Fluorinated Covalent Organic Frameworks for Efficient Drug Delivery

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Experimental section

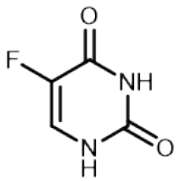
Materials

4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline (TATB), 1,3,5-tri-(4-aminophenyl) benzene (TAPB) and 2,5-difluoroterephthalaldehyde (DFPA) were purchased from Jilin Chinese Academy of Sciences - Yanshen Technology Co., Ltd. The acetic acid, 1,2-dichlorobenzene (*o*-DCB), 1-butanol, Mesitylene, 1,4-Dioxane, N,N-dimethylformamide (DMF), tetrahydrofuran (THF) were purchased from Aladdin Chemicals. 5-Fluorouracil (5-FU) and Captopril (CA) were purchased from Shanghai Dibo Biological Technology Co., Ltd. PBS were purchased from Guangzhou Zhuanyan Biotechnology Co., Ltd. All the other solvents were purchased from Aladdin Chemicals and used as received without further purification.

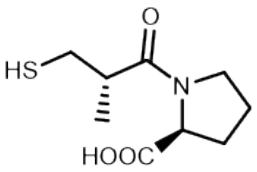
COFs stability test

The COF stability test was examined in acid (1 N HCl) and base (1 N NaOH) aqueous solution. About 20 mg of DF-TAPB-COF was added to 1 N HCl or NaOH aqueous solution, and left at room temperature for 2 days. PXRD was used to test the structure of DF-TAPB-COF, and compared with the untreated sample. The acid or base stability test of DF-TATB-COF is similar to DF-TAPB-COF.

Preparation of drug loaded COFs and *in vitro* drug release



5-fluorouracil (5-FU)



Captopril (CA)

5-fluorouracil (5-FU) and captopril (CA) were used for drug loading and release studies (their chemical structures are shown above). The drug was loaded by immersing solvent-free COF samples in hexane solution of drug with a certain concentration. A typical procedure for loading drug in COFs was as follows: COF (100 mg) was suspended in hexane solution of drug (20 mL, 0.1 M) under stirring for 3 h, while preventing evaporation of hexane by covering with a cap. The drug-loaded sample was

separated from solution by vacuum filtration, washed with hexane, and dried at room temperature. The drug loading capacity was decided by TGA analysis.

A typical procedure for releasing from 5-FU drug-loaded DF-TAPB-COF (5-FU@DF-TAPB-COF) was as follows: 5-FU@DF-TAPB-COF (20 mg) was placed in a vial and dipped in 2 mL of phosphate buffered saline (PBS, pH = 7.4, standard buffer solution from Sigma) at 37 °C. At predetermined time intervals, the dissolution medium was replaced with 2 mL of fresh PBS, and the withdrawn medium was used to determine the 5-FU concentration. The 5-FU concentration was analyzed by UV-Vis spectrophotometry with the help of a calibration curve. The release study was continued until no 5-FU drug was detectable in the withdrawn PBS. The procedure for releasing drug of CA@DF-TAPB-COF, 5-FU@DF-TATB-COF and CA@DF-TATB-COF was similar to 5-FU@DF-TAPB-COF.

In vitro cytotoxicity

To evaluate the biocompatibility, the cytotoxicity of DF-TAPB-COF or DF-TATB-COF was assayed on MCF-7 cell line. MCF-7 cells were planted in 96-well plate at the density of 6×10^3 cells per well and cultured at 37 ℃ within DMEM medium containing 10% fetal bovine serum in 5% CO_2 atmosphere for 24 h. Then different amounts of DF-TAPB-COF or DF-TATB-COF (from 50 to 200 μg/mL) were added into the MCF-7 cells. The cells were incubated with COFs for 48 h. Next, 20 μL of 5% MTT solution in PBS was added to each well and was incubated for another 4 h at 37℃. Then, the culture medium was removed and 200 μL of DMSO was added to each well to dissolve the formazan. The optical density (OD) of each well was recorded at 570 nm. The cytotoxicity of 5-FU, CA, 5-FU@ DF-TAPB-COF, 5-FU@DF-TATB-COF, CA@DF-TAPB-COF and CA@DF-TATB-COF were similarly evaluated by MTT evaluation procedure mentioned-above. The concentrations of 5-FU and CA were at 25, 50, 75 and 100 μg mL^{-1}, respectively. And the concentrations of 5-FU@DF-TAPB-COF, 5-FU@DF-TATB-COF, CA@DF-TAPB-COF and CA@DF-TATB-COF were also at 25, 50, 75 and 100 μg mL^{-1}.

Cellular uptake of COFs

Inverted fluorescence microscope was used to test if DF-TAPB-COF and DF-TATB-COF can be endocytosed by cells. The typical process of Rhodamine B loaded DF-TAPB-COF was as follows: 5 mg of the COF was dispersed in 5 mL Rhodamine B aqueous solution, then the mixture was stirred at room temperature for 10 h. After that, the obtained system was filtered and washed with water, then the Rhodamine B @ TAPB-COF was obtained after dried in vacuum. B16F10 cells were seeded in 24-well plates at a density of 5×10^4 cells per well and incubated in DMEM medium containing 10% fetal bovine serum with 5% CO_2 at 37℃ for 24 h. The Rhodamine B@DF-TAPB-COF at 50 μg mL^{-1} was added into B16F10 cells, and the cells continued to be incubated with Rhodamine B@DF-TAPB-COF for 6 h. Then Cells were visualized using inverted fluorescence microscope (Axio Vert.A1, Zeiss). The sample was excited by a green laser (530–585 nm) with a detection range of 615–4095 nm. Experimental results were observed and recorded under 10x eyepiece and 100x objective lens. The experimental method of DF-TATB-COF was similar to that of DF-TAPB-COF.

Table S1. Drug loading capacity of reported in literatures about COF materials

Entry	COF materials name	Drug name	Loading rate (wt%)	Ref.
1	PI-COF-4, PI-COF-5	IBU	24%, 20%	S1
2	TpASH-FA	5-FU	12%	S2
3	TTI-COF	Quercetin	35%	S3
4	PI-3-COF, PI-2-COF	5-FU	16%, 30%	S4
5	COF-HQ	5-FU	7%	S5
6	Cage-COF-TT	IBU, FLU, CA	18%, 21%, 22%	S6
7	CP-DTCOF	Carboplatin	31%	S7
8	PEG2000-CCM@APTES-COF-1	DOX	10%	S8
9	Fe_3O_4@COF	DOX	33%	S9
10	F-COFs	DOX	36%	S10
11	T-COF-PEG	DOX	29%	S11

Fig. S1. SEM images of DF-TAPB-COF (**A**) and DF-TATB-COF (**B**).

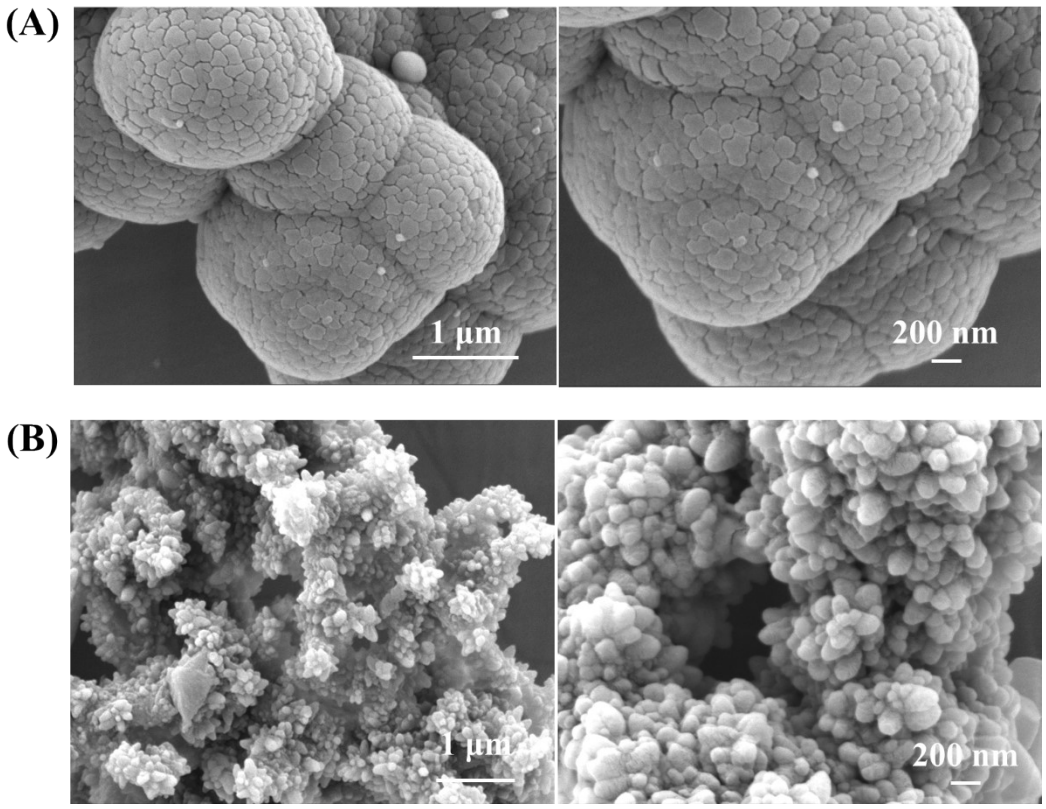


Fig. S2. Elemental distribution mapping of DF-TAPB-COF (**A**) and DF-TATB-COF (**B**).

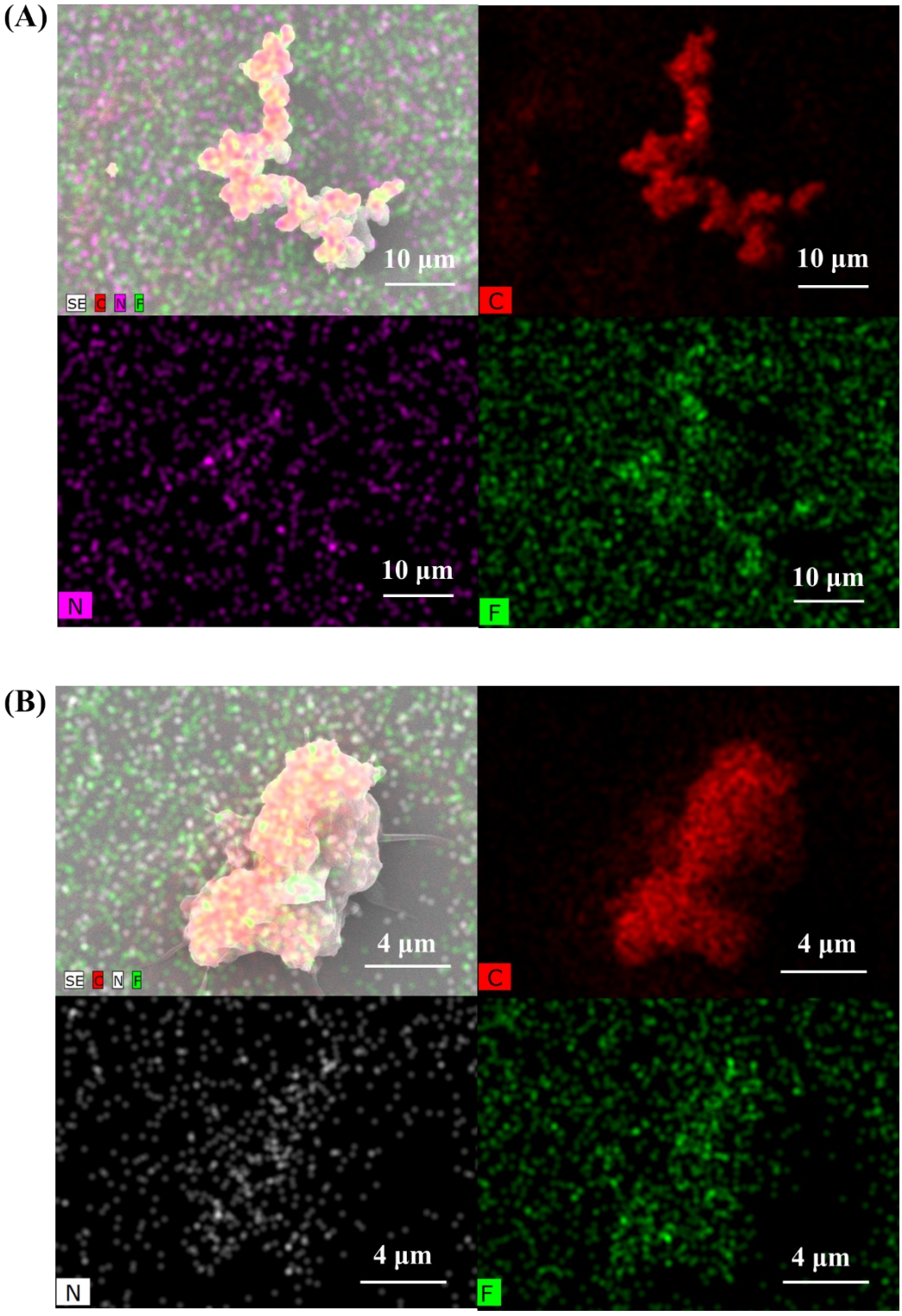


Fig. S3. Thermogravimetric curves of DF-TAPB-COF (black) and DF-TATB-COF (red).

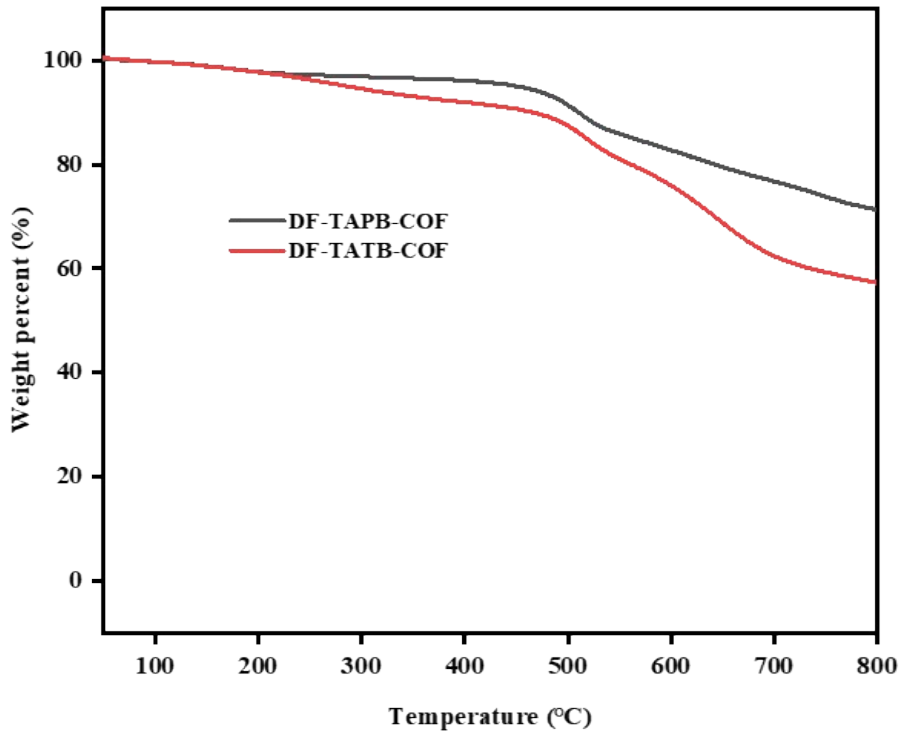


Fig. S4. Chemical stability: PXRD profiles of (a) DF-TAPB-COF, (b) DF-TATB-COF after treatment in HCl (1N, light blue) and NaOH (1N, purple).

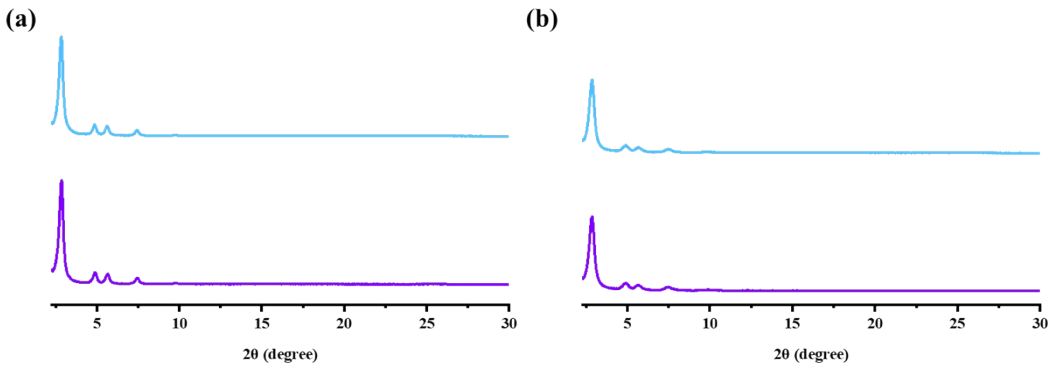


Fig. S5. PXRD patterns of 5-FU@DF-TAPB-COF (A, black), CA@DF-TAPB-COF (A, red), 5-FU @DF-TATB-COF (B, black) and CA@DF-TATB-COF (B, red)

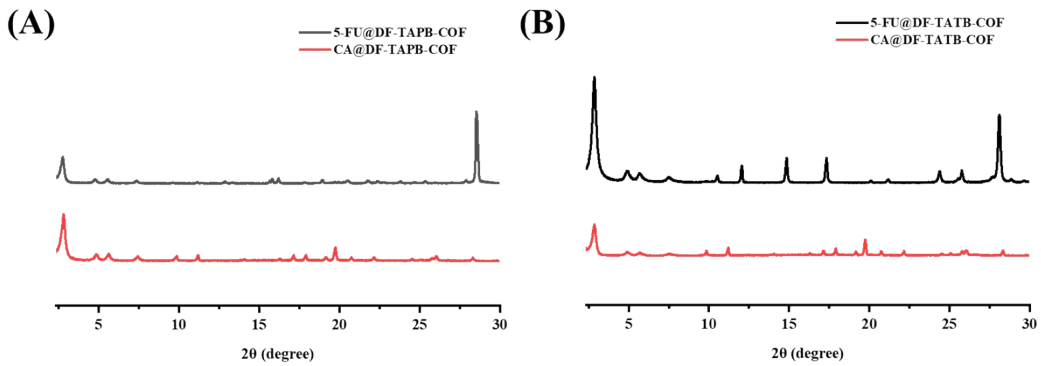


Fig. S6. Thermogravimetric curves of 5-FU (black) and CA (red).

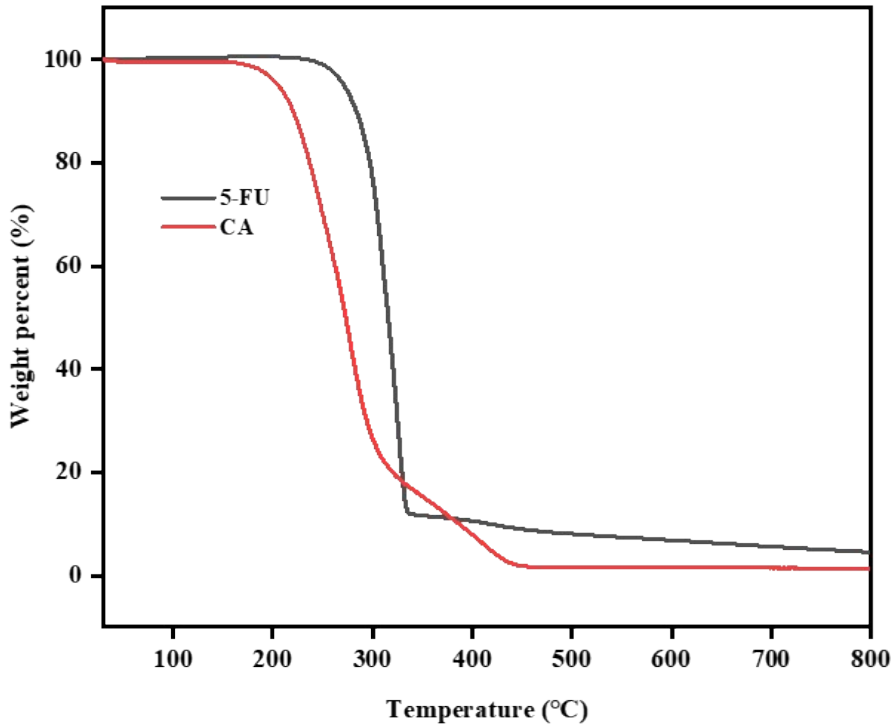


Fig. S7. Thermogravimetric curves of 5-FU@DF-TAPB-COF (**A**), 5-FU@DF-TATB-COF

(**B**), CA@DF-TAPB-COF (**C**) and CA@DF-TATB-COF (**D**).

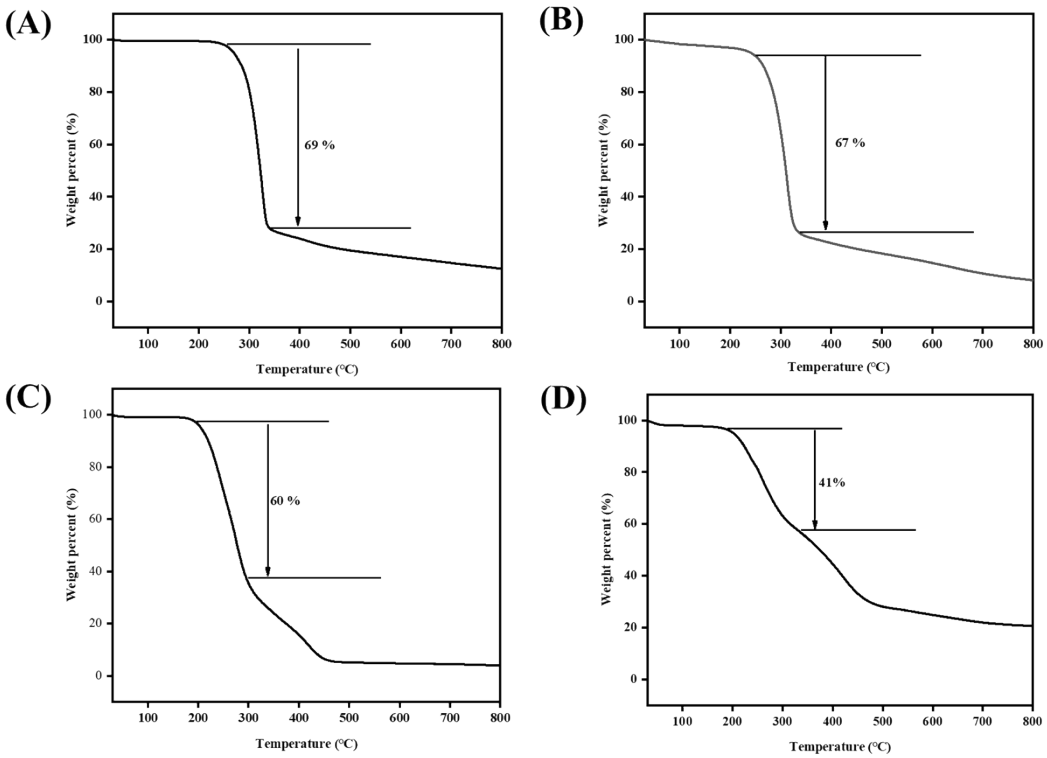


Fig. S8. The chemical structure and PXRD profile of of N-TAPB-OmeTA

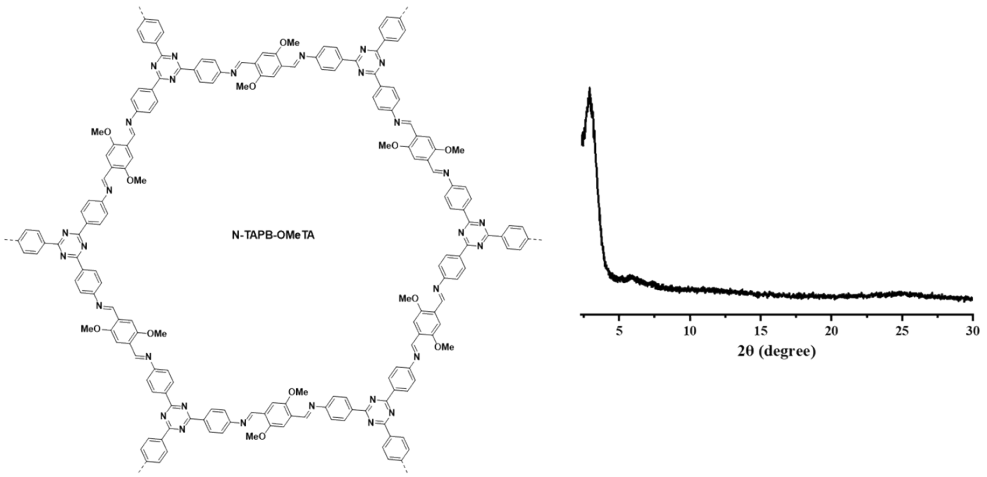


Fig. S9. Thermogravimetric curve of N-TAPB-OMeTA

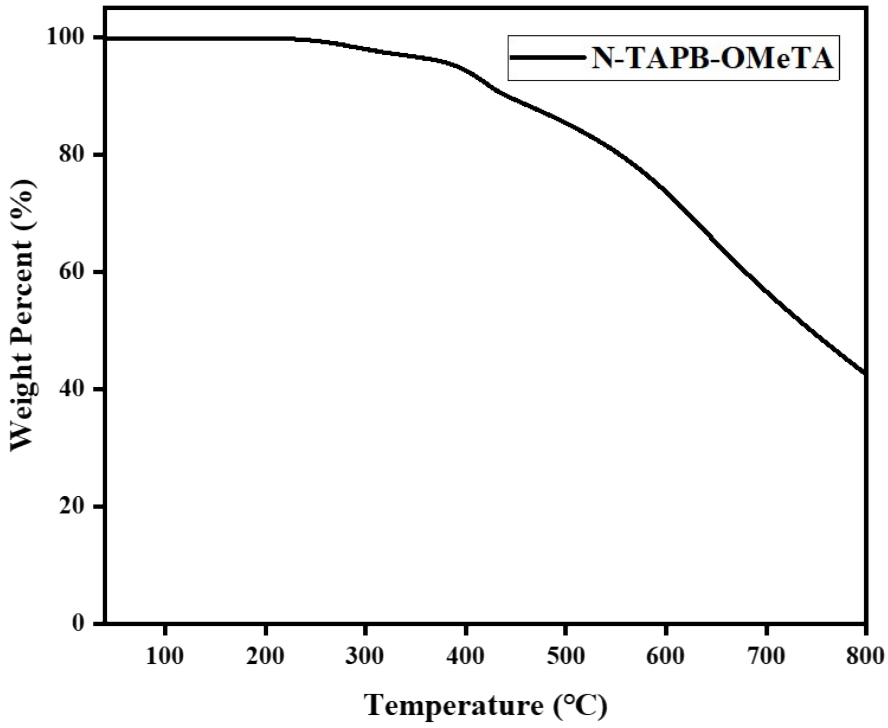


Fig. S10. Thermogravimetric curves of 5-FU@ N-TAPB-OMeTA (**A**) and CA@ N-TAPB-OMeTA (**B**).

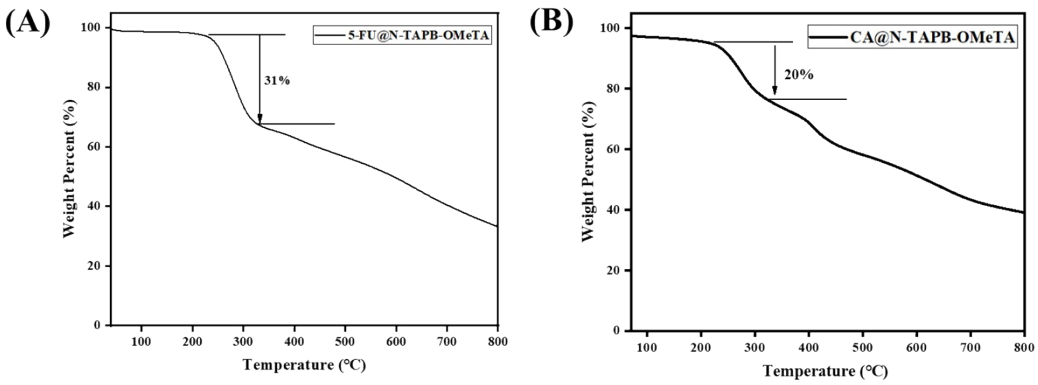


Fig. S11. UV−vis spectra of 5-FU and CA drugs in PBS, and the calibration curve for 5-FU and CA.

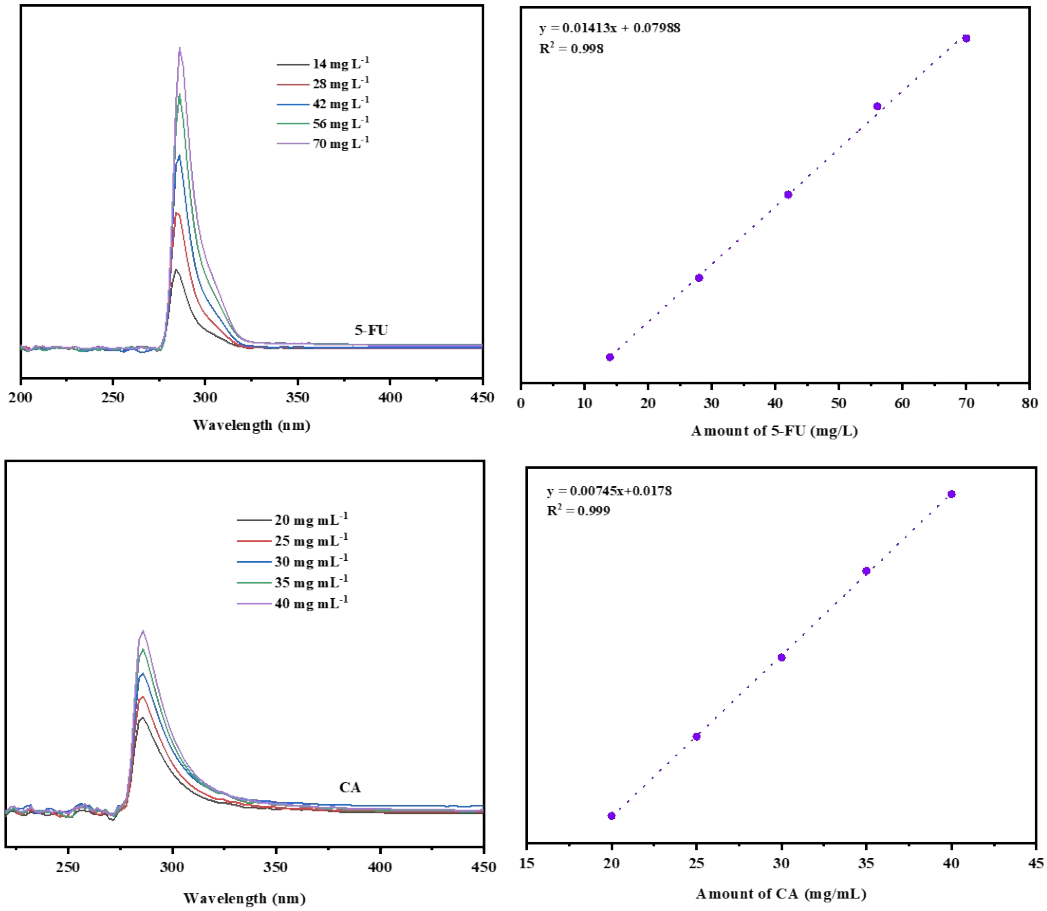


Fig. S12. In vitro cell cytotoxicity of 5-FU and Captopril at 37 ℃ for 48h and their concentration scope was at 25 – 100 μg mL^{-1}.

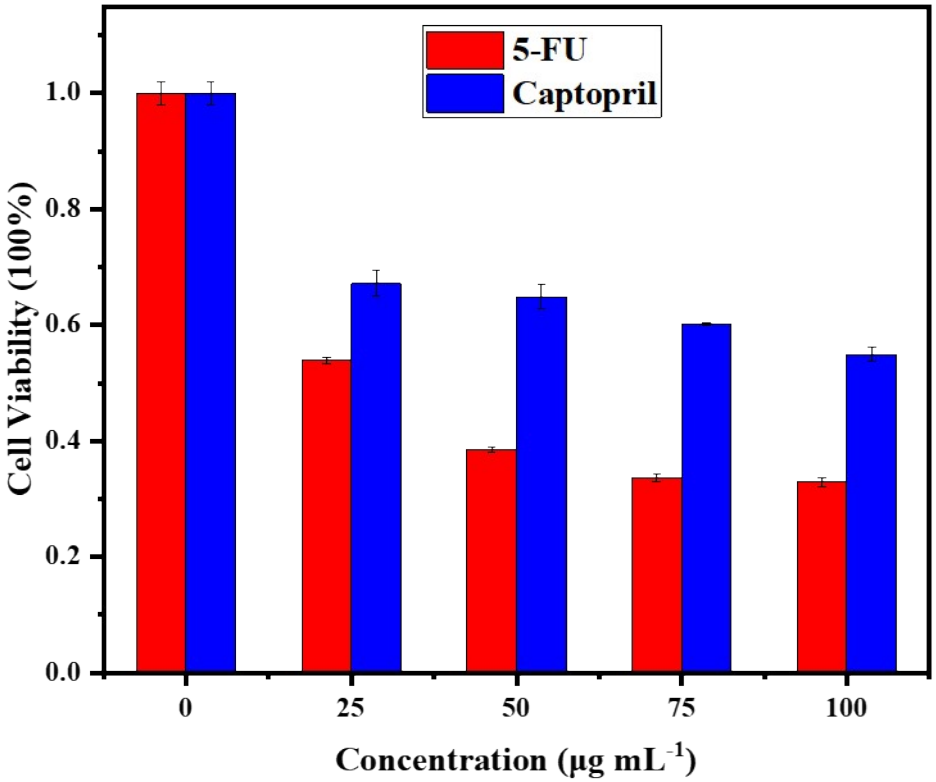
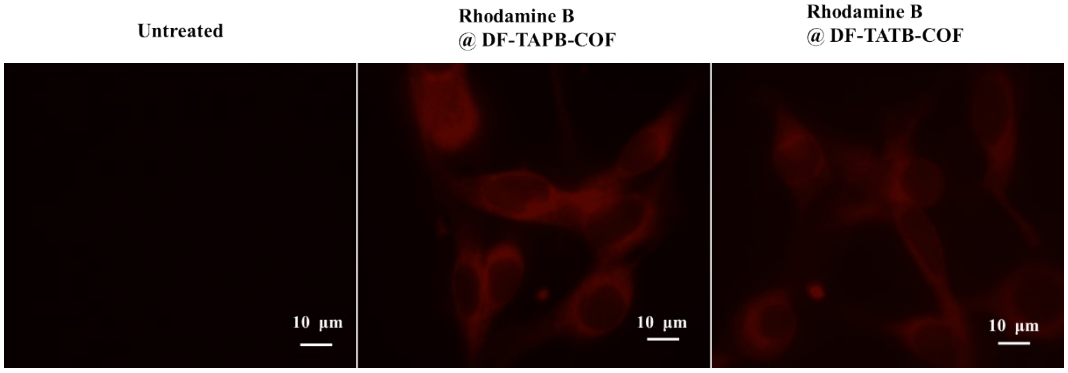


Fig. S13. Inverted fluorescence microscope images of B16F10 cells incubated at 37℃ for 6 h before and after the treatment of Rhodamine B@DF-TAPB-COF and Rhodamine B@DF-TAPB-COF.



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