Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A thiocarbonate-caged fluorescent probe for specific visualization of peroxynitrite in living cells and zebrafish

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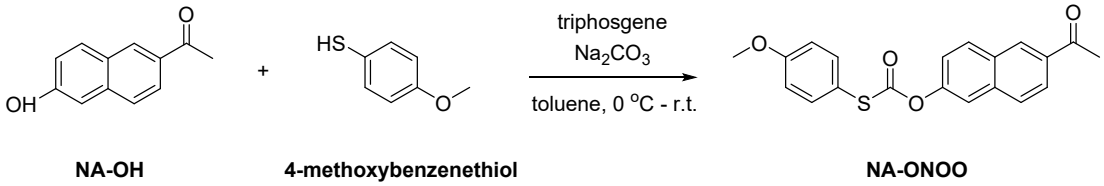
1. Synthetic route of NA-ONOO



Scheme S1. Synthetic route of **NA-ONOO**

2. Preparation of analytes

$ONOO^-$

0.6 M $NaNO_2$ and 0.7 M H_2O_2 (acidified by 0.6 M HCl) were simultaneously and rapidly added into 1.2 M NaOH solution at 0 °C for stirring. After the solution is diluted 10 times, the concentration is calibrated by the absorbance at 301 nm. (Extinction coefficient is 1670 cm^{-1} M^{-1}).

H_2O_2, TBHP, NaClO, $NaNO_2$, KO_2, Hcy, Cys, GSH

The above analytes with a concentration of 10 mM was prepared from the commercial available chemicals and solutions by ultra-pure water.

•OH

The hydroxyl radical was made by Fenton reaction, putting an equivalent amount of hydrogen peroxide (H_2O_2) into the Iron dichloride solution ($FeCl_2$).

NO

Nitric oxide (NO) was generated by sodium nitroprusside.

O^{2-}

Superoxide (O^{2-}) was generated from KO_2 in DMSO.

$^1O_2$

Singlet oxygen ($^1O_2$) was generated from NaOCl and H_2O_2.

3. Cytotoxicity assays

HeLa cells were seeded into 96-well plates at a density of 5 × 10^3 cells per well in culture media (DMEM) at 37 °C, 5% CO2 and 95% air. Then, we cultured 0, 1, 5, 10, 20 and 50 μM (final concentration) **NA-ONOO** with HeLa cells for 24 h. Finally, 10 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg mL^{-1}) was added and cells were cultured for another 4 h. When the purple precipitate was clearly visible under the microscope, 100 μL of DMSO was added to all the wells and swirled gently. Then, the absorbance in each well was measured, including that of the blanks, at 570 nm in a microtiter plate reader (Bio-Rad 680).

4. Characterization of NA-ONOO

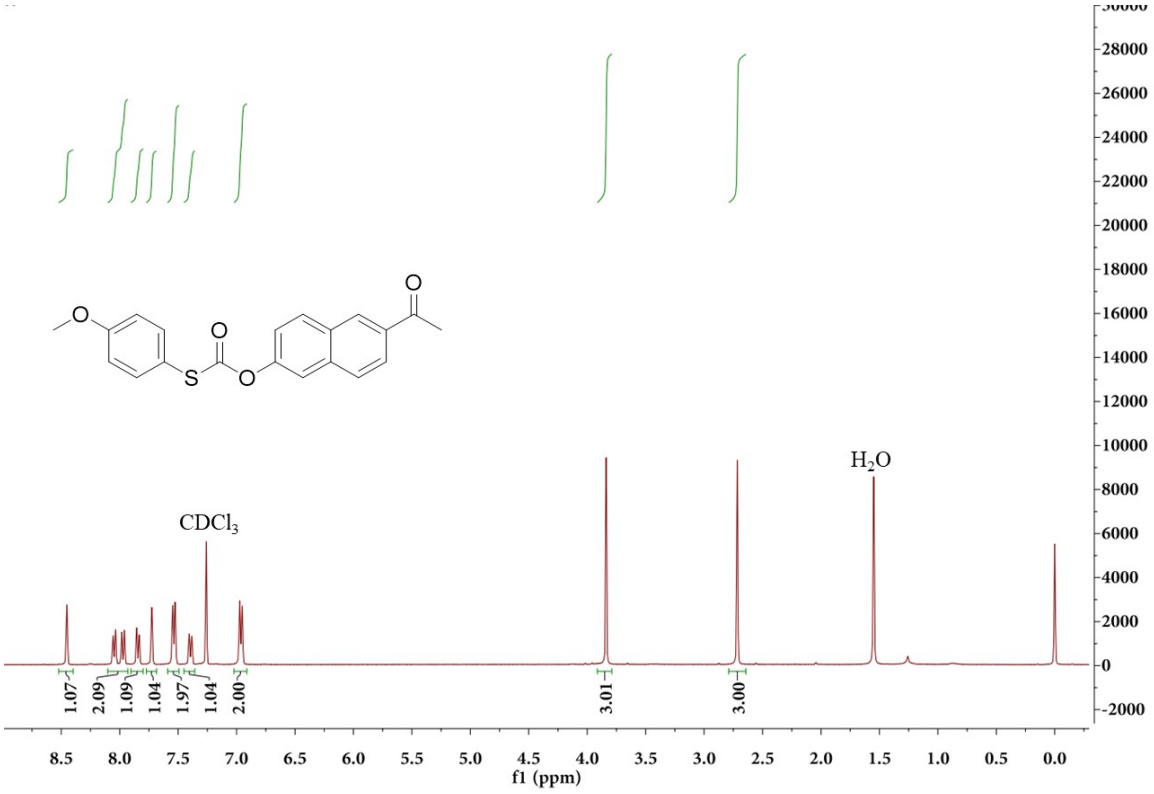


Fig. S1. ^{1}H NMR spectrum of **NA-ONOO**

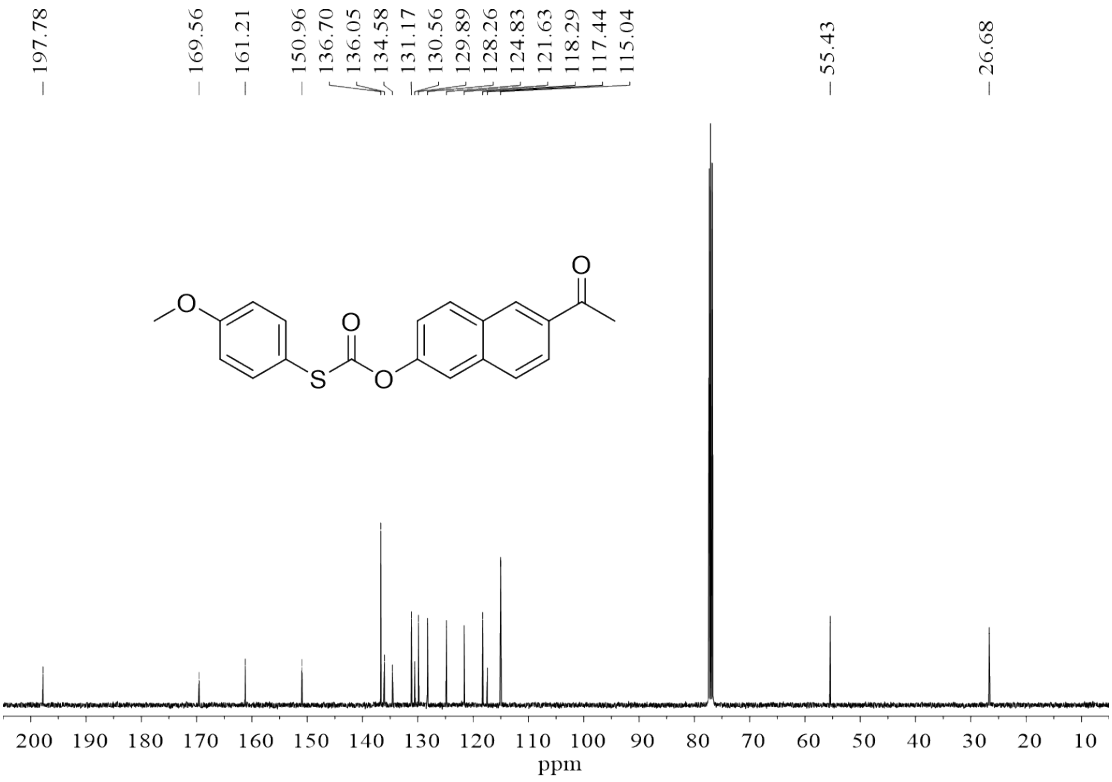
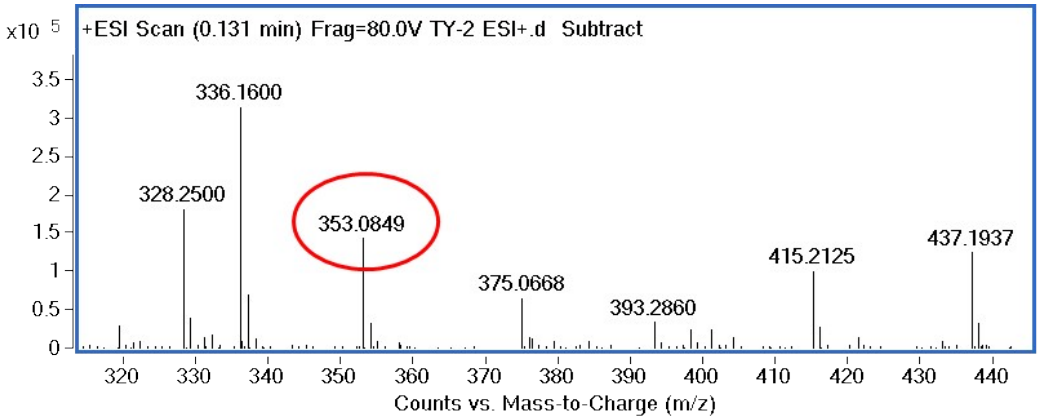


Fig. S2. ^{13}C NMR spectrum of **NA-ONOO**



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g. S3. HR-MS spectrum of **NA-ONOO**

5. The dose-dependent absorption responses of probe NA-ONOO to ONOO⁻

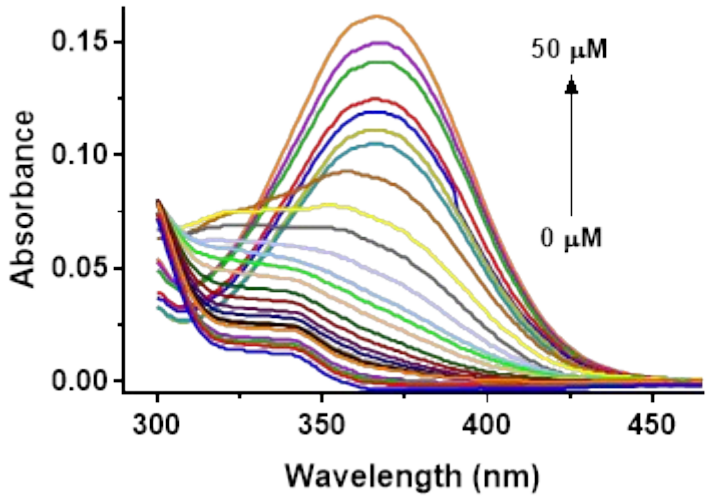

Fig. S4. Absorption spectra of probe **NA-ONOO** (10 μM) in the presence ONOO⁻ (0–50 μM) in EtOH/PBS buffer (10 mM, pH 7.4, 1/1, V/V).

6. The dose-dependent fluorescence responses of probe NA-ONOO to ONOO⁻

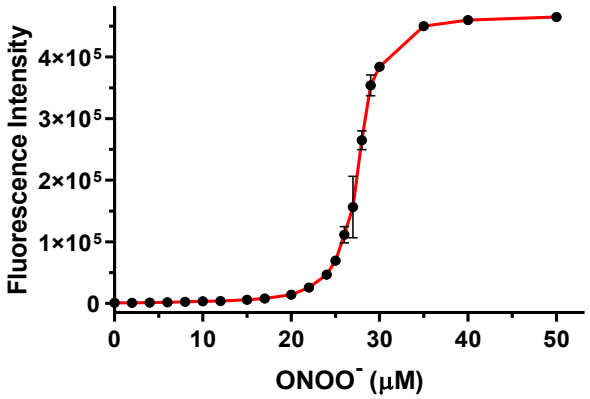

Fig. S5. (a) Fluorescence intensity profiles of **NA-ONOO** with 0–50 μM ONOO⁻.

7. Characterization of NA-ONOO reacting with $ONOO^-$

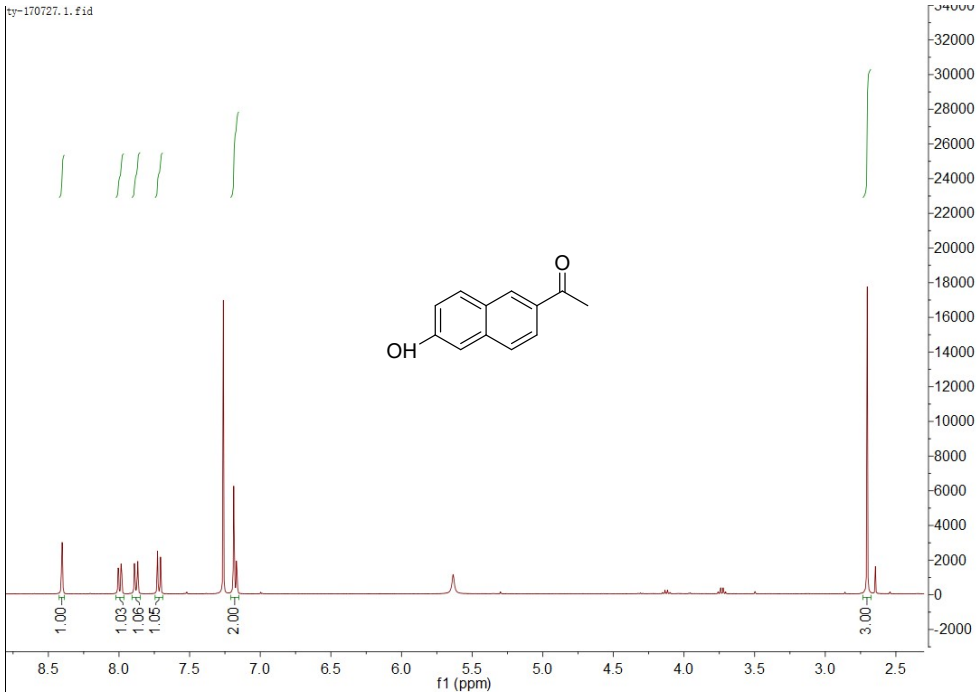

Fig. S6. ^{1}H NMR spectrum of NA-OH

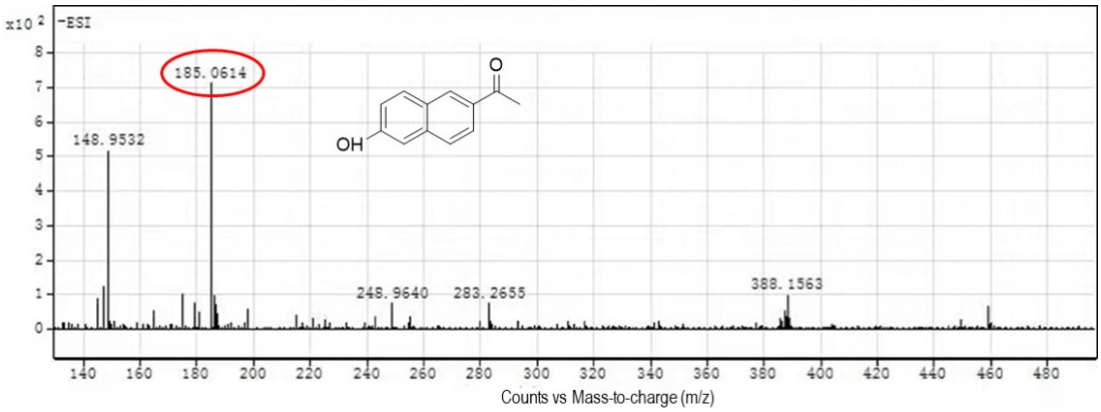

Fig. S7. HR-MS spectrum of NA-OH

8. The interference experiments

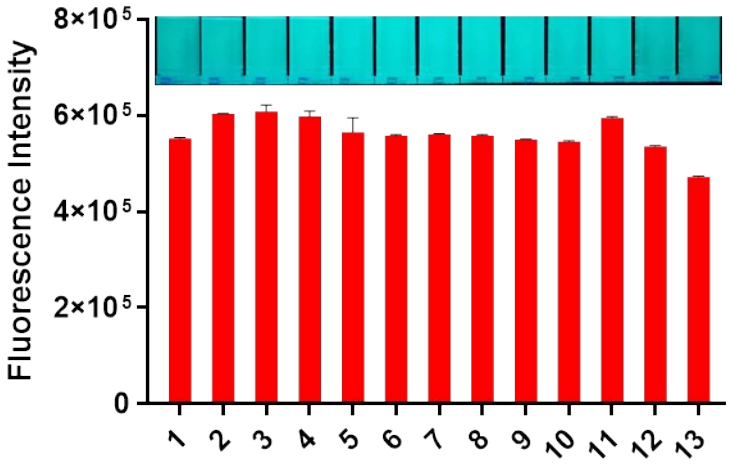

Fig. S8. Fluorescence response of **NA-ONOO** (10 μM) toward $ONOO^-$ (50 μM) in the presence of different analytes (200 μM). (1) Blank; (2) H_2O_2; (3) OCl^-; (4) TBHP; (5) NO; (6) Hcy; (7) Cys; (8) GSH; (9) NO_3^-; (10) NO_2^-; (11) $^1O_2$; (12) $^{\bullet}OH$; (13) O_2^-. Inset: visual photographs of **NA-ONOO** in EtOH/PBS buffer (10 mM, pH 7.4, V/V, 1/1) in the presence of $ONOO^-$ with a series of interference biological reagents under handheld UV lamp.