Supporting information for The ruthenium complex assists in nuclear targeting and selective killing of tumor cells

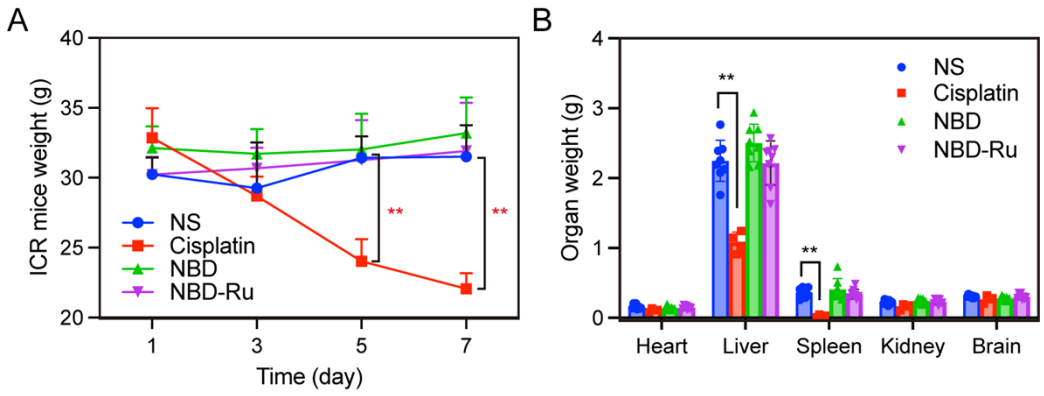
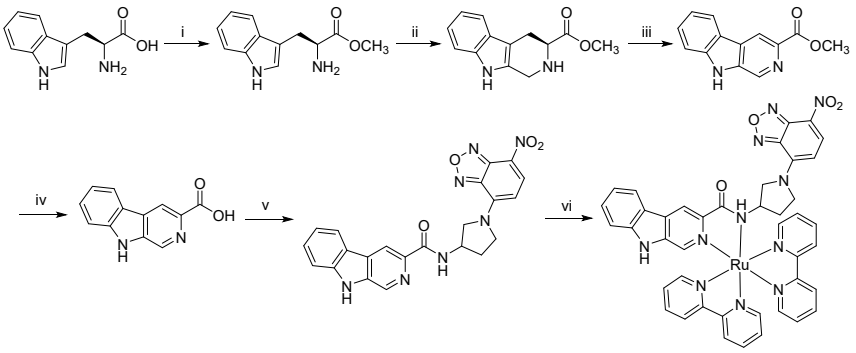


Figure S1. *In vivo* safety evaluation of S180 mouse model. (A) The body weight of ICR mice after treatment for 7 days. (B) Organ weight of ICR mice after treatment with different compounds.



Scheme 1. Synthesis route of **NBD-Ru**. (i) $SOCl_2$, MeOH; (ii) MeOH, H_2SO_4; (iii) $KMnO_4$, acetone/H_2O; (iv) 2N NaOH; (v) 1-(7-nitro-1,3-dihydrobenzo[c][1,2,5]oxadiazol-4-yl)pyrrolidine-3-amine, DCC, HOBt, DMF; (vi) $Ru(bpy)_2Cl_2 \cdot H_2O$, DMF/H_2O.

1. Synthesis of NBD

1.1 Synthesis of *L*-Trp-OCH_3

6 mL of $SOCl_2$ is added dropwise to 80 mL of anhydrous methanol at 0℃. And the reaction mixture is stirred at 0℃ for 0.5 h, and then L-Trp 4.08 g (20 mmol} is added. The reaction mixture is stirred at room temperature for another 24 h, and TLC is used to monitor the reaction. The reaction mixture is concentrated under a vacuum. The resulting crude product is dissolved in 8 ml of methanol and then dried at reduced pressure three times. The residue is again suspended in ether and concentrated under a

vacuum three times. The pure product was obtained by column chromatography. The colorless powder weighed 4.18 g, and the yield was 95.4%.

1.2 Synthesis of methyl (S)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate

The method is similar with that in 1.1, and the difference is that "10.00 g (49 mmol) L-Trp" is replaced with "10.73 g (49 mmol) Trp-OCH_3".^{1}H NMR（300 MHz, d_6-DMSO）, δ = 11.20 ppm (s, 1H), 10.15 ppm (s, 1H), 7.48 ppm (d, 1H, J = 7.8 Hz), 7.38 ppm (d, 1H, J = 8.1 Hz), 7.12 ppm (t, 1H, J = 7.5 Hz), 7.02 ppm (t, 1H, J = 7.5 Hz), 4.62 ppm (dd, 1H, J_1 = 5.1 Hz, J_2 = 9.9 Hz), 4.39 ppm (s, 2H), 3.83 ppm(s, 3H), 3.30 ppm (dd, 1H, J_1 = 14.7 Hz, J_2 = 4.5 Hz), 3.07 ppm (dd, 1H, J_1 = 15.9 Hz, J_2 = 10.2 Hz).

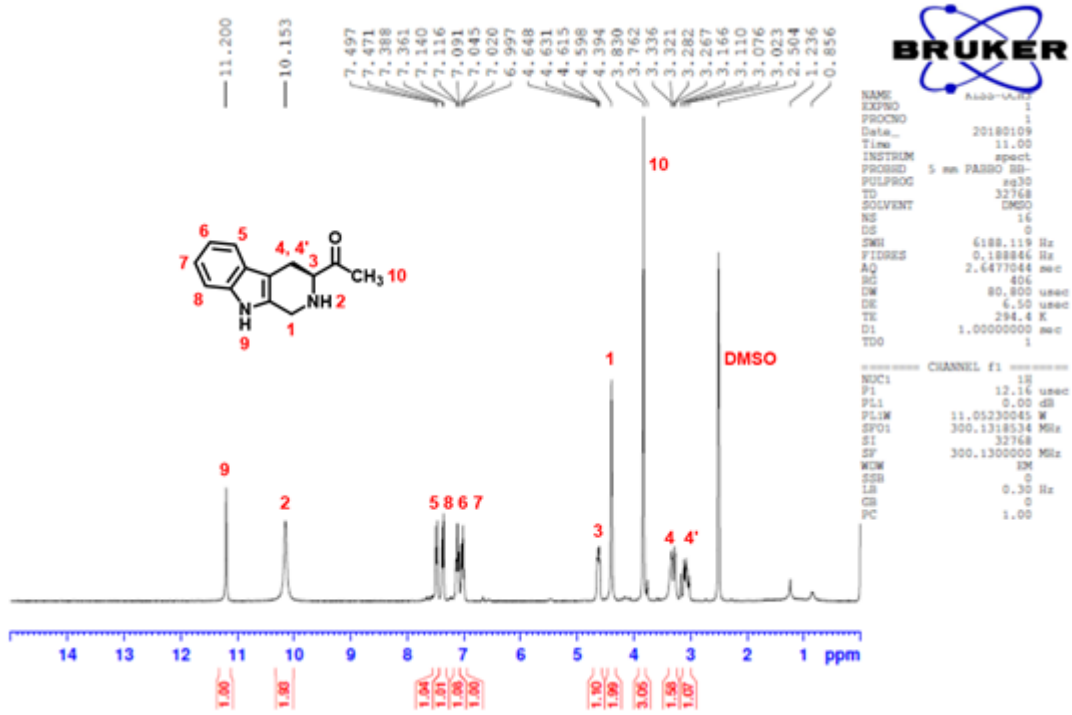


Figure S2. The ^{1}H NMR of **methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate**.

1.3 Synthesis of methyl 9H-pyrido[3,4-b]indole-3-carboxylate

Methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate 460 mg (0.2 mmol) was dissolved in 15 mL of acetone to get solution A. And solution B is made by dissolving $KMnO_4$ 474 mg (0.4 mmol) in 10 mL of water. Then transfer B to A drop by drop at 0 ℃. The reaction mixture is stirred at room temperature overnight, and TLC is used to monitor the reaction. Add ethanol to terminate the reaction. The resulting crude product is filtered to remove MnO_2, and the solvent is removed in a vacuum from the filtrate. The pure product was obtained by column chromatography. The pale-yellow powder weighed 174 mg, and the yield was 38.5%. ESI-MS (m/e): 227.13 $[M + H]^+$. ^{1}H NMR（300 MHz, d_6-DMSO）, δ = 12.09 ppm (s, 1H), 8.97 ppm (s, 1H), 8.94 ppm (s, 1H), 8.41 ppm (d, 1H, J = 7.8 Hz), 7.68 ppm (d,

1H, J = 8.1 Hz), 7.61 ppm (t, 1H, J = 7.5 Hz), 7.32 ppm (t, 1H, J = 7.2 Hz), 3.91 ppm(s, 3H).

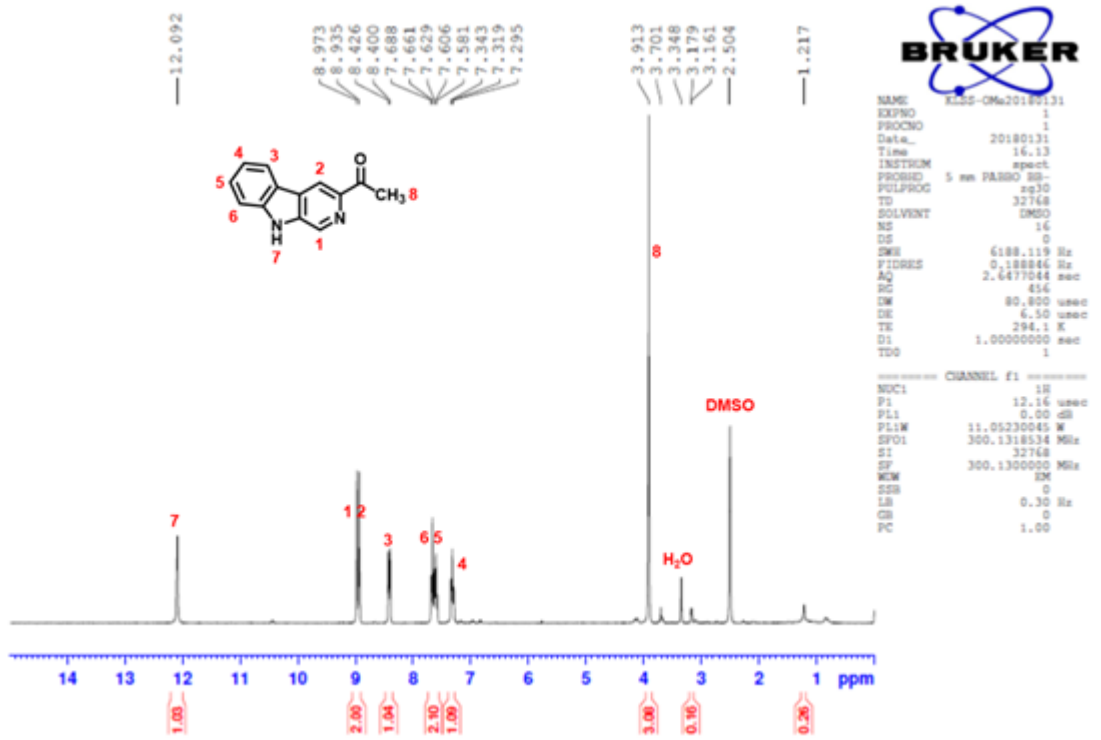


Figure S3. The ^{1}H NMR of **methyl 9H-pyrido[3,4-b]indole-3-carboxylate**.

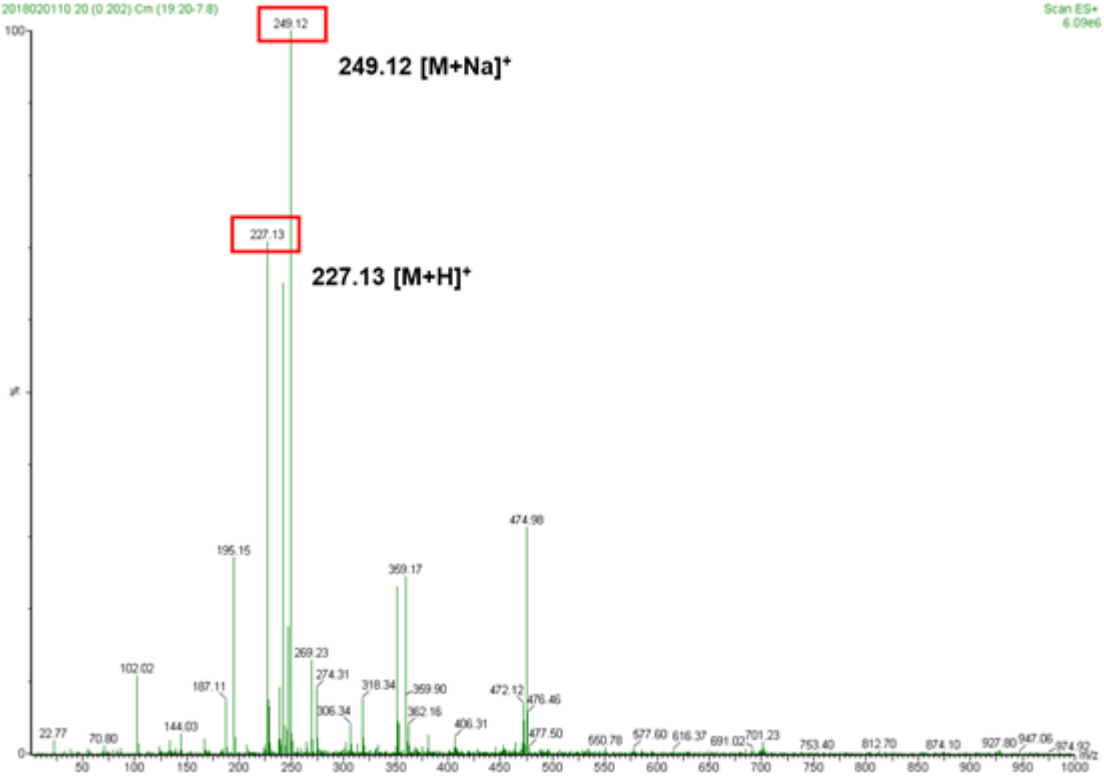


Figure S4. The MS spectrum of **methyl 9H-pyrido[3,4-b]indole-3-carboxylate.**

1.4 Synthesis of 9H-pyrido[3,4-b]indole-3-carboxylic acid

Dissolve methyl 9H-pyrido[3,4-b]indole-3-carboxylate 100 mg (0.044 mmol) in 10 mL of methanol at 0℃. Adjusting the mixture to pH 12 by adding 2N NaOH. The reaction mixture is stirred at 0℃ for 6 h. When the TLC plate indicates a complete disappearance of the raw material (O-KLSS-OMe), quench the reaction by adjusting the reaction to pH 7 with an aqueous $KHSO_4$ solution. After filtration, the filtrate was concentrated under reduced pressure. After a small amount of water is added to the syrup, a brick-red solid is washed out. The title compound was obtained after filtering and drying. The red brick powder weighed 36.6 mg. The filtrate was adjusted to pH 2 by saturated aqueous $KHSO_4$ solution and extracted with ethyl acetate(20 mL*3). The organic phase was washed with saturated aqueous NaCl solution (15mL*3). The organic layer was dried over anhydrous Na_2SO_4 for 2 h. The title compound was obtained after filtration and evaporation under reduced pressure. The colorless powder weighed 16.6 mg. Calculate the gross yield as 56.7%. ESI-MS (m/e): 212.94 $[M + H]^+$. ^1H NMR（300 MHz, d_6-DMSO）, δ = 12.20 ppm (s, 1H), 9.00 ppm (s, 2H), 8.45 ppm (d, 1H, J = 7.8 Hz), 7.71 ppm (d, 1H, J = 8.1 Hz), 7.64 ppm (t, 1H, J = 7.5 Hz), 7.35 ppm (t, 1H, J = 7.2 Hz).

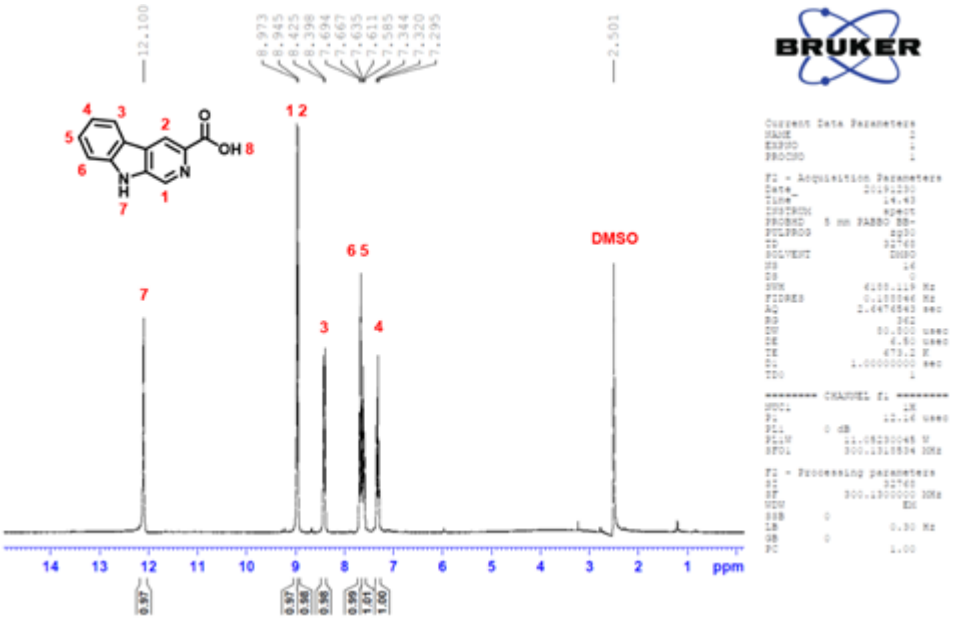


Figure S5. The ^1H NMR of **9H-pyrido[3,4-b]indole-3-carboxylic acid.**

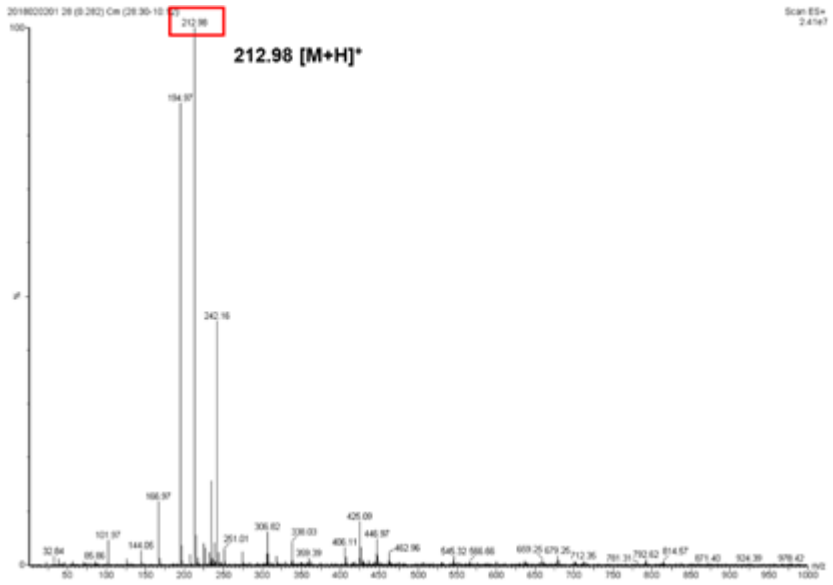


Figure S6. The MS spectrum of **9H-pyrido[3,4-b]indole-3-carboxylic acid**.

1.5 Synthesis of **NBD**

Dissolve 9H-pyrido[3,4-b]indole-3-carboxylic acid 2.12 g (10 mmol), HOBt 1.62 g (12 mmol), and DCC 2.68 g (13 mmol) in 50 mL of anhydrous DMF at 0 °C to get solution A which is stirred for 30 min. And solution B is made by dissolving 1-(7-nitro-1,3-dihydrobenzo[c][1,2,5]oxadiazol-4-yl)pyrrolidine-3-amine 2.74 g (11 mmol) in 10 mL of anhydrous DMF and then adjusting the mixture to pH 7 by adding NMM. Solution A and B are mixed to form solution C, which is stirred vigorously, and the pH of the reaction mixture is kept at 8-9 by adding NMM. The reaction mixture is stirred at room temperature for 6 h, and TLC is used to monitor the reaction. Workup: the resulting crude product is filtered to remove the side product dicyclohexylurea (DCU), and the solvent is removed in a vacuum from the filtrate. The yielded syrup is then dissolved in 150 mL of ethyl acetate to get a solution which is then transferred into a 250 ml separatory funnel and washed successively by saturated aqueous $NaHCO_3$(30 mL*3), saturated aqueous NaCl, 5% aqueous $KHSO_4$ (30 mL*3), 5% aqueous $NaHCO_3$ (30 mL*3) and saturated aqueous NaCl (30 mL*3). The separated ethyl acetate layer is dried by anhydrous Na_2SO_4. After filtration, the filtrate is concentrated at reduced pressure. The pure product was obtained by column chromatography. The orange powder weighed 2.08 g, and the yield was 46.8 %. ESI-MS (m/e): 444.3$[M+H]^+$. ^{1}H NMR (300 MHz, d_6-DMSO), δ（ppm）= 11.93 ppm (s, 1H) , 9.15 ppm (d, 1H, J = 7.17 Hz), 8.87 ppm (s, 2H), 8.50 ppm (d, 1H, J = 8.61 ppm) , 8.40 ppm (d, 1H, J = 7.80 Hz), 7.62 ppm (m, 2H, J = 7.31 Hz), 7.30 ppm (t, 1H, J = 7.04 Hz), 6.33 ppm (d, 1H, J = 8.91 Hz) , 4.88 ppm (s,1H) , 4.42 ppm (m, 2H) , 3.95 ppm (m, 1H), 2.40 ppm (m, 2H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm) = 155.70, 145.44, 145.11,

144.85, 141.52, 149.91, 137.70, 136.81, 132.63, 129.13, 128.65, 122.70, 121.43, 120.50, 120.00, 114.72, 112.73, 102.54, 58.08, 55.91, 49.87, 48.62, 47.98, 33.80, 31.73, 30.30, 24.91; IR: $\nu_{\text{-NH}}$: 3322 cm^{-1}, $\nu_{\text{-NH}}$: 3300~3000 cm^{-1}, $\nu_{\text{-CH2}}$: 2928 cm^{-1}, 2849 cm^{-1}, $\nu_{\text{O=C-NH}}$: 1558 cm^{-1}, $\nu_{\text{C=C}}$: 1615 cm^{-1}, 1496 cm^{-1}, 1459 cm^{-1}, $\nu_{\text{C-OH}}$: 1089 cm^{-1}, $\delta_{\text{=C-H}}$: 732 cm^{-1}; M.p: 216.5 - 217.4 °C.

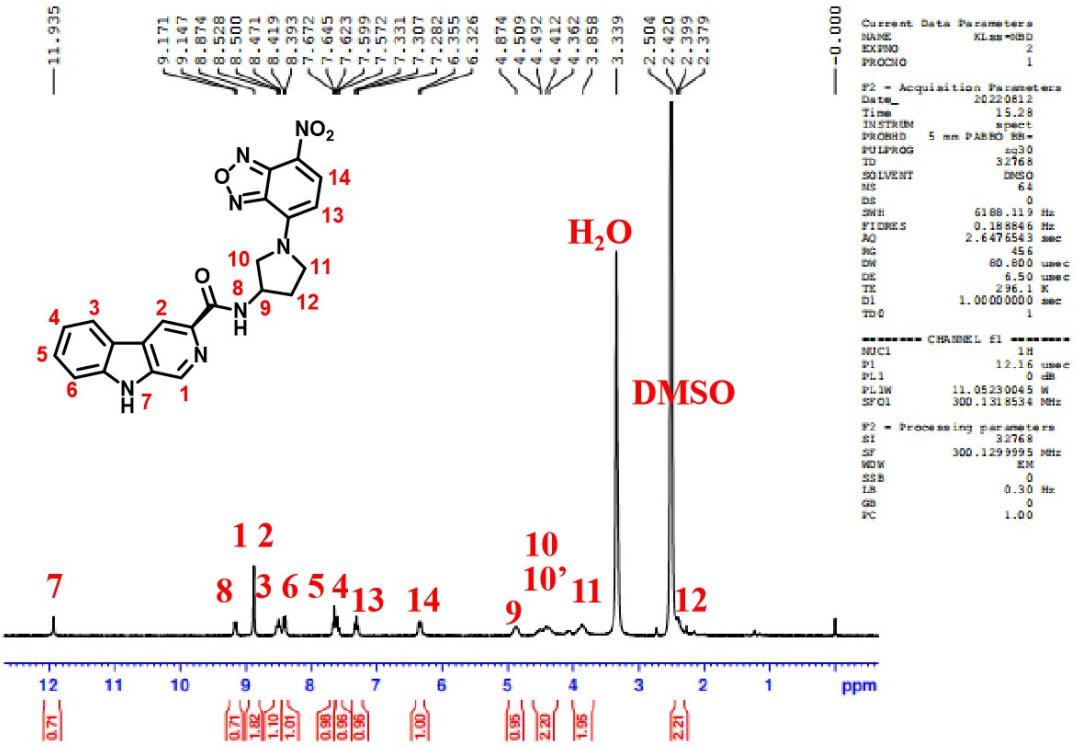
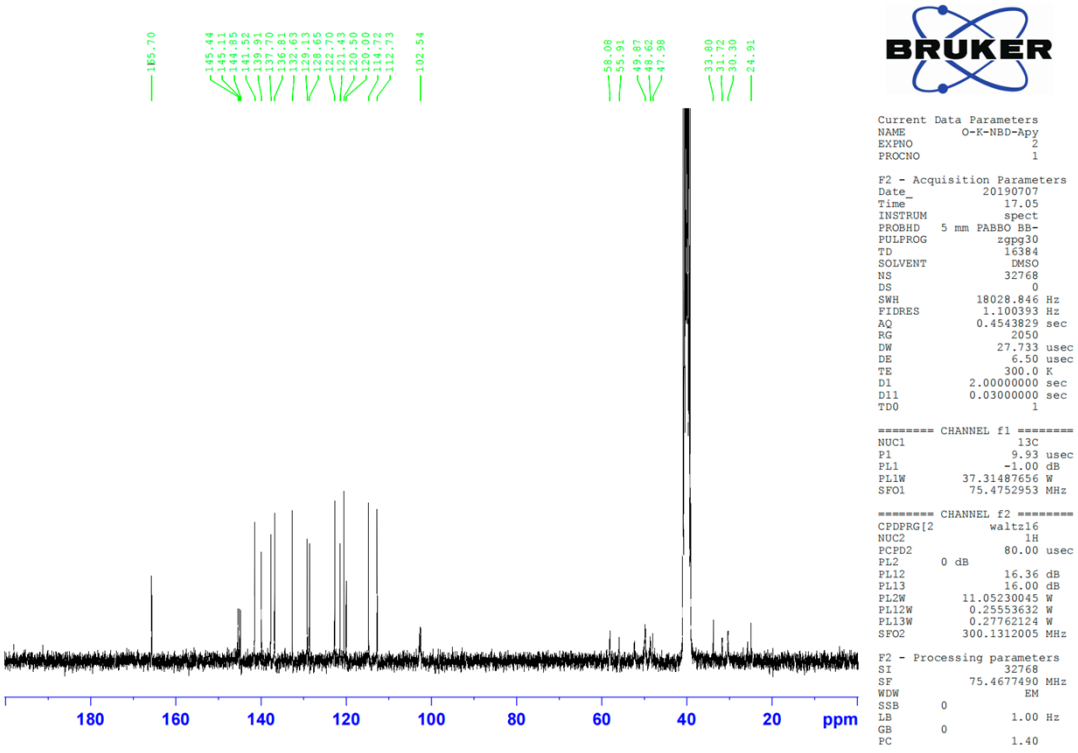


Figure S7. The ^{1}H NMR of **NBD**.



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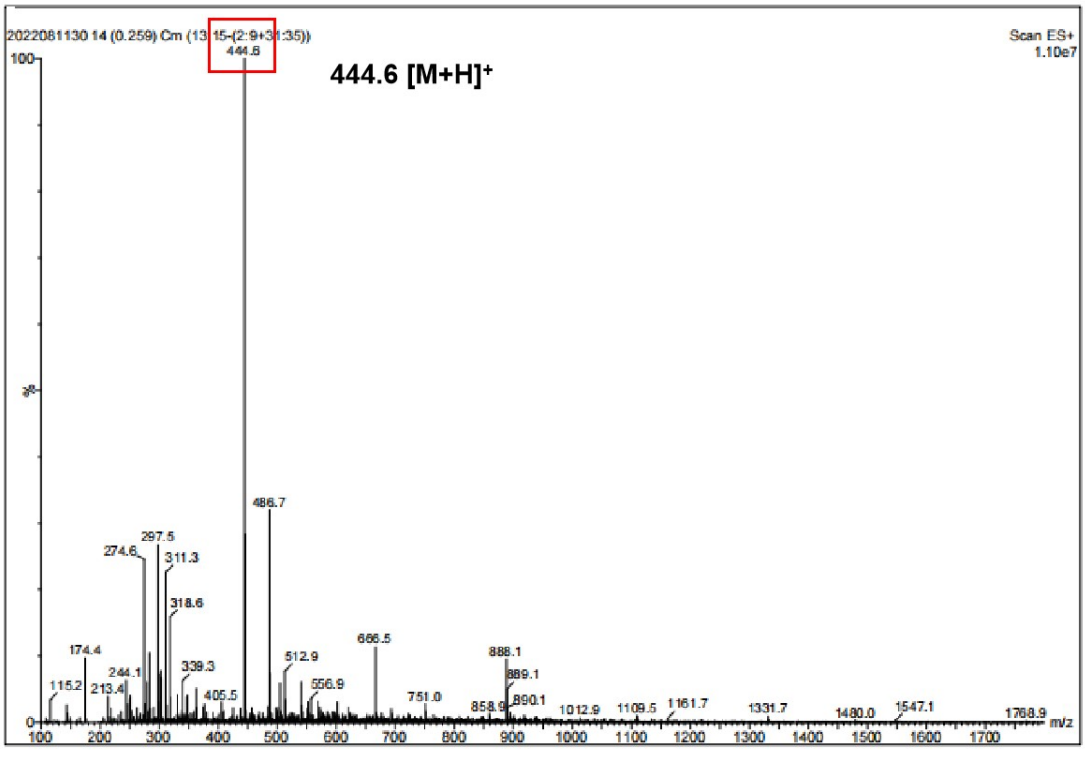
2 Figure S8. The ^{13}C NMR of **NBD**.



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4 Figure S9. The MS spectrum of **NBD**.

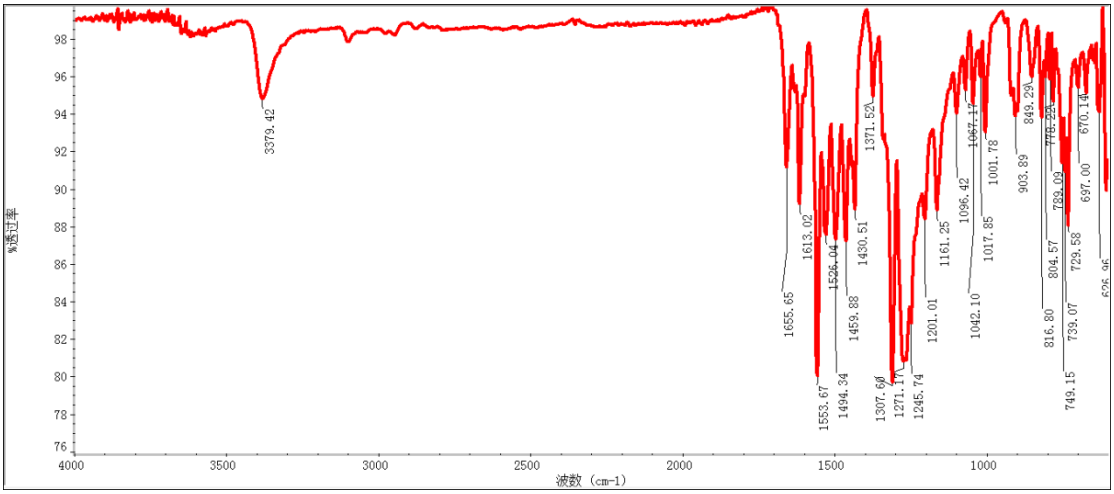
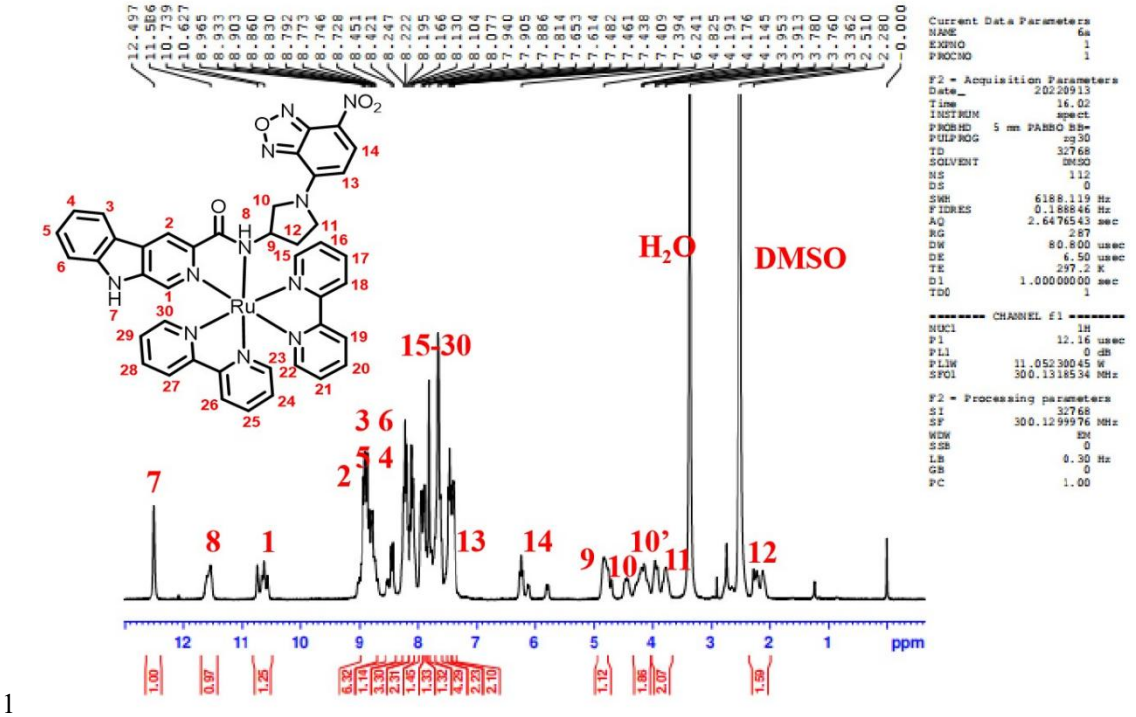


Figure S10. The IR spectrum of **NBD**.

2. Synthesis of NBD-Ru

The synthesis steps of **NBD-Ru** were similar to that of **3a**. The difference was that the synthetic material of NBD-Ru was replaced with 89 mg (0.2 mmol) ligand 6. The reaction solvent was changed to DMF, and the reaction temperature was 80 °C. 22 mg purplish red solid powder (6a). FT-MS (m/z): 856.1677 $[M-H]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.50 (s, 1H), 11.56 (m, 1H), 10.65 (dd, J = 33.5 Hz, 23.5 Hz, 1H), 8.85 (m, 6H), 8.44 (d, J = 8.9 Hz, 1H), 8.21 (m, 3H), 8.10 (t, J = 7.9 Hz, 2H), 7.94 (s, 1H), 7.90 (d, J = 5.6 Hz, 1H), 7.81 (s, 1H), 7.63 (m, 4H), 7.46 (t, J = 6.5 Hz, 2H), 7.40 (m, 2H), 4.83 (m, 1H), 4.17 (m, 2H), 3.86 (m, 2H), 2.20 (m, 2H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.71, 162.76, 160.66, 158.67, 157.11, 153.65, 152.40, 141.52, 139.89, 137.70, 136.81, 134.99, 133.73, 132.64, 129.12, 128.64, 125.75, 125.68, 123.27, 122.92, 122.68, 121.42, 120.49, 119.99, 114.71, 112.74, 47.98, 36.23, 33.80, 31.24, 25.79, 24.90; IR: $\nu_{\text{-NH}}$: 3320 cm^{-1}, $\nu_{\text{-CH2}}$: 2927 cm^{-1}, 2849 cm^{-1}, $\nu_{\text{O=C-NH}}$: 1558 cm^{-1}, $\nu_{\text{C=C}}$: 1623 cm^{-1}, 1496 cm^{-1}, 1460 cm^{-1},$\nu_{\text{C-OH}}$: 1087 cm^{-1}, $\delta_{\text{=C-H}}$: 730 cm^{-1}; M.p.: > 300 °C.



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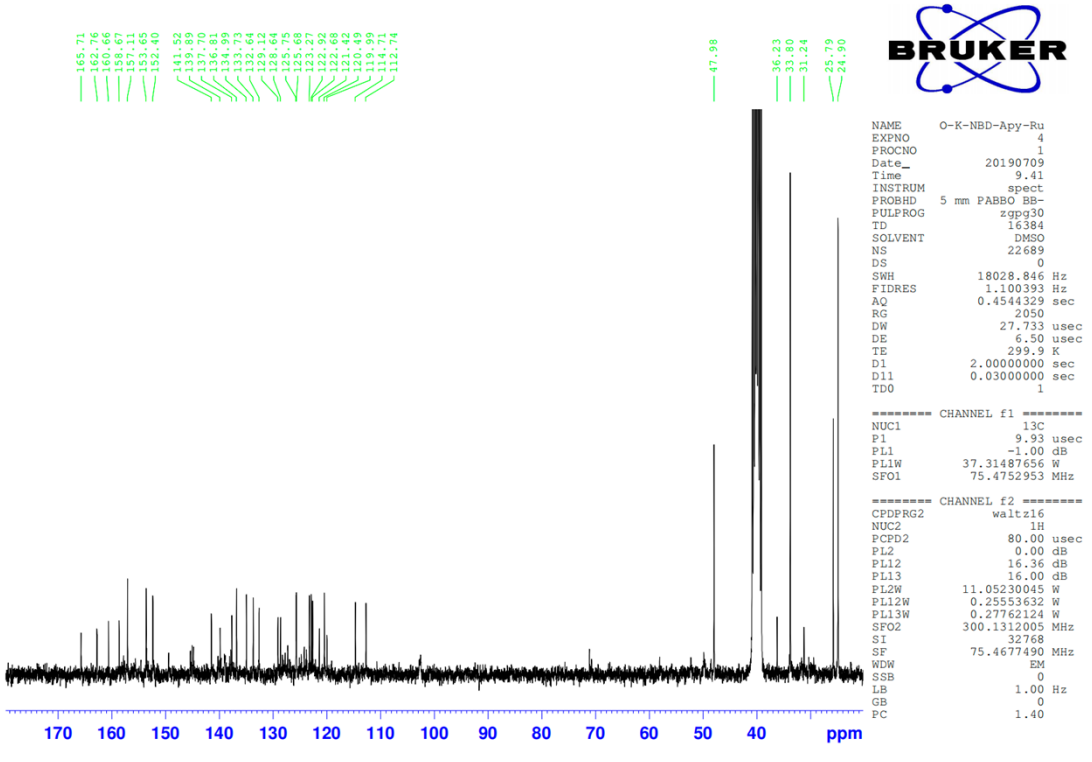
2 Figure S11. The ^{1}H NMR of **NBD-Ru**.



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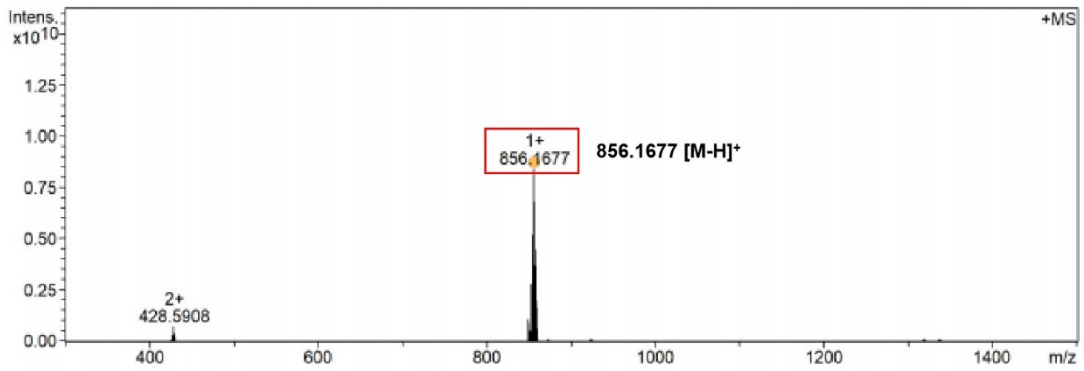
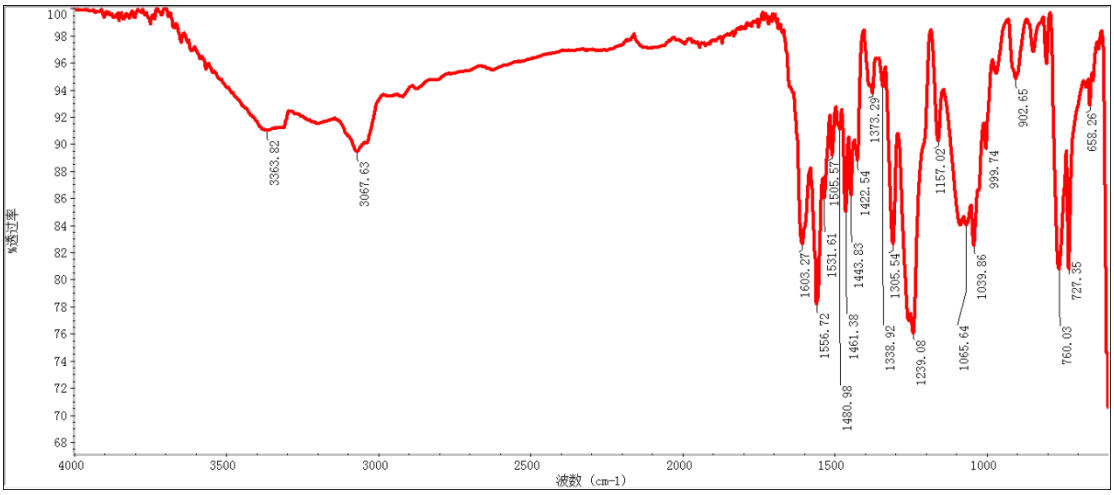
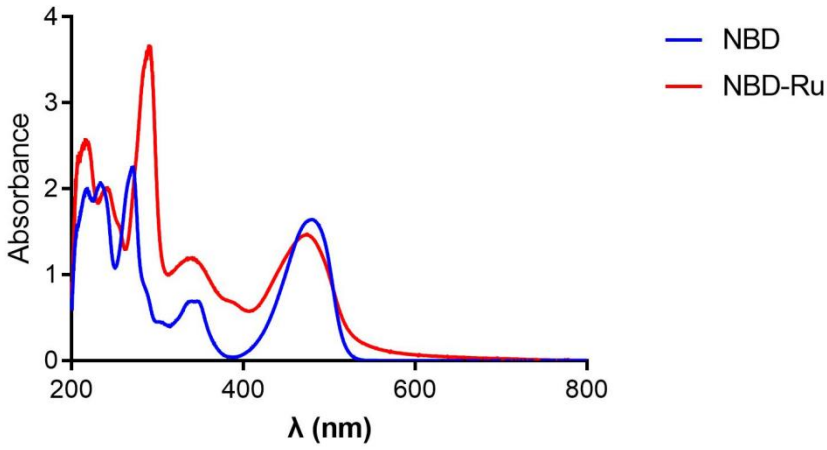
4 Figure S12. The ^{13}C NMR of **NBD-Ru**.

1 

2 Figure S13. The FT-MS spectrum of **NBD-Ru**.

3 

4 Figure S14. The IR spectrum of **NBD-Ru**.

5 

6 Figure S15. The UV spectrum of **NBD** and **NBD-Ru** in MeOH.

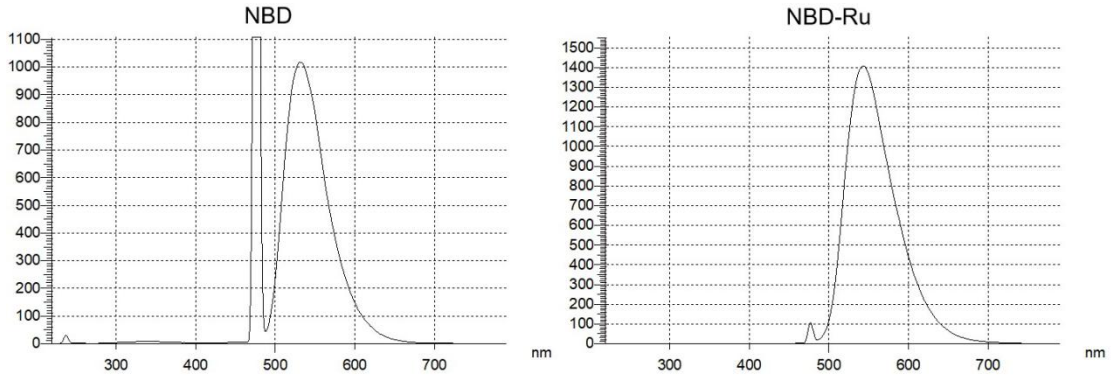


Figure S16. The fluorescence spectra of **NBD** (10^{-4} mg/mL in MeOH) and **NBD-Ru** (0.01 mg/mL in MeOH).

2. Materials

Methanol, Ethanol, CH_2Cl_2, CH_3OH, NaOH, $KHSO_4$, NaCl, Na_2SO_4, DMF, HNO_3, and H_2O_2 (N, N-dimethylformamide) are purchased from Beijing Chemical Reagent Company. *L*-Thr-OBzl (BeijingBoMai*J*ie Technology Co, Ltd), RPMI 1640 medium (Gibco, 31800-022), DMEM medium (Gibco, 12100-046), FBS (Corning, 35-076-CV), Penicillin G Sodium salt (100 U/mL) (Solarbio, P8420), Streptomycin Sulfate (Solarbio, S8290), MTT ((3-(4,5-dimethylthiazol-2-yl)2,5-dipHenyl-tetrazolium bromide) (Aladdin, 298-93-1), 0.25% trypsin (Hyclone, KGY0012), DOX (Sigma-Aldrich, CAS: 23214-92-8), DMSO (Hyclone, 57-68-5), PBS (KeyGEN BioTECH, KGB5001), 4% paraformaldehyde solution (Solarbio, P1110), T25 cell culture flask (Corning, 430639), Human apotransferrin (Sigma-Aldrich, T2252-100MG), calf thymus DNA ctDNA (Sigma-Aldrich, D8899), 200 mesh TEM copper grid (Electron Microscopy China, AZH200), Cell Cycle and Apoptosis Analysis Kit (Byotime Biotechnology, C1052), ALT, AST, Urea, Crea assay kits were purchased from Mindray Bio-Medical Electronics Co., Ltd.

S180 (mouse sarcoma S180 cells), A549 (Human Non-small cell lung cancer cell), LLC (mouse Lewis lung cancer cells), and L02 (normal human embryonic hepatocytes) were purchased from KeyGEN BioTECH. S180 and A549 cancer cells were maintained in RPMI 1640 medium supplemented with 10% FBS, LLC, and L02 cells were cultured in DMEM medium supplemented with 10% FBS.

3. Methods

3.1 MTT

Tumor cells in the logarithmic growth phase were digested with 0.25% trypsin, added to RPMI 1640 medium or DMEM medium supplemented with 10% FBS, plated into 96-well plates (3×10^3 cells/well),

and incubated for 8 hours. The cells were treated with compounds (final concentration: 3.125, 6.25, 12.5, 25, 50, and 100 μM); Cisplatin and NAMI-A were used as standard positive control drugs. Cells in the control wells also acquired the same volume of medium containing 0.1% DMSO. After 48 h, 25 μL of MTT in the PBS (5 mg/mL) was added, and the plates were incubated at 37 °C for 4 hours. The supernatant was discarded, and 150 μL of DMSO was added to each well. The optical density was read with a Spectra Max M3 microplate reader (BioTek, Winooski, VT, USA) at 490 nm and 570 nm. The optical density (OD) value of the compounds-treated well was compared with that of the DMSO-treated well. The proliferation of the cancer cell line was represented by cell viability and IC_{50}. Each measurement was performed in triplicate. Data are presented as mean ± SD.

3.2 Cell cycle

The cells were harvested and centrifuged (5 min at 1000 g) and fixed in 2 mL of 70% aqueous ethanol (v/v). After an incubation of 12 h at 4 °C, cells were centrifuged (10 min at 800 g) and washed twice with ice-cold PBS. Preparation of PI staining solution according to the kit specification. Cells were resuspended with 500μL staining solution containing PI and RNase A. Then the cells were incubated in darkness for 30 minutes and analyzed by EPICS@XL flow cytometry (BECKMAN COULTER, Germany). The number of cells analyzed for each sample was more than 10,000, and the experiments were repeated three times under identical conditions.

3.3 Protocol of ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) combines the high-temperature ionization characteristics of inductively coupled plasma with the low detection limit characteristics of the mass spectrometer to form a solid multi-element simultaneous determination and low detection limit of trace elements analysis technology. Agilent 8800 ICP-MS instrument consists of five parts: a high salt sample injection system, an eight-stage rod collision/reaction cell, a four-stage rod mass filter (Q1, Q2), a plasma radio frequency emitter, electron multiplication detector. It uses a miniature concentric atomizer and maintains a double-pass spray chamber at 2 °C. Readings were up to 1 ng/mL, and the sensitivity limit was 10 pg/mL (95% confidence limit). A range of standard ruthenium solutions (100, 50, 25, 12.5, 6.25, 3.125, and 0 ng/mL) was prepared and placed on the injection rack. Confirm that the washing liquid (ultra-pure water) is sufficient and that the waste liquid barrel has enough space. Open the ventilation system and the high-purity Ar gas cylinder with the pressure indicator at about 0.6. In the computer, we set the theoretical concentration of the standard curve, the internal standard solution Rh^{103}, and the

determination of the element Ru^{101}. The system produces a standard curve based on measured actual and theoretical concentrations. Afterward, we can place all sample solutions on the rack and program them sequentially to determine the ruthenium content.