Electronic Supplementary Information

CO/light dual-activatable Ru(II)-conjugated oligomer agent for lysosome-targeted multimodal cancer therapeutics

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Experimental

Materials. All chemical reagents were commercially obtained from J&K Chemical Ltd. and Aladdin Industrial Corporation and directly used without further purification except special instruction. Organic solvents were dried by standard methods when necessary. CORM-3[$Ru(CO)_3Cl$(glycinate)] and Hemin were purchased from Sigma. LysoTracker® Red DND-99 were obtained from Beyotime. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 and 400 MHz spectrometer. High-resolution mass spectra were measured by a Bruker Microflex LT MALDI TOF. UV-vis absorption spectra were taken on a PerkinElmer Lambda 35 spectrophotometer while fluorescence spectra were recorded on a Hitachi F-7000 spectrophotometer equipped with a xenon lamp excitation source. Confocal laser scanning microscopy of MDA-MB-231 cells were recorded on an Olympus Fluo-view 1200. Two-photon imaging experiments were performed on a Nikon-ARsiMP-LSM-Kit-Legend Elite-USX laser scanning microscope. All solutions were prepared with ultrapure water purified using a Millipore filtration system.

Synthesis of OTE-BN and Ru-OTE.

Compound 1 was synthesized according to the literature. [1]

Synthesis of Compound 2 was synthesized according to the literature. [2] Briefly, N-iodosuccinimide (6.82 g, 30.30 mmol) was added to the solution of 3-(2-(6-bromohexyloxy) ethyl) thiophene (3.68 g, 12.6 mmol) in the mixture of CH_2Cl_2 and CH_3COOH (60 mL, v/v=1/1) at 0 ^oC. Then, the reaction system was warmed to room temperature and stirred for 8 h. After extracted with CH_2Cl_2, the combined organic phase was washed with brine and water followed by dried over $MgSO_4$ and concentrated in vacuum. The residue was purified by silica gel chromatography with CH_2Cl_2/petroleum ether (v/v=1/1) to afford compound **2** (4.9 g, 73%) as

light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.98 (s, 1 H), 3.54 (t, 2 H, *J* = 12 Hz), 3.39-3.45 (m, 4 H), 2.80 (t, 2 H, *J* =13.8 Hz), 1.85 - 1.89 (m, 2 H), 1.56 - 1.62 (m, 2 H), 1.36 - 1.45 (m, 4 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.17, 138.35, 78.01, 75.85, 70.79, 69.73, 33.92, 32.78, 32.47, 29.50, 27.98, 25.41. HRMS (ESI): m/z: 564.8166 ($[M+Na]^+$).

Synthesis of Compound 3. Compound **2** (1.08 g, 2 mmol) and 4-hydroxybenzonitrile (476 mg, 4 mmol) were dissolved in acetone (30 mL). Then, K_2CO_3 (1.1 g, 8 mmol) and 18-Crown-6 (0.26 g, 1 mmol) was added to the flask. The mixture was stirred at 80 ^{o}C for 8 h. After removing the acetone under reduced pressure, the residue was extracted with CH_2Cl_2, the combined organic layer was washed with brine and water followed by dried over $MgSO_4$ and concentrated in vacuum. The residue was was purified by silica gel chromatography with CH_2Cl_2/petroleum ether (v/v=1/1) to give compound **3** (1.1 g, 94%) as yellow viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (t, 1 H *J* = 4.0 Hz), 7.74 (t, 1 H, *J* = 4.0 Hz), 7.11 (s, 1 H), 7.10 (t, 1 H, *J* =8 Hz), 7.08 (t, 1 H, *J* = 4 Hz), 4.04 (t, 2 H, *J* = 12 Hz), 3.49 (t, 2 H, *J* = 12 Hz), 3.37 (t, 2 H, *J* = 12 Hz), 2.70 (t, 2 H, *J* = 12 Hz), 1.68 - 1.75 (m, 2 H), 1.46 - 1.53 (m, 2 H), 1.32 - 1.38 (m, 4 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.44, 146.14, 138.34, 133.96, 119.31, 115.22, 103.69, 78.05, 75.89, 70.84, 69.72, 68.32, 32.47, 29.60, 29.00, 25.97, 25.80. HRMS (ESI): m/z: 603.9275 ($[M+Na]^+$).

Compound 4 was synthesized according to the literature.[3]

Synthesis of Compound 5. 2-Bromo-4-methoxythiophene (2.8 g, 14.5 mmol), 2-bromo-1-propanol (2.7 mL, 29.70 mmol) and $NaHSO_4$ (696 mg, 5.8 mmol) was dissolved in toluene (50 mL). The reaction system was stirred at 100 ^{o}C for 12 h. After cooling down to room temperature, filtration and evaporating toluene, the product was purified by silica gel

chromatography with CH_2Cl_2/hexanes (v/v=1:5) to afford compound **5** as colorless oil (2.3 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.73 (d, 1 H, *J* = 3.0 Hz), 6.16 (d, 1 H, *J* = 3.0 Hz), 4.05 (t, 2 H, *J* = 12.0 Hz), 3.56 (t, 2 H, *J* = 12.0 Hz), 2.26 - 2.30 (m, 2 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.25, 122.50, 111.86, 99.17, 67.26, 32.14, 29.73. HRMS (APCI): 300.8717 m/z: ($[M+H]^+$).

Synthesis of Compound 6. Dimethylamine solution (117 mL, 2 M in THF) was added dropwise to a solution of Compound **5** (1.18 g, 3.9 mmol) in THF (40 mL) at room temperature. After stirring for 12 h, additional dimethylamine solution (70 mL) was added, and the mixture was stirred at room temperature for 12 h. The solvent was then removed under reduced pressure, the solution was extracted with CH_2Cl_2 and washed with saturated NaCl solution. The organic layer was dried over anhydrous $MgSO_4$ for 30 min, and filtered to remove the $MgSO_4$. The solvent was removed and the residual solid was purified by silica gel chromatography with CH_2Cl_2/methanol (v/v=10/1, 0.2% triethylamine) to give compound **6** (1.12 g, 92%) as yellow-brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.73 (d, 1 H, *J* =1.8 Hz), 6.13 (d, 1 H, *J* = 3.0 Hz), 3.95 (t, 2 H, *J* = 12.0 Hz), 2.43 (t, 2 H, *J* =12.0 Hz), 2.25 (s, 6 H), 1.90 - 1.97 (m, 2 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.59, 122.58, 111.60, 98.84, 68.09, 56.22, 45.35, 27.21. HRMS (ESI): m/z: 264.0054 ($[M+H]^+$).

Synthesis of Compound 7. Compound **6** (1.12 g, 4.24 mmol) and triethylamine (40 mL) was dissolved in $CHCl_3$ (20 mL) and the system was degassed with nitrogen gas for 30 min. Then, TMSA (1.19 g, 8.48 mmol), $Pd(PPh_3)_2Cl_2$ (148 mg, 0.42 mmol) and CuI (80 mg, 0.84 mmol) were added to the mixture, which was subsequently heated to 100 ^{o}C and stirred overnight. After cooling down to the room temperature, the solution was extracted with CH_2Cl_2 and washed with saturated NaCl solution. Then, the combined organic layer was dried over

anhydrous $MgSO_4$ for 30 min, and filtered to remove the $MgSO_4$. The solvent was removed and the residual solid was purified by silica gel chromatography with CH_2Cl_2/methanol (v/v=10/1, 0.2% triethylamine) to give compound **7** (950 mg, 79%) as yellow-brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.81 (d, 1 H, *J* =1.8 Hz), 6.21 (d, 1 H, *J* = 1.8 Hz), 4.05 (t, 2 H, *J* = 12.0 Hz), 3.08 - 3.16 (m, 2 H), 2.79 (s, 6 H), 2.30 - 2.37 (m, 2 H), 0.23 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.57, 124.37, 121.99, 100.27, 98.86, 97.95, 68.64, 56.40, 45.59, 27.53. HRMS (ESI): m/z: 182.1343 ($[M+H]^+$).

Synthesis of Compound 8. Compound **7** (947 mg, 3.37 mmol) was dissolved in THF and CH_3OH (60 ml, v/v=1/2) and degassed nitrogen gas for 30 min. Then, K_2CO_3 (697 mg, 5 mmol) was added to the reaction system and stirred for 3 h under nitrogen atmosphere. Removing THF and CH_3OH and the solution was extracted with CH_2Cl_2 and washed with NH_4Cl solution, water, and saturated NaCl, respectively. The organic layer was dried over anhydrous $MgSO_4$ for 30 min, and filtered to remove the $MgSO_4$. After removing the solvent, the residual solid was purified by silica gel chromatography with CH_2Cl_2/methanol (v/v=10/1, 0.2% triethylamine) to give compound **8** (594 mg, 85%) as yellow-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.91 (s, 1 H), 6.21 (s, 1 H), 3.99 (t, 2 H, *J* = 12.0 Hz), 3.30 (s, 1 H), 2.45 (t, 2 H, *J* = 12.0 Hz), 2.26 (s, 6 H), 1.92 - 1.97 (m, 2 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.43, 124.77, 120.65, 100.12, 81.18, 68.50, 56.23, 45.46, 27.40.

Synthesis of Compound 9. Under nitrogen atmosphere, compound **3** (510 mg, 0.85 mmol) and compound **8** (551 mg, 2.63 mmol) were dissolved in degassed diethylamine and $CHCl_3$ (24 mL, v/v=1/2). Then, $Pd(PPh_3)_2Cl_2$ (61.5 mg, 0.08 mmol) and CuI (34 mg, 0.17 mmol) were added and the resulting mixture was stirred at 35 ^{o}C for 2 h under nitrogen atmosphere. The solution was extracted with $CHCl_3$ and the collected organic phase was washed with brine and water,

dried over $MgSO_4$ and concentrated in vacuum. The residue was purified by silica gel chromatography with CH_2Cl_2/methanol (v/v=10/1, with 0.4% triethylamine) to give compound **9** (328 mg, 52%) as yellow viscous liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.73 (d, 2H, *J* = 5.2 Hz,), 7.38 (s, 1 H), 7.13 (d, 1 H, *J* = 1.6 Hz), 7.12 (d, 1 H, *J* = 1.6 Hz), 7.06 (d, 2 H, *J* = 5.2 Hz), 6.78 (d, 1 H, *J* = 1.6 Hz), 6.77 (d, 1 H, *J* = 1.6 Hz), 3.97 - 4.02 (m, 6 H), 3.61 (t, 2 H, *J* = 4.0 Hz), 3.38 -3.41 (m, 2 H), 2.87 (t, 2 H, *J* = 4.0 Hz), 2.34 - 2.39 (m, 4 H), 2.17 (s, 6 H), 2.16 (s, 6 H), 1.81 - 1.85 (m, 4 H), 1.65 - 1.70 (m, 2 H), 1.47 - 1.51 (m, 2 H), 1.28 - 1.39 (m, 4 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.44, 156.74, 144.43, 133.91, 133.54, 123.92, 123.83, 123.02, 121.24, 121.11, 120.62, 119.33, 103.58, 100.77, 100.69, 89.97, 87.61, 86.08, 85.18, 70.73, 69.88, 68.49, 68.30, 56.21, 45.38, 30.08, 29.57, 28.98, 27.30, 25.96, 25.75. HRMS (ESI): m/z: 744.2957 ($[M+H]^+$).

Synthesis of OTE-BN. Compound **9** (39 mg, 0.051 mmol) was dissolved in $CHCl_3$ (5 mL) and the reaction mixture was stirred vigorously to form a homogeneous solution. Then, CH_3I (222 mg, 1.57 mmol) was added and the mixture was stirred at room temperature for 48 h. The solid was collected by filtration, washed with $CHCl_3$ for three times and dried under vacuum to give OTE-BN (51 mg, 98%) as yellow viscous solid. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.62 (d, 2H, *J* = 8.8 Hz,), 7.20 (s, 1 H), 7.02 (s, 2H), 6.99 (d, 2 H, *J* = 9.2 Hz), 6.64 (s, 2H), 4.15 (t, 4 H, *J* = 5.6 Hz), 4.01 (t, 2 H, *J* = 6.4 Hz), 3.70 - 3.63 (m, 6 H), 3.49 (t, 2 H, *J* = 6.4 Hz), 3.25 (s, 18 H), 2.95 (t, 2 H, *J* = 6.4 Hz), 2.36 - 2.29 (m, 4 H), 1.80-1.73 (m, 2 H), 1.61-1.55 (m, 2 H), 1.48 - 1.35 (m, 4 H). ^{13}C NMR (101 MHz, CD_3OD) δ 162.74, 156.13, 156.12, 145.05, 133.78, 133.65, 123.66, 123.54, 122.63, 121.00, 120.89, 120.31, 119.01, 115.18, 102.83, 101.85, 89.59, 87.09, 85.35, 84.52, 70.29, 69.45, 68.19, 66.63, 64.01, 63.98, 63.94, 52.60, 52.57, 52.53, 42.60, 29.64, 29.19, 28.78, 25.68, 25.36, 22.98. HRMS (ESI): m/z: 386.6670 ($[M-2I]^{2+}$).

Synthesis of Ru-OTE-Pre. Ru-OTE-Pre was synthesized according to the literature from Ru-H_2O.[2] Compound Ru-H_2O (155 mg, 0.16 mmol) and compound **9** (270 mg, 0.36 mmol) were dissolved in acetone (10 mL) and the solution was stirred overnight in the dark under nitrogen atmosphere. After removing the solvent, the obtained crude product was purified by silica gel chromatography with CH_2Cl_2/methanol (v/v=50/1) to yield the product as a blue back solid (40 mg, 11%). ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ: 9.10 - 8.97 (m, 6 H), 8.75 - 8.59 (m, 6 H), 8.07 - 7.96 (m, 6 H), 7.54 - 7.43 (m, 6 H), 7.27 - 7.21 (m, 4 H), 7.01 - 6.98 (m, 4 H), 6.90 - 6.87 (m, 4 H), 6.67-6.58 (m, 6 H), 4.12 - 4.08 (m, 8 H), 3.87 (t, 4 H, J = 6.3 Hz), 3.66 (t, 4 H, J = 6.3 Hz), 3.43 (m, 4 H, J = 6.3 Hz), 3.02 - 2.92 (m, 12 H), 2.67 (s, 24 H), 2.13 - 2.09 (m, 8 H), 1.67 (b, 4 H), 1.50 (b, 4 H), 1.36 (b, 8 H). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ 177.46, 177.40, 165.14, 165.11, 165.09, 165.07, 161.44, 161.32, 160.06, 159.66, 158.53, 156.64, 156.63, 152.19, 151.08, 150.25, 149.99, 145.64, 136.99, 136.75, 136.02, 135.17, 134.20, 132.68, 131.01, 130.82, 130.34, 129.90, 129.02, 128.81, 128.51, 128.39, 128.35, 128.01, 127.99, 127.88, 127.60, 127.58, 127.41, 127.28, 126.96, 126.86, 126.54, 125.24, 124.23, 124.14, 122.43, 122.38, 121.30, 120.66, 120.51, 120.27, 120.16, 120.00, 113.91, 101.92, 101.83, 89.80, 87.35, 85.45, 84.64, 70.27, 69.40, 67.98, 67.97, 67.68, 55.96, 43.93, 26.04, 25.80, 25.59, 22.42, 13.46.

Synthesis of Ru-OTE. Ru-OTE-Pre (28 mg, 0.012 mmol) was dissolved in $CHCl_3$ (1 mL) and CH_3OH (1 mL), the reaction mixture was stirred vigorously to form a homogeneous solution. Then, CH_3I (100 mg, 0.70 mmol) was added and the mixture was stirred at room temperature for 48 h. The solid was collected by filtration, washed with $CHCl_3$ for three times and dried under vacuum to give Ru-OTE (34 mg, 98%) as blue back solid. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ: 9.16 - 9.03 (m, 6 H), 8.77 - 8.63 (m, 6 H), 8.08 - 7.99 (m, 6 H), 7.55 - 7.42 (m, 6 H), 7.30 - 7.23 (m, 4 H), 7.04 - 7.01 (m, 4 H), 6.92-6.89 (m, 4H), 6.72 - 6.61 (m, 6 H), 4.22 -

4.19 (m, 8 H), 3.91 - 3.78 (m, 12 H), 3.67 (t, 4 H, J = 6.3 Hz), 3.41 (s, 36 H), 2.97 - 2.94 (m, 8 H), 2.48-2.39 (m, 8 H), 1.66-1.70 (m, 4H), 1.52-1.50 (m, 4 H), 1.37 (b, 8 H). ^{13}C NMR (151 MHz, $(CD_3)_2CO$) δ 184.36, 177.46, 161.45, 161.33, 160.77, 160.46, 160.05, 159.67, 158.54, 156.64, 152.19, 152.18, 151.09, 150.25, 149.99, 145.64, 137.00, 136.75, 136.14, 136.02, 135.45, 135.17, 134.20, 132.74, 131.02, 130.82, 130.34, 129.91, 129.03, 128.82, 128.52, 128.40, 128.35, 128.01, 127.88, 127.61, 127.59, 127.42, 127.29, 126.97, 126.87, 126.55, 125.25, 124.23, 124.14, 122.42, 122.38, 121.30, 120.28, 120.17, 120.16, 120.03, 120.01, 113.91, 101.90, 101.81, 92.91, 89.92, 89.81, 89.19, 87.36, 85.45, 84.64, 70.27, 69.40, 67.99, 67.68, 56.02, 56.00, 44.00, 26.11, 25.80, 25.66, 25.59, 14.30, 13.46. HRMS (ESI): m/z: 360.3232 ($[M-2PF_6-4I]^{6+}$).

Experimental Procedures.

Response of Ru-OTE to Light. 1 μL Ru-OTE (10^{-3} M) was added to 1.0 mL PBS buffer (10 mM, pH 7.4). The solution was irradiated by white light (25 mW/cm^2). The fluorescence intensity of the solution at 438 nm was recorded every 3 min with an excitation wavelength of 371 nm. All the fluorescence spectra were measured at 37 ^{o}C.

Response of Ru-OTE to CO. 1 μL Ru-OTE (10^{-3} M) was added to 1.0 mL PBS buffer (10 mM, pH 7.4). After incubation for 10 min, CORM-3 (from 0 to 10 μM) was added, respectively. Upon incubation for another 15 min, the fluorescence spectra were measured at 37 ^{o}C with the excitation wavelength of 371 nm.

Synergistic Response of Ru-OTE to CO/light. 1.0 μL Ru-OTE (10^{-3} M) was added to 1.0 mL PBS buffer (10 mM, pH 7.4), then 10 μM CORM-3 was added. After incubation for 15 min,

the solution was irradiated with white light (25 mW/cm^2) for 21 min. The fluorescence intensity of the solution at 438 nm was recorded every 3 min with the excitation wavelength of 371 nm.

Reaction Time of Ru-OTE to CO. 1 μL Ru-OTE (10^{-3} M) was added to 1.0 mL PBS buffer (10 mM, pH 7.4). After incubation for 10 min, 10 μM CORM-3 [4] (a CO releasing molecule, 1.0 mol CO can be liberated per mole CORM-3) was added. After incubation for 0, 3, 6, 9, 12, 15, 18 min, respectively, the fluorescence intensity of every solution at 438 nm was measured at 37 ^{o}C with the excitation wavelength of 371 nm.

Selectivity Assay of Ru-OTE. To investigate the selectivity of Ru-OTE to CO, H_2O_2 (hydrogen peroxide), H_2S (hydrogen sulfide), NO (nitric oxide), Cys (cysteine), GSH (glutathione), $ONOO^-$ (peroxynitrite), HCO_3^- (bicarbonates), NaCit (sodium citrate), AA (ascorbic acid) and imidazole were used in the selectivity assay. The fluorescence spectra were recorded at the same condition as above. The concentration of H_2O_2 stock solution was determined by its UV-vis absorbance at 240 nm. HCO_3^- and H_2S stock solution were prepared by directly dissolving $NaHCO_3$ and Na_2S solids into water. NO stock solution was prepared by dissolving nitric oxide (NO) donor (diethylamine nonoate, DEA NONOate) into water. $ONOO^-$ solution was prepared according to the literature. [5] Imidazole solution was prepared by diluting imidazole with H_2O.

$^1O_2$ Quantum Yield Measurements. A commercial photosensitizer (rose bengal, RB) was used as a standard reference. 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) was employed as $^1O_2$-trapping agent. ABDA solution (10 μL,10 mM) was added into 2 mL of OTE-BN and RB solution, which was irradiated by white light with a power density of 5 mW/cm^2. The absorption maxima of RB and OTE-BN was adjusted to ~0.2 OD. The absorbance of

ABDA at 378 nm was recorded at various irradiation times to obtain the decay rate of the photosensitizing process. The $^1O_2$ quantum yield of the OTE-BN in water was calculated using the following equation:

$$\Phi_{OTE\text{-}BN} = \Phi_{RB} \times K_{OTE\text{-}BN} \times A_{RB}/(K_{RB} \times A_{OTE\text{-}BN})$$

where $K_{OTE\text{-}BN}$ and K_{RB} are the decomposition rate constants of ABDA by OTE-BN and RB, respectively, which are determined by the plot $\ln(Abs_0/Abs)$ versus irradiation time. Abs_0 is the initial absorbance of ABDA and Abs is the ABDA absorbance at different irradiation times. $A_{OTE\text{-}BN}$ and A_{RB} represent the light absorbed by OTE-BN and RB, respectively, which are determined by integration of the optical absorption bands in the wavelength range of 300-700 nm. Φ_{RB} is the $^1O_2$ quantum yield of RB, and $\Phi_{RB} = 0.75$ in water. As shown in the **Fig. S3**, the $K_{OTE\text{-}BN}$ values are 0.098, whereas K_{RB} is 0.056. The A values of OTE-BN and RB are calculated to be 9.87 and 7.94, respectively. Thus, the $^1O_2$ quantum yields of OTE-BN in water was calculated to be 1.05 using the above equation.[6]

Cell Experiments. Human breast cancer cells MDA-MB-231 were cultured in DMEM medium (Gibco) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 ^oC in a humidified incubator containing 5% CO_2.

Confocal Laser Scanning Microscopy. MDA-MB-231 cells were seeded in a 20 mm μ-dishes and cultured for 24 h. For endogenous CO detection, the medium was removed and washed twice with sterile PBS (10 mM, pH 7.4) buffer, then fresh medium containing hemin (100 μM) was added followed by incubation for 2 h, 4 h, 6 h, 8 h, respectively. Then, the cells were incubating with Ru-OTE (4.0 μM) for 6 h followed by washed three times with PBS buffer to remove residual complexes, and 1 mL fresh serum-free DMEM medium was added for CLSM imaging.

The procedure for light irradiation imaging experiments was the same as above, except that after incubating the cells with Ru-OTE (4.0 μM) for different times (2 h, 4 h, 6 h, 8 h), cells were irradiated with white light (25 mW/cm^2) for 30 min, then 1 mL fresh serum-free DMEM medium was added immediately for CLSM imaging.

For synergy experiments, the experimental procedure was the same as before, except that after the cells being stimulated with hemin for 6 h, cells were irradiated with white light. Then images were obtained immediately.

The cell imaging experiments of Ru-H_2O and OTE-BN were carried out as the same procedure.

Cell Staining Experiments. The experimental procedure was the same as above, except that after the cells being incubated with Ru-OTE and exposed to light, the prepared LysoTracker® Red DND-99 or acridine orange (AO) dye was added after removing the supernatant, and cells were stained for 30 min for CLSM imaging.

Two-Photon NIR Laser Activable PDT. For two photon laser imaging, 2 × 10^5 MDA-MB-231 cells were seeded in confocal dishes and cultured overnight. Then, the cells were washed with PBS for three times flowed by incubated with Ru-OTE (4.0 μM) for 6 h. Two-photon NIR laser activable PDT experiments were performed on a Nikon-ARsiMP-LSM-Kit-Legend Elite-USX laser scanning microscope. Then Ru-OTE treated cells were irradiated by 800 nm two-photon laser (~100 mW) for different time (0 and 120 s, respectively) within a selected area (20× objective lens).

For NIR PDT, 5 × 10^5 MDA-MB-231 cells were seeded in confocal dishes and cultured overnight. Then, the cells were washed with PBS for three times flowed by incubated with Ru-

OTE (4.0 μM) for 6 h. Two-photon NIR laser activable PDT experiments were performed on a Nikon-ARsiMP-LSM-Kit-Legend Elite-USX laser scanning microscope. Then Ru-OTE treated cells were irradiated by 800 nm two-photon laser (~100 mW) for different time (0-120 s) within a selected 600 μm × 600 μm area (10× objective lens). Live/dead cell staining experiments were conducted after another 24 h incubation.

Cytotoxicity by MTT Assay. The dark cytotoxicity and phototoxicity of Ru-H_2O, OTE-BN and Ru-OTE against MDA-MB-231 cells was evaluated by MTT assay. The MTT assay experiments were performed according to the literature.[7] Briefly, for the dark cytotoxicity test, MDA-MB-231 cells were seeded in a 96-well plate at a density of 5000-7000 cells/well. After incubating for 24 h, the medium was replaced with 100 μL fresh medium containing different concentrations of Ru-OTE, then cells were incubated for another 24 h. 10 μL MTT (5mg/mL) was added to each well followed by incubated for 4 h. The supernatant was removed again and 100 μL DMSO was added to dissolve the produced formazan. After shaking the plate for 10 min, the absorbance of each well at 490/570 nm were measured. The cell viability rate (VR) was calculated according to the following equation:

$$VR = A/A_0 \times 100\%$$

where A was the absorbance of the experimental group and A_0 was the absorbance of the control group, where control groups were carried out without Ru-OTE.

For CO stimulation test, the experimental procedure is the same as above, except that the cells were stimulated with hemin for 6 h first, then incubated for 24 h.

For the phototoxicity experiment, the experimental procedure is the same as above, except that the cells were incubated with Ru-OTE for 6 h and irradiated with white light for 30 min (25 mW/cm^2), and then incubated for 24 h, followed by measuring cell viability.

For synergy experiments, the cell culture method was the same as above, except that the cells

were stimulated with hemin for 6 h firstly and incubated with Ru-OTE, then light irradiation was applied, followed by incubation for 24 h. The cell viability was measured.

The cytotoxicity measurements of Ru-H_2O and OTE-BN were carried out as the same procedure.

Animals and tumor model. In Vivo Mouse Tumor Model. All the animal experiments were conducted in accordance with the protocols approved by the local ethical committee and laboratory animal administration rules of China. BALB/c-nu mice (female, 20-22 g) were commercially purchased from Spaefer Laboratory Animal Technology Co., Ltd (Beijing, China). After adaptive feeding for 7 days, the mouse tumor model was established by injecting 1mL MDA-MB-231 cell suspension with a concentration of 1×10^7 cells/mL on the right armpit. In vivo animal experiments were performed when the tumor volume was above 50 mm^3. At predetermined time points, the tumor size was measured using a caliper and the tumor volume then calculated using the formula: $A \times B^2/2$, where A and B are the lengths of the minor and major axes of the tumor.

***In vivo* antitumor evaluation.** MDA-MB-231 tumor-bearing BALB/c-nu mice were divided into 3 groups with 4 mice per group. The mice were treated with: PBS (100 μL), Ru-OTE (100 μL, 100 μM), Ru-OTE (100 μL, 100 μM) with 30 min visual light irradiation (10 mw/cm^2). After intravenous injection at an equivalent dose for1 h, tumors were illuminated under the white light irradiation for 30 min. The relative tumor volume and body weight of every mice were recorded every 2 days. After 14 days, mice were anesthetized by intraperitoneal injection of 330 μL/100 g 10% chloral hydrate and digital photos were taken. At the end, the tumor tissues were obtained from sacrificed mice for H&E staining.

Supplementary Figures

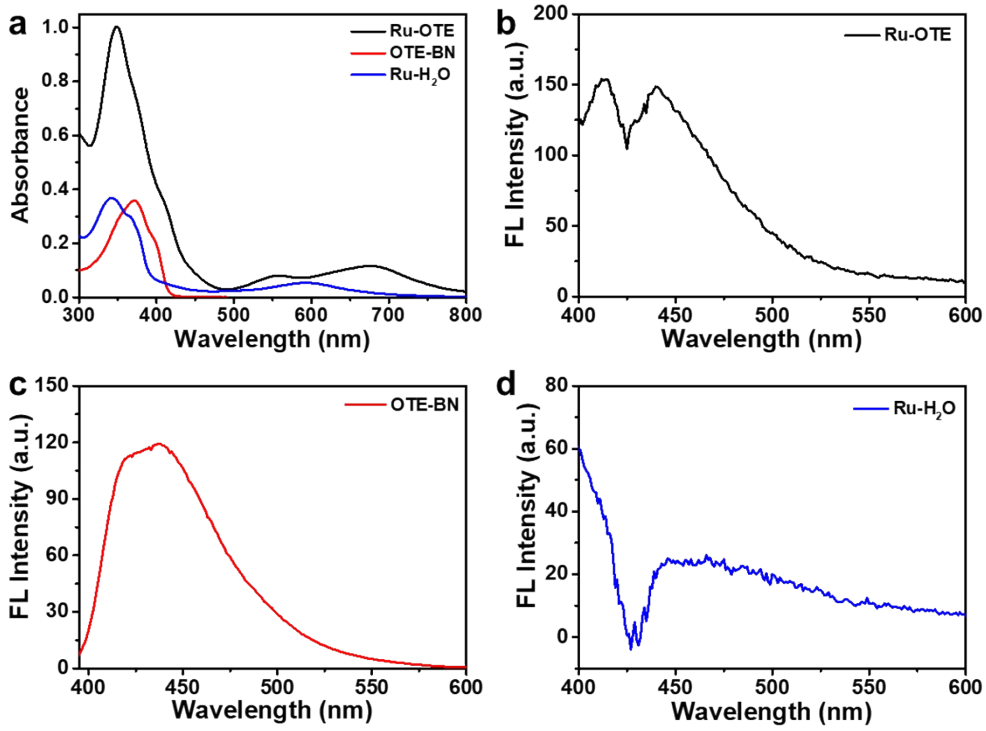

Fig. S1 (a) Absorption spectrum of Ru-OTE, OTE-BN and Ru-H_2O (10 μM) in aqueous solution. Emission spectra of (b) Ru-OTE, (c) OTE-BN and (d) Ru-H_2O in PBS buffer solution. The excitation wavelength is 371 nm, and the concentration of each compound is 1 μM.

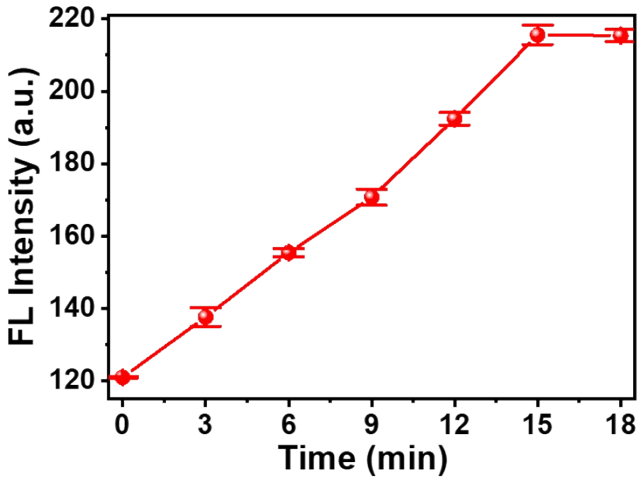

Fig. S2 Response time of the Ru-OTE toward CO. The fluorescence intensity located at 438 nm was counted while the excitation wavelength is 371 nm.

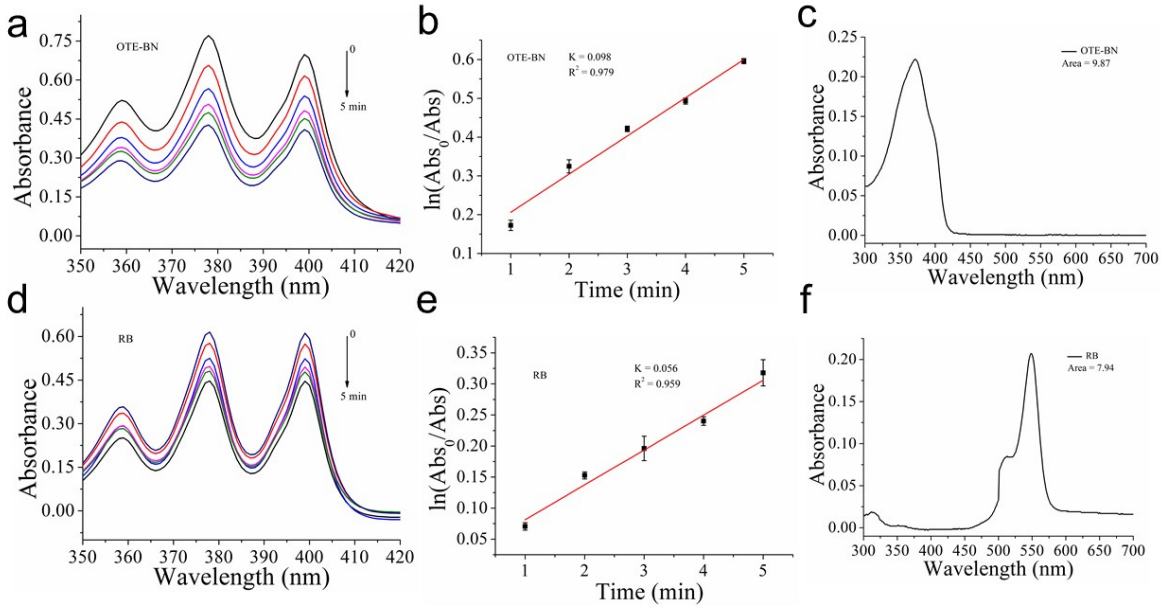

Fig. S3 The UV-vis absorption spectra of ABDA (50 μM) mixed with (a) OTE-BN and (d) RB, and corresponding linear fit curves of (b) OTE-BN and (e) RB at various irradiation time (5 mW/cm^2). The absorption peak area of (c) OTE-BN and (d) RB.

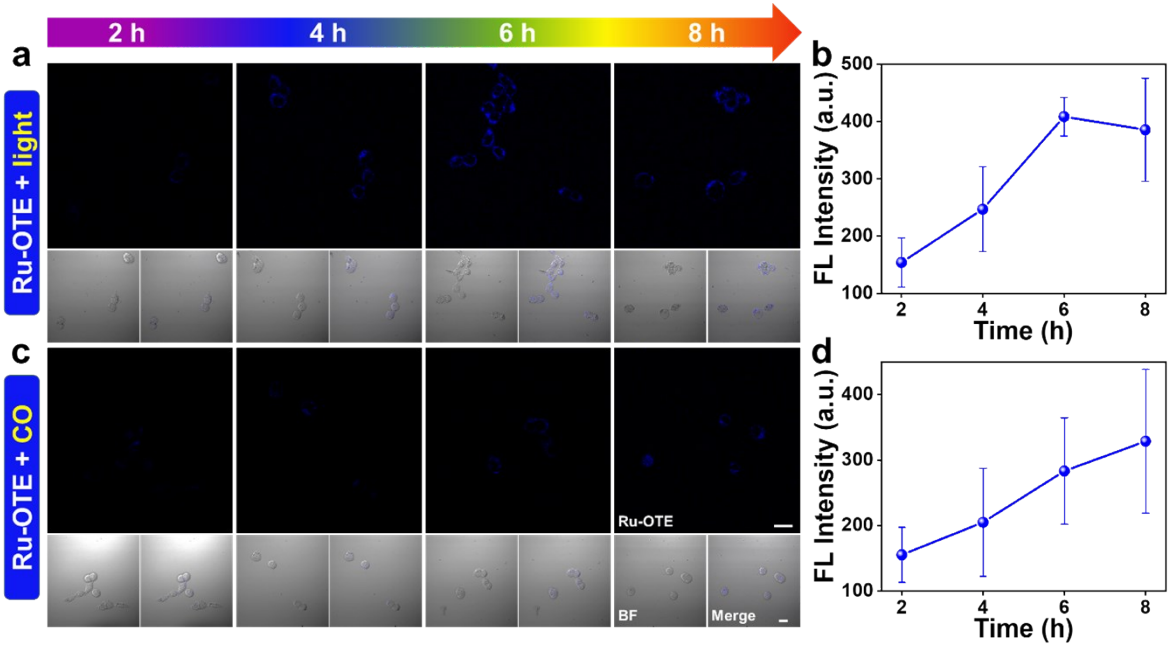

Fig. S4 (a) Confocal laser scanning microscopy imaging of MDA-MB-231 cells and (b) the fluorescence intensity of Ru-OTE inside MDA-MB-231 cells after incubation with Ru-OTE (4.0 μM) for different time and with light irradiation. (c) Confocal laser scanning microscopy

imaging of MDA-MB-231 cells and (d) the fluorescence intensity of Ru-OTE inside MDA-MB-231 cells that stimulated by heme (100 μM) for different time (2 h, 4 h, 6 h or 8 h), and then incubated with Ru-OTE (4.0 μM) for 6 h. The total fluorescence intensity of every confocal images was counted with the Olympus software. The fluorescence imaging of Ru-OTE was collected at 420-460 nm (λ_{ex}: 405 nm). Scale bar: 20 μm.

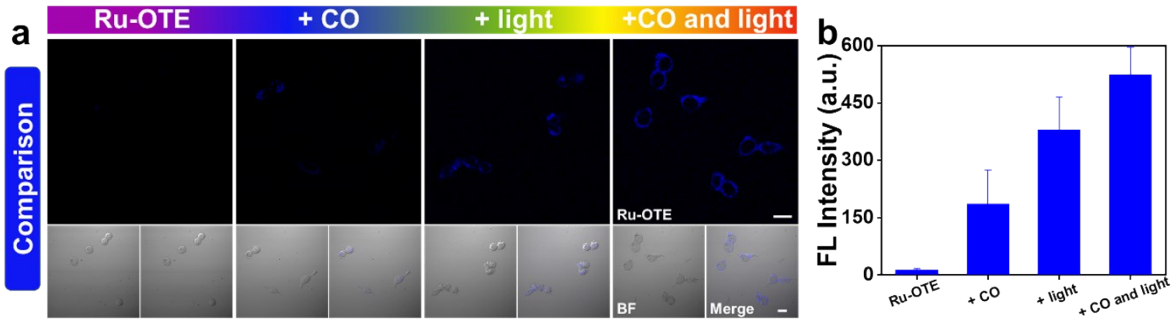

Fig. S5 (a) Confocal laser scanning microscopy imaging of MDA-MB-231 cells and (b) the fluorescence intensity of of Ru-OTE inside MDA-MB-231 cells after incubation with Ru-OTE in the absence and presence of CO, light, and CO + light. The fluorescence imaging of Ru-OTE was collected at 420-460 nm (λ_{ex}: 405 nm). Scale bar: 20 μm.

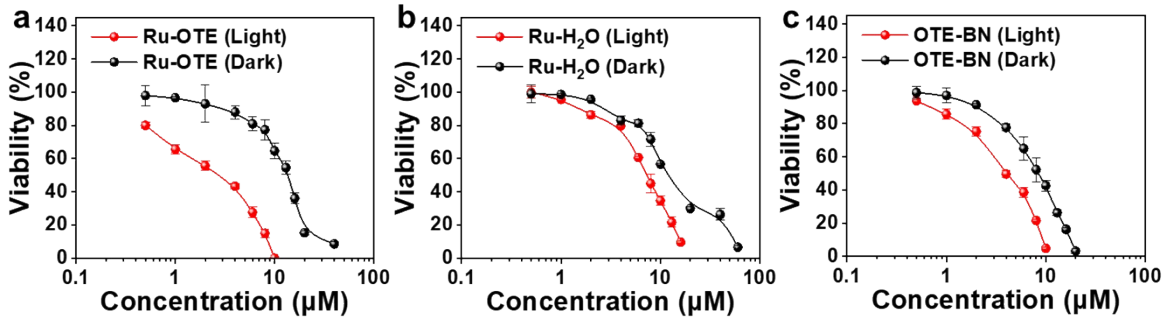

Fig. S6 Viability of MDA-MB-231 cells with different concentrations of Ru-OTE, Ru-H_2O and OTE-BN for 24 h under dark or light conditions. The cells were irradiated with light (25 mW cm^{-2}, 30 min) after 6 h incubation and cell viability were measured after another 24 h incubation.

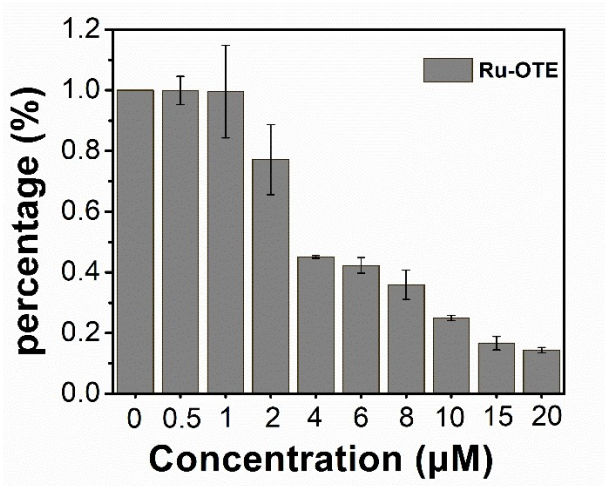

Fig. S7 Viability of HL7702 cells with different concentrations of Ru-OTE for 24 h under dark.

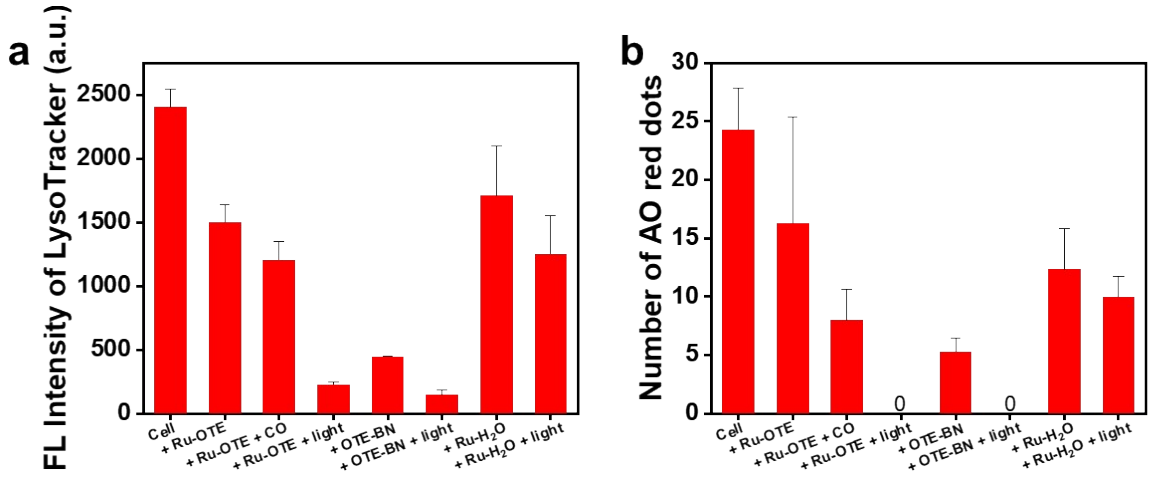

Fig. S8 (a) The fluorescence intensity of LysoTracker red DND 99 and (b) number of AO red dots inside MDA-MB-231 cells after treated with different conditions. The total fluorescence intensity of every confocal images was counted with the Olympus software. The fluorescence imaging of LysoTracker® Red DND-99 was collected at 570-670 nm (λ_{ex}: 559 nm). Fluorescence imaging of AO was collected at 610-640 nm in the red channel (λ_{ex}: 488nm).

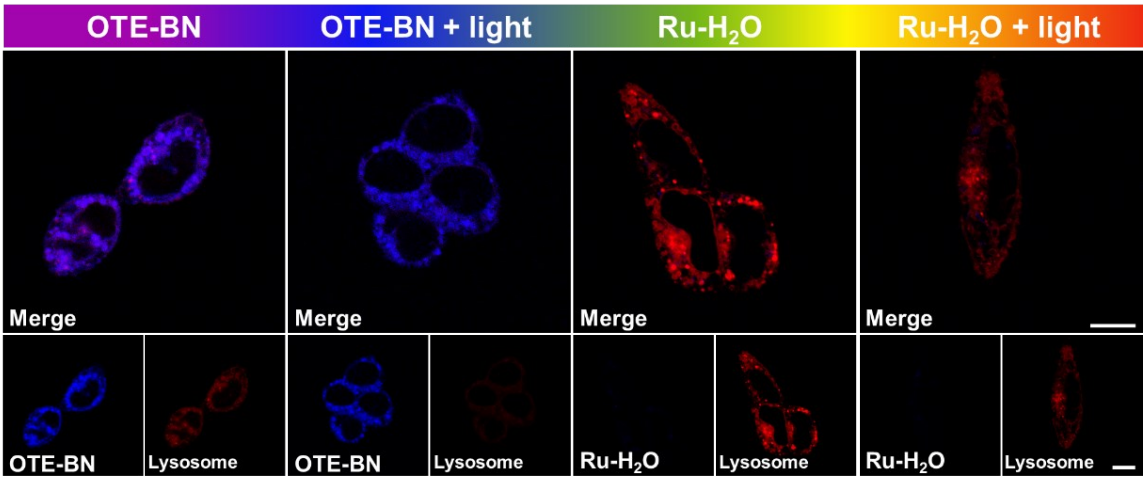

Fig. S9 Confocal laser scanning microscopy images of MDA-MB-231 cells incubated with OTE-BN and Ru-H_2O and stained with LysoTracker red DND 99 with or without light irradiation. The fluorescence imaging of OTE-BN and LysoTracker® Red DND-99 were collected at 420-460 nm (λ_{ex}: 405 nm), 570-670 nm (λ_{ex}: 559 nm), respectively. Scale bar: 10 μm.

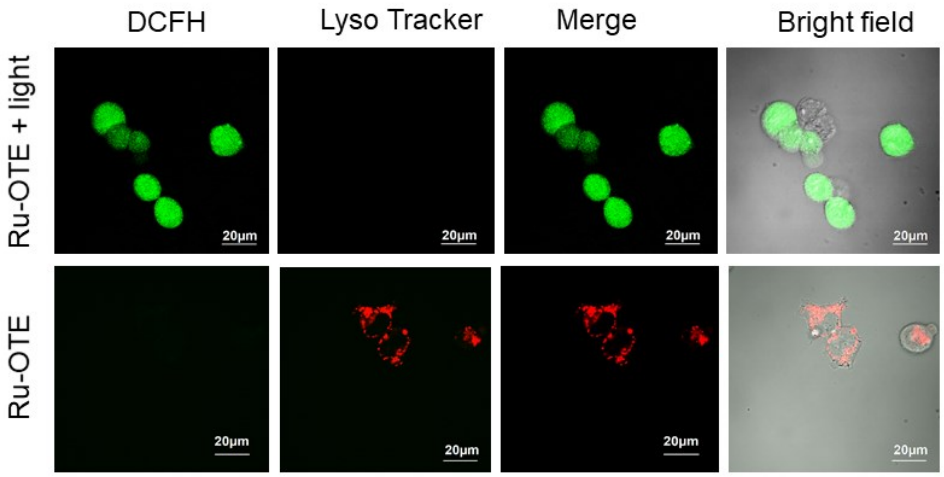

Fig. S10 Confocal laser scanning microscopy images of MDA-MB-231 cells incubated with OTE-BN and stained by 2',7'-dichlorofluorescin diacetate (DCFH-DA) and lysoTracker without or with light irradiation. The fluorescence imaging of DCFH channel was collected at 500-600 nm (λ_{ex}: 488 nm). Fluorescence imaging of LysoTracker channel was collected at 650-750 nm (λ_{ex}: 559 nm). Scale bar: 20 μm.

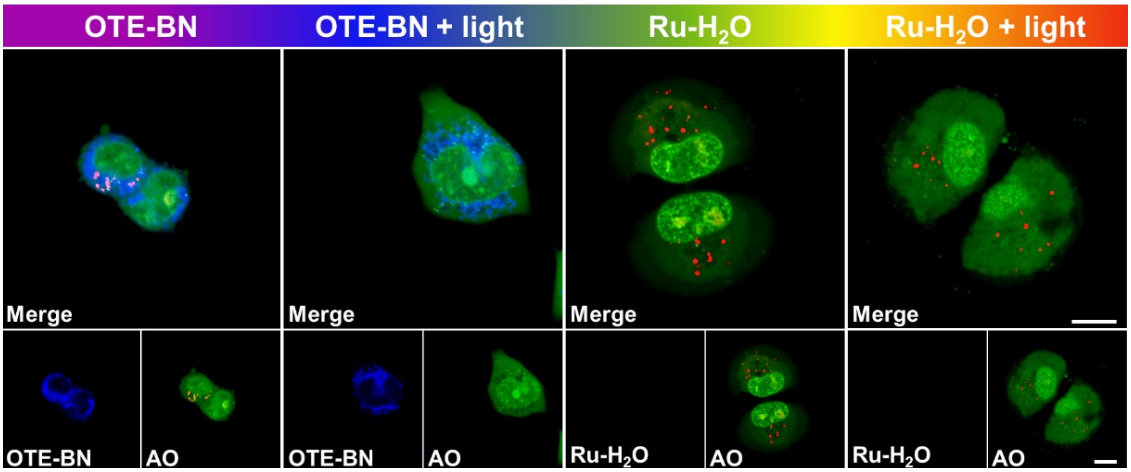

Fig. S11 Confocal laser scanning microscopy images of MDA-MB-231 cells incubated with OTE-BN and Ru-H_2O without or with light irradiation. The fluorescence imaging of OTE-BN was collected at 420-460 nm (λ_{ex}: 405 nm). Fluorescence imaging of AO was collected at 515-545 nm in the green channel and 610-640 nm in the red channel (λ_{ex}: 488nm). Scale bar: 10 μm.

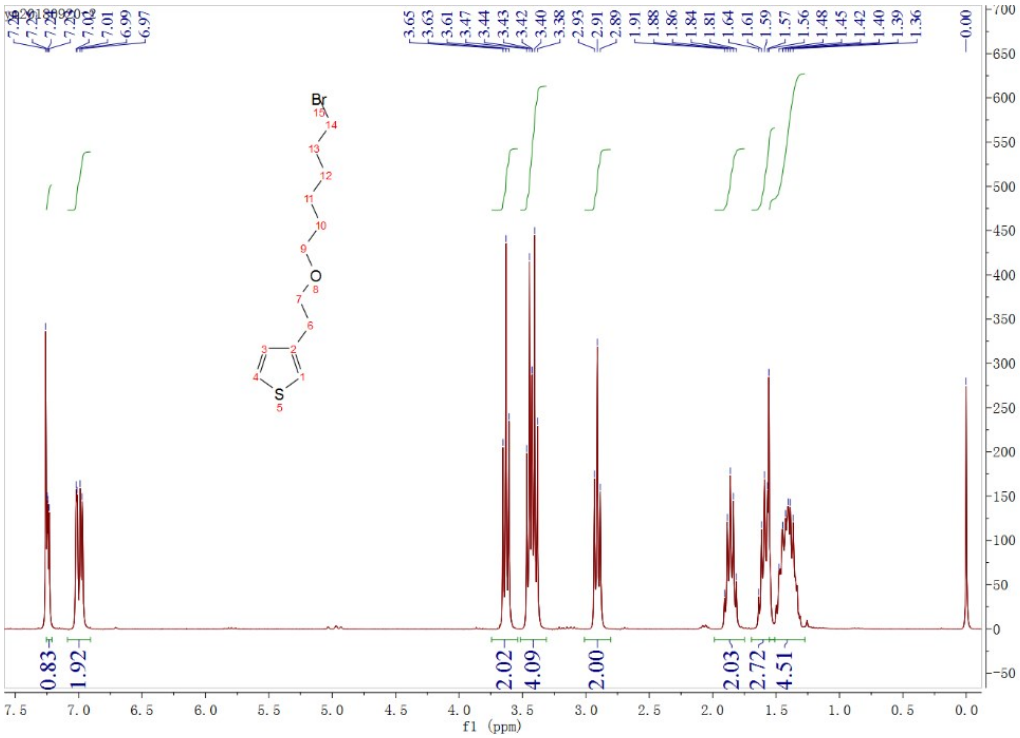

Fig. S12 ^{1}H-NMR spectrum of compound **1** in $CDCl_3$.

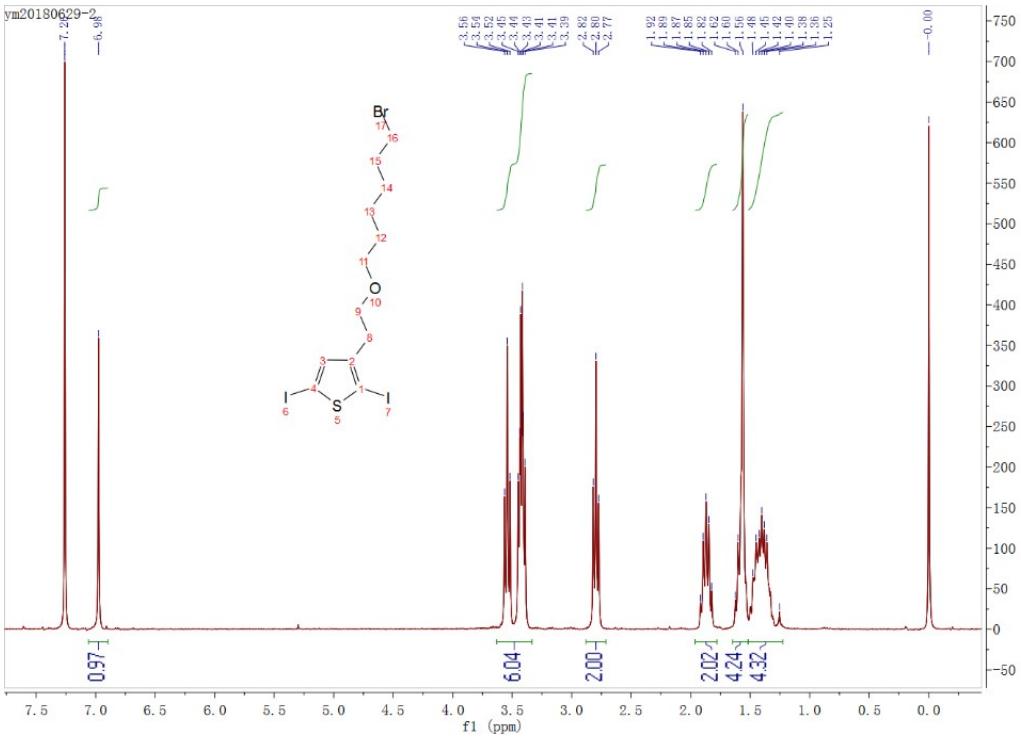

Fig. S13 ^{1}H-NMR spectrum of compound **2** in $CDCl_3$.

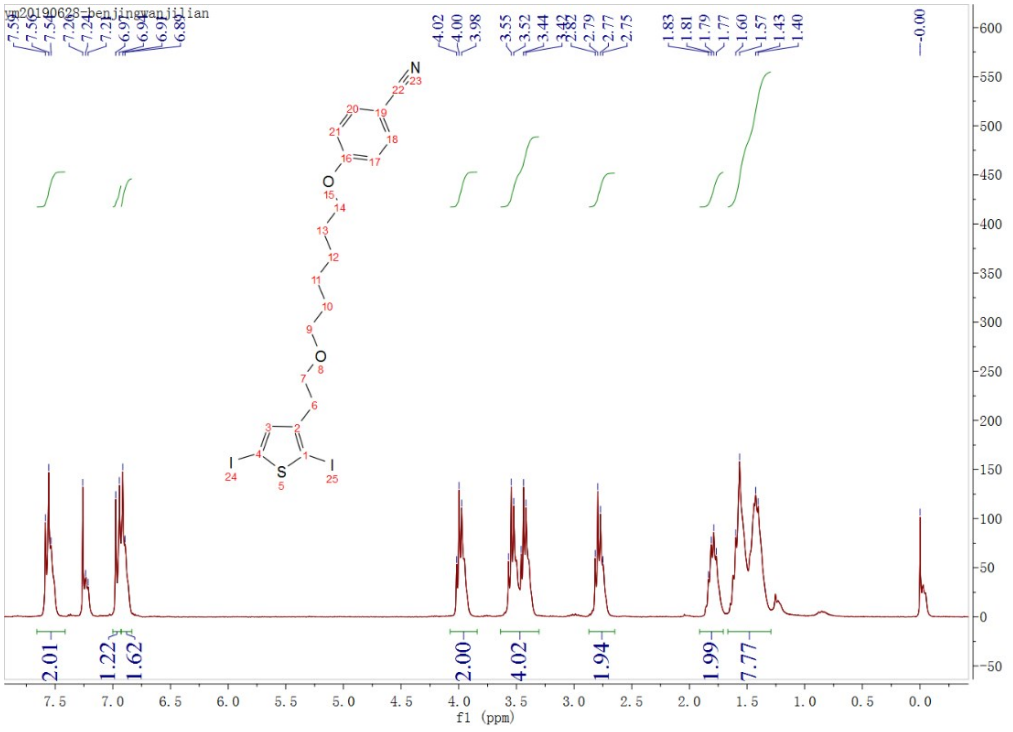

Fig. S14 ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

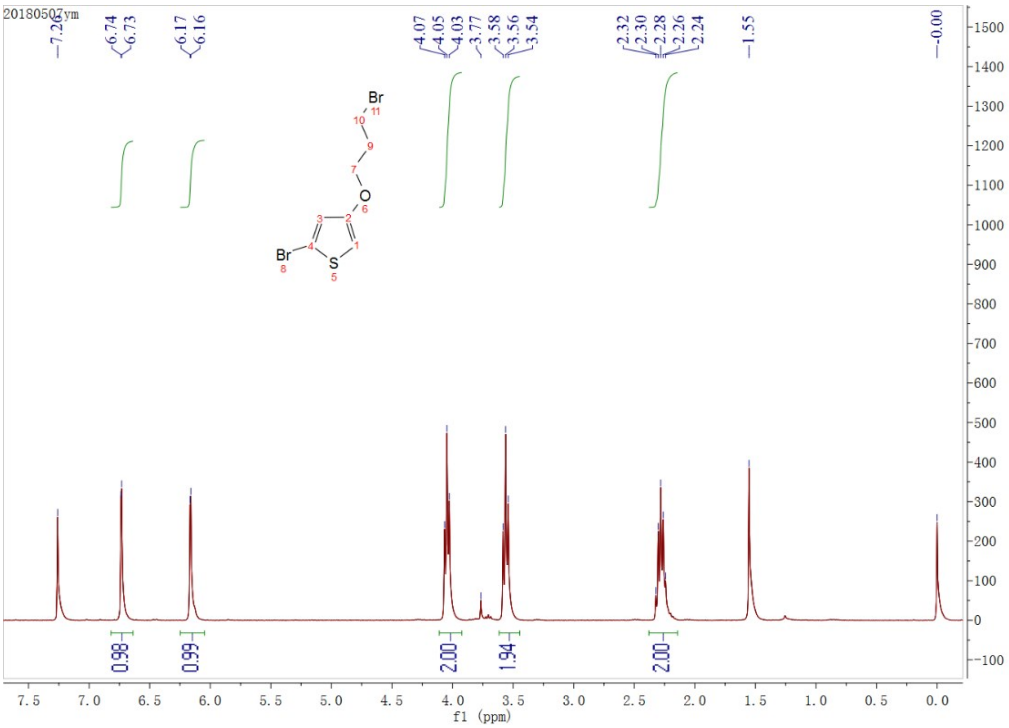

Fig. S15 ^{1}H-NMR spectrum of compound **5** in $CDCl_3$.

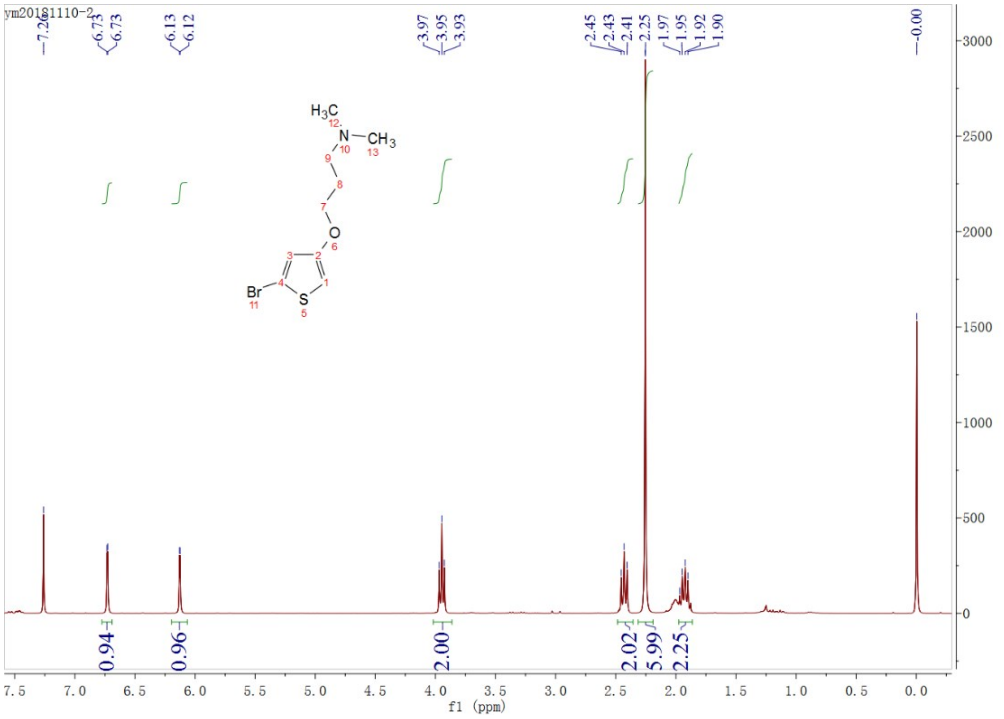

Fig. S16 ^{1}H-NMR spectrum of compound **6** in $CDCl_3$.

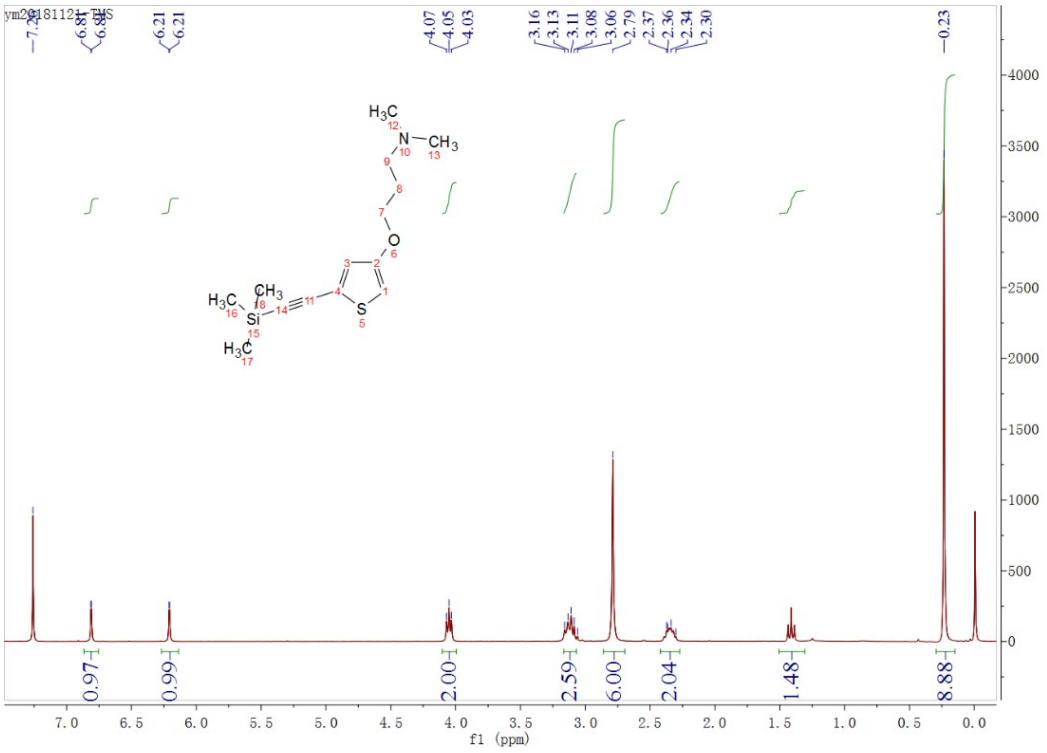

Fig. S17 ^{1}H-NMR spectrum of compound **7** in $CDCl_3$.

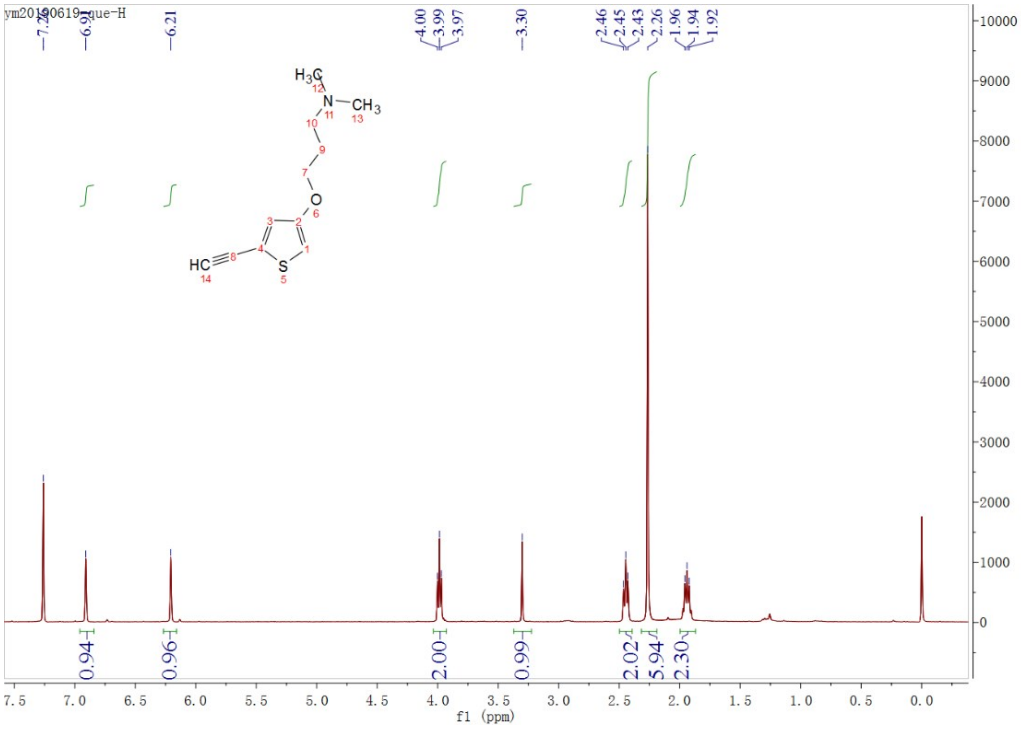

Fig. S18 ^{1}H-NMR spectrum of compound **8** in $CDCl_3$.

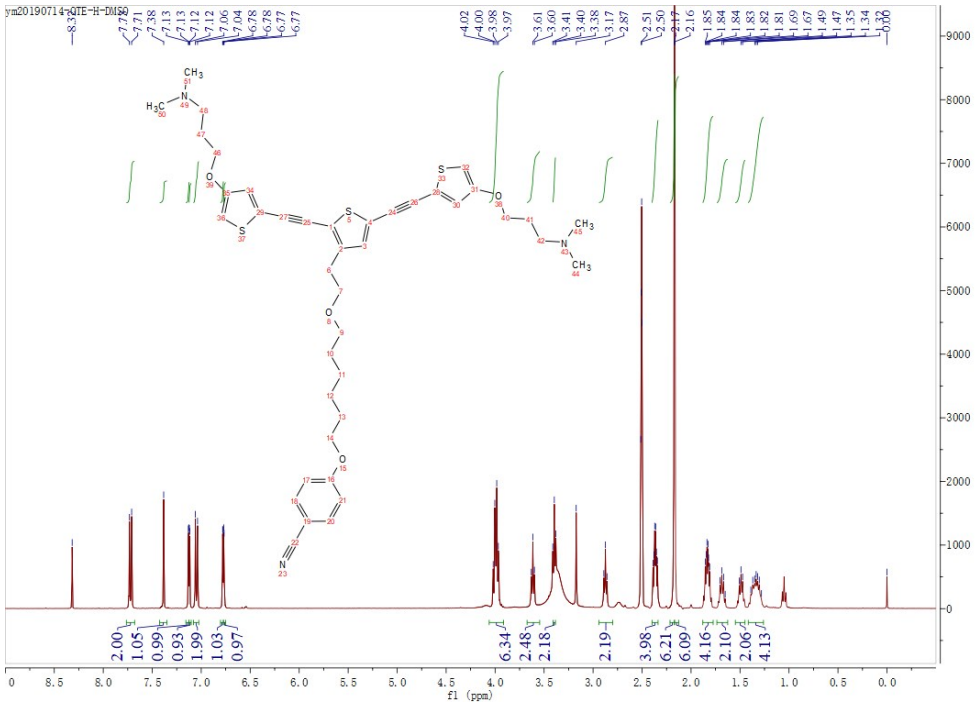

Fig. S19 ^{1}H-NMR spectrum of compound **9** in DMSO-d_6.

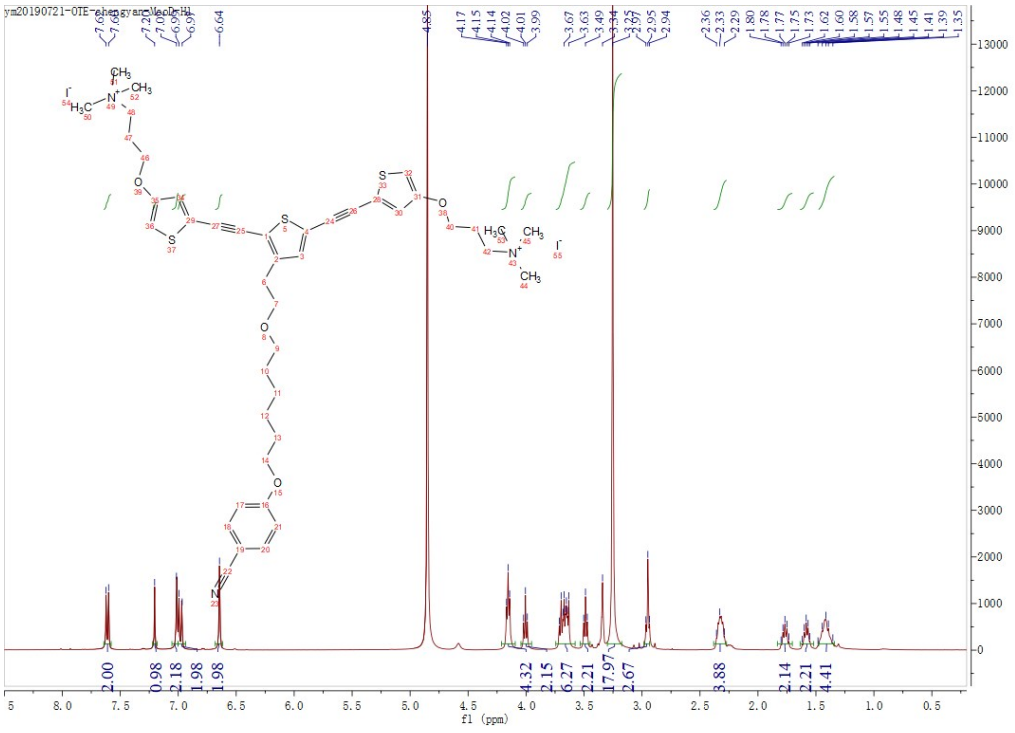

Fig. S20 ^{1}H-NMR spectrum of OTE-BN in CD_3OD.

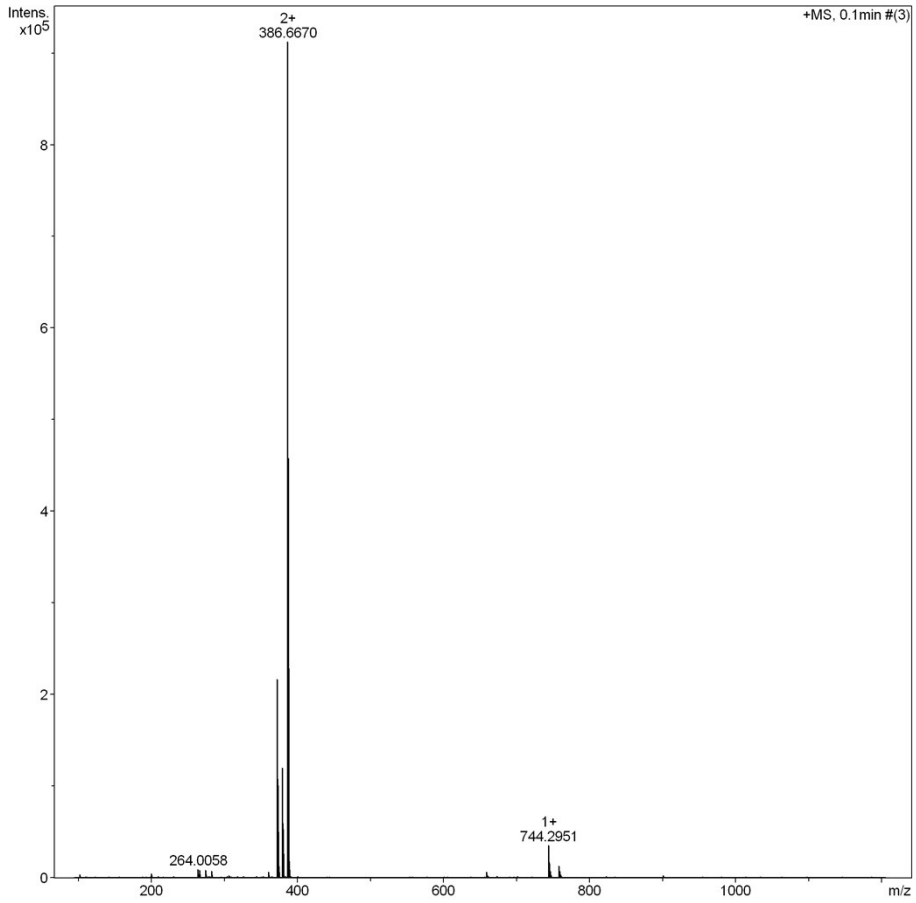

Fig. S21 HRMS spectrum of OTE-BN.

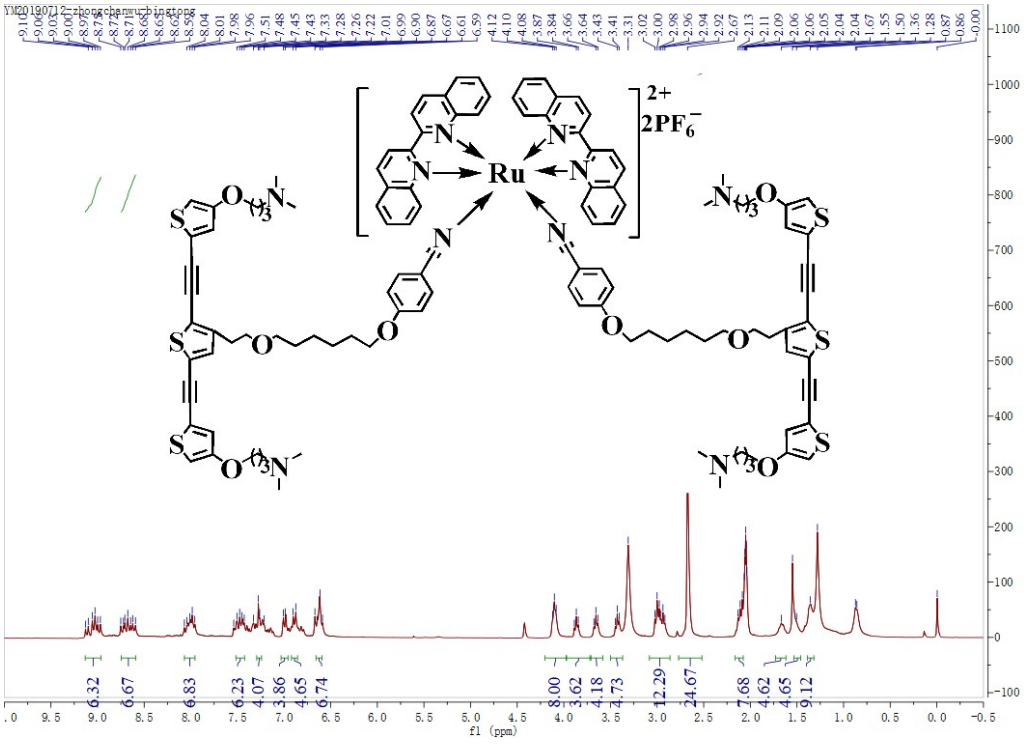

Fig. S22 ^{1}H-NMR spectrum of Ru-OTE-Pre in $(CD_3)_2CO$.

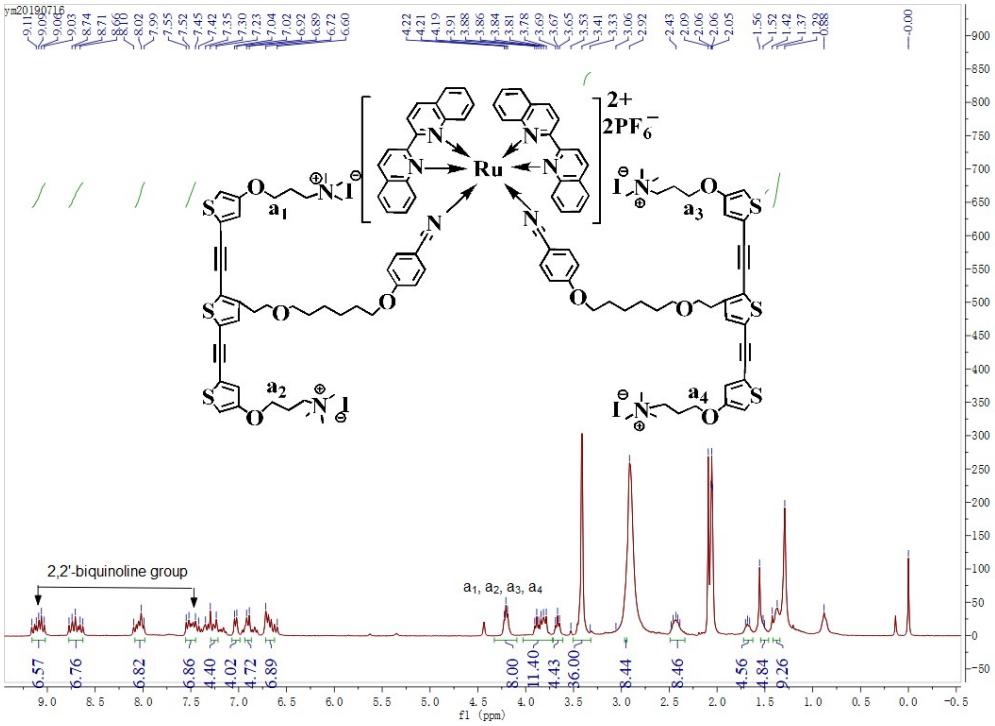

Fig. S23 ^{1}H-NMR spectrum of Ru-OTE in $(CD_3)_2CO$.

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