Supplementary Information

Bioengineered NanoAid synergistically targets inflammatory pro-tumor processes to advance glioblastoma chemotherapy

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1. Abbreviation and description in the manuscript

Abbreviation	Description
PTX	Paclitaxel
lipoPTX	Liposome formulation of PTX
lipoPC	Liposome formulation of PTX and parecoxib
MCM	Macrophage cell membrane
M@lipoPTX	MCM modified lipoPTX
NanoAid	MCM modified lipoPC
COX-2	Cyclooxygenase-2
PCA	Principal component analysis
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
cAMP	Cyclic adenosine monophosphate
MAPK	Mitogen-activated protein kinase
GABAergic	Pertaining to or producing the neurotransmitter GABA
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
CI	Combination index
BBB	Blood-brain barrier
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
PSGL-1	P-selectin glycoprotein ligand-1
LFA-1	Lymphocyte function-associated antigen 1
VLA-4	Very late antigen-4
TEER	Transepithelial electrical resistance
FRET	Fluorescence resonance energy transfer
$AUC_{0-\infty}$	Area under the curve from time 0 extrapolated to infinite time
$t_{1/2}$	Half-life time
CL	Clearance rate
H&E	Hematoxylin and eosin
TUNEL	TdT-mediated dUTP Nick-End Labeling

2. Supplymentary Figures

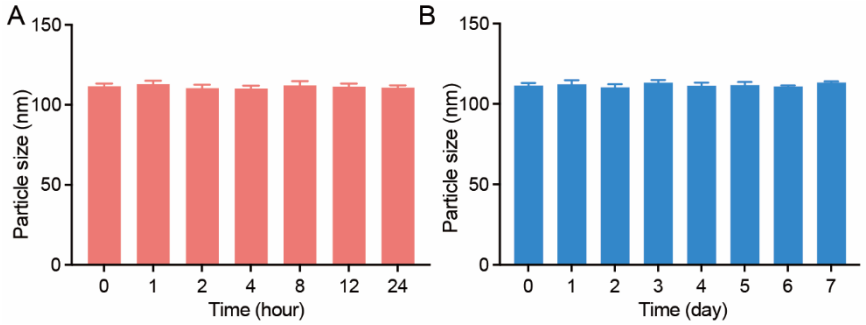


Fig. S1. LipoPC stability assessment. (A) Time-dependent serum stability of lipoPC. The lipoPC stability in serum was measured over a 24-hour period at 37°C using Dynamic Light Scattering (DLS). (B) Long-term storage stability of lipoPC. The long-term storage stability of lipoPC in PBS at 4°C was measured over seven days. The values are expressed as mean ± SD (n = 6).

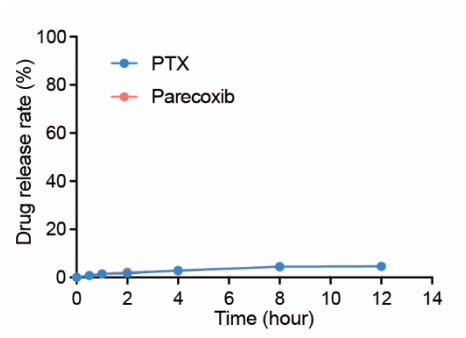


Fig. S2. *In vitro* drug release of PTX and Parecoxib from lipoPC. The controlled release of PTX and parecoxib from lipoPC over 12 hours in PBS was measured. The values are expressed as mean ± SD (n = 6).

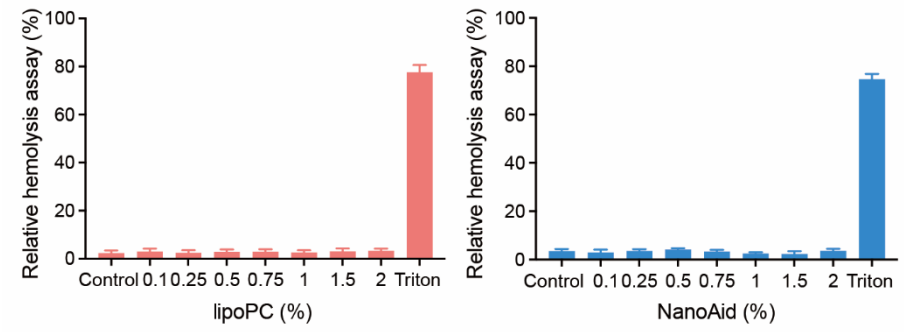


Fig. S3. Hemolysis of lipoPC and NanoAid. The erythrocyte suspension, diluted with pH 7.4 PBS, was incubated with lipoPC or NanoAid at 37°C for 2 hours, after which the relative hemolysis was calculated. The values are expressed as mean ± SD (n = 6).

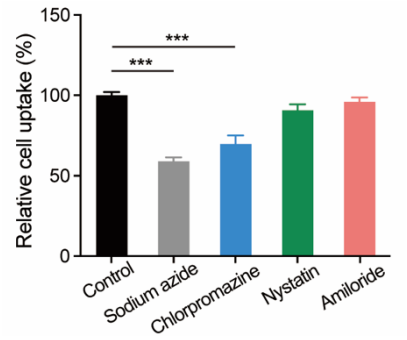


Fig. S4. Cellular uptake mechanism of NanoAid in U87MG cells. U87MG cells were pretreated with various inhibitors, including sodium azide, chlorpromazine, nystatin, and amiloride, followed by incubation with C6-labeled NanoAid for 6 hours. Flow cytometry analysis was used to measure the fluorescent intensity. The values are expressed as mean ± SD (n = 6). ***p < 0.001.

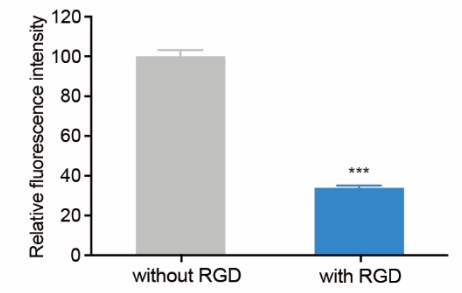


Fig. S5. Cell uptake of cRGD-pretreated U87MG cells after culturing with NanoAid for 6 hours. The values are expressed as mean ± SD (n = 6). ***p < 0.001.

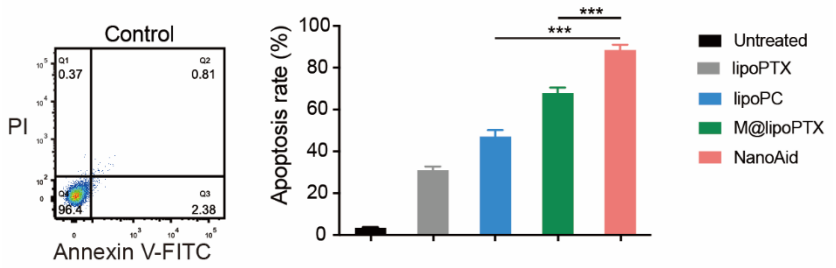


Fig. S6. The cell apoptotic rates of untreated, lipoPTX, lipoPC, M@lipoPTX, and NanoAid were quantified using Annexin V-FITC/PI staining. The values are expressed as mean ± SD (n = 6). ***p < 0.001.

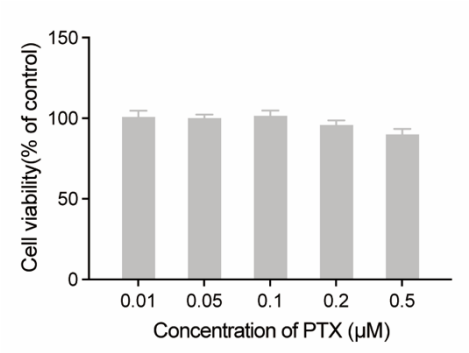
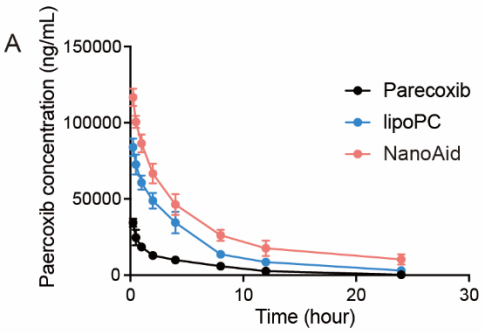


Fig. S7. Biosafety assessment of NanoAid in B.End3 cells. Cell viability was measured after 24-hour exposure to various concentrations of NanoAid. The values are expressed as mean ± SD (n = 6).

A



B

Parameter	Parecoxib	lipoPC	NanoAid
C_{max} (μg/mL)	34.4±2.6	83.9±5.8	116.6±5.7
$t_{1/2}$ (h)	4.0±0.3	7.9±0.4	15.4±0.2
$AUC_{0-\infty}$ (μg/mL*h)	146.6±12.1	430.7±23.9	697.3±39.2
CL (mL/h)	40.9±3.2	13.9±0.9	8.6±0.4

Fig. S8. Pharmacokinetic profile of parecoxib, LipoPC, and NanoAid. (A) Plasma concentration of parecoxib. The time-dependent plasma concentration of parecoxib was determined following the intravenous injection of parecoxib, lipoPC, and NanoAid, all at a dose of 20 mg/kg. (B) Pharmacokinetic parameters of NanoAid. Various key pharmacokinetic parameters for parecoxib were measured and calculated, including the maximum concentration (Cmax) post-injection, plasma half-life ($t_{1/2}$), area under the concentration-time curve ($AUC_{0-\infty}$), and clearance (CL). These values are presented as mean ± SD (n = 5).

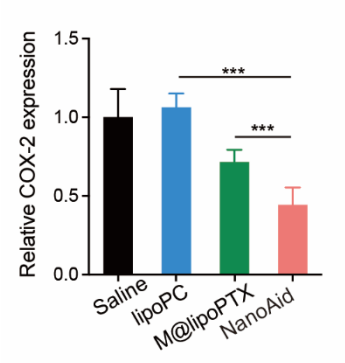


Fig. S9. The quantification of COX-2 in tumor slices collected after treatment with PBS, lipoPC, M@lipoPTX, and NanoAid was performed. The values are expressed as mean ± SD (n = 5).

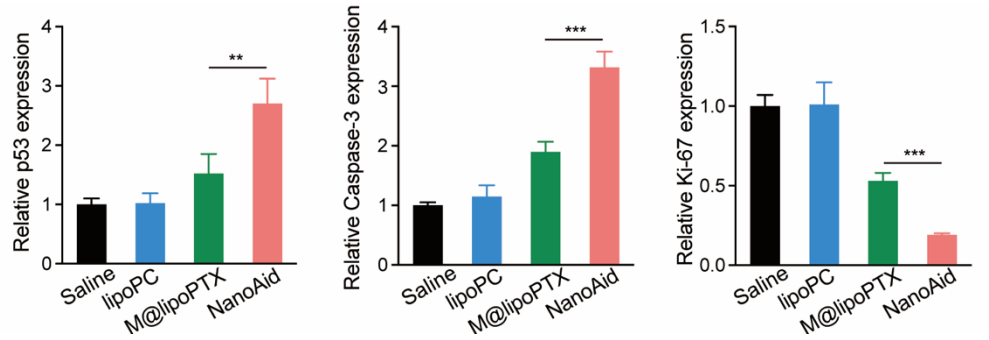


Fig. S10. Measurement of tumor-associated proteins, including the glioma biomarker p53, apoptosis marker Caspase-3, and proliferation marker Ki-67, after 28 days of treatment using an ELISA kit. The values are expressed as mean ± SD (n = 5). **p < 0.05, ***p < 0.001.

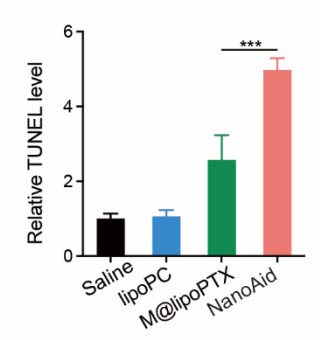


Fig. S11. The quantification of TUNEL staining in tumor slices collected post-treatment with PBS, lipoPC, M@lipoPTX, and NanoAid was conducted. The values are expressed as mean ± SD (n = 5). ***p < 0.001.

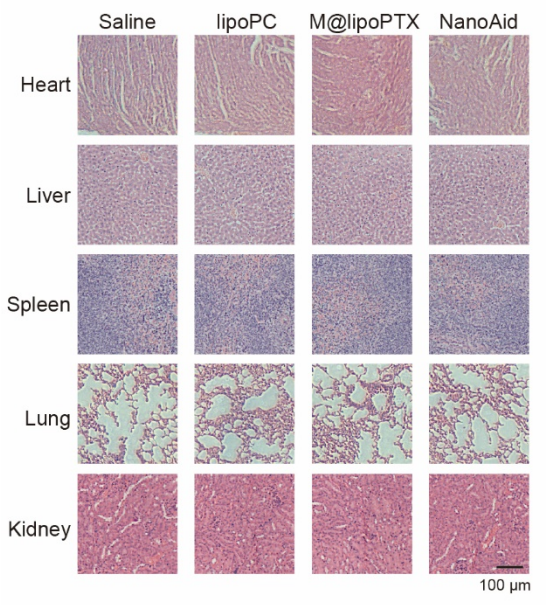


Fig. S12. Hematoxylin and Eosin (H&E) staining of major organs following treatment with PBS, lipoPC, M@lipoPTX, and NanoAid.

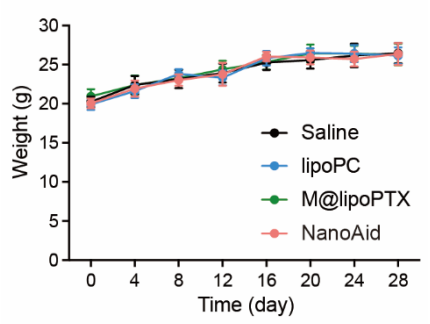


Fig. S13. The weight of tumor-bearing mice was monitored throughout the 28-day treatment period. The values are expressed as mean ± SD (n = 5).

Table S1. Characterization of lipoPC and NanoAid.

Formulation	lipoPC	NanoAid
Particle size (nm)	110.52±1.21	122.62±2.16
Zeta potential (mV)	-23.62±1.34	-19.26±0.27
EE% (PTX)	97.31±0.26	98.26±0.19
DL% (PTX)	2.34±0.06	2.29±0.12
EE% (Parecoxib)	45.89±1.34	46.37±2.13
DL% (Parecoxib)	19.43±0.86	20.03±1.25

Abbreviations: EE (encapsulation efficiency). DL (drug loading). The data are presented as means ± s. d. (n=6).

Table S2. Characterization of C6 or DiR labeled lipoPC and NanoAid.

Formulation	C6- lipoPC	C6-NanoAid	DiR-lipoPC	DiR-NanoAid
Particle size	112.3±3.1	125.2±1.4	113.6±2.3	124.2±0.4
Zeta potential	-23.7±0.2	-18.7±1.3	-23.3±1.3	-19.5±1.3
EE%	97.2±1.4	98.8±0.6	97.8±0.3	97.9±0.8

Abbreviations: EE (encapsulation efficiency). The data are presented as means ± s. d. (n=6).