Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Selectively pericellular hydrogelation by the overexpression of enzyme and membrane receptor

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Contents

S1. Synthesis and characterization

S1.1 Preparation of peptide derivatives.

All peptide derivatives were synthesized via standard phase solid peptide (SPPS) which used 2-chlorotrityl chloride resin as solid phase carrier. N-terminals of amino acids used in peptide synthesis are protected by 9-Fluorenylmethyl N-succinimidyl carbonate (Fmoc) to avoid the appearance of by-products. Further purification of the crude products of peptides obtained by SPPS were by High-performance liquid chromatography (HPLC). The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected. 20% piperidine in anhydrous N, N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using HBTU as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. In the last coupling step, NBD-Alanine was used to produce NBD-peptides. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 2 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of Triisopropylsilane (TIS) and 2.5% of H_2O for 30 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was centrifuged for 10 min at 4 0C at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation using MeOH and H_2O containing 0.05% of TFA as eluents.

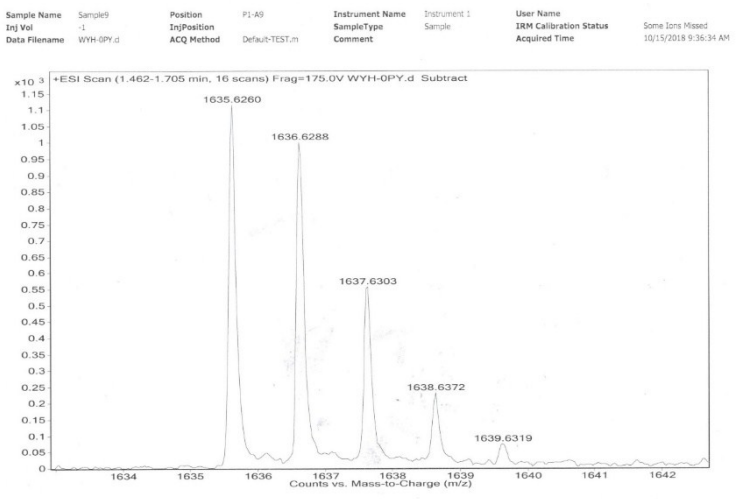

Figure S1. HR-MS spectrum of ***Comp. 1***

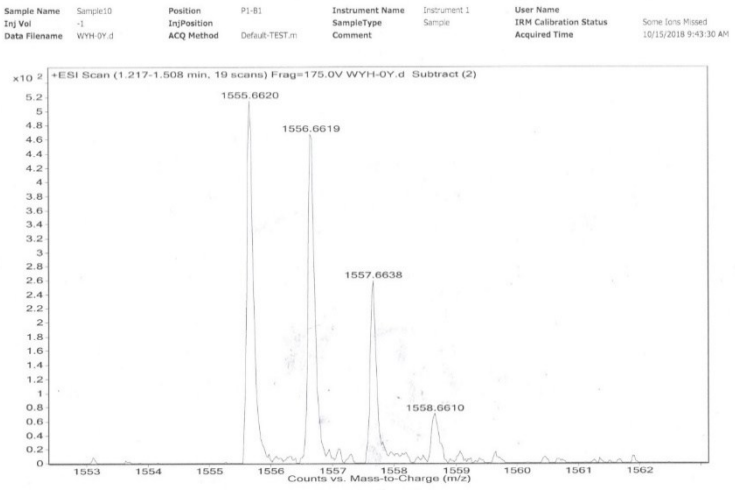

Figure S2. HR-MS spectrum of ***Comp. 2***

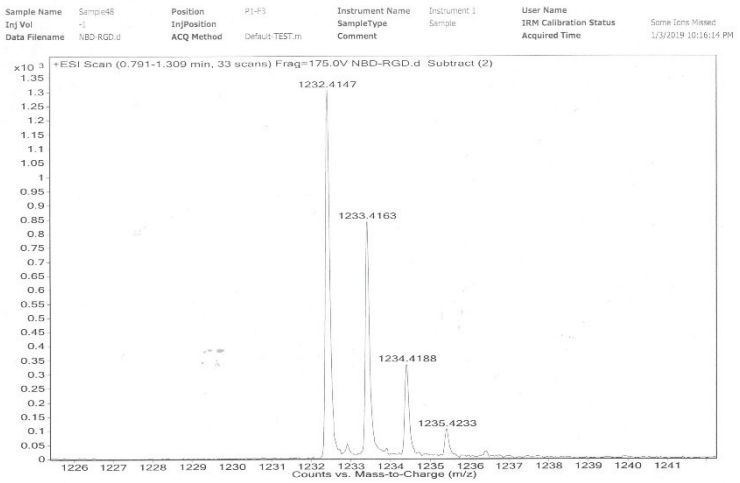

Figure S3. HR-MS spectrum of ***Comp. 3***

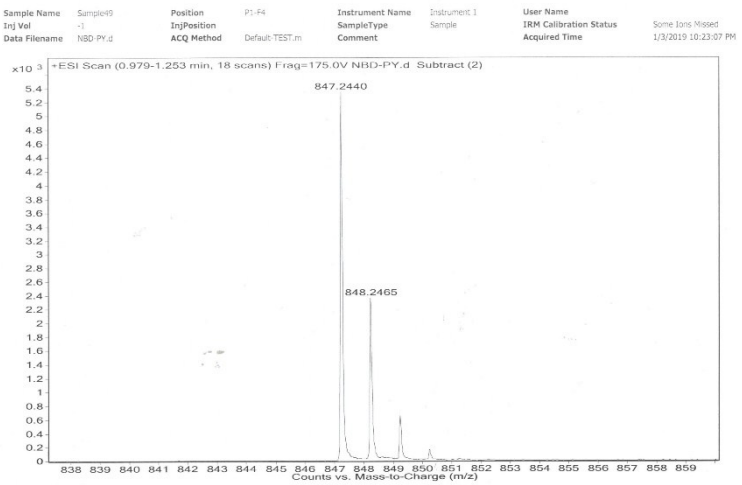

Figure S4. HR-MS spectrum of ***Comp. 4***

S1.2 Synthesis of NBD-β-Alanine.

First, 6 mmol of β-alanine (1.2 equiv.) was mixed with 10 mmol of potassium carbonate (2 equiv.), then make them dissolved completely in the mixture of water and Methanol (appropriate ratio). Next, 5mmol of NBD-Cl (1 equiv.) was sufficiently dissolved in methanol which was added into the mixture mentioned above. The reaction mixture was stirred at room temperature overnight (LC-MS detection). After LC-MS detection indicated that the reaction was almost completed, MeOH of the reaction system was removed by a rotary evaporator. Adjusted the obtained aqueous solution to pH < 3 by HCl (2 M), the brown precipitate would be seen at this time point. The precipitate was collected by filtration and dried in an oven at 70 ℃ to obtain an intermediate product called NBD-β-alanine (80 % yield).

S1.3 Conversion ratio.

The conversion ratio of ***Comp. 1*** into ***Comp. 2*** under the activity of alkaline phosphatase was detected by LC-MS at 220 nm or 254 nm. 1 mg of ***Comp. 1*** was dissolved in 1 mL of PBS, the addition of ALP (5 U/mL) initiated the conversion of ***Comp. 1***, The conversion ratio at different time points was monitored and analyzed by LC-MS.

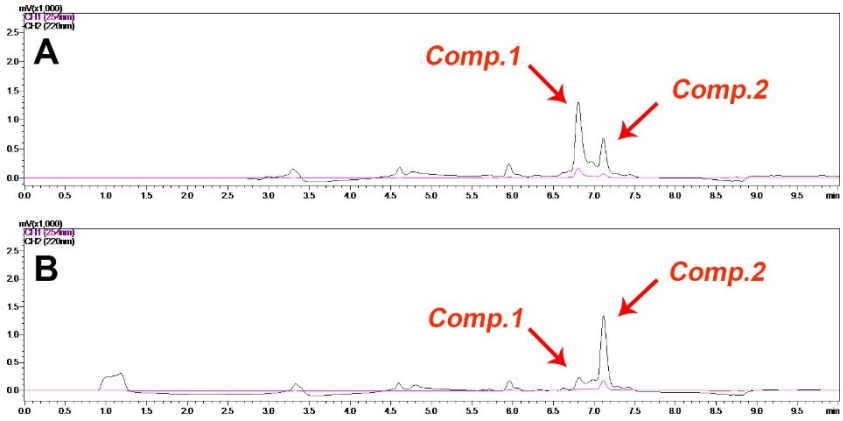

Figure S5. Representative LC-MS spectra to indicate the conversion from ***Comp. 1*** to ***Comp. 2*** by ALP A) at 10-minute and 140-minute time point.

S1.4 Fluorescence.

100 μL solution of ***Comp. 1*** at 200 μM were added in a 96 wells plate. Fluorescence spectrum was acquired on a BioTek SynergyTM 4 Hybrid Microplate Reader, the excitation wavelength was 470 nm.

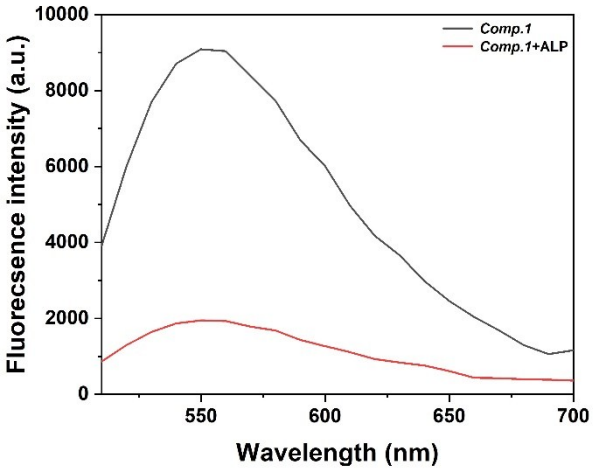

Figure S6. Fluorescence spectra of PBS solution of ***Comp. 1*** with and without addition of ALP (5 U/mL) at 200 μM.

S1.5 Circular Dichroism Spectrum.

Circular dichroism spectrum of the solution of ***Comp. 1*** with or without addition of ALP (5 U/mL) was acquired on a MOS-450 (Biologic).

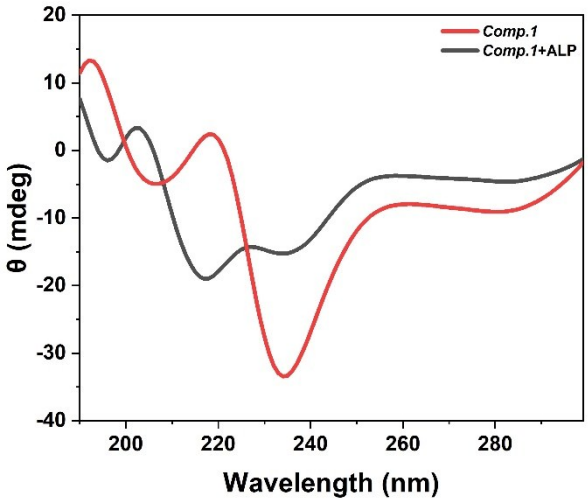

Figure S7. Circular dichroism (CD) spectra of ***Comp. 1*** (0.1 wt%) with or without ALP (5 U/mL).

S1.6 Assembly capacity of precursors and CMC value。

The CMC values of ***Comp. 1*** before and after reaction with ALP (5 U/mL) were determined by dynamic light scattering (DLS). Solutions containing different concentration of compound were tested and the light scattering intensity was recorded for each concentration analyzed. The lower CMC values represent better assembly capacity.

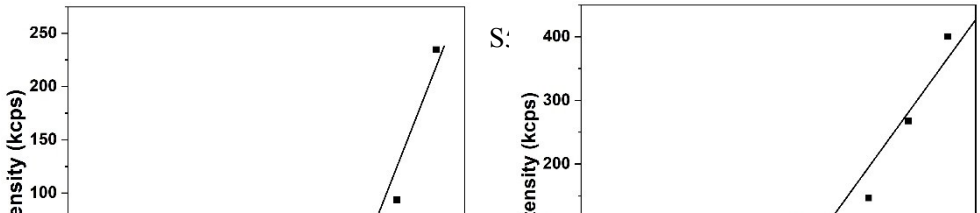

Figure S8. Critical micelle concentration values of ***Comp. 1*** before and after addition of ALP (5 U/mL).

S1.7 Transmission electron microscopy.

The samples of TEM were obtained by dropping 10 μL of a solution or a hydrogel of ***Comp. 2***, ***3***, ***4*** (0.4 wt%) on copper mesh coated with carbon, then excess liquid was removed by filter paper. Negative staining of samples with uranyl acetate is in purpose of view of clearer nanostructure. The samples were then imaged at 200 kV using a Tecnai G2 F20 system.

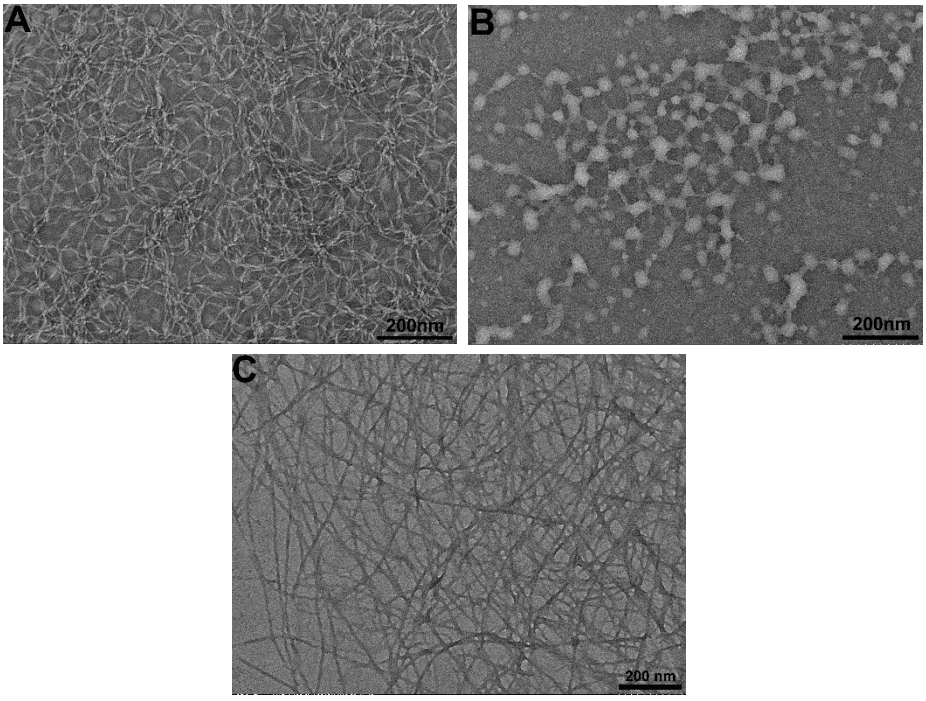

Figure S9. TEM images of (A) a gel of ***Comp. 2*** obtained from self-assembly of peptide derivatives triggered by heating-cooling process, (B) ***3*** and (C) ***4*** are formed by catalysis of alkaline phosphatase (5 U/mL). (Scale bars represent 200 nm.)

S2. Cell experiments

S2.1 Cell experiments Assays of ALP concentration outside and inside cells.

Activity of ALP outside and inside cells was determined by commercially available kit (Beyotime Biotechnology). Different cells were seeded on 96-well plate at a density of 1×10^4 cells, and incubated at 37 ℃ for about 24 h. The upper medium including extracellular ALP was collected and used to detect the activities of extracellular ALP of cells. In addition, cell suspension containing 1×10^5 cells were collected. Cells were lysed by ultrasound and centrifuged at 1.5×10^5 rad/s for 15 min. Appropriate amount of supernatant was collected for determination of activities of ALP expressed inside of cells.

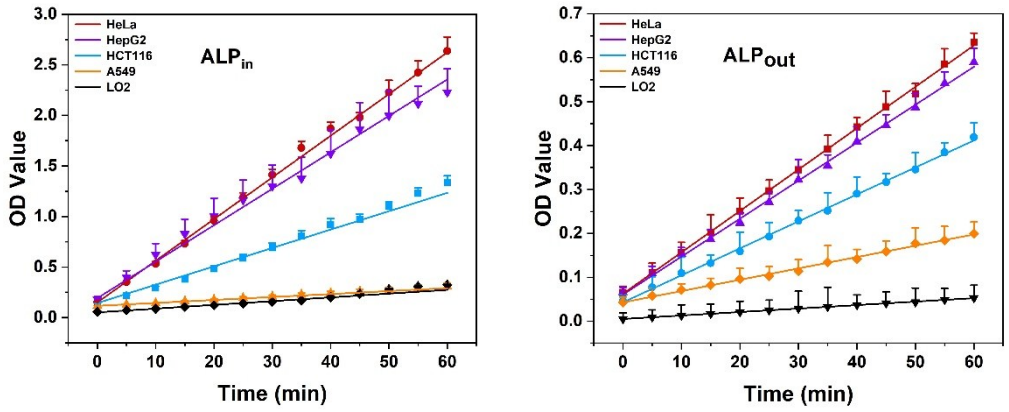

Figure S10. The activities of ALP expressed (A) inside and (B) outside the four cancer cell lines and a normal cell line.

S2.2 Cell experiments Immunofluorescent analysis of expression of CCK2R on cells.

Cells were seeded on the 24-well plate which bottom of wells covered by round coverslips at a density of 5×10^4 cells per well. After cells being attached to the surface of the round coverslip, the medium in wells was removed. Cells attached on coverslips were permeabilized with 0.2 % Triton X-100 in PBS buffer for 5 min at room temperature. Cells were wash three times by PBS buffer for a min each, and fixed with Ethanol (frozen in -20 ℃ for a while) for 10 min. Then, 5 % of goat serum in PBS was used as blocking solution to incubate cells for 1 h. Blocking solution was aspirated and 25 times diluted solution of CCK2R primary antibody in 1 % BSA of solution was added to the cells for 1.5 h at room temperature. Solution of primary antibody was removed and cells were washed three times by PBS buffer. Goat anti-Rabbit IgG (H+L) Secondary Antibody (Alexa Fluor 633) was used to bind to primary antibody specifically at concentration of 4 μg/mL in PBS buffer containing 2 % BSA for 45 min at room temperature. Cells were washed for three times with PBS buffer and then stained with 0.5 μg/mL of DAPI for 4 min at room temperature. All operations need to be protected from light. All images were taken by a laser scanning confocal microscopy (Leica TSC SP5) at the same voltage.

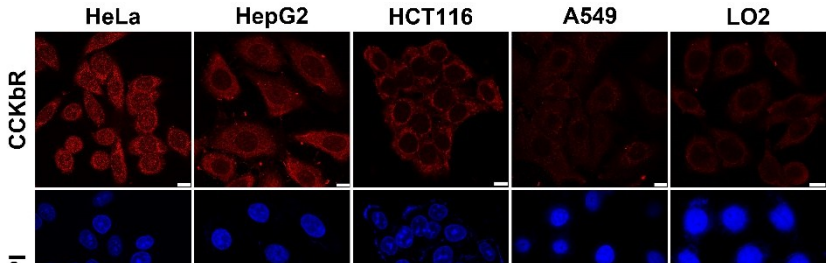

Figure S11. CLSM images of the expression level of CCK2R on four cancer cell lines and a normal cell line. (Scale bars represent 10 μm. λexc. = 633 nm for Red channel indicated CCK2R expression on cells, λexc. = 405 nm for blue channel as nucleus.)

S2.3 Optical images of cells.

Different cells were seeded on the 24-well plate at a density of 1×10^5 cells per well. After cells attached to the bottom of plate, 500 μM of ***Comp. 1-4*** were added to the cells. Medium containing compounds in the wells was removed at 24 h time point. In order to fix the morphology of cells, 4 % of paraformaldehyde solution was added to cells for 10 min. Then, optical images were captured by camera.

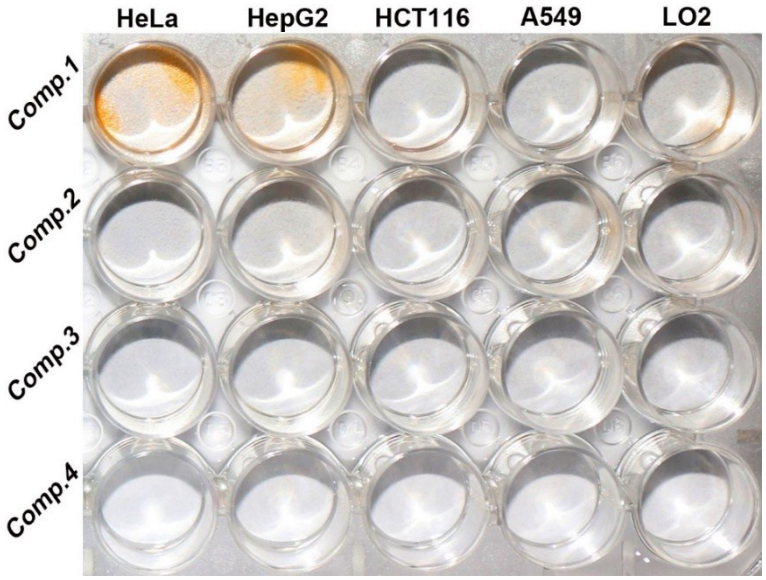

Figure S12. Optical images of four cancer cell lines and a normal cell line seeded on a cell culture plate at a density of 5×10^4 cells per well, incubated with ***Comp. 1-4*** at 500 μM for 8 h respectively.

S2.4 Cell experiments Confocal laser scanning microscopy images of cells.

Different cells were seeded on the 24-well plate with round coverslips on the bottom at a density of 4×10^4 cells per well, the cell suspension is containing quantitative cells and the Dulbecco' s modified Eagle' s medium (DMEM). After cells attached to the bottom of cell culture plate (about 24 h), the medium in the wells were removed and replaced by fresh medium containing 200 μM of compounds. The medium containing compounds was removed at different time points and cells were rinsed several times with PBS buffer. After that, the morphology of the cells was fixed by adding 4 % of paraformaldehyde solution. In purpose of clear determination of the location of cells, 0.5 μg/mL of DAPI was used to incubate cells for 4 minutes at room temperature in the dark for staining nucleus. Then, cells were washed several times by PBS buffer and sealed by fluorescent seals. All operations need to be protected from light. All images were taken by a laser scanning confocal microscopy (Leica TSC SP5) at the same voltage.

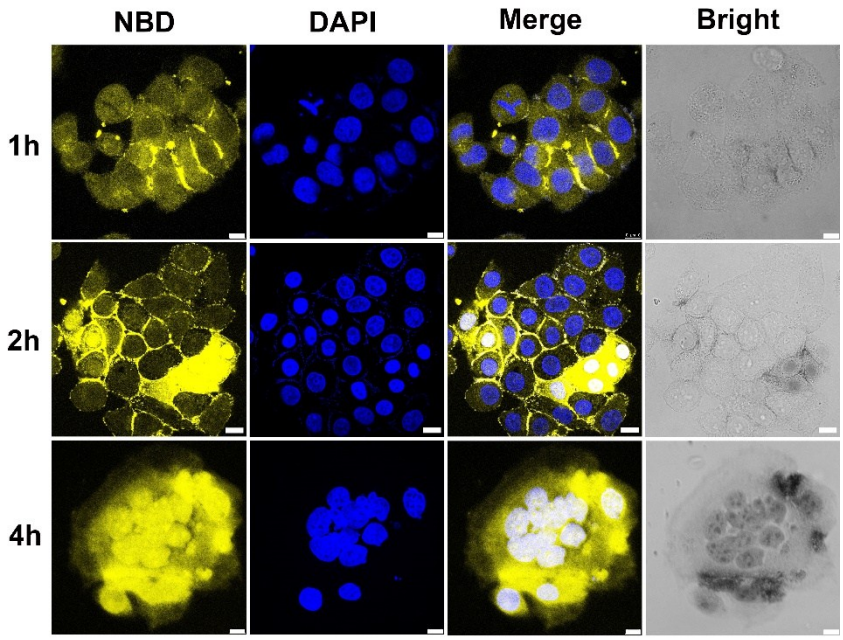

Figure S13. CLSM images of four cancer cell lines and a normal cell line incubated with ***Comp. 1*** at different time points. (Concentration of compound = 200 μM. Scale bars represent 10 μm, λexc. = 405 nm for blue channel, λexc. = 488 nm for yellow channel.)

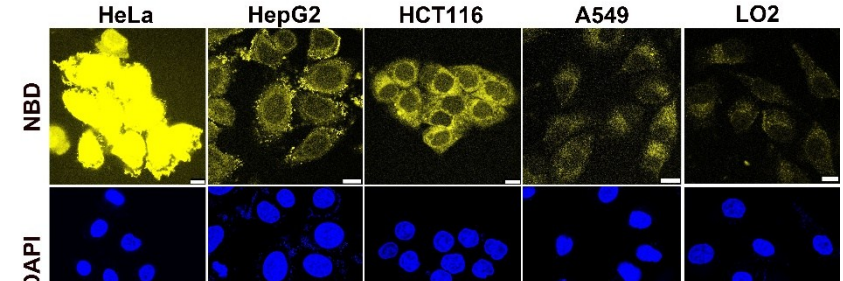

Figure S14. CLSM images of four cancer cell lines and a normal cell line incubated with ***Comp. 1*** for 8 h. (Concentration of compound = 200 μM. Scale bars represent 10 μm, λexc. = 405 nm for blue channel, λexc. = 488 nm for yellow channel.)

S2.5 Pretreatment of proglumide for blocking CCK2R.

HeLa and HepG2 cells were seeded on the 24-well plate with round coverslips on the bottom at a density of 4×10^4 cells per well respectively, the cell suspension is containing quantitative cells and the Dulbecco' s modified Eagle' s medium (DMEM). After cells attached to the bottom of cell culture plate (about 24 h), the medium in the wells was removed, and the fresh medium containing 5 mM of proglumide was added to incubate cells for 24 h. Then, the medium in wells was aspirated, ***Comp. 1*** at concentration of 200 μM was added to incubate with HeLa cells for 24 h. Fixed the morphology of cells with 4% of paraformaldehyde solution for 10 min, and washed three times with PBS buffer. Optical images and CLSM images were then captured by camera and laser scanning confocal microscopy (Leica TSC SP5), respectively.

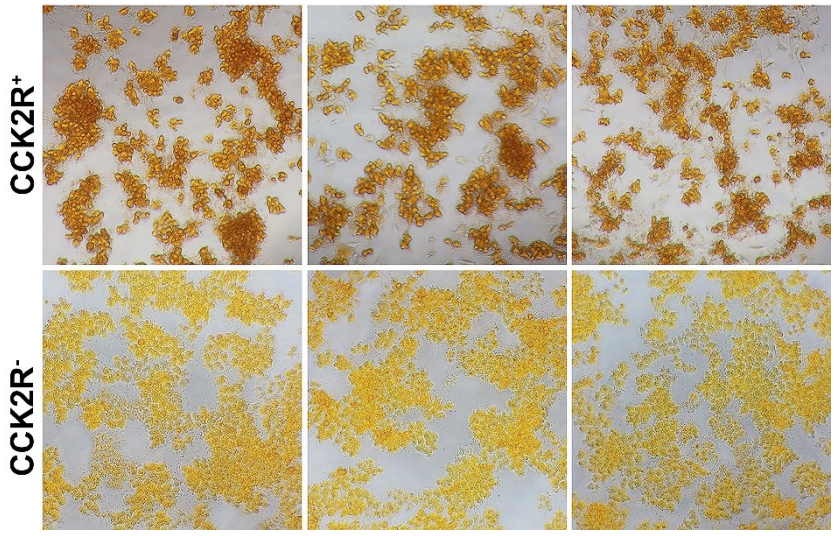

Figure S15. Optical images of HeLa cells incubated with ***Comp. 1*** for 24 h, without (control) or with pretreatment of proglumide (5 mM).

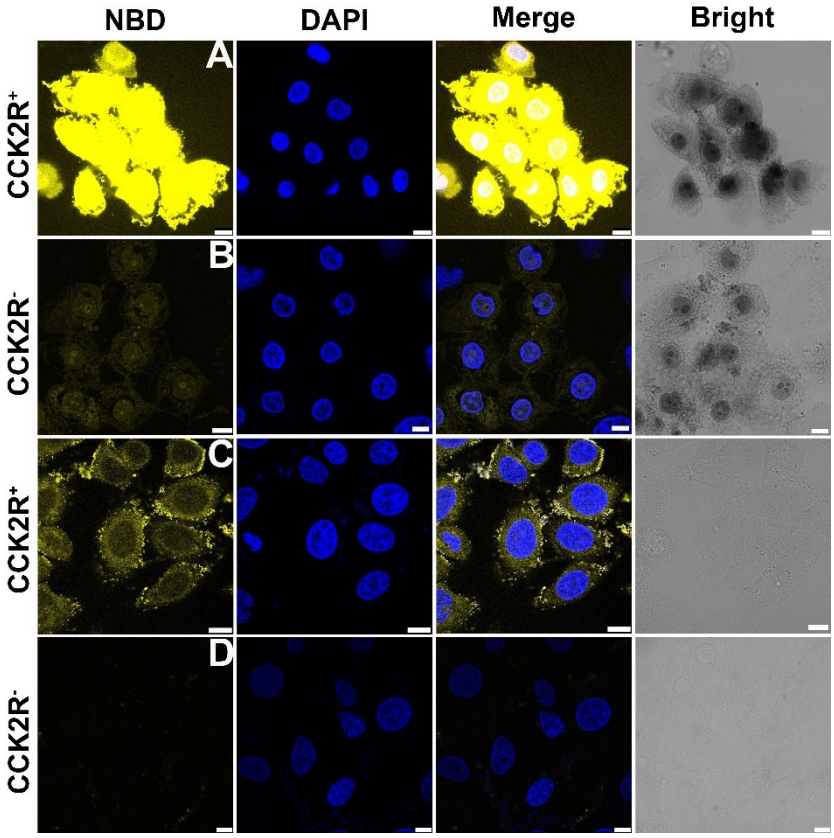

Figure S16. CLSM images of HeLa cells treated with ***Comp. 1*** for 8 h in the (A) absence (control) or (B) presence of the proglumide, and HepG2 cells incubated with ***Comp. 1*** for 8 h, (C) without or (D) with pretreatment of proglumide. (Peptide concentration is 200 μM, concentration of proglumide is 5 mM. Scale bars represent 10 μm, λexc. = 405 nm for blue channel, λexc. = 488 nm for yellow channel.)

S2.6 CLSM images of cells treated with assembly of compound.

The four cancer cell lines were incubated in cell culture dish at a density of $1x10^5$ cells per dish. After 24 h, the DMEM medium containing 200 μM of assembly of ***Comp. 1*** obtained from reaction with ALP (5 U/mL) at 37 ℃ for 4 h was added to the cells. The DMEM solution was removed at 8 h time point, and cells were washed for three times with PBS. Cells were then stained with 0.5 μg/mL of DAPI for 4 minutes at 37 ℃ in dark. After that, the cells were rinsed three times by PBS buffer, and kept in the live cell imaging solution for imaging. We recorded the images by a laser scanning confocal microscopy.

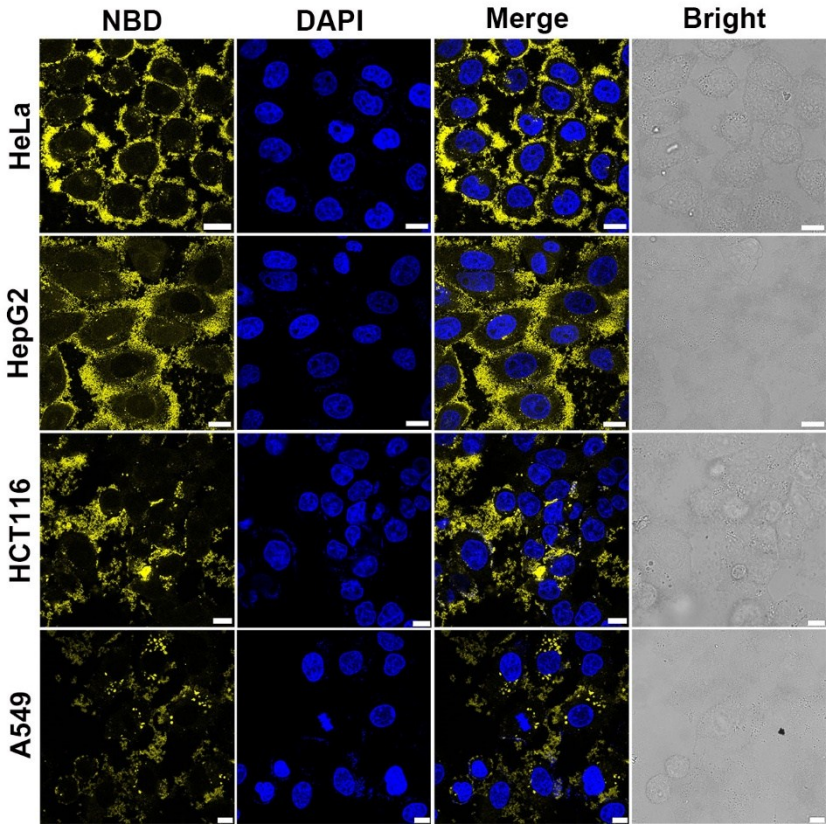

Figure S17. CLSM images of four cancer cell lines incubated with assembly formed from precursor molecule of ***Comp. 1*** which reacted with ALP (5 mg/mL) at 37 ℃ overnight. (Scale bars represent 10 μm, peptide concentration is 200 μM, λexc. = 405 nm for blue channel, λexc. = 488 nm for yellow channel.)

S2.7 Cell Inhibition Analysis.

Five cell lines we studied were seeded in 96-well plates at 1×10^4 cells per well for 24 h followed by culture medium removal and subsequently addition of culture medium containing our compounds at different concentrations. At designated time 48 hours, 10 μL of MTT solution (5 mg/mL) was added to wells and incubated at 37 °C for 4 h, and then the optical density of the solution was measured at 405 nm using a microplate reader (Bio-RAD iMarkTM, America).

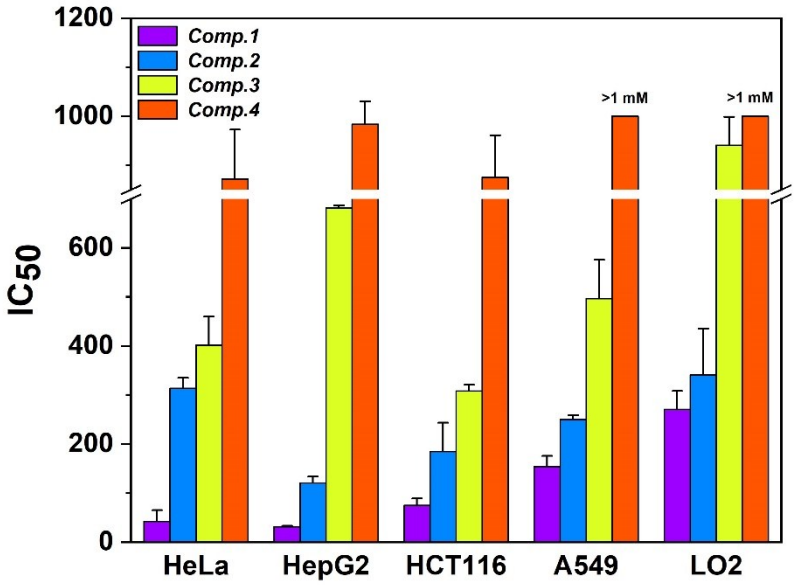

Figure S18. The IC_{50} values of ***Comp. 1-4*** against di□erent cancer cells and a normal cell line. The data shown are mean ± SD (n = 3).