Electronic Supporting Information Materials

High cytotoxic and apoptotic effects of platinum(II) complexes bearing 4-acridinol ligand

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Table S1. Crystal data and structure refinement details for **PtQ**.

Empirical formula	$C_{11}H_{12}ClNO_2PtS$
Formula weight	452.82
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	$Pna2_1$
a/Å	7.28560(10)
b/Å	17.1489(2)
c/Å	9.90310(10)
α/°	90.00
β/°	90.00
γ/°	90.00
Volume/Å^3	1237.29(3)
Z	4
ρ_{calc}g/cm^3	2.431
μ/mm^{-1}	24.695
F(000)	848.0
Crystal size/mm^3	0.1 × 0.1 × 0.1
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	10.32 to 136.34
Index ranges	$-8 \le h \le 8$, $-20 \le k \le 20$, $-11 \le l \le 11$
Reflections collected	10433
Independent reflections	2214 [R_{int} = 0.0669, R_{sigma} = 0.0370]
Data/restraints/parameters	2214/1/157
Goodness-of-fit on F^2	1.074
Final R indexes [I>=2σ (I)]	R_1 = 0.0236, wR_2 = 0.0583
Final R indexes [all data]	R_1 = 0.0245, wR_2 = 0.0592
Largest diff. peak/hole / e Å^{-3}	1.09/-1.15
Flack parameter	0.003(12)

[a] $R_1 = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2]^{1/2}$.

Table S2. Selected bond lengths (Å) for **PtQ**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1	C2	1.373(10)	C7	N1	1.322(9)
C1	C6	1.414(9)	C8	C9	1.363(7)
C1	O1	1.351(8)	C10	S	1.769(5)
C2	C3	1.415(9)	C11	S	1.771(5)
C3	C4	1.387(8)	Cl1	Pt1	2.3013(14)
C4	C5	1.403(8)	N1	Pt1	2.025(6)
C5	C6	1.411(10)	O1	Pt1	2.044(4)
C5	C9	1.414(7)	O2	S	1.472(4)
C6	N1	1.383(8)	Pt1	S	2.2032(14)
C7	C8	1.409(9)			

Table S3. Selected bond angles (°) for **PtQ**.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C2	C1	C6	119.3(6)	C7	N1	C6	118.9(6)
O1	C1	C2	121.8(5)	C7	N1	Pt1	129.8(5)
O1	C1	C6	118.8(6)	C1	O1	Pt1	110.6(4)
C1	C2	C3	119.3(6)	N1	Pt1	Cl1	170.36(16)
C4	C3	C2	121.9(5)	N1	Pt1	O1	82.5(2)
C3	C4	C5	119.4(5)	N1	Pt1	S	99.93(17)
C4	C5	C6	118.6(5)	O1	Pt1	Cl1	88.41(12)
C4	C5	C9	124.2(5)	O1	Pt1	S	177.25(11)
C6	C5	C9	117.3(5)	S	Pt1	Cl1	89.20(5)
C5	C6	C1	121.5(6)	C10	S	C11	100.8(2)
N1	C6	C1	116.6(7)	C10	S	Pt1	109.08(18)
N1	C6	C5	121.9(6)	C11	S	Pt1	110.18(18)
N1	C7	C8	122.1(6)	O2	S	C10	107.2(2)
C9	C8	C7	119.8(5)	O2	S	C11	107.3(2)
C8	C9	C5	119.9(5)	O2	S	Pt1	120.43(18)
C6	N1	Pt1	111.2(5)				

Table S4. Crystal data and structure refinement details for **PtA**.

Empirical formula	$C_{15}H_{14}ClNO_2PtS$
Formula weight	502.87
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	Iba2
a/Å	21.4385(4)
b/Å	16.0457(3)
c/Å	17.8853(3)
α/°	90.00
β/°	90.00
γ/°	90.00
Volume/Å^3	6152.47(19)
Z	16
ρ_{calc}g/cm^3	2.172
μ/mm^{-1}	19.959
F(000)	3807.0
Crystal size/mm^3	0.34 × 0.18 × 0.17
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	6.88 to 136.42
Index ranges	-25 ≤ h ≤ 25, -15 ≤ k ≤ 19, -21 ≤ l ≤ 21
Reflections collected	26871
Independent reflections	5386 [R_{int} = 0.0811, R_{sigma} = 0.0390]
Data/restraints/parameters	5386/1/383
Goodness-of-fit on F^2	1.076
Final R indexes [I>=2σ (I)]	R_1 = 0.0485, wR_2 = 0.1357
Final R indexes [all data]	R_1 = 0.0503, wR_2 = 0.1375
Largest diff. peak/hole / e Å^{-3}	4.90/-1.50
Flack parameter	0.03(2)

[a] $R_1 = \Sigma\, ||F_o| - |F_c||/\Sigma|F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{½}$.

Table S5. Selected bond lengths (Å) for **PtA**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Pt1	Cl	2.325(3)	Pt2	Cl1	2.327(3)
Pt1	S	2.219(3)	Pt2	S1	2.205(3)
Pt1	O4	1.984(8)	Pt2	O3	1.991(7)
Pt1	N3	2.072(8)	Pt2	N4	2.093(8)
S	O5	1.466(7)	S1	O7	1.447(8)
S	C1I	1.773(11)	S1	C1	1.785(14)
S	C11	1.764(11)	S1	C1J	1.756(13)
O4	C1C	1.391(15)	O3	C3	1.328(12)

Atom	Atom	Length/Å	Atom	Atom	Length/Å
N3	C2	1.330(13)	N4	C1D	1.387(15)
N3	C6	1.400(13)	N4	C5	1.339(13)
C	C6	1.400(15)	C1A	C1H	1.41(2)
C	C10	1.434(16)	C1A	C14	1.28(2)
C	C16	1.433(15)	C1B	C1D	1.455(15)
C1C	C2	1.386(15)	C1B	C12	1.387(16)
C1C	C19	1.353(16)	C1B	C13	1.401(17)
C1E	C1F	1.430(18)	C1D	C3	1.380(15)
C1E	C16	1.299(18)	C1G	C3	1.418(15)
C1F	C17	1.376(16)	C1G	C8	1.355(18)
C2	C9	1.467(15)	C1H	C4	1.368(18)
C6	C17	1.426(16)	C4	C5	1.383(16)
C9	C10	1.339(17)	C5	C7	1.430(15)
C9	C15	1.480(17)	C7	C12	1.411(16)
C15	C18	1.338(19)	C7	C14	1.442(17)
C18	C19	1.378(19)	C8	C13	1.37(2)

Table S6. Selected bond angles (°) for **PtA**.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
S	Pt1	Cl	85.16(9)	S1	Pt2	Cl1	83.66(11)
O4	Pt1	Cl	172.9(2)	O3	Pt2	Cl1	172.4(3)
O4	Pt1	S	89.7(2)	O3	Pt2	S1	90.6(2)
O4	Pt1	N3	80.6(3)	O3	Pt2	N4	82.8(3)
N3	Pt1	Cl	104.4(2)	N4	Pt2	Cl1	103.0(2)
N3	Pt1	S	170.2(2)	N4	Pt2	S1	173.3(2)
O5	S	Pt1	117.1(3)	O7	S1	Pt2	118.3(3)
O5	S	C1I	110.3(5)	O7	S1	C1	107.2(6)
O5	S	C11	109.3(5)	O7	S1	C1J	109.3(6)
C1I	S	Pt1	107.7(4)	C1	S1	Pt2	109.3(4)
C11	S	Pt1	107.9(4)	C1J	S1	Pt2	109.3(4)
C11	S	C1I	103.7(6)	C1J	S1	C1	102.2(7)
C1C	O4	Pt1	112.1(6)	C3	O3	Pt2	110.8(6)
C2	N3	Pt1	110.1(7)	C1D	N4	Pt2	107.2(6)
C2	N3	C6	119.5(9)	C5	N4	Pt2	132.5(7)
C6	N3	Pt1	129.7(7)	C5	N4	C1D	120.3(8)
C6	C	C10	117.9(9)	C14	C1A	C1H	123.0(13)
C6	C	C16	119.7(10)	C12	C1B	C1D	114.4(10)
C16	C	C10	122.4(11)	C12	C1B	C13	126.1(10)
C2	C1C	O4	115.8(9)	C13	C1B	C1D	119.4(11)
C19	C1C	O4	124.2(10)	N4	C1D	C1B	123.4(10)

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C19	C1C	C2	120.0(11)	C3	C1D	N4	117.6(9)
C16	C1E	C1F	120.2(10)	C3	C1D	C1B	119.0(10)
C17	C1F	C1E	121.3(11)	C8	C1G	C3	121.6(11)
N3	C2	C1C	118.3(10)	C4	C1H	C1A	117.7(12)
N3	C2	C9	121.0(10)	O3	C3	C1D	120.6(8)
C1C	C2	C9	120.5(10)	O3	C3	C1G	120.0(9)
N3	C6	C	121.6(9)	C1D	C3	C1G	118.9(9)
N3	C6	C17	119.5(9)	C1H	C4	C5	123.2(11)
C	C6	C17	118.8(9)	N4	C5	C4	123.3(10)
C2	C9	C15	115.8(10)	N4	C5	C7	119.7(10)
C10	C9	C2	119.1(10)	C4	C5	C7	117.0(10)
C10	C9	C15	125.1(10)	C5	C7	C14	118.6(11)
C9	C10	C	120.8(10)	C12	C7	C5	119.7(10)
C18	C15	C9	118.7(11)	C12	C7	C14	121.7(11)
C1E	C16	C	121.2(11)	C1G	C8	C13	121.2(11)
C1F	C17	C6	118.6(11)	C1B	C12	C7	122.4(9)
C15	C18	C19	123.4(12)	C8	C13	C1B	119.8(11)
C1C	C19	C18	121.4(12)	C1A	C14	C7	120.6(13)

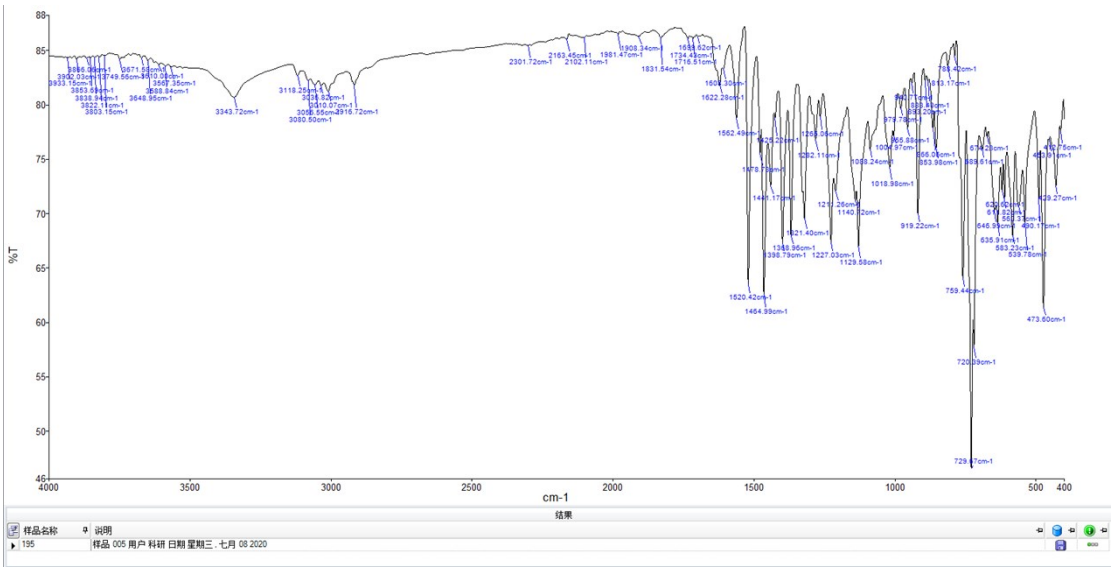

Figure S1. IR (KBr) spectra of **HA**

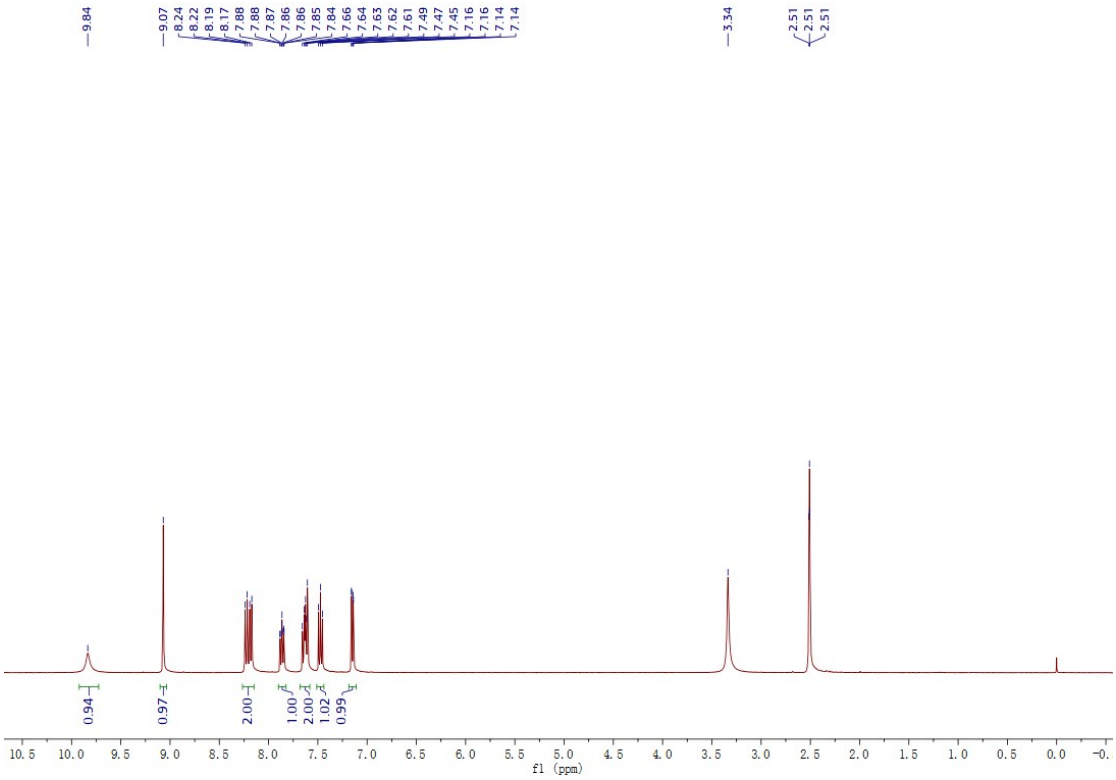

Figure S2. ^{1}H NMR (400MHz, DMSO-d_6) for **HA**.

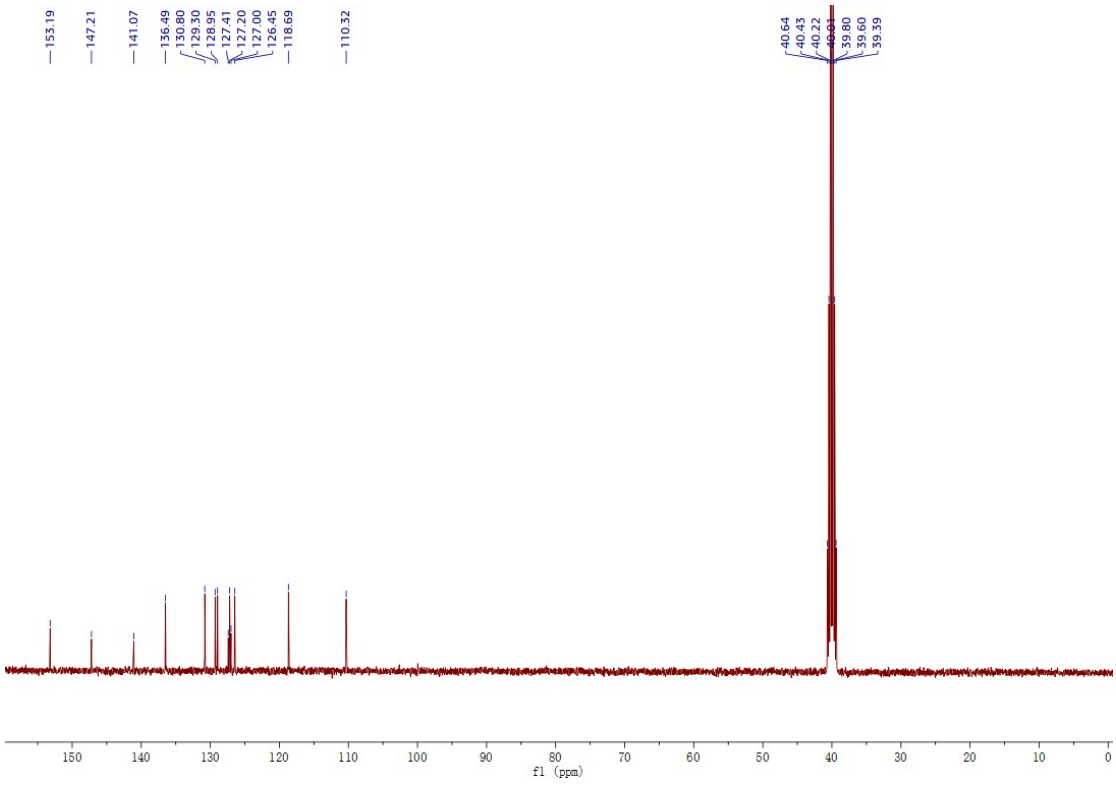

Figure S3. ^{13}C NMR (101MHz, DMSO-d_6) for **HA**.

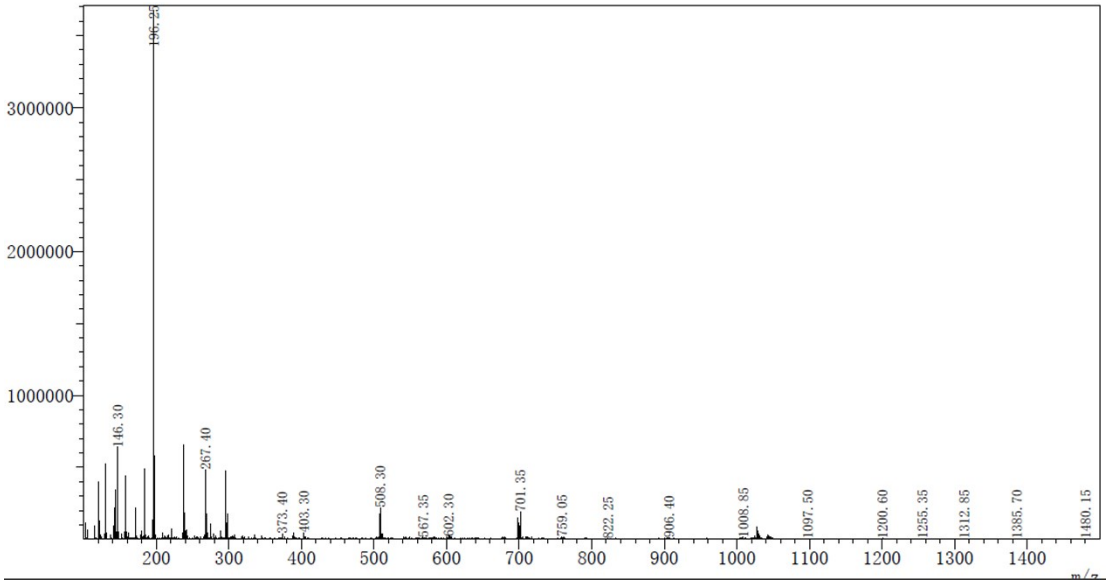

Figure S4. The mass spectra of **HA** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

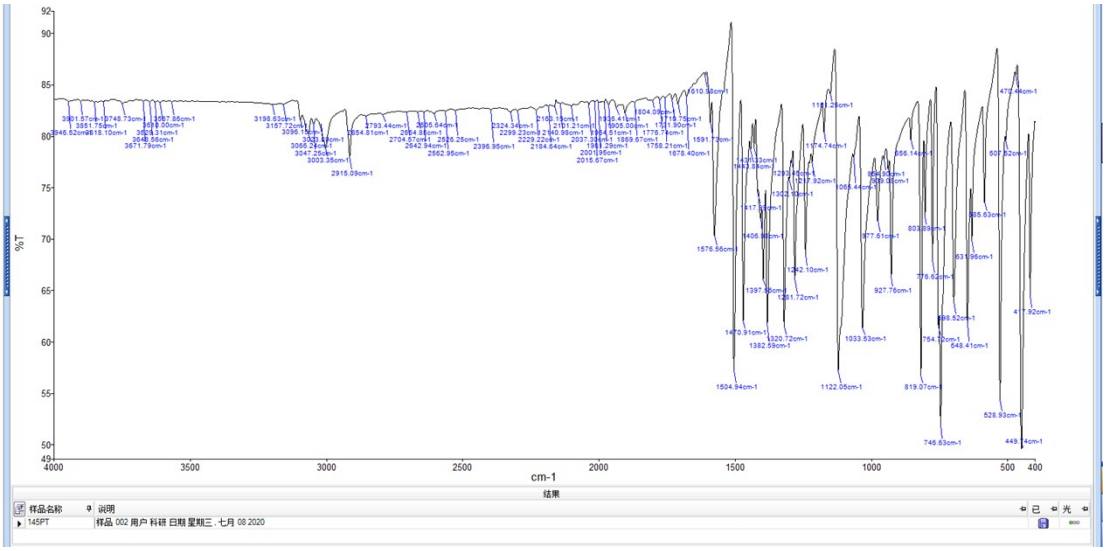

Figure S5. IR (KBr) spectra of **PtQ**

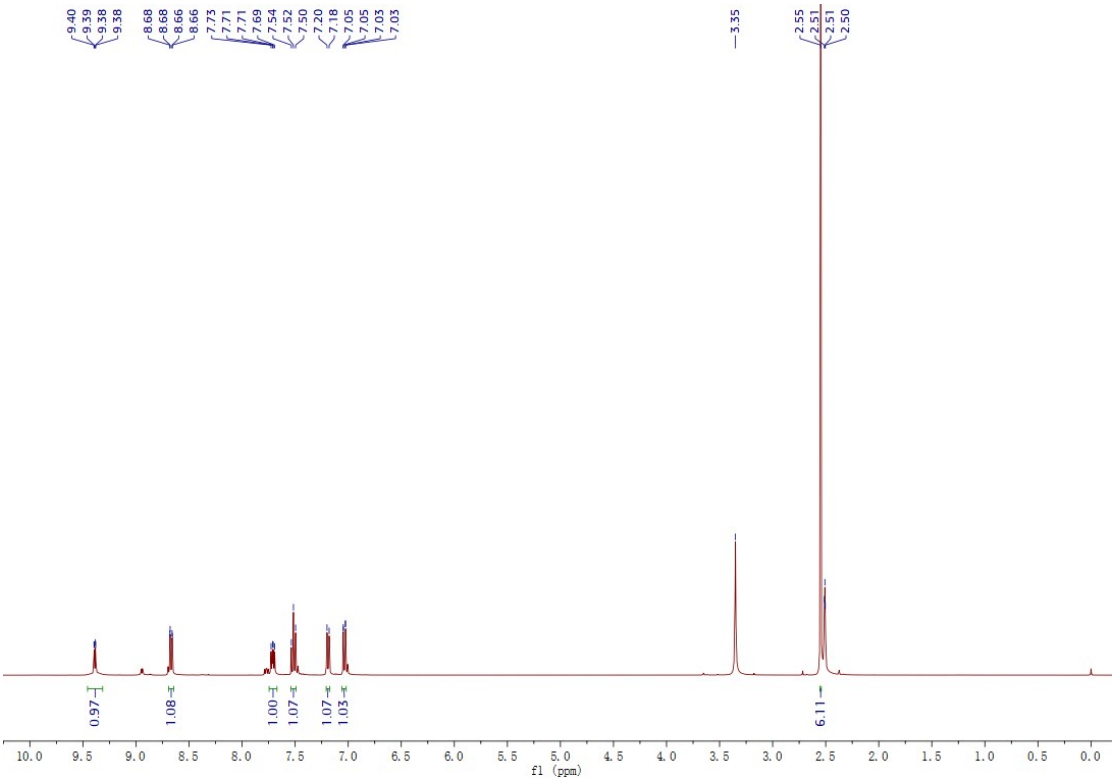

Figure S6. ^{1}H NMR (400MHz, DMSO-d_6) for **PtQ**.

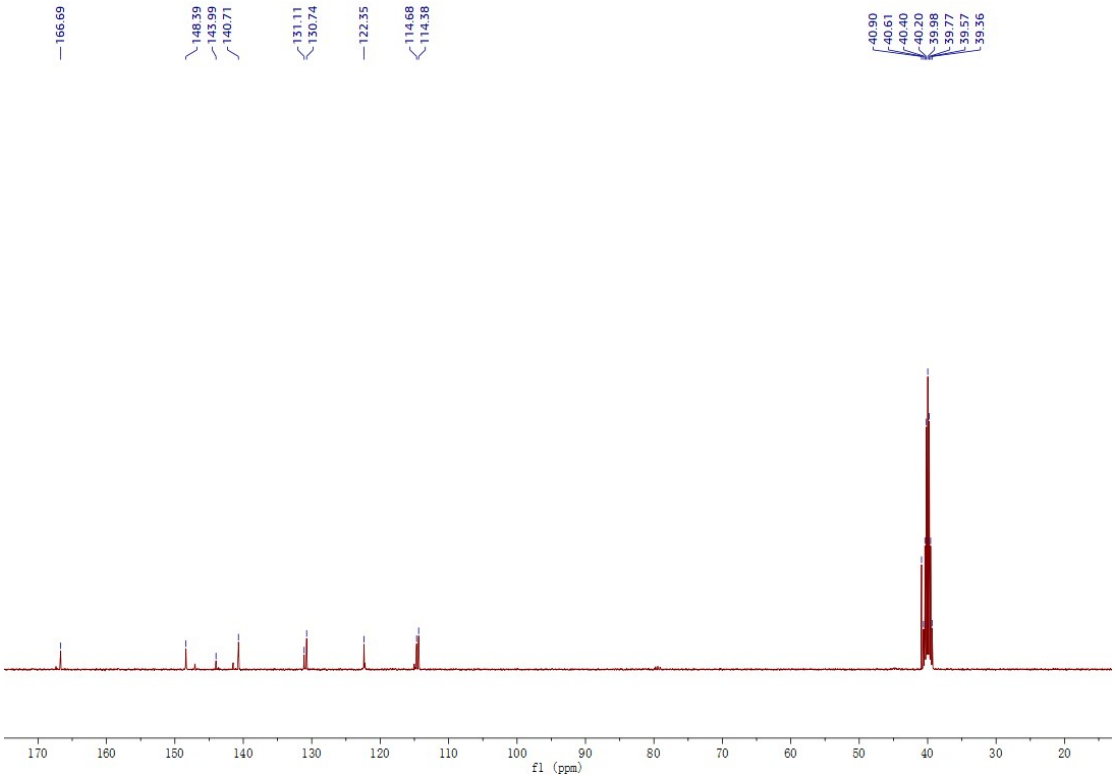

Figure S7. ^{13}C NMR (101MHz, DMSO-d_6) for **PtQ**.

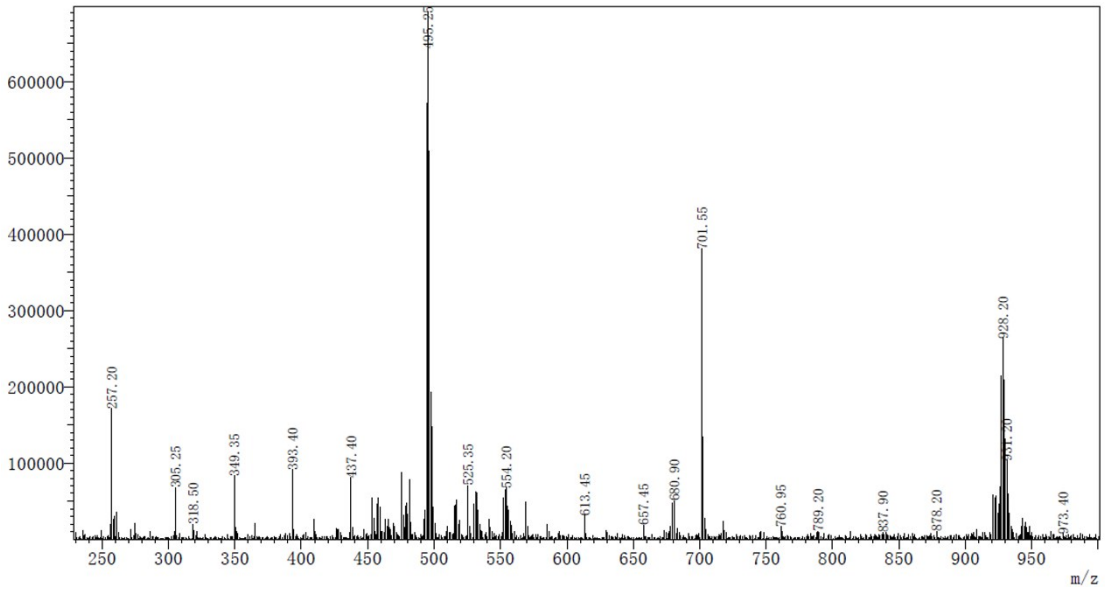

Figure S8. The mass spectra of **PtQ** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

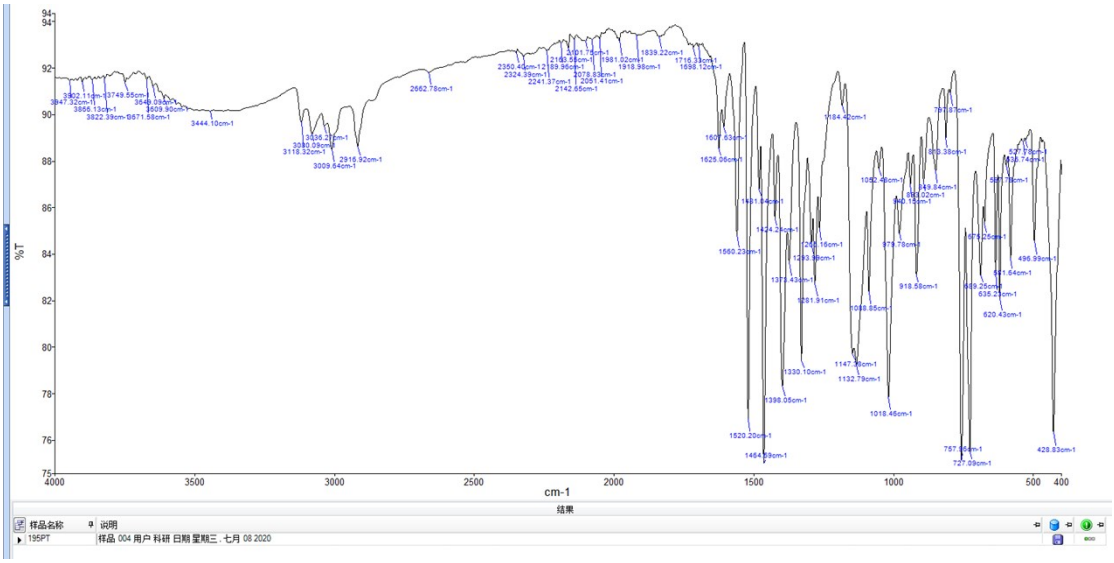

Figure S9. IR (KBr) spectra of **PtA**

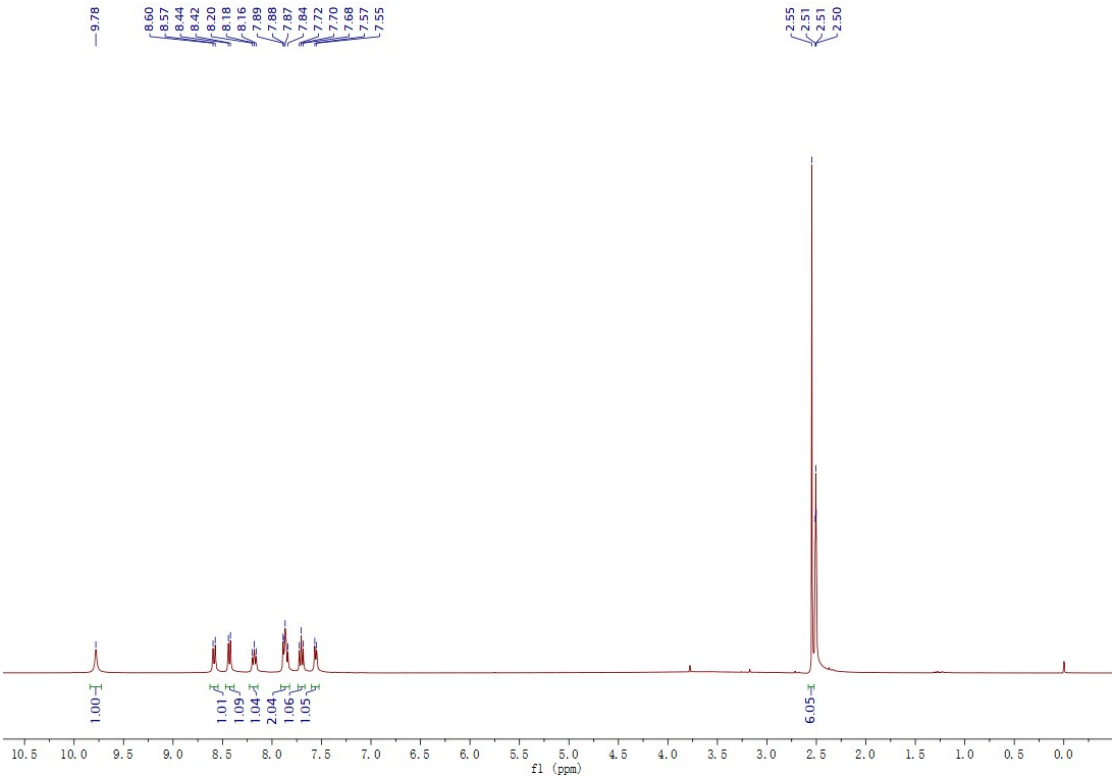

Figure S10. ^{1}H NMR (400MHz, DMSO-d_6) for **PtA**.

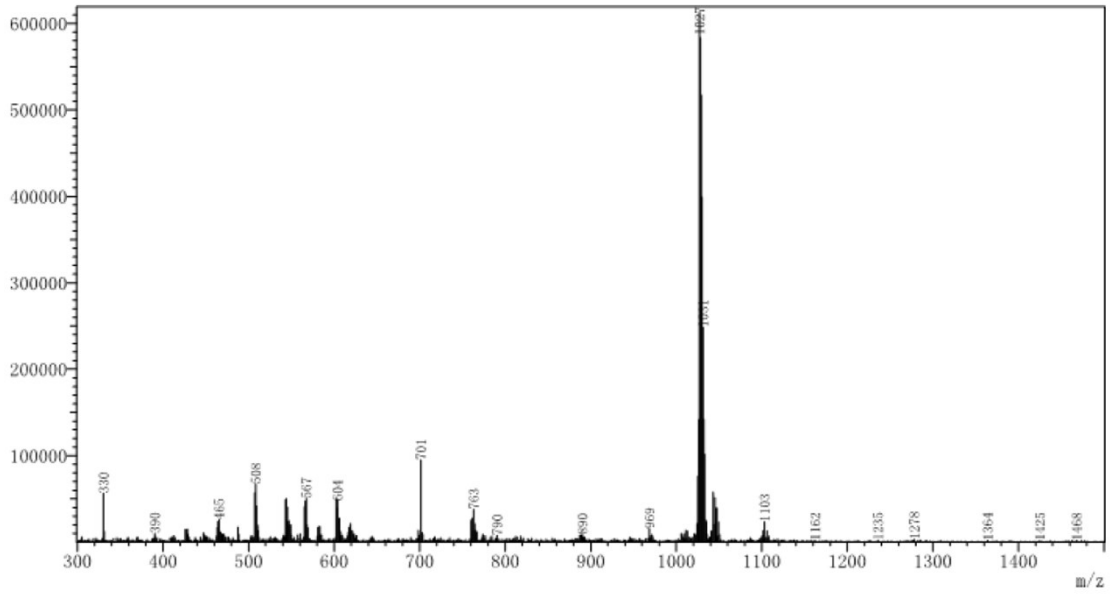

Figure S11. The mass spectra of **PtA** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

Experimental section

Cell Culture

Human SK-OV-3/DDP, SK-OV-3 and HL-7702 cells were grown in DMEM medium, both containing heat-inactivated fetal calf serum (FCS, Sigma, USA) (10%) and antibiotics (penicillin/streptomycin) at 37 °C and CO_2 (5%).

MTT assay

The human cells were seeded in 96-well plates as monolayers with 100 μL of cell solution (approximately 20000 cells) per well and preincubated for 24 h in medium supplemented with 10% FCS. The 4-acridinol derivatives (HQ and HA) ligand and their Pt complexes (**PtQ** and **PtA**) were prepared as 0.5% DMSO solution. Then 100 μL of 4-acridinol derivatives (HQ and HA) ligand and their Pt complexes (**PtQ** and **PtA**) solution was added to each well and the plates were incubated for another 24.0 h. Subsequently, MTT (5.0 mg/mL solution) was added to the cells and the plates were incubated for a further 4.0 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 540 nm using a multiwell plate reader and the fraction of surviving cells was calculated from the absorbance of untreated control cells.

Cell apoptosis

SK-OV-3/DDP cells were seeded in 6-well plates (3×10^5 cells/well) and incubated in the presence or absence of 4-acridinol derivatives Pt complexes **PtQ** (5.08μM) and **PtA** (0.05μM) for 24 h to induce cancer cell apoptosis. After incubation, the cancer cells were harvested and incubated with 5.0 μL of Annexin-V/FITC (Keygen Biotech,

China) in binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$ at pH 7.4) at room temperature for 30.0 min. PI solution was then added to the medium for another 5.0 min of incubation. Almost 10,000 events were collected for each sample and analyzed by flow cytometry (BECKMAN-COULTER). The percentage of apoptotic cells was calculated with EXPO32 ADC Analysis software.

Mitochondrial membrane potential assay

A lipophilic cationic dye, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine (JC-1, Beyotime, China) was used to monitor the level of MMP in the SK-OV-3/DDP cells. At normal state, the MMP is high, and JC-1 appears as aggregates, which is indicated by red fluorescence. However, when apoptosis occurs, the MMP reduced and JC-1 displayed as monomers, which is indicated by green fluorescence. For flow cytometry analysis, SK-OV-3/DDP cancer cells were plated in 6-well plates (3×10^5 cells/well) and grown for 24.0 h and treated with 4-acridinol derivatives Pt complexes **PtQ** (5.08μM) and **PtA** (0.05μM) for 24 h. Then the cells were harvested by centrifugation and incubated with JC-1 solution for 30 min. After briefly washing, the proportion of green and red fluorescence intensity was immediately detected and analyzed by flow cytometry.

Western blot analysis

SK-OV-3/DDP cancer cells seeded in 60.0 mm dishes at a density of 6×10^5 cells/well were incubated with or without 4-acridinol derivatives Pt complexes **PtQ** (5.08μM) and **PtA** (0.05μM) for 24 h. After incubation, these cells were washed twice with ice-cold PBS and then lysed in RIPA lysis buffer containing 150 mM NaCl, 50 mM Tris (pH 7.4), 1% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.1% (w/v) SDS, and 1 mM EDTA (Beyotime, China). The lysates were incubated at 0 °C for 30 min and vortexed every 10.0 min intermittently, and then the total protein was

harvested by centrifuging at 12,500 g for 35.0 min. After the protein concentrations were determined by a BCA Protein Assay Kit (Beyotime, China), the protein extracts were reconstituted in loading buffer containing 62 mM Tris-HCl, 2% SDS, 10% glycerol, and 5% β-mercaptoethanol (Beyotime, China) and boiled at 100 °C for 5.0 min. An equal amount of the proteins (40.0 μg) was separated by 8−12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and was transferred to nitrocellulose membranes (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). After blocking with 5% nonfat dried milk in TBS containing 1% Tween-20 for 90 min at room temperature, the membranes were incubated overnight with specific primary antibodies (CST, USA) at 4 °C. After three washes in TBST, the membranes were incubated with the appropriate HRP-conjugated secondary antibodies at room temperature for 2.5 h. The blots were developed with enhanced chemiluminescence and were detected by an imager.

Measurement of ROS generation

DCFH-DA is a freely permeable tracer specific for ROS. DCFH-DA can be deacetylated by intracellular esterase to the non-fluorescent DCFH which is oxidized by ROS to the fluorescent compound 2',7'-dichloroflorescein (DCF). Thus, the fluorescence intensity of DCF is proportional to the amount of ROS produced by the cells. 6×10^5 SK-OV-3/DDP cells were exposed to 4-acridinol derivatives Pt complexes **PtQ** (5.08μM) and **PtA** (0.05μM) for 24 h. After exposure, the cells were harvested, washed once with ice-cold PBS and incubated with DCFH-DA (100 μM in a final concentration) at 37 °C for 30.0 min in the dark. Then the cells were washed again and maintained in 1 mL PBS. The ROS generation was assessed from 10,000 cells each sample by fluorescence photometer (GC126N, China) with excitation and emission wavelengths of 488 and 525 nm, respectively. The experiment was

performed at least three times.

X-ray Crystallographic Determination

All reflection data were collected on an Agilent Supernova diffractometer (Mo, λ = 0.71073Å) at room temperature. A semiempirical absorption correction by using the SADABS program was applied, and the raw data frame integration was performed with SAINT [1]. The crystal structures were solved by the direct method using the program SHELXS-97[2] and refined by the full-matrix least-squares method on F^2 for all non-hydrogen atoms using SHELXL-97[3] with anisotropic thermal parameters. All hydrogen atoms were located in calculated positions and refined isotropically.

Intracellular free Ca^{2+} detection

The level of intracellular free Ca^{2+} is decided by using a fluorescent dye Fluo-3 AM which can across the cell membrane and be cut into Fluo-3 by intracellular esterase. The Fluo-3 can specifically combine with the Ca^{2+} and has a strong fluorescence with an excitation wavelength of 488 nm. After exposed to 4-acridinol derivatives Pt complexes **PtQ** (5.08μM) and **PtA** (0.05μM) for 24 h, respectively, the SK-OV-3/DDP cells were harvested and washed twice with PBS, then resuspended in Fluo-3 AM (5.0 μM) for 30 min in dark. Detection of intracellular Ca^{2+} was carried by Flow cytometer at 525 nm excitation wavelength.

Statistical Analysis

The experiments have been repeated from three to five times, and the results obtained are presented as means ± standard deviation (SD). Significant changes were assessed by using Student's *t* test for unpaired data, and p values of <0.05 were considered significant.

■ REFERENCES

1 SAINT Software Reference Manual; Bruker AXS: Madison, WI, 1998.

2 G. M. Sheldrick, Phase Annealing in SHELX-90: Direct Methods for Larger Structures. *Acta Crystallogr.* 1990, **A46**, 467.

3 G. M. Sheldrick, SHELXS-97, Program for X-ray Crystal Structure Solution; University of Göttingen: Göttingen, Germany, 1997.