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A boronate-based ratiometric fluorescent probe for fast selective detection of peroxynitrite

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ABSTRACT

Given that peroxynitrite ($ONOO^-$) is profoundly associated with health and diseases, a new fluorescent probe **ABT** was designed and synthesized for detection of $ONOO^-$. **ABT** manifested not only ratiometric fluorescence signals simultaneously in response to concentrations of $ONOO^-$ (within 10 s), but high selectivity and sensitivity towards $ONOO^-$ over other physiological relevant species (detection limit = 26.3 nM). Moreover, **ABT** worked in a broad pH range with biological relevance. Thus, **ABT** could be used to quantitative detection of $ONOO^-$ concentration and has the potential to efficiently monitor $ONOO^-$ in living organisms.

Introduction

Peroxynitrite ($ONOO^-$), a highly reactive oxidant in living organisms, is mostly produced by a diffusion-controlled reaction between nitric oxide (·NO) and superoxide ($\cdot O_2^-$) in mitochondria.[1] On the one hand, as an oxidant and efficient nitrite agent, $ONOO^-$ plays a vital role in diverse pathophysiological conditions like modulating cell signal transduction.[2–6] For example, $ONOO^-$ oxidizes reactive cysteine residues in proteins, as a result, deactivation or activation of the proteins.[7,8] But on the other hand, abnormality level of $ONOO^-$ in living cells can cause serious damage to cellular biomolecules involving lipids, proteins, and nucleic acids, resulting in cell apoptosis or necrosis. Moreover, abnormal concentrations of $ONOO^-$ is associated with some ailments such as cardiovascular diseases, circulatory shock, inflammation, cancers, and so on.[9,10] Thus, it is significant for assays of $ONOO^-$ detection which may be useful for the diagnosis of relative diseases and the exploration of its various pathophysiologies.

Although peroxynitrite in human health and diseases is of significance, the elucidation of the biological functions of peroxynitrite remains a challenge. One of the main obstacles to explore its roles in organisms is short of suitable approaches to detect it in vivo due to its short lifetime (<10 ms) and numerous antioxidant in cell. Fluorescent probe is of course a promising method to detect $ONOO^-$ in vivo.[11] Recently, a lot of fluorescent probes have been developed for peroxynitrite detection in biological systems.[12–20] Nevertheless, most of these probes are based on a single channel with a turn-on or turn-off fluorescence signal, which limits their applications in quantitative measurement of peroxynitrite concentration in vitro and vivo.[21] By contrast, the fluorescent probes on a basis of independent two-channel ratiometric signal enable reducing of the errors caused by various external conditions: excitation power, fluorescence decay and probe distribution.[22,23] However, it is still a heavy task to distinguish $ONOO^-$ from other reactive oxygen species (ROS) such as H_2O_2 and ClO- with similar properties and selectively detect $ONOO^-$ in the presence of reducing molecules such as hydrogen sulfide and glutathione.[24] Therefore, it is indeed urgent to find more excellent fluorescent probes for detecting and monitoring $ONOO^-$ in vivo.

As $ONOO^-$ can react with arylboronates forming corresponding phenols much faster than H_2O_2 and HClO, arylboronate derivatives are considerable sources for the $ONOO^-$ specific fluorescent probes.[25] Moreover, HMBT (2-(2-hydroxy-3-methoxyphenyl) benzothiazole) is regarded as an available fluorophore used in many fields successfully in light of large Stokes shift, good photostability and ratiometric detection capability.[26,27] Consequently, a new ratiometric fluorescent probe named as **ABT** (4-[(2-(2-benzithiazolyl)-5-methoxy) phenoxymethyl] phenylboronic acid pinacol ester), for detecting $ONOO^-$ was designed and synthesized by modifying HMBT with an arylboronate moiety. This probe **ABT** showed ultra-short response time and highly sensitive quality towards $ONOO^-$. Additionally, **ABT** performed prominent selectivity for $ONOO^-$ over other coexisted ROS such as H_2O_2, HClO, and ·OH.

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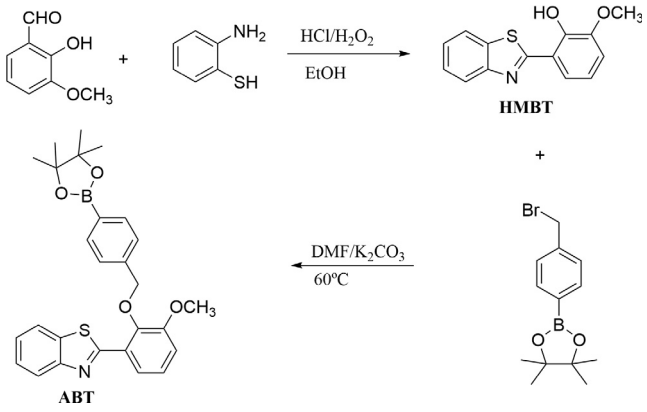

Scheme 1. Synthetic route of the probe **ABT**.

Results and discussion

Synthesis of probe ABT

Probe **ABT** was easily synthesized through two steps outlined in Scheme 1, and the details for synthetic data were given in Supporting information.

Spectral response of the probe to ONOO⁻

As the probe **ABT** and HMBT were in hand, their UV–vis absorption spectra and fluorescence emission spectra were investigated in PBS buffer (10 mM, pH 7.4, 40% ethanol) at room temperature. **ABT** showed maximal absorption/emission bands at 309 nm/405 nm, however, upon addition of $ONOO^-$ (2 equiv), the absorption band at λ = 309 nm was red-shifted to 317 nm and the emission

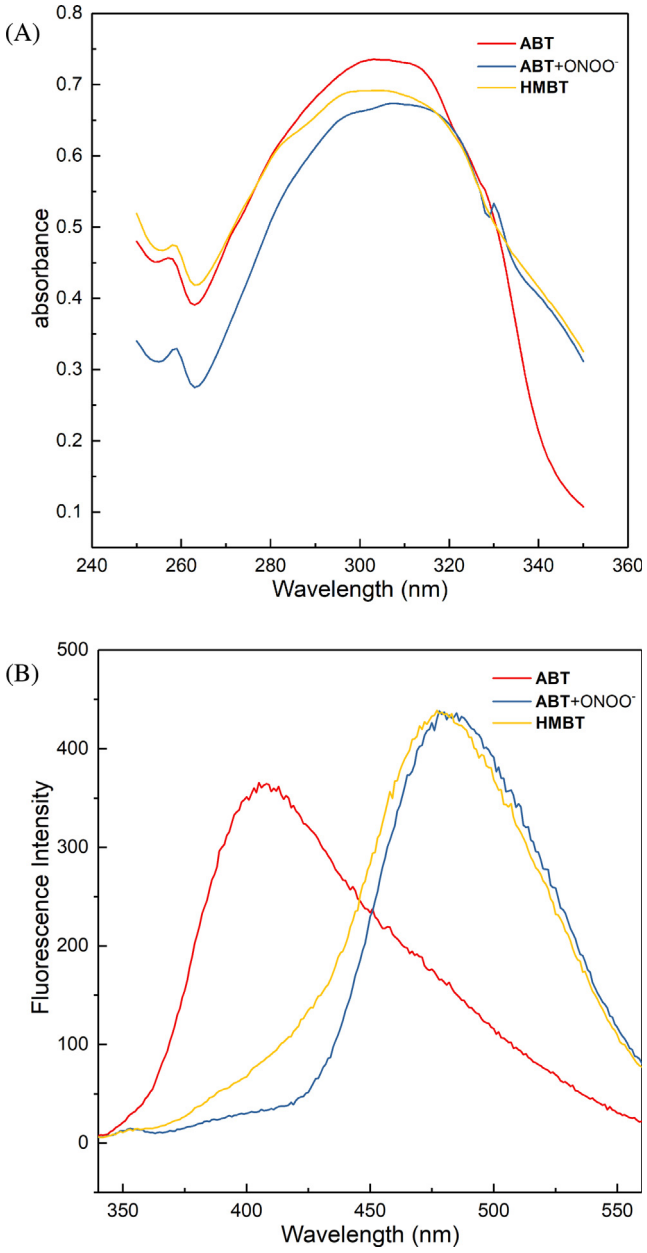

Fig. 1. The spectral profiles were obtained in PBS buffer (10 mM, pH 7.4, 40% ethanol). (A) UV–vis absorption spectra of **ABT** (50 μM), HMBT (50 μM) and **ABT** (50 μM) in the presence of 2 equiv of $ONOO^-$. (B) Fluorescence spectra of **ABT** (5 μM), HMBT (5 μM) and **ABT** (5 μM) in the presence of 2 equiv of $ONOO^-$, $\lambda_{ex.}$ = 317 nm, slits: 5. 5.

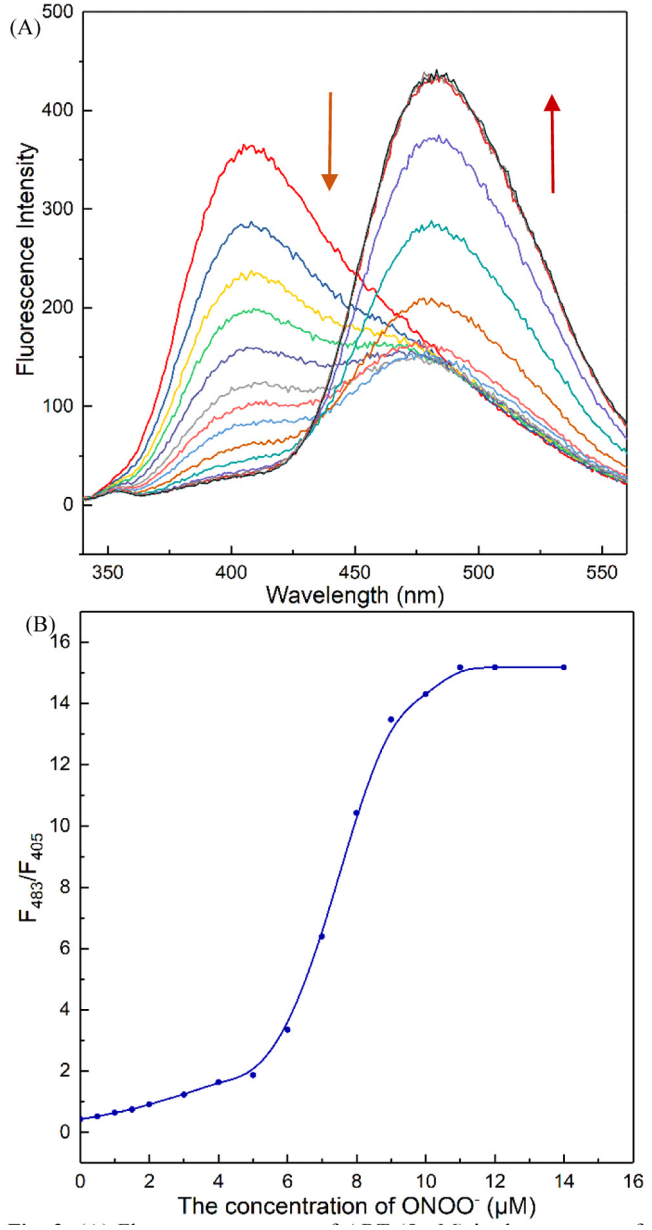

Fig. 2. (A) Fluorescence spectra of **ABT** (5 μM) in the presence of various concentrations of $ONOO^-$ (0–14 μM). (B) The relationship between fluorescence emission intensity ratio (F_{483}/F_{405}) and $ONOO^-$ concentration (0–14 μM). Profiles were obtained in PBS buffer (10 mM, pH 7.4, 40% ethanol), $\lambda_{ex.}$ = 317 nm, slits: 5. 5.

band at 405 nm was also red-shifted to 483 nm. HMBT has two emission bands (enol and keto emission). As modified by an alyboronate group, HMBT exhibited enol-like emission band at 405 nm. But when **ABT** reacted with $ONOO^-$, it would release free HMBT exhibiting keto emission at 483 nm. Moreover, the consequences showed that the UV–vis absorption spectra and emission spectra of HMBT were likely to that of the **ABT** solution in the presence of $ONOO^-$. As a result, it implicated that the binding of **ABT** with $ONOO^-$ furnished HMBT (Fig. 1).

According to the assay of fluorescence titration, an obvious change in ratiometric fluorescence signal (F_{483}/F_{405}) could be observed when $ONOO^-$ was added to the solution of **ABT** and excited at 317 nm. As shown in Fig. 2A, upon incremental addition of $ONOO^-$ (0–14 μM), the emission band of **ABT** at 405 nm dramatically decreased while another emission band at 483 nm rose up and reached a constant value eventually. The fluorescence emission intensity ratio between 483 nm and 405 nm (F_{483}/F_{405}) enhanced to about 35 times from 0.43 to 15.18. Meanwhile, the

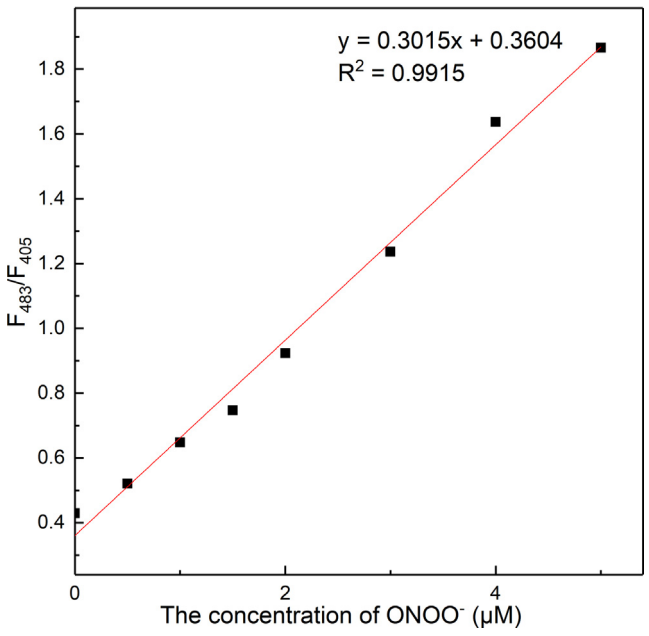

Fig. 3. Linear curve derived from the emission intensity ratio (F_{483}/F_{405}) and $ONOO^-$ concentration (0–5 μM) in PBS buffer (10 mM, pH 7.4, 40% ethanol). $\lambda_{ex.}$ = 317 nm, slits: 5. 5.

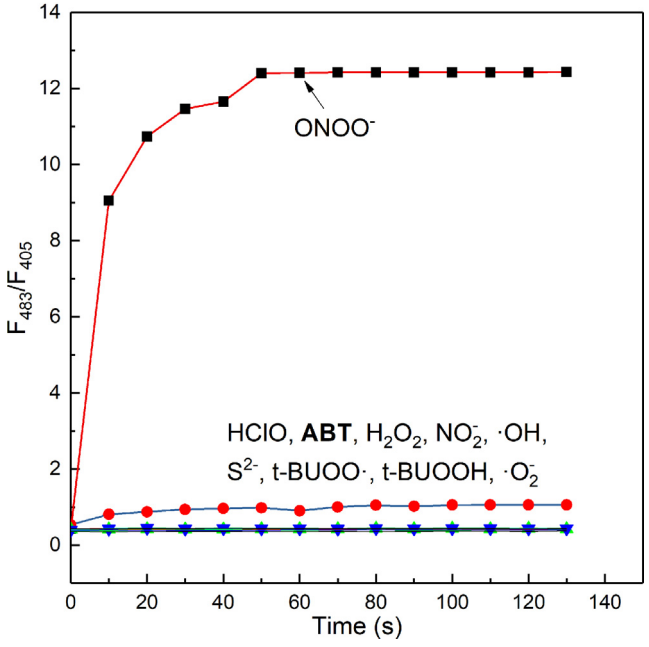

Fig. 4. Time-dependent fluorescence spectra of **ABT** upon addition of $ONOO^-$ (10 μM) and other analytes (50 μM) in PBS buffer (10 mM, pH 7.4, 40% ethanol). $\lambda_{ex.}$ = 317 nm, slits: 5. 5.

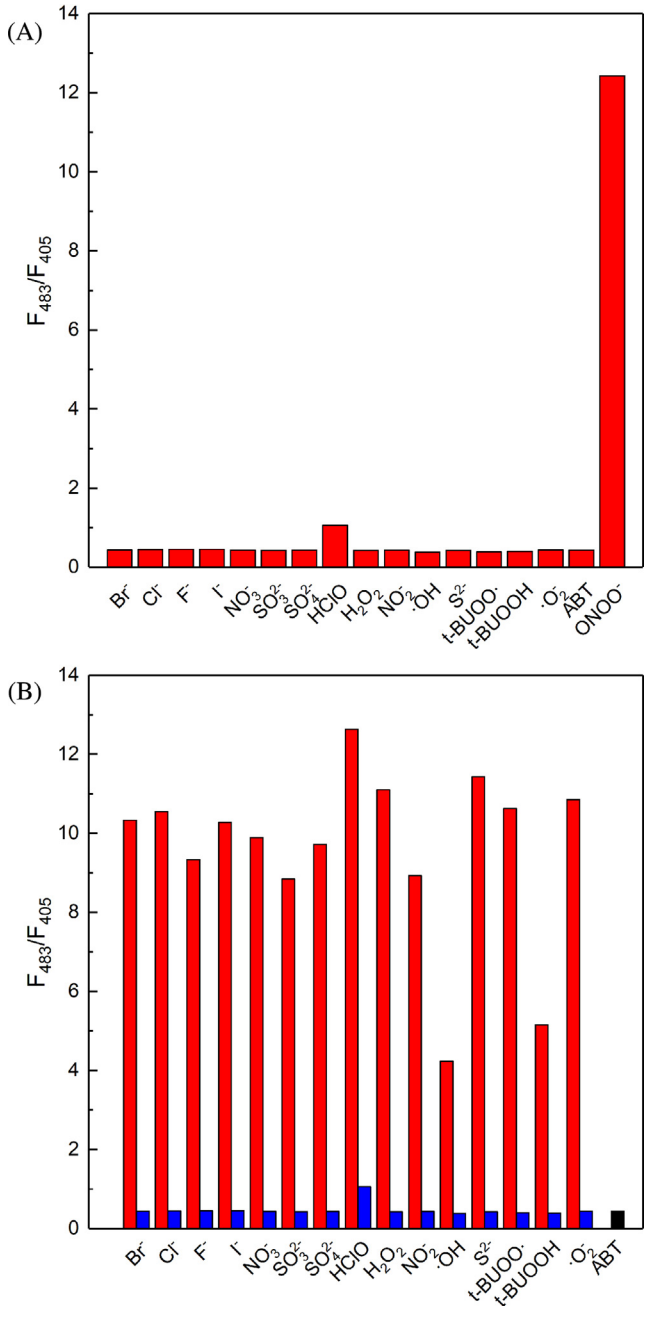

Fig. 5. (A) Fluorescence emission intensity ratio (F_{483}/F_{405}) of **ABT** after treatment with $ONOO^-$ (10 μM) and other analytes (50 μM) including H_2O_2, NO_2^-, ·OH, S^{2-}, *t*-BUOO·, *t*-BUOOH, $\cdot O_2^-$, HClO, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, and SO_3^{2-}. (B) The black column represents the ratio (F_{483}/F_{405}) of **ABT** (5 μM); the blue columns represent the ratio of **ABT** (5 μM) in the presence of other analytes (50 μM) referred to above; the red columns represent the ratio when $ONOO^-$ was added into **ABT** solution containing other analytes. Profiles were obtained in PBS buffer (10 mM, pH 7.4, 40% ethanol), $\lambda_{ex.}$ = 317 nm, slits: 5. 5. The data were gotten after addition of the analytes in two minutes.

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emission intensity ratio (F_{483}/F_{405}) exhibited a good linear relationship over $ONOO^-$ concentration range from 0 to 5 μM or 5 to 10 μM (see Fig. 2B and Supporting information). Hence, the linear curve of the ratio (F_{483}/F_{405}) allowed for the convenient quantitative detection of $ONOO^-$ over this concentration range. In addition, detection limitation of **ABT** for sensing $ONOO^-$ was calculated to be as low as 2.63×10^{-8} mol·L^{-1} in accord with some previously reported probe for $ONOO^-$[24,9,18] (Fig. 3).

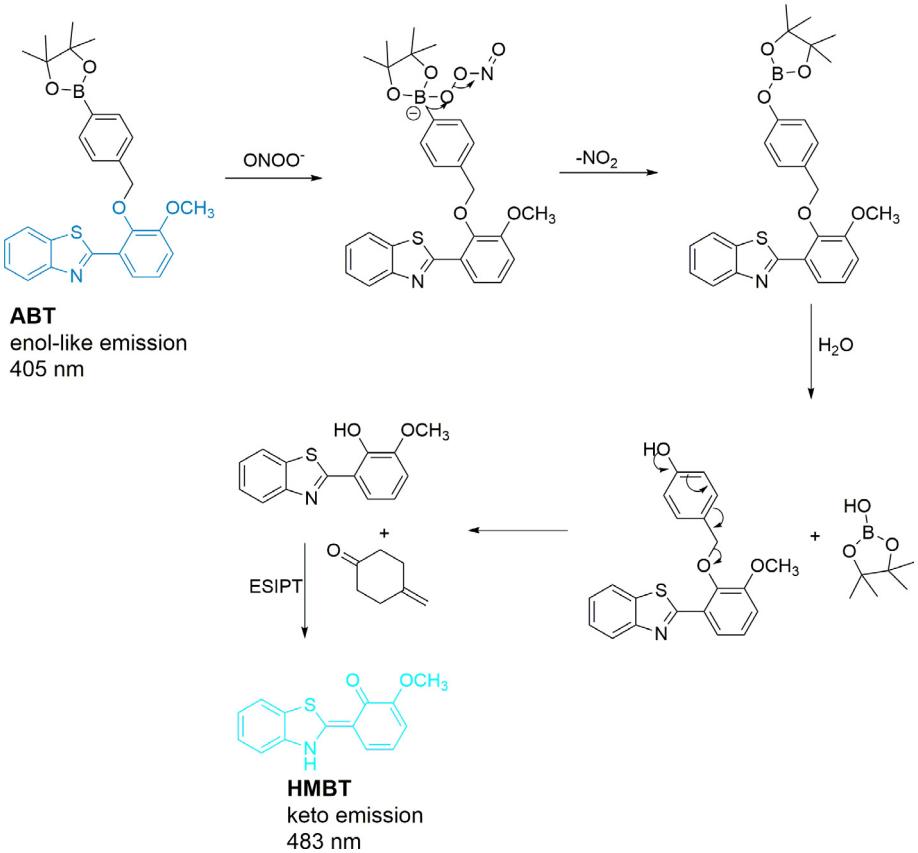
Furthermore, temporal fluorescence responses of **ABT** (5 μM) towards $ONOO^-$ and other analytes were examined as shown in Fig 4. The ratio (F_{483}/F_{405}) rapidly and drastically went up within 10 s, and gradually reached a plateau at 50 s in the presence of $ONOO^-$ (10 μM), while the ratio shows little variety even after 2 mins in the case of other analytes (H_2O_2, NO_2^-, ·OH, S^{2-}, *t*-BUOO·, *t*-BUOOH, and $\cdot O_2^-$) except HClO leading to slight enhancement of the ratio (the concentration of other analytes is all 50 μM). In terms of kinetic studies, the rate constant of the reaction between **ABT** and $ONOO^-$ was 1.16×10^4 M^{-1}·S^{-1}. However, rate constants for the reaction of **ABT** with H_2O_2, and HClO were 0.23 M^{-1}·S^{-1} and 4.81 M^{-1}·S^{-1} respectively. Compared to the former rate constant, the later ones were much smaller. These results suggested that not only **ABT** had great potential to efficiently capture $ONOO^-$ in biological systems, in spite of its short lifetime and high activity, but selectivity of **ABT** for $ONOO^-$ over other analytes referred to above was extremely high.

To evaluate the selectivity and competition of the probe **ABT**, fluorescence spectra were obtained when $ONOO^-$ (10 μM) and other physiologically relevant species (50 μM) including H_2O_2, NO_2^-, ·OH, S^{2-}, *t*-BUOO·, *t*-BUOOH, $\cdot O_2^-$, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, and SO_3^{2-} were added into **ABT** solution (5 μM) respectively. Results observed were analogous to previous time-dependent responses, only $ONOO^-$ made the emission intensity ratio (F_{483}/F_{405}) significant enhancement, but other species tested hardly caused any changes. Although, the ratio increased a little to about 2.5 times in the presence of HClO (Fig. 5A), other analytes barely interfered with the reaction between $ONOO^-$ and **ABT**. The emission intensity ratio (F_{483}/F_{405}) increased to 21–27 times when $ONOO^-$ added into the mixed solution of **ABT** and one of the analytes mentioned above in most cases; even though the ratio only increased to nearly 12 times, as the mixed solution in the presence of HClO, ·OH and *t*-BUOO· (Fig. 5B). May it result from high concentration of HClO, ·OH and *t*-BUOO· reacting with **ABT** in terms of previous reports.[17] In a word, the probe **ABT** displayed high selectivity towards $ONOO^-$ over other physiological species in vitro.

Since the pH values in typical mammalian cells usually range from 4.5 in lysosomes to 8.0 in mitochondria, the effective pH range of **ABT** for peroxynitrite detection was determined to be from 5 to 9, a broad range with biological relevance (see Supporting information).

Proposed sensing mechanism

The results from the spectral response of **ABT** to $ONOO^-$ indicated that HMBT might be the product of reaction between **ABT** and $ONOO^-$. Furthermore, the main product of reaction between **ABT** and $ONOO^-$ was identified by the separate reaction of **ABT** and 3 equiv $ONOO^-$ in DMF (N,N-Dimethylformamide) at room temperature for 1 h and the isolated major product was HMBT which was characterized by ^{1}H NMR spectra. Moreover, the crude reaction mixture of **ABT** and $ONOO^-$ was analyzed using MS showing intense peak of at *m*/*z* 258, consistent with that of compound HMBT. Meanwhile, the intense peak of [**ABT** + H]$^+$ (*m*/*z* 474) was disappeared (see Supporting information). Obviously, it could be proved that the main product was HMBT. So, the sensing mechanism was probably that the reaction of **ABT** with $ONOO^-$ promoted oxidative hydrolysis of the arlyboronate



Scheme 2. Proposed mechanism for **ABT** sensing $ONOO^-$.

group with the rapid elimination of p-quinomethane to generate free HMBT (Scheme 2 for mechanism in detail).[28]

Conclusion

In summary, by combining an ESIPT fluorophore denoted as HMBT with an alyboronate group, a new fluorescent probe **ABT** was designed and synthesized to monitor $ONOO^-$ concentration. The probe **ABT** displayed an emission band at 405 nm, but HMBT produced and its keto emission band at 483 nm emerged once **ABT** reacted with $ONOO^-$. The ratiometric probe exhibited the feature of high selectivity, high sensitivity and fast response for $ONOO^-$ over other common physiological species and worked in a wide pH range from 5 to 9 (favorably adapting to physiological conditions of living systems). Its emission ratio (F_{483}/F_{405}) displayed great dynamic growth to about 35 times and detection limit of 26.3 nM, a very low value, was measured. Hence, **ABT** might be well used for quantitative detection of the $ONOO^-$ in biological systems. But it still need further studies to confirm applicability of **ABT**.

Acknowledgements

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.2017.12.004. These data include MOL files and InChiKeys of the most important compounds described in this article.

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