Supporting Information

Facile synthesis of hydrazone-based zinc(II) complex for ferroptosisaugmented sonodynamic therapy

Dan Li,‡^a Minghui Fan,‡^a Haobing Wang,‡^a, Yongjie Zhu,^a Bole Yu,^c Pingyu Zhang,*^a Huaiyi Huang,*^b

^c Laboratory of life science, Shenzhen Research Institute of The Hong Kong Polytechnic University, Shenzhen, 518057, China

[‡] These authors contributed equally.

^a College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen, 518060, China. E-mail: p.zhang6@szu.edu.cn (P.Z.)

^b School of Pharmaceutical Science (Shenzhen), Shenzhen campus of Sun Yat-sen University, No.66, Gongchang Road, Shenzhen 518107, China.

Experimental section

Content

Fig. S1 ¹H NMR spectrum of AMTC.

Fig. S2 ¹H NMR spectrum of ZnAMTC.

Fig. S3 ¹³C NMR spectrum of ZnAMTC.

Fig. S4 ESI-MS spectrum of ZnAMTC.

Fig. S5 HPLC chromatogram of ZnAMTC.

Fig. S6 The luminescence lifetime of ZnAMTC.

Fig. S7 The dark-stability of ZnAMTC in MeOH

Fig. S8 The dark-stability of ZnAMTC in PBS and FBS

Fig. S9 The sono-stability of ZnAMTC under US irradiation.

Fig. S10 ¹O₂ generation of ZnAMTC measured by DPA probe in the dark.

Fig. S11 The fluorescence intensity of SOSG in the absence and presence of ZnAMTC

under US irradiation.

Fig. S12 The ESR signal of •OH probed by DMPO.

Fig. S13 ROS measurement in 4T1 cells.

Fig. S14 Cellular uptake of ZnAMTC with different incubation time.

Fig. S15 The co-localization images of 4T1 cells treated with ZnAMTC and the commercial dyes.

Fig. S16 The diagrammatic sketch of the sonodynamic therapy assay in the solution and in the cells.

Fig. S17 The sono-cytotoxicities of ZnAMTC toward different types of cancer cells.

Fig. S18 The dark-cytotoxicity of ZnAMTC in L02 normal cells.

Fig. S19 The sono-cytotoxicities of Ce6 toward 4T1 cells.

Fig. S20 GSH depletion by ZnAMTC upon US irradiation.

Fig. S21 Uncropped pictures for western blot analysis.

Fig. S22 Body weight of the mice during the various treatments.

Fig. S23 H&E staining images of the major organs of the treated mice.



Fig. S1 The ¹H NMR spectrum of AMTC (500 MHz, CDCl₃).



Fig. S2 The ¹H NMR spectrum of ZnAMTC (500 MHz, CDCl₃).



220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1 f1 (ppm)

Fig. S3 The ¹³C NMR spectrum of ZnAMTC (151 MHz, DMSO-d₆).



Fig. S4 Positive-ion ESI-HRMS spectrum for ZnAMTC measured in methanol.



Fig. S5 The purity of ZnAMTC examined by HPLC analysis in CH₃OH.



Fig. S6 The emission lifetime spectrum of ZnAMTC (50 μ M) in a water medium (containing 5% DMSO). $\lambda_{ex} = 405$ nm.



Fig. S7 The dark-stability of ZnAMTC in MeOH after storage for 24 h at 298 K examined by HPLC analysis.



Fig. S8 The dark-stability of ZnAMTC (50 μ M) in the mixture of PBS plus 10% fetal bovine serum (FBS) (containing 5% DMSO) after storage for 48 h examined by UV-Vis absorption spectra.



Fig. S9 The sono-stability of ZnAMTC in the CDCl₃ solution under US (1.0 MHz, 3 W cm⁻², 10% duty cycle) irradiation for 20 min examined by ¹H NMR.



Fig. S10 Time-dependent oxidation of DPA by ZnAMTC without US irradiation.



Fig. S11 Time-dependent fluorescence intensity of SOSG (a) in the absence and (b) presence of ZnAMTC (20 μ M) under US irradiation (1.0 MHz, 3 W cm⁻², 10% duty cycle).



Fig. S12 The ESR signal of DMPO for •OH characterization in the presence of **ZnAMTC** under US irradiation (1.0 MHz, 3 W cm⁻², 10% duty cycle).



Fig. S13 (a) Fluorescence microscopy images of 4T1 cells treated with **ZnAMTC** (10 μ M, 4 h) and co-stained with DCFH-DA (10 μ M) under different conditions. DCFH-DA probe: $\lambda_{ex} = 458$ nm, $\lambda_{em} = 540 \pm 30$ nm. Scale bar: 100 μ m. US irradiation: 1.0 MHz, 3 W cm⁻², 10 % duty cycle, 20 min. (b) Confocal laser scanning microscopy (CLSM) images of 4T1 cells treated with **ZnAMTC** (10 μ M, 4 h) and co-stained with SOSG under different conditions. SOSG probe: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 525 \pm 30$ nm. Scale bar: 10 μ m. US irradiation: 1.0 MHz, 3 W cm⁻², 10 % duty cycle.



Fig. S14 Cellular uptake of ZnAMTC (10 μ M) with different incubation time measured by CLSM. $\lambda_{ex} = 488 \text{ nm}, \lambda_{em} = 535 \pm 20 \text{ nm}.$ Scale bar: 20 μ m.



Fig. 15 CLSM images of the living cells treated with ZnAMTC (10 μ M, 4 h) and costained with Lyso-Tracker Deep Red (LTDR, 200 nM, 45 min) or Mito-Tracker Deep Red (MTDR, 200 nM, 45 min). ZnAMTC: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 535 \pm 30$ nm; MTDR: $\lambda_{ex} = 633$ nm, $\lambda_{em} = 680 \pm 30$ nm; LTDR: $\lambda_{ex} = 633$ nm, $\lambda_{em} = 680 \pm 30$ nm. Scale bar: 20 μ m.



For optical spectroscopy assay, the samples were in a plastic tube for US irradiation and then transferred to a cuvette for test.



Fig. S16 The diagrammatic sketch of the sonodynamic therapy assay in the solution and in the cells.



Fig. S17 The sono-cytotoxicities (IC₅₀, μ M) of ZnAMTC toward different types of cancer cells. The data are shown as mean \pm SD (n = 6).



Fig. S18 The dark cytotoxicity of ZnAMTC with various concentrations in L02 normal cells after 4 h incubation. The data are shown as mean \pm SD (n = 6).



Fig. S19 Cell viability of 4T1 cells in the absence or presence of US irradiation for 20 min. Ce6 in different concentration was co-incubated with 4T1 cells for 4 h before the US irradiation (1.0 MHz, 0.3 W cm⁻², 20 min). The data are shown as mean \pm SD (n = 6).



Fig. S20 Irradiation time-dependent depletion of GSH (200 μ M) by **ZnAMTC** (50 μ M) upon US irradiation. The absorption at 412 nm was decreased by increasing the irradiation time.



Fig. S21 Uncropped western blot analysis of β -actin and GPX4 expression for Fig 3c.



Fig. S22 Body weight of the mice during the various treatments. The data are shown as mean \pm SD (n = 5).



Fig. S23 H&E staining images of the major organs (heart, liver, spleen, lung, and kidney) of healthy 4T1 tumor-bearing Balb/c mice after intratumoral injection of **ZnAMTC** (0.66 mg kg⁻¹) at different time points (day 0 and 3). The experiment was repeated three times independently with similar results. Scale bar: 200 μ m.