

## Supplementary Information

### An ultrasensitive fluorogenic probe for revealing the role of glutathione in chemotherapy resistance

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## General Experimental for Chemistry

Dry dichloromethane (DCM) was distilled from  $\text{CaH}_2$ . Other reagents and chemicals for probe synthesis were obtained from commercial suppliers and used without further purification. Reactions were run under a nitrogen atmosphere and monitored by thin-layer chromatography (TLC) carried out on Silica gel 60 F254 plates supplied by Qingdao Puke Separation Material Corporation, and UV light was used as the visualizing agent. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, China.

$^1\text{H}$  NMR spectra were obtained on a Bruker 400 UltraShield<sup>TM</sup> Fourier transform spectrometer (400 MHz) at 25 °C.  $^{13}\text{C}$  NMR spectra were recorded on a Bruker 400 UltraShield<sup>TM</sup> Fourier transform spectrometer (100 MHz) spectrometer. All NMR spectra were calibrated using the residual solvent ( $\text{CDCl}_3$ ) as internal reference ( $^1\text{H}$  NMR = 7.26,  $^{13}\text{C}$  NMR = 77.16). All chemical shifts were reported in parts per million (ppm) and coupling constants ( $J$ ) in Hz. The following abbreviations were used to explain the multiplicities: d = doublet, t = triplet, m = multiplet. IR spectra were taken on a Bruker Vector 22 spectrophotometer as KBr pellets. High resolution mass spectra (HRMS) were measured on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight). UV-Vis spectra were taken on a HITACHI U-3010 Spectrophotometer. Fluorescence measurements were conducted on an Agilent Cary Eclipse Fluorescence Spectrophotometer with slit widths to be 10 and 10 nm for excitement and emission respectively, and the photomultiplier (PMT) detector voltage was set at medium.

## Probe synthesis and characterization

### General procedures for sulfide synthesis

To a stirred solution of *N*-butyl-4-bromo-1, 8-naphthalimide (1.0 eq) and the substituted thiophenol (5.0 eq) in 2-methoxyethanol was added triethylamine (5.0 eq) under nitrogen atmosphere. The reaction was stirred under reflux and monitored by thin-layer chromatography analysis. After the disappearance of the fluorophore which required about 3 hours, the solution was cooled to ambient temperature and poured into water to precipitate a solid, which was collected by filtration, washed with water, and dried. Purification of the crude product by flash column chromatography ( $\text{SiO}_2$ ) to give the product.

### General procedures for sulfoxide synthesis

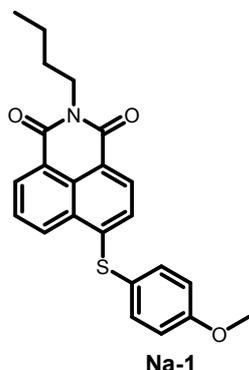
To a stirred solution of the sulfide (1.0 eq) in  $\text{CH}_2\text{Cl}_2$  at 0°C was added mCPBA(1.0 eq) in portions. The reaction was allowed to warm to ambient temperature by removing the ice bath. After completion as shown by thin-layer chromatography analysis which required about 1.0 hour,  $\text{H}_2\text{O}$  was added to quench the reaction and the mixture was diluted with  $\text{CH}_2\text{Cl}_2$ . The biphasic mixture was then transferred to a separatory funnel and the organic layer was washed sequentially with  $\text{H}_2\text{O}$ , saturated  $\text{NaHCO}_3$  and brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. The remaining residue was purified by flash column chromatography ( $\text{SiO}_2$ ) to give the product.

### General procedures for sulfone synthesis

To a stirred solution of the sulfoxide (1.0 eq) in  $\text{CH}_2\text{Cl}_2$  at 0°C was added mCPBA(1.0 eq) in portions. The reaction was then stirred at ambient temperature and monitored by thin-layer

chromatography analysis. After completion, H<sub>2</sub>O was added to quench the reaction and the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>. The mixture was transferred to a separatory funnel and the organic layer was washed sequentially with H<sub>2</sub>O, saturated NaHCO<sub>3</sub> and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The remaining residue was purified by flash column chromatography (SiO<sub>2</sub>) to give the product.

### Characterization



Yellow solid

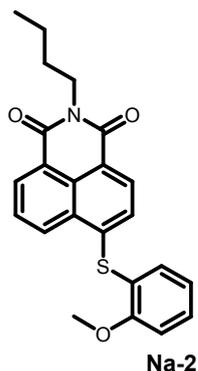
**M.p.:** 127.0-128.3 °C

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ):** 8.64 – 8.57 (m, 2H), 8.29 (d, J = 7.9 Hz, 1H), 7.76 (t, J = 7.9 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 8.1 Hz, 1H), 7.02 (d, J = 8.5 Hz, 2H), 4.16 (t, J = 7.6 Hz, 2H), 3.88 (s, 3H), 1.69 (m, 7.8 Hz, 2H), 1.48 (m, 7.4 Hz, 2H), 0.96 (t, J = 7.2 Hz, 3H).

**<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ):** 164.19, 164.12, 161.21, 147.46, 137.35, 137.35, 131.63, 130.94, 129.82, 128.61, 128.42, 126.77, 123.72, 123.22, 119.84, 119.34, 115.92, 115.92, 55.61, 40.32, 30.32, 20.51, 13.99.

**IR (KBr, cm<sup>-1</sup>):** 3447, 3028, 2956, 2862, 1741, 1701, 1660, 1592, 1351, 1244, 778.

**ESI-HRMS (m/z):** [M+H]<sup>+</sup> calc'd. for C<sub>23</sub>H<sub>21</sub>NO<sub>3</sub>S: 392.1320; found 392.1315



Yellow solid (yield)

**M.p.:** 157.4-158.3 °C

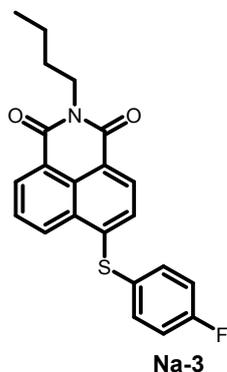
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ):** 8.67 (d, J = 8.4 Hz, 1H), 8.62 (d, J = 7.3 Hz, 1H), 8.33 (d, J = 7.9 Hz, 1H), 7.76 (t, J = 7.9 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.16 (d, J = 7.9 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 7.6 Hz, 1H), 4.16 (t, J = 7.4 Hz, 2H), 3.82 (s, 3H), 1.74 – 1.65 (m, 2H), 1.49 – 1.38 (m, 2H), 0.97 (t, J = 7.3 Hz, 3H).

**<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ):** 164.21, 164.17, 159.48, 144.50, 136.30, 131.66, 131.60,

130.89, 130.46, 129.42, 128.58, 126.82, 125.04, 123.25, 121.90, 119.90, 118.06, 111.80, 56.10, 40.33, 30.33, 20.52, 14.00.

**IR (KBr,  $\text{cm}^{-1}$ ):** 3448, 3026, 2959, 2926, 1639, 1647, 1582, 1361, 1243, 782.

**ESI-HRMS ( $m/z$ ):**  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_3\text{S}$ : 392.1320; found 392.1319



Yellow solid (yield)

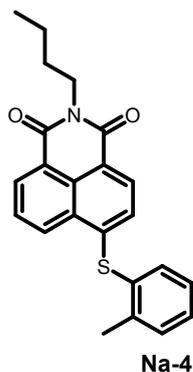
**M.p.:** 125.4-126.2 °C

**$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 8.62 (d,  $J = 7.3$  Hz, 1H), 8.59 (d,  $J = 8.5$  Hz, 1H), 8.33 (d,  $J = 7.9$  Hz, 1H), 7.77 (t,  $J = 7.9$  Hz, 1H), 7.55 (d,  $J = 5.4$  Hz, 1H), 7.53 (d,  $J = 5.3$  Hz, 1H), 7.19-7.15 (m, 3H), 4.5 (t,  $J = 7.6$  Hz, 2H), 1.74-1.66 (m, 2H), 1.48 – 1.38 (m, 2H), 0.96 (t,  $J = 7.3$  Hz, 3H).

**$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 164.06, 163.97, 162.44, 145.42, 137.02, 136.94, 131.75, 130.88, 129.98, 129.07, 128.49, 127.06, 125.73, 125.73( $J = 3.4$  Hz), 123.29, 120.16, 117.67, 117.45, 40.37, 30.30, 20.50, 13.98.

**IR (KBr,  $\text{cm}^{-1}$ ):** 3449, 3030, 2959, 2863, 1688, 1648, 1584, 1495, 1380, 1347, 1227, 778.

**ESI-HRMS ( $m/z$ ):**  $[\text{M}+\text{Na}]^+$  calc'd. for  $\text{C}_{22}\text{H}_{18}\text{FNO}_2\text{S}$ : 402.0940; found 402.0938.



Yellow solid (yield)

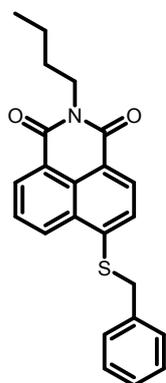
**M.p.:** 103.5-104.2 °C

**$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 8.65 (d,  $J = 2.7$  Hz, 1H), 8.63 (s, 1H), 8.30 (d,  $J = 7.9$  Hz, 1H), 7.79 (t,  $J = 7.9$  Hz, 1H), 7.53 (d,  $J = 7.6$  Hz, 1H), 7.44 – 7.39 (m, 2H), 7.30 (m, 4.3 Hz, 1H), 6.96 (d,  $J = 7.9$  Hz, 1H), 4.15 (t,  $J = 7.6$  Hz, 2H), 2.38 (s, 3H), 1.73-1.66 (m, 2H), 1.49 – 1.37 (m, 2H), 0.96 (t,  $J = 7.3$  Hz, 3H).

**$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 164.17, 164.10, 145.26, 142.63, 136.36, 131.69, 131.56, 131.01, 130.43, 130.08, 129.11, 129.03, 128.60, 127.70, 126.88, 123.91, 123.32, 119.67, 40.34, 30.32, 20.74, 20.51, 13.99.

IR (KBr,  $\text{cm}^{-1}$ ): 3449, 3025, 2958, 2862, 2360, 1697, 1652, 1583, 1511, 1461, 1353, 1231, 1072, 781.

ESI-HRMS ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_2\text{S}$ : 398.1191; found 398.1185.



Na-5

Yellow solid (yield)

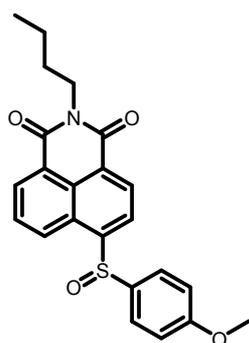
M.p.: 185.6-186.1 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.59 (d,  $J = 7.2$  Hz, 1H), 8.53 (d,  $J = 8.4$  Hz, 1H), 8.41 (d,  $J = 7.8$  Hz, 1H), 7.71 (t,  $J = 7.9$  Hz, 1H), 7.56 (d,  $J = 7.9$  Hz, 1H), 7.40 (d,  $J = 7.2$  Hz, 2H), 7.36 – 7.27 (m, 3H), 4.35 (s, 2H), 4.16 (m,  $J = 7.6$  Hz, 2H), 1.75 – 1.64 (m, 2H), 1.49-1.39 (m, 2H), 0.97 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 164.13, 164.11, 144.50, 135.36, 131.57, 130.82, 130.18, 129.67, 129.03, 129.03, 128.95, 128.95, 127.99, 126.79, 124.06, 123.22, 119.73, 40.35, 37.52, 30.32, 20.52, 14.00.

IR (KBr,  $\text{cm}^{-1}$ ): 3450, 3027, 2959, 2863, 2361, 1695, 1647, 1589, 1504, 1457, 1357, 1232, 1074, 778.

ESI-HRMS ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_2\text{S}$ : 398.1191; found 398.1191.



Na-6

White solid (yield)

M.p.: 158.1-159.1 °C

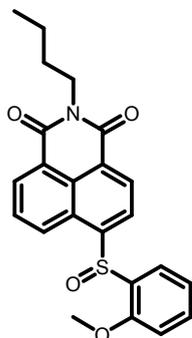
$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.75 (d,  $J = 7.7$  Hz, 1H), 8.58 (d,  $J = 7.3$  Hz, 1H), 8.52 (d,  $J = 7.7$  Hz, 1H), 8.38 (d,  $J = 8.5$  Hz, 1H), 7.74 (t,  $J = 7.9$  Hz, 1H), 7.59 (d,  $J = 8.8$  Hz, 2H), 6.89 (d,  $J = 8.8$  Hz, 2H), 4.15 (t,  $J = 7.4$  Hz, 2H), 3.76 (s, 2H), 1.73 – 1.60 (m, 2H), 1.47-1.37 (7.4 Hz, 2H), 0.95 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 163.64, 163.44, 162.55, 147.86, 135.07, 131.55, 130.74, 128.47, 128.24, 128.18, 128.18, 128.02, 127.38, 124.99, 123.55, 123.55, 115.25, 115.25,

55.63, 40.51, 30.20, 20.43, 13.92.

**IR (KBr,  $\text{cm}^{-1}$ ):** 3447, 3033, 2960, 2865, 2363, 1741, 1704, 1586, 1499, 1356, 1300, 1260, 1082, 758.

**ESI-HRMS ( $m/z$ ):**  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_4\text{S}$ : 408.1270; found 408.1265.



**Na-7**

White solid (yield)

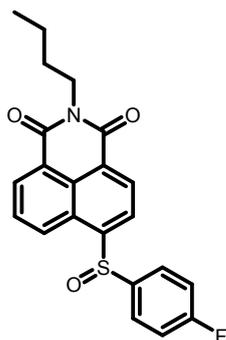
**M.p.:** 173.8-174.5 °C

**$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 8.83 (d,  $J = 8.5$  Hz, 1H), 8.66-8.62 (m, 2H), 8.62 (s, 1H), 8.29 (d,  $J = 7.7$  Hz, 1H), 7.91 (d,  $J = 7.7$  Hz, 1H), 7.82 (t,  $J = 7.9$  Hz, 1H), 7.40 (t,  $J = 7.7$  Hz, 1H), 7.15 (t,  $J = 7.6$  Hz, 1H), 6.82 (d,  $J = 8.3$  Hz, 1H), 4.15 (m,  $J = 7.6$  Hz, 2H), 3.68 (s, 3H), 1.74 – 1.60 (m, 2H), 1.47 – 1.37 (m, 2H), 0.96 (t,  $J = 7.3$  Hz, 3H).

**$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 163.83, 163.53, 155.76, 149.00, 133.13, 132.19, 131.72, 130.80, 129.59, 128.41, 128.31, 127.67, 125.82, 125.16, 124.94, 123.30, 122.16, 111.34, 55.69, 40.53, 30.27, 20.47, 13.93.

**IR (KBr,  $\text{cm}^{-1}$ ):** 3448, 3024, 2925, 2865, 2360, 1694, 1654, 1583, 1475, 1353, 1271, 1233, 1040, 780.

**ESI-HRMS ( $m/z$ ):**  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_4\text{S}$ : 408.1270; found 408.1265.



**Na-8**

White solid (yield)

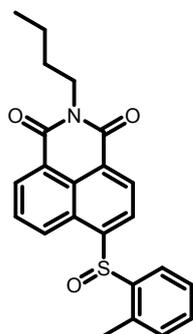
**M.p.:** 130.7-133.5 °C

**$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 8.73 (d,  $J = 7.6$  Hz, 1H), 8.61 (d,  $J = 7.3$  Hz, 1H), 8.52 – 8.42 (m, 2H), 7.79 (t,  $J = 7.9$  Hz, 1H), 7.70-7.67 (m, 2H), 7.10 (t,  $J = 8.2$  Hz, 2H), 4.15 (t,  $J = 7.5$  Hz, 2H), 1.73 – 1.62 (m, 2H), 1.47 – 1.36 (m, 2H), 0.95 (t,  $J = 7.3$  Hz, 3H).

**$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):** 163.53, 163.30, 147.58, 139.91, 139.88, 131.73, 130.72, 128.60, 128.28, 128.06, 128.06, 127.97, 127.58, 125.43, 123.92, 123.76, 117.31, 117.09, 40.57, 30.23, 20.44, 13.90.

IR (KBr,  $\text{cm}^{-1}$ ): 3498, 3027, 2925, 2864, 2361, 1700, 1663, 1583, 1493, 1340, 1299, 1061, 781.

ESI-HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{22}\text{H}_{18}\text{FNO}_3\text{S}$ : 396.1070; found 396.1061.



Na-9

White solid (yield)

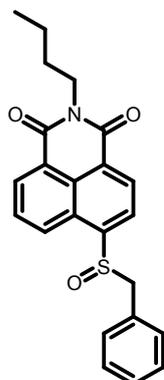
M.p.: 169.2-170.2 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.70 (d,  $J = 7.7$  Hz, 1H), 8.61 (d,  $J = 7.3$  Hz, 1H), 8.47 (d,  $J = 8.5$  Hz, 1H), 8.28 (d,  $J = 7.7$  Hz, 1H), 7.78 (t,  $J = 7.9$  Hz, 1H), 7.68 (d,  $J = 7.7$  Hz, 1H), 7.37 (t,  $J = 7.2$  Hz, 1H), 7.31 (t,  $J = 7.4$  Hz, 1H), 7.23 (d,  $J = 7.4$  Hz, 1H), 4.15 (t,  $J = 7.6$  Hz, 2H), 2.53 (s, 3H), 1.74 – 1.62 (m, 2H), 1.48 – 1.36 (m, 2H), 0.96 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ):  $\delta$  163.62, 163.37, 146.67, 141.56, 136.93, 132.09, 131.73, 131.56, 130.66, 128.51, 128.46, 128.27, 128.24, 127.75, 126.37, 125.81, 125.30, 123.63, 40.56, 30.23, 20.44, 19.07, 13.90.

IR (KBr,  $\text{cm}^{-1}$ ): 3498, 3023, 2958, 2863, 2360, 1705, 1663, 1587, 1343, 1229, 1030, 777.

ESI-HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_3\text{S}$ : 392.1320; found 392.1316.



Na-10

White solid (yield)

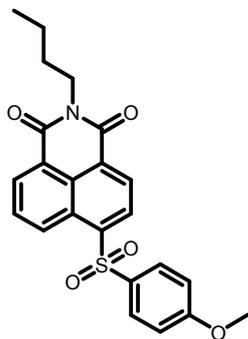
M.p.: 177.9-178.8 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.62 (d,  $J = 7.3$  Hz, 1H), 8.59 (d,  $J = 7.7$  Hz, 1H), 8.12 (d,  $J = 8.4$  Hz, 1H), 8.00 (d,  $J = 7.6$  Hz, 1H), 7.71 (t,  $J = 7.9$  Hz, 1H), 7.22 (t,  $J = 7.3$  Hz, 1H), 7.13 (t,  $J = 7.4$  Hz, 2H), 6.82 (d,  $J = 7.5$  Hz, 2H), 4.29 – 4.20 (m, 2H), 4.16 (d,  $J = 6.5$  Hz, 2H), 1.76-1.68 (m, 2H), 1.50 – 1.39 (m, 2H), 0.98 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 197.94, 169.85, 162.99, 151.44, 150.37, 136.31, 135.00, 134.67, 132.37, 131.43, 130.80, 130.05, 128.36, 126.40, 124.91, 124.25, 122.43, 122.33, 118.97, 26.80, 21.15.

IR (KBr,  $\text{cm}^{-1}$ ): 3448, 3028, 2957, 2862, 2361, 1704, 1656, 1583, 1512, 1352, 1227, 1044, 781.

ESI-HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_3\text{S}$ : 392.1320; found 392.1318.



Na-11

White solid (87% yield)

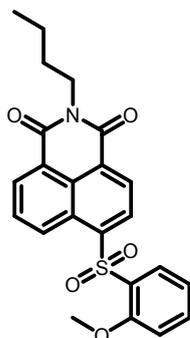
M.p.: 164.2-165.4 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 9.08 (d,  $J = 8.7$  Hz, 1H), 8.67 (d,  $J = 7.7$  Hz, 1H), 8.62 (d,  $J = 7.3$  Hz, 1H), 8.58 (d,  $J = 7.7$  Hz, 1H), 7.92 (d,  $J = 8.9$  Hz, 2H), 7.85 (t,  $J = 8.0$  Hz, 1H), 6.96 (d,  $J = 8.9$  Hz, 2H), 4.15 (t,  $J = 7.76$  Hz, 2H), 3.82 (s, 3H), 1.73 – 1.62 (m, 2H), 1.47 – 1.36 (m, 2H), 0.95 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 163.64, 163.49, 146.23, 131.54, 130.42, 130.33, 130.33, 128.78, 128.63, 128.63, 128.34, 127.99, 127.92, 127.87, 127.43, 124.95, 124.44, 123.54, 62.64, 40.62, 30.27, 20.49, 13.94.

IR (KBr,  $\text{cm}^{-1}$ ): 3449, 3025, 2961, 2926, 2361, 1770, 1705, 1586, 1500, 1353, 1226, 1145, 1023, 789.

ESI-HRMS ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_5\text{S}$ : 424.1219; found 424.1216.



Na-12

White solid (yield)

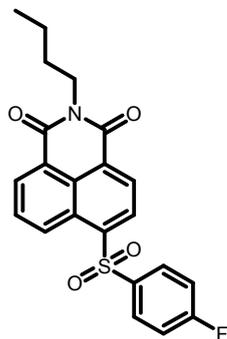
M.p.: 186.2-187.7 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.86 (d,  $J = 8.6$  Hz, 1H), 8.72 (s, 2H), 8.58 (d,  $J = 7.2$  Hz, 1H), 8.36 (d,  $J = 7.8$  Hz, 1H), 7.75 (t,  $J = 8.0$  Hz, 1H), 7.57 (t,  $J = 7.4$  Hz, 1H), 7.21 (t,  $J = 7.6$  Hz, 1H), 6.84 (d,  $J = 8.3$  Hz, 1H), 4.16 (t,  $J = 7.6$  Hz, 2H), 3.60 (s, 3H), 1.71-1.65 (m, 2H), 1.48-1.38 (m, 2H), 0.96 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 163.65, 163.22, 157.30, 141.73, 136.43, 131.58, 131.18, 130.49, 129.91, 129.24, 128.79, 128.67, 128.32, 127.30, 127.13, 123.28, 121.01, 112.73, 55.89, 40.63, 30.21, 20.45, 13.94.

IR (KBr,  $\text{cm}^{-1}$ ): 3449, 3024, 2962, 2926, 2361, 1740, 1705, 1623, 1476, 1374, 1343, 1284, 1147, 790.

ESI-HRMS ( $m/z$ ):  $[M+H]^+$  calc'd. for  $\text{C}_{23}\text{H}_{21}\text{NO}_5\text{S}$ : 424.1219; found 424.1216.



Na-13

White solid (yield)

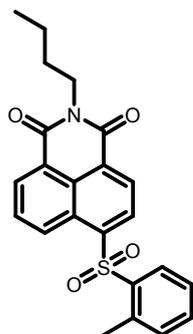
M.p.: 107.2-171.9 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 9.02 (d,  $J = 8.7$  Hz, 1H), 8.70 (d,  $J = 7.7$  Hz, 1H), 8.63 (d,  $J = 7.5$  Hz, 2H), 8.01 (dd,  $J = 8.6, 5.0$  Hz, 2H), 7.86 (t,  $J = 8.0$  Hz, 1H), 7.19 (t,  $J = 8.4$  Hz, 2H), 4.15 (t,  $J = 7.6$  Hz, 2H), 1.73 – 1.62 (m, 2H), 1.48 – 1.35 (m, 2H), 0.95 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 163.43, 162.87, 141.39, 136.71, 136.68, 131.97, 130.77, 130.68, 130.28, 129.77, 129.69, 129.21, 129.11, 127.73, 127.12, 123.53, 117.19, 116.96, 40.67, 30.17, 20.43, 13.92.

IR (KBr,  $\text{cm}^{-1}$ ): 3449, 3026, 2956, 2926, 2862, 2361, 1702, 1663, 1586, 1344, 1232, 1148, 1078, 786.

ESI-HRMS ( $m/z$ ):  $[M+Na]^+$  calc'd. for  $\text{C}_{22}\text{H}_{18}\text{FNO}_4\text{S}$ : 434.0838; found 434.0834.



Na-14

White solid (yield)

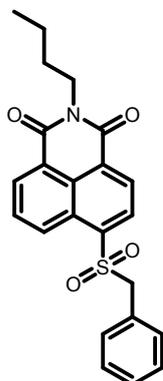
M.p.: 139.4-141.5 °C

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.83 (d,  $J = 8.7$  Hz, 1H), 8.70 (d,  $J = 7.7$  Hz, 1H), 8.59 (t,  $J = 8.2$  Hz, 2H), 8.35 (d,  $J = 7.7$  Hz, 1H), 7.78 (t,  $J = 8.0$  Hz, 1H), 7.54-7.46 (m, 2H), 7.22 (d,  $J = 7.1$  Hz, 1H), 4.16 (t,  $J = 7.6$  Hz, 2H), 2.36 (s, 3H), 1.74 – 1.64 (m, 2H), 1.48 – 1.37 (m, 2H), 0.96 (t,  $J = 7.3$  Hz, 3H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 163.49, 162.98, 141.32, 138.50, 138.34, 134.40, 133.22, 131.88, 130.45, 129.92, 129.47, 129.29, 129.03, 128.93, 127.47, 127.13, 126.97, 123.41, 40.66, 30.19, 20.44, 20.31, 13.94.

**IR (KBr, cm<sup>-1</sup>):** 3449, 3023, 2959, 2867, 2360, 1705, 1661, 1532, 1465, 1355, 1309, 1146, 779.

**ESI-HRMS (m/z):** [M+H]<sup>+</sup> calc'd. for C<sub>23</sub>H<sub>21</sub>NO<sub>4</sub>S: 408.1270; found 408.1267.



**Na-15**

White solid (yield)

**M.p.:** 173.4-174.5°C

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ):** 8.94 (d, J = 8.6 Hz, 1H), 8.67 (d, J = 7.2 Hz, 1H), 8.53 (d, J = 7.7 Hz, 1H), 8.18 (d, J = 7.7 Hz, 1H), 7.82 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 7.4 Hz, 1H), 7.14 (t, J = 7.5 Hz, 2H), 6.92 (d, J = 7.5 Hz, 2H), 4.52 (s, 2H), 4.16 (t, J = 7.6 Hz, 2H), 1.75-1.63 (m, 2H), 1.49-1.38 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H).

**<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ):** 163.48, 162.93, 138.41, 131.90, 131.57, 130.76, 130.41, 129.34, 129.29, 129.14, 128.87, 128.57, 128.20, 127.66, 127.21, 123.35, 63.36, 40.74, 30.18, 20.46, 13.94.

**IR (KBr, cm<sup>-1</sup>):** 3449, 3024, 2962, 2926, 2864, 2361, 1741, 1706, 1547, 1512, 1227, 1073, 784.

**ESI-HRMS (m/z):** [M+H]<sup>+</sup> calc'd. for C<sub>23</sub>H<sub>21</sub>NO<sub>4</sub>S: 408.1270; found 408.1268.

### Fluorometric analysis method

Deionized water was used to prepare all aqueous solutions. All the photophysical characterization experiments were carried out at 37°C. Phosphate buffer saline (PBS, 10 mM, pH 7.4) was purged with nitrogen for 5 min before use. **Na-8** was dissolved in DMSO to make a 5 mM stock solution. GSH and other reactive bio-relevant species were prepared by dissolving commercial reagents in H<sub>2</sub>O. To test the fluorescent responses of **Na-8** towards GSH or other reactive species, aliquots of probe stock solution were diluted with PBS (with 1% cetrimonium bromide) and treated with analytes to make sure both probes and analytes were kept at desired final concentrations. After quick and vigorous shaking, the mixture was allowed standing in the dark at 37°C for desired time and the fluorescent spectra were then recorded under excitation at 405 nm. The emission spectra were scanned from 410 to 650 nm. All fluorometric experiments were performed in triplicate, and data shown were the average.

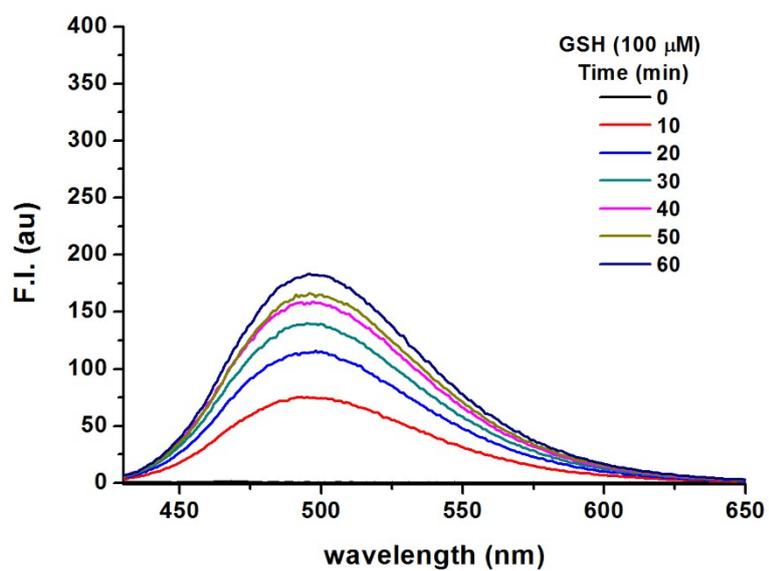


Fig. S1 Fluorescent spectra of Na-8 (5 μM) after the treatment of GSH (100 μM) for various time.

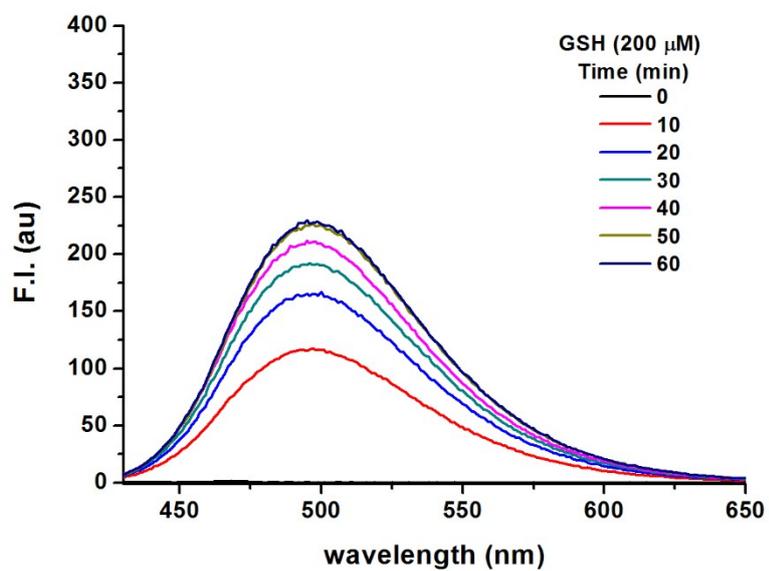


Fig. S2 Fluorescent spectra of Na-8 (5 μM) after the treatment of GSH (200 μM) for various time.

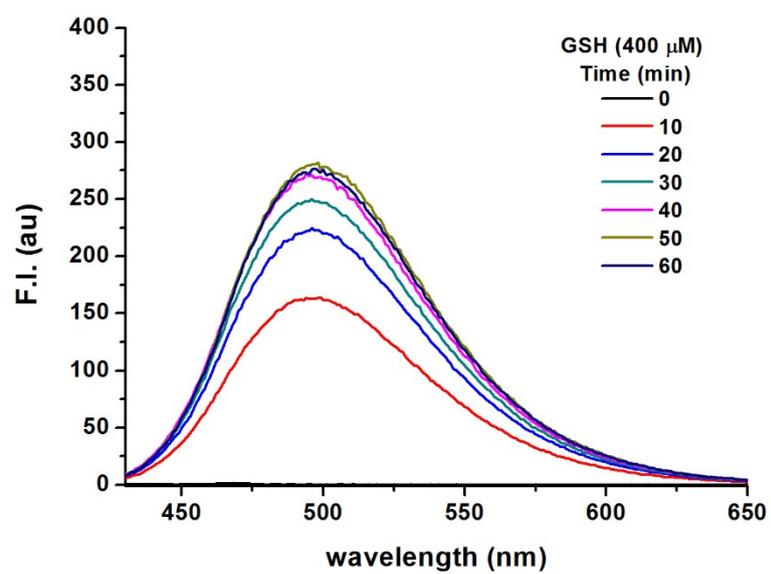


Fig. S3 Fluorescent spectra of Na-8 (5 μM) after the treatment of GSH (400 μM) for various time.

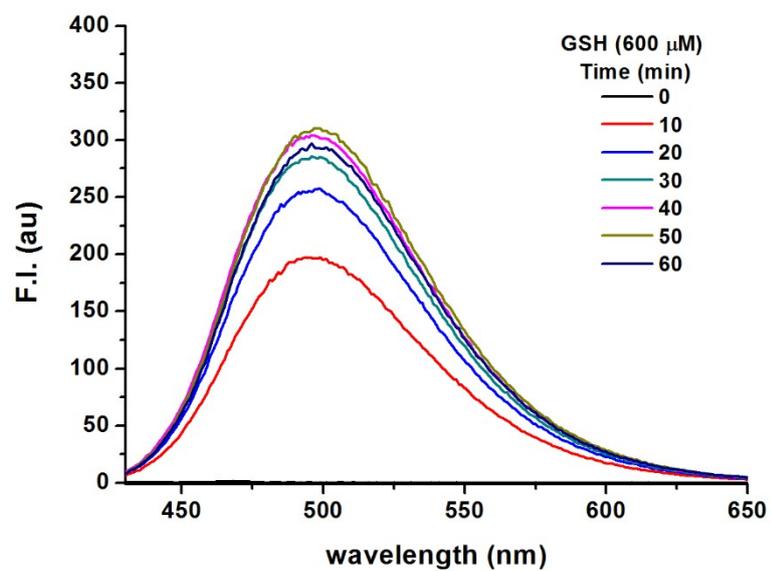


Fig. S4 Fluorescent spectra of Na-8 (5 μM) after the treatment of GSH (600 μM) for various time.

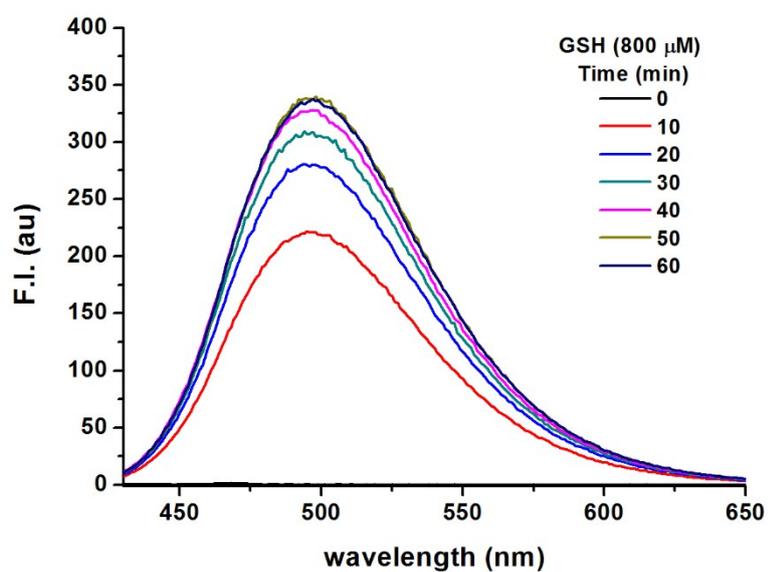


Fig. S5 Fluorescent spectra of Na-8 (5 μM) after the treatment of GSH (800 μM) for various time.

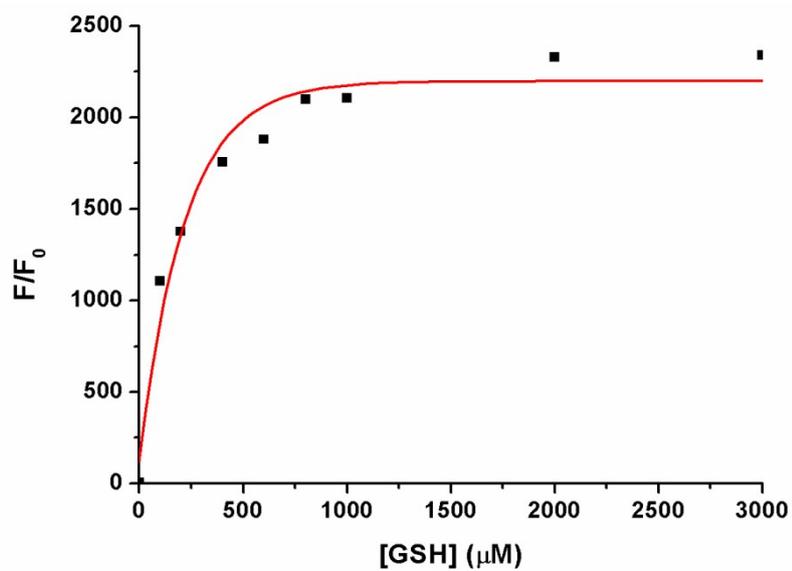
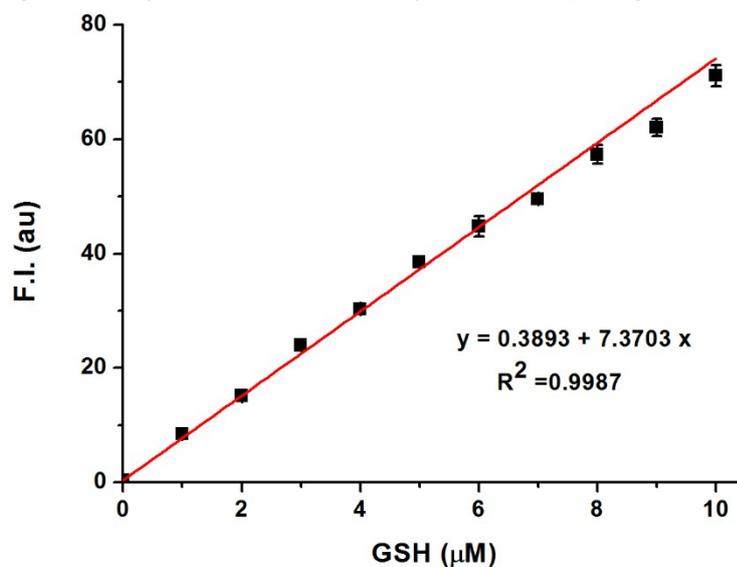


Fig. S6 Plot of Na-8 fluorescence response against GSH concentration. F represented the intensity at 498 nm of Na-8 (5 μM) after GSH treatment of indicated concentration for 1 hour.  $F_0$  was the intensity of Na-8 (5 μM) without GSH treatment,  $\lambda_{ex}$  405 nm.

Step 1: Blank measurements (n=25). Mean:  $F_B = 0.15136 \pm 0.06212$ ;

Step 2: Linear regression analysis on fluorescence intensity and the corresponding GSH concentrations (0-10  $\mu\text{M}$ ):

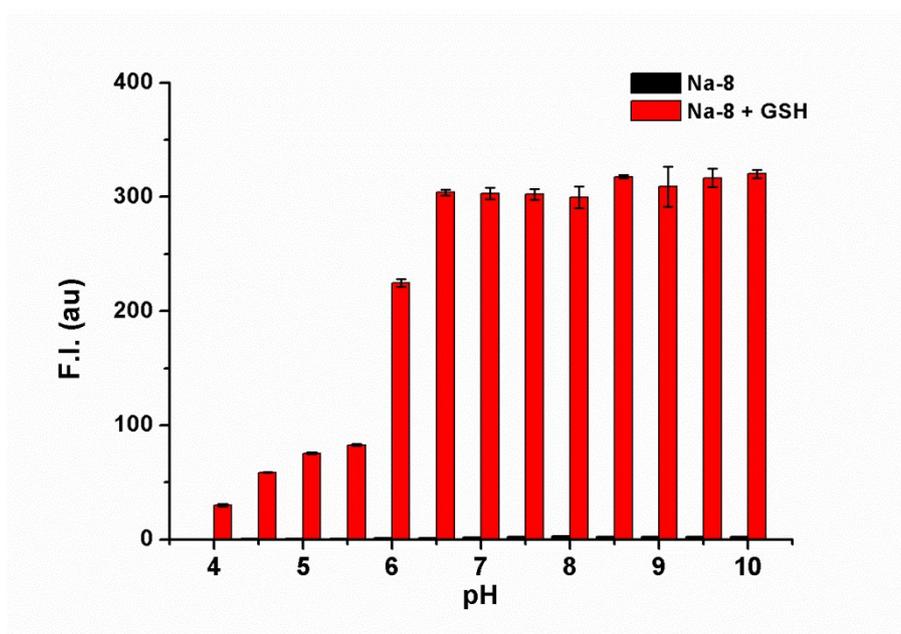


Step 3: Detection limit calculation: 
$$C_L = \frac{3S_B}{m}$$

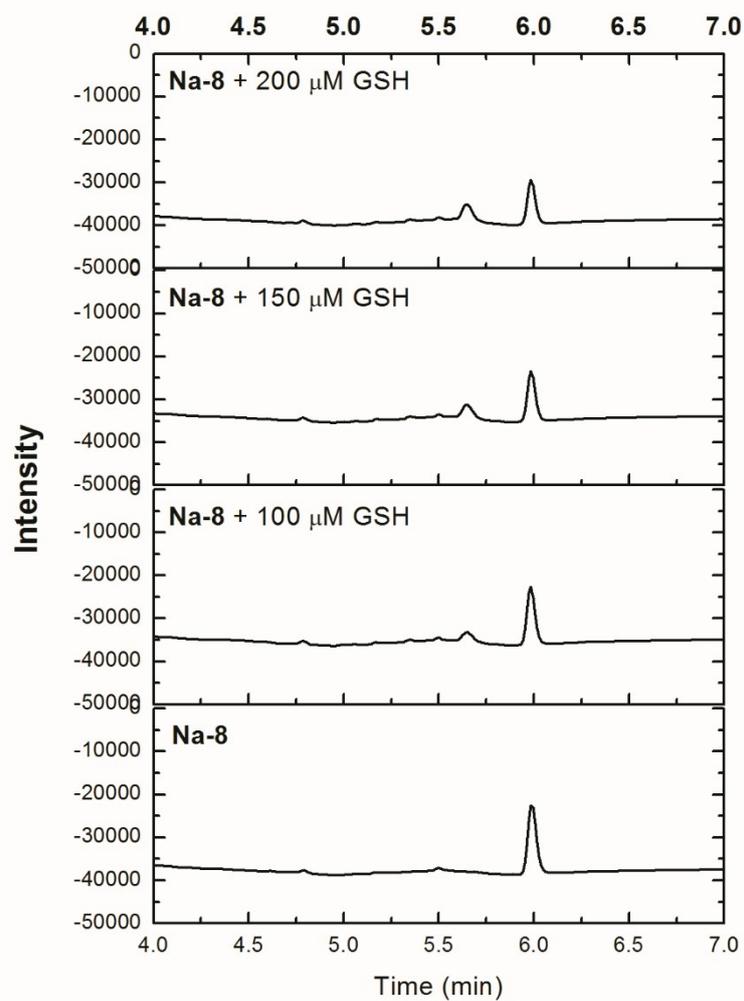
Where  $S_B$  is the standard deviation of the blank measurements;

$m$  is the slope of the calibration curve line obtained from the linear regression analysis.

**Fig. S7** The detection limit determination of **Na-8**. Blank measurements were conducted for 25 times to calculate the standard deviation. **Na-8** was treated with GSH (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10  $\mu\text{M}$ ) for 60 min to record the fluorescence (498 nm) to do the linear regression analysis.

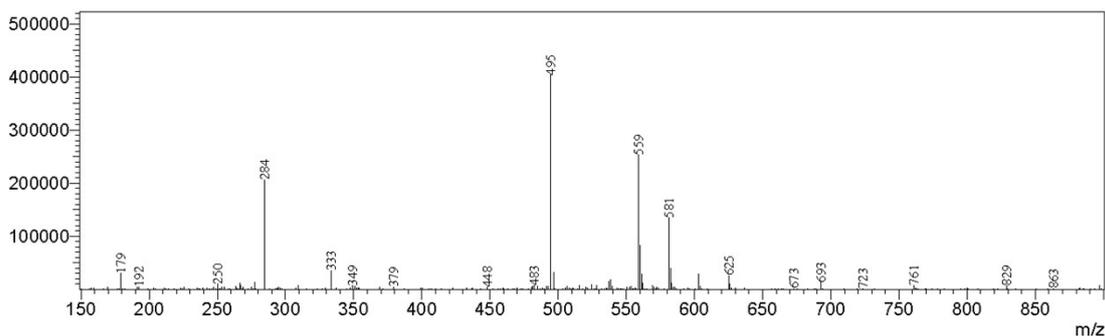


**Fig. S8** Fluorescence intensity at 498 nm of **Na-8** (5  $\mu\text{M}$ ) before or after the treatment of GSH (500  $\mu\text{M}$ ) in PBS of various pH.

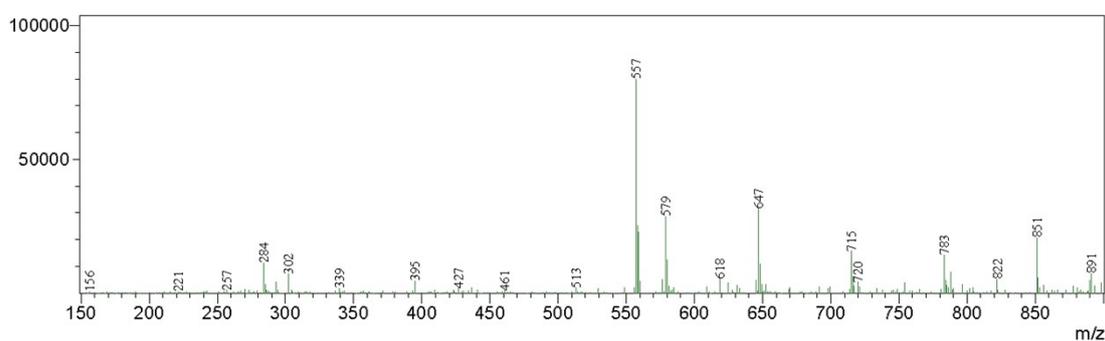


**Fig. S9** The liquid chromatogram (LC) traces of **Na-8** before or after the treatment of various concentrations of GSH for 20 min. The peak corresponding to **Na-8** decreased in a GSH-dose independent way accompanied by the simultaneous increase of a new peak whose structure was elucidated by MS analysis to be the nucleophilic substitution product of **Na-8** by GSH.

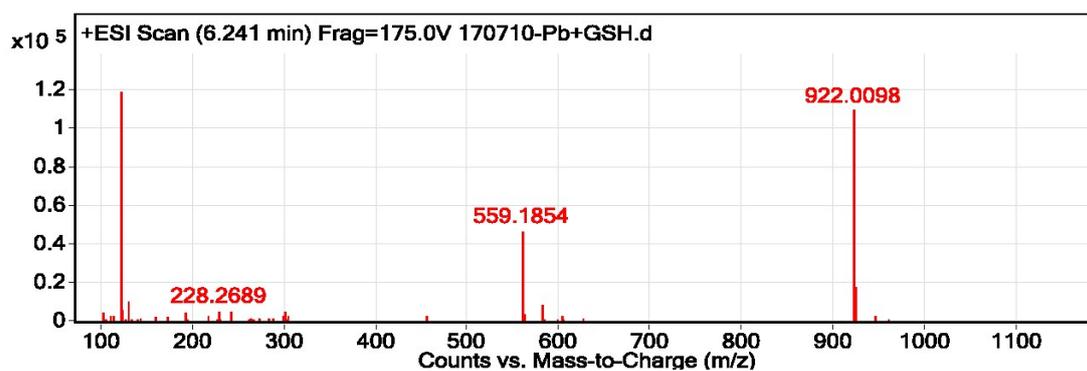
R.Time:5.717(Scan#:447)  
 MassPeaks:262 BasePeak:495(403346)  
 Spectrum Mode:Single 5.717(447)  
 BG Mode:Averaged 4.800-7.333(337-641) Polarity:Positive Segment 1 - Event 1



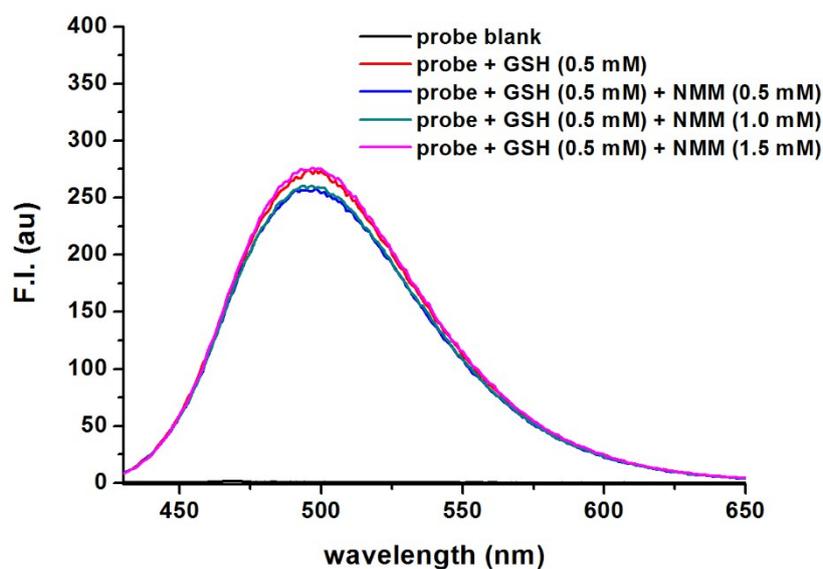
R.Time:5.725(Scan#:448)  
 MassPeaks:301 BasePeak:557(79983)  
 Spectrum Mode:Single 5.725(448)  
 BG Mode:Averaged 4.808-7.341(338-642) Polarity:Negative Segment 1 - Event 2



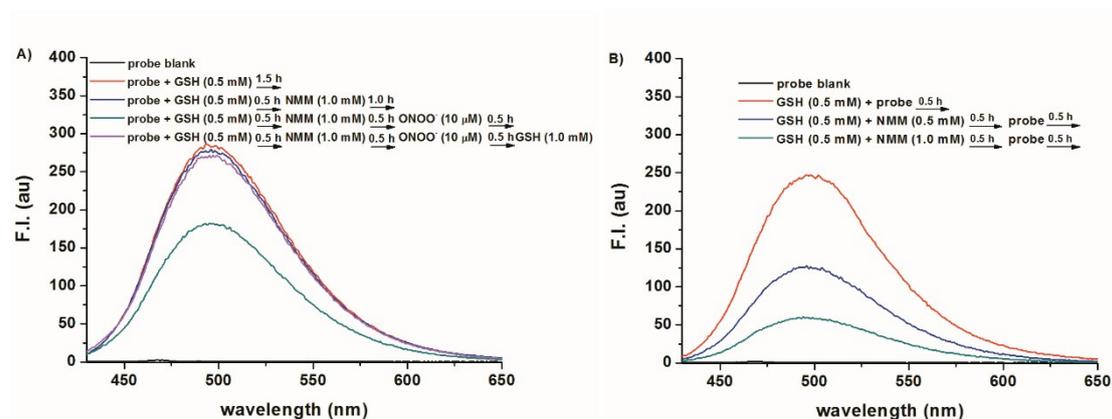
**Fig. S10** MS trace of the new peak in Figure S9. The  $m/z$  signals at 559 (positive, top figure) and 557 (negative, bottom figure) were attributed to  $[M+H]^+$  and  $[M-H]^-$ , respectively.



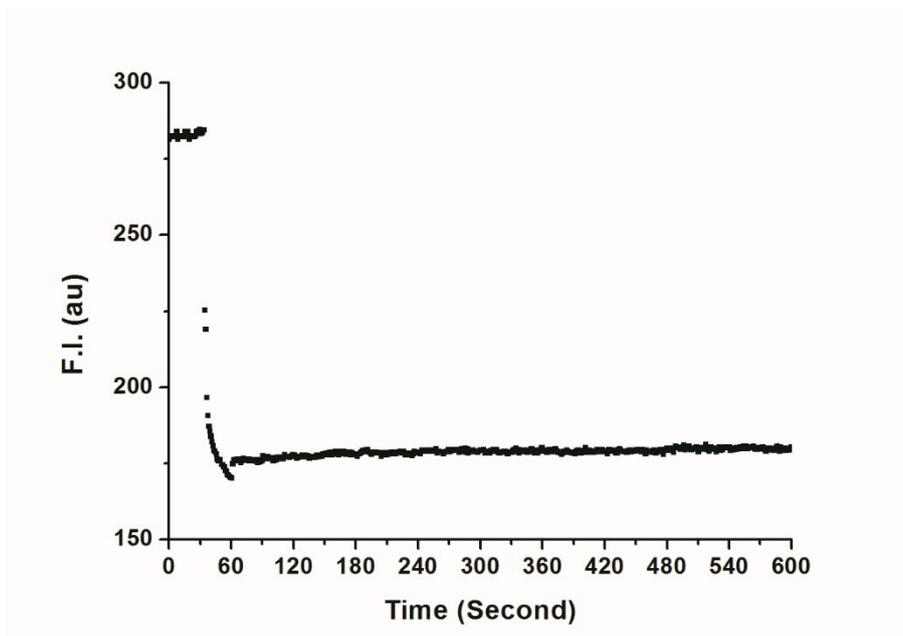
**Fig. S11** HRMS of the new peak in Figure S9. The structure was elucidated to be that shown in Figure 1c. ( $m/z$   $[M+H]^+$  calc'd. for  $C_{26}H_{30}N_4O_8S$ : 559.1863; found 559.1864).



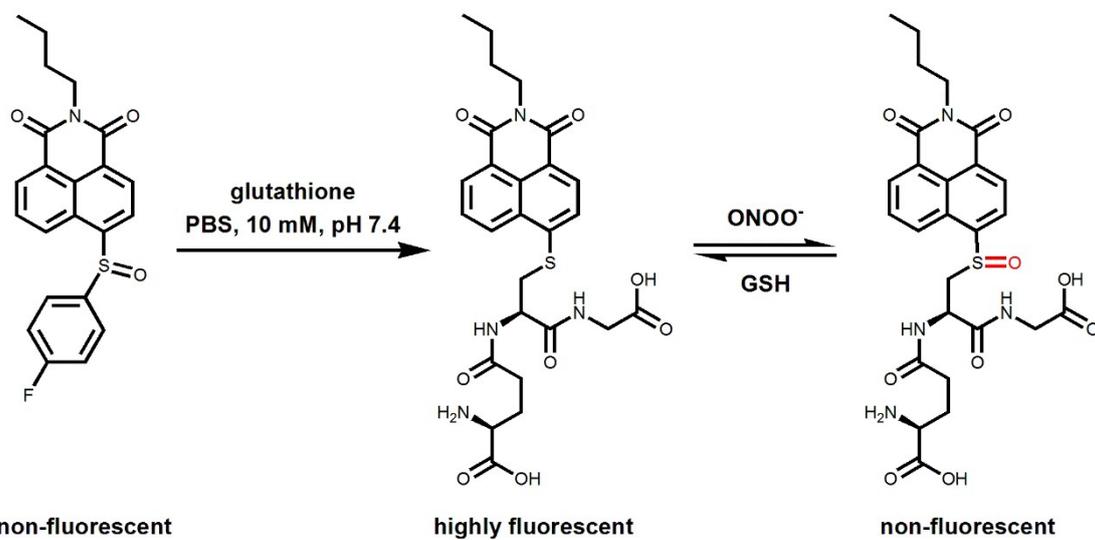
**Fig. S12** Na-8 detects GSH irreversibly. A solution of Na-8 (5  $\mu$ M) was treated with GSH (500  $\mu$ M) for 60 min, and then aliquots of the solution were further treated with *N*-methyl maleimide (NMM) of various concentrations for 30 min to record the emission spectra.



**Fig. S13** Emission spectra of Na-8 (5  $\mu$ M) after various treatment. A) Na-8 (5  $\mu$ M) was treated with GSH (0.5 mM) for 0.5 h. Then an aliquot of the solution was treated with NMM (1.0 mM) to consume the remaining GSH. After another 0.5 h, ONOO<sup>-</sup> (10  $\mu$ M) was added to an aliquot of the solution containing little free GSH and the mixture was allowed to incubate for a further 0.5 h. Next, an aliquot of the ONOO<sup>-</sup> - treated solution was incubated with GSH (1.0 mM) for 0.5 h. detects irreversibly. Finally, all the solutions were recorded for their emission spectra. B) GSH (0.5 mM) without or with the pretreatment of NMM (30 min) induced different degree of Na-8 (5  $\mu$ M).



**Fig. S14** Time-lapsed emission (498 nm) of the naphthalimide-GSH product in response to  $\text{ONOO}^-$  ( $10 \mu\text{M}$ ).



**Fig. S15** Speculated reaction mechanism of the naphthalimide-GSH product to the subsequent treatment of  $\text{ONOO}^-$  and GSH.

## Biological Methods

### Cell culture

L02 and HepG2 cells were cultured in RPMI 1640 (Gibco, 31800022) and DMEM (Gibco, 12100061) growth medium supplemented with 10% FBS (Gibco, Australia) in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air at 37 °C and split when the cells reached 95% confluency.

### Fluorescence imaging

Generally, cells (HepG2, 25000/well; L02, 20000/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. For direct fluorescent quantitation, cells were loaded with probe **Na-8** (10 μM) or monochlorobimane (20 μM) for 30 min. For exogenous GSH detection, HepG2 cells were firstly loaded with the probe **Na-8** (10 μM) in culture medium for 30 min and changed to fresh one containing GSH (Sigma, G4251) of 1.0 mM and 2.0 mM for another 10 min. For endogenous GSH detection, cells were incubated with NEM (Sigma, E3876) of 25 μM and 50 μM for 30 min, after which the probe **Na-8** (10 μM) was added to the medium for further 30 min. After incubation, all the above were washed with PBS twice before image. For NAC treatment, cells were pretreated with NEM (50 μM) for 15 min, then probe **Na-8** (10 μM) was added to culture medium for another 15 min. After that cells were changed to the fresh culture medium containing probe **Na-8** (10 μM) and NAC (100 μM), and imaged at indicated time. The fluorescence images were recorded by Operetta high content imaging system (Perkinelmer, US) at the excitation wavelengths of 360-440 nm. The fluorescence was quantified by columbus analysis system (Perkinelmer, US).

### Cell viability assay

Cell viability was detected by MTS assay as the guidelines. 10 μL per well of MTS/PMS (20:1, Promega Corp) solution was added to each well containing 100 μL of culture medium, followed by a gentle shake. After incubation at 37 °C under 5% CO<sub>2</sub> for 4 h, the absorbance of the solutions was measured at 490 nm, using an M5 microplate reader (Molecular Device, USA).

For drug-resistance assay, cells (L02 10000/well; HepG2 15000/well) were seeded on 96-well microplates in growth medium overnight and exposed to increasing concentration of Cisplatin (0, 0.5, 1, 2.5, 5, 10, 20 μM; Selleck S1166) or Doxorubicin (0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 μg/ml; Selleck S1208) for 72 h.

To test if GSH involved in this process, cells were firstly pretreated with BSO (50 μM; Sigma B2515) for 24 h, followed by exposed to increasing concentration of Doxorubicin (0, 0.05, 0.1, 0.25, 0.5 μg/ml) in absence or in presence of BSO (50 μM) for another 48 h. Or cells were pretreated with Doxorubicin (0.25 μg/ml) for 24 h, followed by exposed to Doxorubicin (0.25 μg/ml) in presence of BSO (0, 12.5, 50 μM) for 48 h. And the Cisplatin (0, 0.5, 1, 2.5, 5, 10, 20 μM; 5 μM) was tested as the same.

For short-term toxicity test, cells (L02 10000/well; HepG2 15000/well) were seeded on 96-well microplates in growth medium overnight and exposed to increasing concentration of probe **Na-8** (1, 5, 10, 20 μM) for 1 h. Then cells were changed to the fresh culture medium for 72 h before MTS assay. For long-term toxicity test, cells (L02 10000/well; HepG2 15000/well)

were seeded on 96-well microplates in growth medium overnight and exposed to increasing concentration of probe **Na-8** (1, 5, 10, 20  $\mu\text{M}$ ) for 48 h. Then cells were changed to the fresh culture medium just before MTS assay.

### ROS detection

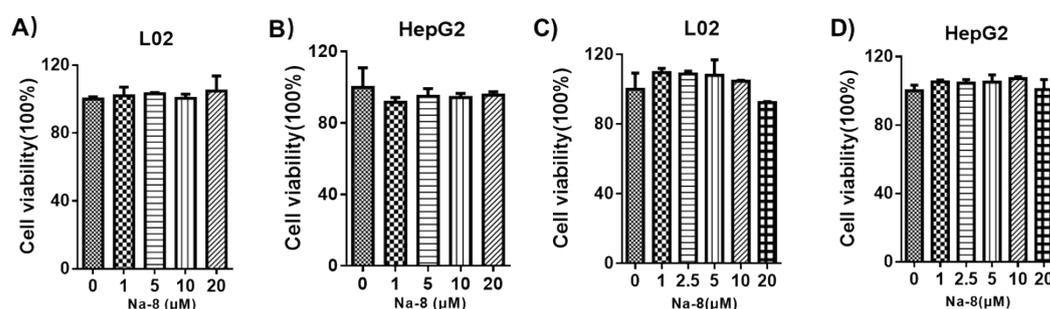
ROS was detected by the Reactive Oxygen Species Assay Kit (Yeasen, 50101ES01). Cells (HepG2, 160000/well; L02, 140000/well) were seeded on a 24-well microplate in growth medium overnight and treated with Doxorubicin (0.25  $\mu\text{g}/\text{ml}$ ) for the indicated time (0,15,30,60 min). After the medium was removed, the cells were loaded with probes DCFH-DA in the phenol red -free medium for 30 min. After a quick wash, cells were trypsinized and analyzed by flow cytometry.

### Dynamic imaging

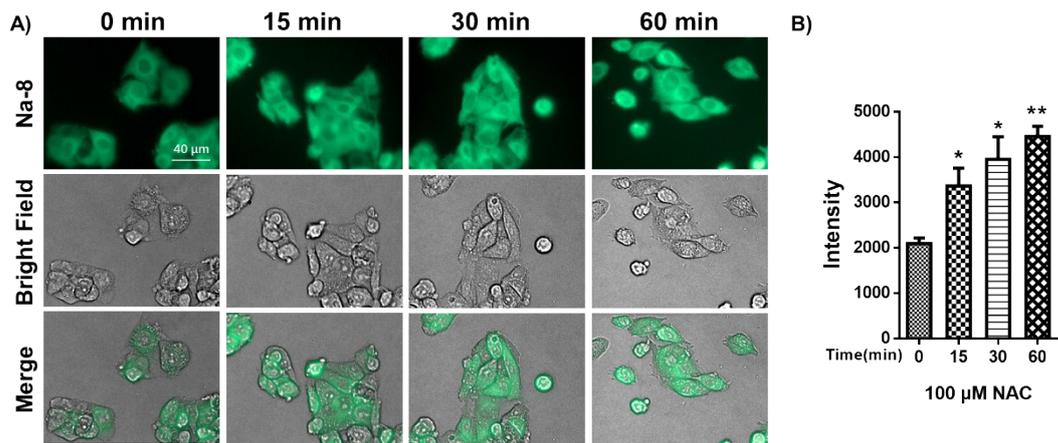
Cells (HepG2, 300000/well; L02, 240000/well) were seeded on a 35 mm dish with optically clear bottom (In vitro science) overnight. HepG2 and L02 cells were incubated with probe **Na-8** (20  $\mu\text{M}$ ) for 30 min, then Doxorubicin (0.25  $\mu\text{g}/\text{ml}$ ) was added to the culture medium. The cells was immediately imaged under confocal laser scanning microscopy (Leica TSC SP8) at the excitation wavelengths of 380-470 nm in time-series (Interval: 5 minutes). The dynamic fluorescence intensity of random 10 cells was measured by Image J. Data are presented as a densitometric ratio change compared with the moment the cells stimulated by compound.

### Statistical analysis

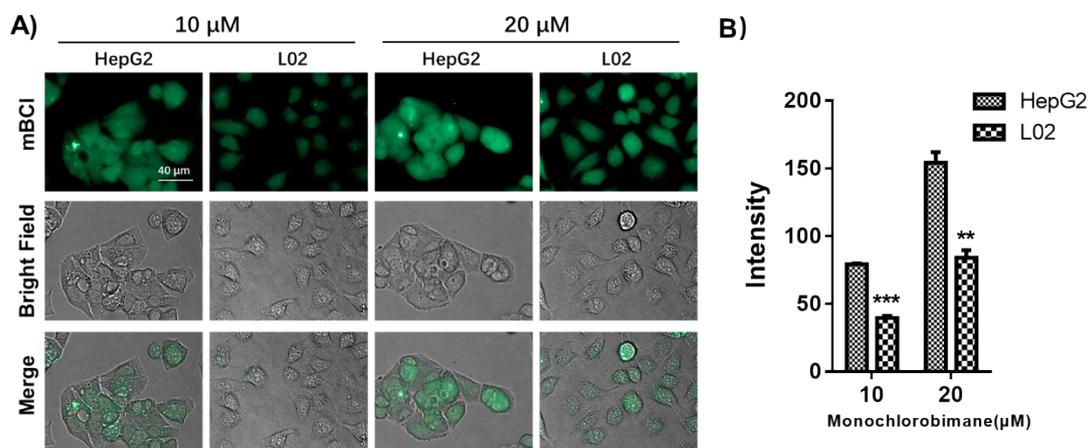
Unpaired T-test was performed to analyze the results using GraphPad Prism software. Results (Figure5-8) are presented as mean  $\pm$  S.D. Results in Figure 9 are presented as mean  $\pm$  SEM. Statistical significance was determined at  $P < 0.05$ (\*);  $P < 0.01$ (\*\*);  $P < 0.001$ (\*\*\*).



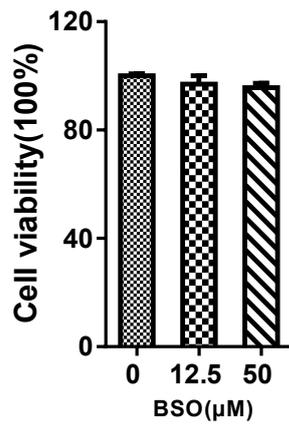
**Fig. S16** Probe **Na-8** had little effect on cell growth. (A-B) L02 (A) and HepG2 (B) cells were incubated with increasing concentrations of probe **Na-8** for 1 h, and then the medium was changed to fresh one. Cell viability was then tested after 72 h of incubation by MTS assay. (C-D) L02 (C) and HepG2 (D) cells were incubated with increasing concentrations of probe **Na-8** for 48 h, and then the medium was changed to fresh one. Cell viability was immediately tested by MTS assay.



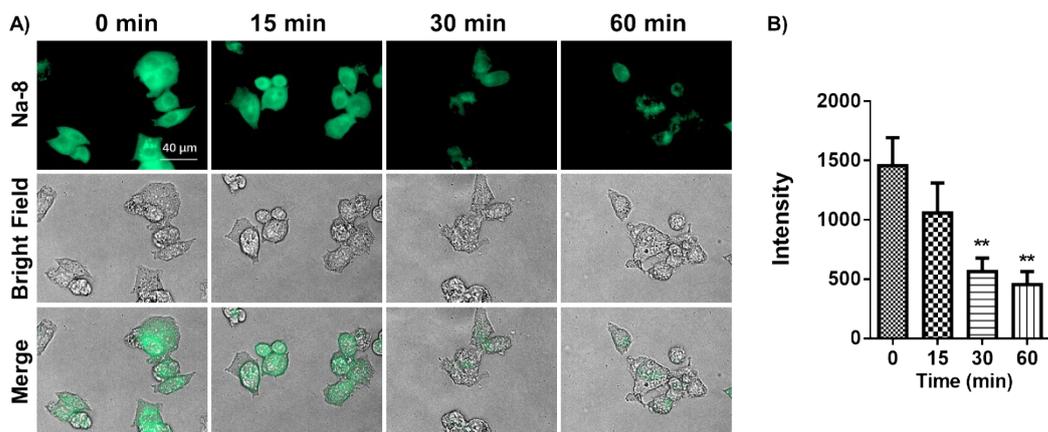
**Fig.S17 Characterization of GSH induced by NAC in hepatocellular carcinoma HepG2 cells.** A) Cells were pretreated with the NEM (50  $\mu$ M) for 15 min, then probe **Na-8** (10  $\mu$ M) was added to culture medium for another 15 min. After that cells were changed to the fresh culture medium containing probe **Na-8** (10  $\mu$ M), and NAC (100  $\mu$ M) was added to the medium at indicated time. At the same time end, all samples were imaged under the same condition. B) Quantified fluorescence intensities of cells as represented in panel (A).



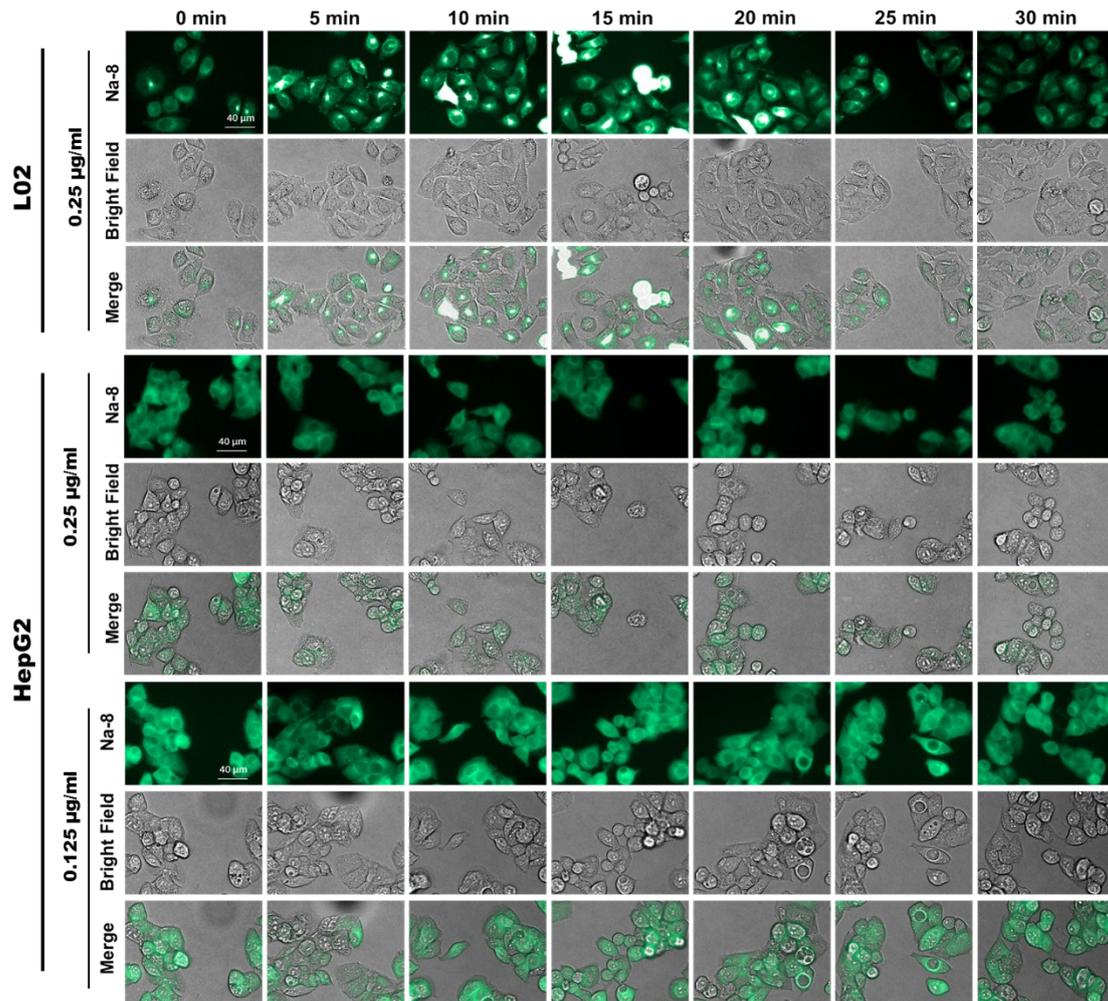
**Fig. S18 A)** Characterization of endogenous GSH by **Monochlorobimane** in HepG2 cells and its normal L02 counterparts. B) Quantified fluorescence intensities of cells as represented in panel (A).



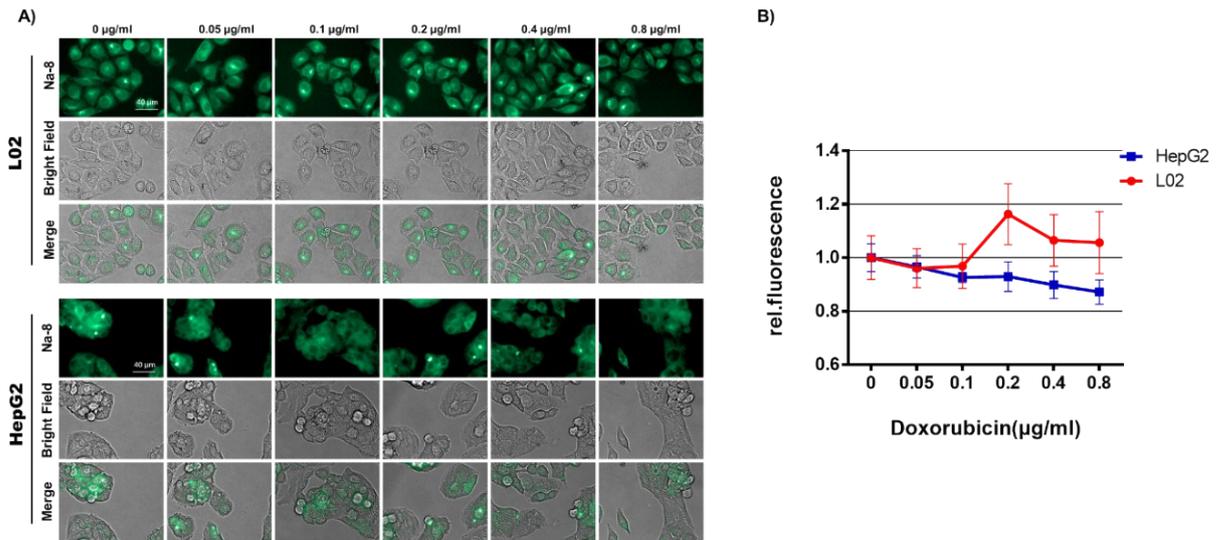
**Fig. S19** BSO had little effect on cell growth. Cells were exposed to increasing concentration of BSO for 48 h before MTS assay.



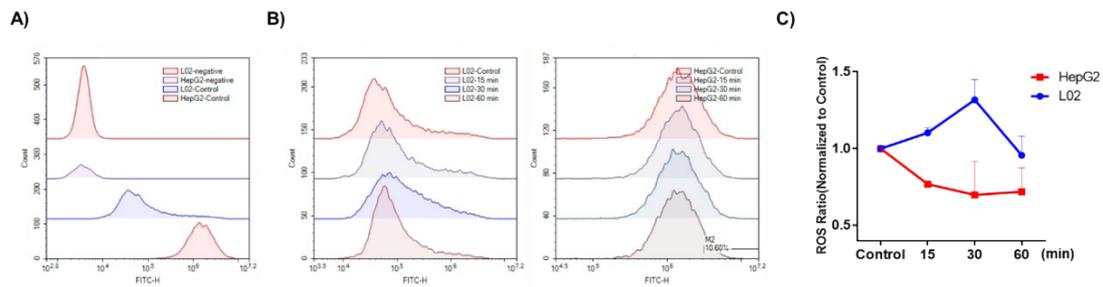
**Fig. S20 Na-8 fluorescence was attenuated by SIN-1.** A) HepG2 cells were loaded with probe Na-8 for 30 min, followed by the treatment of SIN-1 (500 μM) for indicated time, and then imaged. B) Quantified fluorescence intensities of cells as represented in panel (A).



**Fig. S21** Characterization of cellular GSH change by Na-8 (10 μM) in response to Doxorubicin. The images of the bright field and merge related to Fig. 9.



**Fig. S22 Characterization of cellular GSH change by Na-8 (10  $\mu$ M) in response to various doses of Doxorubicin.** A) HepG2 and L02 cells were loaded with probe Na-8 (10  $\mu$ M) for 30 min, followed by increasing concentration of Doxorubicin (0, 0.05, 0.1, 0.2, 0.4, 0.8  $\mu$ g/ml) incubation for 15 min, then imaged. B) The dynamic quantified fluorescence intensities of cells as represented in panel (A). Data are presented as a densitometric ratio change compared with the cells without Doxorubicin treatment.



**Fig. S23 ROS Detection in HepG2 and L02 cells before and after the treatment of Doxorubicin.** ROS level was detected by flow cytometry after DCFH-DA-staining. A) HepG2 cells bear a higher ROS oxidative stress than L02 cells at basal level. B) HepG2 and L02 cells were incubated with Doxorubicin (0.25  $\mu$ g/ml), sampled and detected by flow cytometry at the indicated time point. C) Results represented in panel (B) were normalized to the control, respectively.