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A phenanthroimidazole-based fluorescent probe for hypochlorous acid with high selectivity and its bio-imaging in living cells

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ABSTRACT

In this study, a new compound 2-amino-3-[(4-(1H-phenanthro [9,10-d] imidazol-2-yl) phenyl methylene) amino]-2-butenedinitrile (probe **1**) as a simple fluorescent probe for hypochlorous acid has been synthesized and characterized. It exhibited high selectivity and sensitivity as hypochlorite sensor in mixed aqueous medium. NMR and mass spectral analysis showed that the probe reacted with the hypochlorite ion through de-diaminomaleonitrile reaction which disrupted the ICT mechanism by breaking the donor and acceptor linkage resulting in a remarkable enhancement of the emission intensity. In addition, the probe worked excellently within a wide pH range of 4 to 10 and the detection limit was found to be 1.4×10^{-2} μM. The cell experiments showed the good cell-membrane permeability of probe, and it could be used to image exogenous HOCl.

1. Introduction

Hypochlorous acid (HOCl), one of the important reactive oxygen species (ROS) in living organisms, plays a vital role in many biological processes [1,2]. It is usually produced by myeloperoxidase (MPO)-catalyzed per-oxidation of chloride ions in phagolysosome. Hypochlorite also plays important roles in the human immune defence system, and contributes to the destruction of invading bacteria and pathogens [3–5]. However, the excessive accumulations of this strong oxidative HOCl/OCl^- inside the human body are harmful because these can damage host tissue, and ultimately cause a wide range of diseases, including Parkinson's disease [6], atherosclerosis [7] and certain cancers [8]. Hypochlorite is also widely used in our daily life, for example, household bleach, disinfection of drinking water and cool-water treatment, with a concentration in the range of 10^{-5}–10^{-2} M [9]. Such highly concentrated hypochlorite solution is a potential health hazard to humans and animals. Therefore, it is very important to detect hypochlorous acid in the biological and environmental samples.

Several techniques such as colorimetry, electron paramagnetic resonance, electrochemistry, fluorescence and chemiluminescence, have been developed for the detection of HOCl [10–15]. Among the various analytical techniques above, fluorescence has been considered ideal for detecting hypochlorous acid due to its simplicity, high sensitivity, real-time, non-destructive properties [16–18]. To date, a large number of fluorescent probes for HOCl/OCl^- detection have been developed by utilizing the specific reactions, including dibenzoylhydrazine [19–21], *p*-methoxyphenol [2–24], oxime [25–30], selenide [31–33], thiol compounds (internal thioester, thioether and thiosemicarbazide) [34–36], and other groups [37–42]. The aforementioned fluorescent probes for HOCl exhibit satisfactory properties, but a major limitation is that most of them require complicated multistep synthesis, function only over a basic pH range and are less easily available. Thus, it is of considerable interest to design and develop new probes which can be able to overcome these limitations.

In this work, we designed a new phenanthroimidazole probe integrated with a diaminomaleonitrile unit for selective detection of HOCl. The diaminomaleonitrile functionality has been well established as a sensitive group to hypochlorous acid and provided a suitable signalling platform in the design of fluorescent probes [43–45]. So the probe **1** was synthesized and characterized by ^{1}H NMR, ^{13}C NMR, ESI–MS and IR. Moreover, the detection could be realized in mixed aqueous medium and the detection limit of probe for ClO^- was found to be 0.17 μM. The probe **1** also exhibited rapid detection within 3.0 min and satisfactory selectivity for ClO^- even in the presence of other ROSs, common anions and cations. Furthermore, the probe is cell membrane permeable, and its applicability

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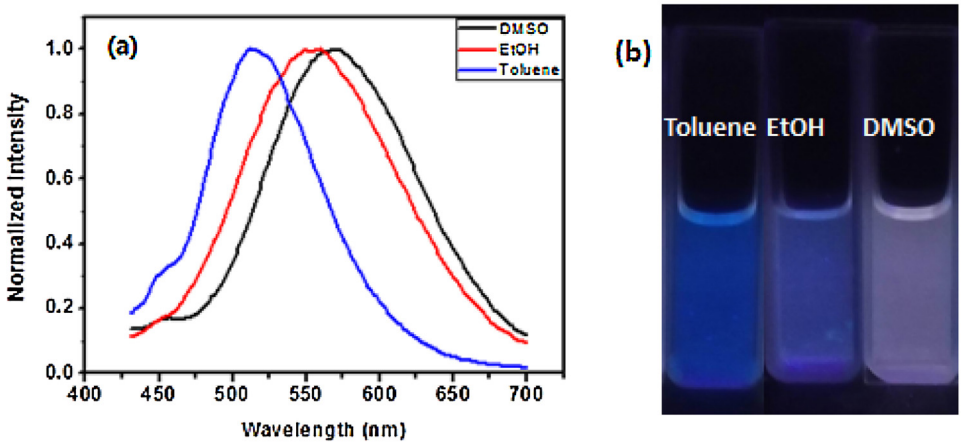
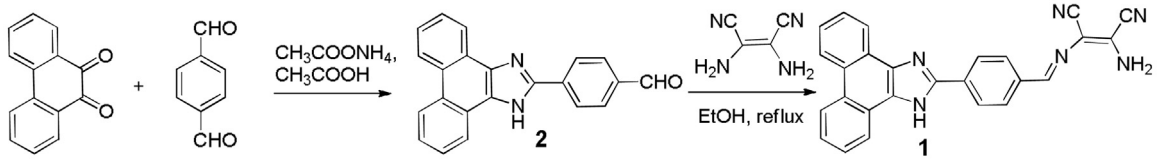


Fig. 1. (a) Solvent-dependent emission spectra of probe **1** (5 μM), λex = 420 nm; slits: (5 nm/5 nm); (b) Photos of probe **1** (5 μM) in different solvents under irradiation with UV light.



Scheme 1. Synthesis of probe **1**.

has been demonstrated for fluorescence imaging of HClO in HeLa cells.

2. Experimental

2.1. General

All reagents and solvents were purchased from commercial source and used without further purification. All reactions were carried out on the magnetic stirrers and their reaction process was monitored on thin layer chromatography (TLC). Absorption spectra were taken on a Varian Carry 4000 spectrophotometer. Fluorescence spectra were taken on Hitachi F-7000 fluorescence spectrometer. ^{1}H NMR and ^{13}C NMR measurements were recorded at 600 and 150 MHz on a Brucker Avance 600-MHz spectrometer. Dimethyl sulfoxide (DMSO – d_6) was solvent, and tetramethylsilane (TMS) was used as internal standard. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; m = multiplet. HRMS were taken on a Fourier transform ion cyclotron resonance mass spectrometry (Varian 7.0 T). The structures of **1** and **2** were characterized by FT – IR, ESI – MS, ^{1}H– and ^{13}C – NMR and the corresponding spectra are shown in Supplementary information.

2.2. Preparation of probe 1

2.2.1. Preparation of 2

A mixture of 9,10 – phenanthroquinone (0.5 g, 2.4 mmol), terephthalaldehyde (0.65 g, 4.8 mmol) and ammonium acetate (2.46 g, 32 mmol) in glacial acetic acid (20 mL) was heated to 100 °C for 2 h. The hot solution was cooled to room temperature, and the resulting yellow solid was collected by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution, and water. The yellow solid was further dried under reduced vacuum, and then purified by silica gel column chromatography using acetone as eluent to afford the pure product **2** as a yellow solid (0.54 g, 70.0%). ^{1}H NMR (600 MHz, DMSO – d_6): δ 10.11 (d, 1H, *J* = 8.2), 8.89 (d, 2H, *J* = 8.4), 8.62 (d, 2H. *J* = 7.2), 8.55 (d, 2H, *J* = 8.4), 8.15 (d, 2H, *J* = 8.4), 7.79 – 7.77 (m, 2H), 7.70 – 7.68 (m, 2H); FT – IR: ν = 3367.18 cm^{-1} (–NH –), 3054.38 cm^{-1} (–(CO)–H), 1667.30 cm^{-1} (C=O).

2.2.2. Preparation of probe 1

2 (0.33 g, 1 mmol) was dissolved in ethanol (10 mL), then diaminomaleonitrile (0.12 g, 1.1 mmol) was added and the reaction mixture heated at reflux for 12 h. Then reaction was cooled to room temperature and the precipitate was collected by filtration, washed with cold ethanol, and dried *in vacuo*. Probe **1** was obtained as a yellow solid in 80% yield (0.34 g). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.89 (d, 2H, *J* = 7.8), 8.61 (d, 2H. *J* = 7.4), 8.42 (d, 2H, *J* = 8.4), 8.36 (s, 1H), 8.27 (d, 2H, *J* = 8.4), 8.12 (s, 2H), 7.78 – 7.76 (m, 2H), 7.69 – 7.66 (m, 2H). ^{13}C NMR (150 MHz, DMSO – d_6): δ 154.6, 148.7, 136.5, 130.1, 128.3, 127.7, 127.6, 126.7, 126.1, 124.5, 122.6, 117.5, 114.9, 114.2, 106.5, 103.2. HRMS (ESI) calcd. for [M+H]$^+$ 413.1509, found 413.1511; FT – IR: ν = 3438.62 cm^{-1}(–NH$_2$), 3346.54 cm^{-1} (–NH–), 2212.12 cm^{-1} (C≡N).

2.3. 1H NMR analysis experiments of probe 1

The solution of probe **1** (3.0×10^3 μM) in DMSO – d_6 (450 μL) was placed in the NMR tube, and NaOCl (60 μM) was added.

2.4. Titration experiments of probe 1

For UV–vis and fluorescence titrations, stock solution of probe **1** was prepared in a EtOH–H$_2$O solution (3: 1, v/v, 10 mM PBS buffer, pH 7.4) to afford a concentration of 5 μM stock solution. The solution of NaOCl was prepared (2×10^2 μM) in pure water. The original volume of probe **1** solution was 2 mL. Detailed preparation of the solutions (10 mM for F^-, NO_3^-, $H_2PO_4^-$, NO_2^-, ClO_4^-, IO_4^-, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, H_2O_2, NO•, $O_2^{\bullet-}$, •OH, ROO•, and ONOO– were described in Supplementary information. The spectra of these solutions were recorded by means of fluorescence method. Fluorescence measurements were carried out with a slit width of 5 nm (λex = 340 nm) in 10 mm quartz cuvettes at room temperature.