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A new turn-on fluorescent probe for sensing 4-methylbenzenethiol in real water samples

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HIGHLIGHTS

- A novel "turn on" fluorescent probe **MBT** was developed to detect *p*-methylbenzenethiol.
- **MBT** possesses good selectivity and sensitivity toward *p*-methylbenzenethiol.
- **MBT** can be used for the detection of *p*-methylbenzenethiol in environmental water samples.

GRAPHICAL ABSTRACT

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ABSTRACT

A new fluorescent probe (MBT) for the detection of 4-methylbenzenethiol (*p*-MePhSH) was developed by using 4-(benzo[*d*]thiazol-2-yl)-3-methoxyphenol as the fluorophore and 2,4-dinitrophenyl ether as the sensing moiety. Probe MBT displayed good selectivity toward *p*-MePhSH in DMSO/PBS buffer (5/5, v/v) solution and anti-interference over other competitive species *via* nucleophilic aromatic substitution. The fluorescence intensities of the probe responded *p*-MePhSH showed a 22-fold enhancement and good linearity with *p*-MePhSH concentration collected in the range of 0–15 μM. Moreover, the probe is sensitive to *p*-MePhSH and the limit of detection is 45 nM. The sensing mechanism of probe MBT was verified by high-resolution mass spectrometry and fluorescence lifetime. Furthermore, the probe was used to the detection of *p*-MePhSH in real water samples.

1. Introduction

Benzenethiol and substituted benzenethiols are important organic synthesis intermediates and have been widely applied in the synthesis of medicine, pesticides [1,2] and antiseptic. Possible production of 4-methylbenzenethiol as an antiseptic may result in its release to the environment through various waste streams. For instance, the concentration of 4-methylbenzenethiol in a raw sludge from a wood preservation operation has been reported to be 0.52 g/L [3]. Because of their high toxicity, exposure to these aromatic thiols may cause many kinds of diseases by damaging central nervous and other biological systems [4,5]. In addition, aromatic thiols can produce aryl disulfides that can cause serious interference on the physiological function of cells [6,7]. Therefore, it is significant to detect aromatic thiols. Although high-performance liquid chromatography and gas chromatography–mass spectrometry as classic approaches have been applied for the determination of thiols, great efforts have been devoted to the developing of fluorescent probes for sensing benzenethiol [8–12]. Typically, fluorescent probes for benzenethiol were based on a nucleophilic aromatic substitution (SNAr). The probes composed of an aromatic amine/hydroxyl fluorophore and a recognition moiety of 2,4-dinitrobenzene-sulfonate [13–17] or 2,4-dinitrophenyl [18–20]. In the sensing process, benzenethiol as a nucleophile performs a nucleophilic aromatic substitution on the probe to release the fluorophore, therefore, the probe turns on. It should be worth noting that other thiols, such as aliphatic thiols and hydrogen sulfide also have the potential to participate in similar SNAr reactions. Therefore, it is enormous challenge to improve the selectivity of

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fluorescent probes. So far, some highly selective fluorescent probes for benzenethiol by subtle modulating the structure of the fluorophores have been developed. However, reported fluorescent probes in general exhibited poor selectivity for benzenethiol over substituted benzenethiol such as 4-methylbenzenethiol.

In present work, we developed a new fluorescent probe for sensing 4-methylbenzenethiol over other thiols even similar benzenethiol.

2. Experimental section

2.1. Apparatus and materials

^{1}H NMR and ^{13}C NMR measurements were performed on a Bruker AV-400 (MHz) spectrometer in DMSO d_6 using TMS as internal standard. IR spectra were recorded by IR spectrophotometer Tensor IIFT-IR (Bruker Optics). Mass spectra were measured on an Impact II spectrograph (Agilent). UV–vis spectra were performed by a Hitachi U-4100 spectrophotometer. Fluorescence spectra were acquired by the excitation at 337 nm, slip 5/3 nm on an LS-55 luminescence spectrophotometer equipped with a 1 cm quartz cell. The fluorescence lifetime and fluorescence quantum yield was performed on an Edinburgh Instruments FLS920 Fluorescence Spectrometer. The pH of systems was measured by a PHS-3C pH meter. Melting points of samples were obtained by an XD-4 digital micro melting point apparatus. Thin-layer chromatography (TLC) was conducted on silica gel 60F254 plates (Merck KGaA).

Unless otherwise stated, all the reagents and solvents used in present work were analytical grade and commercially available, which were used without further purification.

2.2. Synthesis and characterization

The synthetic route of probe **MBT** is depicted in Scheme 1.

2.2.1. Synthesis of 4-(benzo[d]thiazol-2-yl)-3-methoxyphenol (compound **1**)

Compound **1** was synthesized according to the reference [21]. A mixture of *o*-aminothiophenol (2.500 g, 20 mmol) and 4-hydroxy-2-methoxybenzaldehyde (3.043 g, 20 mmol) were dissolved in EtOH (50 mL). Then 30% H_2O_2 (13.604 g) and 37% HCl (5.910 g) were added while stirring at room temperature. After reaction for 1 h, precipitates were filtrated and washed with water and ethanol to obtain compound **1** (3.77 g) in 71.9% yield. Mp: 223–225 ℃; IR (KBr): 3095, 1596, 1532, 1348, 1274, 1150 cm^{-1}; ^{1}H NMR (400 MHz, DMSO d_6), δ (ppm): 10.45 (s, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.47–7.52 (m, 1H), 7.36–7.40 (m, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.61 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 4.01 (s, 3H). ^{13}C NMR (100 MHz, DMSO d_6), δ (ppm): 56.34, 99.81, 109.33, 112.73, 121.82, 122.18, 124.79, 126.65, 130.57, 134.83, 151.21, 159.28, 162.55, 163.43. HRMS (ESI$^+$): m/z (M + H)$^+$ calcd. 258.0583; found 258.0588.

2.2.2. Synthesis of 2-(4-(2,4-dinitrophenoxy)-2-methoxyphenyl)benzo[d]thiazole (probe ***MBT***)

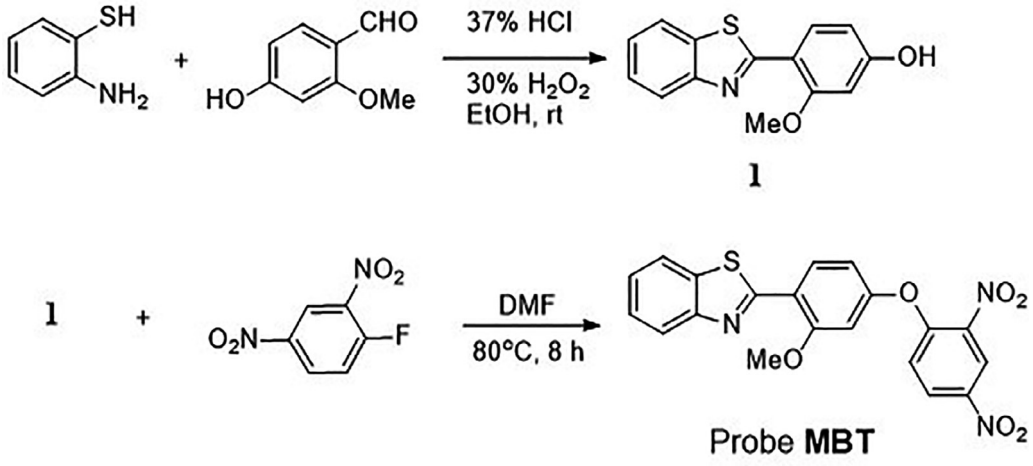
The probe was synthesized according to the literature method [21]. A mixture of compound **1** (1.032 g, 4.0 mmol), 2,4-dinitrofluorobenzene (0.799 g, 4.3 mmol), triethylamine (1.17 g, 11.7 mmol) in dry DMF (30 mL) were stirred for 8.5 h at 80 ℃ under N_2 atmosphere. The reaction mixture was cooled to room temperature, and then poured into a mixture of ice and water (300 mL). Brownish yellow solid precipitated. After workup, probe **MBT** was obtained in 49.3% yield (0.84 g). Mp: 178–180 ℃. IR (KBr): 3012, 1612, 1529, 1439, 1292 cm^{-1}; ^{1}H NMR (400 MHz, DMSO d_6), δ (ppm): 8.95 (d, J = 4.0 Hz, 1H), 8.56 (d, J = 8.0 Hz, 1H), 8.49 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.30–7.58 (m, 4H), 7.04 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 4.08 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 57.21, 105.65, 112.95, 119.48, 120.77, 122.39, 122.42, 122.92, 125.50, 126.86, 130.19, 131.14, 135.62, 140.15, 142.39, 151.96, 154.61, 157.37, 159.05, 161.82. HRMS (ESI$^+$): m/z (M + H)$^+$ calcd. 424.0598; found 424.0589.

2.3. Preparation of stock solution for spectral measurement

The stock solution of probe **MBT** (1×10^{-3} M) was prepared in DMF. PhSNa, *p*-MePhSH were dissolved in DMSO. The anion (S^{2-}, HS^-, SO_3^{2-}, CO_3^{2-}, ClO^-, NO_3^-, $S_2O_3^{2-}$, SCN^-, F^-, Cl^-, Br^-) stock solution and Cys, Hcy, GSH, H_2O_2 were all prepared in deionized water containing phosphate buffered saline (PBS, 1×10^{-2} M, pH = 7.4). Working solutions of probe **MBT** were freshly prepared by diluting the highly concentrated stock solution to the desired concentration prior to the spectroscopic measurements.

2.4. UV–vis and fluorescence-spectral detection

All UV–vis and fluorescence spectral experiments were carried out in $V_{(DMSO)}$:$V_{(PBS)}$ = 50:50 (pH = 7.4) buffer solution. In the fluo-



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

rescence measurements, emission spectra were collected from 350 nm to 500 nm by 337 nm excitation.

3. Results and discussion

3.1. Synthesis and structure characterization of probe ***MBT***

Probe **MBT** was easily synthesized from *o*-aminothiophenol, 4-hydroxy-2-methoxybenzaldehyde and 2,4-dinitrofluorobenzene *via* two steps according to literature (Scheme 1). The structures of compound **1** and probe **MBT** were characterized by IR, NMR and HRMS (Fig. S1-8).

3.2. UV–vis absorption spectral response of probe ***MBT*** toward *p*-MePhSH

The sensing ability of probe **MBT** (20 μM) toward *p*-MePhSH in buffer solution was initially investigated by UV–vis spectroscopy. With the gradual addition of *p*-MePhSH, the maximum absorbance increased and peaked in the presence of 3.5 equiv. *p*-MePhSH. A good linearity between maximum absorbance and concentration of *p*-MePhSH could be obtained. Meanwhile, obvious red shift (21 nm) could be observed (Fig. S9).

3.3. Fluorescence spectral response of probe ***MBT*** toward *p*-MePhSH

The fluorescence spectral response of probe **MBT** (5 μM) toward *p*-MePhSH was performed in the $V_{(DMSO)}$: $V_{(PBS)}$ = 50:50 (pH = 7.4) buffer solution at room temperature. The fluorescence spectral titration of probe **MBT** with different concentration of *p*-MePhSH showed a gradual fluorescence enhancement at 420 nm as *p*-MePhSH concentration increase under the excitation of wavelength at 337 nm. A good linear relationship between fluorescence intensity of probe **MBT** at 420 nm and *p*-MePhSH concentrations in the range of 0—15 μM was observed (Fig. 1). Upon the addition of *p*-MePhSH, the emission intensity could increase 22 times at 420 nm, which showed the phenomenon of an "off–on" switch triggered by *p*-MePhSH. The limit of detection of probe **MBT** for *p*-MePhSH (calculated by 3σ/k) was found to be as low as 45 nM. Moreover, colour change from colourlessness to blue could be observed under 365 nm violet upon the addition of *p*-MePhSH in the PBS/DMSO buffer solution of probe **MBT**, which was almost identical to the colour of compound **1** and easily observed by the naked eye (Fig. S10).

3.4. Selectivity of probe ***MBT*** toward *p*-MePhSH over other analyte and anti-interference

The selectivity of probe **MBT** (5 μM) toward *p*-MePhSH was investigated to assess its potential applications. The fluorescence change of probe **MBT** toward various species including *p*-MePhSH, benzenethiol, biothiol (Cys, Hcy, GSH), some nucleophilic species (S^{2-}, HS^-, SO_3^{2-}, CO_3^{2-}, ClO^-, NO_3^-, $S_2O_3^{2-}$, SCN^-, F^-, Cl^-, Br^-) and reactive oxygen (H_2O_2) showed that probe **MBT** could respond well to *p*-MePhSH and had a weak response to benzenethiol. Meanwhile, the coexistent competing species mentioned above had a negligible interfering effect on the fluorescence intensity of probe **MBT** upon the addition of *p*-MePhSH (Fig. 2). The results suggested that probe **MBT** had high selectivity toward *p*-MePhSH and good anti-interference.

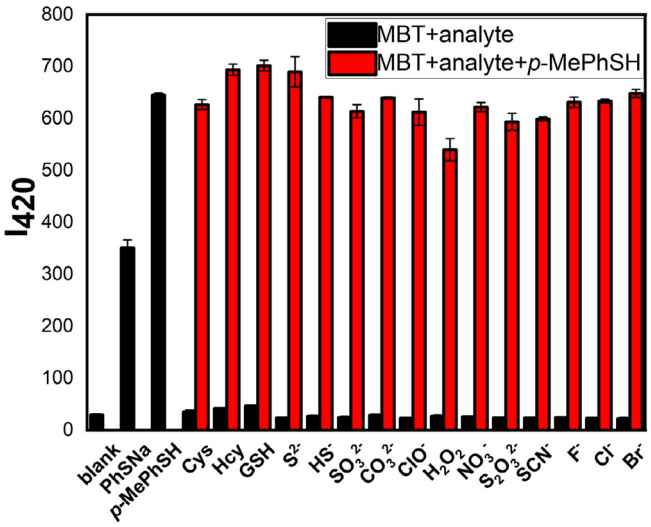


Fig. 2. Fluorescence responses of probe **MBT** (5 μM) toward *p*-MePhSH (25 μM) and various analyte (50 μM) after treated for 10 min. The fluorescence intensity was collected at 420 nm. Excitation: 337 nm. Data are presented as the mean ± SD (n = 3).

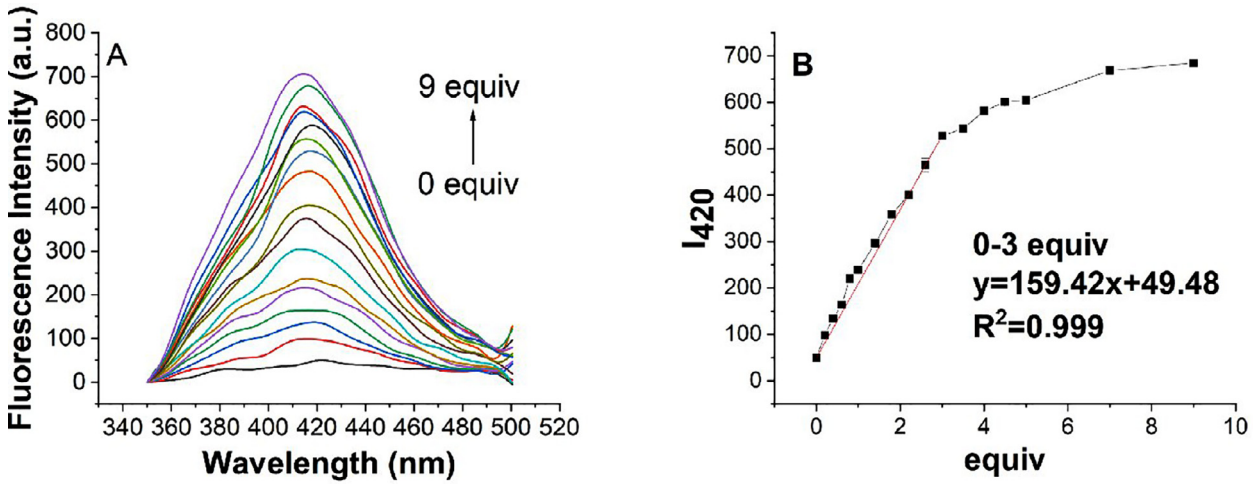


Fig. 1. (A) Fluorescence spectra of probe **MBT** (5 μM) in PBS buffer (pH 7.4, 50% DMSO) at 25℃ for 10 min. Incubated with different concentrations of *p*-MePhSH (0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17.5, 20, 22.5, 25, 35, 45 μM) for 10 min. (B) The linear relationship between fluorescence intensity of probe **MBT** (5 μM) at 420 nm and various *p*-MePhSH concentrations. Excitation: 337 nm. Data are presented as the mean ± SD (n = 3). The linearity was fitted with error bar by Origin 2019b.

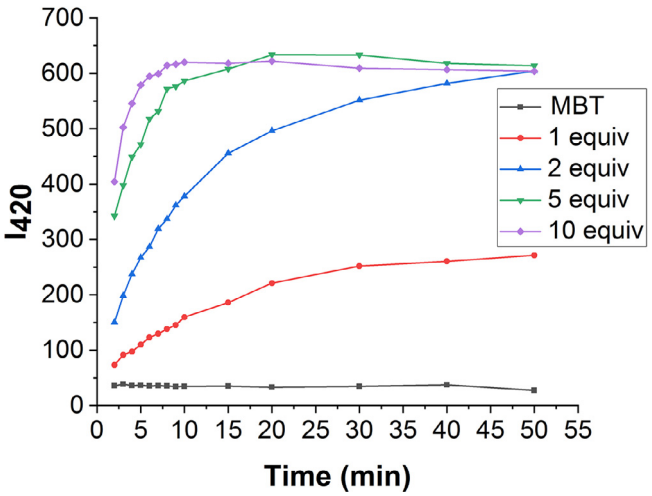


Fig. 3. Time-depending fluorescence spectra of probe **MBT** (5 μM) in PBS buffer (pH 7.4, 50% DMSO) at 25℃. Incubated with different concentrations of *p*-MePhSH (0, 5, 10, 25, 50 μM) after 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 min. Excitation: 337 nm, emission: 350–500 nm. The fluorescence intensity was collected at 420 nm.

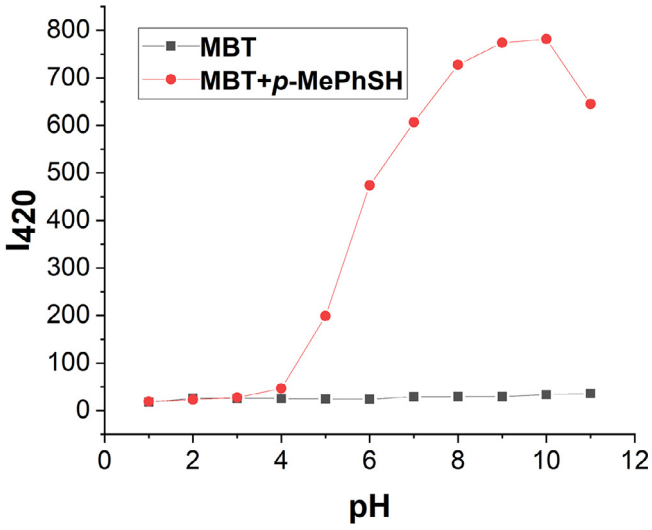


Fig. 4. pH-dependent fluorescence responses of probe **MBT** (5 μM) toward *p*-MePhSH (25 μM) at various pH (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11) after treated for 10 min. Excitation: 337 nm. The fluorescence intensity was collected at 420 nm.

*3.5. Time-dependent fluorescence response of probe **MBT** toward p-MePhSH*

Time-dependent fluorescence response of probe **MBT** toward *p*-MePhSH was determined (Fig. 3). Probe **MBT** showed stable fluorescence in absence of *p*-MePhSH. However, when different equiv. *p*-MePhSH was added, the fluorescence intensity was drastically enhanced within a few minutes and it reached a plateau after 10 min. The results suggested that probe **MBT** could be suitable for the real-time detection of *p*-MePhSH.

*3.6. pH-dependent fluorescence response of probe **MBT** toward p-MePhSH*

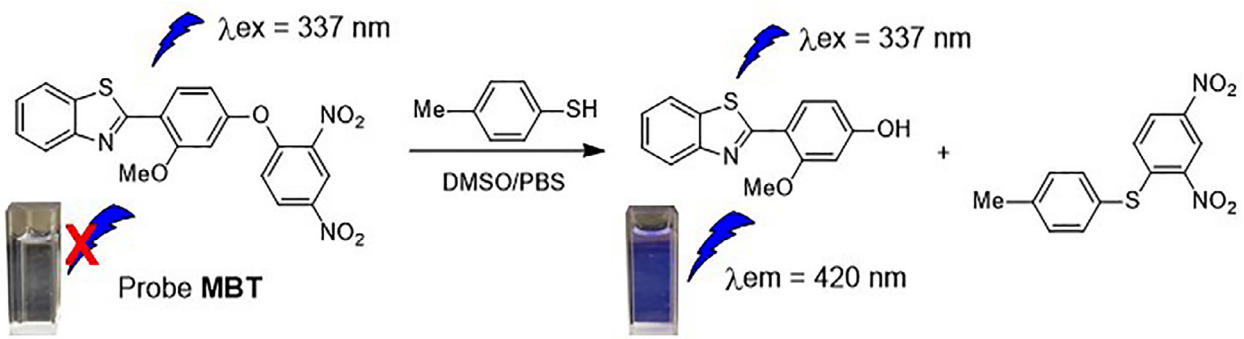
The effect of pH on the fluorescence property of probe **MBT** is also an important factor to be examined for further application. Therefore, fluorescence intensity changes of probe **MBT** in solvent system with different pH values were surveyed in the presence or absence of *p*-MePhSH (Fig. 4). Probe **MBT** alone did not exhibit obvious fluorescence change in the wide pH range of 1–11. In the presence of *p*-MePhSH, fluorescence intensity of the system gradually enhanced from pH 2 and the maximum emission intensity appeared at around pH 8.0. The results implied that probe **MBT** could be a promising tool for the detection of *p*-MePhSH in the pH range of 6–10.

*3.7. Mechanism of probe **MBT** sensing p-MePhSH*

It is known that 2,4-dinitrophenyl aryl ether could be broken by nucleophilic aromatic substitution. *p*-MePhSH as a nucleophile attacked probe **MBT** to release the fluorophore (Scheme 2). The high-resolution mass spectrometry of probe **MBT** reacted with *p*-MePhSH showed a peak at 258.0580, which corresponds to compound **1** (Fig. S11). Compared with the fluorescence lifetime of compound **1** and probe **MBT** responded *p*-MePhSH, fitted perfectly results were observed (Fig. 5, Table 1). Moreover, fluorescence spectra of compound **1** and a mixture of **MBT** with *p*-MePhSH were also fitted (Fig. S12), confirming proposed mechanism. The fluorescence quantum yield of probe **MBT** responded *p*-MePhSH was measured to be 21.84% (Fig. S13).

3.8. Detection of p-MePhSH in real sample

To assess the feasibility of probe **MBT** detecting *p*-MePhSH in a natural water sample, tests of real water samples (tap water, XiaoQing River and Yellow River water Jinan, China) were performed. A total of four concentrations of *p*-MePhSH (0, 5, 10, 15 μM) were spiked in three samples, and then treated with probe



Scheme 2. Sensing mechanism of probe **MBT** for *p*-MePhSH.

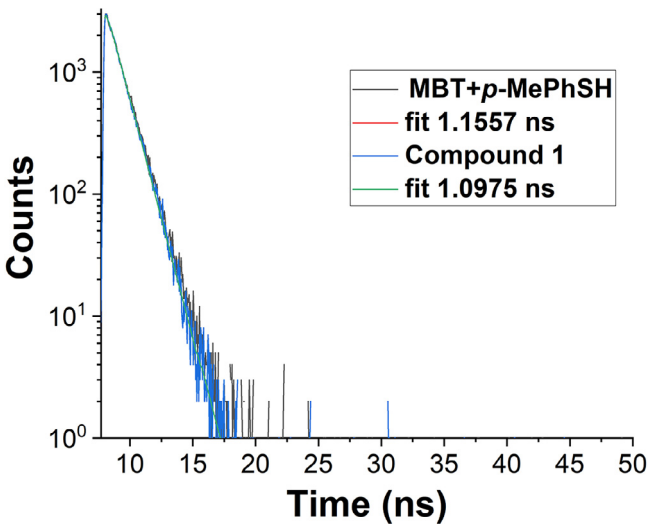


Fig. 5. The fluorescence lifetime of compound **1** and probe **MBT** responded *p*-MePhSH.

Table 1
Fluorescence lifetime of compound **1** (5 μM) and probe **MBT** (5 μM) toward *p*-MePhSH (25 μM).

	τ_1 / ns (α_1 / %)	χ^2
Compound **1**	1.0975 (100%)	1.020
MBT + *p*-MePhSH	1.1557 (100%)	1.024

Table 2
Detection of *p*-MePhSH in real sample by probe **MBT.**

Sample	*p*-MePhSH spiked (μM)	*p*-MePhSH recovered (μM)	Recovery (%)[a]	Standard Deviation
Tap water	0.00	/	/	/
	5.00	5.49	109.79	0.06
	10.00	10.46	104.63	0.02
	15.00	13.54	90.25	0.05
XiaoQing River	0.00	/	/	/
	5.00	4.52	90.39	0.02
	10.00	9.46	94.63	0.07
	15.00	13.04	86.91	0.06
Yellow River water	0.00	/	/	/
	5.00	4.62	92.44	0.10
	10.00	10.04	100.39	0.09
	15.00	13.20	88.00	0.14

[a] The recovered concentrations were calculated by the calibration curve shown in Fig. 1 after deducted the background fluorescence of each kind of water sample.

MBT. The fluorescence intensities of the water samples at 420 nm were measured (Table 2). The results showed that *p*-MePhSH recoveries are between 86.9 and 109.8%, indicating a potential application of probe **MBT** to detect *p*-MePhSH in real water samples.

4. Conclusions

A new fluorescent probe (**MBT**) for the detection of 4-methylbenzenethiol was developed. Probe **MBT** possessed good selectivity, sensitivity toward 4-methylbenzenethiol and anti-interference over other competitive species *via* nucleophilic aromatic substitution. The fluorescence intensities of the probe responded *p*-MePhSH increased 22-fold by a "turn-on" mechanism which was confirmed by HRMS, fluorescence lifetime and fluorescence spectra. A good linearity of probe **MBT** with *p*-MePhSH concentration in the range of 0–15 μM provided a potential method for the detection of *p*-MePhSH in real water samples.

CRediT authorship contribution statement

Jun-Zheng Wang: . **Feng Li:** . **Meng-Min Xiao:** . **Chen-Ran Ma:** . **Guo-Qing Cheng:** . **Bao-Xiang Zhao:** Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.119947.

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