Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information

Fulan Xie, Rui Zhou, Chi Jian, Lizhu Zhang,*[a] and Yonghui He*[a]

[a] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education; Key Laboratory of Natural Products Synthetic Biology of Ethnic Medicinal Endophytes, State Ethnic Affairs Commission; School of Ethnic Medicine, Yunnan Minzu University, Kunming, 650031.

*Corresponding Authors
E-mail: zhanglizhu333@163.com; heyonghui@ymu.edu.cn

Borate-based peroxynitrite fluorescence probe and its application in fluorescence imaging of living cells

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I. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

NaOCl

The concentration of sodium hypochlorite (NaOCl) was determined from the absorbance at 292 nm ($\varepsilon = 350\ M^{-1}\ cm^{-1}$).

H_2O_2

The concentration of hydrogen peroxide (H_2O_2) was determined from the absorption at 240 nm ($\varepsilon = 43.6\ M^{-1}\ cm^{-1}$).

TBHP

Tert-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water.

•OH

Hydroxyl radical (•OH) was generated by Fenton reactions. To prepare •OH solution, hydrogen peroxide (H_2O_2, 10 mM) was added to $FeSO_4$ (1 mM) in deionised water.

$^1O_2$

Hydrogen peroxide (H_2O_2, 10 mM) was added to $NaMoO_4$ (1 mM) in deionised water.

NO

Nitric oxide (NO) was generated from sodium nitroprusside dihydrate.

$ONOO^-$

To a vigorously stirred solution of $NaNO_2$ (1.5 M, 3 mL) and H_2O_2 (0.7 M, 1.5 mL) in deionized H_2O at 0 ℃ was added HCl (0.6 M, 1.5 mL), immediately followed by the rapid addition of NaOH (1.5 M, 3 mL). The concentration of $ONOO^-$ was determined by UV analysis with the extinction coefficient at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$) in 0.1 M NaOH.

II. Response of compounds 3a and 3b to $ONOO^-$ in different ratios of buffer solution

The optimal response ratio of $ONOO^-$ identified by probe **3a** and **3b** in different proportion of CH_3OH-Tris-HCl buffer solution was investigated experimentally. As shown in Fig. S1 (A) and Fig. S1 (B), both **3a** and **3b** obtained the strongest fluorescence emission in CH_3OH-40%Tris-HCl buffer solution after adding $ONOO^-$ buffer solution. Therefore, CH_3OH-40%Tris-HCl buffer solution system was used in the follow-up study.

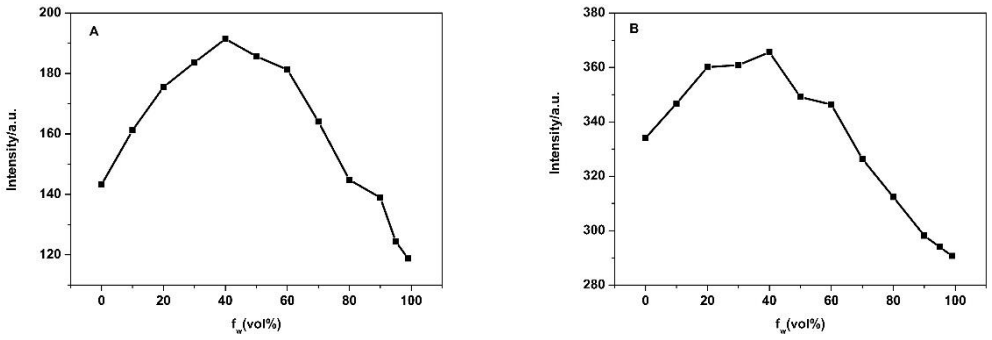


Fig. S1. (A) Fluorescence intensity of **3a** probe (10 μM) in different ratios of Tris-HCl buffer solution / CH_3OH system after interaction with $ONOO^-$ (20 equiv); (B) Fluorescence intensity of **3b** probe (10 μM) in Tris-HCl buffer solution / CH_3OH system of different ratios after interaction with $ONOO^-$ (20 equiv)

III. Quantum yield calculation

The fluorescence quantum yields (Φ) of **3a** and **3b** before and after adding $ONOO^-$ were measured by using the Rhodamine B (Φ = 0.82) as a reference, which was then calculated by using the formula below:

$\Phi F(x) = (\Phi F(s) * As * Fx) / (Ax * Fs)$

Here, A denotes the absorbance at the excitation wavelength, F refers to the area under the corrected fluorescence emission curve. Subscripts X and S represents the test sample and reference, respectively. As expected, the emission spectra of **3a** at 366 nm shows weak fluorescence without $ONOO^-$ (ΦF< 0.1). In contrast, probe **3a** exhibited an immense increase at 510 nm upon addition of $ONOO^-$ (ΦF= 0.23). In addition, the emission spectra of **3b** demonstrated almost no fluorescence in the absence of $ONOO^-$ (ΦF< 0.1). In contrast, probe **3b** exhibited an immense increase at 431 nm upon addition of $ONOO^-$ (ΦF= 0.58).

IV. Endogenous biological imaging in living cells

To stimulate cells to produce ONOO−, further explored the fluorescence imaging of endogenous ONOO−, we added lipopolysaccharide (LPS, 1 μg/mL), interferon-γ (IFN-γ, 100 ng/mL), phorbol-12-myristate-13-acetate (PMA, 10 nM) and **3a** sequentially to co-incubate with HepG2 cells. As shown in Fig. S2, in the blue channel, the stimulation group had far more luminance than both the control group (only incubated with **3a**) and the clear group (incubated with 100 ng/mL N-acetylcysteine and **3a**). We also used the same method to further explore the fluorescence imaging of endogenous $ONOO^-$ for **3b**, the results were encouraging that the probe **3b** was capable of imaging endogenous $ONOO^-$.

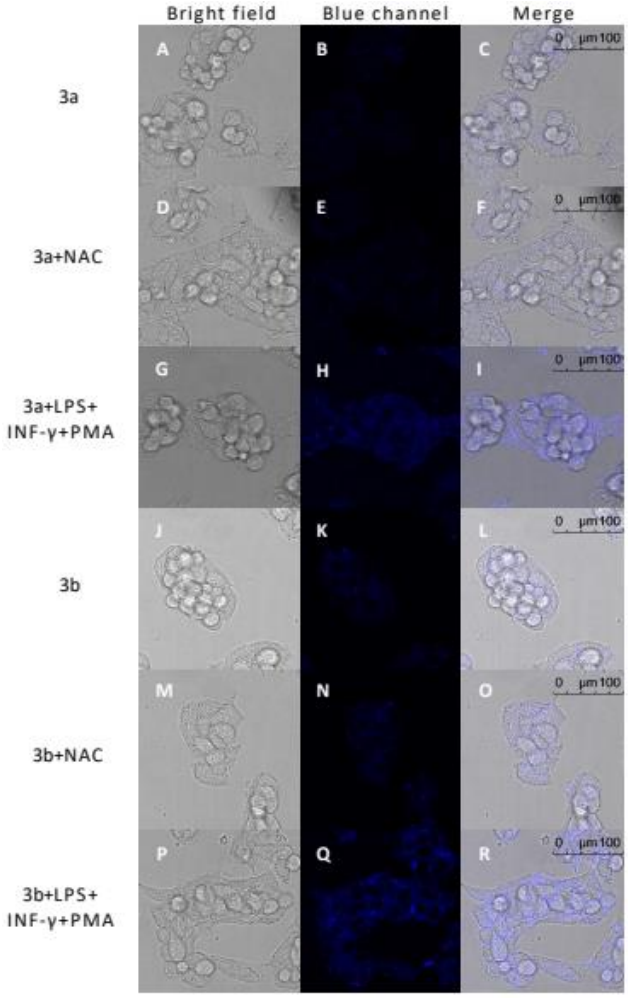
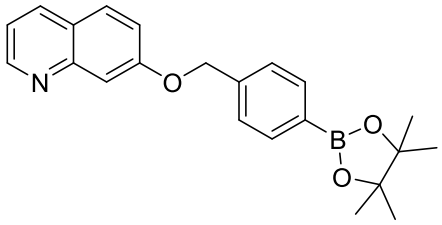


Fig. S2. The bright field (Bright field) is on the left, the fluorescence (Blue channel) is in the middle, and the superposition field of bright field and fluorescence (Merge) is on the right. (A-C) HepG2 cells were treated with **3a**. (D-F) HepG2 cells were initially treated with NAC and **3a**. (G-I) HepG2 cells were treated with IFN-γ and LPS, and then were treated PMA and **3a**. (J-L) HepG2 cells were treated with **3b**. (M-O) HepG2 cells were initially treated with NAC and **3b**. (P-R) HepG2 cells were treated with IFN-γ and LPS, and then were treated PMA and **3b**.

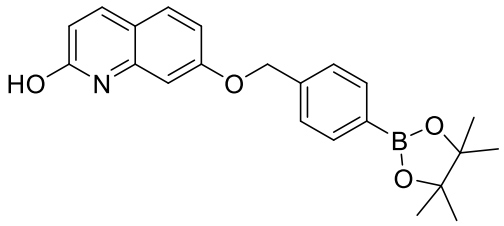
V. Characterization Data

7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)quinoline（3a）



White solid, 88.3% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.70-8.69 (m, 1H), 7.96-7.94 (m, 1H), 7.77-7.75 (m, 2H), 7.60-7.58 (m, 1H), 7.41-7.39 (m, 3H), 7.19-7.13 (m, 2H), 5.11 (s, 2H), 1.24 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.71, 150.40, 149.62, 139.47, 135.94, 135.14, 128.95, 126.83, 125.98, 123.69, 120.23, 119.12, 108.28, 83.87, 70.02, 24.88. HRMS m/z (ESI) calcd for $C_{22}H_{24}BNO_3$ $(M+H)^+$ 362.1922, found 362.1927.

7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)quinolin-2-ol（3b）



White solid, 71.2% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.77(s, 1H), 7.77-7.75 (m, 2H), 7.65-7.63 (m, 1H), 7.38-7.34 (m, 3H), 6.91-6.91 (m, 1H), 6.80-6.77 (m, 1H), 6.51-6.48 (m, 1H), 5.06 (s, 2H), 1.26 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.24, 160.96, 140.93, 140.37, 139.30, 135.08, 129.07, 126.83, 118.02, 114.48, 112.98, 99.54, 83.87, 70.15, 24.89. HRMS m/z (ESI) calcd for $C_{22}H_{24}BNO_4$ $(M+H)^+$ 378.1871, found 378.1866.

VI. MS, ^{1}H NMR and ^{13}C NMR spectra

7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)quinoline（3a）

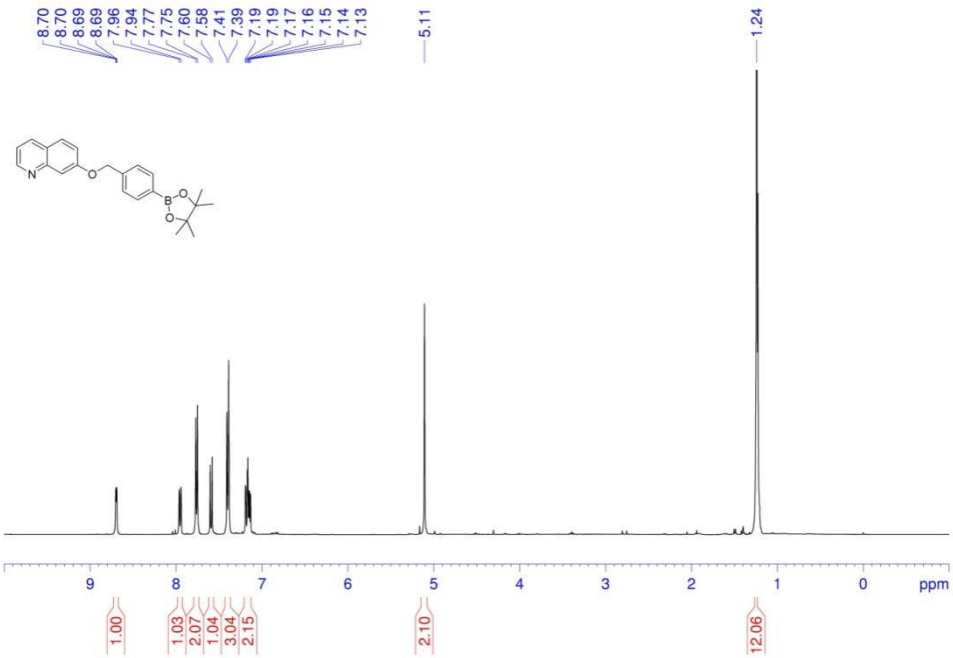


Figure S3. ^{1}H-NMR data of 3a

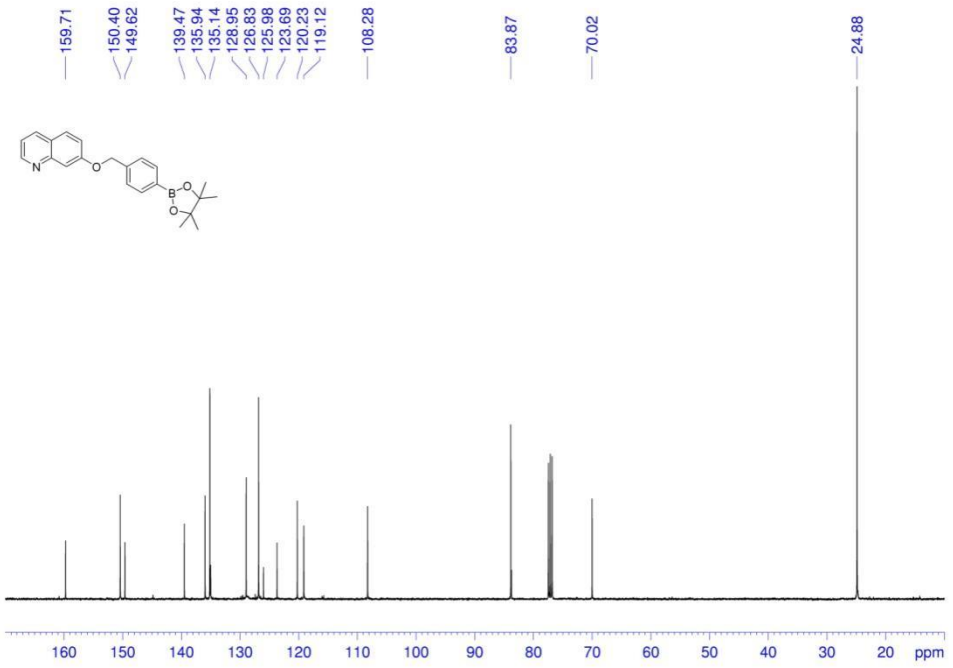


Figure S4. ^{13}C-NMR data of 3a

7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)quinolin-2-ol（3b）

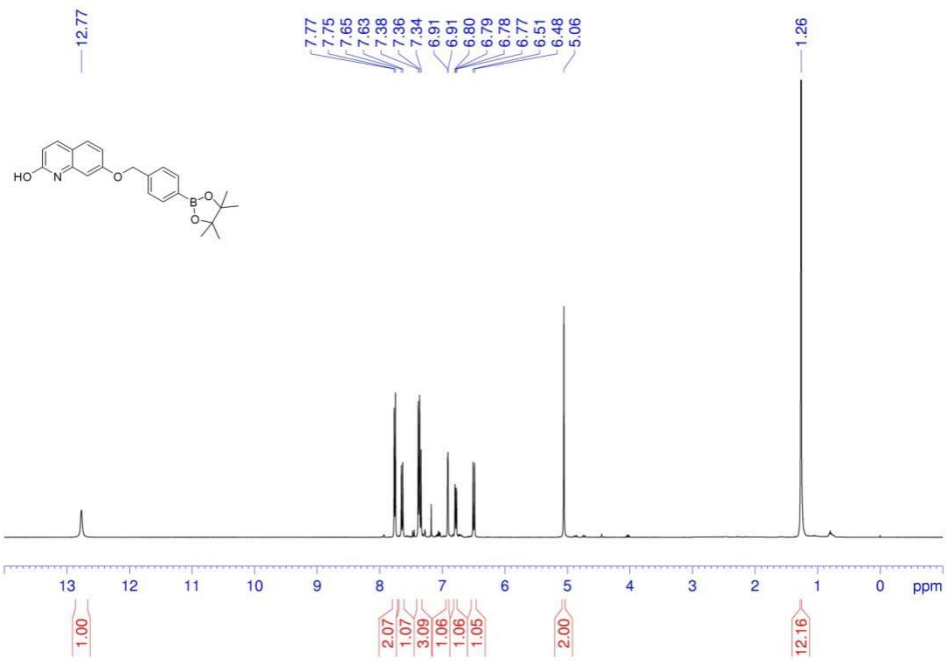


Figure S5. ^{1}H-NMR data of 3b

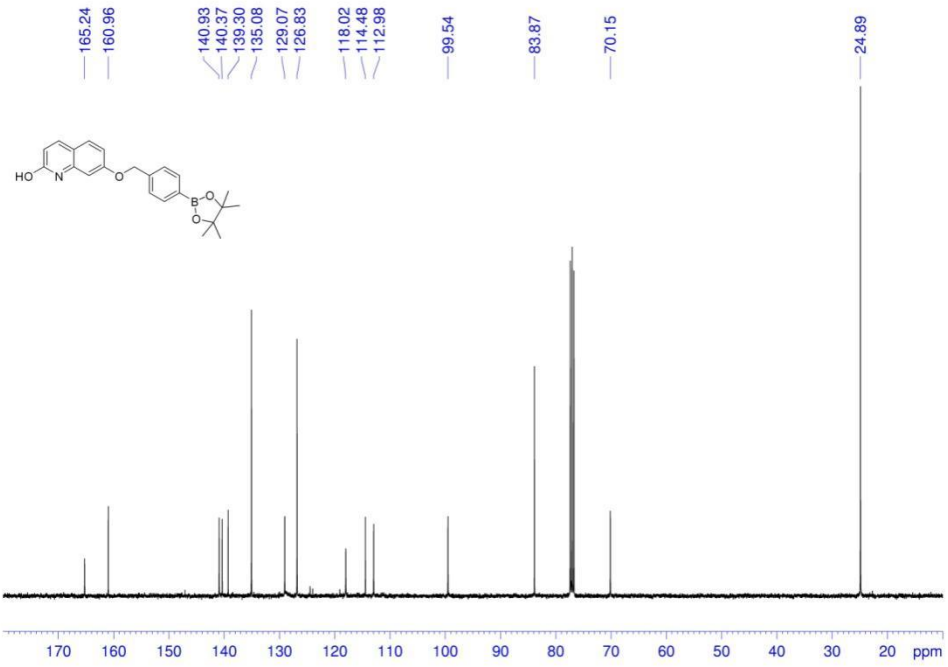


Figure S6. ^{13}C-NMR data of 3b

7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)quinoline（3a）

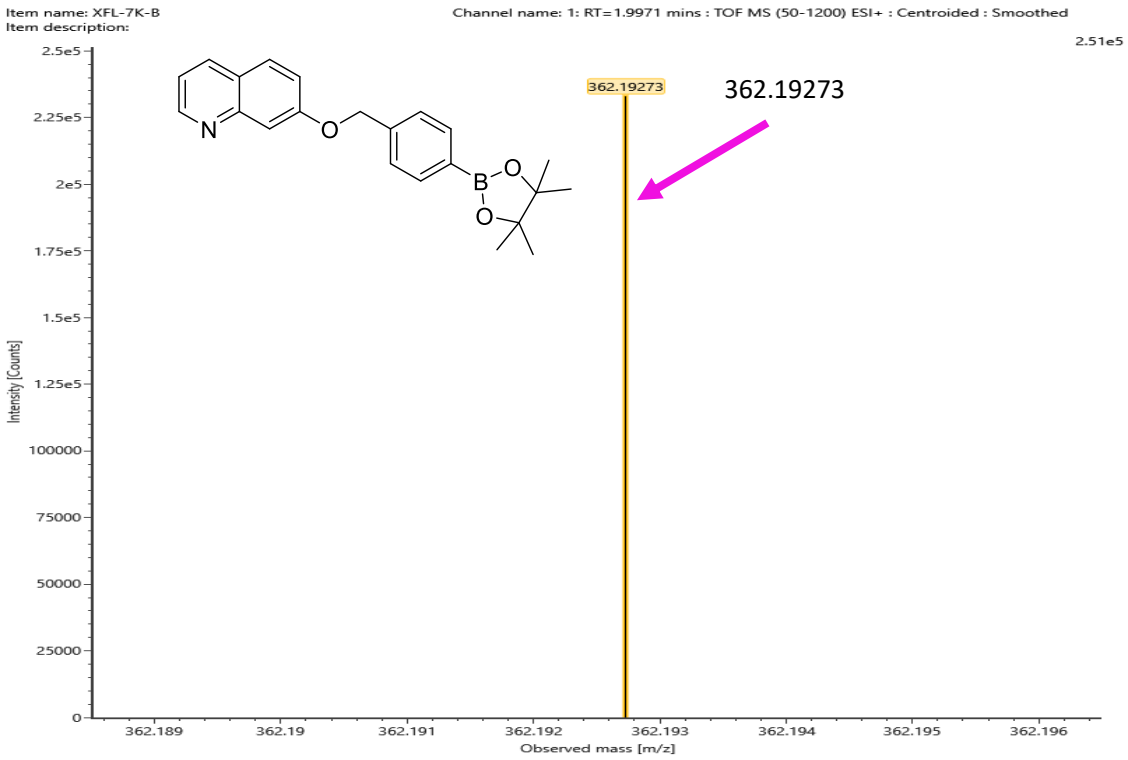


Figure S7. HRMS data of 3a

7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)quinolin-2-ol（3b）

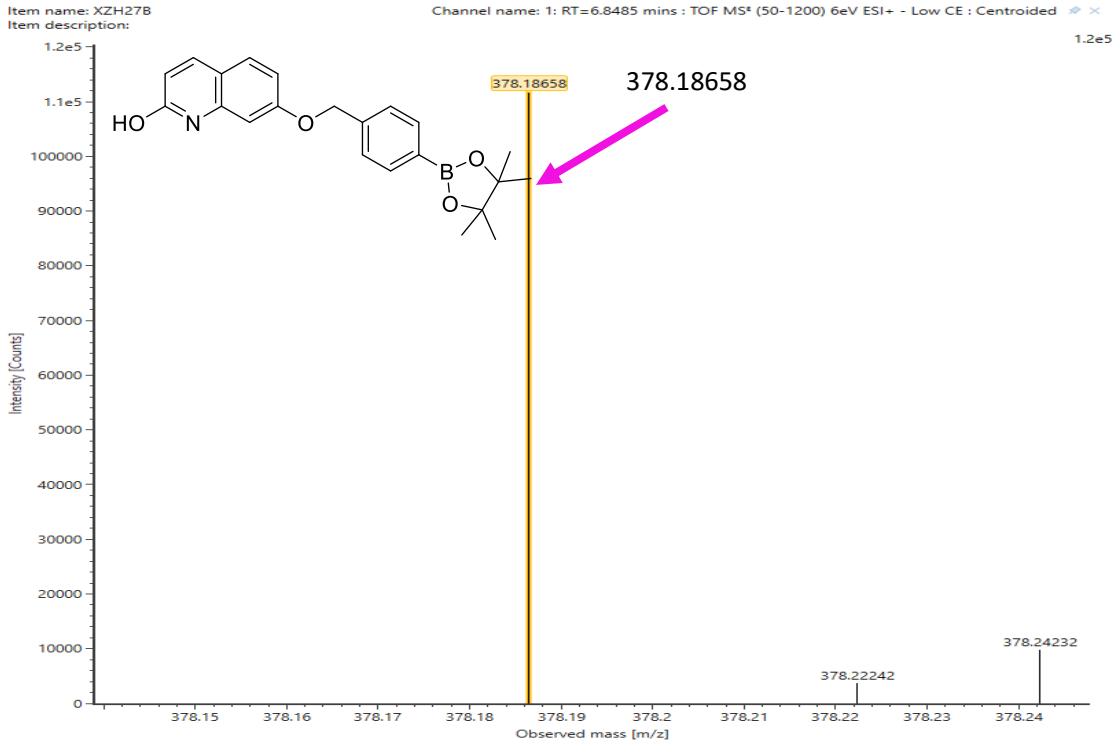
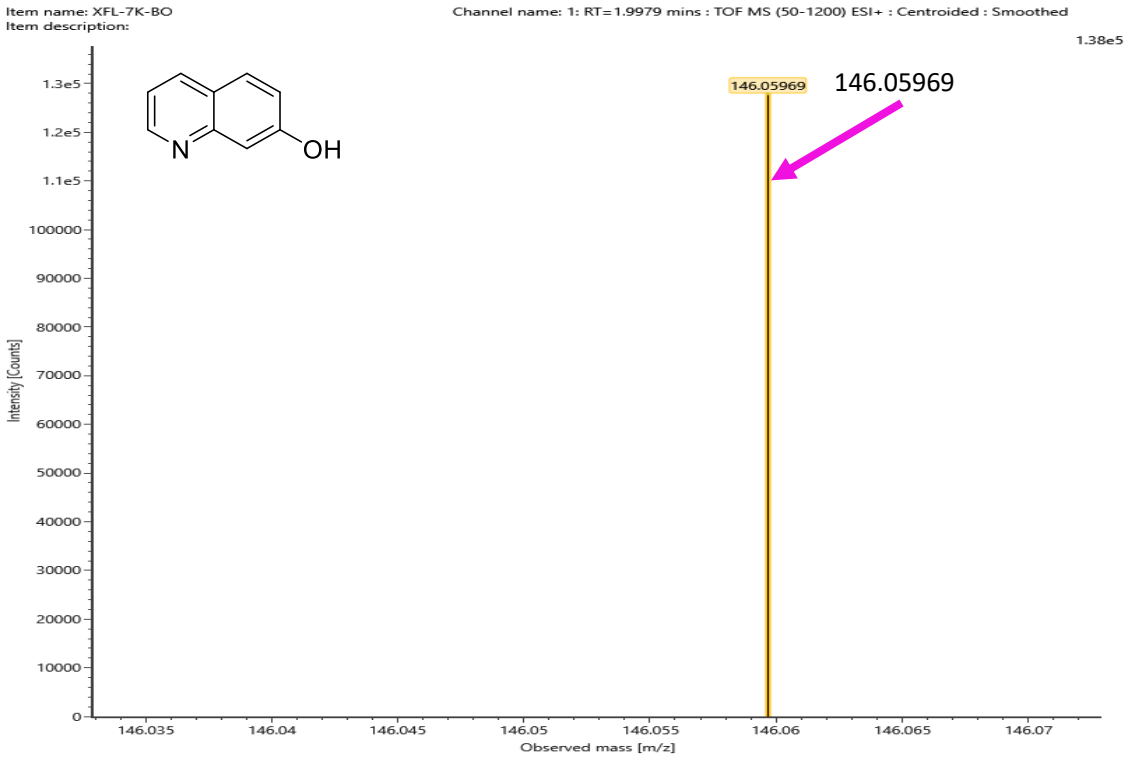


Figure S8. HRMS data of 3b

quinolin-7-ol



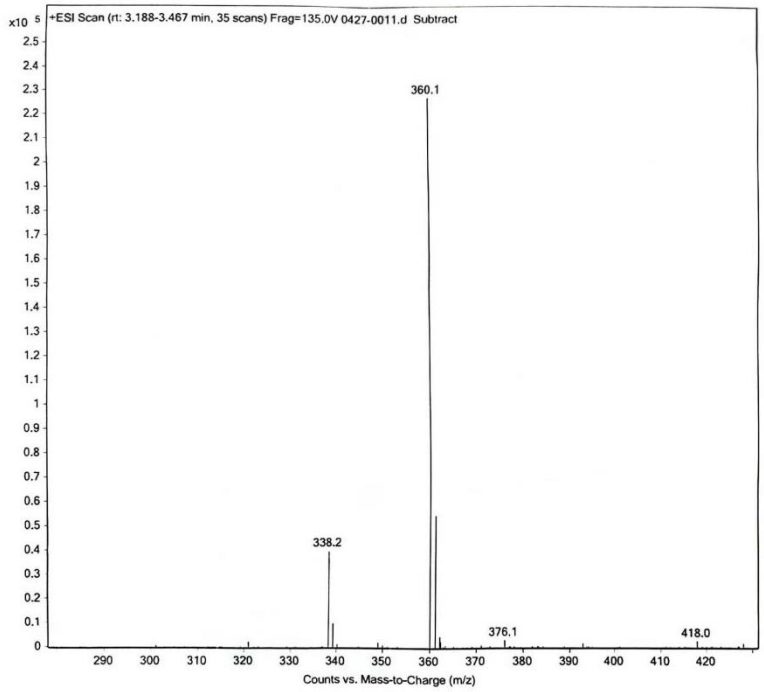


Figure S9. MS data of 3a-OH and 3a-COOH

quinoline-2,7-diol

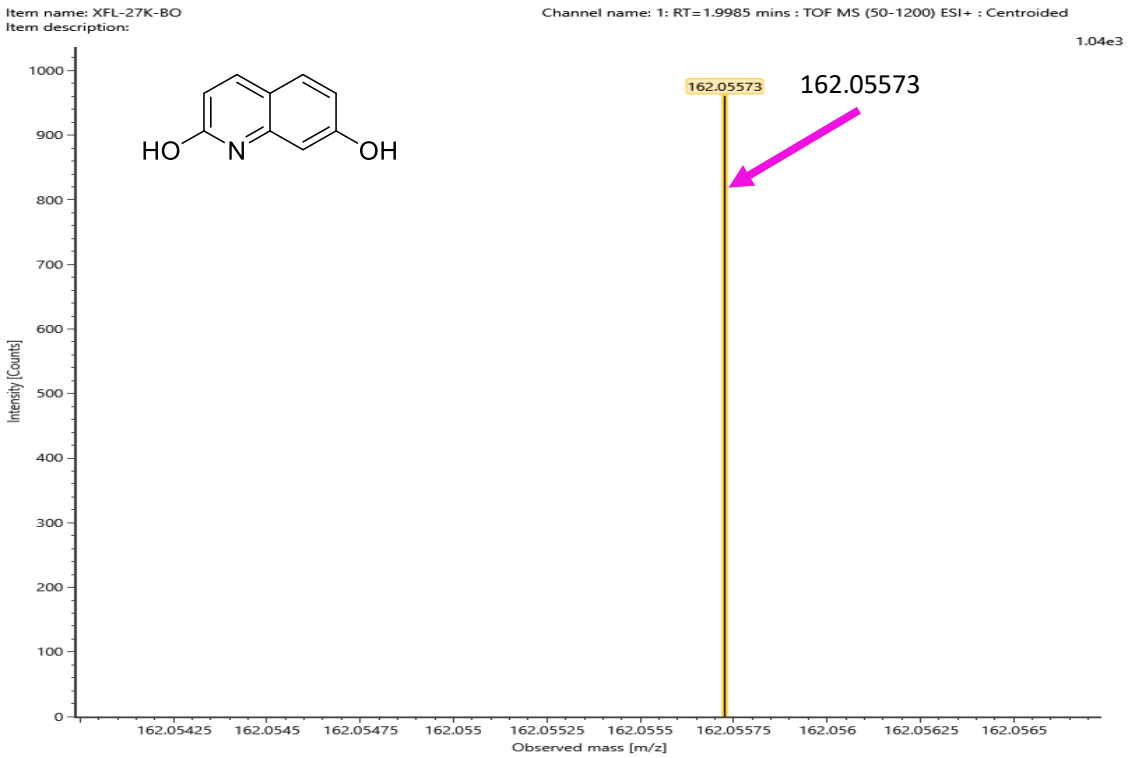


Figure S10. HRMS data of 3b-OH

VII. Fluorescence change diagram

1. With the increase of $ONOO^-$ concentration, the fluorescence intensity of **3a** and **3b** increased significantly（$ONOO^-$: 0-10 equiv）, while with the continuous increase of $ONOO^-$ concentration, the fluorescence intensity decreased gradually（$ONOO^-$: 15-50 equiv）. The fluorescence changes are shown in figure S11-S12.

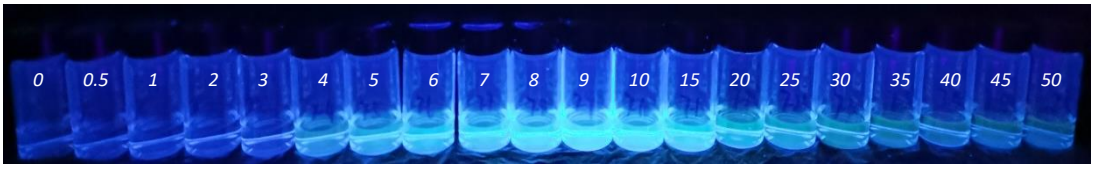


Figure S11. Fluorescence change of **3a** with the increase of $ONOO^-$ concentration

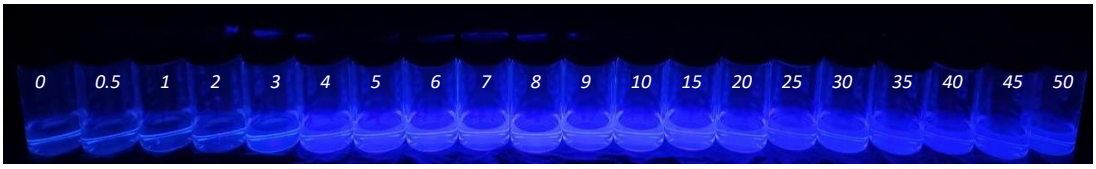


Figure S12. Fluorescence change of **3b** with the increase of $ONOO^-$ concentration

2. Under the condition that other analytes coexist with $ONOO^-$, **3a-ONOO$^-$** can emit light yellow fluorescence and **3b-ONOO$^-$** can emit blue fluorescence. The fluorescence changes are shown in figure S13-S14.

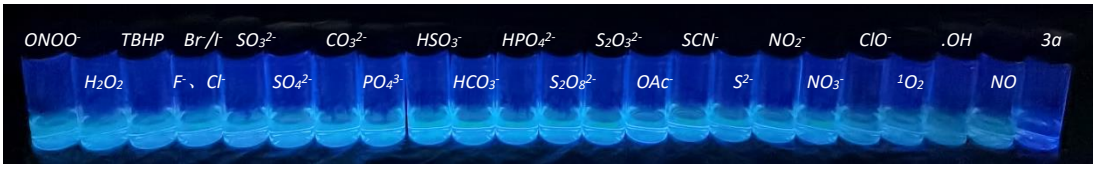


Figure S13-1. **3a-ONOO$^-$** fluorescence changes in coexistence with other active species and anions

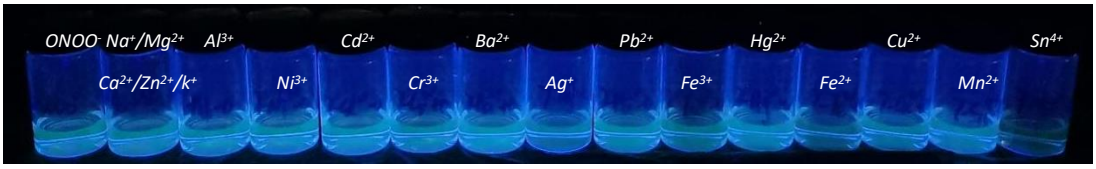


Figure S13-2. **3a-ONOO$^-$** fluorescence change diagram when coexisting with cations

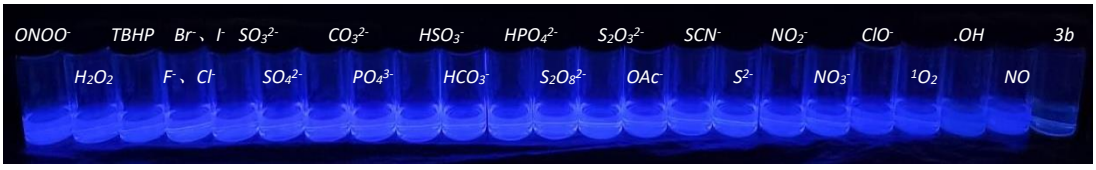


Figure S14-1. **3b-ONOO$^-$** fluorescence changes in coexistence with other active species and anions

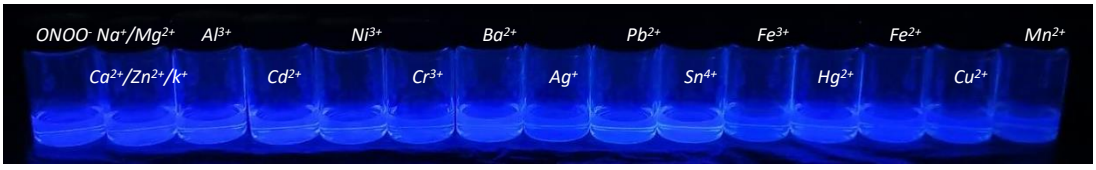


Figure S14-2. **3b-ONOO$^-$** fluorescence change diagram when coexisting with cations