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Supporting information

A Naphthalimide-Based Fluorescent Platform for Endoplasmic Reticulum Targeted Imaging

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Chemicals

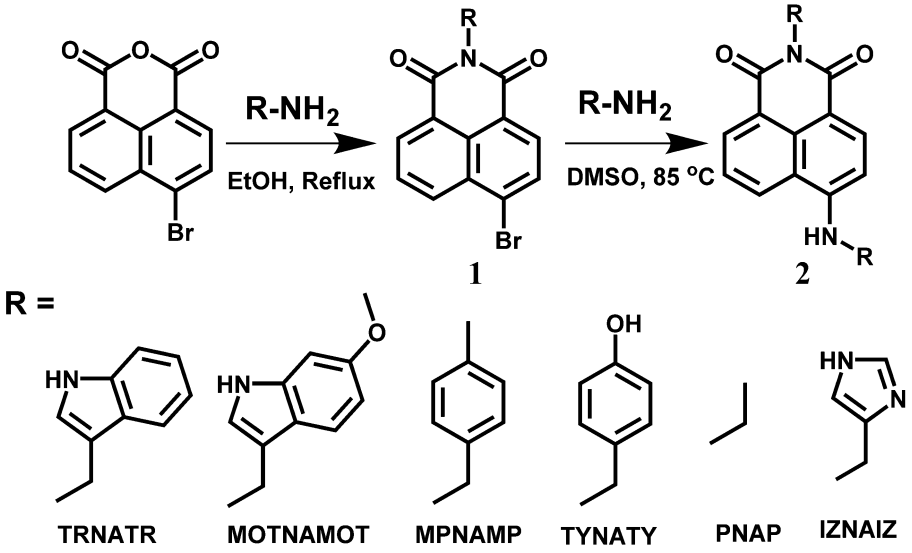
4-Bromo-1,8- naphthalic anhydride was purchased from Liaoning Liangang Dye Chemical Co. Ltd. Tryptamine, 5-methoxytryptamine, 4-methylphenethylamine, tyramine, propylamine, and histamine were purchased from J&K Scientific. Tunicamycin was purchased from Juhemei Biotechnology Co. Ltd. ER-Tracker Red (ER tracker), MitoTracker Orange (Mito tracker) and Cell Counting Kit-8 (CCK-8) were purchased from KeyGEN Biotech. LysoTracker Red (Lyso tracker) were purchased from Beyotime Biotech. RPMI-1640 medium, fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Gibco. HeLa cells was purchased from the cell bank of Shanghai Bioscience Center, Chinese Academy of Sciences. All other reagents were purchased from Beijing Chemical Factory.

Instruments

High-resolution mass spectra (HR-MS ESI) were measured on a solarix FT mass spectrometer (Bruker). ^{1}H NMR and ^{13}C NMR spectra were recorded on Avance III 400, 500 and 600 NMR spectrometer (Bruker). Fluorescence spectra were collected on an F-4600 fluorescent spectrophotometer (Hitachi). Effect of pH on the fluorescence was measured on a SpectraMax M2e Reader (MD). Fluorescence quantum yields were measured on a Horiba FLuoroMax+ spectrophotometer. The absorbance for cell counting kit-8 (CCK-8) analysis was recorded on a SpectraMax M2e Reader (MD). Absorption spectra were recorded on a UH5300 spectrophotometer (Hitachi). Confocal images were recorded on a FV3000-IX83 confocal microscope (Olympus). Cells analysis were collected on a FACScalibur flow cytometer (BD).

Synthesis

Scheme S1. Synthesis route of dyes.



Compound 1: 4-Bromo-1,8- naphthalic anhydride (2.0 g, 7.22 mmol) and 8.38 mmol of different compounds (tryptamine, 5-methoxytryptamine, 4-methylphenethylamine, tyramine, propylamine, and histamine, respectively) were refluxed in EtOH (ethanol) for 2 h. After cooling to room temperature, a lot of precipitates were precipitated when added a large amount of water. These precipitates were collected through vacuum filtration, washed with water and ethanol respectively, and then vacuum dried to obtain a white solid product.

Compound 2 (fluorescent dyes): Different compound 1 (1.1 mmol) and 2.2 mmol of different compounds (tryptamine, 5-methoxytryptamine, 4-methylphenethylamine, tyramine, propylamine,

and histamine, respectively) were dissolved in 10 mL DMSO, then stirred 12 h at 85 °C. After removing the solvent through rotary evaporation, the crude product is purified by column chromatography (dichloromethane/methanol) to obtain a yellow product.

TRNATR (^{1}H NMR (400 MHz, DMSO) δ 10.87 (d, *J* = 11.9 Hz, 1H), 8.71 (d, *J* = 8.0 Hz, 1H), 8.49 (d, *J* = 6.7 Hz, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 7.95 (t, *J* = 5.4 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.71 (dd, *J* = 8.2, 7.5 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.36 (dd, *J* = 8.1, 0.6 Hz, 1H), 7.29 (d, *J* = 2.2 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.13 – 7.06 (m, 1H), 7.05 – 6.96 (m, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 4.34 – 4.25 (m, 1H), 3.70 (dd, *J* = 13.3, 6.9 Hz, 1H), 3.16 (t, *J* = 7.4 Hz, 1H), 3.05 – 2.96 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 164.26, 163.40, 151.06, 136.79, 136.72, 134.84, 131.18, 130.01, 129.09, 127.75, 127.69, 124.75, 123.49, 123.24, 122.43, 121.50, 121.46, 120.66, 118.98, 118.82, 118.71, 112.04, 111.92, 111.85, 108.12, 104.39, 44.24, 24.40. HR-MS (ESI): m/z calcd for $C_{32}H_{25}N_4O_2^-$ $[M]^-$ 497.19830, found, 497.19856).

MOTNAMOT (^{1}H NMR (500 MHz, DMSO) δ 10.69 (d, *J* = 18.8 Hz, 2H), 8.70 (d, *J* = 8.5 Hz, 1H), 8.48 (d, *J* = 7.2 Hz, 1H), 8.32 (d, *J* = 8.5 Hz, 1H), 7.92 (t, *J* = 5.4 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 2.2 Hz, 1H), 7.26 – 7.22 (m, 3H), 7.18 (d, *J* = 2.0 Hz, 1H), 7.03 (d, *J* = 2.2 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 6.73 (ddd, *J* = 8.7, 6.4, 2.4 Hz, 2H), 4.34 – 4.22 (m, 2H), 3.76 (s, 3H), 3.73 – 3.65 (m, 5H), 3.12 (t, *J* = 7.3 Hz, 2H), 3.02 – 2.94 (m, 2H). ^{13}C-NMR (126 MHz, DMSO) δ 164.29, 163.43, 153.51, 153.48, 151.07, 134.83, 131.93, 131.82, 131.20, 130.03, 129.08, 128.12, 128.04, 124.74, 124.14, 123.88, 122.44, 120.67, 112.56, 111.89, 111.64, 111.51, 108.10, 104.40, 100.92, 100.48, 55.74, 55.47, 44.25, 24.41, 24.35. HR-MS (ESI): m/z calcd for $C_{34}H_{29}N_4O_4^-$ $[M]^-$ 557.21943, found, 557.21915).

MPNAMP (^{1}H NMR (600 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 7.3, 0.8 Hz, 1H), 8.48 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.59 (dd, *J* = 8.2, 7.5 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 2H), 7.18 (s, 4H), 7.13 (d, *J* = 7.8 Hz, 2H), 6.78 (d, *J* = 8.4 Hz, 1H), 5.29 (t, *J* = 4.7 Hz, 1H), 4.41 – 4.27 (m, 2H), 3.67 (dd, *J* = 12.2, 6.8 Hz, 2H), 3.07 (t, *J* = 6.9 Hz, 2H), 3.03 – 2.92 (m, 2H), 2.36 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 164.11, 163.25, 150.90, 136.59, 136.32, 135.67, 134.77, 131.15, 129.91, 129.50, 129.44, 129.14, 129.02, 128.94, 124.79, 122.33, 120.62, 108.16, 104.46, 45.04, 41.21, 34.01, 33.76, 21.12. HR-MS (ESI): m/z calcd for $C_{30}H_{27}N_2O_2^-$ $[M]^-$ 447.20780, found, 447.20803).

TYNATY (^{1}H NMR (600 MHz, DMSO) δ 9.21 (s, 2H), 8.67 (d, *J* = 7.8 Hz, 1H), 8.44 (dd, *J* = 7.2, 0.8 Hz, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 7.86 (s, 1H), 7.69 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.09 (dd, *J* = 37.7, 8.4 Hz, 3H), 6.85 (d, *J* = 8.7 Hz, 1H), 6.73 – 6.64 (m, 3H), 4.24 – 4.09 (m, 2H), 3.55 (d, *J* = 8.5 Hz, 2H), 2.95 – 2.84 (m, 2H), 2.82 – 2.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 163.00, 162.15, 155.21, 155.16, 151.72, 149.80, 133.62, 129.99, 129.08, 128.90, 128.79, 128.65, 128.45, 127.88, 123.61, 121.23, 119.50, 114.65, 114.61, 107.04, 103.30, 55.46, 44.26, 40.37, 32.61, 32.31. HR-MS (ESI): m/z calcd for $C_{28}H_{23}N_2O_4^-$ $[M]^-$ 451.16633, found, 451.16661).

PNAP (^{1}H NMR (400 MHz, DMSO) δ 8.71 (d, *J* = 8.4 Hz, 1H), 8.48 – 8.37 (m, 1H), 8.26 (d, *J* = 8.5 Hz, 1H), 7.78 (t, *J* = 5.5 Hz, 1H), 7.68 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 4.07 – 3.90 (m, 2H), 3.47 – 3.27 (m, 3H), 1.67 (ddq, *J* = 41.0, 14.8, 7.4 Hz, 4H), 0.95 (dt, *J* = 35.7, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 164.24, 164.07, 163.42, 163.38, 156.93, 151.24, 134.72, 132.68, 131.91, 131.06, 130.94, 130.05, 129.97, 129.88, 129.41, 125.38, 124.57, 124.53, 122.65, 122.11, 120.55, 113.63, 113.35, 107.64, 104.09, 44.91, 44.81, 41.33, 41.16, 29.46, 28.21, 21.58,

21.42, 21.36, 12.01, 11.85, 11.82. HR-MS (ESI): m/z calcd for $C_{18}H_{19}N_2O_2^-$ $[M]^-$ 295.14520, found, 295.14532).

IZNAIZ (^{1}H NMR (400 MHz, DMSO) δ 14.40 (s, 4H), 9.01 (d, *J* = 16.6 Hz, 2H), 8.67 (d, *J* = 8.3 Hz, 1H), 8.42 (d, *J* = 7.1 Hz, 1H), 8.25 (d, *J* = 8.5 Hz, 1H), 7.93 (s, 1H), 7.75 – 7.65 (m, 1H), 7.53 (s, 1H), 7.44 (s, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 3.72 (s, 2H), 3.06 (dd, *J* = 15.4, 6.3 Hz, 4H), 2.55 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 164.13, 163.26, 158.73, 158.49, 158.23, 151.04, 135.37, 134.68, 131.06, 129.86, 129.24, 124.64, 122.20, 121.15, 120.57, 118.77, 116.39, 114.01, 107.95, 104.19, 43.47, 40.71. HR-MS (ESI): m/z calcd for $C_{22}H_{19}N_6O_2^-$ $[M]^-$ 399.15750, found, 399.15760).

The ^{1}H NMR, ^{13}C NMR and HR-MS spectra of the six different fluorescent dyes are shown in **Fig. S15~S20, Fig. S21~S26** and **Fig. S27~S32**.

General methods

The obtained six fluorescent dyes were dissolved in DMSO to prepare a stock solution with a concentration of 10.0 mM, respectively.

(1) UV-Vis absorption spectra

The six different fluorescent dyes were diluted to 20.0 μM by different solvents (PBS, Acetonitrile, Ethanol, Dichloromethane, N, N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), Methanol, and Ethyl acetate) respectively, and then the UV-Vis absorption spectra were recorded on a UH5300 spectrophotometer.

(2) Fluorescence spectra

The six different fluorescent dyes were diluted to 2.0 μM by different solvents (PBS, Acetonitrile, Ethanol, Dichloromethane, N, N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), Methanol, and Ethyl acetate) respectively, and then the fluorescence spectra were recorded on an F-4600 spectrophotometer (λ_{ex} 445 nm; Ex slit width, 2.5 nm; Em slit width, 5 nm).

(3) Quantum yield

TRNATR and MOTNAMOT were diluted to 3.5 μM in ethyl acetate. MPNAMP, TYNATY, PNAP, and IZNAIZ were diluted to 3.5 μM in DMSO. Then the quantum yields of the six different fluorescent dyes were measured on a Horiba FLuoroMax+ spectrophotometer.

(4) Effect of pH on the fluorescence intensity

To study the effect of pH on the fluorescence spectra, 2.0 μM of six different fluorescent dyes were dissolved in PBS with different pH values. Then, the fluorescence spectra (λ_{ex} 445 nm) were measured on a SpectraMax M2e Reader.

(5) Effect of surfactant types on the fluorescence spectra

SDS (an anionic surfactant), CTAB (a cationic surfactant), and Triton X-100 (a nonionic surfactant) were selected for the study of their effect on the fluorescence spectra of the obtained six dyes. The test samples were prepared by dissolving of different six fluorescent dyes (1.0 μM) in H_2O, SDS (8 mM), CTAB (1 mM), and Triton X-100 (0.3 %) respectively, then the fluorescence spectra were collected (Ex, 445 nm, slit width, 5 nm).

Cell culture and cytotoxicity assay

HeLa cells was cultured in RPMI-1640 cell medium containing 10 % FBS and 1 % penicillin/streptomycin in a cell culture incubator containing 5% CO_2 at 37 ℃. For cytotoxicity assay, HeLa cells were seeded into 96-well plates with 100 μL per well (approximately 5×10^3

cells/well). After incubating overnight, different concentrations of the obtained six fluorescent dyes were added to the wells, respectively. In order to ensure the credibility of the data, three samples were made in parallel for each concentration. After the cells were further incubated in the incubator for 48 h, the cytotoxicity was detected using a CCK-8 method.

Flow cytometry

Flow cytometry was used to study the cellular uptake of different six fluorescent dyes by living cells. HeLa cells were cultured in a 6-well plates for 24 h firstly, and then incubated with different six fluorescent dyes (1.0 μM) for 1 h. After washed 3 times with PBS, the cells were analyzed by flow cytometry.

Cell imaging

HeLa cells (3×10^5 cells/dish) were inoculated and cultured in confocal dishes for 24 h. Then the cells were washed twice with PBS, and incubated with 1.0 μM of different dyes in RPMI-1640 cell medium for 50 min respectively. After washed 3 times with PBS, the cells were imaged on a FV3000-IX83 confocal microscope.

For the colocalization studies, HeLa cells (3×10^5 cells/dish) were inoculated and cultured in confocal dishes for 24 h firstly. Then the cells were washed twice with PBS, and incubated with 1.0 μM of different dyes in RPMI-1640 cell medium for 50 min, respectively. The cells were washed twice with PBS, and incubated with ER tracker, Mito tracker or Lyso tracker for 30 min, respectively. After washed 3 times with PBS, the cells were imaged on a FV3000-IX83 confocal microscope.

For the effect of ER stress study caused by tunicamycin, HeLa cells (3×10^5 cells/dish) were inoculated and cultured in confocal dishes for 24 h firstly. After washing twice with PBS, the cells were incubated with 10 μM of tunicamycin for 24 h. After washing twice, the cells were incubated with 1.0 μM of TRNATR or TYNATY for 50 min, respectively. The cells were washed twice with PBS, and then incubated with ER tracker or Lyso tracker for 30 min, respectively. After washed 3 times with PBS, the cells were imaged on a FV3000-IX83 confocal microscope.

For the study of ER stress caused by nutritional starvation, HeLa cells (3×10^5 cells/dish) were inoculated and cultured in confocal dishes for 24 h firstly. After washing twice with PBS, the cells were incubated in RPMI-1640 cell medium for 24 h. After washing twice, the cells were incubated with 1.0 μM of TRNATR or TYNATY for 50 min, respectively. The cells were washed twice with PBS, and then incubated with ER tracker or Lyso tracker for 30 min, respectively. After washed 3 times with PBS, the cells were imaged on a FV3000-IX83 confocal microscope.

Fluorescence images of the fluorescent dyes were collected in the range of 460-560 nm under excitation of 445 nm laser. Fluorescence images of ER tracker, Mito tracker and Lyso tracker were collected in the range of 575 - 650 nm under excitation of 561 nm laser.

Table S1. Quantum yield and stokes shift of the dyes

Dyes	**Quantum yield (%)**	**Stokes shift (nm)**
TRNATR	54.86*	87
MOTNAMOT	51.94*	85
MPNAMP	80.76	88
TYNATY	85.05	89
PNAP	78.49	85
IZNAIZ	94.01	87

*Ethyl acetate as the solvent for the quantum yield measurement of TRNATR and MOTNAMOT.

All other data measured in DMSO.

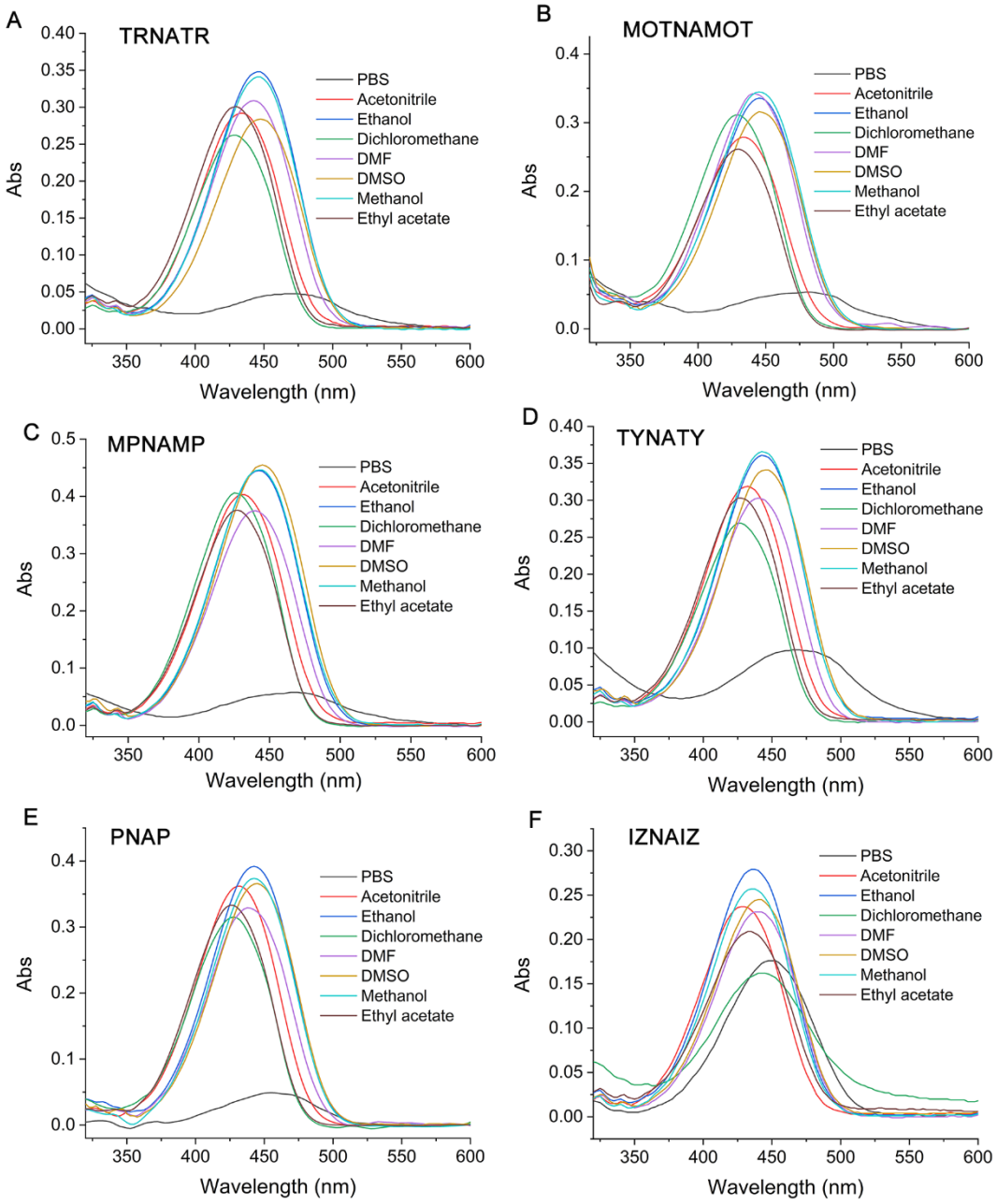


Fig. S1 UV-Vis spectra of different fluorescent dyes in different solvents (dyes, 20.0 μM).

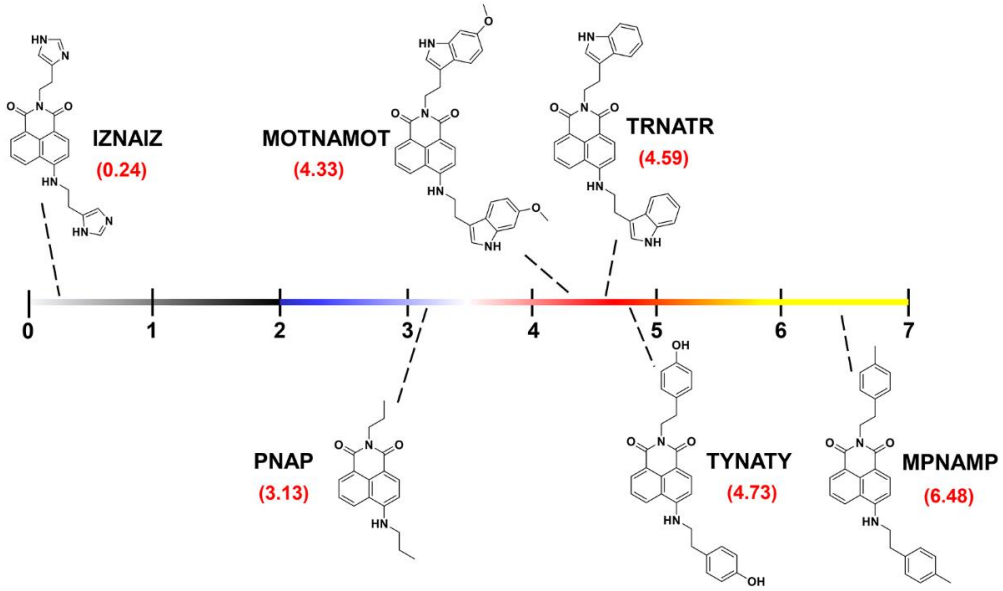


Fig. S2 The clogP values of naphthalimide fluorescent dyes.

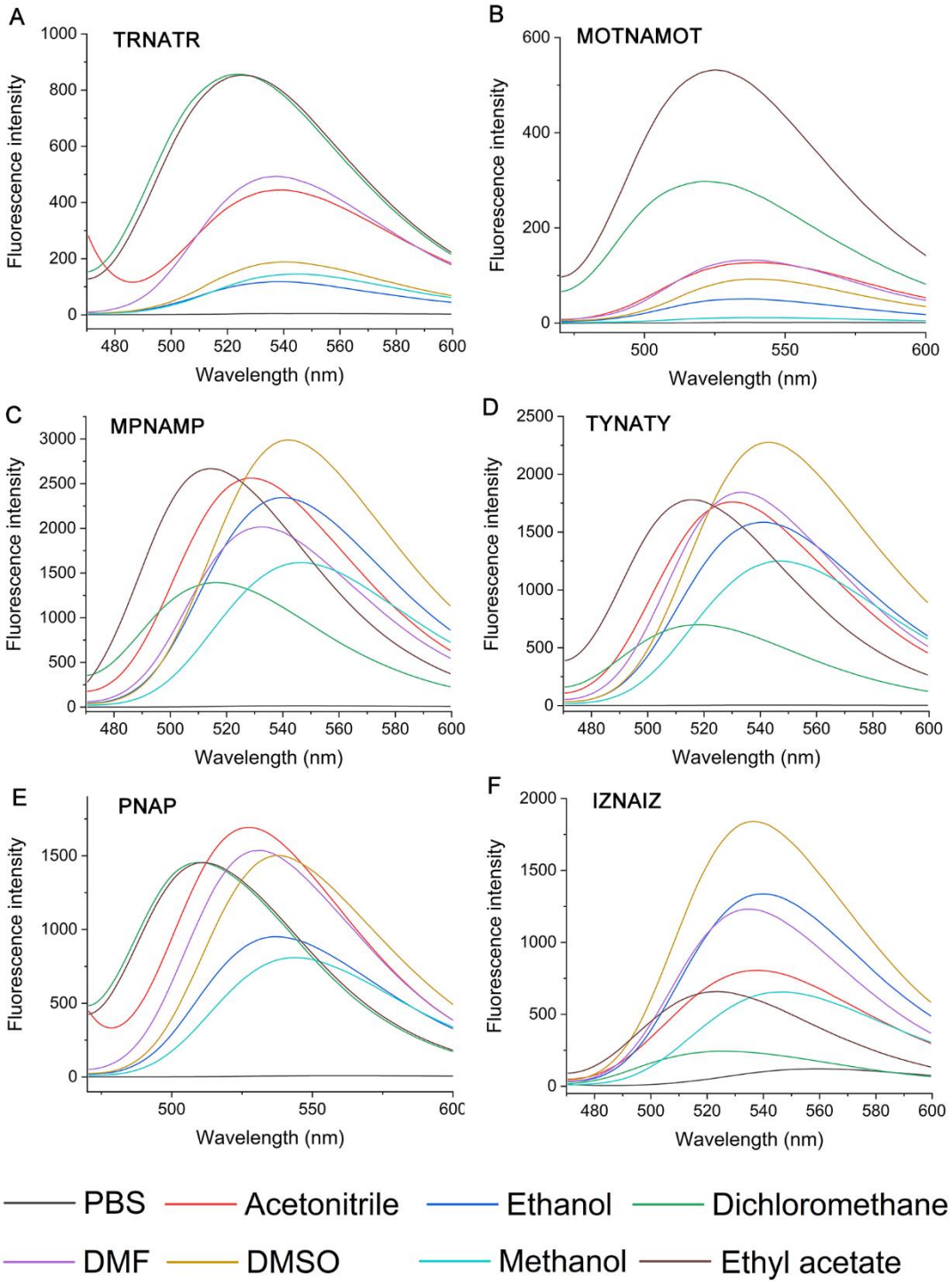


Fig. S3 Fluorescence spectra of different fluorescent dyes in different solvents (dyes, 2.0 μM; Ex, 445 nm; Ex slit width, 2.5 nm; Em slit width, 5 nm).

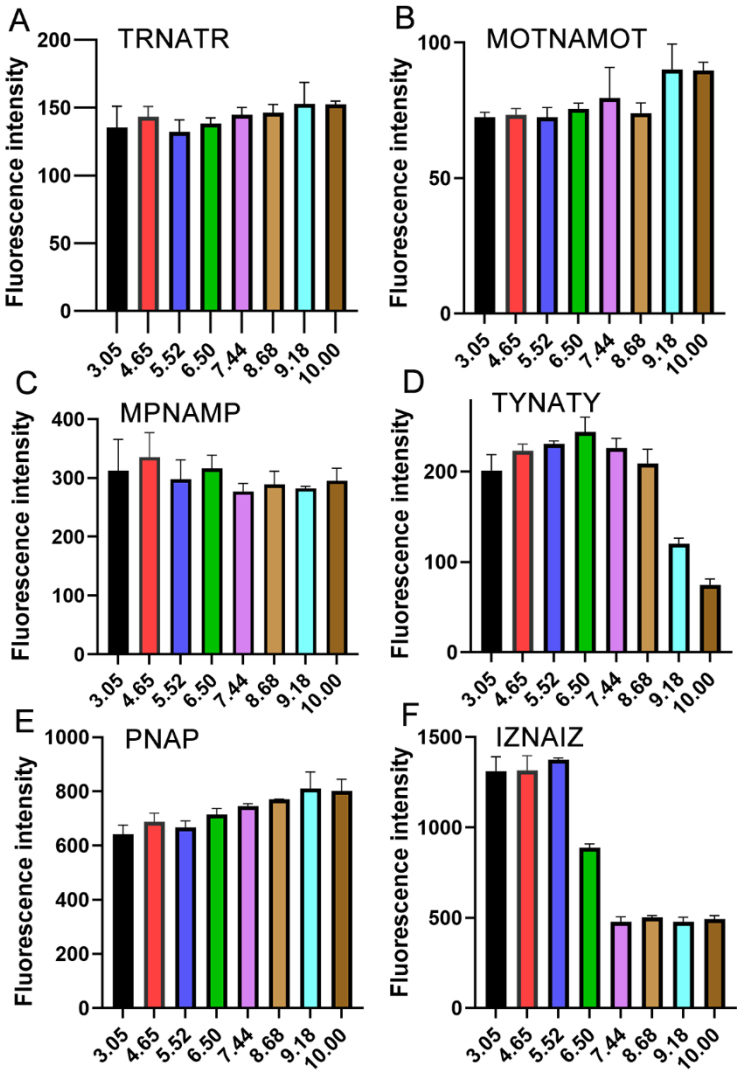


Fig. S4 Effect of pH on the fluorescence of different dyes (dyes, 2.0 μM; Ex, 445 nm).

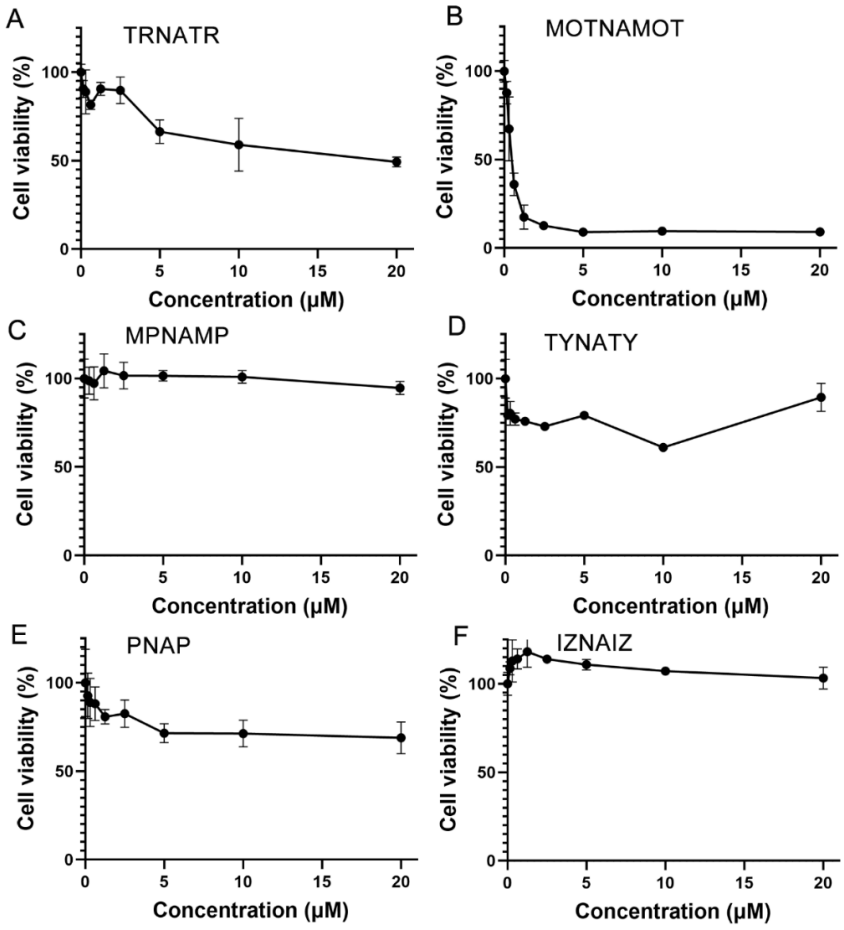


Fig. S5 Cytotoxicity of different fluorescent dyes on HeLa cells.

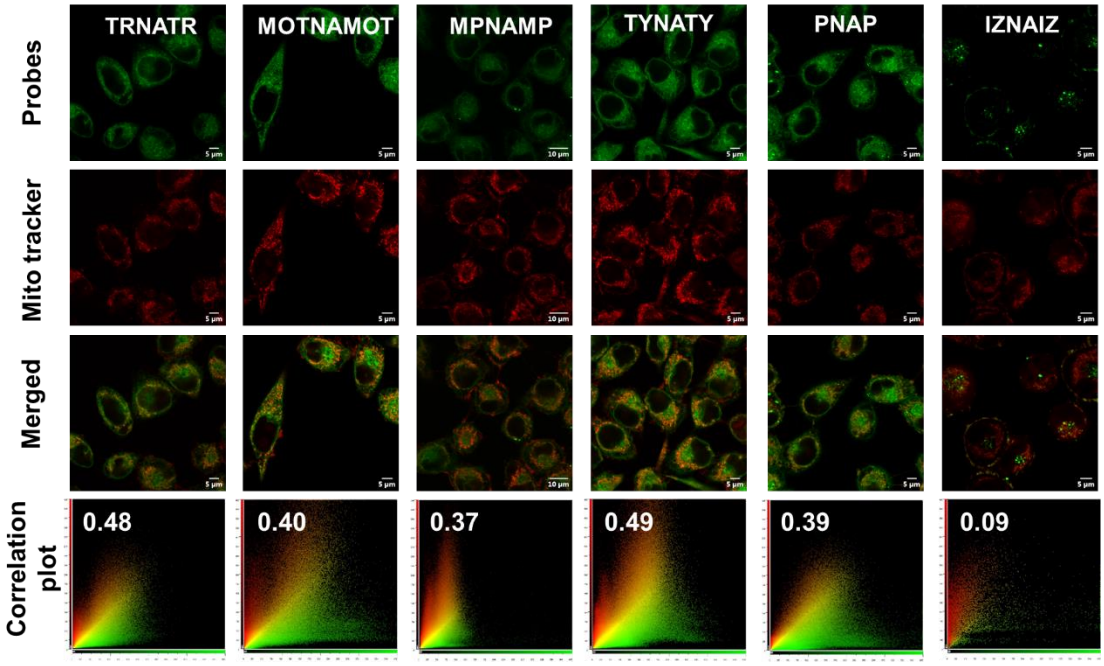


Fig. S6 Colocalization imaging of different dyes with Mito tracker.

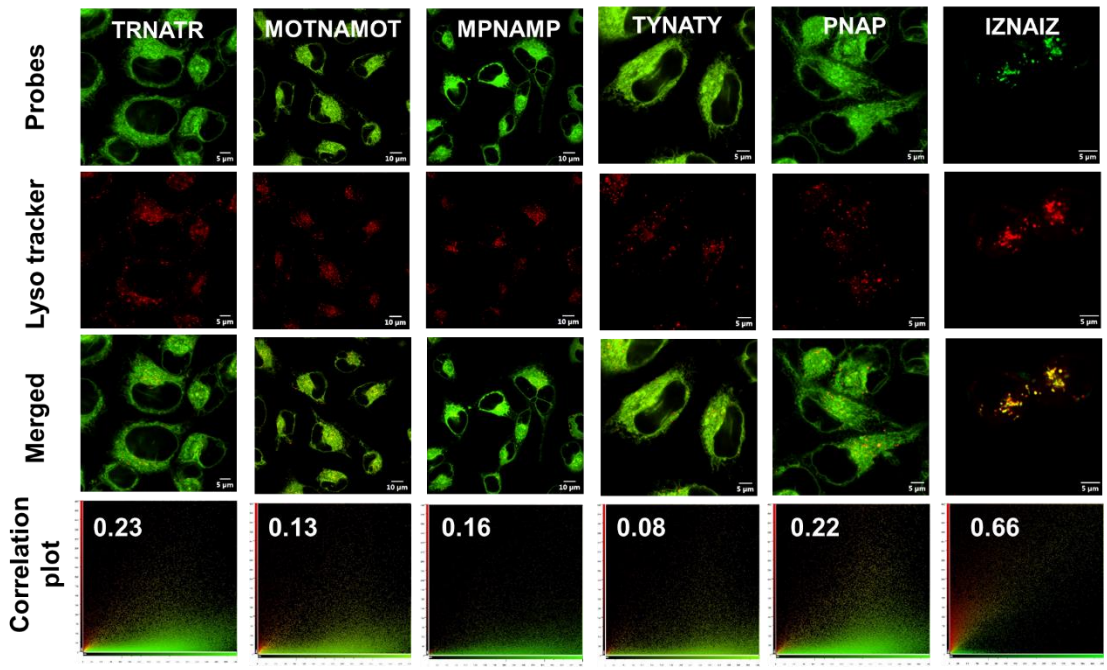


Fig. S7 Colocalization imaging of different dyes with Lyso tracker.

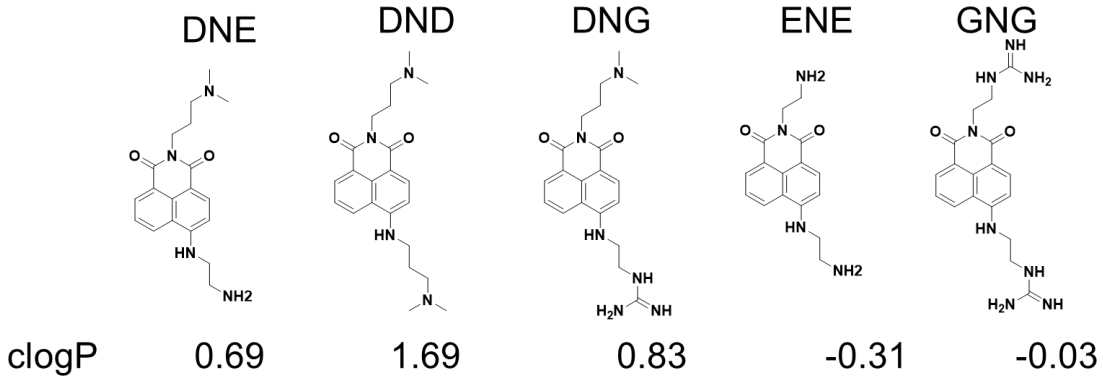


Fig. S8 The structures of naphthalimide compounds with different amine and/or guanidine side chains and their clogP values.

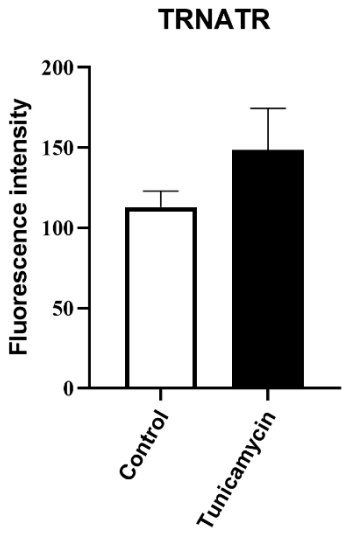


Fig. S9 Fluorescence intensity of TRNATR in confocal images before and after stress treatment by tunicamycin.

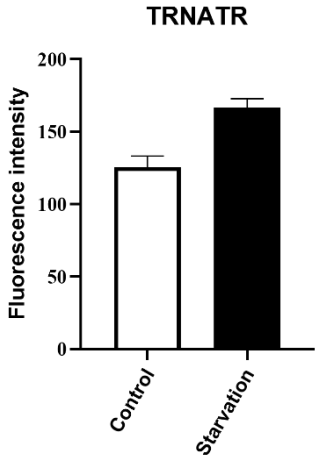


Fig. S10 Fluorescence intensity of TRNATR in confocal images before and after stress treatment by nutritional starvation.

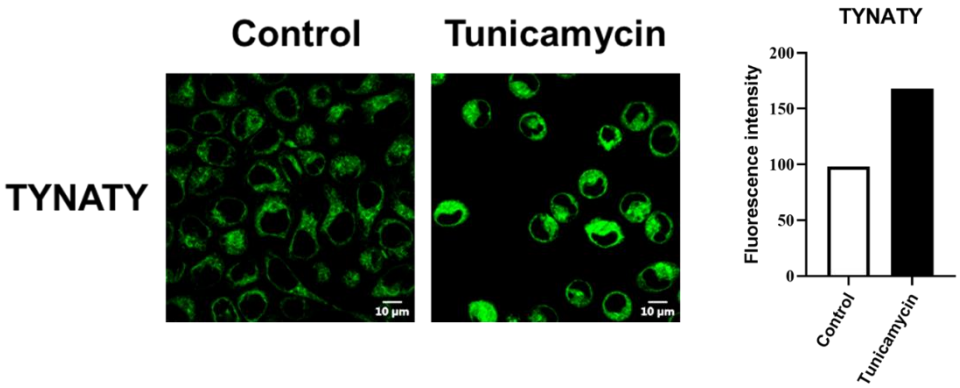


Fig. S11 Confocal images and fluorescence intensity of TYNATY in HeLa cells under ER stress caused by tunicamycin.

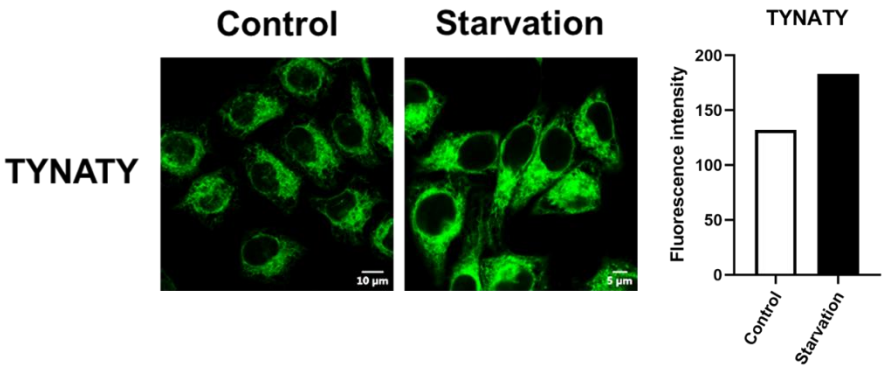


Fig. S12 Confocal images and fluorescence intensity of TYNATY in HeLa cells under ER stress caused by nutritional starvation.

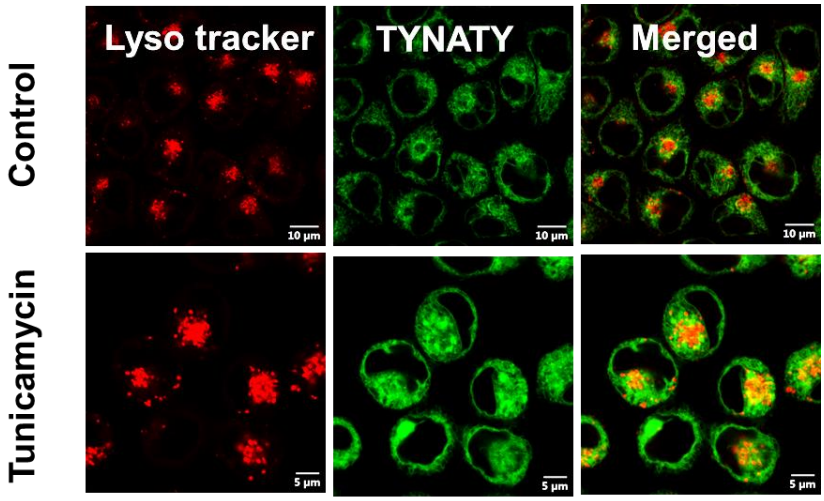


Fig. S13 Colocalization imaging of TYNATY with Lyso tracker under ER stress caused by tunicamycin.

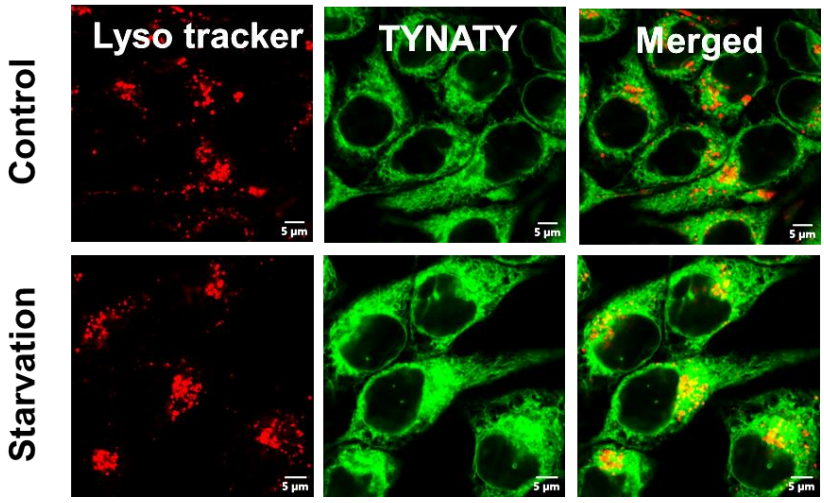


Fig. S14 Colocalization imaging of TYNATY with Lyso tracker under ER stress caused by nutritional starvation.

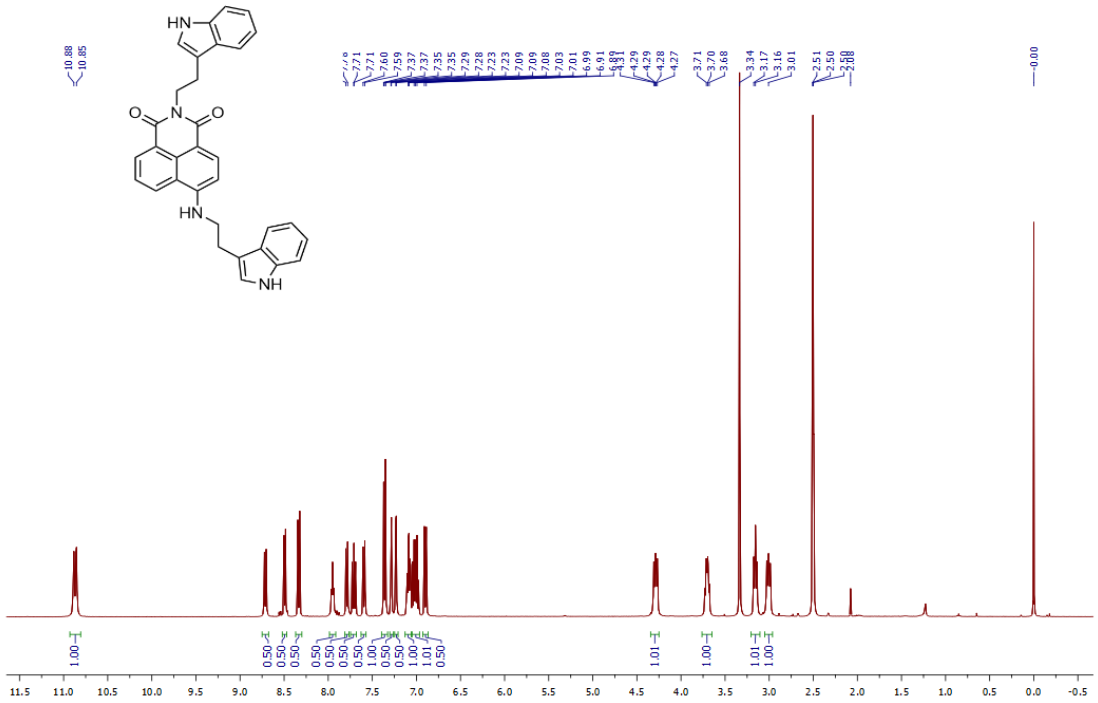


Fig. S15 ^{1}H NMR spectrum of TRNATR.

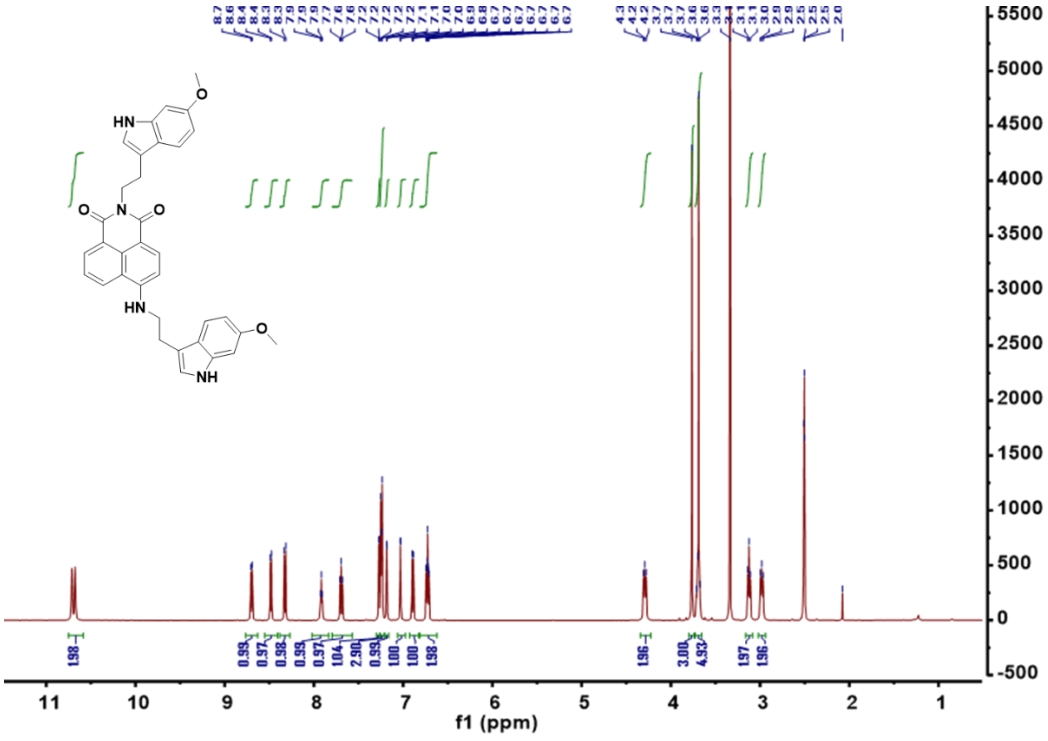


Fig. S16 ^{1}H NMR spectrum of MOTNAMOT.

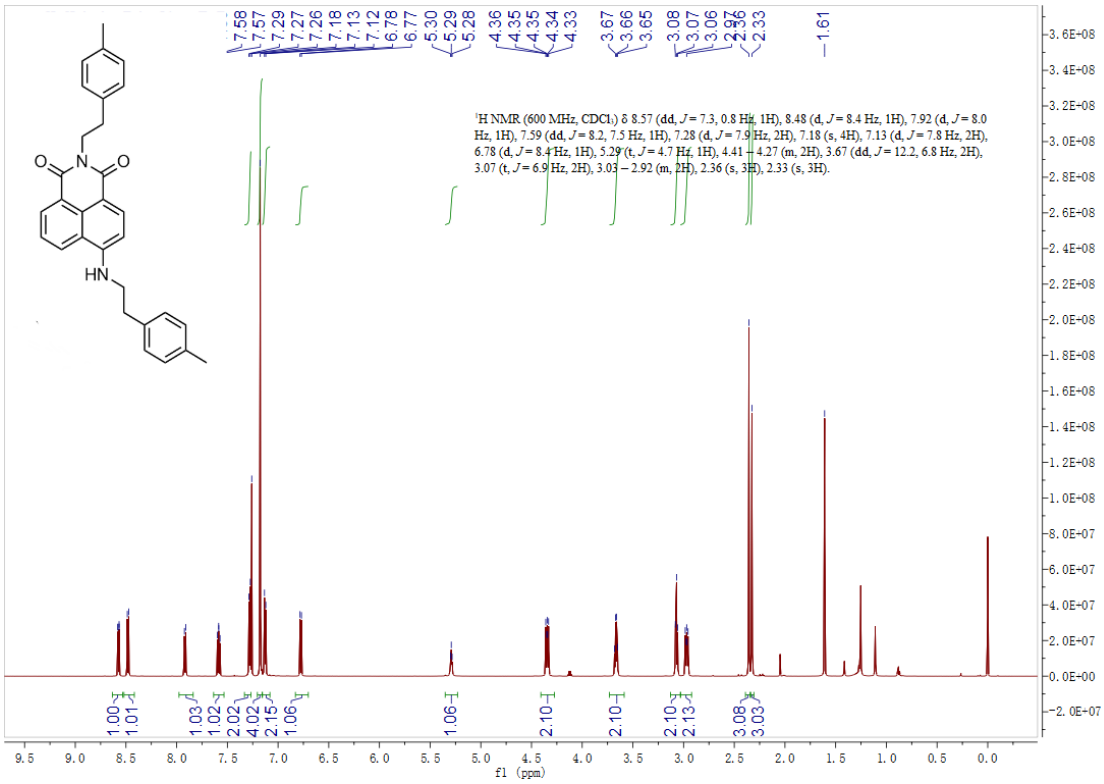


Fig. S17 ^{1}H NMR spectrum of MPNAMP.

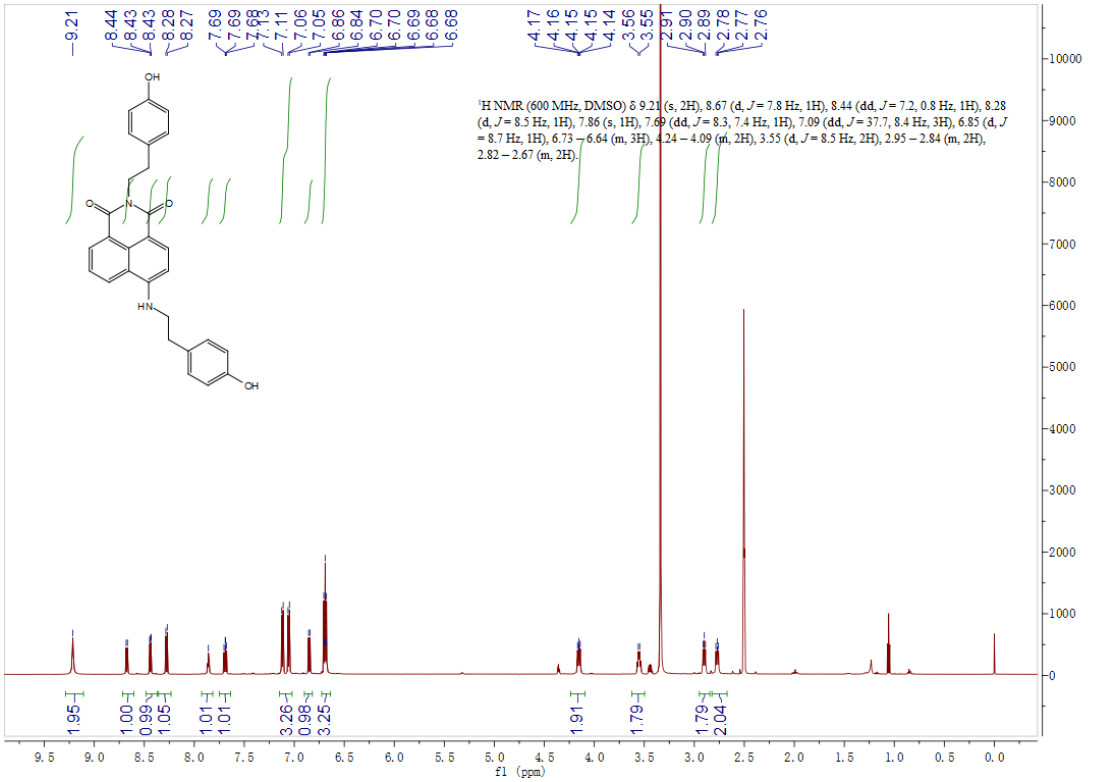


Fig. S18 ^{1}H NMR spectrum of TYNATY.

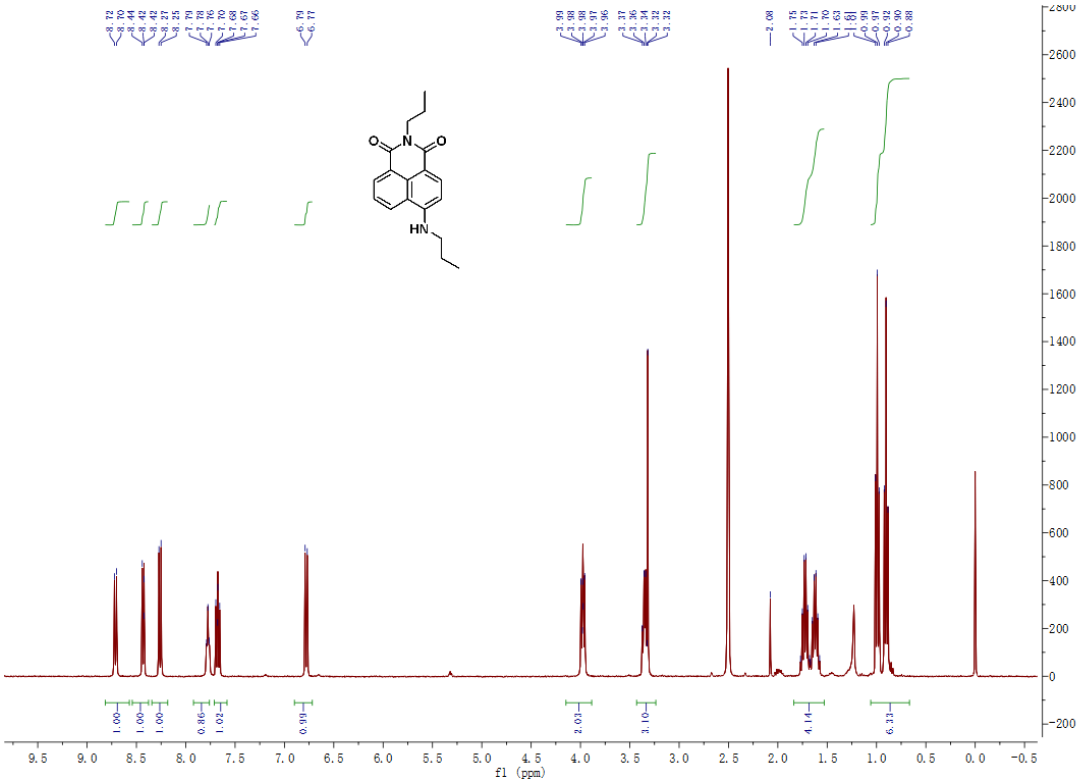


Fig. S19 ^{1}H NMR spectrum of PNAP.

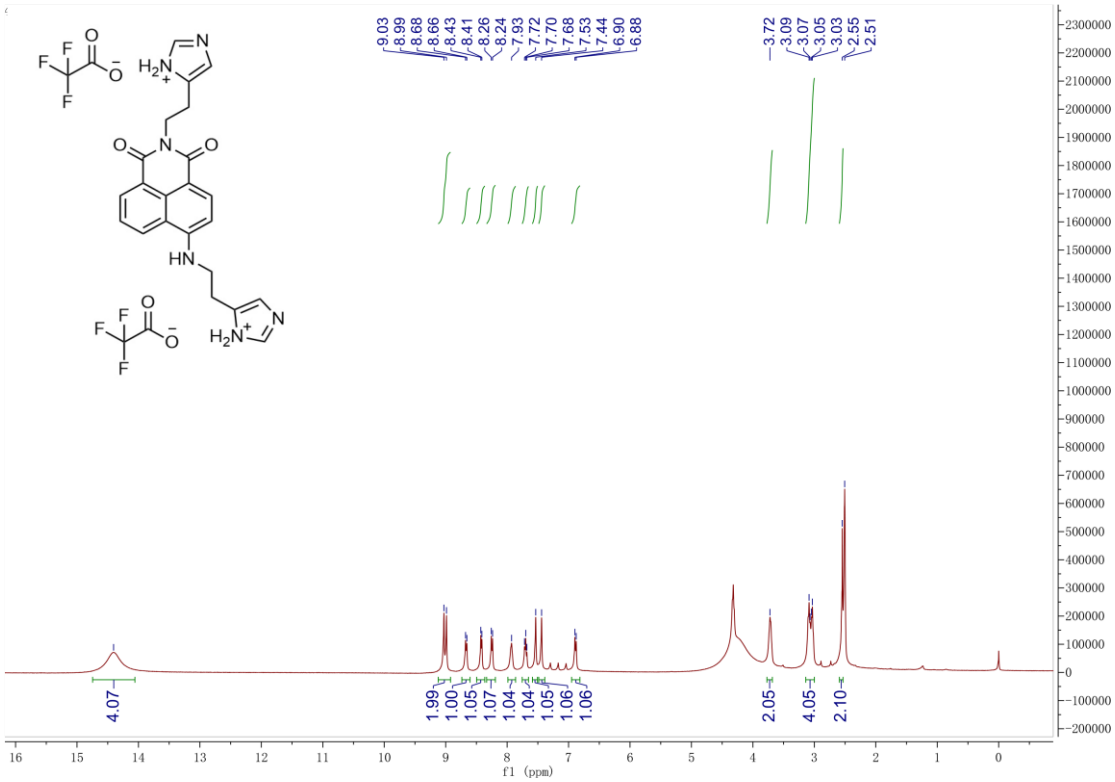


Fig. S20 ^{1}H NMR spectrum of IZNAIZ.

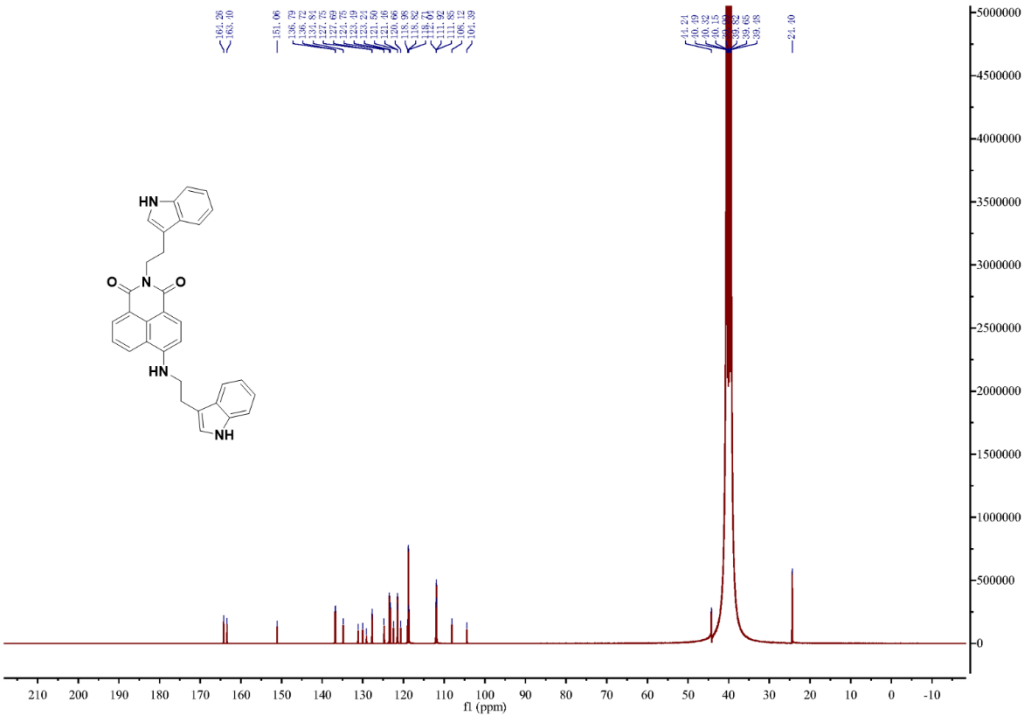


Fig. S21 ^{13}C NMR spectrum of TRNATR.

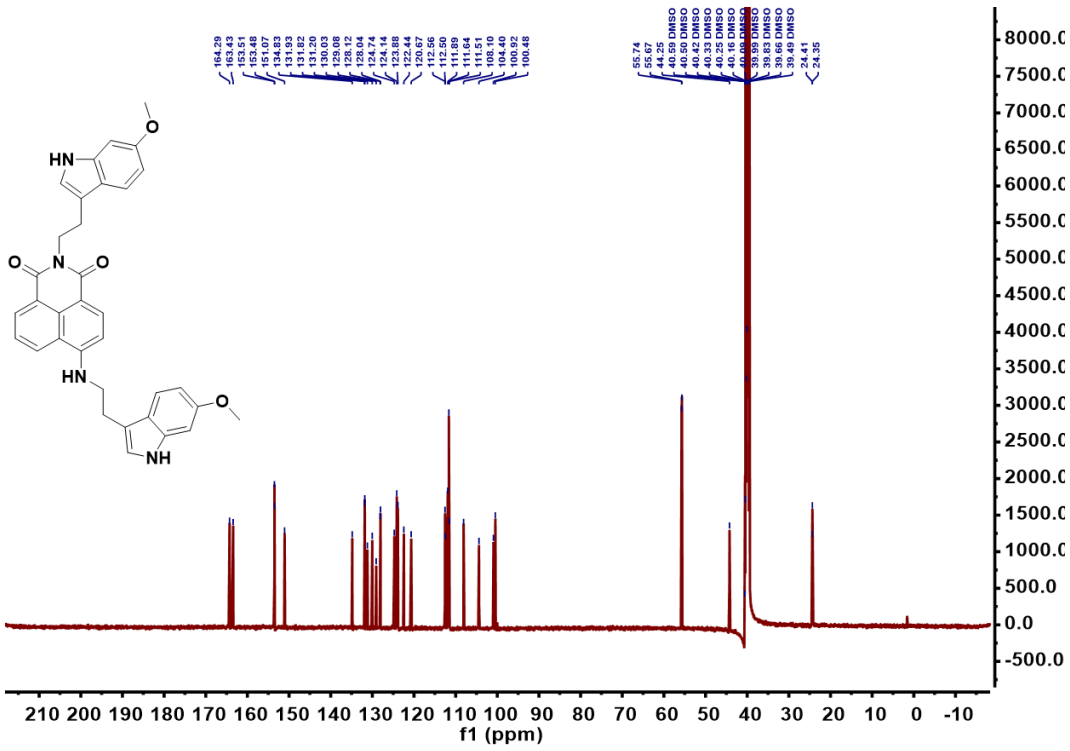


Fig. S22 ^{13}C NMR spectrum of MOTNAMOT.

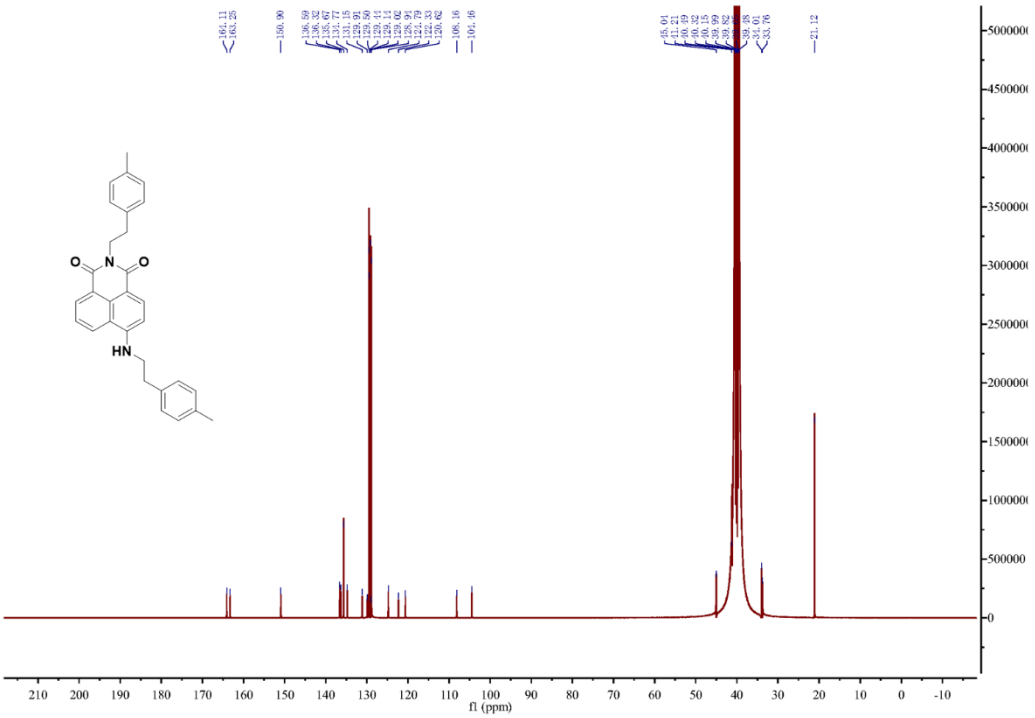


Fig. S23 ^{13}C NMR spectrum of MPNAMP.

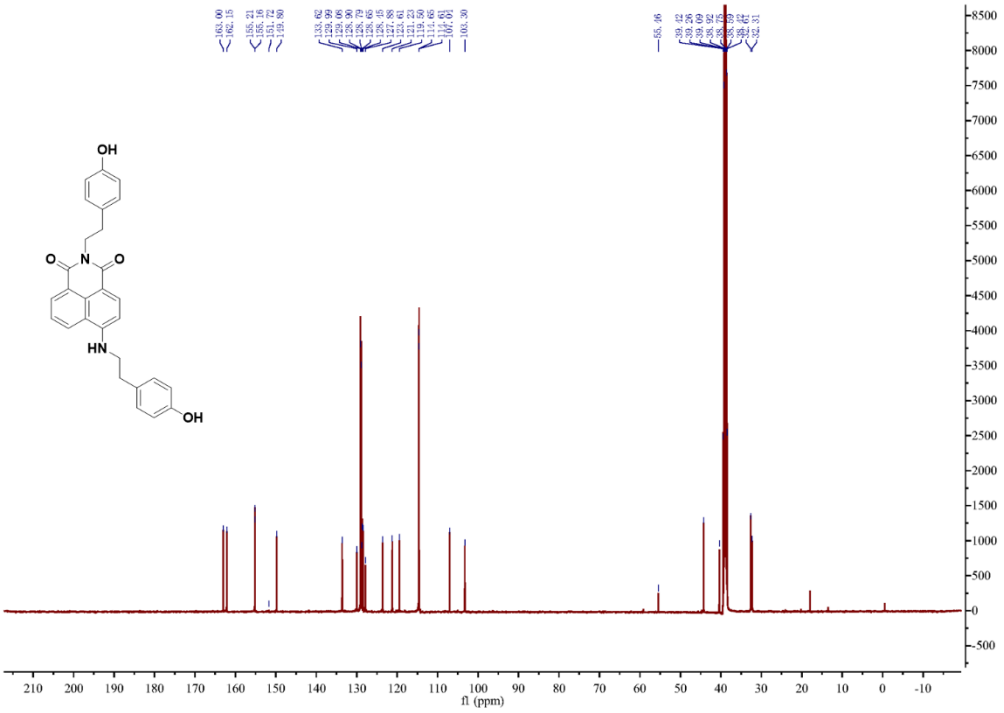


Fig. S24 ^{13}C NMR spectrum of TYNATY.

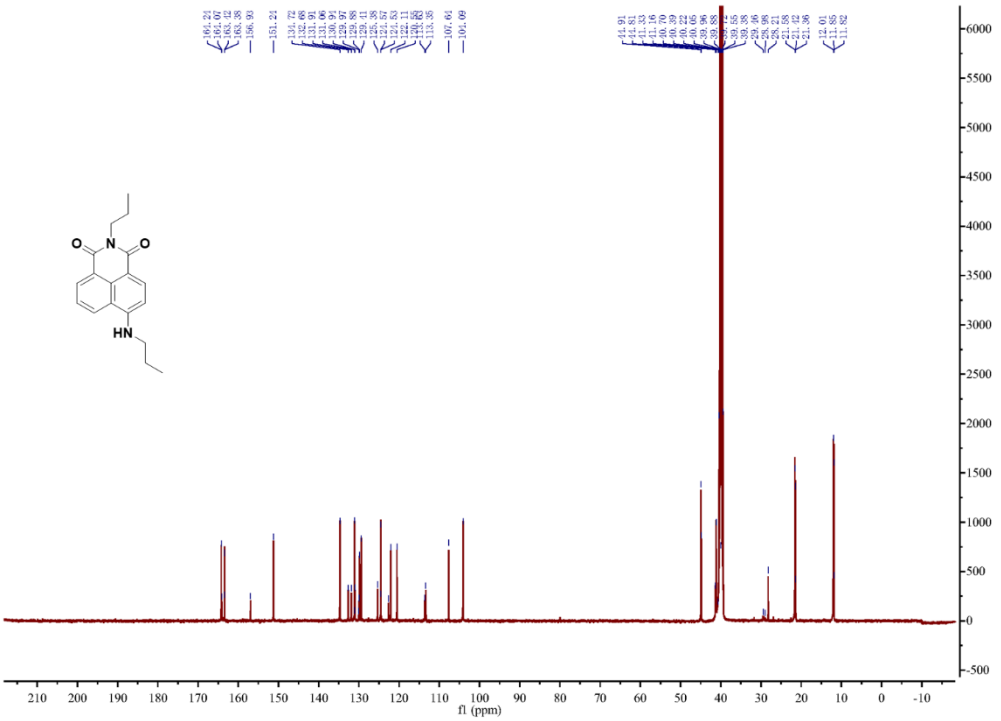


Fig. S25 ^{13}C NMR spectrum of PNAP.

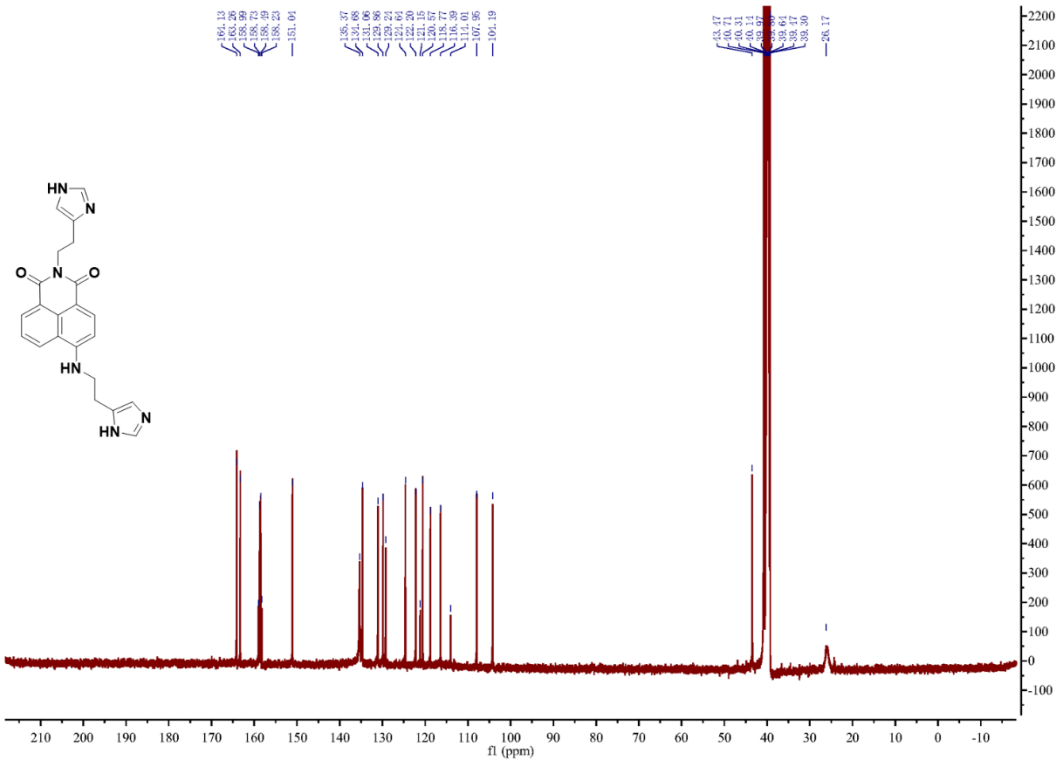


Fig. S26 ^{13}C NMR spectrum of IZNAIZ.

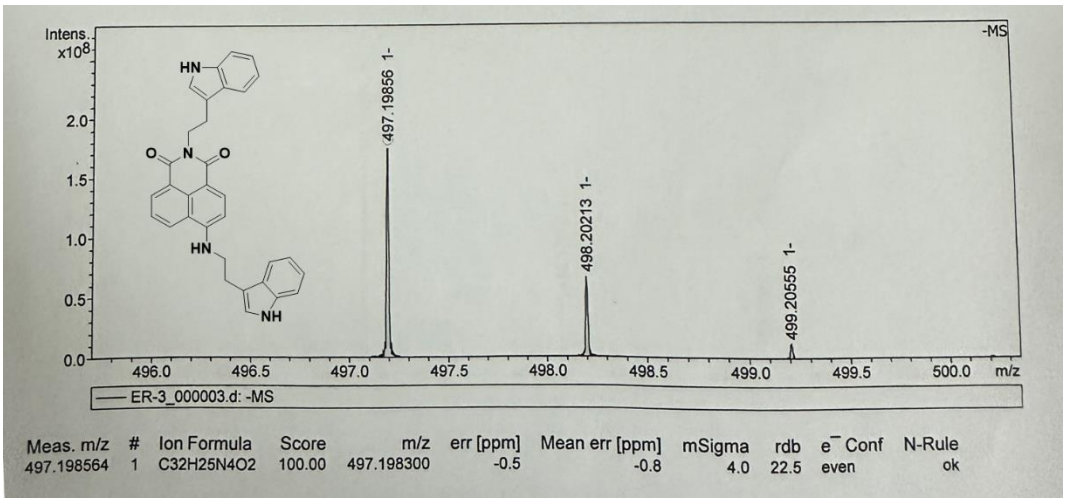


Fig. S27 HR-MS spectrum of TRNATR.

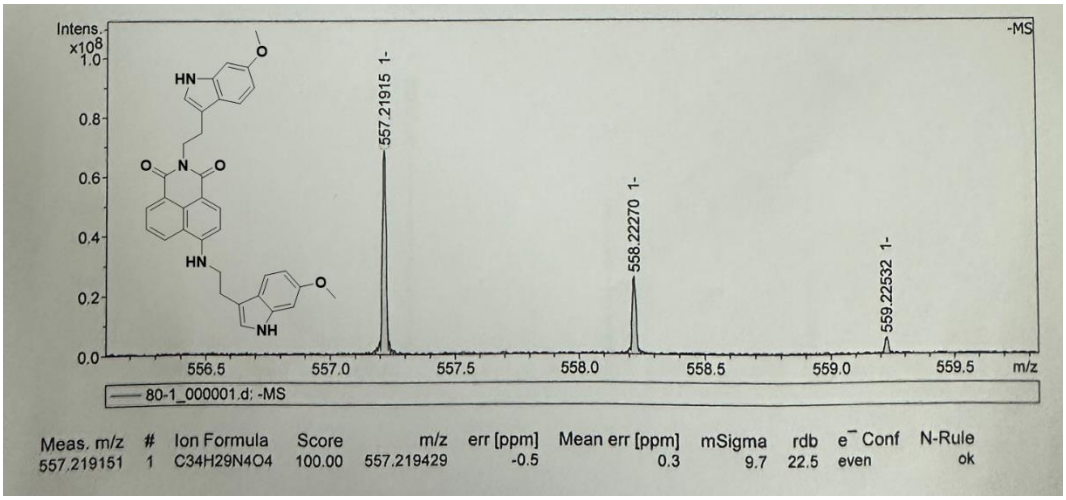


Fig. S28 HR-MS spectrum of MOTNAMOT.

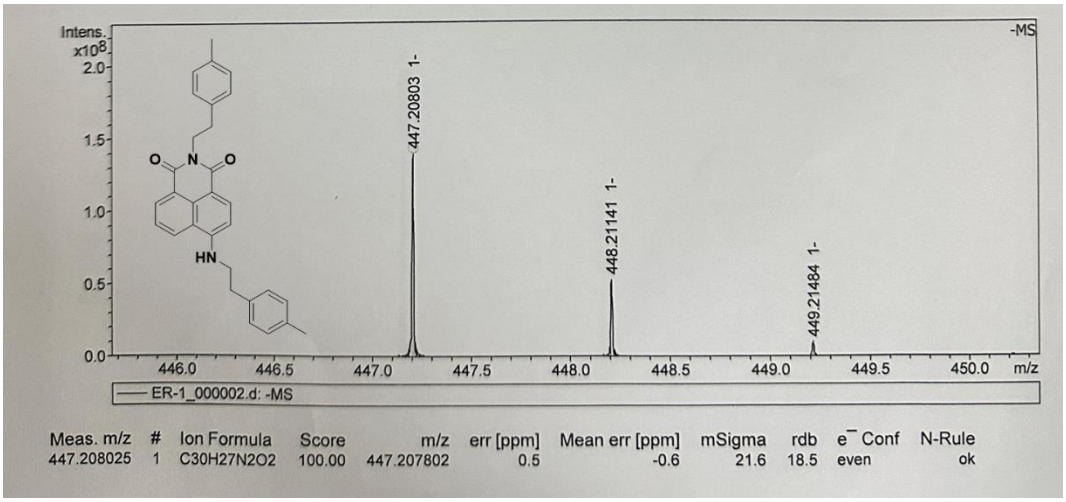


Fig. S29 HR-MS spectrum of MPNAMP.

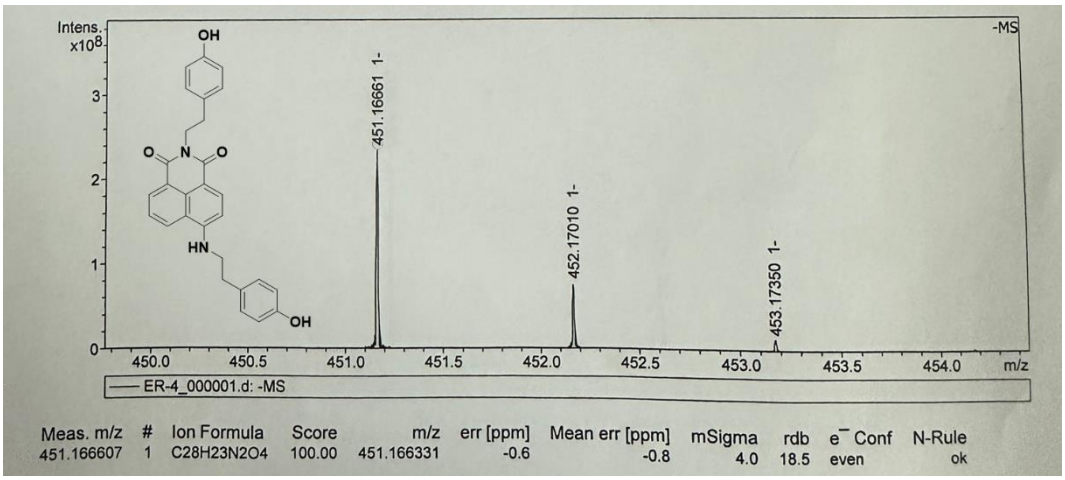


Fig. S30 HR-MS spectrum of TYNATY.

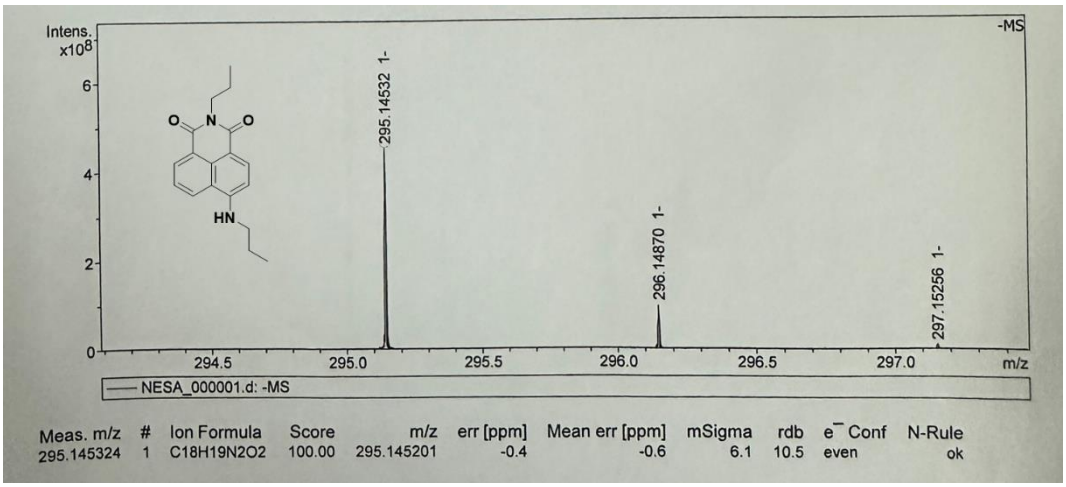


Fig. S31 HR-MS spectrum of PNAP.

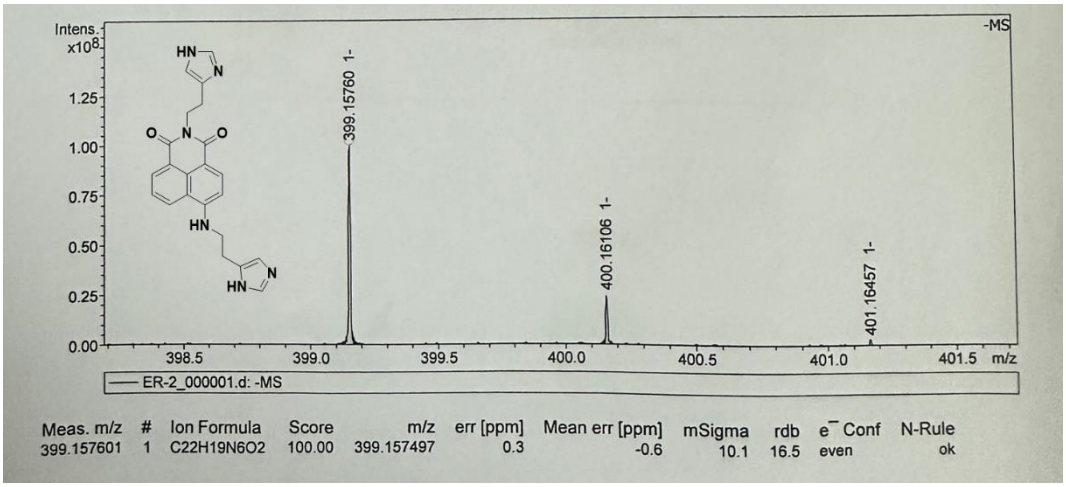


Fig. S32 HR-MS spectrum of IZNAIZ.