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Experimental Procedures

Materials. All amino acid derivatives involved in the synthesis were purchased from GL Biochem (Shanghai) Ltd. N, N-diisopropylethylamine (DIEA) and O-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluorophosphate (HBTU) were purchased from Fisher Scientific. The synthesis of all peptide fragments was based on solid-phase peptide synthesis (SPPS). (R)-3-Methyl-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butan-1-amine hydrochloride was purchased from Bidepharm. The peptides were made *via* the combination of SPPS and solution phase synthesis. All crude compounds were purified by HPLC with the yield of 70-80%. All reagents and solvents were used as received without further purification unless otherwise stated. All cell lines were purchased from ATCC. All media for cell culture were purchased 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. Mitochondria isolation kit was purchased from Thermo Fisher Scientific. The mitochondria were isolated according to the protocol provided by the company. All antibodies were purchased from Abcam. Alkaline phosphatase was purchased from Thermo Fisher Scientific.

Instruments. All peptides were purified by Water Agilent 1100 HPLC system, equipped with an XTerra C18 RP column. LC-MS was operated on a Waters Acquity Ultra Performance LC with Waters MICRO-MASS detector. Transmission electron microscope (TEM) images were taken on Morgagni 268 transmission electron microscope. Fluorescent analysis was performed on Shimadzu RF-5301-PC fluorescence spectrophotometer. Fluorescence images were taken by ZEISS LSM 880 confocal laser scanning microscope. Circular dichroism (CD) spectra were obtained by Jasco J-810 spectropolarimeter.

Peptide Synthesis. The synthesis of **D1p** and **Fmoc-AVPIG-OH** was done by Fmoc-based solid phase synthesis^[1]. The synthesis routes of **1**, **3** and **5** were shown in Scheme S2. Briefly, the carboxylic groups in **Fmoc-AVPIG-OH** were activated by a mixture (1:1:1 molar ratio) of DIEA and HBTU at room temperature for 5 min. After the activation, **D1p** were mixed into the solution. The mixtures were kept at room temperature for overnight. Piperidine was used to remove the Fmoc protection group. HPLC was used to purify the resulted **1**. For the synthesis of **3**, the carboxylic groups in **Fmoc-AVPIG-OH** were activated by a mixture (1:1:1 molar ratio) of DIEA and HBTU at room temperature for 5 min. After the activation, **D1p** was mixed into the solution. The mixtures were kept at room temperature for overnight. After that, HBTU, DIEA and NH₂-NBD (1:1:1) were added into the mixture and stirred for another 8 h. TLC was used to check the formation of product. Eventually, piperidine (20%) was used to remove all protection groups. HPLC was used to purify the final products (**3**).

Determination of Proteolysis Resistance. Peptides (200 μM) of interest were mixed with proteinase K (2 U/mL) and Saos-2 cell lysis (protein concentration 1 mg/mL), respectively and incubated in a 37°C-shaker for 24 h. The results products were analyzed by LC/MS. The Remain% was determined by the weight of intact peptides after incubation/the initial weight of the peptides.

TEM Images. TEM images were taken on Morgagni 268 transmission electron microscope using negative staining. Following an established protocol^[2], samples were dropped on copper grids and dried. Uranium acetate was used to stain the samples. Images were taken by lab members who were properly trained.

Cell Culture. HeLa cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% (vol/vol) FBS, 0.5% (vol/vol) penicillin (10, 000 unit), and 0.5% (vol/vol) streptomycin (10, 000 unit). HS-5 cells were cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% (vol/vol) FBS, 0.5% (vol/vol) penicillin (10, 000 unit), and 0.5% (vol/vol) streptomycin (10, 000 unit). Saos-2 cells were cultured in McCoy's 5a medium supplemented with 15% fetal bovine serum, and 0.5% (vol/vol) streptomycin (10, 000 unit). All cells were maintained at 37 °C in a 5% CO₂ incubator.

Cell Viability Assay. The cell viability was determined by MTT assay. Cells were seeded in 96-well plates (1X10⁴ cells/well) and incubated in a cell incubator for overnight for adhesion. After adhesion, culture medium in each well was removed followed by adding different concentration of the compounds of interests (dissolved in culture medium). To examine the cell viability, 10 μL MTT solution (5 mg/mL) was added into the wells designed for 1, 2 and 3 days incubation, respectively. The plates were incubated in an incubator for another 4 hours. 100 μL SDS-HCl solution was added to stop the MTT-reduction reaction and to dissolve the formazan. After measuring the absorbance of each well at 595 nm with multimode microplate reader, we calculated the cell viability percentage relative to the untreated cells (solvent control). The MTT assay was performed in triplet, and the average value of the three measurements was taken.

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Quantification of Intracellular Fluorescence Intensity. Cells treated by conditions of interest were detached by trypsin incubation for 3-5 min and washed by centrifuge (800 g, 3 min) in PBS for 3 times. The cell suspensions were diluted into 10^5 cells/mL. The diluted cell suspension was added into 96-well plates (200 μ L in each well, at least 5 wells per sample) and incubated at room temperature for 5 min. After the incubation, DTX 880 Multimode Detector (Beckman Coulter Inc.) was used to read the fluorescence in each well.

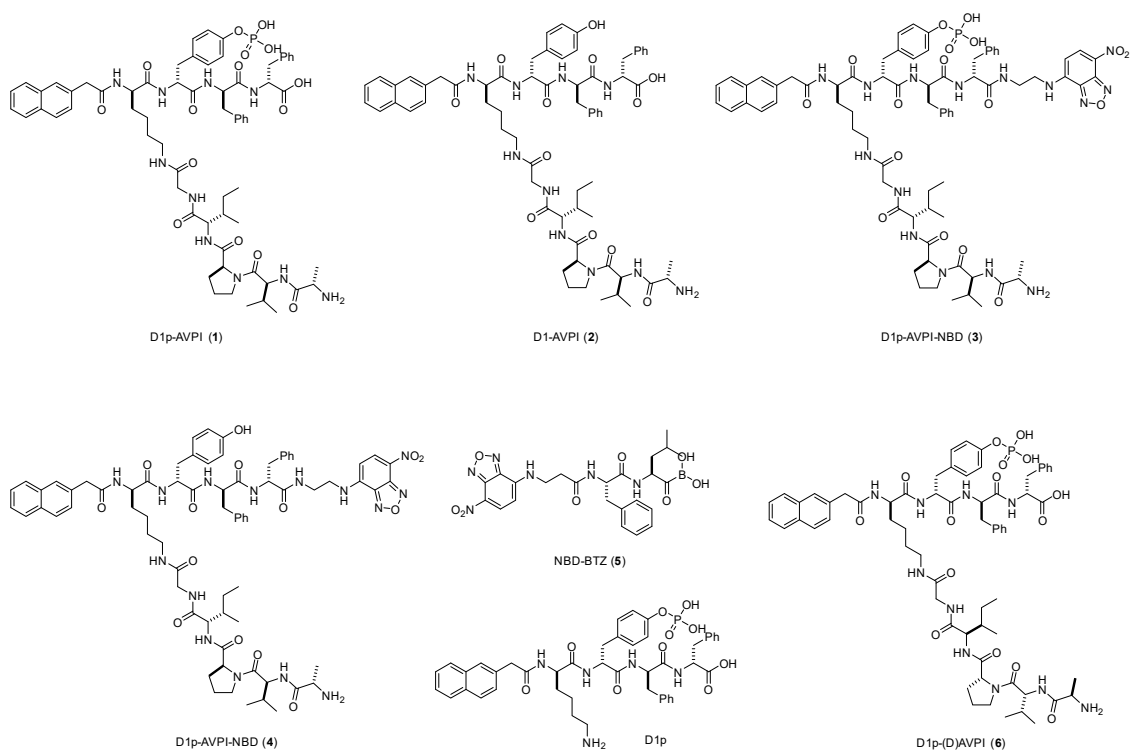
Immunofluorescence Staining. Cells were plated on confocal dishes (CellVis) and fixed in 4 wt% paraformaldehyde for 15 min and permeabilized with 1% BSA and 0.1% Tween 20. Fixed cells were incubated in primary antibody for 15 min, washed three times for 5 min each, incubated in secondary antibody for 1 h, then washed three times for 5 min each.

Western Blot. Total protein extracts were prepared in cell lysis buffer (Cell Signaling Technology, CATALOG # 9803S) followed by 5 freeze-thaw circles. Protein concentration was determined by Coomassie Blue method. Protein extracts (20 μ g per lane) were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Western blotting was performed according to standard protocols^[3]. Gel analysis was conducted using ImageJ.

Semi-Quantification of ALP Density. The ALP density on plasma membrane was semi-quantified by analyzing the immunofluorescent images of ALP in cells using ImageJ. The ALP density was defined by the fluorescence intensity per unit area.

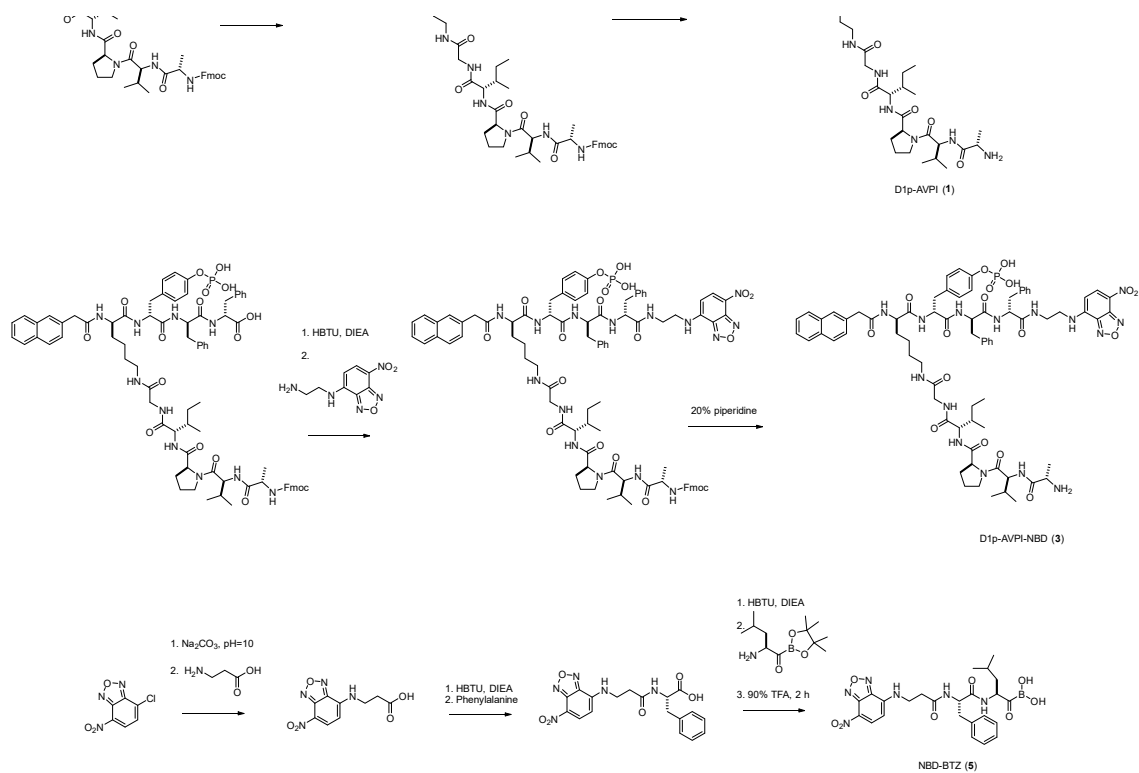
Determination of CMC. The CMC of **1** was determined using pyrene as the fluorescent probe^[4]. Briefly, different concentrations of **1** were prepared in pyrene-saturate solutions. The fluorescence spectra of pyrene solutions with different concentration of **1** were obtained. The intensity ratio of the 1st peak/the 3rd peak (I_1/I_3) was determined. Plot I_1/I_3 against the concentrations of **1**. The concentration at the turning point is the CMC.

Results and Discussion



Scheme S1. Molecular structures.

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Scheme S2. Synthesis routes of 1, 3 and 5.

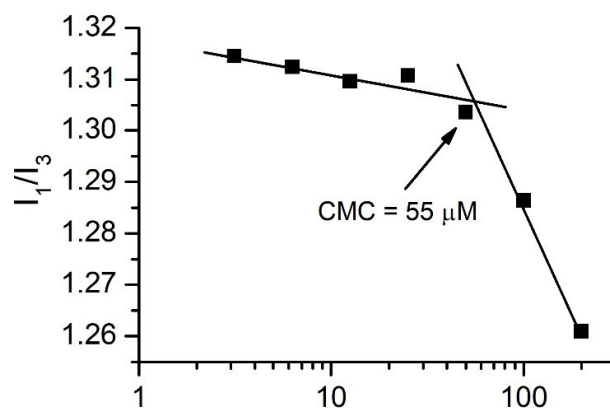


Figure S1. Critical micelle concentration (CMC) of 1.

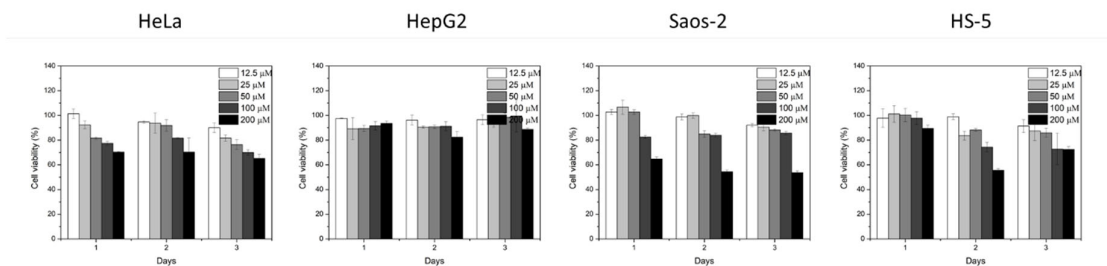


Figure S2. Cytotoxicity of 1 against HeLa, HepG2, Saos-2 and HS-5 cells.

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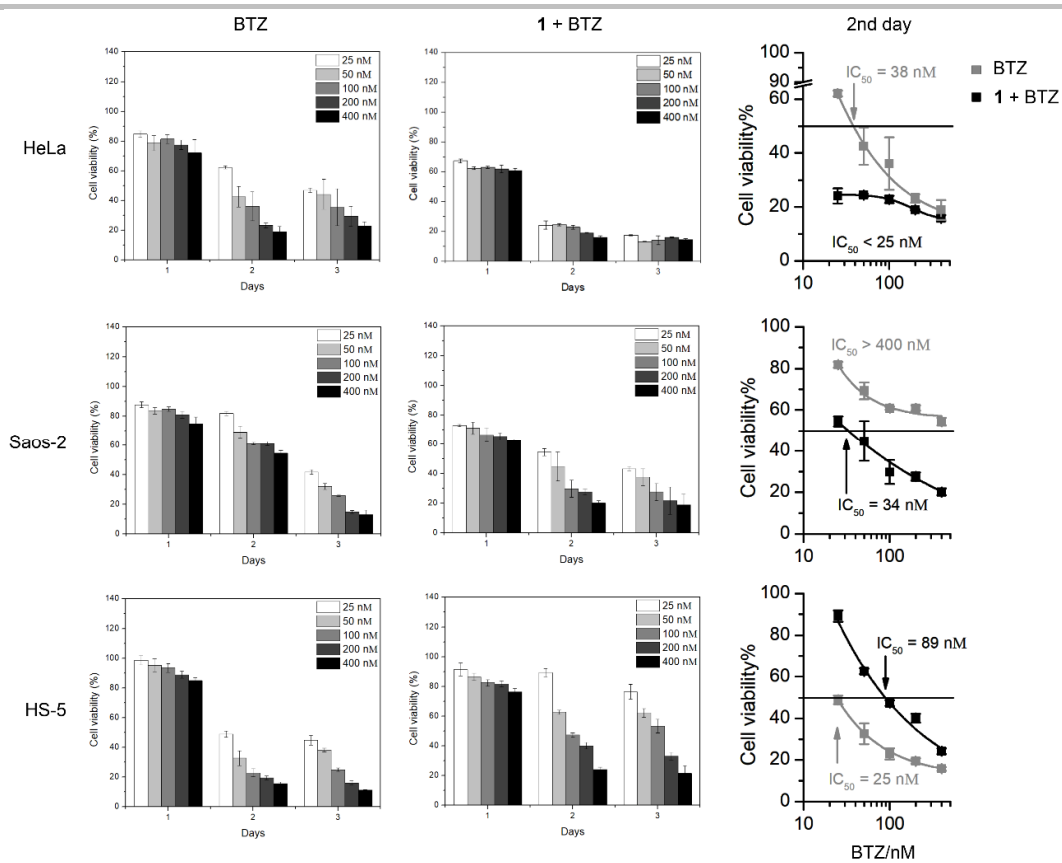
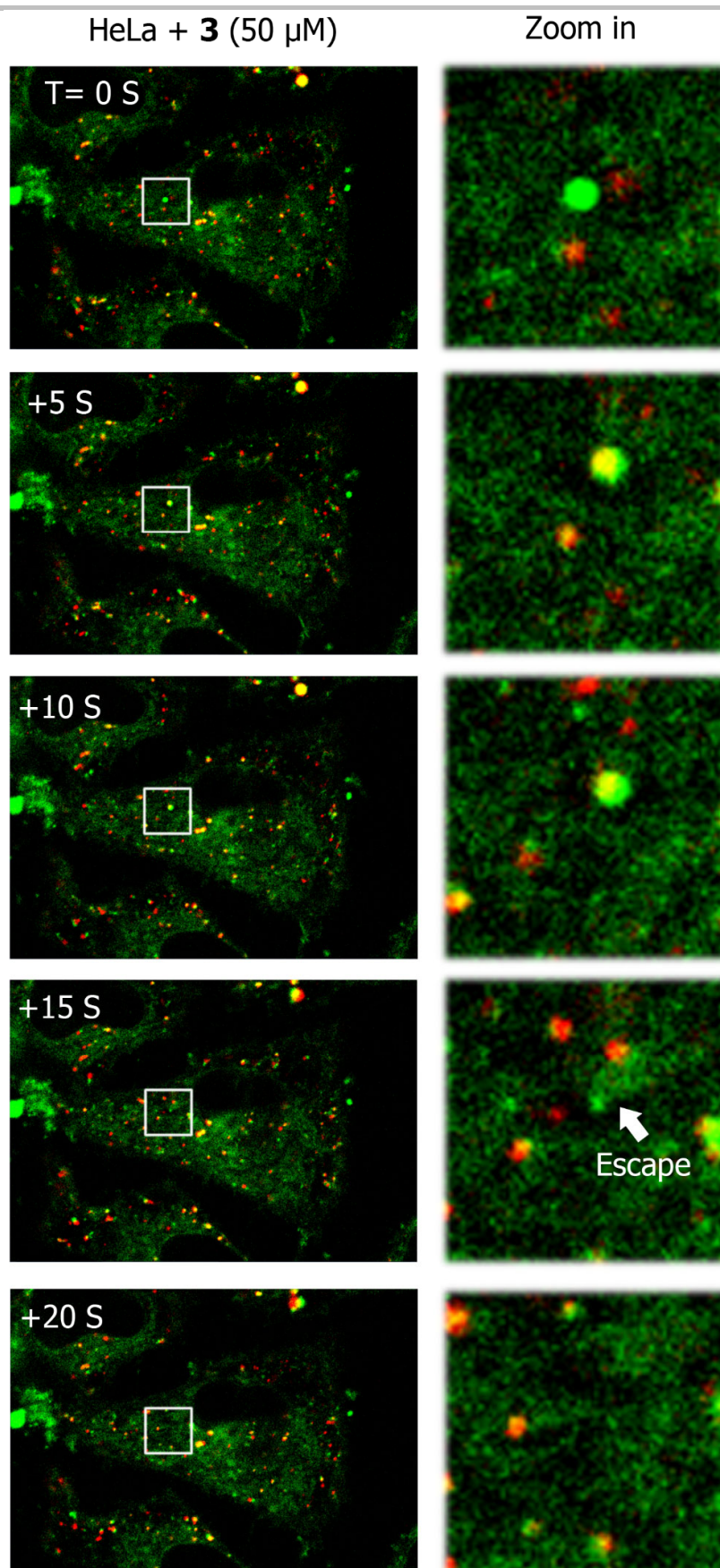


Figure S3. Cytotoxicities of free BTZ and BTZ mixed by 1 (50 μ M) against HeLa, Saos-2 and HS-5 cells.

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Figure S4. Time serial confocal fluorescence image of HeLa cells incubated with **3** (50 μ M). Green = NBD, Red = LysoTracker and Yellow = Overlap. The escape of **3** from lysosome is indicated by arrow.

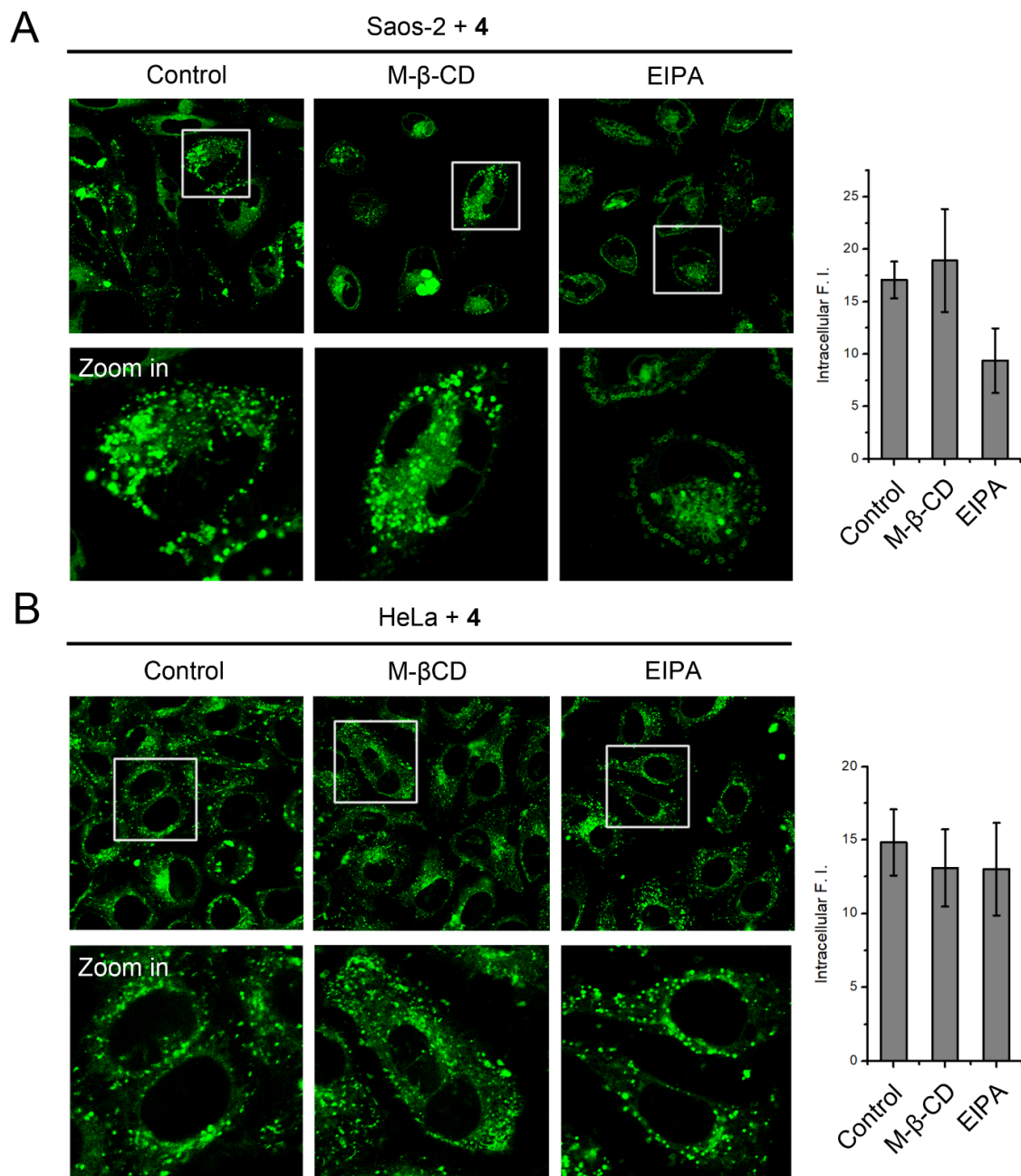


Figure S5. Fluorescent images of (A) Saos-2 and (B) HeLa cells incubated with **4** (50 μ M, 4 h) in the presence of endocytosis inhibitors. M- β -CD (5mM) is for caveolin-dependent endocytosis, EIPA (100 μ M) is for macropinocytosis.

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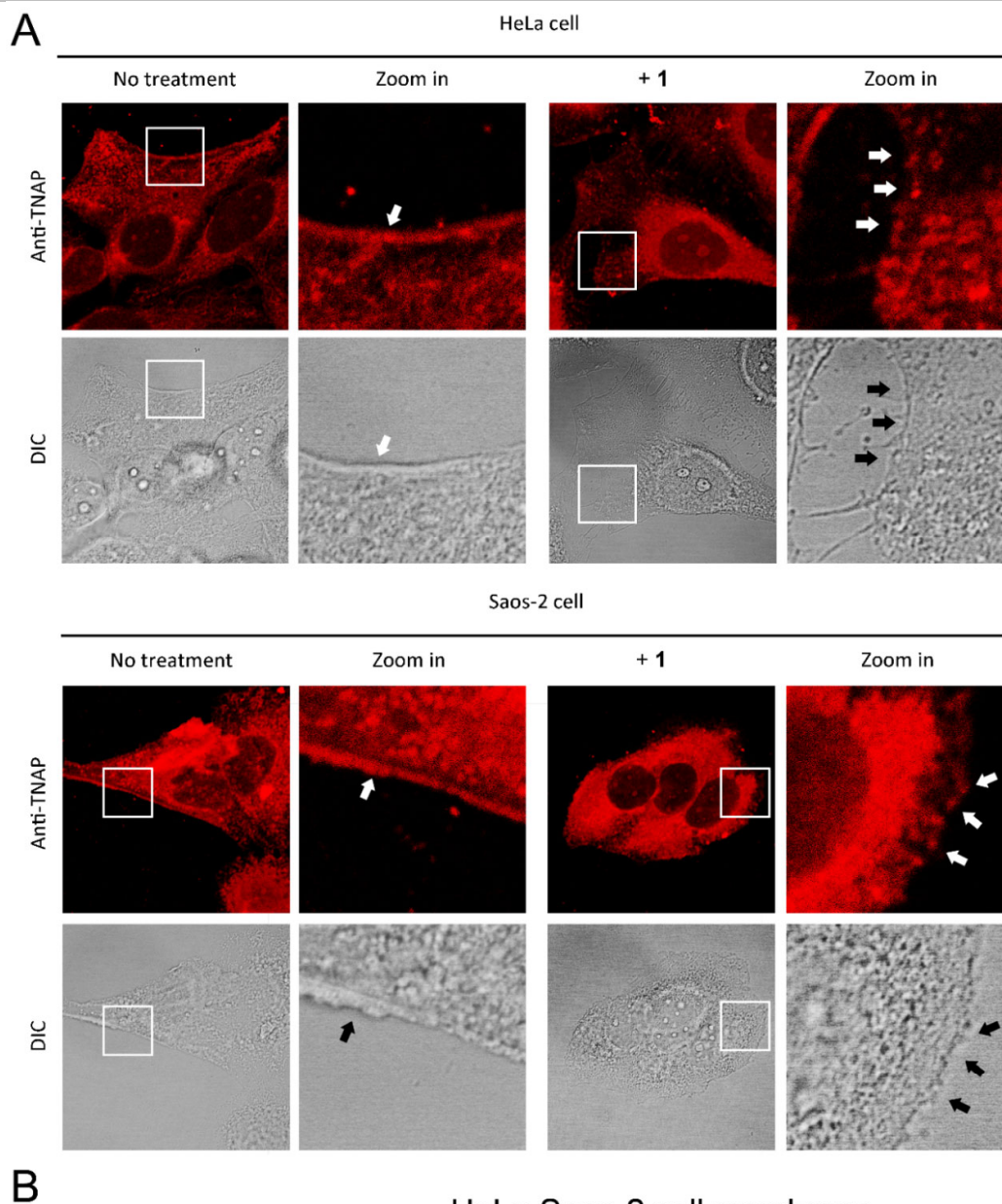


Figure S6. (A) Immunofluorescence staining of TNAP in HeLa and Saos-2 before and after the treatment of **1** (50 μ M, 3 h). The TNAP on plasma membrane was indicated by arrows. (B) Semi-quantification of TNAP density (determined by averaging the fluorescence intensity/unit area) on the plasma membrane of cells with (+)/without (-) the pretreatment of **1** (50 μ M, 3h).

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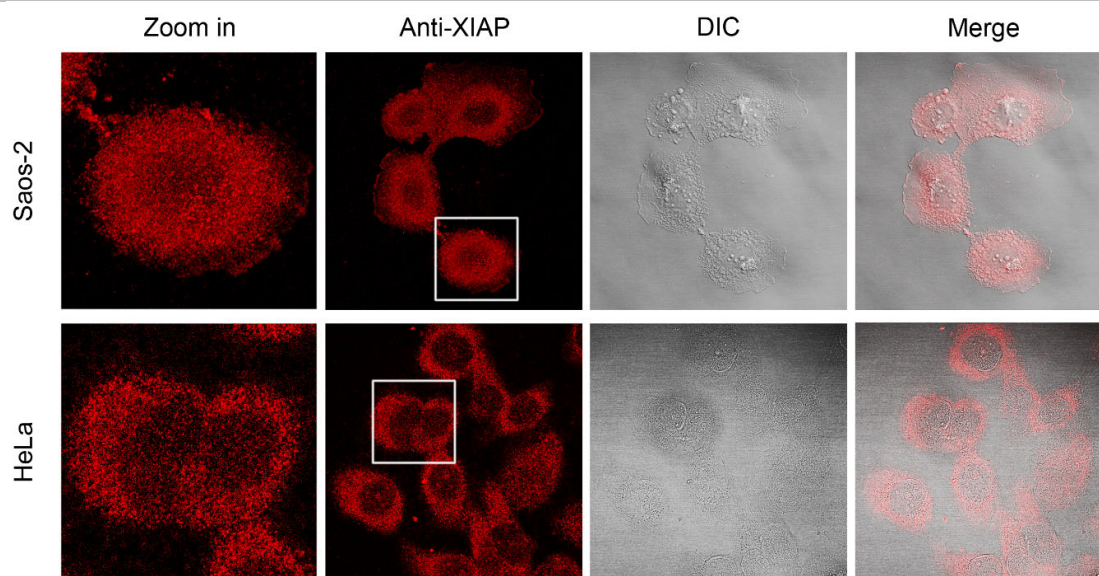


Figure S7. Immunofluorescence staining of HeLa and Saos-2 cell without the pretreatment of **3**.

Table S1. Combination index (CI) determined by CompuSyn^[5] for the combination therapy. CI<1 indicates synergism, CI>1 indicates antagonism (red). The data was collected on the 2nd day.

Drug		CI			
BTZ/nM	1/ μ M	HeLa	HepG2	Saos-2	HS-5
400	50	0.571	0.179	0.631	0.717
200	50	0.287	0.163	0.424	57.969
100	50	0.17	0.102	0.418	26.521
50	50	0.15	0.077	0.652	16.235
25	50	0.337	0.115	0.538	9.279

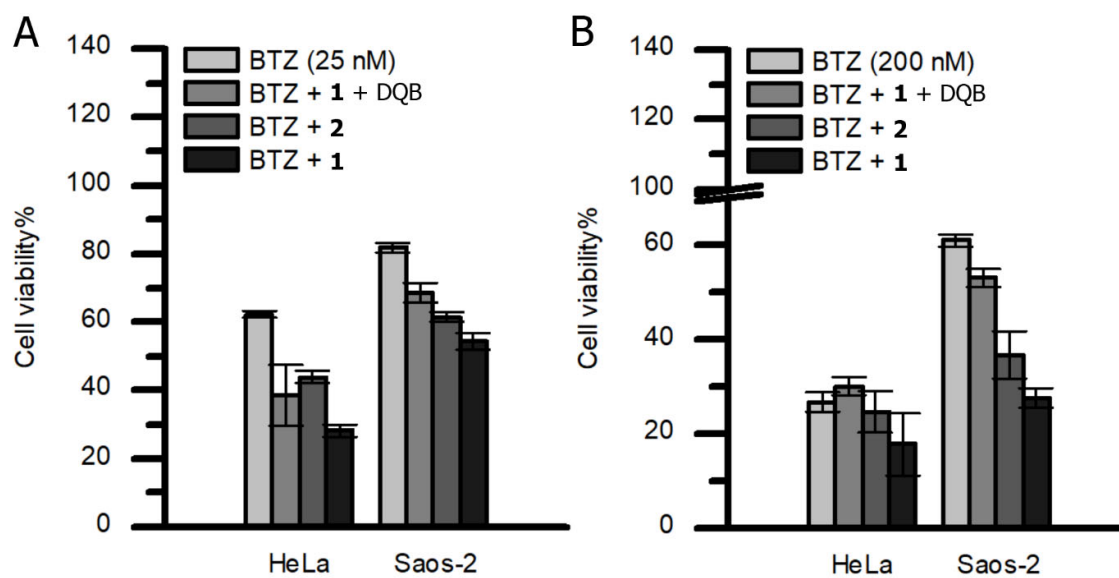


Figure S8. Cell viability of HeLa and Saos-2 cells incubated with BTZ (25 nM or 200 nM) in the presence of PBS (solvent control), **1** (50 μ M), **2** (50 μ M), and the mixture of **1** (50 μ M) and DQB (25 μ M), respectively. The data was collected on the 2nd day.

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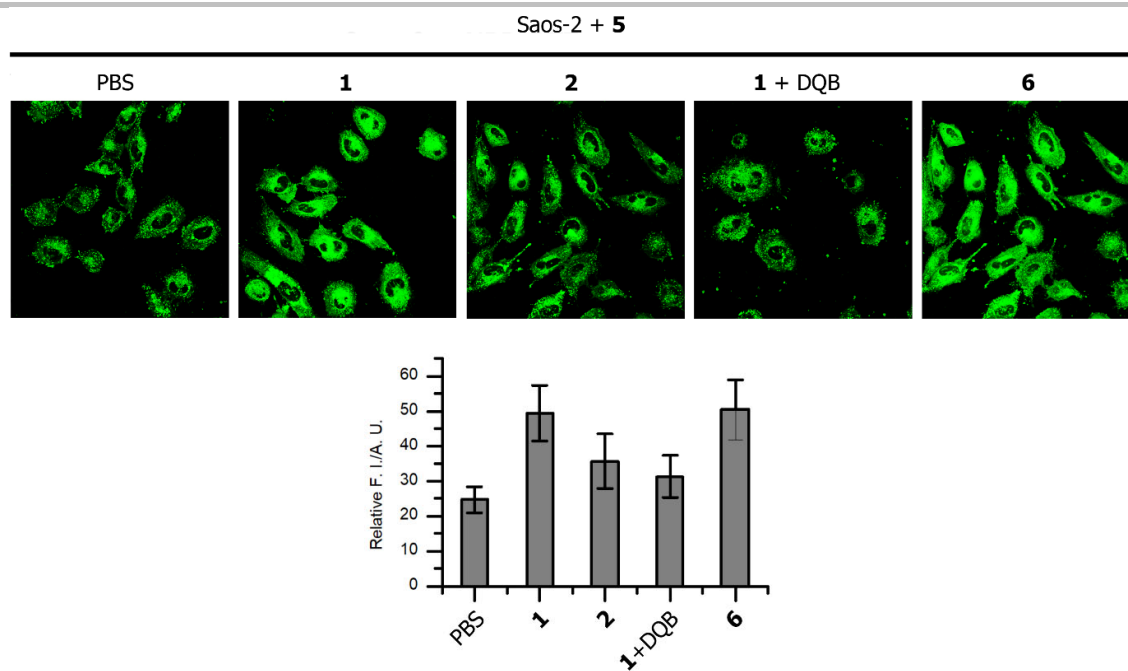


Figure S9. Fluorescence images of Saos-2 cells incubated with **5** (200 nM) in the presence of solvent control (PBS), **1** (50 μ M), **2** (50 μ M), the mixture of **1** (50 μ M) and DQB (25 μ M), and **6** (50 μ M), respectively. The incubation time is 4 h.

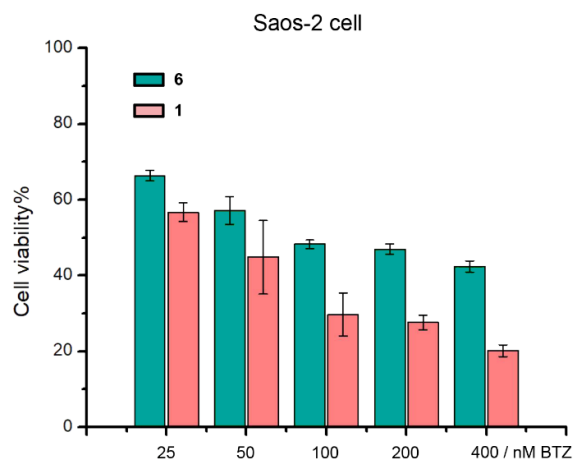


Figure S10. Cell viability of Saos-2 cells incubated with BTZ of different concentrations in the presence of **1** or **6** (50 μ M). Data was collected on the 2nd day.

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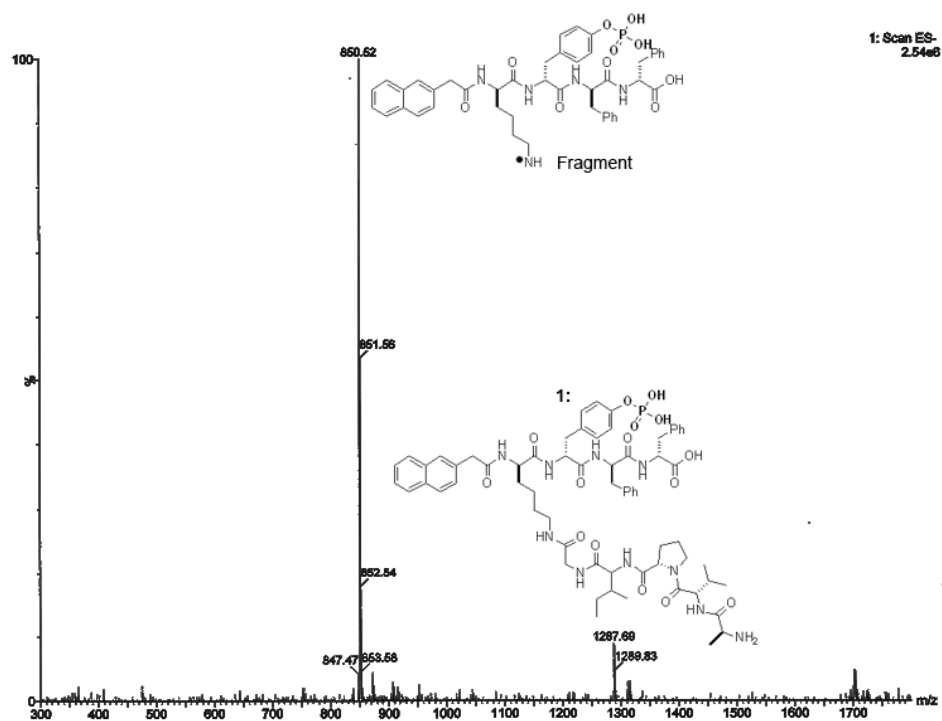


Figure S11. Mass spectrum of 1 (M/Z = 1287.88). The base peak is the fragment (M/Z = 850.52).

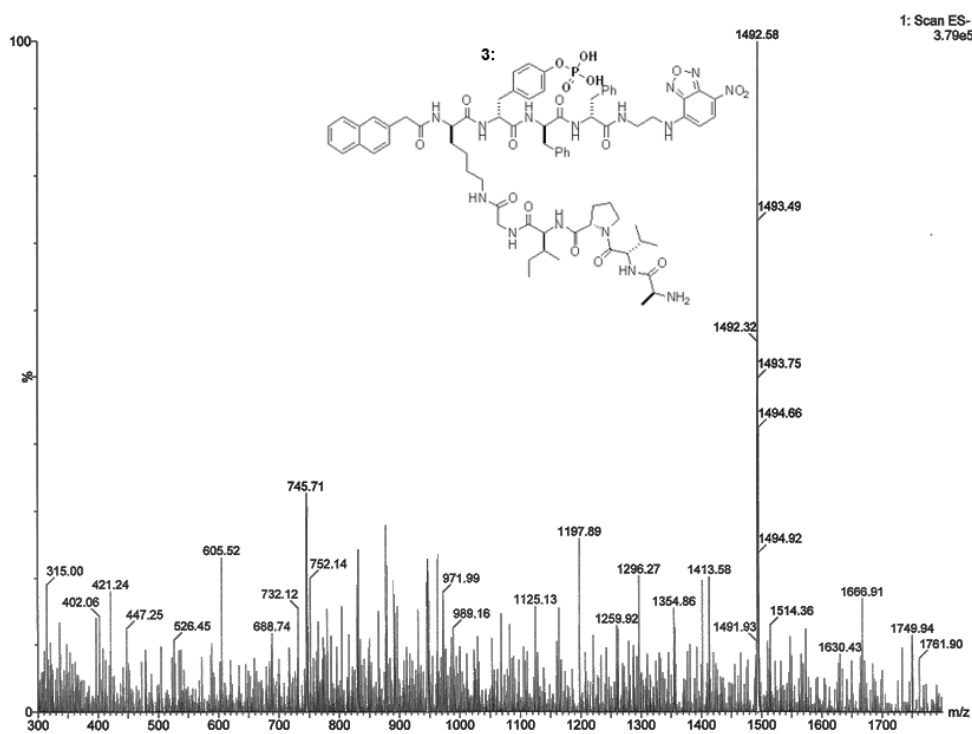


Figure S12. Mass spectrum of 3 (M/Z = 1492.58).

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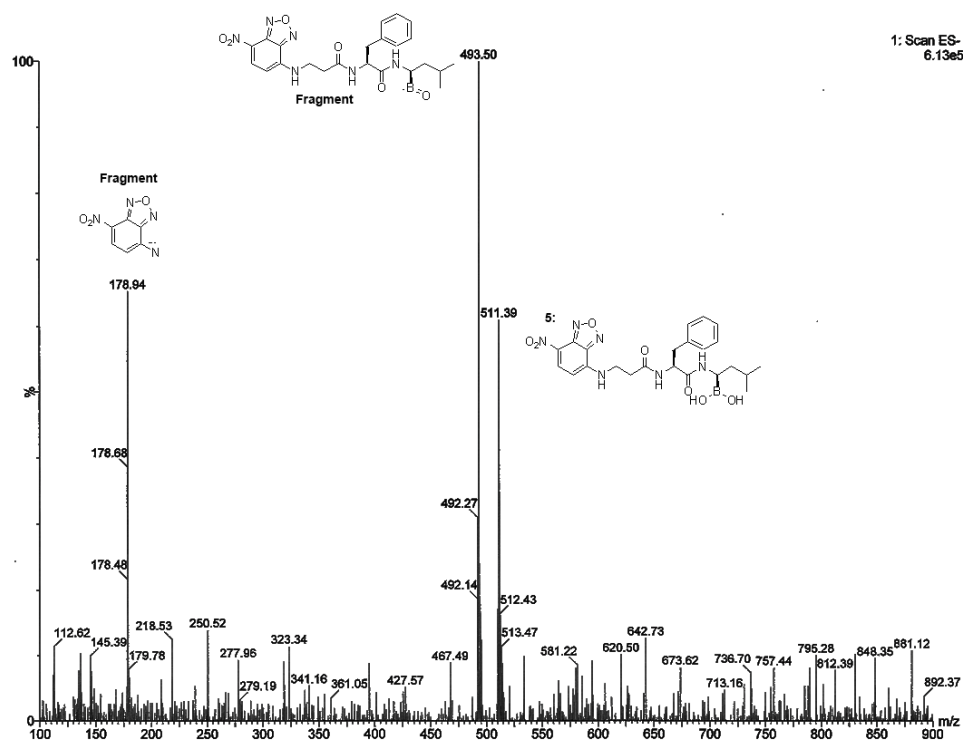


Figure S13. Mass spectrum of 5 ($M/Z = 511.39$). The base peak is the fragment ($M/Z = 493.50$, dehydration).

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- [5] a) T.-C. Chou, P. Talalay, *Adv. Enzyme Regul.* **1984**, *22*, 27-55; b) T. C. Chou, *Pharmacol. Rev.* **2006**, *58*, 621-681.

Author Contributions

H. H. designed and conducted the experiments and wrote the manuscript.
 S. L and D. W. helped with the synthesis of peptides.
 B. X. proposed and supervised the project, and co-wrote and manuscript with H. H.