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Supporting Information

Cholyl-L-Lysine-carboxyl butyryl adriamycin produgs targeting chemically

induced liver injury

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Experimental section

Synthetic Procedure of BCBALys

Preparing N^α-(bile-acyl)-Lys(Fmoc)-OBzl (1)

At 0°C a solution of 4.086 g (10 mmol) Cholic acid (CA), 1.417 g(10.5 mmol) of HOBt, 75 mL of anhydrous THF and 2.472 g(12 mmol) of DCC was stirred for 30 min, to which a solution of 4.815 g (10.5 mmol) of Lys(Fmoc)-OBzl in 25 mL of anhydrous THF was added, and adjusted to pH 8 with NMM. The reaction mixture was stirred at room temperature for 8 h and TLC (CH₂Cl₂:CH₃OH, 15:1) monitored. The formed precipitates of dicyclohexylurea (DCU) were removed by filtration, the filtrate was evaporated under vacuum and the residue was dissolved in 100 mL of ethylacetate. The solution was washed successively with 5% aqueous solution of sodium bicarbonate, 5% aqueous solution of citric acid and saturated aqueous solution of sodium chloride. The ethyl acetate phase was dried with anhydrous sodium sulfate for 12 h. After filtration the filtrate was evaporated under vacuum to provide 6.686 g (78.7%) of the title compound as colorless. ESI-MS (m/z): 849.44 [M + H]⁺.

PreparingN^α-(bile acyl)-Lys (2)

At 0°C a solution of 1.75 g (2.06 mmol) of N^{α}-(bile acyl)-Lys(Fmoc)-OBzl in10 mL solution of 2N NaOH in MeOH was stirredfor12 h. TLC(EA:HAc:H₂O, 5:1:1) indicated the complete disappearance of N^{α}-(bile acyl)-Lys(Fmoc)-OBzl. Saturated aqueous solution of KHSO₄ was used to neutralize the reaction mixture to pH 7 and then evaporated under vacuum. Get filter residue by filtration. After abrasive cleaning1.068 g (96.6%) of the title compound as colorless powders for next reaction directly. ESI-MS (*m*/*z*): 537.39 [M + H]⁺.

Preparingcarbonyl-3-butyryl-adriamycin-4-yl (CBA, 3)

At 0°C a solution of 1.16 g (2mmol) HCl·ADR in 10mLanhydrous DMF, adjusted to pH 8 with 500 μ L DIPEA, add 228 mg (2mmol) GA. The reaction mixture was stirred for 24 hat 0°C, and TLC (CH₂Cl₂:CH₃OH, 15:1) indicated the mostly disappearance of HCl·ADR. The reaction mixture was vaporated under vacuum. Purify by column chromatography (CH₂Cl₂:CH₃OH, 30:1), and then evaporated under vacuum to provide 0.731 g(55.6%) of the title compound as red powders for next reaction directly. ESI-MS (*m*/*z*): 656.43[M-H]⁻.

Preparing (carbonyl-3-butyryl-adriamycin-4-yl)-OSu (CBAOSu, 4)

At 0°C a solution of 1.05 g (1.6 mmol) CBA and 202mg (1.76 mmol) HOSu in 10mLanhydrous DMF, and add 272mg (1.92mmol) DCC. The reaction mixture was stirred for 24 h at 0°C, and TLC (CH₂Cl₂:CH₃OH, 15:1) indicated the complete disappearance of CBA. The reaction mixture was vaporated under vacuum. Purify by column chromatography (CH₂Cl₂:CH₃OH, 30:1), and then evaporated under vacuum to provide0.791 g (32.8%) of the title compound as red powders for next reaction directly. ESI-MS (m/z): 753.33[M-H]⁻.

Preparing N^α-(bile acyl)-N^ε-(carbonyl-3-butyryl-adriamycin-4-yl)-L-Lys (BCBALys, 5)

At 0°Ca solution of 590 mg (1.1 mmol) N $^{\alpha}$ -(bile acyl)-Lysin 20mLdry pyridine, and add 755mg (1 mmol) CBAOSu, adjusted to pH 8 with 500 µL DIPEA. The reaction mixture was stirred for 24 h at 0°C, and TLC (CH₂Cl₂:CH₃OH, 3:1) indicated the complete disappearance of CBAOSu. The

reaction mixture was evaporated under vacuum. Purify by column chromatography (CH₂Cl₂:CH₃OH, 5:1), and then evaporated under vacuum. Purify by semi-preparation column (EtCN/H₂O (1‰ HAc)) and then freeze-drying to provide50 mg (4.3%) of the title compound as red powders. HRMS (ES⁻, m/e) for $C_{62}H_{84}O_3N_{19}$ ⁻, cald: 1174.5705, found: 1174.5643 [M-H]⁻; Mp:

187.5-188.3 °C; $[\alpha]_D^{20} = +157.6$ (C = 1.0, EtCN:H₂O, 1:1);IR (cm⁻¹): 3353.46, 2932.52, 2867.87,

1723.68, 1640.32, 1620.38, 1578.29, 1540.10, 1412.12, 1284.03, 1208.08, 982.46, 792.38, 764.19; ¹H NMR (800 MHz, DMSO-*d*₆)δ/ppm=13.996 (s, 1H), 13.236 (s, 1H), 12.219 (s, 2H), 7.963 (d, *J* = 8.0 Hz, 1H), 7.873 (m,2H), 7.706 (t, J = 11.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.488 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.488 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.488 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.619 (d, J = 8.0 Hz, 1H), 7.688 (d, J = 1.2 Hz, 1H), 7.688 (d, J = 8.0 Hz, 1H), 5.423 (s, 1H), 5.223 (d, J = 3.2 Hz, 1H), 4.919 (t, J = 4.0 Hz, 1H), 4.856 (t, J = 5.6 Hz, 1H), 4.735 (d, J = 3.2 Hz, 1H), 4.584(d, J = 5.6 Hz, 2H), 4.302 (d, J = 4.0 Hz, 1H), 4.166 (q, J = 6.4 Hz, 1H), 4.100 (m, 1H), 4.072(d, J = 3.2 Hz, 1H), 3.986 (d, J = 3.2 Hz, 1H), 3.974 (s, 3H), 3.771 (d, J = 2.4 Hz, 1H), 3.601 (s, 1H), 3.407 (s, 1H), 3.179 (m, 1H), 2.966 (m, 4H), 2.220 (m, 2H), 2.126 (m, 3H), 1.993 (m, 6H), 1.845 (dt, J = 12.8 Hz, J = 4.0 Hz, 1H), 1.774 (m, 2H), 1.714 (m, 1H), 1.638 (m, 6H), 1.537 (m, 1H), 1.431(m, 4H), 1.300 (m, 10H), 1.154 (m, 2H), 1.134(d, J = 6.4 Hz, 3H), 0.939 (m, 1H), 0.917(d, J = 6.4 Hz, 3H), 0.836 (m, 1H), 0.799 (s, 3H), 0.562 3H); ¹³C NMR (200 MHz, DMSO- d_6) δ /ppm =214.25, 186.92, 186.81, 174.34, 173.31, 172.47, 172.03, 171.57, 161.23, 156.56, 154.97, 136.65, 135.96, 135.07, 134.49, 120.40, 120.16, 119.42, 111.19, 111.05, 100.90, 75.43, 71.49, 70.90, 70.36, 68.53, 67.16, 66.71, 64.16, 57.02, 52.11, 46.64, 46.19, 45.38, 41.98, 41.82, 38.63, 37.04, 35.77, 35.61, 35.34, 35.27, 35.20, 34.84, 32.67, 32.53, 32.12, 31.11, 30.86, 30.14, 29.21, 28.99, 27.77, 26.67, 23.38, 23.26, 23.07, 22.08, 21.51, 17.58, 17.49, 12.78. Purity: >98%, mobile phase: EtCN: $H_2O(1\% \text{ HAc}) = 42:58$, retention time: 13.56 min.

HPLC purity of BCBALys

A Waters 2695 HPLC system with a Waters 2996 PDA was used. The sample was analyzed on a Waters XTerra® MS C18 reversed-phase column (column 2.1×150 mm, 5 µm). Onto the column, 5 µL of a solution of BCBALys in acetonitrile was injected. The mobile phase was acetonitrile containing glacial acetic acid (1‰).The flow rate was 0.3 mL/minute. The UV detector was set to a scanning range of 200-600 nm, and a wavelength of 478 nm was used to monitor BCBALys, of which the retention time was13.851 minutes and the purity was 98.48%.

Molecular docking

To elucidate the exact mechanism by which BCBALys activates FXR, molecular docking was performed. The structure of FXR was performed with Autdock 4.0. (The Scripps Research Institute, La Jolla, CA). The crystal structure of rat FXR ligand binding domain (LBD) was used as receptor template, and 3-deoxyCDCA (PDB ID code: 10T7) was used as a validated active ligand. To find the best ligand conformer with the least binding energy, three dimensional structures of BCBALys were built and optimized using ChemDraw Ultra12.0 (Cambridge Soft Corporation, Cambridge, MA). Figures were created with Discovery Studio 4.0 Client (NeoTrident Co., Ltd., China).

Docking BCBALys onto d(CGATCG)₂oligonucleotides

The automated docking studies of BCBALys was performed using the CDOCKER/Ligand

Score in Discovery Studio (DS) Modeling 2.5, d(CGATCG)₂oligonucleotide retrieved from the Protein Data Bank (1Z3F) was used as the interaction model. Energetically the most favorable conformation of the docked structure was selected on the basis of the DOCKING score and visual inspection. Initially hydrogen atoms were added to the protein, considering all the residues at their neutral form. The active site of1Z3F was defined from receptor cavity and was chosen to include not only the active site but also significant portions of surrounding surface. Docking energy -7.14 kcal/mol.



Fig. S1 Interaction details of BCBALysand LBD of FXR.



Fig. S2 The docking interaction of BCBALys with d(CGATCG)₂





ppm



Fig. S6 IR spectrum of BCBALys



Fig. S8 qCID spectra of BCBALys. (A) qcID spectrum of 1589.40002 $m/z(MS4^{3+})$ giving the peaks of the trimer and the monomer; (B) qcID spectrum of 1762.33064 $m/z(MS2^{2-})$ giving the peak of the monomer.



Fig. S9 The structure of BCBALys with the number of the H



Fig. S11 (A) The cytotoxicity of BCBALys against HepG2, HCCLM3, MCF-7, A549, S180 and L02 cell lines. IC₅₀ values ($\bar{x} \pm$ SD μ M); (B) *In vivo* anti-tumor activity of BCBALys on ICR mice transplanted S180 cells.



Fig. S12 (A) UV spectra of CT DNA (final concentration 200 μ M) plus BCBALys (final concentration 0, 5, 10, 15, 20, 25 μ M) in PBS; (B) Hypochromic effect and bathochromic shift occurred in the UV spectra of CT DNA (final concentration 200 μ M) plus ADR (final concentration 0, 5, 10, 15, 20, 25 μ M) in PBS; (C) Fluorescence spectra of BCBALys in PBS (final concentration 20 μ M, $\lambda_{em} = 557$ nm, $\lambda_{ex} = 502$ nm) plus 30 μ L of the solution of CT-DNA in PBS (final concentration 0, 10, 20, 30, 40 μ M); (D) Fluorescence spectra of ADR in PBS (final concentration 20 μ M, $\lambda_{em} = 557$ nm, $\lambda_{ex} = 502$ nm) explain the fluorescence quenching induced by 30 μ L of the solution of CT-DNA in PBS (final concentration 0, T-DNA in PBS (final concentration 20 μ M, $\lambda_{em} = 557$ nm, $\lambda_{ex} = 502$ nm) explain the fluorescence quenching induced by 30 μ L of the solution of CT-DNA in PBS (final concentration 0, 10, 20, 30, 40 μ M).



Fig. S13 Structure of the metabolite of CA-Lys. The theoretical molecular weight of CA – Lys – $3H_2O + Na^+$ is 505.340064; the theoretical predicted molecular weight of CA-Lys – $2H_2O + K^+$ is 539.324566.



Fig. S14 Structure of the metabolite of ADR. The theoretical molecular weight of Doxorubicinol - $2H_2O + Na^+$ is 532.157802; the theoretical molecular weight of Doxorubicinol - $2H_2O + K^+$ is 548.131740.



Fig. S15 FT-MS spectra of homogenates in blood (A), heart (B), spleen (C), kidney (D) and brain (E) of the mice 12 h after CCl_4 injury treated with 2 μ mol/kg of BCBALys.