A Photocleavable Amphiphilic Prodrug Self-Assembled Nanoparticles with Effective Anticancer Activity In Vitro

Ji Chen 1,† Guotao Li 1,† Qihong Liu 1 Yan Liang 2 Miaochang Liu 1,* Huayue Wu 1 and Wenxia Gao 1,*

1 College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325027, China
2 Department of Pharmaceutics, School of Pharmacy, Qingdao University, Qingdao 266021, China
† These authors contributed equally.

Experimental Section

1. Cytotoxicity test

The 4T1 breast cancer cells (5 × 10^3 cells/mL) were harvested and seeded in 96-well plates with 100 μL mediums for 24 h incubation before the tests. MTX-AMC-PEG conjugate in culture mediums were added to the medium-removed 96-well plates with different concentrations and incubated for 48 h. The culture medium was removed and the wells were washed with PBS (pH = 7.4). CCK-8 in DMEM (10%) was added to each well. After the cells were incubated for additional 4 h, the cell viability was determined by measuring the absorption at 450 nm using a microplate reader (Thermo Scientific MK3).

2. In vitro anticancer activity

The 4T1 breast cancer cells (5 × 10^3 cells/mL) were harvested and seeded in 96-well plates with 100 μL medium for 24 h incubation before the tests. 4T1 breast cancer cells were incubated with MTX-AMC-PEG nanoparticles (a certain concentration of MTX 0.4 μg/mL) for 4 h and exposed to laser irradiation with a wavelength of 365 nm laser (5W) for 0, 0.5, 1, 2, and 3 min, respectively. After laser irradiation, the cells were incubated with media for 48 h. The culture medium was removed and the wells were washed with PBS (pH = 7.4). CCK-8 dilution in DMEM (10%) was added to each well. After the cells were incubated for additional 4 h, the cell viability was determined by measuring the absorption at 450 nm using a microplate reader (Thermo Scientific MK3).
Figure S1. $^1$H NMR of compounds after photolysis of MTX-ACM-PEG conjugate, (A) the compound of R$_t$ at 2.57 min and (B) R$_t$ at 7.85 min from HPLC.

Figure S2. The size change of nanoparticles in different pH conditions (A), DLS curve of the nanoparticles in buffer solution after 10 h (B).
Figure S3. The release profiles of nanoparticles in PBS (pH 7.4) with different concentration exposed to laser (365 nm, 5.0 W) for 1 h, (A) MTX-ACM-PEG conjugate in the solution; (B) the photocleavable release profiles of MTX from the nanoparticles. Means±SD (n = 3).

Figure S4. The IC50 of the MTX and MTX-ACM-PEG nanoparticles against 4T1 cells, the incubation time was 48 h, the results were expressed as mean ± SD (n = 3).
Figure S5. Compound 1 $^1$H NMR and $^{13}$C NMR spectra.
Figure S6. Compound 2 \(^1\)H NMR and \(^1\)C NMR spectra.
Figure S7. Compound 3 $^1$H NMR and $^{13}$C NMR spectra.
Figure S8. Compound 4 $^1$H NMR spectra.

Figure S9. IR spectra.

Figure S10. Mass spectra.