Electronic Supporting Information

Visualization of methylglyoxal in living cells and diabetic mice model with a 1, 8-naphthalimide-based two-photon fluorescent probe

Mingwang Yang,[a] Jiangli Fan,*[a] Junwei Zhang [a], Jianjun Du [a] and Xiaojun Peng [a]

State Key Laboratory of Fine Chemicals, Dalian University of Technology, No. 2 Linggong Road, Dalian 116024, P.R. China.

E-mail: fanjl@dlut.edu.cn

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1. Materials and Instruments.

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. The solvents were purified by conventional methods before used. Water was purified and doubly distilled by using a Milli-Q system. ER Tracker Red, Lyso Tracker Red, Mito Tracker Deep Red FM and Golgi Tracker Red were purchased from Invitrogen. The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT), thapsigargin (Tg), dithiothreitol (DTT) were purchased from Sigma Chemical Company. Tunicamycin (Tm) was purchased from Aladdin. Methylglyoxal (MGO), formaldehyde (FA), acetaldehyde (AA), benzaldehyde (BA), glyoxal (GO), glutaraldehyde (GA), glyoxylic acid (GOA) and *o*-phthalaldehude (OPA) were purchased from commercial suppliers. Silica gel (200-300 mesh) used for flash column chromatography was purchased from Qingdao Haiyang Chemical Co., Ltd. ^{1}HNMR and ^{13}CNMR spectra were determined by 400 MHz and 100 MHz using Bruker NMR spectrometers. Chemical shifts (δ) were expressed as parts per million (ppm, in $CDCl_3$ or DMSO, with TMS as the internal standard). Meanwhile, high-resolution mass spectrometry was achieved with ESI-TOF and FTMS-ESI instrument Fluorescence measurements were performed on an Agilent Technologies CARY Eclipse fluorescence spectrophotometer, and absorption spectra were measured on a PerkinElmer Lambda 35 UV−vis spectrophotometer. The pH values of sample solutions were measured with a precise pH-meter pHS-3C. Fluorescence quantum yield was achieved from a C11347-11 Absolute PL Quantum Yield Spectrometer. MTT assays were conducted on the Varioskan LUX Multimode Microplate Reader. The instrument used for imaging living cells and tissues of mice was an Olympus FV 1000 and FV1000-IX81confocal microscopy purchased from Olympus.

2. General procedure for fluorescence spectra detection

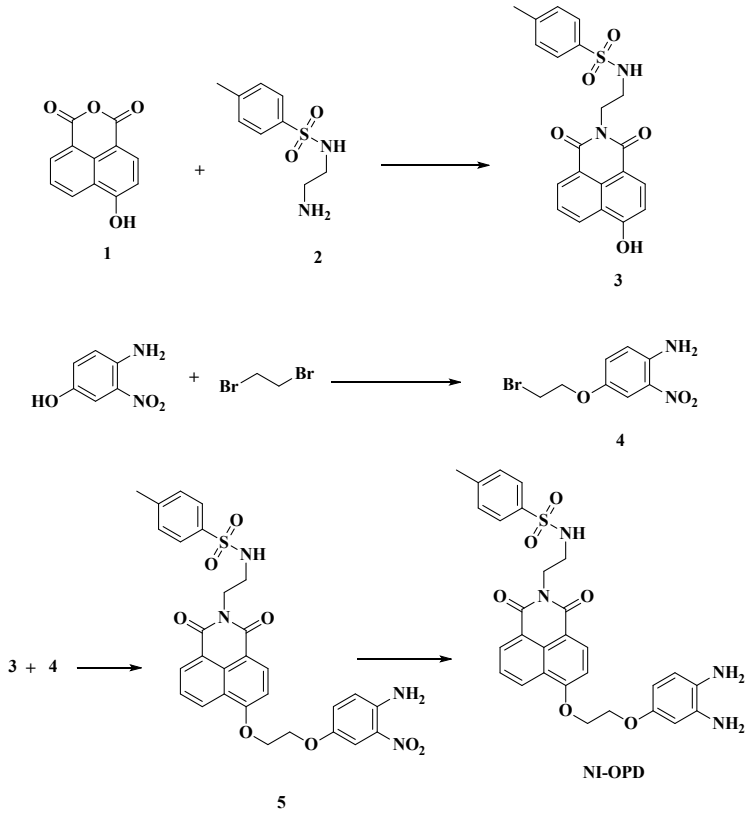
A stock solution of probe **NI-OPD** was prepared in dimethyl sulfoxide (DMSO) at a concentration of 2 mM. The stock solutions of MGO and other aldehydes (10 mM) were made by diluting aqueous solution of aldehydes with ultra pure water. The other

analytes were obtained by traditional methods. For spectroscopic measurement, the probe stock solution was diluted to 10 μM in 10 mM PBS buffer (containing 10% DMF) solutions of different pH values as needed, which was treated with testing species and then kept at 37 °C in an incubator for 2 h. After specified time, the fluorescence spectra were recorded. Unless otherwise stated, the excitation wavelength is 380 nm, and the excitation/emission slit widths are 5/5 nm for all measurements.

3. Determination of detection limits

According to the fluorescence titration data, a linear relationship between the fluorescence intensity ($F_{460\ nm}$) and MGO concentrations was observed, the detection limit was calculated with the following equation: Detection limit = 3σ/k, where σ is the standard deviation of blank measurements (n=10), k is the slop between the fluorescence intensity versus the concentrations of MGO.

4. Synthesis and characterization of probe NI-OPD



Scheme S1 Synthesis of probe **NI-OPD**

Compounds 1 was synthesized following by the previous literature (yield 82.0%). [S1] ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.26 (s, 1H), 8.61 (d, J = 8.4 Hz, 1H), 8.51 (d, J = 7.3 Hz, 1H), 8.40 (d, J = 8.2 Hz, 1H), 7.82 (t, J = 7.9 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 161.92, 161.83, 160.74, 136.13, 133.50, 132.28, 130.62, 126.47, 122.96, 119.01, 110.92, 108.92.

Compounds 2 was synthesized following by the previous literature (yield 69.5%). [S2] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 3.00-2.93 (m, 2H), 2.82-2.74 (m, 2H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 143.51, 137.10, 129.86, 127.24, 45.41, 40.97, 21.65.

Compound 3: Compound 1 (428 mg, 2.0 mmol) and compound 2 (428 mg, 2.0 mmol) were dissolved in 20 mL ethanol under argon atmosphere. The mixture was heated overnight at 80^{o}C. After the reaction completed, the solvent was removed under reduced pressure and the residue was purified by column chromatography with dichloromethane: methanol (30:1) as the eluent to afford the desire product as a deep

brown solid (345 mg, yield 42.1%). ^{1}H NMR (400 MHz, DMSO-*d*6) δ (ppm): 11.87 (s, 1H), 8.55 (dd, *J* = 8.3, 1.1 Hz, 1H), 8.45 (dd, *J* = 7.3, 1.1 Hz,1H), 8.33 (d, *J* = 8.2 Hz, 1H), 7.81-7.74 (m, 1H), 7.71 (d, *J* = 6.4 Hz, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.2 Hz, 1H), 4.09 (t, *J* = 6.6 Hz, 2H), 3.13-3.02 (m, 2H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 164.24, 163.51, 160.90, 142.86, 138.11, 133.97, 131.50, 130.14, 129.90, 129.80, 129.34, 126.99, 126.82, 125.94, 122.89, 122.32, 112.98, 110.39, 21.32.

Compound 4: The 1, 2-dibromoethane **(**1.74 mL, 20.0 mmol) was added to a mixture of 4-amino-3-nitrophenol (616 mg, 4.0 mmol) and K_2CO_3 (1.09 g, 8.0 mmol) in 15.0 mL of CH_3CN. The mixture was refluxed overnight at 85 °C under argon atmosphere. The mixture was filtrated and the filtrate was evaporated to dryness. The crude product was purified by silica gel chromatography with petroleum ether: ethyl acetate (10:1) as the eluent to yield compound 4 as a red solid (641 mg, yield 61.3%). ^{1}H NMR (400 MHz, DMSO–*d*6) δ (ppm): 7.39 (d, *J* = 2.9 Hz, 1H), 7.26 (s, 2H), 7.19 (dd, *J* = 9.2, 3.0 Hz, 1H), 6.99 (d, *J* = 9.2 Hz, 1H), 4.46-4.09 (m, 2H), 3.93-3.53 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 148.08, 142.66, 129.51, 127.90, 121.27, 107.33, 68.98, 31.77.

Compound 5: To a solution containing compound 3 (246 mg, 0.6 mmol) and compound 4 (234 mg, 0.9 mmol) in 10 mL of DMF was added K_2CO_3 (166 mg, 1.2 mmol), KI (100 mg, 0.6 mmol) and tetrabutylammonium iodide (TBAI) (44 mg, 0.12 mmol). The mixture was stirred overnight at 80 °C under argon atmosphere. After the reaction completed, 50 mL of water was added to the mixture and extracted with ethyl acetate for three times, dried over anhydrous Na_2SO_4. After the removal of solvent, the product was purified by silica gel column chromatography with dichloromethane: methanol (50:1) as the eluent to afford the desired product as yellow solid (190 mg, 54.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.50 (d, *J* = 8.4 Hz, 1H), 8.45 (d, *J* = 7.3 Hz, 1H), 8.41 (d, *J* = 8.3 Hz, 1H), 7.81 – 7.74 (m, 1H), 7.71 (t, *J* = 6.4 Hz, 1H), 7.56 (d, J = 8.2 Hz, 2H), 7.52 (d, *J* = 3.0 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.27 (s, 2H), 7.21 (dd, *J* = 11.4, 5.5 Hz, 3H), 7.00 (d, *J* = 9.3 Hz, 1H), 4.66 (s, 2H), 4.48 (s, 2H), 4.08 (t, *J* = 6.4 Hz, 2H), 3.06 (dd, *J* = 13.1, 6.6 Hz, 2H), 2.23 (s, 3H). ^{13}C NMR

(101 MHz, DMSO-d_6) δ (ppm): 164.12, 163.45, 159.77, 148.66, 142.86, 142.63, 138.14, 133.54, 131.48, 129.90, 129.54, 129.19, 128.74, 128.09, 126.80, 123.25, 122.47, 121.29, 115.03, 107.52, 107.14, 68.44, 67.38, 21.31.

Probe NI-OPD: To a solution containing compound 5 (59 mg, 0.1 mmol) in 25 mL of THF, N_2H_4•H_2O (0.5 mL, 98%) and Pd/C (40 mg, 10%) was added to the above solution and refluxed for 1.5 h under argon atmosphere. The mixture was filtrated, evaporated to dryness and the residue re-dissolved in 100 mL of dichloromethane. Then the solution was washed with saturated NaCl solution and saturated $NaHCO_3$ solution, the organic layers were dried over anhydrous Na_2SO_4 and evaporated to the residue, which was further purified by silica gel column chromatography with dichloromethane: methanol (40:1) as the eluent to afford the desired product as light yellow solid (29 mg, 51.8%). ^{1}H NMR (400 MHz, DMSO-*d*6) δ (ppm): 8.53 (d, *J* = 8.4 Hz, 1H), 8.48 (d, *J* = 7.3 Hz, 1H), 8.43 (d, *J* = 8.3 Hz, 1H), 7.86-7.78 (m, 1H), 7.74 (s, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 2H), 6.44 (d, *J* = 8.3 Hz, 1H), 6.25 (d, *J* = 2.7 Hz, 1H), 6.09 (dd, *J* = 8.3, 2.7 Hz, 1H), 4.62 (s, 2H), 4.52 (s, 2H), 4.32 (s, 2H), 4.09 (dd, *J* = 17.2, 10.6 Hz, 4H), 3.08 (d, *J* = 6.1 Hz, 2H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ (ppm): 164.17, 163.50, 159.97, 151.68, 142.88, 138.11, 133.62, 131.51, 129.92, 126.81, 123.33, 114.94, 107.50, 103.09, 102.73, 68.68, 66.82, 21.33, 0.58. HRMS (ESI-TOF): calculated for $C_{29}H_{29}N_4O_6S^+$, $[M+H]^+$, m/z , 561.1808, found: 561.1811.

5. Cell culture and cell cytotoxicity experiments

Human cervical cancer cells (HeLa) and Human breast cancer cells (MCF-7) cells were purchased form Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences. HeLa and MCF-7 cells were cultured in 90% Dulbecco's Modified Eagle Medium (DMEM, Gibico) supplemented with 10% FBS (Gibico) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) in an atmosphere of 37 °C and 5% CO_2. The MTT method was employed to assess the cellular cytotoxicity of **NI-OPD**. Before experiments, HeLa and MCF-7

cells at a density of 1×10^4 cells/well were seeded into 96-well plates and cultured for 24 h. Then the fresh culture contained **NI-OPD** over a range of concentrations (0-30 μM) (n = 6) to substitute the previous media, and further incubation for 24 h. After that, 10 μL of MTT (5 mg/mL in PBS) was added into per well and incubated another 4 h. Finally, 100 μL of DMSO was then added to dissolve formazan. The absorbance at 490 nm and 570 nm was measured in a microplate reader, and the cell viability (%) was calculated according to the following equation: Viability (%) = [OD570 (sample) - OD490 (sample)] / [OD570 (control) - OD490 (control)] × 100.

6. Cell imaging experiments

One day before imaging, the cells were detached and were replanted on glass-bottomed dishes and allowed to adhere for 24 hours. First stage, experiments were performed to confirm the capability of probe for imaging exogenous MGO in HeLa cells. The culture media was replaced with 2 mL of HEPES-Krebs-Ringer's buffer (HKR) (140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES pH 7.4) containing 10 μM fluorescent probe (from a 2 mM stock in DMSO) and the cells were incubated for 1 h. Cells were then washed once with 2 mL HKR before MGO was added (0, 15, 30, or 50 μM final concentration), and the cells were incubated for an additional hour prior to imaging. For inhibition tests, cells were treated with DMEM containing 1 mM aminoguanidine (AG) or N-acetylcysteine (NAC) for 8 hours, then the media was replaced with HKR containing 10 μM fluorescent probe and the cells incubated for 1 h before imaging. To confirm the result, the test cells were further incubated with MGO (50μM) for imaging.

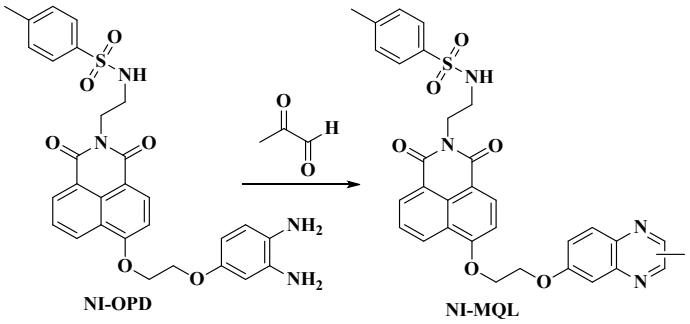
7. Two-photon fluorescence imaging in mice

The BALB/c mice were purchased from Dalian Medical University. All the animal experiments were carried out in accordance with the relevant laws and guidelines approved by Dalian Medical University Animal Care and Use Committee. The diabetic mice were obtained by injecting intraperitoneally streptozotocin (STZ) which

is dissolved freshly in 0.01 M citrate buffer (pH = 4.5) to normal mice after fasting overnight at a dose of 150 mg/kg body weight (BW). Three days after the injection of STZ, the diabetic mice were successfully induced and its blood glucose levels were greater than 16.7 mmol/L. After obtained the diabetic mice, we divided the diabetic mice into three groups. The diabetic mice were intraperitoneally injected with 100 μL saline solution once daily as STZ group (**STZ**). The diabetic mice were intraperitoneally injected with 100 μL aminoguanidine (AG) saline solution at a dose of 250 mg/kg BW once daily as STZ +AG group (**STZ +AG**). The diabetic mice were treated with oral gavage of 100 μL metformin (Metf) saline solution at a dose of 200 mg/kg BW once daily as STZ +Metf group (**STZ +Metf**). The control mice were intraperitoneally injected with 100 μL saline solution once daily as normal group (**Normal**). All mice were reared under normal feed and humidity conditions. After the seventh day of treatment, the mice were anesthetized with chloral hydrate. The different organ were surgically exposed in Normal and STZ group and used to distinguish the MGO level in various organs. For liver and kidney tissue imaging, the liver and kidney were surgically exposed in four groups. All tissue of organ was incubated with 10 μM **NI-OPD** in PBS (10 mM, pH = 7.4) for 2 h, then imaged with two-photon confocal laser scanning microscopy with a 780 nm laser.

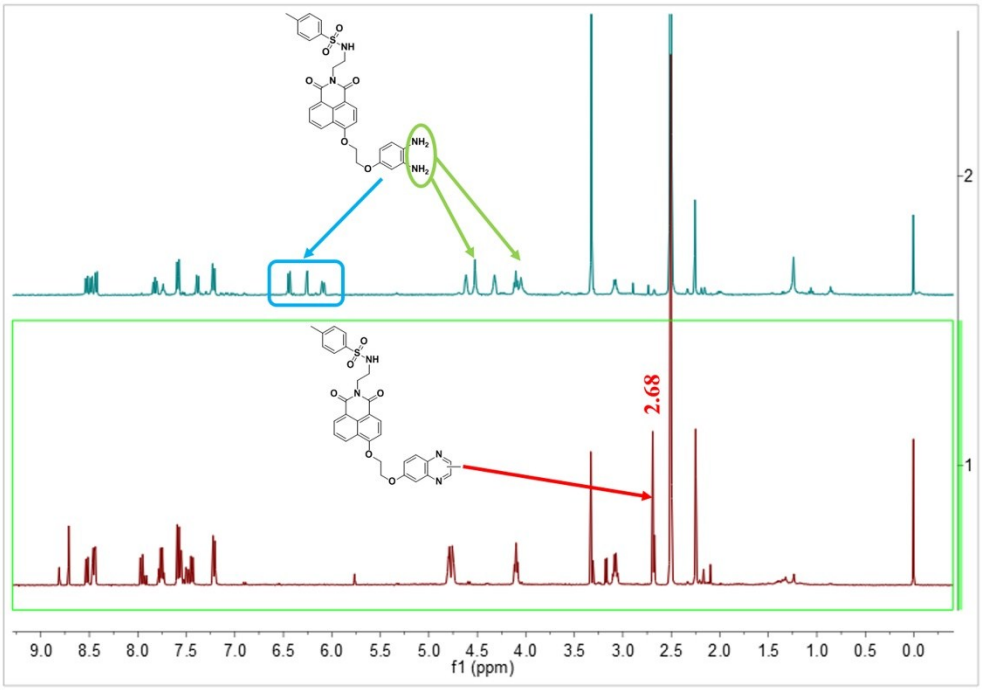
8. Confirm the product of probe and MGO

NI-OPD (28 mg, 0.05 mmol) was dissolved in THF (10 mL) under argon atmosphere, then methylglyoxal (2 mL, 40 % aqueous solution) was added. The mixture was stirred overnight at 37 °C under argon atmosphere. After the reaction completed, the solvent was removed under reduced pressure and the residue was purified by column chromatography with dichloromethane: methanol (40:1) as the eluent to afford the product **NI-MQL** as a light yellow solid (26.9 mg, yield 90.3%).

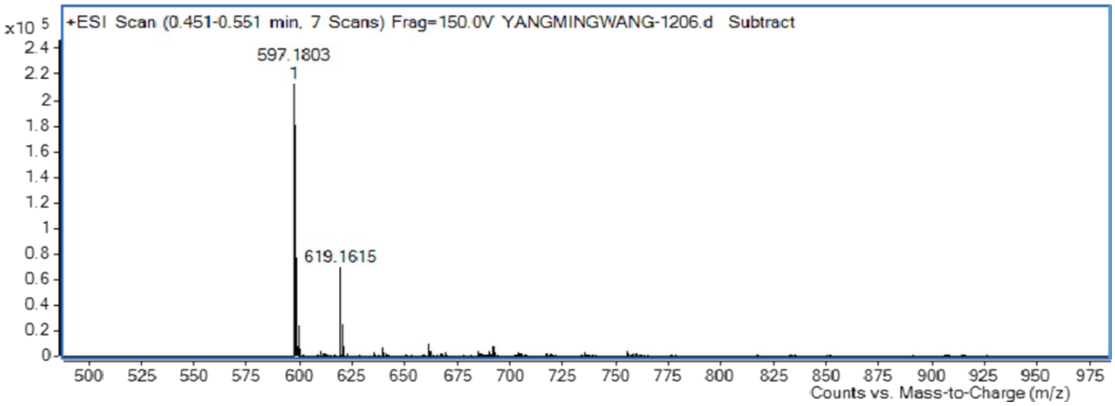


Scheme S2 The reaction of probe **NI-OPD** and methylglyoxal

^{1}H NMR (400 MHz, DMSO-*d*6) δ (ppm): 8.71 (s, 1H), 8.52 (d, *J* = 8.4 Hz, 1H), 8.45 (dd, *J* = 7.7, 3.7 Hz, 2H), 7.94 (dd, *J* = 16.2, 9.1 Hz, 0H), 7.76 (dd, *J* = 14.1, 7.0 Hz, 2H), 7.57 (dd, *J* = 11.3, 5.5 Hz, 3H), 7.49 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 4.77 (dd, *J* = 15.6, 4.4 Hz, 4H), 4.10 (t, *J* = 6.6 Hz, 2H), 3.08 (d, *J* = 6.3 Hz, 2H), 2.68 (d, *J* = 6.5 Hz, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ (ppm): 164.15, 163.49, 159.78, 154.72, 144.42, 143.47, 142.86, 138.12, 136.85, 133.59, 131.50, 130.52, 129.91, 129.23, 128.77, 126.80, 123.28, 122.51, 122.13, 115.12, 108.17, 107.62, 68.33, 67.24, 22.51, 21.31. HRMS (ESI-TOF): calculated for $C_{32}H_{29}N_4O_6S^+$, $[M+H]^+$, m/z , 597.1808, found: 597.1803.



The comparison of **NI-OPD** and **NI-MQL** ^{1}H NMR



HR-MS of product **NI-MQL**

9. The test spectra and images of NI-OPD

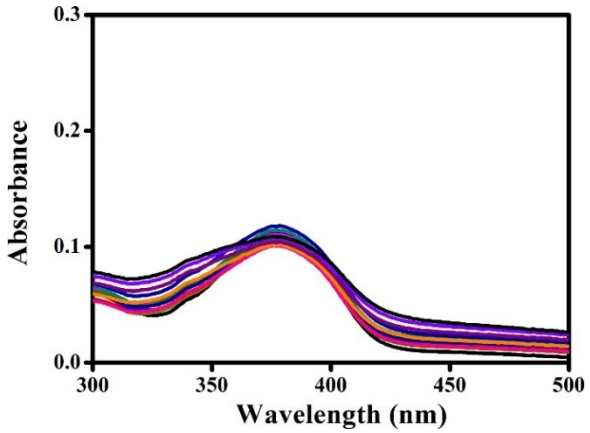


Figure S1 The absorption spectra of **NI-OPD** (10 μM) to different concentration of MGO (0-30 μM) in PBS (pH = 7.4, 10 mM, 10% DMF).

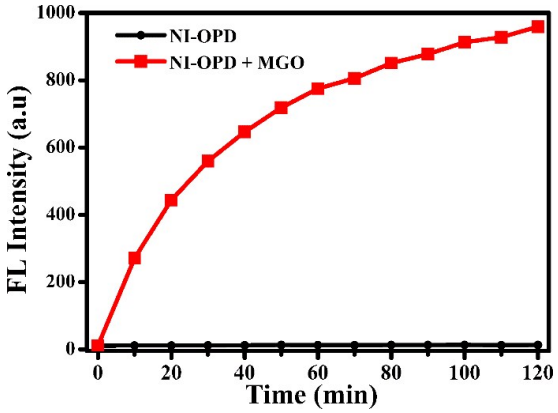


Figure S2 Time-dependent fluorescence intensity changes at 460 nm against reaction time (0–120 min) of **NI-OPD** (10 μM) toward MGO (30 μM).

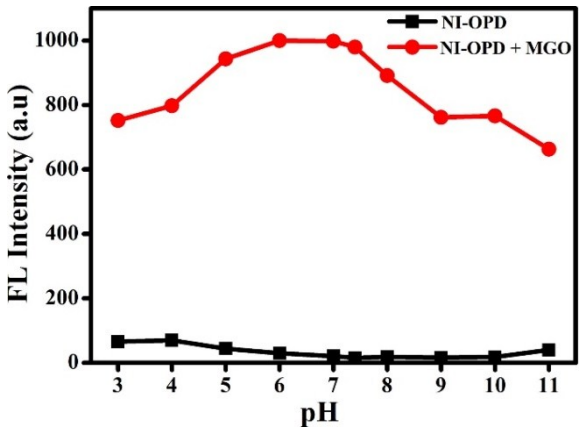


Figure S3 Effect of pH on the fluorescence of **NI-OPD** (10 μM) before and after reacting with MGO (30 μM) in PBS (10 mM, pH=7.4, 10% DMF). λ_{ex} = 380 nm.

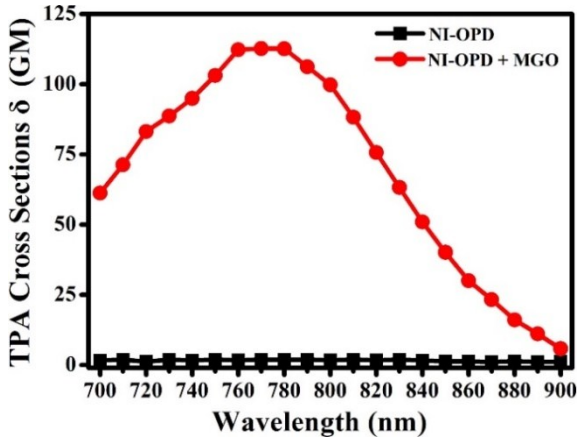


Figure S4 Two-photon excitation spectra of **NI-OPD** (10 μM) in the absence or presence of MGO (30 μM) in PBS (10 mM, pH=7.4, 10% DMF).

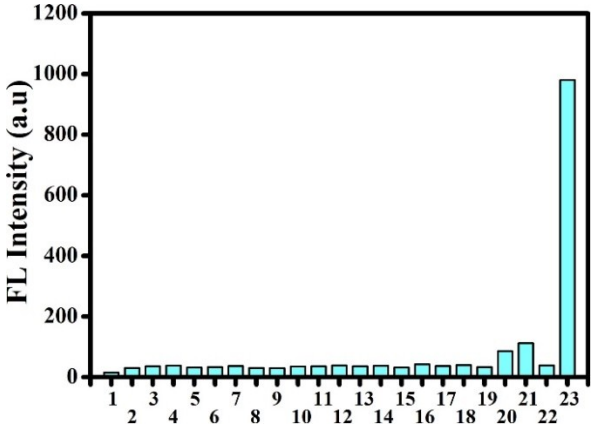


Figure S5 The fluorescence responses of **NI-OPD** (10 μM) to various cation and anion(1) Blank, (2) Na^+, (3) K^+, (4) Mg^{2+}, (5) Pd^{2+}, (6) Ag^+, (7) CO_3^{2-}, (8) HCO_3^-, (9) SO_3^{2-}(10) HSO_3^-, (11) $H_2PO_4^-$, (12) ClO_4^- , (13) OAc^-, (14) F^-, (15) Cl^-, (16) I^-, (17)

SCN^-, (18) NO_2^-, (19) NO_3^-, (20) $ONOO^-$, (21)NO, (22) H_2O_2, (23) MGO. All analytes were evaluated at 100 μM, except MGO at 30 μM. All data were obtained after 2 h incubation at 37 °C in PBS buffer (10 mM, pH ==7.4, 10 % DMF). λ_{ex} = 380 nm.

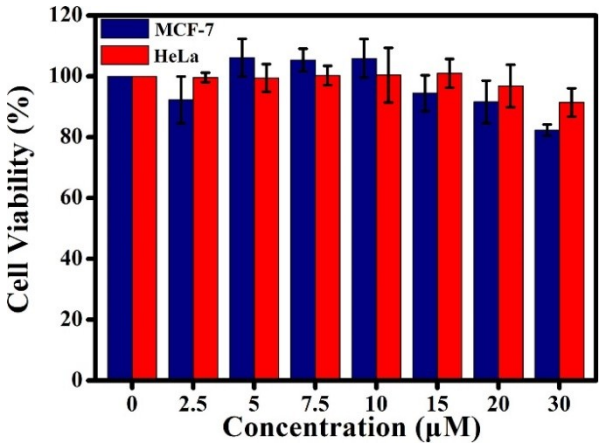


Figure S6 MTT assay of MCF-7 and HeLa cells in different concentration of **NI-OPD** (0-30 μM) for 24 h.

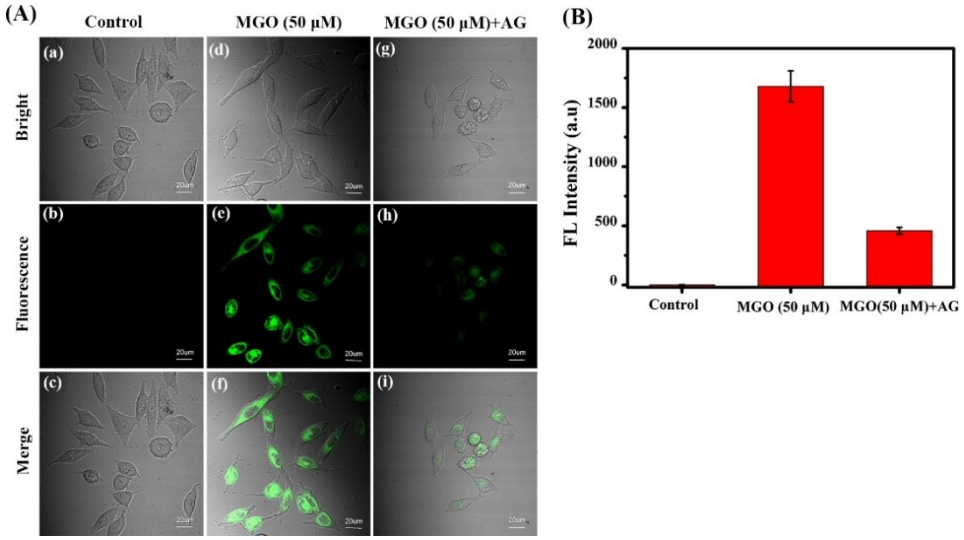


Figure S7 Confocal fluorescence images for monitoring exogenous MGO. (A) MCF-7 cells were incubated first with **NI-OPD** (10 μM), then with different concentrations of MGO ((a-c) 0 μM, (d-f) 50 μM for 2 h. (g-i) Cells were pretreated with MGO (50 μM) and AG (1 mM) sequentially, then with **NI-OPD** (10 μM) for 2 h before imaging. λ_{ex} = 405 nm, collected 420-480 nm. (B) Relative fluorescence intensities output of group (A). Scale bar: 20 μm.

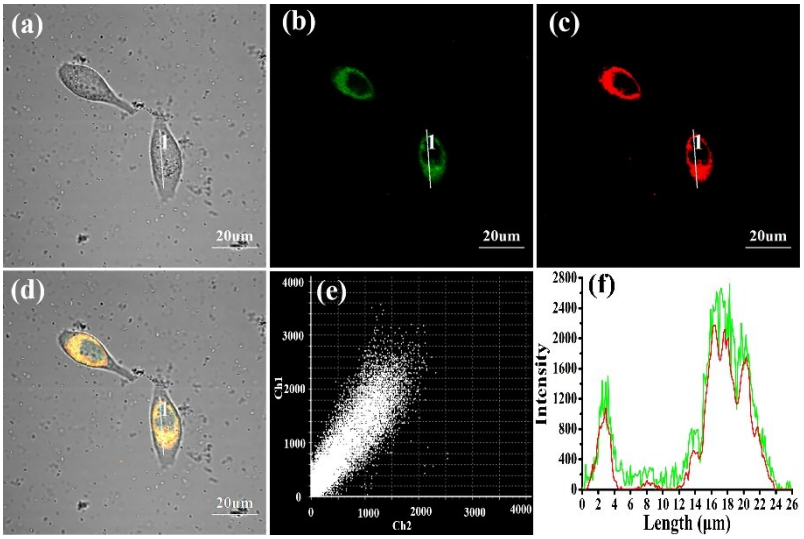


Figure S8 The calocalisation imaging of MCF-7 cells stained with **NI-OPD** (10 μM, λ_{ex} = 405 nm, collected 420-480 nm) with ER-Tracker Red (500 nM, λ_{ex} = 559 nm, collected 590-640 nm). Scale bar: 20 μm.

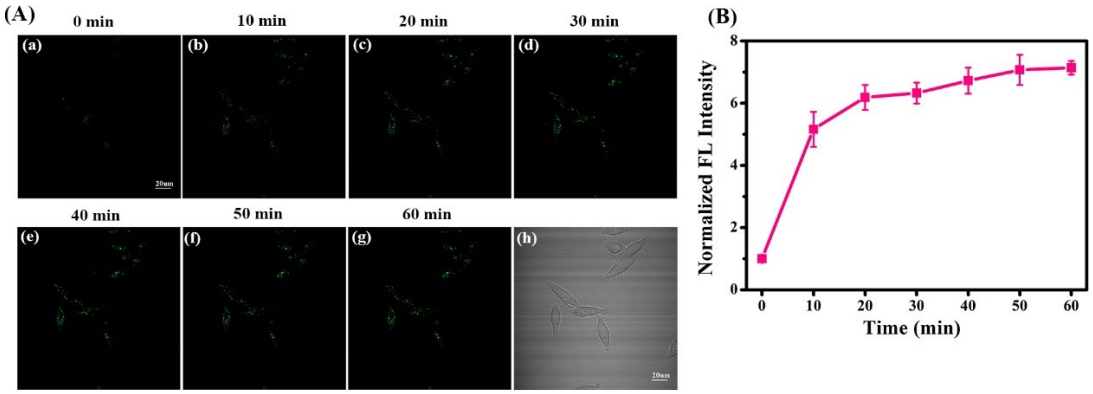


Figure S9 The fluorescence imaging of MGO with **NI-OPD** in living cells stimulated with thapsigargin (Tg) which can induced ER stress. (A) Fluorescence images of **NI-OPD** in MCF-7 cells after treated with Tg (50 μg mL^{-1}). λ_{ex} = 405 nm, collected 420-480 nm. (B) The relative fluorescence intensity output of **NI-OPD** in group (A) at different times. Scale bar: 20 μm.

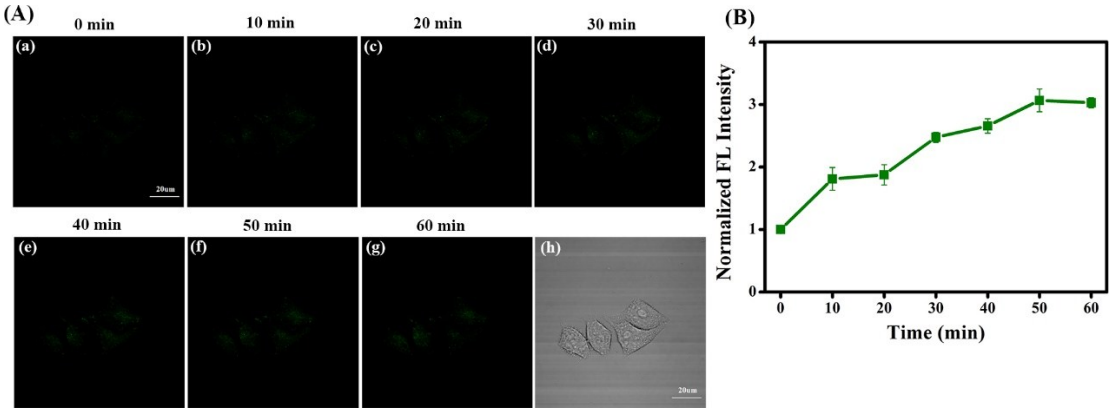


Figure S10 The fluorescence imaging of MGO with **NI-OPD** in living cells stimulated with dithiothreitol (DTT) which can induced ER stress. (A) Fluorescence images of **NI-OPD** in MCF-7 cells after treated with DTT (5 mM). λ_{ex} = 405 nm, collected 420-480 nm. (B) The relative fluorescence intensity output of **NI-OPD** in group (A) at different times. Scale bar: 20 μm.

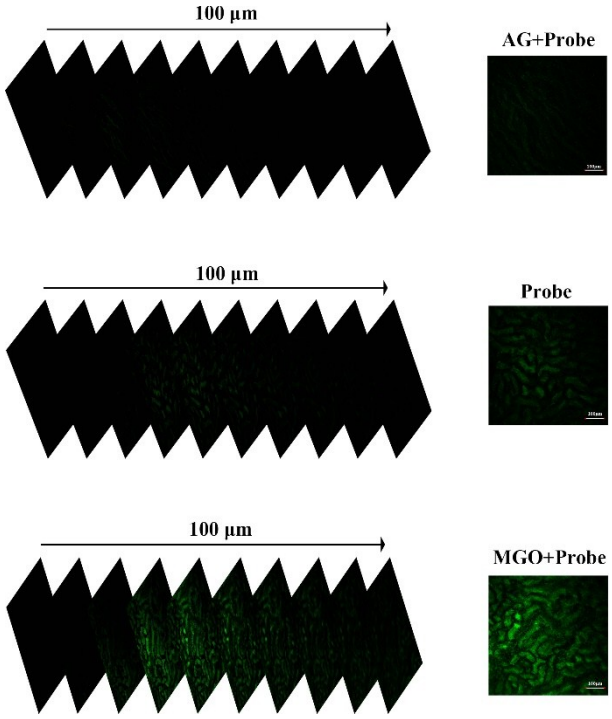


Figure S11 Confocal fluorescence imaging of mouse kidney slices. (a, c, e) The two-photon fluorescence imaging of slices at different depth (0-100 μm) and conditions corresponding to (b, d, f). (b)The tissue was stained with AG (1 mM) for 3 h and with **NI-OPD** (10 μM) for 2 h. (d) The tissue was stained with only **NI-OPD** (10 μM) for 2 h. (f) The tissue was stained with MGO (50 μM) for 3 h and with **NI-OPD** (10 μM) for 2 h. λ_{ex} = 780 nm, collected 420-480 nm. Scale bar: 100 μm.

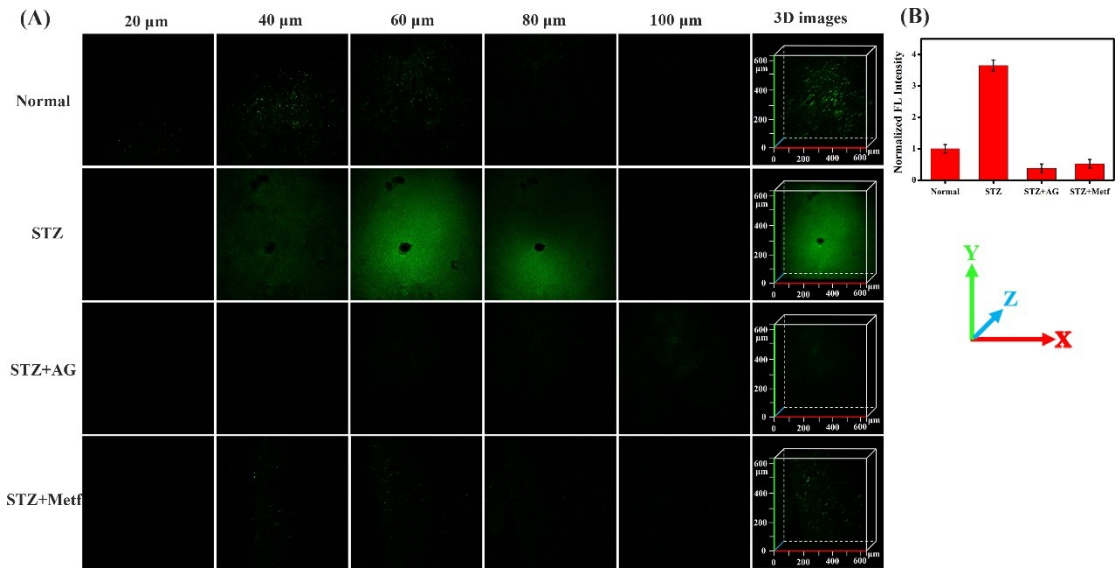
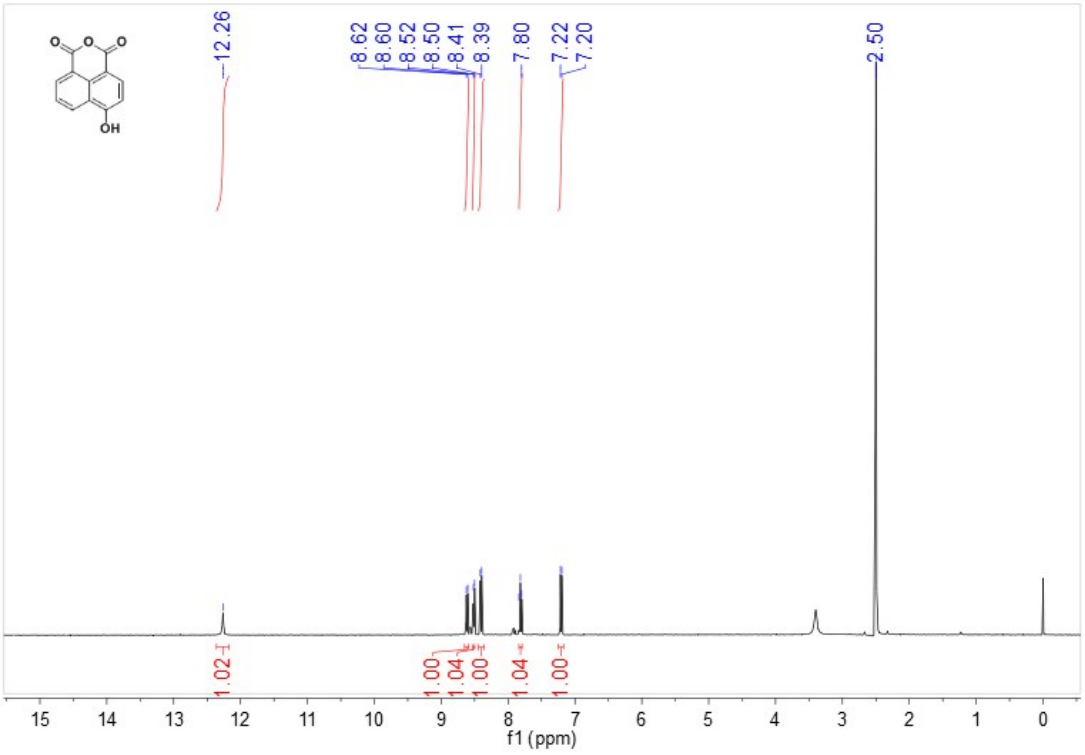
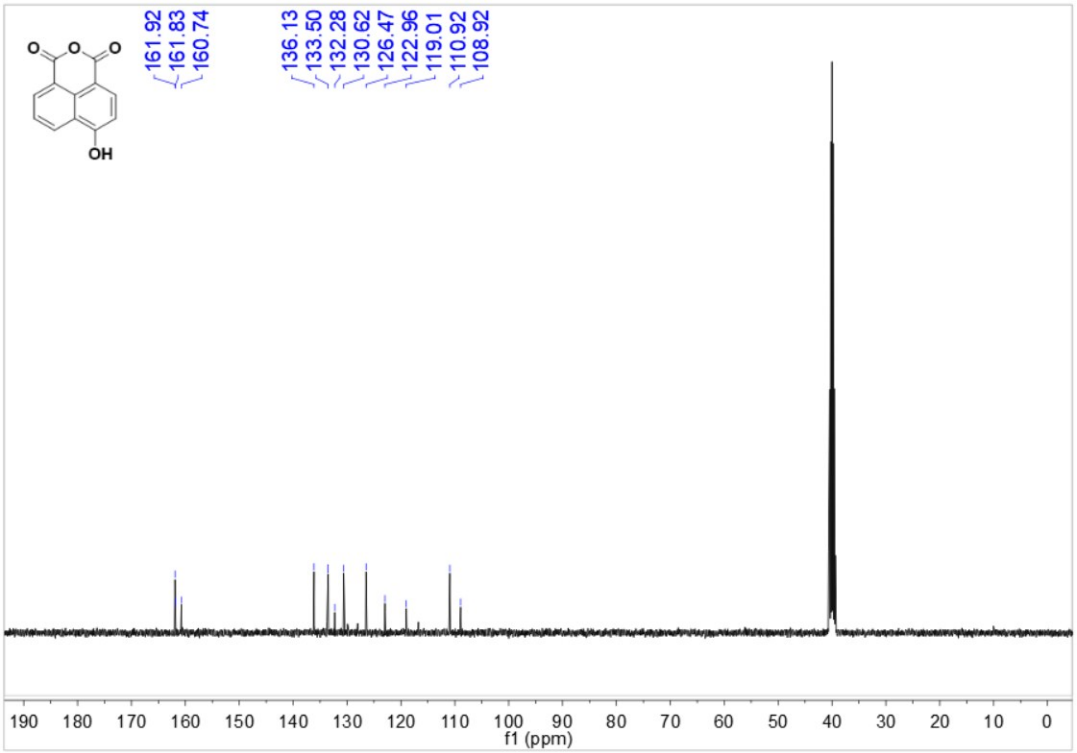


Figure S12 The two-photon fluorescence imaging of MGO in liver tissue of mice. (A) Confocal images of liver tissue stained with **NI-OPD** (10 μM) at different depths and the 3D stack images. (B) The fluorescence intensity statistics was output by five representative regions. λ_{ex} = 780 nm, collected 420-480 nm .

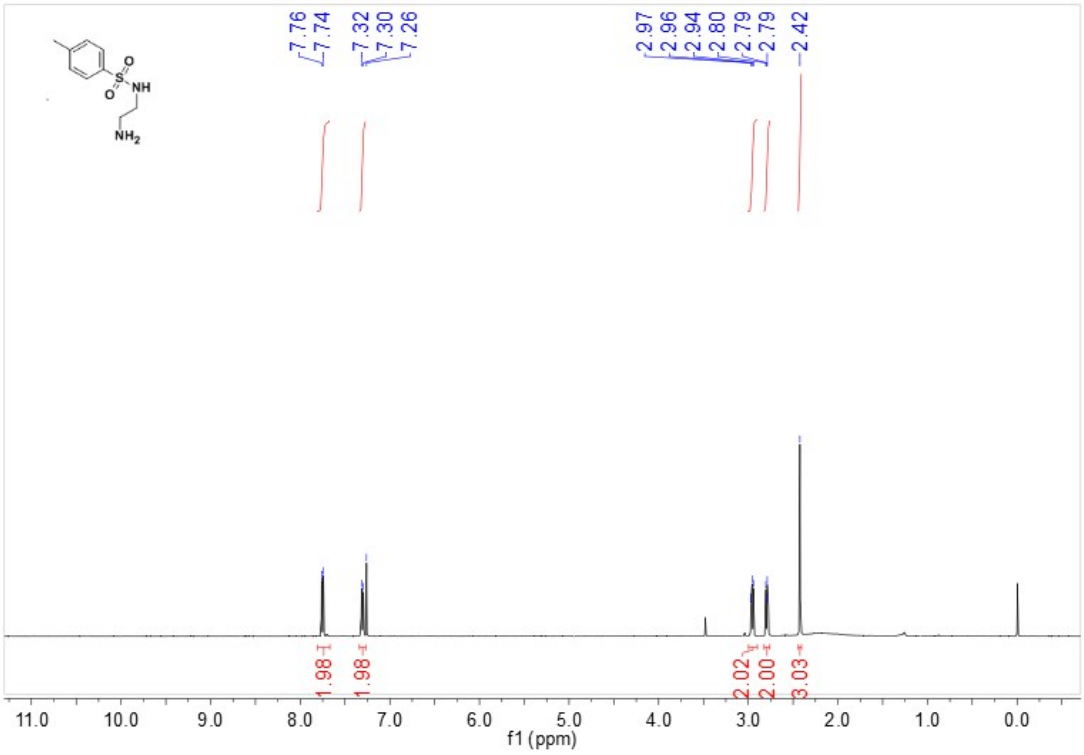
10. NMR and HR-MS Data



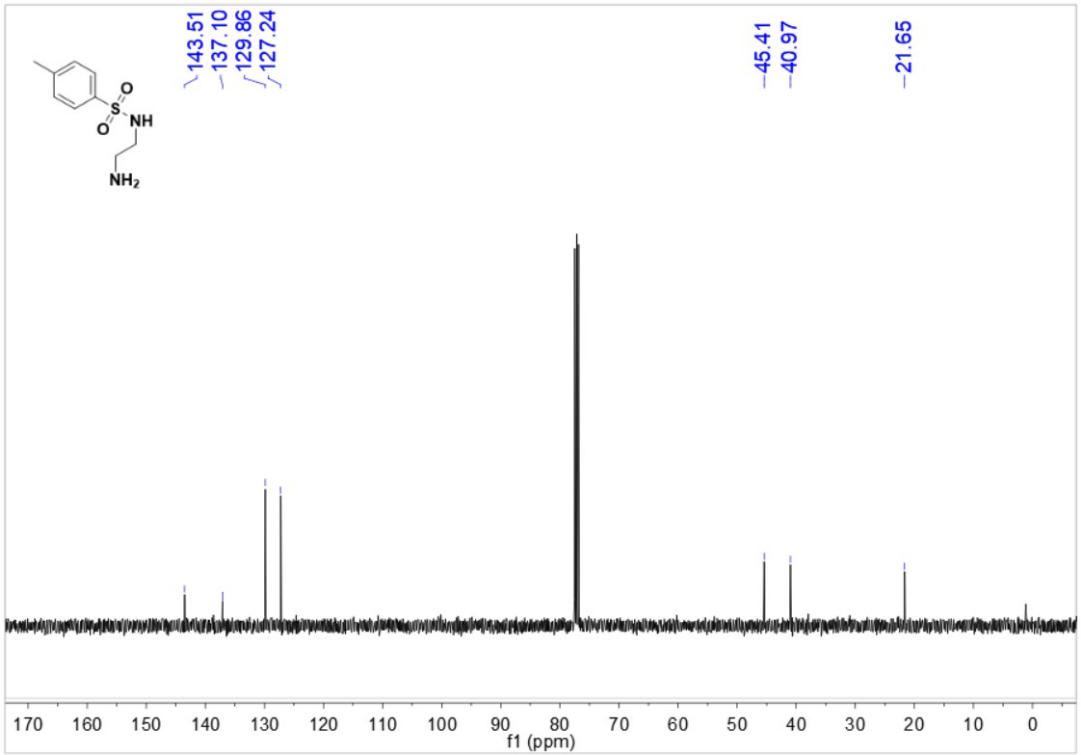
^{1}H NMR of compound **1** in DMSO-d_6



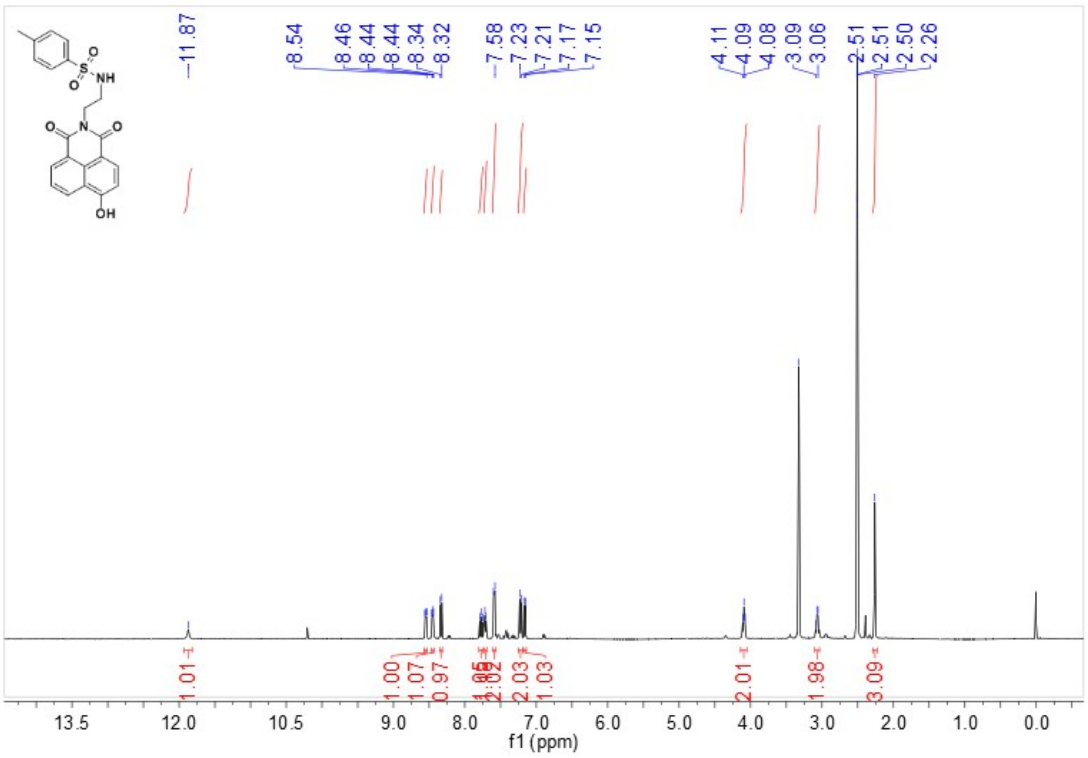
^{13}C NMR of compound **1** in DMSO-d_6



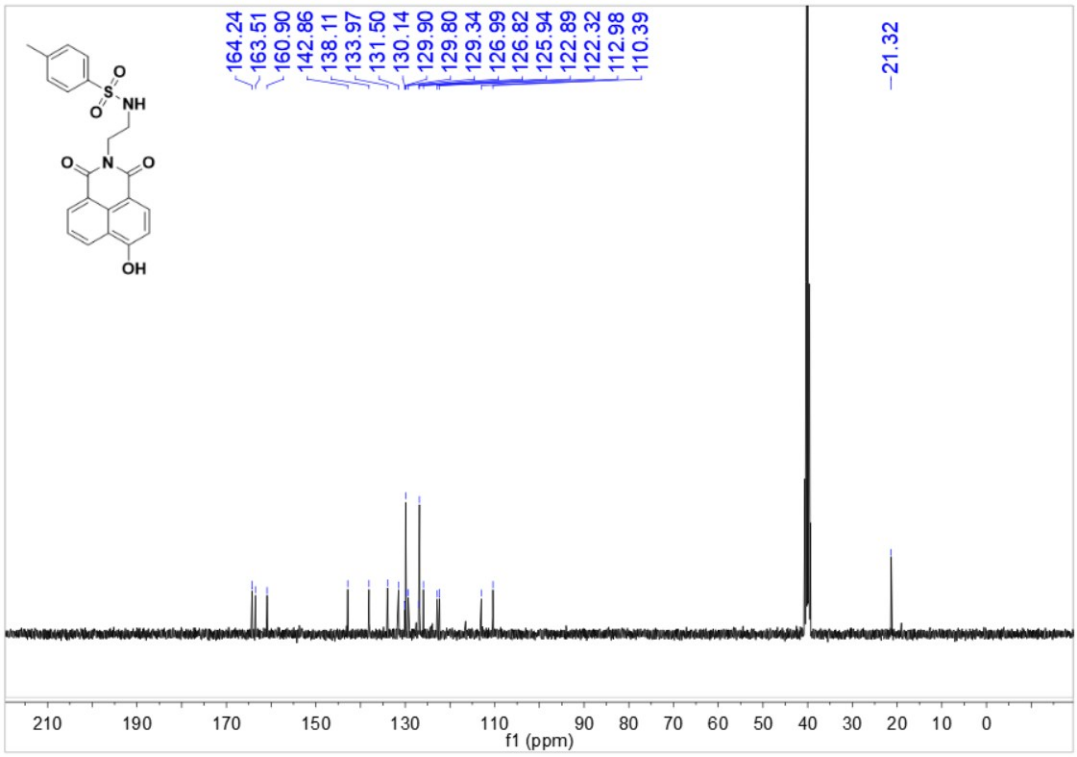
^{1}H NMR of compound **2** in $CDCl_3$



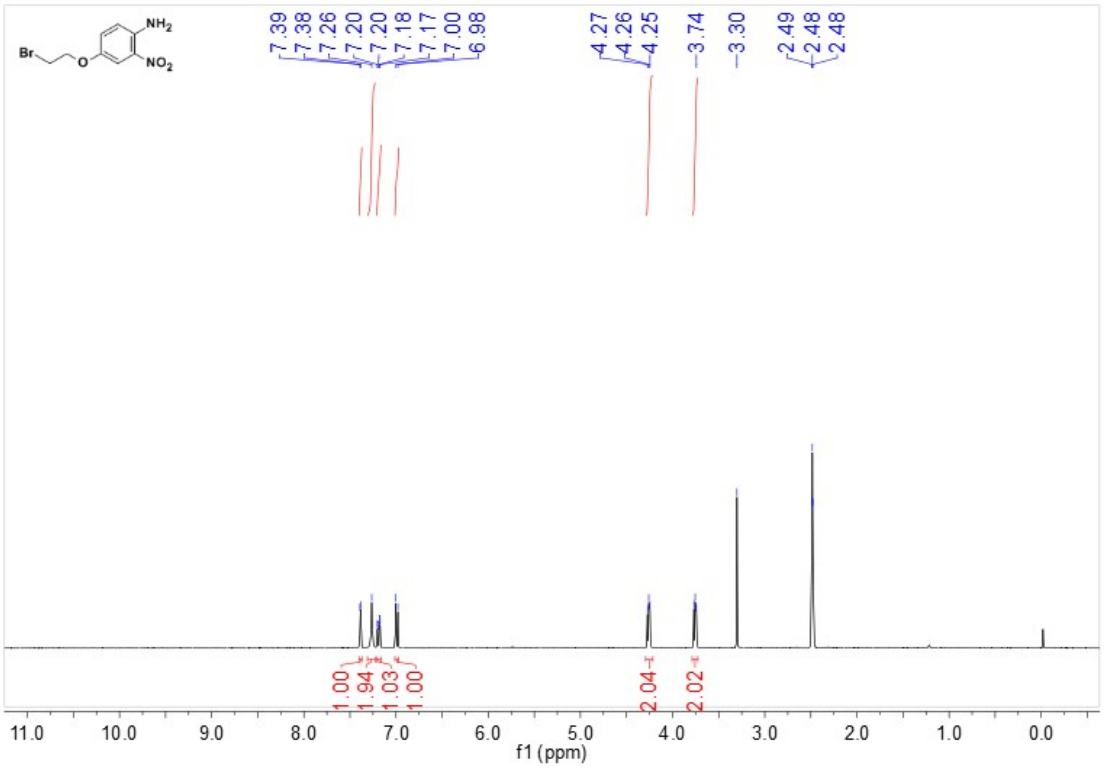
^{13}C NMR of compound **2** in $CDCl_3$



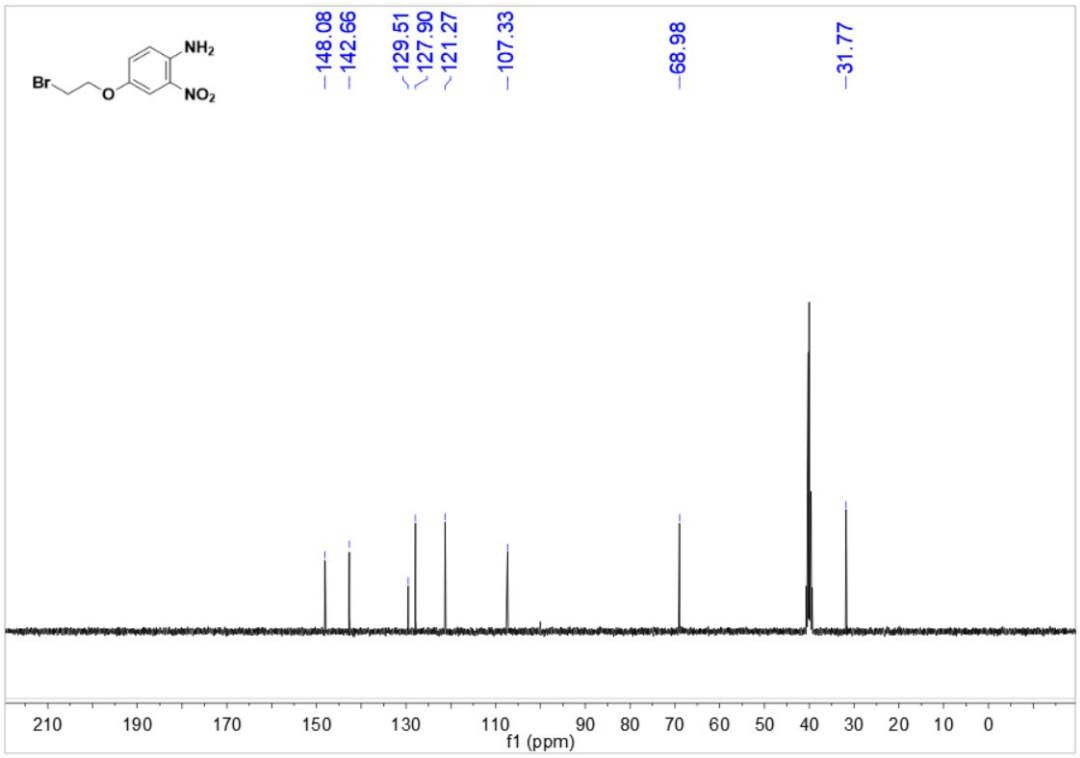
^{1}H NMR of compound **3** in DMSO-d_6



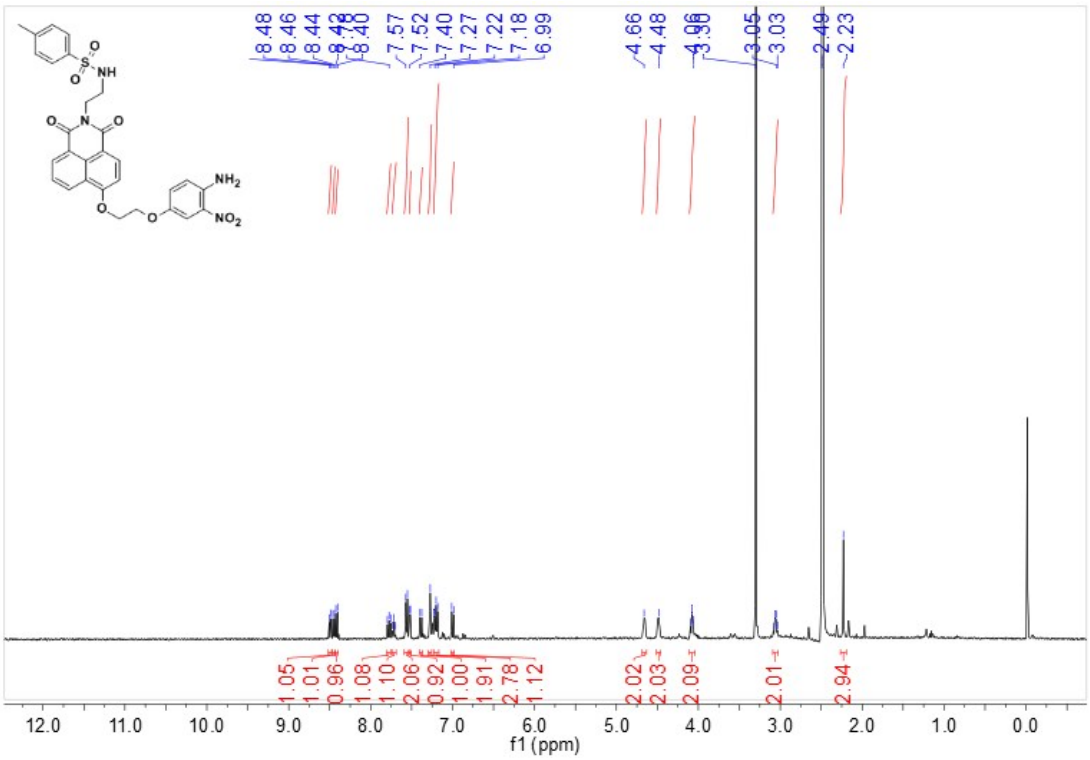
^{13}C NMR of compound **3** in DMSO-d_6



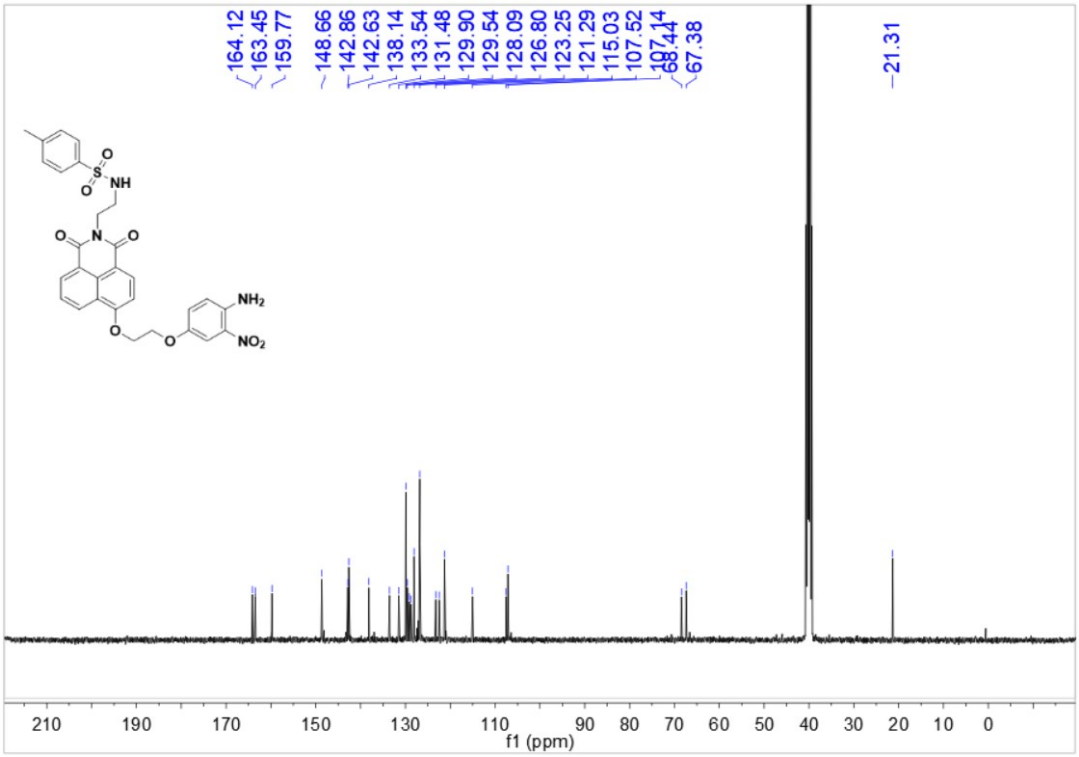
^{1}H NMR of compound **4** in DMSO-d_6



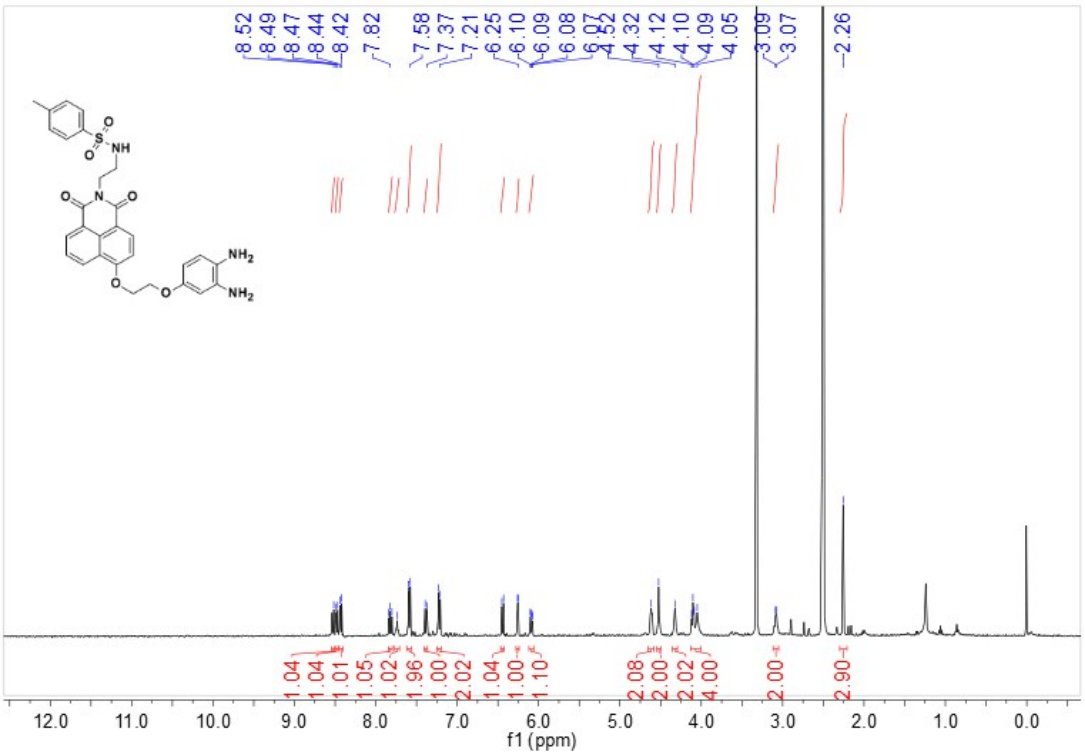
^{13}C NMR of compound **4** in DMSO-d_6



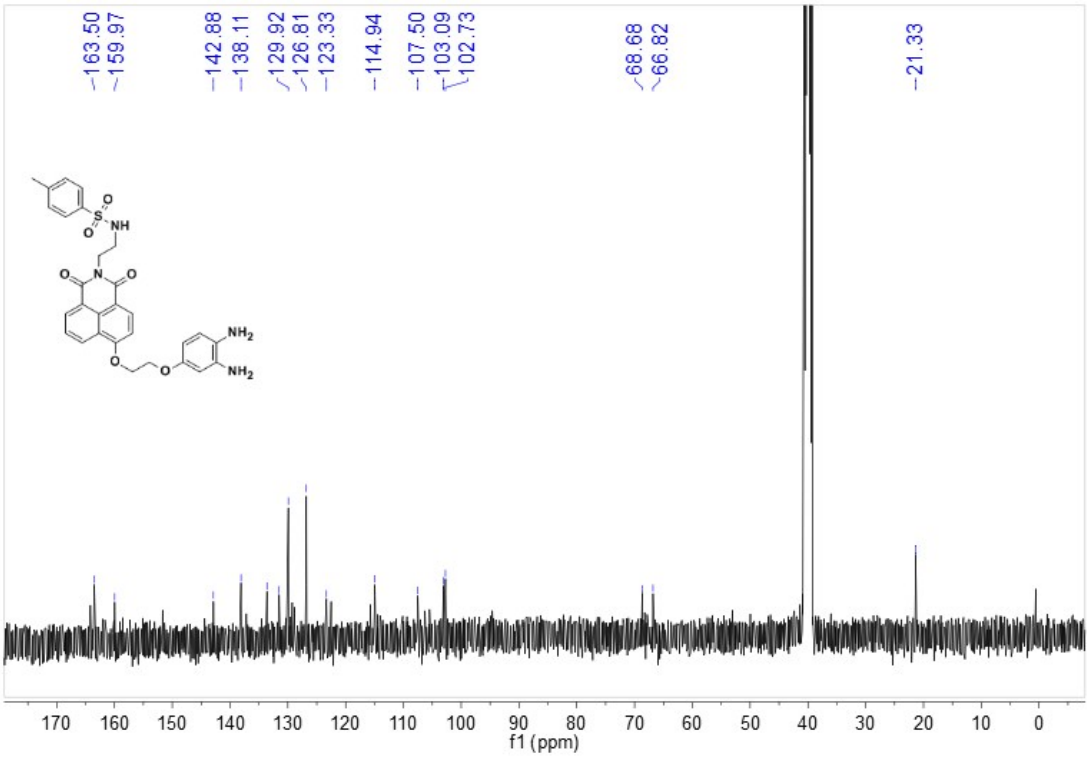
^{1}H NMR of compound **5** in DMSO-d_6



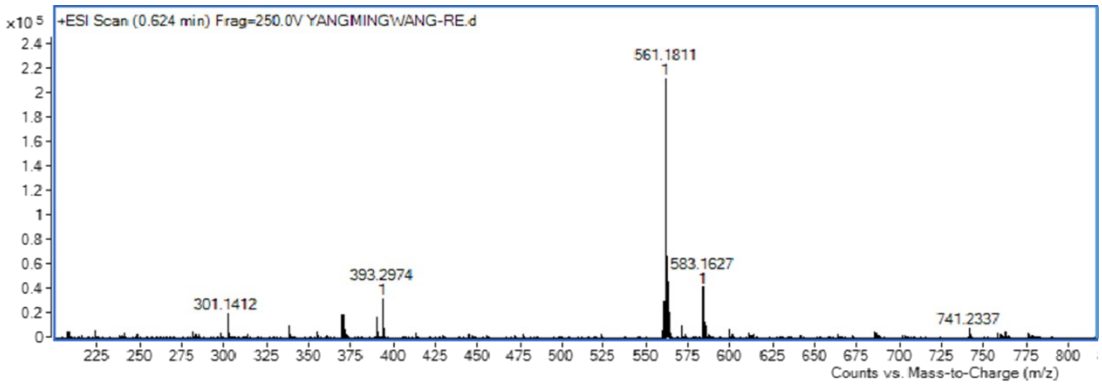
^{13}C NMR of compound **5** in DMSO-d_6



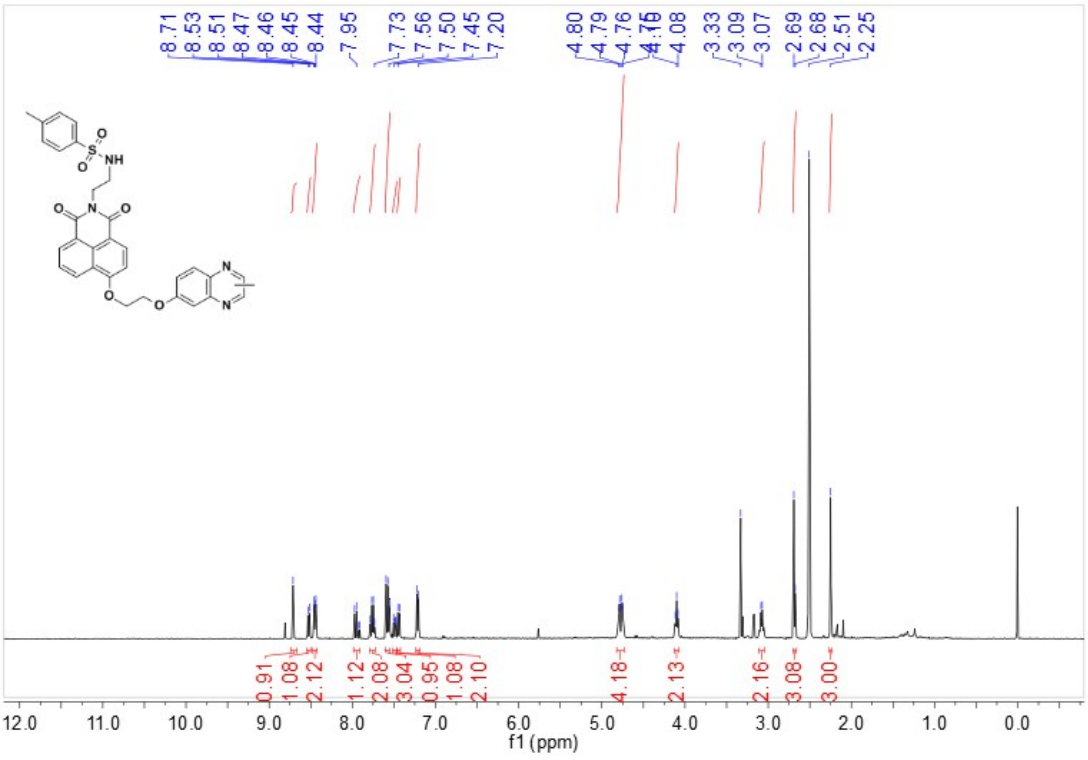
^{1}H NMR of probe **NI-OPD** in DMSO-d_6



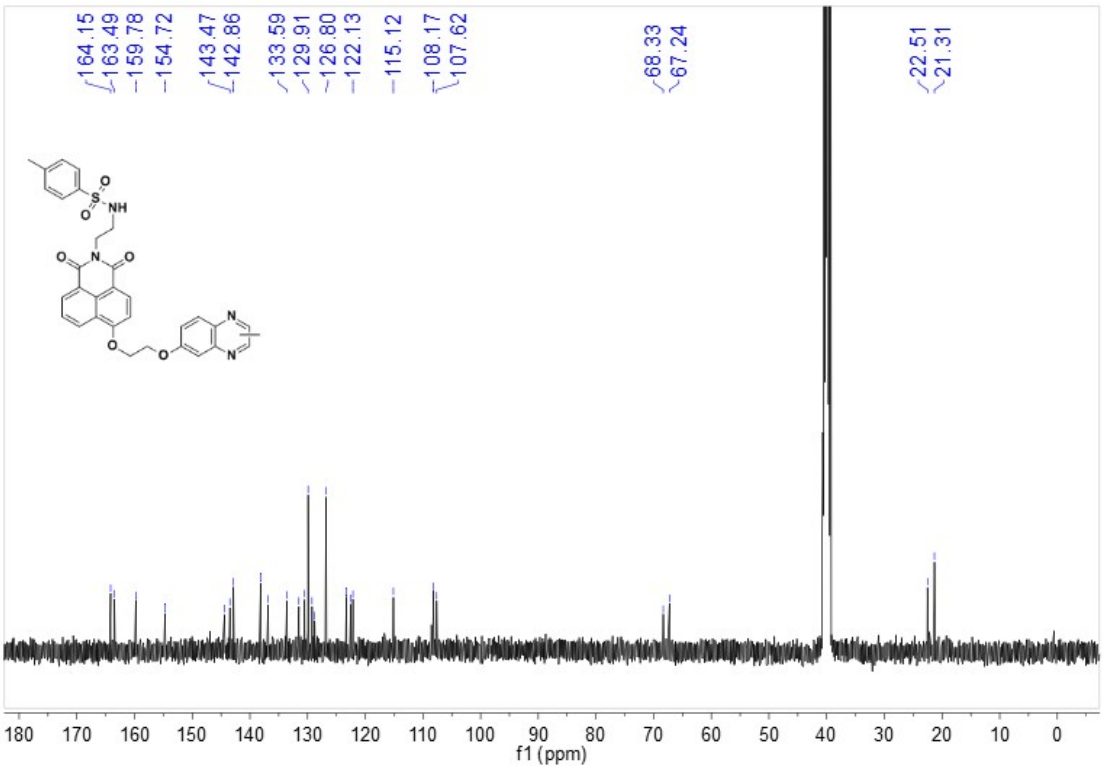
^{13}C NMR of probe **NI-OPD** in DMSO-d_6



HR-MS of probe **NI-OPD**



^{1}H NMR of product **NI-MQL** in DMSO-d_6



^{13}C NMR of product **NI-MQL** in DMSO-d_6

11. Reference

(1) K. Tsukamoto, S. Shimabukuro, M. Mabuchi and H. Maeda, *Chemistry*, 2016, **22**, 8579-8585.

(2) M. T. Barros and F. Sineriz, *Tetrahedron*, 2000, **56**, 4759-4764.