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Supporting Information

Cross-Linked (R)-(+)-Lipoic Acid Nanoparticles with Prodrug Loading for Synergistic Cancer Therapy

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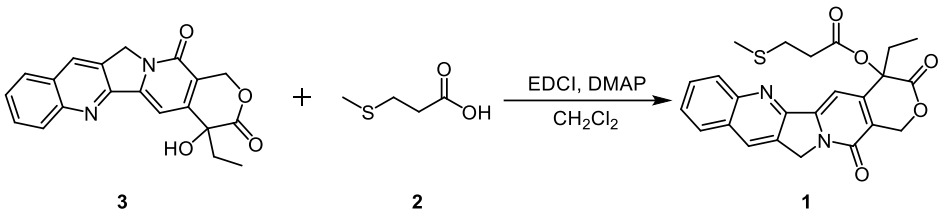
General Method

Routine NMR spectra were obtained on a Bruker AV II-400. The ^{1}H NMR chemical shifts were measured relative to $CDCl_3$ as the internal reference ($CDCl_3$: δ 7.26 ppm). The ^{13}C NMR chemical shifts were given using $CDCl_3$ as the internal standard ($CDCl_3$: δ 77.5 ppm). Mass spectrometry was performed on a Waters Q-Tof premier instrument. The particle sizes were measured with a Dynamic Light Scattering (DLS) Analyzer (Malvern Zetasizer Nano ZS90). Transmission electron microscopy (TEM) studies were carried out using a Tecnai G2F20S-TWIN instrument, operating at 120 kV. The TEM specimens were prepared by gently placing a carbon-coated copper grid on the surface of the sample. The TEM grid was then removed, stained with an aqueous solution of 2% phosphotungstic acid, dried for 0.5 h at room temperature, and then subjected to TEM observation. Gel permeation chromatography (GPC) was performed on an HLC-8320GPC instrument using N,N-Dimethylformamide (DMF) as the mobile phase at a flow rate of 1 mL/min at 40 ℃, and the molecular weights were reported relative to polystyrene (PS) standards. The *in vitro* release experiment of camptothecin (CPT) was performed in sink (AHYQ SHA-C) at 37 ℃. The CPT standard curve was analyzed at 254 nm by high performance liquid chromatography (HPLC, Agilent 1260 LC, Zorbax C18 column 4.6 ×150 mm) with 45% of buffer A and 55% of buffer B within 10 min and flow rate 1.0 mL/min (buffer A: deionized water, buffer B: Methanol). The cell viability was measured using a Thermo Varioskan Flash microplate reader (USA). Cell cycle assay, cell apoptosis assay and cell uptake were measured by flow cytometry (Becto Dickinson, USA). The Western Blotting Detection was measured by a ChemiDoc XR+UV illuminator (Bio-Rad, USA). Mean density of the bands for Bcl-2 and Caspase-3 in HT29 cells was analyzed using the Image J and the β-actin was used as the loading control. The tissue slices were imaged using inverted optical microscopy (Leica DMI4000B, Germany).

Chemicals: Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. All solvents for reactions were freshly distilled prior to use. Deionized water was used in all aqueous experiments.

Synthesis

Scheme S1 Synthesis of compound **1**



3 2 1

Compound 1. Camptothecin (100 mg, 0.287 mmol), 3-(methylthio) propionic acid (69 mg, 0.575 mmol), N-(3-(dimethylamino)-propyl)-N-ethylcarbodiimide hydrochloride (EDCI) (165 mg, 0.86 mmol), 4-dimethylaminopyridine (DMAP) (3.5 mg, 0.029 mmol) were dissolved in 30 mL of dichloromethane (CH_2Cl_2). The mixture was stirred at room temperature for 48 h, and then chloroform (30 mL) was added. The organic phase was washed with water (20 mL), saturated $NaHCO_3$ (10 mL) and brine (20 mL), and then dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified by column chromatography with CH_2Cl_2 – CH_3OH (30:1) to give **1** as a pale yellow solid (64 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.28 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.86 (t, J = 7.7 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.42 (s, 1H), 5.69 (d, J = 17.2 Hz, 1H), 5.40 (d, J = 17.2 Hz, 1H), 5.36 – 5.18 (m, 2H), 2.86 (dd, J = 10.3, 4.3 Hz, 2H), 2.78 (dd, J = 13.5, 7.2 Hz, 2H), 2.25 (ddd, J = 28.7, 14.5, 7.4 Hz, 2H), 2.14 (s, 3H), 1.00 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.03, 167.37, 157.30, 151.95, 148.17, 145.91, 145.67, 131.80, 131.05, 129.11, 128.59, 128.25, 128.21, 120.45, 96.97, 77.24, 76.18, 67.05, 49.98, 34.17, 31.76, 28.59, 15.57, 7.61. High resolution ESI-MS (m/z): calcd for $C_{24}H_{22}N_2O_5SH^+$ $[M+H]^+$ 450.1322, found: 451.1339, calcd for $C_{24}H_{22}N_2O_5SNa^+$ $[M+Na]^+$ 473.1142, found: 473.1134, calcd for $C_{24}H_{22}N_2O_5SK^+$ $[M+K]^+$ 489.1025, found: 489.1041.

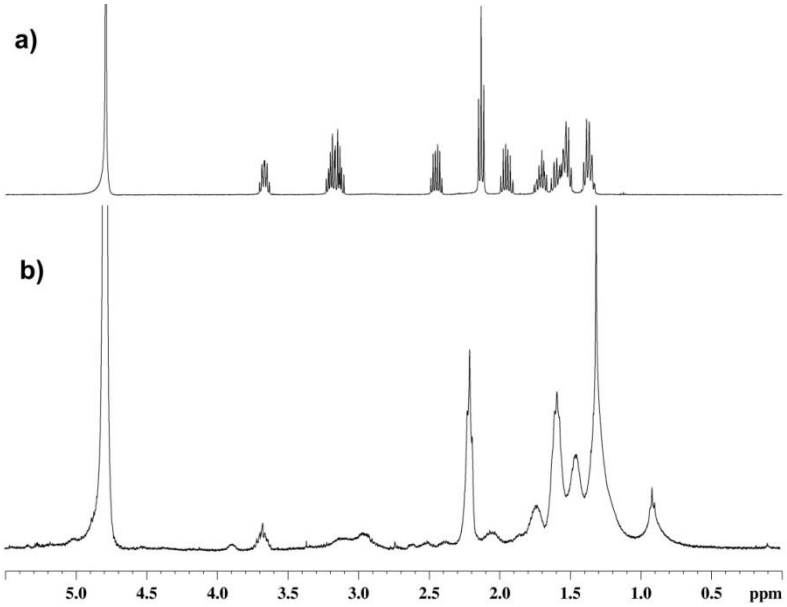


Fig. 1S ^{1}H NMR spectra of sodium lipoic acid queous solution in D_2O before a) and after b) cross-linking.

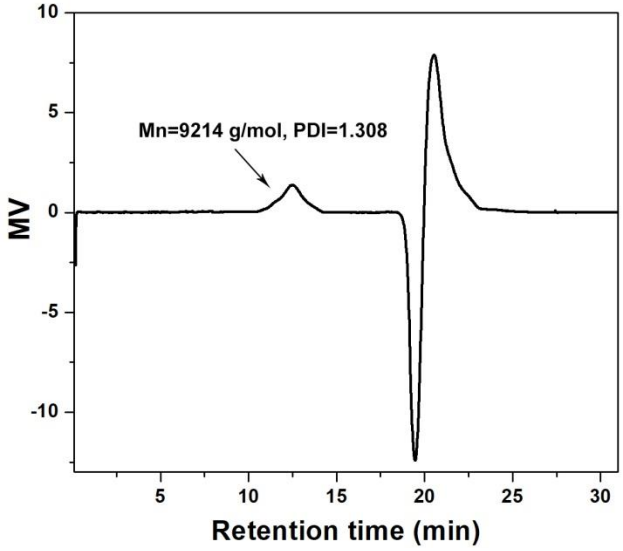


Fig. 2S GPC curve of the cLANs formed by sodium lipoic acid.

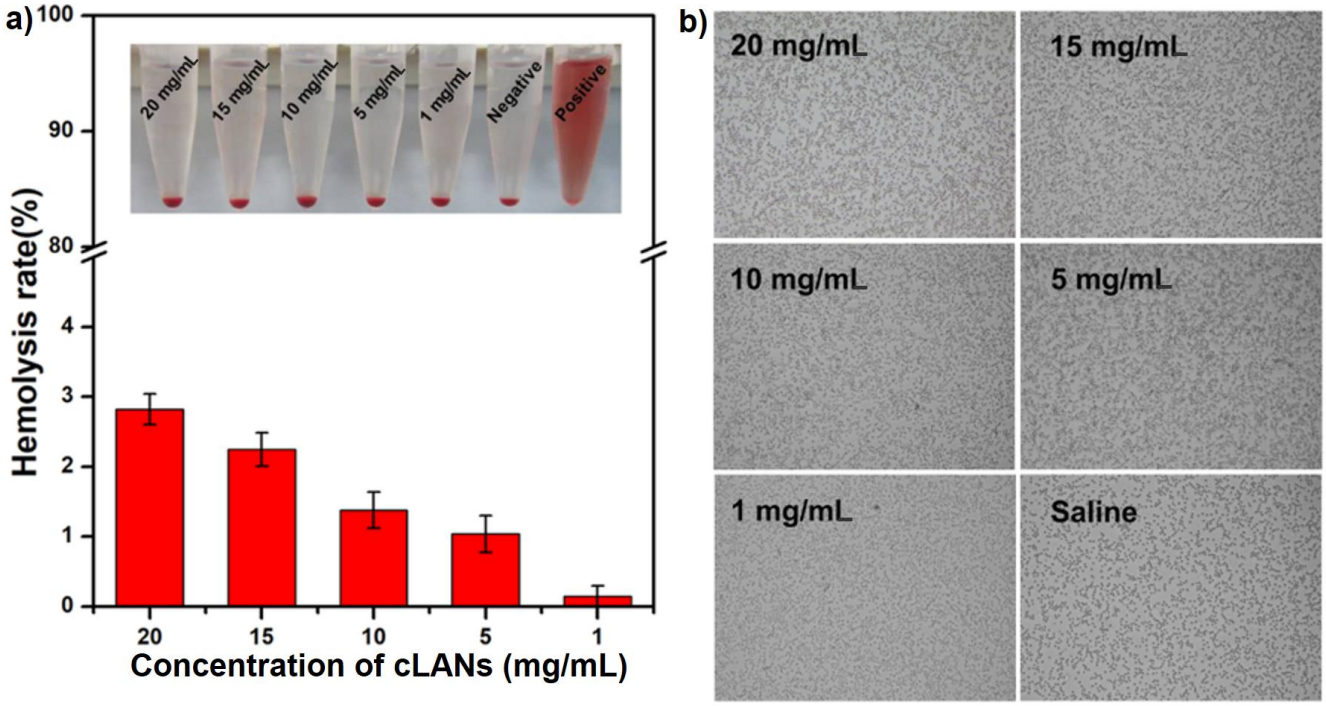


Fig. 3S *In vitro* biocompatibility of cLANs. a) Hemolysis rate of cLANs and the corresponding hemolysis pictures (inset) at different concentrations. b) Hemagglutination assay of cLANs at different concentrations. Saline was used as control. Saline and distilled water were used as negative and positive controls, respectively.

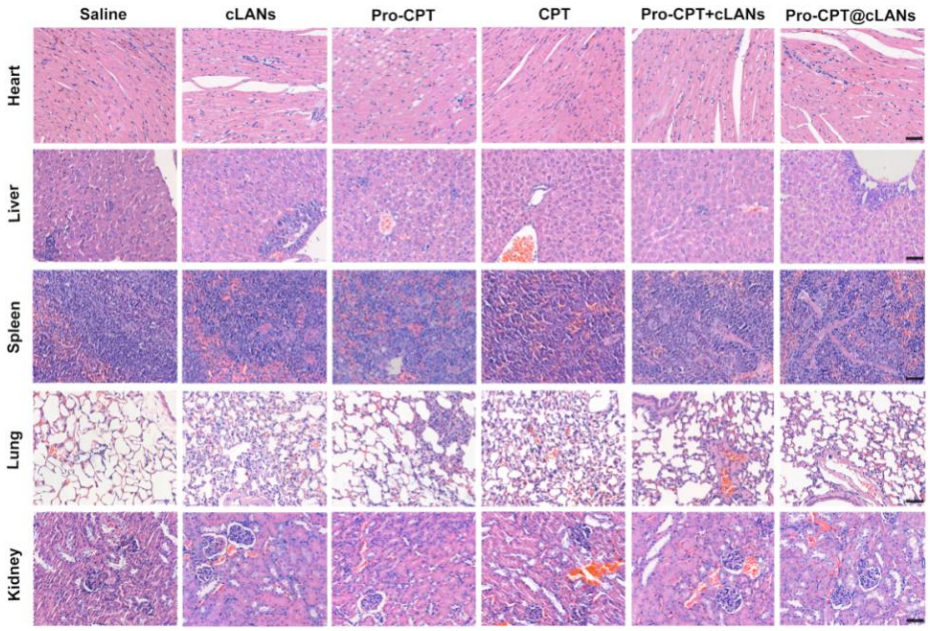


Fig. 4S Histological analysis of mice tissues treated with various treatments. Representative histological microphotographs of heart, liver, spleen, lung and kidney of HT29 tumor bearing nude mice treated with saline, cLANs, CPT, Pro-CPT, [Pro-CPT+cLANs] and Pro-CPT@cLANs, respectively (n = 5).

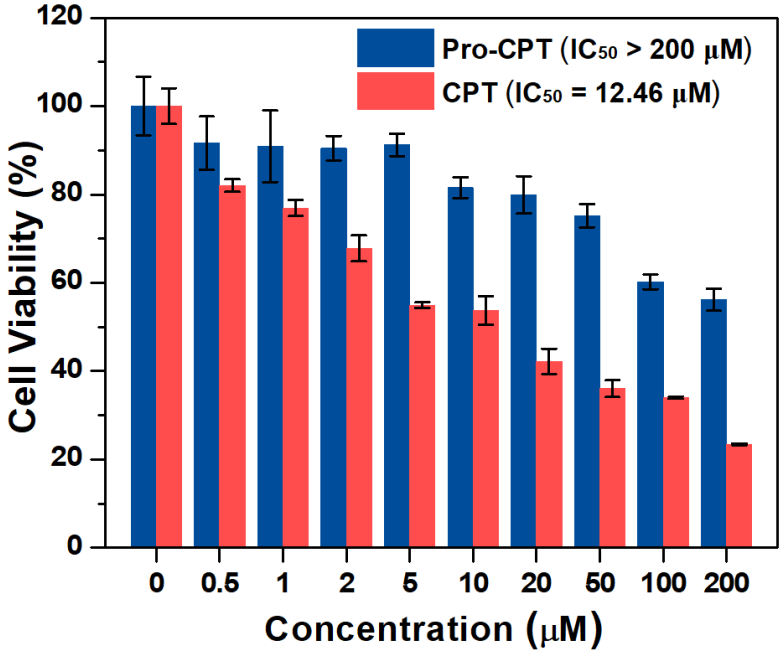


Fig. 5S Cell viability of CPT and Pro-CPT against L929 cells after incubation for 48 h at 37 °C with a series of concentrations.

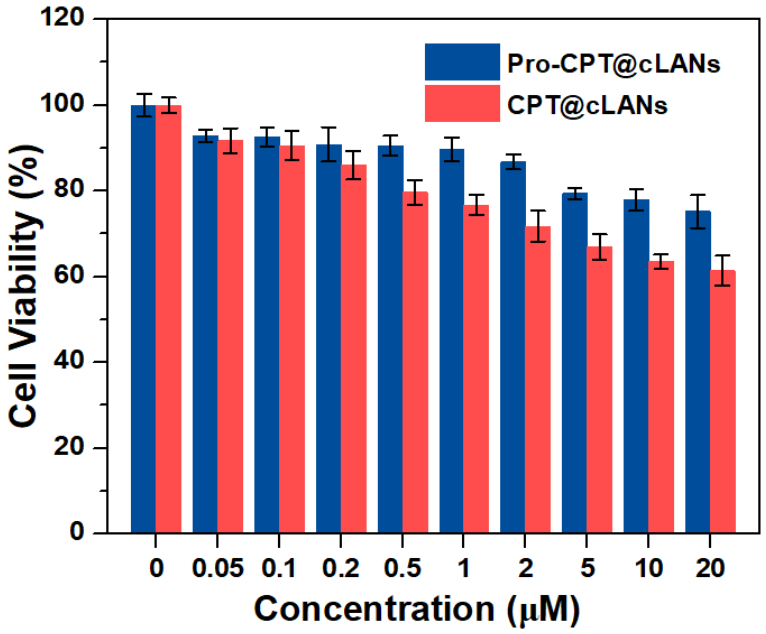


Fig. 6S Cell viability of CPT@cLANs and Pro-CPT@cLANs against L929 cells after incubation for 48 h at 37 °C with a series of concentrations.

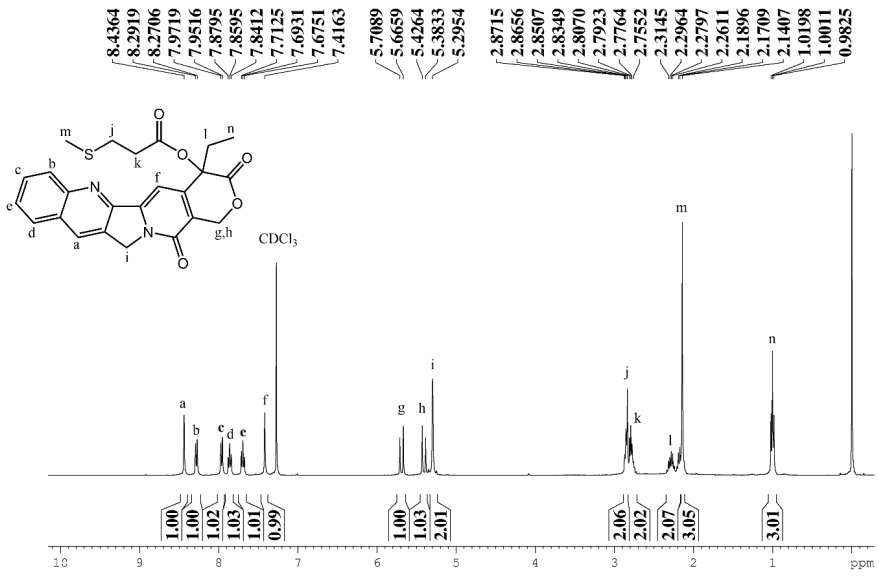


Fig. 7S ^{1}H NMR of Compound **1**.

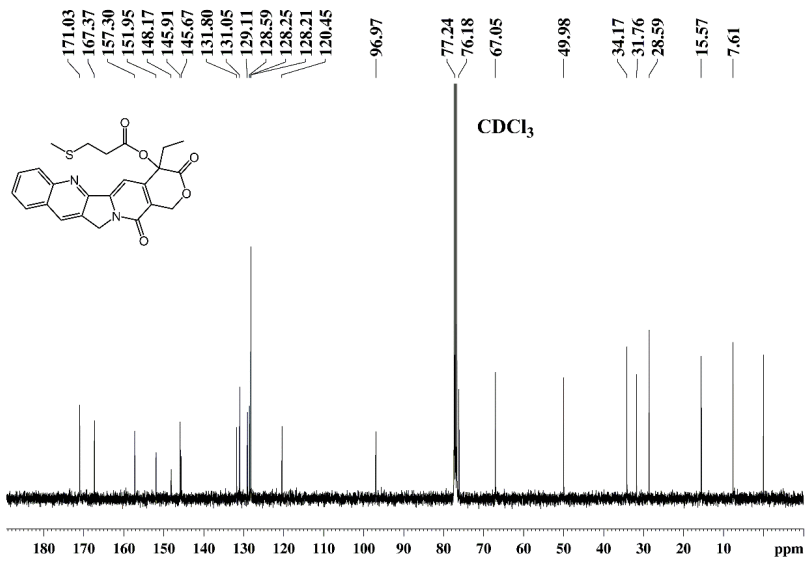


Fig. 8S ^{13}C NMR of Compound **1**.

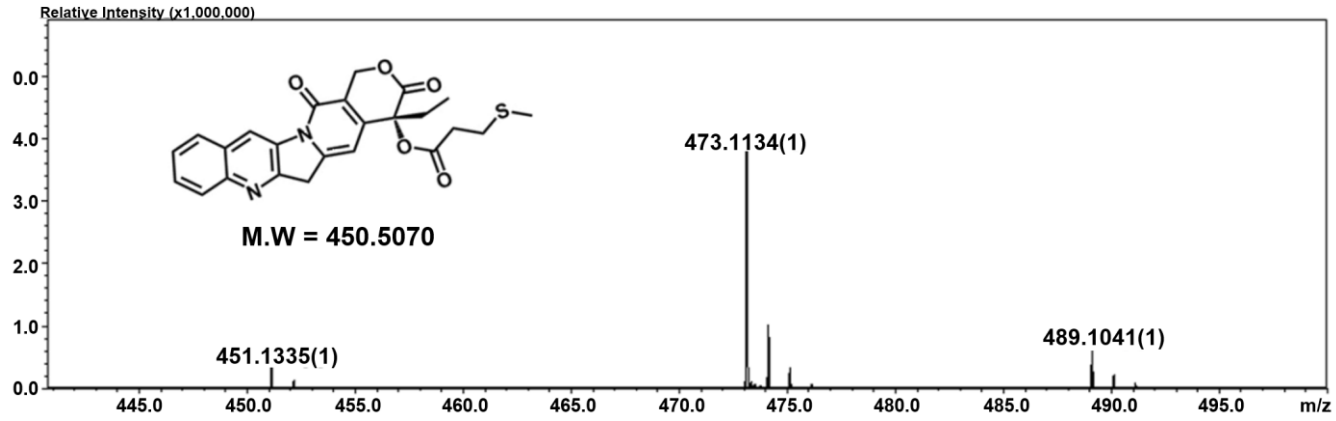


Fig. 9S MS Spectrum of Compound **1**.

Table S1 Zeta potential of the cLANs in PBS buffer and in culture medium of RPMI-1640 containing 10% FBS.

pH	Zeta Potential (mV)	
	PBS	RPMI-1640
7.4	-13.2	-6.7
6.5	-8.9	-3.4