Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Photo-Clickable MicroRNA for In-Situ Fluorescence Labeling and Imaging of MicroRNA in Living Cells

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Materials and Methods

1. General materials and methods

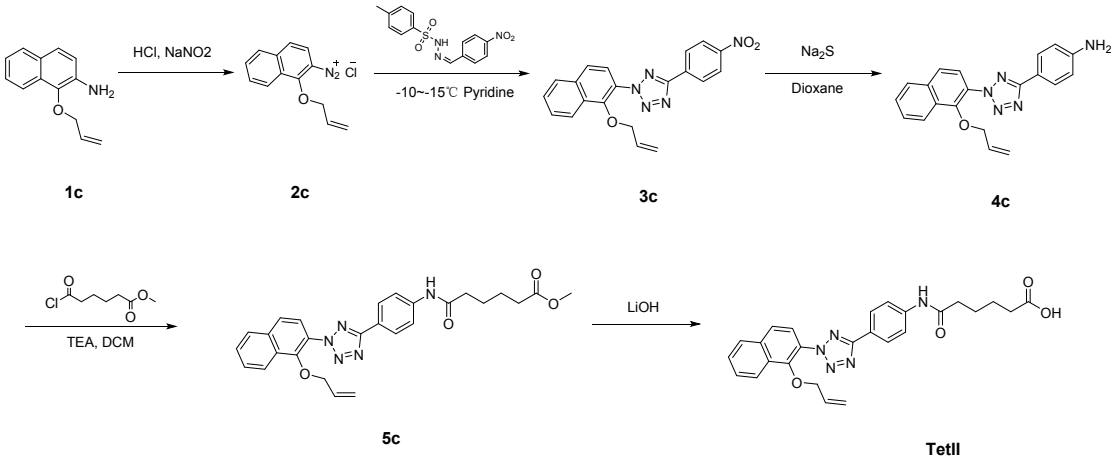
All chemicals and solvents were purchased from J&K chemicals or Sigma-Aldrich. Amine-modified miRNA mimics were purchased from RiboBio. High glucose Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penincilin/streptomycin, Lipofectamine 2000 were purchased from Life Technologies. Luciferase assay kits were purchased from Promega. Luciferase reporter genes were ordered from Genescript. All aqueous solutions were treated by diethy pyrocarbonate (DEPC) before use.

^{1}HNMR and ^{13}CNMR spectra were obtained on a 400 MHz Bruker AVANCE III–400 spectrometer. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). MS and HRMS were done on a SHIMADZULCMS-2020 and Agilent 6550 iFunnel Q-TOF LC/MS. HPLC was carried out on Agilent 1200 LC with CH_3CN/H_2O (0.1 M $CH_3COONH_4^+$) as eluents. Fluorescence images were taken under confocal microscopy (Leica).

An 8 W hand-held 302 nm UV lamp was used as the light source. The irradiation intensity at a distance of 1 m is 28 μW/cm^2. When using light irradiation to trigger intramolecular photo-click reaction, the distance from lamp to samples was ~4-5 cm and the corresponding irradiation intensity was ~ 3.8 mW/cm^2.

2. Synthesis and characterization of chemical compounds

6-((4-(2-(1-(allyloxy)naphthalen-2-yl)-2H-tetrazol-5-yl)phenyl)amino)-6-oxohexanoic acid (TetII):



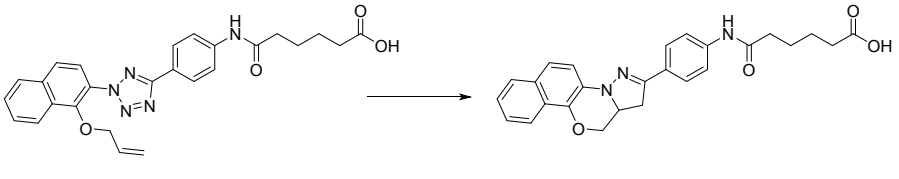
1-(allyloxy)naphthalene-2-diazonium chloride 2c was prepared by adding a cooled solution of sodium nitrite (5 mmol) in 2 mL of water to a solution of 1-(allyloxy)naphthalen-2-amine 1c (5 mmol) and 1.3 mL of concentrated hydrochloric acid in 8 mL of 50% ethanol below 5 °C. The resulted solution of 2c was directly added dropwise over a period of 30 minutes into a solution of 4-methyl-N'-(4-nitrobenzylidene) benzenesulfonohydrazide (5 mmol) in 30 mL pyridine at -10 ~ -

15 °C. The mixture was continued to stir at room temperature for 12 hours and a lot of precipitation formed. The precipitation was filtered and washed with ethyl acetate to give 3c as a yellow solid. Without further purification, 3c was suspended in 12 mL 1, 4-dioxane and was added with Na_2S (6 g, 15 mmol, 3 equiv). The solution was refluxed and stirred for 2 hours. The reaction mixture was extracted with ethyl acetate and water. The ethyl acetate layer was dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 5:1) to give 4c as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 – 8.28 (m, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 8.01 – 7.91 (m, 1H), 7.80 (s, 2H), 7.69 – 7.60 (m, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.14 – 5.92 (m, 1H), 5.37 (d, *J* = 17.1 Hz, 1H), 5.24 (d, *J* = 10.4 Hz, 1H), 4.58 (d, *J* = 5.7 Hz, 2H).

To a solution of 4c (100 mg, 0.3 mmol) dissolved in CH_2Cl_2, triethylamine (100 μL) was added. Methyl-6-chloro-6-oxohexanoate (78 mg, 0.45 mmol) was added dropwise with ice bath, the solution was then stirred at room temperature for 2 hours. TLC showed the reaction was completed and the reaction mixture was extracted with ethyl acetate and water. The ethyl acetate layer was dried with Na_2SO_4. The solvent was removed under reduced pressure to give 5c (140 mg, 99%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 – 8.31 (m, 1H), 8.27 (d, *J* = 8.6 Hz,2H), 8.00 – 7.94 (m, 1H), 7.81 (s, 2H), 7.76 (d, *J* = 8.4 Hz,2H), 7.70 – 7.63 (m, 2H), 7.56 (d, *J* = 13.3 Hz, 1H), 6.05 (ddd, *J* = 16.3, 11.0, 5.8 Hz, 1H), 5.38 (d, *J* = 17.2, 1.3 Hz, 1H), 5.25 (d, *J* = 11.1 Hz, 1H), 4.61 (d, *J* = 5.7 Hz, 2H), 3.72 (s, 3H), 2.54 – 2.35 (m,4H), 1.89 – 1.71 (m, 4H).

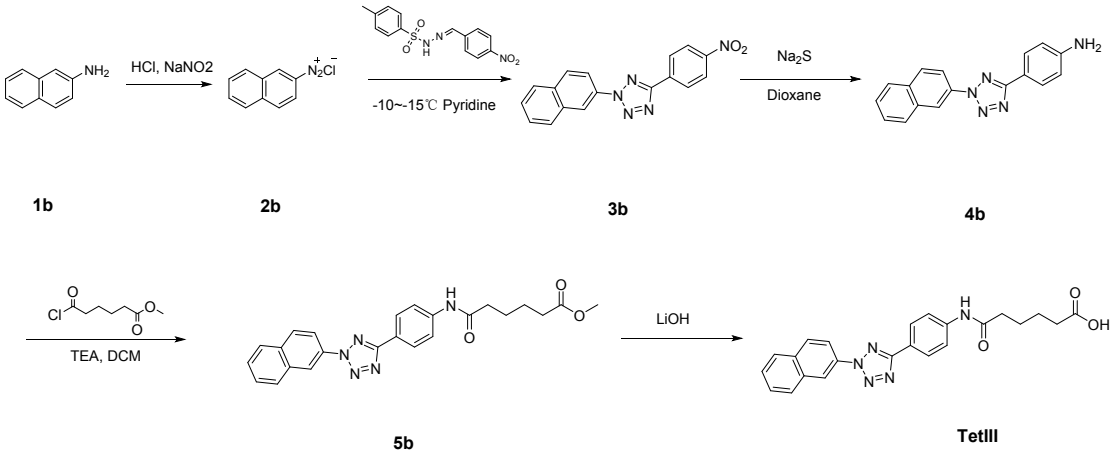
5c (140 mg, 0.3 mmol) was dissolved in THF/H_2O (5 mL/5 mL) and $LiOH·H_2O$ (126 mg, 3 mmol) was added. The solution was stirred at room temperature for 2 hours. TLC showed the reaction was completed and the reaction mixture was extracted with ethyl acetate and water. The ethyl acetate layer was dried with Na_2SO_4. The solvent was removed under reduced pressure to give **TetII** (90 mg, 63%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 12.04 (s, 1H), 10.20 (s, 1H), 8.30 – 8.22 (m, 1H), 8.13 (d, *J* = 8.6 Hz, 2H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.90 – 7.81 (m, 3H), 7.79 – 7.71 (m, 2H), 5.97 (m, 1H), 5.30 (d, *J* = 17.2 Hz, 1H), 5.18 (d, *J* = 10.4 Hz, 1H), 4.55 (d, *J* = 5.6 Hz, 2H), 2.38 (t, *J* = 7.1 Hz, 2H), 2.27 (t, *J* = 7.1 Hz, 2H), 1.69 – 1.51 (m, 4H). ^{13}CNMR (100 MHz, DMSO) δ 174.81, 171.95, 164.69, 149.02, 142.09, 135.37, 133.54, 128.89, 128.43, 128.15, 127.79, 126.56, 125.55, 123.43, 123.31, 121.34, 119.83, 118.69, 76.74, 36.67, 33.90, 25.06, 24.61. HRMS (ESI) calcd for $C_{26}H_{25}N_5O_4Na$ 494.1804 $[M+Na]^+$; found 494.1807.

6-((4-(2-(1-(allyloxy)naphthalen-2-yl)-2H-tetrazol-5-yl)phenyl)amino)-6-oxohexanoic acid (PyrII):



TetII (100 mg, 0.21 mmol) was dissolved in CH_3CN/DCM (10 mL/10 mL). The solution was stirred at room temperature and exposed to irradiation (302 nm) for 2 hours. TLC showed the reaction was completed and the solvent was removed under reduced pressure. The crude product was purified by prep-TLC (DCM/CH_3OH=10/1) to give PyrII (80 mg, 55%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.12 (s, 1H), 8.27 – 8.04 (m, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.84 (dd, *J* = 19.9, 8.3 Hz, 1H), 7.76 – 7.70 (m, 1H), 7.70 – 7.61 (m, 3H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.46 (t, *J* = 7.3 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 4.48 (dd, *J* = 10.5, 3.5 Hz, 1H), 4.27 – 4.10 (m, 1H), 3.46 (dd, *J* = 17.7, 3.7 Hz, 2H), 3.21 (dd, *J* = 17.5, 4.1 Hz, 1H), 2.33 (t, *J* = 7.0 Hz, 2H), 2.22 (t, *J* = 6.9 Hz, 2H), 1.58 (ddd, *J* = 21.2, 14.1, 6.8 Hz, 4H). ^{13}C NMR (100 MHz, DMSO) δ 174.97, 171.75, 150.72, 140.70, 138.13, 129.73, 128.10, 127.17, 126.86, 126.27, 125.43, 124.67, 121.56, 121.27, 120.68, 119.29, 65.14, 56.39, 36.61, 35.79, 34.10, 25.10, 24.68.

6-((4-(2-(naphthalen-2-yl)-2H-tetrazol-5-yl)phenyl)amino)-6-oxohexanoic acid (TetIII):



Naphthalene-2-diazonium chloride 2b was prepared by adding a cooled solution of sodium nitrite (5 mmol) in 2 mL of water to a solution of naphthylamine 1b (5 mmol) and 1.3 mL of concentrated hydrochloric acid in 8 mL of 50% ethanol below 5 °C. The resulted solution of 2b was directly added dropwise over a period of 30 minutes into a solution of 4-methyl-N'-(4-nitrobenzylidene)benzenesulfonohydrazide (5 mmol) in 30 mL pyridine at -10 ~ -15 °C. The mixture was continued to stir at room temperature for 12 hours and a lot of precipitation formed. The precipitation was filtered and washed with ethyl acetate to give 3b as a yellow solid. Without further purification, 3b was suspended in 12 mL 1, 4-dioxane and was added with Na_2S (6 g, 15 mmol, 3 equiv). The solution was refluxed and stirred for 2 hours. The reaction mixture was extracted with ethyl acetate and water. The ethyl acetate layer was dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 5:1) to give 4b as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 1.4 Hz, 1H), 8.35 (dd, *J* = 8.9, 2.0 Hz, 1H), 8.12 (d, *J* = 8.6 Hz, 2H), 8.05 (d, *J* = 9.0 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.95 (d, *J* = 8.9 Hz, 1H), 7.67 – 7.56 (m, 2H), 6.84 (d, *J* = 8.6 Hz, 2H).

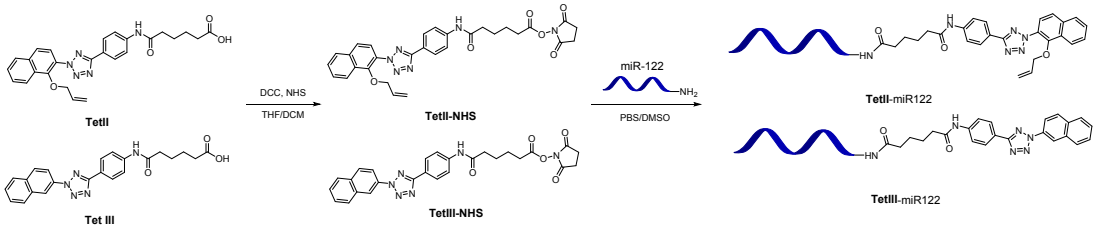
To a solution of 4b (100 mg, 0.35 mmol) dissolved in CH_2Cl_2, triethylamine (100 μL) was added.

Methyl-6-chloro-6-oxohexanoate (100 mg, 0.525 mmol) was added dropwise with ice bath, the solution was then stirred at room temperature for 2 hours. TLC showed the reaction was completed and the reaction mixture was extracted with ethyl acetate and water. The ethyl acetate layer was dried with Na_2SO_4. The solvent was removed under reduced pressure to give 5b (150 mg, 99%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.36 (dd, *J* = 8.9, 2.1 Hz, 1H), 8.29 (d, *J* = 8.6 Hz, 2H), 8.07 (d, *J* = 9.0 Hz, 1H), 8.05 – 8.00 (m, 1H), 7.98 – 7.92 (m, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.67 – 7.59 (m, 2H), 7.55 (s, 1H), 3.73 (s, 3H), 2.52 – 2.37 (m,4H), 1.88 – 1.73 (m,4H)

5b (150 mg, 0.35 mmol) was dissolved in THF/H_2O (5 mL/5 mL) and LiOH·H_2O (147 mg, 3.5 mmol) was added. The solution was stirred at room temperature for 2 hours. TLC showed the reaction was completed and the reaction mixture was extracted with ethyl acetate and water. The ethyl acetate layer was dried with Na_2SO_4. The solvent was removed under reduced pressure to give **TetIII** (80 mg, 55%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 12.04 (s, 1H), 10.20 (s, 1H), 8.74 (s, 1H), 8.25 (M, 3H), 8.15 (d, *J* = 8.7 Hz, 2H), 8.10 – 8.04 (m, 1H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.71 – 7.62 (m, 2H), 2.38 (t, *J* = 7.1 Hz, 2H), 2.27 (t, *J* = 7.1 Hz, 2H), 1.69 – 1.53 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 174.83, 171.96, 164.94, 142.17, 134.08, 133.46, 133.14, 130.71, 129.19, 128.43, 128.19, 128.10, 127.87, 121.26, 119.79, 118.59, 118.25, 36.68, 33.91, 25.05, 24.62. HRMS (ESI) calcd for $C_{23}H_{21}N_5O_3Na$ 438.1542 $[M+Na]^+$; found 438.1543.

3. MiR-122 modification

Scheme S1. 3'-modification of miR-122 with **Tet**



Tet (0.44 mmol), DCC (108 mg, 0.52 mmol) and NHS (60 mg, 0.52 mmol) were dissolved in THF/DCM (5 mL/5 mL). The mixture was stirred at room temperature for 12 hours. The precipitation was filtered and the supernatant was concentrated under reduced pressure to give **Tet**-NHS as a white solid. **Tet**-NHS was then dissolved in DMSO as a stock solution (5 mM). MiR-122 (3'-NH_2) was dissolved in PBS (pH=7.4) and **Tet**-NHS stock solution was then added. The reaction mixture was shaken at 25 °C for 12 hours. The crude was purified by HPLC with CH_3CN/H_2O (0.1 M $CH_3COONH_4^+$) as eluents (CH_3CN from 2% to 40% in 30 min). The collected products were desalted and lyophilized. ESI calcd for single-stranded **TetII**-miR122: 7780.9 $[M+H]^+$; found: 7781.8; ESI calcd for single-stranded **TetIII**-miR122: 7724.8 $[M+H]^+$; found: 7725.6. Single-stranded **Tet**-modified miR-122 was annealed with its passenger strand prior to cellular assays.

4. Cell culture

HepG2 cells were cultured in high glucose DMEM containing 10% fetal bovine serum and 1%

penicillin/streptomycin, and maintained in 5% CO_2 at 37 ^oC.

5. Luciferase assay

HepG2 Cells were seeded on 24 well plates and transfected the following day with 0.5 μg Luciferase reporter plasmids and 0.3 μg β-galactosidase expressing plasmids by using Lipofectamine 2000 according to manufacturer's protocol. β-galactosidase was used as an internal control. After 4 hours, 0.25 μg **Tet**- or **PyrII**-modified miR-122 mimics were further transfected into corresponding cells by using Lipofectamine 2000 according to manufacturer's protocol. Luciferase signals were measured after 48 hours by using Luciferase assay kits according to manufacturer's instruction.

6. Confocal imaging

1×10^5 HepG2 cells were seeded on 35-mm glass-bottom tissue culture dishes. When reaching 80% confluency, 3 μg (~10 μM) **PyrII**-miR122, **TetII**-miR122, **TetIII**-miR122 were respectively transfected into HepG2 cells using Lipofectamine 2000 according to manufacturer's protocol. After 4 hours, the medium was changed to PBS after cells were washed with PBS for three times. The imaging acquisitions were carried out immediately using a Leica confocal microscope. To turn on the fluorescence of pyrazolines, HepG2 cells were irradiated by an 8 W hand-held 302 nm UV lamp. The distance from UV lamp to cells was ~4-5 cm. For spatial controlled imaging, a photo-mask was used. Fluorescence signals from pyrazoline were then acquired (ex, 405 nm or 720 nm; em, 450~550 nm).

Supporting Figures and Tables

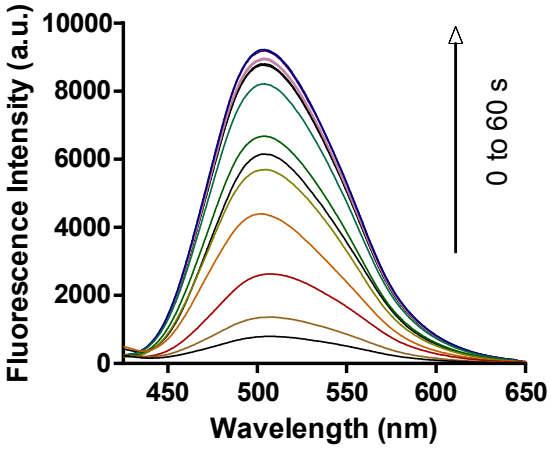


Figure S1. Fluorescence spectra of **TetII** (50 μM) in PBS after irradiation by an 8 W hand-held 302 nm UV lamp for 0 to 60 seconds. Excitation wavelength: 405 nm.

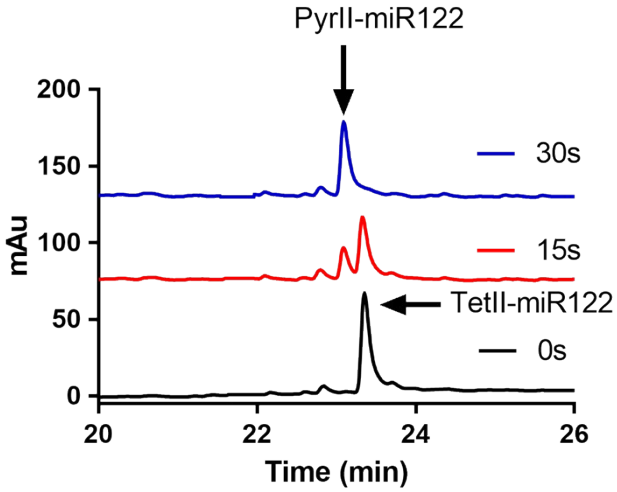


Figure S2. HPLC analysis of reaction mixtures of **TetII**-miR122 (5 μM) in PBS, which was subject to light irradiation by an 8 W hand-held 302 nm UV lamp.

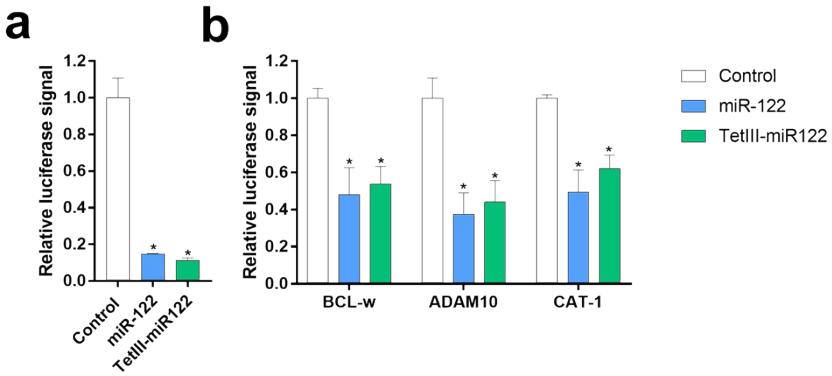


Figure S3. Relative luciferase signal changes upon transfection of miR-122 (30 nM) or **TetIII**-miR122 (30 nM) into HepG2 cells transfected with luciferase reporter genes bearing (**a**) complementary sequence of miR-122 and (**b**) 3'-UTR of BCL-w, ADAM10 and CAT-1. Data are shown as mean ± SEM (n=3). **P* < 0.05, relative to control.

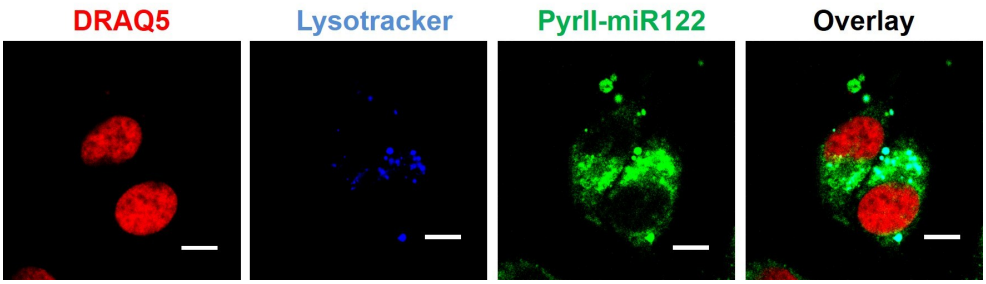


Figure S4. Confocal fluorescence images of HepG2 cells transfected with **PyrII**-miR122 (10 μM). Confocal images were acquired with Ex, 405 nm; Em, 450-550 nm. The nucleus and lysosome were respectively stained with DRAQ5 and Lysotracker. Scale bar: 20 μm.

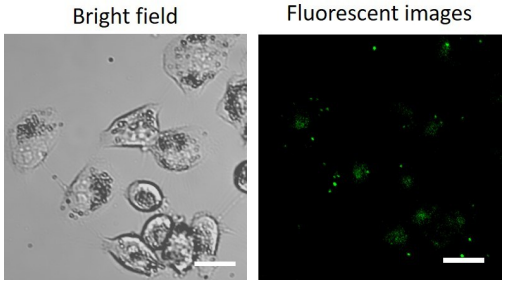


Figure S5. Confocal fluorescence images of HepG2 cells transfected with **TetII**-miR122 (10 μM). Confocal images were acquired with Ex, 405 nm; Em, 450-550 nm. Scale bar: 50 μm.

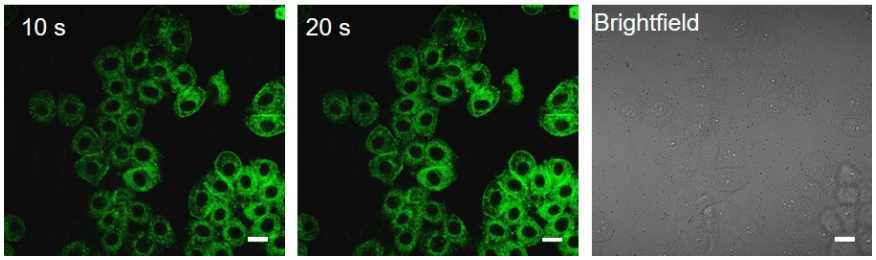


Figure S6. Confocal images of HepG2 cells transfected with **TetII**-miR122 (10 μM) after the cells were irradiated with an 8 W hand-held UV lamp with emitting at 302 nm for 10 and 20 seconds. Confocal images were acquired with Ex, 405 nm; Em, 450-550 nm. Scale bar: 20 μm.

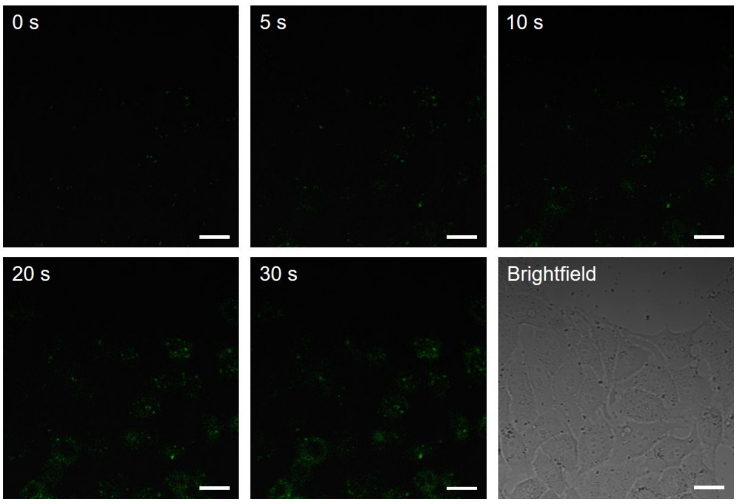


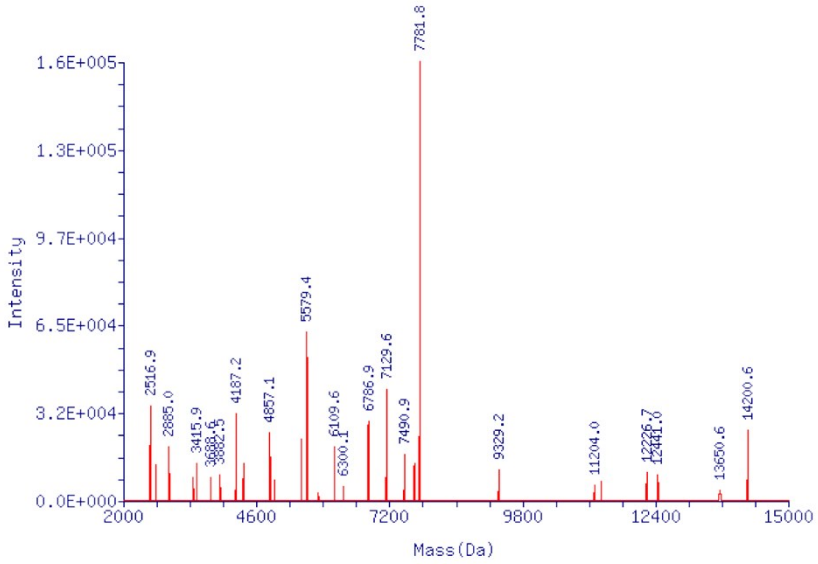
Figure S7. Confocal images of HepG2 cells transfected with **TetIII**-miR122 (10 μM) after cells were irradiated with an 8 W hand-held UV lamp with emitting at 302 nm for 0 to 30 seconds and fluorescence signals of pyrazoline were then acquired at indicated time points (Ex, 405 nm; Em, 450-550 nm). Scale bar: 20 μm.

Table S1. Spectral properties of PyrII. PyrII was dissolved in CH_3CN/PBS (1/1) and the quantum yield was determined using Quinine Sulfate as standard.

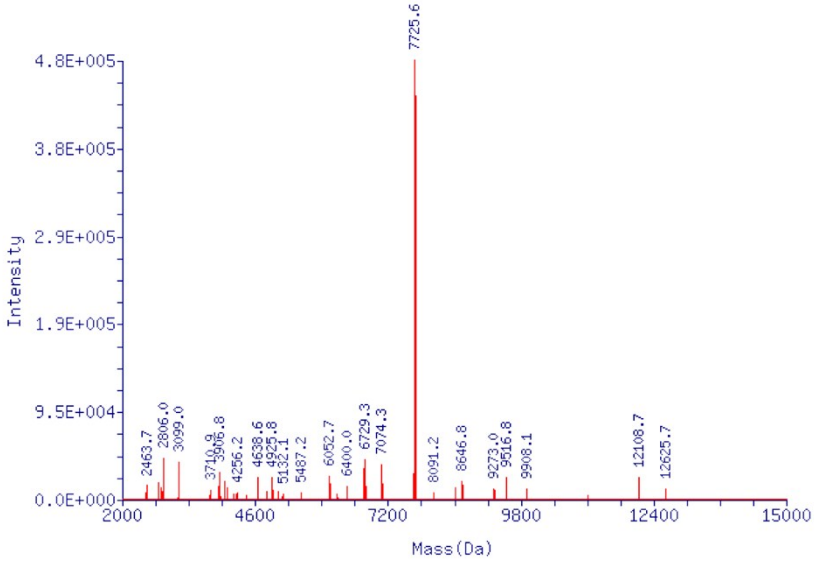
Fluorophore	Ex (nm) Max	Em (nm) Max	Molar extinction coefficient (ε, M^{-1} cm^{-1})	Quantum yield (Φ)
PyrII	340	515	13700	0.25

MS Spectra

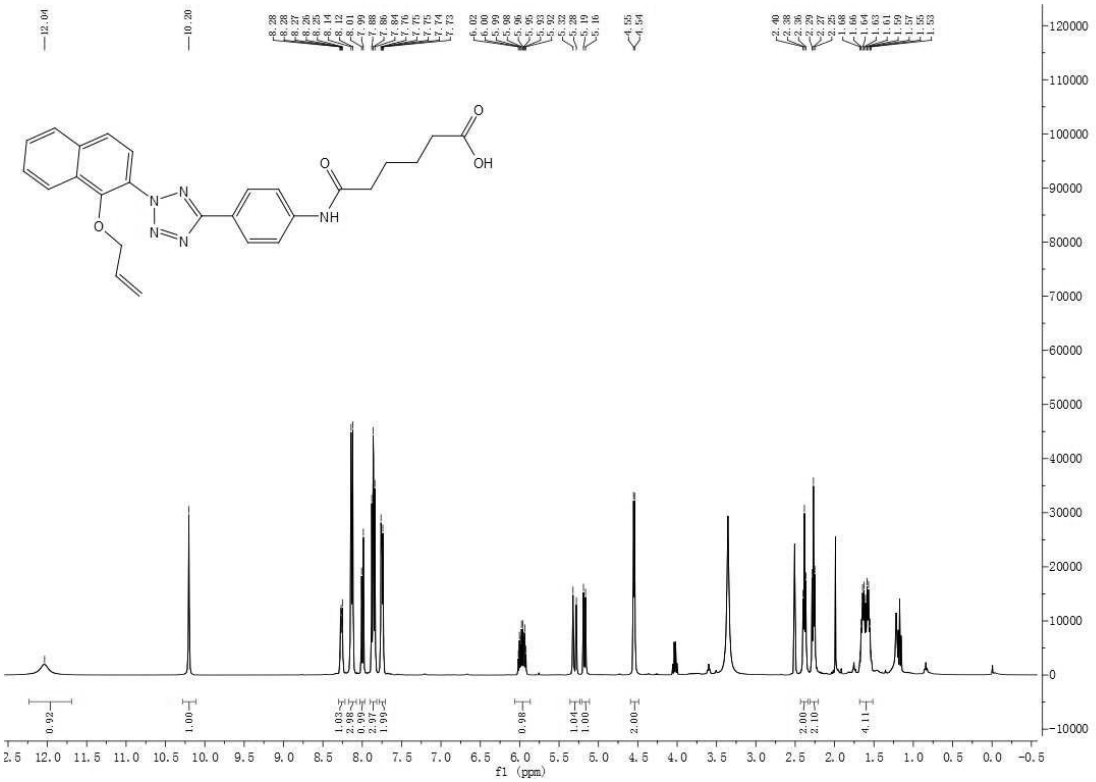
Single-stranded **TetII**-miR122



Single-stranded **TetIII**-miR122



NMR Spectra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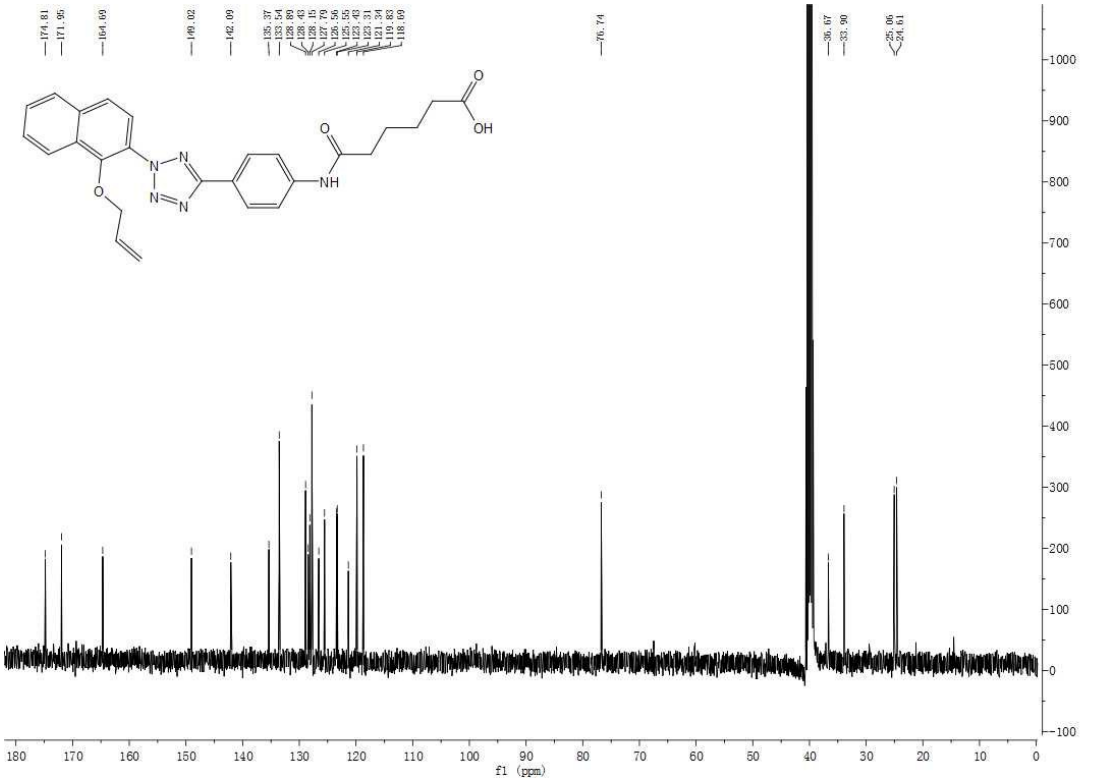
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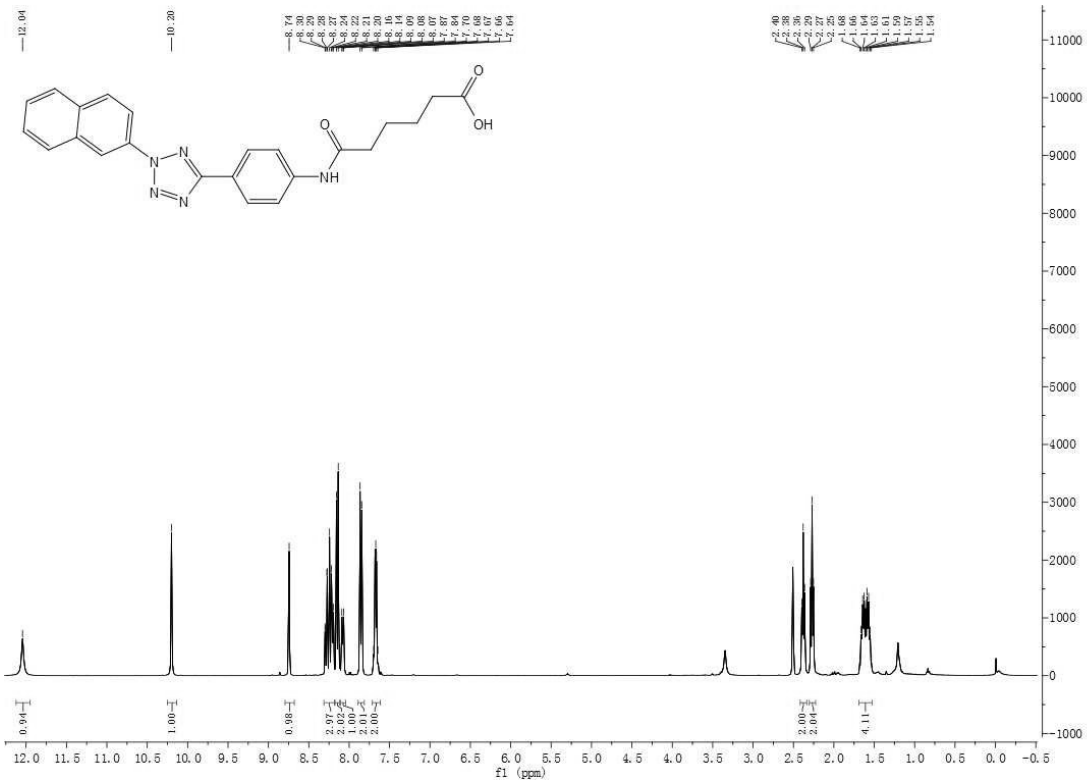
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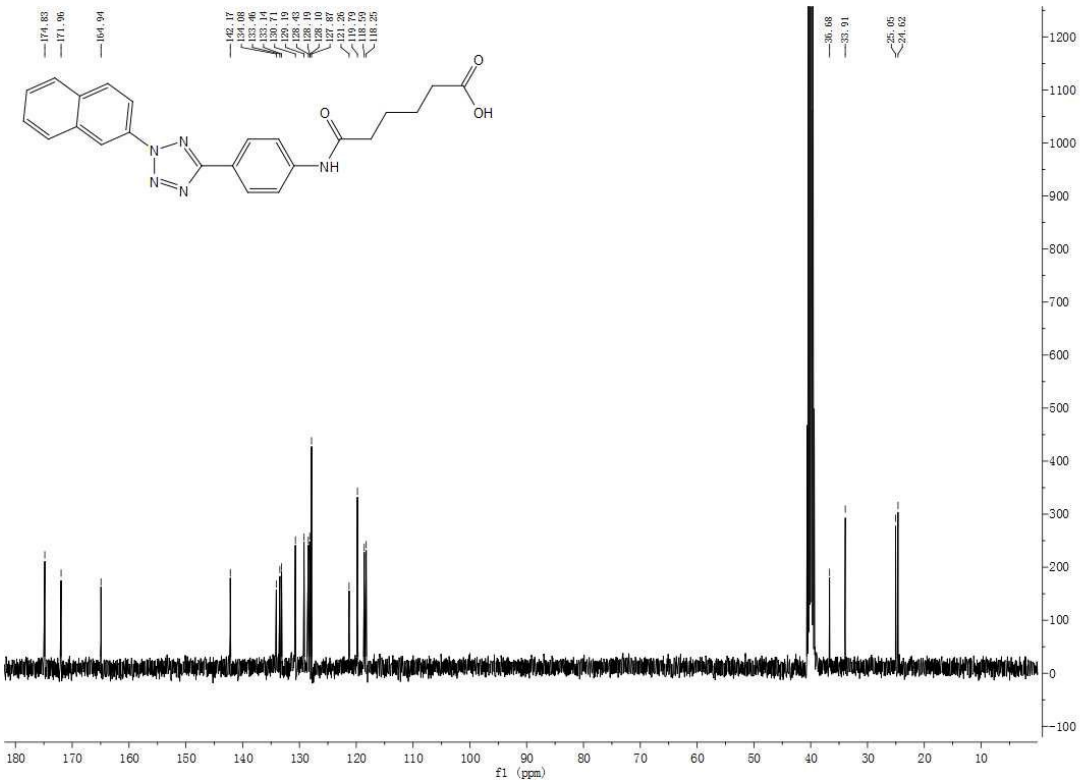
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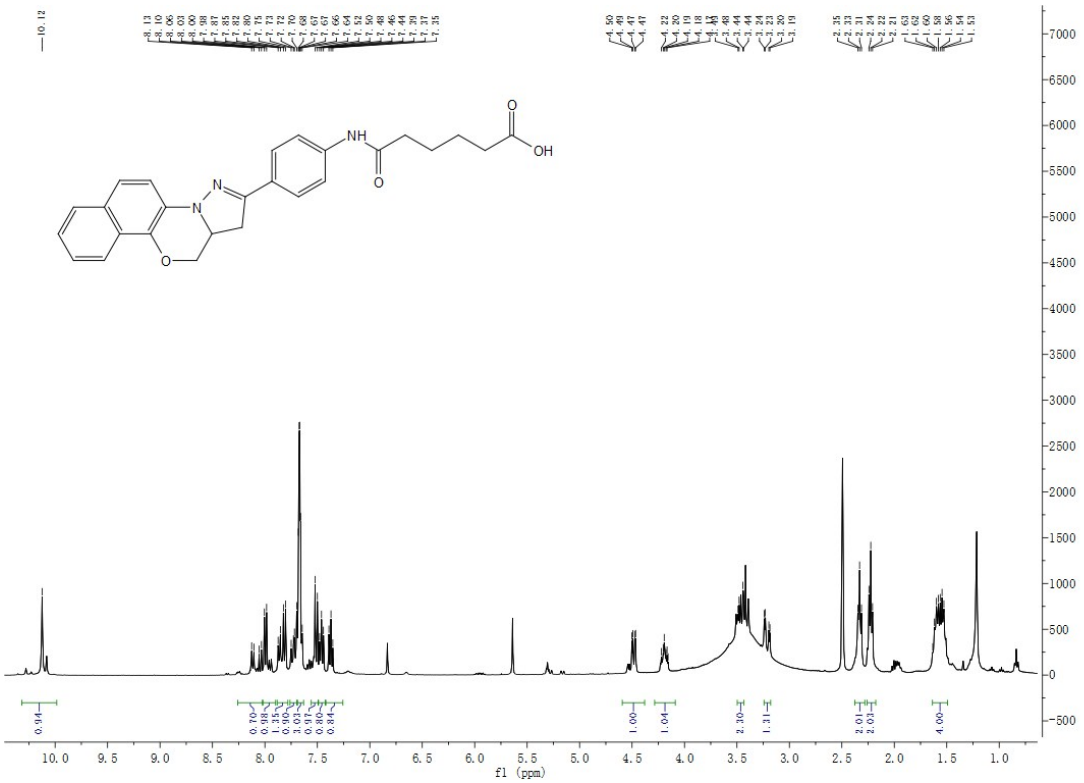
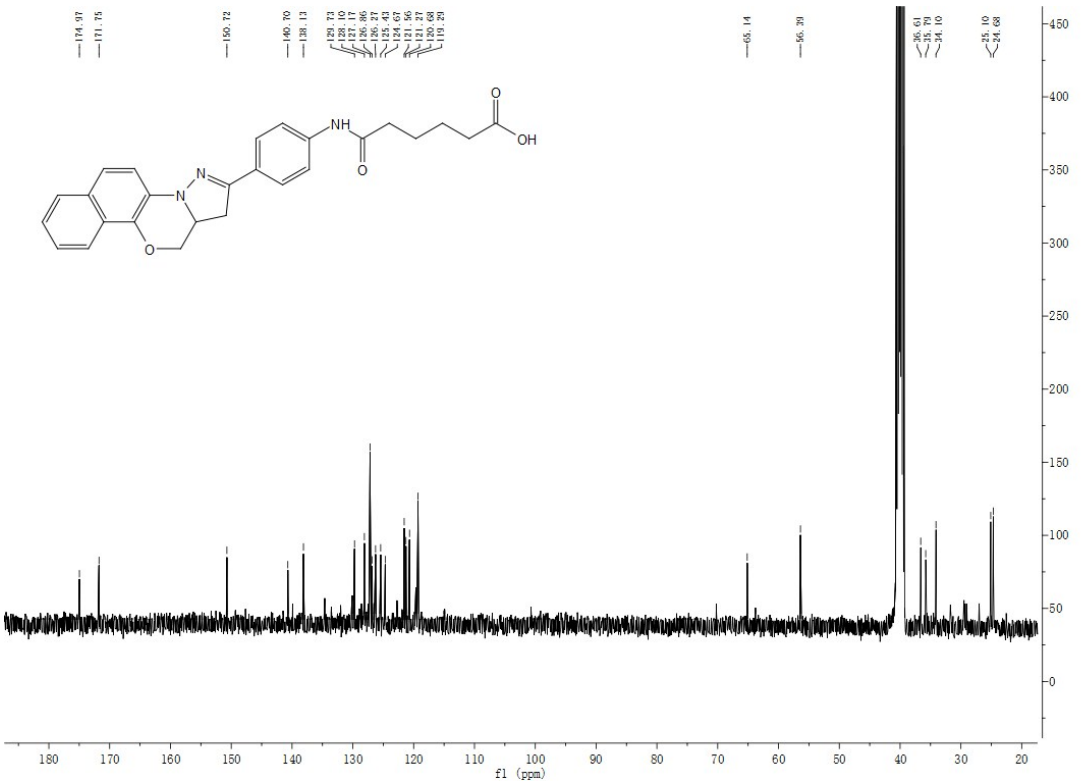
N
f1 (ppm)


NH
O
OH
N
O
f1 (ppm)


NH
O
OH
N
O
f1 (ppm)