Supporting Information for

"AIE+ESIPT" ratiometric fluorescent probe for monitoring sulfur dioxide with distinct ratiometric fluorescence signals in mammalian cells, mouse embryonic fibroblast and zebrafish

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#*Liu and Nie made equal contributions to this work.*

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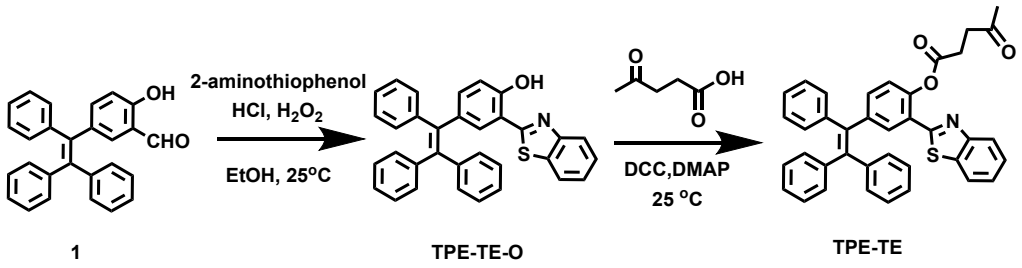
1. Synthesis

The compound **TPE-TE** was synthesized following the synthetic routine in Scheme S1.



Scheme S1. The synthetic routes of the compound **TPE-TE.**

2. Table S1.

Table S1. Properties of the representative SO_2 probes developed and the **TPE-TE** reported in this work.

Ref.	Core structure	Combination of an ESIPT Mechanism and the Aggregate Fluorescence Method	Ratiometric imaging	Detecting environment *In vitro*	Distinguishing imaging capacity of different cells lines	Application
This work	**TPE-TE**	Yes	Yes	PBS buffer solution (pH=7.4)	Yes	Clearly discriminating and monitoring sulfur dioxide in mammalian cells, mouse fibroblast predispose cells and zebrafish
1.	**1**	No	No	H_2O-DMSO (9:1, v/v)	No	Ratiometric Sensing sulfite *in vitro*
2.	**1**	No	No	H_2O-CH_3CN (98:2, v/v)	No	Sensing sulfite *in vitro*
3.	**Anthracene–aldehyde dyad**	No	No	HEPES-DMSO (98:2, v/v)	No	A fast-responding turn-on sensor for sensitive and selective detection of sulfite anions
4.	**1**	No	No	H_2O/CH_3CN (1:1, v/v).	No	Ratiometric Sensing sulfite *in vitro*
5.	**Lev–Rhol**	No	No	HEPES buffer solution (pH=7.5)	No	A continuous spectrophotometric assay for adenosine 50-phosphosulfate reductase activity with sulfite-selective probes
6.	**TSP1–3**	No	No	PBS buffer solution (pH=7.4)	No	Colorimetric and ratiometric fluorescent detection of sulfite in water

7.	**1**	No	No	HEPES-DMF (4:1, v/v)	No	A real-time colorimetric and ratiometric fluorescent probe for sulfite
8.	**C1**	No	No	THF–H_2O (1/99, v/v)	No	Detection of hydrogen sulfite in HeLa cells with turn-on fluorescent methods.
9.	**P-1**	No	No	THF–H_2O (3:7, v/v)	No	Detection of hydrogen sulfite in HeLa cells
10.	**1**	No	No	H_2O-CH_3CN (99:1, v/v)	No	High selectivity and sensitivity for sulfite detection.
11.	**m-PSP**	No	No	PBS buffer solution (pH=7.4)	No	Colorimetric and fluorescent determination of sulfide and sulfite
12.	**1 and 2**	No	No	HEPES buffer solution (pH=7.5)	No	Colorimetric and ratiometric sensing of SO_2 derivatives in living cells
13.	**1**	No	No	PBS buffer solution (pH=7.4)	No	Rapid Detection of Bisulfite through 1,4-Addition Reaction in Aqueous Solution
14.	**2**	No	Yes	PBS buffer solution (pH=7.4)	No	Ratiometric sensing of sulfite in living cells
15.	**1**	No	No	H_2O-DMSO (9:1, v/v)	No	A new fluorescent turn-on probe for highly sensitive and selective detection of sulfite and bisulfite
16.	**1**	No	No	PBS buffer (pH 7.4, 10.0 mM, 1 mM CTAB)	No	A ratiometric fluorescent probe for rapid, sensitive and selective detection of sulfur dioxide
17.	**TP-Mito/Ratio-SO2**	No	Yes	PBS-EtOH (4:1, v/v)	No	Two-photon fluorescent probe for ratiometric visualization of endogenous sulfur dioxide derivatives in mitochondria of living cells and tissues
18.	**HCy-D**	No	Yes	PBS-DMF (7:3, v/v)	No	Ratiometric detection of endogenous sulfur dioxide derivatives in cancer cells
19.	**CZ-Id**	No	No	PBS-DMF (7:3, v/v)	No	Colorimetric and ratiometric fluorescent probe for biological SO_2 derivatives in living cells†
20.	**APCT**	No	No	Tris-DMSO (3:7, v/v)	No	Detection of bisulfite in living cells
21.	**Cl-2**	No	No	PBS buffer solution (pH=7.4)	No	Monitor successfully the concentration change of endogenously generated

						SO2 derivatives in living cells.
22.	**NDB-Id**	No	No	PBS buffer solution (pH=7.4)	No	Imaging sulfur dioxide derivatives in the mitochondria of living cells
23.	**BIFS**	No	No	Glycerol/PBS solution = 4/6 (pH 7.40)	No	Rapid and specific detection of trace biological SO2 derivatives and bio-imaging applications
24.	**BSP1 and BSP2**	No	No	PBS-CTAB	No	Colorimetric/ratiometric fluorescence probes for sulfite in aqueous solution and in living cells
25.	**L**	No	No	In aqueous solution	No	Real-time sensing of SO_3^{2-} and SO_4^{2-} /HSO_4^- in aqueous medium and live cells.

3. Fig. S1.

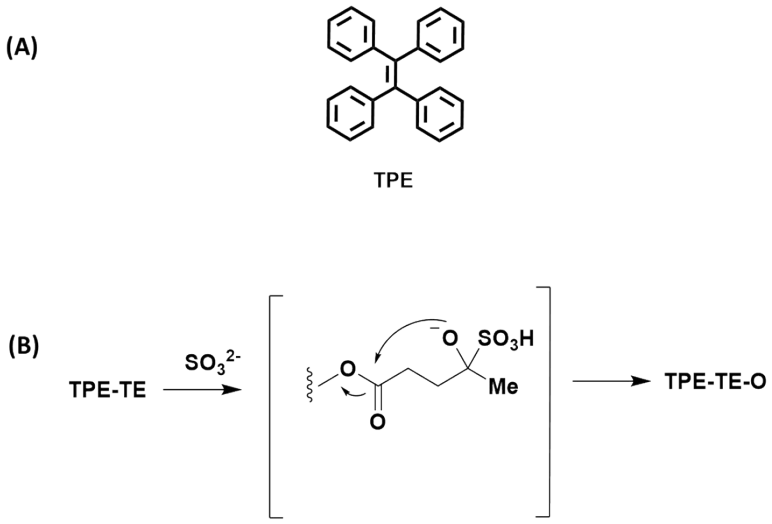


Fig. S1 (A) Structure of the AIE material TPE; (B) Detection mechanism of TPE-TE to SO_2.

4. Table S2.

The photophysical properties of **TPE-TE.**

Slovents	[a]□□nm	[b]□□nm	Stokes shifts	[c]□□□
CH_3CN	306	420	114	0.38
DMF	303	422	119	1.08
DMSO	306	417	111	0.34
MeOH	302	426	124	0.87
CH_2Cl_2	301	422	121	0.40
H_2O	317	465	158	23.0

[a]Maximum absorption wavelength (nm). [b]Maximum emission wavelength (nm). c is fluorescence quantum yield (error limit: 8%) determined by using fluorescein (Φ=0.95) in aqueous NaOH (pH = 13) as the standard.

5. Fig. S2

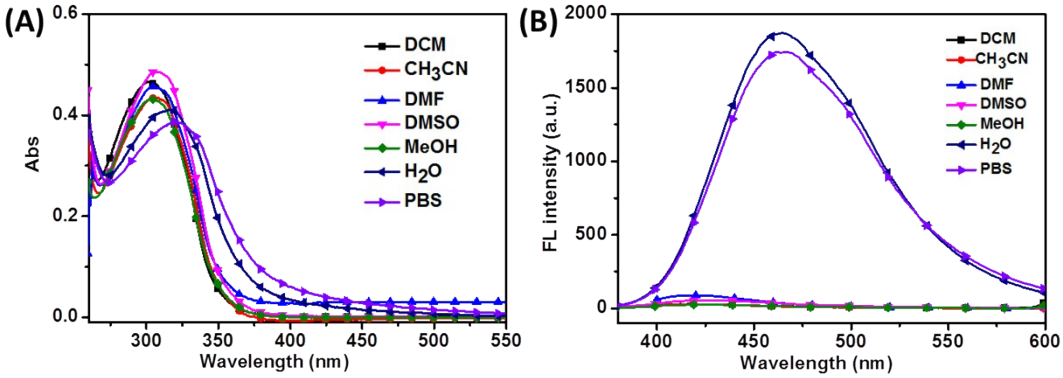


Fig. S2 (A) The UV–vis absorption and (B) fluorescence spectra of **TPE-TE** in aqueous solutions and organic solvents. [**TPE-TE**] = 5.0 μM.

6. Fig. S3

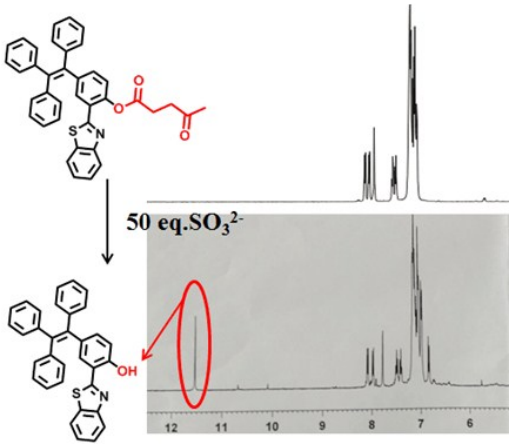


Fig. S3 The ^{1}H NMR spectrum of the hydroxy peak of **TPE-TE** in the absence and presence of SO_2 (50 equiv) in d_6-DMSO/D_2O (4:1).

7. Table S3.

Table S3. Cytotoxicity data of the probe **TPE-TE.**

Incubate concentration(μM)	0	1	5	10	20	30
(% cell survival)	100±4	100±4	95±4	89±4	80±4	83±4

8. Fig. S4

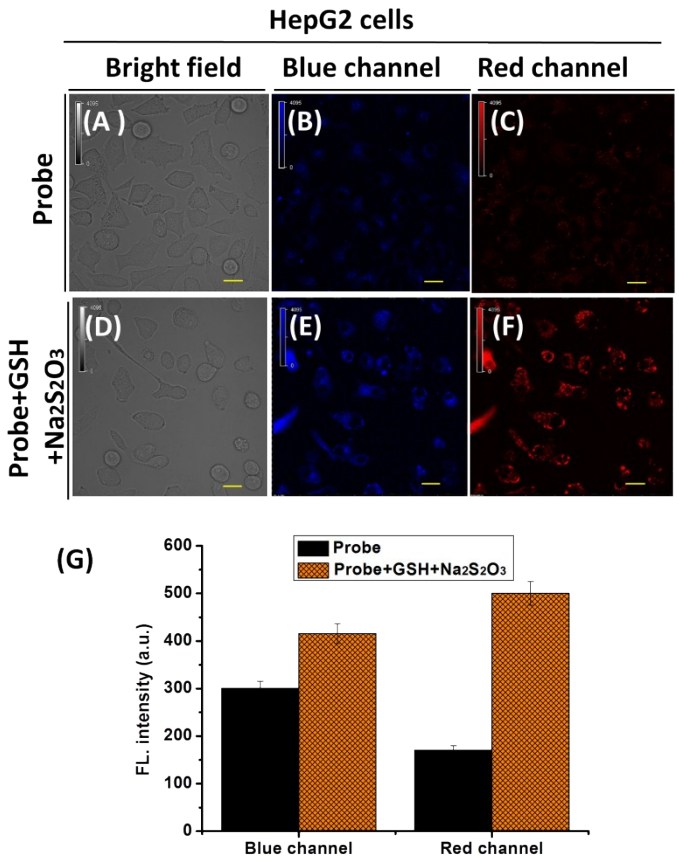


Fig. S4 The first line (parallel): (A-C): HepG2 cells were incubated with **TPE-TE** (5 μM) for 30 min; The second line (D-F): HepG2 cells were incubated with **TPE-TE** (5 μM) for 30 min, and then with 500 μM GSH and 250 μM $Na_2S_2O_3$ for 0.5 h. (G): Fluorescent intensity of **TPE-TE** untreated and treated with GSH and $Na_2S_2O_3$ in blue channel and red channel. Images were acquired from 425 to 475 nm for blue fluorescence, and from 570 to 620 nm for red fluorescence. λ_{ex} = 405 nm. Scale bar = 20 μm.

9. **Fig. S5.**

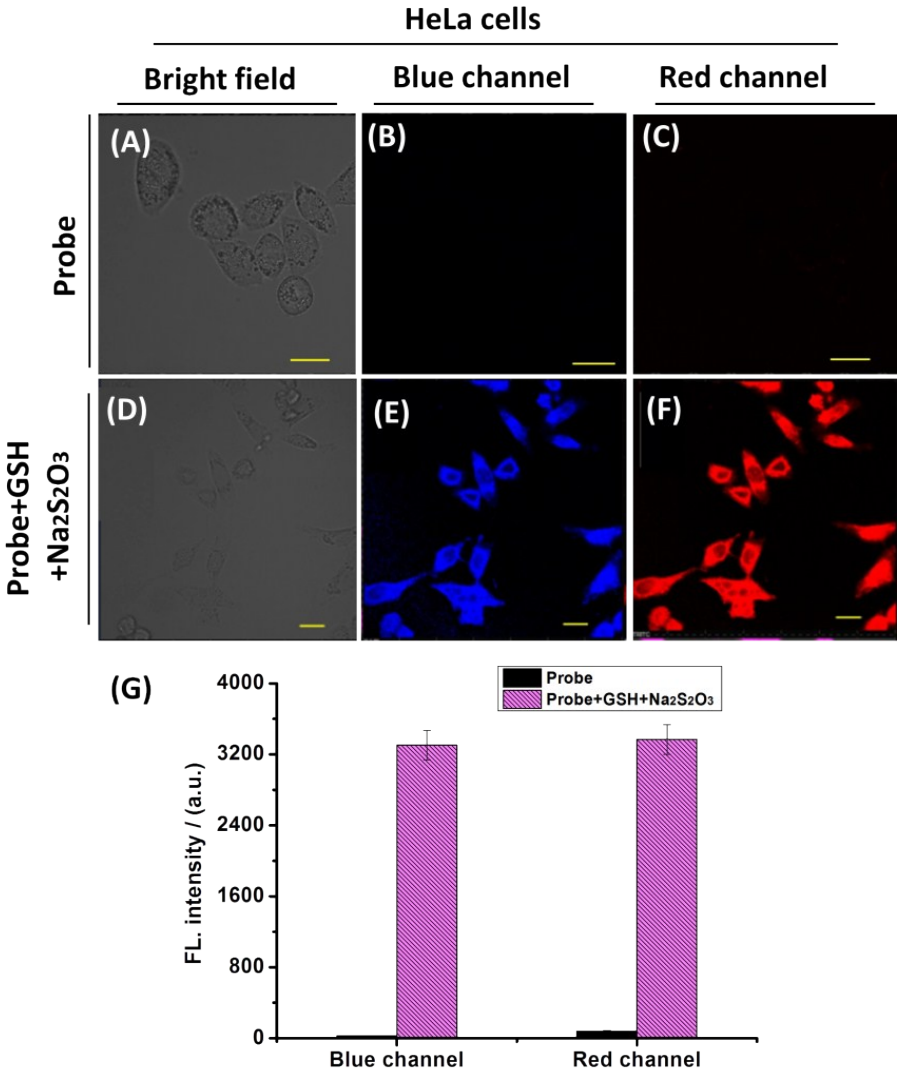


Fig. S5. The first line (parallel): (A-C): HeLa cells were incubated with **TPE-TE** (5 μM) for 30 min; The second line (D-F): HeLa cells were incubated with **TPE-TE** (5 μM) for 30 min, and then with 500 μM GSH and 250 μM $Na_2S_2O_3$ for 0.5 h. (G): Fluorescent intensity of **TPE-TE** untreated and treated with GSH and $Na_2S_2O_3$ in blue channel and red channel. Images were acquired from 425 to 475 nm for blue fluorescence, and from 570 to 620 nm for red fluorescence. λ_{ex} = 405 nm. Scale bar = 20 μm.

10. Fig. S6.

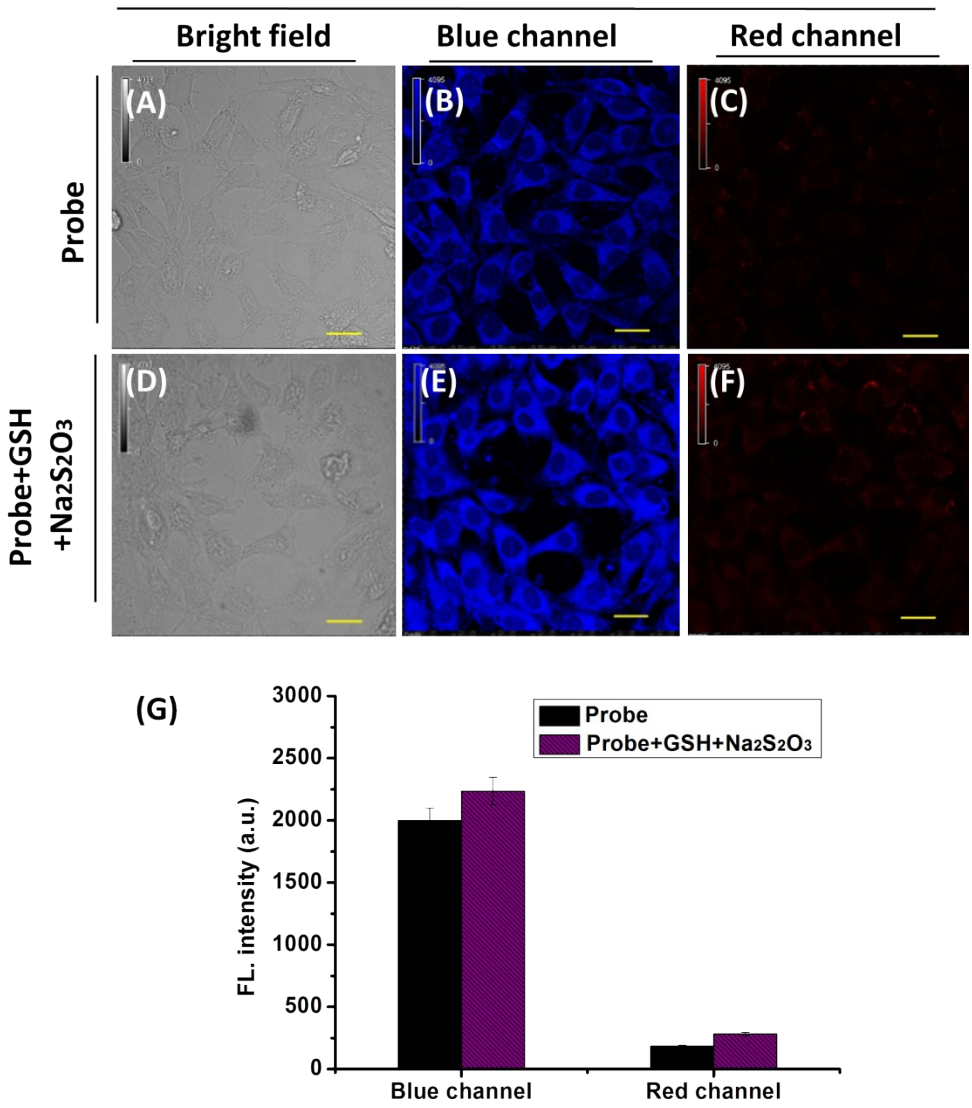


Fig. S6 The first line (parallel): (A-C): 3T3 cells were incubated with **TPE-TE** (5 μM) for 30 min; The second line (D-F): 3T3 cells were incubated with **TPE-TE** (5 μM) for 30 min, and then with 500 μM GSH and 250 μM $Na_2S_2O_3$ for 0.5 h. (G): Fluorescent intensity of **TPE-TE** untreated and treated with GSH and $Na_2S_2O_3$ in blue channel and red channel. Images were acquired from 425 to 475 nm for blue fluorescence, and from 570 to 620 nm for red fluorescence. λ_{ex} = 405 nm. Scale bar = 20 μm.

11. Fig. S7

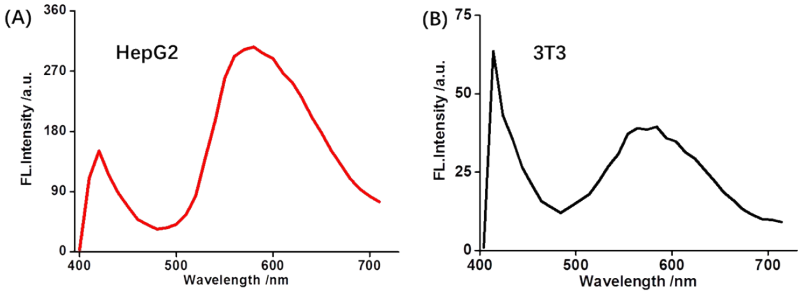


Fig.7 *In situ* fluorescence spectra of the **TPE-TE** (5 μM) HepG2 and 3T3 cells with excitation at 405 nm.

12. Fig. S8

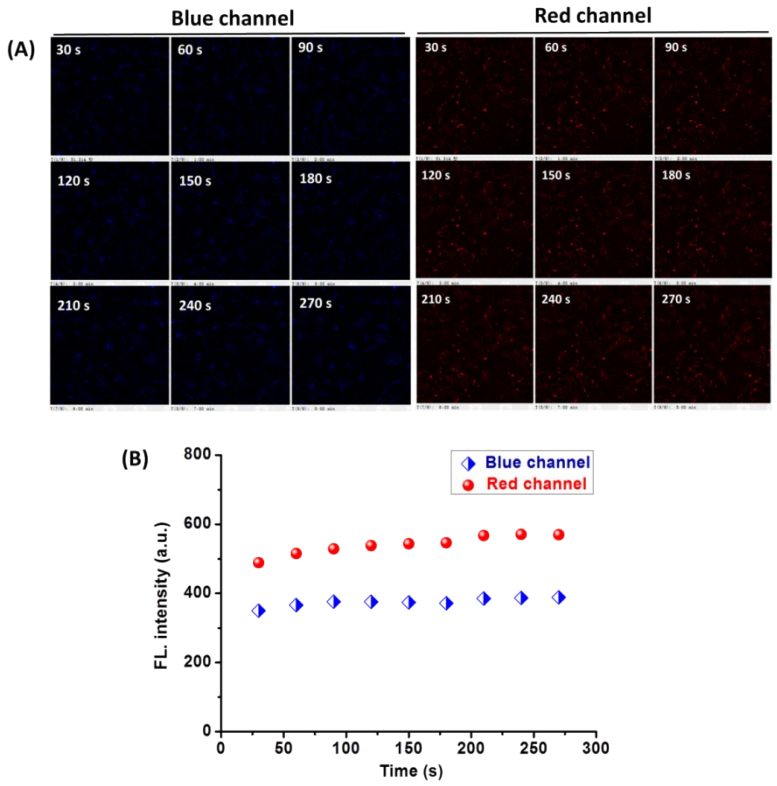


Fig. S8 (A) Fluorescence images (the blue channel and red channel) of HepG2 cells incubated with were incubated with **TPE-TE** (5 μM), 500 μM GSH and 250 μM $Na_2S_2O_3$ acquired at different times under successive excitation. (B) Mean intensities of the cells incubated in the red channel under successive excitation at different times. Images were acquired from 425 to 475 nm for blue fluorescence, and from 570 to 620 nm for red fluorescence. λ_{ex} = 405 nm. Scale bar = 20 μm.

13. Fig. S9

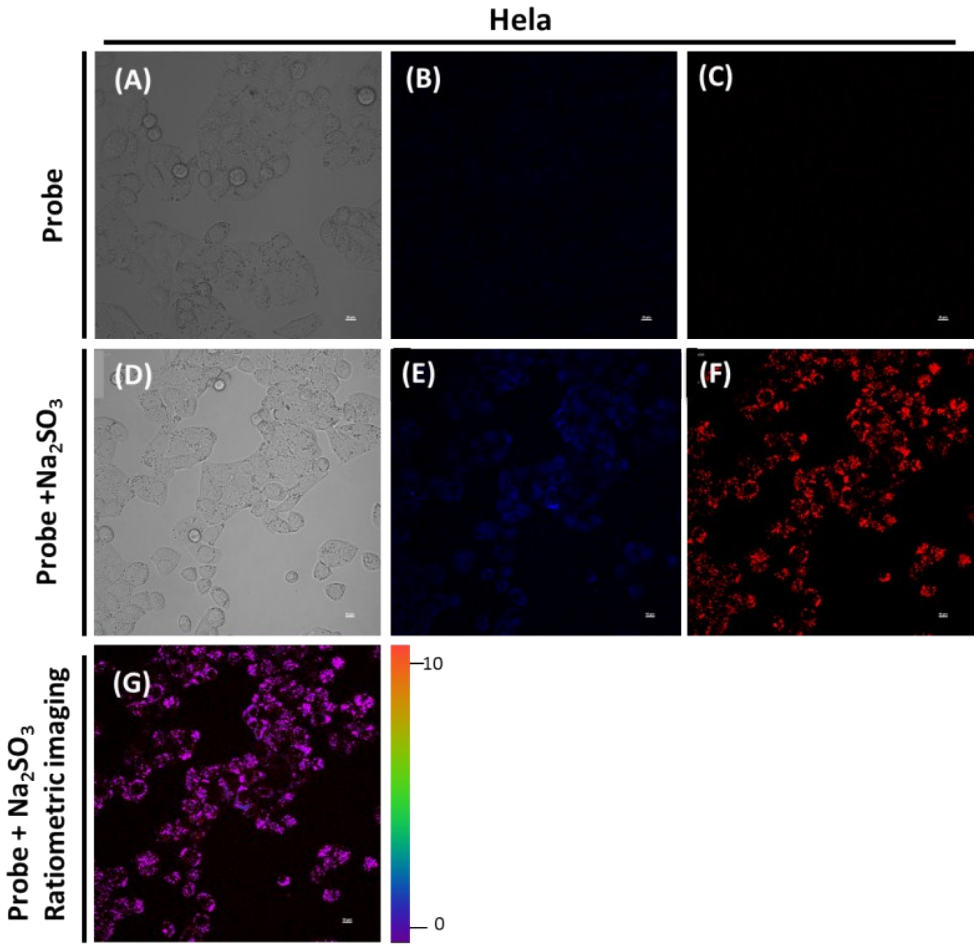


Fig. S9 Cells image of SO_2 in HeLa cells untreated and treated with Na_2SO_3; (A-C) Images of HeLa cells untreated with Na_2SO_3; (B-F) Images of HeLa cells treated with Na_2SO_3 in the blue emission channels (λ_{ex} = 405 nm, λ_{em} = 425‑475 nm); (G) Ratiometric Images of HeLa cells in the blue and red emission channels. Scale bar = 20 μm.

14. Spectral Characterization

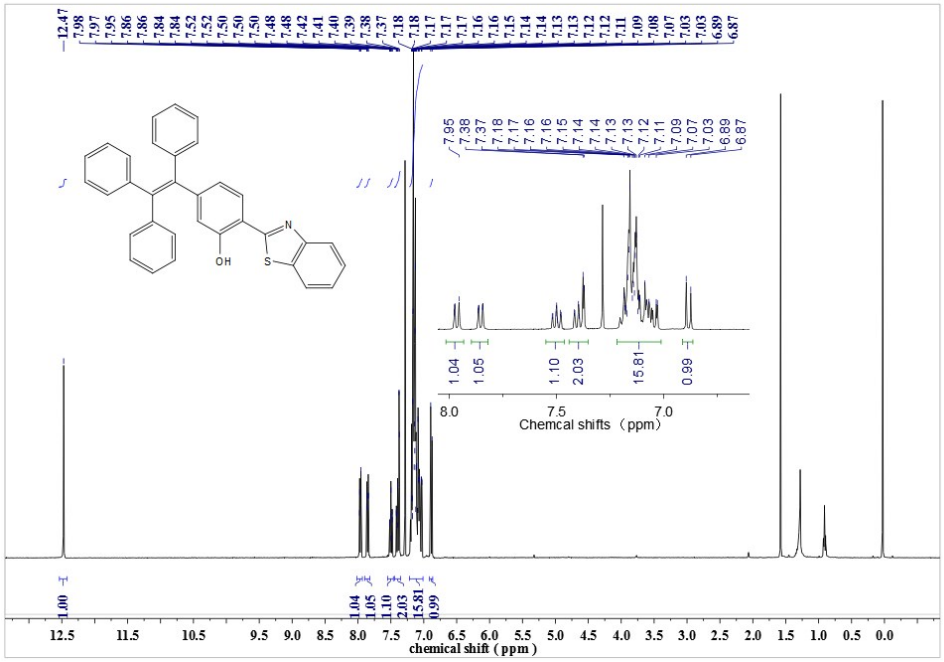


Fig. S10 ^{1}H NMR spectrum of the compound TPE-TEO.

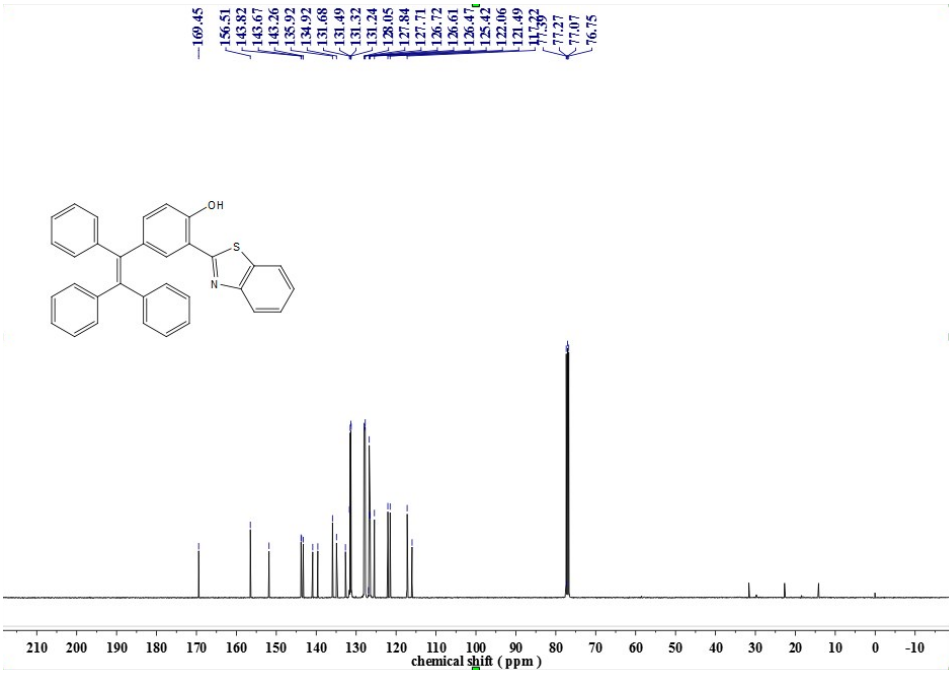


Fig. S11 ^{13}C NMR spectrum of the compound TPE-TEO.

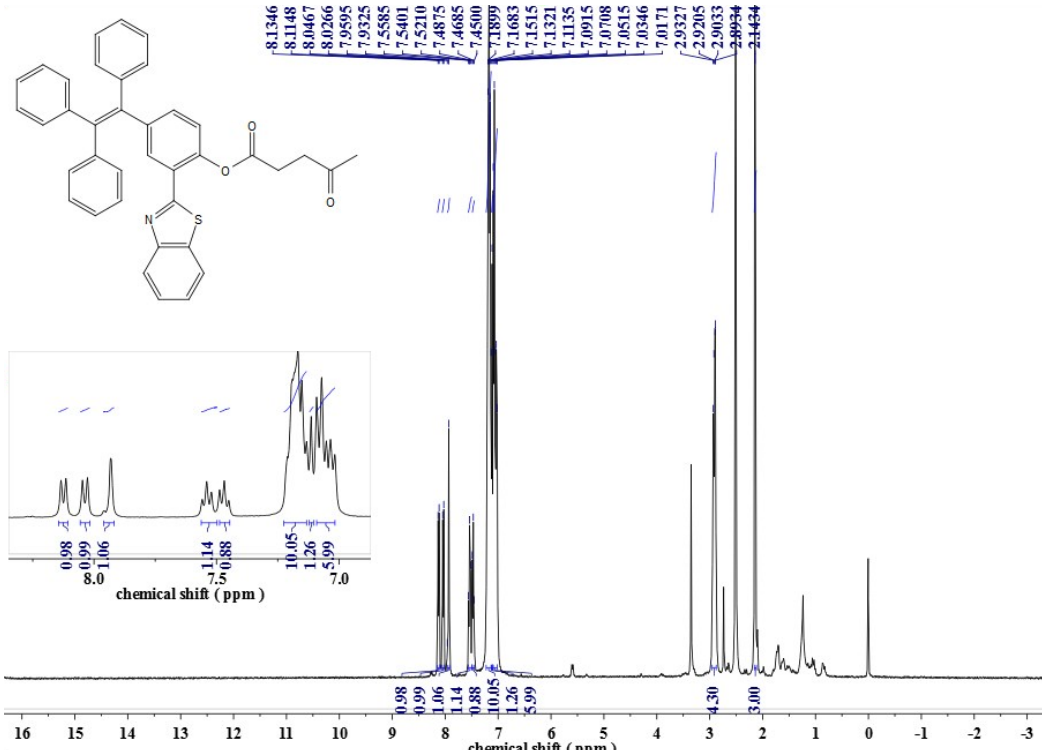


Fig. S12 ^{1}H NMR spectrum of **TPE-TE.**

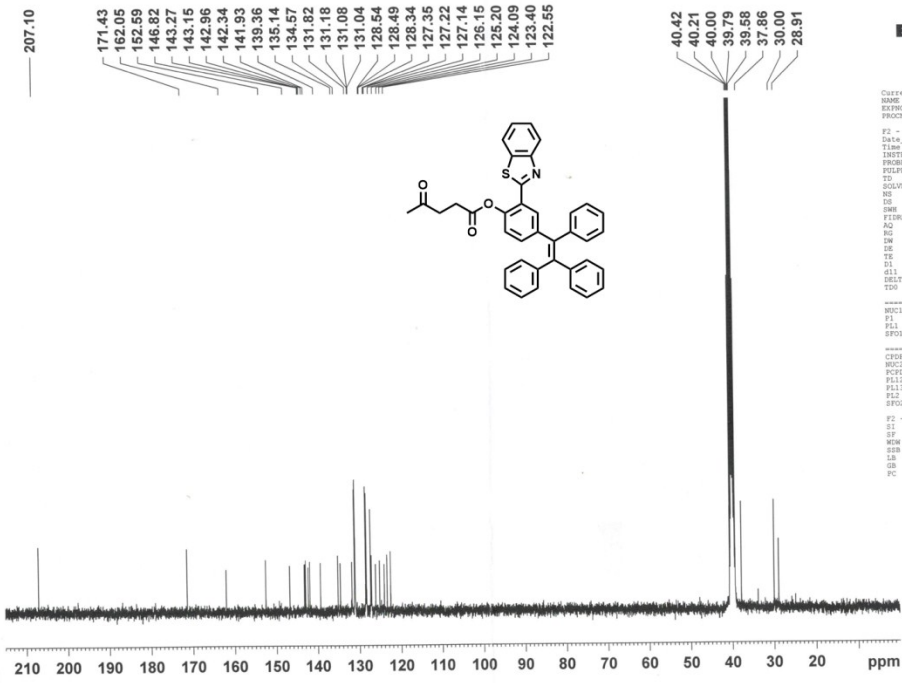


Fig. S13 ^{13}C NMR spectrum of **TPE-TE.**

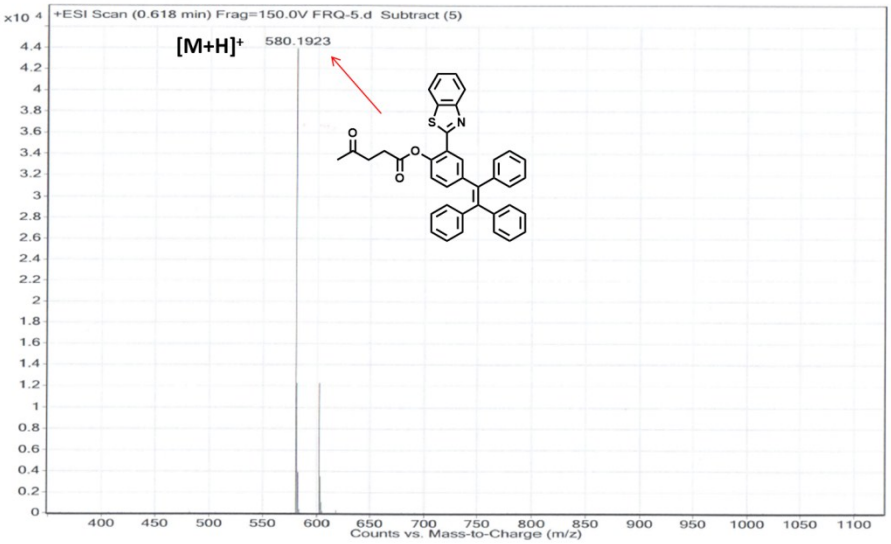


Fig. S14 H RMS spectrum of **TPE-TE.**

15. References

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