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Supporting Information

A lysosome-targeting fluorescent probe to visualize endogenous and exogenous methylglyoxal in live cells and zebrafish

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1. Materials and instrumentation

All chemicals and materials used were purchased from commercial companies without further purification. All progress was monitored by thin layer chromatography (TLC), using GF254 silica gel precoated glass-backed plates and purification was performed by flash column chromatography separations using silica gel (200-300 mesh). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance 400 or Bruker DPX 300 spectrometer. High-resolution mass spectrometry was measured by the micrOTOF-Q II 10203 mass spectrometer with AP-ESI ion source. The confocal microscope model is LSM(Zeiss). Lyso-Tracker Red、Mito-Tracker Red、ER-Tracker Red and 3-(4,5-dimethylthia-zol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were received from Beyotime Biological Technology Co., Ltd. Fetal bovine serum (FBS), DMEM, and RPMI 1640 media were purchased from Thermo Fisher Scientific Co. Ltd (Shanghai, China).

2. Chemistry

*Synthesis of 2-(2-morpholinoethyl)-6-(piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione(**2a**).* To a solution of compound **1a** (100 mg, 0.26 mmol) in MeCN (4mL) was added with piperazine (67mg, 0.77 mmol). The reaction mixture was stirred at 80 °C for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a dark-yellow residue. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH = 10:1v/v) to furnish the desired compound **2a** as a yellow solid (50 mg, 54%).

*Synthesis of 6-(piperazin-1-yl)-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione(**2b**)***.** Compound **1b**(100 mg, 0.26 mmol)**,** MeCN (4mL) and piperazine (67mg, 0.77 mmol) were combined in a procedure analogous to that of 2a. An indentical workup gave 2b as a yellow solid (50 mg, 56%).*Synthesis of N-(4-amino-3-nitrophenyl)-2-chloroacetamide (4).* To a solution of 2-nitrobenzene-1,4-diamine **3** (500 mg, 3.26 mmol) in MeCN (8 mL) was added with K_2CO_3 (1.12 g, 8.15 mmol). The mixture was stirred at 0°C for 20 min and added with 2-chloroacetyl chloride (406 mg, 3.59 mmol). The mixture was stirred at the room temperature for overnight.

The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a dark-yellow residue. The residue was purified by chromatography on silica gel column (PE: EA = 2:1 v/v) to furnish the desired compound **4** as a brown solid (340mg, 45%).

*Synthesis of N-(4-amino-3-nitrophenyl)-2-(4-(2-(2-morpholinoethyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl) piperazin-1-yl) acetamide (**5a**).* To a solution of compound **2a** (95 mg, 0.24 mmol) in MeCN (5mL) was added with K_2CO_3 (179 mg, 0.55 mmol) and compound **4** (50 mg, 0.22 mmol). The mixture was stirred at 80 °C for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a greenish yellow residue. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH = 60:1v/v) to furnish the desired compound **5a** as a greenish yellow solid (62 mg, 48%).

*Synthesis of N-(4-amino-3-nitrophenyl)-2-(4-(1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl) piperazin-1-yl) acetamide (**5b**).* Compound **2b** (100 mg, 0.31 mmol)**,** MeCN (4 mL), K_2CO_3 (183 mg, 0.62 mmol) and compound **4** (70 mg, 0.46 mmol) were combined in a procedure analogous to that of 5a. An indentical workup gave 5b as a yellow solid (72 mg, 45%).

*Synthesis of N-(3,4-diaminophenyl)-2-(4-(2-(2-morpholinoethyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl) piperazin-1-yl) acetamide (**MGO-Naph-A**).* To a solution of compound **5a** (70 mg, 0.12 mmol) in 37%HCl (4 mL) was added with $SnCl_2$ (162 mg, 0.72 mmol). The mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the reaction was neutralized with saturated $NaHCO_3$ aqueous solution and then extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a greenish yellow residue. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH 50:1v/v) to furnish the desired compound ***MGO-Naph-A*** as a greenish yellow solid(56 mg, 85%). 1H NMR (400 MHz, DMSO) δ 9.20 (s, 1H), 8.53 – 8.38 (m, 4H), 7.83 (dd, J = 8.4, 7.4 Hz, 1H), 7.38

(d, J = 8.2 Hz, 1H), 6.88 (d, J = 2.3 Hz, 1H), 6.62 (dd, J = 8.2, 2.3 Hz, 1H), 6.44 (d, J = 8.2 Hz, 1H), 5.75 (s, 1H), 4.18 (t, J = 6.9 Hz, 3H), 3.57 – 3.50 (m, 6H), 2.87 (d, J = 3.8 Hz, 5H), 2.60 – 2.54 (m, 4H), 2.47 (d, J = 4.1 Hz, 4H). (Fig. S9).13C NMR (101 MHz, DMSO) δ 167.22, 163.97, 163.44, 156.07, 135.64, 132.66, 131.52, 131.10, 130.98, 129.77, 129.53, 126.47, 125.70, 122.91, 115.90, 115.52, 114.83, 109.62, 107.51, 66.59, 61.95, 56.05, 53.81, 53.15, 53.02, 49.07, 36.97. (Fig. S10); HR-MS m/z: calcd for 557.2751, found for 558.2853..

*Synthesis of N-(3,4-diaminophenyl)-2-(4-(1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl) piperazin-1-yl) acetamide (**MGO-Naph-B**)*.. To a solution of compound **5b** (50 mg, 0.10 mmol) in 37% HCl (4 mL) was added with $SnCl_2$ (150 mg, 0.6 mmol). The mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the reaction was neutralized with saturated $NaHCO_3$ aqueous solution and then extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a greenish yellow residue. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH 50:1v/v) to furnish the desired compound ***MGO-Naph-B*** as a yellow solid (39 mg, 83%) 1H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 8.53 – 8.39 (m, 3H), 7.86 – 7.78 (m, 1H), 7.37 (d, J = 8.2 Hz, 1H), 6.88 (d, J = 2.3 Hz, 1H), 6.62 (dd, J = 8.2, 2.3 Hz, 1H), 6.44 (d, J = 8.2 Hz, 1H), 4.04 – 3.98 (m, 2H), 3.20 (s, 2H), 2.88 (d, J = 12.6 Hz, 3H), 1.70 – 1.60 (m, 2H), 1.35 (d, J = 6.0 Hz, 1H), 1.23 (d, J = 2.9 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H). (Fig. S14).

*Synthesis of 6-azido-2-(2-morpholinoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**6**)*. To a solution of compound **1a** (100 mg, 0.26 mmol) in DMF (4 mL) was added with sodium azide (25 mg, 0.39 mmol). The mixture was stirred at 80 °C for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a dark-yellow residue. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH = 40:1v/v) to furnish the desired compound **6** as a yellow solid (72 mg, 80%).

*Synthesis of N-(4-bromo-2-nitrophenyl) acetamide (**8**).* Compound **7** (50 mg, 0.23 mmol) was dissolved in acetic acid(2 mL), and acetic anhydride (47 mg, 0.46 mmol) was added dropwise at 0°C, followed by 2 drops of concentrated sulfuric acid. The mixture was stirred at 90 °C for 5h and then was separated out with ice water, dried and used directly without further purification.

*Synthesis of N-(2-nitro-4-((trimethylsilyl) ethynyl) phenyl) acetamide (**9**).* Compound **8** (100 mg, 0.39 mmol), trimethyl ethynyl silicon (76 mg, 0.78 mmol), CuI (7 mg, 0.039 mmol), $(PPh_3)_2PdCl_2$ (14 mg, 0.019 mmol), triphenylphosphine (5 mg, 0.019 mmol) and TEA (1.95 mmol) were dissolved in MeCN (10 ml) under argon protection. The mixture was stirred at 115°C for 2h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was purified by chromatography on silica gel column (PE/EA = 20:1v/v) to furnish the desired compound **8** as yellow solid (75 mg, 63%).

*Synthesis of N-(4-ethynyl-2-nitrophenyl) acetamide (**10**).* Compound **9** (300 mg, 1.1 mmol) and KOH (183 mg, 3.3 mmol) were dissolved in ethanol/water (6 ml 2:1). The mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was purified by chromatography on silica gel column (PE/EA = 20:1v/v) to furnish the desired compound **9** as yellow solid (210 mg, 94%).

*Synthesis of N-(4-(1-(2-(2-morpholinoethyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)-2-nitrophenyl) acetamide (**11**).* Compound **10** (128 mg, 0.33 mmol), compound **6** (100 mg, 0.49 mmol), CuI (6 mg, 0.033 mmol) and sodium ascorbate (33 mg, 0.17 mmol) were dissolved in DMSO (4ml) under argon atmosphere. The mixture was stirred at 80 °C for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a greenish yellow residue. The residue was purified by

chromatography on silica gel column (CH_2Cl_2/MeOH = 50:1v/v) to furnish the desired compound **11** as a greenish yellow solid (310 mg, 87%).

*Synthesis of 6-(4-(3,4-diaminophenyl)-1H-1,2,3-triazol-1-yl)-2-(2-morpholinoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**MGO-Naph-C**).* To a solution of compound **11** (50 mg, 0.097 mmol) in 37% HCl (3mL) was added with $SnCl_2$ (130 mg, 0.58 mmol). The mixture was stirred at 80 °C for 2 h. After cooling to the room temperature, the reaction was neutralized with saturated $NaHCO_3$ aqueous solution and then extracted with EtOAc. The combined organic layers were washed with sat.brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a greenish yellow residue. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH = 30:1v/v) to furnish the desired compound **12** as a greenish yellow solid (24 mg, 55%). 1H NMR (400 MHz, DMSO) δ 8.63 (dd, J = 12.8, 8.4 Hz, 3H), 8.43 (d, J = 7.3 Hz, 2H), 8.23 – 8.15 (m, 2H), 7.65 (t, J = 7.8 Hz, 2H), 7.43 (s, 3H), 6.88 – 6.82 (m, 2H), 4.22 (d, J = 6.6 Hz, 1H), 4.15 (t, J = 6.8 Hz, 4H), 3.54 (s, 6H).(Fig. S15); HR-MS m/z: calcd for 483.2013, found for 484.2096.

3. General procedures for spectral measurements

The solution (10 mM) of **MGO-Naph-A** was prepared in DMSO. The test system was prepared by adding MGO-Naph-A (10 μM) into ultrapure water. The resulting solution was shaken and then fluorescence and UV absorption spectra were measured. Then the relevant test materials (amino acids, cations, anions, reactive oxygen species, etc.) were treated at 37°C for 1h, and the spectra were recorded. For all the measurements, the excitation wavelength was 377 nm, the emission range was 400-640 nm; the excitation slit width and the emission slit width were 5 nm, and the step was 1nm. For the fluorescent titration test, different concentrations of MGO test storage solution were added to the test probe solution and recorded after 60 min of incubation. For the selective spectrum test, requisite amount of analytes test storage solutions was added to the above test probe solutions, and then incubated for 60 min at 37°C. For the pH study, diluted the probe reserve solution to 10 μM with 0.025 mM HEPES buffer of different pH, then added 0.5 mM MGO, and then incubated for 60 min at 37°C.

4. Cytotoxicity determined by MTT assay

A549 cells were inoculated in 96-well plates at a density of 1×10^4 cells per well in RRMI1640 medium with 5% CO_2 and 95% air at 37°C. Then, 0, 0.1, 1,5, 10, 25 and 50μM (final concentration) MGO-Naph-A was cultured with A549 cells for 24 h. Finally, 10 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) was added and cells were cultured for another 4 h. When the purple precipitate was clearly visible under the microscope, 100 μl of DMSO was added to all the wells and swirled gently. Then, the absorbance of each well, including blank holes, was measured at 570 nm in a microplate reader. Cell activity was calculated using the following formula:

$$\text{Cell viability } \% = (OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100\%$$

In equation, OD sample denotes the cells incubated with various of concentrations of the probe, OD control denotes the cells without the probe, and OD blank denotes the wells containing only the culture medium.

5. Fluorescence imaging

For the imaging of exogenous MGO, the A549 cells were seeded in a 35 mm Petri dish with a density of 1×10^4 cells/well for 24 h. Then, A549 cells were incubated with 10μM MGO-Naph-A for 1 h and washed three times with PBS buffer. Subsequently, the cells were treated with various concentrations of MGO (100, 500 μM) for 5 h, respectively, and washed with PBS buffer. The cells were imaged with laser confocal scanning microscope.

For the fluorescence imaging experiment of the endogenous MGO in the living A549 cells, the A549 cells were incubated with the probe MGO-Naph-A (10 μM) for 1h at 37 °C, then washed with PBS buffer three times. For the negative control experiment, the A549 cells were incubated with NAC (1 mM) for 12h at 37 °C, then washed with PBS buffer three times. Subsequently, the cells were incubated with the probe MGO-Naph-A for 1h at 37 °C. For the positive control experiment, MGO (500 μM) was added to the second group and incubated for 5h. The residual probe was removed by washing three times with PBS before the imaging experiment. At the same time, we also imaged endogenous MGO induced by the MGO inhibitor

curcumin. A549 cells were first incubated with different concentrations (0, 10, 20, and 50 μM) of Curcumin for 6h and further treated with MGO-Naph-A (10 μM) for another 1h. Finally, the fluorescence images of the cells were observed with a confocal laser scanning microscope (CLSM).

For the colocation fluorescence imaging experiment, the A549 cells were incubated with 10μM probe MGO-Naph-A for 1h, then the culture medium of the cells was changed to a fresh media containing 100nM Lyso-Tracker Red and further incubated for 15 min. The residual probe was removed by washing three times with PBS before the imaging experiment. Meanwhile, we further verified the colocalization effect by inducing lysosomal rupture with chloroquine. A549 cells were first incubated with MGO-Naph-A (10 μM) for 1 h and further stimulated by 25 and 50 μM chloroquine for another 1 h. After cleaning with PBS for three times, images were obtained by CLSM.

For the imaging of exogenous MGO in zebrafish, 3-day-old zebrafishes were transferred into confocal plates, and 10 μM probe MGO-Naph-A was fed for 60 min; then, 500 μM MGO was added and further incubated for another 60 min. After that, the zebrafishes were transferred into new glass bottom dishes for imaging. The excitation and emission wavelengths were the same as for fluorescence imaging in living cells.

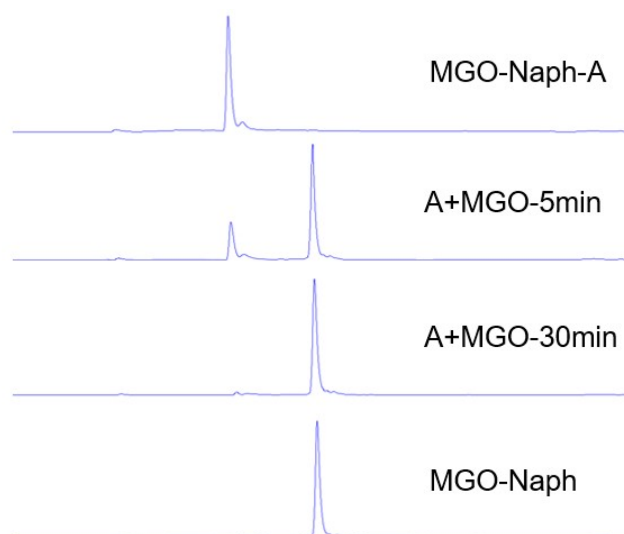

Fig. S1 The reaction process was monitored by HPLC, all compounds were determined by high performance liquid chromatography (HPLC) using a C18 column (Agilent Technologies, 1220

Infinity LC). The mobile phase was a mixture of CH_3CN and H_2O containing 0.1% formic acid. Peaks were detected at 254 nm. The temperature was maintained at 30℃ at a flow rate of 1 mL/min, the injection volume was 10 μl.

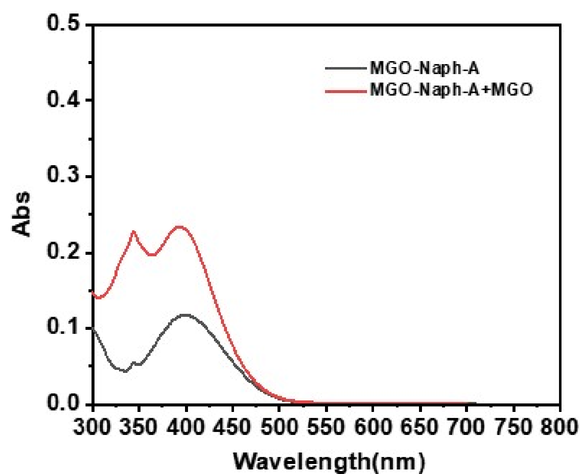

Fig. S2 The absorption and emission spectra of MGO-Naph-A (10 μM) in ultrapure water.

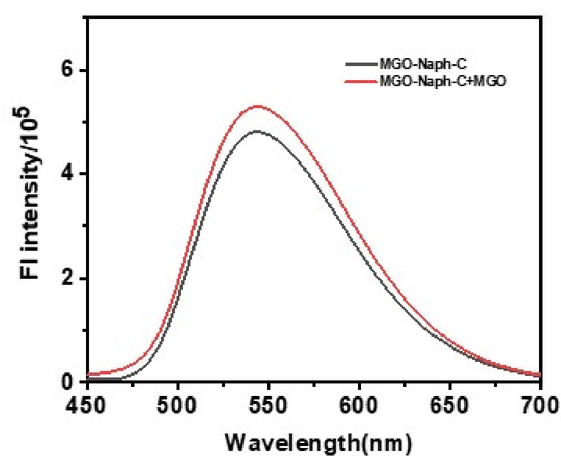

Fig. S3 The Fluorescence spectra of MGO-Naph-C (10 μM) in ultrapure water.

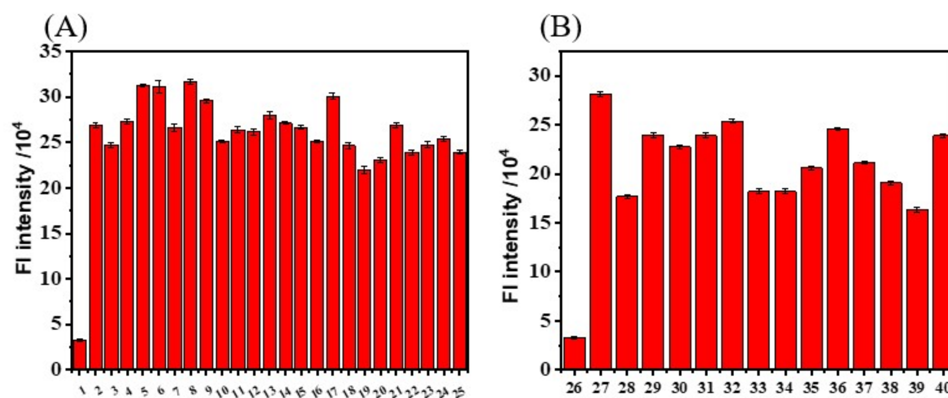

Fig. S4 Fluorescence intensities of MGO-Naph-A upon the addition of various species and

MGO;(A). 1.blank, 2. Ba^{2+}, 3. Ca^{2+}, 4. Cd^{2+}, 5. Co^{2+}, 6. Cr^{2+}, 7. Cu^{2+}, 8. Fe^{2+}, 9. Hg^{2+}, 10. K^{+}, 11. Li^{+}, 12. Mg^{2+}, 13. Mn^{2+}, 14. Ni^{2+}, 15. Pb^{2+}, 16. Sr^{2+}, 17. Zn^{2+}, 18. Br^{-}, 19. F^{-}, 20.NO^{2-}, 21.NO^{3-}, 22.SCN^{-}, 23.SO_3^{2-}, 24.SO_4^{2-}, 25. FA. 26.blank, 27.BA, 28. FA, 29. GO, 30.OPA, 31. Ala, 32. Cys, 33. Leu, 34. Lle, 35. Phe, 36. Pro, 37. Ser, 38. Val, 39. VC, 40.FA.

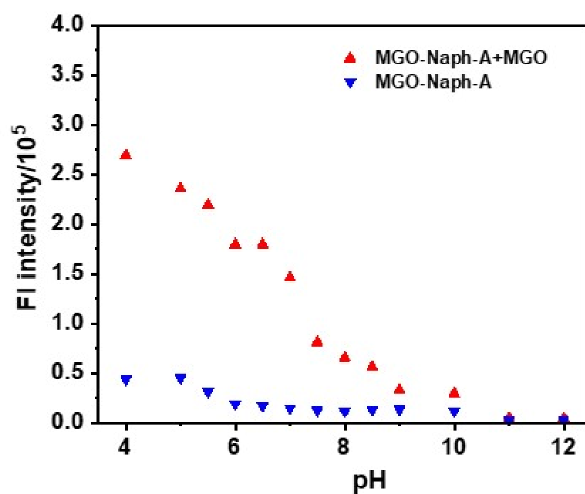

Fig. S5 Effects of pH on the fluorescence of MGO-Naph-A (10 μM) before and after reacting with 500 μM MGO

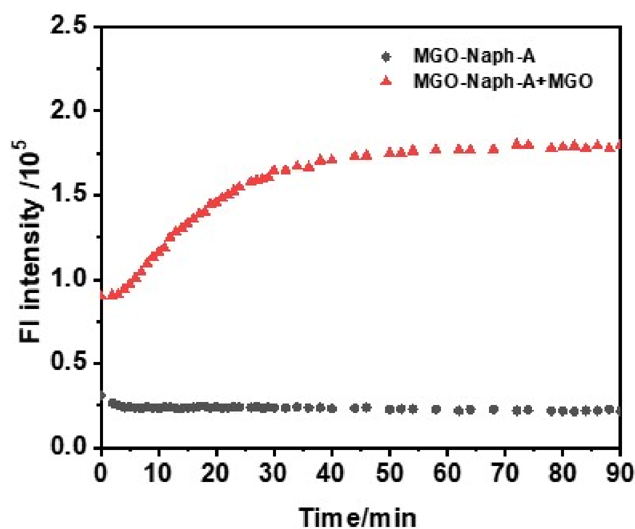

Fig. S6 Effects of reaction time on the fluorescence of MGO-Naph-A (10 μM) in the absence of any MGO or in the presence of 500 μM MGO.

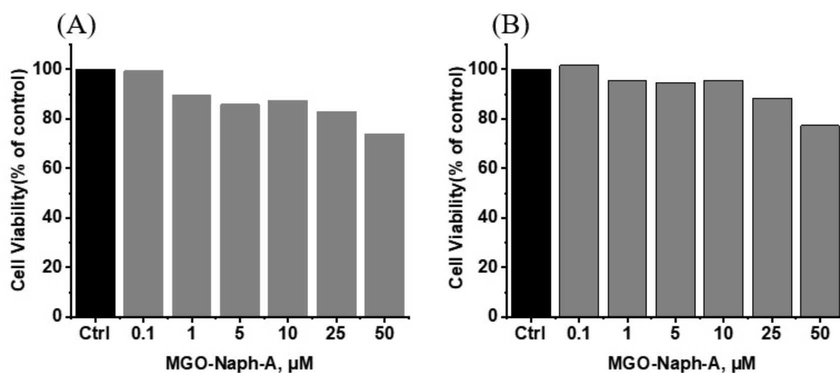

Fig. S7 Viability of A549 (A) and Hela (B) cells treated with MGO-Naph-A at 37°C for 12 h.

6. Characterization data of probe

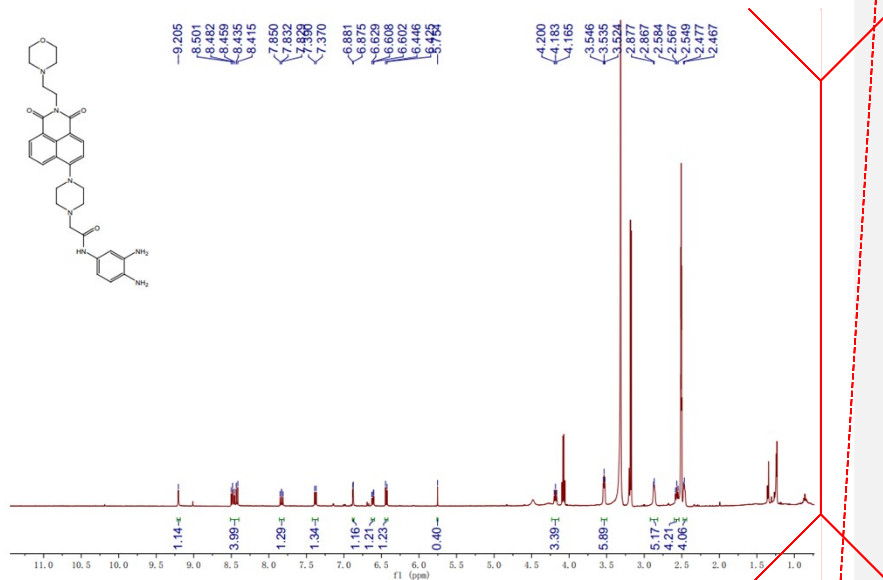

Fig. S9 ^{1}H NMR spectrum of MGO-Naph-A

Comment [LF]: ^{1}H NMR spectrum of MGO-Naph-A

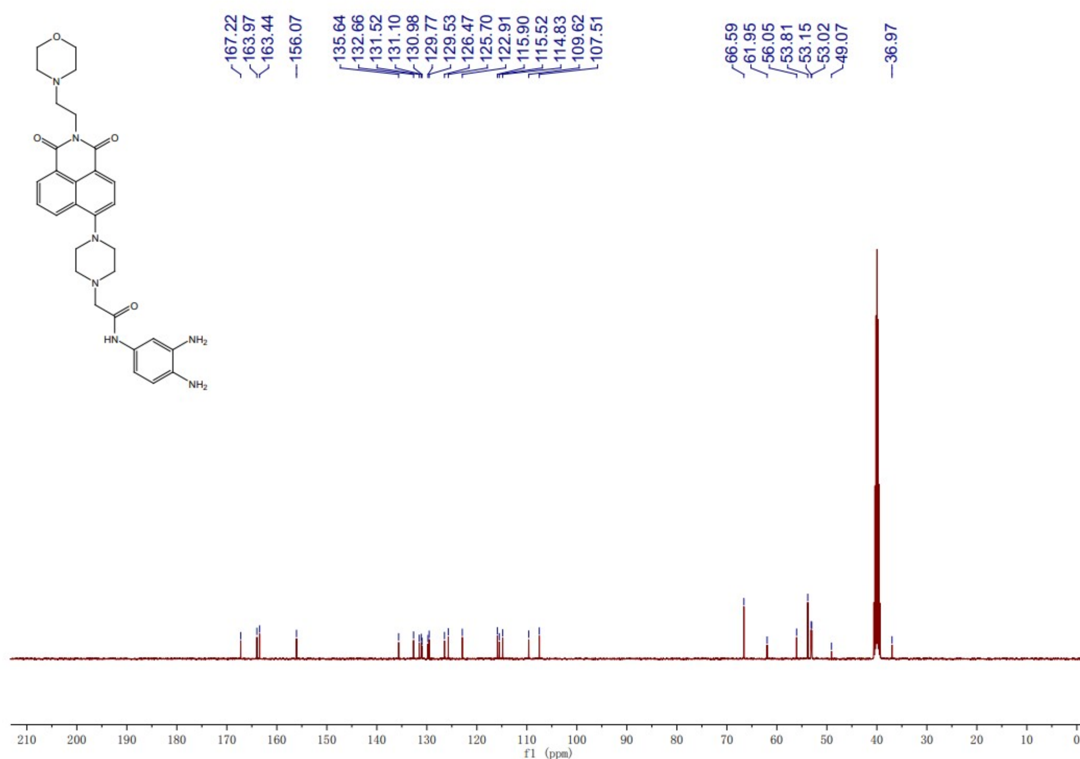

Fig. S10 ^{13}C NMR spectrum of MGO-Naph-A

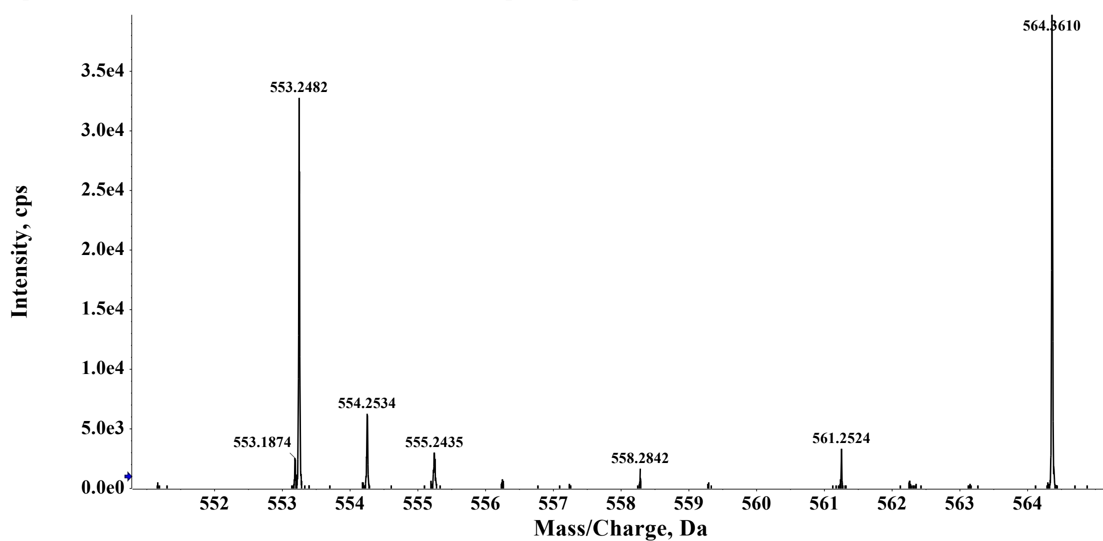

Fig. S10 LC-MS spectra of MGO-Naph-A

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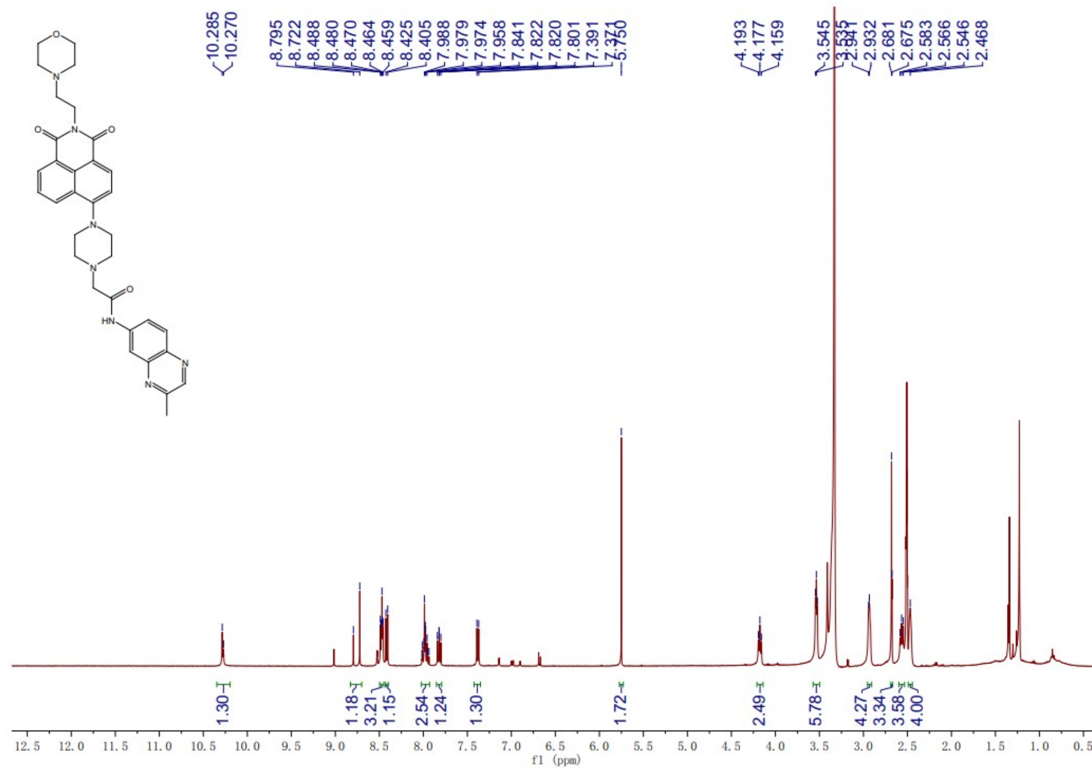

Fig. S11 ^{1}H NMR spectrum of MGO-Naph

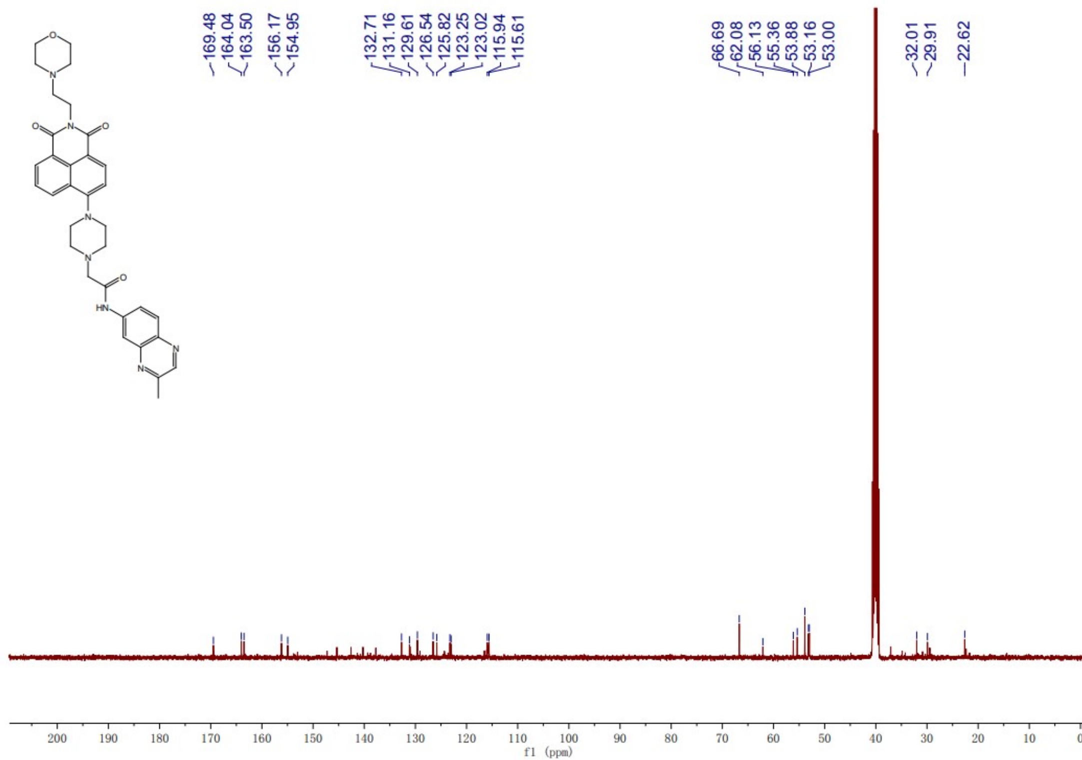

Fig. S12 ^{13}C NMR spectrum of MGO-Naph

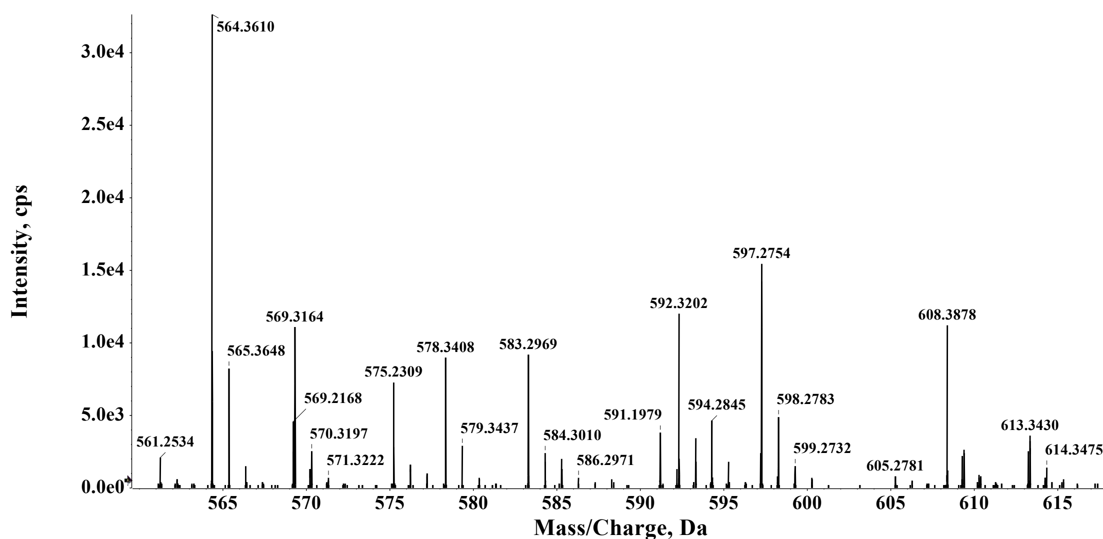

Fig. S13 LC-MS spectra of MGO-Naph

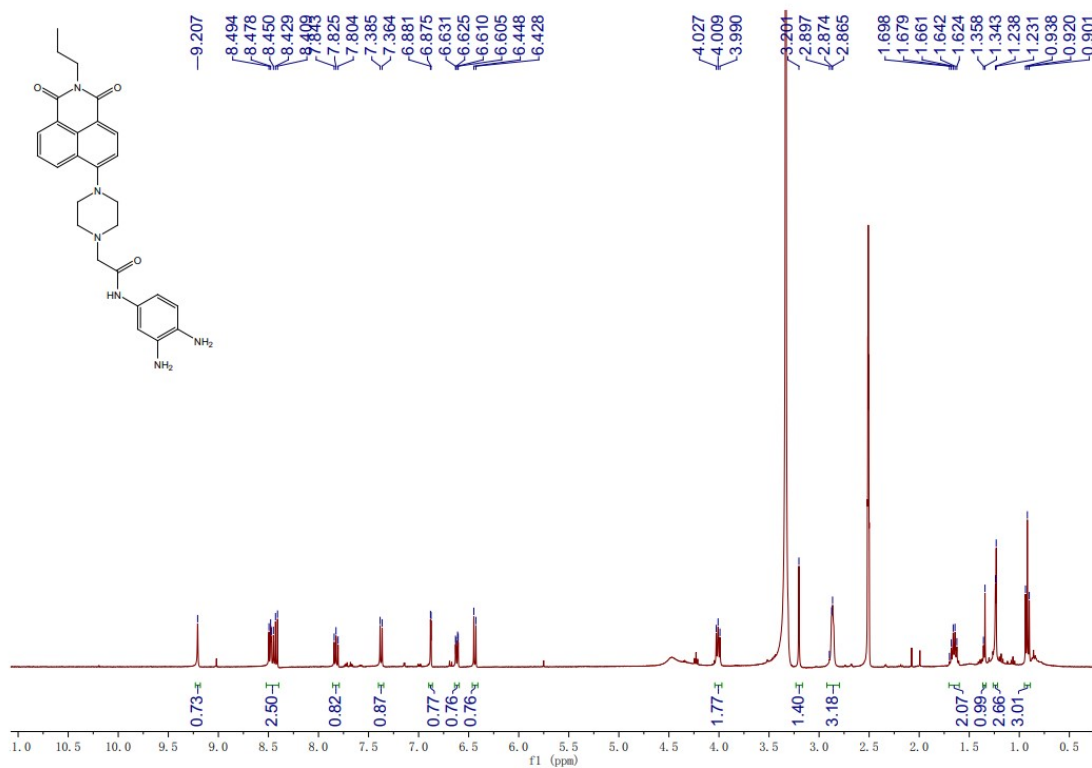

Fig. S14 ^{1}H NMR spectrum of MGO-Naph-B

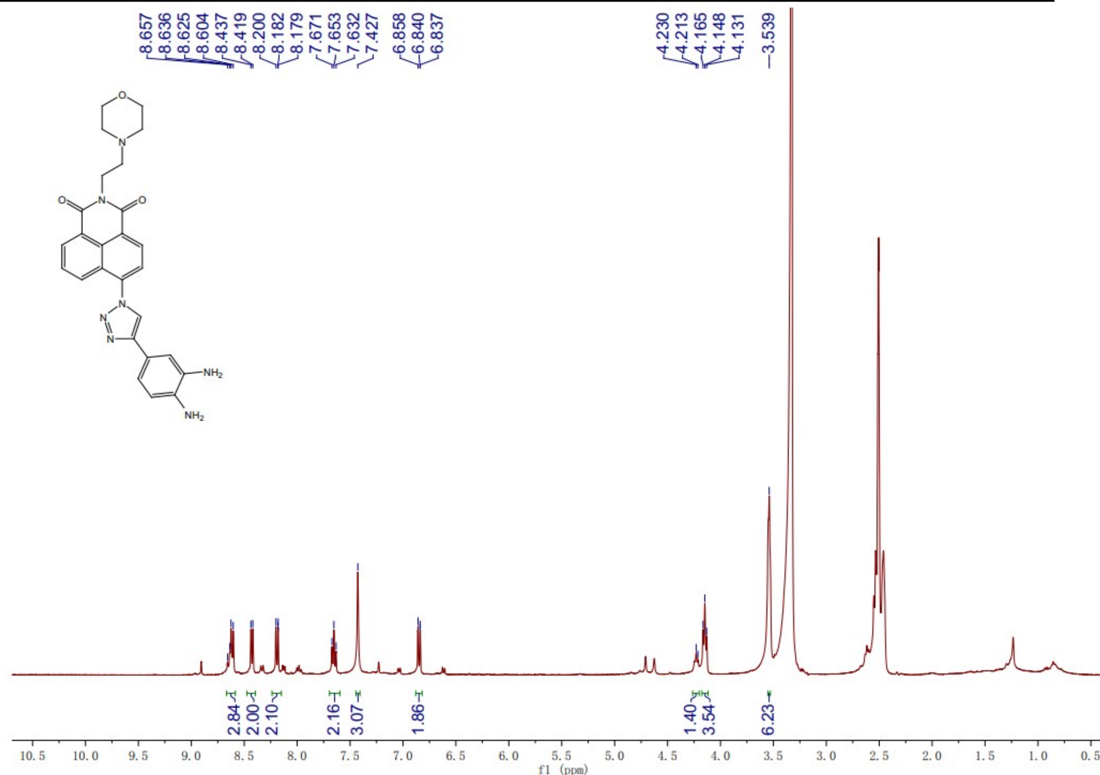

Fig. S15 ^{1}H NMR spectrum of MGO-Naph-C

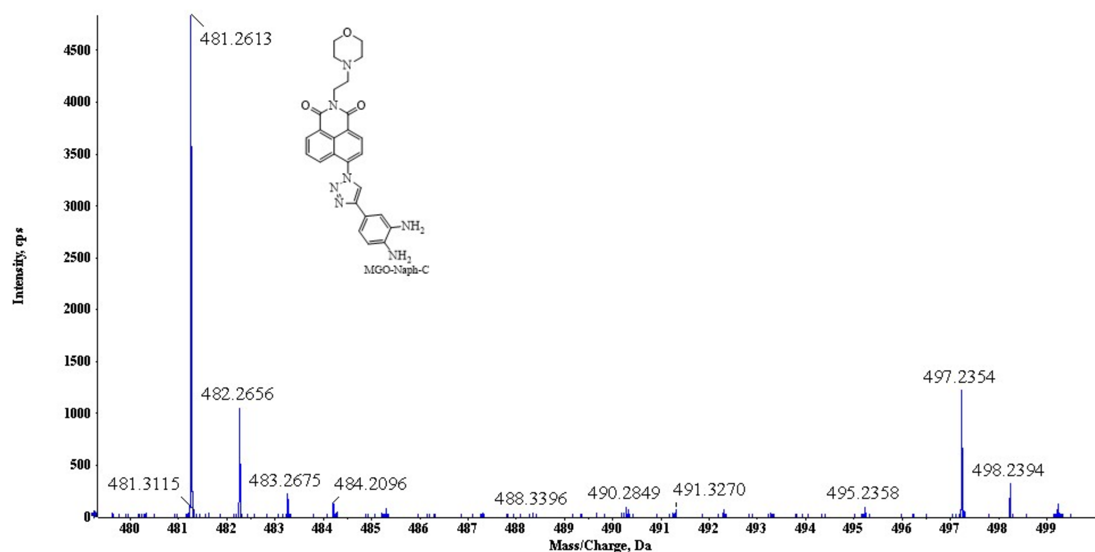

Fig. S16 HRMS spectra of MGO-Naph-C