

Electronic Supplementary Information (ESI) for

# Quinolone-based fluorescent probe for distinguishing detection of Cys and GSH through different fluorescence channels

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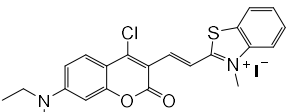
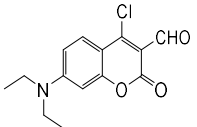
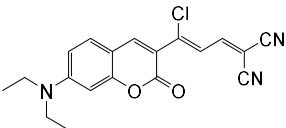
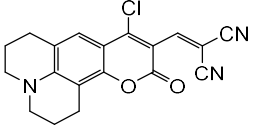
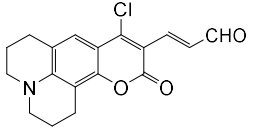
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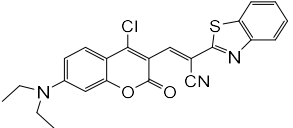
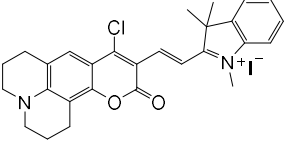
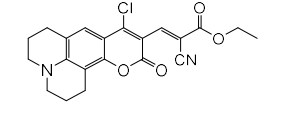
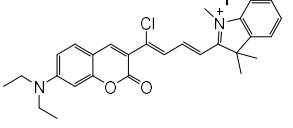
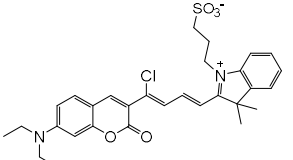
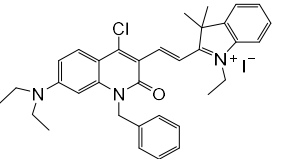
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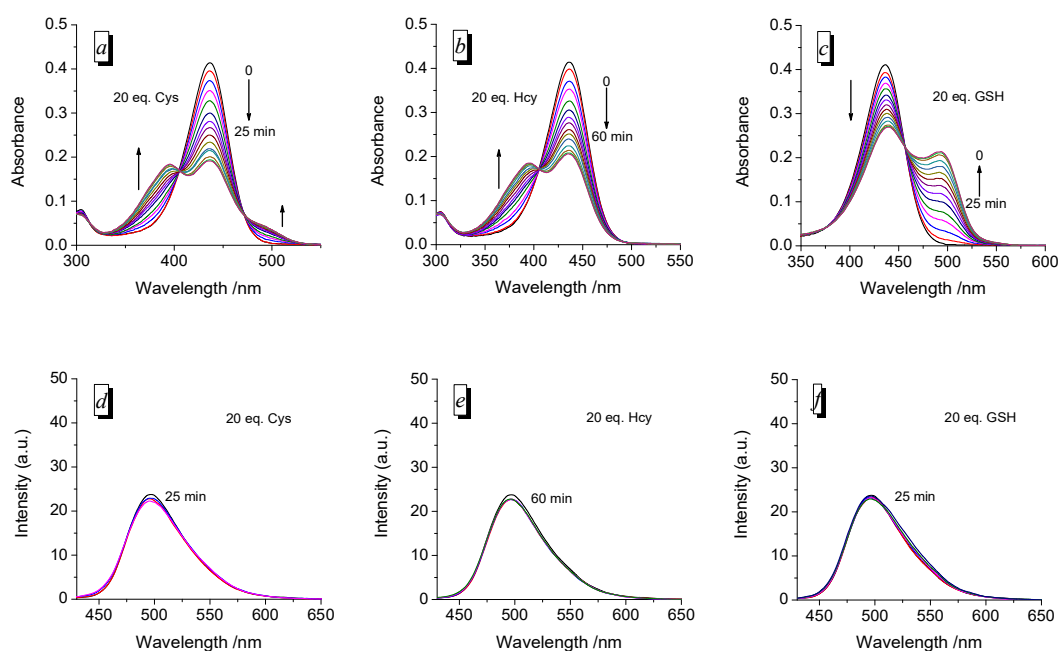
**Table S1.** Summary of coumarin-based fluorescent probes for biothiols

Probes	Testing conditions	Absorption for probes $\lambda_{\max}$ /nm	Distinguishing detection	Emission wavelength /nm	Response time /min	LOD / $\mu$ M	References
	PBS buffer (10 mM, pH 7.4, containing 1 Mm CTAB	535	Cys GSH	420 512	60 60	/ 0.05	J. Am. Chem. Soc. 2014, 136, 574-577
	PBS buffer solution (pH 7.4)	457	Cys Hcy GSH	470 470 546	34 60 40	0.18 0.09 0.08	Anal. Chim. Acta 2015, 900, 103-110
	DMSO/PBS buffer (V/V: 1/1, 10 mM, pH 7.4)	448	GSH	505	7	0.30	Sens. Actuators, B 2017, 253, 42-49
	PBS (pH 7.4, 10 mM, containing 30% acetonitrile	538	Cys GSH	500 560	60 60	0.19 0.43	Biosens. Bioelectron. 2017, 90, 117-124
	PBS (pH 7.4, 10 mM, containing 20% DMSO	500	Cys Hcy	480 542	60 60	1.99 0.61	Chem. Asian J. 2017, 12, 2098-2103

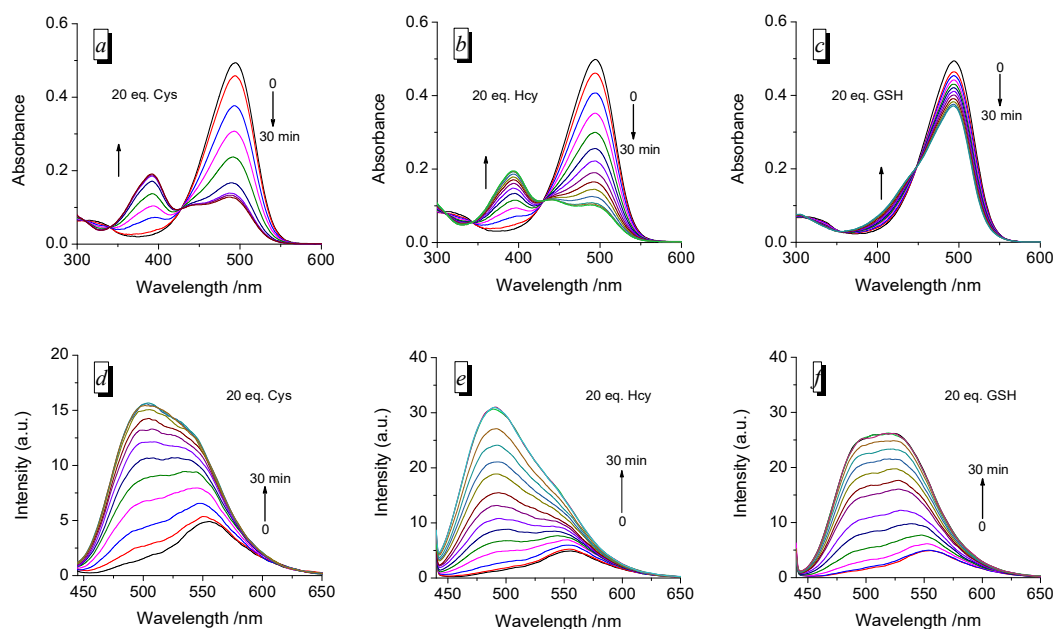
	DMSO/PBS (pH 7.4, 10 mM, v/v, 6/4)	500	Cys Hcy GSH	457 559 529	4 15 5	0.0005 0.0036 0.0069	Angew. Chem. Int. Ed. 2018, 57, 4991-4994
	PBS (pH 7.4, 10 mM, containing 1% DMSO)	614	Cys Hcy GSH	475 475 575	25 55 50	0.014 0.081 0.097	New J. Chem., 2018, 42, 12615-12620
	DMSO-PBS (pH 7.4, 10 mM, v/v, 4/6).	527	Cys Hcy GSH	503 467 568	15 15 15	0.0002 0.0007 0.001	Angew. Chem. Int. Ed. 2019, 58, 4557-4561
	PBS (pH 7.4, 10 mM)/CH <sub>3</sub> OH = 1600/400 (v/v)	578	Cys Hcy GSH	489,564,600 489,564,600 489,564,600	60 90 80	2.965 6.140 6.847	Anal. Chem. 2019, 91, 1472-1478
	Phosphate-buffered saline (pH 7.4, 10 mM).	586	Cys Hcy GSH	498,573,612 498,573,612 498,573,612	60 90 70	2.2 2.08 1.89	J. Mater. Chem. B, 2019, 7, 7723-7728
	10 M PBS buffer pH 7.4, 40% CH <sub>3</sub> CN	573	Cys GSH	420 537	25 40	0.17 0.46	This work

“/” No available data.

## 2. Spectral response of QB-1 and QB-2 toward thiols

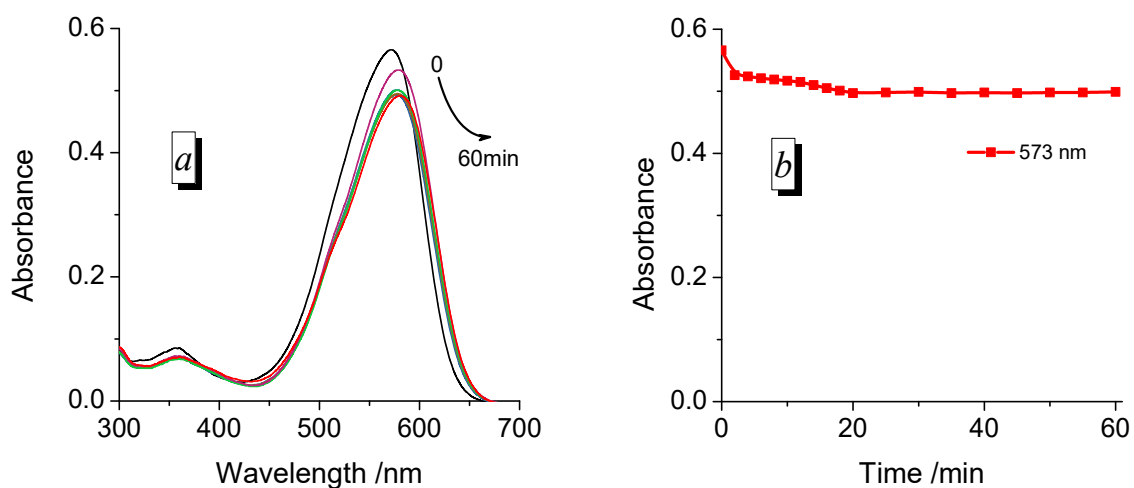


**Figure S1.** Time-dependent absorption spectra (a-c) and fluorescence spectra (d-f) of QB-1 (10 μM) in the presence of 20 equiv of Cys (a, d), Hcy (b, e) and GSH (c, f) in PBS-CH<sub>3</sub>CN (v/v, 3:2, 10 mM, pH 7.4), excited at 405 nm.

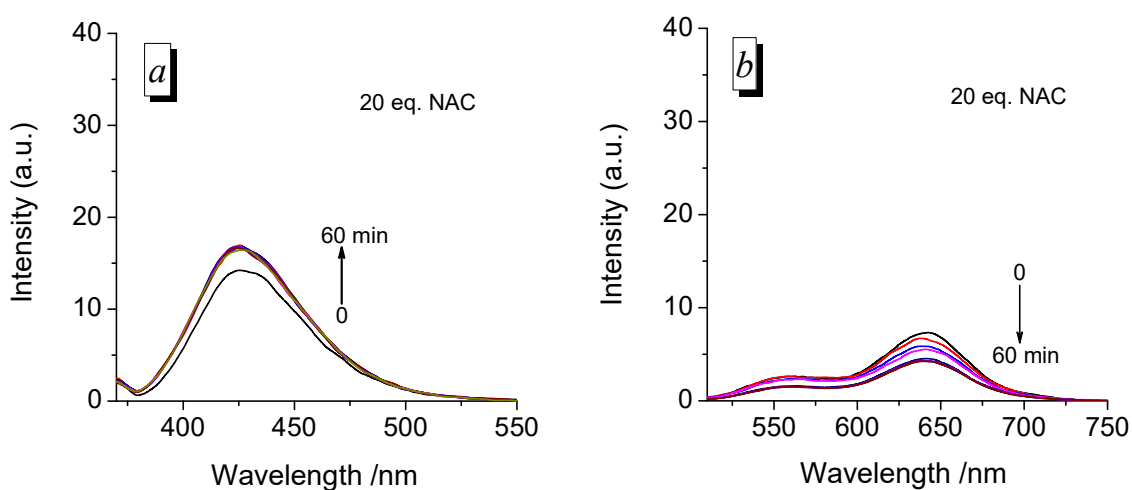


**Figure S2.** Time-dependent absorption spectra (a-c) and fluorescence spectra (d-f) of QB-2 (10 μM) in the presence of 20 equiv of Cys (a, d), Hcy (b, e) and GSH (c, f) in PBS-CH<sub>3</sub>CN (v/v, 3:2, 10 mM, pH 7.4), excited at 430 nm.

### 3. Spectral response of QB-3 toward NAC

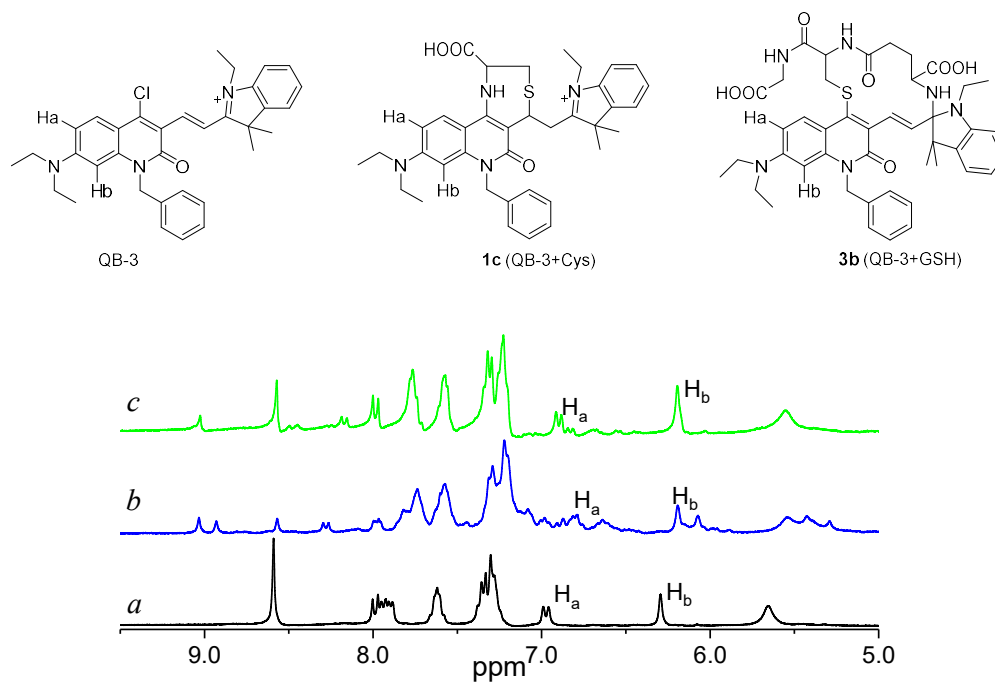


**Figure S3.** (a) Time-dependent absorption spectra of QB-3 (10  $\mu\text{M}$ ) in the presence of 20 equiv of NAC PBS- $\text{CH}_3\text{CN}$  (v/v, 3:2, 10 mM, pH 7.4) and (b) time-dependent absorbance at 573 nm wavelength.

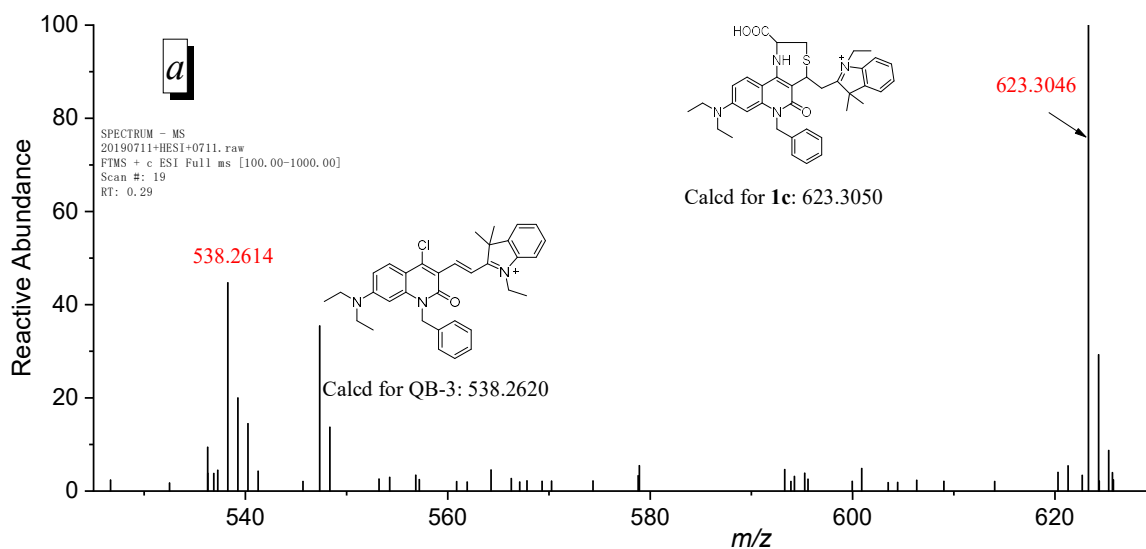


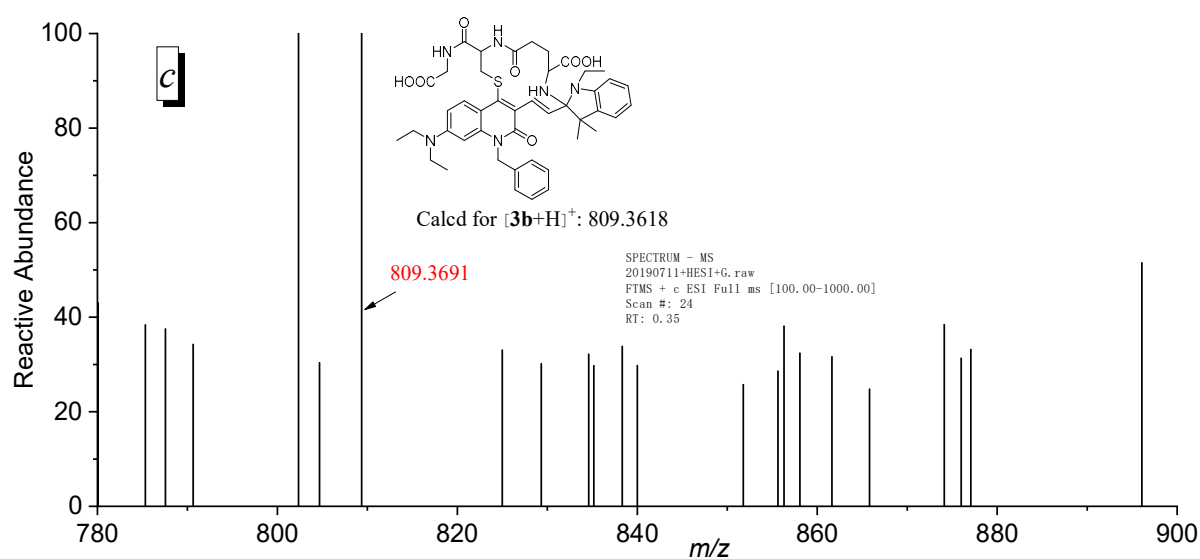
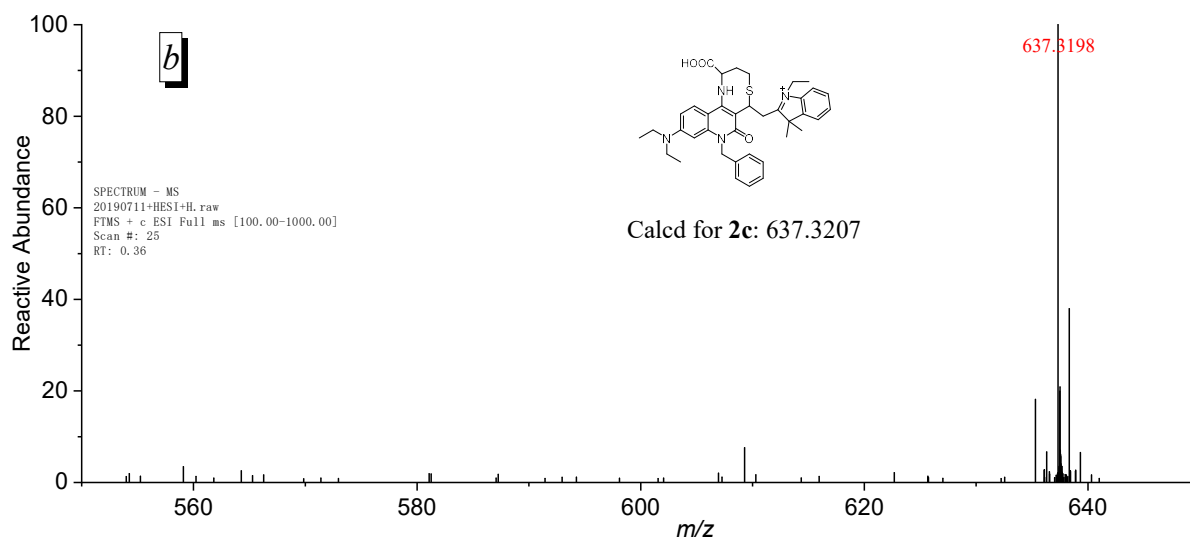
**Figure S4.** (a) Time-dependent fluorescence spectra of QB-3 (10  $\mu\text{M}$ ) in the presence of 20 equiv NAC PBS- $\text{CH}_3\text{CN}$  (v/v, 3:2, 10 mM, pH 7.4), excited at 365 nm and (b) 495 nm.

#### 4. Evidence of the sensing products for QB-3 with Cys/Hcy/GSH



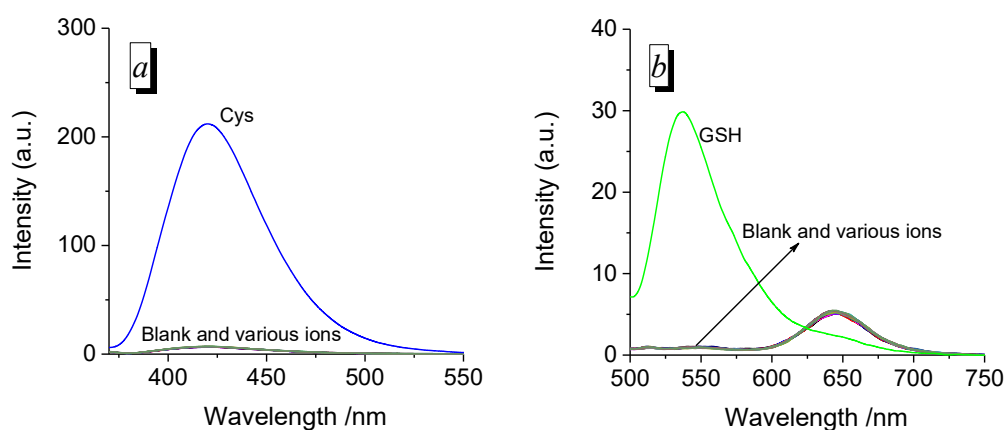
**Figure. S5** Partial <sup>1</sup>H NMR spectra comparison of (a) QB-3, (b) QB-3+Cys and (c) QB-3+GSH. The <sup>1</sup>H NMR spectra of (a) QB-3 was obtained in DMSO-*d*<sub>6</sub>. The <sup>1</sup>H NMR spectra of (b) QB-3+Cys and (c) QB-3+GSH were obtained in DMSO-*d*<sub>6</sub>-D<sub>2</sub>O (3:1, v/v).





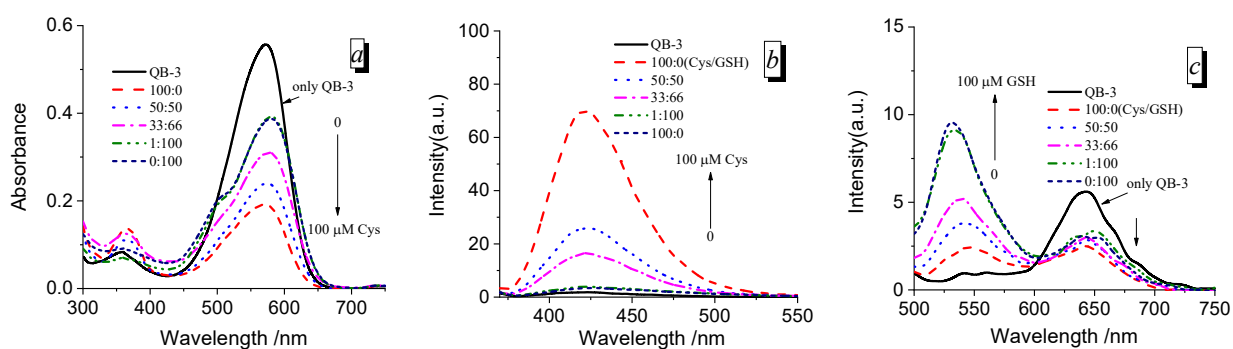
**Figure S6.** High-resolution mass spectrum (HRMS) for the mixture of QB-3 with (a) Cys, (b) Hcy and (c) GSH.

## 5. The selectivity of QB-3 for ions



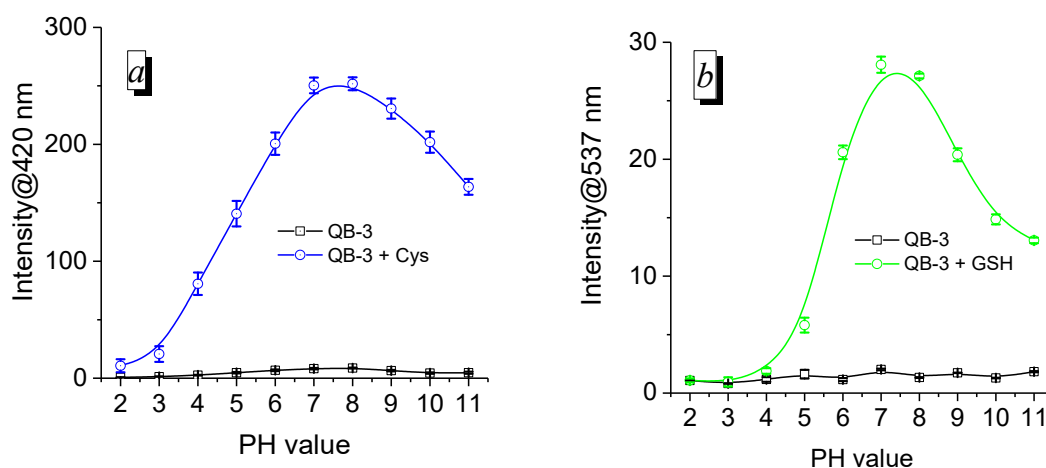
**Figure S7.** Fluorescence spectra of 10  $\mu\text{M}$  QB-3 solutions (PBS- $\text{CH}_3\text{CN}$  v/v, 3:2, 10 mM, pH 7.4) in the presence of 25 equiv of various ions with excitation at (a) 365 nm, (b) 495 nm, including:  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{K}^+$ ,  $\text{Ba}^{2+}$ ,  $\text{I}^-$ ,  $\text{Br}^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{ClO}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{NO}_3^-$ .

## 6. Competitive reaction between two biothiols (Cys/GSH) with QB-3

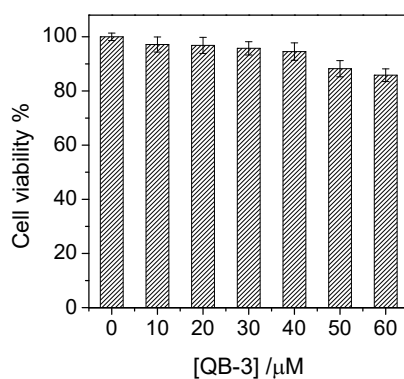


**Figure S8.** UV/Vis absorption spectra (a) and fluorescence spectra (b, c) of QB-3 (10  $\mu\text{M}$ ) after additions of two ratio of biothiols (Cys/GSH,  $\mu\text{M}/\mu\text{M}$ ) of 100:0, 50:50, 33:66, 1:100 and 100:0 for 50 min in PBS/ $\text{CH}_3\text{CN}$  (v/v, 3:2, 10 mM, pH 7.4), excited at 365 nm (b) and 495 nm (c).

## 7. pH effects and cytotoxicity



**Figure S9.** Fluorescence intensities of 10  $\mu\text{M}$  QB-3 and its reaction mixture with 200  $\mu\text{M}$  Cys (a) and GSH (b) in PBS buffer solutions (pH 2–11).



**Figure S10.** MTT assay of HepG2 cells in the presence of different concentrations of QB-3 incubated for 24 h.

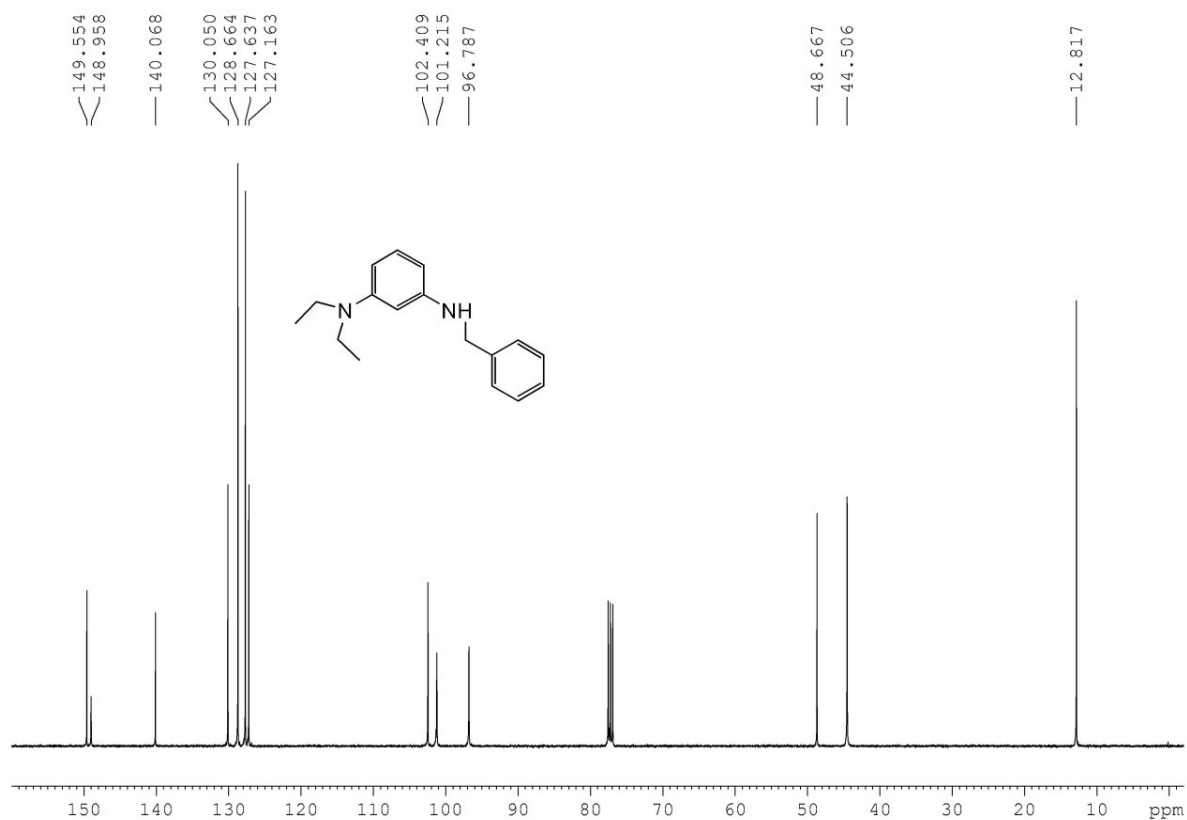


## 8. Copies of NMR spectra for compounds

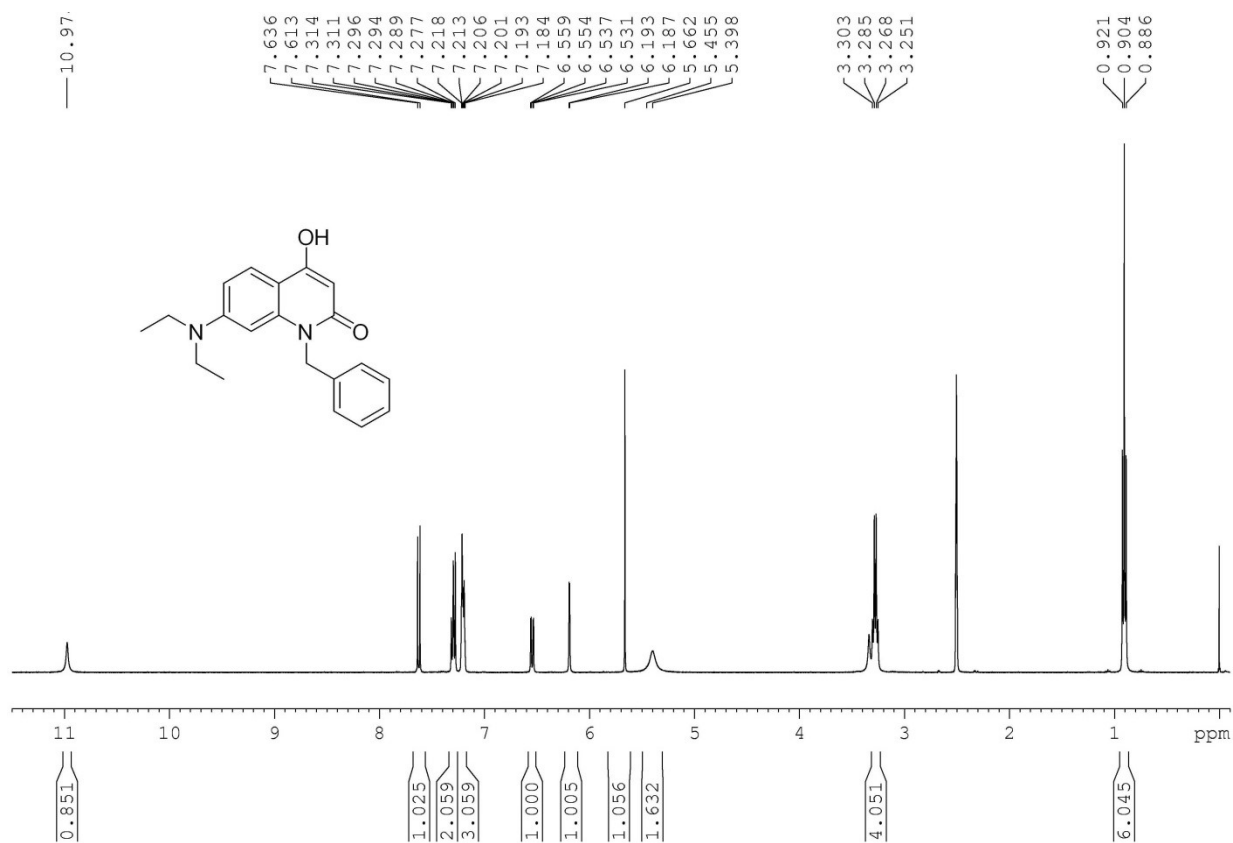
$^1\text{H}$  NMR of **2** in  $\text{CDCl}_3$ , 400 MHz



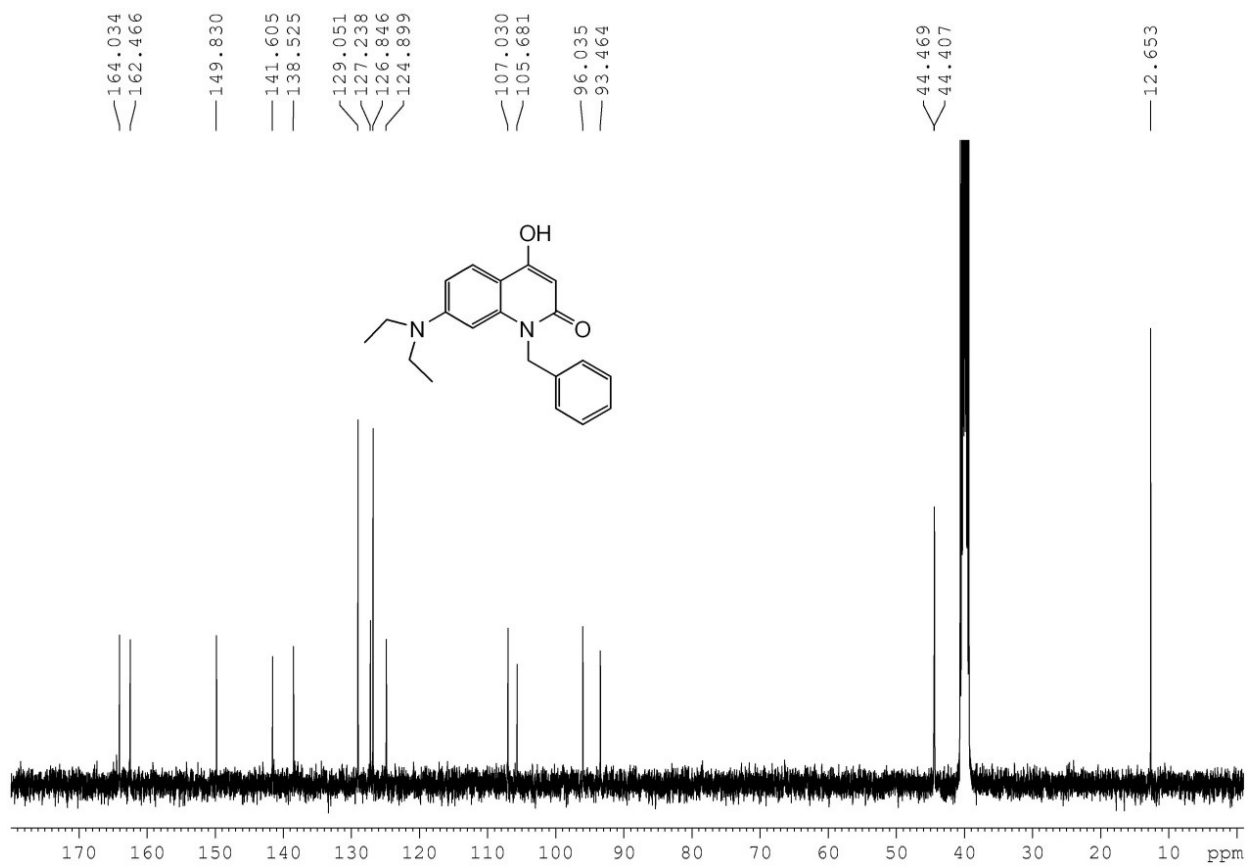
$^{13}\text{C}$  NMR of **2** in  $\text{CDCl}_3$ , 100 MHz



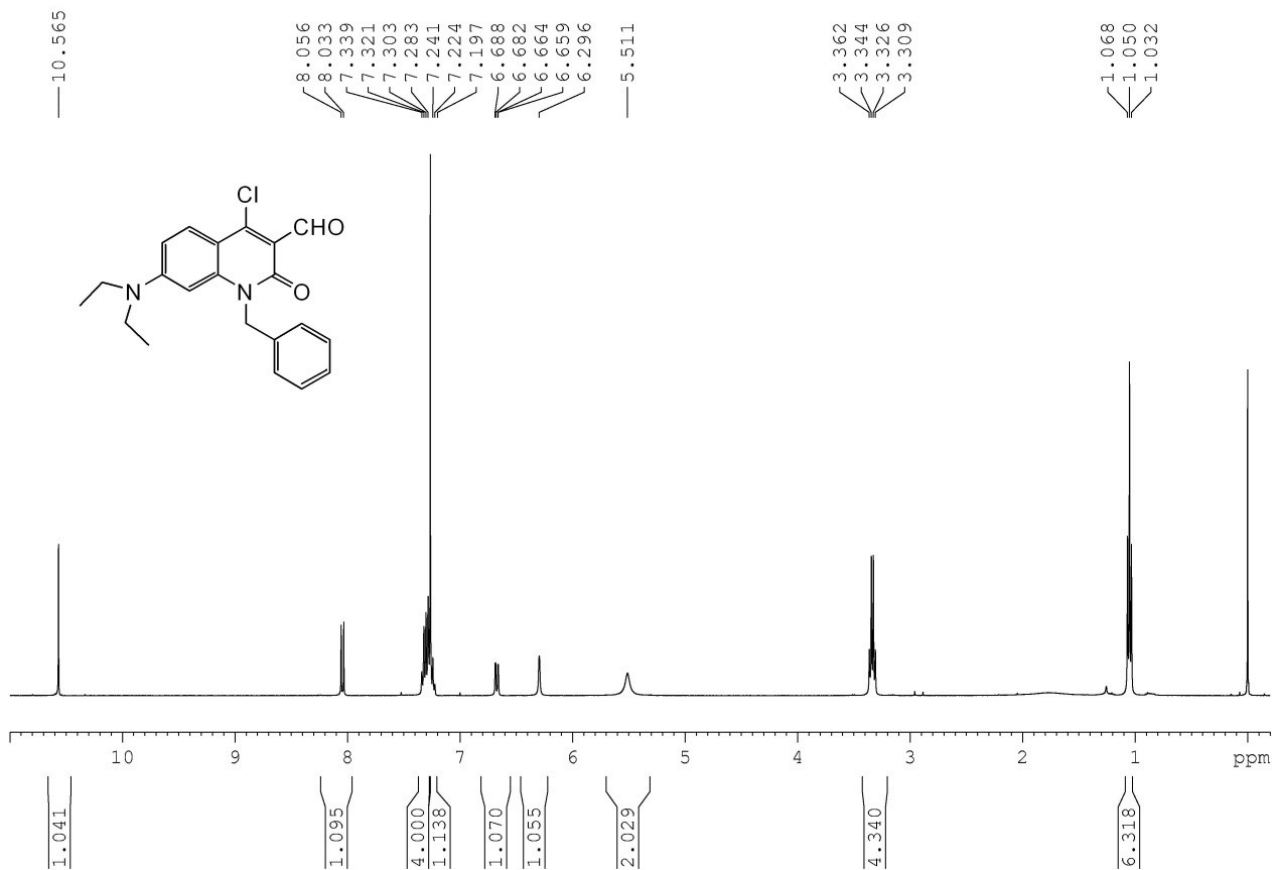
$^1\text{H}$  NMR of **3** in  $\text{DMSO-}d_6$ , 400 MHz



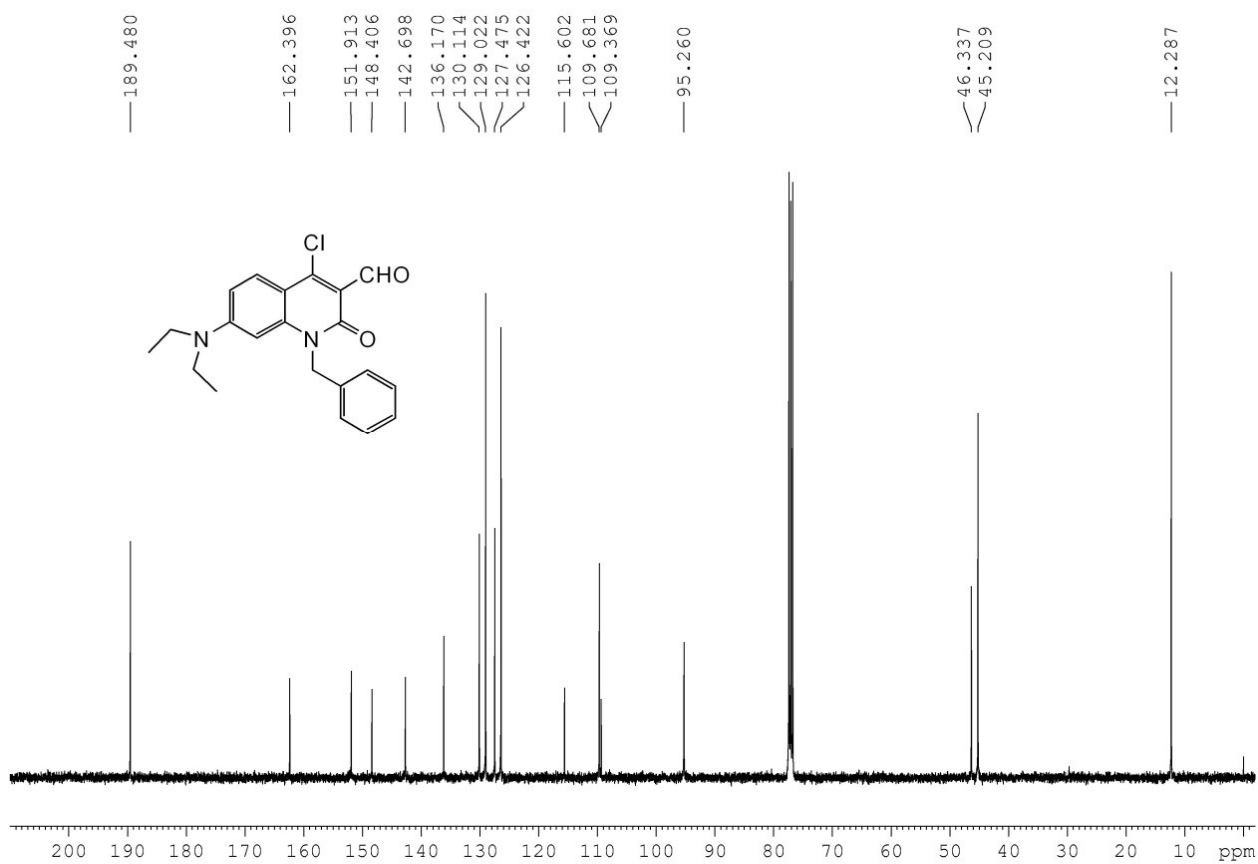
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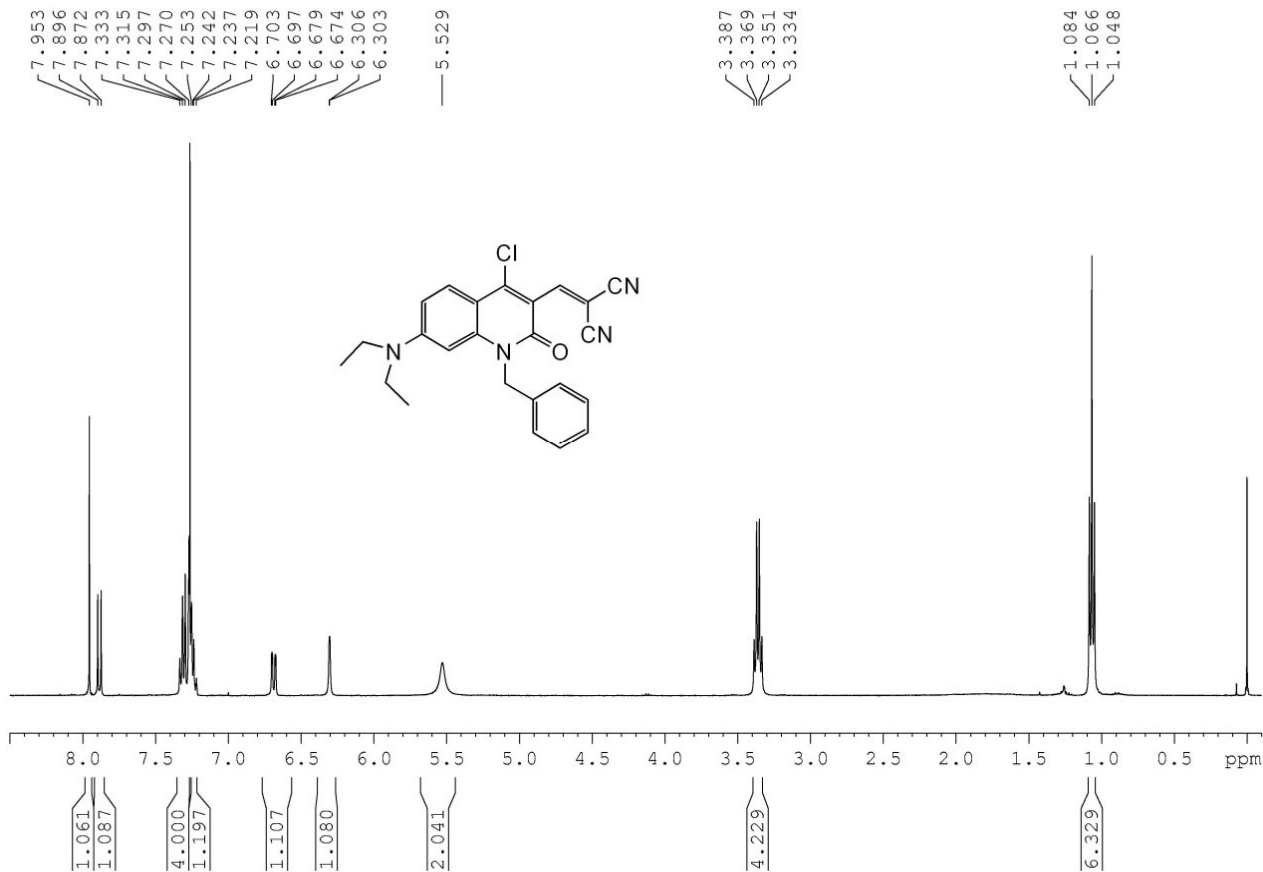
$^1\text{H}$  NMR of QB-1 in  $\text{CDCl}_3$ , 400 MHz



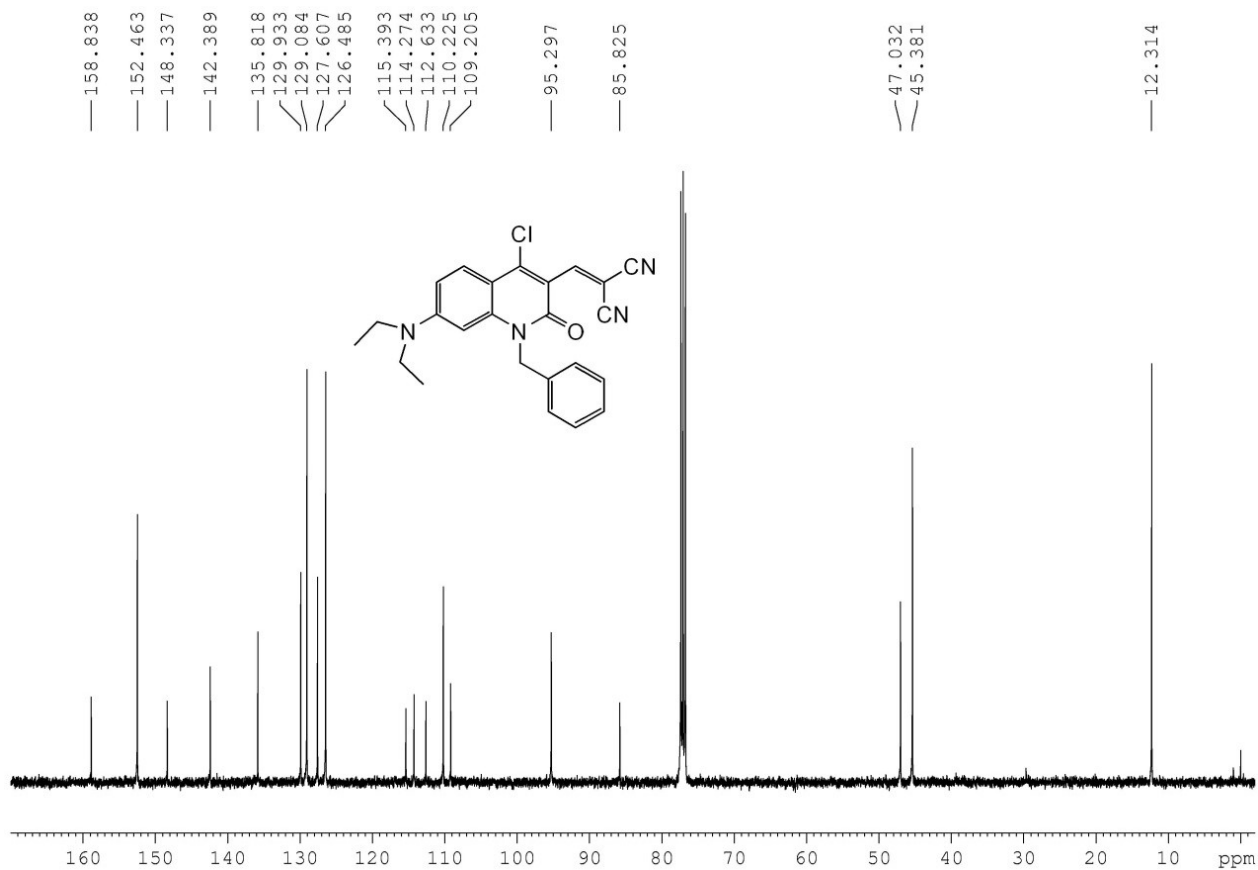
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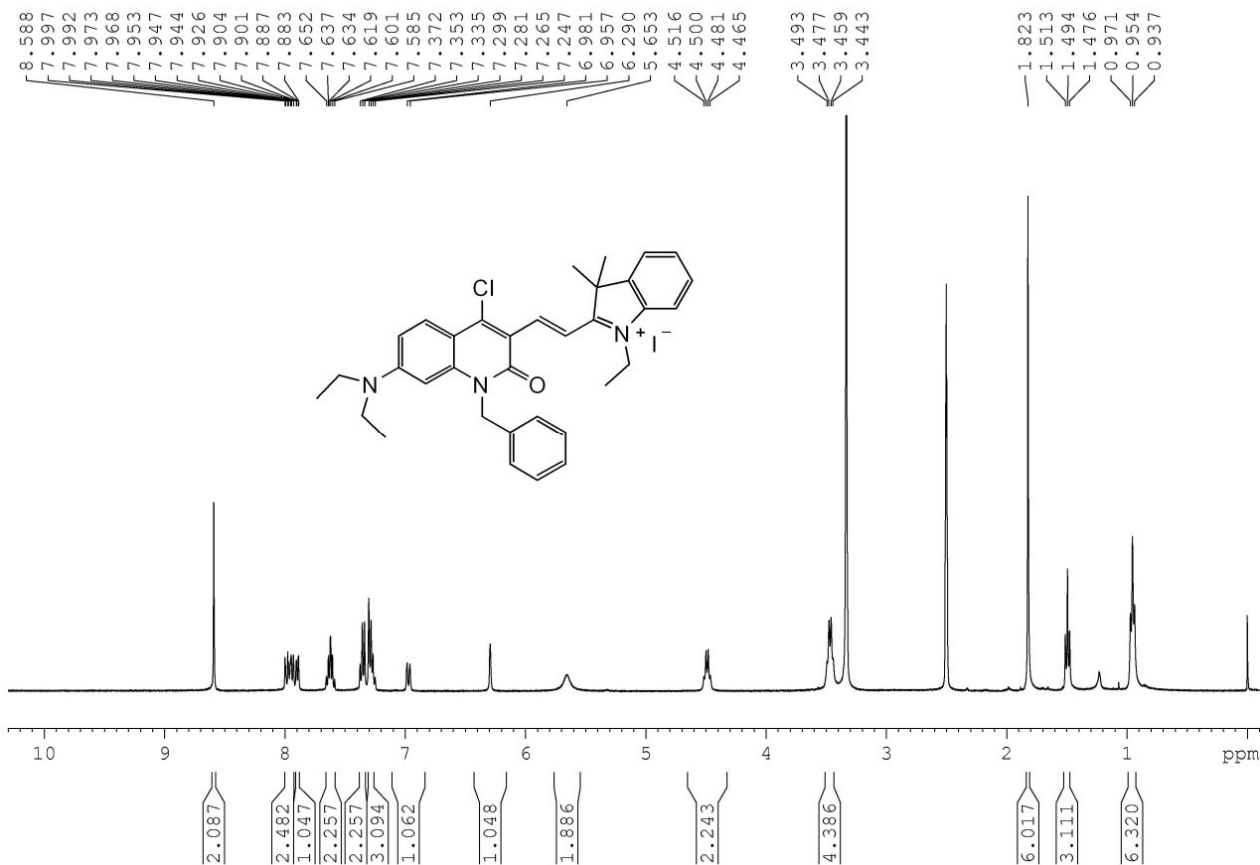
<sup>1</sup>H NMR of QB-2 in CDCl<sub>3</sub>, 400 MHz



<sup>13</sup>C NMR of QB-2 in CDCl<sub>3</sub>, 100 MHz



<sup>1</sup>H NMR of QB-3 in DMSO-*d*<sub>6</sub>, 400 MHz



<sup>13</sup>C NMR of QB-3 in DMSO-*d*<sub>6</sub>, 100 MHz

