Electronic Supplementary Information

Dinuclear phosphorescent rhenium(I) complexes as potential anticancer and photodynamic therapy agents

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Experimental section

Materials and measurements
Re(CO)₅Cl, 1,2-bis(4-pyridyl)ethane (BPE), silver trifluoromethanesulfonate (AgCF₃SO₃), disodium hydrogen phosphate, citric acid, cisplatin, 9,10-anthracenediyl-bis-(methylene)dimalonic acid (ABDA), [Ru(bpy)₃]Cl₂, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acridine orange (AO), Hoechst 33342, H₂DCF-DA, N-acetyl-L-cysteine (NAC) and catalase were purchased from Sigma Aldrich (USA). NH₄PF₆ was purchased from Alfa Aesar (USA). MnTBAP was purchased from Santa Cruz Biotechnology (USA). Dihydroethidium (DHE) was purchased from Beyotime (China). LysoTracker Green DND-26 (LTG,) and MitoTracker Green FM (MTG) were purchased from Life Technologies (USA). All the materials were used as received. For cell-based experiments, the Re(I) complexes were dissolved in DMSO just before the experiments and then diluted to the desired concentrations by cell medium.

¹H and ¹³C NMR spectra of the Re(I) complexes were recorded on a Bruker Avance 400/500 spectrometer. ESI-MS spectra of the Re(I) complexes were recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). Elemental analysis was carried out using an Elemental Vario EL CHNS analyzer (Germany). UV/Vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Steady-state emission spectra were performed on an Edinburgh FLS 920 Spectrometer (UK).

Cell lines and culture conditions
The cell lines and culture conditions used in this study were consistent with those previously reported.¹ A549, A549R, HeLa, MCF-7 and HLF cells were obtained from Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). Cells were kept in Dulbecco’s modified Eagle’s medium (DMEM, Gibco BRL) or Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco BRL), which contained 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin (Gibco BRL), and 100 U/mL penicillin (Gibco BRL). The cells were cultured in a humidified incubator, which provided an atmosphere of 5% CO₂ and 95% air at a constant temperature of 37 °C

Cytotoxicity¹
Cytotoxicity was tested using previously reported methods.¹ A549, A549R, HeLa, MCF-7 and HLF
cells were cultured in 96-well plates were grown to confluence. The compounds were dissolved in DMSO (1%, v/v), and diluted with fresh media immediately. The cells were incubated with a series of concentrations of the tested compounds for 44 h at 37 °C. 20 µL of MTT solution was then added to each well, and the plates were incubated for an additional 4 h. The media was carefully removed, and DMSO was added (150 µL per well) and incubated for 10 min with shaking. The absorbance at 595 nm was measured using a microplate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland).

**Cellular uptake and colocalization assay**

**Cellular uptake:** A549 cells were treated with complexes DRe1 (20 µM) and DRe2 (5 µM) for 1 hour at 37 °C, the culture medium was removed, the cells were washed with PBS for three times, kept in PBS and then visualized immediately by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany). Upon excitation at 405 nm, emission was collected at 550–600 nm and 600–650 nm for DRe1 and DRe2, respectively.

**Colocalization assay:** A549 cells were treated with complexes DRe1 (20 µM) and DRe2 (5 µM) for 0.5 h at 37 °C, and then co-incubated with LTG (150 nM) or MTG (150 nM) for 0.5h. The culture medium was removed, the cells were washed with PBS for three times, kept in PBS and then visualized immediately by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany). Upon excitation at 405 nm, emission was collected at 550–600 nm and 600–650 nm for DRe1 and DRe2, respectively. Upon excitation at 488 nm, emission was collected at 490–530 nm for LTG and MTG.

**AO staining**

A549 cells were treated with different concentrations of DRe2 (1, 2, 4 µM) for 4 h. The old culture medium was removed, and fresh culture medium containing AO (5 µM) was added and cells were incubated for 15 minutes. After removing the culture medium, the cells were washed with PBS for three times, kept in PBS and then visualized immediately by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany). Upon excitation at 488 nm, emission was collected at 490–530 nm (green) and 605–645 nm (red).

**Cellular ROS and superoxide detection**

A549 cells were treated with different concentrations of DRe2 (1, 2, 4 µM) for 4 h at 37 °C. The old
culture medium was removed and cells were washed with PBS for three times, and fresh serum-free RPMI 1640 containing H$_2$DCF-DA (10 μM) or DHE (5 μM) was added and cells were incubated for 20 minutes at 37 °C in the dark. After removing the culture medium, the cells were washed with PBS for three times, kept in PBS and then visualized immediately by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany). Upon excitation at 488 nm, emission was collected at 500–540 nm for DCF. Upon excitation at 514 nm, emission was collected at 570–600 nm for DHE.

**Hoechst 33342 staining**

A549 cells were treated with different concentrations of DRe2 (1, 2, 4 μM) for 24 h. The culture medium was removed, and the cells were washed twice with PBS gently and then fixed with 4% paraformaldehyde for 30 min at room temperature. After removing the 4% paraformaldehyde solution, the cells were washed with PBS for two times, and then PBS containing Hoechst 33342 (5 μg/mL) was added and cells were incubated for 10 minutes in the dark. The cells were washed with PBS for two times, kept in PBS and then visualized immediately by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany). Upon excitation at 405 nm, emission was collected at 440–480 nm.

**Singlet oxygen ($^{1}$O$_{2}$) quantum yield**

The quantum yields for $^{1}$O$_{2}$ production ($\Phi_{\Delta}$) of the dinuclear Re(I) complexes was determined using previously reported methods.$^{2}$

**Phototoxicity**

The phototoxicity of the dinuclear Re(I) complexes was tested using previously reported methods.$^{2}$
Supporting figures and tables

Fig. S1 $^1$H NMR spectrum of DRe1 in DMSO-$d_6$ at 298 K.

Fig. S2 $^1$H NMR spectrum of DRe2 in DMSO-$d_6$ at 298 K.
Fig. S3 $^{13}$C NMR spectrum of DRe1 in DMSO-$d_6$ at 298 K.

Fig. S4 $^{13}$C NMR spectrum of DRe2 in DMSO-$d_6$ at 298 K.
Fig. S5 UV/Vis spectra of DRe1 and DRe2 (10 μM) measured in (a) CH₂Cl₂ and (b) PBS at 25 °C.

Fig. S6 Emission spectra of DRe1 and DRe2 (10 μM) measured in (a) CH₂Cl₂ and (b) PBS at 25 °C. λₘₑₓ = 405 nm.
Fig. S7 Cellular uptake of DRe1 (20 μM, 1 h) and DRe2 (5 μM, 1 h) analyzed by confocal microscopy in A549 cells. All images share the same scale bar: 10 μm.

Fig. S8 A549 cells were incubated with DRe2 (5 μM, 1 h) under different temperatures or pretreated with CCCP (20 μM, 1 h). All images share the same scale bar: 20 μm.
**Fig. S9** Detection of superoxide anion radicals after A549 cells were incubated with different concentrations of DRe2 for 4 h by staining with DHE. DHE: $\lambda_{ex} = 514$ nm; $\lambda_{em} = 570–600$ nm (red). All images share the same the scale bar.
Fig. S10 $^1$H NMR spectra of (a) DRe1 and (b) DRe2 (1 mM) in DMSO-$d_6$ before and after light irradiation (425 nm, 40 mW·cm$^{-2}$) for 15 min.

<table>
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<th>Complex</th>
<th>Medium$^a$</th>
<th>$\lambda_{ex}$/nm</th>
<th>$\lambda_{em,max}$/nm</th>
<th>$\phi^b$/%</th>
<th>$\tau$/µs</th>
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<td>DRe1</td>
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</table>

$^a$ Air-equilibrated solutions. $^b$ The emission quantum yields were determined using [Ru(bpy)$_3$]Cl$_2$ as the standard.
Supporting references
