Supporting Information

The design and synthesis redox-responsive oridonin polymeric prodrug micelle formulation for effective gastric cancer therapy

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Experimental section

Materials

ORI, and 3,3′-dithiodipropionic acid (DTPA) were purchased from Aldrich, and the 3,3′-dithiodipropionic acid anhydride (DTPAA) was prepared according to a previous report.[1] N^{ε}-benzyloxycarbonyl-lysine-N-carboxyanhydride (Lys(Z)-NCA) was obtained from JINJINLE CHEMICAL CO., LTD (Shanghai, China) and was used as received. Methoxy polyethylene glycol functionalized amine (PEG-NH_2, molecular weight [MW]: 5000 Da) was obtained from Aladdin (Shanghai, China) and dehydrated by azeotrope with toluene. Dry *N,N*-dimethylformamide was obtained from Energy Chemical (Shanghai, China). Coumarin-6 was obtained from J&K Scientific Ltd. (Shanghai, China). 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI), 4% paraformaldehyde fix Solution, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased form Beyotime Biotechnology (Shanghai, China).

Cell and animals

Human GC cell lines MGC 803 cells and SGC 7901 cells were cultured in RPMI 1640 containing 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 at 37°C.

Sprague Dawley (SD) rats (male, 250-320 g, 5-6 weeks) and BALB/c-nu mice (male, 18-20 g, 4-6 weeks) were purchased from Beijing Vital River Laboratory Animal Technology CO., Ltd and used under the approval of Animal Care and Use Committee of Nanjing University of Chinese Medicine.

Characterization

The UV spectrum was recorded on a UV-visible spectrophotometer (UV-2450, Shimadzu, Japan). The ^{1}H NMR spectrum was detected by a Bruker (AVANCE) spectrometer (AV-300, Bruker, USA). High performance liquid chromatography (HPLC) analyses were performed using a Shimadzu HPLC system consisting of LC-20 binary pump, SPD-20A UV detector, and an agilenttc-C18 column (250 × 4.6 mm, 5 μm). Methanol/water (55/45, v/v) was used as the mobile phase at 25°C with a flow rate of 1.0 mL/min. The UV detector was set at 262 nm. The size, size distribution, and surface zeta potential of particles in aqueous solution were measured by dynamic light scattering (DLS) carried out on a Malvern Zetasizer Nano ZS90 (UK). Transmission electron microscopy (TEM, H-600, Hitachi, Japan) was explored to visualize the size and shape of micelles.

Critical micelle concentration measurement

Nile Red was employed as the fluorescence probe to investigate the critical micelle concentration (CMC) value of the ORI prodrug. In brief, PEG-*b*-PLL-ss-ORI was dissolved in phosphate buffered saline (PBS) at concentrations ranging from 0.01 to 1000 μg/mL. Then, the Nile Red solution (1.0 mg/mL in DMSO) was added to a final concentration of 0.1 mM. After incubation in the dark at room temperature for 12 h, the fluorescence intensity of these solutions was recorded on a fluorescence spectrometer (F-7000, Shimadzu, Japan).

Hemolysis study

Freshly drawn mouse blood was diluted in saline. Red blood cells (RBCs) were

collected by centrifugation and further diluted by saline. Subsequently, four ORI micelles solutions in saline with various concentration were mixed with the RBCs solution and maintained at 37°C for 2 h in a thermotank. After incubation, the mixture was centrifuged and the supernatant of each sample was collected. The absorbance of the supernatant was read by a microplate reader at 540 nm. Triton X-100 (10 mg/mL) and saline were employed as positive and negative control, respectively. The hemolysis ratio (HR) of RBCs was calculated according to the following equation:

$$HR\% = (A_{sample} - A_{negative}) / (A_{positive} - A_{negative}) \times 100\%$$

where, the A_{smaple}, $A_{negative}$, and $A_{positive}$ indicates the absorbance of sample, negative control, and positive control, respectively.

Supporting figures and tables

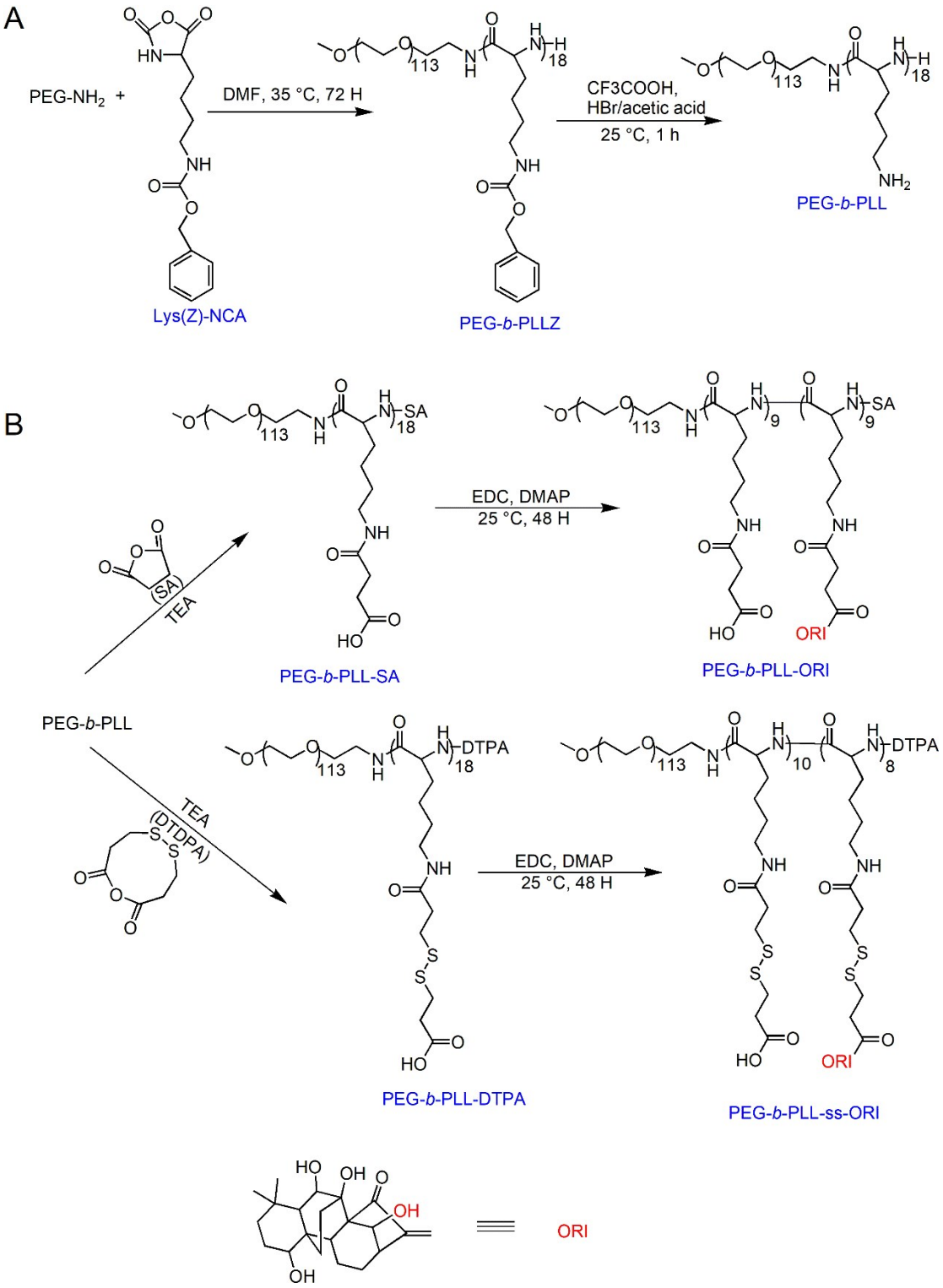

Fig. S1. The synthesis route of ORI polymeric prodrug.

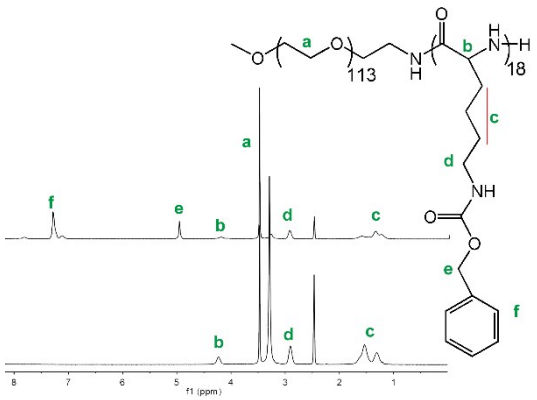

Fig. S2 ^{1}H NMR spectrum of PEG-*b*-PLLZ (above) and PEG-*b*-PLL (below).

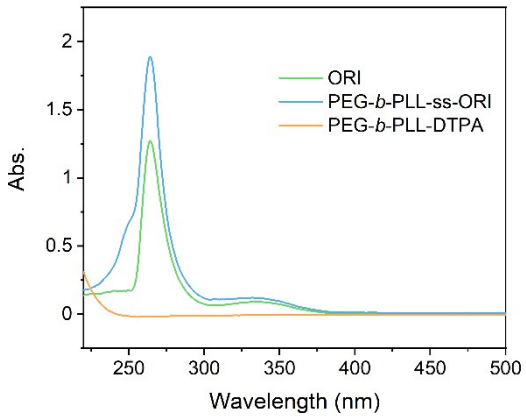

Fig. S3 UV-visible spectrum of ORI, PEG-*b*-PLL-DTPA, and PEG-*b*-PLL-ss-ORI.

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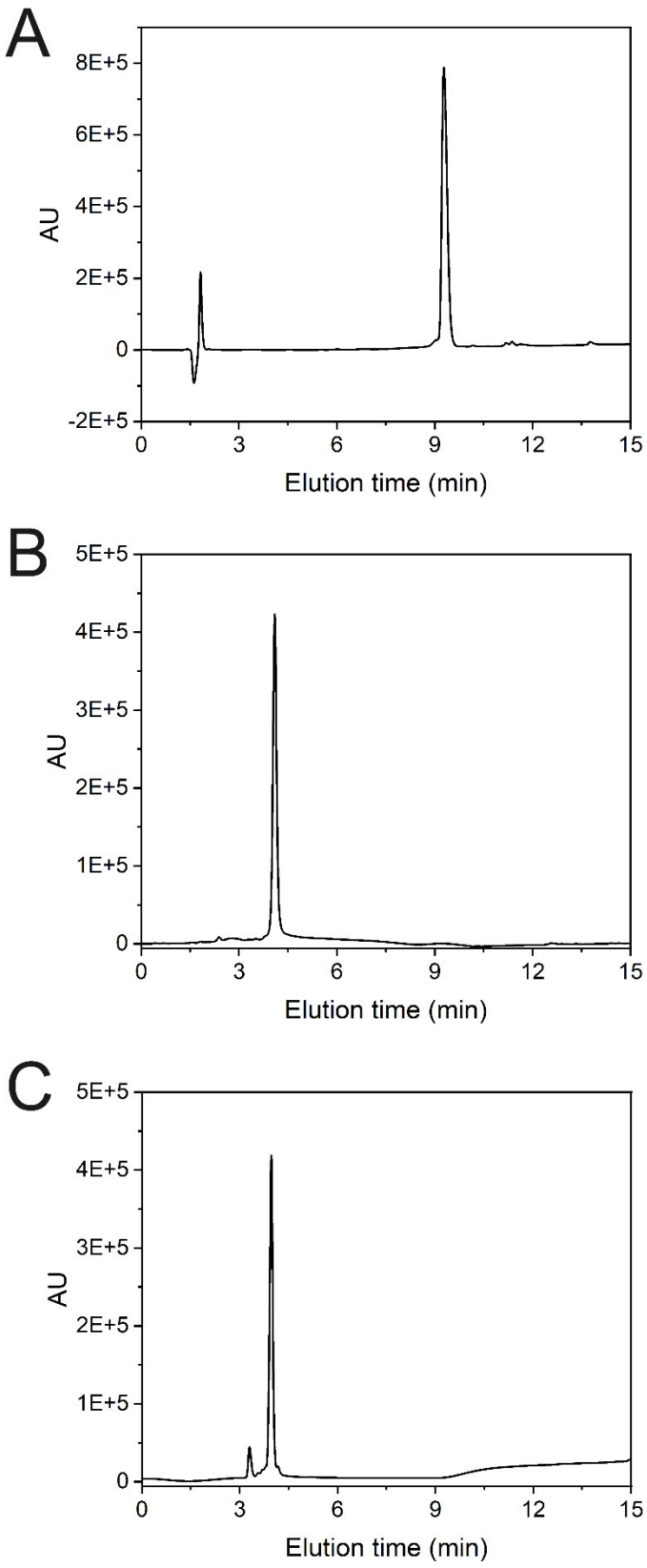

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155 **Fig. S4** The HPLC measurements of free ORI (A, 0.25 mg/mL), PEG-b-PLL-ss-ORI (B, 1.2 mg/mL), and

156 PEG-b-PLL-ORI (C, 1.2 mg/mL).

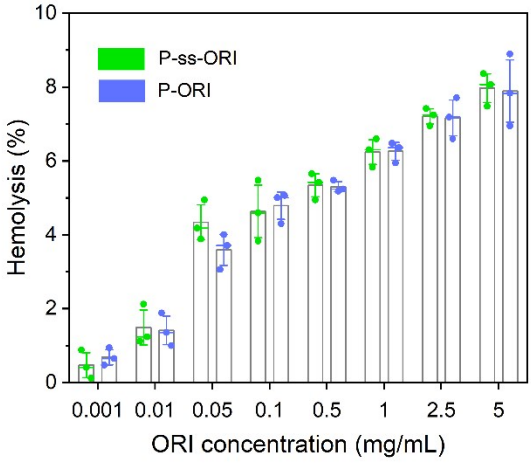

Fig. S5 Hemolysis of P-ss-ORI and P-ORI (*n* = 3).

Table S1. IC50 value of ORI, P-ORI, and P-ss-ORI against SGC7901 and BGC 823 cells (μg/mL).

Cells	ORI	P-ORI	P-ss-ORI
SGC-7901	26.1	81.7	14.2
BGC 823	13.2	29.7	8.6

References:

[1] L. Jia, Z. Li, D. Zhang, Q. Zhang, J. Shen, H. Guo, X. Tian, G. Liu, D. Zheng, L. Qi, *Polym. Chem.* **2013**, *4*, 156-165.