Electronic Supplementary Information

A novel pyrene based dual-multifunctional fluorescence probe for differential sensing of pH and HSO_3^- and its bioimaging in live cells

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455 nm between pH 7.10 and 13.09. Conditions: λex = 370 nm, Ex/Em slit = 2.5/5 nm.

Fig. S8 Effect of pH on the fluorescent intensity of **probe** addition reaction system by bisulfite.

Fig. S9 Time-dependent fluorescence spectra of **probe** (15.0 μM) with HSO_3^- (50.0 nM) in PBS buffer (pH = 5.00, 1.5% DMSO). Conditions: λex = 420 nm, Ex/Em slit = 5/10 nm.

Table S1 Comparison of **probe** with the reported fluorescence probes for HSO_3^-.

Fig. S10 The fluorescence intensity of **probe** (15.0 μM) with HSO_3^- (70.0 nM) and other various analytes (100 equiv.) in the PBS buffer (pH = 5.00, 1.5% DMSO).Conditions: λex = 420 nm, Ex/Em slit = 5/10 nm.

Fig. S11 Fluorescence intensity of **probe** (15.0 μM) at 555 nm in PBS buffer (pH = 5.00, 1.5% DMSO) to various anions (30 equiv.), and it's competition graph with bisulfite. Black bar: **probe** + various species. Red bar: **probe** + various species + bisulfite. λ_{ex} /λ_{em} = 420/555 nm. Ex/Em slit = 5/10 nm.

Fig. S12 Partial ^{1}H NMR spectrum of **probe** and **probe -** OH^- in DMSO-d6.

Figure S13 (a) Absorbance spectral changes of **probe** in DMSO, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b) Fluorescence spectra of **probe** in DMSO upon increasing the concentration of water from 0% to 100%. (Note: the spectra were taken after 18 hours).

Figure S14 (a) Absorbance spectral changes of **probe** in CH_3CN, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b) Fluorescence spectra of **probe** in CH_3CN upon increasing the concentration of water from 0% to 100%. (Note: the spectra were taken after 18 hours).

Table S2 The quantum yield (Φ) of **probe** in DMSO upon increasing the concentration of water

from 0 % to 100%.

Table S3 The quantum yield (Φ) of **probe** in CH_3CN upon increasing the concentration of water from 0 % to 100%.

Fig S15 Cytotoxicity data results obtained from the MTT assay.

1. Cytotoxicity assays in cells

The A549 cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C in DMEM supplemented with 100 units of penicillin, 100 μg mL−1 of streptomycin, and 10% fetal bovine serum. The cytotoxicitie (IC50) of **probe** was determined using a MTT assay, a standard method to detect cell survival fraction, by incubating A549 cells. Briefly, the cells with a density of 1×10^4 cells $well^{-1}$ were cultured in 96-well glass-bottom plates for 48 h under 5% CO_2. Then the cells were incubated with various concentrations of **probe** (0, 1, 5, 10, 15, 20, 40, 60, 80, 100 μM) for 12h. At least six parallel samples were created in each group. After that, the suspension medium was removed and 10 μL (5 mg/mL in PBS pH = 7.40) MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) was added to each well and performed for 4 h. When the incubation was finished, culture supernatants were aspirated away and purple formazan crystals were dissolved into 150 μL of DMSO for additional incubation of 15 min, China). The cell viability was estimated according to the following equation:

Inhibition rate (IR %) = [OD (control) – OD (drug treated cell)]/ [OD(control)] × 100%

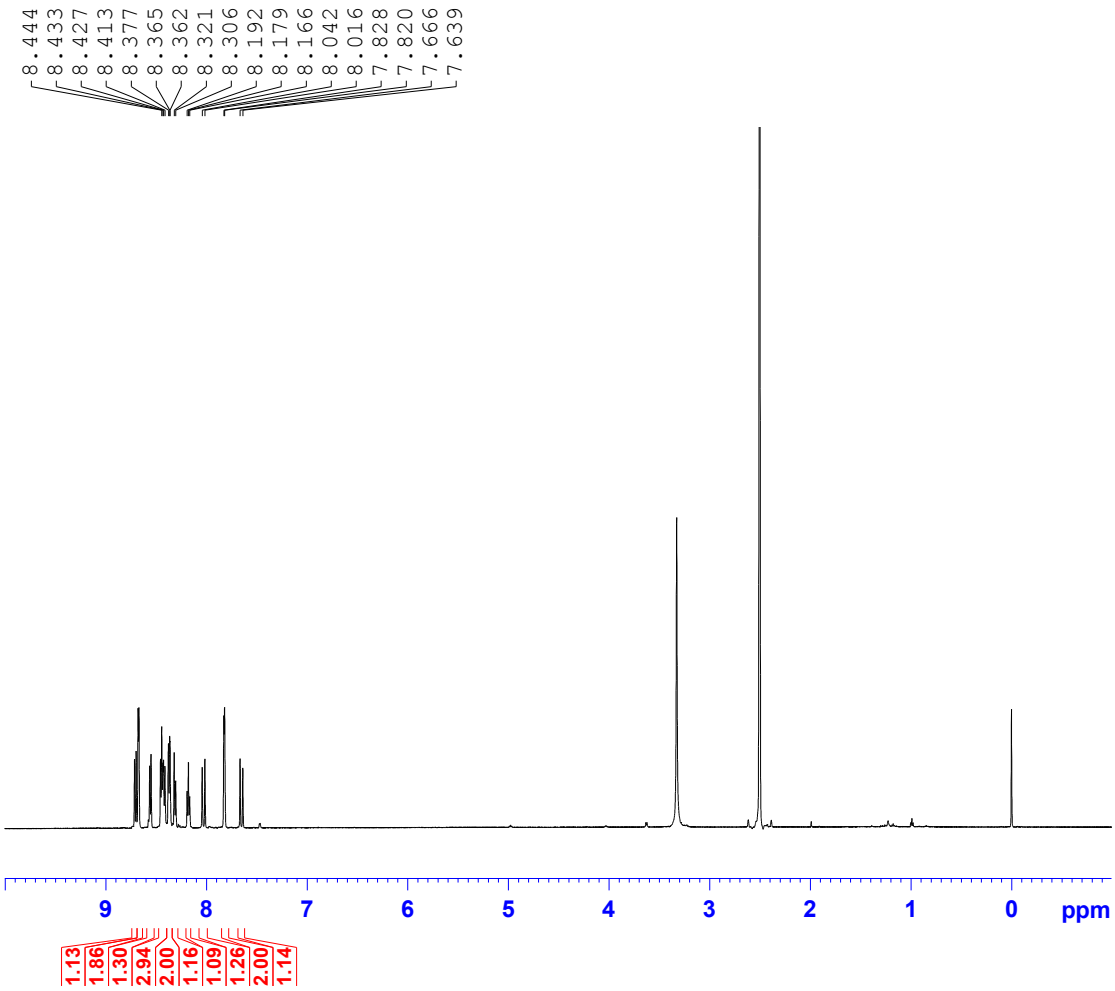
2. Method for determination of the fluorescence quantum yield

For determination fluorescence quantum yields **(**Φ) of **probe**, the quinine sulphate in 0.1 M H_2SO_4 solution was used as a fluorescence standard [1]. The fluorescence quantum yields **(**Φ) were obtained using the following equation:

$$\Phi = \Phi \text{ ref} \times [\text{F sample/F ref}] \times [\text{A ref/A sample}] \times [\eta \text{ sample} / \eta\text{ref}]^2$$

where sample and ref indicated the unknown and standard solution, respectively. Φ = quantum at the exaction wavelength, and η = refractive index of the solvent. Here Φ ref measurements were performed using quinine sulphate in 0.1 M H_2SO_4 as a standard [Φ = 0.546].

3. Figures captions:



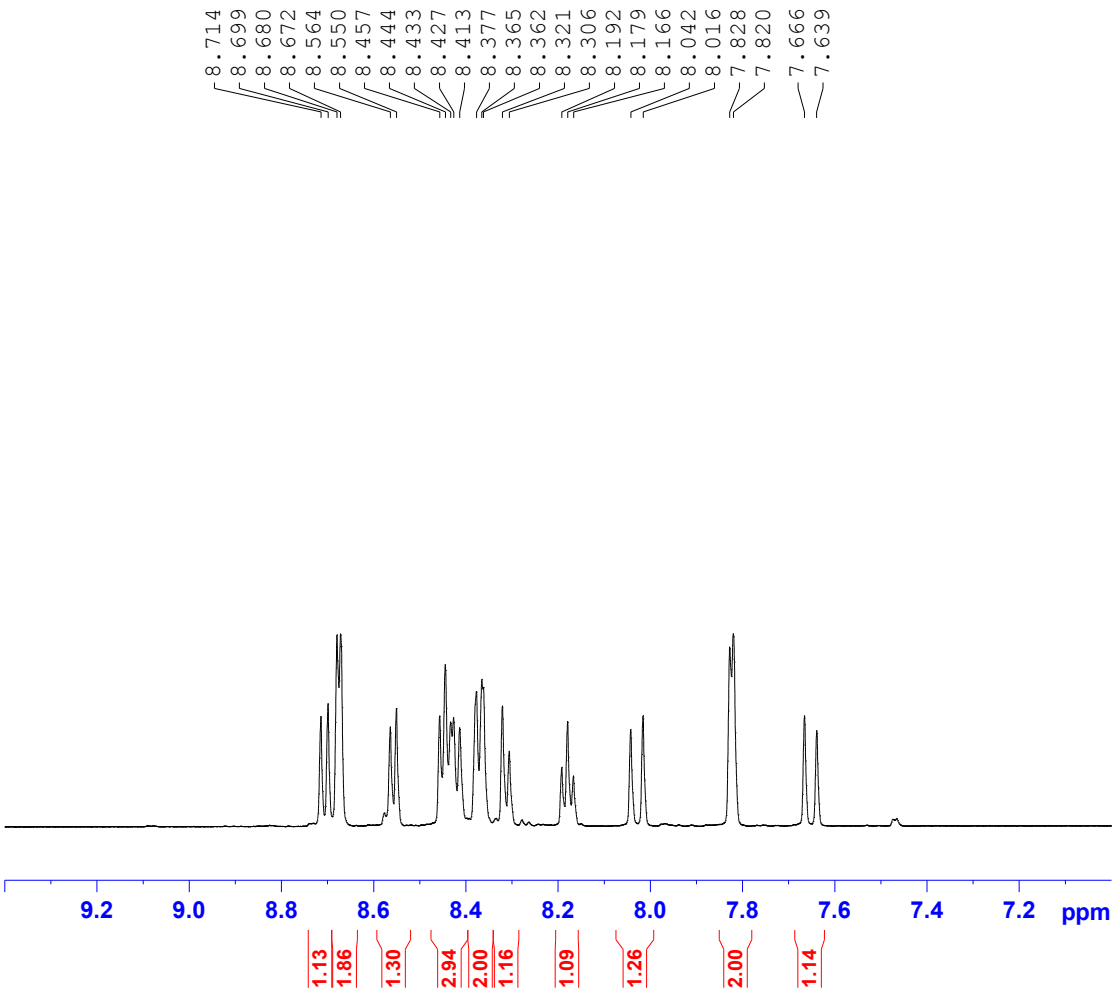
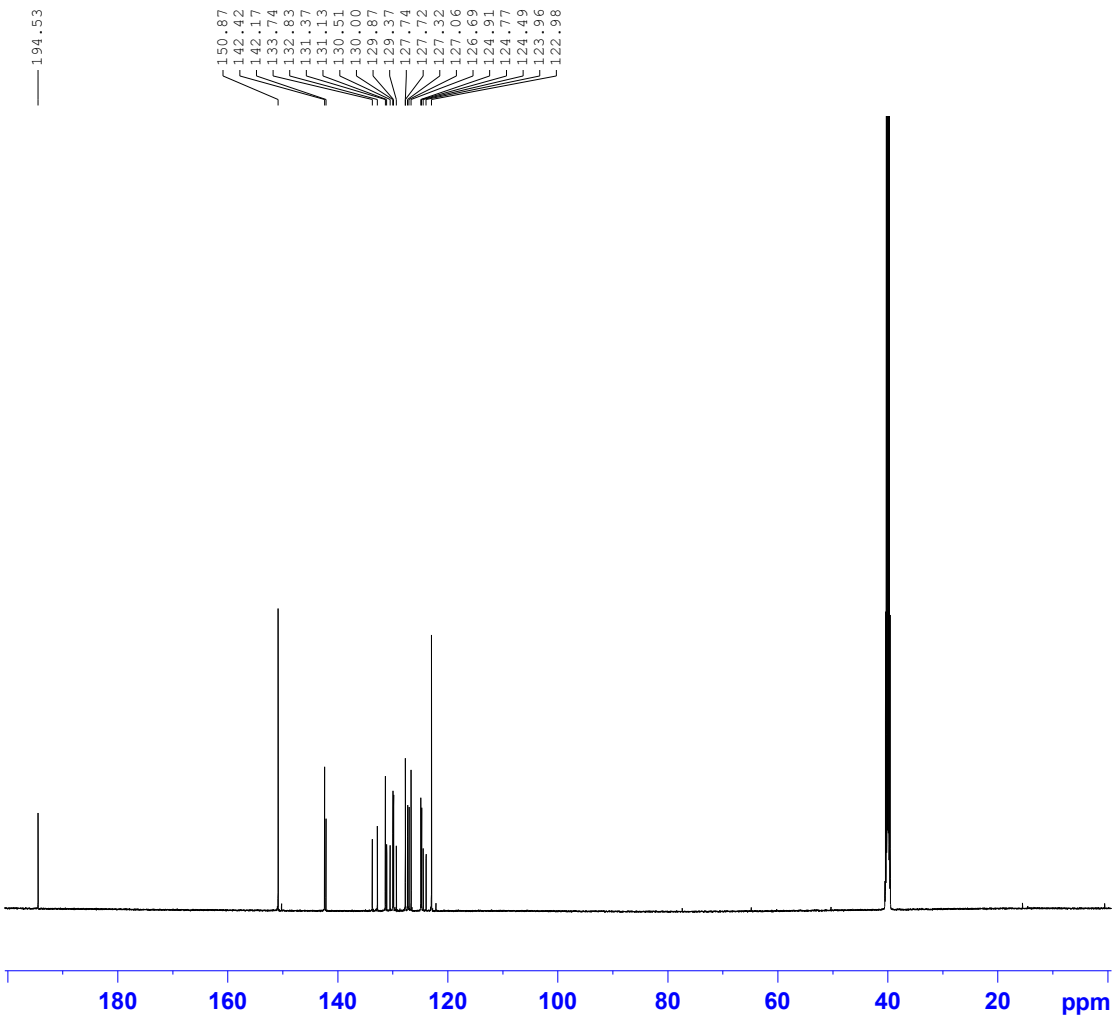


Fig. S1. ^{1}H NMR spectra of **probe** in DMSO-$d_{6.}$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127.32
127.06
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140
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60
40
20
ppm

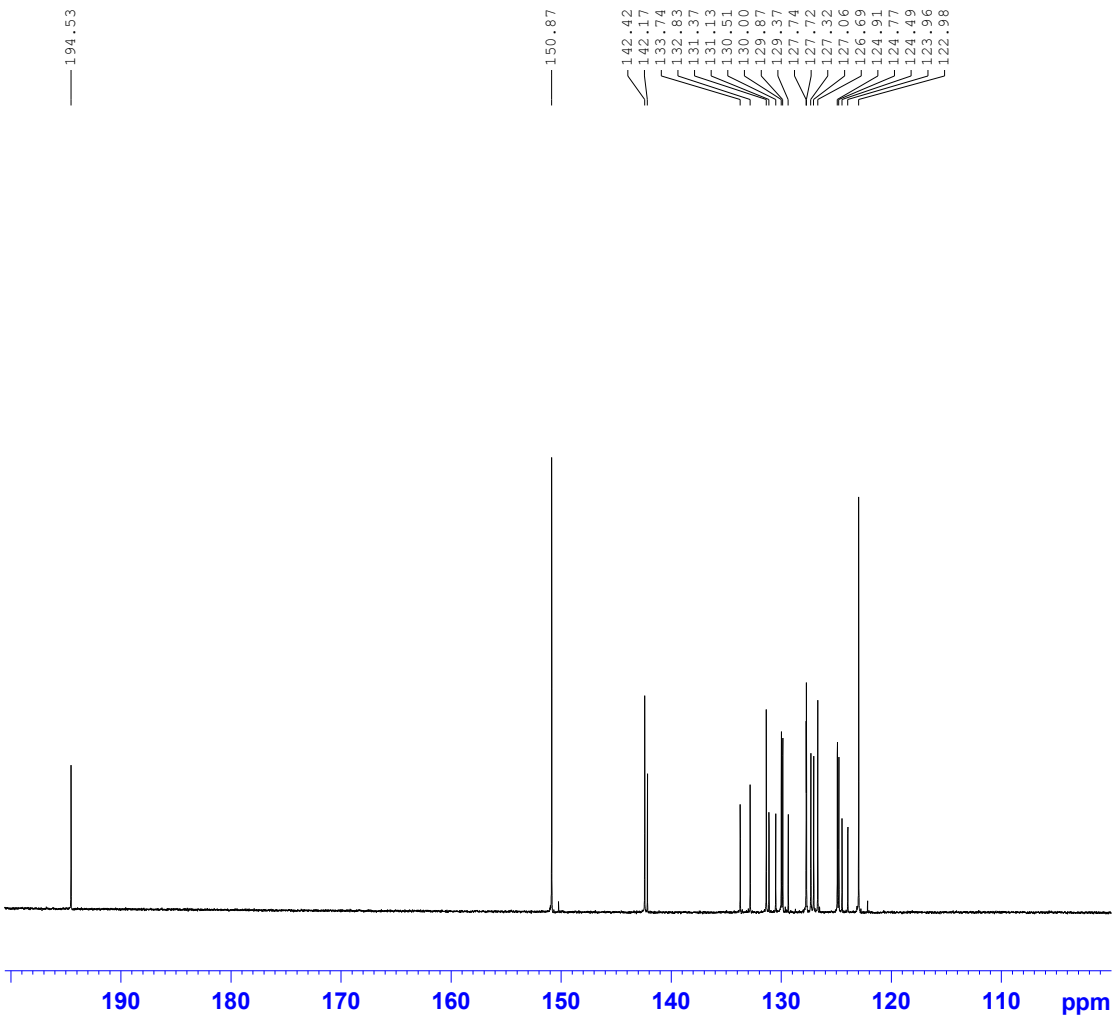


Fig. S2. ^{13}C NMR spectra of **probe** in DMSO-d_6.

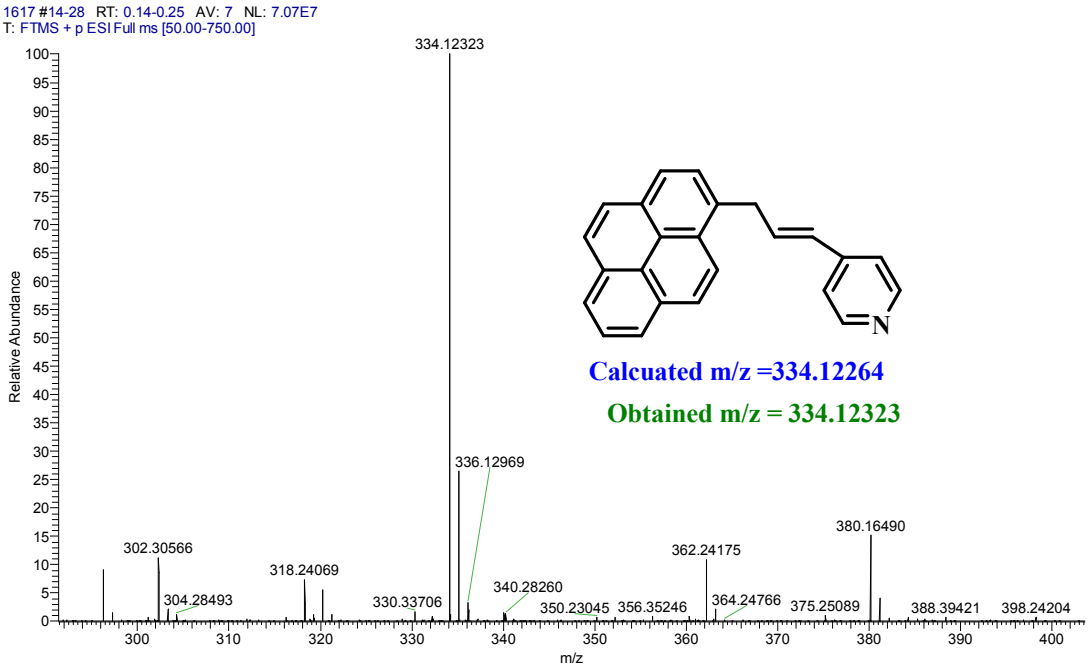


Fig. S3. HRMS spectra of **probe.**

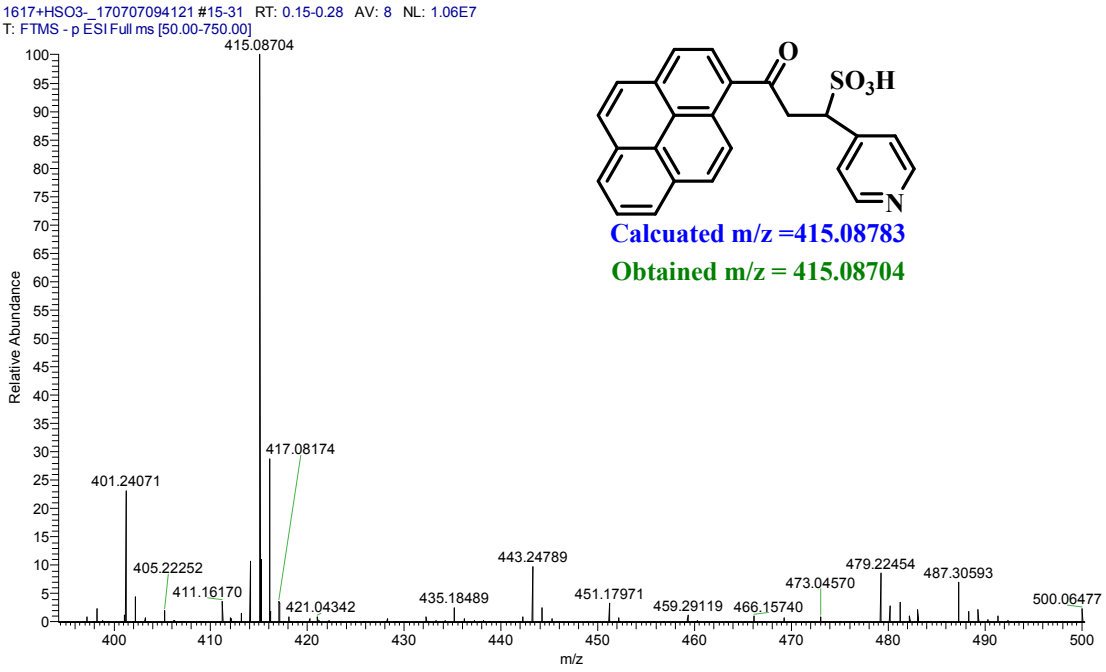


Fig. S4. HRMS spectra of **probe - HSO_3^-**.

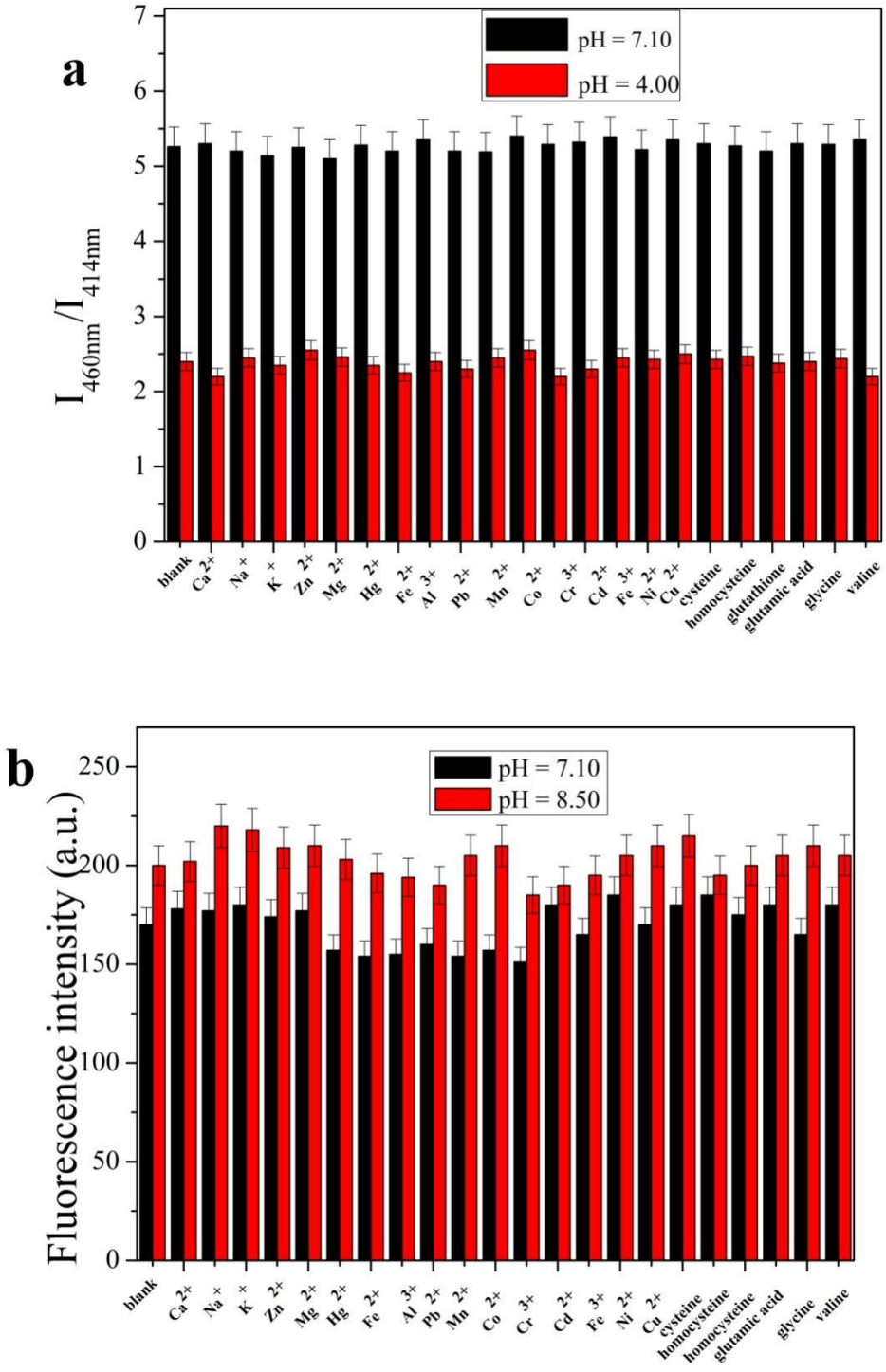
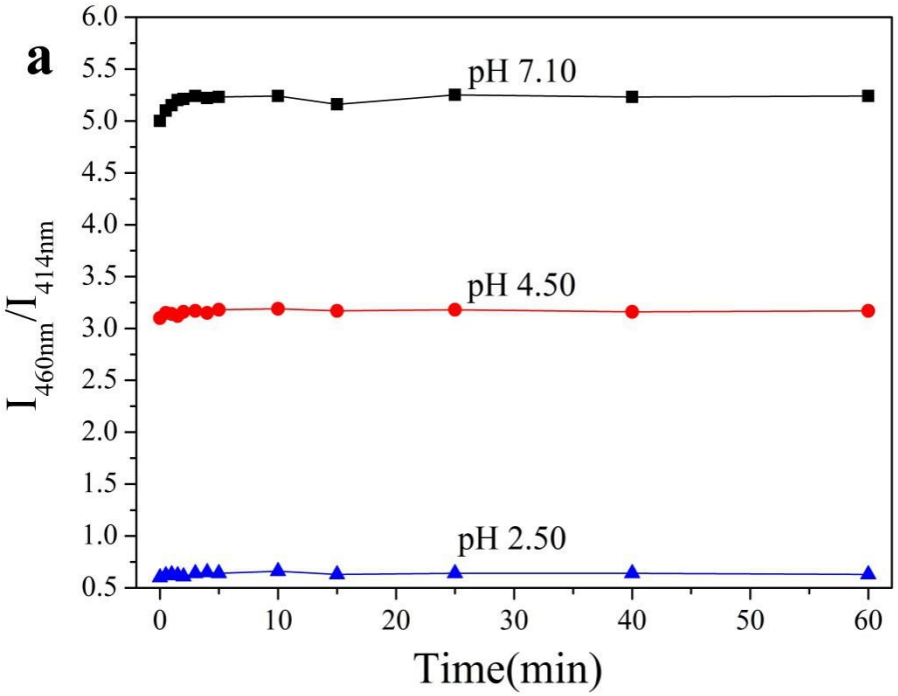


Fig. S5 (a) The ratios of fluorescence intensity of **probe** (15.0 μM) containing diverse species in CH_3CN/ H_2O (1/3, V/V) at pH 7.10 and pH 4.00. Conditions: λex = 370 nm, Ex/Em slit = 5/5 nm. (b) Fluorescence responses (455 nm) of **probe** (15.0 μM) containing diverse species in CH_3CN/ H_2O (1/19, V/V) at pH 7.10 and 8.50. Conditions: λex = 370 nm, Ex/Em slit = 2.5/5 nm. Ca^{2+} (10

mM); Na^+ (150 mM); K^+ (150 mM); other metal ions with 0.2 mM, cysteine (1 mM); homocysteine (1 mM); glutathione (1 mM); glycine (1 mM); valine (1 mM); arginine (1 mM); lysine (1 mM); tyrosine (1 mM).



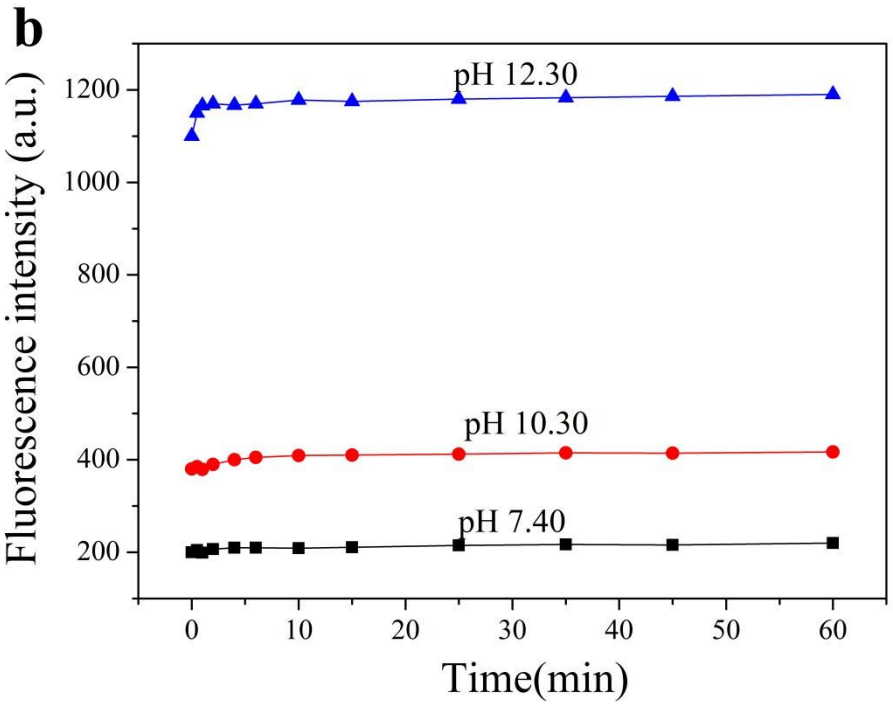


Fig. S6 (a) Time courses of fluorescence emission ratios $I_{460\ nm}/I_{414\ nm}$ in various pH values (7.10, 4.50 and 2.50, respectively). Conditions: λ_{ex} = 370 nm, Ex/Em slit = 5/5 nm. (b) Changes in the fluorescence intensity at 455 nm for **probe** (15.0 μM) in CH_3CN/ H_2O (1/19, V/V) at pH 7.40, 10.30, 12.30, respectively. Conditions: λ_{ex} = 370 nm, Ex/Em slit = 2.5/5 nm.

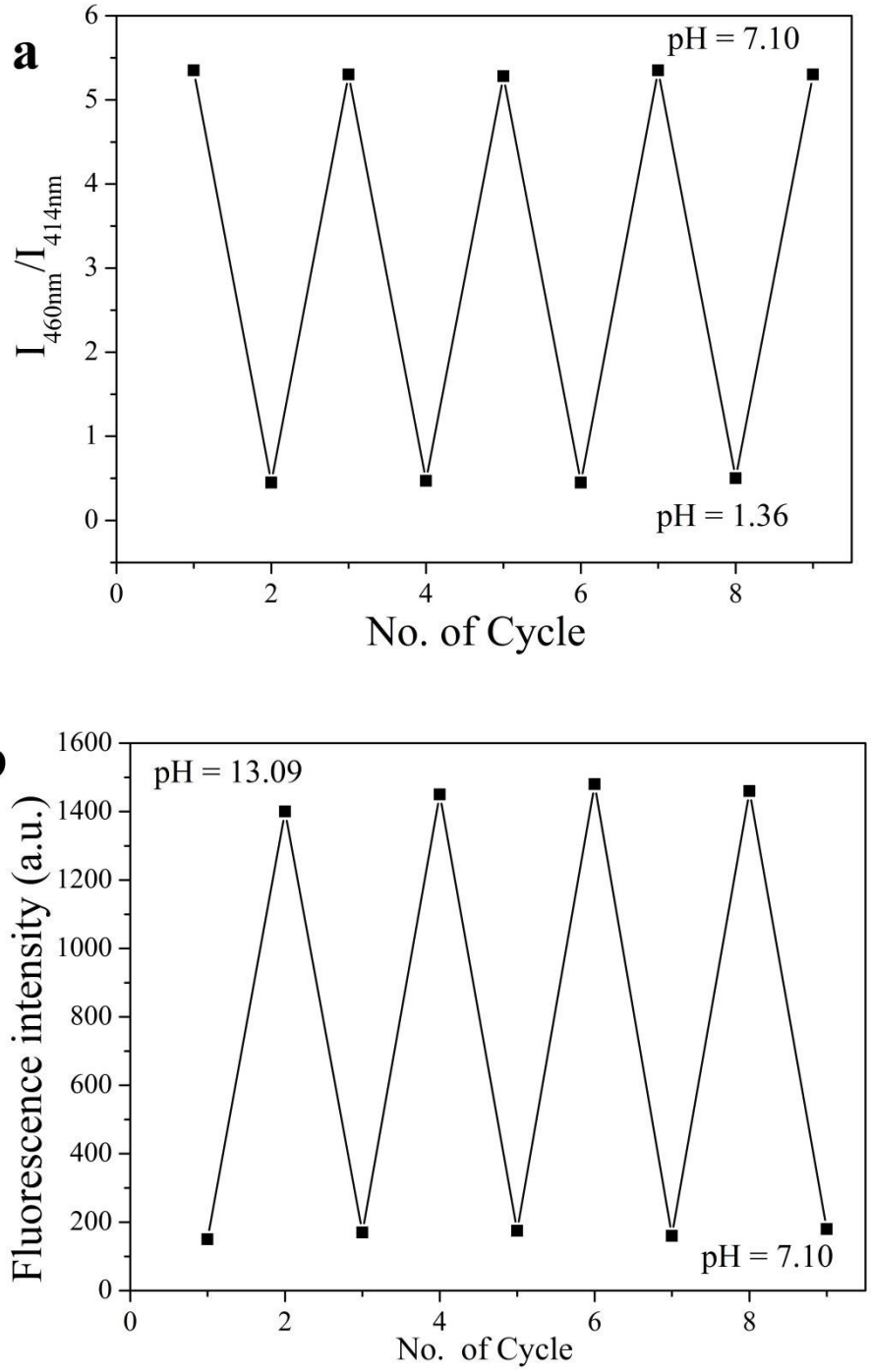


Fig. S7 (a) Reversible changes in the fluorescence emission ratio ($I_{460\ nm}/I_{414\ nm}$) for **probe (**15.0

μM) in CH_3CN/H_2O (1/3, v/v) system between pH 7.10 and 1.36. Conditions: λ_{ex} = 370 nm, Ex/Em slit = 5/5 nm. (b) Changes in the fluorescence intensity of **probe** (15.0 μM) in CH_3CN/H_2O (1/19, v/v) system at 455 nm between pH 7.10 and 13.09. Conditions: λex = 370 nm, Ex/Em slit = 2.5/5 nm.

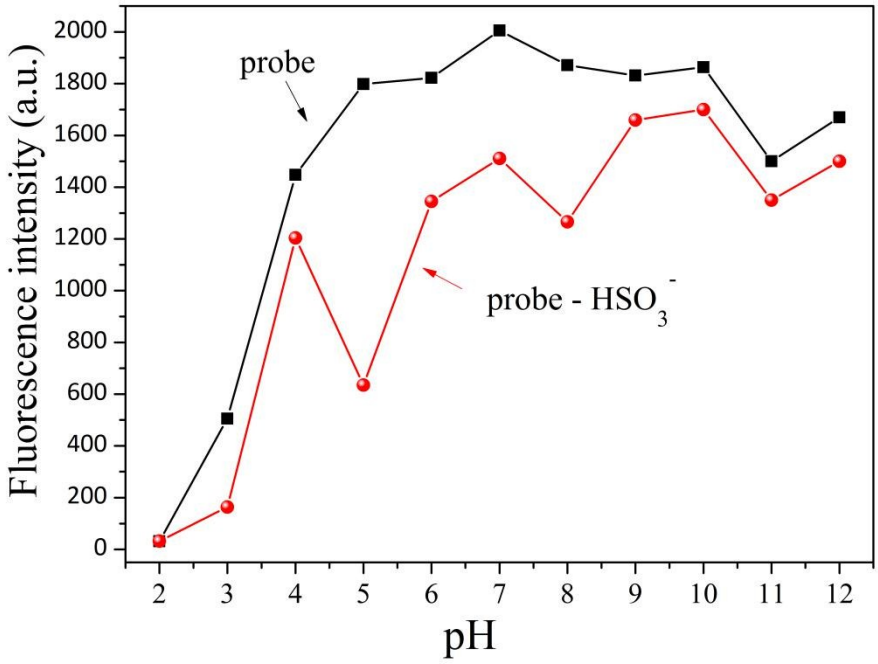


Fig. S8 Effect of pH on the fluorescent intensity of **probe** addition reaction system by bisulfite.

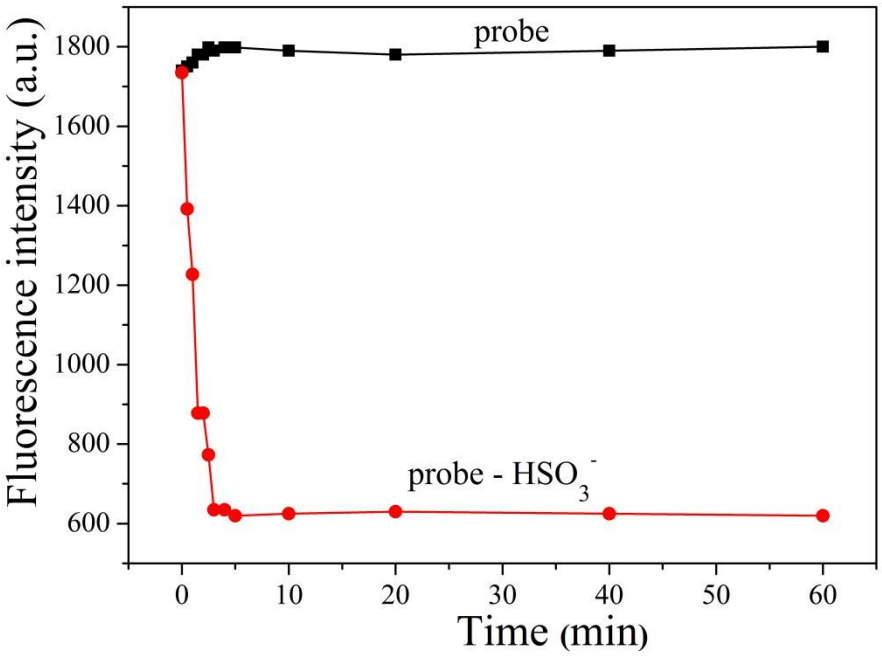
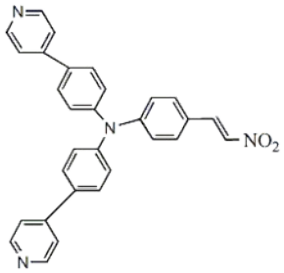
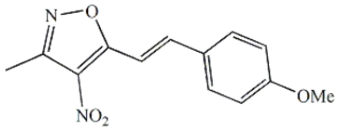
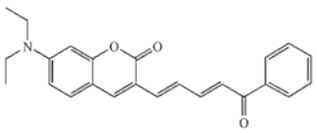
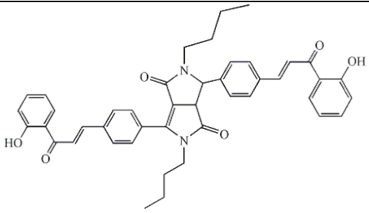
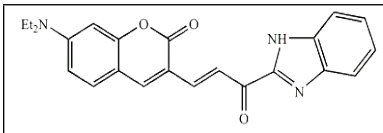
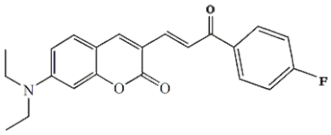
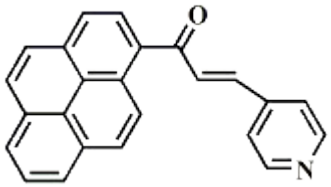


Fig. S9 Time-dependent fluorescence spectra of **probe** (15.0 μM) with HSO_3^- (50.0 nM) in PBS buffer (pH = 5.00, 1.5% DMSO). Conditions: λex = 420 nm, Ex/Em slit = 5/10 nm.

Table S1 Comparison of **probe** with the reported fluorescence probes for HSO_3^-

Probe	Response time	Detection limit	Solution	Reference
	5 min	100 nM	Water-DMSO (99/1,V/V)	Dyes Pigments, 2017 [2]
	a few minutes	28200 nM	Britton-Robinson buffer (20 mM, pH 7)-DMSO (99/1, V/V)	Sens Actuators B:Chemica, 2017 [3]
	7.7 min	3060 nM	Sugar (5.0 g per/100 Ml)	Talanta, 2017 [4]
	20 min	100 nM	HEPES (10 mM, pH 7.40) THF/H_2O (1/1,V/V)	Dyes Pigments, 2017 [5]

	60 min	53 nM	DMF-PBS buffer(10mM,3:7,V/V)	Ana. Chim. Acta, 2015 [6]
	1 h	300 nM	PBS buffer(20 mM) Containing 1mM CTAB	Ana. Chim. Acta, 2013 [7]
	5 min	1.9 nM	PBS (pH 5, 1.5%DMSO)	This work

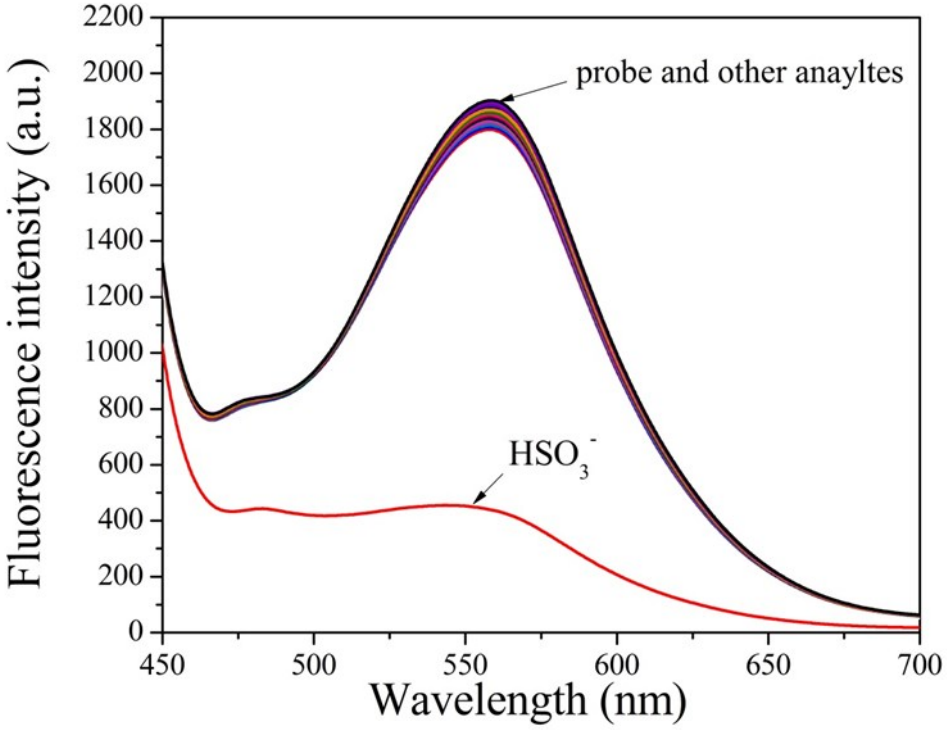


Fig. S10 The fluorescence intensity of **probe** (15.0 μM) with HSO_3^- (70.0 nM) and other various analytes (100 equiv.) in the PBS buffer (pH = 5.00, 1.5% DMSO).Conditions: λex = 420 nm, Ex/Em slit = 5/10 nm.

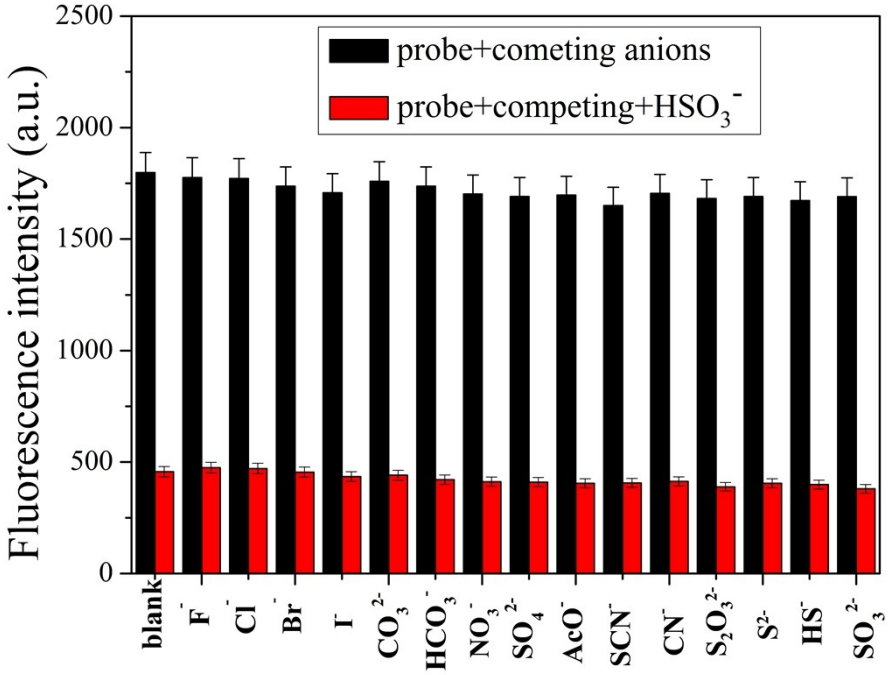
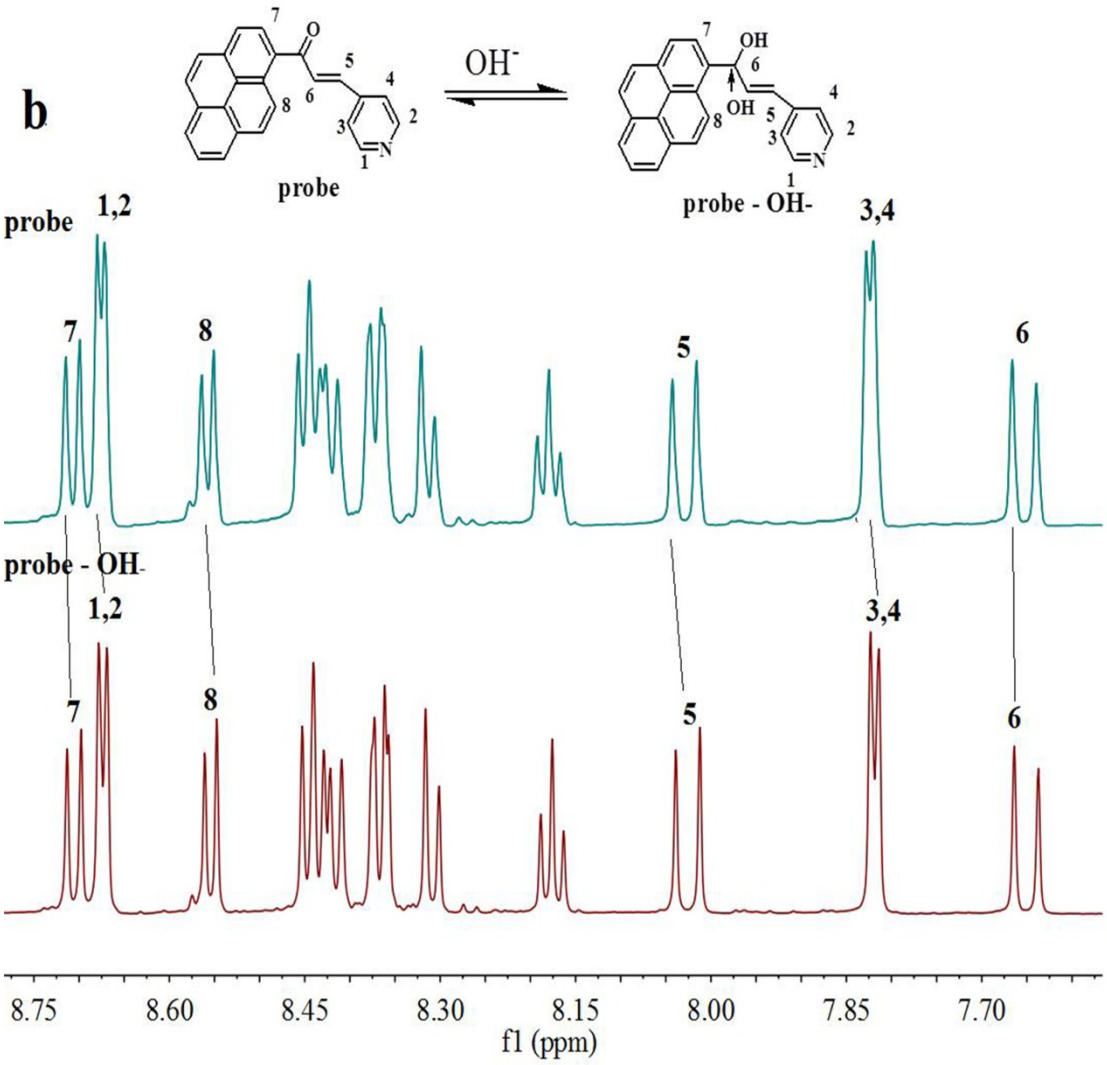
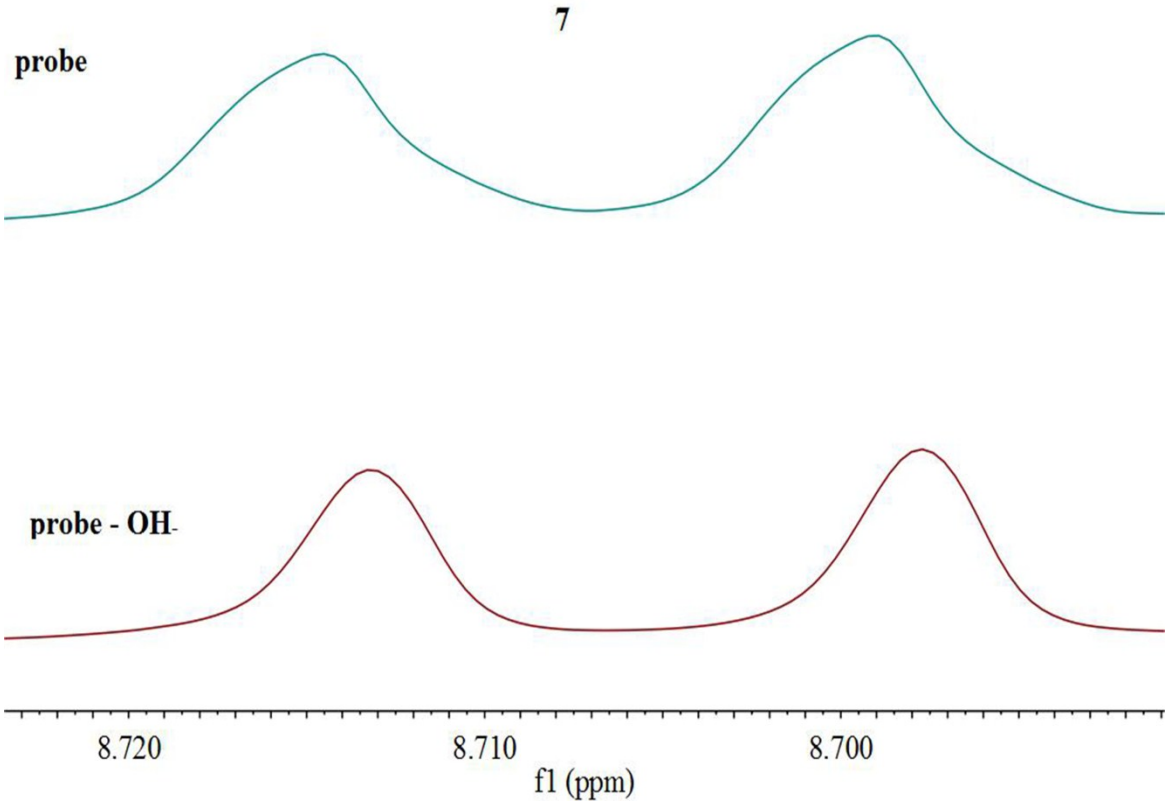
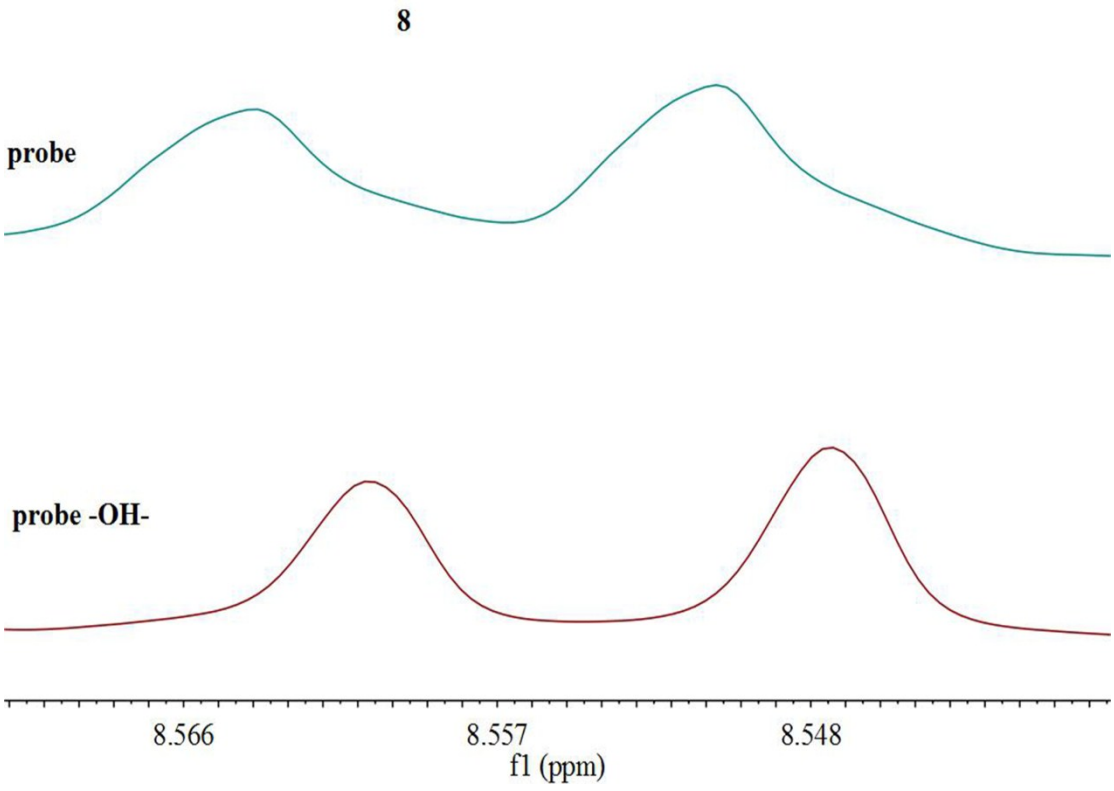
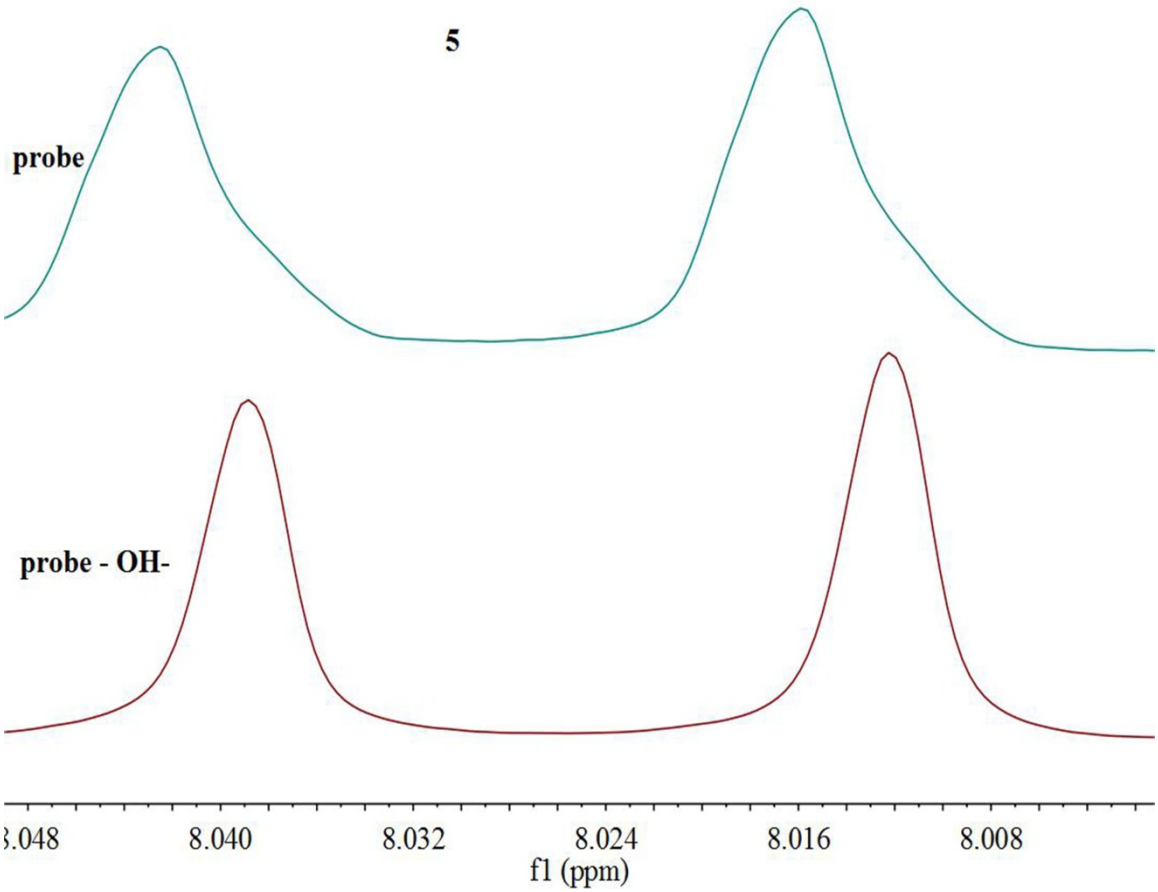


Fig. S11 Fluorescence intensity of **probe** (15.0 μM) at 555 nm in PBS buffer (pH = 5.00, 1.5% DMSO) to various anions (30 equiv.), and it's competition graph with bisulfite. Black bar: **probe** + various species. Red bar: **probe** + various species + bisulfite. λ_{ex} /λ_{em} = 420/555 nm. Ex/Em slit = 5/10 nm.




7
probe
probe - OH-
8.720
8.710
8.700
f1 (ppm)


8
probe
probe -OH-
8.566
8.557
8.548
f1 (ppm)


5
probe
probe - OH-
8.048
8.040
8.032
8.024
8.016
8.008
f1 (ppm)

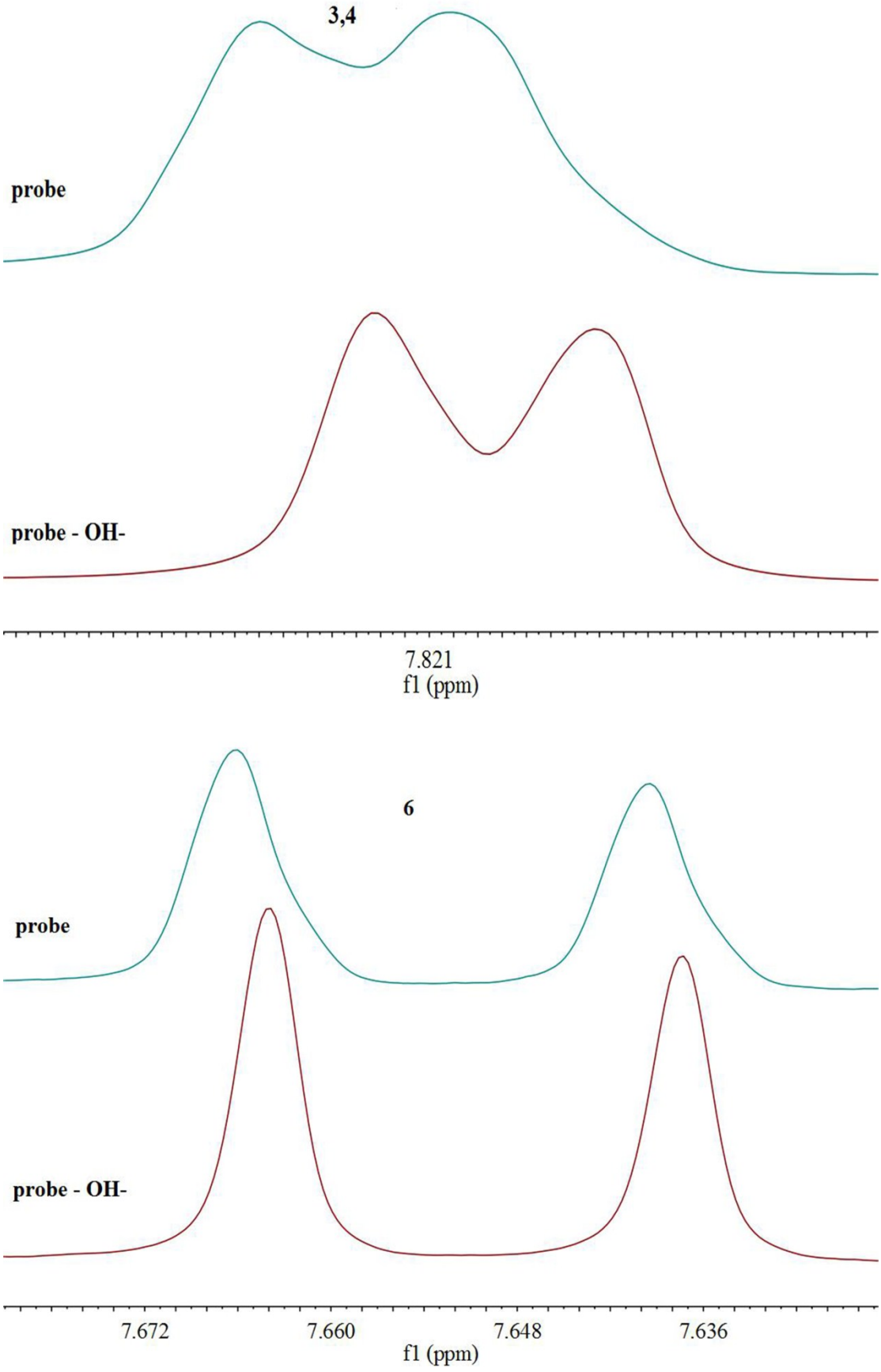


Fig. S12 Partial ^{1}H NMR spectrum of **probe** and **probe -** OH^- in DMSO-d6.

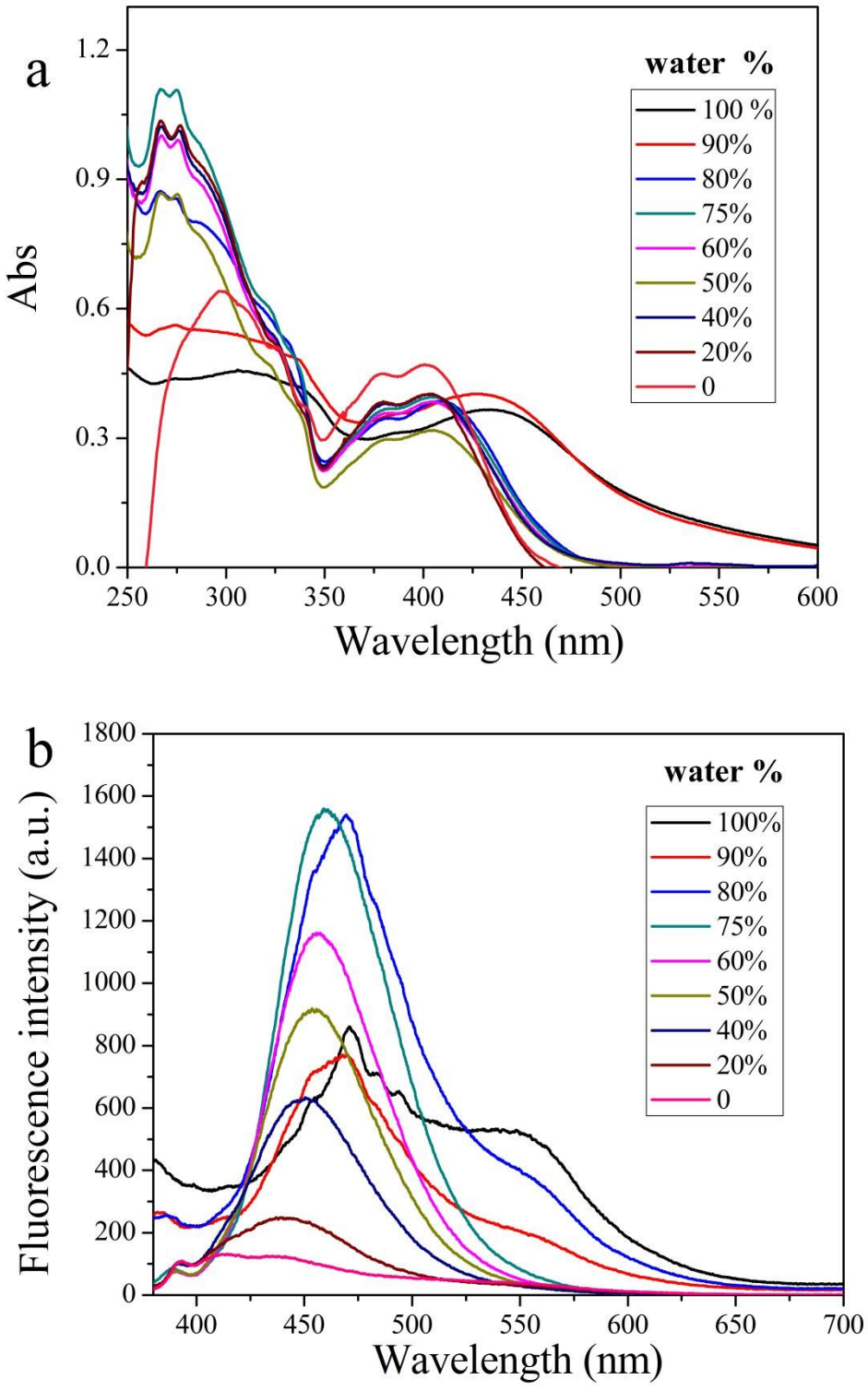


Figure S13 (a) Absorbance spectral changes of **probe** in DMSO, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b) Fluorescence spectra of **probe** in DMSO upon increasing the concentration of water from 0%

to 100%. (Note: the spectra were taken after 18 hours).

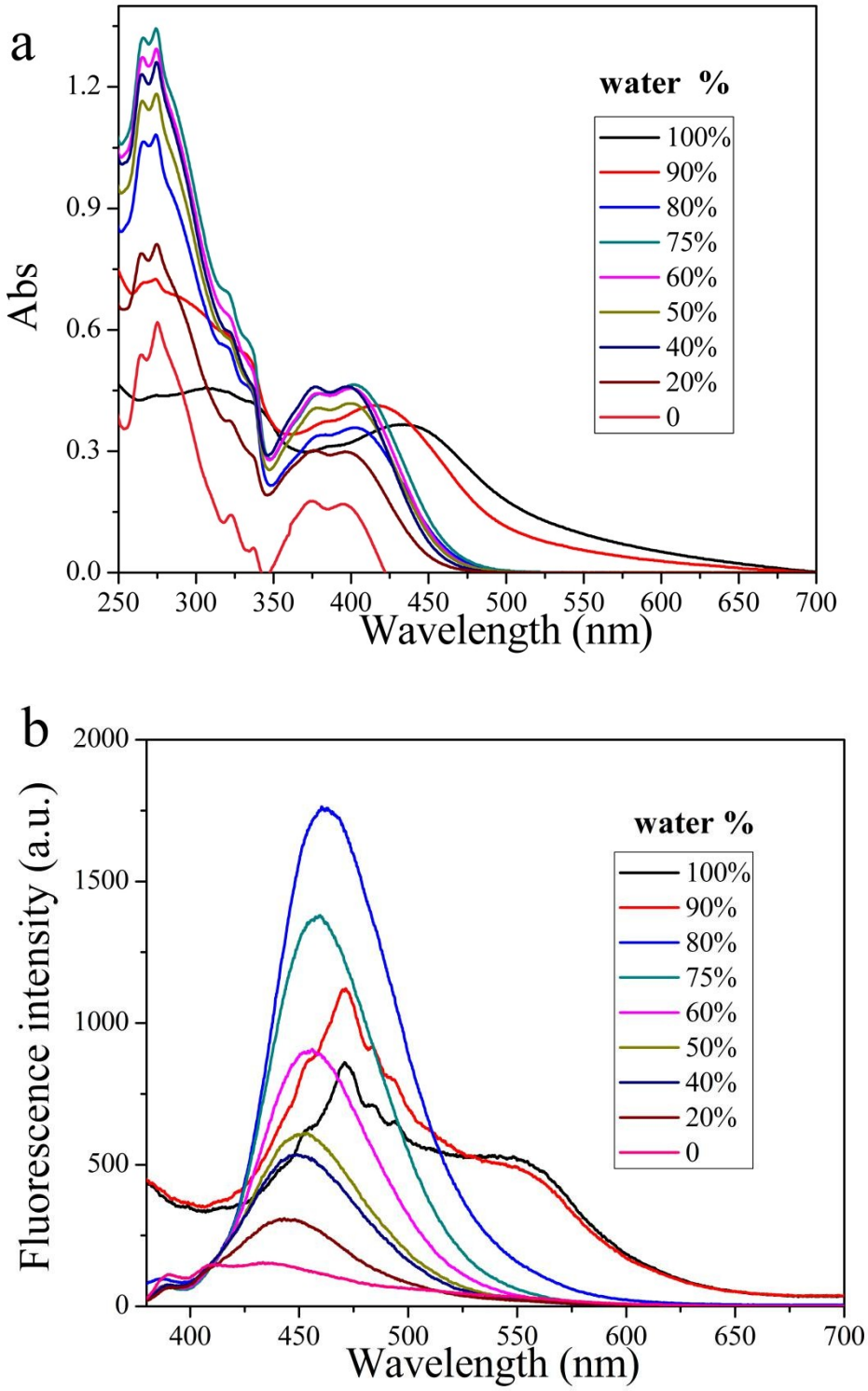


Figure S14 (a) Absorbance spectral changes of **probe** in CH_3CN, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b)

Fluorescence spectra of **probe** in CH_3CN upon increasing the concentration of water from 0 % to100%. (Note: the spectra were taken after 18 hours).

Table S2 The quantum yield (Φ) of **probe** in DMSO upon increasing the concentration of water from 0 % to 100%.

f_w	0%	20%	40%	50%	60%	75%	80%	90%	100%
Φ	0.078	0.060	0.081	0.109	0.173	0.199	0.121	0.065	0.013

Table S3 The quantum yield (Φ) of **probe** in CH_3CN upon increasing the concentration of water from 0 % to 100%.

f_w	0%	20%	40%	50%	60%	75%	80%	90%	100%
Φ	0.029	0.050	0.061	0.066	0.090	0.106	0.100	0.054	0.013

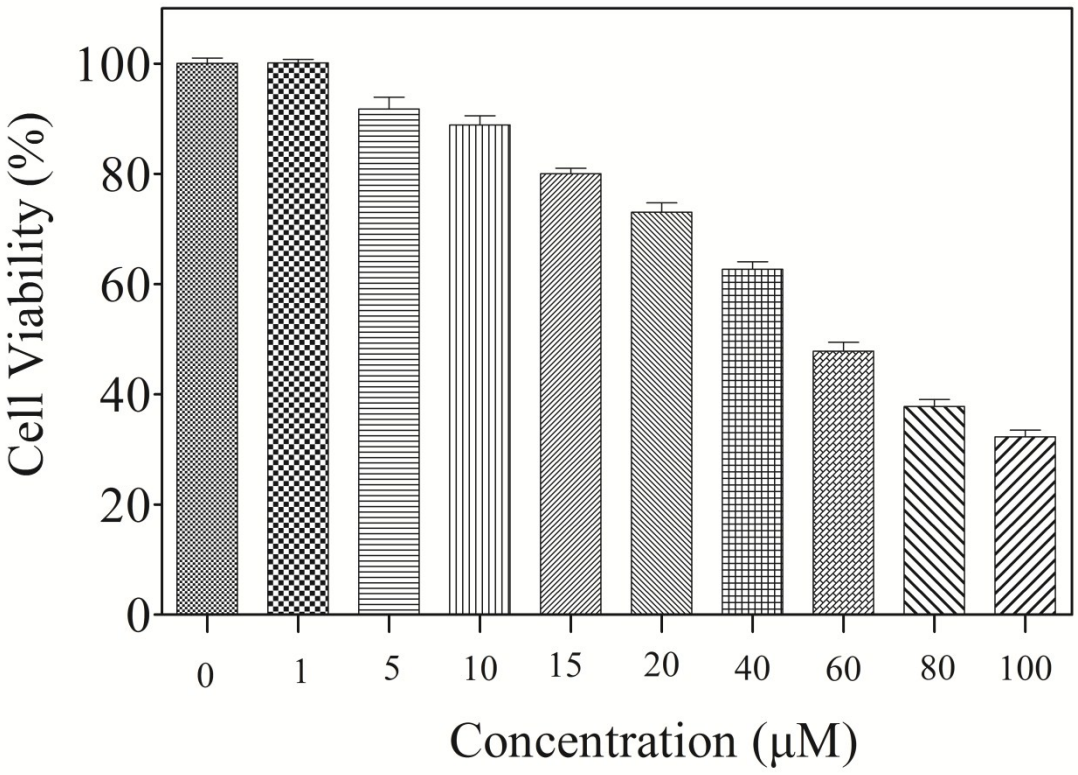


Fig . S15 Cytotoxicity data results obtained from the MTT assay.

References

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7. H. Tian, J. Qian, Q. Sun, H. Bai, W. Zhang, *Ana. Chim. Acta*, **2013**,788, 165-170.