Electronic Supplementary Material (ESI) for Biomaterials Science.


# Supporting Information

## Formulating single thioether-bridged oleate prodrug into self-nanoemulsifying drug delivery system to facilitate oral absorption of docetaxel

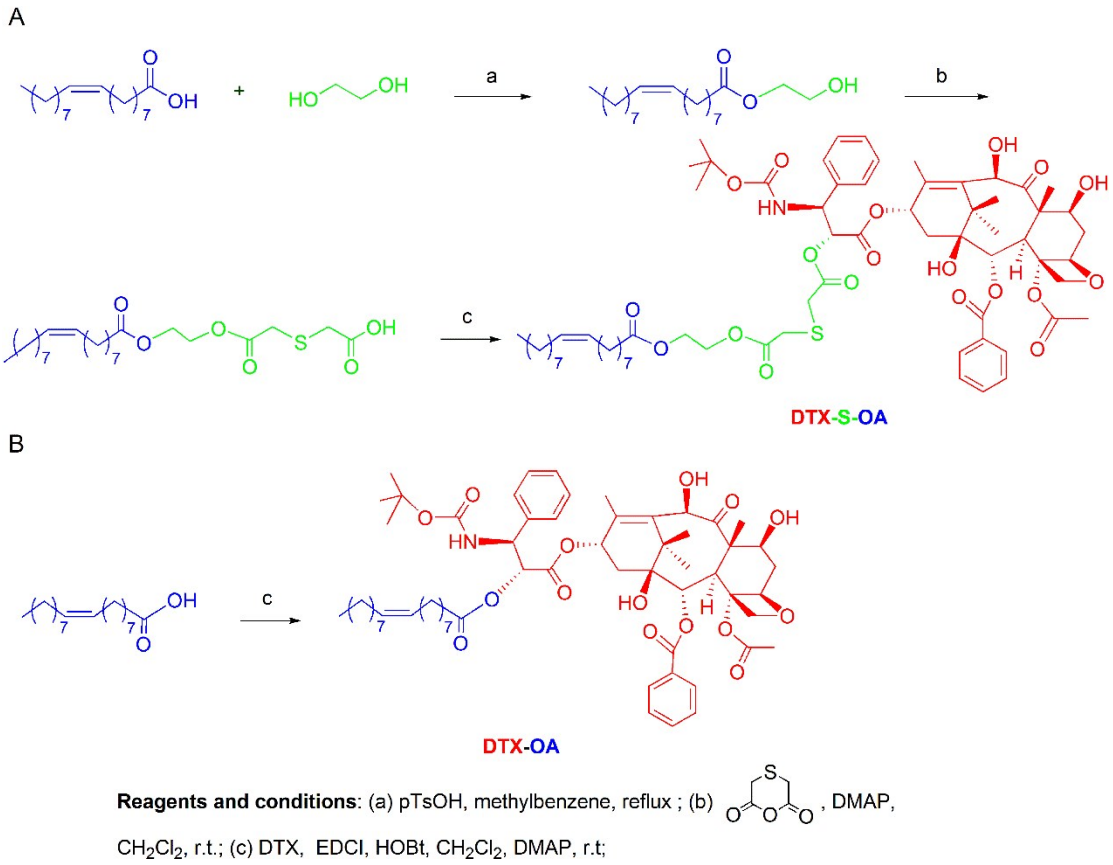


**Figure S1.** Synthetic routes of DTX-OA and DTX-S-OA conjugates.

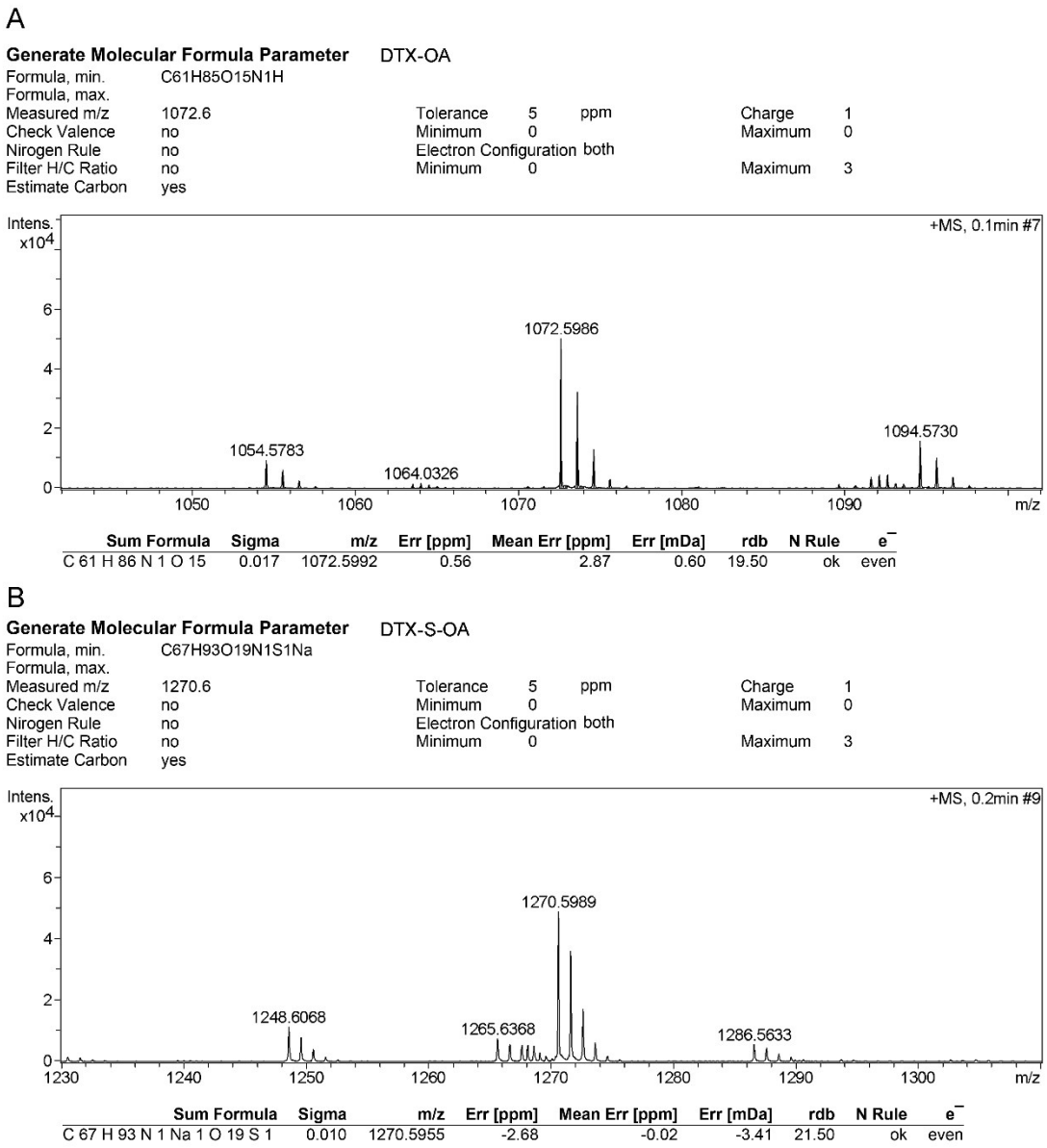


**Figure S2.** TOF-MS spectra of (A) DTX-OA and (B) DTX-S-OA.

The TOF-MS spectra of DTX-OA conjugate showed unimodal and sharp peak at m/z 1094.57 and 1072.60 corresponding to the $[M+Na]^+$ and $[M+H]^+$. The speculative molecular formula and molecular weight were $C_{61}H_{85}N_1O_{15}$ and 1071.60, agreed to the theoretical molecular formula and molecular weight.

The TOF-MS spectra of DTX-S-OA conjugate showed unimodal and sharp peak at m/z 1270.60 and 1248.61 corresponding to the $[M+Na]^+$ and $[M+H]^+$. The speculative molecular formula and molecular weight were $C_{67}H_{93}N_1O_{19}S_1$ and 1247.61, agreed to the theoretical molecular formula and molecular weight.

These outcomes supported the conclusion that DTX-OA and DTX-S-OA had

been successfully synthesized.

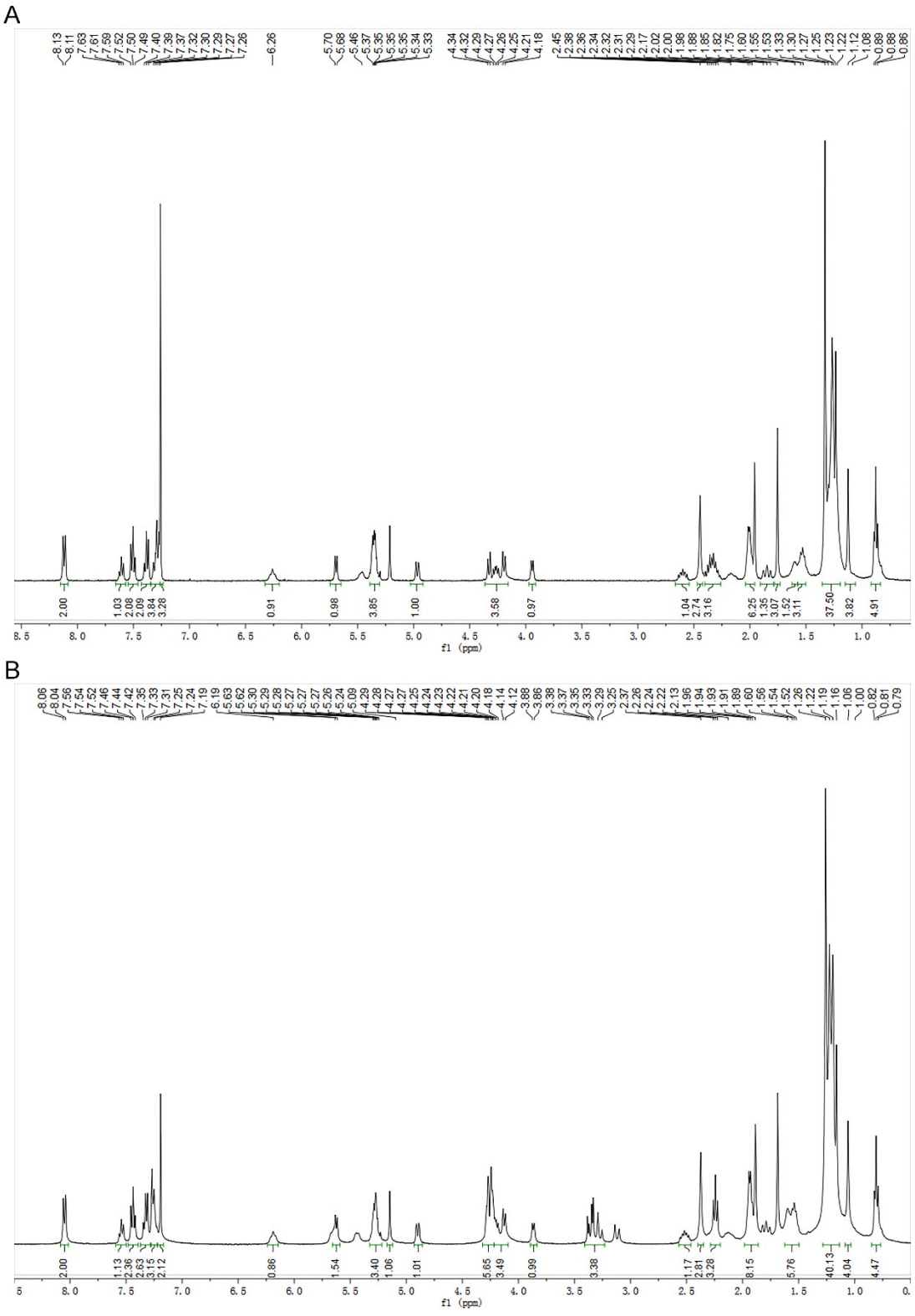


**Figure S3.** $^{1}H$ NMR spectra (600 MHz, $CDCl_3$) of (A) DTX-OA and (B) DTX-S-OA.

**$^{1}$H NMR results:**

The $^{1}$H NMR spectra of DTX-OA showed clearly characteristic peaks of OA: δ 0.88 (t, 3H, -$CH_3$), δ 1.39 – 1.16 (m, 10H, $CH_3$-$(CH_2)_5$-$CH_2$), δ 1.39 – 1.16 (m, 8H, $CH_3$-$(CH_2)_5$-$CH_2$-, -$CH_2$-$(CH_2)_3$-$CH_2$-), δ 1.66 (t, 2H, -$CH_2CH_2CO$-), δ 2.04 (s, 4H, -$CH_2CH$=$CHCH_2$-), δ 2.37 (t, 2H, -$CH_2CO$-), δ 5.36-5.26 (m, 2H, -CH=CH-) and DTX: 1.16 (s, 9H, $-H_{7'-9'}$ ), δ 3.94 (d, 1H, -$H_3$), δ 4.96 (d, 1H, -$H_5$), δ 7.51 – 7.30 (m, 8H, Ph-H), δ 8.12 (d, 2H, -Ar-$H_{25,29}$), demonstrating that the OA was successfully conjugated with DTX.

The $^{1}$H NMR spectra of DTX-S-OA showed distinctly characteristic peaks of OA: δ 0.88 (t, 3H, -$CH_3$), δ 1.38 – 1.19 (m, 10H, $CH_3$-$(CH_2)_5$-$CH_2$), δ 1.38 – 1.16 (m, 8H, $CH_3$-$(CH_2)_5$-$CH_2$-, -$CH_2$-$(CH_2)_3$-$CH_2$-), δ 1.66 (t, 2H, -$CH_2CH_2CO$-), δ 2.02 (s, 4H, -$CH_2CH$=$CHCH_2$-), δ 2.35 (t, 2H, -$CH_2CO$-), δ 5.36-5.26 (m, 2H, -CH=CH-) ,DTX: 1.16 (s, 9H, $-H_{7'-9'}$ ), δ 3.94 (d, 1H, -$H_3$), δ 4.96 (d, 1H, -$H_5$), δ 7.51 – 7.30 (m, 8H, Ph-H), δ 8.12 (d, 2H, -Ar-$H_{25,29}$) and the linkage: δ 3.61 – 3.49 (m,4H,-$CH_2SCH_2$- ), δ 4.37 – 4.16 (m, 4H, -$OCH_2CH_2O$-), demonstrating that DTX-S-OA was successfully synthesized.

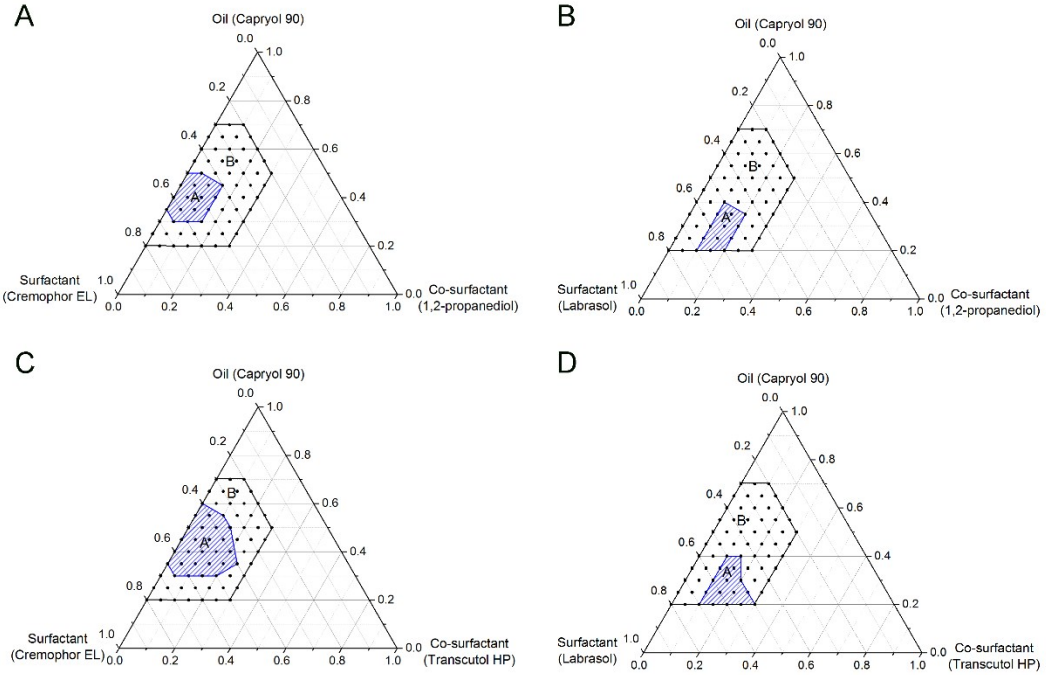

**Figure S4.** Ternary phase diagram of SNEDDS based on (A) Capryol 90/Cremophor EL/1,2-propanediol; (B) Capryol 90/Labrasol/1,2-propanediol; (C) Capryol 90/Cremophor EL/Transcutol HP; (D) Capryol 90/Labrasol/Transcutol HP.

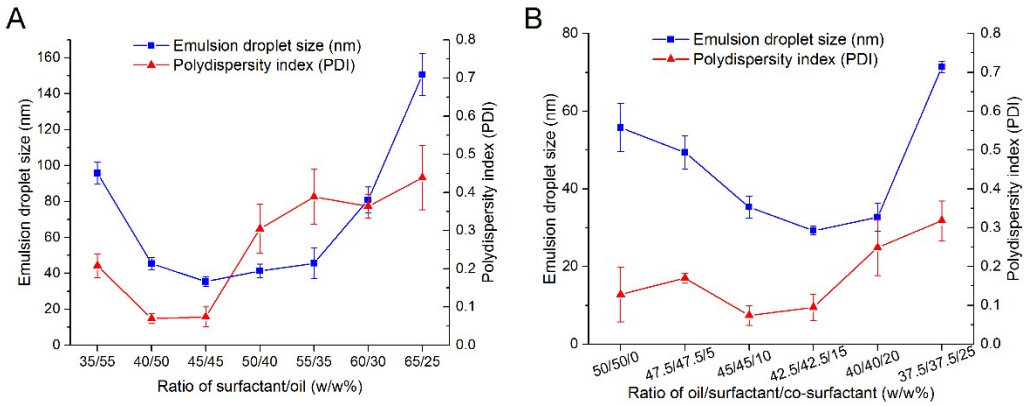


**Figure S5.** Effect of surfactant/oil ratio (A) and oil/surfactant/co-surfactant ratio (B) on

the mean droplet size and PDI of SNEDDS. These emulsions were composed of a 0.1 mL mixture of surfactant/oil and 10 mL of water (n = 3).

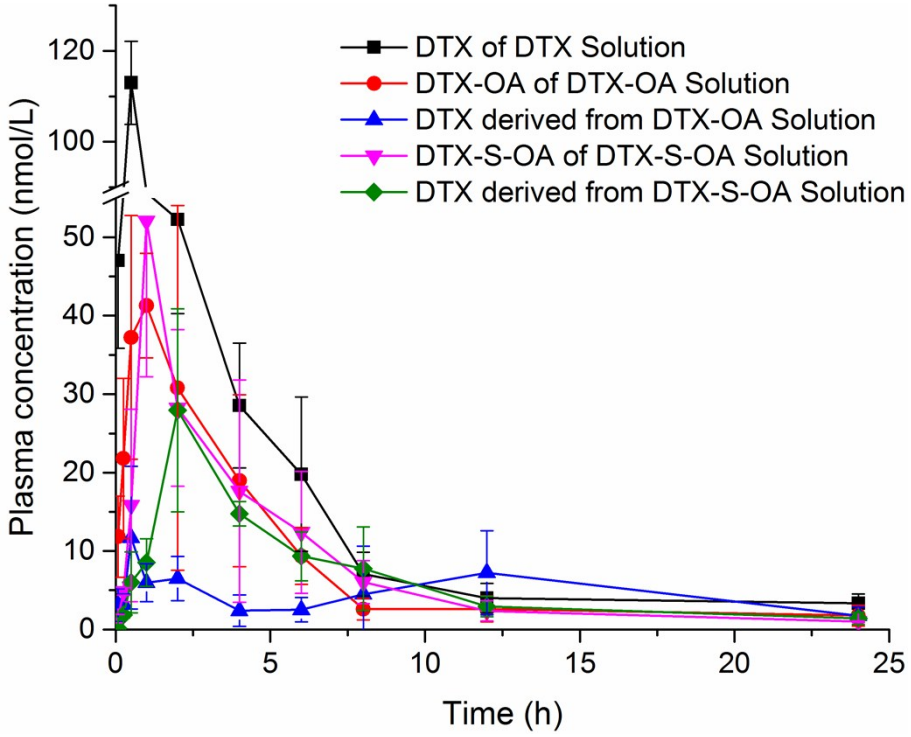


**Figure S6.** *In vivo* plasma concentration-time profiles of DTX and prodrugs after oral administration of DTX solution, DTX-OA solution and DTX-S-OA solution (n = 5).

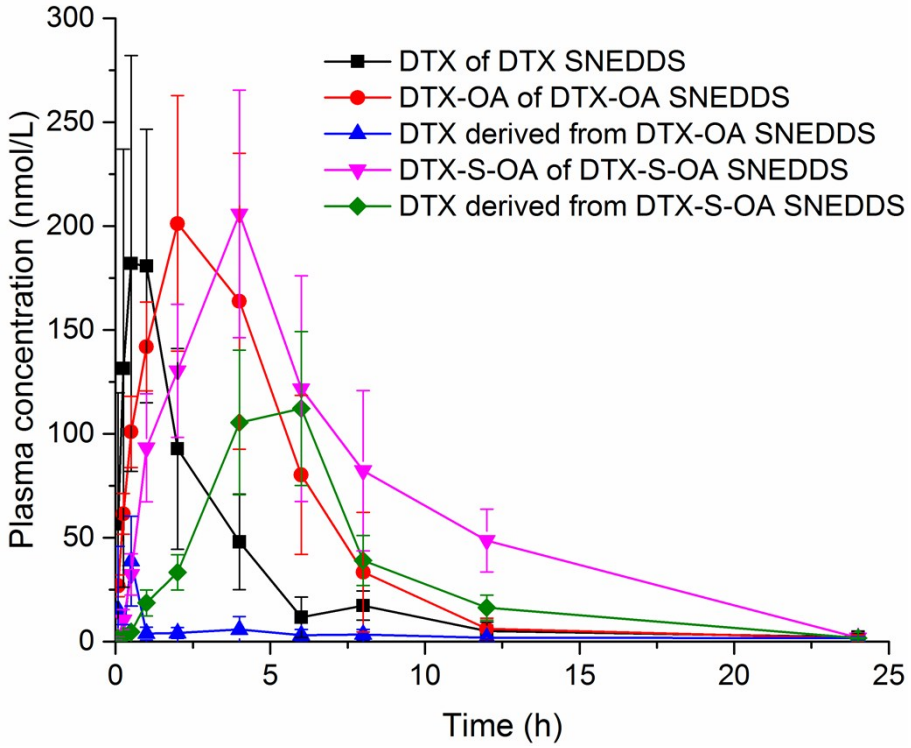


**Figure S7.** *In vivo* plasma concentration-time profiles of DTX and prodrugs after oral administration of DTX SNEDDS, DTX-OA SNEDDS and DTX-S-OA SNEDDS (n = 5).

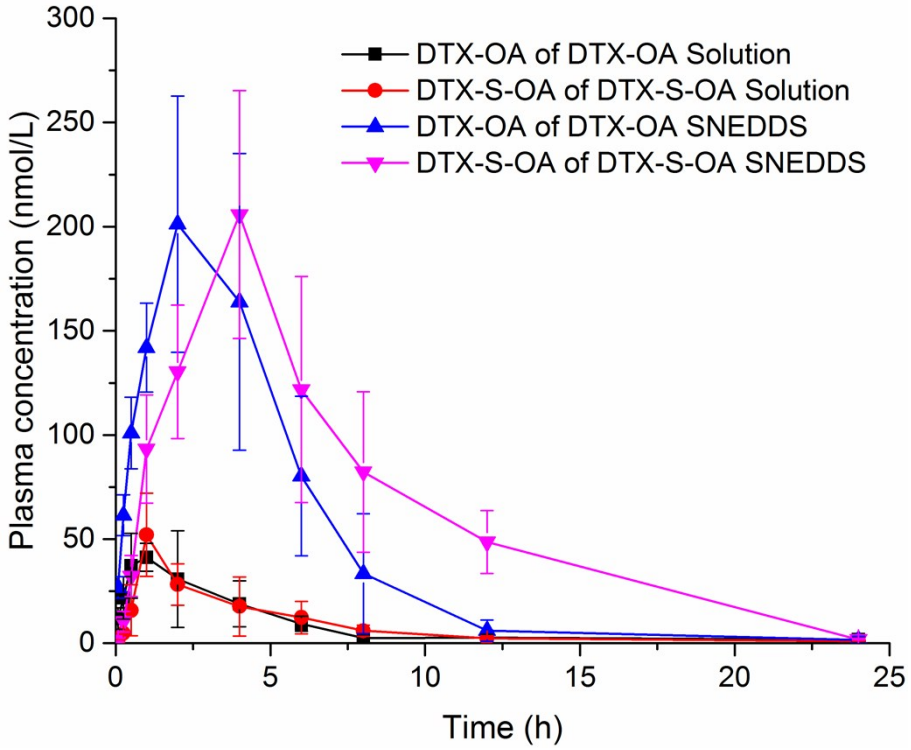


**Figure S8.** *In vivo* plasma concentration-time profiles of prodrugs after oral administration of DTX-OA solution, DTX-S-OA solution, DTX-OA SNEDDS and DTX-S-OA SNEDDS (n = 5).

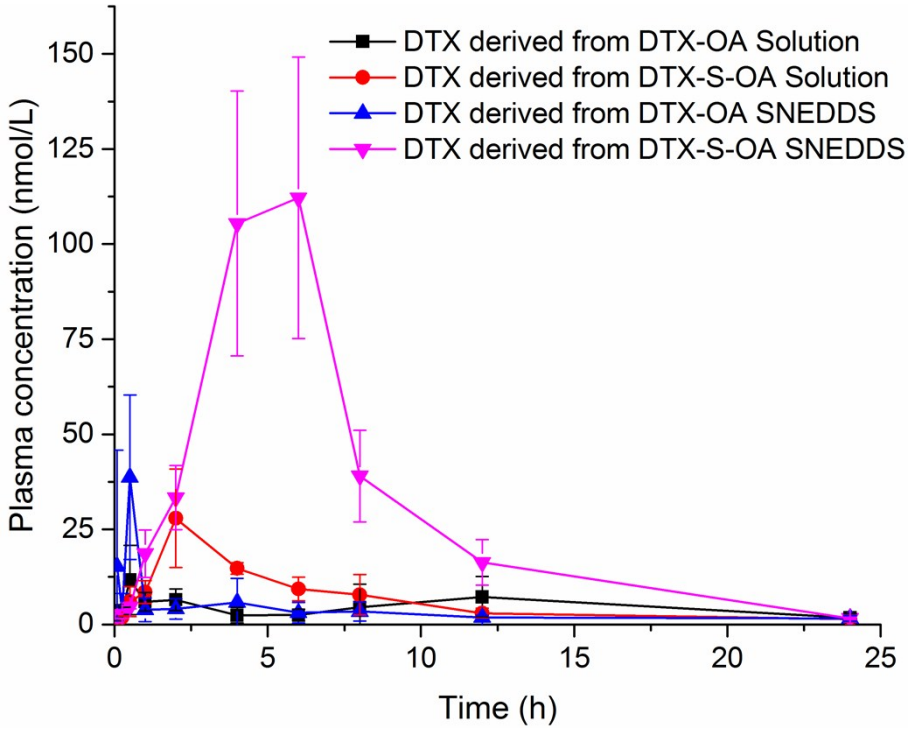


**Figure S9.** *In vivo* plasma concentration-time profiles of DTX derived from DTX-OA solution, DTX-S-OA solution, DTX-OA SNEDDS and DTX-S-OA SNEDDS after oral administration (n = 5).

***In vitro* fluorescer release assay**

The dialysis method was used to investigate in vitro release of DiR and coumarin-6 from SNEDDS. The experiment was performed in accordance with conditions completely consistent with the *in vitro* drug release study. SIF (phosphate buffer, pH 6.8, enzyme-free) were utilized as release media, containing 30% ethanol to achieve sink conditions. The DiR- and coumarin-6-SNEDDS was dispersed in 1 mL of release medium and then filled in the dialysis bags and tightly sealed. The dialysis bags were placed in conical flasks with 30 mL of release media with horizontal shaking at 37 °C. At predetermined time intervals, samples (1.0 mL) of release media were withdrawn and replaced with the same volume of media. The drug amount in these samples was measured using the Varioskan Flash multimode microreader (Thermo Scientific, USA).

As in Figure S9, coumarin-6-SNEDDS and DiR-SNEDDS exhibited sustained drug release behavior in SIF due to the high lipophilicity of the two fluorescent agents. Furthermore, there were no significant differences among the groups of coumarin-6-SNEDDS, DiR-SNEDDS and DTX-S-OA SNEDDS, indicating that the drug release behavior of the fluorescent agents in simulated intestinal fluid was similar to that of DTX-S-OA SNEDDS. Therefore, the biodistribution of DiR-SNEDDS or coumarin-6-SNEDDS can reflect the behavior of DTX-S-OA SNEDDS to some extent.

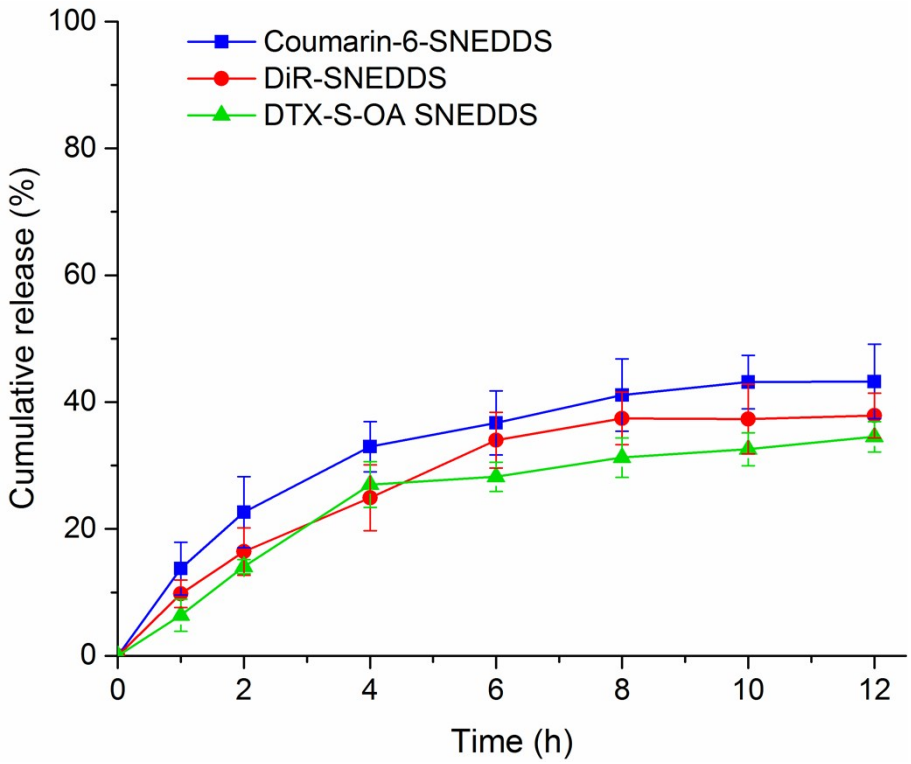


**Figure S10.** *In vitro* drug release of coumarin-6-SNEDDS, DiR-SNEDDS and DTX-S-OA SNEDDS in SIF. (n = 3)

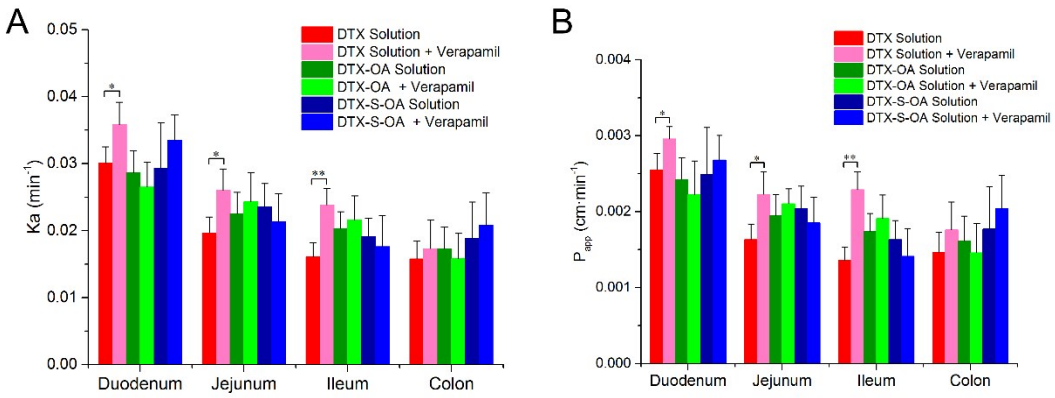


**Figure S11.** In situ single-pass intestinal perfusion (n = 3). $K_a$ (A) and $P_{app}$ (B) of duodenum, jejunum, ileum and colon in the inhibition of P-gp efflux test. Verapamil was used as standard P-gp inhibitor. (* $p < 0.05$, ** $p < 0.01$)

**Table S1.** Experimental logP of DTX, DTX-OA and DTX-S-OA (Mean ± SD, n = 3).

| Compound | DTX | DTX-OA | DTX-S-OA |
|---|---|---|---|
| logP | 4.18 ± 0.28 | 6.47 ± 0.32 | 6.61 ± 0.19 |

**Table S2. Stability results of prodrugs at 37 °C in phosphate buffers of different pH values, SGF, SIF, and plasma (mean ± SD, n = 3)**

| Media | $t_{1/2}$ (h) | |
|---|---|---|
| | DTX-OA | DTX-S-OA |
| pH 1.2 | 41.6 | 36.3 |
| pH 6.8 | 47.5 | 38.9 |
| pH 1.2 SGF with pepsin | 37.9 | 24.5 |
| pH 6.8 SIF with pancreatin | 4.6 | 18.6 |
| Rat plasma | 43.1 | 6.3 |

**Table S3.** Pharmacokinetic parameters of DTX, DTX-OA and DTX-S-OA solution and SNEDDS in rats after oral administration (n = 5).

| Formulations | Determined drug[a] | $T_{max}$ (h) | $C_{max}$ (nmol/L) | $T_{1/2}$ (h) | $AUC_{0-24}$ (nmol·h/L) | $F_{rel}$ (%) | $F_{abs}$ (%) |
|---|---|---|---|---|---|---|---|
| DTX Solution | DTX | 0.6 ± 0.3 | 123.8 ± 9.7 | 4.9 ± 2.3 | 376.1 ± 72.7 | 100.0 | 4.9 |
| DTX SNEDDS | DTX | 0.8 ± 0.4 | 234.9 ± 42.9 | 8.5 ± 5.0 | 662.5 ± 209.2 | 176.2 | 8.6 |
| DTX-OA Solution | DTX | 4.3 ± 5.4 | 17.9 ± 9.1 | 3.1 ± 2.9 | 111.0 ± 27.9 | 80.9 | 4.0 |
| | DTX-OA | 1.2 ± 0.8 | 49.8 ± 12.5 | 4.3 ± 3.4 | 193.0 ± 62.3 | | |
| DTX-OA SNEDDS | DTX | 4.7 ± 2.3 | 55.0 ± 77.6 | 8.9 ± 4.6 | 73.5 ± 36.1 | 314.4 | 15.4 |
| | DTX-OA | 2.8 ± 1.1 | 231.8 ± 49.3 | 12.2 ± 8.1 | 1108.9 ± 247.2 | | |
| DTX-S-OA Solution | DTX | 3.2 ± 2.7 | 28.2 ± 12.6 | 5.7 ± 2.6 | 148.0 ± 17.4 | 90.3 | 4.4 |
| | DTX-S-OA | 2.0 ±2.2 | 52.2 ± 19.7 | 6.9 ± 2.9 | 191.5 ± 46.5 | | |
| DTX-S-OA SNEDDS | DTX | 4.8 ± 1.1 | 113.6 ± 15.7 | 7.2 ± 5.1 | 759.0 ± 66.6 | 622.5 | 30.4 |
| | DTX-S-OA | 4.0 ± 0 | 205.8 ± 19.6 | 9.4 ± 7.2 | 1582.0 ± 162.7 | | |
| DTX Solution (iv) | DTX | - | 846.0 ± 130.3 | 1.3 ± 1.1 | 1282.6 ± 32.0.0 | - | 100.0 |

[a] Conjugates and derived DTX were simultaneously determined.