Supporting Information for

A single small molecule fluorescent probe for imaging RNA distribution and detecting endogenous SO_2 through distinct fluorescence channels

Zhaomin Wang, [a] Yong Liu, [a] Weishan Wang, [a] Chang Zhao, [a] and Weiying Lin *, [a, b]

[a] Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, People's Republic of China.

[b] Guangxi Key Laboratory of Electrochemical Energy Materials, Institute of Optical Materials and Chemical Biology, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P.R. China.

E-mail: weiyinglin2013@163.com

Table of content

1 Materials

Firstly, for all experiment, all reagents of synthesis and analysis experiment were obtained by commercial suppliers. These reagents do not further purification before experiment.

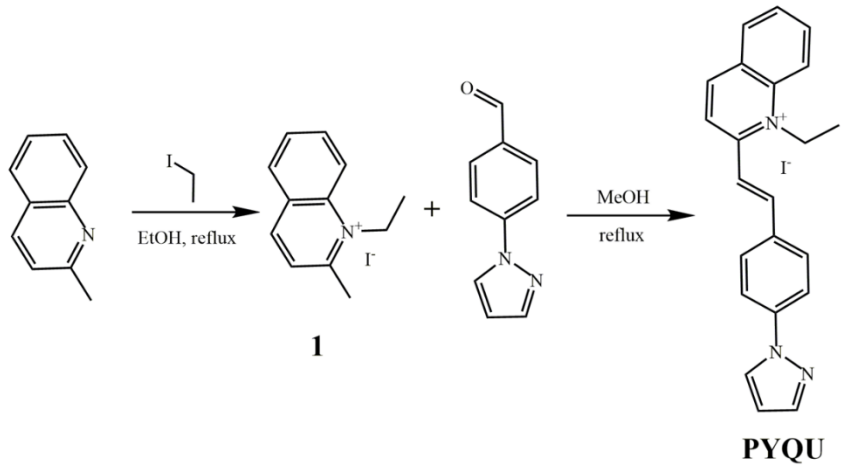
Secondly, for synthesis experiment, all separation and purification of compounds were determined by thin-layer chromatography analysis. This method was performed on silica gel plates; In addition, column chromatography was carried out by silica gel (mesh 200-300); Silica gel was obtained from the Qingdao Ocean Chemicals.

Thirdly, for characterization of compounds, mass spectra were demonstrated by an LCQ advantage ion trap mass spectrometer. It models is Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer; NMR spectra were obtained by the AVANCE III 400 MHz Digital NMR spectrometer.

Fourthly, for analysis experiment, ultraviolet absorption spectra were measured by a Labtech UV Power PC spectrometer; Fluorescence emission spectra were recorded with the HITACHI F4600 fluorescence spectrophotometer.

Fifthly, for biological imaging, fluorescence imaging of the cells and tissues slices was obtained with Nikon A1MP two-photon confocal microscopy. Two-photon imaging was conducted on with Nikon A1MP two-photon confocal microscopy (a Chameleon Vision II: Range 680~1080 nm, a repetition rate of 80 MHz.). In vivo imaging was conducted on IVIS Lumina XR living animal imaging system.

2 Synthesis



1 **PYQU**

Scheme S1 The synthetic route for **PYQU**.

3 Quantum Yields

The fluorescence quantum yields are calculated by the following equation:

$$\Phi_s = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{n_s^2}{n_r^2} \right) \frac{F_s}{F_r}$$

In the above equation, s and r stand for sample and the reference, respectively; ϕ and F is quantum yield and integrated emission intensity, respectively. A and n is absorbance and refractive index, resprctively.

4 Supplementary Figure and Table

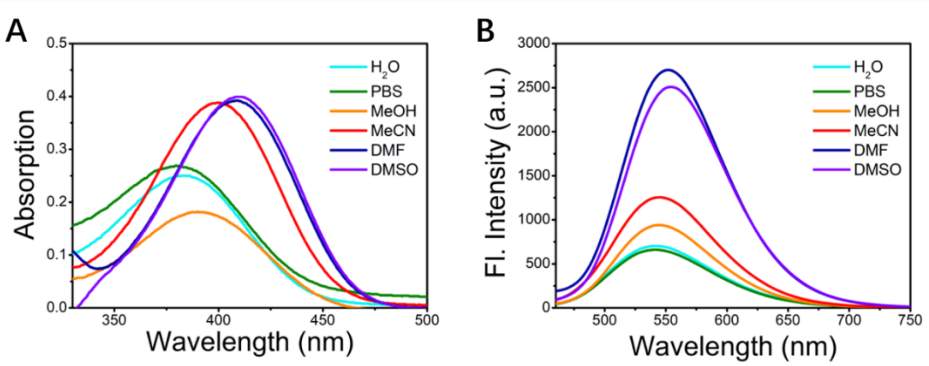


Figure S1 (A) Absorption spectra and (B) fluorescence responses of **PYQU** in various solvents. [**PYQU**]: 10 μM.

Table S1 The photophysical properties of **PYQU** in various solvents

Table S1 The photophysical properties of **PYQU** in various solvents				
Solvents	**λ^a**	**λ^b**	**Stokes shifts**	**$^c\Phi$**
H_2O	380	541	161	0.41%
PBS	380	541	161	0.37%
MeOH	390	545	155	0.71%
MeCN	400	545	145	0.49%
DMF	408	552	144	1.19%
DMSO	410	555	145	1.09%
λ^a is maximum absorption wavelength (nm). λ^b Maximum emission wavelength (nm). $^c\Phi$ is fluorescence quantum yield (error limit: 8%) determined by using Quinine Sulfate (Φ=0.58).				

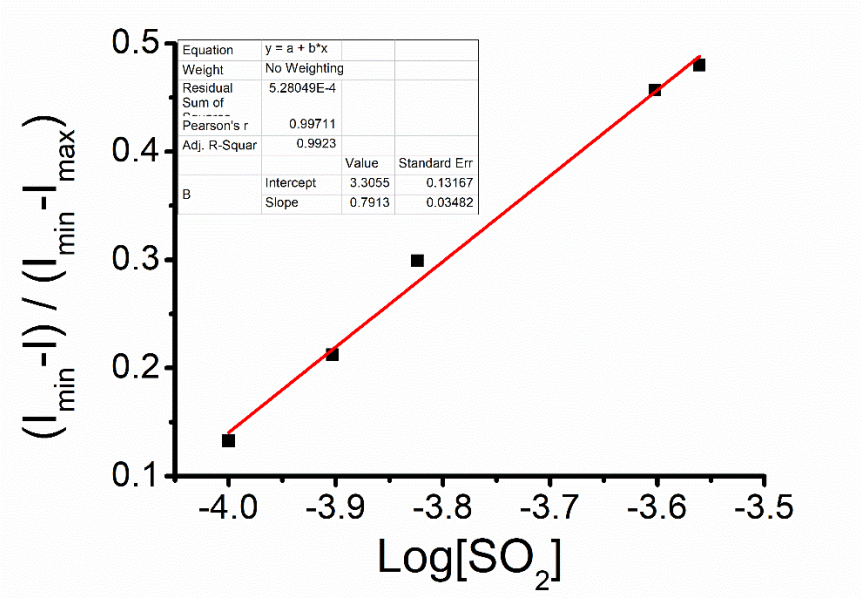


Figure S2 The limit of detection for SO_2.

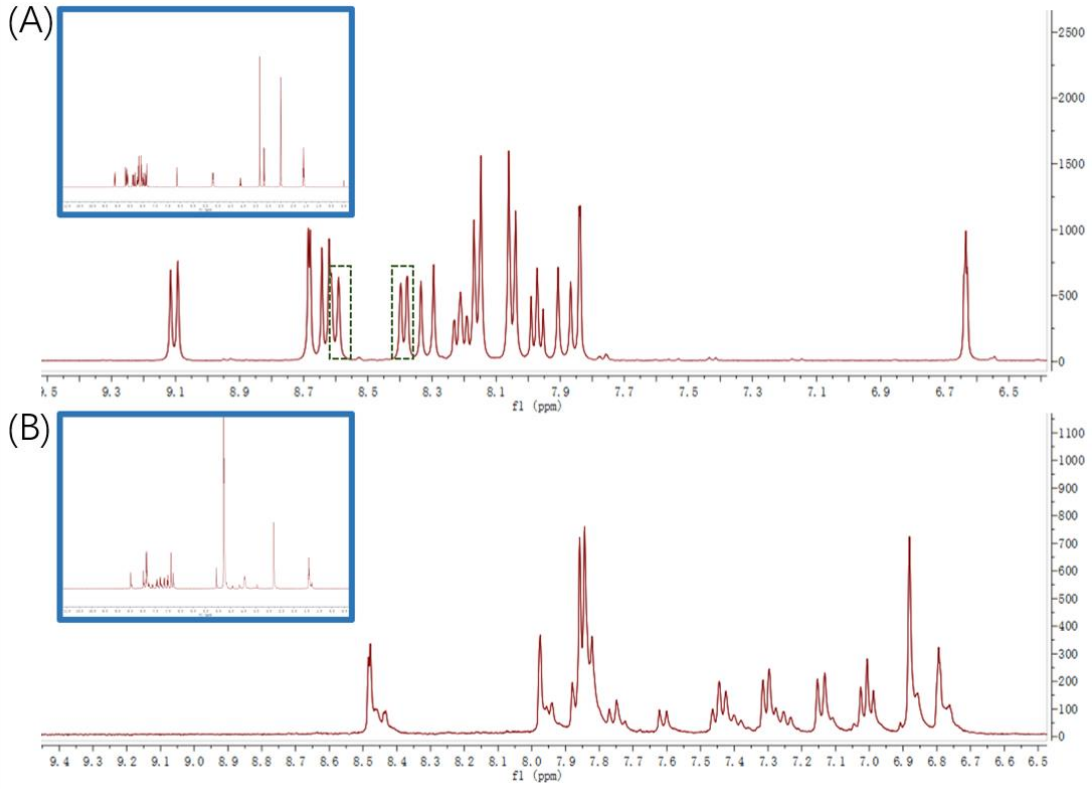


Figure S3 (A) The ^{1}H NMR spectra of **PYQU** in DMSO-*d*$_6$. (B) The ^{1}H NMR spectra of **PYQU** with Na_2SO_3 in DMSO-*d*$_6$ and D_2O (v : v=1:1).

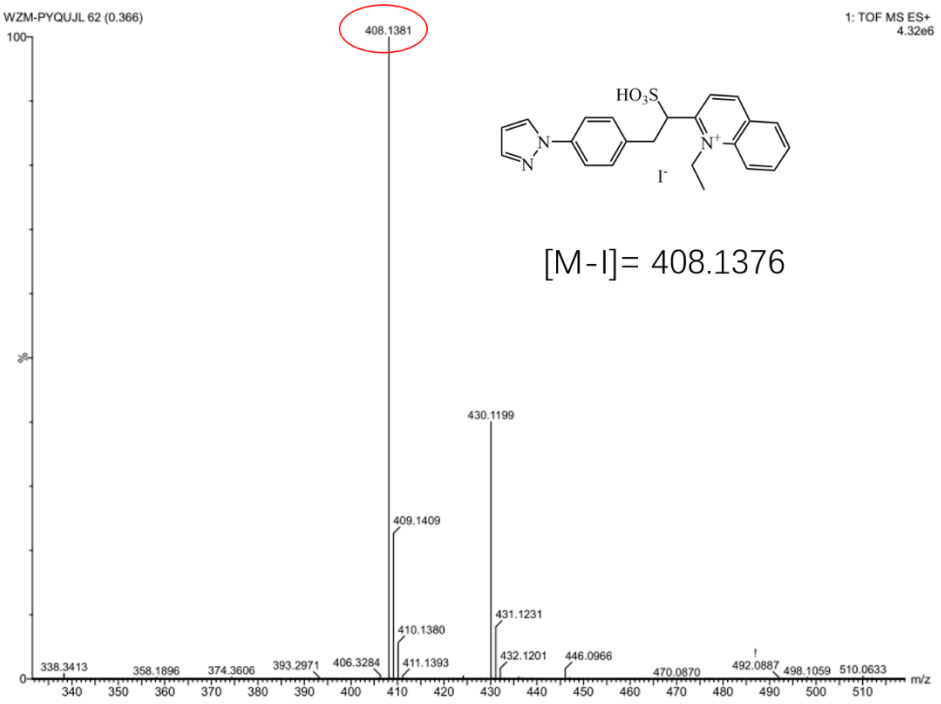


Figure S4 HR-MS spectrum of the **PYQU** in the presence of excessive Na_2SO_3.

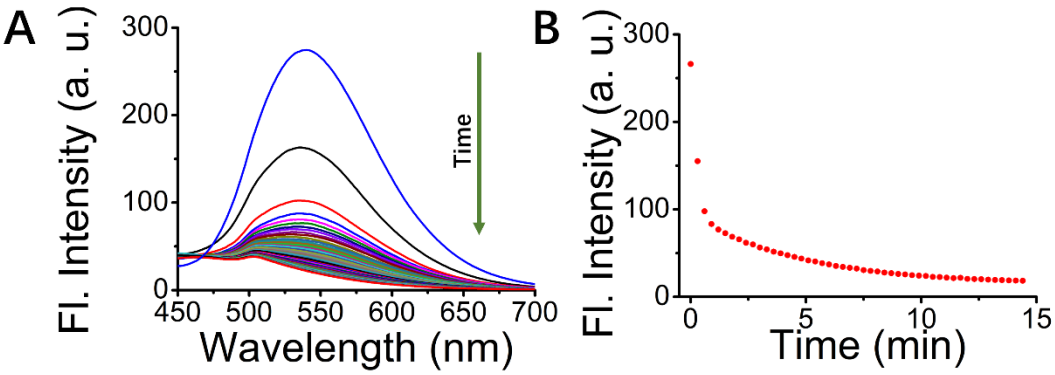


Figure S5 Time-dependent fluorescence response of **PYQU** (5 μM) in the presence of SO_3^{2-} (1 mM) when excited at 425 nm.

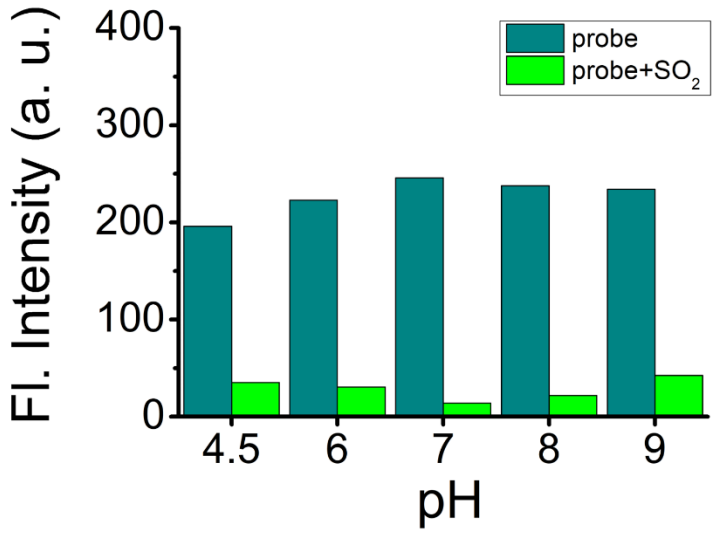


Figure S6 The response of **PYQU** (5 μM) to SO_2 (1 mM) in different pH PBS.

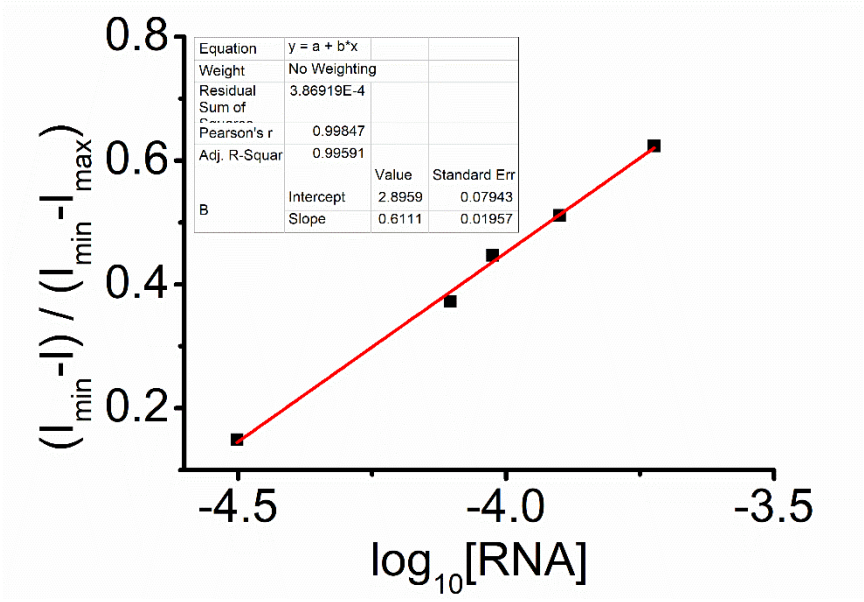


Figure S7 The limit of detection for RNA.

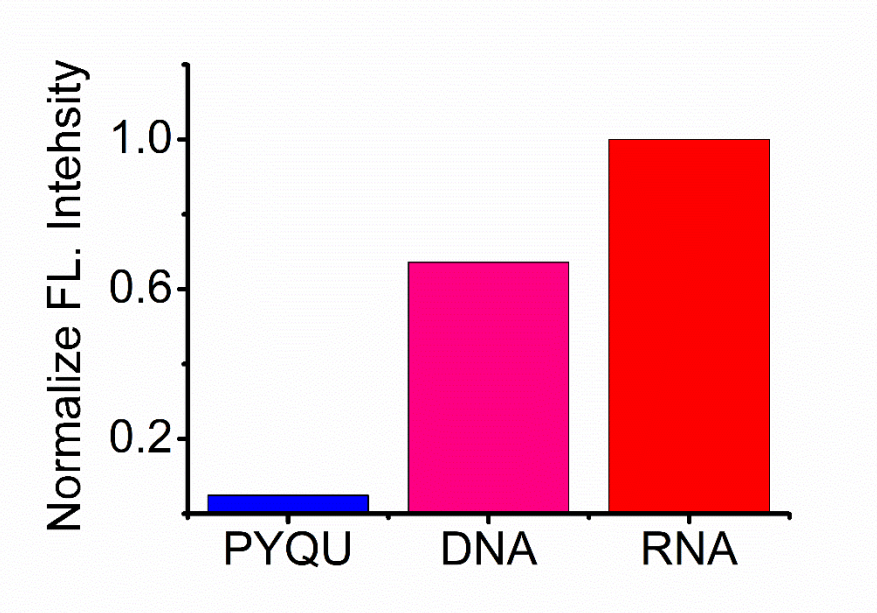


Figure S8 Fluorescence responses of commercial probe to RNA and DNA in Tris-Hcl solution.

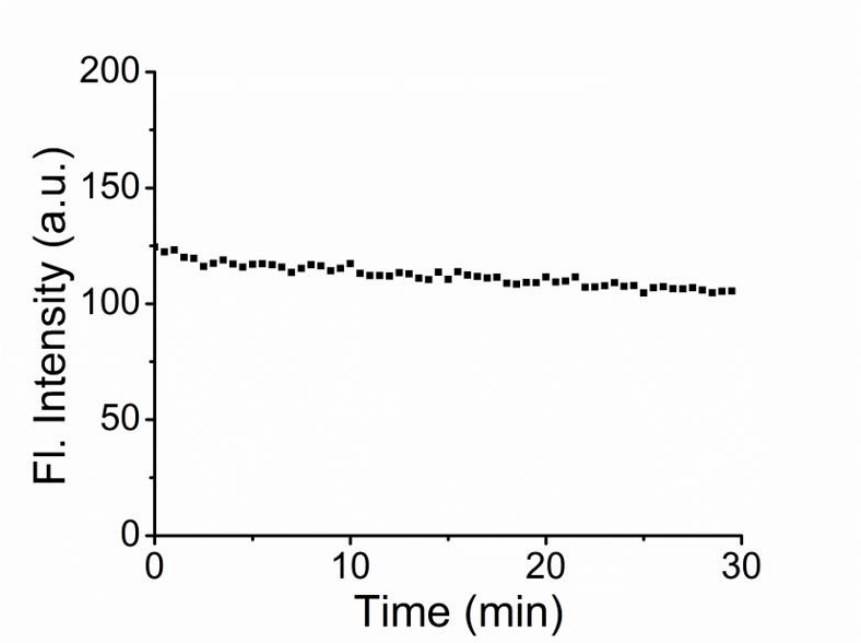


Figure S9 Time courses of fluorescence intensity at 650 nm of **PYQU** (5 μM) containing RNA (2 mM) in buffer solution. λ_{ex} = 560 nm.

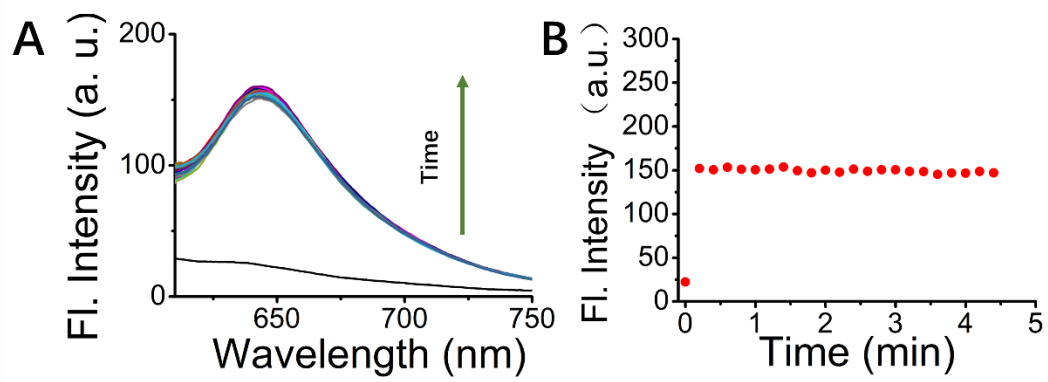


Figure S10 Time-dependent fluorescence response of **PYQU** (5 μM) in the presence of RNA (0.1 mM) when excited at 560 nm.

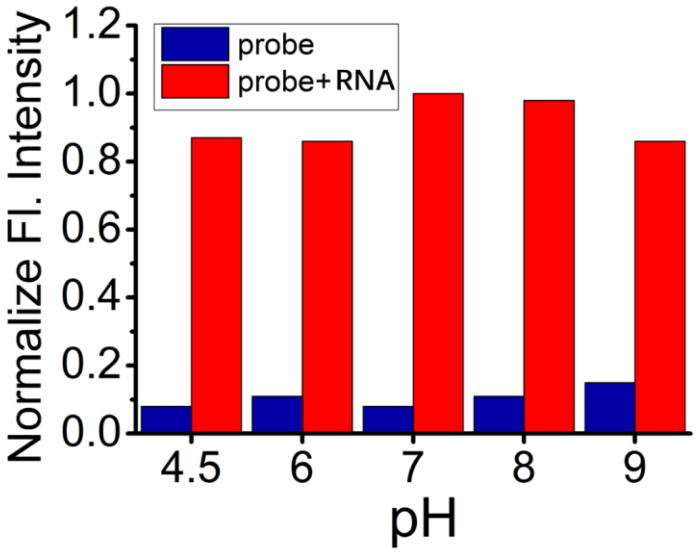


Figure S11 The response of **PYQU** (5 μM) to SO_2 (1 mM) in different pH buffer solution.

Table S2 MTT assay of HeLa cells in the presence of various concentrations of **PYQU**.

[**PYQU**] / μM	0	2	5	10	20	30
cell survival / %	100±4	96±4	96±4	93±4	86±4	85±4

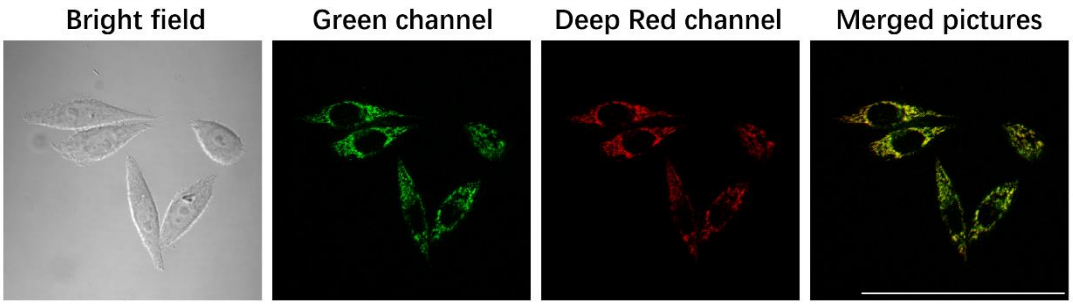


Figure S12 Confocal fluorescence images of living Hela cells incubated with **PYQU** (10 μM). Green fluorescence images were collected under excitation at 405 nm. Deep red fluorescence images were collected under excitation at 561 nm. Scale bar: 100 μm.

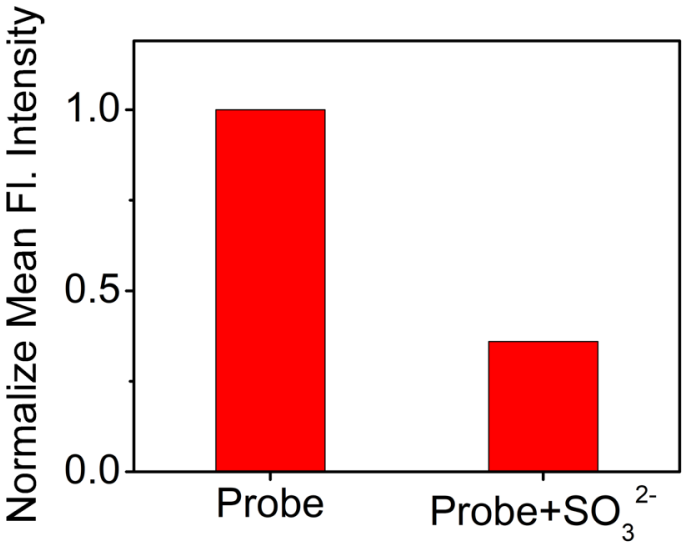


Figure S13 The normalize mean fluorescence intensity in HeLa cells untreated and treated with Na_2SO_3.

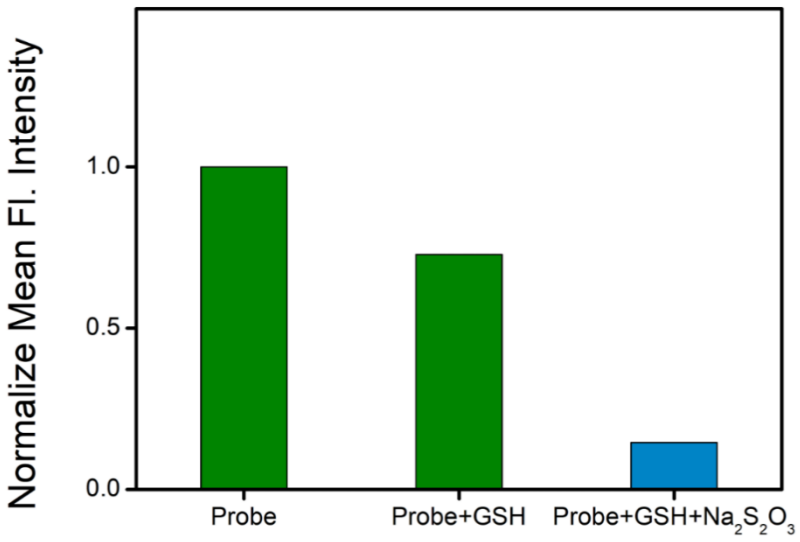


Figure S14 The normalize mean fluorescence intensity in HeLa cells untreated and treated with GSH and $Na_2S_2O_3$.

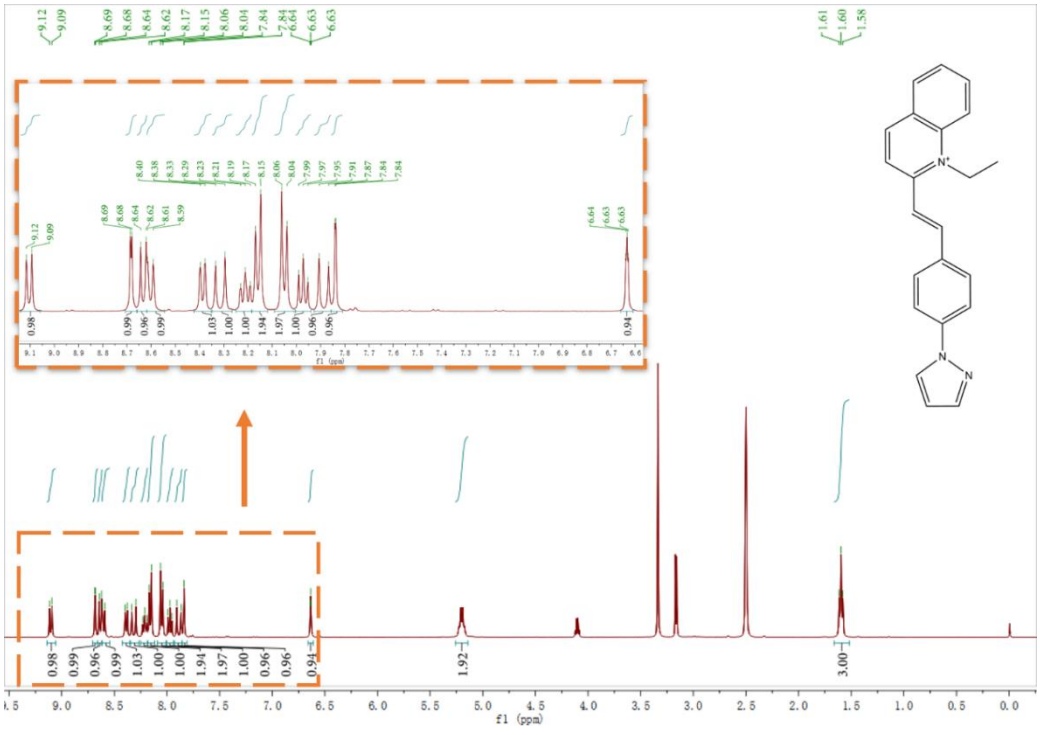


Figure S15 ^{1}H NMR spectrum of the compound **PYQU** in DMSO-*d*$_6$.

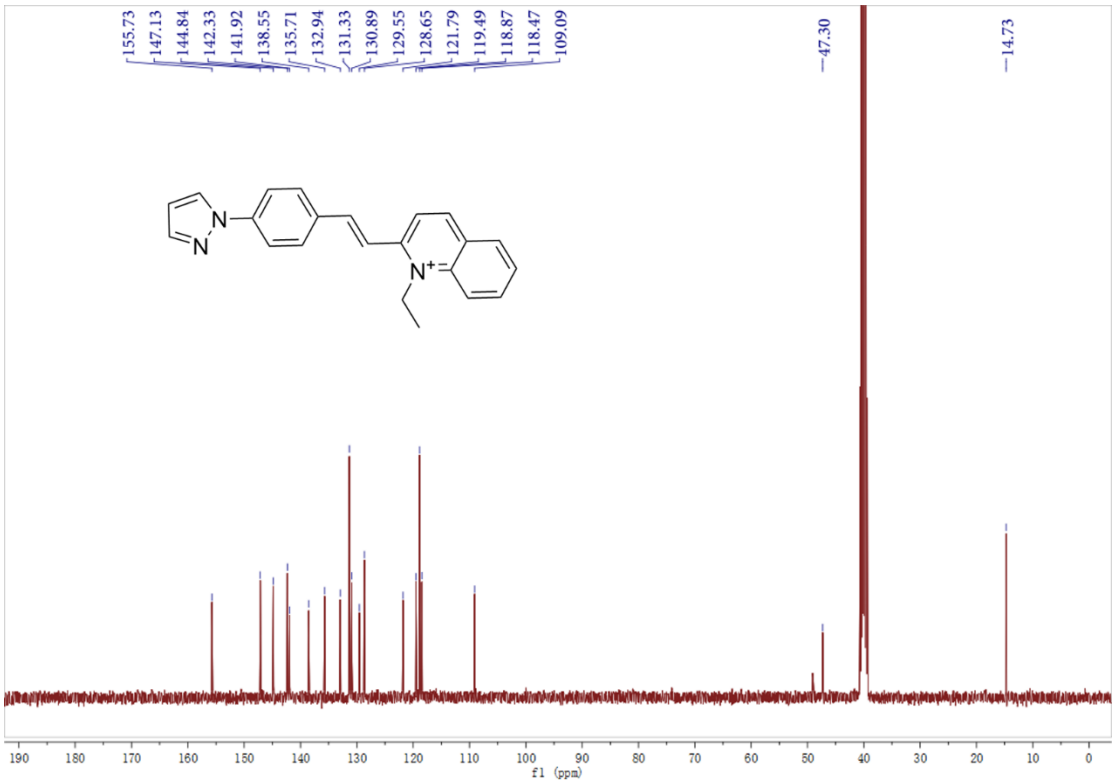


Figure S16 ^{13}C NMR spectrum of the compound **PYQU** in DMSO-d_6.

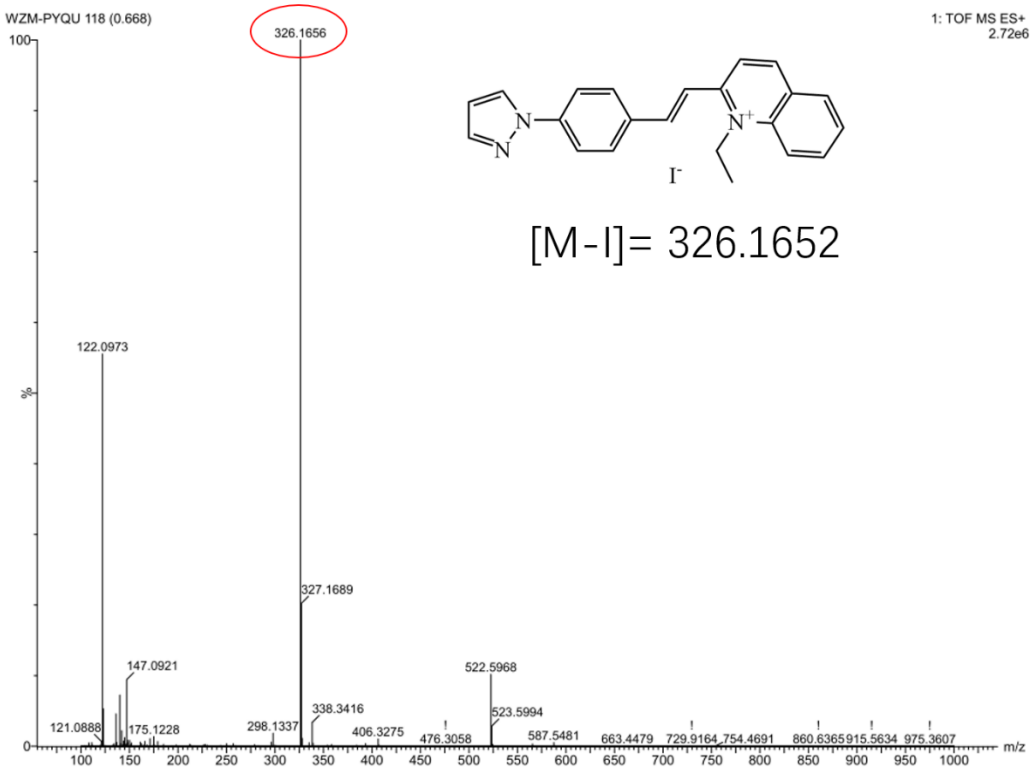


Figure S17 HRMS spectrum of the compound **PYQU.**