

Supplementary Information

A gadolinium-complex-based theranostic prodrug for *in vivo* tumour-targeted magnetic resonance imaging and therapy

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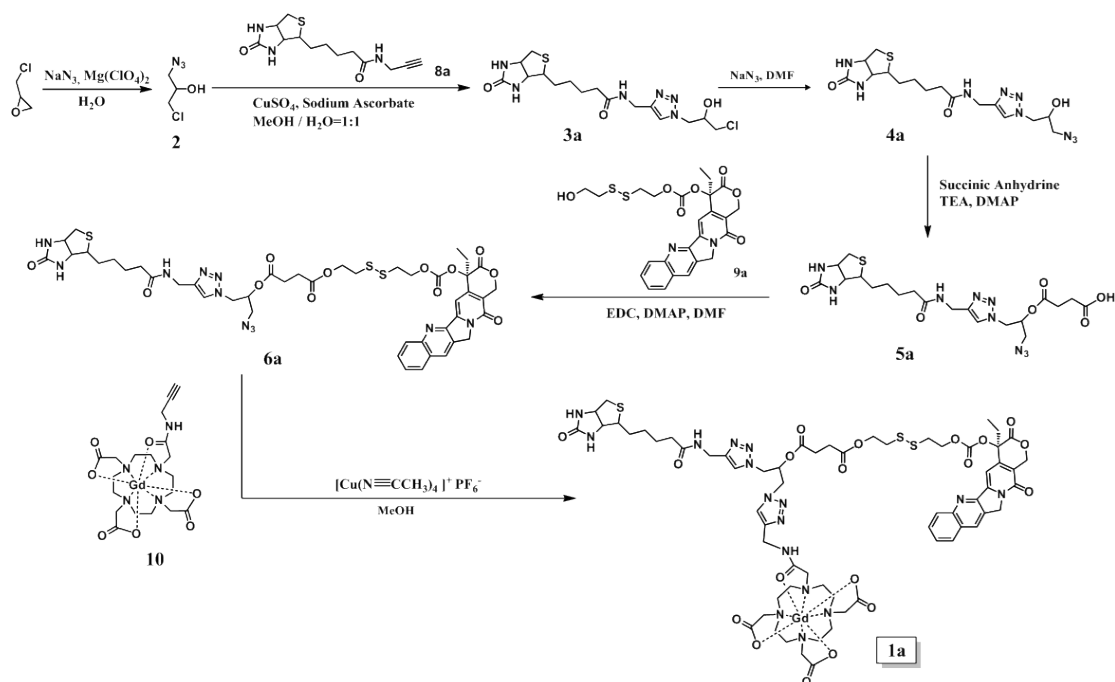
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Materials

Bromoacetyl (98%) was purchased from LuLong Biotech (China); epichlorohydrin (99%), DMAP (99%), 2-hydroxyethyl disulfide (HEDS) (90%) were purchased from Alfa (UK); sodium azide was purchased from Pioneer Chemical (China); magnesium perchlorate, hydroxybenzotriazole (HOBT), triphosgene (98%), propargylamine (98%), DMSO-d6 were purchased from Inno-Chem (China); biotin was purchased from Sangon (Shanghai); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), camptothecin (CPT) (97%), gadolinium oxide (99.9%), sodium iodide (99.5%), tetrakis(acetonitrile)copper(I) hexafluorophosphate were purchased from Aladdin (China); formic acid, succinic anhydride (99%), triethylamine (TEA) (99%) were purchased from J&K Scientific (China). All chemicals were used as received.

Synthesis



Scheme S1. Synthesis of **1a**.

1-Azido-3-chloropropan-2-ol (2). To an ice-bath cooled 100 mL flask containing 50 mL 80% v/v alcohol in water, sodium azide (2.03 g, 31.2 mmol) and magnesium perchlorate (6.96 g, 31.2 mmol) were added. Epichlorohydrin (2.03 mL, 26.1 mmol)

was then added dropwise and the resulting solution was stirred at room temperature for 20 min. The reaction flask was cooled in refrigerator for 4 h and filtered. The filtrate was extracted with diethyl ether. The organic layer was dried with MgSO₄ and concentrated *in vacuo* to give **2** (3.3 g, 95% yield) as a colourless oil, which was stored at 3–5 °C. ¹H NMR (400 MHz, CDCl₃) 4.05–3.93 (1 H, m), 3.67–3.54 (2 H, m), 3.53–3.40 (2 H, m), 2.43 (1 H, dd, *J* = 5.6), 2.04 (1 H, s).

***N*-((1-(3-chloro-2-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)methyl)-5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (3a)**. To a solution of **8a** (0.50 g, 1.78 mmol) and **2** (0.244 g, 1.80 mmol) in water/methanol (1:1, 45 mL), copper sulphates (0.11 g, 0.45 mmol) was added. The reaction mixture was stirred under N₂ at room temperature for 15 min. Sodium ascorbate (0.18 g, 0.9 mmol) in water (3 mL) was added to the reaction mixture quickly. Then the reaction mixture was stirred vigorously overnight. After the reaction was complete as monitored by TLC, the reaction mixture was concentrated *in vacuo* and purified with flash chromatography (DCM/MeOH = 20:1, gradually increased to 12:1) to give **3a** (0.67 g, 90% yield) as a yellow hygroscopic solid: ¹H NMR (400 MHz, DMSO-*d*₆) 8.27 (1 H, t, *J* = 5.5), 7.84 (1 H, s), 6.33–6.45 (2 H, m), 5.68 (1 H, d, *J* = 5.5), 4.49 (1 H, dd, *J* = 13.9, 3.9), 4.40–4.21 (3 H, m), 4.20–4.00 (2 H, m), 3.50–3.66 (2 H, m), 3.09 (1 H, dd, *J* = 11.6, 7.2), 2.82 (1 H, dd, *J* = 12.4, 5.1), 2.57 (1 H, d, *J* = 12.4), 2.10 (2 H, t, *J* = 7.4), 1.70–1.20 (6 H, m). ESI-MS calcd. for C₁₆H₂₅ClN₆O₃S 416.9, found 417.9 ([*M*+*H*]⁺) and 439.6 ([*M*+*Na*]⁺).

***N*-((1-(3-azido-2-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)methyl)-5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (4a)**. To a 50 mL flask, **3a** (0.060 g, 0.144 mmol) in DMF (20 mL) was added, followed by the addition of NaN₃ (0.030 g, 0.461 mmol). A small amount (cat.) of NaI was then added. The reaction was stirred at 80 °C for 16 h and monitored via mass spectrometry until completion. Then filter and the filtrate was concentrated *in vacuo* to give **2**. The faint yellow crude product was purified with flash chromatography (DCM/MeOH = 20:1, gradually increased to

15:1) to give **4a** (0.056 g, 92% yield) as a yellow solid: ESI-MS calcd. for C₁₆H₂₅N₉O₃S 423.5, found 446.5 ([M+Na]⁺).

4-((1-Azido-3-(4-((5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)methyl)-1H-1,2,3-triazol-1-yl)propan-2-yl)oxy)-4-oxobutanoic acid (5a). **4a** (0.050 g, 0.118 mmol) and succinic anhydride (0.012 g, 0.120 mmol) was dissolved in DMF (20 mL) in a 50 mL flask. To this solution was added TEA (0.0364 g, 0.360 mmol) and DMAP (0.004 g, 0.0327 mmol). The reaction mixture was stirred at room temperature overnight and then was concentrated *in vacuo*. The crude product was purified with flash chromatography (DCM/MeOH = 20:1, gradually increased to 10:1) to give **5a** (0.053 g, 86% yield) as a faint yellow solid: ESI-MS calcd. for C₂₀H₂₉N₉O₆S 523.57, found 522.3 ([M-H]⁻).

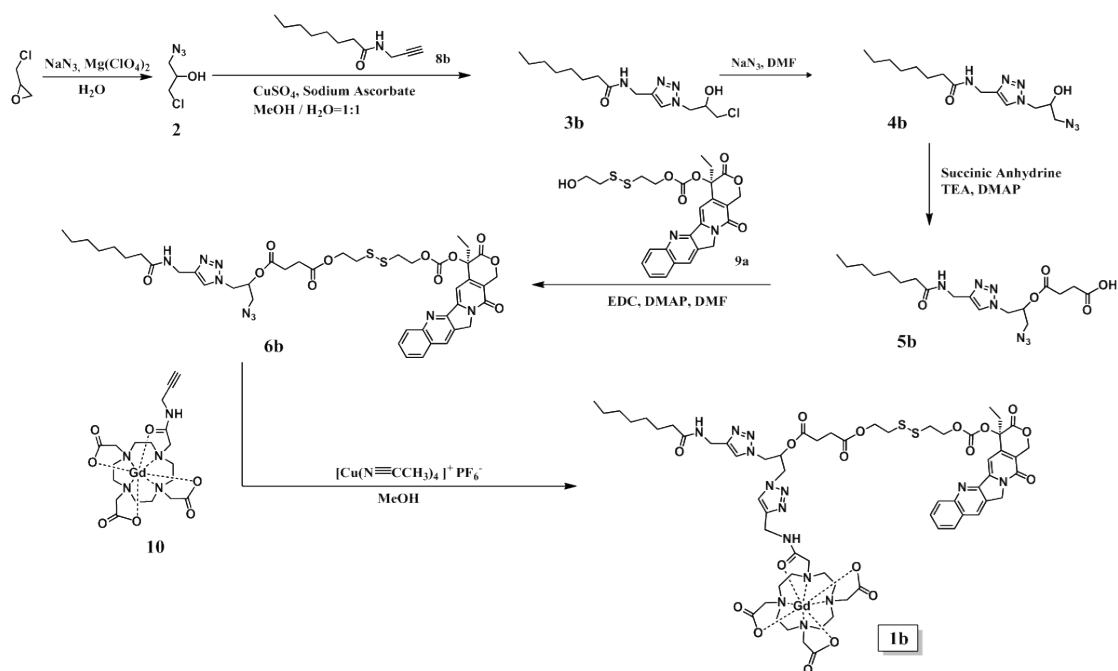
1-Azido-3-(4-((5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)methyl)-1H-1,2,3-triazol-1-yl)propan-2-yl-(2-(((R)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)oxy)ethyl)disulfanyl)ethyl succinate (6a). To a 10 mL flask, CPT-SS-OH (**9a**, 0.04 g, 0.0757 mmol) in 3 mL DMF, EDC (0.0218 g, 0.114 mmol), DMAP (0.0093 g, 0.0327 mmol) and **5a** (40 mg, 0.076 mmol) was added and the reaction mixture was stirred at room temperature for 72 h. The crude product was purified with flash chromatography (DCM/MeOH = 20:1, gradually increased to 12:1) to give **6a** (0.032 g, 42% yield) as a red solid: ¹H NMR (500 MHz, DMSO-d₆) 8.62 (1 H, d, *J* = 7.7), 8.27 (1 H, s), 8.09 (2 H, dd, *J* = 8, 8), 7.93–7.75 (2 H, m), 7.75–7.62 (1 H, m), 7.09 (1 H, s), 6.42 (2 H, s), 5.53 (2 H, s), 5.34–5.12 (3 H, m), 4.68–4.48 (2 H, m), 4.40–4.07 (8 H, m), 3.61 (1 H, dd, *J* = 13.2, 2.9), 3.40 (4 H, dd, *J* = 13.2, 6.3), 3.07 (1 H, d, *J* = 10.5), 3.01 (2 H, s), 2.92 (2 H, t, *J* = 6.1), 2.81 (1 H, dd, *J* = 12.4, 4.9), 2.58 (1 H, d, *J* = 12.4), 2.19 (2 H, dt, *J* = 13.8, 6.8), 2.09 (2 H, t, *J* = 7.2), 1.66–1.39 (4 H, m), 1.38–1.17 (2 H, m), 0.94 (3 H, t, *J* = 7.1), one of the peaks (for 1 H) is obscured by DMSO peaks; ¹³C NMR (126 MHz, DMSO-d₆) 172.51, 171.94, 171.33, 167.52, 163.24, 156.92, 153.30, 152.55, 148.30, 146.66, 145.63, 145.24, 131.97, 130.85, 130.04,

129.39, 128.90, 128.39, 128.15, 124.22, 119.65, 94.89, 78.38, 71.24, 66.84, 62.44, 61.51, 59.70, 55.87, 51.07, 50.69, 50.05, 49.08, 36.70 (2C), 35.47, 34.54, 30.81, 28.96, 28.73, 28.47, 25.64, 8.02; ESI-MS calcd. for $C_{45}H_{51}N_{11}O_{12}S_3$ 1034.15, found 1056.6 ($[M+Na]^+$).

Synthesis of 1a. **6a** (0.020 g, 0.0193 mmol) and **10** (0.023 g, 0.038mmol) dissolved in MeOH (5 mL) and $[Cu(N\equiv CCH_3)_4]^+ PF_6^-$ (0.004 g, 0.0107 mmol) was added to a 10mL flask. The reaction mixture was stirred at room temperature for 16 h and then was concentrated *in vacuo*. The crude product was purified by HPLC to give **1a** (0.026 g, 82% yield) as a white solid: ESI-MS calcd. for $C_{63}H_{77}GdN_{16}O_{19}S_3$ 1629.4111, found 1652.3998 ($[M+Na]^+$).

Synthesis of 10. **10** was synthesized according to a previous report.¹

Synthesis of CPT-SS-OH (9) and CPT-CC-OH (9c). **9** and **9c** were synthesized according to a previous report.²



Scheme S2. Synthesis of **1b**.

5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N-(prop-2-yn-1-yl)pentanamide (8a). Biotin (1.01 g, 4.2 mmol) was dissolved in acetonitrile/water (1:1, 180 mL) and cooled in an ice-bath. 2-propyn-1-amine (0.44 mL, 4.2 mmol), anhydrous hydroxybenzotriazole (HOBt) (0.860 g, 4.2 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1.26 g, 4.2 mmol) was added quickly. The reaction mixture was stirred at room temperature for 6 h and monitored by TLC until completion. The crude product was purified with flash chromatography (DCM/MeOH = 10:1) to give **8a** (1.04 g, 88% yield) as a white solid: ¹H NMR (400 MHz, DMSO-d₆) 8.21 (1 H, s), 6.33-6.45(2 H, m), 4.37-4.26 (1 H, m), 4.22-4.06 (1 H, m), 3.84 (2 H, dd, *J* = 5.4, 2.4), 3.17-2.99 (2 H, m), 2.83 (1 H, dd, *J* = 12.4, 5.1), 2.58 (1 H, d, *J* = 12.4), 2.09 (2 H, t, *J* = 7.4), 1.73-1.17 (6 H, m).

N-(Prop-2-yn-1-yl)octanamide (8b). n-caprylic acid (0.648g, 4.2 mmol) was dissolved in DCM (150 mL) and cooled in an ice-bath. 2-propyn-1-amine (0.44 mL, 4.2 mmol), anhydrous hydroxybenzotriazole (HOBt) (0.860 g, 4.2 mmol), and 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC) (1.26 g, 4.2 mmol) was added quickly. The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was extracted with DCM (100 mL × 2). The combined organic layers was dried with sodium sulphate anhydrous and concentrated *in vacuo*. The crude product was purified with flash chromatography (DCM/MeOH = 20:1) to give **8b** (0.68 g, 90% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) 5.56 (1 H, s), 4.12-3.97 (2 H, m), 2.30-2.04 (3 H, m), 1.72-1.59 (2 H, m), 1.42-1.16 (8 H, m), 0.97-0.77(3 H, m).

N-((1-(3-chloro-2-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)octanamide (3b). To a solution of **8b** (0.322 g, 1.78 mmol) and **2** (0.244 g, 1.78 mmol) in water/THF (1:2, 45 mL), copper sulfate (0.11 g, 0.45 mmol) was added. The reaction mixture was stirred under N₂ at room temperature for 15 min before sodium ascorbate (0.18 g, 0.9 mmol) in water (3 mL) was added quickly. The reaction was stirred vigorously overnight and monitored by TLC until completion. The reaction mixture was then concentrated *in vacuo* and purified with flash chromatography (DCM/MeOH =20:1) to

give **3b** (0.52 g, 92% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃), 6.47 (1 H, s), 4.2-4.9(3 H, m), 4.30 (1 H, s), 3.56 (2 H, s), 2.21 (2 H, s), 1.84 (1 H, s), 1.61 (2 H, s), 1.27 (8 H, s), 0.87 (3 H, t, *J* = 6.7).

***N*-((1-(3-azido-2-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)methyl) octanamide (4b).**

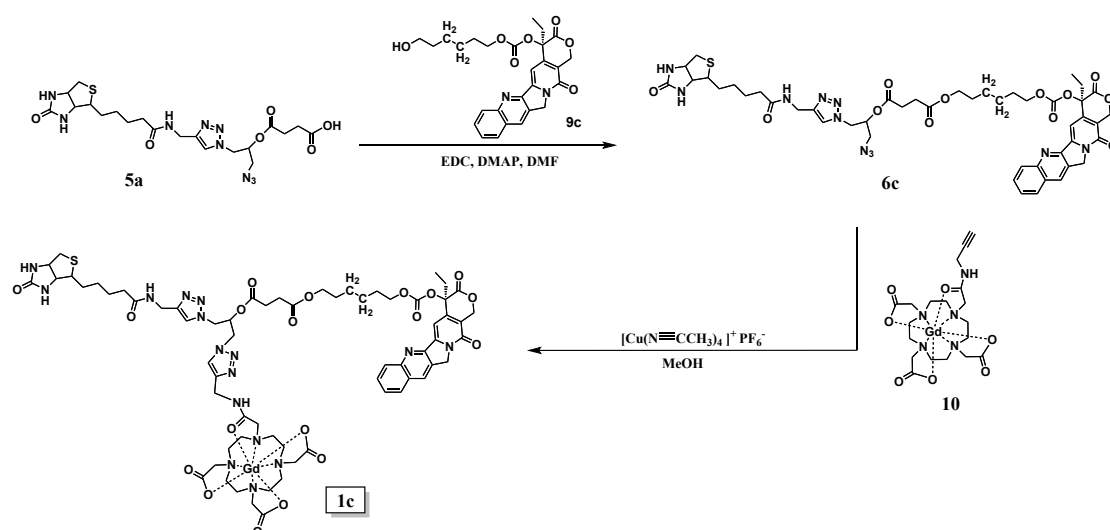
To a 50 mL flask, **3b** (0.455 g, 1.44 mmol) in DMF (20 mL), NaN₃ (0.28 g, 4.32 mmol) and NaI (cat.) were added. The reaction mixture was stirred at room temperature for 12 h and monitored via mass spectrometry until completion. The reaction mixture was concentrated *in vacuo* and then extracted with DCM (100 mL × 3). The combined organic layers were concentrated *in vacuo* to give **4b** (0.441 g, 95% yield) as a white solid product: ESI-MS calcd. for C₁₄H₂₅N₇O₂ 323.21, found 346.2 ([M+Na]⁺).

4-((1-Azido-3-(4-(octanamidomethyl)-1*H*-1,2,3-triazol-1-yl)propan-2-yl)oxy)-4-oxobutanoic acid (5b). **4b** (0.381 g, 1.18 mmol) in DMF (20 mL), succinic anhydride (0.12 g, 1.20 mmol), TEA (0.121 g, 1.20 mmol), and DMAP (0.004 g, 0.0327 mmol) were to a 50 mL flask. The reaction mixture was stirred overnight. The reaction mixture was concentrated *in vacuo* and the crude product was purified with flash chromatography (DCM/MeOH = 10:1) to give **5b** (0.454 g, 91% yield) as a white solid: ESI-MS calcd. for C₁₈H₂₉N₇O₅ 423.22, found 423.9 ([M+H]⁺), 445.9 ([M+Na]⁺), 461.8 ([M+K]⁺).

1-Azido-3-(4-(octanamidomethyl)-1*H*-1,2,3-triazol-1-yl)propan-2-yl (2-((2-(((R)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino [1,2-*b*]quinolin-4-yl)oxy)carbonyl)oxy)ethyl)disulfanyl)ethyl) succinate (6b). To a 10 mL flask, CPT-SS-OH (0.04 g, 0.0757 mmol) in 3 mL DMF, EDCI (0.0218 g, 0.114 mmol), DMAP (0.0093 g, 0.0327 mmol) and **5a** (32 mg, 0.076mmol) was added. The reaction mixture was stirred at room temperature for 72 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified with flash chromatography (DCM/MeOH=10:1) to give **6b** (0.037 g, 52% yield) as a red solid: ¹H NMR (500 MHz, DMSO-*d*₆) 8.63 (1 H, s), 8.29–8.19 (1 H, m), 8.04–8.16 (2 H, *m*), 7.90–7.81 (2 H, *m*),

7.68 (1 H, t, $J = 7.5$), 7.09 (1 H, s), 5.52 (2 H, d, $J = 17.1$), 5.33–5.15 (3 H, m), 4.60 (2 H, dd, $J = 14.5, 5.5$), 4.41–4.15 (6 H, m), 3.61 (1 H, dd, $J = 13.4, 3.5$), 3.44–3.33 (4 H, m), 3.07–2.98 (2 H, m), 2.93 (2 H, t, $J = 6.3$), 2.29–2.12 (2 H, m), 2.07 (2 H, dd, $J = 9.5, 5.5$), 1.48 (2 H, dd, $J = 14.1, 7.1$), 1.32–1.10 (8 H, m), 0.95 (3 H, t, $J = 7.4$), 0.83 (3 H, t, $J = 6.9$), one of the peaks (for 1 H) is obscured by DMSO peaks; ^{13}C NMR (126 MHz, DMSO- d_6) 172.56, 171.91, 171.30, 167.49, 156.92, 153.30, 152.57, 148.32, 146.66, 145.66, 145.24, 131.97, 130.82, 130.06, 129.39, 128.90, 128.4, 128.14, 124.22, 119.66, 94.87, 78.37, 71.24, 66.92, 66.74, 62.44, 51.06, 50.70, 50.03, 36.69, 35.69, 34.52, 31.61, 30.81, 28.942C, 25.66, 22.51, 14.35, 8.01.

Synthesis of 1b. **6b** (0.035 g, 0.038 mmol) and **10** (45 mg, 0.075 mmol) in MeOH (5 mL) and $\text{Cu}(\text{N}\equiv\text{CCH}_3)_4]^+ \text{BF}_6^-$ (0.008 g, 0.0214 mmol) were added to a 10 mL flask. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by HPLC to give **1b** (0.047 g, 82% yield) as a white solid: ESI-MS calcd. for $\text{C}_{62}\text{H}_{79}\text{GdN}_{14}\text{O}_{18}\text{S}_2$ 1529.4379 found 1552.4277($[\text{M}+\text{Na}]^+$).



Scheme S3. Synthesis of **1c**.

1-Azido-3-(4-((5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-

yl)pentanamido)methyl)-1*H*-1,2,3-triazol-1-yl)propan-2-yl-(6-((((*R*)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-4-yl)oxy)carbonyloxy)hexyl) succinate (**6c**). To a 10 mL flask, CPT-CC-OH (0.04 g, 0.0757 mmol) in 3 mL DMF/DCM (1:1), EDC (0.0218 g, 0.114 mmol), DMAP (0.0093 g, 0.0327 mmol) and compound 5a (0.04 mg, 0.076mmol) were added. The reaction mixture was stirred at room temperature for 72 h. The crude product was purified with flash chromatography (DCM/MeOH = 10:1) to give **6b** (0.025 g, 33% yield) as a red solid: ¹H NMR (500 MHz, DMSO-*d*₆) 8.65 (1 H, s), 8.26 (1 H, t, *J* = 5.5), 8.18–8.00 (2 H, m), 7.93–7.77 (2 H, m), 7.69 (1 H, t, *J* = 7.2), 7.04 (1 H, s), 6.33–6.45 (2 H, m), 5.59–5.37 (2 H, m), 5.25 (3 H, s), 4.66–4.45 (2 H, m), 4.38–4.17 (3 H, m), 4.10 (3 H, dd, *J* = 14.0, 7.5), 3.88 (2 H, dd, *J* = 15.1, 9.1), 3.58 (1 H, dd, *J* = 13.8, 11.5), 3.07 (1 H, dt, *J* = 8.4, 6.1), 2.80 (1 H, dd, *J* = 12.4, 5.0), 2.56 (1 H, t, *J* = 11.3), 2.47 (2 H, s), 2.18 (2 H, tq, *J* = 14.2, 7.2), 2.13–2.04 (2 H, m), 1.66–1.38 (8 H, m), 1.37–1.17 (6 H, m), 0.93 (3 H, t, *J* = 7.3); ¹³C NMR (126 MHz, DMSO-*d*₆) 172.49, 172.08, 171.42, 167.64, 163.21, 156.95, 153.51, 152.58, 148.31, 146.66, 145.54 (2C), 132.06, 130.87, 130.14, 129.38, 128.95, 128.44, 128.20, 124.22, 119.61, 94.68, 78.06, 71.20, 69.02, 66.87, 64.41, 61.50, 59.69, 55.86, 51.08, 50.73, 50.05, 35.46, 34.53, 30.77, 29.02, 28.84, 28.68, 28.66–28.11 (3C), 25.64, 25.25 (2C), 25.15, 8.01.

Synthesis of 1c. **6c** (0.038 g, 0.038 mmol) and **10** (0.045 g, 0.075mmol) dissolved in MeOH (5 mL) and Cu(N≡CCH₃)₄⁺ BF₆⁻ (0.008 g, 0.0214 mmol) was added to a 10mL flask. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by HPLC to give **1c** (0.048 g, 80% yield) as a white solid: ESI-MS calcd. for C₆₆H₈₃GdN₁₆O₁₉S 1593.4982 found 1616.4886 ([M+Na]⁺).

Cell culture

A549 human non-small cell lung cancer cells, HeLa human cervical cancer cells, WPMY-1 human normal prostate stromal immortalized cells, HL7702 human normal

hepatocytes, 293T human embryonic kidney cells, MRC-5 human lung fibroblast cells, were cultured in Dulbecco's Modified Eagle's Medium(DMEM), containing 10% fetal bovine serum(FBS) and 1% penicillin-streptomycin. All cell lines used in this work were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China).

Cell cytotoxicity assays

The cytotoxicities of **1a**, **1b**, **1c**, and CPT against A549, HeLa, WPMY-1, MRC-5, HL7702, 293T were evaluated via a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. A549 cells were seeded in 96-well plates at a density of 10000 cells /well and cultured for 24 h. Then the media were replaced by fresh DMEM containing various concentrations of **1a**, **1b**, **1c**, and CPT. Cell viabilities were evaluated according to the standard protocol. The absorbance at 492 nm of each well was measured on a microplate reader (Thermo Scientific, Multiskan FC).

Cellular fluorescence imaging

The cellular targeting efficiency was evaluated via the fluorescence intensity of CPT in biotin receptor (BR) positive or BR negative cells on a confocal fluorescence microscope (Leica, TCS SP5). A549, HeLa, MRC-5 and HL7702 cells were seeded in 3.5 cm dishes and allowed to grow to 70% confluence. All cells were treated with 10 μ M of **1a** in PBS for 15 min, washed with PBS for three times and subjected to confocal fluorescence microscopy. For the blocking experiment, HeLa and A549 cells were pretreated with biotin (1.5 mM in DMEM with 10% FBS) for 1 h, followed by with **1a** (15 μ M for HeLa and 10 μ M for A549) in PBS for 3 h.

***In vitro* cell imaging**

A549 cells were seeded in 10 cm dishes and cultured for 24 h. Then the cell were incubated with fresh DMEM media containing 20 μ M of **1a** for 10 min, 30 min, 1 h, 3 h, 6 h, and 12 h. After incubation, cells were washed with PBS for three times and collected for T_1 -weighted MRI at 0.5 T.

Sanger sequencing

A549 cells was planted in 10 cm culture dish, until the cells fulfilled to 80%, 2 μ M CPT or 2 μ M 1a was added. 12 hours later, cells were digested, centrifuged and resuspended in Trizol. The sample were send to Shanghai Meiji Bio-Phamtech Corporation for the RNA sequencing. The data were analyzed through the free online platform of I-Sanger (www.i-sanger.com).

Animal study

All animal procedures were in accordance with the National Institute of Health Guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Xiamen University. Nude mice (6 weeks old, 18-22g) were purchased from the Laboratory Animal Center of Xiamen University. Tumours were inoculated by injecting A549 human non-small-cell lung cancer cells (1×10^7) into the subcutaneous tissues.

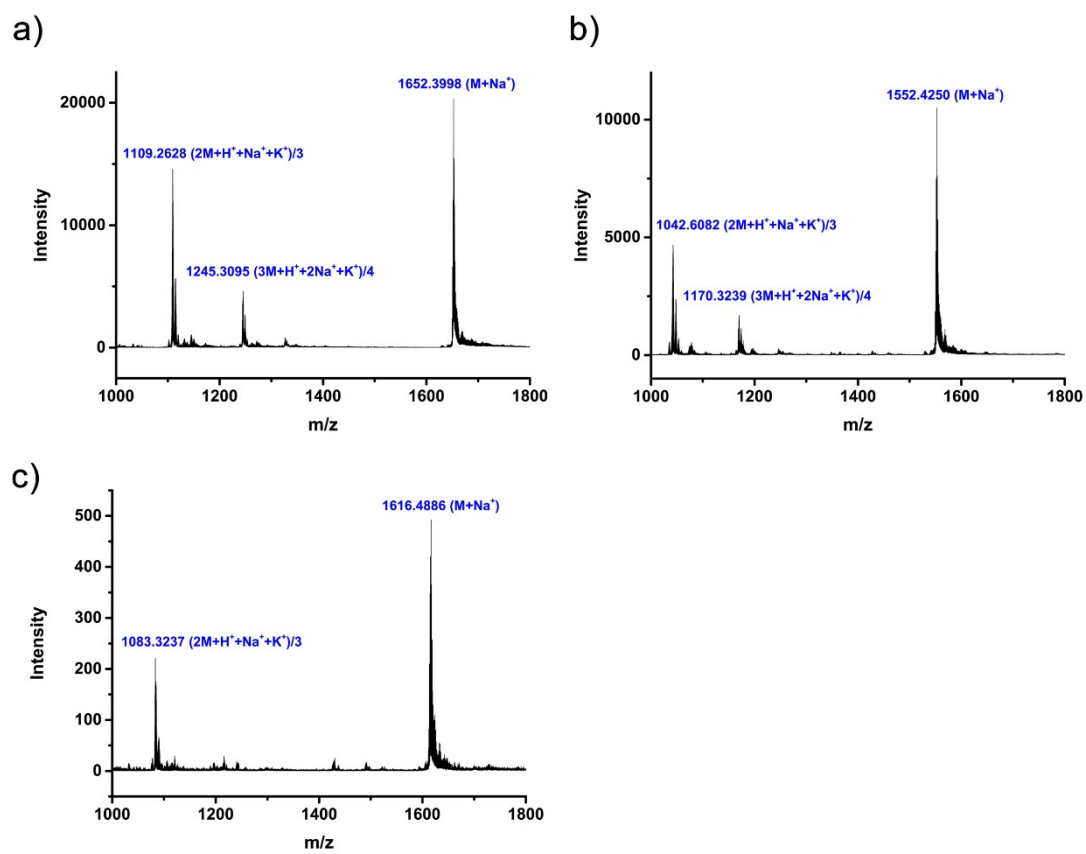


Figure S1. HR-MS spectra of **1a** (a), **1b** (b) and **1c** (c).

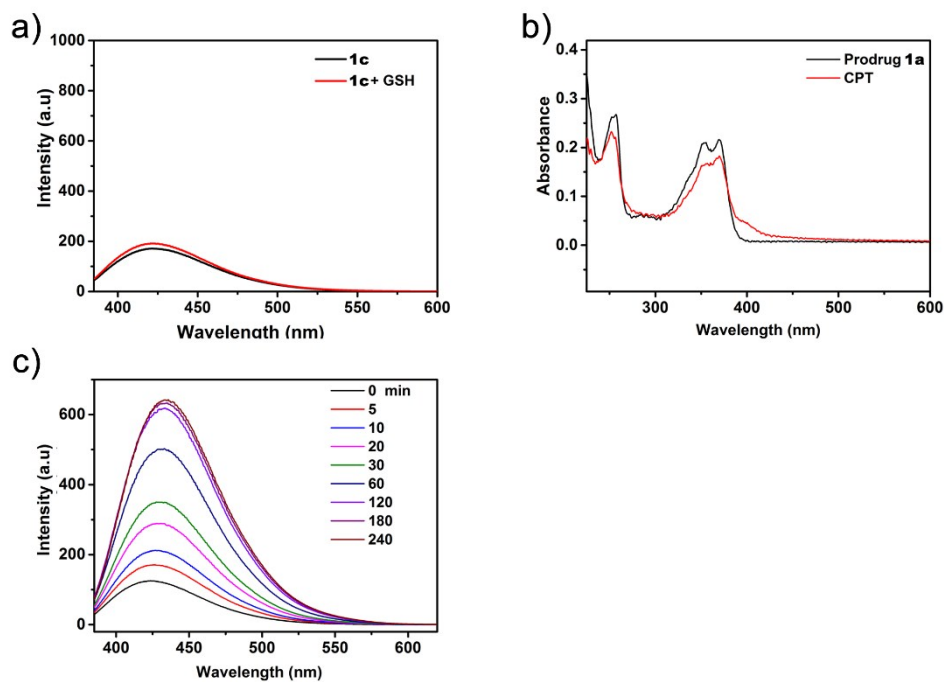


Figure S2. (a) Fluorescence spectra of **1c** (5 μM) incubated with or without GSH (5 mM) in PBS for 3 h. (b) UV-Vis absorption spectra of **1a** and CPT (10 μM , with 25% v/v DMSO in PBS). (c) Fluorescence spectra of **1a** (5 μM) treated with GSH (5 mM) in PBS for indicated times.

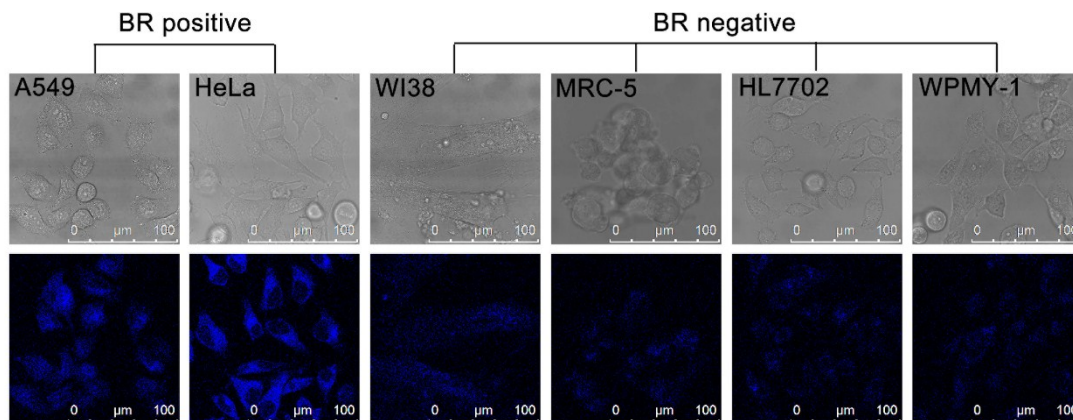


Figure S3. Bright-field (top) and fluorescence (bottom) images of biotin receptor (BR) positive cancer cells (HeLa and A549) and BR negative normal cells (WI38, MRC-5, HL7702, and WPMY-1) after 15 min incubation with **1a** (10 μ M).

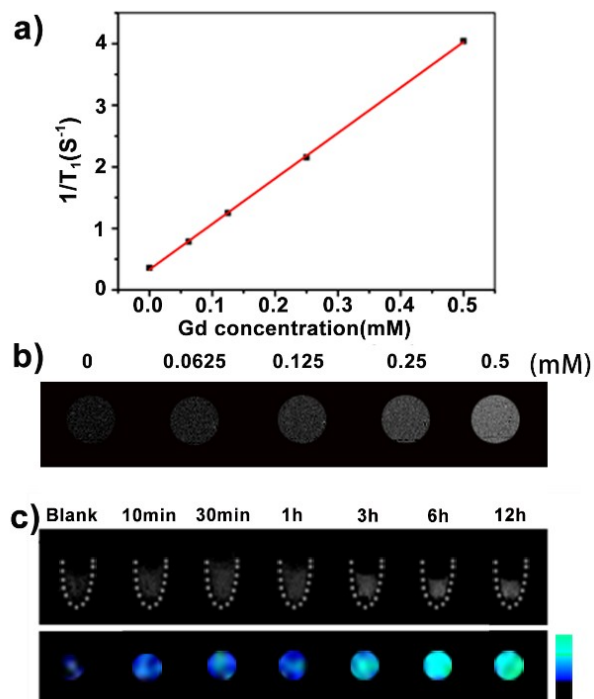


Figure S4. (a) T_1 relaxivity measurement of **1a**. (b) Phantom imaging of **1a** at different concentrations. (c) Figure 2e and corresponding pseudo-color images. Images were acquired on a 0.5 T Niumag NMI20-Analyst system.

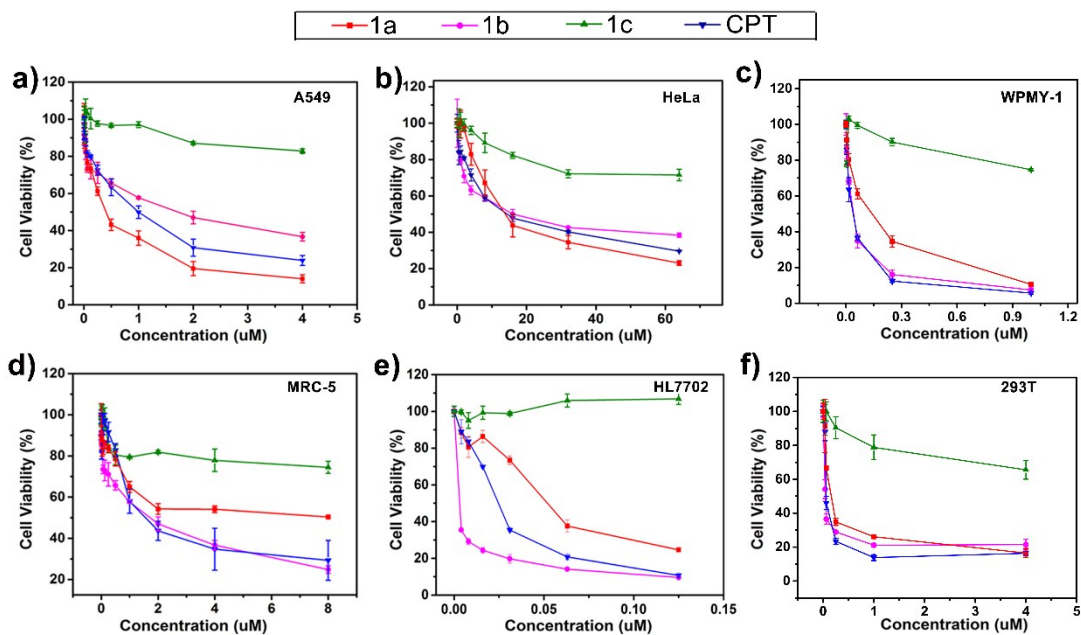


Figure S5. Cytotoxicity evaluation of **1a**, **1b**, **1c**, and CPT against cancer cell lines (HeLa and A549) and normal cell lines (WPMY-1, MRC-5, HL7702, 293T) using MTT assays.

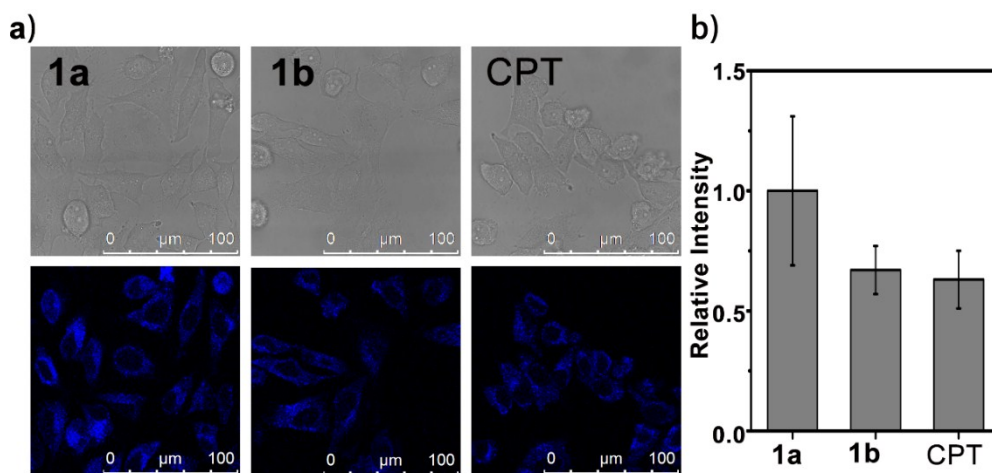


Figure S6. (a) Bright-field and fluorescent images of HeLa cells incubated with **1a**, **1b**, or CPT in PBS for 3 h. (b) Quantitative analysis on the fluorescence intensities. The data were presented as mean \pm SD ($n = 5/\text{group}$).

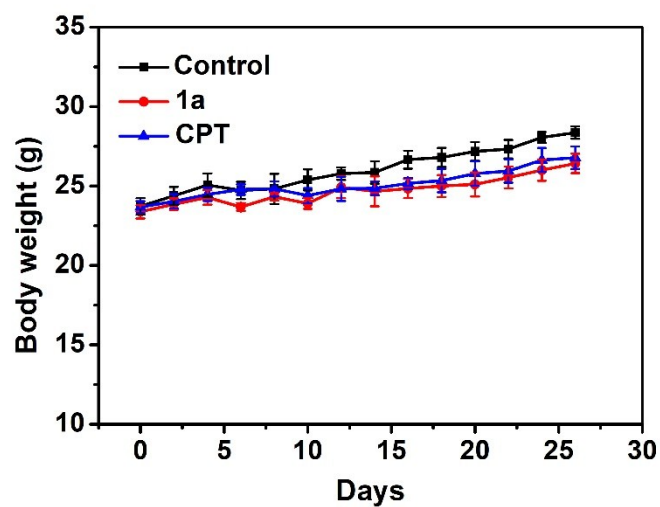


Figure S7. Body weight changes of mice treated with different formulations, measured every two days after the first injection.

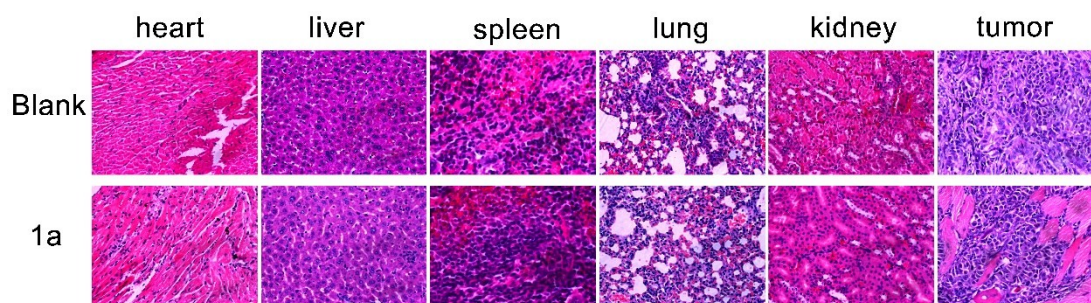


Figure S8. H&E stained slices of tumour and major organs collected from mice after treated with **1a** (3 mg CPT/kg) or PBS for 14 days.

References

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