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S1. Experimental Procedures

S1.1 Materials

All of Fmoc-protected amino acid and O-benzotrazole-N,N,N',N'-tetramethyluronium-hexafluorophosphate-hexafluorophosphate (HBTU) were purchased from Apexbio. Rink amide MBHA resin, pyrene, and β-alanine were purchased from Sigma Aldrich. N,N-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA) were purchased from TCI. N,N-diisopropylethylamine (DIPEA), triethyl amine, trifluoroacetic acid (TFA), acetazolamide, succinic anhydride, triisopropylsilane, 1,6-dibromohexane, and triphenylphosphine, 4-chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl), and 1-pyrenebutyric acid were purchased from TCI. All of the solvents were purchased from SAMCHUN chemicals. Acridine orange staining solution (ab270791) was purchased from abcam. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from ACROS Organics. For cell culture, Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Gibco by Life technologies.

S1.2 Instruments

All of synthesized peptide were purified by Agilent 1100 HPLC system and 1220 HPLC system with Eclipse XDB-C18 column. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF, Ultraflex III, Bruker) was utilized to obtain the mass of peptides. UV-vis absorbance was measured from V-670 by Jasco. Circular dichroism (CD) spectrum was measured from J-815 by Jasco. Fluorescence was measured from F-7000 by Hitachi. Zeta potential was measured from ZEN56000 by Malvern. Western blot image was obtained from Universal hood III by Bio-Rad. JEM-1400 by JEOL and morganini 268 was used to obtain transmission electron microscopy (TEM) image. SU7000 by Hitachi was utilized to obtain scanning electron microscopy (SEM) image. LSM 780 and 880 by ZIESS was utilized to obtain confocal laser scanning microscopy (CLSM) image. MTT assay were performed from SpectraMax M5e Multi Mode Microplate Reader by Molecular Devices.

S1.3 Synthesis for 1-hexyl triphenylphosphonium bromide salt

1-hexyl triphenylphosphonium bromide salt was synthesized by following procedure. Briefly, triphenylphosphine (6.45 g, 24.59 mmol) and 1,6-dibromohexane (18.9 ml, 122.9 mmol) were mixed in a round-bottom flask. After that, the solution was heated at 90°C and stirred for 6 h. Then, the solution was cooled to RT, and two layer was obtained. The remaining 1,6-dibromohexane was decanted off, and the monophosphonium salt was dissolved in dichloromethane. After that, the solution was precipitated with diethyl ether. The paste-like solid was dried and then purified with column chromatography on a silica gel. The product was obtained as a white solid. Yield: 11.5 g (92%). 1H NMR (400 MHz, CDCl3): δ 7.94-7.62 (m, 15H), δ 4.02-3.86 (m, 2H), δ 3.38 (m, 2H), δ 1.87-1.58 (m, 6H), δ 1.49-1.38 (m, 2H)

S1.4 Synthesis for 5-amino-1,3,4-thiadiazole-2-sulfonamide (Az-NH₂)

Hydrochloric acid (2N, 50 ml) was added to a solution of N-(6-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide (5.0 g, 22.5 mmol) in 75 ml methanol. After that, the solution was refluxed for 18 h and then solvent was evaporated. The residue was purified with column chromatography on a silica gel. The product was obtained as a white solid. Yield: 3.27 g (80%). 1H NMR (400 MHz, DMSO-d6): δ 8.05 (s, 2H), δ 7.80 (s, 2H)

S1.5 Synthesis for 4-oxo-4-((5-sulfamoyl-1,3,4-thiadiazol-2-yl)amino)butanoic acid (Az-COOH)

Succinic anhydride (0.22 g, 2.22 mmol) was added to a solution of Az-NH₂ (0.40 g, 2.22 mmol) in N,N-dimethylformamide. After that, the solution was heated at 100°C for 12 h. Then, the solvent was evaporated and the product was utilized in next step without further purification. Yield: 0.60 g (96%). 1H NMR (400 MHz, DMSO-d6): δ 8.29 (s, 2H), δ 2.72 (t, 2H), δ 2.56 (t, 2H)
S1.6 CAC measurement

Critical aggregation concentration (CAC) was measured by steady-state fluorescence pyrene emission method. Different concentrations of peptide solution were prepared in PBS (10 mM, pH 7.4) or sodium acetate buffer (10mM, pH 4.5). 1 µL of pyrene solution in ethanol (2 mM) was added into 999 µL of peptide solutions. The peptide solution containing 2 µM of pyrene was recorded using fluorescence spectroscopy. Excitation wavelength was set to 331.0 nm. The slit widths for excitation and emission were set to 2.5 nm and scan speed was set to 240 nm/min. The ratio between I1 band and I3 band for pyrene was plotted to obtain CAC.

S1.7 Cell culture

HeLa, NIH/3T3, and HEK293T cell line were cultured in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. MDA-MB-468 and 4T1 were cultured in Rosewell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. All cell lines were incubated at 37 °C in a humidified atmosphere of 5% CO₂.

S1.8 Western blot analysis

HeLa and NIH/3T3 cell line were seeded in 6-well plate with the density of 300,000 per well. After 24 h growing, medium were removed and cell line was washed with 1X PBS. Cell line was harvested into microtube using Trypsin-EDTA (0.25%). The harvested cell line was washed with 1X PBS and resuspended into 100 µL of RIPA buffer containing protease inhibitor cocktail at ice for 45 min. Cell lysis supernatant was collected by spin-down with 13,000 rpm for 20 min and total protein concentration was assayed using Bradford reagents. Protein was separated by SDS-PAGE and was transferred into a polyvinylidene difluoride (PVDF) membrane. PVDF membrane was blocked by 5% skim milk solution in TBS-T buffer for 1 h. After blocking step, a primary antibody was incubated at 4°C during overnight. After 3 times wash with TBS-T buffer for 15 min, a secondary antibody labeled with horseradish peroxidase (HRP) was incubated at RT for 1 h. After 3 times wash with TBS-T buffer for 15 min, protein was visualized by BCIP®/NBT solution and observed by ChemiDoc Imaging Systems.

S1.9 Determination of the intracellular concentration of NBD-AT

HeLa and NIH/3T3 cell line were seeded in a 24 well plate (Thermo Fisher Scientific Inc.) with the density of 50,000 per well. After 24 h growing, 20 µM of NBD-AT was incubated with fresh cell culture medium. After 4 h, the medium was removed and the cells were washed by 1X PBS three times, and re-collected as a pellet. Cell pellet was incubated with 200 µL of RIPA cell lysis buffer upon the complete lysis of the cells, and 200 µL of MeOH was added for the clarity of the spectra. The fluorescence of solution was measured using fluorescence spectroscopy.

S1.10 Co-assembly study

HeLa cell line was seeded in 8-well Lab Tek II slide chamber with the density of 10,000 per well. After 24 h growing, HeLa cells was co-incubated with 20 µM of Pep-AT and 10 µM of NBD-AT for 30 min. For negative control group, 10 µM of NBD-AT alone was treated into the cells. Then, the cells were washed with PBS and analyzed by LSM 780 and 880 by ZIESS.
S1.11 Cell death mechanism study

HeLa was seeded in 8-well Lab Tek II slide chamber with the density of 10,000 per well. After 24 h growing, 20 µM of peptide solution diluted in cell culture medium were incubated for 24 h. After that, the medium were removed and the cells was incubated with Alexa Fluor 488 conjugated annexin V and PI working solution (Life Technologies, V13241) at RT for 20 min. Without further washing step, CLSM image directly obtained by LSM 780 and 880 by ZIESS.

For flow cytometry analysis, HeLa was seeded in 24-well plate (Thermo Fisher Scientific Inc.) with the density of 50,000 per well. After growing for 24 h, 20 µM of peptide solution diluted in cell culture medium were incubated for 24 h. Then, HeLa was harvest using Trypsin-EDTA, as followed by washing with 1X PBS. After that, HeLa were stained with Alexa Fluor 488 conjugated annexin V and PI working solution (Life Technologies, V13241) for 15 min at RT. Following the annexin-binding buffer was added into the sample, the fluorescence was measured using flow cytometry (FACScalibur, BD Biosciences, UA) with absorbance emission at 530 nm (i.e., FL1) and >575 nm (i.e., FL3).
S2. Supporting Figures

Scheme S1. Schematic representation for the peptide synthesis of Pep-AT, Pep-T, Pep-A, and Pep.

Figure S1. 1H-NMR spectrum of TPP-C6-Br in CDCl3.
Figure S2. $^1$H-NMR spectrum of Az-NH$_2$ in DMSO-d$_6$.

Figure S3. $^1$H-NMR spectrum of Az-COOH in DMSO-d$_6$. 
Figure S4. $^1$H-NMR spectrum of Pep-AT in DMSO-d$_6$

Figure S5. $^1$H-NMR spectrum of Pep-T in DMSO-d$_6$
Figure S6. $^1$H-NMR spectrum of Pep-A in DMSO-d$_6$

Figure S7. $^1$H-NMR spectrum of Pep in DMSO-d$_6$
Figure S8. Mass spectroscopy for Pep-AT, Pep-T, Pep-A, and Pep. All of peptides are purified by HPLC and characterized by MALDI-TOF/TOF.
Figure S9. The purity of synthesized peptides was analysed by HPLC. All of peptides showed above 95% purity.

Figure S10. $^1$H-NMR spectrum of NBD-AT in DMSO-d$_6$
Figure S11. Mass spectroscopy for NBD-AT. NBD-AT is purified by HPLC and characterized by MALDI-TOF/TOF.

Figure S12. Self-assembly behavior study in pH 7.4. TEM image shows the nanofiber formation for 100 µM solution (PBS, 10 mM, pH 7.4) of Pep-A, Pep-T, and Pep (scale bar = 100 nm).
**Figure S13.** CAC determination for Pep-AT, Pep-T, Pep-A, and Pep by using pyrene emission method in PBS (10mM, pH 7.4). Fluorescence ratio of $I_3/I_1$ of pyrene was plotted, and arrows indicate CAC value.

**Figure S14.** CAC determination of Pep-AT by using pyrene emission method at pH 4.5 (sodium acetate buffer, 10 mM). Fluorescence ratio of $I_3/I_1$ of pyrene was plotted, and arrows indicate CAC value. The CAC at pH 4.5 was determined to be 12 µM. It is lower value than that of pH 7.4 (28 µM), suggesting the self-assembly propensity is changed depending on pH.
**Figure S15.** CD spectroscopy for Pep-AT in 10 µM at pH 7.4 and pH 4.5.

**Figure S16.** Quantification of CAIX expression level analyzed by western blot in HeLa and NIH/3T3.

**Figure S17.** A histogram shows the peak overlap of blue fluorescence and red fluorescence only in HeLa (black arrow).
Figure S18. Co-localization study of Pep-AT with Lysotracker Red and Mitotracker Green after 20 µM treatment of Pep-AT for 4 h and 24 h in HeLa, and its magnified image. White arrows indicate the co-localization between Pep-AT and Lysotracker Red. Pep-AT still existed in lysosome even in 24 h before entering the cell death pathway.

Figure S19. Fluorescence intensity of cell lysate after 20 µM of NBD-AT treatment toward HeLa and NIH/3T3. Data are presented as mean ±SD (n=3).
Figure S20. CLSM image showing the green fluorescence of NBD-AT, and NBD-AT after co-assembly with Pep-AT to investigate self-assembly structure in lysosome. Cellular localization was further confirmed by co-localization with Lysotracker Red (scale bar = 20 µm).

Figure S21. a) Cell viability of Pep-T for 24 h was measured by using MTT assay toward HeLa and NIH/3T3 at normoxia condition. Data are presented as mean ±SD (n=3). b) The graph indicating the selectivity index (IC\(_{50}\) of normal cells/IC\(_{50}\) of cancer cells) of Pep-AT and Pep-T.

Figure S22. Cell viability of a) Pep-A and b) Pep for 24 h was measured by using MTT assay toward HeLa (black line) and NIH/3T3 (red line) at normoxia condition. Data are presented as mean ±SD (n=3).
Figure S23. Cell viability of Pep-AT for 24 h incubation was measured by MTT assay toward HeLa and acetazolamide pre-treated HeLa. Data are presented as mean ±SD (n=3).

Figure S24. Cell viability assay of Pep-AT after 24 h incubation toward HeLa and NIH/3T3 at hypoxia condition. Data are presented as mean ±SD (n=3).

Figure S25. Cell viability of Pep-AT for 24 h was measured by using MTT assay toward a) 4T1, b) MDA-MB-468, and c) HEK293T at normoxia condition. Data are presented as mean ±SD (n=3).
Figure S26. a) CLSM image for Pep-AT-treated-HeLa (20 µM, 24 h) and control HeLa after labelling with PI and Annexin V-FITC to reveal cell death mechanism (scale bar = 20 µm). b) Flow cytometry analysis induced by 20 µM of Pep-AT to investigate the cell death mechanism. c) Cell viability assay of Pep-AT after 24 h incubation toward HeLa and Z-VAD-FMK pre-treated HeLa. Data are presented as mean ±SD (n=3).
S3. Reference

[1] Hill, W. E. Islam, M. Q. Webb T. R. Mcauliffe, C. A. Solution studies of gold(I) complexes of n-hexyldimethylphosphine, n-butyldiphenylphosphine, 1-dimethylphosphino-6-diphenylphosphinohexane, 1,6-bis(dimethylphosphino)hexane and 1,6-bis(diphenylphosphino)hexane. *Inorg. Chim. Acta*, 1989, 157, 215-222