

Electronic Supplementary Information (ESI)  
For  
**pH-Responsive Mitoxantrone (MX) Delivery Using Mesoporous Silica  
Nanoparticles (MSN)**

Yanhang Ma,<sup>a†</sup> Lin Zhou,<sup>b†</sup> Haoquan Zheng,<sup>a</sup> Lei Xing,<sup>a</sup> Chenguang Li,<sup>a</sup> Jinghao Cui<sup>b\*</sup> and Shunai Che<sup>a\*</sup>

<sup>a</sup> School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P. R. China. Fax: +86 21 5474 1297; Tel: +86 21 5474 2852; E-mail: [chesa@sjtu.edu.cn](mailto:chesa@sjtu.edu.cn)

<sup>b</sup> Department of Pharmaceutics, College of Pharmaceutical Science, Soochow University, 199 Renai Road, Suzhou 215123, China. Fax: X+86-512-6588-2077; E-mail: [jhcui@suda.edu.cn](mailto:jhcui@suda.edu.cn)

### 1. Material

Cetyltrimethylammonium bromide (CTAB; SCRC, China), tetraethylorthosilicate (TEOS; TCI, Japan), Mitoxantrone (MX). All materials were used as purchased without further purification.

### 2. Methods

#### 2.1. Synthesis of MCM-41-Type MSN

Typically, 1 g CTAB was first dissolved in the mixture of 480 ml H<sub>2</sub>O and 7 ml NaOH (1.0 M). The temperature of the solution was adjusted to 80 °C. Then 5.0 ml TEOS was added dropwise to the solution, followed by stirring for 2 h at 80 °C to give rise to white precipitation. The solid product was filtered, washed with deionized water and dried at 100 °C overnight. To remove the surfactant, the as-synthesized materials were extracted in the ethanolic solution of 1 M HCl.

#### 2.2. Synthesis of methylate- and mercapto- functionalized MCM-41

The methylate group and mercapto group functionalized MCM-41 nanoparticles were synthesized by post grafting method. Typically, 0.5 g MCM-41 nanoparticles after removal of surfactant were suspended in 20 mL toluene. And then 0.42 mM Methyltriethoxysilane or 3-Mercaptopropyltrimethoxysilane was added dropwise under stirring, followed by refluxing for 12 h.

#### 2.3. Synthesis of carboxyl group functionalized MSN

The carboxyl group functionalized mesoporous nanoparticles were synthesized by co-condensation method. 0.14 g C18-3-1 was dissolved in the mixture of 135 mL deionized water and 5.8 g ethanol at 80 °C. And then 0.174 g CES and 0.78 g TEOS were added simultaneously, followed by stirring for 1 h at 80 °C. The obtained solution was aged at 80 °C for 2 days. To removal the surfactant in the nanoparticles, the synthesized materials were extracted with the mixture of 90 mL THF and 10 mL 35% HCl.

#### 2.4. Characterizations

Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku X-ray diffractometer D/MAX-2200/PC equipped with Cu K<sub>α</sub> radiation (40 kV, 20 mA) at a rate of 1.0°/min over the range of 1–6° (2θ). The morphology of MCM-41 nanoparticles was observed with scanning electron microscope (SEM, JEOL JSM-7401F) with an accelerating voltage of 1.0 kV. High-resolution transmission electron microscopy (HRTEM) images were taken with a JEOL JEM-3010 microscope operating at 300 kV. The nitrogen adsorption/desorption isotherms were measured at –196 °C with a Quantachrome Nova 4200E porosimeter. The surface area was calculated by the Brunauer–Emmett–Teller (BET). The pore size distribution was calculated by Barrett–Joyner–Halenda (BJH) method according to the adsorption branch of the isotherm. The concentration of anti-cancer drugs (MX) in

solution was measured by a UNICO UV-4802 UV-vis double beam spectrophotometer. The zeta-potential of the nanoparticles was measured in a Malvern ZS90 zeta potential analyzer.

### 2.5. Loading of MX in the mesopores of the MSN

The mitoxantrone was first prepared as a solution with the concentration of 200 µg/ml in ethanol. In a typical condition, 0.1 g MCM-41 nanoparticles were added into 10 ml MX solution with stirring for 24 h. The materials were obtained by centrifugation and then would be washed for three times with PBS of pH 7.4.

### 2.6. Release of MX from the MSN in PBS solution

In a typical release experiment, about 25 mg of the MCM-41 materials loading with MX was suspended by vibration in 30.0 ml of PBS solution with pH 4.0-7.4 at 37 °C. In the case of sampling, 2 ml homogenous solution were withdrew to centrifuged, followed by being measured with UV-vis spectrophotometer.

### 2.7. In-vitro cell assay

Cells were seeded in a 96-well plate at a seeding density of 5,000 per well in 100µL of RPMI 1640 medium with 10% FBS and 1% penicillin and streptomycin. After the cells were cultured at 37°C for 24h, the growth medium was removed and fresh growth medium containing the predetermined amount of MX-loaded nanoparticles was added. After a 24h incubation, cells were washed three times with 100µL of PBS, and then 100µL of RPMI 1640 medium with 10% FBS was added. Cytotoxicity was assessed using MTT to measure the viability of the cells. 10µL of MTT solution (5mg/mL) was added to each well. The plates were incubated for an additional 4h and then 100µL of 10%HCl-SDS were added to dissolve the MTT formazan crystals. After the plates were cultured overnight, the absorbance of each well was measured at 570 nm in a microplate reader.

### 2.8. Fluorescent experiments

Cells were seeded in a 6 well plate at a seeding density of  $2 \times 10^5$  per well in 2 mL of RPMI 1640 medium with 10% FBS and 1% penicillin and streptomycin. After the cells were cultured at 37 °C for 24 h with the concentration of CO<sub>2</sub> of 5%, the growth medium was removed and fresh growth medium containing the predetermined amount of Rhodamine-loaded nanoparticles was added. After 24 h incubation, cells were washed three times with 2 mL of PBS and then were observed in a confocal laser microscopy.

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## I. SEM images of MCM-41 nanoparticles

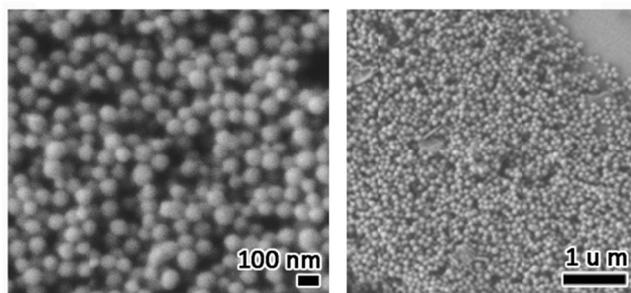


Figure S1. SEM images of MCM-41 nanoparticles.

The SEM image shows that the MSN are spherical shape with the size of 80-100 nm.

## II. N<sub>2</sub> adsorption/desorption results of MCM-41 nanoparticles

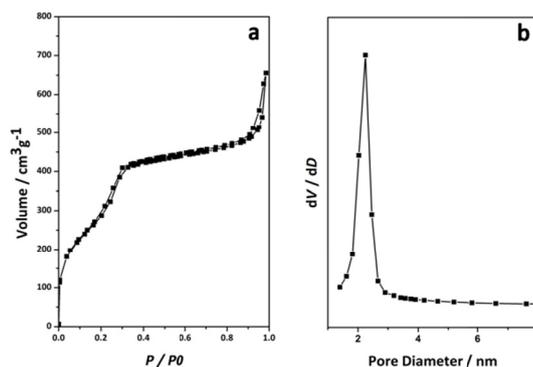


Figure S2. (a) N<sub>2</sub> adsorption/desorption isotherm and (b) pore size distribution of the MCM-41 nanoparticles after calcinations shown in Figure 1.

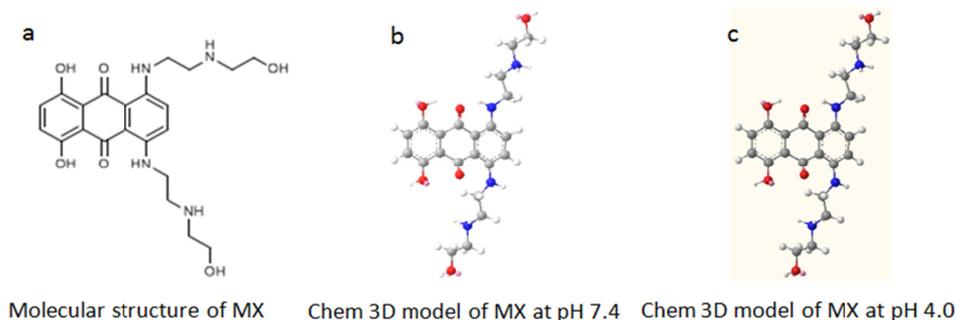
Table S1. Porous and compositional properties of MCM-41 nanoparticles.

	Surface area <sup>a</sup> (m <sup>2</sup> /g)	Pore volume <sup>b</sup> (cm <sup>3</sup> /g)	Pore size <sup>c</sup> (nm)
MCM-41	834	0.73	2.25

<sup>a-c</sup> Calculated from N<sub>2</sub> adsorption/desorption data.

The N<sub>2</sub> adsorption/desorption analysis of the extracted material revealed typical type IV isotherms. The measured BET (Brunauer-Emmett-Teller) surface area was 834 m<sup>2</sup>/g. The corresponding BJH (Barrett-Joyner-Halenda) average pore diameter was 2.25 nm and the pore volume was 0.73 cm<sup>3</sup>/g.

### III. Molecular structures of MX and their Chem3D models.

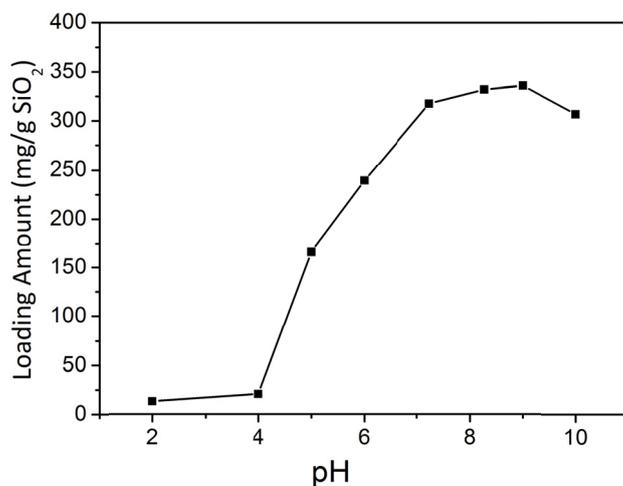


**Figure S3.** (a) Molecular formula of MX and Chem3D model of MX at (b) pH 7.4 and (c) pH 4.0.

5 The two secondary amines on the label chain of MX put the structure of the molecule as shown in Figure S3a. At pH 7.4, only aliphatic amines were positively charged and the molecular size was about 2.07 nm (Fig. S3b). When the pH was changed to 4.0, the aromatic amine groups were also positively charged and molecular size came to be about 2.03 nm (Fig. S3c).

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### 15 IV. Loading amount of MX for the drug delivery system at different pH values.

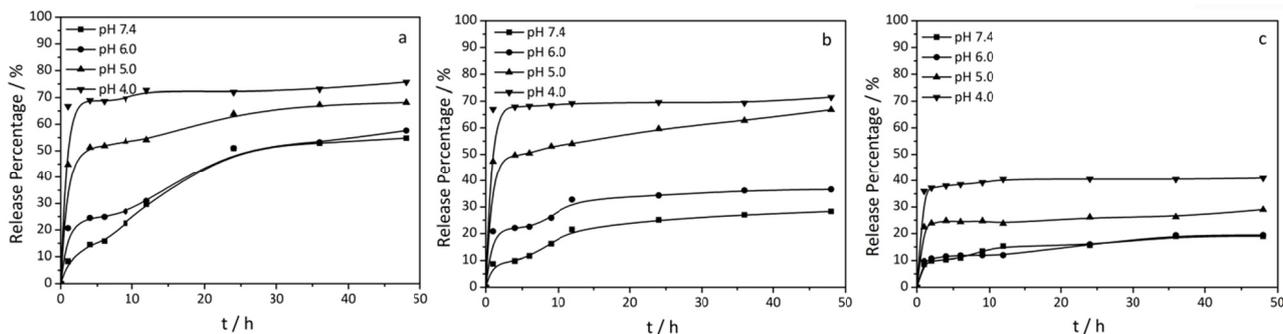


**Figure S4.** Loading amount of MX in the MCM-41 nanoparticles under various pH conditions.

20 The loading amount of MX in the MSN have increased when the pH values increase from 3 to 8, which could be attributed to enhanced negative charge on silanol groups. However, when the pH value reaches above 8, the loading begins to reduce due to the loss of positive charge of MX.

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## V. Release of MX from various organic group functionalized MCM-41 nanoparticles.



5 **Figure S5.** (a) 6% methyl group functionalized MCM-41 nanoparticles; (b) 6% mercapto group functionalized MCM-41 nanoparticles; (c) carboxyl group functionalized mesoporous nanoparticles.

Fig. S5a shows the release profiles of MX in various organic group functionalized MSN. In methyl group functionalized sample, a high release percentage of MX has been observed in PBS solution at 10 pH 7.4. For the second sample (as shown in Fig. S4b), the release profile shows a pH-responsive delivery. These results reveal that organic groups could still interact with organic parts of MX molecules. The pH-responsive delivery of MX in Fig. S4b might be attributed to complicated hydrogen bonding or electrostatic interactions. The quantitative determination of the functional groups was determined by CNS elemental chemical analysis. The results show that the loading amounts of methyl 15 groups and mercapto groups were 6.0 and 6.3 mmol/g SiO<sub>2</sub>, respectively. Careful works would be needed to further investigate the reason. Fig. S4c reveals that MX is hard to be released even at low pH because of a strong electrostatic interaction between carboxyl groups and MX.

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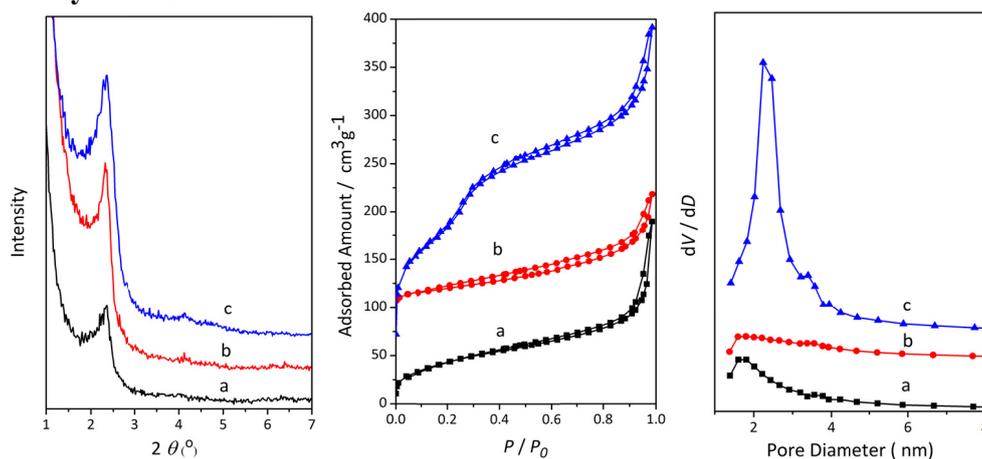
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## VI. The stability of MCM-41 MSN.



**Figure S6.** XRD patterns,  $N_2$  adsorption/desorption isotherms and pore size distribution of (a) MX-loaded MCM-41 nanoparticles; (b) materials released in PBS (pH 7.4) for 48h; (c) materials released in PBS (pH 4.0) for 48h.

**Table S2.** Porous and compositional properties of MCM-41 nanoparticles at various stages.

Materials	Surface area <sup>a</sup> ( $m^2/g$ )	Pore volume <sup>b</sup> ( $cm^3/g$ )	Pore size <sup>c</sup> (nm)
MX loaded MCM-41	158	0.3	1.8
Treated in 7.4 for 48 h	77	0.2	1.8
Treated in 4.0 for 48 h	450	0.538	2.2

<sup>a-c</sup> Calculated from  $N_2$  adsorption/desorption data.

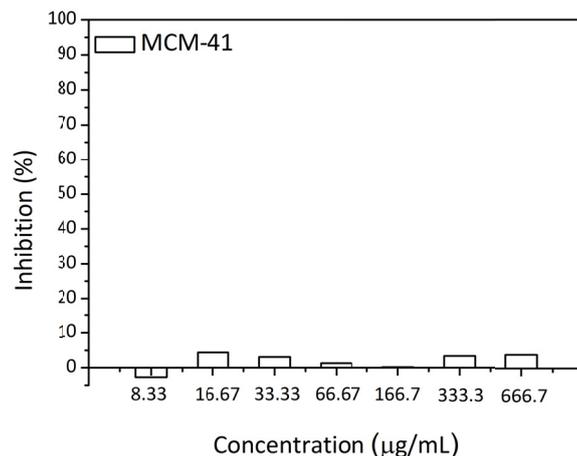
The presence of peak ( $2\theta \sim 2.5^\circ$ ) indicates the maintenance of mesostructure of the nanoparticles after loading and release the drug.  $N_2$  analysis results show that the change of properties of mesopores. The surface area, pore volume and pore size all decrease after treated in PBS solution at pH 7.4 for 24 h and 48 h. However, at pH 4.0, MX would be released and mesopore structure is mostly preserved. An obvious decrease of surface area, about  $676 m^2/g$ , has been found after loading MX, which demonstrates that most of mesopores have been occupied by MX. There is also a decrease of surface area after treated in PBS solution, which might be caused by the collapse of mesopores. As MX will be released at pH 4.0, the surface area of sample in PBS solution at pH 4.0 is larger than that at pH 7.4 after treated for 48 h.

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## VII. Effect of MCM-41 in inhibition of cancer cell.



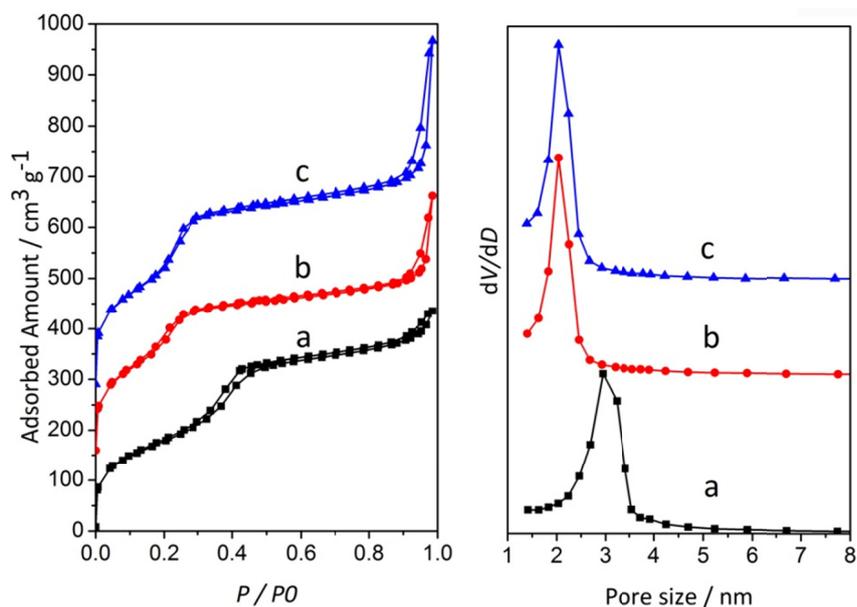
**Figure S7.** Inhibition ratio of SMMC-7721 cells for MCM-41 nanoparticles.

5 This result showed that the MCM-41 nanoparticles show low cytotoxicity and could be employed as a favorable drug carrier for cancer therapy.

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## VIII. N<sub>2</sub> adsorption/desorption results of carboxyl group, methyl and mercapto group functionalized MSN.

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**Figure S8.** N<sub>2</sub> adsorption/desorption isotherms and pore size distribution of (a) carboxyl group functionalized MSN; (b) methyl group functionalized MCM-41; (c) mercapto group functionalized MCM-41.

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**Table S2.** Porous and compositional properties of carboxyl group, methyl group and mercapto group functionalized mesoporous nanoparticles.

Materials	Surface area <sup>a</sup> (m <sup>2</sup> /g)	Pore volume <sup>b</sup> (cm <sup>3</sup> /g)	Pore size <sup>c</sup> (nm)
Carboxyl group functionalized materials	648	0.71	2.94
Methyl group functionalized materials	711	0.8	2.04
Mercapto group functionalized materials	845	0.86	2.04

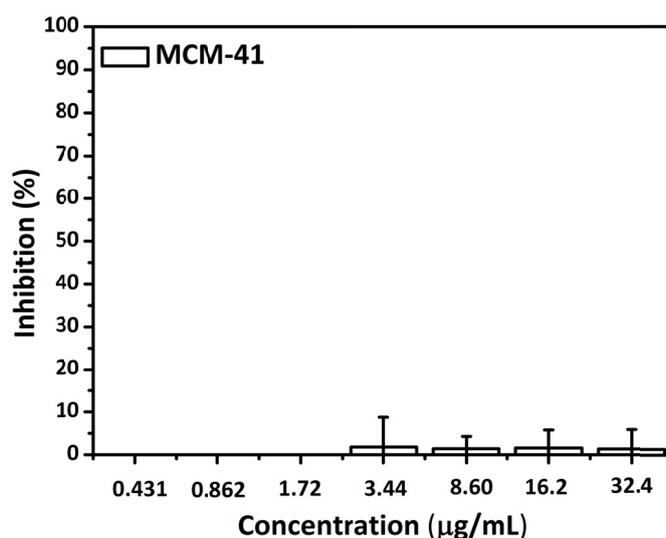
<sup>a-c</sup> Calculated from N<sub>2</sub> adsorption/desorption data.

All the three samples possess a high mesopore surface and pore volume. The carboxyl group functionalized materials have been synthesized based on co-condensation method according to ref. 1. The pore size of 2.94 nm is large enough to hold the drug molecule. The methyl and mercapto group functionalized samples have been synthesized based on post-grafting method. The pore sizes show a slight reduction after functionalization.

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#### IX. Cytotoxicity of MCM-41 nanoparticles to normal cells.

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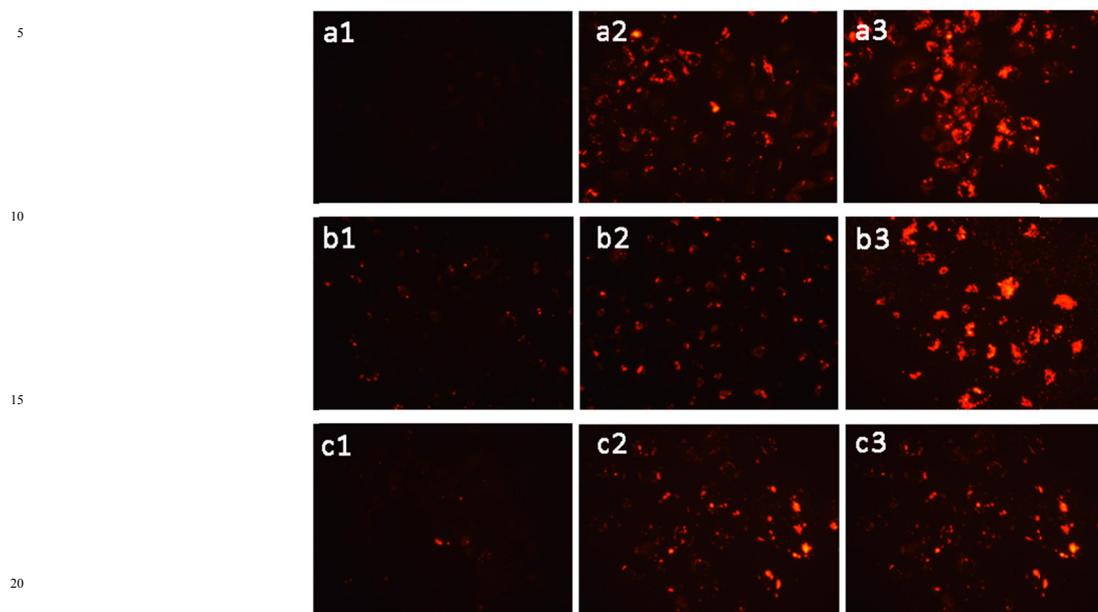
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**Figure S9.** Inhibition ratio of QSF-7701 cells for MCM-41 nanoparticles.

This result showed that the MCM-41 nanoparticles were of low cytotoxicity to normal cells, indicating a good biocompatibility.

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**X. Uptake efficiency of fluorescent MCM-41 in three different cells of A549, SMMC-7721, and MCF-7.**



**Figure S10.** Fluorescent images of a) A549 cells; b) SMMC-7721 cells; c) MCF-7 cells. 1, 2 and 3 represent different concentration of MCM-41, 167 mg/mL, 667 mg/mL and 1000 mg/mL respectively.

To study the uptake efficiencies of MCM-41 nanoparticles in three cell lines, Rhodamine B, a kind of common fluorescent agent, has been used as the dye. As shown in the results, with the increase of concentration of MCM-41, the intensity of fluorescent increases. Besides, the comparison of three groups reveals that the cellular uptake efficiency in A549 is highest, which also accounts for the high inhibition in the *vitro* experiments.