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moPlatinum-AIEgen Coordination Complex for Imaging-Guided Annihilation of Cisplatin-Resistant Cancer Cells

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Contents:

Experimental Section

1. Materials and Instruments

Materials: All organic reagents were purchased from Energy Chemical (China). All solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. Unless noted all reagents and solvents with analytical purity were used as received. Bovine serum albumin (BSA) was purchased from Biosharp. Lysozyme was purchased from Sigma-Aldrich. 3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl tetrazolium bromide (MTT) was purchased from Boster Biological Teachnology Co., Ltd. Lysotracker Red was obtained from Yeasen Biotechnology Co., Ltd. (Shanghai). A549 and A549/DDP cells were purchased from Shanghai Mito Biological Teachnology Co., Ltd. HeLa/DDP cells was purchased from Shcqsw biomart Co., Ltd. Nuclear Extraction Kit was purchased from Beijing Solarbio Science and Technology Co.,Ltd.

Instruments: All nuclear magnetic resonance (NMR) spectra were recorded on an Agilent 400-MR 400 MHz spectrometer operated in the Fourier transform mode. DMSO-d_6 was used as the solvent. High-resolution electrospray mass spectroscpy (HR-ESI-MS) was conducted on a SolariX 7.0T mass spectrometer (BrukerDaltonics, USA). UV-Vis absorption spectra were acquired on a TU-1810DSPC UV/Vis spectrophotometer (Puxi General Instrumental Company, China). TEM images were recorded on transmission electron microscope (Hitachi HT7700, Japan). Fluorescence spectra were recorded on a HITACHI F4600 fluorescence spectrophotometer. Cytotoxicity data were recorded on a microplate reader (Varioskan LUX, Thermo

Scientific, USA). Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was conducted on Agilent 700.

2. Experimental Methods

Synthesis of TPCTP: The intermediate (2Z,2'Z)-2,2'-(1,4-phenylene)bis(3-(4-(bis (4-(pyridin-4-yl)phenyl)amino)phenyl)acrylonitrile) (TPC) was synthesized following a literature procedure.[1] In a 25 mL round-bottom flask, a mixture of cisplatin (10.20 mg, 0.034 mmol) and silver nitrate (5.78 mg, 0.034 mmol) in N,N-dimethylformamide (DMF) (10 mL) was stirred and heated to 60 °C for 24 h under a nitrogen atmosphere. After cooling to room temperature, the reaction mixture was separated by centrifugation. The supernatant was added with TPC (3.32 mg, 0.0034 mmol) and stirred at 60 °C for 24 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was poured into diethyl ether. The precipitate was then washed with diethyl ether three times and dried under reduced pressure to afford the product as an orange powder (6.16 mg, yield: 89%).

The value of water solubility: Frist, the standard curve of TPC and TPCTP in dimethyl sulfoxide (DMSO) have been determined. Then, TPC and TPCTP (0.5 mg) were dissolved in pure water (8 mL), respectively. The aqueous solution was gently swung for 24 h (37 °C) after sonication. Then, 6.8 mL of supernatant was lyophilized after centrifugation. Lyophilized TPC and TPCTP were re-dissolved in DMSO to measure the absorption value. Finally, the values of water solubility were calculated according to the standard curve of TPC and TPCTP, respectively.

Measurement of $^1O_2$ generation: The $^1O_2$ generation of TPC and TPCTP in water

upon white light irradiation (350-800 nm, 1 mW cm^{-2}) was determined using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) as an indicator and Rose Bengal (RB) as the standard reference. [2] The concentration of ABDA was 100 μM and the concentration of the individual solution of cisplatin, TPC, TPCTP, and RB was 5 μM. The absorbance decrease of ABDA at 379 nm was recorded upon different irradiation time. The $^1O_2$ quantum yield was calculated using the following equation

$$\Phi_{sample} = \Phi_{RB} \times \frac{K_{sample} * A_{RB}}{A_{sample} * K_{RB}}$$

where *K* is the slope of the absorbance versus irradiation time. A represents the integral area of absorption by TPC, cisplatin, RB and TPCTP on white irradiation. Φ_{RB} is the $^1O_2$ quantum yield of reference Rose Bengal, which is 0.75 in water. K_{TPCTP}, K_{TPC} and K_{RB} were calculated to be 0.00283, 0.00052 and 0.00135, respectively (Fig. 12D), and A_{TPCTP}, A_{TPC} and A_{RB} during 350-800 nm were determined to be 24.602, 26.231 and 11.772, respectively (Fig. S8).

Cellular Imaging: All cisplatin-resistant and nonresistant cells were cultured in chamber (LAB-TEK, Chambered Cover Glass System) with standard medium containing 10% FBS and 1% antibiotics (37 °C, 5 % CO_2). After 24 h, the medium was replaced with TPCTP in DMEM (5 μM) and incubated for 24 h. Then, the cells were then treated with Lysotraker Red (100×10^{-9} M) and incubated for 30 min. After being washed by phosphate-buffer saline (PBS), the living cells were observed by confocal laser scanning microscope (CLSM, Perkin Elmer & Olympus, UltraVIEW VoX & IX81). The excitation wavelength was 488 nm and signal collection wavelength region was 500-570 nm for TPCTP. The excitation wavelength

was 594 nm and signal collection wavelength region was 610-710 nm for Lysotracker Red.

Flow Cytometry Analysis: All cisplatin-resistant and nonresistant cancer cells were seeded at a density of 2×10^5 cells/mL of the DMEM medium with 10% FBS on 12-well plates to the final volume of 1 mL. The plates were incubated for 24 h and then treated with TPCTP for different time. After incubation under the condition (5% CO_2, 37 °C), cells were collected and washed with PBS for twice. The cell uptake was then quantified by a flow cytometer (CytoFLEX S), immediately.

ICP-OES: HeLa and A549 cells were seeded in 100 mm diameter Petri dish in triplicate with 3.0 million cells and were incubated in standard medium for 24 h (37 ºC, 5% CO_2). Then, the cells were incubated with TPCTP (4 μM) or standard medium in the dark for 24 h. The cells were treated with trypsin and washed with PBS, and their nuclei were isolated using the Nuclear Extraction Kit. The samples were then lyophilized and weighted. Platinum concentrations in all of the fractions were determined by ICP-OES.

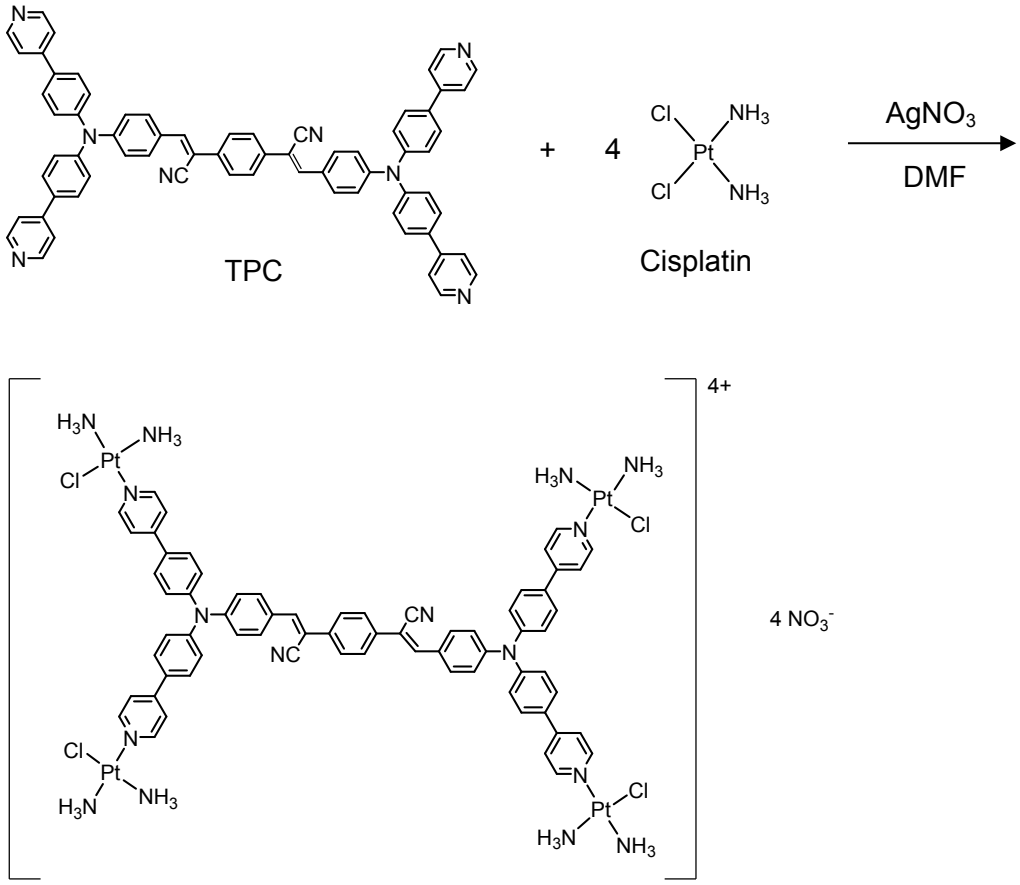
Dark toxicity of TPCTP in Vitro: To evaluate the dark toxicity of TPCTP, the cell viability of TPCTP to various cisplatin-resistant and nonresistant cells were investigated. Cells were seeded in 96-well plates and cultured in standard medium for 24 h (37 ºC, 5% CO_2). The cells were treated with various concentrations of individual drug for 48 h in the dark. After been washed by PBS, 100 μL of freshly prepared MTT solution was added into each well. The MTT solution was carefully removed after 4 h of incubation, and DMSO (130 μL) was added into each well to

dissolve all the formazan formed. The absorbance of MTT at 570 nm was measured by the microplate reader. Cell viability was expressed by the ratio of the cells incubated with different drugs to those incubated with culture normal medium. Each experiment was repeated at least three times.

Phototoxicity of TPCTP in Vitro: The cytotoxicity of different drugs in the presence of white light irradiation was assessed by MTT assays. All cisplatin-resistant and nonresistant cancer cells were seeded in 96-well plates and cultured in standard media for 24 h. The cells were then incubated with drug solutions of various concentrations in the dark for 24 h. The mixtures were exposed to white light irradiation (350-800 nm, 4 mW cm^{-2}) for 30 min. The cells were further cultured for 24 h after irradiation. After being washed by PBS, 100 μL of freshly prepared MTT solution was added into each well. The MTT solution was carefully removed after 4 h of incubation, and DMSO (130 μL) was added into each well to dissolve all the formazan formed. The absorbance of MTT at 570 nm was measured by the microplate reader.

The Value of Combination Index (CI): To verify whether TPCTP has a synergistic effect on PDT and chemotherapy, the CI value of TPCTP was calculated by Calcusyn software. The phototoxicity of TPCTP was caused by the synergy between PDT (TPC + Light group) and chemotherapy (cisplatin group). The calculated CI values are obtained with fraction affected by the dose (Fa) at 0.5.

Supplementary Schemes and Figures



Scheme S1. Synthesis route to TPCTP.

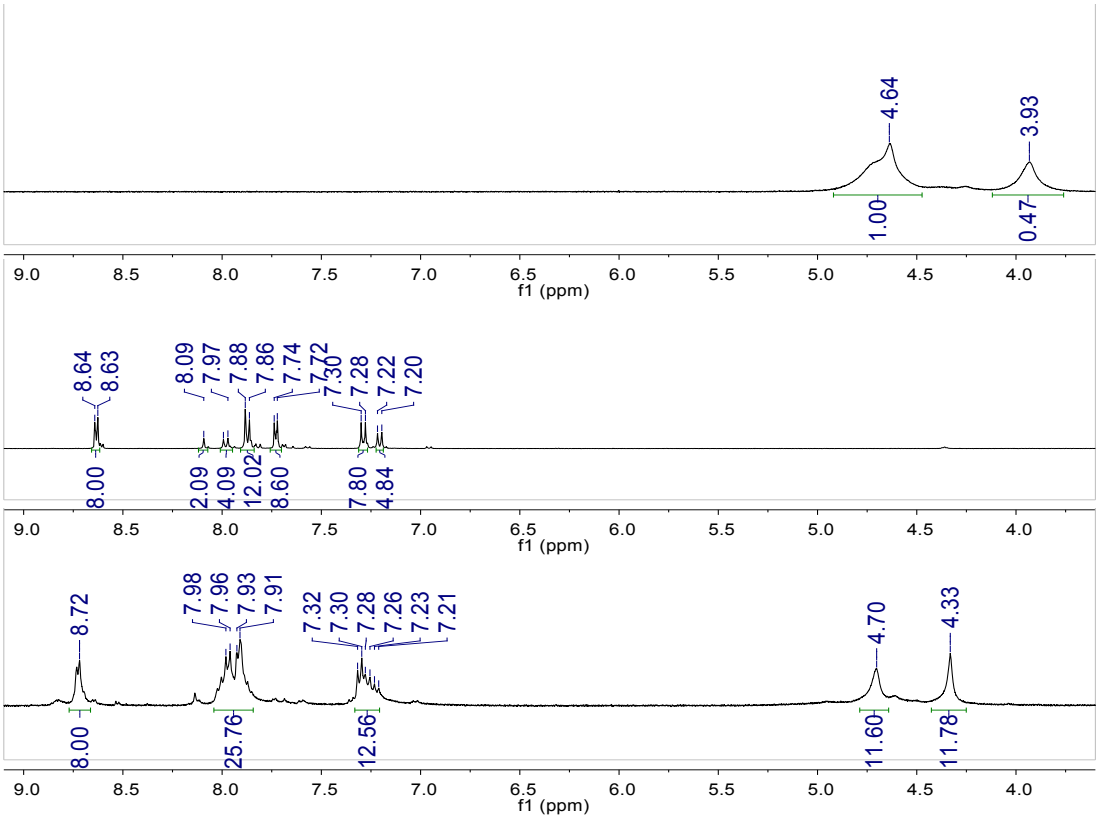


Fig. S1 The ^{1}H NMR spectra of cisplatin, TPC, and TPCTP. Solvent: DMSO-d_6.

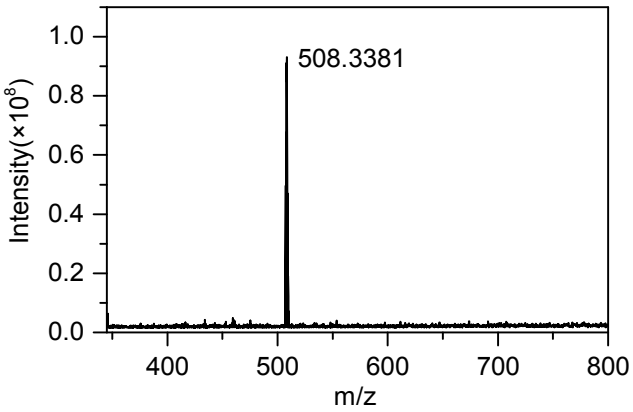


Fig. S2 HR-ESI-MS mass spectrum of TPCTP.

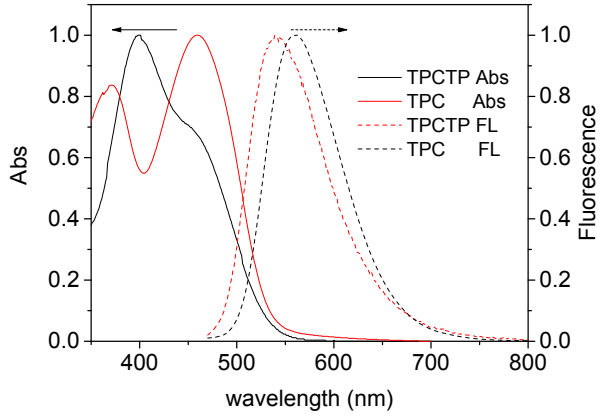


Fig. S3 UV-vis absorption and fluorescence spectra of TPC and TPCTP. Concentration: 5 μM.

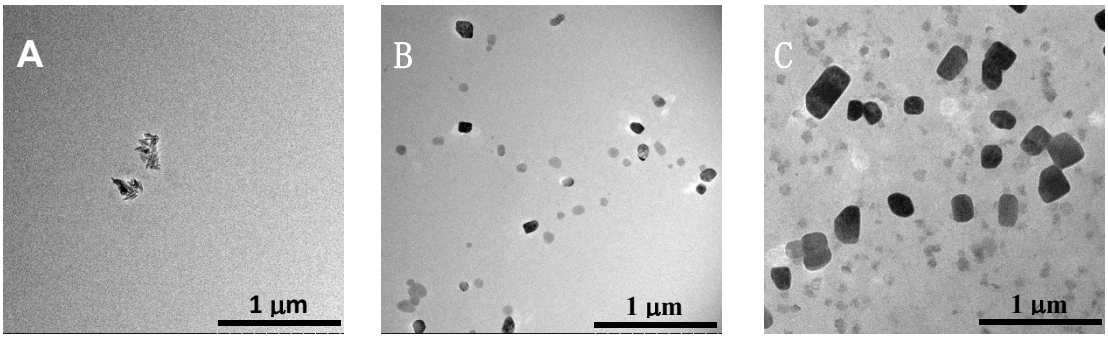


Fig. S4 TEM images of TPCTP with concentrations of 1 μM (A), 10 μM (B) and 50 μM (C), respectively. Scale bar: 1 μm.

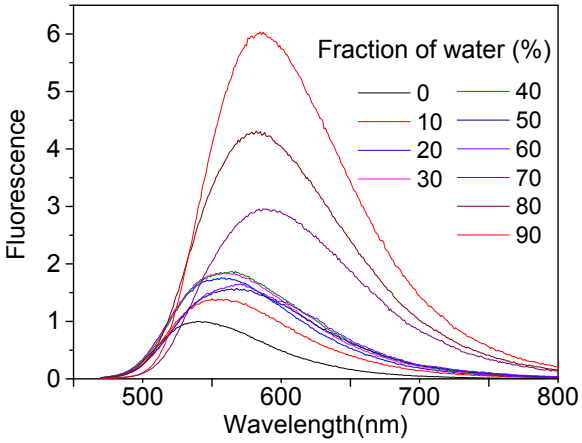


Fig. S5 Fluorescence spectra of TPC (10 μM) in water/THF mixed solvents with

different water fractions. λ_{ex}: 440 nm.

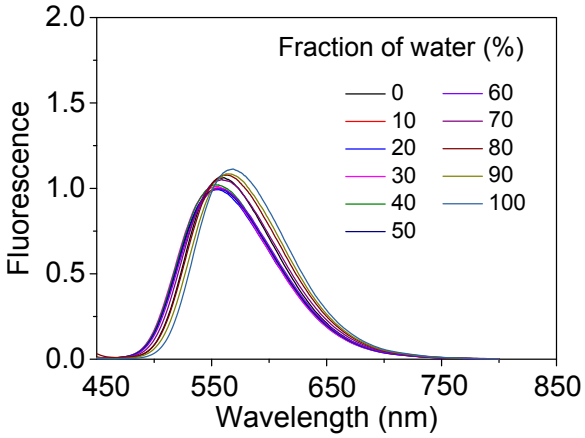


Fig. S6 Fluorescence spectra of TPCTP (1 μM) in water/THF mixed solvents with different water fractions. λ_{ex}: 440 nm.

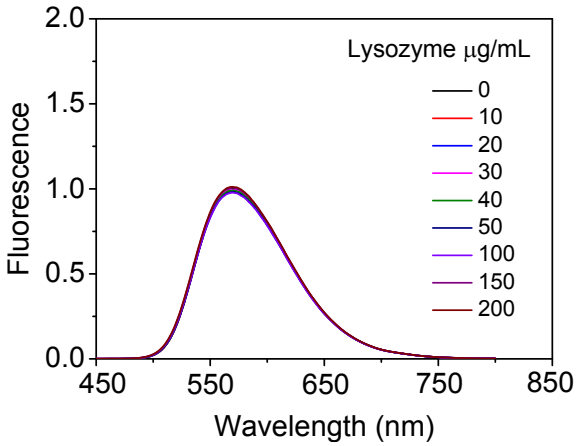


Fig. S7 Fluorescence spectra of TPCTP (10 μM) in the presence of different concentrations (0-200 μg/mL) of lysozyme in aqueous solution. λ_{ex} = 440 nm.

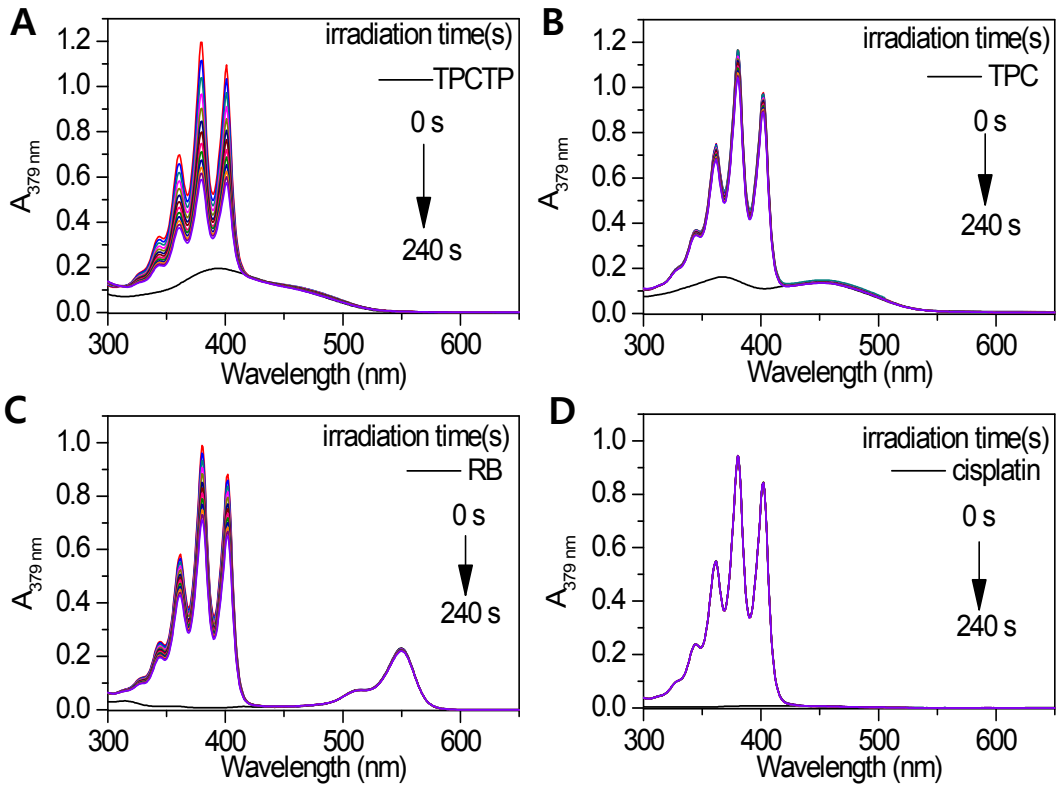


Fig. S8 Absorption spectra of ABDA (100 μM) in the presence of TPCTP (A), TPC (B), RB (C), and cisplatin (D) after being exposed to white light irradiation for different time.

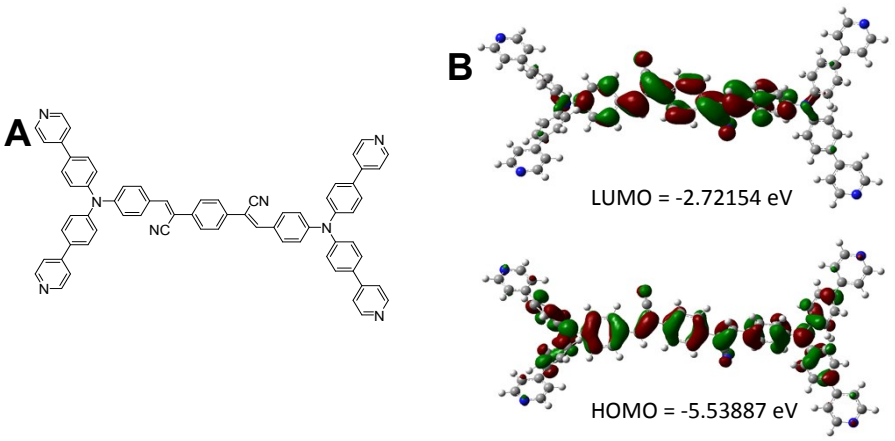


Fig. S9 HOMO-LUMO distribution of TPC. (A) Chemical structure of TPC. (B) Optimized structures of the HOMO and LUMO at S1 were calculated by DFT (Gaussian 09 B3LYP/6-311G(d,p)). HOMO: highest occupied molecular orbital, LUMO: lowest unoccupied molecular orbital. The HOMO-LUMO transition matched well with the absorption for TPC.

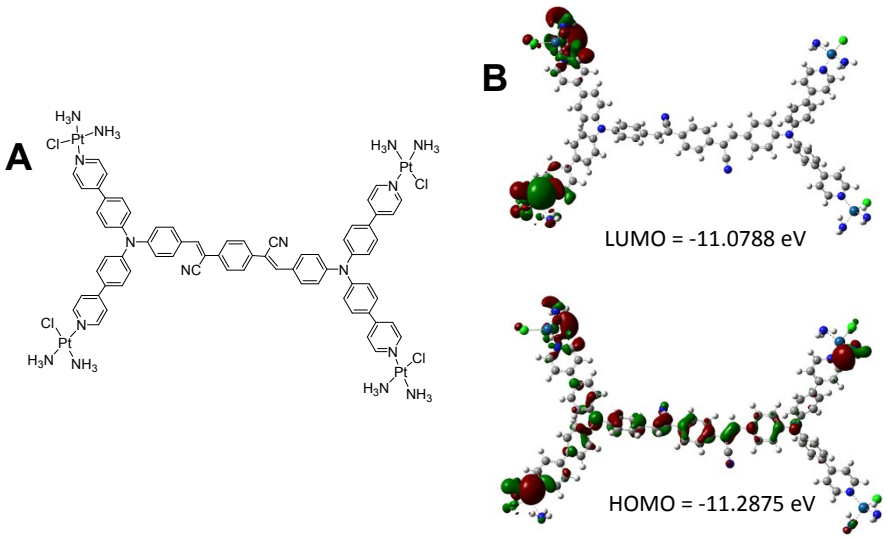


Fig. S10 HOMO-LUMO distribution of TPCTP. (A) Chemical structure of TPCTP. (B) Optimized structures of the HOMO and LUMO at S1 were calculated by DFT (Gaussian 09 B3LYP/6-311G(d,p)). HOMO: highest occupied molecular orbital, LUMO: lowest unoccupied molecular orbital. The interplay became much more

complicated when TPC was coordinated with metals to form the complex, and it was difficult to obtain a reasonable energy and electron density distribution of TPCTP.

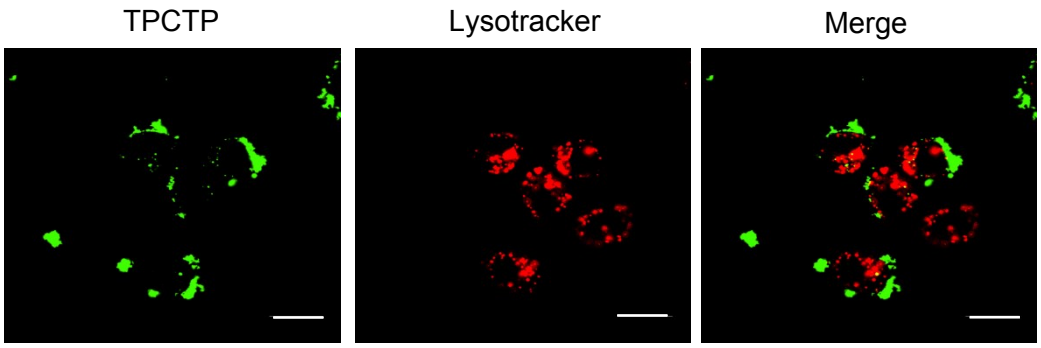


Fig. S11 CLSM images of A549 cells incubated with TPCTP and Lysotracker Red for 24 h. (TPCTP: λ_{ex} = 488 nm, λ_{em} = 510-570 nm; Lysotracker Red: λ_{ex} = 594 nm, λ_{em} = 610-710 nm). Scale bar: 20 μm.

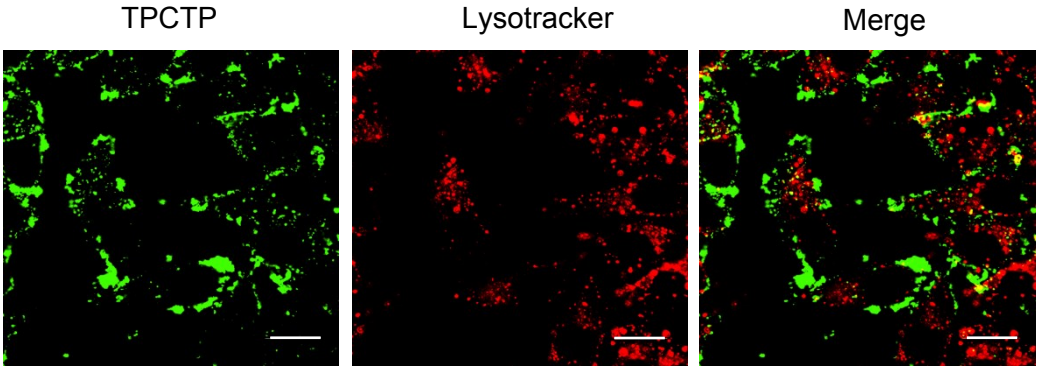


Fig.S12 CLSM images of HeLa cells incubated with TPCTP and Lysotracker Red for 24 h. (TPCTP: λ_{ex} = 488 nm, λ_{em} = 510-570 nm; Lysotracker Red: λ_{ex} = 594 nm, λ_{em} = 610-710 nm). Scale bar: 20 μm.

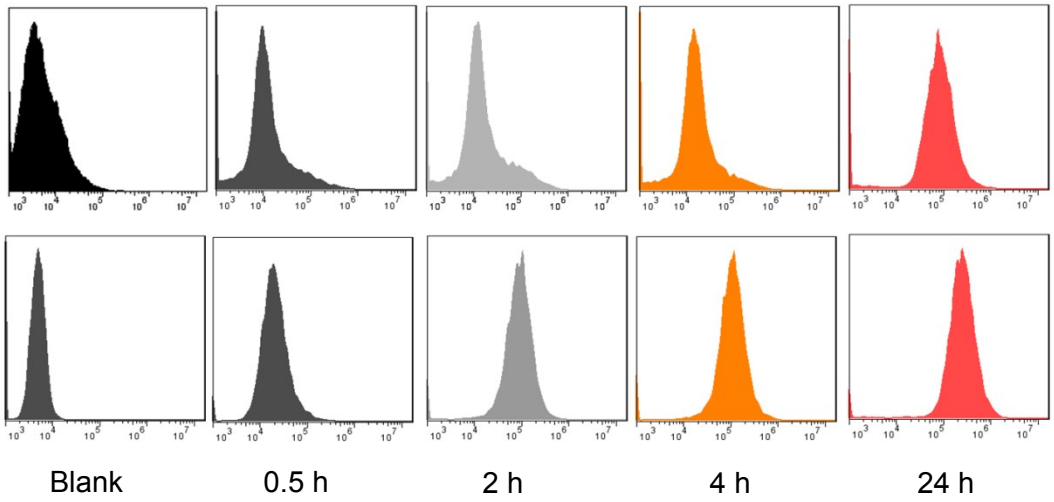


Fig. S13 Flow cytometry analysis of A549 cells (top) and A549/DDP (bottom) treated with TPCTP (5 μM) for different time.

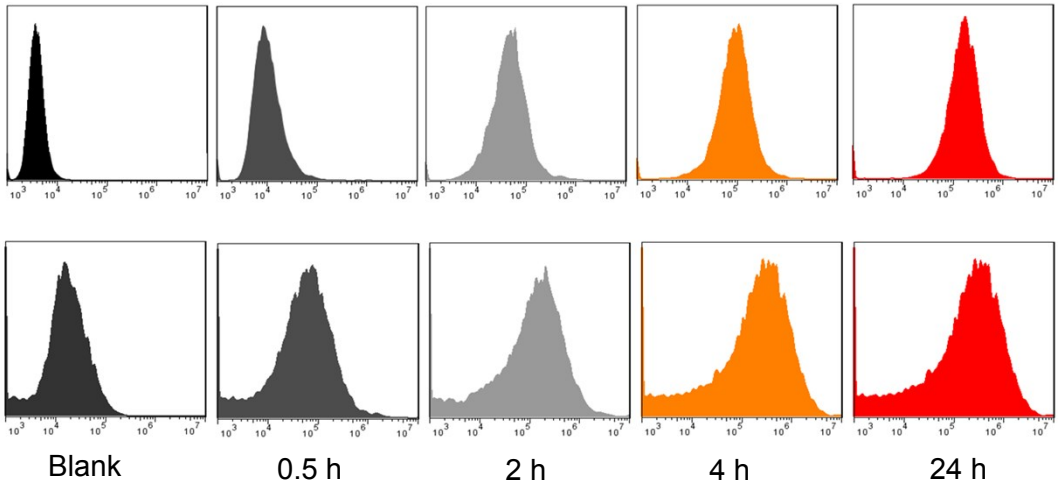


Fig. S14 Flow cytometry study of HeLa cells (top) and HeLa /DDP (bottom) treated with TPCTP (5 μM) for different time.

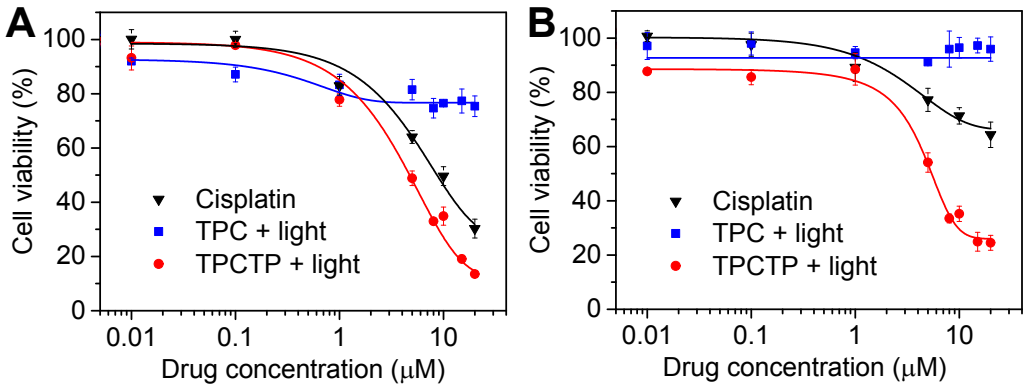


Fig.S15 MTT assays of A549 cells (A) and HeLa cells (B) pretreated with a series doses of cisplatin, TPC and light irradiation, and TPCTP and light irradiation. Light irradiation: 350-800 nm, 4 mW cm^{-2}, 30 min.

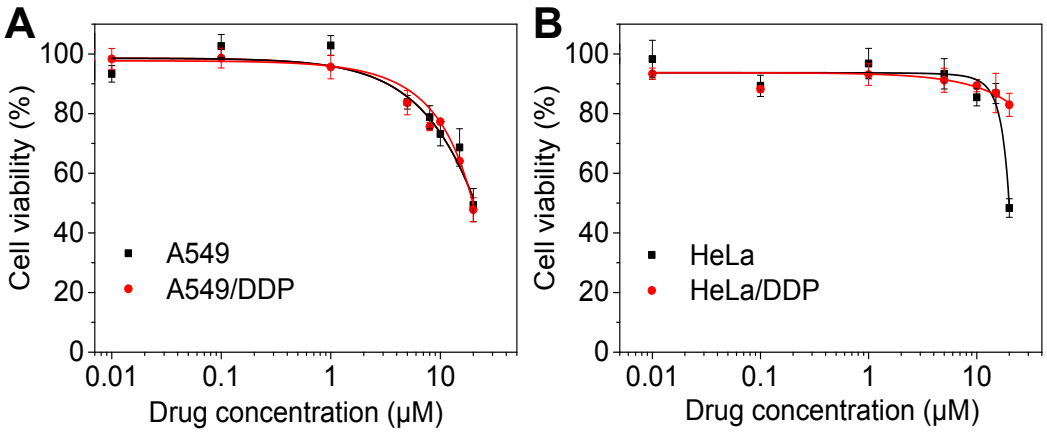


Fig. S16 (A) MTT assays of A549 and A549/DDP cells pretreated with a series of doses of TPCTP in the dark. (B) MTT assays of HeLa and HeLa/DDP cells pretreated with a series of doses of TPCTP in dark.

Table S1. IC_{50} (μM) values of cisplatin and TPCTP without/with irradiation in non- and cisplatin-resistant cancer cells.

Compound	A549	A549/DDP	HeLa	HeLa/DDP
cisplatin	10.1±1.6	32.8±3.95	3.9±0.13	48.2±2.41
TPCTP	>20	19.7	19.9	>>20
TPCTP+L	5.1±0.37	5.6±0.17	1.7±0.09	1.7±0.13

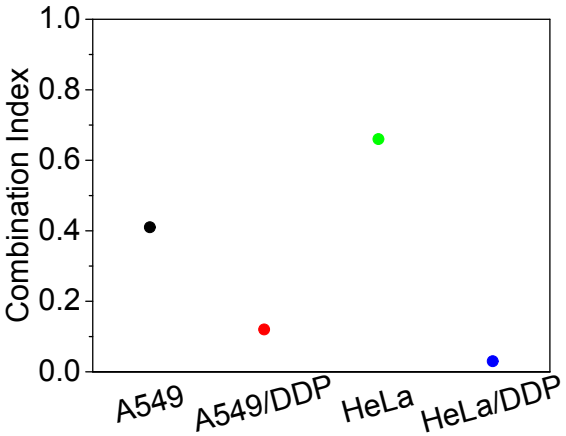


Fig. S17 CI values of TPCTP in cisplatin-resistant and nonresistant A549 and HeLa cells. Light irradiation: 350-800 nm, 4 mW cm^{-2}, 30 min.

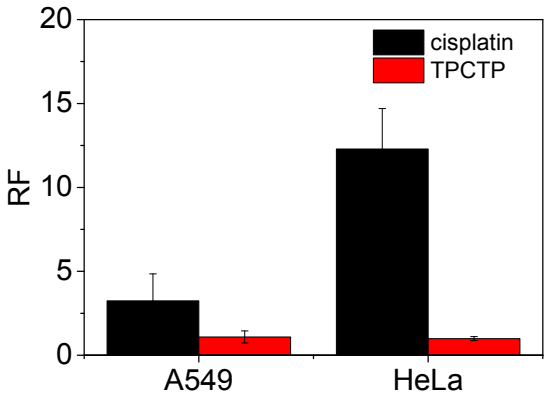


Fig. S18 The RF (resistant factor) values for cisplatin and TPCTP in A549 cells and HeLa cells. RF is defined by the ratio of the IC_{50} value in drug-resistant cells over IC_{50} value in drug nonresistant cells.

Table.S2 Quantification of platinum inside cells after treatment for 24h by ICP-OES measurements

Sample name		Net nuclei/mg	[Pt] (ng/mg)
blank	**HeLa**	1.7	0.8
	A549	3.5	0.6
HeLa nuclei	**1**	0.8	4750.0
	2	1.1	6645.1
	3	0.7	8869.1
	Average		6754.7±1683.4
A549 nuclei	**1**	1.1	842.4
	2	0.9	1751.5
	3	0.7	1070.5
	Average		1221.4±386.2

Reference

[1] Y. Gao, X. Wang, X. He, Z. He, X. Yang, S. Tian, F. Meng, D. Ding, L. Luo, B. Z. Tang, *Advanced Functional Materials* **2019**, *29*, 1902673.

[2] a) Y. Zhu, D. Qiu, G. Yang, M. Wang, Q. Zhang, P. Wang, H. Ming, D. Zhang, Y. Yu, G. Zou, R. Badugu, J. R. Lakowicz, *Biosensors and Bioelectronics* **2016**, *85*, 198-204; b) Y. Zheng, H. Lu, Z. Jiang, Y. Guan, J. Zou, X. Wang, R. Cheng, H. Gao, *Journal of Materials Chemistry B* **2017**, *5*, 6277-6281; c)W. Wu, D. Mao, F. Hu, S. Xu, C. Chen, C. J. Zhang, X. Cheng, Y. Yuan, D. Ding, D. Kong, *Advanced Materials* **2017**, *29*, 1700548.