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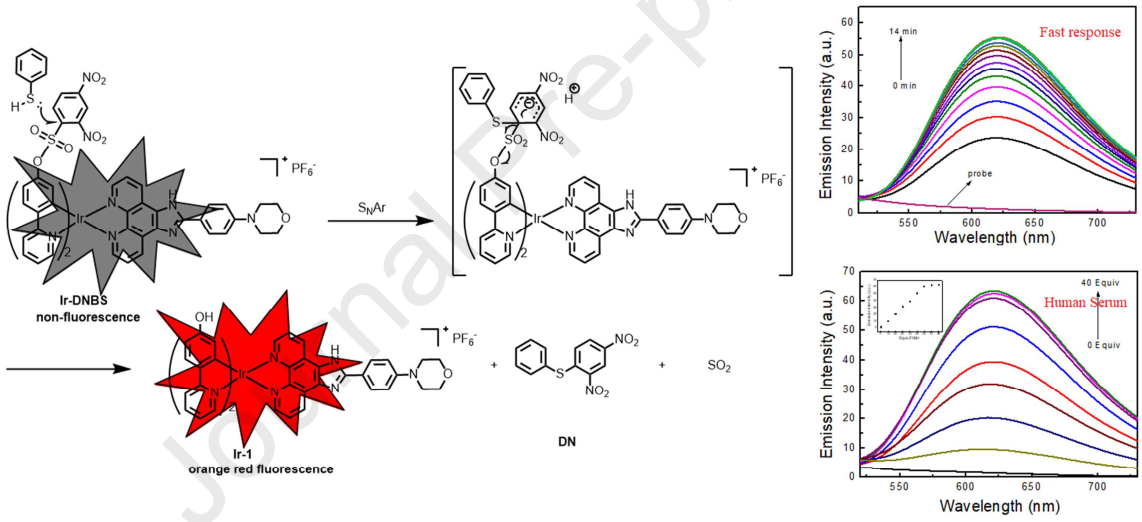

A cycometalated iridium(III) complex-based probe **Ir-DNBS** for fast detection of thiophenol in DMSO/PBS solution, water samples and human serum.

■ Title Page

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Abstract: A new iridium(□) complex-based near-infrared 'turn-on' fluorescent probe **Ir-DNBS** was synthesized for fast (9 min), highly selective and sensitive (LOD: 62.5 nM) thiophenol detection. Photophysical and spectral characterization (NMR, HRMS) results demonstrated that the recognization mechanism of probe for thiophenol was based on thiolate-mediated nucleophilic elimination reaction (S_NAr). Upon addition with thiophenol to PBS/DCM/DMSO solution (pH = 7.4) of probe **Ir-DNBS**, obvious orange red fluorescence with maximum emission wavelength at 620 nm was observed with a large Stokes shift (200 nm) and long lifetime (135 ns). The practical utility of probe **Ir-DNBS** was also investigated by thiophenol detection in real water samples and human serum.

Keywords: Iridium complex, Thiophenol, Near-infrared, Real water samples.

1. Introduction

Thiophenol and its derivatives, as common intermediate in organic synthesis, are widely used in the preparation of pesticides, pharmaceuticals, and polymers.[1-3] Nevertheless, according to the United States Environmental Protection Agency (USEPA waste code: P014),[4] thiophenol has been defined as a major pollutant due to its high toxicity. For instance, the median lethal concentration of thiophenol in fish range from 0.01–0.4 mM,[5] and the range of median lethal dose in mouse is as low as 2.15–46.2 mg kg^{-1}.[6] Moreover, thiophenol in water and soil also can be probably absorbed by human being through various biological and physicochemical pathways, further lead to a series of health problems including the central nervous system injury, muscular weakness, headache, nausea, vomiting, and even death.[7,8] Therefore, it is very necessary to develop an efficient and sensitive method to detect thiophenol in organisms or environment.

In the past few decades, a variety of detection methods have been reported, such as optical analysis, high performance liquid chromatography, gas chromatography, nonlinear spectroscopy and nanophase material sensor.[9-13] Fluorescence sensors, based on changes of fluorescent signal before and after detection, have become the most popular detection approach due to its simple operation, high sensitivity, cheap cost, low detection limit.[14] At present, although many fluorescent probes for thiophenol have been reported, most of them based on organic small molecule,[15-31] and possess some shortcomings like singlet emission and emitting via a short-lived fluorescence modality. In contrast, probes based on transition-metal complexes in the second- and third-row of the d-block typically display triplet emission and exhibit long-lived phosphorescence, as well as high luminescence

quantum efficiency and large Stokes shift.[32]

Zhang and co-workers designed and synthesized a series of Ru(II) complex-based phosphorescent probes to detect thiophenols for the first time.[33] These probes showed large Stokes shift and long luminescence lifetime, due to the triplet metal-to-ligand charge-transfer transitions (^{3}MLCTs) excited state. But long response time (30 min) was their obvious disadvantage. Transition metal complex-based probe for thiophenol have frequently emerged since then.[34-39]

We noted that ruthenium complexes usually only possess ^{3}MLCT excited state, but iridium complexes exhibit multiple excited states, such as ^{3}MLCT, ^{3}LLCT, and ^{3}IL excited states, which lead to their excellent photophysical and photochemical properties with long lifetimes. So the octahedral structure of iridium(□) complexes have attracted more interest as fluorescence probe. [40-43] Thus we designed and synthesized a novel iridium(□) complex-based 'turn-on' fluorescent probe **Ir-DNBS** with two 2,4-dinitrobenzenesulfonate(DNBS) moieties as recognization sites to detect thiophenol and its derivatives. Herein, two electron withdrawing group DNBS in the two C^N ancillary ligands (phenylpyridine) could completely quench the fluorescence of iridium(□) complex-based probe **Ir-DNBS** via internal charge transfer (ICT) mechanism.[44,45] Upon addition of thiophenol to **Ir-DNBS** in PBS/DCM/DMSO solution (pH = 7.4), the two recognition units (DNBS) could be cut off by nucleophilic elimination reaction (S_NAr) to form strong fluorescent complex **Ir-1** with obvious emission band centered at 620 nm. The results demonstrated that the probe **Ir-DNBS** could rapidly (response time: 9 min), sensitively (LOD: 62.5 nM) and selectively detect thiophenol without interference of

aliphatic thiols. Additionally, it also could quantitatively recognize thiophenol in actual water samples and efficiently detect thiophenol in human serum, implying that probe **Ir-DNBS** could be potentially applied in environment and clinic fields.

2. Experiment

2.1. Chemicals and apparatus

Unless otherwise specified, all chemicals used were purchased from commercial suppliers without purification. The human serum was provided by health volunteers. The solvents were dried in standard methods before use. All reactions were carried out under the dry argon atmosphere protection using the Schleck operation. The solution of Ir-DNBS (1 mM) was prepared in chromatographic grade CH_2Cl_2. Standard solution of various analytic samples, such as C_6H_5SH, *p*-Cl-C_6H_5SH, *p*-CH_3-C_6H_5SH, *p*-CH_3O-C_6H_5SH, *p*-NH_3-C_6H_5SH, $HOCH_2CH_2SH$, C_6H_5OH, $(CH_3)_3SH$ and $PhNH_2$, were dissolved in DMSO at concentrations of 10 mM. Solutions of glutathione (GSH), cysteine (Cys), homocysteine (Hcy), alanine (Ala) and glycine (Gly) were respectively prepared in distilled water (10 mM). NMR spectra were recorded in acetone-d_6 and DMSO-d_6 on a Bruker AV400 (400 MHz) spectrometer, the chemical shift (δ) was recorded in parts per million with tetramethylsilane (TMS) as reference. Absorption spectra were determined by an Agilent 8453 UV–vis spectrometer. Infrared spectra dates were analyzed on a Bruker Vertex-70 spectrometer as KBr pellets and were reported in cm^{-1}. HitachiF-4600 fluorescence spectrophotometer was used for measuring fluorescence spectra with excitation and emission slits of 5.0 nm and 5.0 nm. High - resolution mass spectra was performed on a solariX 70 FT-MS instrument. Luminescent decay experiments were completed on Edinburgh FLS980 spectrometer.

2.2. Detection of Thiophenol in real Water Samples and Human Serum

The water samples were obtained from Ganjiang River, Kong Mu Lake and the tap water in Nanchang city, further mixed with sodium phosphate buffer (10 mM, pH = 7.4). Water sample were spiked with different concentrations of thiophenol (50, 100, 150 μM) that had been accurately prepared. The mixtures were incubated for 9 min at room temperature, and the fluorescence was measured at 620 nm. The human serum was diluted (30 fold) with sodium phosphate buffer (10 mM, pH = 7.4) before use. The sensitivity of probe **Ir-DNBS** for thiophenol in human serum was investigated by fluorescence spectral titration.

2.3. Computational Method

The calculations were implemented by using Gaussian 09 program package[46] for complex **Ir-1**. The geometrical structure as isolated molecule in the ground state and the lowest-energy triplet state was firstly optimized, respectively, by the restricted and unrestricted density functional theory (DFT) method with the B3LYP functional.[47] During the optimization process, the convergent values of maximum force, root-mean-square (RMS) force, maximum displacement and RMS displacement were set by default. To analyze the absorption and emission transition properties, 80 singlet and 6 triplet excited-states were calculated, respectively, based on the optimized structures in the ground state and the lowest-energy triplet state to determine the vertical excitation energies by time-dependent density functional theory (TD-DFT)[48-50] with the same functional used in the optimization process. The self-consistent field (SCF) convergence criterions of RMS density matrix and maximum density matrix were set by default in the excited-state calculation. The iterations of excited states continue until the changes on energies of

states were no more than 10^{-7} a.u. between the iterations, and then convergences reached in all the excited states. In the calculation of the ground and excited states, the Solvation Model Based on Density (SMD)[51] method model with DCM/DMSO (v/v =5:1) as mixed solvent (eps = 15.246, epsinf = 2.025) was employed. In these calculations, the Stuttgart-Dresden (SDD)[52] basis set and the effective core potentials (ECPs) including quasi relativistic effect were used to describe the Ir atom, while other non-metal atoms of O, N, C and H were described by the all-electron basis set of 6-31G**.[53] Visualization of the frontier molecular orbitals were performed by GaussView. The contributions of fragments to the orbitals in the electronic excitation process were analyzed by the Ros & Schuit method[54] (C-squared population analysis method, SCPA) in Multiwfn 3.7 program.[55]

2.4. Synthesis

2.4.1. Synthesis of 4-(4-(1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)phenyl)morpholine

1,10-phenanthroline-5,6-dione (0.504 g, 2.4 mmol), ammonium acetate (1.542 g, 20 mmol) and 4-morpholinobenzaldehyde (0.383 g, 2 mmol) were dissolved in a mixture of 20 mL methanol, and stirred at 80 □ for 12 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane / methanol = 10:1) to get a yellow solid (1.2 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, J = 3.7 Hz, 2H), 8.91 (d, J = 8.0 Hz, 2H), 8.15 (d, J = 8.5 Hz, 2H), 7.82 (s, 2H), 7.14 (d, J = 8.6 Hz, 2H), 3.77 (t, 4H, –CH_2–), 3.25 (t, 4H, –CH_2–). ^{13}C NMR (400 MHz, DMSO-d_6) δ151.77, 151.18, 147.49, 129.44, 127.27, 123.70, 123.12, 120.29, 114.50, 65.98 (–CH_2–), 47.60 (–CH_2–).

*2.4.2. Synthesis of compound **1***

A mixture of 2-(4-methoxyphenyl)pyridine (0.92 g, 5 mmol) and iridium chloride hydrate (0.9 g, 5 mmol) in 2-ethoxyethanol (15 mL) and H_2O (5 mL) was stirred at 130 □ for 24 h, and then cooled to room temperature. A yellow precipitate was filtered, and washed with acetone, then dried in vacuo. The crude compound **1** (0.287 g, 40 %) was used for the next step without further purification and characterization.

*2.4.2. Synthesis of compound **2***

A crude compound **1** (0.659 g, 0.55 mmol) was dissolved in the anhydrous dichloromethane (40 mL) and stirred at -78 □ in a nitrogen atmosphere, then BBr_3 (1 mL, 1 M solution in CH_2Cl_2) was added drop wise at dark. The resulting reaction mixture was further stirred at room temperature for 30 h, then was quenched by methanol (10 mL). After cooling to room temperature, the mixture was diluted with water (500 mL) and hexane (20 mL), a green precipitate was collected by filtration, washed with H_2O and hexane, and dried in vacuo to get crude compound **2** (0.229 g, 80%), which was used for the next step without further purification.

*2.4.3. Synthesis of compound **Ir-1***

4-(4-(1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)phenyl)morpholine (0.076 g, 0.2 mmol) and compound **2** (0.119 g, 0.2 mmol) were dissolved in dichloromethane (30 mL) and methanol (30 mL). After being reflux at 55 □ for 24 h, the mixture was cooled to room temperature. Then NH_4PF_6 (0.082 g, 0.5mmol) was added, and stirred for another 2 h. After the solvent was evaporated, crude product was purified by silica gel column chromatography using dichloromethane-methanol (10:1, v/v) as eluent to obtain a yellow

solid (0.091 g, 50%). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.11 (d, 2H), 8.43 (s, 2H), 8.41 (s, 1H), 8.10 (s, 2H), 8.05 (d, *J* = 7.7 Hz, 3H), 7.81 (d, *J* = 8.3 Hz, 4H), 7.58 (s, 2H), 7.11 (d, *J* = 8.3 Hz, 2H), 6.85 (s, 2H, –OH), 6.60 (d, *J* = 8.5 Hz, 2H), 5.97 (s, 2H), 3.82 (t, 4H, $–CH_2–$), 3.30 (t, 4H, $–CH_2–$). ^{13}C NMR (400 MHz, Acetone-d_6) δ 168.08, 159.47, 153.78, 153.53, 153.11, 149.00, 138.12, 135.90, 132.28, 131.75, 130.11, 127.85, 126.83, 123.79, 121.69, 119.73, 118.71, 118.34, 114.79, 110.70, 110.21, 66.44($–CH_2–$), 48.04($–CH_2–$). HRMS: m/z 914.2035 $[M – PF_6^-]^+$. IR (KBr, *υ*, cm^{-1}): 3450 (–OH).

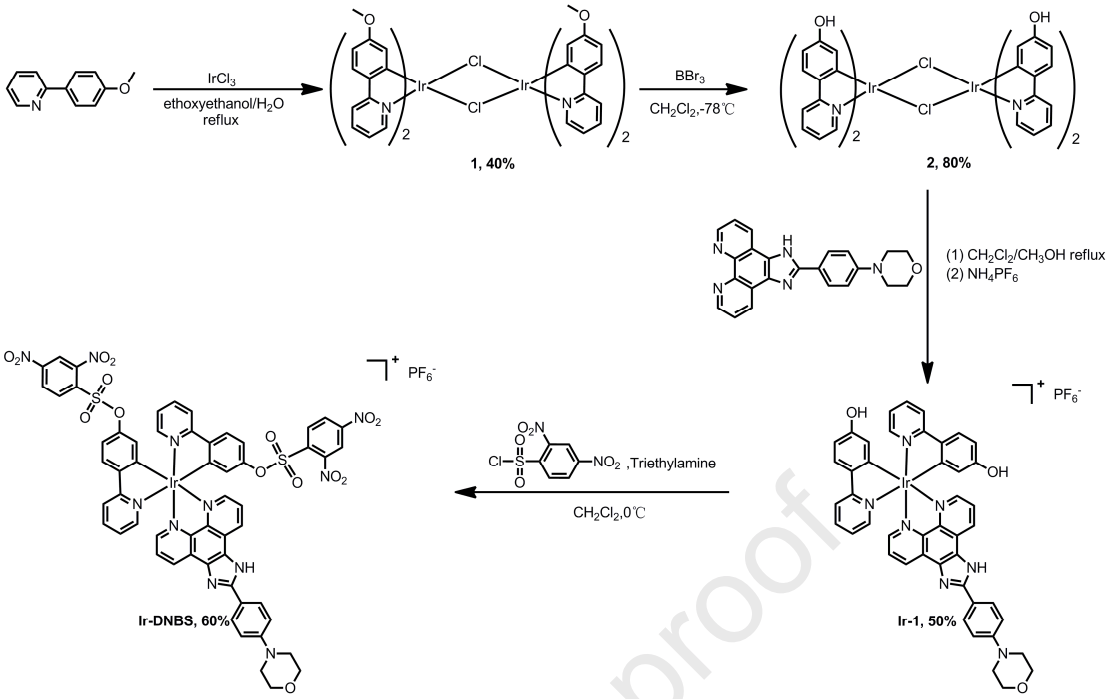
2.4.3. Synthesis of compound ***Ir-DNBS***

After dry dichloromethane (10 mL) of **Ir-1** (0.046 g, 0.05 mmol) was cooled to 0 □, a few of triethylamine (14 μL, 0.1 mmol) was added and stirred for 15 minute. Then 2,4-dinitrobenzene-1-sulfonyl chloride (0.04 g, 0.15 mmol) in 10 mL dry dichloromethane was dropwise added slowly to the mixture at room temperature. The solution was stirred for 6 hour until the reactants were depleted. Finally, the solvent was evaporated under pressure to give an orange red powder. **Ir-DNBS** was separated using a silica gel chromatographic column eluted with dichloromethane/methanol (v/v, 10:1) as eluent to obtain an orange solid (0.052 g, 60%). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.18 (s, 2H), 8.95 (s, 2H), 8.59 (d, *J* = 8.6 Hz, 2H), 8.21 (s, 4H), 8.14 (d, *J* = 8.6 Hz, 6H), 8.00 (d, *J* = 8.3 Hz, 4H), 7.57 (s, 2H), 7.10 (d, *J* = 8.5 Hz, 4H), 7.03 (s, 2H), 5.99 (s, 2H), 3.81 (t, 4H, $–CH_2–$), 3.30 (t, 4H, $–CH_2–$). ^{13}C NMR (400 MHz, Acetone-d_6) δ 169.06, 154.99, 153.43, 153.06, 152.14, 147.62, 142.77, 137.72, 135.30, 131.20, 130.11, 129.66, 127.88, 127.16, 124.01, 123.79, 120.00, 118.05, 69.70($–CH_2–$), 51.30 ($–CH_2–$). HRMS: m/z 1374.0738 $[M – PF_6^-]^+$. IR (KBr, *υ*, cm^{-1}): 1335 (−S=O), 1586 ($-NO_2$).

3. Results and discussion

3.1. Preparation and characterization

The synthetic route of probe **Ir-DNBS** was displayed in Scheme 1. The N^N ancillary ligand 4-(4-(1H-imidazo[4,5-f][1,10]phenanthrolin-2-yl)phenyl)morpholine was obtained by simple Debus-Radziszewski imidazole synthesis using 1,10-phenanthroline-5,6-dione to react with 4-morpholinobenzaldehyde in the presence of ammonium acetate.[56-58] Chloro-bridged dimer Ir(□) complex **1** was synthesized through reaction of 2-(4-methoxyphenyl)pyridine with anhydrous $IrCl_3$ without further purification. Then demethylation of aryl methyl ethers was conducted through adding boron tribromide (BBr_3) to obtain dimer complex **2**, which further reacted with N^N ancillary ligand in refluxing CH_2Cl_2/CH_3OH mixture solution and then exchanged the counter ion using NH_4PF_6 to achieve Ir(□) complex **Ir-1**. Finally, the probe **Ir-DNBS** was synthesized through the reaction between complex **Ir-1** and 3.0 equiv 2,4-dinitrobenzenesulfonyl chloride in the presence of Et_3N under 0 □. All compounds were characterized by NMR, IR and HRMS.



Scheme 1. Synthetic routes of probe **Ir-DNBS**

3.2. UV absorption and Emission spectra

The absorption and emission spectra of probe **Ir-DNBS** and complex **Ir-1** were shown in the Fig. 1. For UV-vis absorption spectra, both of two complexes (**Ir-DNBS** and **Ir-1)** exhibited two intense absorption bands in the UV region shorter than 300 nm, which were ascribed to be intra-ligand-centered $\pi \rightarrow \pi^*$ transitions. The slightly differences of them maybe be caused by two DNBS groups in probe **Ir-DNBS**. Metal to ligand charge transfer (MLCT) transitions from d(Ir) to π^*(C^N and N^N ligands) bands of two complexes occur centered at ca. 347 and ca. 420 nm. The similarity of absorption spectra between **Ir-DNBS** and **Ir-1** suggested that the cyclometalated **Ir-1** was not involved in recognition process. On the contrary, fluorescence spectra of probe **Ir-DNBS** and **Ir-1** in 60% DCM/DMSO (v/v =5:1) PBS buffer (pH = 7.4) were entirely different (Fig. 1b). When excited at 370 nm, **Ir-1** showed obvious fluorescence intensity at 620 nm with large Stokes shift (200 nm). Due to the influence of two electron-withdrawing 2,4-dinitrobenzenesulfonyl

(DNBS) moieties in C^N ligands, the emission of probe **Ir-DNBS** was completely quenched. Meanwhile, the fluorescence lifetime of **Ir-1** in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) was calculated to be 135 ns. (Fig. S6, see ESI).

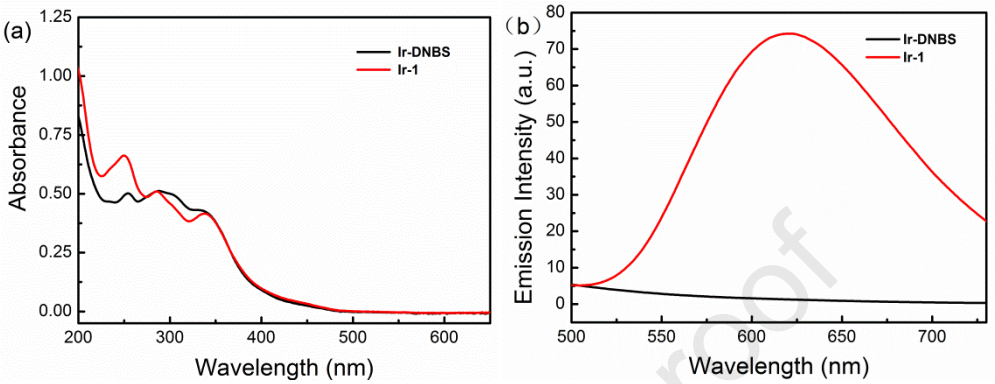


Fig.1 (A) Absorption spectra of probe **Ir-DNBS** (10 μM), complex **Ir-1** (10 μM) in 60% DCM/DMSO (v/v =5:1) at room temperature; (B) Fluorescence sprctra of probe **Ir-DNBS** (10 μM), complex **Ir-1** (10 μM) in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) at room temperature.

3.3. Fluorescence response of probe Ir-DNBS toward thiophenol

To investigate the detection properties of probe **Ir-DNBS** toward thiophenol in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) at room temperature, the fluorescence spectra of **Ir-DNBS** (10 μM) in the absence and presence of thiophenol were recorded firstly. The solution of probe **Ir-DNBS** (10 μM) displayed completely non-fluorescence upon excited 370 nm, then an obvious emission around 620 nm occur when 30 equiv thiophenol (300 μM) was added, accompanying with fluorescent color changed to orange red (Fig. 2a). It was also worth mentioning that the Stokes shift was as large as 200 nm, which could effectively diminish the measurement error caused by the excitation light and scattered light through the large gap between

excitation wavelength and emission wavelength. In addition, time response experiments of probe **Ir-DNBS** for thiophenol was also carried out to calculate the relative kinetic constants and half-time. As depicted in Fig 2b, the fluorescence intensity at 620 nm displayed significant increase within first 4 minutes, and then it increased slowly and finally reached a plateau at 9 min. The kinetics of probe **Ir-DNBS** with addition of thiophenol were observed by monitoring the fluorescence changes at 620 nm (Fig. S7, see ESI), and the kinetic constant (k') and half-time ($t_{1/2}$) were 0.39304 min^{-1} and 1.76 min, respectively. Thus, the above results implied that probe **Ir-DNBS** could detect thiophenol with rapid response and remarkable large Stokes shift.

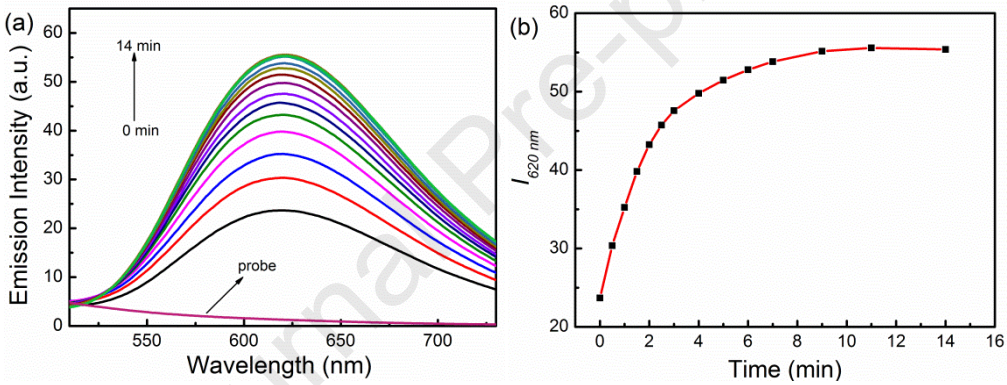


Fig. 2 (A) Changes in fluorescence of probe **Ir-DNBS** (10 μM) in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) induced by 300 μM thiophenol at different reaction time, excited at 370 nm (slit: 5/5); (60 μM); (B) Time response curves at 620 nm of probe **Ir-DNBS** (10 μM) in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) with thiophenol (300 μM).

The fluorescence spectral titration experiments of probe **Ir-DNBS** for thiophenol were carried out in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) to study the detection sensitivity and limit of detection (LOD). Upon gradual addition of thiophenol to solution

of probe **Ir-DNBS** (10 μM) to form complex **Ir-1**, a fluorescence band centered at 620 nm emerged and the intensity progressively increased (Fig. S8, see ESI). Finally, when 30 equiv of thiophenol was added, the emission intensity reached maximum with 35-fold enhancement. A good linear correlation between fluorescence intensity values at 620 nm and concentration of thiophenol in range of 0−150 μM was observed (Fig. 3b), illustrating probe **Ir-DNBS** could be used to quantitatively detect thiophenol. Moreover, the slope of this linear calibration graph was calculated to be 2.49 × 10^{-7} M (R = 0.9968), detection limit of probe **Ir-DNBS** for thiophenol was further determined to be as low as 62.5 nM based on the equation LOD = 3σ/S, where σ was the standard deviation of the blank measurements and S was the slop of emission intensity plotted against thiophenol concentration.[59,60]

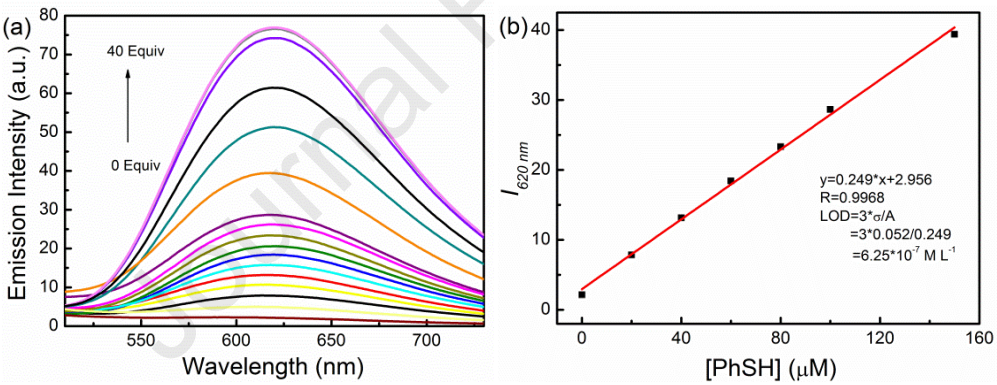


Fig. 3 (A) Changes in fluorescence of probe **Ir-DNBS** (10 μM) in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) induced by different concentrations of thiophenol (0-40 equiv), excited at 370 nm (slit: 5/5). Each spectrum was collected 14 minutes after addition of thiophenol; (B) Linear relationship between fluorescence intensity of probe **Ir-DNBS** (10 μM) at 620 nm and the concentration of thiophenol from 0 to 15 equiv.

3.4. Selectivity of probe Ir-DNBS toward thiophenol

Further to study the selectivity of probe **Ir-DNBS** for thiophenol, the fluorescence response of probe **Ir-DNBS** to different analytes, including thiophenol and its derivatives (*p*-CH_3-C_6H_4SH, *p*-Cl-C_6H_4SH, *p*-NH_2-C_6H_4SH and *p*-CH_3O-C_6H_4SH), aliphatic thiols ($HOCH_2CH_2SH$, $(CH_3)_3CSH$, cysteine (Cys), homocysteine (Hcy) and glutathione (GSH)), and other nucleophilic substances ($C_6H_5NH_2$, C_6H_5OH, glycine (Gly) and alanine (Ala)), was investigated under the same conditions.

As shown in Fig. 4, only the thiophenol and its derivatives could trigger the obviously fluorescent changes. Moreover, the specificity response of thiophenol in probe **Ir-DNBS** solution with co-existed other analytes could not be disturbed. The probe **Ir-DNBS** could discriminate aromatic thiols (thiophenol and its derivatives) from aliphatic thiols maybe attribute to their different pK_a in the neutral medium conditions. In neutral environments (pH = 7.3), thiophenols with lower pK_a (ca. 6.5) could be more efficient dissociated into thiolate species, which triggered S_NAr reaction to form fluorescent complex **Ir-1**, and display more nucleophilic property in comparison with aliphatic thiols (pK_a = ca.8.5) (Scheme 2). The above observations suggested that probe **Ir-DNBS** could be served as a highly selective probe for thiophenols and possess excellent specificity.

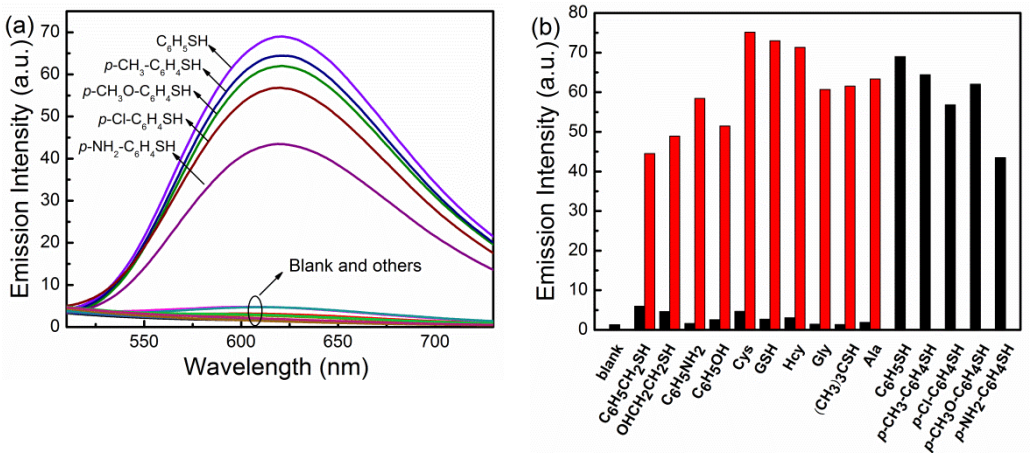
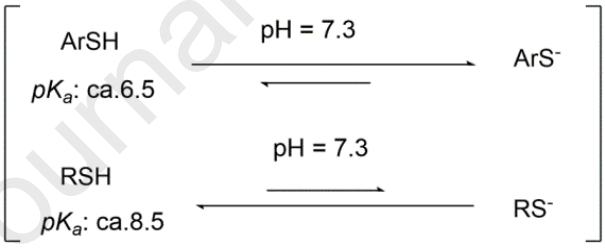


Fig. 4. (A) Emission spectral changes in fluorescence of probe **Ir-DNBS** (10 μM) induced by various analytes (30 equiv) in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1), excited at 370 nm (slit: 5/5). Each spectrum was collected 14 minutes after addition of analyte; (B) Competitive tests on fluorescent responses of probe **Ir-DNBS** at 620 nm to various analytes in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1). Black bars represent the addition of 30 equiv of various analytes to solution of probe **Ir-DNBS**. Red bars represent the addition of thiophenol (30 equiv) to the above solution.

Scheme 2 Dissociation degrees of aliphatic thiols and aromatic thiols under neutral conditions



3.5. Effect of PH

It had been reported that the detection mechanism of probe with DNBS group for thiophenol was based on nucleophilic elimination reaction (S_NAr).[61-64] Usually, the pH value would influence the generation of nucleophilic substances and their nucleophilic capability, further affected the fluorescence response of probe **Ir-DNBS** toward thiophenol. Hence, it was necessary to study the application of probe in the various pH values. As shown in Fig. 5, the fluorescence intensity of probe **Ir-DNBS** at 620 nm remained unchanged in the pH range of 1–14. The fluorescence

intensity at 620 nm of solution after upon the addition of thiophenol displayed a relevant with different pH values, in which emission intensity initially showed gradual increase as pH value ranged from 1.0 to 9.0, and further progressively decrease when pH value was greater than 9. Notably, the reaction between probe **Ir-DNBS** and thiophenol achieved the acceptable results with obvious fluorescence enhancements when pH was between 7.0 and 11.0, implying the probe **Ir-DNBS** could apply in a relatively wide pH range of environments and physiological conditions.[65]

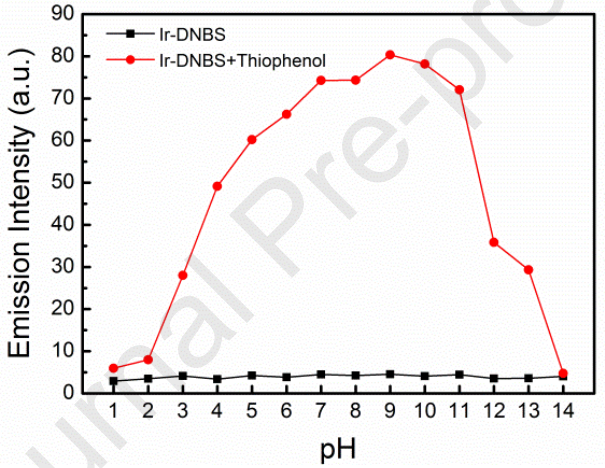


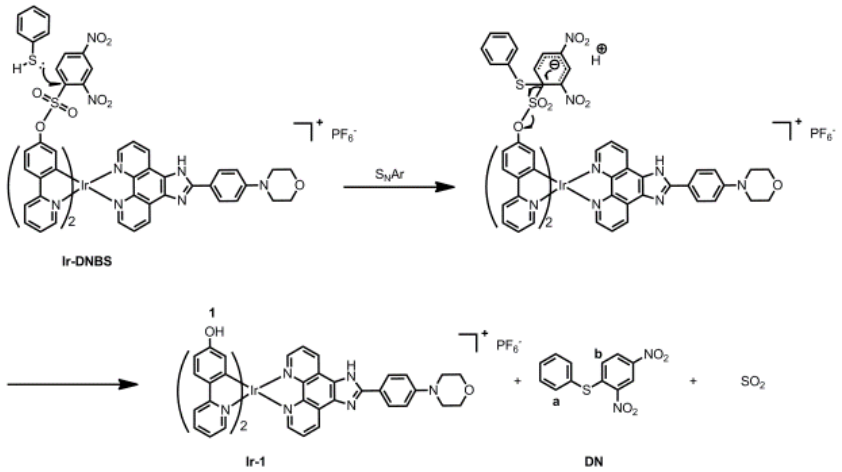
Fig. 5. Emission intensity of **Ir - DNBS** (10 μM) in the absence/presence of thiophenol in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1) at different pH values (1.0–14.0), excited at 370 nm (slit: 5/5)

3.6. Probing mechanism of Ir − DNBS toward thiophenols

In order to elucidate the sensing mechanism of probe **Ir-DNBS** toward thiophenol, ^{1}H NMR, HR-MS spectral analysis and Job′s plot analyses were carried out. As depicted in Fig. 6, with the addition of excess thiophenol, the chemical shift of probe **Ir-DNBS** in acetone-d_6 changed obviously. Proton signals of H_a at 7.57 ppm and H_b at 7.14 ppm which belonged to **DN** emerged,

as well as the characteristic proton signal of **Ir-1** at 6.85 ppm (corresponding to protons 1 in hydroxyl groups) appeared. As expected, both **Ir-1** and **DN** were obtained in the mixtures of **Ir-DNBS** with thiophenol, which indicated the nucleophilic aromatic substitution (S_NAr) occurred during the response of the probe to thiophenol. The results of high - resolution mass spectroscopy (HR-MS) characterization further validated the mechanism (Fig. S5, see ESI). For complexes **Ir-DNBS** and **Ir-1**, their m/z signals appeared on 1374.0738 ([**Ir-DNBS** – PF_6^-]$^+$) and 914.2035 ([**Ir-1** – PF_6^-]$^+$), respectively. When thiophenol was added to the probe **Ir-DNBS**, m/z signals of 914.2224 emerged, corresponding to the [**Ir-DNBS** + $2H^+$ – PF_6^- – 2DNBS]$^+$, implying that reaction product of probe **Ir-DNBS** with thiophenol was nearly identical to the exact molecular weight of [**Ir-1** – PF_6^-]$^+$.

Because probe **Ir-DNBS** possessed two recognition groups (DNBS), the Job′s plot plotting analyses of the reaction between Ir-DNBS and thiophenol was implemented in 10 mM PBS buffer (pH = 7.4) with 60% DCM/DMSO (v/v =5:1), in which the maximum fluorescence intensity occur at 0.67. The result indicated that the stoichiometry of reaction between **Ir-DNBS** and thiophenol was 1:2, and the two luminescence quenchers (DNBS) in the probe **Ir-DNBS** could be both removed through the S_NAr reaction induced by thiophenol. (Fig. S9, see ESI).



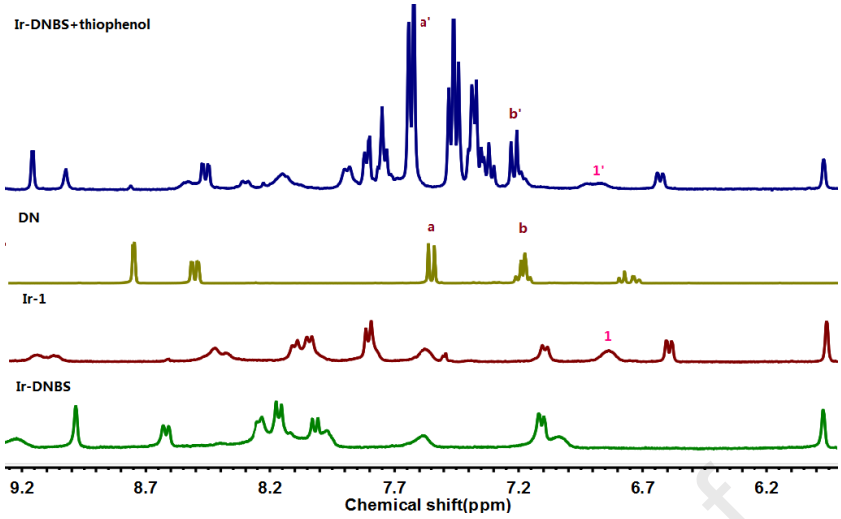


Fig. 6. Partial ^{1}H NMR spectra of probe **Ir-DNBS**, probe **Ir-DNBS** + thiophenol (30 equiv), complex **Ir-1** and **DN** in acetone- d_6

3.7. Detection of Thiophenol in Water Samples and Human Serum

Herein, detection behaviors of probe for thiophenols in water samples and human serum were investigated to study the potential application of probe. As shown in Fig. 7, the result of fluorescence spectral titration in human serum (30-fold dilution by PBS) was similar to that of in DMSO/DCM/PBS solvent. As the amounts of thiophenol increasing, the fluorescence intensity at 620 nm enhanced gradually and finally reached a plateau when 30 equiv of thiophenol was added. Meanwhile, changes in emission intensity at 620 nm displayed a good linear relationship with concentrations of thiophenol. LOD for thiophenol in 30-fold dilution human serum was calculated to be 0.435 μM. It proved the probe can be effectively used for detect thiophenol in human serum.[66-68]

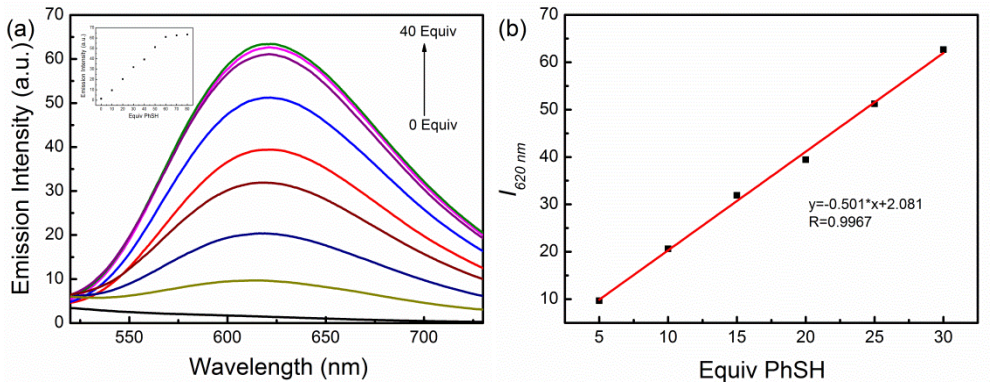


Fig. 7. (A) Changes in fluorescence of probe **Ir-DNBS** (10 μM) in human serum with 60% DCM/DMSO (v/v =5:1) induced by different concentrations of thiophenol (0-40 equiv), excited at 370 nm (slit: 5/5). Each spectrum was collected 14 minutes after addition of thiophenol; (B) Linear relationship between fluorescence intensity of probe **Ir-DNBS** (10 μM) at 620 nm and the concentration of thiophenol from 0 to 30 equiv.

To further verify that the practicability of probe **Ir-DNBS** in environmental science, the probe was used to quantitatively detect thiophenol concentrations in water samples from Ganjiang River, Kong Mu Lake and the tap water in Nanchang city, and the analytical results were listed in Table 1. Referring to the reported analytical methods, the water samples were filtered with 0.2 μm membrane to remove suspended matter and impurities firstly. Secondly, water samples were spiked with different concentrations of thiophenol (50, 100, 150 μM). Then emission intensity at 620 nm of probe **Ir-DNBS** solution mixed with three standard concentrations of thiophenol and original water samples was recorded. And the corresponding detection concentration of thiophenol in various water samples was accurately calculated according to above-mentioned standard line between emission intensity and thiophenol in Fig. 3. The good recovery values (93–105%) demonstrated that this probe had capability of practical detection of thiophenols in water samples.

Table 1 Determination of thiophenol in water samples

Sample	Thiophenol spiked (μM)	Thiophenol recovered (μM)	Recovery (%)
Ganjiang River	0	not detected	
	50	53.84±0.81	107.68±1.63
	100	96.39±3.39	96.93±3.39
	150	149.74±2.08	99.83±1.39
Kong Mu Lake	0	not detected	
	50	45.06±0.94	90.12±1.88
	100	93.94±1.01	93.94±1.02
	150	149.23±0.72	99.49±0.48
Tap water	0	not detected	
	50	52.70±0.36	105.41±0.73
	100	99.55±3.04	99.55±3.04
	150	157.32±1.67	104.88±1.11

[a]Average values ± standard deviation of actual thiophenol concentrations calculated from the fluorescence intensity corresponding to the standard concentration of thiophenol in three parallel experiments in each water sample concentration curve.

[b]The ratio between average values ± standard deviation and standard concentration of thiophenol.

3.8. *Theoretical computational studies*

Time-dependent density functional theory (DFT/TD-DFT) about complex **Ir-1** was also studied to gain deeper insight into the spectroscopic properties. The orbital compositions and

assignments of the absorption and emission transitions are listed in Tables S1−S4 in the ESI.

For the ground state for complex **Ir-1**, HOMO is mainly located on the morpholine unit (76.23) with a moderate population on phenanthroline unit in N^N ligand (23.43%), and HOMO-2 is mostly populated on two C^N ligands. HOMO-4 is also mainly located on the ligands but with a moderate population on Ir centre (ca. 32.26). On the other hand, LUMO/LUMO+1/LUMO+4 is mostly populated on N^N ligand, thus the calculated absorption in the 354-498 nm indeed corresponds to an intraligand $\pi \rightarrow \pi^*$ transitions (HOMO→LUMO/LUMO+1/LUMO+4 and MLCT state from Ir to N^N moieties (HOMO-2 → LUMO). The maximum absorption wavelength which corresponds to state S_1 is calculated to be 498 nm with 2.49 eV.

In the lowest-energy triplet state for complex Ir-1, HOMO is mainly located on N^N ligand (Phen for 29.14 and Morphone for 70.42), HOMO-1 is almost even population on Ir centre (ca. 32.91) and two C^N ligands (ca. 32.83, 32.28). Meanwhile, both of LUMO and LUMO+1 are delocalized on Phen moieties. Thus emission transitions are mixed with ^{3}IL transition (HOMO → LUMO/LUMO+1) and ^{3}MLCT trasntion (HOMO-1 → LUMO). The maximum emission wavelength which corresponds to state T_1 is calculated to be 672 nm with 1.85 eV. Additionally, the calculated Stokes shift is obtained to be 174 nm which is less than that of experiment.

4. Conclusion

A novel ‘turn-on’ fluorescent probe **Ir-DNBS** based on iridium (□) complex with two recognition site (DNBS) for detection of thiophenol had been designed, synthesized and characterized. The two fluorescence quencher 2,4-dinitrobenzenesulfonate (DNBS) in probe Ir-DNBS could completely quench the fluorescence. After upon addition with thiophenol, the two DNBS groups were both removed to form fluorescent complex **Ir-1** and significant fluorescence

enhancement (about 35-fold) was observed. The probe **Ir-DNBS** displayed a large stokes shift (200 nm) high selectivity and high sensitivity with a low detection limit (LOD = 62.5 nM). Furthermore, the new probe could also be suitable for detection of thiophenol in practical water samples and human serum. The rapid response (9 min) indicated that it was possible for probe **Ir-DNBS** to real-time detection of thiophenols.

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Highlights

- A “turn on” probe based on Ir(III) complex with two recognition units (DNBS) for detection of thiphenol was synthesized;
- It could be used to detect thiophenol in real water samples and human serum;
- It could apply in wide pH range (7−11) of environments and physiological conditions.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: