Electronic Supplementary Material (ESI) for Biomaterials Science.

Redox-responsive drug-inhibitor conjugate encapsulated in DSPE-PEG$_{2k}$ micelles for overcoming multidrug resistance of chemotherapy

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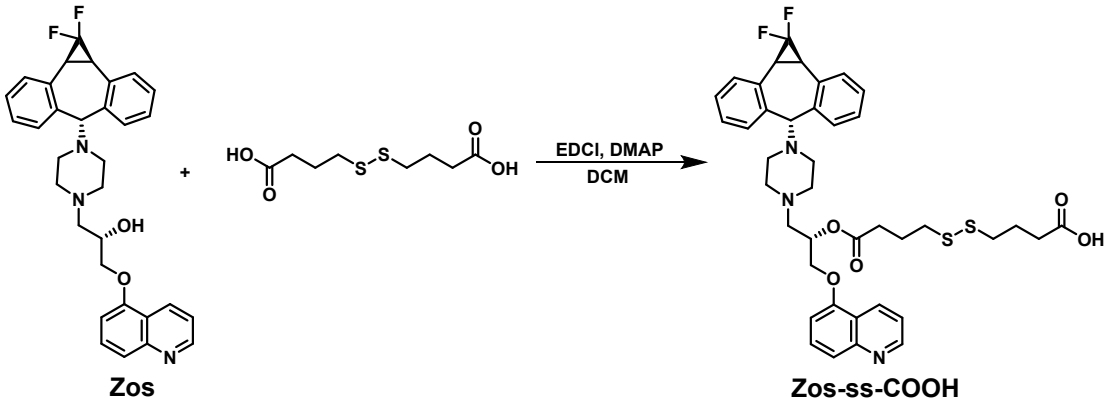
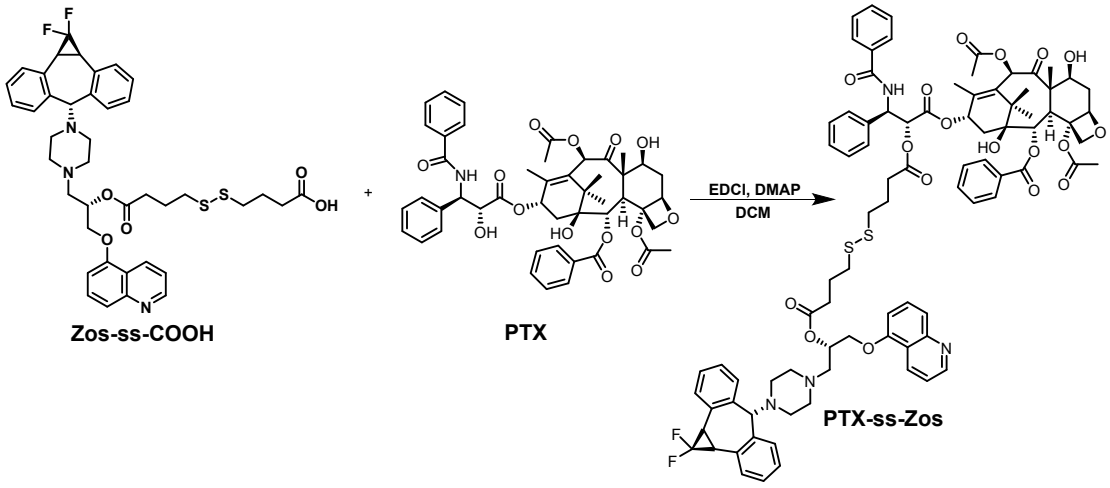
Characterization: ^{1}H, ^{13}C, and ^{19}F NMR spectra were measured by the Bruker AVANCE III HD 400 MHz spectrometer. LC-MS spectra were performed with Acquity UPLC/QTOF Premier (Shimadzu, Japan). UV-Vis absorption spectrum was measured with a Thermo Electron-EV300 UV-Vis spectrophotometer. The size 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. Scanning transmission electron microscopy energy dispersive spectroscopy (STEM-EDS) was carried out by FEI Talos F200X G2 material type field emission transmission electron microscopy (Thermo Fisher, USA). Laser confocal imaging was investigated 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).

1. **Methods**

1.1 **Synthetic route of Zos-ss-COOH.**

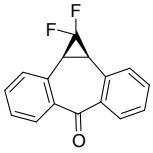


1.2 **Synthetic route of PTX-ss-Zos Conjugate.**



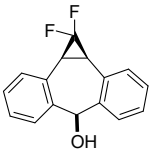
1.3 NMR data of Zos and synthetic intermediates

1,1-Difluoro-1a,10b-dihydrodibenzo[*a,e*]cyclopropa[c]-cyclohepten-6 (1H)-one:



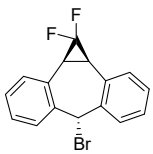
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (dd, J = 7.8, 1.3 Hz, 2H), 7.55 – 7.50 (m, 2H), 7.43 – 7.35 (m, 4H), 3.37 (d, J = 3.3 Hz, 1H), 3.35 (d, J = 3.4 Hz, 1H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -127.03 (d, J = 151.9 Hz, 1F), -151.42 (dd, J = 151.9, 2.3 Hz, 1F). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.41, 197.39, 139.37, 132.27, 131.30, 129.87, 129.74, 127.94, 33.66, 33.56, 33.48.

(1a*α*,6*α*,10b*α*)-1,1-Difluoro-1,1a,6,10b-tetrahydrodibenzo[*a,e*]cyclopropa[c]-cyclohepten-6-ol:



^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (d, J = 7.6 Hz, 2H), 7.23 – 7.18 (m, 2H), 7.16 (d, J = 3.8 Hz, 4H), 6.49 (s, 1H), 6.08 (s, 1H), 3.49 (d, J = 13.5 Hz, 2H). ^{19}F NMR (471 MHz, DMSO-d_6) δ -118.16 (d, J = 143.4 Hz, 1F), -132.60 (d, J = 143.5 Hz, 1F). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.07, 151.05, 136.07, 132.66, 131.93, 131.25, 127.81, 73.11, 32.34, 32.26, 32.17.

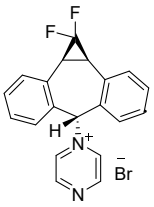
(1a*α*,6*β*,10b*α*)-6-Bromo-1,1-difluoro-1,1a,6,10b-tetrahydro-dibenzo[*a,e*]cyclopropa[c]cycloheptene:



^{1}H NMR (500 MHz, DMSO-d_6) δ 7.55 (d, J = 7.5 Hz, 2H), 7.27 – 7.15 (m, 6H), 6.51 (s, 1H), 3.50 (d, J = 13.5 Hz, 2H). ^{19}F NMR (471 MHz, DMSO-d_6) δ -122.91 (d, J = 143.5 Hz, 1F), -137.35 (d, J = 143.5 Hz, 1F). ^{13}C NMR (126 MHz, DMSO-d_6) δ 146.34, 146.32, 131.34, 127.93, 127.20,

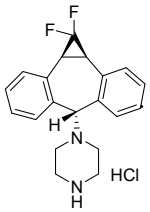
126.51, 123.08, 68.37, 27.60, 27.52, 27.43.

(1aα,6α,10bα)-1-(1,1-Difluoro-1,1a,6,10b-tetrahydrodibenzo[*a,e*]cyclopropa[c]-cycl-ohepten-6-yl)pyrazinium Bromide:



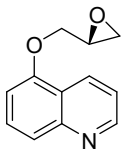
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.50 (dd, J = 3.1, 1.4 Hz, 2H), 8.83 – 8.79 (m, 2H), 7.77 (dd, J = 7.6, 1.0 Hz, 2H), 7.58 – 7.56 (m, 2H), 7.50 (ddd, J = 17.3, 11.8, 4.4 Hz, 4H), 7.26 (s, 1H), 3.22 (d, J = 12.6 Hz, 2H). ^{19}F NMR (471 MHz, DMSO-d_6) δ -123.53 (d, J = 144.5 Hz, 1F), -136.47 (d, J = 144.3 Hz, 1F). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.08, 145.68, 135.37, 135.06, 133.96, 132.79, 131.70, 129.49, 129.29, 76.77, 28.35, 28.26, 28.18.

(1aα,6α,10bα)-1-(1,1-Difluoro-1,1a,6,10b-tetrahydrodibenzo[*a,e*]cyclopropa[c]-cyclohepten-6-yl)-piperazine Hydrochloride:



^{1}H NMR (500 MHz, DMSO-d_6) δ 9.29 (s, 2H), 7.31 – 7.17 (m, 8H), 4.16 (s, 1H), 3.52 (d, J = 12.1 Hz, 2H), 3.11 (s, 4H), 2.47 (s, 4H). ^{19}F NMR (376 MHz, DMSO-d_6) δ -122.54 (d, J = 141.6 Hz, 1F), -136.99 (d, J = 141.5 Hz, 1F). ^{13}C NMR (101 MHz, DMSO-d_6) δ 141.84, 132.99, 130.15, 129.15, 128.61, 128.06, 75.73, 48.59, 43.25, 28.79, 28.70, 28.62.

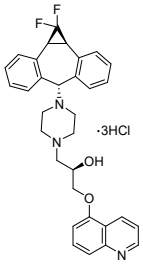
5-[(2*R*)-Oxiranylmethoxy]-quinoline:



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91 (dd, J = 2.8, 1.0 Hz, 1H), 8.62 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.62 – 7.56 (m, 1H), 7.41 – 7.36 (m, 1H), 6.86 (d, J = 7.6 Hz, 1H), 4.44 (dd, J = 11.0, 2.6 Hz, 1H), 4.14 – 4.06 (m, 1H), 3.52 – 3.44 (m, 1H), 3.00 – 2.95 (m, 1H), 2.84-2.83 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.89, 150.81, 149.09, 130.83, 129.23, 122.20, 120.83, 120.34, 105.34, 69.30, 50.07, 44.60.

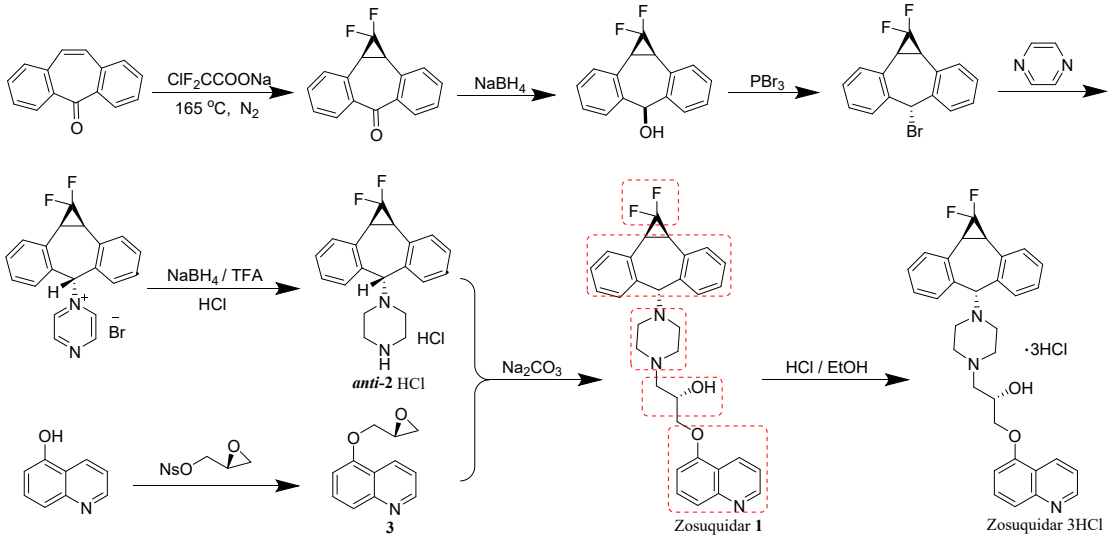
[6(*R*)-(1aα,6α,10bα)]-4-(1,1-Difluoro-1,1a,6,10b)tetrahydrodibenzo[*a,e*]cycloprop-a[c]cyclohepten-6-yl)-α-[(5-quinolinyloxy)methyl]-piperazineethanol, Trihydrochloride Salt

(LY335979·3HCl，Zos·3HCl):



^{1}H NMR (500 MHz, DMSO) δ 10.86 (s, 1H), 9.49 (s, 1H), 9.30 (dd, J = 5.3, 1.4 Hz, 1H), 8.15 – 7.91 (m, 4H), 7.45 – 7.17 (m, 11H), 4.70 – 4.54 (m, 2H), 4.36 – 4.16 (m, 4H), 3.74 – 3.40 (m, 7H), 2.73 (s, 2H), 2.58 (s, 1H). ^{19}F NMR (471 MHz, DMSO) δ -122.48 (d, J = 142.4 Hz, 1F), -136.94 (d, J = 142.3 Hz, 1F). ^{13}C NMR (126 MHz, DMSO) δ 172.43, 154.57, 145.30, 142.24, 138.48, 136.18, 133.22, 131.17, 129.69, 129.30, 128.47, 121.56, 121.47, 112.56, 109.18, 71.59, 64.26, 60.23, 56.47, 48.02, 29.22, 21.60, 21.25, 19.00, 14.55.

2. **Scheme and Figures**



Scheme S1. Synthetic route of Zosuquidar. The specific and detailed experimental operations were carried out according to the literature reported by Barnett [1] in 2004.

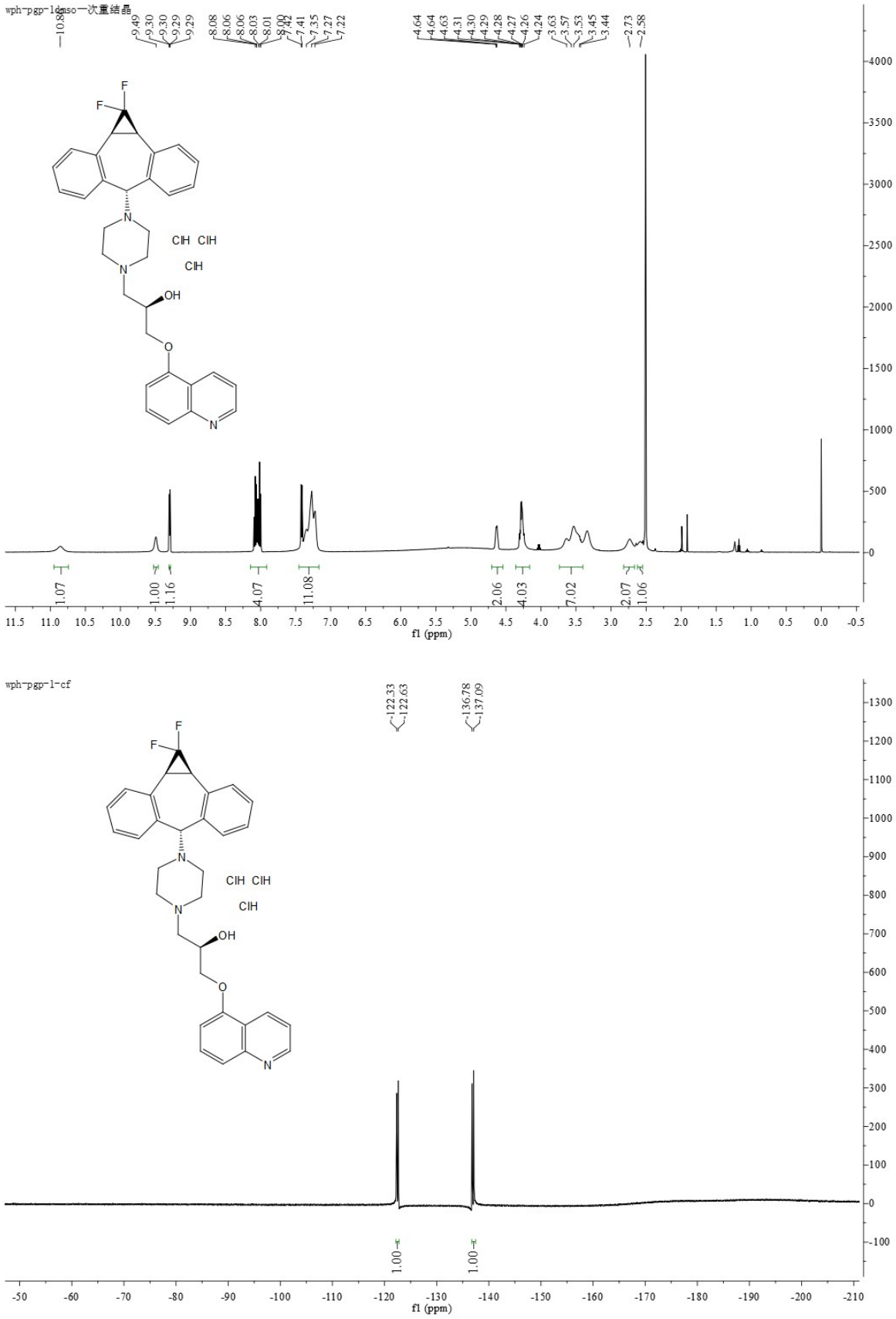


Figure S1. The ^{1}H and ^{19}F NMR spectra of Zos·3HCl in DMSO-d_6.

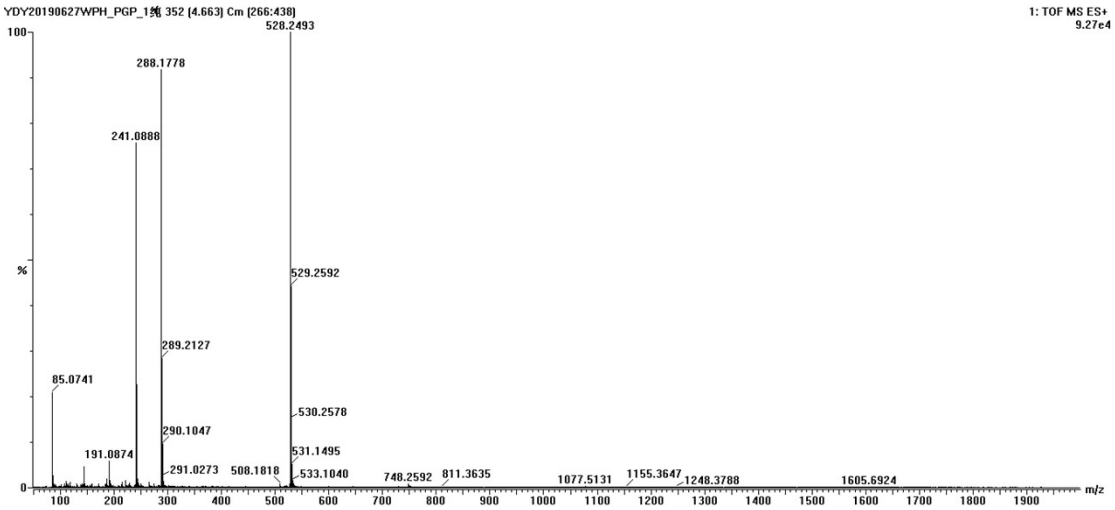


Figure S2. HRMS spectrum of Zos (calculated for $C_{32}H_{31}F_2N_3O_2$, $[M + H]^+$m/z 528.2493, found 528.2457).

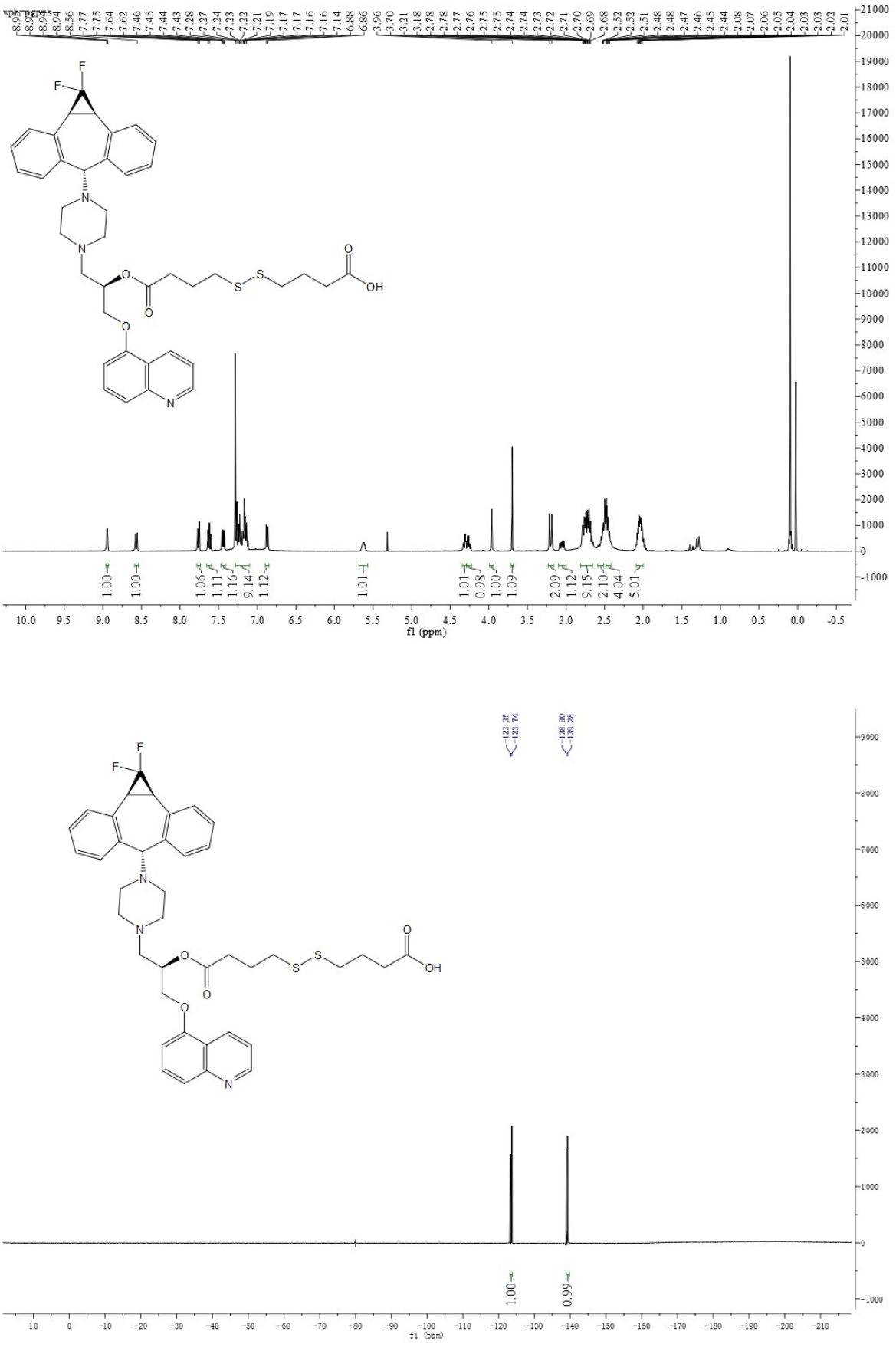


Figure S3. The ^{1}H and ^{19}F NMR spectra of Zos-ss-COOH in $CDCl_3$.

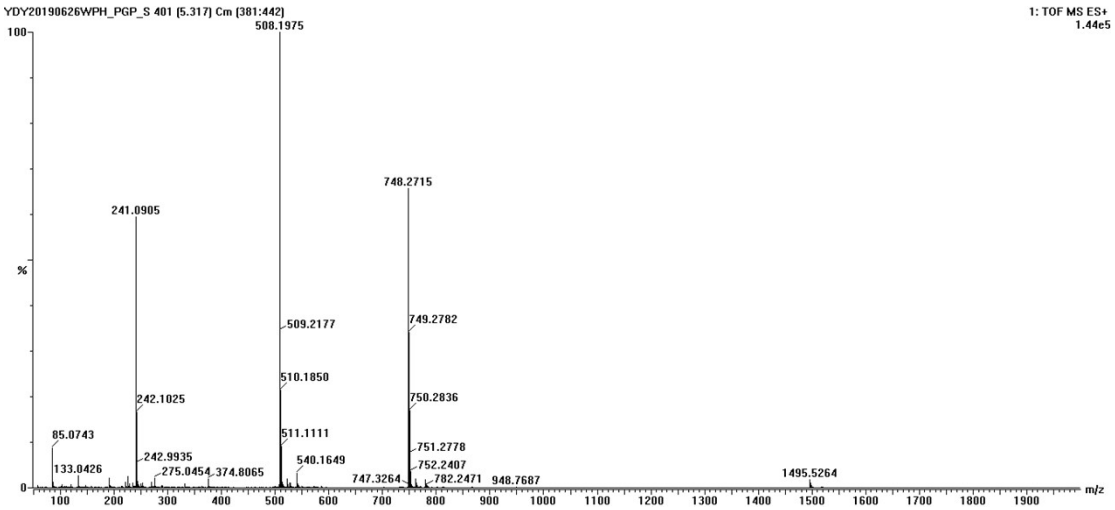
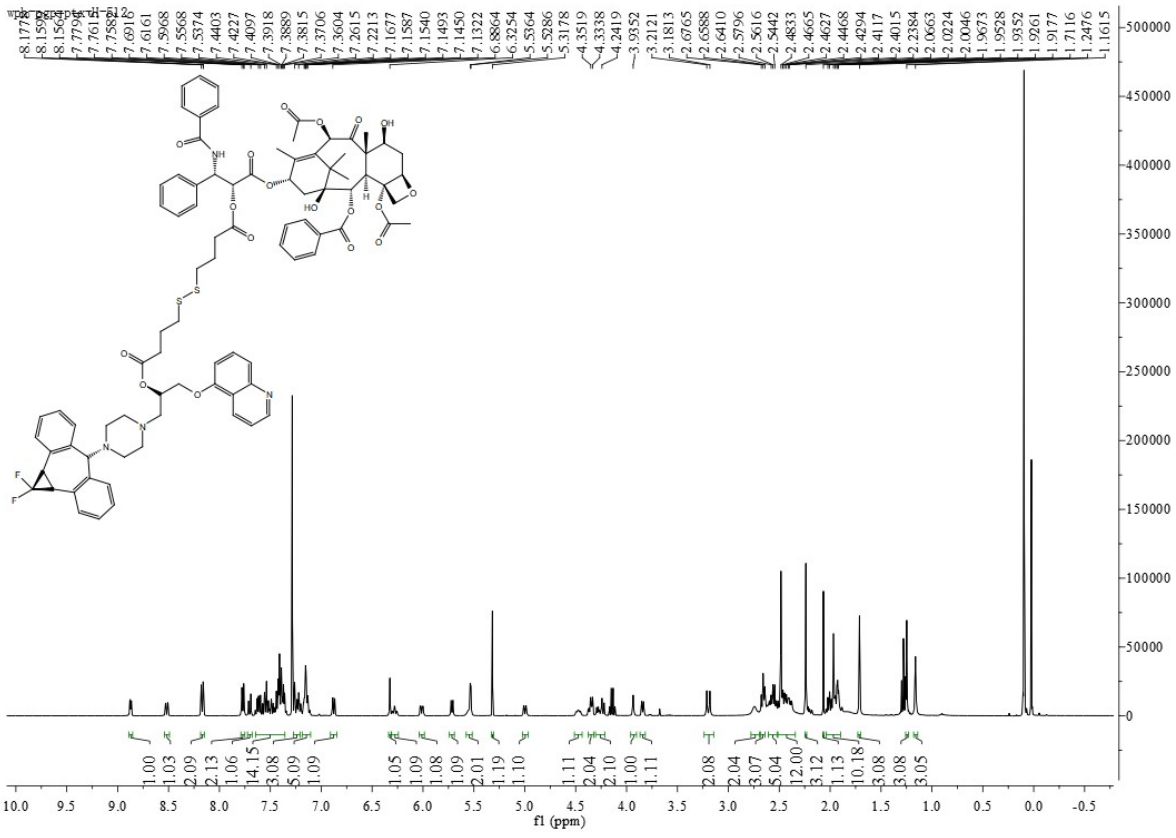


Figure S4. HRMS spectrum of Zos-ss-COOH (calculated for $C_{40}H_{43}F_2N_3O_5S_2$, $[M + H]^+$m/z 748.2715, found 748.2685).



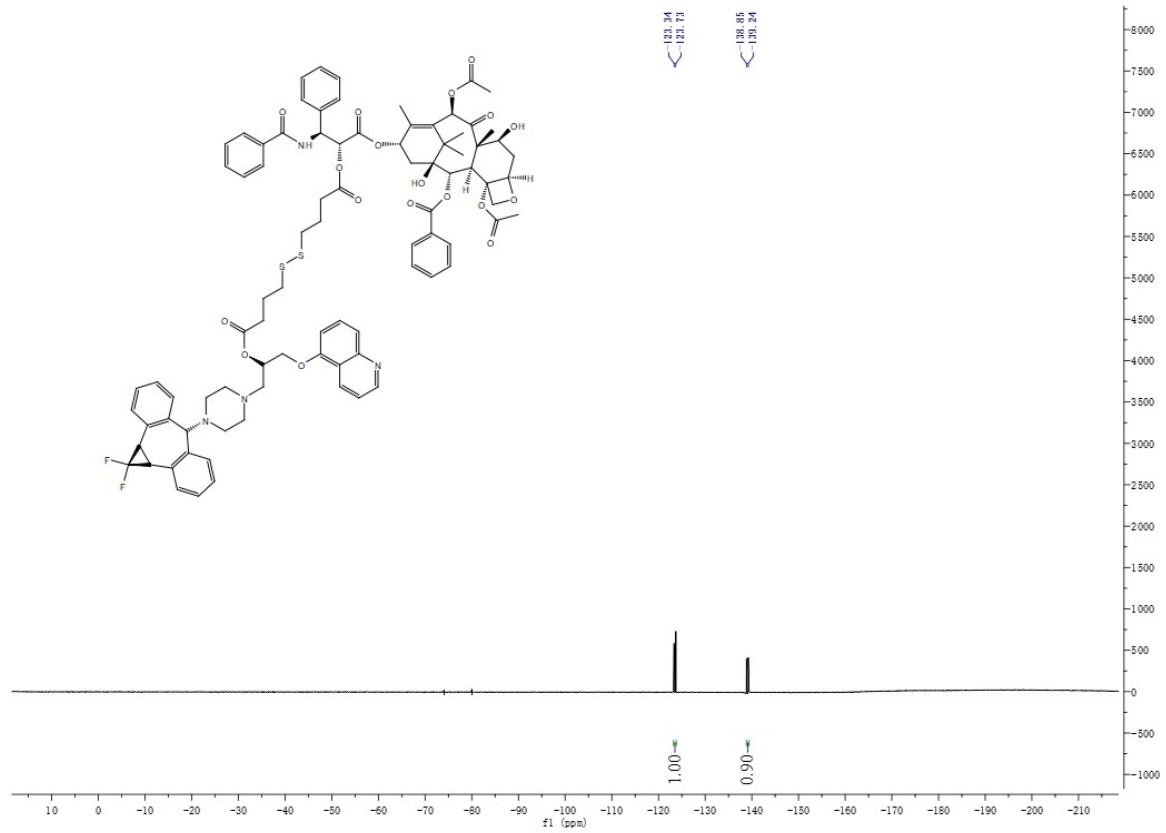


Figure S5. The ^{1}H and ^{19}F NMR spectra of PTX-ss-Zos in $CDCl_3$.

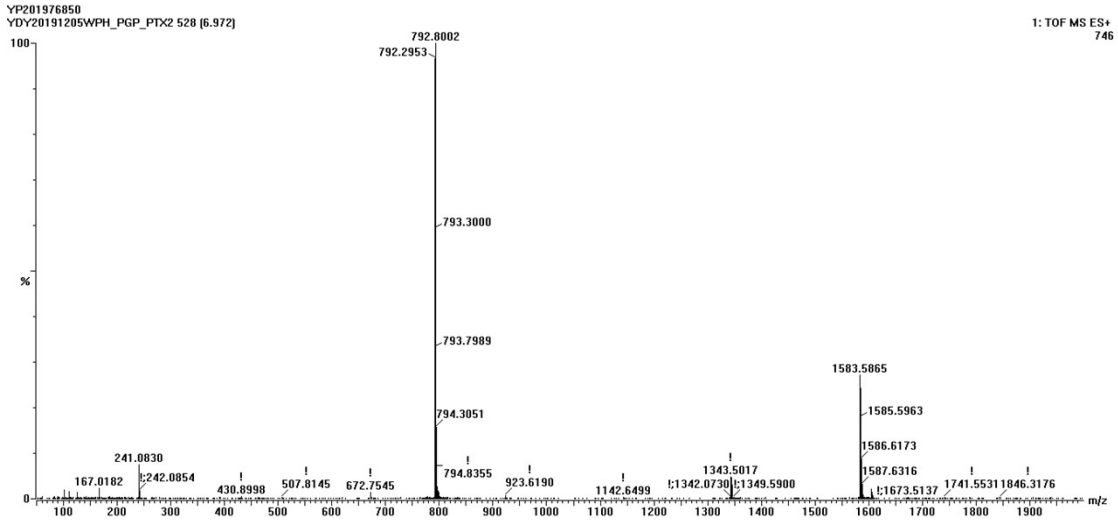


Figure S6. HRMS spectrum of PTX-ss-Zos (calculated for $C_{87}H_{92}F_2N_4O_{18}S_2$, $[M + H]^+$m/z 1583.5865, found 1583.5889).

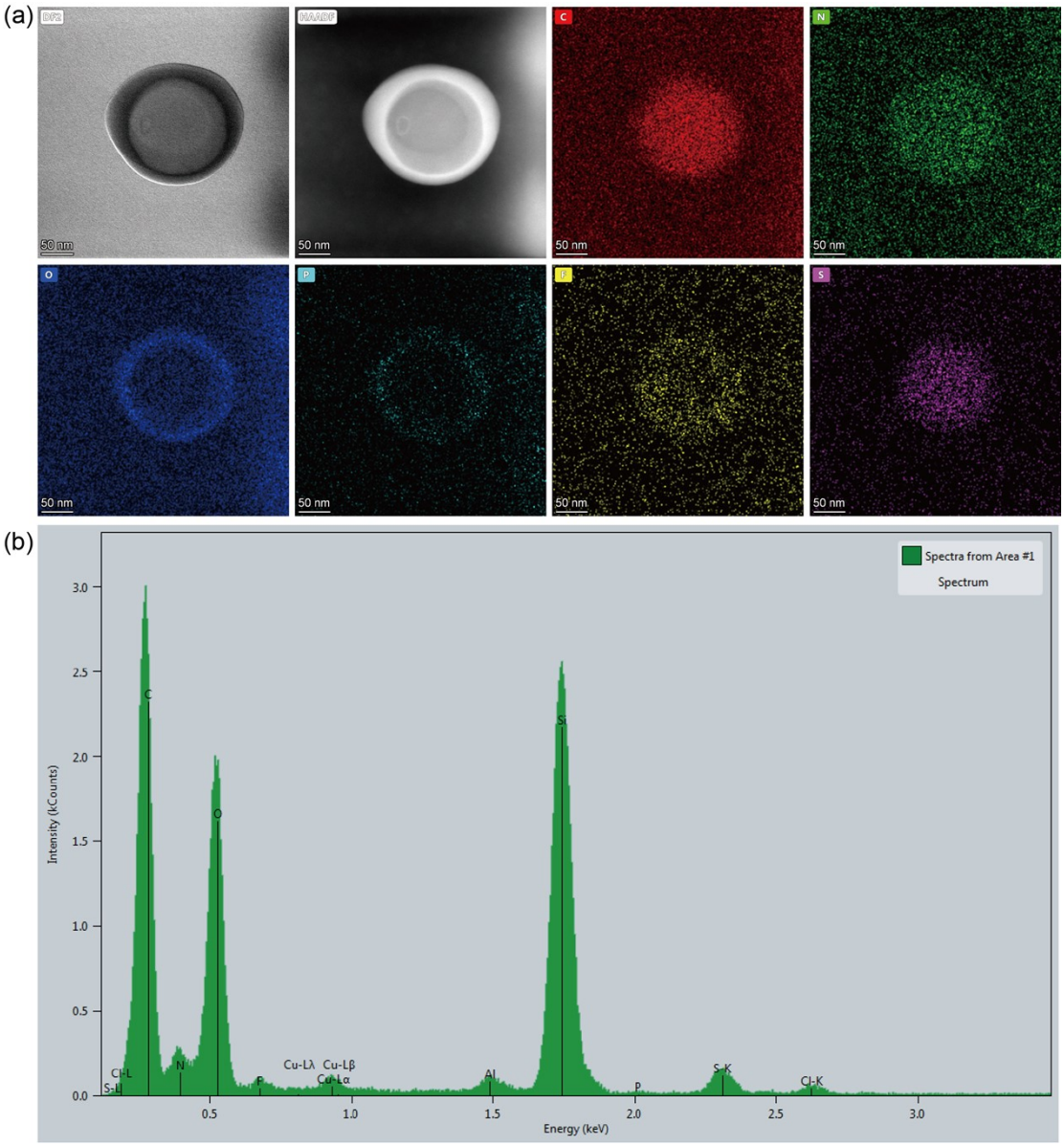


Figure S7. (a) STEM images of PTX-ss-Zos@DSPE-PEG$_{2k}$ NPs and related elements (C, N, O, P, F, S) in NPs. (b) Energy Dispersive Spectroscopy (EDS) of PTX-ss-Zos@DSPE-PEG2k NPs.

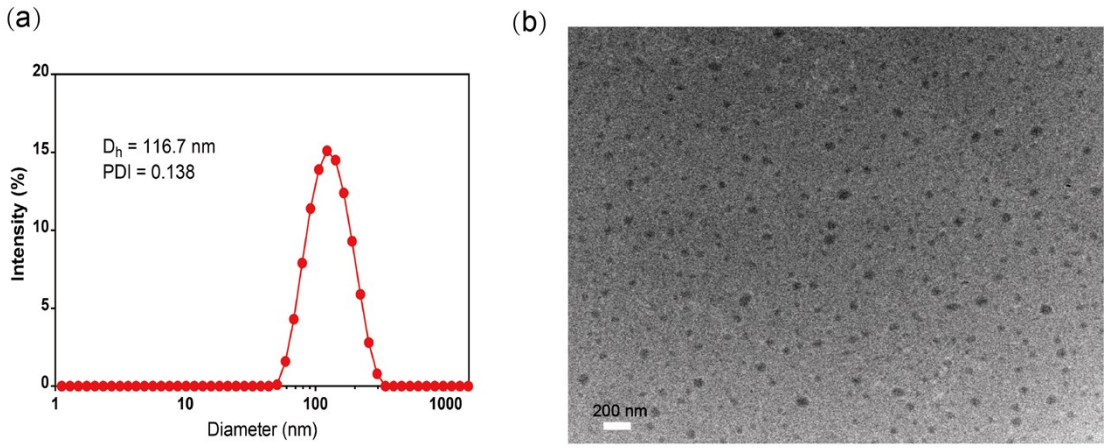


Figure S8. (a) DLS measurement and (b) TEM image of Cy5.5-loaded PTX-ss-Zos@DSPE-PEG_{2k} NPs.

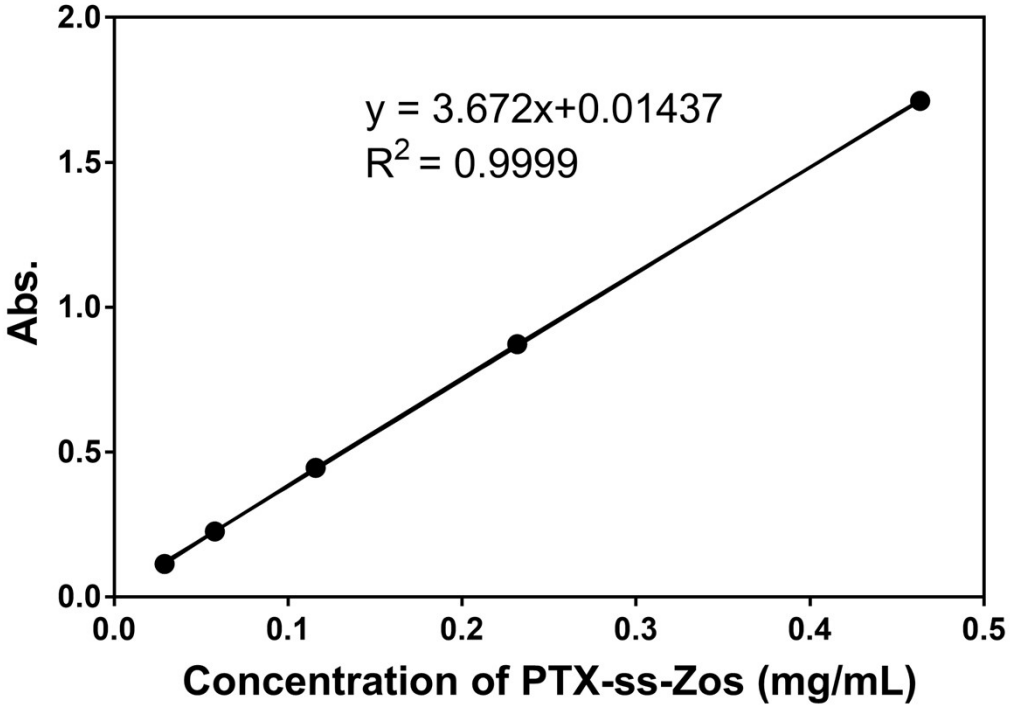


Figure S9. The standard concentration-absorbance curve of PTX-ss-Zos conjugate based on UV-Vis absorption at 303 nm.

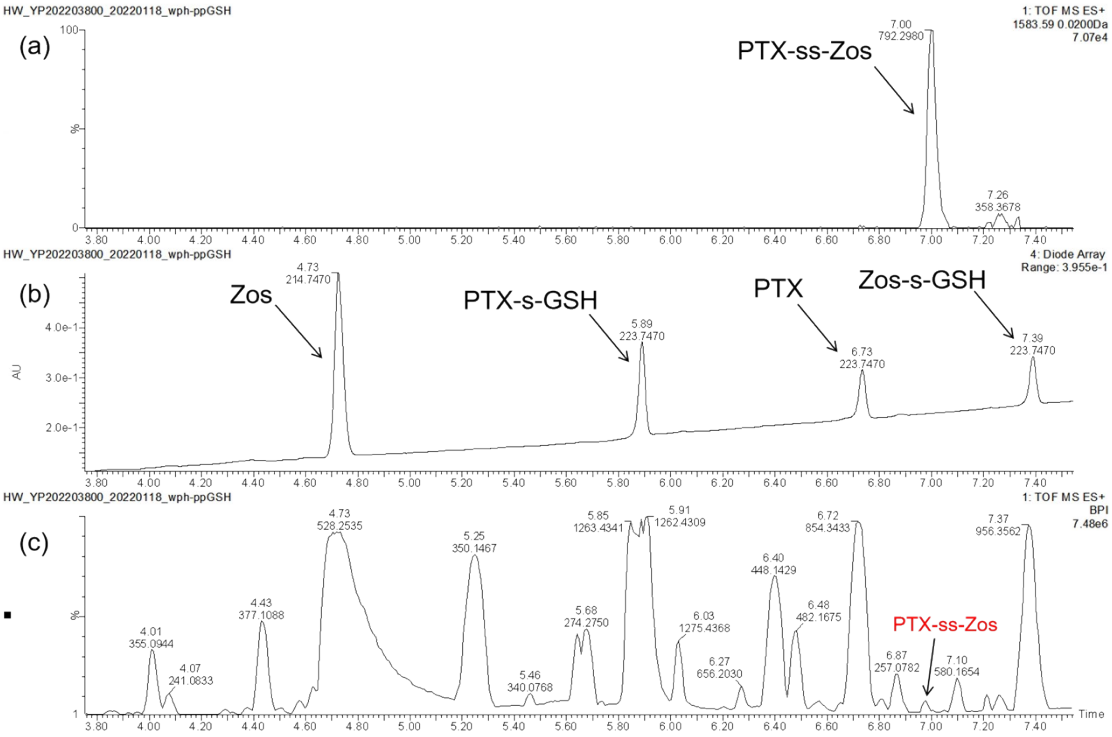


Figure S10. The LC-MS spectra of a solution of PTX-ss-Zos@DSPE-PEG_{2k} NPs treated with GSH for 72 h at 37 ℃ acquired by UPLC-Q-TOF-MS with positive mode. (a) Extract of ion

chromatogram (EIC) of PTX-ss-Zos conjugate. (b) LC profile spectrum. (c) Based peak intensity (BPI) chromatogram.

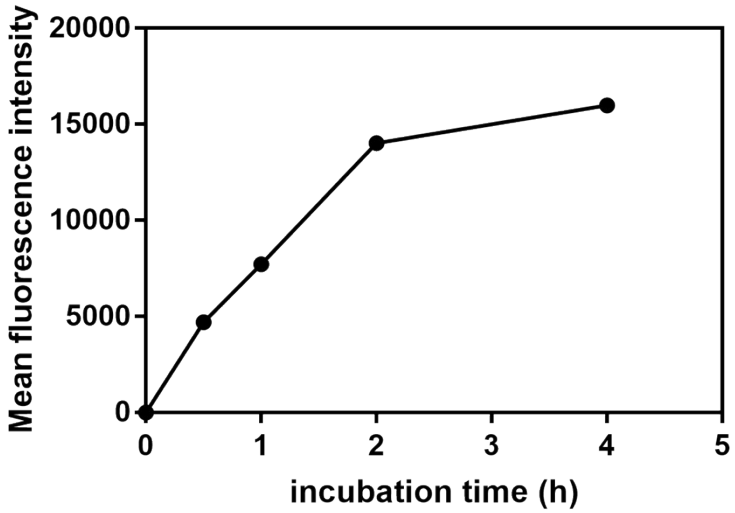


Figure S11. Time-dependent profiles of Cy5.5-loaded PTX-ss-Zos@DSPE-PEG$_{2k}$ NPs fluorescence intensity in the HeLa/PTX cells.

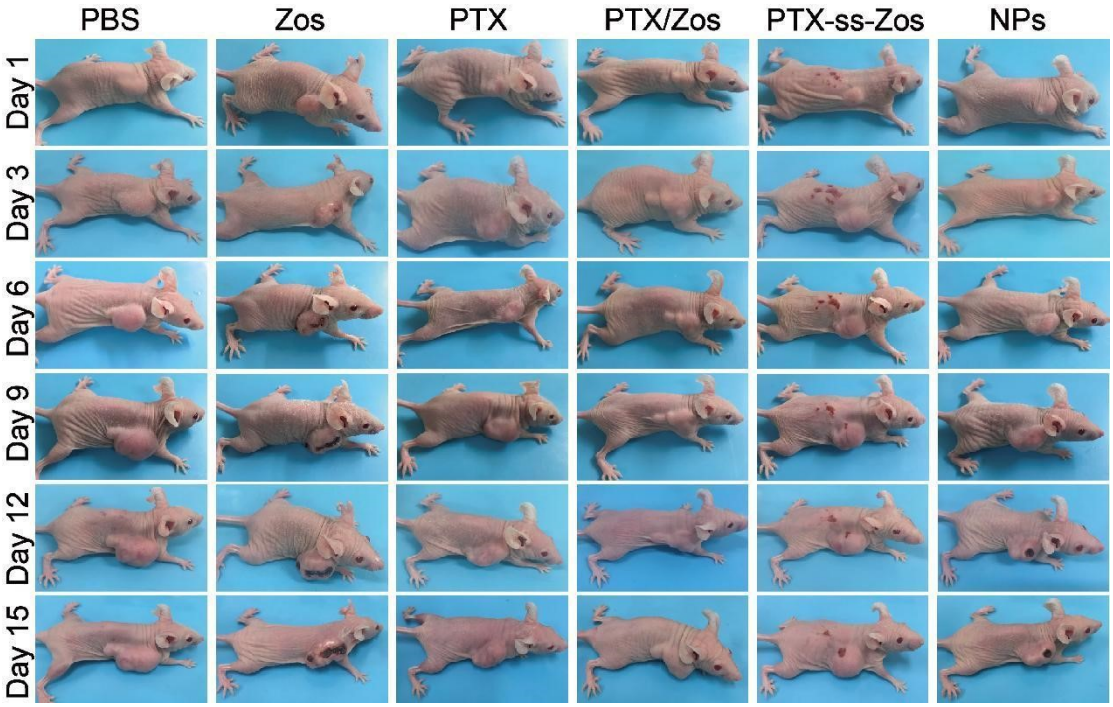


Figure S12. Images of the HeLa/PTX tumor-bearing nude mice during the predetermined treatments.

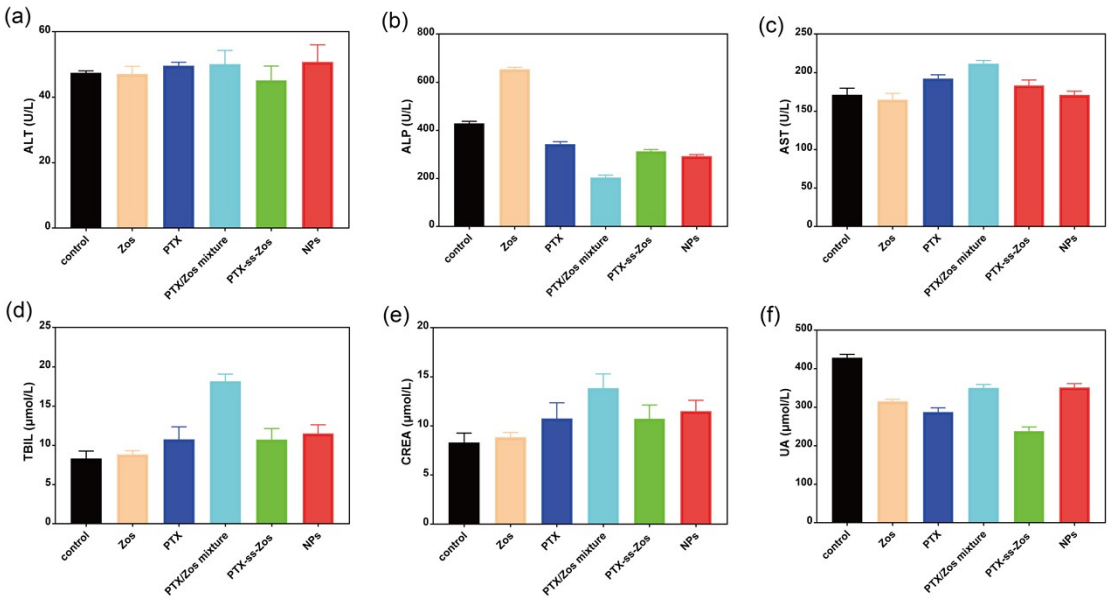


Figure S13. Serum biochemistry assays of liver function parameters included (a) alanine aminotransferase (ALT), (b) alkaline phosphatase (ALP), (c) aspartate aminotransferase (AST), and (d) serum total bilirubin (TBIL) and kidney function parameters included (e) creatinine (CREA) and (f) uric acid (UA).

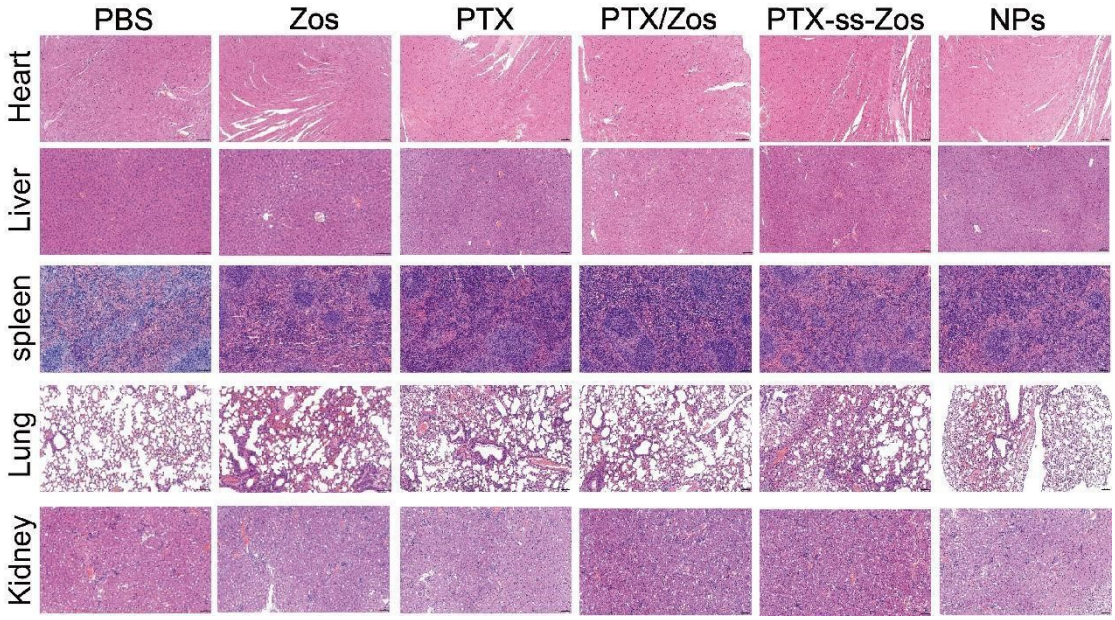


Figure S14. H&E staining of major organs (heart, liver, spleen, lung, kidney) of different drug formulations after 15 days of treatment. Scale bars: 100 μm.

References

[1] C. J. Barnett, B. Huff, M. E. Kobierski, M. Letourneau, T. M. Wilson, J. Org.

Chem. 2004, 69, 7653-7660.