Supporting Information

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

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Experimental section

Content

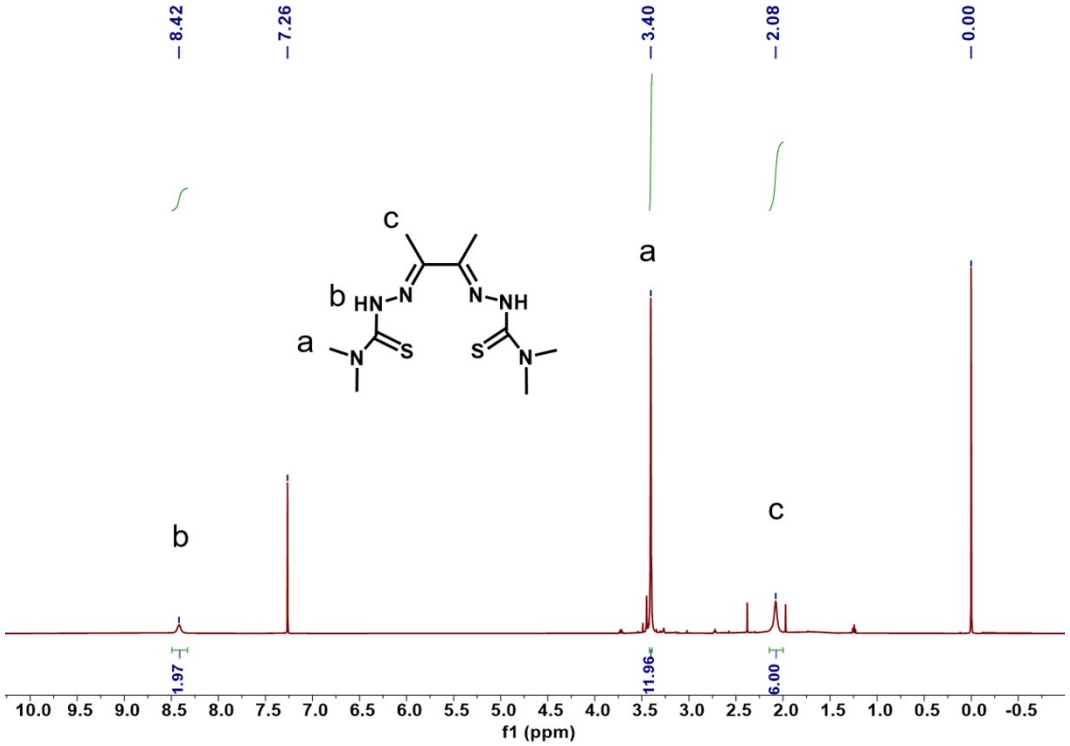

Fig. S1 The ^{1}H NMR spectrum of **AMTC** (500 MHz, $CDCl_3$).

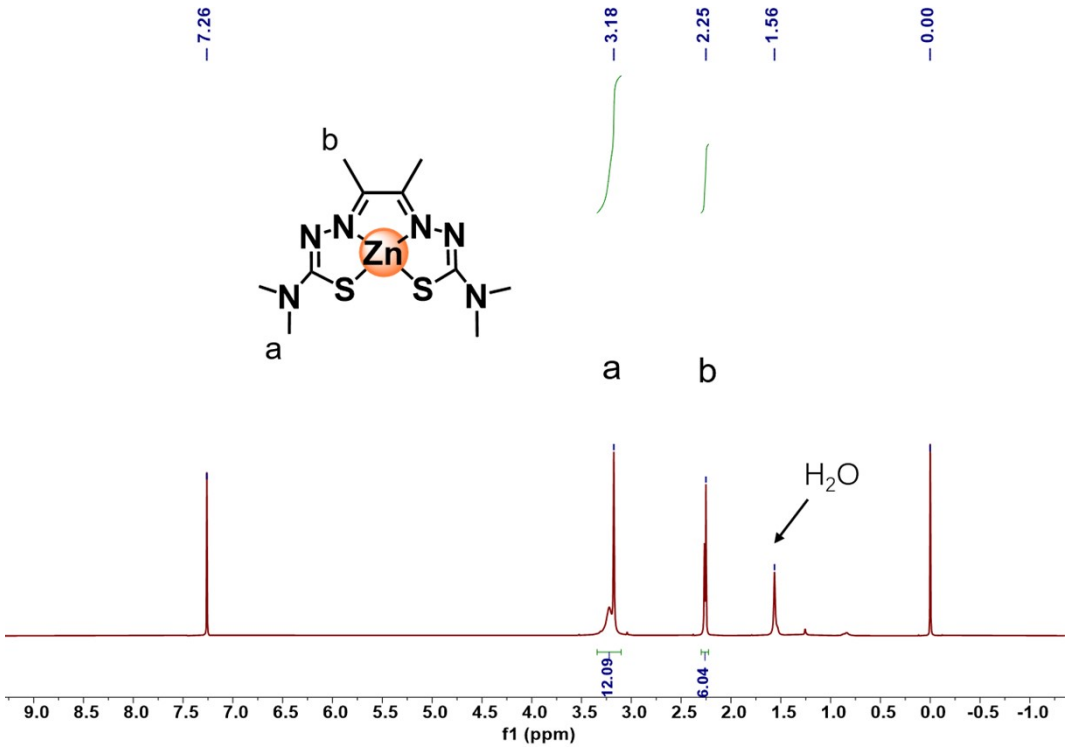

Fig. S2 The ^{1}H NMR spectrum of **ZnAMTC** (500 MHz, $CDCl_3$).

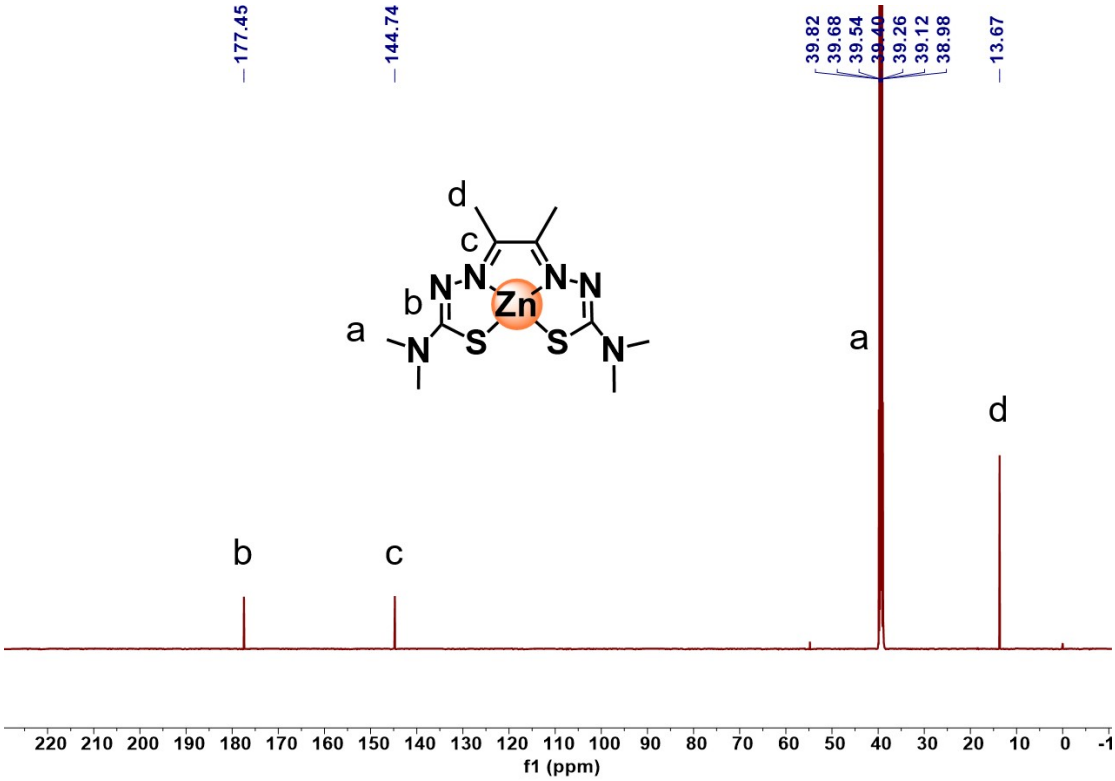

Fig. S3 The ^{13}C NMR spectrum of **ZnAMTC** (151 MHz, DMSO-d_6).

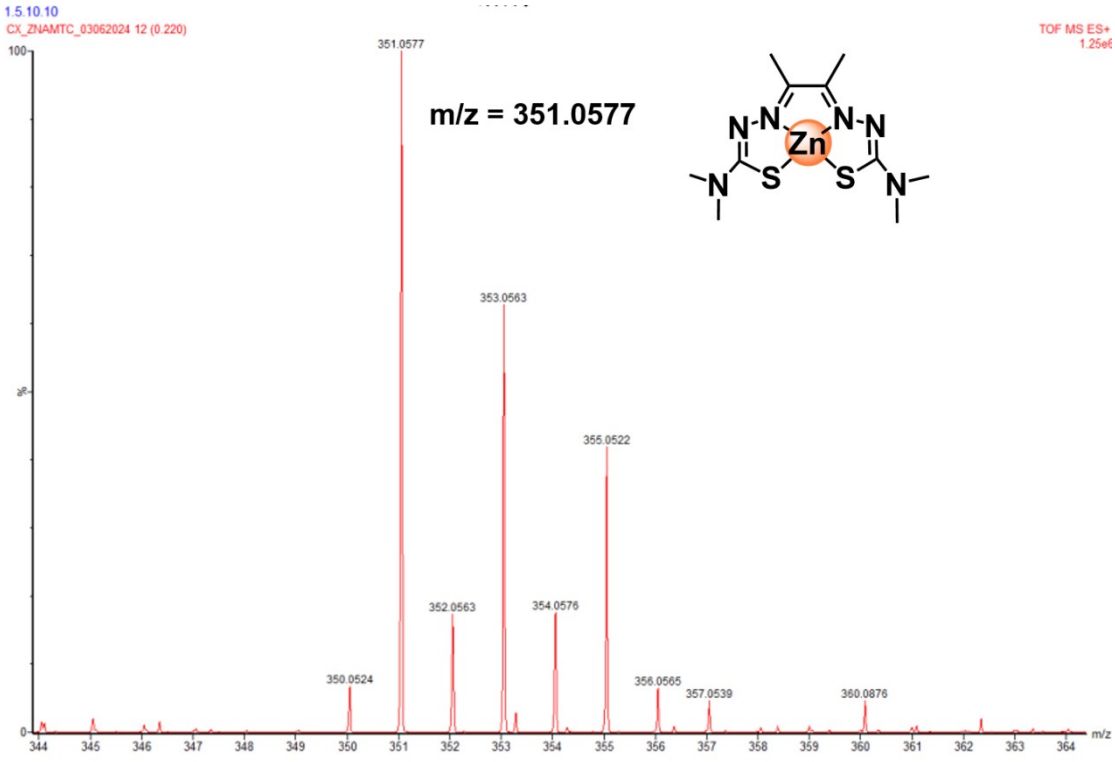

Fig. S4 Positive-ion ESI-HRMS spectrum for **ZnAMTC** measured in methanol.

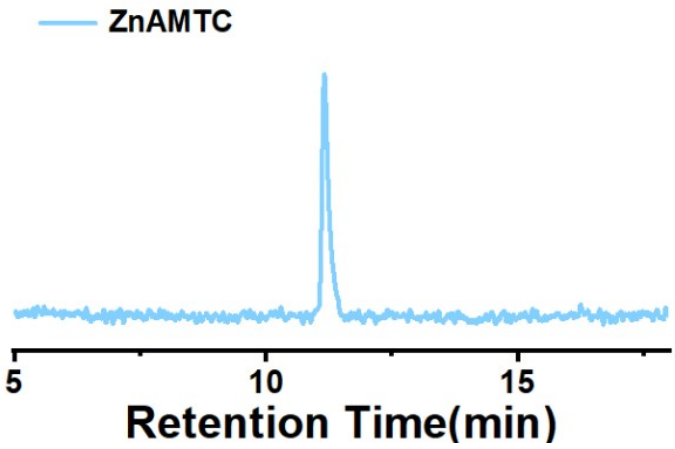

Fig. S5 The purity of **ZnAMTC** examined by HPLC analysis in CH_3OH.

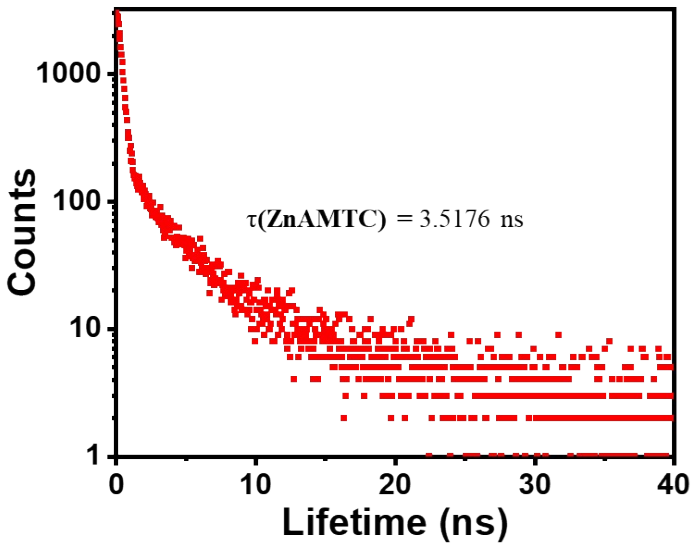

Fig. S6 The emission lifetime spectrum of **ZnAMTC** (50 μM) in a water medium (containing 5% DMSO). λ_{ex} = 405 nm.

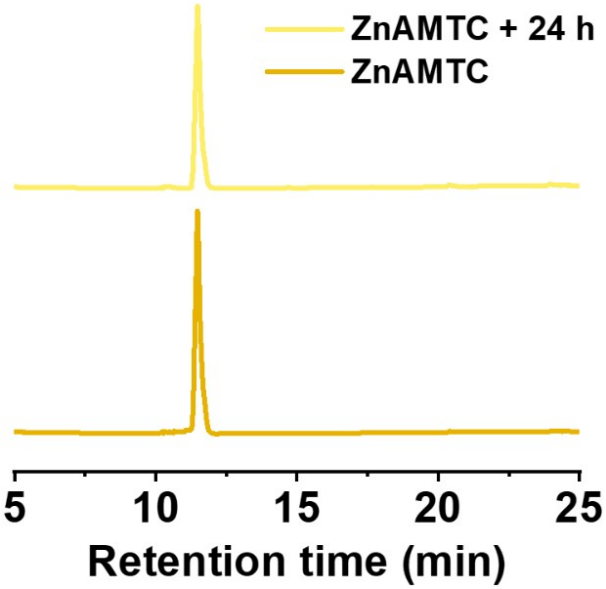

Fig. S7 The dark-stability of **ZnAMTC** in MeOH after storage for 24 h at 298 K examined by HPLC analysis.

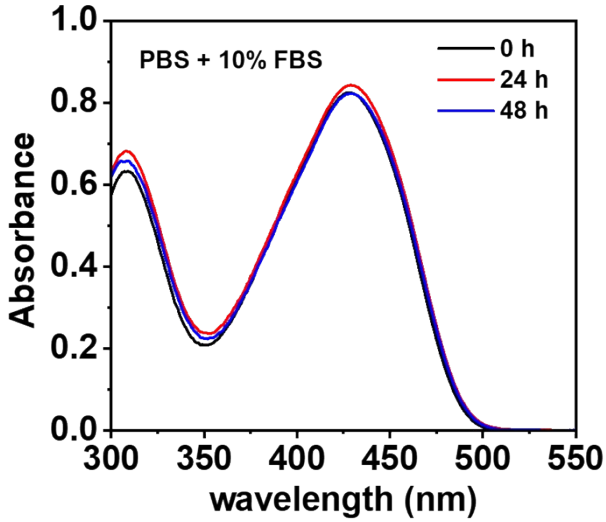

Fig. S8 The dark-stability of **ZnAMTC** (50 μM) in the mixture of PBS plus 10% fetal bovine serum (FBS) (containing 5% DMSO) after storage for 48 h examined by UV-Vis absorption spectra.

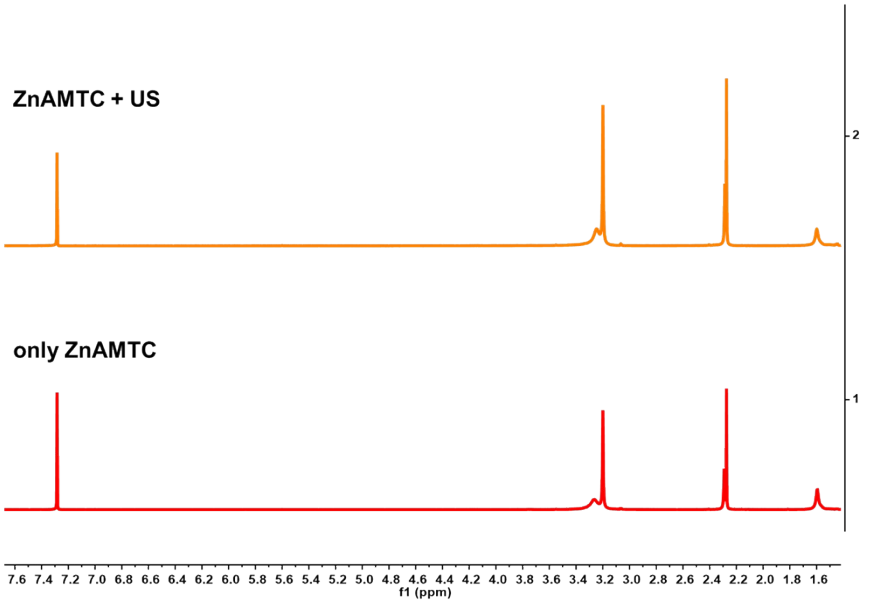

Fig. S9 The sono-stability of **ZnAMTC** in the $CDCl_3$ solution under US (1.0 MHz, 3 W cm^{-2}, 10% duty cycle) irradiation for 20 min examined by ^1H NMR.

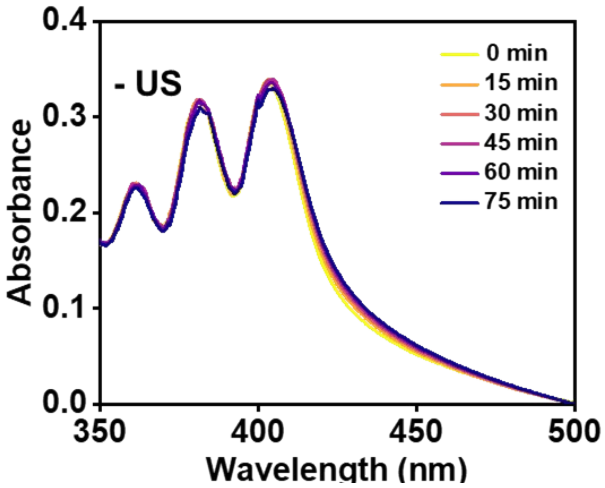

Fig. S10 Time-dependent oxidation of DPA by **ZnAMTC** without US irradiation.

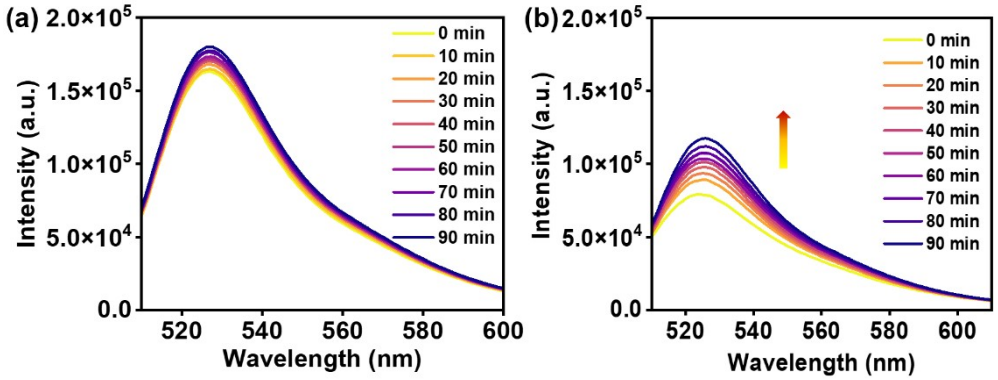

Fig. S11 Time-dependent fluorescence intensity of SOSG (a) in the absence and (b) presence of **ZnAMTC** (20 μM) under US irradiation (1.0 MHz, 3 W cm^{-2}, 10% duty cycle).

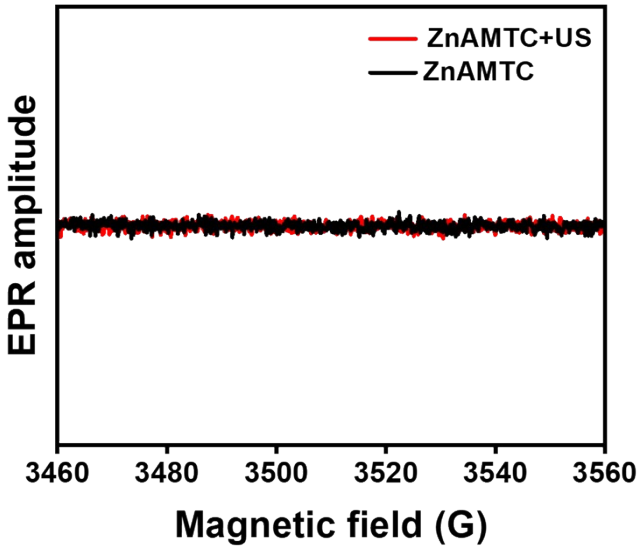

Fig. S12 The ESR signal of DMPO for •OH characterization in the presence of **ZnAMTC** under US irradiation (1.0 MHz, 3 W cm^{-2}, 10% duty cycle).

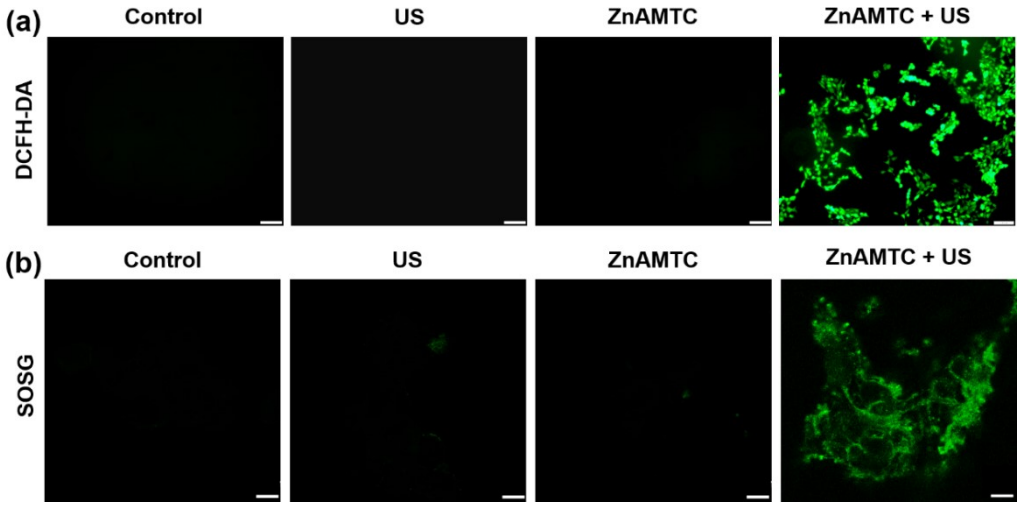

Fig. S13 (a) Fluorescence microscopy images of 4T1 cells treated with **ZnAMTC** (10 μM, 4 h) and co-stained with DCFH-DA (10 μM) under different conditions. DCFH-DA probe: λ_{ex} = 458 nm, λ_{em} = 540 ± 30 nm. Scale bar: 100 μm. US irradiation: 1.0 MHz, 3 W cm^{-2}, 10 % duty cycle, 20 min. (b) Confocal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

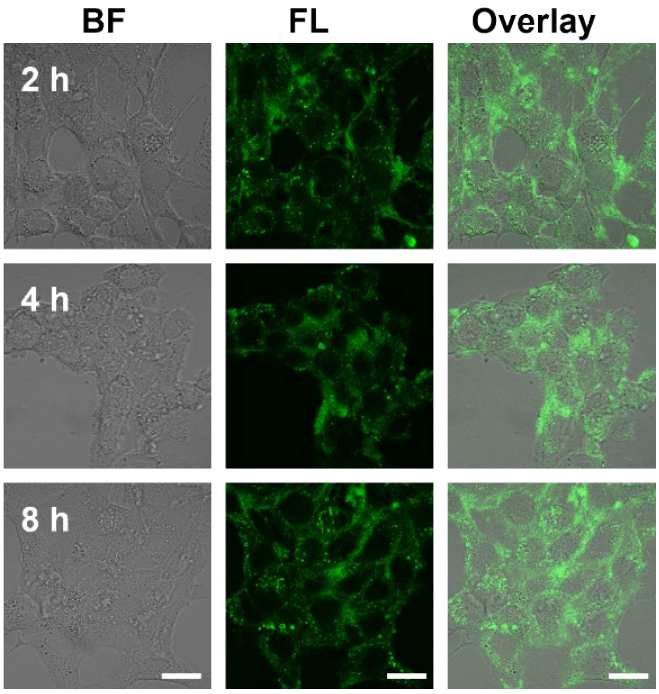

Fig. S14 Cellular uptake of **ZnAMTC** (10 μM) with different incubation time measured by CLSM. λ_{ex} = 488 nm, λ_{em} = 535 ± 20 nm. Scale bar: 20 μm.

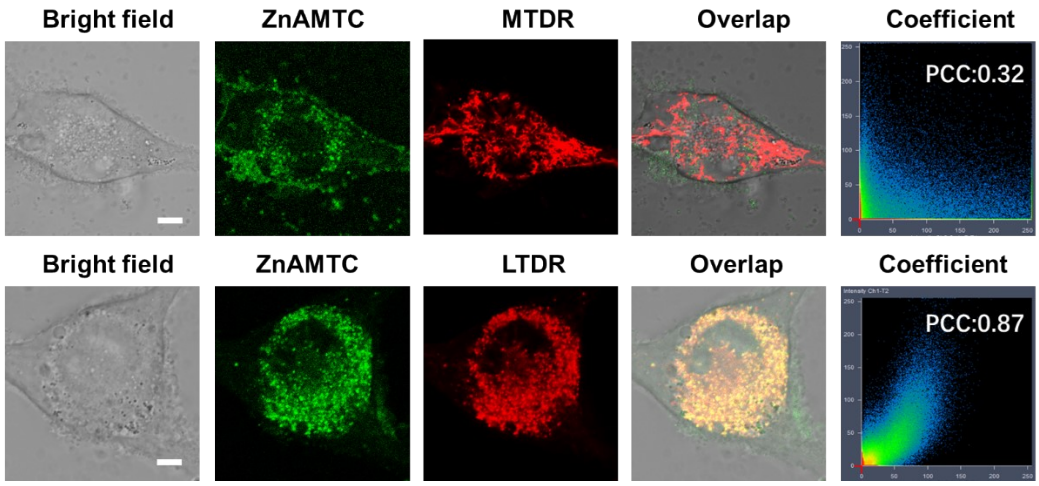

Fig. 15 CLSM images of the living cells treated with **ZnAMTC** (10 μM, 4 h) and co-stained with Lyso-Tracker Deep Red (LTDR, 200 nM, 45 min) or Mito-Tracker Deep Red (MTDR, 200 nM, 45 min). **ZnAMTC**: λ_{ex} = 488 nm, λ_{em} = 535 ± 30 nm; MTDR: λ_{ex} = 633 nm, λ_{em} = 680 ± 30 nm; LTDR: λ_{ex} = 633 nm, λ_{em} = 680 ± 30 nm. Scale bar: 20 μm.

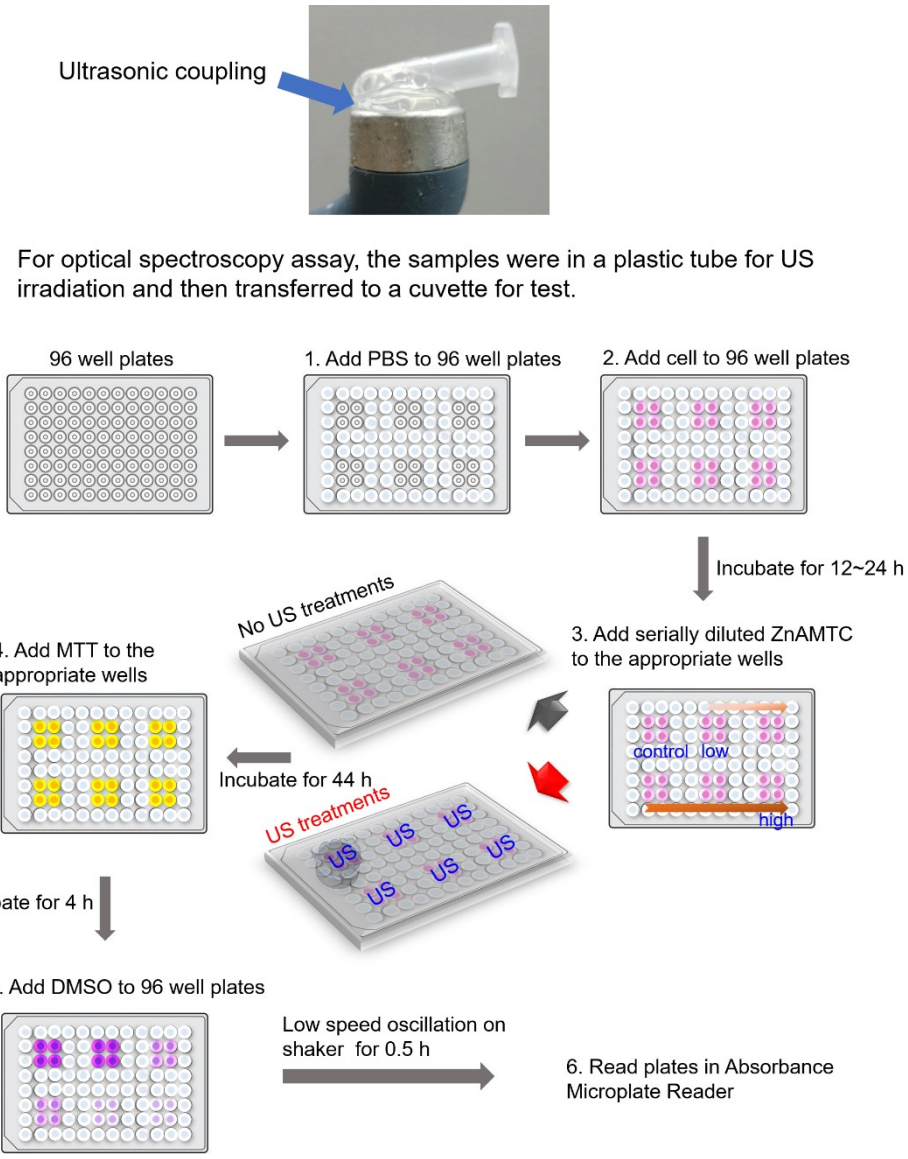

Fig. S16 The diagrammatic sketch of the sonodynamic therapy assay in the solution and in the cells.

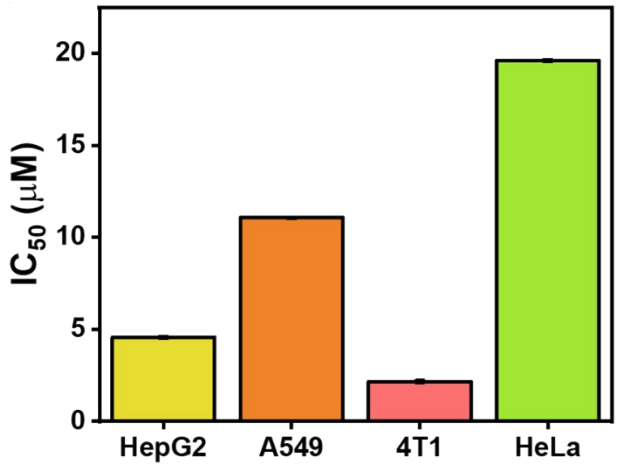

Fig. S17 The sono-cytotoxicities (IC_{50}, μM) of **ZnAMTC** toward different types of cancer cells. The data are shown as mean ± SD (n = 6).

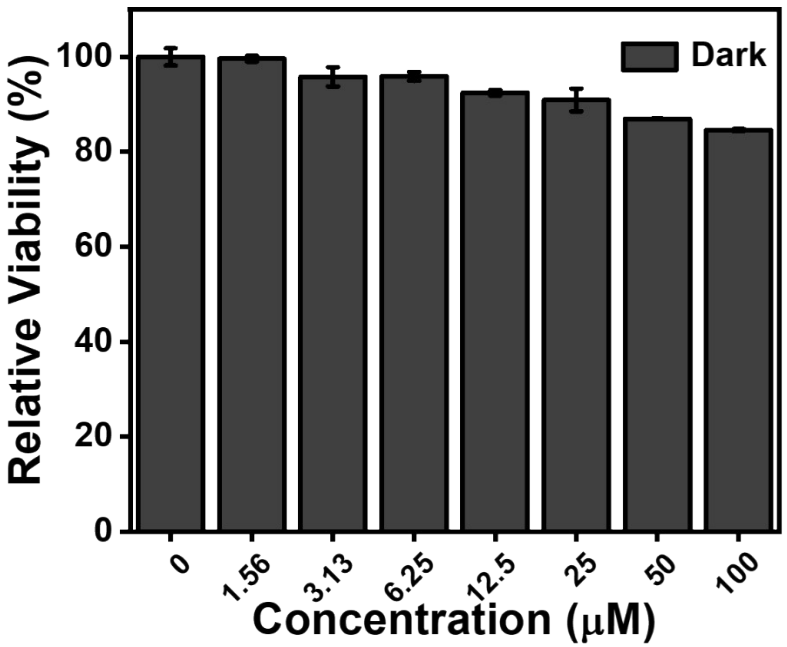

Fig. S18 The dark cytotoxicity of **ZnAMTC** with various concentrations in L02 normal cells after 4 h incubation. The data are shown as mean ± SD (n = 6).

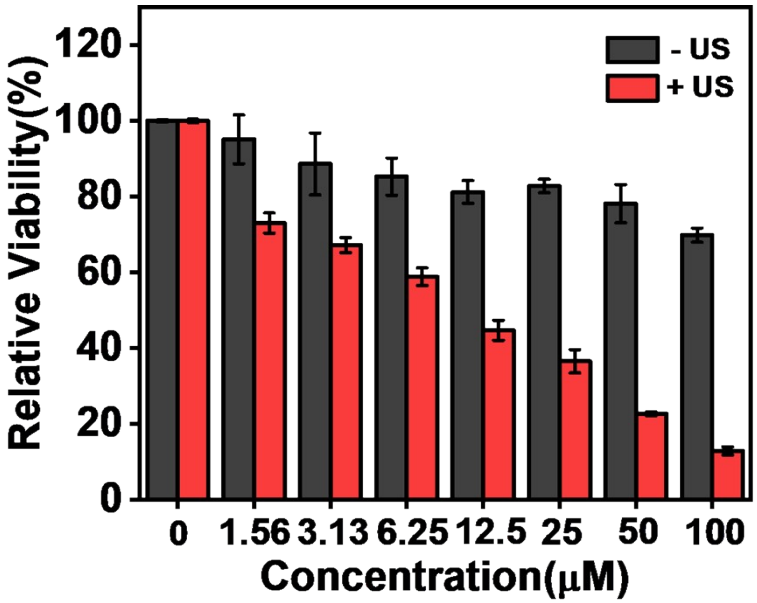

Fig. S19 Cell viability of 4T1 cells in the absence or presence of US irradiation for 20 min. Ce6 in different concentration was co-incubated with 4T1 cells for 4 h before the US irradiation (1.0 MHz, 0.3 W cm^{-2}, 20 min). The data are shown as mean ± SD (n = 6).

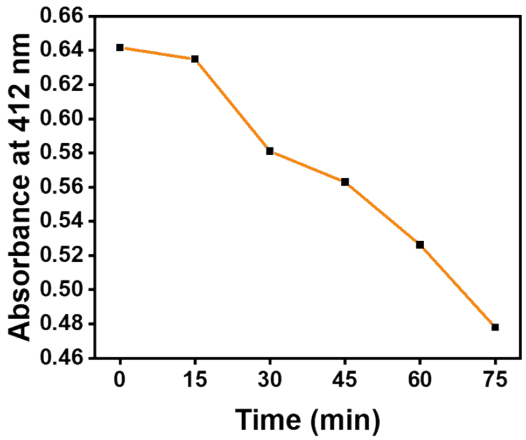

Fig. S20 Irradiation time-dependent depletion of GSH (200 μM) by **ZnAMTC** (50 μM) upon US irradiation. The absorption at 412 nm was decreased by increasing the irradiation time.

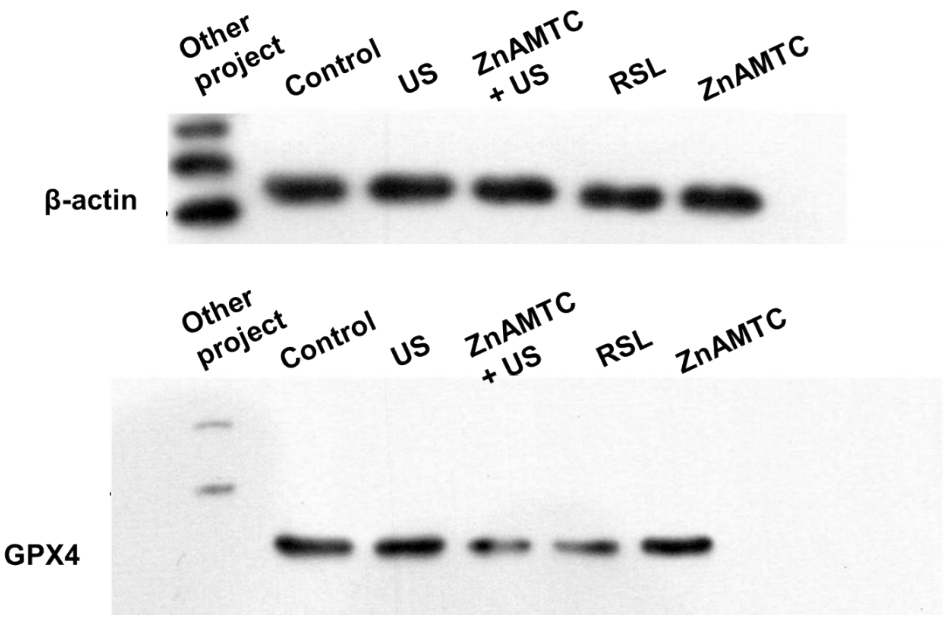

Fig. S21 Uncropped western blot analysis of β-actin and GPX4 expression for Fig 3c.

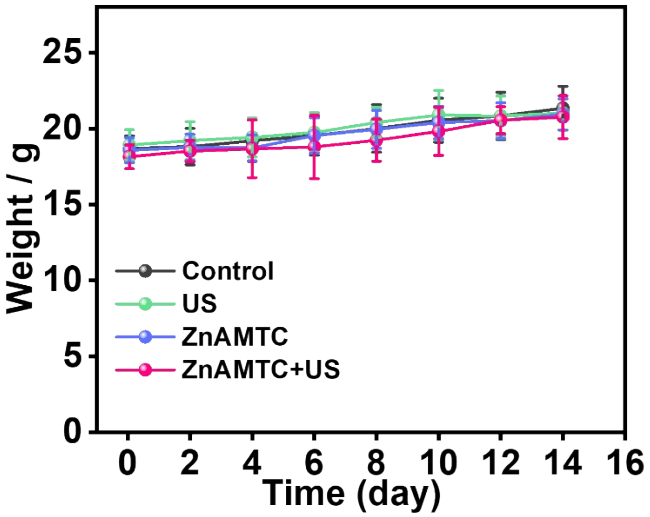

Fig. S22 Body weight of the mice during the various treatments. The data are shown as mean ± SD (n = 5).

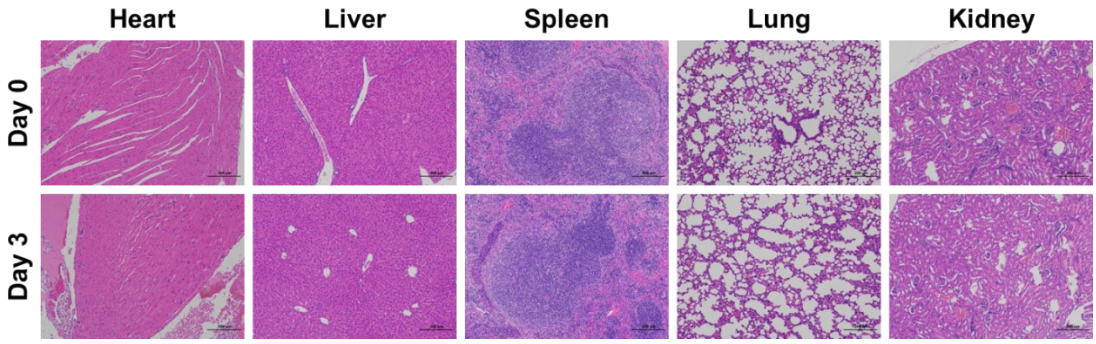

Fig. S23 H&E staining images of the major organs (heart, liver, spleen, lung, and kidney) of healthy 4T1 tumor-bearing Balb/c mice after intratumoral injection of **ZnAMTC** (0.66 mg kg^{-1}) at different time points (day 0 and 3). The experiment was repeated three times independently with similar results.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