Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

CD44-Targeted Cu (II) delivery nanoplatform for Sensitized disulfiram Chemotherapy to Triple-Negative breast cancer

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Experimental Section

Materials

Hyaluronic Acid (HA, Mw: 20000) were purchased from Ruixi Biological Technology Co., Ltd. Meso-tetra(4-carboxyphenyl) porphyrin (TCPP) was purchased from Frontier Scientific. Probe Assay Kit was purchased from Heliosense Biotechnologies, Inc. (Xiamen, China). All chemical reagents were commercially available from Sigma-Aldrich Chemical Reagent Co., Ltd and used as received without further purification.

Characterization

The morphology of the samples was observed with field-emission scanning electron microscope (FE-SEM), transmission electron microscopy (TEM, JEOL JEM-1010 microscope operated at 100 kV) and atomic force microscopy (AFM, Dimension ICON with Nanoscope V controller, Bruker, USA). Powder X-ray diffraction (XRD) patterns were recorded with a Shimadzu XRD-6000 powder X-ray diffractometer, using Cu Kα radiation (λ=1.5406 Å) at a scanning rate of 2° per min. UV-vis absorption spectra were recorded on a Lambda 25 UV-visible spectrophotometer. Fluorescence (FL) spectra were obtained with a FluoroMax-4 spectrofluorometer with xenon discharge lamp excitation. X-ray photoelectron spectroscopy (XPS) analysis was performed on a VG ESCALAB 220i-XL spectrometer under ultrahigh vacuum (6×10^{-9} mbar), equipped with a monochromatic Al Kα (1486.7 eV) X-ray source. The contents of Cu and Sm were obtained by ICP-MS measurement using a PerkinElmer NexION 300X. The kinetic energy discrimination (KED) mode was against a standard curve of known copper and phosphorus concentrations (CMS-5, Inorganic Ventures), with Ga (20 μg/L, Inorganic Ventures) as an internal standard. The Zeta potential of the nanosheets were determined by a Malvern Zetasizer Nano-ZS instrument (ZEN3600, Malvern Instruments).

Synthesis of Sm-TCPP(Cu)@HA nanosheets

The synthesis of Sm-TCPP nanosheets were performed by hydrothermal method according to our previous work [S1]. Then, Sm-TCPP nanosheets were activated under vacuum drying overnight at 150℃. 20 mg of activated Sm-TCPP and 15 mg of copper acetate were dissolved into 5 mL of DMF for continuous stirring for 20 min, which then was transferred into 20 mL of Teflon-lined autoclave to heat at 120 ℃ for 16 h. After the reaction system was cooled to room temperature, the crude product was collected by repeated centrifugation and washing by DMF and water. The obtained Sm-TCPP(Cu) (2 mg/mL) was mixed with HA (0.1 mg/mL) solution and stirred at 25℃ for 24 h under dark conditions. The Sm-TCPP(Cu)@HA nanosheets were purified by centrifugation and washing using water, which was then dried under vacuum overnight for further characterization.

Stability assessment of Sm-TCPP(Cu)@HA nanosheets

20 mg of Sm-TCPP(Cu)@HA were dissolved into 10 mL of DMEM media and stirring for 24 h at 37℃. At desirable time, 0.5 mL of aliquots were fetched and replaced with fresh media; The sample was isolated by centrifugation and the supernatant were

performed by ICP-MS analysis for Sm and Cu element. After 24 h of incubation, the rest of Sm-TCPP(Cu)@HA was collected by centrifugation followed by performing XRD analysis.

Cell culture

In our study, MDA-MB-231 human breast carcinoma cells and MCF-10A human normal breast epithelial cells were cultured in DMEM medium with 10% of FBS. The incubation of both cells was implemented under a 37 °C and 5% carbon dioxide atmosphere.

Cu (II) delivery efficacy of Sm-TCPP(Cu)@HA nanosheets

MDA-MB-231 and MCF-10A cells were seeded into 6-wells at a density of 1×10^5 /well. After 24 h of incubation, cells were incubated with different concentration of Sm-TCPP(Cu)@HA and TCPP(Cu) at same dose of Cu for 3 h. For the HA competition experiments, cells were pretreated by HA for 1 h and washed by fresh media, followed by Sm-TCPP(Cu)@HA and TCPP(Cu) treatment at fixed concentration (5 μM) for 3h. For temperature-dependent Cu delivery, the incubation temperature changed from 37 to 4 °C according to demand; Then, Cells were washed by fresh media, and then rinsed by EDTA to remove non- internalized copper, followed by adding 300 μL of nitric acid. The dissolved cell breaking fluid was analyzed by ICP-MS.

In vitro image with R6G to copper

MDA-MB-231 cells were seeded into confocal dishes at a density of 1×10^5 /well. Cell were performed by Sm-TCPP(Cu)@HA treatment with or without pretreatment of HA for 1h. Then, the waste media were removed and replaced with PBS containing R6G (10 μM); And cell nucleus were stained with classic DAPI. Finally, the dishes were observed using CLSM.

MTT assay

Cells were seeded into 96-wells at a density of 8×10^3 /well and incubated for 24 h. The waste complete media in each well was superseded equivalently by fresh media containing Sm-TCPP(Cu)@HA at indicated concentration. For GSH competition experiment, cells were pretreated with L-BSO for 3h, and then incubated using Sm-TCPP(Cu)@HA under same conditions. After 24 of incubation, the solution was removed and replaced with 200 μL of PBS containing MTT (0.5 mg/mL) for another incubation (4 h). Then, PBS was replaced with 200 μL DMSO and cells were shaken for 5 min to fully resolve blue formazan. Finally, an ELISA reader was used to measure the absorbance at 570 nm of each well. Cell viability was calculated by comparing with the absorbance of the cells in control group and expressed as the percentage histogram. For enhanced cytotoxicity of DSF induced by Cu, MDA-MB-231 and MCF-10A Cells were treated by different concentration of DSF (up to 200 μg/mL) to assess its nontoxicity. Then, MDA-MB-cells were incubated with copper gluconate (20 μM), fixed concentration of DSF (100 μM) plus different concentration of copper gluconate (5, 10, 15, 20 μM) or Sm-TCPP(Cu)@HA at same Cu dose for 24 h. Then, MTT assay was used to obtain results like above operation.

Biodistribution and pharmacokinetics

ALL animals were received from Nanjing KeyGEN Biotech. And tumor models were conducted in accordance with policies of the National Ministry of Health. In brief, the

MDA-MB-231 cells tumor model were established by injecting 0.1 mL of collected cells at a concentration of 1×10^6 cells into armpit of right forelimb. When tumor volume was about 150 mm^3, mice were injected by Sm-TCPP(Cu)@HA at 0.8 mg Cu per kilogram of body. For pharmacokinetics, the blood samples were extracted at desirable time and digested by hydrochloric acid solution (4.8 mM/60% ethanol). The copper contents were determined by ICP-MS. For tissue biodistribution, after 6 and 24 h, mice were executed and major tissues including heart, liver, spleen, lung, kidney, and tumor tissues were harvested, grinded and digested by aqua regia for Cu detection by ICP-MS.

For security assessment of Sm-TCPP(Cu)@HA in vivo, tumor-bearing mice were injected by PBS and same concentration of TCPP(Cu) and Sm-TCPP(Cu)@HA at 0.8 mg Cu per kilogram of body. After 6 h, the mice were harvested and liver tissues were harvested for routine hematoxylin and eosin (H&E) assay.

In vivo antitumor and histology study

Tumor-bearing mice, with about 150 mm^3 and a body weight of about 20.5 g, were randomly divided into 4 groups: 1) PBS, 2) Sm-TCPP(Cu)@HA; 3) DSF, 4) Sm-TCPP(Cu)@HA plus DSF. Noting that DSF treatment were performed by using oral dose at 50 mg/kg every day, while Sm-TCPP(Cu)@HA treatment was by tail vein injection at 0.8 mg Cu per kilogram of body by every other day. The body weight and tumor volume of the mice were recorded during the 15-day observation period. Tumor volume (V) was calculate according to the equation: $V = (L*W^2) *0.5$, where L and W represented the length and width of the tumor, respectively. At the end of the 15 days, the mice were sacrificed and the main organs including heart, liver, spleen, lung, kidney, and tumor tissues were collected for routine hematoxylin and eosin (H&E) assay.

References

[S1] Gao, Z.; Chen, F.; Li, Y.; Zhang, Y.; Cheng, K.; An, P.; Sun, B. Dalton Trans. 2019, 2019,48, 16861-16868

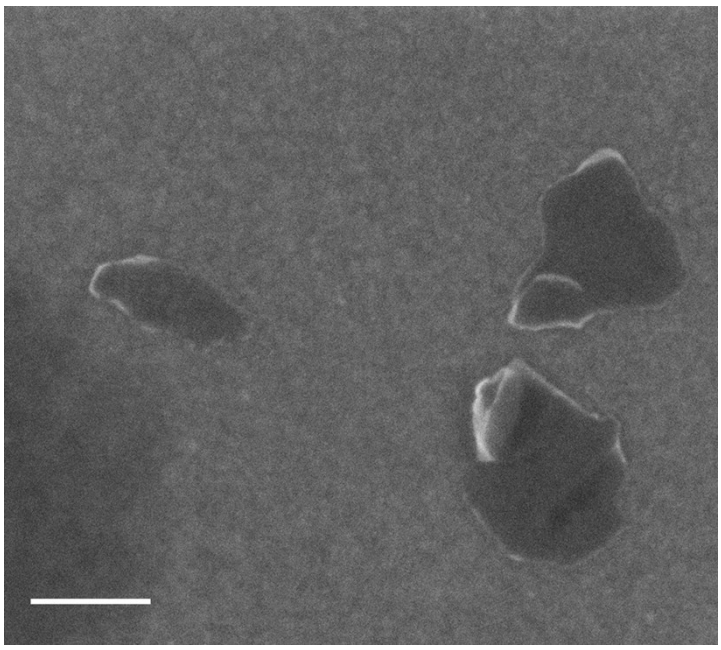

Figure S1: SEM image of Sm-TCPP(Cu) nanosheets. Scale bar: 200 nm.

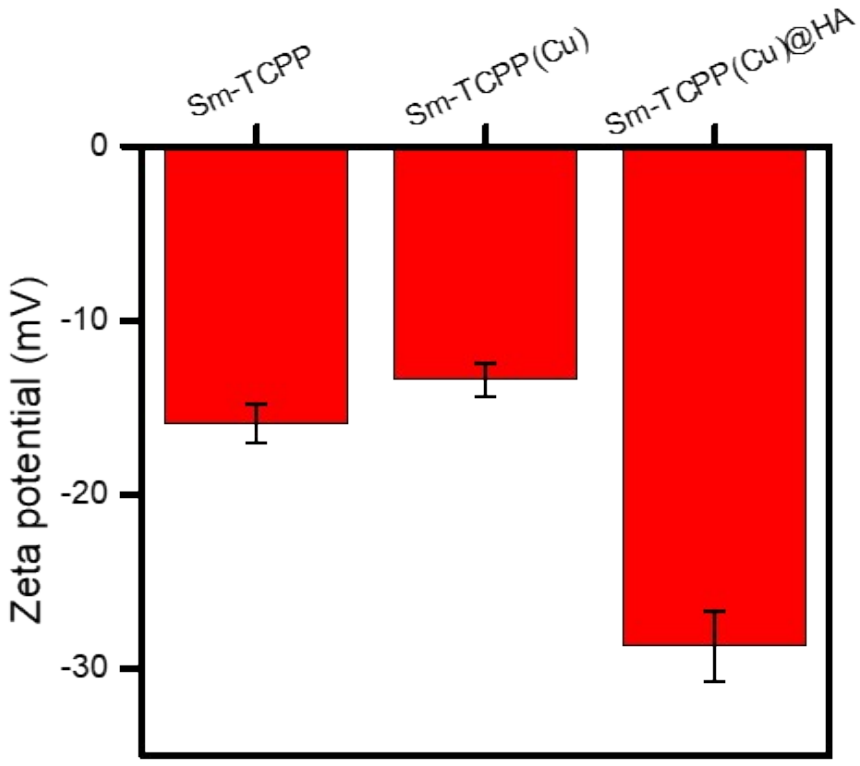

Figure S2: Zeta potential of Sm-TCPP, Sm-TCPP(Cu), and Sm-TCPP(Cu)@HA nanosheets.

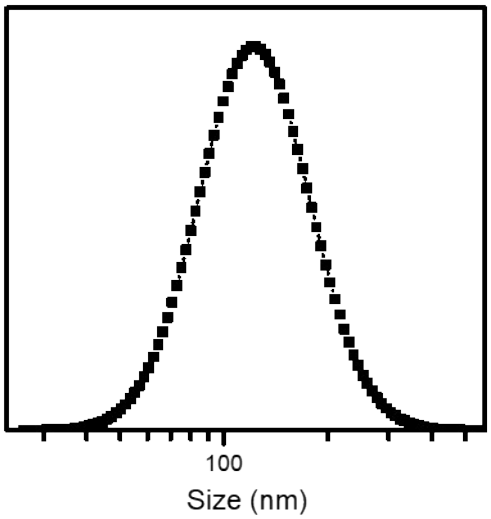

Figure S3: Size distribution of Sm-TCPP(Cu)@HA measured by DLS.

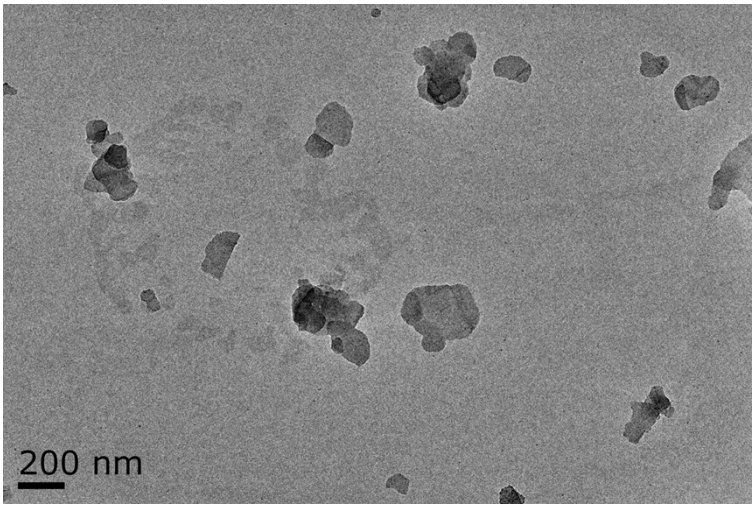

Figure S4: TEM image of Sm-TCPP(Cu)@HA nanosheets.

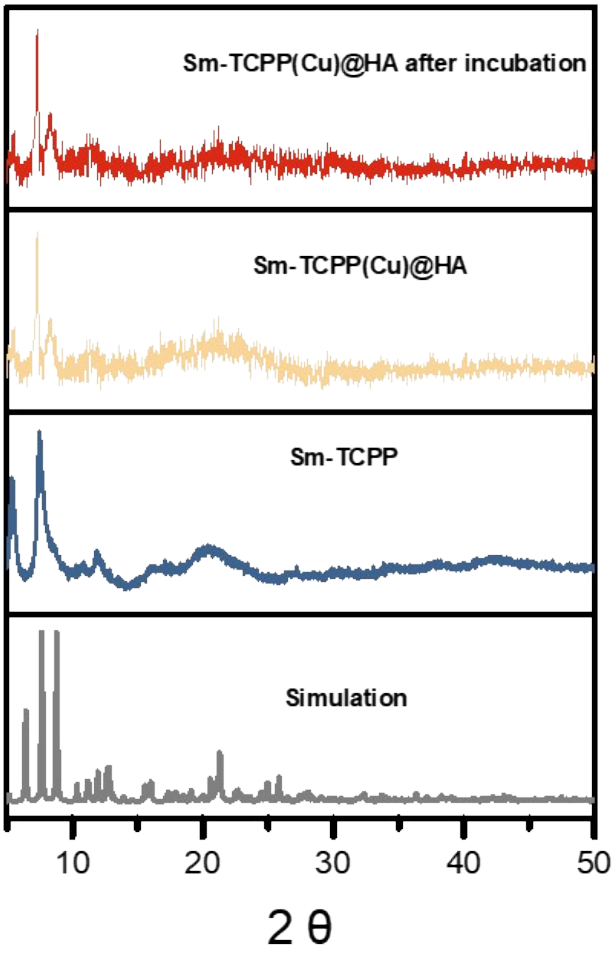

Figure S5: XRD pattern of Sm-TCPP, Sm-TCPP(Cu)@HA, and Sm-TCPP(Cu)@HA incubated by DMEM media for 24 h.

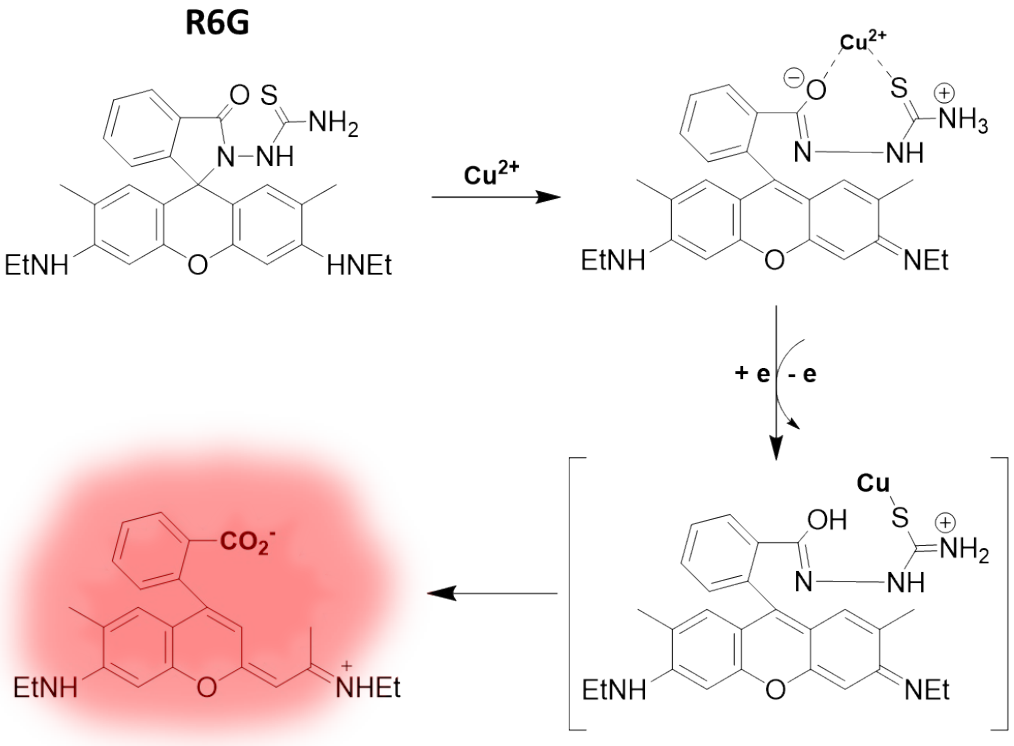

Figure S6: Detection mechanism of R6G binding Cu^{2+} to give bioluminescent signal.

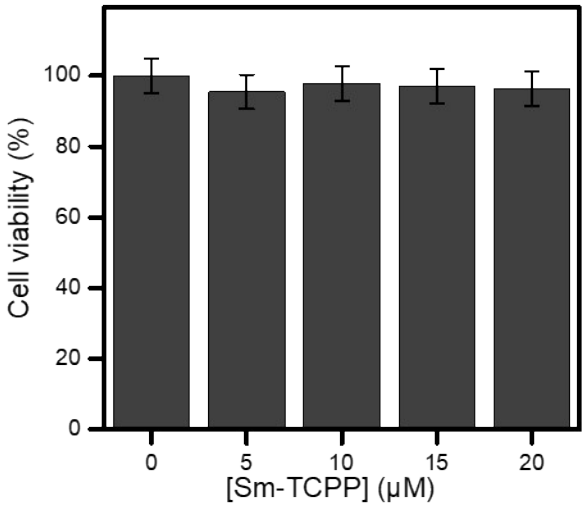

Figure S7: Cell viability for MDA-MB-231 cells treated with DSF (20 μM) and Sm-TCPP after 24 h.

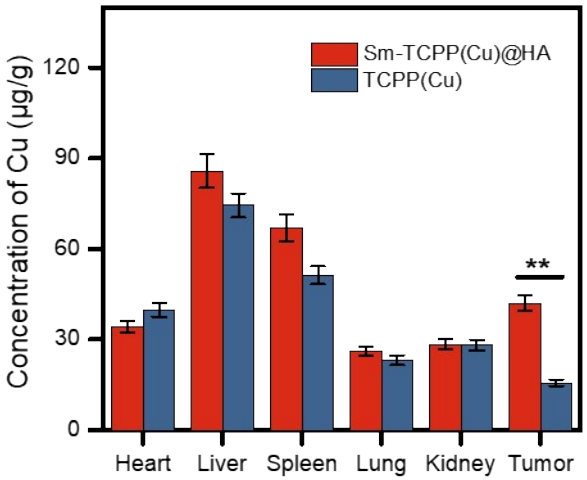

Figure S8: The Cu tissue distribution in major tissues including heart, liver, spleen, lung, kidney, and tumor after 24 h of treatment with Sm-TCPP(Cu)@HA.

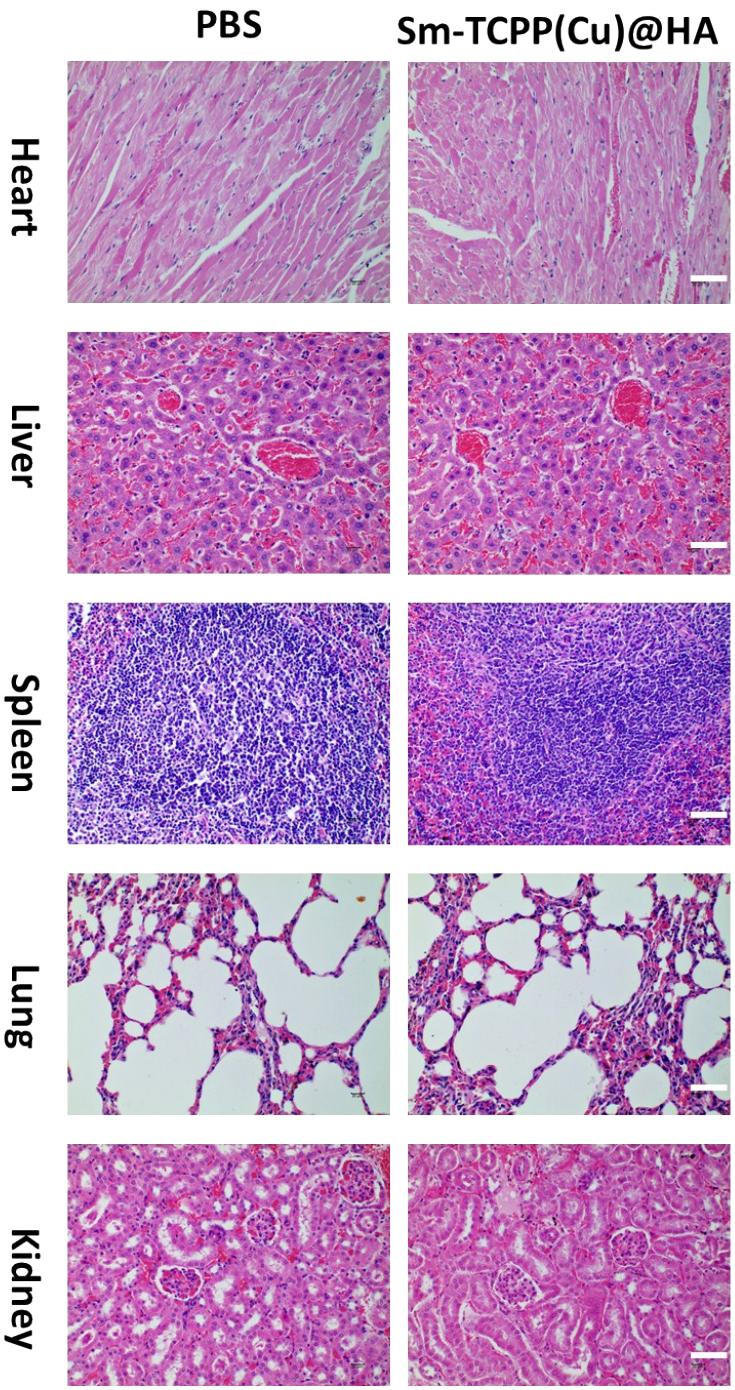

Figure S9. H&E dying analysis of heart, liver, spleen, lung, and kidney tissues after various treatments. Scale Bar: 50 μm.

Table S1 IC50 value of different treatments after 72 h of incubation for MDA-MB-231

	DSF	Cu	**DSF/Cu**	Cisplatin
IC50/μM	>20	>20	**0.7±0.1**	7.3±1.5

Cu source was copper gluconate.