

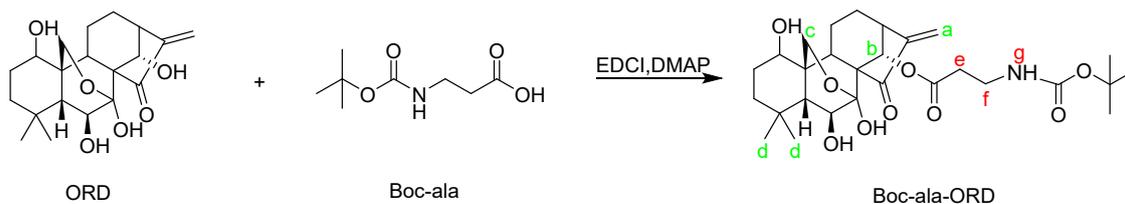
Resolving the hepatic fibrosis via suppressing oxidative stress and inflammatory response by novel hyaluronic acid modified nanocomplex

Lianjie Ren,^{‡^{u,b,e}} Jingjing Li,^{‡^{u,b}} Lisha Liu,^{‡^{u,b}} Wantao Wu,^{a,b} Di Zhao,^{a,b} Kai Zhang,^{a,b} Xiaofei Xin,^{a,b} Lei Yang^{*^{a,b}} and Lifang Yin^{*^{a,b,c,d}}

- a. Jiangsu Province Engineering Research Center for R&D and Evaluation of Intelligent Drugs and Key Functional Excipients, China Pharmaceutical University, Nanjing 210009, China
- b. Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China
- c. State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China
- d. Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, Nanjing 210009, China
- e. Center for Drug Evaluation, NMPA, Beijing 100022, China

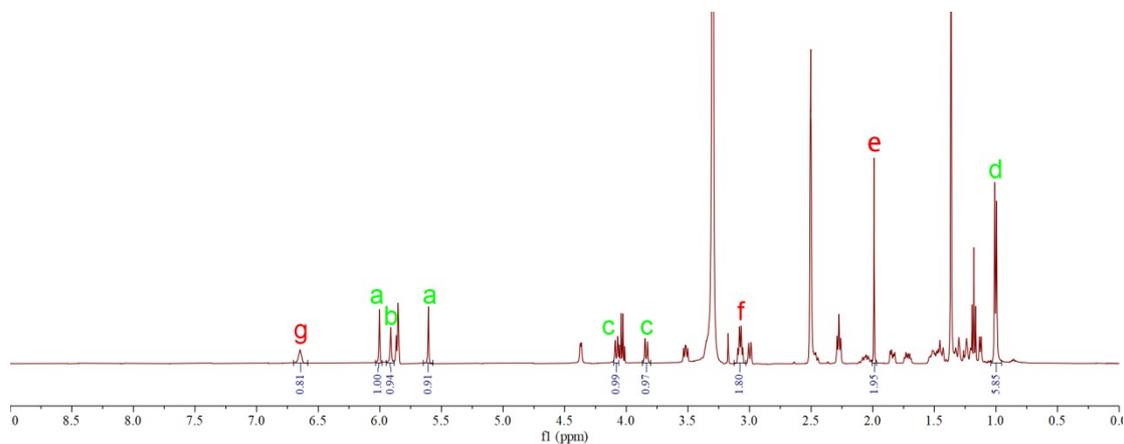
*Corresponding Authors: Lei Yang, E-mail: yangleisypu@126.com; Lifang Yin, E-mail: lifangyin_@163.com.

‡These authors contributed equally to this article.

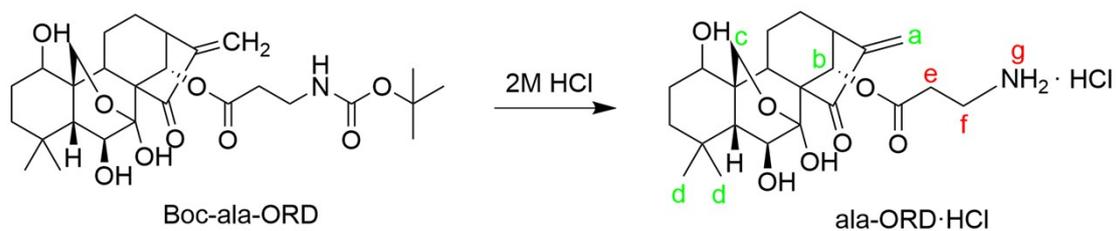


Supplementary Figure 1. The synthesis of Boc-ala-ORD Oridonin (500mg, 1.37mmole) and Boc-ala (311mg, 1.64 mmole) was dissolved in 30ml of CH_2Cl_2 . After that, EDCI (263.01mg, 1.37mmole) was added to the solution. After 10 minutes of mixture by stirring the solution, DMAP (167mg, 1.372mole) was also added to the solution. The mixed solution was stirred at room temperature overnight. Subsequently, the solution of the mixture was purified by the column chromatography with a constant elution (ethyl acetate : petroleum ether = 5:1 by volume) and confirmed by $^1\text{H-NMR}$ analysis. 638mg of Boc-ala-ORD as a white powder were collected after purification and conformation, yield 84%.

$^1\text{H-NMR}$ analysis:

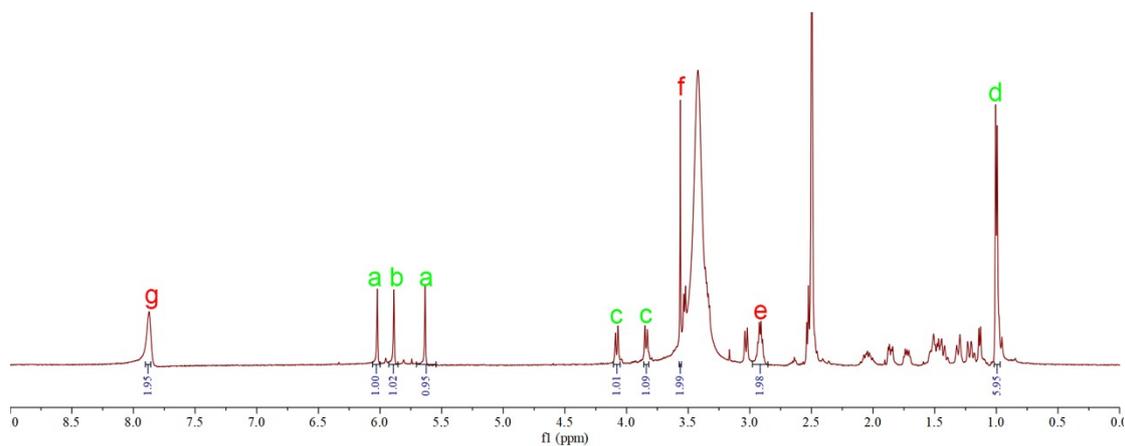


$^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$, δ) : 6.65 (s, 1H), 6.00 (s, 1H), 5.91 (s, 1H), 5.61 (s, 1H), 4.08 (d, J = 10.2 Hz, 1H), 3.84 (d, J = 10.2 Hz, 1H), 3.07 (q, J = 6.8 Hz, 2H), 1.99 (s, 2H), 1.00 (d, J = 7.1 Hz, 6H).

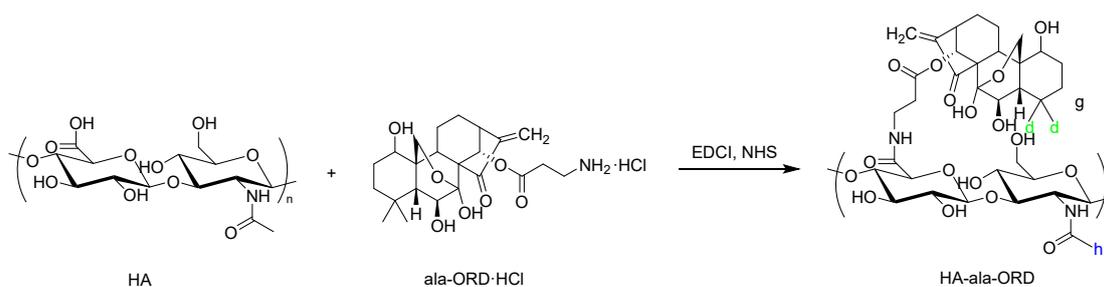


Supplementary Figure 2. The synthesis of ala-ORD·HCl Boc-ala-ORD (638mg, 1.15mmole) was put into the 2M HCl in 1,4-Dioxane solution to put off tert-butyl groups. The solution was stirred at room temperature for 24 hours. The resulting solution was evaporated in vacuum chamber for 12hours and 540 mg ala-ORD·HCl was collected, yield 100%.

¹H-NMR analysis:

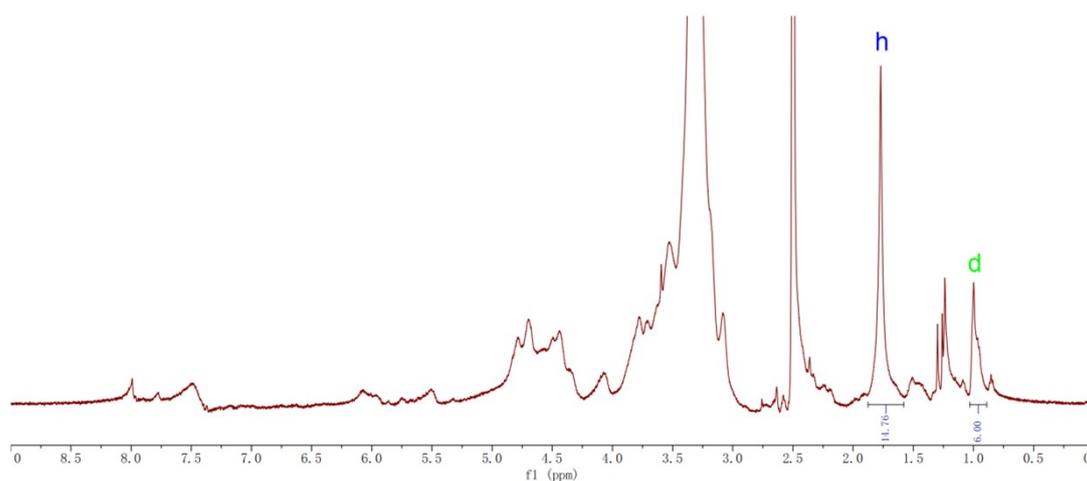


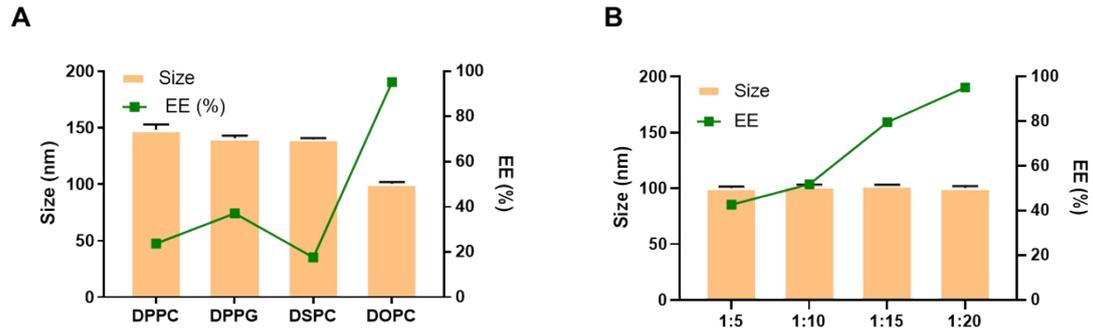
¹H NMR (500 MHz, DMSO-*d*₆, δ) : 7.88 (d, *J* = 9.8 Hz, 2H), 6.02 (s, 1H), 5.89 (s, 1H), 5.63 (s, 1H), 4.08 (d, *J* = 10.3 Hz, 1H), 3.84 (d, *J* = 10.2 Hz, 1H), 3.56 (s, 2H), 2.92 (h, *J* = 7.0, 6.4 Hz, 2H), 1.00 (d, *J* = 6.6 Hz, 6H).



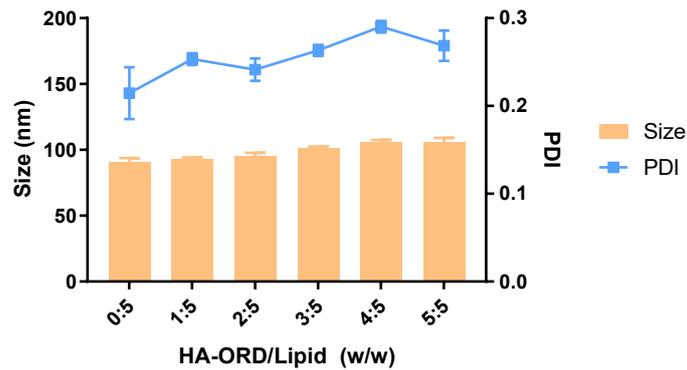
Supplementary Figure 3. The synthesis of HA-ala-ORD hyaluronic acid (100mg, 0.26mmole as a carboxyl group equivalent) and ala-ORD·HCl (247mg, 0.53mmole) was dissolved in 20 mL of formamide. EDCI (253mg, 1.32mmole) and NHS (152mg, 1.32mmole) were added to the formamide solution and stirred at room temperature overnight. After the overnight stirring, the mixture was dialyzed in an excess amount of deionized water with a nominal molecular weight of 3.5kDa for 48 hours. The resulting solution was lyophilized to get the HA-ala-ORD as a white powder with 98 mg, yield 46%.

¹H-NMR analysis:

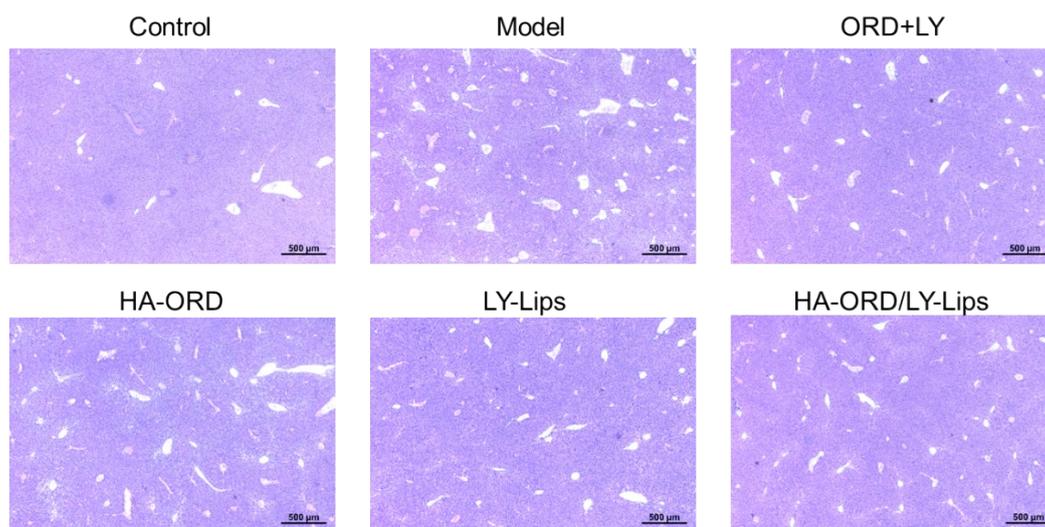




Supplementary Figure 4. Preparation and characterization of different LY-Lips particles. (A) The effect of different phospholipids on size distribution and PDI of LY-Lips. (B) The effect of different drug/DOPC ratio on size distribution and entrapment efficiency of LY-Lips.



Supplementary Figure 5. Preparation and characterization of different HA-ORD/LY-Lips. (A) The effect of different HA/Lipid ratios on size distribution and PDI. (B) The effect of different HA/Lipid ratios on zeta potential.



Supplementary Figure 6. The H&E staining results of liver tissue section after different tested formulations.

Supporting Table 1. Dosing regimen for the therapeutic efficacy study

Group	Name	Dosing Regimen
1	Healthy control (C)	Without any treatment
2	Model control (M)	Model mice
3	ORD + LY solution	Model mice, 10 mg/kg LY and 5 mg/kg ORD, twice a week
4	HA-ORD prodrug	Model mice, 5 mg/kg ORD, twice a week
5	LY/Lips	Model mice, 10 mg/kg LY, twice a week
6	HA-ORD/LY-Lips	Model mice, 10 mg/kg LY and 5 mg/kg ORD, twice a week