Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for the manuscript:

A Fabricated Material with Divergent Chemical Handles Based on UiO-66 for Targeted Photodynamic Therapy

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Section 1. Experimental Procedures

Materials

All reagents and solvents unless otherwise stated were obtained from commercial sources (Alfa Aesar, Sigma Aldrich, Aladdin) and used without further purification.

The ligand 2-Azido-1,4-benzenedicarboxylic acid (N_3-BDC) and the photosensitizer 2- carboxy substitutive phthalocyanine zinc (Pc) were synthesized following the published procedure.

Instrumentation

The morphology of the sample was investigated by field emission scanning electron microscopy (SEM) (Hitachi S4800, Tokyo, Japan). TEM images were taken on a high-resolution transmission electron microscopy (HR-TEM, Tecnai G2 F20 S-TWIN, 200 kV, FEI Company, USA) operated at an acceleration voltage of 200 keV by dropping solution onto a carbon-coated copper grid. Powder X-ray diffraction (PXRD) was carried out on a Bruker D8-Focus Bragg-Brentano X-ray powder Diffractometer equipped with a Cu Ka1 radiation (k = 1.5406 Å) in 5°to 50° 2θ range with a scan speed of $2^{o}min^{-1}$. Energy-dispersive X-ray spectroscopy (EDS) characterization were taken on a high-resolution transmission electron microscopy (HR-TEM, Tecnai G2 F20 S-TWIN, 200 kV, FEI Company, USA) operates at 15 keV. X-ray photoelectron spectroscopy (XPS) data were obtained on Thermo ESCALAB250 instrument with a monochromatized Al Kα line source (200 W). Fourier transformed infrared (FTIR) spectra were recorded on BioRad FTS 6000 spectrometer using KBr pellet in 400-4000 cm^{-1}range. Inductively coupled plasma mass spectrometry (ICP-MS) were performed on XSERIES 2.UV-Vis spectra were recorded on Beijing PuXi Tu-1901 spectrophotometer. Fluorescence spectra were recorded on a Varian carye clipse spectrometer with Xe lamp as the excitation source at room temperature. The singlet oxygen quantum yields were determined on UV-Vis spectrophotometer (TU-1901). The fluorescence intensity of zinc phthalocyanine in the cells was monitored by flow cytometry (C6, BD BioSciences). Cell Counting Kit-8 (CCK-8) was obtained from Beyotime Institut of Biotechnology. Confocal laser scanning microscopy (CLSM) images were performed on an Olympus FV1000-IX81 CLSM and a Leica TCS SP confocal system (Leica, Germany).

Experimental Section

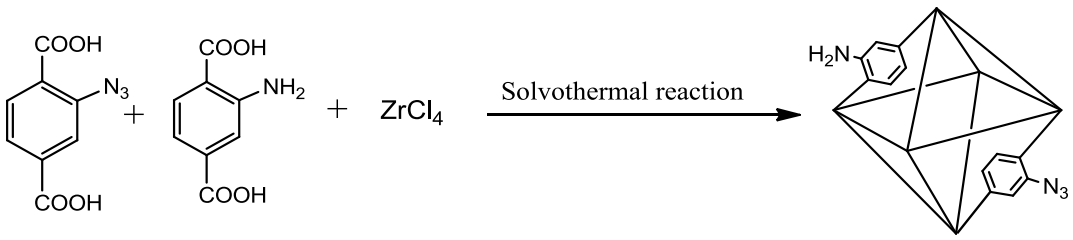
Synthesis of N_3-BDC

Caution ! Organic azides are potentially explosive materials–proper safety precautions should be employed. Synthesis of 2-Azido-1,4-benzenedicarboxylic acid was carried out under conditions reported by Kim et al. [1]

Synthesis of 2-carboxy substituted phthalocyanine zinc

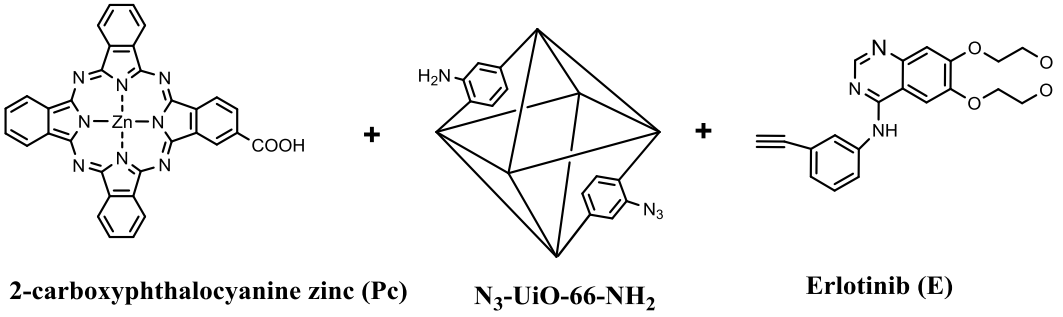
Synthesis of 2-carboxyphthalocyanine zinc was carried out under conditions reported by Huang et al. [2]

Synthesis of N_3-UiO-66-NH_2

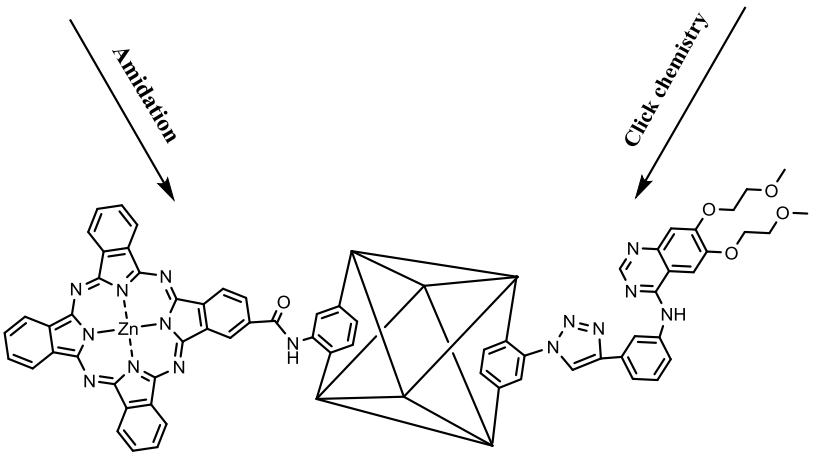


N_3-BDC (27.3 mg, 0.15 mmol) and NH_2-BDC (31.1 mg, 0.15 mmol) were dissolved in *N,N* Dimethylformamide (DMF) (3 mL). In a separate vial, zirconyl chloride octahydrate (26.3mg, 0.083 mmol) was dissolved in DMF(2 mL). The two solutions were mixed together in a 10 mL scintillation vial, and acetic acid (0.5 mL) was added to the reaction mixture. The solution was heated at 90˚C for 18 h to yield N_3 -UiO-66-NH_2. Then the sample was purified and collected by centrifugation (15000 rpm, 60 min) followed by solvent exchange (3 x DMF and 3 x ethyl alcohol), then the N_3-UiO-66-NH_2 powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and analysis.

Synthesis of E-UiO-66-Pc

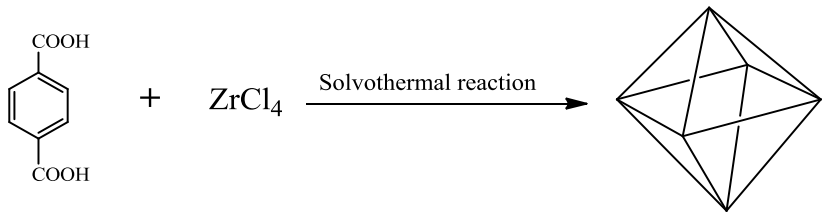


2-carboxyphthalocyanine zinc (Pc) **N_3-UiO-66-NH_2** **Erlotinib (E)**



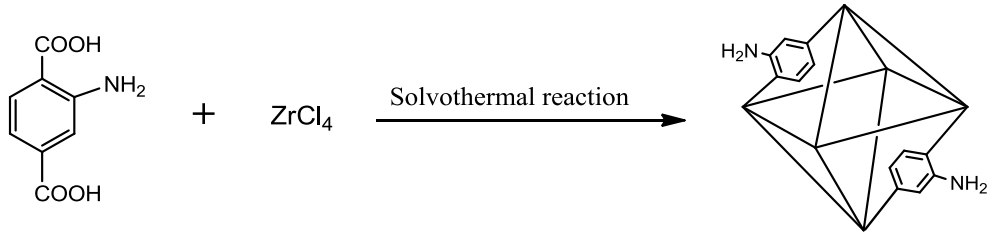
N_3-UiO-66-NH_2 (76.2 mg), Erlotinib (114.3 mg), $CuSO_4.5H_2O$ (7.62 mg), sodium ascorbate (9.84 mg) in THF (6.4 mL), H_2O (3.2 mL) and t-BuOH (6.4 mL) was stirred at 40˚C under an atmosphere of nitrogen for 5 h. The volatiles were evaporated under reduced pressure, then the E-UiO-66-NH_2 was purified and collected by centrifugation (15000 rpm, 90 min) followed by solvent exchange (3 x DMF and 3 x NANOpure H_2O and 3 x ethyl alcohol), then make the sample suspended in DMF for characterization and functionalization with Pc.
2-carboxyphthalocyanine zinc （20 mg）was dissolved in DMF (2 mL), In a separate vial, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI,8.5 mg) and 1-Hydroxybenzotriazole（HOBT,6.0 mg） were dissolved in DMF (2 mL). The two solutions were mixed together in a 10 mL round-bottom flask, and stirred at 0˚C for 30 min, then 2 drops of triethylamine were added, finally when the temperature was raised to room temperature, E-UiO-66-NH_2 (30 mg) was added and stirred overnight. The E-UiO-66-Pc was purified by centrifugation (15000 rpm, 90 min) followed by solvent exchange (3 x DMF and 3 x NANOpure H_2O and 3 x ethyl alcohol), then the sample powder was dried under vacuum at ambient temperature. Finally the sample was stored for further characterization and in vitro test.

Synthesis of UiO-66



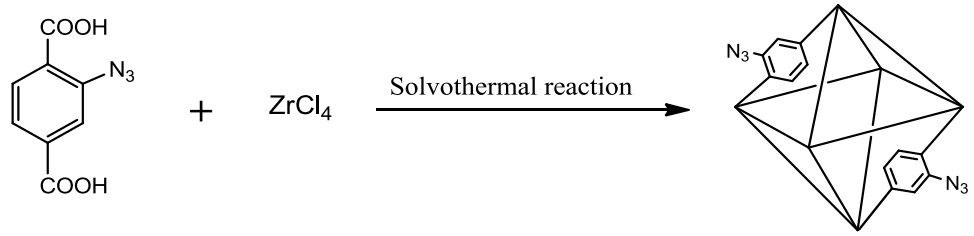
Terephthalic acid（BDC）(49.8 mg, 0.3 mmol) was dissolved in DMF (3 mL). In a separate vial, zirconyl chloride octahydrate (26.3 mg, 0.083 mmol) was dissolved in DMF (2 mL). The two solutions were mixed together in a 10 mL scintillation vial, and acetic acid (0.5 mL) was added to the reaction mixture. The solution was heated at 90˚C for 18 h to yield UiO-66. Then the sample was purified and collected by centrifugation (15000 rpm, 60 min) followed by solvent exchange (3 x DMF and 3 x ethyl alcohol),then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and analysis.

Synthesis of UiO-66-NH_2



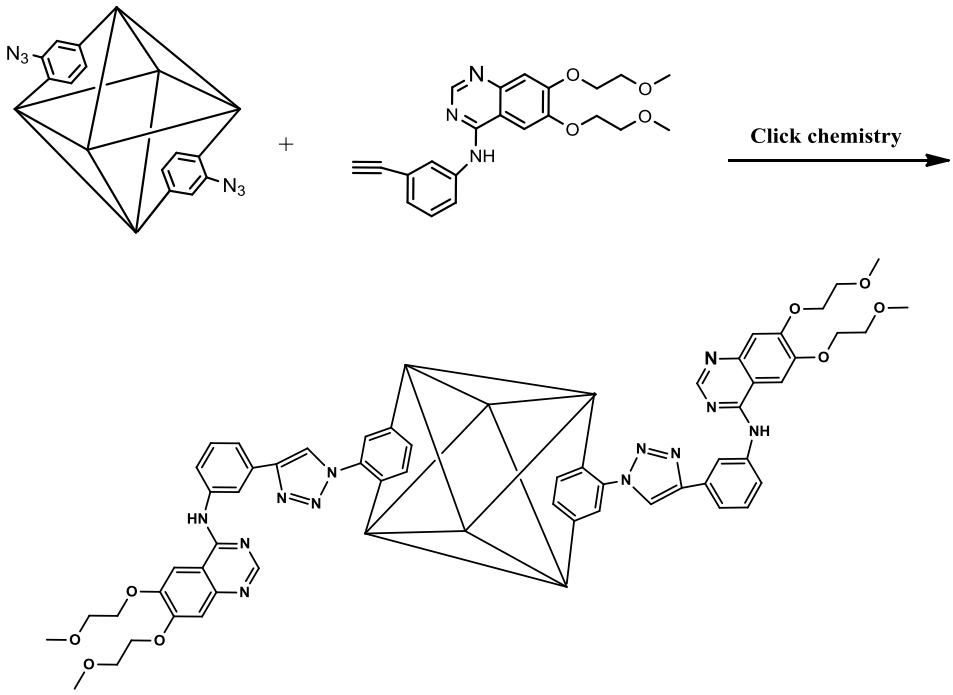
2-Aminoterephthalic acid (NH_2-BDC) (54.3 mg, 0.3 mmol) was dissolved in DMF (3 mL). In a separate vial, zirconyl chloride octahydrate (26.3mg, 0.083 mmol) was dissolved in DMF (2 mL). The two solutions were mixed together in a 10 mL scintillation vial, and acetic acid (0.5 mL) was added to the reaction mixture. The solution was heated at 90˚C for 18 h to yield UiO-66. Then the sample was purified and collected by centrifugation (15000 rpm, 60 min) followed by solvent exchange (3 x DMF and 3 x ethyl alcohol) ,then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and analysis.

Synthesis of UiO-66-N_3



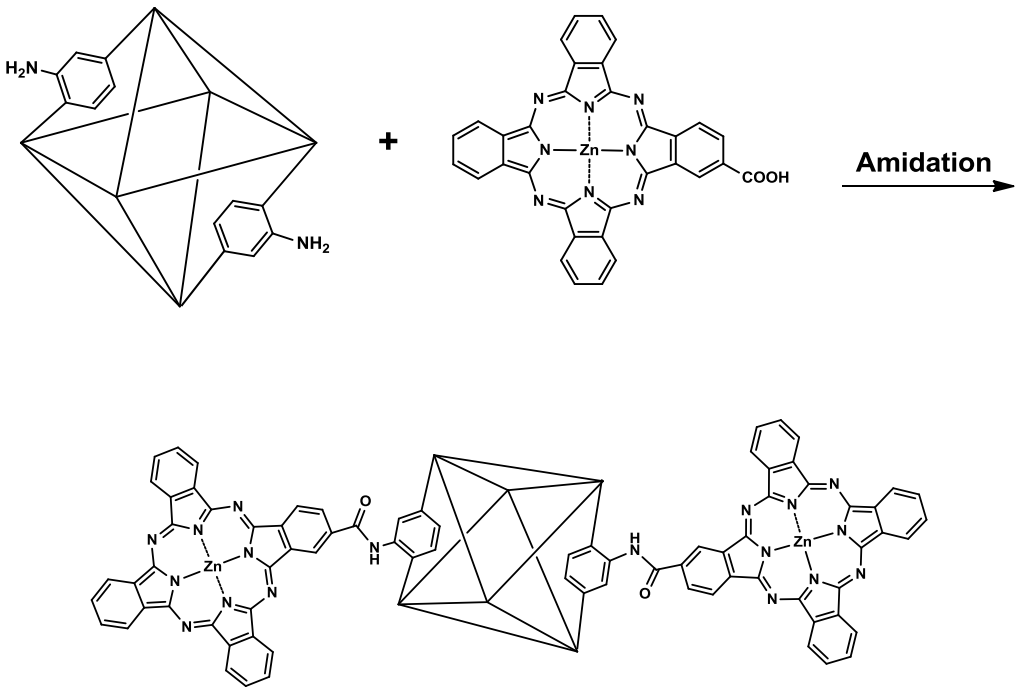
2-Azido-1,4-benzenedicarboxylic acid(N_3-BDC) (62.1 mg, 0.3 mmol) was dissolved in DMF (3 mL). In a separate vial, zirconyl chloride octahydrate (26.3mg, 0.083 mmol) was dissolved in DMF (2 mL). The two solutions were mixed together in a 10 mL scintillation vial, and acetic acid (0.5 mL) was added to the reaction mixture. The solution was heated at 90˚C for 18 h to yield UiO-66. Then the sample was purified and collected by centrifugation (15000 rpm, 60 min) followed by solvent exchange (3 x DMF and 3 x ethyl alcohol),then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and analysis.

Synthesis of UiO-66-E



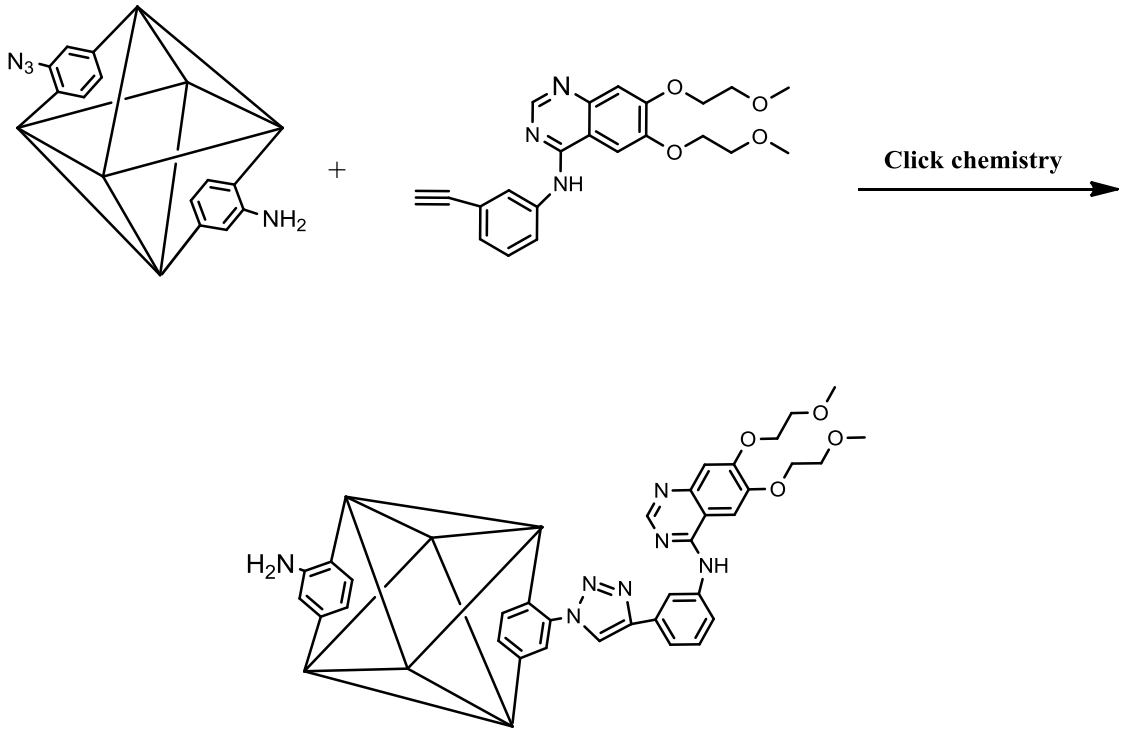
N_3-UiO-66 (38.1 mg), Erlotinib (114.3 mg), $CuSO_4.5H_2O$ (7.62 mg), sodium ascorbate (9.84 mg) in THF (6.4 mL), H_2O (3.2 mL) and t-BuOH (6.4 mL) was stirred at 40˚C under an atmosphere of nitrogen for 5 h. The volatiles were evaporated under reduced pressure, Then the sample was purified and collected by centrifugation (15000 rpm, 90 min) followed by solvent exchange (3 x DMF and 3 x NANOpure H_2O and 3 x ethyl alcohol), then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and toxicity test.

Synthesis of UiO-66-Pc



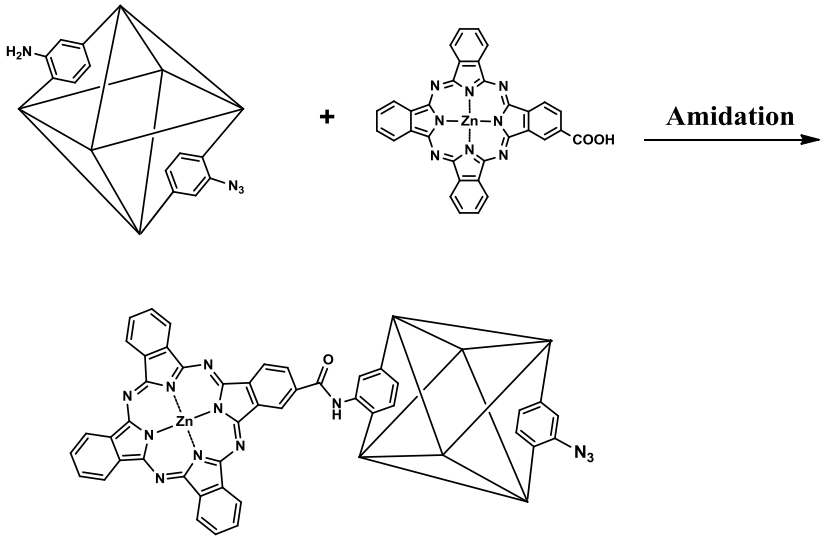
2-carboxyphthalocyanine zinc （ 20 mg ） was dissolved in DMF (2 mL) in a separate vial, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI,8.5 mg) and 1-Hydroxybenzotriazole（HOBT,6.0 mg） were dissolved in DMF (2 mL) in a separate vial. The two solutions were mixed together in a 10 mL round-bottom flask, and stirred at 0˚C for 30 min, then 2 drops of triethylamine were added, finally when the temperature was raised to room temperature, UiO-66-NH_2 (30 mg) was added and stirred overnight. After the reaction is done, the sample were purified and collected by centrifugation (15000 rpm, 90 min) followed by solvent exchange (3 x DMF and 3 x NANOpure H_2O and 3 x ethyl alcohol),then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and toxicity test.

Synthesis of E-UiO-66-NH_2



N_3-UiO-66-NH_2 (76.2 mg), Erlotinib (114.3 mg), $CuSO_4.5H_2O$ (7.62 mg), sodium ascorbate (9.84 mg) in THF (6.4 mL), H_2O (3.2 mL) and t-BuOH (6.4 mL) was stirred at 40˚C under an atmosphere of nitrogen for 5 h. The volatiles were evaporated under reduced pressure, Then the sample was purified and collected by centrifugation (15000 rpm, 90 min) followed by solvent exchange (3 x DMF and 3 x NANOpure H_2O and 3 x ethyl alcohol), then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and toxicity test.

Synthesis of N_3-UiO-66-Pc



2-carboxyphthalocyanine zinc （ 20 mg ） was dissolved in DMF (2 mL) in a separate vial, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDCI,8.5 mg) and 1-Hydroxybenzotriazole （HOBT,6.0 mg） were dissolved in DMF (2 mL) in a separate vial. The two solutions were mixed together in a 10 mL round-bottom flask, and stirred at 0˚C for 30 min, then 2 drops of triethylamine were added, finally when the temperature was raised to room temperature, N_3-UiO-66-NH_2 (60 mg) was added and stirred overnight. Then the sample were purified and collected by centrifugation (15000 rpm, 90 min) followed by solvent exchange (3 x DMF and 3 x NANOpure H_2O and 3 x ethyl alcohol), then the sample powder was dried under vacuum at ambient temperature, finally the sample was stored for further characterization and toxicity test.

Section 2 . SEM / TEM and photograph characterizations

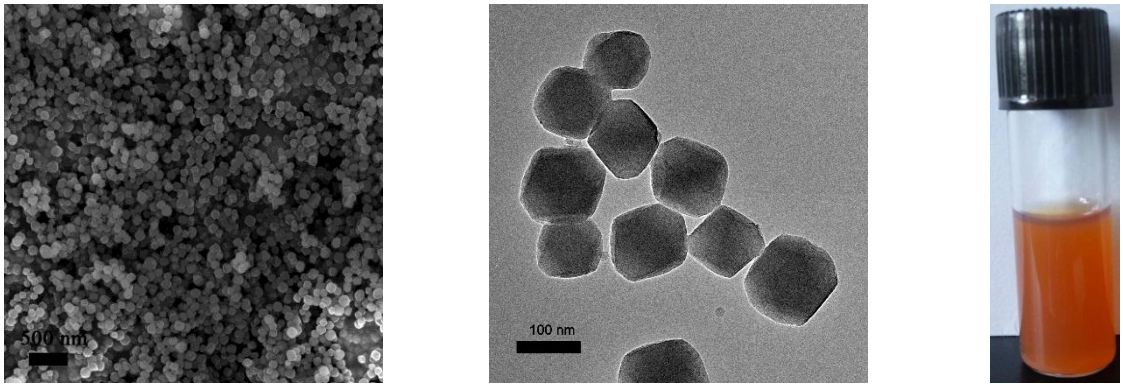


Figure S1. The SEM image, TEM image and photograph of N_3-UiO-66-NH_2 from left to right.

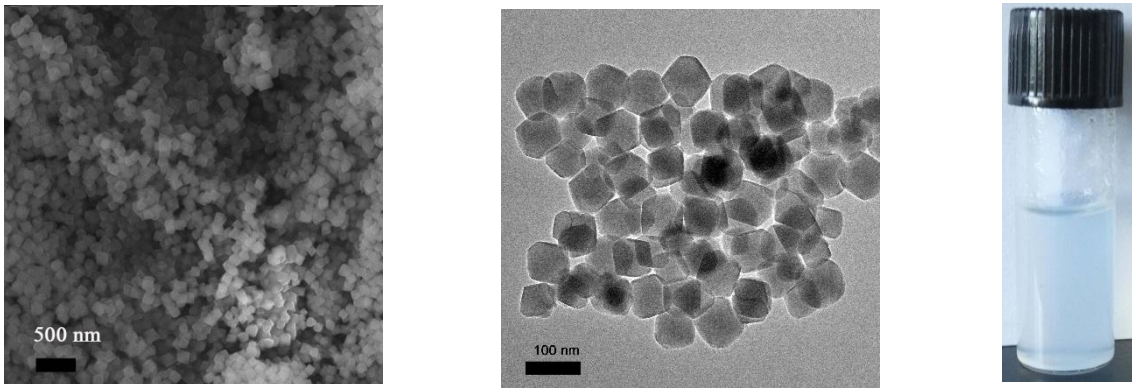


Figure S2. The SEM image, TEM image and photograph of UiO-66 from left to right.

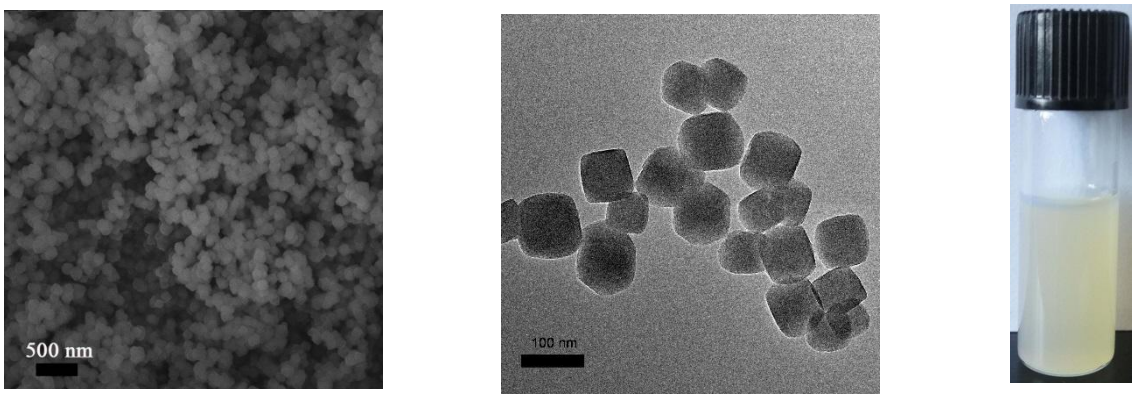


Figure S3. The SEM image, TEM image and photograph of UiO-66-NH_2 from left to right.

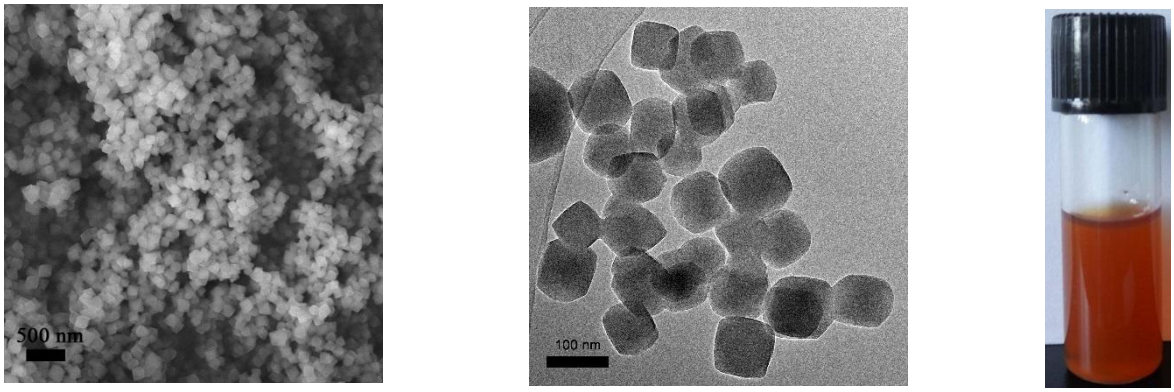


Figure S4. The SEM image, TEM image and photograph of N_3-UiO-66 from left to right.

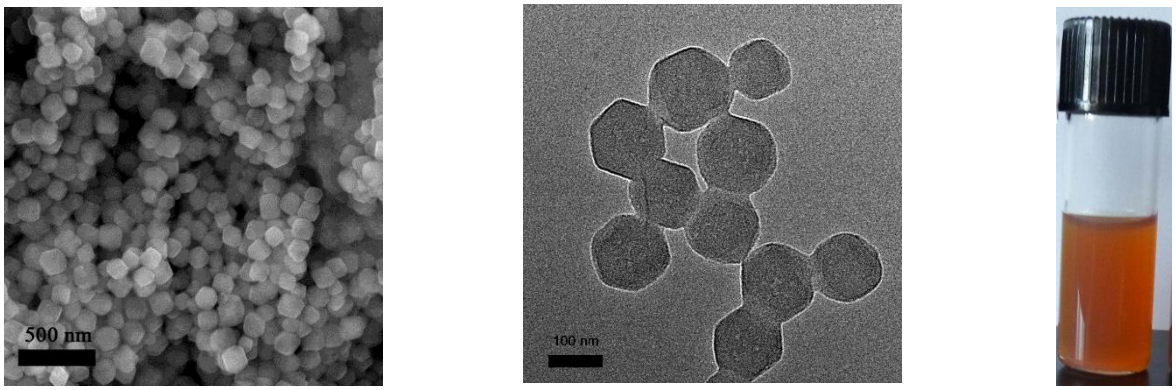


Figure S5. The SEM image, TEM image and photograph of UiO-66-E from left to right.

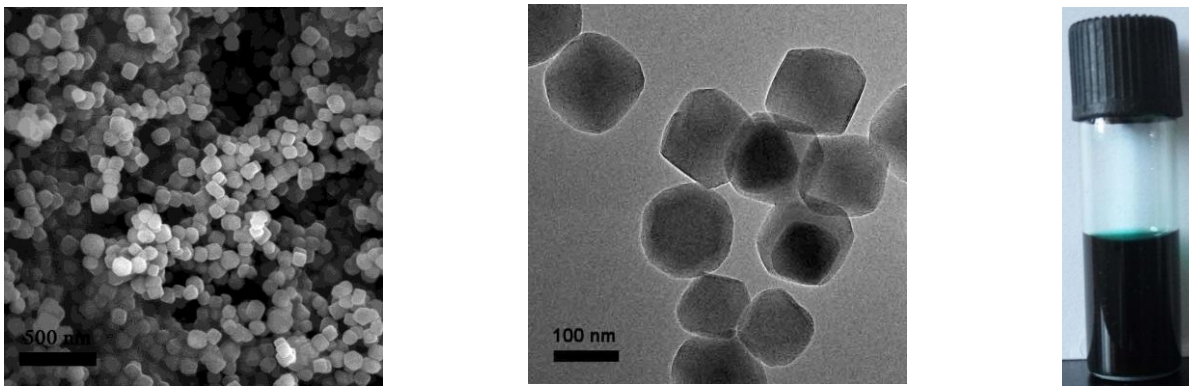


Figure S6. The SEM image, TEM image and photograph of UiO-66-Pc from left to right.

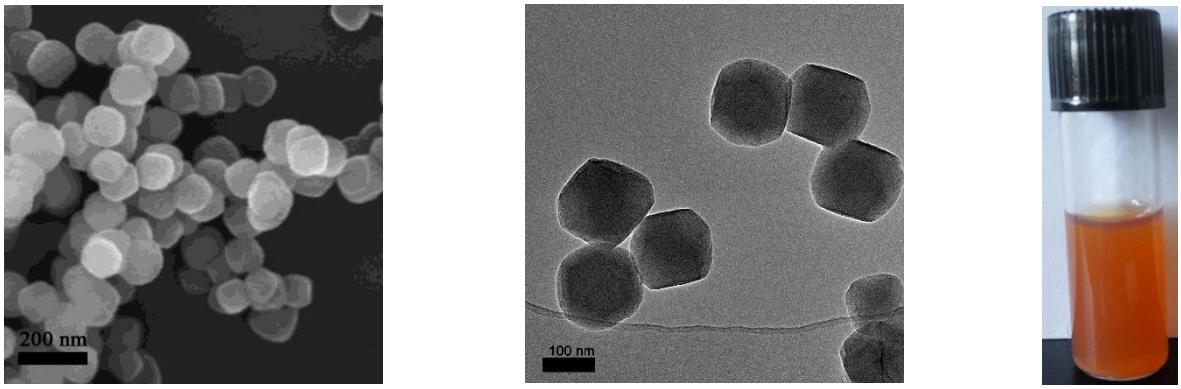


Figure S7. The SEM image, TEM image and photograph of E-UiO-66-NH_2 from left to right.

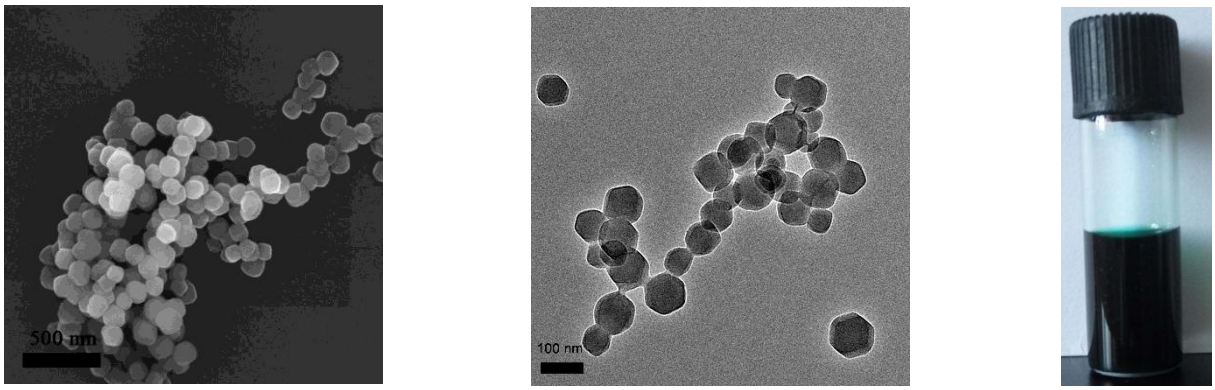
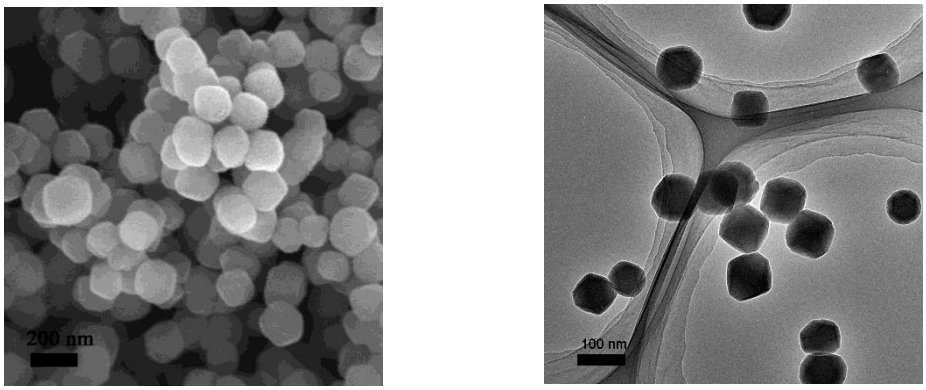


Figure S8. The SEM image, TEM image and photograph of N_3-UiO-66-Pc from left to right.

a) **b)**



c) d)

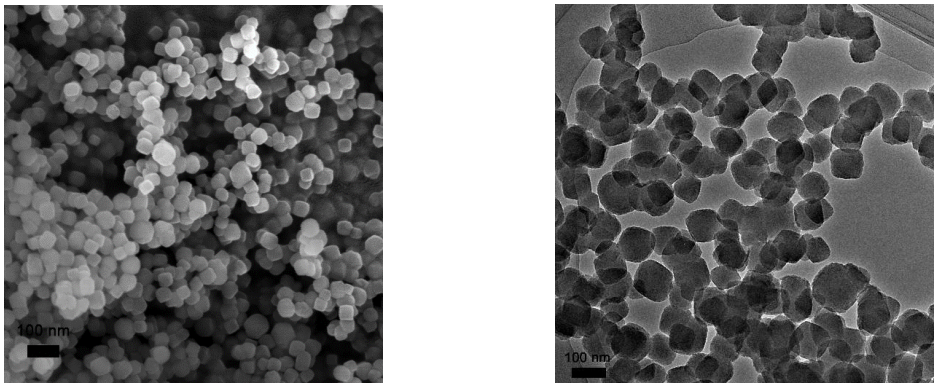


Figure S9. The SEM and TEM images of E-UiO-66-Pc before (a and b) and after (c and d) treated in medium for 24h.

Section 3 . DLS characterizations

Table S 1 .The size distribution of samples by DLS

Samples	d DLS, water[nm]	Samples	d DLS, water[nm]
UiO-66	62-225 (87)	**N_3-UiO-66-Pc**	88-268 (105)
UiO-66-NH_2	57-201 (90)	**UiO-66-E**	64-338 (114)
UiO-66-N_3	70-281 (94)	**E-UiO-66-NH_2**	81-415 (121)
N_3-UiO-66-NH_2	68-350 (102)	**E-UiO-66-Pc**	74-307 (125)
UiO-66-Pc	67-236 (107)	**After treatment**	95-359 (134)

Data for particle sizes from dynamic light scattering (DLS) measurements
(the maximum of the distribution is given in parentheses) in water (d DLS, water)

Section 4.TEM/EDS mapping characterizations

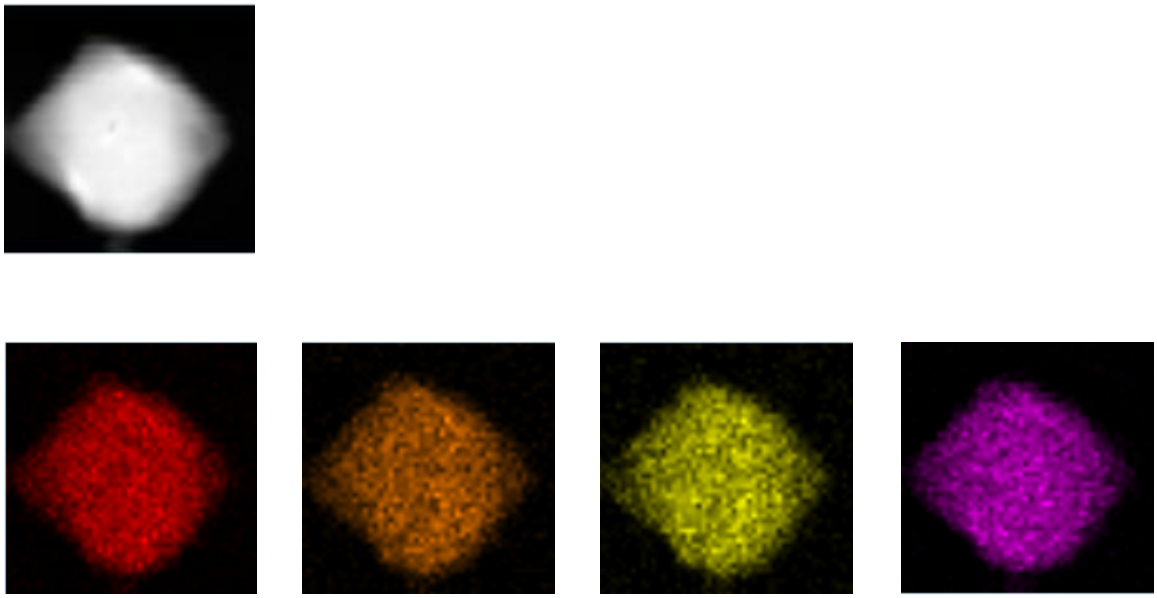


Figure S10. TEM image (up) of N_3-UiO-66-NH_2 and corresponding EDS-mappings (down) of C, N, O, Zr from left to right.

Section 5 . Powder X-ray Diffraction

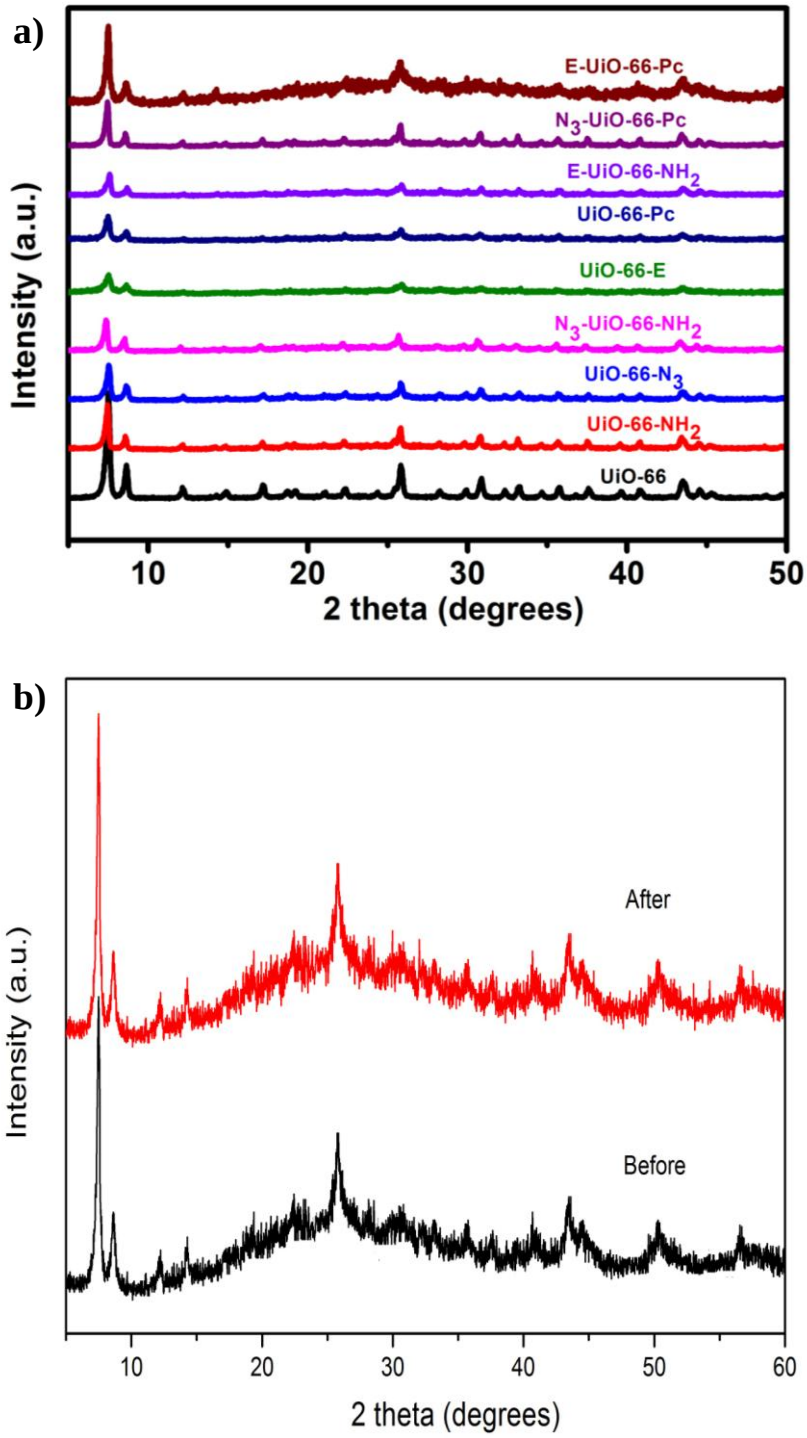
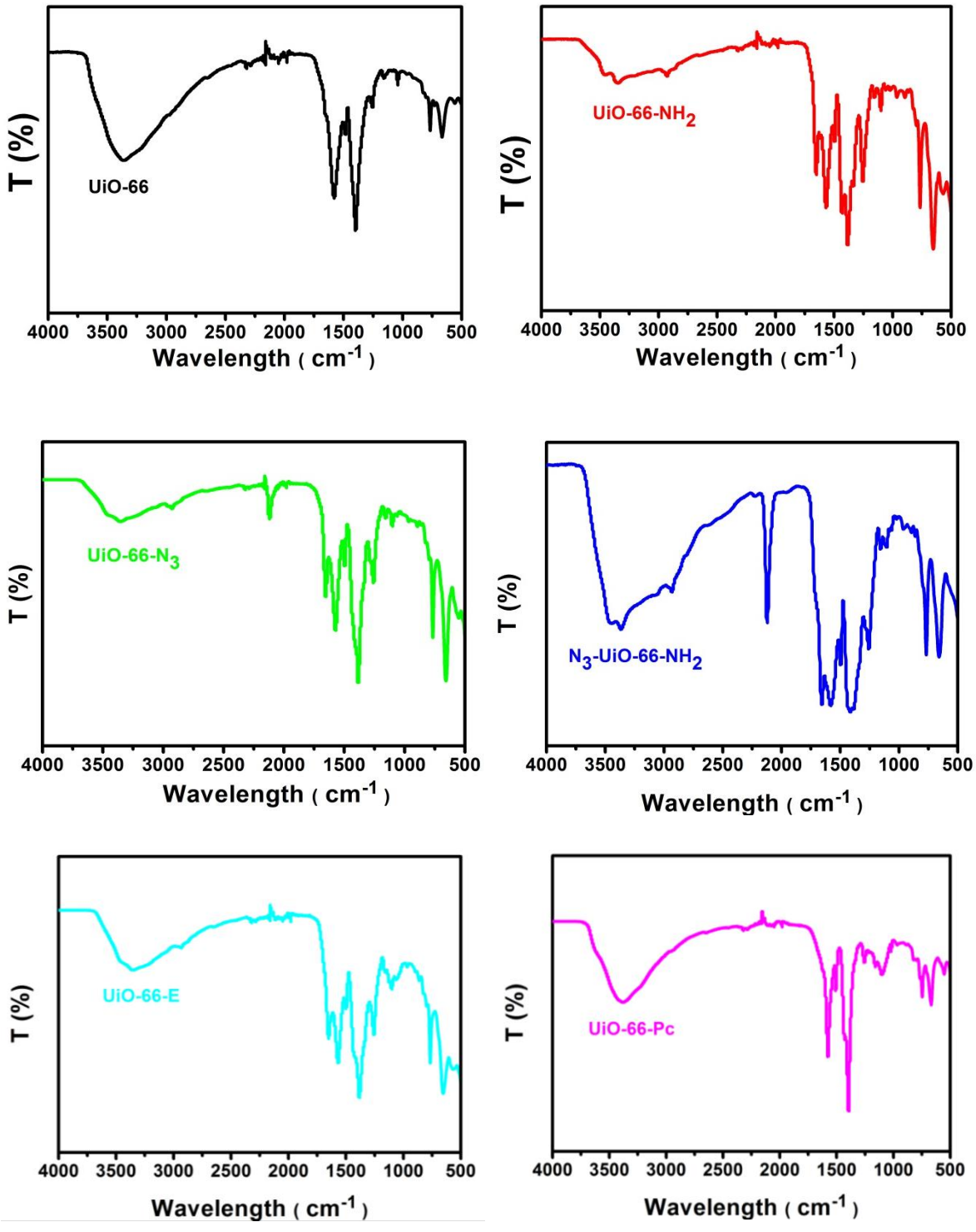


Figure S11. a) The PXRD patterns for all MOFs synthesized in this work, b) The PXRD patterns for E-UiO-66-Pc treated in medium for 24 h.

Section 6. Fourier transformed infrared (FTIR)



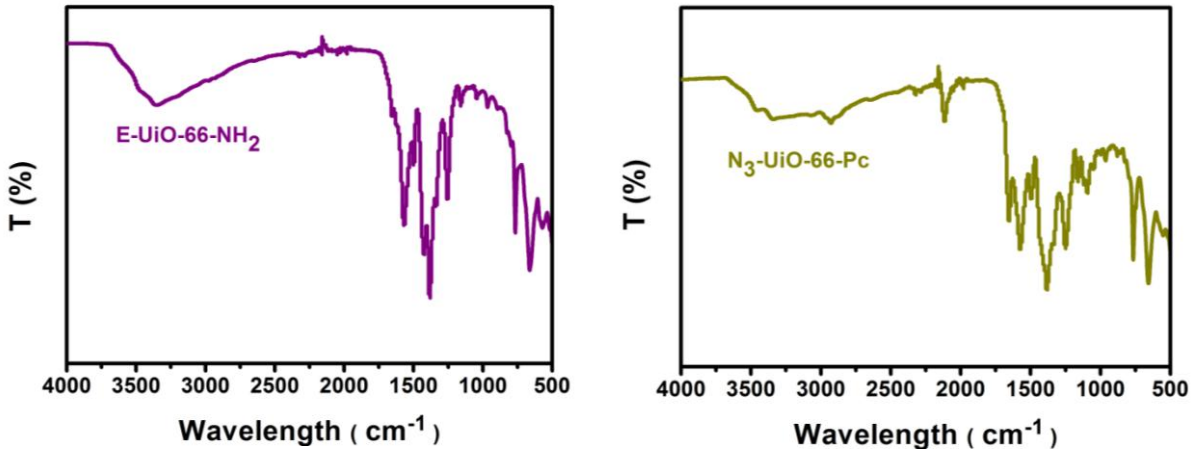


Figure S12. The FTIR spectra of all MOFs synthesized in this work.

Section 7. X-ray photoelectron spectroscopy (XPS) characterization.

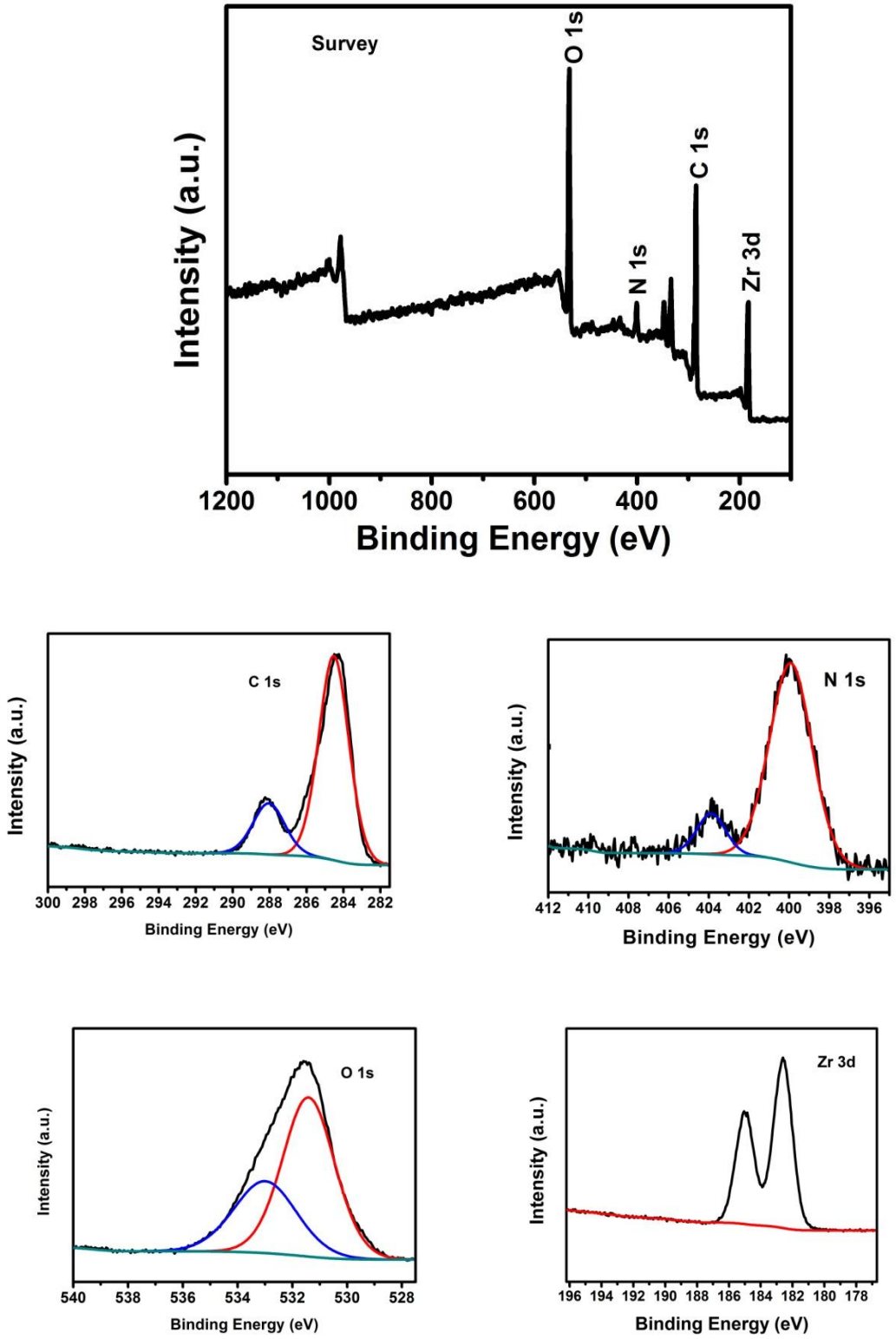


Figure S13. The XPS spectra of N_3-UiO-66-NH_2.

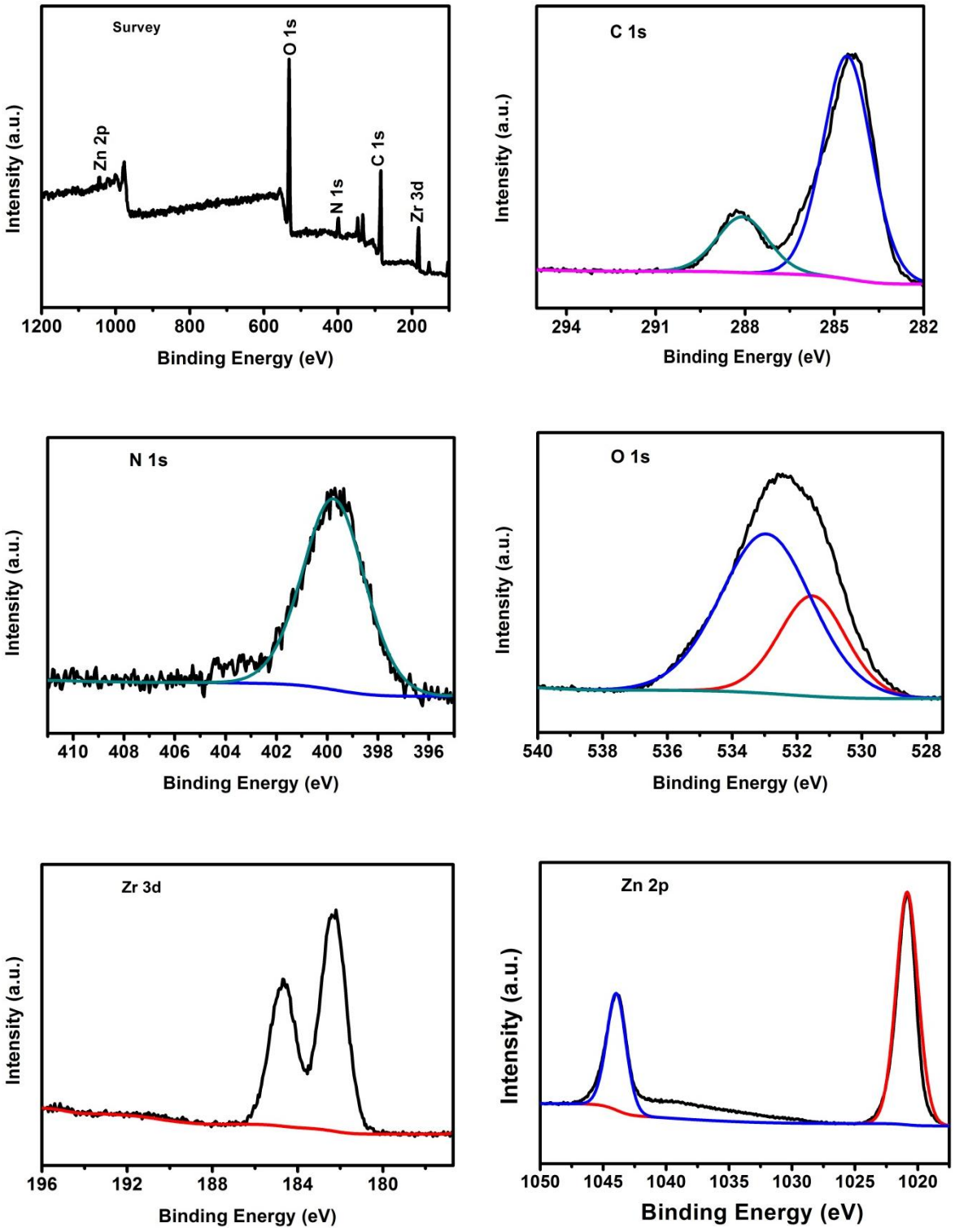


Figure S14. The XPS spectra of E-UiO-66-Pc.

Section 8 . Calibration curves

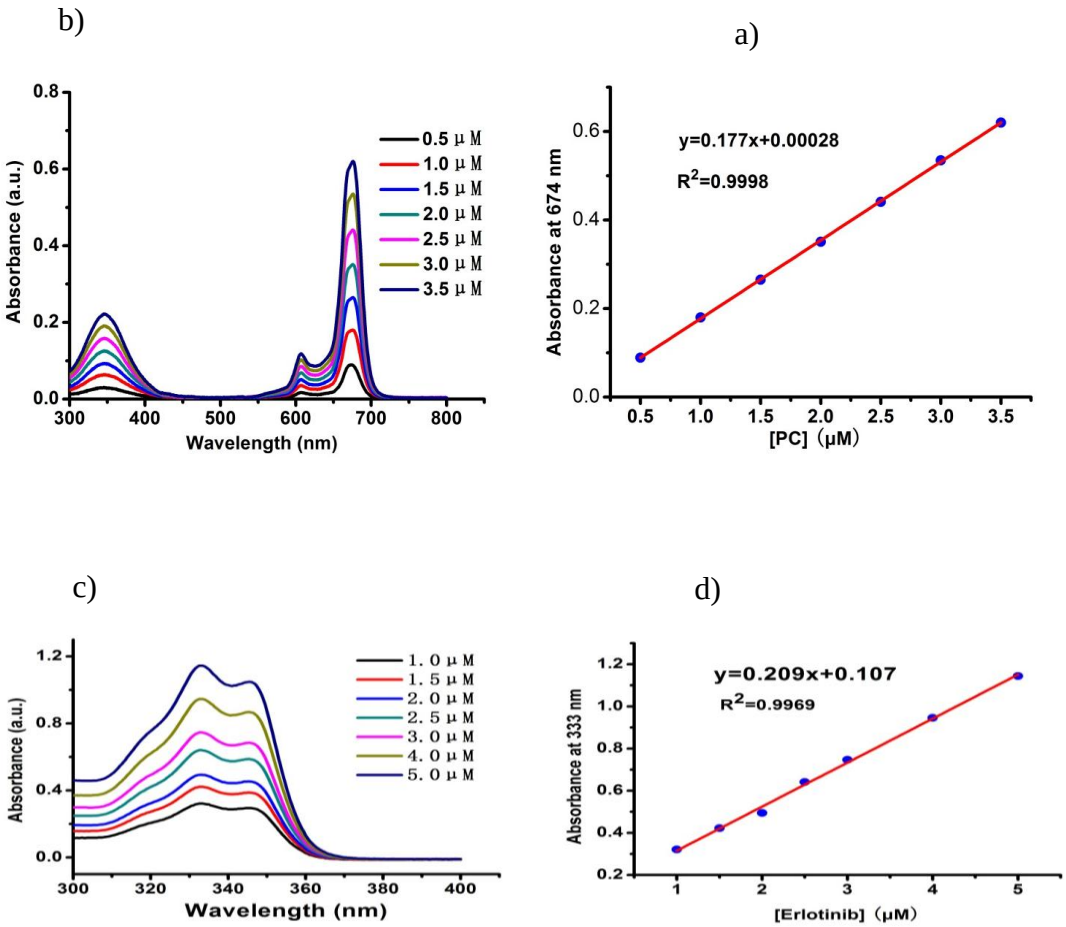


Figure S15. The UV-Vis spectra of Pc at various concentrations in DMF (a), the Calibration curve of Pc in DMF at 674 nm (b). The UV-Vis spectra of Erlotinib at various concentrations in DMF (c), the Calibration curve of Erlotinib in DMF at 333 nm(d).

Section 9. Inductively coupled plasma mass spectrometry (ICP-MS) characterization

Table S2. The zinc content in the UiO-66-Pc measured by ICM-MS methods.

Run	Time	64Zn(ppb)	Pc(ppm)
1	16:23:05	M 15.890	/
2	16:23:28	M 16.320	/
3	16:23:52	M 16.820	/
X		M 16.340	M 0.16
		M 0.464	/
%RSD		M 2.839	/

Table S3 The zinc content in the N_3-UiO-66-Pc measured by ICM-MS methods.

Run	Time	64Zn(ppb)	Pc(ppm)
1	16:37:08	M 7.725	/
2	16:37:31	M 7.970	/
3	16:37:55	M 7.984	/
X		M 7.893	M 0.077
		M 0.145	/
%RSD		M 1.843	/

Table S4 The zinc content in the E-UiO-66-Pc measured by ICM-MS methods.

Run	Time	64Zn(ppb)	Pc(ppm)
1	16:51:11	M 6.994	/
2	16:51:33	M 7.421	/
3	16:51:57	M 7.619	/
X		M 7.345	0.071
		M 0.319	/
%RSD		M 4.349	/

Section 10. Singlet oxygen quantum yield.

Singlet oxygen quantum yield (Φ_Δ) values were measured by comparative method using 1, 3-diphenylisobenzofuran (DPBF) as singlet oxygen chemical quencher in DMF, [3] (Eq.(1) in air)

$$\Phi_\Delta = \Phi_\Delta^{std} \frac{kI_{abs}^{std}}{k^{std} I_{abs}} \qquad (1)$$

Where Φ_Δ^{std} is the singlet oxygen quantum yield for the unsubstituted zinc phthalocyanine(ZnPc) standard[4] (Φ_Δ^{std} = 0.56 in DMF). k and k^{std} are the DPBF photobleaching rates in the presence of carboxyl substitutive zinc phthalocyanine, E-UiO-66-Pc and ZnPc, respectively. I_{abs} and I_{abs}^{std} are the rates of light absorption by synthetic phthalocyanines and reference substance.

The reduction of the solutions was supervised at 415 nm, and DPBF concentrations were reduced to 0.1 mmol·L^{-1}. Figure S16 shown that Φ_Δ value of carboxyl substitutive zinc phthalocyanine and E-UiO-66-Pc is 0.63 and 0.73, respectively.

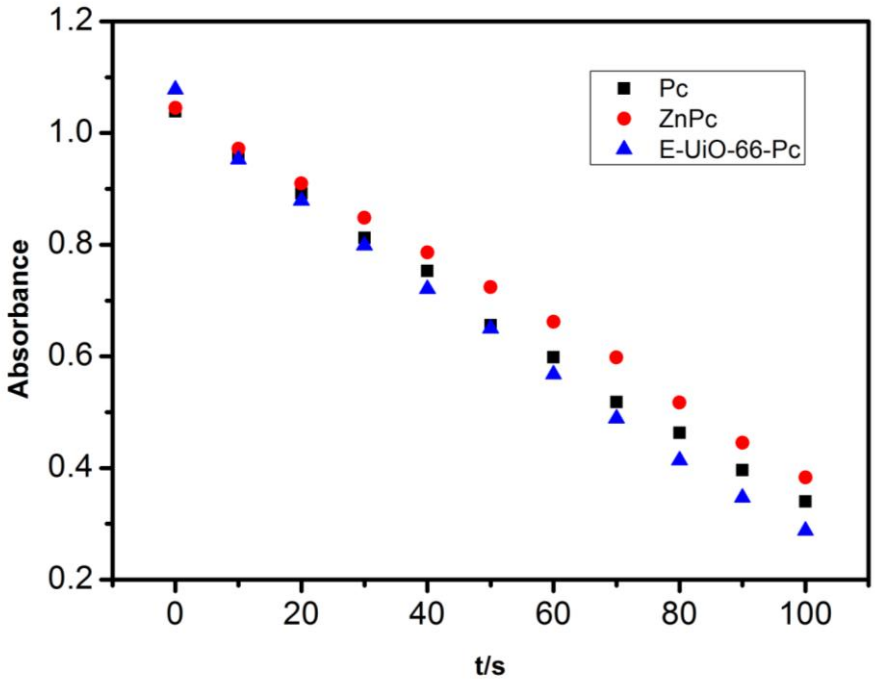


Figure S16. The absorbance of DPBF at 415nm versus the time.

Section 11. In vitro test

Cell Culture. HepG2 cells and HELF cells (from ATCC) were maintained in RPMI medium 1640 (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and Primocin antibiotic (Invitrogen). The cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere. All the compounds required the preparation of a concentrated DMSO stock solution (5% CEL), which were then diluted with medium and the final DMSO concentration was 1% in medium.

Cytotoxicity Assay. To assess the cytotoxic effect of the phthalocyanines, about 1.0×10^4 HepG2 cells per well in the culture medium were seeded in 96-multiwell plates and incubated at 37 °C for 24 h in a humidified 5% CO_2 atmosphere. Drugs were first dissolved in DMF to give 10 mM solutions, which were diluted to 1 mM with the culture medium in the presence of 0.5% Cremophor EL. These served as the stock solutions for the following in vitro studies. For cytotoxicity studies, the solutions were further diluted with the culture medium. The cells, after being rinsed with phosphate buffered saline (PBS), were incubated with 100 μL of the diluted drug solutions for 2 h at 37 °C under 5% CO_2. The cells were then rinsed again with PBS and refed with 100 μL of the culture medium before being illuminated at ambient temperature. For dark cytotoxicity, drugs were diluted and added to tetraplicate wells. 24 h later, the added compounds were removed by fresh medium and were incubated for another 24 h. The cell survival was assessed using the MTT assay. For light cytotoxicity, after incubated with phthalocyanines for about 24 h, the cells were exposed to light (λ = 670 nm) at a dose of 1.5 $J\cdot cm^{-2}$ and then incubated again for 24 h and finally the MTT cell viability assay was performed and each experiment was performed in triplicate.

Cell viability was determined by means of the colormetric MTT assay. [5] After illumination, the cells were incubated at 37 °C under 5% CO_2 overnight. An MTT (Sigma) solution in PBS (3 mg mL^{-1}, 50 μL) was added to each well followed by incubation for 2 h under the same environment. A solution of sodium dodecyl sulfate (SDS; Sigma, 10% by weight, 50 μL) was then added to each well. The plate was incubated in an oven at 60 °C for 30 min, and then 80 μL of isopropyl alcohol was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 10 s before the absorbance at 540 nm at each well was taken. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the equation: % Viability = [$\sum$ (Ai/Acontrol× 100)] /n, where Ai is the absorbance of the ith data (i = 1, 2, ..., n), Acontrol is the average absorbance of the control wells, in which the drug was absent, and n (= 4) is the number of data points.

Table S5 IC_{50} Values for compound against HepG2 Cells

Compound	IC_{50} (μg·mL^{-1})	
	In dark	In light
E-UiO-66-Pc	114.5	4.14
UiO-66	a	a
UiO-66-E	100.1	b
UiO-66-Pc	a	2.39

[a]Noncytotoxic up to 200 μg·mL^{-1}. [b]Not determined

Cellular Uptake1. HepG2 and HELF cells (5×105cells/well) were seeded in 6-well plates in RPMI-1640 medium respectively for 24 h before further manipulation. Then cells were incubated with UiO-66 or E-UiO-66-Pc (10μg mL^{-1}) for 24h. The treated cells were washed with phosphate buffered saline(PBS,pH 7.4) twice to removed the un-loaded drug. After that the PBS were replaced with 500μL RPMI-1640 medium without serum and phenol red. Finally, the fluorescence intensity of the cells was monitored by flow cytometry (C6, BD BioSciences) with excitation at 640 nm and emission at 690 nm.

Table S6 The fluorescence intensities(FI) of the Pc in HELF and HepG2 cells

	CELL（FI）	UiO-66-Pc（FI）	E-UiO-66-Pc（FI）
HELF	2795±86	61473±2202	7249±1023
HepG2	639±10	76507±2495	9731±224
R		1.29	2.04

R= HepG2 (FI) - HepG2 CELL（FI） / HELF (FI) - HELF CELL（FI） .

Cellular Uptake2. The HELF and HepG2 cells suspension were plated on a culture dish (10 0000 : 10 0000 cells) and incubated overnight at 37 °C under 5% CO_2. Then the cells were exposed to 10 $g \cdot ml^{-1}$ E-UiO-66-Pc and incubated for 24 h. After incubation, the cells were rinsed with PBS for three times and the intracellular fluorescence caused by phthalocyanines(excited at 633 nm, monitored at 650-750 nm) was recorded and statistically analyzed by Confocal laser scanning microscope. Figure S16 shows comparison of relative intracellular average fluorescence intensity of phthalocyanines in HepG2 and HELF cells (measured in the ROIs).

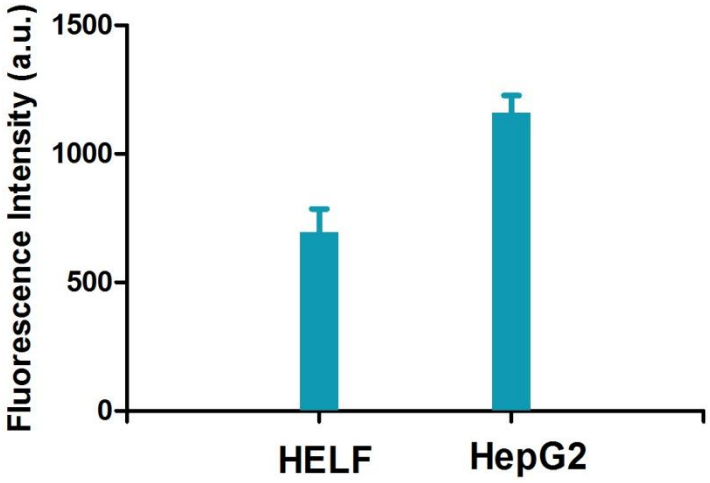
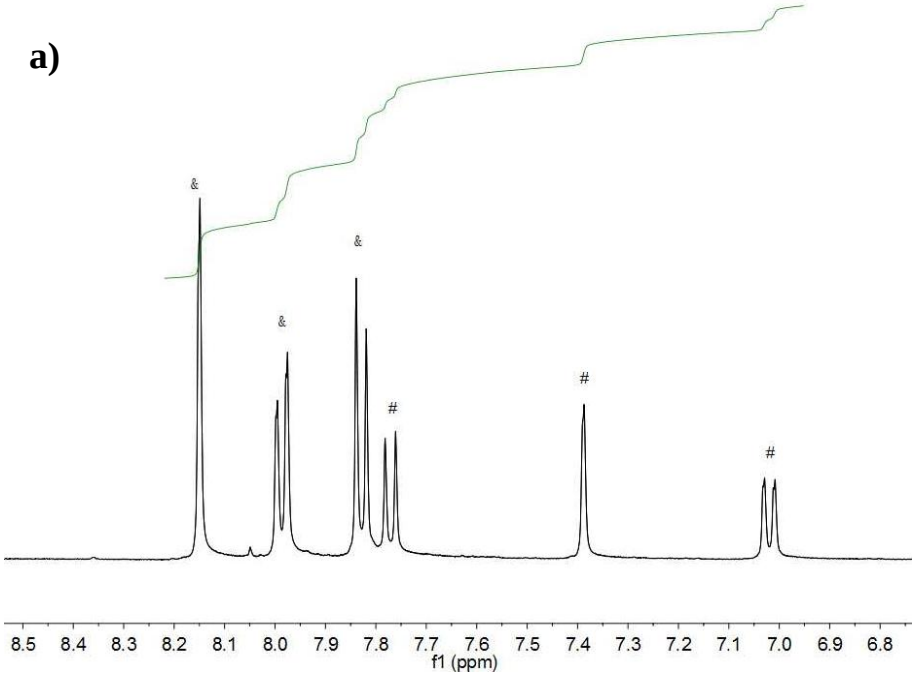


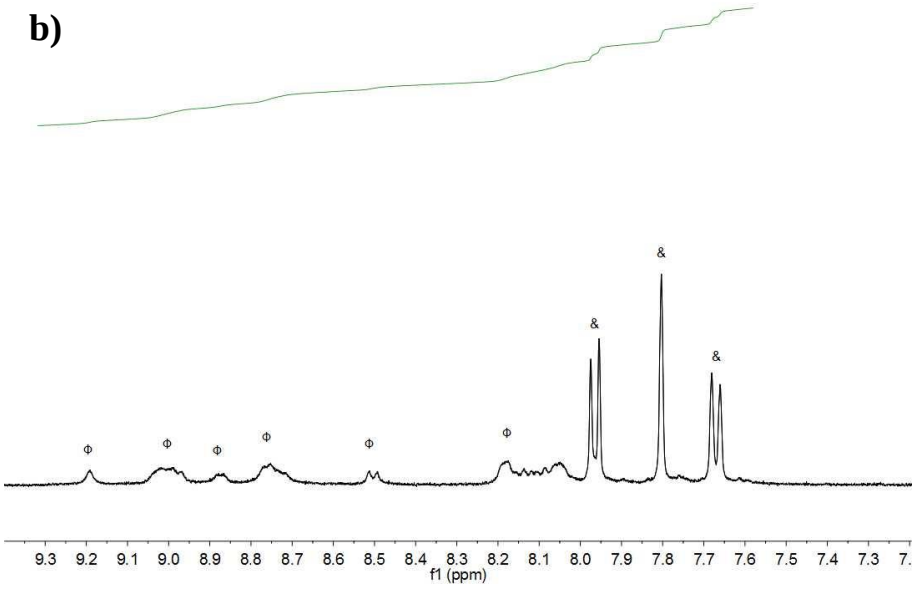
Figure S17. Comparison of the relative intracellular fluorescence intensity of Pc in HELF and HepG2 cells.

Section 12. ^{1}H NMR Spectrum

a)



b)



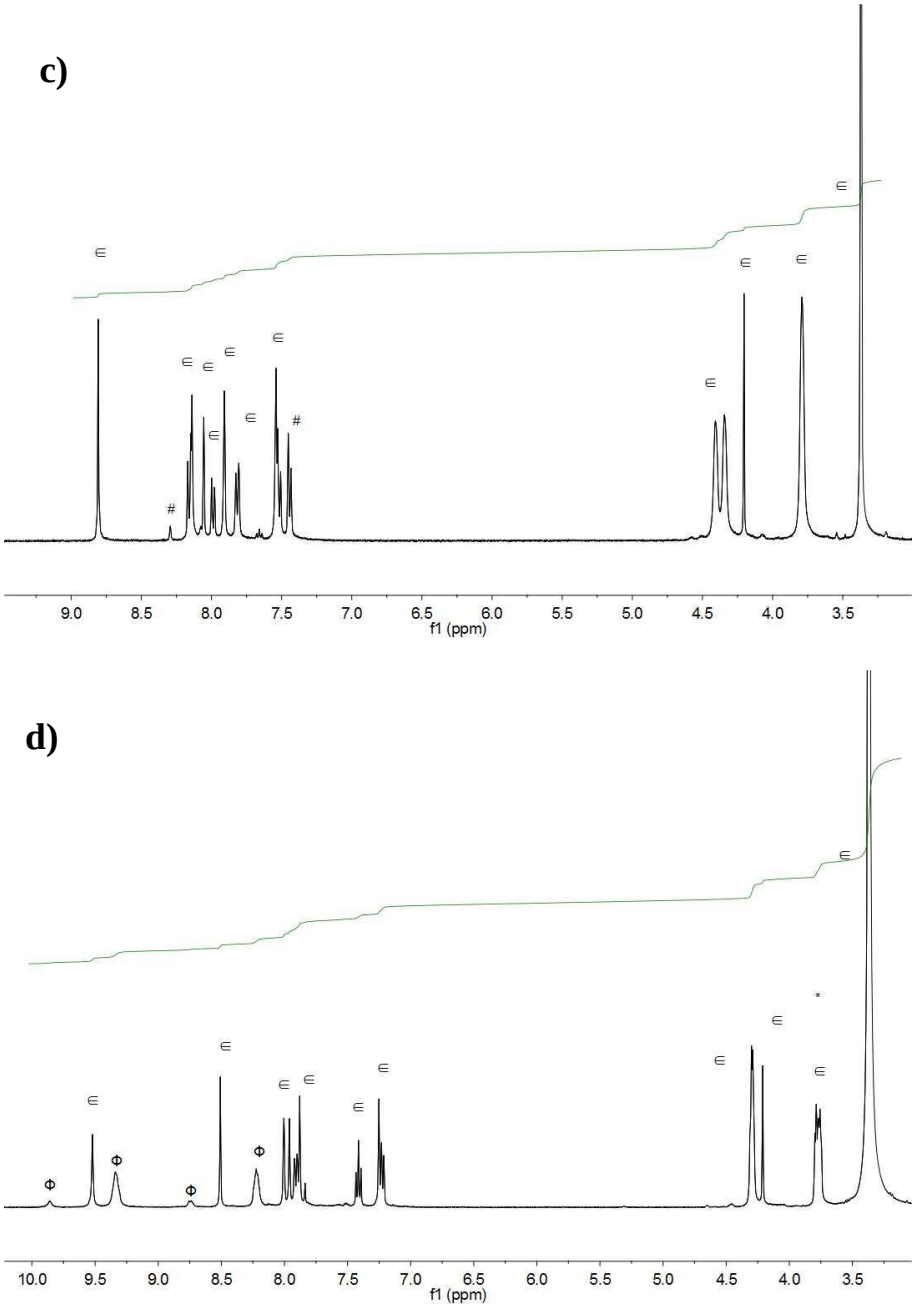


Figure S18. ^{1}H NMR Spectrum (400 MHz, room temperature) of a) N_3-UiO-66-NH_2 b) UiO-66-Pc c) UiO-66-E d) E-UiO-66-Pdissolved in a warm 1:6 mixture of D2SO4 and DMSO-d6. &Signals of NH_2-BDC. #Signals of N3-BDC. Φ Signals of ZnPc. ∈ Signals of erlotinib.

Section 13. References

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