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Pressure-controlled drug release in a Zr-cluster-based MOF

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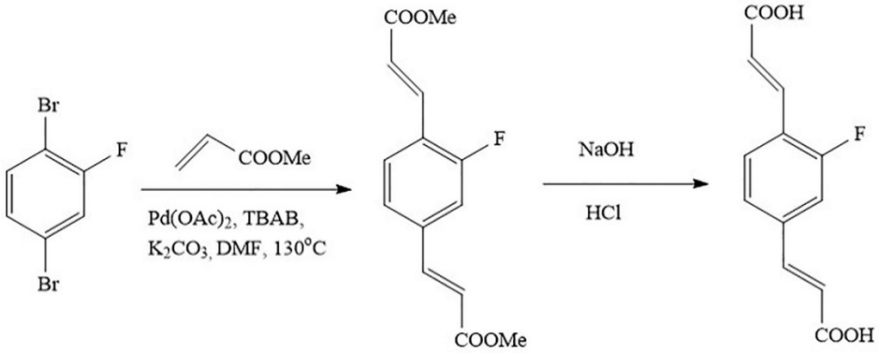
S1. Materials and Measurements

All the initial materials and other reagents were obtained from commercial sources without further purification. PXRD data were taken on a Shimadzu XRD7000 powder X-ray diffractometer with the recording rate 5^{o} min^{-1} in the 2θ=3-50 degree at room temperature. The morphology of the samples was imaged through a Hitachi S-4800 field emission scanning electron microscope. The 77k N_2 adsorption measurements were taken on an ASAP (accelerated surface area and porosimetry) 2460 system. The structure of ligand was measured by a ^{1}H NMR spectra recorded on a Bruker Avance DMX 500 MHz spectrometer using tetramethylsilane (TMS) as internal standard. The drug loading capacity was calculated by a ^{1}H NMR spectra taken on a Bruker Avance DMX 600 MHz spectrometer with tetramethylsilane (TMS) as aninternal standard. It was verified by ionic chromatography (Dionex, ICS-1000) and Thermogravimetric analyses (Netzsch TG209F3). YP-2 tablet machine was used for pressure treatment. The drug release analysis was carried out by high performance liquid chromatography (HPLC) using the Agilent 1200 chromatographic system. FV-1000 confocal laser scanning microscope was used for visualizing the cell proliferation and the cytotoxicity of the crystal.

S2. Synthesis routes to the organic linker F-H_2PDA

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



Scheme S1: Synthetic routes to the organic linker F-H_2PDA

Synthesis of (2E,2'E)-3,3'-(2-fluoro-1,4-phenylene) diacrylic acid (2): 1,4-dibromo-2-fluorobenzene (8 g, 33.9 mmol), ethyl acrylate (9 ml, 84.6 mmol), K_2CO_3 (6.63 g, 48 mmol), tetrabutyl ammonium bromide (TBAB) (2.06 g, 6.4 mmol), $Pd(OAc)_2$ (0.2 g, 0.82 mmol), and DMF (100 ml) were added in a 250 ml round-bottomed flask. The mixture was stirred at 130 ^oC for 24h. The mixture was extracted with ethyl acetate and water three times after the reaction was cooled down. Anhydrous $MgSO_4$ was used for drying the organic phase and the organic phase was removed under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum/ethyl acetate, 4:1 v/v) to get the pure product **(1)**. Yield: 5 g (62.5%). And the product **1** (2 g, 6.8 mmol) was suspended in a mixture of 100 ml of 10 M NaOH aqueous solution and 1, 4-dioxane (20 mL). After the mixture was stirred at 100 ^oC overnight, the pH value of the mixture was adjusted to 3 by concentrated HCl. The solid was collected by filtration, washed with water until the pH value up to 7, and dried at 65 ^oC to get a white powder. Yield: 1.8 g (90%); ^1H NMR (500 MHz, DMSO), δ = 6.68 (q, 2H), 7.60 (q, 2H), 7.65 (d, 1H), 7.72 (d, 1H) 7.89 (t, 1H).

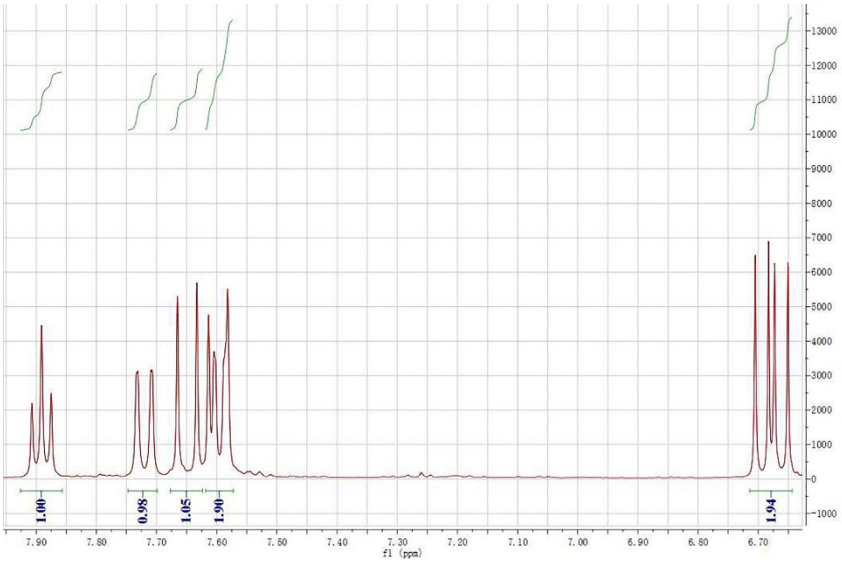


Figure S1. ^1H NMR for ligand

S3. Synthesis of crystal and Characterization

ZJU-800 was synthesized according to Omar K. Farha with a slight modification.[2] 60 mg (0.186 mmol) of $ZrOCl_2.8H_2O$ was dissolved in 5 ml of DMF and 0.18 ml of formic acid under 5 min sonication, while 50 mg (0.212 mmol) of the ligand was added, followed by 15 min sonication and heating at 70 ^oC for 96 h. The resulting solid was collected by centrifugation at 10000 rpm for 10 minutes and the washed with DMF and acetone three times. The white product was soaking in acetone overnight and then dried at 60 ^oC to remove the solvents. The porosity of ZJU-800 was analysed by N_2 adsorption at 77K (Figure S2). The BET area of the synthesized ZJU-800 is 1625 m^2/g.

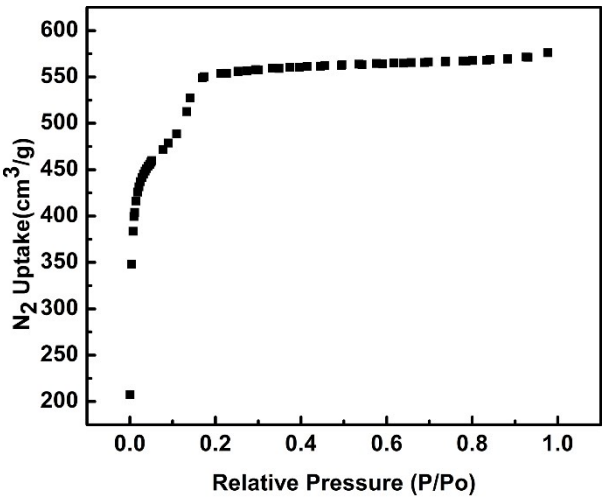


Figure S2. N_2 adsorption isotherms at 77 K on activated ZJU-800

S4 Drug loading experiments

Calculating the DS loading capacity by ^{1}H NMR

The diclofenac sodium **(**DS**)** was performed by immersing 50 mg of ZJU-800 into 5 mL of ethanol DS solution (20 mg mL^{-1}) at 25 ^oC under stirring for 2 days. The loaded ZJU-800 was collected by centrifugation at 5000 rpm for 10 minutes, washed three times with ethanol and dried in the vacuum oven to remove the solvent. The amount of drug loaded was quantified by ^{1}H NMR 600 MHz. In the ^{1}H NMR spectrum of DS-loading ZJU-800, the sp^2 protons in the benzene ring of the ligand F-H_2PDA were presented in the peaks 7.68 ppm (1H), 7.48 ppm (1H) and 7.44 ppm (1H). The spectrum also displayed the proton signals of the drug DS, and the peaks of -CH_2- is 3.5 ppm (2H). Therefore, the ratio of the ligand to drug DS was 1.08:0.695 by calculating the peak areas of 7.48 ppm (ligand) and 3.5 ppm (DS). The amount of loaded DS was about 58.80%.

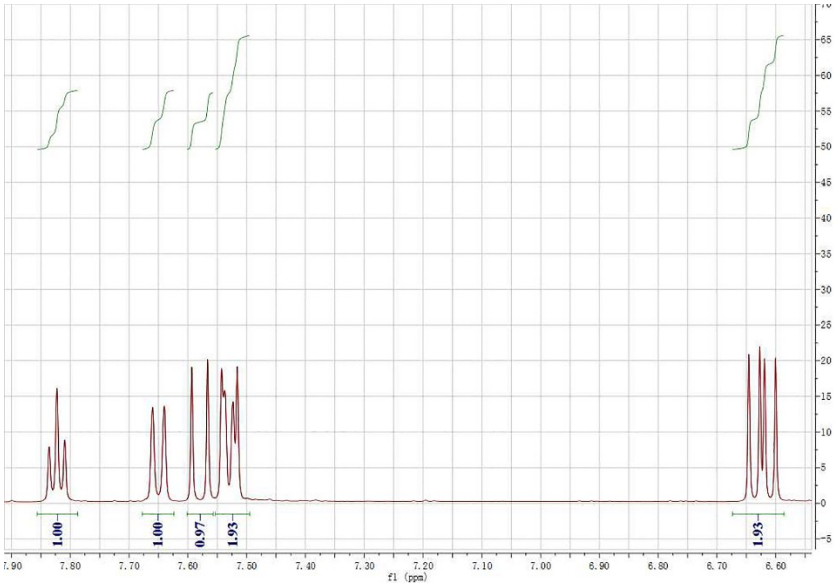


Figure S3. ^{1}H NMR for ZJU-800

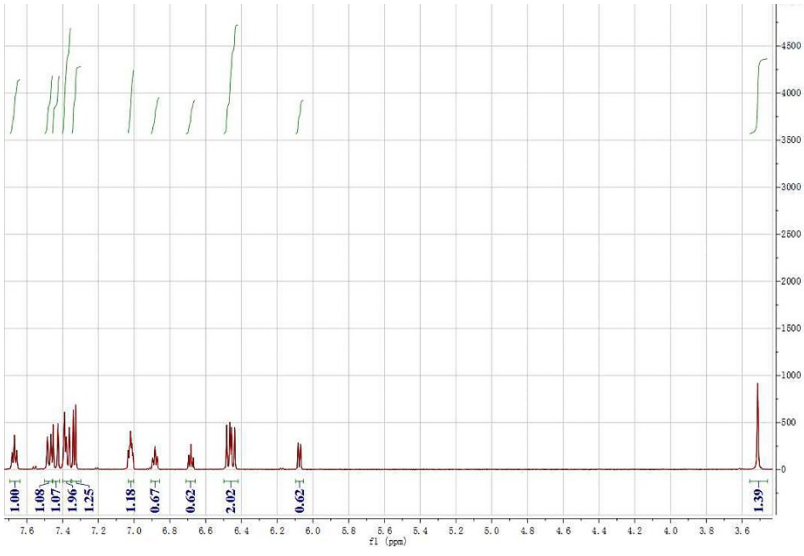


Figure S4. ^{1}H NMR for ZJU-800 encapsulated DS

Calculating the DS loading capacity by Ionic chromatography

The Ionic chromatography analysis of ZJU-800 and DS@ZJU-800 are listed in Table 1 and Table 2. Therefore, the molar ratio of the ligand to drug DS was 24:15.32 by calculating the element Cl and F. Finally, the amount of loaded DS was about 58.42%.

Table S1. Ionic chromatography analysis of ZJU-800

NO.	Ret.Time (min)	Peak Name	Height (μS)	Area (μS*min)	Rel.Area (%)	Amount	Type
1	4.03	F^-	4.492	3.897	88.18	9.025	BMB*
2	6.75	Cl^-	2.308	0.523	11.82	1.632	BMB*
Total			6.800	4.419	100.00	10.657	

Table S2. Ionic chromatography analysis of DS@ZJU-800

NO.	Ret.Time (min)	Peak Name	Height (μS)	Area (μS*min)	Rel.Area (%)	Amount	Type
1	3.96	F^-	2.778	1.854	28.42	4.293	BMB*
2	6.80	Cl^-	19.192	4.669	71.58	14.578	BMB*
Total			21.970	6.522	100.00	18.871	

Calculating the DS loading capacity by TGA

From figure S5c and -d, we conclude that the initial weight loss 20% observed in the temperature range 240-300^{o}C should be the first decomposition of the drug DS. And from 300-

800°C, the weight loss from figure S5d belongs to the decomposition of MOF and the drug DS. Therefore, the amount of loaded drug DS was calculated to be about 60.40% quite consistent with the aforementioned ^{1}H NMR and Elemental analysis.

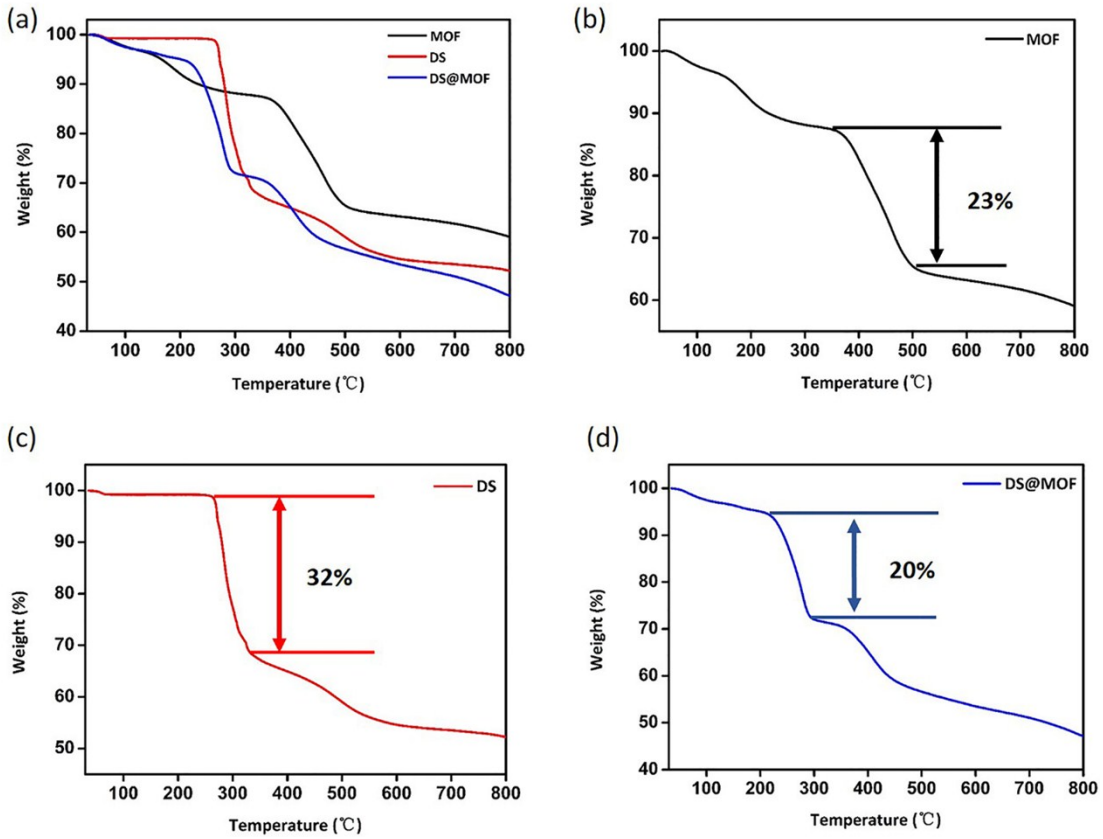


Figure S5. (a) TGA curves of the MOF, the DS and DS@MOF; (b) TGA curve of the MOF; (c) TGA curve of the drug DS; (d) TGA curve of the DS@MOF

S5. Pressure treatment

20 mg of DS loaded ZJU-800 was compacted in the pressure of 10 MPa and hold for 1 minute to get a slice. And the procedure was the same for the 30 MPa. After that, the slice was crumbled into fragments and 10 mg was taken for drug delivery assay.

S6. Delivery assays

The drug DS release experiments were taken in the phosphate buffered saline (PBS) at pH 7.4 and 37 °C for simulating physiological conditions. 10 mg of drug-loaded particles were soaked in 20 mL PBS solution. At different intervals, 0.2 mL of PBS was taken and replaced with 0.2 mL of fresh PBS. The amount of drug delivery was measured by high performance liquid chromatography (HPLC). The Zorbax Eclipse XDB-C 18 reverse-phase column (5m, 4.6 mm* 250 mm) is supplied by water. The mobile phase was compose of 20% phosphoric acid and 40% distilled water in methanol. And the effluent was determined at 276nm with the 25 ul injection

volume at the flow rate of 1.0 mL/min. The release profile can be fitted as a function of $y = A - B * C^x$

with the high correlation coefficient.

Material	Equation	R^2
ZJU-800@DS	C (wt%) = 88.577- 83.640 × 0.8734 ^ t	0.99935
ZJU-800@DS@10MPa	C (wt%) = 92.501- 90.440 × 0.9715 ^ t	0.99833
ZJU-800@DS@30MPa	C (wt%) = 87.816- 85.237 × 0.9863 ^ t	0.99448

S7. Cell Culture, imaging, and Cytotoxicity of ZJU-800

PC 12 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM, Neuronbc) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S, Boster) for 3 days in a cell incubator (37 ^oC, 5% CO_2). PC12 cell line is a widely used cellular model to assess the cytotoxicity of nanomaterials because of the adhesion nature of PC12 cells to culture plate.[3] What's more, the morphology can reflect their state visualized by microscopy due to the enriched neuritis in PC12 neuron cells. And in this method, the MTT dye can be reduced into formazan by mitochondrial enzymes presenting in metabolically active live cells. The amount of formazan is corresponding to the number of active cells. The cytotoxicity of ZJU-800 was evaluated through standard MTT assay in a 96-well plate. PC12 cells were incubated in the humidified incubator with ZJU-800 at different concentrations (5, 50, 100, and 150 μg/mL). 50 μL of 1 × MTT solutions were added to each well and incubated for 4 h. After that, 200 μL of the media were removed and 150 μL of dimethyl sulfoxide was added to each well. The absorbance of each sample was measured at 490 nm using a microplate reader. The experiments were sextuplicated, and the results were averaged. Besides, the imaging experiments were seeded in a 24-well plate with a coverslip (0.17 mm in thickness), and cells were incubated with ZJU-800 for 24 h at the concentration of 5 and 50 μg/mL. After that, cells were washed twice with PBS solutions (pH = 7.4) and fixed with 4% paraformaldehyde for 15 minutes at room temperature. Besides, the cells were permeabilized by Triton X-100 (0.5% in PBS) for 2 h at room temperature blocked by FBS (2% in PBS) with bovine serum albumin (BSA, 2% in PBS) for another 2 h. Then Alexa-Fluor 647-cnjugated β-tubulin (1:500 in PBS, Cell signalling) was used for staining the microtubules of the PC12 cells at room temperature for 2 h, followed by DAPI (1 μg/mL, Sigma-Aldrich) staining for 15 minutes to visualize nuclear DNA. Fluorescence imaging of PC12 cells was taken on by a confocal laser scanning microscopes at room temperature in a two-channel mode with laser excitation wavelengths of 405 nm for DAPI and 633 nm for Alexa-Fluor 647-conjugated β-tubulin.

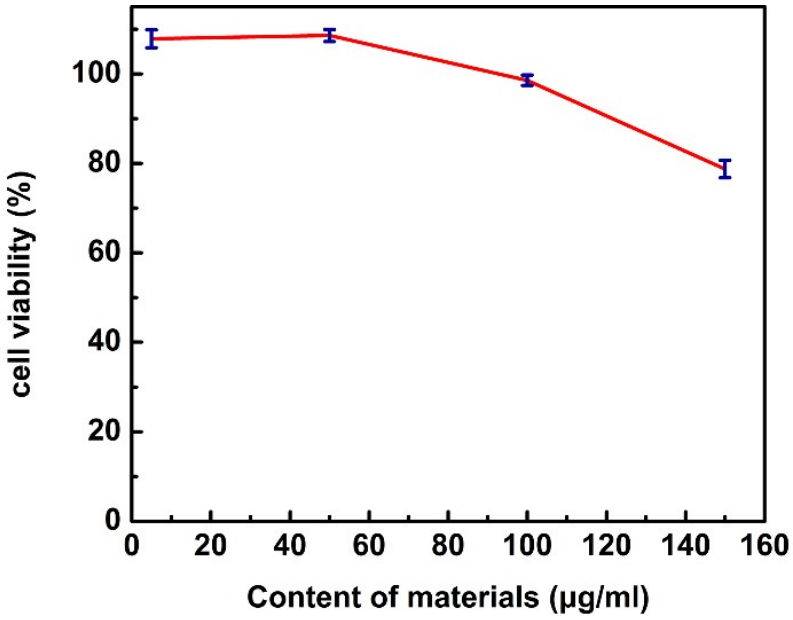


Figure S6. MTT assay of ZJU-800 obtained from incubation with PC12 Cells

WST-1 assay: PC12 cells were incubated in the humidified incubator with ZJU-800 at different concentrations (5, 20, 50, 80 and 100 μg/mL) for 24 h. 10 μL of WST-1 was added to each well and incubated for 2 h. The absorbance of each sample was measured at 450 nm using a microplate reader. The experiments were sextuplicated, and the results were averaged.

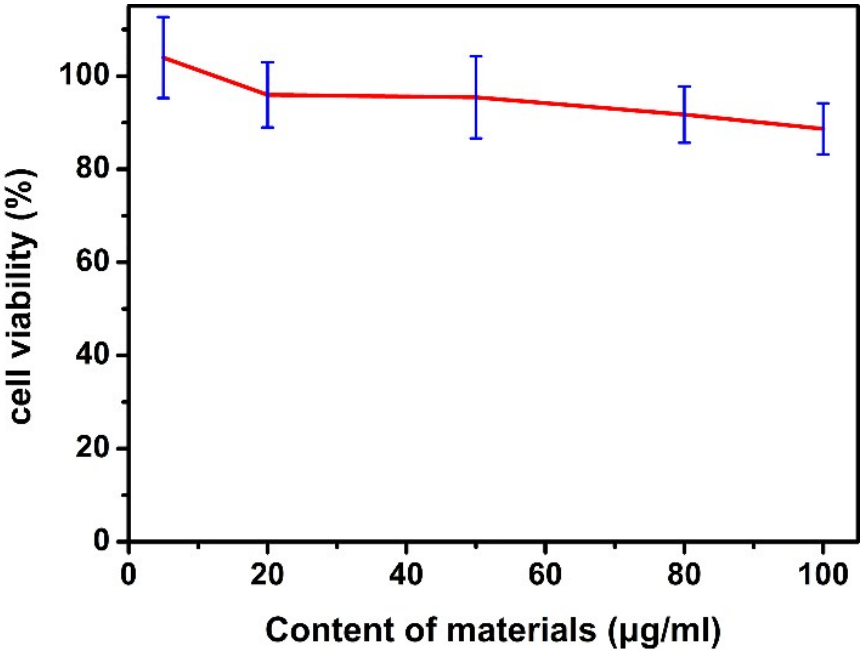


Figure S7. WST-1 assay of ZJU-800 obtained from incubation with PC12 Cells

Confocal microscope image by DAPI: The DAPI staining experiments were proceeded in a 24-well plate with a coverslip (0.17 mm in thickness), and cells were incubated with ZJU-800 for 24 h at the concentration of 0 and 50 μg/mL. The 24-well plate was incubated in the incubator

for 24 hours. After incubation, the cells were washed twice with 400μL of Phosphate Buttered Saline (PBS). After that, the PBS containing 4% paraformaldehyde was putted into the plate slowly and kept for 15 minutes in darkness, and then washed with 400μL PBS for twice. And then the cells were incubated with DAPI for five minutes at room temperature, and still washed with PBS for twice. After the above-mentioned procedure, the morphologies of all cells were observed using a confocal laser scanning microscope.

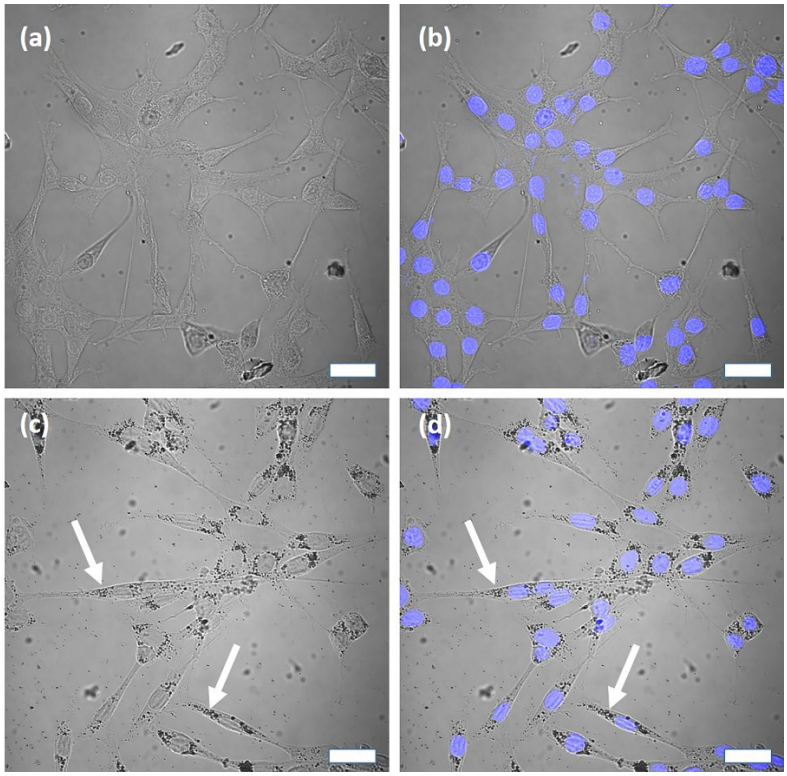


Figure S8. Confocal microscopy images of PC12 cells without ZJU-800 (a, b) and confocal microscopy images of PC12 cells incubated with ZJU-800 of 50 μg mL^{-1} for 24h (c, d) and the nuclei (blue) are fluorescently stained by DAPI (b and d). And the arrow refers to ZJU-800.Scale bar, 20 μm.

References

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