Supporting Information

Fast response two-photon fluorogenic probe based on Schiff base derivatives for monitoring nitric oxide level in living cells and Zebrafishes

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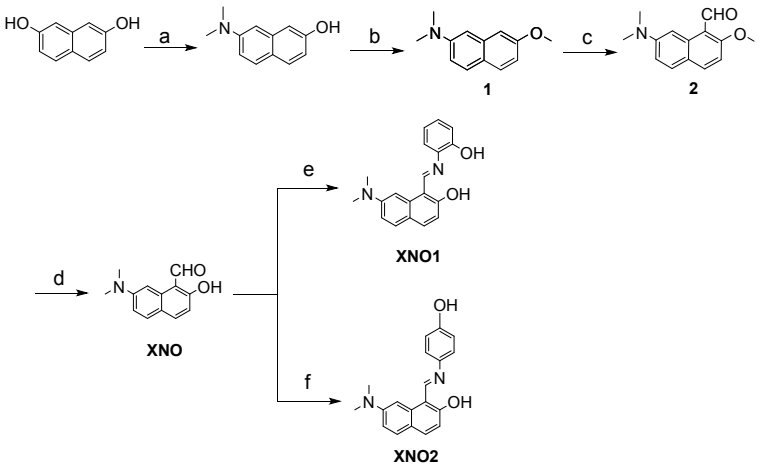
1. Experimental Section

1.1 Materials and instruments

All the chemical reagents were purchased from Aldrich or TCI. Commercially available reagents were used without further purification. Anhydrous solvents for organic synthesis were distilled over CaH_2. Reaction progress was monitored by TLC on pre-coated silica plates (250 μM thickness) and spots were visualized by UV light or iodine. ^1H and ^{13}C NMR spectra were measured with an Avance AV-300 spectrometer and DRX-500. Coupling constants (*J* value) are reported in Hertz. Photoluminescent spectra were recorded using BioTek Cytatio5 Cell Imaging Multi-Mode Reader. All the measurements were performed at room temperature. The liquid chromatography-mass spectrometry (LC-MS) was carried out with Acquity UPLC H-ClASS Waters UPLCH-CL systems equipped with an autosampler, using Isogradient elution-phase Acquity uplc HSS T3 1.8 μm C18 2.1 × 100 mm columns. Mass spectra were recorded on a TargetLynx XS™ spectrometer. High resolution mass spectra (HRMS) were obtained using an LTQ Orbitrap XL hybrid FTMS (Fourier Transform Mass Spectrometer) by Thermo Fisher Scientific. All images were acquired on Zeiss LSM880 NLO (2+1 with BIG) Confocal Microscope System equipped with objective LD C-Apochromat 63x/1.15 W Corr M27, cell incubator with temperature control resolution ± 0.1 ^oC, 405 nm Diode laser, Argon ion laser (458, 488 and 514 nm), HeNe laser (543 and 594 nm), Rack LSM 880 incl. 633 nm laser, and a Spectra Physics femtosecond Ti: sapphire laser (~4 W at 820 nm) which corresponded to approximately 1% (~40 mW at 820 nm, the output laser pulses have a tunable center wavelength from 690 nm to 1040 nm with pulse duration of 150 < fs and a repetition rate of 80 MHz) average power in the focal plane as the excitation source, with main beam splitter wheel VIS equipped for ROGB lasers/Axio imager beam coupling optics for NLO and 405 nm laser and 8 channels AOTF for simultaneous control of 8 laser lines. A PMT detector ranging from 420 nm to 700 nm for steady-state fluorescence and non-descanted detectors (BiG.2) for the two-photon excited fluorescence were used. Internal photomultiplier tubes were used to collect the signals in 8-bit unsigned 1024×1024 pixels at a scan speed of 200 Hz. Images were processed with Zeiss User PC Advanced for LSM system (BLUE).

1.2 The synthesis of XNO1 and XNO2.



Scheme S1. Synthesis route of **XNO1** and **XNO2**. Reagents and conditions. a) $Na_2S_2O_5$, Me_2NH, H_2O, 140 °C, 5 h, 60%; b) $CsCO_3$, MeI, acetone, 80 °C 3 h, 80%; c) CH_2Cl_2,Cl_2CHOCH_3, $TiCl_4$, 25 °C, 75%; d) BBr_3, CH_2Cl_2, 25 °C, 67%; e) 2-aminophenol, piperidine, EtOH, reflux, 4 h, 80%; f) 4-aminophenol, piperidine, EtOH, reflux, 4 h, 90%.

The synthesis of [7-(methoxymethoxy)naphthalen-2-yl]dimethylamine (1):

Compound **1** was synthesized according to the previously reported method with some modification.[1] Briefly, A solution of dimethylamine (10.5 mL, 93.5 mmol) was added to a mixture of 2,7-dihydroxynaphthalene (3.0 g, 18.7 mmol), sodium metabisulfite (7.11 g, 37.4 mmol), and H_2O (10 mL) in a microwave tube. The reaction mixture was reacted at 140 °C for 5 h. After being cooled to room temperature, the pH value of the reaction was adjusted to 4 to dissolve the solid, and then the dichloromethane (50 mL) was added three times for extraction and the organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (eluent: 20% EtOAc in petroleum ether) to afford 8-dimethylamino-2-naphthalenol as a white solid (2.10 g, 60%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.57 (m, 2H), 6.97-7.01 (m, 1H), 6.93 (d, J = 3 Hz, 1H) , 6.68 (m, 2H), 2.98 (s, 6H).

To a solution of 8-dimethylamino-2-naphthalenol (1.0 g, 5.34 mmol) in acetone (10 mL), and the cesium carbonate (5.21 g, 15 mmol) was added at room temperature and then the methyl iodide (1.36 mL, 10.68 mmol) was added dropwise. The resulting mixture was reacted at 80 °C for 3 h. After being cooled to room temperature and the pH value of the reaction was adjusted to 4 to dissolve the solid, and then the dichloromethane (20 mL) was added three times for extraction and the organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (eluent: 5% EtOAc in petroleum ether) to afford compound **1** as a white solid (988 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (m, 2H), 7.02 (m, 2H), 6.92 (m, 2H), 3.01 (s, 3H), 3.05 (s, 6H). ^{13}C NMR (75 MHz, CD_3OD): δ 159.68, δ 137.62, δ 129.86, δ 125.76, δ 116.96, δ 115.31, δ 106.34, δ 105.69, δ 41.65. MS (*m*/*z*): calcd $[M+H]^+$ for $C_{13}H_{15}NO$: 202.11; found, 202.18.

The synthesis of (dimethylamino)-2-methoxy-1-naphthaldehyde (2):

Compound **2** was synthesized according to the previously reported method with some modification.[1] To a solution of compound **1** (635 mg, 3.155 mmol) in dichloromethane (5 mL) was added $TiCl_4$ (6.5 mL, 1 M solution in dichloromethane) at -20 °C and the resulting mixture was stirred at the same temperature for 10 min. To this mixture was added a solution of dichloromethyl methyl ether (0.30 mL, 3.47 mmol) in dichloromethane (5 mL) dropwise, and the resulting mixture was stirred at -20 °C for 30 min and then at room temperature 24 h. The reaction mixture was treated with saturated NH_4Cl (15 mL), and the product was extracted with dichloromethane (20 mL) three times. The combined organic phase was dried over Na_2SO_4 and concentrated, and the reside was purified by silica gel column chromatography (eluent: 20% EtOAc in petroleum ether) to give compound **2** as a yellow solid (0.54g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.87 (m, 1H), 8.62 (m, 2H), 7.89 (d, J = 9 Hz, 1H), 7.63 (d, J = 9 Hz, 1H), 7.06 (d, J = 9 Hz, 1H), 6.95 (d, J = 9 Hz,

1H), 4.01 (s, 3H), 3.13 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 119.13, δ 165.42, δ 137.48, δ 133.58, δ 129.93, δ 119.65, δ 119.13, δ 114.77, δ 56.37, δ 41.17. MS (*m*/*z*): calcd $[M+H]^+$ for $C_{14}H_{15}NO_2$: 230.11; found, 230.19.

The synthesis of 7-(Dimethylamino)-2-hydroxy-1-naphthaldehyde (XNO):

To a solution of compound **2** (1.08 g, 4.71 mmol) in dichloromethane (50 mL) was added BBr_3 (7.1 mL, 1.0 M in dichloromethane) at -20 °C. After being stirred for 1 h at same temperature, the reaction was stirred for another 24 h at room temperature. The mixture was treated with a saturated solution of $NaHCO_3$ slowly and then stirred at 0 °C for 30 min. The reaction mixture was extracted with dichloromethane (20 mL) three times. The combined organic phase was dried over Na_2SO_4 and concentrated, and the reside was purified by silica gel column chromatography (eluent: 10% EtOAc in petroleum ether), compound **XNO** was obtained as a yellow solid (680 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.22 (s, 1H), 10.72 (s, 1H), 7.79 (d, *J* = 4.5 Hz, 1H), 7.62 (d, *J* = 4.5 Hz, 1H), 7.36 (s, 1H), 6.97 (d, *J* = 4.5 Hz, 1H), 6.80 (d, *J* = 3 Hz, 1H), 3.12 (s, 1H). HRMS (*m*/*z*): calcd $[M+H]^+$ for $C_{13}H_{13}NO_2$: 216.0946; found, 216.1027.

The synthesis of (E)-7-(dimethylamino)-1-(((2-hydroxyphenyl) imino) methyl) naphthalen-2-ol (XNO1):

To a solution of compound **XNO** (100 mg, 0.46 mmol) in EtOH (5 mL), three drops of piperidine and 2-aminophenol (52 mg, 0.48 mmol) were added under argon atmosphere. The reaction solution was refluxed for 4 h. After being cooled to room temperature, a red precipitate rapidly appeared and was filtered, then further washed with EtOH for three times. Compound **XNO1** was obtained as a dark red solid (114 mg, 80%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 15.70 (s, 1H), 10.21 (s, 1H), δ 9.35 (s, 1H), 7.84 (d, *J* = 5 Hz, 1H), 7.60 (d, *J* = 10 Hz, 1H), 7.47 (d, *J* = 10 Hz, 1H), 7.33 (d, *J* = 5 Hz, 1H), 7.06-7.10 (m, 1H), 6.92-6.99 (m, 1 H), 6.79-6.81 (dd, J_1 = 10 Hz, J_2 = 5 Hz, 2H). 6.44 (d, *J* = 10 Hz, 1H), δ 3.07 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 177.90, δ 150.49, δ 149.32, δ 148.43, δ 138.09, δ 135.60, δ 130.08, δ 126.29, δ 119.75, δ 117.75, δ 115.87, δ 110.59, δ 107.46, δ 100.06, δ 40.32. HRMS (m/z): calcd $[M+H]^+$ for $C_{19}H_{18}N_2O_2$: 307.1368; found, 307.1454.

The synthesis of (E)-7-(dimethylamino)-1-(((4-hydroxyphenyl)imino)methyl)naphthalen-2-ol (XNO2):

To solution of compound **XNO** (100 mg, 0.46 mmol) in EtOH (5 mL), three drops of piperidine and 4-aminophenol (52 mg, 0.48 mmol) were added under argon atmosphere. The reaction solution was refluxed for 4 h. After being cooled to room temperature, a red precipitate rapidly appeared and was filtered, then further washed with EtOH for 3 times. Compound **XNO2** was obtained as a dark red solid (129 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 16.06 (s, 1H),

δ 9.64 (s, 1H), δ 9.47 (s, 1H), δ 7.67 (d, *J* = 10 Hz, 1H), δ 7.58 (d, *J* = 10 Hz, 1H), δ 7.45 (d, *J* = 10 Hz, 2H), δ 7.35 (s, 1H), δ 6.93 (d, *J* = 5 Hz, 1H), δ 6.88 (d, *J* = 10 Hz, 2H), δ 6.64 (d, *J* = 10 Hz, 1H), δ 3.07 (s, 6H). ^{13}C NMR (125 MHz, DMSO-*d6*): δ 169.35, δ 156.30, δ 154.02, δ 150.13, δ 136.33, δ 135.76, δ 134.86, δ 129.97, δ 121.92, δ 116.42, δ 116.06, δ 111.76, δ 107.60, δ 99.35, δ 40.31. MS (*m/z*): calcd $[M+H]^+$ for $C_{19}H_{18}N_2O_2$: 307.1368; found, 307.1452.

1.3 Quantum yield and two-photon absorption cross-section measurement

The fluorescence quantum yields were measured in DMSO by using rhodamine B as a standard (Φ_f = 0.70). The fluorescence quantum yield of **XNO1** was calculated in terms of the following equation (eq 1):

$$\Phi_S = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{n_s^2}{n_r^2} \right) \frac{\int F_s}{\int F_r} \quad (1)$$

Where the subscript r stands for the reference molecule and subscript s stands for the sample, Φ is the fluorescence quantum yield, A is the absorbance of the solution, n is the refractive index of the solvent, and F is fluorescence intensity of the solution at the excitation wavelength. The absorption maximum was kept during 0.02-0.05. Quantum yield of rhodamine B is 0.70 in absolutely ethanol.[2]

The two-photon absorption cross-section (σ) and action cross-section (Φσ) was measured by using the two-photon induced fluorescence measurement technique with the following equation.[3]

$$\sigma_S = \sigma_r \frac{F_s \Phi_r C_r \eta_r}{F_r \Phi_s C_s \eta_s} \quad (2)$$

The subscripts "s" and "r" stand for the sample and reference molecules respectively. F is the integrated fluorescence intensities measured at the same power of the excitation beam. Φ is the fluorescence quantum yield. n is refractive index. The number density of the molecules in the solution was denoted as c. σ_r is the two-photon absorption cross section of the reference molecule. In this experiment, **Flu1** was selected as reference two-photon dye (The above results were summarized in Table S1).[4]

1.4 Preparation of NO Stock Solutions.

The preparation of NO and its concentration were determined by the Griess method reported previously.[5] Since O_2 can rapidly oxidize NO to form NO_2, all apparatus were carefully degassed with argon for 30 min to remove O_2. The NO gas was bubbled through a saturated NaOH solution to eliminate NO_2 generated from the reaction of NO with O_2. To produce NO stock solution,

this gas was bubbled through 10 mL of deoxygenated deionized water for 30 min and kept under an NO atmosphere. The concentration of this stock solution was determined by the Griess method.

1.5 The fluorescence responses of the probe XNO1 to NO and other analytes.

A stock solution of **XNO1** was prepared in DMSO, the analytes solution various ROS, RNS, and ions were prepared based on the method reported in the previous literature.[6,7] The fluorescence emission spectrum of 20 μM **XNO1** react with different amounts of NO and other analytes were measured after reaction for 1 min in the PBS buffer (10 mM, 7.4, containing 5% DMSO and 0.2% Triton X-100) at 37 ^{o}C.

1.6 The effect of pH.

Different pH values (from 4 to 9) buffer were prepared that contained 5% DMSO and 0.2% Triton X-100. The fluorescence emission spectrum of the probe **XNO1** (20 μM) and the product **XNO** (20 μM) with 1 equiv. of NO were measured after incubating for 1 min in various pH solutions. To investigate the pH stability, the time-dependent fluorescence emission spectrum of the probe **XNO1** (20 μM) were measured with/without 1.0 equiv. of NO in different pH buffer solution that contained 5% DMSO and 0.2% Triton X-100.

1.7 The mechanism study.

Before the LC-MS experiment, the probe **XNO1** (20 μM) was dissolved in methanol and then divided into two groups averagely. After that, the first sample (10 μL) was directly carried out by the LC-MS automatically. In the second group, the probe solution was added with 5.0 equiv. of NO solution and then stirred for 1 min. After that, the reacted solution (10 μL) was directly carried out by the LC-MS automatically. In addition, the **XNO** and pyrocatechol (Commercialize available) were dissolved in methanol and the solution (10 μL) was directly carried out by the LC-MS automatically as a contrast. The flow rate was set as 0.3 mL/min. The detailed elution method was 60% ACN and 0.1% formic acid in water for 10 min.

1.8 Cell culture and imaging.

SH-SY5Y cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), containing 10% fetal bovine serum (FBS), 100.0 mg mL^{-1} streptomycin, and 100 IU mL^{-1} penicillin. SH-SY5Y cells were seeded in glass-bottom dishes (Mattek) and grown to 60-70% confluency. The cytotoxicity activities of the probes were determined using an XTT reagent (Sigma) following the manufacturer guidelines. Briefly, 5000-10000 cells were seeded (Since they will reach 60~70% confluency within 24 to 36 h in the absence of compounds) in 96-well plates under the conditions described above. The medium was aspirated, washed with PBS, and then treated, in duplicate, with 0.1 mL of the medium containing different concentrations of XNO1 (0-35 μM). Probes were applied from DMSO stocks, whereby DMSO never exceeded 1% in the final solution. The same volume of DMSO was used as a negative control, while the same volume of staurosporine (STS, 200 nM) was

used as a positive control. After a total treatment time of 24 h, proliferation was assayed using the XTT reagent (Sigma).

Briefly, the cell experiment can be divided into three groups. The first group is that SH-SY5Y cells were incubated with 20 μM **XNO1** for 2 h. Then SH-SY5Y cells were washed by PBS buffer before imaging. In the second group, SH-SY5Y cells were incubated 20 μM **XNO1** for 2 h and washed by PBS buffer three times and then incubated exogenous NO (SNP) solution (20 μM) for 2 h and then washed by PBS buffer three times before imaging. In the third group, the SH-SY5Y cells were pre-incubated with LPS (15 μg mL^{-1}) for 12 h and then washed by PBS buffer three times and subsequently incubated with 20 μM **XNO1** for 2 h. Background signals of all images were verified to be nearly zero by imaging the same cells treated with a negative control. Confocal fluorescence images of SH-SY5Y cells incubated with 20 μM **XNO1** for 2 h and in the presence of exogenous NO (Sodium nitroprusside dihydrate, SNP, NO donor) solution (20 μM) for 2 h and then stained with LysoTracker™ for 15 min. In order to investigate the pH effect in lysosome, we performed the colocalization assay by removing the NO with 1 h pretreatment of NO inhibitor (L-nitroarginine-methyl-ester, L-NAME, 1.0 mM). One- and two-photon fluorescence images of cells were obtained by exciting samples with laser source set at wavelength of 405 nm and 820 nm, and recorded at 500-650 nm.

1.9 Zebrafish culture and imaging by two-photon fluorescence microscopy (TPFM).

For imaging of Zebrafishes, all Zebrafish embryos were passed through three successive washes of buffer solution before observation. Before imaging, the fish was anesthetized because we use 0.01%-0.02% tricaine in egg water to imprison it. Zebrafishes were cultured for 4-day-old for two-photon fluorescence bioimaging *in vivo*. Subsequently, 4-day-old Zebrafishes were raised with **XNO1** (20 μM) at 28 °C. Two-photon fluorescence images of Zebrafishes were obtained by exciting samples with laser source set at wavelength of 820 nm for different time points, respectively. In addition, in order to regulate the NO level in Zebrafishes, we also pretreated the Zebrafishes with different amounts of L-NAME (2.0 mM and 4.0 mM) or NO donor for 1 h and then incubated with **XNO1** (20 μM) for 2 h. A staining free group was used as a control. All images were acquired at the same way.

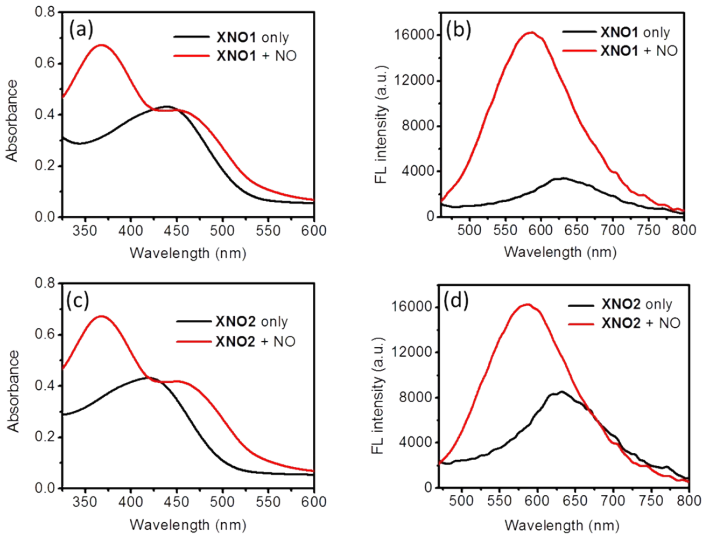


Fig. S1. Spectral profiles of **XNO1** and **XNO2**. UV-vis absorption (a) and fluorescence spectrum (b) of **XNO1** (20 μM) with/without NO (20 μM). UV-vis absorption (c) and fluorescence spectrum (d) of **XNO2** (20 μM) with/without NO (20 μM). The spectrum were recorded in PBS buffer (10 mM, pH 7.4, containing 5% DMSO and 0.2% Triton X-100) after 1 min. λ_{ex} = 360 nm.

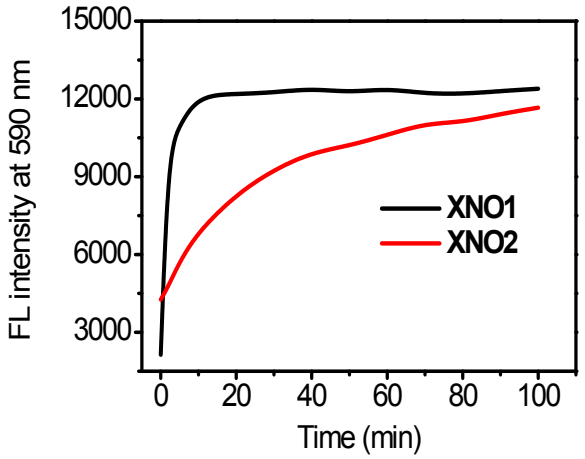


Fig. S2. Time-dependent fluorescence intensities at 590 nm of **XNO1** (20 μM) and **XNO2** (20 μM) reacted with NO (1.0 equiv.) in PBS buffer (10 mM, pH 7.4, containing 5% DMSO and 0.2% Triton X-100). λ_{ex} = 360 nm.

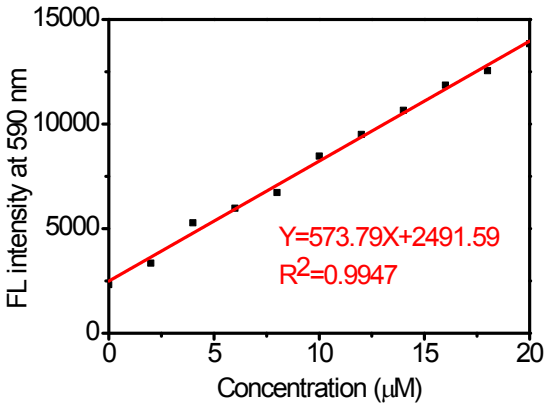


Fig. S3. Fluorescence intensity at 590 nm of **XNO1** (20 μM) as a function of NO concentration in PBS buffer (10 mM, pH7.4, containing 5% DMSO and 0.2% Triton X-100). λ_{ex} = 360 nm.

The method for determining the limit of detection (LOD)

First the calibration curve was obtained from the plot of fluorescence intensity at 590 nm, as a function of the NO concentration. The regression curve equation was then obtained for the lower concentration part.

The detection limit = 3 × S.D. / k

Where k is the slope of the curve equation, and S.D. represents the standard deviation for the probe **XNO1** solution's fluorescence intensity in the absence of NO.[8]

I_{590} = 2491 + 573.79[NO] (R = 0.9947)

LOD = 3 × 0.0187 / 573.515 = 97.81 nM

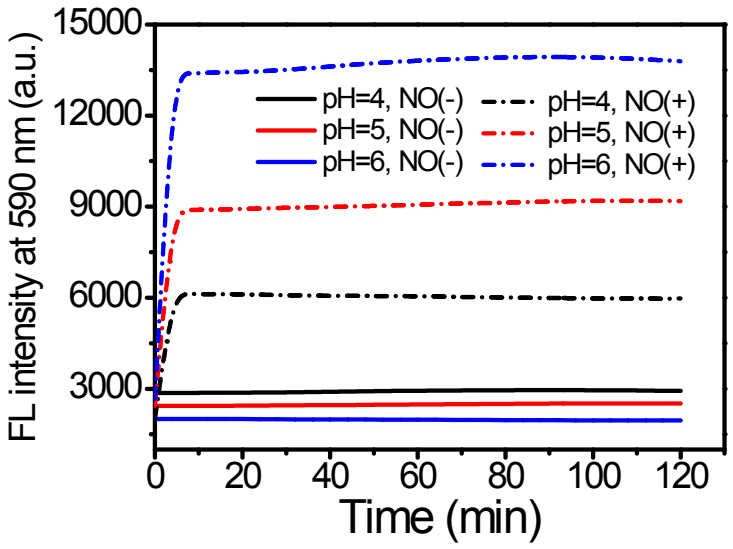
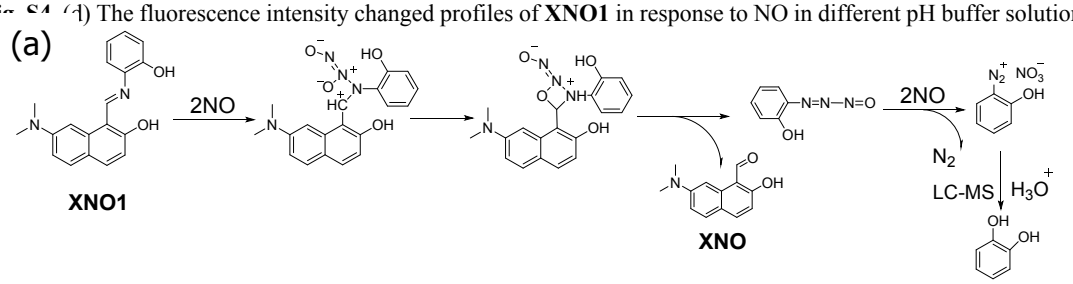
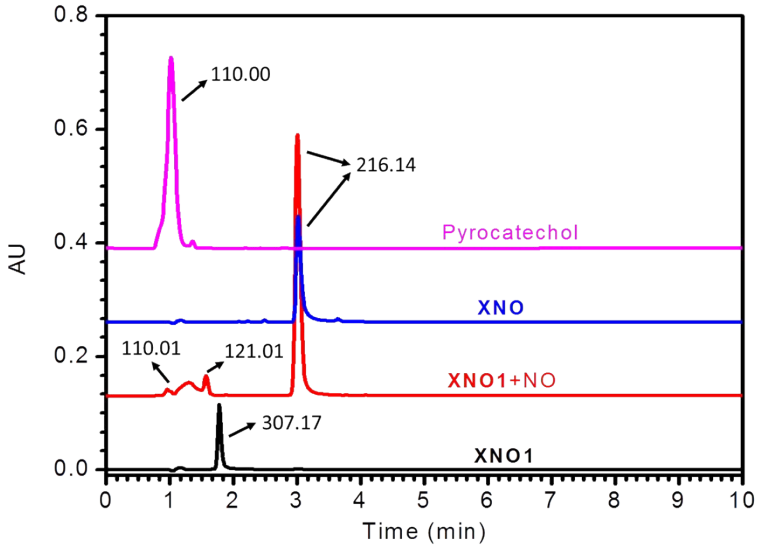
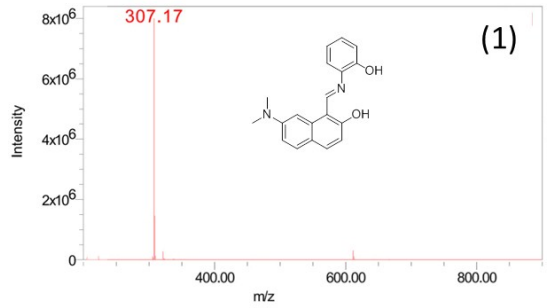


Fig. S4 (a) The fluorescence intensity changed profiles of **XNO1** in response to NO in different pH buffer solution.



(c)


110.00
216.14
Pyrocatechol
XNO
110.01
121.01
XNO1+NO
307.17
XNO1
AU
Time (min)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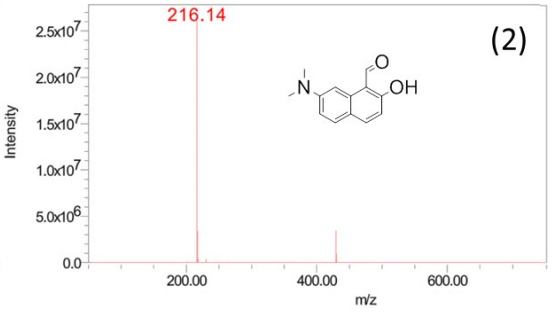
(1)
Intensity
m/z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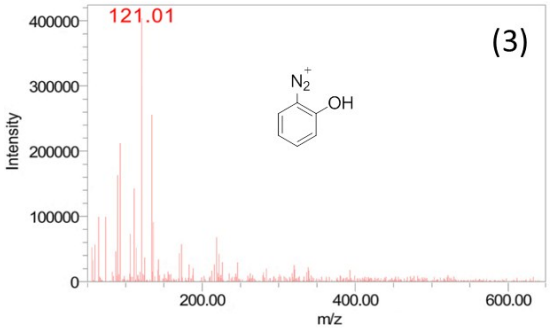
Intensity
m/z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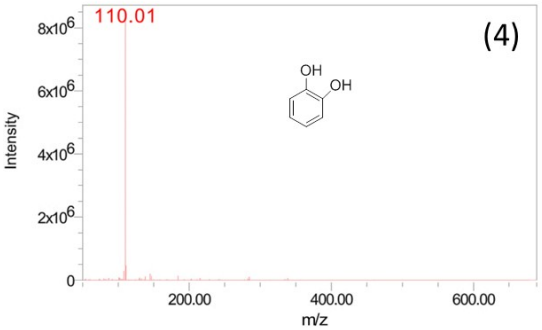
Intensity
m/z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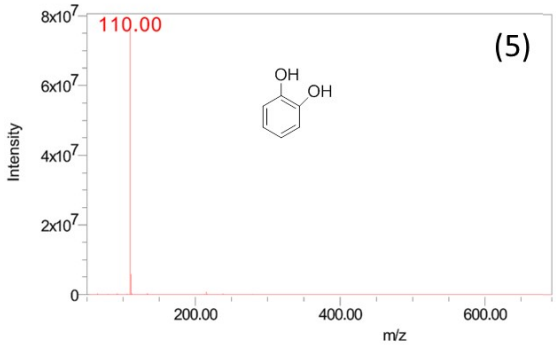
Intensity
m/z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Intensity
m/z

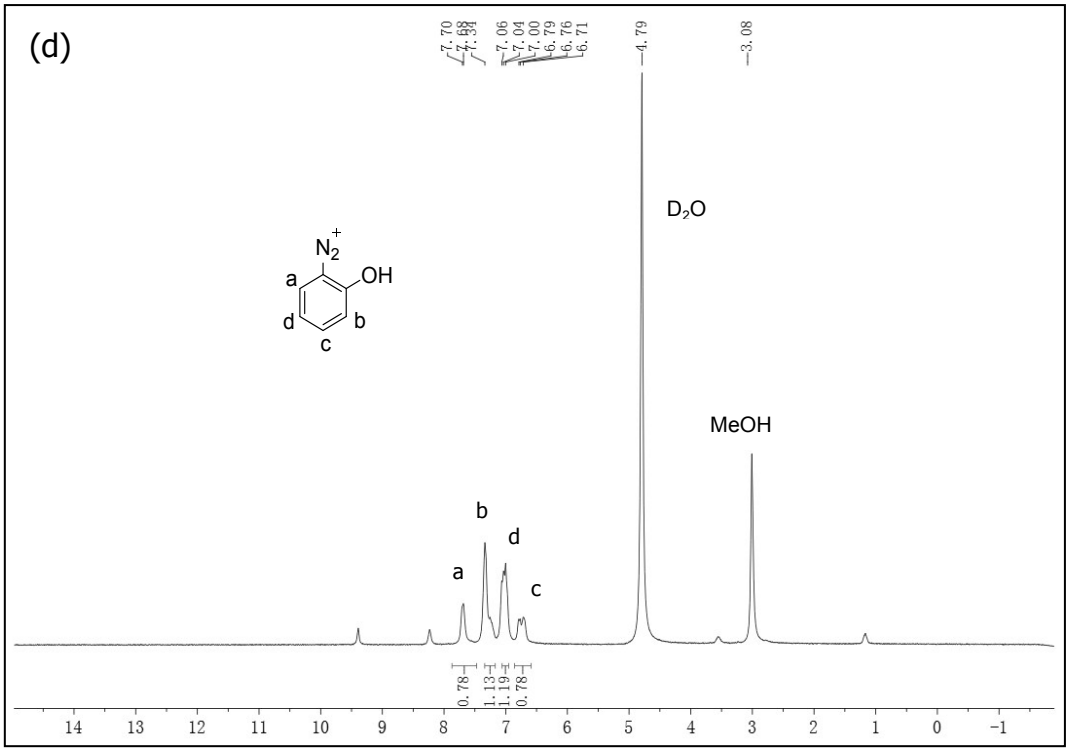


Fig. S5. (a) The proposed reaction mechanism of **XNO1** response to NO; (b) the LC spectra of the probe **XNO1**, **XNO1** response to NO for 1 min, **XNO** and pyrocatechol; (c) the corresponding mass spectra and results of (b); (d) ^{1}H NMR spectra of the product 2-hydroxybenzenediazonium from the reaction of **XNO1** with NO.

To demonstrate the sensing mechanism of the probe **XNO1** response to NO, the detailed LC-MS experiment was performed. Before the LC-MS experiment, the probe **XNO1** (20 μM) was dissolved in methanol and then divided into two group averagely. After that, the first sample (10 μL) was directly carried out by the LC-MS automatically. In the second sample, the probe solution was added with 5.0 equiv. of NO aqueous solution and then stirred for 1 min. After that, the reacted solution (10 μL) was directly carried out by the LC-MS automatically. In addition, as a contrast, the **XNO** and pyrocatechol (commercialize available) were dissolved in methanol and the solution (10 μL) was directly carried out by the LC-MS, respectively. As shown in Fig. S5b and S5c, upon addition of NO solution for 1 min, the MS with *m/z* = 216.14, *m/z* =121.01 and *m/z* = 110.01 were appeared simultaneously, which are corresponding to the product **XNO**, 2-hydroxybenzenediazonium and pyrocatechol, respectively. Due to its instability under the mild acid condition of LC-MS experiment (60% ACN and 0.1% formic acid in water), the 2-hydroxybenzenediazonium could easily produce pyrocatechol by hydrolysis reaction.[9] Thus, the peaks of 2-hydroxybenzenediazonium and pyrocatechol were simultaneously appeared in the LC-MS experiment. In order to further confirm the final product 2-hydroxybenzenediazonium, we next performed ^{1}H NMR measurement to investigate its structure (Fig. S5d). The 2-hydroxybenzenediazonium was synthesized according to the previously reported method with some modification.[10] **XNO1** (0.60 g) was dissolved in ether (70 mL), the solution was placed in bottle and deoxygenated with nitrogen thoroughly. The preparation of NO and its concentration were determined by the Griess method reported previously.[5] Before the NO bubbled into the reaction solution using a long needles, the intake system was deoxygenated with nitrogen for 5 min as well. And then the reaction was stirred 1 h under the NO bubbling persistently whilst an orange red precipitate formed. The excess gas was swept away with nitrogen and then placed into the 4 ^{o}C to make it precipitated completely after the completion of the reaction. After that, the orange red precipitate was filtered and recrystallized from icy (4 ^{o}C) methanol/ether for 5 times to yield compound 2-hydroxybenzenediazonium (143 mg; 60%) as dark orange solid. ^{1}H NMR (300 MHz, D_2O) δ 7.70 (d, J = 6 Hz, 1H), 7.28 (m, 1H), 7.04 (d, J =18 Hz, 1H)7.70 (t, J = 24 Hz, 1H).

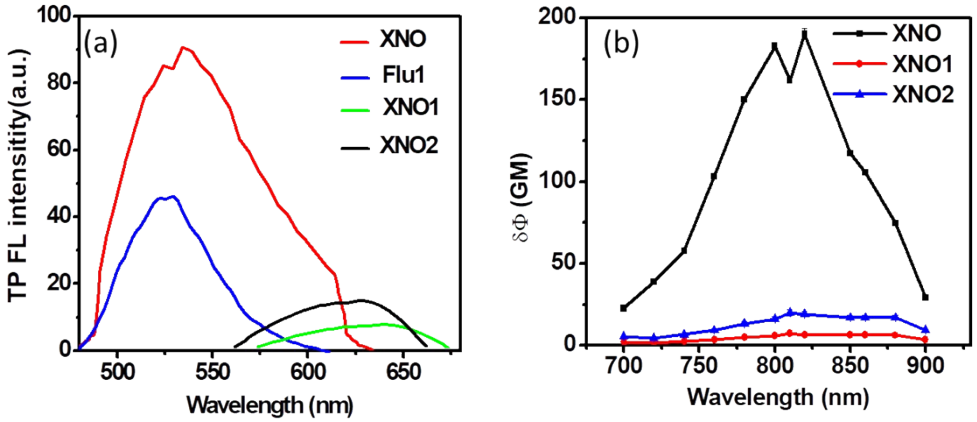


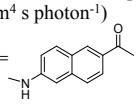
Fig. S6. (a) TP emission spectra of **XNO** (10 μM) (red), **XNO1** (20 μM) (green), **XNO2** (20 μM) (black) and **Flul** (10 μM) (blue) in DMSO solution. λ_{ex} = 820 nm; (b) The calculated values of two-photon action cross-section of **XNO**, **XNO1** and **XNO2**.

Table S1. Summaries of spectroscopic properties of XNO1 and XNO

Compounds	λ*	*Φ*fl (%)	ε*Φ*	δ*Φ*‡ / GM
XNO1	450/620	7.9	661.1	15.9
XNO2	430/620	9.8	1157.8	19.8
XNO	360/590	22.4	8034.5	190.3
Flu1[4]	352/498	19.0	4480.0	128.0

* Peak position of the longest absorption/emission band.
† Brightness upon the absorption maximum of **XNO1/XNO2/XNO/Flu1.**
‡ The maxima two-photon action cross-section values upon excitation wavelength of **Flu1** from 700 to 900 nm in GM (1 GM = 10^{-50} cm^4 s $photon^{-1}$)

Flu1 =



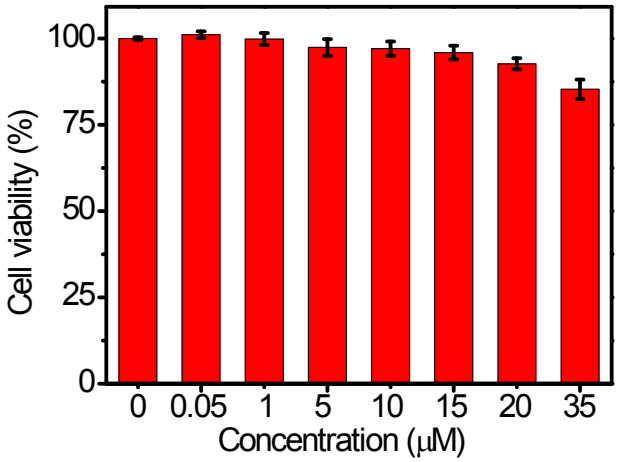


Fig. S7. XTT assay of SH-SY5Y cells treated with **XNO1** at different concentrations for 24 h.

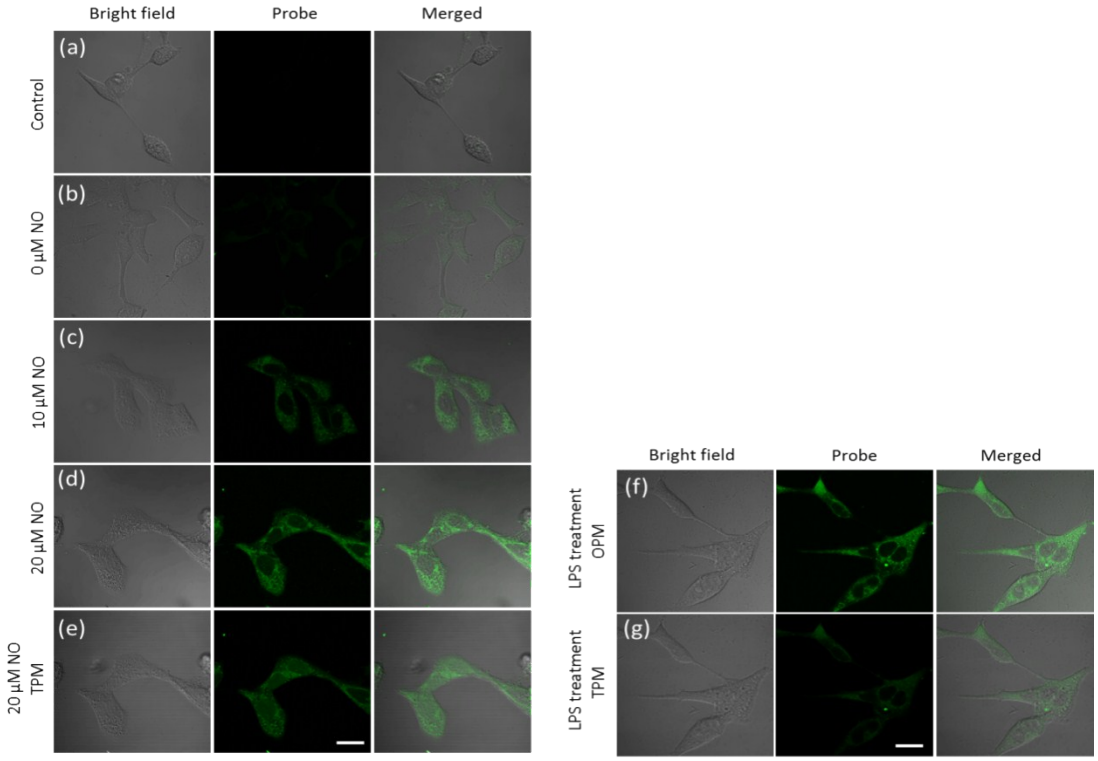


Fig. S8. Confocal images of live SH-SY5Y cells incubated with **XNO1** (20 µM) for 2 h and then treated NO donor at 0 µM (b), 10 µM (c) and 20 µM (d). (e) TPFM image of (c). OP (f) and TP (g) images of SH-SY5Y cells pretreated with NO stimulations (LPS 15 µg mL^{-1}, 12 h) and subsequently incubated with **XNO1** for 2 h. The fluorescence emission was collected at 500-650 nm upon the excitation at 405 nm (OP) and 820 nm (TP). Control = cells without any treatment. Scale bar = 20 µm.

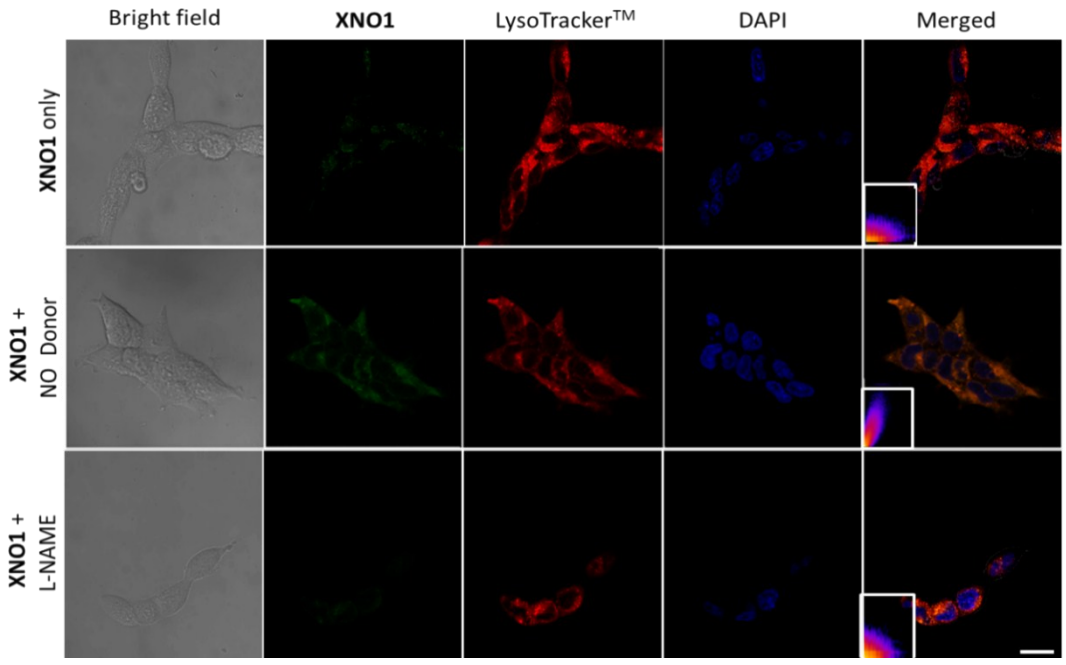


Fig. S9. Confocal images of live SH-SY5Y cells incubated with **XNO1** (20 µM) only for 2 h (Top), **XNO1** (20 µM) for 2 h then treated NO donor at 20 µM for 30 min (Middle) and 1-h pretreatment of L-NAME (1.0 mM) then incubated with **XNO1** (20 µM) for 2 h (Bottom). After that, cells were stained with LysoTracker™ and DAPI before imaging. The fluorescence emission was collected at 500-650 nm upon the excitation at 405 nm. The Pearson's R value was calculated to be 0.3, 0.83 and 0.26 between the probe (shown in Green channel) and LysoTracker™, respectively. Scale bar = 20 µm.

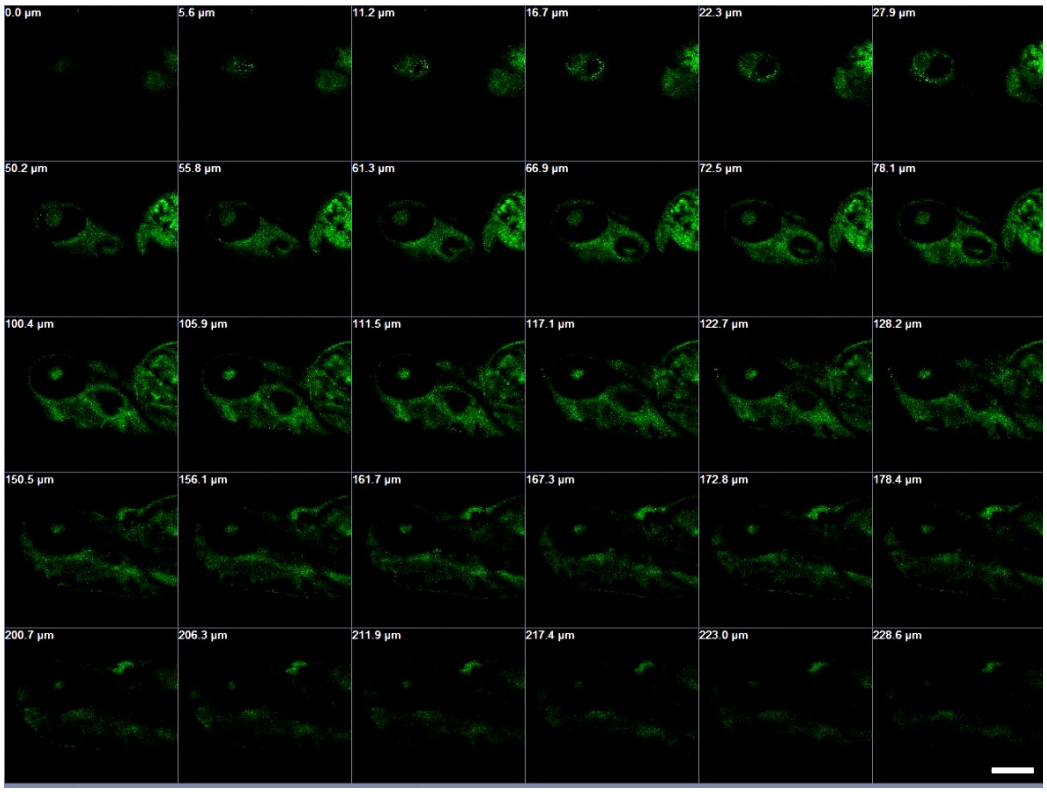


Fig. S10. *Z*-scan fluorescence images of Zebrafish's head. The scan depth is from 0 to 228.6 µM. The fluorescence images were collected at 500-650 nm upon the excitation at 820 nm (TP). Scale bar = 200 µm.

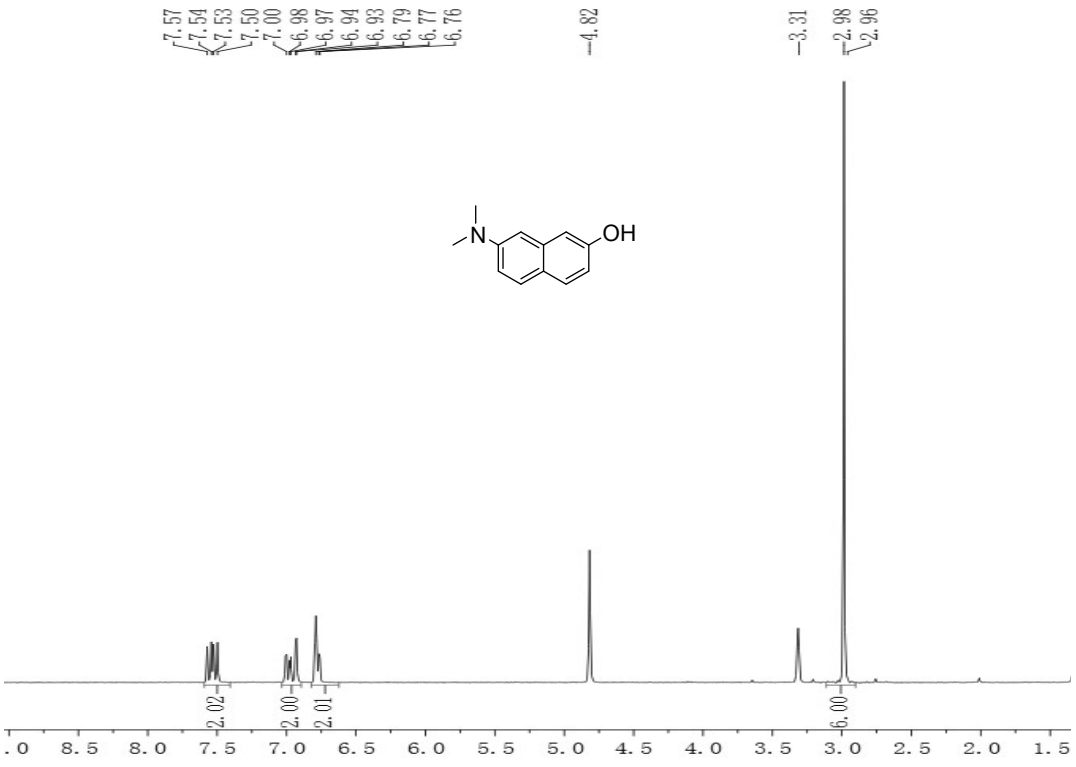


Fig. S11. [1]H NMR spectra of 8-Dimethylamino-2-Naphthalenol

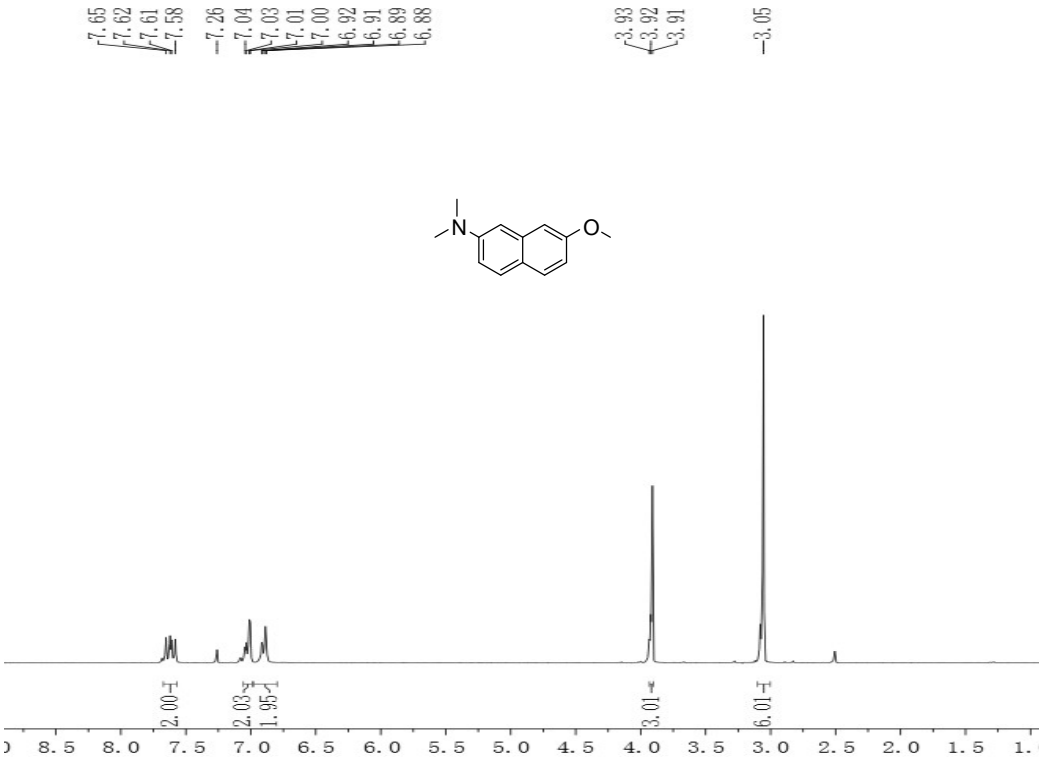


Fig. S12. [1]H NMR spectra of compound **1**

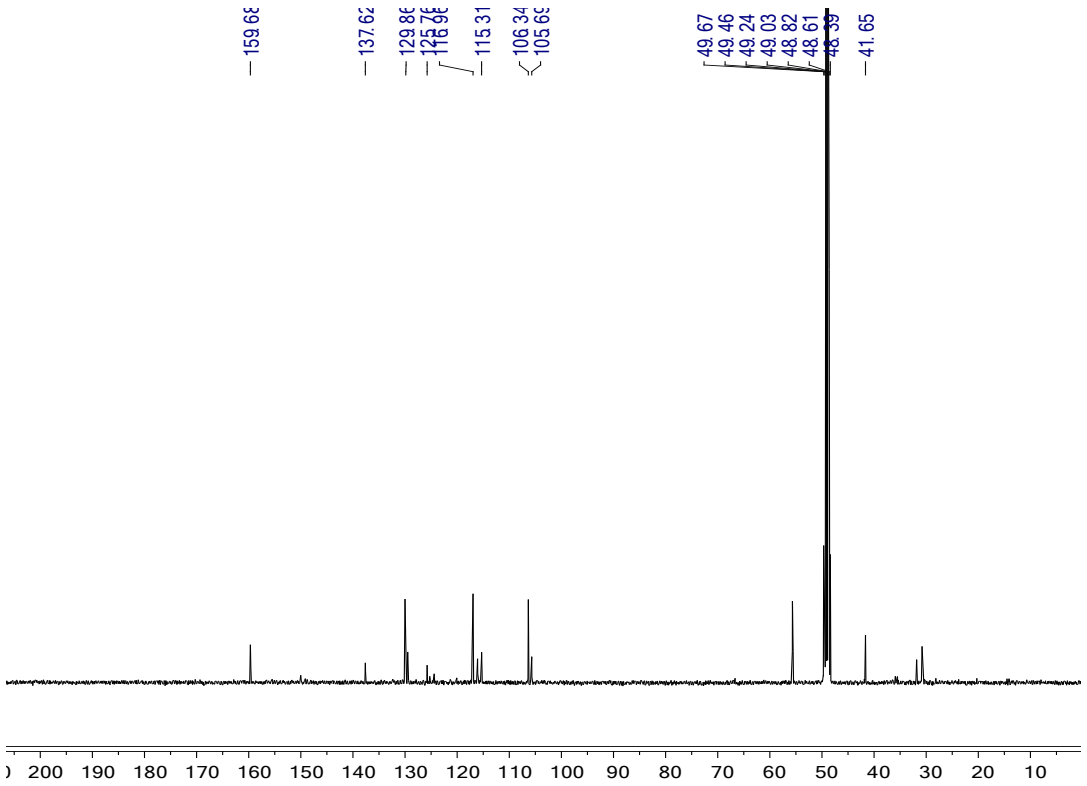


Fig. S13. ^{13}C NMR spectra of compound **1**

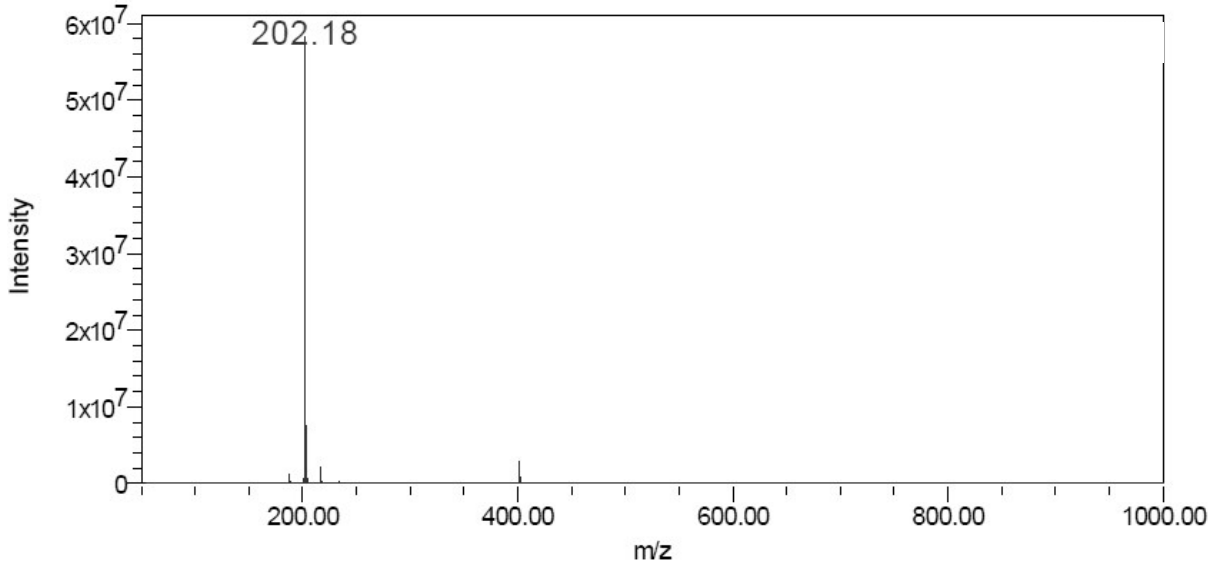


Fig. S14. MS data of compound **1**

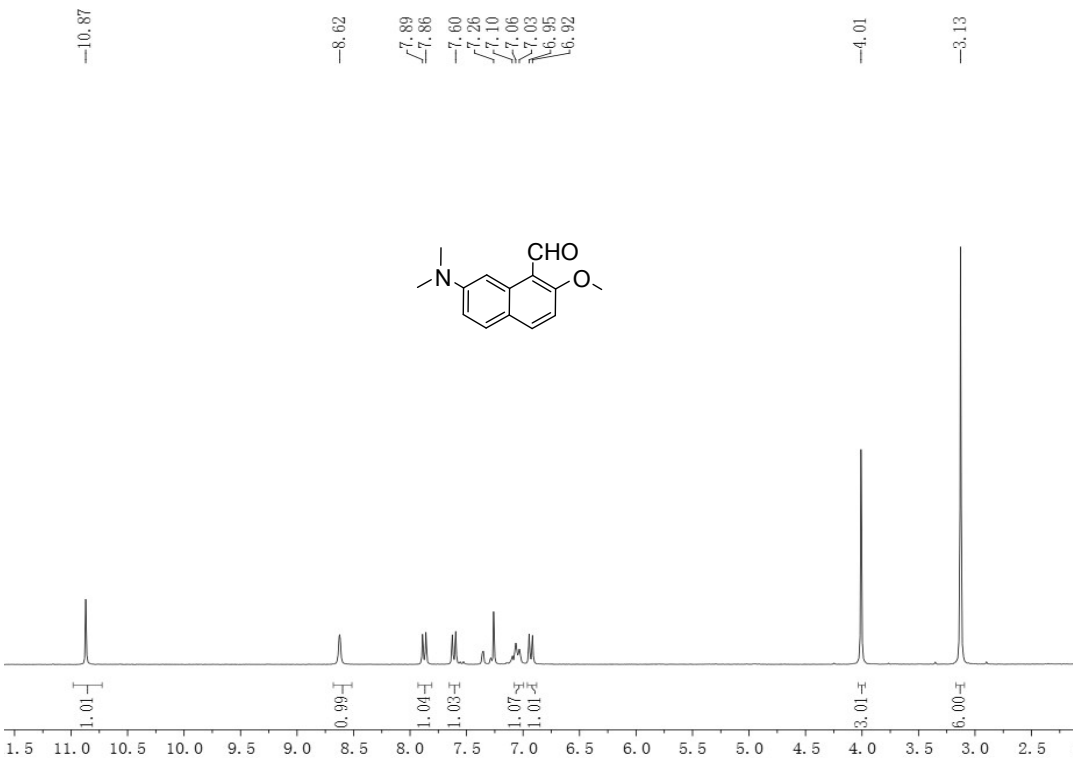


Fig. S15. ^{1}H NMR spectra of compound **2**

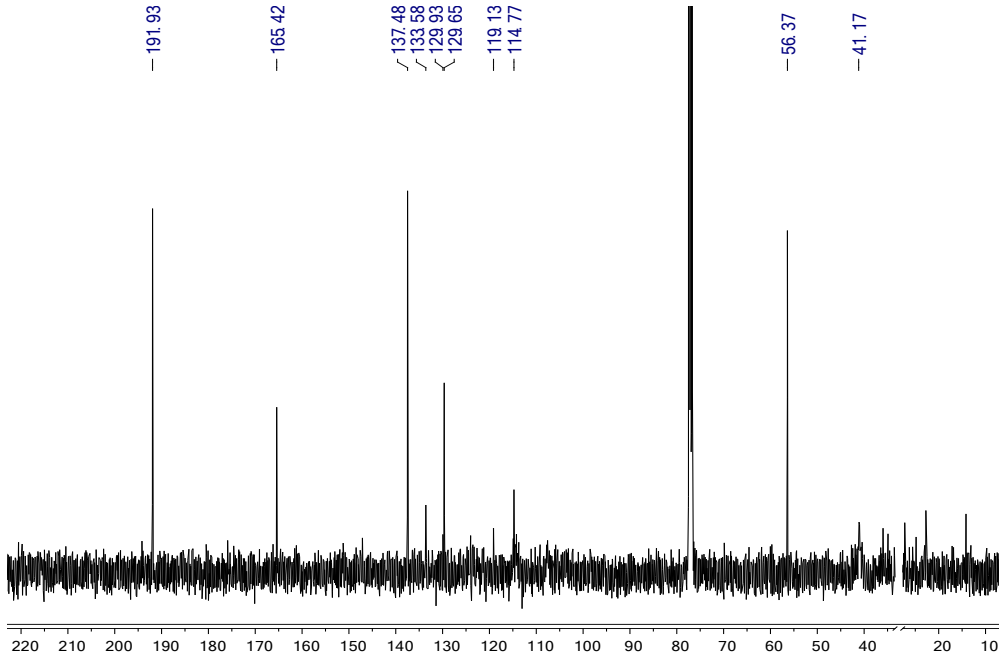


Fig. S16. ^{13}C NMR spectra of compound **2**

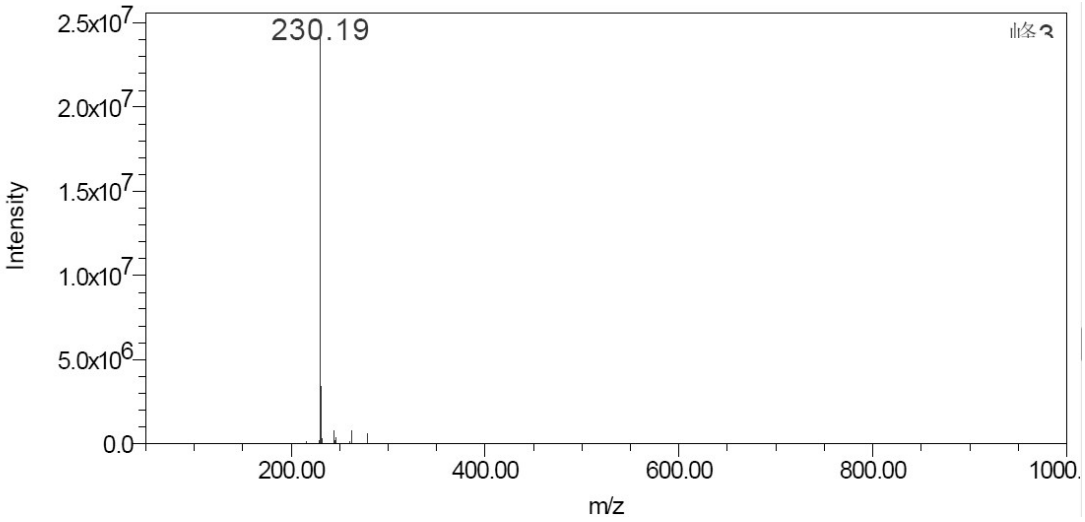


Fig. S17. MS data of compound **2**

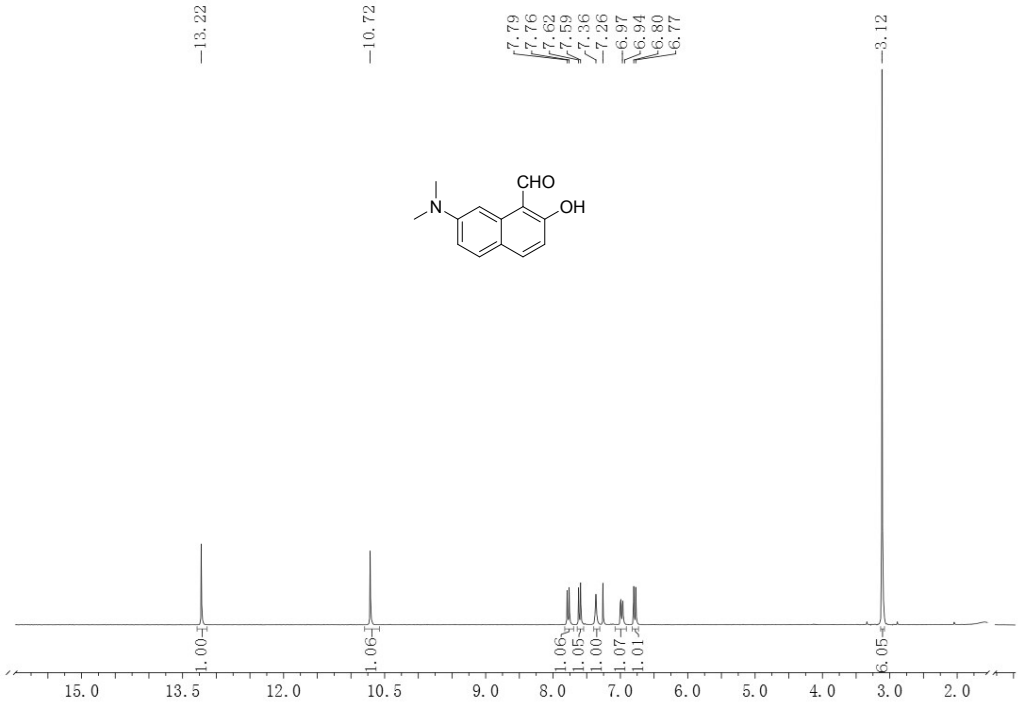


Fig. S18. ^{1}H NMR spectra of **XNO**

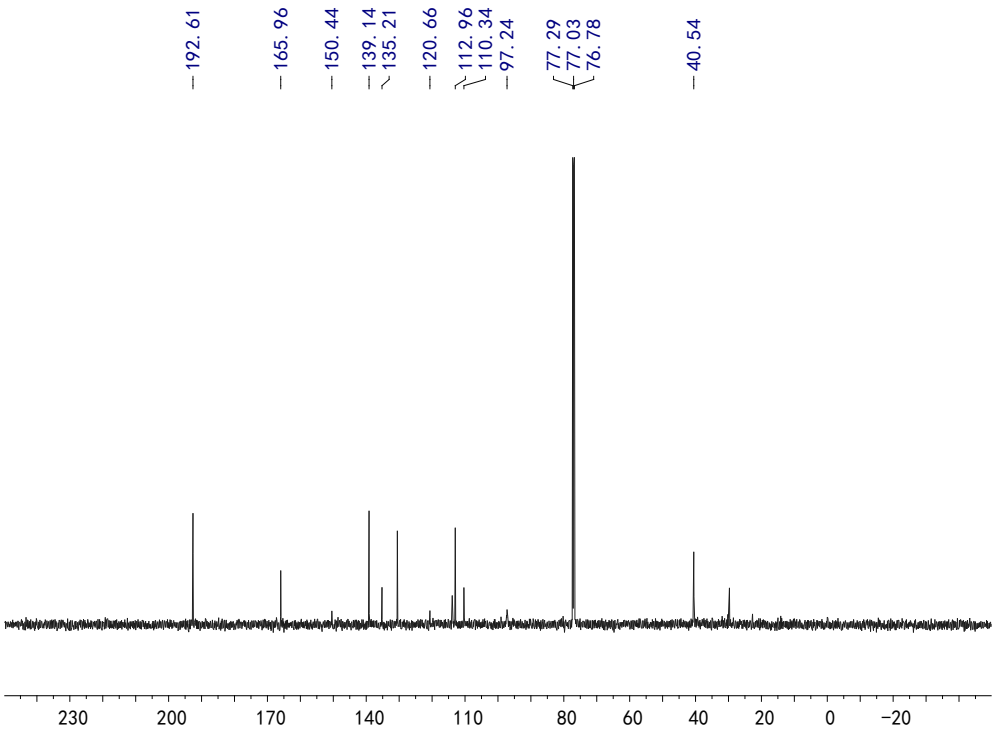


Fig. S19. ^{13}C NMR spectra of **XNO**

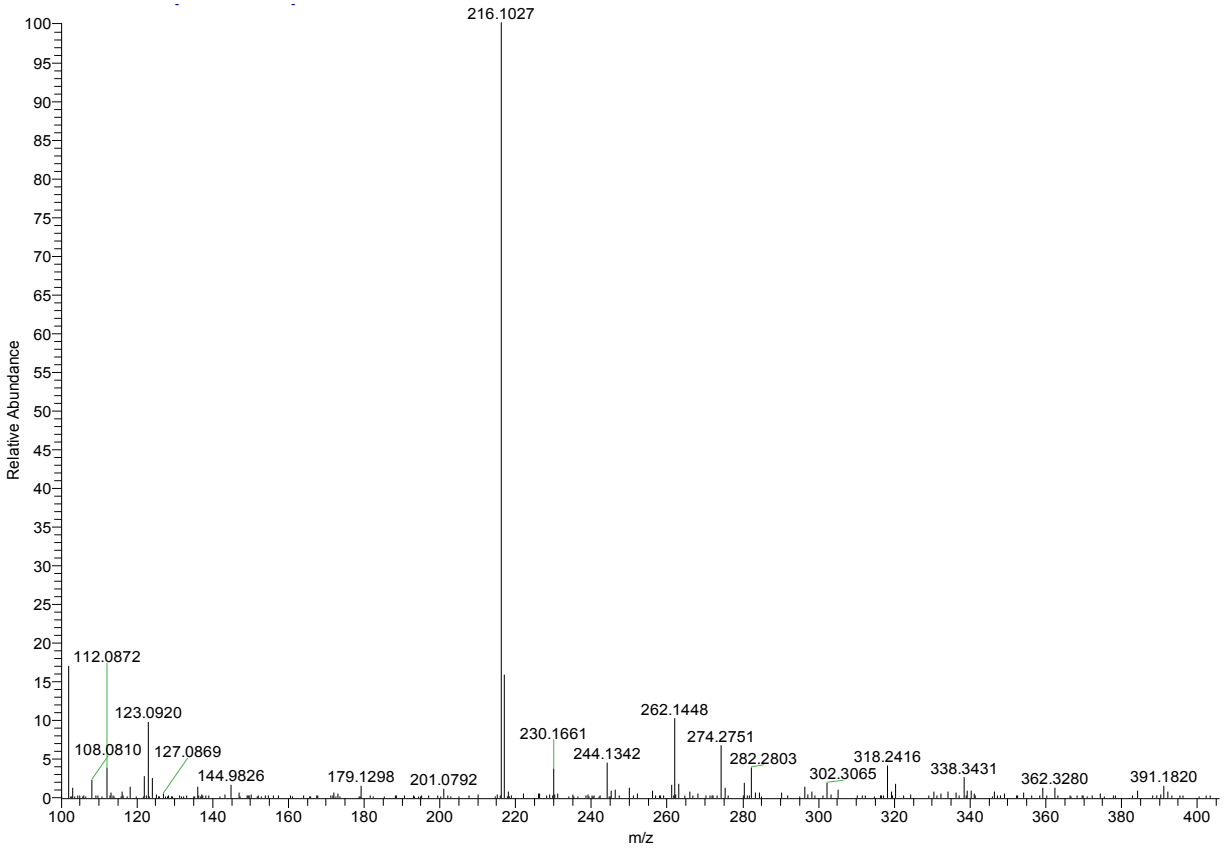


Fig. S20. HRMS spectra of **XNO**

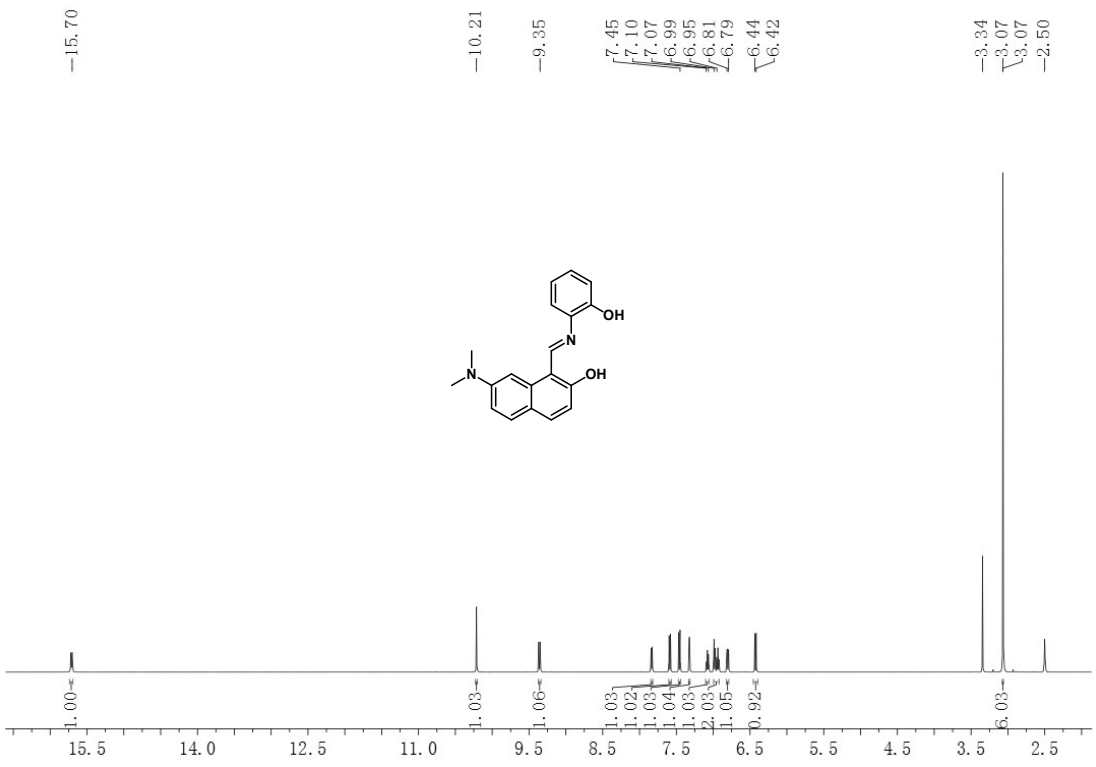


Fig. S21. [1]H NMR spectra of **XNO1**

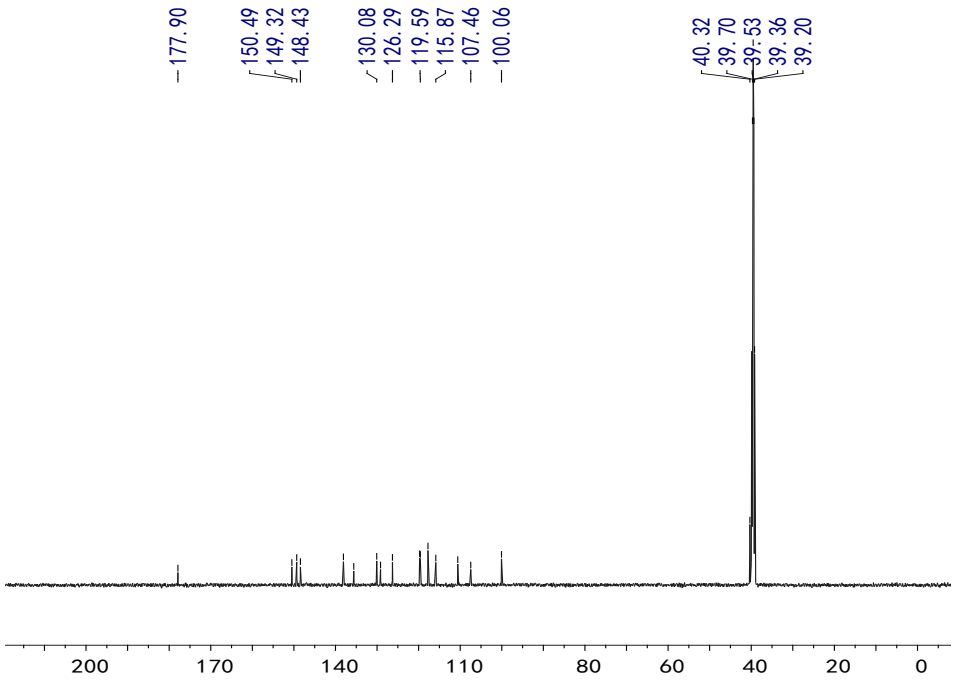


Fig. S22. [13]C NMR spectra of **XNO1**

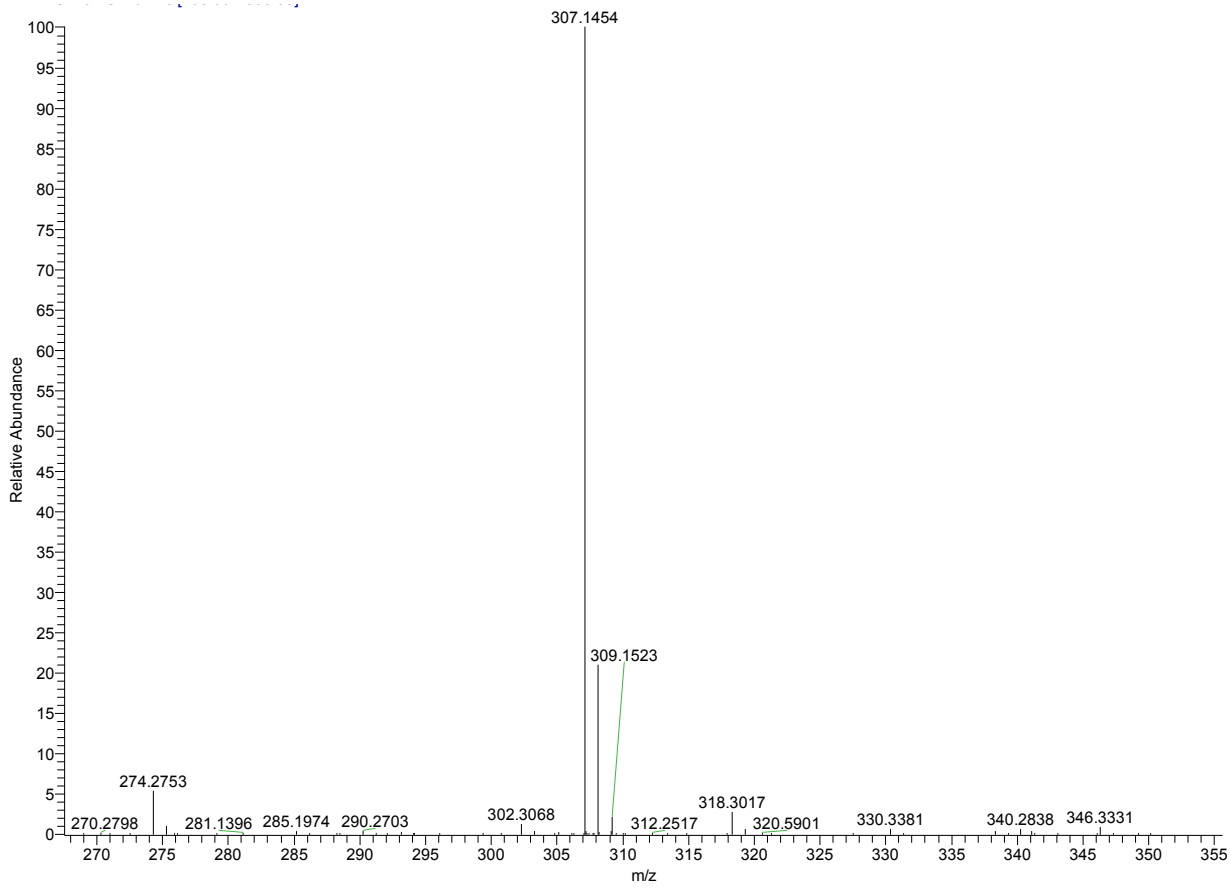


Fig. S23. HRMS spectra of **XNO1**

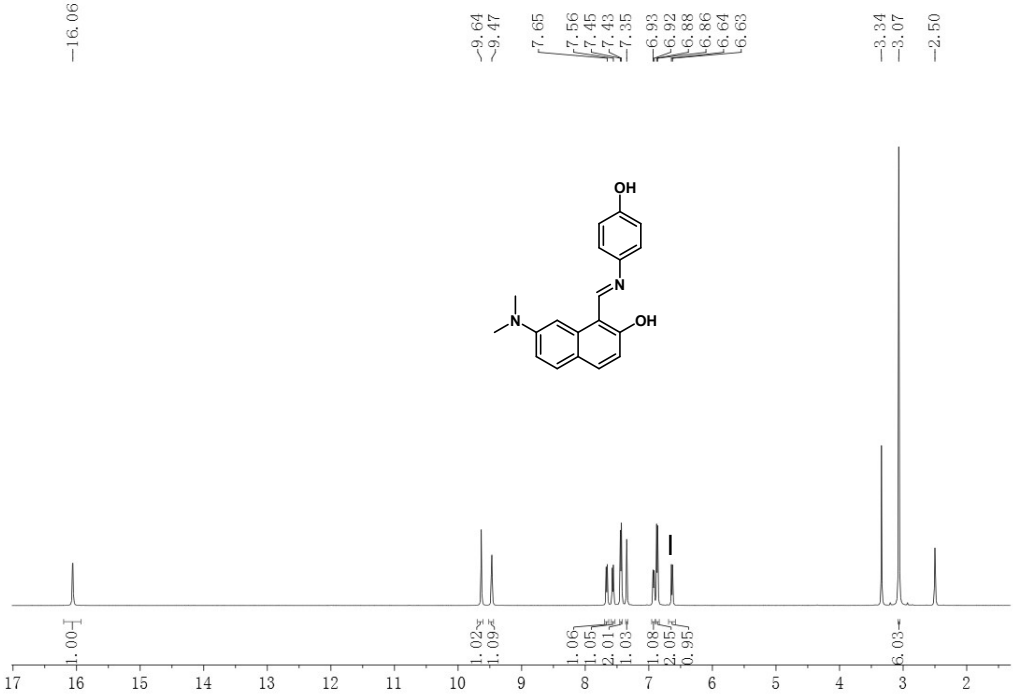


Fig. S24. [1]H NMR spectra of **XNO2**

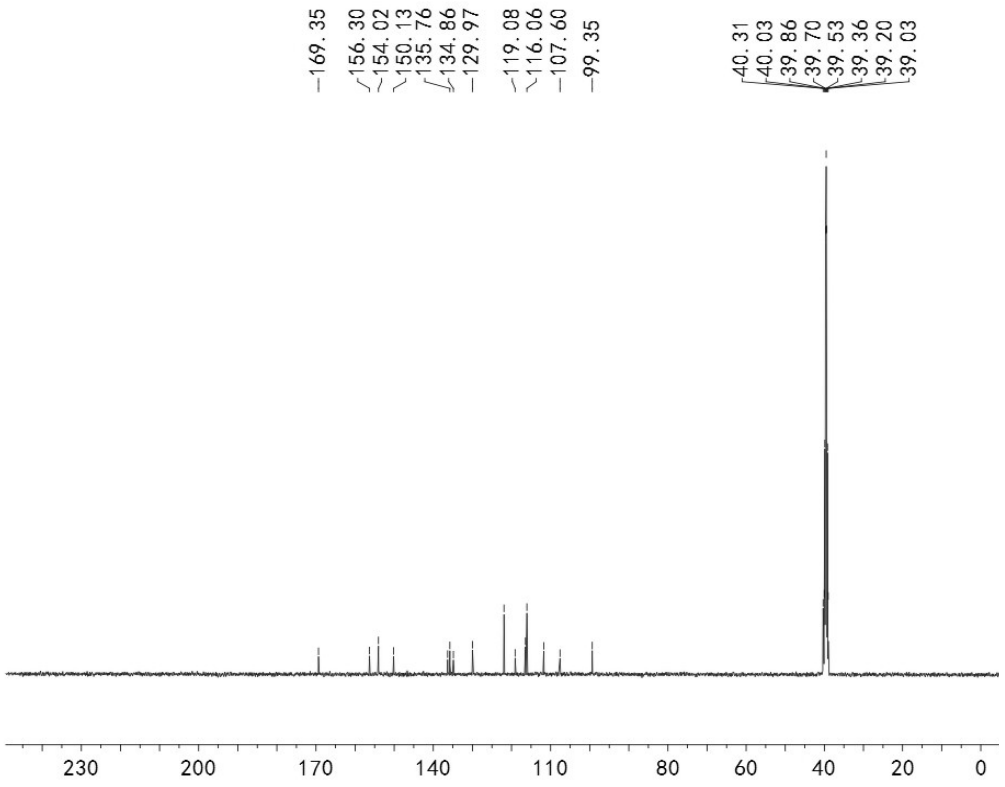


Fig. S25. ^{13}C NMR spectra of **XNO2**

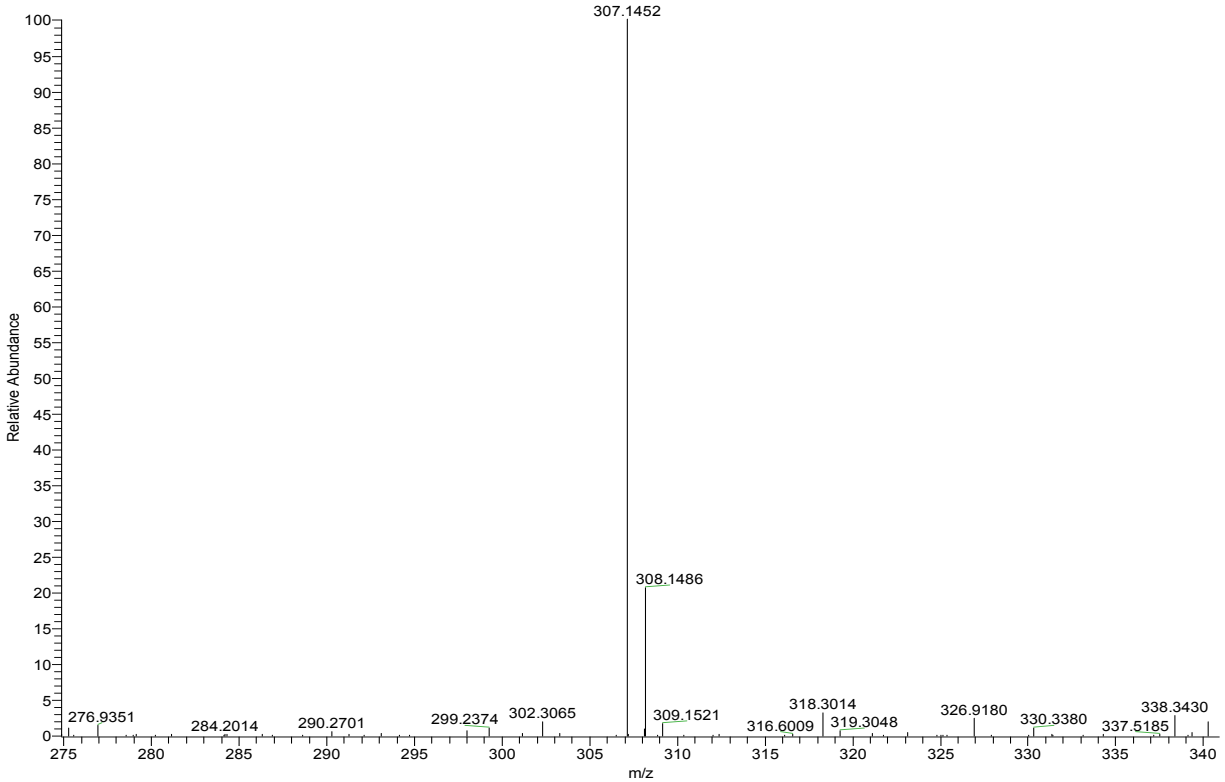


Fig. S26. HRMS spectra of **XNO2**

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