Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Direct fluorescence labelling of NO inside the plant cells

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1. Table S1. Comparison table

Analytes	Sensor type	Detection method	Application	References
NO	2-hydroxynapthaldehyde	**Naked-eye, UV-Vis, Fluorescence**	**Detection of NO in endogenous plant cell**	**Present manuscript**
NO	1,8-naphthalimide	UV-Vis	fluorescence imaging of NO in HT29 cells (Human colon adenocarcinoma grade II cell line)	Dyes and Pigments 2013, 96, 333-337
NO	Pyrene based	UV-Vis, Fluorescence	Detection of TNP by the test strip method under day light and UV lamp	ACS Omega 2018, 3, 8, 10306–10316
NO, Histidine	Rhodamine-based copper complex	Naked-eye, UV-Vis	fluorescence images of RAW 264.7 macrophages	Chempluschem 2014, 79, 1761-1766
NO	Anilino-benzothiazole	UV-Vis, Fluorescence	Detection in HeLa cells. Fluorescence images were acquired by confocal microscopy.	Sensors and Actuators B: Chemical 2018,259, 347-353.
NO	N-nitrosation-based	UV-Vis, Fluorescence	Fluorescence imaging endogenous NO in living cells of zebrafish	Sensors and Actuators B: Chemical 2021, 329, 129147
NO	Rhodamine based	UV-Vis, Fluorescence	live-cellular imaging	Dyes and Pigments, 2023 215, 111284
NO	1,8-naphthalimide	UV-Vis, Fluorescence	the detection of intracellular nitric oxide concentrations in macrophages and endothelial cells	Journal of Photochemistry and Photobiology B: Biology 2022, 234, 112512
NO	Hemicyanine-based	UV-Vis, on-off Fluorescence	imaging NO in living cells	Dyes and Pigments 2022,197, 109871

2. NMR Studies

^{1}H NMR of NPO in DMSO-d_6

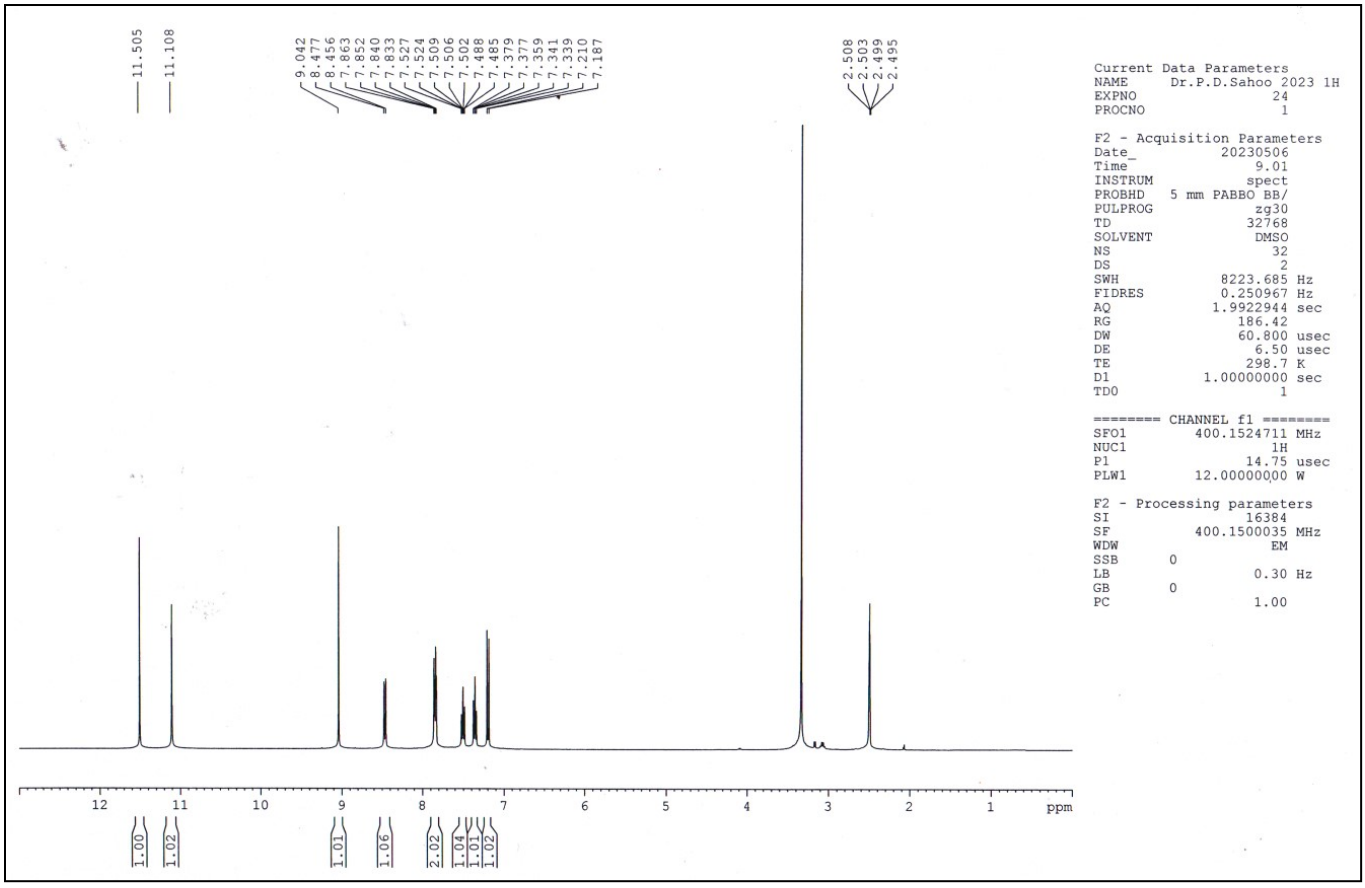

Figure S1. ^{1}H NMR of **NPO** in DMSO-d_6 (400 MHz).

^{13}C NMR of NPO in DMSO-d_6:

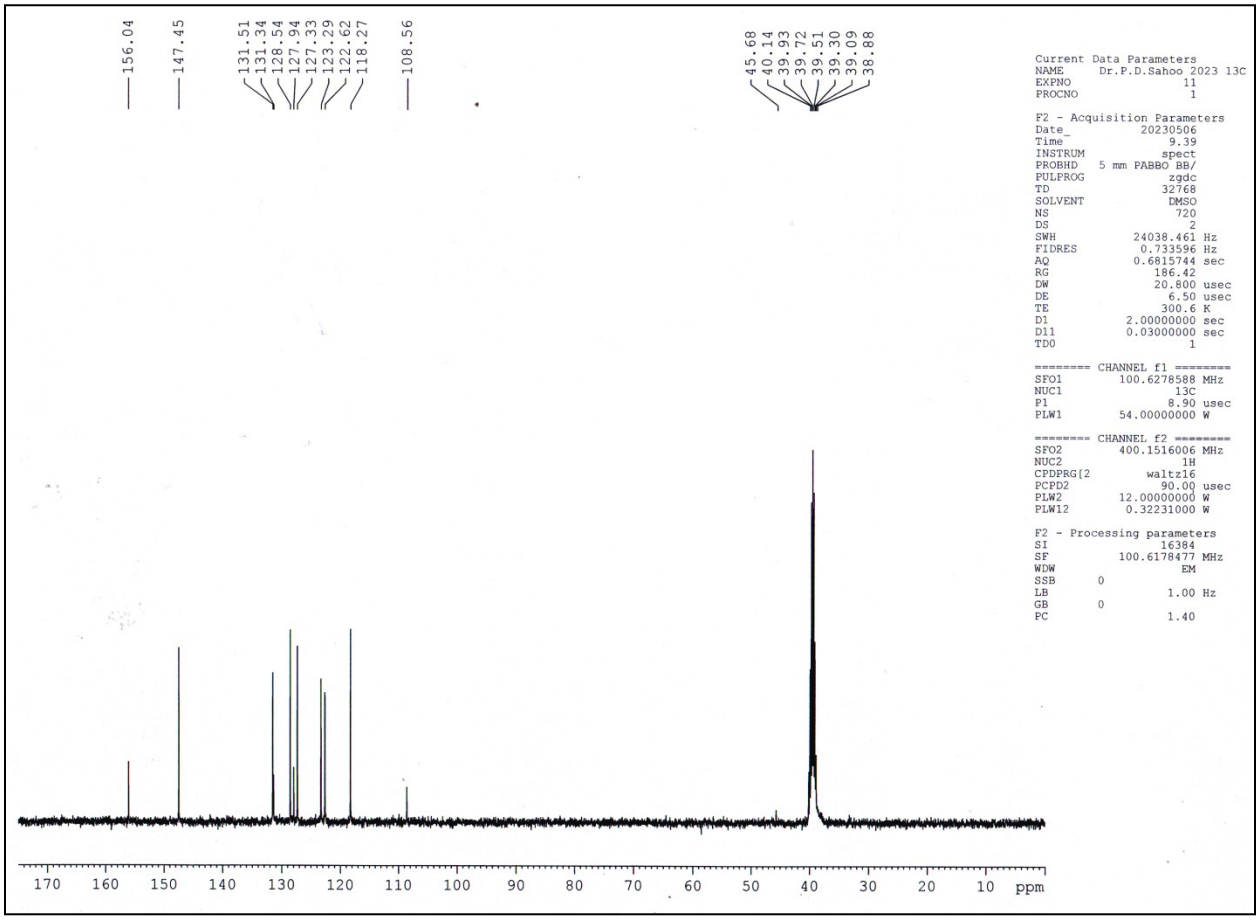

Figure S2. ^{13}C NMR of **NPO** in DMSO-d_6 (100 MHz).

3. UV-Vis and fluorescence titration studies

UV-vis spectral studies:

A stock solution of **NPO** (1×10^{-5} M) was prepared in water-DMSO (4:1, v/v). **NO** solution of concentration 1×10^{-4} M was prepared. All experiments were carried out in aqueous medium at neutral pH. During the titration, each time a 1×10^{-5} M solution of **NPO** was filled in a quartz optical cell of 1 cm optical path length and **NO** stock solution was added into the quartz optical cell gradually by using a micropipette.

Fluorescence spectral studies:

A stock solution of **NPO** (1×10^{-5} M) was prepared in water-DMSO (4:1, v/v). **NO** solution of concentration 1×10^{-4} M was prepared. All experiments were carried out in aqueous medium at neutral pH. During titration, each time a 1×10^{-5} M solution of **NPO** was filled in a quartz optical cell of 1 cm optical path length and **NO** stock solution was added into the quartz optical cell gradually by using a micropipette. For all fluorescence measurements, excitations were provided at 310 nm, and emissions were collected from 350 to 530 nm.

4. Selectivity

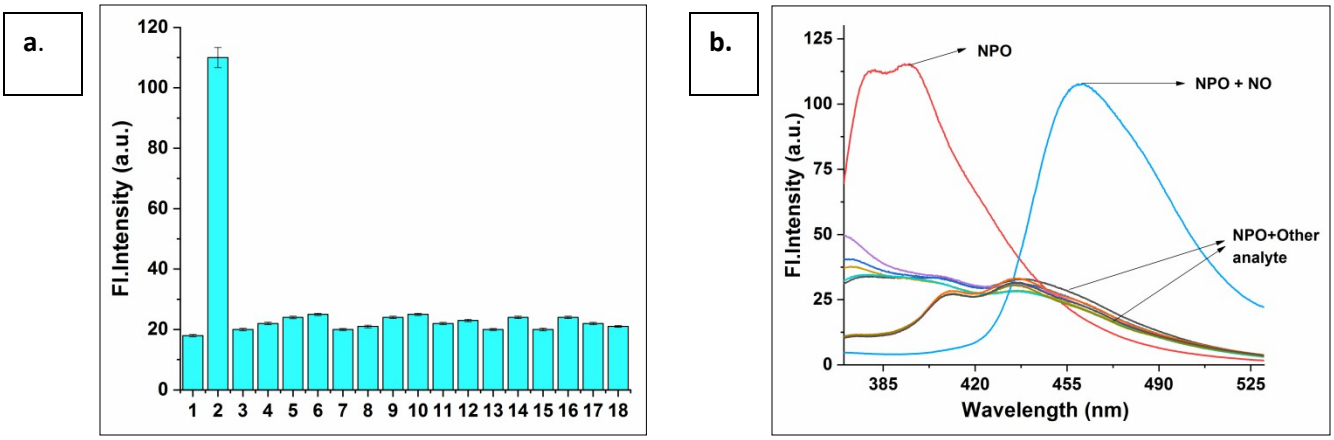

Figure S3. (a) fluorescence spectra of **NPO** in presence of different analytes (4 x 10^{-5} M) at 460 nm (λ_{ex}= 310 nm) in water-DMSO (4:1, v/v) at neutral pH. [1) Blank, 2) NO, 3) O_2, 4) •OH, 5) O_2^-, 6) ONOO−, 7) OCl^-, 8) SO_4^{2-}, 9) NO_3^-, 10) $H_2PO_4^-$, 11) Br^-, 12) F^-, 13) Cl^- , 14) I^-, 15) NO_2^- , 16) CO_3^{2-}, 17) SO_3^{2-} , 18) H_2O_2. **(b)** Fluorescence spectra of NPO with different analytes.

5. Competitive selectivity in presence of other analytes

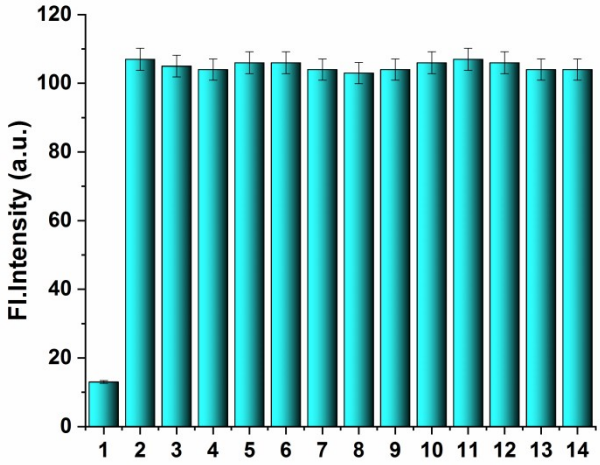

Figure S4. fluorescence spectra of **NPO** in presence of different analytes (10^{-5} M) at 460 nm (λ_{ex}= 310 nm) in water-DMSO (4:1, v/v) at neutral pH. [1) Blank, 2) NO, 3) NO + Zn^{2+}, 4) NO + Cu^{2+}, 5) NO + Pb^{2+}, 6) NO + NO_2^-, 7) NO +OCl^-, 8) NO + CN^-, 9) NO + NO_3^-, 10) NO +SO_4^{2-}, 11) NO + Br^-, 12) NO + F^-, 13) NO + Cl^- and 14) NO + I^- ,

6. Calculation of limit of detection (LOD) of NPO with NO:

The detection limit of the chemosensor **NPO** for **NO** was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without **NO** was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of **NPO** for sensing **NO** was determined from the following equation[2-3]:

$$LOD = K \times SD/S$$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

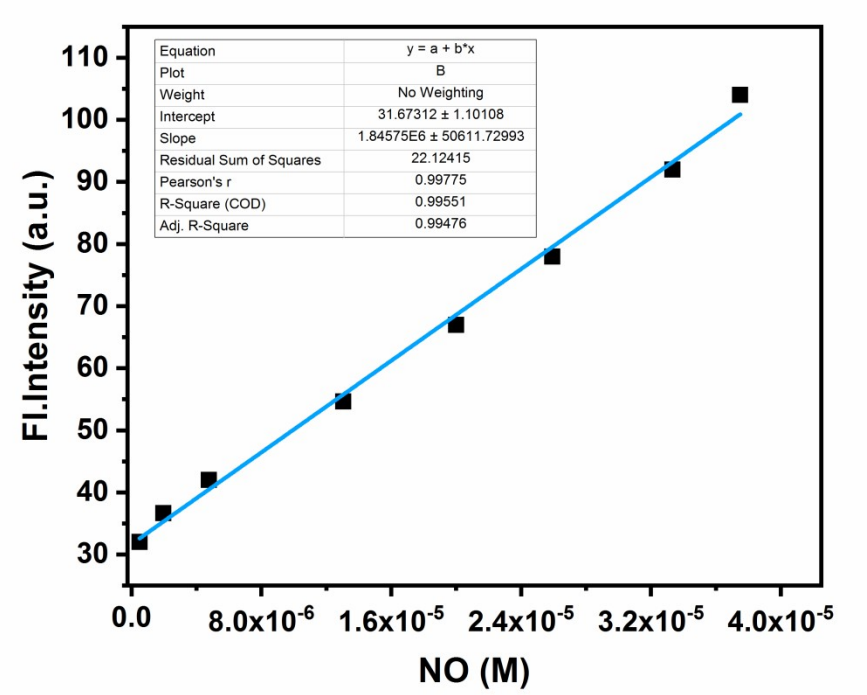

Figure S5. Linear fit curve of **NPO** at 460 nm with respect to **NO** concentration (10^{-4} M).

For **NPO** with **NO:**

From the linear fit graph we get slope = 1.84575×10^6, and SD value is 0.2134.

Thus using the above formula we get the Limit of Detection = 0.11 x 10^{-6} M or 0.11 μM. Therefore **NPO** can detect **NO** up to this very lower concentration by fluorescence technique.

SD Calculation

Blank Reading (NPO)	Fluorescence Intensities at 460nm(X)	Mean (x)	Standard Deviation $(\sigma) = \sqrt{\frac{\sum\|X - x\|^2}{N}}$
Reading 1	18.57	18.44	0.2134
Reading 2	18.31		
Reading 3	18.16		
Reading 4	18.42		
Reading 5	18.78		

7. Job's Plot

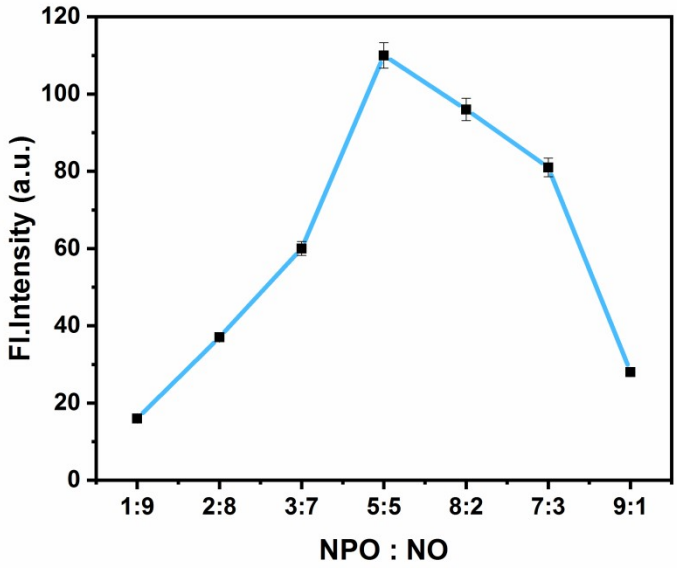

Figure S6. Job's plot of **NPO** (10^{-5} M) with **NO** in water-DMSO (4:1, v/v), at neutral pH, by fluorescence method, which indicate 1:2 stoichiometry for **NPO** with **NO**. Standard deviations are represented by error bar (n=3).

8. Time-dependent fluorescence spectra of NPO in the presence of NO.

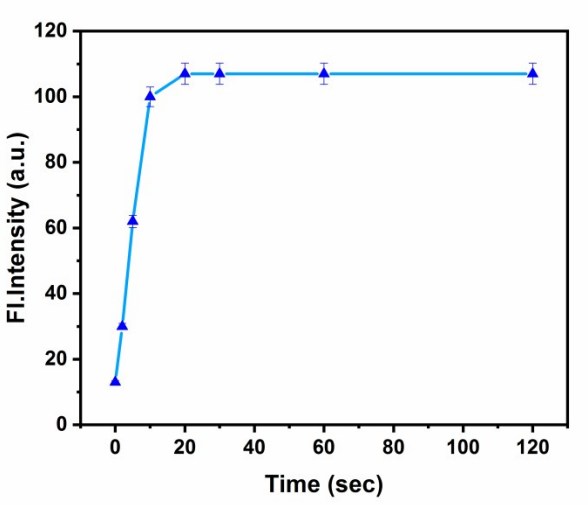

Figure S7. Time-dependent fluorescence spectra of NPO (10^{-5} M) in the presence of NO (10^{-4} M) in water-DMSO (4:1, v/v) at neutral pH.

9. pH titration study:

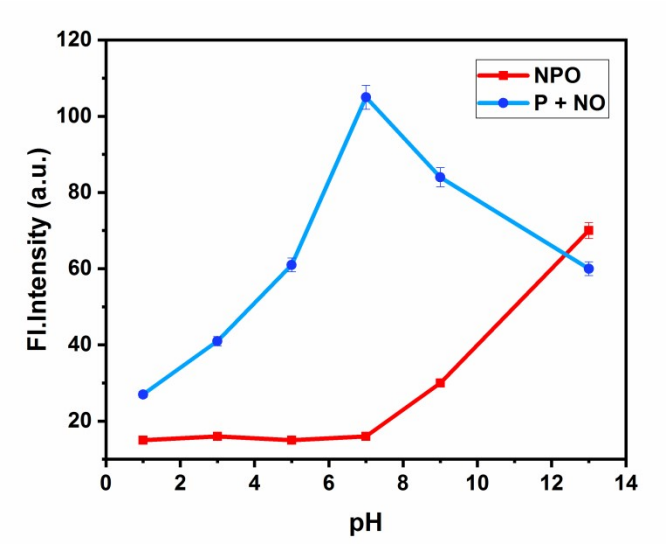

Figure S8. Effect of pH on the fluorescence intensity of **NPO** (10^{-5} M) in the absence of **NO** (redline) and in the presence of **NO** (10^{-4} M, blue line).

10. DFT study

TDDFT- Calculations

Table S2. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of CPLC. The data were calculated by TDDFT//B3LYP/6-31G(d,p) based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c] (%)
NPO	$S_0 \rightarrow S_1$	3.7896 eV 327.17 nm	0.1680	H → L (68.1%)

Table S3. Details of the geometry optimization in Gaussian 09 program

Details	**NPO**	**NO^+**	**NPO+NO complex**	**2 H**
Calculation method	B3LYP	B3LYP	B3LYP	B3LYP
Basis set	6-31G**	6-31G**	6-31G**	6-31G**
E(CAM-B3LYP) (a.u.)	-629.74	-129.65	-758.53	-1.00
Charge, Multiplicity	0, 1	0,1	-1,1	0,1
Solvent (CPCM)	Water	Water	Water	Water

11. HRMS

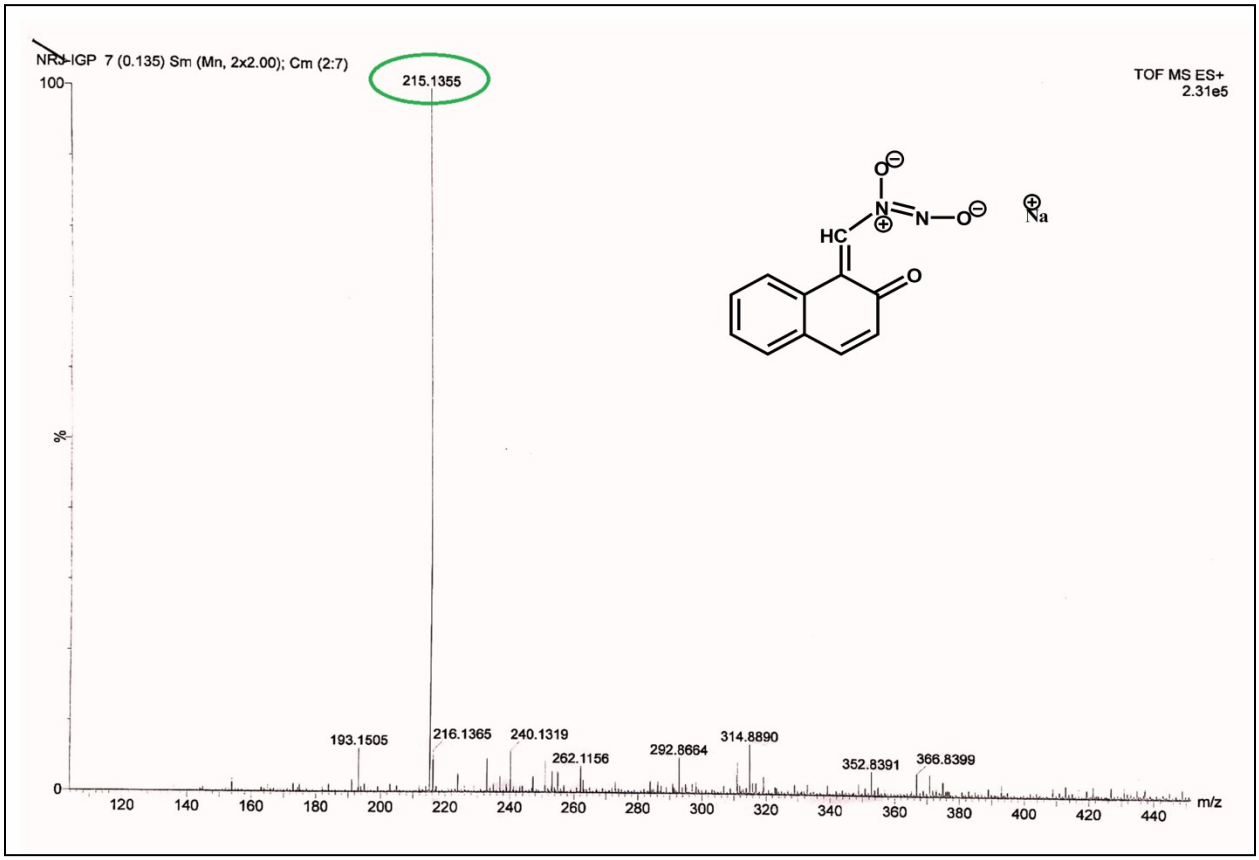

Figure S9. HRMS spectra of NPO+ NO complex ($C_{11}H_7N_2O_3$)

12. Confocal images of the stem and root of chickpea saplings after 36 hours treatment with NPO

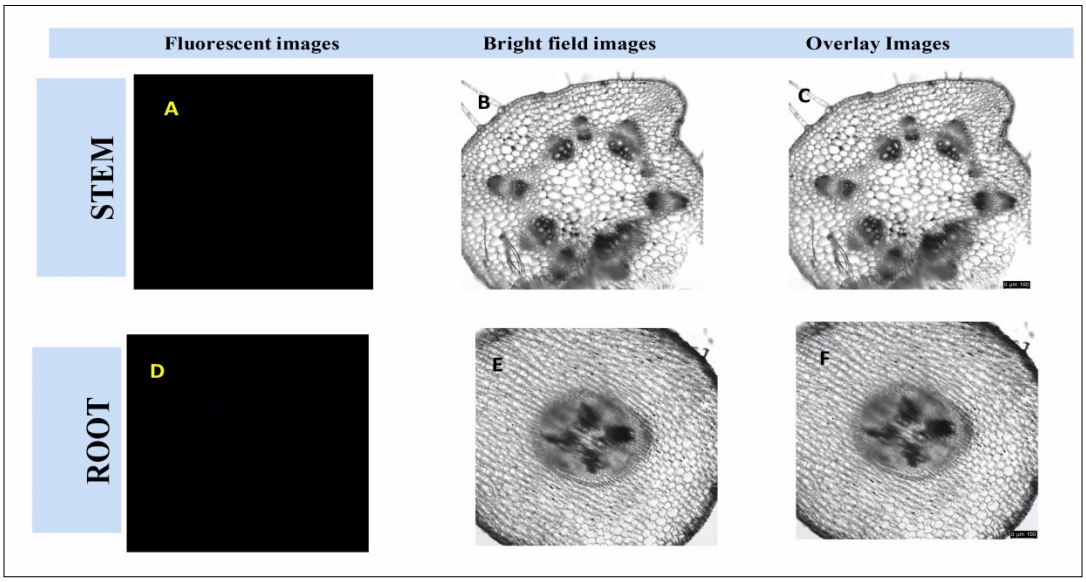

Figure S10. Confocal microscopic images of the stem and root of chickpea saplings after 36 hours treatment with NPO. (A,D) are fluorescent images, (B,E) are the bright field images and (C,F) are overlay images of stem and root sections respectively. Scale bars are 100 µm.

13. Synthetic scheme

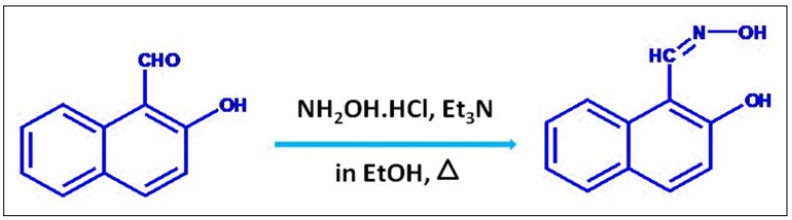

Figure S11. Synthesis of NPO