Supplementary Information

{2-[1-(3-Methoxycarbonylmethyl-1*H*-indol-2-yl)-1-methyl-ethyl]-1*H*-indol-3- yl}-acetic acid methyl ester (MIAM): Its anti-cancer efficacy and intercalation mechanism identified via multi-model systems

Wenjing Wang, Ming Zhao, Yuji Wang, Jiawang Liu, Jianhui Wu, Guifeng Kang, Shiqi Peng**

College of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, P.R. China

1. Measuring MIAM inside cells with HPLC/MS

To explore Hela-selectivity, the relative concentration of MIAM inside cells was measured with HPLC-MS method. High performance liquid chromatography (HPLC) of analytical gradient normal phase was performed on a Waters SymmetryShield RP18 (3.0 × 150 5μm) and the column temperature was 22 °C. The mobile phase consisted of aqueous solution of ammonium formate (5 mM, A) and methanol (B). The flow rate was 400 μL/min. The ESI source potentials were capillary 3 kV, lens 0.5 kV, extractor 3V and cone voltage (CV) 30 V. The source temperature was 120 ºC and the desolvation temperature was 200 ºC. Nitrogen was used as the desolvation and cove gas at flow rate of 350 and 50 L/h, respectively. Mass spectra were measured with a ZQ2000 Waters mass spectrometer (ESI) and were recorded in the mass range

100~500.

S180, K-562, HeLa, HepG2, or MCF-7 cells were cultured in DMEM medium containing 10% (v/v) fetal bovine serum, penicillin (10,000 U/mL) and streptomycin (100 μg/mL). Cell suspension (100 μL, 5×10^4 cell/mL) were seeded in 96-well plates and incubated at 37 ºC in a humidified atmosphere of 5% CO_2 for 4 h. To each well, 100 μL of medium containing the solution of MIAM in DMSO (final concentration 70 μM) was added. After incubation for 48 h the cells of each cell line of five wells were collected, washed with ultrapure water (50 μL × 2). The cells were suspended in 50 μL of ultrapure water and broken by ultrasonic vibration, centrifuged at 15000 rpm for 15 min, 20 μL of the supernatants was loaded on the column, and the relative concentrations of MIAM inside Hela, MCF-7, S180, HepG2 or K562 were measured and are shown in Figure S1. The data indicate that the concentration of MIAM inside Hela is 5-, 7-, 16- and 26-fold higher than that of it inside MCF-7, S180, HepG2 and K562, respectively.

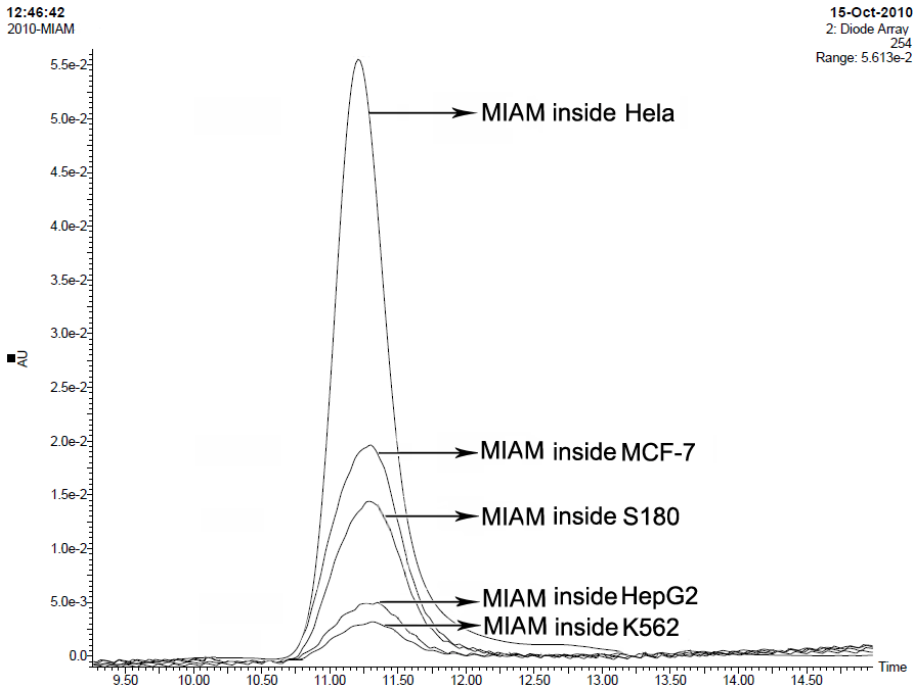

Figure S1 HPLC spectra of MIAM inside Hela, MCF-7, S180, HepG2 and K562.

2. Agarose electrophoresis measurements of pBR322 DNA with doxorubicin

The solution of 250 ng of supercoiled (SC) pBR322 DNA (Promega, USA) in 10 μL of TAE buffer and the solutions of 250 ng of SC pBR322 DNA plus 0.1, 1.0, 10.0, and 100 ng of doxorubicin in 10 μL of TAE buffer were incubated at 37 ºC for 24 h, then loaded in 1.0% agarose gel electrophoresis (110V, 60 mA, 0.5 h). After postelectrophoretic staining with ethidium bromide the pictures were taken and are shown in Figure S2.

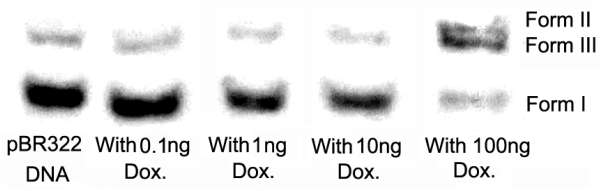

Figure S2 Cleavage of supercoiled pBR322 DNA by doxorubicin in TAE buffer at 37 ºC. Cleavage condition: supercoiled pBR322 DNA, 50 mg/L; 37 ºC for 24 h. Wherein Form I represents the supercoiled monomer, Form II represents the nicked monomer, and Form III represents the linear monomer.