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S1. Experiment materials and instruments

Fmoc-OSu, Fmoc-amino acid with protected group on the side chain and 2-Cl-trityl chloride resin (1.0 mmol/g) were purchased from GL Biochem (Shanghai, China); N, N-diisopropylethylamine (DIPEA), HBTU, trimethylsilyl chloride and other chemical reagents and solvents were obtained from Fisher Scientific; alkaline phosphatase was purchased from Biomatik (Cat. No. A1130, alkaline phosphatase [ALP], >1300 U/mg, in 50% glycerol.). All chemical reagents and solvents were used as received from commercial sources without further purification. Water was purified using a Millipore system. Dulbecco's Modified Eagle's medium (DMEM) and McCoy's 5A medium obtained from ATCC, fetal bovine serum (FBS) and penicillin/streptomycin from Gibco by Life Technologies, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from ACROS Organics. Cell culture related dishes, 96 cell well and confocal dish, were purchased from Corning Inc. Water Delta600 HPLC system, equipped with an XTerra C18 RP column, was used to purify the compound. LC-MS spectrum was obtained on Waters Acquity Ultra Performance LC with Waters MICROMASS detector, and TEM images on Morgagni 268 transmission electron microscope. Carbon-coated copper grids (400 meshes) from Pacific Grid-Tech.

S2. Transmission electron microscopic

After the grids (400 mesh copper grids coated with continuous thick carbon film ~35 nm in thickness) were glow discharged for 30 seconds, we placed sample solution on the grid (5 µL, sufficient to cover the grid surface). About 25 s later, we placed three large drops of ddH2O on parafilm to let the grid touch the water drops (each for 5 seconds) with the sample-loaded surface facing the parafilm, and then tilted the grid and gently absorbed water from the edge of the grid using a filter paper. Immediately after rinsing, we placed three large drops of uranyl acetate (UA) staining solution on

parafilm to let the grid touch the staining solution drops with the sample-loaded surface facing the parafilm, and then tilted the grid and gently absorbed the stain solutions from the edge of the grid using a filter paper. After drying the grid, we examined the nanostructure on Morgagni 268 Microscope

S3. Cell culture

Saos-2 and HS-5 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Saos-2 cell lines were cultured in McCoy's 5A (Invitrogen Life Technologies) with 15% FBS and 1% P/S (100 U mL-1 penicillin and 100 µg mL^{-1} streptomycin, Invitrogen Life Technologies). HS-5 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen Life Technologies) with 10% FBS and 1% P/S. Co-cultured cells of Saos-2 and HS-5 were cultured in McCoy's 5A with 15% FBS and 1% P/S. The incubation conditions for all the cells were at 37 °C in a humidified atmosphere of 5% CO2. The color of Hoechst 33342 is blue. We switched the blue color to red for better readability.

S4. Cell viability assay

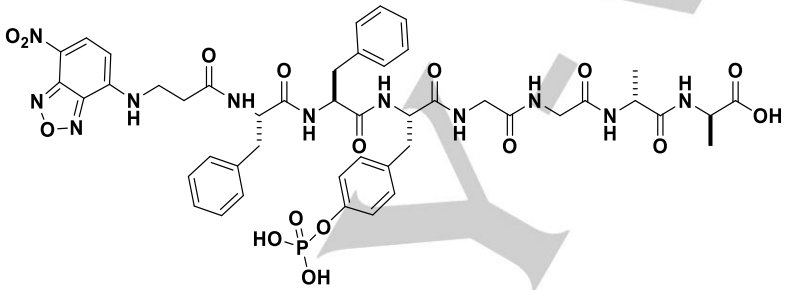
All cells were seeded in a 96-well plate with the density of 3×10^4 cells per-well (total medium volume of 100 µL). 24 hours post seeding, the solutions with a serial of concentrations (5 concentrations) of different precursors were added to each well. Cells without the treatment of the precursors were used as the control. At designated time (24/48/72 hours), 10 µL MTT solution (5 mg/mL) was added to each well and incubated at 37°C for another 4 h, and then 100 µL of SDS-HCl solution was added to stop the reduction reaction and dissolve the purple formazan. The absorbance of each well at 595 nm was measured by a multimode microplate reader. For 3D cell viability upon the treatment of different chemotherapy drugs, we first induced cell spheroid formation by 1P:2, and then replaced the culture medium with a serial of concentration of drugs. As comparing, 2D cell culture with the same cell number was used for parallel test. The cytotoxicity assay was performed three times, and the average value of the three measurements was taken.

S5. Live-dead assay

Cell viability was performed by a live/dead assay. At designated times, the cells after the treatment were washed three times with PBS buffer, and then 100 µL of the Live/Dead solution containing 4 µM EthD-1 (ethidium homodimer-1) and 2 µM calcein AM was added on top of each sample for 30 min in an incubator at 37 °C with 5% CO2. After removing the staining solution, we used CLSM to view the sample with excitation filters of 450–490 nm (green, Calcein AM) and 510–560 nm (red, EthE). Green represented live cells, while red indicated dead cells.

S6. Time-dependent western blot

After the co-cultured Saos-2 and HS-5 cells reached to about 75% confluence in 10 cm culture dish, cells were washed with PBS buffer for three times and then added 500 µL cell lysis buffer for 5 min. Cell scraper was used for harvesting all the lysis solution from the cell culture dish. The collected cell lysates were mixed with 5 µL of 100× proteinase inhibitor cocktail and then were snap-frozen and thawed for three cycles to lyse the cells. The cell lysates were then centrifuged at 12,000× g for 20 min at 4 °C, and the supernatant was collected. After quantification of proteins in cell lysates by the Bradford reagent, the same amount of proteins from the samples of different ratio of Saos-2 and HS-5 were mixed with the same volume of 2× Laemmli buffer before SDS-PAGE. After finishing the gel electrophoresis and transferring the proteins on the gels to the PVDF membranes for overnight, the membranes were incubated with primary antibodies (1:1000) for overnight. The membranes were then incubated with secondary antibodies (1:2000) for 1 h, followed by the reaction with ECL solutions for 10 min and detected by developing X-ray film.



Scheme S1. Molecular structure of **NBD-1P**

S7. Supplemental figures

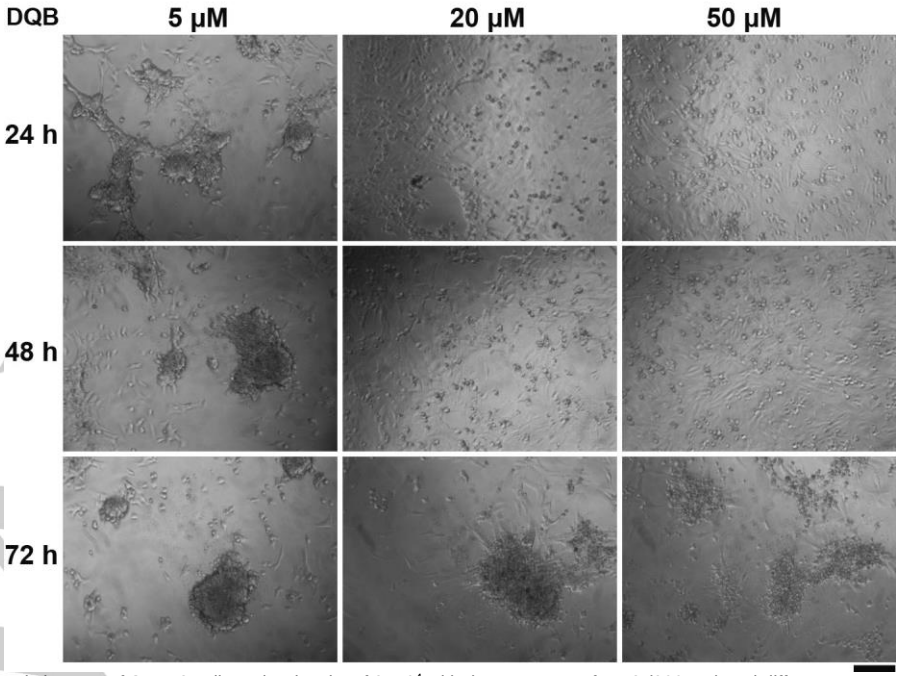


Figure S1. Optical microscopic images of Saos-2 cells at the density of 3×10^4 with the treatment of **1P:2** (300 µM) and different concentrations of DQB for 24, 48 or 72 h. Scale bar is 150 µm.

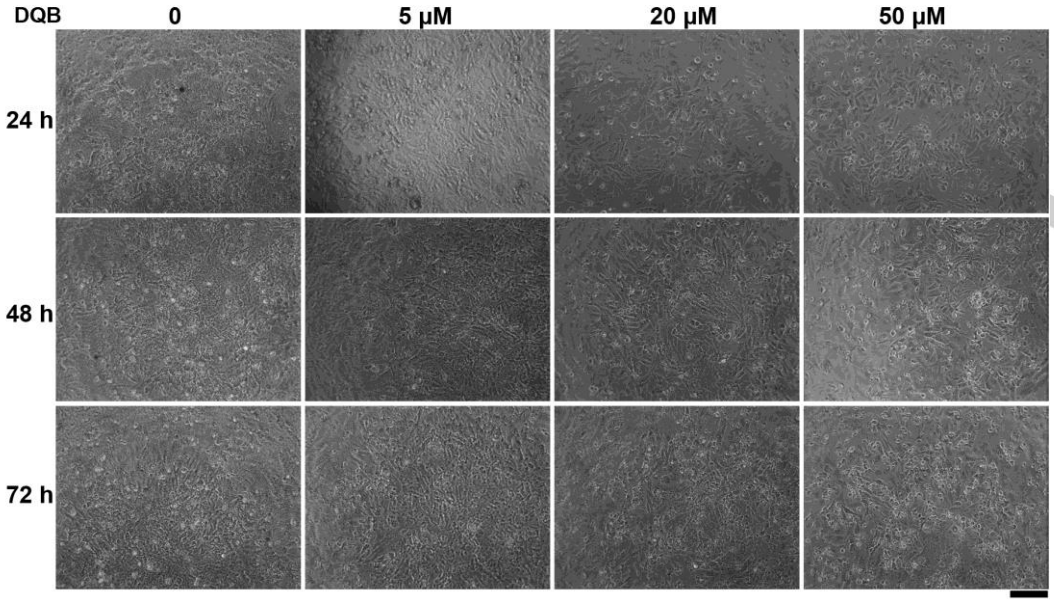


Figure S2. Optical microscopic images of Saos-2 cells at the density of 3×10^4 with the treatment of **1P** (300 μM) and different concentrations of DQB for 24, 48 or 72 h. Scale bar is 150 μm.

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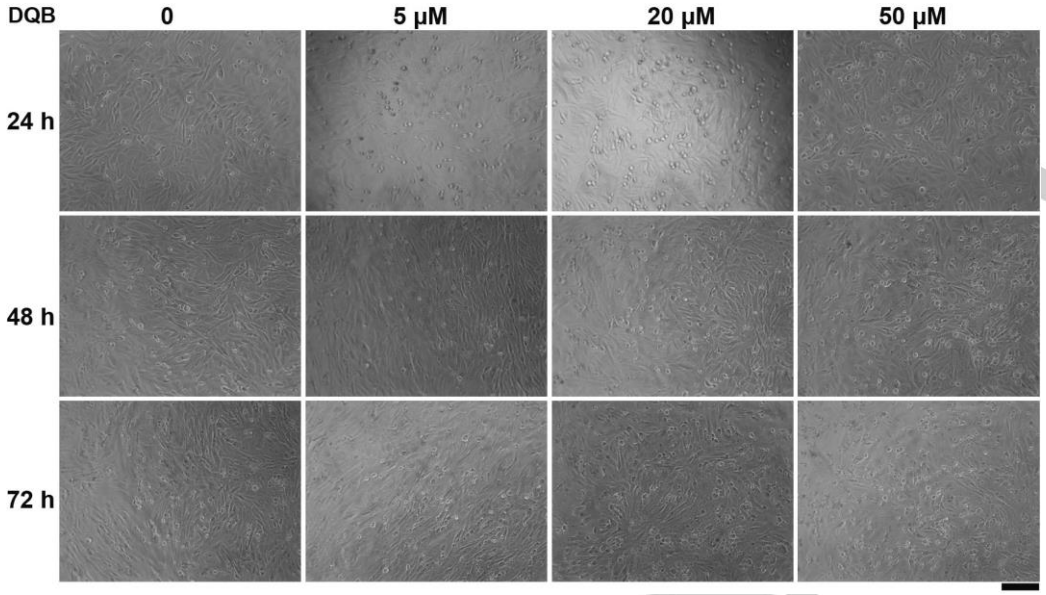

Figure S3. Optical microscopic images of Saos-2 cells at the density of 3×10^4 with the treatment of **2** (300 μM) and different concentrations of DQB for 24, 48 or 72 h. Scale bar is 150 μm.

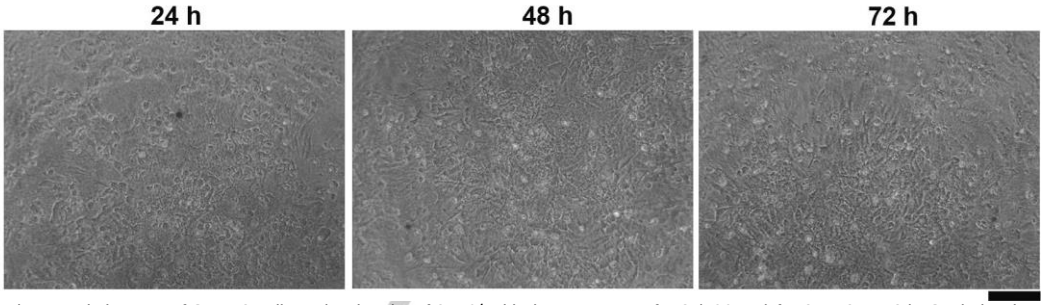

Figure S4. Optical microscopic images of Saos-2 cells at the density of 3×10^4 with the treatment of **1:2** (500 μM) for 24, 48 or 72 h. Scale bar is 150 μm.

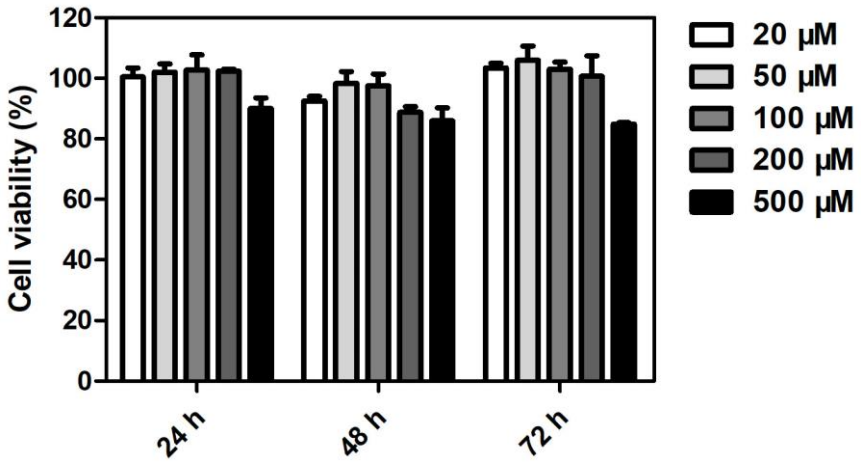

Figure S5. Cytotoxicity of **1:2** against Saos-2 cells for 24, 48 and 72 h.

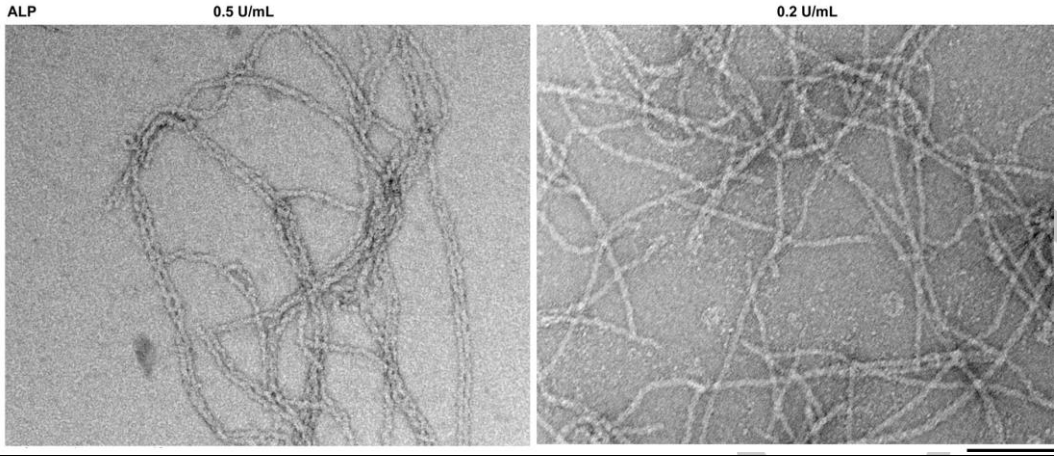

Figure S6. TEM images of **1P:2** (300 μM) after treated by a phosphatase (ALP, 24 h) at 0.5 U/mL or 0.2 U/mL. Scale bar is 100 nm.

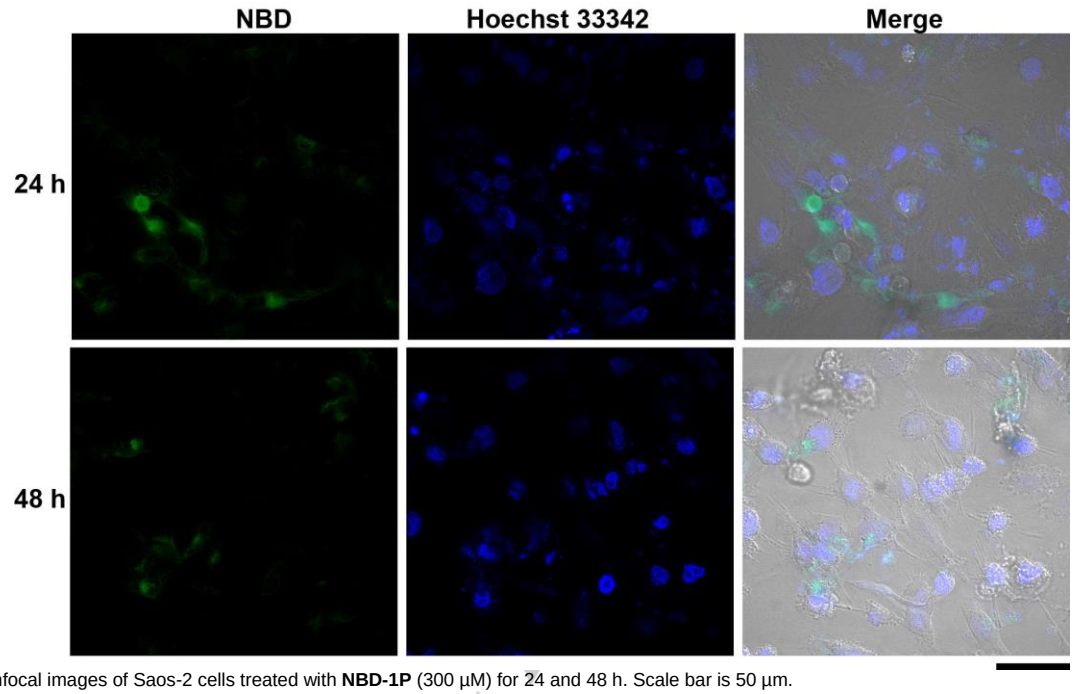

Figure S7. Confocal images of Saos-2 cells treated with **NBD-1P** (300 μM) for 24 and 48 h. Scale bar is 50 μm.

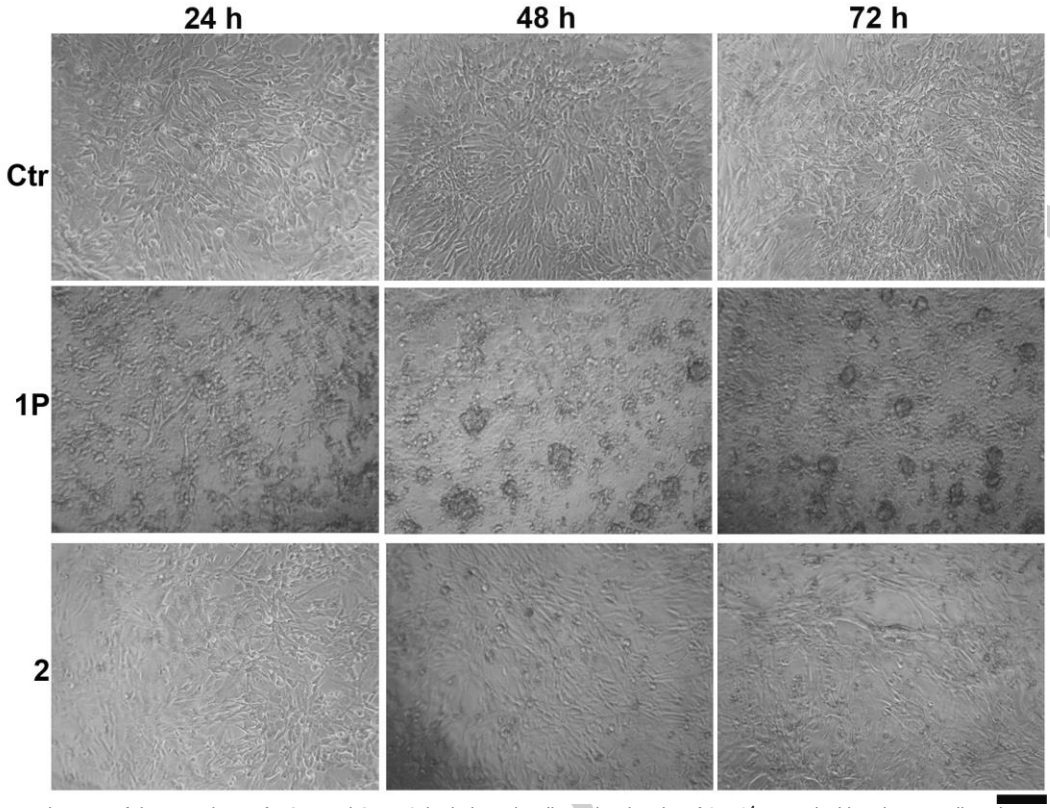

Figure S8. Microscope images of the co-culture of HS-5 and Saos-2 (ratio is 1:1) cells at the density of 3×10^4 treated with culture medium (control), **1P** or **2** for 24, 48 and 72 h.

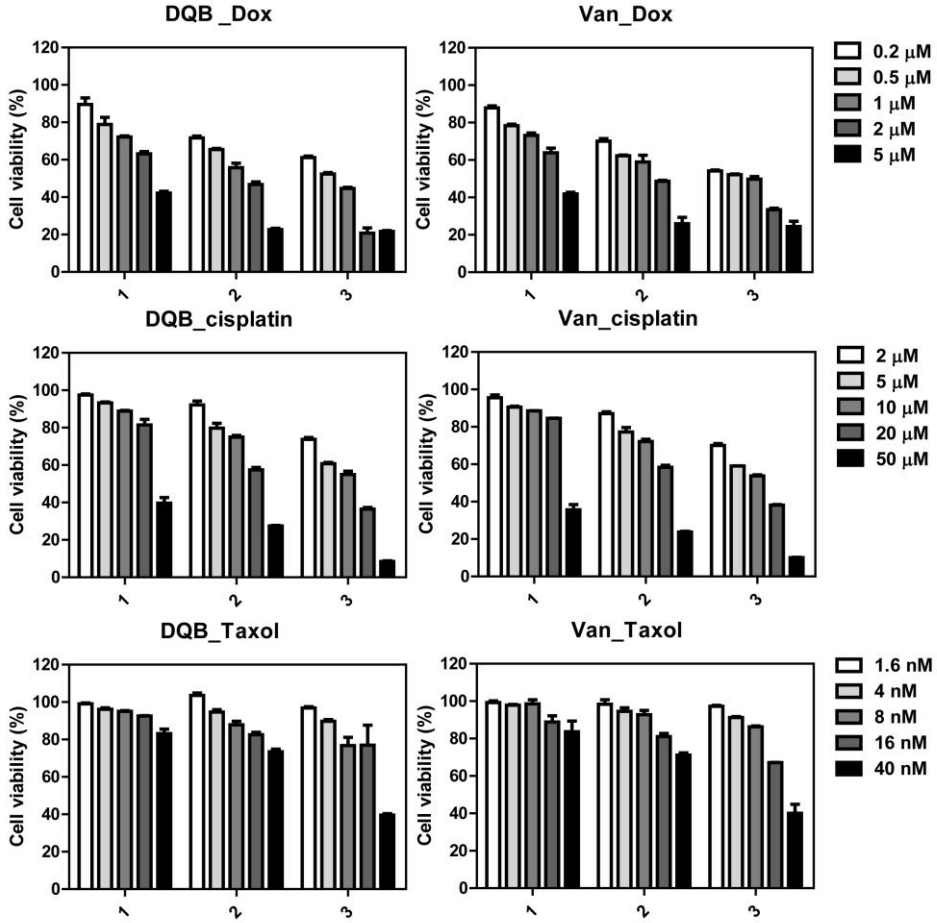

Figure S9. Cytotoxicity of three representative chemotherapy drugs doxorubicin, cisplatin, and taxol on the Saos-2 cell with addition of DQB (5 μM) or Vancomycin (300 μM)

S8. Supplemental Movies

Movie S1: Constructed Z-scan of Saos-2 cells treated with (**NBD-1P:2**) at concentration of 300 μM for 48 h.

Movie S2: Constructed Z-scan of Saos-2 cells treated with (**NBD-1P:2**, 300 μM) and DQB (5 μM) for 48 h.

Movie S3: Constructed Z-scan of co-cultured HS-5 and Saos-2 cells treated with (**1P:2**, 300 μM) and DQB (5 μM) for 48 h and then stained with live-dead assay.

Movie S4: Constructed Z-scan of co-cultured HS-5 and Saos-2 cells treated with (1P:2, 300 μM) and DQB (5 μM) for 48 h. HS-5 cell lines was firstly treated with Hochest 33342 (red) for 10 minutes and then co-cultured with the Saos-2 cell firstly treated with membrane probe (green)[1] for 1 h.

S9. Supplemental References

[1] H. Wang, Z. Feng, S. J. Del Signore, A. A. Rodal, B. Xu, *J. Am. Chem. Soc.* **2018**, *140*, 3505-3509.

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