Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Cystine-responsive cyano-functionalized acenaphthopyrazine derivative for tumor microenvironment modulation-based chemotherapy sensitization and side effect reduction†

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General information and methods

Materials

All chemicals for synthesis were obtained from Shanghai Titan Scientific Co., ltd and used without further purification unless otherwise specified.

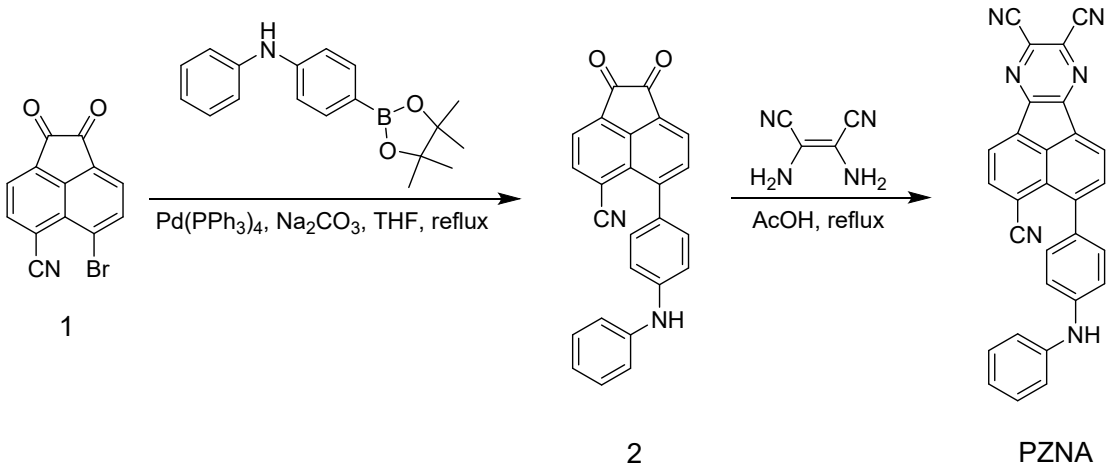
Murine 4T1 cancer cells were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). JC-1, Hoechst33342, ThiolTracker™ Violet, Trizol and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Shanghai, China). PBS buffer and DMEM (Dulbecco's modified Eagle medium) were purchased from Servicebio (Wuhan, China). The chemotherapy drug cisplatin (DDP), reductant vitamin C (Vc), oleic acid (OA), Penicillin-Streptomycin and paraformaldehyde (PFA, 4%) were purchased at Aladdin (Shanghai, China). 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) and 2-(4,5-Dimethylthiazol-2-yl)-3,5-diphenyl-2H-tetrazol-3-ium bromide (MTT) were purchased from Bide Pharmatech Ltd Shanghai, China). BODIPY 493/503, Calreticulin rabbit monoclonal antibody, Alexa Fluor 488 secondary antibody, Annexin V-FITC Apoptosis Detection Kit, Calcein AM (AM) and Propidium iodide (PI) were purchased from Beyotime Biotechnology (Shanghai, China). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}) was purchased from Ponsure (Shanghai, China). Protein-free rapid closure solution was purchased from Applygen Technologies Inc (Beijing,China). The female BALB/c mice (7-8 weeks old) were purchased from Hunan Boryxin Biotechnology Co, Ltd, China.

Measurements

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE NEO 500 spectrometer, using locking to the deuterated solvent ($CDCl_3$) and using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were measured on Thermoscientific Q-Exactive. UV-vis absorption spectra were measured on a UV-2600i spectrophotometer (Shimadzu, Japan)

by using a 1 cm glass cuvette. Fluorescence emission spectra were collected on a F-7100 fluorescence spectrophotometer (Hitachi, China). Zeta potential measurements and particle size measurements were conducted on dynamic light scattering (NanoBrook, Brokhaven, USA). Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (TI2-CTRE, Nikon, Japan). The absorbance for MTT analysis was recorded on a microplate reader (Epoch, BioTek, Germany). The flow assay was performed by flow cytometer (BD LSRFortessa, BD Biosciences, USA).

Synthetic procedures and characterization data for the compounds



Scheme S1 Synthetic route of PZNA.

The starting material **1** was synthesized according to literature.[1]

Synthesis of 2

Compound **1** (2.5 g, 9.0 mmol), *N*-phenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (2.65 g, 9.0 mmol) and $Pd(PPh_3)_4$ (0.416 g, 0.36 mmol) were dissolved in 140 mL THF under an argon atmosphere. After stirring the resultant mixture at 50 ^oC for 10 min, 20 mL of degassed 2 M K_2CO_3 aqueous was added and then refluxed overnight. The reaction mixture was cooled to room temperature and THF, toluene was removed under vacuum condition. The solid residue was dissolved in 100 mL of dichloromethane and washed with water (3 × 100 mL). The organic layer was separated and dried

with anhydrous Na_2SO_4 and concentrated by rotary evaporation. The residue solid was purified by column chromatography (silica gel) using petroleum ether/dichloromethane (1/6, v/v) as eluent to give a red solid. Yield: 0.99 g (30%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.41 (d, *J* = 7.2 Hz, 1H), 8.23 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 7.1 Hz, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.29 (t, *J* = 7.7 Hz, 2H), 7.19 (dd, *J* = 12.0, 8.4 Hz, 4H), 6.90 (t, *J* = 7.2 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.43 (s), 187.95 (s), 186.00 (s), 146.81 (s), 145.71 (s), 145.07 (s), 141.94 (s), 137.61 (s), 132.61 (s), 131.79 (d, *J* = 18.2 Hz), 131.07 (s), 130.70 (s), 129.80 (s), 129.48 (s), 129.06 (d, *J* = 11.5 Hz), 128.41 (s), 123.48 (s), 122.16 (s), 121.91 (s), 120.68 (s), 119.27 (s), 116.37 (s), 114.55 (s).

Synthesis of PZNA

2 (663 mg, 1.77 mmol) was taken and suspended in degassed acetic acid (40 mL) along with 2,3-diaminomaleonitrile (229 mg, 2.12 mmol, 1.20 equiv) and heated to reflux overnight under argon. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was then heated to reflux for 3 hours and suspended in ethanol/hexane v/v 1:1 (500 mL). The mixture was heat-filtered and the filtrate was washed sequentially with boiling ethanol (3 × 100 mL), boiling hexane (2 × 50 mL) and pentane (3 × 50 mL). The filtrate was then dissolved in DCM and passed through a thick silica gel pad (eluent: DCM). After vacuum drying, PZNA was obtained as black powder. Yield: 452 mg (57.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1H NMR (500 MHz, DMSO) δ 9.84 (s, 1H), 8.71 (d, *J* = 7.3 Hz, 1H), 8.65 (d, *J* = 7.3 Hz, 1H), 8.52 (d, *J* = 7.3 Hz, 1H), 8.43 (s, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.13 (dd, *J* = 10.6, 8.8 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.74 (s), 153.06 (s), 152.36 (s), 147.14 (s), 145.96 (s), 144.08 (s), 141.27 (s), 140.84 (s), 136.90 (s), 132.30 (s), 131.62 (s), 130.43 (s), 130.12 (s), 128.45 (t, *J* = 5.3 Hz), 126.96 (s), 126.77 (s), 126.17 (s), 124.79 (s), 123.62 (s), 121.19 (s), 120.52 (s), 118.29 (s), 117.12 (s), 115.33 (d, *J* = 11.4 Hz), 113.52 (s), 112.82 (s). HRMS (ESI) for $C_{29}H_{14}N_6$ $[M]^+$: calcd

446.4730, found 447.1361.

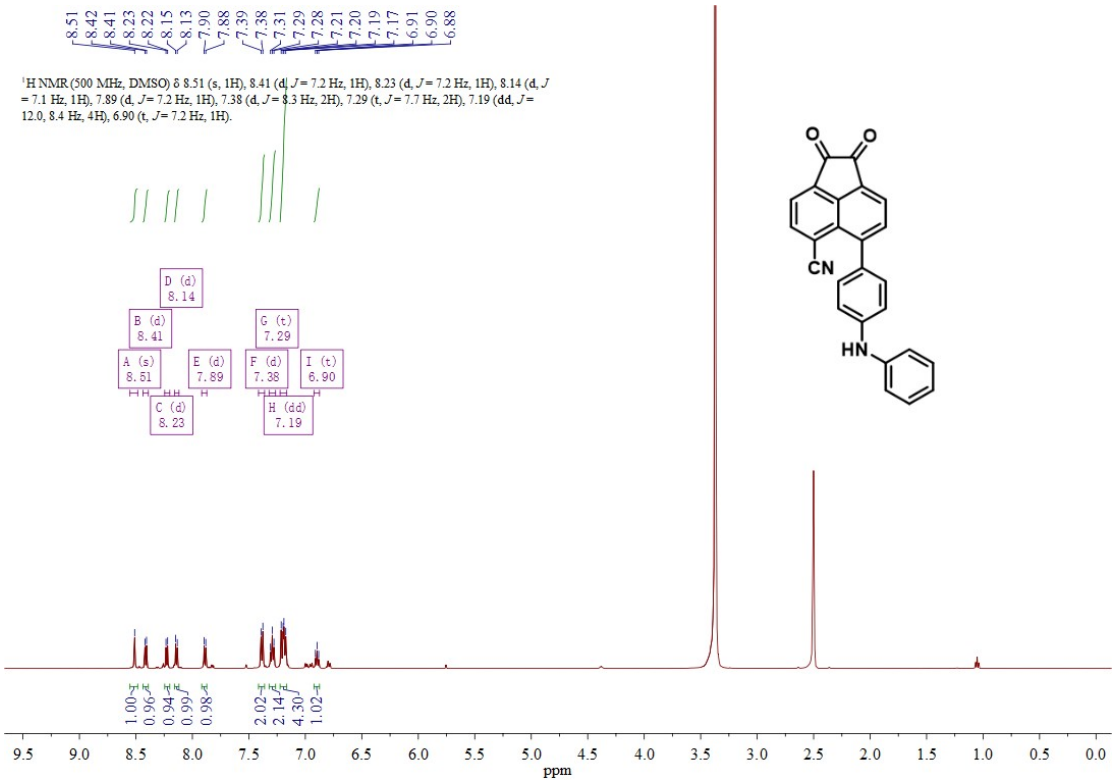


Fig. S1 ^{1}H NMR spectrum of **2**.

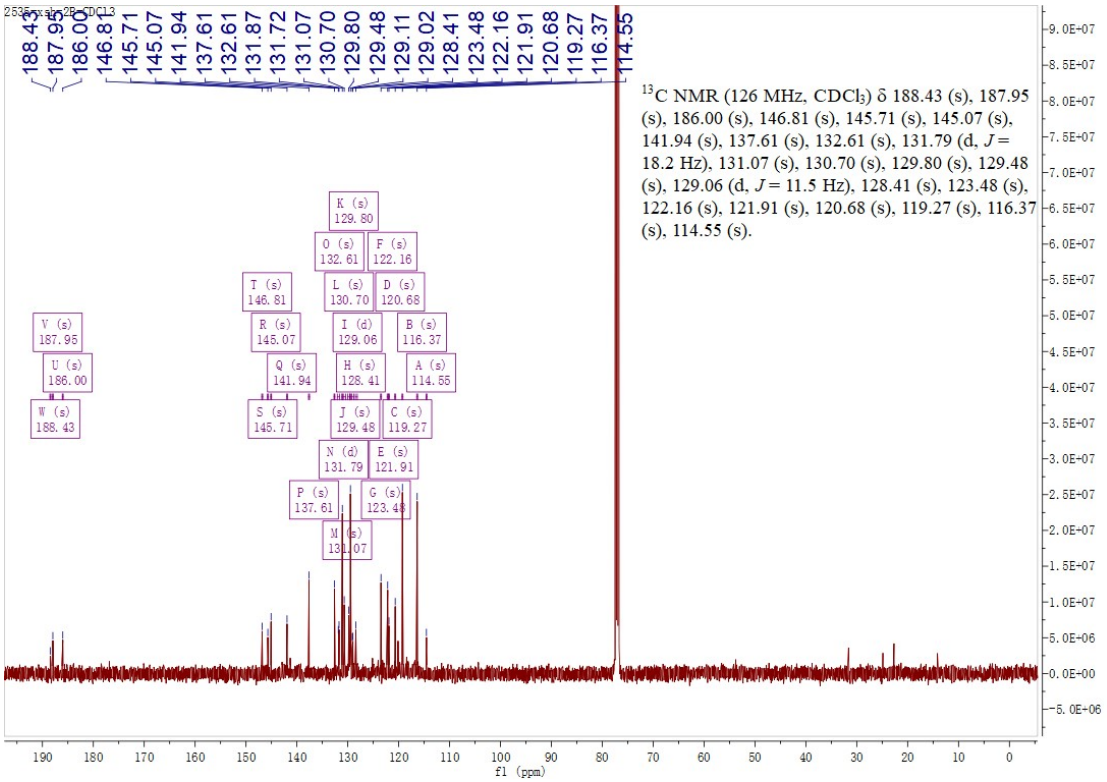


Fig. S2 ^{13}C NMR spectrum of **2**.

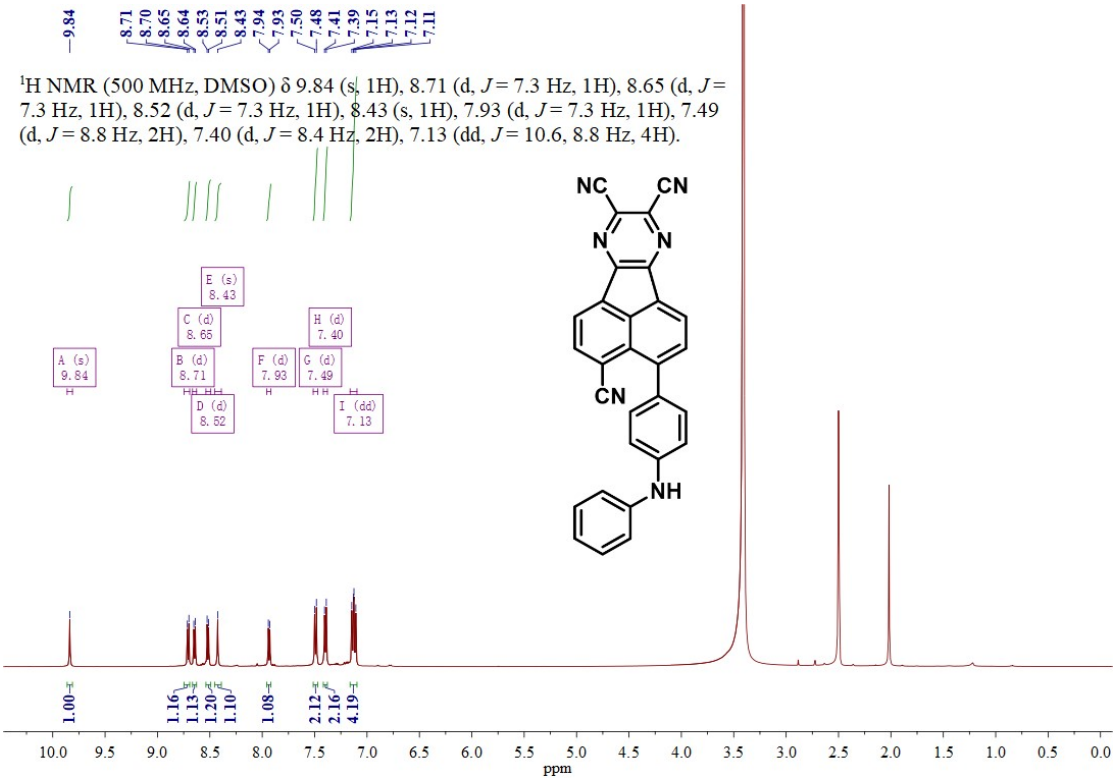


Fig. S3 ^{1}H NMR spectrum of PZNA.

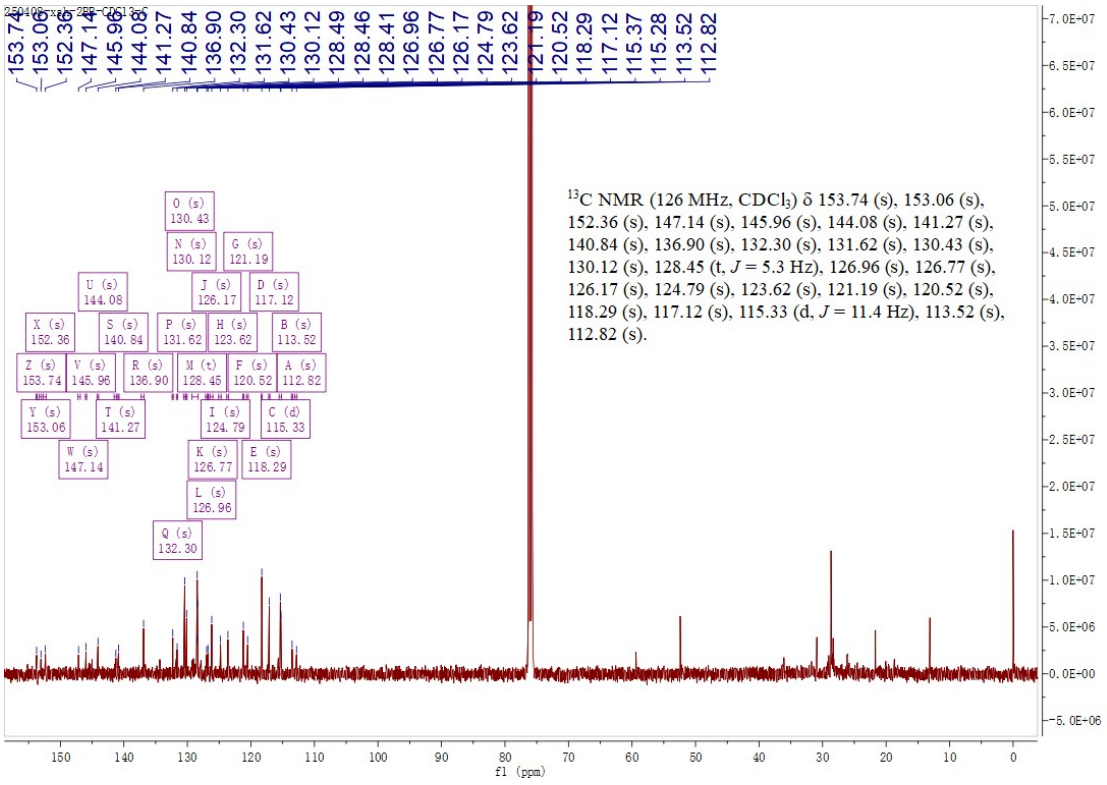


Fig. S4 ^{13}C NMR spectrum of **PZNA**.

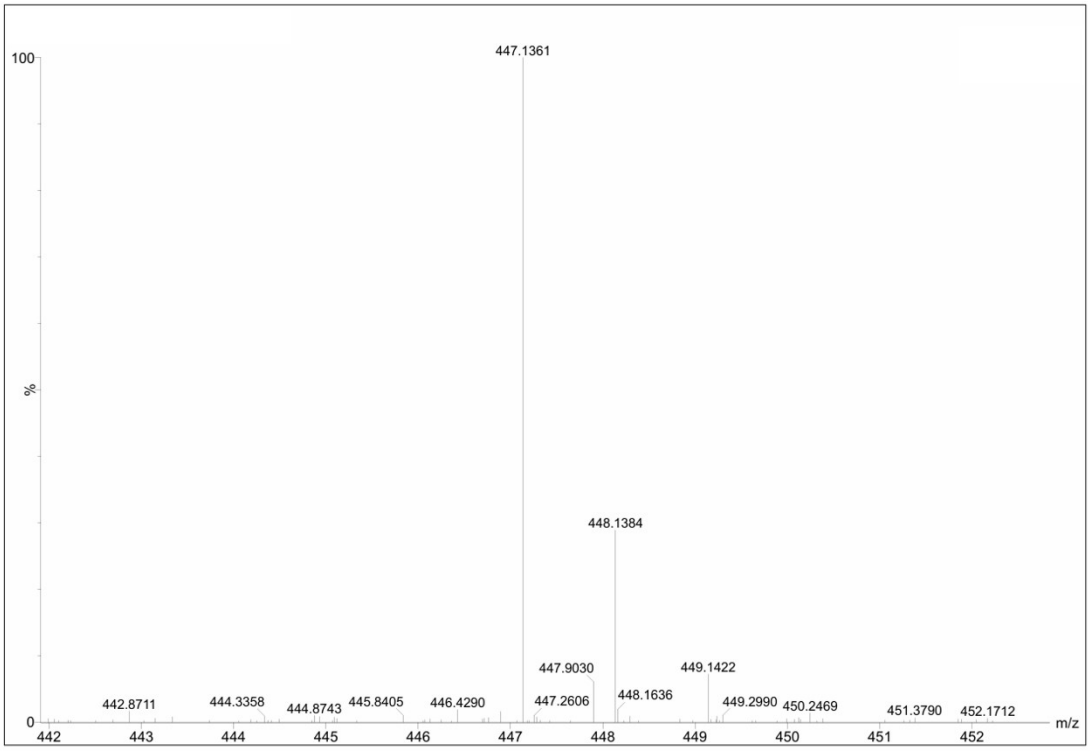


Fig. S5 HRMS spectrum of **PZNA**.

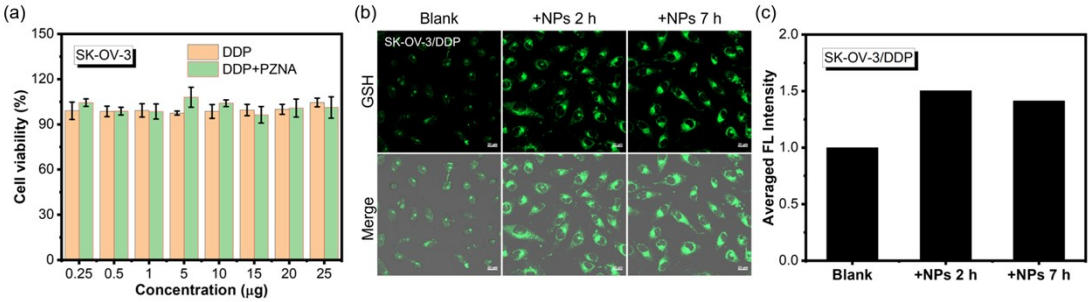


Fig. S6 a) SK-PV-3/DDP cells after pretreatment with and without PZNA NPs (8 μM/mL) for 10 h, and incubation with different concentrations of DDP for 14 h (n = 6). b) Glutathione changes in SK-PV-3/DDP cells of different treatment groups after incubation with PZNA NPs for different times, co-stained with ThiolTracker (18 μM/mL, 30 min) respectively. Scale bar: 20 μm. c) Changes in averaged fluorescence intensity of GSH after different treatments. Data are expressed as mean ± S.D. (standard deviation).

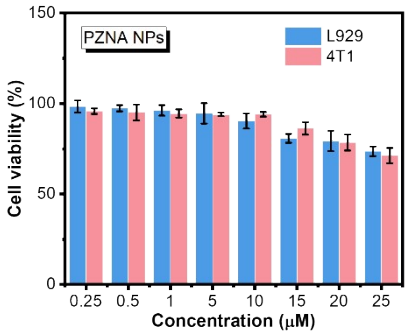


Fig. S7 Cell viability after 12 h of culture in 4T1 and L929 cells with different concentrations of PZNA NPs, respectively.

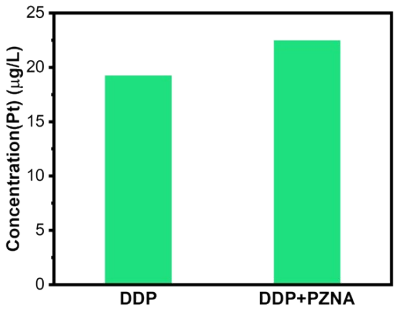


Fig. S8 Changes in intracellular platinum metal content after different treatments. 4T1 cells in DDP group were incubated with 8 μg/mL for 20 h, and DDP + NPs group were incubated with NPs 50 μM/mL, DDP 8 μg/mL for 20 h (n = 1).

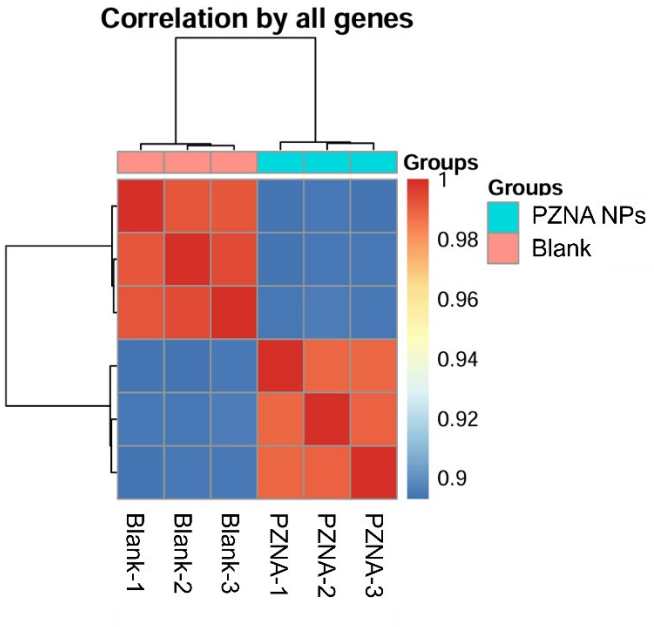


Fig. S9 Heatmap summarizing the Pearson's correlation coefficients between the different RNA-seq samples (n = 3).

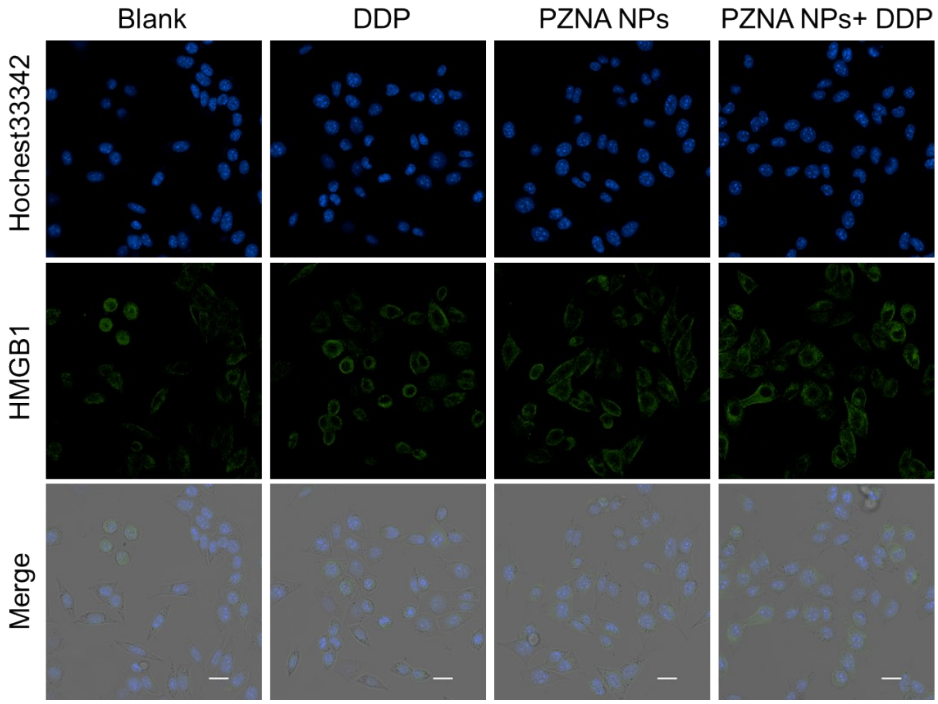


Fig. S10 CLSM images and quantified data of 4T1 cells with immunofluorescence staining of HMGB1.

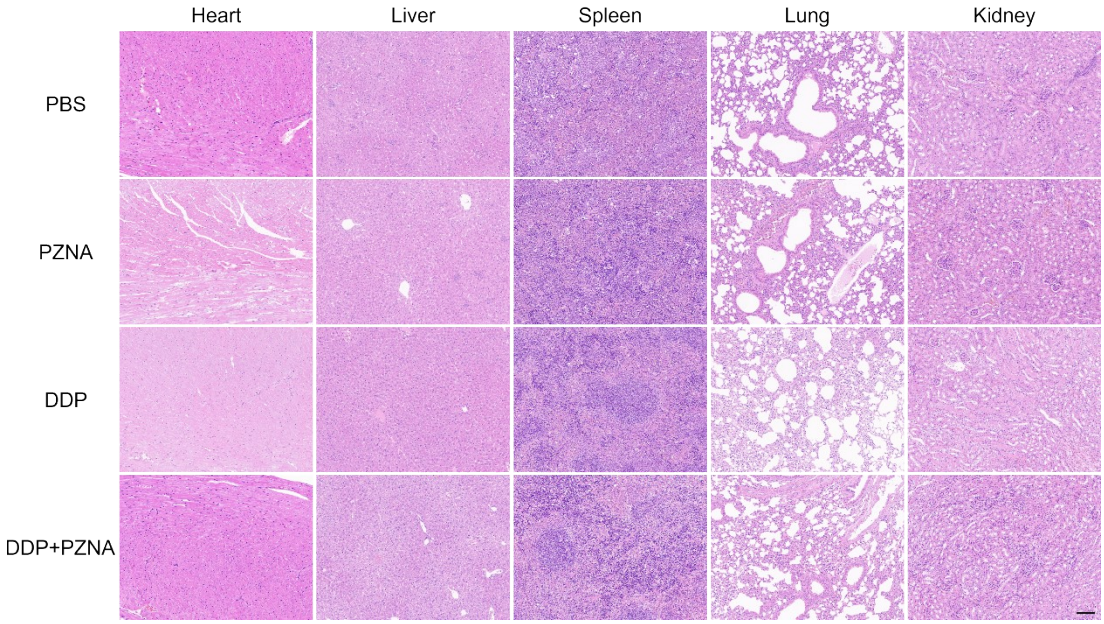


Fig. S11 H&E staining images of organs with different treatments. Scale bar: 50 μm.

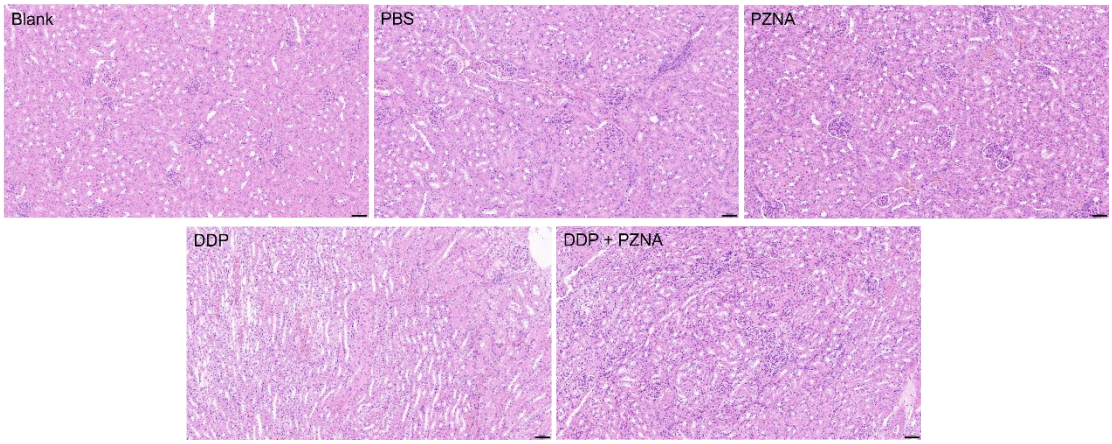


Fig. S12 H&E staining image of renal medulla in different groups. Scale bar: 50 μm.

[1] D. G. Congrave, B. H. Drummond, P. J. Conaghan, H. Francis, S. T. E. Jones, C. P. Grey, N. C. Greenham and D. Credgington, H. Bronstein, A Simple Molecular Design Strategy for Delayed Fluorescence toward 1000 nm, *J. Am. Chem. Soc.* 2019, **141**, 18390-18394.