Supporting Information

Experimental Section

General: All reactions were carried out in a dry nitrogen atmosphere. Solvents were dried by standard procedures, distilled, and deaerated prior to use. All chemicals were obtained from Aldrich Chemical Company and, where appropriate, degassed before use. 5-Bromo-1,10-phenanthroline, (M. Hissler, W. B. Connick, D. K. Geiger, J. E. McGarrah, D. Lipa, R. J. Lachicotte, R, Eisenberg, *Inorg. Chem.* **2000**, *39,* 447-457) 2,2'-dipyrroylmethane, (B. J. Littler, M. A. Miller, C.-H. Hung, R. W. Wagner, D. F. O'Shea, P. D. Boyle, J. S. Lindsey, *J. Org. Chem.* **1999**, *64*, 1391-1396) and *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$ (B. P. Sullivan, D. J. Salmon, T. J. Meyer, *Inorg. Chem.* **1978**, *17*, 3334-3341) were synthesized according to literature procedures. NMR spectra were recorded with a Bruker Ultrashield 400 Plus NMR spectrometer. ^{1}H NMR chemical shifts were referenced to internal $CDCl_3$ and then re-referenced to TMS (δ = 0.00 ppm). High-resolution mass spectra, reported as *m*/*z*, were obtained with a Bruker Autoflex MALDI-TOF mass spectrometer.

Synthesis of PZn-Ru: Figure S1 shows the chemical structure and the strategy for the synthesis of **PZn-Ru**. The precursor **PZn-Phen** was prepared in *ca.* 75% yield via Sonogashira cross-coupling reaction between 5-ethynyl-1,10-phenanthroline and 5-bromo-10,15,20-tri(3',4',5'-trimethoxyphenyl)porphyrinato zinc(II) (**BrPZn**). **PZn-Phen** was then reacted with *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$ in THF/ethanol at 80^{o}C to give the title product **PZn-Ru** in 72% yield. The synthesis of 5-ethynyl-1,10-phenanthroline was reported by R. Ziessel et al. via a two-step reaction with a total yield of *ca.* 51%. (R.

Ziessel, J. Suffert, M. T. Youinou, *J. Org. Chem.* **1996**, *61*, 6535-6546)

However, we directly obtained 5-ethynyl-1,10-phenanthroline in good yield (69%) using a Sonogashira cross-coupling reaction between 5-bromo-1,10-phenanthroline and the trimethylsilane protected alkyne in the presence of diisopropylamine (DIPA), followed by sonication with KCN in water.

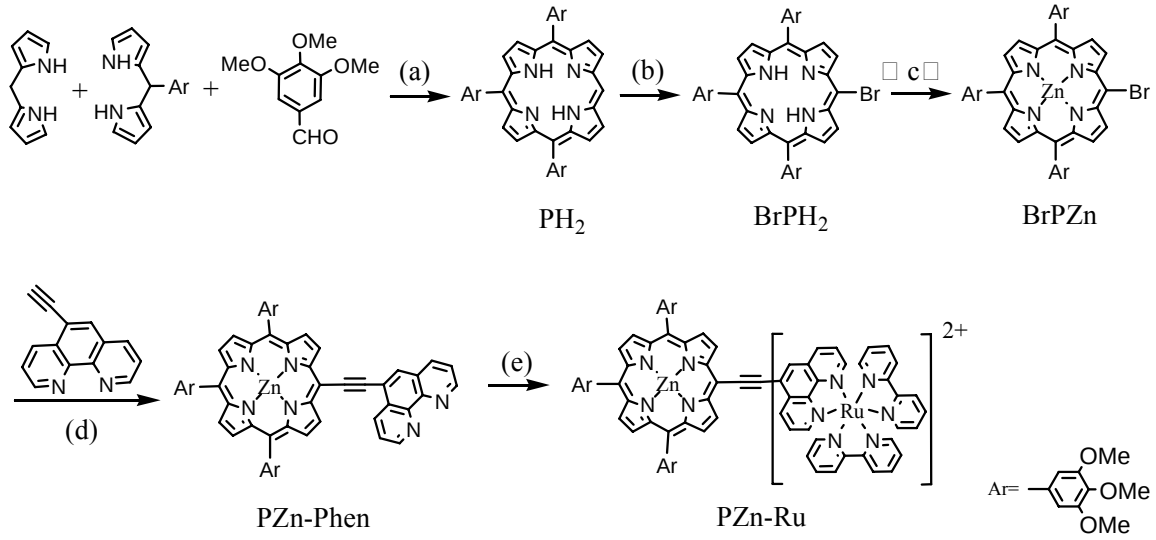

Figure S1. Preparation of **PZn-Ru**: (a) 1. TFA, $CHCl_3$, r.t.; 2. DDQ, r.t.; (b) NBS, 0°C; (c) $Zn(OAc)_2 \cdot 2H_2O$, CH_2Cl_2, reflux; (d) $Pd(PPh_3)_4$, CuI, THF/DIPA, 45°C; (e) *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$, THF/ ethanol, 80°C.

5-Ethynyl-1,10-phenanthroline: Under a nitrogen atmosphere, a 100 ml Schlenk flask was charged with 5-bromo-1,10-phenanthroline (1000 mg, 3.9 mmol), $Pd(PPh_3)_4$ (400 mg, 0.37 mmol), CuI (110 mg, 0.58 mmol), (trimethylsilyl)acetylene (2.8 mL, 20 mmol), diisopropylamine (15 mL), and THF (15 mL). After stirring for 24 h at 45°C, the solvents were distillated under vacuum and the residue dissolved in methanol (50 mL). After addition of KCN (200 mg, 3.1 mmol) in water (20 mL), the solution was sonicated for 2 h. After removing most of methanol under vacuum, the product extracted with CH_2Cl_2 (3

×30 mL). The organic layer was dried over $MgSO_4$. After filtration and evaporation of the solvent, the crude material was chromatographed on silica gel (CH_2Cl_2/CH_3OH 100/8) to give the pure product in 69% yield (550 mg). MALDI-TOF HRMS: calcd. for $[M+1]^+$ 205.0760; obsd: 205.0765. ^1H NMR (400 MHz, $CDCl_3$): δ 3.55 (s, 1 H), 7.69 (m, 2 H), 8.10 (s, 1 H), 8.23 (dd, J = 8.1 Hz, 1.9 Hz, 1 H), 8.76 (dd, J = 8.2 Hz, 1.7 Hz, 1 H), 9.22 (m, 2 H).

3,4,5-Trimethoxyphenyldipyrromethane: Pyrrole (347 mL, 5.00 mol) and 3,4,5-trimethoxybenzaldehyde (9.81 g, 50.0 mmol) were added to a 500 mL single-neck round-bottomed flask containing a magnetic stir bar. The solution was degassed with a stream of nitrogen for 10 min. Trifluoroacetic acid (TFA, 0.39 mL, 5.00 mmol) was added, and the mixture was stirred under argon at room temperature for 1.5 h. The mixture turned yellow during the course of the reaction. NaOH (6.0 g, 0.15 mol) was added to quench the reaction. Stirring for 45 min afforded a pale yellow mixture. The mixture was filtered. The crude product obtained after removal of pyrrole was purified via chromatography on silica gel using hexanes/CH_2Cl_2/ethyl acetate (7:2:1) as the eluent. The yield was 5.22 g (33%).

5,10,15-Tri(3',4',5'-trimethoxyphenyl)porphyrin (PH_2): A solution of *meso*-unsubstituted dipyrromethane (1.46 g, 10.0 mmol), 3,4,5-trimethoxyphenyl dipyrromethane (3.13 g, 10.0mmol), and 3,4,5-trimethoxybenzaldehyde (3.92 g, 20.0 mmol) in $CHCl_3$ (1L) was degassed by bubbling with nitrogen for 30 min. Then trifluoroacetic acid (TFA, 1.54 mL, 20.0 mmol) was added. After the solution was stirred for 2 h at room temperature under nitrogen, 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 7.26 g, 32.0 mmol) was added, and the reaction mixture was stirred for a further 2

h. Triethylamine (30 mL) was added. The solvent was evaporated and the crude product was purified by silica chromatography using $CHCl_3$/ethyl acetate/acetone/triethylamine (100:20:20:3) as the eluent, to give a purple product (2.50 g, 31%). MALDI-TOF HRMS: calcd. for $[M]^+$ 808.3103; obsd: 808.3105. ^{1}H NMR($CDCl_3$): δ -3.01 (br, 2H), 3.97 (s, 6H), 4.00 (s, 12H), 4.19 (s, 3H), 4.20 (s, 6H), 7.48 (s, 2H), 7.50 (s, 4H), 9.00 (d, *J* = 4.8 Hz, 2H), 9.03 (d, *J* = 4.8 Hz, 2H), 9.13 (d, *J* = 4.6 Hz, 2H), 9.36 (d, *J* = 4.6 Hz, 2H), 10.24 (s, 1H) ppm. UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 418 (5.53), 510 (4.13), 548 (4.00), 581 (3.69), 638 (3.39).

5-Bromo-10,15,20-tri(3',4',5'-trimethoxyphenyl)porphyrin ($BrPH_2$): A solution of **PH_2** (808 mg, 1.00 mmol), NBS (178 mg, 1.00 mmol) and pyridine (1mL) in CH_2Cl_2 (200 mL) was stirred at 0^{o}C for 15 min. Acetone (20 mL) was added, and the solvent was removed in vacuo. The residue was purified via chromatography on silica gel using $CHCl_3$/ethyl acetate (2:1) as the eluent. The yield was 835 mg (94%). MALDI-TOF HRMS: calcd. for $[M]^+$ 888.2198; obsd: 888.2242. ^{1}H NMR($CDCl_3$): δ -2.76 (br, 2H), 3.96 (s, 6H), 3.98 (s, 12H), 4.17 (s, 3H), 4.19 (s, 6H), 7.43 (s, 2H), 7.45 (s, 4H), 8.91 (m, 4H), 9.01 (d, *J* = 4.6 Hz, 2H), 9.69 (d, *J* = 4.6 Hz, 2H) ppm. UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 425 (5.41), 517 (4.23), 557 (4.11), 593 (3.96), 650 (3.83).

5-Bromo-10,15,20-tri(3',4',5'-trimethoxyphenyl)porphyrinatoZinc(II) (BrPZn): **$BrPH_2$** (266 mg, 0.3 mmol) and zinc acetate (657 mg, 3 mmol) were refluxed in $CHCl_3$ (200 mL) for 4 h. The solvent was removed and the residue was purified via chromatography on silica gel using CH_2Cl_2 as the eluent, to give the product in quantitative yield. MALDI-TOF HRMS: calcd. for $[M]^+$ 950.1326, obsd: 950.1381. ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 6H), 3.94 (s, 12H), 4.12 (s, 9H), 7.42 (s, 2H), 7.43 (s, 4H), 9.01

(m, 4H), 9.11 (d, *J* = 4.7 Hz, 2H), 9.80 (d, *J* = 4.7 Hz, 2H) ppm. UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 428 (5.60), 561 (4.33), 600 (4.05).

PZn-Phen: Under a nitrogen atmosphere, 5-ethynyl-1,10-phenanthroline (126 mg, 0.61 mmol), **BrPZn (**234 mg, 0.246 mmol), $Pd(PPh_3)_4$ (28 mg, 0.025 mmol), CuI (7 mg, 0.037 mmol), THF (13 mL) and diisopropylamine (13 mL) were added to a Schlenk containing a magnetic stir bar. After stirring for 24 h at 45°C, the solvents were distillated under vacuum and the residue dissolved in methanol (25 mL). After addition of KCN (80 mg, 1.23 mmol) in water (8mL), the solution was sonicated for 2 h. After removing most of solvents under vacuum, the product extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed twice with water and dried over $MgSO_4$. After filtration and evaporation of the solvent, the crude product was chromatographed on silica gel (CH_2Cl_2/CH_3OH 10/2) and recrystallized from $CHCl_3$/methanol to give the pure product in 75% yield (200 mg). MALDI-TOF HRMS: calcd. for $[M]^+$ 1072.2769, obsd: 1072.2738. ^1H NMR ($CDCl_3$): δ 3.98 (s, 6H), 4.05 (s, 12H), 4.18 (s, 3H), 4.23 (s, 6H), 7.49 (s, 2H), 7.53 (dd, *J* = 8.0 Hz, 4.2 Hz, 1H), 7.56 (s, 4H), 7.87 (dd, *J* = 8.0 Hz, 4.2 Hz, 1H), 8.20 (m, 2H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.48 (s, 1H), 9.01 (m, 4H), 9.03 (d, *J* = 4.5 Hz, 2H), 9.35 (d, *J* = 8.0 Hz, 1H), 9.52 (d, *J* = 4.5 Hz, 2H) ppm. UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 283 (4.38), 450 (5.09), 574 (4.02), 635 (4.36).

PZn-Ru: A solution of **PZn-Phen** (7) (100 mg, 0.093 mmol) and *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$ (96 mg, 0.186 mmol) in 40 mL THF/ethanol (1:3) was degassed by bubbling with nitrogen for 10 min. The mixture was heated at 88°C for 16 h. The solvents were distillated under vacuum and the product was purified by column chromatography on alumina using $CHCl_3$/MeOH (3:1) as the eluent. The yield was 100 mg (72%). MALDI-

TOF HRMS: calcd. for $[M]^+$ 1488.3199, obsd: 1488.3159. ^{1}H NMR (CD_3OD): δ 3.87 (s, 6H), 3.89 (s, 12H), 4.00 (s, 3H), 4.02 (s, 6H), 7.33 (m, 2H), 7.40 (s, 2H), 7.44 (s, 4H), 7.51 (m, 2H), 7.73 (d, *J* = 5.5 Hz, 2H), 7.84 (m, 2H), 7.92 (t, *J* = 4.7 Hz, 2H), 8.02-8.17 (m, 6H), 8.30 (d, *J* = 4.7 Hz, 2H), 8.67-8.73 (m, 4H), 8.77-8.81 (m, 5H), 9.00 (m, 3H), 9.57 (d, *J* = 8.3 Hz, 1H), 9.82 (d, *J* = 4.6 Hz, 2H) ppm. UV–vis ($CHCl_3$): λ_{max} (nm, log ε) = 287 (4.87), 450 (5.22), 575 (4.17), 635 (4.52).

Linear Photophysical Properties: Electronic absorption spectra in the UV/Vis region were recorded with a Varian Cary 100 UV/Vis spectrophotometer, steady-state visible fluorescence and photoluminescence excitation spectra were recorded with a Photon Technology International (PTI) Alphascan spectrofluorimeter, and quantum yields of the visible emissions were determined according to the literature method (V. Chauke, A. Ogunsipe, M. Durmus, T. Nyokong, *Polyhedron* **2007**, *26*, 2663-2671) using H_2TPP as reference standard (Φ_{em} = 0.11 in benzene). (P. G. Seybold, M. Gouterman, *J. Mol. Spectrosc.* **1969**, *31*, 1-13)

Singlet Oxygen Quantum Yield: Singlet oxygen was detected directly by its phosphorescence emission at 1270 nm using an InGaAs detector on the PTI QM4 luminescence spectrometer. The singlet oxygen quantum yields (Φ_Δ) of all compounds were determined in $CHCl_3$ by comparing the singlet oxygen emission intensity of the sample solution to that of a reference material (H_2TPP, Φ_Δ = 0.55 in $CHCl_3$) (R. Schmidt, E. Afshari, *J. Phys. Chem.* **1990**, *94*, 4377-4378) according to equation (1) (Y. Li, T. M. Pritchett, J. Huang, M. Ke, P. Shao, W. Sun, *J. Phys. Chem. A* **2008**, *112,* 7200-7207)

$$\Phi_{\Delta}^{S} = \Phi_{\Delta}^{REF} \times \left(\frac{n_S}{n_{REF}} \right)^2 \frac{G_{\Delta}^{S}}{G_{\Delta}^{REF}} \times \frac{A_{REF}}{A_S} \qquad (1)$$

where Φ_{Δ} is the singlet oxygen quantum yield, G_{Δ} is the integrated emission intensity, A is the absorbance at the excitation wavelength, n is the refractive index of the solvent. Superscripts REF and S correspond to the reference and sample, respectively. In all cases, the $^1O_2$ emission spectra were measured after excitation with the absorbance set at 0.05 in order to minimize re-absorption of the emitted light.

Two-Photon Absorption Measurements: The two-photon absorption spectra were measured at 800 nm by the open-aperture *Z*-scan method using 100-fs laser pulses from an optical parametric amplifier operating at a 1 kHz repetition rate generated from a Ti:sapphire regenerative amplifier system. The laser beam was split into two parts by a beam splitter. One was monitored by a photodiode (D1) as the incident intensity reference, I_0, and the other was detected as the transmitted intensity by another photodiode (D2). After passing through a lens with f = 10 cm, the laser beam was focused and passed through a quartz cell. The position of the sample cell, z, was moved along the laser-beam direction (z axis) by a computer-controlled translatable table so that the local power density within the sample cell could be changed under the constant incident intensity laser power level. Finally, the transmitted intensity from the sample cell was detected by the photodiode (D2). The photodiode (D2) was interfaced to a computer for signal acquisition and averaging. Each transmitted intensity data represent the average of over 100 measurements. Assuming a Gaussian beam profile, the nonlinear absorption coefficient, β, can be obtained by curve fitting to the observed open-aperture traces, $T(z)$,

with equation (2), (K. Wang, C.-T. Poon, W.-K. Wong, W.-Y. Wong, C. Y. Choi, D. W. J. Kwong, H. Zhang, Z.-Y. Li, *Eur. J. Inorg. Chem.* **2009**, 922-928) where a_0 is the linear absorption coefficient, l is the sample length (1 mm quartz cell) and z_0 is the diffraction length of the incident beam.

$$T(z) = 1 - \frac{\beta I_0 (1 - e^{-\alpha_0 l})}{2a_0 \left(1 + (z / z_0)\right)^2} \qquad (2)$$

After obtaining the nonlinear absorption coefficient β, the TPA cross-section σ_2 of the sample molecule (in units of GM, where 1 GM = 10^{-50} cm^4 s $photon^{-1}$) can be calculated using equation (3) (K. Wang, C.-T. Poon, W.-K. Wong, W.-Y. Wong, C. Y. Choi, D. W. J. Kwong, H. Zhang, Z.-Y. Li, *Eur. J. Inorg. Chem.* **2009**, 922-928), where N_A is the Avogadro's constant, d is the concentration of the sample compound in solution, h is the Planck's constant, and v is the frequency of the incident laser beam.

$$\sigma_2 = \frac{1000 \beta h v}{N_A d} \qquad (3)$$

Cell culture: Human HK-1 nasopharyngeal carcinoma cells were maintained in RPMI 1640. The medium was supplemented with 10% FBS (Gibco) and antibiotics (penicillin 50 U/mL; streptomycin 50 μg/mL). The cell lines were incubated at 37 ^oC in a humidified incubator with 5% CO_2. Human cervical carcinoma (HeLa) cells were maintained in an RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in 5% CO_2.

Photosensitizer: A stock solution of **PZn-Ru** (4×10^{-4} M) was prepared in dimethylsulfoxide (DMSO), sterile filtered and stored in the dark at 4^{o}C.

Flow cytometric analysis of cellular uptake: HK-1 cells (3×10^{5}/well) were seeded onto coverslip in a 35 mm Petri dish for overnight. The cells were incubated with PZn-Ru (1 μM) for 6 hours or 24 hours in dark. The cells were trypsinized and washed with PBS for twice. The fluorescence profiles of the treated cells were analyzed using FACSCalibur (Becton Dickinson). The 488 nm laser line was used for the excitation of **PZn-Ru** and the fluorescence signal was collected using the FL-3 channels equipped with long pass filter (> 650 nm). At least 10,000 events were counted.

Photodynamic treatment (PDT) assay: HK-1 cells (1×10^{4}/well) were incubated with various concentrations of **PZn-Ru** for 24 h in 96-well flat bottom tissue culture plates. Then the treated cells were washed with fresh medium and exposed to yellow light (0-12 J/cm^2) from a 400 W tungsten lamp fitted with heat-isolation filter and 500 nm long-pass filter at an intensity of 5.6 mW/cm^2, as measured with a power meter (OPHIR). The cells were incubated for another 24 h. Viability was then assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reduction assay. The optical density (OD) of dissolved formazan crystal was measured using the iEMS Analyzer (Lab-system, Type 1401) at 570 and 690 nm. The percentage of cytotoxicity was calculated using the following equation:

$$\text{cytotoxicity (\%)} = (OD_{\text{control group}} - OD_{\text{treatment group}})/OD_{\text{control group}} \times 100$$

where $OD = OD_{570\text{-}690\ nm}$.

Confocal microscopic analysis of subceollular localization of PZn-Ru: HK-1 cells (5 × 10^4/well) were seeded onto cover slip in a 35 mm Petri dish for overnight and incubated with **PZn-Ru** (1 μM) for 24 hours. The cells were then rinsed with PBS and stained with organelle-specific probes, namely, mitochondria-specific probe Mito-Tracker Green FM dye M7514 (50 nM) for 30 minutes at 37^{o}C. The cells were then rinsed with PBS. Subcellular localizations of **PZn-Ru** were examined using an Olympus FV1000 confocal microscope. A 488 nm argon-ion laser was used for excitation of the **PZn-Ru** and Mito-Tracker. A diode laser line at 405 nm line was used for excitation of the Mito-Tracker and the **PZn-Ru**. Pinhole size of 100-120 μm was selected to exclude fluorescence light emitted from out-of-focus planes above and below the focusing plane. A 60X objective was used for image capturing. Images were processed and analyzed using the FV10-ASW software (Olympus).For the two-photon *in-vitro* imaging, the cells were imaged in the tissue culture chamber (5% CO_2, 37^{o}C) using a Leica SP5 (upright configuration) confocal microscope equipped with a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent). The excitation beam produced by the femtosecond laser, which was tunable from 680-1050 nm, (λ_{ex} = 850 nm, ~ 8mW) and focused on coverslip-adherent cells using a 40 x oil immersion objective.

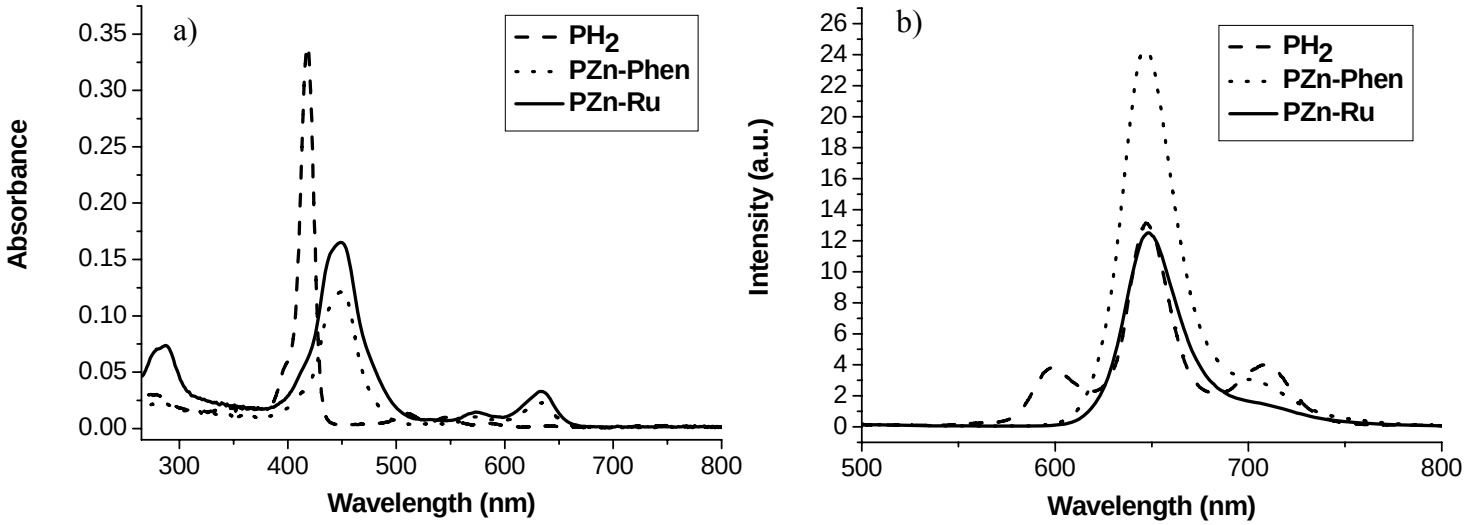

Figure S2. (a) absorption spectra and (b) Fluorescence of **PZn-Ru**, **PZn-Phen** and **PH_2** in $CHCl_3$ (10^{-6} M).

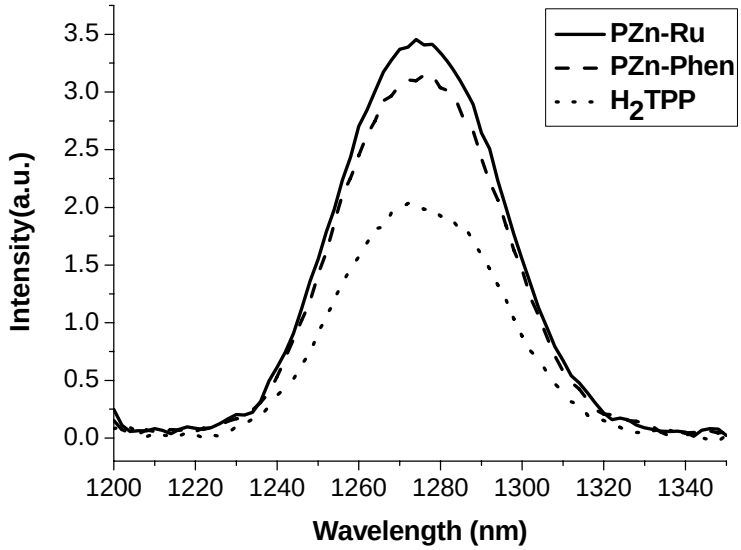

Figure S3. Phosphorescence spectra of $^1O_2$ generated from **PZn-Phen**, **PZn-Ru** and H_2TPP in $CHCl_3$ under identical irradiation conditions [λ_{exc} = 424 nm; abs (λ_{exc}) = 0.05].