Electronic Supplementary Information (ESI †)

For

A highly selective ICT-based fluorescent probe for cysteine sensing and its application in living cells imaging

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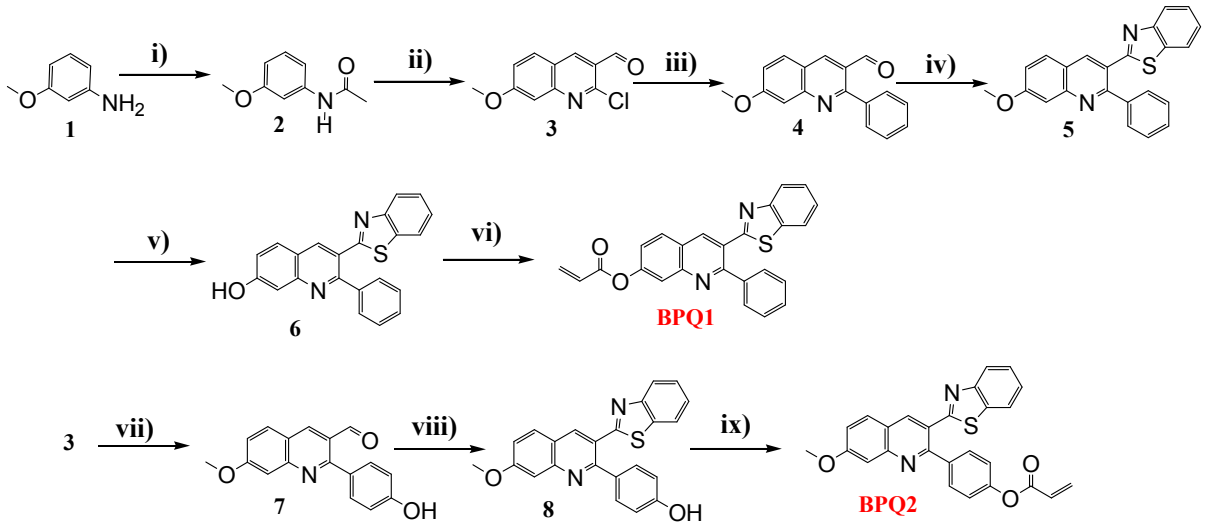
Synthesis and characterization:



Reagent & condition: (**i**) Ac_2O, DMAP, DIPEA, DCM, 2h, rt, 93% ; (ii**)** $POCl_3$, DMF, 110 ^oC, 2.5h, 59.8%; (iii) phenyl boronic acid, $Pd(PPh_3)_2Cl_2$, 2M Na_2CO_3, DME, 90 ^oC, overnight, 75.6%; (iv) 2-Aminothiophenol, $KHSO_4$, reflux, 12h, 57%; (v) Na_2S, NMP, 140 ^oC, overnight, 62%; (vi) Acryolyl chloride, Et_3N, DCM, 5h, 43%; (vii) 4-Hydroxy phenyl boronic acid, $Pd(PPh_3)_2Cl_2$, 2M Na_2CO_3, DME, 90 ^oC, overnight, 79% ; (viii) 2-Aminothiophenol, $KHSO_4$, reflux, overnight, 54%; (ix) Acryolyl chloride, Et_3N, DCM, 5h, 46%.

Analytical data of compounds and intermediates

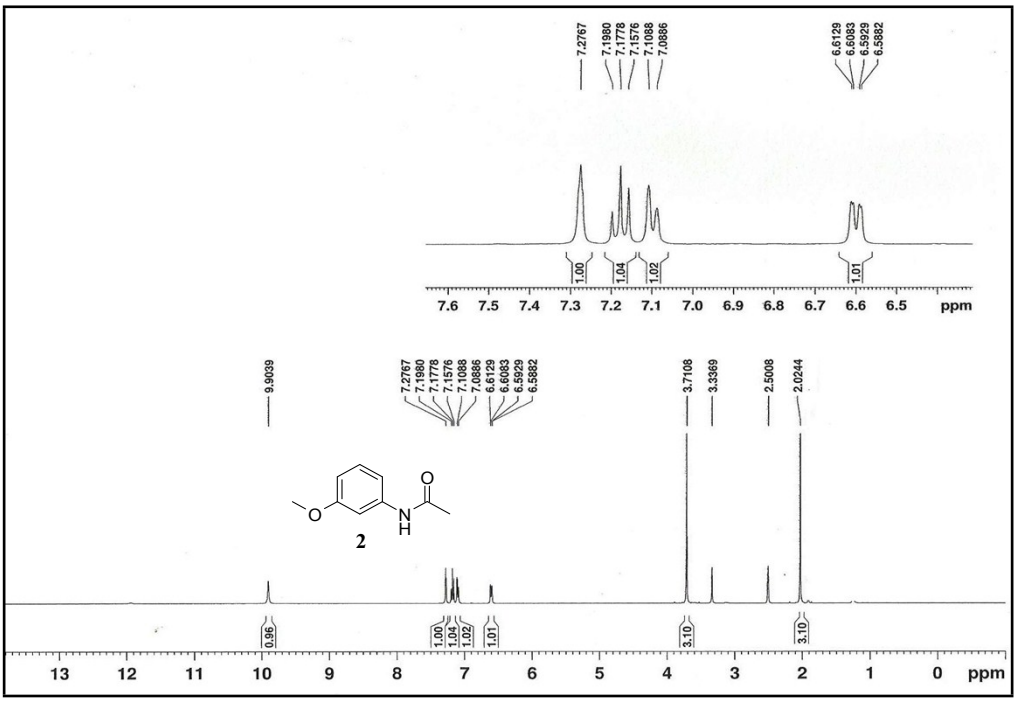


Fig. S1: ^{1}H NMR of compound **2** in DMSO-d_6**.**

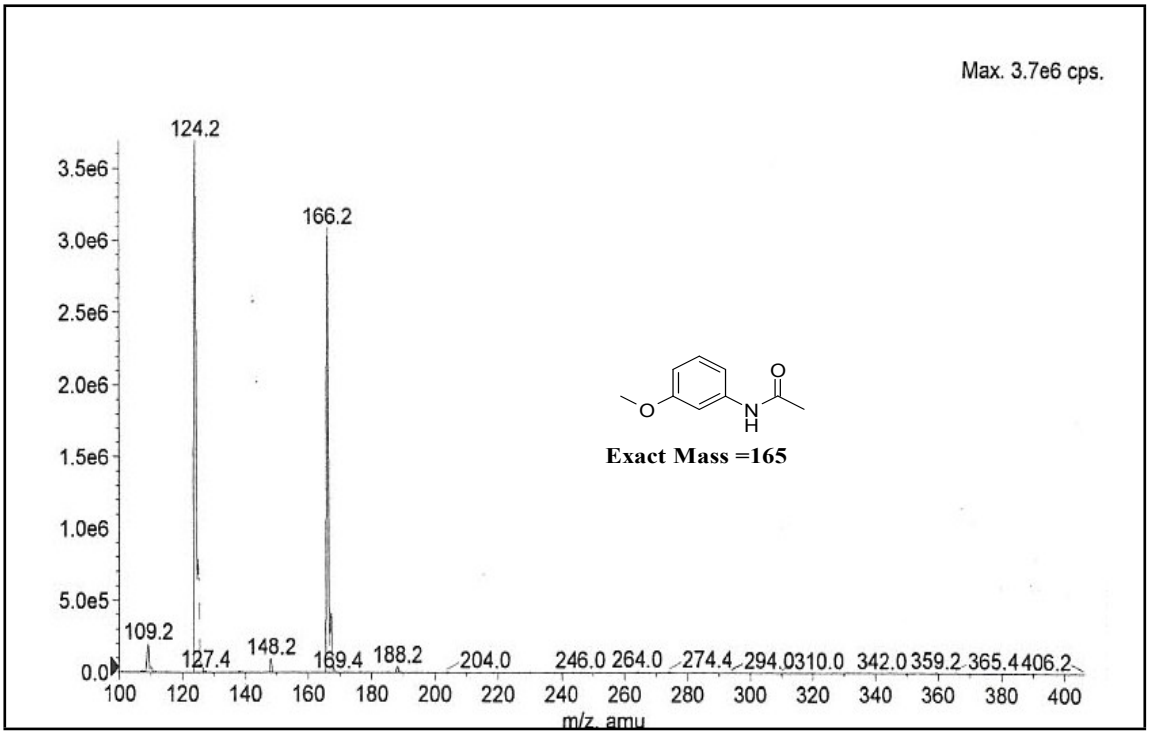


Fig. S2: ESI-MS of compound **2**

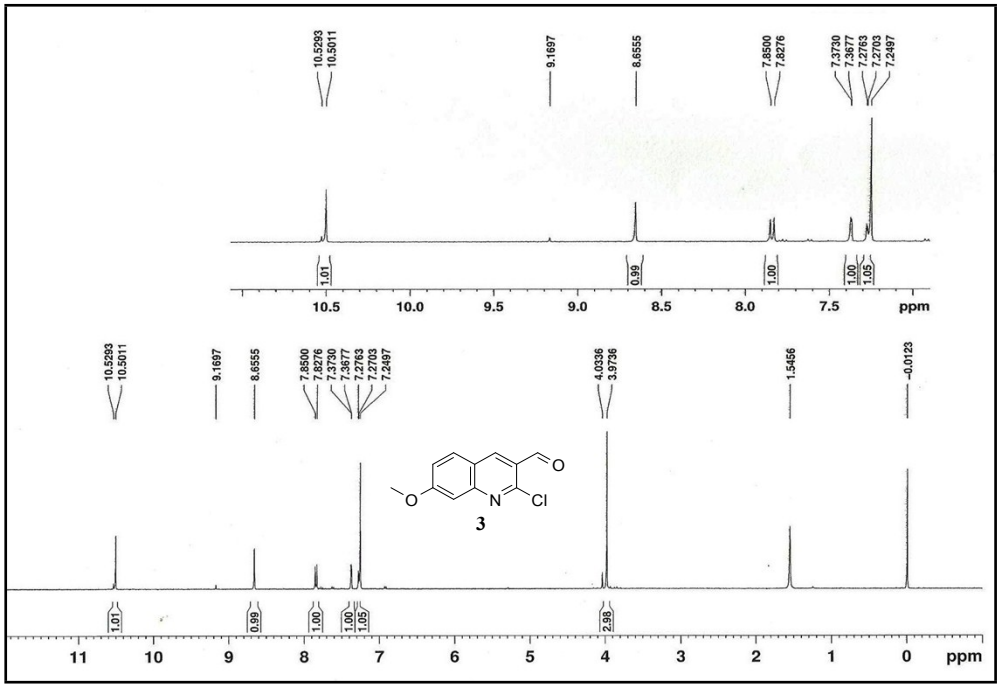


Fig. S3: ^{1}H NMR of compound **3** in $CDCl_3$

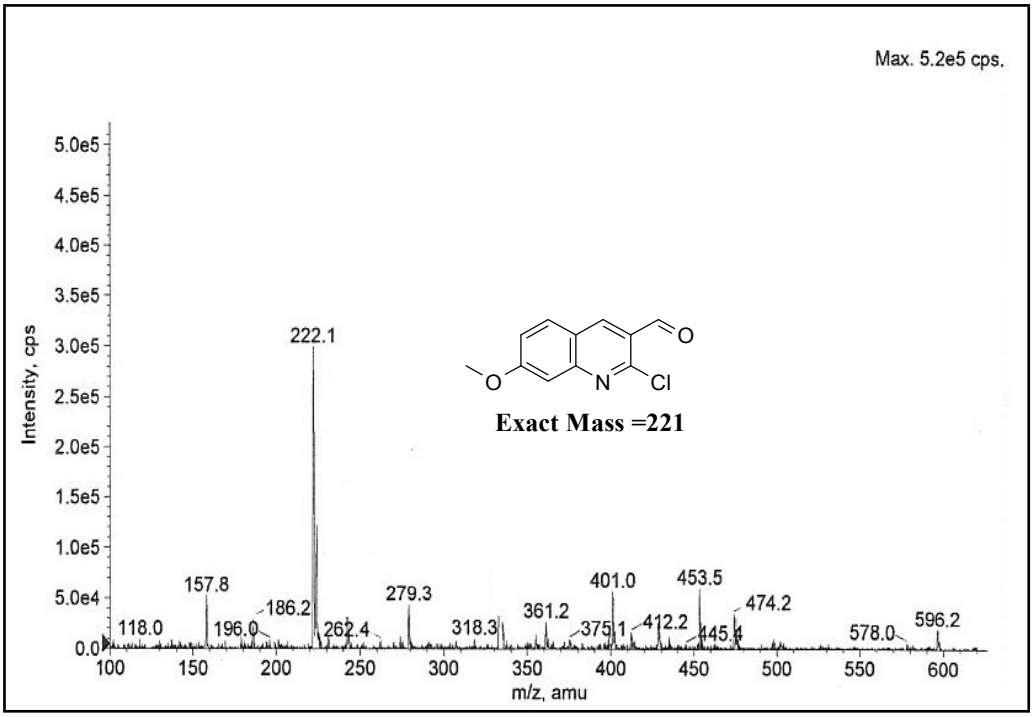


Fig. S4: ESI-MS of compound **3**

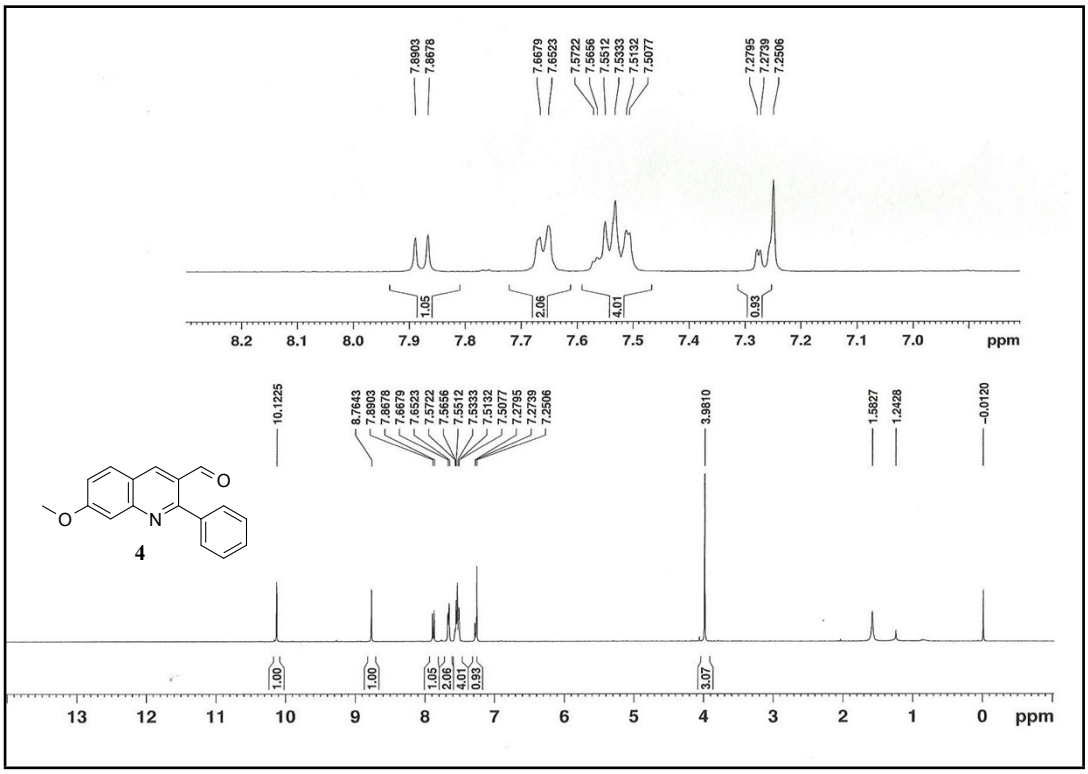


Fig. S5: ^{1}H NMR of compound **4** in $CDCl_3$

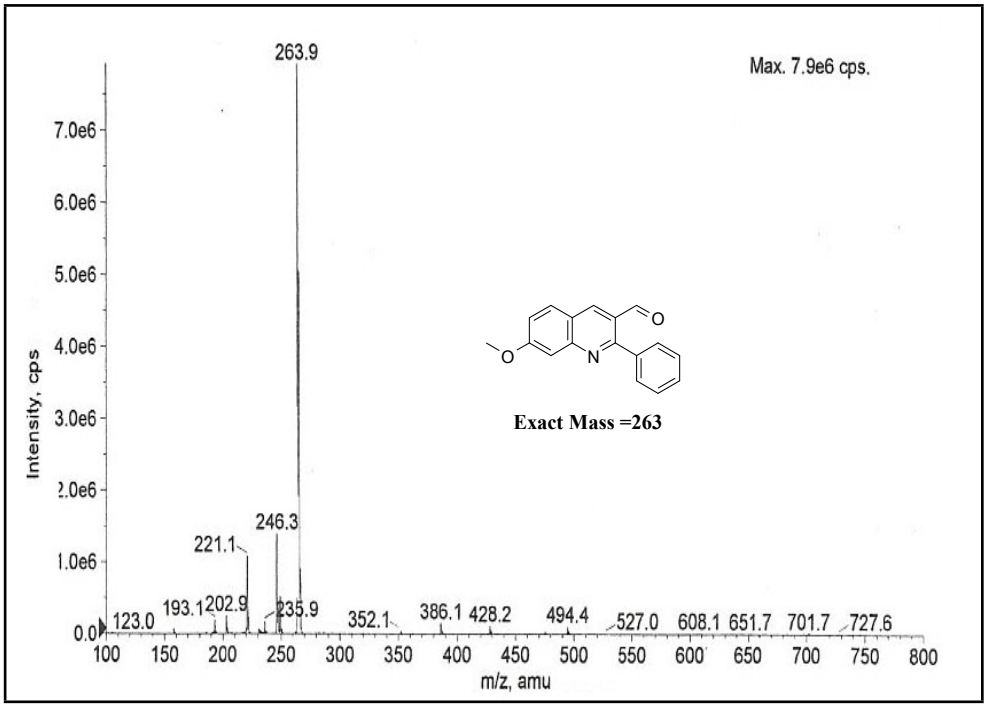


Fig. S6: ESI-MS of compound **4**

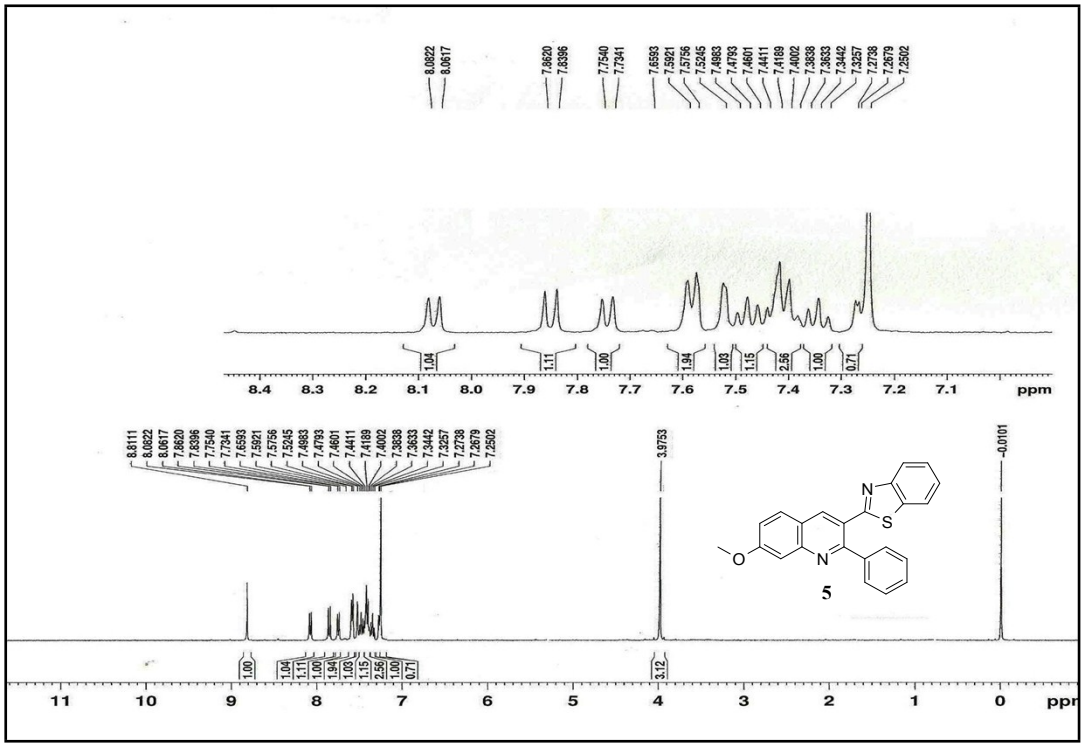


Fig. S7: ^{1}H NMR of compound **5** in $CDCl_3$

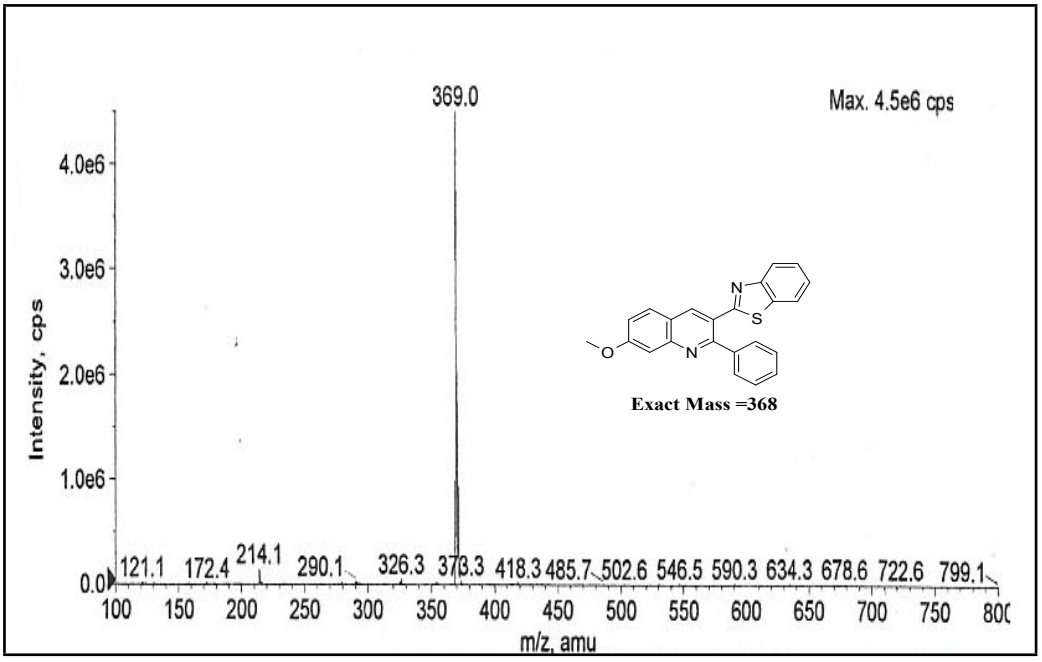


Fig. S8: ESI-MS of Compound **5**

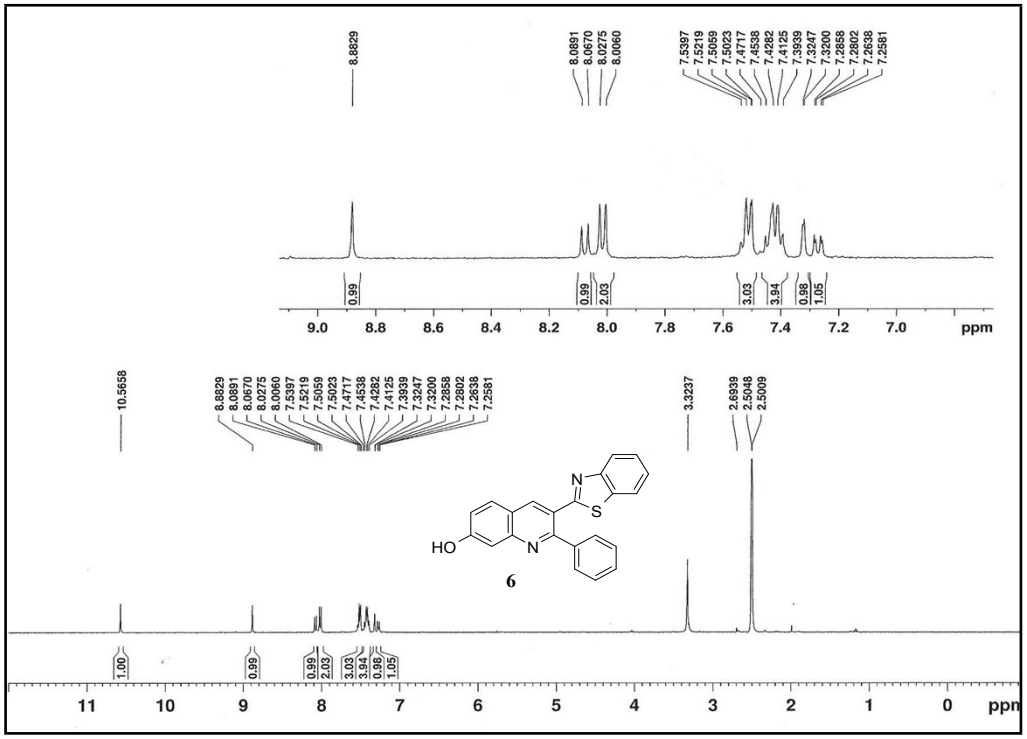


Fig. S9: ^{1}H NMR of compound **6** in DMSO-d_6

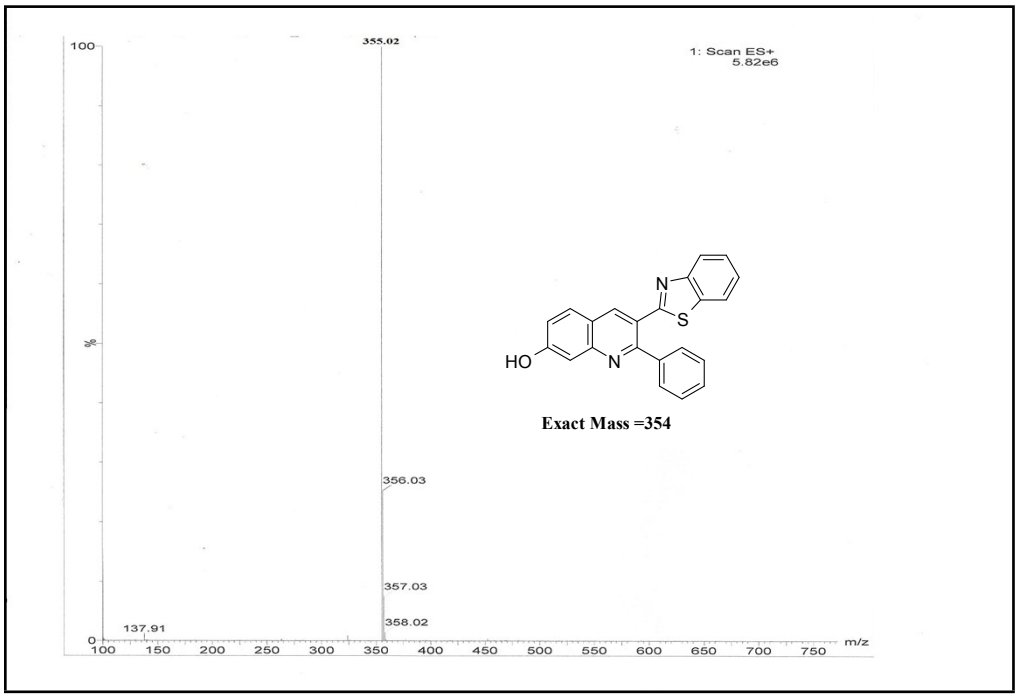


Fig. S10: ESI-MS of Compound **6**

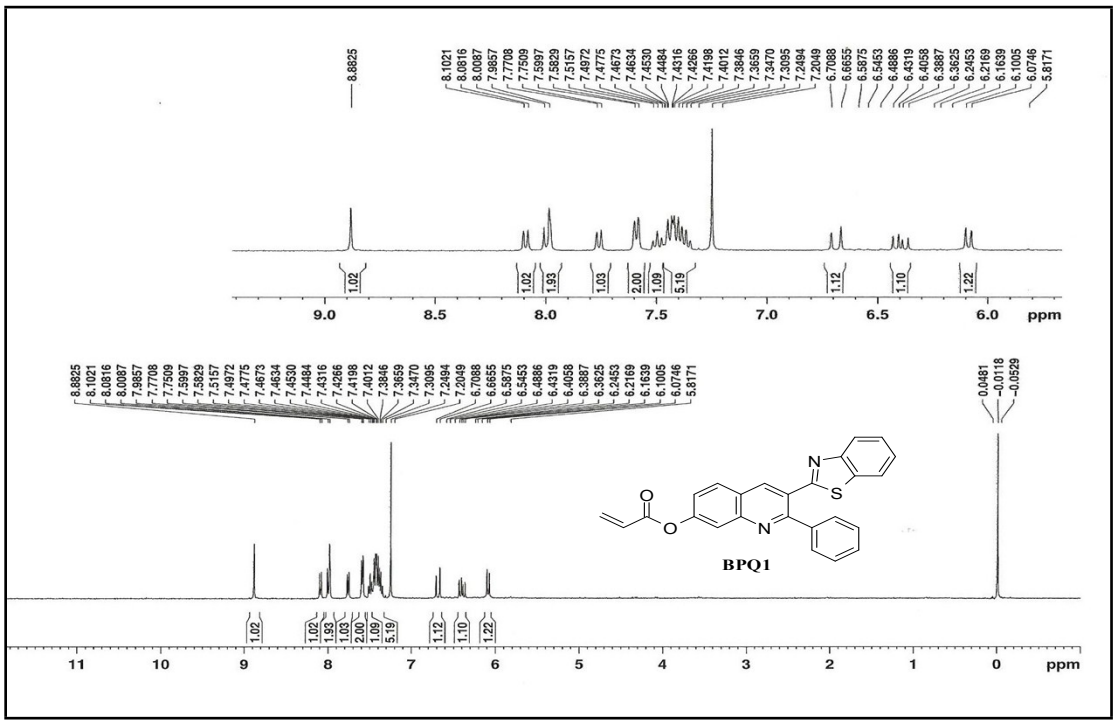


Fig. S11: ^{1}H NMR of probe **BPQ1** in $CDCl_3$

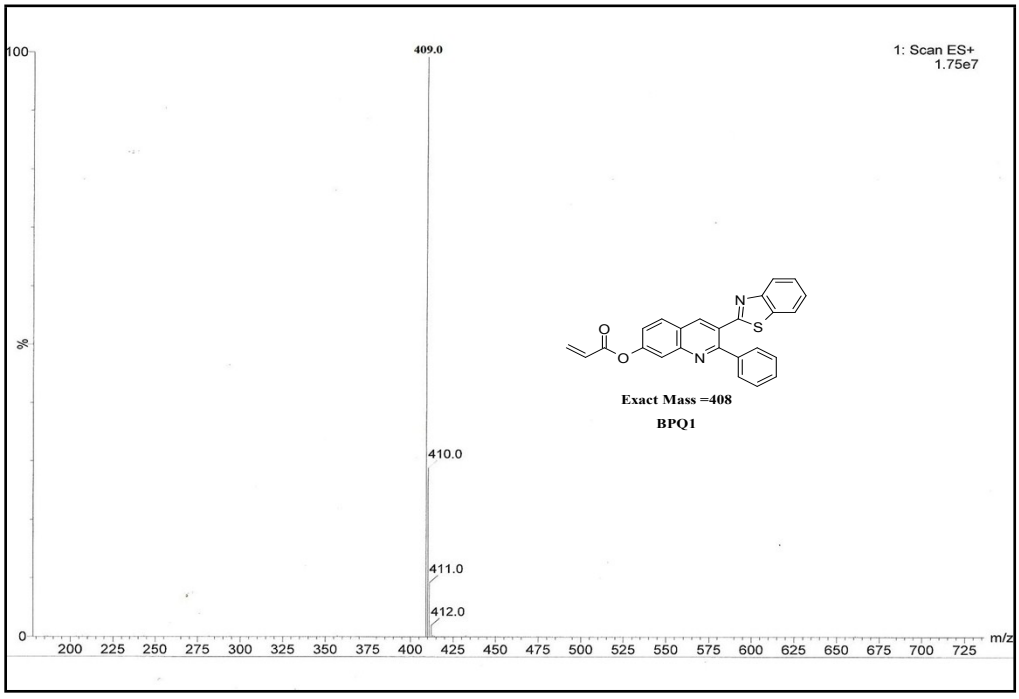


Fig. S12: ESI-MS of probe **BPQ1**

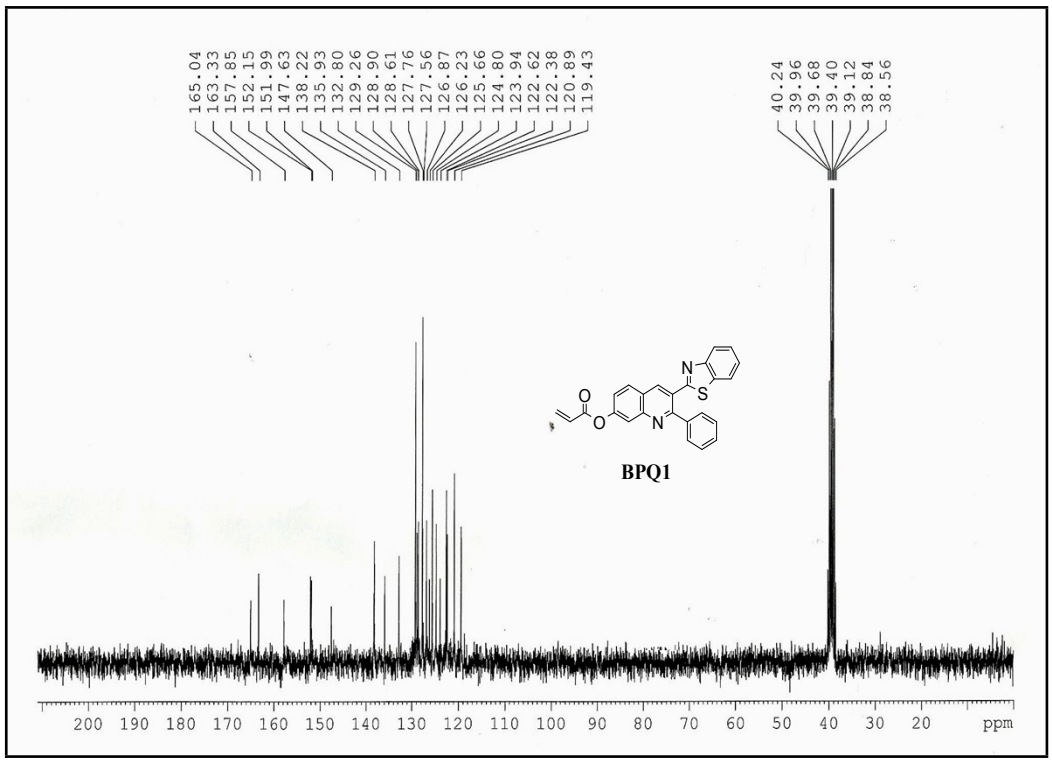


Fig. S13: ^{13}C NMR spectra of **BPQ1** in $CDCl_3$

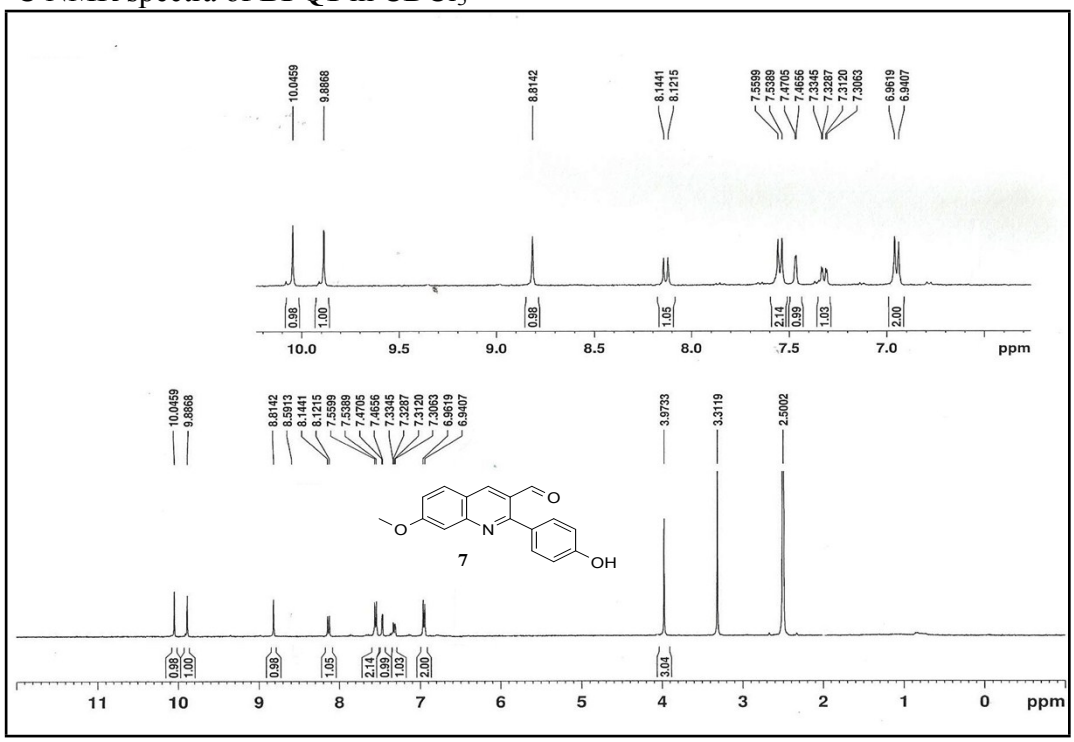


Fig. S14: ^{1}H NMR of compound **7** in DMSO-d_6

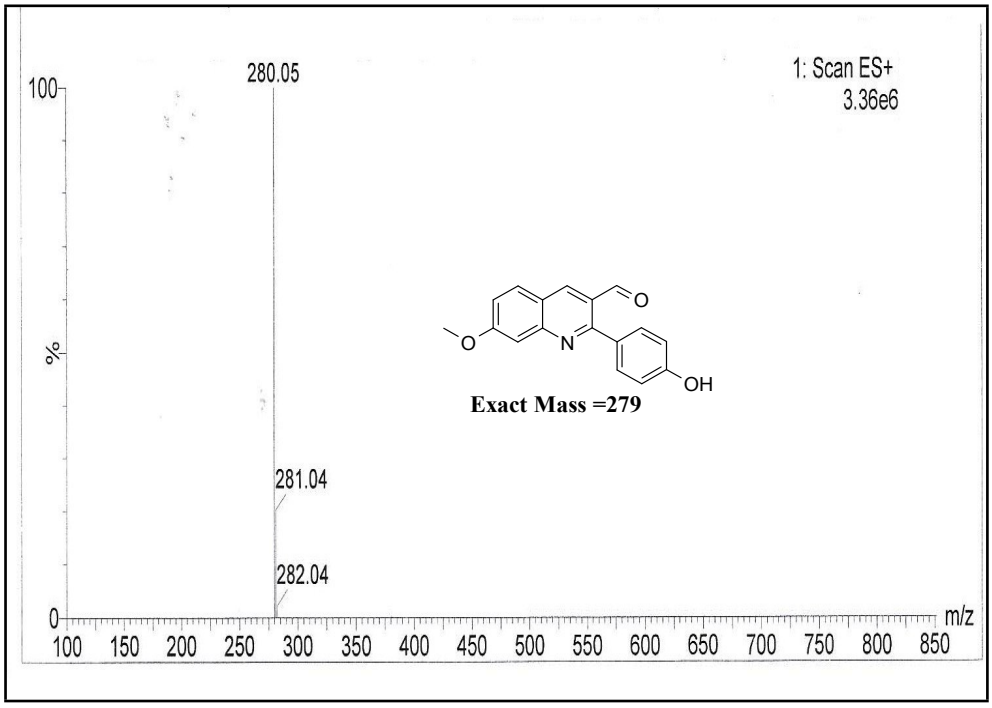


Fig. S15: ESI-MS of Compound **7**

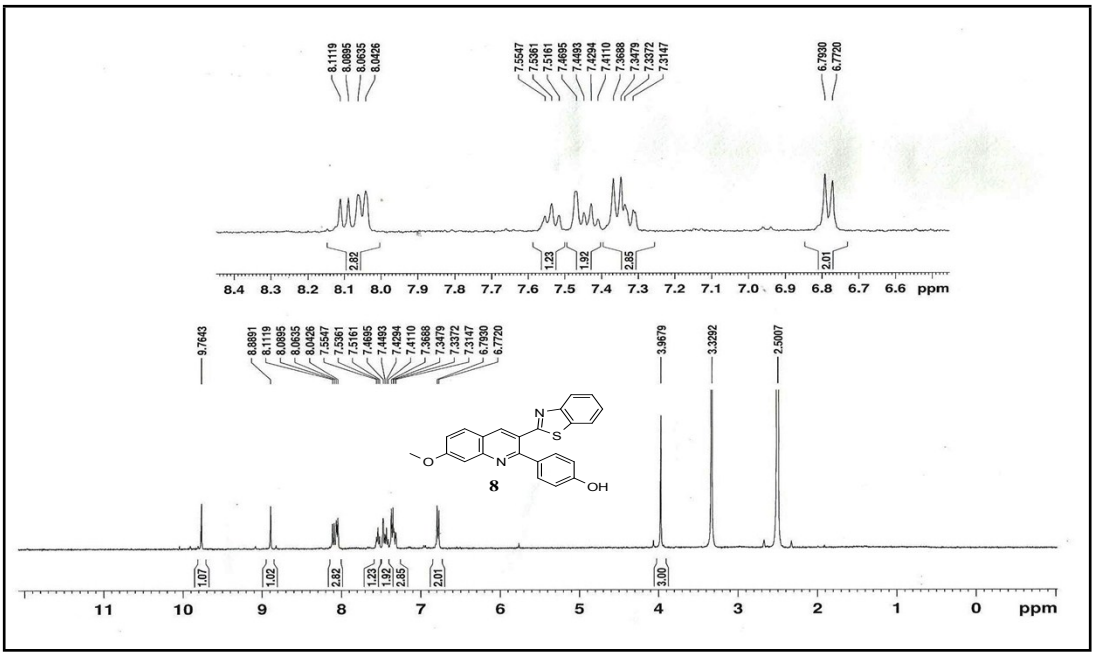


Fig. S16: ^{1}H NMR of compound **8** in DMSO-d_6

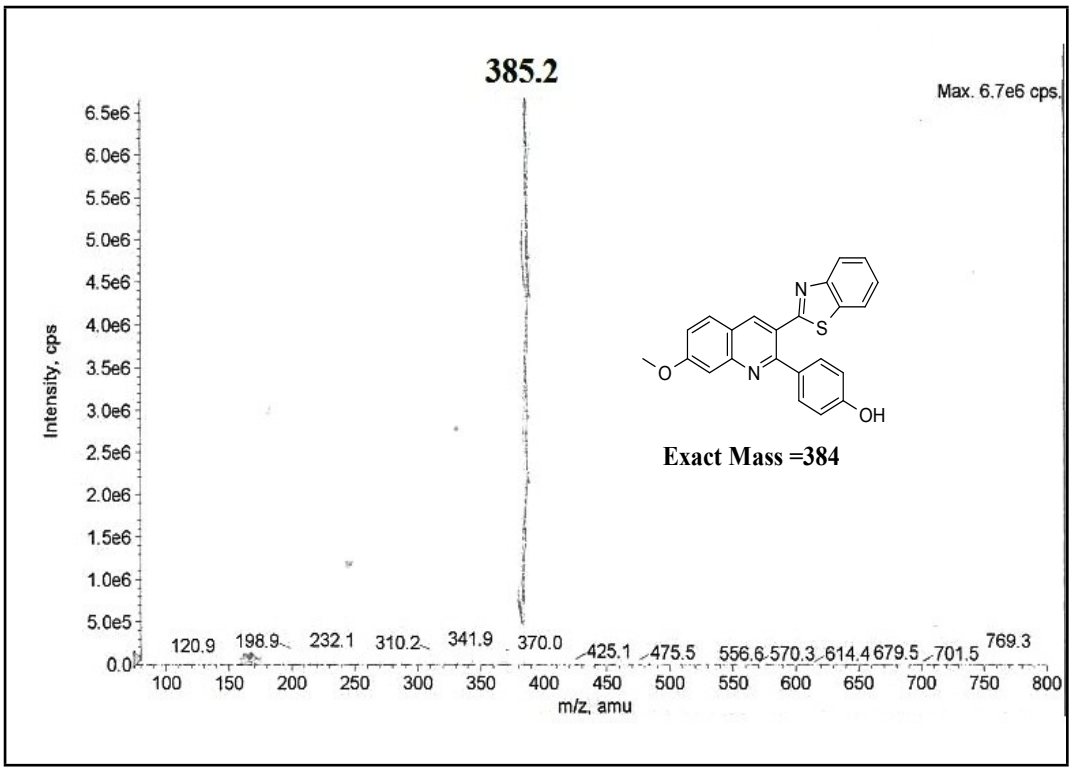


Fig. S17: ESI-MS of Compound **8**

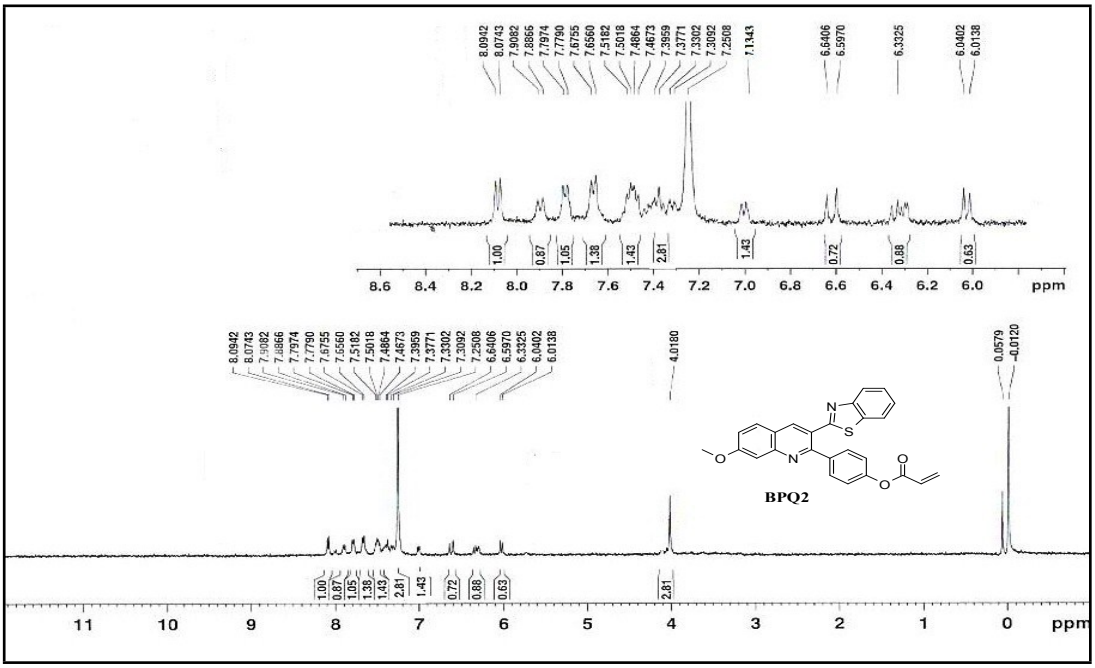


Fig. S18: ^{1}H NMR of probe **BPQ2** in $CDCl_3$

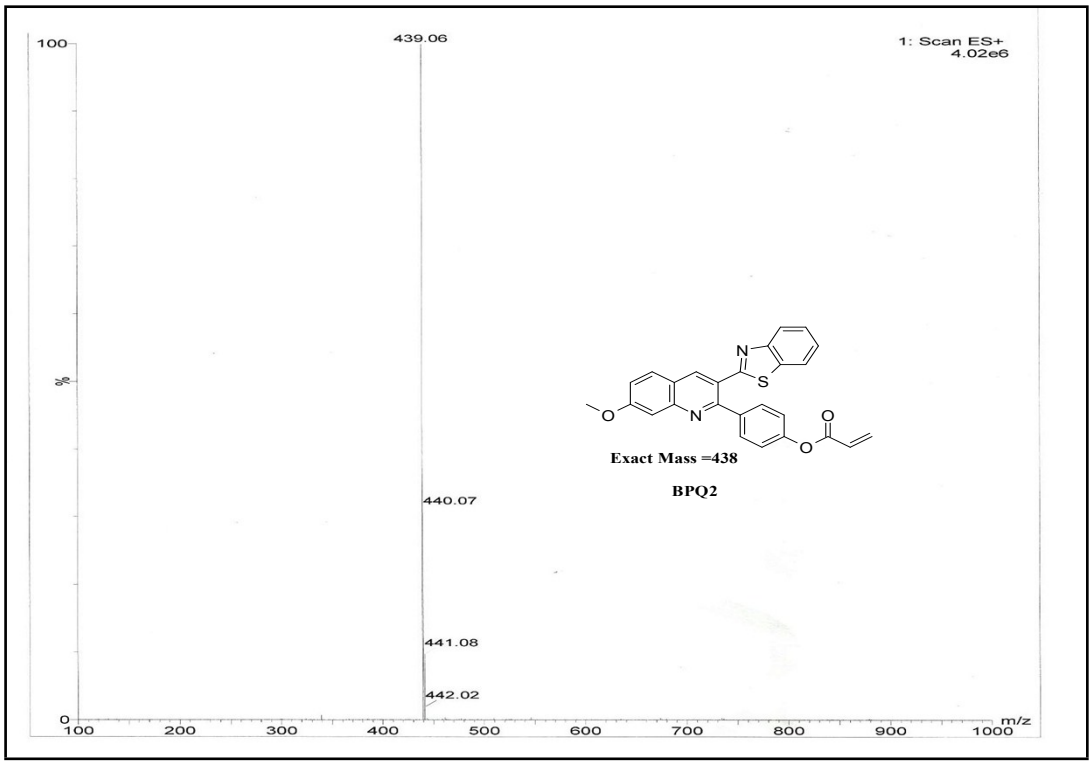


Fig. S19: ESI-MS of probe **BPQ2**

Calculations for detection limit:

The detection limit (DL) of Probe **BPQ1** was determined from the following equation:
DL = K* Sb1/S

Where K = 2 or 3 (we take 2 in this case); Sb1 is the standard deviation of the blank solution;

S is the slope of the calibration curve.

From graph Sb1=3.80209, S=1.60535 x 10^8

Detection limit = 2 x 3.80209/1.60535 x 10^8 = 4.7x 10^{-8} = **0.047 ppm = 47 ppb.**

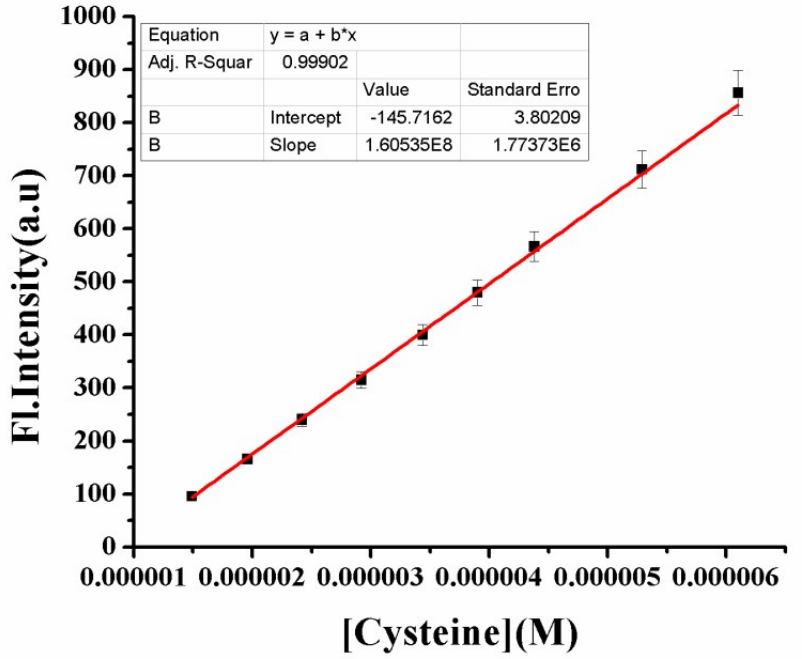


Fig. S20. Calibration curve for Fluorescence titration of **BPQ1** at 506 nm with Cys.

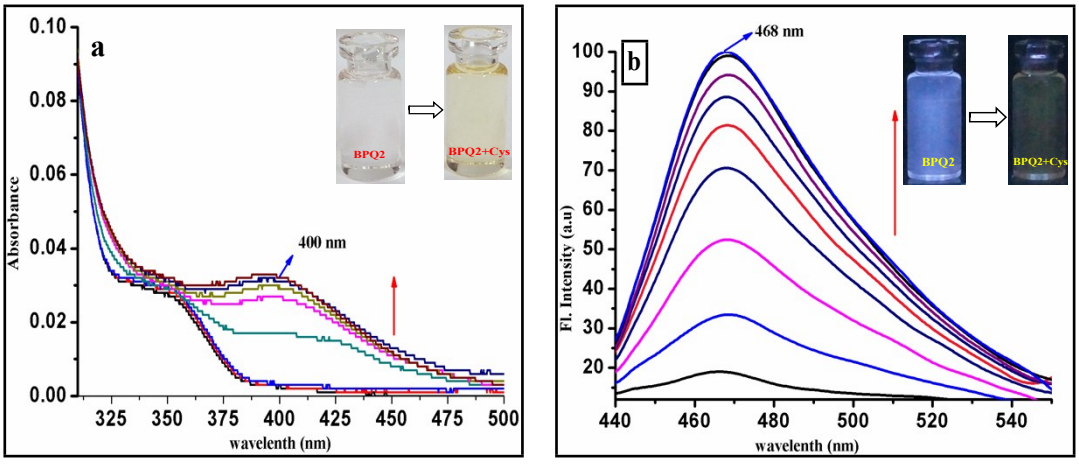


Fig. S21. (a) UV-vis spectra of **BPQ2** (10 μM) in DMSO: H_2O (7:3, v/v) using 10 mM buffer pH 7.4 in presence of Cys (0-100 μM); (b)Emission spectra changes of **BPQ2** (10 μM) in aqueous DMSO (7:3, v/v, 10 mM HEPES Buffer pH 7.4) upon addition of Cys (0–1.25 equiv)

with excitation at 400 nm. Attach photos are the photographs of the solution of **BPQ2** in the absence (left) and presence of Cys (right) for both (a & b).

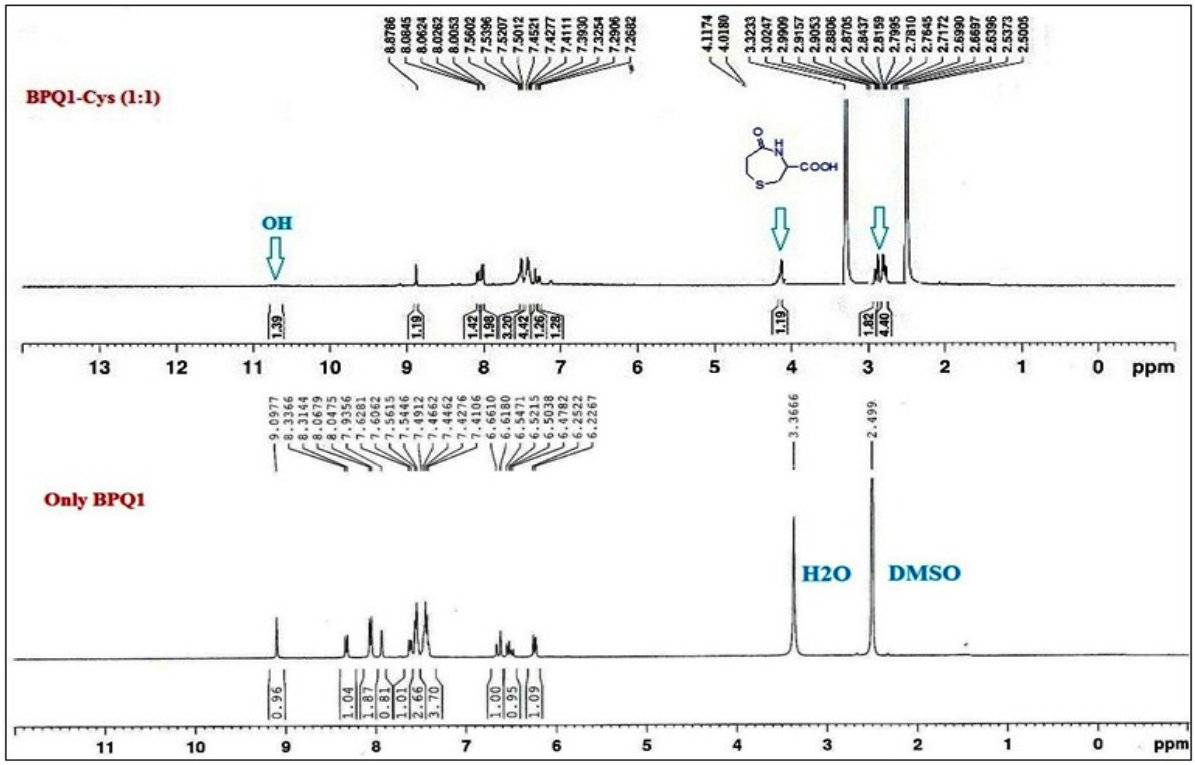


Fig. S22. BPQ1 showed the signals of the acryloyl protons of 3 appear at 6.64, 6.52 and 6.23 ppm in DMSO-d_6 as solvent. Upon addition of Cys to **BPQ1** (1:1) in DMSO-d_6, these 3 protons disappeared and a new peak appeared at around 10.65 ppm corresponding to phenolic moiety of 6 was distinctly observed and showed lactam moiety as byproduct.

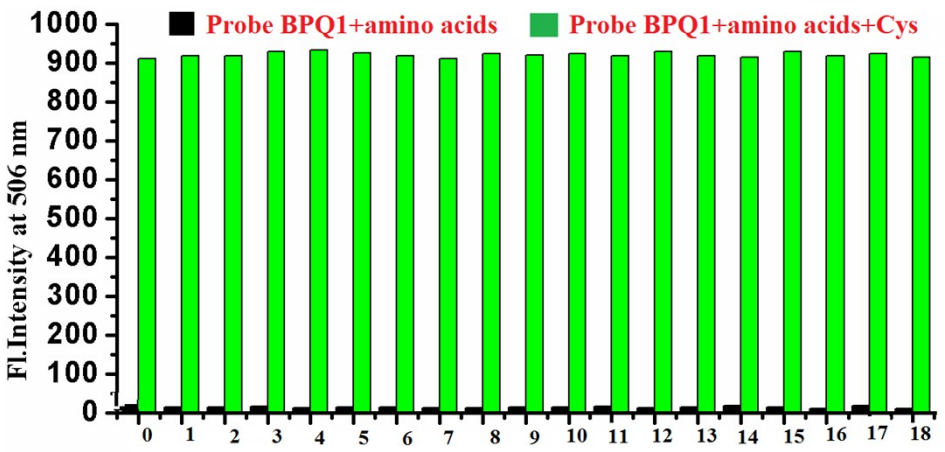


Fig. S23 (b) Fluorescence selectivity of the probe **BPQ1** (10 μM) to Cys in the absence and presence of various amino acids (100 μM) in aqueous DMSO (DMSO: H_2O = 7:3, v/v, 10 mM HEPES buffer, pH = 7.4). The black bars represent the emission changes of **BPQ1** in the presence of other amino acids (all were 100 μM). The green bars represent the emission changes of **BPQ1** with Cys in the presence of other amino acids. Various amino acids including: 0-Blank, 1- Ala, 2- Glu, 3- Arg, 4- Hcy, 5- GSH, 6- Lys, 7- Asp, 8- Gly, 9- Leu, 10- Tyr, 11- His, 12- Trp, 13- Ile, 14- Thr, 15- Phe, 16- Pro, 17- Met, 18-Val. The intensities were recorded at 506 nm.

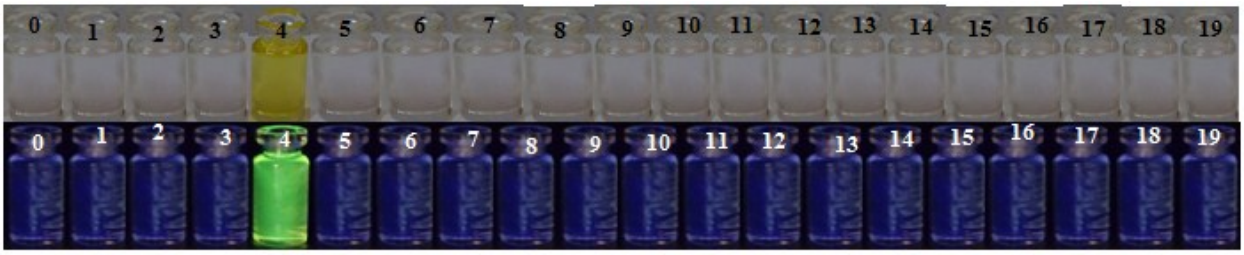


Fig. S24 Photograph taken in presence of various aminoacids in day light (top) and uv lamp (bottom) of **BPQ1** (10 μM) in DMSO: H_2O (7:3, 10 mM HEPES Buffer pH 7.4) in presence of Cys (100 μM). From 0-19: blank, Ala, Glu, Arg, Cys, Hcy, GSH, Lys, Asp, Gly, Leu, Tyr, His, Trp, Ile, Thr, Phe, Pro, Met and val.

Kinetic Studies:

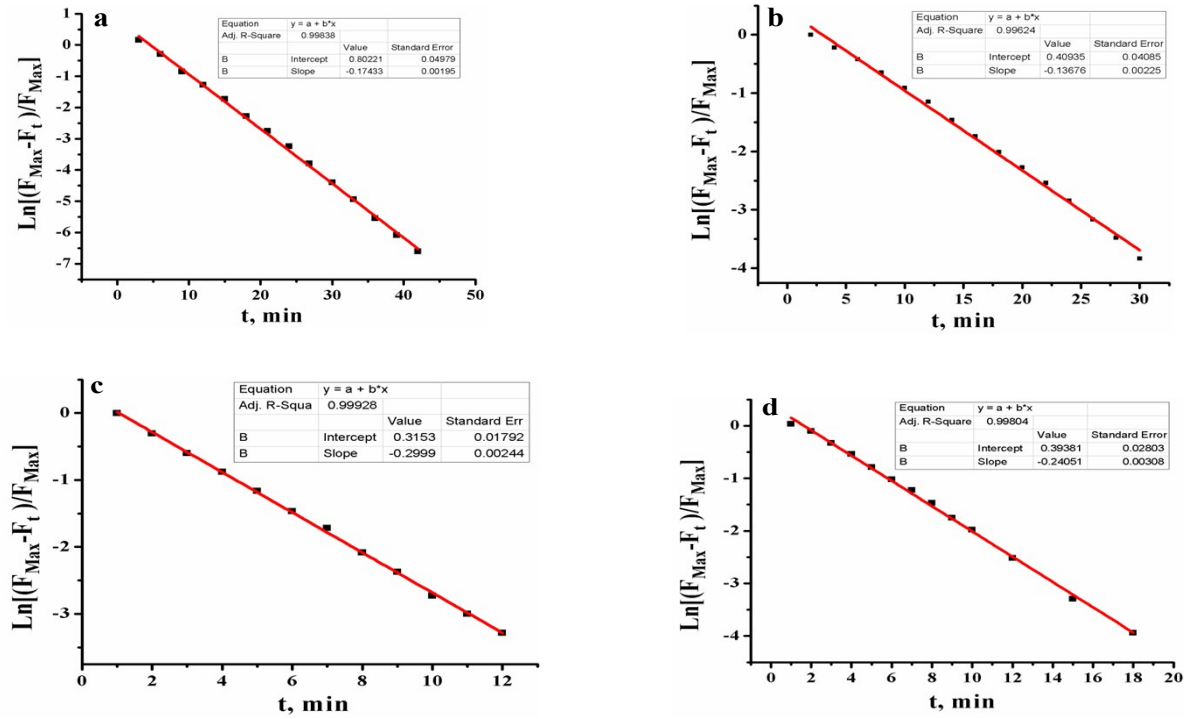


Fig. S25 Pseudo first-order kinetic plot of reaction of **BPQ1** (10 μM) with Cys (100 equiv.), slope=**-0.17433 min^{-1}** (b) Kinetic plot of **BPQ1** with 25 equiv. Cys (c) Kinetic plot of **BPQ1** with 50 equiv. Cys (d) Kinetic plot of **BPQ1** with 80 equiv. Cys in DMSO: H_2O (7:3, 10 mM HEPES Buffer, pH 7.4)

The second-order rate constant for this reaction is thus the slope of a linear plot of k/ versus the concentration of Cys (Fig.26): k = **29.76171 M^{-1} min^{-1}**

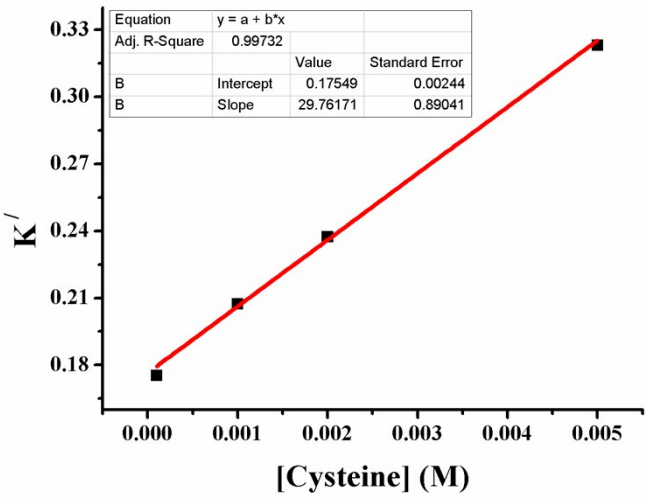


Fig. S26 Plot of the observed **k/** versus the concentration of Cys for the pseudo first-order reaction of **BPQ1** (10 μM) with varying concentration of Cys (10-150 Eq). **Slope = 29.76171 M^{-1} min^{-1}**

Cell viability assay:

To determine the cell viability, MG-63 cells were treated with 10 μM probe **BPQ1** for 1 h followed by 1 h treatment with 100 μM Cys as described previously. After treatment, cell viability was measure using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) solution as per manufacturer protocol (Himedia Laboratories, India).

Cytotoxic effect of **BPQ1** and Cys was determined by cell viability assay. The standard MTT assays revealed that **BPQ1** as well as Cys had low cytotoxic effect on MG-63 cells (Fig. S27). The cell viability were 84.41%, 75.42%, 65.23%, 61.23% and 51.52% for 10 mM, 20 mM, 30

mM, 40 mM and 50 mM respectively. This result revealed that either **BPQ1** or both **BPQ1** and Cys were less cytotoxic effect to the living cells.

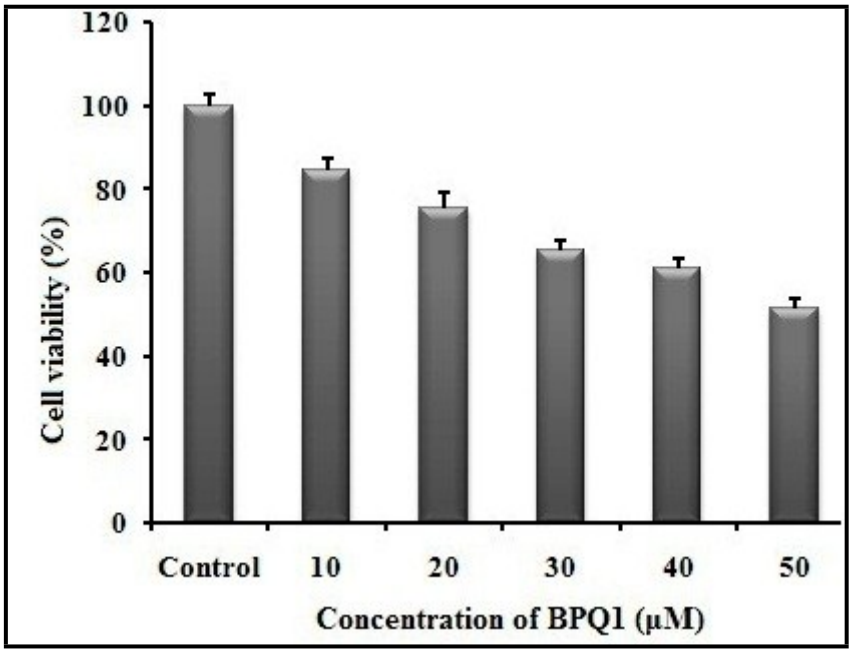


Fig. S27 Cell viability assay of MG63 cells to observe the cytotoxic effect of **BPQ1** and Cysteine.

Computational studies:

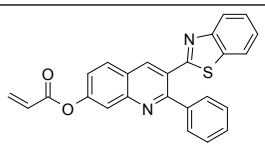
Table S1: Selected electronic excitation energies (eV), oscillator strengths (f) and main configurations of **BPQ1** and **BPQ1+Cys**. The data were calculated by TDDFT//B3LYP/6-311+G (*d,p*) based on the optimized ground state geometries.[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength. [c] H stands for HOMO and L stands for LUMO

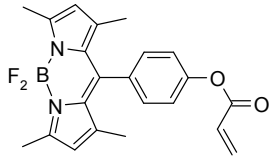
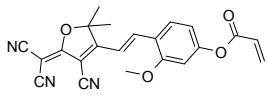
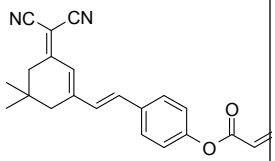
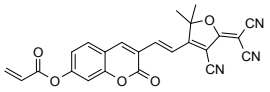
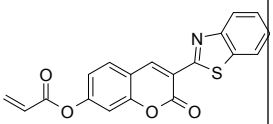
Molecules	Excitation Energy[a]	f[b]	Composition[c]	(composition) %
BPQ1	3.6750 eV 337.37 nm	0.3388	H → L	62.9
	4.3062 eV 287.92 nm	0.1532	H -2→ L+1	58.48
BPQ1+Cys	3.8929eV 401.49 nm	0.1917	H → L	65.51
	4.1252V 339.55 nm	0.1054	H → L+1	51.76

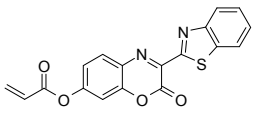
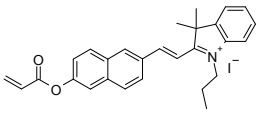
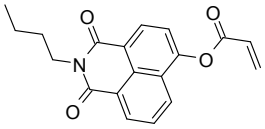
Table S2: Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of **BPQ1** and **BPQ1 + Cys.**

Species	**E_{HOMO} (a.u)**	**E_{LUMO}(a.u)**	**ΔE(a.u)**	**ΔE(eV)**	**ΔE(kcal/mol)**
BPQ1	-0.29381	-0.07501	0.2188	5.953	137.29
BPQ1+Cys	-0.22247	-0.06865	0.1538	4.184	96.50

Table-1 (Comparison of this work with some reported Cys probes)

Probe		**Fluorescence enhancement (fold)**	**Detection limit (μM)**	**Reaction time (mins)**	**Analyte**	**Strokes shift (nm)**	**Ref**
1	 Ex=410 nm, Em=506 nm	992	0.047	12	Cys	96	This work

2	 **Ex=480 nm, Em=517 nm**	23	0.05	5	Cys	37	[1]
3	 **(Ex=550 nm, Em=610 nm)**	NA	0.3	10	Cys	60	[2]
4	 **(Ex=557nm,Em=673 nm)**	NA	0.16	20	Cys	116	[3]
5	 **(Ex=574nm,Em=675 nm)**	60	0.2	10	Cys Hcy GSH	101	[4]
6	 **(Ex=458nm,Em=498 nm)**	NA	0.012	10	Cys Hcy GSH	40	[5]

7	 Ex=527nm, Em=560nm)	10	0.120	3	Cys	33	[6]
8	 (Ex=410nm,Em=595nm)	4	NA	25	Cys	185	[7]
9	 (Ex=452nm,Em=547 nm)	60	1.8	7	Cys Hcy	95	[8]

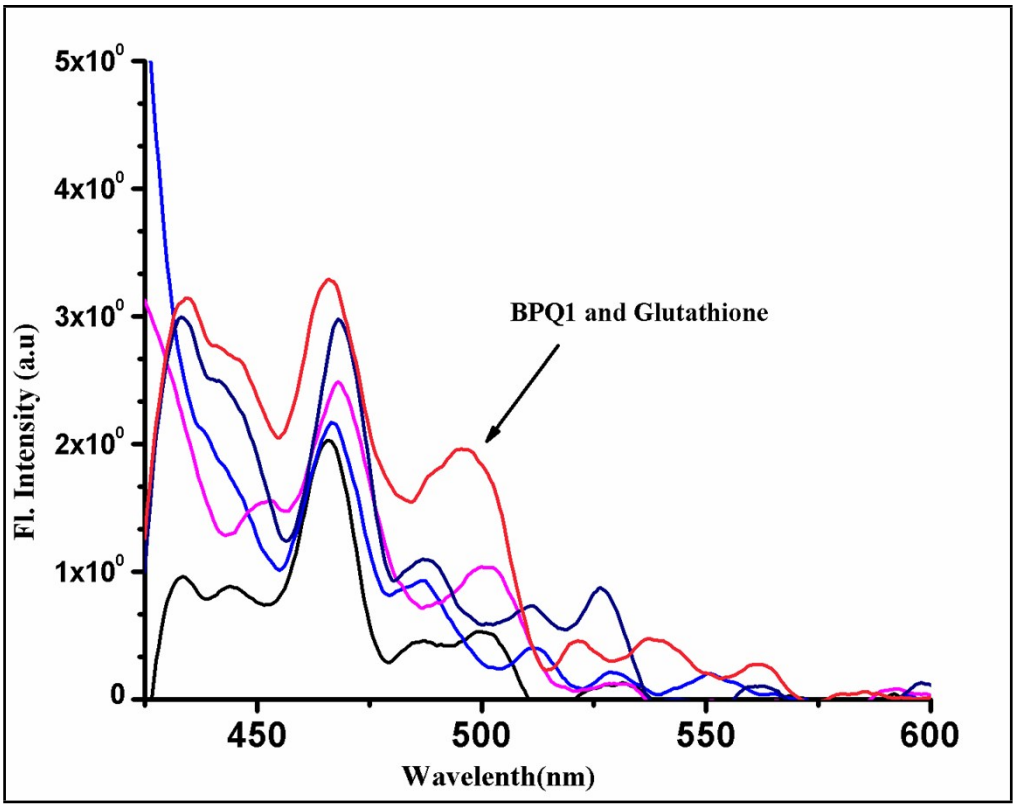


Fig. S28 (a) Fluorescence intensity changes of **BPQ1** (10 μM) using aqueous DMSO (7:3, v/v, 10 mM HEPES Buffer pH 7.4) upon addition of Glutathione (0-200 μM) with excitation at 410 nm.

References

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