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Supplementary Materials

Chain-Shattering Polymeric Sulfur Dioxide Prodrug Micelles for Redox-Triggered Gas Therapy of Osteosarcoma

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Synthesis of compound 2.

Compound 1 was synthesized according to the previous report. Compound 1 (5g, 12.6 mmol) and triethylamine (TEA, 2.63 mL, 18.9 mmol) were dissolved in 50 mL of dichloromethane (DCM). The mixture was stirred at 0°C, to which 2,4-dinitrobenzenesulfonyl chloride (5g, 18.9 mmol) was added. The reaction was allowed to proceed overnight at room temperature, after which the DCM was removed. The residue was purified by chromatography (hexane: ethyl acetate 10:1) to afford slightly yellow solid (yield 58%).

Synthesis of DN

Compound 2 (0.5 g, 0.79 mmol) was dissolved in 10 mL of methanol, and then *p*-toluenesulfonic acid monohydrate (31.8 mg, 0.16 mmol) was added. The mixture was stirred for 1h at room temperature, after which the methanol was removed. The residue was purified by chromatography (hexane: ethyl acetate 3:1) to afford white solid (yield 74%).

Characterization

Chemical structures of the synthesized products were characterized by ^{1}H NMR spectroscopy on a Varian 600 MHz spectrometer, using deuterated chloroform or dimethyl sulfoxide as solvent. The molecular weights distribution of $mPEG_{2000}$-P(HDI-DN)$_{20}$ treated by GSH were characterized using a Waters' gel permeation chromatography (GPC) system equipped with a 2414 refractive index detector, and DMF as mobile phase at 35°C with a

flow rate of 1.0 mL/min. The size and zeta potential of micelles in PBS were measured by Malvern Zetasizer (Zetasizer Nano ZS). Critical micelle concentration (CMC) of mPEG-P(HDI-DN) micelles was determined using pyrene as fluorescence probe. The morphology of mPEG-P(HDI-DN) micelles was estimated by field emission transmission electron microscopy (JEOL JEM-2100F) after drying a drop of micelles solution on carbon-coated copper grids.

Detection of intracellular ROS by flow cytometry

143B cells were seeded in a 6-well plate and cultured overnight at 37°C. The medium was replaced with fresh DMEM medium containing $mPEG_{2000}$-$P(HDI\text{-}DN)_{20}$ micelles with different concentrations (0, 0.2, 0.4, 0.8 mg/ml). After co-incubation for 18 h, the cultural medium was removed. The cells were washed and stained with DCFH-DA following with protocol. Finally, flow cytometry was utilized to measure the fluorescence signal of each group.

Blood routine test

Female BALB/c nude mice (4 weeks old) were purchased from Guangdong Medical Laboratory Animal Center. Animal care and handling procedures were in accordance with the guidelines evaluated and approved by the ethics committee of Shenzhen TopBiotech Co.,Ltd. The mice were randomly divided into 4 groups (n=4). Saline, $mPEG_{2000}$-$P(HDI\text{-}DN)_{20}$ micelles (10 mg/kg), $mPEG_{2000}$-$P(HDI\text{-}DN)_{20}$ micelles (20 mg/kg) and $mPEG_{2000}$-

$P(HDI\text{-}DN)_{20}$ micelles (40 mg/kg) were given by subcutaneous injection, respectively. After 3 days, the mice were sacrificed, and the blood was collected for blood routine test.

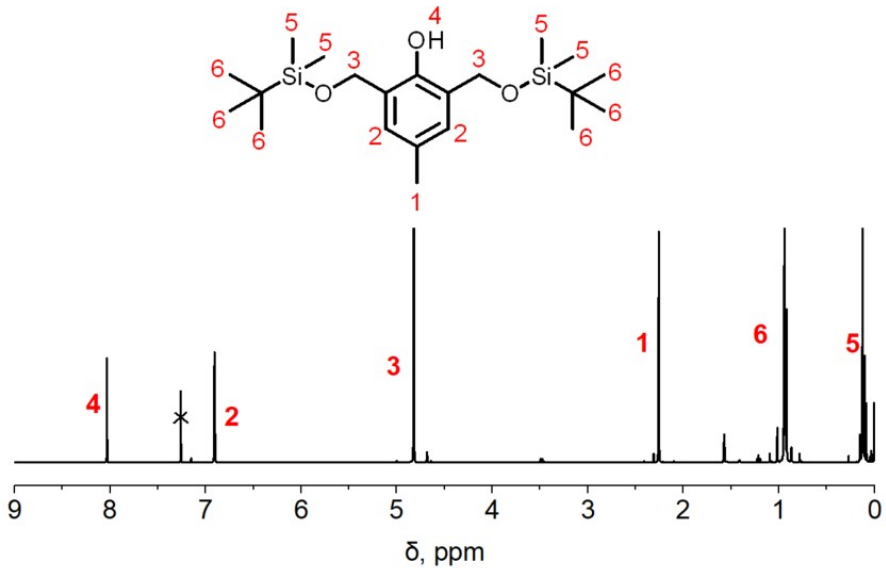

Figure S1. ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

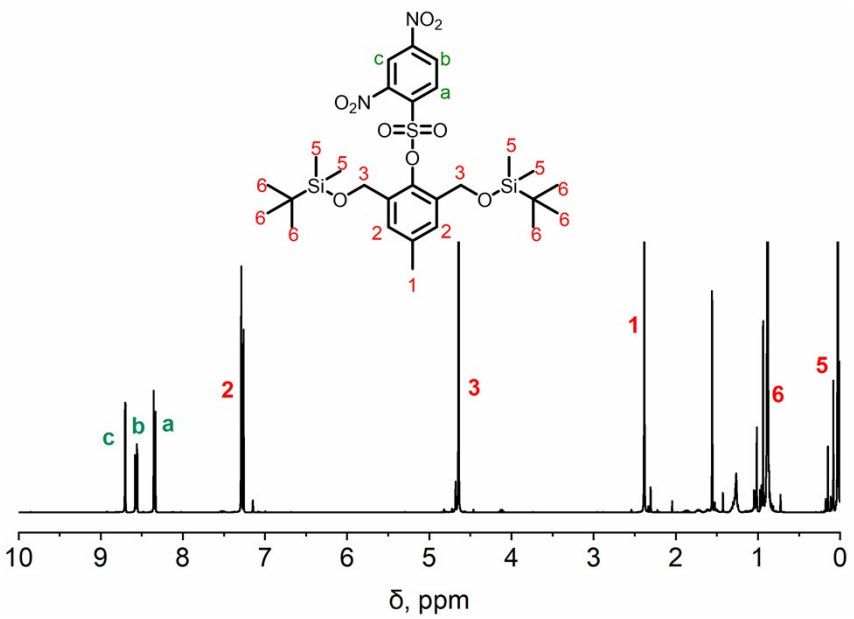

Figure S2. ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

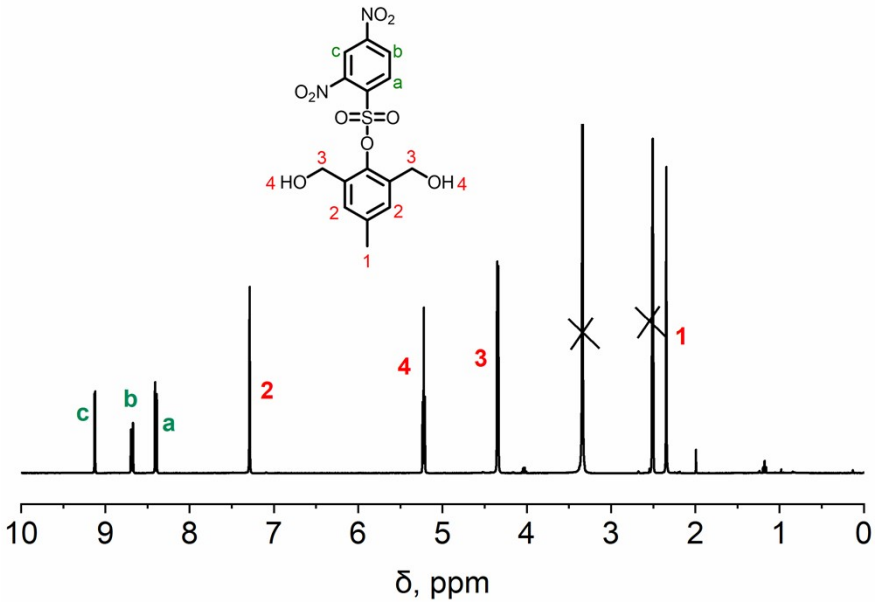

Figure S3. ^{1}H NMR spectrum of compound DN in DMSO-d6.

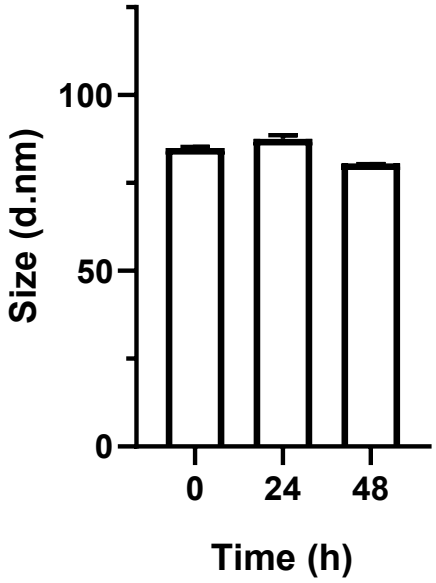

Figure S4. Size change of PEG_{2000}-P(HDI-$DN)_{20}$ micelles at 37℃.

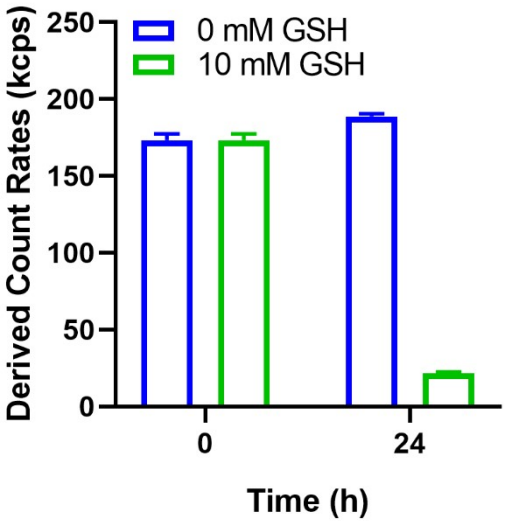

Figure S5. Derived count rates change of $mPEG_{2000}$-P(HDI-$DN)_{20}$ micelles after received different treatments.

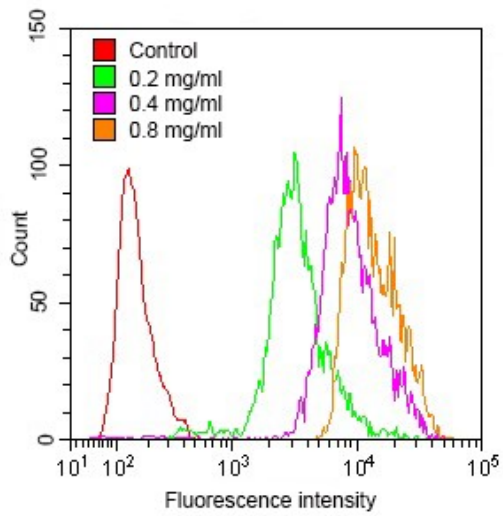

Figure S6. Cellular ROS level of 143B cells after treatment by $mPEG_{2000}$-$P(HDI\text{-}DN)_{20}$ determined by flow cytometry.

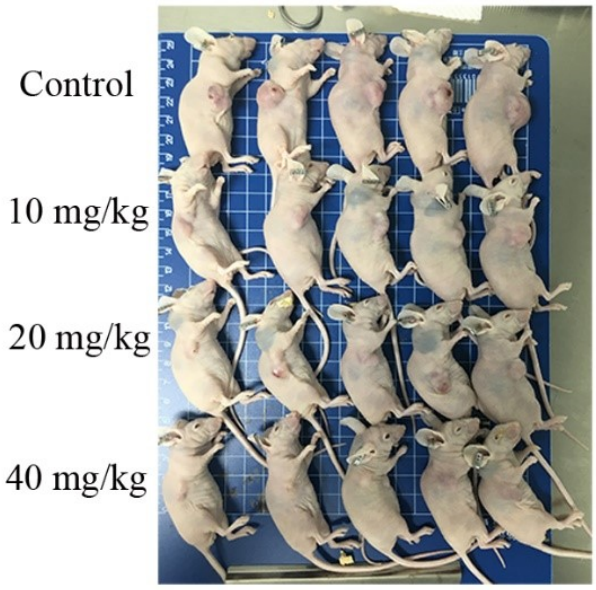

Figure S7. Photographs of mice after received different treatments for 16 days.

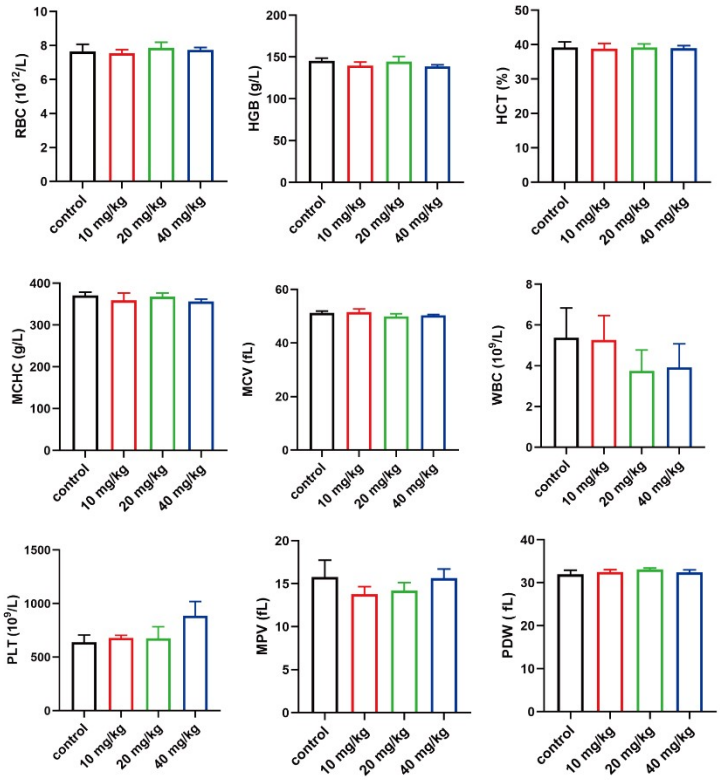

Figure S8. Routine blood tests of mice after received different treatments.