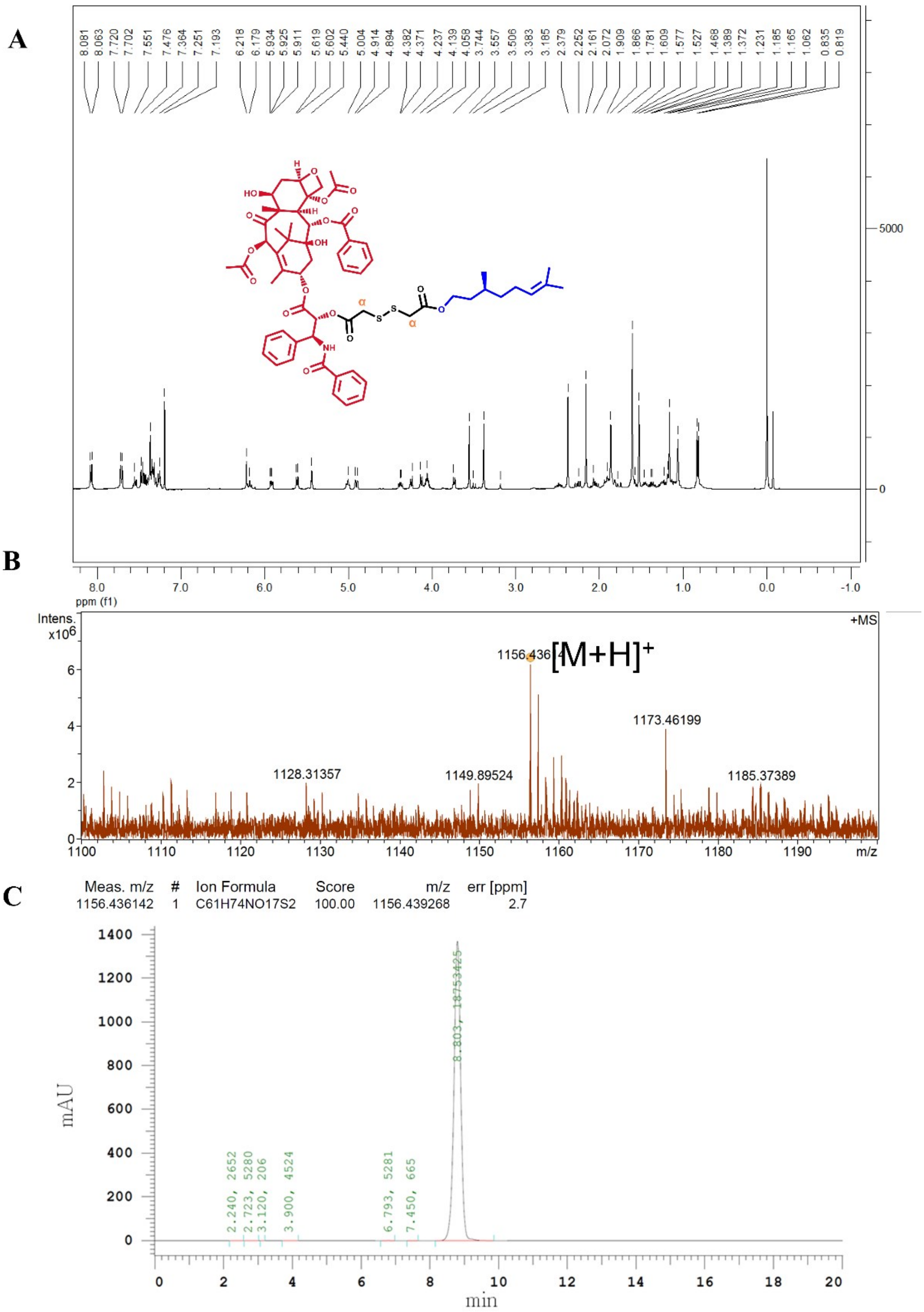
Supporting Information

The Length of Disulfide Bond-Containing Linkages Impacts the Oral Absorption and Antitumor Activity of Paclitaxel Prodrugs-Loaded Nanoemulsions

*Yanlin Gao, Shiyi Zuo, Lingxiao Li, Tian Liu, Fudan Dong, Xin Wang, Xuanbo Zhang, Zhonggui He, Yinglei Zhai *, Bingjun Sun *, Jin Sun **



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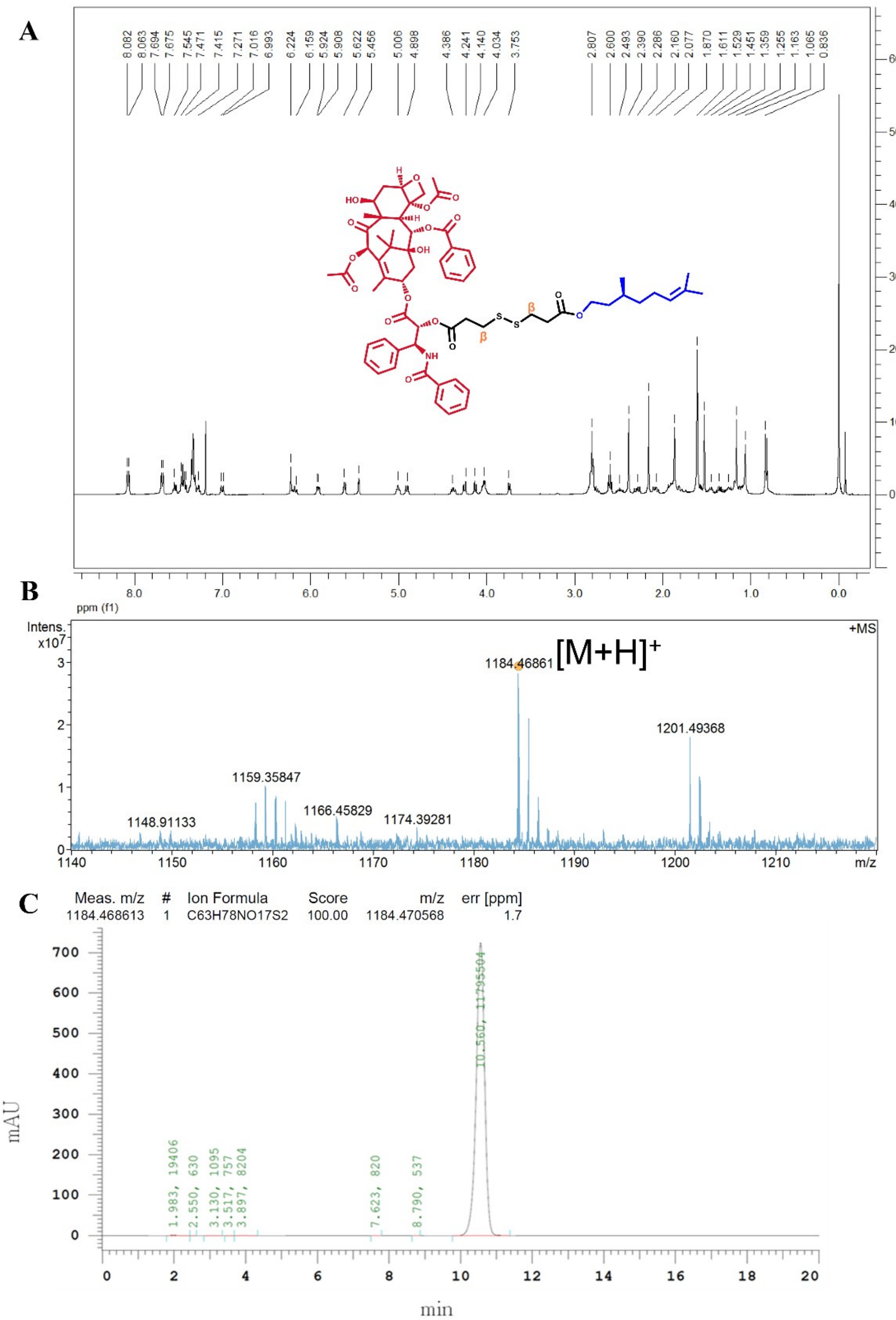
3	3.120	206	0.001	BB
4	3.900	4524	0.024	BB
5	6.793	5281	0.028	BB
6	7.450	665	0.004	BB
7	8.803	18753425	99.901	BB
		18772033	100.000	

Figure S1. Structure confirmation of α-PTX-SS-CIT. (A) ^{1}H NMR. (B) MS. (C) Purity.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.081 (d, 2H, Ar-H, *J*=7.3Hz), 7.720 (d, 2H, Ar-H, *J*=7.4Hz), 7.551 (t, 1H, Ar-H, *J*=7.4Hz), 7.476-7.251 (Ar-H, 10H), 6.218 (s, 1H, 10-H), 6.179 (t, 1H, *J*=8.6Hz, 13-H), 5.934 (dd, 1H, *J*=3.0Hz, *J*=6.4Hz, 3'-H), 5.619 (d, 1H, *J*=7.0Hz, 2-H), 5.440 (d, *J* = 3.5Hz, 1H, H-2', PTX), 5.004 (t, 1H, $(CH_3)_2C\underline{CH}$), 4.914 (d, 1H, *J*=8.0Hz, 5-H), 4.382 (dd, 1H, *J*=6.5Hz, *J*=4.2Hz, 7-H), 4.237 (d, 1H, *J*=8.4Hz, 20α-H), 4.139 (d, 1H, *J*=8.4Hz, 20β-H), 4.058 (m, 2H, $\underline{CH_2}OCO$), 3.744 (d, 1H, *J*=7.0Hz, 3-H), 3.557 (dd, 4H, $\underline{CH_2}SS\underline{CH_2}$), 3.383 (m, 1H, 6α-H), 2.379 (s, 3H, 4-$COCH_3$), 2.252 (m, 2H, 14-H), 2.161 (s, 3H, 10-$COCH_3$), 1.866 (m, 2H, $\underline{CH_2}CH_2OH$), 2.072 (t, 1H, 6β-H), 1.781 (s, 3H, 18-CH_3), 1.609 (s, 3H, 19-CH_3), 1.511 (s, 3H, $(\underline{CH_3})_2CCH$, cis-), 1.527 (s, 3H, $(\underline{CH_3})_2CCH$, trans-), 1.468 (m, 2H, $(CH_3)_2CCH\underline{CH_2}$), 1.389 (m, 2H, $\underline{CH_2}CH(CH_3)\ CH_2$), 1.185 (s, 3H, 17-$CH_3$), 1.165 (s, 3H, 16-$CH_3$), 1.062 (m, 1H, $CH_2\underline{CH}(CH_3)CH_2$), 0.835 (d, 3H ,$CH_2CH(\underline{CH_3})CH_2$).
MS (ESI) for α-PTX-SS-CIT $[M+H]^+$=1156.43614.



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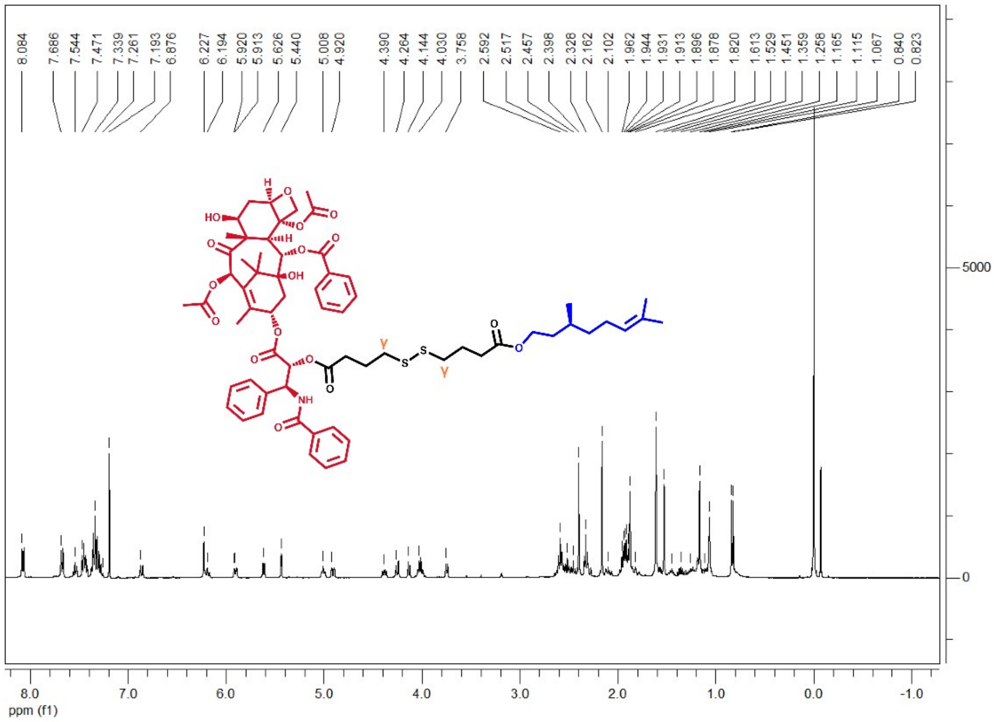
1	1. 983	19406	0. 164	BV
2	2. 550	630	0. 005	TBB
3	3. 130	1095	0. 009	BB
4	3. 517	757	0. 006	BB
5	3. 897	8204	0. 069	BB
6	7. 623	820	0. 007	BB
7	8. 790	537	0. 005	BB
8	10. 560	11795504	99. 734	BB
		11826953	100. 000	

Figure S2. Structure confirmation of β-PTX-SS-CIT. (A) ^{1}H NMR. (B) MS. (C) Purity.

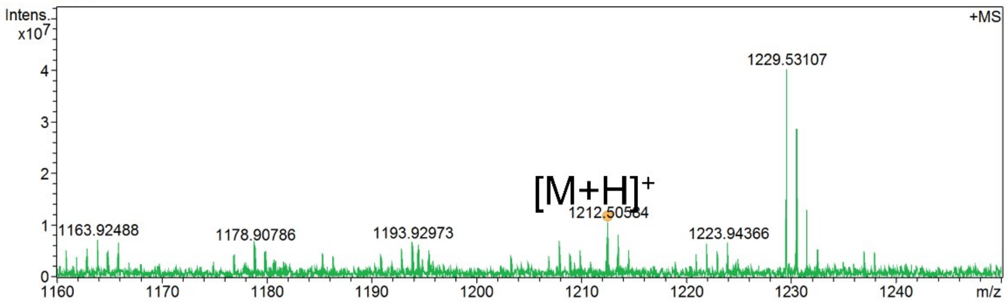
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.082 (d, 2H, Ar-H, J=7.3Hz), 7.694 (d, 2H, Ar-H, J=7.4Hz), 7.545 (t, 1H, Ar-H, J=7.4Hz), 7.471-7.271 (Ar-H, 10H), 7.016 (d, 1H, J=9.2Hz, -NH-), 6.224 (s, 1H, 10-H), 6.159 (t, 1H, J=8.6Hz, 13-H), 5.924 (dd, 1H, J=3.0Hz, J=6.4Hz, 3'-H), 5.622 (d, 1H, J=7.0Hz, 2-H), 5.456 (d, J = 3.5Hz, 1H, H-2', PTX), 5.006 (t, 1H, $(CH_3)_2CCH$), 4.898 (d, 1H, J=8.0Hz, 5-H), 4.386 (dd, 1H, J=6.5Hz, J=4.2Hz, 7-H), 4.241 (d, 1H, J=8.4Hz, 20α-H), 4.140 (d, 1H, J=8.4Hz, 20β-H), 4.034 (m, 2H, CH_2OCO), 3.753 (d, 1H, J=7.0Hz, 3-H), 2.807 (t, 4H, $CH_2CH_2SSCH_2CH_2$), 2.600 (t, 4H, $CH_2CH_2SSCH_2CH_2$), 2.493 (m, 1H, 6α-H), 2.390 (s, 3H, 4-$COCH_3$), 2.286 (m, 2H, 14-H), 2.160(s, 3H, 10-$COCH_3$), 2.077 (m, 2H, CH_2CH_2OH), 1.87 (t, 1H, 6β-H), 1.611 (s, 3H, 18-CH_3), 1.529 (s, 3H, 19-CH_3), 1.451 (m, 2H, $(CH_3)_2CCHCH_2$), 1.359 (m, 2H, $CH_2CH(CH_3)CH_2$), 1.163 (s, 3H, 17-CH_3), 1.065 (s, 3H, 16-CH_3), 1.255 (m, 1H, $CH_2CH(CH_3)CH_2$), 0.836 (d, 3H, $CH_2CH(CH_3)CH_2$).

MS (ESI) for β-PTX-SS-CIT $[M+H]^+$= 1184.46861

A

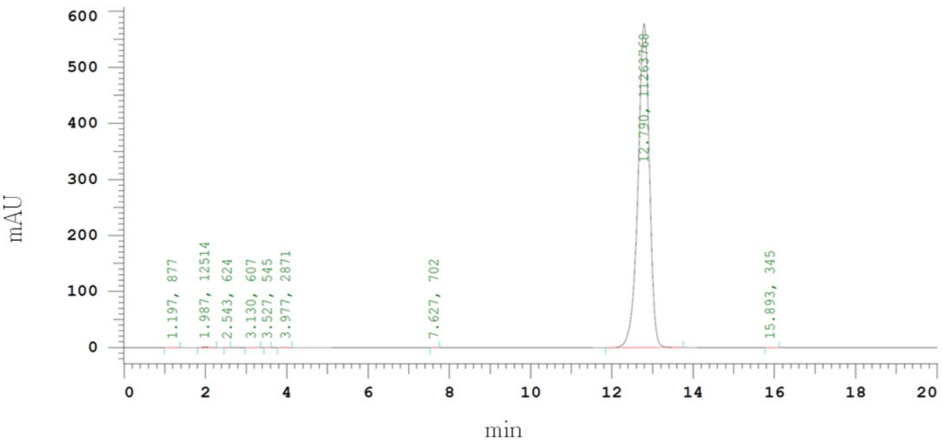


B



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]
1212.505838	1	C65H82NO17S2	100.00	1212.501869	-3.3

C



No.	RT	Area	%Area	BC
1	1.197	877	0.008	BB
2	1.987	12514	0.111	BB
3	2.543	624	0.006	BB
4	3.130	607	0.005	BB
5	3.527	545	0.005	BB
6	3.977	2871	0.025	BB
7	7.627	702	0.006	BB
8	12.790	11263768	99.831	BB
9	15.893	345	0.003	BB
		11282853	100.000	

Figure S3. Structure confirmation of γ-PTX-SS-CIT. (A) ^{1}H NMR. (B) MS. (C) Purity.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.084 (d, 2H, Ar-H, *J*=7.3Hz), 7.686 (d, 2H, Ar-H, *J*=7.4Hz), 7.544 (t, 1H, Ar-H, *J*=7.4Hz), 7.471-7.261 (Ar-H, 10H), 6.876 (d, 1H, *J*=9.2Hz, -NH-), 6.227 (s, 1H, 10-H), 6.194 (t, 1H, *J*=8.6Hz, 13-H), 5.920 (dd, 1H, *J*=3.0Hz, *J*=6.4Hz, 3'-H), 5.626 (d, 1H, *J*=7.0Hz, 2-H), 5.440 (d, *J*=3.5Hz, 1H, H-2', PTX), 5.008 (t, 1H, $(CH_3)_2C\underline{CH}$), 4.920 (d, 1H, *J*=8.0Hz, 5-H), 4.390 (dd, 1H, *J*=6.5Hz, *J*=4.2Hz, 7-H), 4.264 (d, 1H, *J*=8.4Hz, 20α-H), 4.144 (d, 1H, *J*=8.4Hz, 20β-H), 4.030 (m, 2H, $\underline{CH_2}OCO$), 3.758 (d, 1H, *J*=7.0Hz, 3-H), 2.592 (t, 4H, $CH_2CH_2\underline{CH_2}SS\underline{CH_2}CH_2CH_2$), 2.517 (t, 4H, $\underline{CH_2}CH_2CH_2SSCH_2CH_2\underline{CH_2}$), 2.457 (m, 1H, 6α-H), 2.398 (s, 3H, 4-$COCH_3$), 2.328 (m, 2H, 14-H), 2.162 (s, 3H, 10-$COCH_3$), 1.962 (t, 4H, $CH_2\underline{CH_2}CH_2SSCH_2\underline{CH_2}CH_2$), 1.944 (m, 2H, $\underline{CH_2}CH_2OH$), 2.102 (t, 1H, 6β-H), 1.878 (s, 3H, 18-CH_3), 1.820 (s, 3H, 19-CH_3), 1.613 (s, 3H, $(\underline{CH_3})_2CCH$, cis-), 1.529 (s, 3H, $(\underline{CH_3})_2CCH$, trans-), 1.451 (m, 2H, $(CH_3)_2CCH\underline{CH_2}$), 1.359 (m, 2H, $\underline{CH_2}CH(CH_3)CH_2$), 1.115 (s, 3H, 17-$CH_3$), 1.076 (s, 3H, 16-$CH_3$), 1.115 (m, 1H, $CH_2CH(CH_3)CH_2$), 0.840 (d, 3H, $CH_2CH(CH_3)CH_2$).

MS (ESI) for γ-PTX-SS-CIT $[M+H]^+$=1212.50584

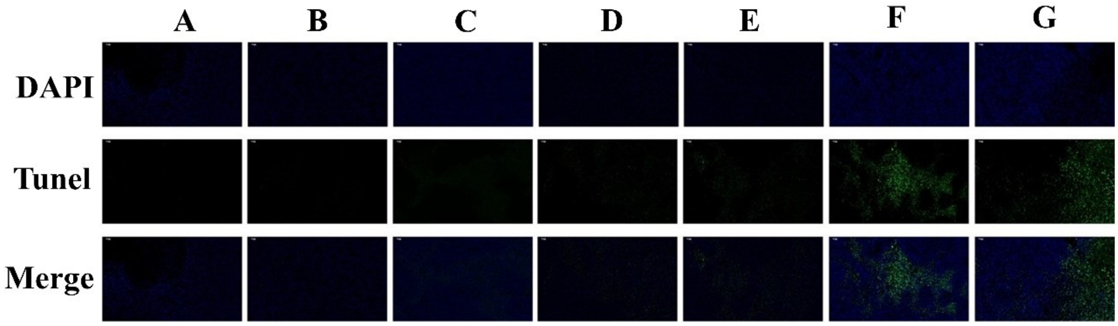


Figure S4. Apoptosis fluorescence micrographs of tumor cells. (A) Saline. (B) Taxol (p.o.) (30 mg kg^{-1}). (C) γ-PTX-SS-CIT NEs (30 mg kg^{-1}). (D) β-PTX-SS-CIT NEs (30 mg kg^{-1}). (E) α-PTX-SS-CIT NEs (30 mg kg^{-1}). (F) α-PTX-SS-CIT NEs (50 mg kg^{-1}). (G) Taxol (i.v., 10mg kg^{-1}).

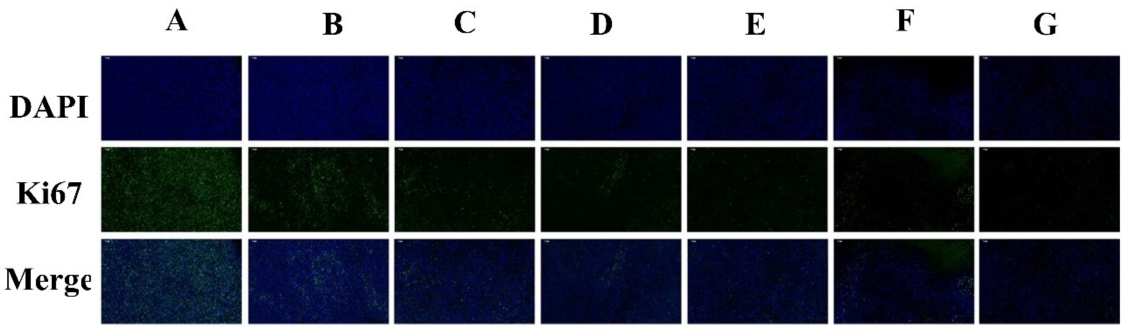


Figure S5. Proliferation fluorescence micrographs of tumor cells. (A) Saline. (B) Taxol (p.o.) (30 mg kg^{-1}). (C) γ-PTX-SS-CIT NEs (30 mg kg^{-1}). (D) β-PTX-SS-CIT NEs (30 mg kg^{-1}). (E) α-PTX-SS-CIT NEs (30 mg kg^{-1}). (F) α-PTX-SS-CIT NEs (50 mg kg^{-1}). (G) Taxol (i.v., 10mg kg^{-1}).

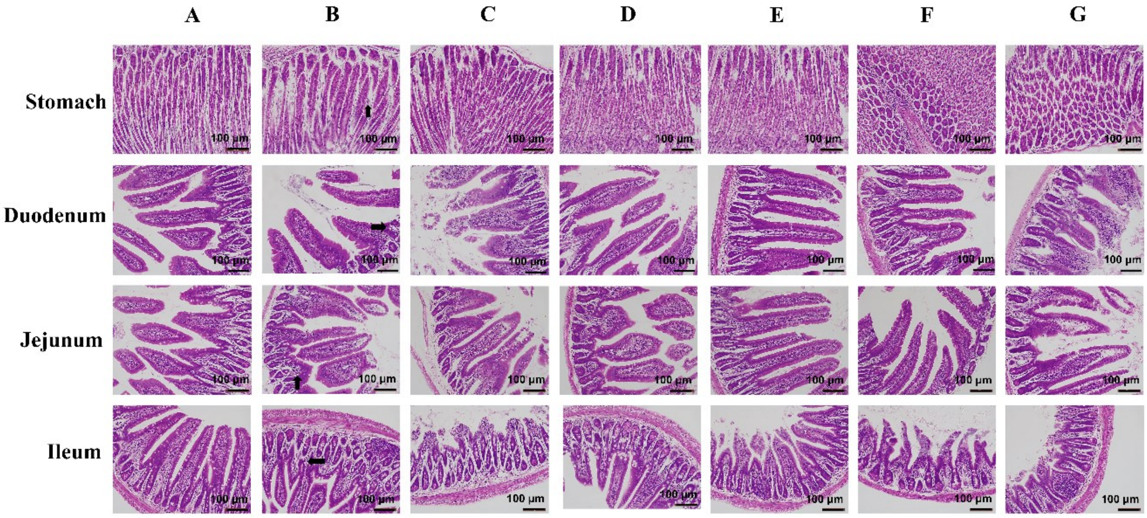


Figure S6. H&E staining of the major tissues of gastrointestinal tract after treatments. (A) Saline. (B) Taxol. (i.v., 10mg kg^{-1}). (C) α-PTX-SS-CIT NEs (50 mg kg^{-1}). (D) α-PTX-SS-CIT NEs (30 mg kg^{-1}). (E) β-PTX-SS-CIT NEs (30 mg kg^{-1}). (F) γ-PTX-SS-CIT NEs (30 mg kg^{-1}). (G) Taxol (p.o.) (30 mg kg^{-1}).

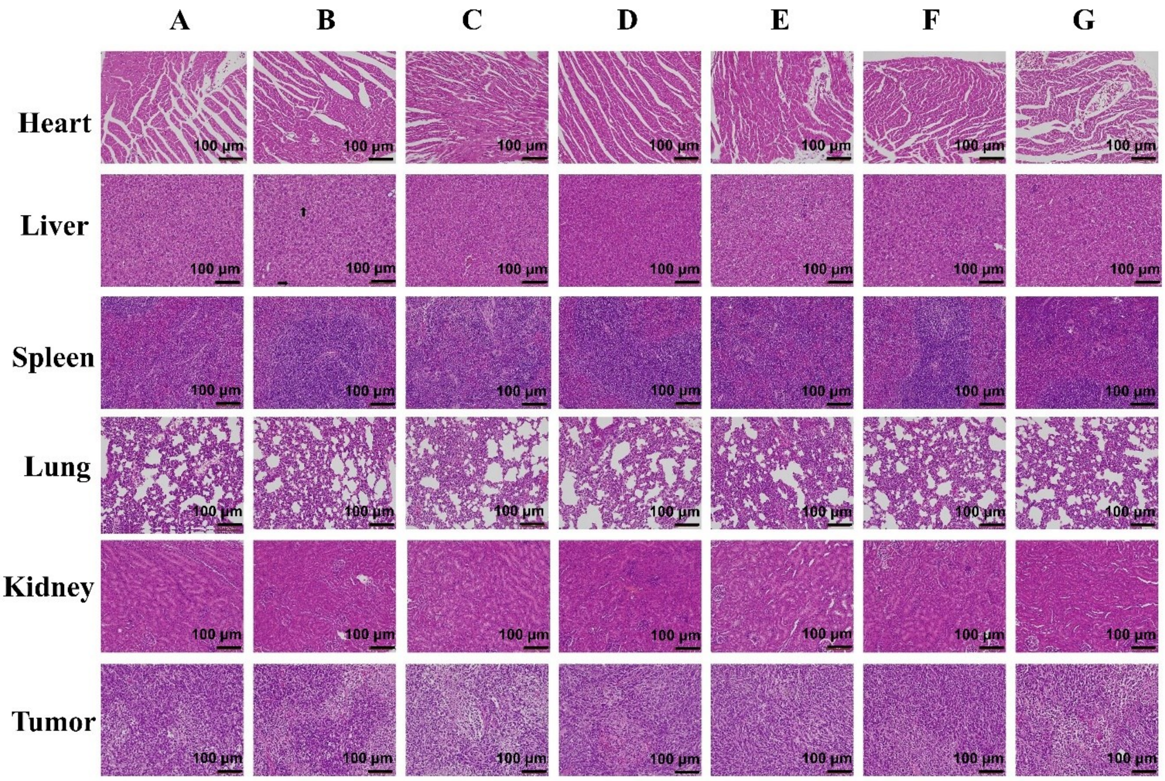


Figure S7. H&E staining of the major tissues of gastrointestinal tract after treatments. (A) Saline. (B) Taxol. (i.v., 10mg kg^{-1}). (C) α-PTX-SS-CIT NEs (50 mg kg^{-1}). (D) α-PTX-SS-CIT NEs (30 mg kg^{-1}). (E) β-PTX-SS-CIT NEs (30 mg kg^{-1}). (F) γ-PTX-SS-CIT NEs (30 mg kg^{-1}). (G) Taxol (p.o.) (30 mg kg^{-1}).

Table S1. IC_{50} values (nmol/L) of PTX NEs, Taxol, and prodrug NEs to 4T1 cell line.

Formulations	IC50 (nmol/L)
Taxol	27.9
PTX NEs	26.4
α-PTX-SS-CIT NEs	48.5
β-PTX-SS-CIT NEs	241.5
γ-PTX-SS-CIT NEs	119.0

Table S2. The screening process of the amount of emulsifier.

Mixed oil	Egg yolk lecithin (mg)	Sodium deoxycholate (mg)	Size (nm)	PDI
Soybean oil: MCT=1:4	120	40	145.7±0.5	0.234±0.02
Soybean oil: MCT=1:4	200	40	149.8±1.1	0.112±0.01
Soybean oil: MCT=1:4	240	40	164.4±1.2	0.195±0.12
Soybean oil: MCT=1:4	120	100	145.2±0.2	0.189±0.15
Soybean oil: MCT=1:4	120	130	169.4±0.9	0.170±0.10
Soybean oil: MCT=1:4	200	100	188.6±0.8	0.175±0.02

Table S3. The screening process of ratio of oil and water(w/w).

Ratio of oil and water (w/w)	Egg yolk lecithin (mg)	Sodium deoxycholate (mg)	Size (nm)	PDI
Oil: water=1:5	200	40	146.5±0.4	0.240±0.12
Oil: water=1:6	200	40	147.0±1.6	0.211±0.14
Oil: water=1:7	200	40	135.4±1.2	0.145±0.18
Oil: water=1:8	200	40	126.2±0.1	0.197±0.11
Oil: water=1:9	200	40	133.0±1.3	0.158±0.12
Oil: water=1:10	200	40	149.8±0.6	0.112±0.02