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Electronic Supplementary Information

Carbazole-based near-infrared emitted fluorescence probe for the detection of bisulfite in live animals and real food samples

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Materials

9-Phenyl-9H-carbazol-3-ylboronic acid, 5-bromothiophene-2-carbaldehyde, 1,3-indanedione and tetrabutylammonium cyanide, dithiothreitol, thioglycol and mercaptopurine were received from Aladdin reagent Co. (Shanghai, China). Potassium carbonate (K_2CO_3), hydrogen peroxide (H_2O_2), piperidine, anions (sodium salts), hypochlorite (NaOCl), thiophenol and sodium cetyltrimethylammonium bromide (CTAB) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All of the experimental live nude mice (6 to 8-week-old) and zebrafish were obtained from Liaoning Changsheng Biotechnology Co., Ltd., and the experiments were performed in compliance with the relevant laws and guidelines. Unless otherwise stated, solvents and reagents were of analytical grade from commercial suppliers and were used without further purification.

Apparatus

^{1}H NMR and ^{13}C NMR spectra were recorded with an AVANCE 400 MH_Z spectrometer (Bruker) with chemical shifts reported as ppm (in Chloroform-*d*, DMSO-d_6 and D_2O, TMS as an internal standard). Mass spectra were recorded on an Agilent 6530 QTOF spectrometer. Absorption spectra were tested with a Perkin Elmer Lambda 900 UV/VIS/NIR spectrophotometer. Fluorescence spectra were recorded with a Spectrofluorometer FS5 luminescence spectrometer. All pH measurements were made with an OHAUS Starter 3100/f meter. Imaging of HSO_3^- in zebrafish and mice were performed on a SPECTRAL Ami Imaging Systems (Spectral Instruments Imaging, LLC, Tucson, AZ) with an excitation filter 500 nm and an

emission filter 710 nm. Amiview Analysis software (Version 1.7.06) was used to calculate fluorescence intensity in region of interest (ROI), and values are presented as the mean ± SD for each group of three experiments.

Quantum yield measurement.

The relative fluorescence quantum yields of **QPM** before and after reacting with HSO_3^- were determined by using fluorescein as the reference (Φ_f = 0.85 in 0.1 M NaOH aqueous solutions). After the measurements of UV-Vis and fluorescence spectra (λ_{ex}=490 nm), the quantum yield of the corresponding compounds were determined using the equation[S1].

$$\Phi_{unk} = \Phi_{std} \frac{(F_{unk}/A_{unk})}{(F_{std}/A_{std})} \left(\frac{\eta_{unk}}{\eta_{std}} \right)^2$$

Where Φ_{std}, F_{std}, A_{std}, and η_{std} are the quantum yield, integrated fluorescence intensity, absorbance, and refraction of the standard solution, respectively. Φ_{unk}, F_{unk}, A_{unk}, and η_{unk} represent the quantum yield, integrated fluorescence intensity, absorbance, and refraction of the sample solution, respectively. All of the spectroscopic measurements were performed in triplicate and averaged.

(1) Calculation of fluorescence quantum yield (Φ_1) of **QPM**:

The measured data of **QPM** were Φ_{std}=0.85, F_{std}=165646394.50, A_{std}=0.2849, η_{std}=1.3812, F_{unk}=108469230.27, A_{unk}=0.2993 and η_{unk}= 1.3853, respectively. Put the above data into the formula and calculate Φ_1=0.5329.

(2) Calculation of fluorescence quantum yield (Φ_2) of **QPM-**SO_3H:

The measured data of **QPM-**SO_3H were Φ_{std}=0.85, F_{std}=165646394.5, A_{std}=0.2849, η_{std}=1.3812, F_{unk}=219667.32, A_{unk}=0.0391 and η_{unk}=1.3546, respectively. Put the

above data into the formula and calculate Φ_1=0.0079.

MTT assay of cytotoxicity of QPM in A549 cells

The cytotoxicity of **QPM** to live A549 cells was investigated by MTT assays[S2]. The following formula was used to calculate the viability of cell growth: Viability (%)=(mean of absorbance value of treatment group-blank)/(mean absorbance value of control-blank)×100.

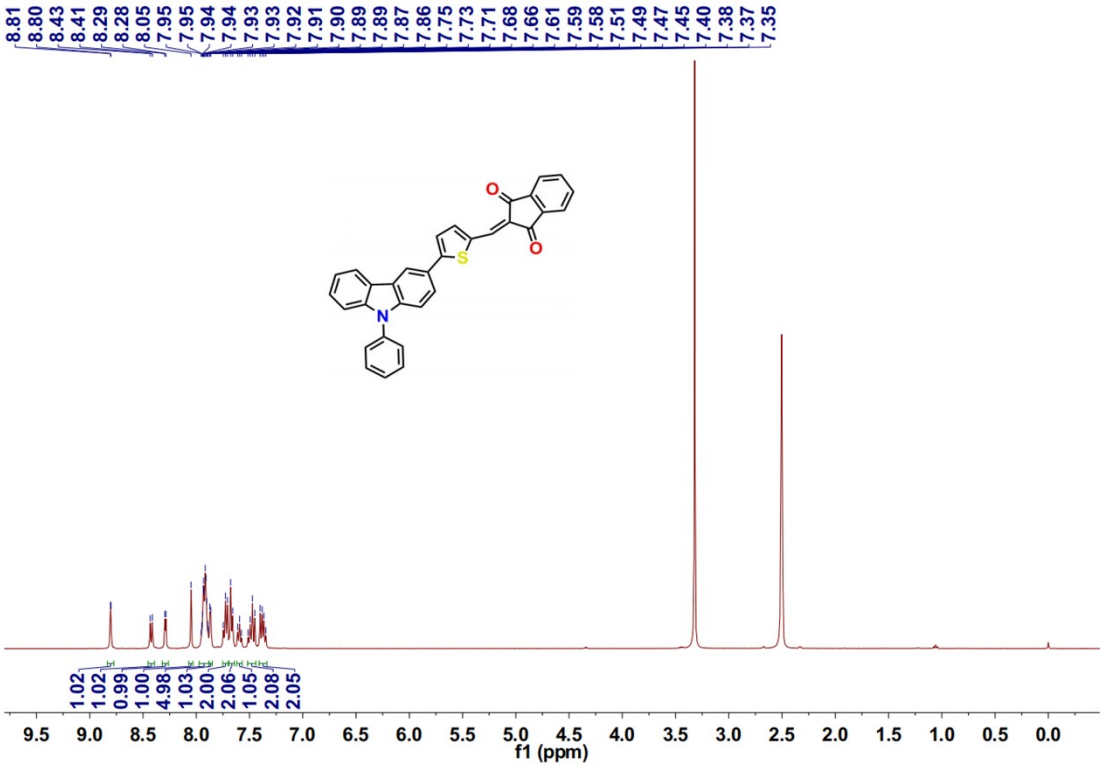

Fig. S1 ^{1}H NMR of **QPM** (DMSO-d_6, 400 MHz).

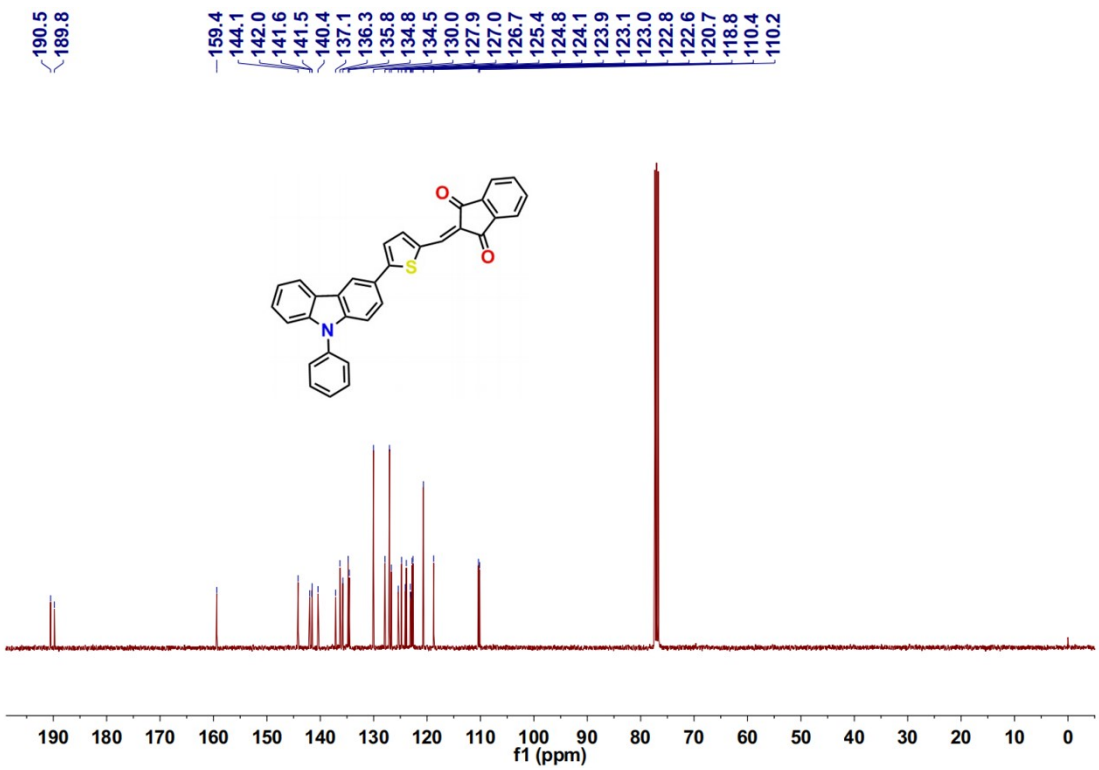

Fig. S2 ^{13}C NMR of **QPM** (Chloroform-*d*, 101 MHz).

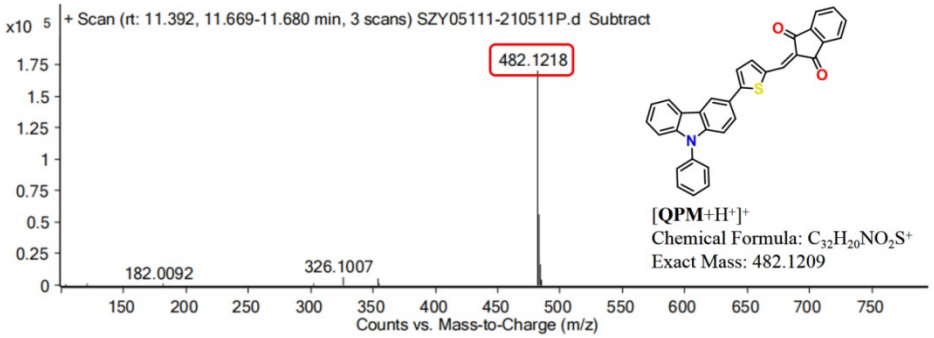

Fig. S3 HR-MS of **QPM**.

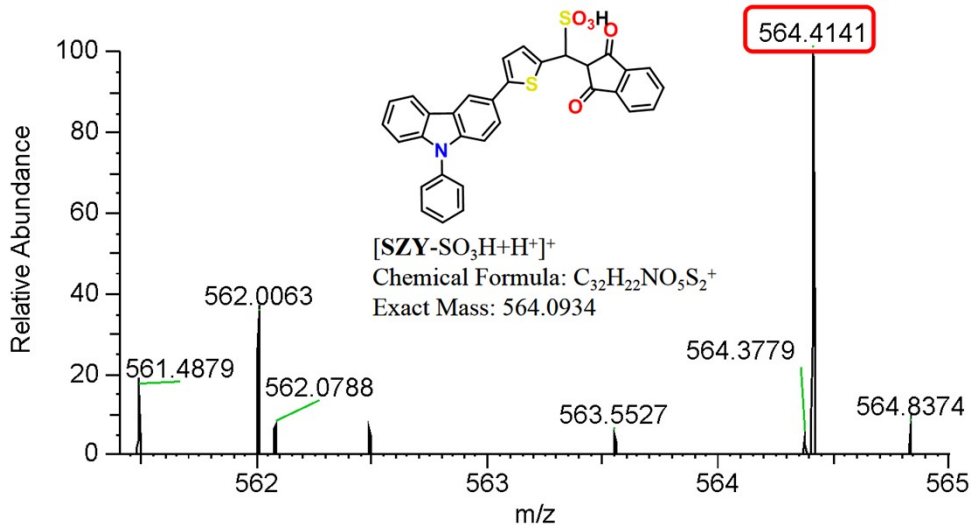

Fig. S4 HR-MS of **QPM** (10 μM) in the presence of HSO_3^- in Tris buffer solution (Tris:DMSO=7:3, pH=7.4, containing 1 mM CTAB).

Theoretical computations

The ground state structures of **QPM** and **QPM**-SO_3H were optimized using the density functional theory (DFT) with the Beck's three-parameter hybrid functional with the Lee-Yang-Parr correlation functional (B3LYP). All atoms were treated with 6-31G(d,p) basis set. All quantum chemistry calculations were performed using the Gaussian 16 package.

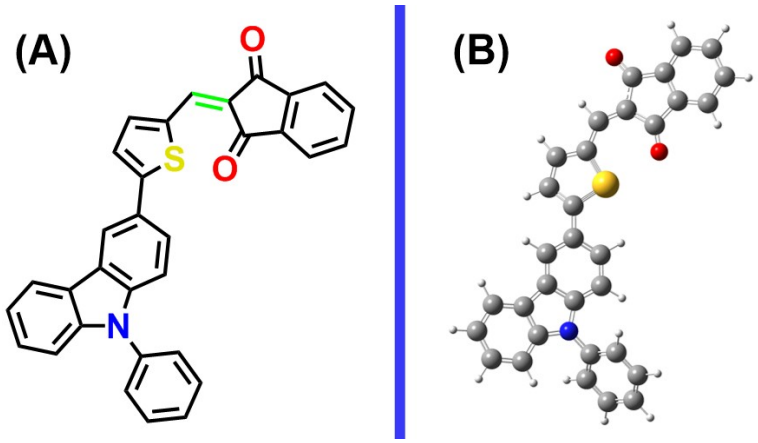

Fig. S5 (A) Molecular structure of **QPM**, and (B) optimized molecular geometry of **QPM** in the ground state (S_0) obtained from DFT calculations at B3LYP//6-31G(d,p) level of theory.

Table S1 Cartesian coordinates of **QPM** in the ground state (S_0).

Coordinates (ground state S_0)				
Center Number	**Atomic Number**	**Forces(Hartrees/Bohr)**		
		X	**Y**	**Z**
1	6	-0.000002693	-0.000003431	0.000000188
2	6	-0.000001361	0.000003464	0.000001056
3	6	0.000004169	-0.000001227	-0.000000776
4	6	-0.000006465	-0.000000831	0.000001469
5	6	-0.000004745	0.000000755	-0.000000249
6	6	0.000003905	0.000000896	-0.000000829
7	7	0.000010728	0.000019077	0.000000482
8	6	-0.000012214	-0.000011440	0.000002363
9	6	0.000007267	0.000000851	0.000001030
10	6	0.000002786	0.000000590	-0.000001038
11	6	-0.000001562	0.000000748	0.000000843
12	6	0.000001491	-0.000001337	-0.000001293
13	6	-0.000001760	0.000000975	-0.000001060
14	6	0.000002917	-0.000013654	-0.000001435
15	6	-0.000001584	0.000003021	0.000001550
16	6	0.000000417	-0.000001018	0.000001426
17	6	0.000000878	-0.000000995	0.000001402

18	6	0.000000393	-0.000000542	0.000000661
19	6	0.000000498	0.000002140	0.000001031
20	6	-0.000004594	-0.000001101	-0.000001356
21	16	0.000004681	0.000003211	-0.000000312
22	6	0.000000827	-0.000001619	0.000004255
23	6	-0.000000129	0.000001361	-0.000001491
24	6	-0.000000731	-0.000000925	-0.000001247
25	6	-0.000002742	-0.000002336	-0.000002368
26	6	0.000002631	0.000002742	-0.000003528
27	6	-0.000001120	-0.000004307	-0.000000568
28	6	0.000000344	0.000003708	0.000001481
29	6	-0.000001451	-0.000002941	0.000001287
30	6	0.000002728	0.000002264	0.000000312
31	6	-0.000003665	0.000001635	-0.000002072
32	6	0.000001848	-0.000003680	-0.000000136
33	6	0.000002343	0.000004225	-0.000000194
34	6	-0.000003199	-0.000000365	-0.000001512
35	8	-0.000000276	-0.000001064	-0.000000067
36	8	-0.000000468	0.000002148	-0.000001628
37	1	0.000000088	0.000000266	-0.000000399
38	1	0.000000005	-0.000000820	0.000000053
39	1	-0.000000556	-0.000000350	0.000000835
40	1	-0.000000367	-0.000000041	-0.000000229
41	1	0.000000845	-0.000000021	0.000000567
42	1	0.000000145	-0.000001094	-0.000000179
43	1	-0.000000664	-0.000000206	-0.000000038
44	1	0.000000375	-0.000000268	0.000000628
45	1	0.000000714	0.000000047	0.000001412
46	1	0.000000441	-0.000000364	0.000001684
47	1	0.000000165	-0.000000760	0.000001169
48	1	0.000000191	-0.000000773	0.000000659
49	1	-0.000000624	-0.000000035	-0.000000666
50	1	-0.000000586	0.000000755	-0.000000769
51	1	0.000000095	0.000000536	-0.000001492
52	1	0.000000020	0.000000500	-0.000000594
53	1	-0.000000336	0.000001109	-0.000000515
54	1	-0.000000275	0.000000117	-0.000000126
55	1	0.000000231	0.000000404	0.000000320

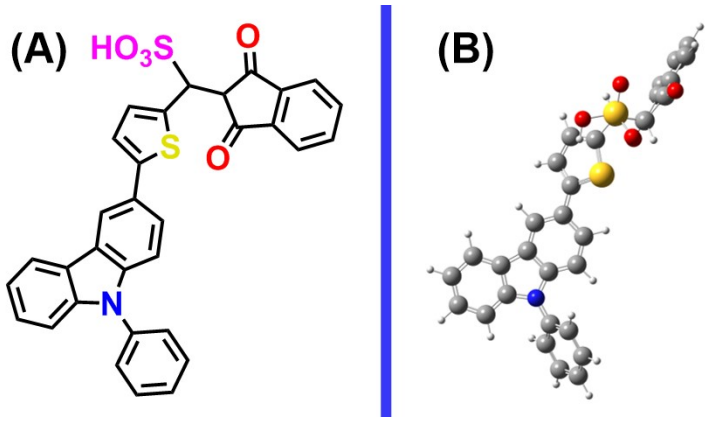

Fig. S6 (A) Molecular structure of **QPM-**SO_3H, and (B) optimized molecular geometry of **QPM-**SO_3H in the ground state (S_0) obtained from DFT calculations at B3LYP//6-31G(d,p) level of theory.

Table S2 Cartesian coordinates of **QPM-**SO_3H in the ground state (S_0).

Coordinates (ground state S_0)

Center Number	Atomic Number	Forces(Hartrees/Bohr)		
		X	Y	Z
1	6	0.000086206	-0.000097580	-0.000040240
2	6	0.000036091	0.000129648	-0.000011567
3	6	0.000134083	0.000094185	-0.000003643
4	6	-0.000331713	0.000196627	0.000227075
5	6	0.000105981	-0.000244753	0.000345223
6	6	-0.000102467	-0.000042826	-0.000239690
7	7	-0.000084028	0.000033936	0.000375645
8	6	0.000547240	0.000599816	-0.000009580
9	6	-0.000178439	0.000285314	-0.000439098
10	6	-0.000050290	0.000021810	0.000112656
11	6	0.000021008	-0.000365910	-0.000081372
12	6	-0.000181714	-0.000044448	-0.000102771
13	6	0.000063293	-0.000161610	0.000201165
14	6	-0.000146194	-0.000786052	-0.000565446
15	6	-0.000100779	-0.000040123	0.000024927
16	6	-0.000063514	0.000047217	0.000031086
17	6	-0.000039241	0.000130766	0.000023503
18	6	0.000191598	-0.000040665	0.000009296
19	6	0.000173592	0.000040991	0.000046261
20	6	0.000639446	0.000106654	-0.000146534
21	16	-0.000338508	0.000772302	0.000296345
22	6	-0.000147084	-0.000381415	0.000429400
23	6	0.000252200	-0.000157797	0.000075888
24	6	0.000079193	0.000164993	-0.000134023
25	6	-0.000655531	-0.000329924	-0.000375630
26	6	0.000124184	0.000029624	0.000006026
27	6	0.000116724	0.000163546	0.000346176

28	6	0.000021425	0.000268854	-0.000021835
29	6	-0.000143015	0.000200717	0.000135274
30	6	0.000277367	-0.000635531	-0.000486060
31	6	0.000139515	-0.000072882	-0.000128212
32	6	-0.000058585	-0.000138103	0.000081326
33	6	-0.000008781	-0.000172638	0.000117683
34	6	-0.000010333	0.000088616	-0.000084139
35	8	-0.000155546	0.000346667	0.000339740
36	8	-0.000260626	0.000048389	-0.000210769
37	16	0.000355687	-0.000427344	0.000349200
38	8	-0.000152972	0.000157242	-0.000203823
39	8	-0.000268989	0.000291707	0.000002285
40	8	0.000334778	0.000100172	-0.000101311
41	1	-0.000011979	0.000024309	-0.000015743
42	1	-0.000017863	-0.000008514	0.000022203
43	1	0.000029901	-0.000040642	-0.000067518
44	1	-0.000013454	0.000017034	0.000048968
45	1	-0.000074655	-0.000065465	-0.000034801
46	1	-0.000145302	0.000063349	-0.000059161
47	1	0.000153299	0.000138029	0.000066109
48	1	-0.000001297	0.000052252	0.000011498
49	1	0.000024912	-0.000022087	-0.000032201
50	1	-0.000015122	-0.000005518	-0.000006705
51	1	-0.000028302	-0.000043969	-0.000001550
52	1	-0.000041471	0.000026103	0.000030654
53	1	0.000027671	0.000011575	0.000023866
54	1	-0.000046644	-0.000165514	-0.000038023
55	1	0.000104949	0.000126129	-0.000173731
56	1	0.000186906	-0.000106686	0.000159647
57	1	-0.000045966	-0.000009112	-0.000012467
58	1	-0.000009269	0.000027330	0.000000108
59	1	0.000017984	-0.000002649	-0.000014249
60	1	0.000038561	0.000006383	-0.000004156
61	1	-0.000354124	-0.000202530	-0.000093186

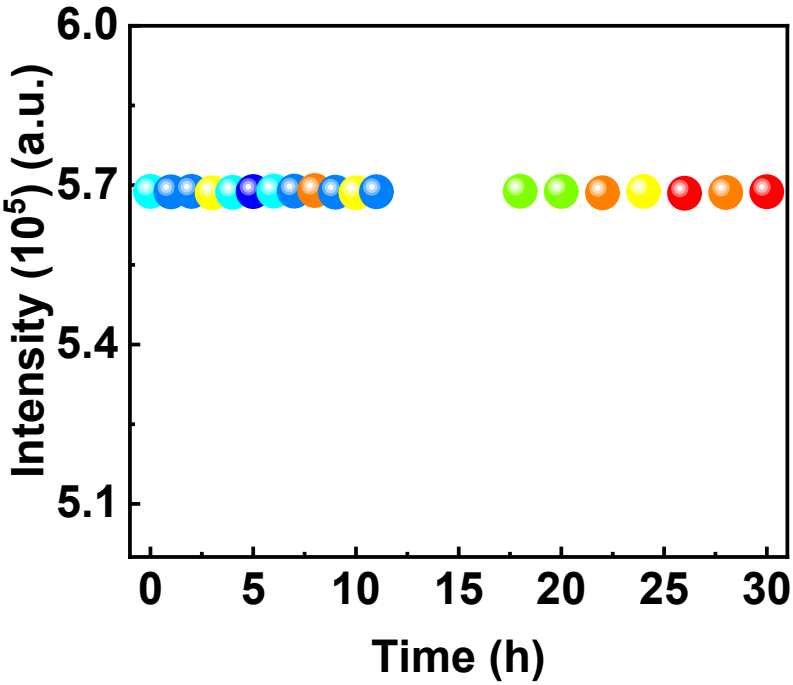

Fig. S7 Fluorescence changes of **QPM** (10 μM) at different time in Tris buffer solution (Tris:DMSO=7:3, pH=7.4, containing 1 mM CTAB). The intensities were recorded at 710 nm, excitation was performed at 490 nm.

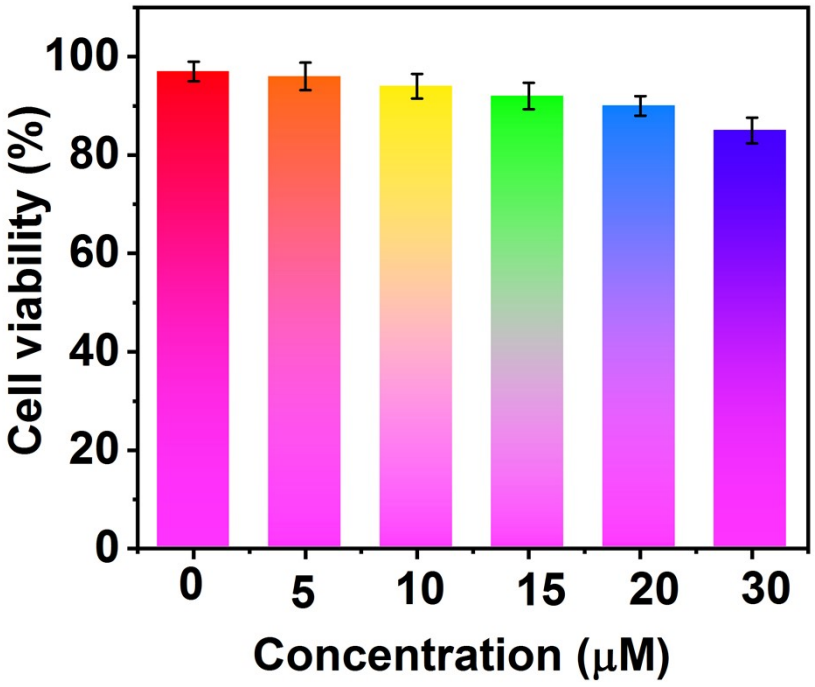

Fig. S8 The viability of A549 cells incubated with **QPM** (0−30 μM) for 24 h.

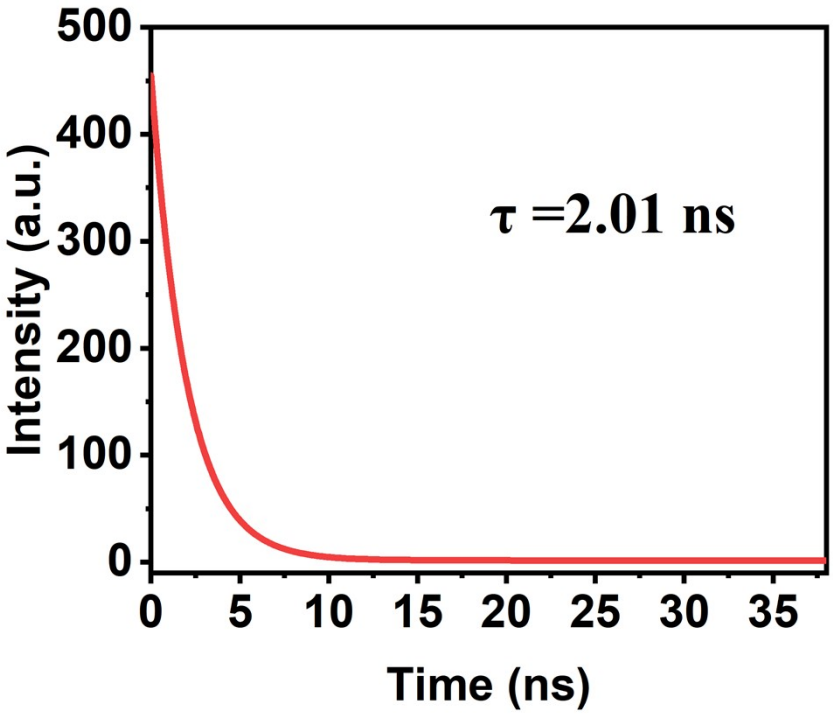

Fig. S9 The photoexcited decay curves of **QPM**.

Table S3. Comparison of this work with reported fluorescent probes for HSO_3^- detection.

Probes	Emission wavelength	Detection limit	Response time	Detection of HSO_3^- in live animals	Detection of HSO_3^- in food samples	Ref.
BFO-SFT	515 nm	152 nM	20 min	Mouse	No	S3
NG-TCF	550 nm/670 nm	1.53 μM	7 min	No	No	S4
probe 1	530 nm/590 nm	0.37 μM	30 s	Zebrafish	Yes	S5
PCPT	568 nm/648 nm	80.5 nM	15 min	No	No	S6
NBIS	534 nm/610 nm	16.2 nM	12 min	No	No	S7
BCVTI	608 nm	3.3 nM	4 min	No	No	S8
HDI	460 nm/565 nm	80 nM	2 min	No	No	S9
DQ	620 nm	0.11 μM	15 s	No	No	S10
CMQ	640 nm	15.6 nM	5 s	Zebrafish	No	S11

probe 1	450 nm/594 nm	3.21 μM	2.5 min	No	No	S12
Ru-azo	635 nm	0.69 μM	60 min	No	No	S13
probe 1	514 nm	22.8 nM	10 s	No	No	S14
MS-Bindol	490 nm	0.2 nM	90 s	No	Yes	S15
MITO-TPE	455 nm	27.22 μM	20 s	Zebrafish	No	S16
Hcy-Mo	596 nm	80 nM	30 s	Mouse	No	S17
RBC	456 nm/583 nm	6.6×10^{-8} M	35 s	No	No	S18
TBQN	514 nm	3.19×10^{-8} M	3 min	No	No	S19
Q5	485 nm/650 nm	89 nM	within 0.5 h	No	No	S20
QPM	710 nm	58 nM	45 s	Zebrafish/mouse	Yes	**This work**

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