Supporting Information

A Water-Soluble Near-Infrared Probe forColorimetric and Ratiometric Sensing of SO_2Derivatives in Living Cells

Ming-Yu Wu, Kun Li*, Chun-Yan Li, Ji-Ting Hou, Xiao-Qi Yu*

Key Laboratory of Green Chemistry and Technology,Ministry of Education, College of Chemistry, Sichuan University, Chengdu, 610064, China Fax: (86)-28-85415886 ; Tel: (86)-28-85415886; E-mail: xqyu@scu.edu.cn; kli@scu.edu.cn.

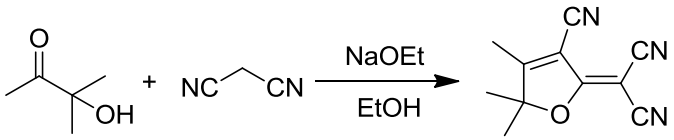
Content:

1. General information and methods.

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies. TLC analyses were performed on silica gel GF 254. Column chromatographic purifications were carried out on silica gel (HG/T2354-92). NMR spectra were measured on a Bruker AMX-400. The ^{1}H NMR (400 MHz) chemical shifts were given in ppm relative to the internal reference TMS. The ^{13}C NMR (100 MHz) chemical shifts were given using $CDCl_3$ and DMSO-d_6 as the internal standard. ESI-MS and HR-MS spectral data were recorded on a Finnigan LCQDECA and a BrukerDaltonics Bio TOF mass spectrometer, respectively. Fluorescence excitation and emission spectra were obtained using FluoroMax-4 Spectrofluoro photometer (HORIBA JobinYvon). UV-Vis absorption spectra were recorded on a Hitachi PharmaSpec UV-1900 UV-Visible spectrophotometer. A stock solution of the probe **1**, and **2**, (0.5 mM) were prepared in DMSO. All the stock solutions of anions and reactive sulfur were prepared with corresponding sodium salts in deionized water at a concentration of 20 mM. Test solutions were prepared by placing 25 μL of the probe stock solution into a test tube, diluting the solution to 2.5 mL with HEPES pH 7.4 buffer solution, and adding an appropriate aliquot of each anion stock. Fluorescence spectra were measured after 90 seconds upon the addition of anions. Fluorescent quantum yields were determined by standard methods, using fluorescein (Φ = 0.85 in 0.1 N NaOH) as a standard.

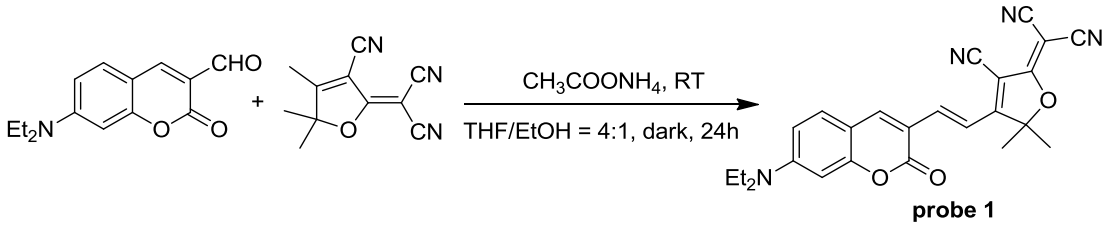
2. Synthesis of probe 1 and 2

2-(3-cyano-4,5,5-trimethylfuran-2(5H)-ylidene)malononitrile (TCF):[1]



To 900 mg of sodium ethoxide (13 mmol, 0.15 equiv) dissolved in 10 mL of absolute EtOH in a room temperature water bath was added 9 g 3-hydroxy-3-methyl-2-butanone (88 mmol) and 12 g freshly distilled malononitrile (181 mmol, 2.05 equiv) with stirring. After 1 hr 30 mL of absolute EtOH was added and heated at reflux for 1 additional hr. This is cooled in a refrigerator and the solid filter, washed with a minimal amount of cold EtOH, and then air dryed giving a first crop of 11.6 g of off-white crystalline solid (65% yield). Concentration of the filtrate and cooling gave a second crop of 0.48 g product (total yield 68%). ^{1}H NMR (400 MHz, $CDCl_3$),δ2.36 (s, 3H), 1.63 (s, 6H); ^{13}C NM (100 MHz, $CDCl_3$), δ 182.7, 175.3, 111.0, 110.4, 109.0, 104.8, 99.8, 58.4, 24.3, 14.2.ESI MS: 222.1 $[M + Na]^+$

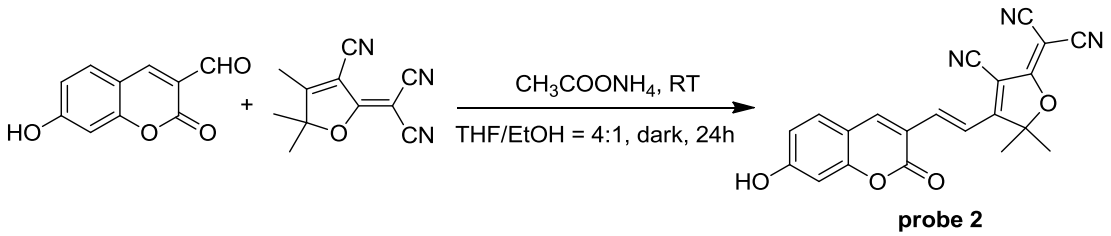
(E)-2-(3-cyano-4-(2-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)vinyl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (probe 1):



probe 1

245.1 mg (1 mmol) of 7-diethylamine coumarin aldehyde, 219 mg TCF (1.1 mmol, 1.1 equiv) and 85 mg ammonium acetate (1.1 mmol, 1.1 equiv.) were dissolved in 10 ml THF/EtOH = 4:1 mixture solvents, and stired under dark for 24 hr, greyish-green solid were obtained through filtrating and recrystallizing from EtOH with 370 mg (87% yield). ^{1}H NMR (400 MHz, DMSO-d_6),δ 8.53(S, 1H), 7.76 (d, 1H,*J* = 16.0 Hz), 7.62 (d, 1H,*J* = 16.0 Hz), 7.54 (d, 1H,*J* = 9.2 Hz), 6.86 (dd, 1H, *J* = 2.0 Hz, *J* = 9.0 Hz), 6.67 (d, 1H, *J* = 1.6 Hz), 3.54 (q, 4H, *J* = 6.8 Hz), 1.79 (S, 1H), 1.16 (t, 6H, *J* = 6.8 Hz); ^{13}C NM (100 MHz, $CDCl_3$), δ177.5, 176.3, 159.6, 157.6, 153.8, 149.5, 145.0, 132.3, 114.4, 113.5, 113.1, 112.7, 111.6, 111.3, 109.6, 99.0, 97.4, 96.9, 53.4, 45.1, 26.1, 12.9.HRMS (ESI): m/z $[M + Na]^+$calcd for $C_{25}H_{22}N_4NaO_3$:449.1584; found 449.1589.

(E)-2-(3-cyano-4-(2-(7-hydroxy-2-oxo-2H-chromen-3-yl)vinyl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (probe 2):



probe 2

190 mg (1 mmol) of 7-hydroxy coumarin aldehyde, 219 mg TCF (1.1 mmol, 1.1 equiv) and 85 mg ammonium acetate (1.1 mmol, 1.1 equiv.) were dissolved in 10 ml THF/EtOH = 4:1 mixture solvents, and stired under dark for 24 hr. After distilling the solvent, the residues were extracted with 30 ml ethyl acetate three times. The combined organic layers were dried over Na2SO4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on a silica gel column using DCM /EA = 2:1 as the mobile phase, affording probe 2 as a red powder 185.1 mg (50% yield).^{1}H NMR (400 MHz, DMSO-d_6),δ 11.26 (s, 1H),8.67(S, 1H), 7.74 (s, 2H,), 7.66 (d, 1H,*J* = 8.8 Hz), 6.90 (dd, 1H, *J* = 2.0 Hz, *J* = 8.8 Hz), 6.80 (d, 1H, *J* = 2.0 Hz), 1.81 (S, 1H),; ^{13}C NM (100 MHz, $CDCl_3$), δ.177.0, 175.4, 164.4, 158.8, 156.1, 149.3, 143.0, 131.9, 116.8, 116.6, 114.7, 112.8, 112.0, 110.8, 102.2, 100.0, 99.1, 54.3, 25.4. ^{1}H NMR (400 MHz, CD_3COCD_3),δ 11.02 (s, 1H, br), 8.59(S, 1H), 7.90 (d, 1H,*J* = 16.4 Hz), 7.84 (d, 1H,*J* = 16.2 Hz), 7.66 (d, 1H,*J* = 8.6 Hz), 6.93 (d, 1H, *J* = 8.6 Hz), 6.84 (s, 1H,), 1.91 (S, 1H);HRMS (ESI): m/z $[M + Na]^+$calcd for$C_{21}H_{13}N_3NaO_4$:394.0798; found 394.0803.

Ref. 1: M. C. Davis, A. P. Chafin, R. A. Hollins, L. C. Baldwin, E. D. Erickson, P. Zarras, E. Drury, Synth. Commun. 34, 3419-3429.

3. Quantum Yields.

Quantum yields were determined using fluorescein as a standard according to the published method.[1] The quantum yield was calculated according to the equation: ($\Phi_{sample} = \Phi_{standard} * (I_{sample} /$

$I_{standard}$) * (A_{sample} / $A_{standard}$)); where Φ is the quantum yield, $\Phi_{standard}$ = 0.85 in 0.1 M NaOH; I_{sample} and $I_{standard}$ are the integrated fluorescence intensities of the sample and the standard, A_{sample} and $A_{standard}$ are the optical densities, at the excitation wavelength, of the sample and the standard, respectively.

Quantum yield of Probe 1: Φ = 0.049. Quantum yield of Probe 2: Φ = 0.0007

After the complete reaction with sulfite, the Quantum yield of Probe 2: Φ = 0.0044

4. The absorption and emission spectrum of probe 1 in different solvents

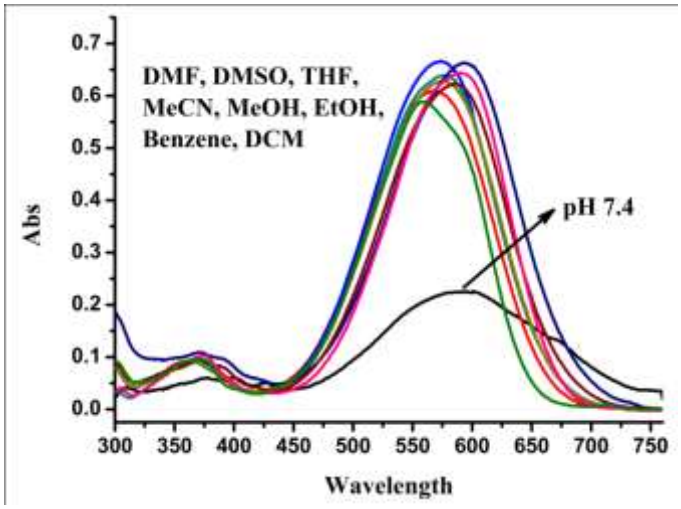


Figure S1. The absorption spectra of probe 1 (2 μM) in different solvents.

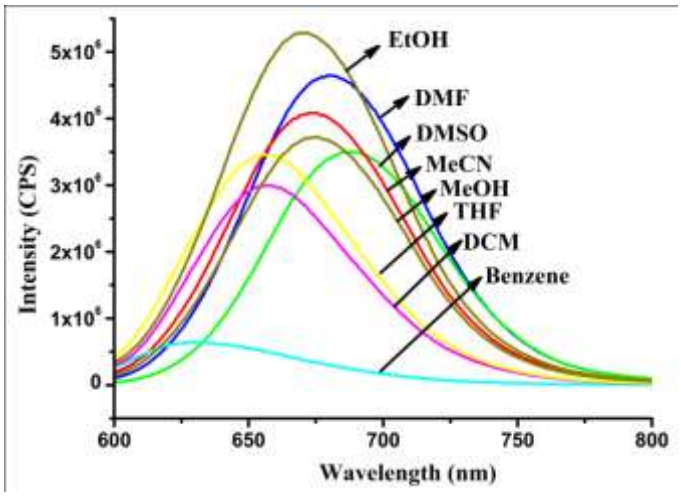


Figure S2. The emission spectra of probe 1 (2 μM) in different solvents.

5. The fluorescent spectra of probe 1 in different water content solutions

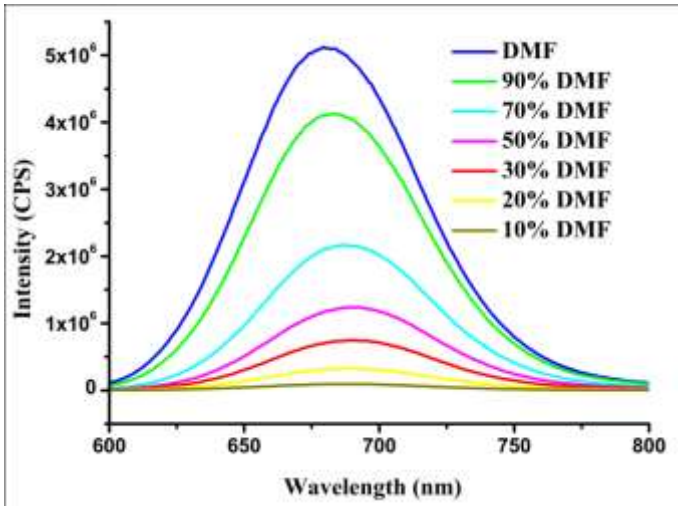


Figure S3. The influence of water content to the fluorescent intensity of probe **1** (2 μM).

6. The absorption and emission spectra of probe 2 in different solvents

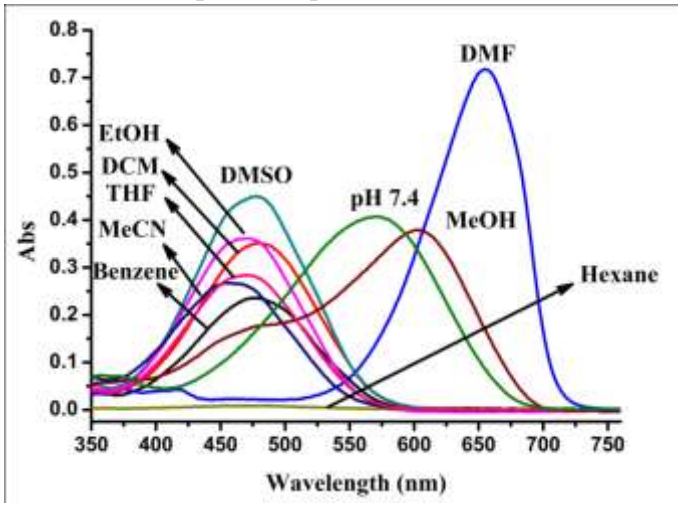


Figure S4. The absorption spectra of probe 2 (10μM) in different solvents.

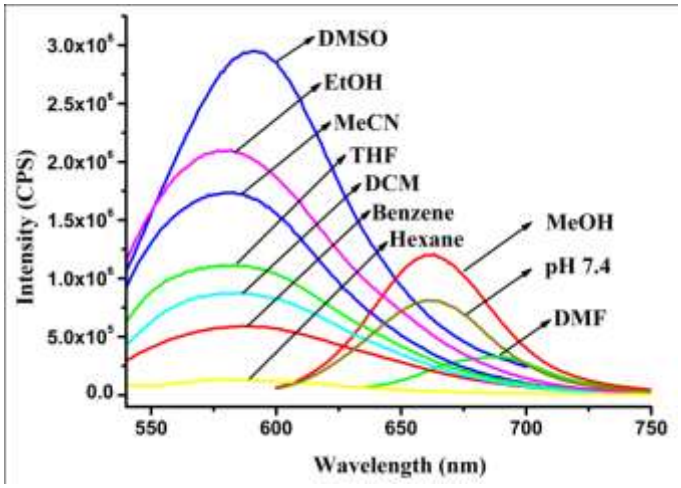


Figure S5.The emission spectrum of probe 2 (5 μM) in different solvents.

7. Photophysical properties of probe 1 and probe 2 in various solvents

Table S1.

compound	solvent	□ □abs. max nm	□ □em. max nm
Probe 1	Benzene	560	628
Probe 1	DCM	590	658
Probe 1	DMF	585	681
Probe 1	DMSO	594	688
Probe 1	EtOH	575	670
Probe 1	MeOH	574	676
Probe 1	MeCN	576	673
Probe 1	THF	568	658
Probe 1	buffer[a]	527	676
Probe 2	Benzene	477	589
Probe 2	DCM	480	585
Probe 2	DMF	655	689
Probe 2	DMSO	478	592
Probe 2	EtOH	471	582
Probe 2	MeOH	602	663
Probe 2	MeCN	458	585
Probe 2	THF	470	583
Probe 2	buffer[a]	570	663
Probe 2	Hexene	472	581
Probe 2 + Na_2SO_3	buffer[a]	473, 330	523

Data were obtained in [a]20 mM pH 7.4 HEPES buffer solution.

8. The photobleachingof probe 2 undervisibleand UV light

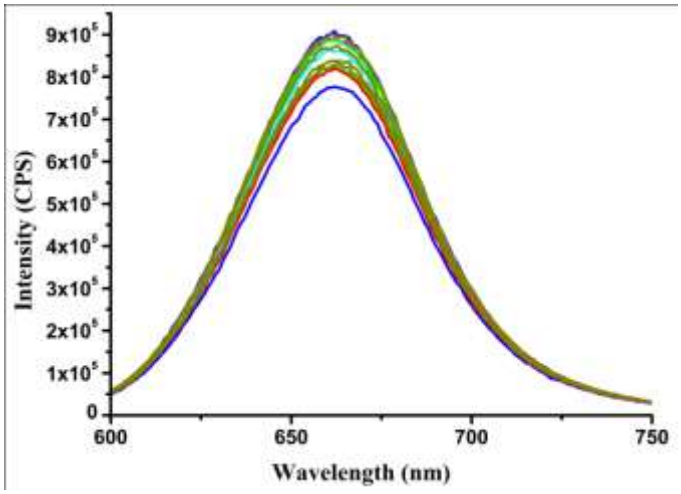


Figure S6. The stability and photobleaching of probe 2 under the shining of the sunshine at 0-6 hours, λ_{ex} = 580 nm, Slit: 5 nm/5 nm.

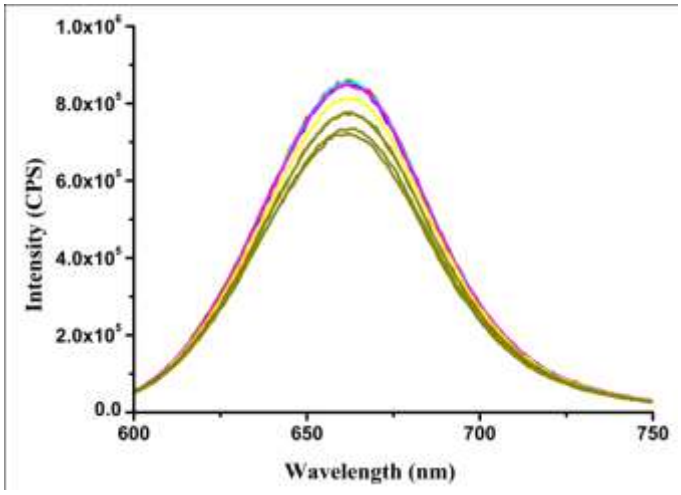
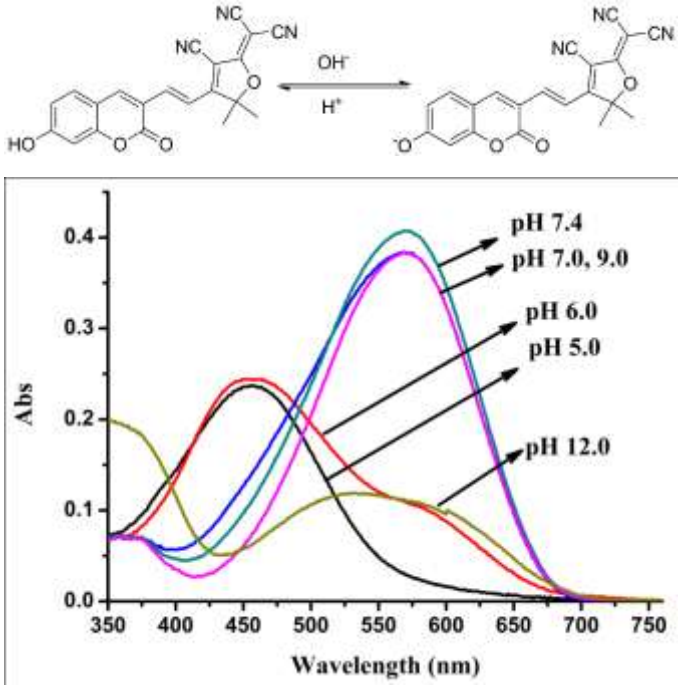


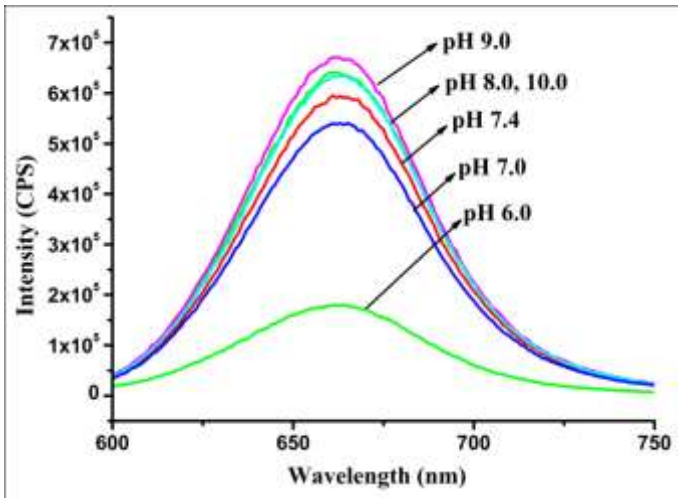
Figure S7. The stability and photobleaching under the UV-lamp (254 and 365 nm at the same time) at 0-6 hours, λ_{ex} = 580 nm, Slit: 5 nm/5 nm.

9. The absorption and emission spectra of probe 2 in different pH buffer solution

The Britton-Robinson buffer solution was chosen in this part for its wider range from acidity to alkalinity pH. In 100 ml 0.04 mol/L phosphoric acid, acetic acid and boric acid mixture system, 0.2 mol/LNaOH solution was added to adjust the pH from 4 to 12.



FigureS8.The influence of pH value to the absorption spectrum of probe **2**(10 μM).



FigureS9. The influence of pH value to the emission spectrum of probe **2**(5μM), λ_{ex} = 580 nm, Slit: 5 nm/5 nm.

10. The time course of probe 2 with sulfite and bisulfite

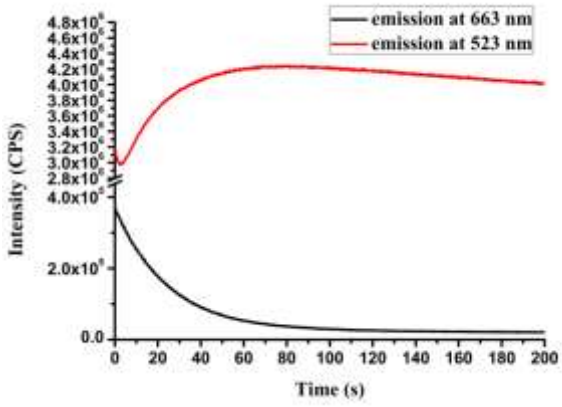


Figure S10. The kinetic response of the sulfute (8 equiv.) to the probe 2 (5 μM) in pH 7.4 HEPES buffer solution (for black line: λ_{ex} = 580 nm,λ_{em} = 663 nm, Slit: 5 nm/5 nm; for red line:λ_{ex} = 466 nm, λ_{em} = 523 nm, Slit: 5 nm/5 nm).

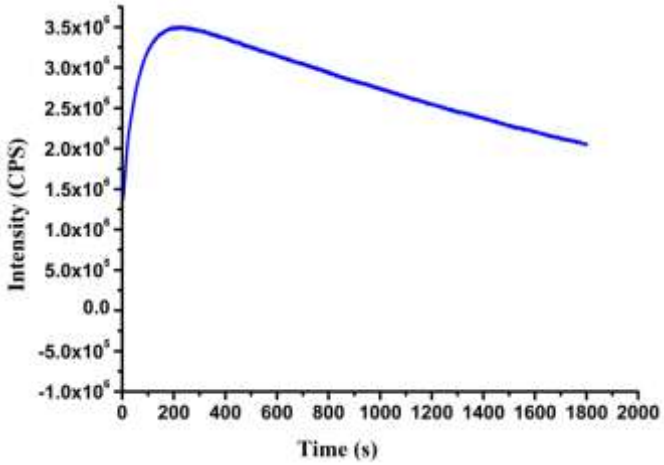


Figure S11. The kinetic response of the sulfute (4 equiv.) to the probe 2 (5 μM) in pH 7.4 HEPES buffer solution (λ_{ex} = 580 nm,λ_{em} = 663 nm, Slit: 5 nm/5 nm)

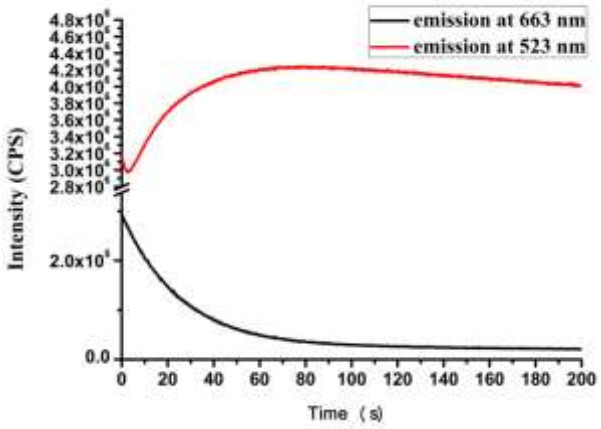


Figure S12. The kinetic response of the bisulfute (8 equiv.) to the probe 2 (5 μM) in pH 7.4 HEPES buffer solution (for black line:λ_{ex} = 580 nm, λ_{em} = 663 nm, Slit: 5 nm/5 nm; for red line: λ_{ex} = 466 nm, λ_{em} = 523 nm, Slit: 5 nm/5 nm).

11. The influence of pH value to emission spectrum and intensity ratio changes of the reaction between probe 2 with sulfite

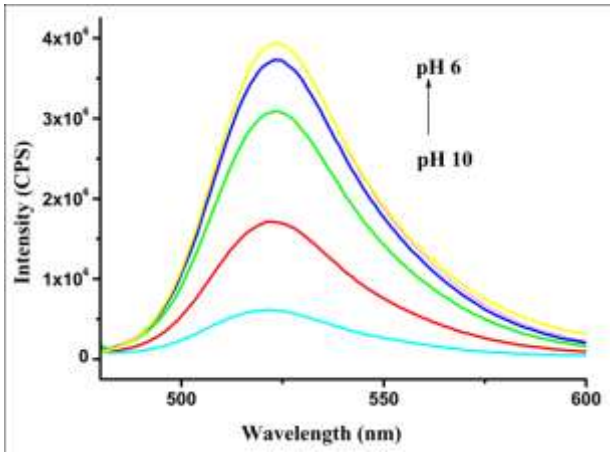


Figure 13. The influence of pH value to the reaction of probe **2**(5μM)with sulfite (40 μM), λ_{ex} = 466 nm. Slit: 5 nm/5 nm.

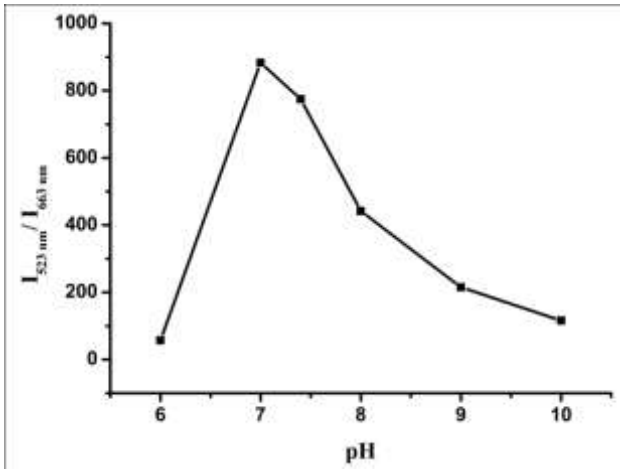


Figure 14. The influence of pH value to intensity ratio at 523 nm and 663 nm.

12. The detection limit of probe tosulfite[2]

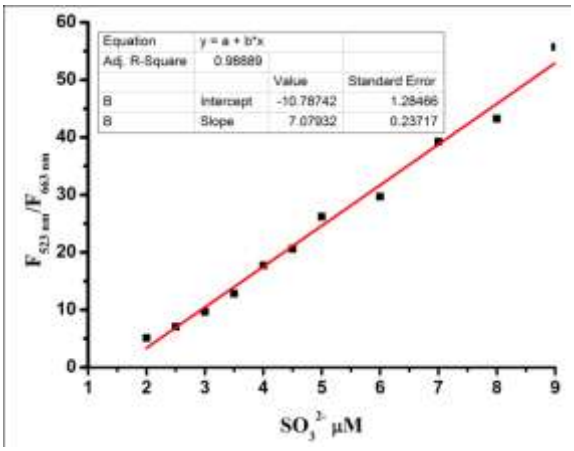


Figure S15.The line relationship between the fluorescent intensity ratio of the probe **2** (5 μM at 523 nm and 663 nm) and the concentration of the sulfite at 2 to 9 μM in 20 mmol pH 7.4 buffer solution.

Ref. 2: B. P. Joshi, J. Park, W. I. Lee and K. Lee, *Talanta*.**2009**, *78*, 903-909.

13. The fluorescent titration and detection limit of probe 2 with bisulfite

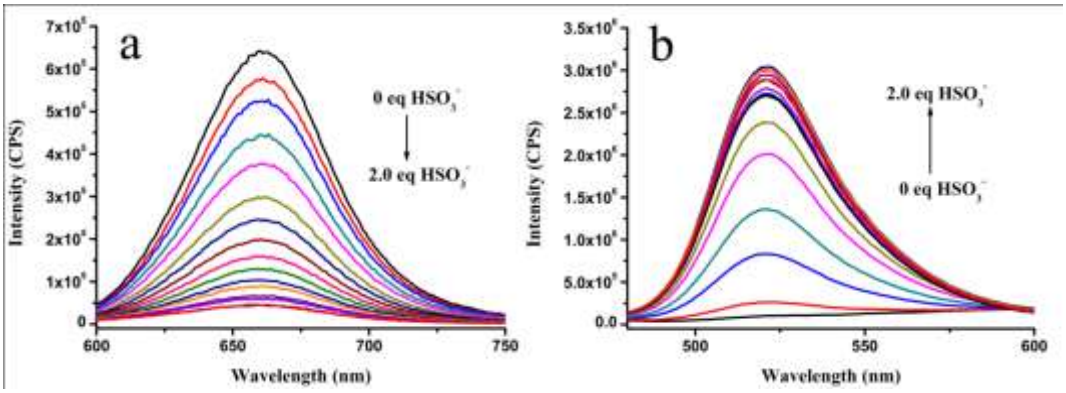


Figure S16.Fluorescence titration of probe **2** (5 μM) with HSO_3^- (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 μM, 0-2 equiv.) (for a: λ_{ex} = 580 nm. Slit: 5 nm/5 nm; for b: λ_{ex} = 466 nm. Slit: 5 nm/5 nm,) in 20 mM pH 7.4 HEPES buffer solution.

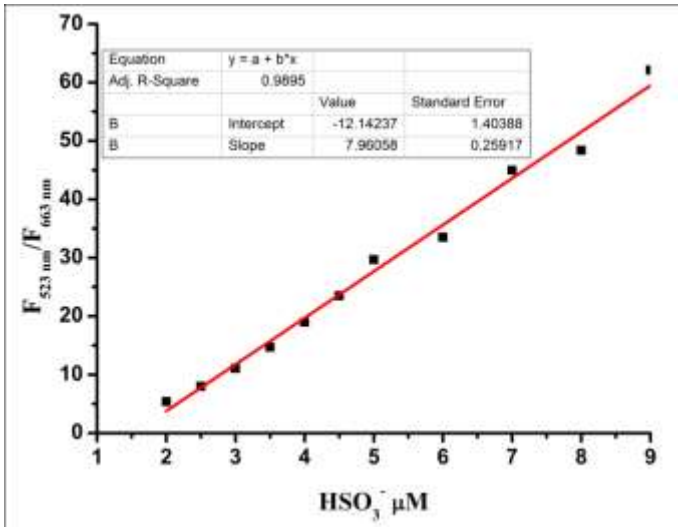
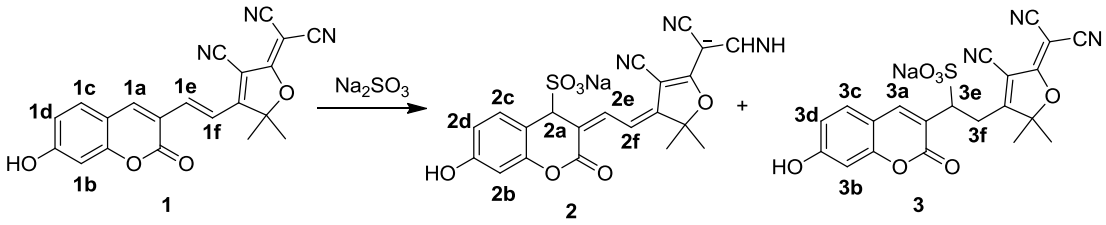


Figure S17.The line relationship between the fluorescent intensity ratio of the probe **2** (5 μM at 523 nm and 663 nm) and the concentration of the sulfite at 2 to 9 μM in 20 mmol pH 7.4 buffer solution.

14. The high resolution mass spectrum and ^{1}H NMR spectrum of probe 2 with sulfite



Scheme S1.The proposed reaction mechanism of probe **2** with sulfite

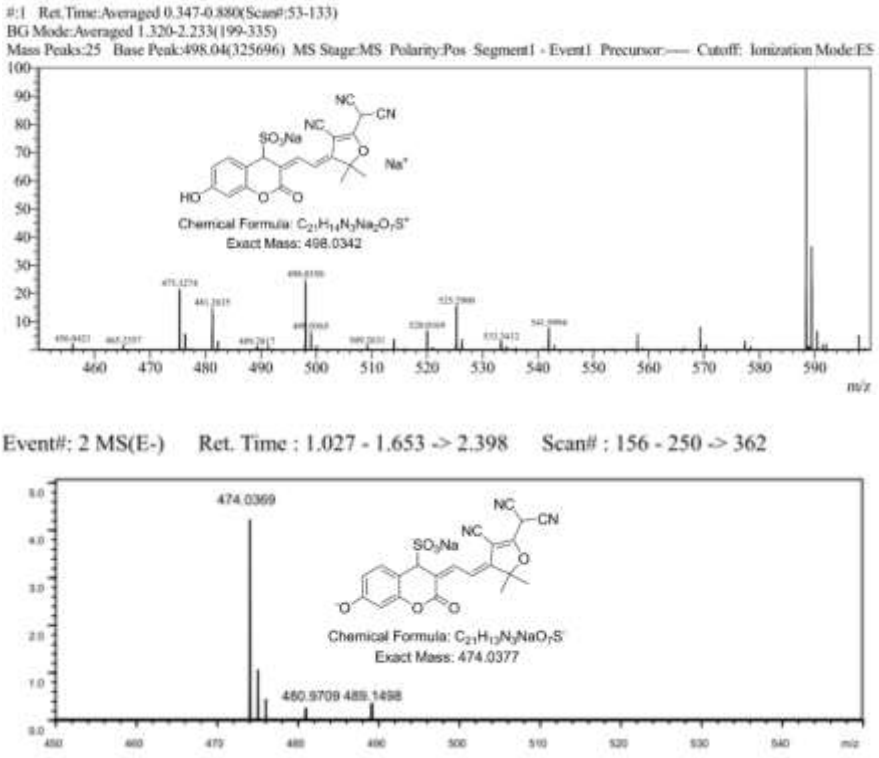


Figure S18. The high resolution mass spectrum of probe 2 with sulfite.

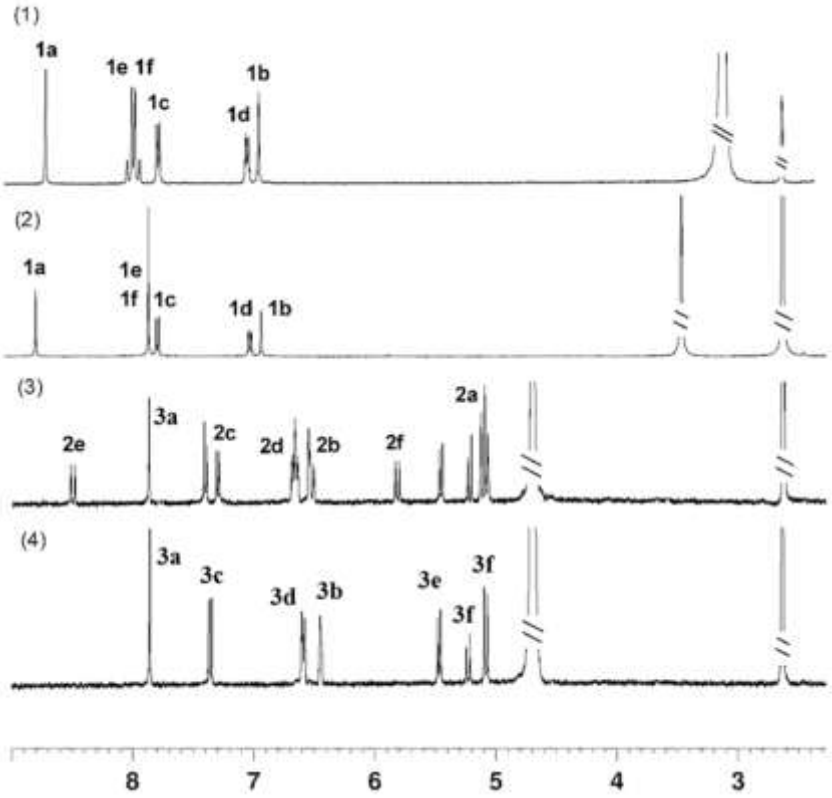


Figure19.^{1}H NMRspectra of 5 mMprobe 2 (1:in 0.5 ml CD_3COCD_3, and 50 μL DMSO-d_6 as the co-solvent; 2: in DMSO-d_6), and 5 mM probe 2 with 2 equiv. sulfite in DMSO-d_6: D_2O = 1:4 (3: 2h, 4: 24h).

15. The absorption spectra of TCF, probe 2 and the reaction of probe 2 with sulfite

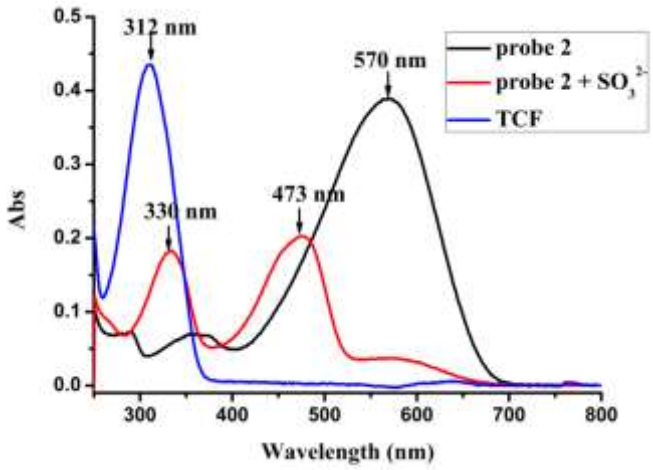
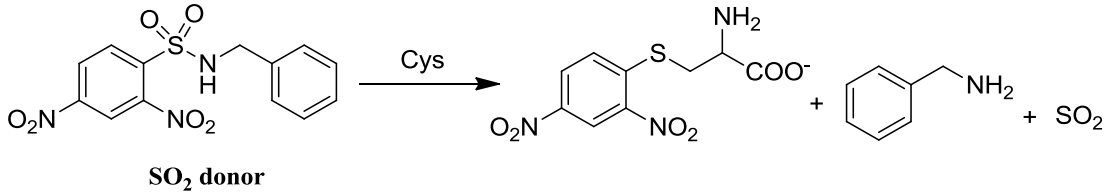


Figure S20. The absorption spectra of TCF, probe 2 and the reaction between probe **2** (10 μM) with 8 equiv sulfite in 20 mmol pH 7.4 buffer solution.

16. The response of SO2 releasing agent to probe 2



SO_2 donor

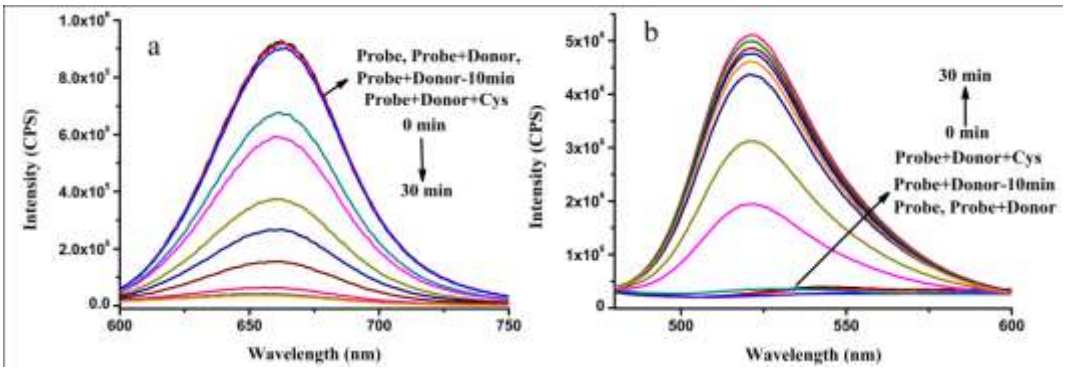


Figure S21. Time-dependent fluorescent spectra of probe (5 μM) + SO2 donor (40 μM) upon addition of 400μM Cys in 20 mool pH 7.4 HEPES buffer solution (for a: λ_{ex} = 580 nm. Slit: 5 nm/5 nm, $\lambda scan$ = 600 – 750 nm; for b: λ_{ex} = 466 nm. Slit: 5 nm/5 nm, $\lambda scan$ = 480 – 600 nm).

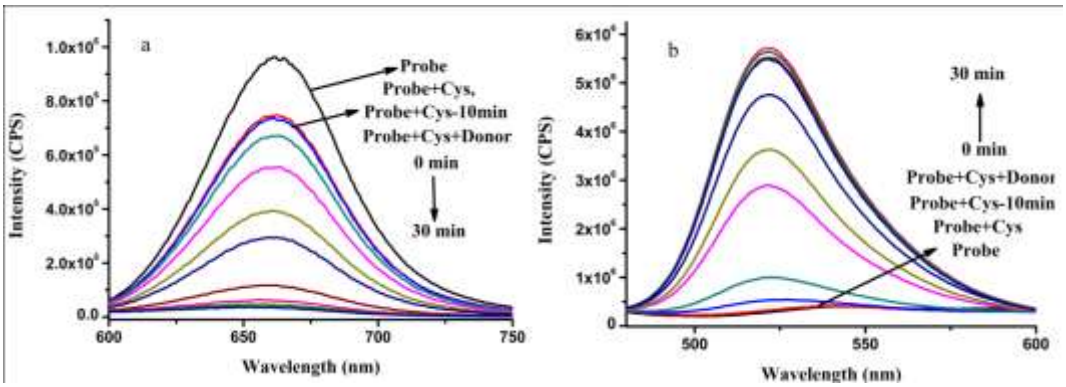


Figure S22. Time-dependent fluorescent spectra of probe (5 μM) + 400μM Cys upon addition of SO_2 donor (40 μM) in 20 mool pH 7.4 HEPES buffer solution (for a: λ_{ex} = 580 nm. Slit: 5 nm/5 nm; for b: λ_{ex} = 466 nm. Slit: 5 nm/5 nm,).

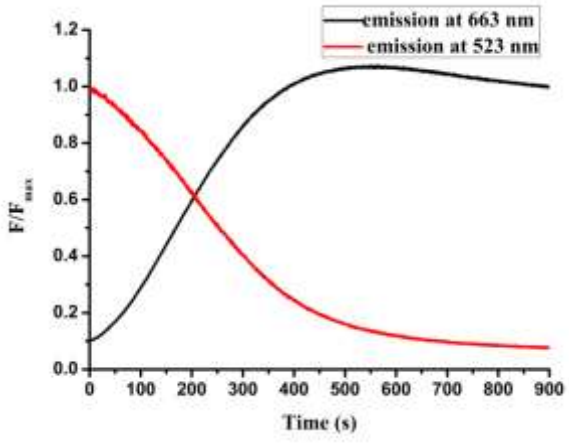


Figure S23. The time course of the response between probe 2 with the SO_2 donor and Cys(for black line:λ_{ex} = 580 nm, λ_{em} = 663 nm, Slit: 5 nm/5 nm; for red line: λ_{ex} = 466 nm, λ_{em} = 523 nm, Slit: 5 nm/5 nm).

17. Cell toxicity test.

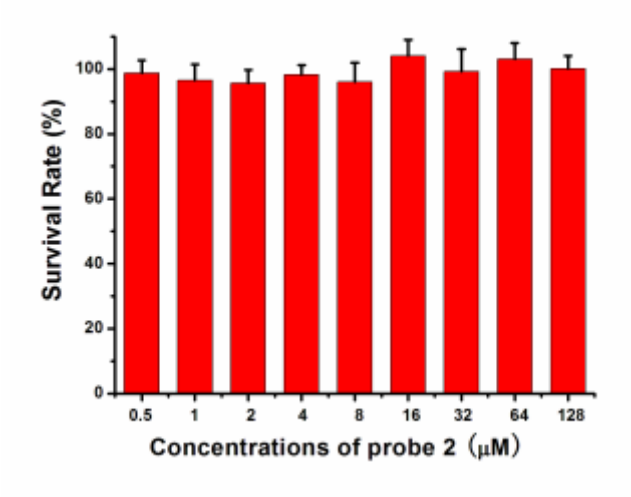
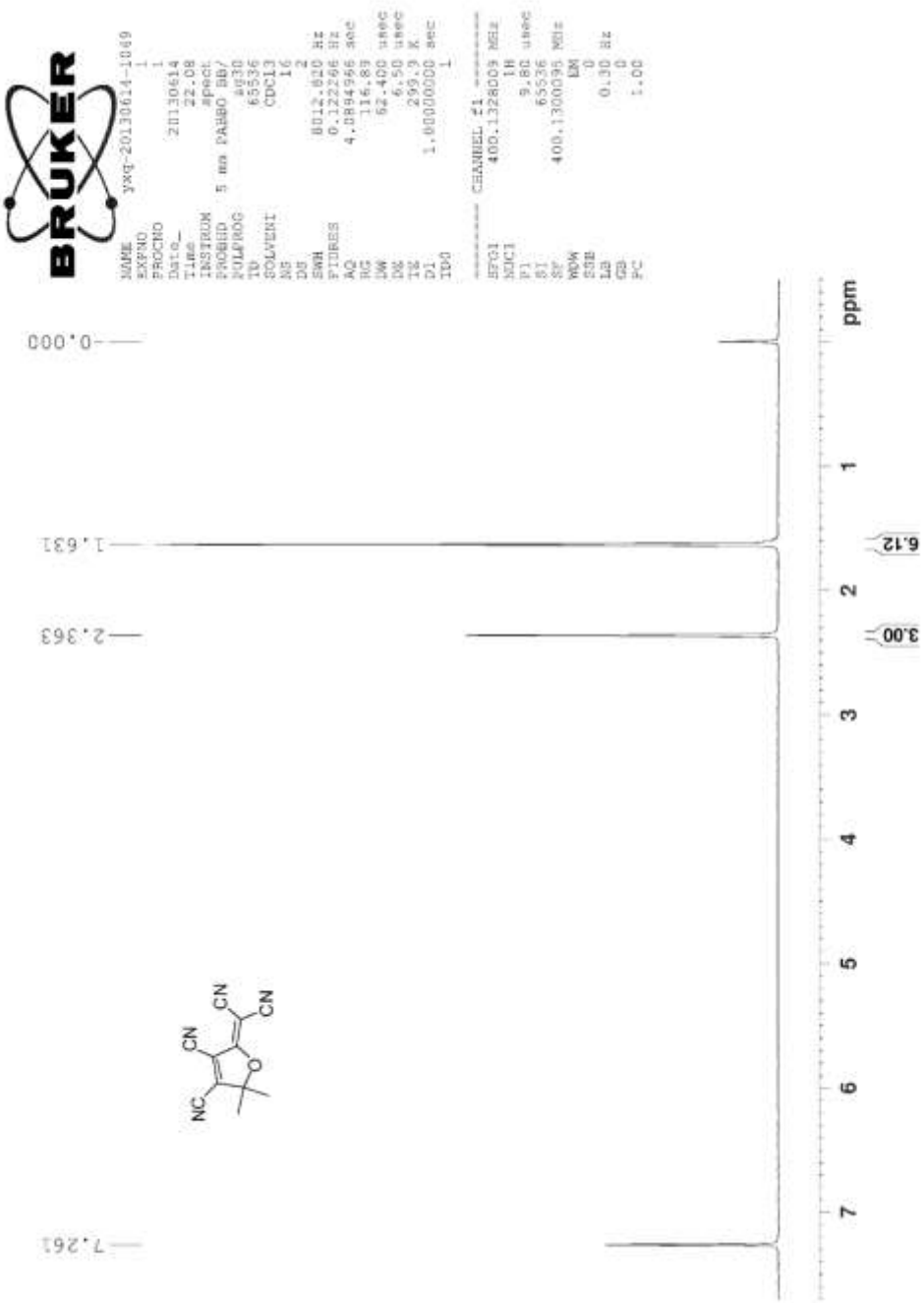


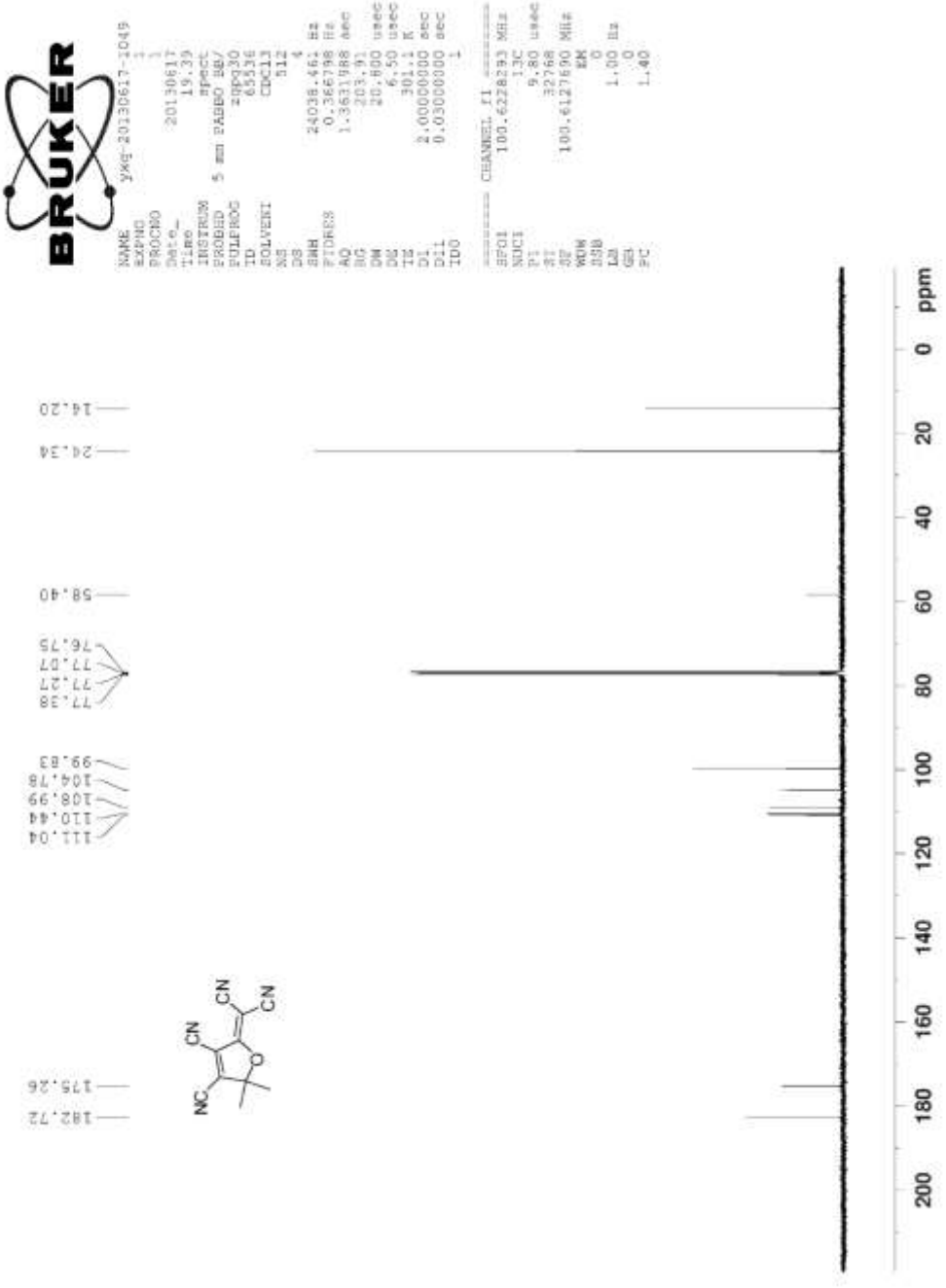
Figure S24.Cell viability by a standard MTT assay, the experiment was repeated three times and the data are shown as mean (±S.D.).

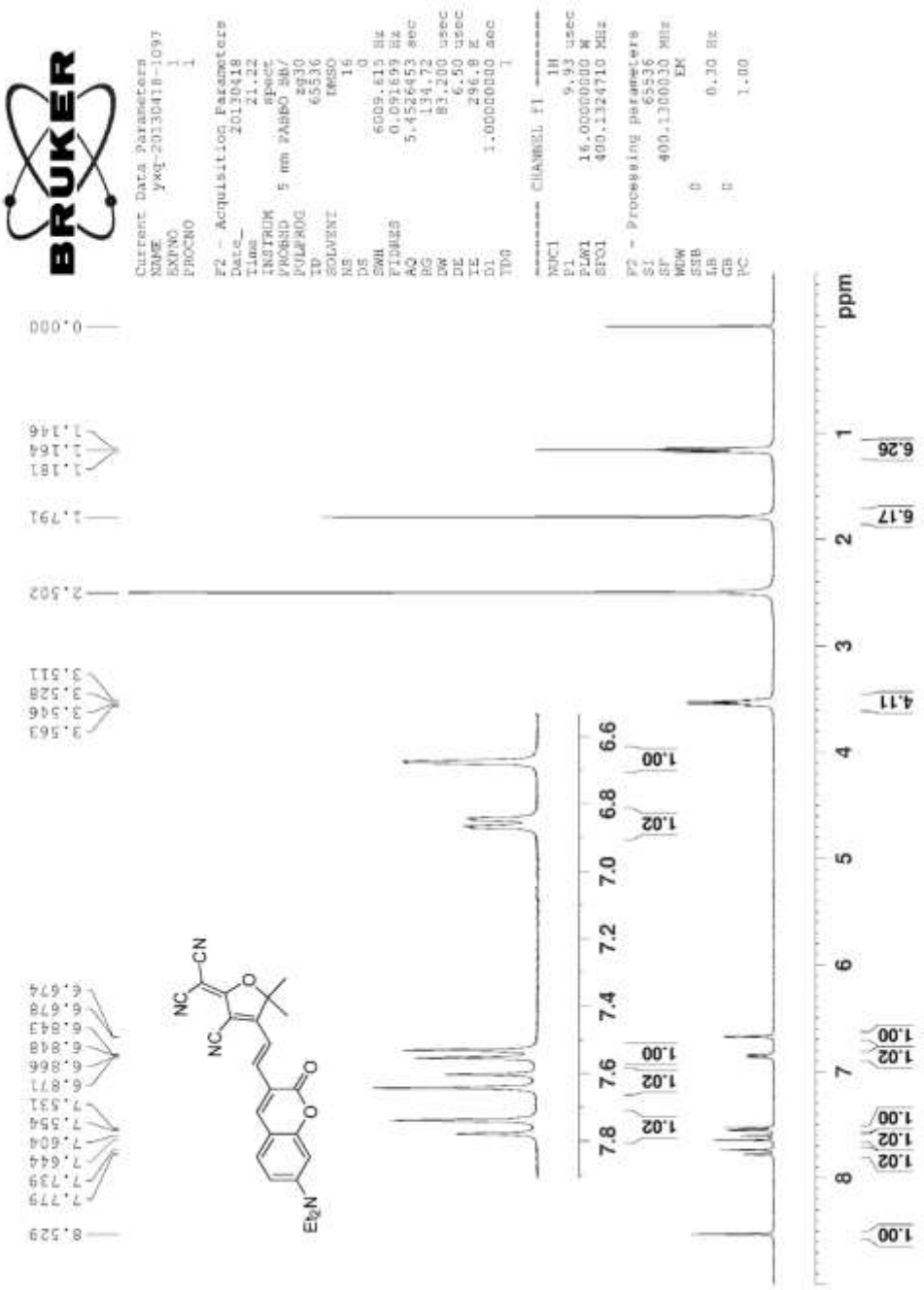
18. Cell culture fluorescence imaging

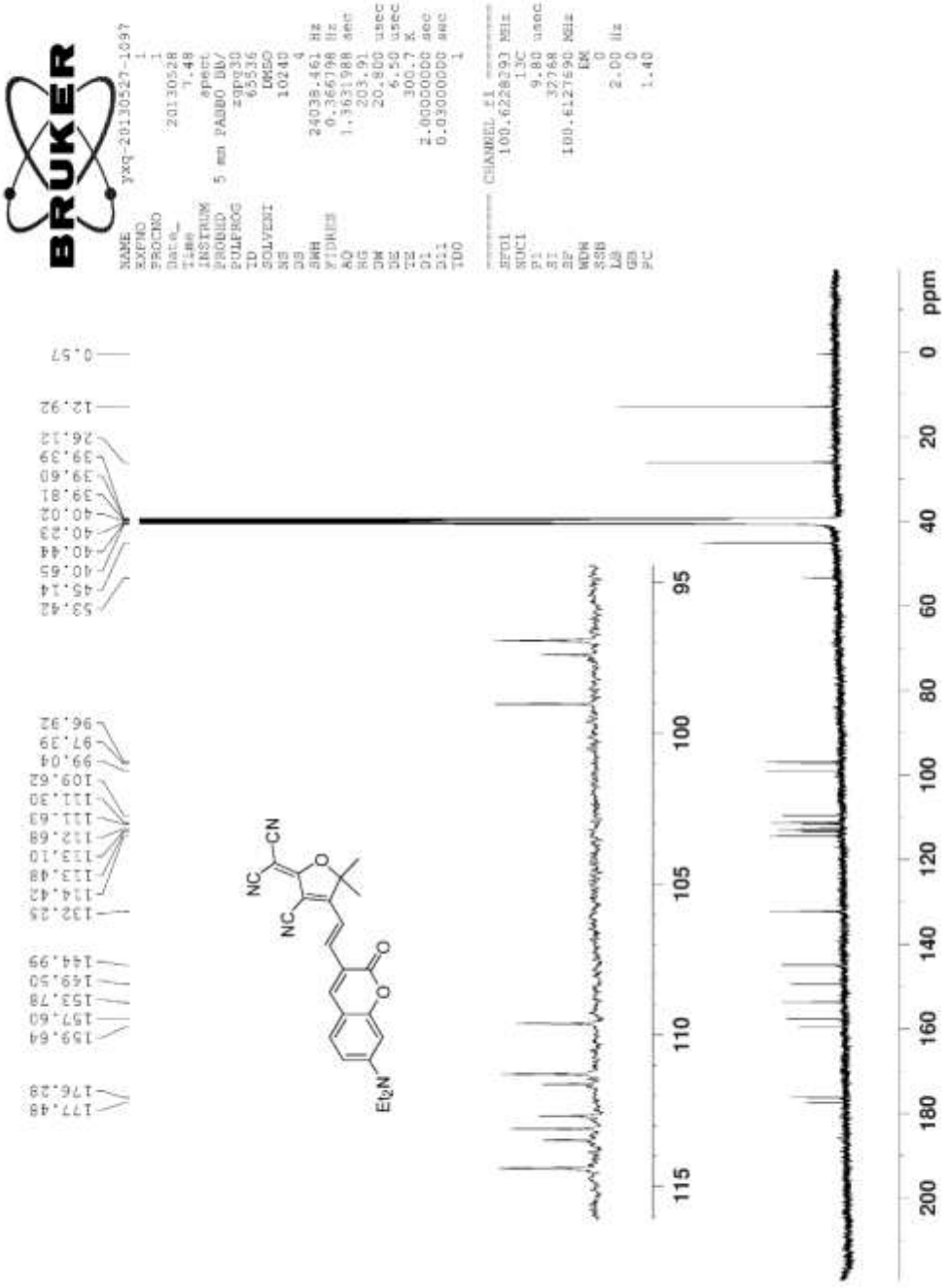
The U-2OS cell were grown in RPMI 1640 medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Cells were plated on 6-well plate at 5×106 cells per well and allowed to adhere for 12 hours. Fluorescence imaging was performed with a LEICA TCS-SP5 Laser Scanning Confocal Microscope with a 40 × oil-immersion objective lens. Before the experiments, cells were washed with PBS and then incubated with probe **2** (10 μM) in PBS for 30 min at 37 °C. Experiments to assess SO_3^{2-} uptake were performed in the same media supplemented with 80 μM Na_2SO_3or 80 μM SO_2 donor and 800 μM Cys for 30 min at 37 °C. Cell imaging was then carried out after washing cells with physiological saline. Emission was collected at 465~530 nm (excited at 488 nm) for green channel and at 600~700 nm (excited at 543 nm) for red channel.

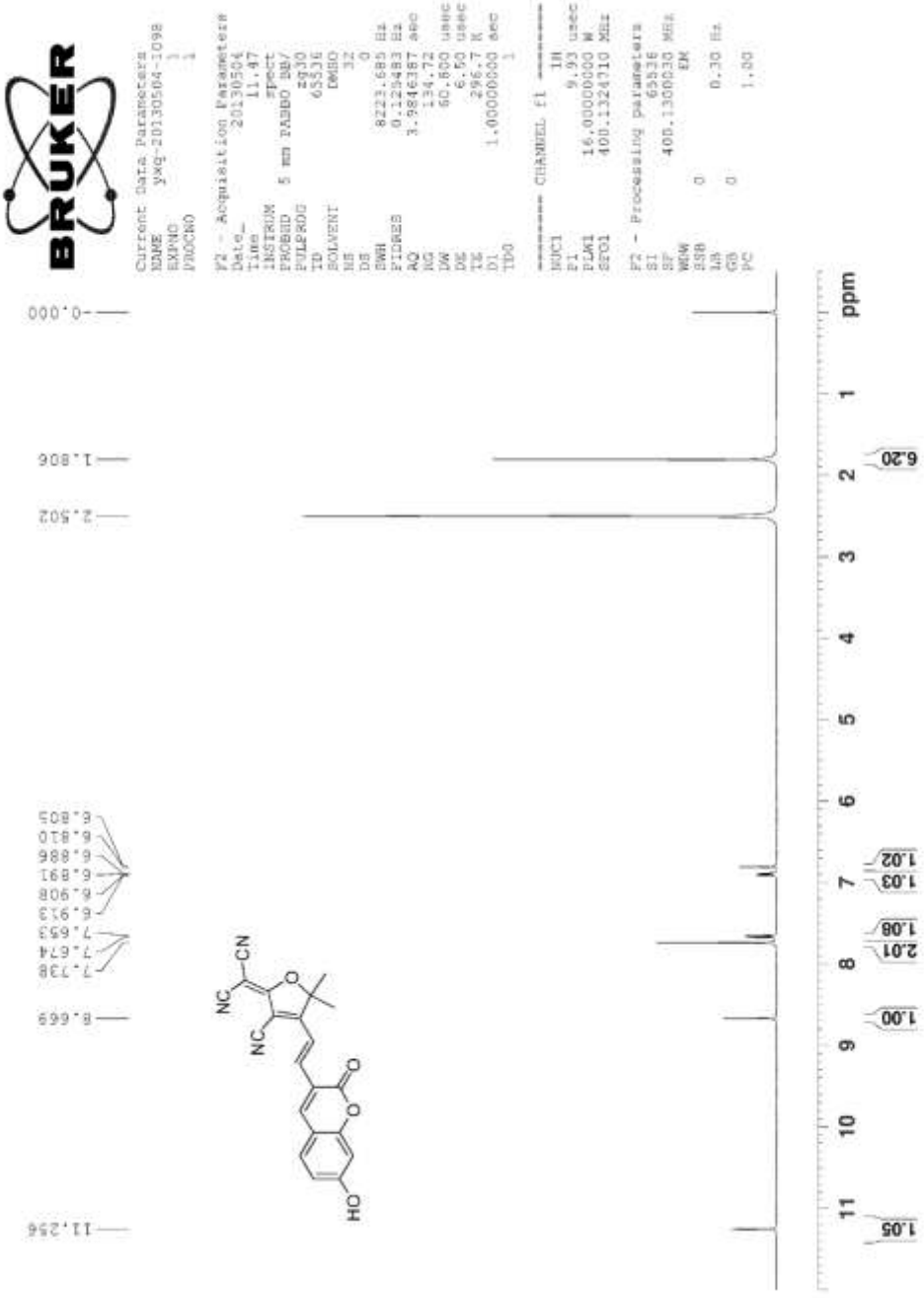
19. ^{1}H NMR and ^{13}C NMR spectrums.

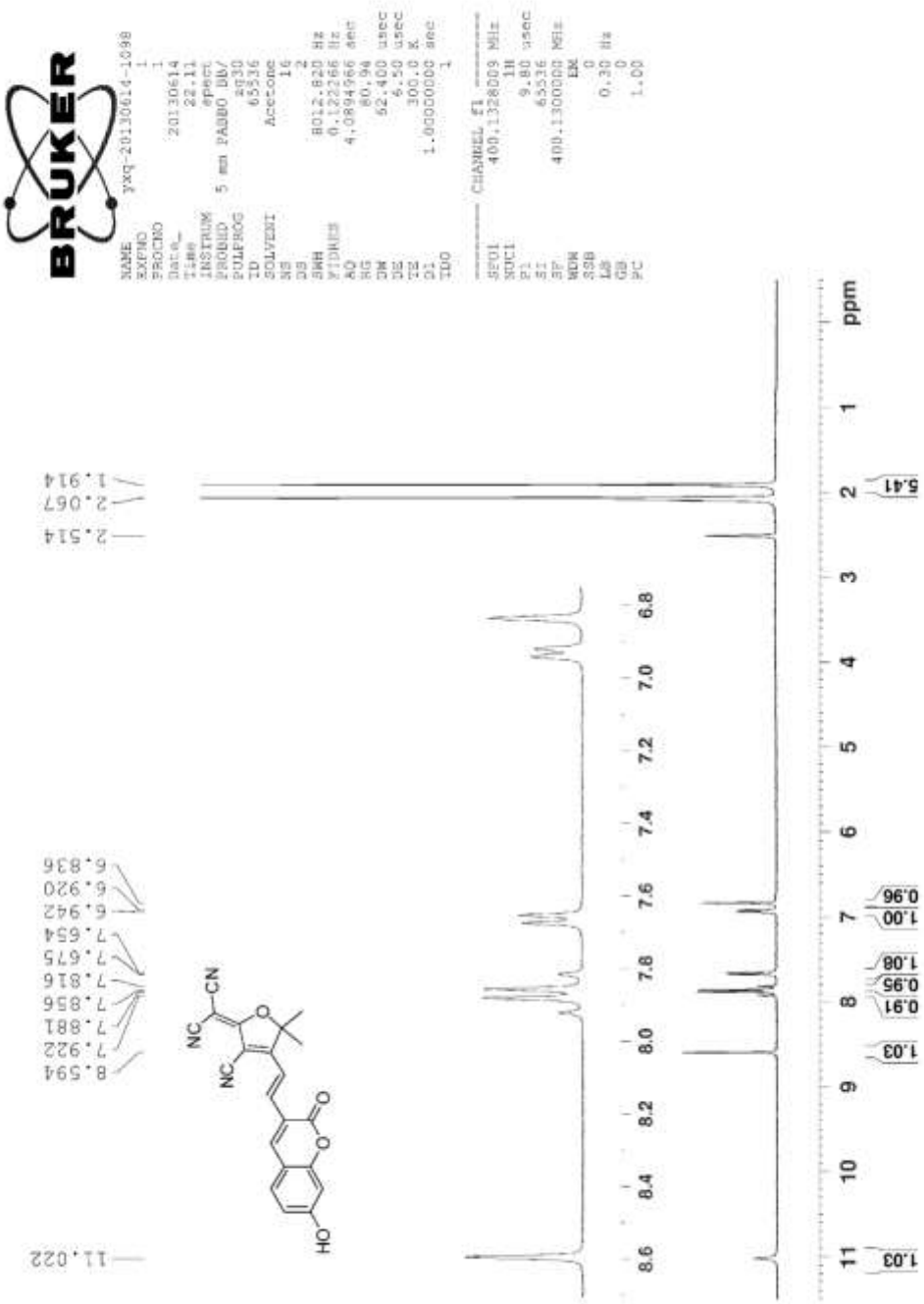


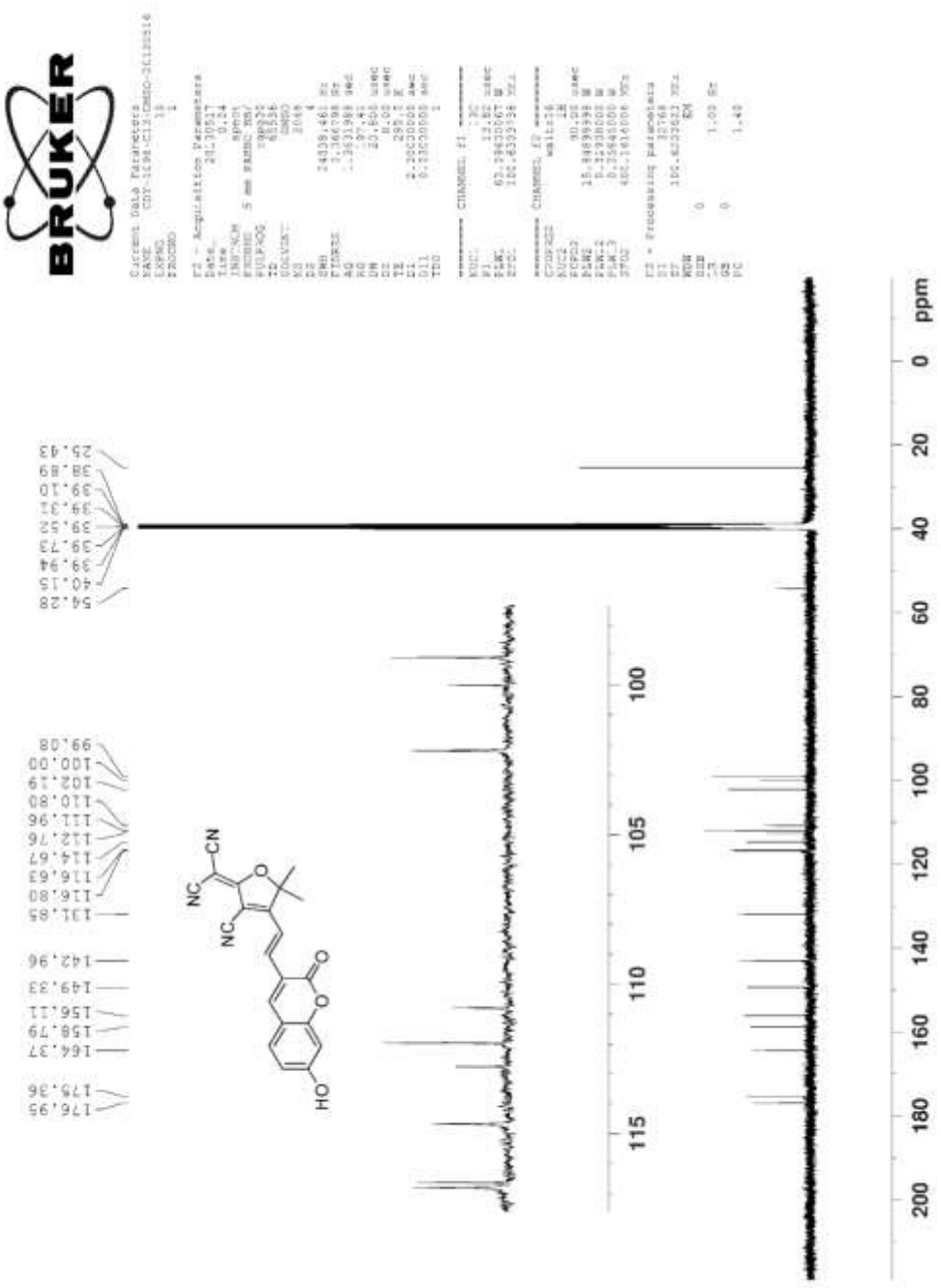

BRUKER
14.20
24.34
58.40
76.75
77.07
77.27
77.38
99.83
104.78
108.99
110.44
111.04
175.26
182.72
CN
CN
CN
NC
O
200
180
160
140
120
100
80
60
40
20
0
ppm

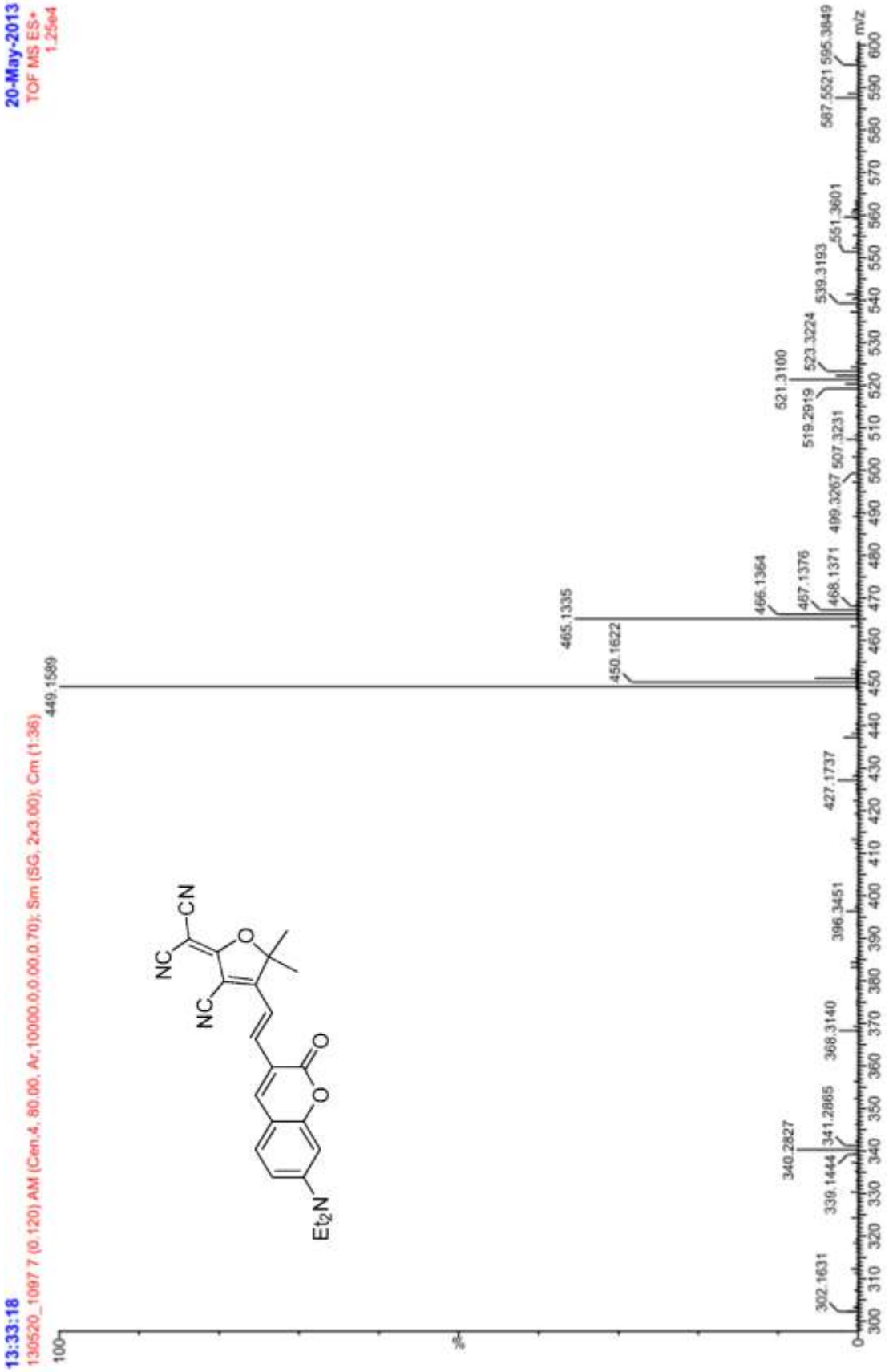

BRUKER
ppm
NC
CN
NC
O
O
O
Et2N

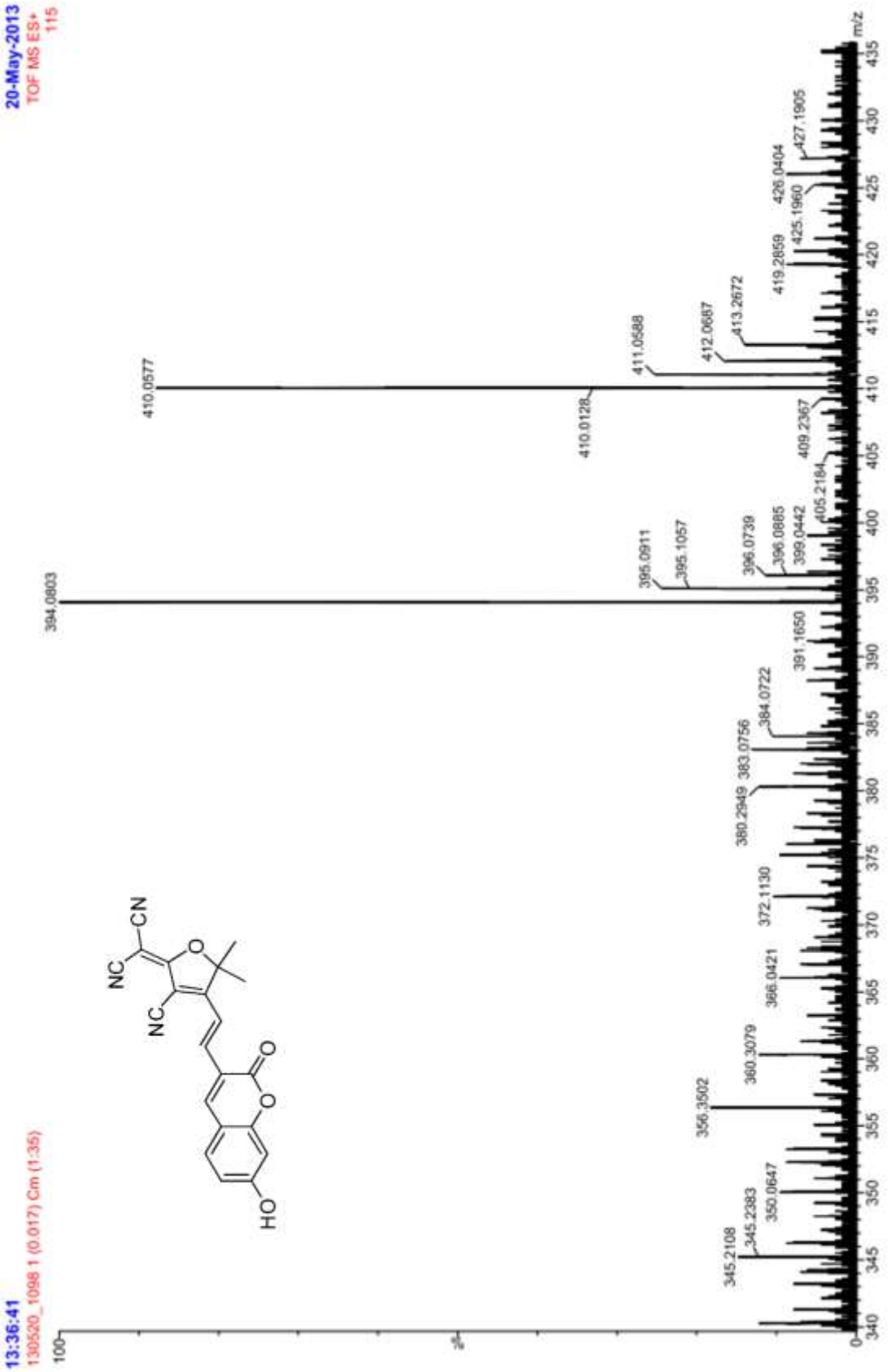

BRUKER
NC
CN
NC
O
O
O
Et2N
0.57
12.92
26.12
39.39
39.60
39.81
40.02
40.23
40.44
40.65
45.14
53.42
96.92
97.39
99.04
109.62
111.30
111.63
112.68
113.10
113.48
114.43
132.25
144.99
149.50
153.78
157.60
159.64
176.29
177.48
ppm


BRUKER
ppm
11.256
8.669
7.738
7.674
7.653
6.913
6.908
6.891
6.886
6.810
6.805
1.806
2.502
0.000
6.20
1.02
1.03
2.01
1.08
1.00
1.05
NC
CN
NC
O
O
HO


BRUKER
NAME yxq-20130614-1698
EXPNO 1
PROCNO 1
DATA_ 20130614
TIME 22.11
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT Acetone
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 80.64
DW 62.400 usec
DE 6.50 usec
TE 300.0 K
D1 1.0000000 sec
TD0 1
CHANNEL f1
SFO1 400.1328009 MHz
NUC1 1H
P1 9.80 usec
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
NC
CN
NC
O
O
HO
11.022
8.594
7.922
7.881
7.856
7.816
7.675
7.654
6.942
6.920
6.836
2.514
2.067
1.914
5.41
1.03
0.91
0.95
1.08
1.00
0.96
1.03


BRUKER
25.43
38.89
39.10
39.31
39.52
39.73
39.94
40.15
54.28
99.08
100.00
102.19
110.80
111.96
112.76
114.67
116.63
116.80
131.85
142.96
149.33
156.11
158.79
164.37
175.36
176.95
NC
CN
NC
O
O
HO
100
105
110
115
0
20
40
60
80
100
120
140
160
180
200
ppm


20-May-2013
TOF MS ES+
1.25e4
13:33:18
130520_1097 7 (0.120) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (1:36)
100
%
449.1589
465.1335
450.1622
466.1364
467.1376
468.1371
499.3267
507.3231
519.2919
521.3100
523.3224
539.3193
551.3601
587.5521
595.3849
427.1737
396.3451
368.3140
340.2827
341.2865
339.1444
302.1631
m/z
NC
CN
NC
O
O
O
Et2N


20-May-2013
TOF MS ES+
115
13:36:41
130520_1098 1 (0.017) Cm (1:35)
100
%
0
m/z
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
345.2108
345.2383
350.0647
356.3502
360.3079
366.0421
372.1130
380.2949
383.0756
384.0722
391.1650
394.0803
395.0911
395.1057
396.0739
396.0885
399.0442
405.2184
409.2367
410.0128
410.0577
411.0588
412.0667
413.2672
419.2859
425.1960
426.0404
427.1905
NC
CN
NC
O
O
O
HO