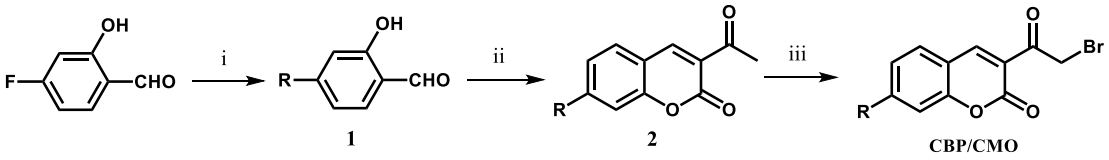
Supporting Information

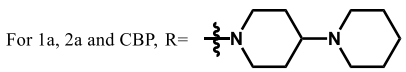
A coumarin-based biosensor for human hepatocellular carcinoma diagnosis with enhanced brightness and water solubility

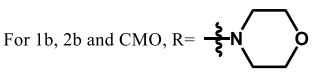
General information.

Unless otherwise noted, chemicals and solvents were purchased and used without further purification. Purification of products was conducted by column chromatography on silica gel (200-300 mesh). NMR spectra were measured on a Bruker ARX400 (^{1}H at 400 MHz, ^{13}C at 101 MHz) magnetic resonance spectrometer. Chemical shifts (δ) are reported in ppm using tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, m = multiplet), and coupling constants (*J*) were reported in Hertz (Hz). HRMS data were obtained on a VG ZAB-HS mass spectrometer, Brucker Apex IV FTMS spectrometer. UV-visible spectra were measured on a Quawell UV/Vis spectrophotometer (Q5000) by using droplet measurement with a sample volume of 10 μL. Fluorescence spectra were acquired on a HITACHI F-4600 fluorescence spectrophotometer using fluorescence cuvettes (Fisher Scientific) with a sample volume of 1.5 mL. MTT data were collected from micro plate spectrophotometer (Thermo Scientific Fluoroskan). The confocal fluorescence images were taken with a Leica TCS SP8 fluorescence microscope. The human colon cancer cell line (Hela) is obtained from ATCC. Human hepatocellular carcinoma tissues were obtained from the Fourth Affiliated Hospital of Henan University of Science and Technology. Tissue slices were prepared using a Leica CM1950 slicer. The Ethics Committee of Anyang Normal University and Fourth Affiliated Hospital of Henan University of Science and Technology approved this study. All surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee in Fourth Affiliated Hospital of Henan University of Science and Technology, Anyang, China.

General Procedure:







1a and **1b** were obtained by following previously reported procedure[1-7].

(**2a**):4-([1,4'-bipiperidin]-1'-yl)-2-hydroxybenzaldehyde (**1a**) (1.23 g, 4.27 mmol), ethyl

acetoacetate (0.61 g, 4.70 mmol) and 0.1 mL piperidine and 0.2 mL acetic acid were dissolved in 20 mL absolute ethanol. Then, the mixture was refluxed under nitrogen overnight. After cooling to room temperature, the bright orange precipitate was purified by silica gel column chromatography to afford compound **2a.** (1.07 g, 3.05 mmol). Yield: 71.4%.

(**CBP**): A solution of CuBr (0.44 g, 2.0 mmol) in ethanol (12 mL) was added dropwise to a solution of compound **2a** (0.354 g, 1.0 mmol) in ethanol (15 mL), the mixture was stirred vigorously at 80 °C under nitrogen. After overnight reaction, the mixture was cooled to room temperature. The resulting precipitate was filtered and purified by silica gel column chromatography to afford probe **CBP** (0.116 g, 32.8% yield) as a brown solid.

4-([1,4'-bipiperidin]-1'-yl)-2-hydroxybenzaldehyde (**1a**)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.51 (s, 1H), 9.54 (s, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 6.44 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 3.99 (dt, *J* = 13.3, 2.7 Hz, 2H), 3.02 – 2.85 (m, 2H), 2.51 (t, *J* = 5.4 Hz, 4H), 1.91 (dt, *J* = 13.3, 2.7 Hz, 2H), 1.63 – 1.55 (m, 5H), 1.44 (dd, *J* = 7.7, 4.1 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 192.47, 164.30, 156.20, 135.23, 112.37, 106.15, 99.21, 62.35, 50.25, 46.87, 27.52, 26.32, 24.71. HRMS (ESI): calcd for $C17H25N2O2^{+}$ $[M+H]^{+}$: 289.1911; found: 289.1913.

7-([1,4'-bipiperidin]-1'-yl)-3-acetyl-2H-chromen-2-one (**2a**)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.43 (s, 1H), 7.46 – 7.37 (m, 1H), 6.80 (d, *J* = 9.0 Hz, 1H), 6.65 (s, 1H), 4.00 (d, *J* = 13.2 Hz, 2H), 3.00 (t, *J* = 12.7 Hz, 2H), 2.69 (s, 3H), 2.52 (s, 5H), 1.96 (d, *J* = 12.9 Hz, 2H), 1.67 (s, 1H), 1.60 (q, *J* = 9.1, 6.1 Hz, 6H), 1.49 – 1.41 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 195.75, 160.61, 158.51, 154.91, 147.69, 131.65, 117.61, 111.64, 109.21, 98.99, 62.13, 50.30, 47.07, 30.63, 27.52, 26.27, 24.65. HRMS (ESI): calcd for $C21H27N2O3^{+}$ $[M+H]^{+}$: 355.2016; found: 355.2019.

7-([1,4'-bipiperidin]-1'-yl)-3-(2-bromoacetyl)-2H-chromen-2-one (**CBP**)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.53 (s, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 6.82 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.64 (d, *J* = 2.4 Hz, 1H), 4.76 (s, 2H), 4.04 (d, *J* = 13.1 Hz, 2H), 3.09 – 3.01 (m, 2H), 2.60 (s, 4H), 2.07 – 2.00 (m, 2H), 1.72 – 1.61 (m, 7H), 1.48 (s, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 188.63, 158.55, 155.47, 150.47, 149.47, 131.99, 131.63, 129.36, 120.58, 116.27, 115.34, 111.72, 109.77, 99.10, 48.75, 46.85, 36.60, 30.65. HRMS (ESI): calcd for $C21H25BrN2O3^{+}$ $[M+H]^{+}$: 433.1121; found: 433.1126.

2-hydroxy-4-morpholino-benzaldehyde (**1b**)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.48 (s, 1H), 9.61 (s, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 6.47 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.29 (d, *J* = 2.4 Hz, 1H), 3.87 – 3.81 (m, 4H), 3.40 – 3.35 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 193.12, 164.15, 156.86, 135.20, 113.31, 106.03, 99.70, 66.45, 46.96. HRMS (ESI): calcd for $C11H13NO3^{+}$ $[M+H]^{+}$: 208.0968; found: 208.0963.

3-acetyl-7-morpholino-2H-chromen -2-one (**2b**)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.45 (s, 1H), 7.48 (d, *J* = 8.9 Hz, 1H), 6.83 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.68 (d, *J* = 2.4 Hz, 1H), 3.90 – 3.85 (m, 4H), 3.43 – 3.38 (m, 4H), 2.70 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 195.68, 160.35, 158.16, 155.44, 147.69, 131.55, 118.72, 111.43, 110.10, 99.37,

66.32, 47.06, 30.63. HRMS (ESI): calcd for $C_{15}H_{16}NO_4^+$ $[M+H]^+$: 274.1074; found: 274.1071.

3-(2-bromoacetyl)-7- morpholino-2H-chromen-2-one (**CMO**)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.57 (s, 1H), 7.51 (d, *J* = 9.0 Hz, 1H), 6.85 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.68 (d, *J* = 2.4 Hz, 1H), 4.77 (s, 2H), 3.91 – 3.86 (m, 5H), 3.44 (t, *J* = 5.0 Hz, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 188.67, 160.09, 158.45, 155.86, 149.52, 131.94, 115.74, 111.57, 110.06, 99.17, 66.29, 46.97, 36.57. HRMS (ESI): calcd for $C_{15}H_{15}BrNO_4^+$ $[M+H]^+$: 352.0179; found: 352.0181.

ImageJ pixel quantification

For the quantification of fluorescence images, software ImageJ was used. First, draw a region of interest (ROI) around the object with one of the drawing tools (in the toolbar) and then Analyze - Measure will limit its measurement to that area. Use Edit - Selection - Restore Selection to copy/paste that area onto another image to analyze the same size/shape area in another image. Check the boxes next to the information you want. You can get information on area, diameter, perimeter and other factors as well as information about intensity. (ImageJ is a Java based application for analyzing images. It's free and available at https://imagej.nih.gov/ij)

References

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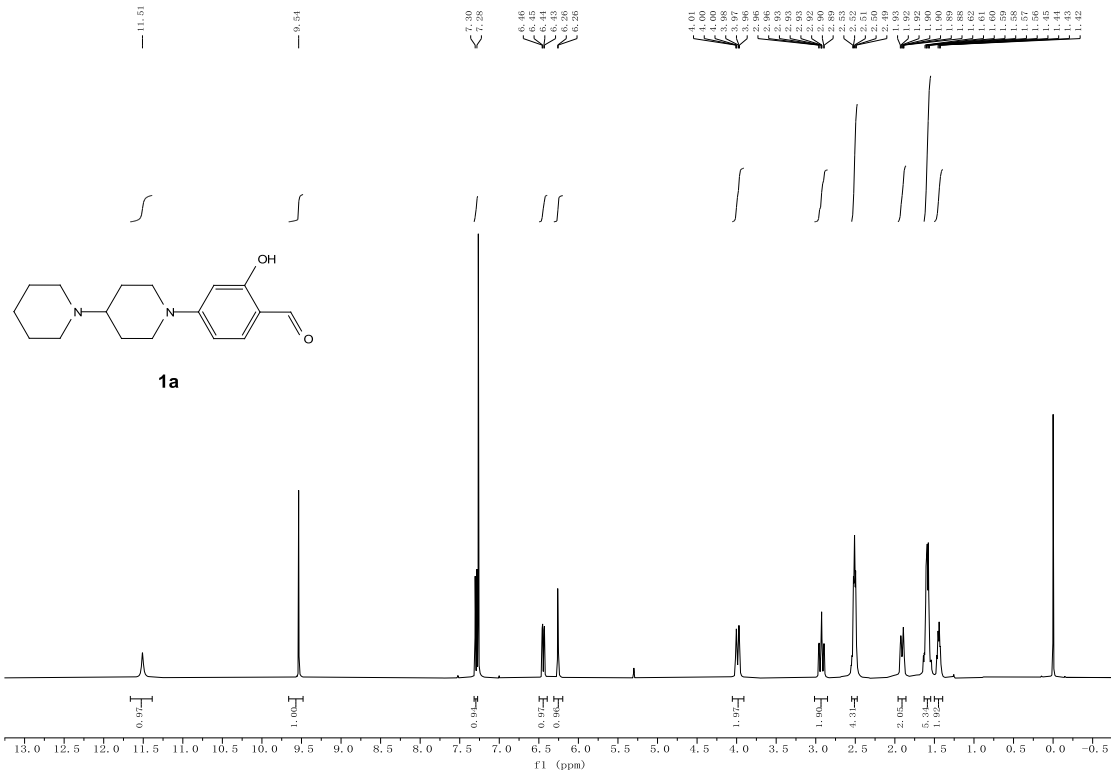


Figure S1. Proton NMR of compound **1a**

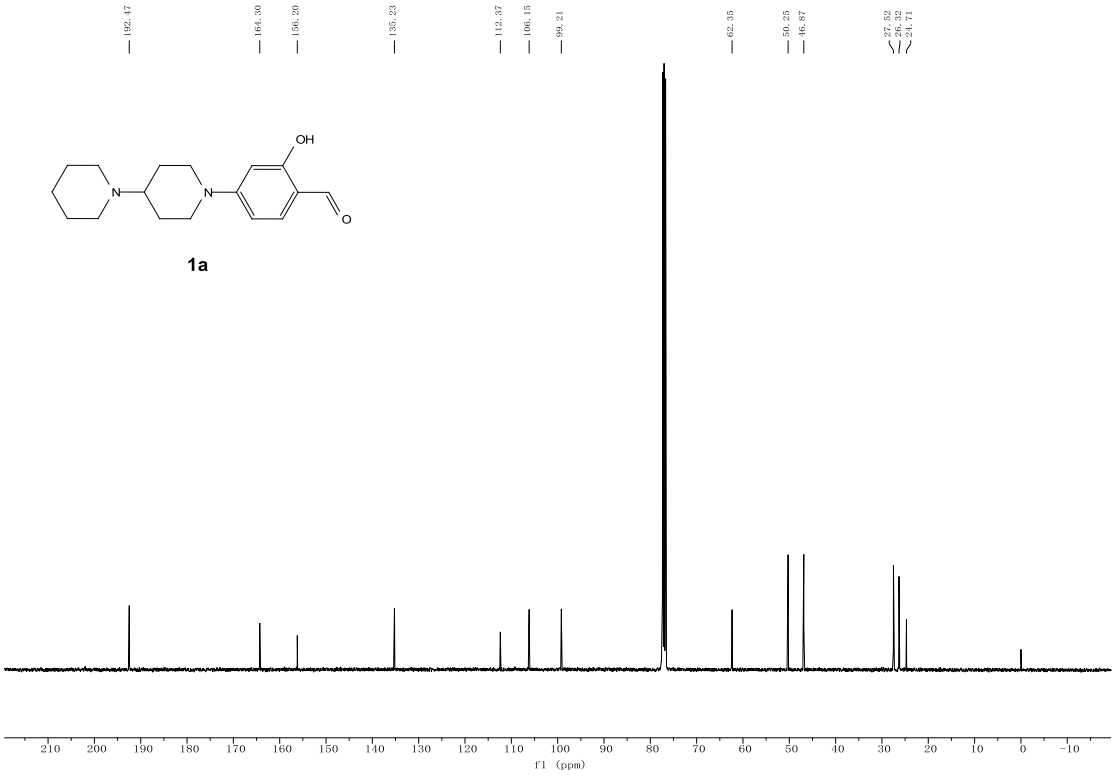


Figure S2. Carbon NMR of compound **1a.**

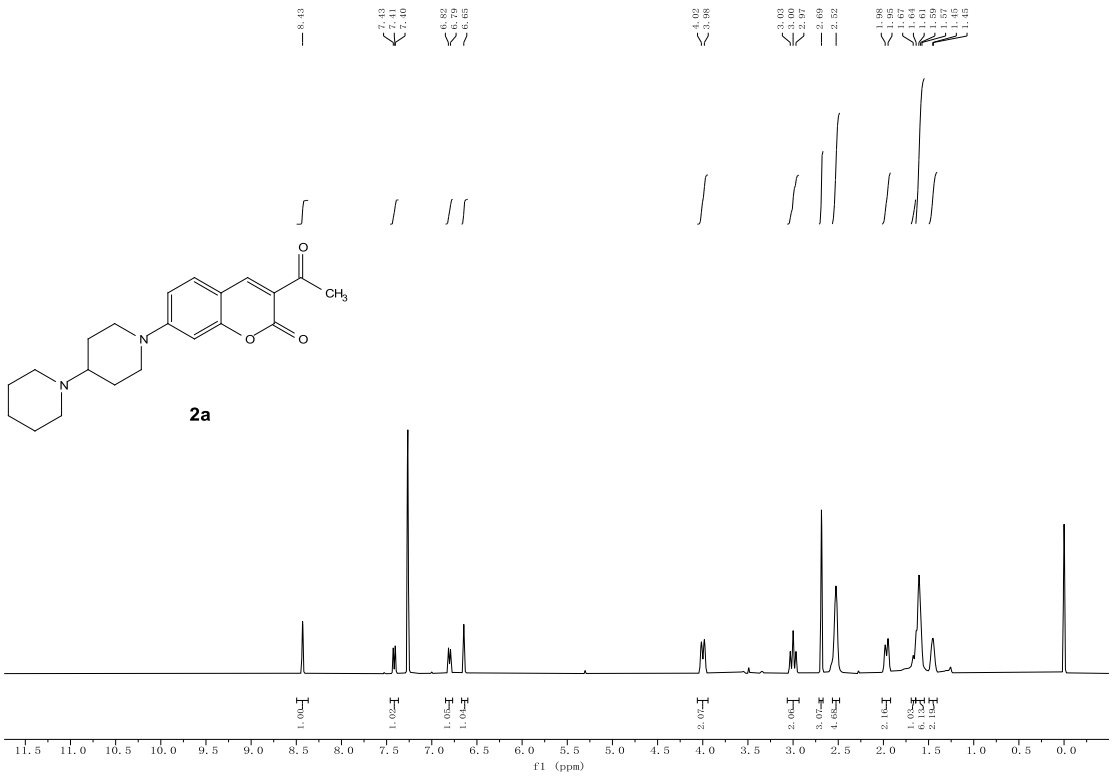


Figure S3. Proton NMR of compound **2a**

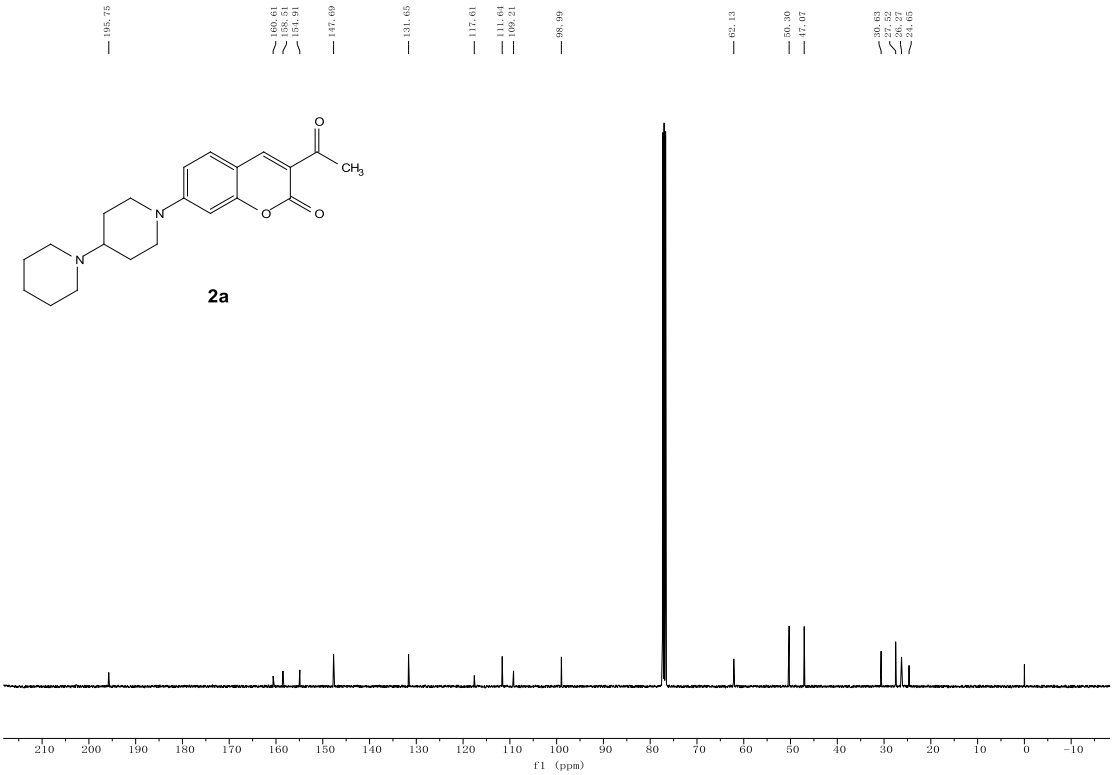


Figure S4. Carbon NMR of compound **2a.**

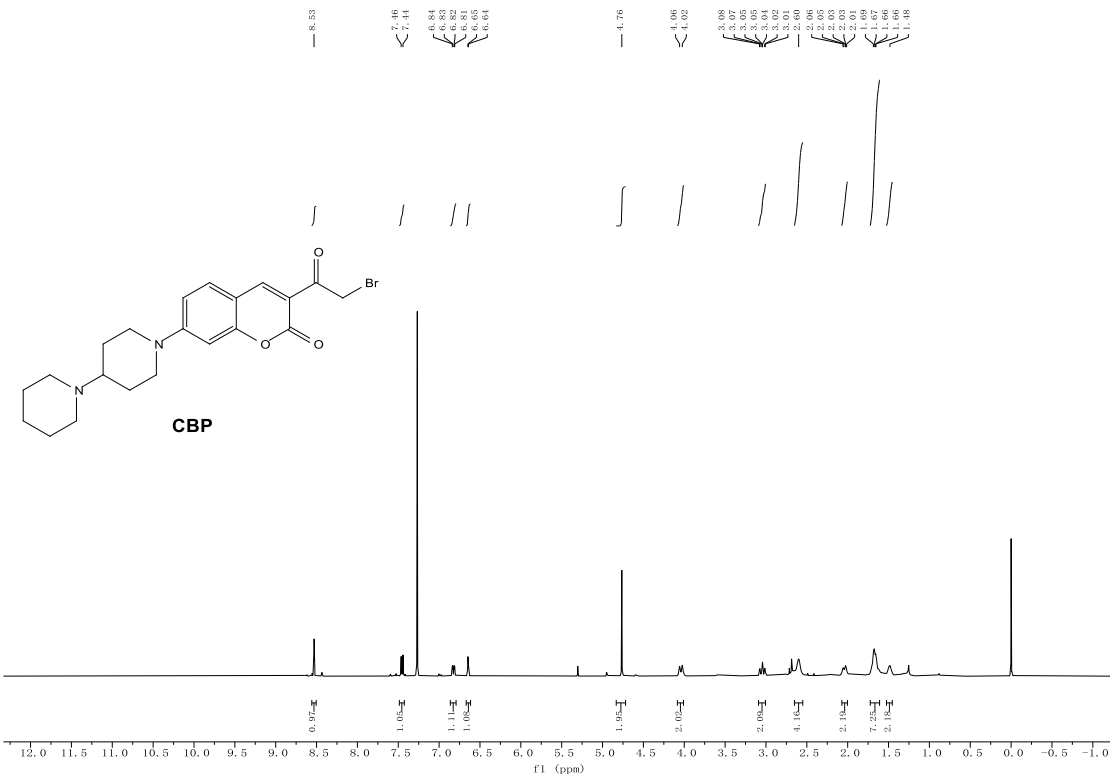


Figure S5. Proton NMR of compound **CBP**

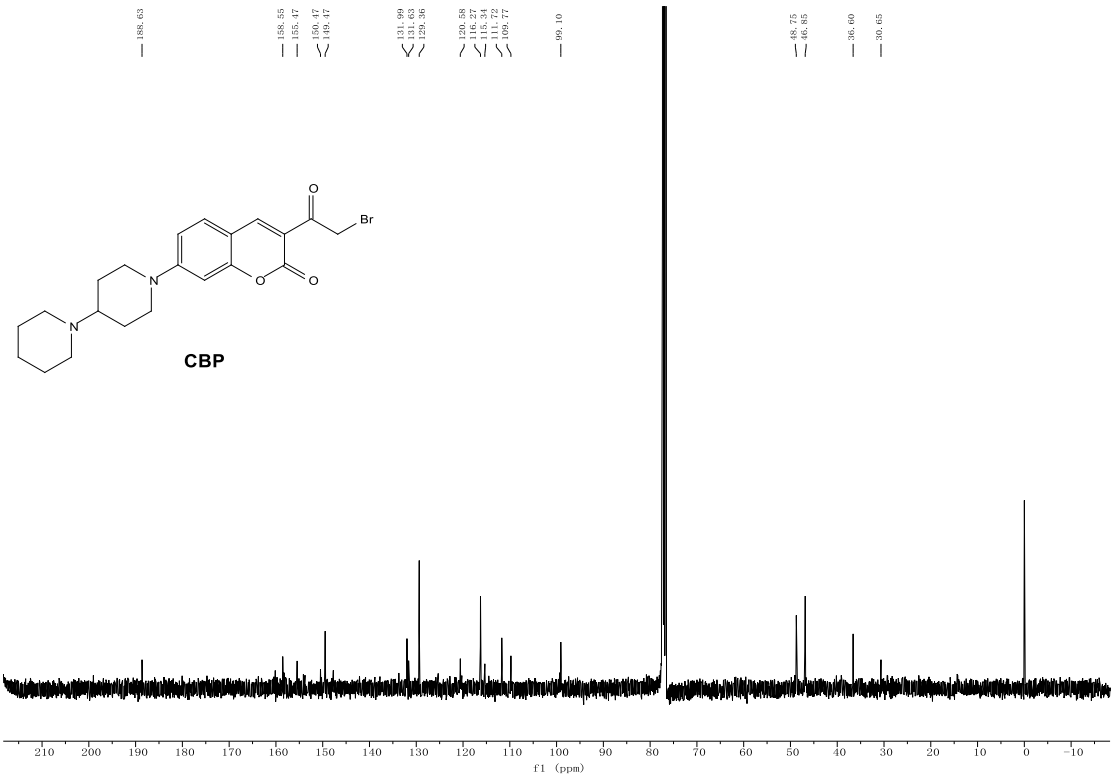


Figure S6. Carbon NMR of compound **CBP**

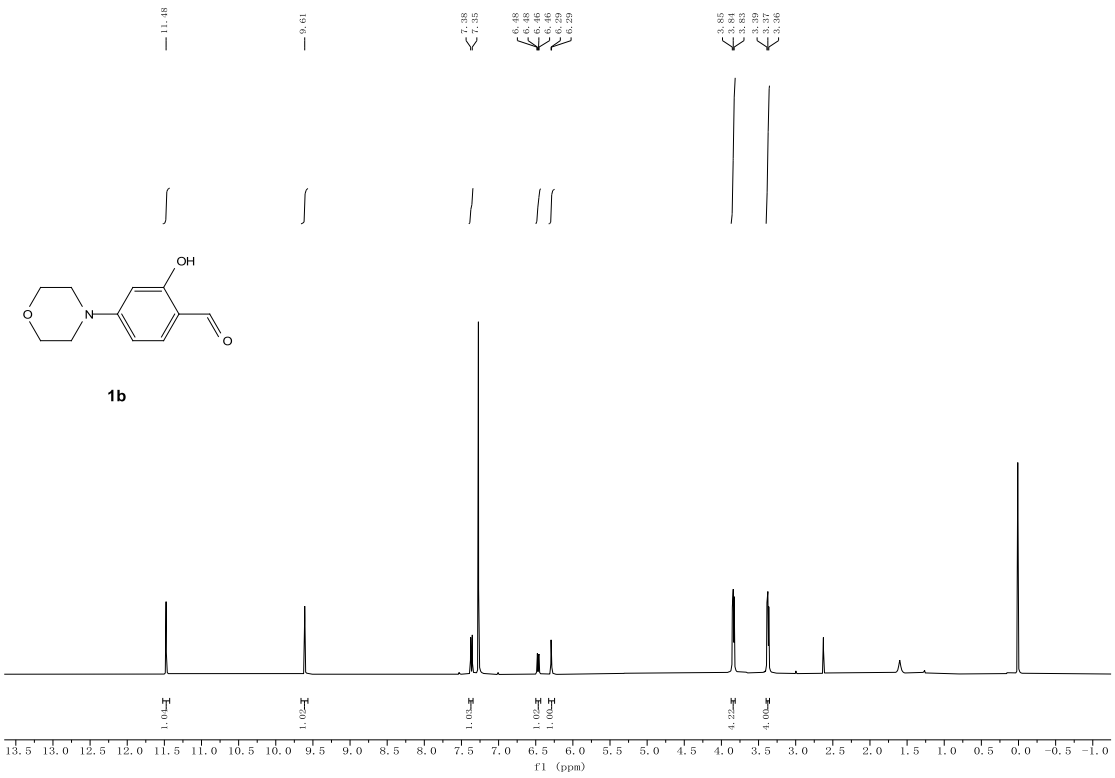


Figure S7. Proton NMR of compound **1b**.

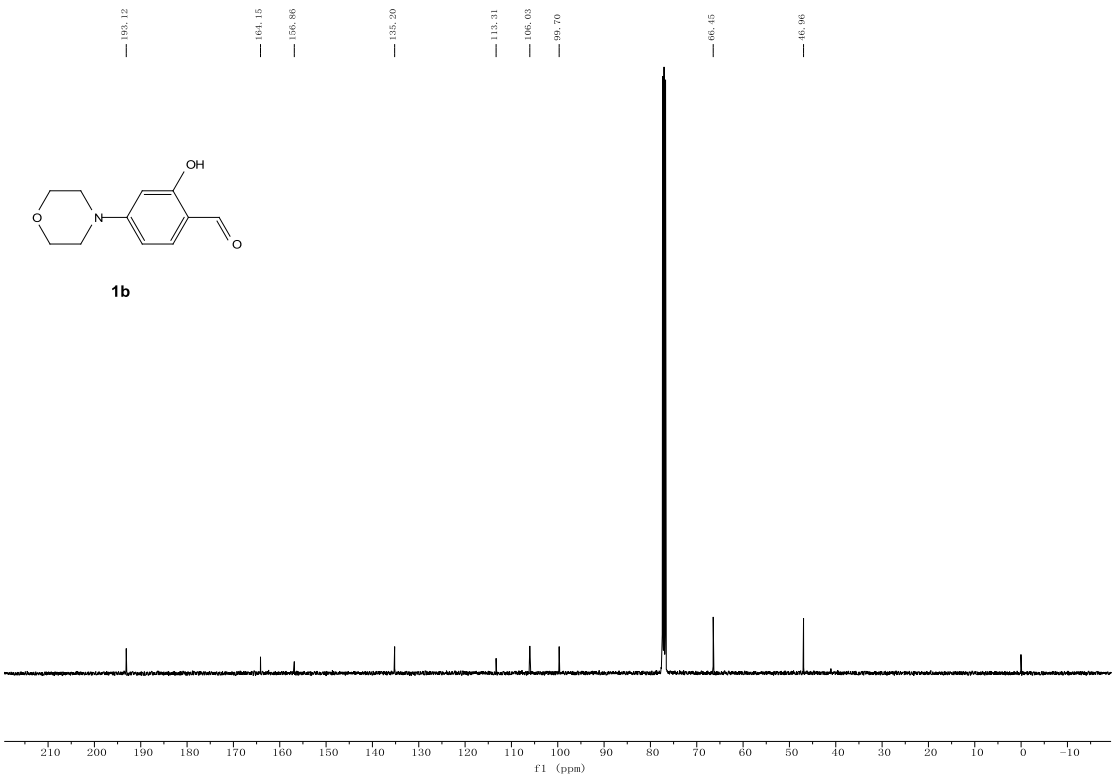


Figure S8. Carbon NMR of compound **1b**.

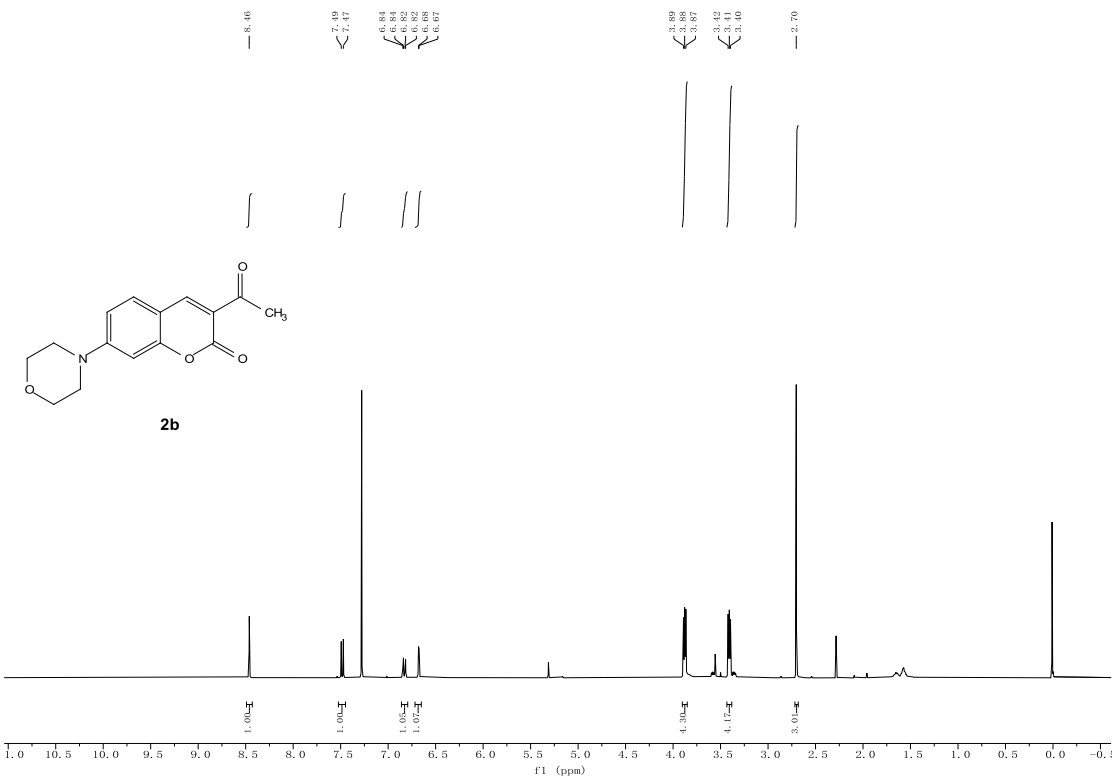


Figure S9. Proton NMR of compound **2b**.

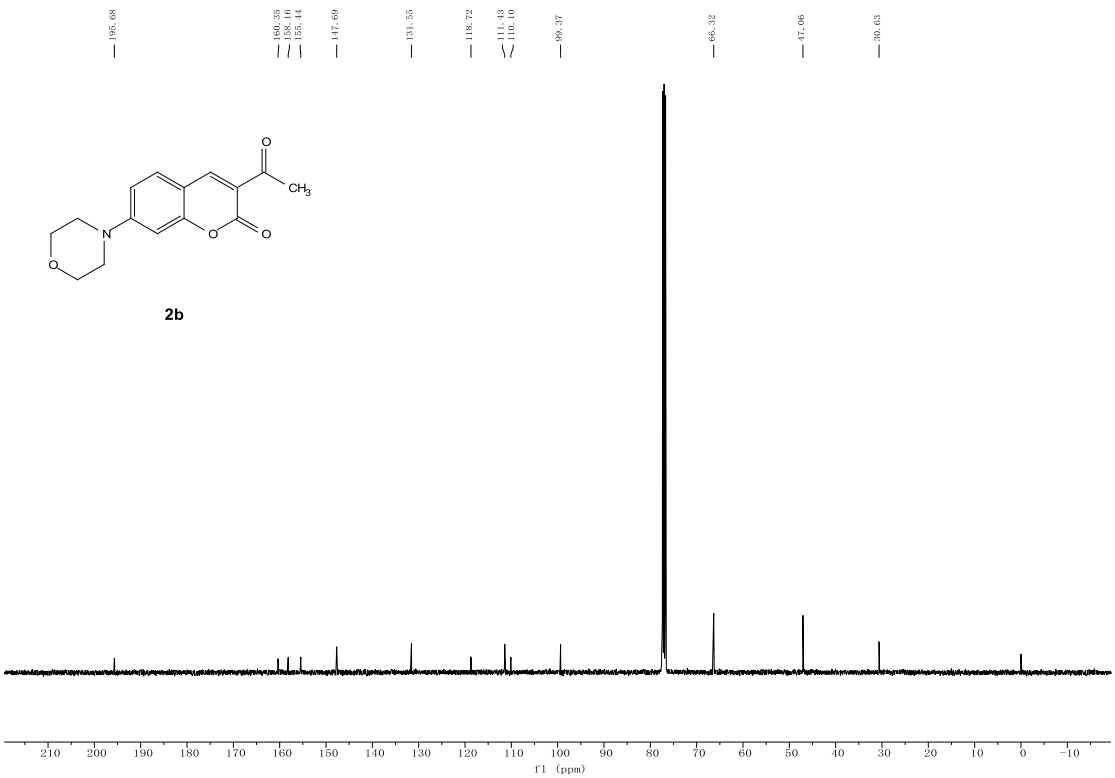


Figure S10. Carbon NMR of compound **2b**.

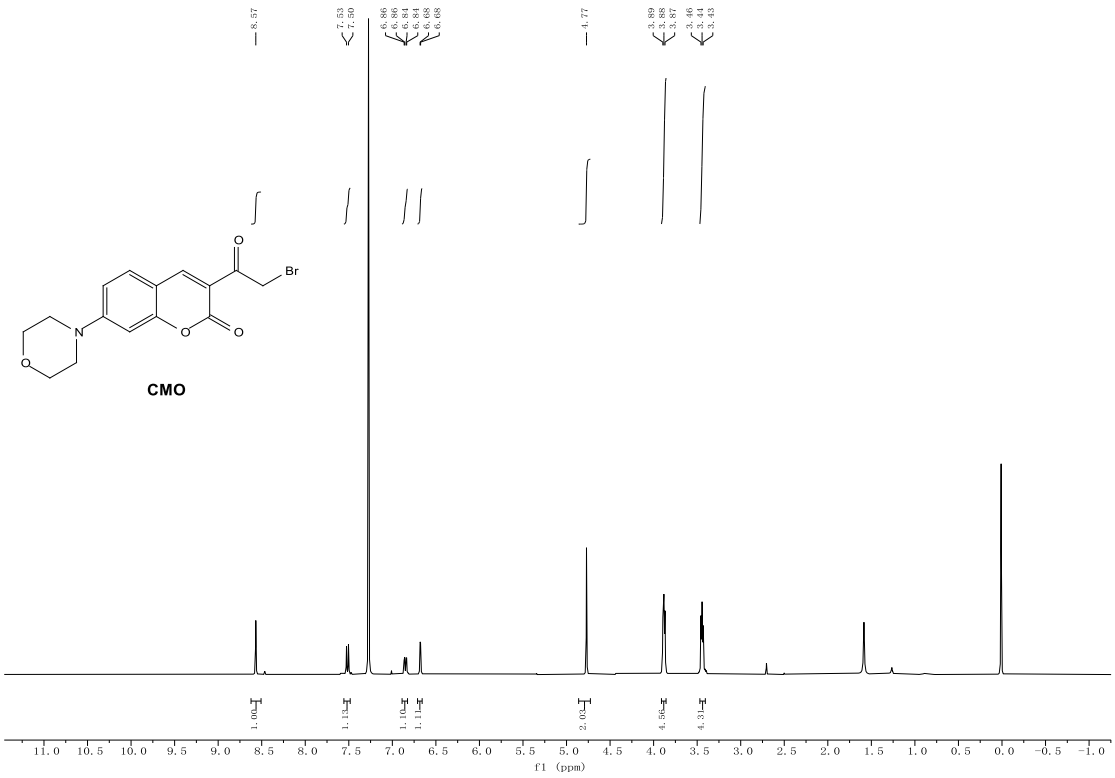


Figure S11. Proton NMR of compound **CMO**

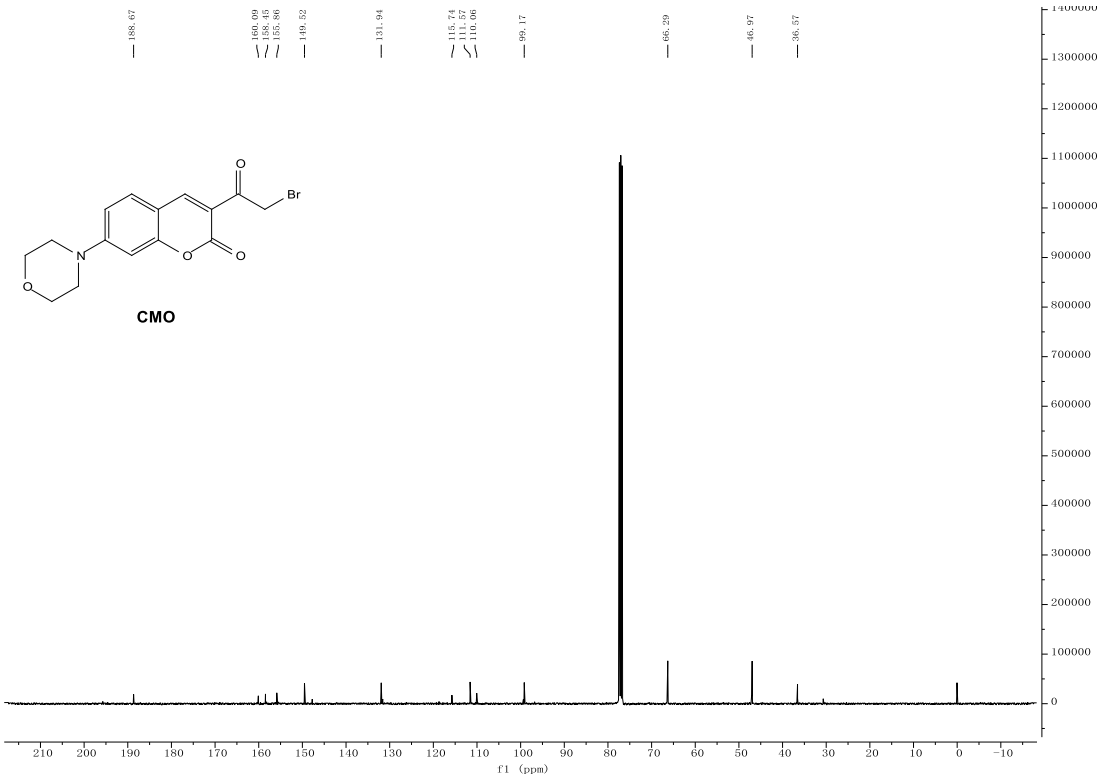


Figure S12. Carbon NMR of compound **CMO**

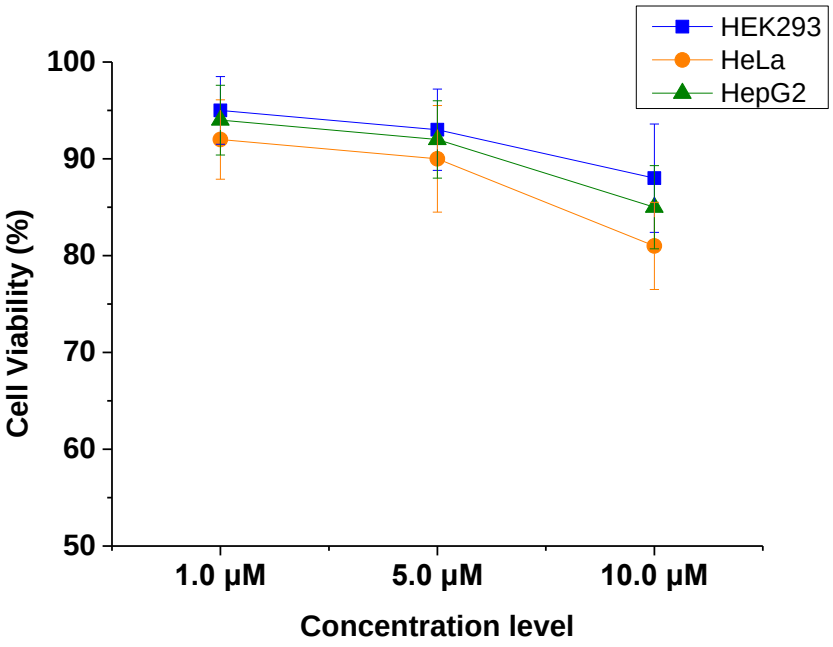


Figure S13. Cell viability with HEK293, Hela and HepG2 cell lines. Cell lines were treated with different concentrations (1-10 μM) of probe for 24 hrs as described in experimental protocols. Cell viability was measured employing MTT assay for three times.

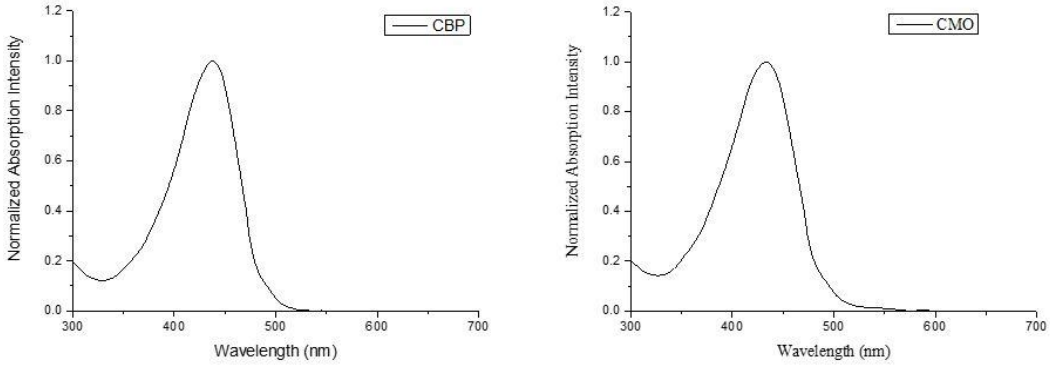


Figure S14. Normalized absorption spectra of **CBP** and **CMO**.

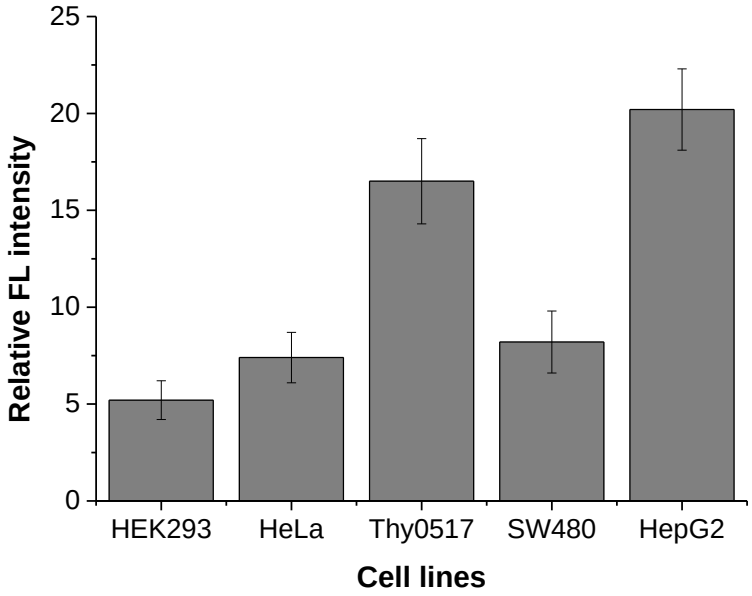


Figure S15. Probe selectivity towards GSH within different cell lines. Each cell line was treated with probe CBP (5 μM) for 30 min. Results are presented as means ± SE.

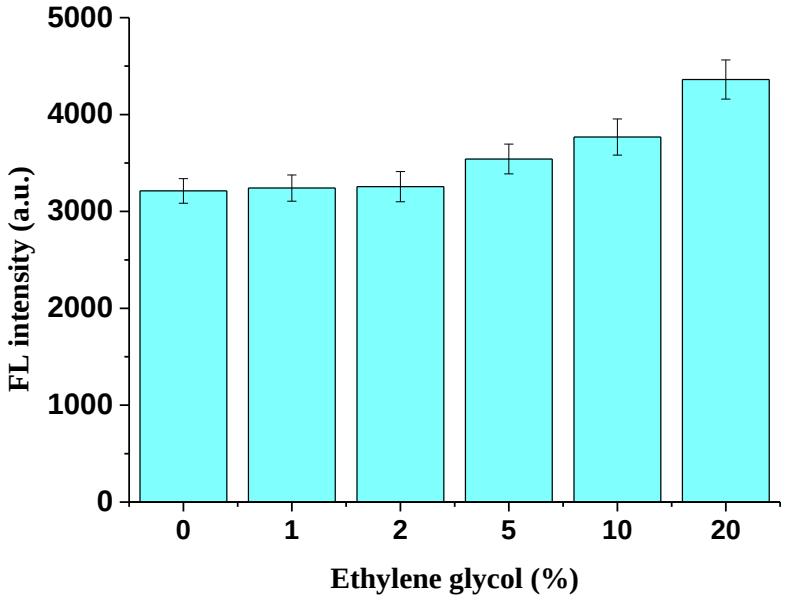
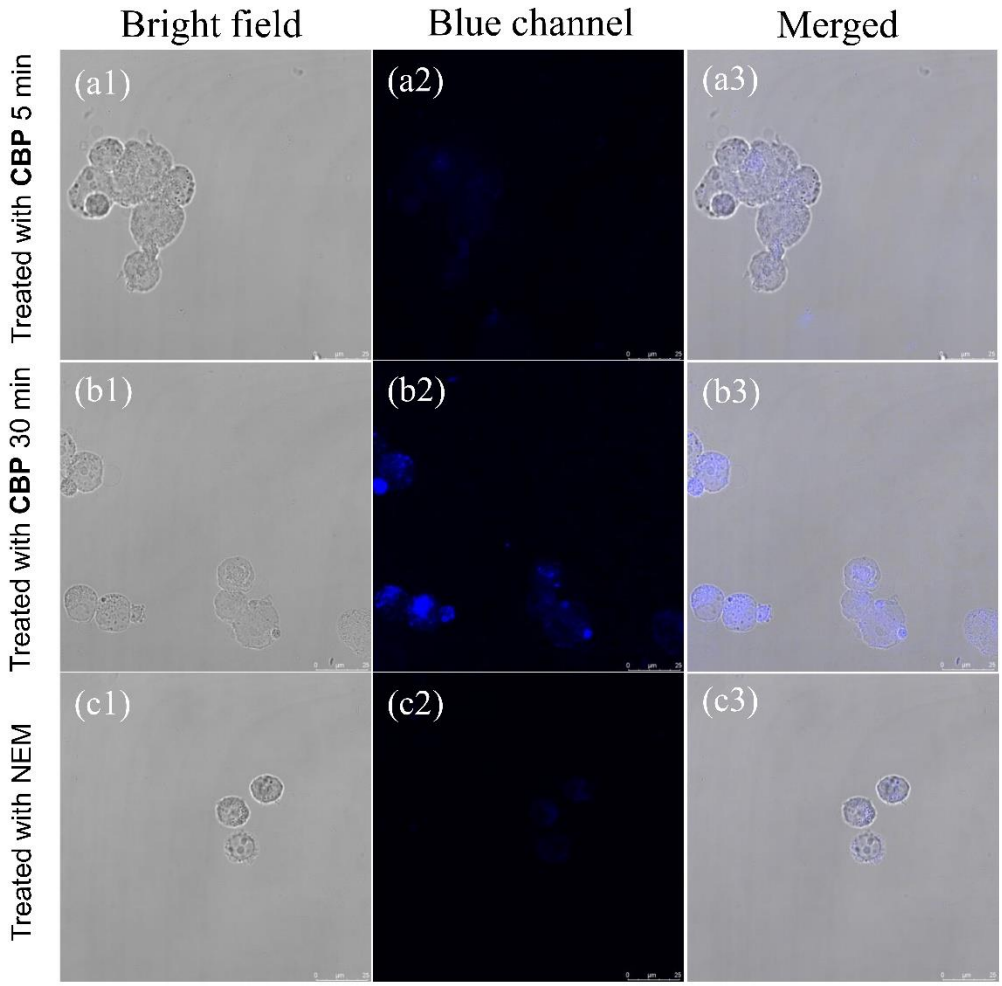


Figure S16. Fluorescence intensity of probe **CBP** (5 μM) in a different fraction of ethylene glycol from 0 to 20%.



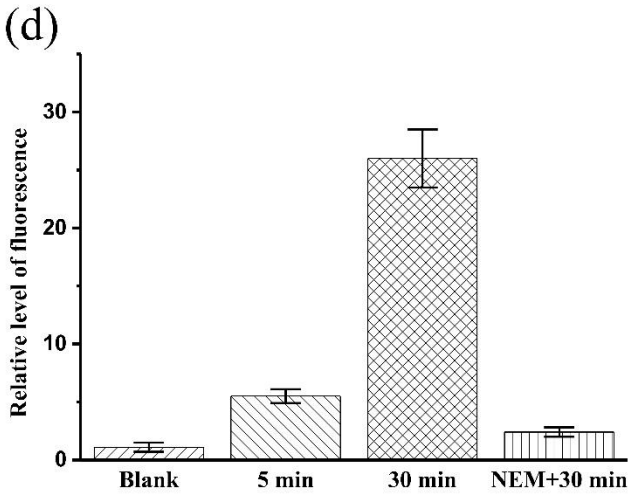


Figure S17. Confocal fluorescence images for the detection of GSH in Hela cell lines. (a 1-3). Images of Hela cells pretreated with 100 μM GSH, followed by treating with **CBP** (10 μM) for 5 min. (b1-3) Images of Hela cells pretreated with 100 μM GSH, followed by treating with **CBP** (10 μM) for 30 min. (c1-3) image of Hela cells pretreat ed with 20 mM NEM for 30 min and then added **CBP** (10 μM) and incubated for 30 min. (d). Fluorescence intensity quantitation. Images were obtained using 405 nm excitation and emission channels of 490-520 nm (Leica TCS SP8). Scale bar = 25 μm. Results are presented as means ± SE.