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# Sequential detection of hypochlorous acid and sulfur dioxide derivatives by a red-emitting fluorescent probe and bioimaging applications *in vitro* and *in vivo*†

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Hypochlorous acid (HOCl) and sulfur dioxide derivatives ( $\text{SO}_3^{2-}/\text{HSO}_3^-$ ) play critical roles in complex signal transduction and oxidation pathways. Therefore, it is meaningful and valuable to detect both HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  derivatives in biosystems by a fluorescence imaging assay. In this work, we developed a red-emitting fluorescent probe (DP) by the condensation of malononitrile and phenothiazine derivatives through a C=C double bond. DP was designed with a donor- $\pi$ -acceptor (D- $\pi$ -A) structure, which enables absorption and emission in the long wavelength region. In the presence of HOCl, specific oxidation of the thioether of phenothiazine in DP to a sulfoxide derivative (DP=O) occurs, resulting in a hypochromic shift (572 nm to 482 nm) of the absorption spectra and "OFF-ON" response of the maximum emission at 608 nm. After the activation of the C=C double bond by oxidation, DP=O reacts specifically with  $\text{SO}_3^{2-}/\text{HSO}_3^-$  via a 1,4-nucleophilic addition reaction leading to a decrease in the intensity of the absorption and emission spectra, which enabled the realization of sequential detection of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  by a single fluorescent probe. The detection limits of DP for HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  were calculated to be 81.3 nM and 70.8 nM/65.1 nM, respectively. The results of fluorescence microscopic imaging indicated that DP shows potential for the detection of intracellular HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$ . Using adult zebrafish and nude mice as live animal models, DP was successfully used for the fluorescence imaging of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  *in vivo*.

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## Introduction

Reactive oxygen species (ROS), including mainly HOCl,  $\text{H}_2\text{O}_2$ , HO, and  $\text{O}_2^-$ , play crucial roles in various regular biochemical approaches and irregular pathological pathways.<sup>1–3</sup> Among the ROS, hypochlorous acid (HOCl) and the dissociated hypochlorite ion ( $\text{OCl}^-$ ) are strong oxidants found in human bodies while being widely used in our daily life as a bleach.<sup>4–6</sup> Endogenous HOCl is usually generated from hydrogen peroxide and chloride ions by secreted myeloperoxidase (MPO) *in vivo* in response to

inflammatory stimuli.<sup>7,8</sup> The physiologically relevant levels of HOCl in living organisms are 5–25  $\mu\text{M}$ .<sup>9</sup> It has been evidenced that excessively generated HOCl is related to extensive oxidative stress and oxidative damage, leading to numerous diseases such as neurodegeneration including Parkinson's disease and Alzheimer's disease, inflammatory diseases, atherosclerosis and cancer.<sup>10–14</sup>

Cells often possess an elaborate antioxidant defense system to regulate their internal redox equilibrium.<sup>15–17</sup> Sulfur dioxide ( $\text{SO}_2$ ), one of the reactive sulfur species (RSS), participates actively in equilibrating the redox status as an antioxidant against ROS.<sup>18–20</sup>  $\text{SO}_2$  forms two reductive derivatives: sulfite ( $\text{SO}_3^{2-}$ ) and bisulfite ( $\text{HSO}_3^-$ ) (3 : 1 M/M, in neutral fluids).<sup>21,22</sup> It is believed that  $\text{SO}_2$  derivatives play crucial roles in a wide range of physiological and pathological processes such as vaso-relaxant, antihypertensive, antiatherogenic, and antioxidative effects.<sup>23–25</sup> However, excessive intake of  $\text{SO}_3^{2-}/\text{HSO}_3^-$  is associated with a variety of adverse effects and acute symptoms such as flushing, hypotension, diarrhea, urticaria, and abdominal pain.<sup>26–29</sup> In addition, excessive intake of  $\text{HSO}_3^-$  can lead to a variety of diseases such as cancer, nervous system disorders, asthma and allergic reactions, and even tissue damage.<sup>30</sup> Food

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and Agriculture Organization (FAO) and World Health Organization (WHO) have recommended that the daily intake of sulfites/bisulfites per person be less than  $0.7 \text{ mg kg}^{-1}$  (body weight) in view of the serious health risks associated with excessive intake of  $\text{HSO}_3^-$ .<sup>31</sup> In view of the important roles of ROS/RSS-regulated redox homeostasis in biosystems, it is meaningful and valuable to develop efficient methods for monitoring  $\text{HOCl}/\text{SO}_2$  derivatives regulating redox cycle processes *in vitro* and *in vivo*, which is vital for biological research as well as clinical diagnoses.

Among the various bioanalytical methods, fluorescence imaging technology has been regarded as a powerful visual methodology for the detection and monitoring of various biological components due to its advantages such as high sensitivity, good selectivity, little invasiveness and real-time detection.<sup>32–35</sup> To date, number of fluorescent probes have been designed and reported for the individual detection and visualization of  $\text{HOCl}$  and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  in biosystems and real water or food samples.<sup>36–46</sup> However, a fluorescent probe specifically for detecting and monitoring both  $\text{HOCl}$  and  $\text{SO}_2$  derivatives in biosystems and visually detecting  $\text{SO}_2$  derivatives in real samples is rarely reported.<sup>47,48</sup> Herein, we developed a red-emitting fluorescent probe (**DP**) by linking malononitrile and phenothiazine derivatives through a  $\text{C}=\text{C}$  double bond. In the presence of  $\text{HOCl}$ , specific oxidation of the thioether of **DP** to a sulfoxide derivative (**DP=O**) occurs, resulting in a hypochromic shift (574 nm to 480 nm) of the absorption spectra and “OFF–ON” response of the emission. After the activation of the  $\text{C}=\text{C}$  double bond by oxidation, the oxidation product (**DP=O**) reacts with  $\text{SO}_3^{2-}/\text{HSO}_3^-$  via a 1,4-nucleophilic addition reaction leading to a decrease in the intensity of the absorption and emission spectra, which enabled the realization of sequential detection of  $\text{HOCl}$  and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  by a single fluorescent probe (Scheme 1).

## Results and discussion

### Design, synthesis and characterizations of fluorescent probe (**DP**)

The probe **DP** was synthesized *via* a one-step condensation reaction between 2-[3-cyano-4,5,5-trimethylfuran-2(5*H*)-ylidene] malononitrile (DCDHF) and 10-ethyl-10*H*-phenothiazine-3-carbaldehyde in the presence of catalytic amounts of piperidine in methanol (Scheme S1†). The chemical structure of **DP** was characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS (Fig. S1–S3†). **DP** was designed with a donor– $\pi$ –acceptor ( $\text{D}-\pi-\text{A}$ ) structure, in which a phenothiazine unit acts as an electron-donating group (EDG), while DCDHF acts as an electron-

withdrawing group (EWG). The large  $\pi$ -conjugation structure enables **DP** to absorb and emit in the long wavelength region. **DP** features outstanding water solubility, allowing its application in 90% PBS aqueous solutions ( $\text{DMF}:\text{H}_2\text{O} = 1:9$ , v/v, 20 mM,  $\text{pH} = 7.4$ ). In addition, only small fluctuations in the fluorescence intensity of **DP** at 608 nm were found within 30 h in the HEPES aqueous buffer (Fig. S4†), suggesting the high stability of **DP** under simulated physiological conditions. The proposed reaction mechanisms of **DP** towards  $\text{HOCl}$  and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  were first studied by HR MS titration measurement. As shown in Fig. S5,† after addition of  $\text{HOCl}$ , the molecular ion peak assigned to **DP** at 451.15936 ( $[\text{DP}+\text{H}]^+$ ,  $m/z$ , calcd.: 451.15926) disappeared and a new peak belonging to the oxidized sulfoxide product (**DP=O**) emerged at  $m/z$  467.1558 ( $[\text{DP}=\text{O}+\text{H}]^+$ ,  $m/z$ , calcd. 467.1536). Sequentially, upon the addition of  $\text{HSO}_3^-$  to the solution of **DP=O**, a new peak assigned to the addition product **DP=O-SO<sub>3</sub>** ( $[\text{DP}=\text{O-SO}_3]^-$ ,  $m/z$ , calcd. 547.1115) emerged at  $m/z$  547.1191 (Fig. S6†). Similarly, a new peak assigned to the addition product **DP=O-SO<sub>3</sub>** also appeared at  $m/z$  547.1135 in the presence of  $\text{SO}_3^{2-}$  (Fig. S7†).

### Spectroscopic responses of **DP** towards $\text{HOCl}$

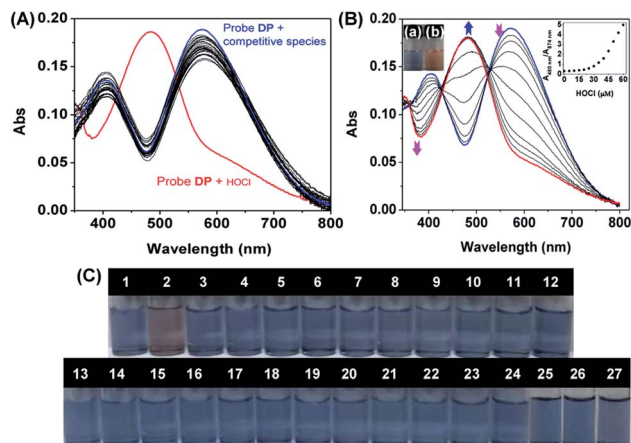
With probe **DP** in hand, we first tested the spectroscopic response of **DP** (10  $\mu\text{M}$ ) in stimulated physiological media ( $\text{PBS}:\text{H}_2\text{O} = 1:9$ , v/v, 20 mM,  $\text{pH} = 7.4$ ). In PBS aqueous solutions, **DP** displayed a main absorption band centered at 574 nm. Upon the addition of various analytes such as reactive oxygen species ( $\text{HOCl}$ ,  $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ ,  $\text{ONOO}^-$ ,  $\text{OH}^\cdot$  and  $\text{O}_2^-$ ), reactive sulfur species (Cys, GSH, Hcy,  $\text{S}^{2-}$ ,  $\text{SO}_3^{2-}$ ,  $\text{HSO}_3^-$ ,  $\text{HSO}_4^-$  and  $\text{SO}_4^{2-}$ ) and biologically relevant anions ( $\text{Br}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{P}_2\text{O}_7^{4-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{OH}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ) to the solution of **DP**, only  $\text{HOCl}$  contributed to the major absorption peak of **DP** at 574 nm, which gradually decreased accompanied by a new absorption peak appearing at 480 nm. Significant changes were not observed in the absorption spectra upon the addition of the aforementioned competitive species (Fig. 1A). The absorption spectra response of **DP** towards  $\text{HOCl}$  was then investigated by a titration experiment. As presented in Fig. 1B, upon the addition of increasing amounts of  $\text{HOCl}$  (0–60  $\mu\text{M}$ ), the major peak of **DP** centered at 574 nm declined notably and an intense absorption band centred at 480 nm appeared and enhanced gradually. The new absorption was attributed to the  $\text{HOCl}$ -induced oxidation product (**DP=O**). The absorbance ratio ( $A_{480 \text{ nm}}/A_{574 \text{ nm}}$ ) of **DP** became constant and the blue solution of **DP** turned to yellowish-brown when the concentration of  $\text{HOCl}$  reached 50  $\mu\text{M}$  (Fig. 1B, inset). Furthermore, two clear isosbestic points at 426 nm and 525 nm emerged in the presence of  $\text{HOCl}$ , which indicated the quantitative reaction of **DP** with  $\text{HOCl}$ . The specificity of **DP** toward  $\text{HOCl}$  was then validated by a “naked-eye” colorimetric assay. As shown in Fig. 1C, the color of the **DP** solution was changed from blue to yellowish-brown specifically in the presence of  $\text{HOCl}$ , suggesting that **DP** can serve as a potential “naked-eye” indicator for  $\text{HOCl}$  detection.

The sensing behavior of **DP** toward various analytes was further tested with fluorescence spectra. The solution of free **DP**



Scheme 1 Proposed sensing mechanism of **DP** for the sequential detection of  $\text{HOCl}$  and  $\text{SO}_2$  derivatives ( $\text{SO}_3^{2-}/\text{HSO}_3^-$ ).

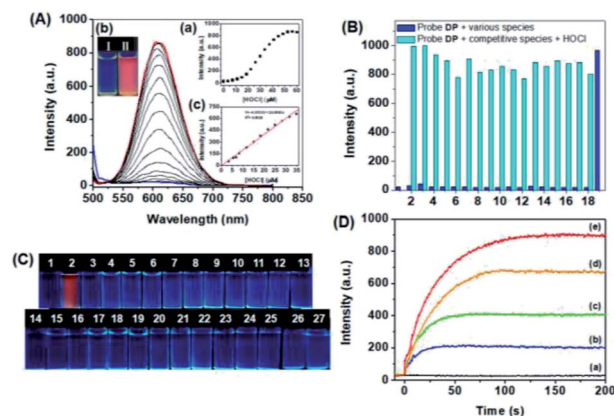




**Fig. 1** (A) Absorption spectra of probe DP (10  $\mu\text{M}$ ) in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4) upon addition of various analytes (60  $\mu\text{M}$ ). (B) UV-vis absorption spectra of probe DP (10  $\mu\text{M}$ ) in the presence of different amounts of HOCl (0–60  $\mu\text{M}$ ) in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). Inset: absorbance ratio of probe DP at 480 nm and 574 nm as a function of HOCl concentration. Colorimetric changes of probe DP (a) in the absence and (b) in the presence of HOCl (60  $\mu\text{M}$ ). (C) Colorimetric changes of probe DP (10  $\mu\text{M}$ ) in the presence of various analytes (60  $\mu\text{M}$ ): (1) free DP, (2) HOCl, (3)  $\text{Br}^-$ , (4)  $\text{F}^-$ , (5)  $\text{Cl}^-$ , (6)  $\text{P}_2\text{O}_7^{4-}$ , (7)  $\text{PO}_4^{3-}$ , (8)  $\text{H}_2\text{PO}_4^-$ , (9) Cys, (10) GSH, (11) Hcy, (12)  $\text{S}^{2-}$ , (13)  $\text{SO}_3^{2-}$ , (14)  $\text{HSO}_3^-$ , (15)  $\text{H}_2\text{O}_2$ , (16)  $^1\text{O}_2$ , (17)  $\text{OH}^-$ , (18)  $\text{CH}_3\text{COO}^-$ , (19)  $\text{HCO}_3^-$ , (20)  $\text{NO}_3^-$ , (21)  $\text{ONOO}^-$ , (22)  $\text{NO}_2^-$ , (23)  $\text{NO}_3^-$ , (24)  $\text{HSO}_4^-$ , (25)  $\text{SO}_4^{2-}$ , (26)  $\text{OH}^-$  and (27)  $\text{O}_2^-$ .

shows no fluorescence emission in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). Subsequently, upon increasing the amount of HOCl (0–60  $\mu\text{M}$ ), the fluorescence emission band at 608 nm increased dramatically with excitation at 480 nm (Fig. 2A). The fluorescence intensity of DP reached platform until the concentration of HOCl reached 5.0 equiv., resulting in a fluorescence color change from colourless to red (Fig. 2A(a and b)). To further understand the fluorescence enhancement mechanism of DP in the presence of HOCl, density functional theory (DFT) calculations were performed using Materials Studio 2019 (Fig. S8, Tables S1 and S2†). The optimized molecular frontier orbital distribution and corresponding electric energies of DP and DP=O are presented in Fig. S8.† The highest occupied molecular orbital (HOMO) of DP is mainly localized to the phenothiazine moiety, while the lowest unoccupied molecular orbital (LUMO) is primarily localized to the malononitrile (DCDHF) unit. Thus, an intra-molecular charge transfer (ICT) took place through the C=C conjugated bridge from phenothiazine to the DCDHF moiety, which results in a silent fluorescence emission of DP.<sup>49</sup> However, after HOCl-triggered oxidation of the thioether of phenothiazine to the sulfoxide unit, the HOMO of DP=O is distributed throughout the molecule of DP=O. As a result, the ICT-induced quenching effect is inhibited, resulting in the enhancement of the fluorescence emission.<sup>50</sup>

The fluorescence emission increase of DP at 608 nm showed a good linear relationship with the concentration of HOCl (3.2–35  $\mu\text{M}$ ) (Fig. 2A(c)), and the detection of limit was calculated to



**Fig. 2** (A) Fluorescence spectra of probe DP (10  $\mu\text{M}$ ) in the presence of different amounts of HOCl (0–60  $\mu\text{M}$ ) in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). Inset: (a) fluorescence intensities of probe DP at 608 nm as a function of HOCl concentration. (b) Fluorescence color changes of probe DP (I) in the absence and (II) in the presence of HOCl. (c) Linear relationship between the fluorescence intensity of DP (5  $\mu\text{M}$ ) at 608 nm and HOCl concentration (3.2–35  $\mu\text{M}$ ). (B) Changes in the fluorescence intensity of probe DP (10  $\mu\text{M}$ ) towards HOCl (60  $\mu\text{M}$ ) in the presence of various competing analytes (60  $\mu\text{M}$ ): (1) blank, (2)  $\text{Br}^-$ , (3)  $\text{F}^-$ , (4)  $\text{Cl}^-$ , (5)  $\text{P}_2\text{O}_7^{4-}$ , (6)  $\text{PO}_4^{3-}$ , (7)  $\text{H}_2\text{PO}_4^-$ , (8)  $^1\text{O}_2$ , (9)  $\text{OH}^-$ , (10)  $\text{CH}_3\text{COO}^-$ , (11)  $\text{HCO}_3^-$ , (12)  $\text{NO}_3^-$ , (13)  $\text{ONOO}^-$ , (14)  $\text{NO}_2^-$ , (15)  $\text{HSO}_4^-$ , (16)  $\text{SO}_4^{2-}$ , (17)  $\text{OH}^-$ , (18)  $\text{O}_2^-$ , (19) HOCl. (C) Fluorescence colour images of probe DP (10  $\mu\text{M}$ ) in the presence of various analytes (60  $\mu\text{M}$ ) under UV light of 365 nm. (1) Free DP, (2) HOCl, (3)  $\text{Br}^-$ , (4)  $\text{F}^-$ , (5)  $\text{Cl}^-$ , (6)  $\text{P}_2\text{O}_7^{4-}$ , (7)  $\text{PO}_4^{3-}$ , (8)  $\text{H}_2\text{PO}_4^-$ , (9) Cys, (10) GSH, (11) Hcy, (12)  $\text{S}^{2-}$ , (13)  $\text{SO}_3^{2-}$ , (14)  $\text{HSO}_3^-$ , (15)  $\text{H}_2\text{O}_2$ , (16)  $^1\text{O}_2$ , (17)  $\text{OH}^-$ , (18)  $\text{CH}_3\text{COO}^-$ , (19)  $\text{HCO}_3^-$ , (20)  $\text{NO}_3^-$ , (21)  $\text{ONOO}^-$ , (22)  $\text{NO}_2^-$ , (23)  $\text{NO}_3^-$ , (24)  $\text{HSO}_4^-$ , (25)  $\text{SO}_4^{2-}$ , (26)  $\text{OH}^-$  and (27)  $\text{O}_2^-$ . (D) Time-profile fluorescence enhancement at 608 nm of probe DP in the presence of (a) 0  $\mu\text{M}$ , (b) 20  $\mu\text{M}$ , (c) 25  $\mu\text{M}$ , (d) 35  $\mu\text{M}$  and (e) 50  $\mu\text{M}$  HOCl in PBS aqueous solutions. Excitation was performed at 480 nm.

be 81.3 nM according to the reported method defined by IUPAC.<sup>51</sup> Then, the selectivity of DP toward various analytes was evaluated. The fluorescence intensity at 608 nm was measured in the presence of analytes, and the result is shown in Fig. 2B. All these representative interfering species such as  $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ ,  $\text{ONOO}^-$ ,  $\text{OH}^-$ ,  $\text{O}_2^-$ , Cys, GSH, Hcy,  $\text{S}^{2-}$ ,  $\text{SO}_3^{2-}$ ,  $\text{HSO}_3^-$ ,  $\text{HSO}_4^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Br}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{P}_2\text{O}_7^{4-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{OH}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  lead to a negligible change in the fluorescence spectra of probe DP (Fig. S9†). In contrast, the addition of HOCl resulted in a significant enhancement of the fluorescence intensity of DP at around 608 nm. To further verify the utility of the probe, competition experiments were subsequently performed. The fluorescence intensity of probe DP towards HOCl in the presence of various competing analytes was recorded at 608 nm. As shown in Fig. 2B, DP exhibited a similar fluorescence response toward HOCl with the coexistence of other interfering species, indicating high specificity of DP for HOCl even in a complicated system. The selectivity of DP towards HOCl was also verified by the “naked-eye” fluorescence colorimetric assay. The colourless fluorescence turned red exclusively in the presence of HOCl (Fig. 2C). Due to the high selectivity of DP towards HOCl and observable color responses,

**DP** then serves as a potential indicator for HOCl detection in water samples. In the presence of increasing concentrations of HOCl from 0 to 60  $\mu\text{M}$ , the solution of **DP** gradually changed from blue to yellowish-brown and the silent fluorescence color turned to red (Fig. S10†). Accordingly, **DP** can be used for the semi-quantitative analysis of the level of HOCl by “naked eye”.

Considering the high reactivity of HOCl in live organisms, a fluorescent probe with fast response towards HOCl is highly desirable.<sup>52</sup> We then investigated time-profile fluorescence enhancement at 608 nm of probe **DP** in the presence of 0–50  $\mu\text{M}$  HOCl. As shown in Fig. 2D, **DP** displayed quenching and stable fluorescence emission with excitation at 480 nm. In the presence of 20  $\mu\text{M}$  HOCl, the emission of **DP** at 608 nm increased rapidly and reached a plateau within 30 s, and then maintained a steady level. In addition, a high concentration of HOCl took a longer time to achieve fluorescence intensity saturation. When 50  $\mu\text{M}$  of HOCl was added, the emission of **DP** at 608 nm reached an intensity plateau within 120 s. Overall, the rapid response of **DP** toward HOCl enables the potential application in tracking HOCl in biological systems.

### Spectroscopic responses of **DP=O** towards sulfur dioxide derivatives ( $\text{SO}_3^{2-}/\text{HSO}_3^-$ )

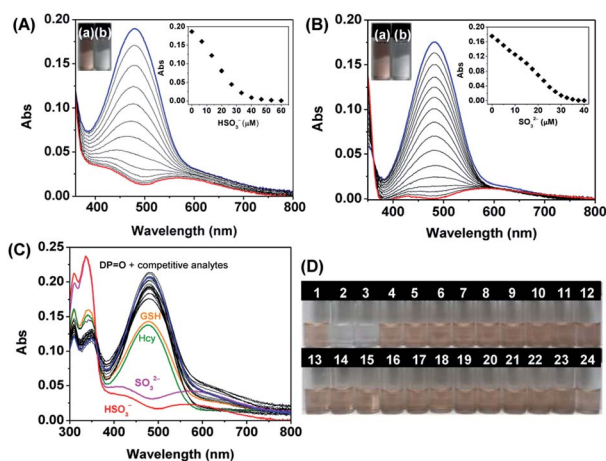
Then, the sensing performances of the HOCl-induced oxidation product (**DP=O**) towards  $\text{SO}_3^{2-}/\text{HSO}_3^-$  were investigated by spectroscopic titration in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). Fig. 3A and B show the changes in the

UV-visible spectra and solution colors. The main absorption band of **DP=O** at 480 nm gradually decreased in the presence of  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$ , which was accompanied by a clear visual change in the color of the **DP=O** solution from yellowish-brown to colorless. The absorbance of **DP=O** at 480 nm decreased remarkably with incremental addition of  $\text{SO}_3^{2-}$  and  $\text{HSO}_3^-$  and then reached the plateaus with adding 30  $\mu\text{M}$   $\text{HSO}_3^-$  and 40  $\mu\text{M}$   $\text{SO}_3^{2-}$ .

The nucleophilic addition reaction of the C=C double bond of **DP=O** towards  $\text{SO}_3^{2-}/\text{HSO}_3^-$  takes on high specificity. The interferences deriving from various potential analytes including anions ( $\text{HCO}_3^-$ ,  $\text{Br}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{P}_2\text{O}_7^{4-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{OH}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{NO}_3^-$ ,  $\text{ONOO}^-$ ,  $\text{NO}_2^-$ ,  $\text{HSO}_4^-$ , and  $\text{SO}_4^{2-}$ ), ROS ( $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ , and  $\text{ONOO}^-$ ) and RSS (Cys,  $\text{S}^{2-}$ ,  $\text{SO}_3^{2-}$ , and  $\text{HSO}_3^-$ ) induced silent responses towards **DP=O**. Although GSH and Hcy caused a slight decline of the absorption compared to that of  $\text{SO}_3^{2-}/\text{HSO}_3^-$  (Fig. 3C), it is still enough to distinguish between  $\text{SO}_3^{2-}/\text{HSO}_3^-$  from the interferences. The specificity of **DP=O** towards  $\text{SO}_3^{2-}/\text{HSO}_3^-$  was further confirmed by a “naked-eye” colorimetric assay. The colors of the **DP=O** solution were changed exclusively after the addition of  $\text{SO}_3^{2-}/\text{HSO}_3^-$  (Fig. 3D). The results indicated that **DP=O** was specific towards  $\text{SO}_3^{2-}/\text{HSO}_3^-$  detection over other competitive species.

The specific responses of **DP=O** in the presence of increasing amounts of  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$  were then investigated by fluorescence titration in PBS aqueous solutions. With an increase in the amount of  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$  added to the solution of **DP=O**, the fluorescence emission peak centred at 608 nm gradually decreased. The fluorescence intensity remained constant and the red fluorescence colors completely quenched when over 5.0 eq. of  $\text{HSO}_3^-$  and 3.0 eq. of  $\text{SO}_3^{2-}$  were added (Fig. 4A(a and b), Fig. 4B(a and b)), respectively. The fluorescence quenching of **DP=O** could be attributed to the intercept of the conjugation system after the 1,4-addition reaction of the C=C double bond of **DP=O** with  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$ .<sup>53</sup> The detection limits ( $3\sigma/K$ ) of **DP=O** towards  $\text{SO}_3^{2-}$  and  $\text{HSO}_3^-$  were then calculated to be 70.8 nM and 65.1 nM according to the IUPAC standard (Fig. 4A(c) and B(c)).

To verify the specificity of **DP=O** toward  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$ , the emission responses were carried out in the presence of various competitive species, such as  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{P}_2\text{O}_7^{4-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{H}_2\text{PO}_4^-$ , Cys, GSH, Hcy,  $\text{S}^{2-}$ ,  $\text{HSO}_4^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ ,  $\text{OH}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$ ,  $\text{ONOO}^-$ ,  $\text{NO}_2^-$  and  $\text{Br}^-$ . As shown in Fig. 4C,  $\text{SO}_3^{2-}/\text{HSO}_3^-$  elicited an obvious decrease in the fluorescence at 608 nm. However, other analytes induced negligible fluorescence changes except for slight quenching in the presence of Hcy and GSH. Furthermore, the strong red fluorescence emission exclusively quenched for the addition of  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$  to the **DP=O** solution, and other competitive analytes caused unobvious changes in the fluorescence color (Fig. 4D). These results indicated the desired selectivity of **DP=O** towards  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$  over other analytes. Then, the response kinetic profiles of **DP=O** towards  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$  were investigated to verify its real-time performance. When different concentrations of  $\text{HSO}_3^-$  and  $\text{SO}_3^{2-}$  were added into the solution of **DP=O**, the fluorescence intensity at 608 nm significantly declined within 600 seconds for  $\text{SO}_3^{2-}$  and 200 seconds for  $\text{HSO}_3^-$  (Fig. S11†). The results indicated the fast response times



**Fig. 3** UV-vis absorption spectra of **DP=O** (10  $\mu\text{M}$ ) in the presence of different amounts of (A)  $\text{HSO}_3^-$  (0–60  $\mu\text{M}$ ) and (B)  $\text{SO}_3^{2-}$  (0–40  $\mu\text{M}$ ) in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). Inset: colorimetric changes of **DP=O** (a) in the absence and (b) in the presence of  $\text{HSO}_3^-$  (60  $\mu\text{M}$ ) and  $\text{SO}_3^{2-}$  (40  $\mu\text{M}$ ). Absorbances of **DP=O** at 480 nm as a function of  $\text{HSO}_3^-/\text{SO}_3^{2-}$  concentrations. (C) UV-vis absorption spectra of **DP=O** (10  $\mu\text{M}$ ) in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4) in the presence of various analytes (60  $\mu\text{M}$ ). (D) Colorimetric changes of **DP=O** (10  $\mu\text{M}$ ) in the presence of various analytes (60  $\mu\text{M}$ ) in PBS aqueous solutions (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). (1) free **DP=O**. (2)  $\text{HSO}_3^-$ , (3)  $\text{SO}_3^{2-}$ , (4)  $\text{F}^-$ , (5)  $\text{Cl}^-$ , (6)  $\text{P}_2\text{O}_7^{4-}$ , (7)  $\text{PO}_4^{3-}$ , (8)  $\text{H}_2\text{PO}_4^-$ , (9) Cys, (10) GSH, (11) Hcy, (12)  $\text{S}^{2-}$ , (13)  $\text{HSO}_4^-$ , (14)  $\text{SO}_4^{2-}$ , (15)  $\text{H}_2\text{O}_2$ , (16)  $^1\text{O}_2$ , (17)  $\text{OH}^-$ , (18)  $\text{CH}_3\text{COO}^-$ , (19)  $\text{HCO}_3^-$ , (20)  $\text{NO}_3^-$ , (21)  $\text{ONOO}^-$ , (22)  $\text{NO}_2^-$ , (23)  $\text{Br}^-$ , (24)  $\text{OH}^-$ .



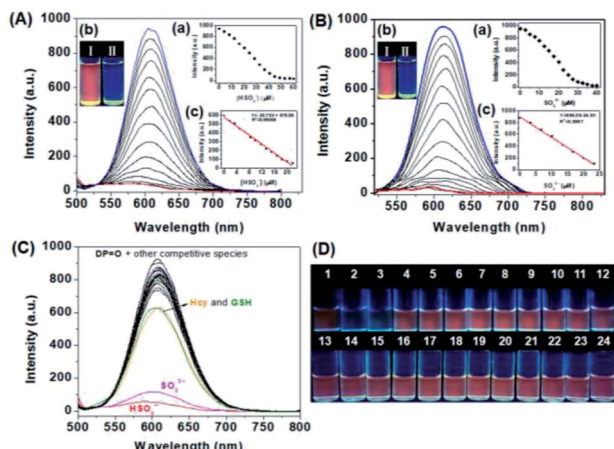


Fig. 4 (A) Fluorescence spectra of DP=O (10 μM) in the presence of different amounts of (A) HSO<sub>3</sub><sup>-</sup> (0–60 μM) in PBS aqueous solutions (DMF : H<sub>2</sub>O = 1 : 9, v/v, 20 mM, pH = 7.4). Inset: (a) fluorescence intensities of DP=O as a function of HSO<sub>3</sub><sup>-</sup> concentrations. (b) Fluorescence color photos of DP=O (10 μM) in the presence of HSO<sub>3</sub><sup>-</sup> (60 μM) under UV light. (c) Linear relationship between the fluorescence intensity of DP=O (5 μM) and HSO<sub>3</sub><sup>-</sup> concentration (0–22 μM). (B) Fluorescence spectra of DP=O (10 μM) in the presence of different amounts of SO<sub>3</sub><sup>2-</sup> (0–40 μM) in PBS aqueous solutions (DMF : H<sub>2</sub>O = 1 : 9, v/v, 20 mM, pH = 7.4). Inset: (a) fluorescence intensities of DP=O as a function of SO<sub>3</sub><sup>2-</sup> concentrations. (b) Fluorescence color photos of DP=O (10 μM) in the presence of SO<sub>3</sub><sup>2-</sup> (40 μM) under UV light. (c) Linear relationship between the fluorescence intensity of DP=O (5 μM) and SO<sub>3</sub><sup>2-</sup> concentration (0–23 μM). (C) Fluorescence spectra of DP=O (10 μM) in PBS aqueous solutions (DMF : H<sub>2</sub>O = 1 : 9, v/v, 20 mM, pH = 7.4) in the presence of all kinds of analytes (60 μM). (D) Fluorescence color images of DP=O (10 μM) in the presence of various analytes (60 μM) under UV light in PBS aqueous solutions (DMF : H<sub>2</sub>O = 1 : 9, v/v, 20 mM, pH = 7.4). (1) Free DP=O, (2) HSO<sub>3</sub><sup>-</sup>, (3) SO<sub>3</sub><sup>2-</sup>, (4) F<sup>-</sup>, (5) Cl<sup>-</sup>, (6) P<sub>2</sub>O<sub>7</sub><sup>4-</sup>, (7) PO<sub>4</sub><sup>3-</sup>, (8) H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, (9) Cys, (10) GSH, (11) Hcy, (12) S<sup>2-</sup>, (13) HSO<sub>4</sub><sup>-</sup>, (14) SO<sub>4</sub><sup>2-</sup>, (15) H<sub>2</sub>O<sub>2</sub>, (16) <sup>1</sup>O<sub>2</sub>, (17) OH<sup>-</sup>, (18) CH<sub>3</sub>COO<sup>-</sup>, (19) HCO<sub>3</sub><sup>-</sup>, (20) NO<sub>3</sub><sup>-</sup>, (21) ONOO<sup>-</sup>, (22) NO<sub>2</sub><sup>-</sup>, (23) Br<sup>-</sup>, (24) OH<sup>-</sup>. The intensities were recorded at 608 nm, and excitation was performed at 480 nm.

of DP=O toward HSO<sub>3</sub><sup>-</sup> and SO<sub>3</sub><sup>2-</sup> compared to those of the previously reported SO<sub>2</sub> derivative fluorescent probes.<sup>54,55</sup>

DP=O was also used for the semi-quantitative analysis of the levels of HSO<sub>3</sub><sup>-</sup> and SO<sub>3</sub><sup>2-</sup> in aqueous solutions by “naked eye” (Fig. S12†).

In addition, the responses of DP in the presence of HSO<sub>3</sub><sup>-</sup> and SO<sub>3</sub><sup>2-</sup> have also been measured and compared by spectroscopic titration. As shown in Fig. S13,† no remarkable responses of DP appeared in the presence of HSO<sub>3</sub><sup>-</sup> and SO<sub>3</sub><sup>2-</sup>, indicating the inert reaction of the C=C double bond with HSO<sub>3</sub><sup>-</sup> and SO<sub>3</sub><sup>2-</sup>. However, after the oxidation of the thioether of DP to a sulfoxide product (DP=O), the C=C double bond of DP=O was activated. Then, SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> reacts with DP=O via a 1,4-nucleophilic addition reaction mechanism leading to a decrease in the intensity of absorption and emission spectra (Fig. S14†).

### Fluorescence imaging *in vitro* and *in vivo*

To verify the reliability of DP in the physiological pH range, the reactivity of DP towards HOCl and DP=O to SO<sub>3</sub><sup>2-</sup>/

HSO<sub>3</sub><sup>-</sup> under different pH values was tested. As shown in Fig. S15,† the fluorescence intensity of DP at 608 nm remained constant in the pH range of 5.8–8.0. Upon the addition of HOCl, the emission increased dramatically in the same pH range. Furthermore, the fluorescence responses of DP=O towards SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> were slightly affected in the pH range from 5.8 to 7.0 and reached a constant value over the pH range 7.0–8.0. The results indicated that DP could be used to sequentially detect HOCl and SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> in the physiological pH range. Then, the cytotoxicity of DP was measured by an MTT assay using a HeLa cell line.<sup>56</sup> It was found that 89% of HeLa cells were still alive after incubation with up to 10 μM DP for 24 h and the HeLa cell viability was still greater than 82.4% at a higher concentration of DP (30 μM) after 24 h of incubation (Fig. S16†), suggesting the low cytotoxicity of DP.

Encouraged by the outstanding performances of DP under simulated physiological conditions, we then explored the capabilities of DP to image HOCl and SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> in live cells. The application of DP in the sequential fluorescence imaging of HOCl and SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> *in vitro* was evaluated with live HeLa cells. As shown in Fig. 5a, the HeLa cells incubated with DP at 37 °C for 20 min present silent intracellular fluorescence. However, upon addition of HOCl and culturing for 25 min, the red fluorescence signal emerged significantly (Fig. 5d). The results indicated that DP is cell-permeable and capable of monitoring HOCl in live cells. In addition, when SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> was added to the above stained cells for further 30 min, the red fluorescence was quenched again (Fig. 5g), indicating the formation of the addition product DP=O-SO<sub>3</sub> in live cells.

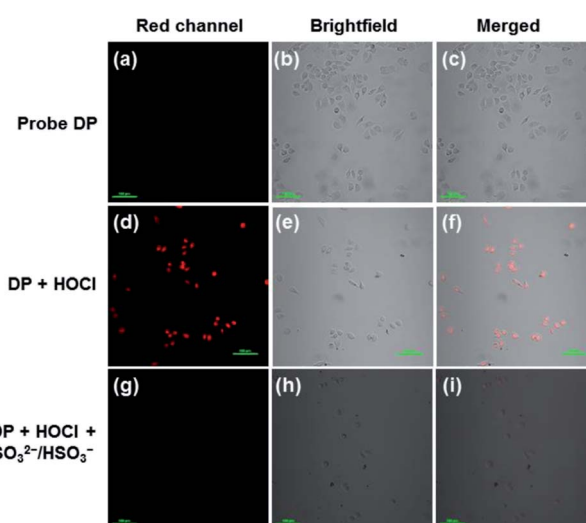


Fig. 5 Sequential fluorescence imaging of HOCl and SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> in live HeLa cells. (a–c) HeLa cells treated with DP (2 μM) for 20 min. (d–f) HeLa cells pre-treated with HOCl (10 μM) and then incubated with DP (2 μM) for further 25 min. (g–i) A549 cells pre-treated with HOCl (10 μM) and then incubated with DP (2 μM) for 25 min, and finally incubated with SO<sub>3</sub><sup>2-</sup>/HSO<sub>3</sub><sup>-</sup> (10 μM) for further 30 min. The images were acquired using a confocal microscope at 465 nm excitation and 610 nm emission. Scale bar = 100 μm.

Then, fluorescence imaging of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  *in vivo* was investigated in adult zebrafish and nude mice. Endogenous HOCl was generated in zebrafish by stimulation with lipopolysaccharides (LPS). Free zebrafish and LPS-stimulated zebrafish were used as the control groups. As shown in Fig. S17,† no fluorescence was observed in the control groups. However, intense red fluorescence on the gill and abdomen in zebrafish gradually increased over time when the LPS-stimulated zebrafish was further stained with DP. The results indicated that LPS stimulation-generated HOCl reacted with DP to generate the oxidized product (DP=O) in live zebrafish. Furthermore, after the DP=O loaded zebrafish were sequentially stimulated with  $\text{SO}_3^{2-}/\text{HSO}_3^-$ , the red fluorescence signal in zebrafish was then quenched over time (Fig. S18 and S19†), which demonstrated the occurrence of the addition reaction of DP=O with  $\text{SO}_3^{2-}$  and  $\text{HSO}_3^-$ .

It has been reported that long-emission wavelength light has advantages including minimum photodamage, deep penetration into tissues, and low interference from autofluorescence. Due to the long emission wavelength (608 nm) of DP, the capability of DP for the sequential visualization of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  *in vivo* was further verified in live nude mice (6–8 week). DP was subcutaneously injected into the hind limbs of 6–8 week-old nude mice, followed by the sequential treatment of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  in the same area. Fluorescence imaging was then performed at different time courses after the administration. As shown in Fig. 6, the free mouse and DP-injected mice showed no fluorescence signal. By contrast, an intense fluorescence signal was noted over time after the mouse was treated with HOCl.

Then, the *in situ* produced DP=O can be employed as an imaging agent for visualizing  $\text{SO}_3^{2-}/\text{HSO}_3^-$  *in vivo*. As shown in Fig. 7, the right hind limbs of mice displayed a robust fluorescence signal compared with the control group after DP=O was *in situ* generated in the injected area. Then, the fluorescence signal decreased gradually until it vanished when  $\text{SO}_3^{2-}/\text{HSO}_3^-$  was subcutaneously injected into the same areas. The fluorescence imaging studies in adult zebrafish and nude mouse adequately indicated that DP can be used as an effective tool for the sequential monitoring of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  *in vivo*.

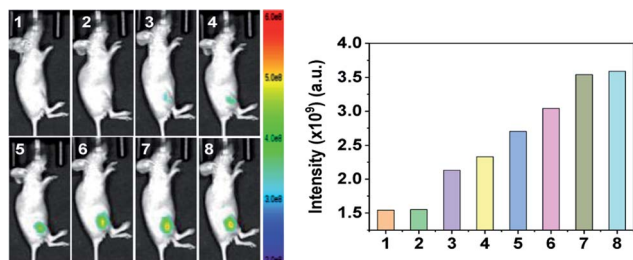


Fig. 6 Fluorescence imaging of exogenous HOCl in live mice. (1) Nude mouse; (2) DP (20  $\mu\text{M}$ , 125  $\mu\text{L}$ ) was subcutaneously injected into the mouse; (3) followed by the injection of 25  $\mu\text{L}$  HOCl (0.1 mM) to this area. Images were then recorded at different time points: (4) 5 min, (5) 10 min, (6) 15 min, (7) 20 min and (8) 25 min. The mouse was imaged using an excitation filter (465 nm) and an emission filter (610 nm).

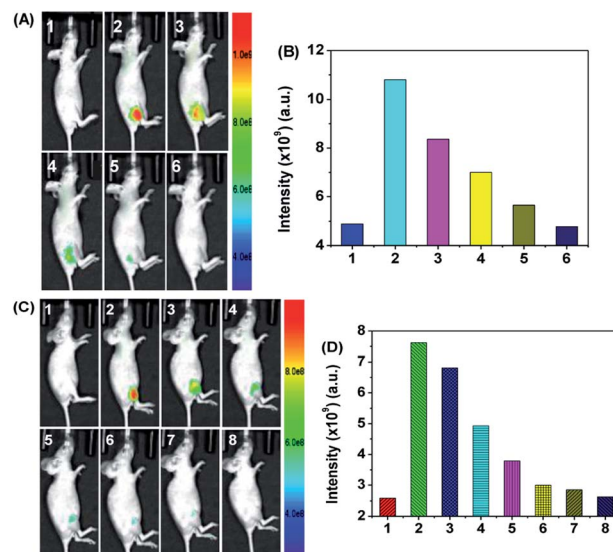


Fig. 7 Fluorescence imaging  $\text{SO}_3^{2-}/\text{HSO}_3^-$  intake in mice. (A) (1) Control group; (2) 20  $\mu\text{M}$  (125  $\mu\text{L}$ ) DP=O was subcutaneously injected into the left hind limbs of the mouse; then  $\text{SO}_3^{2-}$  (0.1 mM) was injected into the same areas of interest: (3) 5 min, (4) 10 min, (5) 15 min, (6) 20 min, (7) 25 min and (8) 30 min post injection. (C) (1) Control group; (2) 20  $\mu\text{M}$  (125  $\mu\text{L}$ ) DP=O was subcutaneously injected into the left hind limbs of the mouse; then  $\text{HSO}_3^-$  (0.1 mM) was injected into the same areas of interest for (3) 5 min, (4) 10 min, (5) 20 min and (6) 30 min post injection. (B) and (D) Mean fluorescence intensity of interested areas at different time points shown in (A) and (C). The mice were imaged with an excitation filter at 465 nm and an emission filter at 610 nm.

## Experimental

### Synthesis of the fluorescent probe DP

2-[3-Cyano-4,5,5-trimethylfuran-2(5H)-ylidene]malononitrile (DCDHF)<sup>57</sup> (0.199 g, 1 mmol) and 10-ethyl-10H-phenothiazine-3-carbaldehyde<sup>58</sup> (0.269 g, 1 mmol) were dissolved in hot ethanol, respectively. Then, the two solutions were mixed and two drops of piperidine were added. The reaction mixture was refluxed for 6 hours in an argon atmosphere. The precipitate was filtrated, washed with cold ethanol and dried under vacuum. The crude product was further purified by recrystallization in acetonitrile to get the purple-black solid DP with 65% yield. <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 7.76–7.91 (Ar-H, 3H), 7.06–7.28 (Ar-H and C=C-H, 6H), 3.97 (–CH<sub>2</sub>, 2H), 1.68–1.83 (–CH<sub>3</sub> and –CH<sub>2</sub>, 8H), 1.01 (CH<sub>3</sub>, 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>): 177.7, 175.8, 148.9, 147.1, 143.3, 131.3, 129.1, 128.4, 128.0, 127.7, 124.1, 122.7, 117.0, 116.3, 113.4, 113.3, 112.6, 111.7, 99.5, 97.5, 53.9, 49.1, 25.7, 19.9, 11.3. HRMS-API (positive mode, *m/z*) for [DP+H]<sup>+</sup>: calcd 451.15926, found: 451.15936. Mp: 184.4–185.2 °C.

### General procedures of spectra detection

Stock solutions of DP were prepared at 0.5 mM in DMF, and the solution of DP was diluted with a PBS solution to 10  $\mu\text{M}$  (DMF : H<sub>2</sub>O = 1 : 9, v/v, 20 mM, pH = 7.4). The ROS species were prepared in water according to the procedure previously



reported in the literature.<sup>59</sup> The solutions of various testing analytes (20 mM) were prepared in distilled water. For spectroscopic response of **DP** to HOCl and **DP=O** to  $\text{SO}_3^{2-}/\text{HSO}_3^-$ , HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  at different concentrations were added into the probe solution (total volume: 3 mL), followed by immediate spectroscopic measurements. Excitation and emission slits are 5 nm, respectively.

### Confocal fluorescence imaging in HeLa cells

Confocal fluorescence imaging was carried out at the School of Forensic Medicine, Xinxiang Medical University. For the visualisation of exogenous HOCl in live HeLa cells, HeLa cells were seeded at a density of  $1.5 \times 10^5$  cells per mL in 22 mm coverglass bottom culture dishes. After replacement of the culture medium, the cells were incubated with fresh PBS containing **DP** (2  $\mu\text{M}$ ). After 20 min incubation, the cells were sequentially treated with 10  $\mu\text{M}$  HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  (10  $\mu\text{M}$ ) for further 25 min, respectively. The cells were washed three times with PBS to remove excess analytes and then subjected to confocal microscope fluorescence imaging. All the A549 cells were imaged using an excitation filter (465 nm) and an emission filter (610 nm). Scale bar = 100  $\mu\text{m}$ .

### Fluorescence imaging of endogenous HOCl generation in adult zebrafish<sup>60</sup>

Adult zebrafish was stimulated with LPS (2  $\mu\text{L mL}^{-1}$ ) for 3 h, and then stained with **DP** (10  $\mu\text{M}$ ) for 5 min, 10 min and 15 min, respectively. Blank zebrafish was chosen as the control group. Zebrafish was imaged with an excitation filter (465 nm) and an emission filter (610 nm).

### Fluorescence imaging of $\text{SO}_3^{2-}/\text{HSO}_3^-$ in adult zebrafish

**DP=O** (10  $\mu\text{M}$ ) was prepared by addition of 5 equiv. HOCl into a PBS aqueous solution (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). Adult zebrafish was first stimulated with **DP=O** (10  $\mu\text{M}$ ) for 30 min, and then stained with  $\text{SO}_3^{2-}$  (40  $\mu\text{M}$ ) and  $\text{HSO}_3^-$  (40  $\mu\text{M}$ ) for 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min and 40 min, respectively. Zebrafish were imaged with an excitation filter (465 nm) and an emission filter (610 nm).

### Fluorescence imaging of exogenous HOCl in live mice

Nude mice (6–8 week old mice) were anesthetized with isoflurane in a flow of oxygen throughout the experiments. For imaging of exogenous HOCl in live mice, **DP** (20  $\mu\text{M}$ , 125  $\mu\text{L}$ ) was subcutaneously injected into mice, followed by the injection of 25  $\mu\text{L}$  (0.1 mM) HOCl in the same area. Imaging for the injection area was performed every 5 min within 25 min with an excitation filter at 465 nm and an emission filter at 610 nm.

### Fluorescence visualization of $\text{SO}_3^{2-}$ and $\text{HSO}_3^-$ intake in mice

Nude mice were anesthetized with isoflurane in a flow of oxygen. **DP=O** (10  $\mu\text{M}$ ) was prepared by addition of 5 equiv. HOCl into a PBS aqueous solution (DMF :  $\text{H}_2\text{O}$  = 1 : 9, v/v, 20 mM, pH = 7.4). For the imaging of exogenous  $\text{SO}_3^{2-}$  and  $\text{HSO}_3^-$  in live mice, **DP=O** (20  $\mu\text{M}$  125  $\mu\text{L}$ ) was subcutaneously

injected into the right hind limbs of the mouse; then  $\text{SO}_3^{2-}$  (0.1 mM) and  $\text{HSO}_3^-$  (0.1 mM) were injected into the same areas of interest. Imaging for the injection area was performed every 5 min within 30 min with an excitation filter at 465 nm and an emission filter at 610 nm.

## Conclusions

In summary, we have successfully developed a red-emitting fluorescent probe (**DP**) for the sequential detection of hypochlorous acid (HOCl) and sulfur dioxide derivatives ( $\text{SO}_3^{2-}/\text{HSO}_3^-$ ) *in vitro* and *in vivo*. **DP** possesses a donor- $\pi$ -acceptor (D- $\pi$ -A) structure, in which the phenothiazine unit acts as the electron-donating group (EDG), while DCDHF acts as the electron-withdrawing group (EWG). The thioether moiety of **DP** can be exclusively oxidized to sulfoxide (**DP=O**) in the presence of HOCl, resulting in the hypochromic shift of the absorption spectra and “OFF-ON” response of the emission. Furthermore, after the activation of the C=C double bond by oxidation, **DP=O** reacts with  $\text{SO}_3^{2-}/\text{HSO}_3^-$  via a nucleophilic addition reaction leading to a decrease in the intensity of absorption and emission spectra, which enabled the realization of sequential detection of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  by a single fluorescent probe. The proposed reaction mechanisms of **DP** towards HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  have been confirmed by spectroscopic titration and high-resolution mass spectroscopy (HRMS). **DP** has the advantages of high water solubility, reliability in the physiological pH range, red-emission, outstanding selectivity and low cytotoxicity, which enabled its practical values in biological fields. **DP**'s application in biological systems was demonstrated by the monitoring of HOCl and  $\text{SO}_3^{2-}/\text{HSO}_3^-$  in live cells and live animal models. The successful applications of the fluorescent probe prove that it would be a useful tool for the detection and monitoring of the redox equilibrium regulation-related bioactive species in living systems.

## Ethical statement

All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Dalian Medical University and experiments were approved by the Animal Ethics Committee of the University of Science and Technology Liaoning.

## Author contributions

Q. T. Meng and Z. Q. Zhang designed and guided the study, analyzed all the data and wrote the manuscript. J. H. Liu, H. Y. Yin and Z. Y. Shang performed the synthesis, spectroscopic titration measurements and data processing. P. Gu and G. J. He performed the fluorescence microscopy imaging in live cells. R. Zhang contributed to review and polish the manuscript. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.



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