Supporting Information

Aliphatic Group-Tethered Iridium Complex as a Theranostic Agent against Malignant Melanoma Metastasis

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Methods

General experimental. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^{1}H and ^{13}C NMR were recorded on a Bruker Advance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (Acetonitrile-d_3: ^{1}H, δ1.94, ^{13}Cδ118.7; Acetone-d_6: ^{1}H δ2.05, ^{13}Cδ29.7). Chemical shifts (d) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ± 0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. All NMR data was acquired and processed using standard Bruker software (Topspin).

Chemicals and reagents: Dual-Luciferase® Reporter Assay System was purchased from (Promega, Madison, WI, USA). p53-Luciferase plasmid was purchased from Beyotime (Beyotime, Shanghai, China). Iridium chloride hydrate ($IrCl_3 \cdot xH2O$) and rhodium chloride hydrate ($RhCl_3 \cdot xH2O$) were purchased from Precious Metals Online (Australia). The synthesis and stability experiments of compounds were described in SI Materials and Methods. Transfection Reagent was obtained from Thermo Scientific (Thermo Scientific, St Leon-Rot, Germany). p53 antibody, S100B antibody were purchased from Abcam (Abcam Inc., Cambridge, MA, USA), MMP-2 antibody, p21 antibody, Bax antibody, and GAPDH antibody were purchased from CST (CST Inc., Massachusetts, USA). S100B protein was purchased from R&D Systems (R&D Systems Inc., Minneapolis, Minnesota, USA). p53 peptide was purchased from GL Biochem Ltd. (GL Biochem Ltd., Shanghai, China). All the compounds were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO is less than 0.1%. Human malignant melanoma (A375 cells) was obtained from American Type Culture Collection. The hepatocyte cells (LO2 cells) was obtained from Chinese Academy of Science (Cell Biology of Shanghai Institute, Shanghai, China).

Synthesis of rhodium(III) and Iridium(III) complexes

Preparation of the precursor complexes [$M_2(C^N)_4Cl_2$]. A solution of rhodium(III)/iridium(III) chloride (1 eq.) and corresponding C^N ligands (2.1 eq.) in a mixture of methoxyethanol:water (3:1, 48 mL) was heated under reflux overnight under a nitrogen atmosphere. The reaction mixture was cooled to room temperature. The solid was collected by filtration, washed with additional portions of water (2 × 100 mL) and diethyl ether (2 × 50 mL) and dried to yield the corresponding precursor complexes.

Preparation of the final complexes. A suspension of cyclometallated dimer complex [$Rh_2(C^N)_4Cl_2$] or [$Ir_2(C^N)_4Cl_2$] (1 eq.) and the corresponding N^N ligands (2.1 eq.) in a mixture of methanol:dichloromethane (1:1, 20 mL) was refluxed overnight under a nitrogen atmosphere. The resulting solution was then allowed to cool to room temperature, and filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaporation until precipitation of the crude product occurred. The precipitate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compounds.

Complex 1: (Yield: 52%) ^{1}H NMR (400 MHz, Acetone-d6) δ 8.50 (s, 4H), 8.43 (d, J = 2.0 Hz, 2H), 8.24 (d, J = 8.0 Hz, 2H), 8.21 (d, J = 6.0 Hz, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.47-7.43 (m, 2H), 7.37 (d, J = 8.8 Hz, 2H), 7.18-7.14 (m, 2H), 7.11-7.08 (m, 2H), 6.84-6.80 (m, 2H), 6.57 (d, J = 7.6 Hz, 2H), 1.29 (s, 18H). ^{13}C NMR (101 MHz, Acetone-d6) δ 171.4, 165.2, 156.6, 152.6, 148.7, 148.5, 147.0, 141.1 135.3, 131.7, 131.4, 130.2, 128.9, 128.3, 127.6, 126.1, 125.7, 123.7, 122.0, 118.9, 36.3, 29.9. MALDI-TOF-HRMS: calcd. for $C_{48}H_{44}IrN_4[M–PF_6]^+$: 869.3195 Found: 869.3153 Anal.: ($C_{48}H_{44}IrN_4PF_6$) C, H, N: calcd. 56.85, 4.37, 5.52; found 56.63, 4.41, 5.62 (Figure S1).

Complex 2: Reported.[1]

Complex 3: Reported.[2]

Complex 4: (Yield: 59%) ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.80 (d, J = 2.0 Hz, 2H), 8.59-8.49 (m, 4H), 8.28-8.19 (m, 4H), 8.00-7.92 (m, 4H), 7.48 (t, J = 8.0 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.20 (m, 4H), 6.84 (t, J = 6.4 Hz, 2H), 6.55 (d, J = 8.0 Hz, 2H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.97, 156.51, 150.87, 149.70, 148.16, 146.70, 141.41, 136.71, 135.23, 132.90, 132.16, 131.57, 130.31, 128.95, 128.86, 128.37, 127.77, 125.55, 124.00, 118.95. MALDI-TOF-HRMS: calcd. for $C_{38}H_{32}IrN_4$ $[M–PF_6]^+$: 915.0123 Found: 915.0135. Anal.: ($C_{40}H_{26}Br_2F_6IrN_4P$) C, H, N: calcd. 45.34, 2.82, 5.29; found 45.43, 2.82, 5.01 (Figure S2).

Complex 5: Reported.[1]

Complex 6: Reported.[3]

Complex 7: Reported.[4]

Complex 8: (Yield: 62.3%) ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.89 (d, J = 0.8 Hz, 2H), 8.72 (d, J = 3.2 Hz, 2H), 8.18 (d, J = 5.6 Hz, 2H), 7.74 (dd, J = 6.0, 2.0 Hz, 2H), 7.66 (dd, J = 8.0, 0.8 Hz, 2H), 7.18 (d, J = 2.0 Hz, 2H), 7.10-7.06 (m, 2H), 6.91-6.87 (m, 2H), 6.72-6.70 (m, 2H), 6.36 (dd, J = 7.2, 1.2 Hz, 2H), 1.46 (s, 18H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 165.1, 157.4, 151.5, 144.3, 139.3,

134.0, 133.7, 128.8, 127.4, 126.0, 124.0, 122.6, 112.8, 109.2, 36.4, 30.5; MALDI-TOF-HRMS: Calcd. for $C_{36}H_{38}IrN_6[M–PF_6]^+$: 747.2787, found: 747.2714; Anal. ($C_{36}H_{38}IrN_6PF_6$) C, H, N: calcd. 48.48, 4.29, 9.42, found 48.42, 4.19, 9.30 (Figure S3).

Complex 9: Reported.[5]

Complex 10: Reported.[6]

Complex 11: Reported.[7]

Complex 12: Reported.[1]

Complex 13: Reported.[8]

Complex 14: (Yield: 61%) ^{1}H NMR (400 MHz, Acetone-d6) δ 9.08 (dd, J = 6.5, 3.0 Hz, 2H), 8.91 (d, J = 1.6 Hz, 2H), 8.46 (d, J = 7.9 Hz, 2H), 8.10 – 8.05 (m, 2H), 7.98 – 7.89 (m, 4H), 7.83 (d, J = 5.7 Hz, 2H), 7.72 – 7.59 (m, 6H), 7.21 (td, J = 7.2 Hz, 1.2 Hz, 2H), 6.98 (td, J = 7.5, 1.3 Hz, 2H), 6.29 (d, J = 7.7 Hz, 2H), 1.40 (s, 18H). 13C NMR (101 MHz, Acetone-d6) δ 171.45, 171.33, 171.14, 166.56, 165.81, 165.64, 164.31, 159.36, 154.67, 154.57, 149.69, 149.65, 145.61, 142.56, 140.44, 140.38, 137.23, 137.20, 132.79, 132.49, 131.91, 131.80, 130.30, 130.10, 129.57, 129.36, 128.46, 127.96, 127.11, 126.56, 126.50, 126.27, 125.50, 124.90, 124.86, 123.20, 122.03, 121.77, 121.70, 121.40, 35.50. MALDI-TOF-HRMS: calcd. for $C_{48}H_{44}RhN_4$ $[M–PF_6]^+$: 779.2616 Found: 779.2645. Anal.: ($C_{48}H_{44}F_6RhN_4P$+0.5H_2O) C, H, N: calcd. 61.74, 4.86, 6.00; found 61.73, 4.90, 5.99 (Figure S4).

UV/Vis absorbance spectroscopy: Complex **7** (5 μM) was added to 0, 0.01, 0.1, 1, 10 mM of Ca^{2+} in 20 mM Tris-HCl buffer (pH = 7.4) at 25 °C. Absorption spectra were recorded on Cary UV-100 Spectrophotometer at a range of 200 nm to 800 nm. The absorbance was corrected by subtraction of 20 mM Tris-HCl buffer (pH = 7.4) as the background absorbance.

Stability experiments. For ^{1}H NMR, complex **7** (5 mM) was dissolved in 90% [d_6]DMSO/10% D_2O at 25 °C over 7 days. ^{1}H NMR measurements were carried out on a 400 MHz Bruker instrument. For UV-Visible spectrometry, complex **7** (5 μM) was dissolved in 80% acetonitrile/20% 20 mM Tris-HCl buffer (pH = 7.4) at 25 °C over 7 days. Absorption spectra were recorded on a Cary UV-100 Spectrophotometer at a range of 200 nm to 800 nm. The absorbance of complex **7** was corrected by subtraction of 80% acetonitrile/20% 20 mM Tris-HCl buffer (pH = 7.4) as the background absorbance.

Cyclic voltammetry (CV). Cyclic voltammetry measurements of complexes **7** and **14** were carried out on a CHI 650E electrochemical workstation (Chenhua, Shanghai, China) with a conventional three-electrode system consisting of a modified working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode. Meantime, the electrochemical characteristics of the rhodium/ iridium complexes were investigated in Tris-buffer by using CV experiments from -1.5 V to 1.5 V.

Cell cultures: The cells were cultivated in DMEM medium with 1% penicillin (100 units/mL)/streptomycin (100 μg/mL) and 10% fetal bovine serum (FBS). Cells were maintained at a density of 6 × 10^5 cell/mL in 5% CO_2 at 37 °C.

Flow cytometry protein interaction assay. The EZ-Link Sulfo-NHS-LC-Biotinylation Kit was used to conjugate biotin and S100B protein to polystyrene beads in Ca^{2+} buffer. The procedure was performed as described.[9] After conjugating S100B protein to the beads, human p53 peptide labeled with FITC was added, and FITC fluorescence was detected by flow cytometry**.**

Co-IP assay: The co-IP assay was performed as previously described.[10-11] Briefly, A375 cells were seeded at a density of 2 × 10^6 cells in a six-well plate. Cells were treated with the 0.1 and 1.0 μM complex **7** or 10 μM SBi1 for 12 h. Cells were lysed and protein samples were collected. The concentration of protein samples was calculated using the Pierce BCA protein assay kit. 30 μg of each protein sample were incubated overnight with 10 μL pre-incubated anti-p53 magnetic beads according to the manufacturer's protocol. The complex was washed 5 times to elute non-specific and non-cross-linked antibodies. Then, the precipitated proteins were subjected to SDS-PAGE and analyzed by Western blotting with the indicated antibodies.

Western blotting: A375 cells were seeded at a density of 6 × 10^5 cell in a 6-well plate overnight. Cells were treated with complex **7** (in 0.1% DMSO), SBi1, or vehicle control in 1% FBS medium for an additional 12 h. Cells were lysed, and protein samples were collected. Western blotting analysis was performed as described.[12]

Transient transfection: A375 cells were seeded in a culture dish overnight before transfection. p53-luciferase plasmid, and TurboFect reagent were mixed together in Dulbecco's modified eagle medium (DMEM) without FBS and the resulting solution was incubated for 20 min at 37 °C. The mixture was then added to the A375 cells in the wells. The cells were incubated for 12 h at 37 °C in a CO_2 incubator before use.

Luciferase reporter assay: The inhibition of p53 activity was assayed by a luciferase reporter assay system (Promega, Madison, WI, USA). Briefly, A375 cells were seed at a density of 6 × 10^5 cell in a culture dish overnight. The cells were co-transfected with p53-luciferase plasmid in serum-free DMEM medium using TurboFect Transfection Reagent. Then, the transfected cells were seeded in a 24-well plate and treated with an indicated concentration of **7** in 1% FBS medium for 6h. The transfected cells were lysed by the addition of 160 μL Passive Lysis Buffer (PLB). 50 μL of the cell lysates were transferred to a 96-well white plate followed by adding 50 μL of

luciferase reporter reagent (LAR). The transcriptional activity was determined by measuring the activity of firefly luciferase in SpectraMax M5 microplate reader (Molecular Devices).

Inductively coupled plasma mass spectrometry (ICP-MS): 500 μL of the cell samples from mitochondria isolation, nuclear extraction or whole cell lysate was then combined with 1500 μL of a 68% HNO_3: H_2O_2 (v/v = 4:1) solution, while the remainder of the lysate was quantified for protein by a bicinchoninic assay (BCA). The 2% HNO_3 solution was analyzed for iridium uptake levels on a Thermo iCAP Qs ICP-MS. Iridium uptake levels in each sample were normalized to the concentration of corresponding protein and calculated each value of ng [Iridium]/mg [protein].

Knockdown assay: A375 cells were seeded in 6-well plate at 80% confluences in DMEM medium for 24 h. Lipo3000 reagent and S100B siRNA (5′ - GAA CAU GAG UGA GAU UAG ATT - 3′ (sense), 5′ - UCU AAU CUC ACU CAU GUU CTT - 3′ (antisense)) was gently mixed and incubated for 20 min at room temperature. Then, 500 μL of the Lipo3000/siRNA mixture were added to each well. Cells were incubated at 37 °C in a CO_2 incubator for 48 h post-transfection before further research.

Cellular thermal shift assay: Cellular thermal shift assay was performed to monitor the target engagement of **7** in A375 cell lysates. Briefly, cell lysates from 2 × 10^6 A375 cells were collected, diluted in PBS and separated in the same aliquots. Each aliquot was treated with **7** (10.0 μM) or DMSO 30 min after incubation at room temperature, the compound-treated lysates were divided into 50 μL in each of PCR tubes and heated individually at different temperatures (Veriti thermal cycler, Applied Biosystems/Life Technologies). The heated lysates were centrifuged and the supernatants were analyzed by SDS-PAGE followed by immunoblotting analysis by probing with the indicated antibody.

Protein thermal shift assay: To evaluate binding affinity of complex **7** with S100B protein, the GloMelt™ Thermal Shift Protein Stability Kit was used. Briefly, after incubating S100B protein with complex **7** for 30 min, GloMelt dye was added. Reactions were performed in triplicate and fluorescence signals were recorded in am Applied Biosystems™ ViiA™ 7 system. The data were exported to Excel and the fluorescence signal of each samples was plotted. A significant increase in slope corresponds to the melting temperature of the protein.

Chromatin immunoprecipitation (ChIP) assay: A375 cells were treated with **7**, SBi1 or vehicle for 12 h. The cells were cross-linked by incubating with 1% (v/v) formaldehyde-containing medium for 10 minutes at 37 °C. S100B antibody was used to precipitate protein-DNA complexes. The protein-DNA complexes were collected with Protein A Sepharose beads (Millipore), eluted, and reverse cross-linked. Complexes were purified with ChIP DNA Purification Kit (Active Motif, No. 58002). Recovered DNAs were analyzed by real-time qPCR (ViiA™ 7 System, ABI). The PCR primers for the target promoters are listed in Supplementary Table S1. ChIP-PCR analysis was performed following a published protocol.[13]

Immunofluorescence: After washing with phosphate-buffered saline (PBS) three times, the cells were treated with 4% PFA for 30 min followed by washing with PBS three times. Then, cells were incubated with 0.5% Triton X-100 for 30 min at room temperature, then washed with PBS three times. After blocking with 5% BSA for 30 min, cells were incubated with S100B antibody overnight at 4 °C (primary antibody) and then with secondary antibody for 3 h at room temperature.

Cell imaging: A375 cells were seeded into a glass-bottomed dish (35 mm dish with 20 mm wells) for 24 h. Cells were then incubated with complex **7** for the indicated time periods and concentrations and then washed with phosphate-buffered saline three times. Luminescence imaging was performed on a Leica TCS SP8 confocal laser scanning microscope system. The excitation wavelength was 500-700 nm.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR): To an eppendorf was added 1 μL of RNA, 4× gDNA Wisper Mix 4 μL, Oligo-(dT)23 VN (50 μM) 1 μL, random hexamers (50 ng/uL) 1 μl, and RNAse-free ddH_2O added to 16 μL. The mixture was incubated at 42 °C for 2 min. Then, 2 μL of 10× RT Mix and 2 μl of Hiscript II enzyme Mix were added, and then the mixture was sequentially heated at 25 °C for 5 min, 50 °C for 15 min, and 85 °C for 2 min to configure cDNA. The cDNA was quantified using the BioTek Take Micro-Volume Plate, with 2 μL of cDNA added per well. The cDNA concentration was diluted to 20 ng/μL using ddH_2O. For qRT-PCR, a 10 μL reaction contained a 3 mixture of two reverse transcriptases: 10 μL of 2× AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co.,Ltd) with Hot Start Taq polymerase, 0.5 μL of primers, and 0.5 μL (20 ng/mL) of template. The StepOnePlus™ System was used for all real-time PCR assays.[14] The reaction was activated the AceTaq® DNA Polymerase at 95°C for 5 min. This was then amplified for 40 cycles at 95 °C for 3 s for denaturation, annealing, and acquisition at 60°C for 40 s. It was finally elongated at 95°C for 15 s. Fluorescence can be measured after the annealing phase. With an Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plate, 10 μL of the reaction mix was added, as well as the 96-SYBR-Green assays on the StepOnePlus™ Real Time System. To prepare the assay, all of the reagents were kept either on a cooling block or on ice. The ΔΔCt method was used in

calculations. For each sample three independent qPCR experiments were performed. Each experiment involved three replicates for each gene. Expression of GAPDH was used as an internal control.

Transwell assay: A375 cells were seeded at 10^5 cells per well in the transwell inserts. The transwell inserts and the 24-well plates were incubated 5% CO_2 at 37 °C for 12h.

MTT assay: A375 and LO2 cells were seeded at 5000 cells per well in a 96-well plates and incubated overnight at 37 °C. The cells were treated with **7** at a final concentration from 0.01 to 10 μM for 72 h. Then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was added to each well at a final concentration of 0.5 mg/mL for a further 4 h. After that, the medium was replaced with 100 μL DMSO. The viability of the cells was measured by recording the absorbance of each well at 490 nm using a SpectraMax M5 microplate reader after shaking the plate for 10 min at room temperature in the dark.

Melanoma xenograft assay: NOD.CB17-Prkdcscid / NcrCrl (NOD / SCID) female mice were purchased from BioLASCO Experimental Animal Center (Taiwan Co., Ltd)., and injected with A375 cells at 8 weeks of age. The injection site was sanitized using 70% ethyl alcohol. 1×10^6 A375 cells dissolved in 0.1 mL of PBS were subcutaneously implanted into mice by using a 27-gauge (G) needle. The mice were observed for 7 to 10 days, until the tumor grew to an appropriate size (about 100 mm^3). The testing drug was administrated via subcutaneous injection to mice and observe its therapeutic effect on melanoma tumors [1].

Metastasis tumor assay: BALB/C mice were purchased from BioLASCO Experimental Animal Center (Taiwan Co., Ltd), and injected with B16F10 cells at 8 weeks of age. B16F10 cells were detached by trypsin, and 2×10^7 cells were placed in an eppendorf tube, rinsed once with serum-free medium, and centrifuged at 400 g for 5 minutes to remove the supernatant. 1 ml of Diluent C and 4 μL of PKH26 dye were added and the mixture was allowed to stand for 5-10 minutes, after which an equal volume of serum was added. 5×10^5 cells were withdrawn and the suspension was centrifuged at 400 g for 10 minutes. The supernatant was removed and 150 μL of PBS was added to resuspend the cells, before injection into the tail vein of the mouse. After the tumor development to be visible by the IVIS system (12 days), complex **7** was delivered via intravenous injection at 1 mg/kg twice a week to mice until 36 day. The degree of metastasis of B16F10 was observed by 2D optical tomography using the Caliper IVIS Spectrum System one week later. The mice were anesthetized with the XGI-8 Anesthesia system and then placed in the IVIS machine with the internal wafer cooled to -90 °C for fluorescence imaging.

Sample preparation from animal tissues: Mouse tumor tissues were chopped and rinsed in ice-cold saline solution. The tissue was immersed with 10 times the volume of RIPA, and then homogenized with a Mixer Mill MM 300 homogenizer (Qiagen, Chatsworth, CA). The mixture was further incubated with RIPA for 10 min on ice and then centrifuged at 12000 g for 30 min, and the supernatant was collected and analyzed by Western blotting.

Statistical analysis: For statistical analysis, all data were analyzed with one-way analysis of variance (ANOVA) followed by the Dunnett's method for multiple comparisons by using GraphPad Prism 5.0.

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Supplementary Tables

Table S1. Primer sequences used in this paper.

Name	sense	antisense
p21 [15]	5′-CGCTCTACATCTTCTGCCTT -3′	5′-GACAGCGCTGGGAAGGAGC -3′
Bax [15]	5′-TCAGCACAGATTAGTTTCTG -3′	5′- GGGATTACAGGCATGAGCTA -3′
MMP-2[16]	5'- TGACACGGAGACAGGAAGTG-3'	5'- GCATCTTGGTGAGGTGATGA-3'

Supplementary Figures

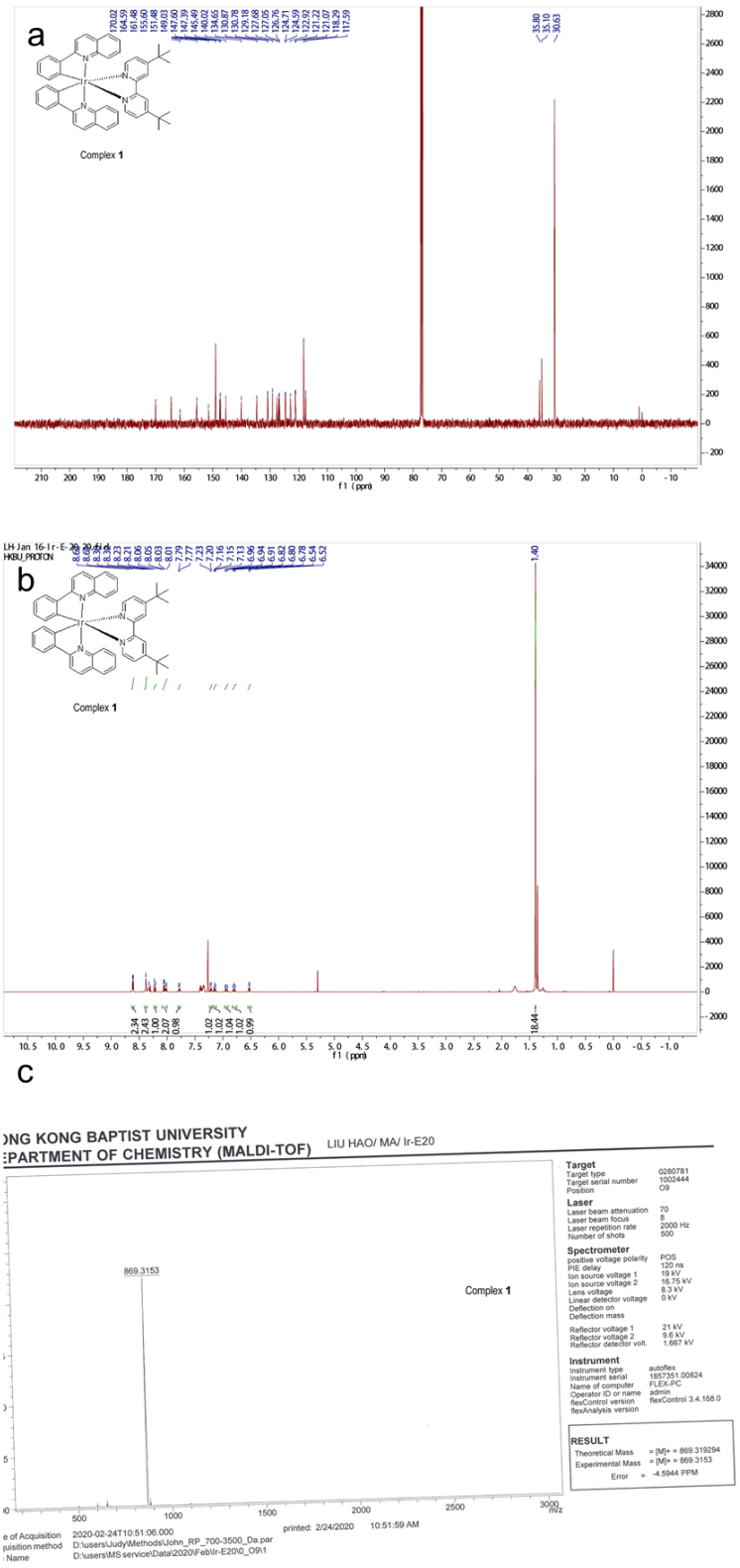

Figure S1. (a) ^{1}C NMR of complex **1**, (b) ^{1}H NMR of complex **1**, (c) HRMS of complex **1**.

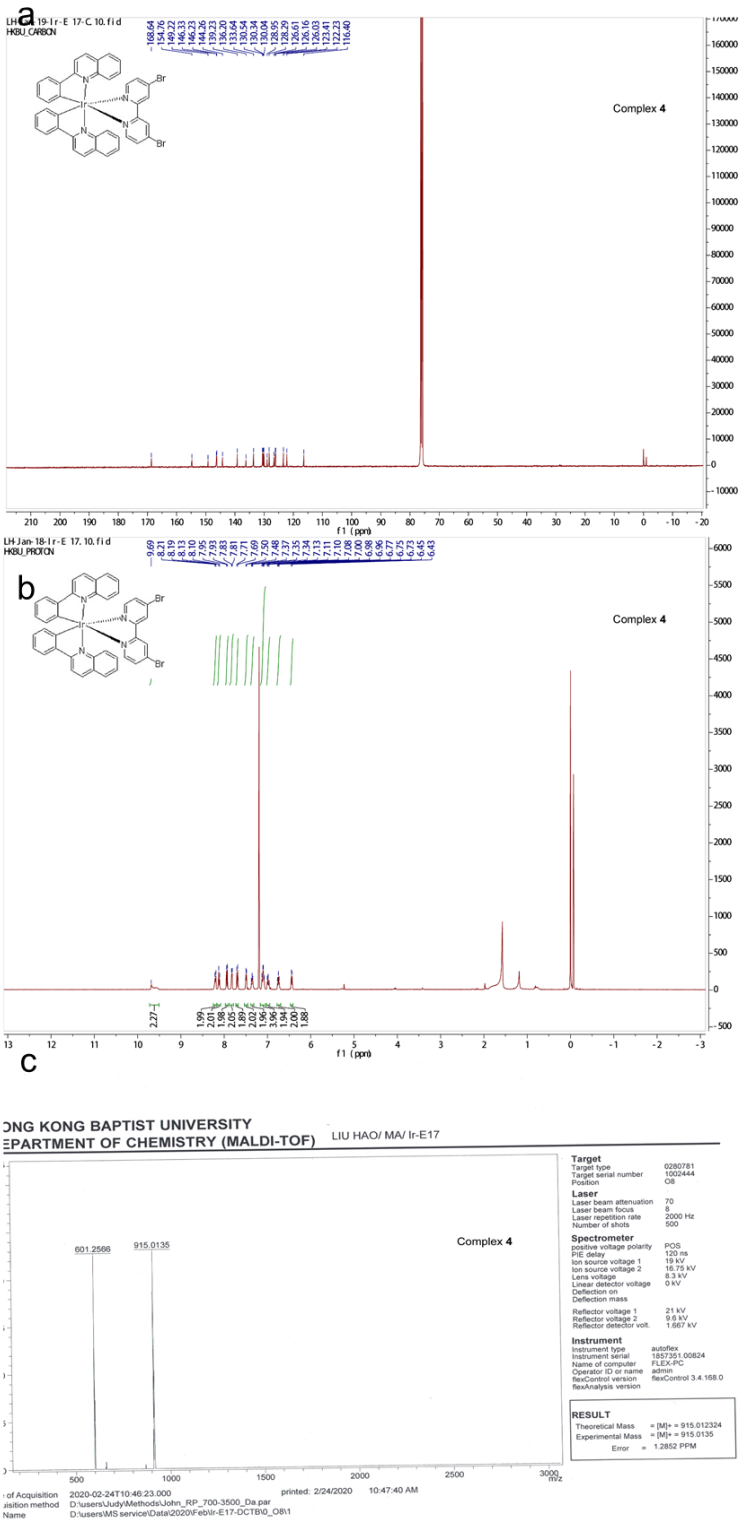

Figure S2. (a) ^{1}C NMR of complex **4**, (b) ^{1}H NMR of complex **4**, (c) HRMS of complex **4**.

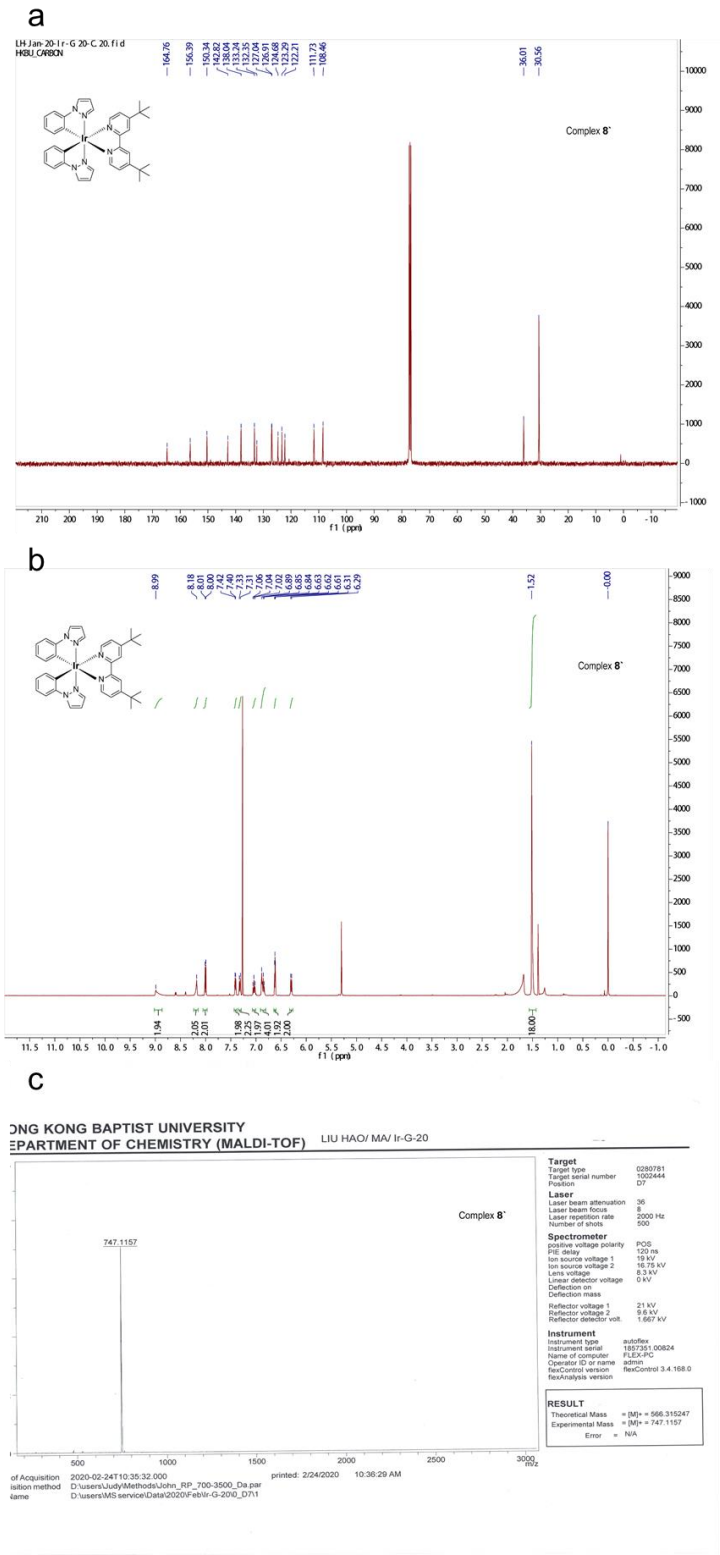

Figure S3. (a) ^{1}C NMR of complex **8**, (b) ^{1}H NMR of complex **8**, (c) HRMS of complex **8**

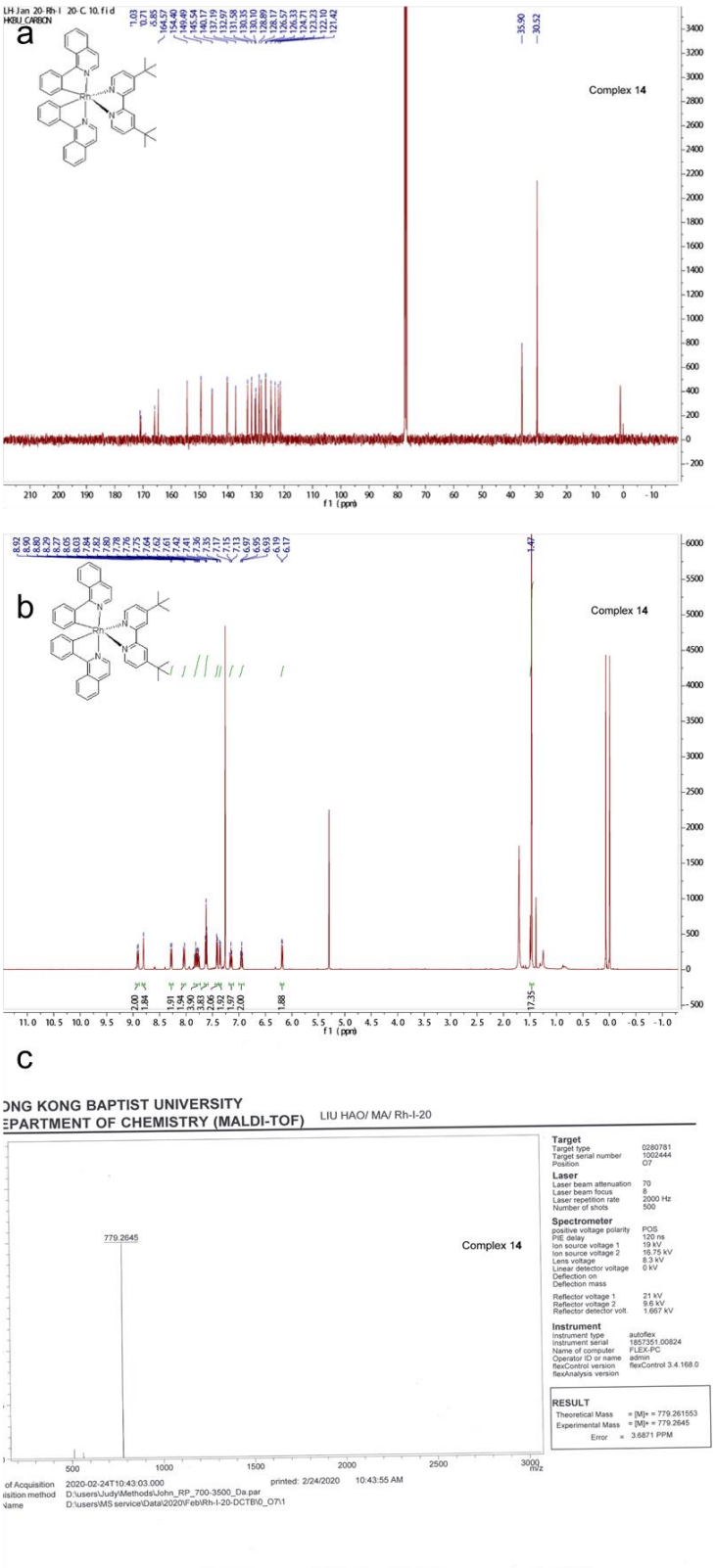

Figure S4. (a) ^{1}C NMR of complex **14**, (b) ^{1}H NMR of complex **14**, (c) HRMS of complex **14**

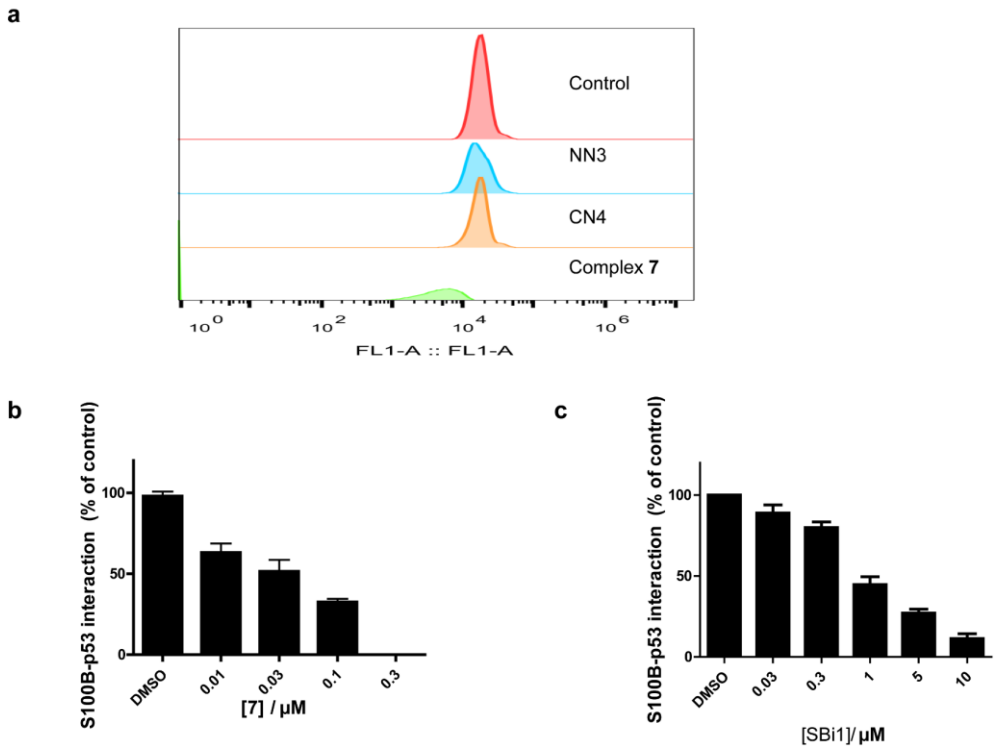

Figure S5. Complex **7** selectively inhibits the S100B/p53 PPI. (a) Effects of NN3 and CN4 ligands (10 μM) on the inhibition of S100B/p53 PPI by FCPIA. (b, c) Effects of complex **7** and SBi1 on the S100B/p53 PPI by FCPIA. Error bars represent standard deviation of the means of the results from three independent experiments.

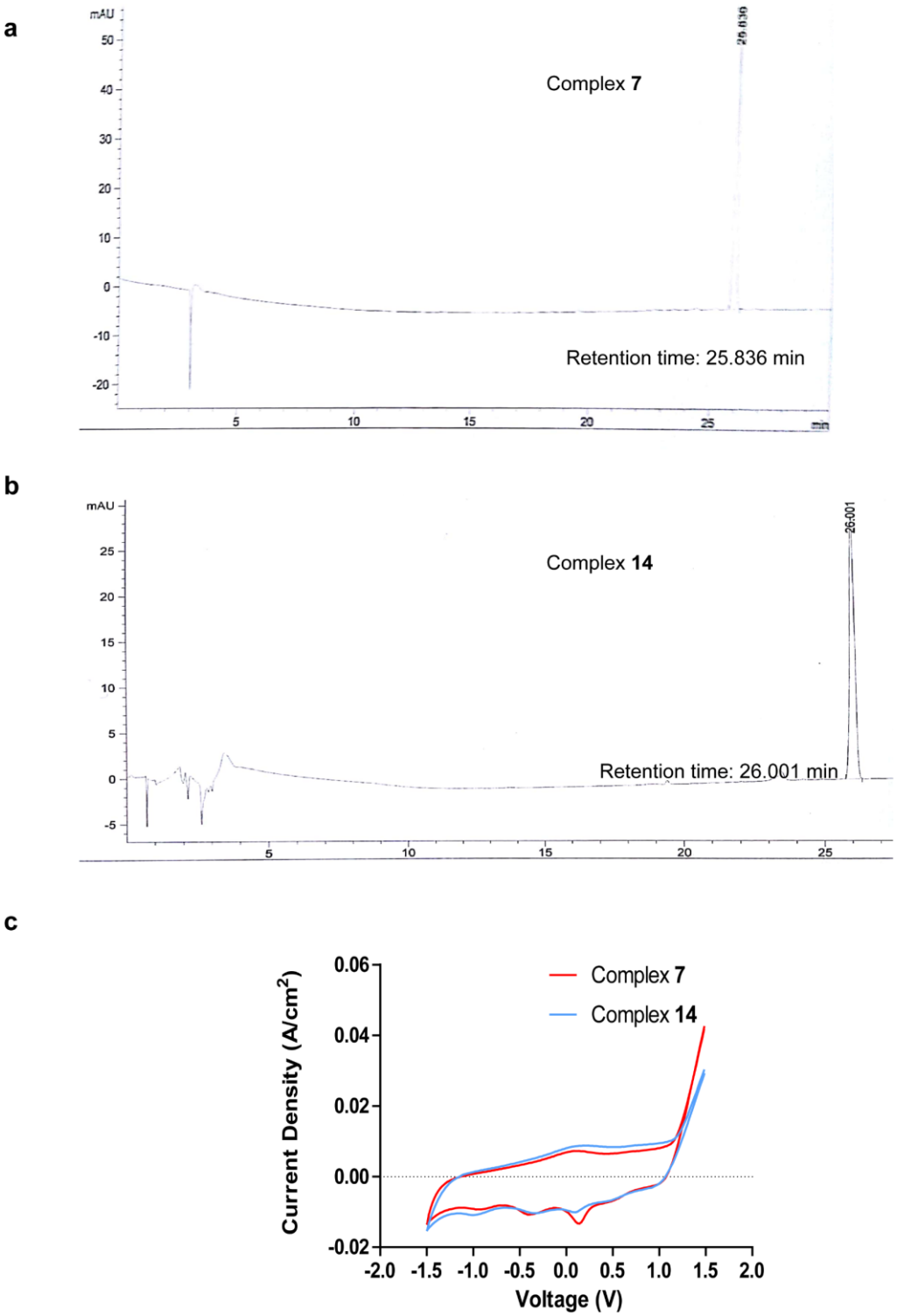

Figure S6. Retention times and HPLC spectra for complexes **7** and **14** were detected by reverse phase HPLC. Retention time: (a) complex **7**: 25.836 min, and (b) complex **14**: 26.001 min. (c) Cyclic voltammetry of complexes **7** and **14**. Data were obtained at a scan rate of 20 V s^{-1} in Tris-buffer with a platinum wire counter electrode and an Ag/AgCl as reference electrode.

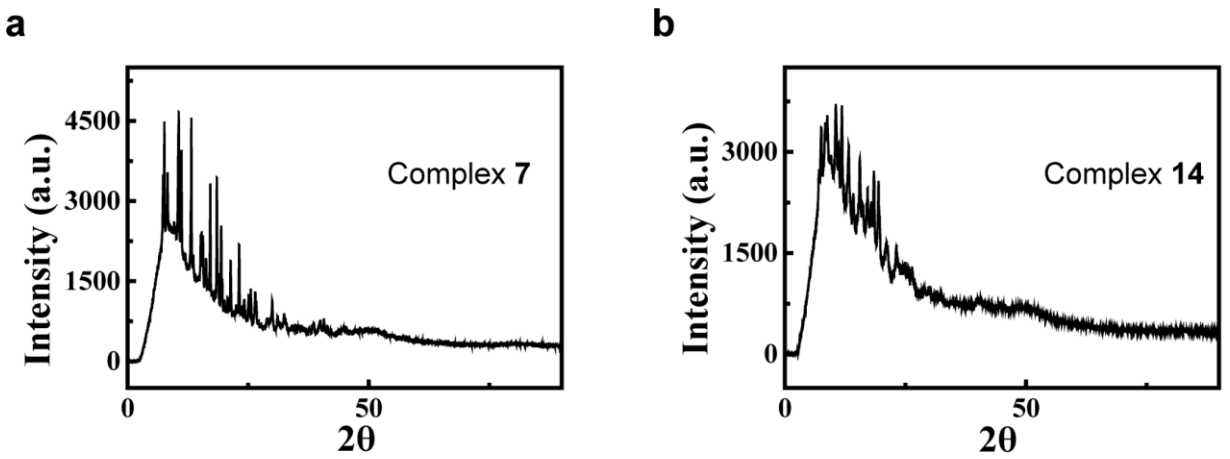

Figure S7. (a, b) X-ray powder diffraction patterns of complexes **7** and **14**.

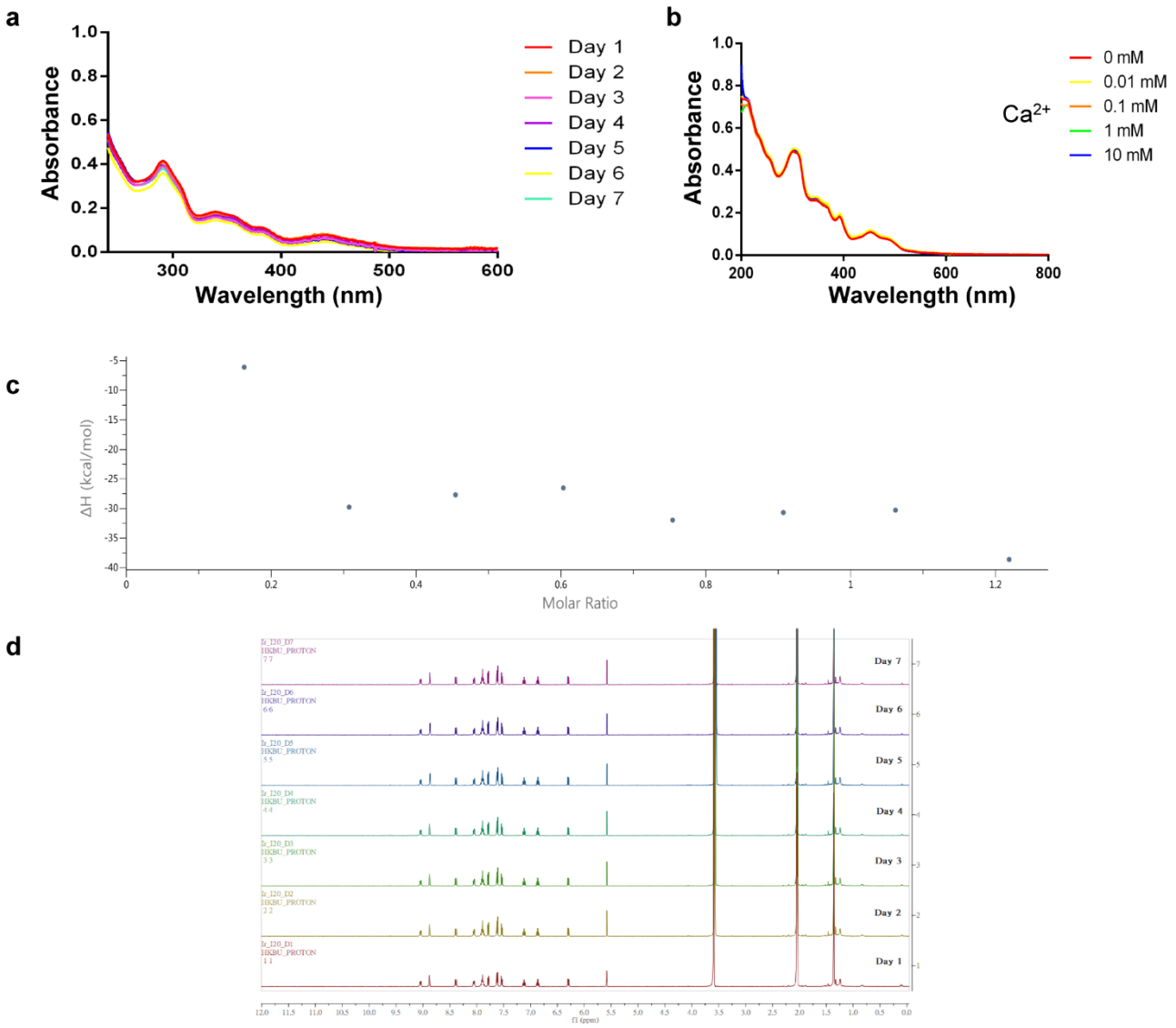

Figure S8. Stability of complex **7.** (a) UV-Vis absorption of complex **7** at 5 μM in 80% acetonitrile/20% 20 mM Tris-HCl buffer (pH=7.4) at 25 °C over 7 days. (b) UV/Vis absorption of the compound **7** at 5 μM in 20 mM Tris-HCl buffer (pH=7.4) at 25°C in the absence or presence of Ca^{2+}. (c) Representative ITC data of Ca^{2+} titrated into complex **7**. (d) ^{1}H NMR spectra of complex **7** at 5 mM in 90%[d_6]DMSO/10% D_2O at 25 °C over 7 days.

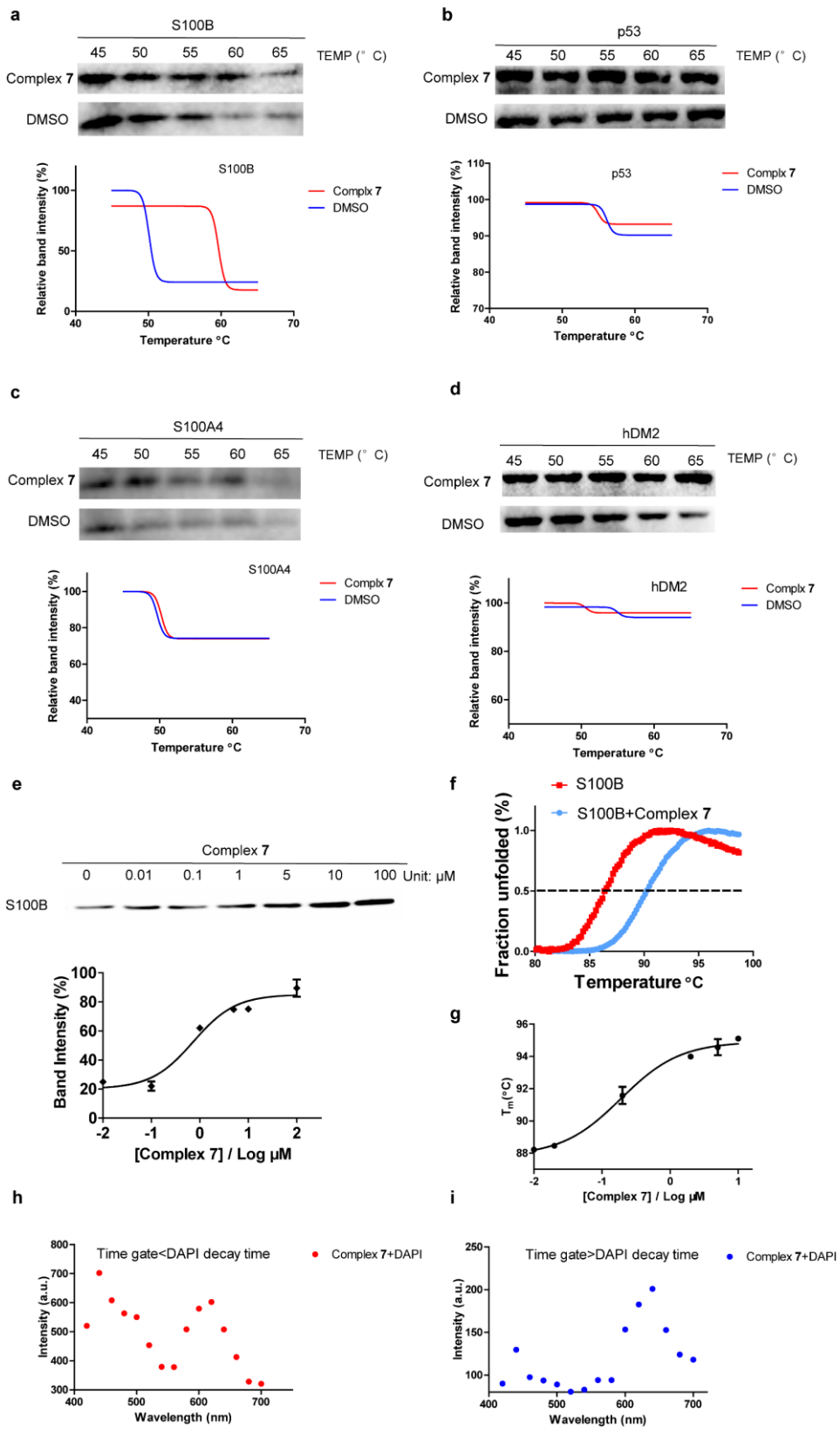

Figure S9. Complex **7** engages S100B protein in cell lysates and *in vitro*. (a)The effects of complex **7** on the thermal stability of S100B. (b) The effects of complex **7** on the thermal stability of p53. (c)The effects of complex **7** on the thermal stability of S100A4. (d) The effects of complex **7** on the thermal stability of hDM2. (e) $ITDRF_{CETSA}$ curves of S100B protein in the presence of complex **7** (0-100μM). The protein samples were detected by Western blotting and the band density were analyzed. (f) Melt profile of S100B protein with or without complex **7** (1 μM) by protein thermal shift assay. (g) The dependence of the melting temperature of S100B protein on the concentration of complex **7** (0-10 μM) by protein thermal shift assay. (h) Time-resolved spectra of complex **7** (1 μM) and the nuclear dye DAPI (1 μM) in A375 cells with time gate set to less than the DAPI decay time. (i) Time-resolved spectra of complex **7** (1 μM) and the nuclear dye DAPI (1 μM) in A375 cells with time gate set to greater than the DAPI decay time.

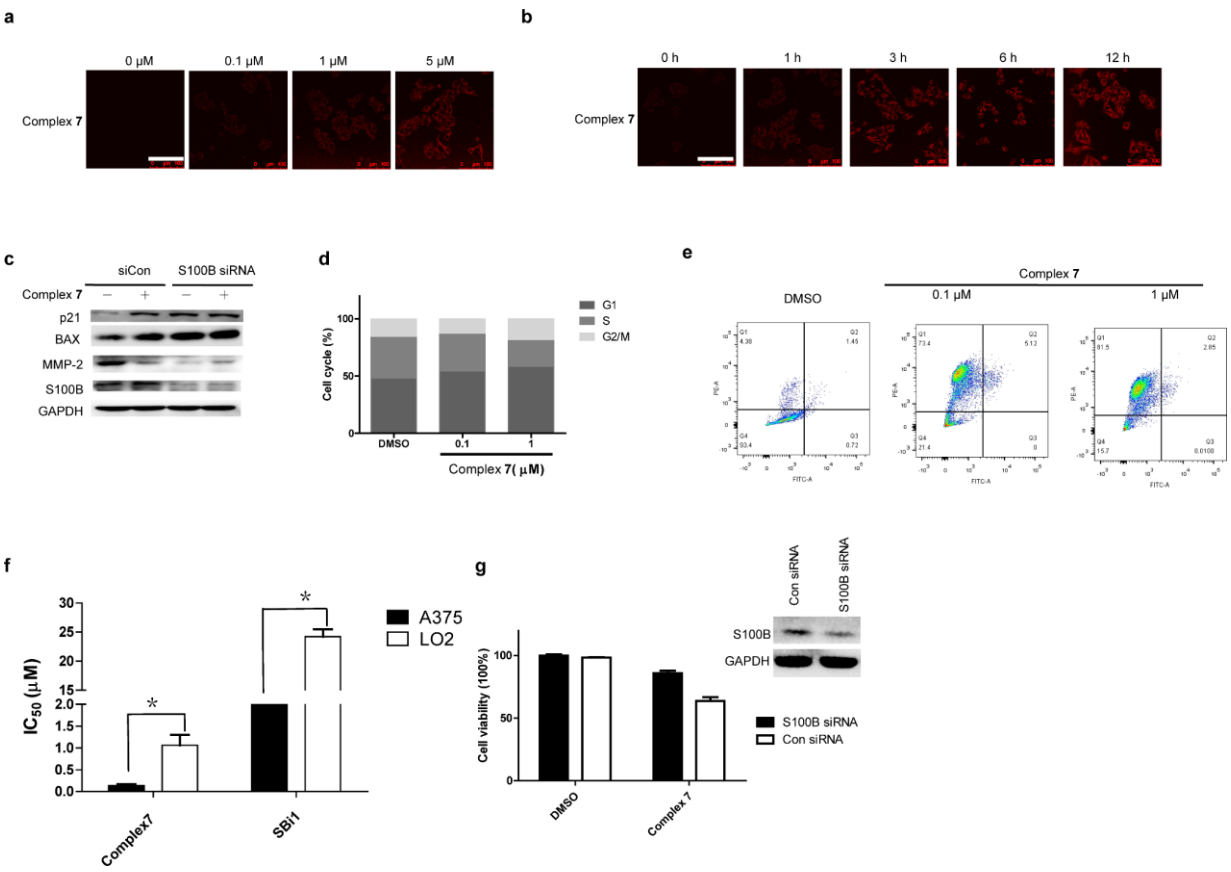

Figure S10. (a) Confocal imaging of A375 cells incubated with complex **7** (0-5 μM) for 1 h at 37 °C. (b). Confocal imaging of A375 cells incubated with complex **7** (1 μM) for 0-12 h at 37 °C. (c) The effect of complex **7** on MMP-2, p21 and Bax protein expression after S100B knockdown by S100B siRNA in A375 cells. (d) The effect of complex **7** on the cell cycle in A375 cells. (e) The effect of complex **7** on apoptosis in A375 cells. (f) The effect of complex **7** on cell viability in LO2 and A375 cells. (g) The effect of complex **7** on A375 cell viability after knockdown S100B by S100B siRNA. Error bars represent the standard deviations of the results from three independent experiments. *$p<0.05$ compared with control group.

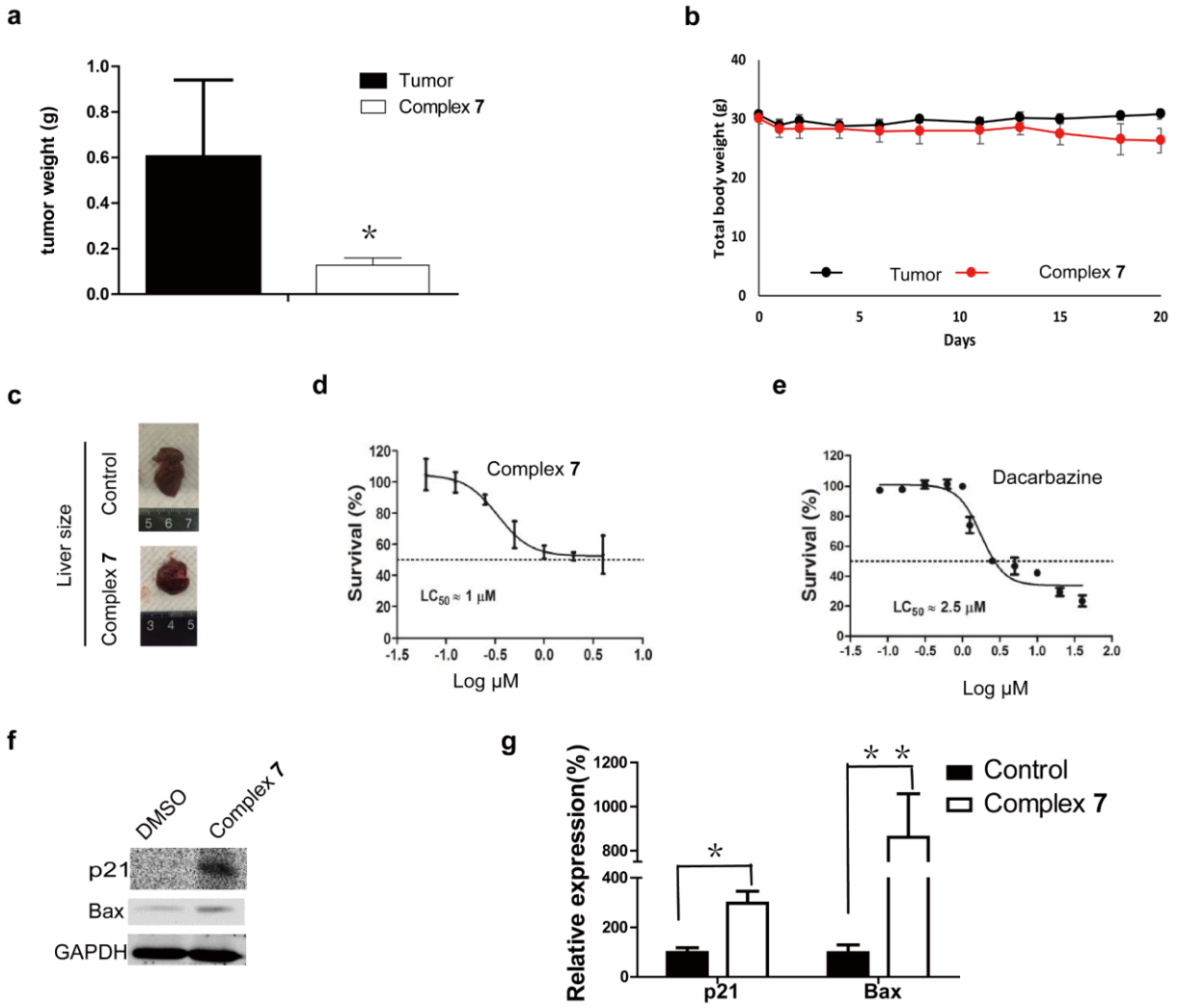

Figure S11. Effects of complex **7** on A375 and B16F10 xenografts model. (a) Effects of complex **7** on tumor weight in A375 xenografts model. (b) Effects of complex **7** on mice body weight in A375 xenografts model. (c) Representative images of dissected liver tissue from each group in A375 xenografts model. (d, e) After 48 h of continuous administration, the LC_{50} of complex **7** and Dacarbazine were detected in A375 xenografts model. (f) Effect of complex **7** on p21 and Bax protein levels in A375 xenograft model.(g) Effect of complex **7** on p21 and Bax RNA levels in A375 xenograft model. Error bars represent the standard deviations of the results from three independent experiments. *p<0.05, **p<0.01 compared with control group.