Supporting Information

A rhodamine hydrazide-based fluorescent probe for sensitive and selective detection of hypochlorous acid and its application in living cells

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1. UV-vis absorption spectra of probe **RGNH** with different levels of HOCl

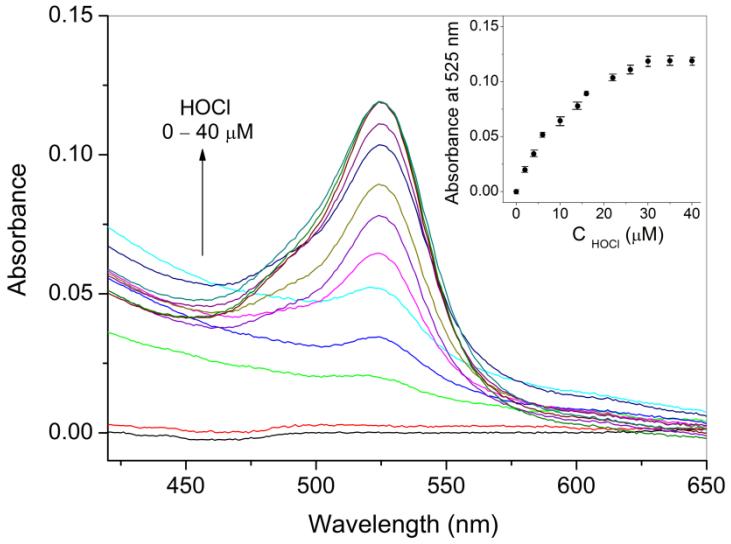


Fig. S1 UV-vis absorption spectra of probe **RGNH** (10 μM) with different levels of HOCl (bottom to top: 0, 2, 4, 6, 10, 14, 16, 22, 26, 30, 35, 40 μM) in PBS (10 mM, pH 7.4, 1% ethanol, v/v). Inset: Absorbance changes of probe **RGNH** (10 μM) at 525 nm as a function of HOCl concentration.

2. Effect of the ratio of ethanol to water on the fluorescence response of probe **RGNH** to HOCl

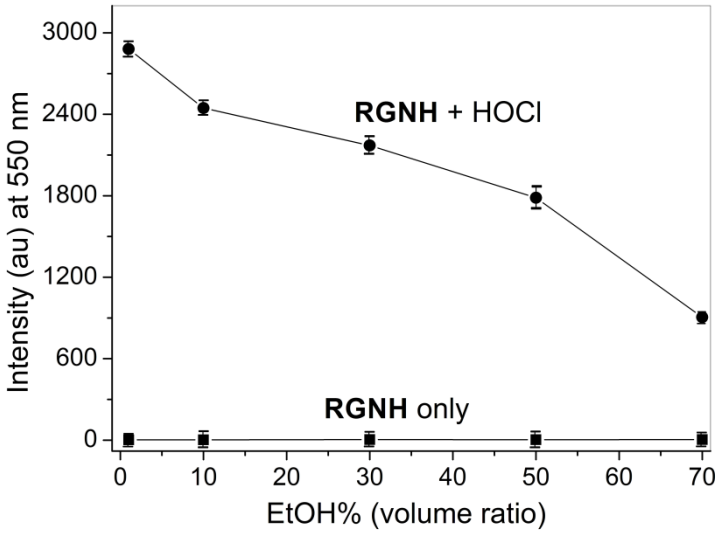


Fig. S2 Effect of the ratio of ethanol to water on the fluorescence intensity (550 nm) of probe **RGNH** (10 μM) in the absence and presence of HOCl (20 μM) in PBS (10 mM, pH 7.4). λ_{ex} = 500 nm.

3. Kinetic behavior of the fluorescence intensity changes of probe **RGNH** with HOCl

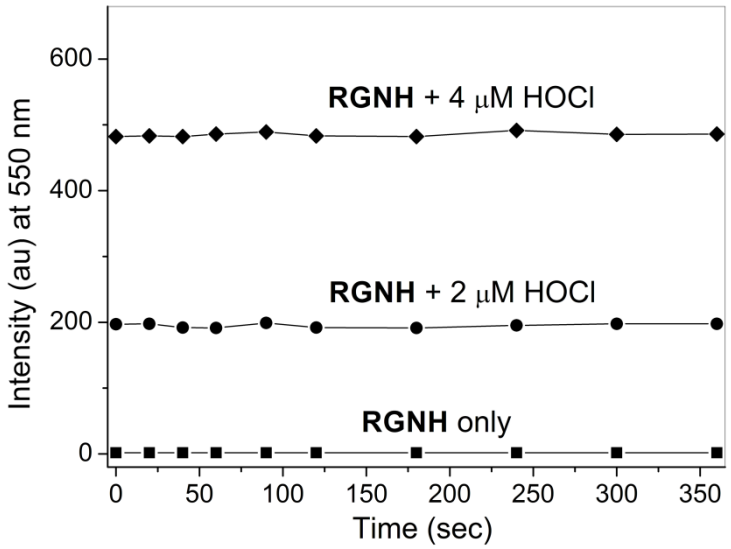


Fig. S3 Kinetic behavior of the fluorescence intensity changes (550 nm) of probe **RGNH** (10 μM) in the absence and presence of HOCl (2, or 4 μM) in PBS (10 mM, pH 7.4, 1% ethanol, v/v). λ_{ex} = 500 nm.

4. Fluorescence intensity changes of probe **RGNH** with HOCl and other ROS/RNS

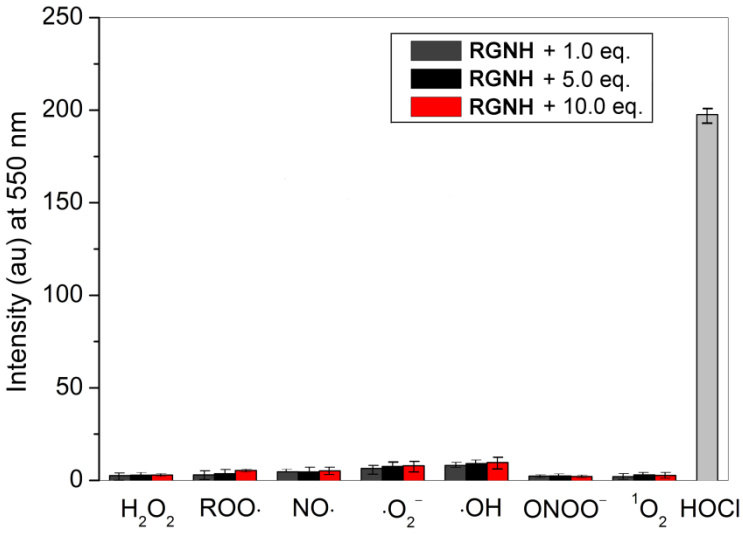
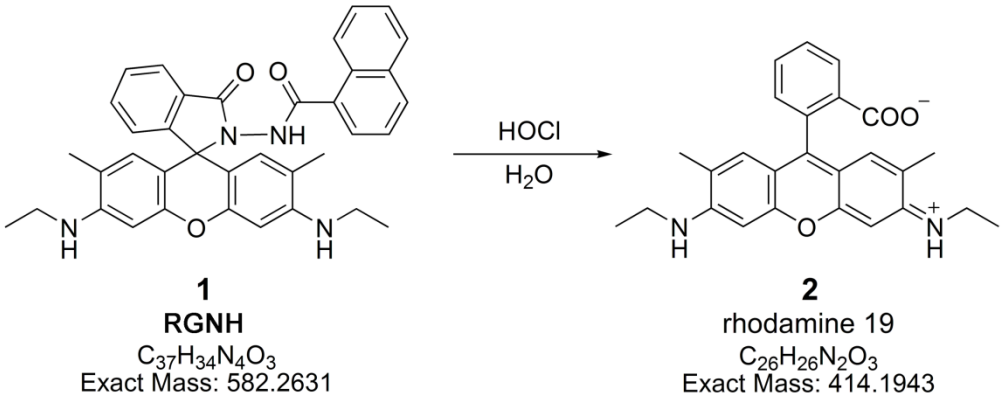


Fig. S4 Fluorescence intensity changes (550 nm) of probe **RGNH** (10 μM) upon addition of HOCl (2 μM) or other ROS/RNS (10, 50, 100 μM) in PBS (10 mM, pH 7.4, 1% ethanol, v/v). λ_{ex} = 500 nm.

5. Color changes of probe **RGNH** with HOCl and other ROS/RNS

Fig. S5 Color changes of probe **RGNH** (10 μM) upon addition of HOCl (20 μM), other ROS/RNS (100 μM), or HOCl (20 μM) plus L-methionine (200 μM).

6. Analysis of the reaction product between probe **RGNH** and HOCl by high-resolution ESI-MS



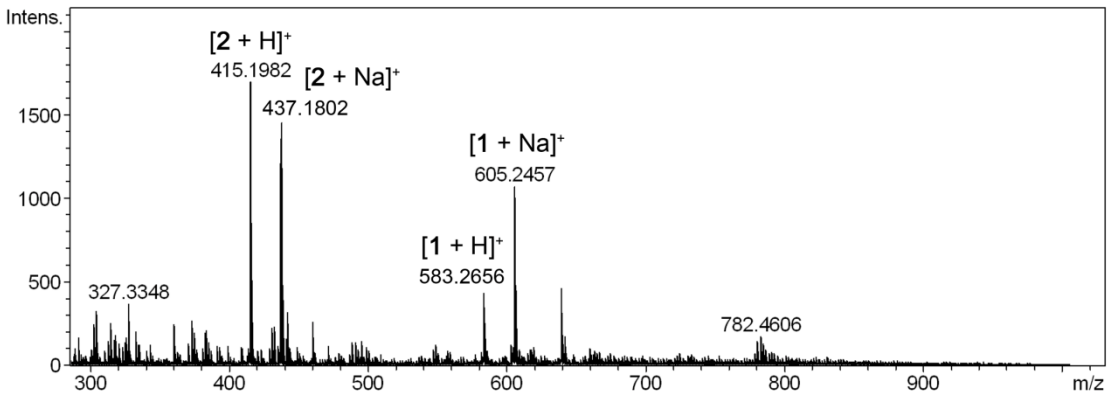
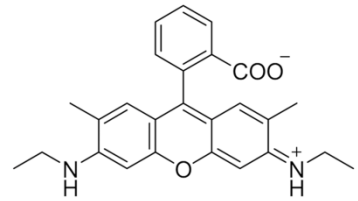


Fig. S6 High-resolution ESI-MS spectrum of probe **RGNH** in the presence of HOCl.

7. ESI-MS and ^{1}H NMR spectra of rhodamine 19, the product isolated from the reaction of probe **RGNH** with HOCl via column chromatography



rhodamine 19
$C_{26}H_{26}N_2O_3$
Mol. Wt.: 414.5

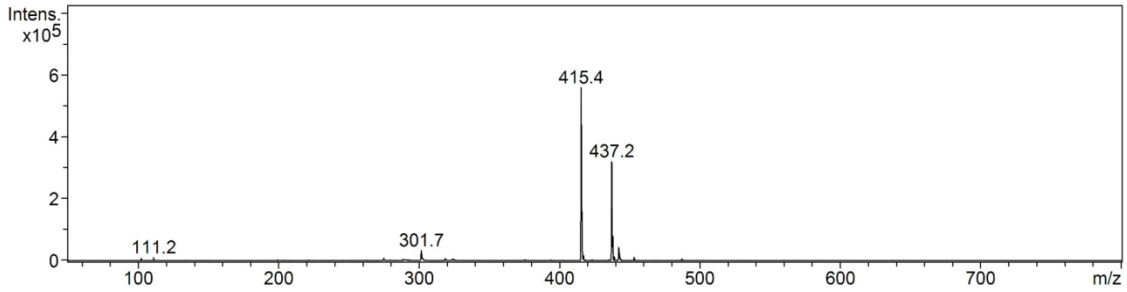


Fig. S7 ESI-MS spectrum of rhodamine 19.

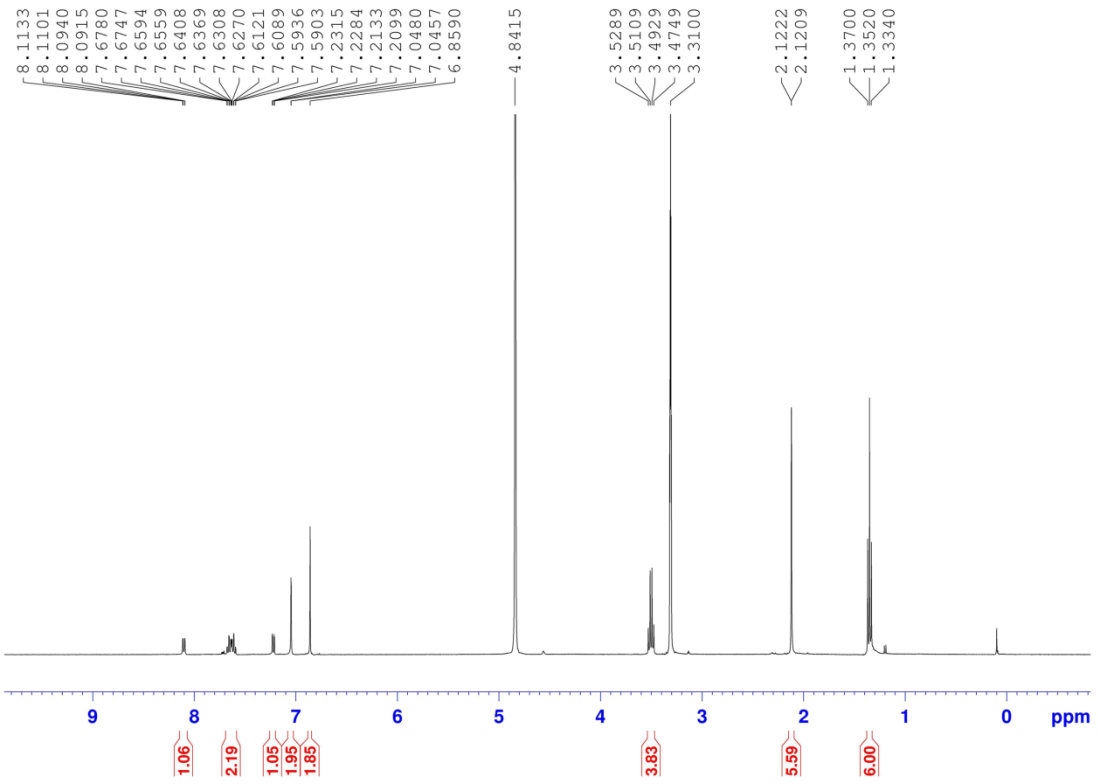
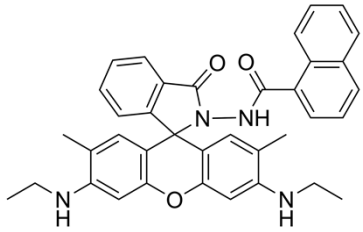


Fig. S8 ^{1}H NMR spectrum of rhodamine 19.

8. ESI-MS, high-resolution ESI-MS, ^{1}H NMR, and ^{13}C NMR spectra of probe **RGNH**



RGNH
$C_{37}H_{34}N_4O_3$
Mol. Wt.: 582.7
Exact Mass: 582.2631

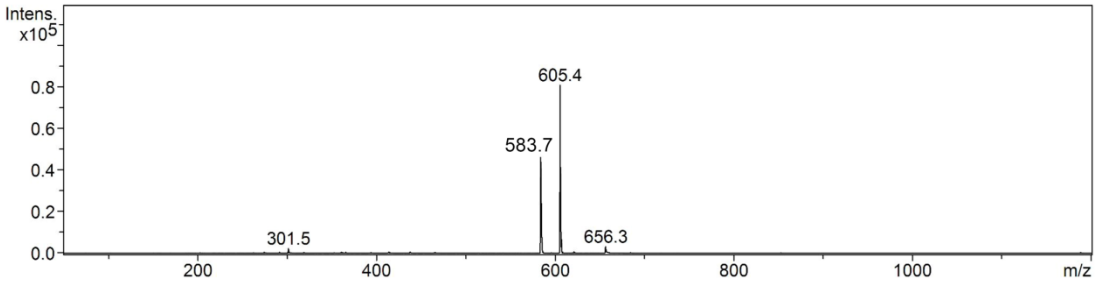


Fig. S9 ESI-MS spectrum of **RGNH**.

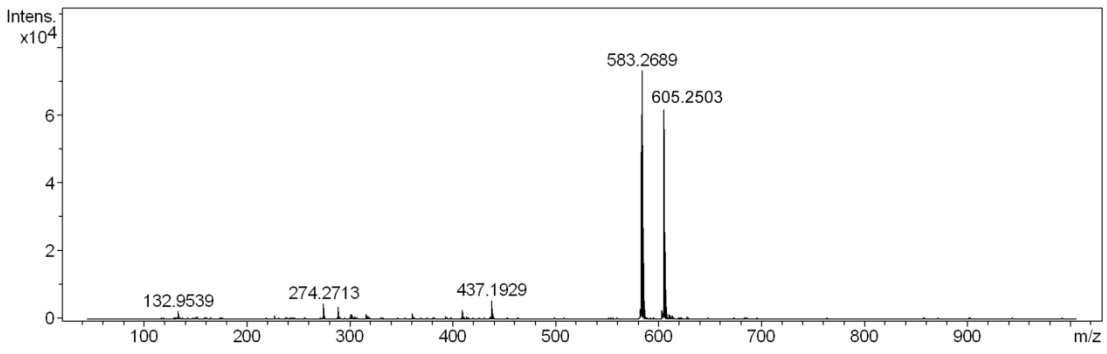


Fig. S10 High-resolution ESI-MS spectrum of **RGNH**.

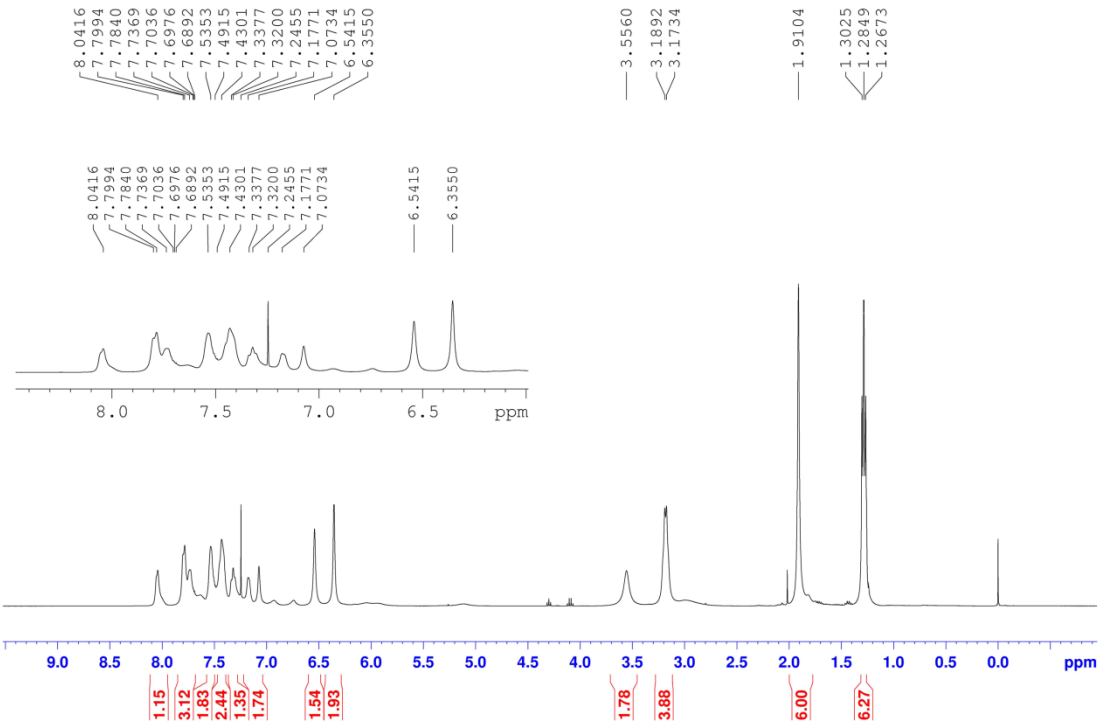


Fig. S11 [1]H NMR spectrum of **RGNH**.

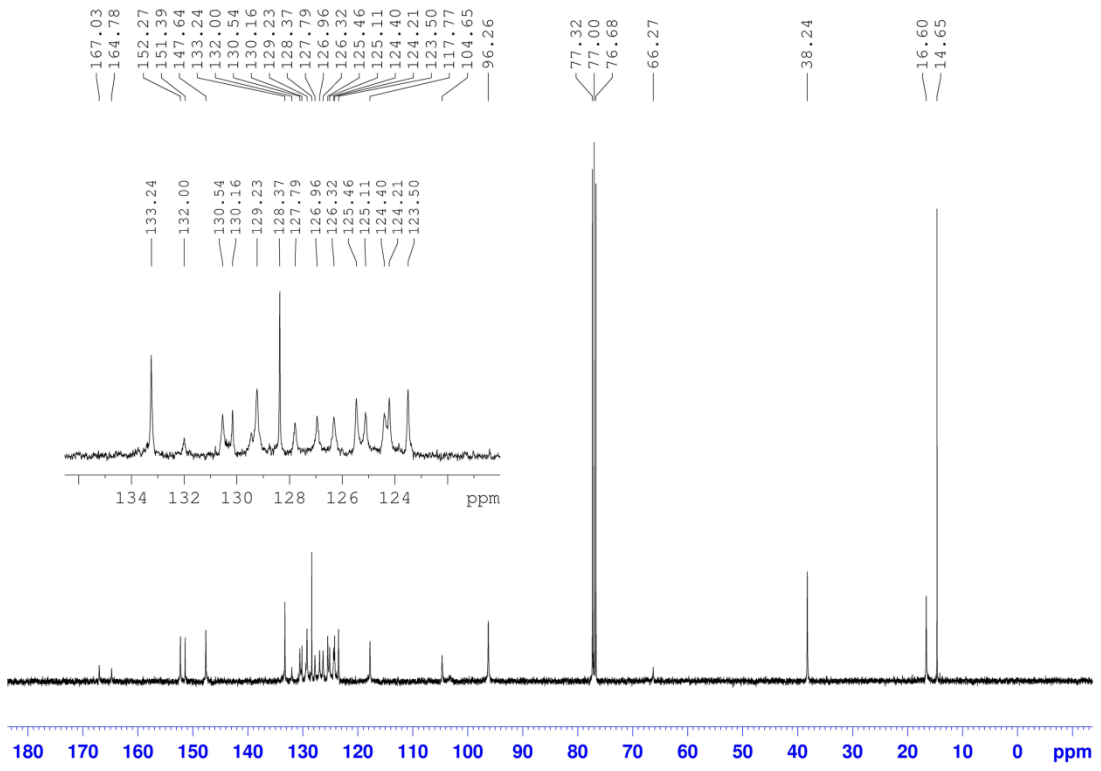
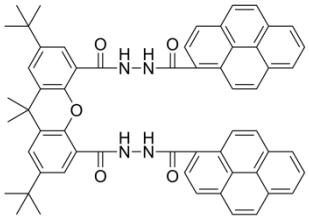
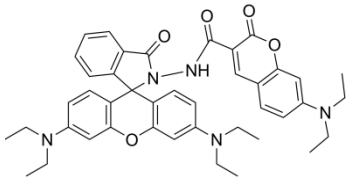
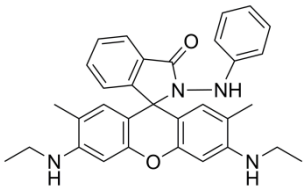
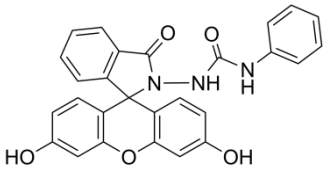
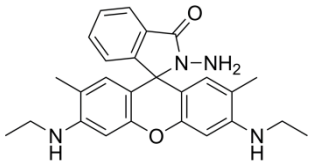
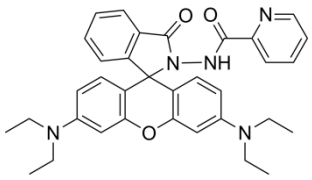
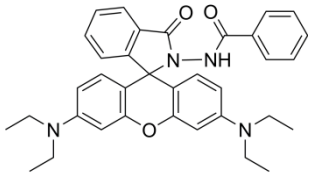
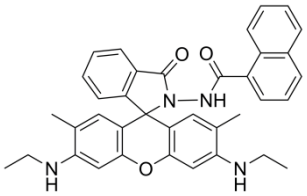


Fig. S12 [13]C NMR spectrum of **RGNH**.

9. Comparison of the recently reported hydrazide-based fluorescent probes for HOCl or OCl^-

Table S1. Comparison of the recently reported hydrazide-based fluorescent probes for HOCl or OCl^-.

Probe	Response time	Detection limit	pH span of detection	Comments
 *Anal. Methods*, 2014, **6**, 609	30 min	0.35 μM	5–9	HEPES buffer (pH 7.4) containing 50% (v/v) ethanol I_{375}/I_{495} = 152 fold Ratiometric fluorescent probe
 *Anal. Chim. Acta*, 2013, **775**, 100	within 1 min	0.024 μM	7.5–11	PBS buffer (pH 8.5) containing 40% (v/v) DMF I_{578}/I_{501} = 3955 fold Ratiometric fluorescent probe
 *Anal. Methods*, 2012, **4**, 616	more than 60 min	5.0 nM	6–11.3	water Fluorescent turn-on response I_{548} = over 82 fold 10 equiv Cu^{2+} and Hg^{2+} induced around 10-fold enhancement at 548 nm
 *Anal. Methods*, 2012, **4**, 4334	within 2 min	0.33 μM	no data available	PBS buffer (pH 7.4) with less than 1% ethanol Fluorescent turn-on response I_{528} = 156 fold Hg^{2+} induced 80-fold enhancement at 530 nm

Probe	Response time	Detection limit	pH span of detection	Comments
 *Talanta*, 2011, **85**, 779	10 min	0.06 μM	4.5–8	HEPES buffer (pH 7.4) containing 1% (v/v) DMF Fluorescent turn-on response I_{550} = 270 fold Cu^{2+} induced 100-fold enhancement at 550 nm Proton (especially under acid conditions, pH < 6) induced great enhancement at 550 nm Imaging both exogenous and endogenous HOCl in living cells Low cytotoxicity when probe concentration below 100 μM
 *Org. Biomol. Chem.*, 2011, **9**, 432	10 s	~ 1.0 nM	4–13	$Na_2B_4O_7$/NaOH buffer (pH 12.0) containing 70% (v/v) methanol Fluorescent turn-on response I_{580} > 420 fold
 *Chem. Eur. J.*, 2008, **14**, 4719	30 min	27 nM	9.2–12.7	$Na_2B_4O_7$/NaOH buffer (pH 12.0) containing 30% (v/v) THF Fluorescent turn-on response centered at 578 nm
 *This work*, **RGNH**	completed immediately after the sample solutions were prepared	3.3 nM	5–14	PBS buffer (pH 7.4) with 1% (v/v) ethanol Fluorescent turn-on response I_{550} = 1890 fold Imaging both exogenous and endogenous HOCl in living cells Flow cytometry analysis of endogenous HOCl in living cells Low cytotoxicity when probe concentration below 100 μM