Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

A frog-derived bionic Peptide with discriminative Inhibition of Tumor based on Integrin αvβ3 Identification

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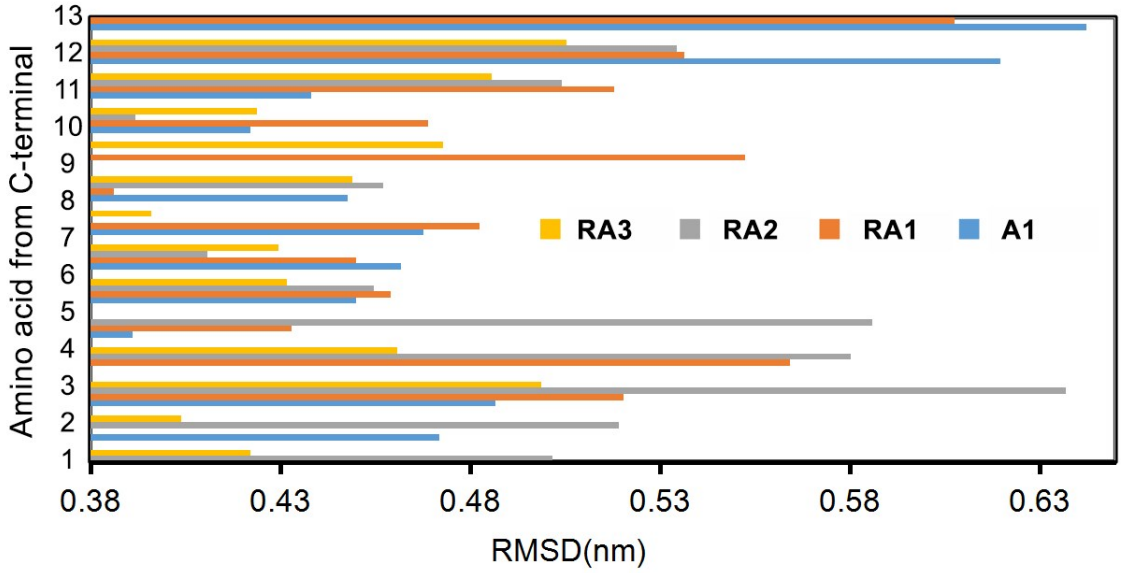


Figure S1. The average RMSD variation of the amino acids of the four peptide sequences.

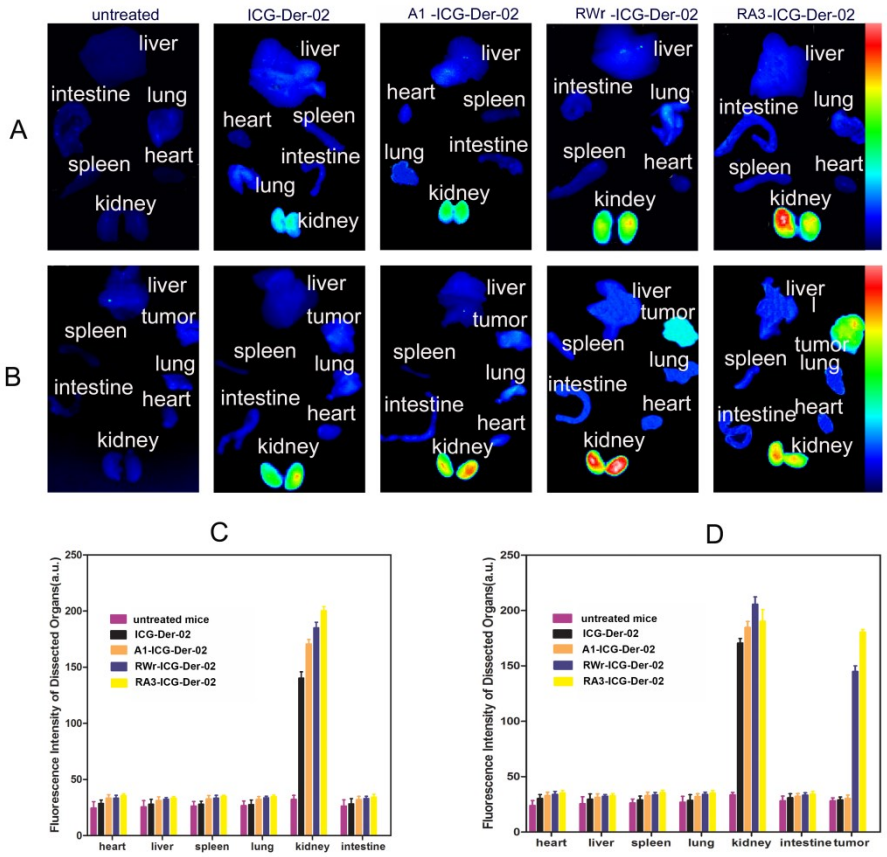


Figure S2: Metabolism of peptide molecules in tumor-free mice and U87MG tumor-bearing mice. (A) The non-tumor beading mice were injected drug with metabolism lasted for 8 hours, then dissect various organs and collect the uptake of peptide-icg-der-02. (B) U87MG tumor-bearing mice were injected drug with metabolism lasted for 8 hours, then dissect various organs and collect the uptake of peptide-icg-der-02. (C) Semi-quantitative statistics of the uptake of organs in normal mice. (D) Semi-quantitative statistics of the uptake of U87MG tumor-bearing mice.

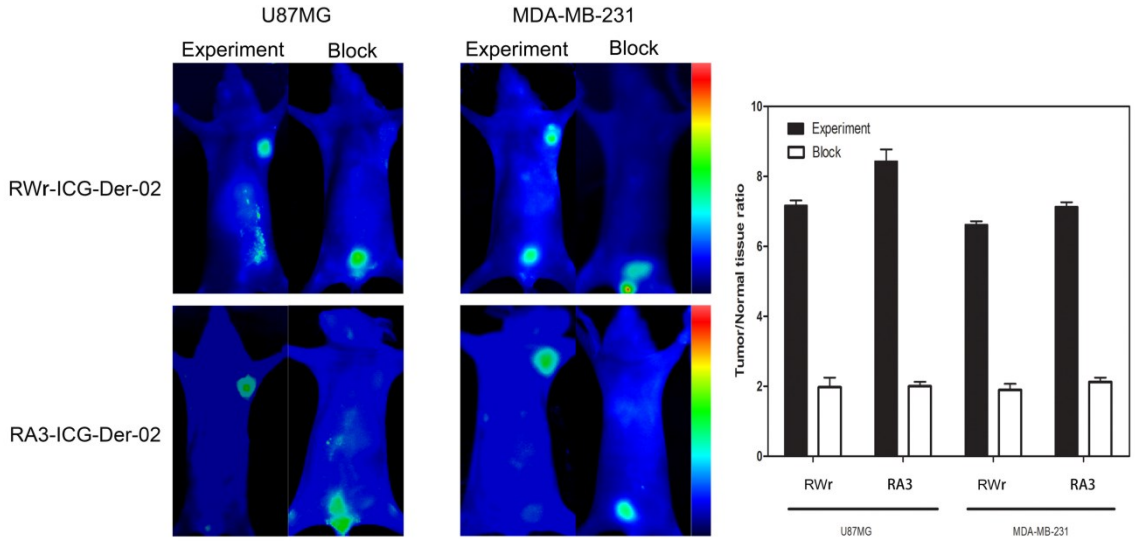


Figure S3: Treatment of tumor-bearing mice after RWr blocking. (A) Targeting of U87MG and MDA-MB-231 tumor-bearing mice. (B) Semi-quantitative statistics of the uptake of block and experiment groups.

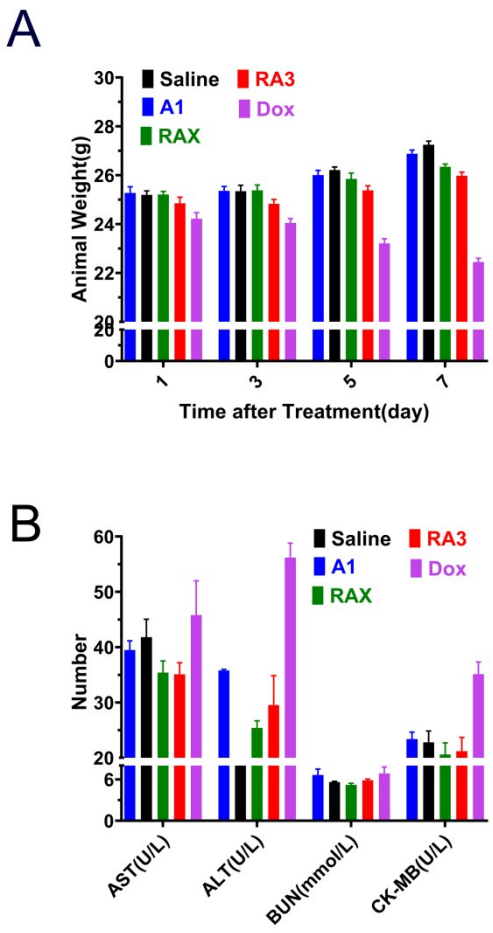


Figure S4: The acute toxicity of the therapeutic dose in normal mice. (A) Changes in body weight of mice in different groups. (B) Liver injury, kidney injury, and cardiotoxicity index in different groups of mice.

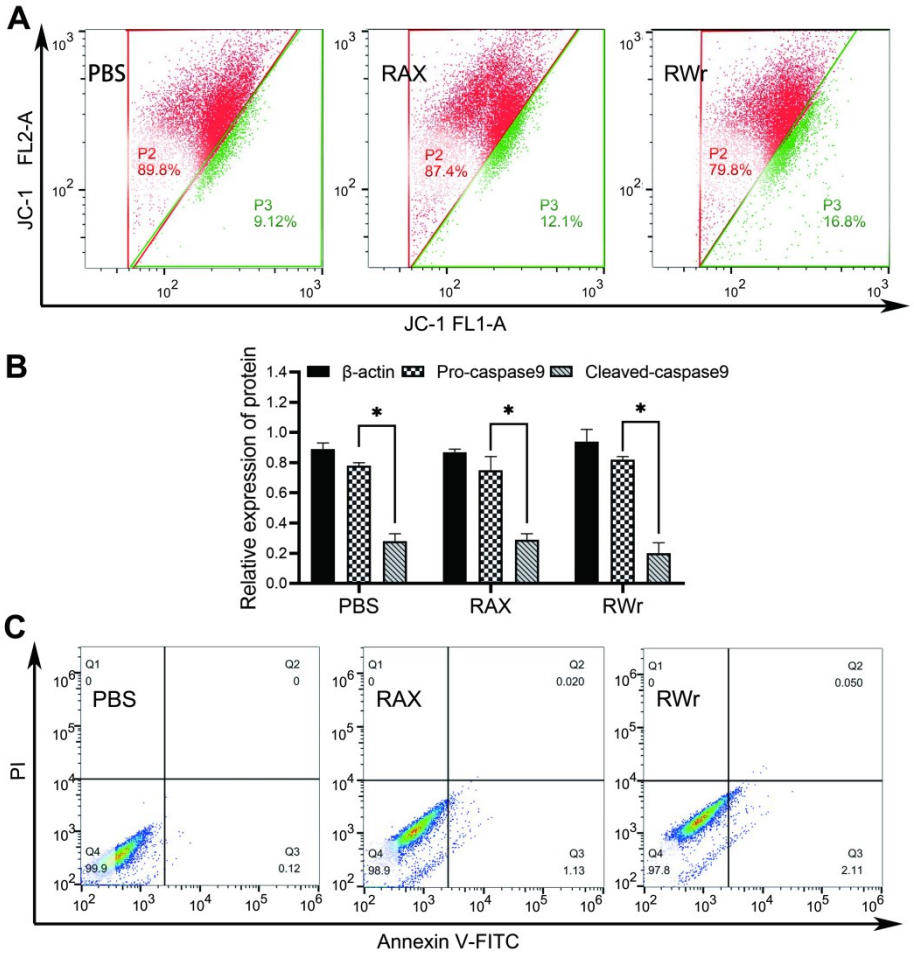
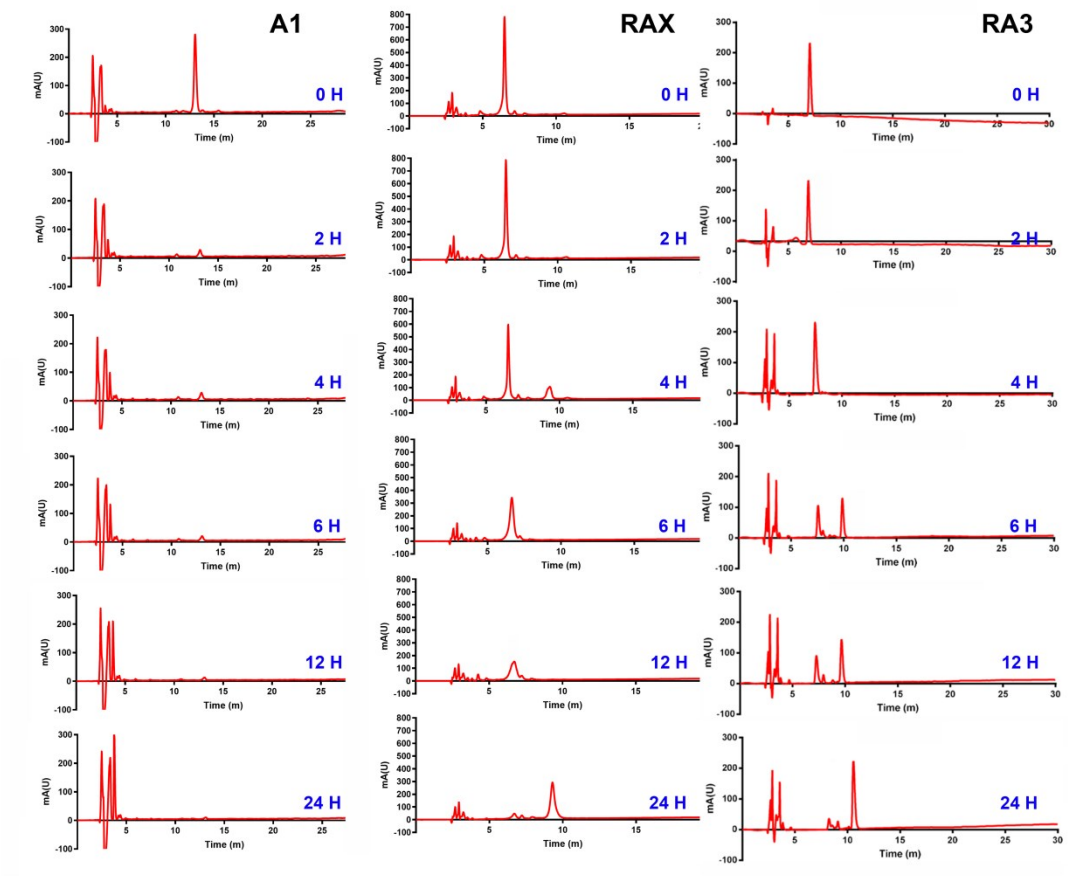


Figure S5. Apoptosis induced by membrane rupture for RAX and RWr peptides. (A) Analysis of mitochondrial membrane potential by JC-1. For quantification, flow cytometry was used to measure the relative fluorescence of free JC-1 (green) or aggregates JC-1 (red) (B) Semi-quantitative statistics of western blot. p values <0.05 were considered significant, and p values

<0.01 were considered highly significant (*p < 0.05; **p < 0.01) (C) Analysis of apoptosis by Annexin V-PI.

A


A1
RAX
RA3
0 H
2 H
4 H
6 H
12 H
24 H
mA(U)
Time (m)

B

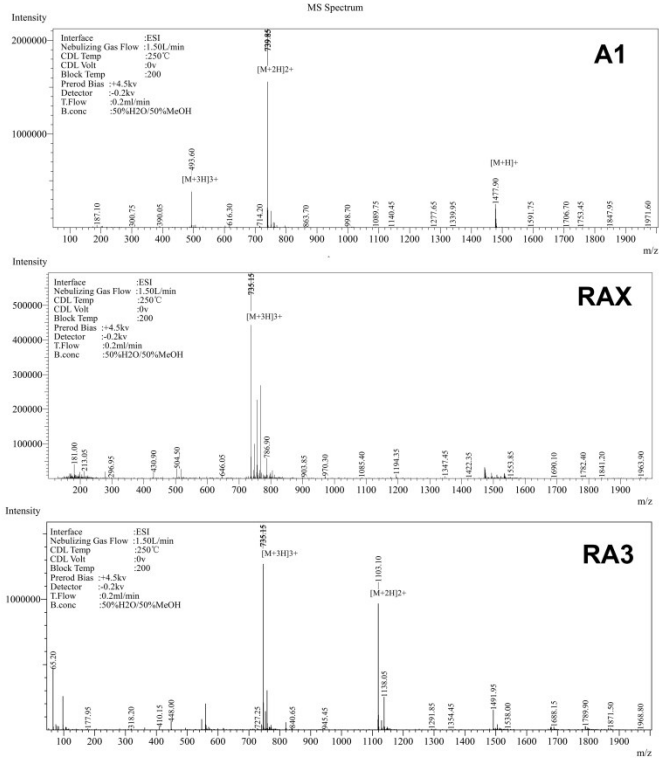
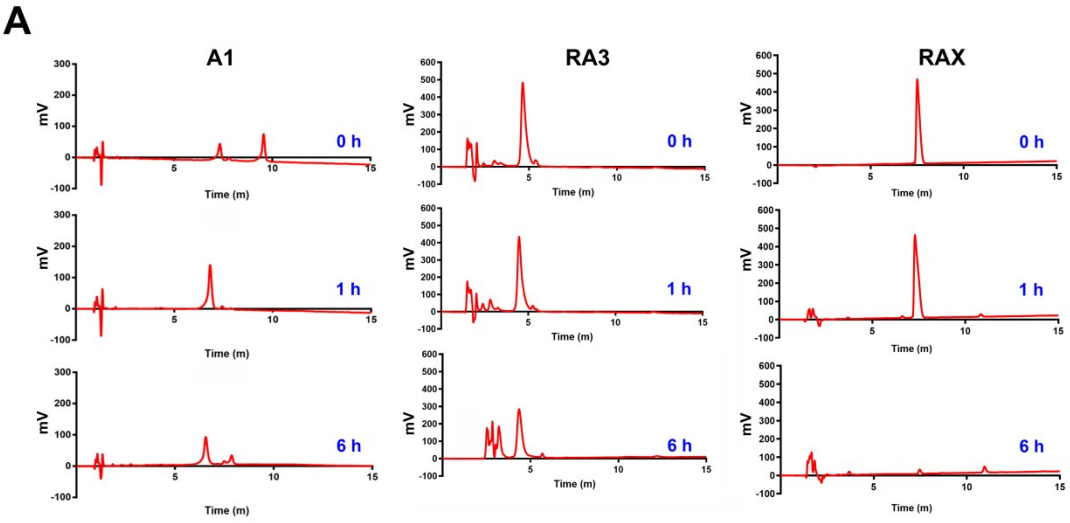

MS Spectrum
A1
RAX
RA3
Intensity
m/z
[M+2H]2+
[M+3H]3+
[M+H]+

Figure S6. In vitro stabilities of A1, RA3 and RAX peptides. (A) In vitro stabilities of peptides in plasm, respectively, at different times after incubation. (B) MS spectrum of peptides A1, RAX and RA3 (MW:1477.90, 2206.25, 2206.83)



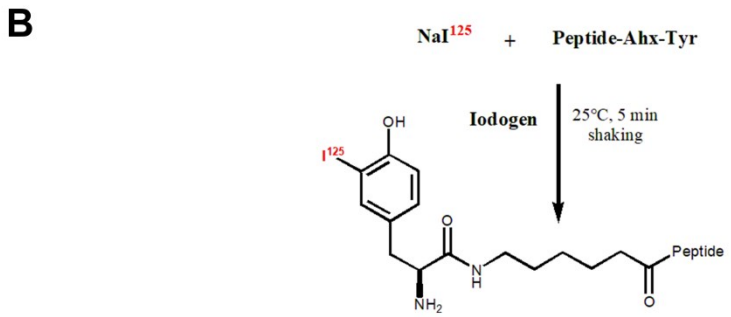


Figure S7. In vivo metabolic stabilities of A1, RA3 and RAX peptides. (A) In vivo metabolic stabilities of peptides in urine, respectively, at different times after injection. (B) Structure and synthetic route of peptides-Ahx-Tyr-I^{125}

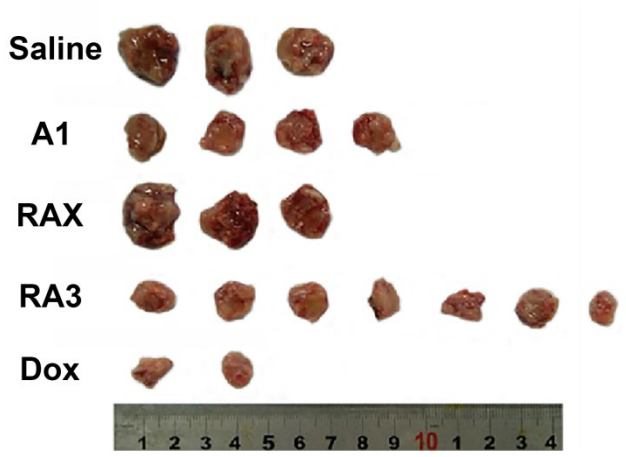


Figure S8. Digital photos of tumors for the five groups of mice

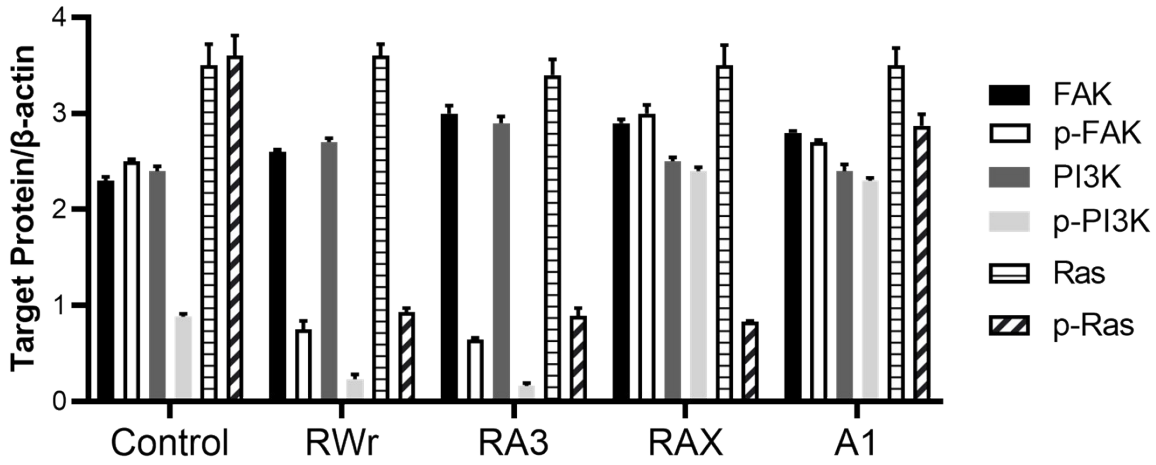


Figure S9. Semi-quantitative statistics of western blot in figure 3E.

Supplementary method

Regeants

RhodamineB (RhB), Doxorubicin hydrochloride, Hochest 33342, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma-Aldrich Co. (St. L uis, MO,USA). ICG-Der-02 was prepared in our laboratory. Peptide, peptide-RhB, peptide-ICG-Der-02 were purchased from Karebay Biochem Ltd (Ningbo, China), the purity>98%. RPMI-1640, DMEM medium, Penicillin−streptomycin and fetal bovine serum (FBS) was purchased from Gibco (Tokyo, Japan). All other reagents used in the study were certified analytical reagent grade (Shanghai Chemical Reagent Company, Shanghai, China).

Instruments

Flow Cytometer（BD,USA）,Confocal microscopy(LCFM, FluoView™ FV1000, Olympus, Japan), NIR fluorescence imaging system[an excitation laser, high-sensitivity NIR CCD camera (PIXIS 512B, Princeton Instrumentation), 800nm long pass filte, UV-vis spectrophotometer(Perkin elmer,USA), Analytical HPLC analysis(Waters, USA), Semipreparative HPLC(Bio-Rad, USA), pH meter(Sartorrius,Germany).

Cell culture

The human cell lines U87MG (glioblastoma cells), MDA-MB-231 (breast cancer cells), MCF-7(breast cancer cells), and one L02(normal liver cell) were purchased from American Type Culture Collection (ATCC; Manassas, USA). These cells were cultured in DMEM and RPMI- 1640 with 1% L-glutamine, and 10% FCS, 100 IU/mL penicillin, and 100 IU/mL streptomycin and all the cells were cultivated at 37 ℃ in a 5% CO2 incubator.

Tumor-bearing nude mouse model

Female athymic nude aged 4-6 weeks(purchased from Charles River Laboratories) were inoculated subcutaneously with U87MG, MDA-MB-231 and MCF-7 cells (5×106 cells in 0.2mL PBS) at the flanks, respectively. When the tumors reached an appropriate size (600

mm3), mice were maintained on a folate-free diet (Test Diet) for one week prior to and for the duration of the studies.

Histopathological Analyses

The tumors, hearts, and kidneys were extracted from tumor-bearing mice and fixed in a 4% paraformaldehyde solution. The tumors and organs were embedded in paraffin, sectioned and processed for hematoxylin and eosin (HE).

Toxicology Study

PBS, A1, RAX, DOX and RA3 were administered into healthy mice via tail vein at doses of 20 mg/kg body weight(equivalent to 1 mg/kg) to examine their toxicities. Animals were given enough food and water. The changes of the body weights were examined every other day for 1 week. The blood samples were centrifuged at 4000 rpm for 10 min to collect serum. Animal serum ALT, AST, CK-MB and BUN were determined for the evaluation of the functions of livers, hearts, and kidneys, respectively.

Preparation of 125I-Tyr-Ayx-Peptides

The Iodogen method was used to label Tyr-Ayx-Peptides (purchased from GL Biochem, Shanghai, China) with 125I (purchased from Shanghai Xinke, Shanghai, China). In the labelling reaction, 25 μL of Tyr-Ayx-Peptides (2 mg/mL) and 5 μL of Na125I in 1×phosphate-buffered saline (PBS) (20 mCi/mL) were sequentially added to a reaction tube coated with Iodogen and mixed gently. The tube was sealed, and the reaction was allowed to proceed for 20 min at room temperature.

After the reaction was completed, the labelling efficiency was determined using a mixture of methanol and water (volume methanol:volume water=85∶15) as the developing solvent and Whatman 3 mm chromatography paper as the stationary phase, with Rf values of 0 and 0.8 for

125I-Tyr-Ayx-Peptides and Na125I, respectively. The final labelling rate of 125I-Tyr-Ayx-Peptides was approximately 95%, and the radiochemical purity was greater than 95%.