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ARTICLE TYPE

A specific and rapid "on-off" acenaphthequinone-based probe for HOCl detection and imaging in living cells

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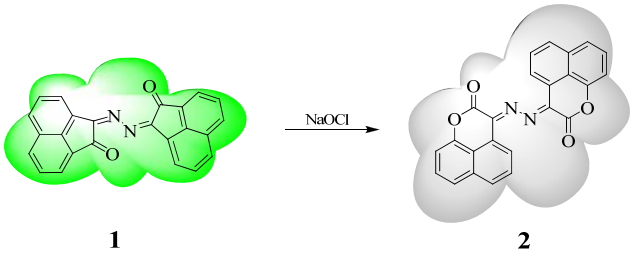
A fluorescent probe exhibiting specific and sensitive properties to HOCl was successfully developed on the basis of Baeyer-Villiger reaction. It worked well both in solutions and on test papers, allowed facile monitoring of hypochlorite. The probe was further imaged in living cells.

Hypochlorous acid (OCl^-/HOCl, *pKa* = 7.46), a powerful microbicidal agent, plays a crucial role in daily life and mediates various biological processes in the immune system[1] and inflammation.[2] Excessive hypochlorite solutions are involved in some diseases such as cardiovascular disease，inflammatory disease[3] and cancer.[4] Many efforts have been devoted into developing novel fluorescent probes[5] to image HOCl due to their superior sensitivity and specificity for labeling and bioanalysis.[6] To develop satisfactory HOCl probes, different frameworks have been reported for HOCl determination,[7] including rhodamine and rhodamine derivative ring-opening,[8] oxidative cleavage,[9] the oxidation reactions of BODIPY-based probes, dihydrofluorescein-ether, the oxime group, naphthalene, heptamethine cyanine dyes and so on.[10] Despite the recent advances, there is still much room for further improvement of hypochlorite probes in terms of application. In this contribution, compound **1** is reported as a selective fluorescent probe for HOCl with a strong emission in the visible region (538 nm) based on Baeyer-Villiger reaction promoted by OCl^- (Scheme 1). Moreover, it was double checked that **1** reacted with a standard peroxide reagent 3-Chloroperoxybenzoic acid (MCPBA) (Fig. S11). The fluorescence responses to various Reactive Oxygen Species (ROS) and imaging on the test papers as well as within living cells were studied.



Scheme 1 The proposed reaction of **1** to hypochlorite.

To explore the sensitivity of compound **1** to HOCl, it was treated with various concentrations of hypochlorite. Considering the practical application, we tested the optical properties of **1** at pH 7.4, in PBS/THF (1/9, V/V). It showed single-wavelength fluorescence decrease (a highly specific and rapid "turn-off" response for HOCl) after addition of NaOCl (Fig. 1). When NaOCl was titrated from 0 to 50 μM, a good linear relationship (FL at 538 nm against NaOCl concentration) was obtained with a lower detection limit (0.34 μM, 3s/k), indicating that **1** was suitable for accurate analysis of HOCl (Fig. S2). In comparison, the detection limit for an oxidative cleavage-based near-infrared fluorescent probe for HOCl is 0.70 μM,[9a] for a heptamethine cyanine dye is 1.95×10^{-8} M.[10r] One of the BODIPY-based probe can give a detection limit of 0.52 μM,[10l] another probe offer a detection limit of 3.7 μM.[10f] The changes of the UV/vis spectra of **1** in the presence of NaOCl were also studied. The absorption at 400 nm decreased and a new absorption band appeared at 336 nm with an isosbestic point at 350 nm during the titration (Fig. S3). Time course studies were not investigated because the reaction occurred rapidly at room temperature. This can be attributed to the reaction between **1** and HOCl (Scheme 1), which was confirmed by ESI-MS (Fig. S10).

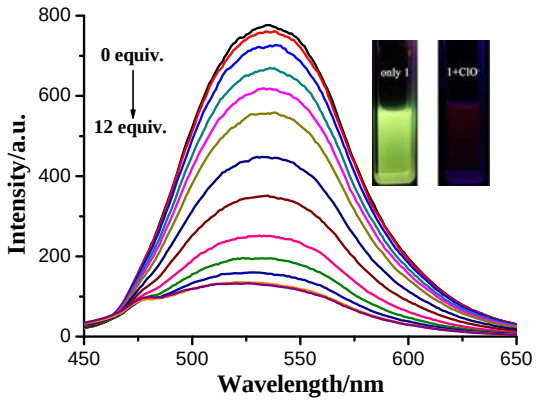


Fig. 1 The emission of **1** (5 μM) in PBS/THF (1/9, V/V) with the addition of ClO^-.

To give some further evidence, compound **1** was examined both in the absence and in the presence of OCl^- in different pH solutions. As shown in Fig. S4, **1** was quite stable in aqueous media and exhibited significant emission over the pH range of 3-10, and then the fluorescence emission decreased significantly along with the increase in the pH value. In comparison, it showed weak fluorescence in the presence of OCl^- over the pH range of 6-8 (Fig. S5). The interference of alkalinity can be eliminated by

control of the pH (Fig. S6).

To check the selectivity, **1** was treated with several relevant ROS such as H_2O_2 (hydrogen peroxide), $^1O_2$ (singlet oxygen), $^{\bullet}OH$ (hydroxyl radicals), $ONOO^-$ (peroxynitrite), $O_2^{\bullet-}$ (superoxide), TBHP (*tert*-butyl hydroperoxide), $TBO^{\bullet}$ (*tert*-butoxy radical)and $^{\bullet}NO$ (nitric oxide). Not any analyte led to a significant response on the fluorescence (Fig. 2). Interference studies of other ions including Li^+, K^+, Cu^{2+}, Hg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, F^-, Cl^-, Br^-, I^-, ClO_4^-, NO_2^-, $S_2O_3^{2-}$ and SCN^- were also carried out (Fig. 2). As expected, none of the cations and anions showed noticeable fluorescence changes.

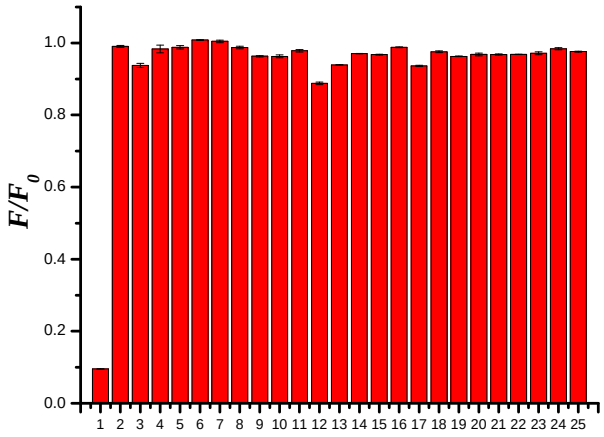


Fig. 2 Fluorescence ratio (F/F_0) at 538 nm of **1** (5 μM) in PBS/THF (1/9, V/V) in the presence of 1. NaOCl, 2. H_2O_2, 3. $^1O_2$, 4. $^{\bullet}OH$, 5. $ONOO^-$, 6. $O_2^{\bullet-}$, 7. TBHP, 8. $TBO^{\bullet}$, 9. $^{\bullet}NO$, 10. Li^+, 11. K^+, 12. Cu^{2+}, 13. Hg^{2+}, 14. Mn^{2+}, 15. Fe^{2+}, 16. Fe^{3+}, 17. Al^{3+}, 18. F^-, 19. Cl^-, 20. Br^-, 21. I^-, 22. ClO_4^-, 23. NO_2^-, 24. $S_2O_3^{2-}$, 25. SCN^-.

Furthermore, filter papers were immersed in tetrahydrofuran solution of **1** (5×10^{-5} M) and then dried in air. In the practical detection, after the tested aqueous solution of OCl^- was dropped on the prepared test papers for a few seconds (in 3-5 S), the color of the test papers changed. As shown in Fig. 3, under UV light (365 nm) irradiation, the blank paper remained green, while those treated with OCl^- became non-fluorescent. The OCl^- was detectable at a concentration of 5×10^{-5} M. The result illustrated that compound **1** can be used as a potential fluorescent probe for OCl^- detection in practical applications.

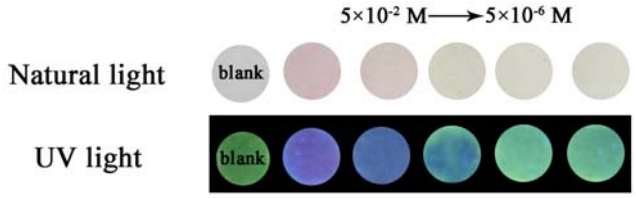


Fig. 3 Fluorescence responses of **1** (5×10^{-5} M) in response to OCl^- (under UV light at 365 nm) on the test papers. OCl^- concentrations from left to right: none; 5×10^{-2} M; 5×10^{-3} M; 5×10^{-4} M; 5×10^{-5} M; 5×10^{-6} M.

Next, we checked the detecting process within living cells. BEL-7402 cells were grown at 37 °C under 5% CO_2 in RPMI-1640 medium. As controls, the cells were incubated with **1** (5 μM) for 20 min at 37 °C and washed three times to remove excess **1**. Subsequently, some cells were further treated 50 μM NaOCl for 0.5 h before imaging. The strong fluorescence in the absence of hypochlorite can be observed. However, the fluorescence intensity decreased immediately (Fig. 4) after addition of hypochlorite. This is consistent with the fluorescence change in Fig. 1, indicated that **1** penetrated the cell and worked in the living cell.

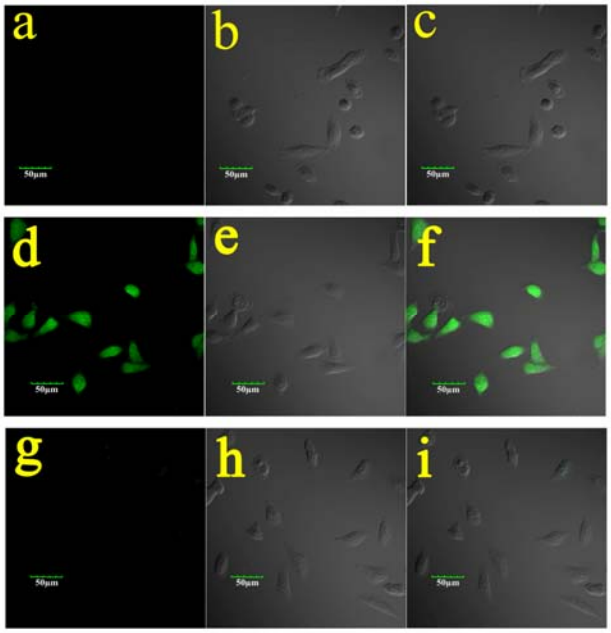


Fig. 4 Confocal fluorescence images of BEL-7402 cells. Cells incubated in RPMI-1640 medium (top); then treated with **1** (5 μM) for 20 min (middle); image of cells after treatment with **1** (5 μM) for 20 min and subsequent treatment with 50 μM hypochlorite for 0.5 h (bottom). The columns represent scale bar (50 μm) of the cell image. (a), (d), (g) Dark field images. (b), (e), (h) Bright field images. (c), (f), (i) Merged images. λ_{ex} = 408 nm.

MTT assays[11] in the BEL-7402 cell lines are carried out to check the cytotoxicity of **1** (Figure S12), the results demonstrated that for the 24 h measurement, more than 90% of the cells remain alive even at a high concentration of **1 (**10 μM), indicates the low cytotoxicity **of 1**.

In conclusion, a new selective and sensitive fluorescent probe was developed; it can be used to visualize the hypochlorite both in solutions and on test papers. Moreover, **1** can respond to hypochlorite in live cells.

Experimental section

Materials

Acenaphthenequinone, malononitrile, hydrazine, Potassium Nitroferricyanide (III) Dihydrate, KO_2, $FeCl_2$, *Tert*-Butyl Hydroperoxide, MCPBA and hypochlorite reagents were purchased from Aladdin (China). Tetrahydrofuran was purified with sodium. Other chemicals and solvents were used as received unless specifically noted.

Synthesis

Compound **1** was synthesized and characterized in our previous work.[12] ^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 8.42 (d, J = 7.0 Hz, 1H), 8.28 (d, J = 7.1 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H). ^{13}CNMR (125 MHz, DMSO-d_6) δ (ppm): 178.71, 134.02,

133.38, 130.87, 129.99, 128.26, 127.50, 126.91, 126.83, 122.76, 56.53, 19.00. ESI-MS: m/z = 382.89, $[M + Na]^+$, calc. $C_{24}H_{12}N_2O_2Na$ = 383.09.

NaOCl was added dropwise to a solution of **1** in 3 mL THF, and then the mixture was stirred at room temperature. After a few minutes, solvents were removed under reduced pressure, and the residue was purified by silica gel chromatography to afford **2**. ^{1}HNMR (500 MHz, TFA) δ (ppm): 9.65 (d, J = 7.5 Hz, 1H), 9.40 (d, J = 7.4 Hz, 1H), 9.02 (d, J = 7.9 Hz, 1H), 8.82 (d, J = 8.1 Hz, 1H), 8.33 (t, J = 7.7 Hz, 1H), 8.28 (t, J = 7.7 Hz, 1H). ESI-MS: m/z = 802.75, $[2M+H_2O+H]^+$, calc. $C_{48}H_{27}N_4O_9$ = 803.16.

To prove our hypothesis, MCPBA was added to a solution of **1** in CH_3CN, indicating that the formula of the main product was $C_{24}H_{12}N_2O_4$ (m/z = 431.9, $[M + K]^+$, calc. $C_{24}H_{12}N_2O_4K$ = 431.46).

Sample preparation and titration

Stock solutions of hypochlorite and relative chemicals were prepared with a concentration of 5×10^{-2} M. A stock solution of compound **1** (5×10^{-3} M) was prepared in DMSO and further diluted to 5.0×10^{-6} M for titration experiments.

Cell incubation

BEL-7402 cells were seeded on 35 mm glass-bottomed dishes (NEST) and incubated in RPMI-1640 in an incubator (37 °C, 5% CO_2 and 20% O_2) for 24 h. The cells were rinsed slightly 3 times with fresh RPMI-1640 and incubated in RPMI-1640 medium spiked with or without **1** (5 μM) for 10 min, respectively. The cells treated with **1** were further incubated in fresh RPMI-1640 containing 50 μM NaOCl for 0.5 h. Cells were then analyzed by Laser Scanning Confocal Microscope.

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Notes and references

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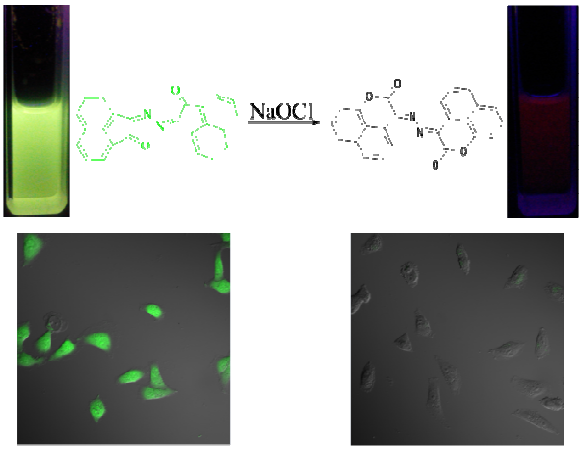

A facile sensor monitoring hypochlorite both on test papers and living cells has been proposed.