Methods

Synthesis of OH-ss-CPT. To a mixture of CPT (70 mg, 0.2 mmol) and triphosgene (24 mg, 0.08 mmol) in 30 mL of dry chloroform was added DMAP (60 mg, 0.48 mmol) in CH$_2$Cl$_2$ dropwise. The solution was allowed to react for 4 h, then flushed with argon for 5 min, following by adding of 2,2'-dithiodiethanol (1 mmol, 154 mg) and DIPEA (25 μL, 0.1 mmol) in 6 ml anhydrous THF. The reaction mixture was allowed to stir for 5 h. Then the solvent was evaporated, the resulted solid was washed with water for three times. The crude product was purified over silica gel using MeOH / CH$_2$Cl$_2$ (v/v, 1:40) as the eluent to yield OH-ss-CPT as yellowish solid (86 mg, 82%). $^1$H NMR (CDCl$_3$, 500 MHz): δ8.46 (s, 1H), 8.27 (d, $J$ = 8.4 Hz, 1H), 7.99 (d, $J$ = 8.2 Hz, 1H), 7.91-7.87 (m, 1H), 7.74-7.70 (m, 1H), 7.47 (s, 1H), 5.72 (d, $J$ = 7.6 Hz, 1H), 5.42 (d, $J$ = 7.6 Hz, 1H), 5.34 (s, 2H), 4.46-4.35 (m, 2H), 3.94 (s, 2H), 3.34 (s, 1H), 3.07-2.86 (m, 4H), 2.37-2.15 (m, 2H), 1.05 (t, 3H, $J$ = 7.4 Hz). Mass spectrometry (HR-MS, m/z): [M + H$^+$] calcd for C$_{25}$H$_{25}$N$_2$O$_7$S$_2$, 529.1103; found 529.1079.

Synthesis of Biotin-ss-CPT. OH-ss-CPT (53 mg, 0.1 mmol), DAMP (15 mg, 0.12 mmol), EDC (21 mg, 0.11 mmol) and biotin (25 mg, 0.1 mmol) was suspended in 30 ml CH$_2$Cl$_2$, the mixture was allowed to stirred for 13 h at room temperature. The solvent was evaporated and washed with 10% hydrochloric acid and water twice respectively. The resulting crude product was subject to HPLC to yield Biotin-ss-CPT as white solid (45 mg, 60%). Purity: 99.7% (Figure S19). HPLC: Agilent 1260 infinity pre-system, YMC-Pack ODS-A Column (250*20 mml. D. S-5μm, 12nm). Mobile phase A: H$_2$O, B: MeOH. 0-26 min: 30%-70% B, 26-34 min: 70%-100% B. Absorption wavelength, 365
nm (based on the UV spectrum in Figure S20). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$8.46 (s, 1H), 8.29 (d, $J$ = 8.6 Hz, 1H), 7.98 (d, $J$ = 8.4 Hz, 1H) 7.88-7.85 (m, 1H), 7.71-7.69 (m, 1H), 7.44 (s, 1H), 5.68(d, $J$ = 7.6 Hz, 1H), 5.41 (d, $J$ = 7.6 Hz, 1H), 5.34 (s, 2H), 4.53-4.51 (m, 1H), 4.42-4.32 (m, 3H), 4.27-4.23 (m, 2H), 3.17-3.16 (m, 1H), 2.98-2.88 (m, 5H), 2.72 (d, $J$ = 6.3 Hz, 1H), 2.33-2.26 (m, 3H), 2.20-2.13 (m, 1H), 1.71-1.60 (m, 4H), 1.47-1.39 (m, 2H), 1.01 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz) : $\delta$173.2, 167.3, 162.9, 157.3, 153.5, 151.9, 148.3, 146.1, 145.7, 131.8, 131.0, 129.2, 128.7, 128.3, 120.4, 96.64, 78.1, 67.1, 66.6, 61.97, 61.96, 60.2, 55.2, 53.4, 52.7, 50.2, 40.5, 37.5, 36.6, 33.7, 31.9, 28.2, 24.7, 7.7. Mass spectrometry (HR-MS, m/z): [M + H$^+$] calcd for C$_{35}$H$_{39}$N$_4$O$_9$S$_3$, 755.1879; found 755.1862.

**Synthesis of OH-cc-CPT.** To a mixture of CPT (70 mg, 0.2 mmol) and triphosgene (24 mg, 0.08 mmol) in 30 mL of dry chloroform was added DMAP (60 mg, 0.48 mmol) in CH$_2$Cl$_2$ dropwise. The solution was allowed to react for 4 h, then flushed with argon for 5 min, following by adding of 1,6-hexanediol (1 mmol, 118 mg) and DIPEA (25 $\mu$L, 0.1 mmol) in 6 ml anhydrous THF. The reaction mixture was stirred for 5 h. Then the solvent was evaporated. Then the solvent was evaporated, the resulted solid was washed with water for three times. The crude product was purified over silica gel using MeOH / CH$_2$Cl$_2$ (v/v, 1:55) as the eluent to yield OH-cc-CPT as yellowish solid (76 mg, 78%). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$8.51 (s, 1H), 8.38 (d, $J$ = 8.4 Hz, 1H), 8.00 (d, $J$ = 8.2 Hz, 1H), 7.94-7.88 (m, 1H), 7.77-7.72 (m, 1H), 7.62 (s, 1H), 5.70 (d, $J$ = 7.6 Hz, 1H), 5.45 (s, $J$ = 7.6 Hz, 1H), 5.35 (s, 2H), 4.16 (m, 2H), 3.63 (t, $J$ = 6.8 Hz, 2H), 3.27 (s, 1H), 2.38-2.15 (m, 2H), 1.72-1.67 (m, 2H), 1.61-1.52 (m, 2H), 1.45-1.36 (m, 4H), 1.03 (t, $J$ = 7.4 Hz, 3H). Mass spectrometry (HR-MS, m/z): [M + H$^+$] calcd for C$_{27}$H$_{29}$N$_2$O$_7$, 493.1975; found 493.1926.

**Synthesis of Biotin-cc-CPT.** OH-cc-CPT (49 mg, 0.1 mmol), DAMP (15 mg, 0.12 mmol), EDC (21 mg, 0.11 mmol) and biotin (25 mg, 0.1 mmol) was suspended in 30 ml CH$_2$Cl$_2$, the mixture was allowed to stirred for 13 h at room temperature. The solvent was evaporated and washed with 10% hydrochloric acid and water twice respectively. The resulting crude product was subject to HPLC to yield Biotin-cc-CPT as white solid (42 mg, 58%). Purity: 98.9%. HPLC: Agilent 1260 infinity pre
system, YMC-Pack ODS-A Column (250*20 mml. D. S-5μm, 12nm). Mobile phase
A: H2O, B: MeOH. 0-27 min: 30%-70% B, 27-35 min: 70%-100% B. Absorption
wavelength, 365 nm (based on the UV spectrum in Figure S19). 1H NMR (CDCl3,
500 MHz): δ8.45 (s, 1H), 8.28 (d, J = 8.6 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.89-7.86
(m, 1H), 7.73-7.69 (m, 1H), 7.40 (s, 1H), 5.72 (d, J = 7.6 Hz, 1H), 5.44 (d, J = 7.6 Hz,
1H), 5.34 (s, 2H), 4.56-4.53 (m, 1H), 4.36-4.33 (m, 1H), 4.20-4.09 (m, 2H), 4.02 (t, J
= 6.6 Hz, 2H), 3.20-3.16 (m, 1H), 2.95 (dd, J = 2.6, 6.8 Hz, 1H), 2.76 (d, J = 6.9 Hz,
1H), 2.33-2.27 (m, 3H), 2.22-2.15 (m, 1H), 2.14-1.59 (m, 8H), 1.48-1.35 (m, 6H),
1.03 (t, J = 7.4 Hz, 3H). 13C NMR (CDCl3, 125 MHz): δ173.6, 167.5, 157.3, 153.8,
152.2, 148.7, 146.3, 145.9, 131.5, 130.9, 129.5, 128.6, 128.2, 128.2, 120.4, 96.3, 77.7,
77.2, 76.8, 69.0, 67.1, 64.2, 62.0, 60.2, 55.3, 50.1, 40.5, 33.9, 31.9, 28.4, 28.4, 28.3,
28.3, 25.5, 25.2, 24.8, 7.7. Mass spectrometry (HR-MS, m/z): (M + H+) calcd for
C32H43N4O9S 719.2751; found 719.2717.

Results

Scheme S1. Synthetic route to Biotin-cc-CPT and Biotin-ss-CPT.
Figure S1. HRMS for OH-ss-CPT

Figure S2. $^1$H NMR spectrum of OH-ss-CPT in CDCl$_3$. 
Figure S3. HRMS for **Biotin-ss-CPT**

Figure S4. $^1$H NMR spectrum of **Biotin-ss-CPT** in CDCl$_3$. 
Figure S5. $^{13}$C NMR spectrum of Biotin-ss-CPT in CDCl$_3$.

Figure S6. HR-MS for OH-cc-CPT
Figure S7. $^1$H NMR spectrum of OH-cc-CPT in CDCl$_3$.

Figure S8. HRMS for Biotin-cc-CPT.
Figure S9. $^1$H NMR spectrum of Biocin-cc-CPT in CDCl$_3$.

Figure S10. $^{13}$C NMR spectrum of Biocin-cc-CPT in CDCl$_3$. 
Figure S11. Excitation (A) and Emission spectrum (B) of Biotin-cc-CPT in CHCl$_3$.

Figure S12. Excitation (A) and Emission spectrum (B) of Biotin-ss-CPT in CHCl$_2$.

Figure S13. Excitation (A) and Emission spectrum (B) of CPT in CHCl$_2$. 
Figure S1. HPLC analysis (A) and fluorescence spectra of CPT release from the Biotin-ss-CPT (B) and Biotin-cc-CPT (C) at different equivalent of GSH. Peak area ratio rise of CPT release form Biotin-ss-CPT in HPLC and fluorescence enhancement of Biotin-ss-CPT witnessed on treatment with increasing concentrations of GSH (0–8 equiv) in fluorescence spectra respectively. Biotin-ss-CPT (20 µM) was treated with GSH in mixed solution of PBS buffer and DMSO (v/v: 4/1) at pH=7.4. Peaks in the chromatograms were detected by monitoring the UV/Vis absorption at 365 nm. HPLC: Agilent 1260 infinity II system, Agilent ZORBAX SB C18 (250*4.6mm, 5µm) Mobile phase A: H$_2$O, B: CH$_3$CN. 0-20 min: 50%-100% B. Flow rate: 1 ml/min.
Figure S15. Fluorescence spectra of Biotin-ss-CPT (20.0 µM) toward 10 equiv of Homocysteine (Hcy) (A), Cysteine (Cys) (B) and some amino acids (C). The data were recorded 2 h after incubation with Hcy, Cys (at interval of 30 min) or amino acids in mixed solution of PBS buffer and DMSO (v/v:4/1) at pH=7.4. Excitation was set at 365 nm.
Figure S16. (A) Stability of Biotin-ss-CPT in Milli-Q water, PBS, culture medium and human plasma measured by fluorescence spectra. (B) Reverse-phase HPLC chromatograms analysis of Biotin-ss-CPT (30 µM) in plasma after incubation for 0, 24, 48 and 72 h respectively. Peaks in the chromatograms were detected by monitoring the UV/Vis absorption at 365 nm. HPLC: Agilent 1260 infinity II system, Agilent ZORBAX SB C18 (250*4.6 mm, 5 µm) Mobile phase A: H₂O, B: CH₃CN. 0-20 min: 50%-100% B. Flow rate: 1 ml/min.
Figure S17. The inhibitory effects of Biotin-cc-CPT, Biotin-ss-CPT and CPT on the proliferation of tumor cells MGC803, NCM460, HepG2, Hela and MCF-7 and normal cells GS1, SW620 and L02. Cell viability was determined by MTT assay after treatment for 72 h.
Quantitative analysis of the migrated cells subjected to Biotin-ss-CPT, Biotin-cc-CPT and CPT at 24 h by manual counting. Values expressed are the mean ±SD of 3 independent experiments.

Figure S18.
Figure S19. Effects of biotin on the anticancer activity of CPT (A) or Biotin-ss-CPT (B). The cells were pretreated with different concentration of biotin (0-20 µM) in 96-well plates for 2 h then exposed to CPT (5 µM) or Biotin-ss-CPT (5 µM) for 72 h. Cell viability was measured by MTT assay. (C) Effects of biotin on the fluorescence intensity of CPT or Biotin-ss-CPT. The cells were pretreated with biotin (20 µM) in 2-cm dish for 2 h then exposed to CPT (10 µM) or Biotin-ss-CPT (10 µM) for 5 h then the fluorescence was measured under fluorescence microscope.
Figure S20. Rate of body weight change. Values represented were means ± SD of triplicates.

Figure S21. The quantitative analysis of ADCs.