Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Hydrogen Sulfide Induced Supramolecular Self-Assembly in Living Cells

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1. General methods

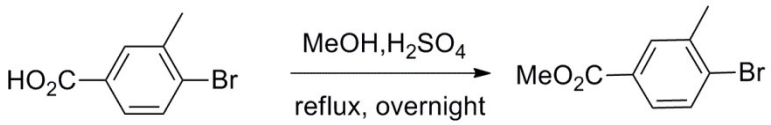
All chemicals were purchased from Sigma-Aldrich and used without further purification unless otherwise stated. NMR spectra were recorded on a Bruker 400 MHz Fourier transform spectrometer. For TEM samples preparation, 10 μL of the gel were deposited onto a carbon-coated copper grid for 10 min, and the excess liquid was removed with filter paper. Uranyl acetate (5 % w/v) solution was added for negative staining. TEM images were obtained on a JEM 2100 transmission electron microscope (JEOL ltd, Japan). For the rheological measurements, the dynamic strain sweep and dynamic frequency sweep experiments were carried on a strain-controlled rheometer (Anton Paar MCR302) using parallel-plate geometry with a 0.50 mm gap. The gel (1.0 wt %) was deposited on the sample cell holder carefully. The experiments were performed at 25°C. High Resolution Mass Spectrum (HRMS) were recorded on a Thermo Fisher Scientific mass spectrometer (Exactive). The confocal images were confirmed by confocal microscope (Zeiss 710). The UV spectra were obtained on a Shimadzu UV-2600. The fluorescence spectra were recorded on a F98 fluorometer.

2. Synthesis methods

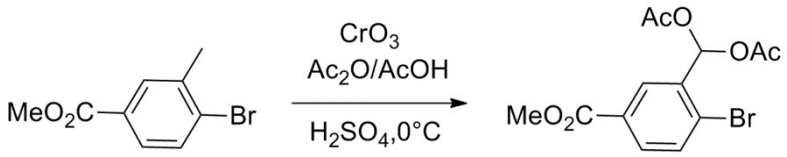
2.1 Synthesis of the peptide

All the peptides were prepared by solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (100~200 mesh, ~1.0 mmol/g) and Fmoc-protected amino acids. The resin was swelled in dry dichloromethane (DCM) for 5 minutes, then the first amino acid was loaded onto resin at its C-terminal with Fmoc-protected amino acid (1.1 equiv.) and N, N-diisopropylethylamine (DIPEA) in DMF for 1 hour. After washed with DMF (3× 3 mL), the resin was agitated with the blocking solution (16:3:1 of DCM/MeOH/DIPEA) for 20 minutes to deactivate the unreacted sites. Then the resins were treated with 20% piperidine (in DMF) for 20 minutes to remove the protecting Fmoc group, followed by coupling next Fmoc-protected amino acid (3 equiv.) to the free amino group on the resin using HBTU and DIPEA as coupling reagents. These two steps were repeated to elongate the peptide chain, and the resin was washed with DMF for 3~5 times after each step. Finally, the peptide was cleaved from resin with TFA (5 mL) for 2 hours and the resulted crude products were purified by reverse phase HPLC.

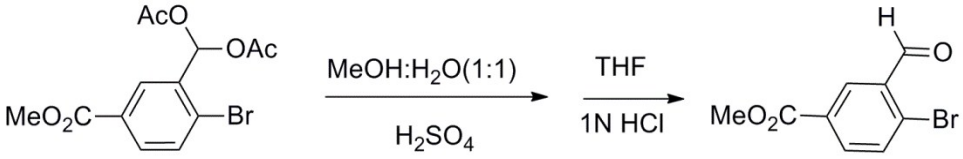
2.2 Synthesis of the target compound 1.



4-Bromo-3-methyl-benzoic acid (6.53 g, 30 mmol) was heated at reflux in MeOH (40 mL) containing H_2SO_4 (1 mL) overnight. The solvent was removed by rotary evaporation and the syrupy residue taken up in EtOAc (150 mL), washed with saturated aqueous $NaHCO_3$, H_2O and brine, then dried over Na_2SO_4. The solvent was removed by rotary evaporation and the solid taken up in CH_2Cl_2. The solution was passed through a plug of SiO_2 and the solvent again removed to give the product as a pink solid. Yield 5.87 g (85 %). **^{1}H NMR** (400 MHz; $CDCl_3$) δ 2.44 (s, 3 H), 3.91 (s, 3 H), 7.58-7.62 (m, 1 H), 7.67-7.72 (m, 1 H), 7.88-7.92 (m, 1 H).

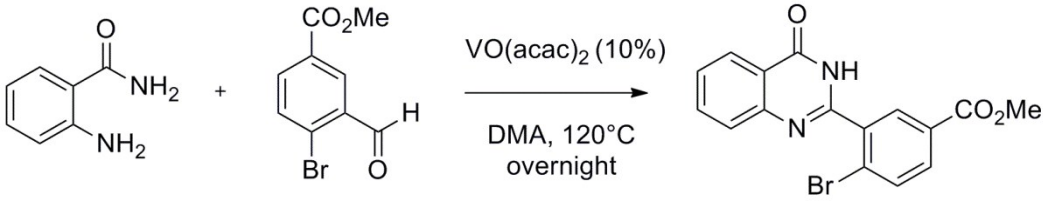


CrO_3 (6.00 g, 60 mmol) was added in portions over 30 min to 4-bromo-3-methyl-benzoic acid methyl ester (4.60 g, 20 mmol) dissolved in AcOH (33 mL) and Ac_2O (34 mL) containing H_2SO_4 (5 mL) cooled to ice-bath temperature. The mixture was stirred for another hour as the ice bath expired. The reaction mixture was poured onto chilled water (300 mL) and stirred vigorously for 20 min before collecting the precipitated solid by filtration and washing with water. Yield 4.21 g (61 %). **^{1}H NMR** (400 MHz; $CDCl_3$) δ 2.16 (s, 6 H), 3.91 (s, 3 H), 7.65-7.70 (m, 1 H), 7.90-7.93 (m, 1 H), 8.17-8.22 (m, 1 H).

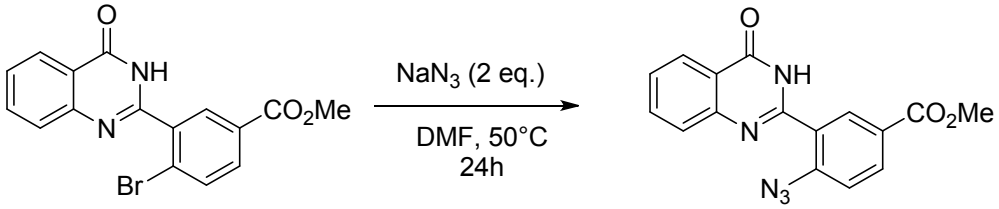


4-Bromo-3-diacetoxymethyl-benzoic acid methyl ester (4.21 g, 12.2 mmol) was heated at reflux in MeOH-H_2O (1:1, 40 mL) containing H_2SO_4 (0.5 mL) for 30 min. The reaction mixture was then diluted with H_2O (100 mL) and extracted with EtOAc, the combined organic layer was washed with H_2O and brine, dried over Na_2SO_4. The solvent was removed by rotary evaporation to give light yellow oil. This was a mixture of 4-bromo-3-formylbenzoic acid methyl

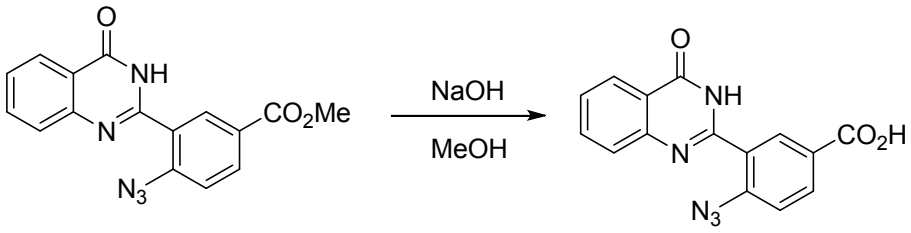
ester and the dimethyl acetal as found by ^{1}H NMR spectroscopy. The oil was taken up in THF (30 mL) and 1N HCl (8 mL) and the mixture heated at reflux until complete conversion to the aldehyde was achieved as determined by TLC. The THF was removed by rotary evaporation and the product isolated as above. Yield 1.77 g (59 %). **^{1}H NMR** (400 MHz; $CDCl_3$) δ 3.94 (s, 3 H), 7.72-7.77 (m, 1 H), 8.06-8.11 (m, 1 H), 8.51-8.55 (m, 1 H), 10.37 (s, 1 H).



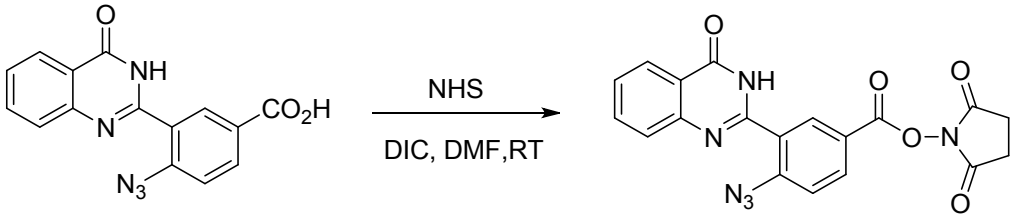
In air, 2-aminobenzamide (381.7 mg, 2.8 mmol), methyl 4-bromo-3-formylbenzoate (620 mg, 2.55 mmol) and $VO(acac)_2$ (67.6 mg, 0.28 mmol) were heated to 120℃ in DMA (10 mL) overnight. The reaction mixture was then diluted with H_2O (100 mL) and extracted with EtOAc, the combined organic layer was washed with H_2O and brine, dried over Na_2SO_4. The solvent was removed by rotary evaporation, and the residue was subjected to silica gel column chromatography to give purified product (485.5 mg, yield: 53%). **^{1}H NMR** (400 MHz; DMSO-d6) δ 3.88 (s, 3 H),7.55-7.62 (m, 1 H), 7.70-7.75 (m, 1 H), 7.84-7.90 (m, 1 H), 7.93-7.97 (m, 1 H), 7.98-8.02 (m, 1 H), 8.15-8.21 (m, 2 H), 12.68 (s, 1 H).



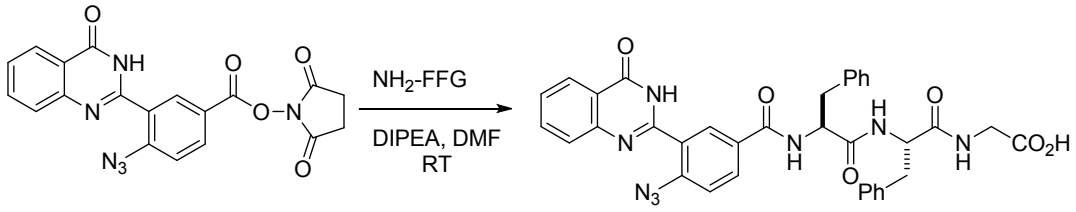
Methyl 4-bromo-3-(4-oxo-3,4-dihydroquinazolin-2-yl) benzoate (102 mg, 0.28 mmol) and NaN_3(36.9 mg, 2.0 mmol) were heat to 50℃ in DMF (5 mL) for 24 h, The reaction mixture was then diluted with H_2O (10 mL) and extracted with EtOAc, the combined organic layer was washed with H_2O and brine, then dried over Na_2SO_4. The solvent was removed by rotary evaporation, and the residue was subjected to silica gel column chromatography to give product (48.3 mg, yield: 54%). **^{1}H NMR** (400 MHz; DMSO-d6) δ 3.88 (s, 3 H), 7.55-7.60 (m, 1 H), 7.62-7.66 (m, 1 H), 7.72-7.76 (m, 1 H), 7.83-7.88 (m, 1 H), 8.14-8.19 (m, 2H), 8.21-8.23 (m, 1 H), 12.48 (s, 1 H).



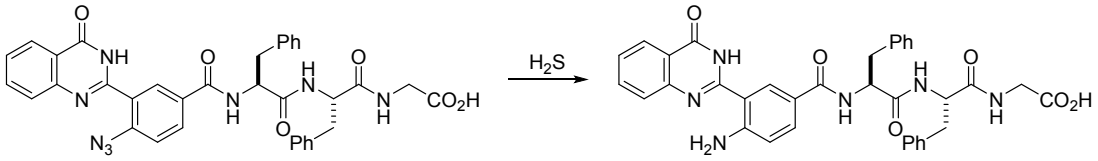
1 N NaOH (1.8 mL, 18 mmol) was added to methyl 4-azido-3-(4-oxo-3, 4-dihydroquinazolin-2-yl) benzoate (47.9 mg, 0.15 mmol) dissolved in MeOH (5 mL) and the mixture stirred for 4 h. The solvent was then removed by rotary evaporation and replaced with 0.33 N NaOH (30 mL). The resulting solution was filtered and then acidified (pH = 1) through addition of 1 N HCl and the precipitated solid collected by filtration and washed with H_2O and oven dried. Yield 40.9 mg (89%). **^{1}H NMR** (400 MHz; DMSO-d_6) δ 7.52-7.62 (m, 2 H), 7.70-7.79 (m, 1 H), 7.81-7.90 (m, 1 H), 8.10-8.21 (m, 3H), 12.48 (s, 1 H).



4-azido-3-(4-oxo-3,4-dihydroquinazolin-2-yl) benzoic acid (40.9 mg, 0.13 mmol) was mixed with N-hydroxysuccinimide (23.0 mg, 0.2 mmol), then 3 mL of DMF was added to obtain a well-dispersed solution. After N, N'-Dicylcohexylcarbodiimide (41.2 mg, 0.2 mmol) was added into the mixture, the solution was stirred overnight at room temperature. The reaction mixture was then diluted with H_2O (10 mL) and extracted with EtOAc, the combined organic layer was washed with H_2O and brine, dried over Na_2SO_4. The solvent was removed by rotary evaporation, and the residue was subjected to silica gel column chromatography to give product (45.2 mg, yield: 76%). **^{1}H NMR** (400 MHz; DMSO-d6) δ 3.91 (s, 4 H), 7.56-7.61 (m, 1 H), 7.74-7.79 (m, 1 H), 7.84-7.90 (m, 1 H), 8.16-8.21 (m, 2H), 8.27-8.31 (m, 1 H), 8.24-8.37 (m, 1 H), 12.56(s, 1 H).



Peptide FFG (148 mg, 0.4 mmol) was dissolved in 0.5 mL of DMF and DIPEA (103.4 mg, 0.8 mmol) was added to DMF (0.5 mL) solution of 2,5-dioxopyrrolidin-1-yl4-azido-3-(4-oxo-3,4-dihydroquinazolin-2-yl) benzoate (45.2 mg, 0.11 mmol), The mixture was stirred at room temperature for 12 hrs. The reaction mixture was subjected to HPLC purification. Compound was purified with water and CH_3CN eluent (from 7:3 to 1:9) to give the product (21.2 mg, yield: 25%). ^{1}H NMR (400 MHz; DMSO-d6) δ 2.81-2.95 (m, 2 H), 3.05-3.08 (m, 2 H), 3.78-3.79 (m, 2 H), 4.56-4.62 (m, 1 H), 4.72-4.78 (m, 1 H), 7.11-7.29 (m, 10 H), 7.56-7.60 (m, 2 H), 7.74-7.76(m, 1 H), 7.85-7.89(m, 1 H), 7.99-8.02 (m, 1 H), 8.09-8.10 (m, 1H), 8.17-8.20 (m, 2 H), 8.32-8.34 (m, 1 H), 8.68-8.70 (m, 1 H); ^{13}C NMR (100 MHz, DMSO-d6) δ 171.7, 171.6, 171.5, 165.0, 161.8, 151.9, 149.0, 141.4, 138.7, 138.1, 135.1, 131.4, 130.7, 130.6, 129.7, 129.5, 128.5, 127.9, 126.6, 126.7, 126.6, 126.3, 125.9, 121.5, 120.1, 55.1, 54.2, 41.1, 38.1, 37.2; HRMS (ESI): Calcd. For $[C35H29O6N8]^-$($[M-H]^-$): 657.2216; Found: 657.2199.



After treated with NaHS, N_3-quinazolinone-FFG (**1**) was transformed to NH_2-quinazolinone-FFG (**2**). ^{1}H NMR (400 MHz; DMSO-d6) δ 2.80-2.93 (m, 2 H), 3.04-3.14 (m, 2 H), 3.78-3.80 (m, 2 H), 4.57-4.62 (m, 1 H), 4.75-4.81 (m, 1 H), 6.83-6.85 (m, 1 H), 7.06-7.28 (m, 10 H), 7.50-7.54 (m, 1 H), 7.61-7.63 (m, 1 H), 7.77-7.79 (m, 1 H), 7.82-7.86(m, 1 H), 8.07-8.08 (m, 2H), 8.15-8.17 (m, 1 H), 8.24-8.29 (m, 2 H); HRMS (ESI): Calcd. for $[C35H31O6N6]^-$ ($[M-H]^-$): 631.2311, found 631.2290.

3. Cell experiments

3.1 Cytotoxicity assays

HeLa cells and HUVEC cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 and grown continuously in DMEM medium supplemented with 10% heat-inactivated FBS (fetal bovine serum). Cells were plated out in flat bottom 96-well plates at a density of 1×10^4 cells/well and allowed to attach for 12 h, followed by addition of different concentrations of **N_3-quinazolinone-FFG** (**1**) or **2** (100μM~500μM). The cells were then incubated at 37 °C in an atmosphere of 5% CO_2 for 24 h, 48 h, and 72 h, followed by an MTT assay. 10 μL Thiazolyl Blue Tetrazolium Bromide (MTT) (Solarbio) and 90 μL DMEM media were freshly added to each well. After incubation for 4 h, the media was gently removed. The formed formazan crystals were dissolved in 110 μL DMSO, subsequently the absorbance was measured with a plate reader (Perkin Elmer) at 490 nm.

3.2 Imaging of H_2S in different cell lines

HeLa cells, HUVEC cells, MCF-7 cells and MCF-10A cells were seeded on glass coverslip for confocal imaging in DMEM supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO_2 at 37 ºC, To start the experiment, HeLa cells were preloaded with or without PMA (1 μg/mL) for 30 minutes, and then **N_3-quinazolinone-FFG** at a concentration of 500 μM were added and further incubation for 4h. The cells were washed three times, LysoTracker Red (100 nM) and MitoTracker Red (100 nM) were used for staining lysosome and mitochondria, respectively.

4. Supplementary Figures

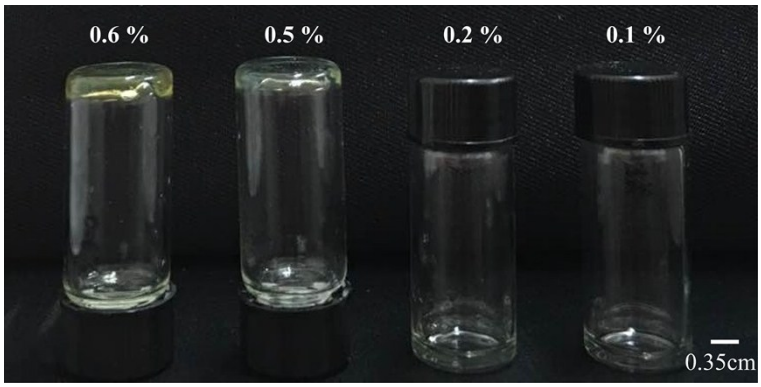


Figure S1. Critical gelation concentration of N_3-quinazolinone-FFG (**1**) after treated with NaHS. The critical gelation concentration was 0.5 wt %.

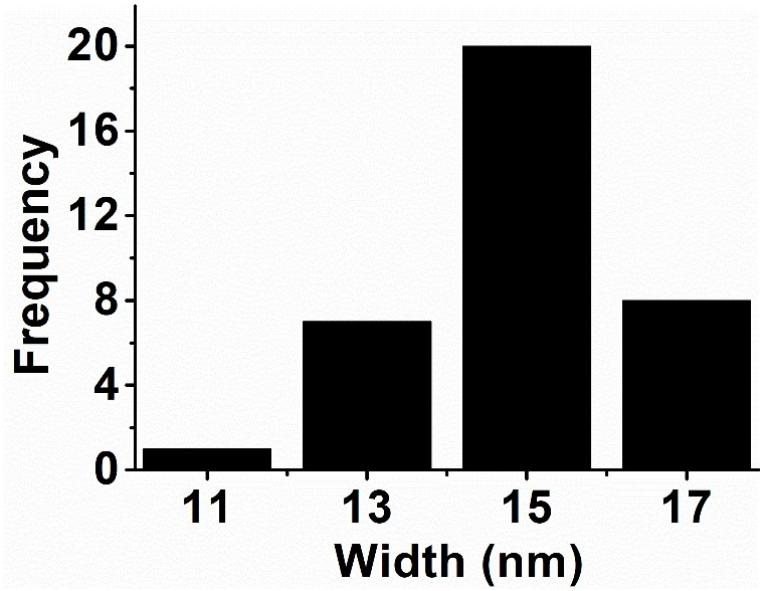


Figure S2. The width distribution of the *in vitro* nanofibers.

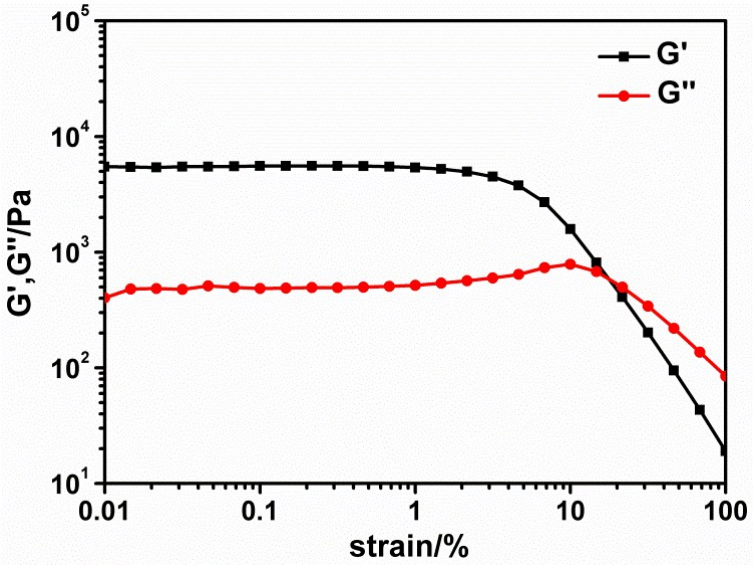


Figure S3. Dynamic strain sweep of **1** after it was treated by 13 equiv. of NaHS. The concentration of **1** was 1.0 wt % and the pH value was maintained around 7.4.

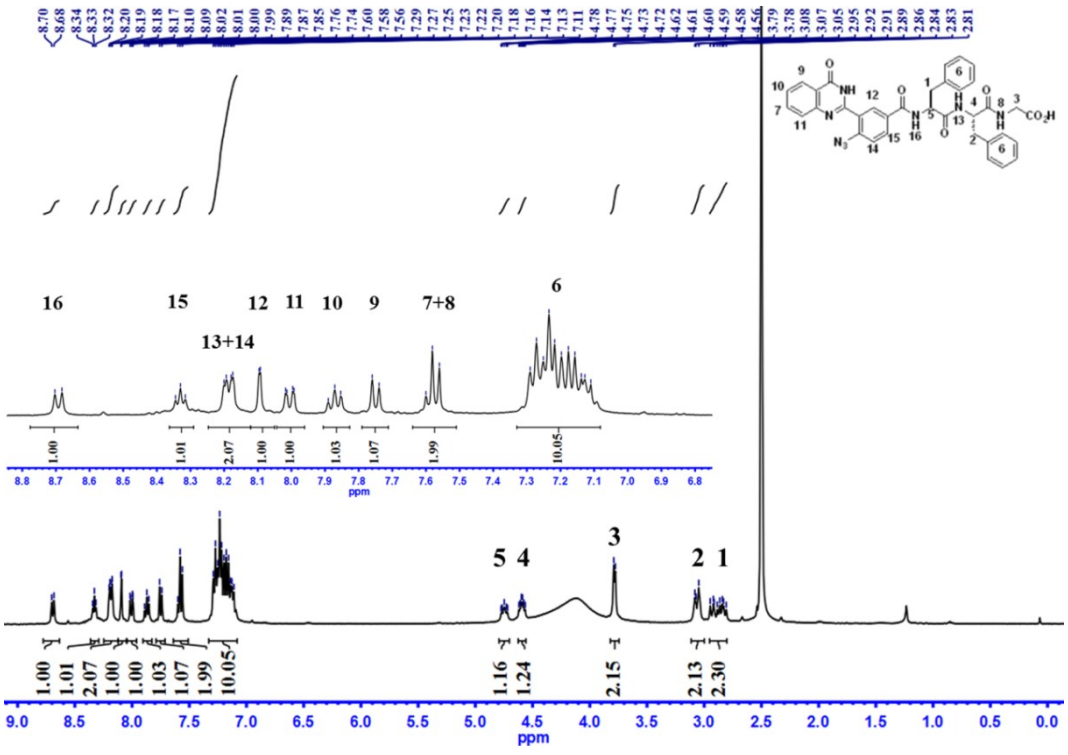


Figure S4. ^{1}H NMR (400 MHz; DMSO-d6) of **1**.

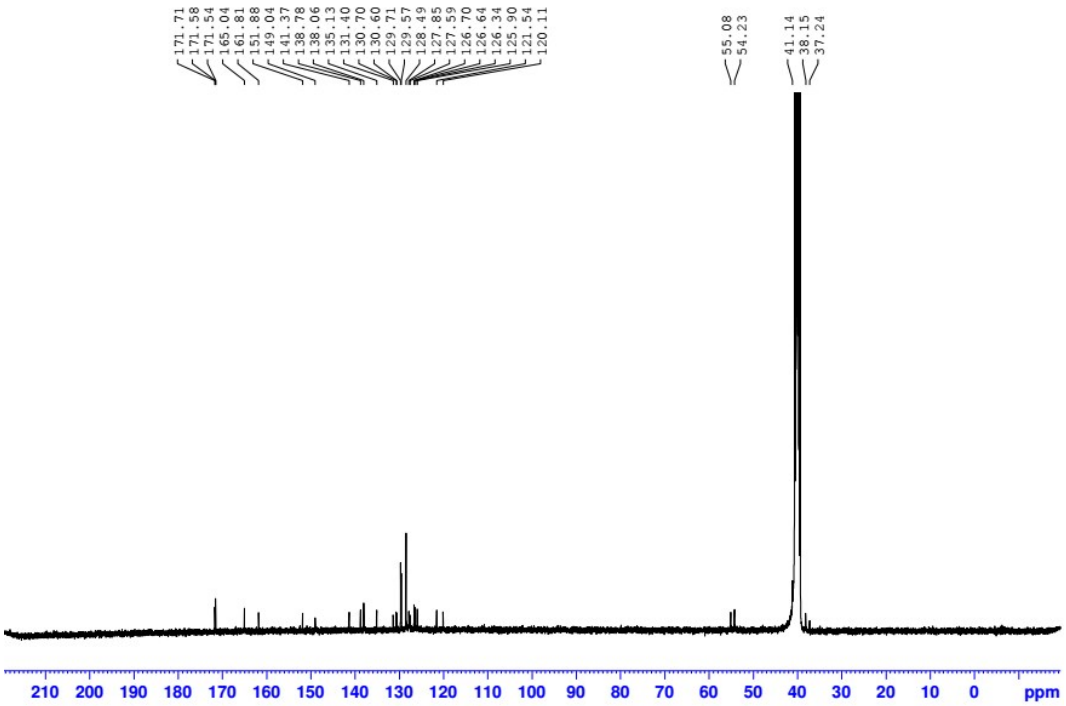


Figure S5. ^{13}C NMR (100 MHz, DMSO-d6) of **1**.

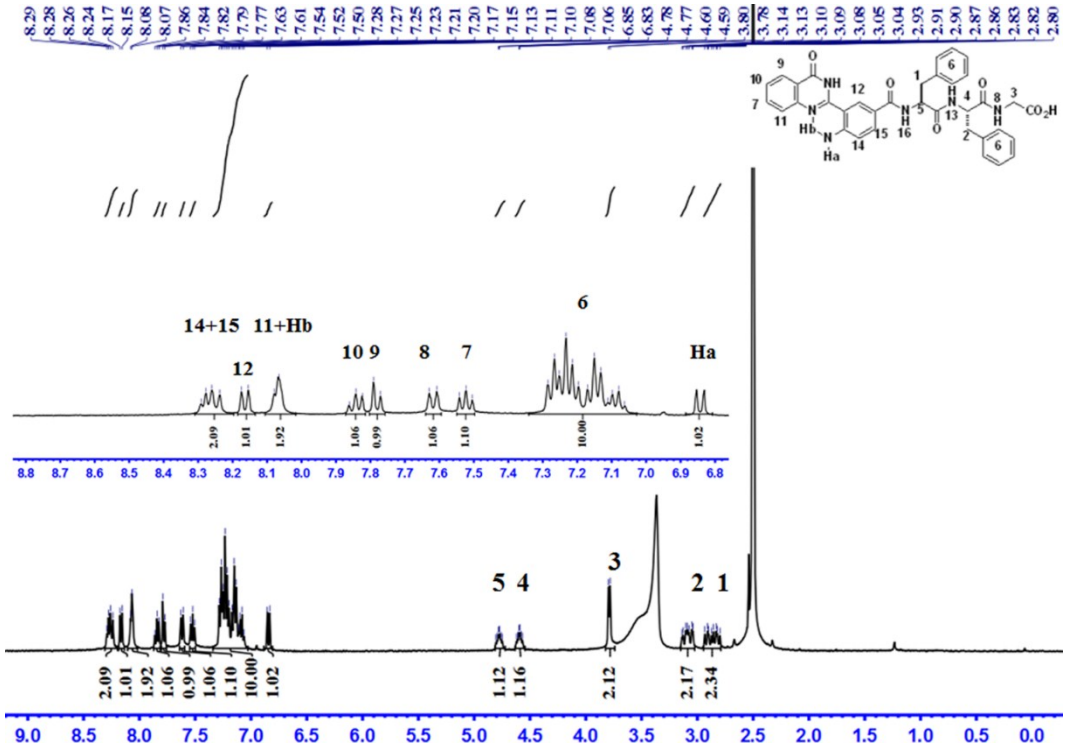


Figure S6. ^{1}H NMR (400 MHz; DMSO-d6) of **2**.

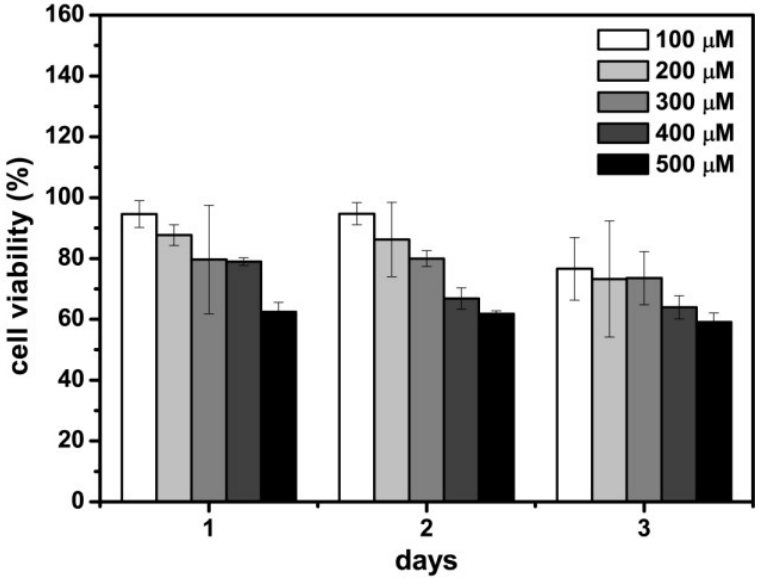


Figure S7. Viability of HeLa cell treated with **1** at different concentrations (100-500 μM). Cell viability was determined by MTT assay with an initial cell number of 10,000 cells/well.

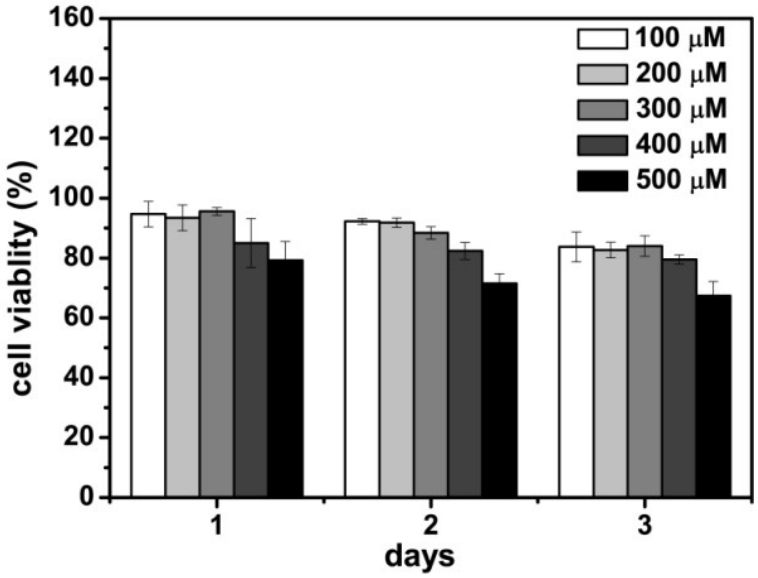


Figure S8. Viability of HUVEC cell treated with **1** at different concentrations (100-500 μM). The cell viability was determined by MTT assay with an initial cell number of 10,000 cells/well.

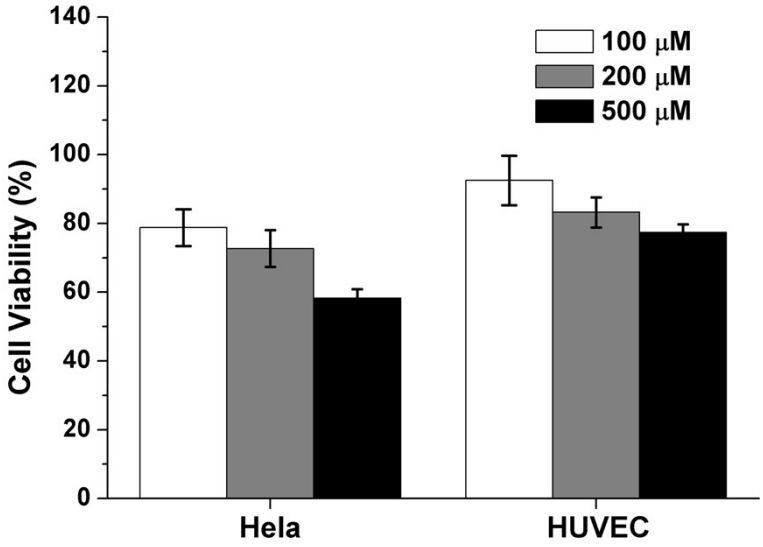


Figure S9. Viability of HeLa and HUVEC cells treated with **2** at different concentrations (100 μM, 200 μM and 500 μM). The cell viability was determined by MTT assay with an initial cell number of 10,000 cells/well. The culture time was 3 days.

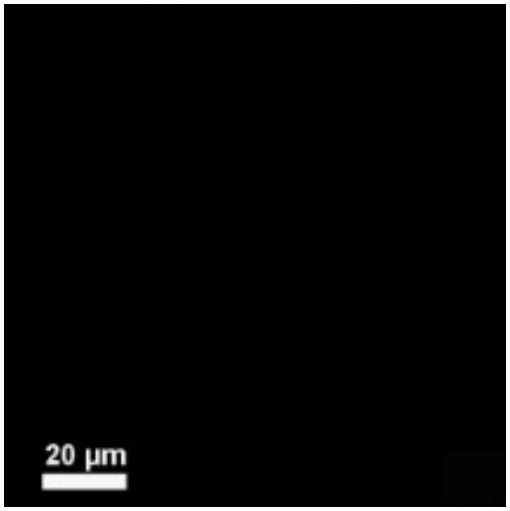


Figure S10. HeLa cells were firstly incubated with PMA (1 μg/mL) for 30 min, and then treated with **1** (500 μM) for another 4 h. No fluorescent assemblies formed inside HeLa cells.

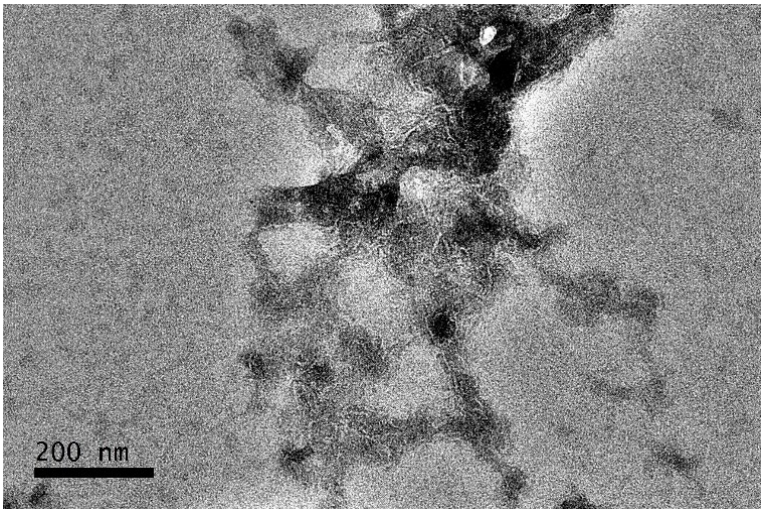


Figure S11. TEM image showed the nanofibers in the fraction of HeLa cells pre-incubated with **1** (500 μM) for 4 h.

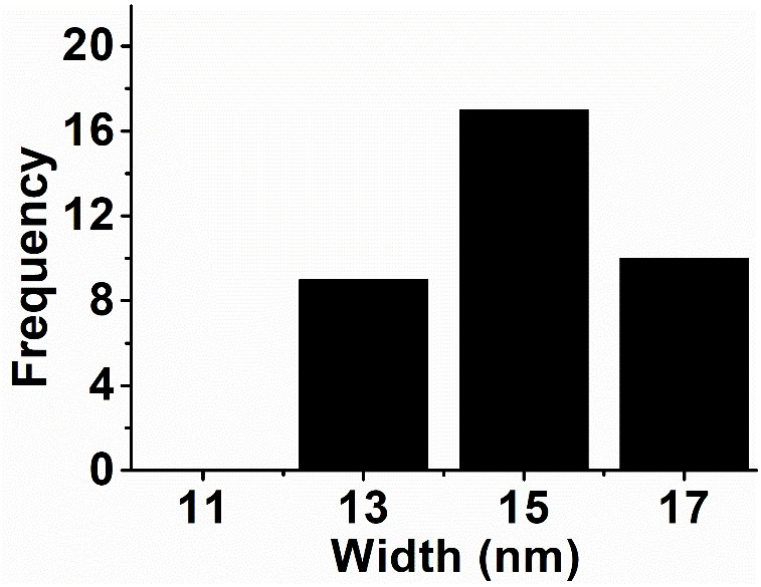


Figure S12. The width distribution of the nanofibers in the fraction of HeLa cells.

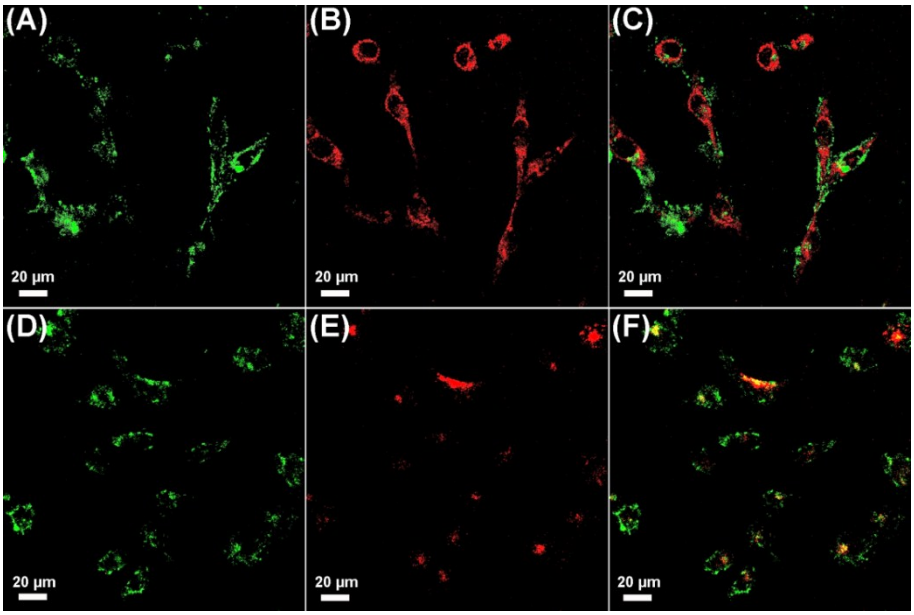


Figure S13. Confocal images of **1** co-localized with mitochondria and lysosome of HUVEC cells. (A) **1** (500 μM, λ_{ex} = 405 nm); (B) Mito-Tracker Red (100 nM, λ_{ex} =561 nm, λ_{em}= 570-620 nm); (C) Overlay of A and B; (D) **1** (500 μM, λ_{ex} = 405 nm); (E) Lyso-Tracker Red (100 nM, λ_{ex} = 561 nm); (F) Overlay of D and E.

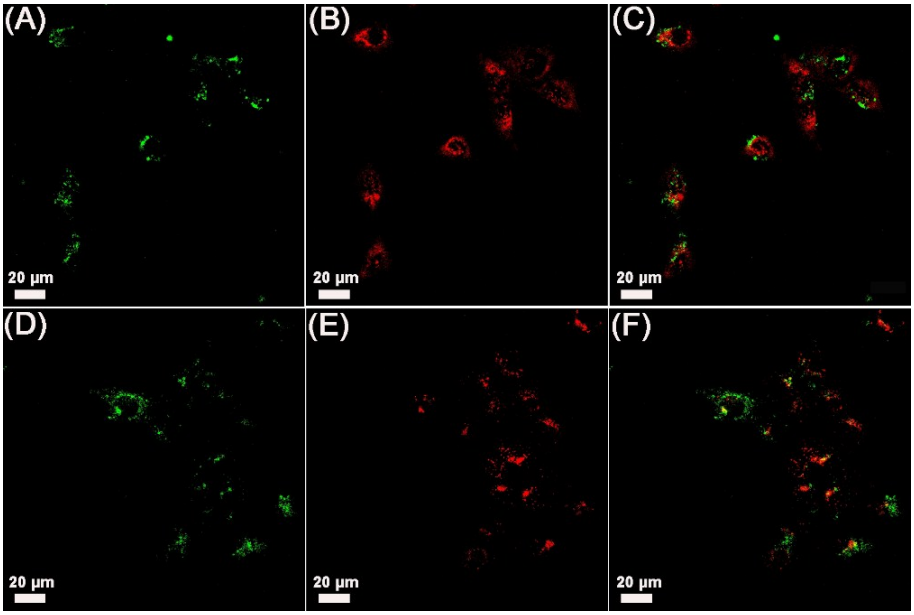


Figure S14. Confocal images of **1** co-localized with mitochondria and lysosome of MCF-7 cells. (A) **1** (500 μM, λ_{ex} = 405 nm); (B) Mito-Tracker Red (100 nM, λ_{ex} =543 nm, λ_{em}= 570-620 nm); (C) Overlay of A and B; (D) **1** (500 μM, λ_{ex} = 405 nm); (E) Lyso-Tracker Red (100 nM, λ_{ex} = 543 nm); (F) Overlay of D and E.

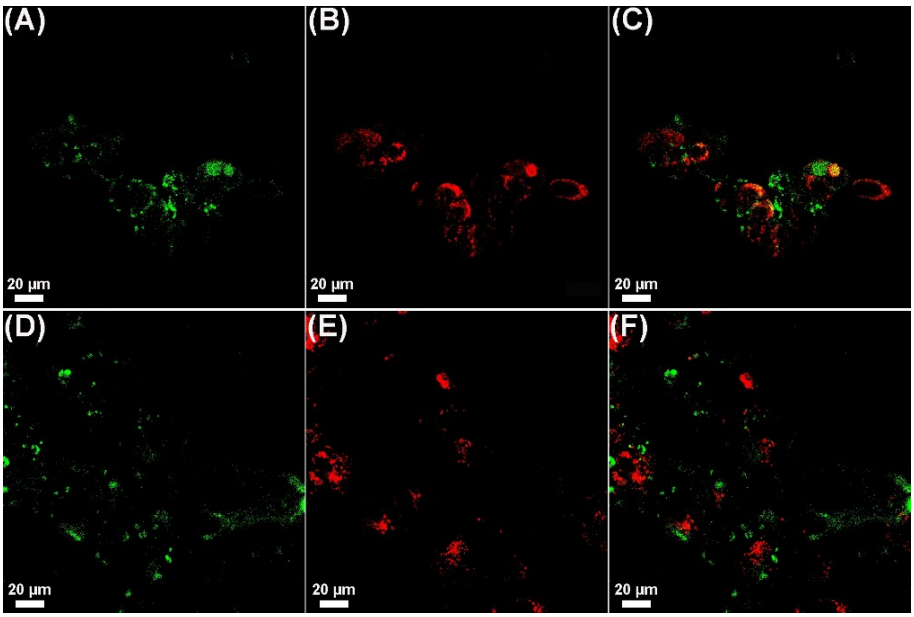


Figure S15. Confocal images of **1** co-localized with mitochondria and lysosome of MCF-10A cells. (A) **1** (500 μM, λ_{ex} = 405 nm); (B) Mito-Tracker Red (100 nM, λ_{ex} =543 nm, λ_{em}= 570-620 nm); (C) Overlay of A and B; (D) **1** (500 μM, λ_{ex} = 405 nm); (E) Lyso-Tracker Red (100 nM, λ_{ex} = 543 nm); (F) Overlay of D and E.

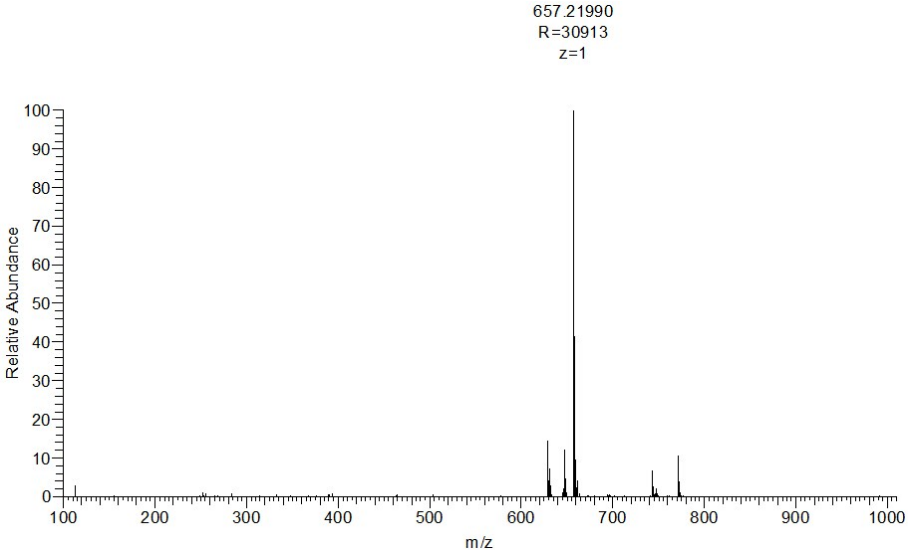


Figure S16. HRMS spectrum of N_3-quinazolinone-FFG (**1**).

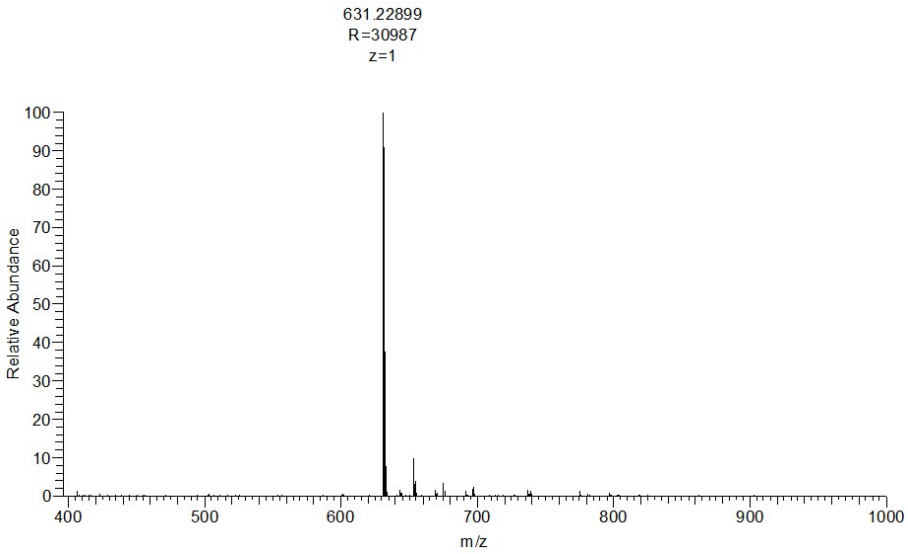


Figure S17. HRMS spectrum of **2**.