Dyes and Pigments 190 (2021) 109289

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: http://www.elsevier.com/locate/dyepig

A red-to-near-infrared fluorescent probe for the detection of thiophenol based on a novel hydroxylflavone-quinoline-amino molecular system with large Stokes shift

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ARTICLE INFO

Keywords:
Quinoline
Hydroxylflavone
Fluorescence
Probe
Thiophenol

ABSTRACT

In this work, we synthesized a novel long-wavelength-emitting fluorophore **FQ-OH** based on a novel designed hydroxylflavone-quinoline-amino molecular system with both intramolecular charge transfer (ICT) and excited-state intramolecular proton transfer (ESIPT) process, enabling **FQ-OH** with strong fluorescence in a wide range of 550–800 nm, covering red-to-near-infrared emission region and large stokes shift as much as 162 nm. Due to the promising spectra property, **FQ-OH** was then fabricated into a red-to-near-infrared fluorescent probe **FQ-DNP** for the selective detection of thiophenol via aromatic nucleophilic substitution (S_NAr) reaction mechanism. Spectra assays in the solution demonstrated that **FQ-DNP** displayed prominent turn-on fluorescence response to thiophenol in 550–800 nm with emission peak at 627 nm, excellent selectivity, and exceptional sensitivity (detection limit as low as 7.2 nM). In addition, thiophenol in vapor form could be detected by **FQ-DNP** coated test papers enabling naked eye detection. Moreover, **FQ-DNP** was utilized for detecting thiophenol in environmental samples and showed great recovery results. Furthermore, biological application of **FQ-DNP** in living cells through cell imaging study demonstrated apparent intracellular fluorescence enhancement before/after thiophenol addition.

1. Introduction

Thiophenol (PhSH) is an indispensable organic compound which is extensively applied in the production of polymer materials, agrochemicals and pharmaceuticals [1,2]. However, thiophenol is highly toxic besides its noxious odor due to the autoxidation of thiophenol into aromatic disulphides which would generate harmful hydrogen peroxide and superoxide radicals [3]. The median lethal dose (LD) of thiophenol is 0.01–0.4 mM in fish and the US National Institute for Occupational Safety and Health (NIOSH) has an exposure limit of 0.1 ppm under 15 min [4]. Furthermore, glutathione could produce active oxygen species in the presence of disulphides, which may lead to the damage of erythrocyte cells and induce a series of diseases such as tachypnea, muscular weakness, nervous system damage and even death [5,6]. Therefore, there has been great interests in developing efficient methods to monitor thiophenol in the environmental and biological system.

Fluorescent probing technology has become a prevalent tool for the detection of a variety of analytes ascribe to its swift response, superior selectivity, low detection limit, and noninvasiveness compared to traditional methods [7–9]. Besides, fluorescent probe has the potential to be directly applied to investigate the biological process [10]. Up to now, a number of fluorescent probes toward thiophenol have been reported with good sensing performance [11–32,38]. Nonetheless, some of them suffer from sluggish response, some needed better sensitivity, some displayed short-wavelength-emission (<600 nm) and small stokes shift (<100 nm) (Table 1). Short-wavelength-emission probes are normally handicapped by shallow tissue penetration, undesirable photodamage to biological samples and fluorescence disturbance during bio-imaging process. Fluorophore with small stokes shift can be easily perturbed by self-absorption and auto-fluorescence, which may vastly

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https://doi.org/10.1016/j.dyepig.2021.109289
Received 6 January 2021; Received in revised form 24 February 2021; Accepted 28 February 2021
Available online 9 March 2021

Table 1
Comparison of **FQ-DNP** with other thiophenol fluorescent probes.

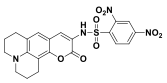
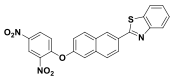
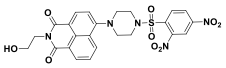
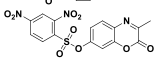
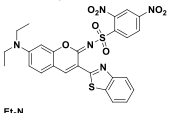
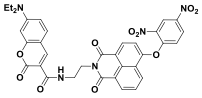
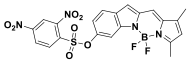
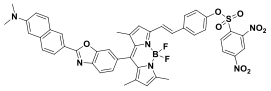
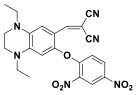
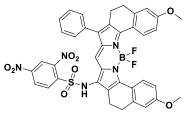
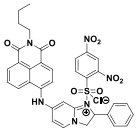
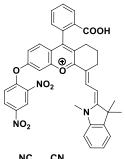
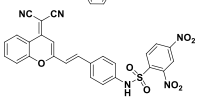
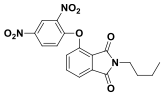
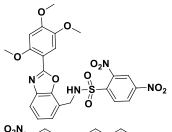
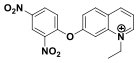
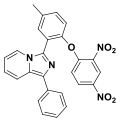
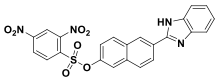
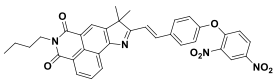
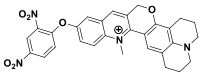
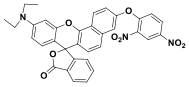
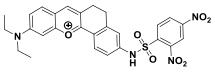
Probe structure	λ_{ex}/λ_{em} (nm)	Stokes shift (nm)	Detection limit (nM)	Ref
	380/535	155	4.5	[11]
	402/505	103	9.6	[12]
	380/517	137	10.3	[13]
	354/538	184	24	[14]
	478/524	46	9	[15]
	425/554	129	120	[16]
	600/629	29	950	[17]
	570/586	16	4.9	[18]
	477/606	129	8.2	[19]
	608/633	25	37	[20]
	481/590	109	20	[21]
	680/706	26	34	[22]
	490/670	180	150	[23]
	355/516	161	3.5	[24]
	335/403	68	200	[25]
	398/498	100	8	[26]
	305/478	173	5.6	[27]
	330/460	130	6	[28]

Table 1 (*continued*)

Probe structure	λ_{ex}/λ_{em} (nm)	Stokes shift (nm)	Detection limit (nM)	Ref
	450/590	140	36	[29]
	502/610	108	8.1	[30]
	595/653	58	15	[31]
	570/626	56	363	[32]
FQ-DNP	**465/627**	**162**	**7.2**	**this work**

affect the sensitivity and reliability. Presently, only a few thiophenol probes with both long-wavelength emission and large stokes shift [19,23,30] are reported, as a result, exploring excellent probe in this direction is still a significant challenge.

3-hydroxylflavone is being widely used to design fluorescent probes due to its outstanding excited-state intramolecular proton transfer (ESIPT) process. ESIPT process is favorable for extending fluorescence spectra to longer region, as well as large stokes shift by means of structure conversion from *enol* to *keto* form under excitation [33,34]. To our best knowledge, very few 3-hydroxylflavone-derived fluorophore with long emission (emission peak >600 nm) is reported. In addition, D-π-A molecular system, composed of electron-drawing moiety (A), π-conjugation and electron-donating moiety (D), is an attractive skeleton for constructing dyes with long emission spectra due to the characteristic intramolecular charge transfer (ICT) process [35].

In this work, we creatively extended the commonly used 3-hydroxylflavone fluorophore by introducing an amino-substituted quinoline skeleton, forming a novel and unreported hydroxyflavone-quinoline-amino molecular system **FQ-OH** (chart 1). **FQ-OH** is expected to have ESIPT property due to the existence of 3-hydroxylflavone moiety. Meanwhile, **FQ-OH** is a typical D-π-A molecule with strong ICT process from N, *N*-diethyl amino group to 3-hydroxylflavone moiety, with quinoline as π-conjugation part. Therefore, **FQ-OH** should own both ESIPT and ICT process enabling the fluorescence spectra to be expected in the long-emission region. To further confirm this assumption, UV–vis

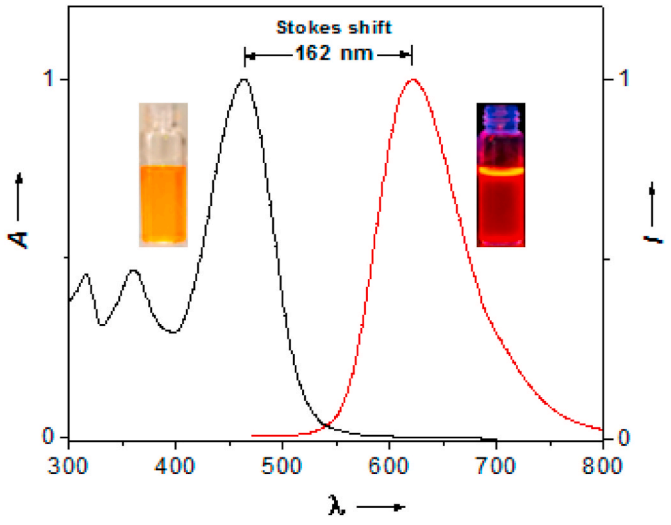


Fig. 1. Normalized UV-absorption (black line) and fluorescence (red line) spectra of **FQ-OH** in the pH 7.2 solution (DMF/PBS v/v 1:3).

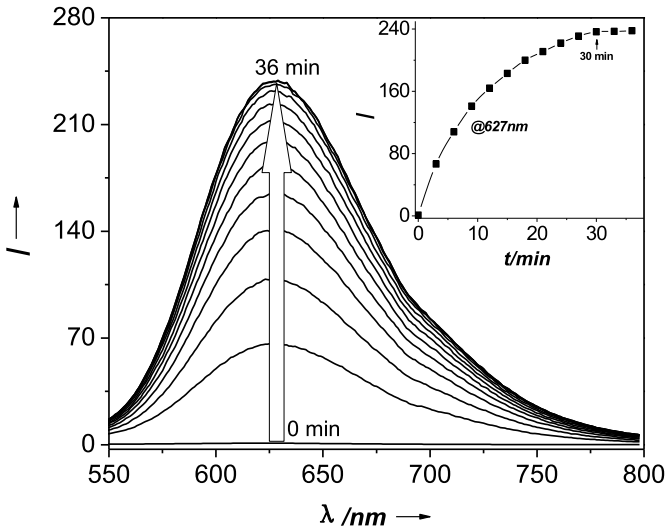


Fig. 2. Fluorescence response of **FQ-DNP** (8 μM) toward 10.0 equiv of thiophenol in the pH 7.2 solution (DMF/PBS v/v 1:3) from 0 to 36 min under excitation wavelength at 465 nm, inset: fluorescence intensity at 627 nm as a function of time.

absorption and fluorescence spectra of **FQ-OH** in the aqueous solution were performed and shown in Fig. 1. The results are in line with expectations, **FQ-OH** shows strong fluorescence output ($\Phi = 0.18$) in the range of 550–800 nm involving red to near-infrared emission with maximum (λ_{em}) at 627 nm and strong absorption below 550 nm with absorption maximum (λ_{ex}) at 465 nm, the stokes shift (λ_{em}-λ_{ex}) is approximately 162 nm. Following these exciting spectroscopic features, a red-to-near-infrared fluorescent probe **FQ-DNP** on the base of **FQ-OH** was constructed for the selective detection of thiophenol via aromatic nucleophilic substitution (S_NAr) reaction mechanism. **FQ-DNP** displayed prominent turn-on fluorescence response to thiophenol in 550–800 nm with excellent selectivity and exceptional sensitivity. Furthermore, detection of thiophenol in vapor form, environmental samples and living cells was demonstrated.

2. Experimental section

2.1. Materials and methods

Unless otherwise stated, chemicals for the synthesis of target compounds were bought from commercial suppliers and used without further purification. Solvents of technical quality for the preparation of sample solutions were distilled prior to use. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra of **FQ-OH** and **FQ-DNP** in $CDCl_3$ were measured with a Varian INOVA-400MHz NMR spectrometer, with chemical shifts recorded in ppm based on tetramethylsilane (TMS) as the internal standard. Mass spectra was obtained with a Waters LCT Premier XE mass spectrometer and a Bruke AUTOFLEX SPEED mass spectrometer. Fluorescence emission and UV–vis absorption spectra were carried out using an Agilent Cary Eclipse Fluorescence Spectrometer and a PerkinElmer LAMBDA 365 UV/vis spectrometer, respectively.

2.2. Preparation of sample solutions

Sample solutions for spectral assays were prepared in DMF/buffer (v/v 1:3) binary solvent (0.1 M disodium hydrogen phosphate-0.1 M citric acid buffer for pH values 3–8, and 0.1 M $NaHCO_3$-0.1 M Na_2CO_3 buffer for pH 9 and 10). Water used was purified by a Millipore Elix Essential Water Purification System. All pH values were measured and made accurate on a Mettler Toledo FiveEasy Plus pH meter.

For spectral measurement, a stock solution of **FQ-DNP** (2 mM) in DMF was prepared in advance. Sample solutions of the probe with a concentration of 8 μM were achieved by adding 10 μL of the stock solution into 2.5 ml DMF/buffer (v/v 1:3) solvent in quartz cuvettes (1 cm × 1 cm) through micro syringes. After certain amount of thiophenol or other analytes solution (20 mM) in DMF was added, sensing behavior of **FQ-DNP** was recorded through fluorescence spectra.

2.3. Kinetic analysis

The sensing kinetic profile of **FQ-DNP** to thiophenol exhibits *pseudo-first-order* reaction conditions with the rate constant k_{obs} obtained based on the following equation:

$$\ln[(I - I_{max})/(I_0 - I_{max})] = -k_{obs}t$$

where I_0 is the fluorescence emission value at 627 nm of the solution before addition of thiophenol, I is that value at certain time after addition of thiophenol and I_{max} is that value at complete reaction of the solution. t is the sensing reaction time. Furthermore, a second-order rate constant k is obtained in terms of the equation, $k_{obs} = k$ [thiophenol], in which [thiophenol] is the concentration of thiophenol for each kinetic assay.

2.4. Cell culture and MTT assay

MDA-MB-231 cells were grown in Dulbecco's modified eagle

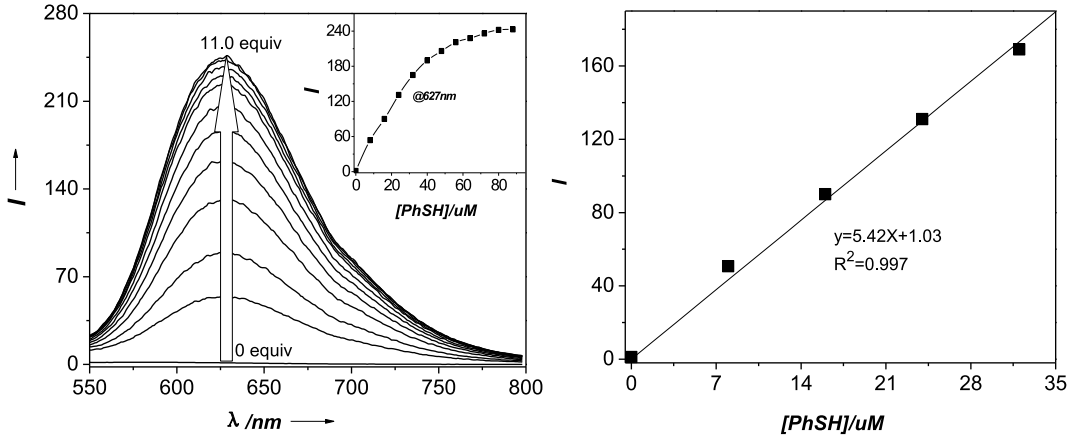


Fig. 3. Left: fluorescence response of **FQ-DNP** (8 μM) toward thiophenol of different concentrations from 0 to 11.0 equiv in the pH 7.2 solution (DMF/PBS v/v 1:3) under excitation wavelength at 465 nm, inset: emission intensity at 627 nm as a function of thiophenol concentration. Right: linear fitting of the intensity at 627 nm of **FQ-DNP** (8 μM) as a function of thiophenol concentration from 0 to 32 μM.

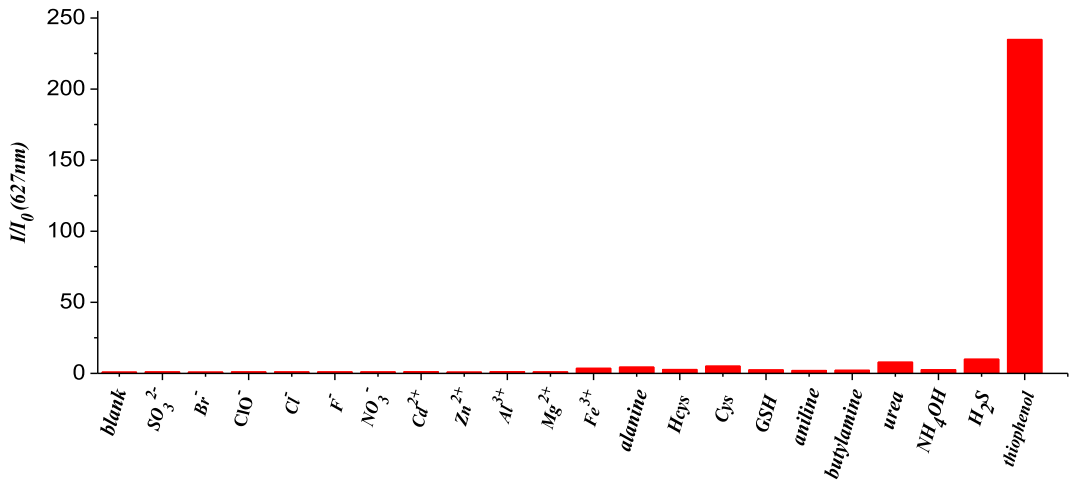


Fig. 4. I_{627nm} enhance-fold of **FQ-DNP** (8 μM) after treated with 10.0 equiv of different analytes for 30 min in the pH 7.2 solution, analytes include 1. blank, 2. SO_3^{2-}, 3. Br^-, 4. ClO^-, 5. Cl^-, 6. F^-, 7. NO_3^-, 8. Cd^{2+}, 9. Zn^{2+}, 10. Al^{2+}, 11. Mg^{2+}, 12. Fe^{3+}, 13. alanine, 14. Hcys, 15. Cys, 16. GSH, 17. aniline, 18. butylamine, 19. urea, 20. NH_4OH, 21. H_2S, 22. thiophenol.

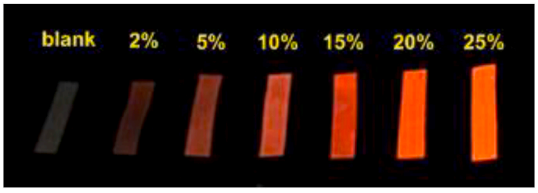


Fig. 5. Fluorescent photograph of **FQ-DNP**-coated filter papers after exposed to gaseous thiophenol vapored from thiophenol solution in THF with various concentrations.

medium at 37 °C, supplemented with 10% fetal calf serum under humidified air with 5% CO_2. The operation of MTT assay was described in detail in our previous work [36].

2.5. Fluorescence imaging in living cells

A laser scanning confocal microscope (Zeiss 710) was applied to perform the fluorescence imaging of MDA-MB-231 cells. The cells of one group were cultured with 16 μM **FQ-DNP** for 30 min, for another group, cells were incubated with 16 μM **FQ-DNP** for 30 min and then with 160 μM thiophenol for another 30 min. Fluorescence imaging was performed after the cells were washed with pH 7.4 PBS solutions three times. Intracellular fluorescence signal was collected from the red channel (600–800 nm) under excitation at 450 nm.

2.6. Synthesis and characterization

2.6.1. Synthesis of 6-(diethylamino)quinoline-2-carbaldehyde (QA)

Compound **QA** was synthesized according to the procedure in reference [37].

2.6.2. Synthesis of 2-(6-(diethylamino)quinolin-2-yl)-3-hydroxy-4H-chromen-4-one (FQ-OH)

The compound **QA** (0.20 g, 0.88 mmol), 2′-hydroxyacetophenone (0.12 g, 0.88 mmol) and NaOH (88 mg, 2.2 mmol) were added to a solution of CH_3OH (8 ml) and then stirred under reflux for 6 h. After

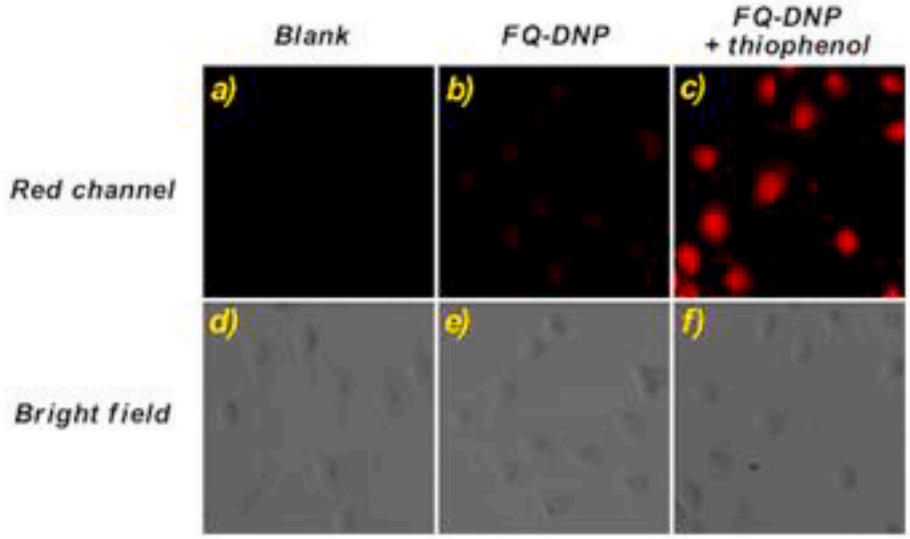


Fig. 6. Confocal fluorescence image of MDA-MB-231 cells under excitation at 450 nm with emission signal recorded from red channel (600–700 nm); (a) fluorescence image of the blank cells. (b) Fluorescence image of the cells treated with 10 μM **FQ-DNP** for 30 min. (c) Fluorescence image of the cells incubated with 10 μM **FQ-DNP** for 30 min and then with 100 μM thiophenol for 30 min (d, e and f) image of bright field.

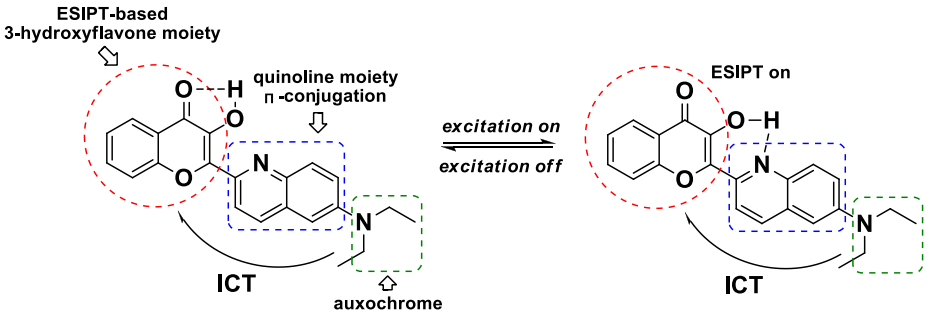


Chart 1. Designing strategy of the long-wavelength-emitting fluorophore **FQ-OH**.

that, stirring was continued at room temperature for another 12 h 5 ml NaOH solution (0.5 N) and 0.5 ml H_2O_2 solution (30%) were added to the mixture and stirred for 6 h. After reaction, the mixture was poured into 80 ml ice-water and acidified with acetic acid to pH around 5. The solid precipitated out was filtered. Purification via column chromatography (silica gel-H, CH_2Cl_2/ethyl acetate 3:1) gave **FQ-OH** (0.10 g, 30.7%) as orange-red solid. ^{1}H NMR (400 MHz, $DCCl_3$, 25 °C, TMS): δ = 8.34–8.32 (d, 1H, J = 8.0 Hz, ArH), 8.11–8.02 (m, 2H, ArH), 7.85–7.83 (d, 1H, J = 8.0 Hz, ArH), 7.68–7.64 (m, 1H, ArH), 7.55–7.53 (d, 1H, J = 8.0 Hz, ArH), 7.38–7.31 (m, 2H, ArH), 6.75 (s, 1H, ArH), 3.52–3.47 (m, 4H, CH_2), 1.28–1.25 ppm (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 173.6, 154.5, 135.2, 133.1, 128.8, 126.1, 124.0, 123.4, 120.0, 117.8, 117.2, 103.2, 44.7, 12.6 ppm; FTMS(ESI): $C_{22}H_{20}N_2O_3$+H^+, m/z: found 361.1428.

2.6.3. Synthesis of the probe *FQ-DNP*

To a solution of **FQ-OH** (0.10 g, 0.28 mmol) in DMF (5 ml) was added 2, 4-dinitro-chlorobenzene (84 mg, 0.42 mmol) and K_2CO_3 (78 mg, 0.56 mmol). The reaction mixture was stirred for 48 h at room temperature. After reaction completed, 80 ml CH_2Cl_2 was added and then washed with water (60 ml, pH 7.4), dried with anhydrous Mg_2SO_4, condensed and purified via column chromatography (silica gel-H, CH_2Cl_2/ethyl acetate 3:1) to afford **FQ-DNP** (52 mg, 35.3%) as orange-red solid. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.94 (d, 1H, ArH), 8.34–8.32 (d, 1H, J = 8.0 Hz, ArH), 8.19–8.17 (d, 1H, J = 8.0 Hz, ArH), 8.13–8.11 (d, 1H, J = 8.0 Hz, ArH), 8.04–8.02 (d, 1H, J = 8.0 Hz, ArH), 7.94–7.93 (d, 2H, ArH), 7.63–7.56 (m, 2H, ArH), 7.37–7.35 (d, 1H, J = 8.0 Hz, ArH), 7.26–7.24 (d, 1H, J = 8.0 Hz, ArH), 6.87 (s, 1H, ArH), 3.46–3.44 (m, 4H, CH_2), 1.13–1.10 ppm (m, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS): δ = 172.5, 156.3, 155.8, 155.0, 147.6, 142.5, 141.3, 140.8, 138.4, 135.3, 134.5, 134.2, 130.9, 129.4, 126.2, 125.6, 124.0, 122.0, 120.4, 119.3, 118.3, 102.8, 44.4, 12.9 ppm; FTMS (ESI): $C_{28}H_{22}N_4O_7$+H^+, m/z: found 527.1690.

3. Results and discussion

3.1. Synthesis of the probe *FQ-DNP*

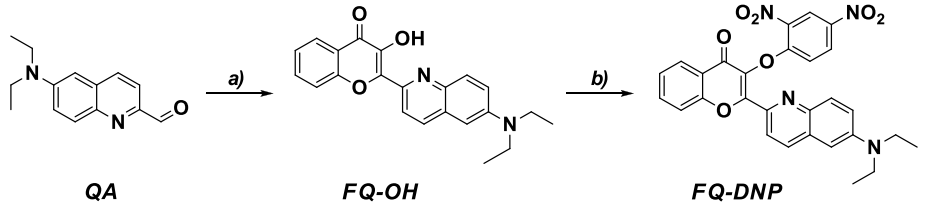
The probe **FQ-DNP** was synthesized via a simple two-step synthesis route (Scheme 1) with 6-(diethylamino)quinoline-2-carbaldehyde (**QA**) as starting material, which was obtained according to our previous work [37]. Reaction of **QA** with 2′-hydroxyacetophenone in the presence of NaOH and the oxidant H_2O_2 yielded the fluorophore **FQ-OH**. Further reaction of **FQ-OH** with 2, 4-dinitro-chlorobenzene in the presence of K_2CO_3 in DMF solution rendered the target probe **FQ-DNP**. Molecular structure of **FQ-OH** and **FQ-DNP** was confirmed by NMR and mass spectra.

3.2. Sensing mechanism and spectral response of the probe to thiophenol

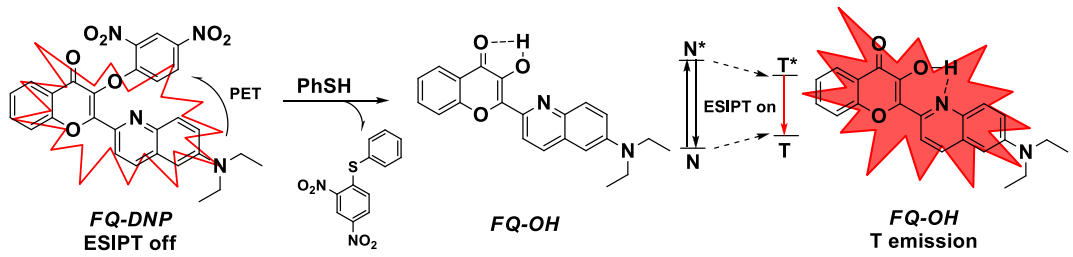
FQ-DNP in the pH 7.2 solution was nearly non-fluorescent due to the photo-induced electron transfer (PET) behavior from amino group to 2, 4-dinitro-chlorobenzene moiety. After incubated with thiophenol over 10 min, strong red fluorescence appeared. This obvious fluorescence color change could be directly observed by naked eyes via a hand-held UV lamp with excitation at 365 nm, indicating that sensing reaction of **FQ-DNP** with thiophenol occurs. It is considered that 2, 4-dinitro-chlorobenzene moiety in **FQ-DNP** was attacked by nucleophilic thiophenol via aromatic nucleophilic substitution (S_NAr) mechanism, same as the reported probes (Table 1). During this process, phenyl ether bond in **FQ-DNP** was cleaved and structure of the fluorophore **FQ-OH** was recovered, along with its fluorescence property (Scheme 2).

The reaction of **FQ-DNP** with thiophenol was further traced by mass spectra and shown in Fig. S1. The peak signal at 361.100 and 383.107 were assigned to the released fluorophore **FQ-DNP** with the formula [$C_{22}H_{20}N_2O_3$+H^+] and [$C_{22}H_{20}N_2O_3$+Na^+], respectively. Signal at 277.892 was ascribed to the formula of S_NAr side product, [$C_{12}H_8N_2O_4S$ + H^+]. These results confirm the S_NAr mechanism for sensing reaction of **FQ-DNP** toward thiophenol.

Fluorescence spectral response of **FQ-DNP** toward thiophenol was performed to further analyze the sensing behavior in detail. As shown in Fig. 2, the aqueous **FQ-DNP** solution (DMF/PBS v/v 1:3, pH 7.2) was nearly non-fluorescent (Φ = 0.00089), after treated with 10.0 equiv of thiophenol, fluorescence emission intensity in a large range of 550–800 nm (centered at 627 nm) gradually increased under excitation at 465 nm and achieved the equilibrium within 30 min. The results indicated that **FQ-DNP** displayed *turn-on* fluorescence response to thiophenol. Fluorescence intensity at 627 nm (I_{627nm}) enhanced from initial 1.03 to 238.75, about 234-fold increasement in 30 min, providing a high signal-to-noise ratio for thiophenol detection. Accordingly, **FQ-DNP** could detect thiophenol with large spectra response, long-wavelength-emission and large stokes shift.



Scheme 1. Synthesis of the fluorophore **FQ-OH** and the probe **FQ-DNP**. (a) (i) **QA**, 2′-hydroxyacetophenone, NaOH, CH_3OH, reflux, 6 h, rt, 12 h; (ii) 0.5 N NaOH, 30% H_2O_2, rt, 6 h; (b) 2,4-dinitro-chlorobenzene, DMF, K_2CO_3, rt, 48 h.



Scheme 2. Proposed sensing mechanism of **FQ-DNP** to thiophenol.

Furthermore, kinetic profiles of the response were analyzed in the presence of thiophenol with different concentrations in terms of fluorescence spectra (Fig. S2), which revealed *pseudo-first-order* conditions. Several *pseudo-first-order* rate constants k_{obs} were obtained and linear-fit against thiophenol concentration to give the second-order rate constant, with the value of 12.2 $M^{-1}s^{-1}$.

3.3. Sensitivity of *FQ-DNP* to thiophenol

Fluorescence response of **FQ-DNP** solution treated with thiophenol of various concentrations was measured to study the sensitivity of the probe. As shown in Fig. 3, fluorescence emission in the range of 550–800 nm gradually increased with increasing thiophenol concentration under excitation at 465 nm. Fluorescence intensity at 627 nm (I_{627nm}) exhibited an excellent linear relationship with thiophenol concentration from 0 to 4.0 equiv, suggesting **FQ-DNP** can afford quantitative determination of thiophenol. The detection limit was determined, with the value as low as 7.2 nM based on the general 3σ/k method.

We further compared **FQ-DNP** with other reported thiophenol fluorescent probes in detail (Table 1), by comparison, **FQ-DNP** in this work displayed a few outstanding advantages involving: (1) novel molecular structure. (2) long-wavelength fluorescence response covering both red and near-infrared emission region. (3) large stokes shift over 160 nm. (4) very low detection limit. These promising qualities enable **FQ-DNP** with more desirable application values.

3.4. Selectivity of *FQ-DNP* to thiophenol

To evaluate the selectivity of **FQ-DNP** toward thiophenol, fluorescence response in the presence of other possible interfering analytes such as anions (SO_3^{2-}, Br^-, ClO^-, Cl– F^-, NO_3^-), metal cations (Cd^{2+}, Zn^{2+}, Al^{3+}, Mg^{2+}, Fe^{3+}) and a series of nucleophiles (alanine, Cys, Hcys, GSH, aniline, butylamine, urea, NH_4OH, H_2S) was investigated. The results show that no obvious spectra change for these analytes except thiophenol (Fig. 4, Fig. S3). Accordingly, it can be concluded that **FQ-DNP** has good selectivity to thiophenol.

3.5. pH effect on the response of *FQ-DNP* to thiophenol

Spectral response of **FQ-DNP** solutions with different pH from 3.0 to 10.0 was performed and shown in Fig. S4. In the acidic region of pH < 5.0, there is nearly no response. With increasing solution pH value to alkaline region, spectra response became more and more obvious. This pH effect is attributed to the nucleophilicity alternation of thiophenol in different pH solutions because high pH condition is favorable for the dissociation of neutral thiophenol to its anion form (PhS^-), which possesses much stronger nucleophilicity. However, with further increasing pH above 8.0, the response sharply descended. This is due to the hydroxyl group of the sensing product **FQ-OH** dissociates to form the anion, inhibiting ESIPT process resulting the weakened fluorescence. Overall, **FQ-DNP** can perform decently to detect thiophenol under physiological conditions, suggesting the potential application in biological samples.

3.6. Detection of gaseous thiophenol with probe-coated test paper

A piece of filter paper was cut into strips and then immersed into the solution of **FQ-DNP** (8 μM) in DMF/PBS (v/v 1:1, pH 7.2) for 5 min. The probe-coated paper strips were then taken out and exposed to thiophenol vapor by being hung in sealed bottles containing different concentrations of thiophenol (blank, 2%, 5%, 10%, 15%, 20%, 25%) in THF for 30 min. As shown in Fig. 5, with increasing thiophenol concentration, fluorescence intensity on test paper strips gradually enhanced when excited with a hand-held UV lamp. These results indicate that **FQ-DNP**-loaded filter papers could be applied for the detection of thiophenol vapor.

3.7. Detection of thiophenol in environmental samples

The probe **FQ-DNP** was also investigated for detecting thiophenol in environmental samples. Two types of water samples were selected, which were obtained from tap water and Wenshan Lake of Shenzhen University, respectively. As shown in Table 2, there was no thiophenol detected in the two blank samples. When the water samples were treated with standard thiophenol solution and then analyzed with **FQ-DNP**, the recovery was determined three times according to the standard addition method and in the range of 96–107%. The results suggest that **FQ-DNP** could be potentially applied for the detection of thiophenol in real-world samples.

3.8. Fluorescence imaging

3.8.1. Cell toxicity

MDA-MB-231 cells were utilized to evaluate cytotoxicity of **FQ-DNP** by MTT experiment. The probes with concentrations from 0 μM to 30 μM were injected into the living cells and then incubated for 24 h. The resulted cell viability is desired, ranging from 95% to 85% as the probe concentration increased, implying **FQ-DNP** has low toxicity towards the cultured cells (Fig. S5).

3.8.2. Fluorescence imaging of cells

Biological application of **FQ-DNP** was demonstrated by detecting thiophenol in MDA-MB-231 cells (Fig. 6). Cells in one group were incubated with 10 μM **FQ-DNP** for 30 min and then washed with PBS

Table 2
Detection of thiophenol in environmental samples.

Samples	Spiked (μM)	Detected (μM)	Recovery (%)	RSD (%)
Tap water	0	Not detected	–	–
	8.0	8.21	102.6	2.4
	16.0	16.79	104.9	2.2
	32.0	31.02	96.9	4.7
Wenshan Lake of Shenzhen University	0	Not detected	–	–
	8.0	8.54	106.7	2.8
	16.0	17.11	106.9	4.2
	32.0	33.27	104.0	3.8

solution, confocal microscope exhibited nearly no emission signal in the red channel for the intracellular probe. In contrast, cells in another group were incubated with **FQ-DNP** for 30 min first, washed with culture solution and then treated with 100 μM thiophenol for another 30 min. It could be observed that the living cells emitted bright red fluorescence, indicating the fluorophore **FQ-OH** was formed following sensing reaction of **FQ-DNP** with thiophenol. Additionally, under the same experimental conditions, no red fluorescence for cells in the control group (treated without thiophenol) was observed. The cell assays demonstrate that **FQ-DNP** has good cell-permeation and thiophenol-sensing in the living cells could be achieved.

4. Conclusions

In summary, a novel long-wavelength-emitting fluorophore **FQ-OH** based on the uniquely designed hydroxylflavone-quinoline-amino molecular system was obtained in this study. **FQ-OH** has a wide emission range of 550–800 nm, covering both red and near-infrared region and large stokes shift as much as 162 nm due to the ESIPT and ICT process. **FQ-OH** was fabricated into a red-to-near-infrared fluorescent probe **FQ-DNP** for the selective detection of thiophenol via S_NAr mechanism. **FQ-DNP** displayed remarkable turn-on fluorescence response to thiophenol with emission peak at 627 nm, excellent selectivity and exceptional sensitivity (detection limit as low as 7.2 nM). The probe-coated test papers were made and applied for detecting thiophenol vapor with good fluorescence response. Furthermore, **FQ-DNP** was utilized for detecting thiophenol in environmental samples and the results well matched with the standard data. Finally, biological application of **FQ-DNP** in living cells was demonstrated by cell imaging study in which obvious intracellular fluorescence enhancement was observed before/after thiophenol addition.

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.

Acknowledgment

This work was supported by National Science Foundation of China (51603126) and Fundamental Research Funds for the Central Universities of China (2682019CX08).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2021.109289.

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