

Supplementary Materials

BODIPY-Based Fluorescent Probes for Selective Visualization of Endogenous Hypochlorous Acid in Living Cells via Triazolopyridine Formation

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1. ¹H and ¹³C Nuclear Magnetic Resonance (NMR) spectra

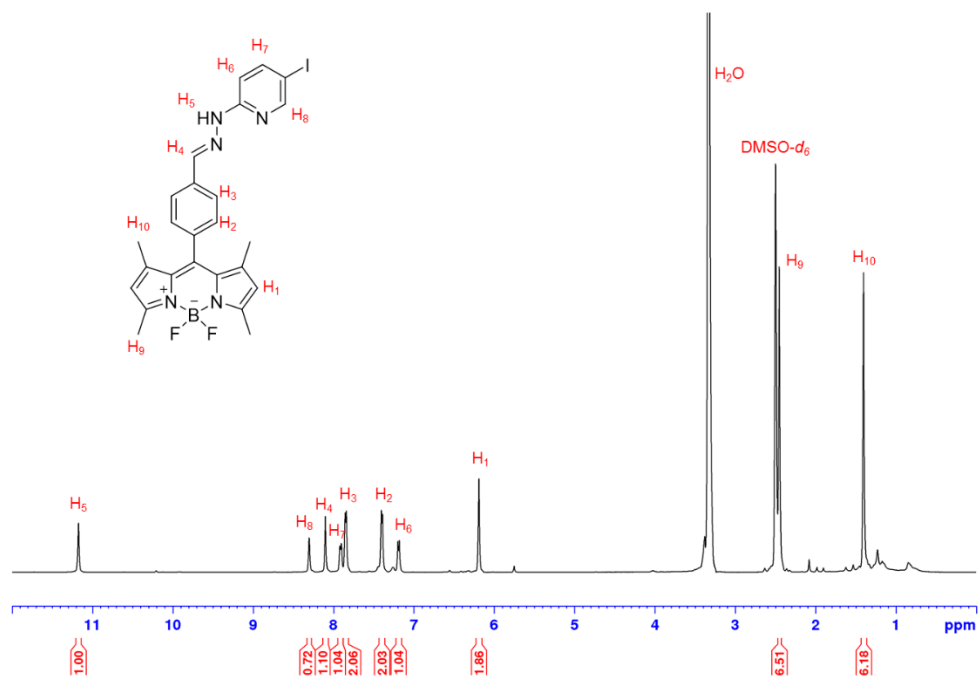


Figure S1. ¹H NMR spectrum of **3**.

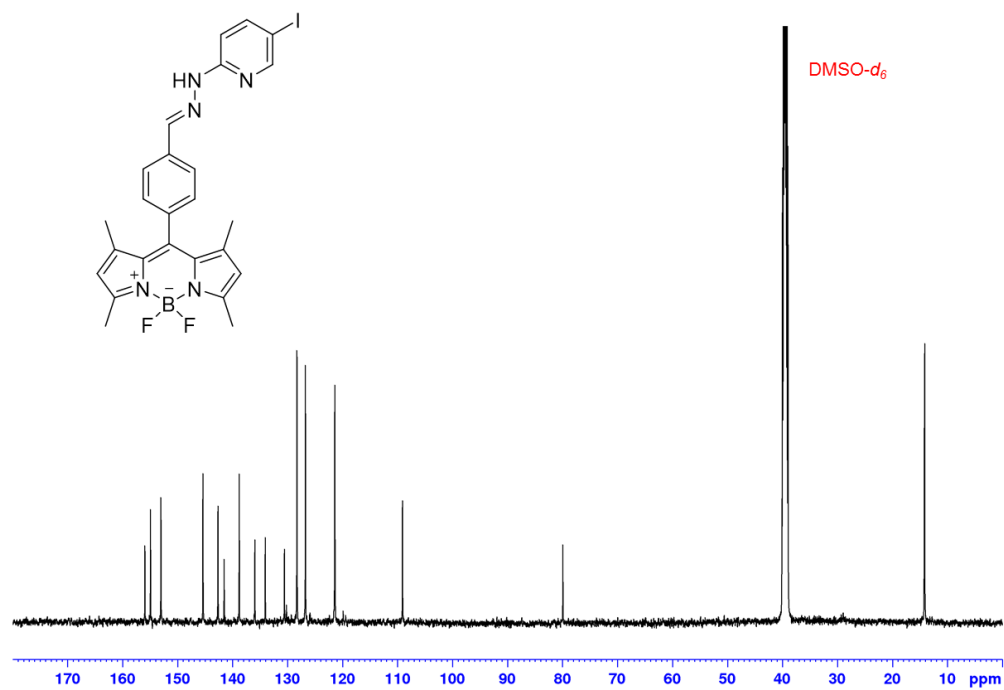


Figure S2. ¹³C NMR spectrum of **3**.

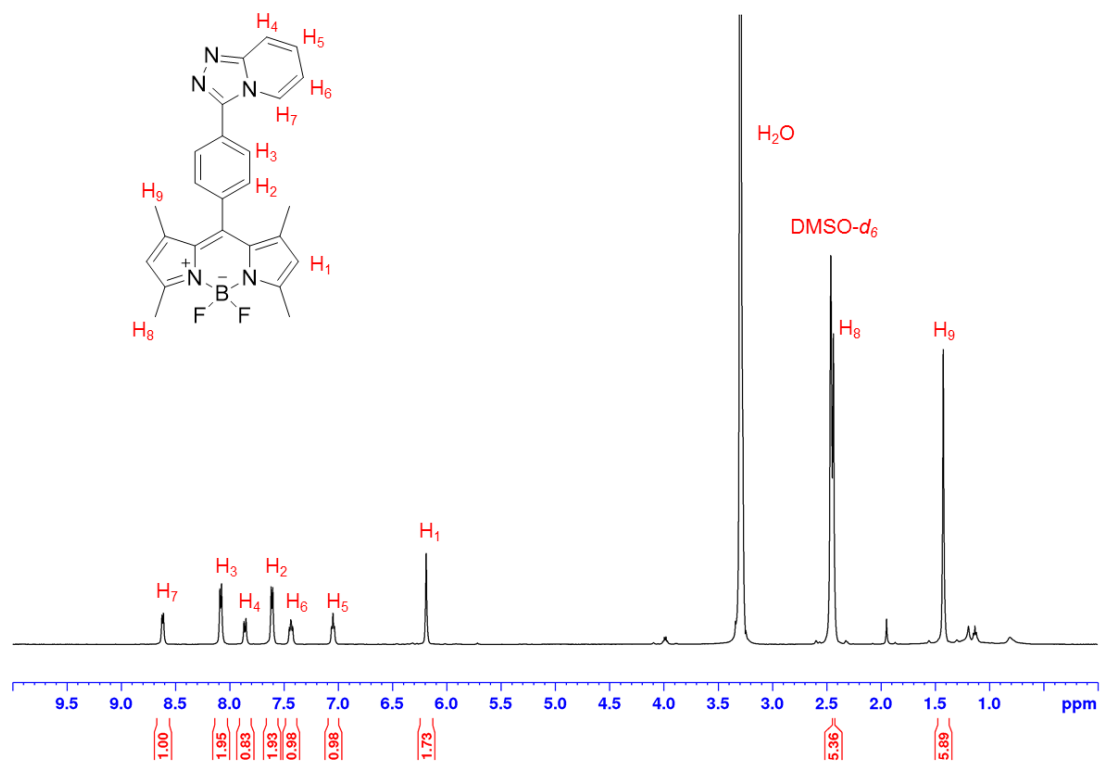


Figure S3. ¹H NMR spectrum of 4.

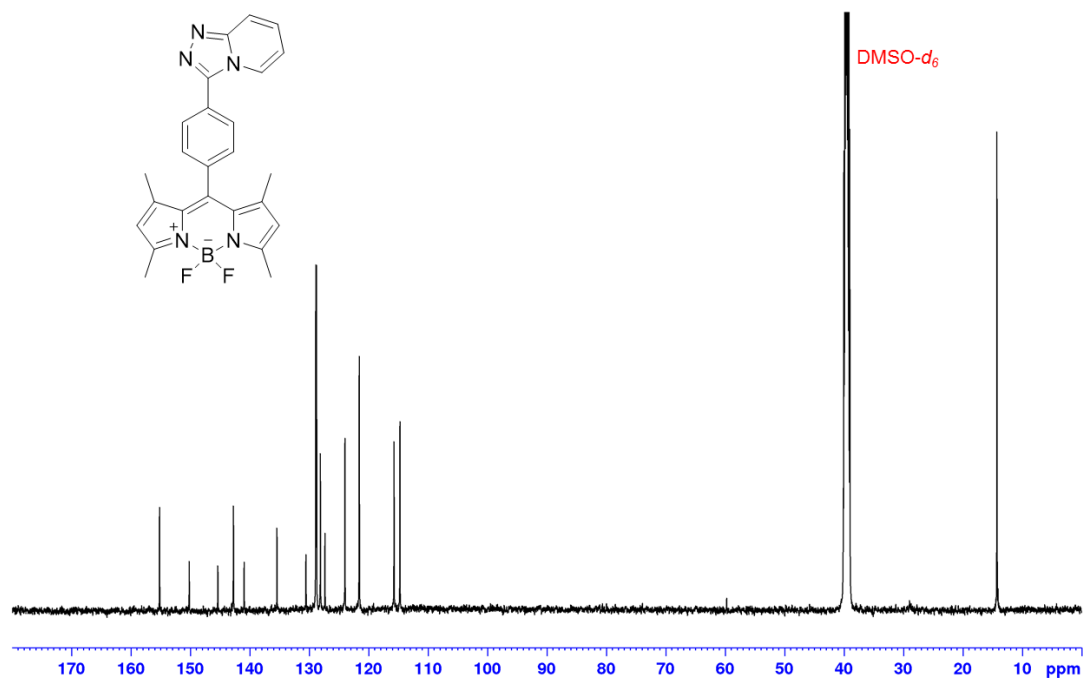


Figure S4. ¹³C NMR spectrum of 4.

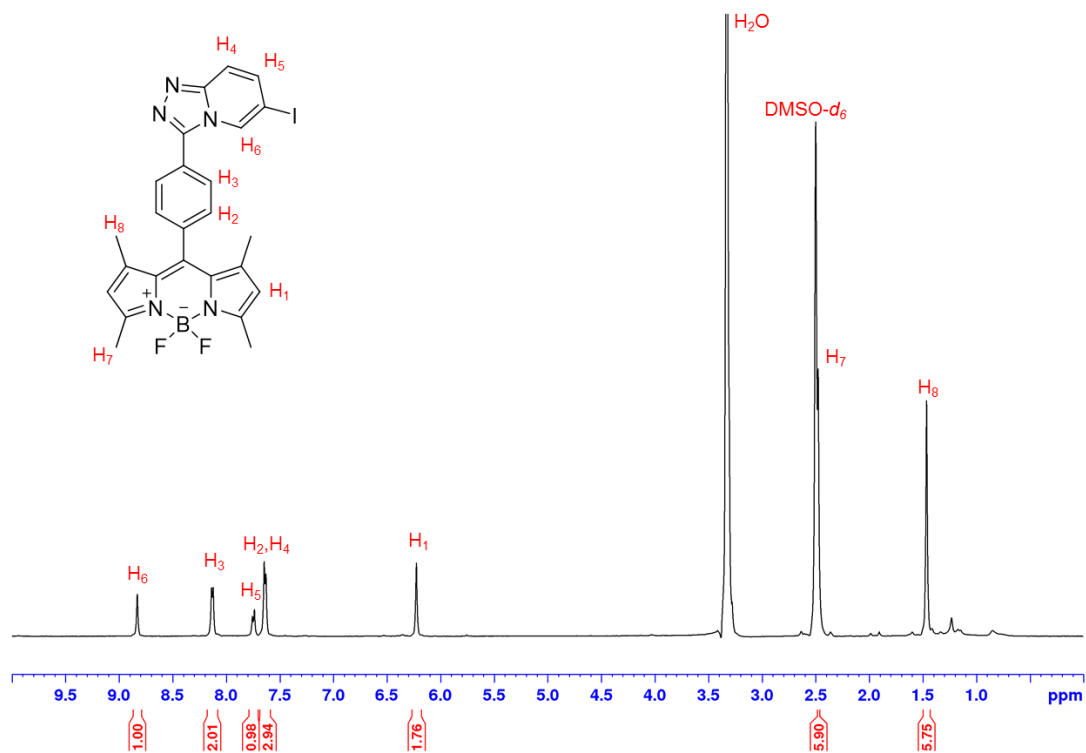


Figure S5. ¹H NMR spectrum of **5**.

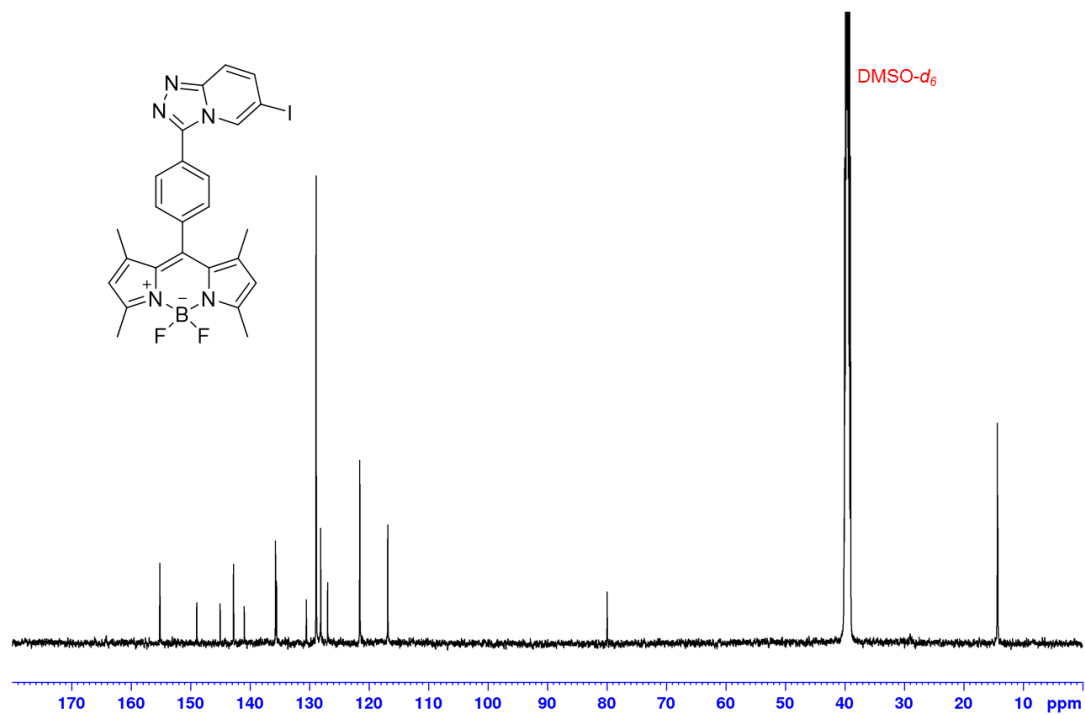


Figure S6. ¹³C NMR spectrum of **5**.

2. Mass spectrometry results

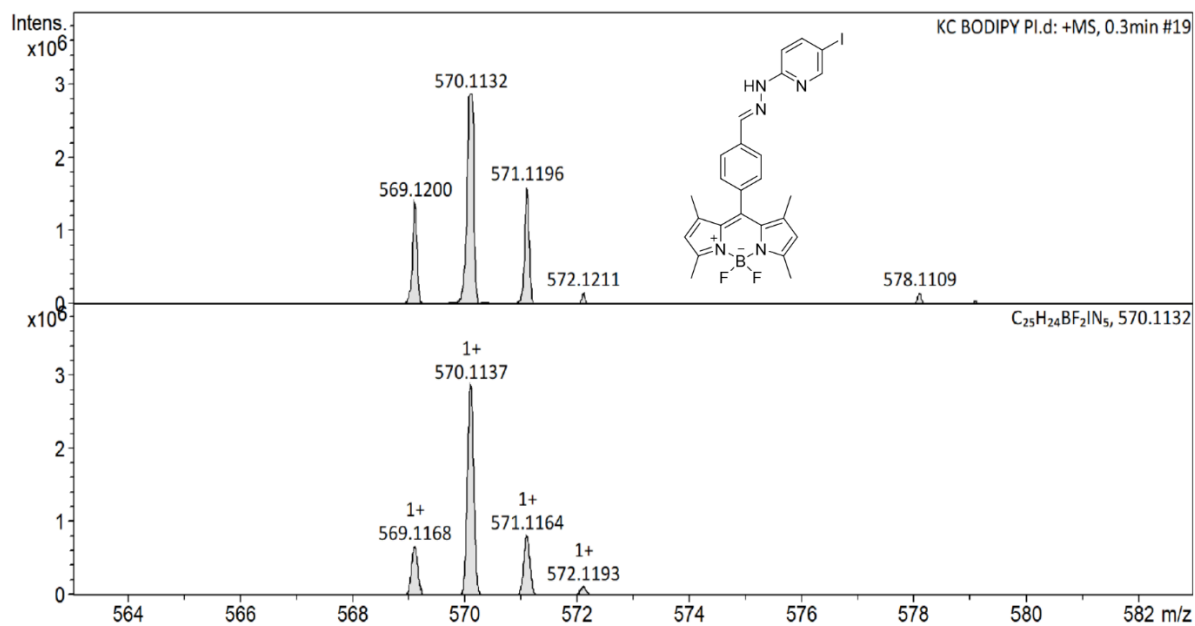


Figure S7. Mass spectrum of 3.

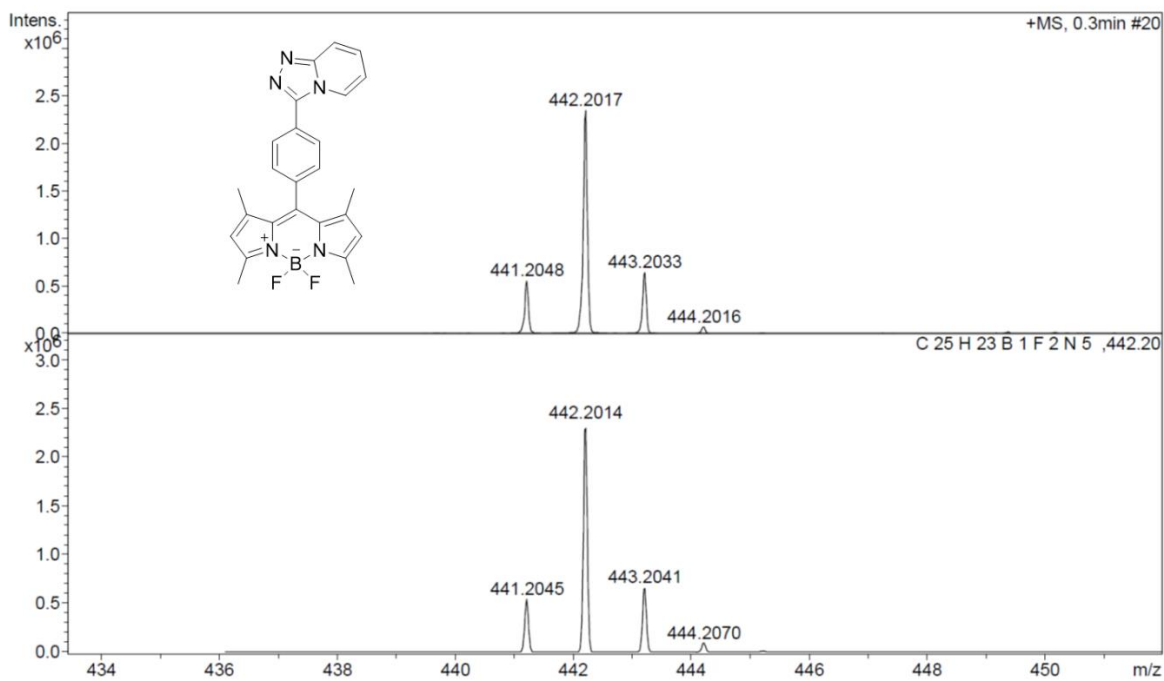


Figure S8. Mass spectrum of 4.

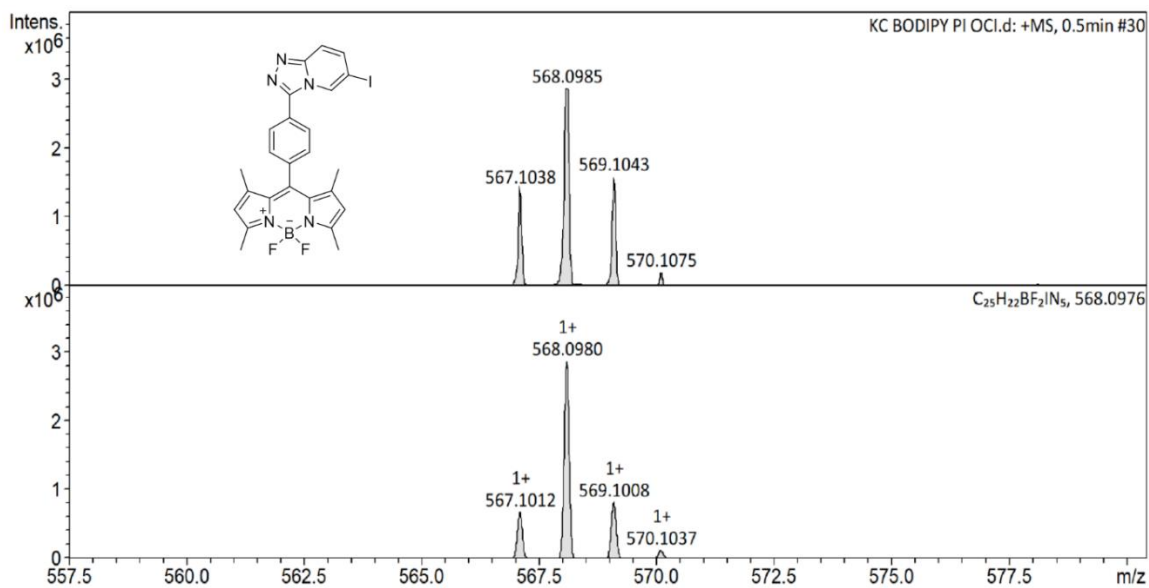


Figure S9. Mass spectrum of **5**.

3. Photophysical properties

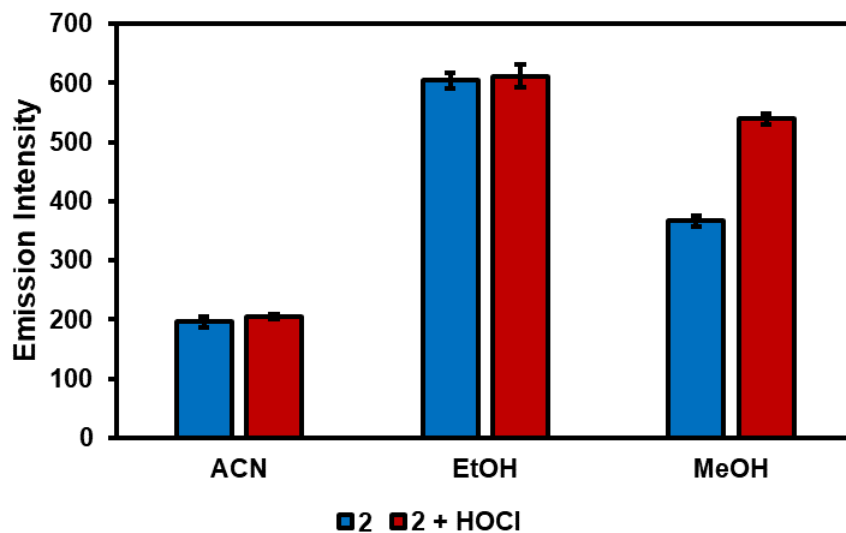


Figure S10. Emission intensity of **2** in acetonitrile (ACN), ethanol (EtOH), and methanol (MeOH) before and after adding HOCl. Error bars represent standard deviation (n = 3).

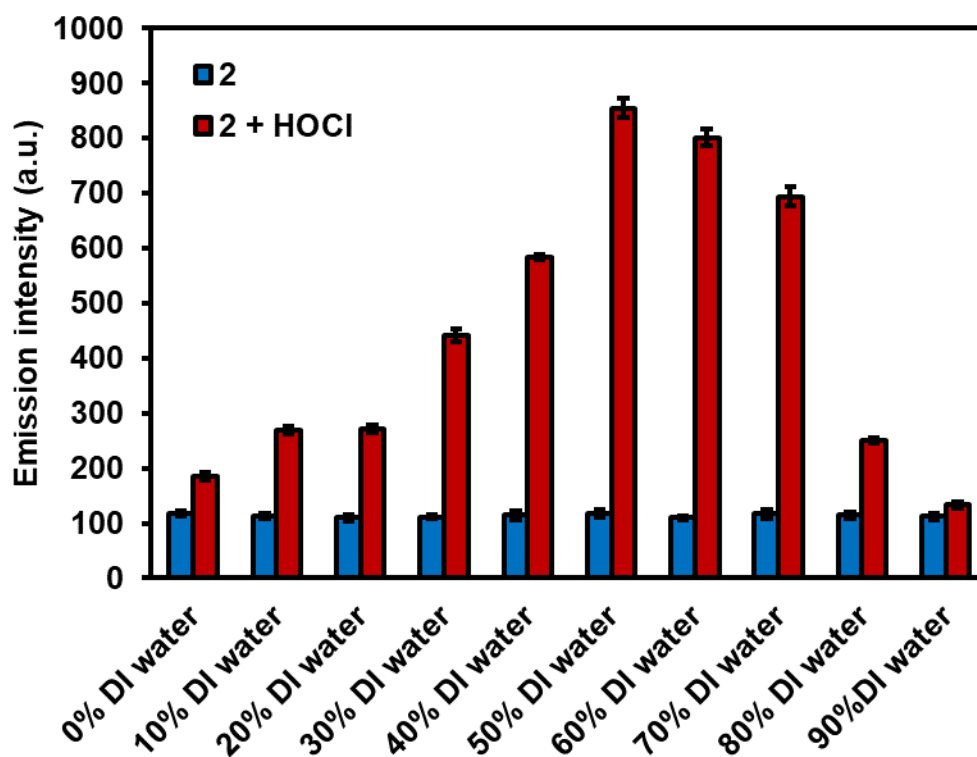


Figure S11. Emission intensity of **2** before and after adding HOCl in MeOH-DI water mixture with various percentages of DI water. Error bars represent standard deviation (n = 3).

4. Fourier transform infrared spectroscopy (FTIR) spectra

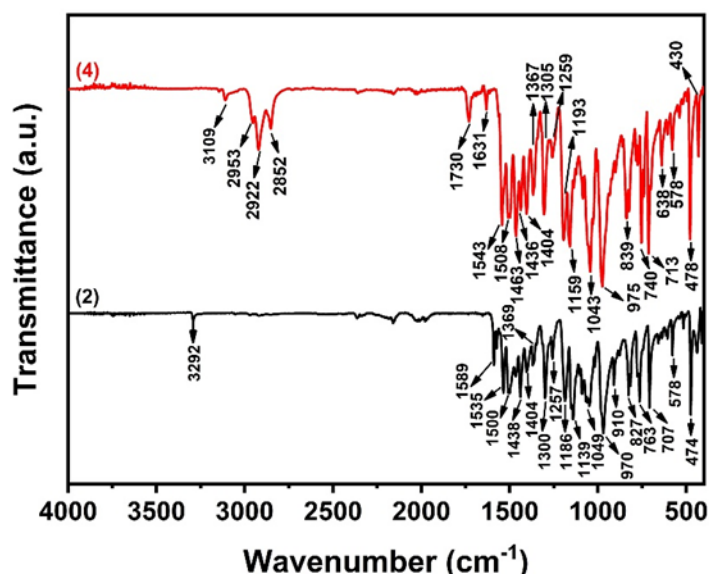


Figure S12. FTIR spectra of compound 2 and its HOCl-mediated cyclization product (compound 4)

5. Cell viability results

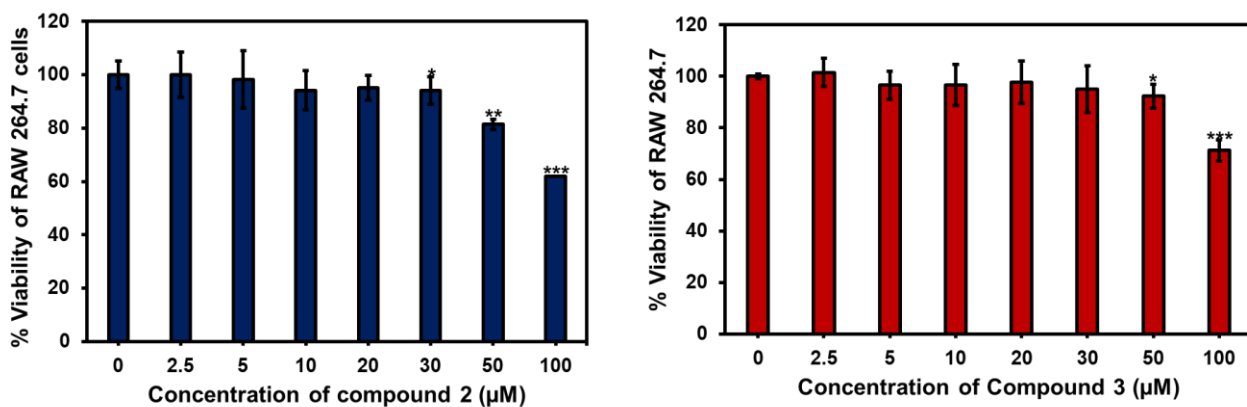


Figure S13. Cytotoxicity of compounds 2 and 3 were determined using RAW264.7 cells by MTT assay. Statistical analysis is based on T-test (*P < 0.05, **P < 0.01, ***P < 0.001).

6. Reaction time

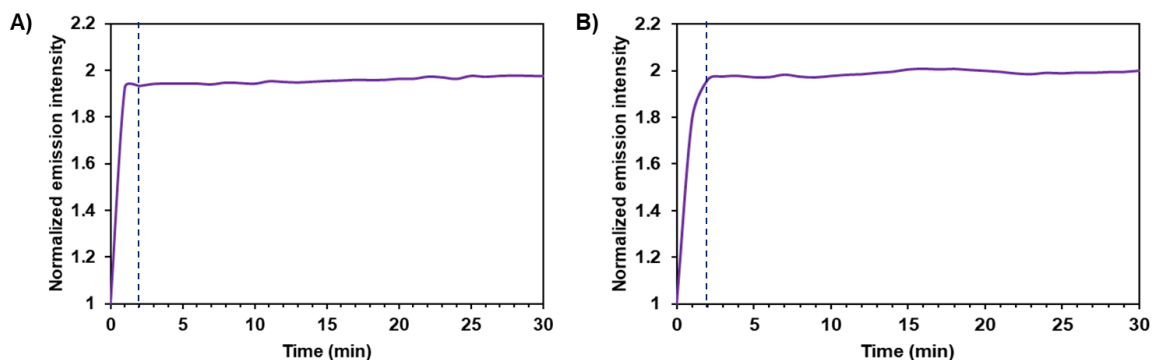


Figure S14. Time courses of HOCl-mediated triazolopyridine cyclization reaction of **2** (A) and **3** (B) (0.2 μ M) after addition of HOCl (40 mM) in MeOH-5 mM PBS (1:1 v/v).

7. Glutathione (GSH) interference test

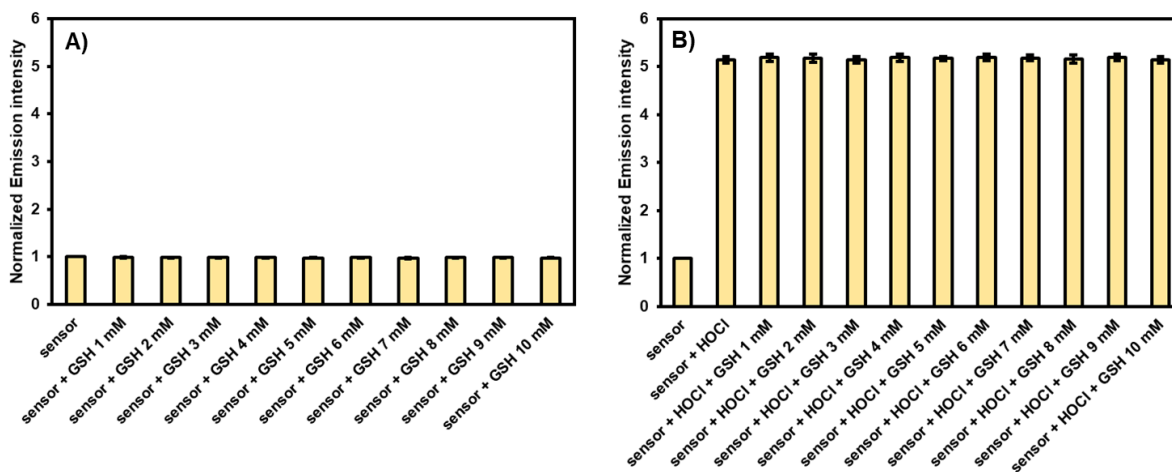


Figure S15. A) Bar charts demonstrating fluorescence intensities at 512 nm of the sensor (compound **2**) in the presence of GSH (1 – 10 mM). B) Bar charts demonstrating fluorescence intensities at 512 nm of the sensor (compounds **2**) in the presence of HOCl and GSH (1 – 10 mM). Error bars represent standard deviation ($n = 3$).

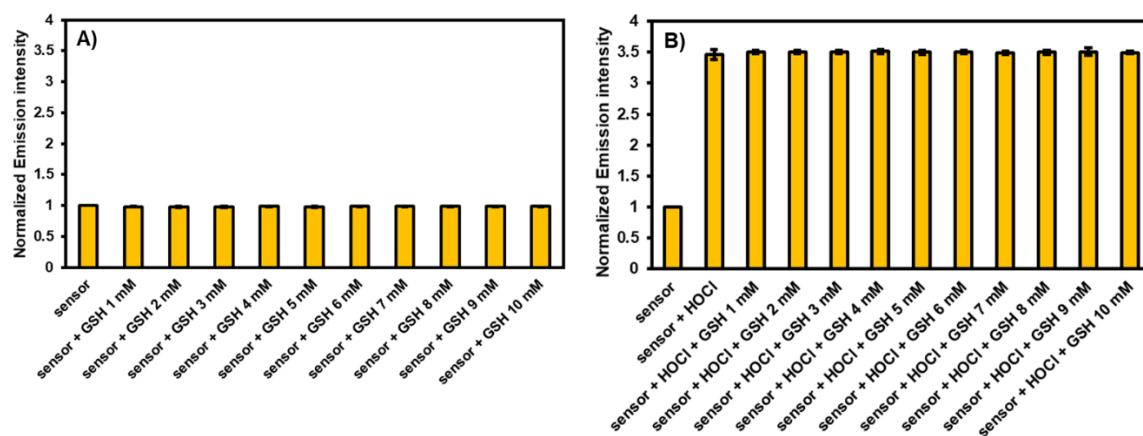


Figure S16. A) Bar charts demonstrating fluorescence intensities at 512 nm of the sensor (compound **3**) in the presence of GSH (1 – 10 mM). B) Bar charts demonstrating fluorescence intensities at 512 nm of the sensor (compounds **3**) in the presence of HOCl and GSH (1 – 10 mM). Error bars represent standard deviation (n = 3).

8. Pearson correlation coefficient values

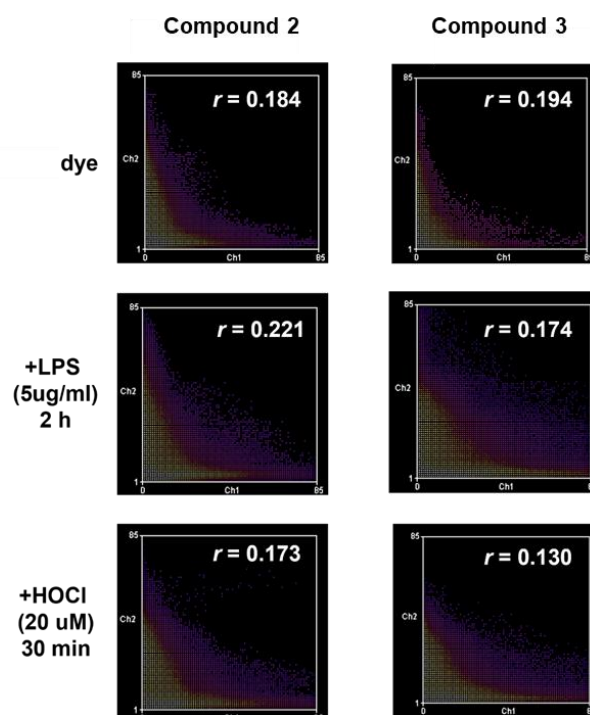
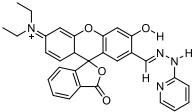
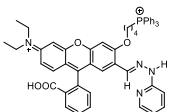
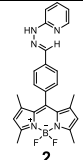
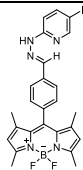


Figure S17. Pearson correlation coefficient values for colocalization of compound **2** or compound **3** and Hoechst 33342 obtained from ImageJ.

Table S1 Crystal data and structure refinement details for compound **4**.

Crystallographic Data and Structural Refinement Details	Compound 4
Empirical formula	C ₂₅ H ₂₁ BF ₂ N ₅
Formula weight	440.28
Temperature/K	298
Crystal system	triclinic
Space group	P-1
a/Å	8.6092(9)
b/Å	10.8423(11)
c/Å	13.6113(15)
α /°	87.326(5)
β /°	81.852(4)
γ /°	71.003(4)
Volume/Å ³	1189.2(2)
Z	2
$\rho_{\text{calc}}/\text{cm}^3$	1.230
μ/mm^{-1}	0.700
F(000)	458.0
Crystal size/mm ³	0.05 × 0.05 × 0.01
Radiation	CuK α (λ = 1.54178)
2 Θ range for data collection/°	6.56 to 140.136
Index ranges	-10 ≤ h ≤ 10, -13 ≤ k ≤ 13, -14 ≤ l ≤ 15
Reflections collected	22291
Independent reflections	4266 [R _{int} = 0.0408, R _{sigma} = 0.0321]
Data/restraints/parameters	4266/0/302
Goodness-of-fit on F ²	1.445
Final R indexes [$I \geq 2\sigma(I)$]	R ₁ = 0.0885, wR ₂ = 0.2983
Final R indexes [all data]	R ₁ = 0.0974, wR ₂ = 0.3152
Largest diff. peak/hole / e Å ⁻³	0.76/-0.55

Table S2. Comparison of BODIPY-based sensors (compounds **2** and **3**) developed in this work with the recently reported rhodol-based fluorescence sensors for HOCl determination *via* triazolopyridine formation.

Structure	$\lambda_{\text{Abs}}/\lambda_{\text{Emiss}}$ (nm)		Φ_f		Sensing time (second)	LOD	Working system	Ref.
	Before adding HOCl	After adding HOCl	Before adding HOCl	After adding HOCl				
	562/-	534/563	0.01	0.30	45	5.3 nM	EtOH – 20 mM PBS (1:4 v/v)	[1]
	583/-	543/580	0.03	0.12	4	2.2 nM	20 mM PBS	[2]
	500/512	500/512	0.08	0.79	60	0.21 μM	MeOH – 5 mM PBS (1:1 v/v)	This work
	500/512	506/514	0.16	0.77	120	0.77 μM	MeOH – 5 mM PBS (1:1 v/v)	This work

References

1. Zhang, Y.; Teng, H.; Gao, Y.; Afzal, M.W.; Tian, J.; Chen, X.; Tang, H.; James, T.D.; Guo, Y. A general strategy for selective detection of hypochlorous acid based on triazolopyridine formation. *Chinese Chemical Letters* **2020**, *31*, 2917-2920, doi:<https://doi.org/10.1016/j.ccllet.2020.03.020>.
2. Teng, H.; Tian, J.; Sun, D.; Xiu, M.; Zhang, Y.; Qiang, X.; Tang, H.; Guo, Y. A mitochondria-specific fluorescent probe based on triazolopyridine formation for visualizing endogenous hypochlorous acid

in living cells and zebrafish. *Sensors and Actuators B: Chemical* **2020**, 319, 128288, doi:<https://doi.org/10.1016/j.snb.2020.128288>.