Supporting Information

A platinated prodrug leveraging PROTAC technology for targeted protein degradation and enhanced antitumor efficacy

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Contents

Experimental Procedures

Materials, instruments, and reagents

Unless otherwise noted, all the reactions were carried out under normal atmospheric conditions with protection from light. All chemicals and solvents were purchased from commercial resources. Carboplatin was purchased from Shandong Boyuan Pharmaceutical Co., Ltd. ARV771 and VHL ligand was purchased from Furun Pharmatech Ltd. 1H, 13C, and 195Pt NMR spectra were obtained on a Bruker AVANCE III 400 MHz NMR spectrometer or a Bruker Ascend AVANCE III HD 600 MHz spectrometer with a BBO probe. ESI-MS tests were performed on an Agilent API-3200 Q-Trap mass spectrometry. The high-resolution MS data were recorded with Sciex X500R Q-TOF. High-performance liquid chromatography (HPLC) experiments were conducted on a Shimadzu Prominence HPLC system. For analysis, a Phenomenex column (Gemini, 5 μm, C18, 110 Å, 250 mm × 4.6 mm) was used. The samples were monitored by UV absorbance at 254 nm. The samples were eluted by using a program as follows: 0% B (0 min)--100% B (9 min)--100% (11 min)--0% (12 min)--0% (16 min). Pt content was measured by an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (PE Optima 8000) or an inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (PE Nexion 2000). Apoptosis and cell cycle analyses were conducted on a flow cytometer (BD Bioscience FACS Calibur). Chemiluminescence of Western blot was imaged by ChemiDoc touch System. MTT was measured with Molecular Devices SpectraMAX ID5 Microplate Reader. A Leica SPE laser Confocal Scanning Microscope was employed to image the immunofluorescence. Cell images were taken by Nikon ECLIPSE Ts2 inverted microscope. Unless stated otherwise, all chemicals and solvents were procured from authentic commercial suppliers and used without further purification. All the reactions were carried out under normal atmospheric pressure, under the exclusion of light by tin foil. Ham's F-12K (Kaighn's) medium, Dulbecco's modified eagle medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, trypsin, phosphate-buffered saline (PBS, pH 7.4), penicillin and streptomycin, sodium pyruvate, and fetal bovine serum (FBS) were obtained from ThermoFisher.

Synthesis and characterizations

Synthesis of Complex 1: 500 mg carboplatin was suspended in 100 mL of acetic acid before 2.5 mL of hydrogen peroxide (50% in H_2O) was added. The mixture was stirred at room temperature for 0.5 h. The reaction mixture was poured into 400 mL of cold ethyl ether (Et_2O) to precipitate a white solid. The formed solid was collected by centrifugation and washed with acetone, dichloromethane (DCM), and Et_2O (510 mg, 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ

6.19 – 5.65 (m, 6H), 2.56 (d, J = 7.8 Hz, 2H), 2.46 (d, J = 8.1 Hz, 2H), 1.87 (s, 3H), 1.79 (t, J = 7.8 Hz, 2H).

Synthesis of PROTAC-Pt(IV): In an argon-purged environment, ARV771 (32 mg, 1 equivalent) and pyridine (8 μL, 4 equivalents) were dissolved in DCM (0.5 mL). A separate solution of triphosgene (bis(trichloromethyl) carbonate (BTC); 38.4 mg, 4 equivalents in 0.5 mL DCM) was added to the mixture at 0 °C. The reaction was allowed to warm to room temperature and stirred for 2 h. After removing the solvent under reduced pressure, complex 1 (13.2 mg, 1 equivalent) in 0.2 mL dimethylformamide (DMF) was added. The reaction mixture was stirred overnight at room temperature. Purification was achieved via preparative thin-layer chromatography (TLC) followed by HPLC (5 mg, 11%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.46 (d, J = 7.7 Hz, 1H), 8.28 (t, J = 5.7 Hz, 1H), 7.48 (d, J = 8.7 Hz, 2H), 7.44 – 7.41 (m, 4H), 7.39 (d, J = 9.4 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 6.35 (s, 6H), 4.90 (d, J = 6.7 Hz, 2H), 4.50 (dd, J = 8.0, 6.1 Hz, 1H), 4.47 – 4.42 (m, 2H), 4.09 (d, J = 15.3 Hz, 1H), 4.01 (d, J = 11.8 Hz, 1H), 3.96 (d, J = 15.3 Hz, 1H), 3.65 (d, J = 8.4 Hz, 1H), 3.56 (dt, J = 6.5, 3.2 Hz, 2H), 3.50 (s, 2H), 3.31 – 3.19 (m, 6H), 2.59 (s, 3H), 2.45 (s, 3H), 2.40 (s, 3H), 2.24 (dd, J = 13.7, 8.1 Hz, 1H), 2.07 (s, 1H), 1.91 (s, 3H), 1.83 – 1.78 (m, 4H), 1.62 (s, 3H), 1.36 (d, J = 7.0 Hz, 3H), 0.94 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.66, 176.59, 170.34, 170.15, 169.49, 163.48, 158.04, 155.59, 151.96, 148.24, 145.07, 137.24, 135.70, 132.73, 131.58, 131.18, 130.61, 130.31, 130.20, 130.05, 129.33, 128.92, 126.77, 69.39, 68.46, 67.56, 58.54, 55.97, 54.31, 48.30, 37.97, 35.47, 33.35, 29.86, 26.60, 22.91, 16.46, 16.22, 14.52, 13.16, 11.77. ^{195}Pt NMR (129 MHz, DMSO-d_6) δ 1968.14. ESI-HRMS (positive mode) m/z: $[M+H]^+$ calcd. for $C_{58}H_{75}ClN_{11}O_{15}PtS_2$ 1460.4221, found: 1460.4211.

Synthesis of JQ1-Pt(IV): 20.2 mg of complex 1 was suspended in 0.2 mL of DMF, to which JQ-1 (20 mg, 0.8 equivalents), hexafluorophosphate benzotriazole tetramethyl uronium (HBTU, 38 mg, 2 equivalents), and N,N-diisopropylethylamine (DIEA, 18 μL, 2 equivalents) were added. The mixture was stirred at room temperature overnight, after which the DMF was removed under reduced pressure. The crude product was immediately subjected to HPLC for purification (16 mg, 39%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.51 – 7.43 (m, 4H), 6.38 (d, J = 42.8 Hz, 6H), 4.41 (dd, J = 7.6, 5.8 Hz, 1H), 3.49 (dd, J = 16.6, 5.8 Hz, 1H), 3.41 – 3.38 (m, 2H), 2.60 (s, 3H), 2.54 (d, J = 8.0 Hz, 2H), 2.47 (t, J = 8.4 Hz, 2H), 2.43 – 2.40 (m, 3H), 1.91 (s, 3H), 1.82 – 1.74 (m, 2H), 1.63 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 177.81, 177.65, 176.77, 176.73, 163.30, 155.48, 150.31, 137.29, 135.60, 132.70, 131.14, 130.84, 130.33, 130.04, 128.88, 55.99, 54.40, 38.21, 32.43, 30.98, 22.95, 16.21, 14.55, 13.16, 11.78. ^{195}Pt NMR

(129 MHz, DMSO-d_6) δ 1956.23. ESI-HRMS (positive mode) m/z: $[M+H]^+$ calcd. for $C_{27}H_3ClN_6O_8PtS$ 831.1320, found: 831.1272.

Cell lines and cell culture conditions

A2780, A2780cisR cells (human ovarian carcinoma), and 4T1 (murine breast carcinoma) were cultured in Roswell Park Memorial Institute (RMPI) 1640 medium with 10% FBS and 100 units of penicillin and streptomycin. MCF-7 (human breast carcinoma), HeLa (human cervical carcinoma), A549, and A549cisR (human lung adenocarcinoma) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 100 units of penicillin and streptomycin. MRC-5 (human lung normal) cells were cultured in MEM supplemented with 12% FBS, 1% L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate, and 100 units of penicillin and streptomycin. All cells were incubated at 37 °C in 5% CO_2.

Stability and reduction

The stability of PROTAC-Pt(IV) (10 μM) was monitored by RP-HPLC with an absorption at 254 nm in a PBS buffer (pH 7.4) with or without 5 mM sodium ascorbate, and Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS. The DMF content in all the test samples was 1%. The solutions were kept at 37 °C in a water bath with protection from light by tin foil. 50 μL of the samples were injected into the RP-HPLC system at different time points until 24 h. The newly formed products were separated by HPLC and characterized by ESI-HRMS.

Cytotoxicity by MTT assay

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on A549, A549cisR, A2780, A2780cisR, HeLa, 4T1, MCF7, MRC5 cells. These cells were plated in 96-well plates at 2500, 3000, 2500, 3000, 2000, 1500, 2500, and 3000 cells each well. After 24 h, they were treated with different concentrations of PROTAC-Pt(IV), ARV771, carboplatin, JQ1-Pt(IV), and ARV771 with carboplatin containing 0.5% DMF. Following 72 h incubation, the medium was removed, and the cells were treated with 200 μL of MTT solution (1 mg/mL in serum-free media) and incubated in the dark for 2 h. Upon removal of the MTT solution, 200 μL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm and 630 nm using a microplate reader. IC_{50} values were calculated from the dose-response curves by the GraphPad Prism 7 software.

Colony formation assay

500 A2780 cells were seeded on a 6-well plate and incubated overnight. After attachment, incubate with 40 nM of PROTAC-Pt(IV), carboplatin, ARV771, JQ1-Pt(IV), and carboplatin+ARV771 containing 0.1% DMF for 14 days. The medium was replaced every 3 days. After that, the medium was removed, washed with PBS, and fixed with 4% formaldehyde for 30 min. The fixed cells were stained with 0.5% crystal violet for 30 min, washed with tap water, and finally imaged.

3D multicellular spheroid

2000 A2780cisR cells were cultured in an ultra-low attachment 96-well plate with continuous shaking during the following 12 h. Fresh medium was replaced every 3 days for spheroid formation. After 7 days, tumor spheroids were treated with 100 nM of PROTAC-Pt(IV), ARV771, carboplatin, JQ1-Pt(IV), and ARV771+carboplatin for another 7 days. The size and morphology of the tumor spheroids were monitored by an inverted microscope.

Cellular accumulation

A2780 cells were plated at a density of 4 x 10^5 cells in six-well plates and incubated at 37 °C overnight. Subsequently, the cells were exposed to 10 μM of PROTAC-Pt(IV), carboplatin, and JQ1-Pt(IV) for 6 h. Post-incubation, cells were washed with ice-cold PBS three times and then harvested by trypsinization. The cell pellets were collected by centrifuge at 1000 g for 5 min and washed with PBS three times. After the cells were counted, concentrated nitric acid was added and digested overnight at 70 °C. Upon dilution to 1 mL volume with Milli-Q water, the platinum concentrations were ascertained by ICP-MS.

Western blot

A2780, A2780cisR, or A549 cells were seeded in a 6-well plate and incubated at 37 °C for 24 h. The cells were treated with 1 μM of different compounds for 24 h. Then, the media was removed, and the cells were washed with PBS, followed by harvested by cell scrapper in lysis buffer (Beyotime P0013) containing protease and phosphatase inhibitors cocktail (Thermo Scientific 78442). After centrifugation (14000 rpm, 4 °C), the protein concentration of the supernatant was determined by a BCA assay (Beyotime P0009). The protein samples were denatured by the addition of 5x SDS-PAGE loading buffer (Beyotime P0015L) at 95 °C for 10 min. After being separated by electrophoresis on an 8-12% sodium dodecyl sulfate (SDS) polyacrylamide gel (SDS-PAGE, 90V, 1.5 h), proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (230 mA, 120 min). The membrane was then blocked by 4%

(w/v) non-fat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) buffer for 2 h at room temperature and incubated with primary antibody: BRD4 (Cell Signaling Technology (CST), E2A7X; abcam, ab128874, 1/200), PARP (CST, 9532s, 1/1000), p21 (CST, 2947s, 1/1000), caspase-3 (abcam, ab13585, 1/1000), p53 (CST, 2524s, 1/1000), Actin (CST, 4970s, 1/5000), GAPDH (CST, 2118, 1/10000), cyclin-A2 (Beyotime, AF2524, 1/1000), cyclin-B1 (Beyotime, AF1606, 1/1000), and PD-L1 (abcam, ab213524, 1/200) at 4 °C overnight. Then the membrane was washed with TBST buffer three times and incubated with a secondary antibody with horseradish peroxidase (HRP): against rabbit (CST, 7074s, 1/2000), or against mouse (CST, 7076s, 1/2000) at room temperature for 2 hours. The membrane was washed with TBST buffer three times and incubated with an enhanced chemiluminescence (Bio-Rad) solution for 5 min. Finally, the protein bands were imaged using a Bio-Rad ChemiDoc Touch Imaging System.

Immunofluorescence

A549 or A2780 cells were seeded at an 8-well chamber slide with a density of 2 x 10^4 per well and incubated for 24 h. The cells were treated with 1 μM of ARV771 and PROTAC-Pt(IV) containing 0.1% DMF as supporting solvent for 12 h. Afterward, the cells were washed with PBS three times and fixed with 4% paraformaldehyde at room temperature for half an hour. After permeabilized in PBS with 0.1% Triton X-100 (pH 7.4) for 30 min, the cells were then blocked in Phosphate-buffered saline containing 0.1% Tween 20 (PBST) buffer with 4% BSA for 2 h at room temperature. Then, the cells were treated with primary BRD4 antibody (abcam, ab128874, 1:100 in 1% BSA PBST) at 4 °C overnight. After washing with PBST three times, the cells were incubated with a secondary antibody (Anti-rabbit Alexa 488, abcam, ab15077, 1/200) at room temperature for 1 h. After washing with PBST three times, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min before being imaged by a laser confocal scanning microscope (BRD4 Ex: 488 nm, Em: 510-580 nm; DAPI: Ex: 405 nm, Em: 420-500 nm).

Wound healing assay

A2780 cells were seeded in a 6-well plate and incubated for 48 h. The cells were allowed to attach and grow to form a confluent monolayer. Wounds were created perpendicularly by the tips, and the unattached cells were removed by washing with PBS. The cells were incubated with a medium containing 1% FBS and 40 nM of the compounds containing 0.1% DMF. Photos were taken at 0 and 48 h with 4X magnification by an inverted microscope. The areas of wounds

were quantified using ImageJ. Wound closure ratio (%) = [(original wound area – wound area at 48 h)/original wound area] * 100%.

Cell cycle arrest

A2780 cells were seeded in a 6-well plate with a density of 4 x 10^5 per well and incubated for 24 h. After incubation with 40 nM of the PROTAC-Pt(IV), ARV771, carboplatin, JQ1-Pt(IV), and carboplatin+ARV771, the cells were washed by PBS three times and harvested by trypsinization. The cells were washed with PBS once and fixed in 70% ethanol overnight at 4 °C. The fixed cells were stained with propidium iodide (PI) solution (0.1% Triton-X, 0.2 mg/L RNase A, 0.02 mg/mL PI) for 15 min in the dark before being analyzed by flow cytometer.

Annexin V/PI double staining assay

4 x 10^5 A2780 cells were seeded in a 6-well plate and incubated for 24 h to allow attachment. The cells were incubated with 40 nM of PROTAC-Pt(IV), ARV771, carboplatin, JQ1-Pt(IV), and ARV771+carboplatin for 48 h. The cells were then washed with PBS three times and collected by trypsinization. Subsequently, the cells were washed with PBS once and Annexin-binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH = 7.4) twice gently. After that, the cell pellets were suspended in 100 μL binding buffer and costained with Annexin V-FITC conjugate and PI for 15 min at room temperature. The cell suspensions were diluted with 400 μL binding buffer and analyzed by a flow cytometer**.**

Animal ethics

All mice were purchased from the City University of Hong Kong and cultured under pathogen-free conditions. All animal-related experiments were carried out following the Guidelines for the Care and Use of Laboratory Animals of the City University of Hong Kong and approved by the Animal Research Ethics Sub-Committee of the City University of Hong Kong (AN-STA-00000308).

Blood concentration profile determination

15 BALB/c mice were divided into three groups randomly. These mice were intravenously injected with carboplatin and PROTAC-Pt(IV) (n = 5) at a single dose of 2 mg-Pt/kg. The same molar concentration of ARV771 was administrated (10.1 mg-ARV771/kg). 0.9% NaCl + 1% DMF + 20% kolliphor solution worked as the vehicle. The volume of intravenous injection was determined by the mice's body weight, with 0.2 mL per 20 g. At different time points post-

compound administration, mice were anesthetized, and blood samples were collected via submandibular venipuncture. The volume of blood samples was carefully quantified, and the Pt content in them was determined using ICP-MS after digestion with HNO_3 at 70 °C overnight. The concentration of ARV771 was determined by LC-HRMS with the establishment of a standard curve. The blood concentration-time profiles were analyzed by the PKsolver 2.0 software, and the curve fit the Intravenous Bolus Administration One-Compartment Model for analysis.

Maximum tolerated dose (MTD) determination

To determine the biocompatibility of PROTAC-Pt(IV) with systematic administration, BALB/c mice at the age of 6-8 weeks were randomly divided into 4 groups (n = 3). All the mice were numbered with ear tags, and subsequently anesthetized in a chamber filled with 2% isoflurane in oxygen. Each group was intravenously injected with various dosages of PROTAC-Pt(IV) (0 mg-Pt/kg, 1 mg-Pt/kg, 2 mg-Pt/kg, and 3 mg-Pt/kg in 0.9% NaCl containing 1% DMF and 20% kolliphor). The body weight of the mice was monitored every 1-2 days for a total of 14 days.

***In vivo* antitumor evaluation**

To evaluate the anticancer activity of PROTAC-Pt(IV), a xenograft model was established on BALB/c mice. The 4T1 cells (2×10^6 per mouse) were subcutaneously implanted in the right flank of 24 BALB/c mice (6-8 weeks old). When the tumor volumes reached about 100 mm^3, the mice were divided into 4 groups, with 6 mice per group, to ensure the average tumor volume of each group was close to each other. All the mice were then numbered with ear tags and intravenously injected with (i) 0.9% NaCl + 1% DMF + 20% kolliphor, (ii) carboplatin, (iii) carboplatin mixed with ligand ARV771 (molar ratio 1:1), (iv) PROTAC-Pt(IV). 0.9% NaCl + 1% DMF + 20% kolliphor was used as the vehicle to dissolve all the tested compounds. The complexes were injected every 3 days for a total of 5 injections. After the first treatment, the tumor dimensions and body weight were measured every two days. After 20 days, all the mice were sacrificed, and the tumors and the major organs (heart, liver, spleen, lung, and kidney) were collected for further investigation.

Tissue staining

The collected tumor and vital organs were fixed in 10% paraformaldehyde, followed by dehydration with gradient ethanol (50, 70, 80, 95, and 100%). The dehydrated tissues were cleared with xylene and embedded in paraffin. The embedded tissues were cut into 5 μm-thick

sections, which were then mounted on glass slides, deparaffinized, and subjected to staining with H&E or immunohistology analysis. The mean fluorescence intensity of TUNEL, CD3, and CD4 were quantified using ImageJ.

Statistical analysis

All the results were presented as Mean ± SD where applicable. GraphPad Prism 9.5 was used for statistical analysis.

Table S1. IC_{50} value (nM) of various complexes against different cell lines by MTT assay.

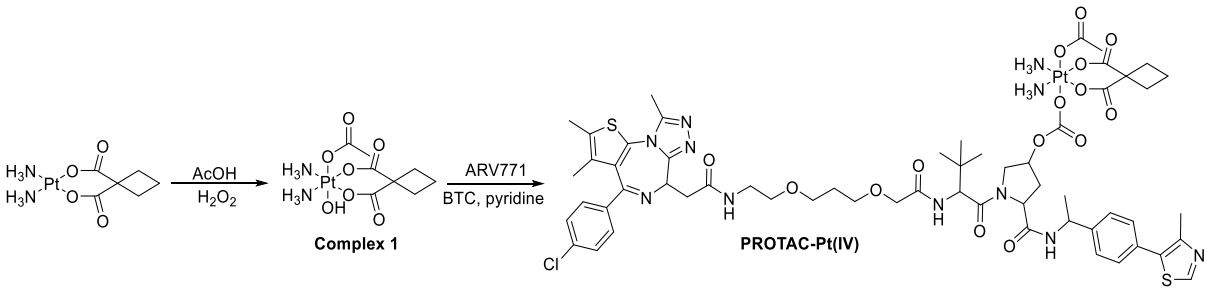
Cell line	carboplatin	JQ1-Pt(IV)	ARV771	ARV771+ carboplatin	PROTAC-Pt(IV)
A2780	19,300 ± 3,400	>25,000	1.5 ± 0.6	1.2 ± 0.3	11.0 ± 2.7
MCF7	47,200 ± 6,800	>25,000	7.7 ± 1.3	8.4 ± 1.3	35.4 ± 17.9
HeLa	77,500 ± 5,300	>25,000	10.5 ± 4.8	14.4 ± 1.8	102.5 ± 8.0
A2780cisR (RF[a])	48,700 ± 4,200 (2.5)	>25,000	16.8 ± 5.6 (11.2)	11.2 ± 0.6 (9.3)	128.8 ± 19.5 (11.7)
MRC5	19,400 ± 4,000	>25,000	150 ± 30	120 ± 60	2,800 ± 300
4T1	11,100 ± 2,700	>25,000	500 ± 200	400 ± 100	4,500 ± 400
A549	33,300 ± 7,100	>25,000	300 ± 80	300 ± 200	5,100 ± 1,900
A549cisR (RF[a])	124,100 ± 33,500 (3.7)	>25,000	1200 ± 400 (4.0)	1000 ± 250 (3.7)	6,700 ± 2,000 (1.3)

[a)] Resistance factor (RF) = IC_{50} value of A549cisR (A2780cisR) / IC_{50} value of A549 (A2780).

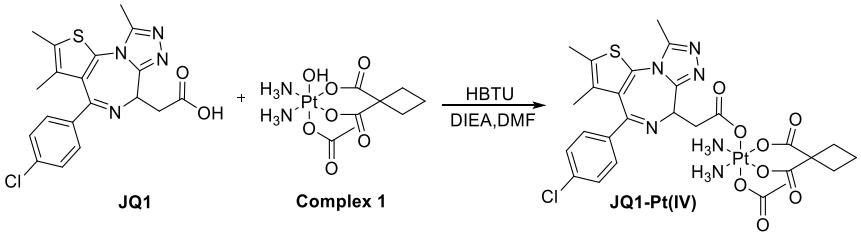
Table S2. Calculated pharmacokinetics parameters for single *i.v.* administration of PROTAC-Pt(IV), ARV771, and carboplatin. 2 mg-Pt/kg for platinum complexes, and 10.1 mg/kg for ARV771 (same molar concentration as Pt).

	Carboplatin	**ARV771**	**PROTAC-Pt(IV)**
CL [mL/h][a]	6.98	15.54	2.65
Vss [mL][b]	30.85	38.54	6.74
$AUC_{0\text{-}inf}$ [h·μM][c]	46.87	28.01	338.20
$t_{1/2}$ [h][d]	0.82	0.31	0.43

[a)] Clearance, the volume of plasma or blood that is completely cleared of the drug per unit of time. [b)] Volume of Distribution at Steady State, the apparent volume into which a drug distributes in the body at a steady state. [c)] Area Under the Curve to Infinity, the area under the drug concentration-time curve from the time of administration to infinity. [d)] Half-life, the time it takes for the concentration of a drug in the body to decrease by 50% during the elimination phase



Scheme S1. The synthetic route of PROTAC-Pt(IV).



Scheme S2. The synthetic route of JQ1-Pt(IV).

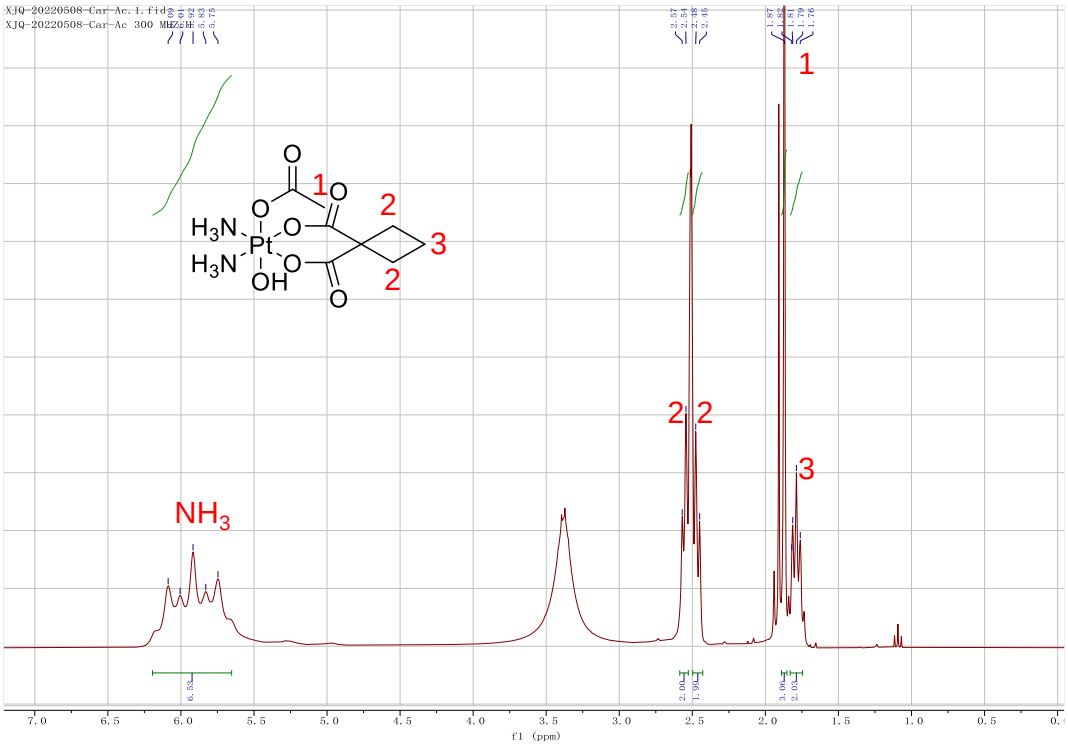


Figure S1. ^{1}H NMR spectra of complex **1** in DMSO-d_6.

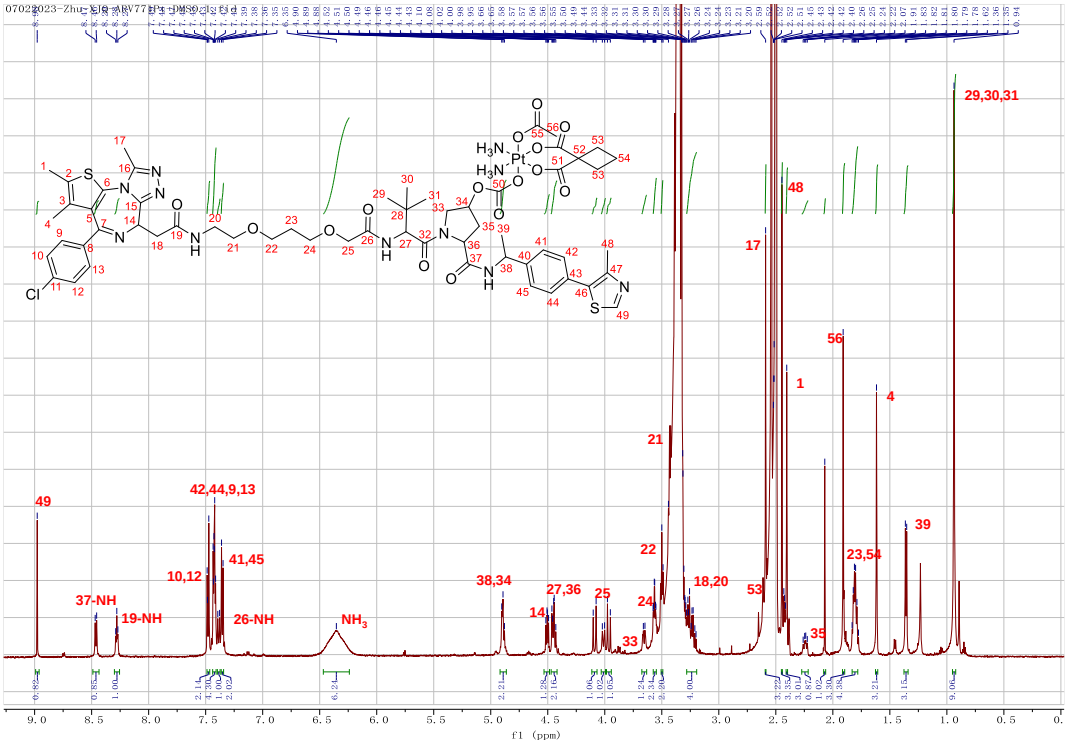


Figure S2. ^{1}H NMR spectra of PROTAC-Pt(IV) in DMSO-d_6.

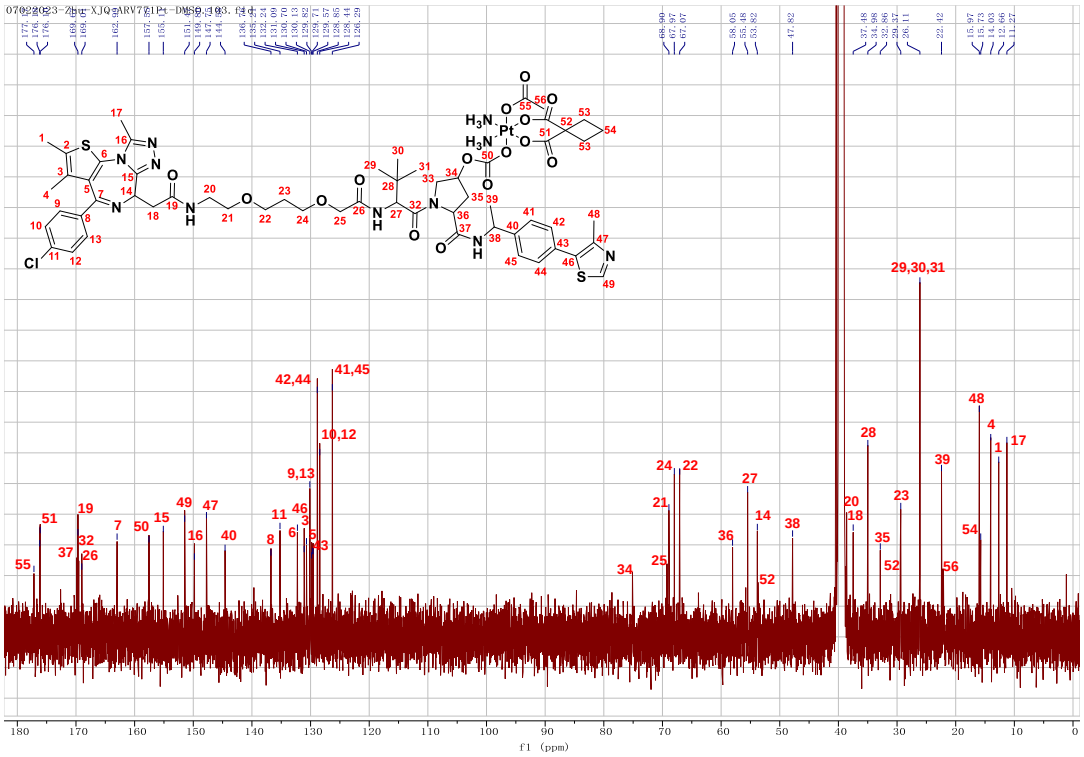


Figure S3. ^{13}C NMR spectrum of PROTAC-Pt(IV) in DMSO-d_6.

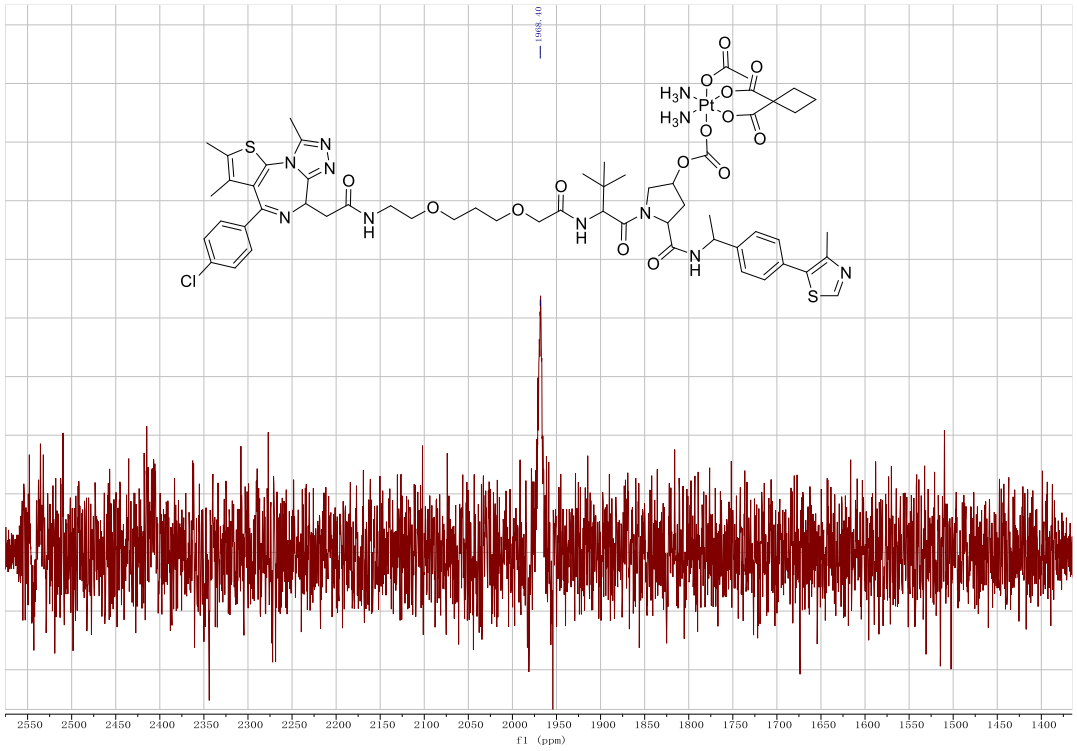


Figure S4. ^{195}Pt NMR spectrum of PROTAC-Pt(IV) in DMSO-d_6.

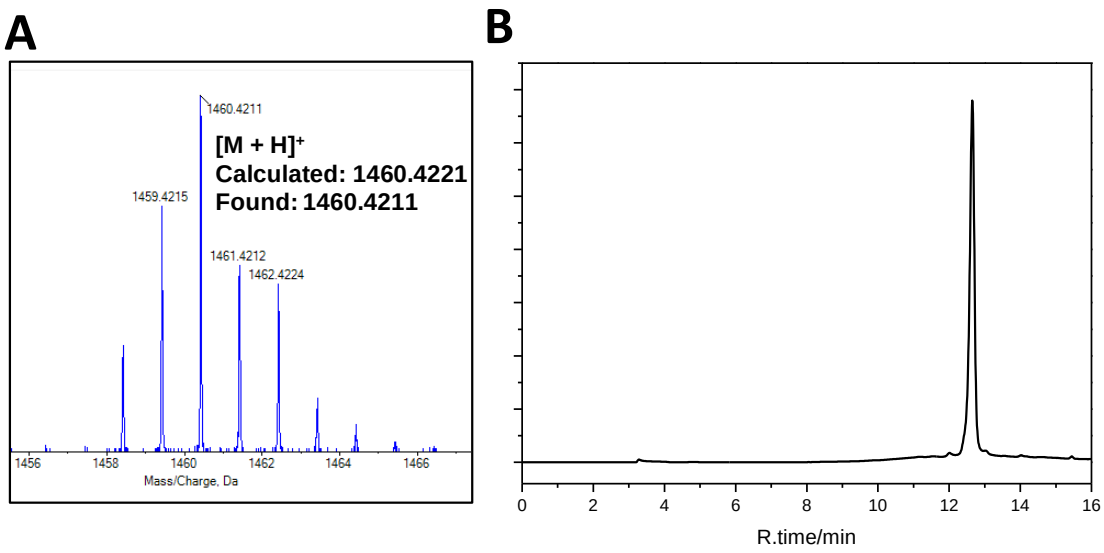


Figure S5. (A) ESI-HRMS spectrum and (B) HPLC chromatogram of PROTAC-Pt(IV) (purity: 98%).

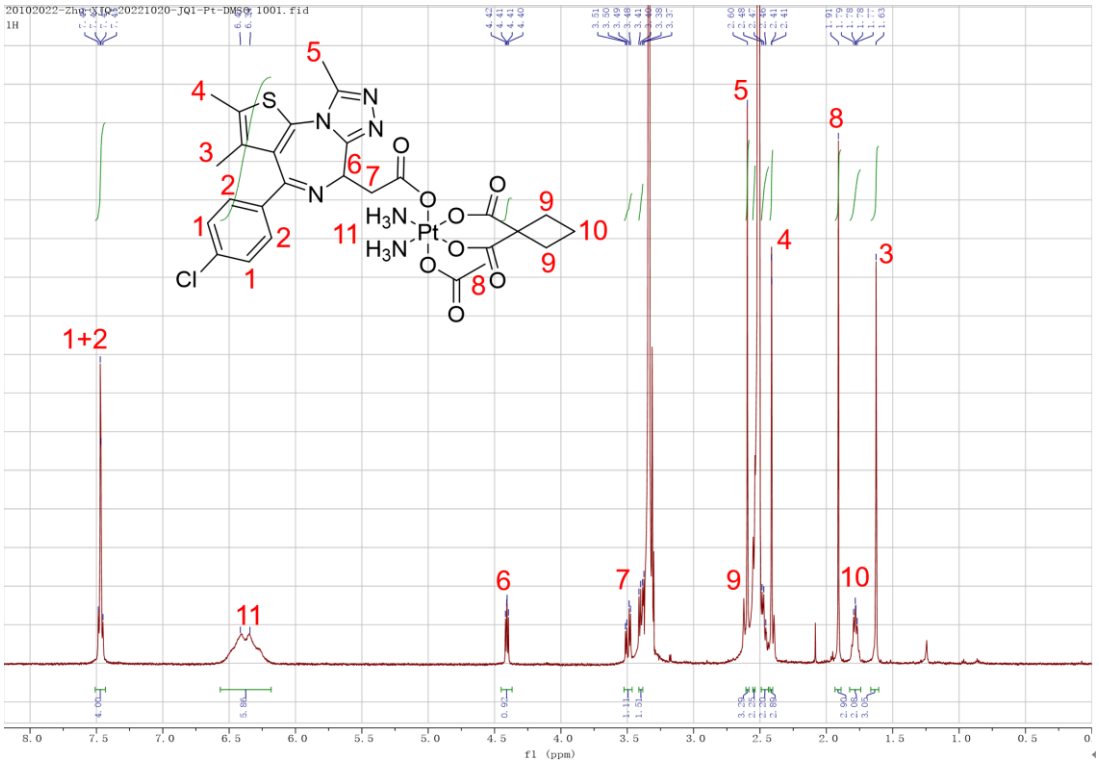


Figure S6. ^{1}H NMR spectrum of JQ1-Pt(IV) in DMSO-d_6.

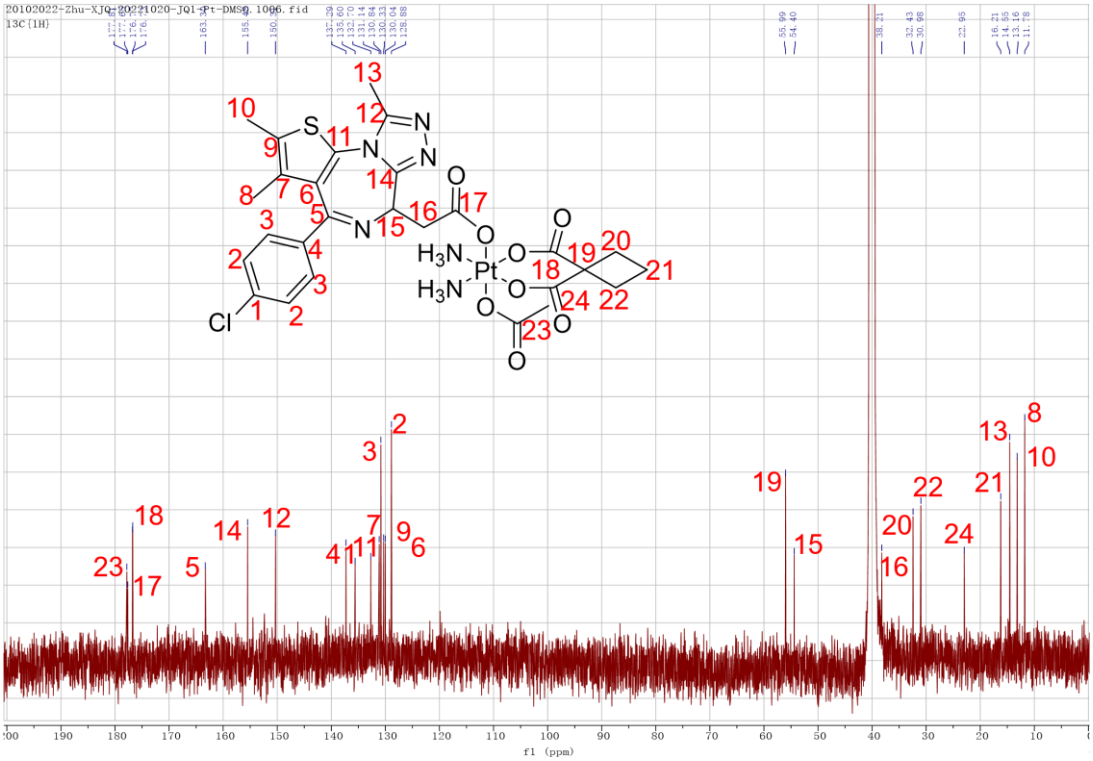


Figure S7. ^{13}C NMR spectrum of JQ1-Pt(IV) in DMSO-d_6.

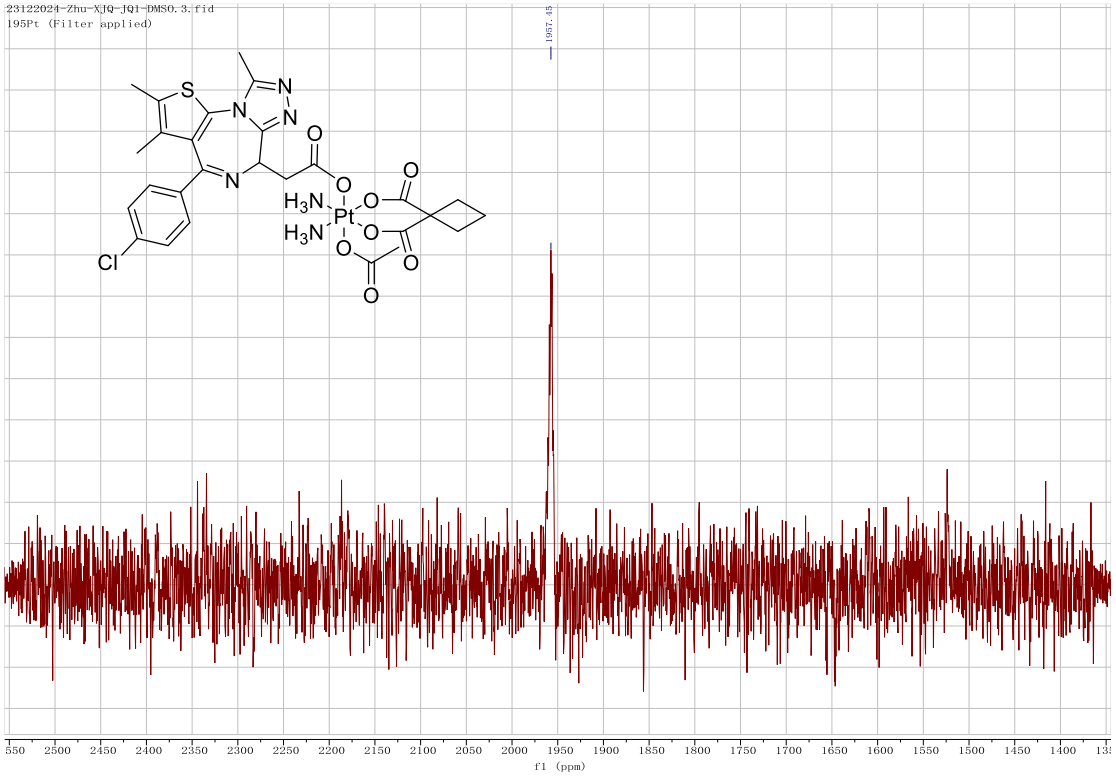


Figure S8. ^{195}Pt NMR spectrum of JQ1-Pt(IV) in DMSO-d_6.

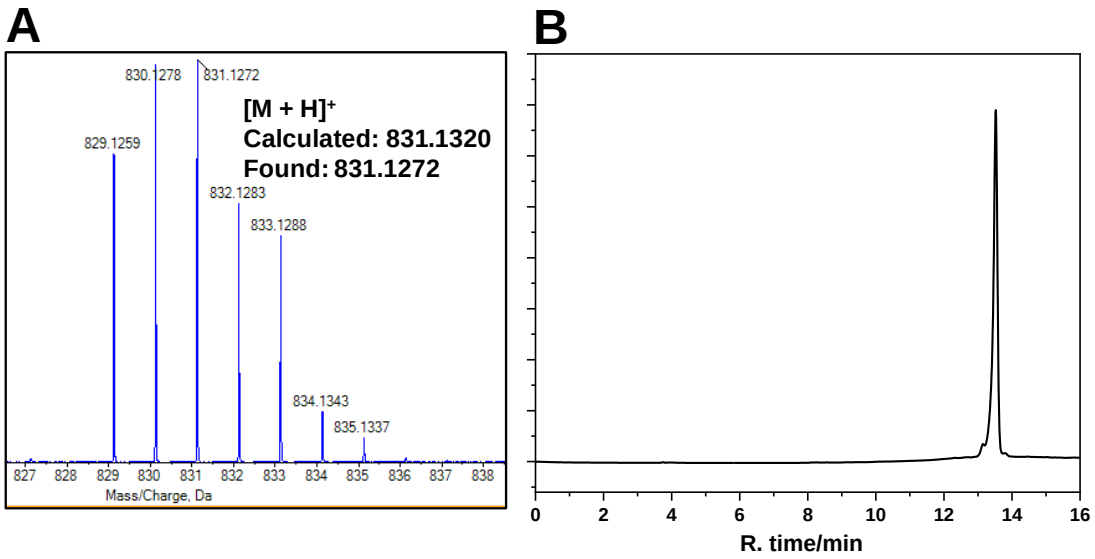


Figure S9. (A) ESI-HRMS spectrum and (B) HPLC chromatogram of JQ1-Pt(IV) (purity: 97%).

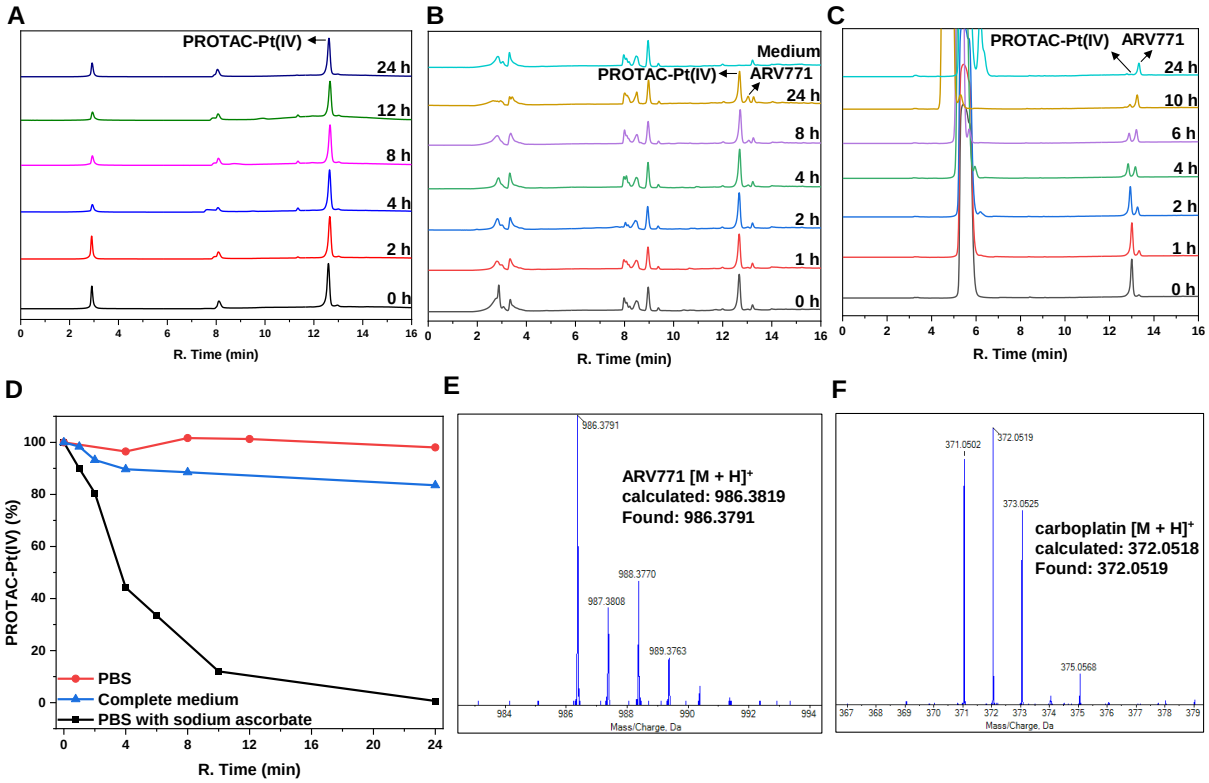


Figure S10. Stability of PROTAC-Pt(IV) in PBS, complete medium, and PBS with sodium ascorbate, monitored by HPLC (254 nm). (A) Chromatograms of PROTAC-Pt(IV) in PBS buffer (pH 7.4). (B) Chromatograms of PROTAC-Pt(IV) in complete DMEM medium. (C) Chromatograms of PROTAC-Pt(IV) in PBS buffer (pH 7.4) containing sodium ascorbate. (D) Percentages of PROTAC-Pt(IV) remained in different buffers. (E) HRMS spectrum of ARV771 as the reduced product. (F) HRMS spectrum of carboplatin as the reduced product.

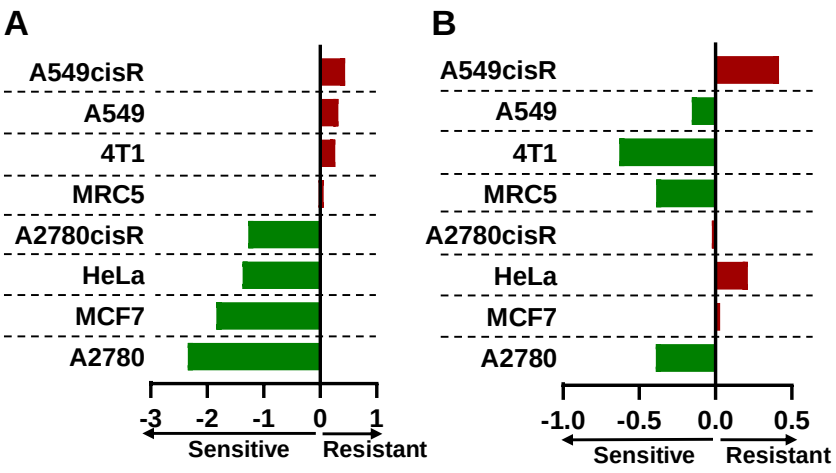


Figure S11. Comparative analysis of cytotoxicity of (A) PROTAC-Pt(IV) and (B) carboplatin. The values were calculated as log(IC_{50} of relative cell line under irradiation) – log(mean IC_{50}). A negative or positive value means the cell lines are sensitive or resistant to the compound, respectively.

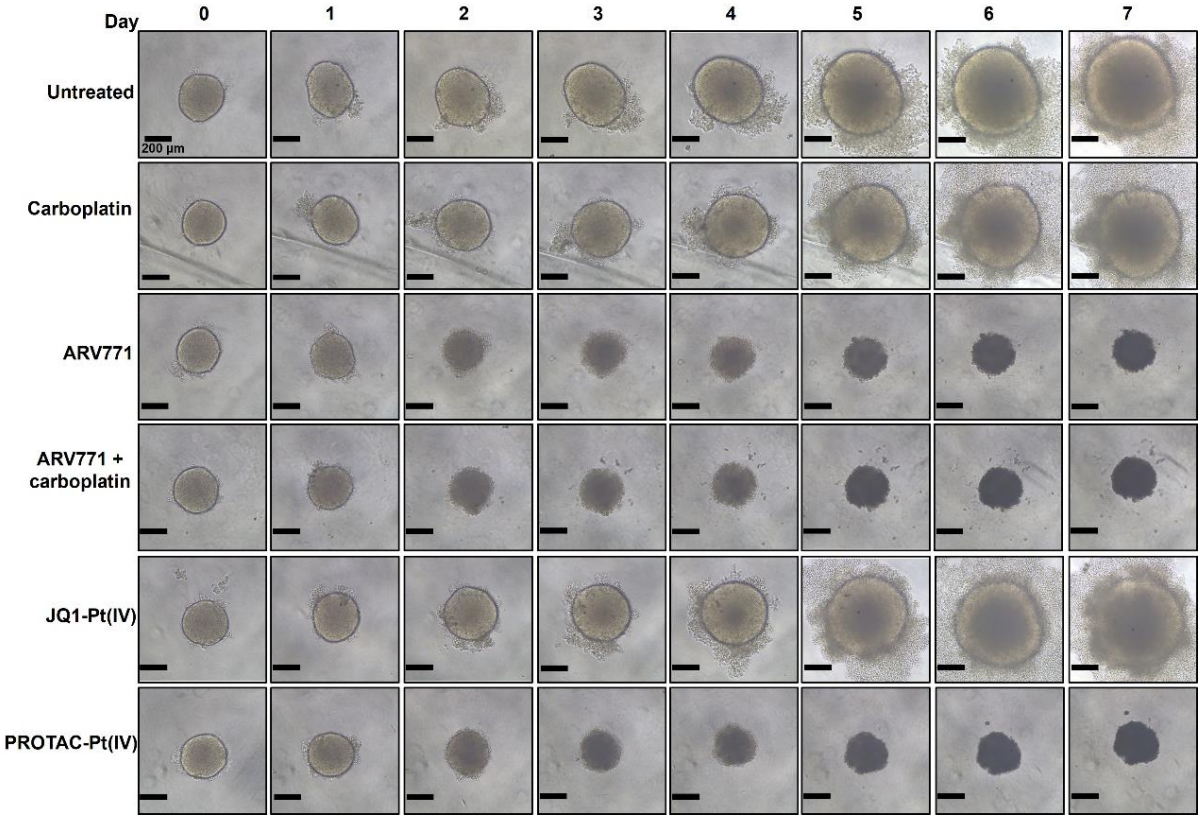


Figure S12. Images of A2780cisR spheroids treated with carboplatin, ARV771, ARV771+carboplatin, JQ1-Pt(IV), and PROTAC-Pt(IV) for 7 days.

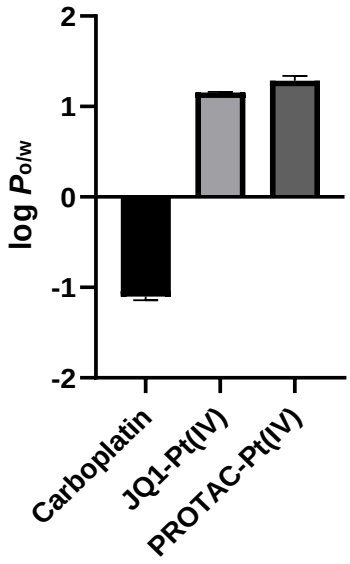


Figure S13. Partition coefficients of carboplatin, JQ1-Pt(IV), and PROTAC-Pt(IV).

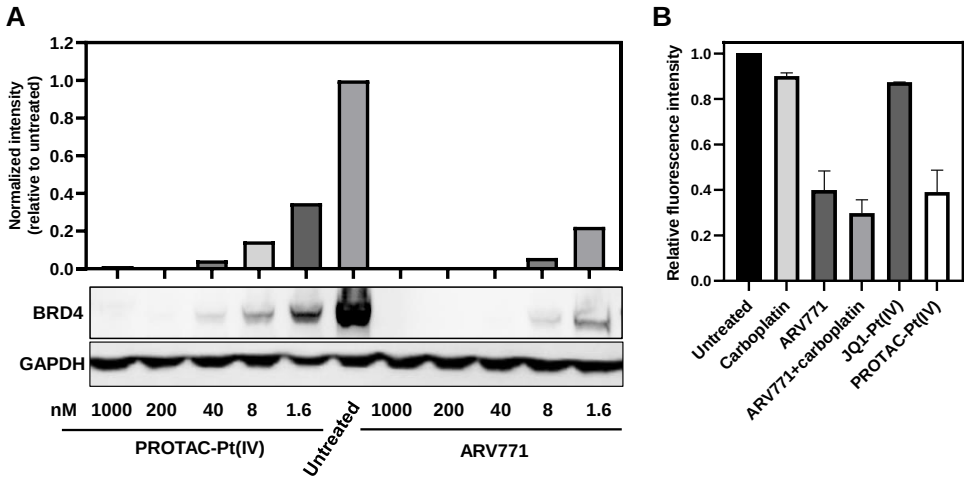


Figure S14. (A) Western blotting images and quantification of BRD4 in A2780 cells upon treatment with different concentrations of PROTAC-Pt(IV) and ARV771. (B) Quantitative analysis of fluorescence intensity from the confocal images in Figure 3D.

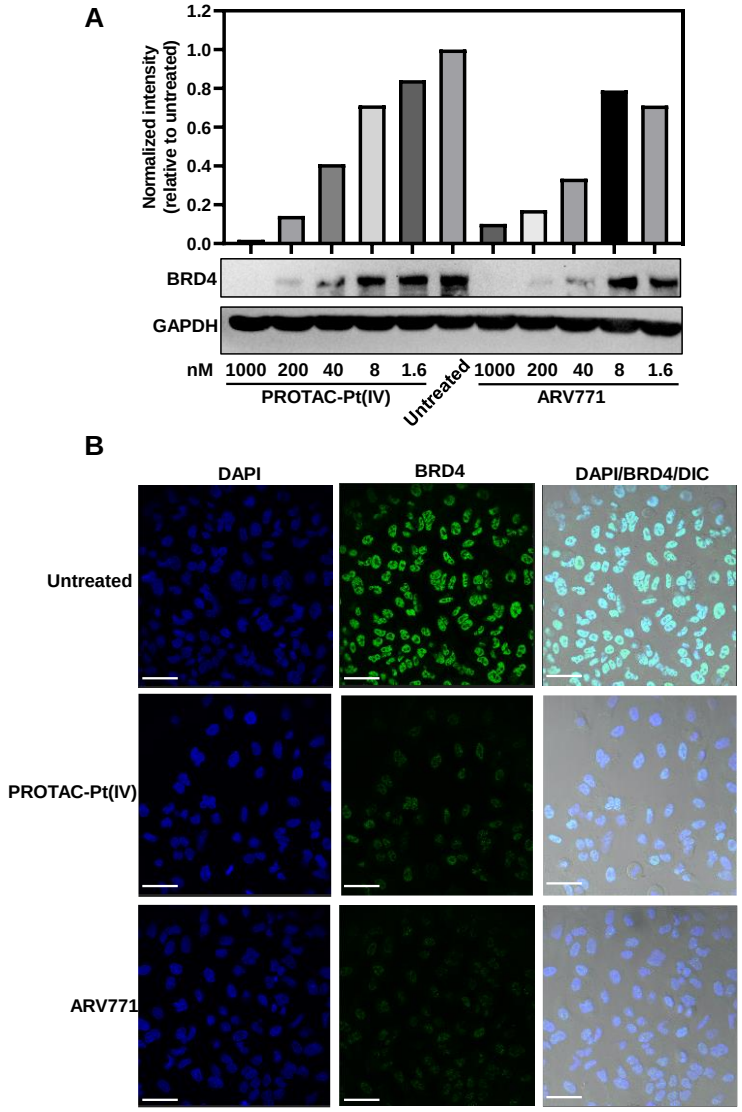


Figure S15. BRD4 degradation in A549 cells. (A) Western blot images and quantification of BRD4 in A549 cells upon treatments with different complexes. (B) Confocal images of immunofluorescence of BRD4 in A549 cells upon treatment of different complexes. Scale bars, 50 μm.

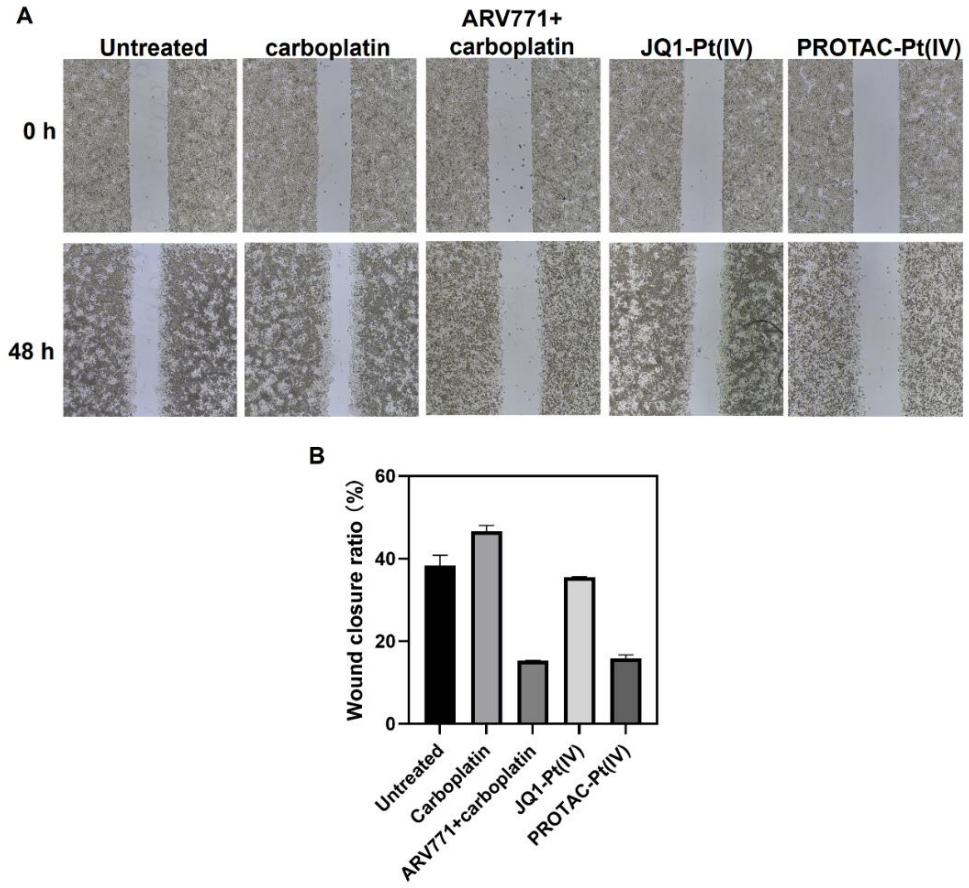


Figure S16. (A) Images of cell wound healing assay in A2780 cells treated with 40 nM of PROTAC-Pt(IV), carboplatin, ARV771+carboplatin, and JQ1-Pt(IV). (B) Quantitative analysis of the wound healing ratio.

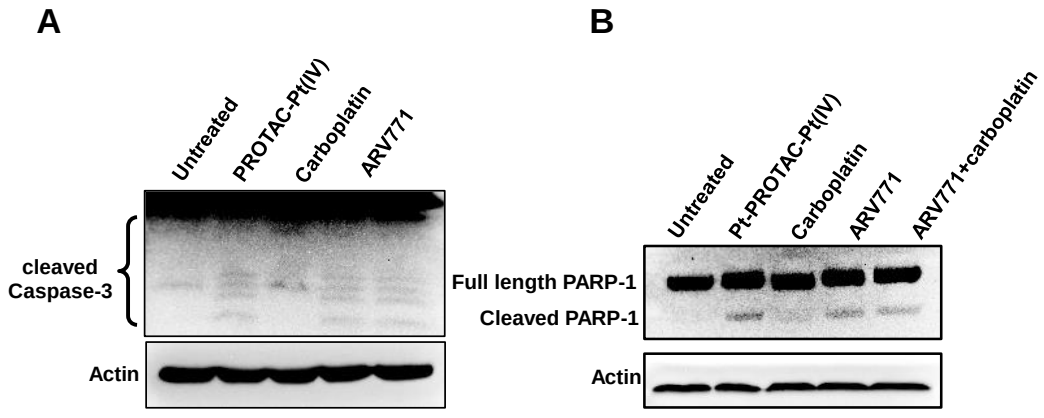


Figure S17. Western blot images of (A) cleaved caspase-3, and (B) cleaved PARP-1 upon treatment with different complexes.

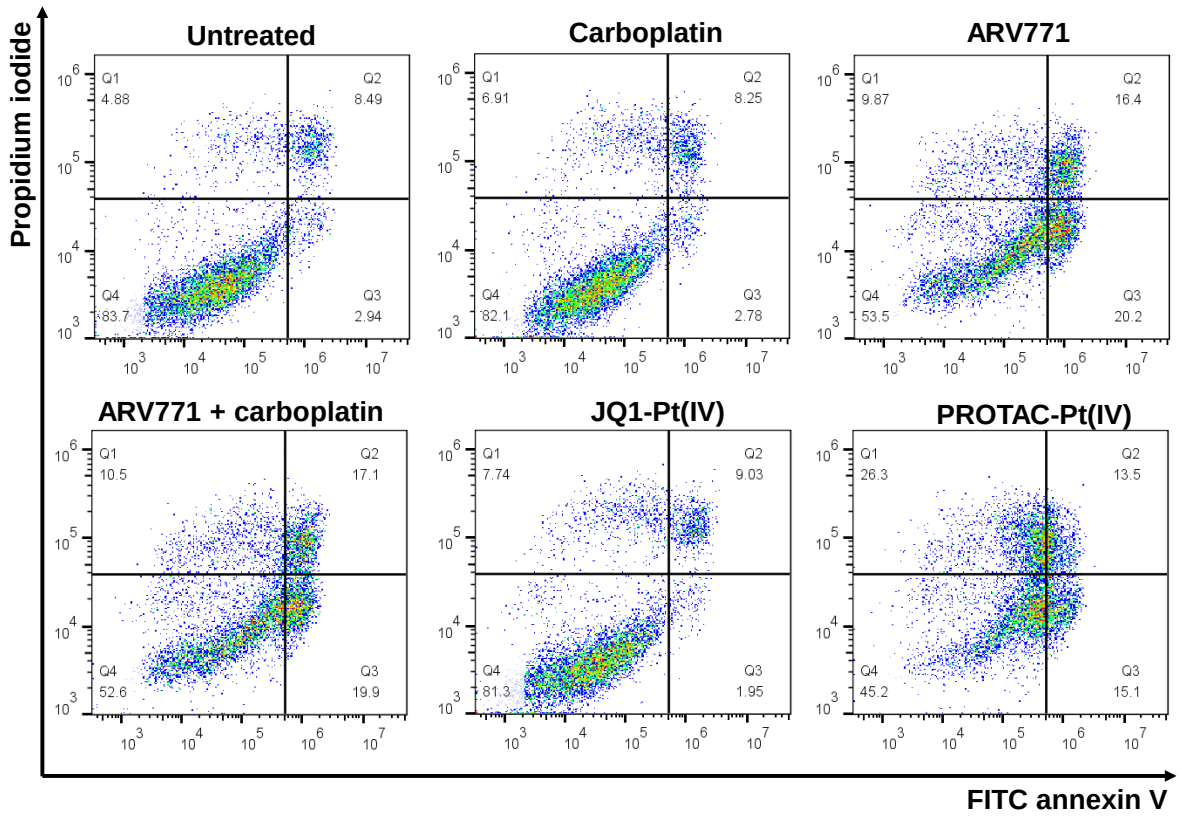


Figure S18. Apoptosis of A2780 cells treated for 48 h with 40 nM of PROTAC-Pt(IV), carboplatin, ARV771, JQ1-Pt(IV), as well as the 1:1 mixture of ARV771 and carboplatin. Analyzed with PI/annexin V double staining by flow cytometry.

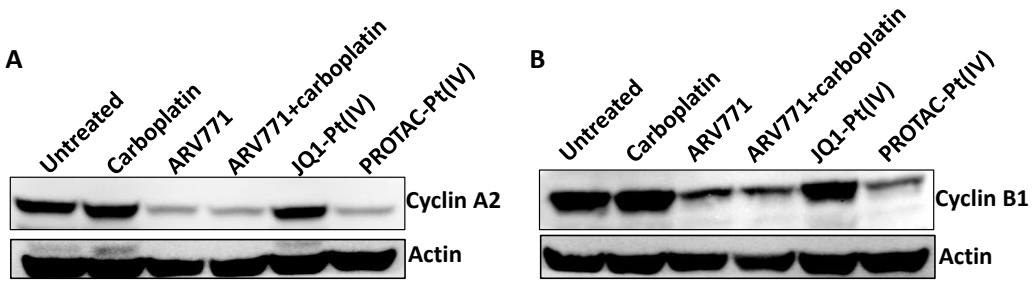


Figure S19. Western blot images of (A) cyclin A2, and (B) cyclin B1 in A2780 cells upon treatment with different complexes.

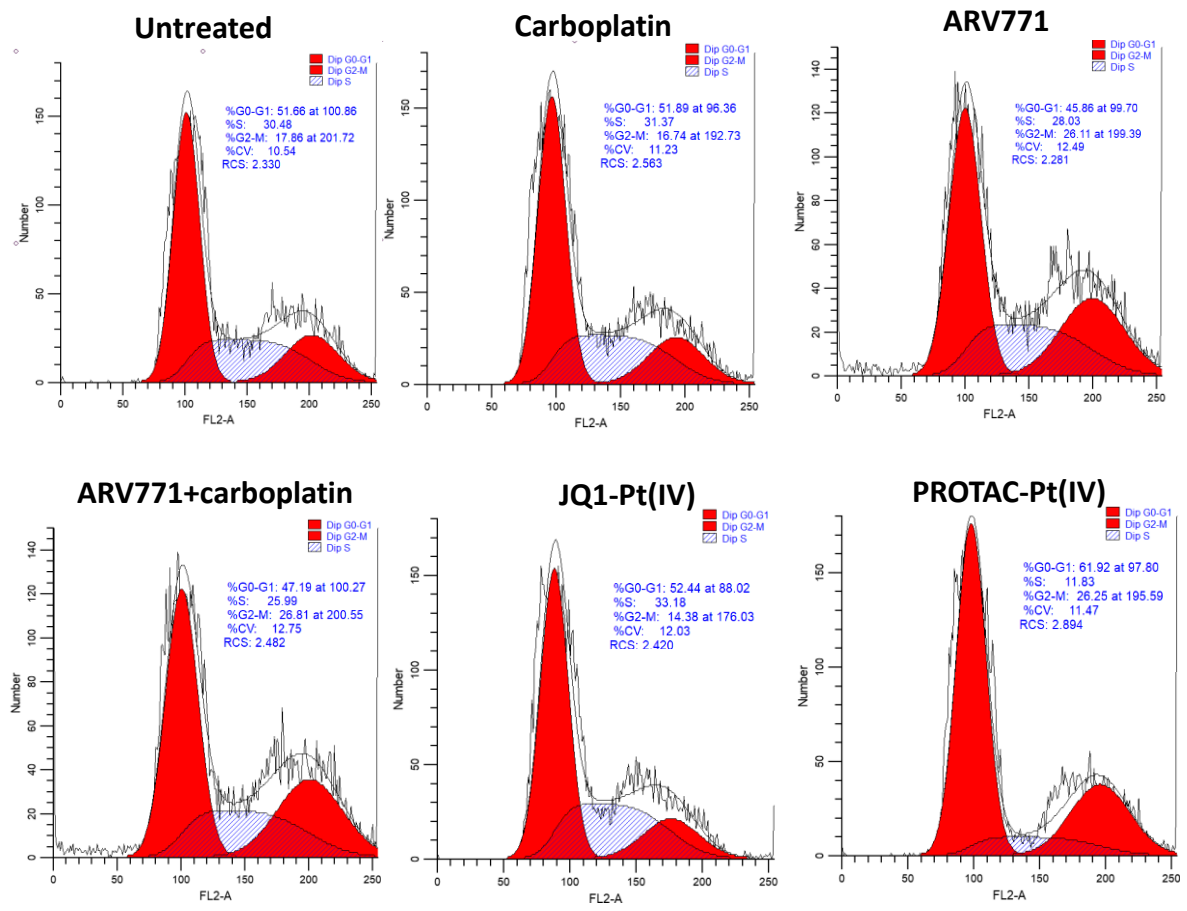


Figure S20. Histograms of A2780 cells staining PI upon different treatments.

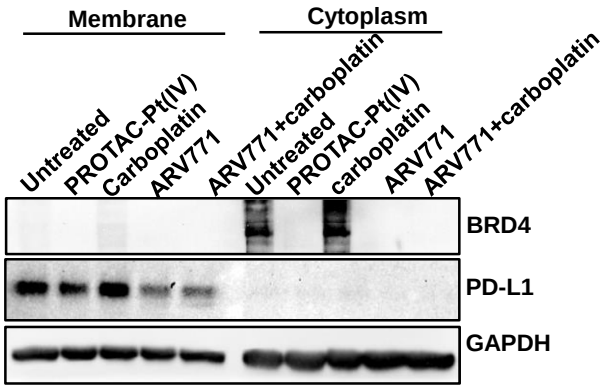


Figure S21. Western blot images of BRD4 and PD-L1 in the membrane and cytoplasm fractions of A2780cisR cells upon treatment with different complexes.

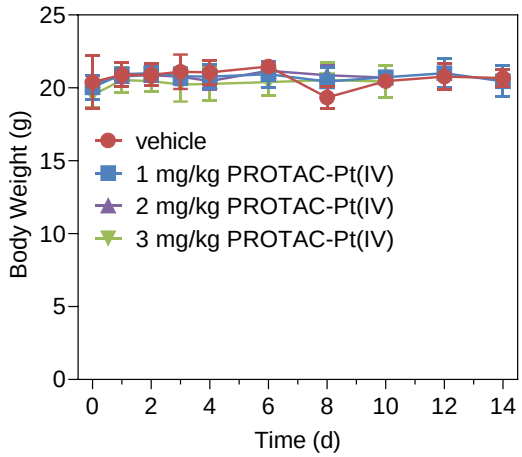


Figure S22. The change of mice body weight after treatments with various doses of PROTAC-Pt(IV) for toxicity test.

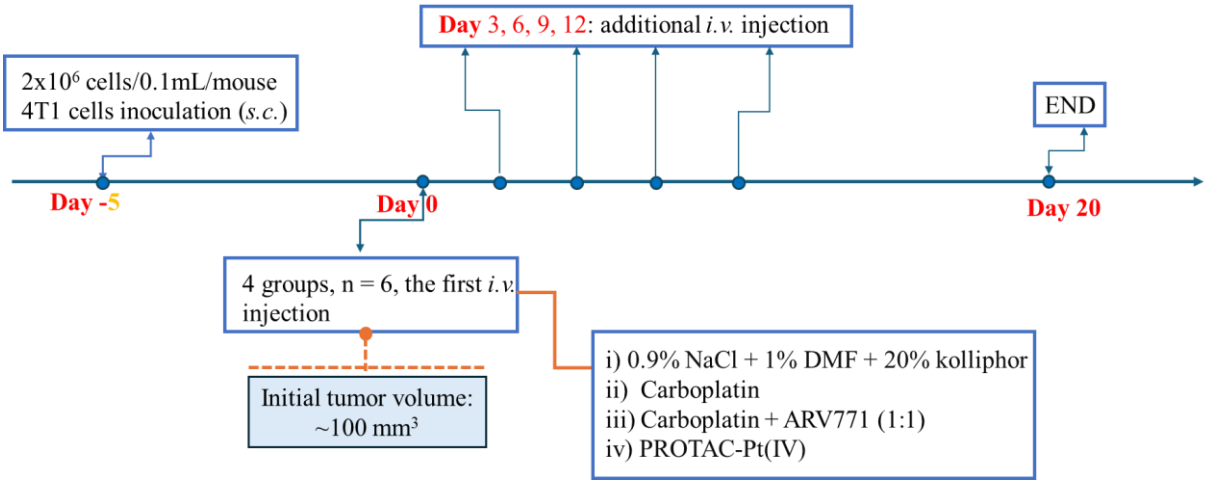


Figure S23. The time schedule for antitumor activity evaluation PROTAC-Pt(IV) in 4T1 xenograft mouse model (4 groups, n = 6). When the tumor volume reached around 100 mm^3, the treatment started at day 0. Additional four *i.v.* injections were given on day 3, 6, 9, and 12, respectively.

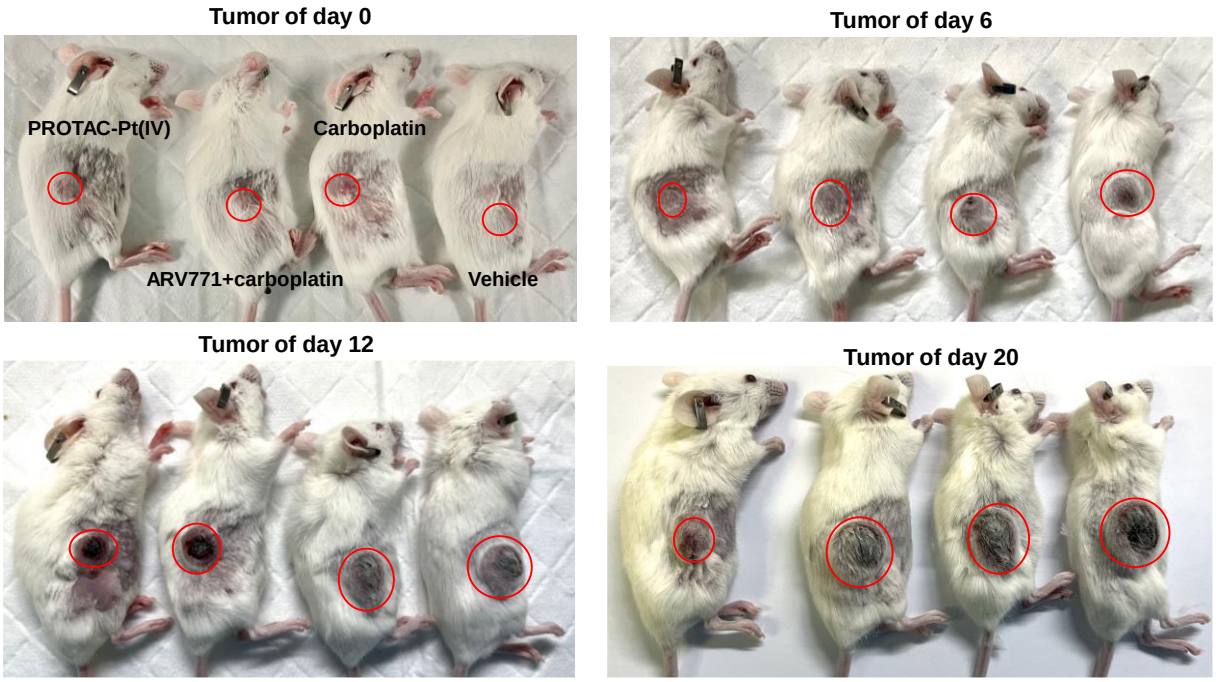


Figure S24. Representative photos of mice from each group during the treatment.

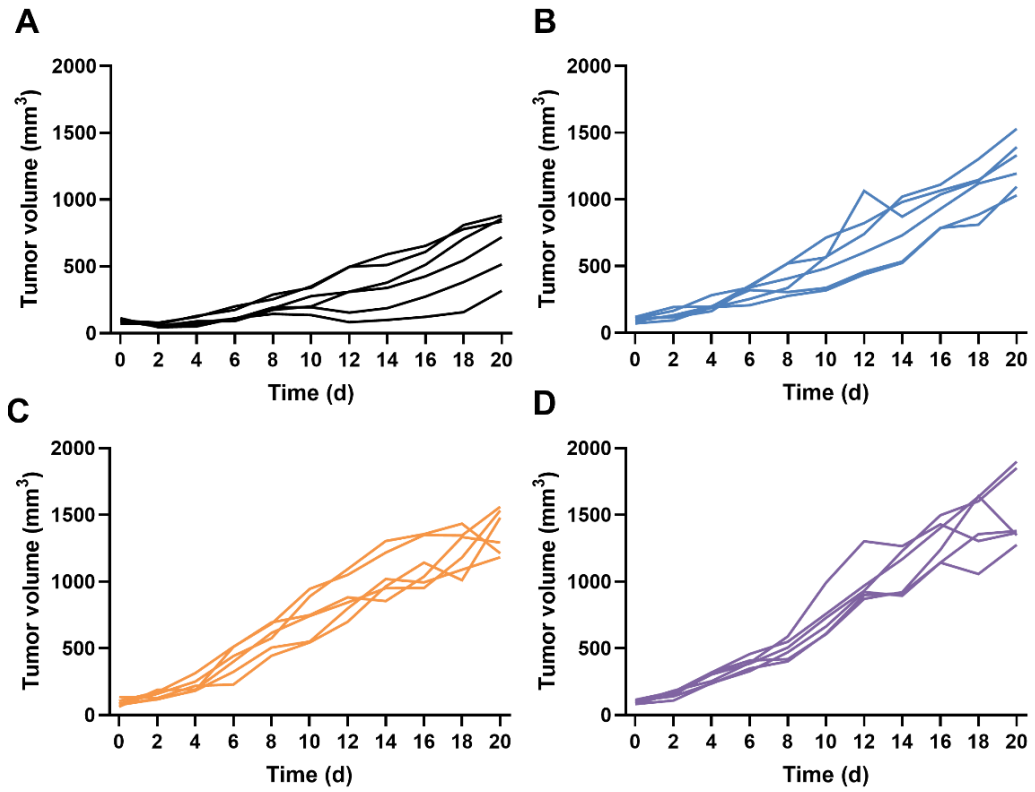


Figure S25. Tumor growth curve of individual mice treated with (A) PROTAC-Pt(IV), (B) ARV771+carboplatin, (C) carboplatin, and (D) vehicle.

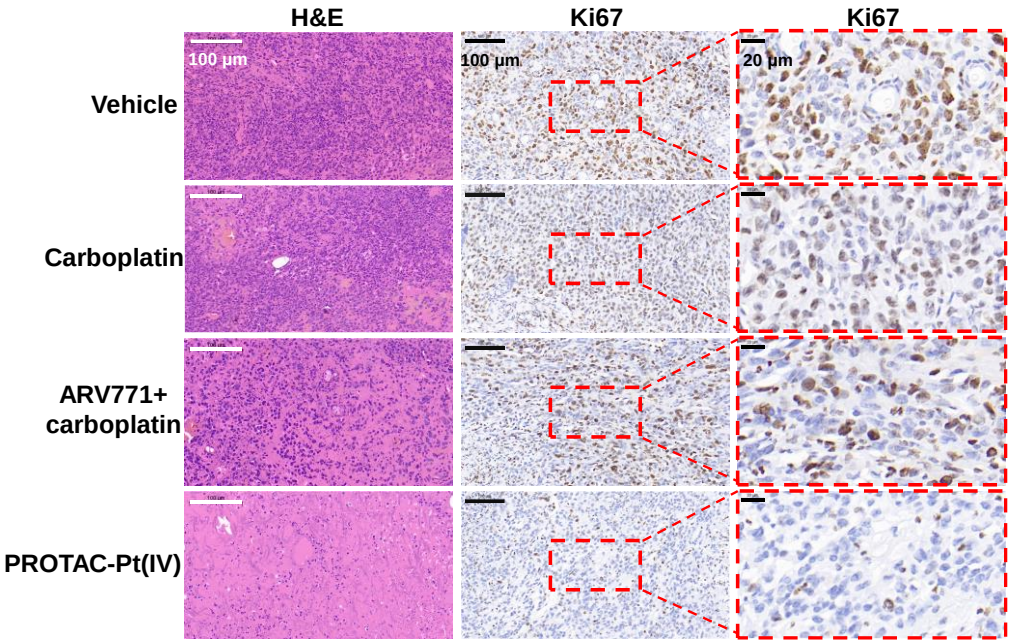


Figure S26. Hematoxylin and eosin (H&E) staining and Ki67 staining for dissected tumors in different groups.

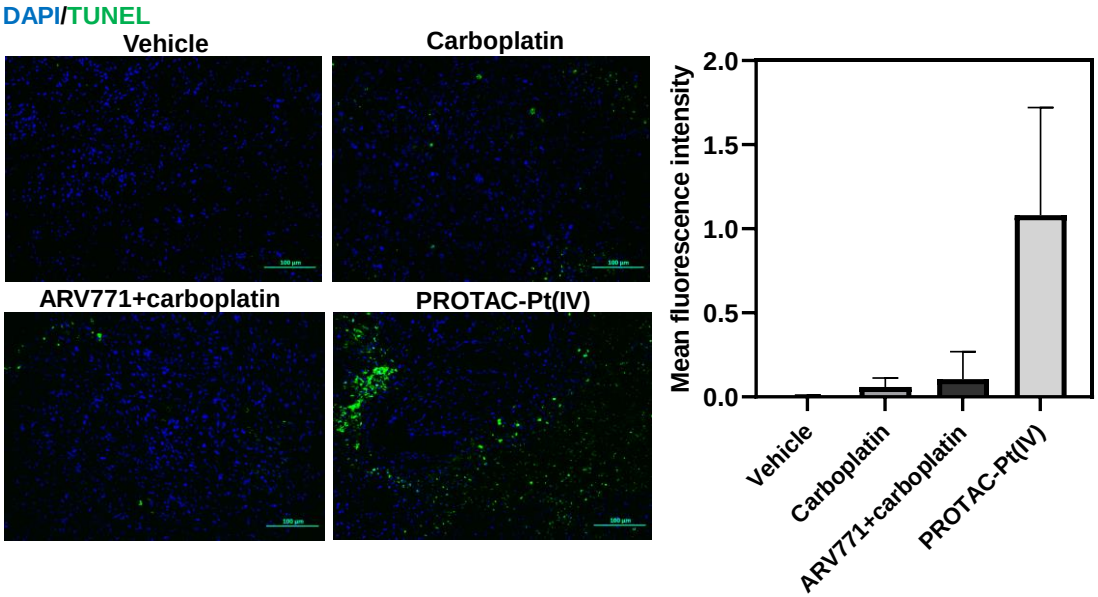


Figure S27. TUNEL staining and quantitative analysis for tumors from each group.

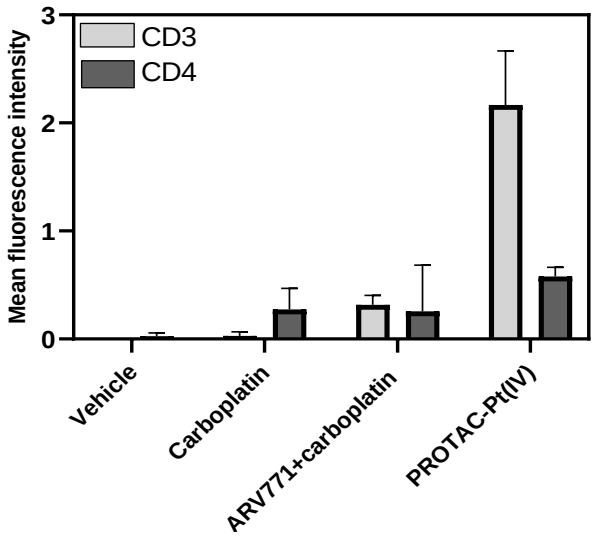


Figure S28. Quantitative analysis of immunofluorescence staining of CD3 and CD4 for tumors from each group.

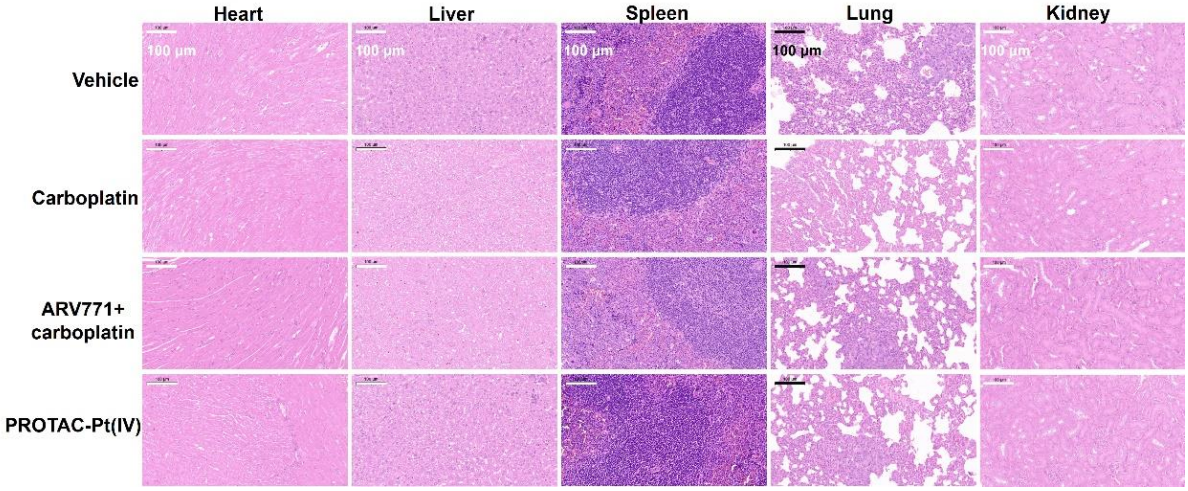
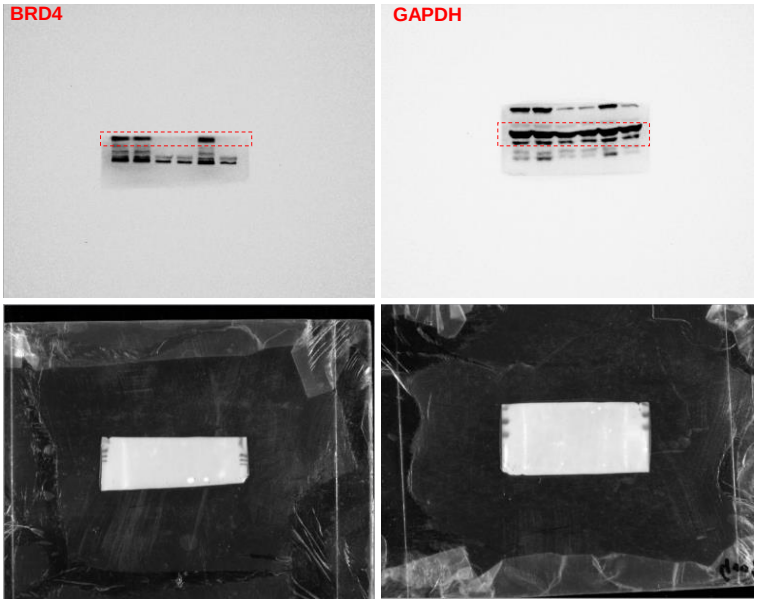
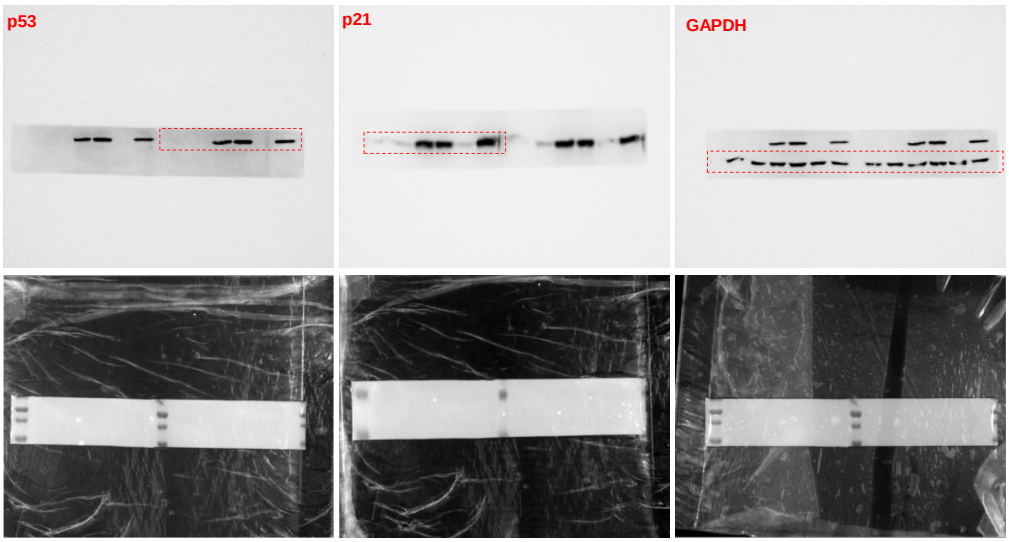


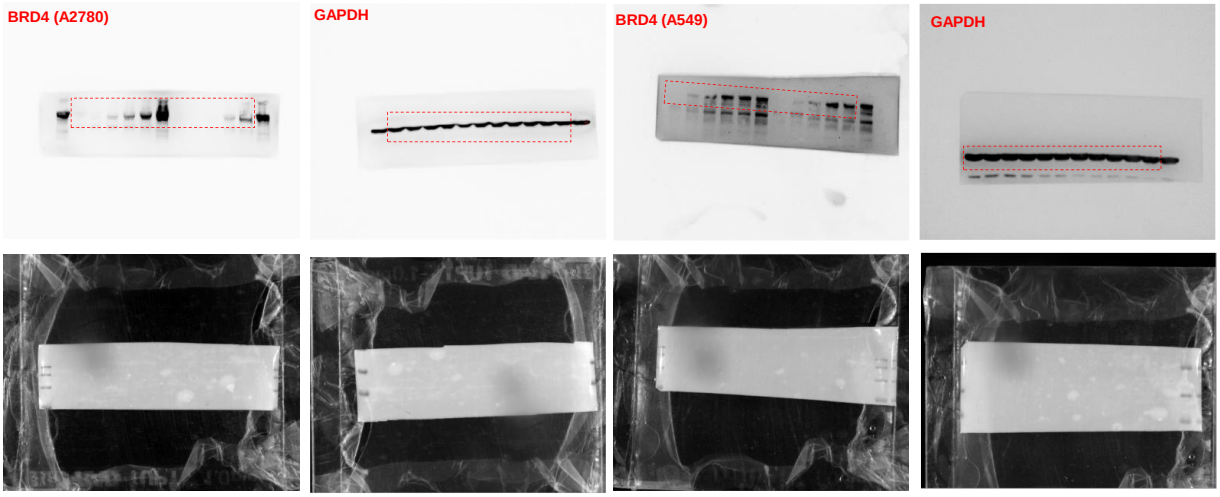
Figure S29. H&E Staining of tissue sections from excised organs of 4T1 xenograft mice after the treatments with vehicle, carboplatin, ARV771+carboplatin (1:1), and PROTAC-Pt(IV) for 20 days. The scale bar is 100 μm.



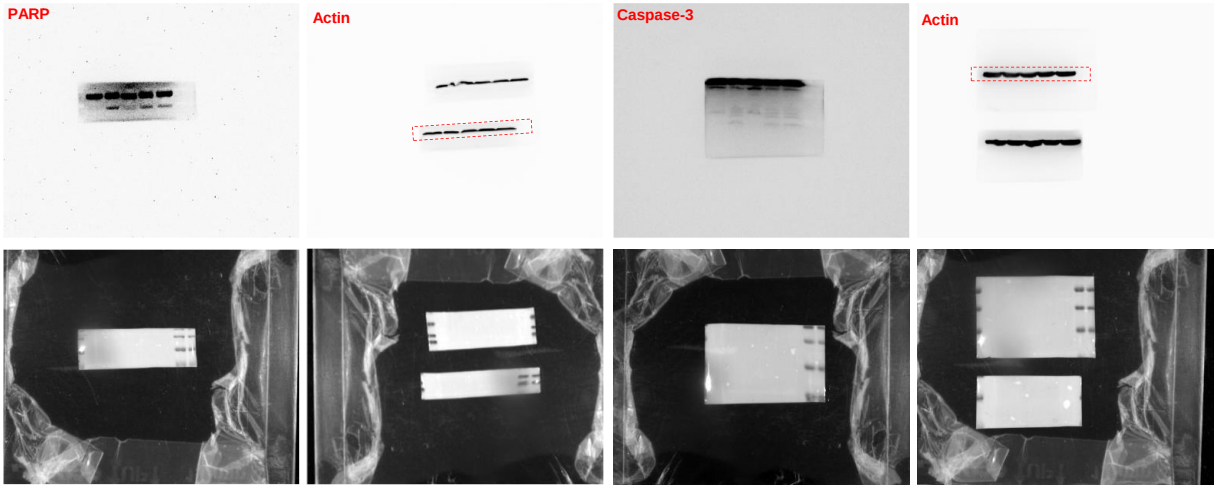
The full images of Figure 3C.



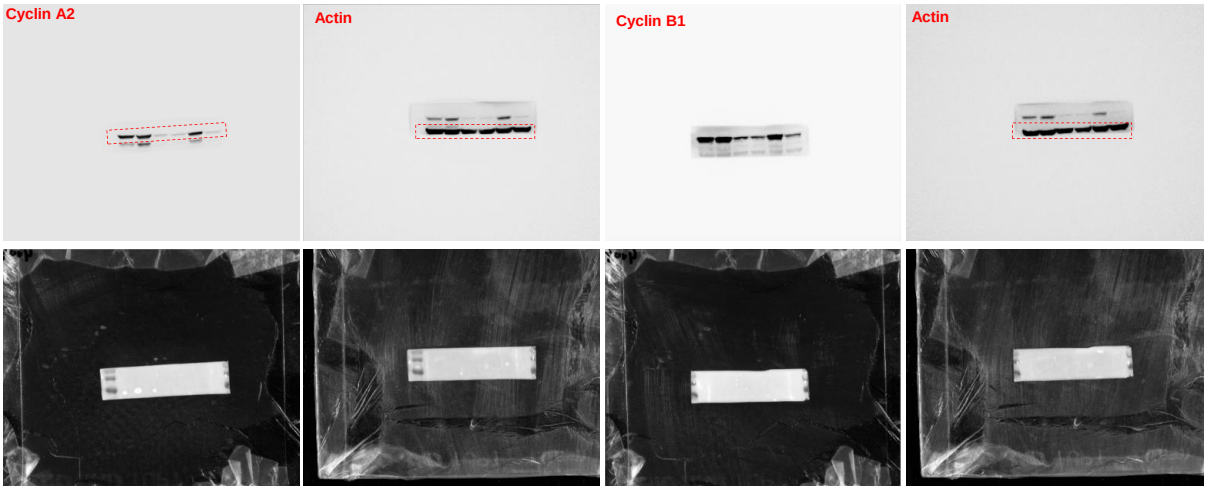
The full images of Figures 4A and 4C.



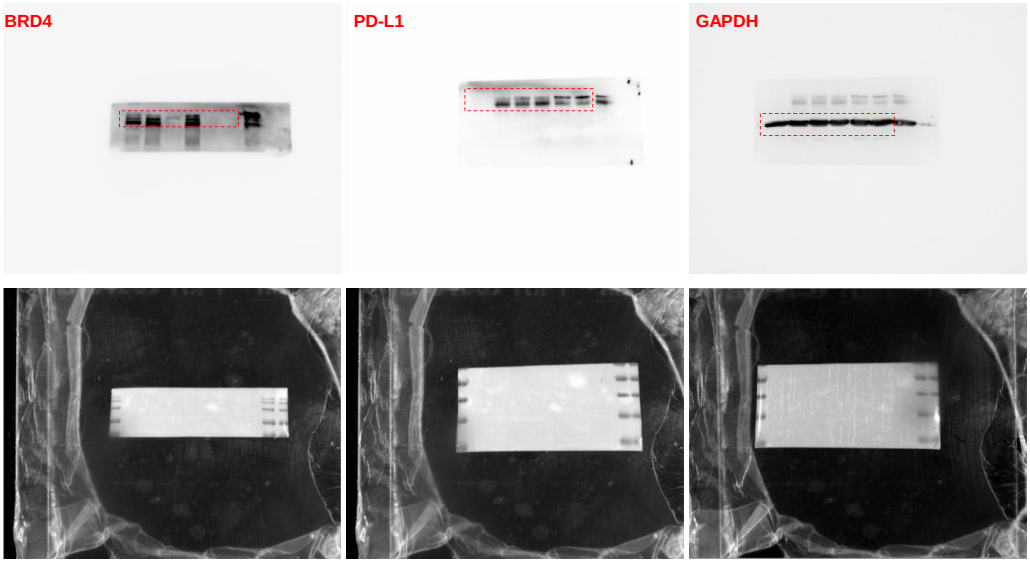
The full images of Figures S14 and S15



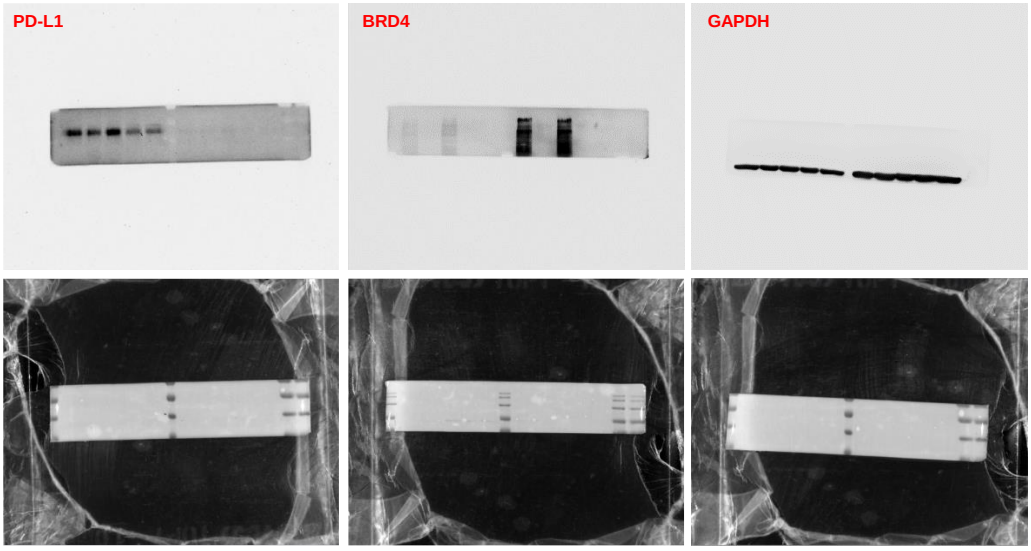
The full images of Figure S17.



The full images of Figure S19.



The full images of Figure 4E.



The full images of Figure S21.