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
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Fig. S4 $^{13}$C NMR (MeOH-$d_4$) of Ru-L
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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 (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)
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Fluorescence Decay of two complexes

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

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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.
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 solution (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}Pr^{3+}: 517.3364; found for 517.1522).