Electronic Supplementary Material (ESI) for Analyst.

Electronic Supporting Information

Fluorescence turn-on for the highly selective detection of nitric oxide in vitro and in living cells

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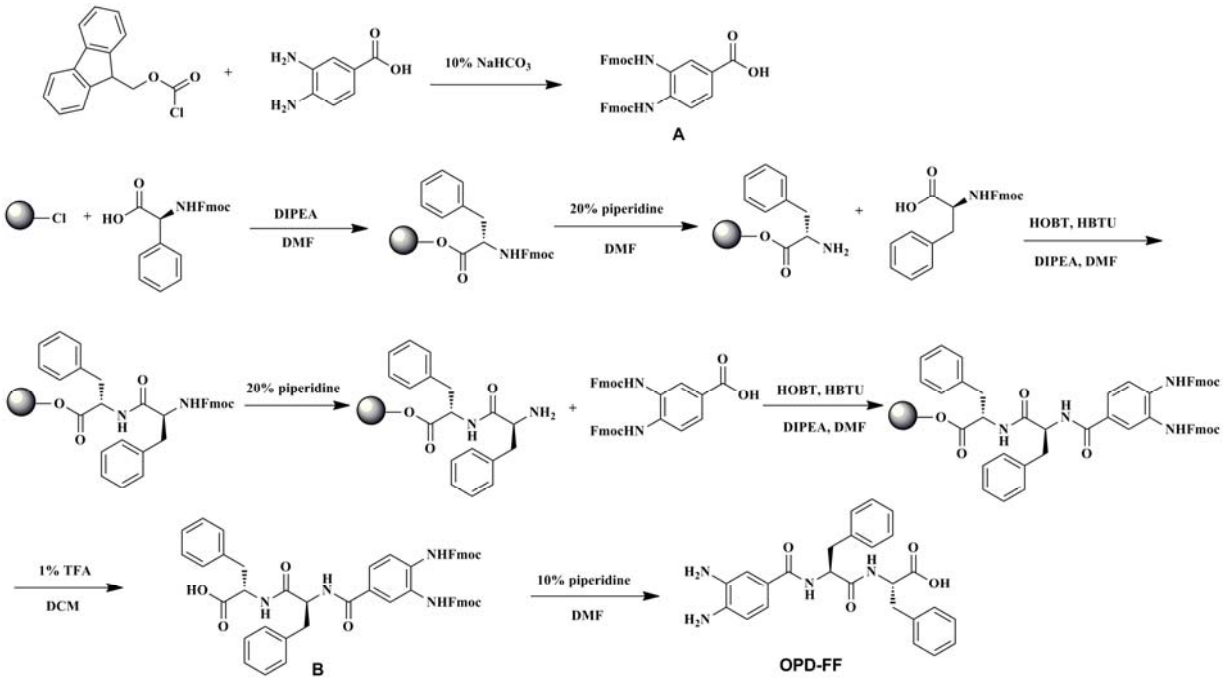
1. Synthetic route for 1

Scheme S1. Synthetic route for **1**.



2. Characterizations of 1

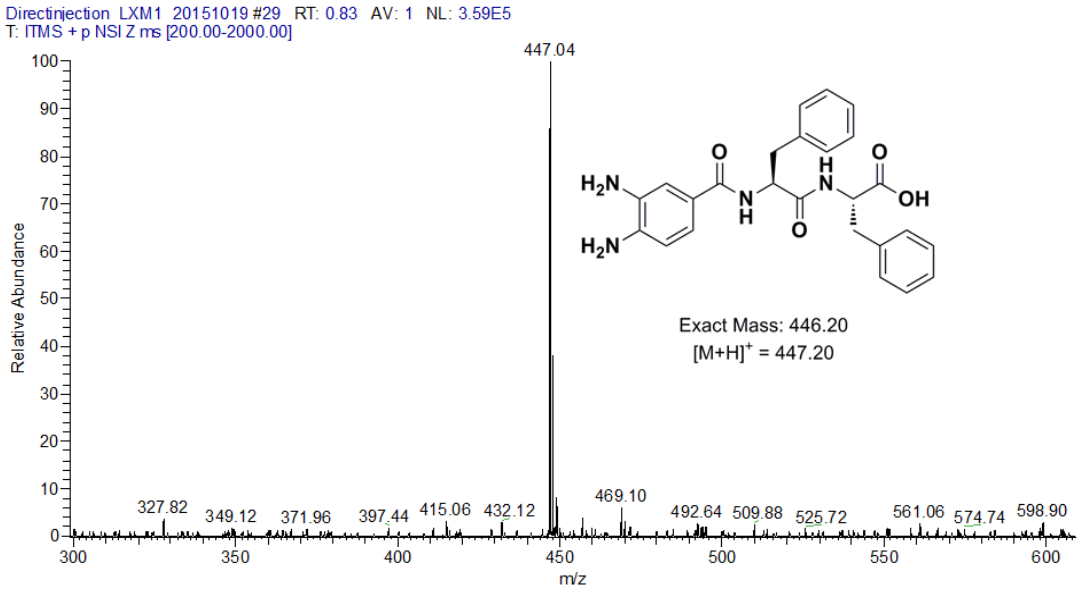


Figure S1. ESI/MS spectrum of **1**.

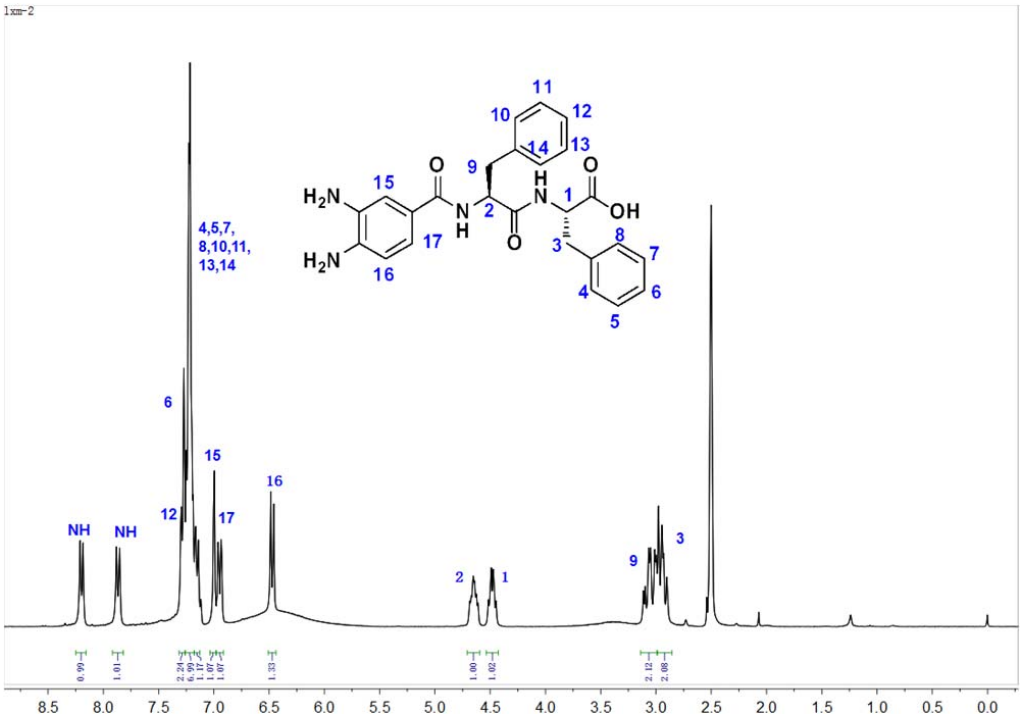


Figure S2. ^{1}H NMR spectrum of **1**.

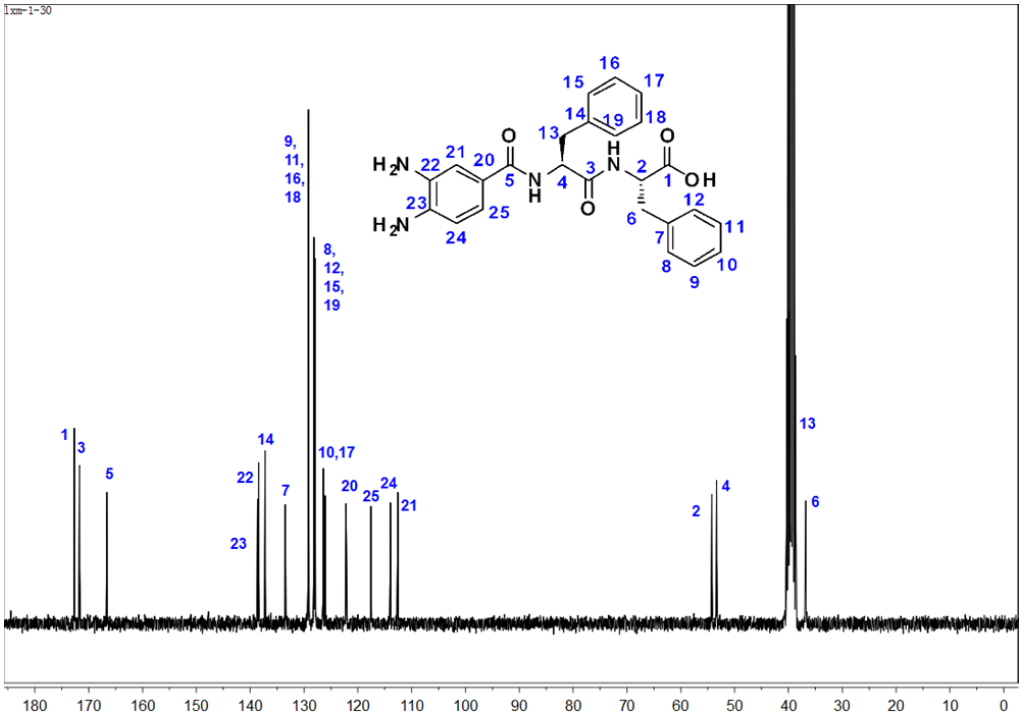


Figure S3. ^{13}C NMR spectrum of **1**.

3. Supporting figures and tables

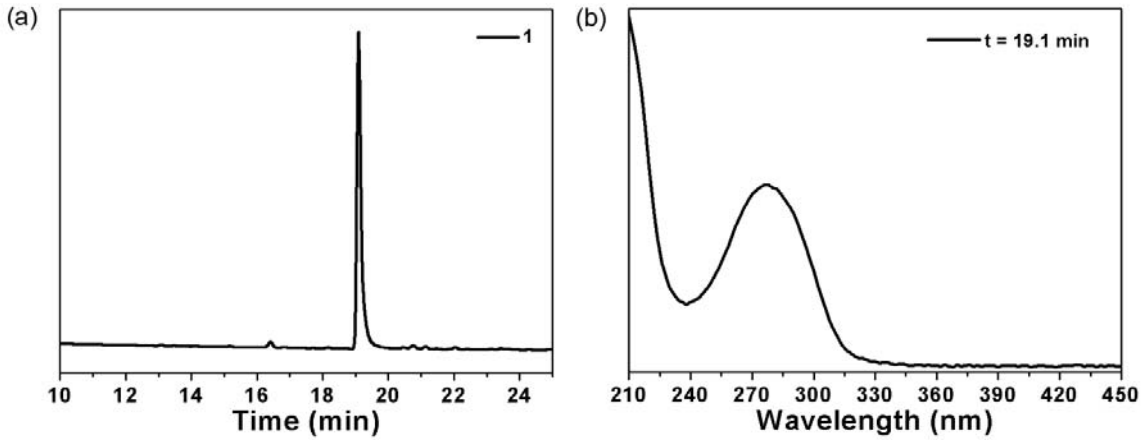


Figure S4. (a) HPLC Trace and (b) absorption spectrum of **1** at the retention time of 19.1 min.

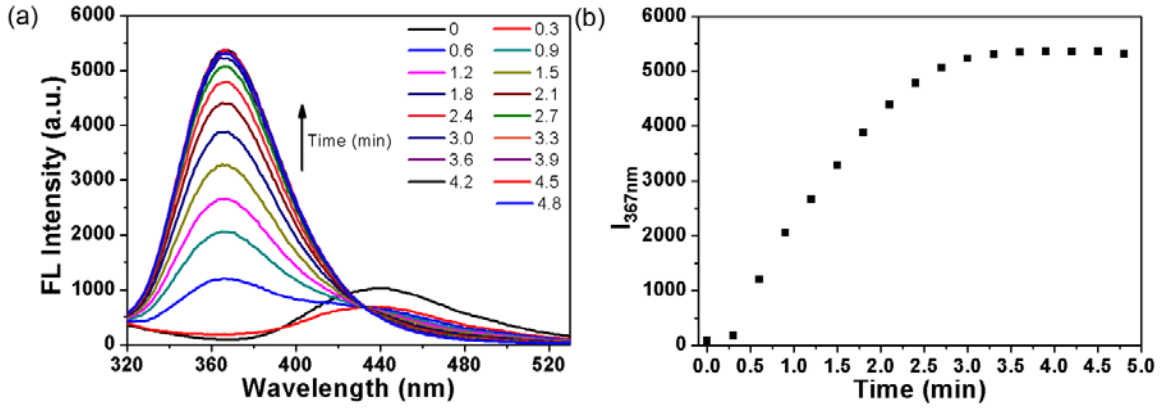


Figure S5. (a) Illumination time-dependent fluorescence spectra of 10 μM **1** in the presence of 5 equiv. of NO donor in PBS buffer (0.2 M, pH 7.4) at RT. (b) The corresponding changes of fluorescence intensity at 367 nm as a function of time in a. Illumination wavelength: 275 nm.

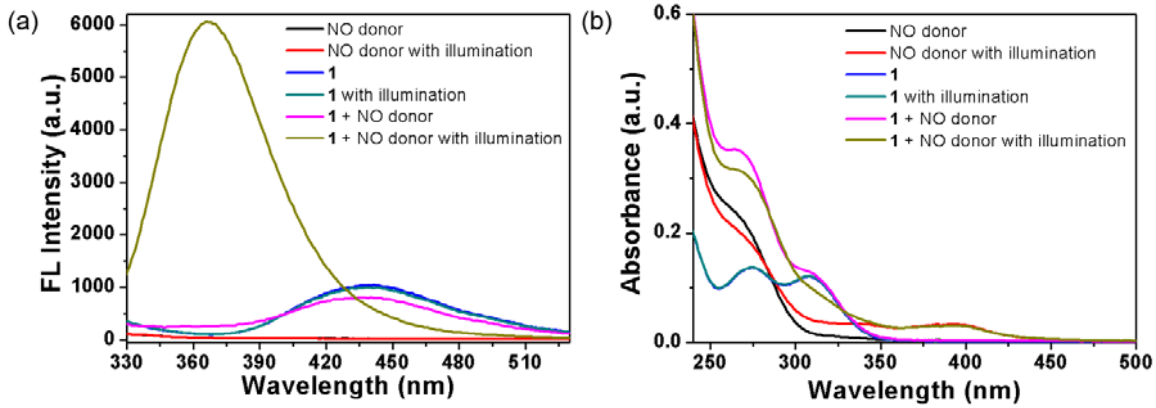


Figure S6. (a) Fluorescence spectra of 50 μM NO donor with or without 3 min illumination, 10 μM **1** with or without illumination, added with 5 equiv. of NO donor with or without 3 min illumination. Excitation wavelength: 275 nm. (b) Absorption spectra of 50 μM NO donor with or without 3 min illumination, 10 μM **1** with or without illumination, added with 5 equiv. of NO donor with or without 3 min illumination.

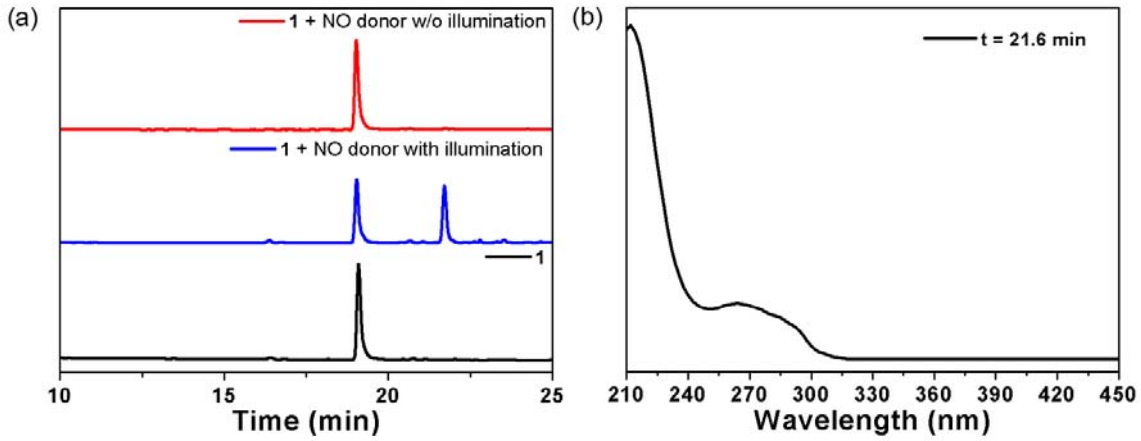


Figure S7. (a) HPLC Traces of **1** (black), **1** added with 5 equiv. of NO solution in PBS buffer (0.2 M, pH 7.4) at RT with (blue) or without (red) 3 min illumination. (b) Absorption spectrum of **1** with 5 equiv. of NO donor after 3 min illumination at HPLC retention time of 21.6 min.

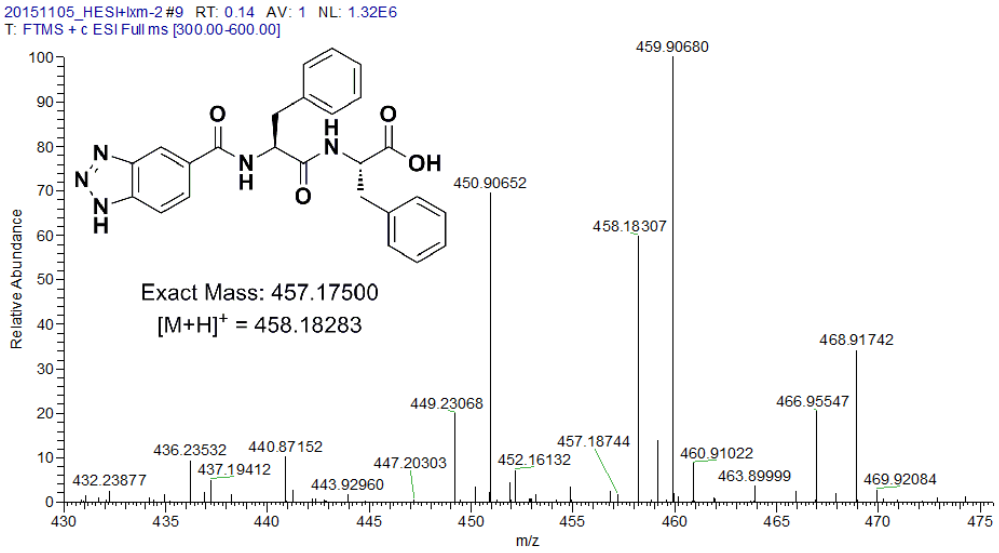


Figure S8. HR-ESI mass spectrum of HPLC peak at retention time of 21.6 min in Figure S7a.

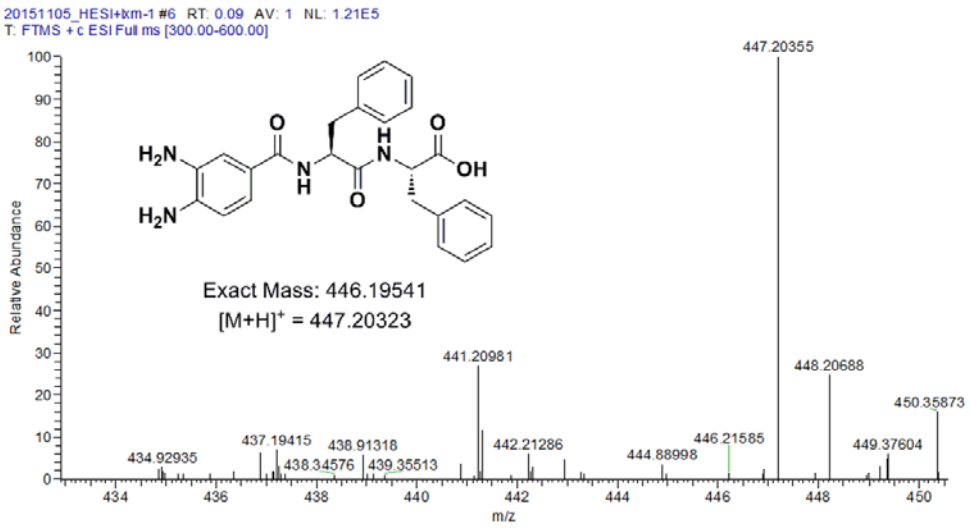


Figure S9. HR-ESI mass spectrum of HPLC peak at retention time of 19.1 min in Figure S7a.

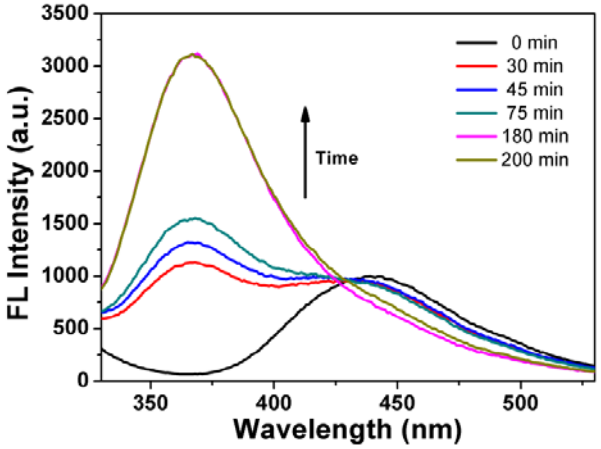


Figure S10. Time-dependent fluorescence spectra of 10 μM **1** in the presence of 5 equiv. of DEA·NONOate in PBS buffer (0.2 M, pH 7.4) at RT. Excitation wavelength: 275 nm.

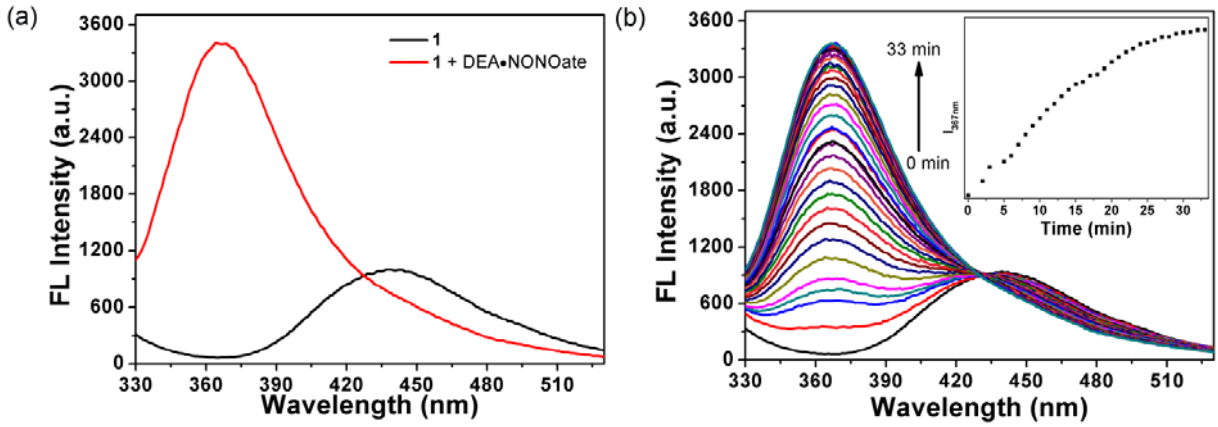


Figure S11. (a) Fluorescence spectra of 10 μM **1** (black), added with 5 equiv. of DEA·NONOate (red) after 30 min illumination. Excitation wavelength: 275 nm. (b) Illumination time-dependent fluorescence spectra of 10 μM **1** in the presence of 5 equiv. of DEA·NONOate in PBS buffer (0.2 M, pH 7.4) at RT. Inset: the corresponding changes of fluorescence intensity at 367 nm as a function of time. Excitation wavelength: 275 nm.

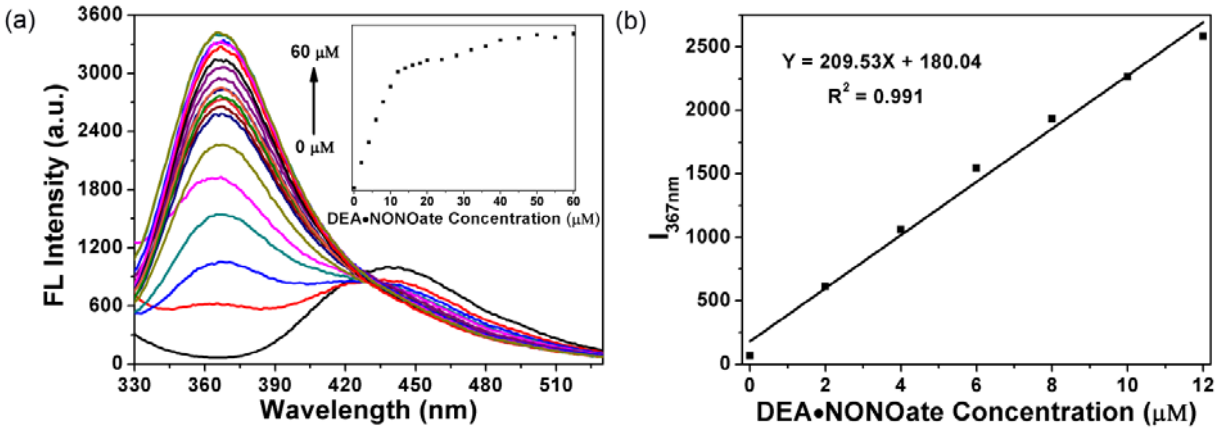


Figure S12. (a) Fluorescence spectra of 10 μM **1** upon addition of various concentrations of DEA·NONOate at 0, 2, 4, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32, 36, 40, 45, 50, 55, 60 μM in PBS buffer (0.2 M, pH 7.4) and 30 min illumination at RT. Inset: the corresponding fluorescence responses of **1** at 367 nm upon addition of different concentrations of DEA·NONOate. (b) Fitted calibration curve of the fluorescence intensities at 367 nm. Excitation wavelength: 275 nm.

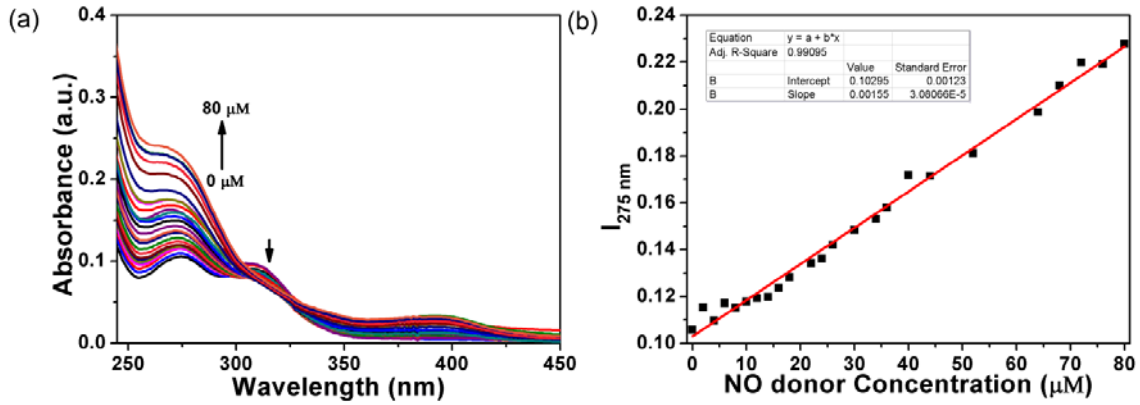


Figure S13. (a) UV-Vis absorption spectra of 10 μM **1** upon addition of different concentrations of NO donor at 0, 2, 4, 8, 10, 12, 14, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, 80 μM in PBS buffer (0.2 M, pH 7.4) at RT. (b) Fitted calibration curve of the absorbance at 275 nm. Absorbance is collected after 3 min photo illumination at wavelength of 275 nm.

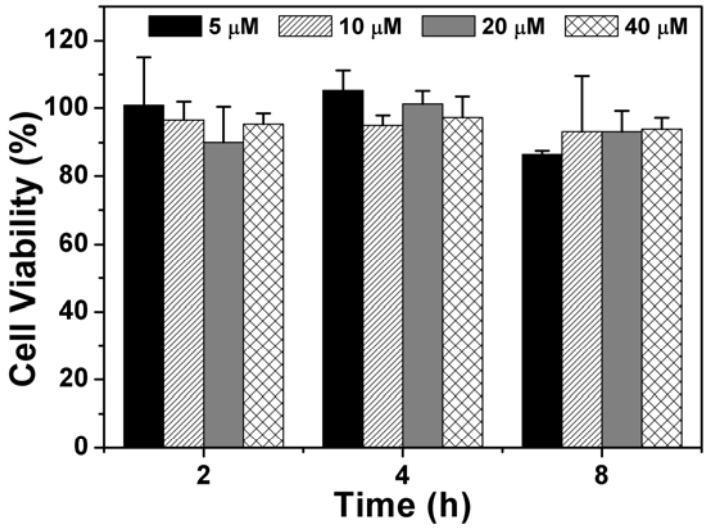


Figure S14. MTT assay of **1** on HepG2 cells. Cell viability values (%) estimated by MTT proliferation test at concentrations of 5, 10, 20 and 40 μM of **1**. HepG2 cells were cultured in the presence of **1** for 2, 4 and 8 h at 37 °C under 5% CO_2. These experiments were performed in triplicate. Results are representative of three independent experiments. Error bars represent standard deviations.

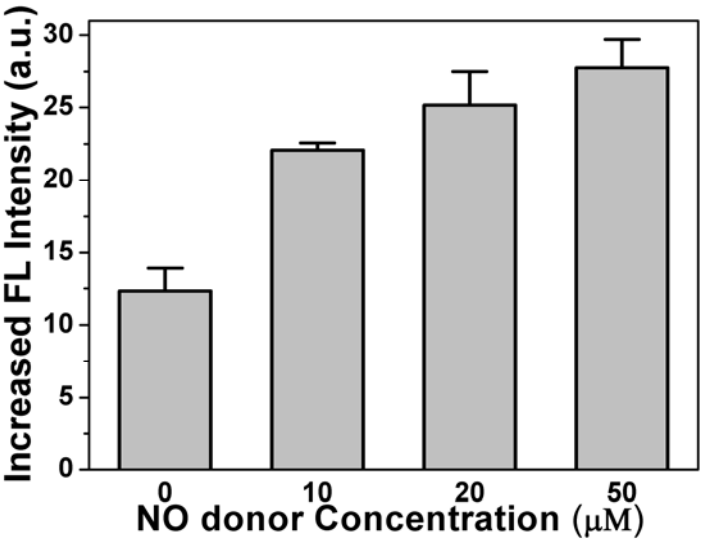


Figure S15. The average increased fluorescence intensity at 367 nm of HepG2 cells incubated with 10 μM **1** in serum-free medium in 96-well for 1 h at 37 °C, washed with PBS for three times, then incubated with 0, 10, 20 or 50 μM of NO donor (SNP) for 0.5 h at 37 °C and illuminated for 2 min at 254 nm proir to detection, respectively.

Table S1. HPLC condition for the purification of compound **1**.

Time (minute)	Flow (mL/min.)	H_2O % (0.1 % TFA)	CH_3CN% (0.1 % TFA)
0	3.0	90	10
3	3.0	90	10
35	3.0	30	70
37	3.0	30	70
38	3.0	90	10
40	3.0	90	10

Table S2. Statistics of sensitivity of **OPD-FF** (**1**) in this work and other reported probes for NO detection.

Probe	**LOD**	**Response time**	**Buffer solution**
OPD-FF	6 nM	3 min	0.2 M PBS buffer, pH 7.4
DAR-2[1]	7 nM	five accumulations	0.1 M PBS buffer, pH 7.4 (10 mM DMSO stock solution)
DAF-2[2]	5 nM	seven accumulations,15 min	0.1 M PBS buffer, pH 7.4
BANPBO-H[3]	2.1 nM	20 min	CH_3CN/PBS (3:2, v/v), pH 7.4
Probe **1** by Guo *et al.*[4]	12 nM	30 min	50 mM PB buffer, 30% CH_3CN, pH 7.4
Probe **2** by Guo *et al.*[5]	30 nM	30 min	20 mM EtOH/PBS buffer (1:1, v/v), pH 7.4
Mito-Rh-NO[6]	4 nM	30 min	CH_3CN/PBS (20:80, v/v), pH 7.4
Lyso-NINO[7]	5 nM	15 min	CH_3CN/PB (20:80, v/v), pH 5.0
$[Ru(bpy)_2(dabpy)][PF_6]_2$[8]	270 nM	2 min	0.1 M borate buffer, pH 7.4

4. References

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