Supplementary Information

Precise Regulation of MOF Morphology and Structure via Organic Linker Ratio Adjustment for Enhanced Tumor-Specific Therapy

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Supplementary Materials and Methods

Materials

Ferric chloride ($FeCl_3 \cdot 6H_2O$, 99.9%) and N,N-Dimethylformamide (DMF, AR grade 99.5%) were both purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). 4,4'-dithiodibenzoic acid (DTBA) and 4-((4-carboxybenzyl) amino) (CBAB) benzoic acid were obtained from Jilin Zhongke Science and Technology Co., Ltd. (Jilin, China). All the chemicals were used as received without further purification. Ultrapure water (Millipore Milli-Q grade) with a resistivity of 18.2 MΩ was used in all the experiments.

Characterizations

Powder X-ray diffraction (XRD) patterns were recorded on a D/MAX-TTRIII (CBO) and Xeuss SAXS/WAXS system with Cu Kα radiation (λ = 1.542 Å) operating at 40 kV and 40 mA. Scanning electron microscope (SEM) images of the samples were taken on a Regulus 8100 field emission gun-scanning electron microscope at an accelerating voltage of 0.5-30.0 kV. UV-vis absorption spectra were measured using a U-3900 spectrophotometer (Hitachi). Confocal laser scanning microscopy (CLSM) images were obtained using a Nikon Ti-E inverted microscope.

Preparation of MOFs with different ligand doping ratios

$FeCl_3 \cdot 6H_2O$ (0.024 mol, 5.59 mg), DTBA (0.024 mol, 7.352 mg) or CBAB (0.024 mol, 7.652 mg), and DMF (1.6 mL) were added into a Pyrex vial. The mixture was heated in an oven at 85ºC for 60 h. To obtain MOFs with different morphologies, varying proportions of the two ligands were used, namely, 20% DTBA + 80% CBAB, 40% DTBA + 60% CBAB, 60% DTBA + 40% CBAB, and 80% DTBA + 20% CBAB.

ROS activity testing of MOFs structures with different doping ratios in *vitro*

The reactive oxygen species (ROS) activity of MOFs structures with different doping ratios was evaluated using 3,5,3',5'-tetramethylbenzidine (TMB) as the substrate in a HAc-NaAc buffer (pH 7.0) at 25°C. Absorbance was measured at 650 nm using a Bio-Rad 680 microplate reader.

Michaelis-Menten Kinetics

To investigate the enzymatic kinetics of different doping ratios MOFs, different concentrations of H_2O_2 (40, 80, 120, 160and 200 mM) were mixed with TMB (1 mM) and different doping ratios MOFs in PBS solution. The reaction was monitored using a UV–vis spectrometer by measuring the absorbance change at 650 nm over time. The absorbance values were then converted to initial reaction velocities using the Beer–Lambert law, enabling the construction of a Michaelis–Menten kinetic curve. The Michaelis–Menten constant (K_M) and the maximum velocity V_{max} were determined using a Lineweaver–Burk double reciprocal plot. The kinetic parameters were calculated based on the following equations.

$$A = kbc \quad (1)$$

$$v_0 = \frac{V_{max}[S]}{K_M} \quad (2)$$

$$\frac{1}{v_0} = \frac{K_M}{V_{max}}\left(\frac{1}{[S]} + \frac{1}{K_M}\right) \quad (3)$$

where v_o was the initial velocity, and [S] was the concentrations of substrates.

In *vitro* cytotoxicity assay

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Invitrogen) at 37°C, with 5% CO_2 and 10% humidity. To evaluate the cytotoxicity, the cells were seeded in triplicate at a density of 8000 cells/well in 96-well microplates and incubated for 24 h. Subsequently, the culture medium was replaced, and the cells were treated with various concentrations (0, 0.86, 2.59, 7.78, 23.3, 70.0 or 210 μg mL^{-1}) of different formulations, including 100% CBAB, 40% DTBA + 60% CBAB, 80% DTBA + 20% CBAB, and 100% DTBA, at 37°C for another 24 h. The cytotoxicity was assessed at the end of the incubation period using a standard MTT assay.

Determination of ROS generation at cellular level

To evaluate the intracellular ROS production, Hela cells were seeded in 6-well plates at a density of 1×10^5 cells per well and incubated at 37°C for 24 h in the dark.

The cells were then co-incubated with various formulations, including 100% CBAB, 40% DTBA + 60% CBAB, 80% DTBA + 20% CBAB, and 100% DTBA, for an additional 24 h. Following incubation, the medium was removed, and the cells were gently washed three times with PBS. Subsequently, DCFH-DA was added to each well and incubated for 1 h. The fluorescent morphology of the cells was observed using a confocal laser scanning microscopy (CLSM, Nikon C2+, China), and the intracellular fluorescence intensity was then quantified to assess ROS production.

Live/dead assay

Hela cells were seeded in 6-well plates at a density of 1×10^5 cells per well and cultured overnight at 37°C in a 5% CO_2 atmosphere. The cells were then treated as described under the specified above conditions for 24 h. After the treatment period, the cell culture medium was discarded and replaced with fresh medium containing calcein-AM (10 μg mL^{-1}) and propidium iodide (PI, 10 μg mL^{-1}). After incubation for 20 min, the cells were washed with PBS, and imaged using an inverted fluorescence microscope (Nikon C2+, China).

Statistical Analysis

All experiments were presented as the mean ± standard deviation (SD). Statistical comparisons between groups were performed using a one-way analysis of variance (ANOVA) in SPSS 25.0. Each experiment was performed independently at least three times. A P value < 0.05 was considered statistically significant.

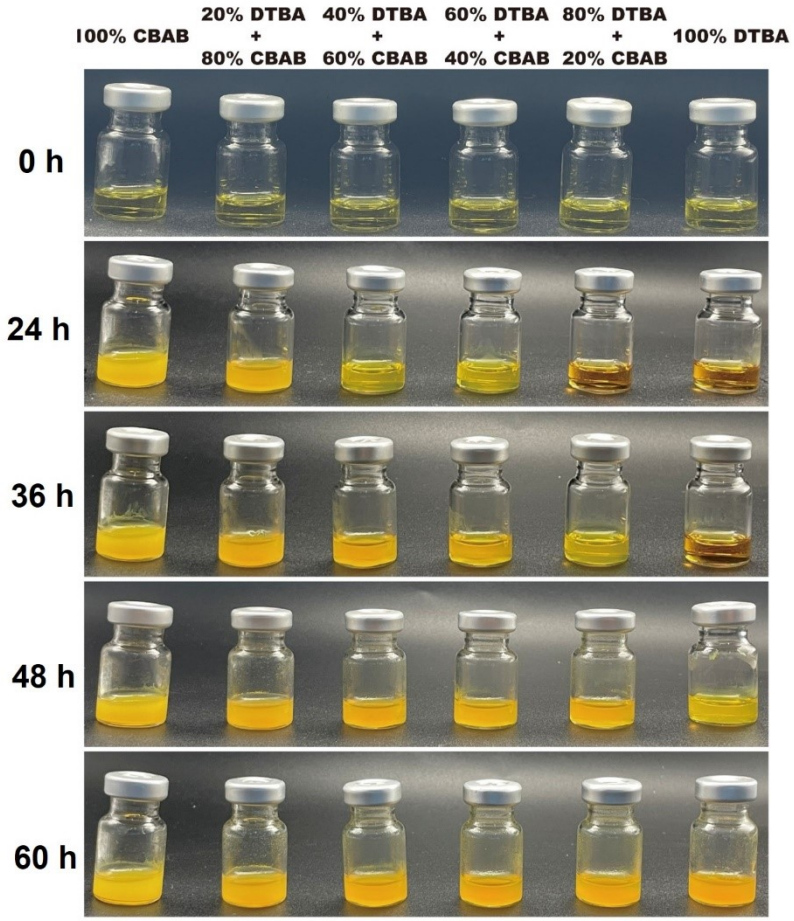

Figure S1. Reaction diagram of different experimental groups at different time.

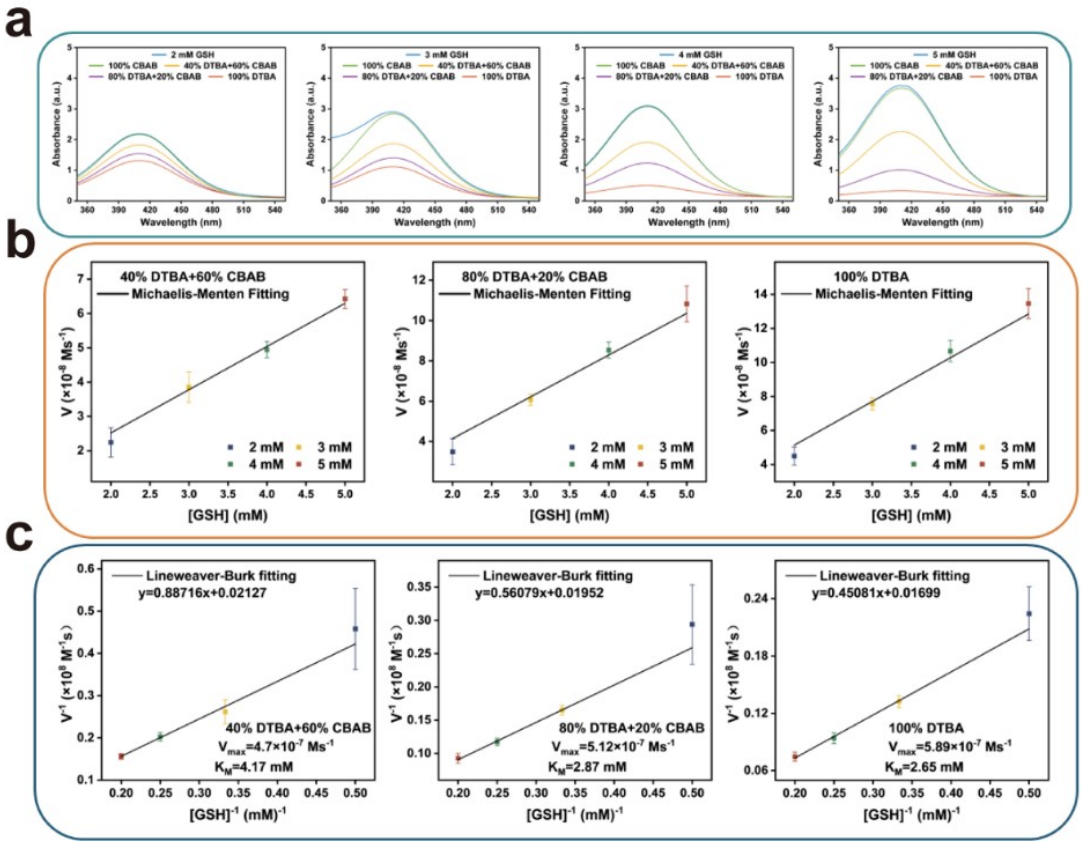

Figure S2. The GSH consumption rate of MOFs with different doping proportions was determined by DTNB method. a) Ultraviolet absorption curve, b) Michaelis-Menten kinetic analysis, c) Lineweaver-Burk plotting method.

We systematically analyzed and validated the GSH depletion performance of MOFs with varying DTBA doping ratios using the classical Michaelis–Menten steady-state kinetic model. As shown in Figure S2, GSH was used as the substrate at concentrations of 2, 3, 4, and 5 mM. The change in absorbance before and after the reaction was recorded for each group, from which the average initial reaction rates were calculated (Figure S2a). According to the Beer–Lambert law, these absorbance changes were converted into initial ROS generation rates (V_0), which were then fitted to the Michaelis–Menten equation to obtain the corresponding kinetic curves (Figure S2b). To derive key kinetic parameters, a Lineweaver–Burk double reciprocal plot was further constructed (Figure S2c), from which the Michaelis–Menten constant (K_M) and maximum reaction rate (V_{max}) were calculated. The experimental results clearly demonstrated a progressive enhancement in GSH depletion ability with increasing DTBA content.

Specifically, the V_{max} reached 4.17 × 10^{-7} M·s^{-1} when the DTBA doping ratio was 40%, increased to 5.12 × 10^{-7} M·s^{-1} at 80%, and peaked at 5.89 × 10^{-7} M·s^{-1} for MOFs composed entirely of DTBA. Concurrently, the K_M values exhibited a continuous downward trend, indicating an increasing affinity of MOFs toward the GSH substrate. These findings further confirm the potential of the DTBA-doped MOF system in regulating intracellular GSH and enhancing oxidative stress.

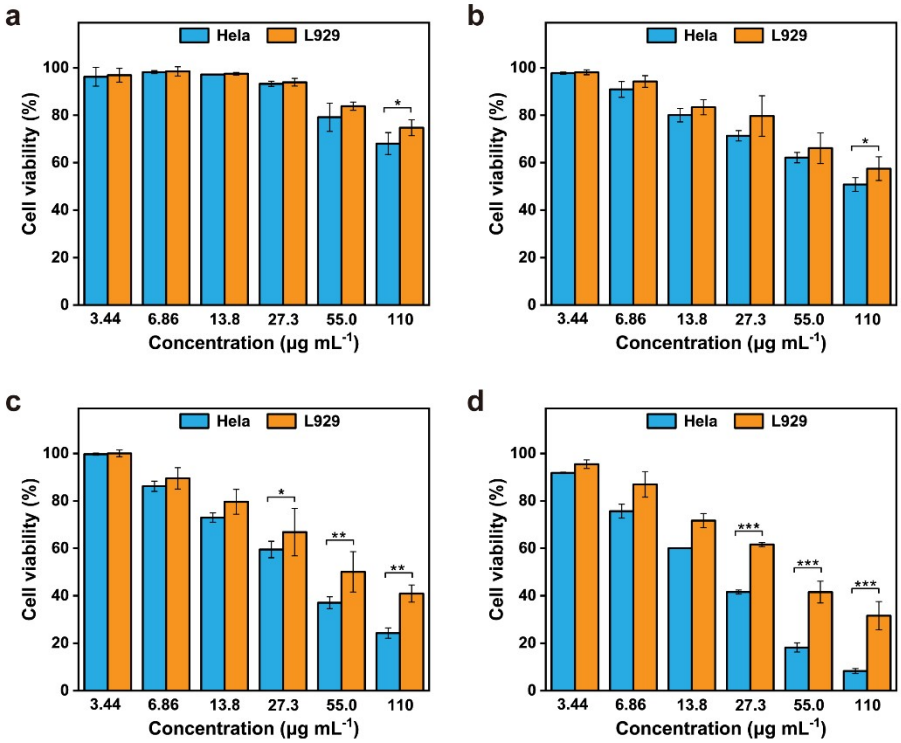

Figure S3. MTT comparison between Hela and L929. a) 100% CBAB, b) 40% DTBA+60% CBAB, c) 80% DTBA+20% CBAB, d) 100% DTBA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

As shown in Figure S3, MOFs with progressively higher DTBA doping ratios exhibited significantly increased cytotoxicity toward HeLa tumor cells relative to normal L929 cells, indicating effective selectivity for the tumor microenvironment. Notably, the 100% DTBA-MOF treatment group demonstrated the most pronounced difference in cytotoxicity between HeLa and L929 cells, particularly at higher concentrations, highlighting its superior tumor-specific cytotoxicity.

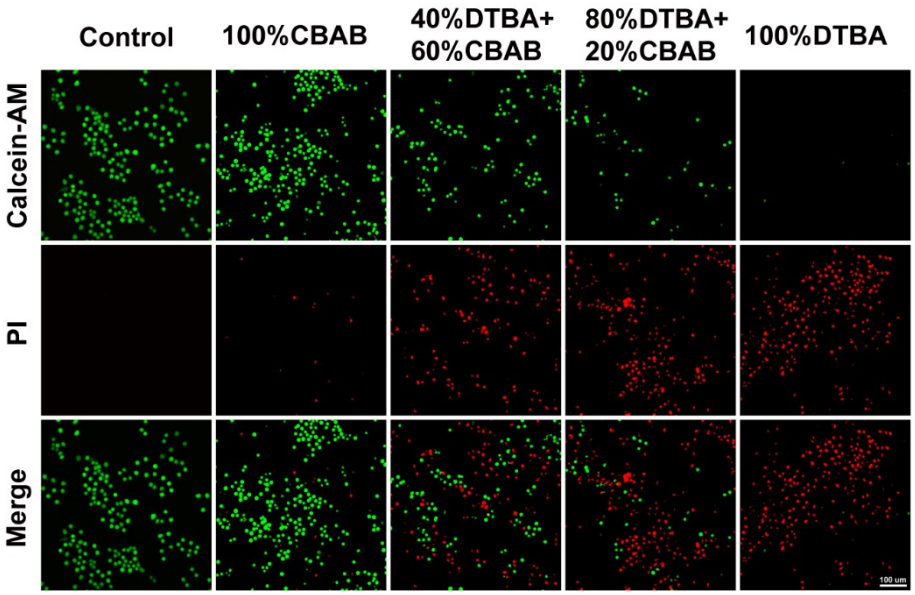

Figure S4. Fluorescence images of Hela cells costained with Calcein AM/PI after different treatment groups. Scale bar: 100 μm.