Supplementary Information

For

Fluorescent 'turn-on' Chemodosimeter Probe for Nitrosyl (HNO)

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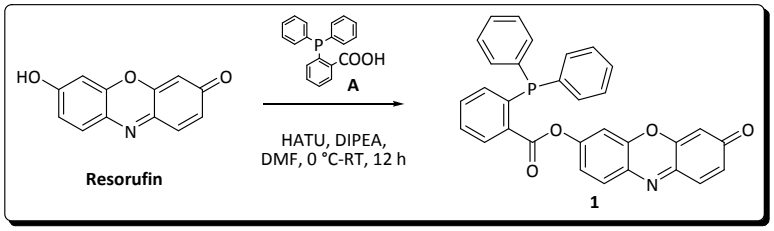
Materials, methods and instrumentations

Resorufin (TCI), 2-(Diphenylphosphino) benzoic acid (Alfa-aesar), HATU (Alfa-aesar), DIPEA (Alfa- aesar), DMF (Aldrich), Diethyl ether (Loba chem., India), Angeli's salt ($Na_2N_2O_3$, Cayman Chemical) and DCM (Loba chem. India) were purchased commercially and used without further purification. Analytical thin layer chromatography was performed using silica gel 60 (pre coated sheets with 0.25 mm thickness). Mass spectra were recorded on anion SpecHiRes ESI mass spectrometer. NMR spectra were collected on a 400 MHz spectrometer (Bruker, Germany).

Reaction Scheme:



Synthesis of probe 1

To a solution of 2-(diphenylphosphino) benzoic acid (287 mg, 0.938 mmol) in DMF (10 mL) at 0℃ DIPEA (0.19 mL, 1.05 mmol), resorufin (200 mg, 0.938 mmol) and HATU (375 mg, 0.985 mmol) were added. The reaction was continued to stir for 12 h at RT. The reaction was monitored by TLC. After completion of reaction, reaction mixture was diluted with water and stirred for 15 min. The precipitated solid was filtered, washed with water and dried in vacuo. The crude product was purified by column chromatography on neutral alumina using ethyl acetate in hexane (3:7) as eluent to obtain probe, **1** as pale brown solid (260 mg; 55.31 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.24 (q, 1H); 7.73 (d, 1H); 7.50 (t, 2H); 7.45 (d, 2H); 7.36-7.29 (m, 9H); 7.03-6.98 (m, 3H); 6.87-6.84 (dd, 1H); 6.32 (d, 2H).^{13}C-NMR (100 MHz, DMSO-d_6): 186.21, 164.62, 153.29, 149.89, 148.70, 144.42, 137.45, 137.36, 135.60, 135.19, 134.30, 134.11, 133.94, 133.76, 131.88, 131.58, 131.29, 129.60, 129.45,129.35, 129.29, 119.90, 110.31, 106.53. ESI-HRMS *m/z* (M+H): calcd. 501.110, found 502.125

Absorption and fluorescence studies

All fluorescence and UV-Visible spectra were obtained with F-4500 FL spectrometer with a 1 cm standard quartz cell and UV-1800 spectrophotometer, respectively. Angeli's salt ($Na_2N_2O_3$) was used as the source of HNO, because it decomposes rapidly (t1/2 = 3 min) in aqueous solutions at pH = 7 to produce HNO and $NaNO_2$.[1] Stock solutions (1 mM) of various analytes (HNO, H_2O_2, $HO^{\cdot}$, GSH, NO, NO^-, NO_2^-, GSNO, AA, NaOCl, $FeCl_3$) were prepared in deionized water. The stock solution of probe, 1 was prepared in PBS buffer (pH = 7.4) with 20% DMSO. Excitation was carried out at 530 nm with excitation and emission slit widths is 3 nm each. The Fluorescence experiments (solution test) of probe, 1 (5.0 μM) recorded in the presence of increasing concentrations of HNO (0–25 eq.) in PBS buffer (pH = 7.4) with 20% DMSO. Probe, 1 was incubated with HNO for 30 min at 37 °C. The quantum yield of the probe **1** in presence and absence of HNO were measured in sodium phosphate buffer (pH 5.0 or 7.4). For determination of each Φ_F fluorescein in 0.1 N NaOH (Φ_F = 0.79) was used as a fluorescence standard.

Culture of CHO cells and Fluorescent Imaging

CHO was cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5%CO_2 and 95% air at 37 °C. The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 ^oC under 5%CO_2. Then the cells were incubated with 75.0 μM, 125 μM of AS an atmosphere of 5% CO_2 and 95% air for 2 h at 37 °C, respectively. Then the cells were incubated with 5.0 μM probe, **1** in PBS for 1h .Washed the cells twice with 1mL deionized water at room temperature, Cells were imaged using an Olympus BX51 inverted fluorescence microscopy.

Culture of *C. elegans* and Fluorescent Imaging

The *C. elegans* wild type strain N_2 was acquired from the Key Biological Laboratory Center (Yunnan University). The larval stage 4 (L4) *C. elegans* was used. The L4 stage nematodes were washed three times with deionized water by centrifugation at 2500 rpm for 2 minutes. For imaging of accumulations of HNO (AS) in the nematode, the previously exposed worms were incubated in centrifuge tube filled with 2 mL of deionized water, containing 5 μM (in PBS) of

probe **1** at 20 °C for 1 h. Again the nematodes were washed three times with deionized water by centrifuging at 2500 rpm for 2 minutes before being mounted onto a slide glass.

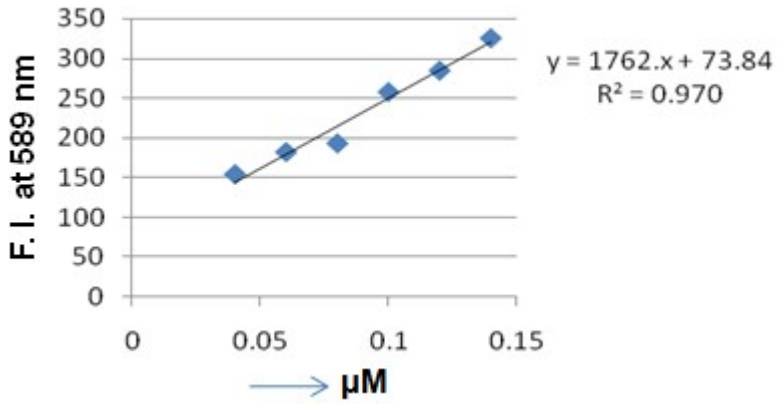


Fig.S1 Fluorescence intensity of the probe, **1** (**5** μM) normalized for the minimum fluorescence intensity in the absence or presence of HNO. Excitation was effected at 565 nm and emission at 590 nm with the excitation and emission slit widths both set at 3 nm.

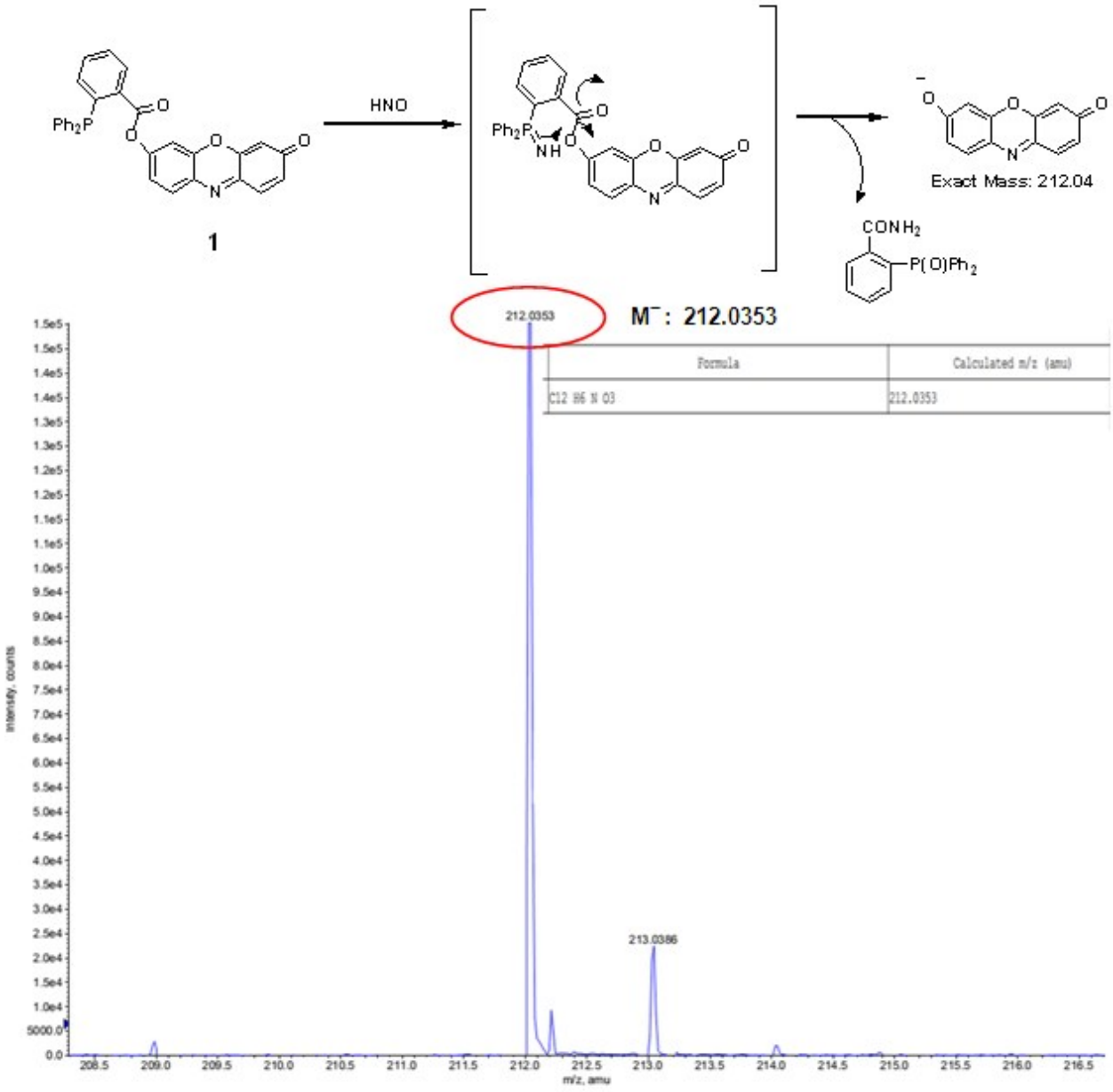


Fig.S2 The HRMS spectra was recorded after 5 mg of probe, **1** in 5ml DMSO-PBS buffer (V/V=1:4) in HNO atmosphere for 1h. HRMS (ESI): calcd for $C_{12}H_6NO_3$=212.0353, found *m/z* 212.0353.

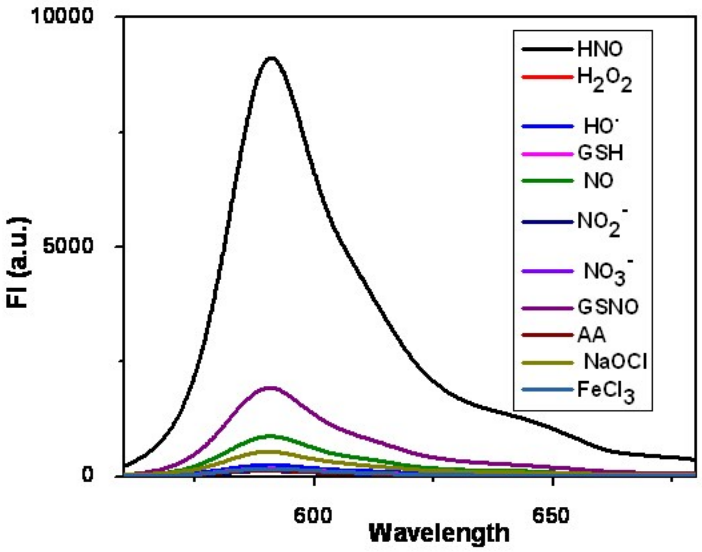


Fig.S3. Fluorescence responses of probe, 1 (5 μM) in presence of various analytes in aqueous buffer solutions (in PBS, 20% DMSO) at 37 °C. Excitation was effected at 565 nm with the excitation and emission slit widths both set at 3 nm.

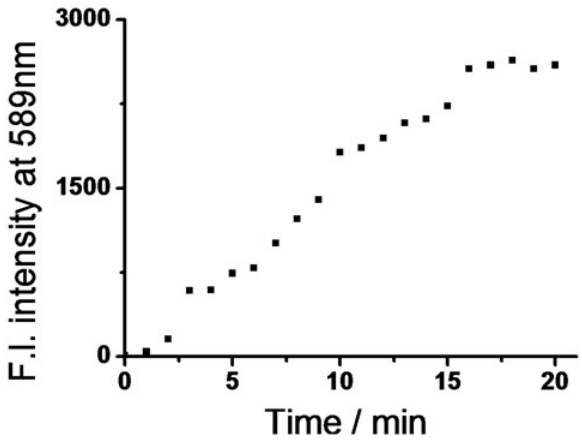


Fig.S4 Time-trace plots of HNO monitored by the emission of 589 nm. Probe, **1** (5 μM) in PBS buffer with 20% DMSO in HNO (100 μM) atmosphere, excitation at 565 nm with the excitation and emission slit widths both set at 1.5 nm.

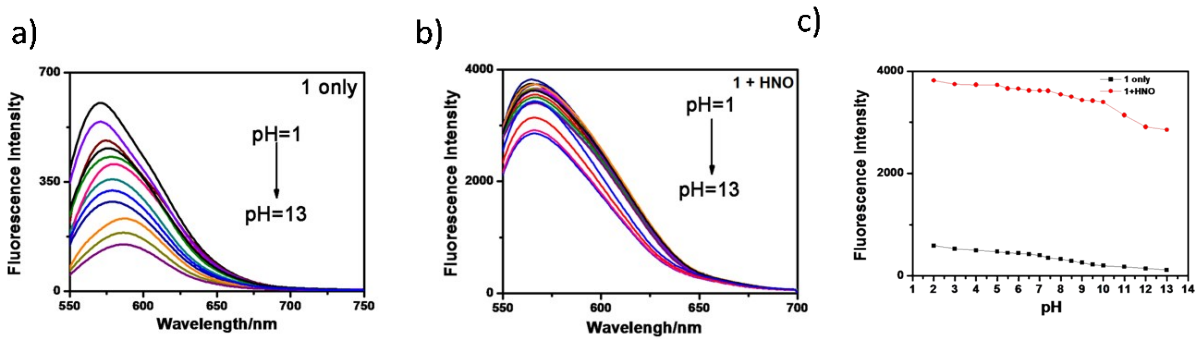


Fig.S5. Changes of fluorescence intensity of probe, **1** (05 μM) in variable pH (1-13) (Φ_F = 0.012) in absence (a) and in presence (b) of HNO (50 μM); c) Pattern of fluorescence intensity of probe **1** in presence and absence of HNO. Excitation at 565 nm, emission at 595 nm in PBS buffer (10 mM) with 1 % DMSO. (Slit widths: ex 1.5 / em 1.5)

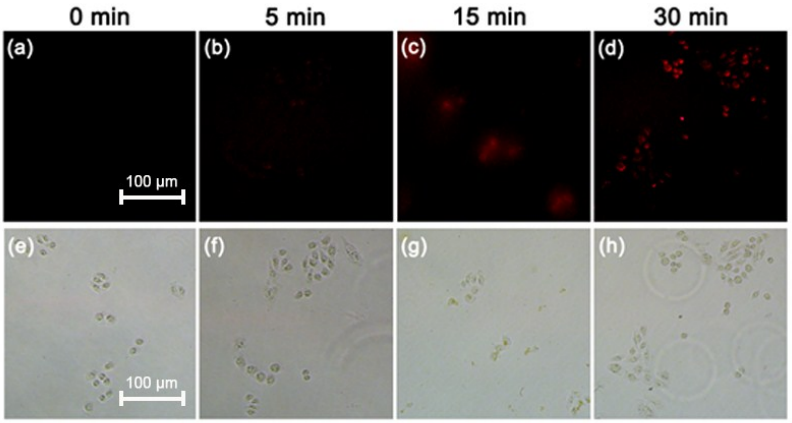


Fig.S6 Time tracking of fluorescent imaging (top) and phase contrast (bottom) for HNO (125 μM) in CHO cells. (a) 0 min; (b) 5 min; (c) 15 min; (d) 30 min. All images share the same scale bar (100 μM). Cells were using excitation wave length of 540 nm and emission length of 570-630 nm.

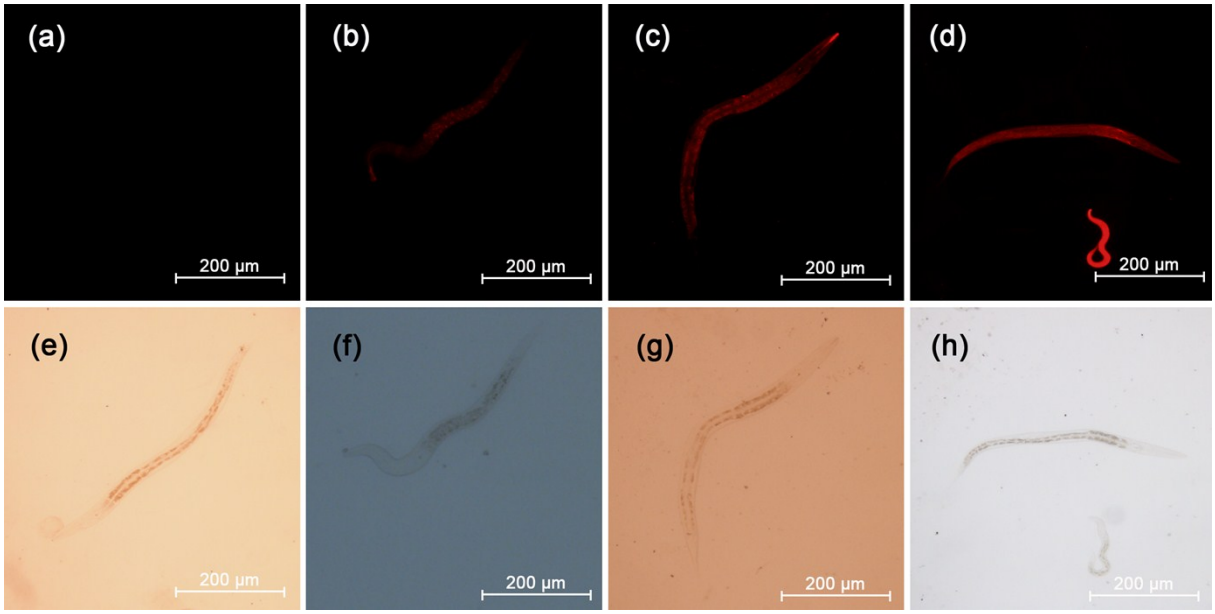


Fig.S7 Fluorescent imaging (top) and phase contrast (bottom) for HNO in *C. elegans*. (a) Probe, **1** (5 μM in PBS), HNO (AS) (300 μM), 0min; (b) Probe **1** (5 μM), HNO (AS) (300 μM), 30min; (c) Probe, **1** (5 μM), HNO (As) (300 μM), 60 min; (d) Probe **1** (5 μM), HNO (300 μM), 90min. *C. elegans* images were obtained using an Olympus BX51 inverted fluorescence microscopy. All images share the same scale bar (200 μM). Cells were using excitation wave length of 540 nm and emission length of 570-630 nm.

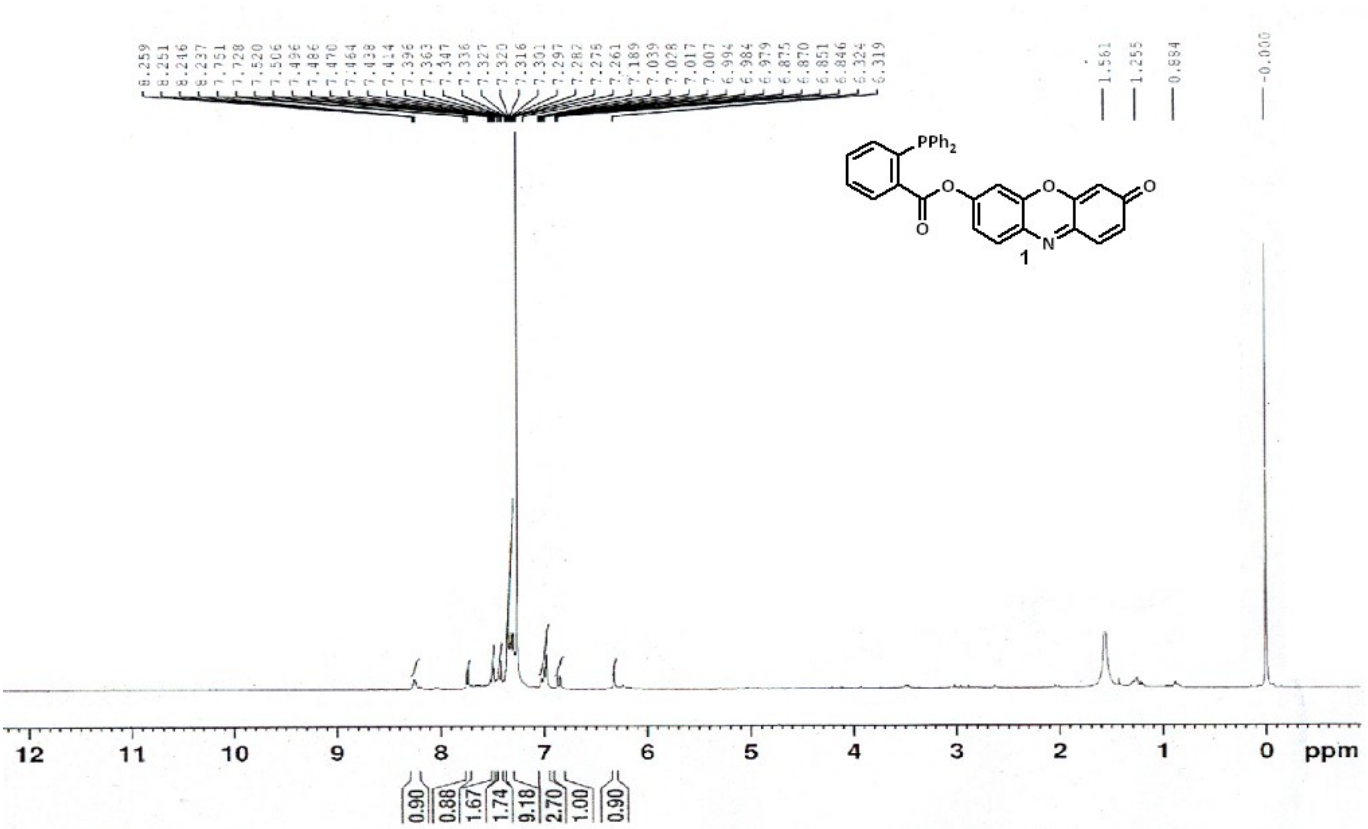


Fig.S8 ^{1}H-NMR of **1** in $CDCl_3$

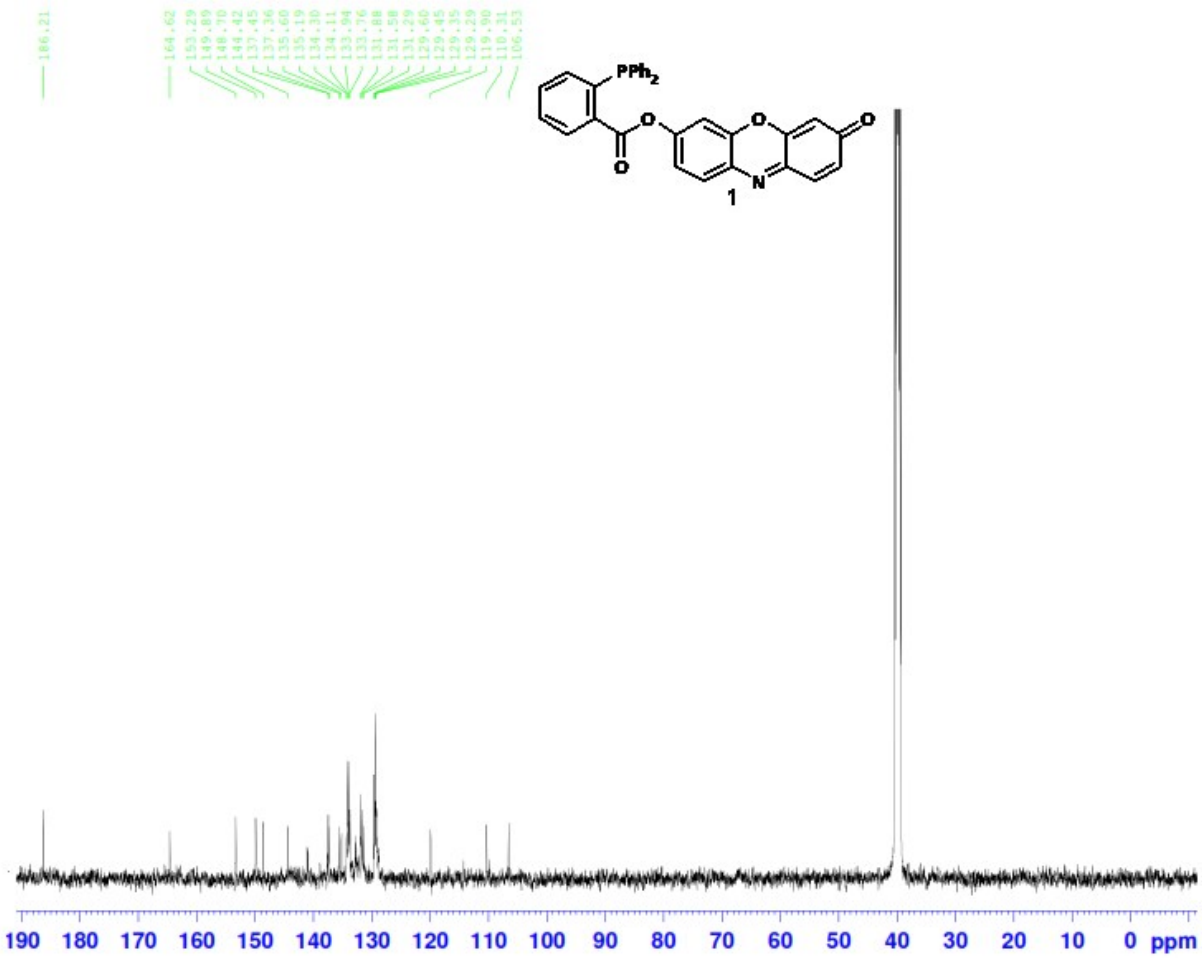


Fig. S9 ^{13}C-NMR of **1** in DMSO-d_6

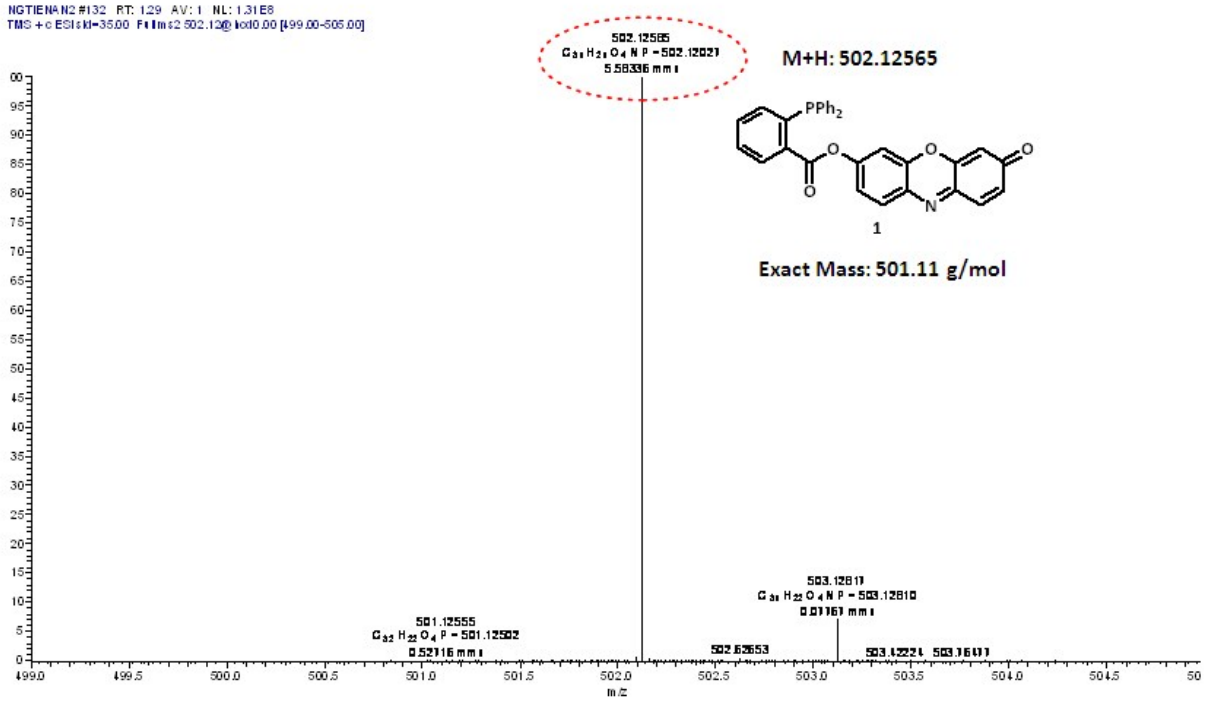
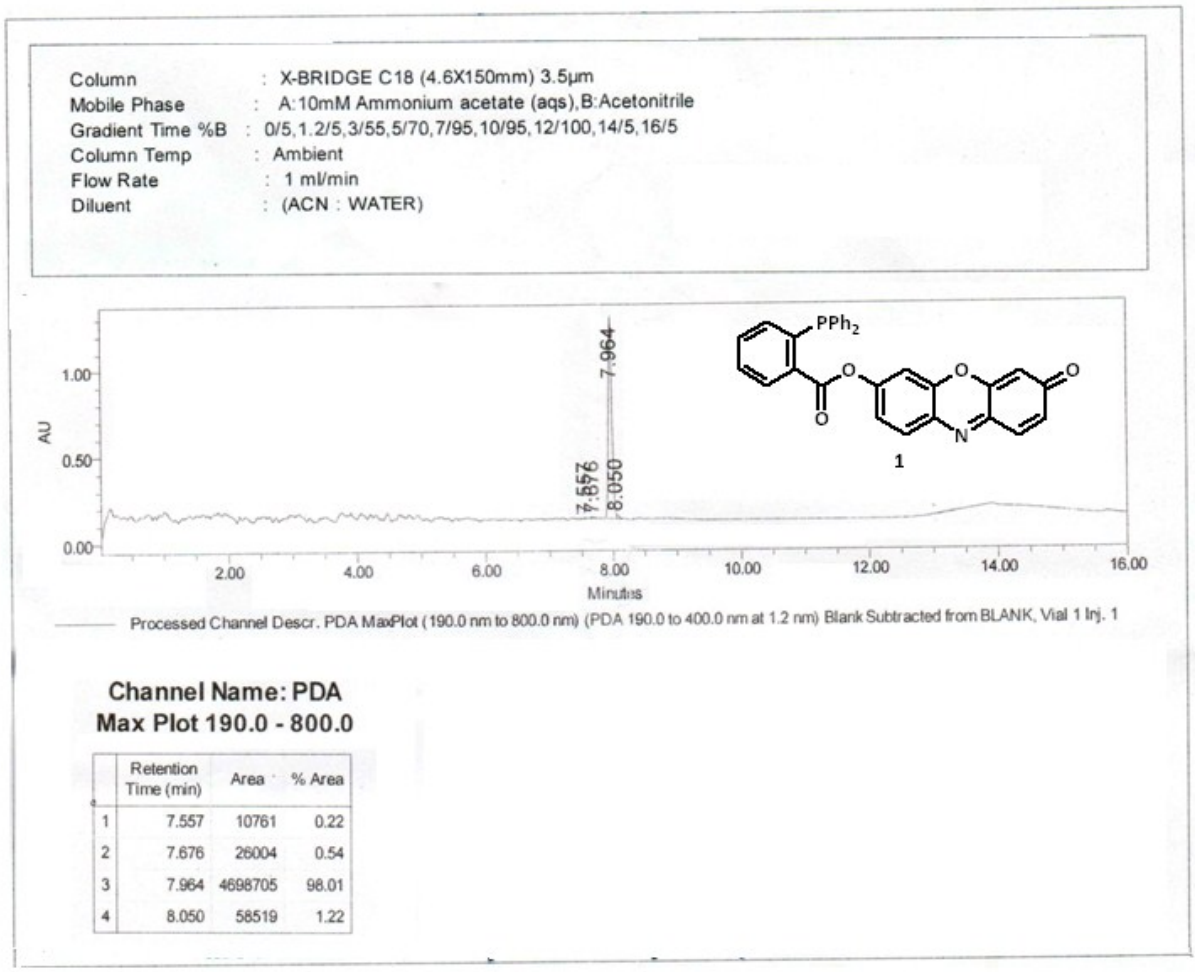


Fig.S10 HRMS of **1**



	Retention Time (min)	Area	% Area
1	7.557	10761	0.22
2	7.676	26004	0.54
3	7.964	4698705	98.01
4	8.050	58519	1.22

Fig.S11 HPLC of **1**

References

1. (*a*) K. M. Miranda, H. T. Nagasawa and J. P. Toscano, *Curr.Top. Med. Chem*., 2005, **5**, 649−664; (*b*) A. S. Dutton, J. M. Fukuto and K. N. Houk, *J. Am. Chem. Soc*., 2004, **126**, 3795–3800.