Electronic Supplementary Material (ESI) for Chemical Communications.

ESIPT-based fluorescence probe for the rapid detection of peroxynitrite 'AND' biological thiols

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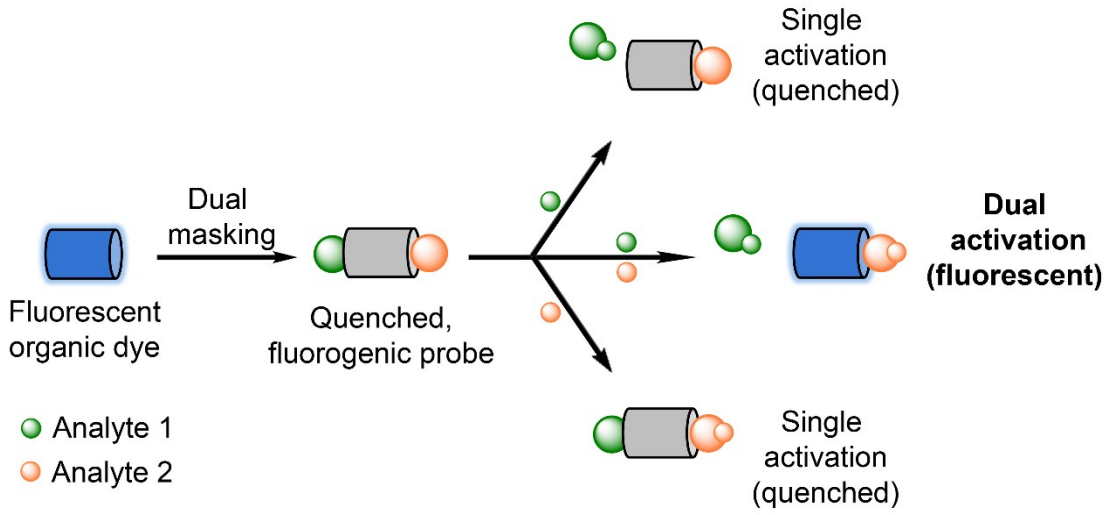
1. Design concept for AND logic based fluorogenic probe



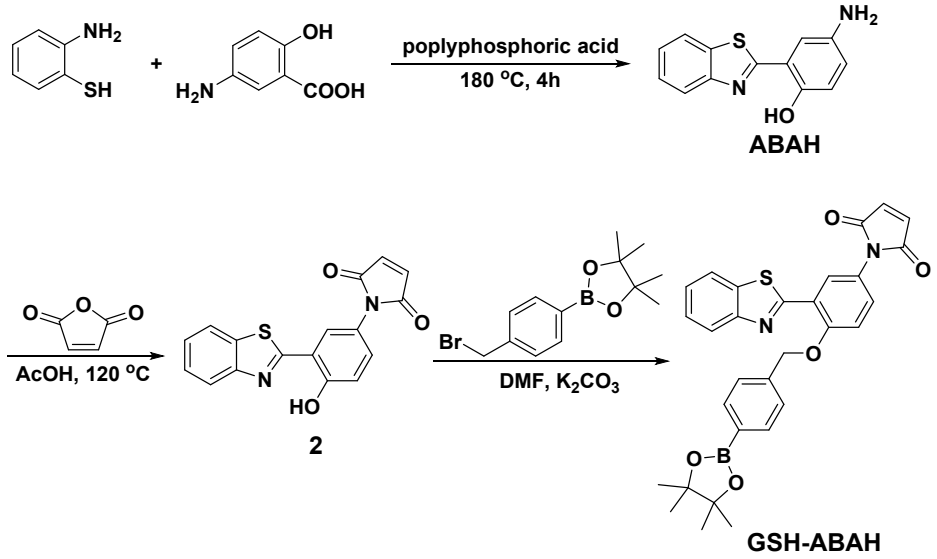
Scheme S1 Design concept for AND logic based fluorogenic probe. A fluorescence dye is masked by two functional groups, which respond to two different analytes. The fluorogenic probe requires both analytes to be present or to work in tandem in order to produce a response.

2. Experimental

2.1. Synthetic experiments: material and apparatus

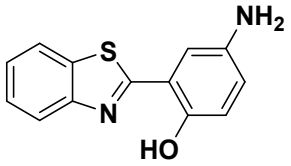
All chemical reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel plates and visualized by UV. Column chromatography was performed using silica gel (Sigma-Aldrich) 200-400 mesh. ^{1}H and ^{13}C NMR spectra were recorded employing a Bruker AV-500 spectrometer with chemical shifts expressed in parts per million (in DMSO-d_6, $CDCl_3$. Me_4Si as internal standard). Electrospray ionization (ESI) mass spectrometry was performed using a Bruker MicrTOF spectrometer.

2.2. Synthetic experiments: synthetic procedures



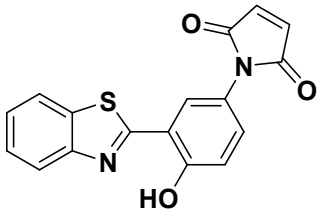
Scheme S2. Synthesis of target probe **GSH-ABAH**.

4-amino-2-(benzo[d]thiazol-2-yl)phenol (ABAH).



ABAH was synthesized according to the similar procedures.[1, 2] In brief, a mixture of 2-aminothiophenol (1.00 g, 8.0 mmol) and 5-aminosalicylic acid (1.23 g, 8.0 mmol) was stirred in 30 mL polyphosphoric acid (PPA) at 185 ^{o}C for 4 h. Then the reaction mixture was poured into cold water to give a yellow precipitate. After filtering, the yellow precipitate was washed by 10% Na_2CO_3 and the product changes to green (1.27 g, 66% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.11 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 15.1 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.64 (s), 151.94 (s), 150.46 (s), 134.77 (s), 126.87 (s), 125.44 (s), 122.80 (s), 122.45 (s), 122.39 (s), 118.77 (s), 118.14 (s), 117.18 (s), 115.56 (s). HRMS (ES$^+$): calc. for $C_{13}H_{10}N_2OS$ $[M+H]^+$ 243.0587, found 243.0577.

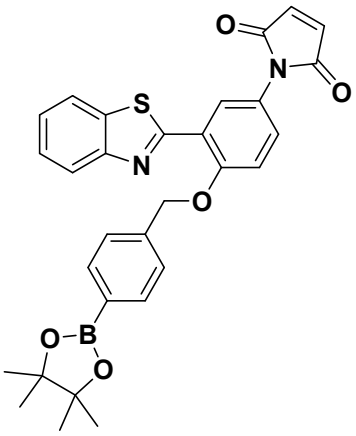
1-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)-1H-pyrrole-2,5-dione (compound 2).



2

Compound **2** was synthesized according to the reported procedures[2]. A mixture of **ABAH** (0.97 g, 4 mmol) and maleic anhydride (0.45 g, 5.0 mmol) in glacial acetic acid (40 mL) was heated under reflux for 4 h. The reaction mixture was left to cool, and poured into 150 mL cold water to give a yellow precipitate. The yellow solid obtained was filtered and washed with sodium carbonate solution. After drying, the residue was purified by silica gel chromatography using CH_2Cl_2 as the eluent to give **2** as a yellow solid (0.99 g, 77%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.75 (s, 1H), 8.20 (d, J = 2.6 Hz, 1H), 8.15 (d, J = 7.5 Hz,1H), 8.04 (d, J = 8.1 Hz, 1H), 7.58 – 7.51 (m, 1H), 7.48 – 7.41 (m, 1H), 7.34 (dd, J = 8.7, 2.6 Hz, 1H), 7.23 – 7.08 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.64 (s), 163.84 (s), 151.82 (s), 151.77 (s), 135.32 (s), 135.11 (s), 131.42 (s), 127.44 (s), 126.89 (s), 125.53 (s), 122.67 (s), 122.48 (s), 119.53 (s), 117.74 (s), 109.99 (s). HRMS (ES$^+$): calc. for $C_{17}H_{10}N_2O_3S$ $[M+H]^+$ 323.0485, found 323.0513.

1-(3-(benzo[d]thiazol-2-yl)-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)phenyl)-1H-pyrrole-2,5-dione (GSH-ABAH)



To a solution of compound **2** (0.65g, 2.02 mmol) in dry DMF 2-(4-(Bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.72 g, 2.42 mmol) was added under an argon atmosphere, the reaction mixture was stirred for about 2 h, then the reaction mixture was concentrated under reduced pressure. The obtained crude product was purified by chromatography on silica gel eluting with petrol ether and ethyl acetate to afford a pale light yellow solid (293 mg, 27% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, J = 2.7 Hz, 1H), 8.06 (d, J = 8.1 Hz, 1H), 7.89 (t, J = 8.7 Hz, 3H), 7.55 (d, J = 8.0 Hz, 2H), 7.50 – 7.46 (m, 1H), 7.39 – 7.33 (m, 2H), 7.16 (d, J = 8.9 Hz, 1H), 6.87 (s, 2H), 5.39 (s, 2H), 1.37 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) 169.49 (s), 161.72 (s), 155.64 (s), 151.91 (s), 138.62 (s), 136.20 (s), 135.16 (s), 134.22 (s), 129.46 (s), 127.86 (s), 126.97 (s), 126.00 (s), 124.80 (s), 124.67 (s), 123.51 (s), 122.93 (s), 121.35 (s), 113.61 (s), 83.92 (s), 71.37 (s), 24.88 (s). HRMS (ES^+): calc. for $C_{30}H_{27}BN_2O_5S$ $[M+H]^+$ 539.1796, found 539.1812.

3. Generation of various ROS

ROO•

ROO• was generated from 2,2'-azobis (2-amidinopropane) dihydrochloride. AAPH (2, 2' azobis (2-amidinopropane) dihydrochloride,1 M) was added into deionizer water, and then stirred at 37 °C for 30min.

$•O_2^-$

Superoxide was generated from KO_2. KO_2 and 18-crown-6 ether (2.5 eq) was dissolved in DMSO to afford a 0.25 M solution.

•HO

Hydroxyl radical was generated by the Fenton reaction. To prepare •OH solution, hydrogen peroxide(H_2O_2, 10 eq) was added to $Fe(ClO_4)_2$ in deionised water.

$^1O_2$: $NaMoO_4$ (20 mM) and H_2O_2 (20 mM) were prepared in deionized water. Equal aliquots of these two solutions were then mixed to yield $^1O_2$ of 10 mM.

$ONOO^-$

Simultaneously, 0.6 M KNO_2, 0.6 M in HC1, 0.7 M in H_2O_2 was added at to a 3 M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using extinction co-efficient of 1670 cm^{-1} M^{-1} at 302 nm in 0.1 M sodium hydroxide aqueous solutions.

^-OCl

The concentration of ^-OCl was determined from the absorption at 292 nm (ε = 350 M^{-1} cm^{-1}).

H_2O_2

The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}).

4. UV-Vis and fluorescence analysis of GSH-ABAH

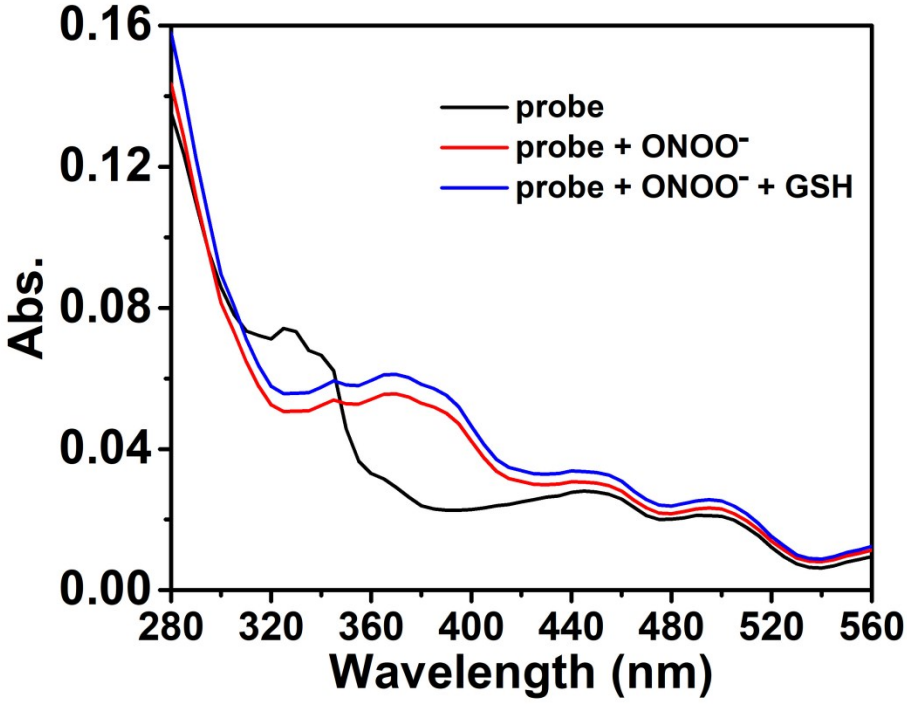


Figure S1. Absorption spectrum of **GSH-ABAH** (2 μM) with and without $ONOO^-$ (4 μM), and **GSH-ABAH** (2 μM) with addition of $ONOO^-$ (4 μM) wait 1 min then addition of GSH (2 μM) in in buffer solution [8% DMSO, 1 mM CTAB] (pH 8.20 at 25 ^oC).

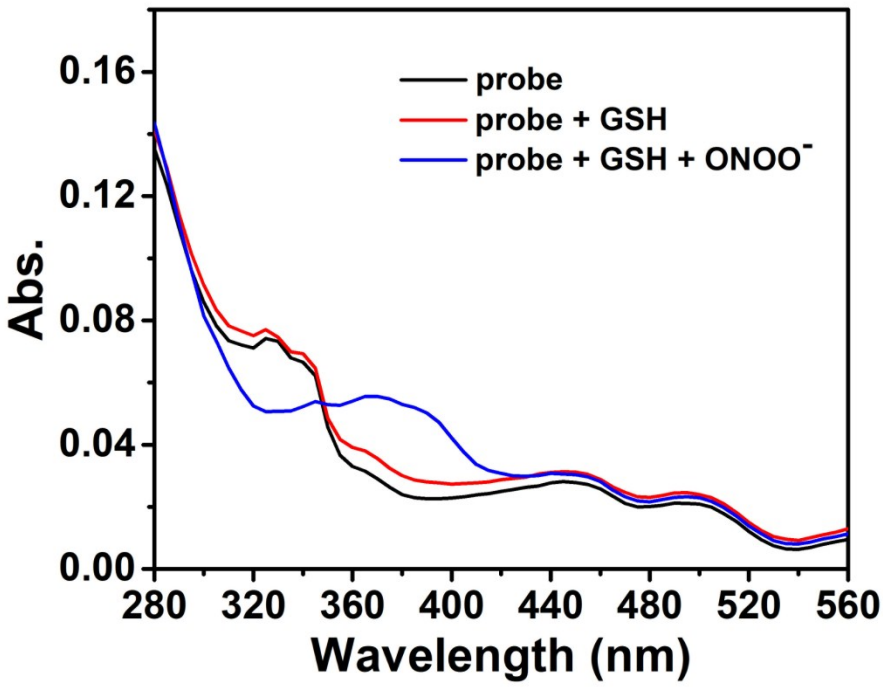


Figure S2. Absorption spectrum of **GSH-ABAH** (2 μM) with and without GSH (5 μM), and **GSH-ABAH** (2 μM) with addition of GSH (5 μM) wait 1 min then addition of $ONOO^-$ (14 μM) in in buffer solution [8% DMSO, 1 mM CTAB] (pH 8.20 at 25 °C).

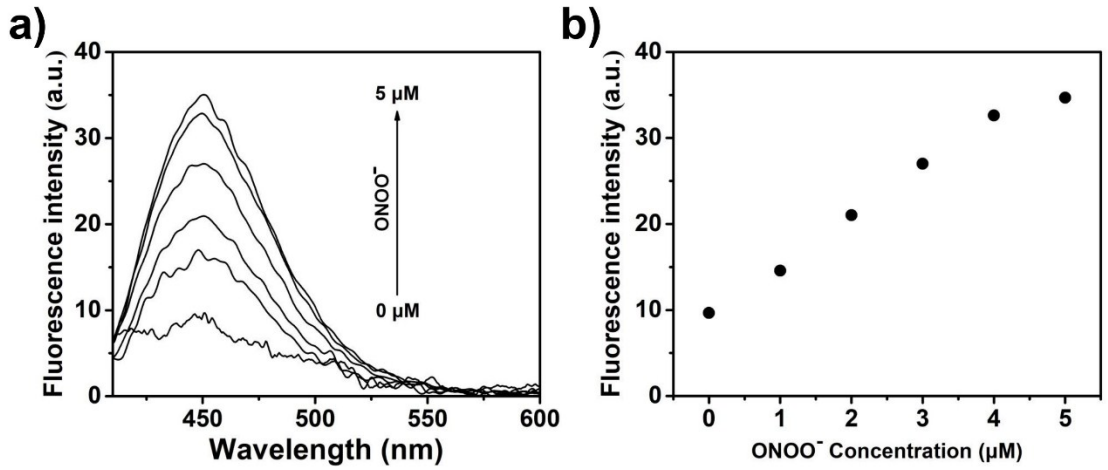


Figure S3. (a) Emission at 451 nm of **GSH-ABAH** (2 μM) with increasing addition of $ONOO^-$ (from 0 to 5 μM) in PBS buffer (pH 8.2, containing 8% DMSO, 1 mM CTAB) after 1 min. λ_{ex} = 390 nm. (b) A plot of fluorescence intensity changes (based on the peak heights at the maxima, 451 nm) depending on $ONOO^-$ concentration. λ_{ex} = 390 nm. Slit widths: ex = 4 nm, em = 4 nm.

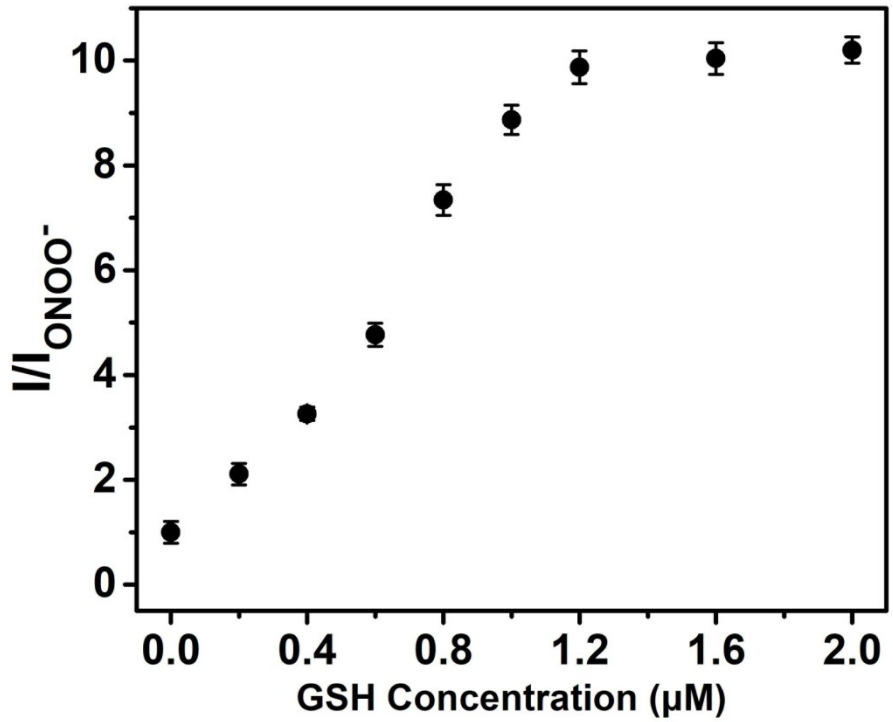


Figure S4. Fluorescence intensity changes (I/I_{ONOO^-}) for **GSH-ABAH** (2 μM) with addition of $ONOO^-$ (4 μM) followed by the addition of GSH (0 - 2 μM), 1 min wait between addition in buffer solution [8% DMSO, 1 mM CTAB]. pH = 8.20 at 25 °C. Fluorescence intensities were measured with λ_{ex} = 390 nm/ λ_{em} = 451 nm with slit widths Ex slit: 4 nm and Em slit: 4 nm.

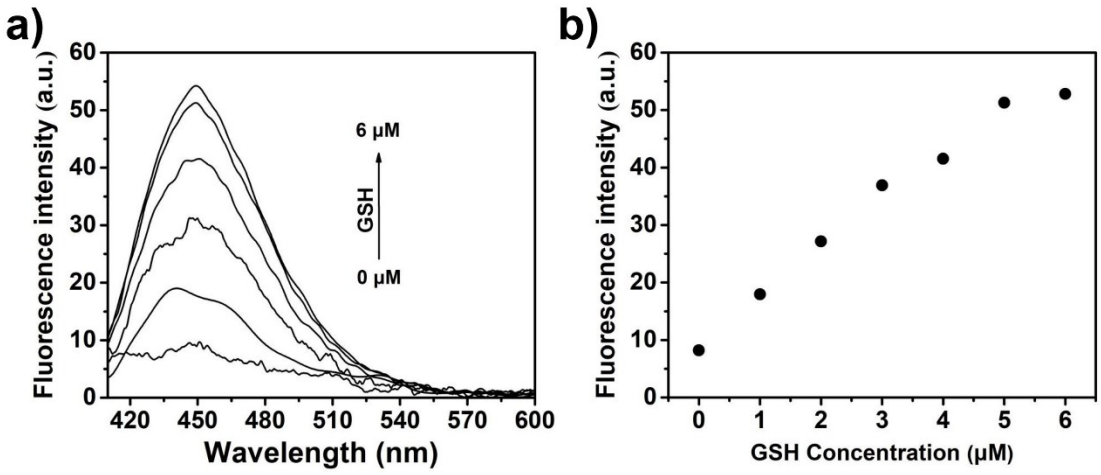


Figure S5. (a) Emission at 451 nm of **GSH-ABAH** (2 μM) with increasing addition of GSH (from 0 to 6 μM) in PBS buffer (pH 8.2, containing 8% DMSO, 1 mM CTAB) after 1 min. λ_{ex} = 390 nm. (b) A plot of fluorescence intensity changes (based on the peak heights at the maxima, 451 nm) depending on GSH concentration. λ_{ex} = 390 nm. Slit widths: ex = 4 nm, em = 4 nm.

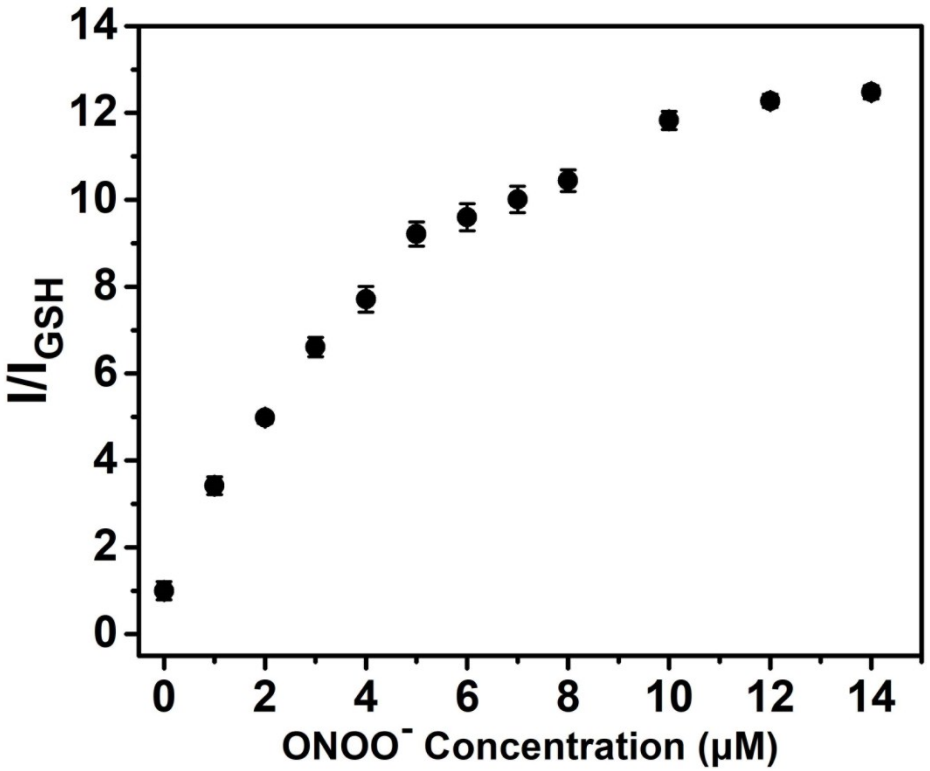


Figure S6. Fluorescence intensity changes (I/I_{GSH}) for **GSH-ABAH** (2 μM) with addition of GSH (5 μM) followed by the addition of $ONOO^-$ (0 - 14 μM), 1 min wait between addition in buffer solution [8% DMSO, 1 mM CTAB]. pH = 8.20 at 25 ^oC. Fluorescence intensities were measured with λ_{ex} = 390 nm/ λ_{em} = 451 nm with slit widths Ex slit: 4 nm and Em slit: 4 nm.

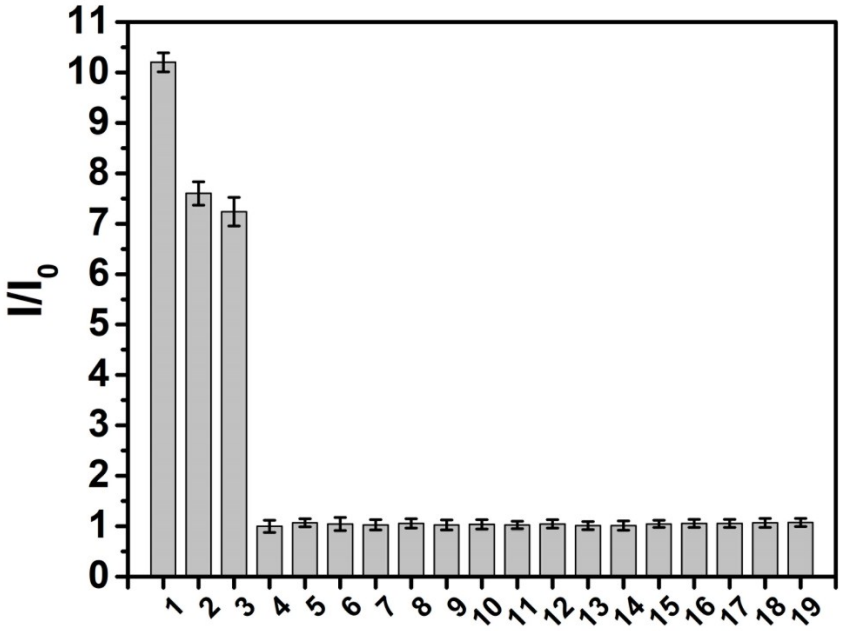


Figure S7. Selectivity bar chart of **GSH-ABAH** (2 μM) with addition of $ONOO^-$ (4 μM) then addition of various amino acids – 50 μM (1 – GSH (4 μM), 2 – Cys (4 μM), 3 – Hcy (4 μM), 4 – Blank, 5 – Ala, 6 – Asn, 7 – Arg, 8 – Asp, 9 – Glu, 10 – Gln, 11 – Gly, 12 – Leu, 13 – Lys, 14 – Met, 15 – Phe, 16, – Pro, 17 – Ser, 18 – Thr, 19, – Trp). 2 min wait before measurement in buffer solution [8% DMSO, 1 mM CTAB] (pH 8.20 at 25 ^oC). Fluorescence intensities were measured with λ_{ex} = 390 nm/ λ_{em} = 451 nm with slit widths ex slit: 4 nm and em slit: 4 nm.

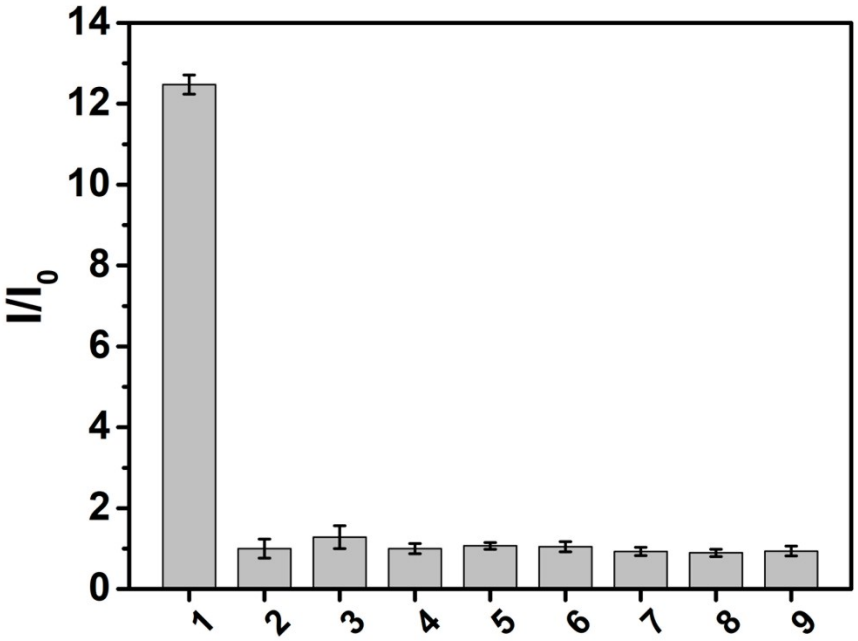


Figure S8. Selectivity bar chart of **GSH-ABAH** (2 μM) with addition of GSH (5 μM) wait 2 min then addition of various ROS –100 μM (1 – $ONOO^-$, 2 – blank, 3 – ClO^-, 4 – H_2O_2, 5 – •OH, 6 – $•O_2^-$, 7 – $^1O_2$, 8. ROO•, 9. NO). 1 min wait before measurement in buffer solution [8% DMSO, 1 mM CTAB] (pH 8.20 at 25 ^oC). Fluorescence intensities were measured with λ_{ex} = 390 nm/ λ_{em} = 451 nm with slit widths ex slit: 4 nm and em slit: 4 nm.

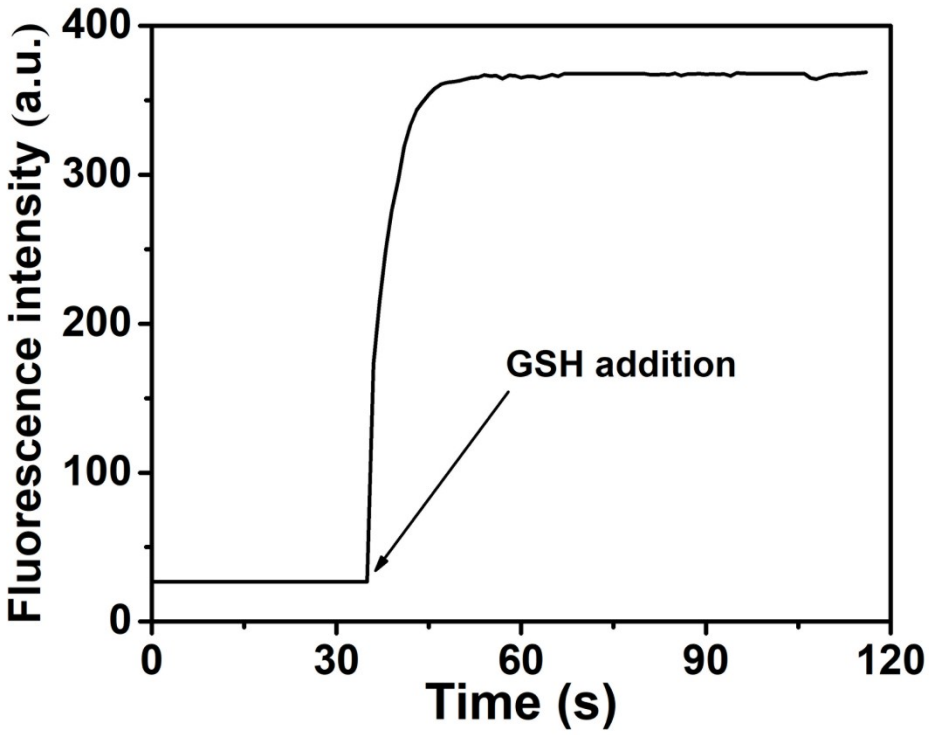


Figure S9. Fluorescence intensity over time of **GSH-ABAH** (2 μM) with $ONOO^-$ (4 μM), followed by the the addition of GSH (2 μM) in buffer solution [8% DMSO, 1 mM CTAB] (pH 8.20 at 25 °C). λ_{ex} = 390 nm/ λ_{em} = 451 nm.

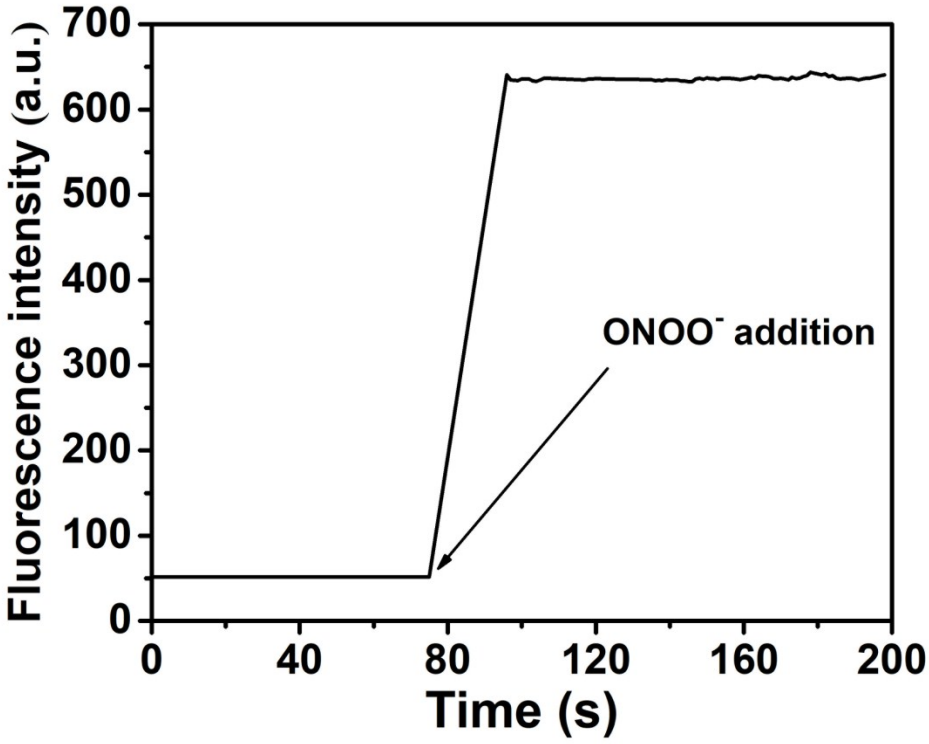
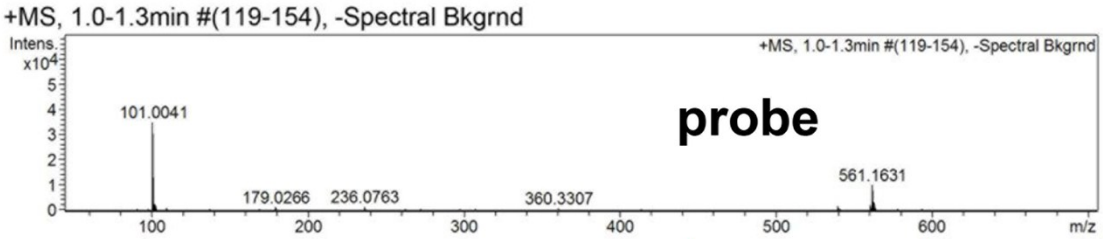


Figure S10. Fluorescence intensity over time of **GSH-ABAH** (2 μM) with GSH (5 μM), followed by the the addition of $ONOO^-$ (14 μM) in in buffer solution [8% DMSO, 1 mM CTAB] (pH 8.20 at 25 °C). λ_{ex} = 390 nm/ λ_{em} = 451 nm.

5. Mass spec analysis of GSH-ABAH



#	m/z	I	I %	Area	S/N
1	101.0041	34751	100.0	375	18212.0
2	102.0093	2436	7.0	23	1243.0
3	102.9996	1951	5.6	24	970.4
4	109.9796	859	2.5	15	362.9
5	179.0266	1220	3.5	21	2211.3
6	236.0763	1485	4.3	30	1503.6
7	539.1800	1830	5.3	153	643.0
8	560.1680	2173	6.3	191	713.3
9	561.1631	10131	29.2	1000	3389.1
10	562.1672	3223	9.3	331	1099.1

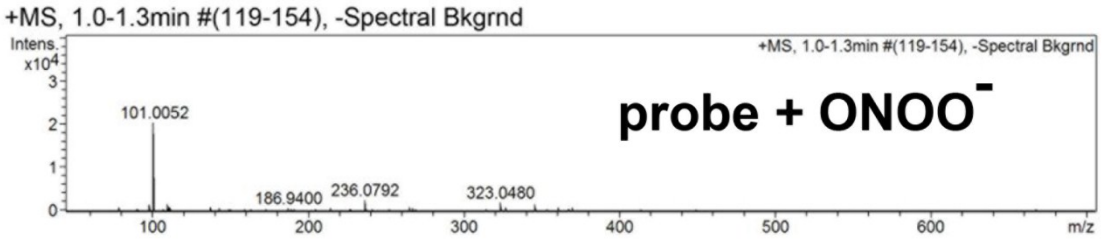
Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C30H27BN2O5S **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	561.1631	561.1631	0.00	0.0166	C 30 H 27 B 1 N 2 Na 1 O 5 S 1

Figure S11. HRMS spectrum of probe **GSH-ABAH** before the addition of analyte.



#	m/z	I	I %	Area	S/N
1	97.9953	1463	7.2	29	794.9
2	101.0052	20273	100.0	132	10062.8
3	109.9801	1391	6.9	32	548.9
4	110.9778	818	4.0	16	315.9
5	137.0043	880	4.3	22	525.2
6	236.0792	2516	12.4	40	2069.2
7	264.9529	800	3.9	34	710.0
8	323.0480	1842	9.1	87	1141.8
9	345.0335	1569	7.7	79	858.7
10	369.2267	880	4.3	47	446.2

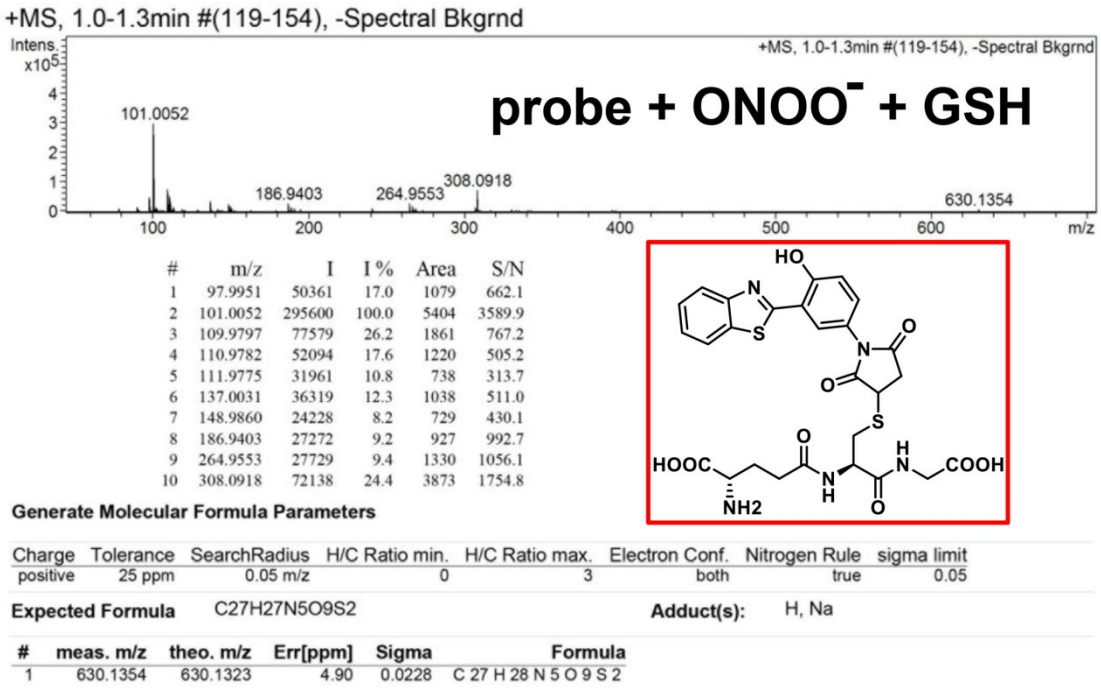
Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C17H10N2O3S **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	345.0335	345.0304	8.90	0.0379	C 17 H 10 N 2 Na 1 O 3 S 1

Figure S12. HRMS spectrum of **GSH-ABAH** + **ONOO$^-$**.



#	m/z	I	I %	Area	S/N
1	97.9951	50361	17.0	1079	662.1
2	101.0052	295600	100.0	5404	3589.9
3	109.9797	77579	26.2	1861	767.2
4	110.9782	52094	17.6	1220	505.2
5	111.9775	31961	10.8	738	313.7
6	137.0031	36319	12.3	1038	511.0
7	148.9860	24228	8.2	729	430.1
8	186.9403	27272	9.2	927	992.7
9	264.9553	27729	9.4	1330	1056.1
10	308.0918	72138	24.4	3873	1754.8

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C27H27N5O9S2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	630.1354	630.1323	4.90	0.0228	C 27 H 28 N 5 O 9 S 2

Figure S13. HRMS spectrum of **GSH-ABAH** + **ONOO**$^-$ + **GSH** followed by addition of **GSH**.

6. Detailed protocols for cell culture

Cell culture. RAW 264.7 cells were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5% CO2 and 95% air at 37 ºC and split when the cells reached 90% confluency.

7. Fluorescence imaging in live cells and MTS assay

Fluorescence imaging of cells. Cells were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. The cells were pre-incubated with N-ethylmaleimide (NEM, GSH scavenger) for 1 h. For SIN-1/GSH imaging experiments, the cells were incubated with **GSH-ABAH** (20 μM) for 30 min, followed by incubation with SIN-1 (500 μM), or GSH (300 μM), or the mixture of SIN-1 (500 μM) and GSH (300 μM) for 30 min. Then, cells were washed with PBS (phosphate buffered saline) three times. The fluorescence images were recorded using an Operetta high content imaging system (Perkinelmer, US) at an excitation wavelength of 360-400 nm and an emission wavelength of 410-480 nm and quantified and plotted by columbus analysis system (Perkinelmer, US).

Confocal laser scanning microscopy. Cells cultured in growth medium supplemented with 10% FBS were added to a 24-well microplate. Cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C overnight, and then were pre-incubated with N-ethylmaleimide (NEM, GSH scavenger) for 1 h, the cells were incubated with **GSH-ABAH** (20 μM) for 30 min, followed by incubation with SIN-1 (500 μM), or GSH (300 μM), or the mixture of SIN-1 (500 μM) and GSH (300 μM) for 30 min. After three rinses with PBS, the fluorescence was detected and photographed with confocal laser scanning microscopy.

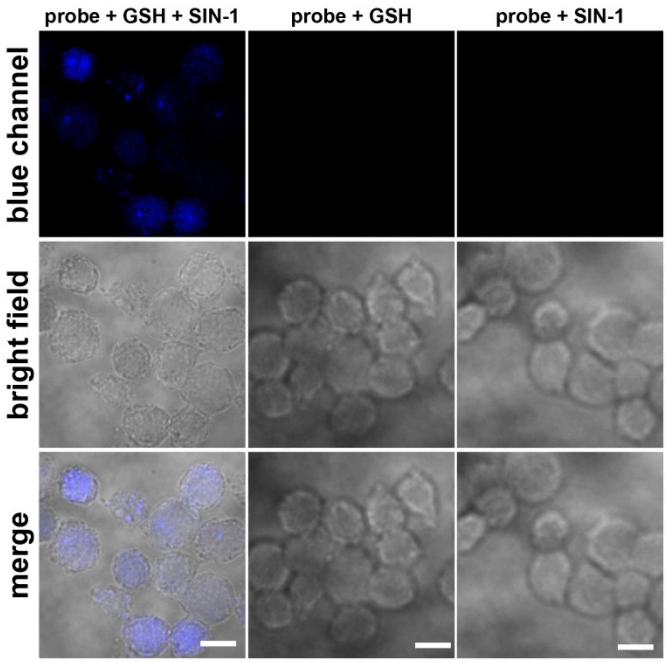


Figure S14. Confocal imaging of RAW264.7 cells with **GSH-ABAH** (20 μM) in the presence of exogenously added GSH (300 μM) and/or SIN-1 (500 μM) with 1% DMSO. Excitation 405 nm, emission channel filtered = 410-480 nm. Scale bar = 10 μm. Note: The cells were pre-incubated with N-ethylmaleimide (NEM, GSH scavenger)

Cell viability assay. Raw264.7 cells were seeded on a 96-well plate. After 24 h, cells were incubated with **GSH-ABAH** of different concentrations for 48 h. Then, the cell viabilities were determined through the MTS cell proliferation assay using 1% DMSO as the control.

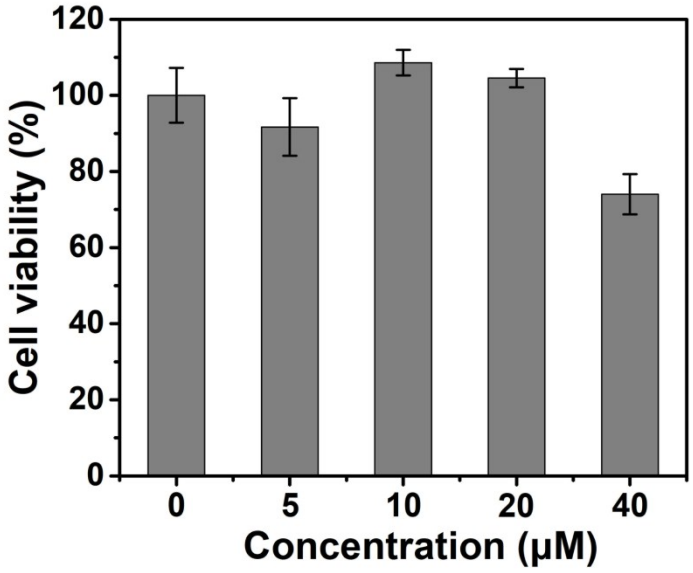


Figure S15. Cell toxicity of **GSH-ABAH** (from 0 to 40 μM) when the incubation time was 48 h. Error bar represents s.d.

8. NMR spectrum

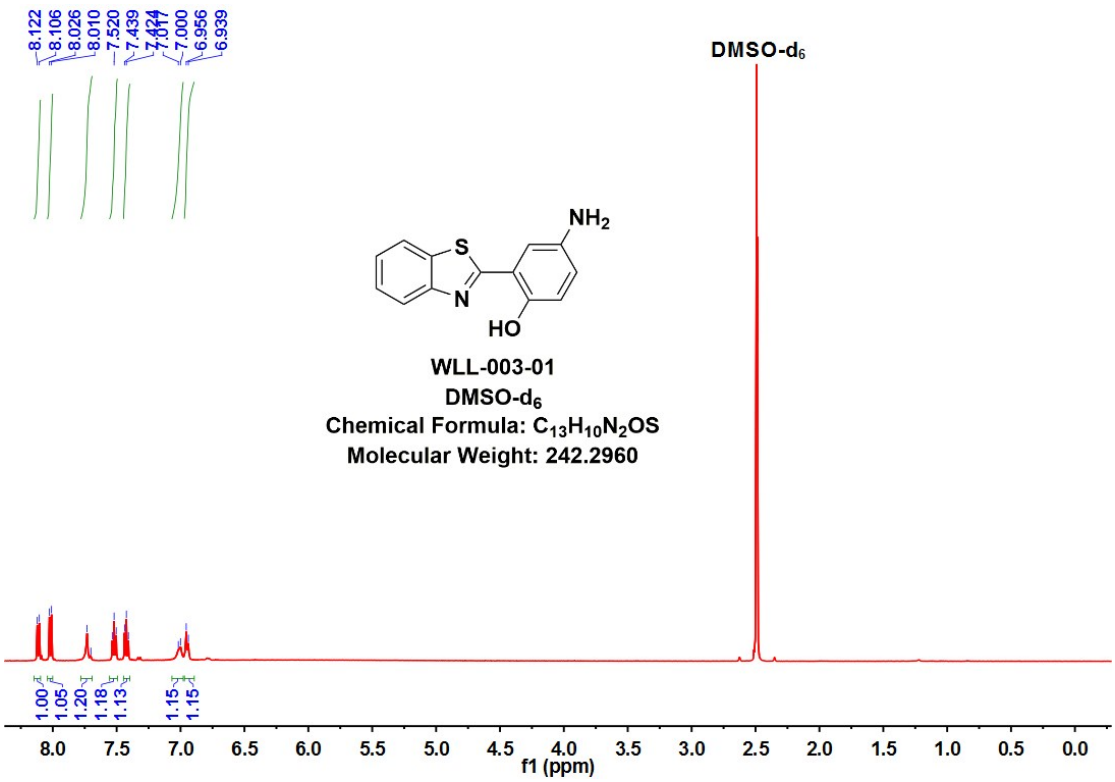


Figure S16. ^{1}H NMR spectrum of **ABAH**.

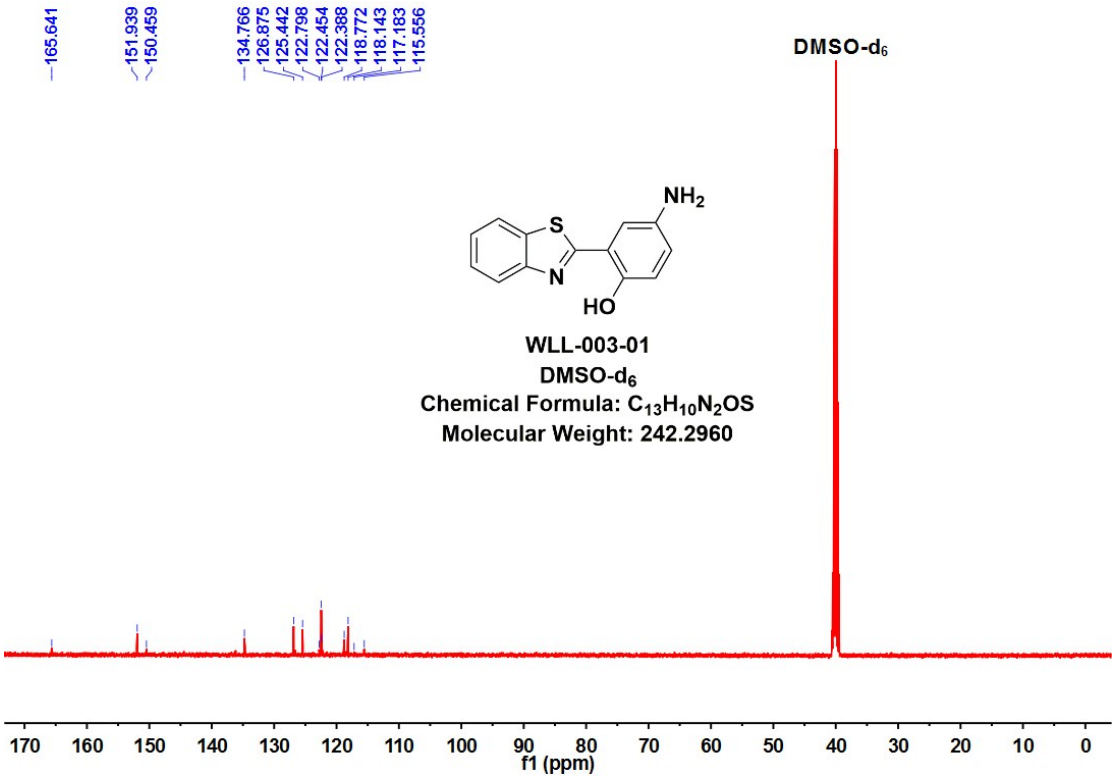


Figure S17. ^{13}C NMR spectrum of **ABAH**.

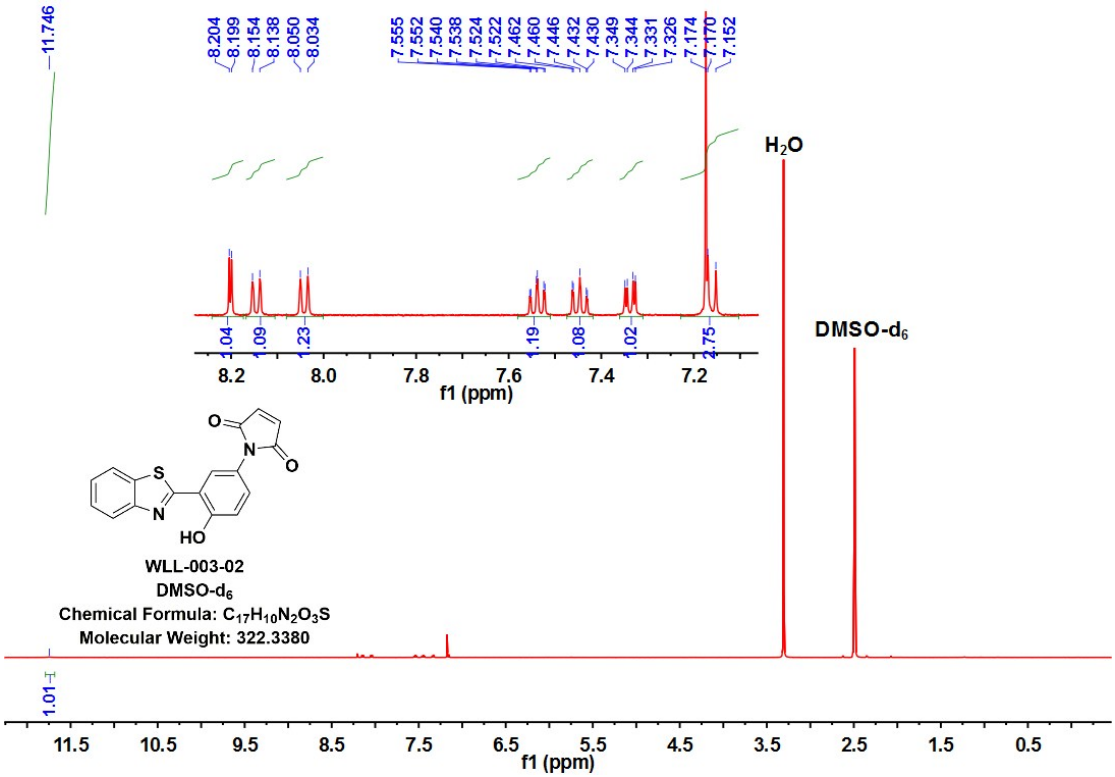


Figure S18. ^{1}H NMR spectrum of compound **2**.

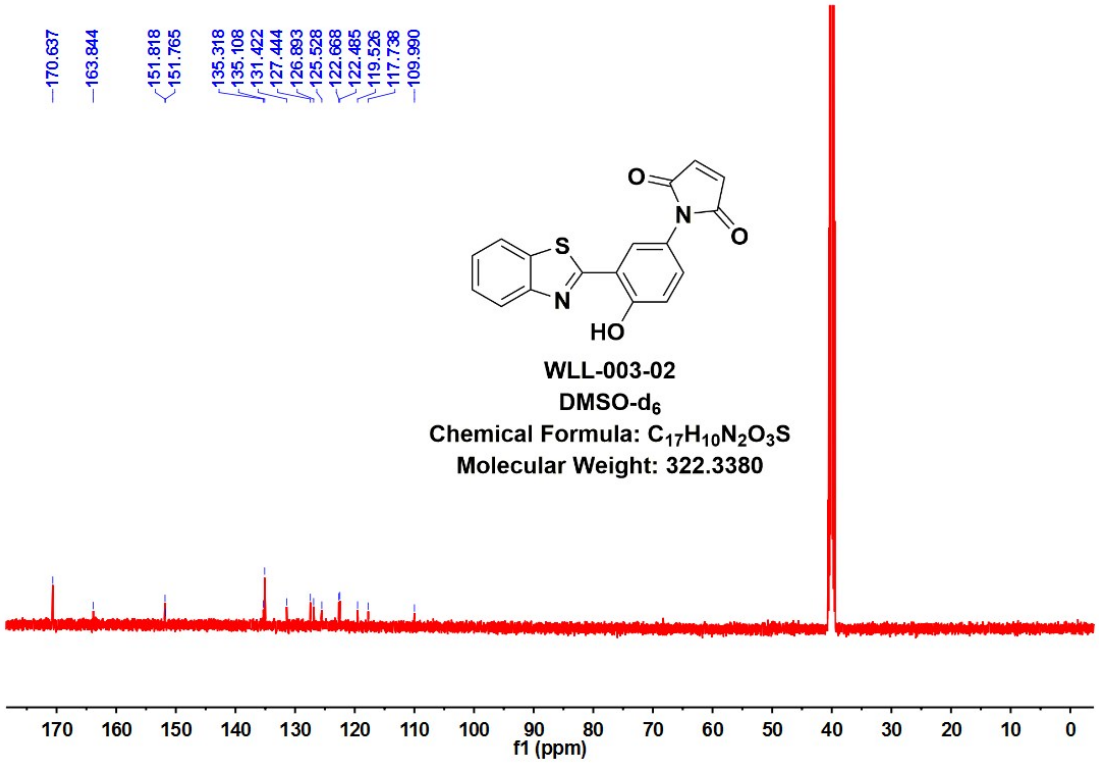


Figure S19. ^{13}C NMR spectrum of compound **2**.

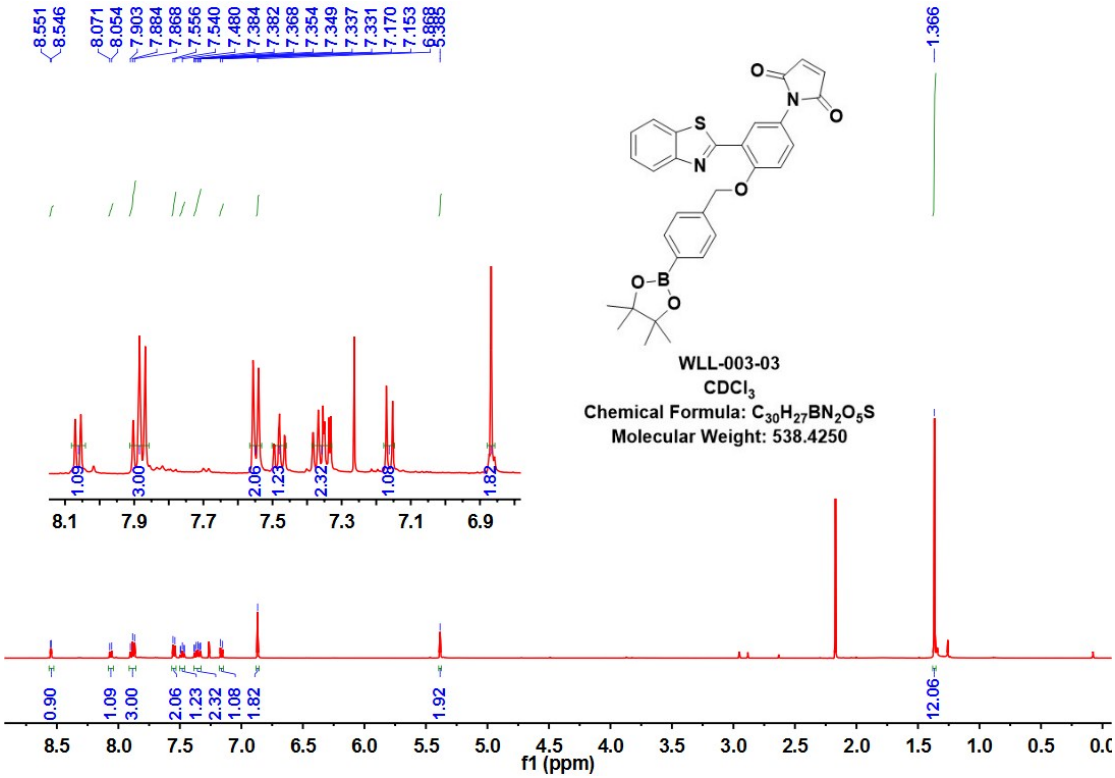


Figure S20. ^{1}H NMR spectrum of target probe **GSH-ABAH**.

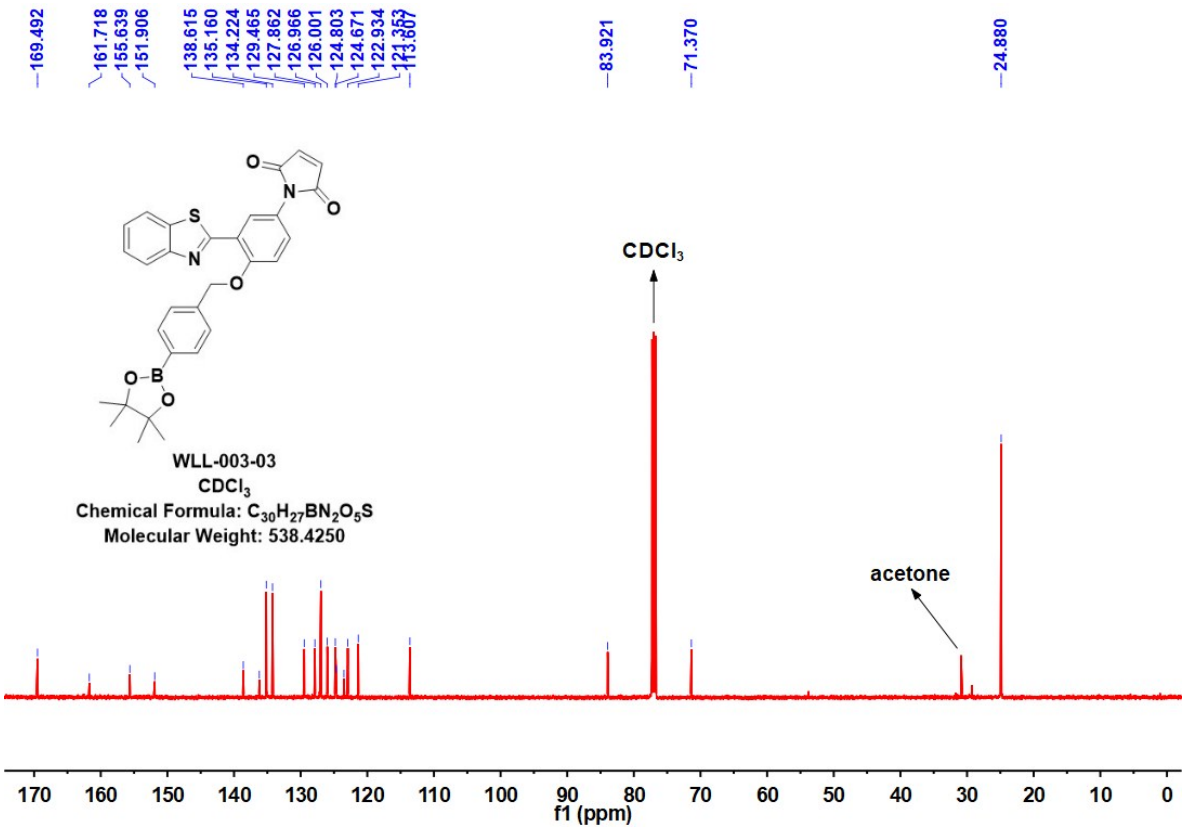


Figure S21. ^{13}C NMR spectrum of target probe **GSH-ABAH**.

9. References

1. Cui, L.; Zhu, W.; Xu, Y.; Qian, X., A novel ratiometric sensor for the fast detection of palladium species with large red-shift and high resolution both in aqueous solution and solid state. *Analytica Chimica Acta* 2013, 786, 139-145.

2. Zhao, Y.; Xue, Y.; Li, H.; Zhu, R.; Ren, Y.; Shi, Q.; Wang, S.; Guo, W., An excited state intramolecular proton transfer dye based fluorescence turn-on probe for fast detection of thiols and its applications in bioimaging. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2017, 175, 215-221.