Supporting Information for

Synthesis and Evaluation of Novel O^2-Derived Diazeniumdiolates as Photochemical and Real-time Monitoring Nitric Oxide Delivery Agents

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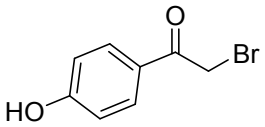
Contents

1. General information

All reagents and solvents (analytical grade) were purchased commercially and used without further purification unless otherwise noted. Ultraviolet lighter (150W, 0.02 W/cm^2, TLS3009-X105) was used to supply UV light (365-400 nm), and visible lighter (20W, 0.05 W/cm^2, HL-2000) was used as visible light (≥ 410 nm) source. Melting points were determined on a X-4 precision micro-melting point detector. Infrared (IR) spectra (KBr) were recorded on a Nicolet iS10 FT-IR instrument (KBr pellet). ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 303 K, using TMS as an internal standard. MS spectra were recorded on a Mariner Mass Spectrum (ESI). High resolution mass measurement was performed on Agilent QTOF 6520 mass spectrometer with electron spray ionization (ESI) as the ion source. Analytical and preparative TLC was performed on silica gel (200−300 mesh) GF/UV 254 plates, and the chromatograms were visualized under UV light at 254 and 365 nm. Analytical reversed phase high performance liquid chromatography (RP-HPLC) was performed on an Shimadzu LC-20AT system, and monitored using a diode array UV/Vis detector. A Venusil MP C_{18} column (250 mm × 4.6 mm × 5 μm) was used for purity analysis at a flow rate of 1 mL/min. Solutions after reactions and extractions were concentrated using a rotary evaporator operating at a reduced pressure of ca. 20 Torr. The excitation and emission spectrum were determined by Infinite® 200 Pro.

2. Preparation procedures and characterization data for compounds 1-4, 6, 10-12

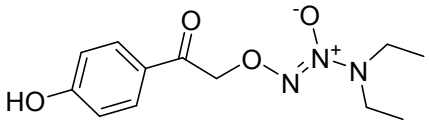
2-bromo-1-(4-hydroxyphenyl) ethan-1-one (6). [1]



6

To a solution of 4-hydroxyacetophenone (**5,** 1.36g, 10 mmol) in ethyl acetate (100 mL), $CuBr_2$ (3.5g, 15 mmol) was added slowly. The reaction mixture was stirred at room temperature for about 24h. The precipitate was removed by filtration. The obtained clear solution was washed with brine (50 mL × 3), and dried over anhydrous Na_2SO_4. After filtration, the organic solution was concentrated, and the residue was purified by recrystalization with ethanol to yield of 2-bromo-1-(4-hydroxyphenyl) ethan-1-one (1.5 g, 69.7%). mp: 124-125 °C. ^{1}H NMR ($DMSO_{d6}$, 300 MHz): 4.79 (s, 2H, CH_2Br), 6.89 (dd, 2H, J =16, 9 Hz, 2ArH), 7.88 (dd, 2H, J =16, 9 Hz, 2ArH), 10.56 (s, 1H, OH). IR (KBr, cm^{-1}): 3271, 1676, 1222, 698. ESI-MS: $[M + H]^+$, found 216 m/z $C_8H_7BrO_2$ requires 215.

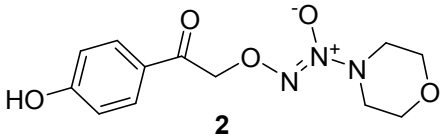
O^2-(*p*-Hydroxyphenacyl)-1-(*N,N*-diethylamino)diazen-1-ium-1,2-diolate (1).



1

To a solution of 1-(*N,N*-Diethylamino)diazen-1-ium-1,2-diolate (**7**) [2,3] (155 mg, 1 mmol) in dry *N,N*–dimethylformamide (DMF, 2 mL) was added compound **6** (215 mg, 1 mmol) under an inert atmosphere at -10 °C. The obtained solution was allowed to stir at this condition for 2.5h. After completion of the reaction (as monitored by TLC), DMF was removed under vacuum. The resulting crimson oil was dissolved in dichloromethane (20 mL) and washed with brine (20 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation to yield the crude product, which was purified on a silica column using 20 % ethyl acetate in petroleum ether to afford **2** as a white solid (59.8 mg, 22.4%). mp: 144-145 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.09 (t, *J* =7.1 Hz, 6 H, CH_3 × 2), 3.15 (q, *J* = 7.1 Hz, 4 H, NCH_2 × 2), 5.37 (s, 2 H, OCH_2), 6.88-6.92 (m, 2H, 2ArH), 7.76-7.80 (m, 2H, 2ArH). ^{13}C NMR (75 MHz, CDCl3): δ 10.9, 48.2, 73.9, 115.4, 126.3, 130.1, 161.1, 191.2. IR (KBr, cm^{-1}): 3174, 1671, 1229, 1096. ESI-MS: $[M - H]^-$, found 266.1 $C_{12}H_{17}N_3O_4$ requires 267.2. HRMS m/z calcd for $C_{12}H_{16}N_3O_4$ $[M - H]^-$, 266.1141. found 266.1136. ppm error 1.9.

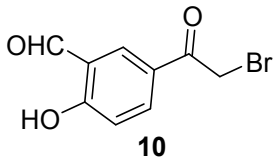
O^2-(*p*-hydroxyphenacyl)-1-(*N,N*-morpholine)diazen-1-ium-1,2-diolate (2).



2

The title compound was prepared from the reaction of **6** with 1-(*N,N*-morpholine)diazen-1-ium-1,2-diolate **8** by using the same method as compound **1**, as a white solid (90 mg, 32%). mp: 166-167 °C. ^{1}H NMR ($DMSO_{d6}$, 300 MHz): 3.28 (t, *J* = 4.8 Hz, 4H, NCH_2 × 2), 3.71 (t, *J* = 4.8 Hz, 4H, OCH_2 × 2), 5.53 (s, 2H, OCH_2), 6.87 (d, *J* = 8.7 Hz, 2H, 2ArH), 7.83 (d, *J* = 8.7 Hz, 2H, 2ArH), 10.51 (s, 1H, OH). ^{13}C NMR ($DMSO_{d6}$, 75 MHz): δ 51.6, 74.6, 115.9, 126.2, 130.9, 163.1, 192.2. IR (KBr, cm-1): 3173, 1676, 1243, 1112,1090. ESI-MS: $[M-H]^-$, found 280.1. $C_{12}H_{15}N_3O_5$ requires 281.1. HRMS m/z calcd for $C_{12}H_{14}N_3O_5$ $[M-H]^-$: 280.0933. found 280.0939. ppm error 2.1.

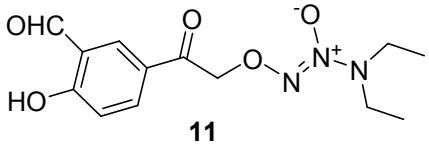
5-(2-bromoacetyl)-2-hydroxybenzaldehyde (10). [4]



10

To a suspension of aluminum chloride ($AlCl_3$) (1.06 g, 8 mmol) in 60 mL of dichloromethane (DCM), bromoacetyl bromide (480 mg, 2.4 mmol) was added slowly at 10 °C. The temperature of the mixture was brought to 30 °C and the mixture was stirred for an hour. Then a solution of salicylaldehyde **(9)** (245 mg, 2 mmol) in DCM (10 mL) was added slowly. The obtained mixture was stirred at rt for 15h. Then the reaction was quenched by adding cool water (50 mL). The DCM layer was separated and the aqueous layer was extracted with DCM (30 mL × 2), and dried over anhydrous Na_2SO_4. Removal of solvent generated crude slurry, which was added n-heptane (20 mL) and stirred for 15 min. The precipitate was filtrated, washed with n-heptane and dried at 50 °C, to yield compound **10** as a yellow solid (192 mg; 60%)**.** mp 57-58 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 4.40 (s, 2H, CH_2Br), 7.10 (d, J = 8.8 Hz, 1H, ArH), 8.17 (1H, dd, J = 8.8, 2.2 Hz, ArH), 8.30 (d, J = 2.2 Hz, 1H, ArH), 9.99 (s, 1H, OH), 11.53 (s, 1H, CHO). IR (KBr, cm^{-1}): 3250, 2877, 1725, 1681, 1197, 662. ESI-MS: $[M+Cl]^-$, found 278 m/z $C_9H_8BrO_3$ requires 243.1.

***O*^{2}-(5-(2-acetoxy)-2-hydroxybenzaldehyde)-1-(*N,N*-diethylamino)diazen-1-ium-1,2-diolate (11).**

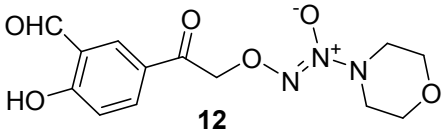


11

To a solution of 1-(*N,N*-Diethylamino)diazen-1-ium-1,2-diolate (**7**) [2,3] (155 mg, 1 mmol) in dry tetrahydrofuran (THF, 3 mL) and dimethyl sulfoxide (DMSO, 0.5 mL) was added compound **10** (243mg, 1 mmol) under an inert atmosphere at -10 °C. The obtained solution was allowed to stir at this condition for 3.5h. After completion of the reaction (as monitored by TLC), DMF was removed under vacuum. The resulting crimson oil was dissolved in dichloromethane (20 mL) and washed with brine (20 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation to yield the crude product, which was purified on a silica column using 20 % ethyl acetate in petroleum ether to afford **11** as a white acicular crystal (77.3 mg, 26.2%). mp: 91-92 °C. ^{1}H NMR (300MHz, $CDCl_3$) 1.03 (t, J = 7.1 Hz, 6H, CH_3 × 2), 3.10 (q, J = 7.1 Hz, 4H, CH_2 × 2), 5.34 (s, 2H, OCH_2), 7.05 (d, 1H, J = 8.8 Hz, ArH), 8.11(dd, 2H, J = 8.8, 2.2 Hz, ArH), 8.27(d, J = 2.2 Hz, 1H, ArH), 9.95 (s, 1H, OH), 11.46 (s, 1H, CHO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.4, 48.5, 74.7, 118.4, 120.1, 126.9, 135.0, 136.4, 165.6, 191.0, 196.3. IR (KBr,

cm^{-1}): 3427, 2853, 1654, 1230, 1091. ESI-MS: $[M + Na]^+$, found 318.1 m/z $C_{13}H_{17}N_3O_5$ requires 295.1. HRMS m/z calcd for $C_{13}H_{17}N_3O_5Na$ $[M + Na]^+$: 318.1066. found 318.1059. ppm error 2.1.

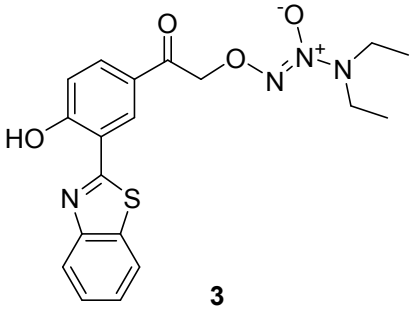
***O*²-(5-(2-acetoxy)-2-hydroxybenzaldehyde)-1-(*N,N*-morpholine)diazen-1-ium-1,2-diolate(12).**



12

The title compound **12** was prepared from the reaction of **10** with 1-(*N,N*-morpholine)diazen-1-ium-1,2-diolate **8** by using the same method as compound **11**, as a white solid (102.6 mg, 33.2%). mp: 249-250 °C. ^{1}H NMR (300 MHz, $CDCl_3$) 3.34 (d, J = 4.4 Hz, 4H, $NCH_2\times$ 2), 3.73 (d, J = 4.4 Hz, 4H, $OCH_2 \times 2$), 5.24 (s, 2H, OCH_2), 7.01(d, J = 8.8 Hz, 1H, ArH), 8.08 (d, J = 8.8 Hz, 1H, ArH), 8.25 (s, 1H, ArH), 9.90 (s, 1H, OH), 11.43 (s, 1H, CHO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 51.5, 65.5, 74.8, 118.5, 120.2, 126.8, 135.1, 136.5, 165.7, 191.1, 196.3. IR (KBr, cm^{-1}): 3447, 2857, 1693, 1225, 1180, 1103. ESI-MS: $[M + Na]^+$, found 332.1 m/z $C_{13}H_{15}N_3O_6$ requires 309.1. HRMS m/z calcd for $C_{13}H_{15}N_3O_6Na$ $[M + Na]^+$, 332.0859 found 332.0865. ppm error 1.8.

***O*²-(*p*-hydroxyphenacyl–benzothiazole)-1-(*N,N*-diethylamino)diazen-1-ium-1,2-diolate(3).**

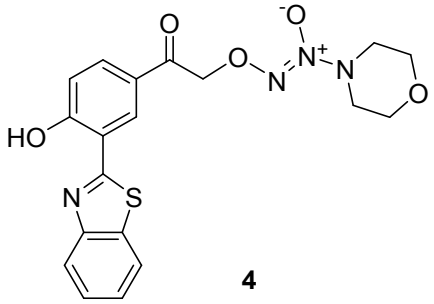


3

To a solution of **11** (50 mg, 0.17 mmol) in DMSO (3 mL) was added 2-aminobenzothiol (21 mg, 0.17 mmol). The mixture was stirred at reflux for 2h. The reaction was quenched by adding cool water (20 mL), and then was extracted with ethyl acetate (10 mL × 3). Organic layer was collected, dried over anhydrous Na_2SO_4, and the solvent was removed to produce a brown solid residue, which was purified by column chromatography using 20 % ethyl acetate in petroleum ether to give **11** as a white solid (67.8 mg, 50%). mp: 217-218 °C. ^{1}H NMR (300MHz, $CDCl_3$) 1.00 (t, J = 7.1 Hz, 6H, $CH_3 \times 2$), 3.03 (q, J = 7.1 Hz, 4H, $CH_2 \times 2$), 5.34 (s, 2H, OCH_2), 7.06 (d, J = 8.7 Hz, 1H, ArH), 7.34-7.47 (m, 2H, ArH), 7.84-7.93 (m, 3H, ArH), 8.29 (d, J =1.9 Hz, 1H, ArH), 13.14 (s, 1H, OH). ^{13}C NMR (75 MHz, CDC^{l3}): δ 11.4, 48.7, 74.6, 117, 118.2, 121.7, 122.3, 126.1, 126.4, 127, 129.4, 132.4, 132.7,162.4, 168.4, 191.1. IR (KBr, cm^{-1}): 3448, 1694, 1624, 1503, 1220, 1099. ESI-MS$^+$: $[M + H]^+$, found 401.1 m/z $C_{19}H_{20}N_4O_4S$ requires 400.1 HRMS m/z calcd

for $C_{19}H_{21}N_4O_4S$ [M + H]$^+$: 401.1284. found 401.1289. ppm error 1.2.

***O*^{2}-(*p*-hydroxyphenacyl–benzothiazole)-1-(*N,N*- Morpholine)diazen-1-ium-1, 2-diolate(4).**



4

The title compound **4** was prepared from the reaction of **12** with 2-aminobenzothiol by using the same method as compound **3**, as a white solid (80.4 mg, 62.3%). mp: 289-290 °C. ^{1}H NMR (300 MHz, $CDCl_3$) 3.42 (t, *J* = 4.8 Hz, 4H, NCH_2 × 2), 3.80 (t, *J* = 4.8 Hz, 4H, OCH_2 × 2), 5.35 (s, 2H, OCH_2), 7.16 (d, *J* = 8.7 Hz, 1H, ArH), 7.43-7.56 (m, 2H, ArH), 7.79-8.02 (m, 3H, ArH), 8.40 (d, J = 1.9 Hz, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 51.5, 65.6, 74.8, 117.1, 118.3, 121.7, 122.3, 126.1, 126.4, 127, 129.5, 132.5, 132.7, 151.4, 162.6, 168.4, 191.2. IR (KBr, cm^{-1}): 3422, 1695, 1654, 1498, 1226, 1114, 1022. ESI-MS, found [M - H]$^-$, 413.1. m/z $C_{19}H_{18}N_4O_5S$ requires 414.1 HRMS m/z calcd for $C_{19}H_{17}N_4O_5S$ [M - H]$^-$, 413.0920. found 413.0926. ppm error 1.45.

3. MTT Assay

Human lung cancer A549 cells, human colon carcinoma HCT116 cells, HT29 cells, human acute monocytic leukemia THP-1 cells, human leukemia HL60 cells, human liver carcinoma HepG2 cells, human normal colon epithelial CCD-18Co cells were purchased from American Tissue Culture Collection (ATCC, Rockville, MD, USA). Human normal liver LO2 cells were purchased from Shanghai Institute of Cell Research. All cells were cultured in Roswell Park Memorial Institute medium (RPMI-1640, Thermo, USA) and Fetal bovine serum (FBS, GIBCO, Invitrogen Corporation, NY, USA) at 37 °C under 5% CO_2 atmosphere. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl- 2-H-tetrazolium bromide (MTT) were obtained from Sheng Xing Biotechnology Company.

Intermittent light exposure protocol for Table 1 in MS. Cells were incubated in a 96 well plate with a concentration of 10^4 cells/well and cultured in 37 °C 5% CO_2 for 24 h. Then cells were respectively treated with or without test compounds containing EMEM, and intermittently exposed under ultraviolet light (365-400 nm, 0.02 W/cm^2, UV lamp TLS3009-X105) or under visible light (≥410 nm, 0.05 W/cm^2, HL-2000) for 20 mins at 0h, 1h, 2h, 4h, 6h, 8h, 12h, 24h, 36h time points, or under dark. Each concentration was repeated 3 times in parallel. After incubation for 44 h, MTT (20 μL, 5 mg/mL) was added into each well, and the cells were incubated for additional 4h. Then the medium was carefully removed. Dimethyl sulfoxide (150 μL/well) was added and the 96 well plate was oscillated gently until crystal dissolved. The absorbance at 570 nm was measured using a microplate reader. The cell viability was expressed as a percentage of OD570 and the corresponding IC_{50} values are summarized in Table 1 in MS. It was found that the cancer cell viability was not affected by intermittent light exposure (Table S1).

Table S1. The proliferation inhibitory rates (%) of intermittent ultraviolet light exposure (without treatment of **1** or **2**) or intermittent visible light exposure (without treatment of **3** or **4**) to A549, HCT116, THP-1, and HL-60 cells

	Without 1	**Without 2**	**Without 3**	**Without 4**
A549	0.26 ± 0.06	3.85 ± 0.98	0.68 ± 1.55	1.48 ± 2.32
HCT116	0.13 ± 0.04	1.15 ± 0.26	-1.32 ± 1.60	-1.68 ± 2.73
THP-1	1.37 ± 0.33	1.24 ± 0.26	1.13 ± 4.20	2.71 ± 2.62
HL60	1.50 ± 0.23	1.82 ± 0.52	-2.04 ± 2.02	0.85 ± 1.14

Continuous visible light exposure protocol. HL-60 cells were incubated in a 96 well plate with a concentration of 10^4 cells/well and cultured in 37 °C 5% CO_2 for 24 h. Then cells were treated with the compound **3** at different concentrations containing EMEM for 4h in the dark.

Then the cells were exposed by continuous visible light (≥410 nm, 0.05 W/cm^2, HL-2000) for 1h, and then kept in the dark for some hours. The total incubation time were 24, 48 or 72h. Each concentration was repeated 3 times in parallel.

Table S2. IC_{50} values (μM) of **3** under intermittent or continuous visible light against HL-60 cells with different incubation time.

	The intermittent visible light		The continuous visible light	
	Light	Dark	Light	Dark
24h	3.25 ± 0.15	679.1 ± 13.46	2.98 ± 0.17	706.5 ± 11.49
48h	0.64 ± 0.03	205 ± 2.6	0.59 ± 0.06	207.1 ± 3.47
72h	0.47 ± 0.05	131.7 ± 3.52	0.47 ± 0.08	136.0 ± 3.94

Table S3. IC_{50} values (μM) of **3** under continuous visible light exposure or in dark against HT29, CCD-18Co, HepG2, and LO2 cells with total incubation time for 72h.

	HepG2	**LO2**	**HT29**	**CCD-18Co**
3 in the dark	202.3 ± 5.69	123.8 ± 7.64	163.5 ± 5.98	125 ± 6.61
3 under visible light	1.226 ± 0.051	124.2 ± 5.51	1.121 ± 0.09	95.38 ± 2.51

4. The NO release and photolytic decomposition kinetics studies of 1 and 3 in PBS

A solution of **1** (50 μM) in PBS (50 mM, containing 5% DMSO) was exposed ultraviolet light (365-400 nm) for 360 mins (keeping the quartz cuvette 6 cm away from the light source, 0.02 W/cm^2) or in dark at 37 ℃. At 0, 5, 10, 30, 60, 120, 240, 360 min, 200 μL of sample was take out. 150 μL of sample was mixed with 50 μL Griess reagent and incubated for addition 5 mins at 37 ℃ to determine the concentrations of NO_2^-, while 50 μL of sample was analyzed by RP-HPLC using Venusil MP C18 (250 mm × 4.6 mm × 5 μm) at a flow rate of 1mL/min with a detection wavelength of 254 nm to determine the concentration of **1** remaining. The experiment was repeated three times and the results are shown in Figure 2A in the manuscript.

A solution of **3** (50 μM) in PBS (50 mM, containing 5% DMSO) were exposed visible light (≥ 410 nm) for 30 mins (keeping the quartz cuvette 6 cm away from the light source, 0.05 W/cm^2) or in dark at 37 ℃. At 0, 5, 10, 15, 30 min, 200 μL of sample was take out. 150 μL of sample was mixed with 50 μL Griess reagent and incubated for addition 5 mins at 37 ℃ to determine the concentrations of NO_2^-, while 50 μL of sample was analyzed by RP-HPLC using Venusil MP C18 (250 mm × 4.6 mm × 5 μm) at a flow rate of 1mL/min with a detection wavelength of 254 nm to

determine the concentration of **3** remaining. The experiment was repeated three times and the results are shown in Figure 2B in the manuscript.

A solution of **1** (50 μM) in PBS (50 mM, containing 5% DMSO) and a solution of **3** (50 μM) in PBS (50 mM, containing 5% DMSO) were incubated in dark at 37 ℃ for 6h and 30 min, respectively. 50 μL of samples were analyzed by HPLC to determine the stability of **1** and **3** in PBS in dark (Figure S1). It was found that compounds **1** and **3** were stable in dark in PBS solution.

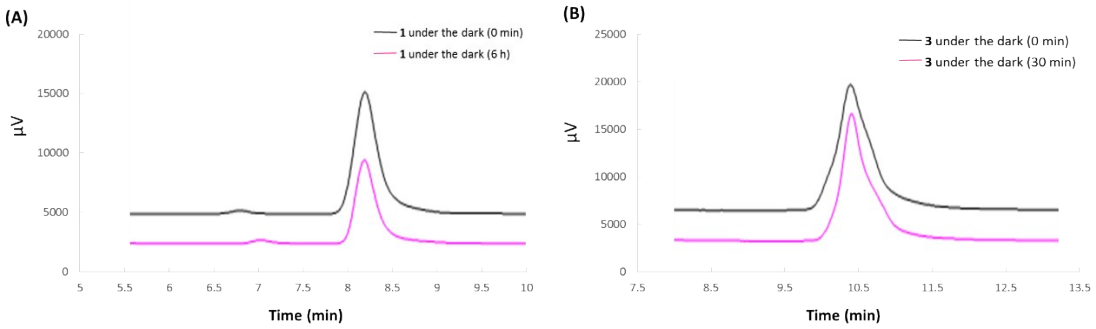


Figure S1. (A) Overlay of HPLC chromatograms of **1** under the dark at 0 min, 6h. (B) Overlay of HPLC chromatograms of **3** under the dark at 0 min, 30 min.

5. NO release measurement in HL60 cells

DAF-FM DA (Beyotime) was used as a fluorescent indicator of intracellular NO release. HL-60 cells were treated with **1** (0.4 μM) under intermittent ultraviolet light (365-400 nm, 0.02 W/cm^2) exposure for 20 mins at 0, 1, 2, 4, 6, 8, 12, 24, 36h time points, or in the dark for total 48h. The cells were then collected and resuspended with DAF-FM DA (1.5 mL, 5 μM) 37 °C for 20 mins in the dark. The cells were rinsed three times with PBS and then analyzed by fluorescence-activated cell sorting (FACS). NO production was measured with the flow cytometer with excitation and emission wavelengths of 495 and 515 nm, respectively. BD Accuri™ C6 flow cytometer was used, and 10,000 cells was gated for each sample. HL-60 cells were treated with **3** (0.6 μM) under the same conditions as **1** except using visible light (≥ 410 nm, 0.05 W/cm^2).

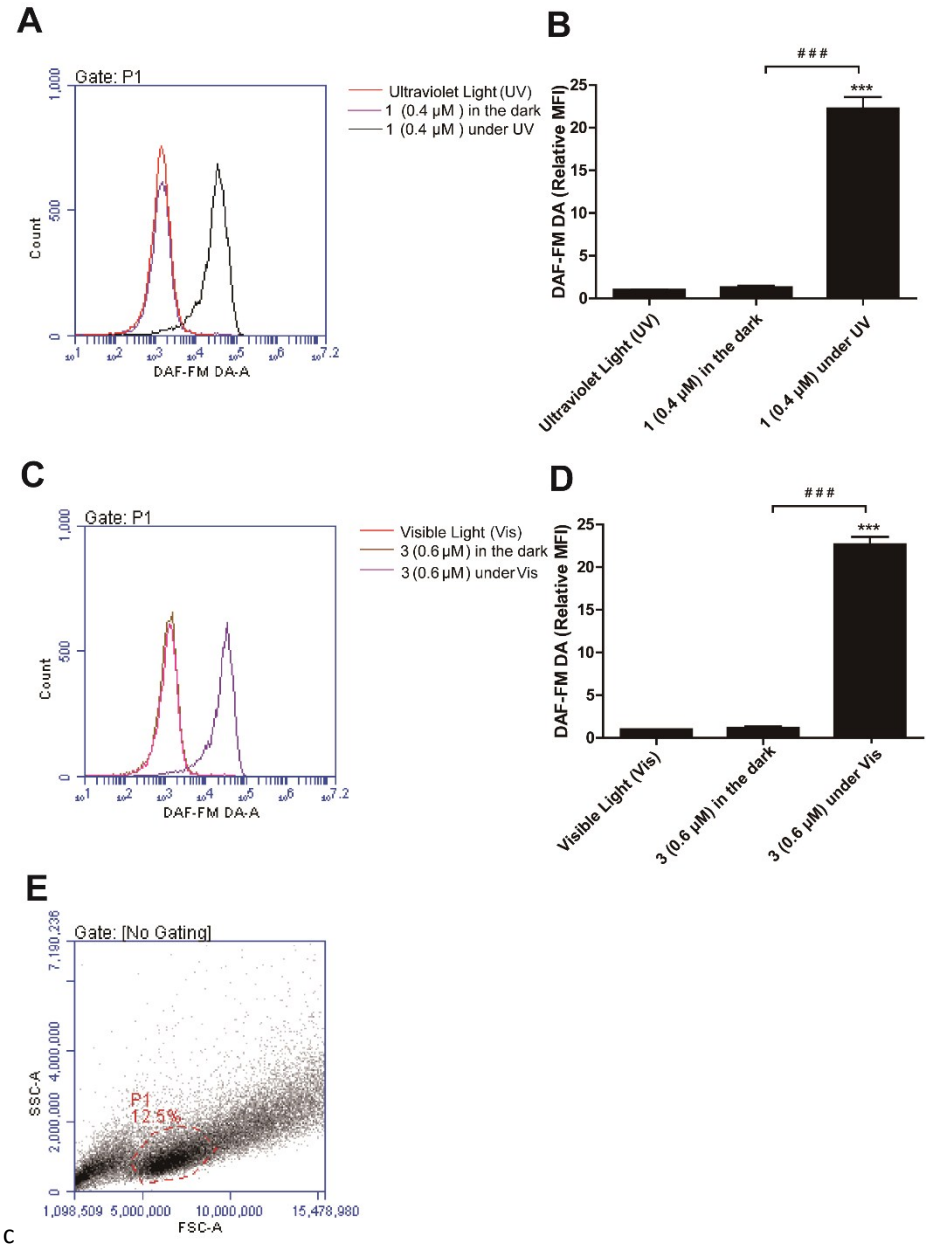


Figure S2. (A) and (B) HL-60 cells were treated with **1** (0.4 μM) under intermittent ultraviolet light (365-400 nm, 0.02 W/cm^2) exposure or in the dark for 48h. The cells were then collected and resuspended with DAF-FM DA (5 μM) for 20 mins in the dark, and then analyzed by fluorescence-activated cell sorting (FACS). (C) and (D) HL-60 cells were treated with **3** (0.6 μM) under the same conditions as (A and B) except using visible light (≥ 410 nm, 0.05 W/cm^2). (E) Plot of applied gate in the flow cytometry.

6. The excitation and emission spectrum of compound 3

Compound **3** was dissolved in DMSO and diluted to 30 μM under dark. The excitation (Ex) and emission (Em) spectra of **3** (30 μM) in DMSO were recorded at 0 and 30 mins under visible light exposure (≥410 nm, 0.05 W/cm^2, HL-2000) by using Tecan Infinite® 200 Pro.

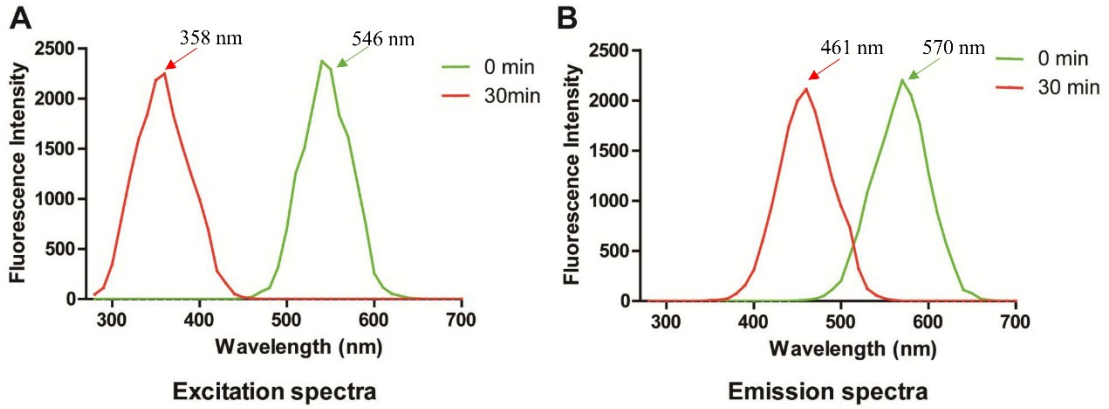


Figure S3. (A) The excitation spectra and (B) emission spectra of compound **3** (30 μM in DMSO) under visible light exposure for 30 mins.

7. Confocal microscopy studies of HL60 cells internalization of 3

Real-time monitoring of NO release from **3** in HL60 cells under visible light (≥ 410 nm) irradiation was studied by a Zeiss LSM confocal scanning system. HL60 cells were treated with 0.3 μM of **3** under dark at 37 °C for 8h. The cells were washed twice with PBS, and incubated with DAF-FM-DA (5.0 μM) under dark for 20 min. After washing, compound **3**-treated cells were irradiated with visible light (≥ 410 nm, 0.05 W/cm^2, HL-2000, 20W) for 15 min. Druing the incubation, fluorescent images are taken every 5 minutes. For the compound **3**, excitation wavelength (Ex) was carried out with lasers at λ = 546 nm and emissions wavelength (Em) were recorded in 570 nm, respectively. For DAF-FM DA, Ex/Em: 495 nm/515 nm and for the decomposition product (benzothiazole-2-yl)-4-hydroxy phenylacetic acid, Ex/Em: 358 nm/461 nm. All images are normalized to have the same brightness to ensure that any changes in image brightness is due to biological/chemical phenomena. As to clearly distinguish the fluorescence colour changes, ZEN 2012 software, which comes with the microscope, was used to set Ex/Em: 546/570 nm in green, 358/461 nm in blue and 495/515 nm in red, respectively.

8. Absorbance spectra (200-500 nm) of 3 under visible light (≥ 410 nm) for 30 mins

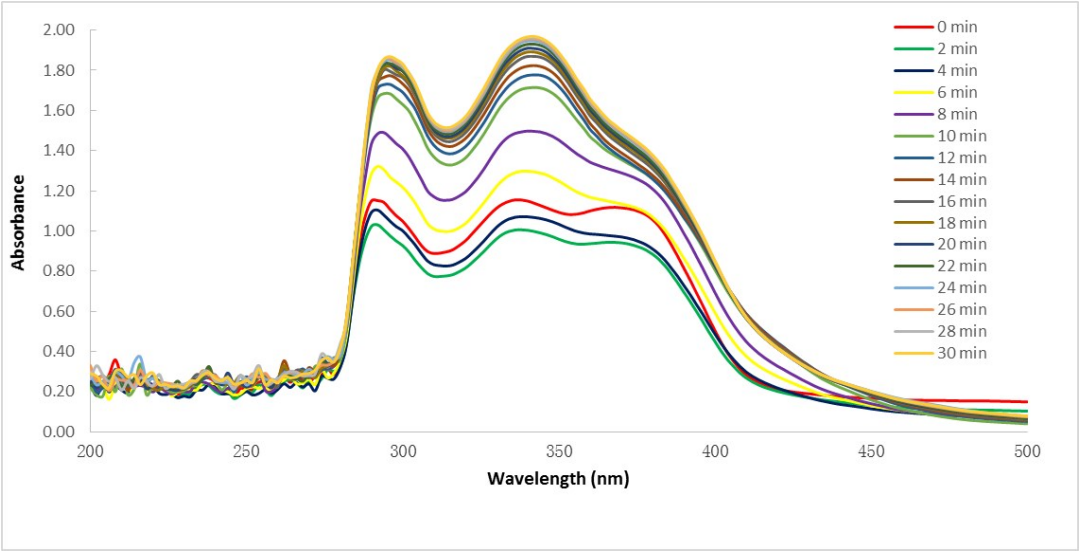
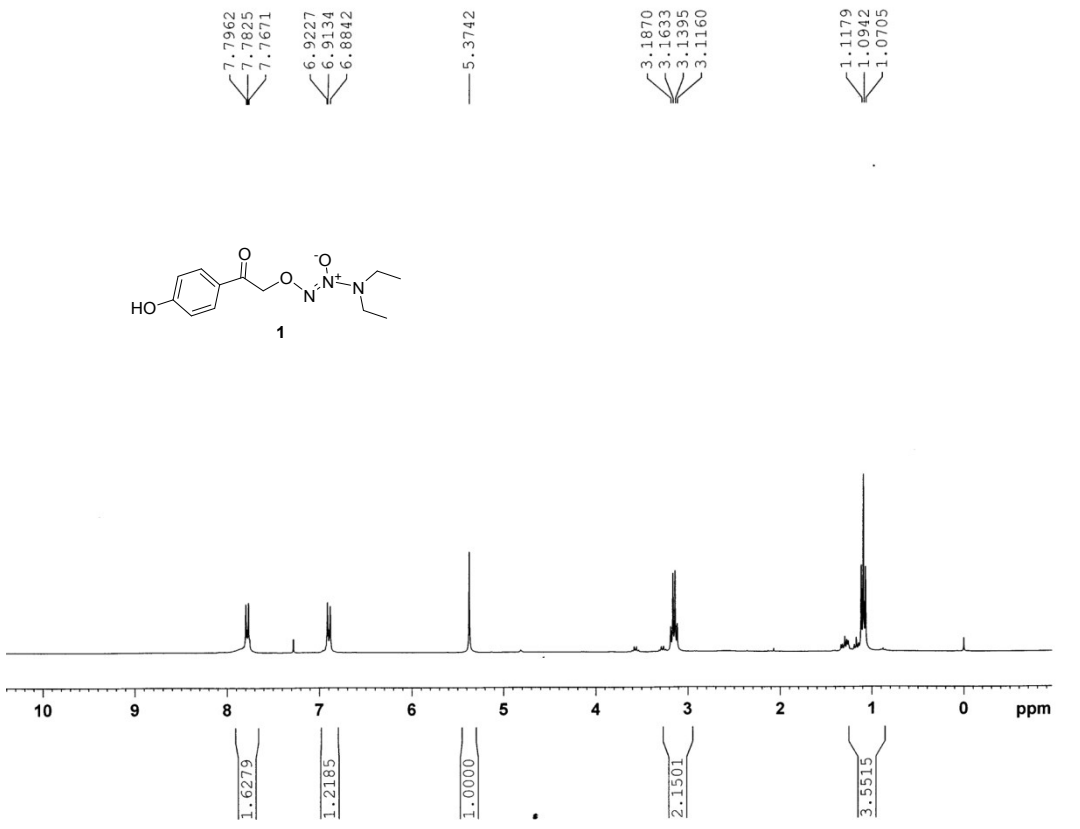
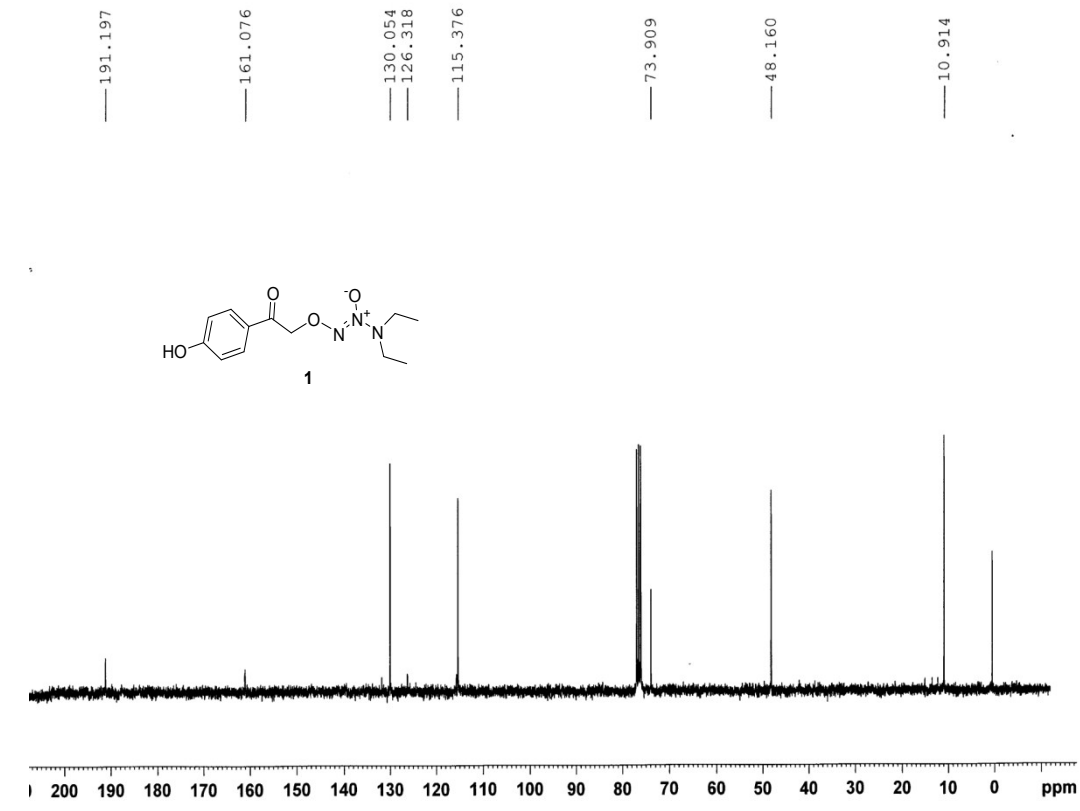
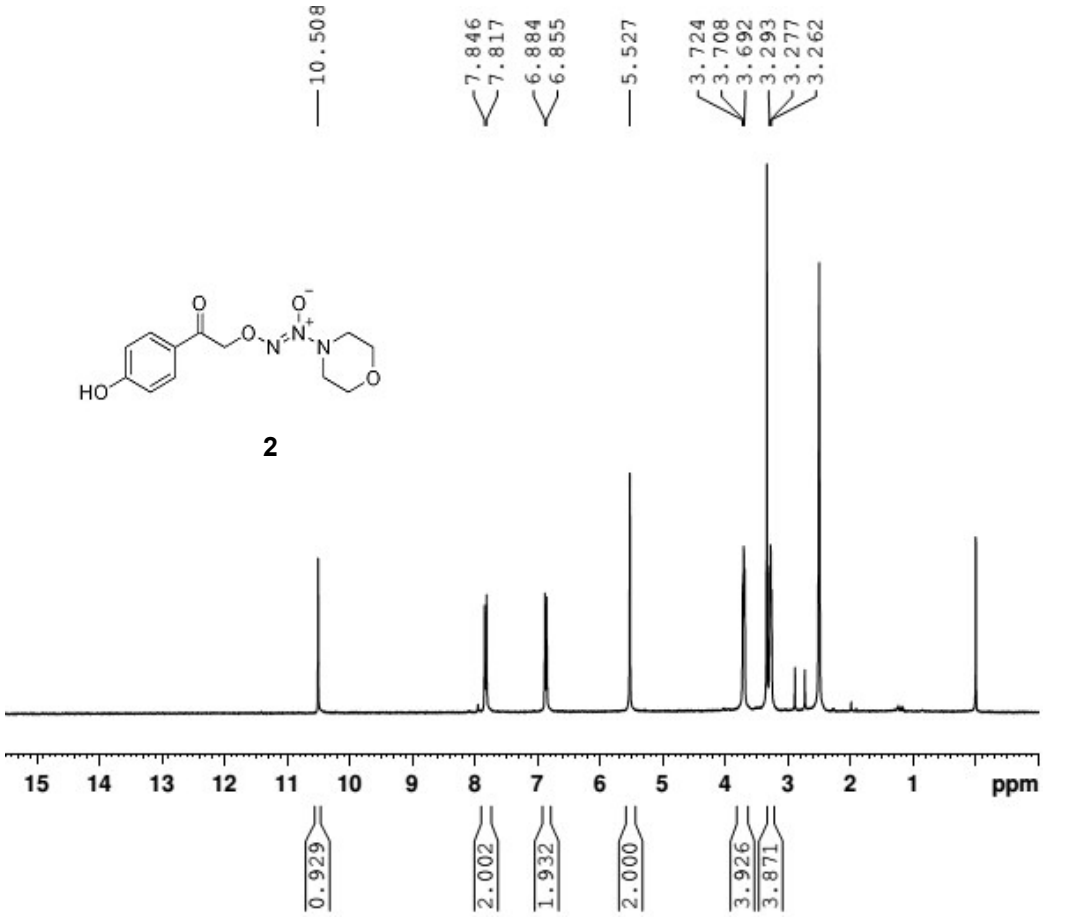


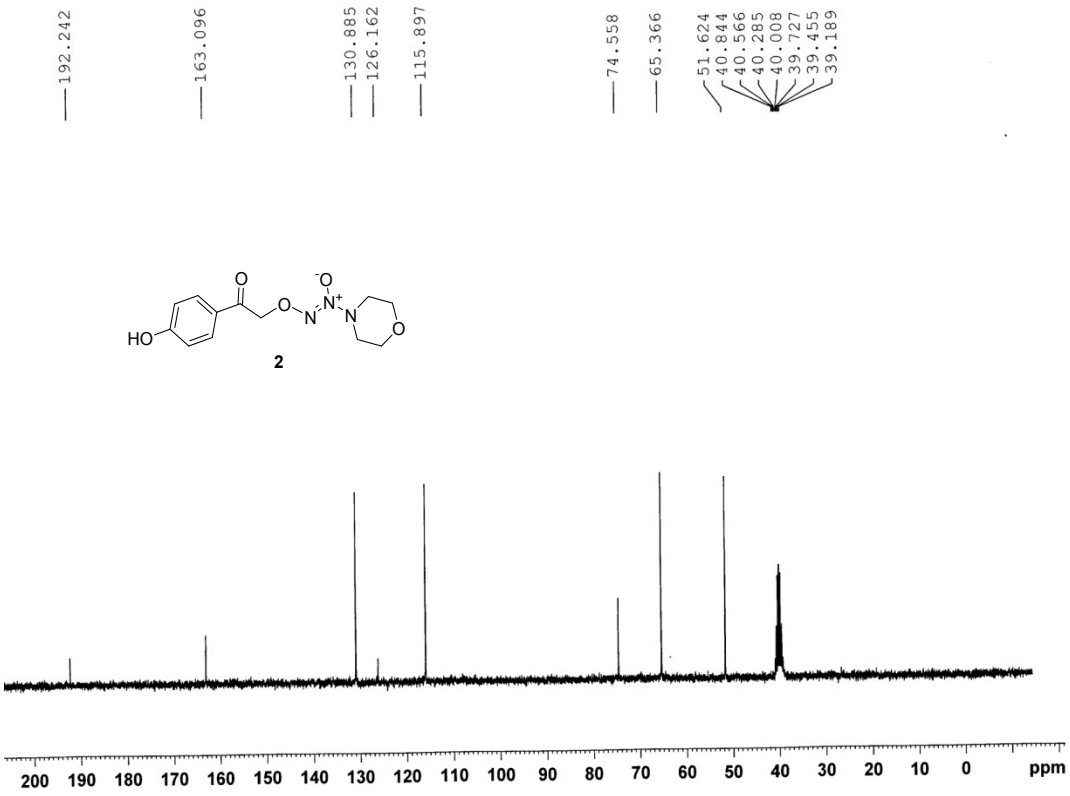
Figure S4. The absorbance spectra (200-500 nm) of **3** (200 μM) in PBS (10 % DMSO) at intervals of 2 mins under visible light (≥ 410 nm) for 30 mins.

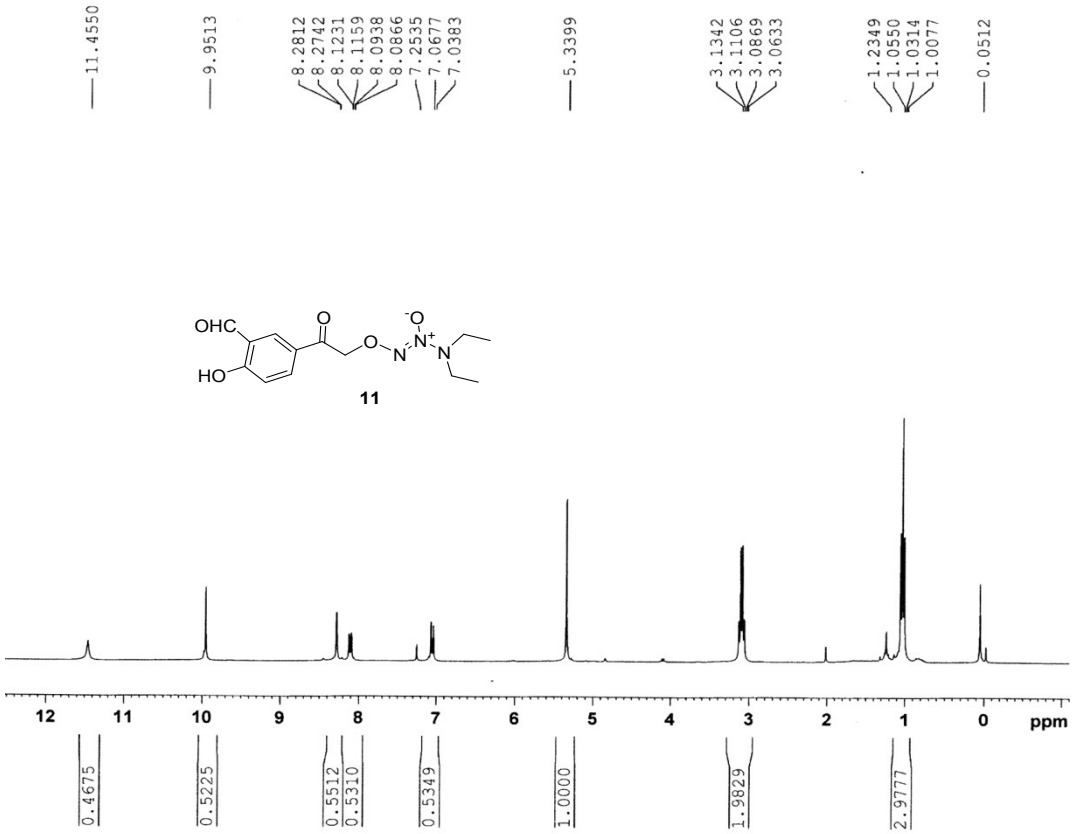
9. NMR Spectra Scanning files

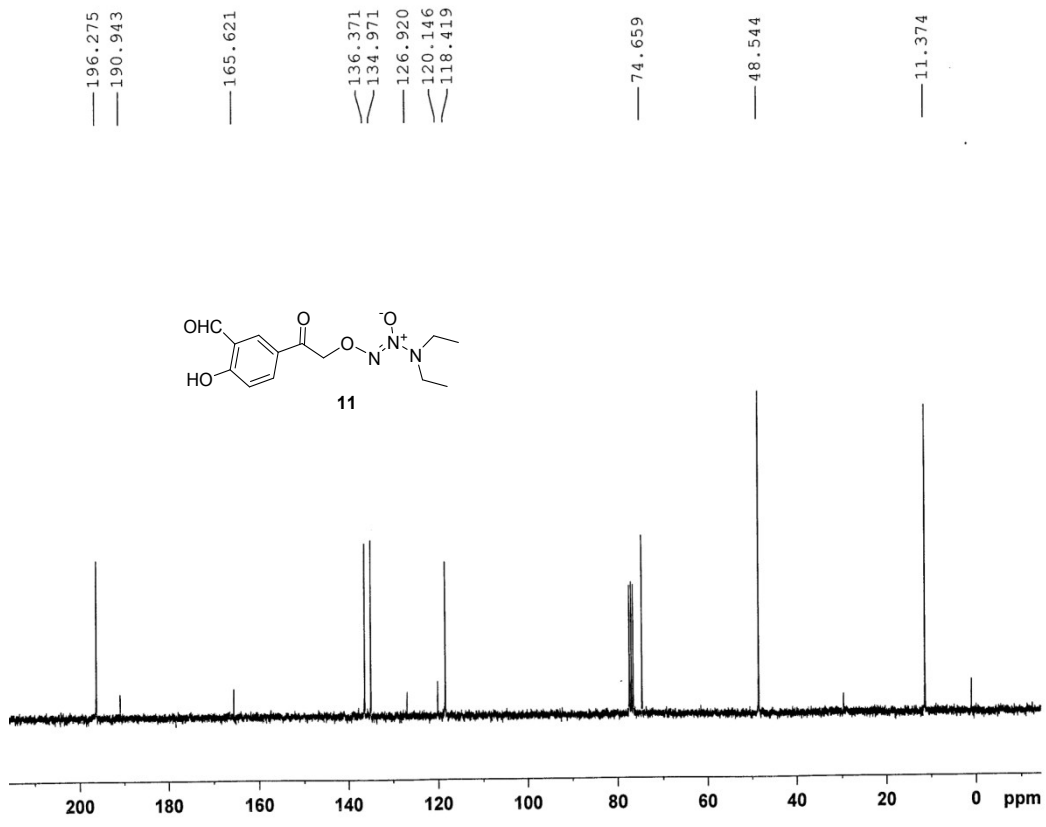


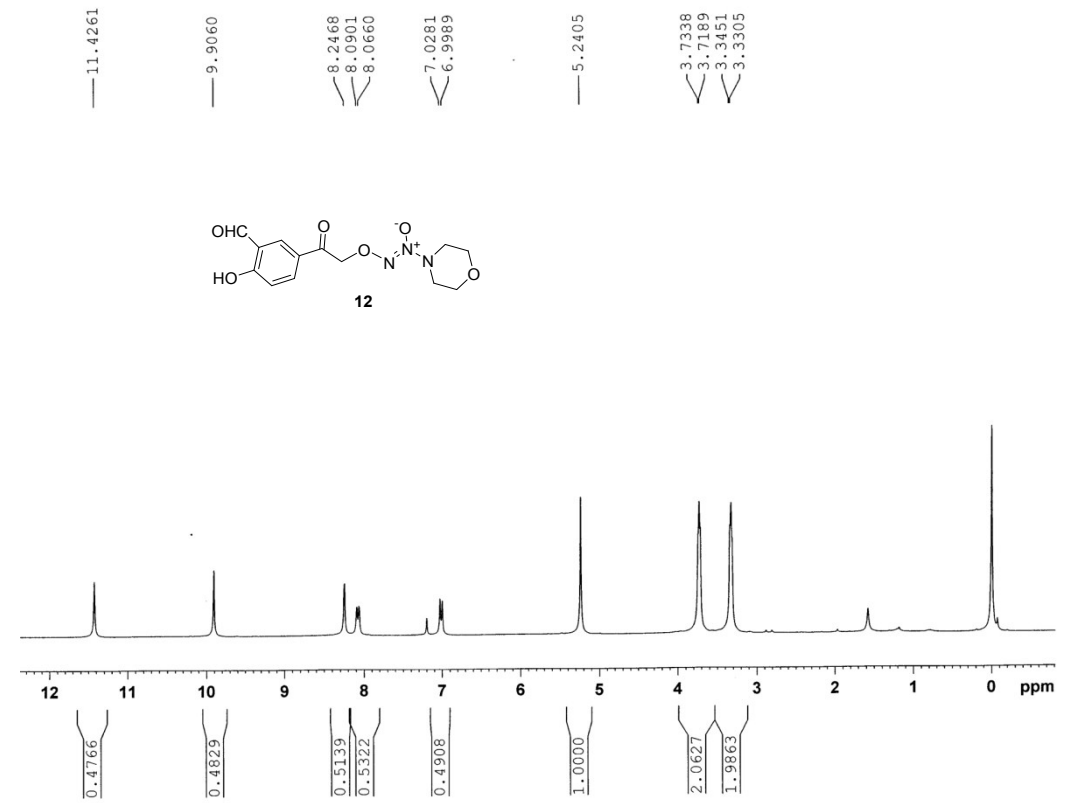


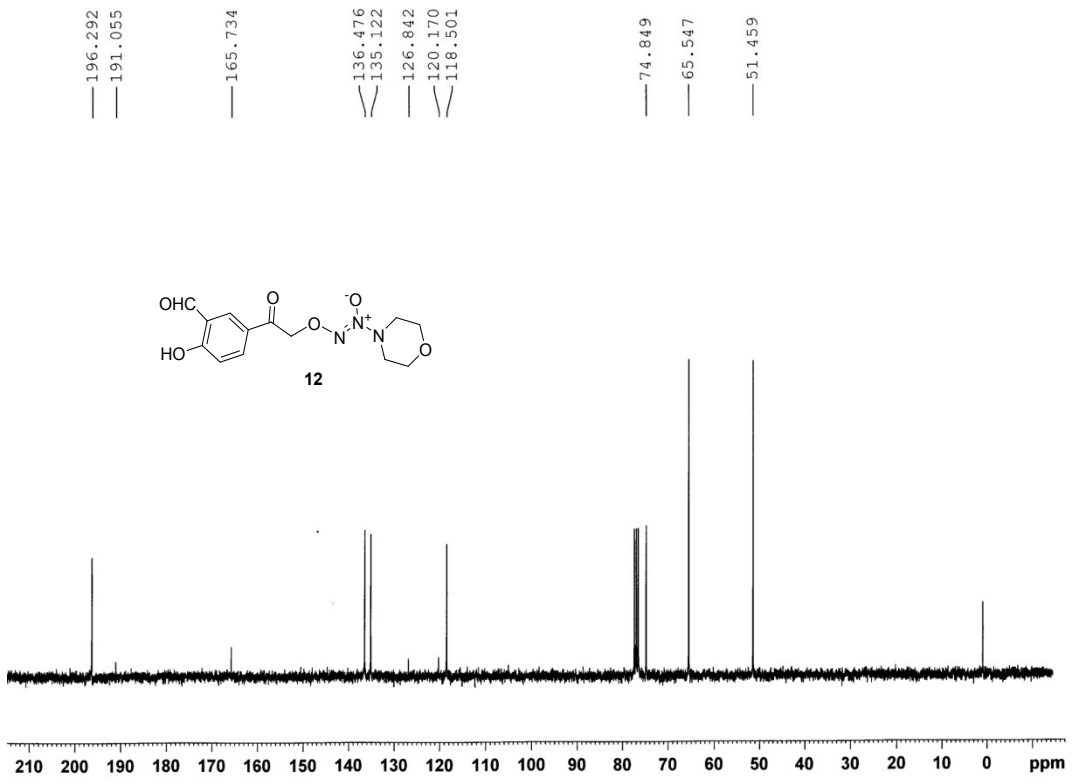

10.508
7.846
7.817
6.884
6.855
5.527
3.724
3.708
3.692
3.293
3.277
3.262
HO
2
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.929
2.002
1.932
2.000
3.926
3.871

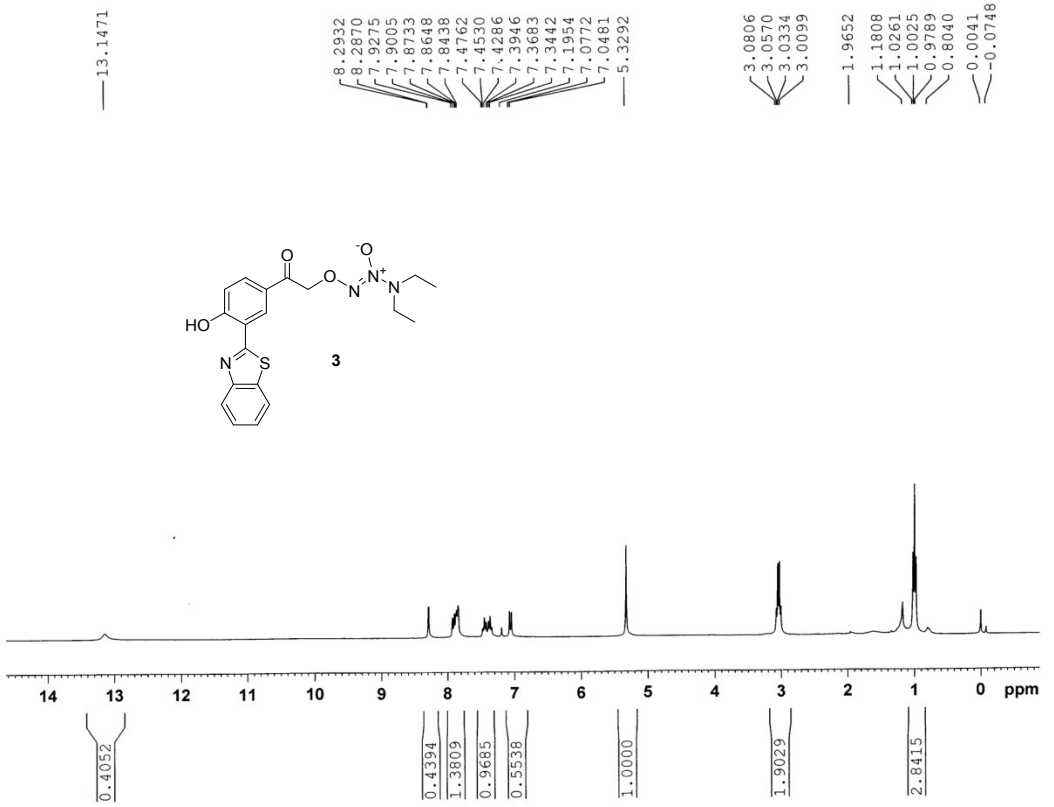

192.242
163.096
130.885
126.162
115.897
74.558
65.366
51.624
40.844
40.566
40.285
40.008
39.727
39.455
39.189
HO
2
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

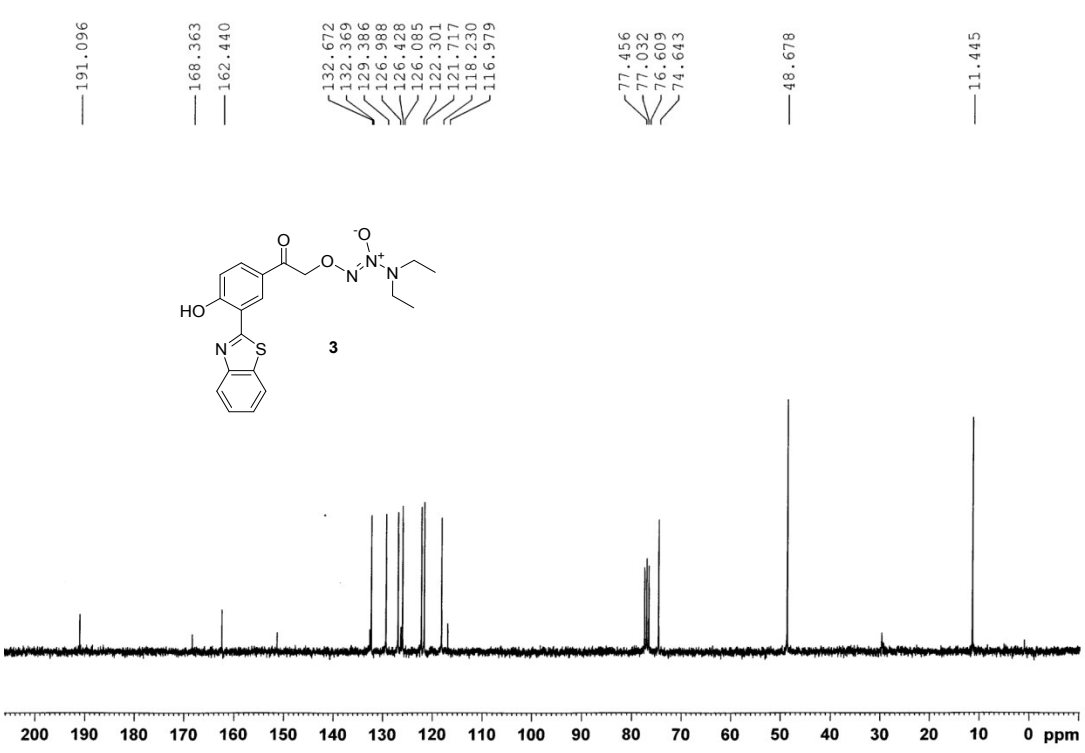

11.4550
9.9513
8.2812
8.2742
8.1231
8.1159
8.0938
8.0866
7.2535
7.0677
7.0383
5.3399
3.1342
3.1106
3.0869
3.0633
1.2349
1.0550
1.0314
1.0077
0.0512
OHC
HO
11
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.4675
0.5225
0.5512
0.5310
0.5349
1.0000
1.9829
2.9777


196.275
190.943
165.621
136.371
134.971
126.920
120.146
118.419
74.659
48.544
11.374
OHC
HO
11
200
180
160
140
120
100
80
60
40
20
0
ppm

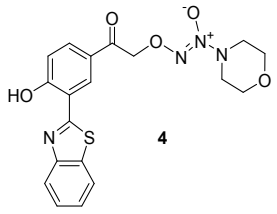

11.4261
9.9060
8.2468
8.0901
8.0660
7.0281
6.9989
5.2405
3.7338
3.7189
3.3451
3.3305
OHC
HO
O
O
N
N
O
12
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.4766
0.4829
0.5139
0.5322
0.4908
1.0000
2.0627
1.9863


196.292
191.055
165.734
136.476
135.122
126.842
120.170
118.501
74.849
65.547
51.459
OHC
HO
O
O
N
N
O
12
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

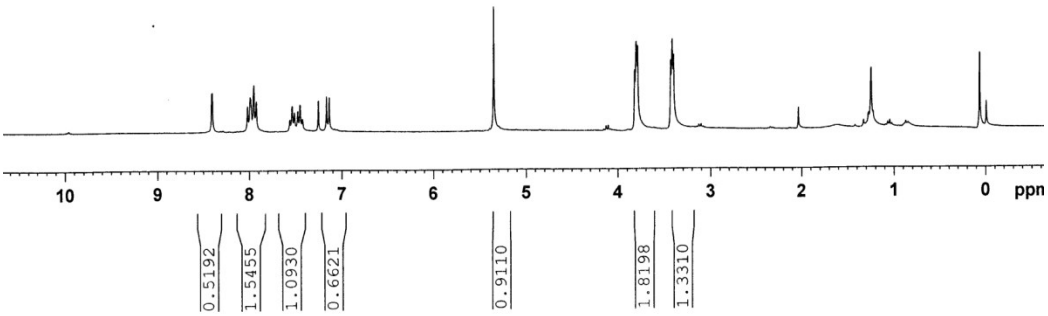

13.1471
8.2932
8.2870
7.9275
7.9005
7.8733
7.8648
7.8438
7.4762
7.4530
7.4286
7.3946
7.3683
7.3442
7.1954
7.0772
7.0481
5.3292
3.0806
3.0570
3.0334
3.0099
1.9652
1.1808
1.0261
1.0025
0.9789
0.8040
0.0041
-0.0748
HO
N
S
O
N
3
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.4052
0.4394
1.3809
0.9685
0.5538
1.0000
1.9029
2.8415

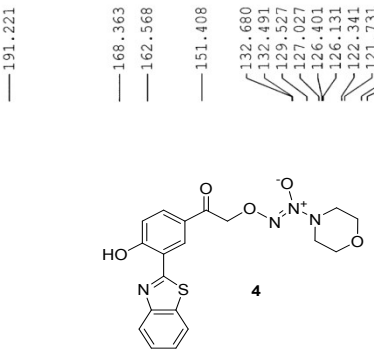

191.096
168.363
162.440
132.672
132.369
129.386
126.988
126.428
126.085
122.301
121.717
118.230
116.979
77.456
77.032
76.609
74.643
48.678
11.445
HO
N
S
O
N
3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


8.4093
8.4030
8.0208
7.9934
7.9872
7.9802
7.9515
7.9273
7.5637
7.5399
7.5158
7.4786
7.4548
7.4310
7.2601
7.1709
7.1418
5.3553
3.8201
3.8047
3.7886
3.4348
3.4187
3.4035
2.0371
1.3321
1.2775
1.2528
1.2301
1.0501
0.0704
-0.0029


HO
N
S
O
O
N
N
O
O

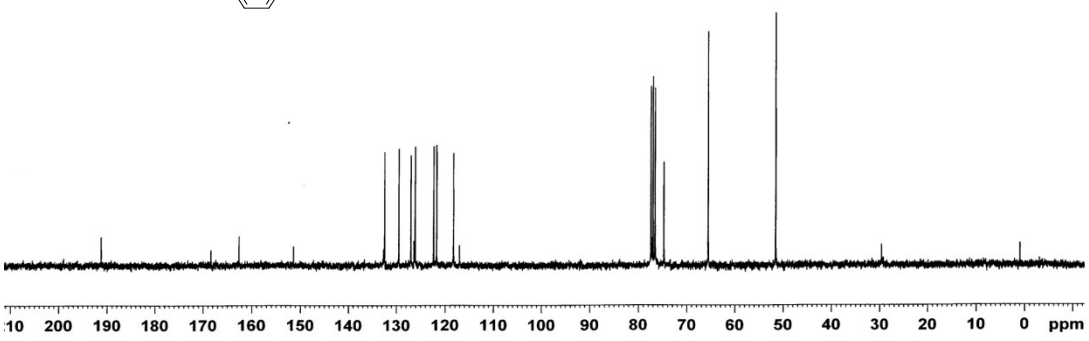
4


10
9
8
7
6
5
4
3
2
1
0
ppm
0.5192
1.5455
1.0930
0.6621
0.9110
1.8198
1.3310

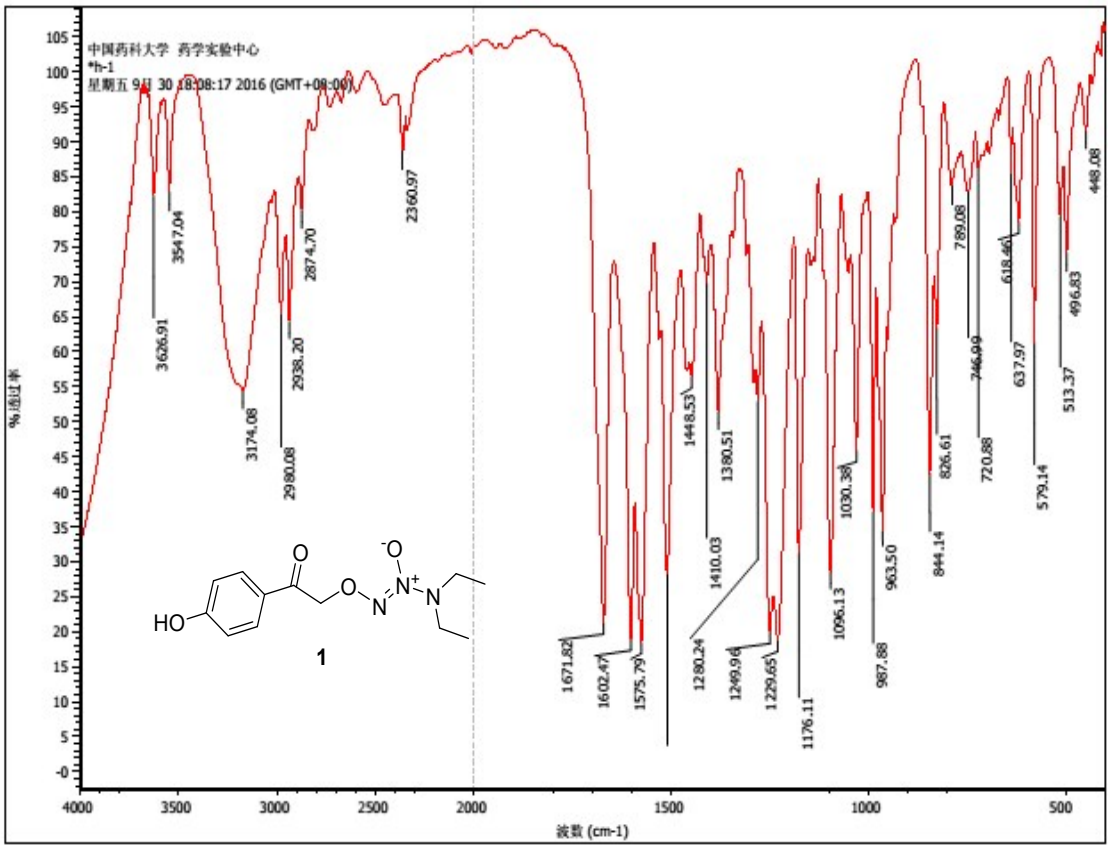

191.221
168.363
162.568
151.408
132.680
132.491
129.527
127.027
126.401
126.131
122.341
121.731
118.299
117.066
74.789
65.592
51.544

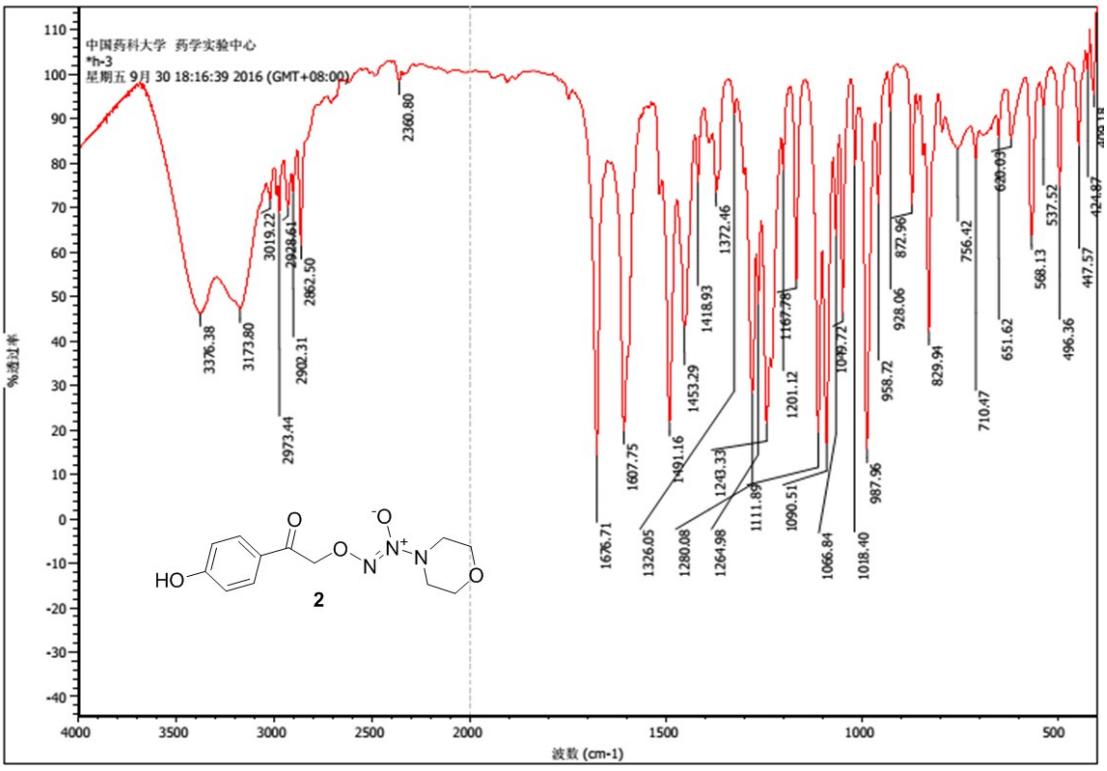
HO
N
S
O
O
N
N
O
O

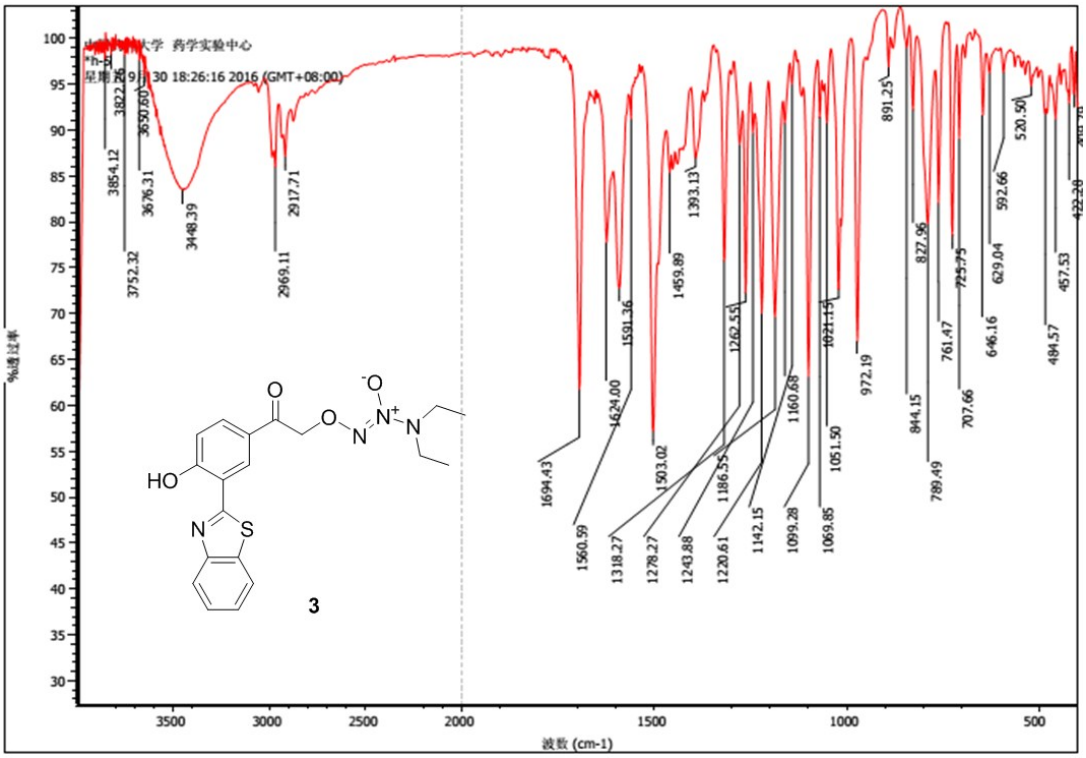
4

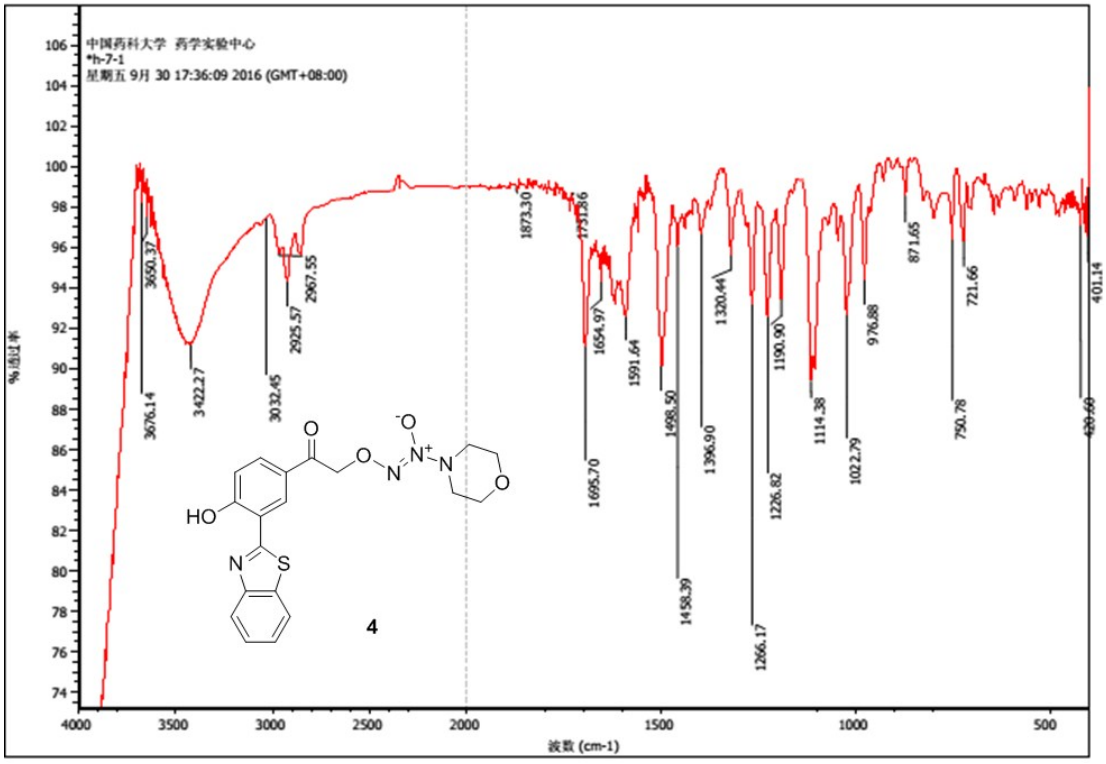

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

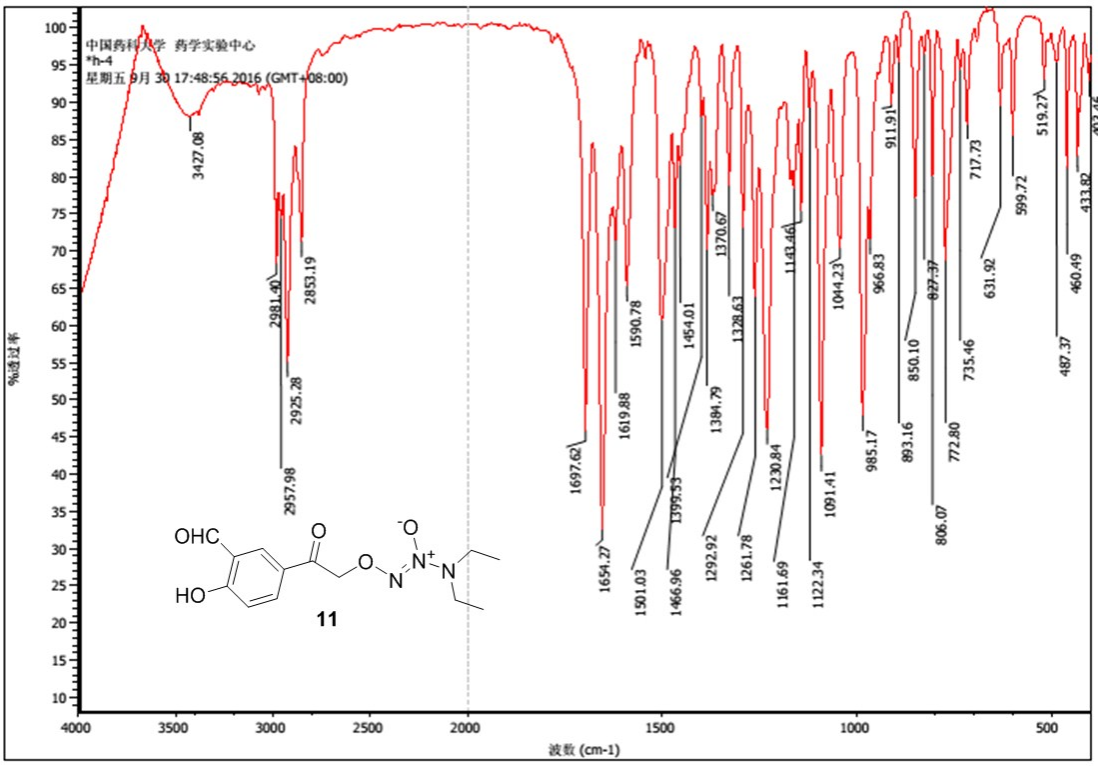
8. FTIR of compounds 1-4,11,12

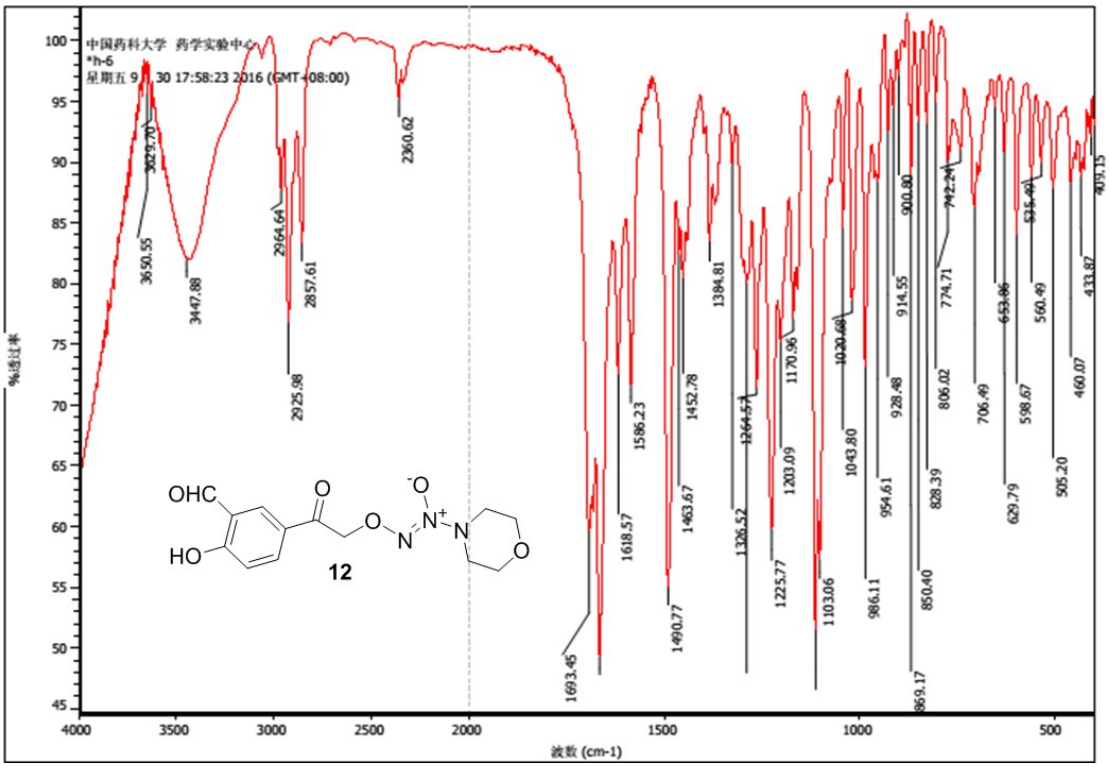




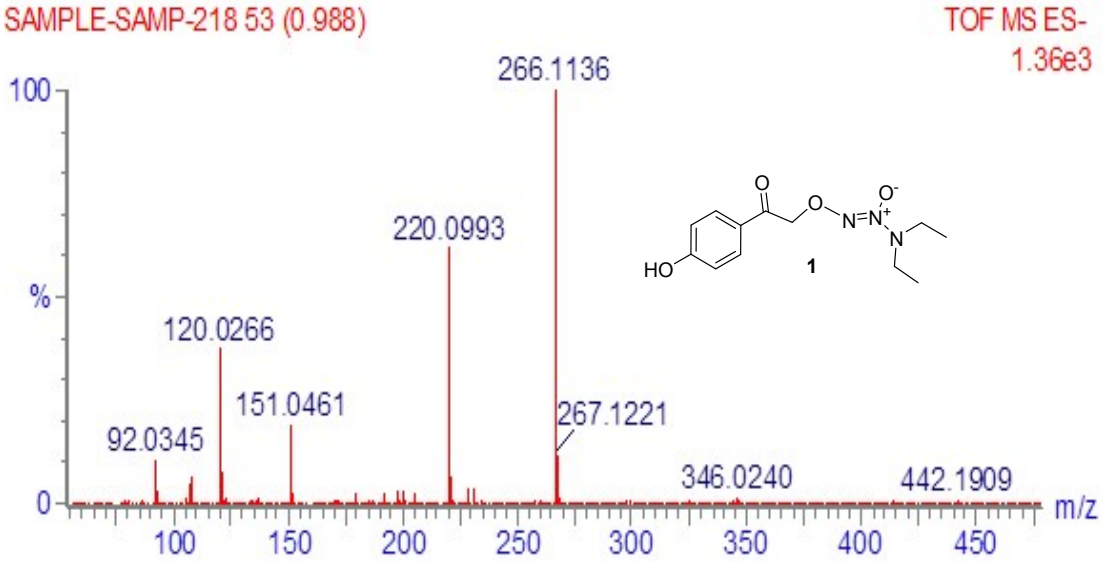

中国药科大学 药学实验中心
星期五 9月 30 18:26:16 2016 (GMT+08:00)
3854.12
3822.76
3650.60
3676.31
3752.32
3448.39
2969.11
2917.71
1694.43
1624.00
1591.36
1560.99
1503.02
1459.89
1393.13
1318.27
1278.27
1262.55
1243.88
1220.61
1186.55
1160.68
1142.15
1099.28
1069.85
1051.50
1021.15
972.19
891.25
844.15
827.96
789.49
761.47
725.75
707.66
646.16
629.04
592.66
520.50
484.57
457.53
422.30
HO
N
S
O
N
O
N
N
3
%透过率
波数 (cm-1)

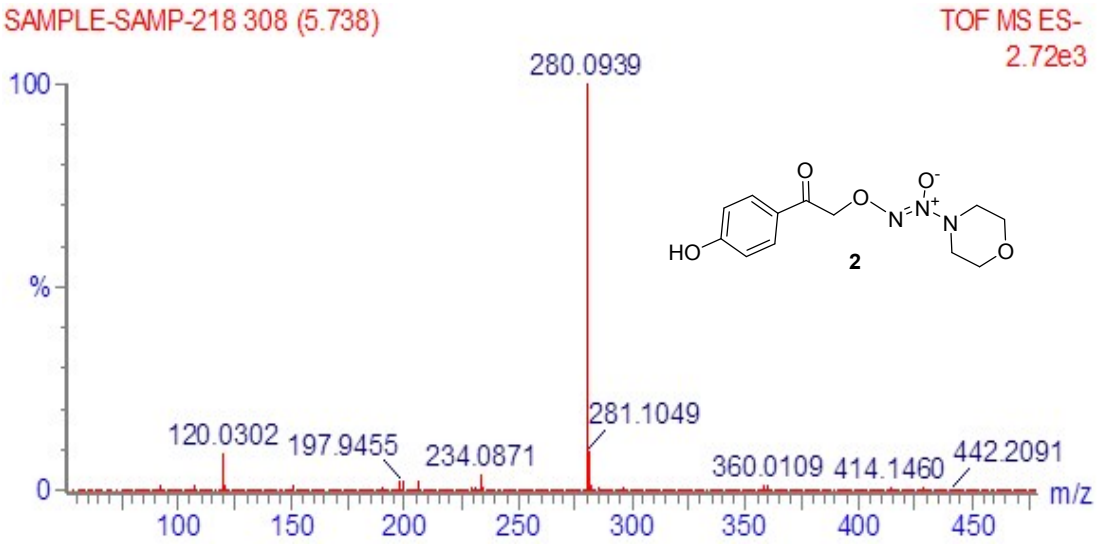

中国药科大学 药学实验中心
*h-7-1
星期五 9月 30 17:36:09 2016 (GMT+08:00)
3650.37
3676.14
3422.27
3032.45
2925.57
2967.55
1873.30
1751.86
1695.70
1654.97
1591.64
1498.50
1458.39
1396.90
1320.44
1266.17
1226.82
1190.90
1114.38
1022.79
976.88
871.65
750.78
721.66
420.69
401.14
HO
N
S
O
N
O
N
N
O
4
%透过率
波数 (cm-1)

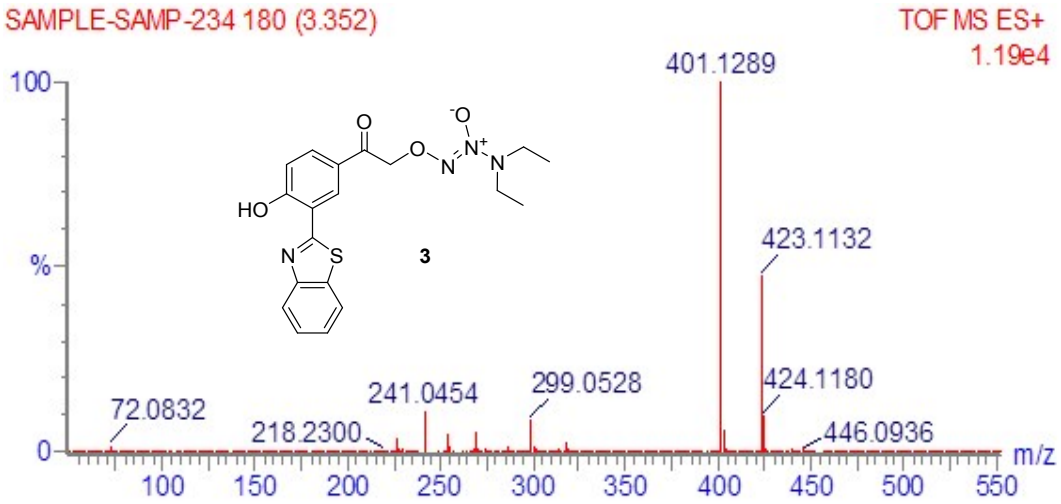

中国药科大学 药学实验中心
*h-4
星期五 9月 30 17:48:56 2016 (GMT+08:00)
%透过率
波数 (cm-1)
3427.08
2981.40
2925.28
2853.19
1697.62
1654.27
1619.88
1590.78
1501.03
1466.96
1454.01
1399.53
1384.79
1370.67
1328.63
1292.92
1261.78
1230.84
1161.69
1143.46
1122.34
1091.41
1044.23
985.17
966.83
911.91
893.16
850.10
827.37
806.07
772.80
735.46
717.73
631.92
599.72
519.27
487.37
460.49
433.82
OHC
HO
O
N
N+
-O
11

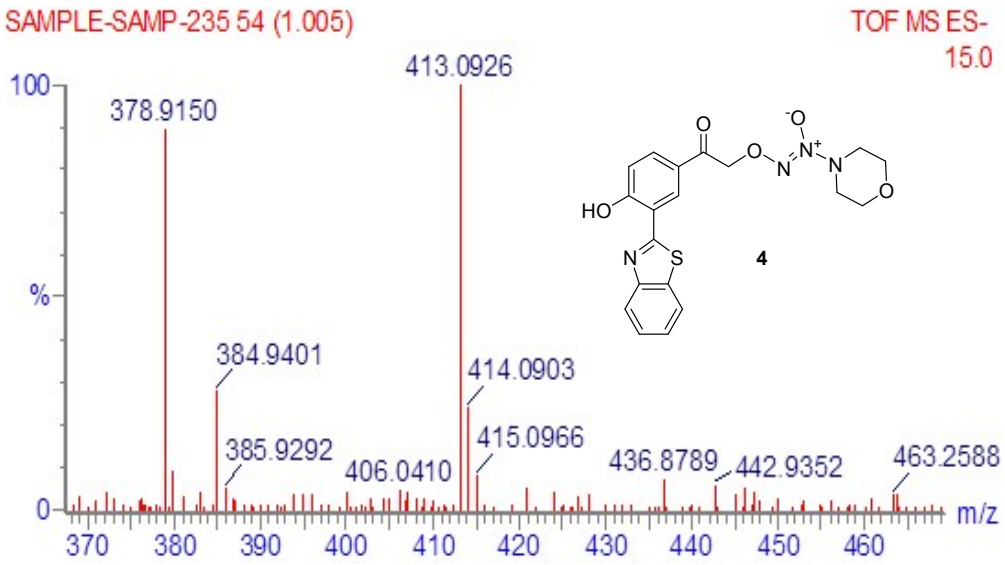

中国药科大学 药学实验中心
*h-6
%透过率
波数 (cm-1)
3650.55
3629.70
3447.88
2964.64
2925.88
2857.61
2360.62
1693.45
1618.57
1586.23
1490.77
1463.67
1452.78
1384.81
1326.52
1264.57
1225.77
1203.09
1170.96
1103.06
1043.80
1020.68
986.11
954.61
928.48
914.55
900.80
869.17
850.40
828.39
806.02
774.71
742.24
706.49
653.86
629.79
598.67
560.49
535.40
505.20
460.07
433.87
409.15
OHC
HO
O
N
N+
-O
12

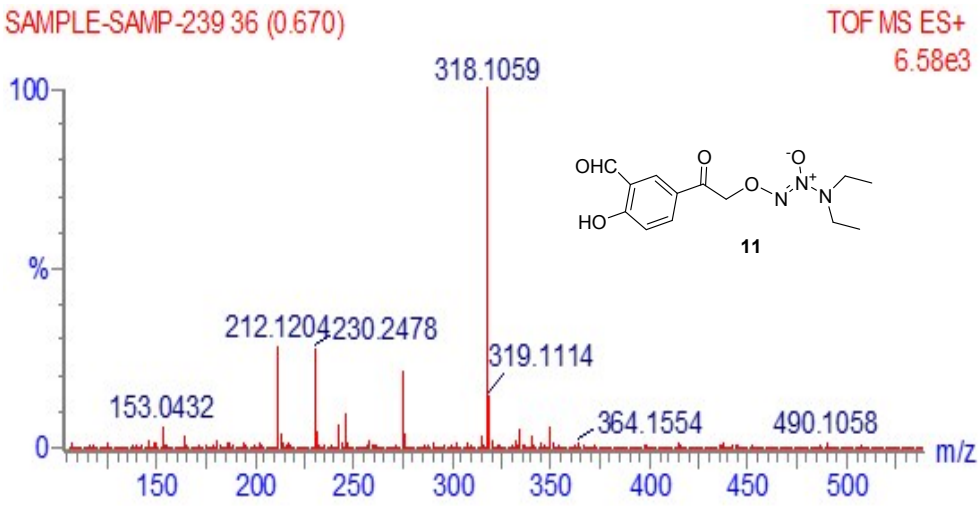
10. HRMS of compounds 1-4, 11-12

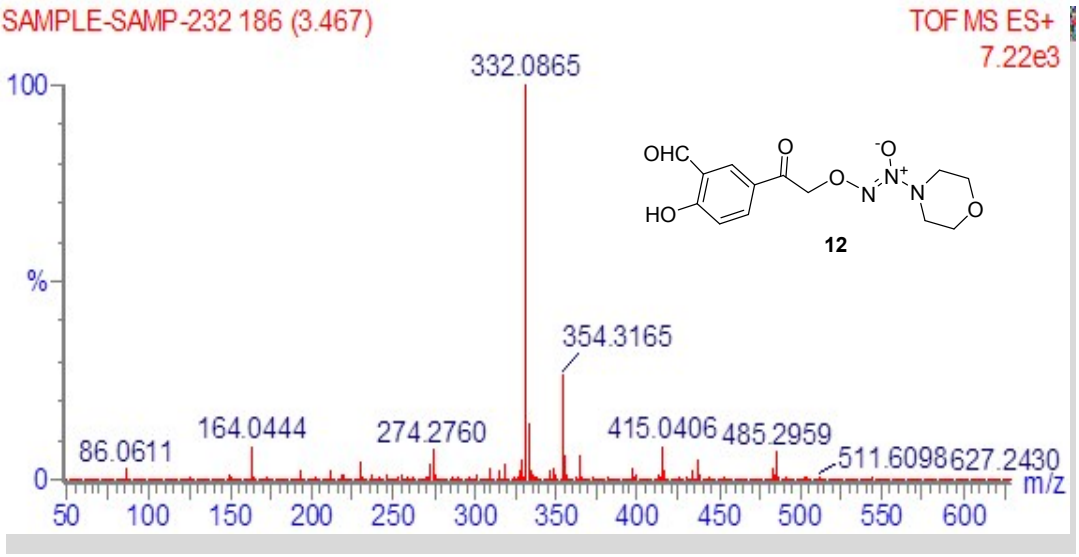



SAMPLE-SAMP-218 308 (5.738)
TOF MS ES-
2.72e3
280.0939
100
%
0
120.0302
197.9455
234.0871
281.1049
360.0109
414.1460
442.2091
m/z
100
150
200
250
300
350
400
450
HO
O
O
N
N
O
O
2


SAMPLE-SAMP-234 180 (3.352)
TOF MS ES+
1.19e4
401.1289
423.1132
424.1180
446.0936
299.0528
241.0454
218.2300
72.0832
100
%
0
m/z
100
150
200
250
300
350
400
450
500
550
HO
O
O
N
N+
-O
N
S
3


SAMPLE-SAMP-235 54 (1.005)
TOF MS ES-
15.0
100
%
0
378.9150
413.0926
384.9401
414.0903
385.9292
415.0966
406.0410
436.8789
442.9352
463.2588
370
380
390
400
410
420
430
440
450
460
m/z
HO
O
O
N
N+
O-
N
S
O
4


SAMPLE-SAMP-239 36 (0.670)
TOF MS ES+
6.58e3
318.1059
100
%
0
212.1204
230.2478
319.1114
153.0432
364.1554
490.1058
m/z
150
200
250
300
350
400
450
500
OHC
HO
O
O
N
N+
N
11


SAMPLE-SAMP-232 186 (3.467)
TOF MS ES+
7.22e3
332.0865
100
%
0
354.3165
164.0444
274.2760
86.0611
415.0406
485.2959
511.6098
627.2430
m/z
50
100
150
200
250
300
350
400
450
500
550
600
OHC
HO
O
O
N
N+
O-
N
O
12

11. HPLC assessment of compound purity

All tested compounds **1 - 4**with a purity of > 97% were used for subsequent biological assays. We provided the spectra of HPLC assays as below.

Column: Venusil MP C18（ 250 mm × 4.6 mm × 5 um）;

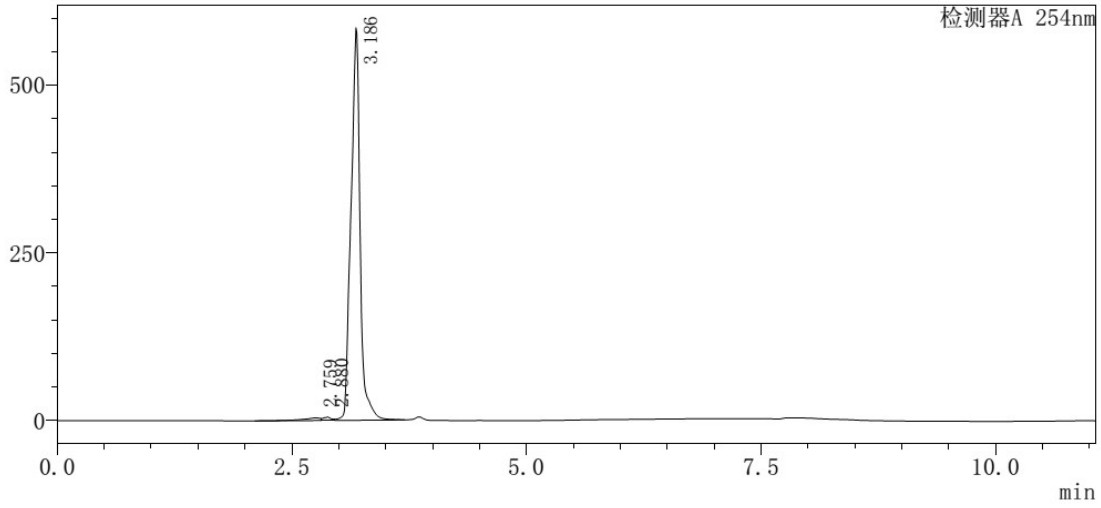
Mobile phase: The compound **1-2**: Methanol-Water (50 to 50, v/v); **3-4**: Acetonitrile-Water (65 to 35, v/v).

Wavelength: 254 nm;

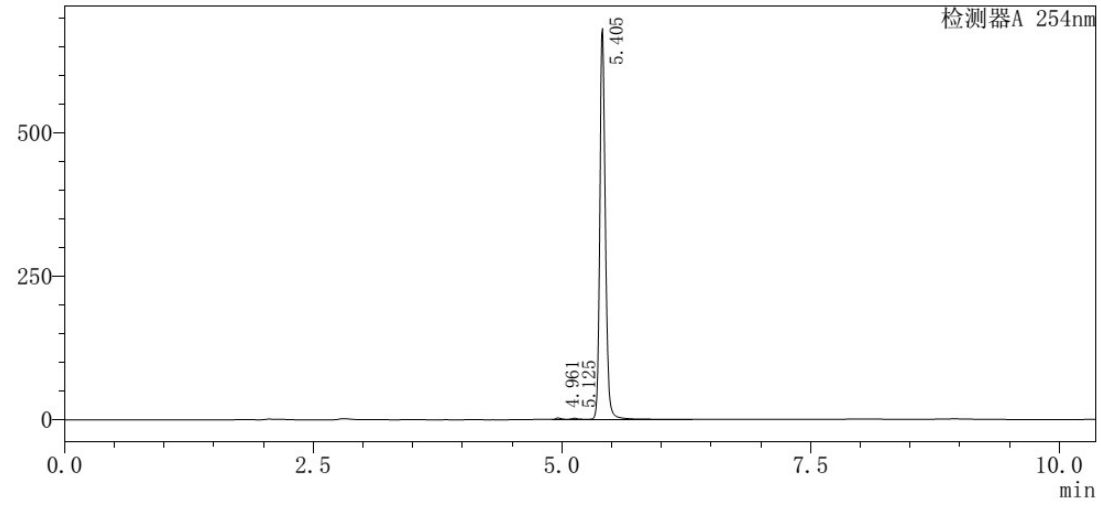
Rate: 1 mL/min;

Temperature: 25 °C;

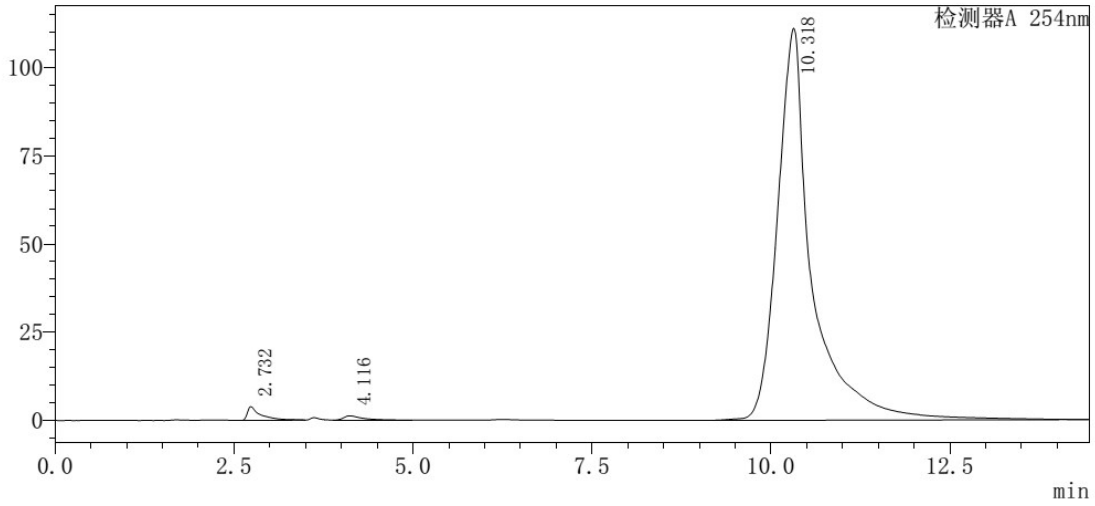
1, 97.83%



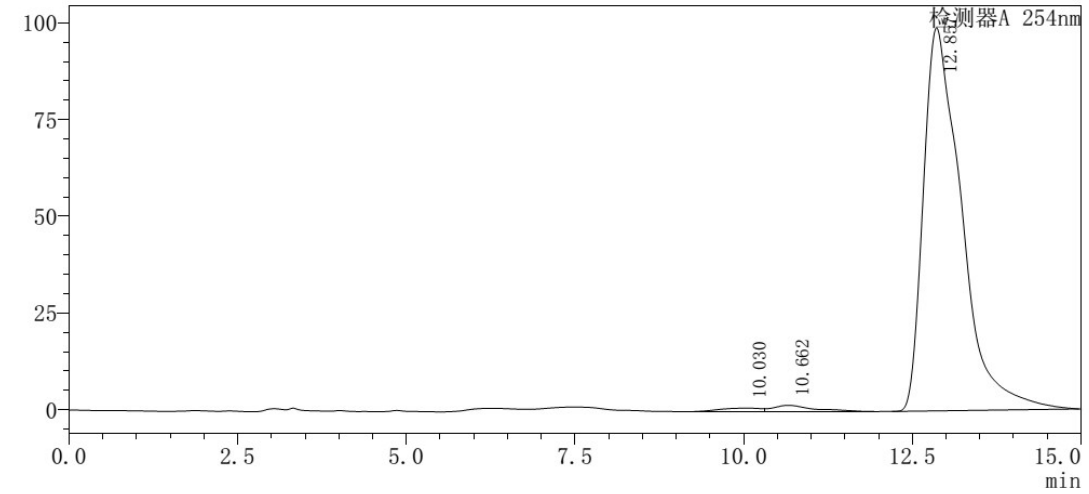
2, 99.31%



3, 98.06%



4, 97.25%



12. Reference

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