Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Fluorescent detection of endogenous bisulfite in liver cancer cells using an effective ESIPT enhanced FRET platform

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General Section

Apparatus and chemicals

^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded on Bruker Avance 300 (or 400) spectrometer using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. HR-MS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). IR spectra were recorded by use of an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). Melting points were measured on an XD-4 digital micro-melting point apparatus. Thin-layer chromatography (TLC) was conducted on silica gel 60 F_{254} plates (Merck KGaA) and column chromatography was conducted over silica gel (mesh 200-300). Fluorescence measurements were recorded on a Perkin-Elmer LS-55 luminescence spectrophotometer, and UV-vis spectra were recorded on a U-4100 UV-Vis-NIR Spectrometer (Hitachi). Quartz cuvettes with a 1-cm path length and 3-mL volume were involved in fluorescence and UV-vis measurements. The pH values were measured by use of a PHS-3C digital pH-meter (YouKe, Shanghai). All reagents were purchased from J&K, Aladdin or Sinopharm Chemical Reagent Co. and used without further purification.

Preparation for UV-vis and fluorescence measurements

PBS buffer (10 mM, pH 7.4) was used throughout the measurements. L-HF1 (or L-HF2) was dissolved in DMF to get the stock solution (1 mM). Distilled water was used to prepare stock solutions (10^{-2} M) of NaF, NaCl, NaBr, KI, $NaHCO_3$, KNO_3, $NaNO_2$, NaN_3, NaOAc, NaH_2PO_4, NaClO, $KClO_3$, Na_2SO_4, $NaHSO_4$, NaHS, KSCN, $Na_2S_2O_3$, Na_2SO_3 and $NaHSO_3$. Stock solutions (10^{-1} M) of cysteine, homocysteine, glutathione, glutamate, histidine, lysine, tryptophan, valine, arginine, sarcosine and aspartic acid were also prepared in distilled water, to which hydrochloric acid was added to assist the dissolution of tryptophan and aspartic acid. Stock solutions of $NaHSO_3$ and Na_2SO_3 were freshly prepared each time before use. Test solution was

prepared by placing 50 μL stock solution of L-HF1 (or L-HF2) into a 10-mL volumetric flask with PBS buffer (10 mM, pH 7.4) containing 25% DMF (v/v), then an appropriate aliquot solution of the analyte was added, after which the test solution was diluted to 10 mL with PBS buffer (10 mM, pH 7.4) containing 25% DMF (v/v). Excitation wavelength was 345 nm otherwise stated.

Cell imaging

Cells were cultured in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37℃. Probe L-HF1 was dissolved in DMSO to get the stock solution (10 mM) and diluted to 5 μM each time before use. Hela cells were incubated with L-HF1 for 1 h, then with bisulfite (0.025, 0.1, 0.5 or 1 mM) for 0.5 h. HepG2 cells or L-02 cells were incubated with L-HF1 for 1 h, then with a mixture of GSH (500 μM) and $Na_2S_2O_3$ (250 μM) for 0.5 h. In control experiments, probe loaded HepG2 cells were incubated with GSH (500 μM) for 0.5 h before images were taken. Probe loaded HepG2 cells were also pretreated with TNBS (10 mM) for 0.5 h, and then with GSH (500 μM) and $Na_2S_2O_3$ (250 μM) for another 0.5 h. Excited at 405 nm, images were taken under a confocal microscope (LSM 700). Green fluorescence was collected from channel 405-555 nm and red fluorescence from channel 560-700 nm, respectively. Cytotoxicity of probe L-HF1 was researched by SRB assays using HeLa cells incubated with L-HF1 (1, 5 or 10 μM) for 6 h.

Calculation of energy transfer efficiency

Energy transfer efficiency (*E*) was calculated using the following equation:

$$E = 1 - F_{DA}/F_D$$

Where, F_{DA} and F_D denote the donor fluorescence intensity with and without an acceptor, respectively.

Theoretical Calculation methods

All the calculations were implemented with the Gaussian 09 program package. The geometric structure, absorption spectra, emission spectra and the excited-state proton transfer energy profiles of those compounds in both the ground and the lowest singlet excited state have been calculated by density functional theory (DFT) and time-dependent density functional theory (TDDFT) method at B3LYP/6-31g (d,p) level.

To investigate the proton transfer process, a truncated model of moiety D was constructed and fully optimized. Based on the optimized geometries, the proton transfer processes on ground and the lowest singlet excited state were scanned with a decrement of 0.02 Å.

Synthesis

Probe L-HF1

Probe L-HF1 was synthesized via condensation of compound 3 and 4 (see ESI). The mixture of compound 3 (220 mg, 0.5 mmol) and compound 4 (180 mg, 0.6 mmol) in absolute ethanol (20 mL) was refluxed under N_2 atmosphere until compound 3 was consumed (TLC). The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (CH_2Cl_2 : MeOH = 30:1 to 15:1) to afford a red solid (285 mg, 79%).^{1}H NMR (400 MHz) 8.37 (d, *J* = 8.4 Hz, 2H), 8.27 (d, (d, *J* =7.6 Hz, 1H), 8.24 (d, *J* = 7.2 Hz, 2H), 8.09 (d, *J* = 15.6 Hz, 1H), 7.75-7.63 (m, 5H), 7.53-7.45 (m, 5H), 7.14 (s, 1H), 6.99 (d, *J* = 6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3, 173.5, 169.7, 155.3, 154.7, 154.5, 143.6, 142.2, 141.6, 138.9, 136.2, 134.9, 134.0, 132.7, 129.3, 128.5, 127.9, 127.5, 125.3, 124.7, 124.1, 122.4, 120.5, 118.4, 114.1, 113.5, 107.2, 51.45, 46.84, 35.79, 27.43; IR (KBr, cm^{-1}): 3014, 2919, 2850, 1729, 1611, 1571, 1522, 1466, 1384, 1297, 1237, 1190, 1115, 1004; HR-MS (ESI): m/z calculated for $C_{39}H_{36}N_3O_4^+$ 610.2706, found 610.2702.

2.4.2. Probe L-HF2

The mixture of compound 3 (0.446 g, 1 mmol), compound 4 (301 mg, 1mmol), sodium acetate (82 mg, 1mmol) and acetic anhydride (2 mL) was stirred under 80℃ for 3 h. After Cooling to room temperature, the mixture was diluted with CH_2Cl_2 (100 mL) and washed with brine for three times. The organic layer was dried over anhydrous sodium sulphate. After filtration, the solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (CH_2Cl_2 : MeOH = 30:1) to afford a red solid (580 mg, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.26 (dd, *J* = 7.8 Hz, 1.5Hz, 1H), 8.20 (d, *J* = 8.7 Hz, 2H), 8.09 (d, *J* = 15.6 Hz, 1H), 7.98 (d, *J* = 8.4 Hz, 2H), 7.77-7.72 (m, 1H), 7.67-7.55 (m, 4H), 7.54-7.43 (m, 5H), 7.01 (d, *J* = 8.7 Hz, 2H), 4.31 (s, 3H), 3.95-3.64 (m, 8H), 2.38 (s, 3H), 1.80 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.4, 172.0, 169.4, 167.9, 155.6, 155.0, 154.7, 154.5, 142.2, 141.6, 137.6, 135.0, 134.2, 134.1, 131.6, 129.4, 128.7, 128.5, 127.6, 126.0, 125.4, 124.2, 123.5, 122.4, 118.2, 114.2, 113.5, 107.3, 51.4, 35.8, 27.4, 20.7; IR (KBr, cm^{-1}): 3012, 2974, 2922, 2854, 1774, 1729, 1642, 1572, 1523, 1477, 1395, 1297, 1238, 1189, 1004; HR-MS (ESI): m/z calculated for $C_{41}H_{38}N_3O_5^+$ 652.2811, found 652.2819.

Compound 1

To a solution of ethanol (30 mL) and water (10 mL) in an ice bath was added 2-hydroxyacetophenone (1.36 g, 0.01 mol), 4-carboxybenzaldehyde (1.5 g, 0.01 mol) and then sodium hydroxide (2.0 g, 0.05 mol). The mixture was stirred for 0.5 h at 0℃, then for 7 h at room temperature. The mixture was poured into ice-water (100 mL). Hydrochloric acid was added dropwise to adjust pH 6. The precipitate was collected and dried. The solid (2.15 g, 0.08 mol) was dispersed in ethanol (60 mL). Sodium hydroxide (1.6 g, 0.04 mol) in water (20 mL) was added under 0℃. Then hydrogen peroxide (30%, 8 mL) was added dropwise over a period of 15 min. The mixture was allowed to warm up to room temperature and stirred for 12 h, then poured into ice-water (100 mL). Yellow precipitate was collected and dried to afford compound 1 in a total 66% yield. Compound 1 was pure enough and used without further purification for next step.

Compound 3

Compound 1 (100 mg, 0.35 mmol), compound 2 (64 mg, 0.35 mmol), EDC (67 mg, 0.35 mmol) and DMAP (4 mg, 0.035 mmol) were added into CH_2Cl_2 (10 mL) and stirred at room temperature until the starting materials were consumed (about 8 h). CH_2Cl_2 (30mL) was added to the mixture which was then washed with brine for three times. Organic layer was separated and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (CH_2Cl_2: ethyl acetate = 1:1) to afford a bright yellow solid (128 mg, 82%). Mp: 238-239℃. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.83 (s, 1H), 8.36 (d, *J* = 8.7 Hz, 2H), 8.27 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.75-7.72 (m, 1H),7.64-7.61 (m, 3H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.12 (s, 1H), 6.97 (d, *J* = 8.7 Hz, 2H), 3.97-3.49 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 190.4, 173.5, 169.7, 155.4, 154.5, 143.5, 138.9, 136.4, 134.0, 132.7, 131.8, 128.0, 127.9, 127.4, 125.5, 124.7, 120.6, 118.3, 114.1; IR (KBr, cm^{-1}): 3270, 3000, 2918, 2850, 2809, 2724, 1731, 1664, 1633, 1603, 1568, 1483, 1421, 1288, 1227, 1159, 1006, 753; HR-MS (ESI): m/z calculated for $C_{27}H_{23}N_2O_5^+$ 455.1607, found 455.1602.

Compound D

Compound 1 (840 mg, 3 mmol), diethyl amine (328 mg, 4.5 mmol), EDC (859 mg, 4.5 mmol) and HOBt (607 mg, 4.5 mmol) were added into CH_2Cl_2 (20 mL). The mixture was stirred for 8 h at room temperature. CH_2Cl_2 (30mL) was added to the mixture which was then washed with brine for three times. Organic layer was separated and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (petroleum ether: ethyl acetate = 1:1) to afford a yellow solid (452 mg, 45%). Mp: 183-184℃. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.32 (d, *J* = 8.1 Hz, 2H), 8.26 (dd, *J* = 8.1 Hz, 1.5 Hz, 1H), 7.76-7.70 (m, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.10 (br, 1H, *OH*), 3.57 (s, 2H), 3.32 (s, 2H), 1.24 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.5, 170.5, 155.4, 143.9, 138.7, 133.8, 131.8, 127.7, 126.6, 125.5, 124.6, 120.6, 118.3, 43.32, 39.40, 14.29, 12.89; IR (KBr, cm^{-1}): 3275, 2972, 2937,

2877, 1612, 1568, 1476, 1425, 1301, 1213, 1100, 749; HR-MS (ESI): m/z calculated for $C_{20}H_{20}NO_4^+$ 338.1392, found 338.1391.

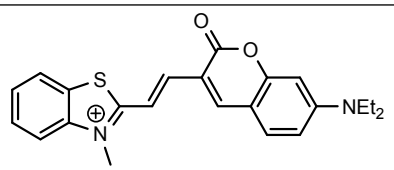
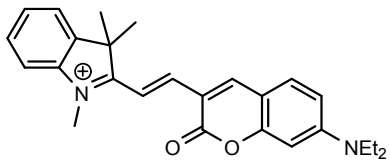
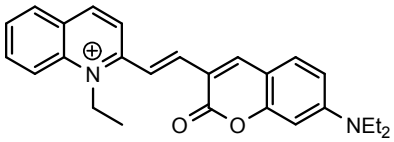
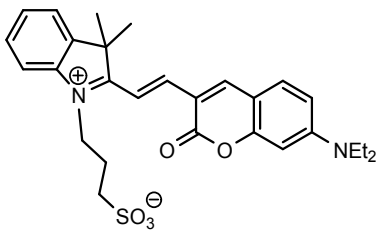
Tables, Schemes and Figures

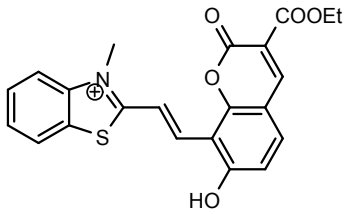
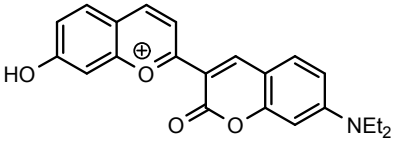
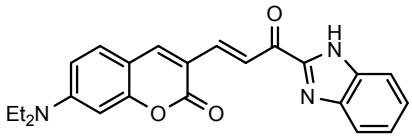
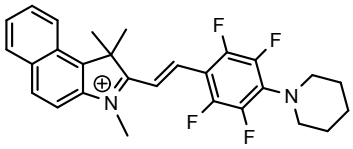
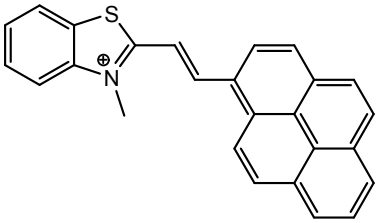
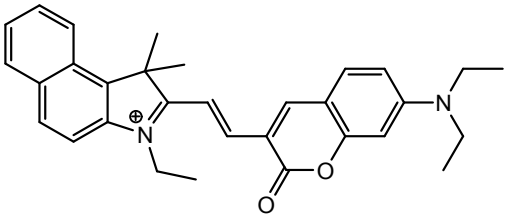
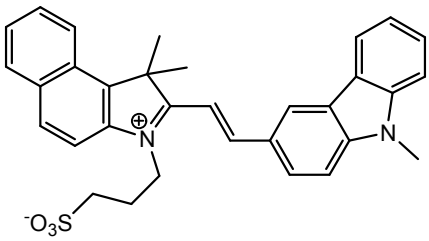
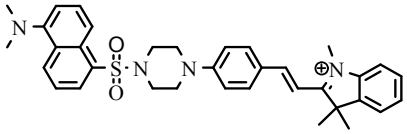
Table S1 Comparison of different FRET operating modes

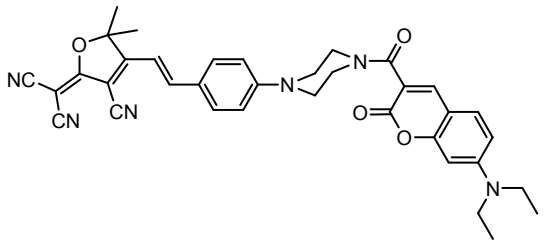
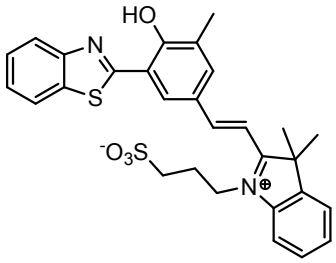
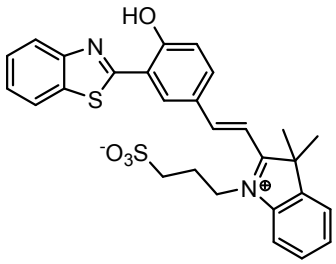
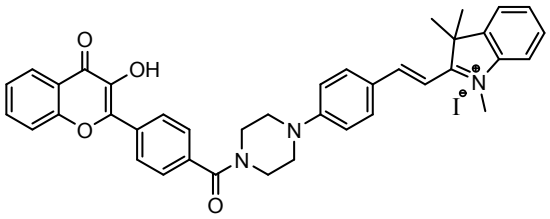
FRET process	Distance[1]	Donor structure	Acceptor structure
Off--on	Unchanged	Unchanged	Changed
On--off	Unchanged	Unchanged	Changed
On--off	Changed	Unchanged	Changed

[1] The distance between two fluorophores (donor and acceptor).

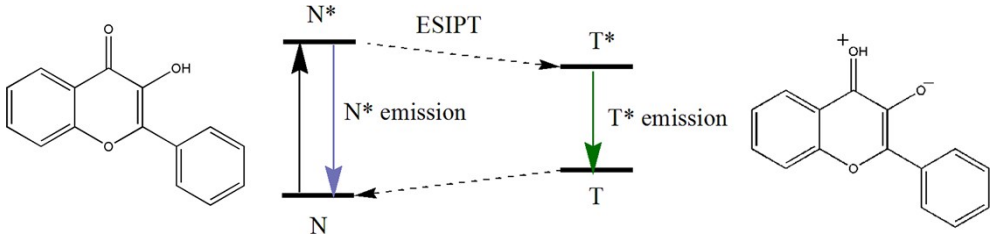
Table S2 Comparison of ratiometric fluorescent probes for HSO_3^-/SO_3^{2-}.

Entry	Probe structures	Stokes shifts (nm)[1]	Detection limits (nM)	Interaction Mechanisms	Ref.
1		23/188	380	ICT	12*a*
2		75/245	90	ICT	12*b*
3		68/160	89	ICT	12*c*
4		35/117	27	ICT	12*d*

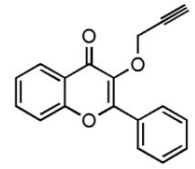
5		45/100	--	ICT	12*e*
6		55/35	34	PET	12*f*
7		70/190	53	ICT	12*g*
8		138/125	3	TICT	12*h*
9		51/270	89	ICT	12*i*
10		70/160	27.6	ICT	12*j*
11		58/220	58	ICT	12*k*
12		120/170	100	FRET-ICT	7*c*

13		80/242	45	FRET	12*l*
14		78/210	56	ESIPT-ICT	10*a*
15		60/200	340	ESIPT-ICT	10*b*
16		185/245	16	FRET-ESIPT	This work

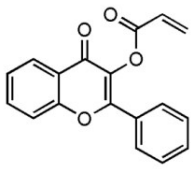
[1] The former data represent the distances between the excitation wavelengths and the short wavelength emission peaks; the later data represent the distances between excitation wavelengths and the longer wavelength emission peaks.



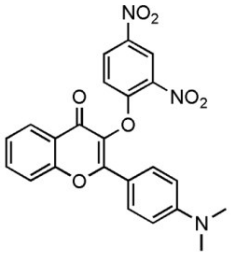
Scheme S1 Schematic of the ESIPT process of 3-hydroxyflavone.



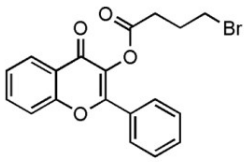
Chem. Commun., 2012



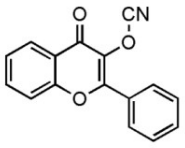
ACS Appl. Mater. Interfaces, 2014



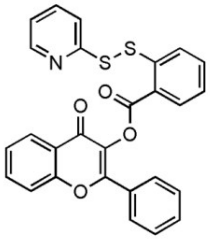
Org. Biomol. Chem., 2014



Sens. Actuators B-Chem., 2015

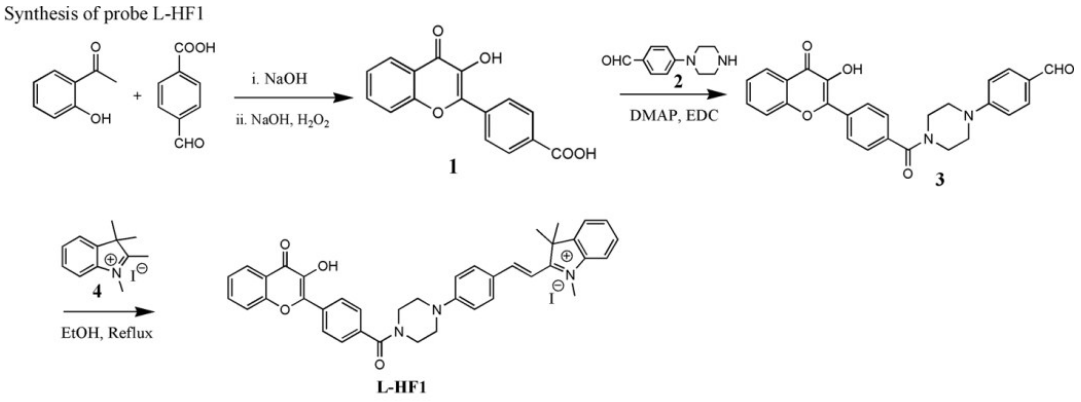


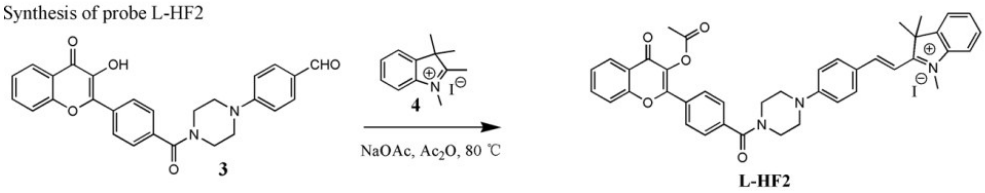
Anal. Chem., 2016

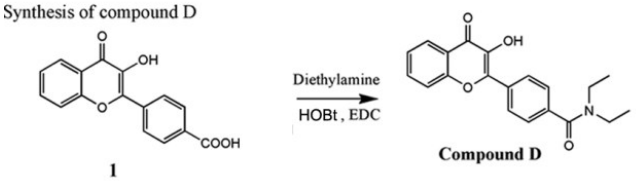


Sens. Actuators B-Chem., 2016

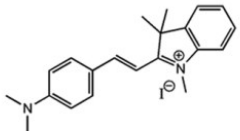
Scheme S2 Structures of probes designed on flavone fluorophore.



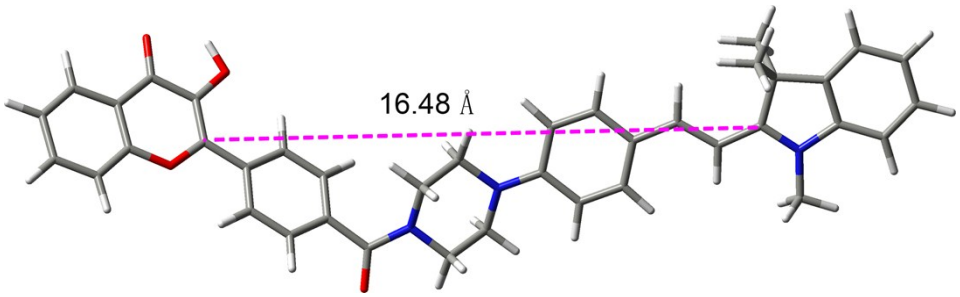




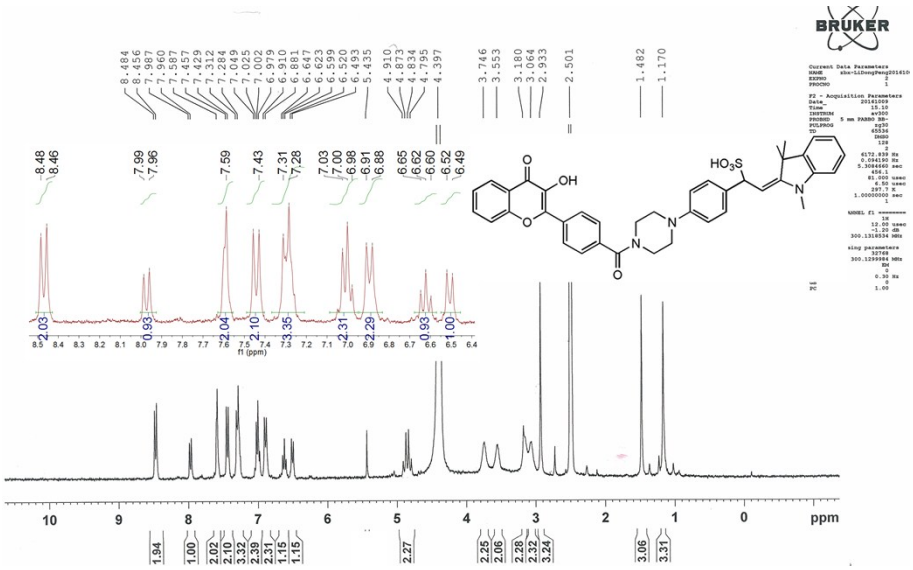
Structure of compound A



Scheme S3 Synthesis and structures of compounds L-HF1, L-HF2, D and A.



Scheme S4 Optimized structure of probe L-HF1 gotten by using a suite of Gaussian 09 programs. Calculated C-C bond distance (as indicated in the scheme): 16.48 Å.



Scheme S5 The ^{1}H NMR spectrum of the reaction product of L-HF1 with excess amount of Na_2SO_3 in DMSO-*d6*-D_2O (4:6, v/v).

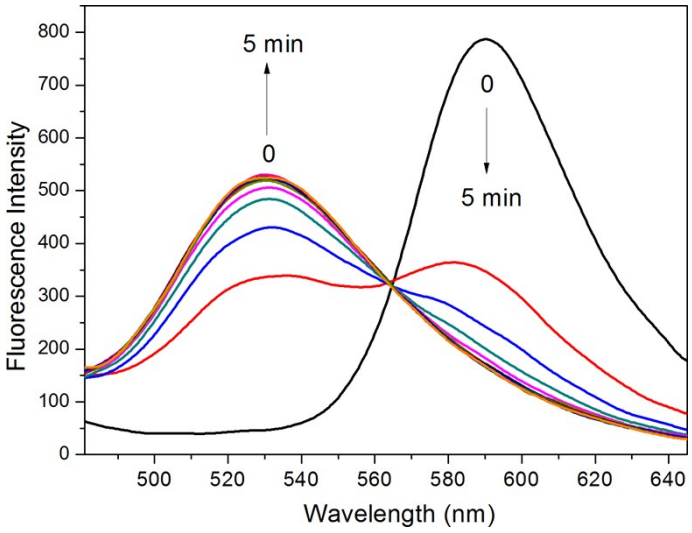


Fig. S1 Time-dependent fluorescence spectra of L-HF1 (5 μM) in the presence of bisulfite (10 equiv.) in PBS (pH = 7.4, 10 mM, containing 25% DMF). λ_{ex} = 345 nm, slits: 10 nm/10 nm.

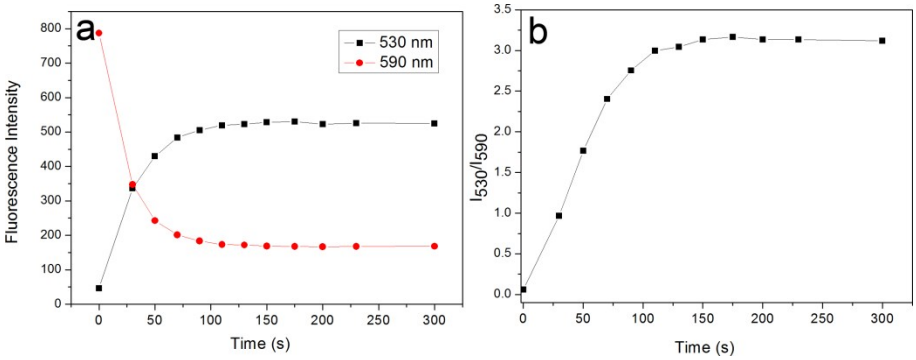


Fig. S2 Time-dependent fluorescence changes of L-HF1 (5 μM) in the presence of bisulfite (10 equiv.). All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF). λ_{ex} = 345 nm, slits: 10 nm/10 nm.

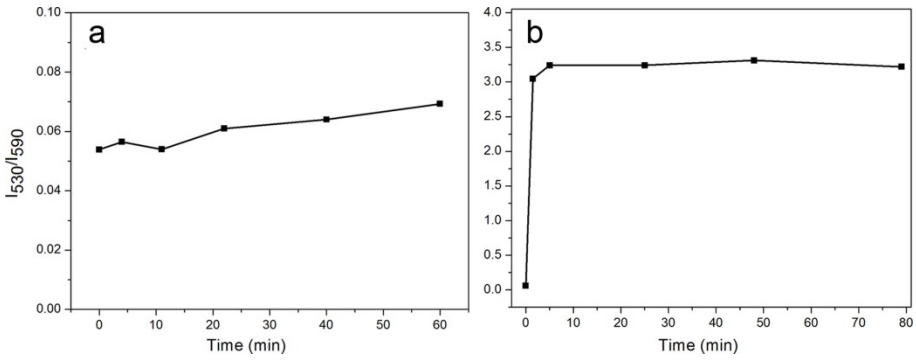


Fig. S3 Stability of probe L-HF1 (5 μM) in the absence (**a**) or presence (**b**) of bisulfite

(10 equiv.) in test solutions. All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF). λ_{ex} = 345 nm.

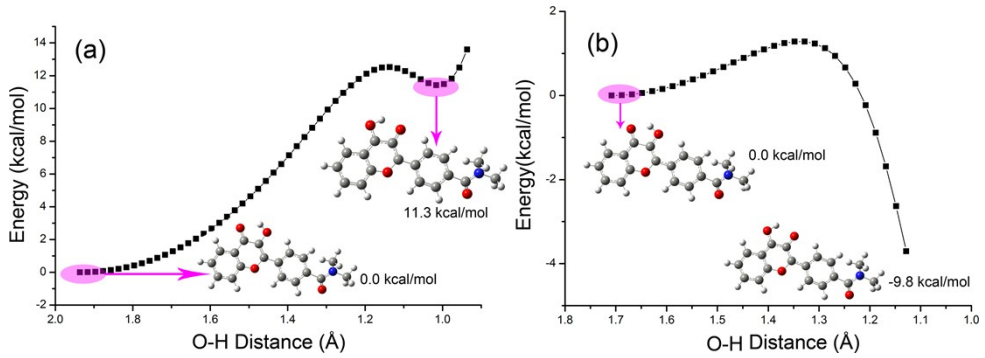


Fig. S4 The ground state (a) and excited-state (b) proton transfer energy profiles.

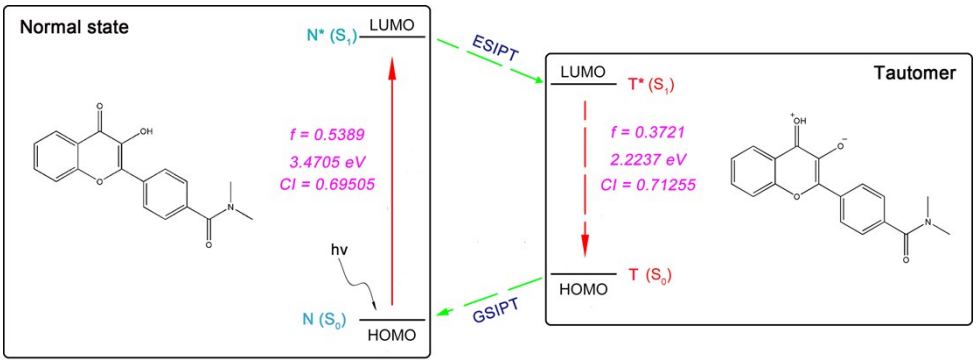


Fig. S5 Schematic illustration of the ESIPT process of moiety Data were gotten via theoretical calculations.

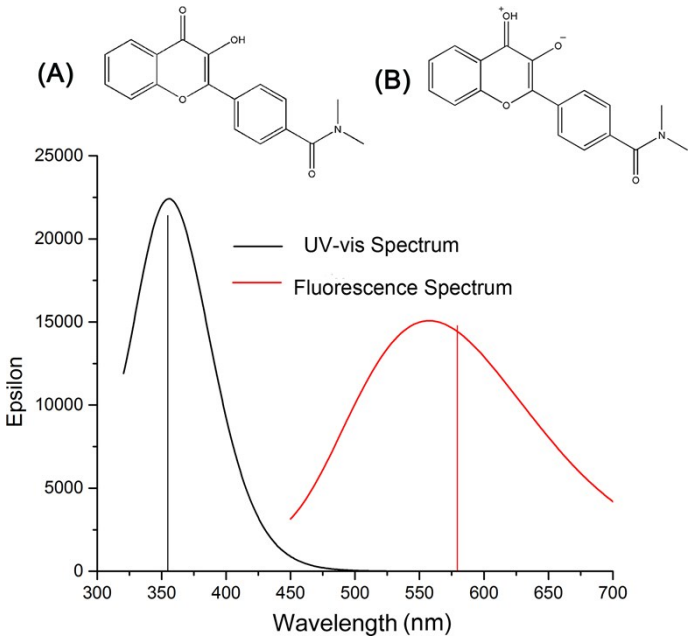


Fig. S6 The simulated absorption spectrum of structure A (black line) and fluorescence spectrum of the tautomer B (red line).

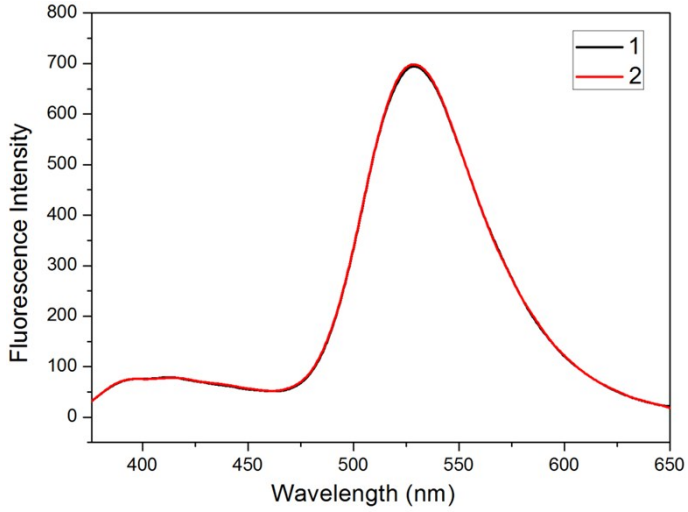


Fig. S7 Fluorescence spectra of compound D in the absence (line 1) or presence (line 2) of bisulfite (10 equiv.). Data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 10 min. λ_{ex} = 345 nm, slits: 10 nm/6 nm.

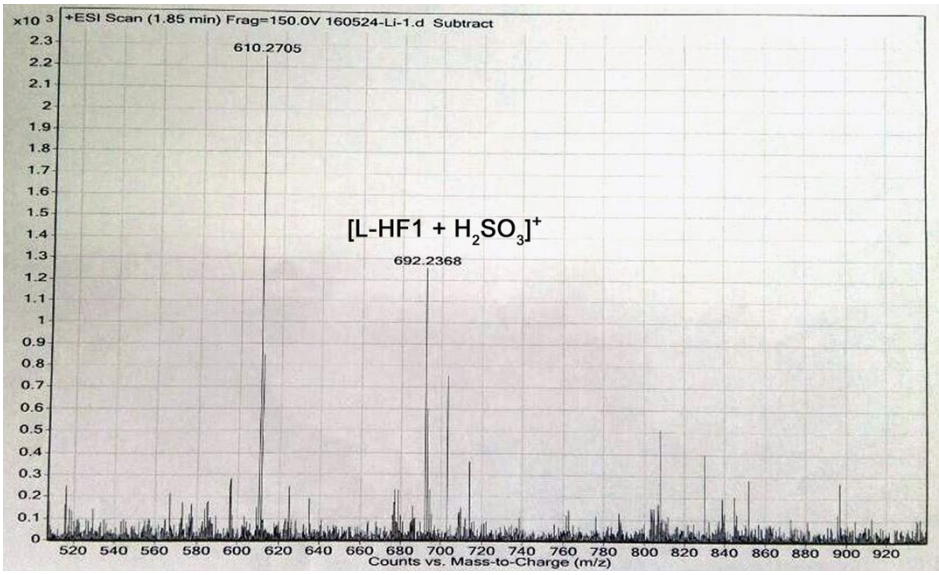


Fig. S8 High Resolution Mass Spectroscopy (HR-MS) of L-HF1 in the presence of bisulfite (10 equiv.) in PBS (pH = 7.4, 10 mM, containing 25% DMF).

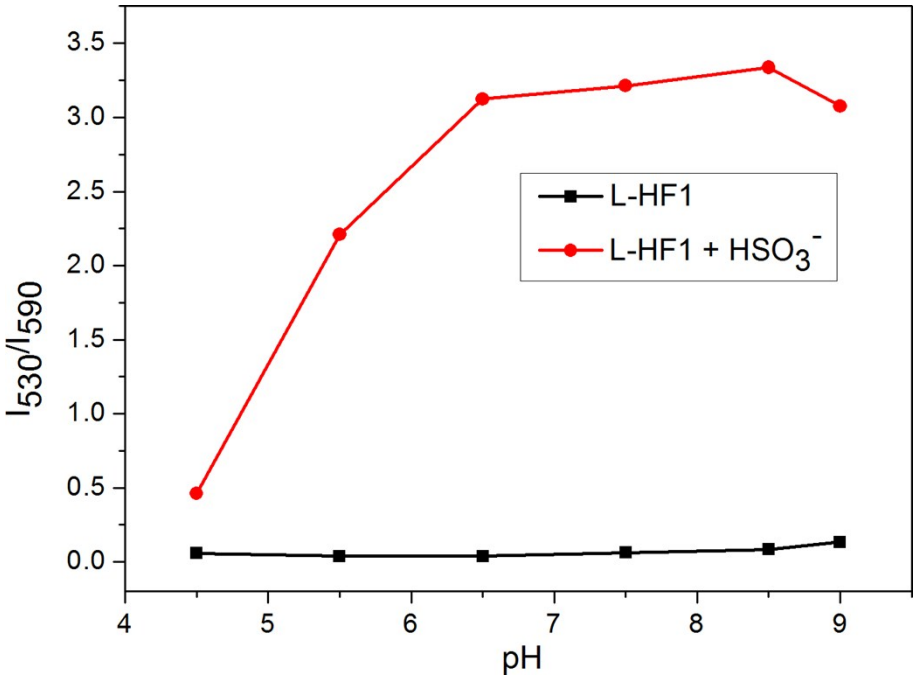


Fig. S9 Effect of pH values of test solutions on L-HF1 (5 μM) in absence (black line) or presence (red line) of bisulfite (10 equiv.). All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 5 min. λ_{ex} = 345 nm.

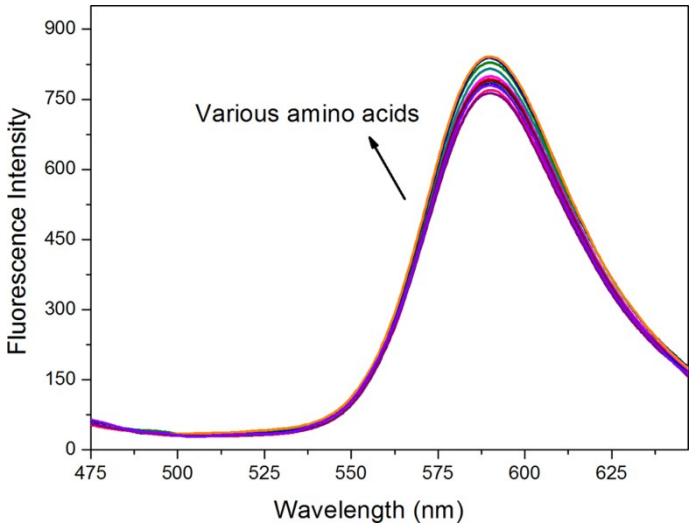


Fig. S10 Fluorescence spectra of L-HF1 (5 μM) in the presence of various amino acids (1 mM) including glutamate, histidine, lysine, tryptophan, valine, arginine, sarcosine, aspartic acid, cysteine, homocysteine, glutathione. All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 5 min. λ_{ex} = 345 nm, slits: 10 nm/10 nm.

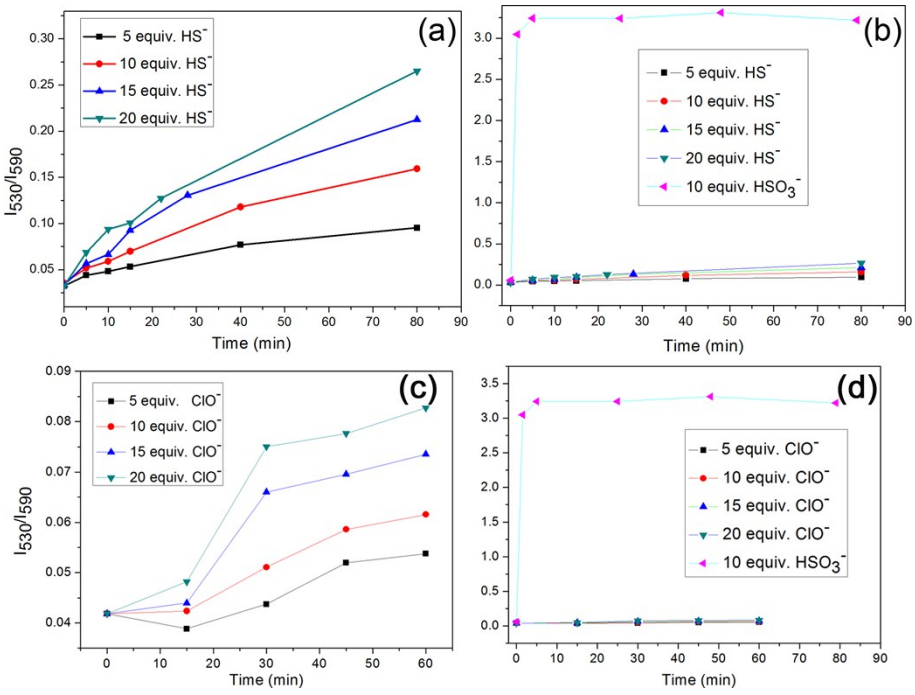


Fig. S11 Fluorescence response of probe L-HF1 (5 μM) toward different equivalents of HS^- or ClO^- with different reaction time. NaHS and NaClO were used here to provide HS^- and ClO^-, respectively. All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF). λ_{ex} = 345 nm.

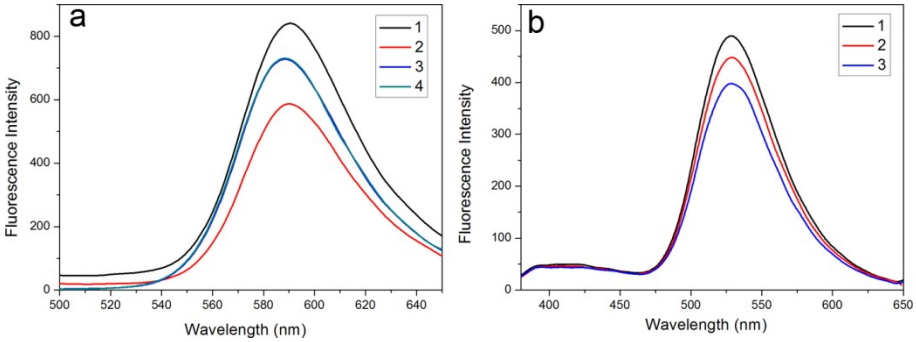


Fig. S12 (a) Fluorescence spectra of L-HF1 (5 μM) in the absence or presence of ClO^- (10 equiv.). Line 1: λ_{ex} = 345 nm, 0 equiv. ClO^-; Line 2: λ_{ex} = 345 nm, 10 equiv. ClO^-; Line 3: λ_{ex} = 480 nm, 0 equiv. ClO^-; Line 4: λ_{ex} = 480 nm, 10 equiv. ClO^-. (b) Fluorescence spectra of compound D in the presence of ClO^-. Line 1: 0 equiv.; Line 2: 10 equiv.; Line 3: 20 equiv.. λ_{ex} = 345 nm. All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 5 min.

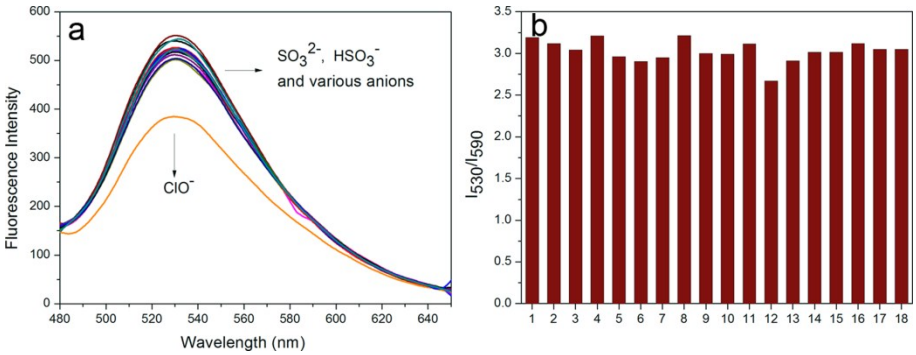


Fig. S13 Fluorescence response of L-HF1 (5 μM) to bisulftie (10 equiv.) in the presence of different anions: 1. vacant; 2. F^-; 3. Cl^-; 4. Br^-; 5. I^-; 6. HCO_3^-; 7. NO_3^-; 8. NO_2^-; 9. N_3^-; 10. AcO^-; 11. $H_2PO_4^-$; 12. ClO^-; 13. ClO_3^-; 14. SO_4^{2-}; 15. HSO_4^-; 16. HS^-; 17. SCN^-; 18. $S_2O_3^{2-}$. All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 5 min. λ_{ex} = 345 nm, slit: 10 nm/10 nm.

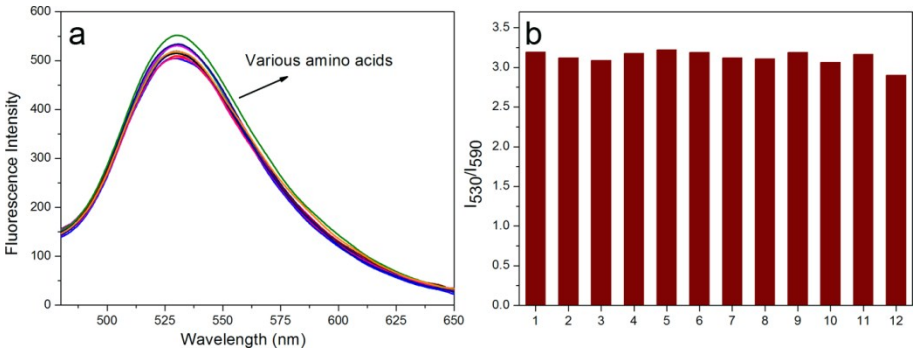


Fig. S14 Fluorescence response of L-HF1 (5 μM) to bisulftie (10 equiv.) in the presence of different amino acids: 1. vacant; 2. glutamate; 3. histidine; 4. lysine; 5. tryptophan; 6. valine; 7. arginine; 8. sarcosine; 9. aspartic acid; 10. cysteine; 11. homocysteine; 12. glutathione. All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 5 min. λ_{ex} = 345 nm, slit: 10 nm/10 nm.

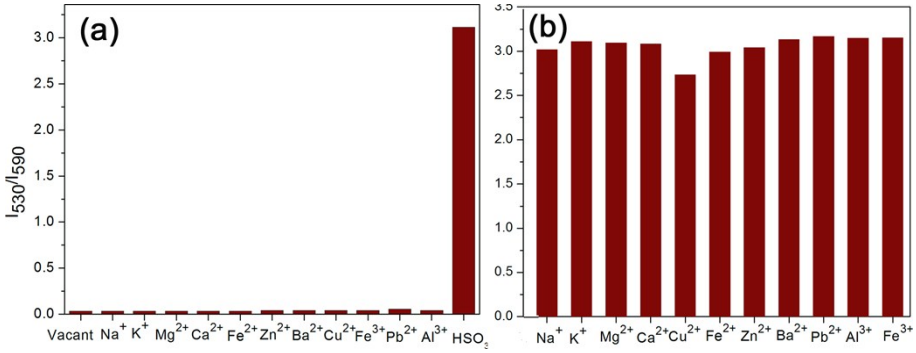


Fig. S15 Selectivity of probe L-HF1 (5 μM) toward metal ions (100 μM), and the interference study of metal ions (50 μM) on detection of bisulfite (50 μM). All data were acquired in PBS (pH = 7.4, 10 mM, containing 25% DMF) in 5 min. λ_{ex} = 345 nm.

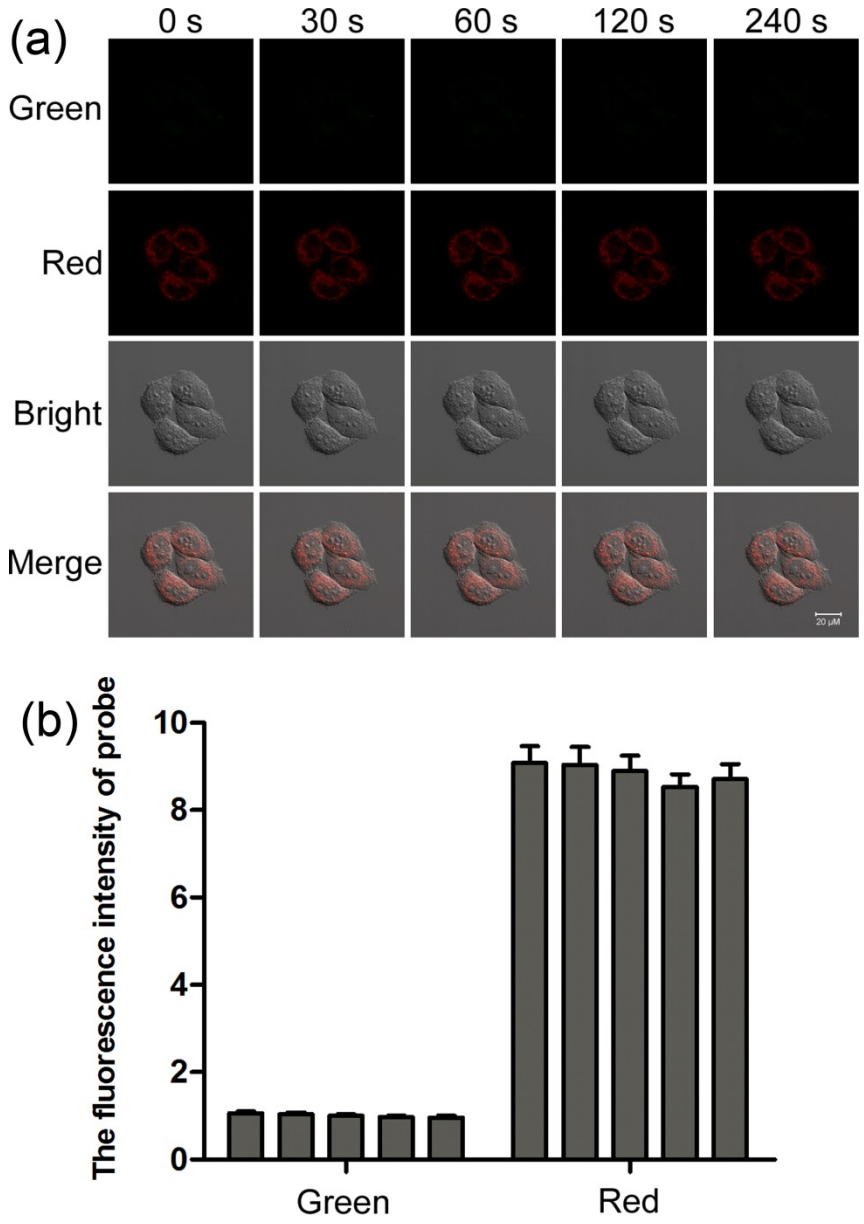


Fig. S16 Photostability of probe L-HF1 in living HeLa cells. (a) Fluorescence and bright field images of L-HF1 loaded HeLa cells; (b) Statistical data of fluorescence intensity from the green and red channel, respectively. Cells were incubated with L-HF1 (5 μM) for 1 h beforehand.

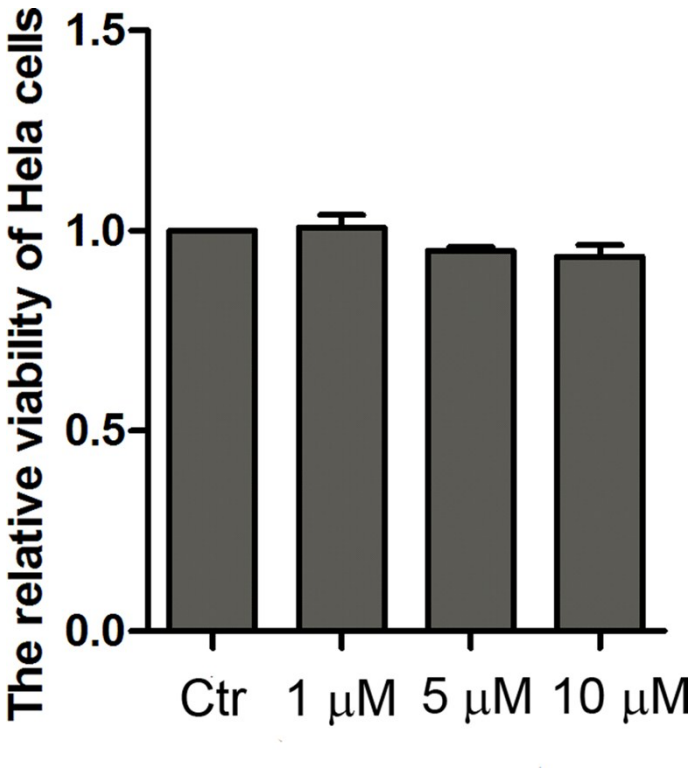


Fig. S17 Cell viability by a standard SRB assay. HeLa cells were incubated with L-HF1 (0, 1 , 5 or 10 μM) for 6 h beforehand.

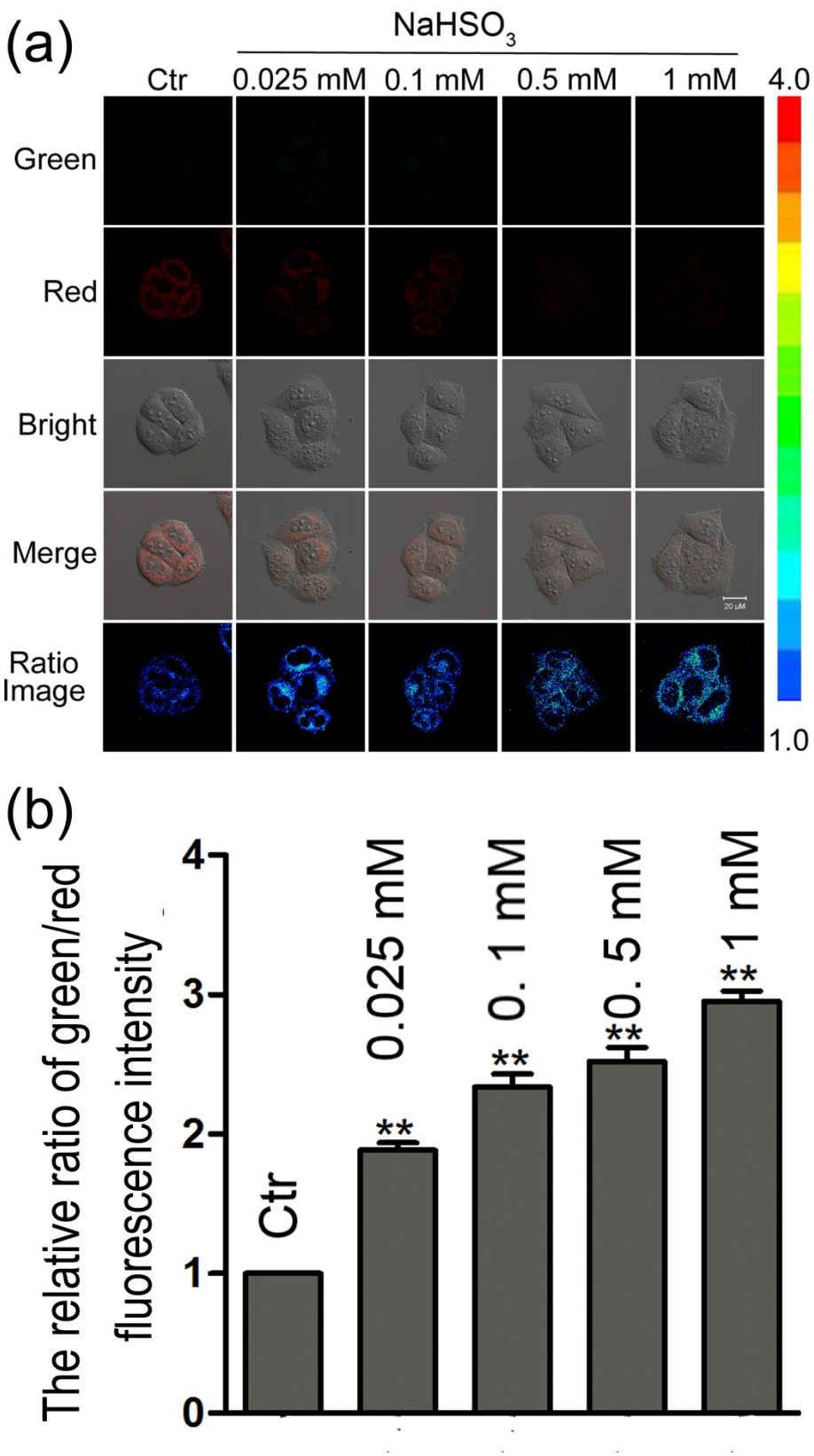


Fig. S18 (a) HeLa cells incubated with L-HF1 (5 μM) for 1 h, then with $NaHSO_3$ (0, 0.025, 0.1, 0.5 or 1 mM) for 0.5 h before images were taken. (b) The relative ratio of green/red fluorescence intensity. Images were acquired from 405-555 nm for green

fluorescence, and from 560-700 nm for red fluorescence.

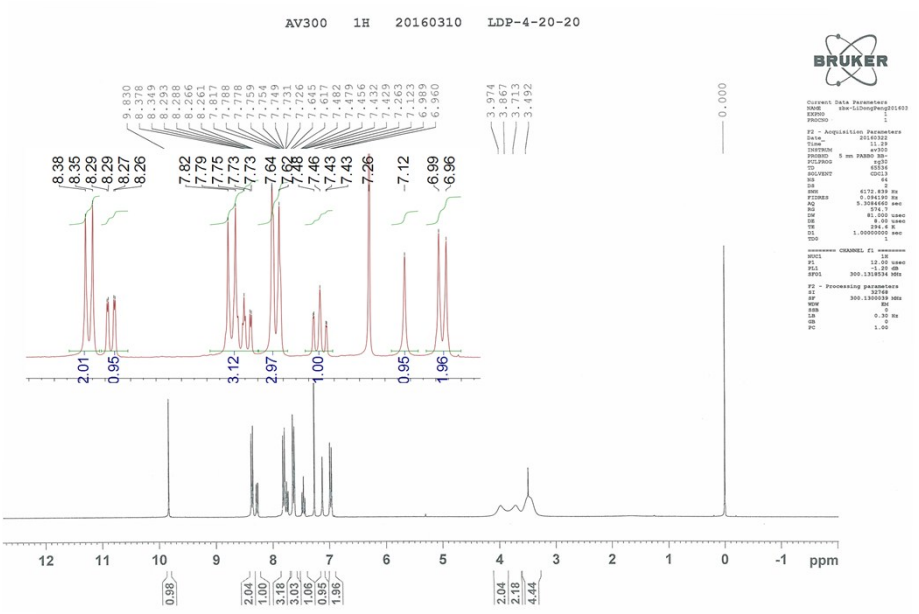


Fig. S19 ^{1}H NMR spectrum of compound 3.

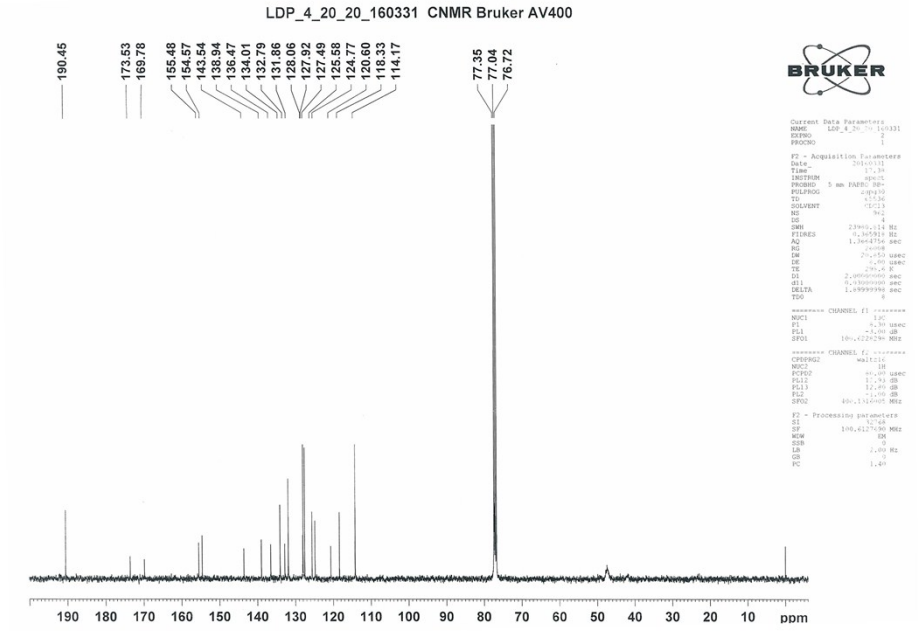


Fig. S20 ^{13}C NMR spectrum of compound 3.

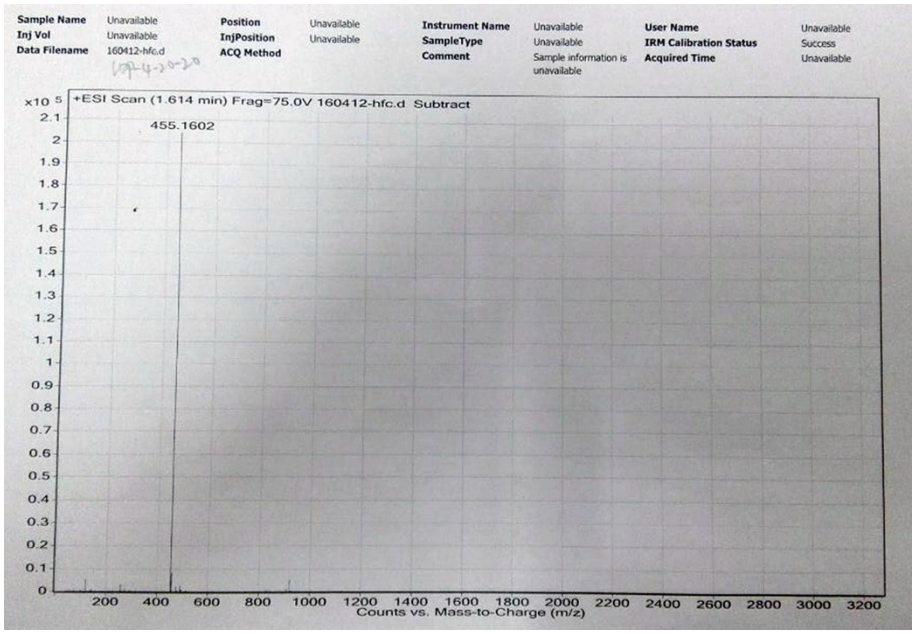


Fig. S21 HRMS spectrum of compound 3.

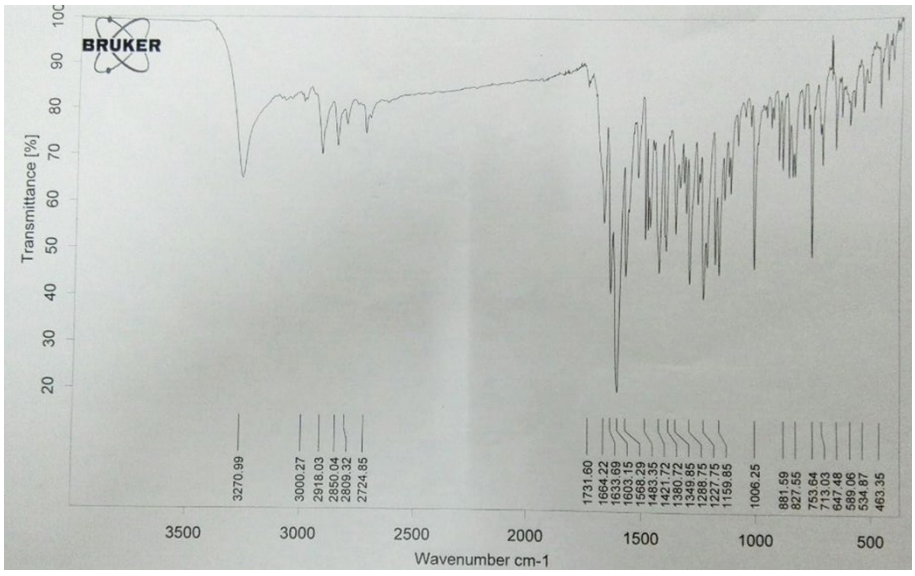


Fig. S22 FT-IR spectrum of compound 3.

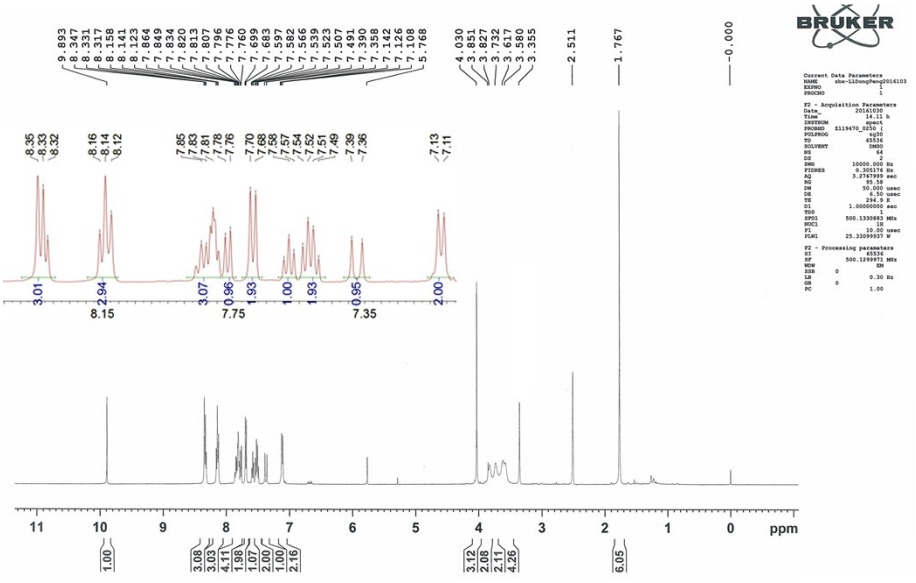


Fig. S23 ^{1}H NMR spectrum of L-HF1.

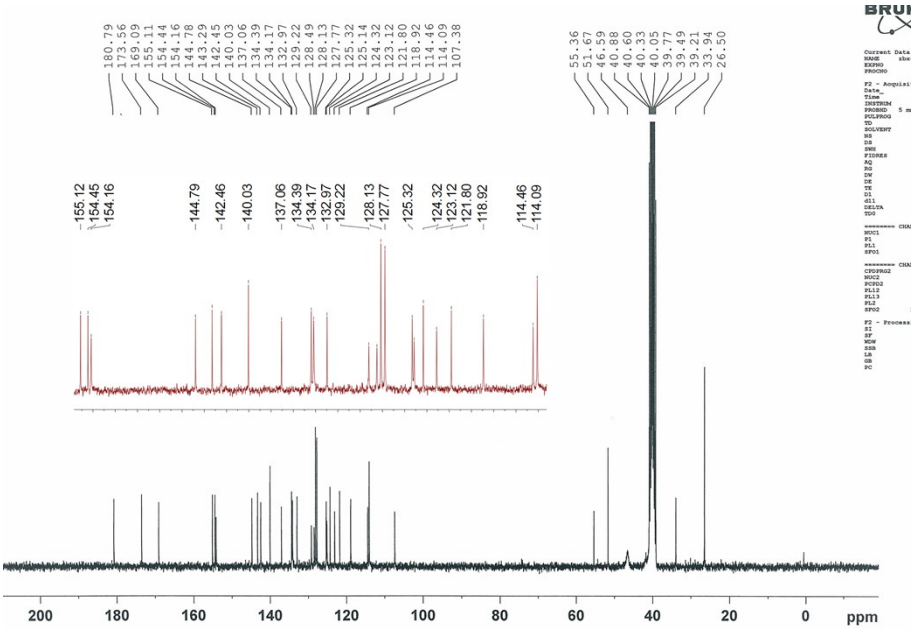


Fig. S24 ^{13}C NMR spectrum of L-HF1.

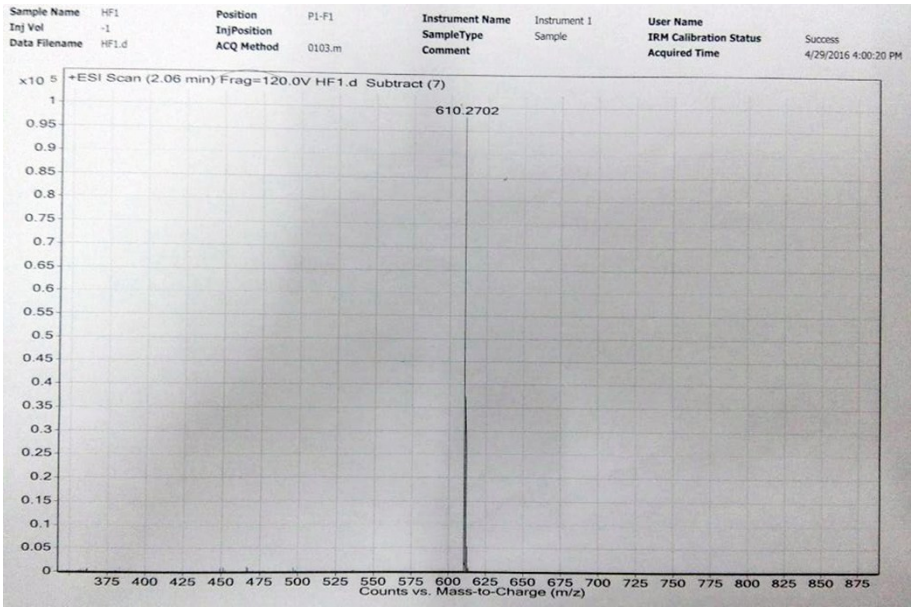


Fig. S25 HRMS spectrum of L-HF1.

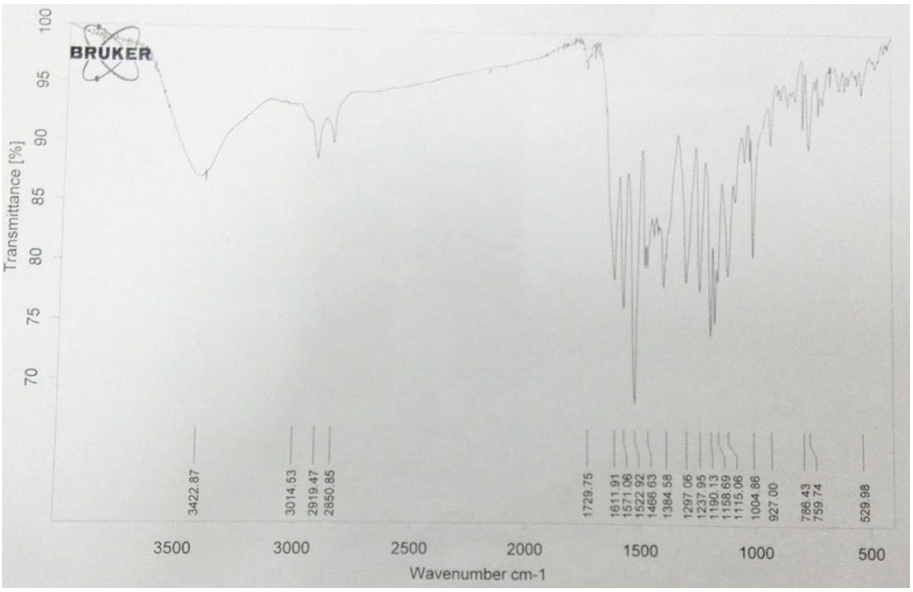


Fig. S26 FT-IR spectrum of L-HF1.

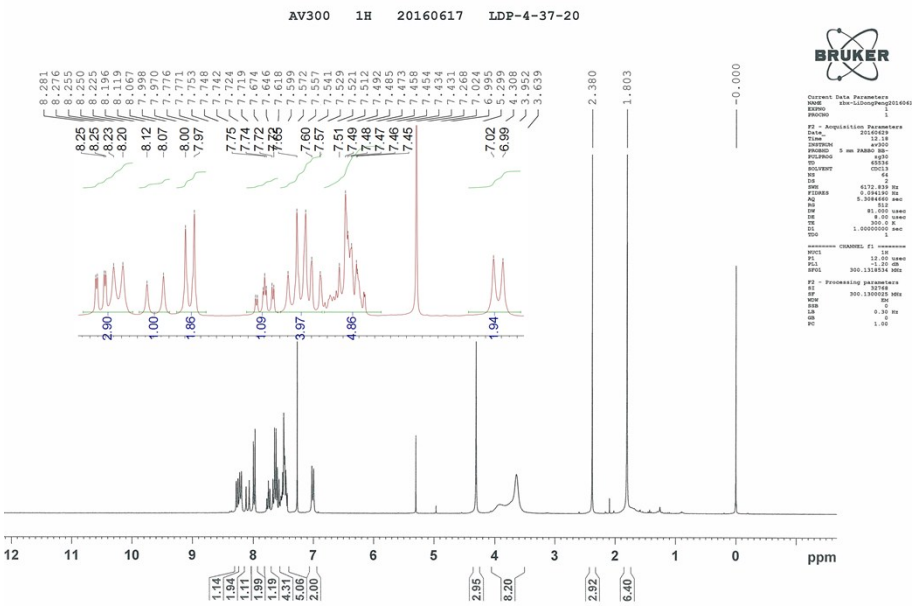


Fig. S27 ^{1}H NMR spectrum of L-HF2.

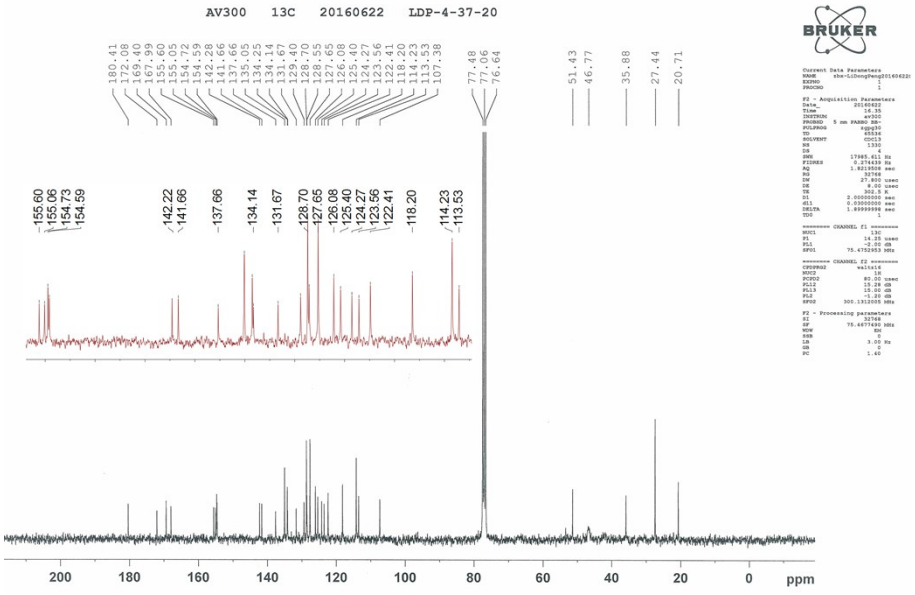


Fig. S28 ^{13}C NMR spectrum of L-HF2.

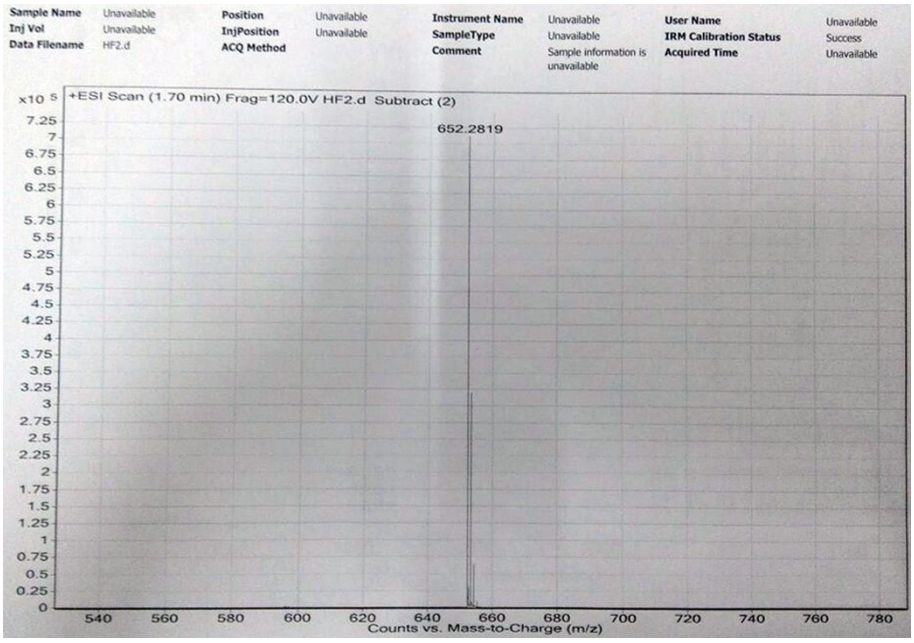


Fig. S29 HRMS spectrum of L-HF2.

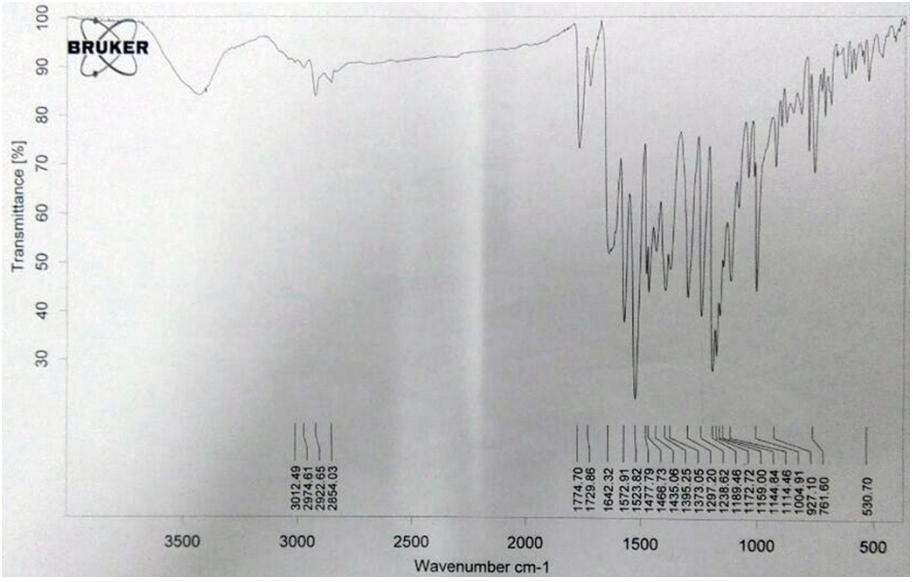


Fig. S30 FT-IR spectrum of L-HF2.

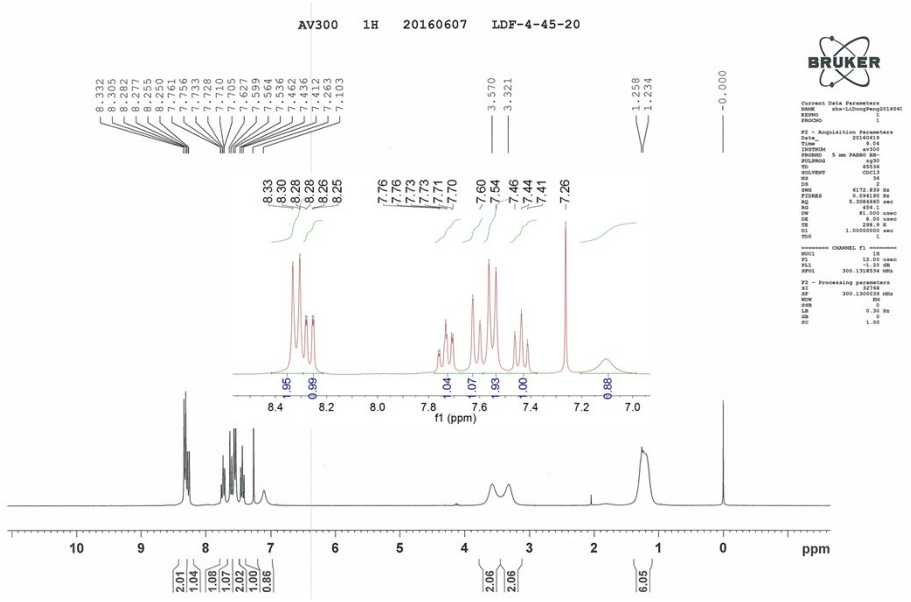


Fig. S31 ^{1}H NMR spectrum of compound D.

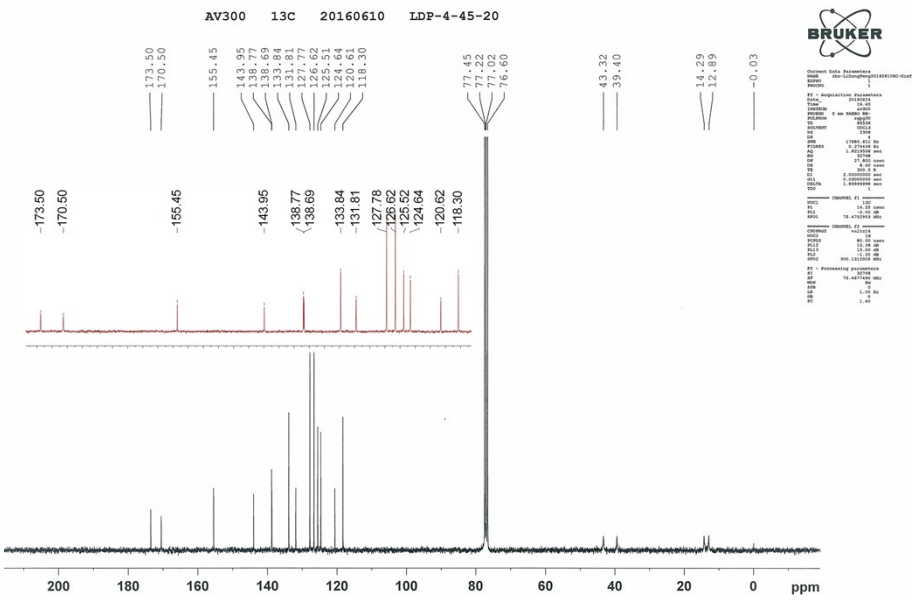


Fig. S32 ^{13}C NMR spectrum of compound D.

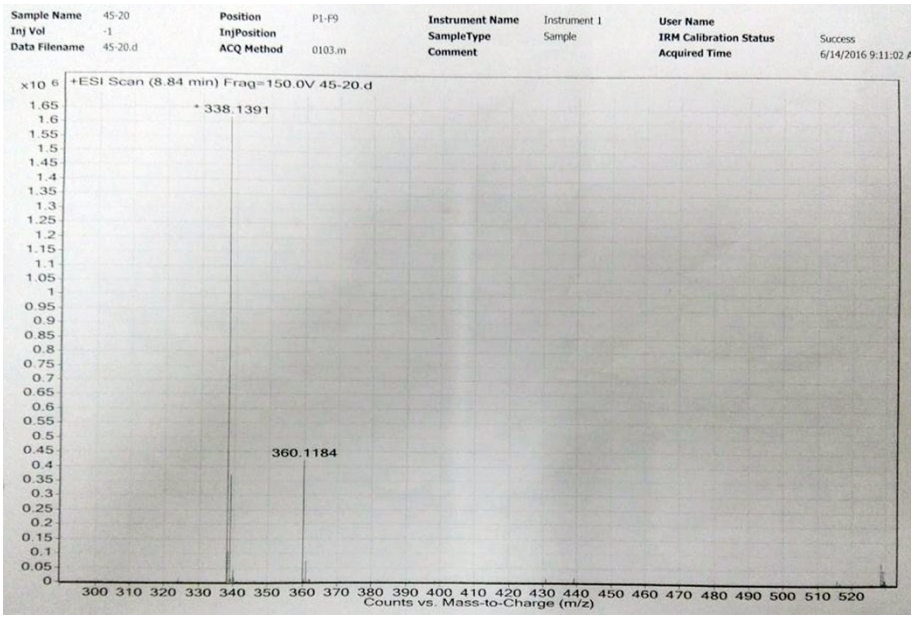


Fig. S33 HRMS spectrum of compound D.

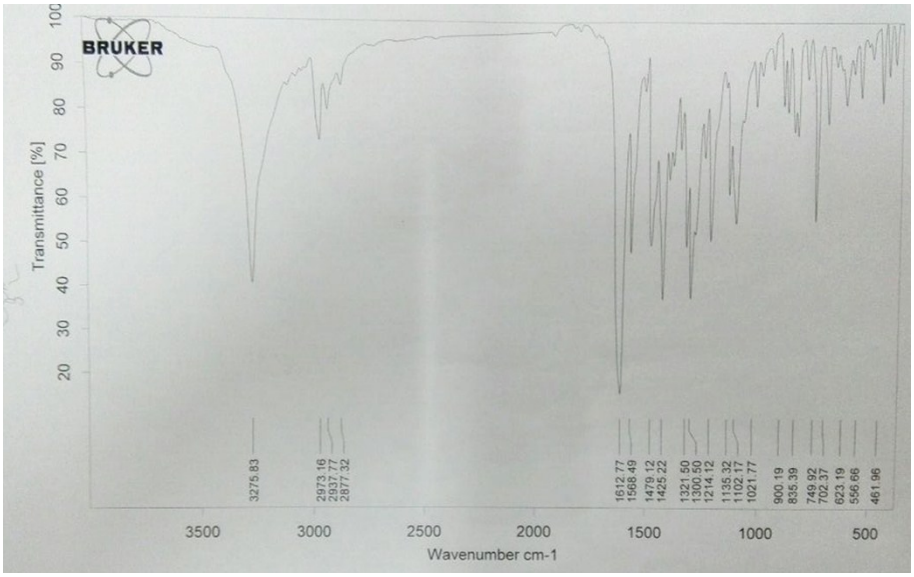


Fig. S34 FT-IR spectrum of compound D.