Supporting Information

A two-photon fluorescent probe for specific detection of hydrogen sulfide based on a familiar ESIPT fluorophore bearing AIE characteristics

Liyan Chen,[a] Di Wu,[a*] Chang Su Lim,[b] Dayoung Kim,[a] Sang-Jip Nam,[a] Woolin Lee,[a] Gyungmi Kim,[a] Hwan Myung Kim,[b*] and Juyoung Yoon[a*]

[a]Department of Chemistry and Nano Science, EwhaWomans University, Seoul, 120-750, Korea

[b]Department of Chemistry and Energy Systems Research, Ajou University, Suwon, Korea

E-mail: jyoon@ewha.ac.kr; wudi19871208@163.com; kimhm@ajou.ac.kr

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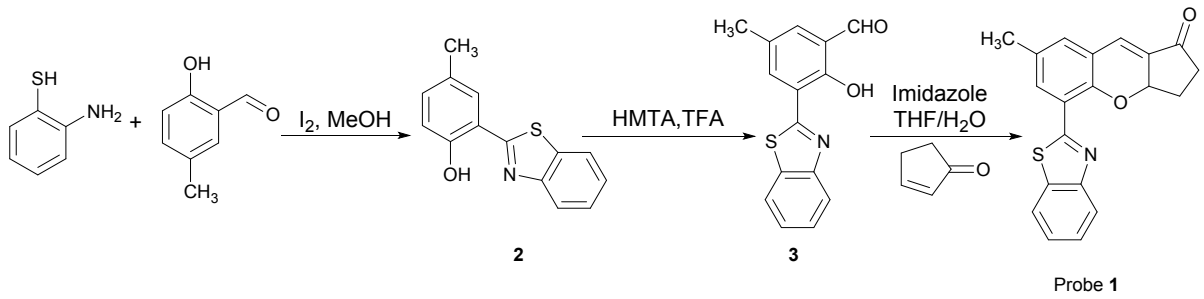
1. General Information

Unless otherwise noted, materials were purchased from commercial suppliers and used without further purification. All the solvents were treated according to general methods. Flash column chromatography was performed using 200-300 mesh silica gel. Chemical shifts were reported on the delta (δ) scale in parts per million (ppm) relative to the singlet (0 ppm) for tetramethylsilane (TMS). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR spectra were recorded at 75 MHz with complete proton decoupling. Chemical shifts are reported in ppm relative to the central line of the triplet at 77.0 ppm for $CDCl_3$. Fluorescence emission

Spectra were obtained using a RF-5301/PC spectrofluorophotometer (Shimadzu). UV absorption spectroscopy measurements were carried out on Scinco S-3100 using a 1 cm optical path length cell at room temperature. Confocal microscopy experiment was conducted with a confocal laser scanning biological microscope (Olympus, FV1200, Japan) and super resolution STED laser (stimulated emission depletion) confocal microscopy (Leica TCS SP8, Germany), excitation source: 405 nm diode laser. The high resolution mass spectra (HRMS) were measured on a Bruker Ultraflex Xtreme MALDI-TOF/TOF mass spectrometer by ESI.

2. Synthetic pathway of probe 1.



Scheme S1. Synthetic pathway of probe **1**.

Synthesis of compounds 2 and 3:

The intermediates **2** and **3** were synthesized according to the literature 1.

Synthesis of probe 1:

The compound **3** (670 mg, 0.25 mmol, 1 equiv.) and imidazole (255 mg, 0.38 mmol, 1.5 equiv.) were totally dissolved in 40 mL the mixture of tetrahydrofuran and distilled water (v:v = 1:1) under Ar. Then 2-cyclopenten-1-one (410 mg, 0.5 mmol, 2 equiv.) was added. The mixture was warm to 40 ^{o}C. Upon completion of the reaction as monitored by TLC, the final mixture was diluted by 1 M HCl (20 ml) and extracted with ethyl acetate (3×15 ml). The organic layer was concentrated under reduced pressure. After these, chromatography of the crude product on silica gel, using DCM and *n*-hexane as eluant, gave pure the probe **1**, 357 mg (43 %). Probe **1** was synthesized according to the literature 2.

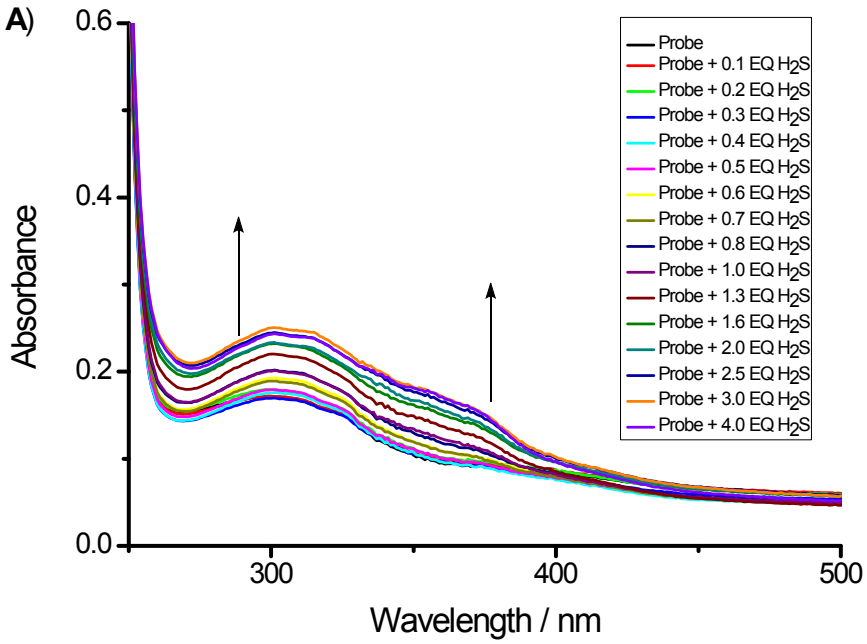
Compound **3**: δ H (300 MHz, $CDCl_3$): 12.94 (1 H, s), 10.42 (1 H, s), 7.94 (1 H, d, *J* = 8.1), 7.87 (1 H, d, *J* = 7.8), 7.77 (1 H, s), 7.62 (1 H, s), 7.45-7.55 (1 H, m), 7.31-7.42 (1 H, m), 2.33 (3 H, s); δ C (75 MHz, $CDCl_3$): 190.61, 166.82, 158.38, 151.37, 134.92, 132.98, 132.30, 128.74, 126.72 , 125.70 , 123.61 , 122.24, 121.46, 118.48, 20.21.

Probe **1**: δ H (300 MHz, $CDCl_3$): 8.35 (1 H, s), 8.17 – 8.08 (1 H, m), 7.98-7.95 (1 H, m), 7.60 – 7.47 (1 H, m), 7.48 – 7.36 (1 H, m), 7.26 (1 H, d, *J* = 2.5), 7.21 (1 H, s), 5.46 (1 H, t, *J* = 7.8), 3.01 – 2.83 (1 H, m), 2.80 – 2.59 (1 H, m), 2.48 (1 H, dd, *J* = 10.6, 8.9), 2.42 (3 H, d, *J* = 1.6), 2.38 (1 H, d, *J* = 12.1); δ C (75 MHz, $CDCl_3$): 201.02, 162.19, 152.06, 150.84, 136.09, 133.13, 132.17, 132.11, 132.04, 127.44, 126.12, 124.94, 122.90, 122.55, 121.53, 121.28,76.28, 37.18, 27.92, 20.46; HRMS (ESI) m/z = 334.0895, calcd for $[M+H]^+$ $C_{20}H_{16}NO_2S$ = 334.0902.

3. Fluorescence Quantum Yield Measurement

Fluorescence quantum yield of probe **1** and probe **1** after additon of 3 equivalents of Na_2S was determined in the reference of fluoresceinin 0.1 M aqueous NaOH. The quantum yield of probe **1** and probe **1** with Na_2S are calculated according to the equation: $\Phi_x=\Phi_s(A_sS_x)/(A_xS_s)$, where Φ_s is the fluorescence quantum yield of fluorescein (Φ = 0.98), A_x is the absorbance of probe **1** and probe **1** after addition with Na_2S, respetively. A_s is the absorbance of the fluorescein. S_x is integrated fluorescence emission intensity of probe **1** and probe **1** with Na_2S while the S_s is integrated fluorescence emission intensity of fluorescein.

4. UV-vis spectrum and detection limit.



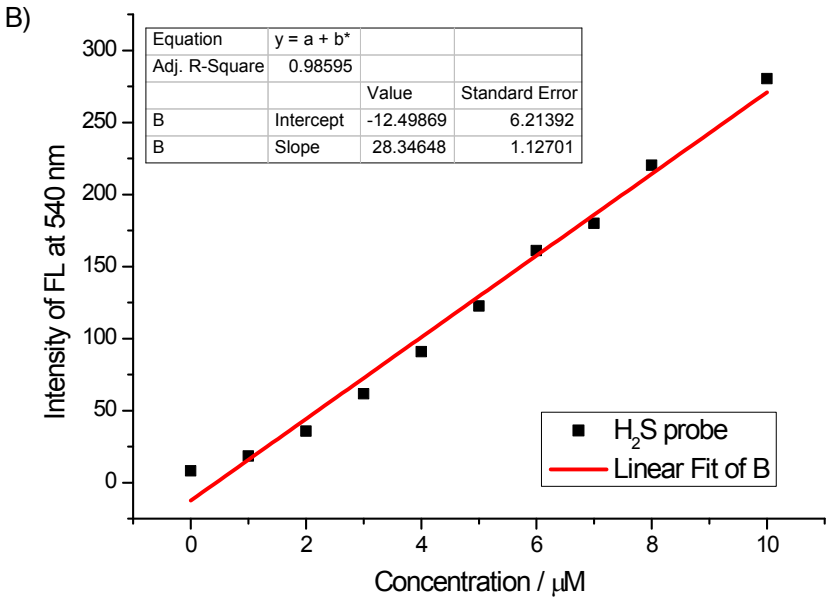


Fig S1. A) UV-vis spectra of probe (10 μM) in PBS buffer (1.0 mM, pH = 7.4, containing 10% DMF) unpon addtion different concentration of H_2S. B) Detection limit of probe **1** response to H_2S. Spectra were recorded after incubation of H_2S for 30 min.

5. Reaction mechanism of probe reacted with H_2S.

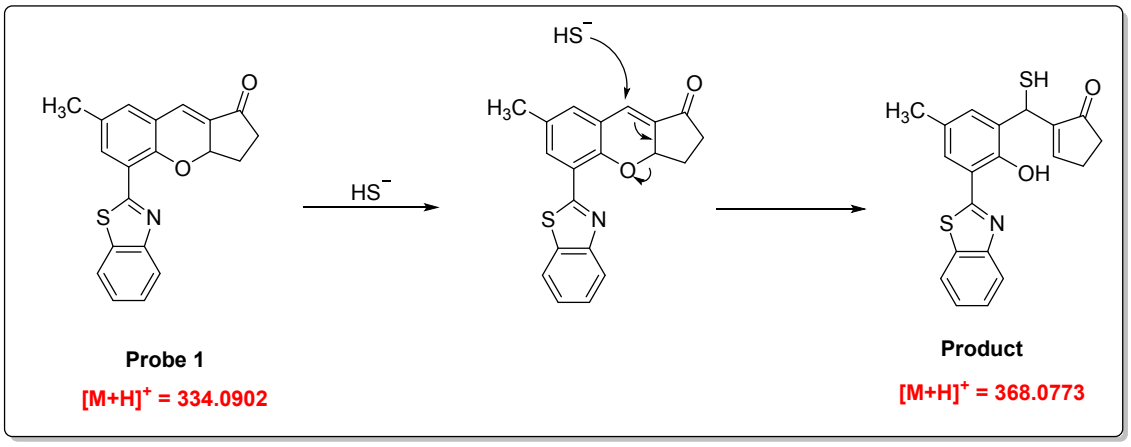


Fig S2. Reaction mechanism of probe **1** and H_2S.

Procedure:

The probe **1** (9.0 mg, 0.025 mmol, 1 equiv.) and Na_2S (10 mg, 0.125 mmol, 5.0 equiv.) were totally dissolved in the mixture of PBS (pH = 7.4) and DMF (v:v = 4:1). The reaction was stirred at room temperature for 2 hours. The mixture was diluted and extracted with DCM (3×8 ml). The organic layer was concentrated under reduced pressure. After these, chromatography of the crude product on silica gel, using DCM and *n*-hexane as eluant, gave pure the product, 7 mg (81 %).

Product: δ H (300 MHz, $CDCl_3$): 12.72 (1 H, s), 7.98 – 7.92 (1 H, m), 7.90 – 7.80 (2 H, m), 7.47 – 7.35 (3 H, m), 7.25 – 7.21 (1 H, m), 5.41 (1 H, d, J = 1.1), 2.68 (2 H, s), 2.40-2.50 (2 H, m), 2.23 (3 H, s), 2.07 (1 H, s); δ C (75 MHz, $CDCl_3$): 207.26, 169.34, 161.52, 153.10, 151.61, 145.26, 133.06, 132.53, 128.33, 127.49, 127.03, 126.55, 125.31, 121.83, 121.37, 115.97, 37.69, 35.08, 26.64, 20.55; HRMS (ESI) m/z = 368.0773, calcd for $[M+H]^+$ $C_{20}H_{17}NO_2S_2$ = 368.0770.

6. pH-dependence of probe 1 and probe after addition of H_2S

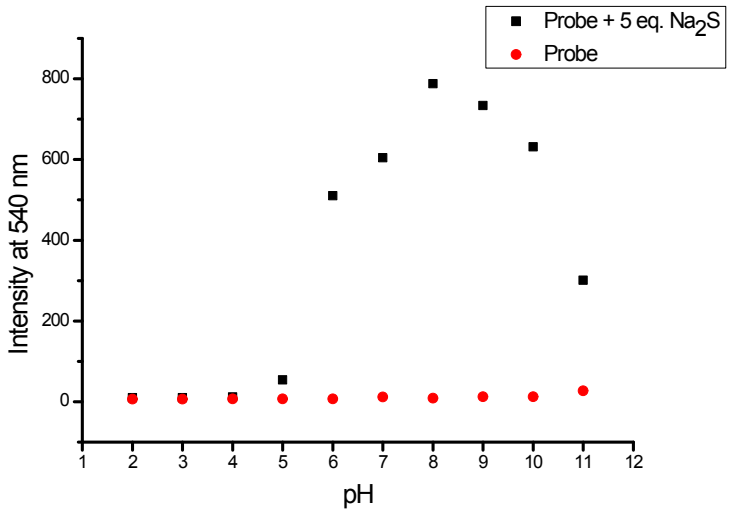


Fig S3. Fluorescence intensity of probe **1** (10 μM) at 540 nm under different pH values in the absence and the presence of 3 equiv. of H_2S in PBS buffer (1.0 mM, pH = 7.4, containing 10% DMF). Spectra were recorded after incubation of H_2S for 30 min.

7. Selectivity of probe after addition biothiols and sulfite.

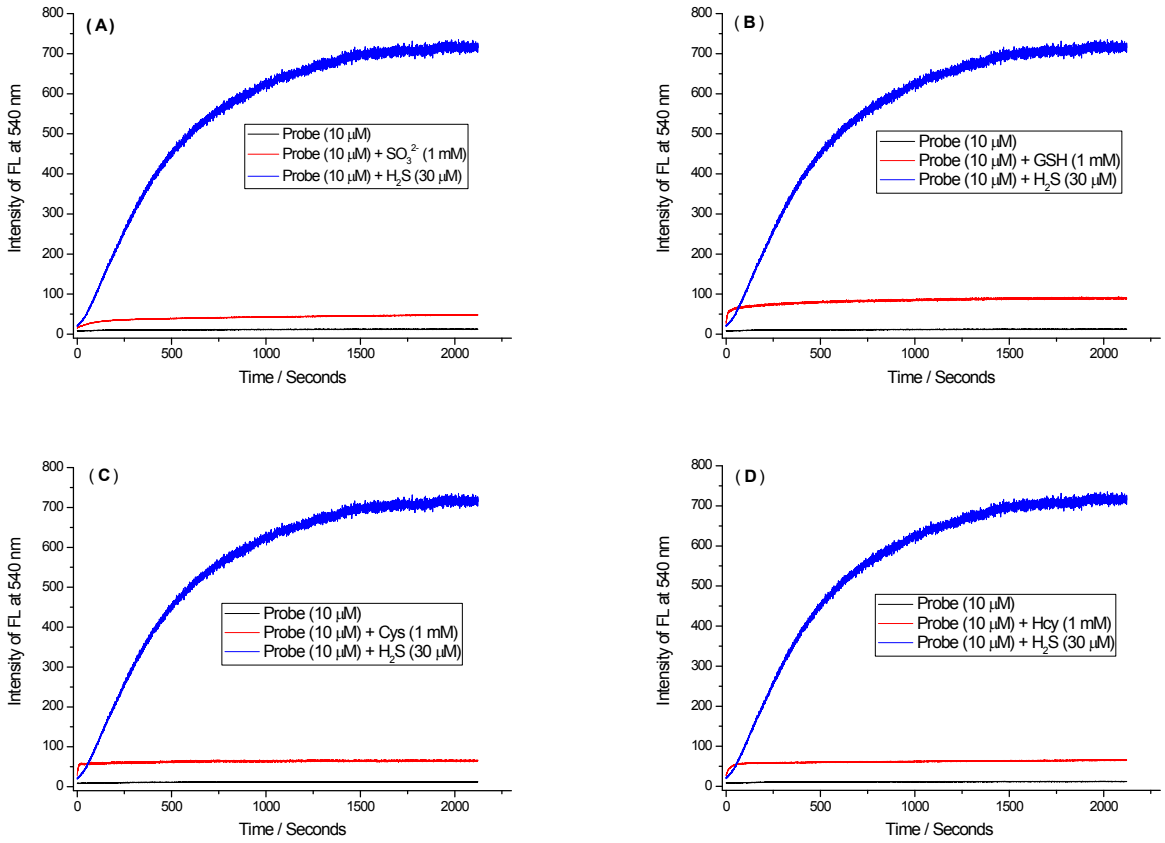


Fig S4. Time course experiment of **1** (10 μM) with (A) Na_2SO_3 (1 mM), (B) GSH (1 mM), (C) Cys (1 mM) and (D) Hcy (1 mM) in PBS buffer (1.0 mM, pH 7.4, 10% DMF) relative to the time course of **1** (10 μM) with H_2S (30 μM) in the same condition.

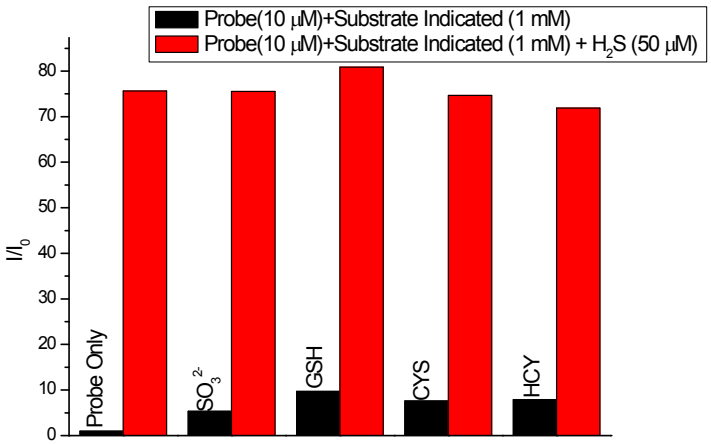


Fig S5. Fluorescent spectra of probe (10 μM) in PBS buffer (1.0 mM, pH = 7.4, containing 10% DMF) unpon addtion of 1 mM of sulfite, GSH, Cys and Hcy. Fluorescent response of H_2S (50 μM) with probe **1** (10 μM) in the presence of these analytes. Spectra were recorded after incubation of H_2S for 30 min.

8. Response of probe after addition of H_2S in two different concentration of serum.

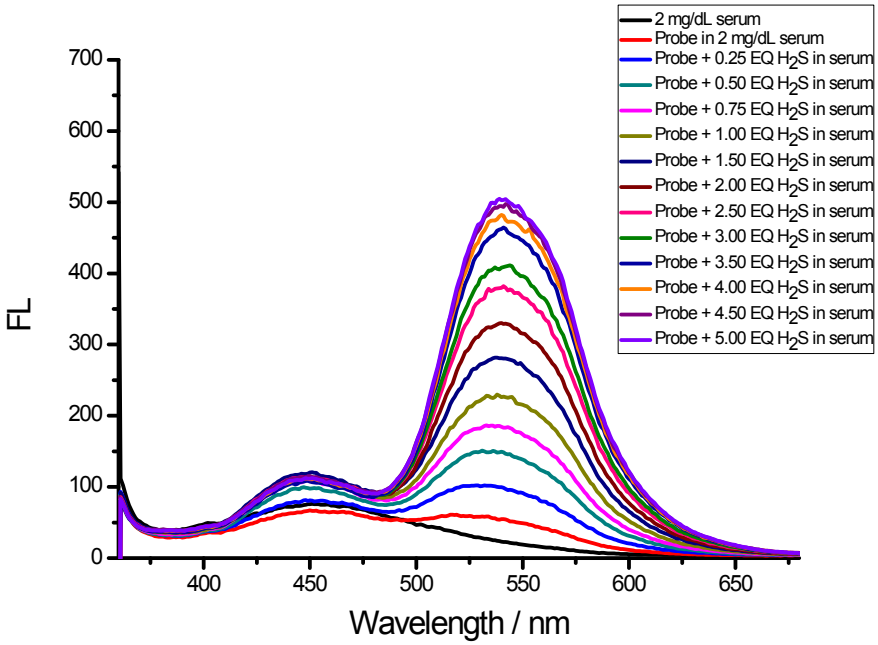


Fig S6. Fluorescent spectra of probe **1** (10 μM) in serum (**2 mg/ dL**) with 10% DMF as co-solvent. Excitation Wavelength = 350 nm. Slit width = 5*5 nm. Reaction time = 30 min.

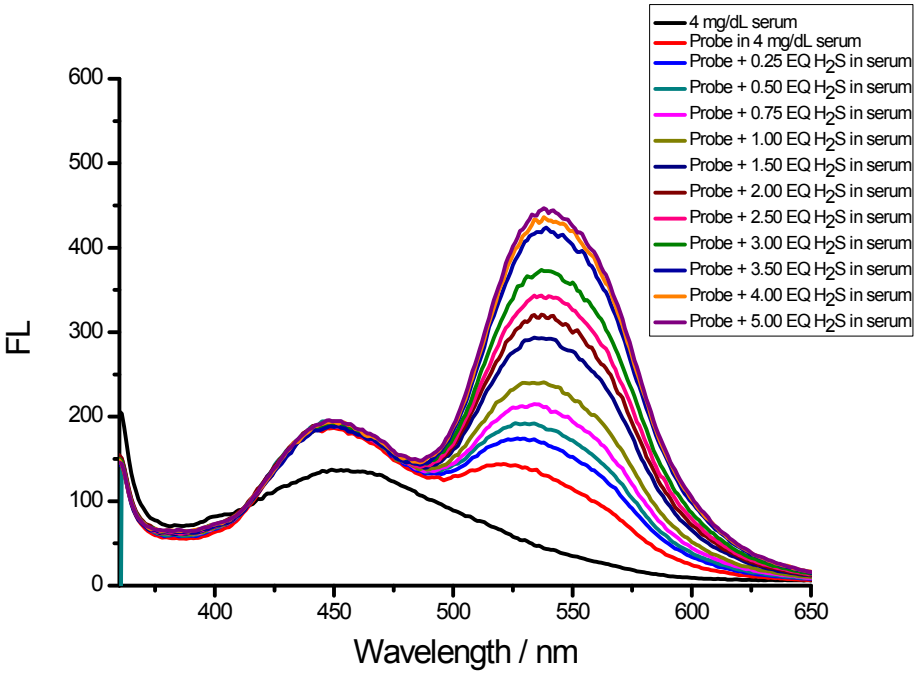
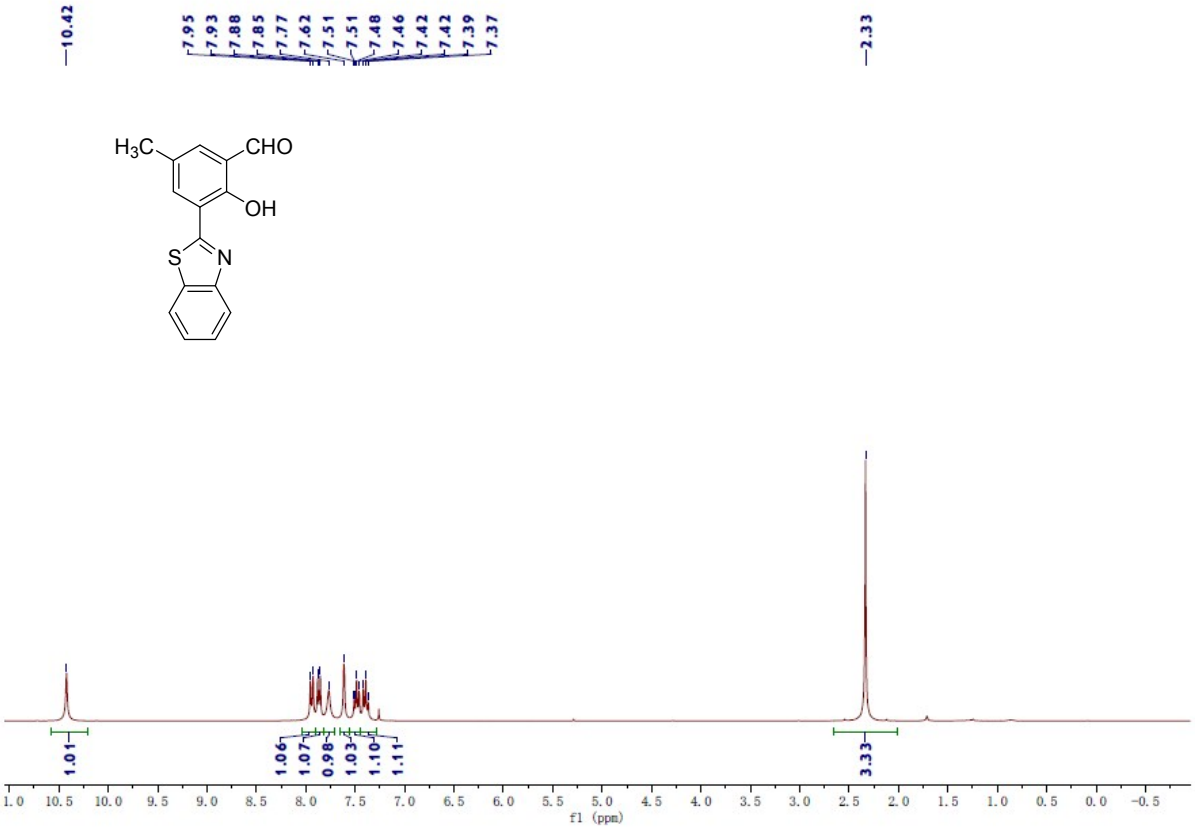


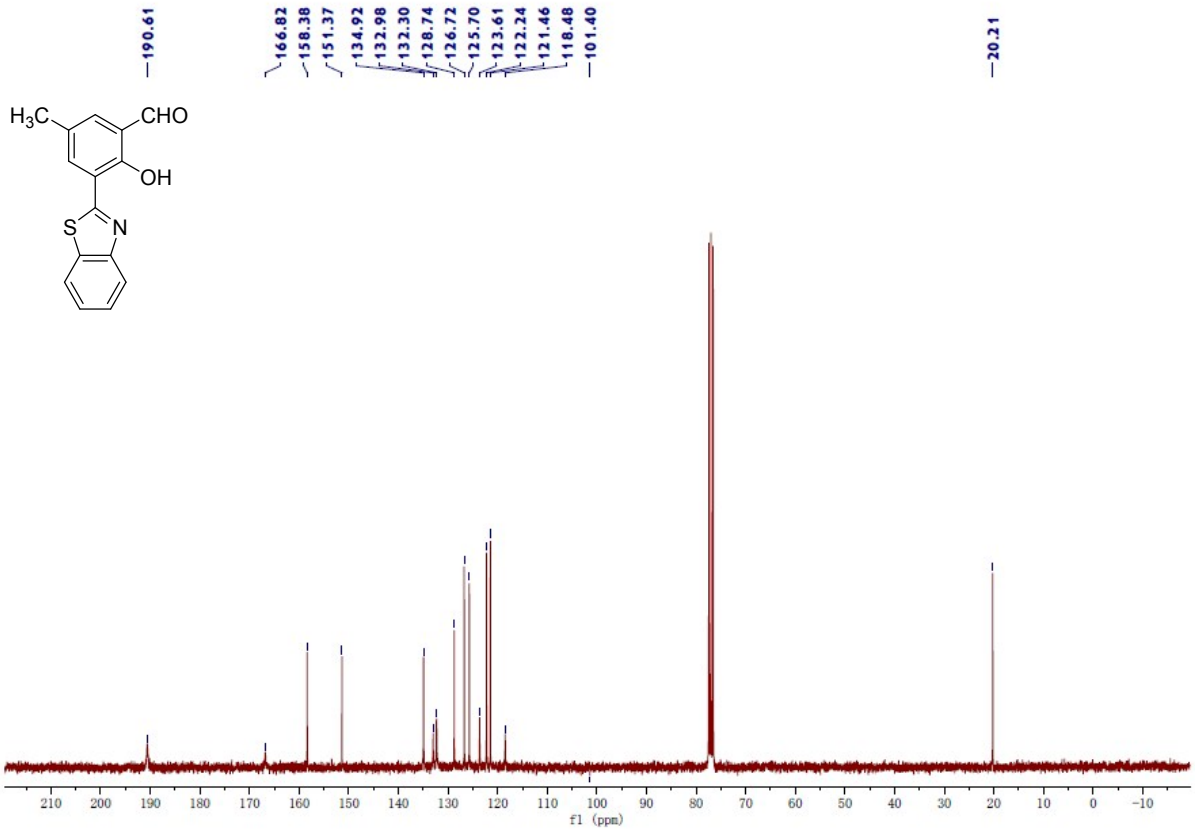
Fig S7. Fluorescent spectra of probe (10 μM) in serum (**4 mg/ dL**) with 10% DMF as co-solvent. Excitation Wavelength = 350 nm. Slit width = 5*5 nm. Reaction time = 30 min.

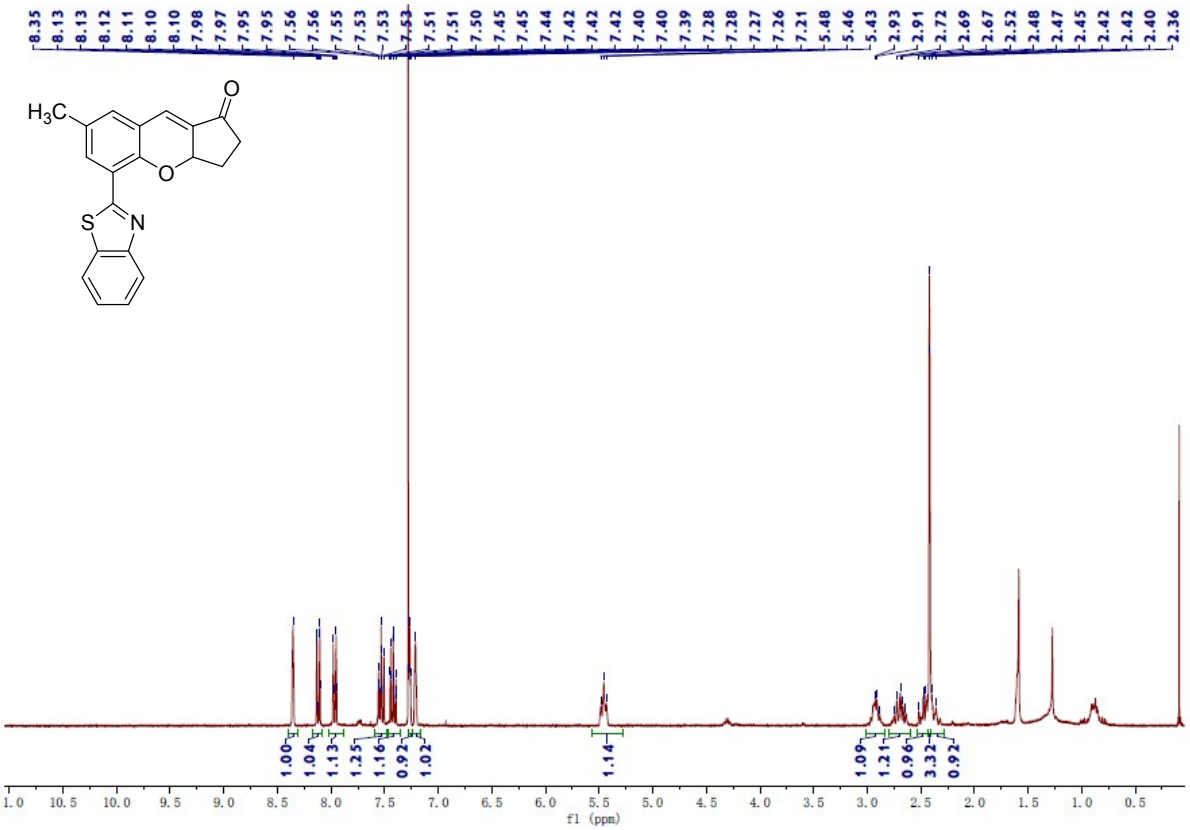
9. Two-Photon Fluorescence Microscopy.

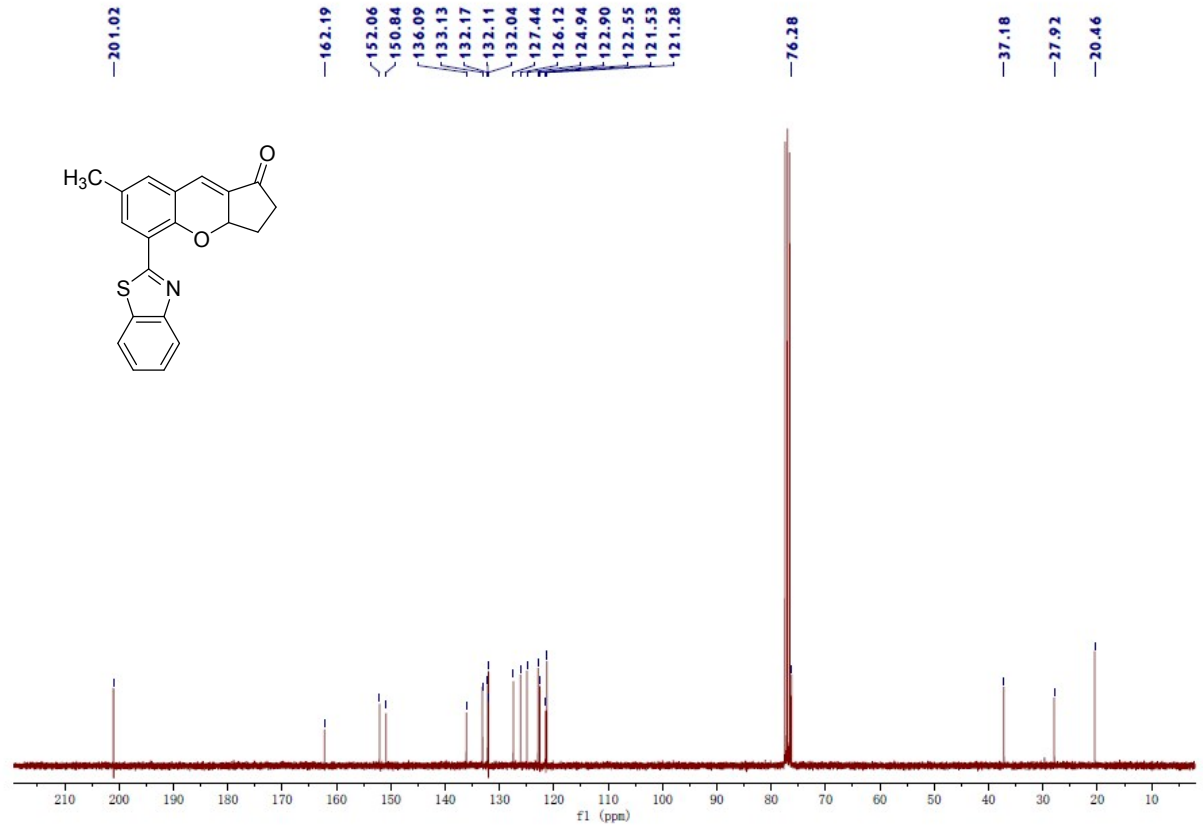
Two-photon fluorescence microscopy images of probe **1**-labeled cells were obtained with spectral confocal and multiphoton microscopes (Leica TCS SP2 MP) with ×100 oil objectives, numerical aperture (NA) = 1.30. The two-photon fluorescence microscopy images were obtained with a DMI6000B Microscope (Leica) by exciting the probes with a mode-locked titanium-sapphire laser source (Chameleon, 90 MHz, 200 fs; Coherent Inc., Santa Clara, CA, USA) set at wavelength 740 nm and output power 1305 mW, which corresponded to approximately 1.44×10^9 mW/cm^2 average power in the focal plane. To obtain images at 500−600 nm range, internal PMTs were used to collect the signals in an 8 bit unsigned 1024 × 1024 pixels at 200 Hz scan speed, respectively. Flurorescence intensity analysis was carried out using Leica software.

10. Copies of ^{1}H NMR, ^{13}C NMR and Mass Spectra

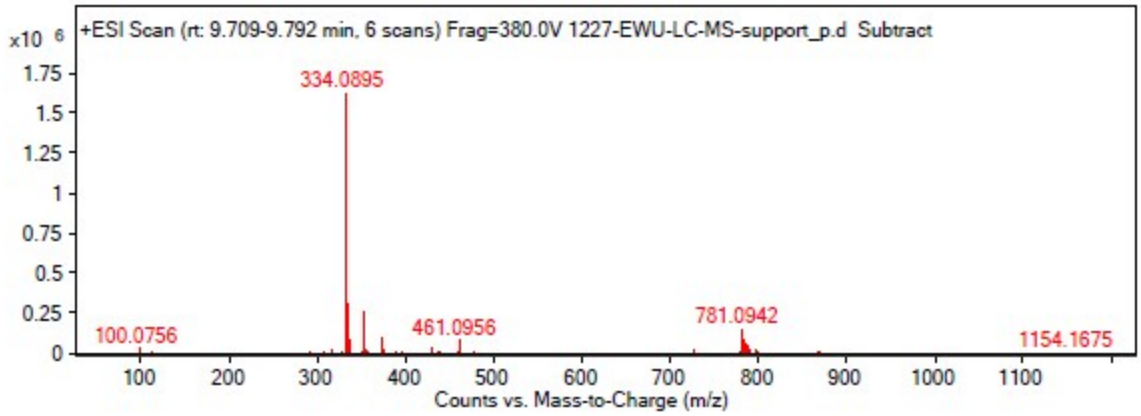





8.35 8.13 8.13 8.12 8.11 8.10 8.10 7.98 7.97 7.95 7.95 7.56 7.56 7.55 7.53 7.53 7.53 7.51 7.51 7.50 7.45 7.45 7.44 7.42 7.42 7.42 7.40 7.40 7.39 7.28 7.28 7.27 7.26 7.21 5.48 5.46 5.43 2.93 2.91 2.72 2.69 2.67 2.52 2.48 2.47 2.45 2.42 2.42 2.40 2.36
H3C
O
O
S
N
1.00 1.04 1.13 1.25 1.16 0.92 1.02 1.14 1.09 1.21 0.96 3.32 0.92
f1 (ppm)

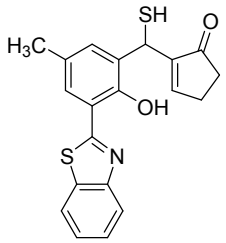

201.02 162.19 152.06 150.84 136.09 133.13 132.17 132.11 132.04 127.44 126.12 124.94 122.90 122.55 121.53 121.28 76.28 37.18 27.92 20.46
H3C
O
O
S
N
f1 (ppm)

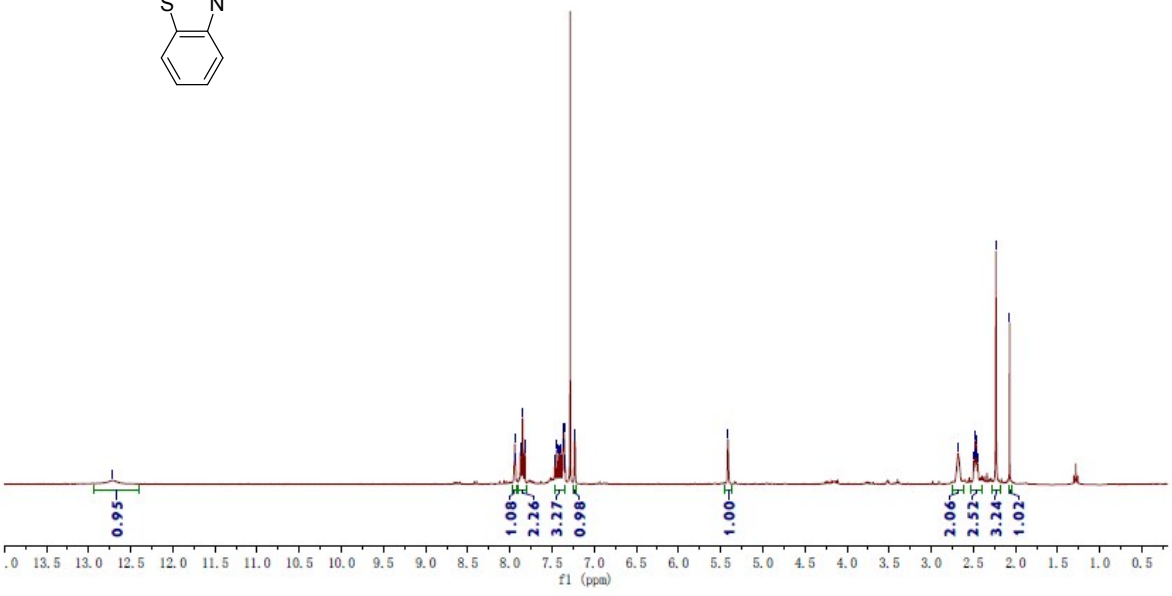
Fragmentor Voltage	Collision Energy	Ionization Mode
380	0	ESI

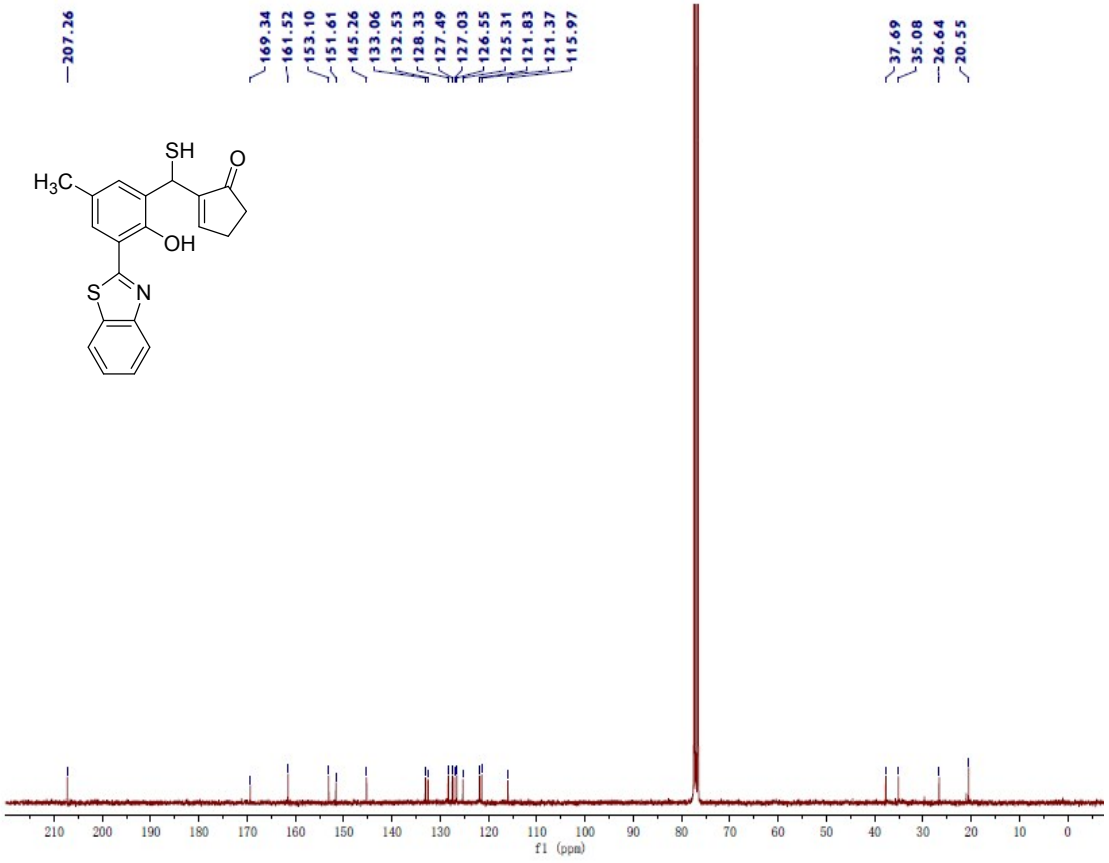


Peak List

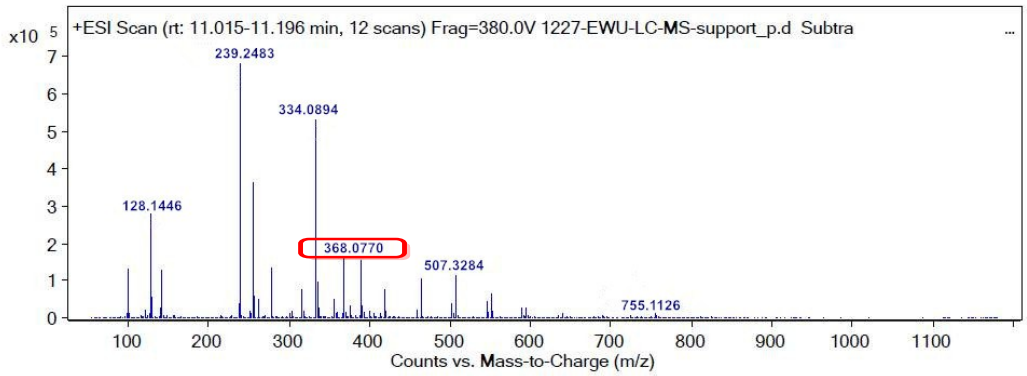
m/z	z	Abund
334.0895	1	1621846.88
335.0928	1	315831.53
336.0898	1	78982.23
352.0999	1	255282.89
353.1027	1	57848.79
374.0815	1	92827.06
461.0956	1	73542.48
781.0942	1	146153.95
782.0965	1	67712.17
783.0918	1	81919.3
786.0881	1	61895.8
788.0871	1	37055.74







Fragmentor Voltage	Collision Energy	Ionization Mode
380	0	ESI



Peak List

m/z	z	Abund
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239.2483	1	626872.13
255.243	1	364857.22
277.2247	1	136082.66
334.0894	1	475987.03
335.0929	1	99437.27
368.077	1	163218.75
390.059	1	153628.47
463.3021	1	105833.5
507.3284	1	113842.14

11. Reference

1. Z. J. Huang, S. S. Ding, D. H. Yu, F. H. Huang and G. Q. Feng, *Chem. Commun.*, 2014, **50**, 9185.

2. a) X. Chen, S.-K. Ko, M. J. Kim, I. Shin and J. Yoon, *Chem. Commun.,* 2010, **46**, 2751; b) F.-J. Huo, Y.-Q. Sun, J. Su, J.-B. Chao, H.-J. Zhi and C.-X. Yin, *Org. Lett.,* 2009, **11**, 4918; c) F.-J. Huo, Y.-Q. Sun, J. Su, Y.-T. Yang, C.-X. Yin and J.-B. Chao, *Org. Lett.,* 2010, **12**, 4756; d) Y.-M. Dong, Y. Peng, M. Dong and Y.-W. Wang, *J. Org. Chem.,* 2011, **76**, 6962.