



Cite this: *Chem. Commun.*, 2018, 54, 8522

Received 8th May 2018,  
Accepted 28th June 2018

DOI: 10.1039/c8cc03717e

rsc.li/chemcomm

## ESIPT-based fluorescence probe for the rapid detection of hypochlorite (HOCl/CIO<sup>−</sup>)†

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ESIPT-based fluorescence probes are emerging as an attractive tool for the detection of biologically relevant analytes owing to their unique photophysical properties. In this work, we have developed an ESIPT-based fluorescence probe (TCBT-OMe) for the detection of HOCl/CIO<sup>−</sup> through the attachment of a bioorthogonal dimethylthiocarbamate linker. TCBT-OMe was shown to rapidly detect HOCl/CIO<sup>−</sup> (<10 s) at biologically relevant concentrations (LoD = 0.16 nM) and have an excellent selectivity towards others ROS/RNS and amino acids. Therefore, TCBT-OMe was tested in live cells and was successfully shown to be able to detect endogenous and exogenous HOCl/CIO<sup>−</sup> in HeLa cells. Additionally, TCBT-OMe acts as a dual input logic gate for Hg<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>. Interestingly, Hg<sup>2+</sup> alone gradually causes a fluorescence response but requires >30 min to produce a fluorescence response. Test strips containing TCBT-OMe were prepared and were demonstrated as an effective way to detect HOCl/CIO<sup>−</sup> in water. Furthermore, TCBT-OMe was shown to detect exogenously added HOCl/CIO<sup>−</sup> in three different water samples with little interference thus demonstrating the effectiveness as a method for the detection of HOCl/CIO<sup>−</sup> in drinking water samples.

Hypochlorous acid (HOCl) is a biologically important reactive oxygen species (ROS), which partially dissociates to form its hypochlorite anion (CIO<sup>−</sup>) under physiological conditions. In biological systems, myeloperoxidase, an enzyme found in leukocytes produces HOCl/CIO<sup>−</sup> by catalysing the reaction between Cl + H<sub>2</sub>O<sub>2</sub> → HOCl.<sup>1</sup> This vital ROS is used in immune defence systems due to its microbicidal properties.<sup>1</sup> Unfortunately, excessive

production of HOCl/CIO<sup>−</sup> can lead to the damage of a range of biological targets such as amino acids, proteins, carbohydrates and lipids.<sup>2,3</sup> As a consequence, HOCl/CIO<sup>−</sup> has been associated with a number of diseases causing cell and tissue damage.<sup>4</sup>

In addition to its role in biological systems, HOCl/CIO<sup>−</sup> is produced by the chlorination of water (Cl<sub>2</sub> + H<sub>2</sub>O → HOCl), which is the most common method for the treatment of water especially in public swimming pools.<sup>5</sup> NaOCl (Bleach) is also extensively used as a disinfectant for both domestic and industrial purposes. Unfortunately, over-exposure to HOCl/CIO<sup>−</sup>, results in swimming pool-associated asthma, irritation to the oesophagus, throat and spontaneous vomiting ([http://www.who.int/water\\_sanitation\\_health/dwq/chlorine.pdf](http://www.who.int/water_sanitation_health/dwq/chlorine.pdf)).<sup>6</sup> Additionally, there is an increased risk of bladder cancer associated with chlorinated by-products produced from chlorinated water.<sup>7,8</sup> Therefore, given the potential health hazard towards animals and humans, the development of an effective method for HOCl/CIO<sup>−</sup> detection is required.

Within our research group, we are interested in developing reaction-based fluorescence sensors for the detection of biologically important analytes.<sup>9–13</sup> Small-molecule fluorescence probes are a particular attractive tool owing to their high sensitivity, selectivity and high spatial and temporal resolution.<sup>14</sup> In particular, we are interested in using Excited State Intramolecular Proton Transfer (ESIPT)-based fluorescence probes due to their excellent photophysical properties, which include intense luminescence, photostability and a large Stokes shift.<sup>15,16</sup> Previously, we reported an ESIPT-based fluorescence probe for the detection of peroxynitrite (ONOO<sup>−</sup>) through the use of a benzyl boronic ester protecting group (Scheme 1).<sup>15</sup> This protecting group blocked the ESIPT process and therefore a low fluorescence intensity was observed. The addition of ONOO<sup>−</sup>, resulted in the fluorophore's deprotection and an increase in fluorescence intensity was observed.

In this work, we believed a methoxy-hydroxybenzothiazole (HBT-OMe) fluorophore would provide an effective ESIPT fluorescence probe for the detection of HOCl/CIO<sup>−</sup> (see ESI,† S1).<sup>17,18</sup>

To obtain TCBT-OMe we first prepared HBT-OMe by the addition of a 2 : 1 H<sub>2</sub>O<sub>2</sub>–(30% in H<sub>2</sub>O)/HCl solution to 2-aminothiophenol

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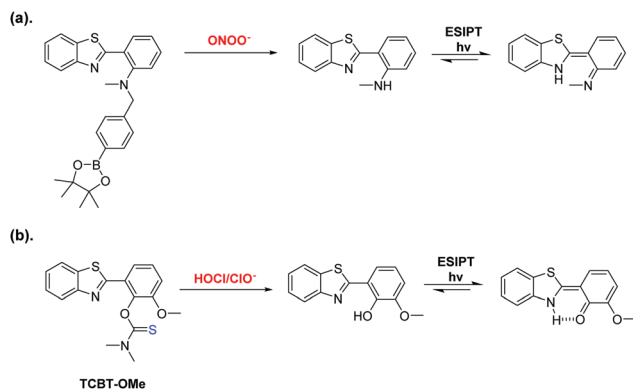
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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8cc03717e



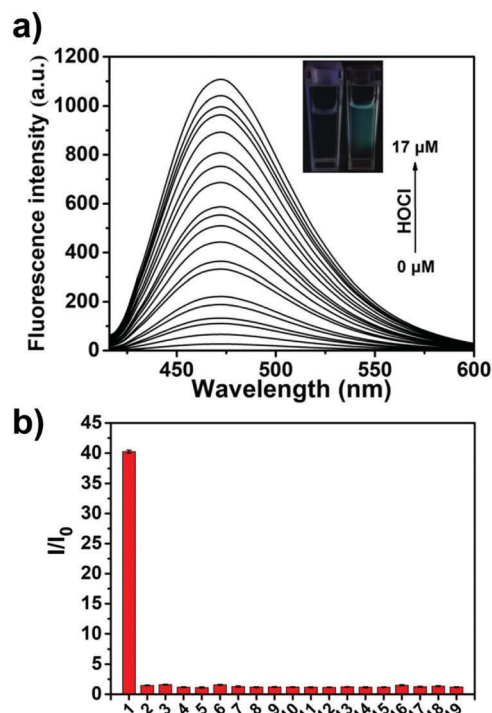


**Scheme 1** (a) Our previously reported ESIPT probe for the detection of  $\text{ONOO}^-$ . (b) This work – a thiocarbamate linker-based ESIPT **TCBT-OMe** for the detection of  $\text{HOCl/CIO}^-$ .

and *O*-vanillin in EtOH. This reaction proceeded quickly and smoothly, in a good yield (68%). With **HBT-OMe** in hand, four equivalents of dimethylthiocarbamoyl chloride was then added slowly to a solution of **HBT-OMe** in DCM. DIPEA was subsequently added dropwise to the reaction, which produced **TCBT-OMe** in excellent yield (72%).

We then evaluated the UV-Vis of **TCBT-OMe** with the addition of  $\text{HOCl/CIO}^-$  (10  $\mu\text{M}$ ), which resulted in the formation of a UV absorption peak at  $\sim 310$  nm (see ESI,† Fig. S1). Bhattacharyya *et al.* have reported that the fluorescence emission of the ESIPT process can be effected by intermolecular hydrogen bonding.<sup>19,20</sup> Therefore, evaluation of ESIPT-based fluorescence probes are commonly carried out in the presence of the surfactant cetyl trimethylammonium bromide (CTAB, 1 mM) or by using a large ratio of organic solvent.<sup>19,21–23</sup> It is believed that the formation of a micellar environment creates a hydrophobic pocket that aids the ESIPT process. Therefore, we evaluated the ability of **TCBT-OMe** to detect  $\text{HOCl/CIO}^-$  by fluorescence in the presence of CTAB, 1 mM. As shown in Fig. 1a, **TCBT-OMe** was found to be very sensitive towards  $\text{HOCl/CIO}^-$  reacting with micromolar concentrations to produce a large increase in fluorescence ( $\sim 42$  fold – Fig. S3, ESI†). **TCBT-OMe** was shown to rapidly react with  $\text{HOCl/CIO}^-$  producing a fluorescence response within less than 10 s (see ESI,† Fig. S4) and have a very low Limit of Detection (LoD) of 0.16 nM (see ESI,† Fig. S5).  $\text{HOCl/CIO}^-$  (35  $\mu\text{M}$ ) was added to **TCBT-OMe** at different pH values and a bell-shaped curve was observed. The largest fluorescence response was seen at the  $\text{pK}_a$  of  $\text{HOCl/CIO}^- = 7.53$  (Fig. S5, ESI†) suggestive of general acid–base catalysis being in operation. (see ESI,† Scheme S1 for proposed mechanism).

We then evaluated the selectivity of **TCBT-OMe** towards other reactive oxygen/nitrogen species (ROS/RNS) and amino acids (Fig. 1b). Remarkably, **TCBT-OMe** had an excellent selectivity towards  $\text{HOCl/CIO}^-$  therefore permitting its use as a fluorescence probe for the detection of  $\text{HOCl/CIO}^-$  in live cells. As shown in Fig. 2, **TCBT-OMe** was successfully used to visualise endogenously stimulated  $\text{HOCl/CIO}^-$  in HeLa cells using phorbol 12-myristate 13-acetate (**PMA**, which is a ROS stimulant that induces the production of  $\text{HOCl/CIO}^-$ ). Separately, HeLa cells were



**Fig. 1** (a) Fluorescence spectra of **TCBT-OMe** (5  $\mu\text{M}$ ) with increasing additions of  $\text{HOCl/CIO}^-$  (from 0 to 17  $\mu\text{M}$ ) in PBS buffer (pH 7.4, containing 1% DMSO, 1 mM CTAB). Measurements were taken after 1 min.  $\lambda_{\text{ex}} = 310$  nm. Slit widths: ex = 6 nm em = 4 nm. (b) Selectivity bar chart of **TCBT-OMe** in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB with  $\text{HClO}$  (15  $\mu\text{M}$ ) and other interfering reagents (ROS/RNS and various amino acids). 1,  $\text{HClO}$ ; 2, blank; 3,  $\text{ONOO}^-$ ; 4,  $\text{H}_2\text{O}_2$ ; 5,  $\text{ROO}^\bullet$ ; 6,  $\bullet\text{OH}$ ; 7,  $\bullet\text{O}_2^-$ ; 8,  $^1\text{O}_2$ ; 9, NO; 10, glycine; 11, asparagine; 12, cysteine; 13, homocysteine; 14, glutathione; 15, arginine; 16, histidine; 17, serine; 18, glycine; 19, threonine. Note: the concentration of **TCBT-OMe** and each interfering species are 5  $\mu\text{M}$  and 100  $\mu\text{M}$  respectively, 30 min wait before measurement in buffer solution.  $\lambda_{\text{ex}} = 310$  nm/ $\lambda_{\text{em}} = 472$  nm error bars represent s.d. Measurements were taken after 30 min.  $\lambda_{\text{ex}} = 310$  nm. Slit widths: ex = 6 nm, em = 4 nm.

also pretreated with 4-aminobenzoic acid hydrazide (**ABAH**, which is a specific inhibitor of MPO which suppressed the generation of  $\text{HOCl}$ ) and as expected only weak fluorescence was observed. **TCBT-OMe** was also able to detect  $\text{HOCl/CIO}^-$  added exogenously to the HeLa cells.

The dimethylthiocarbamate linker of **TCBT-OMe** has previously been used in the construction of dual input molecular logic gate<sup>24</sup> for the detection of  $\text{Hg}^{2+}$  'AND'  $\text{H}_2\text{O}_2$  (see ESI† Scheme S2 for proposed mechanism).<sup>25,26</sup> Therefore, we evaluated the ability of **TCBT-OMe** to perform molecular logic with the input of  $\text{Hg}^{2+}$  and  $\text{H}_2\text{O}_2$ . The presence of solely  $\text{H}_2\text{O}_2$  (120  $\mu\text{M}$ ) led to a small increase in fluorescence intensity (dashed line), however, with subsequent additions of  $\text{Hg}^{2+}$  (0–9  $\mu\text{M}$ ) a large fluorescence response was observed (Fig. 3a). To demonstrate that both analytes are required,  $\text{Hg}^{2+}$  was added first, followed by the addition of  $\text{H}_2\text{O}_2$  (0–180  $\mu\text{M}$ ). As shown in Fig. 3b, the subsequent addition of  $\text{H}_2\text{O}_2$  rapidly led to an increase in fluorescence intensity. **TCBT-OMe** was shown to be selective towards  $\text{Hg}^{2+}$  over other metal cations in the presence of  $\text{H}_2\text{O}_2$  (see ESI,† Fig. S9). Interestingly,  $\text{Hg}^{2+}$  alone resulted in a slow increase in fluorescence intensity (see ESI,† Fig. S10). This is believed to be





Fig. 2 (a) From top to bottom: HeLa cells were pretreated with **TCBT-OMe** (40  $\mu\text{M}$ ) for 30 min; HeLa cells pretreated with **TCBT-OMe** (40  $\mu\text{M}$ ) were then left for 30 min after preincubation with PMA (1.2  $\mu\text{g mL}^{-1}$ ) for 90 min; HeLa cells pretreated with **TCBT-OMe** (40  $\mu\text{M}$ ) were then left for 30 min after preincubation with 250  $\mu\text{M}$  ABAH for 70 min; HeLa cells loaded with **TCBT-OMe** (40  $\mu\text{M}$ ) for 30 min followed by the exogenous addition of 8  $\mu\text{M}$  NaOCl for 5 min. Scale bar: 25  $\mu\text{m}$   $\lambda_{\text{ex}}$  = 420 nm/ $\lambda_{\text{em}}$  = 420–590 nm. (b) The histogram shows the semi-quantitative calculation of averaged fluorescence intensity (FI) of each fluorescence panel in the displayed images by ImageJ software.

due to the instability of the dimethylcarbonate formed from the reaction of **TCBT-OMe** with  $\text{Hg}^{2+}$ .

Despite this interesting dual responsive reactivity of **TCBT-OMe**, this 'AND' logic requires minutes to fully react, whereas  $\text{HOCl}/\text{ClO}^-$  reacts with **TCBT-OMe** within seconds. Therefore, due to the significantly greater reactivity of **TCBT-OMe** towards  $\text{HOCl}/\text{ClO}^-$  over  $\text{Hg}^{2+}$ , we believed we could use it as an effective method for the detection of  $\text{HOCl}/\text{ClO}^-$  in drinking water sources.

We produced test strips by simply soaking a commercially available test strip in water containing **TCBT-OMe** (0.8 mM). After drying, test strips impregnated with **TCBT-OMe** were placed in water containing  $\text{HClO}/\text{ClO}^-$  (0–200  $\mu\text{M}$ ). As shown in Fig. 4, there is a clear colour/intensity difference in the test strips that have been dipped into water containing various concentrations of  $\text{HClO}/\text{ClO}^-$ .

In addition to detecting  $\text{HClO}/\text{ClO}^-$  in water, **TCBT-OMe** was added into three different water samples containing 1 mM CTAB (Sample A, tap water from University of Bath; Sample B, water from the Avon River (Bath); Sample C, water from Roman spa in Bath). Interestingly, little interference was observed for the exogenous addition of  $\text{HClO}/\text{ClO}^-$  to each water sample (>95% recovery) – see ESI,† Table S1.

In summary, we have developed an ESIPT-based fluorescence **TCBT-OMe** for the detection of  $\text{HClO}/\text{ClO}^-$ . **TCBT-OMe** was shown to have a very high sensitivity and selectivity towards  $\text{HClO}/\text{ClO}^-$  fully reacting within 10 s and having a LoD of 0.16  $\mu\text{M}$ . Significantly, **TCBT-OMe** was able to detect endogenous and

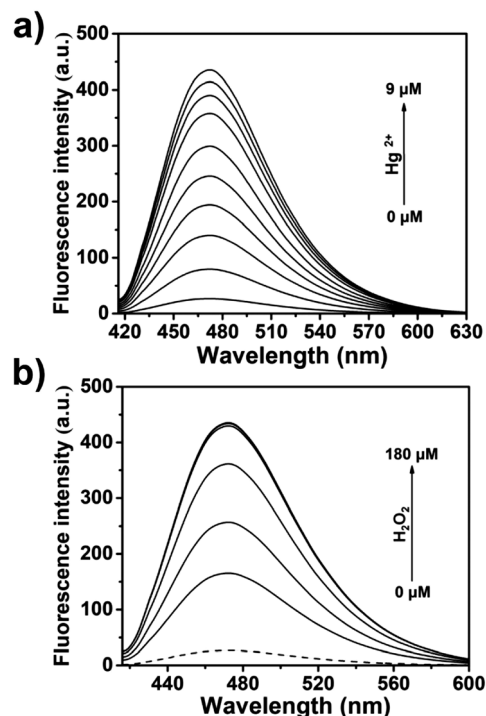


Fig. 3 (a) Fluorescence spectra of **TCBT-OMe** (5  $\mu\text{M}$ ) in the presence of  $\text{H}_2\text{O}_2$  (120  $\mu\text{M}$ ) – (dashed line represent probe and  $\text{H}_2\text{O}_2$ ) with increasing concentrations of  $\text{Hg}^{2+}$  (0–9  $\mu\text{M}$ ) in buffer solution pH 7.4, 1% DMSO, 1 mM CTAB 14 min wait between measurement.  $\lambda_{\text{ex}}$  = 310 nm. Slit widths: ex = 6 nm em = 4 nm. (b) Fluorescence spectra of **TCBT-OMe** (5  $\mu\text{M}$ ) in the presence of  $\text{Hg}^{2+}$  (9  $\mu\text{M}$ ) – (dashed line represents probe and  $\text{Hg}^{2+}$ ) with increasing concentrations of  $\text{H}_2\text{O}_2$  (final concentration: 0, 20, 40, 80, 100, 120, 140  $\mu\text{M}$  and 180  $\mu\text{M}$ ) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB. 14 min wait between measurement in buffer solution.  $\lambda_{\text{ex}}$  = 310 nm. Slit widths: ex = 6 nm em = 4 nm.



Fig. 4 Photograph showing the colour changes of **TCBT-OMe** impregnated test strips after addition to water samples containing different concentrations of  $\text{HClO}/\text{ClO}^-$  under UV light (365 nm).

exogenous  $\text{HClO}/\text{ClO}^-$  in HeLa cells. Additionally, **TCBT-OMe** was shown as a dual input logic gate with  $\text{Hg}^{2+}$  and  $\text{H}_2\text{O}_2$  as inputs. Interestingly,  $\text{Hg}^{2+}$  alone gradually produced a fluorescence response but required >30 min to produce a significant fluorescence response. Test strips containing **TCBT-OMe** were developed and shown to be an effective way to detect  $\text{HClO}/\text{ClO}^-$  in water. Furthermore, **TCBT-OMe** was shown to detect exogenously added  $\text{HClO}/\text{ClO}^-$  in three different water samples with little interference demonstrating its effectiveness as a method to detect  $\text{HClO}/\text{ClO}^-$  in drinking water samples.



LW wishes to thank China Scholarship Council and the University of Bath for supporting his PhD work in the UK. We would like to thank the EPSRC and the University of Bath for funding. ACS thanks the EPSRC for a studentship. AJC wishes to thank the Royal Society for a University Research Fellowship. TDJ wishes to thank the Royal Society for a Wolfson Research Merit Award and Sophia University for a visiting professorship. NMR characterisation facilities were provided through the Chemical Characterisation and Analysis Facility (CCAF) at the University of Bath ([www.bath.ac.uk/ccaf](http://www.bath.ac.uk/ccaf)). The EPSRC UK National Mass Spectrometry Facility at Swansea University is thanked for analyses. All data supporting this study are provided as supplementary information accompanying this paper (ESI†).

## Conflicts of interest

No conflicts of interest.

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