Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Synthesis, Photophysical Property and *In Vitro* Evaluation of Chlorambucil Conjugated Ruthenium (II) Complex for Combined Chemo-Photodynamic Therapy against HeLa Cell

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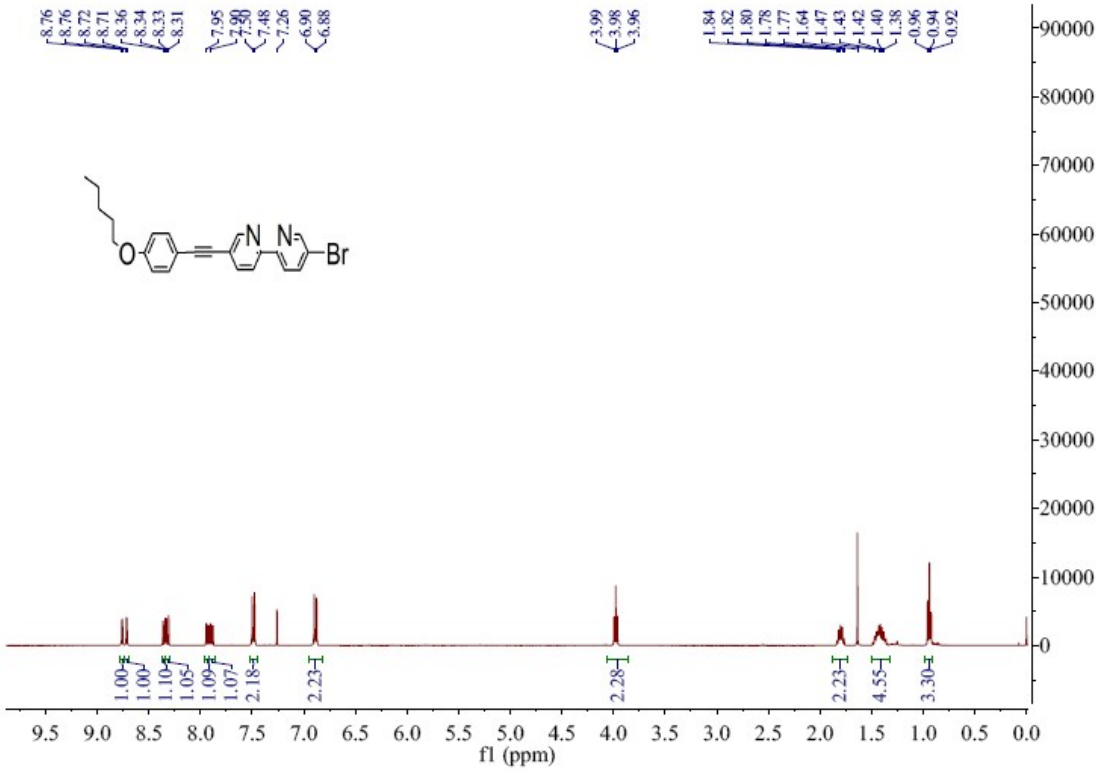


Fig. S1 ^{1}H NMR ($CDCl_3$) of **5–bromo–5'– (2''– (4'''– (pentyloxy)phenyl)ethynyl)–2,2'–bipyridine (1)**

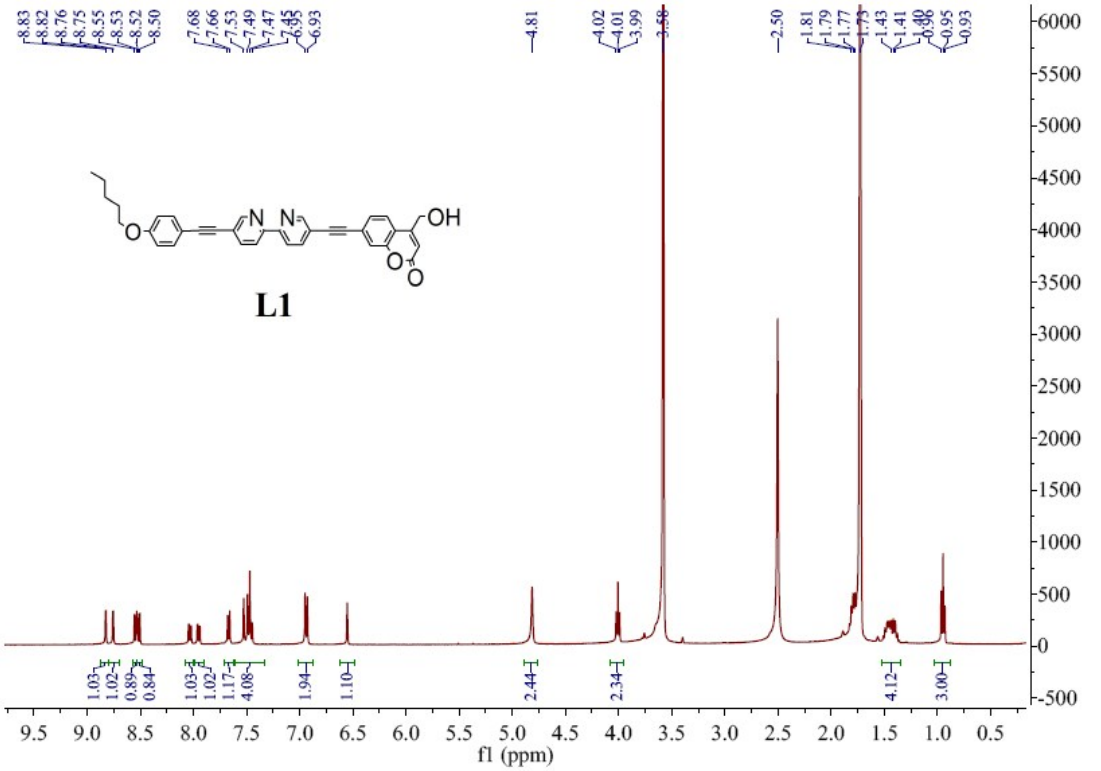


Fig. S2 ^{1}H NMR (THF-d_8) of **L1**

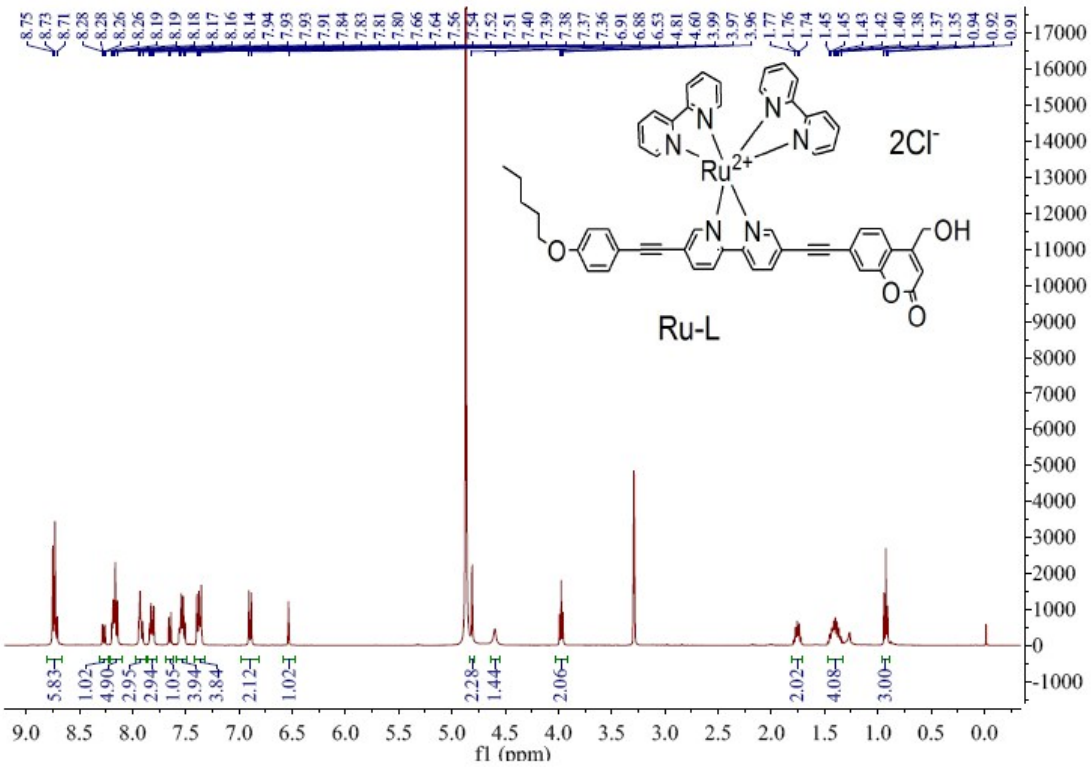


Fig. S3 ^{1}H NMR (MeOH-d_4) of **Ru-L**

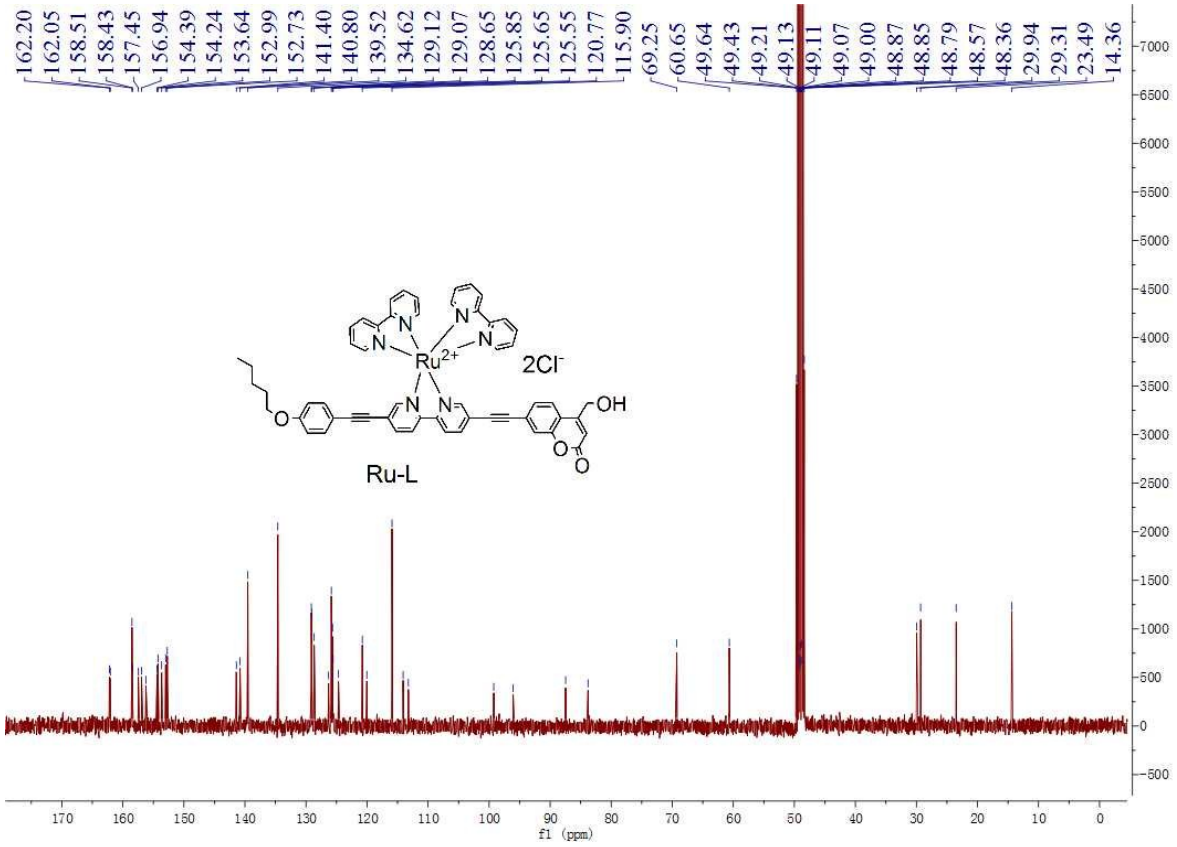


Fig. S4 ^{13}C NMR (MeOH-d_4) of **Ru-L**

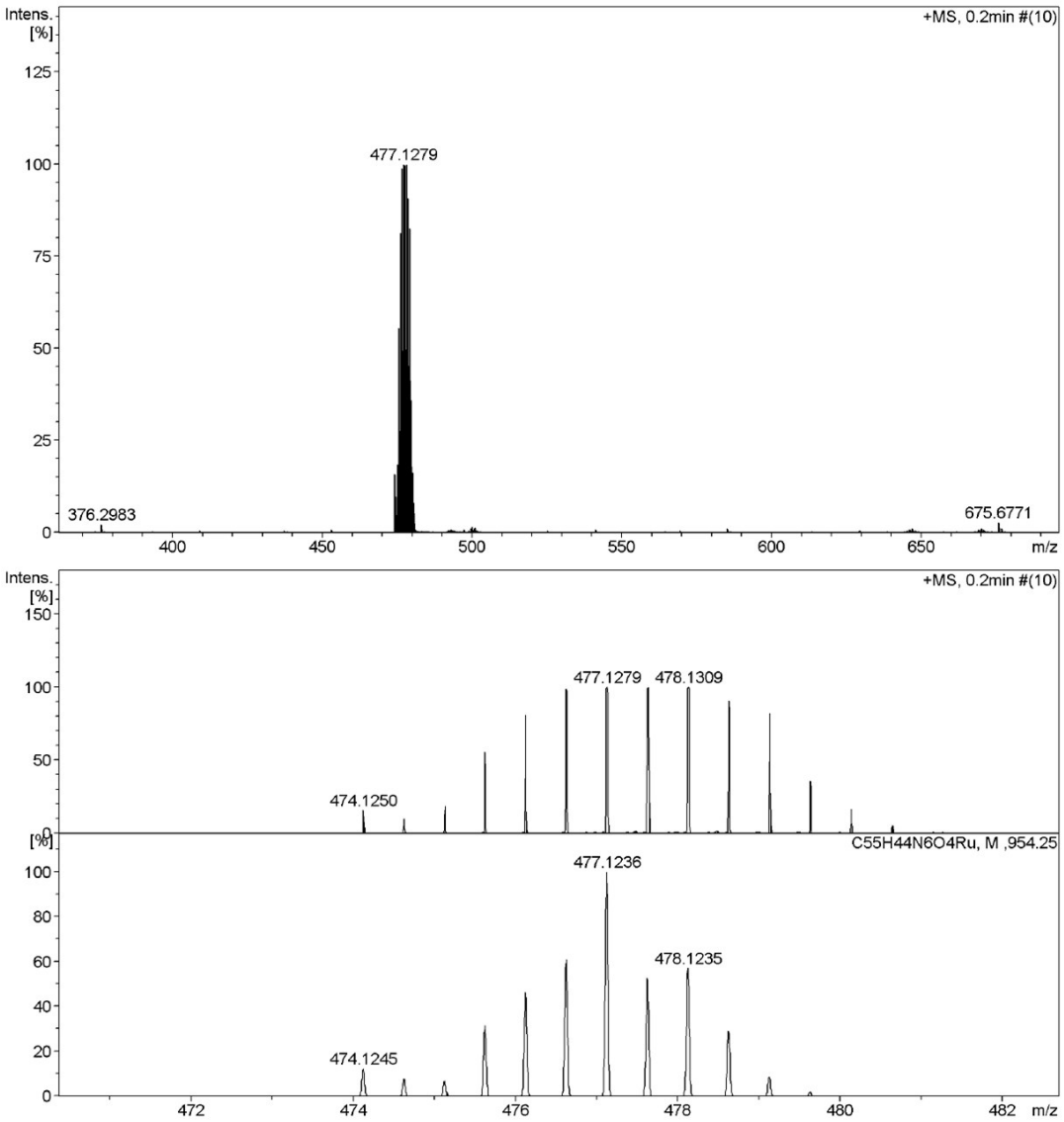


Fig. S5 Experimental and Simulated HR-ESI Mass Spectrum of **Ru-L**

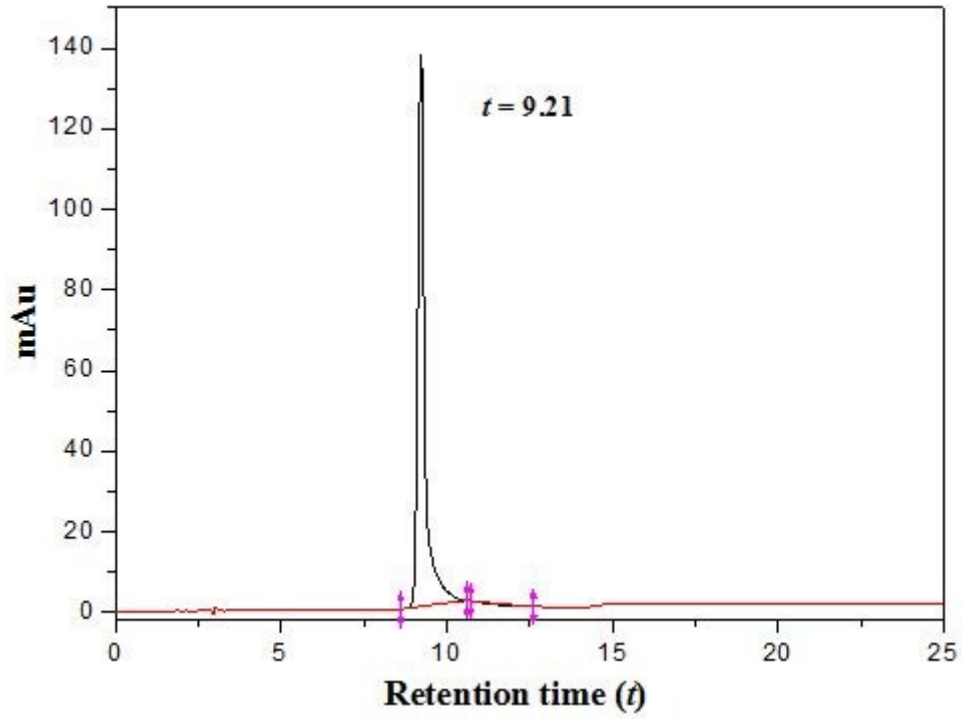


Fig. S6 HPLC chromatogram of **Ru-L**: gradient mobile phase = 30% acetonitrile for 10 min, 90% acetonitrile for 10 min, then 30% acetonitrile for 10 min, flow rate = 1 mL/min, detection at 254 nm = t_r (retention time) =9.21 min; purity = 99.0 %.

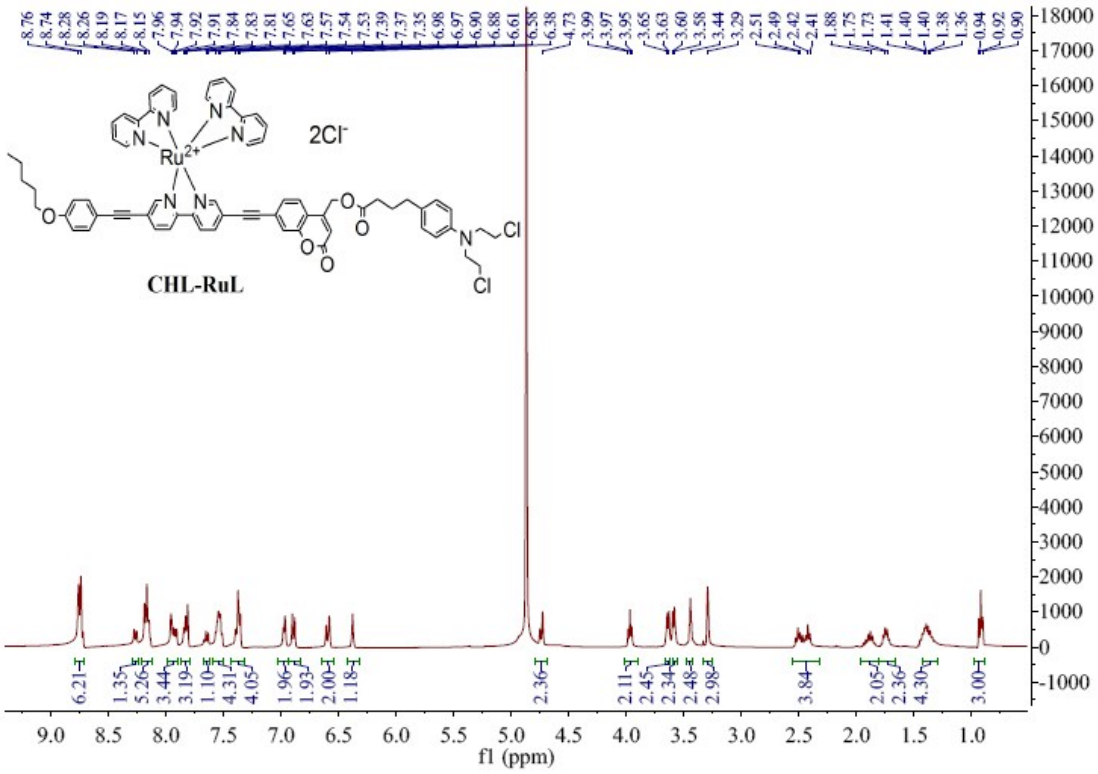


Fig. S7 ^{1}H NMR (MeOH-d_4) of **CHL-RuL**.

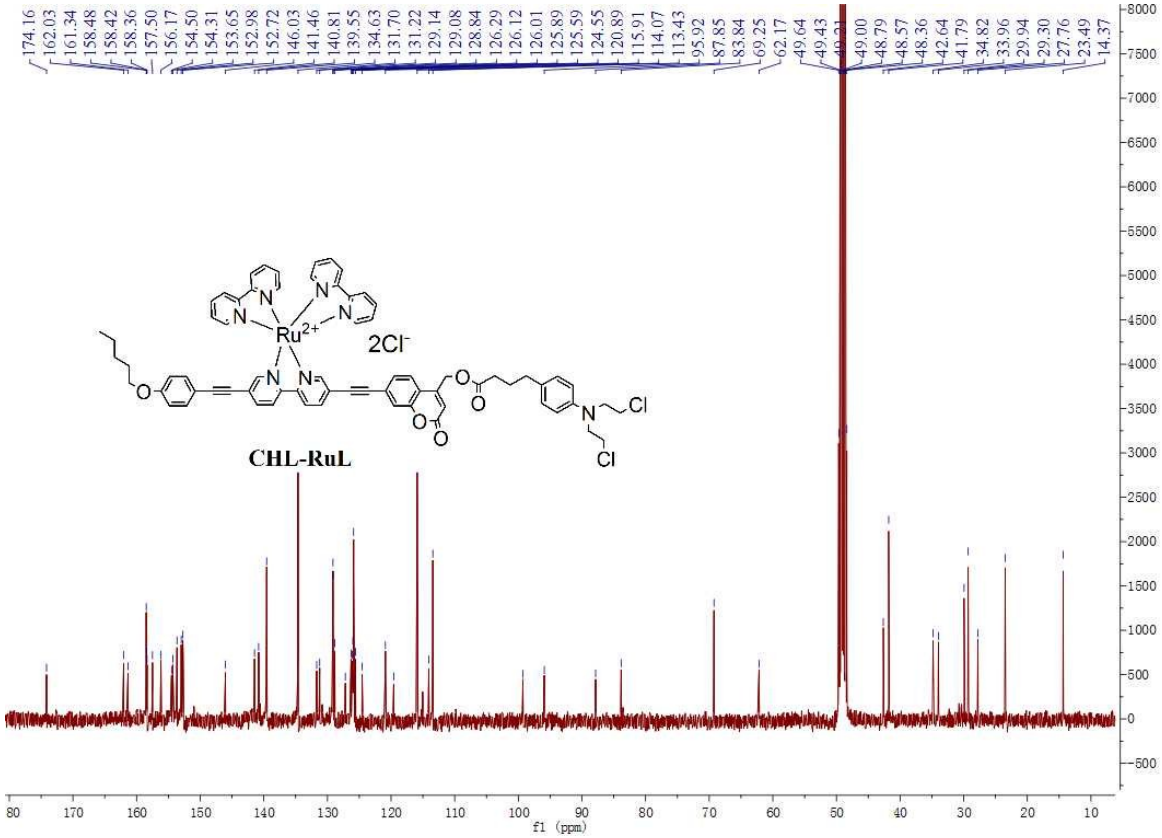
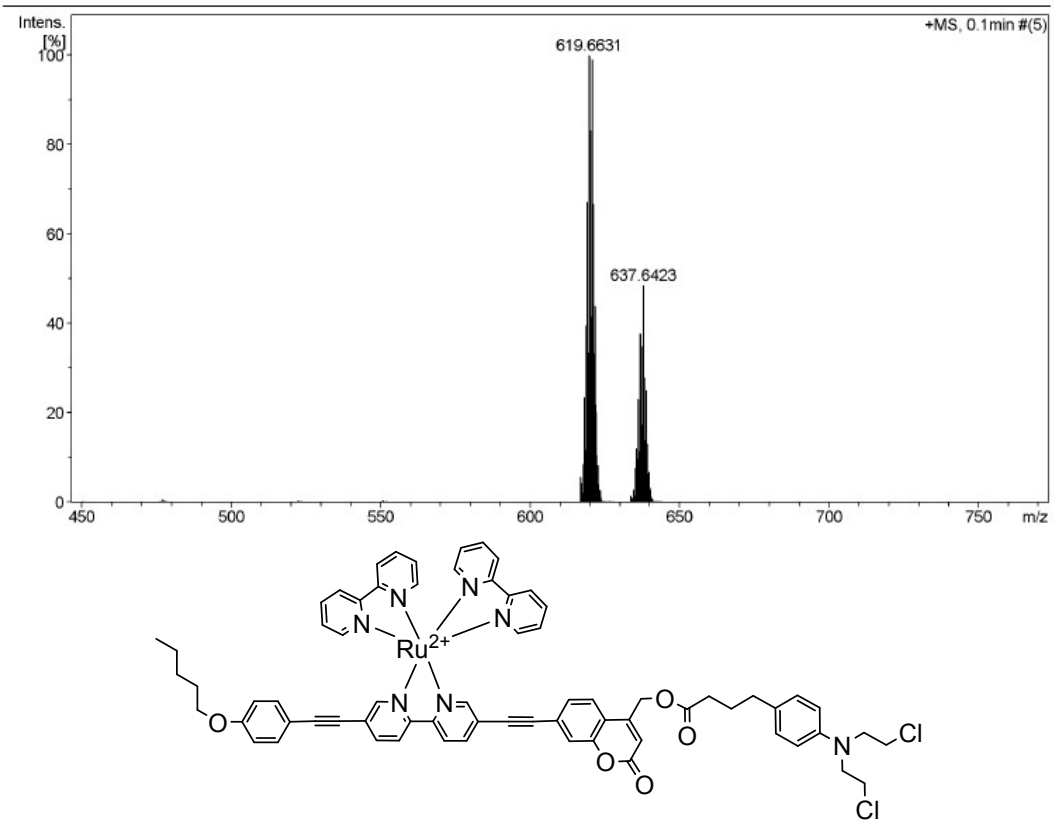
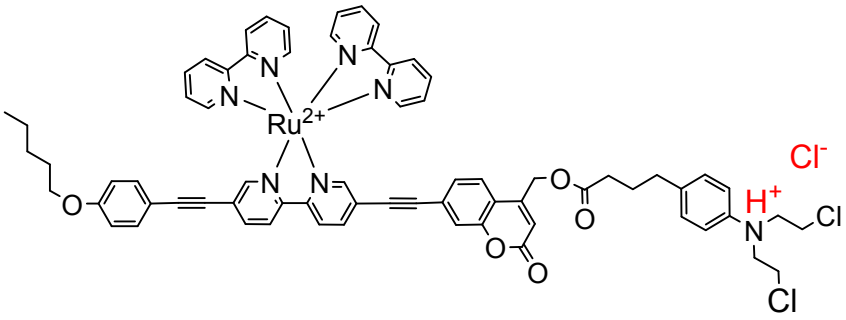


Fig. S8 ^{13}C NMR (MeOH-d_4) of **CHL-RuL**.



Calculated(m/z): $C_{69}H_{61}Cl_2N_7O_5Ru^{2+}$, 619.6578; Found: 619.6631.



Calculated(m/z): $C_{69}H_{62}Cl_3N_7O_5Ru^{2+}$, 637.6455; Found: 637.6423.

Fig. S9 Experimental HR-ESI Mass Spectrum of **CHL-RuL** and corresponding structure fragment.

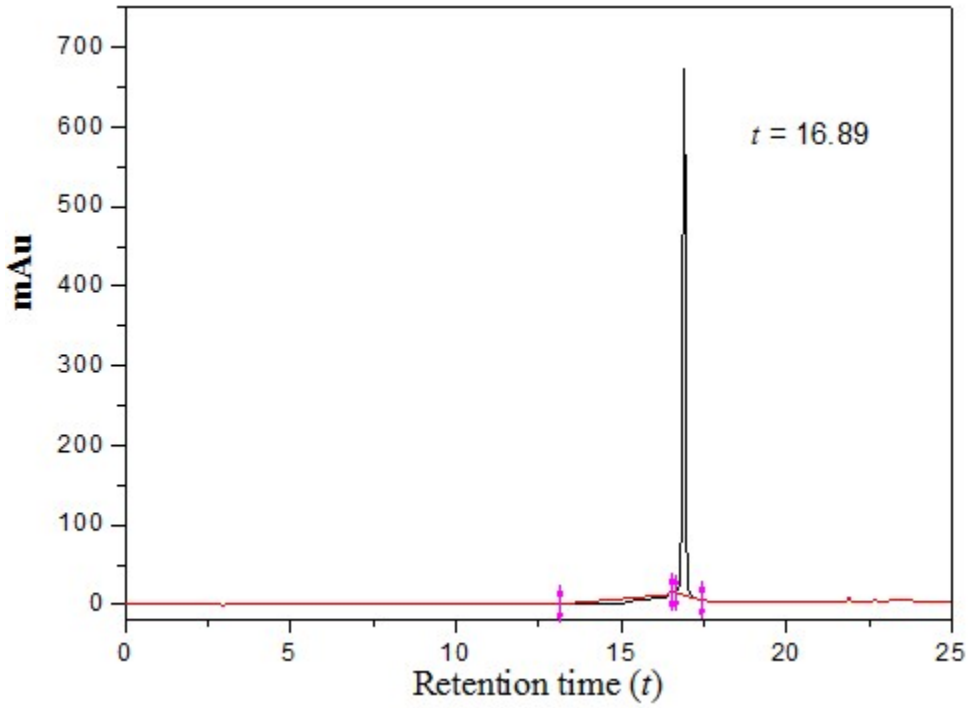


Fig. S10 HPLC chromatogram of **CHL-RuL**: gradient mobile phase = 30% acetonitrile for 10 min, 90% acetonitrile for 10 min, then 30% acetonitrile for 10 min, flow rate = 1 mL/min, detection at 254 nm = t_r (retention time) =16.89 min; purity = 98.6 %.

Singlet Oxygen Quantum Yield Measurements

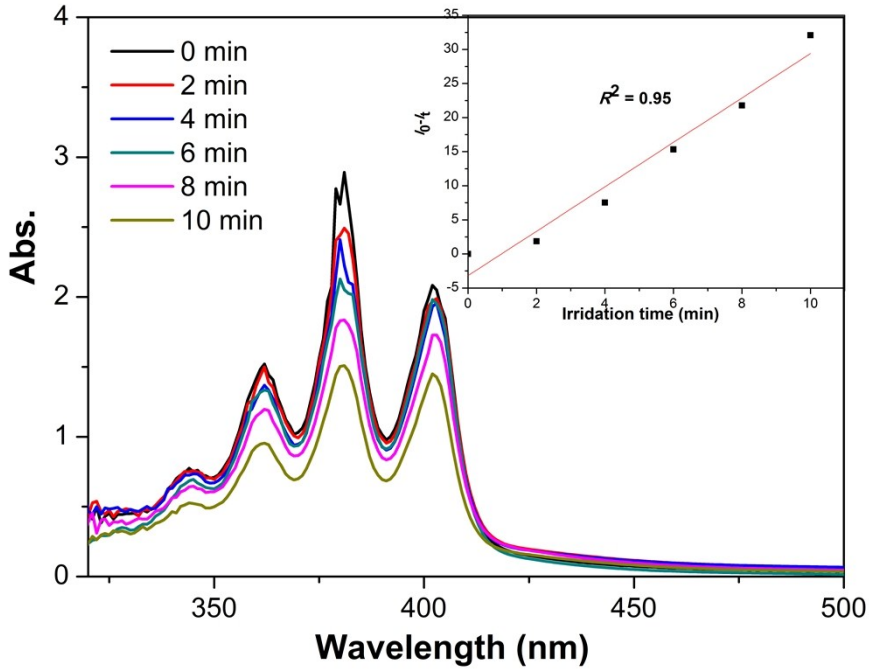


Fig. S11 ABDA Absorption decrease in the DMSO-PBS (1/199) solution containing **Ru-L** (10μM) and ABDA (200μM) with exposed to a visible light in different times (Inset: linear relationship of (I_t-I_0) *vs* t, *I* is integration of absorption of ABDA, *t* is duration time of illumination)

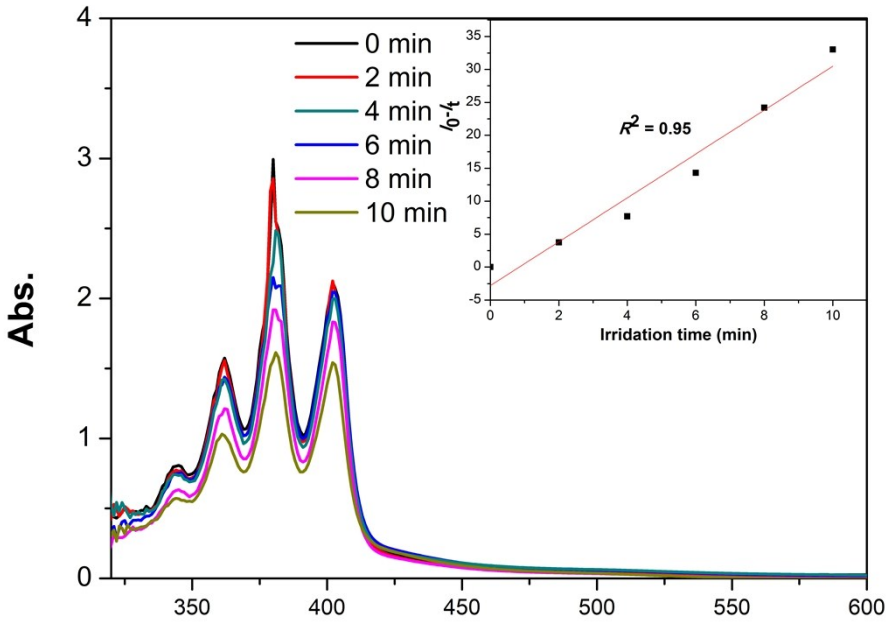


Fig. S12 ABDA Absorption decrease in the DMSO-PBS (1/199) solution containing CHL-**Ru-L** (10 μM) and ABDA (200 μM) with exposed to a visible light in different times (Inset: linear relationship of (I_t-I_0) *vs* t, *I* is integration of absorption of ABDA, *t* is duration time of illumination)

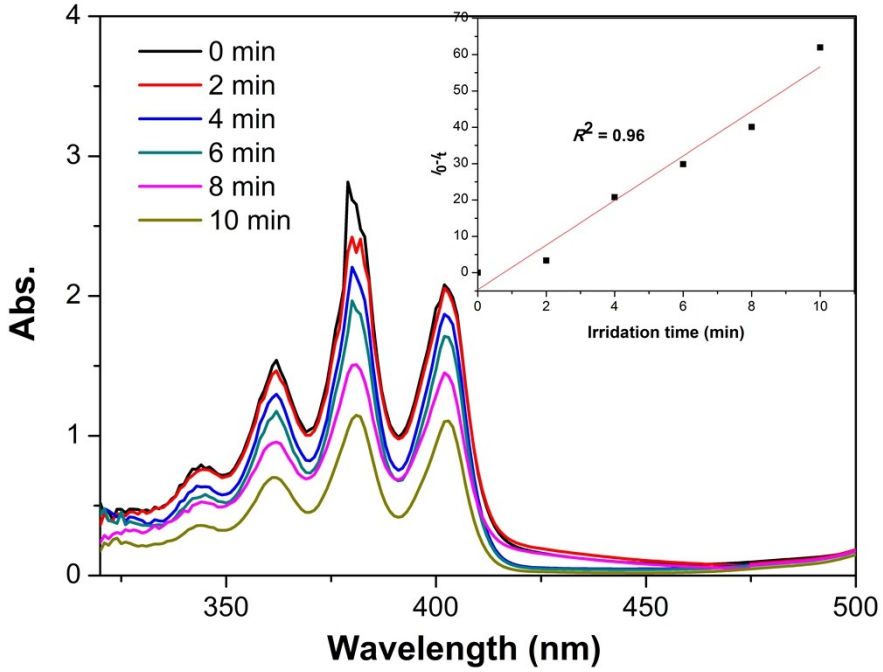


Fig. S13 ABDA Absorption decrease in the DMSO-PBS (1/199) solution containing standard **Rose Bengal** (10μM) and ABDA (200μM) with exposed to a visible light in different times (Inset: linear relationship of (I_t-I_0) *vs* t, *I* is integration of absorption of ABDA, *t* is duration time of illumination)

Fluorescence Decay of two complexes

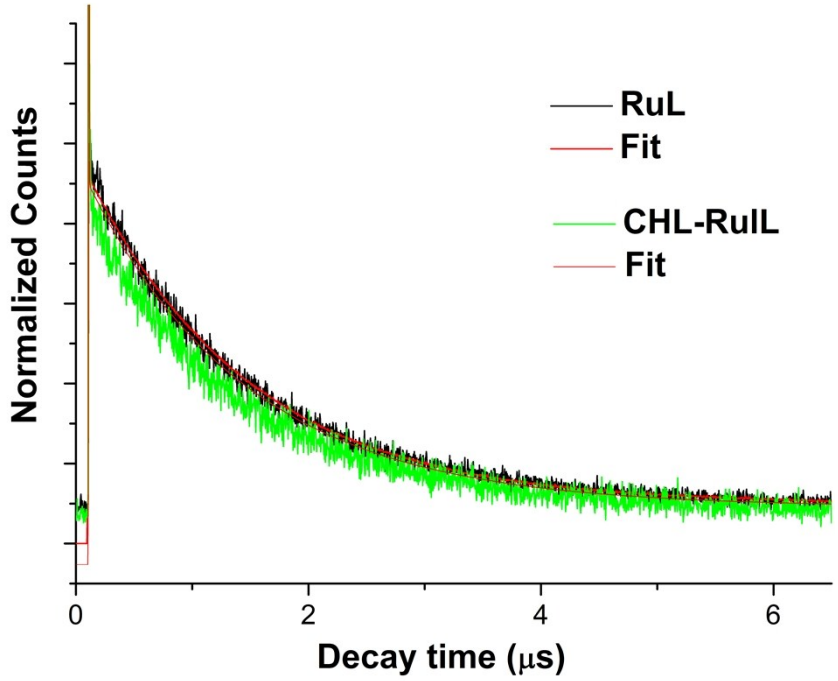


Fig. S14 Luminescence Decay curves of two Ru (II) complexes in deoxygenated aqueous-glycerol (1:1) solution (1×10^{-5} mol/L, λ_{ex} = 390 nm, λ_{em} = 702 nm) at 77 K.

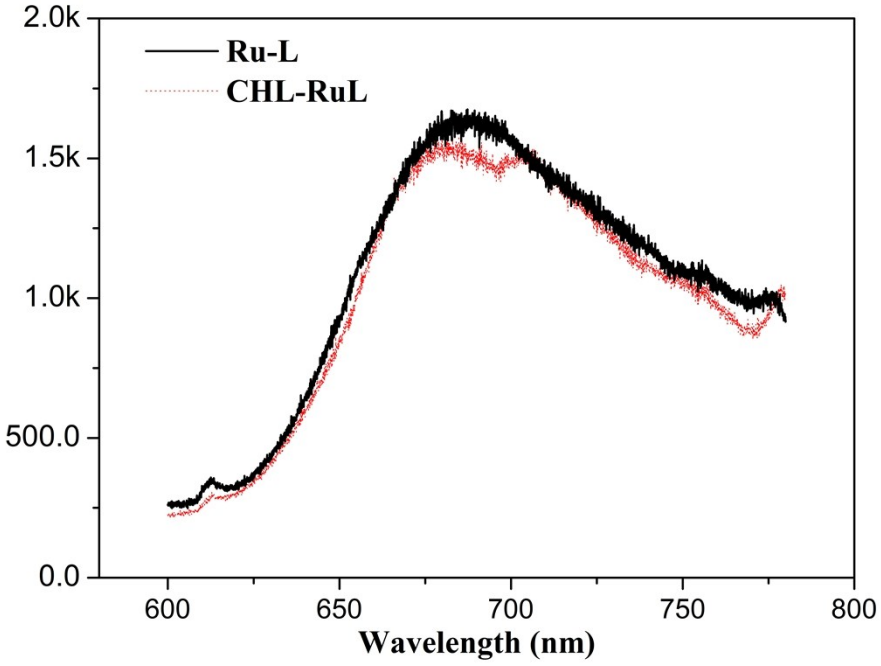


Fig. S15 Two-photon induced emission of **Ru-L** and **CHL-RuL** in methanol solution (λ_{ex} = 850 nm, 1×10^{-4} M) at 298 K.

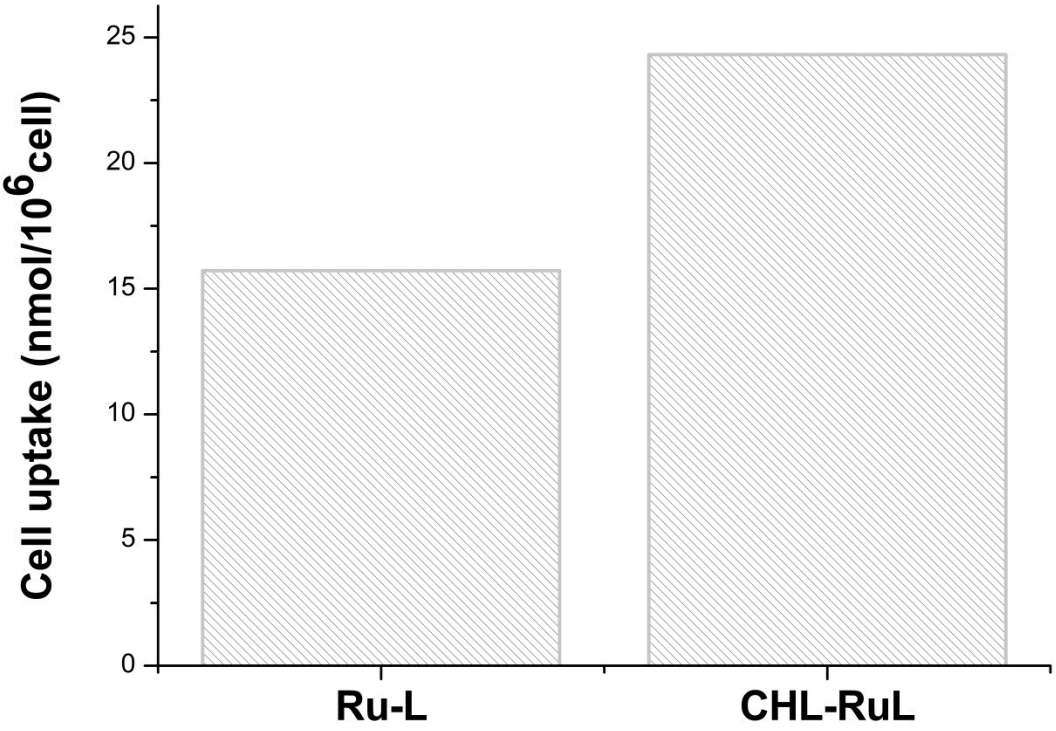


Fig. S16 Fluorometric analysis of cellular uptake extent of 5 μM **Ru-L** and **CHL-RuL** incubated with HeLa cells (2×10^5 cell/well) for 24 h.

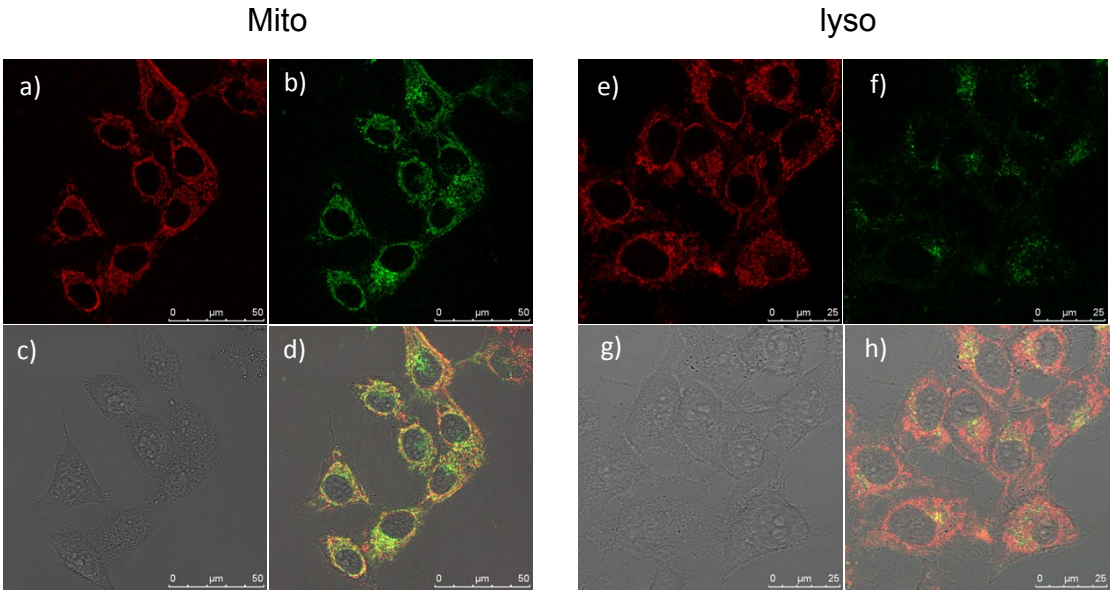
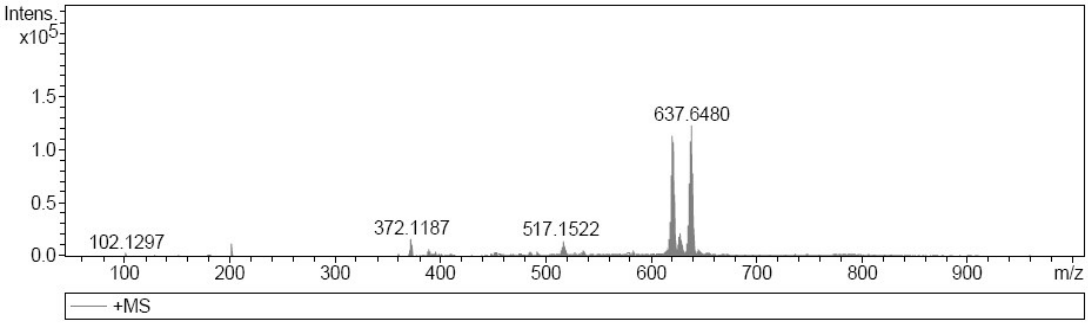
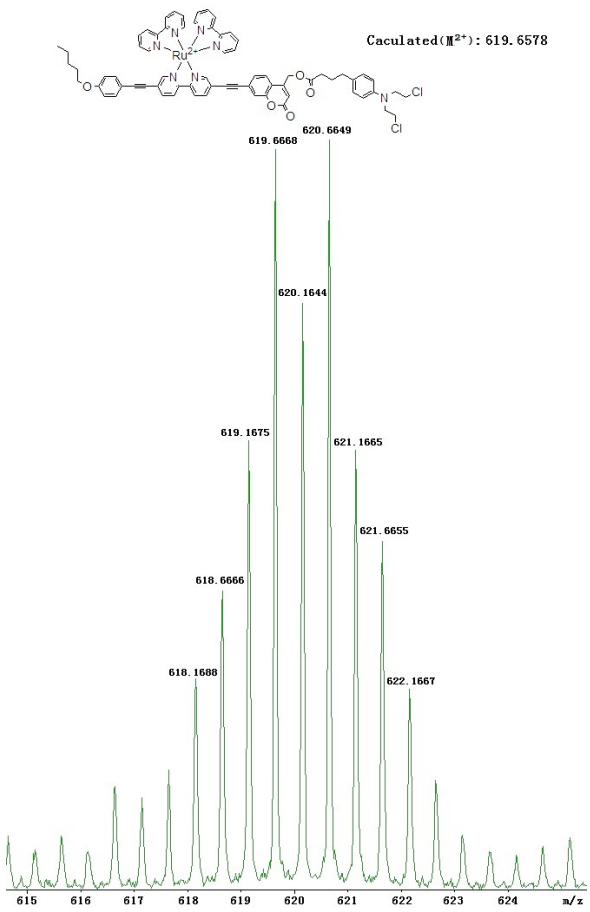


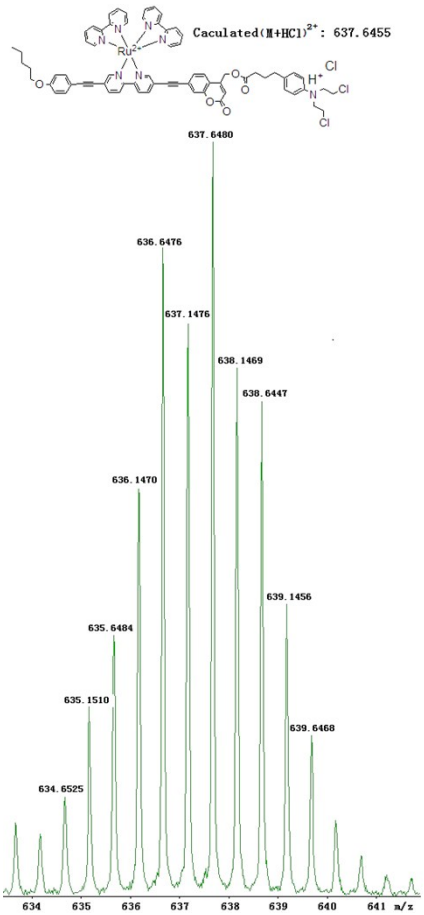
Fig. S17 Positive (mitochondria, a-d) and negative (lysosome, e-h) co-localization experiments of **Ru-L**. (a, e) HeLa cells treated with **Ru-L** (dose concen.= 2 μM, incubation time = 6 h, λ_{ex} = 405 nm, band pass filter > 600 nm); (b)Treated with Green mitochondria marker – Invitrogen M7514 (50 nM, λ_{ex} = 488 nm); (f) Treated with Green lysosome marker – Invitrogen L7526 (50 nM, λ_{ex} = 488 nm); (c, g) Bright field images; (d, h) Merged images.


Intens.
x10^5
1.5
1.0
0.5
0.0
637.6480
372.1187
517.1522
102.1297
100
200
300
400
500
600
700
800
900
m/z
+MS

(a)


Caculated(M2+): 619.6578
619.6668
620.6649
620.1644
619.1675
621.1665
621.6655
618.6666
618.1688
622.1667
615
616
617
618
619
620
621
622
623
624
m/z

(b)


Caculated(M+HCl)2+: 637.6455
637.6480
636.6476
637.1476
638.1469
638.6447
636.1470
639.1456
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639.6468
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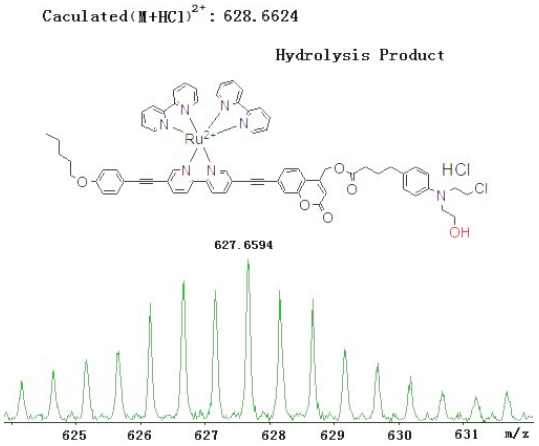
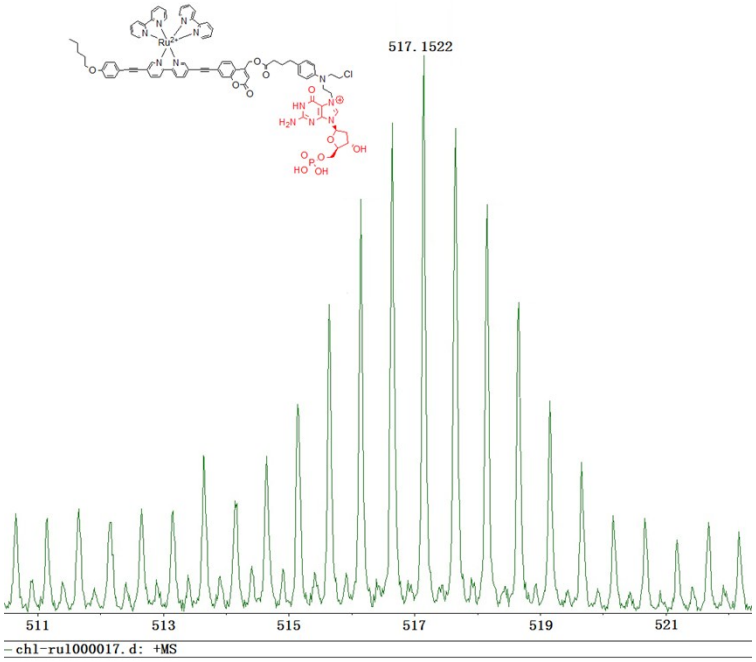
635
636
637
638
639
640
641
m/z

(c)



(d)



(e)

Fig. S18 ESI mass spectrum of the mixed solution of 2-deoxyguanosine 5'-monophosphate (dGMP) and **CHL-RuL** after 10 h incubations at pH = 7.0 PBS soulution (a) and the ionic peak of **CHL-RuL** (b,c), hydrolysis product (d) and also the very weak signal of mono-adduct of dGMP-**CHL-RuL** (e) (m/z calculated for $C_{79}H_{75}ClN_{12}O_{12}PRu^{3+}$: 517.3364; found for 517.1522).