Supplementary Information

A smart P-gp inhibitor-drug conjugate nanomedicine overcomes administration challenges and multidrug resistance in breast cancer therapy

Yingchun Liu,[a] Penghui Wang,[a] Xuelin Xia,[b] Wenhui Gao,[a] Yao Wang,[d] Wei Huang,[*a, c] and Deyue Yan[*a, c]

[a] School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, People's Republic of China

[b] State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

[c] XIANGFU Laboratory, Jiaxing, Zhejiang, 314102, China

[d] Institute of Molecular Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

AUTHOR INFORMATION

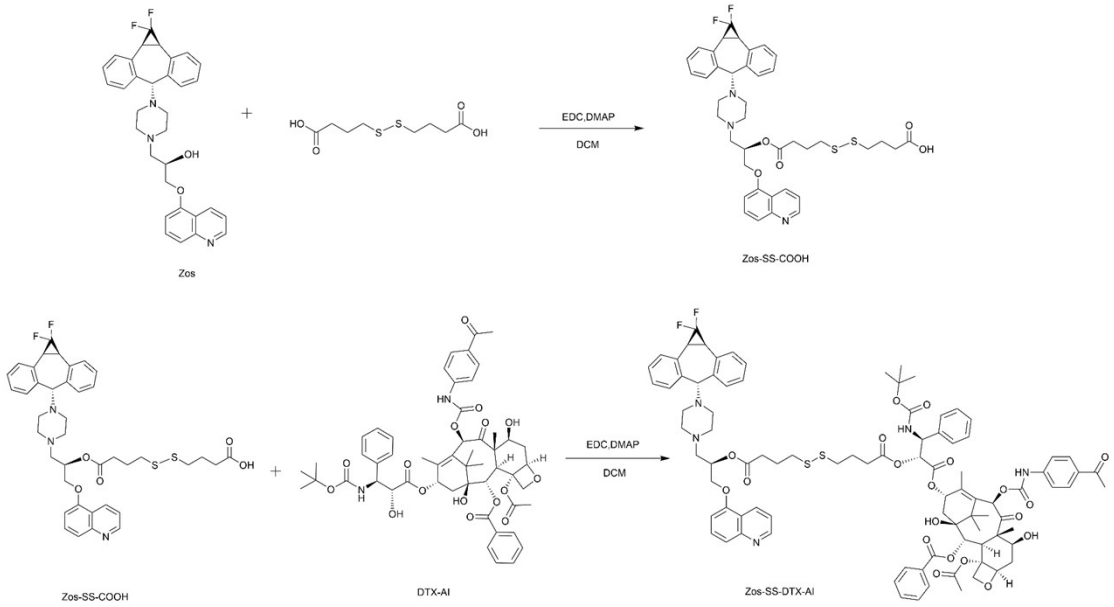
Corresponding Authors

Prof. Deyue Yan, E-mail: hw66@sjtu.edu.cn

Prof. Wei Huang, E-mail: dyyan@sjtu.edu.cn

Characterization: ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded using the Bruker AVANCE III HD 400 MHz or 500 MHz spectrometer using DMSO-d_6 or $CDCl_3$ as solvents. LC-MS spectra were obtained using Acquity UPLC/QTOF Premier (Shimadzu, Japan). UV-Vis absorption spectra were measured using a Thermo Electron-EV300 UV-Vis spectrophotometer. The size and distribution of the assembly was measured using a dynamic light scattering (DLS) apparatus (Malvern Zetasizer Nano S, UK). The zeta potential was investigated by a Brookhaven NanoBrook-Omni High Sensitivity Zeta Potentiometer and Particle Size Analyzer. Transmission electron microscopy (TEM) was performed using a Tecnai G2 Spirit Biotwin instrument (Thermo Fisher, USA) at 120 kV. Laser confocal imaging was conducted on a Leica TCS SP8 STED 3X Super-resolution multiphoton confocal microscope (Leica, Germany). The flow cytometry analysis was performed on a BD LSR Fortessa flow cytometer (BD, USA)

Scheme and Figures



Scheme S1. Synthetic route of Zos-SS-DTX-AI.

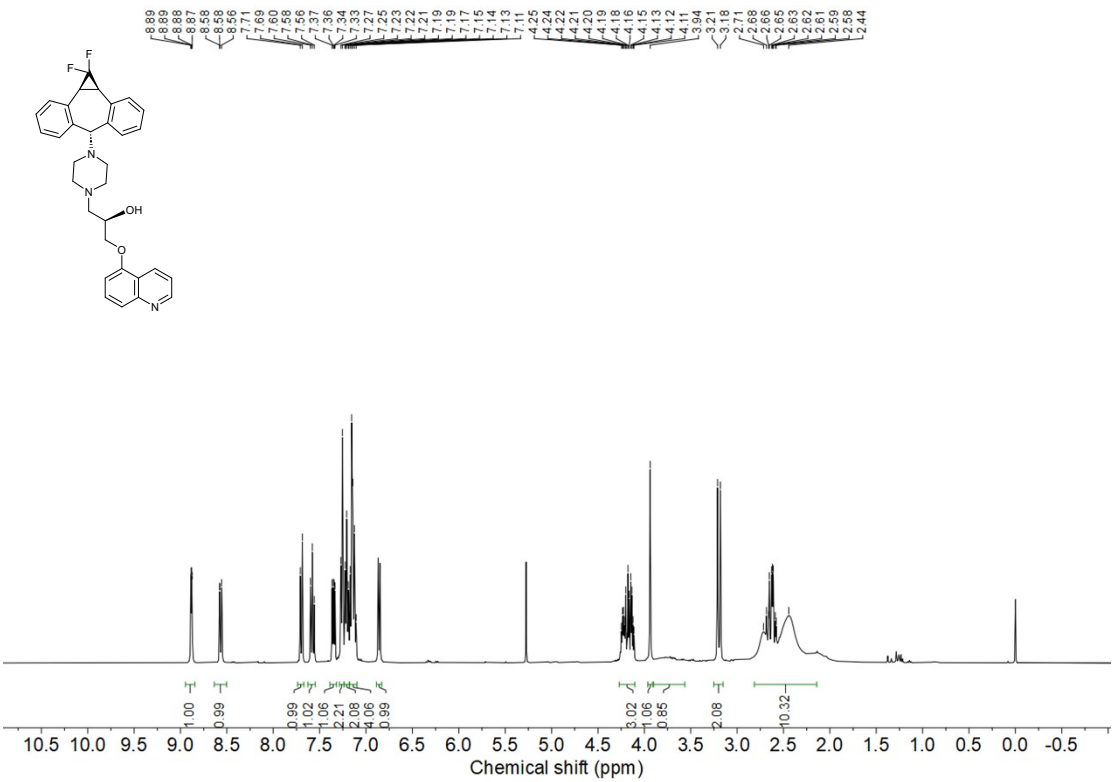


Fig. S1 ^{1}H NMR spectrum of Zos in $CDCl_3$.

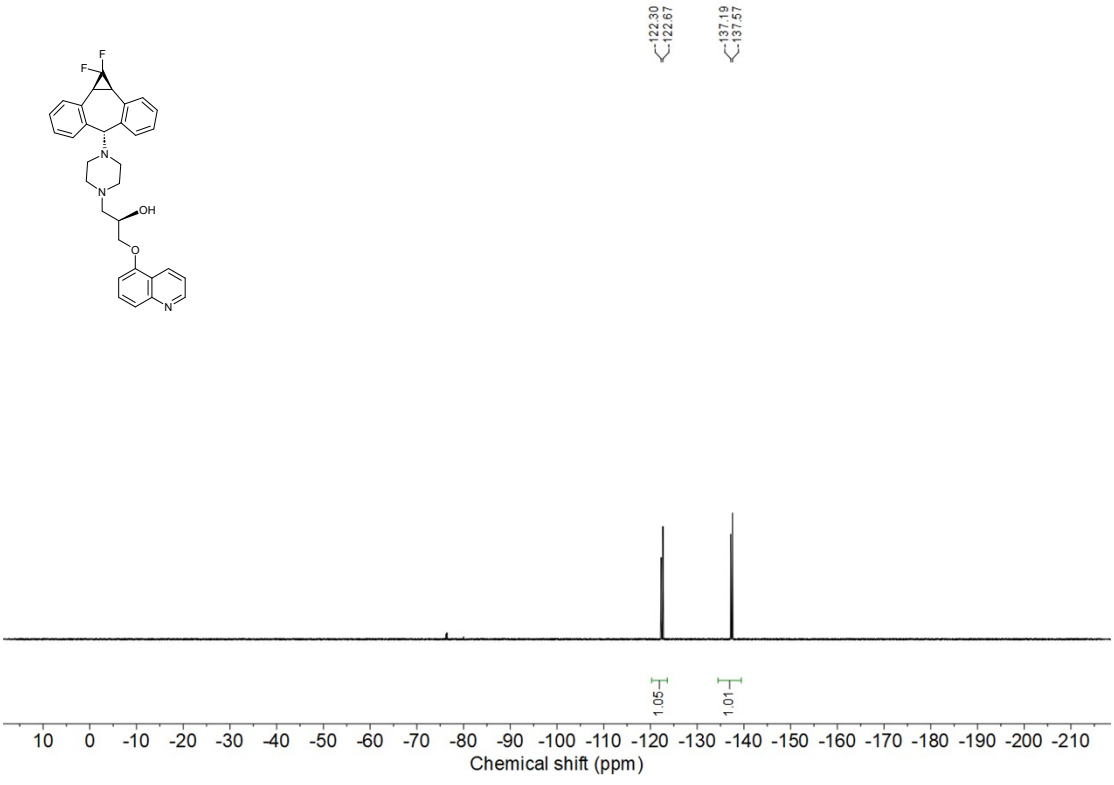


Fig. S2 ^{19}F NMR spectrum of Zos in DMSO-d_6.

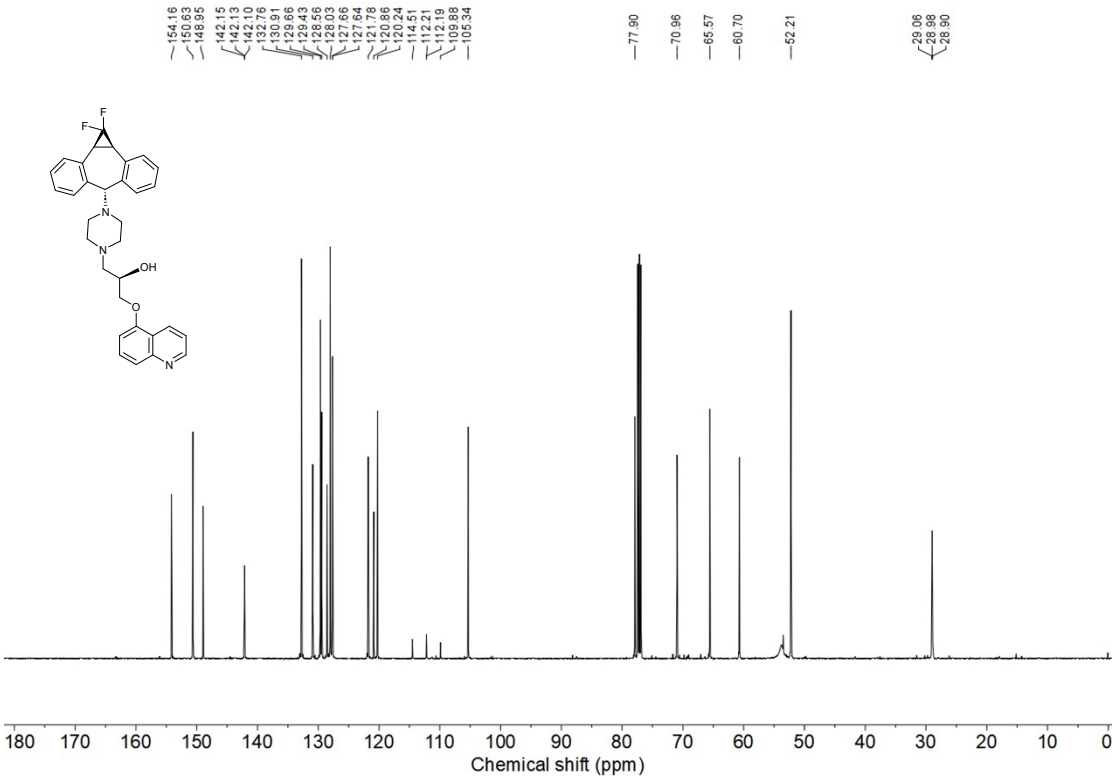


Fig. S3 ^{13}C NMR spectrum of Zos in $CDCl_3$.

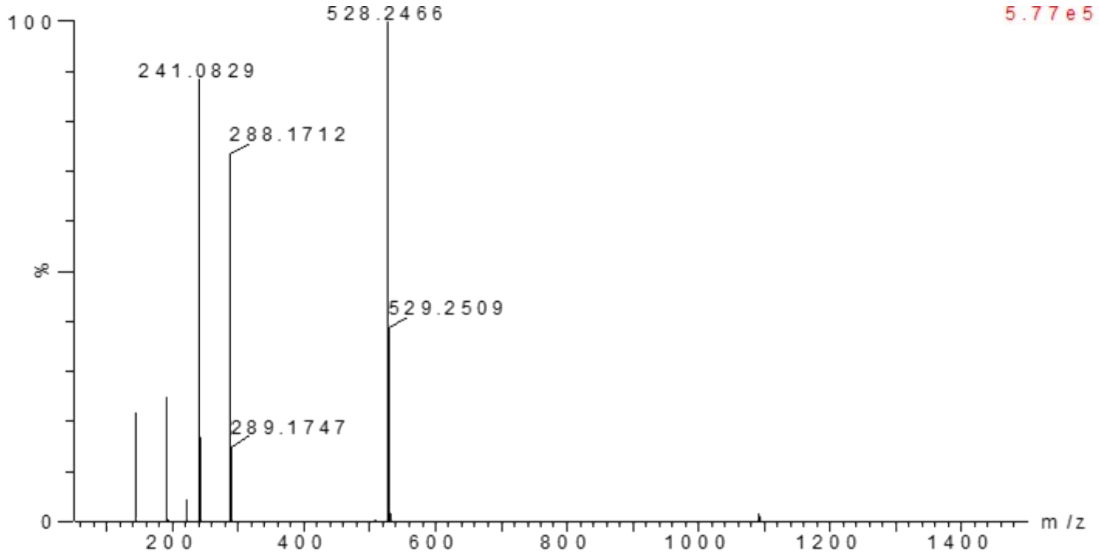


Fig. S4 HRMS spectrum of Zos (calcd for $C_{32}H_{31}F_2N_3O_2$, $[M + H]^+$ m/z 528.2463, found 528.2466)

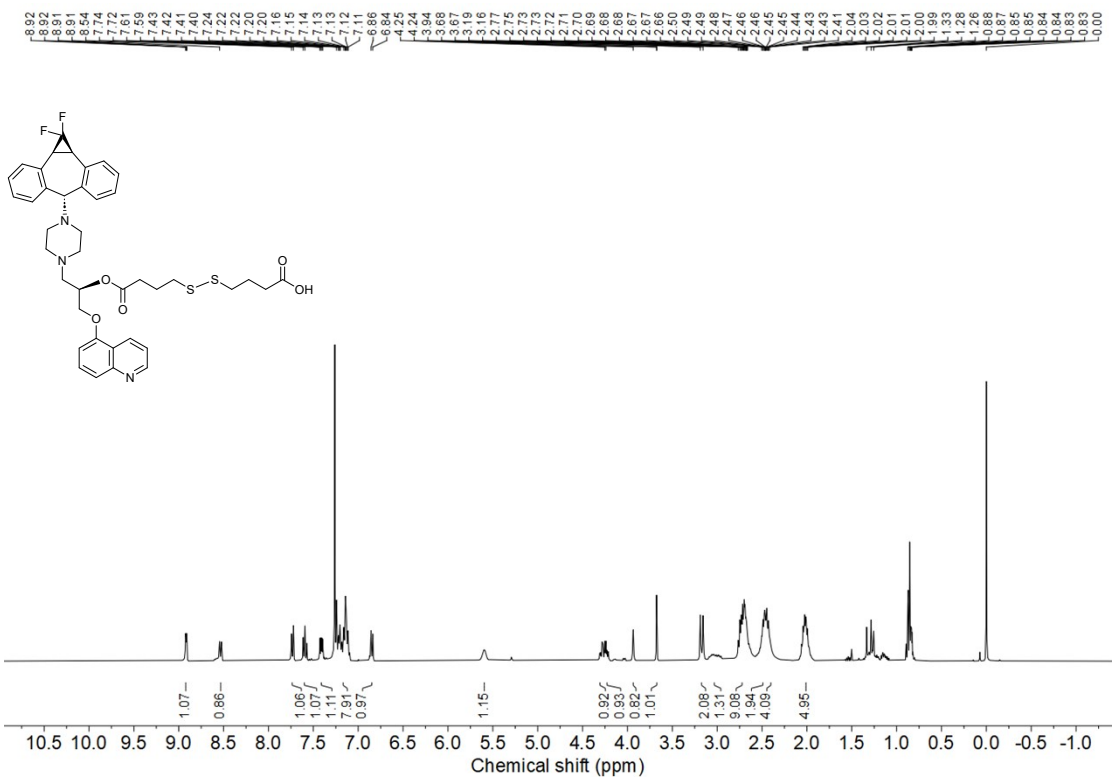


Fig. S5[1]H NMR spectrum of Zos-SS-COOH in $CDCl_3$.

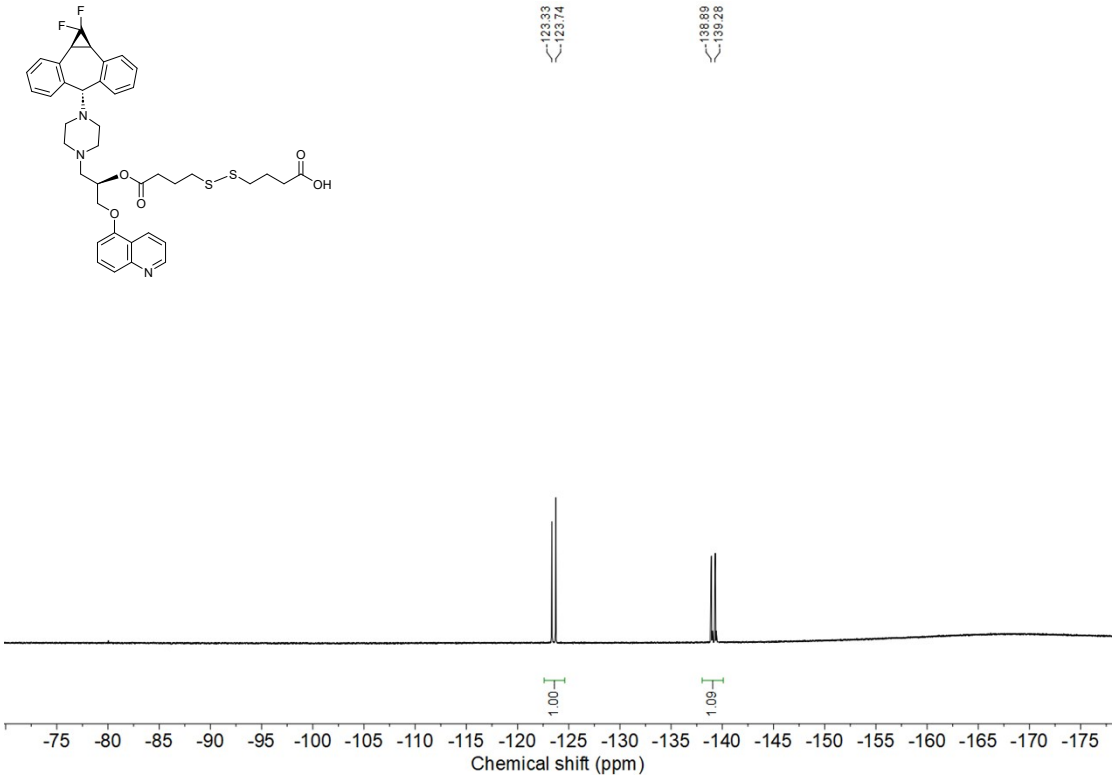


Fig. S6 [19]F NMR spectrum of Zos-SS-COOH in $CDCl_3$.

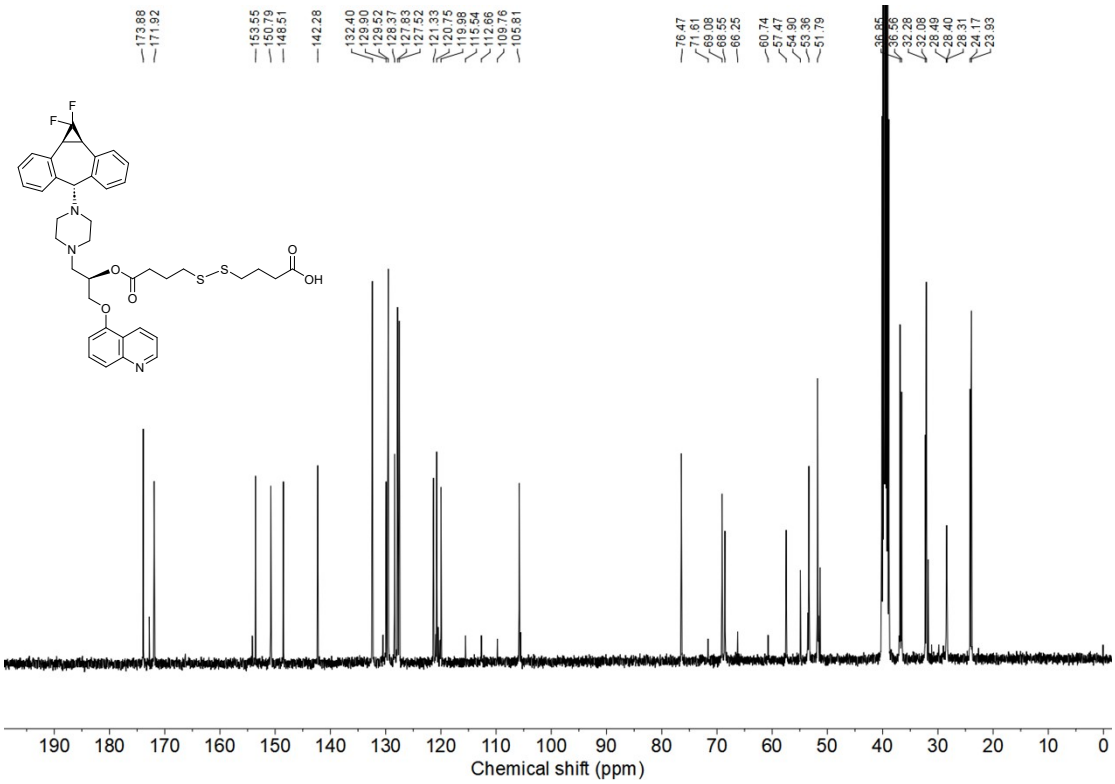


Fig. S7 ^{13}C NMR spectrum of Zos-SS-COOH in DMSO-d_6.

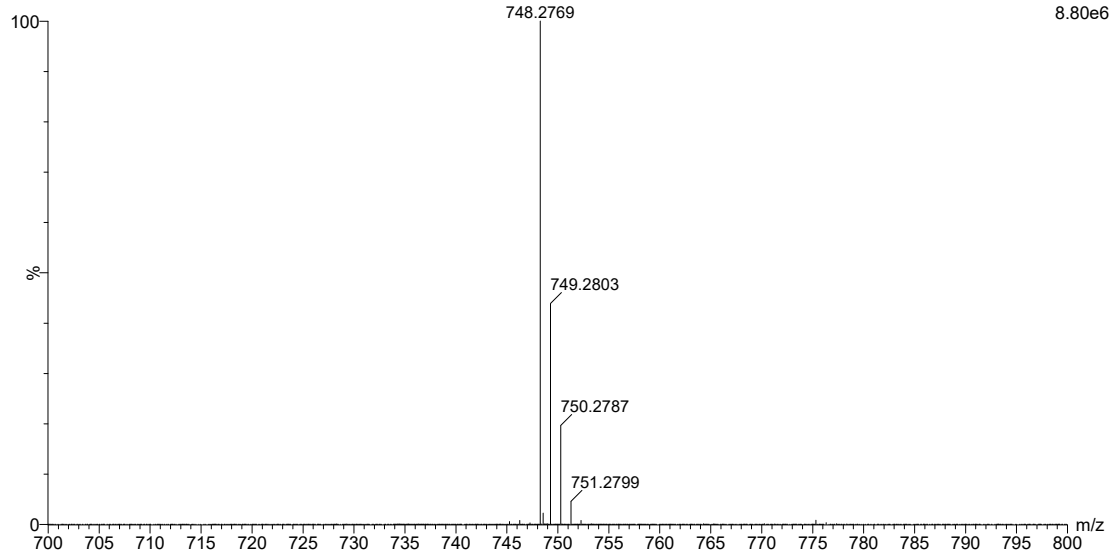


Fig. S8 HRMS spectrum of Zos-SS-COOH (calcd for $C_{40}H_{43}F_2N_3O_5S_2$, $[M + H]^+$ m/z 748.2690; found 748.2769)

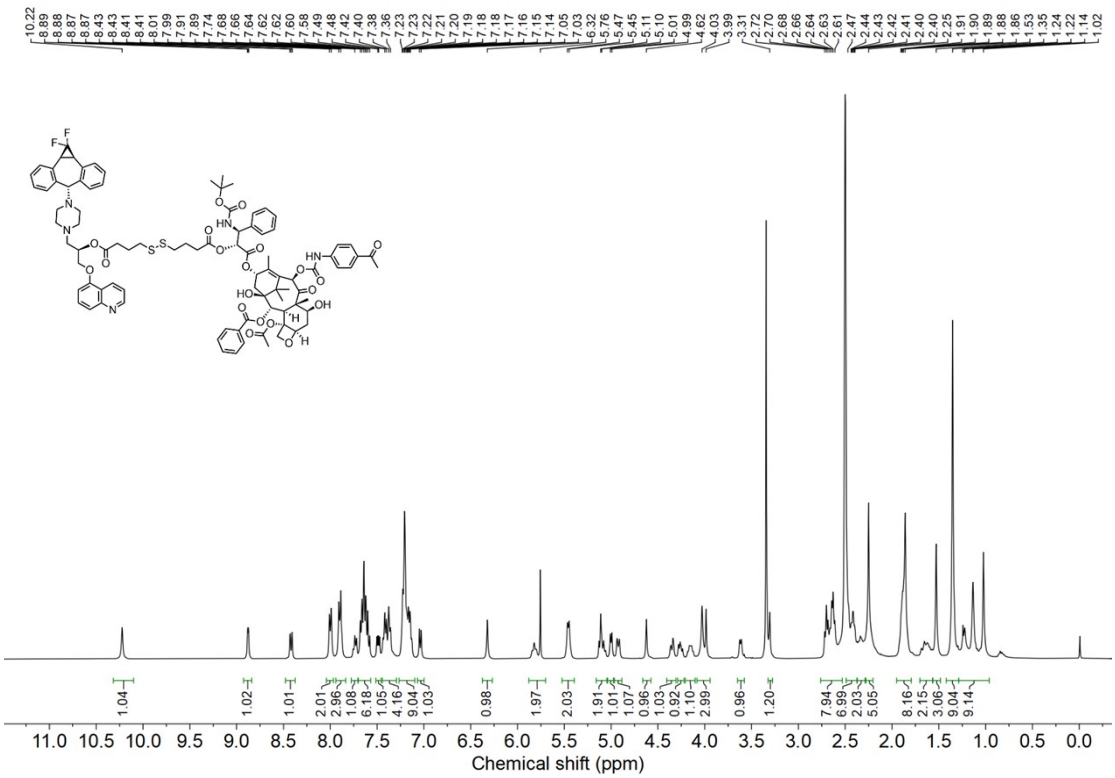


Fig. S9 ^{1}H NMR spectrum of Zos-SS-DTX-AI in DMSO-d_6.

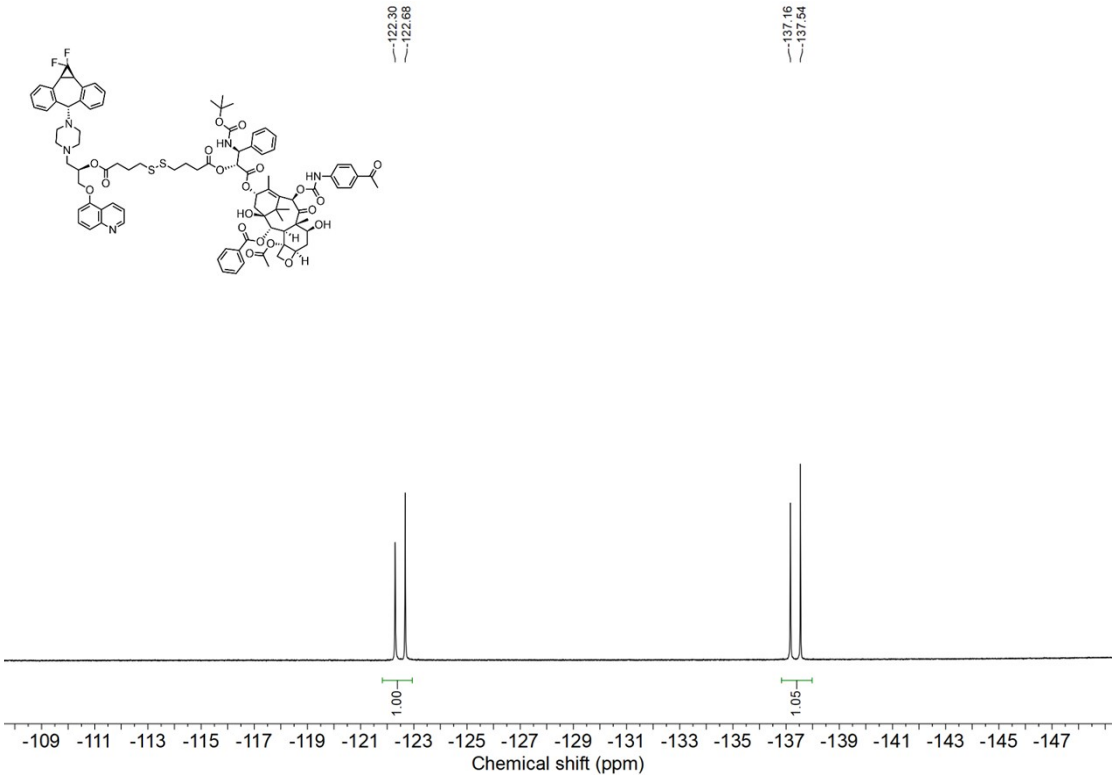


Fig. S10 ^{19}F NMR spectrum of Zos-SS-DTX-AI in DMSO-d_6.

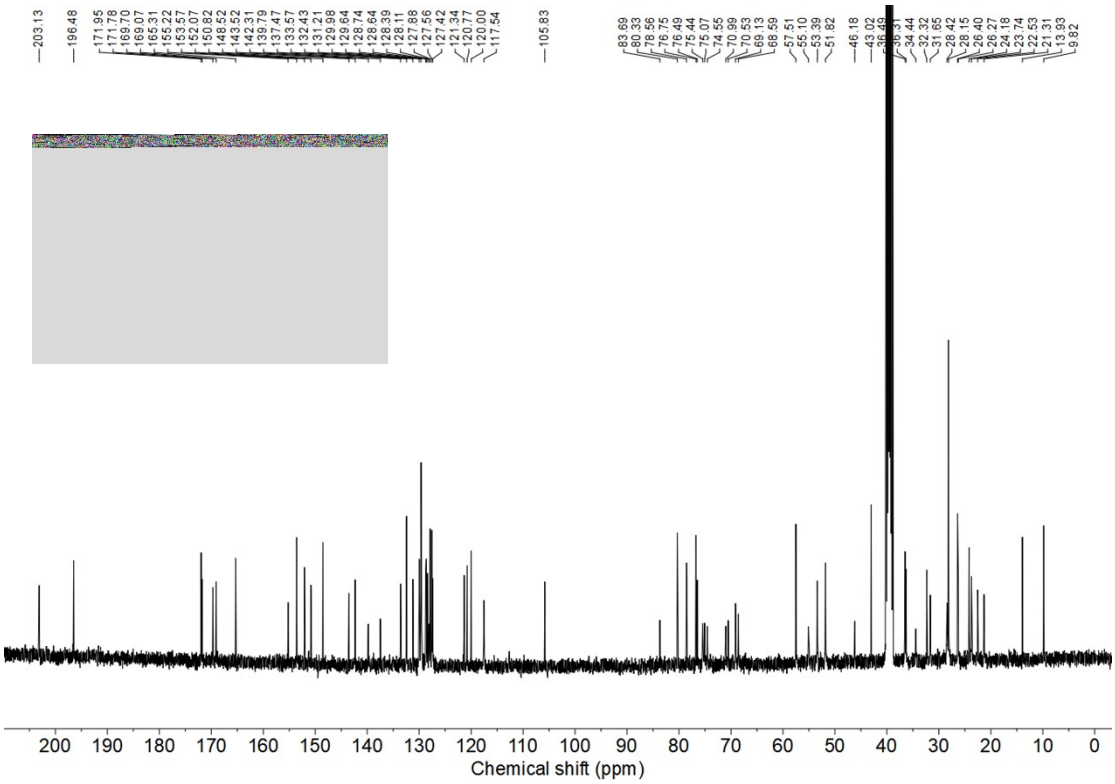


Fig. S11 ^{13}C NMR spectrum of Zos-SS-DTX-AI in DMSO-d_6.

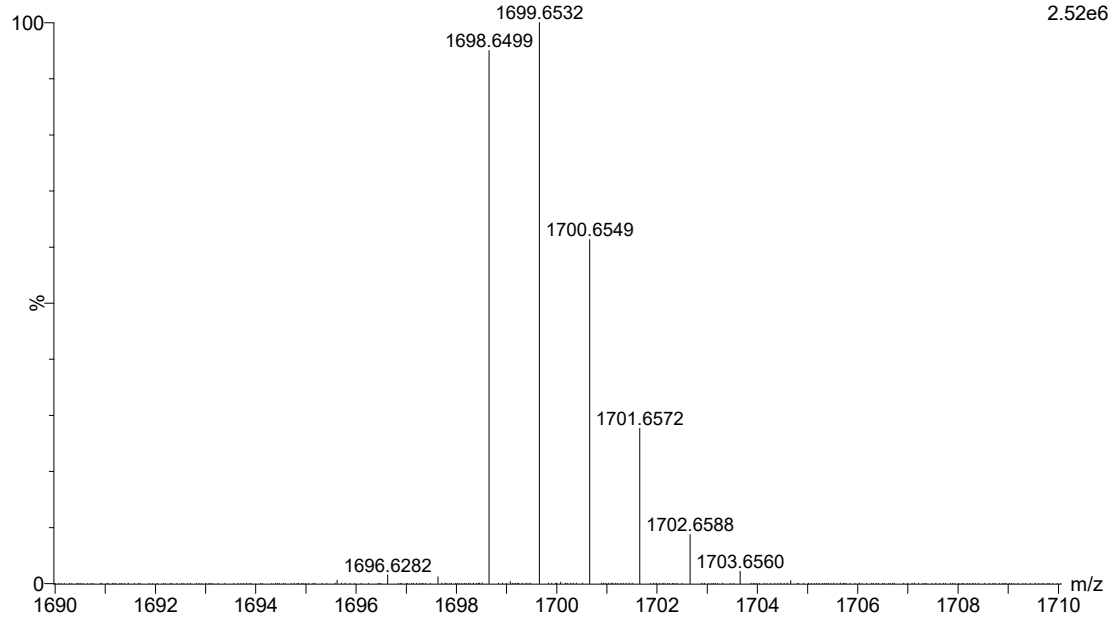


Fig. S12 HRMS spectrum of Zos-SS-DTX-AI (calcd for $C_{92}H_{101}F_2N_5O_{20}S_2$, $[M+H]^+$ m/z 1698.6528; found 1698.6499)

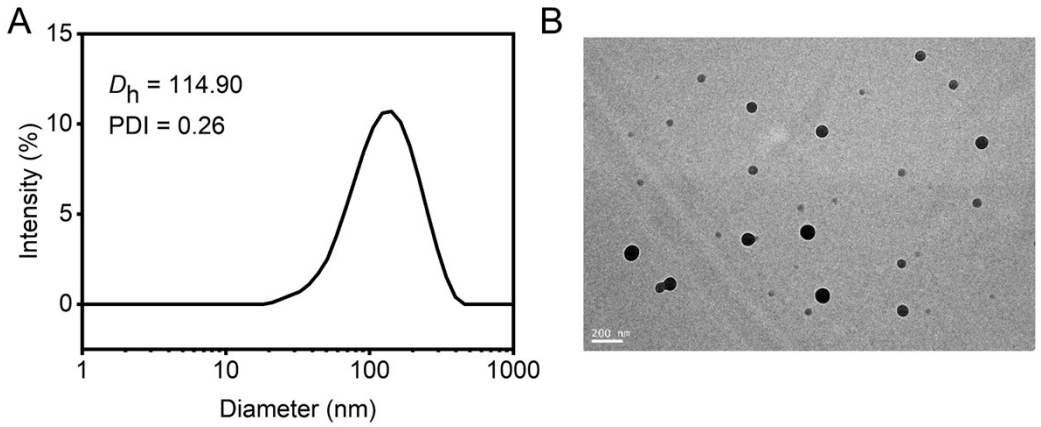


Fig. S13 (A) DLS curve and (B) TEM image of Cy5.5-loaded Zos-SS-DTX-AI@DSPE-PEG$_{2k}$ NPs (scale bar: 200 nm)

Table S1. The IC_{50} values and resistance index (RI)[a] of DTX-AI against MCF-7 and MCF-7/PTX cells.

Cell line	IC_{50} (μg mL^{-1})	RI
MCF-7	0.28	—
MCF-7/PTX	9.29	34.41

[a]RI = IC_{50} (MCF-7/PTX)/IC_{50} (MCF-7)

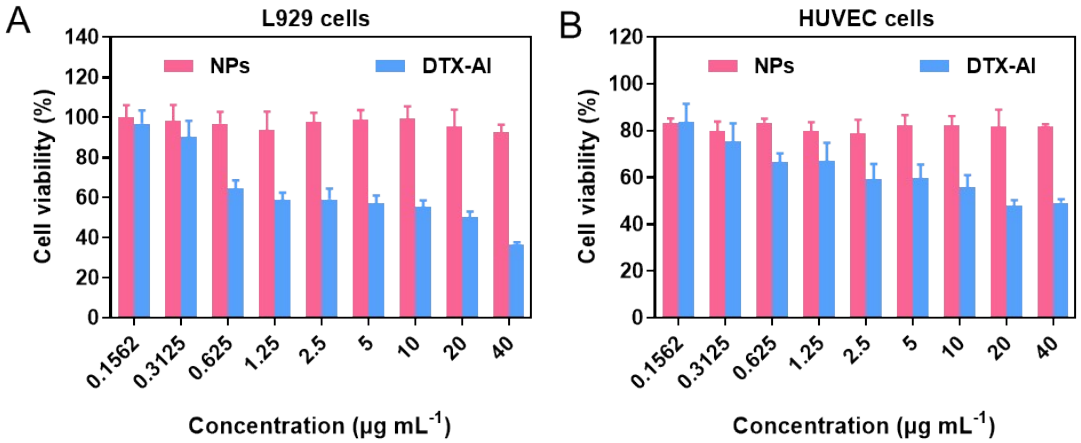


Fig. S14 Cell viability of (A) L929 cells and (B) HUVEC cells treated with Zos-SS-DTX-AI@DSPE-PEG$_{2k}$ NPs and free DTX-AI, respectively, at the same concentration of DTX-AI.

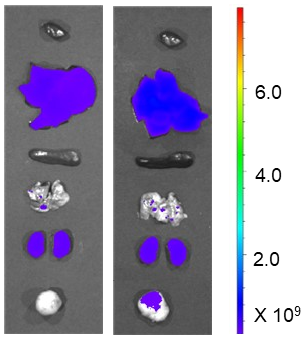


Fig. S15 Representative *ex vivo* fluorescence images of tumors and major organs excised from MCF-7/PTX tumor-bearing nude mice 8 h after intravenous administration of free Cy5.5 and Cy5.5-loaded Zos-SS-DTX-AI@DSPE-PEG$_{2k}$ NPs.

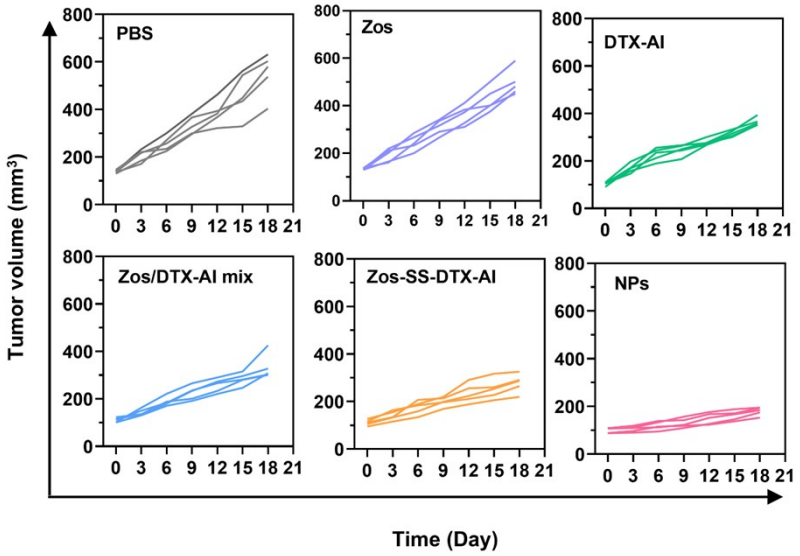


Fig. S16 MCF-7/PTX tumor growth curves of each mouse in various treatment groups (n = 5)

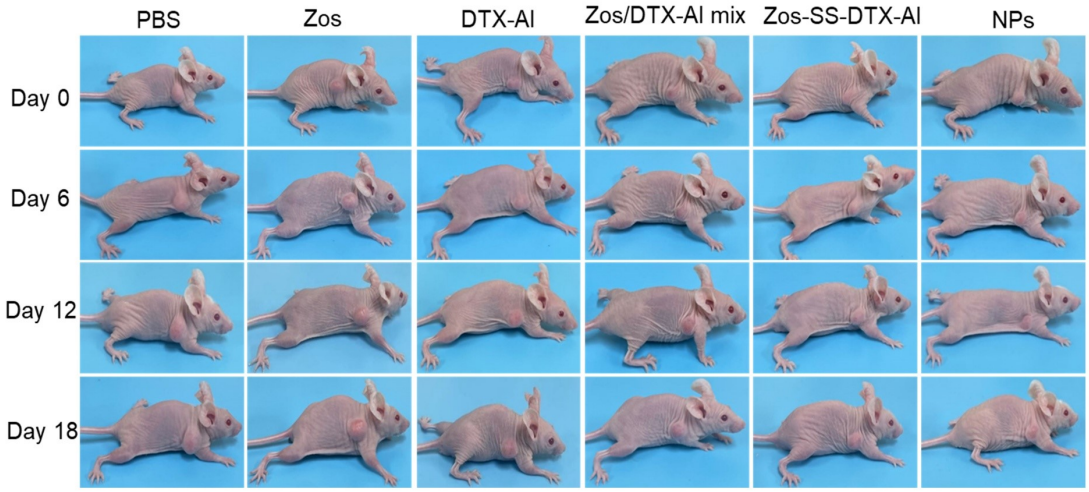


Fig. S17 Representative photographs of MCF-7/PTX tumor-bearing nude mice in various treatment groups.