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Electronic Supplementary Information

A new ratiometric fluorescent probe for detecting endogenous HClO in living cells

Li-Jie Zhang [a, c, 1], Jun-Ya Ning [b,1], Jun-Ying Miao [b], Jin-Ting Liu [a, *], Bao-Xiang Zhao [a, *]

[a] *Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, P.R. China.*
[b] *Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100,P.R. China.*
[c] *School of Chemistry, Sun Yat-Sen University, Guangzhou 510220, P.R. China.*

[1] The two authors contributed equally.

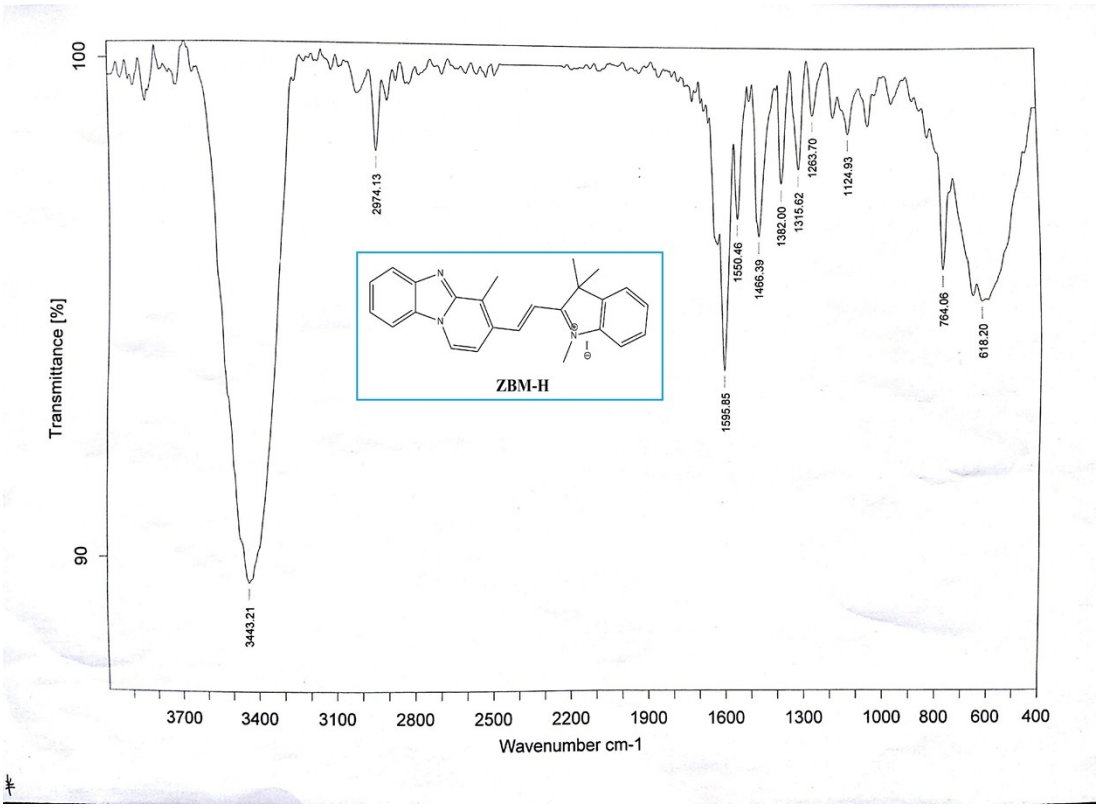


Fig. S1 Infrared spectrum of **ZBM-H**.

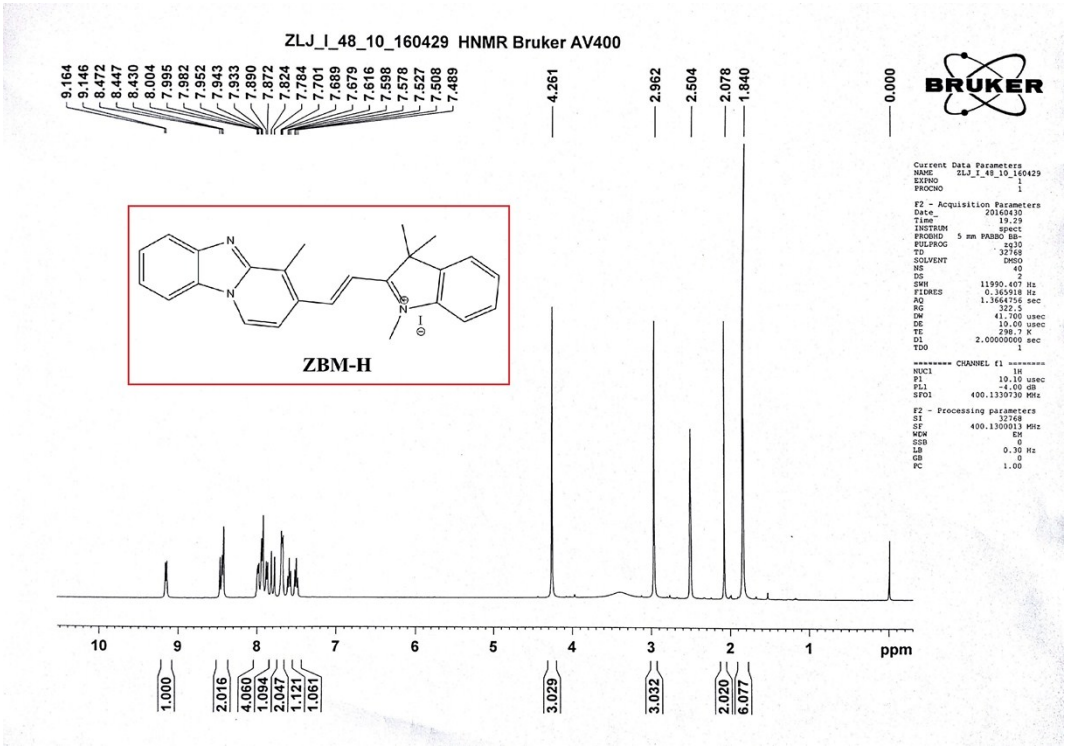


Fig. S2 ^{1}H NMR spectrum of **ZBM-H**.

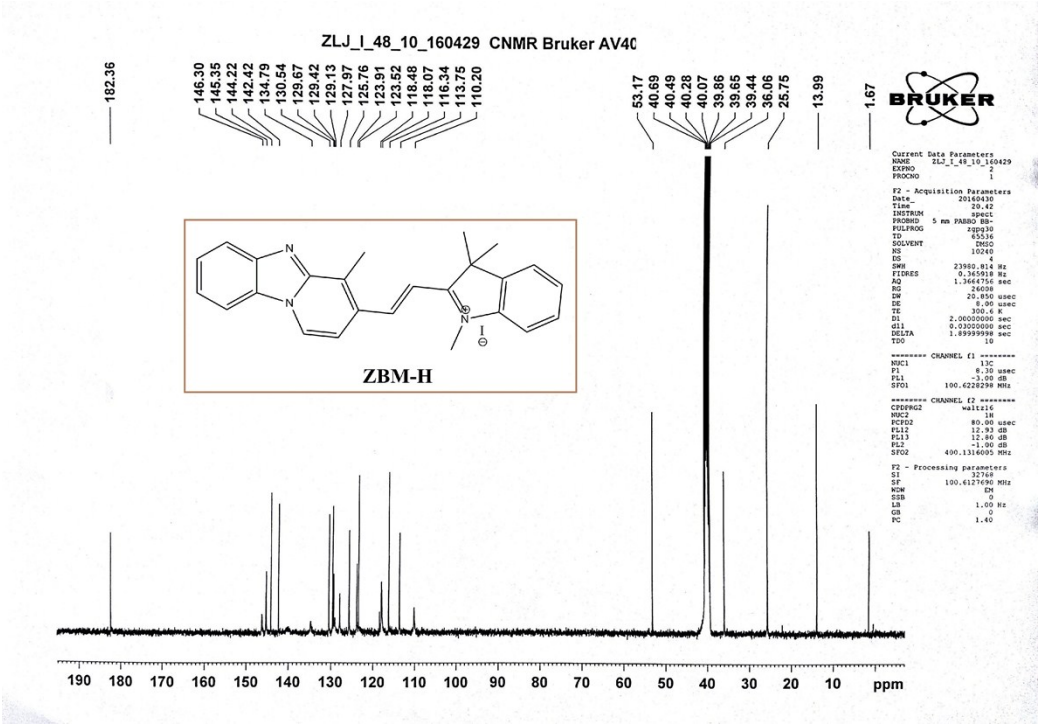


Fig. S3 ^{13}C NMR spectrum of **ZBM-H**.

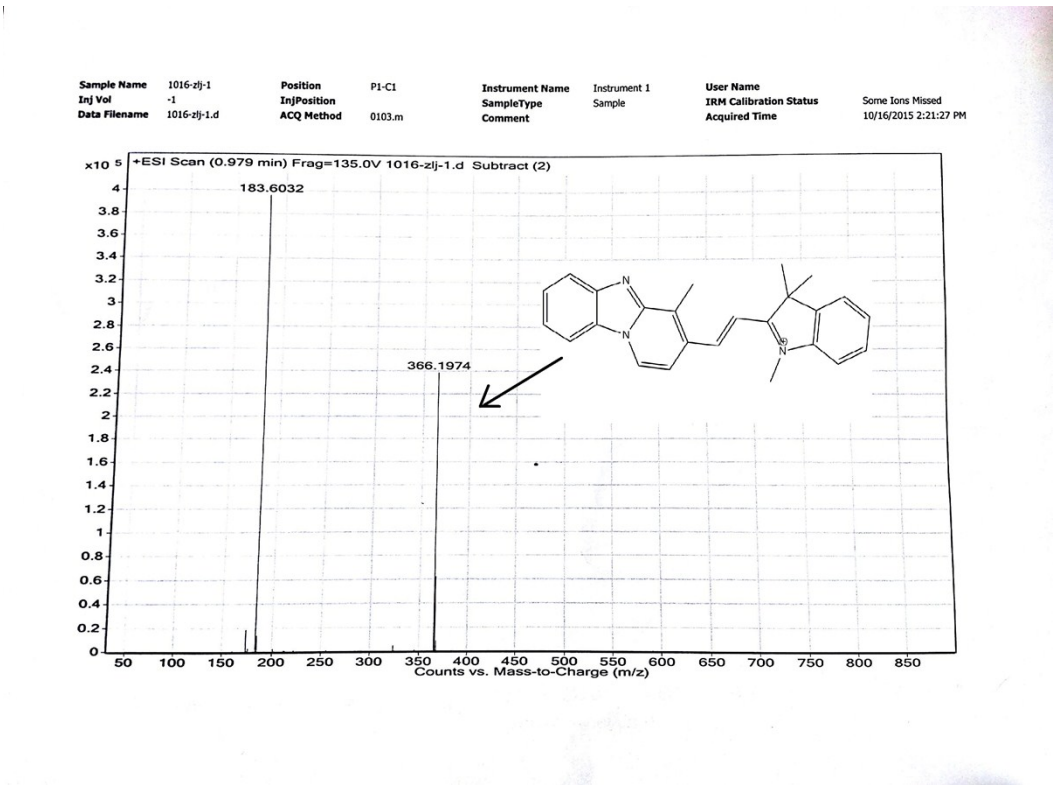


Fig. S4 Mass spectrum of **ZBM-H**.

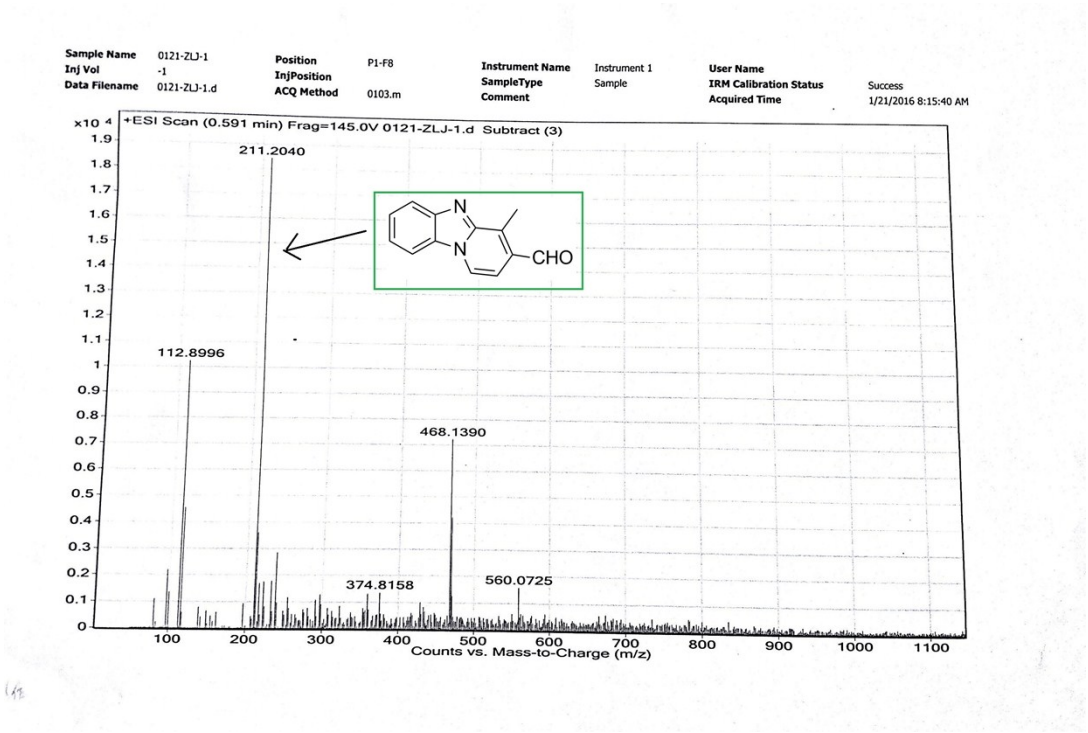


Fig. S5 Mass spectrum of the crude product of **ZBM-H** with NaClO (15 equiv.).

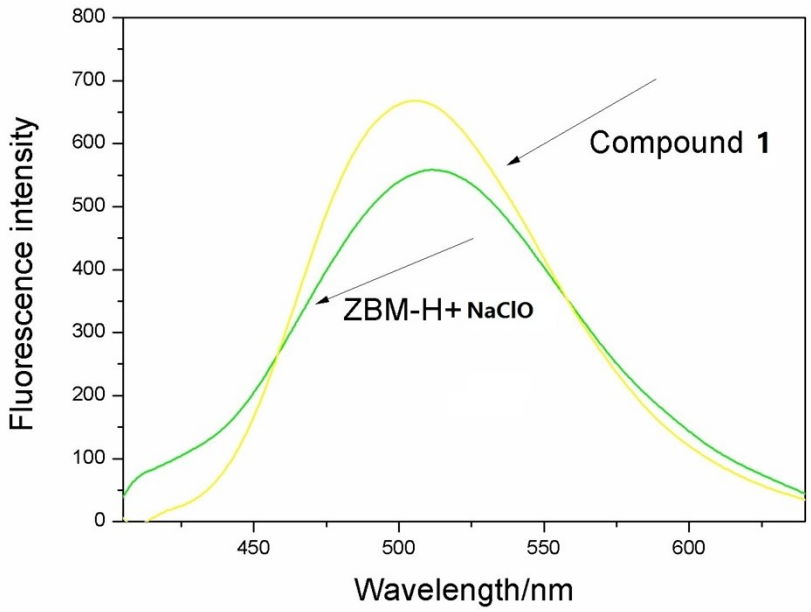


Fig. S6 The fluorescence spectrum of **ZBM-H** (10 μM) toward HClO (add 15 equiv. NaClO) and compound **1** (10 μM) in PBS buffer solution (0.01 M, pH = 6, containing 50% CH_3CN).

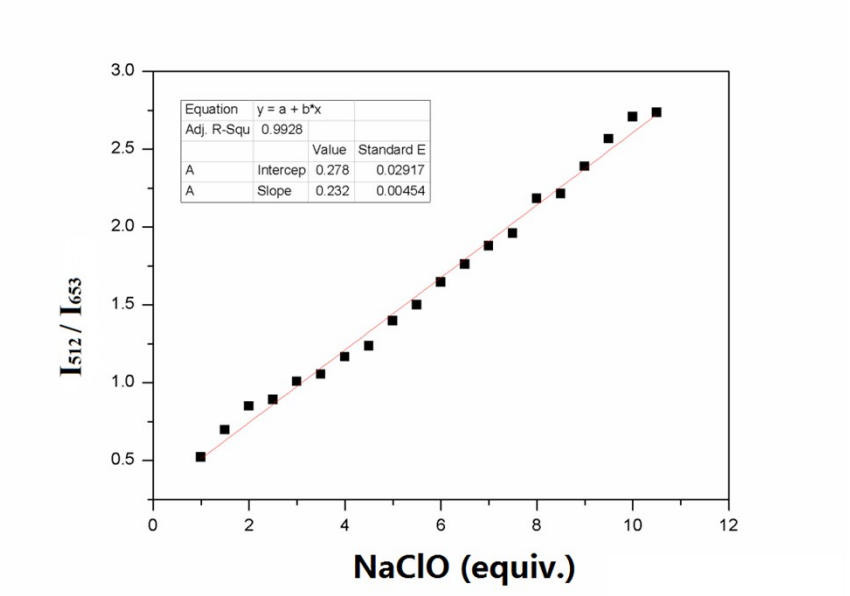


Fig. S7 Linearity of the fluorescence intensity ratio (I_{512}/I_{653}) of **ZBM-H** (10 μM) toward HClO (add 0~10.5 equiv. NaClO) in PBS (0.01 M, pH = 6):CH_3CN = 1:1 (v/v), R^2 = 0.9928.

Calculation of the detection limit (LOD)

$$LOD = 3\sigma/k$$

Where, σ is the standard deviation of the blank solution and k is the slope of the linear calibration plot between the fluorescence emission intensity and the concentration of hypochlorite.

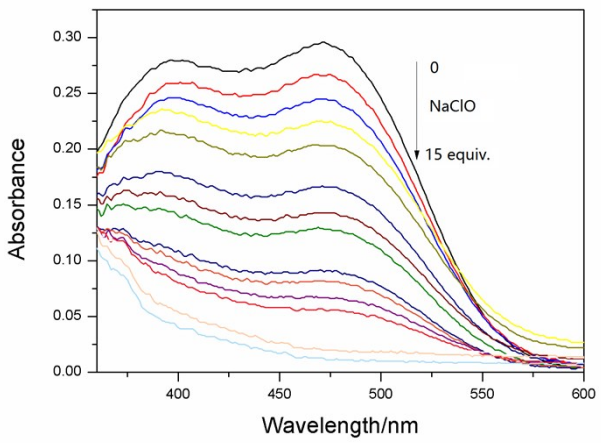


Fig. S8 The absorption spectrum of **ZBM-H** (30 μM) toward HClO (add 0-15 equiv. NaClO), CH_3CN:PBS =1:1 (v/v), pH = 6.

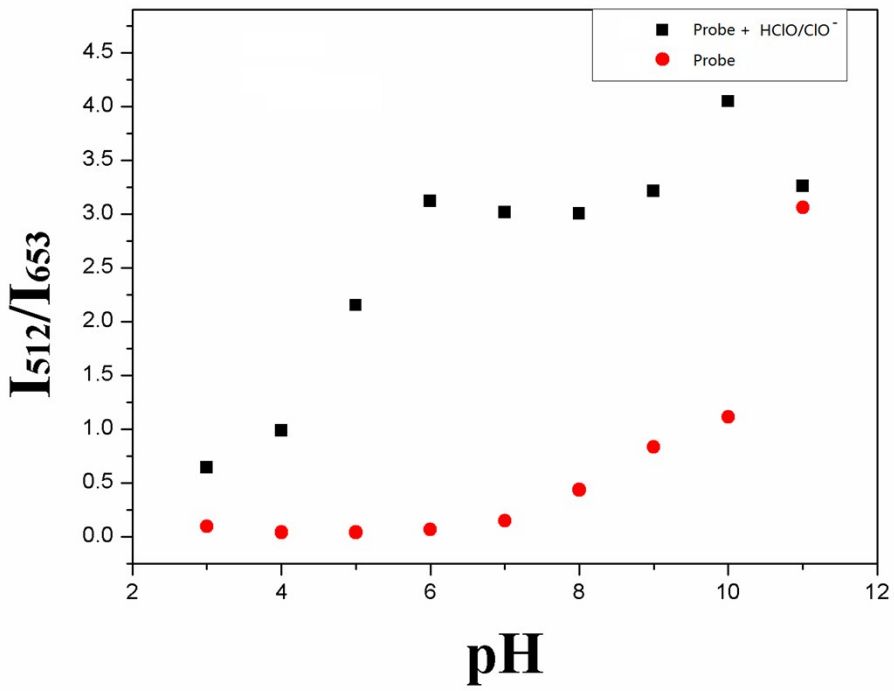


Fig. S9 pH-dependent fluorescence intensity ratio changes of **ZBM-H** (10 μM) in the presence or absence of NaClO/HClO (add 15 equiv. NaClO), CH_3CN:PBS =1:1 (v/v).

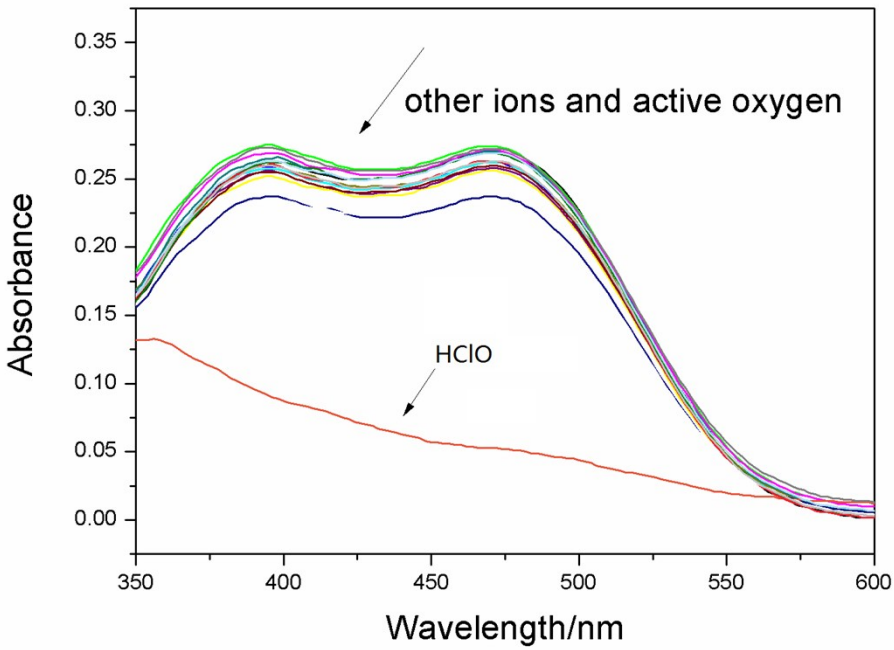


Fig. S10 The absorption spectra of **ZBM-H** (30 μM) toward various biologically relevant species including $CH_3CO_2^-$, HCO_3^-, I^-, SCN^-, $S_2O_3^{2-}$, NO_2^-, SO_4^{2-}, Ag^+, Ba^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Fe^{3+}, Pb^{2+}, $ONOO^-$, H_2O_2, $^1O_2$, *t*-BuO•, •OH, HClO (50 equiv.), CH_3CN:PBS =1:1 (v/v), pH = 6.

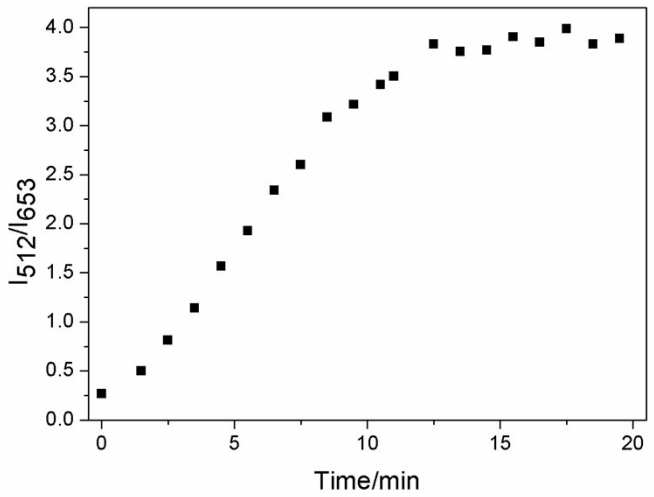


Fig. S11 Fluorescence response time of **ZBM-H** (10 μM) in the presence of HClO (add 15 equiv. NaClO). The fluorescence intensity ratio (I_{512}/I_{653}) was continuously monitored at time intervals, CH_3CN:PBS =1:1 (v/v), pH = 6.

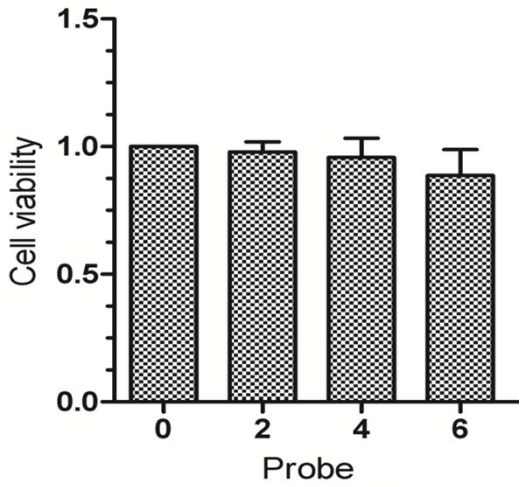


Fig. S12 Cytotoxicity of **ZBM-H** was evaluated in RAW264.7 cells by the standard SRB assay. The cells were incubated with **ZBM-H** (0, 2, 4, 6 μM) for 4 h.

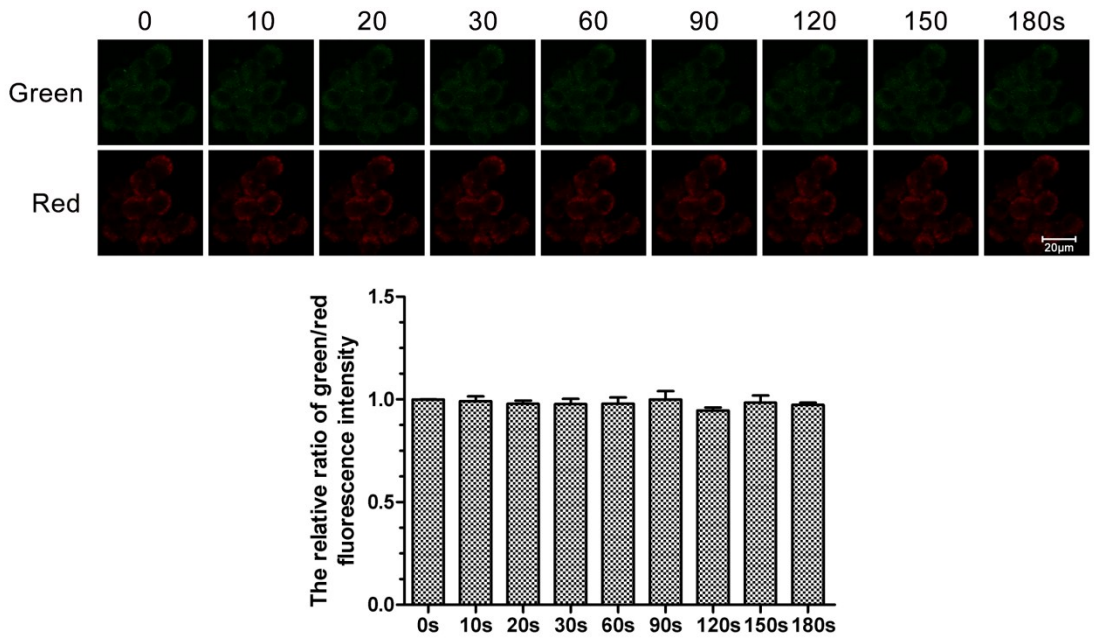


Fig. S13 (a) Photostability of **ZBM-H** in RAW264.7 cells. The cells incubated with **ZBM-H** (2 μM) were irradiated continuously for 0, 10, 20, 30, 60, 90, 120, 150 and 180 s. Images were acquired from 450 nm to 555 nm for green fluorescence, and from 640 nm to 700 nm for red fluorescence. (b) The green/red relative fluorescence intensity ratio of **ZBM-H** in RAW264.7 cells at different periods of time (the initial fluorescence intensity was defined as 1.0).

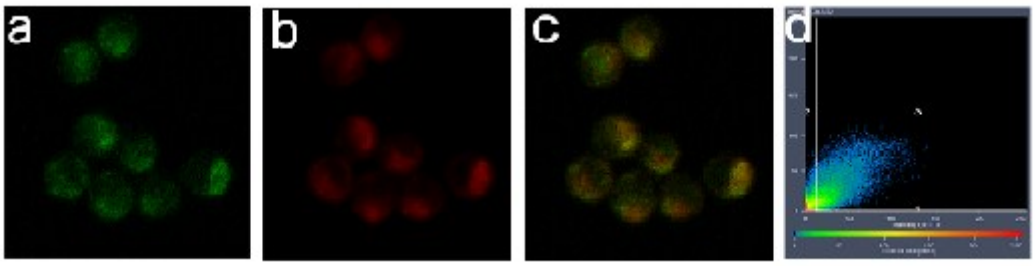
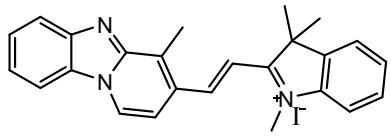
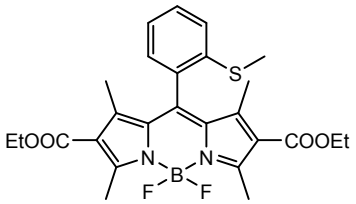
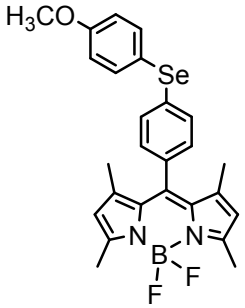
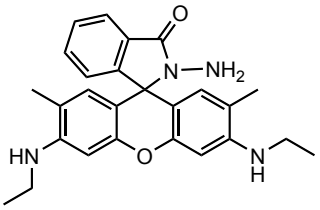
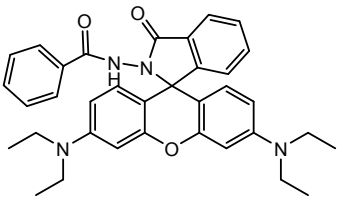


Fig. S14 RAW264.7 cells were incubated with **ZBM-H** (5 μM) for 15 min, followed by Lyso-Tracker Deep Red (0.5 μM) for 30 min. (a) The fluorescence image of **ZBM-H**. λex = 405 nm, green channel: 450-555 nm. (b) The fluorescence image of Lyso-Tracker Deep Red. λex = 639 nm, red channel = 640-700 nm. (c) Merged images of (a) and (b). (d) The colocalization coefficient of **ZBM-H** and Lyso-Tracker Deep Red was 0.86.

Table S1 Comparison of probe **ZBM-H with** other kind of fluorescent probe in some performances

Probe	Reaction time	Detection limit	Concentration of probe for cell image	References
	13 min	56 nM	2.0 μM	This work
	>40 min	23.7 nM	10.0 μM	[1]
	20 min	No data	10.0 μM	[2]
	5 min	60 nM	10 μM	[3]
	30 min	27 nM	No cell-image	[4]

[1] S. R. Liu, M. Vedamalai, S. P. Wu, Analytica Chimica Acta 2013, **800**, 71-76.

[2] B. S. Wang, P. Li, F. B. Yu, P. Song, X. F. Sun, S. Q. Yang, Z. R. Lou, K. L. Han, Chem. Commun., 2013, **49**, 1014-1016.

[3] Z. Zhang, Y. Zheng, W. Hang, X. M. Yan, Y. F. Zhao, Talanta 2011, **85**, 779-786.

[4] X.Q. Chen, X. C. Wang, S. J. Wang, W. Shi, K. Wang, H. M. Ma, Chem. Eur. J. 2008, **14**, 4719-4724.