Electronic Supporting Information

Dinuclear ruthenium(II) polypyridyl complexes as one and two-photon luminescence cellular imaging probes

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Experimental section

Instruments

Microanalysis (C, H, and N) was carried out with a Vario EL cube elemental analyzer. ^{1}H NMR spectra were recorded on a Bruke 500 Nuclear Magnetic Resonance Spectrometer at 25 ^{o}C. All chemical shifts are given relative to tetramethylsilane (TMS). Electrospray mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA). Fast atom bombardment mass spectra (FAB-MS) were acquired on a VG ZAB-HS spectrometer in a 3-nitrobenzyl alcohol matrix. UV–Vis spectra were recorded on a PerkinElmer Lambda 850 spectrophotometer. Emission spectra were recorded on a PerkinElmer LS 55 fluorescence spectrometer at room temperature. Time-resolved emission measurements were conducted on an FLS 920 combined fluorescence lifetime and steady state spectrometer. Quantum yields of luminescence at room temperature were calculated according to literature procedures,[1] using an aerated aqueous solution of $[Ru(bpy)_3]^{2+}$ ($\phi = 0.042$)[2] as reference emitter. All data were dealed with using the OriginPro 7.5 software package.

Materials

All materials were commercially available and of reagent grade. PBS buffer (pH 7.4) was prepared using doubly distilled water.

Synthesis

The compounds 1,10-phenanthroline-5,6-dione,[3] and *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$[4] and were synthesized according to the literature methods.

Syntheses of 1,3-bis(1-phenyl-1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)benzene (L$_1$)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ， aniline (0.27 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A yellow precipitate was obtained and washed by water and ether. The crude product was recrystallized from CH_3OH and produced a yellow powder. Yield: 85%. Anal. Calcd for $C_{44}H_{26}N_8$: C, 79.26; H, 3.93; N, 16.81. Found: C, 79.12; H, 3.45; N, 24.39%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.11 (d, J = 5.0 Hz, 2H), δ 9.03 (d, J = 5.0 Hz, 2H), δ 8.97 (d, J = 5.0 Hz, 2H), 8.17 (s, 1H), 7.93 (m, 2H), 7.76 (m, 8H), 7.66 (m, 2H), 7.56 (d, J = 5.0 Hz, 2H), 7.48 (d, J = 5.0 Hz, 2H), 7.36 (t, J_1 = J_2=5.0 Hz, 2H) , 7.28 (t, J_1=J_2=5.0 Hz, 1H). FAB-MS: m/z = 667 [M+1].

Syntheses of 1,3-bis(1-p-tolyl-1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)benzene (L$_2$)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ，p-toluidine (0.32 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A dark yellow precipitate was obtained and washed by water and ether. The crude product was recrystallized from CH_3OH and produced a dark yellow powder. Yield: 83%. Anal. Calcd for $C_{46}H_{30}N_8$: C, 79.52; H, 4.35; N, 16.13. Found: C, 79.48; H, 4.42; N, 16.10%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.10 (d, J = 5.0 Hz, 2H), δ 9.01 (d, J = 5.0 Hz, 2H), δ 8.96 (d, J = 5.0 Hz, 2H), 8.25 (s, 1H), 7.91 (m, 2H), 7.63 (d, J =5.0 Hz, 4H), 7.53 (d, J =5.0 Hz, 4H), 7.51 (m, 2H), 7.47 (d, J = 5.0 Hz, 2H), 7.40 (d, J = 5.0 Hz, 2H), 7.30 (t, J_1=J_2=5.0 Hz, 1H) , 2.52 (s, 6H). FAB-MS: m/z = 695 [M+1].

Syntheses of 1,3-bis(1-(4-tert-butylphenyl)-1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)benzene (L$_3$)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ， 4-tert-butylaniline (0.45 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A pale yellow precipitate was obtained and washed by water and ether. The crude product was recrystallized from

CH_3OH and produced a pale yellow powder. Yield: 80%. Anal. Calcd for $C_{52}H_{42}N_8$: C, 80.18; H, 5.43; N, 14.39. Found: C, 80.09; H, 5.48; N, 14.43%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.11 (d, J = 5.0 Hz, 2H), δ 9.03 (d, J = 5.0 Hz, 2H), δ 8.97 (d, J = 5.0 Hz, 2H), 8.00 (s, 1H), 7.91 (m, 2H), 7.72 (d, J =5.0 Hz, 4H), 7.62 (d, J =5.0 Hz, 2H), 7.52 (m, 2H), 7.46 (d, J = 5.0 Hz, 2H), 7.39 (d, J = 5.0 Hz, 1H), 7.33 (t, J_1=J_2=5.0 Hz, 1H), 7.29 (d, J = 5.0 Hz, 3H), 1.25 (s, 18H). FAB-MS: m/z = 779 [M+1].

Syntheses of 1,3-bis(1-(4-fluorophenyl)-1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)benzene (L$_4$)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ，4-fluoroaniline (0.33 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A light blue precipitate was obtained and washed by water and ether. The crude product was recrystallized from CH_3OH and produced a light blue powder. Yield: 73%. Anal. Calcd for $C_{44}H_{24}N_8F_2$: C, 75.20; H, 3.44; N, 15. 95. Found: C, 75.18; H, 3.48; N, 15.93%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.98 (d, J = 25.0 Hz, 2H), δ 9.06 (m, 4H), 8.13 (s, 1H), 7.96 (d, J =5.0 Hz, 2H), 7.90 (d, J =5.0 Hz, 2H), 7.85 (d, J =5.0 Hz, 2H), 7.63 (t, J_1=J_2=5.0 Hz, 1H),7.58 (m, 4H), 7.45 (d, J = 5.0 Hz, 2H), 7.11 (m, 4H). FAB-MS: m/z = 703 [M+1].

Syntheses of 1,3-bis(1-(4-methoxyphenyl)-1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)benzene (L$_5$)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ，4-methoxyaniline (0.37 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A dark blue precipitate was obtained and washed by water and ether. The crude product was recrystallized from CH_3OH and produced a dark blue powder. Yield: 75%. Anal. Calcd for $C_{46}H_{30}N_8O_2$: C, 76.02; H, 4.16; N, 15.42. Found: C, 76.06; H, 4.12; N, 15.38%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.10 (d, J = 5.0 Hz, 2H), δ 9.01 (d, J = 5.0 Hz, 2H), δ 8.96 (d, J = 5.0 Hz, 2H), 8.25 (s, 1H), 7.91 (m, 2H), 7.68 (d, J =10.0 Hz, 4H), 7.53 (m, 4H), 7.44 (d, J = 5.0 Hz, 2H), 7.34 (t, J_1=J_2=5.0 Hz, 1H), 7.28 (m, 4H), 3.92 (s, 6H). FAB-MS: m/z = 727 [M+1].

Syntheses of 4,4'-(2,2'-(1,3-phenylene)bis(1H-imidazo[4,5-f][1,10]phenanthroline-2,1-diyl))-bis(N,N-dimethylaniline) (L_6)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ， N_1,N_1-dimethylbenzene-1,4-diamine (0.41 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A dark red precipitate was obtained and washed by water and ether. The crude product was recrystallized from CH_3OH and produced a dark red powder. Yield: 72%. Anal. Calcd for $C_{48}H_{36}N_{10}$: C, 76.58; H, 4.82; N, 18.60. Found: C, 76.66; H, 4.78; N, 18.56%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.10 (d, J = 5.0 Hz, 2H), δ 9.01 (d, J = 5.0 Hz, 2H), δ 8.96 (d, J = 5.0 Hz, 2H), 8.38 (s, 1H), 7.90 (m, 2H), 7.56 (m, 8H), 7.47 (d, J = 10.0 Hz, 2H), 7.34 (t, J_1=J_2=5.0 Hz, 1H), 6.95 (m, 4H), 3.06 (s, 12H). FAB-MS: m/z = 753 [M+1].

Syntheses of 1,3-bis(1-(4-phenoxyphenyl)-1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)benzene (L_7)

A mixture of 1,10-phenanthroline-5,6-dione (0.63 g, 3 mmol), ammonium acetate (4.62 g, 60 mmol), isophthalic aldehyde (0.201 g, 1.5 mmol) ， 4-phenoxyaniline (0.56 g, 3 mmol) and glacial acetic acid (35 cm^3) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and poured into water (50 cm^3). The solution was neutralized with a 25% NH_3 solution. A blue precipitate was obtained and washed by water and ether. The crude product was recrystallized from CH_3OH and produced a blue powder. Yield: 70%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.10 (d, J = 5.0 Hz, 2H), δ 9.06 (d, J = 5.0 Hz, 2H), δ 9.01 (d, J = 5.0 Hz, 2H), 7.99 (s, 1H), 7.86 (m, 2H), 7.75 (d, J = 5.0 Hz, 2H), 7.69 (t, J_1=J_2=5.0 Hz, 1H), 7.62 (m, 4H), 7.58 (t, J_1=J_2=5.0 Hz, 2H) , 7.48 (m, 2H) , 7.33 (m, 4H) , 7.26 (m, 4H) , 7.07 (m, 4H) , 6.96 (m, 2H). Anal. Calcd for $C_{56}H_{34}N_8O_2$: C, 79.04; H, 4.03; N, 13.17. Found: C, 79.01; H, 4.11; N, 13.12%. FAB-MS: m/z = 851 [M+1].

Syntheses of [(phen)$_2$Ru(L_1)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL_1)

A mixture of *cis*-[Ru(phen)$_2$Cl$_2$]·2H$_2$O (0.104 g, 0.2 mmol) and $\mathbf{L_1}$ (0.068 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown

red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (3:1, v/v) as eluent. Yield: 63%. Anal. Calcd for $C_{92}H_{58}Cl_4N_{16}O_{16}Ru_2$: C, 55.60; H, 2.94; N, 11.28. Found: C, 55.83; H, 2.91; N, 11.39%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.39 (d, J = 10.0 Hz, 8H), 8.11 (m, 7H), 8.08 (d, J =5.0 Hz, 2H), 8.04 (t, J = 5.0 Hz, 2H), 7.98(d, J = 10.0 Hz, 2H), 7.88(d, J = 15.0 Hz, 2H), 7.78 (m, 12H), 7.75 (m, 6H), 7.59 (d, J = 10.0 Hz, 2H), 7.51 (d, J = 15.0 Hz, 2H), 7.41 (m, 3H). ES-MS [CH_3CN, m/z]: 397 ([M-4ClO_4]$^{4+}$), 563 ([M-3ClO_4]$^{3+}$).

Syntheses of [(phen)$_2$Ru(L$_2$)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL$_2$)

A mixture of *cis*-[Ru(phen)$_2$Cl$_2$]·2H$_2$O (0.104 g, 0.2 mmol) and **L$_2$** (0.070 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (7:2, v/v) as eluent. Yield: 66%. Anal. Calcd for $C_{94}H_{62}Cl_4N_{16}O_{16}Ru_2$: C, 56.01; H, 3.10; N, 11.12. Found: C, 55.93; H, 3.13; N, 11.20%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.39 (d, J = 10.0 Hz, 8H), 8.24(s, 1H), 8.11 (m, 6H), 8.08 (d, J =5.0 Hz, 2H), 8.04 (t, J = 5.0 Hz, 2H), 7.98(d, J = 10.0 Hz, 2H), 7.88(d, J = 15.0 Hz, 2H), 7.76 (m, 8H), 7.66(d, J = 5.0 Hz, 2H), 7.60 (m, 4H), 7.53 (m, 6H), 7.45 (d, J = 5.0 Hz, 2H), 7.42 (t, J_1=J_2=10.0 Hz, 1H), 2.08(s, 6H). ES-MS [CH_3CN, m/z]: 404.5 ([M-4ClO_4]$^{4+}$), 573 ([M-3ClO_4]$^{3+}$).

Syntheses of [(phen)$_2$Ru(L$_3$)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL$_3$)

A mixture of *cis*-[Ru(phen)$_2$Cl$_2$]·2H$_2$O (0.104 g, 0.2 mmol) and **L$_3$** (0.078 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (3:1, v/v) as eluent. Yield: 65%. Anal. Calcd for $C_{100}H_{74}Cl_4N_{16}O_{16}Ru_2$: C, 57.20; H, 3.55; N, 10.67. Found: C, 57.08; H, 3.60; N, 10.72%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.39 (d, J = 10.0 Hz, 8H), 8.11 (m, 6H), 8.06 (d, J =5.0 Hz, 4H), 7.99 (t, J = 5.0 Hz, 3H), 7.88(d, J = 15.0 Hz, 2H), 7.80 (m, 4H), 7.76 (m, 6H), 7.68(m, 4H), 7.63(d, J = 10.0 Hz, 2H), 7.56 (d, J = 5.0 Hz, 2H), 7.50

(d, J = 5.0 Hz, 2H), 7.47 (d, J = 5.0 Hz, 2H), 7.38 (t, J_1=J_2=10.0 Hz, 1H), 1.25(s, 18H). ES-MS [CH_3CN, m/z]: 425.5 ($[M\text{-}4ClO_4]^{4+}$), 600.5 ($[M\text{-}3ClO_4]^{3+}$), 950 ($[M\text{-}2ClO_4]^{2+}$).

Syntheses of [(phen)$_2$Ru(L$_4$)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL$_4$)

A mixture of *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ (0.104 g, 0.2 mmol) and **L$_4$** (0.070 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (3:1, v/v) as eluent. Yield: 68%. Anal. Calcd for $C_{92}H_{56}Cl_4F_2N_{16}O_{16}Ru_2$: C, 54.61; H, 2.79; N, 11.08. Found: C, 54.58; H, 2.73; N, 11.22%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.40 (d, J = 10.0 Hz, 8H), 8.10 (m, 6H), 8.08 (d, J =5.0 Hz, 2H), 8.05 (t, J = *5*.0 Hz, 3H), 8.01(d, J = 5.0 Hz, 2H), 7.89(d, J = 5.0 Hz, 2H), 7. 87 (m, 4H), 7.80 (m, *4*H), 7.76 (m, *4*H), 7.63 (m, *6*H), 7.56(d, J = 10.0 Hz, 2H), 7.50(m,3H). ES-MS [CH_3CN, m/z]: 407 ($[M\text{-}4ClO_4]^{4+}$), 576 ($[M\text{-}3ClO_4]^{3+}$).

Syntheses of [(phen)$_2$Ru(L$_5$)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL$_5$)

A mixture of *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ (0.104 g, 0.2 mmol) and **L$_5$** (0.073 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (4:1, v/v) as eluent. Yield: 60%. Anal. Calcd for $C_{94}H_{62}Cl_4N_{16}O_{18}Ru_2$: C, 55.14; H, 3.05; N, 10.95. Found: C, 55.11; H, 3.08; N, 10.89%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.40 (d, J = 10.0 Hz, 8H), 8.24(s, 1H), 8.09 (m, 6H), 8.08 (d, J =5.0 Hz, 2H), 8.05 (t, J = *10*.0 Hz, 2H), 7.99(d, J = 10.0 Hz, 2H), 7.88(d, J = 15.0 Hz, 2H), 7.77 (m, 8H), 7.69 (m, 4H),7.62(d, J = 10.0 Hz, 2H), 7.55 (d, J = 5.0 Hz, 2H), 7.50 (d, J = 10.0 Hz, 2H), 7.45 (t, J_1=J_2=10.0 Hz, 1H), 7.28 (m, 4H), 3.88(s, 6H). ES-MS [CH_3CN, m/z]: 413 ($[M\text{-}4ClO_4]^{4+}$), 583 ($[M\text{-}3ClO_4]^{3+}$).

Syntheses of [(phen)$_2$Ru(L$_6$)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL$_6$)

A mixture of *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ (0.104 g, 0.2 mmol) and **L$_6$** (0.075 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown

red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (5:2, v/v) as eluent. Yield: 56%. Anal. Calcd for $C_{96}H_{68}Cl_4N_{18}O_{16}Ru_2$: C, 55.60; H, 3.31; N, 12.16. Found: C, 55.52; H, 3.38; N, 12.18%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.40 (d, J = 15.0 Hz, 8H), 8.09 (m, 10H), 7.98(d, J = 5.0 Hz, 2H), 7.87(d, J = 10.0 Hz, 2H), 7.77 (m, 8H), 7.62 (d, J = 10.0 Hz, 2H), 7.57 (d, J = 5.0 Hz, 2H), 7.52 (m, 2H), 7.46 (m, 3H), 7.24 (s, 1H), 6.95 (m, 4H), 3.03(s, 12H). ES-MS [CH_3CN, m/z]: 419 ([M-4ClO_4]$^{4+}$), 592 ([M-3ClO_4]$^{3+}$).

Syntheses of [(phen)$_2$Ru(L$_7$)Ru(phen)$_2$](ClO)$_4$)$_4$ (RuL$_7$)

A mixture of *cis*-[Ru(phen)$_2$Cl$_2$]·2H$_2$O (0.104 g, 0.2 mmol) and **L$_7$** (0.085 g, 0.1 mmol) in ethylene glycol (12 cm^3) was refluxed under argon for 12 h to give a clear red solution. Upon cooling, a brown red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on alumina with CH_3CN-Toluene (4:1, v/v) as eluent. Yield: 58%. Anal. Calcd for $C_{104}H_{66}Cl_4N_{16}O_{18}Ru_2$: C, 57.52; H, 3.06; N, 10.32. Found: C, 57.48; H, 3.12; N, 10.29%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, J = 10.0 Hz, 2H), 8.78 (m, 8H), 8.40 (d, J = 10.0 Hz, 8H), 8.10 (m, 10H), 8.02(d, J = 5.0 Hz, 2H), 7.84(d, J = 10.0 Hz, 2H), 7.77 (m, 11H), 7.68 (d, J = 5.0 Hz, 2H), 7.63 (m, 4H), 7.55 (d, J_1=J_2=10.0 Hz, 1H), 7.35 (m, 4H), 7.25 (m, 6H), 7.06 (m, 6H). ES-MS [CH_3CN, m/z]: 444 ([M-4ClO_4]$^{4+}$), 625 ([M-3ClO_4]$^{3+}$) .

Determination of two-photon absorption cross sections

The two-photon absorption spectra of probes determined over a broad spectral region by the typical two-photon induced fluorescence (TPF) method relative to Rhodamine B in methanol as standard.[5] The two-photon fluorescence data were acquired using a Opolette^{TM} 355II (pulse width $\leqslant$ 100 fs, 80 MHz repetition rate, tuning range 750-1000 nm, Spectra Physics Inc., USA). Two-photon fluorescence measurements were performed in fluorometric quartz cuvettes with **RuL$_{1\text{-}7}$** at 2×10^{-4} M in in aqueous media (DMSO/H_2O, v/v = 1:99) at 298K. The experimental fluorescence excitation and detection conditions were conducted with negligible reabsorption processes which can affect TPA measurements. The quadratic dependence of two-photon induced fluorescence intensity on the excitation power was

verified for excitation wavelength at 830 nm. The two-photon absorption cross section of the probes was calculated at each wavelength according to equation (1)[6]

$$\delta_2 = \delta_1 \frac{\phi_1 C_1 I_2 n_2}{\phi_2 C_2 I_1 n_1} \quad (1)$$

where I is the integrated fluorescence intensity, C is the concentration, n is the refractive index, and Φ is the quantum yield, subscript '1' stands for reference samples, '2' stands for samples.

Cell line and cell culture

HeLa cells were obtained from the Cell Bank (Cell Institute, Sinica Academica Shanghai, Shanghai, China). All cell lines were maintained in either RPMI-1640 or DMEM media supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37 °C in CO_2 incubator (95% relative humidity, 5% CO_2).

Flow cytometry analysis

HeLa cells in a density of 1×10^5 cells/mL were cultured in 6-well plates for 24 h in incubator, then **RuL$_4$** (10 μM) was added with fresh DMEM and were further incubated for 2 h in incubator. Then the cells were trypsinized and washed with PBS. The cell uptake samples were analyzed with FACSCanto II (BD Biosciences, USA).

ICP-MS analysis

HeLa cells were plated at a density of 1×10^5 cells per ml in a volume of 5 mL of DMEM medium, **RuL$_{1\text{-}7}$** (10 μM) was added to the culture medium and incubated for varying amounts of time at 37 ºC. After digestion, HeLa cells were counted and divided into two parts. One part: the nuclei were extracted using a nucleus extraction kit; the second part: the cytoplasms were extracted using a cytoplasm extraction kit (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd.). The samples were digested by 60% HNO_3 at RT for one day. Each sample diluted with DDW to obtain 2% HNO_3 sample solutions. The ruthenium concentration in the two parts was determined by an inductively coupled plasma mass spectrometry (ICP-MS Thermo Elemental Co., Ltd.).

MTT cell viability assay[7]

Cells were plated in 96-well microassay culture plates (1×10^4 cells per well) and grown overnight at 37 ^oC in a 5%CO_2 incubator. Test compounds were then added to the wells to achieve final concentrations ranging from 10^{-6} to 10^{-4} M. Control wells were prepared by addition of culture medium (100 μL). Wells containing culture medium without cells were used as blanks. The plates were incubated at 37 ^oC in a 5%CO_2 incubator for 24 h. Upon completion of the incubation, stock MTT dye solution (20 μL, 5 mg/mL) was added to each well. After 4 h incubation, buffer (100 μL) containing N,N-dimethylformamide (50%) and sodium dodecyl sulfate (20%) was added to solubilize the MTT formazan. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 590 nm.

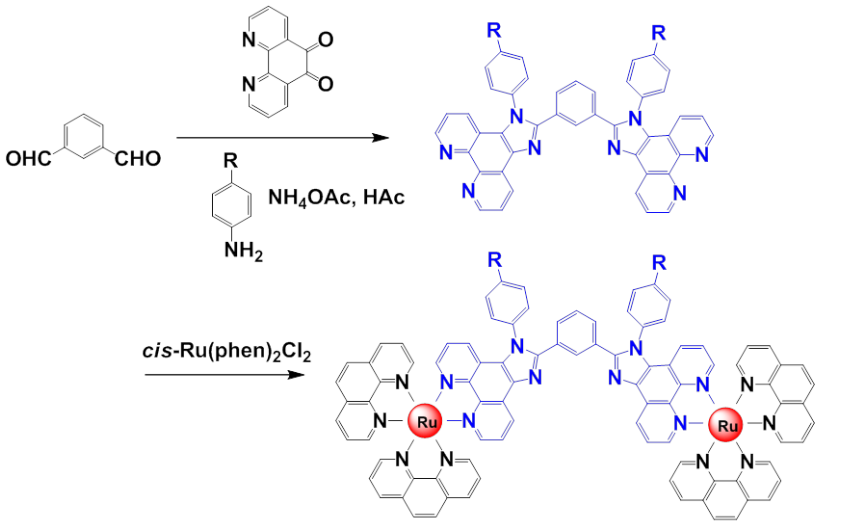
One- and two-photon luminescent imaging

HeLa cell lines were incubated with **RuL$_{1\text{-}7}$** (10 μM) for 2 h at 37 ^oC. After being washed with fresh PBS (pH = 7.4) three times, the cells were imaged on a Zeiss LSM 710 NLO confocal microscope (63×/NA 1.4 oil immersion objective). Excitation wavelength of laser was 458 nm, and emission spectra were integrated over the range 580-630 nm (single channel). For two-photon images, excitation wavelength of laser was 800/830 nm.

Table S1 Photophysical data of **RuL**$_{1\text{-}7}$ in DMSO/H_2O[a]

Probe	$\lambda_{max}/\lambda_{max}$[b]	ε_{max}[c]	ϕ[d]	τ/ns[e]	δ(GM)[f]
RuL$_1$	263, 282, 455/603	16.4, 10.2, 2.94	0.045	989.09	322
RuL$_2$	263, 281, 456/602	17.3, 11.0, 3.10	0.041	1002.10	368
RuL$_3$	263, 280, 457/607	14.1, 9.77, 2.57	0.050	1000.56	357
RuL$_4$	263, 280, 455/606	18.3, 11.8, 3.25	0.044	1006.65	361
RuL$_5$	263, 279, 455/608	14.3, 7.64, 2.08	0.050	987.71	386
RuL$_6$	264, 280, 456/607	14.2, 8.92, 2.28	0.054	1113.69	355
RuL$_7$	263, 279, 456/608	14.8, 10.4, 2.64	0.048	1075.99	330

[a]All data were measured in aqueous media (DMSO/H_2O, v/v = 1:99) at 298K. [b]λ_{max} values of the one-photon absorption and emission spectra in nm. [c]Extinction coefficient in 1×10^4 $M^{-1}\cdot cm^{-1}$. [d]Luminescence quantum yield. [e]Life time. [f]Two-photon absorption cross section at 830 nm for **RuL$_4$**, 800 nm for **RuL$_{1\text{-}7}$**.



	R		R
RuL$_1$	**H**	**RuL$_5$**	**OMe**
RuL$_2$	**CH_3**	**RuL$_6$**	**$N(CH_3)_2$**
RuL$_3$	**$C(CH_3)_3$**	**RuL$_7$**	
RuL$_4$	**F**		

Scheme S1. Synthesis of ligands **L$_{1\text{-}7}$** and complexes **RuL$_{1\text{-}7}$**.

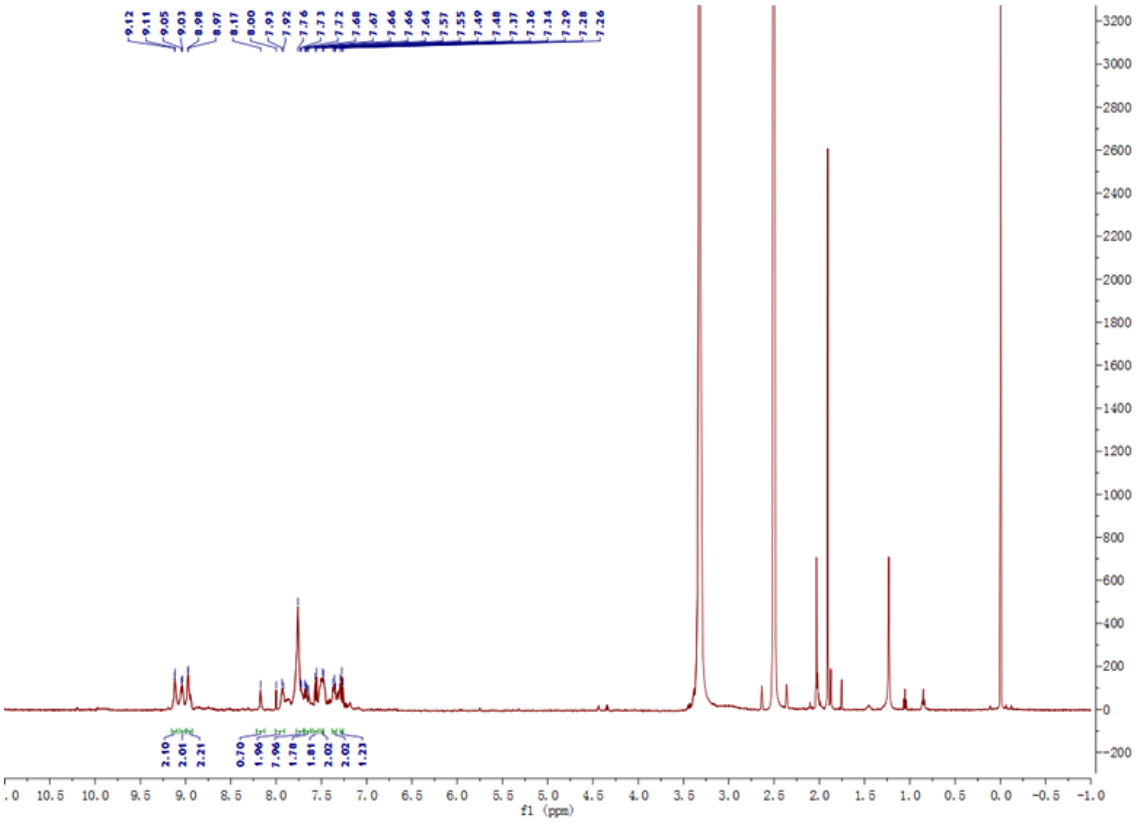


Fig. S1 ^{1}H NMR spectrum of $\mathbf{L_1}$.

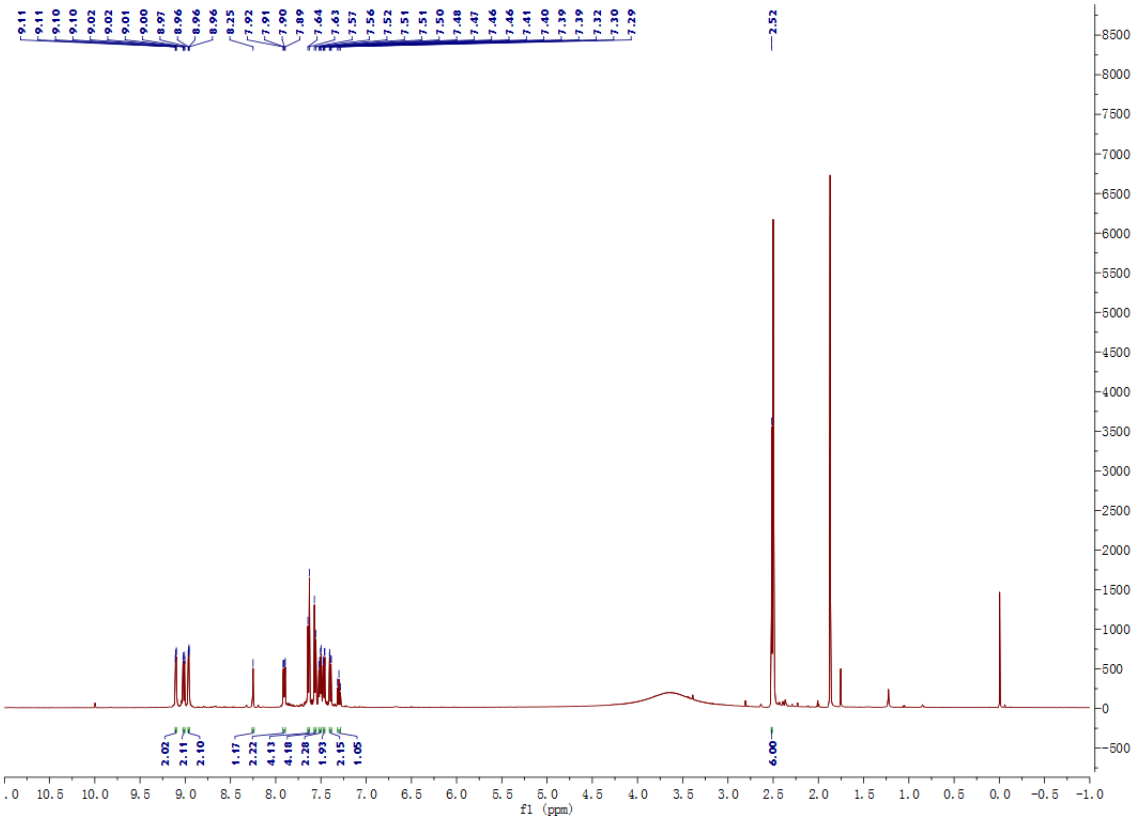


Fig. S2 ^{1}H NMR spectrum of $\mathbf{L_2}$.

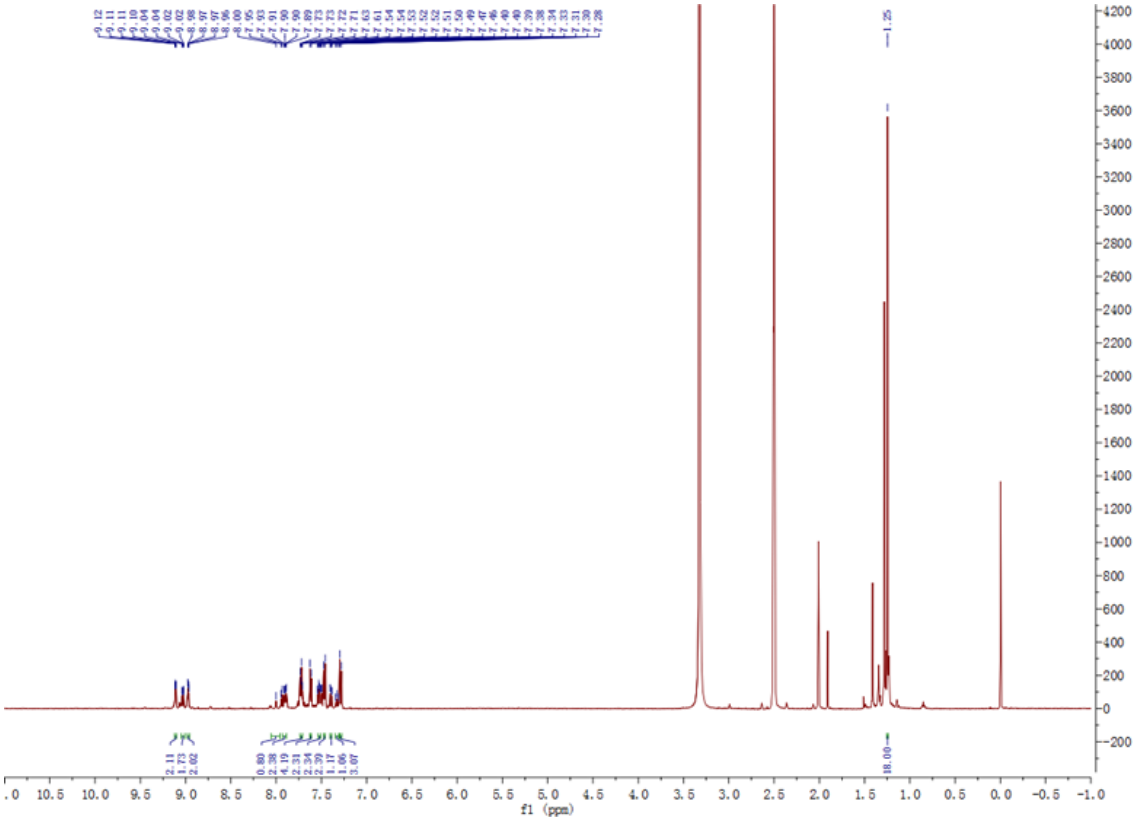


Fig. S3 ^{1}H NMR spectrum of $\mathbf{L_3}$.

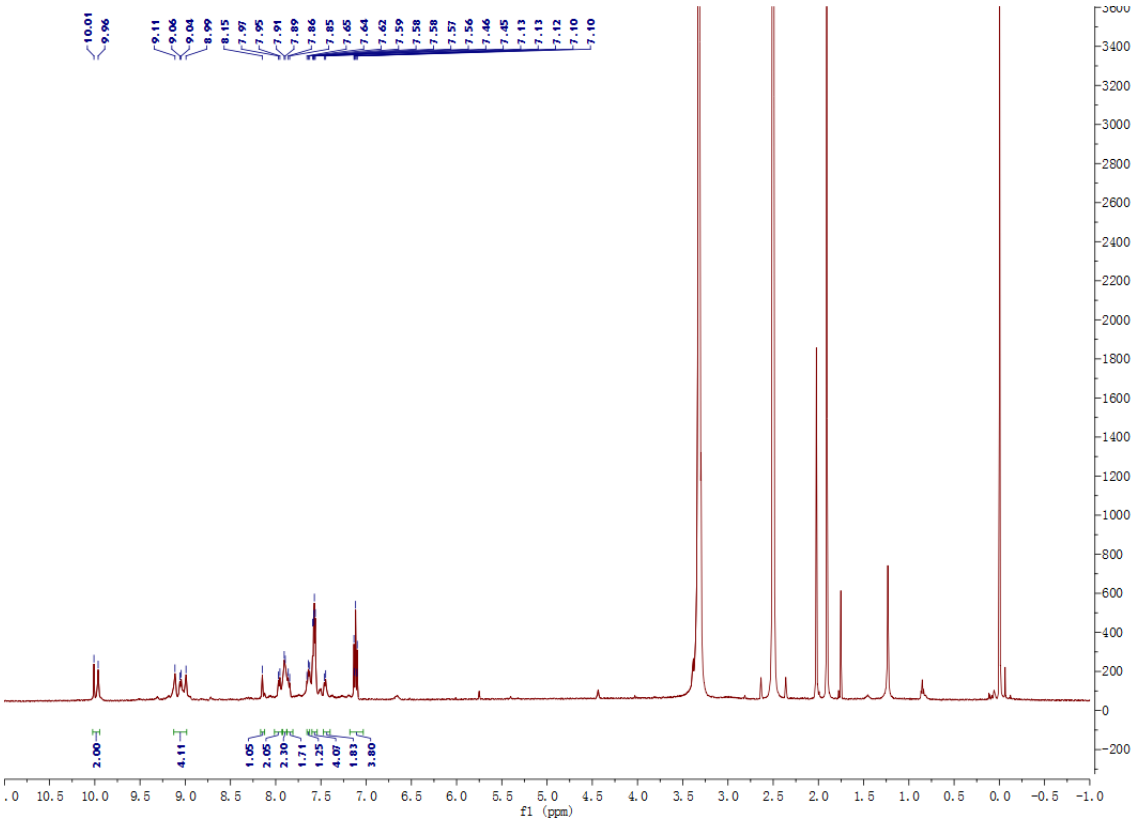


Fig. S4 ^{1}H NMR spectrum of $\mathbf{L_4}$.

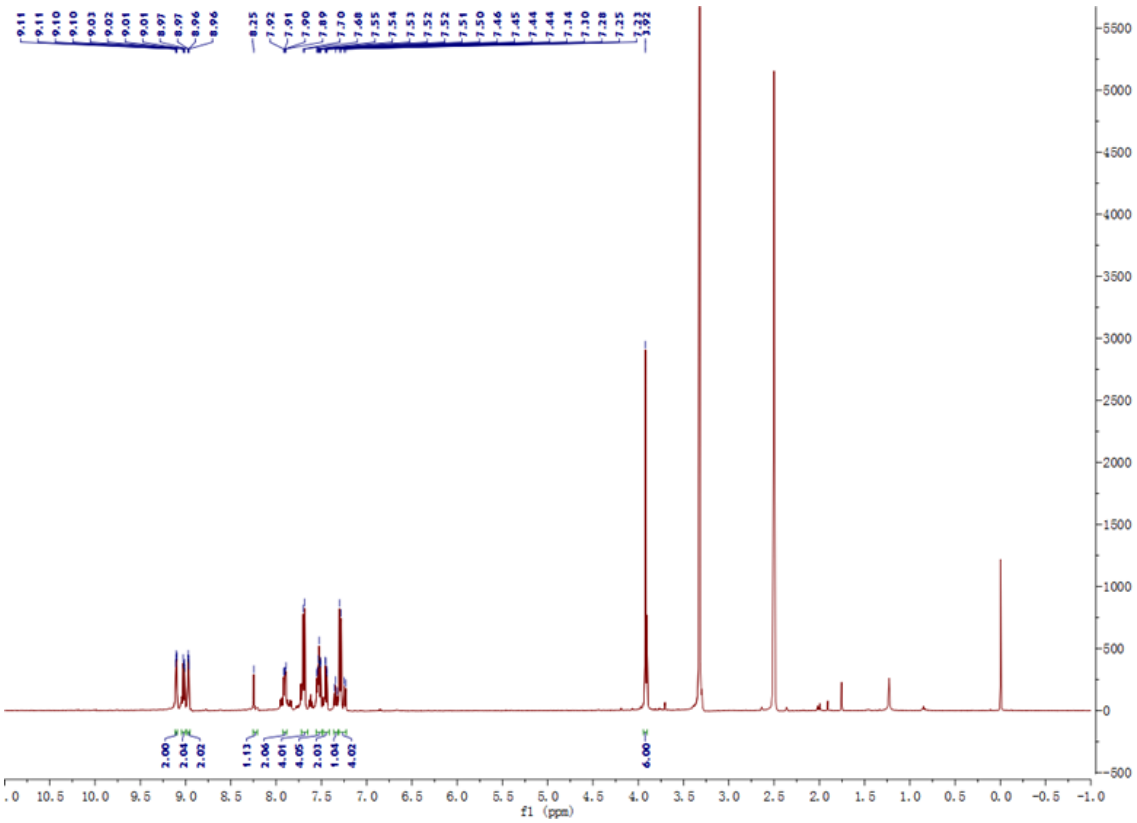


Fig. S5 ^{1}H NMR spectrum of $\mathbf{L_5}$.

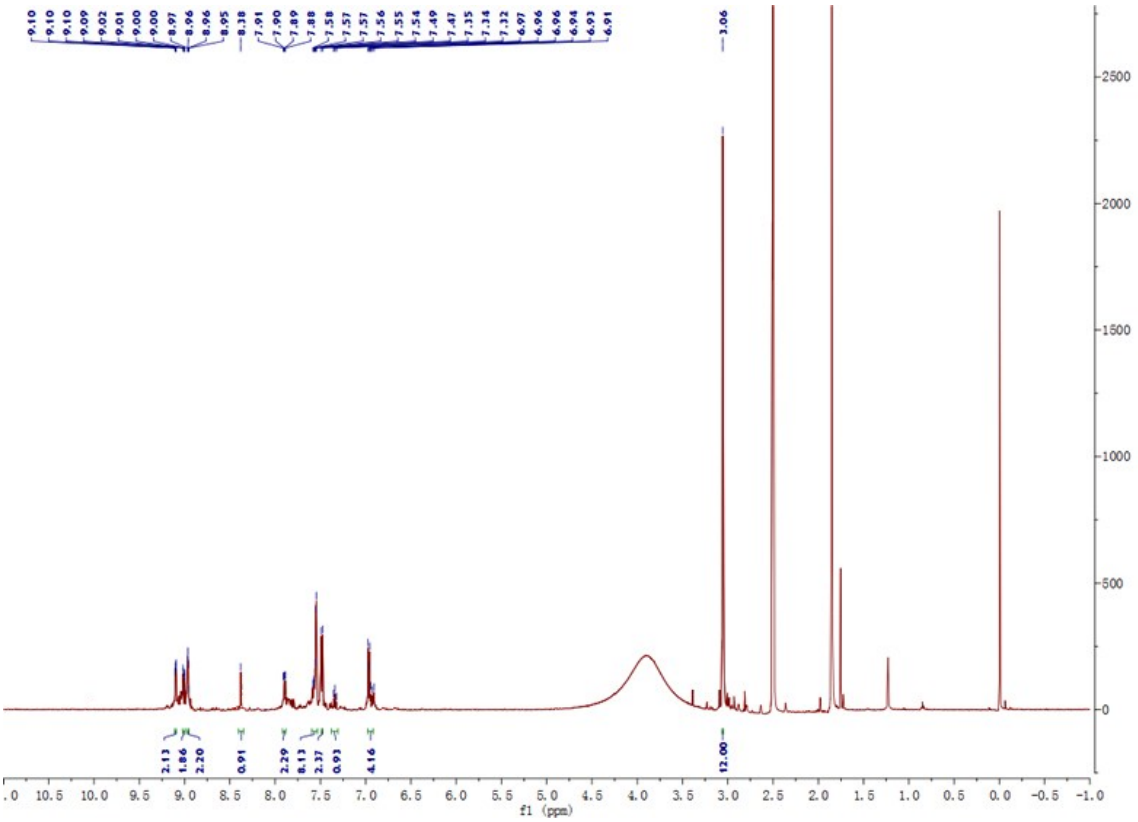


Fig. S6 ^{1}H NMR spectrum of $\mathbf{L_6}$.

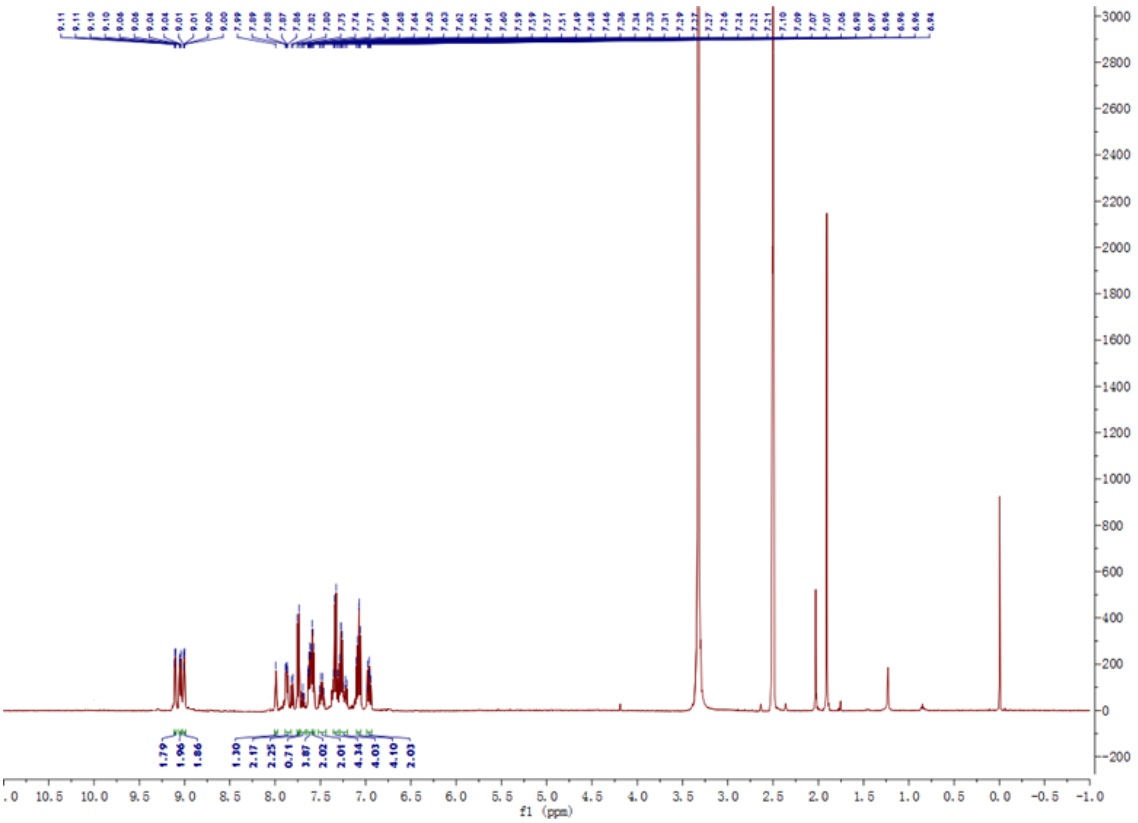


Fig. S7 ^{1}H NMR spectrum of $\mathbf{L_7}$.

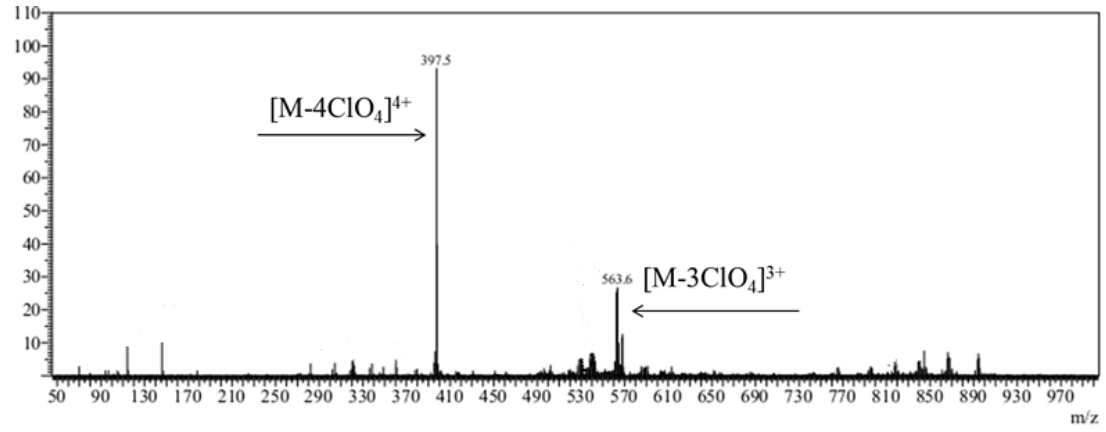


Fig. S8 ES-MS spectrum of **RuL$_1$**.

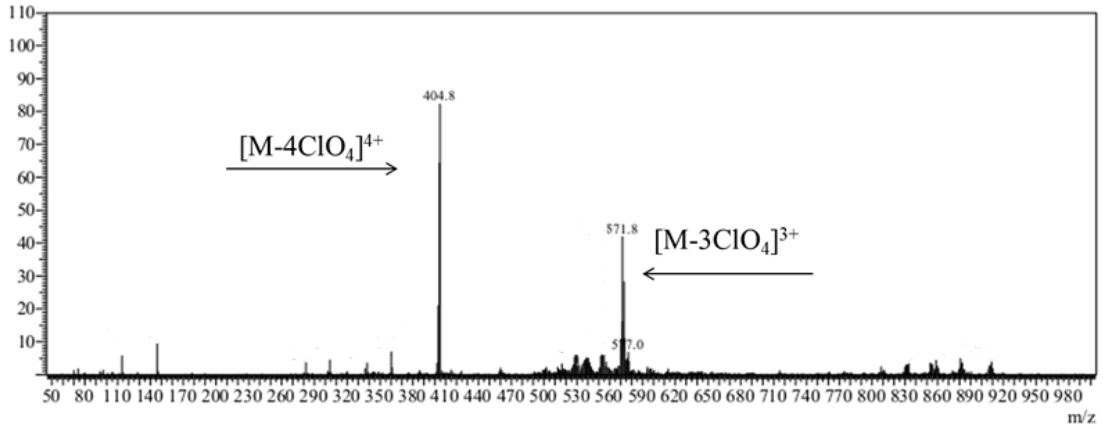


Fig. S9 ES-MS spectrum of **RuL$_2$**.

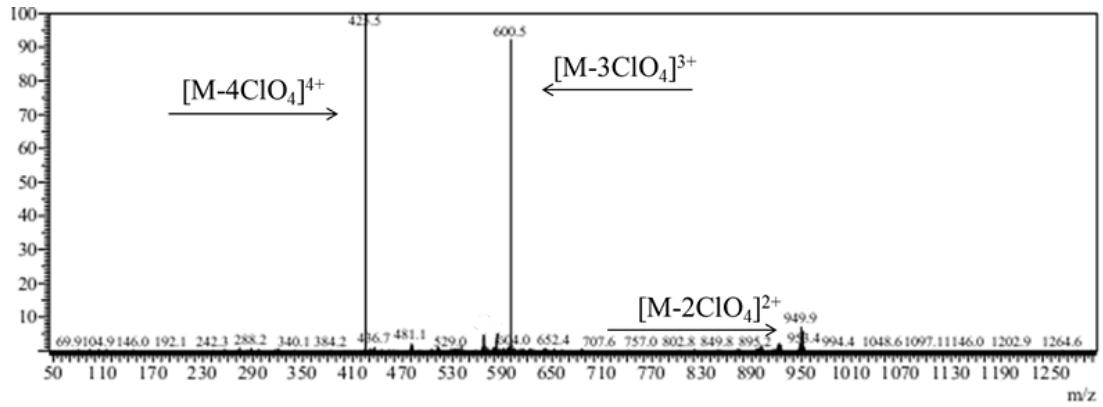


Fig. S10 ES-MS spectrum of **RuL$_3$**.

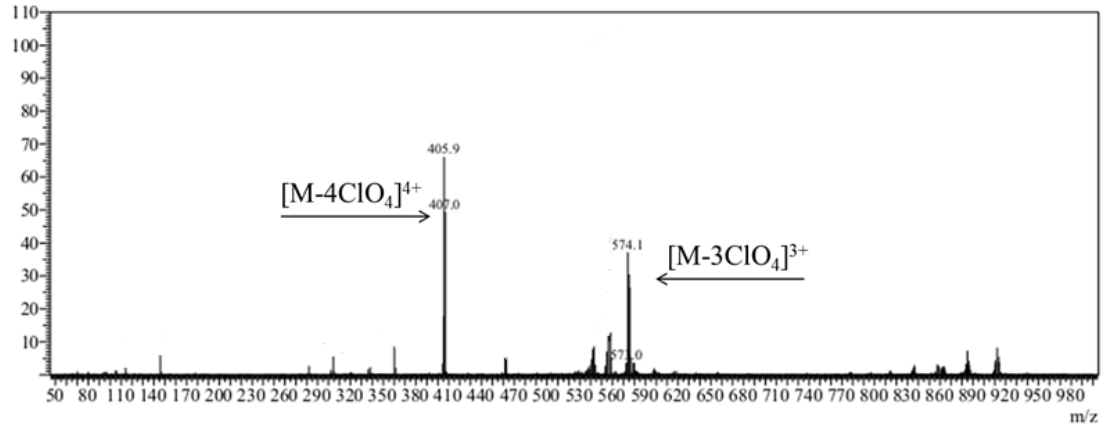


Fig. S11 ES-MS spectrum of **RuL$_4$**.

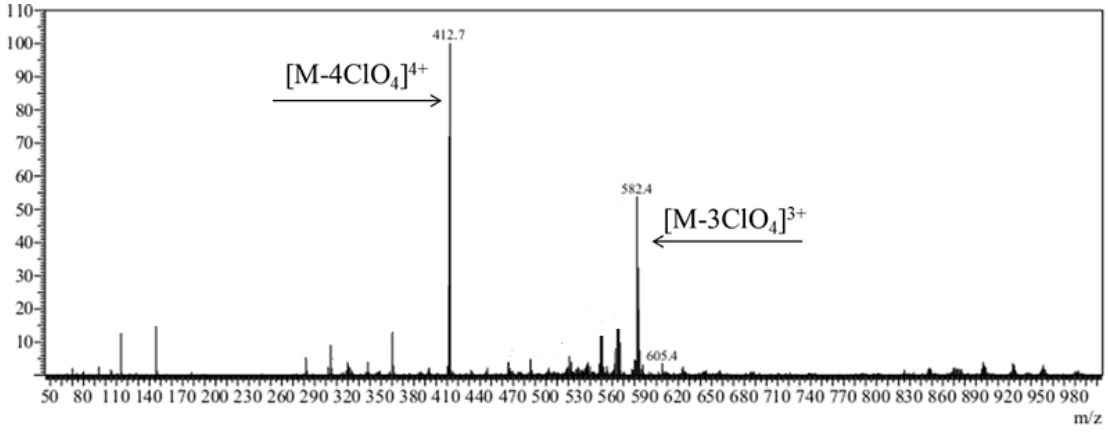


Fig. S12 ES-MS spectrum of **RuL$_5$**.

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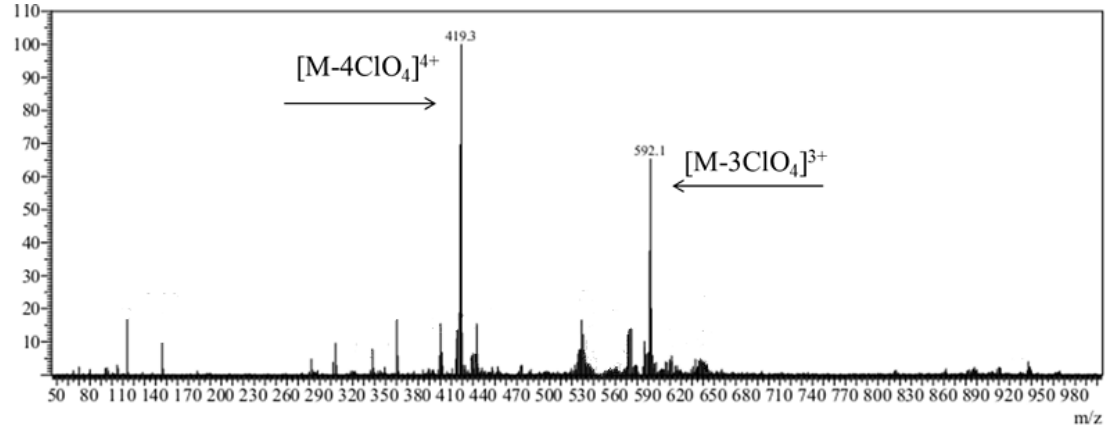

Fig. S13 ES-MS spectrum of **RuL$_6$**.

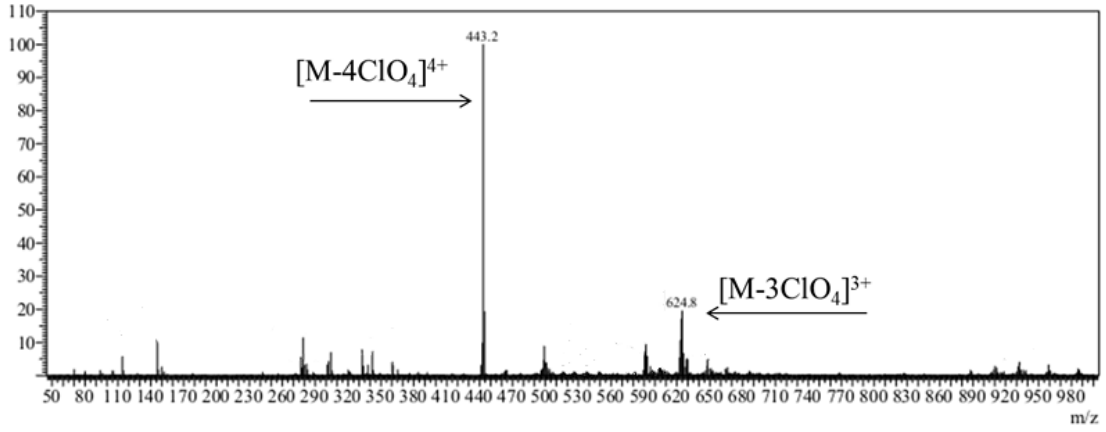

Fig. S14 ES-MS spectrum of **RuL**$_7$.

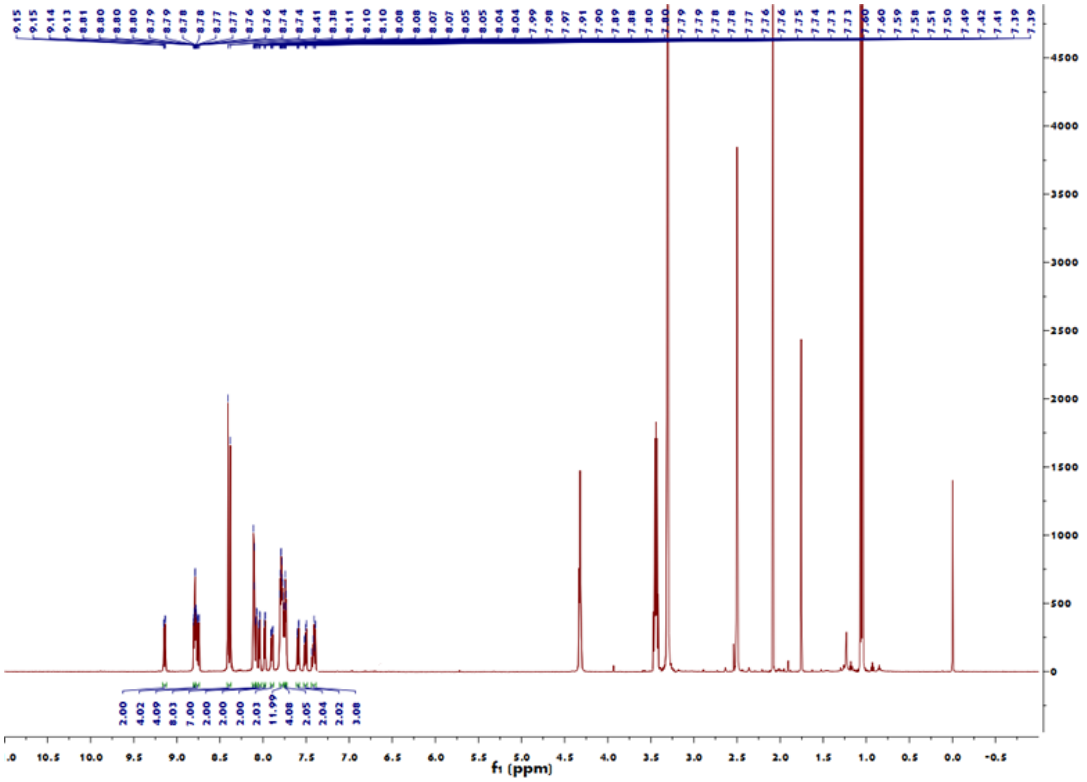

Fig. S15 ^{1}H NMR spectrum of **RuL**$_1$.

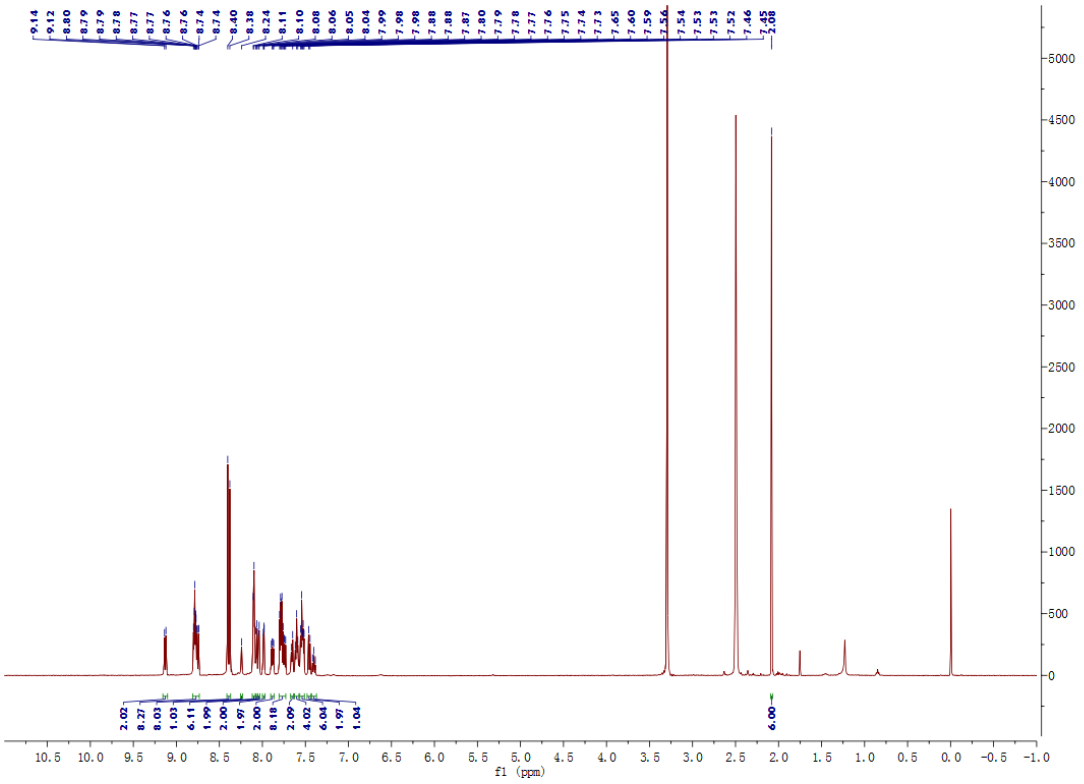

Fig. S16 ^{1}H NMR spectrum of **RuL$_2$**.

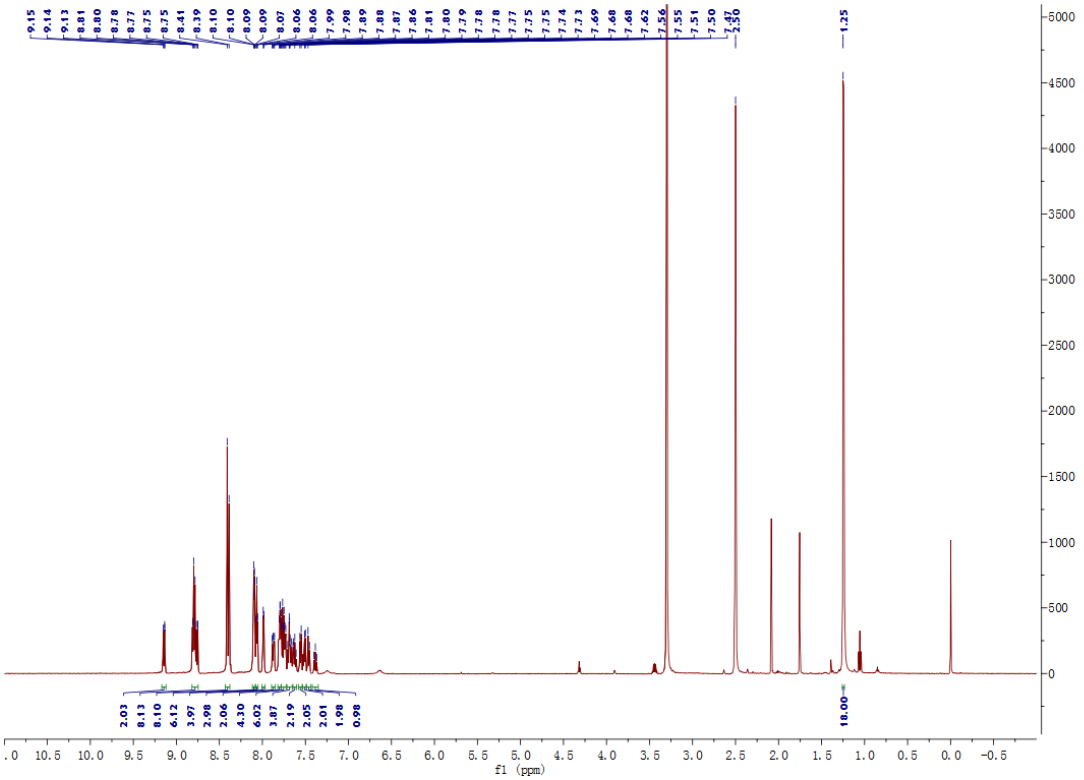

Fig. S17 ^{1}H NMR spectrum of **RuL$_3$**.

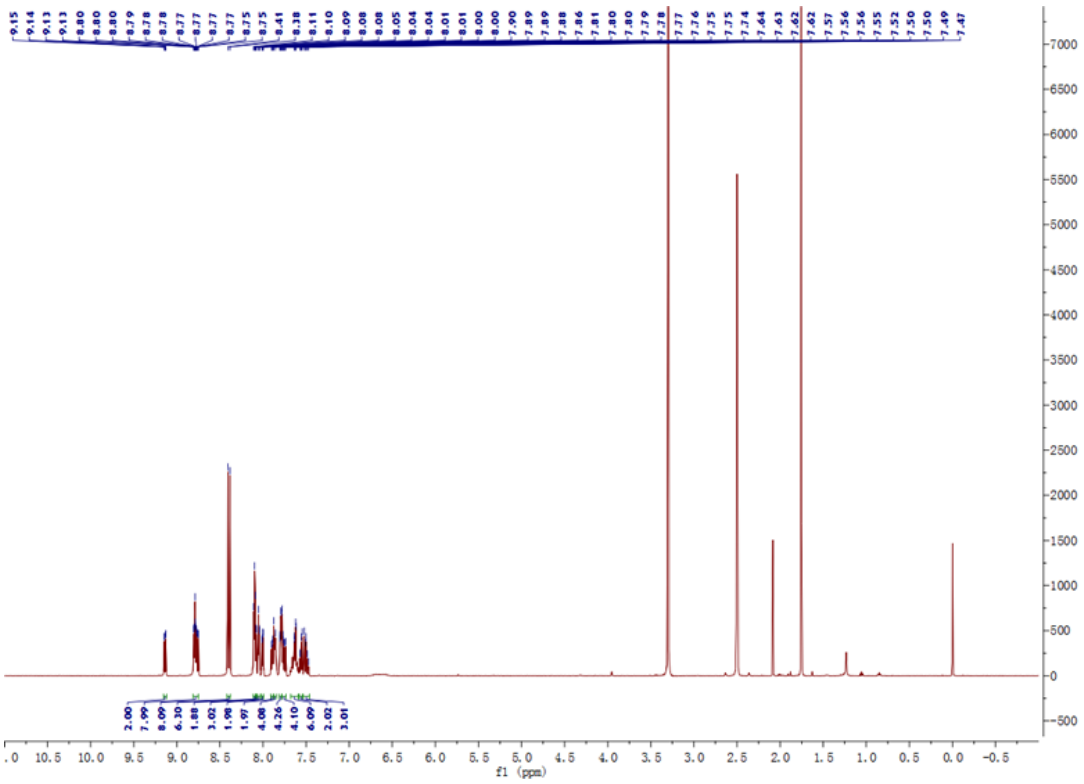

Fig. S18 ^{1}H NMR spectrum of **RuL$_4$**.

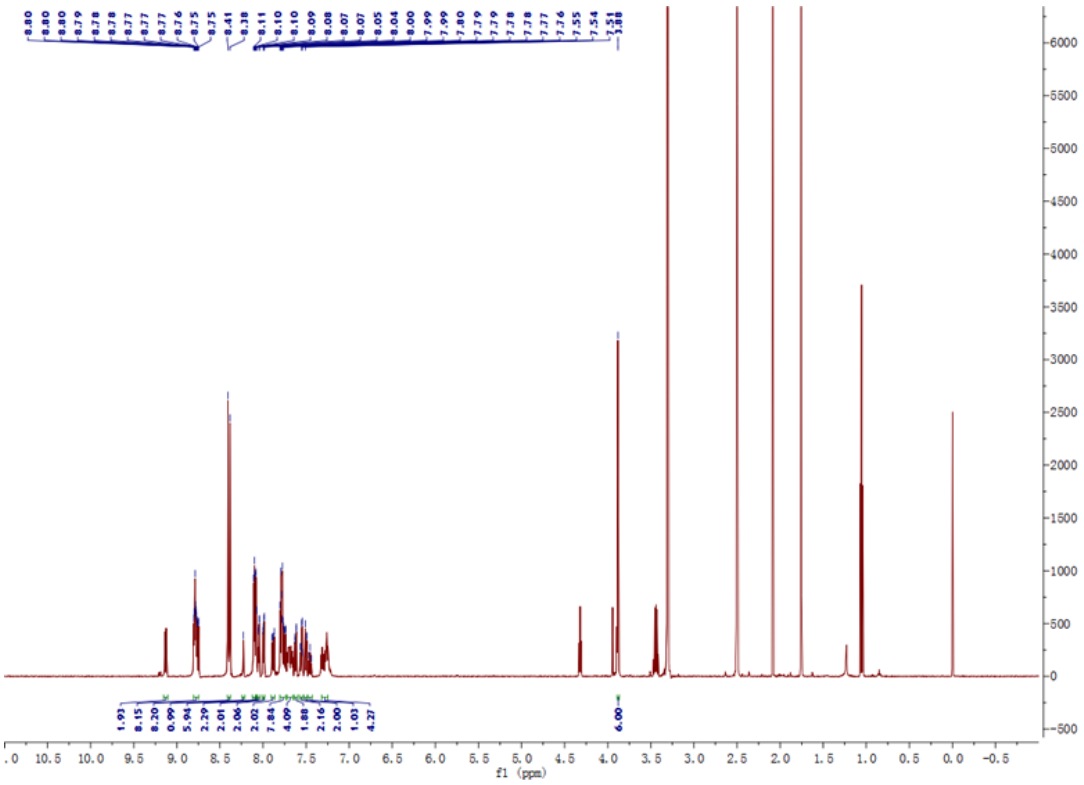

Fig. S19 ^{1}H NMR spectrum of **RuL$_5$**.

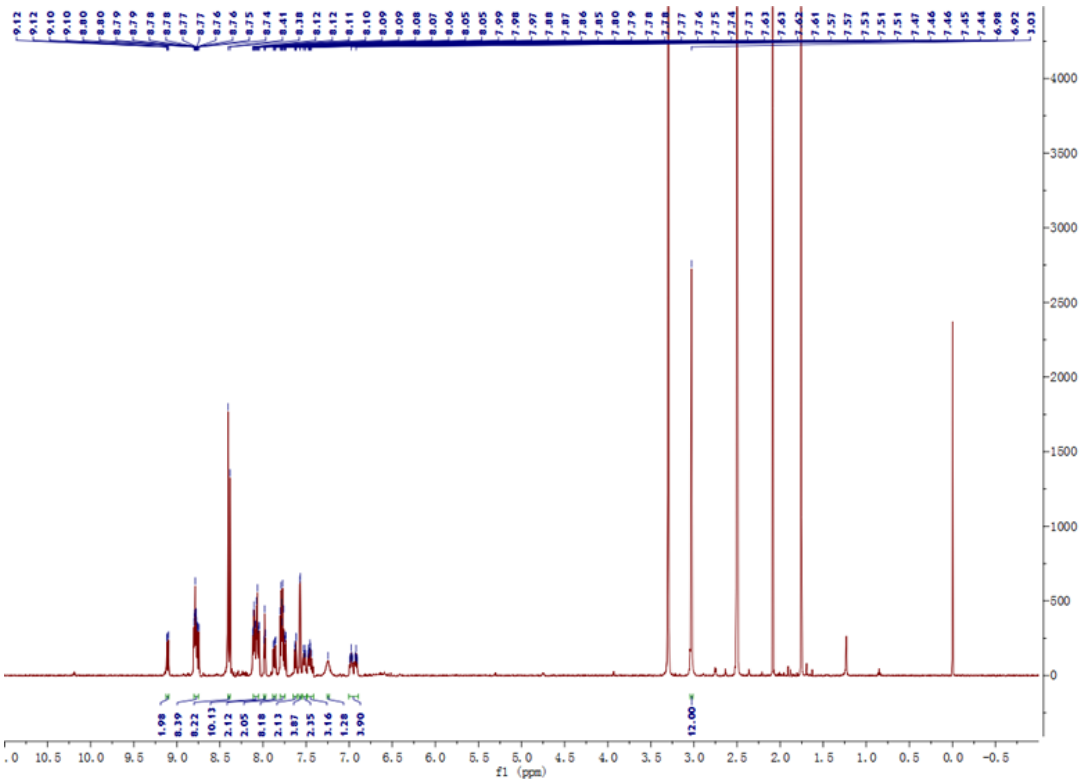

Fig. S20 ^{1}H NMR spectrum of **RuL$_6$**.

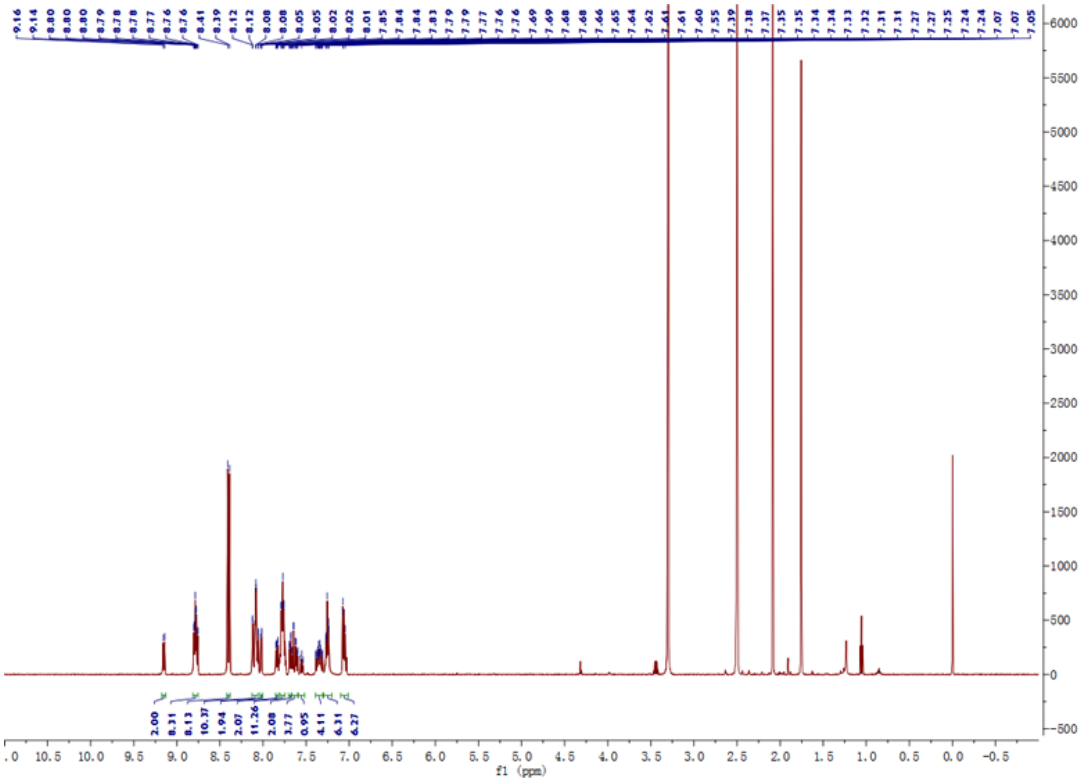

Fig. S21 ^{1}H NMR spectrum of **RuL$_7$**.

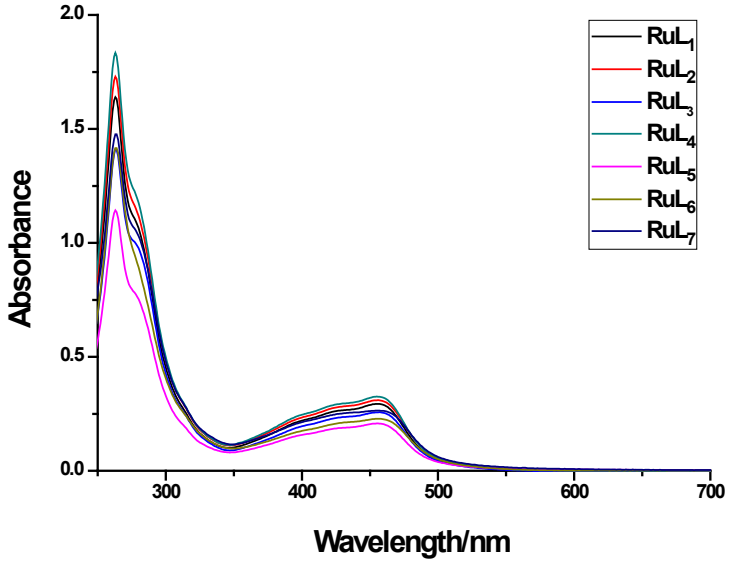

Fig. S22 Absorption spectra of **RuL**$_{1\text{-}7}$ (10 μM) in DMSO/H_2O (v/v = 1:99) solution at 298K.

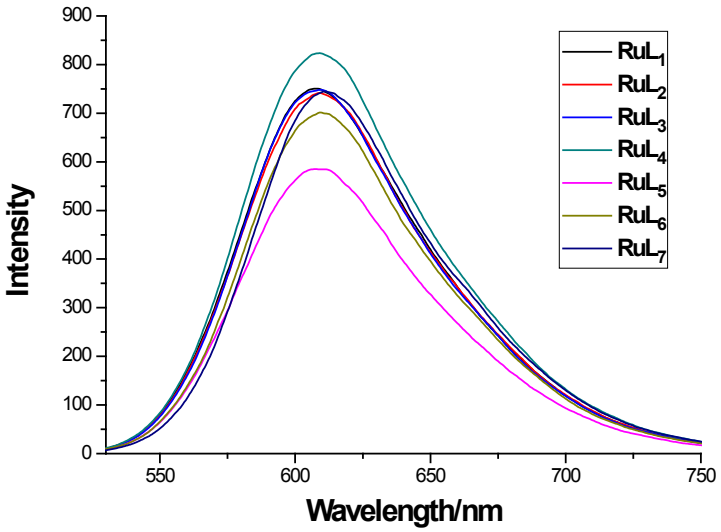

Fig. S23 Emission spectra of **RuL**$_{1\text{-}7}$ (10 μM) DMSO/H_2O (v/v = 1:99) solution at 298K.

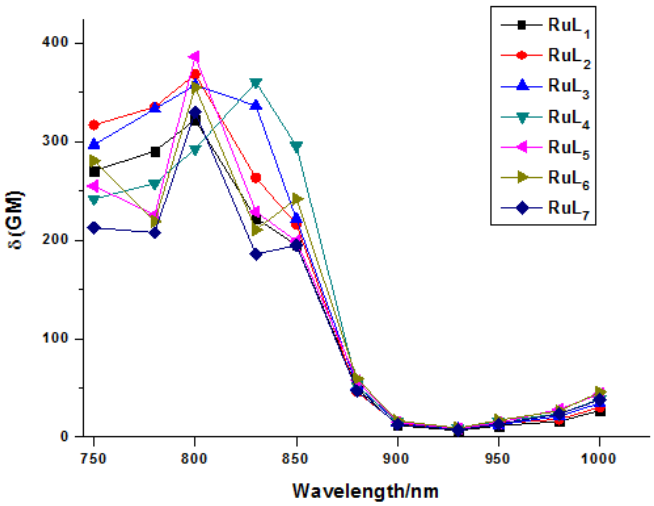

Fig. S24 Two-photon excited spectra of **RuL**$_{1\text{-}7}$ at different excitation wavelengths from 750 to 1000 nm.

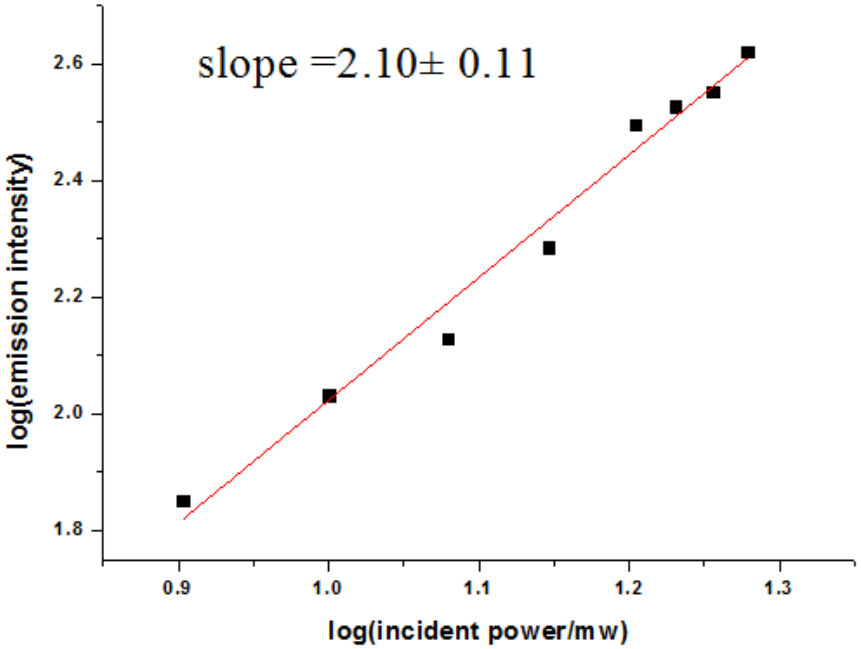

Fig. S25 The logarithmic plots of the power dependence of relative two-photon induced luminescence intensity of **RuL**$_4$ as a function of pump power at an excitation wavelength of 830 nm, respectively. The solid lines are the best-fit straight lines with gradient, n = 2.10± 0.11, indicating that **RuL**$_4$ is two-photon excitation active.

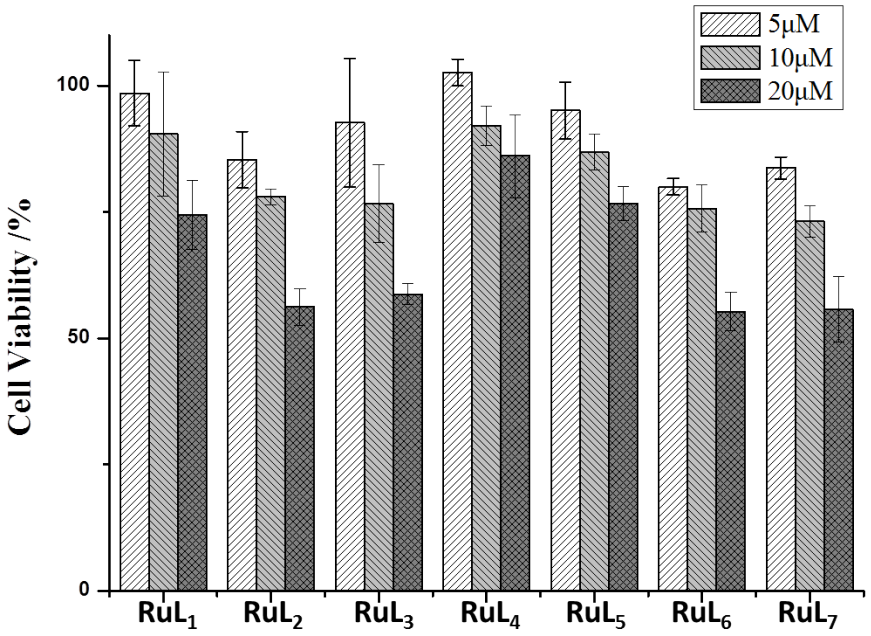

Fig. S26 Cell viability of HeLa cells with $\mathbf{RuL_{1\text{-}7}}$.

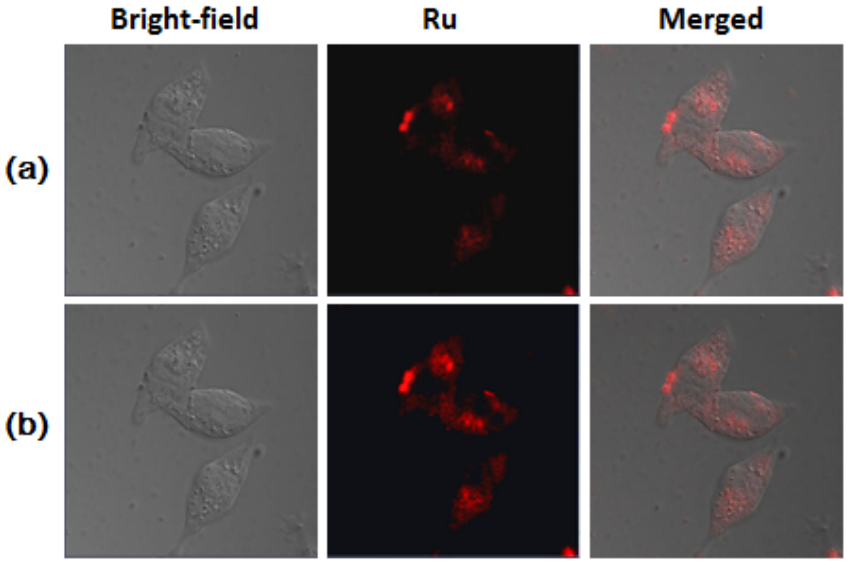

Fig. S27 OPM (a) and **TPM** (b) images of HeLa cells incubated with $\mathbf{RuL_1}$ (10 μM) for 2 h at 37 °C. The wavelengths for one- and two-photon excitation were 458 and 800 nm, respectively.

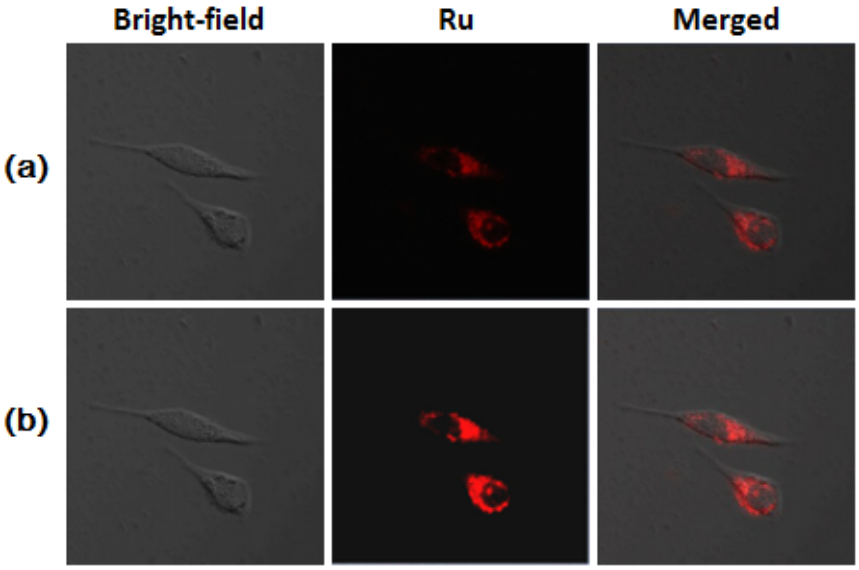

Fig. S28 OPM (a) and **TPM** (b) images of HeLa cells incubated with $\mathbf{RuL_2}$ (10 μM) for 2h at 37 °C. The wavelengths for one- and two-photon excitation were 458 and 800 nm, respectively.

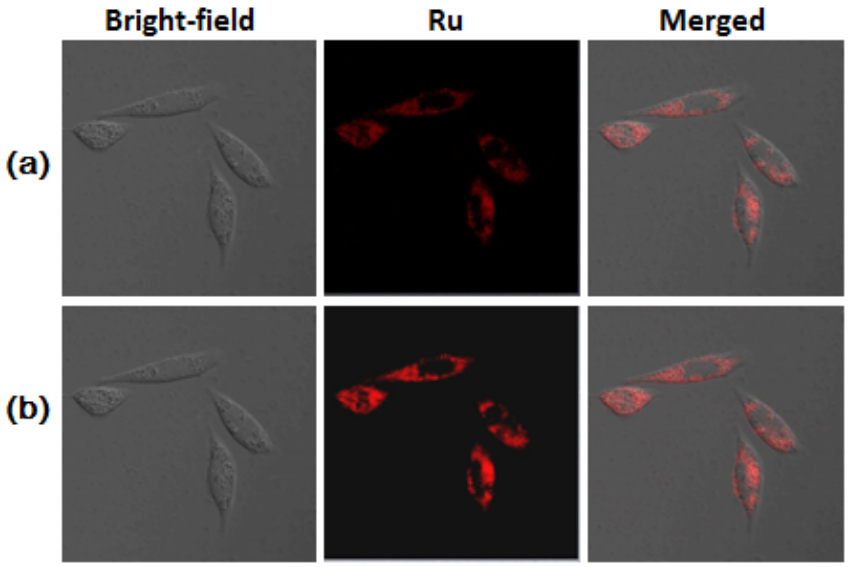

Fig. S29 OPM (a) and **TPM** (b) images of HeLa cells incubated with $\mathbf{RuL_3}$ (10 μM) for 2h at 37 °C. The wavelengths for one- and two-photon excitation were 458 and 800 nm, respectively.

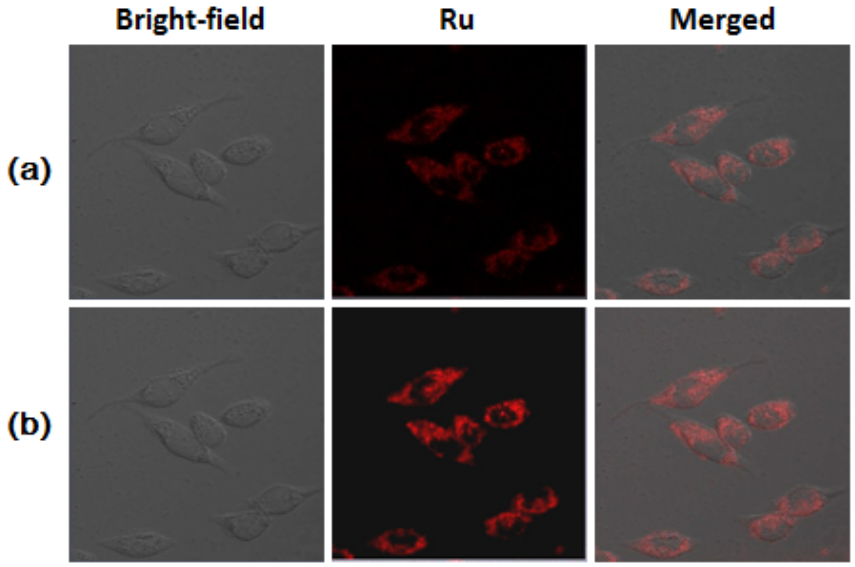

Fig. S30 OPM (a) and **TPM** (b) images of HeLa cells incubated with **RuL$_5$** (10 μM) for 2h at 37 °C. The wavelengths for one- and two-photon excitation were 458 and 800 nm, respectively.

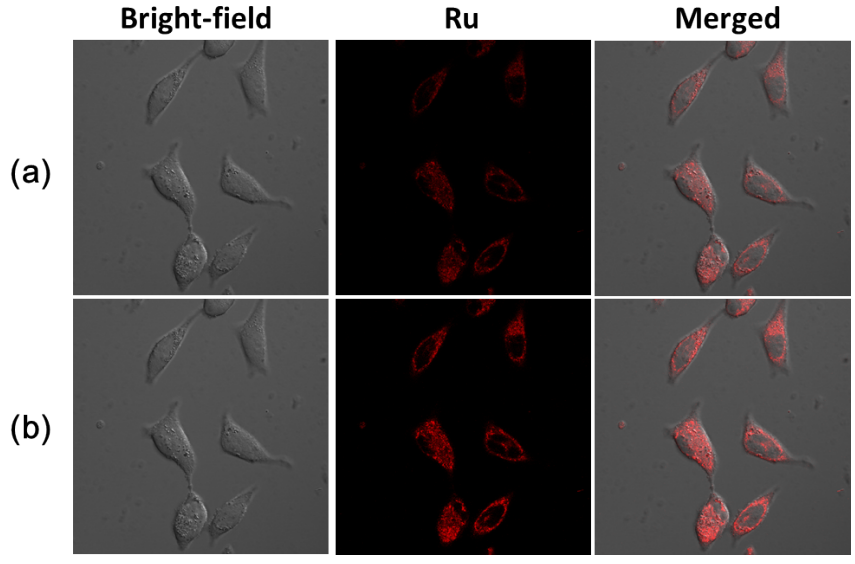

Fig. S31 OPM (a) and **TPM** (b) images of HeLa cells incubated with **RuL$_6$** (10 μM) for 2 h at 37 °C. The wavelengths for one- and two-photon excitation were 458 and 800 nm, respectively.

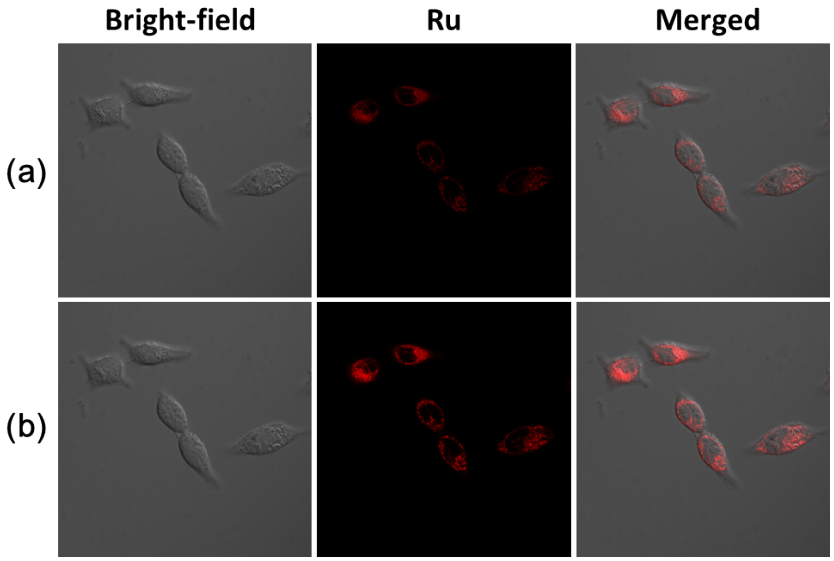

Fig. S32 OPM (a) and **TPM** (b) images of HeLa cells incubated with **RuL$_7$** (10 μM) for 2h at 37 °C. The wavelengths for one- and two-photon excitation were 458 and 800 nm, respectively.

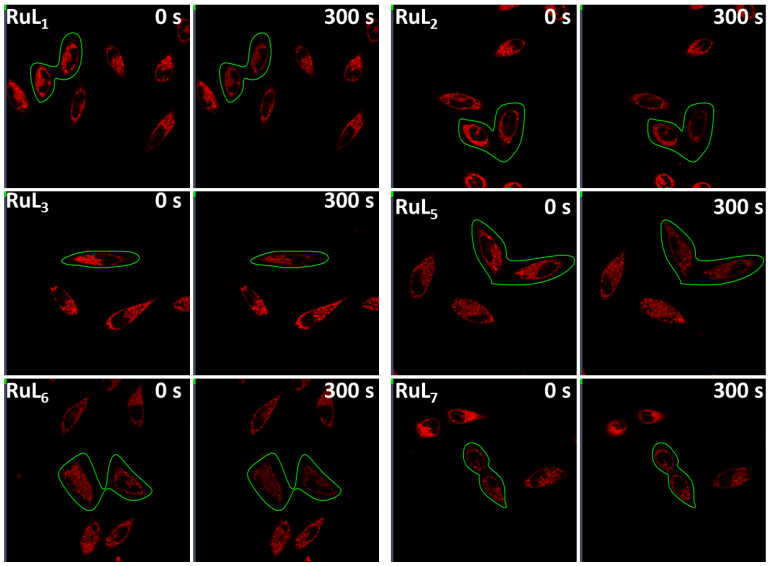

Fig. S33 Photostability experiments of **RuL$_{1\text{-}7}$**. The images were taken under successive irradiation. The wavelength for **RuL$_{1\text{-}7}$** was 458 nm .

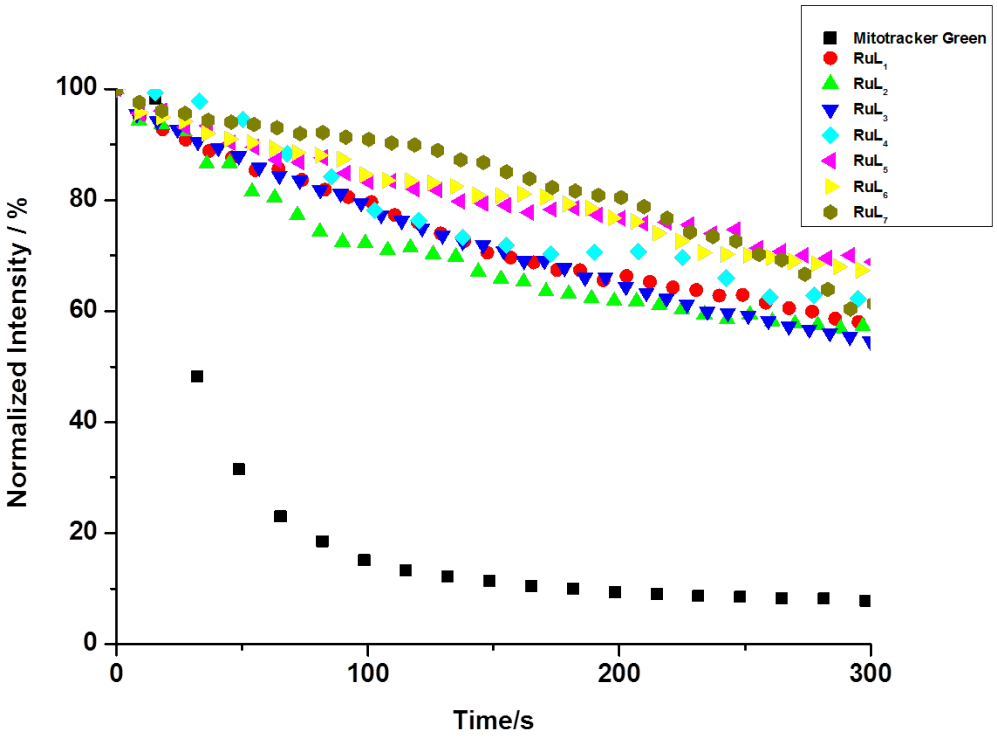

Fig. S34 Normalized fluorescence intensity curves of **RuL$_{1\text{-}7}$** and Mitotracker Green under successive irradiation. The wavelengths for **RuL$_{1\text{-}7}$** and Mitotracker Green irradiation were 458 and 488 nm, respectively.

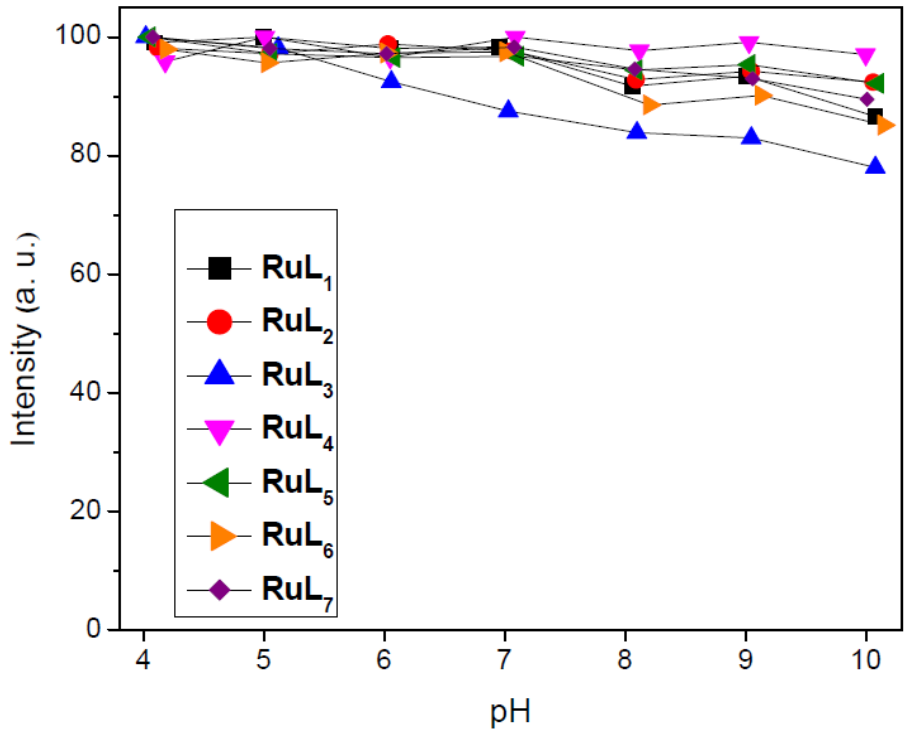

Fig. S1 The emission intensity of 10 μM **RuL$_{1\text{-}7}$** at 608 nm under different pH in Britton-Robinson buffer.

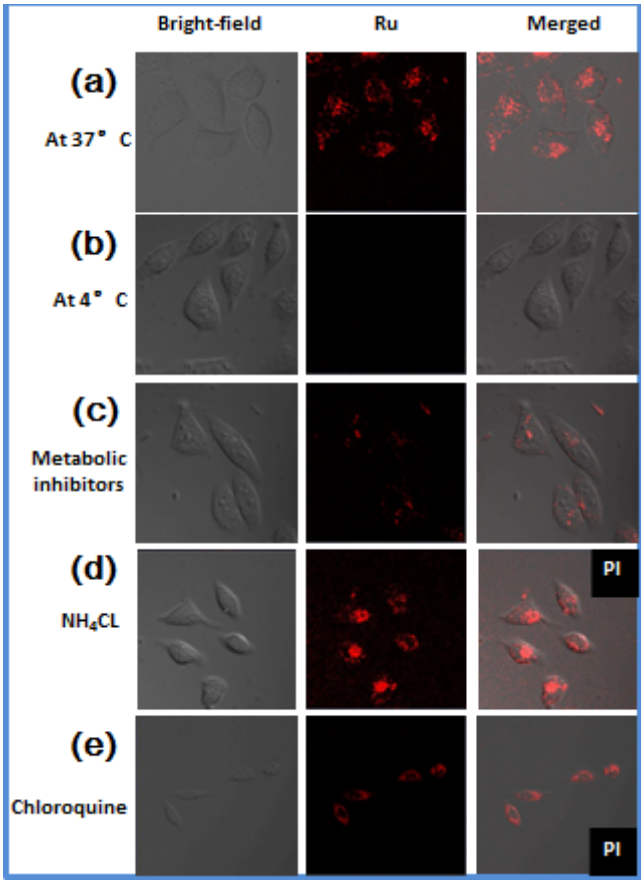

Fig. S36. OPM images of living HeLa cells incubated with 10 μM **RuL$_4$** under different conditions. (a) The cells were incubated with 10 μM **RuL$_4$** at 37 ^{o}C for 2 h. (b) The cells were incubated with 10 μM **RuL$_4$** at 4 °C for 2 h. (c) The cells were preincubated with 50 mM 2-deoxy-D-glucose and 5 μM oligomycin in PBS for 1 h at 37 ^{o}C and then incubated with 10 μM **RuL$_4$** at 37 °C for 2 h. (d and e) The cells were pretreated with endocytic inhibitors NH_4Cl (50 mM), and chloroquine (50 μM) respectively, and then incubated with 10 μM **RuL$_4$** at 37 ^{o}C for 2 h. PI staining is included for each to indicate cell viability.

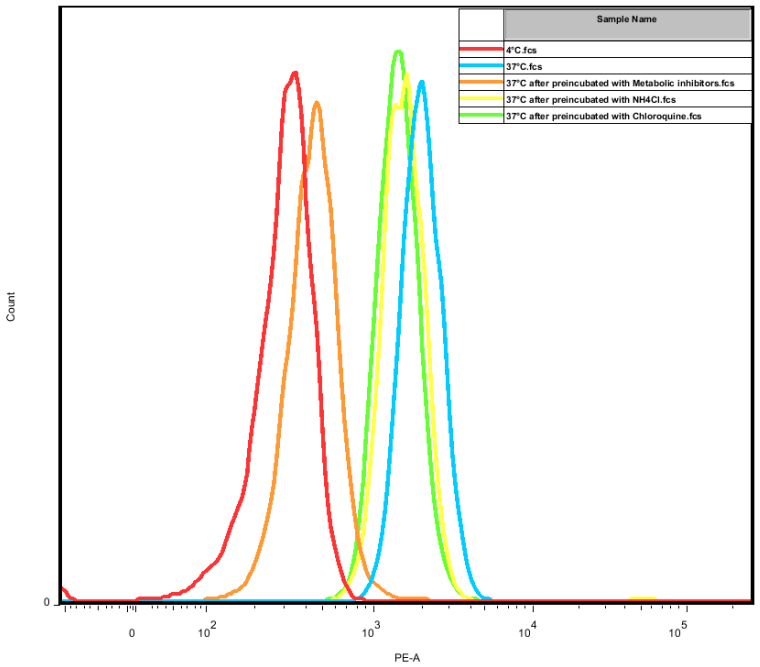

Fig. S37. Flow cytometric histogram profile of cellular uptake of **RuL$_4$** in HeLa cells. HeLa cells were incubated with 10 μM **RuL$_4$** for 2 h at 37 ℃ (blue), 4 ℃ (red), and 37 ℃ after the cells had been preincubated with metabolic inhibitors 2-deoxy-D-glucose (50 mM) and oligomycin (5 μM) in PBS for 1 h at 37℃ (orange), endocytic inhibitors NH_4Cl (50 mM) (yellow) and chloroquine (50 μM) (green) in PBS for 1 h at 37℃ respectively.

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