Electronic Supplementary Information for

Mitochondria targeted and NADH triggered photodynamic activity of chloromethyl modified Ru(II) complexes under hypoxic condition

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Experimental section

Materials

$RuCl_3 \cdot 3H_2O$, 2,2'-bipyridine (bpy), 4,4'-bis(chloromethyl)-2,2'-bipyridine(bcm-bpy), 4,4'-dimethyl-2,2'-dipyridine (dmbpy), 1,10-phenanthroline monohydrate (phen), gel loading buffer, tris-hydroxymethyl-aminomethane (Tris base) were purchased from Sigma Aldrich. The supercoiled pBR322 plasmid DNA was obtained from TaKaRa Biotechology Company. Dulbecco's modification of Eagle's medium (DMEM), penicillin, streptomycin, fetal bovine serum, and 10 mM PBS (pH = 7.4) were purchased from Corning.

Instruments

^1H NMR spectra were determined on a Bruker DMX-400 MHz spectrophotometer. High-resolution ESI mass spectrometry (HR ESI-MS) spectra were recorded on a Brucker APEX IV (7.0T) FT_MS. UV-vis absorption spectra were obtained on a Shimadzu UV-1601 spectrophotometer.

EPR spectra were measured on a Bruker ESP-300E spectrometer at 9.8 GHz, X-band, with 100 Hz field modulation. Samples were injected quantitatively into quartz capillaries, purged with argon for 20 min in the dark, and illuminated in the cavity of the EPR spectrometer with a mercury lamp (> 400nm).

For other assays needed for irradiation, an LED lamp (470 ± 10nm) was used as light source.

Laser confocal scanning microscope images were collected on a Olympus FV1000.

Purity of 1-3 was verified by high performance liquid chromatography (HPLC). HPLC analysis was done on an HITACHI series instrument using a WH–C–18 column (5 μm, 4 mm × 150 mm). Flow: 1 mL/min with 100% CH_3CN; Pump: Chromaster 5110; autosampler: Chromaster 5210; UV detector: Chromaster 5420.

log Po/wmeasurements

The *n*-octanol/water partition coefficients were determined at room temperature following a reported method.[1] **1-3** (30 μM) were added in a mixed solution containing 1 mL water and 1mL *n*-octanol, then sonicated for 30 min and centrifuged at 4000 rpm for 10 min. The concentrations of complexes in two phases were measured by UV-vis. Each measurement was repeated for 3 times.

Cell Culture in hypoxia.

SKOV-3 cells were cultured with DMEM and 10% FBS at 37 °C in an anaerobic incubator, with 5% $CO_2$and 3% O_2(N_2 was another gas source to control O_2partial pressure).

Cellular uptake

SKOV-3 cells were cultured in 25 cm^2bottle for 24h, then incubated with **1-3 (**1μM)for 4 h. Cells were washed with PBS, and obtained by trypsinization, then washed for another two times. The cell organelles were obtained as the procedure given by the Mitochondria Isolation Kit from Invent. After chemical digestion for 24 h, they were diluted with 2% HNO_3 to take ICP-MS measurements.

Laser confocal scanning microscopy

SKOV-3 cells in 20mm^2 glass bottom dishes were treated with **1-3**(5 μM)for 4 h, then washed with PBS and then incubated with Mito-tracker Green (50 nM) for 10 min. After rinsed 3 times with PBS, the cells were imaged on a CLSM(Olympus FV1000). Mito-tracker Green was excited at 470 nm and monitored at 510-530 nm, while **1-3** were excited at 488 nm and monitored at 610-

650 nm.

MTT assay

Cells were seeded in 96 well plates at the density of 5000-8000 cells per well for 24 h, then incubated with different concentrations of **1-3** for 4 h. Light groups were irradiated for 30 min (470 nm, 22.5 mW/cm^2), and cells were cultivated for another 20 h. Cell medium was discarded and MTT (1 mg/mL) was added. 4 h later, MTT solution was removed and DMSO was added. And obtained the results by a Thermo MK3Multiscanmicroplate reader at 570 nm.

Apoptotosis and JC-1 staining assay

Annexin V-FITC staining of the membranes was performed by using the annexin V-FITC and PI apoptosis detection kit. SKOV-3 cells were incubated with **3** for 4 h, then irradiated with 470nm LED lamp (22.5 mW/cm^2) for 30 min.Cells were harvested after another 6h,stained with annexin V-FITC/PI and analyzed by flow cytometry. Similar procedure was used for JC-1 staining assay.

SKOV-3 cellular NADH measurement

10^5 SKOV-3 cells were seeded in 6 well plates and incubated with **1-3** for 4 h. Light groups were irradiated for 30 min (470 nm, 22.5 mW/cm^2).Then cells were washed with cold PBS and homogenized with 100 μL NADH extraction buffer. The extracts were heated for 5 min at 60°C, then 20 μL Assay Buffer and 100 μL NAD extraction buffer were added. After centrifugation with 14000 rpm for 5 min, the supernatant was used for NADH detection.

Synthesis and characterization of 1-3

$Ru(phen)_2Cl_2$ and $Ru(bcm\text{-}bpy)_2Cl_2$ were synthesized by the reported methods.[2]

Synthesis and characterization of 1

100 mg $Ru(phen)_2Cl_2$ (0.19 mmol) and bcm-bpy (50.6 mg, 0.2 mmol) were refluxed in CH_3OH overnight under a N_2 atmosphere. Solvent was removed by rotary evaporator. Complex **1** was purified on silica gel using $CH_3CN/H_2O/KNO_3$(10:4:1) as eluent and precipitated by excess NH_4PF_6. Yield: 80%.^{1}H NMR (400 MHz, CD_3CN) δ 8.70 (d, *J* = 8.2 Hz, 2H), 8.65 – 8.55 (m, 4H), 8.33 – 8.19 (m, 6H), 7.89 (t, *J* = 8.8 Hz, 2H), 7.83 (dd, *J* = 8.2, 5.3 Hz, 2H), 7.70 (d, *J* = 5.8 Hz, 2H), 7.59 (dd, *J* = 8.2, 5.3 Hz, 2H), 7.35 (d, *J* = 5.1 Hz, 2H), 4.82 (s, 4H). HR ESI-MS: m/z = 357.0315 for $(M\text{-}2PF_6^-)^{2+}$. HPLC purity > 95%.

Synthesis and characterization of 2

100 mg $Ru(bcm\text{-}bpy)_2Cl_2$ (0.15 mmol) and 1,10-phenanthroline (28.8 mg, 0.16 mmol) were refluxed in CH_3OH overnight under N_2 atmosphere. Solvent was removed by rotary evaporator. Complex **2** was purified on silica gel using $CH_3CN/H_2O/KNO_3$(10:4:1) as eluent and precipitated by excess NH_4PF_6. Yield: 65%.^{1}H NMR (400 MHz, CD_3CN) δ 8.69–8.61 (m, 4H), 8.59 (s, 2H), 8.28 (s, 2H), 8.10 (s, 2H), 7.87 (d, *J* = 6.0 Hz, 2H), 7.78 (s, 2H), 7.53 (d, *J* = 5.2 Hz, 4H), 7.29 (s, 2H), 4.87 (s, 4H), 4.78 (s, 4H). HR ESI-MS: m/z = 394.0017 for $(M\text{-}2PF_6^-)^{2+}$. HPLC purity > 95%.

Synthesis and characterization of 3

$RuCl_3{\cdot}xH_2O$ (50 mg, 0.19 mmol) and bcm-bpy (152 mg, 0.6 mmol) were refluxed in DMF overnight under a N_2 atmosphere. Solvent was removed by rotary evaporator. Complex **3** was purified on silica gel using $CH_3CN/H_2O/ KNO_3$ (10:4:1) as eluent and precipitated by excess NH_4PF_6. Yield: 55%.^{1}H NMR (400 MHz, CD_3CN) δ 8.49 (s, 6H), 7.61 (d, *J* = 5.8 Hz, 6H), 7.37 (d, *J* = 5.9 Hz, 6H), 4.73 (s, 12H). HR ESI-MS: m/z = 429.9834 for $(M\text{-}2PF_6^-)^{2+}$. HPLC purity > 95%.

Table S1. Log $P_{o/w}$ and SKOV-3 cellular uptake levels of **1-3.**

	1	**2**	**3**
Log $P_{O/W}$	-1.41 ± 0.02	-1.26 ± 0.05	-0.97 ± 0.03
pmol/10^6cell [a]	32.1 ± 1.4	35.1 ± 2.1	65.4 ± 3.0

[a] measured by inductively coupled plasma-mass spectrometry (ICP-MS).

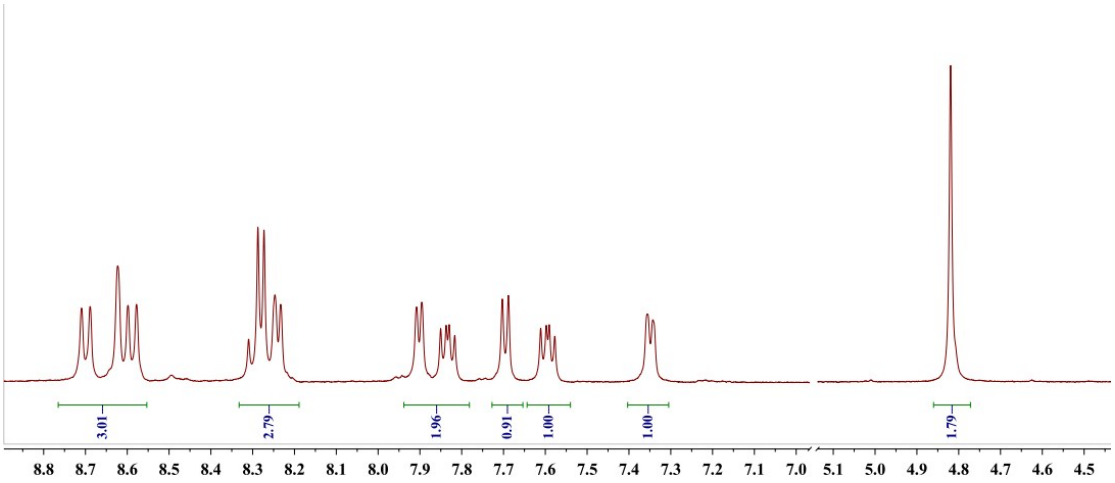

Fig. S1 ^{1}H NMR spectrum of **1** in CD_3CN**.**

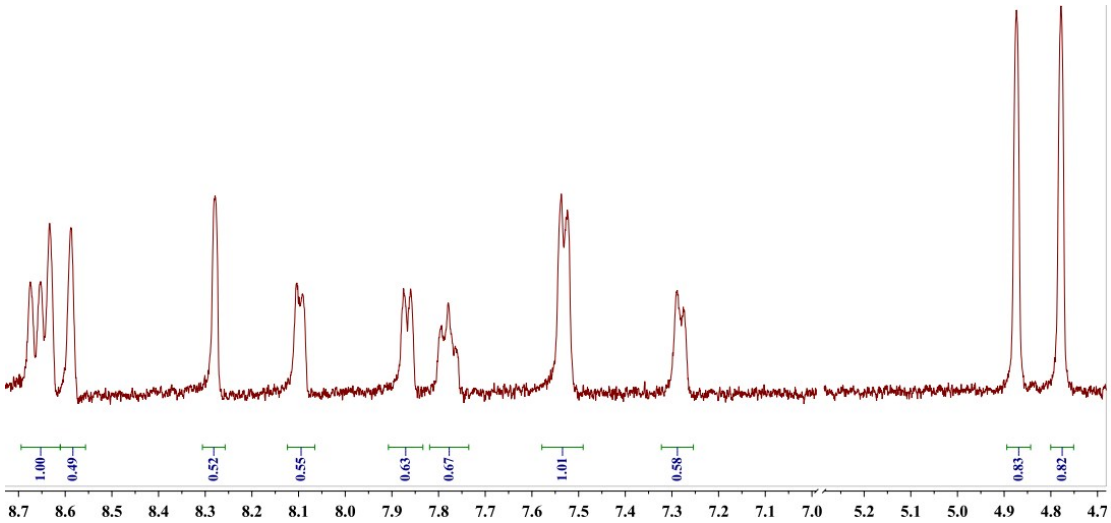

Fig. S2 ^{1}H NMR spectrum of **2** in CD_3CN**.**

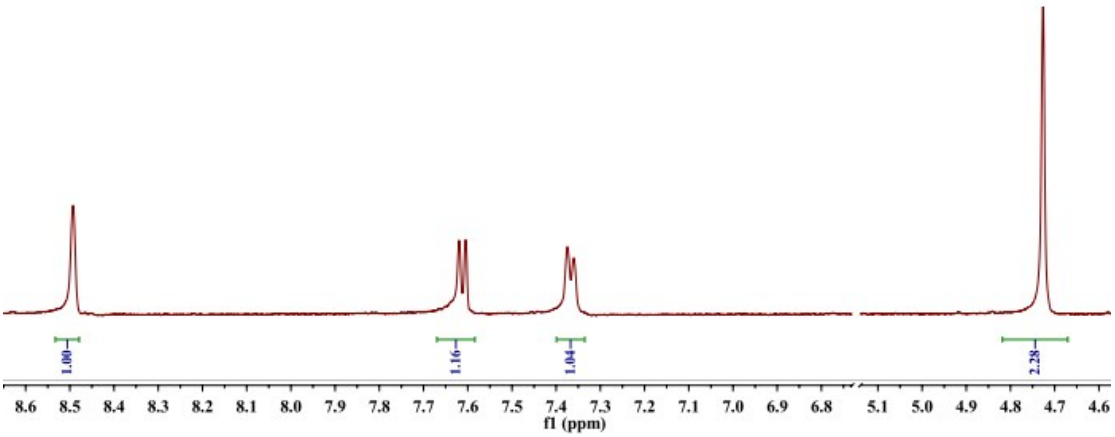

Fig. S3 ^{1}H NMR spectrum of **3** in CD_3CN.

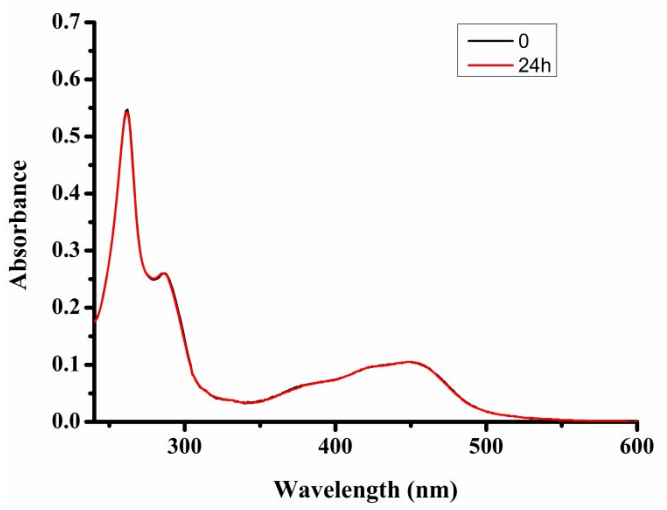

Fig. S4 Absorption spectra changes of **1** (10 μM) in H_2O for 24 h in the dark.

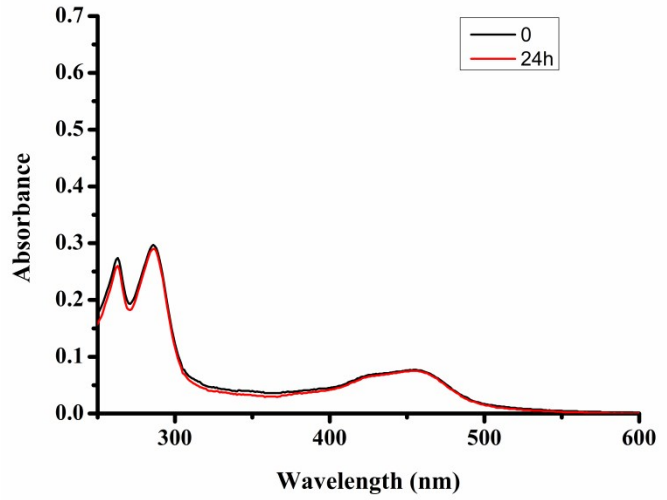

Fig. S5 Absorption spectra changes of **2** (10 μM) in H_2O for 24 h in the dark.

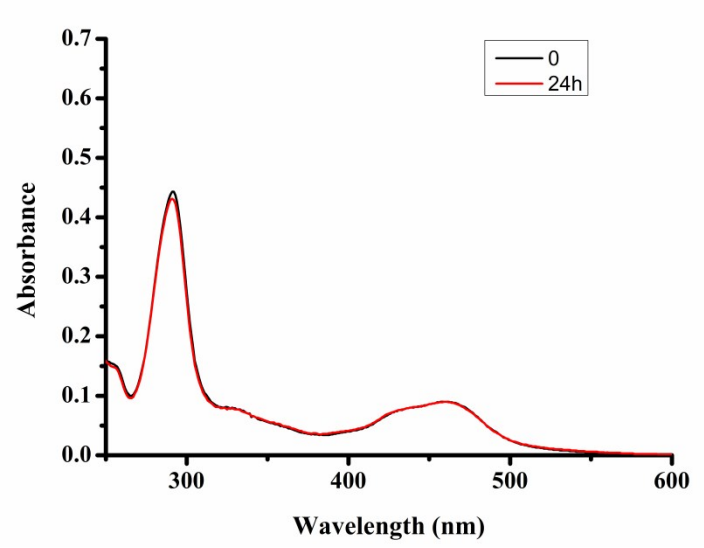

Fig. S6 Absorption spectra changes of **3** (10 μM) in H_2O for 24 h in the dark.

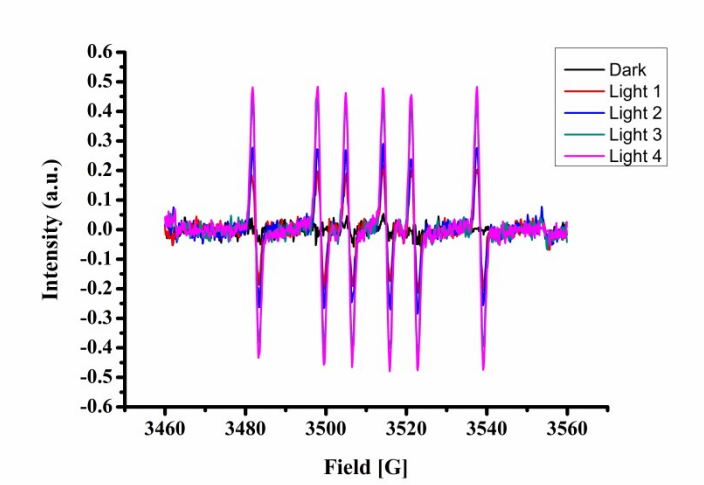

Fig. S7 EPR signals obtained upon irradiation (>400 nm) of **1** (1 mM) in Ar-saturated H_2O solution in the presence of NADH (120 mM) and DMPO (50 mM).

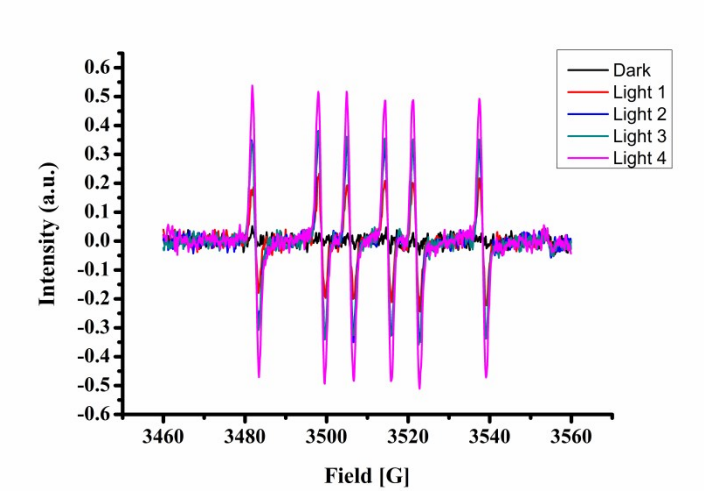

Fig. S8 EPR signals obtained upon irradiation (>400 nm) of **2** (1 mM) in Ar-saturated H_2O solution in the presence of NADH (120 mM) and DMPO (50 mM).

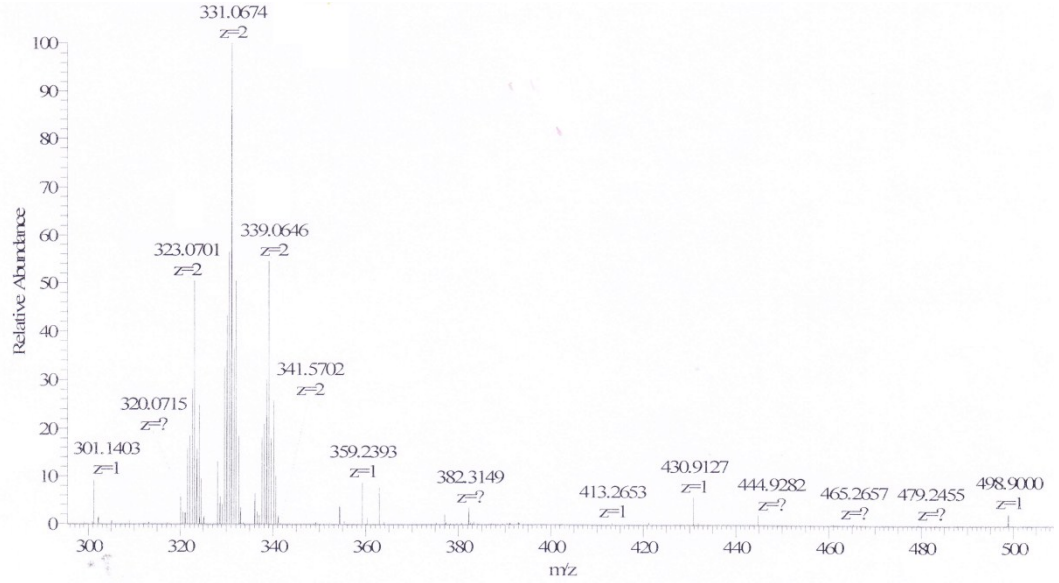

Fig. S9 HR ESI-MS of complex **1** (1 mM) in Ar-saturated H_2O in the presence of NADH (120 mM) after 470 nm LED irradiation for 5 min. The m/z peak of 323.0701 can be ascribed to the product in which two chloromethyl groups were transformed into methyl groups (cal. m/z = 323.0709). Similarly, the m/z peak of 331.0674 can be ascribed to the product with one methyl group and one hydroxymethyl group (cal. m/z = 331.0684).

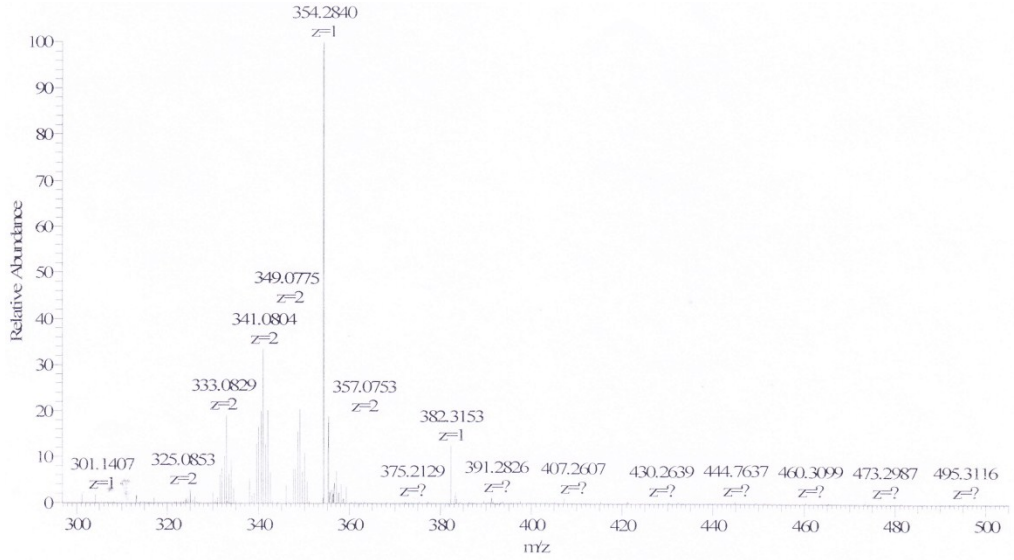

Fig. S10 HR ESI-MS of complex **2** (1 mM) in Ar-saturated H_2O in the presence of NADH (120 mM) after 470 nm LED irradiation for 5 min. The m/z peak of 325.0853 can be ascribed to the product with four methyl groups (cal. m/z = 325.0866); m/z peak of 333.0829 for the product with three methyl groups and one hydroxymethyl group (cal. m/z = 333.0840); m/z peak of 341.0804 for the product with two methyl groups and two hydroxymethyl groups (cal. m/z = 341.0815); m/z peak of 349.0775 for the product with one methyl group and three hydroxymethyl groups (cal. m/z = 349.0789).

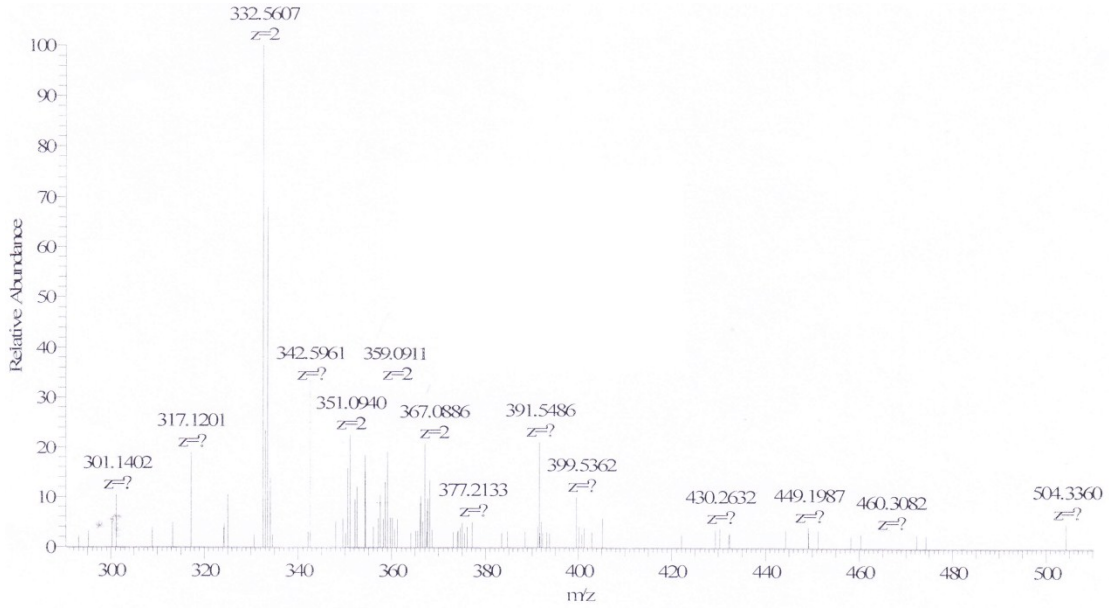

Fig. S11 HR ESI-MS of complex **3** (1 mM) in Ar-saturated H_2O in the presence of NADH (120 mM) after 470 nm LED irradiation for 5 min. The m/z peak of 351.0940 can be ascribed to the product with three methyl groups and three hydroxymethyl groups (cal. m/z = 351.0946); m/z peak of 359.0911 for the product with two methyl groups and four hydroxymethyl groups (cal. m/z = 359.0921); m/z peak of 367.0886 for the product with one methyl group and five hydroxymethyl groups (cal. m/z = 367.0896).

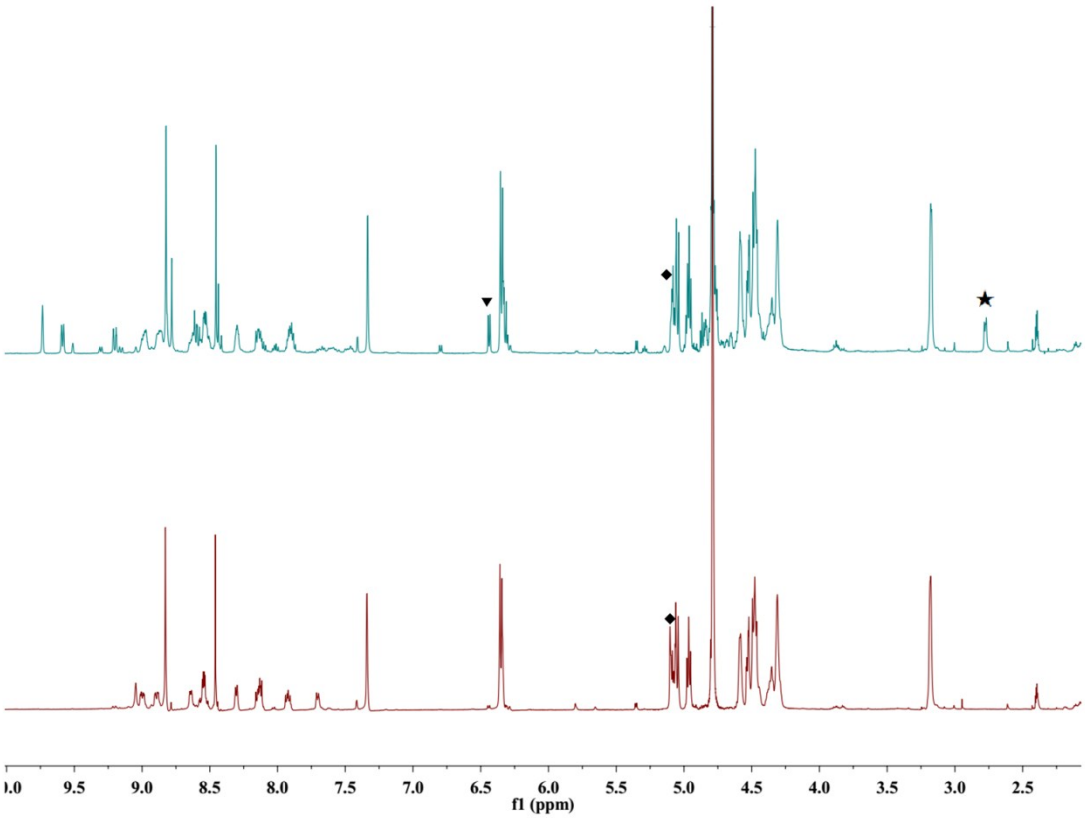

Fig. S12 ^{1}H NMR spectra of complex **1** in Ar-saturated CD_3COCD_3/D_2O (1:1, v/v) solution in the

presence of NADH before (bottom) and after (top) 470 nm LED irradiation. The resulted methyl groups were indicated by ★, which were verified by comparison with that of $[Ru(phen)_2(bmbpy)]^{2+}$ (not shown). The signal of the methylene groups were indicated by ◆, and the new signal at about 6.5 ppm (indicated by▼) after light irradiation can be ascribed to NAD^+ by comparison with the NMR spectrum of NAD^+ in the same solvent (see Fig. S31).

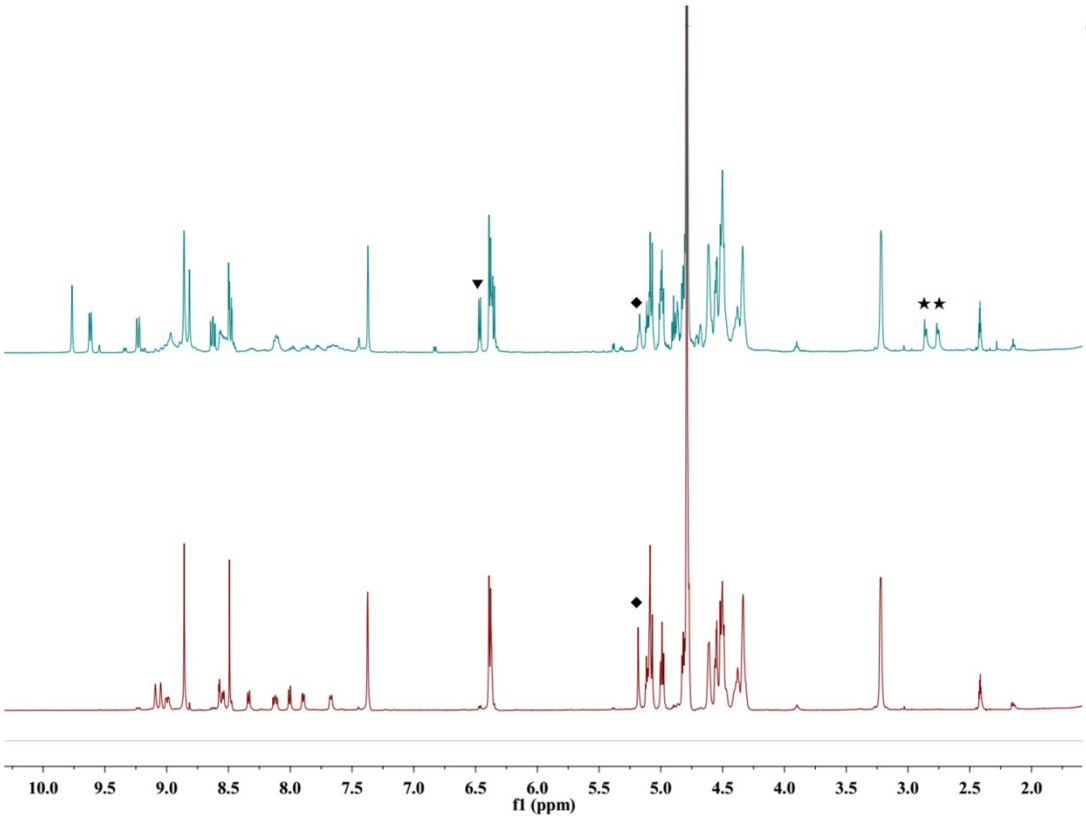

Fig. S13 H NMR spectra of complex **2** in Ar-saturated CD_3COCD_3/D_2O (1:1, v/v) solution in the presence of NADH before (bottom) and after (top) 470 nm LED irradiation. The resulted methyl groups were indicated by ★. The signal of the methylene groups were indicated by ◆, and the new signal at about 6.5 ppm (indicated by▼) after light irradiation can be ascribed to NAD^+ by comparison with the NMR spectrum of NAD^+ in the same solvent (see Fig. S31).

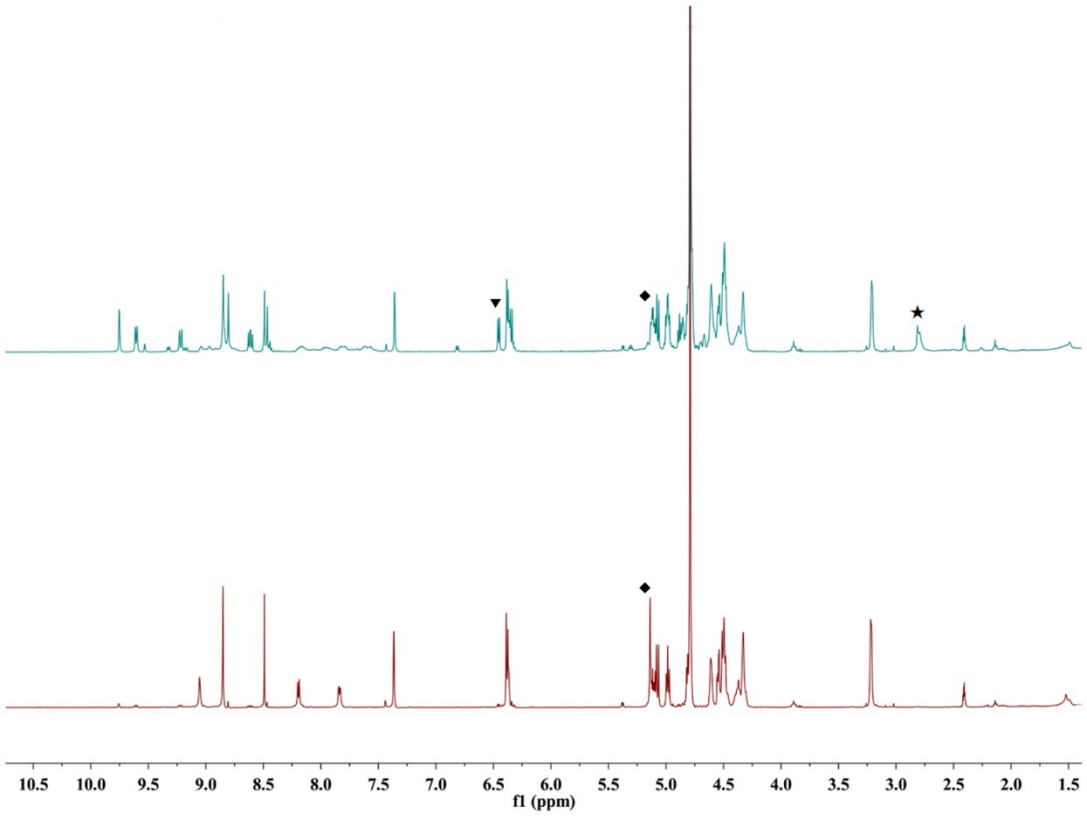

Fig. S14 ^{1}H NMR spectra of complex **3** in Ar-saturated CD_3COCD_3/D_2O (1:1, v/v) solution in the presence of NADH before (bottom) and after (top) 470 nm LED irradiation. The resulted methyl groups were indicated by ★. The signal of the methylene groups were indicated by ◆, and the new signal at about 6.5 ppm (indicated by▼) after light irradiation can be ascribed to NAD^+ by comparison with the NMR spectrum of NAD^+ in the same solvent (see Fig. S31).

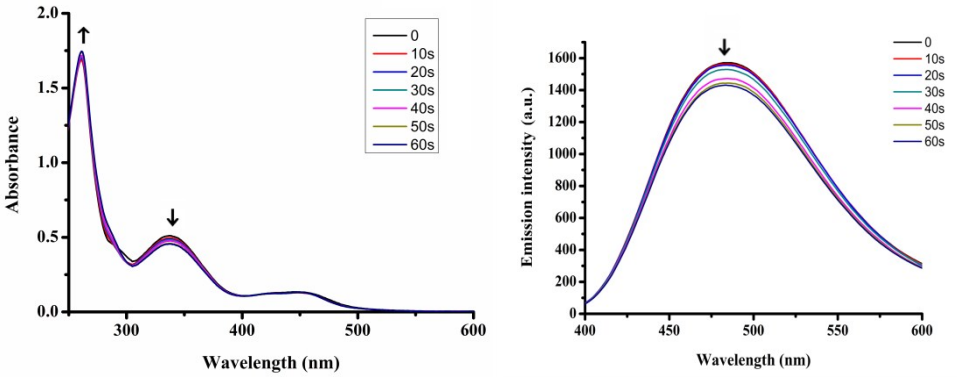

Fig. S15 Absorption and emission (λ_{ex} = 360 nm) spectra changes of mixed **1** (10 μM) and NADH (70 μM) in Ar-saturated H_2O solution upon 470 nm LED irradiation.

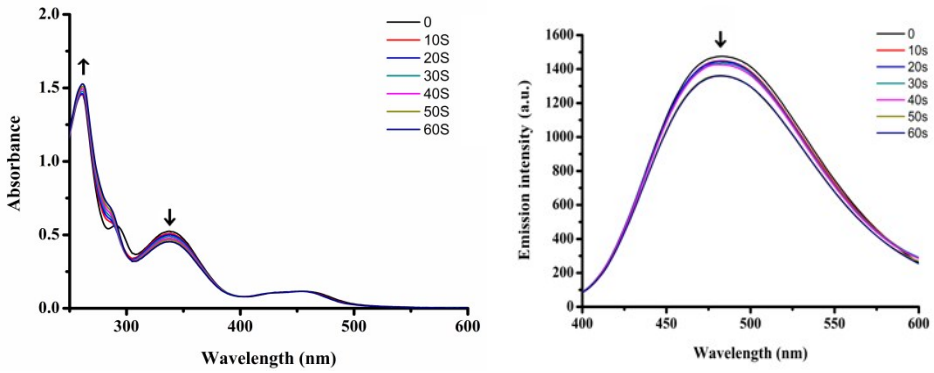

Fig. S16 Absorption and emission (λ_{ex} = 360 nm) spectra changes of mixed **2** (10 μM) and NADH (70 μM) in Ar-saturated H_2O solution upon 470 nm LED irradiation.

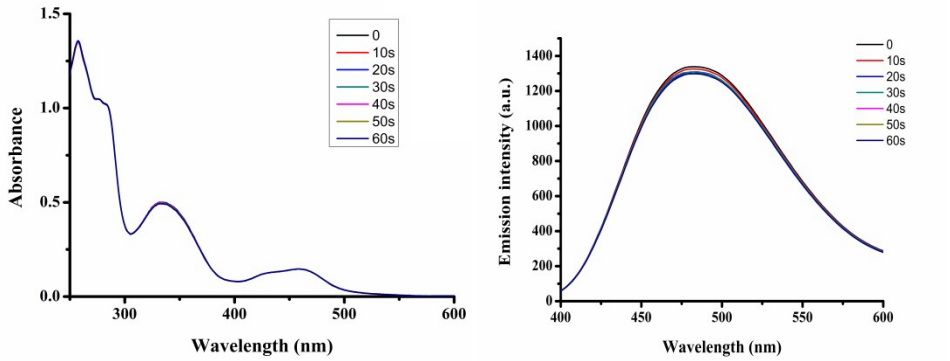

Fig. S17 Absorption and emission (λ_{ex} = 360 nm) spectra changes of mixed $[Ru(bmbpy)_3]^{2+}$ (bmbpy = 4,4'-bismethyl-2,2'-bipyridine) (10 μM) and NADH (70 μM) in Ar-saturated H_2O solution upon 470 nm LED irradiation.

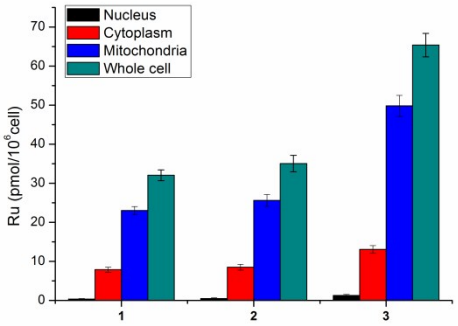

Fig. S18 ICP-MS quantifications of **1-3** in different subcellular organelles.

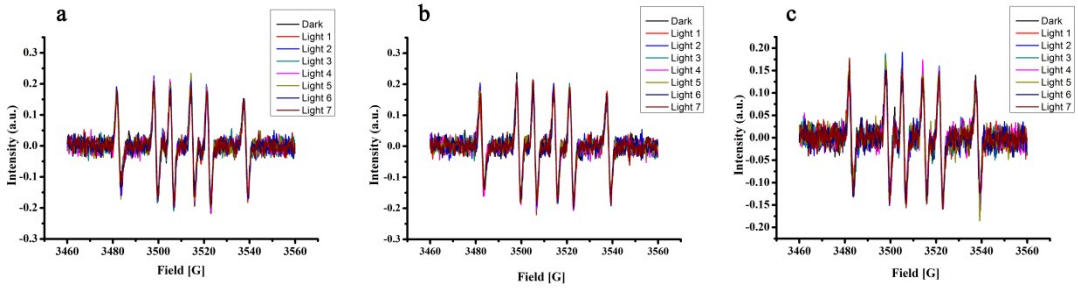

Fig. S19 EPR signals obtained upon irradiation (> 400 nm) of **1-3** (a-c, 1 mM) in Ar-saturated

DNA buffer solutions in the presence of DMPO (50 mM).

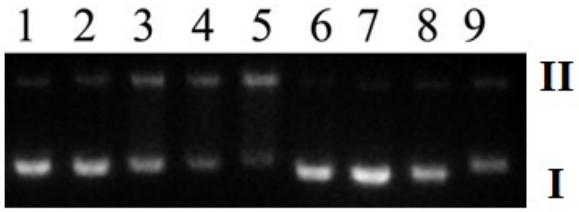

Fig. S20 Agarose gel electrophoresis pattern of pBR322 DNA (100 mM in base pairs) in Ar-saturated Tris-EDTA (pH = 7.5) upon irradiation (470 nm) for 5 min in the presence of complexes **1-2** with varied concentrations. Lane 1: DNA control; Lane 2: DNA+ **2** (80 μM) in the dark; Lane 3-5: Light + **2** with concentration of 20, 50 and 80 μM, respectively; Lane 6: DNA+ **1** (80 μM) in the dark; Lane 7-9: Light + **1** with concentration of 20, 50 and 80 μM, respectively.

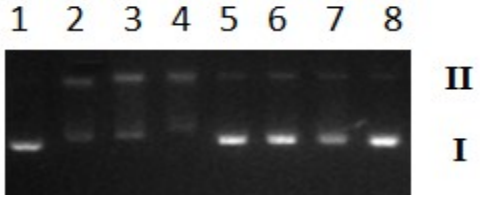

Fig. S21 Agarose gel electrophoresis pattern of pBR322 DNA (100 mM in base pairs) in Ar-saturated Tris–EDTA (pH = 7.5) upon irradiation (470 nm) for 1.5 min in the presence of 80 μM Ru complexes. Lane 1: DNA; Lane 2-4: **1-3** + Light; Lane 5-7: **1-3** + Dark; Lane 8: $[Ru(bmbpy)_3]^{2+}$ + Light.

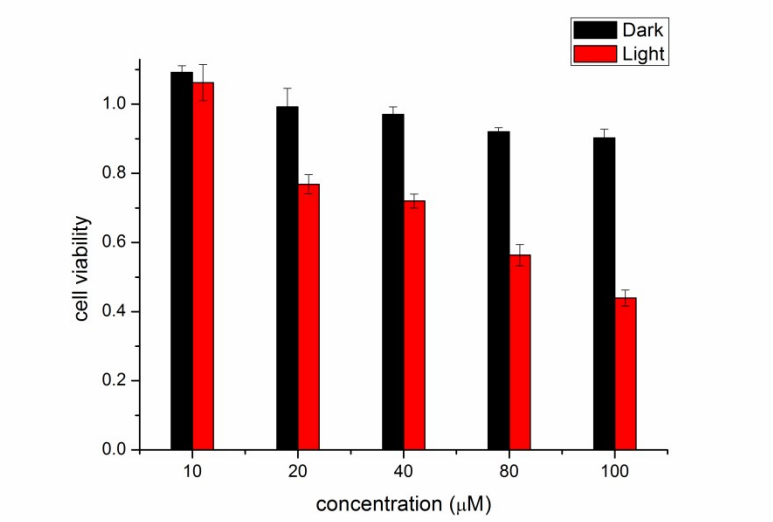

Fig. S22 Cytotoxicity of **1** towards SKOV-3 cells under hypoxic condition (3% O_2) in the dark or irradiated with 470 nm LED for 30 min (22.5 mW/cm^2).

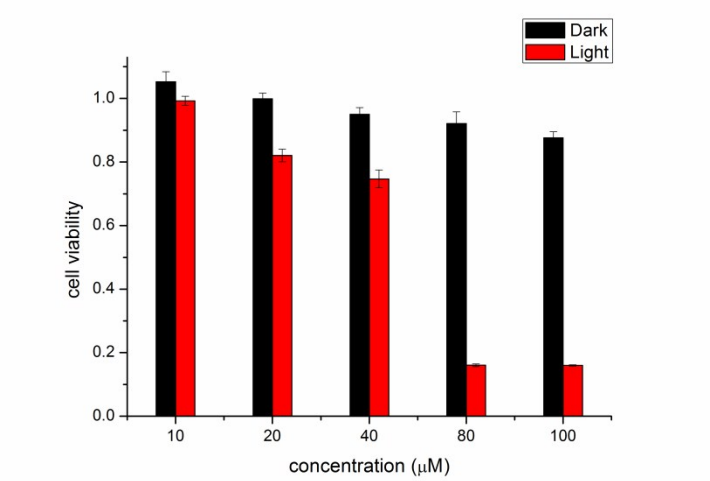

Fig. S23 Cytotoxicity of **2** towards SKOV-3 cells under hypoxic condition (3% O_2) in the dark or irradiated with 470 nm LED for 30 min (22.5 mW/cm^2).

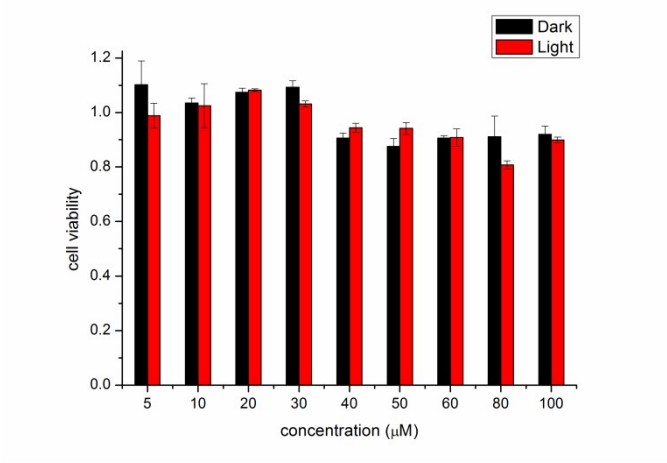

Fig. S24 Cytotoxicity of $[Ru(bmbpy)_3]^{2+}$ towards SKOV-3 cells under hypoxic condition (3% O_2) in the dark or irradiated with 470 nm LED for 30 min (22.5 mW/cm^2).

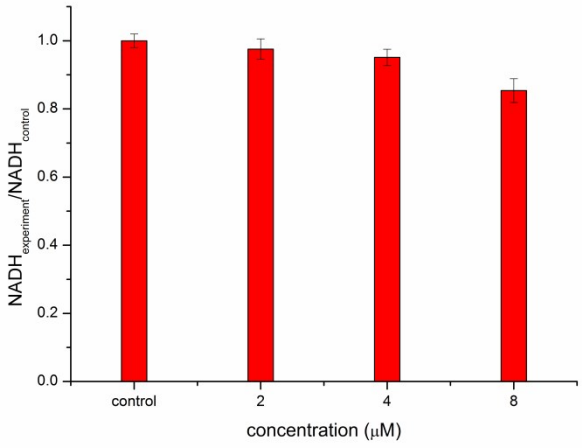

Fig. S25 SKOV-3 cellular NADH changes in the presence of **1** with different concentrations in the dark and under hypoxic condition (3% O_2).

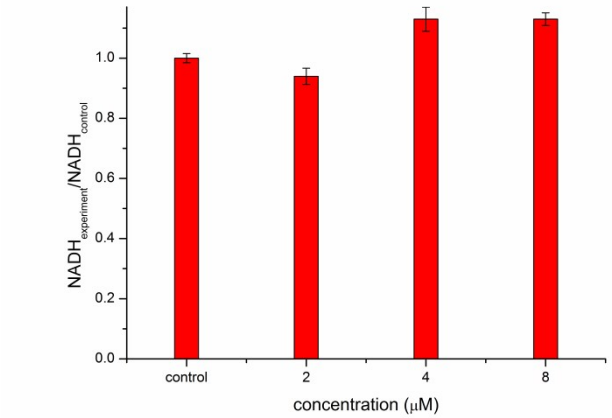

Fig. S26 SKOV-3 cellular NADH changes in the presence of **2** with different concentrations in the dark and under hypoxic condition (3% O_2).

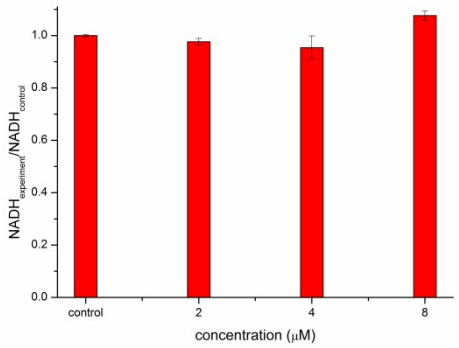

Fig. S27 SKOV-3 cellular NADH changes in the presence of **3** with different concentrations in the dark and under hypoxic condition (3% O_2).

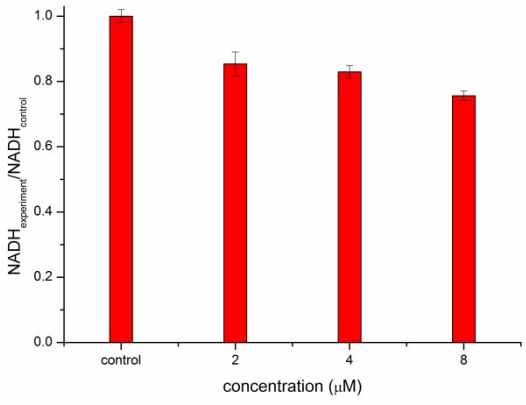

Fig. S28 SKOV-3 cellular NADH changes in the presence of **1** with different concentrations upon 470 nm LED irradiation for 30 min (22.5 mW/cm^2) under hypoxic condition (3% O_2).

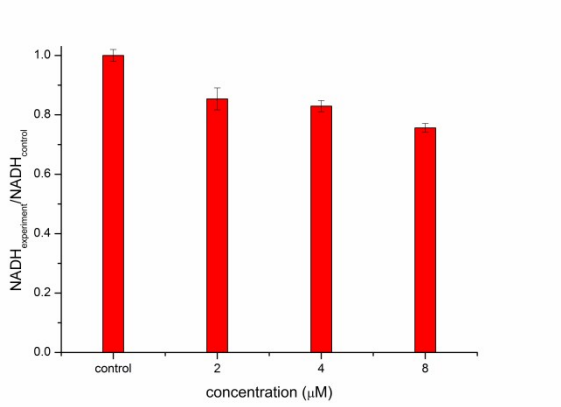

Fig. S29 SKOV-3 cellular NADH changes in the presence of **2** with different concentrations upon 470 nm LED irradiation for 30 min (22.5 mW/cm^2) under hypoxic condition (3% O_2).

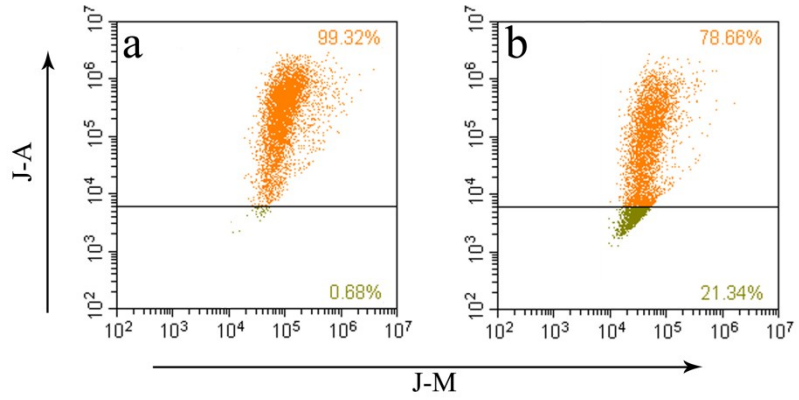

Fig. S30 Effects of **3** (5 μM) on mitochondrial membrane potential of SKOV-3 cells analyzed by flow cytometry. a: dark control; b: irradiated with 470 nm LED for 30 min (22.5 mW/cm^2) under hypoxic condition (3% O_2).

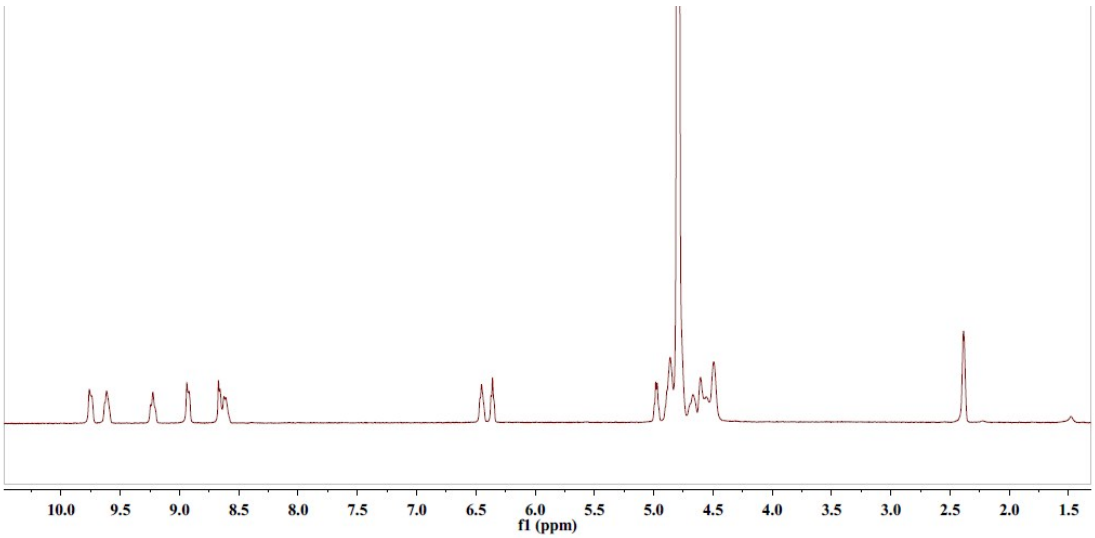

Fig. S31 ^{1}H NMR spectrum of NAD^+ in CD_3COCD_3/D_2O (1:1, v/v) solution.

Reference

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