Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for

Synthesis, molecular docking calculation, fluorescence and bioimaging of mitochondria-targeted ratiometric fluorescent probe for sensing hypochlorite *in vivo*

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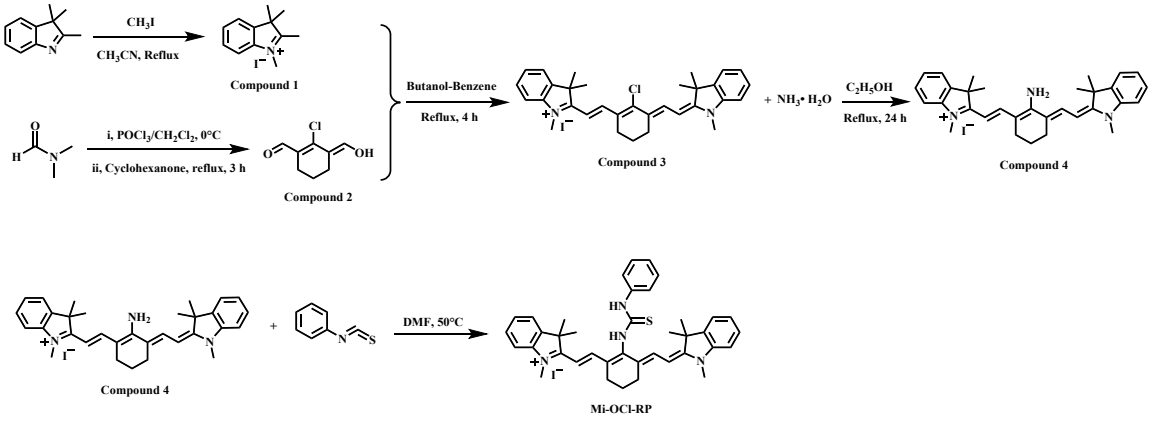
1. Materials and methods

All reagents were obtained from commercial suppliers without further purification. All experiments used ultra-pure water. Solvents were purified by standard methods prior. Ultra-pure water is using by ULPURE. The pH measurements were performed with PHS-3E pH meter. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope. CCK-8 was purchased from Fluorescence imaging experiments were performed with TransGen Biotechnology. TLC analysis was carried out on silica gel plates, and column chromatography was conducted over silica gel (mesh 200-300); both of them were purchased from the Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity 600 spectrometer. High resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T.

2. The synthesis of the probe Mi-OCl-RP



Scheme S1. The synthetic route of **Mi-OCl-RP**.

Compounds **1**, **2** and **3** were prepared by the literature procedure.[1] Synthesis of other compounds are described below.

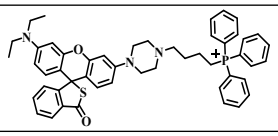
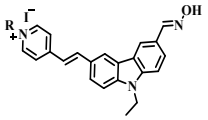
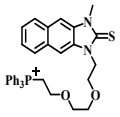
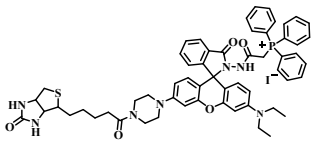
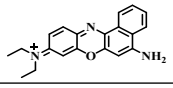
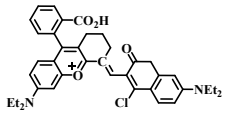
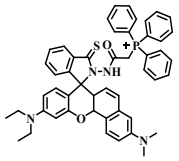
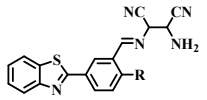
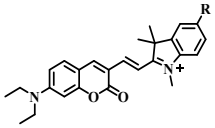
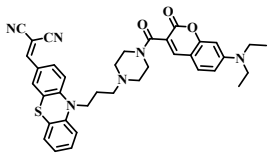
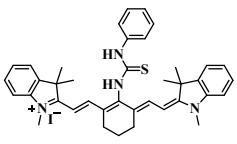
Synthesis of compound 4. Compound **3** (100 mg, 0.16 mmol) was dissolved in C_2H_5OH (20 mL). Then the solution was added slowly into the ammonium hydroxide. The mixture solution was then stirred at 85 ℃ for 24 h. After cool to room temperature, the residue was purified by silica chromatography using CH_2Cl_2/PE (10:1, V/V) as the eluent, and compound **4** was obtained as dark red solid (48 mg, 50%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.29 (s, 1H), 7.86 (d, J = 12.8 Hz, 1H), 7.27–7.21 (m, 3H), 7.20–7.15 (m, 1H), 6.97 (t, J = 7.4 Hz, 1H), 6.75 (d, J = 7.9 Hz, 1H), 5.56–5.46 (m, 1H), 5.32 (s, 1H), 3.30–3.19 (m, 4H), 2.63–2.60 (m, 2H), 2.56 (s, 1H), 2.52 (d, J = 6.1 Hz, 2H), 1.96–1.87 (m, 1H), 1.83 (d, J = 3.7 Hz, 2H), 1.80 (dd, J = 12.4, 6.2 Hz, 2H), 1.75 (d, J = 7.3 Hz, 2H), 1.69 (s, 5H), 1.33–1.26 (m, 1H), 0.91 (t, J = 6.9 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.85 (s), 162.92 (s), 148.70 (s), 144.45 (s), 139.20 (s), 131.15 (s), 128.65 (s), 127.90 (s), 127.64 (s), 127.61 (s), 125.72 (s), 123.34 (s), 121.77 (s), 121.65 (s), 120.89 (s), 119.33 (s), 117.78 (s), 106.78 (s), 105.64 (s), 92.93 (s), 92.50 (s), 53.45 (s), 46.44 (s), 45.67 (s), 44.79 (s), 29.41 (s), 28.32 (s), 26.70 (d), 24.61 (s), 20.95 (d). Anal. Calcd for $C_{32}H_{38}IN_3$: 591.58. Found: ESI-MS m/z ($[C_{32}H_{38}IN_3]$ + H)$^{+}$: 592.2372; ($[C_{32}H_{38}IN_3]$ + Na)$^{+}$: 614.2021.

Synthesis of the Probe Mi-OCl-RP. Compound **4** (50 mg, 0.084 mmol) was dissolved in DMF (5 mL). Then the solution was added slowly into the phenyl isothiocyanate. The mixture solution was then stirred at 50 ℃ for 24 h. After cooling to room temperature, the residue was purified by silica chromatography using CH_2Cl_2/MeOH (10:1, V/V) as the eluent, and the probe **Mi-OCl-RP** was obtained as dark red solid (15 mg, 24%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.66 (d, *J* = 11.9 Hz, 1H), 7.89 (s, 1H), 7.80 (d, *J* = 11.4 Hz, 1H), 7.57 (d, *J* = 5.3 Hz, 3H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 7.2 Hz, 2H), 7.35 (s, 3H), 7.24 (d, *J* = 7.4 Hz, 1H), 7.16 (s, 1H), 7.12 (s, 1H), 7.03 (dd, *J* = 16.1, 8.8 Hz, 2H), 4.09 (s, 3H), 2.97 (s, 2H), 2.76 (s, 2H), 1.89 (s, 2H), 1.79 (d, *J* = 16.7 Hz, 6H), 1.27 (s, 6H), 0.89 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.72 (s), 164.25 (d, *J* = 3.1 Hz), 142.83 (s), 141.04 (s), 140.40 (s), 140.07 (s), 138.79 (s), 136.86 (s), 135.41 (s), 129.57 (s), 129.40 (s), 129.18 (d, *J* = 5.0 Hz), 127.33 (s), 126.50 (s), 126.14 (s), 125.75 (s), 123.76 (s), 123.06 (s), 122.27 (s), 120.02 (s), 118.29 (s), 117.59 (s), 116.34 (s), 115.17 (s), 111.75 (s), 109.97 (s), 100.00 (s), 92.17 (s), 51.96 (s), 35.70 (s), 31.93 (s), 31.66 (s), 31.43 (s), 29.70 (s), 29.37 (s), 28.25 (s), 25.79 (s), 24.75 (s), 22.70 (s), 21.68 (s). Anal. Calcd for $C_{39}H_{43}IN_4S$: 726.77. Found: ESI-MS m/z ($[C_{39}H_{43}IN_4S] + H)^+$: 727.2386.

3. Cell culture and cytotoxicity assays

A549 cells, MCF-7 cells and RAW264.7 were cultured in DMEM or 1640 (Dulbecco's modified Eagle's medium or Roswell Park Memorial Institue medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ℃. In vitro cytotoxicity was measured using CCK-8 assay on A549 cells, MCF-7 cells and RAW264.7. These Cells were seeded into a 96-well tissue culture plate in the presence of completed Dulbecco's modified Eagle's medium (DMEM) or Roswell Park Memorial Institue medium (1640) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere overnight followed by incubation for 24 h in the presence of **Mi-OCl-RP** at different concentrations (5, 10, 20, 30, 40 μM). A commercial cell counting kit-8 (CCK-8) (TransGen Biotechnology, China) was used to detecting the cell viability and the assay was run following the manufactures' instructions. The cell viability was determined by assuming 100% cell viability for cells without **Mi-OCl-RP**.

Table S1 Literature on mitochondria-targeted OCl^--sensitive probes and our probe.

Reagents	Molecular calculation	Response time	Stokes shift	Ratio imaging	Reference
	No	-	27 nm	No	2
	No	-	104 nm	No	3
	No	< 3 min	122 nm	No	4
	No	< 10 s	15 nm	No	5
	No	< 5 s	72 nm	No	6
	No	< 3 min	200 nm	No	7
	No	1 min	45 nm	No	8
	No	≤ 8 s	52 nm	No	9
	No	120 s	230 nm	Yes	10
	No	< 40 s	209 nm	Yes	11
	Yes	< 7 s	278 nm	Yes	This work

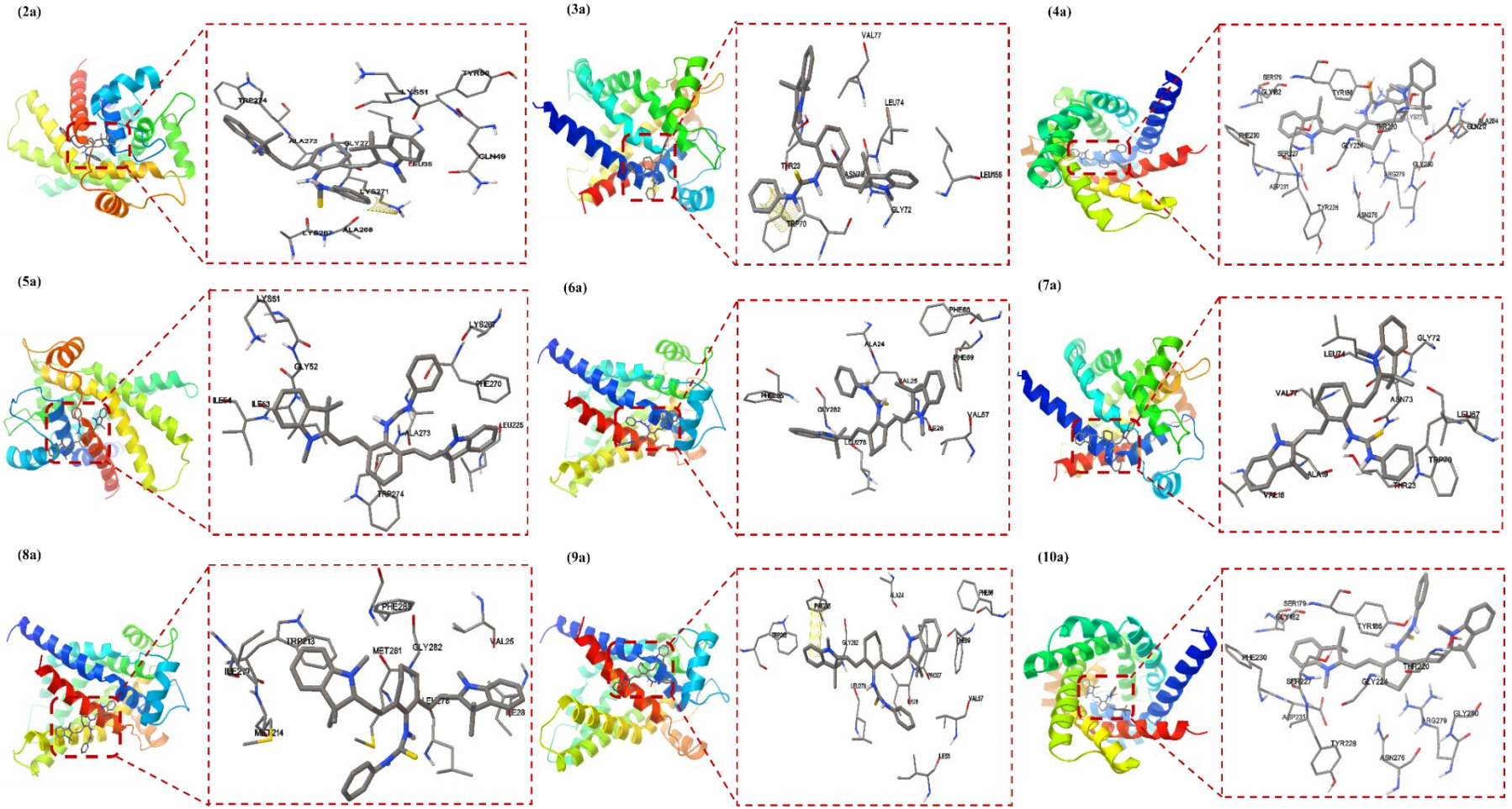


Figure S1 Molecular binding modeling of conformation **2-10**. Tertiary structure of ANT, actives site and residues of (2a-10a). The π-cation and π-π interactions is indicated by golden block.

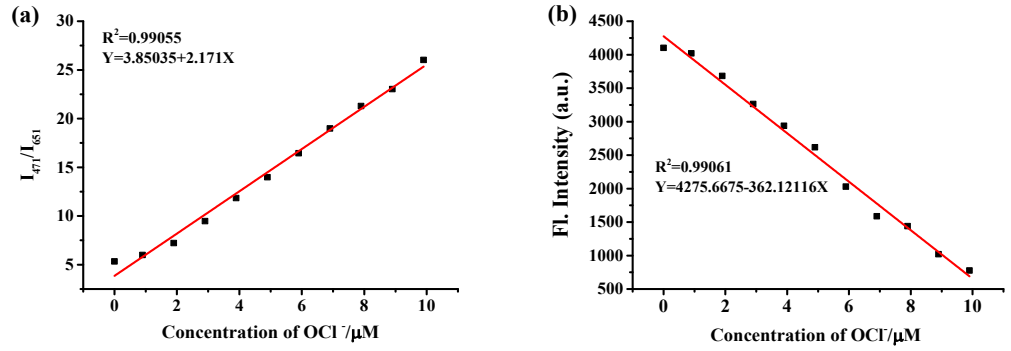


Figure S2 Linear relationship of fluorescence intensity of **Mi-OCl-RP** in different concentration of OCl^- (0-10 μM) (a: λ_{ex} = 373 nm; b: λ_{ex} = 588 nm).

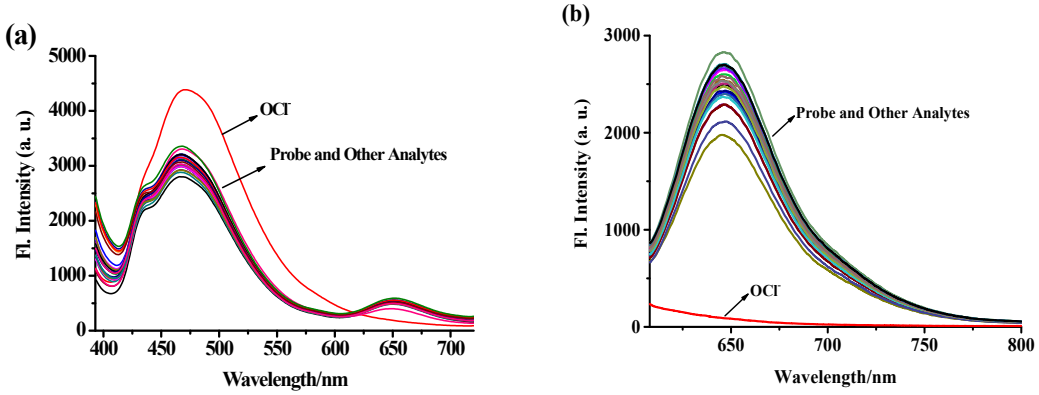


Figure S3 Fluorescence intensity of **Mi-OCl-RP** (10 μM) with OCl^- and various relevant species (10 μM): Hydroxyl radicals; Cys; Hcy; Glutathione; CH_3COOOH; H_2O_2; t-butylhydroperoxide; NO; Ca^{2+}; Zn^{2+}; Co^{2+}; Cu^{2+}; Fe^{2+}; Mg^{2+}; F^-; Cl^-; Br^-; I^-; HCO_3^-; SO_3^{2-}; HSO_3^-; and NO_2^- in pH 7.4 PBS buffer (a: λ_{ex} = 373 nm; b: λ_{ex} = 588 nm).

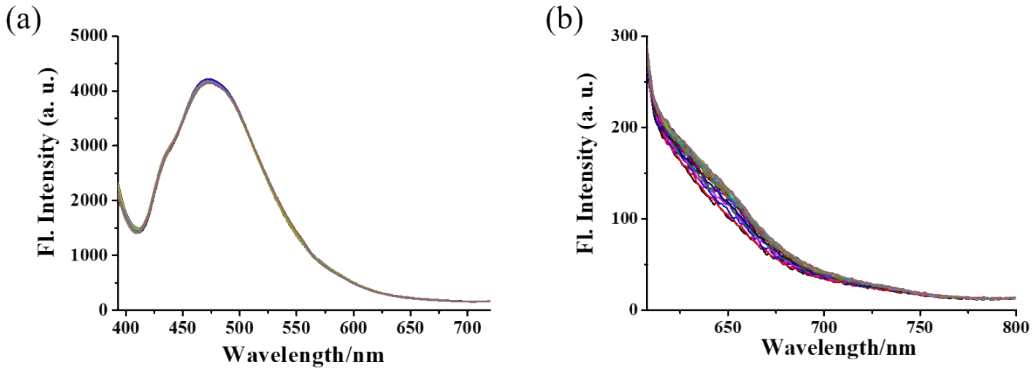


Figure S4 Fluorescence spectra stability of 10 μM **Mi-OCl-RP** in pH 7.4 PBS buffer (a: λ_{ex} = 373 nm; b: λ_{ex} = 588 nm).

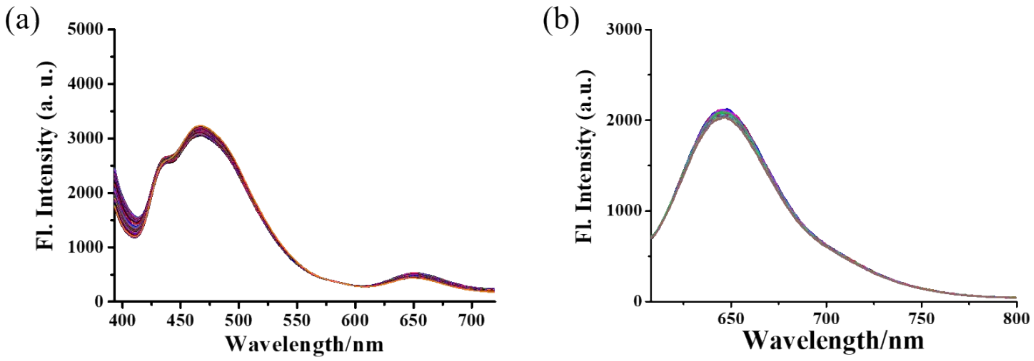


Figure S5 Fluorescence spectra stability of 10 μM **Mi-OCl-RP** in the presence of OCl^- (10 μM) in pH 7.4 PBS buffer (a: λ_{ex} = 373 nm; b: λ_{ex} = 588 nm).

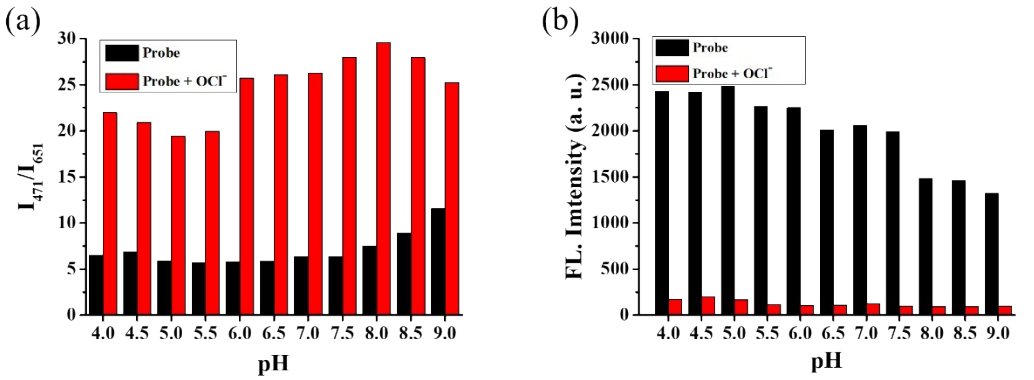


Figure S6 pH dependency of **Mi-OCl-RP** (10 μM) in the absence or presence of OCl^- (10 μM) in PBS buffer (a: λ_{ex} = 373 nm; b: λ_{ex} = 588 nm).

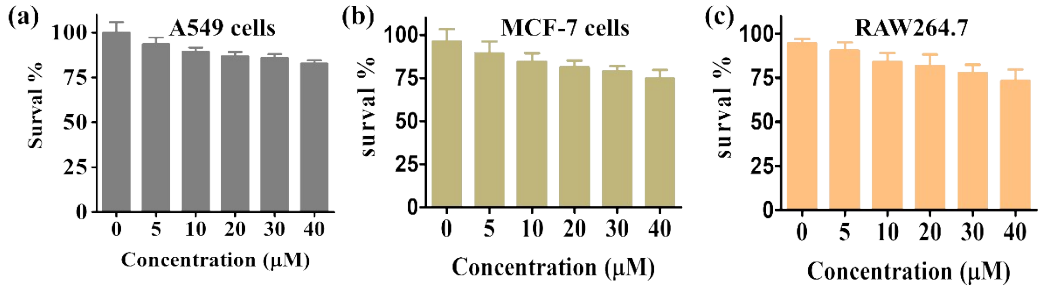


Figure S7 Cytotoxicity of A549 cells, MCF-7 cells and RAW264.7 measured CCK-8 assay after treatment with different concentrations of **Mi-OCl-RP**.

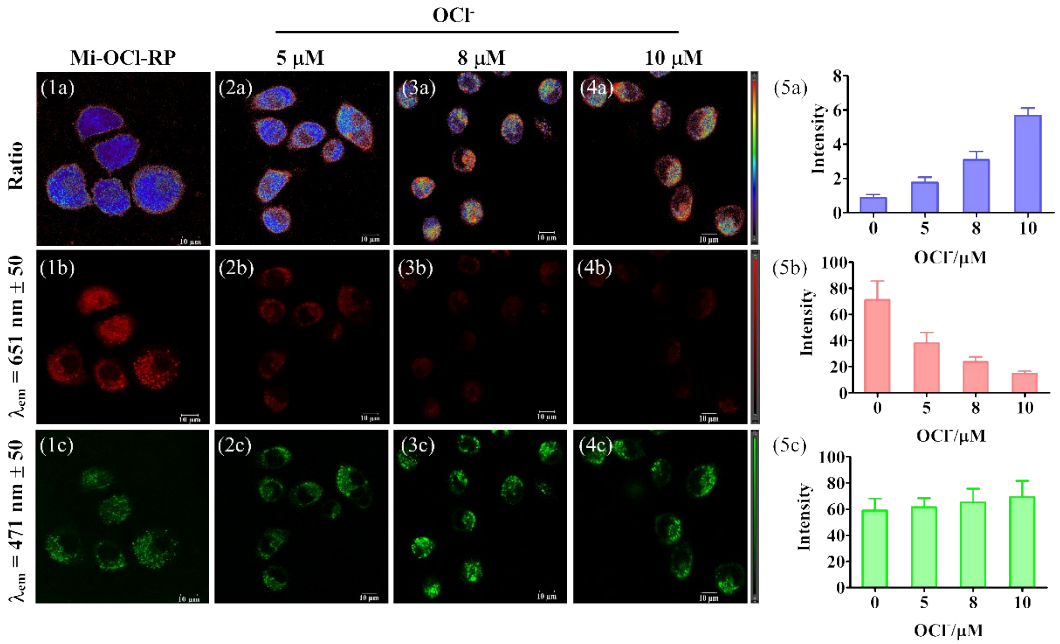


Figure S8 Confocal fluorescence images of **Mi-OCl-RP** responding to OCl^- in MCF-7 cells. (1a-1c) MCF-7 cells incubated with **Mi-OCl-RP** (10 μM, 20 min); (2a-4c) MCF-7 cells incubated with OCl^- (5, 8 and 10 μM) for 10 min and then incubated with **Mi-OCl-RP** (10 μM, 20 min). First column: ratios images of excitation at 405 and 588 nm; second column: excitation at 588 nm; third column: excitation at 405 nm. (5a-5c) Quantified relative fluorescence intensity of images 1a-4c. Scale bar: 10 μm.

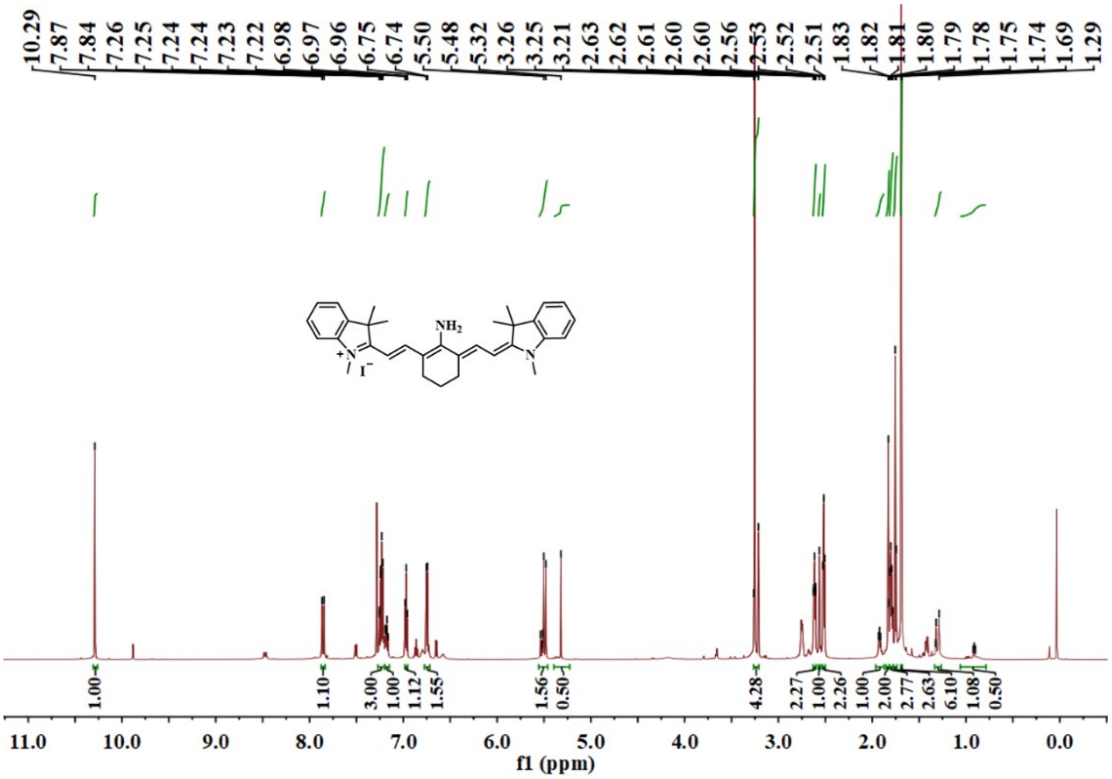


Figure S9 ^{1}H NMR spectra of compound **2** in $CDCl_3$.

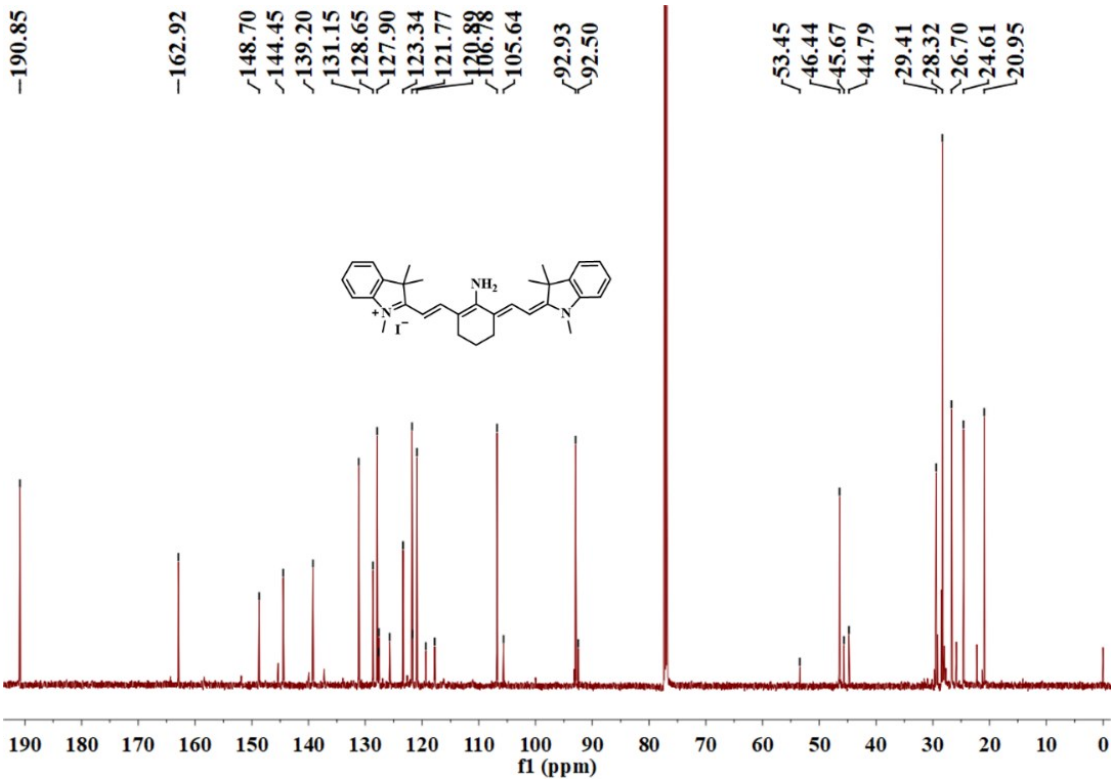


Figure S10 ^{13}C NMR spectra of compound **2** in $CDCl_3$.

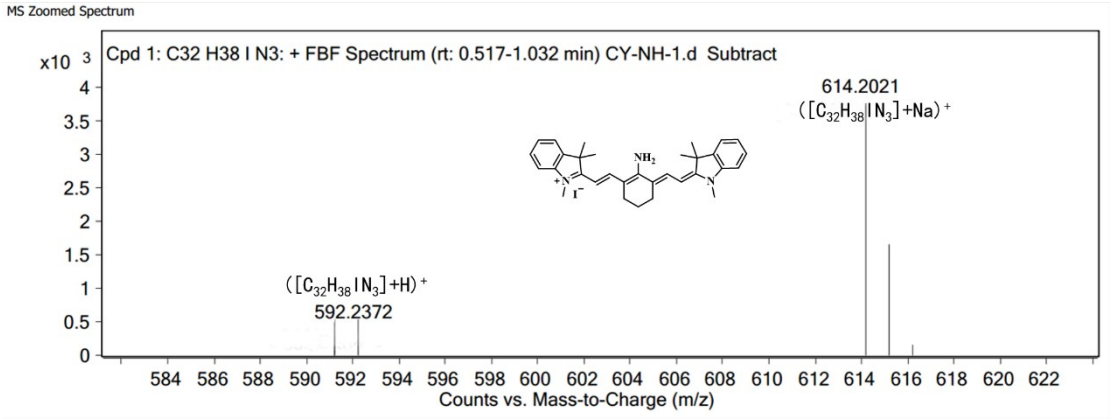


Figure S11 HRMS of compound **2**.

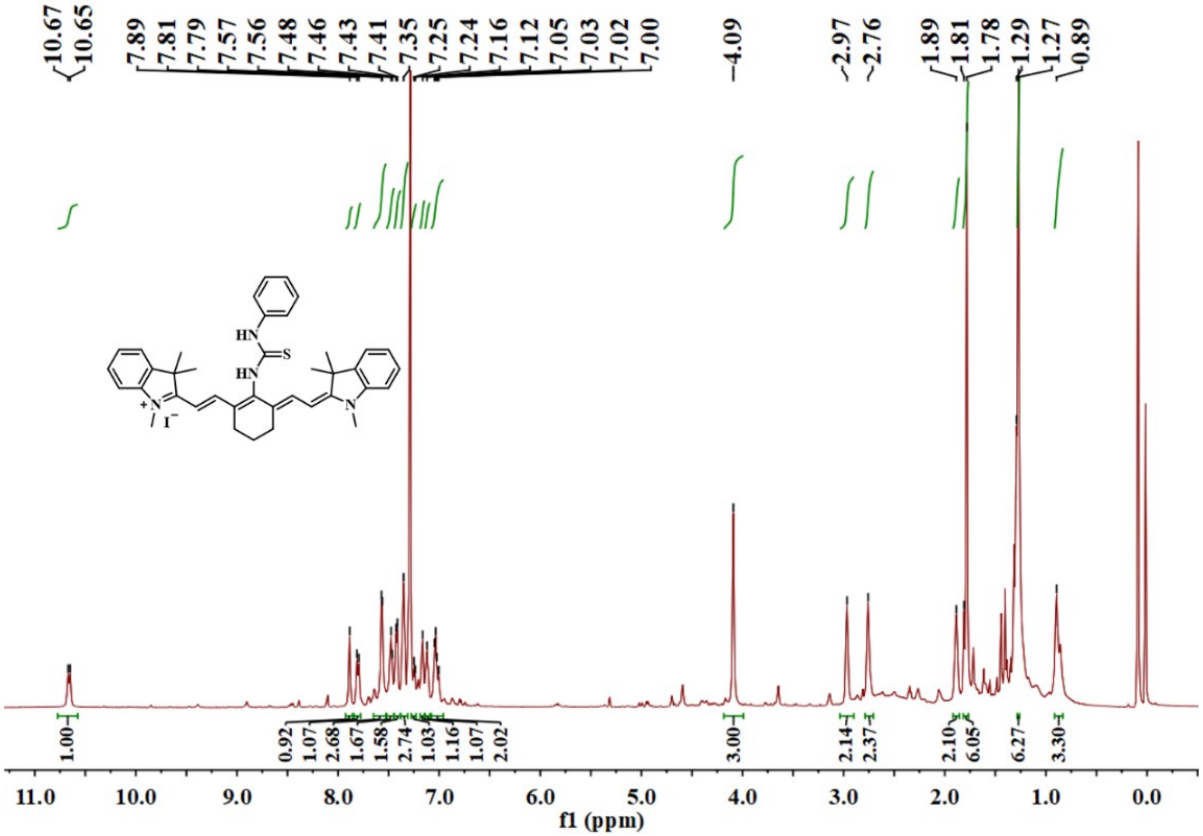


Figure S12 ^{1}H NMR spectra of **Mi-OCl-RP** in $CDCl_3$.

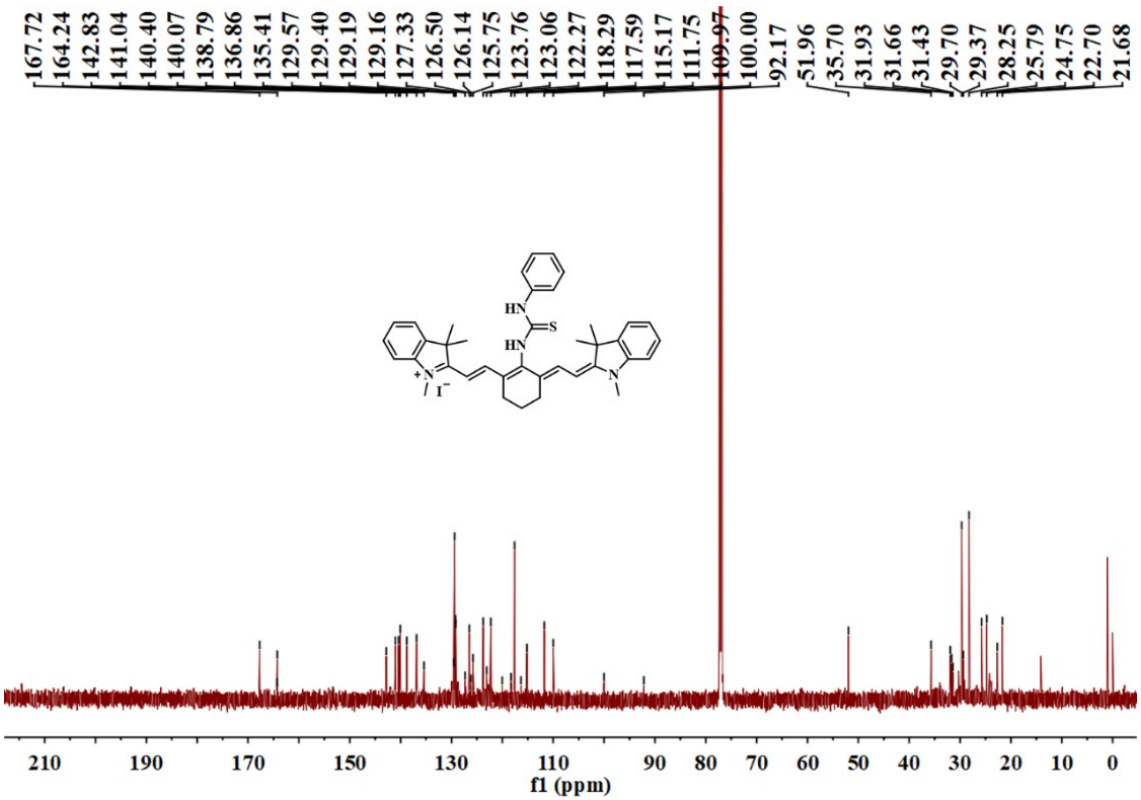


Figure S13 ^{13}C NMR spectra of **Mi-OCl-RP** in $CDCl_3$.

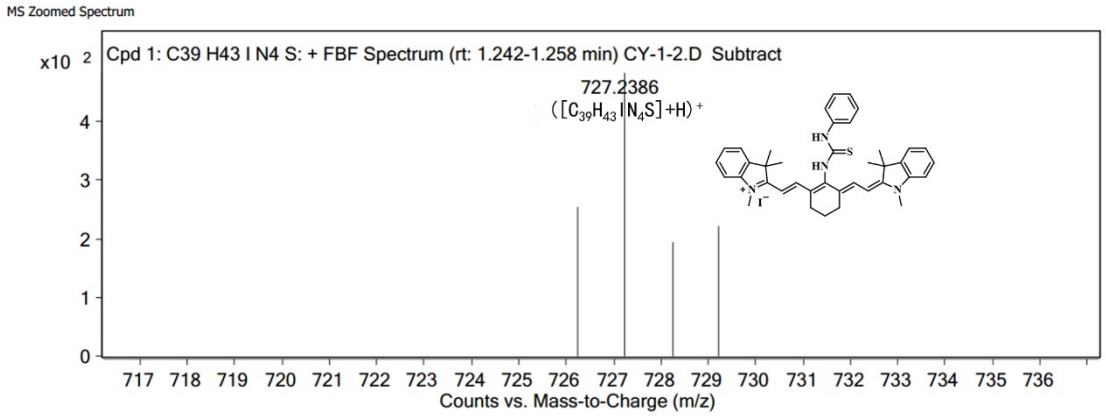


Figure S14 HRMS of **Mi-OCl-RP**.

References

1. D. L. Gallaher Jr. and M. E. Johnson, *Analyst*, 1999, **124**, 1541–1546.
2. J. Zhou, L. Li, W. Shi, X. Gao, X. Li and H. Ma, *Chem. Sci.* 2015, **6**, 4884–4888.
3. D. Li, Y. Feng, J. Lin, M. Chen, S. Wang, X. Wang, H. Sheng, Z. Shao, M. Zhu and X. Meng, *Sensors and Actuators B-Chem.* 2016, **222**, 483–491.
4. Q. Xu, C. H. Heo, J. A. Kim, H. S. Lee, Y. Hu, D. mKim, K. M. K. Swamy, G. Kim, S. J. Nam, H. M. Kim and J. Yoon, *Anal. Chem.* 2016, **88**, 6615–6620.
5. K. Li, J. Hou, J. Yang and X. Yu, *Chem. Commun.* 2017, **53**, 5539–5541.
6. B. Zhu, L. Wu, M. Zhang, Y. Wang, C. Liu, Z. Wang, Q. Duan and Pan. Jia, *Biosens. Bioelectorn.* 2018, **107**, 218–223.
7. H. Niu, K. Chen, J. Xu, X. Zhu, W. Cao, Z. Wang, Y. Ye and Y. Zhao, *Sensor. Actuat. B-Chem.* 2019, **29**, 126938.
8. G. Mao, G. Gao, Z. Liang, Y. Wang, L. Su, Z. Wang, H. Zhang, Q. Ma and G. Zhang, *Anal Chim. Acta* 2019, **1081**, 184–192.
9. X. Zhong, Q. Yang, Y. Chen, Y. Jiang and Z. Dai, *J. Mater. Chem. B* 2020, DOI: 10.1039/d0tb01496f.
10. J. Lan, L. Liu, R. Zeng, Y. Qin, Y. Liu, X. Jiang, A. Aihemaiti, Y. Ding, T. Zhang and R. J. Y. Ho, *Chem. Commun.* 2020, **56**, 1219–1222.
11. W. Wang, J. Ning, J. Liu, J. Miao and B. Zhao, *Dyes Pigments* 2019, **177**, 107708.