Supporting Information

Selenium-containing metallodrug overcomes cervical cancer radioresistance by physical-chemical dual sensitization

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1 Experimental Procedures

1.1 General Information

All reagents and solvents were obtained commercially and used as received. Ruthenium trichloride, 4,5-bis(diphenylphosphino)-9,9- dimethylxanthene, and 5,6-diamino-1,10-phenanthroline were purchased from Sarn Chemical Technology (Shanghai) Co. Ruthenium trichloride, 4,5-bis(diphenylphosphino)-9,9- dimethylxanthene, and 5,6-diamino-1,10-phenanthroline were purchased from Sarn Chemical Technology (Shanghai) Co. selenium dioxide were purchased from Shanghai Aladdin Biotechnology Co. Imidazo[4,5-f]1,10-phenanthroline and phenanthroline triazole were purchased from Changsha Ruizheng Co. Imidazo[4,5-f]1,10-phenanthroline and phenanthroline triazole were purchased from Changsha Ruixinhecheng Biotechnology Co. Thiazolyl blue tetrazolium bromide (MTT), propidium iodide (PI), and 2',7 '-dichlorofluorescin (DCFH-DA), JC-1 probe purchased from Merck. FBS was purchased from Gibco. HeLa (cervical cancer) was purchased from American Type Culture Collection (ATCC). HeLa (cervical cancer) was purchased from American Type Culture Collection (ATCC), U.S.A. R-HeLa was used as an experimental construct, prepared by irradiating HeLa with 6 Gy, 4 times. NMR spectra were recorded on a Bruker Ascend 300 spectrometer operating at 300 MHz /400 MHz for ^{1}H acquisitions. High-resolution mass spectra were obtained using Agilent LC-UV-TOF and Agilent LC-UV-TOF. The UV-Vis spectra of the compounds were obtained using the UH4150 UV-Visible (Hitachi, Japan). The UV-Vis spectra of the compounds were obtained using the UH4150 UV-Visible (Hitachi, Japan). The UV-Vis spectra of the compounds were obtained using the UH4150 UV-Visible (Hitachi, Japan). Cell Viability analyzed by Cell Imaging Multi-Mode Reader (Bio Tek, USA). Cell cycle analysis was detected by Cyto FLEX Flow Cytometer (Beckman Coulter, USA).

1.2 Synthesis

1.2.1. Synthesis of $Ru(PPh_3)_3Cl_2$

In the reaction flask, 1.00 g of weighed ruthenium trichloride hydrate (3.83 mmol) was added, some methanol was added, and after part of the reactants were dissolved, 6.00 g of weighed triphenylphosphine (22.9 mmol) was added, and the rest of the methanol was added, and a total of 50 mL was added. 75 ℃ stirring and refluxing reaction was carried out for 4 h. After the completion of the reaction, the reaction was allowed to stand at room temperature, and filtration was carried out, and the reddish-brown solid obtained was washed with ethyl ether and dried. The red-brown solid was washed with ether and dried to obtain $Ru(PPh_3)_3Cl_2$; the yield was up to 98 %.

1.2.2. Synthesis of $Ru[(PPh_3)_3(POP)]Cl_2$, Ru (POP)

Ru (POP) was synthesized according to our previous study[1-5]. Weighing 4,5-bisdiphenylphosphine-9,9-dimethylxanthene (0.5mmol) 0.27 g, tris(triphenylphosphine)ruthenium(II) dichloride (0.5 mmol) 0.45 g was dissolved in 10 mL of acetone, and stirred and refluxed at 65 ℃, the reaction was shut down for 2 h, and the reaction was cooled down at room temperature, and filtered to obtain an orange-yellow solid, which was washed by ethyl ether, and then recrystallized from methylene chloride-ether and dried. The product precursor Ru (POP); the yield was 72 %.

1.2.3. Synthesis of PhenSe

Weigh 0.21 g (1 mmol) of 5,6-diamino-1,10-phenanthroline was dissolved in 5 ml of glacial acetic acid to obtain solution A. Weigh 0.11 g (1 mmol) of selenium dioxide was dissolved in 3 ml of ultrapure water to obtain solution B. Solution A and solution B were mixed, and the reaction was stirred for 30 min, then about 7 mL of ammonia were added and filtered, and the resulting precipitate was washed with ultrapure water and dried; Then dissolved with dichloromethane-methanol (1:1) organic solution, natural evaporation and crystallization, the product pink needle-like crystals were collected to obtain the ligand PhenSe ; the yield was up to 75 %.

1.2.4. Synthesis and Characterization of [Ru(POP) (PhenC)Cl]Cl ,Ru-C

Weigh 0.10 g (0.1 mmol) of Ru(POP), 0.022 g (0.1 mmol) of imidazo[4,5-f]1,10-phenanthroline in a reaction flask, add 10 mL of ethylene glycol ethyl ether, the temperature was set to 100 ℃, stirring and refluxing the reaction for 4 h. After the reaction was completed, the reaction was cooled to room temperature naturally, and if there was any insoluble material, it was filtered to remove it, and then a yellow solid was precipitated by addition of ethyl ether. After the reaction was completed, any insoluble material was removed by filtration with natural cooling at room temperature, then ether was added, and a yellow solid was precipitated, filtered, and washed with ether to obtain an orange-yellow solid, which was recrystallized from dichloromethane-ether to obtain the complex [Ru(POP) (PhenC)Cl]Cl, and was subsequently abbreviated as Ru-C. The yield was up to 74 %. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.29 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 7.1 Hz , 9H), 7.64 , 9H), 7.64 (d, J = 4.9 Hz, 4H), 7.61 - 7.38 (m, 13H), 6.86 (d, J = 13.9 Hz, 4H), 1.90 (s, 3H), 1.87 (s, 3H). MS (ESI) m/z: 935.1445 $[Ru(POP)(PhenC)Cl]^+$; Molecular formula for Ru-C: $[Ru(POP) (PhenC)Cl]Cl(H_2O)_3$ Elemental analysis for Ru-C: calculated C 60.94, H 4.52, N 5.47; found C 60.89, H 4.607, N 5.47.

1.2.5. Synthesis and Characterization of [Ru(POP)(PhenN)Cl]Cl ,Ru-N

0.10 g (0.1 mmol) of Ru (POP) and 0.022 g (0.1 mmol) of phenanthroline triazole were mixed in 10 mL of ethylene glycol ethyl ether. The reaction was stirred at 100 ℃ for 4 h. After the reaction was completed, the reaction was cooled naturally. Insoluble matter, if any, was removed by filtration, then 50 mL of ether was added, and a brown solid precipitated out, which was filtered, and washed with ether. The product Ru-N was recrystallized from dichloromethane-ether to give about 0.075 g in 77.3 % yield. ^{1}H NMR (300 MHz, DMSO-d6) δ 9.76 (d, J = 4.7 Hz, 1H), 8.98 (d, J = 6.7 Hz, 1H), 8.59 (s, 1H), 8.08 (s, 10H), 7.74 - 7.38 (m, 18H), 6.83 (s, 2H), 1.90 (s, 3H), 1.88 (s, 3H). MS (ESI) m/z: 936.1391 $[Ru(POP)(PhenN)Cl]]^+$; Molecular formula for Ru-N: $[Ru(POP)(PhenN)Cl]Cl(H_2O)_3$ Elemental analysis for Ru-N: calculated C 59.71, H 4.42, N 6.83; found C 59.49, H 4.853, N 6.22;

1.2.6. Synthesis and Characterization of [Ru(POP)(PhenSe)Cl]Cl ,Ru-Se

Ru (POP) 0.10 g (0.1 mmol), 0.028 g (0.1 mmol) o-phenanthroline selenadiazole (PhenSe) was weighed in a reaction flask, 10 mL of ethylene glycol ether was added, and the temperature was set to 100 ℃, and the reaction was stirred and refluxed for 4 h. After completion of the reaction, the reaction was cooled down to room temperature naturally, and if there were any insoluble matter, it was filtered to remove it, and then ethyl ether was added, and a yellow solid was precipitated out, and it was filtered. Then a yellow

solid precipitated out, filtered, washed with ether, a brown-red solid was obtained, and the selenium-containing ruthenium complex was obtained after recrystallization with dichloromethane-ether. The complex was called complex [Ru(POP)(PhenSe) Cl]]Cl, subsequently abbreviated as Ru-Se. The yield was up to 67 %.^{1}H NMR (400 MHz, DMSO-d6) δ 9.87 (d, J = 5.8 Hz, 1H), 8.99 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 14.2 Hz, 8H), 7.62 - 7.46 (m, 10H), 6.58 (s, 6H), 6.01 (s, 6H), 1.87 (s, 6H). MS (ESI) m/z: 1001.0459 [Ru(POP)(PhenSe) Cl]]$^{+}$; Molecular formula for Ru-Se:[Ru(POP)(PhenSe)Cl]Cl$(H_2O)_4$ Elemental analysis for Ru-Se: calculated C 55.29, H 4.19, N 5.06 ; found C 55.89, H 5.290, N 4.87.

1.3 The characterization of enzyme mimics

The catalyst 2.5 mM, substrate 1000 mM, solvent and oxidant 100 mM were added sequentially and then stirred in the sample bottle at room temperature for 12 h. At the end of the reaction, the solution was aspirated, filtered, transferred to a liquid phase bottle, and detected and analyzed by gas chromatography.Sample yields were determined on an Agilent 8860 gas chromatograph. According to the oxidation chromatographic conditions of benzyl alcohol and styrene: the conversion rate of each component was determined by internal standard method.

1.4 Cell biological research

1.4.1. Cell culture and cytotoxicity tests

HeLa (cervical cancer) was purchased from American Type Culture Collection (ATCC), U.S.A. R-HeLa was used as an experimental construct, which was prepared by irradiating HeLa for 6 Gy, 4 times. The cell culture medium was DMED medium, which consisted of 10 % fetal bovine serum, 100 units/mL penicillin and 50 units/mL streptomycin. The cells were cultured in a 37 ℃ incubator with a relative humidity of 95 % and a CO_2 concentration of 5 %.

Logarithmic growth phase cells were taken and inoculated in 96-well plates at a density of 2×10^4 cells/mL, placed in an incubator for 24 h. Different drug concentrations were added for 48 h, respectively. 30 μL of MTT solution (5 mg/mL, PBS solution) was added to each well, and after 3 hours, the upper layer of culture medium was withdrawn, 150 μL of DMSO (dimethyl sulfoxide) was added, and placed on a shaker and shaken slowly for 15 minutes, and waited for the purple crystals to dissolve completely. The absorbance value of each well of the well plate at 570 nm was measured by multifunctional enzyme labeling instrument, and the half inhibitory concentration IC_{50} was calculated. cell viability (%) = (OD_{570} experimental group/OD_{570} control group) × 100 % [6].

1.4.2. Cloning formation experiment

Logarithmic growth phase cells were taken and inoculated in 6-well plates at a density of 2×10^4 cells/mL. When the cell attachment was completed, different concentrations of drugs were added, and 4 h after the addition of drugs, one plate was subjected to 4 Gy irradiation in order to compare with the control group. After irradiation, the cells were put back into the incubator for culture, and the liquid was changed every three days, with gentle movements to avoid sucking off the adherent cells. 8 days later,

the cell cloning was observed. After cell cloning was completed, the supernatant was removed, the medium was washed away with PBS, and the cells were fixed with 4 % paraformaldehyde for 15 min. The cells were washed with PBS to remove the paraformaldehyde, and 0.5 % crystal violet ethanol solution (wt/vol) was added to form a colony staining for the cells, and the excess crystal violet was rinsed off slowly with running water after 15 min. After washing, the 6-well plate was placed in a dry place and after the 6-well plate was free from water staining, the cell colonies were recorded and counted using a camera and microscope.

1.4.3. Live cell staining

Cells in the logarithmic growth phase were taken and inoculated in 6-well plates (density of 8×10^4 cells/mL), and after 24 h, different drugs were added, and the incubation was continued for four hours before X-ray irradiation, and after 48 h, cells were washed. After washing, add 1mL of Calcein AM assay working solution and incubate for 30min at 37℃ away from light. Following the incubation period, the survival of cells was observed under a fluorescence microscope.[7]

1.4.4. Cell cycle analysis

The logarithmic growth phase cells were taken and inoculated in 6-well plates at a density of 2×10^4 cells/mL and incubated in the incubator for 24 h. Different drug concentrations were added separately. After continuing the incubation for four hours, X-Ray irradiation was performed and the cells were collected after 72 h. The cells were then incubated in the incubator for 24 h and then the different drug concentrations were added. The cell supernatant was withdrawn and placed in a centrifuge tube, and the excess medium was washed away by PBS, the washing process should be gentle and careful. Add 600 μL of trypsin for digestion, and terminate digestion after complete digestion. Transfer the liquid in the six-well plate to a centrifuge tube for centrifugation, pour off the supernatant, gently blow the cells centrifuged to the bottom with PBS, and centrifuge. After pouring off the supernatant, the cells were blown to the bottom of the centrifuge tube with pre-cooled 70 % ethanol and fixed in the refrigerator at -20 ℃ for 24 h. The cells were centrifuged every other day, the supernatant ethanol was removed, the cells were washed with PBS and snap-dried, and 500 μL of PI dye was added and incubated in an incubator in the absence of light for 30 minutes. The samples were filtered through a 400 mesh sieve and analyzed on a flow cytometer. The number of counted cells in each sample was 10,000, and the proportion of distribution of tumor cells in each cycle was analyzed by Multi Cycle software.[8]

1.4.5. Evaluation of mitochondrial function

HeLa cells in logarithmic growth phase were taken and inoculated in 6-well plates (density of 4×10^4 cells/mL,) and after the cell attachment was completed, the cells were added with different drugs to continue incubation for four hours, X-Ray irradiation was performed, and the cells were collected and washed 48 h later. After washing was completed, the water was controlled dry, and the configured JC-1 working solution was added to the cells for resuspension. The cells were placed in the incubator to continue incubation for 10 min and were detected on a flow cytometer.[9]

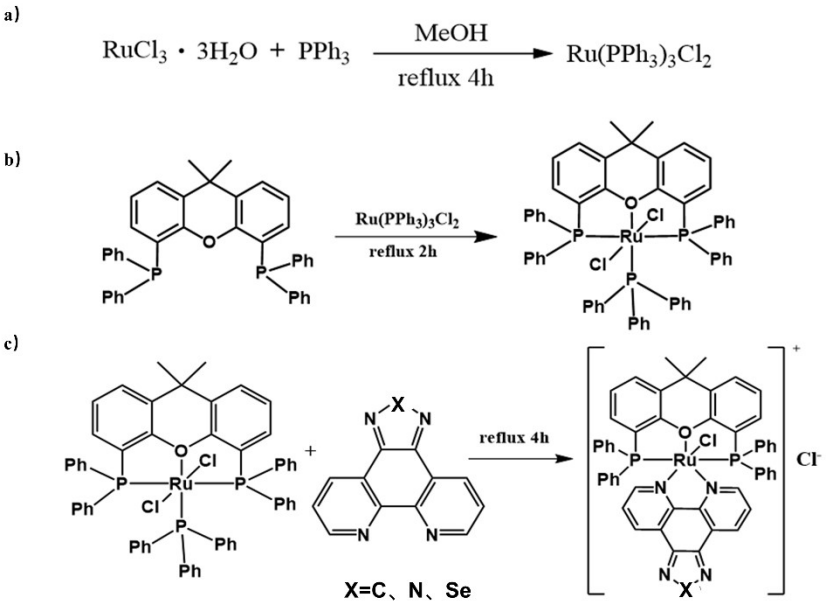
1.4.6. Western blotting experiments

Take the logarithmic growth phase HeLa cells inoculated in a 10 cm dish (density of

1 × 10^5 cells/mL, 10 mL/dish), after the completion of cell attachment, add drugs to continue to cultivate for four hours, then X-Ray irradiation, 48 h, collect the cells, remove the supernatant of the cells, wash the plate with PBS for three times, deduct the water, the dish was put on ice, and add RIPA to protein After ten minutes, the proteins were quickly scraped off with a spatula and stored in the refrigerator at -20 ℃. The cells were shaken and centrifuged every other day to detect the protein content. Cooking protein. After the preparation of gel, the proteins will be sampled, and the proteins will be separated and electrophoresed. After electrophoresis, membrane transfer was performed: PVDF membrane was soaked in methanol for 2~3 min before use, and the protein was transferred to the PVDF membrane, then the membrane was closed with 5 % skimmed milk for 2 h. The membrane was incubated with primary antibody overnight, and then recovered on the next day, and then recovered after incubation with secondary antibody. After incubation, the membrane was washed with TBST buffer, evenly coated with luminescent chromogenic solution, and color developed by Kodak luminescence detector.[10]

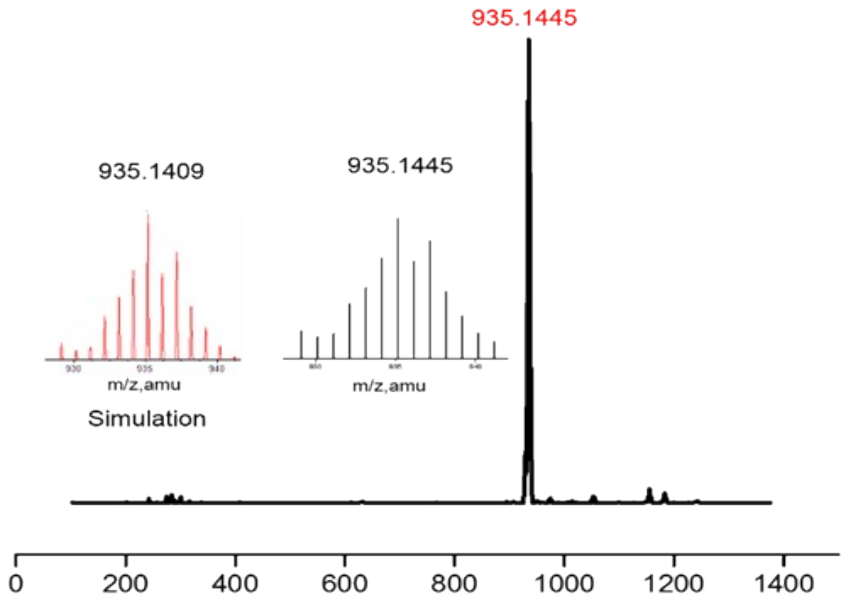
2 Results

2.1 Figure S1 Synthesis routes of Ru-C, Ru-N, Ru-Se

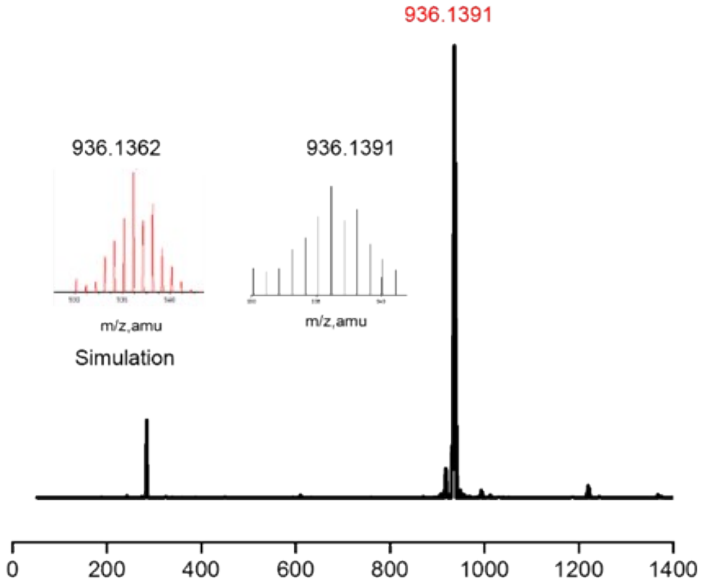


(a) Synthetic of $Ru(PPh_3)_3Cl_2$.(b) Synthetic of Ru(POP).(c) Synthetic of Ru-C, Ru-N, Ru-Se

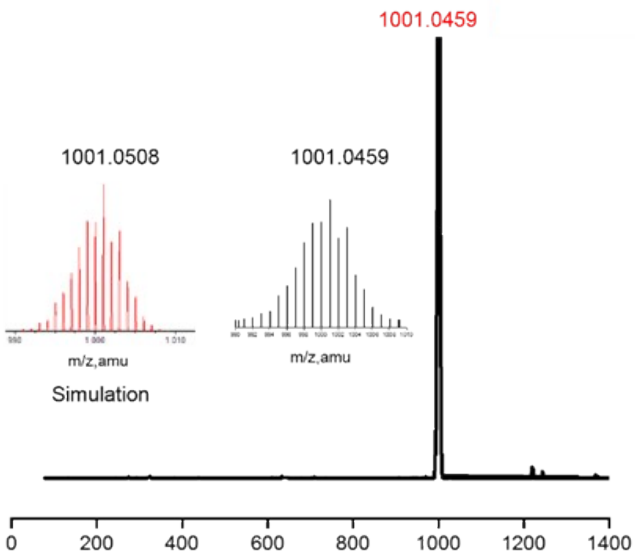
2.2 Figure S2 Mass spectrum of Ru-C in methanol.



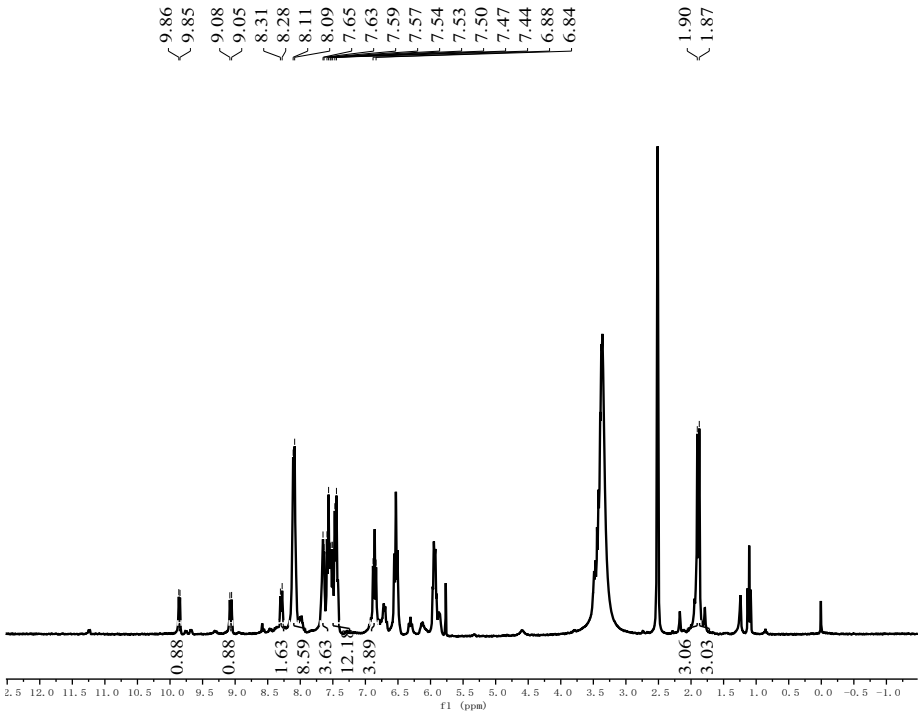
2.3 Figure S3 Mass spectrum of Ru-N in methanol.



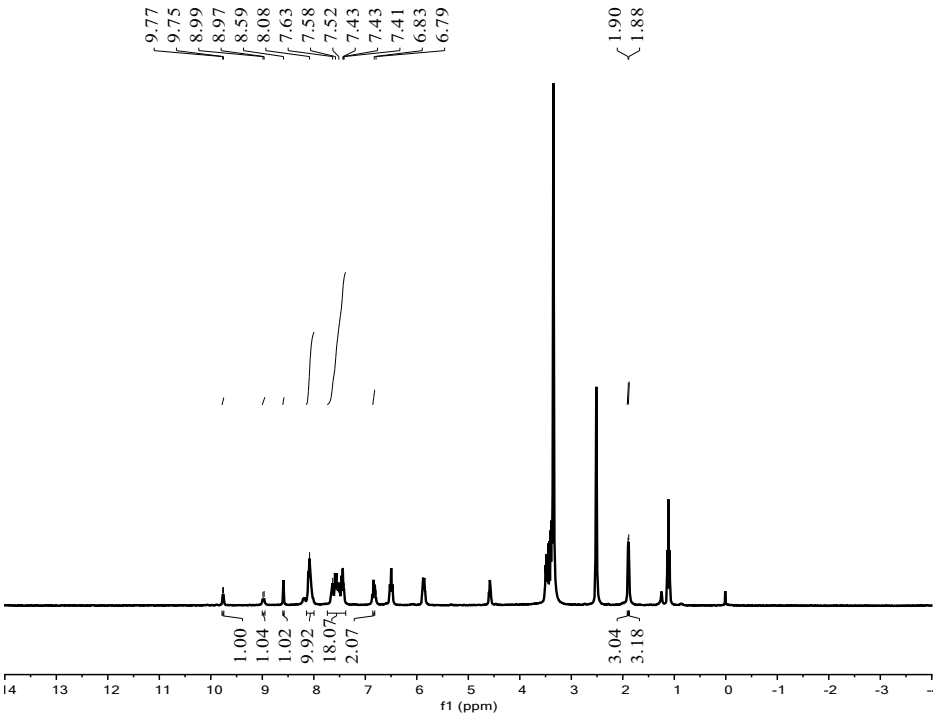
2.4 Figure S4 Mass spectrum of Ru-Se in methanol.



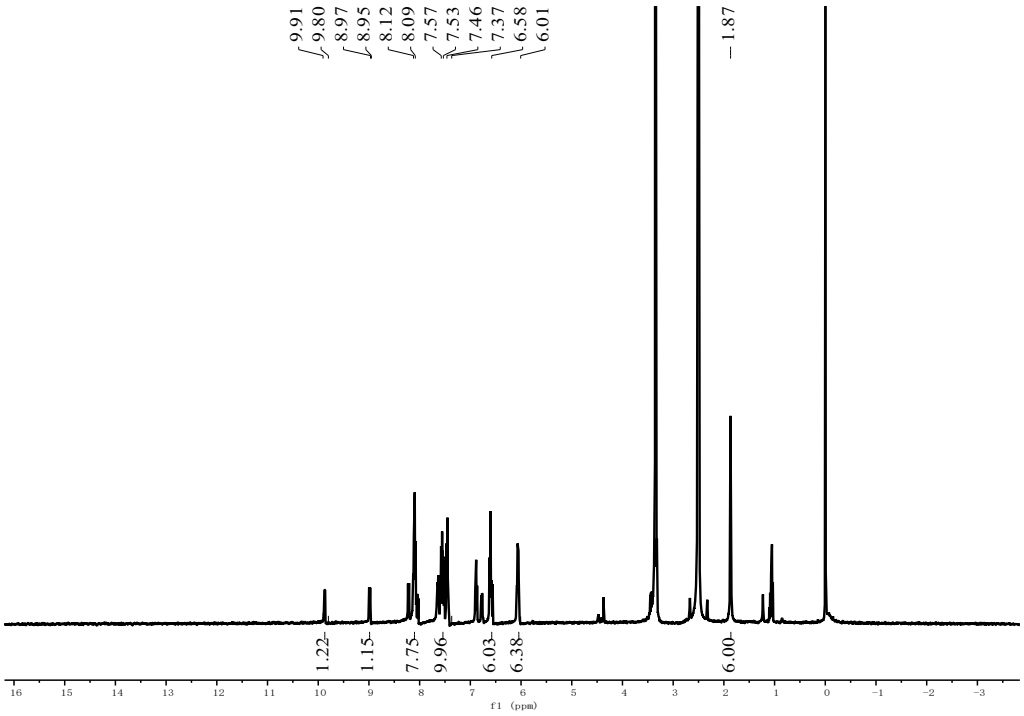
2.5 Figure S5 ^{1}H NMR data assignments for Ru-C in DMSO-d6



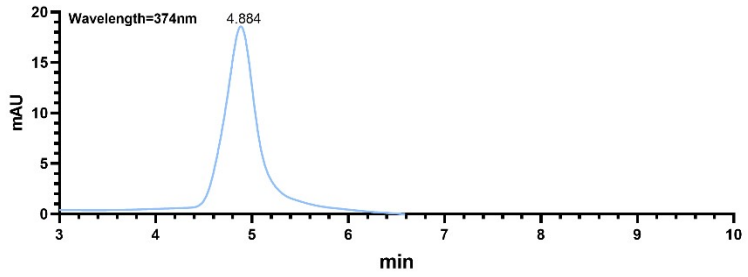
2.6 Figure S6 ^{1}H NMR data assignments for Ru-N in DMSO-d6



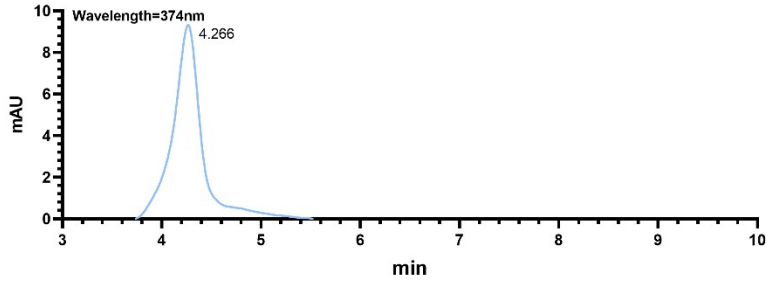
2.7 Figure S7 ^{1}H NMR data assignments for Ru-Se in DMSO-d6



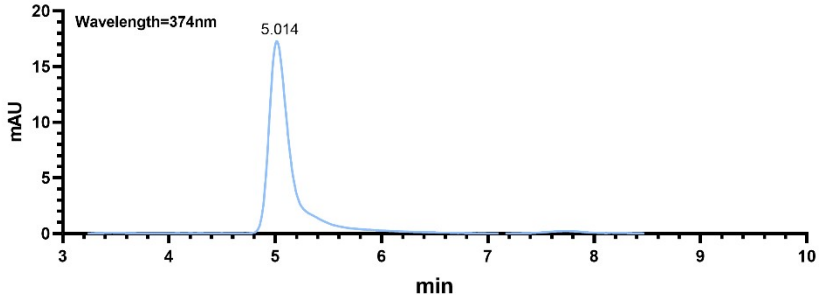
2.8 Figure S8 The HPLC of the complex Ru-C



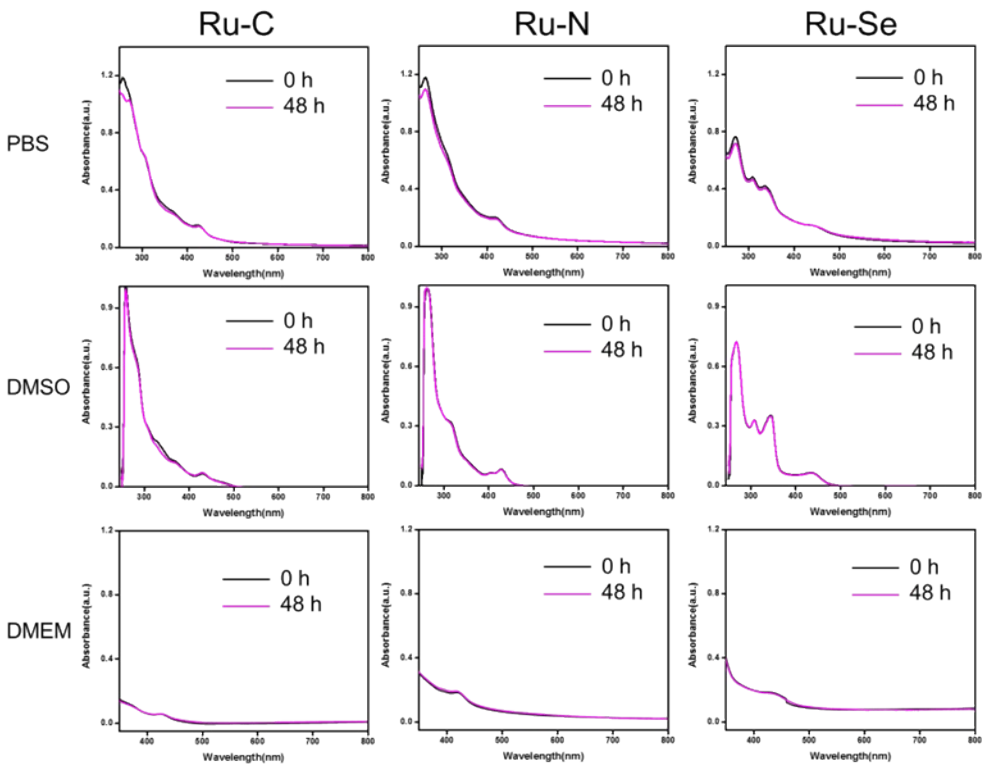
2.9 Figure S9 The HPLC of the complex Ru-N



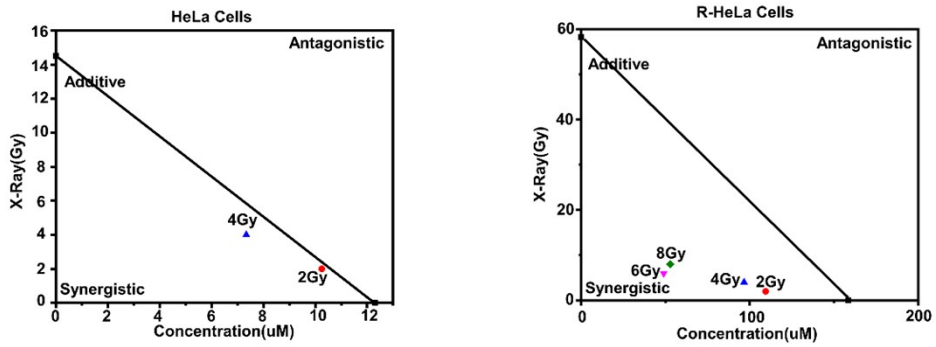
2.10 Figure S10 The HPLC of the complex Ru-Se



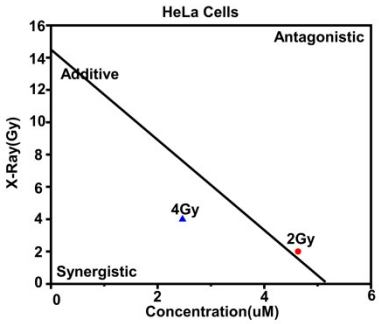
2.11 Figure S11 Stability testing of ruthenium complexes

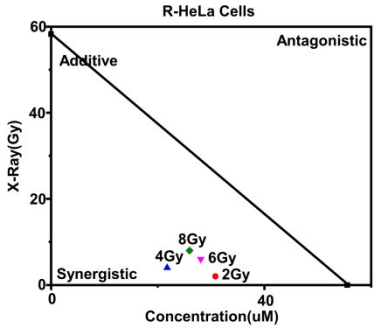


2.12 Figure S12 Isobologram analysis of the synergistic antiproliferative effect of the combined treatment of X-ray and Ru-C



2.13 Figure S13 Isobologram analysis of the synergistic antiproliferative effect of the combined treatment of X-ray and Ru-N

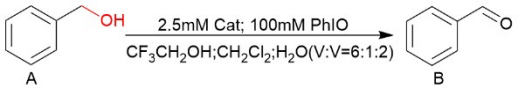


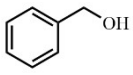
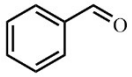
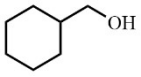
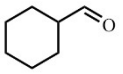
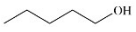
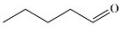
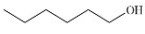
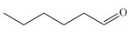


2.14 Table S1 The half-wave potential of Ru-C, Ru-N, Ru-Se

	Ru-C	Ru-N	Ru-Se
Ru^{II}/Ru^{III}(V)	0.971	0.965	0.892
Ru^{III}/Ru^{IV}(V)	1.159	1.297	1.220

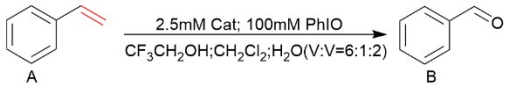
2.15 Table S2 Performance of alcohol catalyzed by different ruthenium complexes [a]



Catalyst	Reactant A	Product B	Yield（%）[b]
\			37
Ru-C			99
Ru-N			97
Ru-Se			100
Ru-C			100
Ru-N			100
Ru-Se			100
Ru-C			100
Ru-N			100
Ru-Se			100
Ru-C			100
Ru-N			100
Ru-Se			100
Ru-C			100
Ru-N			100
Ru-Se			100

[a] Reaction conditions: 2.5 mM catalyst and 1000 mM substrate
[b] Yield was calculated based on oxidant.

2.16 Table S3 Performance of olefins catalyzed by different ruthenium complexes [a]



Catalyst	Reactant A	Product B	Yield（%）[b]
\			0.8
Ru-C			87.6
Ru-N			89.2
Ru-Se			92
Ru-C			24.8
Ru-N			21.1
Ru-Se			25.9
Ru-C			80
Ru-N			54.4
Ru-Se			43.5
Ru-C			85.4
Ru-N			65.1
Ru-Se			70.5

[a] Reaction conditions: 2.5 mM catalyst and 1000 mM substrate

[b] Yield was calculated based on oxidant.

3 References

1. W. Cao, W. Zheng and T. Chen, Ruthenium polypyridyl complex inhibits growth and metastasis of breast cancer cells by suppressing FAK signaling with enhancement of TRAIL-induced apoptosis, *Scientific Reports*, 2015, **5**, 9157.
2. L. Chan, Y. Huang and T. Chen, Cancer-targeted tri-block copolymer nanoparticles as payloads of metal complexes to achieve enhanced cancer theranosis, *Journal of Materials Chemistry B*, 2016, **4**, 4517-4525.
3. Q. Chen, L. He, X. Li, L. Xu and T. Chen, Ruthenium complexes boost NK cell immunotherapy via sensitizing triple-negative breast cancer and shaping immuno-microenvironment, *Biomaterials*, 2022, **281**, 121371.
4. J. Liu, H. Lai, Z. Xiong, B. Chen and T. Chen, Functionalization and cancer-targeting design of ruthenium complexes for precise cancer therapy, *Chemical Communications*, 2019, **55**, 9904-9914.
5. C. Shi, Z. Yuan, T. Liu, L. Chan, T. Chen and J. Zhao, X-ray sensitive selenium-containing Ru complexes sensitize nasopharyngeal carcinoma cells for radio/chemotherapy, *Journal of Materials Chemistry B*, 2023, **11**, 5607-5618.
6. F. Denizot and R. J. J. o. i. m. Lang, Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, *Journal of immunological methods*, 1986, **89**, 271-277.
7. J. F. Kerr, A. H. Wyllie and A. R. J. B. j. o. c. Currie, Apoptosis: a basic biological phenomenon with wideranging implications in tissue kinetics, *British journal of cancer*, 1972, **26**, 239-257.
8. P. J. C. Nurse, Ordering S phase and M phase in the cell cycle, *British journal of cancer*, 1994, **79**, 547-550.
9. R. C. Scaduto and L. W. J. B. j. Grotyohann, Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives, *Biophysical journal*, 1999, **76**, 469-477.
10. H. Towbin, T. Staehelin and J. J. P. o. t. n. a. o. s. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, *Proceedings of the national academy of sciences* 1979, **76**, 4350-4354.