Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

COF-based nanoreactors for click-activated prodrug delivery and precise anti-vascular therapy

Peng Wang*[a,b], Mili Li[a], Fang Zhou[a,b], Yue Yang[a], Xia Yin*[a], Xiao-Bing Zhang[a] and Guosheng Song*[a]

a. Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, China.

b. The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, Zhejiang 310022, China.

E-mail: chemwangp@hnu.edu.cn; yinxia@hnu.edu.cn; songgs@hnu.edu.cn

Experimental Section

Materials and instrumentation. All chemicals were obtained from commercial suppliers and used without purification. Ultrapure water was utilized from a Milli-Q reference system (Millipore). TEM images were recorded using JEM-2100F (JEOL). DLS measurements were made on a Zetasizer 3000Hs (Malvern). The UV-vis absorption spectra were collected on a UV-2450 UV-vis spectrometer (Shimadzu). Cell viability was monitored by measuring the absorption at 490 nm using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT). Fluorescence bioimages of live cells were picked up through an inverted Nikon A1Si laser scanning confocal microscope (Nikon).

Synthesis of click-activated prodrug CA4V. The newly designed prodrug CA4V was synthesized according to our optimized methodology: $Cu(OAc)_2$ (72 mg, 0.4 mmol) was added to a round-bottomed flask containing dichloromethane (DCM, 20 mL). The mixture was stirred at room temperature for 15 min. 2,4,6-Trivinyl cyclotriboroxane-pyridine complex (96 mg, 0.4 mmol), CA4 (126 mg, 0.4 mmol), and pyridine (320 mg, 4 mmol) were added, and the reaction mixture was stirred at room temperature for 24 h. The reaction solution was concentrated in vacuo. The residue was separated by chromatography (DCM/MeOH (v/v) = 100:1) and the product CA4V (97 mg, 71%) was obtained as a white solid.

Synthesis of the CA4V/ZIF-90. The optimized one-pot synthetic methodology for CA4V/ZIF-90 is described as follows: ICA (40 mg, 0.416 mmol) was added to DMF (20 mL), heated at 60 °C until completely dissolved, and then cooled to room temperature. The DMF solution (10 mL) of $Zn(NO_3)_2 \cdot 6H_2O$ (44.6 mg, 0.150 mmol) was added to the ICA solution containing CA4V (3.5 mg, 0.01 mmol) with vigorous stirring, followed by the addition of DMF solution (10 mL) containing trioctylamine (172 μL, 0.394 mmol). After 1 min, the reaction was quenched with ethanol (20 mL). CA4V/ZIF-90 was isolated by centrifugation at 8,000 rpm for 10 min and washed with DMF, ethanol, and ether, until no significant UV-vis absorbance signal was detected in the supernatant.

Synthesis of the CA4V/ZIF-90@COF. CA4V/ZIF-90@TzCOF was synthesized

according to our optimized methodology: Tz (9.3 mg, 0.035 mmol) and Tp (4.2 mg, 0.02 mmol) were dispersed into the 1,4-dioxane/mesitylene mixture (1 mL, V/V = 4:1) and then sonicated, until completely dissolved. CA4V/ZIF-90 (6 mg) was added to the above solution and sonicated for 15 min, followed by the addition of scandium(III) trifluoromethanesulfonate ($Sc(OTf)_3$, 1.2 mg, 2.5 μmol). The resulted suspension was sonicated for 30 min. CA4V/ZIF-90@TzCOF was isolated by centrifugation at 8,000 rpm for 5 min and washed with DMF, ethanol, and acetone. Tph (9.1 mg, 0.035 mmol) was used for CA4V/ZIF-90@TphCOF synthesis, and other conditions remained unchanged.

Synthesis of CyV/ZIF-90@TzCOF@Apt. CA4V/ZIF-90@TzCOF (1 mg) and polyXQ-2d (4 mg) were mixed in DPBS (600 μL) and then shaken at 400 rpm overnight at room temperature. CA4V/ZIF-90@TzCOF@Apt was isolated by centrifugation at 6,000 rpm for 10 min. The control DNA polymer was used for CA4V/ZIF-90@TzCOF@Con synthesis; the other conditions remained unchanged.

Response monitoring of nanoreactors by HPLC. CA4V/ZIF-90@TzCOF (1 mg) or CA4V/ZIF-90@TphCOF (1 mg) were added to acetonitrile/PBS (pH 7.4, 1 mL, v/v = 1:1) and acetonitrile/PBS (pH 5.5, 1 mL, v/v = 1:1), respectively. After ultrasonic dispersion, the solution was shaken at 300 × g for 12 h at room temperature. The resulting supernatant was obtained by centrifugation at 8,000 rpm for 5 min and then concentrated by lyophilization. CA4V standard solution (200 μM), CA4 standard solution (200 μM), and the reaction solution obtained above were directly purified using a C-18 column by reverse phase HPLC (Agilent 1260 Infinity) equipped with a UV-vis detector. The process was performed according to the following program:

Time (min)	A (0.1 M TEAA)	B (acetonitrile)
0	95	5
3	80	20
8	40	60
20	5	95

30	5	95

Detection: UV-vis (300 nm) detector. Flow rate: 1 mL/min. T: 25 °C. Injection volume: 100 μL.

Cellular uptake and live cell imaging. DU145 cells were first incubated with DMEM in a 30 mm optical culture dish for 24 h at 37 °C, then the used DMEM was replaced and different nanoreactors were added into the fresh DMEM for incubating another required time at 37 °C. After washing three times with DPBS, and staining with DAPI, fluorescence images were recorded at 60 × magnification using confocal laser scanning microscopy. The fluorescence signals of DAPI and Cy5 were excited by a 403 nm laser and a 639 nm laser, respectively.

To investigate subcellular trafficking, the cells were first incubated with CA4V/ZIF-90@TzCOF@Apt for 4 h and then stained with LysoTracker Red for another 30 min. After washing three times with DPBS and staining with DAPI, fluorescence images were recorded. The fluorescence signals of DAPI, LysoTracker Red, and Cy5 were excited by a 403 nm laser, 560 nm laser, and 639 nm laser, respectively.

Microtubule inhibition analysis: DU145 cells were incubated with different nanoreactors for 24 h at 37 °C. At the pre-assigned time, the cells were washed, fixed in 4% paraformaldehyde, and stained with DAPI, Actin-Tracker Green (Beyotime), and Tubulin-Tracker Red (Beyotime) according to their standard protocols before confocal laser scanning microscopy analysis.

Cell apoptosis experiments: DU145 cells were first incubated with DMEM in a 24-well plate for 24 h at 37 °C, after which DMEM was replaced and different nanoreactors (100 μg/mL) were added into the fresh DMEM for another 24 h at 37 °C. After washing with DPBS, the cells were stained with Annexin V-FITC/PI apoptosis kit (for C6, MultiSciences) according to the standard protocol. The adherent cells were washed with DPBS three times and then incubated with 2 mL 0.2% EDTA in DPBS for 3-5 min. After removing the EDTA solution, the cells were carefully washed twice with 2 mL DPBS (Note: after treatment with 0.2% EDTA, the cells were prone to detachment from the culture dish). Finally, the cells were collected by repeated pipetting and

suspended in DPBS for flow cytometric analysis (BD Accuri C6 Plus).

Animal experiments. Female Babl/c nude mice (4-6 weeks) were purchased from Hunan SJA Laboratory Animal Co. Ltd. (Changsha, China). The tumor model was established by subcutaneous injection of DU145 cells (1.0×10^7 cells) in the right axilla. Tumors were allowed to grow for 10 days. The mice were randomly divided into three groups (n = 4 in each group) and intravenously injected with: (1) DPBS (50 μL); (2) CA4V/ZIF-90@TzCOF@Apt (40 mg kg^{-1} on CA4V basis, 50 μL); and (3) CA4V/ZIF-90@TphCOF@Apt (40 mg kg^{-1} on CA4V basis, 50 μL). From day 0, the tumor size and body weight were measured every other day, and the tumor volume was calculated as the volume = (tumor length) × (tumor width)^2/2. The relative tumor volumes were calculated as V/V_0 (V_0 was the tumor volume when the treatment was initiated). After 24 h post treatment, representative tumors were collected from various groups and fixed in 4 % paraformaldehyde for TUNEL, CD31 and HIF-1α immunofluorescence staining using the standard protocols. After two weeks of treatment, the mice were sacrificed, and their major organs (heart, liver, spleen, lung, and kidneys) were excised for H&E staining.

All animal operations were carried out in compliance with the relevant laws and approved by the Institutional Animal Care and Use Committee of Hunan University (Changsha, China).

Supplemental Figures

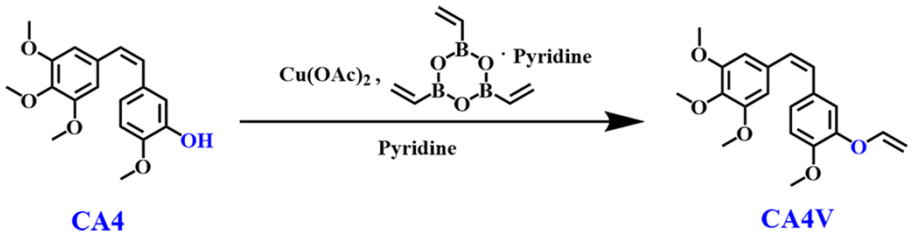

Figure S1. The general design route of click-activated prodrug CA4V.

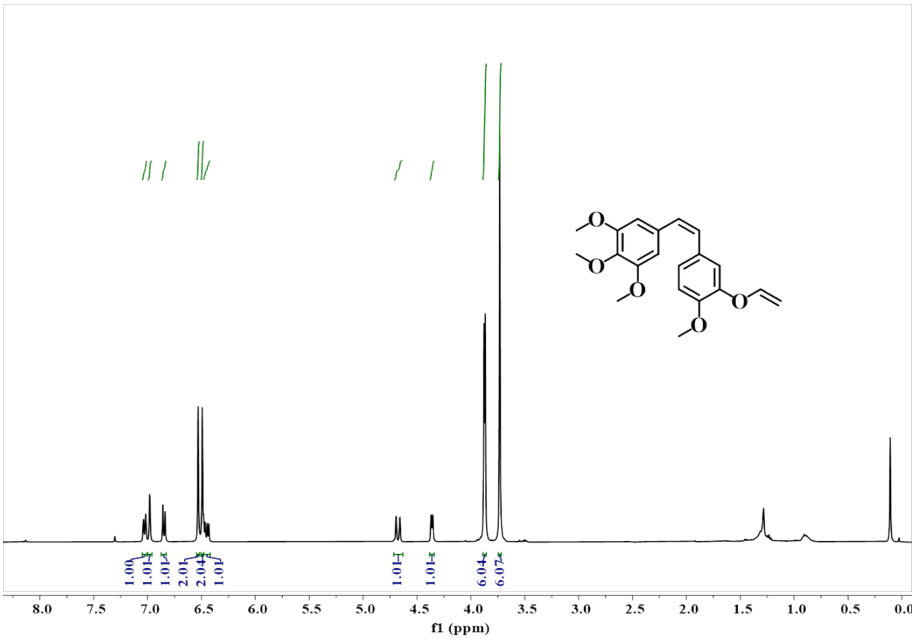

Figure S2. ^{1}H NMR spectrum of CA4V**.**

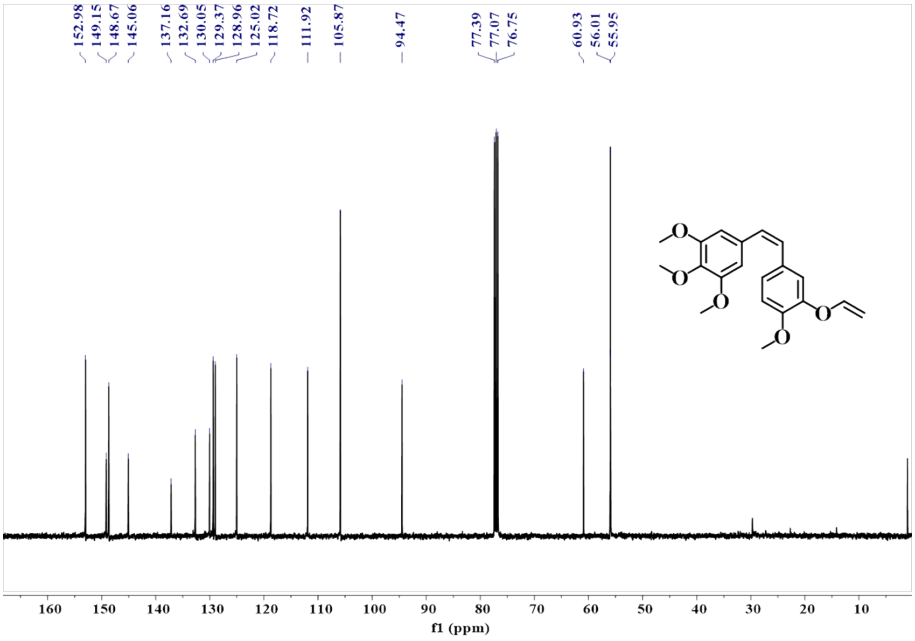

Figure S3. ^{13}C NMR spectrum of CA4V.

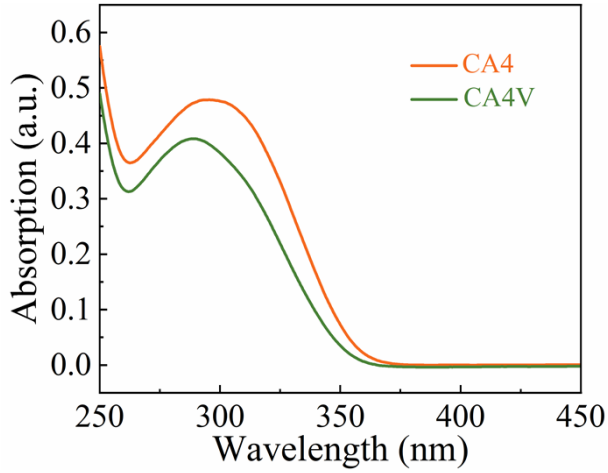

Figure S4. The UV-vis absorption spectra of CA4 and CA4V (10 μM) in acetonitrile/PBS (pH 7.4, v/v = 1:1).

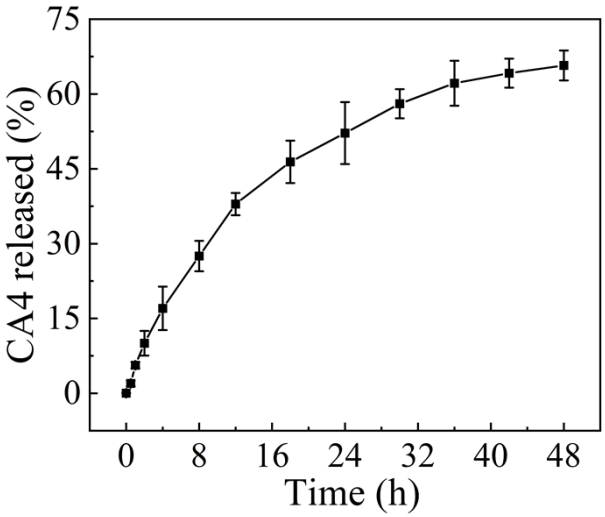

Figure S5. Release profiles of CA4 from the CA4V (10 μM) in the presence of the tetrazine monomer (50 μM) by HPLC.

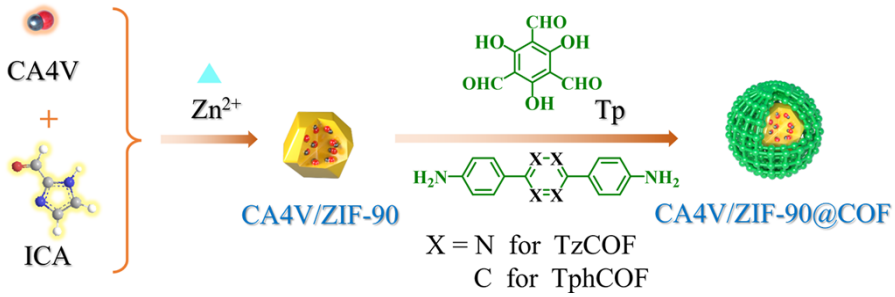

Figure S6. The general design route of nanoreactor CA4V/ZIF-90@COF.

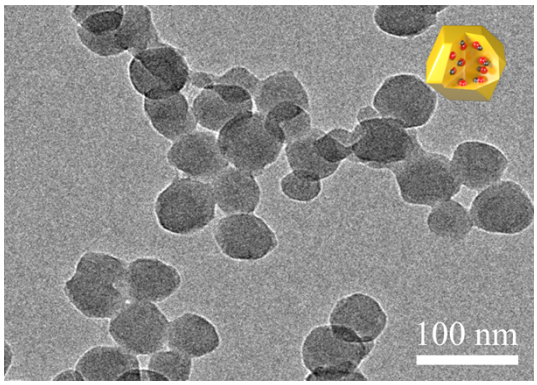

Figure S7. TEM images of CA4V/ZIF-90.

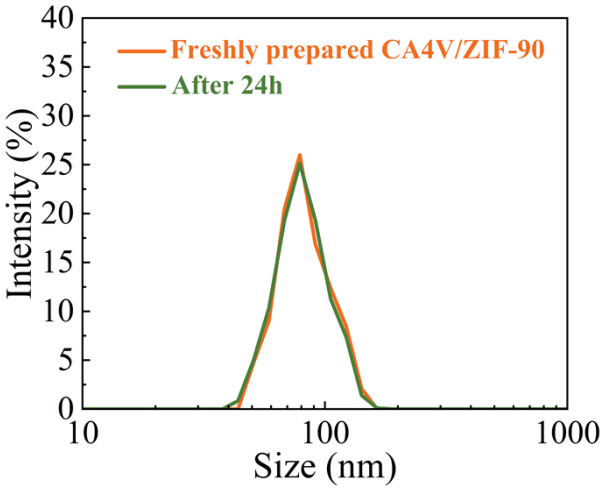

Figure S8. DLS profiles of CA4V/ZIF-90 treated with PBS (pH = 7.4) after 24 h.

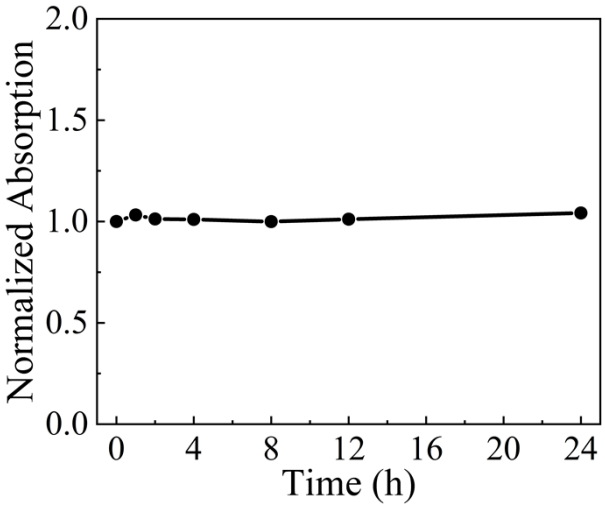

Figure S9. The stability investigation of CA4V/ZIF-90 by time-dependent absorption spectra.

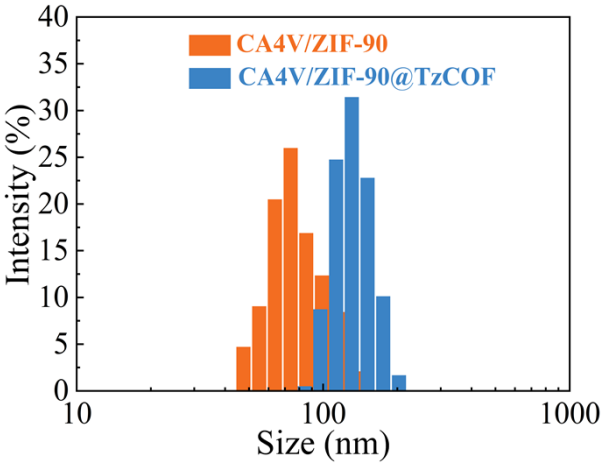

Figure S10. The size distribution of CA4V/ZIF-90 and CA4V/ZIF-90@TzCOF by DLS.

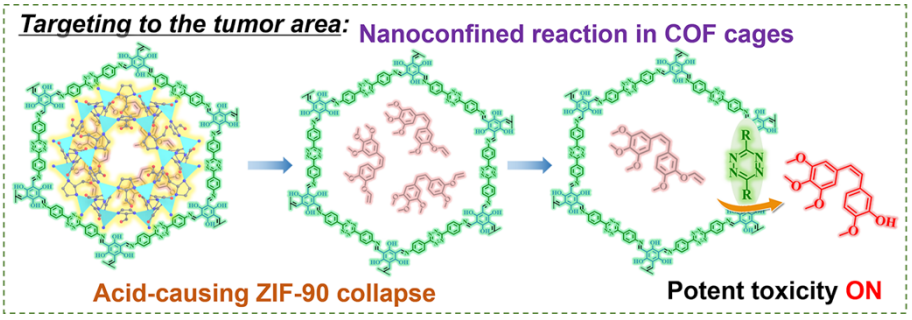

Figure S11. The response process of nanoreactor CA4V/ZIF-90@COF.

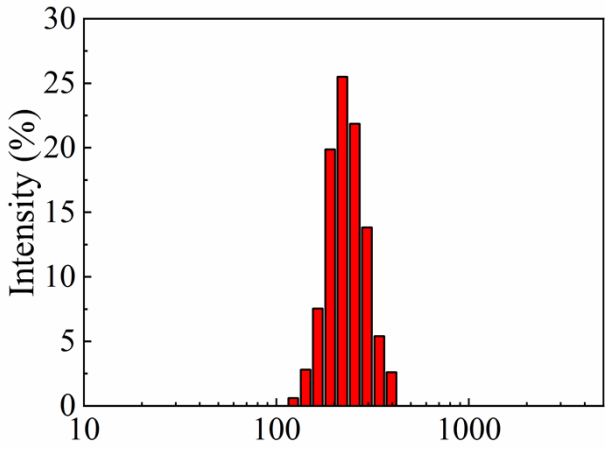

Figure S12. The size distribution of CA4V/ZIF-90@TzCOF@Apt by DLS.

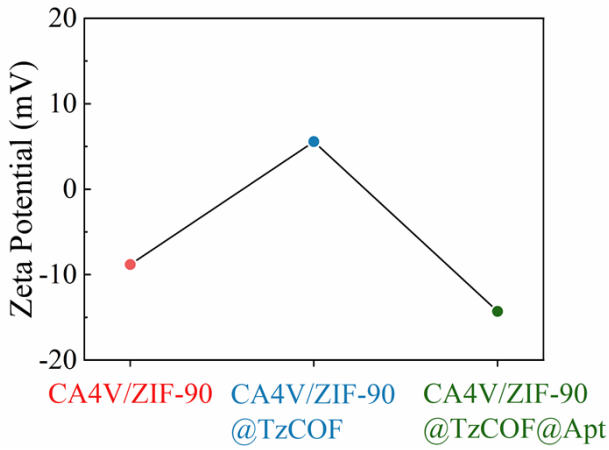

Figure S13. Comparison of zeta potential between CA4V/ZIF-90, CA4V/ZIF-90@TzCOF and CA4V/ZIF-90@TzCOF@Apt.

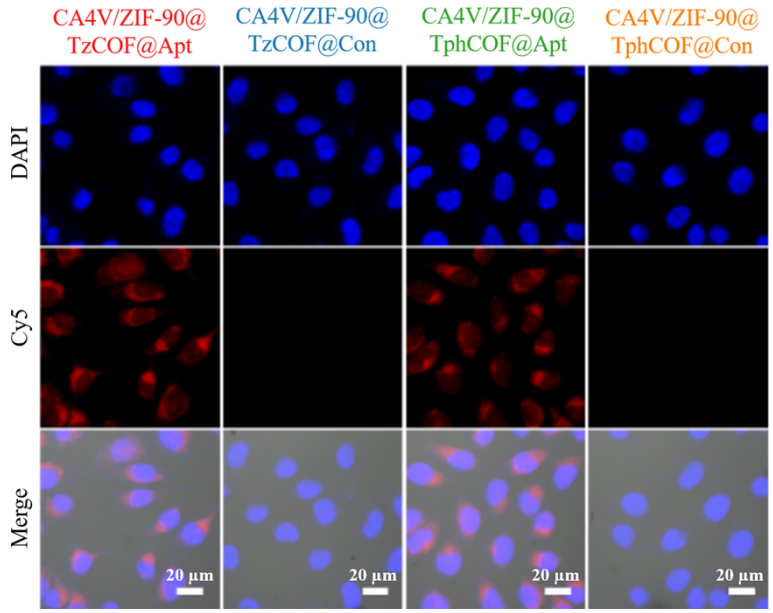

Figure S14. Confocal fluorescence images of DU145 cells with different treatment. Scale bar, 20 μm.

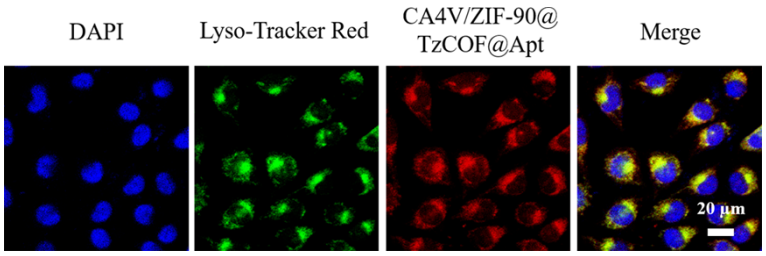

Figure S15. The subcellular localization of CA4V/ZIF-90@TzCOF@Apt in DU145 cells. Scale bar, 20 μm.

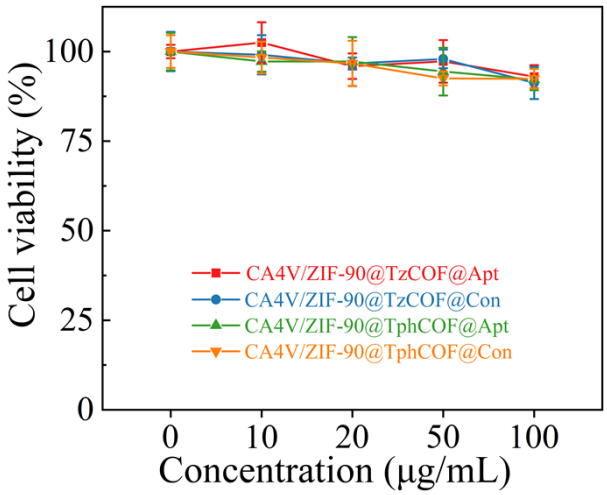

Figure S16. Cytotoxicity assay of HEK293 cells with different treatment.

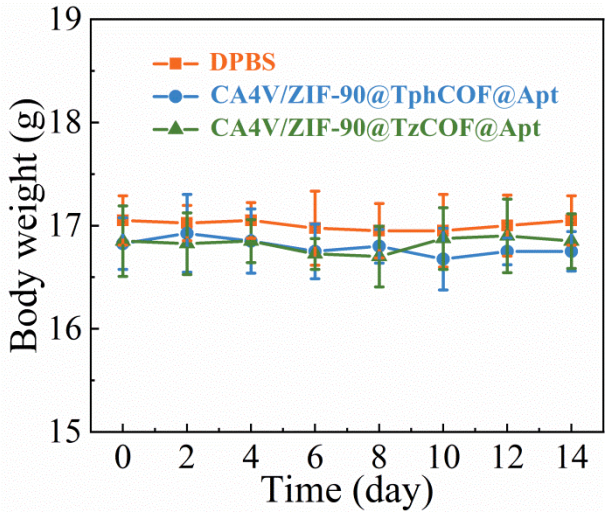

Figure S17. The body weight data after 14 days with different treatment.

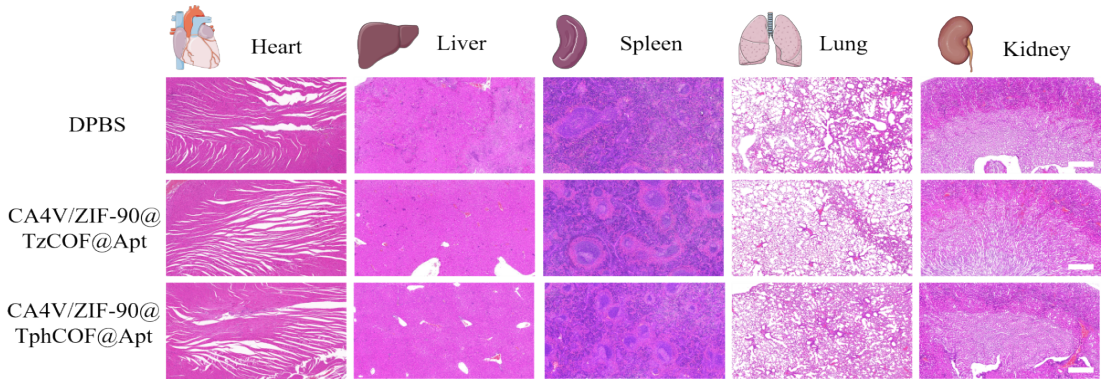

Figure S18. H&E stained images of major organs with different treatment after 14 days. Scale bar, 500 μm.

Table S1. Aptamer and control DNA used in this study.

Name	Sequences and modifications (5’-3’)
XQ-2d-DBCO	DBCO-GCT CAT AGG GTT AGG GGC TGC TGG CC A GAT ACT CAG ATG GTA GGG TTA CTA TGA GC DBCO-GCT CAT AGG GTT AGG GGC TGC TGG CC A GAT ACT CAG ATG GTA GGG TTA CTA TGA GC-FAM DBCO-GCT CAT AGG GTT AGG GGC TGC TGG CC A GAT ACT CAG ATG GTA GGG TTA CTA TGA GC-Cy5
ConDNA	DBCO-AGC TCA TAG GGT TTA TTA TTA TTA TTA TTA TTA TTA ATG AGC DBCO-AGC TCA TAG GGT TTA TTA TTA TTA TTA TTA TTA TTA ATG AGC-FAM DBCO-AGC TCA TAG GGT TTA TTA TTA TTA TTA TTA TTA TTA ATG AGC-Cy5

Table S2. The binding interface analysis between CA4, CA4V and tubulin.

	CA4	CA4V
Tubulin residues	LYS-254	ASN-349
	LEU-252	PRO-348
	LEU-242	ILE-347
	ALA-250	LYS-352
	LEU-255	ASN-258
	CYS-241	LEU-255
	LEU-248	LYS-254
	ASN-258	
	THR-240	
	VAL-238	
	TYR-202	
	GLY-237	
	MET-259	
	ALA-316	
	VAL-315	
	LYS-352	

Table S3. The calculated binding energy of CA4 or CA4V with tubulin.

	Binding energy (kcal/mol)	Intermolecular Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Internal Energy (kcal/mol)	Torsional Free Energy (kcal/mol)
CA4	-10.26	-12.05	0.05	-1.13	1.79
CA4V	-1.31	3.10	-0.06	-1.60	1.79

Table S4. Complete blood tests of control mice and mice after 24 h intravenous injection of CA4V/ZIF-90@TzCOF@Apt at the therapeutic dose.

Parameter	Control	i.v. 24 h	Unit	Reference range
WBC	4.85 ± 0.21	5.75 ± 0.31	10^9/L	0.8-6.8
Lymph	2.58 ± 0.91	3.93 ± 0.74	10^9/L	0.7-5.7
Mon	0.2 ± 0.05	0.225 ± 0.05	10^9/L	0.0-0.3
Gran	1.15 ± 0.19	1.18 ± 0.22	10^9/L	0.1-1.8
RBC	8.12 ± 0.24	8.32 ± 0.40	10^{12}/L	6.36-9.42
HGB	121.5 ± 2.08	124.25 ± 2.75	g/l	110-143
HCT	39.78 ± 2.22	40.23 ± 1.55	%	34.6-44.6
MCV	52.55 ± 2.29	51.15 ± 1.20	Fl	48.2-58.3
MCH	17.48 ± 0.64	17.58 ± 0.36	Pg	15.8-19
MCHC	329.50 ± 14.20	331.75 ± 6.13	g/l	302-353
RDW	14.95 ± 1.04	15.025 ± 0.28	%	13-17
PLT	1133.25 ± 130.38	1178.00 ± 168.39	10^9/L	450-1590
MPV	5.35 ± 0.61	5.43 ± 0.43	Fl	3.8-6.0
PDW	16.55 ± 0.45	16.60 ± 0.34		
PCT	0.21 ± 0.07	0.20 ± 0.09	%	

WBC: white blood cells; Lymph: lymphocyte; Mon: monocytes; Gran: granulocyte; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: red cell distribution width; PLT: Platelets; WPV: mean platelet volume; PWD: platelet distribution width; PCT: thrombocytocrit.

Table S5. Blood biochemical parameters of control mice and mice after 24 h intravenous injection of CA4V/ZIF-90@TzCOF@Apt at the therapeutic dose.

Parameter	Control	i.v. 24 h	Unit	Reference range
ALT	41.94 ± 18.62	61.88 ± 21.85	U/L	10.06-96.47
AST	81.41 ± 17.66	96.72 ± 10.74	U/L	36.31-235.48
DBIL	2.59 ± 0.97	3.09 ± 0.37	μmol/L	0.45-33.89
TBIL	14.85 ± 3.31	15.91 ± 3.49	μmol/L	6.09-53.06
ALB	26.30 ± 3.24	27.63 ± 3.41	g/L	21.22-39.15
ALP	38.50 ± 6.23	46.79 ± 9.76	U/L	22.52-474.35
CR	17.81 ± 1.25	18.22 ± 3.16	μmol/L	10.91-85.09
UA	152.44 ± 20.38	154.32 ± 22.49	μmol/L	44.42-224.77

ALT: aminotransferase; AST: aspartate aminotransferase; DBIL: Direct Bilirubin; TBIL: total bilirubin; ALB: Serum albumin; ALP: alkaline phosphatase; CR: creatinine; UA: uric acid; GLU: glucose.