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ARTICLE

Design and synthesis of a novel colorimetric fluorescent probe for selective detection of sulfur dioxide in SH-SY5Y neuroblastoma cells and its application in Traditional Chinese Medicines

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Sulfur fumigation has attracted more and more attention as one of the important post-harvest processing methods for some Traditional Chinese Medicines (TCMs) in the last decade. However, sulfur-fumigated TCMs have recently emerged as a controversial topic due to their potential detrimental effect on the safety and efficacy, as some sulfur-fumigated TCMs contain lots of sulfur dioxide derivatives. Additionally, high levels of the sulfur dioxide derivatives could cause some diseases and dangerous environmental pollutant. In this work, a fast response time, low limit of detection and high fluorescence quantum yield probe **DTCC** was designed and synthesized to detect SO_2 derivatives based on coumarin-thiophene dye which was fused with a coumarin moiety and 2-thiophenecarboxaldehyde. Probe **DTCC** exhibited fast response time (less than 10 s), satisfactory selectivity for SO_2 derivatives in the presence of other ROS and excellent sensitivity for SO_2 derivatives with low limit of detection (0.23 μM) and widely linear range (0～100 μM). Furthermore, probe **DTCC** was successfully applied in fluorescent imaging in SH-SY5Y neurobalstoma cells with excellent membrane permeability and stability. It was also employed for monitoring the total SO_2 derivatives in several real TCMs sample. These results illustrated that probe **DTCC** has an excellent capability for monitoring SO_2 derivatives in living cells and real TCMs sample.

1. Introduction

Reactive Sulfur Species (RSS) are associated with numerous physiological and pathophysiological processes, which are produced by various biochemical and physiological oxidative processes in the body. RSS play a major role in the pathogenesis of various human diseases. At low concentrations, RSS exhibit beneficial effects by regulating intracellular signaling and homeostasis; at high levels, however, RSS play a major role in the damage of proteins, lipids, DNA and carcinogenesis.[1] Among RSS, endogenous sulfur dioxide and its derivatives have received recently increasing attention because they are involved in various physiology and pathological processed as a possible gasotransmitter[2,3] following nitro oxide,[4,5] carbon monoxide[6,7] and hydrogen sulfide.[8] On the other hand, exogenous sulfur dioxide derivatives (SO_3^{2-}/HSO_3^{2-}) are widely used as a kind of preservative in foods, beverages and pharmaceutical products due to their capacity to inhibit the growth of microorganisms and prevent mildew.[9] However, high levels of the sulfur dioxide derivatives could cause some diseases, such as rheumatoid arthritis, Parkinson's disease, Alzheimer's disease[10,11] and lung cancer.[12,13]

For the sake of preserve moisture, colour and freshness, and to prevent problems with insects and mold,[14] sulfur fumigation has attracted more and more attention as one of the important post-harvest processing methods for some Traditional Chinese Medicines (TCMs) in the last decade. However, sulfur-fumigated TCMs have recently emerged as a controversial topic due to their potential detrimental effect on the safety and efficacy.[15] So residual sulfur dioxide content is officially used as the exclusive indicator for the

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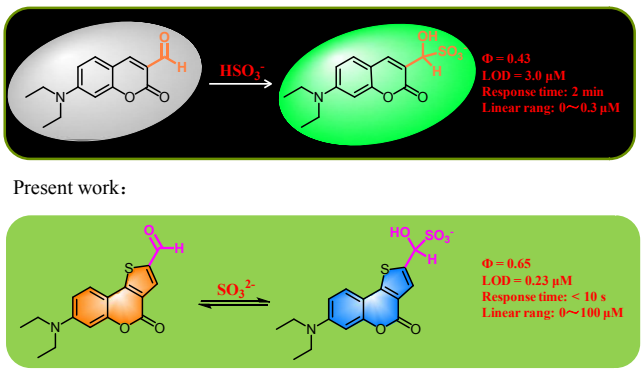
safety evaluation of sulfur-fumigated products worldwide, and the residual content is expected to be controlled as low as possible.[16] Therefore, it is of extremely emergency to develop a convenient, efficient and reliable method for monitoring sulfur dioxide derivatives in sulfur-fumigated TCMs. At present, several traditional analytical methods have been used to detect SO_2 and its derivatives, such as spectrophotometry,[17] high-performance liquid chromatography (HPLC),[18,19] electrochemistry,[20,21] capillary electrophoresis,[22] flow injection analysis[23,24] and so on. However, these methods depend on high-cost instruments, complex pre-treatment processes and are time-consuming, which limit their practical application.

To solve these problems, fluorescent probes have been extensively used as promising detection tools due to its non-invasiveness, high sensitivity and selectivity, fast response, as well as high temporal and spatial resolution.[25,26] Accordingly, some fluorescent and chromogenic chemosensors have been developed for the monitor of sulfur dioxide derivatives based on familiar mechanism contained nucleophilic reaction with aldehyde,[27] Michael addition,[28] dequenching of levulinate[29] and coordinative interactions.[30] However, these works have several drawbacks, such as long detecting time (up to 10 h), poor water solubility and interference by hydrogen sulfide or biothiols.[31] Therefore, it is utmost important to design a fast response, widely linear range and the sophisticated background sample application to detect SO_2 derivatives.

Coumarin scaffold is frequently used as fluorescence dyes for the detection of RSS due to their less toxicity, good water solubility and convenience to be modified for sensing of different analytes.[32] In addition, aromatic heterocyclic compounds containing a five-membered furan or thiophene unit have been extensively studied in recent years due to their impressive optical properties and excellent charge-transport properties.[33,34] To the best of our knowledge, there has been no report on the properties and applications of thiophene modified coumarin derivatives as the fluorescence probe. [35] Herein, a fast response time, low limit of detection and high fluorescence quantum yield probe **DTCC** was designed and synthesized to detect SO_2 derivatives based on coumarin-thiophene dye which was fused with a coumarin moiety and 2-thiophenecarboxaldehyde. Probe **DTCC** exhibited fast response time (less than 10 s), satisfactory selectivity for SO_2 in the presence of other RSS and excellent sensitivity for SO_2 derivatives with low limit of detection (0.23 μM) and widely linear range (0～100 μM). Furthermore, probe **DTCC** was successfully applied in fluorescent imaging in SH-SY5Y neurobalstoma cells with excellent membrane permeability and stability. For practical application, probe **DTCC** was employed for monitoring the total SO_2 derivatives in several real TCM sample (**Scheme 1**).



Scheme 1 Strategies to response of probe **DTCC.**

2. Materials and methods

2.1. Materials

All chemical reagents and solvents were purchased from commercial sources with analytical grades, and used without further purification. All the reactions were carried out in dried glassware with magnetic stirring and the progress of reactions was monitored by thin layer chromatography (TLC). TLC was performed by using Merck F254 gel-60 plates. Silica gel (200 mesh) was used as the solid phase for column chromatography. Stock solutions (1.0 Mm) of Cys, GSH, $S_2O_5^{2-}$, $S_2O_8^{2-}$, NO_2^-, NO_3^-, F^-, Cl^-, I^-, Br^-, SO_4^{2-}, HSO_4^-, CO_3^{2-}, AcO^- were prepared by direct dissolution of proper amounts of sodium salts. The Hepes buffer solution was prepared from 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (2.3800 g) in 100 mLdeionized water and 20% NaOH solution. The deionized water was prepared from a Millipore water purification system. All glassware were cleaned with deionized water for three times and dried before use.

2.2. Instrumentation and methods

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The UV–vis absorption spectra were determined at room temperature on a Shimadzu UV-2550 spectrophotometer in a 1 cm quartz. The fluorescence spectra were taken on a Hitachi F-7000 Fluorescence spectrophotometer using a 5.0 nm slit width in a 1×1 cm quartz cell. The cell fluorescence imaging was obtained by laser scanning confocal fluorescence microscopy Olympus FV1000 (Olympus Corporation, Tokyo, Japan). HRMS spectra were recorded on a micro TOF-QII mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained from a Varian Gemini 2000 DMX600 MHz FT NMR spectrometer using $CDCl_3$/DMSO-d_6 as solvent and TMS as internal standard. The chemical shifts were represented by ppm. The quantum yield was measured at room temperature referenced to fluorescein in aqueous solution of 0.1 M sodium hydroxide. The structures of synthesized compounds were fully characterized by HRMS, ^{1}H NMR, ^{13}C NMR and the related spectra are shown in the Supporting Information.

2.3. Synthesis of compound bis-(2, 4, 6-trichlorophenyl) malonate (1)

A mixture of commercial available malonic acid (0.5200 g, 5.0 mmol, 0.63 equiv.) and 2,4,6-trichlorophenol (1.5800 g, 8.0 mmol, 1.0 equiv.) in toluene (20 mL) and $POCl_3$ (1 mL, 10.5 mmol, 1.31 equiv.) was heated at 100 ℃ for 4 h in oil bath. After cooling to room temperature, the mixture was poured into ice water and extracted with ethyl acetate (3×50 mL). The combined organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to give bis-(2, 4, 6-trichlorophenol) malonate **1** as a white solid, which was used directly in the next step without further purification (Yield: 43%, R_f = 0.64, PE: EtOAc = 5:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 (s, 4H), 4.05 (s, 2H). HRMS ($C_{15}H_6Cl_6O_4$): calcd. For $[M+H]^+$ 460.8475; found: $[M+H]^+$ 460.8470 (**Fig. S2**).

2.4. Synthesis of 7-(diethylamino)-4-hydroxy-2H-chromen-2-one (2)

3-Diethylamino- phenol (0.8150 g, 5 mmol, 1.0 equiv.) was added to a solution of compound **1** (2.2840 g, 5 mmol, 1.0 equiv.) in dry toluene (5 mL). The mixture was heated under reflux at 110 ℃ for 2 h. Then the reaction mixture was cooled to room temperature and filtered. The resultant grey solid was washed with toluene and dried under high vacum to generate 7-(diethylamino)-4-hydroxy-2H-chromen-2-one **2**, which was used directly in the next step without further purification (Yield: 70%, R_f = 0.2, PE:EtOAc = 1:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.89 (s, 1H), 7.54 (d, *J*=9.0, 1H), 6.65 (d, *J*=9.0, 2.5, 1H), 6.44 (s, 1H), 5.25 (s, 1H), 3.40 (q, *J*=7.0, 4H), 1.11 (s, 6H). HRMS ($C_{13}H_{15}NO_3$): calcd. for $[M+H]^+$ 234.1130; found: $[M+H]^+$ 234.1134 (**Fig. S3**).

2.5. Synthesis of 4-chloro-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (3)

Fresh distilled DMF (2.6 mL) was added dropwise to $POCl_3$ (2.6 mL) at room temperature and was stirred for 30 min to obtain a red solution under nitrogen. Then a portion of 7-(diethylamino)-4-hydroxy-2H-chromen-2-one (2.0000 g, 8.6 mmol, 1.0 equiv., dissolved in 9 mL DMF) was added dropwise to the above solution and yield a scarlet suspension. After the mixture was stirred at 60 ℃ for 12 h, it was poured into 100 mL of ice water. NaOH solution (20%) was used to adjust the pH to obtain a large amount of precipitate. The crude product was subjected to column chromatography (PE:EtOAc=1:4) to give 4-chloro-7-(diethylamino)-2-oxo-2H –chromene-3-carbaldehyde as an orange solid (Yield: 74%, R_f = 0.75, PE:EtOAc=1:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.26 (s, 1H), 7.80 (d, *J*=9.0, 1H), 6.67 (d, *J*=9.0, 2.5, 1H), 6.41 (d, *J*=2.5, 1H), 3.45 (q, *J*=7.1, 4H), 1.25 (t, *J*=7.0, 6H). HRMS ($C_{14}H_{14}ClNO_3$): calcd. For $[M+H]^+$ 280.0740; found: $[M+H]^+$ 280.0784 (**Fig. S4**).

2.6. Synthesis of 7-(diethylamino)-4-oxo-4H-thieno [3, 2-c] chromene-2-carbaldehyde (DTCC)

A solution of $Na_2S\bullet 9H_2O$ (0.0470 g, 0.2 mmol, 1.1 equiv.) was added 4-chloro-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (0.0500 g, 0.2 mmol, 1.0 equiv.) in DMF (3 mL). The mixture was stirred at 60 ℃ for 2 h. Then chloroacetaldehyde (0.013 mL, 0.2 mmol, 1.1 equiv.) was added rapidly and the reaction was stirred during 3 h at 60 ℃, K_2CO_3 (0.0280 g, 0.2 mmol, 1.1 equiv.) was dissolved in water (1.0 mL) and added to the reaction solution. The mixture was stirred for 10 min at 60 ℃, cooled at room temperature and quenched in water. The aqueous phase was extracted with EtOAc (3×50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (PE:EtOAc=5:1) to obtain the desired compound **DTCC** (Yield: 40%, R_f = 0.83, PE:EtOAc=1:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.96 (d, 1H), 8.39 (s, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 6.76 (d, *J* = 8.9 Hz, 1H), 6.62 (s, 1H), 3.46 (dd, *J* = 7.0 Hz,

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4H), 1.14 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.55, 154.98, 154.30, 151.15, 139.08, 125.98, 120.62, 109.70, 103.81, 97.00, 67.38, 44.17, 12.30. HRMS ($C_{16}H_{15}NO_3S$): calcd. For $[M+Na]^+$ 324.0773; found: $[M+Na]^+$ 324.0664 (**Fig. S5-S7**).

2.7. Titration experiments of probe DTCC

For UV-vis and fluorescence titrations, Sodium sulphite and sodium bisulfite (Na_2SO_3/$NaHSO_3$) solution was prepared by dissolving Na_2SO_3/$NaHSO_3$ in deionized water (5.0 mM). Probe stock solution (1.0 mM) was prepared by dissolving 15.068 mg **DTCC** in 50 mL absolute ethanol. Different portions of the stock solution were then diluted to different concentrations for further usage. Generally, 25 μL of the **DTCC** solution was added into 5 mL of buffer solution (0.1 mM Hepes buffer, pH =7.4, 20% CH_3CN) in a colorimetric tube. The other ions (Cys, GSH, $S_2O_5^{2-}$, $S_2O_8^{2-}$, NO_2^-, NO_3^-, F^-, Cl^-, I^-, Br^-, SO_4^{2-}, HSO_4^-, CO_3^{2-}, AcO^-) was dissolved in water and added to the probe solution under the same condition. The spectra of these solutions were recorded by means of fluorescence method. Fluorescence measurements were carried out with a slit width of 5 nm (λ_{ex} = 385 nm) in 10 mm quartz cuvettes at room temperature and the scan rate was 1200 nm/min.

2.8. Quantum calculations

Geometries of probe **DTCC** in the ground state (S_0) were optimized by the density functional theory (DFT) using B3LYP with 6-311G(d) basis set.[36] The excited state geometries and related optical properties of all molecules were calculated by TD-DFT at the same level of theory. Vibrational analysis calculations were done in order to confirm that optimized geometries corresponded to local minima on the potential energy surfaces of the whole molecules. The olarisable continuum model (PCM)[37,38] using the integral equation formalism variant (IEFPCM) was employed in DMF to describe the solvent effects throughout theoretical calculations. All calculations were performed using the Gaussian 09 package.[39]

2.9. Cytotoxicity experiments

Cell counting kit-8 (CCK-8) was used to detect the cytotoxicity of probe **DTCC**. SH-SY5Y cells were purchased from the ATCC Cell Bank. Cells were seeded in 96-well plates at a concentration of 1 × 10^5 cells per well and cultured for 24 h in DMEM (supplemented with 10% fetal bovine serum (FBS)) in an incubator (37 ℃, 5% CO_2). After the cells were incubated with probe **DTCC** at different concentrations (0, 5, 10, 15 and 20 μM) for 24 h, CCK-8 (10 μL) was added to each well of the 96-well plate for 2 h at 37 ℃, the absorbance was measured at 450 nm using a microplate reader. Five replicates were done for each treatment group.

2.10. Cell imaging of probe DTCC

For intracellular imaging of SO_3^{2-}, SH-SY5Y neuroblastoma cells were placed on a 20 mm diameter glass bottom cell culture dish and allowed to adhere for 24 h. Before confocal scanning imaging, these cells were incubated with 10 μM of probe **DTCC** at 37 ℃ for 10 min followed by washing three times with phosphate buffered saline (PBS, pH = 7.4). Subsequently, the cells were washed with PBS for three times to remove excess **DTCC**, then in situ treated with different concentrations of Na_2SO_3 solution (0, 5, 10, 15, 20 μM, respectively). Cells were then visualized with an FV1000 laser scanning confocal microscope. The excitation wavelength was set to 405 nm and the emission collected in the range 450-550 nm.

3. Results and discussion

3.1. Synthesis of probe DTCC

The synthesis of probe **DTCC** was achieved in four steps as shown in **Scheme 2**. Treatment of 2, 4, 6-trichlorophenol with malonic acid in $POCl_3$ at 100 ℃ gave the compound **1**. The compound **1** reacted with 3-diethylaminophenol in anhydrous toluene to give coumarin derivative **2**. The intermediate aldehyde **3** was prepared by the Vilsmeier-Haack reaction, and it was further used to synthesize the compound **DTCC** through condensation with sodium ulphide in DMF in the presence of chloroacetaldehyde and K_2CO_3 at 60 ℃ without purification.[40,41] The detailed synthetic procedures were presented in the Materials and methods section. All synthetic compounds were carefully characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

3.2. Photophysical properties of probe DTCC

With probe **DTCC** in hand, the emission properties of probe **DTCC** were investigated in 20% CH_3CN solution (0.1 mM Hepes buffer, pH 7.4) at room temperature. As a new designed fluorescent probe, **DTCC** was evaluated the spectroscopic properties in different polarity solvents, such a DCM, DMF, EtOH, DMSO and so on.

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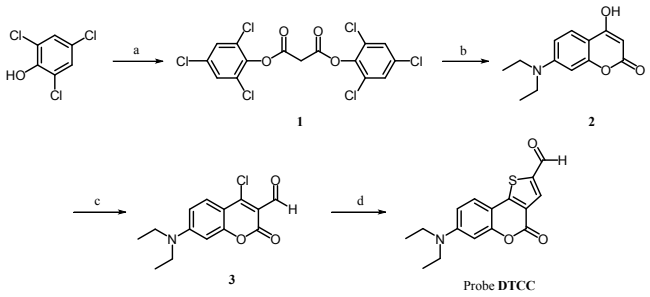

Scheme 2 Reagents and conditions: a) Malonic acid, $POCl_3$, 100 ℃, reflux, 4 h, 43%; b) 3-Diethylaminophenol, toluene, 100 ℃, 70%; c) DMF, $POCl_3$, 74%; d) DMF, $Na_2S•9H_2O$, Chloroacetaldehyde, K_2CO_3, 40%.

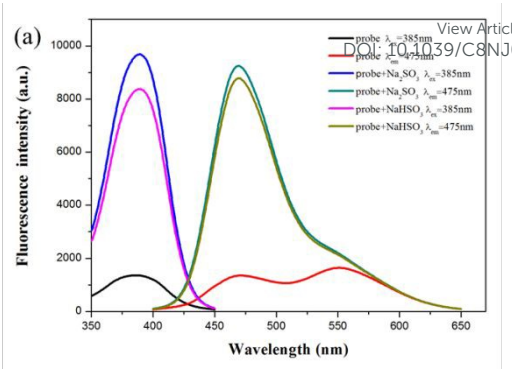

Fig. 1 Fluorescence spectra of probe **DTCC** reaction with Na_2SO_3 and $NaHSO_3$ in a 20% CH_3CN solution (10 mM Hepes buffer, pH 7.4) at room temperature. λ_{ex}= 385 nm, λ_{em}= 475 nm, slits: 5.0 nm / 5.0 nm.

Meanwhile, the fluorescence quantum yield of **DTCC**-hydrogen sulphite adduct was carefully measured using fluorescein in aqueous solution of 0.1 M sodium hydroxide (Φ_f = 0.79) as the standard reference compound. The photophysical data for fluorophore **DTCC** were depicted in **Table S2**.

In the fluorescence emission study, only very weak fluorescence was observed for **DTCC** in 550 nm when excited at 385 nm. A new fluorescence emission peak at 475 nm could be readily observed after reaction with SO_3^{2-} as shown in **Fig. 1**. At the same time, the colour of solution was noticeable changed from earthy yellow to blue under fluorescent lamp (**Fig. 3a**, inset). These results illustrated that probe **DTCC** could be used as a sensitive probe for detection of SO_3^{2-}.

3.3. Fluorescence response of probe DTCC towards SO_3^{2-}

The fluorescence emission spectra of probe **DTCC** (10 μM) after addition of SO_3^{2-} in a 20% CH_3CN water solution (10 mM Hepes buffer, pH 7.4) were shown in **Fig. 2**. Probe **DTCC** exhibited weak fluorescence emission at 550 nm under the excitation wavelength of 385 nm, since the fluorescence could be nearly completely quenched in polar solvents when an aldehyde group was introduced to coumarin. However, a significant enhancement of the emission intensity positioned at 475 nm when SO_3^{2-} was added to the probe solution. An excellent linear relationship (R^2 = 0.9974) was obtained with SO_3^{2-} concentrations ranging from 0 to 100 μM by plotting the corresponding fluorescence intensity ration and the

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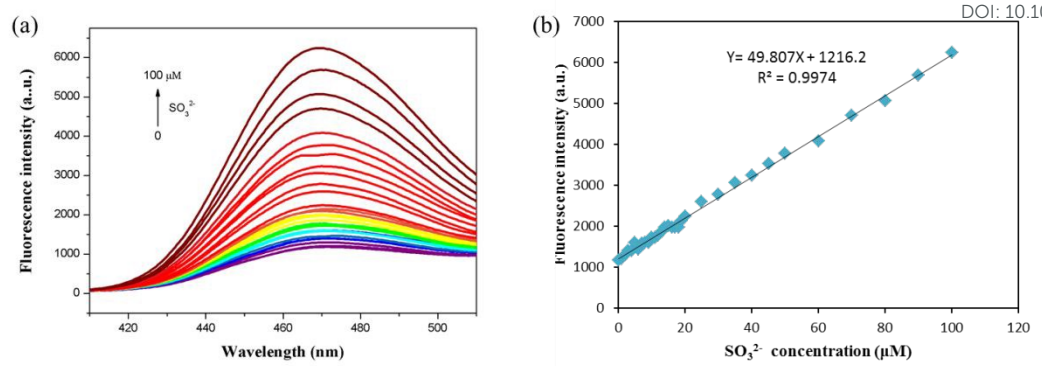

Fig. 2 (a) Fluorescence spectra responses of probe **DTCC** (10 μM) in the presence of different concentrations of SO_3^{2-} (0 ~ 100 μM) in a 20% CH_3CN solution (10 mM Hepes buffer, pH 7.4) at room temperature; Error bars are ± SD, n = 3; (b) Linear relationships between fluorescence response at 475 nm and concentrations of SO_3^{2-}. λ_{ex}= 385 nm, λ_{em}= 475 nm, slits: 5.0 nm / 5.0 nm.

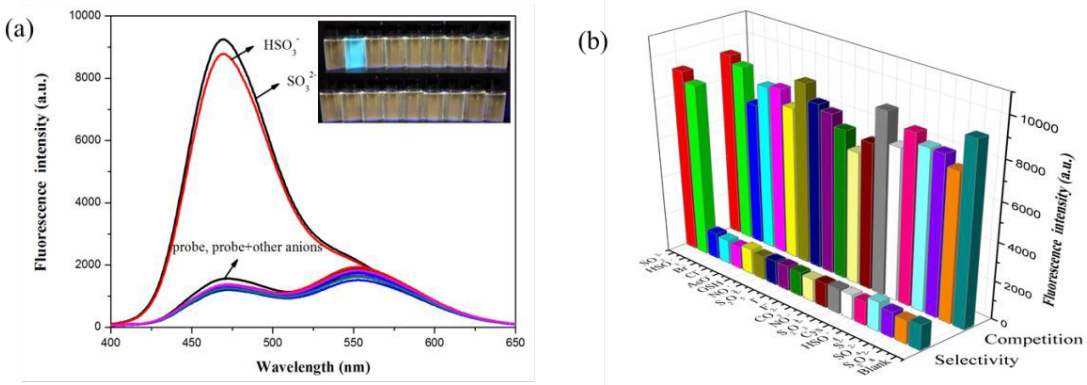

Fig. 3 (a) Fluorescence spectra of probe **DTCC** (10 μM) upon addition of 100 μM SO_3^{2-} and 19 kinds of other ions in a 20% CH_3CN solution (10 mM Hepes buffer, pH 7.4). (b) The pillars in the front row represent fluorescence response of the probe to the varying ions of interest. The pillars in the back row represent the subsequent addition 100 μM SO_3^{2-} to the solution containing probe and the various anions 100 μM, respectively. The detection medium was in a 20% CH_3CN solution (10 mM Hepes buffer, pH 7.4). λ_{ex}= 385 nm, λ_{em}= 475 nm, slits: 5.0 nm / 5.0 nm.

concentration of SO_3^{2-}. The detection limit of **DTCC** for SO_3^{2-} was calculated to be 0.23 μM according to signal to noise ratio (*S/N* = 3). These results illustrated that the probe **DTCC** possesses excellent capability for quantitative determination of SO_3^{2-} with high sensitivity in 20% CH_3CN aqueous media.

3.4. Selectivity experiment

Various biologically relevant species were investigated including ROS, RSS, reactive nitrogen species (RNS), anions and cations for evaluating the selectivity of probe **DTCC** toward SO_3^{2-}. As shown in **Fig. 3a**, the fluorescence intensity ration of the probe **DTCC** significantly increased after the addition of SO_3^{2-}. On the contrary, no obvious change of the fluorescence signal was detected upon addition of other analytes even in considerable concentrations.

Then the anti-interference ability of **DTCC** to other analytes has been examined as shown in **Fig. 3b**, all the competing analytes did not interfere the detection of SO_3^{2-}, these results demonstrated that probe **DTCC** has excellent selectivity for SO_3^{2-} over a variety of interfering species that could be present in cells and TCM sample, which enable it to detect SO_3^{2-} in complex biological system and other sophisticated background sample.

3.5. Influences of time and pH-dependent of probe DTCC

The time-dependent fluorescence changes of probe **DTCC** toward SO_3^{2-} were investigated using kinetic study. As displayed in **Fig. 4a**,

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the fluorescence intensity at 475 nm increased dramatically and reached a plateau within 10 s. In addition, the fluorescence intensity of probe **DTCC** almost kept unchanged after the reaction. The time-dependent absorption changes were in consistent with the time-dependent fluorescence changes (**Fig. S1**). The results illustrate that probe **DTCC** could fast monitor SO_3^{2-} change in biological environment and TCM sample.

For SO_3^{2-} detection, pH change was an important factor in various environmental. The different pH range of the free probe **DTCC** and probe **DTCC** with SO_3^{2-} was studied in 20% CH_3CN mixed solution with addition of buffers prepared in the relevant pH range from 3.0 to 11.0, respectively (**Fig. 4b**). The fluorescence intensity of free probe **DTCC** could not undergo any notable changes in the pH range from 3.0 to 11.0. Probe **DTCC** itself was stable in the pH range of 3.0 to 11.0. After addition of SO_3^{2-} to the **DTCC** solution, fluorescence intensity increased significantly in the pH range from 4.0 to 10.0, these results indicated that probe **DTCC** had a wide range of tolerance to pH change in sophisticated sample.

3.6. Proposed recognition mechanism and theoretical computation.

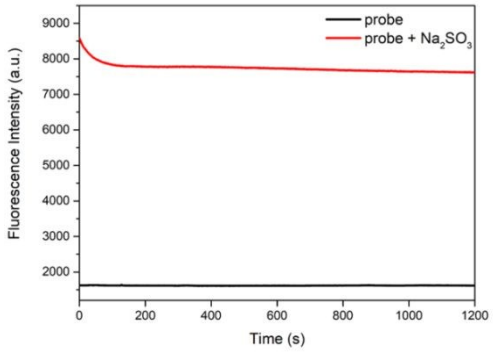
The proposed mechanism for the detection of SO_3^{2-} was interpreted in **Scheme 3**. The probe itself has a conjugated system that showed weak fluorescence activity, ICT effect was open. When the probe coexists with SO_3^{2-} that aldehyde-hydrogen sulphite adduct

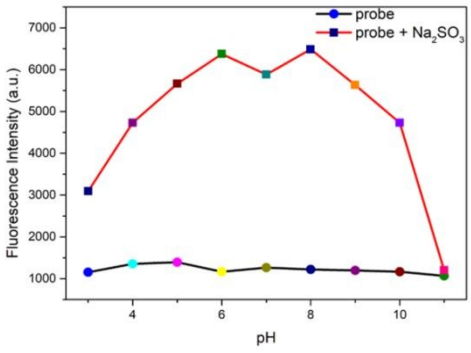
Fig. 4 (a) Time-dependent fluorescence intensity of free probe (10 μM) and probe (10 μM) with SO_3^{2-}(100 μM) in Hepes buffer (10 mM, pH 7.4, containing 20% CH_3CN, 25℃) solution. (b) pH-dependent changes of fluorescence intensity of probe (10 μM) in the absence and presence of SO_3^{2-} (100 μM) in Hepes buffer (10 mM, pH 7.4, containing 20% CH_3CN, 25℃) solution. Different pH values were adjusted by NaOH and HCl aqueous solution. λ_{ex} = 385 nm, λ_{em}= 475 nm, slit=5.0nm.

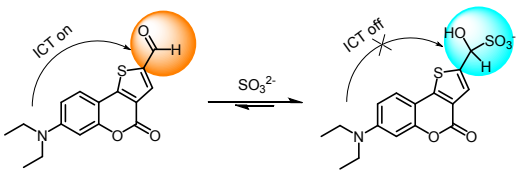
fluorescence was remarkably enhanced by the nucleophilic addition reaction of sulfhydryl toward the α, β-unsaturated aldehyde in probe **DTCC**. To prove the above proposed sensing mechanism, we initially attempted to isolate the aldehyde-hydrogen sulphite adduct from the mixture of probe **DTCC** with sodium sulphite. Unfortunately, it was observed that the intermediate is unstable in aqueous solution, and TLC analysis shows that it has been recovered to its starting material gradually, indicating this addition reaction could be reversible.[42]

In order to verify our hypothesis, the reaction of **DTCC** （100 μM ）and SO_3^{2-} （150 μM）was performed at room temperature for 3 min in MeOH solution, then the reaction solution was diluted appropriately and subjected to mass spectroscopy, where an expected peak at m/z 382.0417, corresponding to aldehyde-hydrogen sulphite adduct ($C_{16}H_{16}NO_6S_2^-$, Exact Mass: $[M]^-$ 382.0425) was observed (**Fig. S8**).

To further investigate the optical property upon addition of SO_3^{2-} in probe **DTCC** solution, DFT calculations were performed using







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Scheme 3 Proposed mechanism of the reaction of **DTCC** with SO_3^{2-}.

B3LYP/6-311+G(d) level of Gaussian 09 program. The optimized structures of **DTCC** and the adduct **DTCC**-SO_3^{2-} were shown in **Fig. S9**. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) in **DTCC** were shown in **Fig. 5**. Obviously, there was no ICT process occurred in the adduct **DTCC**-SO_3^{2-}, in that a sp^3 hybridized carbon atom existed in quaternary carbon moiety of thiophene ring. The interruption of the ICT process also resulted in the increased HOMO-LUMO energy gap of the adduct **DTCC**-SO_3^{2-} (3.60 eV) in comparison with that of the **DTCC** (3.12 eV), which brought about a blue-shift in the emission spectra observed. Therefore, these DFT calculations verified that SO_3^{2-} was bound to the aldehyde group of probe **DTCC** and are in good agreement with the experimental results.

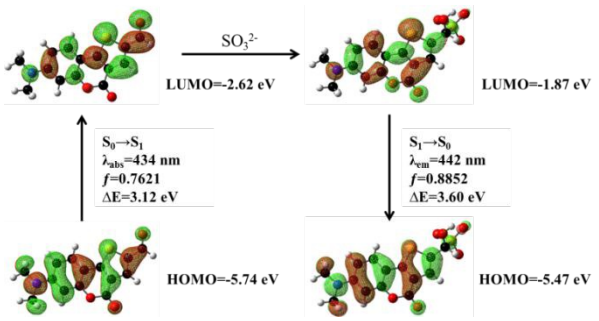

Fig. 5 HOMO-LUMO energy levels and frontier molecular orbital of probe **DTCC** and the adduct **DTCC**-SO_3^{2-}.

3.7. Cytotoxicity and fluorescence imaging for living cells

CCK-8 was used to evaluate cytotoxicity of probe **DTCC** before intracellular fluorescence imaging for living cells. As shown in **Fig. S10**, after 24 h of incubation with 5 μM probe, more than 95% of the SH-SY5Y neuroblastoma cells remained viable. In addition, the cell viability was as high as 85% when 20 μM probes were incubated for 24 h. These data indicated that probe **DTCC** exhibits low cytotoxicity and good biocompatibility at concentrations of 0 ~ 20 μM in SH-SY5Y neuroblastoma cells.

With these preliminary data in hand, the potential application of the probe **DTCC** for imaging SO_3^{2-} in living cells was explored using a laser confocal microscope. When the SH-SY5Y cells were incubated with probe **DTCC** (10 μM) for 5 min, no fluorescence was displayed at the blue channel (λ_{ex}= 405 nm). On the contrary, certainly strong fluorescence intensity was clearly observed in the cytoplasm after adding SO_3^{2-} (5 μM) and then incubating for another 5 min at 37 ℃, indicating that probe **DTCC** could penetrate the cell membranes and react with exogenous SO_3^{2-} in the cellular environment at extremely low concentrations. As shown in **Fig. 6**, the fluorescence intensity of the cells was gradually increased when the concentration of probe **DTCC** changed from 5 μM to 20 μM. At the same time, the fluorescence intensity of the cells was basically stable and barely any fluorescence quenching was observed until 30 min. The above results proved that probe **DTCC** has the ability to visualize and quantitatively detect SO_3^{2-} in SH-SY5Y neuroblastoma cells.

3.8. Practical application of probe DTCC in Traditional Chinese Medicine

There was an excellent linear relationship between the fluorescence intensity and the concentration of SO_3^{2-} from 0 to 100 μM. In order to further explore the practical application of probe **DTCC** in realistic sample, positive sample was collected and detected the concentration of SO_3^{2-} in Traditional Chinese Medicine according to the excellent properties of probe **DTCC**. The fluorescent titration spectra of **DTCC** with different concentrations of SO_3^{2-} showed an increase at 475 nm in real TCM sample. The detection results illustrate that probe **DTCC** was able to detect the

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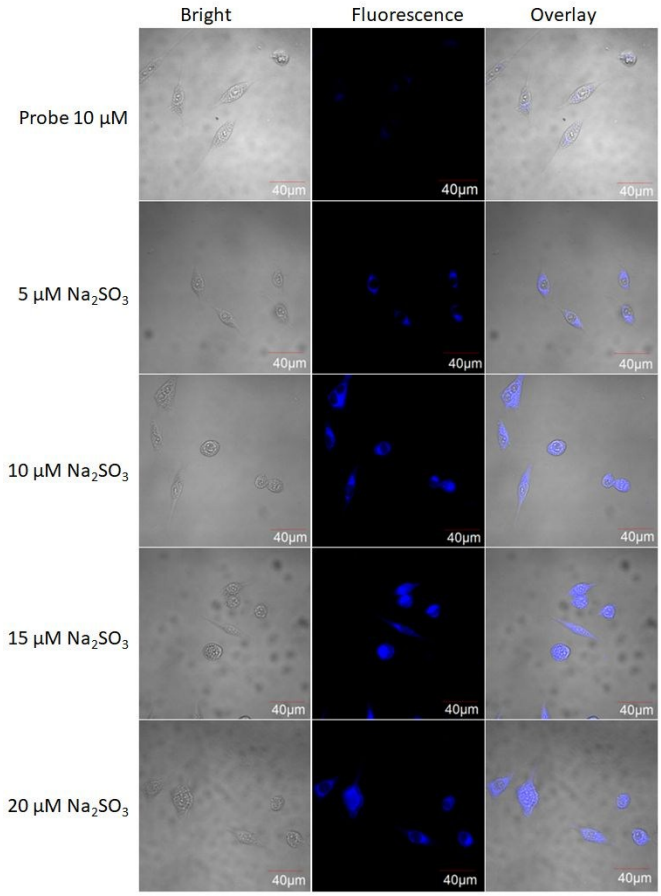

Fig. 6 Confocal fluorescence microscopic images of SH-SY5Y cells incubated by probe **DTCC** (10 μM) and observed under bright field (a), blue channel (b), overlay (c), then further incubation with Na_2SO_3 (5、10、15、20 μM) for 5 min at 37℃ and observed under bright field, blue channel, overlay.

Table 1 Determination of sulfite in negative TCM sample.

Sample	Added (μM)	Found (μM)	Recovery (%)
Dioscotea opposite Thunb	0	1.01	-
	40.00	40.70	101.75
	80.00	82.86	103.58
Angelica sinensis (Oliv.) Diels	0	1.05	-
	40.00	41.56	103.9
	80.00	84.02	105.02
Lycium barbarum L.	0	1.10	-
	40.00	38.14	95.35
	80.00	77.89	97.36

Table 2 Comparison of analysis results for sulfite in TCM sample by fluorescent probe DTCC with a traditional method. (n=5)

Sample	Batch No.	Pharmacopoeia method (mg/kg)	Fluorescence method (mg/kg)
Dioscotea opposite Thunb	201701001[a]	386	361
	170301	361	372
	201701001[b]	390	363
Angelica sinensis (Oliv.) Diels	161001	5	7
	170301	5	4
	160801	3	6
Lycium barbarum L.	20150416	51	59
	17042601	23	21
	170403	36	34

a, b: The two samples with the same batch No. come from the different regions.

concentration of SO_3^{2-} in Traditional Chinese Medicine with good recovery (**Table 1**) and excellent consistent with the traditional experimental values according to the working curve, respectively (**Table 2**). More importantly, the fluorescent probe **DTCC** methods for quantitative analysis of SO_3^{2-} in TCM involve short analysis time, simple sample preparations and easy operation.

4. Conclusions

In summary, a novel colorimetric fluorescent probe was successfully designed and synthesized based on coumarin scaffold for highly selective and sensitive detection of SO_3^{2-} with fast response time, low limit of detection and wide pH range in applications. Moreover, the probe could be applied to sense exogenous imaging of SO_3^{2-} in SH-SY5Y neuroblastoma cells with negligible cytotoxicity, excellent cell membrane permeability and good biocompatibility. Additionally, the probe could be acted as an effective tool for the detection of SO_3^{2-} in Traditional Chinese Medicine and other sophisticated sample. Furthermore, we envision that such a novel probe **DTCC** could be applied to further reveal essential information about SO_3^{2-} in extensive biological systems and in real samples.

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Conflicts of interest

There are no conflicts to declare.

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References

1 A. Acharya, I. Das, D. Chandhok and T. Saha, *Oxid. Med. Cell. Longev.*, 2010, **3**, 23–34.

2 D. Liu, Y. Huang, D. Bu, A.-D. Liu, L. Holmberg and Y. Jia, *Cell Death Dis.*, 2014, **5**, e1251.

3 Y. F. Zong, Y. Q. Huang, S. Y. Chen, M. Z. Zhu, C. S. Tang and J. B. Du, *Oxid. Med. Cell. Longev.*, 2015, **2015**, 1–11.

4 E. Culotta, D. E. Koshland, *Science*, 1992, **258**, 1862–1865.

5 L. J. Ignarro, *Angew. Chem. Int. Ed.*, 1999, **38**, 1882–1892.

6 M. Bilban, A. Haschemi, B. Wegiel, B. Y. Chin, O. Wagner and L. E. Otterbein, *J. Mol. Med.*, 2008, **86**, 267–279.

7 C. A. Piantadosi, *Free Radic. Biol. Med.*, 2008, **45**, 562–569.

8 T. Hohn, *Proc. Natl. Acad. Sci USA*, 2007, **104**, 17905–17906.

9 C. Winkler, B. Frick, K. Schroecksnadel, H. Schennach and D. Fuchs, *Food Chem. Toxicol.*, 2006, **44**, 2003–2007.

10 T. Finkel, N. J. Holbrook, *Nature*, 2000, **408**, 239–247.

11 M. H. Stipanuk, J. E. Dominy, J. I. Lee and R. M. Coloso, *J Nutr.*, 2006, **136**, 1652S–1659S.

12 W. Beeson, D. Abbey and S. Knutsen, *Environ. Health Perspect.*, 1998, **106**, 813–823.

13 W. Lee, K. Teschke, T. Kauppinen, A. Andersen, P. Jappinen, and I. Szadkowskastanczyk, *Environ. Health Perspect.*, 2002, **110**, 991–995.

14 J. J. Liu, X. Liu, S. L. Li, B. C. Cai and H Cai, *Chin. Tradit. Herb Drugs*, 2010, **41**, 1403–1406.

15 X. Jiang, L.-F. Huang, S.-H. Zheng and S.-L. Chen, *Phytomedicine*, 2013, **20**, 97–105.

16 (a) European Pharmacopoeia 8.0 (2014). Vol. 1. Strasbourg: The Directorate for the Quality of Medicines & HealthCare of the Council of Europe. (b) Pharmacopoeia of People's Republic of China (2010). Vol. 1. Beijing: China Medical Science Press. (c) United States Pharmacopoeia 38 (2014). Rockville: United States Pharmacopeial Convention Inc.

17 P. West and G. Gaeke, *Anal. Chem.*, 1956, **28**, 1816–1819.

18 Y. Zhao, W. Qiu, C.-Y. Yang and J.-N. Wang, *Energy Fuels*, 2017, **31**, 693–698.

19 Z. Zhong, G. Li, B. Zhu, Z. Luo, L. Huang and X. Wu, *Food Chem.*, 2012, **131**, 1044–1050.

20 U. T. Yilmaz and G. Somer, *Anal. Chim. Acta.*, 2007, 603, 30–35.

21 M. H. Pournaghi-Azar, M. Hydarpour and H. Dastangoo, *Anal. Chim. Acta.*, 2003, **497**, 133–141.

22 B. Palenzuela, B. Simonet, A. Ríos and M. Valcarcel, *Anal. Chim. Acta.*, 2005, **535**, 65–72.

23 J. J. Sullivan, T. A. Hollingworth, M. M. Wekell, R. T. Newton and J. E. Larose, *J. Assoc. Off. Anal. Chem.*, 1986, **69**, 542–546.

24 S. S. Hassan, M. S. Hamza and A. H. Mohamed, *Anal. Chim. Acta.*, 2006, **570**, 232–239.

25 (a) L. Yuan, W. Lin, K. Zheng and S. Zhu, *Acc. Chem. Res.*, 2013, **46**, 1462–1473. (b) P. A. Gale and C. Caltagirone, *Chem. Soc. Rev.*, 2015, **44**, 4212–4227. (c) D. H. Qu, Q. C. Wang, Q. W. Zhang, X. Ma and H. Tian, *Chem. Rev.*, 2015, **115**, 7543–7588. (d) Y. Tang, D. Lee, J. Wang, G. Li, J. Yu and W. Lin, *Chem. Soc. Rev.*, 2015, **44**, 5003–5015. (e) T. Ueno and T. Nagano, *Nat. Methods*, 2011, **8**, 642–645.

26 X. Gu, C. Liu, Y.-C. Zhu and Y.-Z. Zhu, *J. Agric. Food Chem.*, 2011, **59**, 11935–11939.

27 (a) H. Agarwalla, S. Pal, A. Paul, Y. W. Jun, J. Bae and K. H. Ahn, *J. Mater. Chem. B*, 2016, **4**, 7888–7894. (b) C. Yin, X. Li, Y. Yue, J. Chao, Y. Zhang and F. Huo, *Sensor. Actuat. B-Chem.*, 2017, **246**, 615–622.

28 (a) Y.-Q. Sun, J. Liu, J. Zhang, T. Yang and W. Guo, *Chem. Commun.*, 2013, **49**, 2637–2639. (b) H. Tian, J. Qian, Q. Sun, H. Bai and Zhang W. *Anal. Chim. Acta.*, 2013, **788**, 165–170. (c) J. Xu, J. Pan, X. Jiang, C. Qin, L. Zeng and H. Zhang, *Biosens. Bioelectron.*, 2016, **77**, 725–732.

29 (a) H.-Y. Zhang, S.-H. Xue and G.-Q. Feng. *Sensor. Actuat. B-*

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Chem., 2016, **231**, 752–758. (b) H. Paritala and K. S. Carroll, *Anal. Biochem.*, 2013, **440**, 32–39.

30 (a) Y.-M. Sun, C. Zhong, R. Gong, H. Mu and E. Fu, *J. Org. Chem.*, 2009, **74**, 7943–7946. (b) C. Wang, S. Feng, L. Wu, S. Yan, C. Zhong and P. Guo, *Sensor. Actuat. B-Chem.*, 2014, **190**, 792–799.

31 O. Rusin, N. N. St. Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang and I. M. Warner, *J. Am. Chem. Soc.*, 2004, **126**, 438–439.

32 K. N. Venugopala, V. Rashmi and B. Odhav, *BioMed. Res. Int.*, 2013, **2013**, 963248–963262.

33 N. J. Greco and Y. Tor, *Tetrahedron*, 2007, **63**, 3515–3527.

34 S. C. Rasmussen, S. J. Evenson and C. B. Mccausland, *Chem. Commun.*, 2015, **51**, 4528–4543.

35 (a) Y. Y. Ning, J. H. Cui, X. Q. Wang, C. N. Xiao, S. P. Wu, J. L. Li and Y. M. Zhang, *Sensor. Actuat. B-Chem.*, 2018, **269**, 322–330. (b) Y. Y. Ning, X. Q. Wang, L. L. Yang, C. N. Xiao, S. P. Wu, J. L. Li and Y. M. Zhang, *New J. Chem.*, 2018, **42**, 14510–14516.

36 C. Herbivo, A. Comel, G. Kirsch, M. Manuela and M. Raposo, *Tetrahedron*, 2009, **65**, 2079–2086.

37 M. Cossi, V. Barone, R. Cammi and J. Tomasi, *Chem. Phys. Lett.*, 1996, **255**, 327–335.

38 S. Miertuš, E. Scrocco, and J. Tomasi, *Chem. Phys.*, 1981, **55**, 117–129.

39 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. V. Cheeseman, B. Barone, G. A. Mennucci, H. Petersson, M. Nakatsuji, X. Caricato, H.-P. Li, A. F. Hratchian, J. Izmaylov, G. Bloino, J.-L. Zheng, M. Sonnenberg, M. Hada, K. Ehara, R. Toyota, J. Fukuda, M. Hasegawa, T. Ishida, Y. Nakajima, O. Honda, H. Kitao, T. Nakai, J. A. Vreven, J. J. Montgomery, E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, GAUSSIAN 09, Revision E.01 Gaussian, Inc, Wallingford, CT, 2009.

40 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, 37, 785–789.

41 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652.

42 A. D. McLean and G. S, *J. Chem. Phys.*, 1980, **72**, 5639–5648.

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Design and synthesis of a novel colorimetric fluorescent probe for selective detection of sulfur dioxide in SH-SY5Y neuroblastoma cells and its application in Traditional Chinese Medicines

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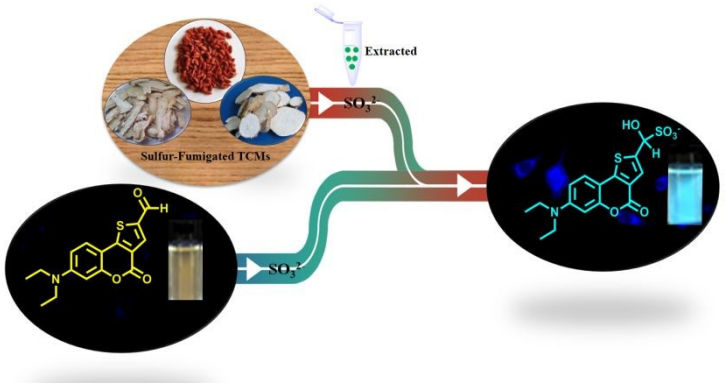

Keywords: Coumarin scaffold, Fluorescent probe, Sulfur dioxide, Fast responsive, Bioimaging, Traditional Chinese Medicines

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