# Low molecular weight heparin-based reduction-sensitive nanoparticles for antitumor and anti-metastasis of orthotopic breast cancer

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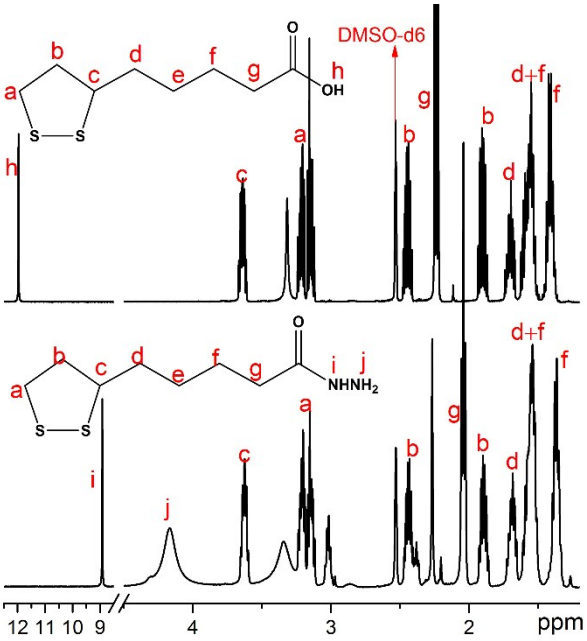


**Fig. S1** $^{1}$H NMR spectra of LA and LA-hydraizde.

The structure was characterized by $^{1}$H NMR (500 MHz, DMSO-$d_6$): δ (ppm) = 8.92 (s，1H，-C(=O)N***H***$NH_2$)，4.17 (br，2H，-C(=O)NHN***H*****$_2$**)，3.60-3.65 (m，1H，-SSC***H***-)，3.12-3.24 (m，2H，-SSC***H*****$_2$**$CH_2$-)，2.38-2.46 (m，1H，-$SSCH_2$C***H*****$_2$**-)，2.03-2.06 (m，2H，-C***H*****$_2$**C(=O)NH-)，1.86-1.98 (m，1H，-$SSCH_2$C***H*****$_2$**-), 1.51-1.73 (m，4H，-C***H*****$_2$**$CH_2$C***H*****$_2$**$CH_2$C(=O)NH-)，1.34-1.39 (m，2H，-$CH_2$C***H*****$_2$**$CH_2CH_2$C(=O)NH-).

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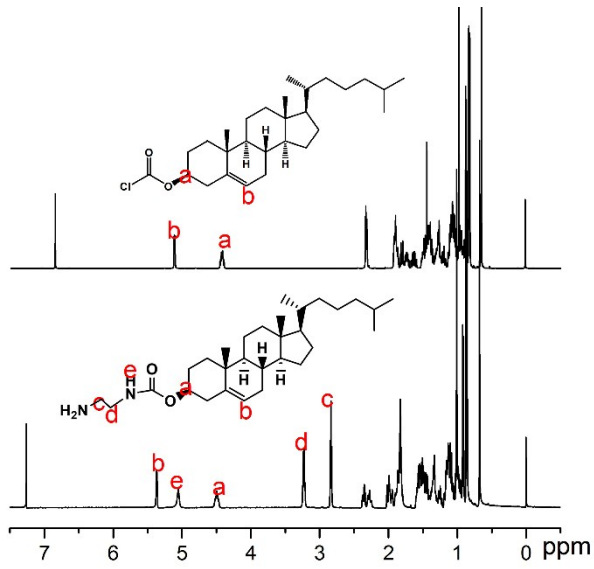


**Fig. S2** $^{1}$H NMR spectra of Chol and Chol-eda.

The Chol-eda was confrmed by $^{1}$H NMR in $CDCl_3$ (500 MHz, $CDCl_3$): δ (ppm) 2.85 (t, 2H, $NH_2C\underline{\boldsymbol{H_2}}CH_2$-), 3.24 (q, 2H, -NHC$\underline{\boldsymbol{H_2}}CH_2$-), 4.50 (s, 1H, -C$\underline{\boldsymbol{H}}(CH_2\text{-})_2$), 5.05 (s, 1H, -C(=O)N$\underline{\boldsymbol{H}}$-), 5.36 (s, 1H, $CH_2C\underline{\boldsymbol{H}}$=).

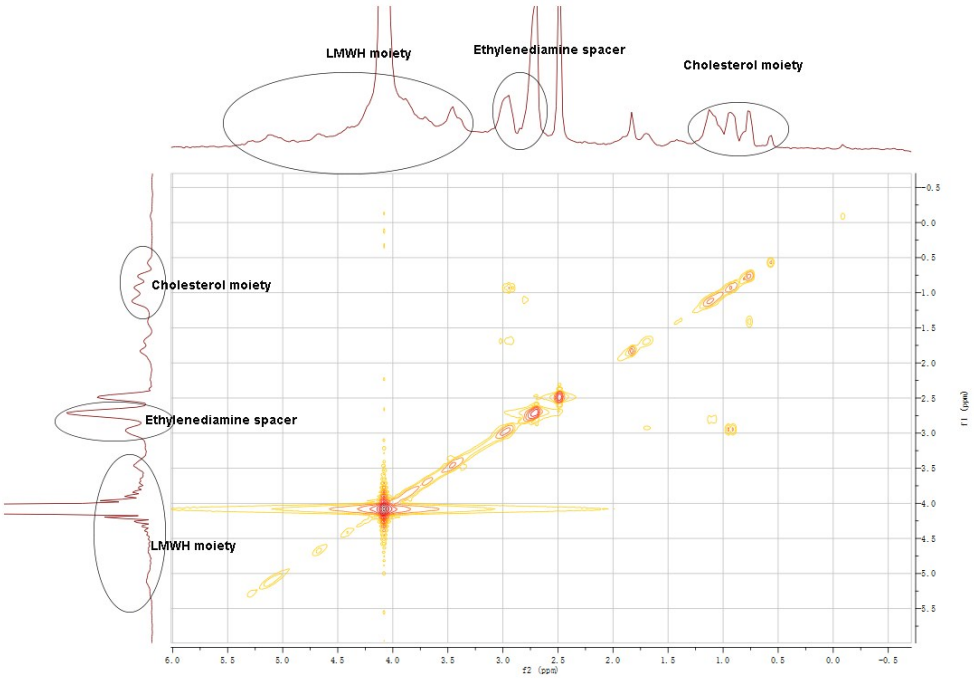


**Fig. S3** $^{1}$H-$^{1}$H COSY spectrum of $LHC_2$

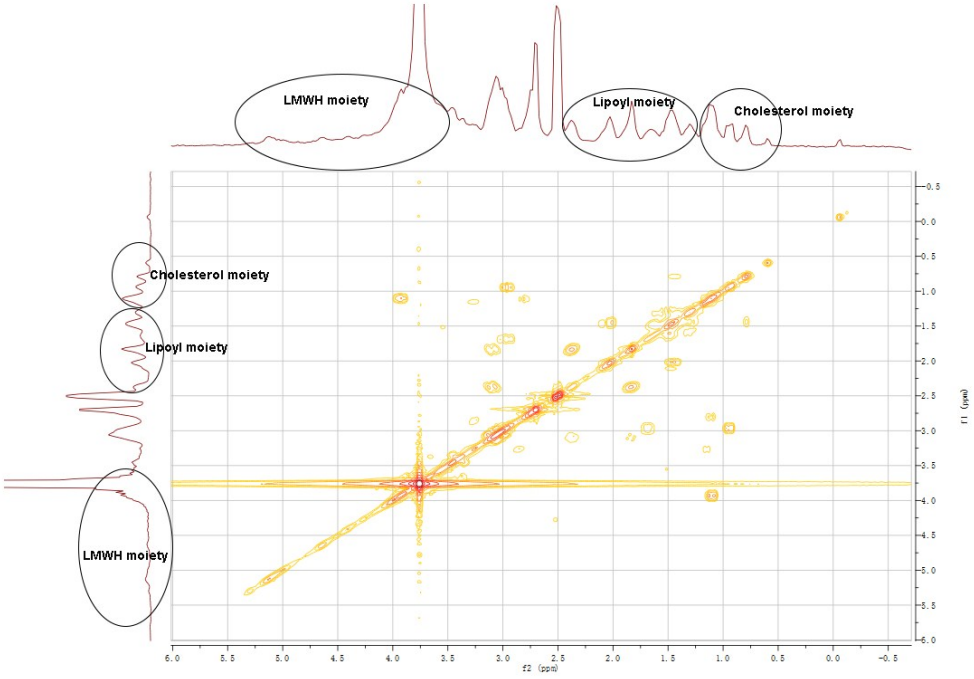


**Fig. S4** $^{1}H$-$^{1}H$ COSY spectrum of $LLHC_2$

The Cholesterol modified LMWH was confirmed by the characteristic peaks of the methyl protons in Chol-eda at 0.57-1.13 ppm. Additionally, ethylenediamine spacer also appeared at 2.73-2.89 ppm, indicating the successful conjugation of Chol-eda to LMWH (**Fig. S3**). For $LLHC_2$ polymer, the characteristic peaks of lipoyl moiety protons appeared at 1.30, 1.47, 1.69, 1.83, 2.03 and 1.83 ppm and the peaks at 0.59-1.17 ppm of Chol-eda protons also emerged (**Fig. S4**). These results indicate that LMWH has been successfully conjugated to cholesterol and lipoic acid.

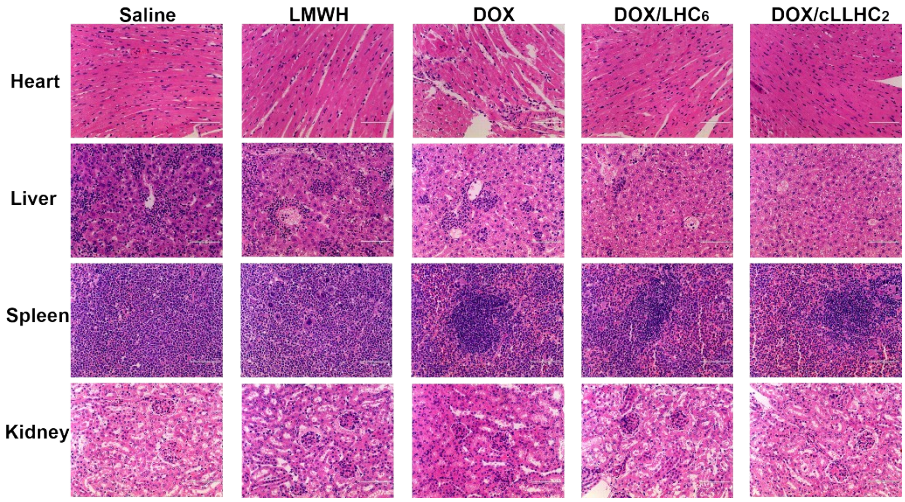


**Fig. S5** H&E analysis of heart, liver, spleen and kidney tissues from mice treated with

the different formulations. Scale bar reads 400 μm.

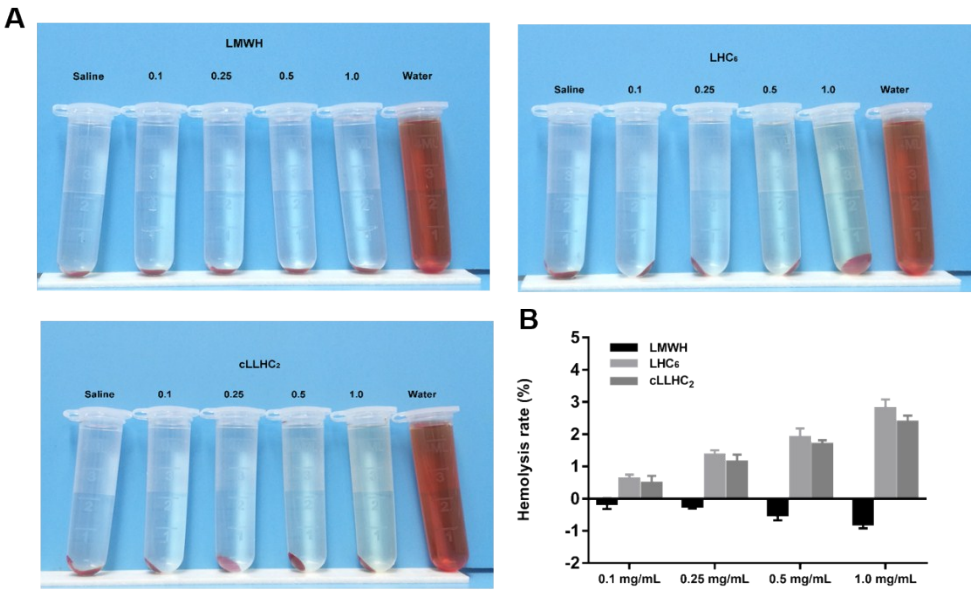


**Fig. S6** (A) Images of the hemolysis of red blood cells treated with different polymers after centrifugation; (B) The hemolysis of different groups at different concentrations ($n$ = 3).

**Table S1** Summary of pharmacokinetic parameters for DOX, DOX/$LHC_6$ and DOX/$cLLHC_2$

| Parameters | DOX | DOX/$LHC_6$ | DOX/$cLLHC_2$ |
|---|---|---|---|
| $AUC_{0-t}$ (μg/mL * h) | 2.35 ± 0.18 | 61.42 ± 8.93** | 78.57 ± 15.77** |
| $t_{1/2\alpha}$ (h) | 0.28 ± 0.04 | 0.58 ± 0.05** | 0.57 ± 0.08** |
| $t_{1/2\beta}$ (h) | 3.60 ± 1.28 | 7.43 ± 0.68* | 11.63 ± 4.10* |
| CL (L/h/kg) | 1.70 ± 0.03 | 0.08 ± 0.01** | 0.06 ± 0.01** |
| $K_{10}$ (1/h) | 0.91 ± 0.17 | 0.60 ± 0.03* | 0.57 ± 0.10* |
| $K_{12}$ (1/h) | 1.42 ± 0.84 | 0.39 ± 0.34 | 0.60 ± 0.24 |
| $K_{21}$ (1/h) | 0.64 ± 0.21 | 0.16 ± 0.01* | 0.13 ± 0.002* |

*$p$ < 0.05 *vs* free DOX, **$p$ < 0.01 *vs* free DOX, $n$ = 3.