

## Supporting Information

# **Directed co-assembly of herbal small molecules into carrier-free nanodrugs for enhanced synergistic antitumor efficacy**

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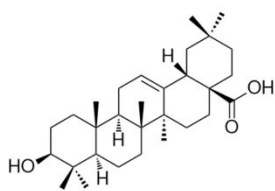
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Street, Nan Gang District, Harbin, Heilongjiang, 150001, P.R.China

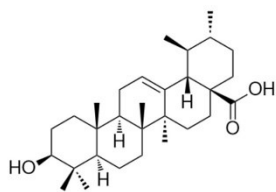
b. Department of Ophthalmology, the Second Hospital, Jilin University, No.4026 Yatai street, Nangan

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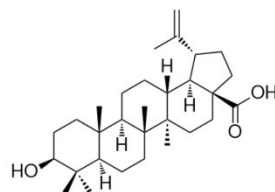
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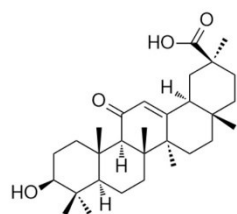
**Oleanolic acid**



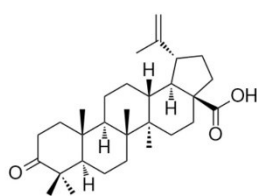
**Ursolic acid**



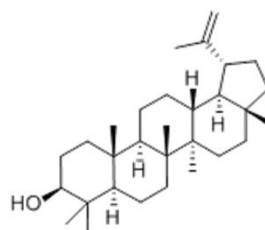
**Betulinic acid**



**Glycyrrhetic acid**



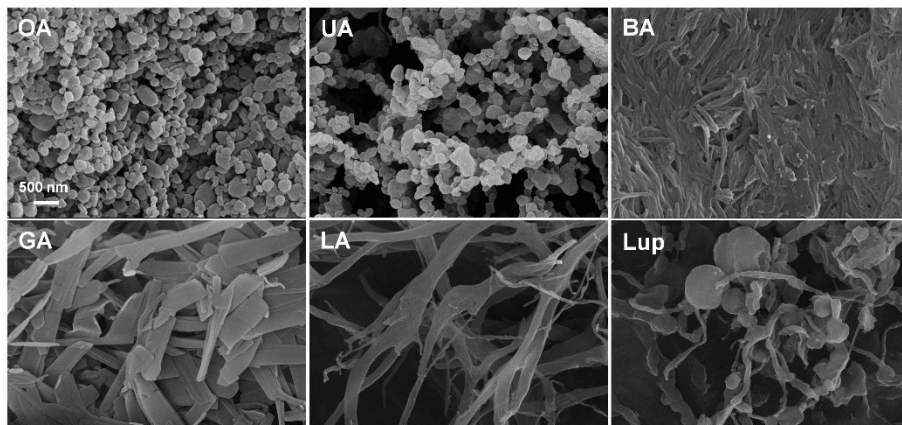
**Liquidambaric acid**



**Lupeol**

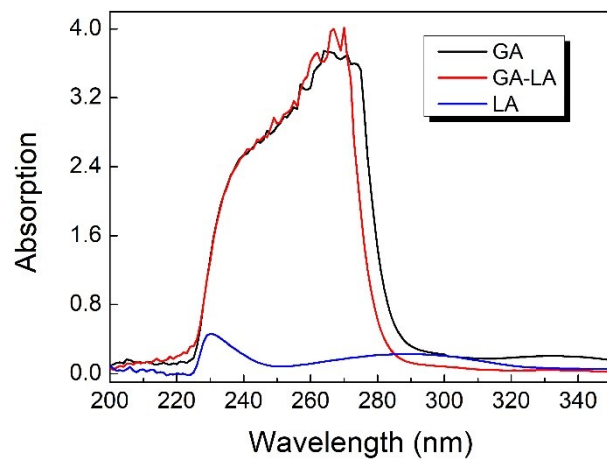
**Scheme S1.** Structural formula of of pentacyclic triterpenoids

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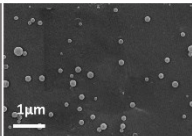
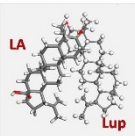
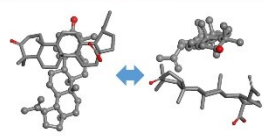
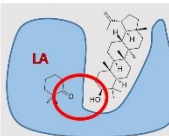
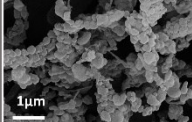
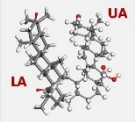
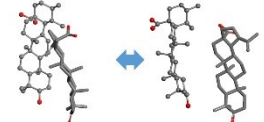
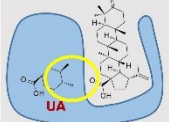
**Figure S1** SEM images of self-assembled nanoparticles of pentacyclic triterpenoids

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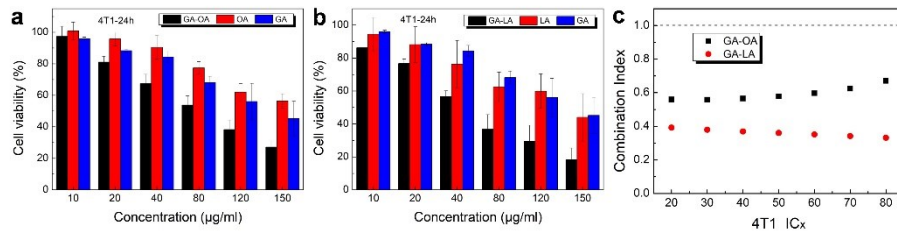


**Figure S2** Overlay of UV-visible spectrum of GA, LA and GA-LA

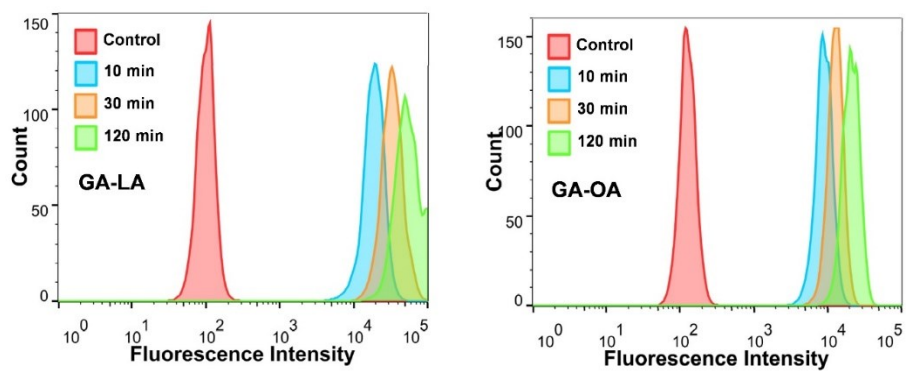
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Group		Micro-morphology	Molecular configurations	Molecular skeleton configuration	Hydrophobic surface
Co-assembly	LA + Lup				
Non-co-assembly	UA + LA				

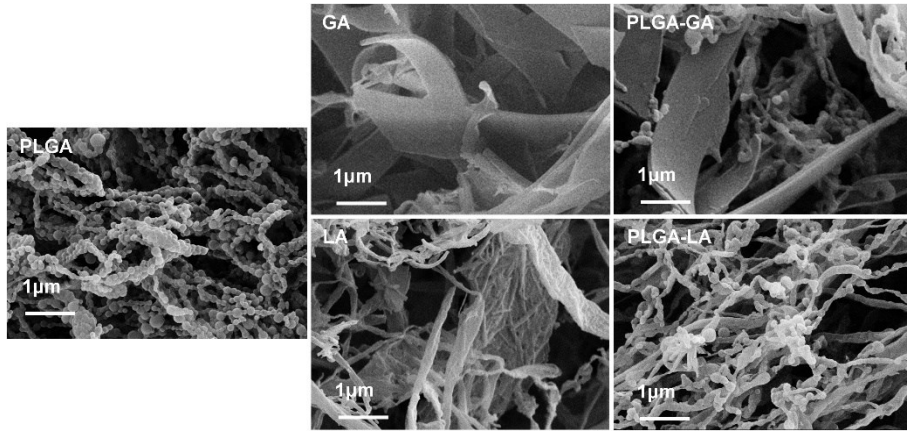
**Figure S3** Analysis of molecular dynamics simulation results. Cluster analysis of co-assembled and non-co-assembled NPs. Two molecules in the co-assembled nanoparticles form a parallel plane space configuration, while the two molecules in the non-co-assembled nanoparticles form a spatial configuration. Red circle represents hydrophilic chain; yellow circle represents hydrophobic chain



**Figure S4** Cytotoxicity evaluation of GA-OA and GA-LA NPs. **a.b** The growth inhibitory effects of GA-OA and GA-LA NPs on 4T1 cells for 24h **c.** Combination index (CI) of GA-OA and GA-LA on 4T1 cells. All of the CI values of GA-OA and GA-LA NPs are less than 1.0 indicate synergy



**Figure S5** Cellular uptake capability of NPs in MCF-7 cells. Flow cytometry analysis of cell phagocytosis of GA-LA and GA-OA NPs



**Figure S6** SEM images of SEM images of PLGA loading GA and PLGA loading LA

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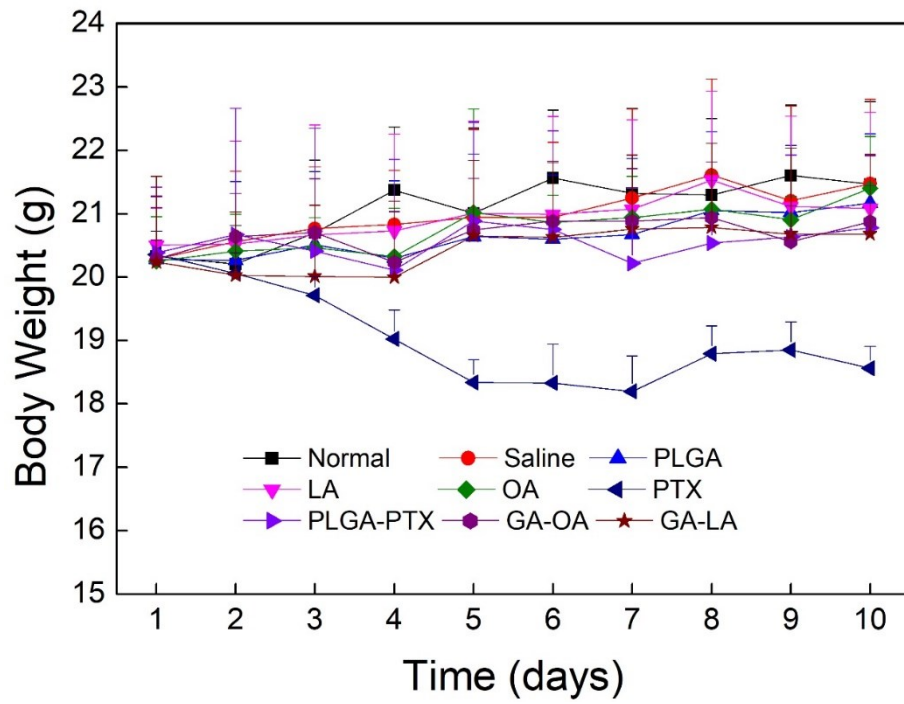
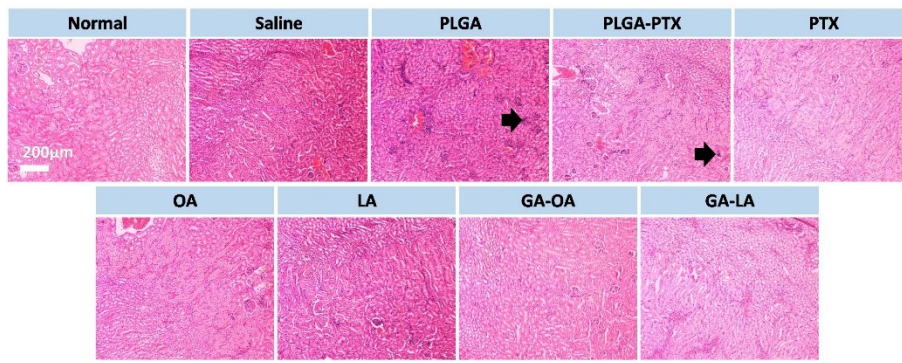


Figure S7 The weight change of the mice during the treatment with various drug formulations



**Figure S8** Representative images of H&E-stained sections of kidneys (Black arrows represent inflammatory cell infiltration)

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Formula Abbreviation	Diameter (nm)	PDI	Zeta potential (mV)
OA	233 ± 2	0.05 ± 0.03	-20.5 ± 1.6
UA	275 ± 5	0.05 ± 0.01	-21.3 ± 1.2
BA	1811 ± 280	0.81 ± 0.11	-13.3 ± 0.4
GA	543 ± 26	0.32 ± 0.06	-12.9 ± 1.1
LA	878 ± 18	0.43 ± 0.11	-8.1 ± 0.9
Lup	334 ± 22	0.32±0.11	-11.4 ± 1.4

**Table S1** Particle size, polydispersity index (PDI) and *Zeta* potentials of self-assembly NPs measured by DLS

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Formula Abbreviation	Diameter (nm)	PDI	Zeta potential (Mv)
OA-BA	341 ± 18	0.20 ± 0.04	-15.6 ± 0.7
GA-LA	228 ± 10	0.03 ± 0.02	-16.5 ± 1.3
LA-Lup	265 ± 3	0.12 ± 0.03	-9.3 ± 0.6
GA-OA	172±12	0.05±0.03	-22.5±1.8

**Table S2** Particle size, polydispersity index (PDI) and *Zeta* potentials of co-assembly NPs measured by DLS

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Group	Cell cycle distribution (%)		
	G0-G1	S	G2-M
Control	37.84	56.68	5.49
OA	8.58	59.3	32.12
GA	27.54	55.3	17.16
LA	23.23	72.59	4.18
GA-OA	7.9	58.06	34.04
GA-LA	22.96	62.37	14.68

**Table S3.** Cell cycle distribution was detected by flow cytometry

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Tumor tissue (Average fluorescence intensity)						
Time (h)	1	2	4	8	12	24
GA-OA	14990	14283	11678	7044	5099	1862
GA-LA	17290	15579	11718	8005	5133	1897

**Table S4.** Fluorescence intensity of tumor tissue at different time

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Group	Main tissues (Average fluorescence intensity)					
	Heart	Liver	Spleen	Lung	Kidney	Tumor
GA-OA	84.1	479.1	156.6	183.2	653.8	476.5
GA-LA	139.8	391.2	161.3	172.6	468.0	675.6

**Table S5.** The fluorescence intensity of main tissues at 24 hours after injection

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