Supporting Information for

Mild dealkylative N-nitrosation of N,N-dialkylaniline derivatives for convenient preparation of photo-triggered and photo-calibrated NO donors

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General Methods

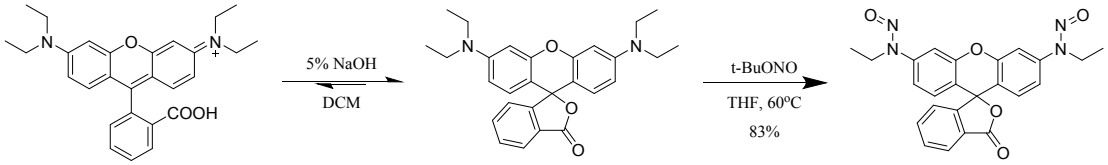
Chemicals and solvents of analytical grade were used in experiments as purchased without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were taken over a Bruker AV-400 spectrometer and chemical shifts were given in ppm with the residue solvent peaks as reference. ESI and EI HRMS spectra were taken on a Micromass GCT spectrometer.

Spectroscopic methods. UV-Vis absorption spectra were taken on a SHIMADZU UV-2600 spectrophotometer while fluorescence spectra were collected using a PTI-QM4 steady-state fluorimeter, which is equipped with a model 810 type PMT and a75 W Xeon arc lamp. Voltage of the PMT was set to 950 V. All the excitation and emission slits were set at 0.5 nm.

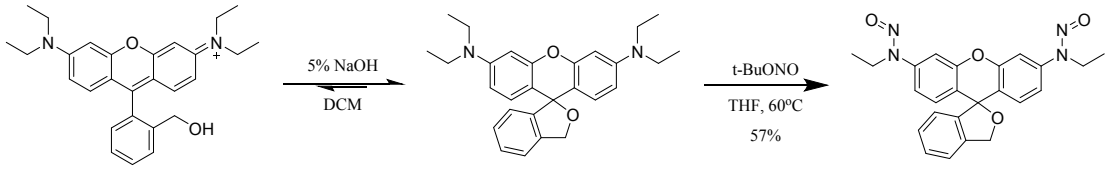
Photodegradation experiment. Three photo-controlled NO donors were dissolved in phosphate buffer (50 mM at pH = 7.4) with 5% DMSO as co-solvent in a 1-cm quartz cuvette equipped with a closing screw (10 μM for **N1**, **N2** and 50 μM for **N3**). The cuvette was put in a water bath, stirred on a magnetic stirrer and irradiated with a 375 nm mercury lamp. The change of the UV−vis absorption as well as the fluorescence turn-on were recorded every one minute.

Confocal Imaging. The supernatant of the substratum was discarded after the finishment of the cell culture. Fresh medium was added to the substratum followed with 5 μM **N1** and **N2** in DMSO. 10 mins later, the culture mediums were washed 3 times with PBS buffer to remove the excess photo-controlled NO donors. Confocal images were recorded one shutter per second on a Leica ICS SP5 Ⅱ confocal microscope using a 488 nm laser.

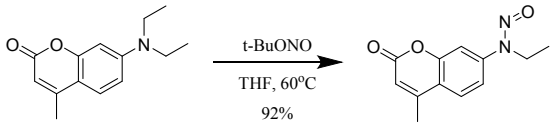
Synthetic scheme, procedures and characterizations.



N,N'-(3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-diyl)bis(N-ethylnitrous amide) (N1). Rhodamine B (1.00 g, 2.25 mmol) was dissolved in 100 mL dichloromethane and diluted with 5% NaOH (aq.) (100 mL, 3 times). The organic layer was dried with Na_2SO_4, filtered, and evaporated under reduced pressure to afford a yellow oil. To the stirred solution of the yellow oil in 50 mL THF was added butyl nitrite (0.70 g, 6.76 mmol). The mixture was heated to 60 ◦C and stirred for 10 hours before evaporated under reduced pressure. Crude product was purified by a column chromatography with PE and EA (20:1 v/v) as the eluent to afford a white solid (0.83 g) in an 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 6.7, 0.9 Hz, 1H), 7.73 (td, J = 7.4, 1.4 Hz, 1H), 7.68 (td, J = 7.4, 1.1 Hz, 1H), 7.52 (d, J = 2.2 Hz, 2H), 7.34 (dd, J = 8.7, 2.3 Hz, 2H), 7.23 (d, J = 8.7 Hz, 2H), 4.07 (q, J = 7.2 Hz, 4H), 1.18 (t, J = 7.2 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.18, 152.84, 151.83, 143.45, 135.61, 130.42, 129.51, 126.23, 125.59, 123.94, 117.26, 114.44, 106.78, 81.49, 38.58, 11.83. HRMS (ES^+) Calcd for $[M+Na]^+$, 467.1326; Found, 467.1330.

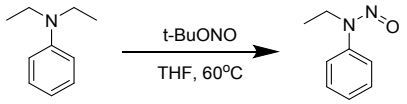


N,N'-(3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-diyl)bis(N-ethylnitrous amide) (N2). This compound was synthesized in a manner analogous to that of **N1**, from 9-(2-hydroxylmethylphenyl)-3, 6-bis(diethylamino)- xanthylium chloride (1.00 g, 2.33 mmol) and butyl nitrite (0.72 g, 7.00 mmol). The compound **N2** (0.73g) was obtained as a yellow solid and recrystallized with methanol to afford a white solid (0.58 g) in a 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 2.4 Hz, 4H), 7.35 (dd, J_1 = 8.7 Hz, J_2 = 2.2 Hz, 1H), 7.14 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 7.6 Hz, 1H), 5.42 (s, 2H), 4.08 (q, J = 7.2 Hz, 4H), 1.19 (t, J = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.95, 144.47, 142.31, 138.80, 130.28, 128.87, 128.80, 123.83, 123.21, 121.13, 114.52, 106.76, 83.09, 73.02, 38.85, 11.86. HRMS (ES^+) Calcd for $[M+H]^+$, 431.1714; Found, 431.1718.

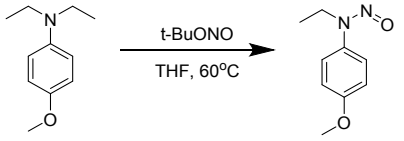


N-ethyl-N-(4-methyl-2-oxo-2H-chromen-7-yl)nitrous amide (N3).To the stirred solution of 7-Diethylamino-4-methylcoumarin (1.00 g, 4.32 mmol) in 50 mL THF was added butyl nitrite(1.34g, 12.97 mmol). The mixture was heated to 60 ◦C and stirred for 2 hours before evaporated under reduced pressure. Crude product was purified by a column chromatography with PE and EA (20:1 v/v) as the eluent to afford a white solid (0.92 g) in an 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 8.7 Hz, 1H), 7.63 (dd, J = 8.7, 2.1 Hz, 1H), 7.44 (d, J = 2.1 Hz, 1H), 4.07 (q, J = 7.2 Hz, 2H), 2.46 (d, J = 1.1 Hz, 3H), 1.17 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.49, 154.44, 151.98, 144.10, 125.98, 118.38, 114.81, 114.16, 106.01, 38.36, 18.77,

11.78. HRMS (ES$^+$) Calcd for $[M+Na]^+$, 255.0740; Found, 255.0747.

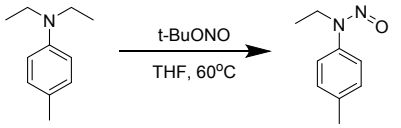


N-ethyl-N-phenylnitrous amide (3) was prepared from N,N-diethylaniline (1.00 g, 6.70 mmol) as a yellow solid (0.87 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.52 (m, 2H), 7.50 – 7.45 (m, 2H), 7.39 – 7.33 (m, 1H), 4.08 (q, *J* = 7.2 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 3H).

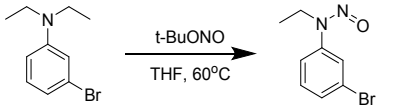


N-ethyl-N-(4-methoxyphenyl)nitrous amide (4). To the stirred solution of N,N-diethyl-4-methoxyaniline (1.00 g, 5.58 mmol) in 50 mL THF was added butyl nitrite(1.73 g, 16.74 mmol). The mixture was heated to 60 ◦C and stirred for 4 hours before evaporated under reduced pressure. Crude product was purified by a column chromatography with PE and EA (20:1 v/v) as the eluent to afford a brown oil (0.80 g) in an 80% yield.

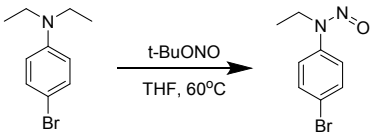
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (m, 2H), 7.02 – 6.96 (m, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.85 (s, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).



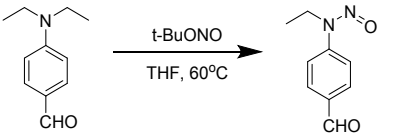
N-ethyl-N-(p-tolyl)nitrous amide (5) was prepared from N,N-diethyl-4-methylaniline (1.00 g, 6.13 mmol) as a yellow solid (0.90 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.3 Hz, 2H), 4.06 (q, *J* = 7.2 Hz, 2H), 2.40 (s, 3H), 1.16 (t, *J* = 7.2 Hz, 3H).



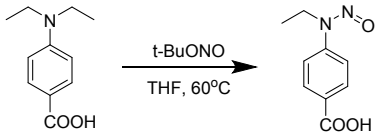
N-(3-bromophenyl)-N-ethylnitrous amide (6) was prepared from 3-bromo-N,N -diethylaniline (1.00 g, 4.38 mmol) as a yellow solid (0.88 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (t, *J* = 2.0 Hz, 1H), 7.48 – 7.42 (m, 2H), 7.31 (dd, *J* = 9.1, 7.1 Hz, 1H), 4.01 (q, *J* = 7.2 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H).



N-(4-bromophenyl)-N-ethylnitrous amide(7) was prepared from 4-bromo-N,N -diethylaniline (1.00 g, 4.38 mmol) as a yellow solid (0.92 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.56 (m, 2H), 7.47 – 7.39 (m, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 1.16 (t, *J* = 7.2 Hz, 3H).



N-ethyl-N-(4-formylphenyl)nitrous amide (8) was prepared from 4-(diethylamino)benzaldehyde (1.00 g, 5.64 mmol) as a yellow solid (0.95 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 8.7 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ190.90, 146.01, 134.50, 131.24, 118.17, 38.21, 11.72.



4-(Ethyl(nitroso)amino)benzoic acid (**9**) was prepared from 4-(diethylamino)benzoic acid (1.00 g, 5.17 mmol) as a yellow solid (0.96 g, 96%). ^{1}H NMR (400 MHz, DMSO) δ 8.09 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.7 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 1.06 (t, *J* = 7.1 Hz, 3H).

Table S1. Crystal data and structure refinement for **N1.**

Identification code	d8v17683	
Empirical formula	C24 H20 N4 O5	
Formula weight	444.44	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 13.1450(5) Å	α= 90°.
	b = 14.0218(6) Å	β= 114.4160(10)°.
	c = 12.9264(5) Å	γ = 90°.
Volume	2169.47(15) $Å^3$	
Z	4	
Density (calculated)	1.361 Mg/m^3	
Absorption coefficient	0.098 mm^{-1}	
F(000)	928	
Crystal size	0.180 x 0.150 x 0.100 mm^3	
Theta range for data collection	2.359 to 26.000°.	
Index ranges	-16<=h<=16, -17<=k<=17, -15<=l<=15	
Reflections collected	47168	
Independent reflections	4256 [R(int) = 0.0732]	
Completeness to theta = 25.242°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7456 and 0.6733	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4256 / 0 / 301	
Goodness-of-fit on F^2	1.028	
Final R indices [I>2sigma(I)]	R1 = 0.0411, wR2 = 0.0889	
R indices (all data)	R1 = 0.0592, wR2 = 0.1004	
Extinction coefficient	0.0152(12)	
Largest diff. peak and hole	0.205 and -0.196 $e.Å^{-3}$	

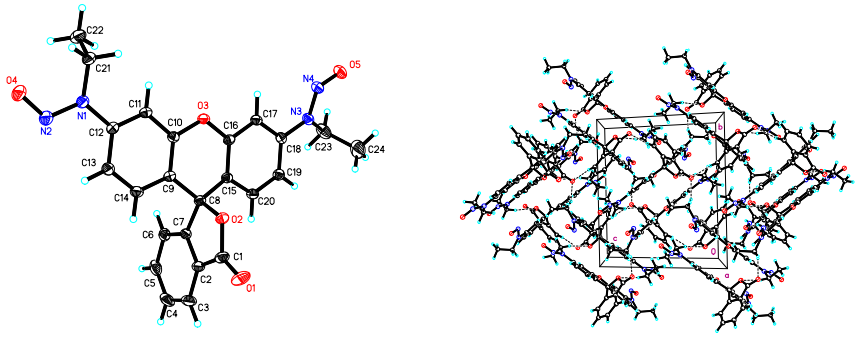


Figure S1. Ortep drawings and the packing mode of **N1** in its solid state.

Table S2. Crystal data and structure refinement for **N2**.

Identification code	d8v17444	
Empirical formula	C24 H22 N4 O4	
Formula weight	430.45	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 18.2546(6) Å	α= 90°.
	b = 9.1982(3) Å	β= 108.2310(10)°.
	c = 13.3199(4) Å	γ = 90°.
Volume	2124.27(12) Å^3	
Z	4	
Density (calculated)	1.346 Mg/m^3	
Absorption coefficient	0.094 mm^{-1}	
F(000)	904	
Crystal size	0.160 x 0.140 x 0.100 mm^3	
Theta range for data collection	2.738 to 25.495°.	
Index ranges	-22<=h<=21, -11<=k<=10, -16<=l<=16	
Reflections collected	31544	
Independent reflections	3944 [R(int) = 0.0522]	
Completeness to theta = 25.242°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7456 and 0.6975	

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3944 / 0 / 292
Goodness-of-fit on F^2	1.038
Final R indices [I>2sigma(I)]	R1 = 0.0494, wR2 = 0.1184
R indices (all data)	R1 = 0.0736, wR2 = 0.1352
Extinction coefficient	0.015(2)
Largest diff. peak and hole	0.310 and -0.275 e.$Å^{-3}$

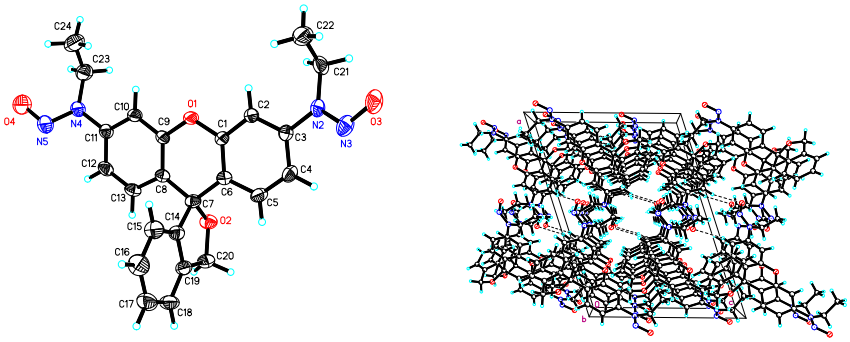


Figure S2. Ortep drawings and the packing mode of **N2** in its solid state.

Table S3. Crystal data and structure refinement for **N3**.

Identification code	d8v17769	
Empirical formula	C12 H12 N2 O3	
Formula weight	232.24	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 11.2278(5) Å	α= 90°.
	b = 13.7389(6) Å	β= 107.1730(10)°.
	c = 7.4364(3) Å	γ = 90°.
Volume	1095.98(8) $Å^3$	
Z	4	
Density (calculated)	1.407 Mg/m^3	
Absorption coefficient	0.103 mm^{-1}	
F(000)	488	

Crystal size	0.200 x 0.160 x 0.100 mm^3
Theta range for data collection	2.409 to 25.996°.
Index ranges	-13<=h<=12, -16<=k<=16, -9<=l<=9
Reflections collected	18997
Independent reflections	2146 [R(int) = 0.0617]
Completeness to theta = 25.242°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7456 and 0.6637
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2146 / 0 / 157
Goodness-of-fit on F^2	1.042
Final R indices [I>2sigma(I)]	R1 = 0.0387, wR2 = 0.0914
R indices (all data)	R1 = 0.0500, wR2 = 0.0998
Extinction coefficient	0.032(6)
Largest diff. peak and hole	0.211 and -0.154 e.$Å^{-3}$

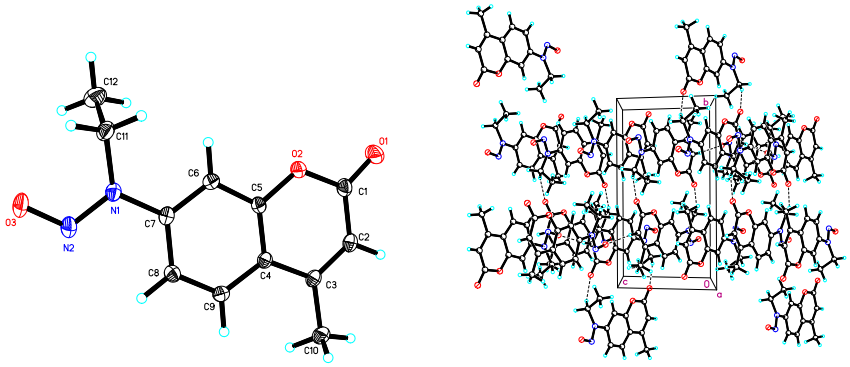


Figure S3. Ortep drawings and the packing mode of **N3** in its solid state.

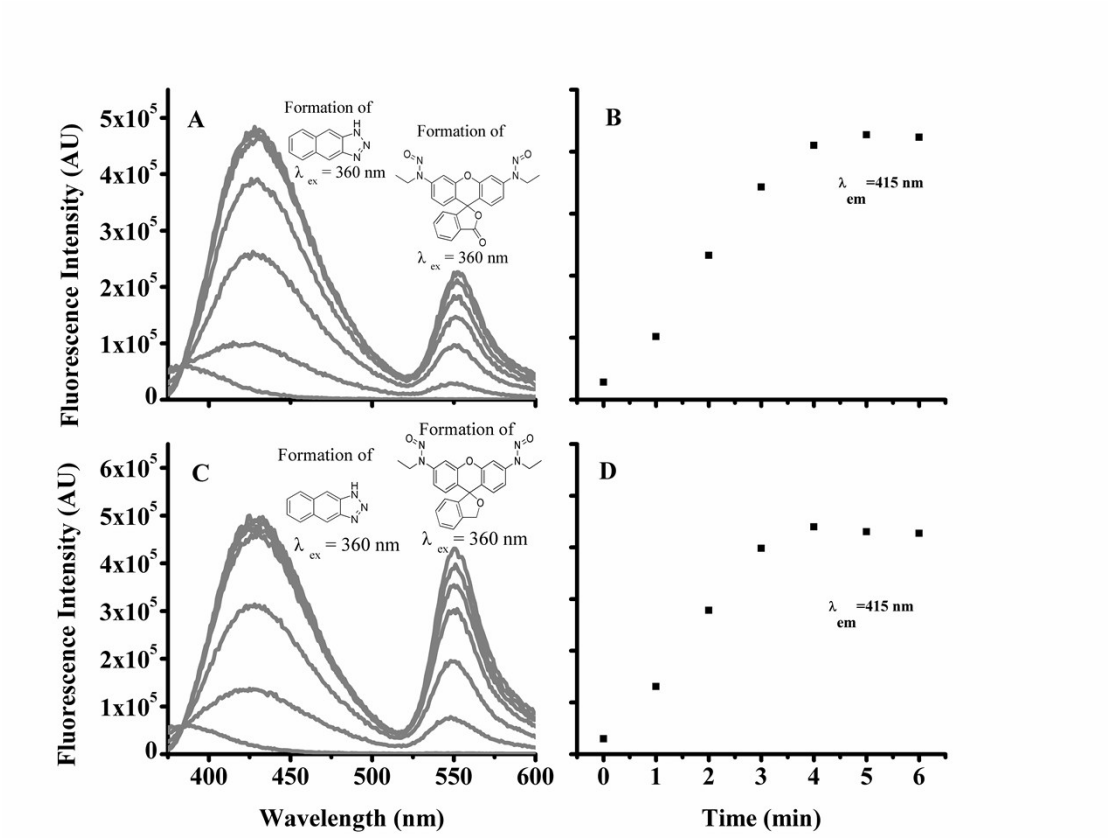


Figure S4. Fluorescence turn-on monitoring of the formation of NAT and corresponding dyes upon excitation of **N1** (A) and **N2** (C) with a 375 nm mercury lamp. The enhancement of the NAT'fluorescence emission intensity at 415 nm by excitation at 360 nm upon solvents of DAN and **N1** (C), **N2** (D).

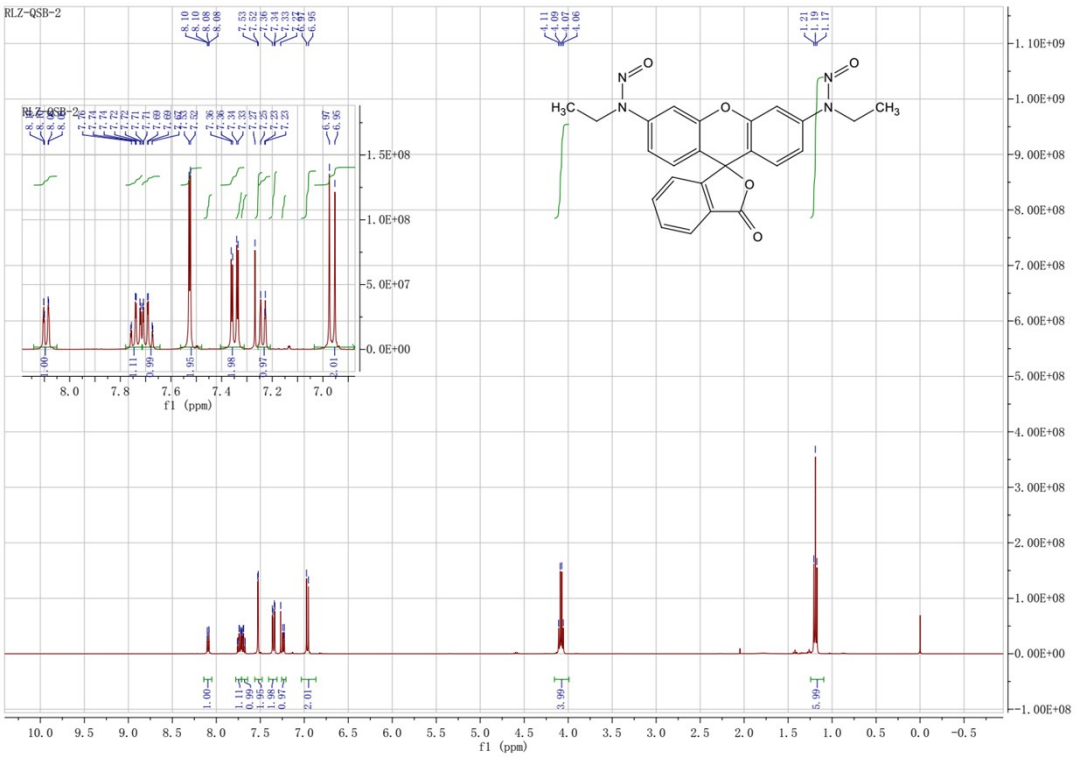


Fig S5. The ^{1}H NMR of compound N1 in $CDCl_3$

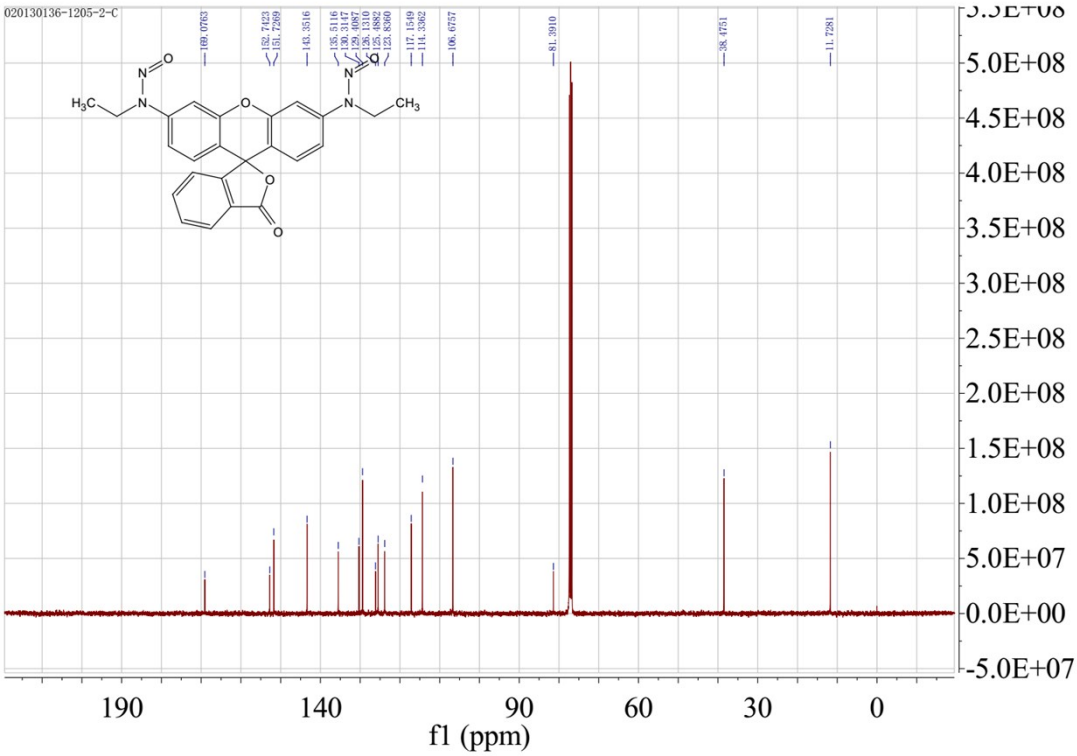


Fig S6. The ^{1}H NMR of compound C2 in $CDCl_3$

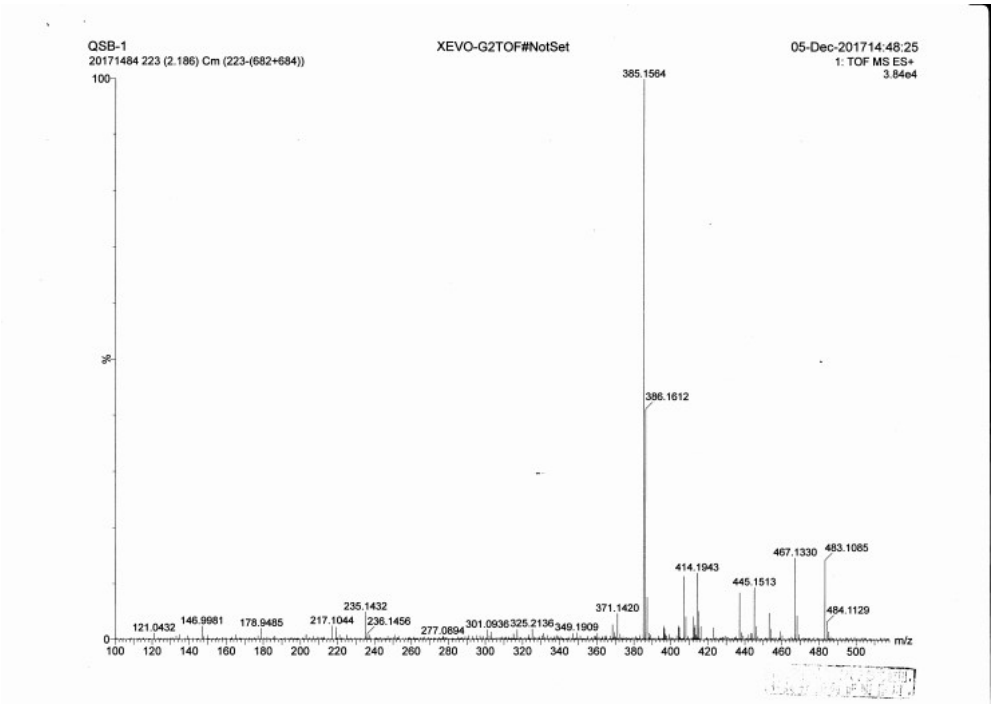


Fig S7. The HRMS(ESI) of compound N1

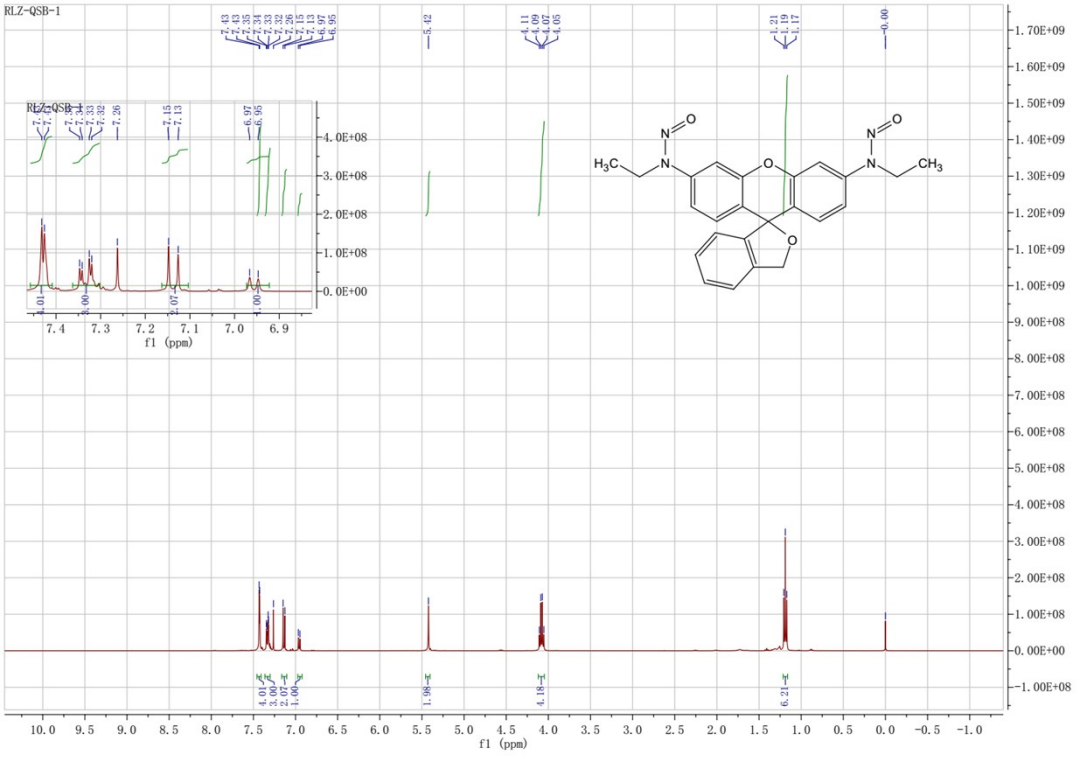


Fig S8. The ^{1}H NMR of compound N2 in $CDCl_3$

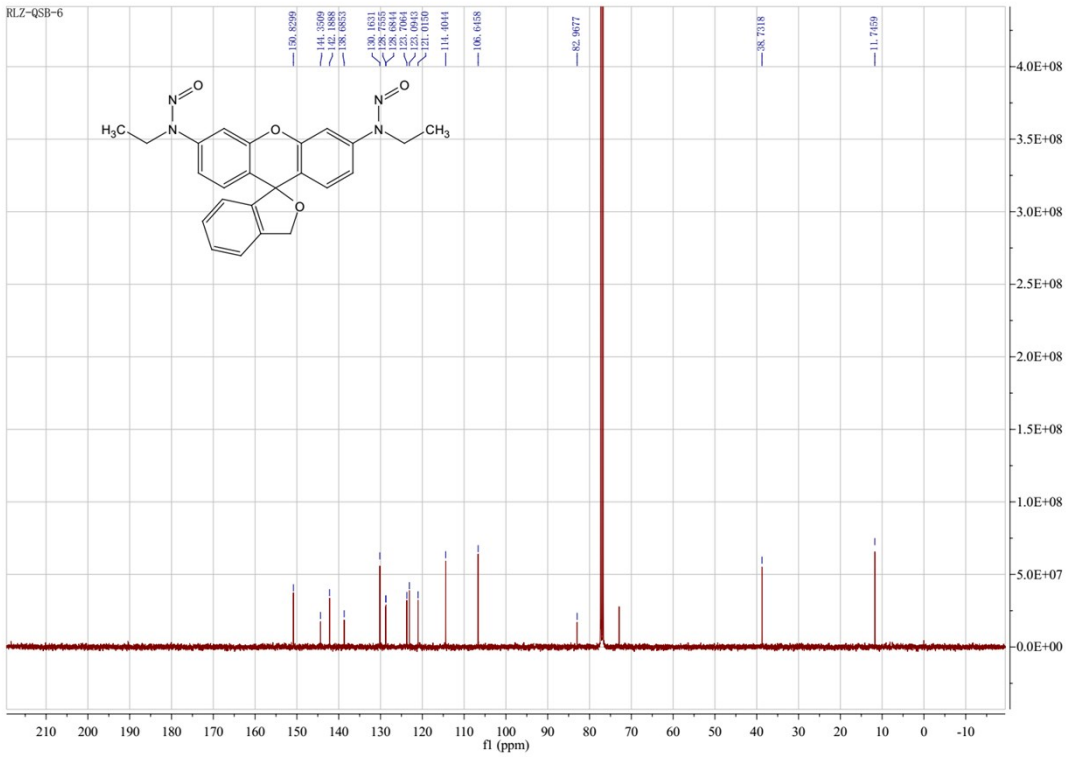


Fig S9. The ^{13}C NMR of compound N2 in $CDCl_3$

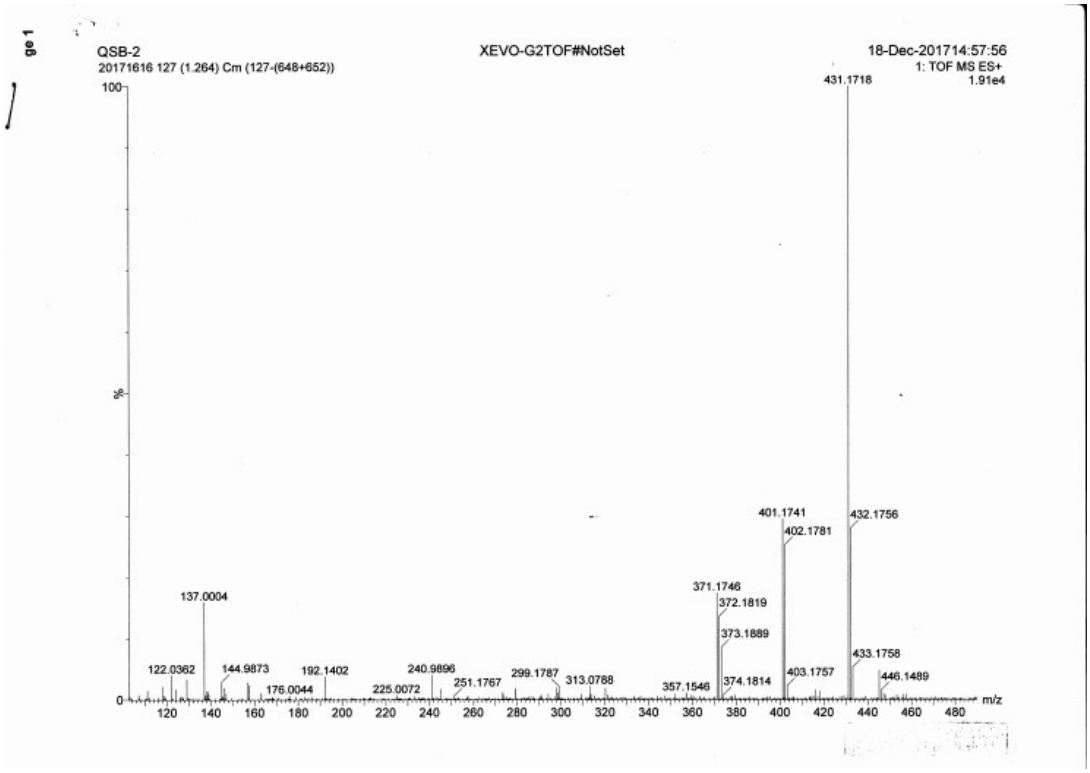


Fig S10. The HRMS(ESI) of compound N2 in $CDCl_3$

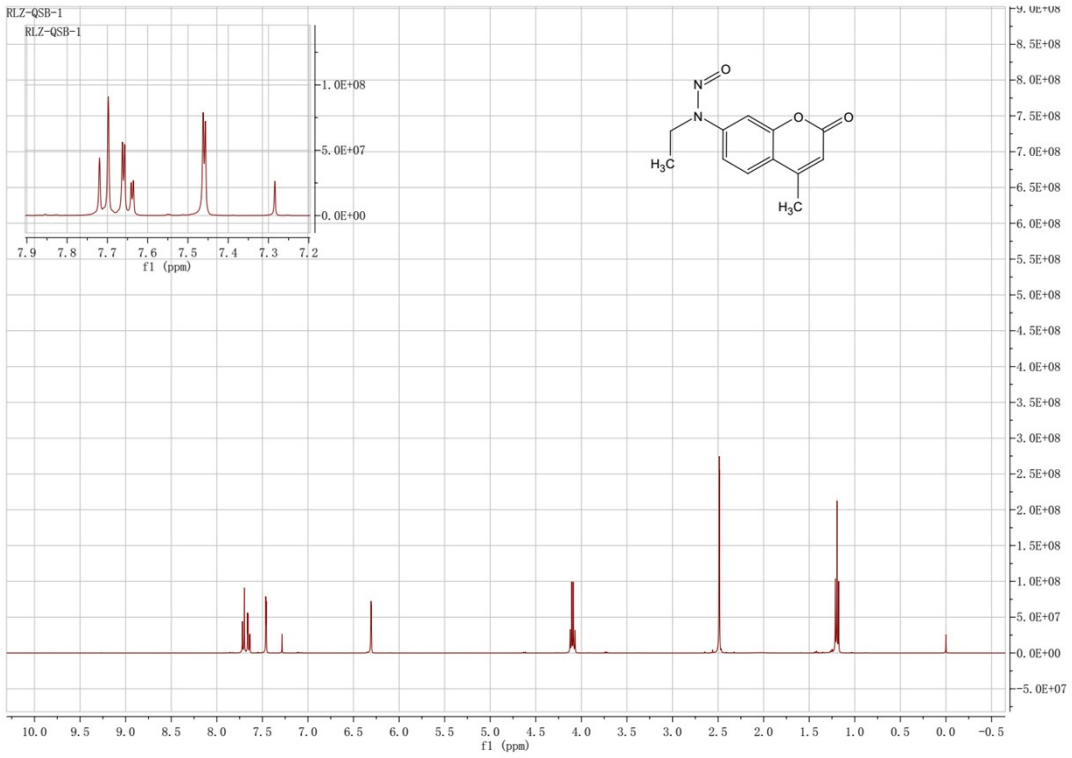


Fig S11. The ^{1}H NMR of compound N3 in $CDCl_3$

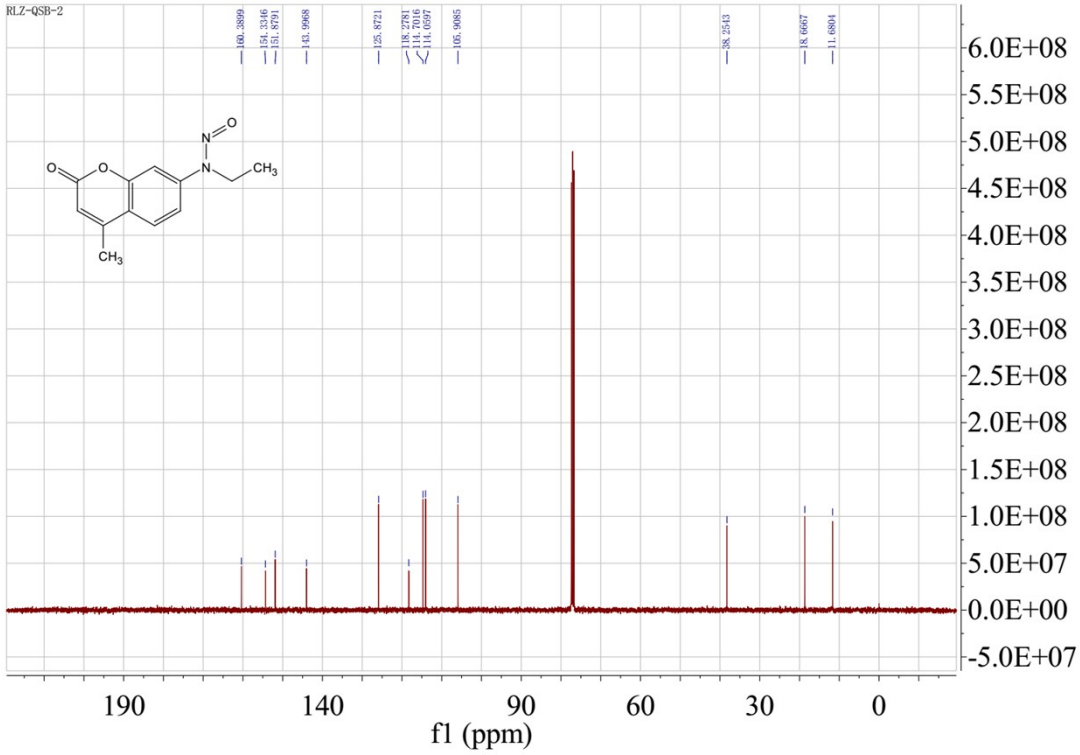


Fig S12. The ^{13}C NMR of compound N3 in $CDCl_3$

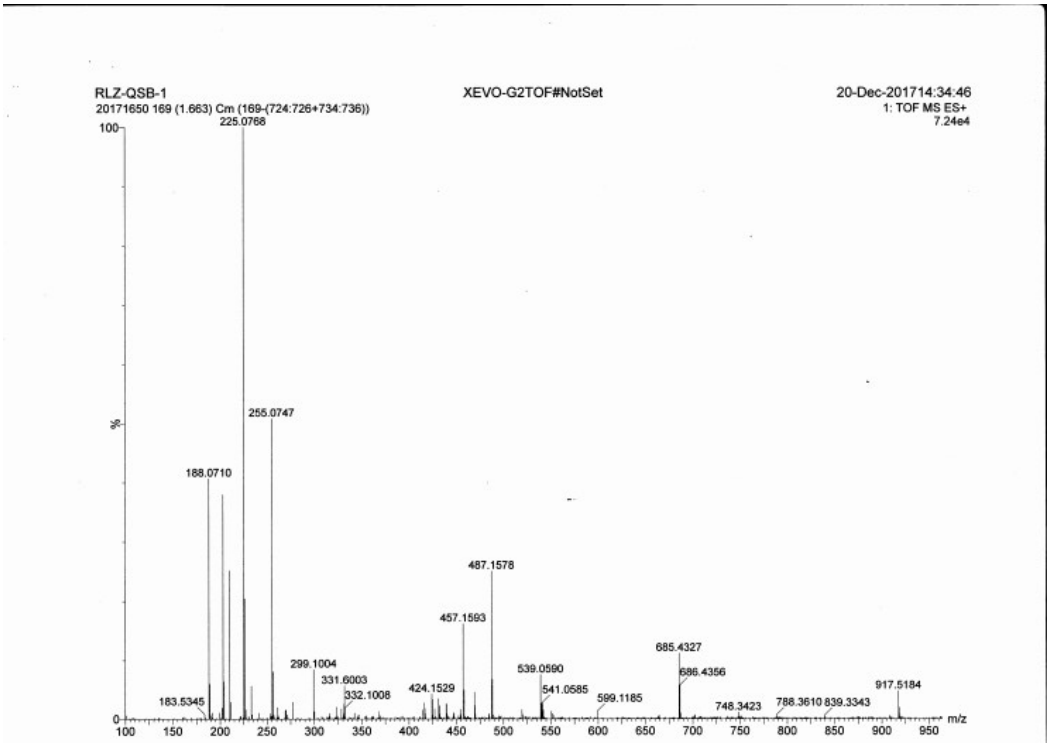


Fig S13. The HRMS(ESI) of compound N3

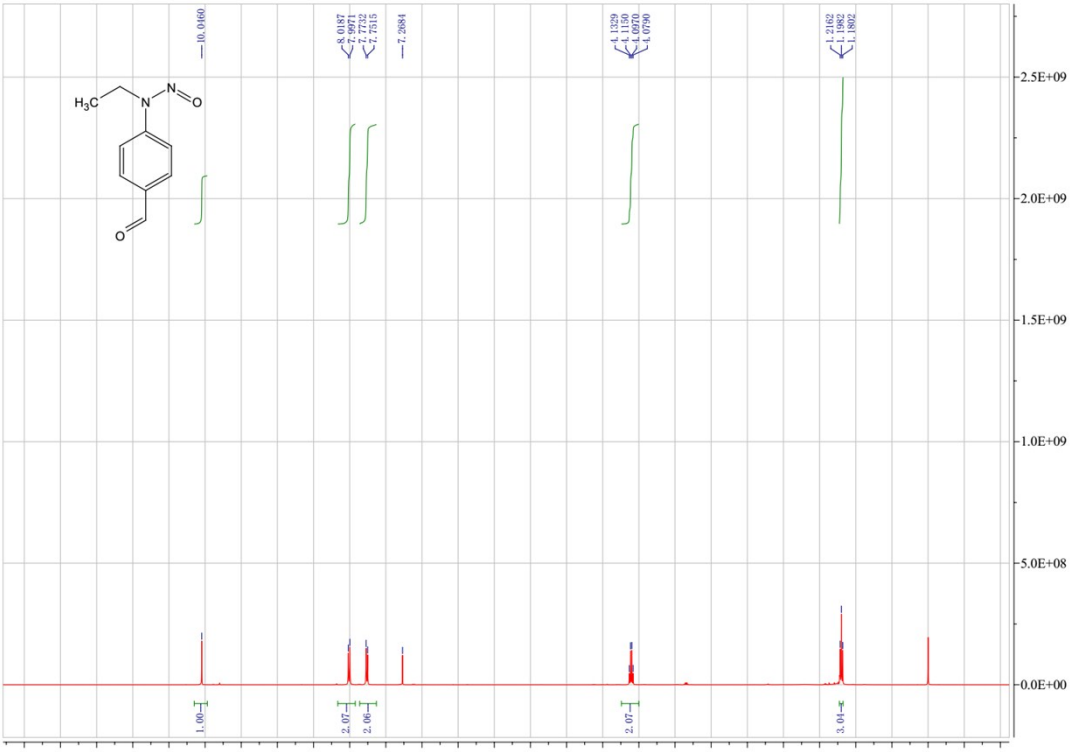


Fig S14. The ^{1}H NMR of compound 8 in $CDCl_3$

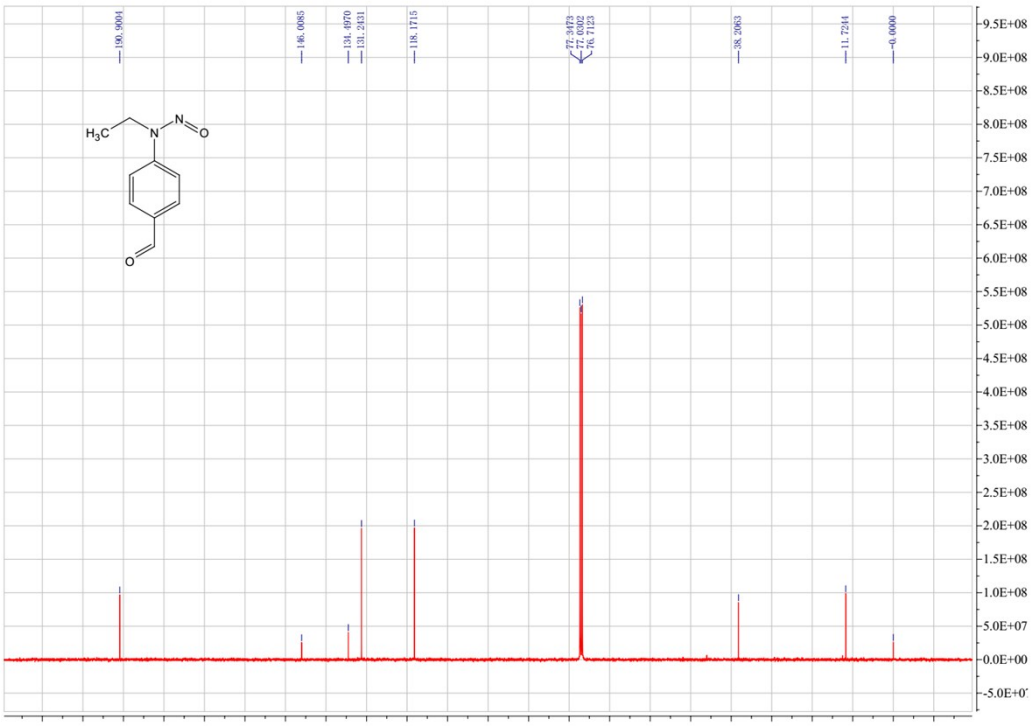


Fig S15. The ^{13}C NMR of compound 8 in $CDCl_3$

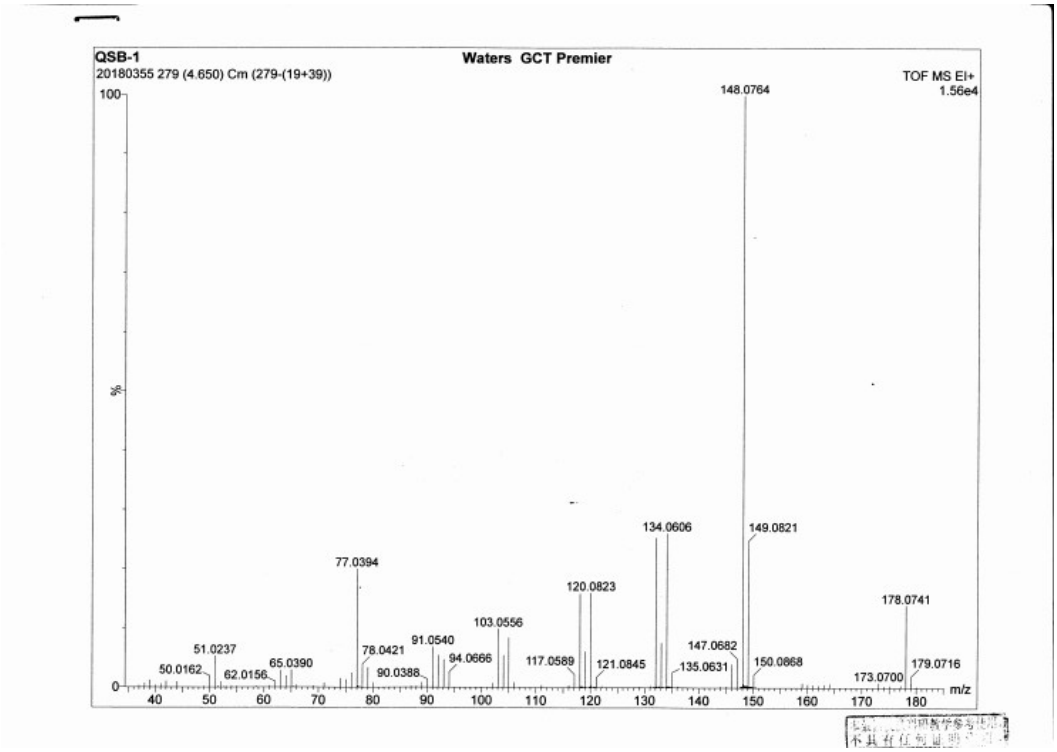


Fig S16. The HRMS(EI) of compound 8

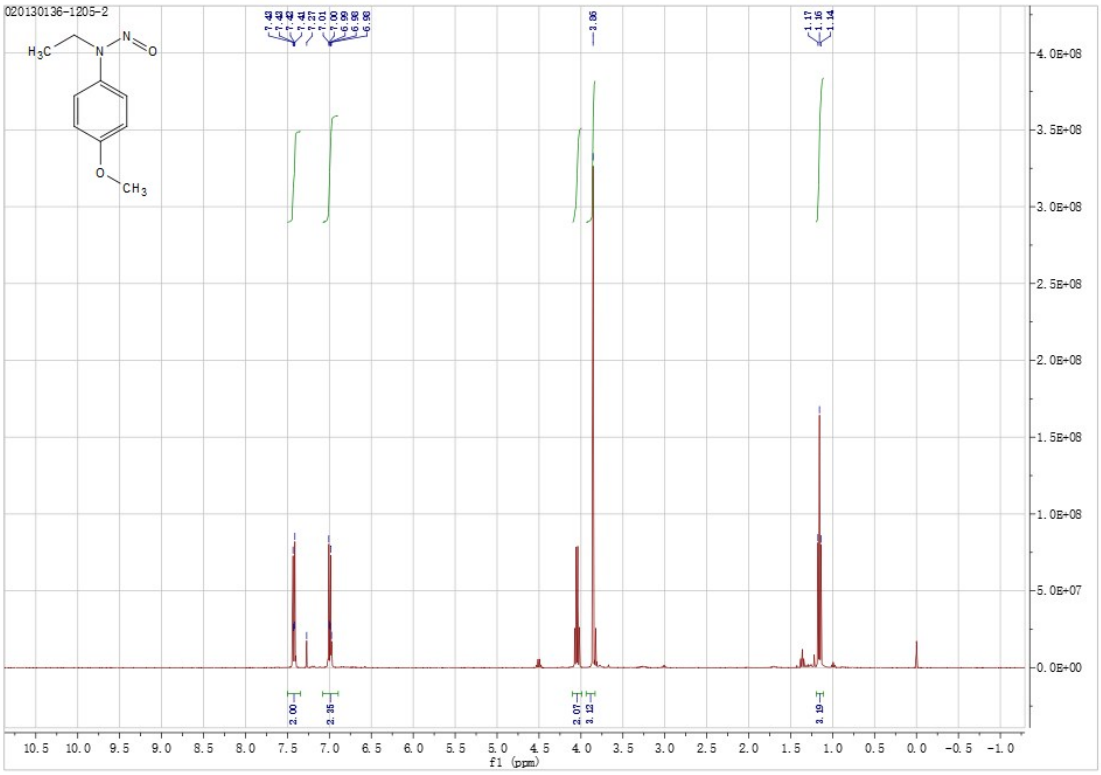


Fig S17. The ^{1}H NMR of compound 4 in $CDCl_3$

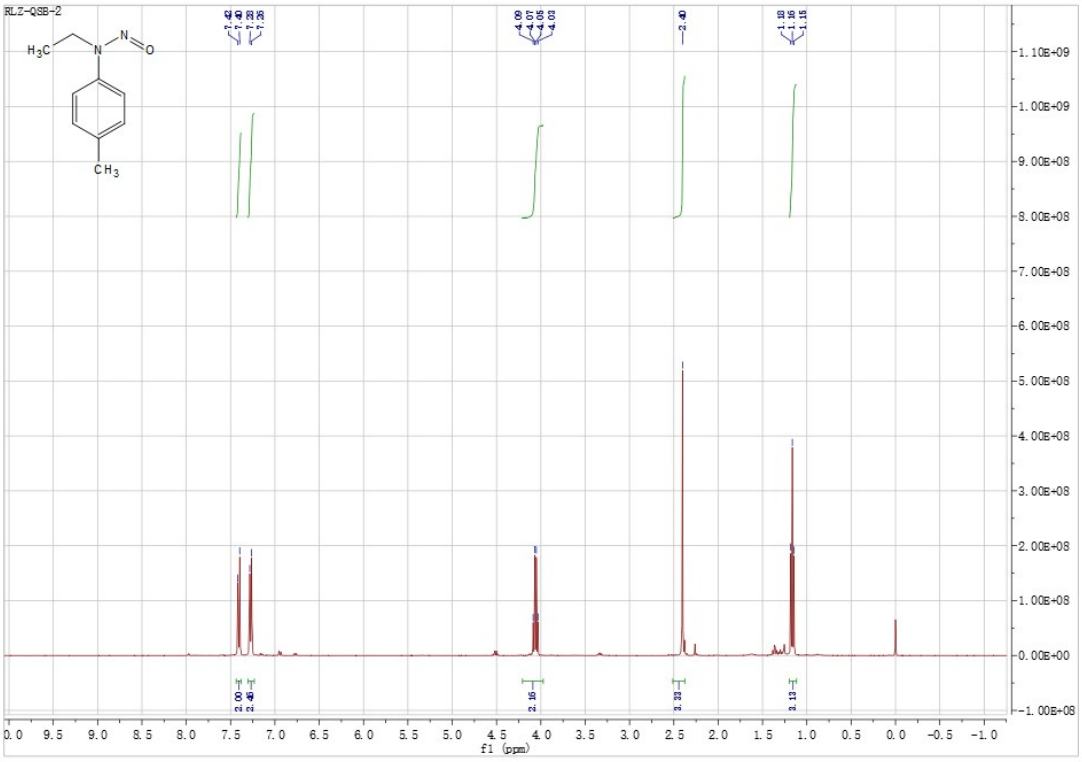


Fig S18. The ^{1}H NMR of compound 5 in $CDCl_3$

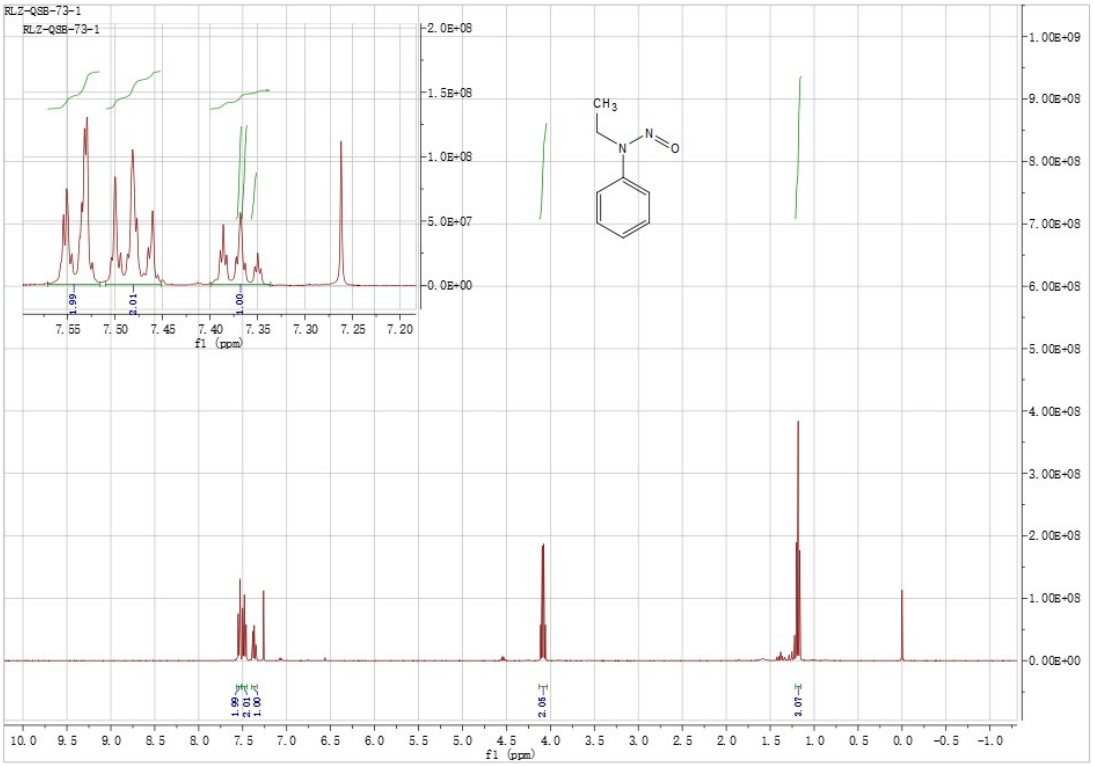


Fig S19. The ^{1}H NMR of compound 3 in $CDCl_3$

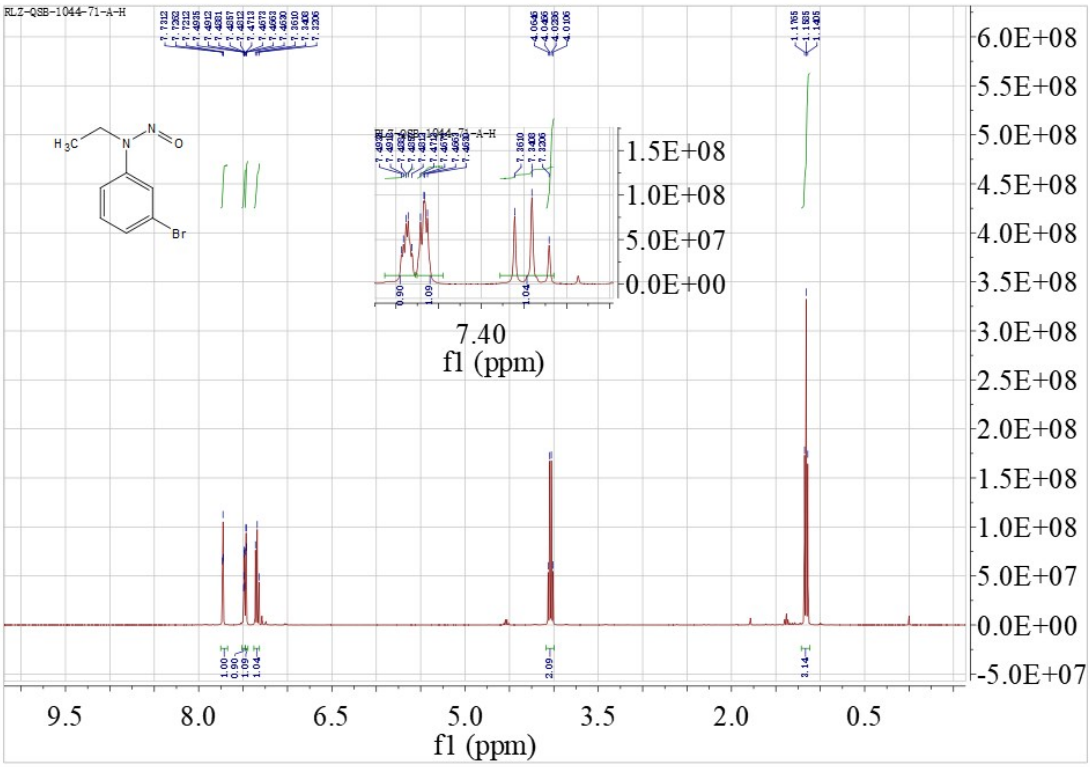


Fig S20. The ^{1}H NMR of compound 6 in $CDCl_3$

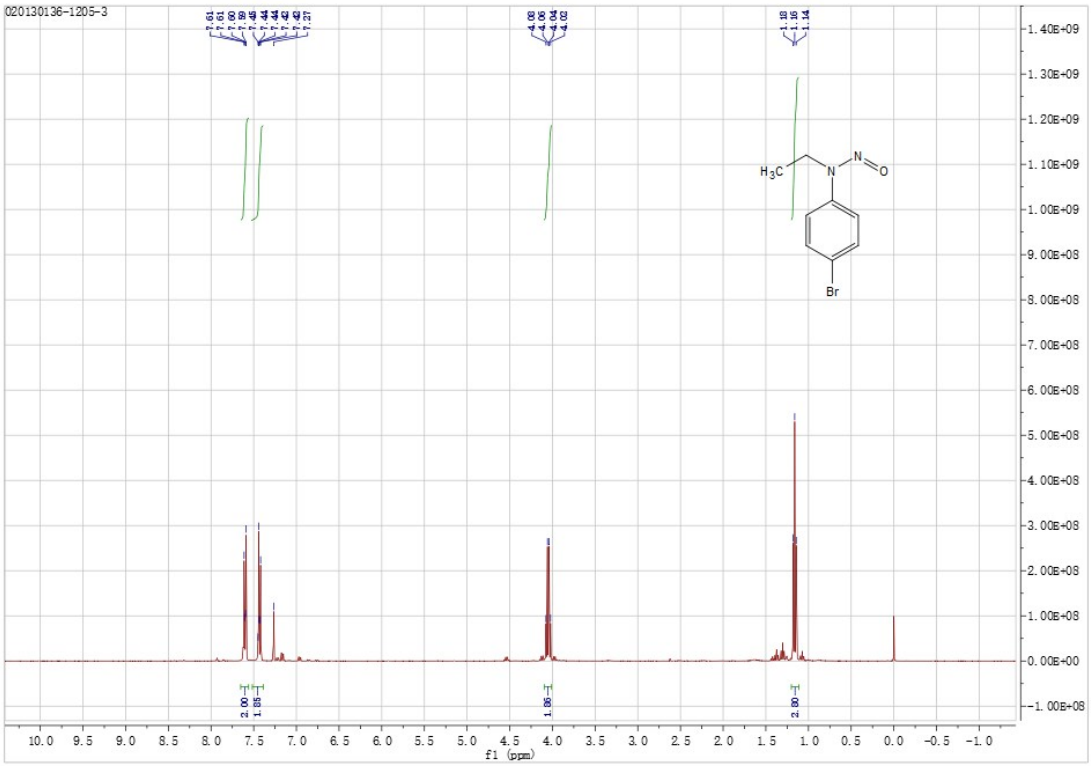


Fig S21. The ^{1}H NMR of compound 7 in $CDCl_3$

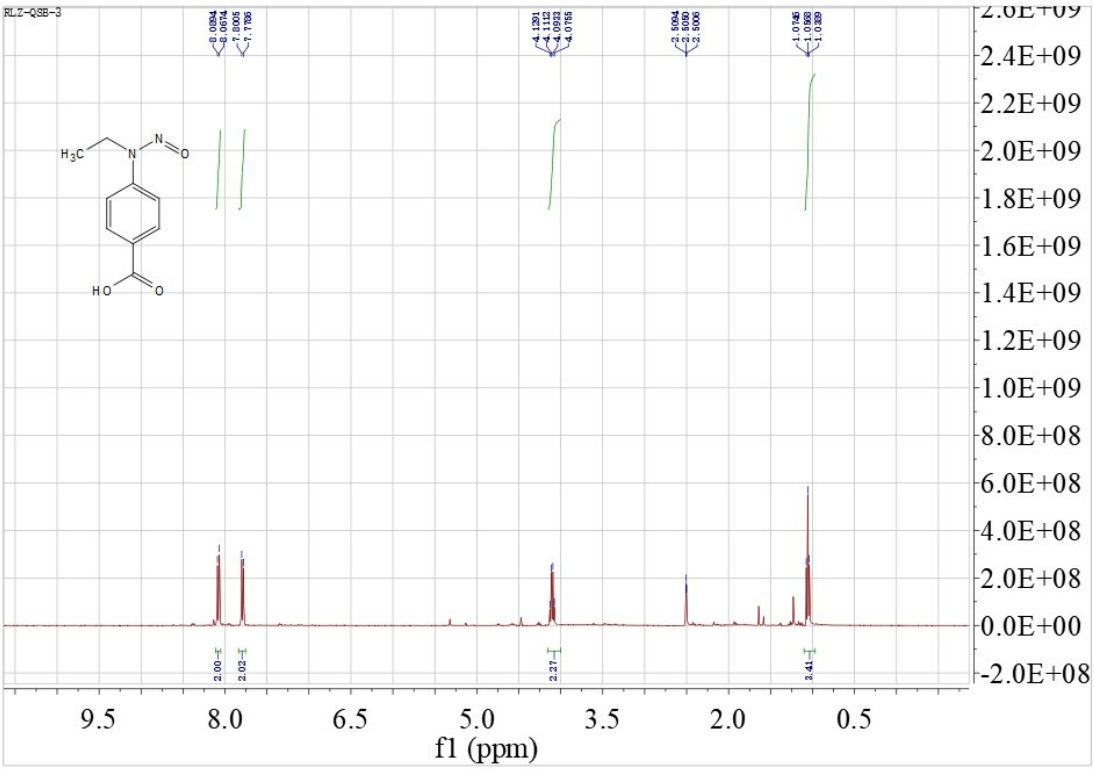


Fig S22. The ^{1}H NMR of compound 8 in DMSO