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Materials

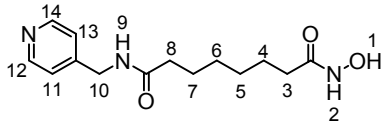
$Re(CO)_5Cl$ (Sigma Aldrich), 4,7-diphenyl-1,10-phenanthroline (DIP, Sigma Aldrich), 4-(aminomethyl)pyridine (Sigma Aldrich), methyl 8-chloro-8-oxooctanoate (Sigma Aldrich), NH_2OH (50% in H_2O) (J&K Scientific Ltd.), DMAP (4-dimethylaminopyridine, J&K Scientific Ltd.), NH_4PF_6 (Alfa Aesar), silver trifluoromethanesulfonate (Sigma Aldrich), cisplatin (Sigma Aldrich), SAHA (Sigma Aldrich), DMSO (dimethyl sulfoxide, Sigma Aldrich), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich), MTDR (MitoTracker Deep Red FM, Life Technologies, USA), LTDR (LysoTracker Deep Red FM, Life Technologies, USA), Hoechst 33342 (2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-1H,3'H-2,5'-bibenzimidazole, Sigma Aldrich), JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide, Sigma Aldrich), H_2DCFDA (2',7'-dichlorodihydrofluorescein diacetate, Sigma Aldrich), NAC (N-acetylcysteine, Sigma Aldrich), CCCP (carbonyl cyanide-m-chlorophenylhydrazone) and z-VAD-fmk (Sigma Aldrich) were used as received. Caspase-3/7 activity assay kit was purchased from Promega (USA). The fluorescent HDACs activity assay kit was purchased from Millipore (USA). The HDAC class IIa Fluorogenic Assay Kit and human recombinant HDAC7 were purchased from BPS Bioscience (USA). All the tested compounds were dissolved in DMSO just before the experiments, and the concentration of DMSO was 1% (v/v). The solution of the rhenium complex in PBS was proved to be stable for at least 48 h at room temperature as monitored by UV/Vis spectroscopy.

NMR spectra were recorded on a Bruker Avance 400 spectrometer (Germany). ESI-MS were recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). Microanalysis (C, H, and N) was carried out using an Elemental Vario EL CHNS analyzer (Germany). UV/Vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Emission measurements were conducted on an FLS 920 combined fluorescence lifetime and steady state spectrometer (Japan). Quantum

yields of luminescence at room temperature were calculated according to literature procedures using $[Ru(bpy)_3](PF_6)_2$ as the reference.[1]

Synthetic protocols and characterizations

Synthetic procedure of ligand L: 4-(aminomethyl)pyridine (3.72 mmol) and DMAP (0.037 mmol) were dissolved in dry DMF (45 mL), and methyl 8-chloro-8-oxooctanoate (4.839 mmol) in dry DMF was then added. The mixture was stirred under nitrogen for 12 h at room temperature, and then concentrated to ~ 3 mL. The concentrated liquid was poured into ether (30 mL) while stirring to afford a beige precipitate, which was filtered and suspended in a 1:1 (v/v) mixture of methanol and hydroxylamine (50% in H_2O, aq), then 1 N NaOH (2 mL) was added. After 30 min stirring, the mixture became homogeneous. The solution was neutralized by addition of 1 N HCl. The resulting precipitate was filtered and dried, giving **L** as a beige solid. Yield: 0.467 g (45%).

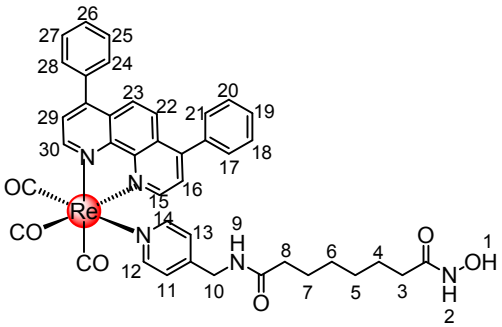


^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.37 (s, 1H; H1), 8.68 (s, 1H; H9), 8.48 (m, 3H; H2, H12 and H14), 7.23 (d, J = 5.6 Hz, 2H; H11 and H13), 4.28 (d, J = 6.0 Hz, 2H; H10×2), 2.16 (t, J = 7.5 Hz, 2H; H3×2), 1.94 (t, J = 7.3 Hz, 2H; H8×2), 1.56 – 1.44 (m, 4H; H4×2 and H7×2), 1.29 – 1.21 (m, 4H; H5×2 and H6×2). ESI-MS (CH_3OH): *m/z* 280.0 $[M+H]^+$. Elemental analysis: calcd (%) for $C_{14}H_{21}N_3O_3$: C, 60.20; H, 7.58; N, 15.04; found: C, 60.32; H, 7.66; N, 15.10.

Synthetic procedure of [Re(DIP)(CO)$_3$(CH$_3$CN)](PF$_6$): [Re(DIP)(CO)$_3$Cl] was prepared according to a literature procedure.[2] It was then converted to $[Re(DIP)(CO)_3(CH_3CN)](PF_6)$ following a standard method with slight modifications.[3] To a suspension of [Re(DIP)(CO)$_3$Cl] (0.80 mmol) in 200 mL of CH_3CN was added $AgCF_3SO_3$ (0.80 mmol). The mixture was refluxed under nitrogen for

24 h in the dark. After removed off-white AgCl precipitate, a 6-fold excess of NH_4PF_6 was added, the resulting yellow precipitate was collected by centrifugation and washed with water then with diethylether, which can be used directly for the next step without further purification.

Synthetic procedure of [Re(DIP)(CO)$_3$(L)](PF$_6$): A mixture of [Re(DIP)(CO)$_3$(CH$_3$CN)](PF$_6$) (0.35 mmol) and ligand **L** (0.35 mmol) in THF (50 mL) was refluxed under an inert atmosphere of nitrogen for 4 h. The mixture was then evaporated to dryness, and purified by column chromatography on silica gel using CH$_3$CN:H$_2$O:sat. aq. NaNO$_3$ (100:9:1) as the eluent. The PF$_6$ salt of the complex was again formed by addition of a saturated aqueous solution of NH_4PF_6. Then, the complex was further recrystallized from a mixture of CH$_3$CN and diethyl ether. Yield: 0.223 g (62%).



^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 10.33 (s, 1H; H1), 9.82 (d, J = 5.4 Hz, 2H; H15 and H30), 8.67 (s, 1H; H9), 8.54 (d, J = 6.4 Hz, 2H; H12 and H14), 8.35 (t, J = 5.8 Hz, 1H; H2), 8.21 (d, J = 5.4 Hz, 2H; H22 and H23), 8.16 (s, 2H; H16 and H29), 7.70 (m, 10H; H17, H18, H19, H20, H21, H24, H25, H26, H27 and H28), 7.22 (d, J = 6.4 Hz, 2H; H11 and H13), 4.18 (d, J = 5.7 Hz, 2H; H10×2), 2.08 (t, J = 7.5 Hz, 2H; H3×2), 1.89 (t, J = 7.3 Hz, 2H; H8×2), 1.43 (m, 4H; H4×2 and H7×2), 1.17 (m, 4H; H5×2 and H6×2). ESI-MS (CH$_3$CN): *m/z* 882.3 [M−PF$_6$]$^+$. Elemental analysis: calcd (%) for $C_{41}H_{37}F_6N_5O_6PRe$: C, 47.95; H, 3.63; N, 6.82; found: C, 47.86; H, 3.72; N, 6.75.

Cell lines and culture conditions

Human cervical cancer (HeLa), human lung adenocarcinoma epithelial (A549), cisplatin-resistant

A549 (A549R), human hepatocellular liver carcinoma (HepG2) and human normal liver (LO2) cells were obtained from Experimental Animal Center of Sun Yat-sen University (Guangzhou, China) and cultured in a humidified incubator at 37 °C under 5% CO_2. DMEM (Dulbecco's modified Eagle's medium, Gibco BRL) or RPMI 1640 (Roswell Park Memorial Institute 1640, Gibco BRL) medium were supplemented with 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL). A549R cells were cultured in a medium containing increasing concentrations of cisplatin to maintain the resistance. In each experiment, cells treated with vehicle DMSO (1%, v/v) were used as the reference group.

Cytotoxicity Assay

Growth inhibition effect of the tested compounds was determined by MTT assay as previously described.[4] For the cytotoxicity assay in the presence of the inhibitors, HeLa cells were preincubated with 50 μM z-VAD-FMK or 10 mM NAC for 1 h before the complexes were added. Percentage of cell viability was determined by MTT.

Cellular uptake studies

HeLa cells were treated with **ReLMito** (20 μM) at 37 °C for 2 h, then washed three times with PBS and viewed immediately under a confocal laser-scanning microscope (LSM 710, Carl Zeiss, Göttingen, Germany) by excitation at 405 nm. Emission was collected at 564 ± 20 nm.

Colocalization assay

HeLa cells were seeded in 35 mm dishes for 24 h and then incubated with **ReLMito** (20 μM) at 37 °C for 1.5 h. The cells were further co-incubated with MTDR (100 nM) or LTDR (50 nM) at 37 °C for 0.5 h. Cells were washed three times with PBS and visualized by a confocal microscope (LSM 710, Carl Zeiss, Göttingen, Germany) immediately. **ReLMito** was excited at 405 nm, MTDR and

LTDR were excited at 633 nm. Emission was collected at 564 ± 20 nm, 665 ± 20 nm and 668 ± 20 nm for **ReLMito**, MTDR and LTDR, respectively.

Live cell imaging after treatment with metabolic or endocytic inhibitors

HeLa cells were seeded in 35 mm dishes for 24 h and preincubated with CCCP (30 μM) or chloroquine (50 μM) for 1 h. The medium was removed and the cells were then incubated with **ReLMito** (20 μM) for 2 h. The cells were washed three times with PBS and visualize by a confocal microscope (LSM 710, Carl Zeiss, Göttingen, Germany) immediately.

HDAC enzyme inhibition assay

Pan-HDAC inhibition assay. Pan-HDAC inhibition assay was performed using a fluorescent HDACs activity assay kit (Millipore, USA), following the manufacturer's instructions.

Human recombinant HDAC7 inhibition assay. The inhibition assay for HDAC7 was performed using a fluorogenic HDAC class IIa assay kit (BPS Bioscience Incorporated), according to the manufacturer's instructions. Briefly, the tested compounds were incubated with human recombinant HDAC7 enzyme (BPS Bioscience, USA). The reaction was initiated by addition of the Fluor-de-Lys substrate. Samples were incubated for 30 min at 37 °C, followed by the addition of the developer solution to stop the reaction. The mixture was incubated for another 10 min at 25 °C. Fluorescence was quantified with a TECAN Infinite M200 station.

Western blot analysis

Western blotting was performed as previously described.[5] Briefly, HeLa cells were seeded into 60 mm tissue culture dishes (Corning) and incubated for 24 h, and then treated with **ReLMito** at different concentrations for 24 h. Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer. The proteins were separated on SDS-polyacrylamide gel electrophoresis and then transferred onto

polyvinylidene difluoride membranes (Millipore, MA, USA). The membrane was blocked and incubated with the primary antibodies (Cell Signaling Technology, MA, USA) at 4 °C overnight. After a subsequent washing step, the membrane was incubated with the HRP-conjugated secondary antibody for 1 h at room temperature. The signals were detected using the enhanced chemiluminescence (ECL) kit (Amersham Inc, USA). Images were captured on FluorChem M (ProteinSimple, Santa Clara, CA).

Cell cycle analysis

Cell cycle distribution was analyzed by flow cytometry and PI staining as previously described.[4]

Transmission electron microscopy

HeLa cells were treated with **ReLMito** (20 μM) at 37 ºC for 24 h. Cells were washed twice and fixed overnight at 4 ºC in phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde. The cells were then treated with osmium tetroxide, stained with uranyl acetate and lead citrate, and visualised under a transmission electron microscope (JEM 100 CX, JEOL, Tokyo, Japan). Images were photographed using the Eversmart Jazz program (Scitex).

Hoechst staining

The morphological changes of HeLa cells treated with **ReLMito** (20 μM, 24 h) were studied by Hoechst 33342 staining according to the method previously reported.[4]

Caspase-3/7 activity assay

Caspase-3/7 activity was measured using the Caspase-Glo® Assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, cells were cultured in 48-well plates and treated with different concentration of **ReLMito** for 6 h, and then 50 μL cell lysate was added to each well,

followed by the addition of 50 μL Caspase-Glo® 3/7 reagent. The mixture was incubated at room temperature for 30 min and then the luminescence was measured using a TECAN Infinite M200 station.

Analysis of mitochondrial membrane potential (MMP)

The impact of **ReLMito** and SAHA on MMP was determined as previously described.[4] Briefly, HeLa cells were treated with **ReLMito** and SAHA at the indicated concentrations for 6 h. The cells were then collected and stained with 5 μg/mL JC-1, then analyzed immediately in a flow cytometer (FACSCalibur™, Becton Dickinson, Franklin Lakes, NJ, USA). Red and green mean fluorescence intensities were analyzed using FlowJo 7.6 software (Tree Star, OR, USA). 10, 000 events were acquired for each sample.

Measurement of intracellular reactive oxygen species (ROS)

The impact of **ReLMito** and SAHA on ROS levels was determined as previously described.[4] Briefly, cells were treated with **ReLMito** and SAHA at the indicated concentrations for 6 h and then incubated with 10 μM H_2DCFDA in serum-free DMEM for 15 min at 37 °C in the dark. The fluorescence intensity of the cells was measured immediately by flow cytometry (FACSCalibur™, Becton Dickinson, Franklin Lakes, NJ, USA) with excitation at 488 nm and emission at 530 nm. Green mean fluorescence intensities were analyzed using FlowJo 7.6 software (Tree Star, OR, USA).

Mitochondrial real-time tracking

HeLa cells were seeded in 35 mm dishes for 24 h and then incubated with **ReLMito** (20 μM) at 37 °C for varying amounts of time. The cells were then washed three times with PBS and visualized by a confocal microscope (LSM 710, Carl Zeiss, Göttingen, Germany) immediately. Emission was collected at 564 ± 20 nm upon excitation at 405 nm.

Statistical analysis

All biological experiments were performed at least twice with triplicates in each experiment. Representative results were depicted in this report and data were presented as means ± standard deviations.

Supporting Figures and Tables

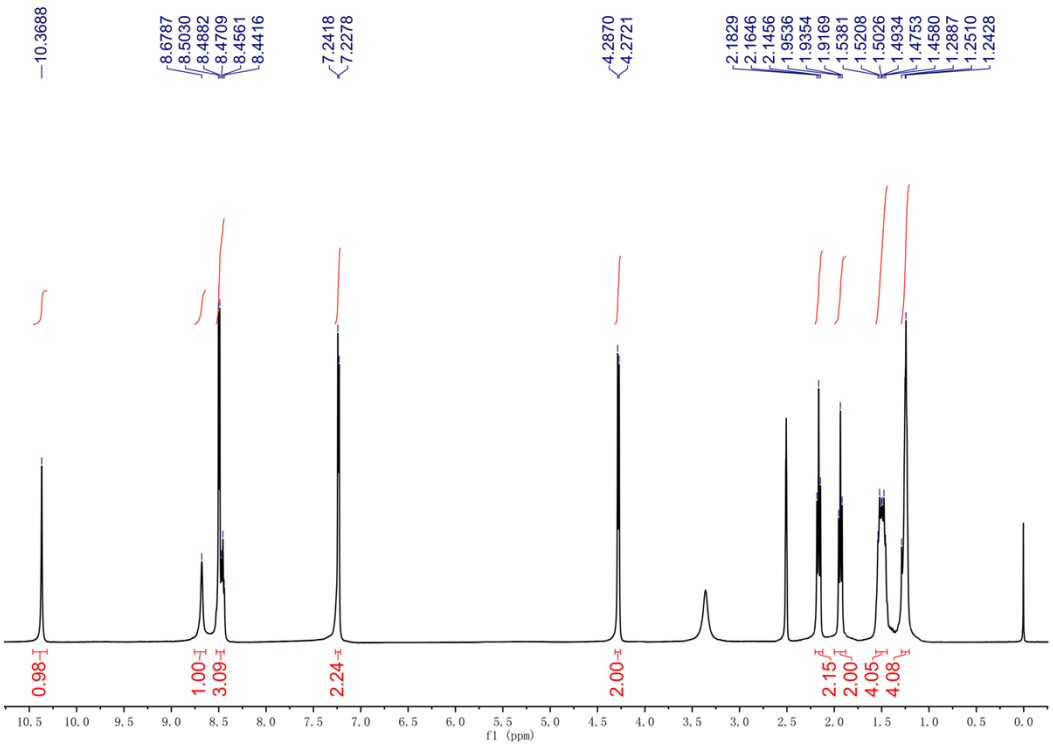


Fig. S1 ^{1}H NMR spectrum of **L**.

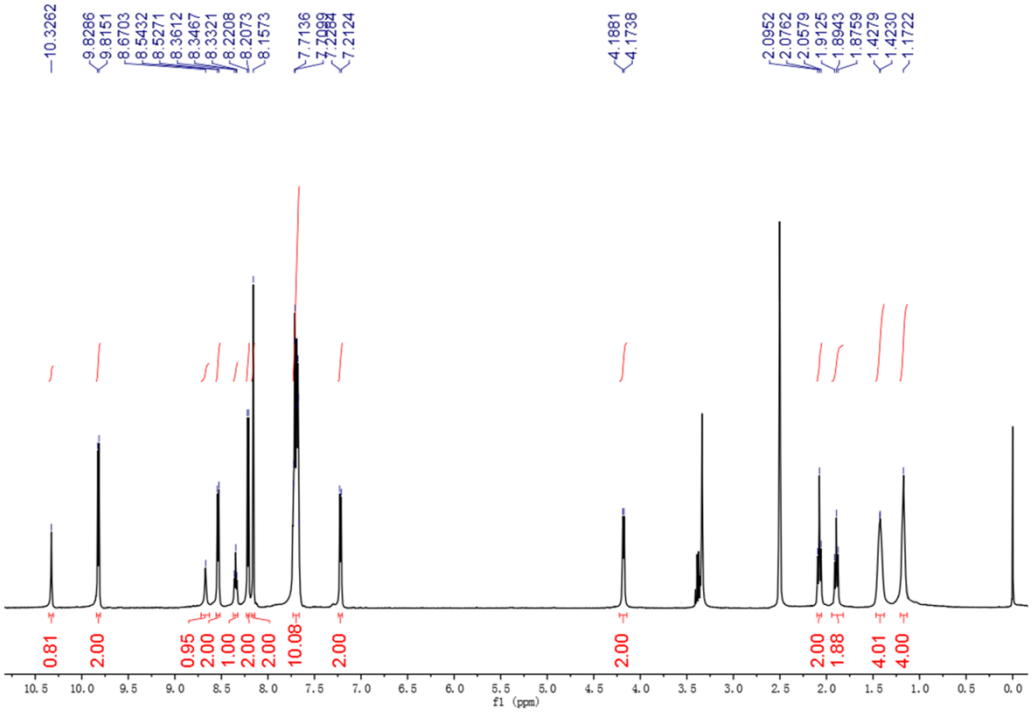


Fig. S2 ^{1}H NMR spectrum of **ReLMito**.

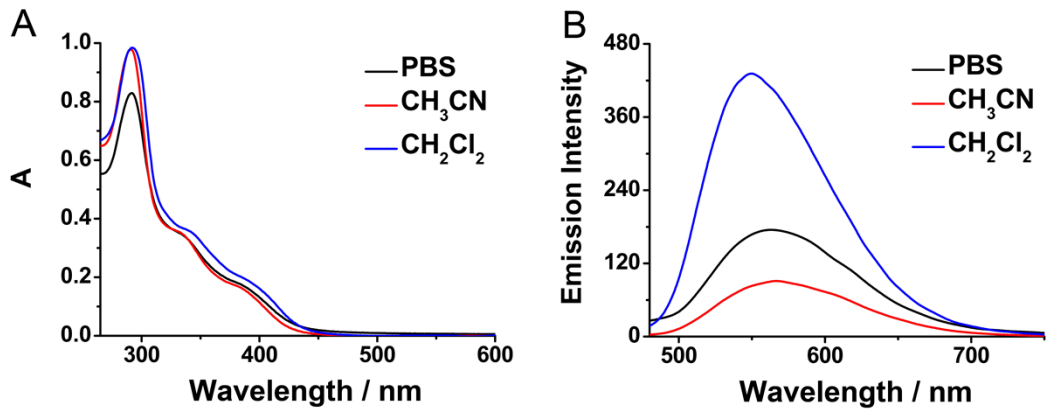


Fig. S3 UV/Vis spectra (A) and emission spectra (B) of **ReLMito** (2×10^{-5} M) measured in degassed solvents at 298 K.

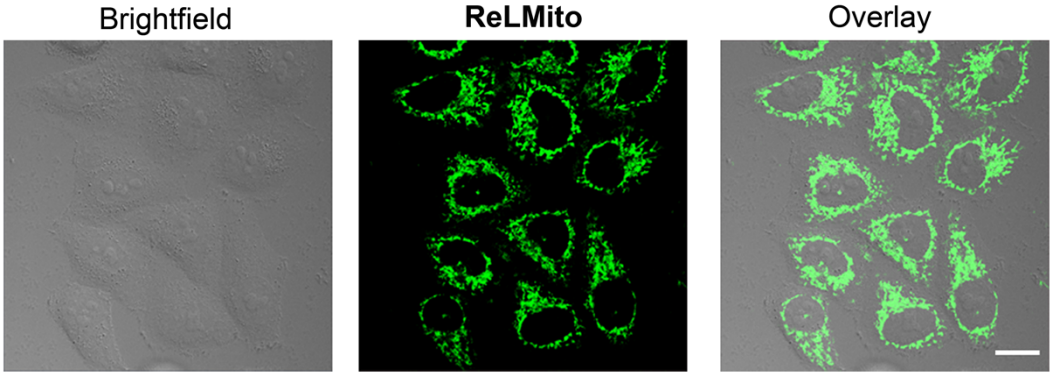


Fig. S4 Confocal microscopic images of HeLa cells incubated with **ReLMito** (20 μM) at 37 °C for 2 h. (λ_{ex} = 405 nm, λ_{em} = 564 nm ± 20 nm). Scale bar: 20 μm.

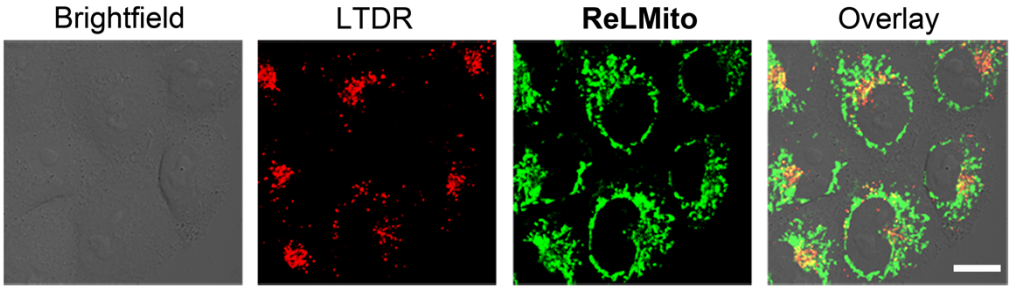


Fig. S5 Confocal microscopic images of HeLa cells after incubation with **ReLMito** (20 μM, 2 h, λ_{ex} = 405 nm, λ_{em} = 564 ± 20 nm). The cells were co-stained with LTDR (50 nM, 0.5 h, λ_{ex} = 633 nm, λ_{em} = 668 ± 20 nm). Scale bar: 20 μm.

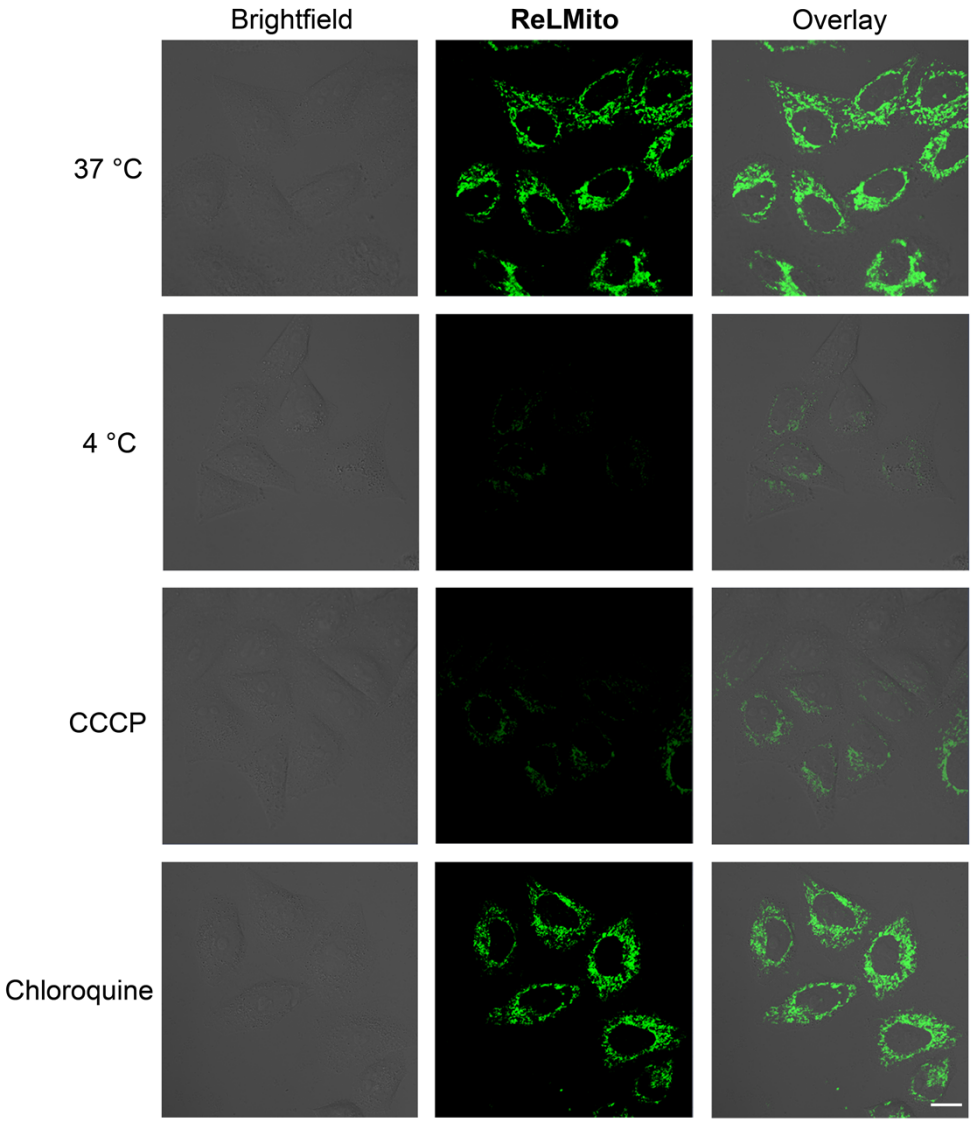


Fig. S6 Effect of incubation temperature (37 ºC and 4 ºC), metabolic inhibitor (CCCP, 30 μM) and chloroquine (50 μM) on cellular uptake of **ReLMito** (20 μM, 2 h) measured by confocal microscopy. **ReLMito** was excited at 405 nm and emission was collected at 564 ± 20 nm. Scale bar: 20 μm.

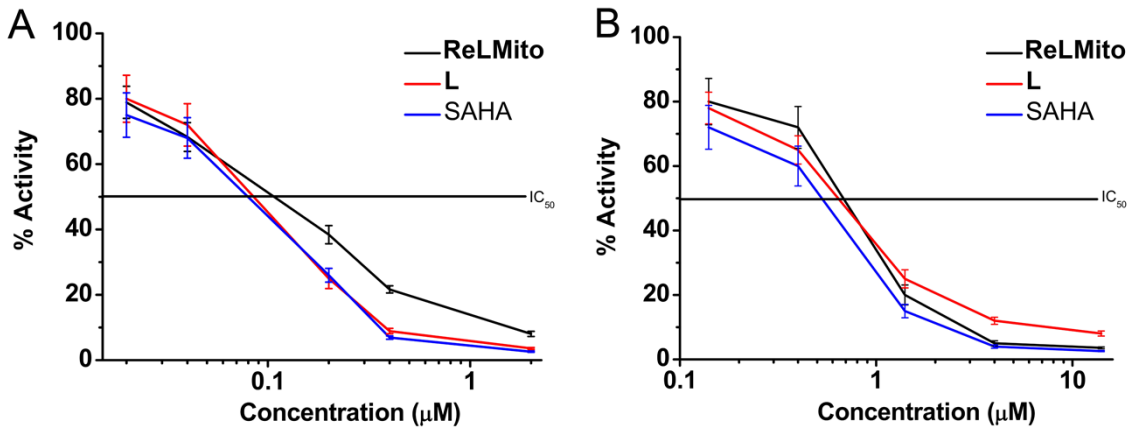


Fig. S7 Inhibition of HDACs activity by **ReLMito**, **L** and SAHA. (A) Inhibition of HDACs in HeLa cells nuclear extracts. (B) Inhibition of human recombinant HDAC7. Data were expressed as the mean of three independent experiments.

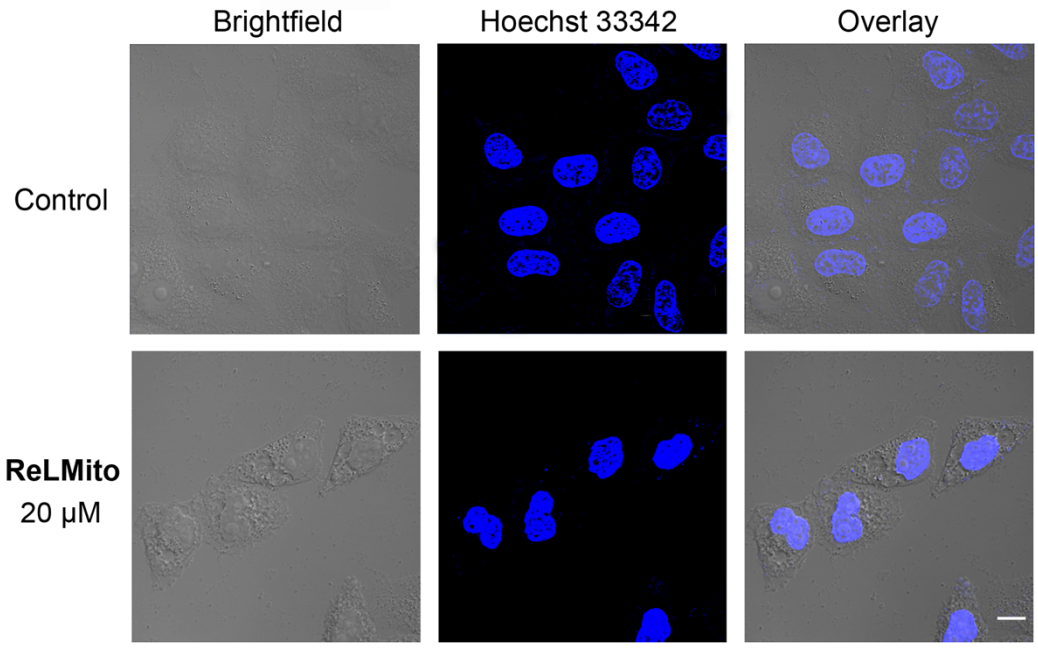


Fig. S8 Hoechst 33342 stained HeLa cells after treatment of **ReLMito** (20 μM) for 24 h. Scale bar: 20 μm.

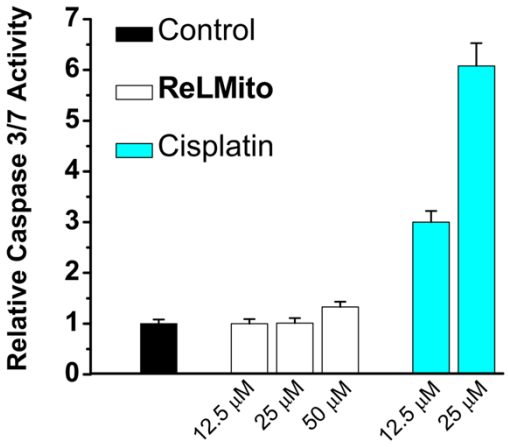


Fig. S9 The activation of caspase-3/7 in HeLa cells treated with **ReLMito**, SAHA or cisplatin at the indicated concentrations for 6 h.

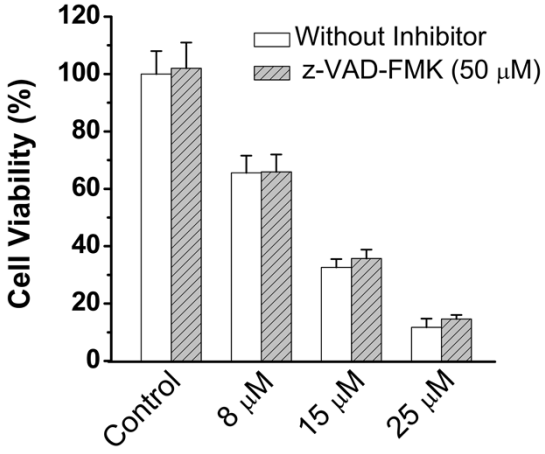


Fig. S10 The impact of z-VAD-FMK on the cytotoxicity of **ReLMito**. HeLa cells were treated with **ReLMito** for 48 h at the indicated concentrations in the absence or presence of z-VAD-FMK. Cell viability was measured by MTT assay.

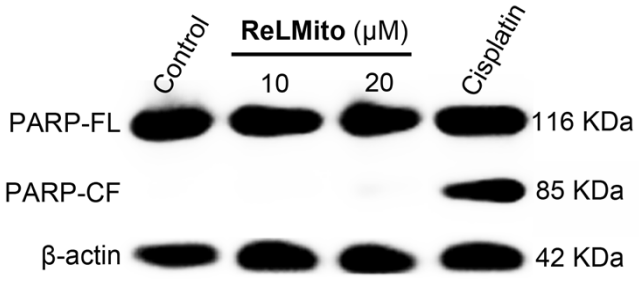


Fig. S11 Effects of **ReLMito** on PARP cleavage. HeLa cells were treated with vehicle, **ReLMito** at

different concentrations or cisplatin (25 μM) for 24 h. The full-length and cleaved protein fragments of PARP were detected by western blot (FL: full length; CF: cleaved form).

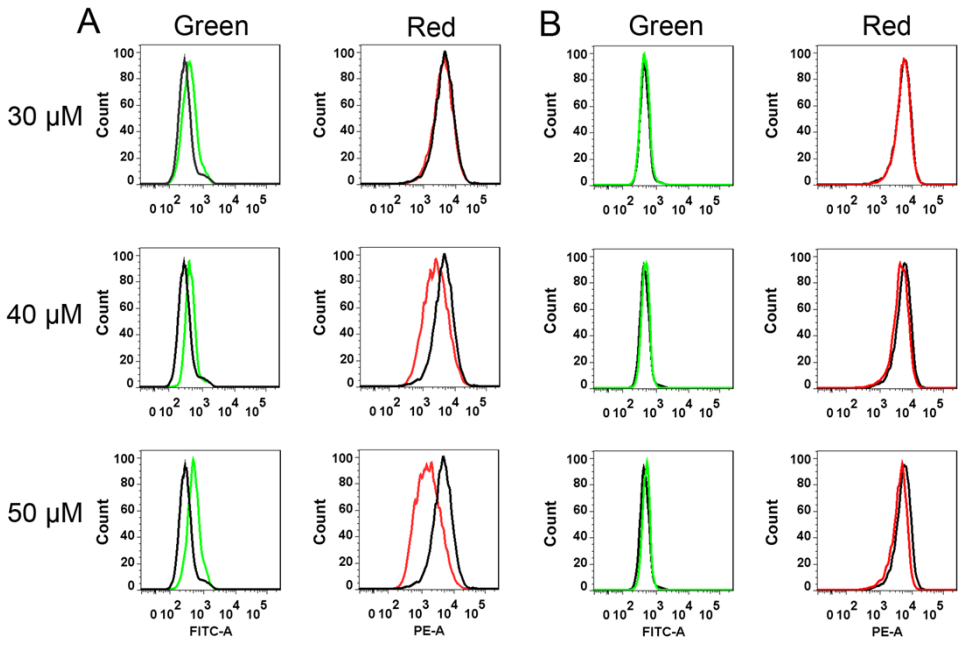


Fig. S12 Effects of **ReLMito** (A) and SAHA (B) on MMP. Cells were treated with vehicle, **ReLMito** or SAHA at the indicated concentrations for 6 h. Black curve: cells treated with vehicle (1% DMSO); red and green curves: cells treated with **ReLMito** or SAHA.

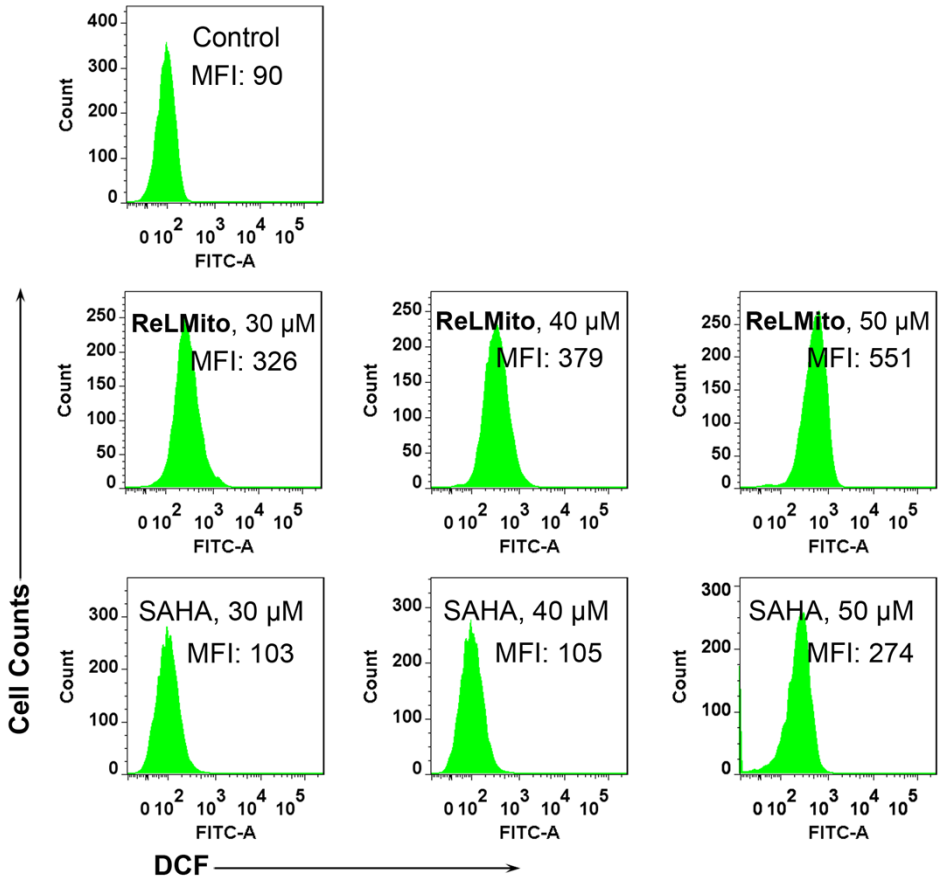


Fig. S13 Effects of **ReLMito** and SAHA on ROS generation. HeLa cells incubated with **ReLMito** or SAHA at 37 °C for 6 h, after which they were labeled with H_2DCFDA and analyzed by flow cytometry (reflected by the mean fluorescence intensity (MFI) of DCF; excitation at 488 nm and emission at 525 nm).

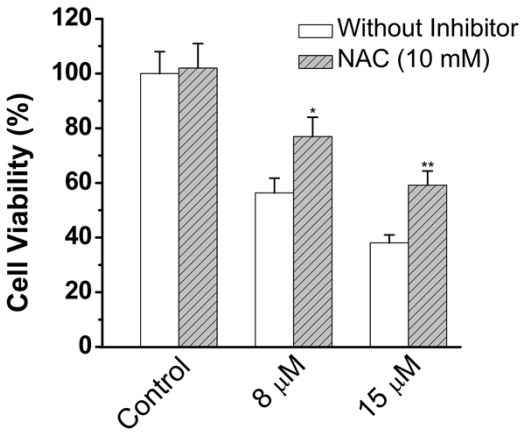


Fig. S14 The impact of NAC on the cytotoxicity of **ReLMito**. HeLa cells were treated with

ReLMito for 48 h at the indicated concentrations in the absence or presence of NAC. Cell viability was measured by MTT assay. Data are represented as means ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$.

Table S1 Photophysical data of **ReLMito** in degassed medium[a]

Compounds	Medium	$\lambda_{abs, max}$ (nm)	$\lambda_{em, max}$ (nm)	Φ_{em}[b]	τ_{av}[c] (ns)
ReLMito	PBS	385	564	0.029	985.33
	CH_3CN	385	570	0.058	405.55
	CH_2Cl_2	394	550	0.070	1057.12

[a] All emission decays were obtained on freshly prepared samples placed in quartz cuvettes. Samples were 2×10^{-5} M in concentration. [b] Solutions of $[Ru(bpy)_3](PF_6)_2$ were used as the standard, PBS ($\Phi_{em} = 0.042$)[6], CH_3CN ($\Phi_{em} = 0.062$)[7] and CH_2Cl_2 ($\Phi_{em} = 0.059$)[8]. [c] Decay curves of compounds were recorded by an Edinburgh FLS 920 Spectrometer. All curves were fitted into a two exponential formula $F(t) = A + B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) + B_3 \exp(-t/\tau_3)$; $\tau_{ay} = \frac{B_1\tau_1^2 + B_2\tau_2^2 + B_3\tau_3^2}{B_1\tau_1 + B_2\tau_2 + B_3\tau_3}$

Table S2 IC_{50} values of tested compounds towards different cell lines[a]

Compounds	IC_{50} (μM)				
	HeLa	A549	A549R	HepG2	LO2
ReLMito	8.2 ± 0.7	10.6 ± 1.1	12.2 ± 1.5	7.5 ± 0.8	51.6 ± 4.8
L	> 100	> 100	> 100	> 100	> 100
SAHA	20.5 ± 2.0	4.8 ± 0.5	3.2 ± 0.3	26.4 ± 2.5	> 100
cisplatin	25.1 ± 2.1	13.5 ± 1.1	75.6 ± 6.2	14.8 ± 1.5	33.1 ± 2.9

[a] IC_{50} values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviations obtained in at least three independent experiments and drug treatment period was 48 h.

Supporting References

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