Supporting Information

Luminescent Ru(II)-thiols modified silver nanoparticles for lysosome targeted theranostics

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Experimental section

1. Materials.

All reagents and solvents were obtained commercially and used without any further purification unless otherwise specified, and Ultrapure MilliQ water was used in this study. AgNPs were purchased from Nanjing Nanoeast Biotech Co., Ltd (China). Hoechst 33342 and Lyso-Tracker Green were purchased from Thermo Fisher Scientific (USA). CCK-8, DCF-DA, JC-1, FBS, PBS, DMEM and DMSO were purchased from Beyotime (China). Hela cells were purchased from Chinese Academy of Sciences Cell Bank.

2. Physical measurements.

^{1}H NMR spectra were recorded on Varian Mercury-Plus 400 NMR instrument (^{1}H 400 MHz). Mass spectra were measured on an agilent TOF-G6230B mass spectrometer. UV-vis spectra were obtained by a UV/Vis spectrophotometer (Hitachi U-3900) in wave-length range of 200–800 nm, at a resolution of 1 nm using a 10 mm path length quartz cuvette. Transmission electron microscope (TEM) analysis was performed on a JEM-2100 TEM with an accelerating voltage of 200 kV. TEM samples were prepared by drop casting on a carbon-coated copper grid and the grid dried before observations. Zeta potential and dynamic light scattering (DLS) measurements were conducted on a Zetasizer Nauo ZS90 (Malvern) instrument at a room temperature.

3. Synthesis and characterization.

The precursor complex cis-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$ and cis-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ were prepared according to the literature methods.[1-3] The main ligand was synthesized mainly in three steps:

Main ligand 11-mercapto-N-(1,10-phenanthrolin-5-yl) undecanamide.

(1). 11-(benzoylthio)undecanoic acid. Under N_2, 11-Mercaptoundecanoic acid (0.0023 mol，0.5 g, 1.0 equiv) was first dissolved in anhydrous DCM, then the

solution was cooled to 0 °C by means of an ice bath. In such a condition, benzoyl chloride (0.00345 mol，0.4 ml, 1.5 equiv) and trimethylamine (0.0069 mol，1.0 ml, 3.0 equiv) were added into the above solution. The mixture was stirred overnight at room temperature (monitored by TLC, EtOAc/PE = 1:5). After completion of the reaction, the solvent was removed under vacuum. The obtained residue was purified by column chromatography on silica gel (EtOAc/PE = 1:10) to afford the compound as white powder.

(2). S-(11-chloro-11-oxoundecyl) benzothioate. Under N_2, 11-(benzoylthio)undecanoic acid was dissolved in anhydrous DCM. The temperature was cooled to 0 °C by means of an ice bath, then oxalyl chloride was added. The ice bath was removed and the mixture was stirred at room temperature for 1 h (monitored by TLC, EtOAc/PE = 1:5). Then, the solvent was removed under reduced pressure.

(3). 11-mercapto-N-(1,10-phenanthrolin-5-yl) undecanamide. Under N_2, 1,10-phenanthrolin-5-amine (0.001244 mol ,0.243 g, 0.4 equiv) was dissolved in DCM, then in ice bath, trimethylamine (0.00933 mol, 1.29 mL, 3.0 equiv) was added. After 0.5-1 h stirring, the solid was dissolved and orange solution was gained by dropwise addition of acryl chloride (0.00311 mol, 1.06 g, 1.0 equiv) which was obtained in the previous step. The temperature was raised to room temperature and the mixture was stirred overnight under N_2. After completion (monitored by TLC, DCM : MeOH = 10:1), MeOH (2.0 mL) was added. Then, the solvent was removed under reduced pressure access to beige solid (0.42 g, 86%). ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H), 9.12 (dd, *J* = 4.2, 1.4 Hz, 1H), 9.02 (dd, *J* = 4.2, 1.4 Hz, 1H), 8.61 (d, *J* = 8.2 Hz, 1H), 8.45 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.17 (s, 1H), 7.81 (dd, *J* = 8.2, 4.2 Hz, 1H), 7.73 (dd, *J* = 8.2, 4.2 Hz, 1H), 2.66 (t, *J* = 7.1 Hz, 2H), 1.68-1.15 (m, 16H). HRMS (ESI) calculated m/z for $C_{23}H_{30}N_3OS$ 396.2110 ($[M+H]^+$), found 396. 2042 ($[M+H]^+$).

Ru(11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide)(bpy)$_2$(PF$_6$)$_2$ (Ru1).

11-mercapto-N-(1,10-phenanthrolin-5-yl) undecanamide (0.253 mmol, 0.1 g, 1.0 equiv) and $Ru(bpy)_2Cl_2{\cdot}2H_2O$ (0.253 mmol, 0.122 g, 1.0 equiv) were dissolved in 15 mL of EtOH/H_2O (4:1, v/v), and the mixture was degassed through bubbling with nitrogen for 15 minutes. Then, the reaction mixture was heated at 150 °C for 40 minutes under microwave irradiation (monitored by TLC, MeCN : H_2O: $NaNO_3$ (sat.) = 40:4:1). After cooling, the reaction mixture was filtered to remove solid impurities. Saturated aqueous NH_4PF_6 solution was then added to the filtrate under stirring and filtered again. The brown solid was collected and washed with EtOH/H_2O (4:1, v/v), then dried under vacuum to afford brown solid (0.19 g, 68%). ^{1}H NMR (400 MHz, MeOD) δ 8.78 – 8.66 (m, 5H), 8.62 (dd, *J* = 7.8, 3.3 Hz, 1H), 8.50 (t, *J* = 3.3 Hz, 1H), 8.23 (d, *J* = 4.2 Hz, 1H), 8.14 (dd, *J* = 18.5, 13.1 Hz, 3H), 8.07 (t, *J* = 7.9 Hz, 2H), 7.94 (t, *J* = 4.6 Hz, 2H), 7.87 (dd, *J* = 8.5, 5.3 Hz, 1H), 7.79 (ddd, *J* = 8.4, 5.2, 3.3 Hz, 1H), 7.65 (d, *J* = 5.5 Hz, 2H), 7.55 (dd, *J* = 7.3, 5.9 Hz, 2H), 7.34 (t, *J* = 6.6 Hz, 2H), 2.74 – 2.60 (m, 4H), 1.844 – 1.31 (m, 16H). HRMS (ESI) calculated m/z for $C_{43}H_{45}N_7ORuS$ 404.6225 ($[M]^{2+}$/2), found 404.1268.

Ru(11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide)(phen)$_2$(PF$_6$)$_2$ (Ru2).

This complex was synthesized in a manner identical to that described for complex Ru1, with $Ru(phen)_2Cl_2{\cdot}2H_2O$ (0.253 mmol,0.135 g) in place of $Ru(bpy)_2Cl_2.2H_2O$

(0.253 mmol, 0.122 g). Yield: 0.18 g, 62%. ^{1}H NMR (400 MHz, MeOD) δ 8.78 – 8.64 (m, 5H), 8.64 – 8.56 (m, 1H), 8.50 (s, 1H), 8.33 – 8.32 (m, 4H), 8.19 – 8.04 (m, 6H), 7.78 – 7.63 (m, 6H), 2.73 – 1.31 (m, 16H). HRMS (ESI) calculated m/z for $C_{47}H_{45}N_7ORuS$ 428.6225 ($[M]^{2+}/2$), found 428.1133 ($[M]^{2+}/2$).

Ru1-2·AgNPs.

AgNPs (600 μL, 0.1 mg/ml) was mixed with Ru1 or Ru2 (375 μL, 400 μM) and PB (525 μL,10 mM) for 3 h with vibrating at 25 °C. After incubation at least 24 h at the dark, the solution was purified by centrifugation (40 min, 12000 rpm). The supernatant was discarded and then the resulting conjugate precipitate was redispersed in PB or cell culture medium (protecting from light) for spectroscopy, morphology, and biological studies.

4. Cell Culture conditions.

Hela cells were cultured in DMEM medium with 10% Fetal Bovine Serum (FBS). Cultures were maintained at 37 ˚C in a humidified atmosphere containing 5% CO_2 and 95% air.

5. Live Cell Confocal Microscopy.

Hela cells were cultured in 10 mm glass bottom dishes overnight. Ru1-2·AgNPs (20 μg/ml) was added to the culture medium (final DMSO concentration, 0.1% v/v) and incubated for several hours at 37 °C. After incubation, nuclear staining was performed by Hoechst 33342 (10 μM) for 30 min or lysosomes staining was performed by Lysosome tracker green (20 μM) for 30 min. The cells were then washed three times with cold PBS. Confocal images were captured with a confocal laser scanning microscope (Leica TCS SP5 II) using a 63 × oil-immersion objective lens immediately.

6. TEM Imaging of Cell Sections.

Hela cells (2×10^6 cells/mL) were incubated with Ru1-2·AgNPs (20 μg/ml) for 4 h. After incubation, the cells were thoroughly washed with PBS to eliminate the unbound Ru1-2·AgNPs. Cells were trypsinized, washed 3 times in phosphate buffer, centrifuged and the culture medium was decanted. For ultrastructural analysis, 1 mm^3 thick were cut from the specimen with a razor blade and immediately fixed in 2.5% glutaraldehyde in 0.1 mol L^{-1} phosphate buffer (pH 7.2) at 4°C for 3 h. Cells were then washed with phosphate three times and postfixed in 1% OsO4 at 4°C for 3 h, washed with phosphate buffer three times, then dehydrated in a graded acetone series and embedded in Spurr's resin. For electron microscopy, ultra-thin sections, 70-nm thick, were cut from samples. The sections were then mounted on formvar-coated copper mesh grids and doubly stained with saturated uranyl acetate for 30 min followed by lead citrate for 15 min. Ultra-thin sections on the grids were examined in a JEOL JEM-1230 transmission electron microscope at 80 kV.

7. CCK-8 assay.

Cytotoxicity was assessed by the CCK-8 kit (Cell Counting Assay Kit-8). Hela cells were seeded in triplicate in 96 micro-well plates at a density of 5×10^3 cells per well

in 100 μL of medium and incubated overnight at 37 °C in 5% CO_2. Then the medium was replaced with the respective medium containing the compounds at various concentrations and incubated 48 h. Add 10 μL of CCK-8 solution to the each well and incubated for 2 h. Cell viability was measured by absorbance at 450 nm with a reference wavelength of 650 nm, using a micro plate reader (pectra Max M5).

8. Cellular Uptake

Hela cells were cultured in a six-well plate at a saturated density. After incubation with different concentrations of AgNPs and Ru1-2·AgNPs for 12h and 24h, all of the cells were washed thoroughly with PBS, digested by trypsine-EDTA and centrifuged. The cell precipitations were digested with aqua regia, and then the solutions were diluted by Mili-Q water. The total Ag content in the solution was determined using the ICP-OES. The same procedure was employed to measure the control groups.

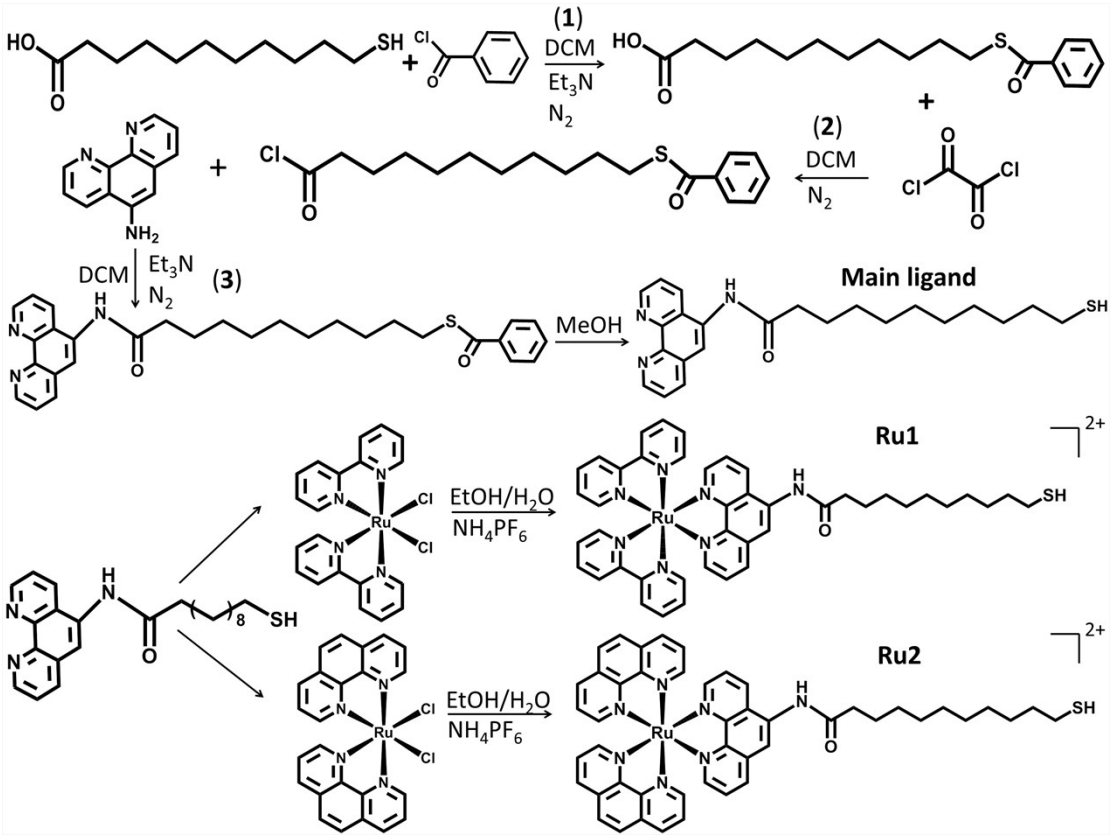
9. Detection of intracellular ROS generation.

The intracellular ROS in Hela cells were detected with DCF-DA assay. Hela cells were seeded in a six-well plate then treated with various concentrations of Ru1-2·AgNPs under dark. After 8 h exposure of Ru1-2·AgNPs, cells were harvested and washed twice with PBS, and incubated with 10 mM DCF-DA at 37 °C for 20 min in the dark. Cells were then washed twice in serum-free medium. The fluorescence of cells was examined by a laser scanning confocal microscopy with excitation and emission wavelengths at 488 and 525 nm, respectively (Leica TCS SP5 II) and flow cytometry (FACS Verse, BD Biosciences). For each sample, 10000 cells were collected.

10.Measurement of MMP ($\Delta\Psi_m$).

The decrease of MMP was measured by the fluorescent probe JC-1 (Beyotime, China). HeLa cells were seeded into 12-well culture plates at a density of 1×10^5 cells per well and incubated at 37 °C for 24 h and then treated with various concentrations of compounds. After 48 h incubation, cells were washed twice with PBS and incubated with JC-1 working solution in the dark 20 min. JC-1 working solutions prepared according to the manufactures instructions. The stained cells were then washed with JC-1 staining buffer and resuspended in culture medium. Images were obtained with a laser scanning microscope (Leica TCS SP5 II) at 490 nm excitation and 530 nm emission for green fluorescence, and at 525 nm excitation and 590 nm emission for red fluorescence.

Supplementary Figures



Scheme S1. Synthetic route to main ligand and complexes Ru1-2.

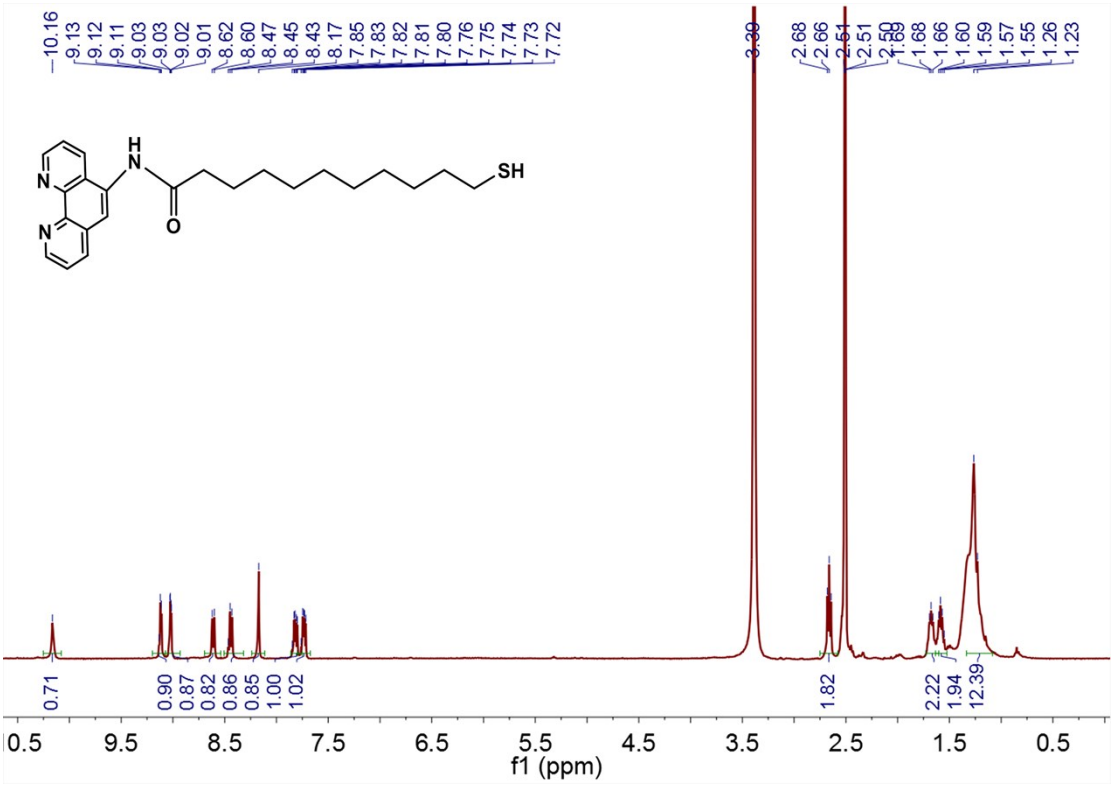


Fig. S1 ^{1}H NMR (400 MHz) of main ligand in DMSO.

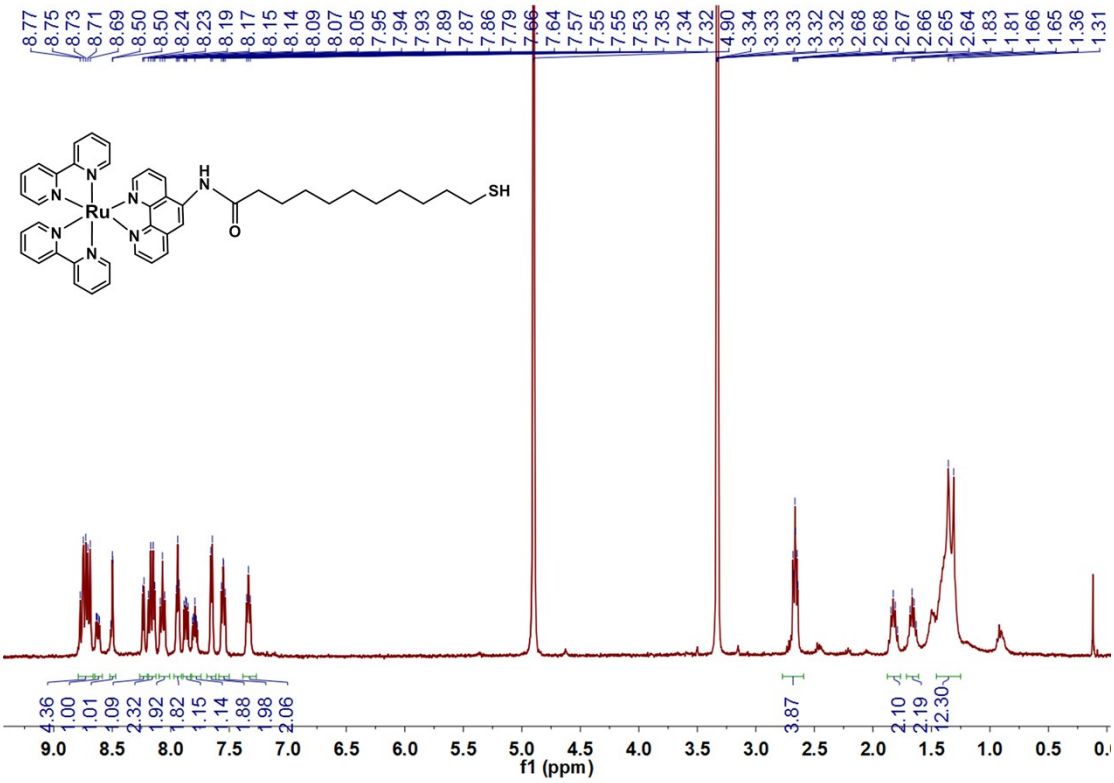


Fig. S2 ^{1}H NMR (400 MHz) of Ru1 in MeOD.

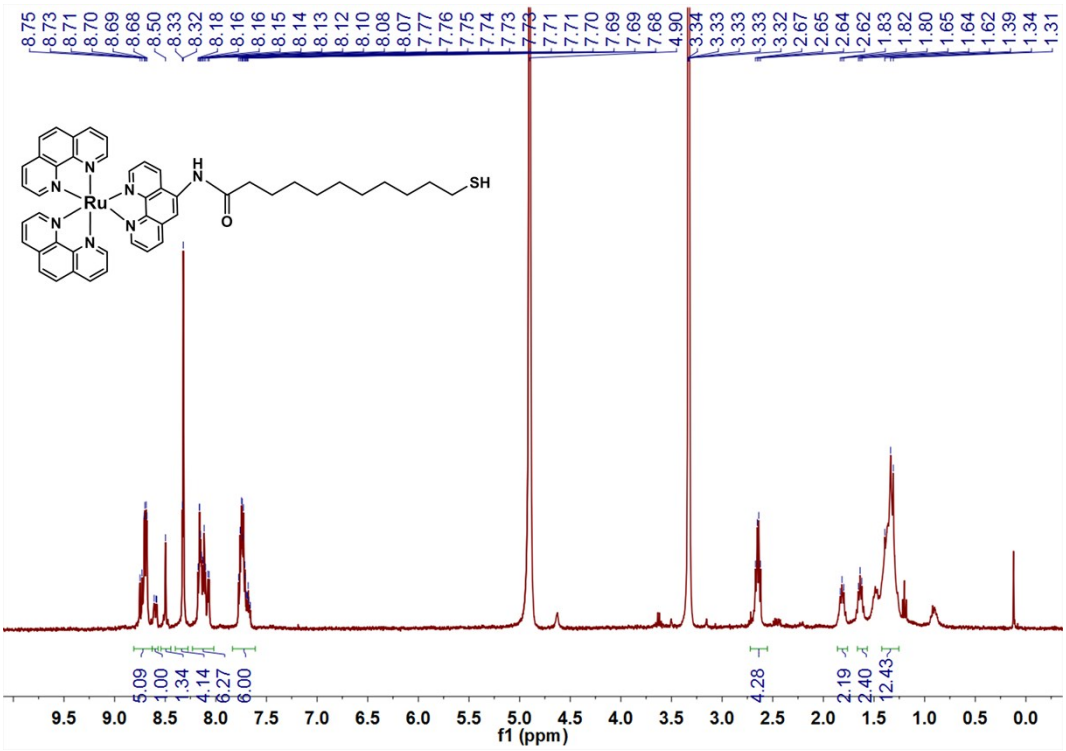


Fig. S3 ^{1}H NMR (400 MHz) of Ru2 in MeOD.

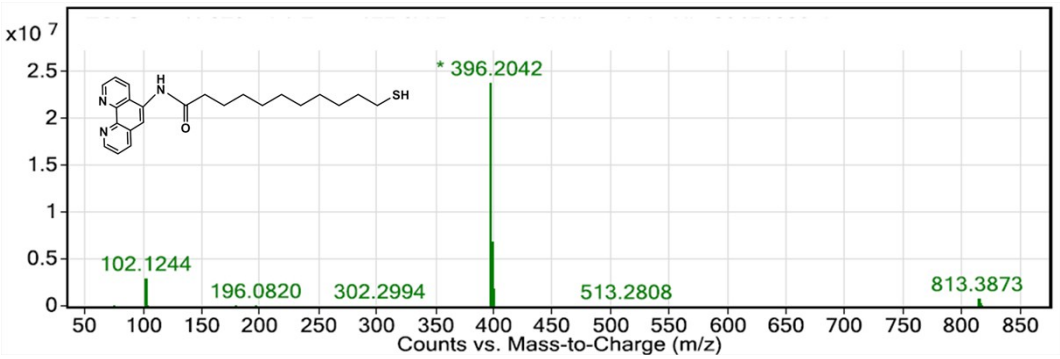


Fig. S4 ($C_{23}H_{30}N_3OS$) HRMS (ESI): 396. 2042 ($[M+H]^+$).

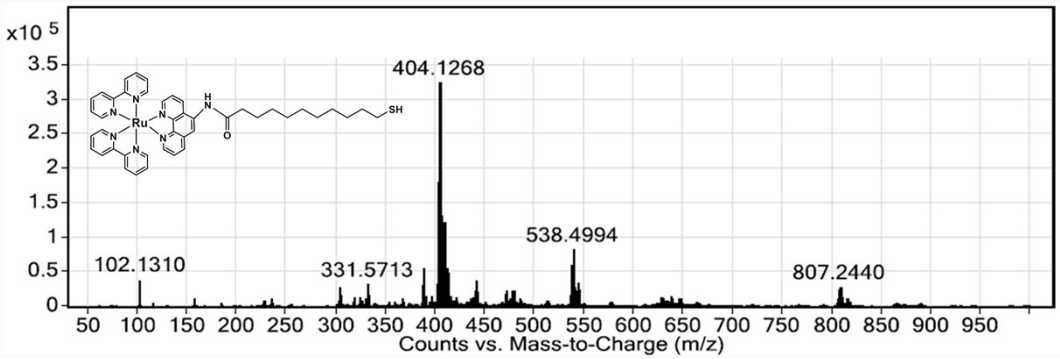


Fig. S5 ($C_{43}H_{45}N_7ORuS$) HRMS (ESI): 404.1268 ($[M]^{2+}/2$).

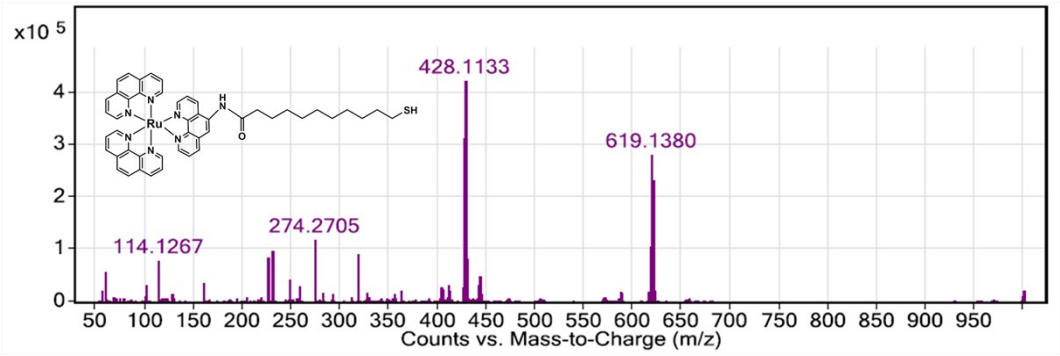


Fig. S6 ($C_{47}H_{45}N_7ORuS$) HRMS (ESI): 428.1133 ($[M]^{2+}/2$).

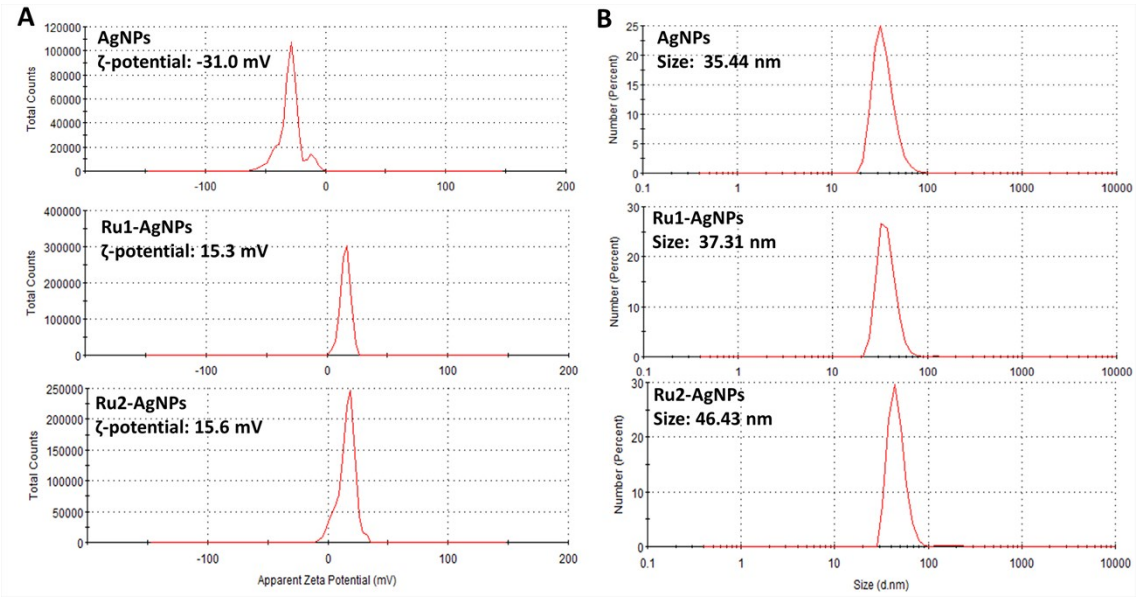


Fig. S7 Zeta potential (A) and hydrodynamic size (B) of AgNPs and Ru1-2·AgNPs in ultrapure water.

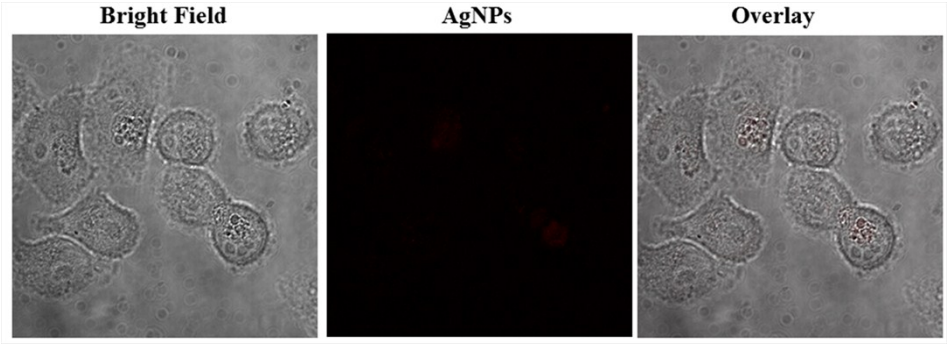
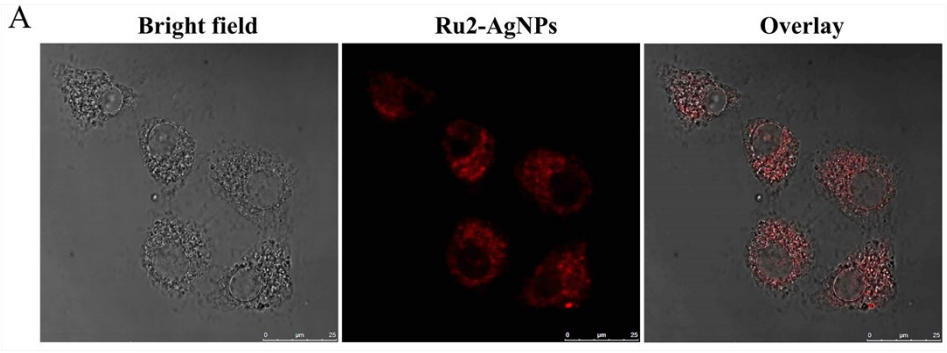


Fig. S8 Confocal fluorescence images of HeLa cells incubated with 20 μg/ml AgNPs for 4 h (λ_{ex} = 458 nm, λ_{em} = 570-620 nm, red).



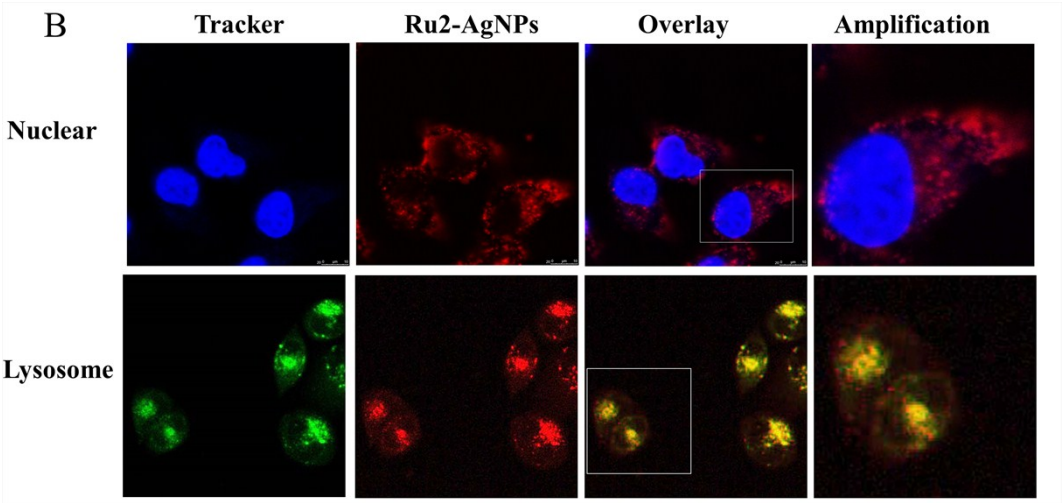


Fig. S9 Confocal fluorescence images of HeLa cells (A) Cells incubated with 20 μg/ml Ru2-AgNPs for 4 h (λ_{ex} = 458 nm, λ_{em} = 570-620 nm, red) (B) Cells co-stained with Ru2-AgNPs (20 μg/ml, 4 h) and Hoechst 33342 (λ_{ex} = 405 nm, λ_{em} = 420-480 nm, blue) and Lyso-tracker green (λ_{ex} = 488 nm, λ_{em} = 500-550 nm, green).

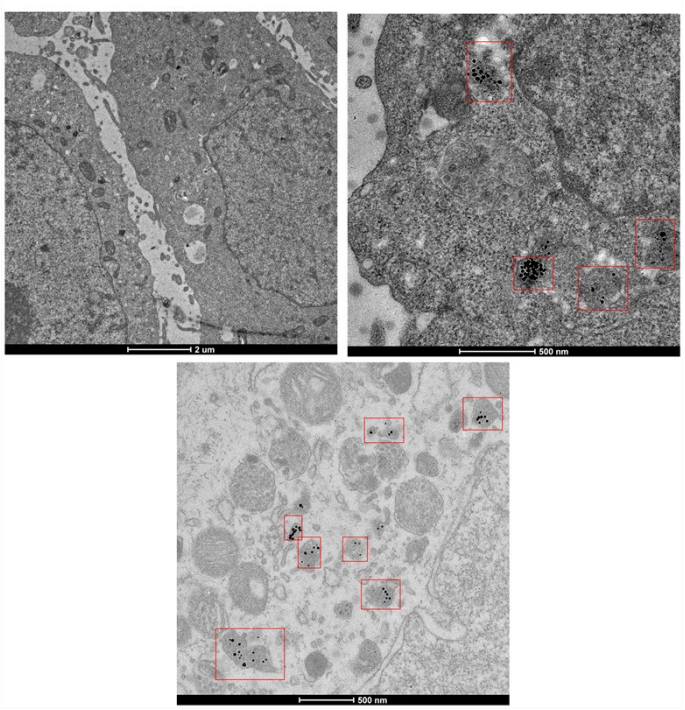


Fig. S10 TEM images of Ru2-AgNPs (20 μg/ml, 4 h) internalized into Hela cells. Red squares indicate Ru2-AgNPs accumulated in lysosome.

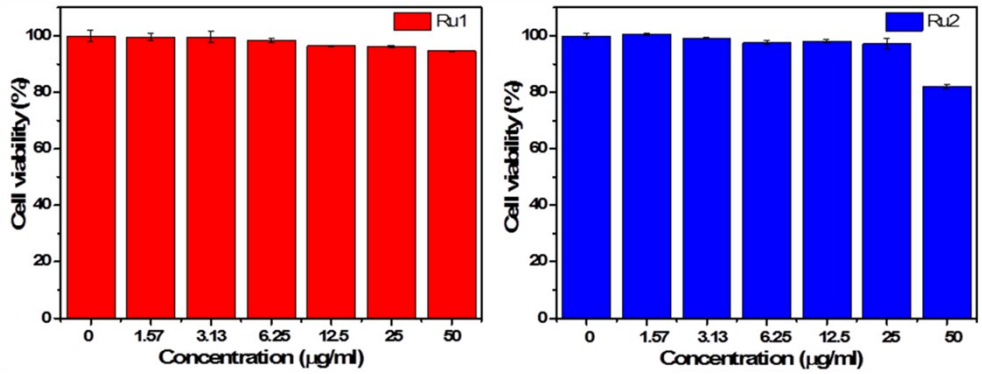


Fig. S11 Effects of different concentrations of Ru1 and Ru2 on Hela cell viability (48 h).

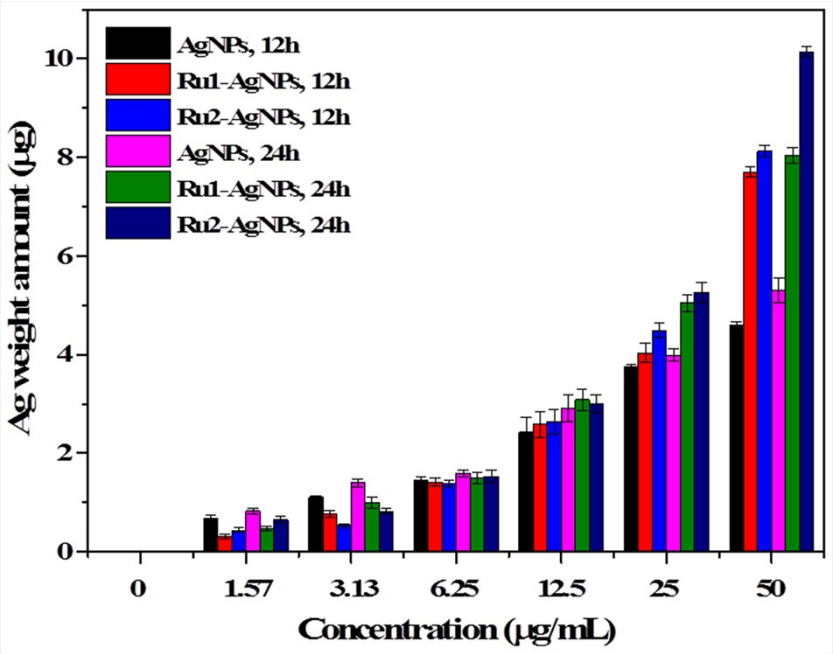


Fig. S12 Uptake of AgNPs and Ru1-2·AgNPs by Hela cells. The cells were treated with increasing concentrations of NPs for 12h and 24h. The cellular Ag content was analyzed by ICP-OES.

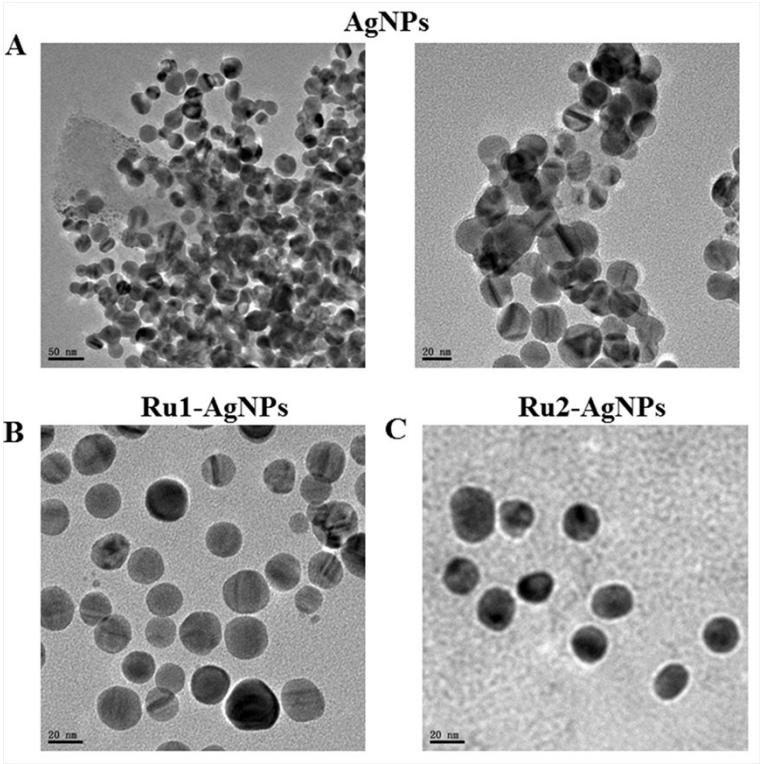


Fig. S13 TEM images of the AgNPs and Ru1-2·AgNPs (50 μg/mL) dispersion in the cell growth medium that reveal the sizes, shapes and agglomeration states of the NPs. (A) AgNPs, (B) Ru1-AgNPs, (C) Ru2-AgNPs.

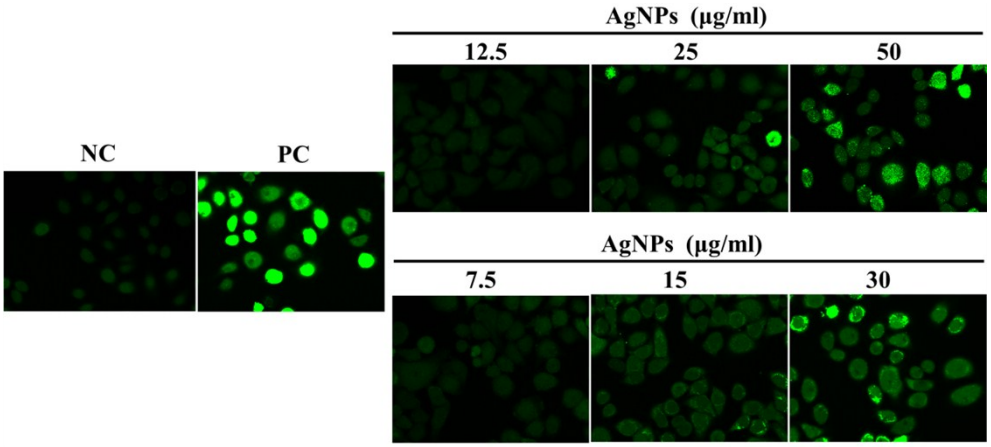


Fig. S14 Analysis of ROS generation by confocal microscopy. Hela cells were incubated with different concentrations of AgNPs for 8 h and stained with DCFH-DA.

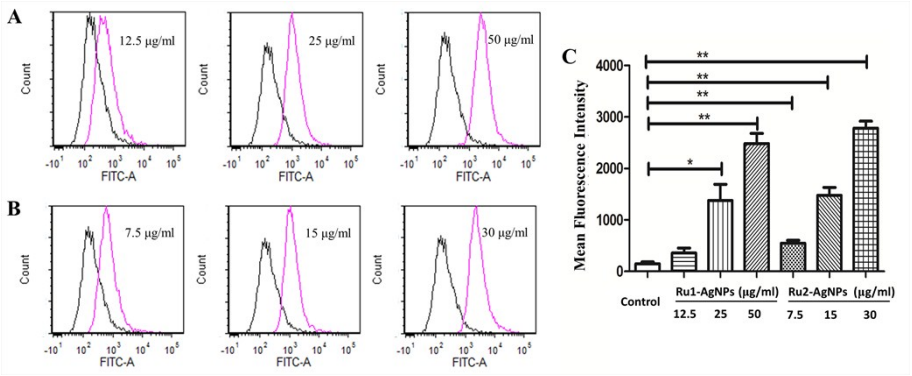


Fig. S15 Analysis of ROS generation by flow cytometry, (A) Ru1-AgNPs; (B) Ru2-AgNPs; (C) Mean fluorescence intensity (MFI) of DCF. Hela cells were incubated with different concentrations of Ru1-2·AgNPs for 8 h and stained with DCFH-DA.

References

1. W. Paw and R. Eisenberg, *Inorg. Chem.,* 1997, **36**, 2287.
2. J. Bolger, A. Gourdon, E. Ishow and J.-P. Launay, *Inorg. Chem.,* 1996, **35**, 2937.
3. B. P. Sullivan, D. J. Salmon and T. J. Meyer, *Inorg. Chem.,* 1978, **17**, 3334.