2**.

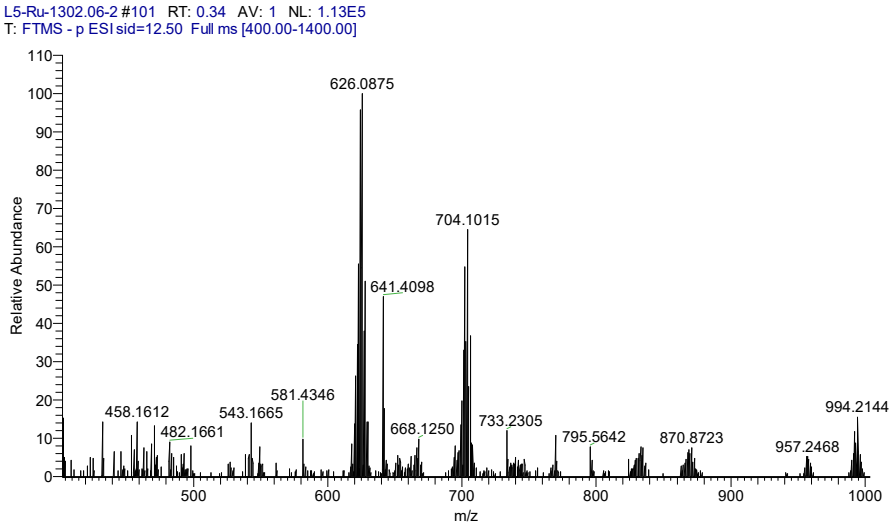

Fig. S7. The HRMS of the complex **3**.

Tabel S2 Crystallographic data and refinements of complexes **1**–**3.**

Complex code	1	**2**	**3**
Chemical formula	$C_{27.5}H_{28}Cl_3N_6O_{2.5}Ru$	$C_{17}H_{20}Cl_3N_6ORu$	$C_{50}H_{73}Cl_6N_{12}O_{2.5}Ru_2$
Formula Weight	689.98	531.81	1297.04
Temperature(K)	293(2)	293(2)	292(5)
Crystal system, space group	*Monoclinic, P21/c*	*monoclinic, C2/c*	*Triclinic, P-1*
a/b/c	*a*=17.9942(3)Å	*a*=15.1044(6)Å	*a*=13.3340(3)Å
	b=7.55123(19)Å	*b*=11.9266(4)Å	*b*=14.3744(3)Å
	c = 20.9800(3)Å	*c* =24.2173(11)Å	*c* =15.7555(4)Å
α/β/γ	*α*= 90.00°	*α*= 90°	*α*= 78.483(2)°
	β =95.3962(8)	*β* =105.309(5)°	*β* =83.943(2)°
	γ = 90.00°	*γ* =90°	*γ* =81.607(2)°
Volume(Å^3)	2838.11(7)	4207.8(3)	2918.24(12)
Z, Caculated density(g.cm^{-3})	4, 1.615	8, 1.679	2, 1.476
μ/ (mm^{-1})	7.401	1.147	7.115
F(000)	1400.0	2136.0	1334.0
Crystal size (mm^3)	0.15×0.13×0.12	0.31×0.26×0.12	0.29×0.22×0.19
Radiation	CuKα (λ = 1.54184)	MoKα (λ = 0.71073)	Cu Kα (λ = 1.54184)
Theta range for data collection	4.932 to 151.206	6.832 to 51.992	6.328 to 148.978
Limiting indices	–21 ≤ h ≤ 22, –9≤ k ≤ 8, –26≤ l ≤ 25	–18 ≤ h ≤ 18,–14 ≤ k ≤ 14,–29≤ l ≤ 29	–16 ≤ h ≤ 15,–17 ≤ k ≤ 17,–18≤ l ≤ 19
Reflections collected / unique	19463/5665[R(int) =0.0523]	15070 / 4122 [R(int) = 0.0352]	38925 / 11583 [R(int) = 0.0338]
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraint/parameters	5665/16/382	4122/2/258	11583/53/693

Goodness-of-fit on F^2	1.033	1.075	1.018
Final R indices [I>2sigma(I)]	R_1 = 0.0697, wR_2 = 0.1496	R1 = 0.0525, wR2 = 0.1233	R1 = 0.0521, wR2 = 0.1396
R (all data)	R_1 =0.1399, wR_2 = 0.1846	R1 = 0.0706, wR2 = 0.1362	R1 = 0.0632, wR2 = 0.1478
Largest diff. peak/hole(e/Å^3)	1.71 and -0.48	1.85 and -0.98	1.10 and -0.99

Table S3. Selected bond lengths (Å) and angles (º) for **1-3**.

1			
Cl1-Ru1	2.352 (3)	Cl2-Ru1	2.331 (3)
Cl3-Ru1	2.340 (3)	N1-Ru1	2.026 (8)
N3-Ru1	2.077 (7)	O1-Ru1	2.112 (6)
Cl2-Ru1-Cl1	91.76 (11)	Cl2-Ru1-Cl3	176.54 (11)
Cl3-Ru1-Cl1	90.83 (11)	N1-Ru1-Cl1	101.8 (2)
N1-Ru1-Cl2	90.0 (2)	N1-Ru1-N3	77.4 (3)
N1-Ru1-Cl3	91.8 (2)	N1-Ru1-O1	174.3 (3)
N3-Ru1-Cl1	179.2 (2)	N3-Ru1-Cl3	89.4 (2)
N3-Ru1-Cl2	88.1 (2)	N3-Ru1-O1	96.9 (3)
O1-Ru1-Cl1	83.92 (19)	O1-Ru1-Cl3	88.4 (2)
O1-Ru1-Cl2	89.5 (2)		
2			
Cl1-Ru1	2.3268 (16)	Cl2-Ru1	2.3403 (15)
Cl3-Ru1	2.3410 (16)	N3-Ru1	2.029 (4)
N1-Ru1	1.985 (4)	O1-Ru1	2.075 (4)
Cl1-Ru1-Cl2	92.62 (6)	Cl2-Ru1-Cl3	176.35 (6)
Cl1-Ru1-Cl3	90.91 (6)	N1-Ru1-Cl1	101.50 (13)
N1-Ru1-Cl2	89.11 (14)	N1-Ru1-N3	78.39 (17)
N1-Ru1-Cl3	91.11 (14)	N1-Ru1-O1	175.42 (18)
N3-Ru1-Cl1	178.99 (14)	N3-Ru1-Cl3	88.09 (13)
N3-Ru1-Cl2	88.39 (13)	N3-Ru1-O1	97.17 (16)
O1-Ru1-Cl1	82.96 (12)	O1-Ru1-Cl3	89.87 (15)
O1-Ru1-Cl2	89.63 (15)		
3			
Ru1-Cl1	2.3428 (15)	Ru2-Cl4	2.3202 (14)
Ru1-Cl2	2.3796 (15)	Ru2-Cl5	2.3633 (15)
Ru1-Cl3	2.3316 (14)	Ru2-Cl6	2.3589 (13)

Ru1-O1	2.091 (3)	Ru2-O2	2.123 (3)
Ru1-N1	2.060 (4)	Ru2-N3	2.071 (3)
Ru1-N2	2.016 (4)	Ru2-N4	2.015 (4)
Cl1-Ru1-Cl2	92.81 (6)	Cl4-Ru2-Cl5	93.83 (6)
Cl3-Ru1-Cl1	174.71 (6)	Cl4-Ru2-Cl6	174.51 (5)
Cl3-Ru1-Cl2	91.60 (5)	Cl6-Ru2-Cl5	90.78 (6)
O1-Ru1-Cl1	89.78 (12)	O2-Ru2-Cl4	87.18 (13)
O1-Ru1-Cl2	83.47 (13)	O2-Ru2-Cl5	84.79 (11)
O1-Ru1-Cl3	87.82 (12)	O2-Ru2-Cl6	90.29 (13)
N1-Ru1-Cl1	90.60 (12)	N3-Ru2-Cl4	89.23 (10)
N1-Ru1-Cl2	176.10 (12)	N3-Ru2-Cl5	176.67 (10)
N1-Ru1-Cl3	85.10 (12)	N3-Ru2-Cl6	86.23 (10)
N1-Ru1-O1	98.45 (16)	N3-Ru2-O2	96.68 (13)
N2-Ru1-Cl1	91.21 (13)	N4-Ru2-Cl4	90.18 (13)
N2-Ru1-Cl2	100.33 (13)	N4-Ru2-Cl5	101.50 (11)
N2-Ru1-Cl3	90.87 (13)	N4-Ru2-Cl6	91.80 (12)
N2-Ru1-O1	176.02 (17)	N4-Ru2-O2	173.34 (15)
N2-Ru1-N1	77.68 (16)	N4-Ru2-N3	77.15 (13)

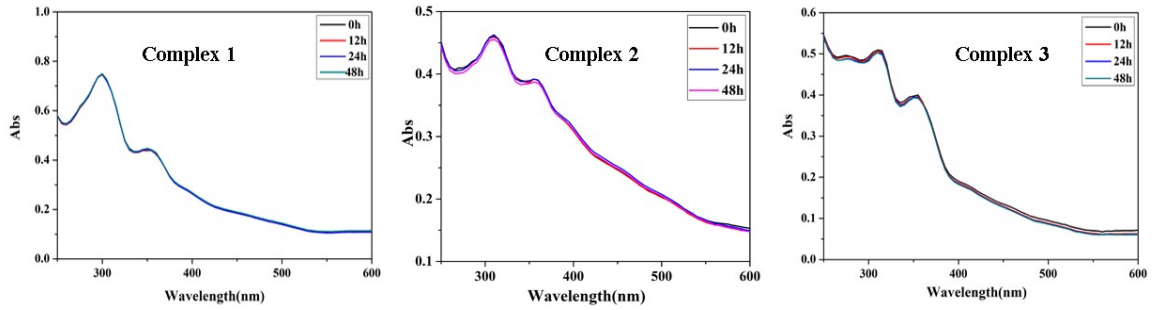

Fig. S8. The UV- visible spectroscopy of the complexes **1**–**3.**

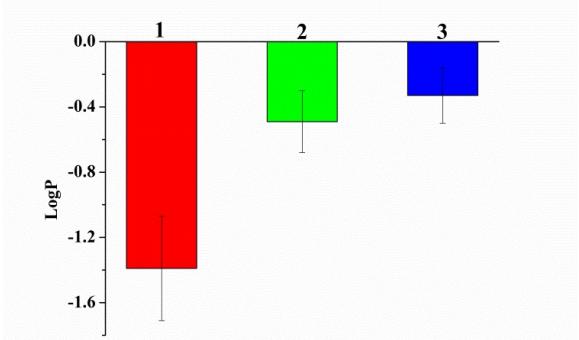

Fig. S9. The octanol/water partition coefficients of complexes **1**–**3.**

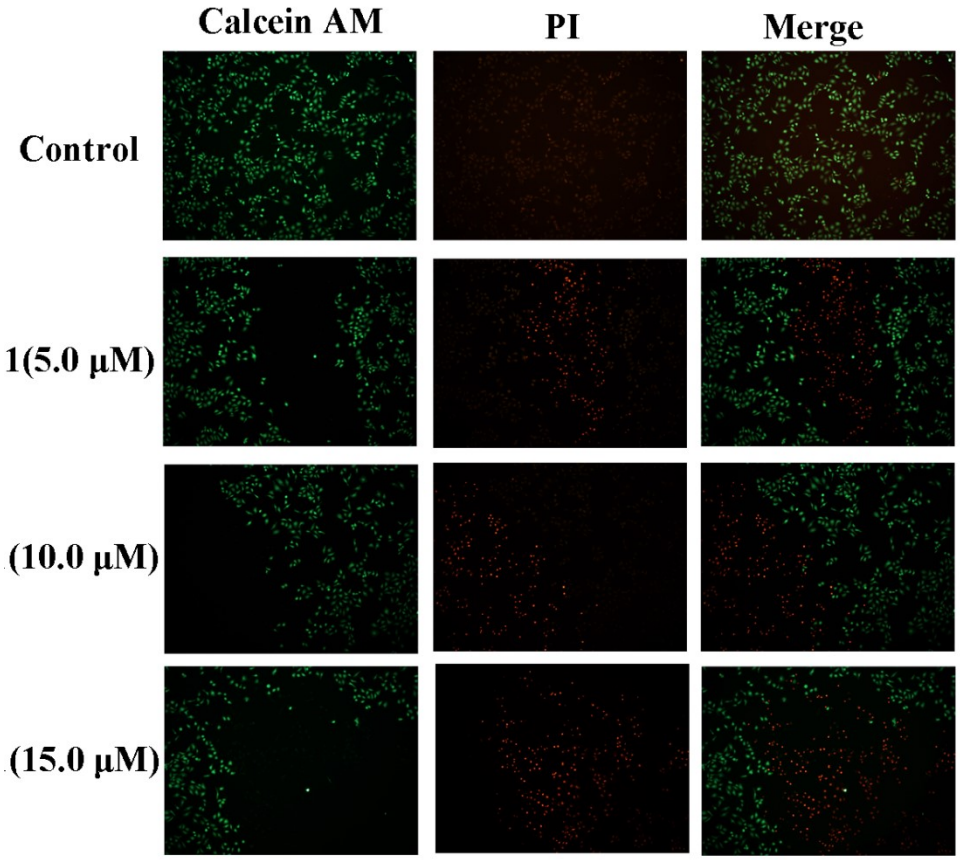

Fig. S10. Representative images of SK-OV-3 cells stained with Calcein-AM (green) and PI (red) (10×).

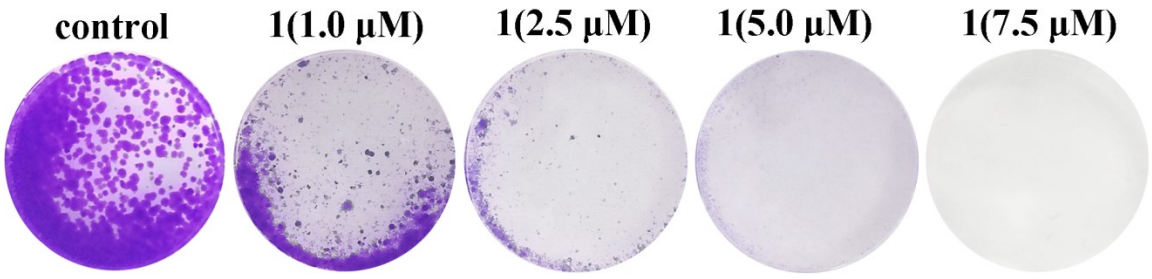

Fig. S11. Colony formation assays of SK-OV-3 treated with complex **1**.

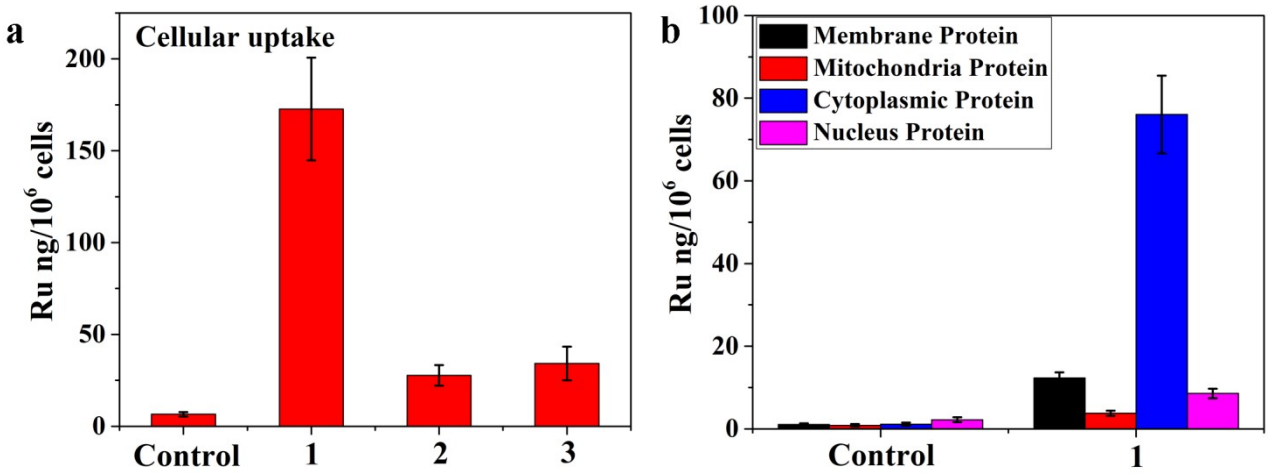

Fig. S12. (A) Ruthenium content in whole cells after SK-OV-3 cells were treated with complexes **1**–**3** (10.0 μM) **for** 10 h. (B) Ruthenium content different fraction after SK-OV-3 cells were treated with complexes **1**(10.0 μM) **for** 10 h. Values are given as means ± SDs from three independent experiments performed in triplicates.

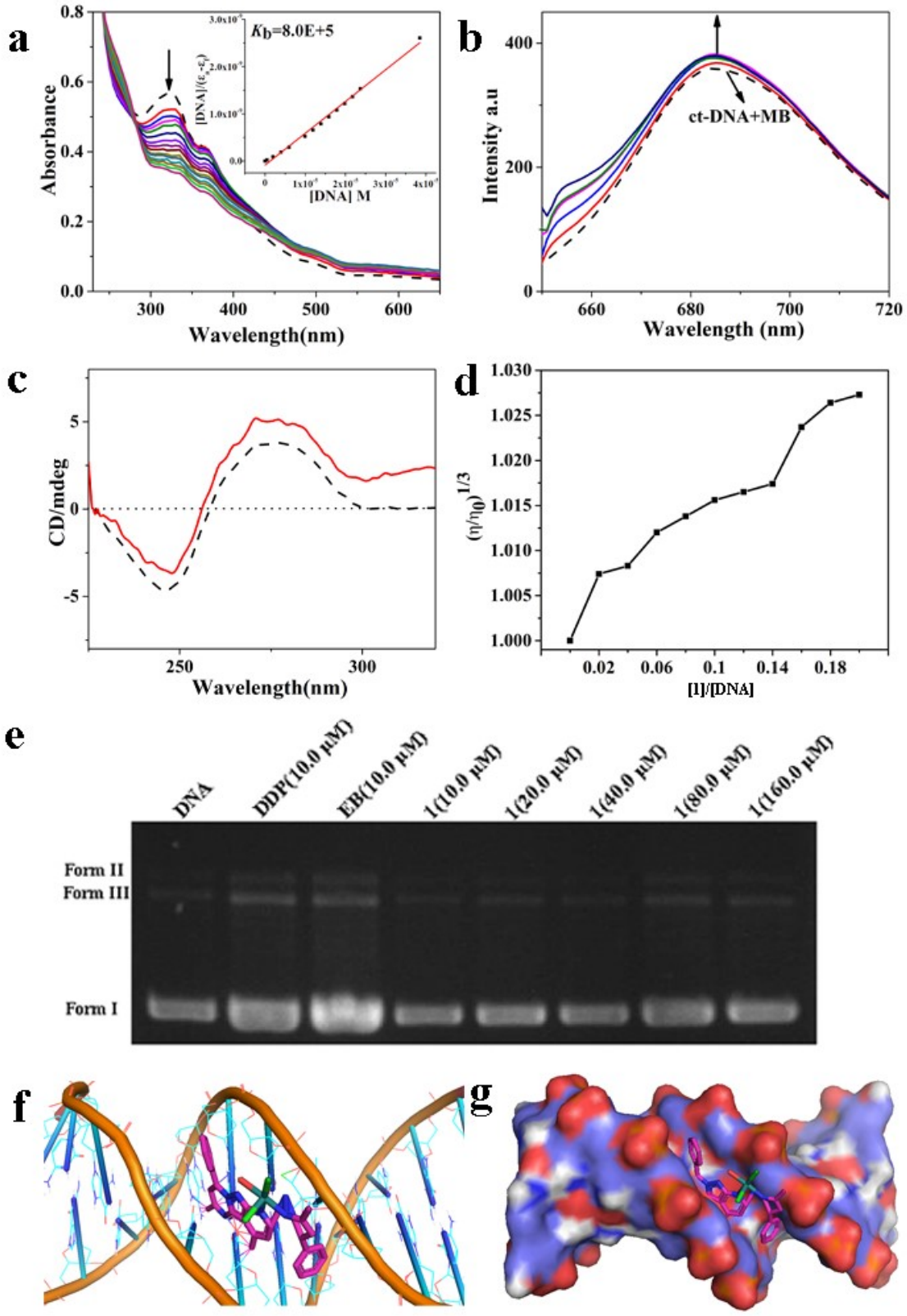

Fig. S13. (a) UV spectrum of complex **1** (2×10^{-5} mol/L, black dotted line) treated with increasing amounts of ct-DNA. (C_{DNA} = 0 to 3.36×10^{-5} mol/L). (b) Competitive displacement analysis between the complex and MB in a pre-treatment MB-DNA conjugate ($C_{MB}=5\times10^{-6}$ mol/L, $C_{DNA}=2\times10^{-4}$ mol/L, $C_{complex}$ = 0 to 2×10^{-5} mol/L) in 50 mmol/L Tris-HCl buffer (pH 7.4). (c) CD spectrum of complex **1** bound to DNA (black dotted line). [DNA] =

50 μM, [1] = 5 μM. (d) Effect of increasing amounts of complex **1** on the viscosity of ct-DNA (1×10^{-3} M) in 50 mM Tris-HCl buffer. (e) Agarose gel electrophoresis results for cleavage of pBR322 DNA by complex **1** at pH 7.0 for 4 h at 37 ℃. Lane 1: DNA alone, Lane 1: DNA +DDP, Lane 1: DNA +EB, Lanes 4−8: DNA with **1** at concentrations of 10, 20, 40, 80 and 160 μM. (f-g) Docking studies of **1** with DNA.

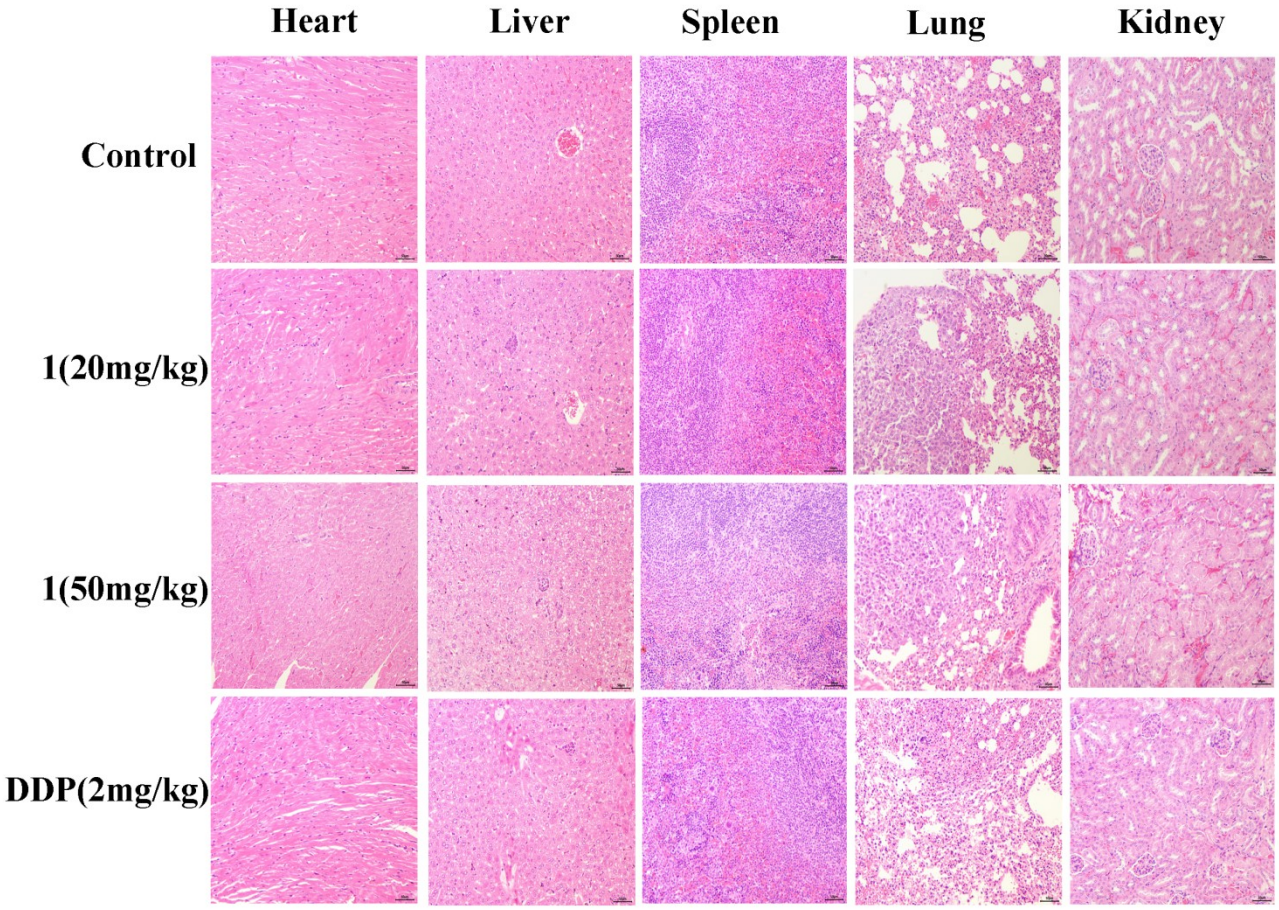

Fig. S14. The heart, liver, spleen, lung and kidney of mice were harvested after the treatment for 14 days. The cells were then stained by H&E (400×).

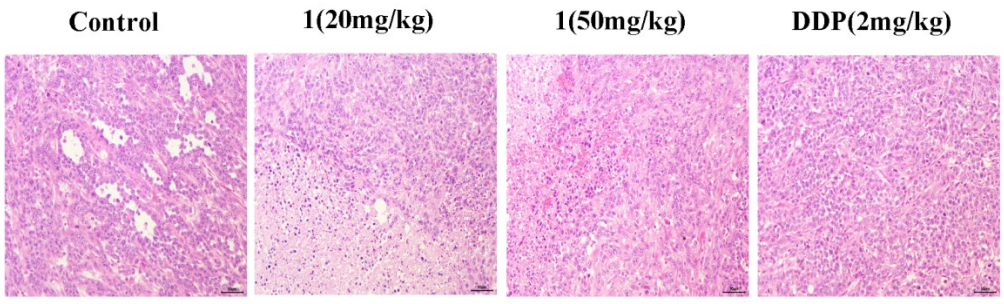

Fig. S15. Tumor tissue section of different concentration complex **1** treatment.