Supporting Information

Outstanding drug loading capacity by water stable microporous MOF: A potential drug carrier

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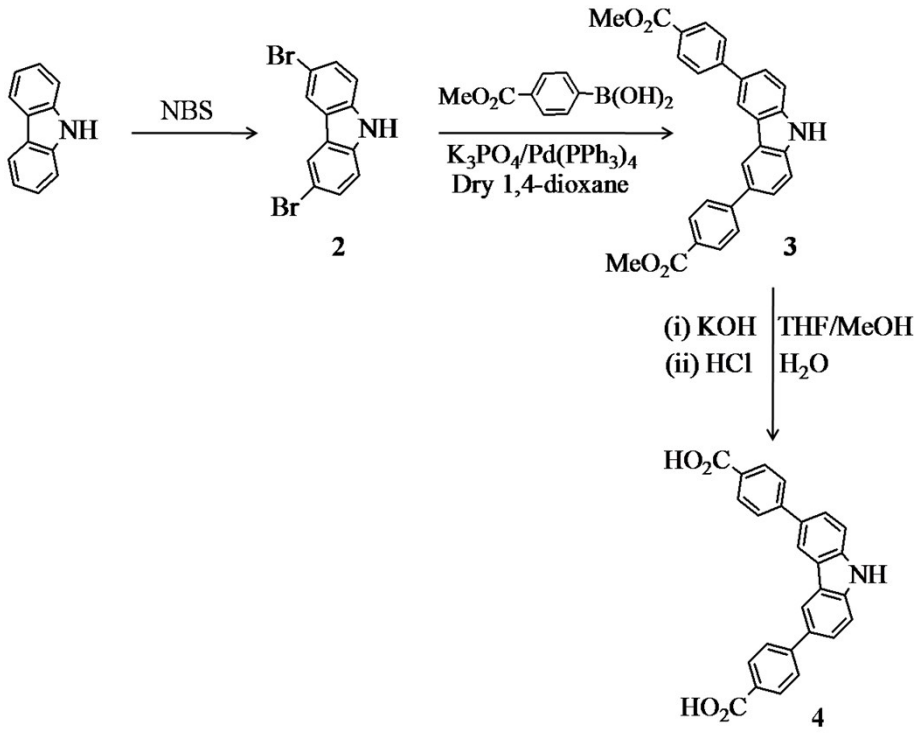
S1. Materials and Measurements

All the starting materials and solvents were obtained from commercial sources, and were used without further purification. Elemental analyses (C, H and N) were measured on a Elementar Vario EL III analyzer. IR spectrum was performed in the range 4000–400 cm^{-1} using KBr pellets on an PerkinElmer Spectrum. Powder X-ray diffraction (PXRD) data were collected on a Rigaku MiniFlex2 diffractometer working with Cu Kα radiation (λ = 1.5418 Å), and the recording speed was 5° min^{-1} over the 2θ range of 5 − 50° at room temperature. Low-pressure gas adsorption measurements were carried out on an ASAP (accelerated surface area and porosimetry) 2020 system. The Scanning Electron Microscopy (SEM) images are taken on a JEOL JSM-6700F instrument with gold spray. Thermogravimetric analysis (TGA) of the samples was performed using a NETZSCH STA 449C unit heated from room temperature to 900 ºC at a heating rate of 10 °C min^{-1} under flowing nitrogen atmosphere. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker Avance III spectrometer (400 MHz; the chemical shifts were referenced to TMS in the solvent signal in DMSO-d_6. Fluorescence spectroscopy data were recorded on a FLS920 fluorescence spectrophotometer. The simulated powder patterns were calculated using Mercury 2.0. The purity and homogeneity of the bulk products were determined by comparison of the simulated and experimental X-ray powder diffraction patterns. The images of the crystals were captured under a microscope (CEWEI PXS6555, Sanghai Cewei Photoelectric Technology Company).

S2. Synthesis of Ligand

The ligand was synthesized according to modified procedure of the reported literature.[1]



Scheme1. Synthetic pathway of the targeted ligand **4**.

Synthesis of 3,6-Dibromo-9H-carbazole (**2**)**:** A solution of N-bromosuccinimide (22.35 g, 0.125 mol) in 50 mL DMF was slowly added with stirring to a solution of carbazole (10 g, 0.059 mol) in 20 mL DMF with ice bath. After reacting for 2 h, the reaction mixture was poured into 600 mL ice water, and the crude product was collected by filtration to give white powder (19.24 g, 99% yield). Recrystallization from EtOH/H_2O to afford white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.49 (d, J = 6.9 Hz, 2H), 7.54 (d, J = 6.9 Hz, 2H), 8.44 (s, 2H), 11.60 (s, 1H, ―NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 111.09, 112.99, 123.01, 123.58, 129.31, 139.41.

Synthesis of 4,4́-(9H-carbazole-3,6-diyl)dibenzoic acid dimethyl ester (**3**)**:** To a 250 mL Schlenk flask, **2** (2 g, 6.15 mmol), 4-methoxycarbonylphenylboronic acid (2.768 g, 11.1 mmol), and K_3PO_4 (21.2 g, 15.38 mmol) were mixed in 1,4-dioxane (100 mL), and the mixture was deaerated using N_2 for 30 min. $Pd(PPh_3)_4$ (163.56 mg, 0.14 mmol) was added to the stirred reaction mixture and the mixture was heated at 90 ˚C for 48 h under N_2 after which 1,4-dioxane was removed under a vacuum. The resultant solid was washed with water (30 mL) and methylene dichloride (60 mL), respectively, and then dried under a vacuum. The pure 4,4́-(9H-carbazole-3,6-diyl)dibenzoic acid dimethyl ester (**3**, 1.5 g) was

filtered and dried after refluxing in MeOH for 24 h. Yield 55.9%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm = 3.96 (s, 6H, —OMe), 7.55 (d, J = 7.6 Hz, 2H), 7.75 (d, J = 7.6 Hz, 2H), 7.80 (d, J = 6.8 Hz, 4H), 8.15 (d, J = 7.2 Hz, 4H), 8.23 (s, 2H), 8.4 (s, 1H, —NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 51. 09, 111.09, 115.99, 118.29, 119.45, 127.01, 129.11, 129.52, 129.81, 131.54, 168.41.

Synthesis of 4,4́-(9H-carbazole-3,6-diyl)dibenzoic acid (4): 3 (1.5 g, 3.44 mmol) was then suspended in a mixture of THF (25 mL) and MeOH (25 mL), to which 15 mL of 2 N KOH aqueous solution was added. The mixture was stirred under reflux overnight and the THF and MeOH were removed under a vacuum. Dilute HCl was added to the remaining aqueous solution until the solution was at pH = 3. The solid was collected by filtration, washed with water and MeOH, and dried to give H_2CDBA (1.3 g, 92.7% yield). ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm = 7.63 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 8.4 Hz, 4H), 8.07 (d, J = 8.0 Hz, 4H), 8.74 (s, 2H), 11.55 (s, 1H, —NH), 12.91 (s, 2H, —CO_2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 111.29, 116.19, 118.49, 119.15, 127.31, 129.41, 129.82, 129.89, 132.24, 169.11.

Synthesis of (1): A mixture of $Zn(NO_3)_2 \cdot 6H_2O$ (14.57 mg, 0.049 mmol), H_2CDDB (10 mg, 0.0245 mmol), and 1 mL of DMF/EtOH (1:1) was stirred for 10 min. The mixture was then transferred and sealed in a Teflon reactor (18 mL) and heated at 100 °C for 3 d. After the mixture was cooled to room temperature at 5.83 °C h^{-1}, colorless crystals of **1** were obtained (60.5% yield based on H_2CDDB). Elemental analysis (%) calcd. for **1** : C, 56.16; H, 5.34; N, 6.38. Found: C, 56.31; H, 5.32; N, 6.45.

Table S1. Nature of packing, pore size, drug loading capacity of different MOFs for 5-Fu

Nature of packing	Materials		Pore Size/Diameter(Å)	Drug loading (wt%)
	Organic part	Inorganic Part		
2D	4,4'-(9-H Carbazole-3,6-diyl)dibenzoic acid	Zn	28.1 × 23.17	**53.3** **This work**
3D	5-(prop-2-ynyloxy)isophthalic acid, 2,6-lutidine	Cu	—	4.38[12#]
3D	5,5′,5′′-(1,3,5-triazine-2,4,6-triyl)tris(azanediyl)triisophthalate	Zn	6.3 × 10.5 and 14.3 × 11.5	33.3[13]
3D	5-NH2-m-benzenedicarboxilic acid	Cu	23.18 × 32.63	23.76[14]

3D	4,5-di(1H-tetrazol-5-yl)-2H-1,2,3-triazole	Zn	12 (diameter)	30.48[15]
3D	5-aminoisophthalic acid	Mg	15.14 × 12.84	21.06[16]
3D	Benzenetricarboxilic acid	Zn	14.509 × 13.908 and 19.727 × 20.751	14.49[17]
3D	1,2,3,4,5,6-hexakis(3-carboxyphenyloxymethylene) benzene	Zn	11.0 × 11.0	26.93[18]
3D	diphenylmethane-4,4'-dicarboxylic acid and 4,4′-bipyridine	Cu	—	21.56[19]
3D	1,1′,4′,1″,4″,1‴-quaterphenyl-3,5,3‴,5‴-tetracarboxylic acid 1,3,5-benzenetrisbenzoate	Zn	14.2 × 14.5	22.5[20]

The mentioned references from 12–20, are belongs to main draft.

S3. Single crystal X-ray diffraction; data collection, structure solution and refinement procedures.

Single-crystal X-ray diffraction data this MOF **1** was collected on a SuperNova, Dual, Cu at zero, Atlas diffractometer. The crystals were kept at 100(16) K during data collection. The structures were solved with the SHELXS[2] structure solution program using Direct Methods and refined with the SHELXL[3] refinement package using Least Squares minimisation. All non-hydrogen atoms were refined with anisotropic displacement parameters. The positions of hydrogen atoms attached to carbon atoms were generated geometrically. Attempts to locate and model the disordered solvent molecules in the pores were unsuccessful. Therefore, we employed PLATON/SQUEEZE[4] to calculate the contribution to diffraction from the solvent region and thereby produced a set of solvent-free diffraction intensities. The coordinated H2O and DMF molecules in this structure are determined according to charge balance and rational experimental bond and angle parameters. The final formula was calculated from the SQUEEZE results combined with elemental analysis data and TGA data. More details on the crystallographic studies as well as atomic displacement parameters are given in the ESI as CIF files. Crystallographic data for the structures reported in this study has been deposited. The following crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number (CCDC no. 1410694) for compounds **1**.

Preparation of desolvated MOF 1 (active)

A fresh MOF **1** was soaked in methanol for 24 h and the extract was discarded. Fresh methanol was subsequently added, and the sample was allowed to soak for another 24 h to remove H_2O and DMF.

The sample was then treated with dichloromethane using the same procedures to remove methanol solvates; after the removal of dichloromethane by decanting, the sample was activated by employing a dynamic vacuum ($<10^{-3}$ Torr) at 100 ºC for 12 h. This sample activated sample was used for drug loading experiment.

S4. Drug Loading and Release

To load 5-fluorouracil (5-FU) into the pores of **1**, dehydrated **1** (20 mg) was dispersed in a 5- FU (25 mg) containing methanol solution (5 mL) and stirred for 1 days yielding homogenous light white solution. Then the mixture was centrifuged (12000 r/min for 20 min) and then the solid was washed extensively with chloroform[5] to obtain the drug-loaded **1**. The adsorbed amount of 5-Fu into the porous solids was estimated by taking the mass of the sample and thermogravimetric analysis (TGA).

Determination of the 5-Fluorouracil Content

Optimization of 5-Fu adsorption

Table S2. Estimated f-Fu content = f(several impregnation parameters).

Impregnation parameters		g 5-FU/g dehydrated MOF
5-FU/material weight ratio	1:1	-
	2:1	0.05
	3:1	0.1
	4:1	0.15
	5:1	0.2
	1:2	-
	3:2	0.2
	5:2	0.4
	2:3	0.111
	4:3	0.222
	5:3	0.444
	5:4	0.666

15 mg of drug-loaded **1** was dispersed into 1.5 mL of PBS buffer solution (pH = 7.4), and loaded into a dialysis bag (MWCO = 1000), which was dialyzed against 3 mL of deionized water at 37°C.

During each time interval, *ca.* 1 mL of the solution was pulled out to test, and then decanted back when the test was over. The content of 5-FU in the samples taken out was monitored by fluorometry, in which the detection wavelength was 388 nm (λ_{ex} = 311 nm). (We have done this experiment in two different wavelength; one is at λ_{em} = 388 nm, when λ_{ex} = 311 nm and other is at λ_{em} = 454 nm, when λ_{ex} = 400 nm. Between these two wavelength former was more clear, so we use this for all the measurements).

S5. The Supporting Figures

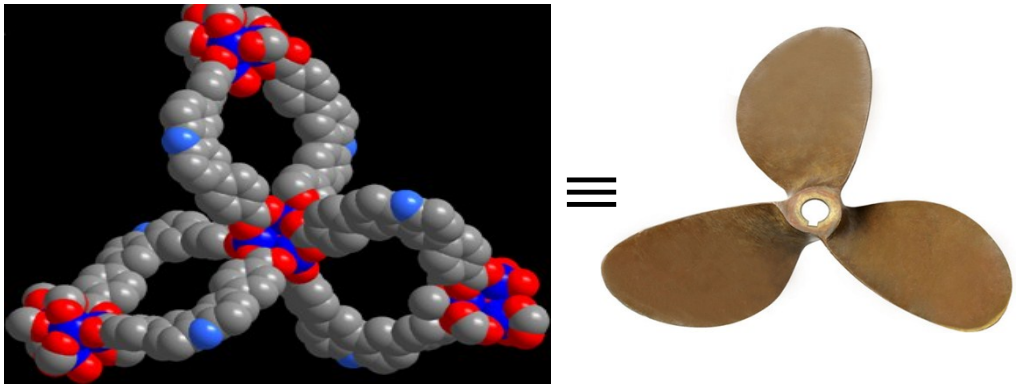


Figure S1. Propeller type packing of two SBU in the crystal structure.

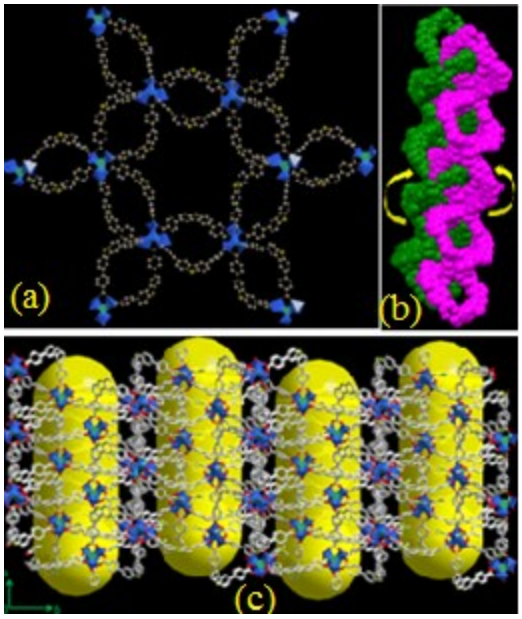


Figure S2. (a) 2D hexagonal ring where two different SBU act as alternative vertex and the bridging ligands CDDB as edge. (b) Helical packing through (101) plane. (c) Side view of 1D channel through *a*-axis having sixe 28.1 Å × 23.17 Å, indicated by a yellow cylinder, formed by double interpenetrating 2-fold 2D network.

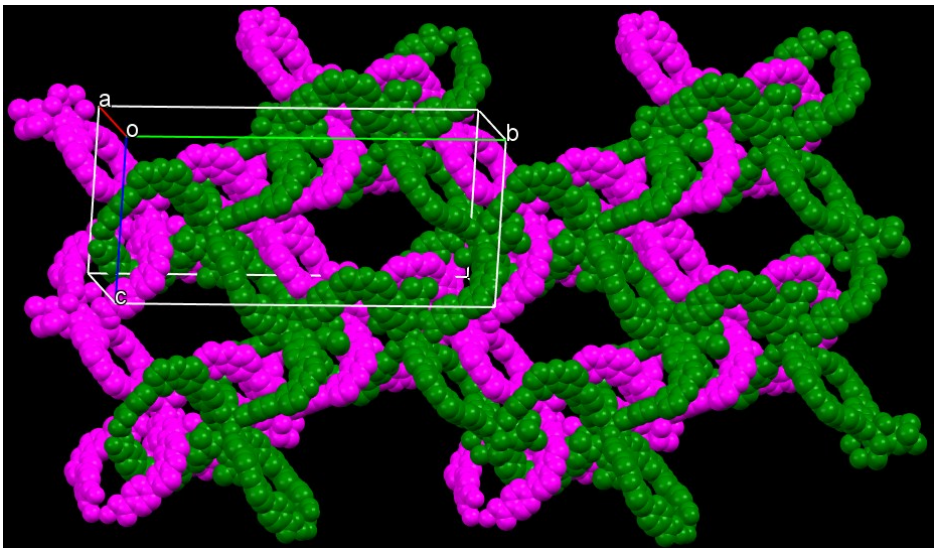
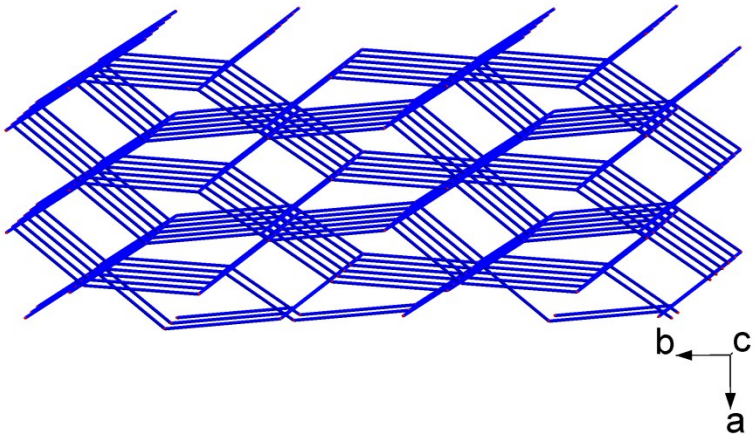
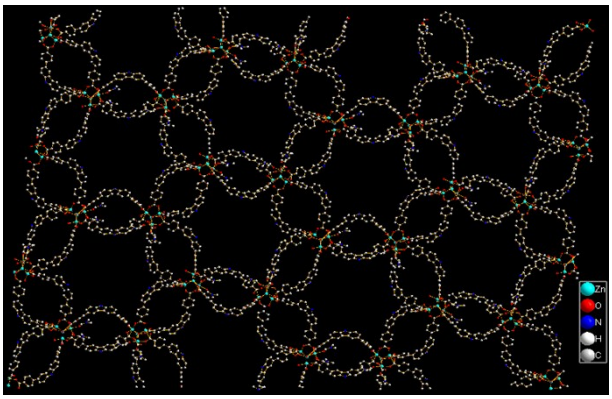


Figure S3. Double 2-fold interpenetration among the 2D chain, forming an 1D channel. (different 2D chains are denoted by different colour).



(a)



(b)

Figure S4. (a) Perspective view of (6,3) net of $[Zn_8(CDDB)_6(DMF)_4(H_2O)]$ $(DMF)_9(H_2O)_{13}$, where CDDB is a tetradentate ligand. (b) For clarity, a portion of structure (without interpenetration), where it can be clearly visualized the (6,3) net. (the two DMF and one water molecules, are not considered for topology calculations).

S6. N_2 Adsorption Study

The permanent microporosity of the activated sample was confirmed by the N_2 sorption experiment at 77 K. As shown in Figure S4, the fully activated sample reveals a reversible typical type-I behavior as expected for microporous materials, which is coincidental with the crystal structure. Derived from N_2 adsorption, the Langmuir surface area of **1** is 283 $m^2 g^{-1}$, corresponding to a BET surface area of 209.8 $m^2 g^{-1}$. Even though **1** shows small BET surface area, it shows outstanding drug loading capability. The strong hydrogen bonding and other interactions are mainly responsible for enormous amount of solid drug loading whereas those strong interactions are absent during gas adsorption.

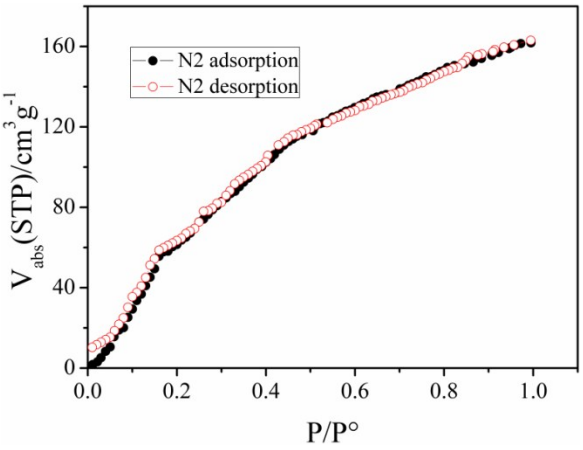


Figure S5. Experimental N_2 isotherm at 77 K for **1**; filled and open symbols represent adsorption and desorption data, respectively.

Thermogravimetric Analyses

Table S3. Comparison table of calculated (single crystal structure) and experimental value of TGA data.

	Calculated value (single crystal structure)	**Experimental value (TGA)**
1st weight loss of **1**	14 H_2O	15 H_2O
2nd weight loss of **1**	13 DMF	15 DMF

The discrepancy of the experimental value with calculated one is due to few non-assigned solvent molecules, for which we employed PLATON/SQUEEZE.[4]

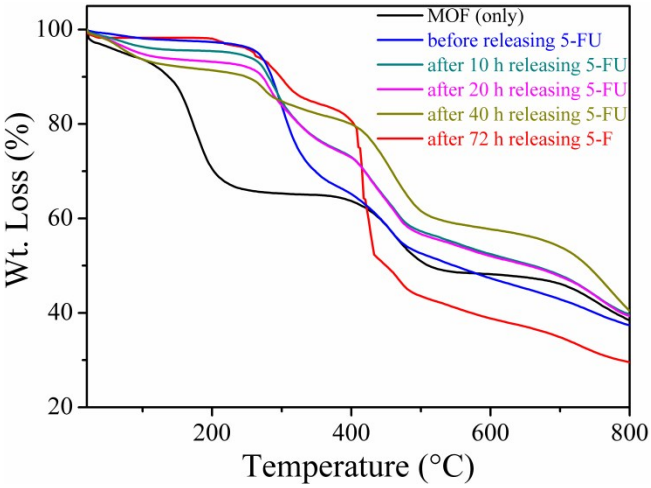


Figure S6. TGA under nitrogen gas of MOF (black) and 5-Fu-containing MOF (red) and remaining are after releasing at different time intervals. Notice that, there are two weight losses are observed within the range 220-350 °C and 415-500 °C for the 5-Fu-containing **1** sample. The two weight losses correspond to the departure of 5-Fu and the destruction of the porous solid, respectively. The remaining four curves clearly indicate that at the beginning, weight loss is slowly decreasing with increasing the 5-FU release time as the amount of 5-FU decreases with increasing time.

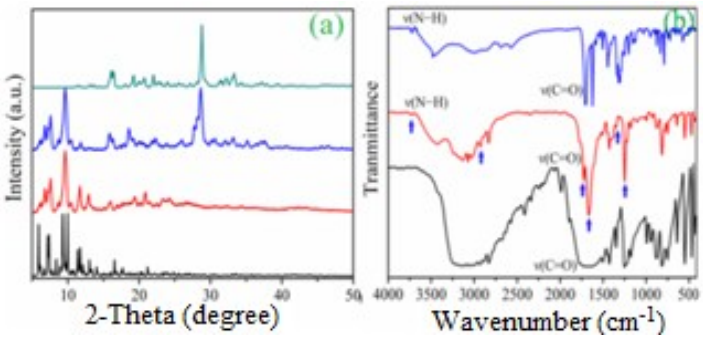


Figure S7. (a) PXRD patterns for 1 (simulated, black; desolvated 1, red; 5-FU-loaded 1, blue; 5-FU, dark cyan) (b) IR spectra of 5-FU, black; 1 loaded with 5-FU, red and activated 1, blue.

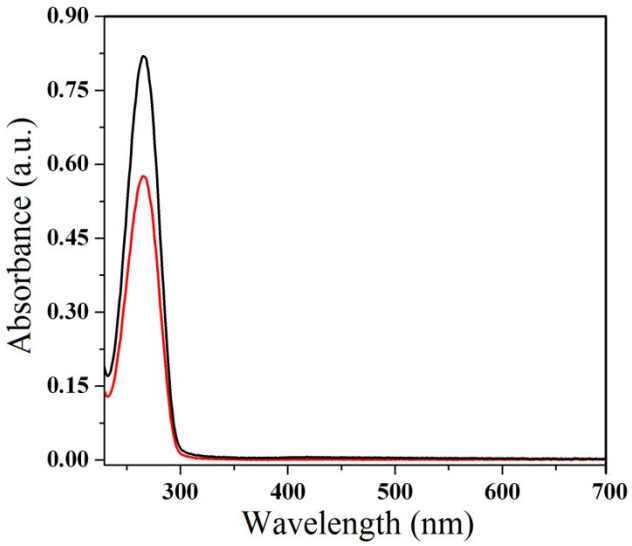


Figure S8. The UV/Vis absorption spectra of 5-FU in methanol solution before (black) and after (red) drug loaded when diluted.

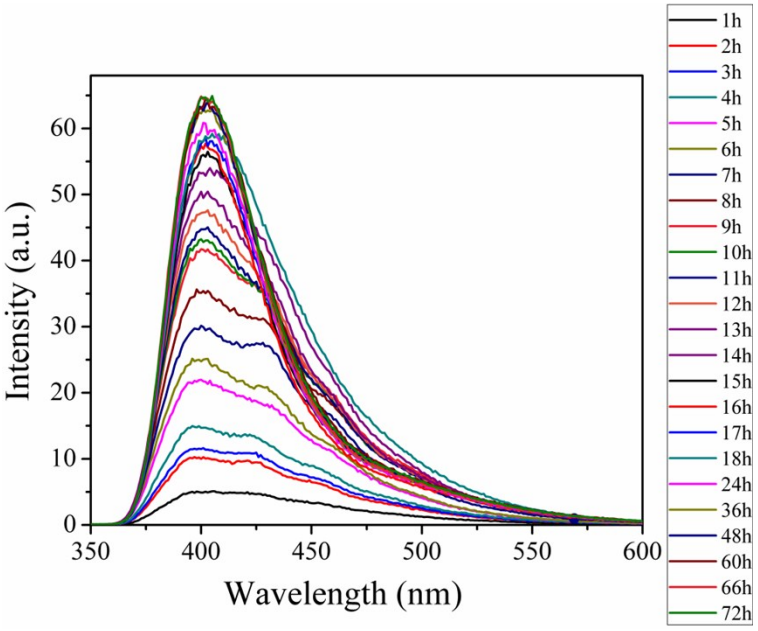


Figure S9. Luminescence spectra of 5-FU releasing from MOF **1** in PBS (pH 7.4).

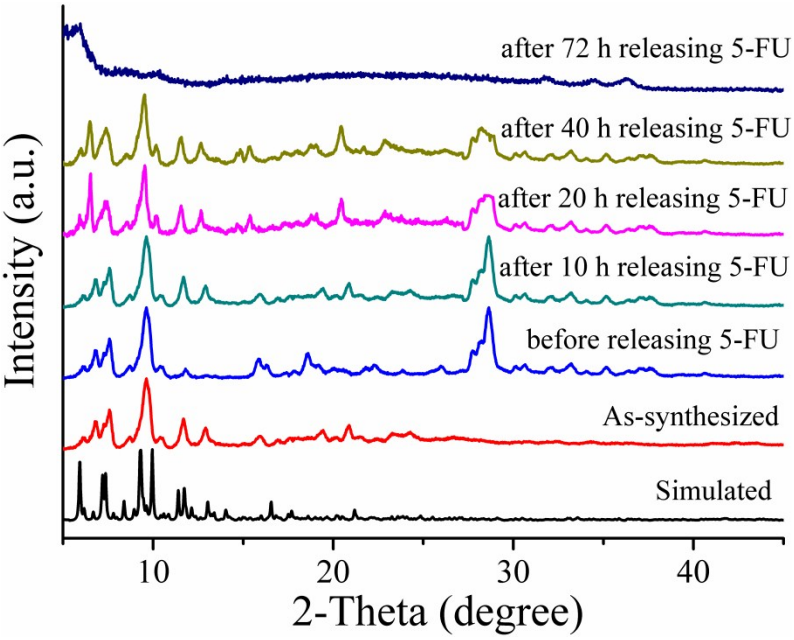


Figure S10. Experimental PXRD pattern of **1** and 5-FU loaded **1** after releasing at different time intervals; where it is clearly indicate that the crystallinity of MOF was retained little after complete drug releasing also.

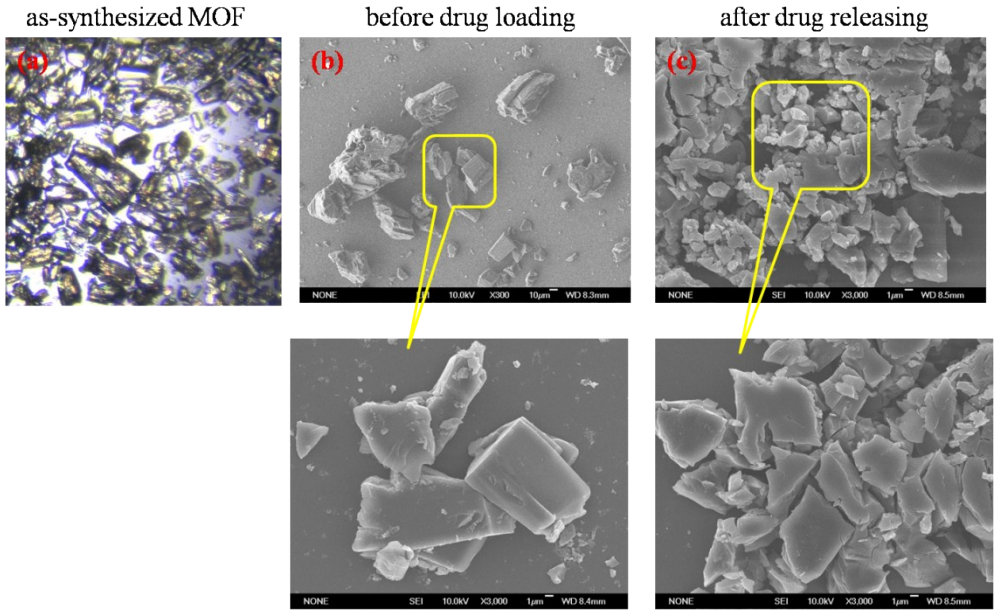


Figure S11. (a) Photography of crystals. SEM image of these crystals (b) before drug loading (activated **1**) and (c) after drug releasing, where the crystalline property is observed clearly and it is also noticed that after drug releasing the size of the crystals become narrow.

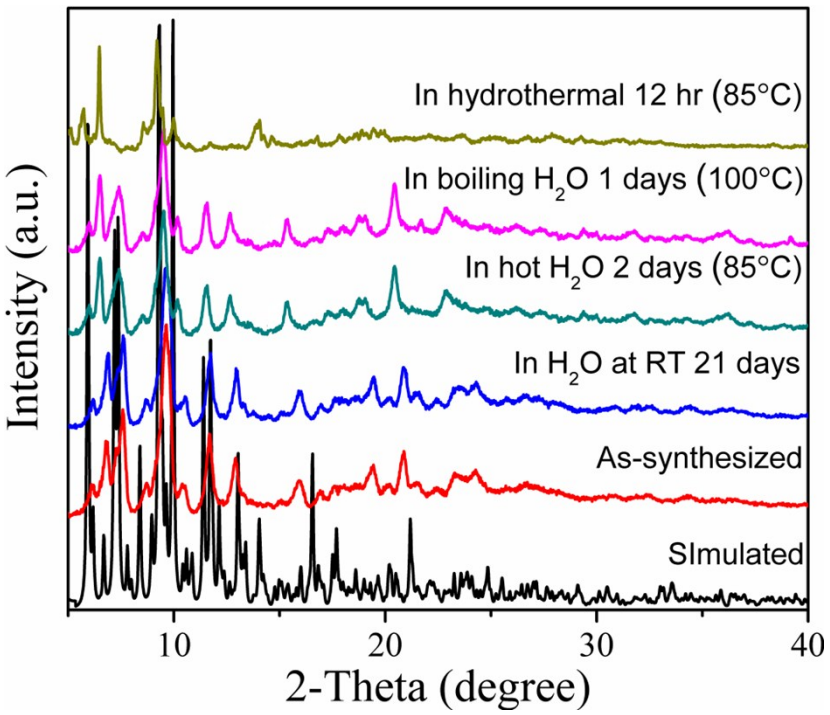


Figure S12. Experimental PXRD pattern to show the water stability and phase purity of MOF **1** in deionised water at different temperature for 12 hr to 21 days time period.

S7. *In vitro* Cytotoxicity Test

Cell Culture

The cell lines used in current study were human hepatoblastoma cell line (HepG2) and human breast ductal carcinoma cell line (MDA-MB-435S). They were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and were routinely maintained in RPMI-1640 (GIBCO BRL), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37℃ under humidified air containing 5% CO_2. The viability of cells was determined by Trypan blue dye exclusion. Cells were maintained in logarithmic phase with viability >95%.

Cytotoxicity experiment of MOF

Cells (HepG2 or MDA-MB-435S) at a density of 3×10^5 per ml in a volume of 200 μl per well were placed in 96-multiwell plates and incubated overnight. The cells were incubated with MOF or MOF/5-FU at concentrations of 80 μg/ml or 100 μg/ml for 12 h. One control column in the plate was filled with only culture medium as a blank. The cells were then washed with sterile culture medium before fresh medium was added. After another 12 h incubation, the cell viability was measured on the

microplate reader using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay measured at 490 nm using a microplate reader (Synergy 4, BioTek Instruments). Four replicates in each dosage were tested for the cell line and each experiment was repeated three times. MOF/5-FU was measured in parallel for comparison.

References

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