Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting information

Superoxide Targeted "*Turn-On*" Fluorescence Sensing Enabled by Diselenide Based Quinoline Probe and *In Vitro* Anticancer Activity against Breast Cancer (MCF-7) and Human Lung Carcinoma (A549) Cells

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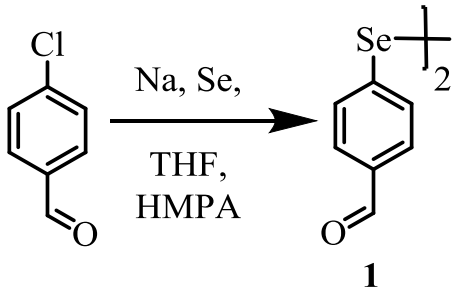
Synthesis



1

*Synthesis of 4,4'-diselanediyldibenzaldehyde (**1**):-*

4,4'-diselanediyldibenzaldehyde (**1**) was prepared by previously reported procedure.[1] Sodium diselenide (25 mmol) (in situ) was synthesized from sodium and selenium in the presence of catalytic amount of naphthalene in dry THF (30 mL). p-chlorobenzaldehyde (3.51 g, 25 mmol) and HMPA (10 mL) were added to this solution, stirred the reaction mixture for 6 hours and refluxed for 12 hours. After completion, the reaction was cooled to room temperature and quenched with small portion of water. The solvent was evaporated completely under vacuum and the crude was washed several times with water to get pure yellow solid and was recrystallized from DCM. Yield:- 3.62 g (28%). Spectroscopic data: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 9.95 (s, 2H), 7.76 (d, 4H), 7.61 (d, 4H).

Characterization

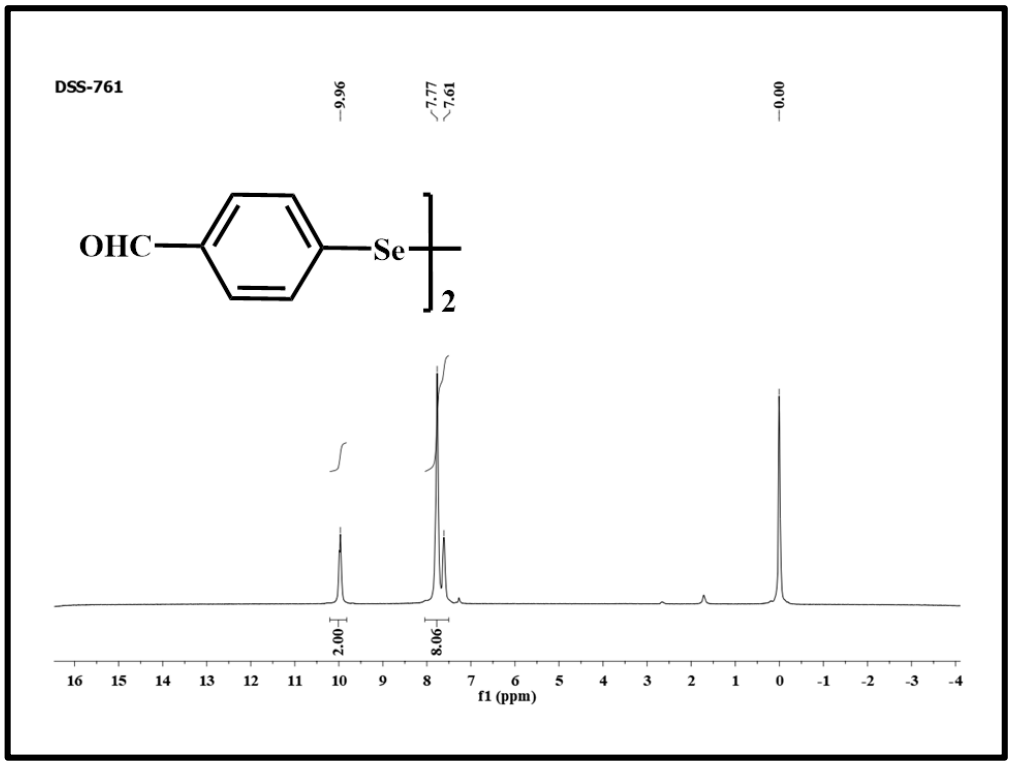


Figure:-S1. ^{1}H NMR spectrum of Compound **1**.

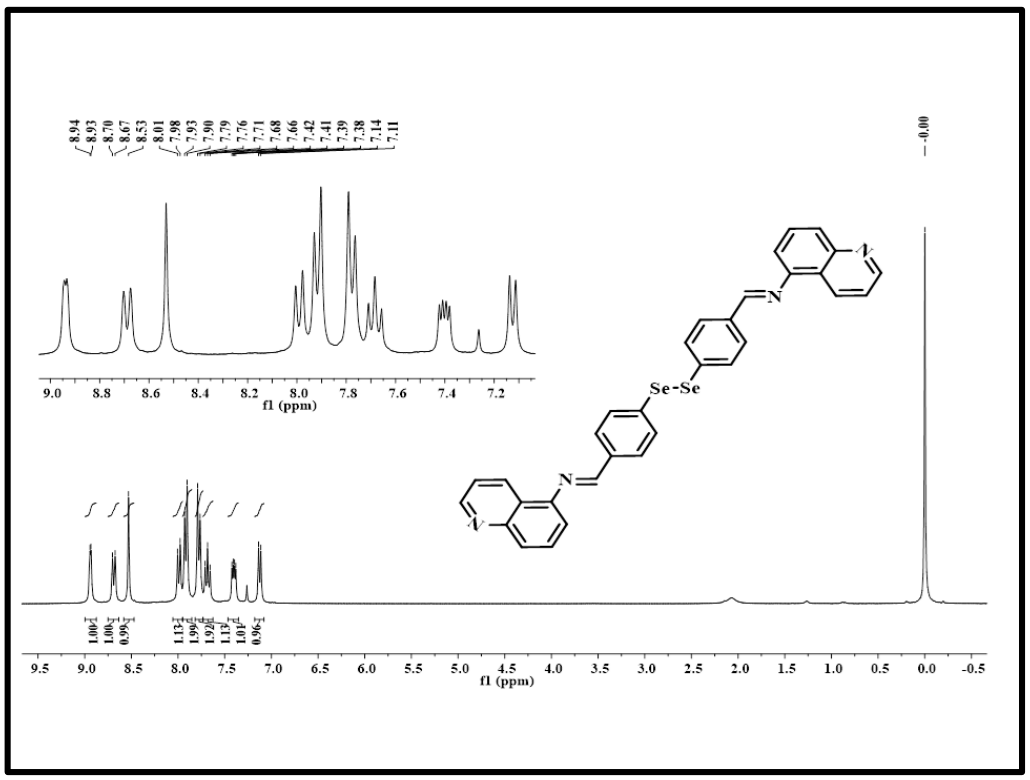


Figure:-S2. ^{1}H NMR spectrum of the probe.

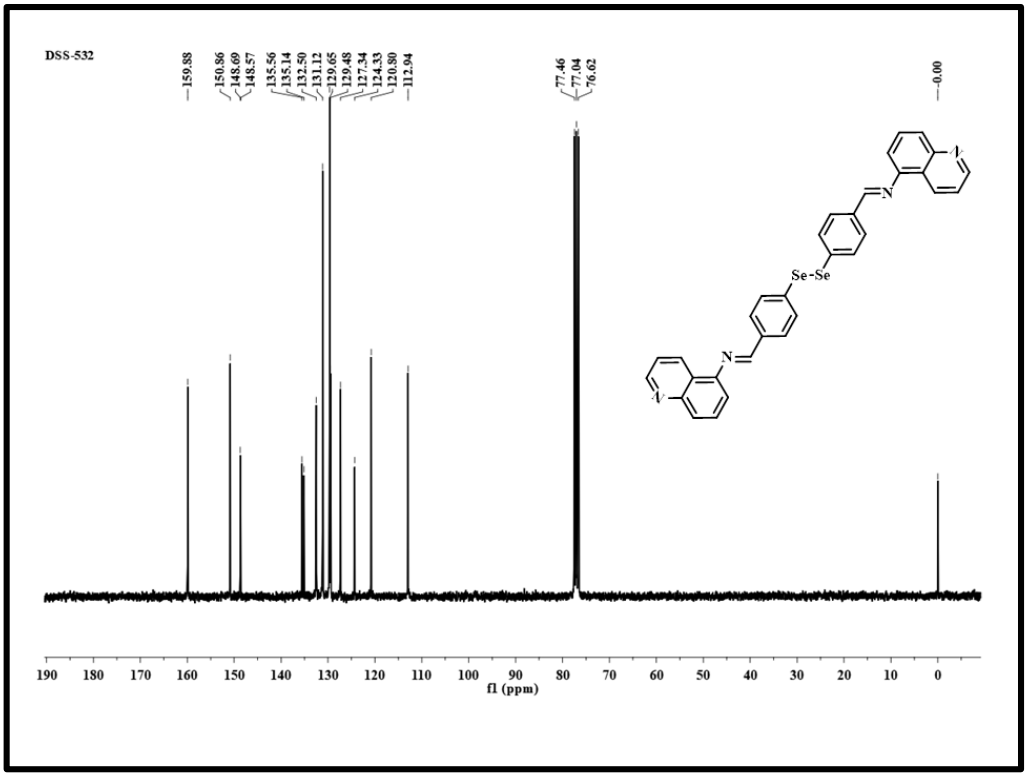


Figure:-S3. ^{13}C NMR spectrum of the probe.

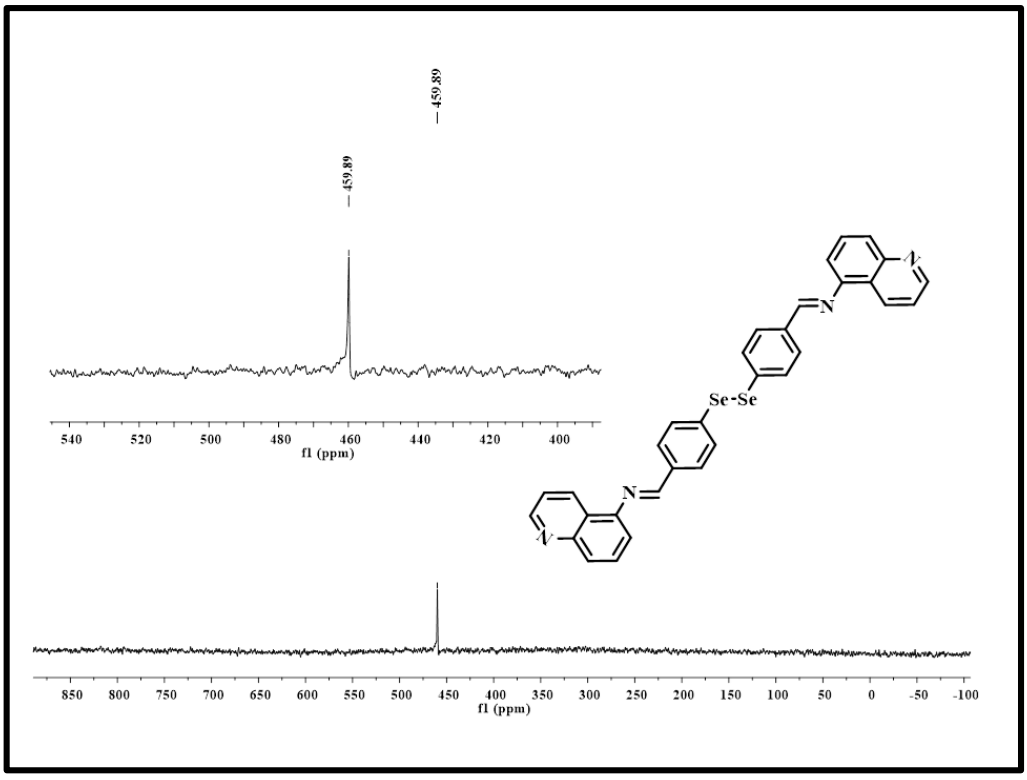


Figure:-S4. ^{77}Se NMR spectrum of the probe.

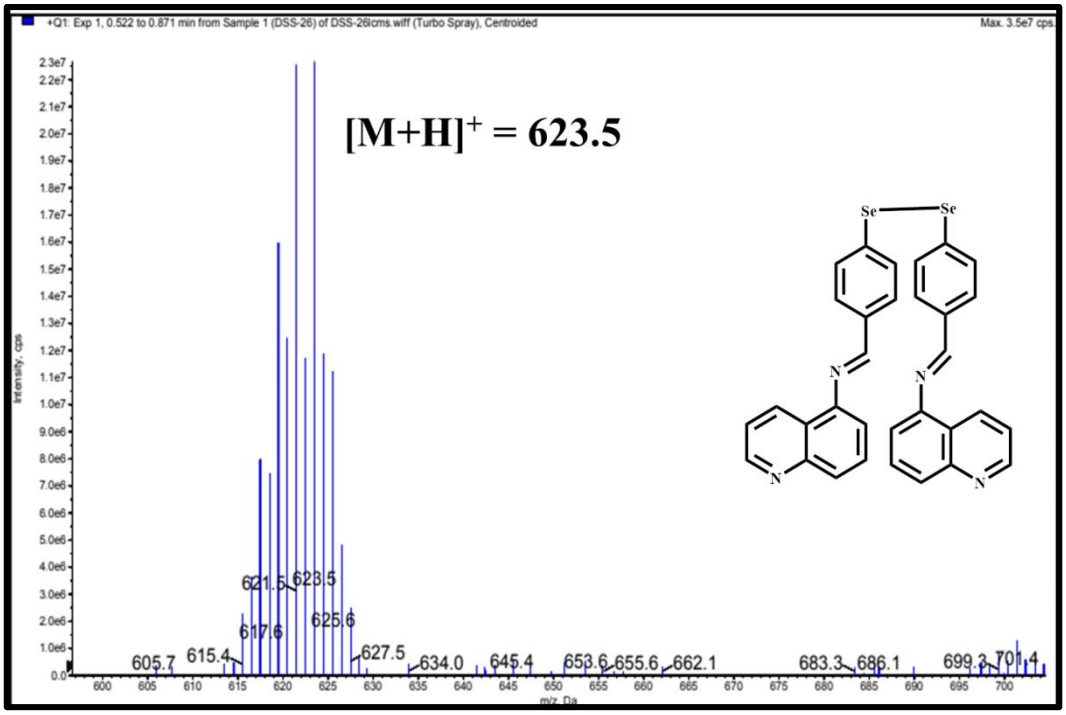


Figure:-S5. Mass spectra of the probe.

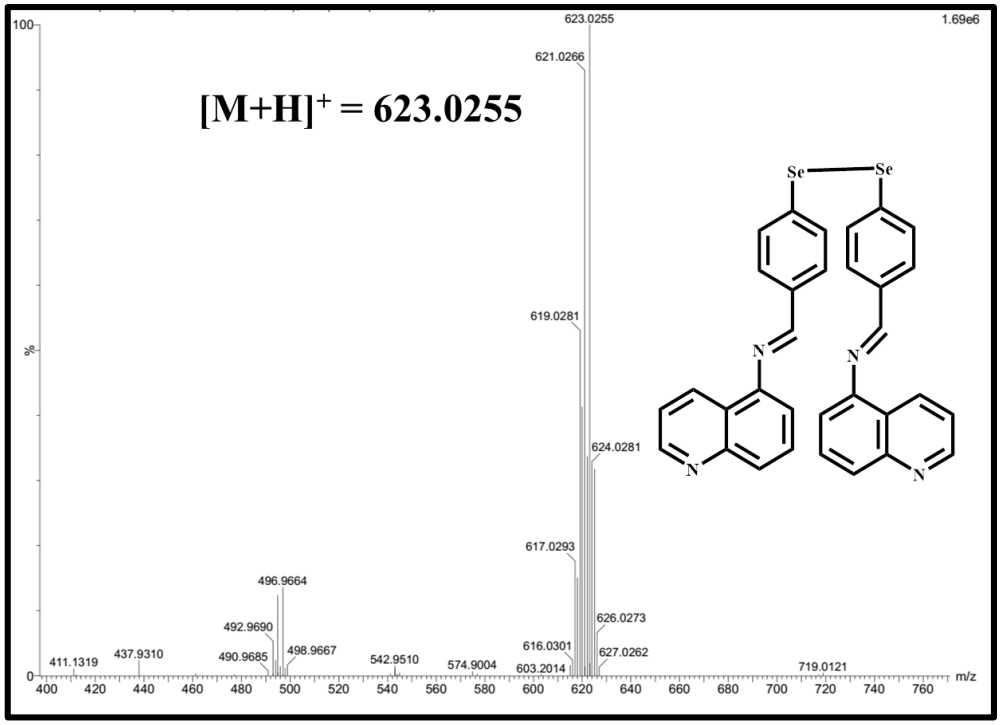


Figure:-S6. HRMS spectra of the probe.

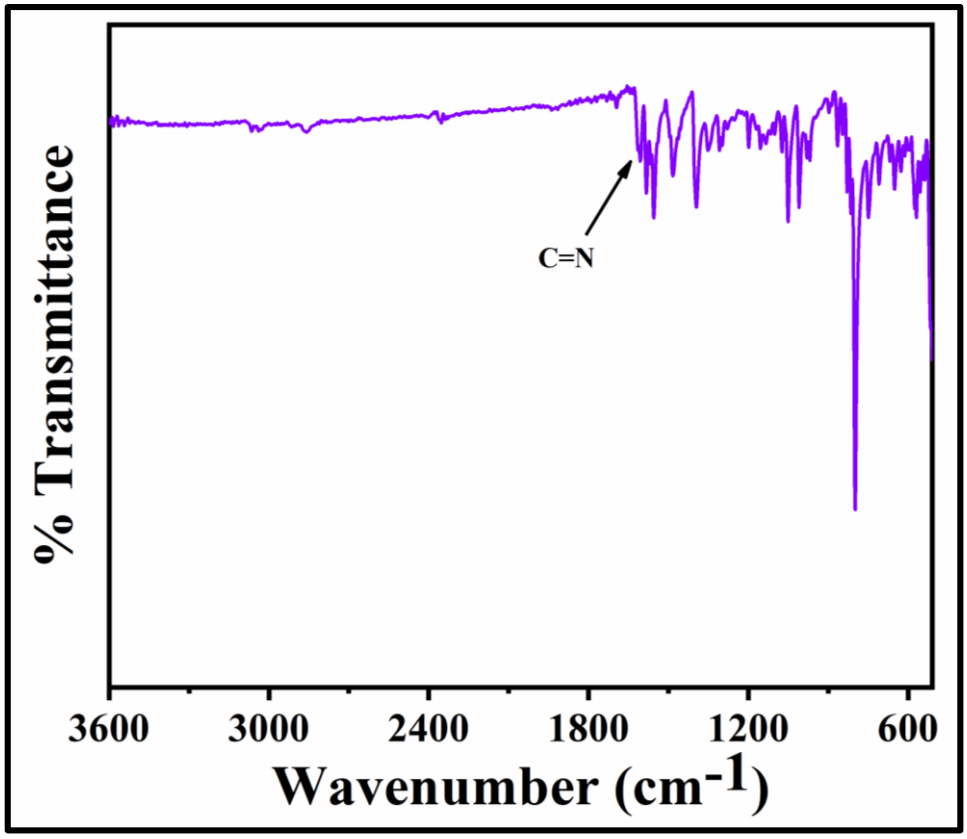


Figure:-S7. FTIR spectra of the probe.

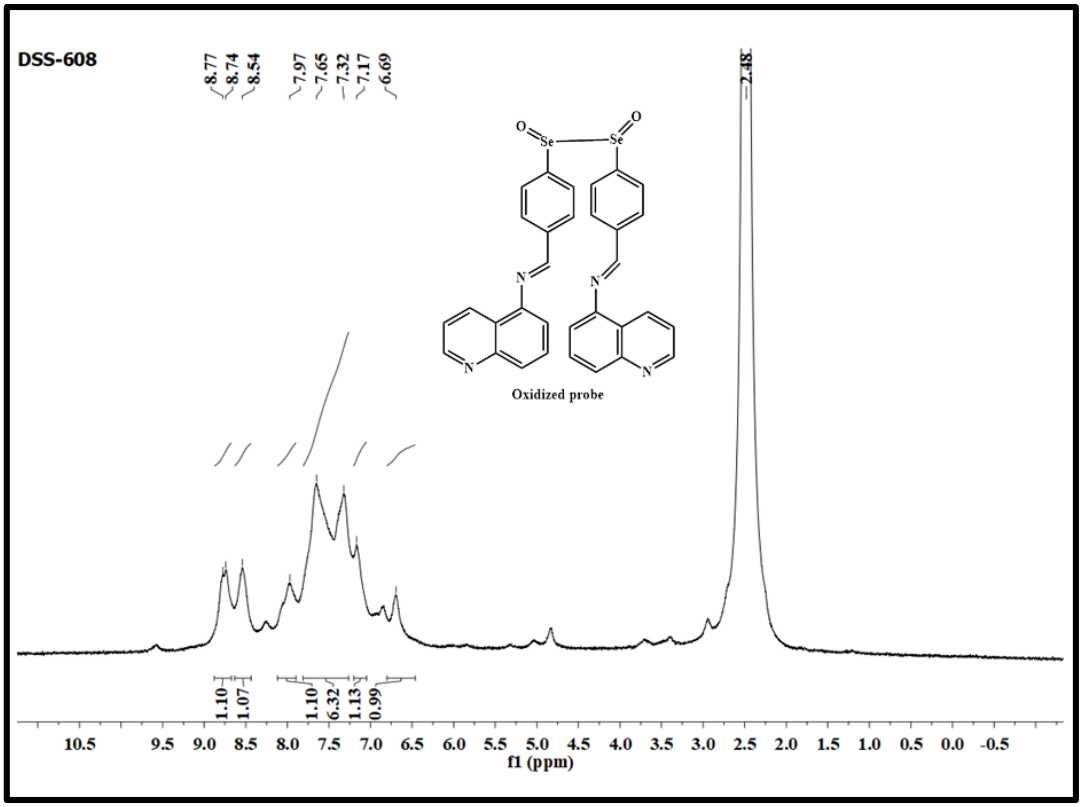


Figure:-S8. ^{1}H NMR spectrum of oxidized probe.

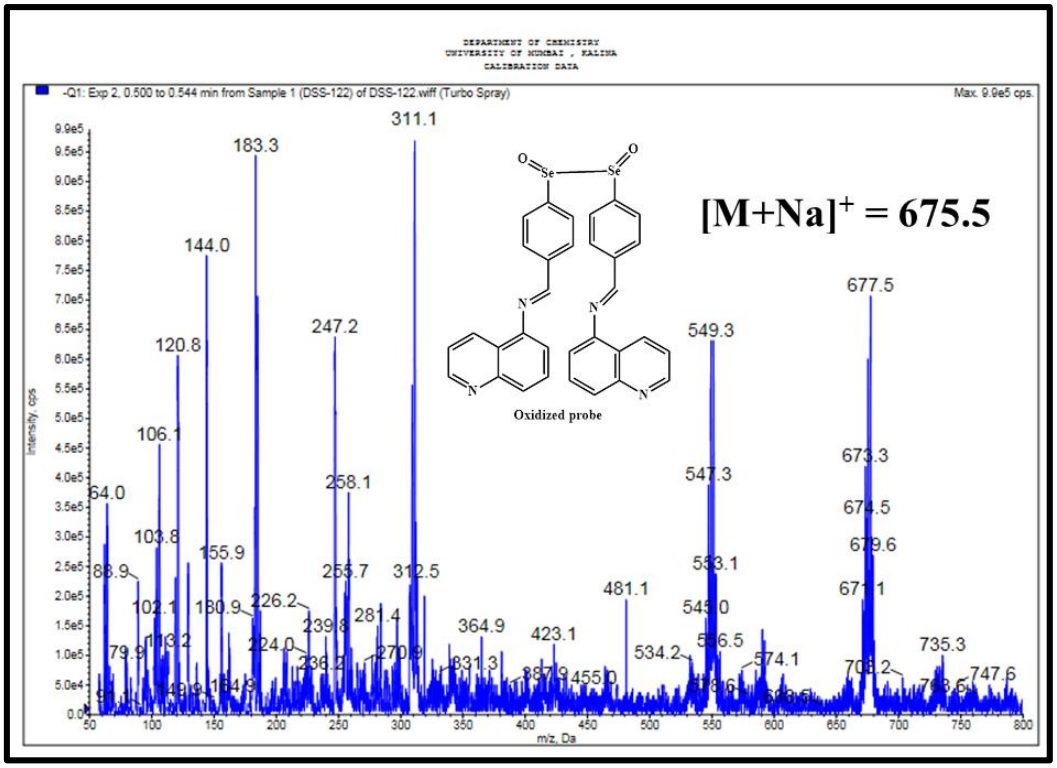


Figure:-S9. Mass spectra of oxidized probe.

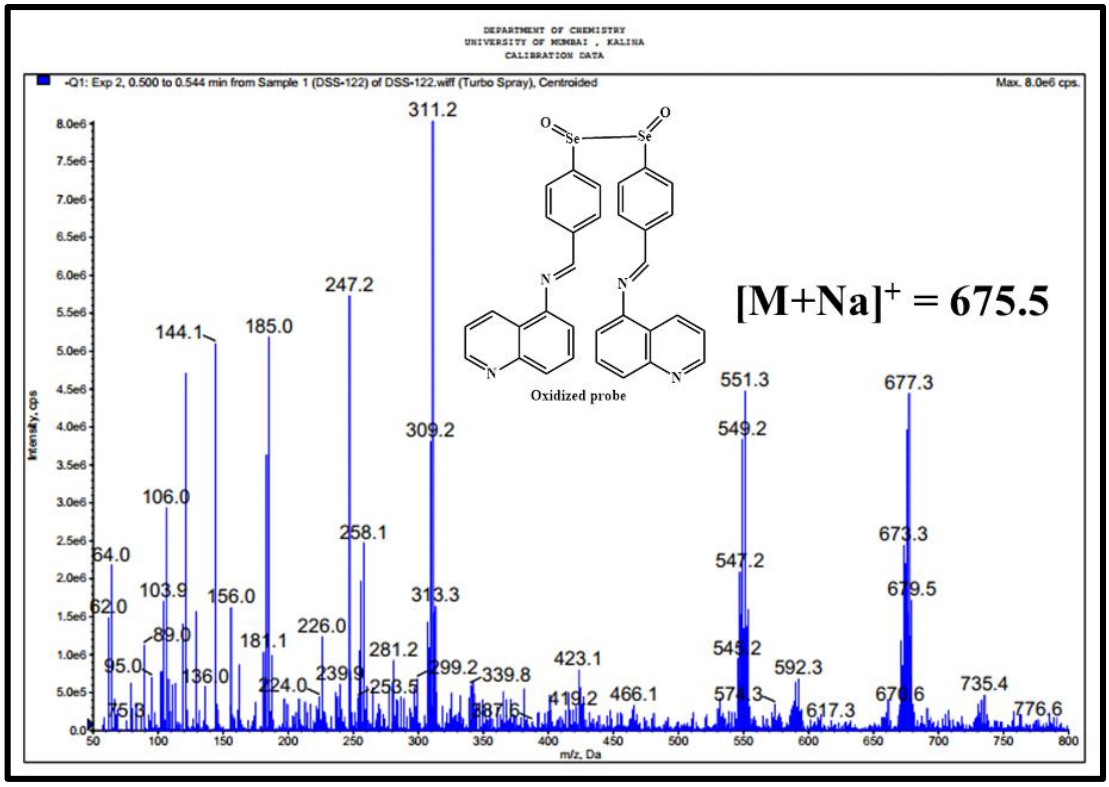


Figure:-S10. Mass spectra (Centroid) of oxidized probe.

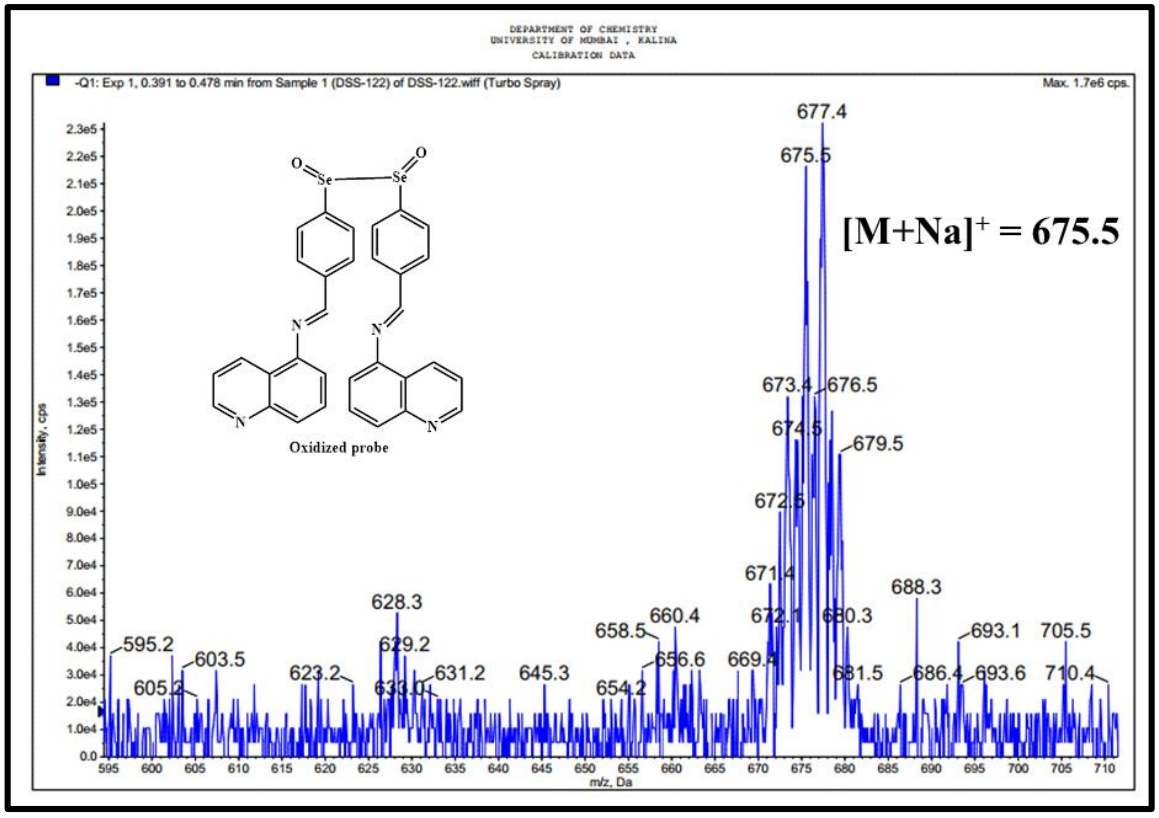


Figure:-S11. Expanded mass spectra of oxidized probe.

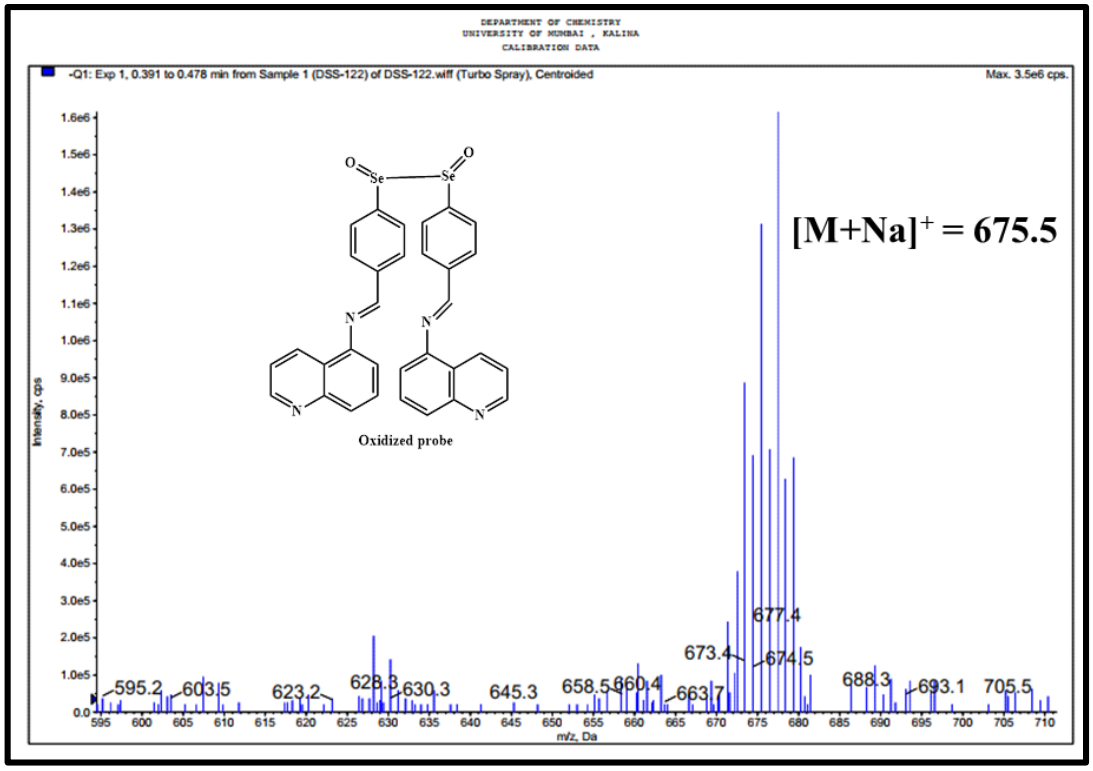


Figure:-S12. Expanded mass spectra (Centroid) of oxidized probe.

Photophysical Study

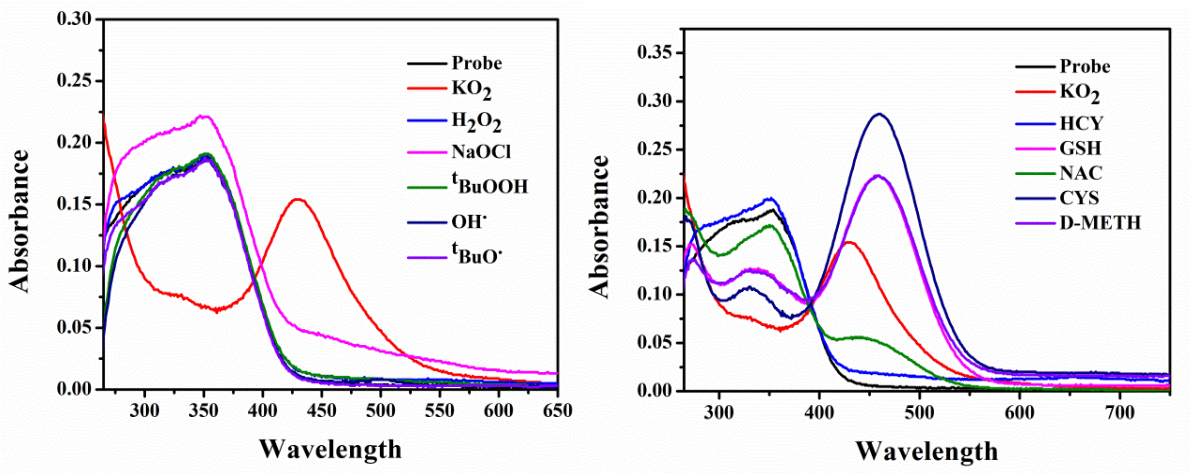


Figure:-S13. UV-visible absorption spectra of the probe (5 μM) with (a) ROS (Probe, $O_2^{\bullet-}$, NaOCl, H_2O_2, $^tBuO_2H$, $^{\bullet}OH$ and $^tBuO^{\bullet}$; 667 μM) and (b) Biothiols (Probe, $O_2^{\bullet-}$, HCY, GSH, NAC, CYS and D-METH; 667 μM) incubated for 40 min at rt.

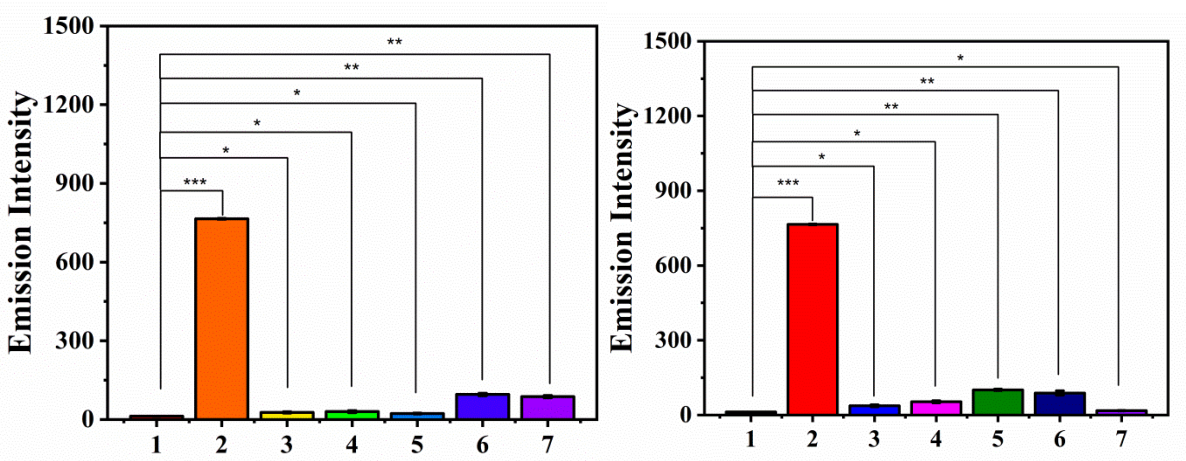


Figure:-S14. Selectivity study bar graph for fluorescence emission of the probe (5 µM) with (a) (1 = Probe, 2 = $O_2^{\bullet-}$,, 3 = NaOCl, 4 = H_2O_2, 5 = $^tBuO_2H$, 6 = $^{\bullet}OH$ and 7 = $^tBuO^{\bullet}$) and (b) Biothiols (1 = Probe, 2 = $O_2^{\bullet-}$, 3 = HCY, 4 = CYS, 5 = GSH, 6 = NAC, 7 = D-Meth) 667 µM incubated for 40 min at rt (λ_{ex} = 352 nm, λ_{em} = 511 nm), slit width 5/5 nm. Asterisks denote statistical significance *p<0.05, **p<0.01 and ***p<0.001.

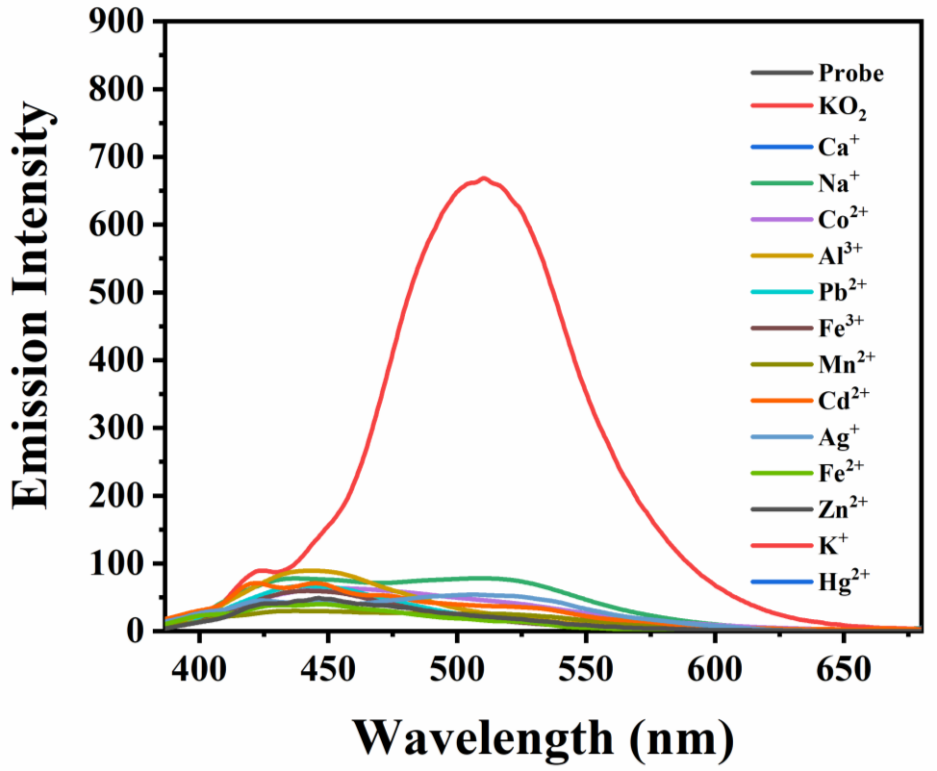


Figure:-S15. Emission spectra of the probe (5 μM) in DMSO with Metal ions ($O_2^{\bullet-}$, Ca^+, Na^+, Co^{2+}, Al^{3+}, Pb^{2+}, Fe^{3+}, Mn^{2+}, Cd^{2+}, Ag^+, Fe^{2+}, Zn^{2+}, K^+, Hg^{2+}) of 667 μM concentration after incubation time of 40 min at rt (λ_{ex} = 352 nm, λ_{em} = 511 nm), slit width 5/5 nm respectively.

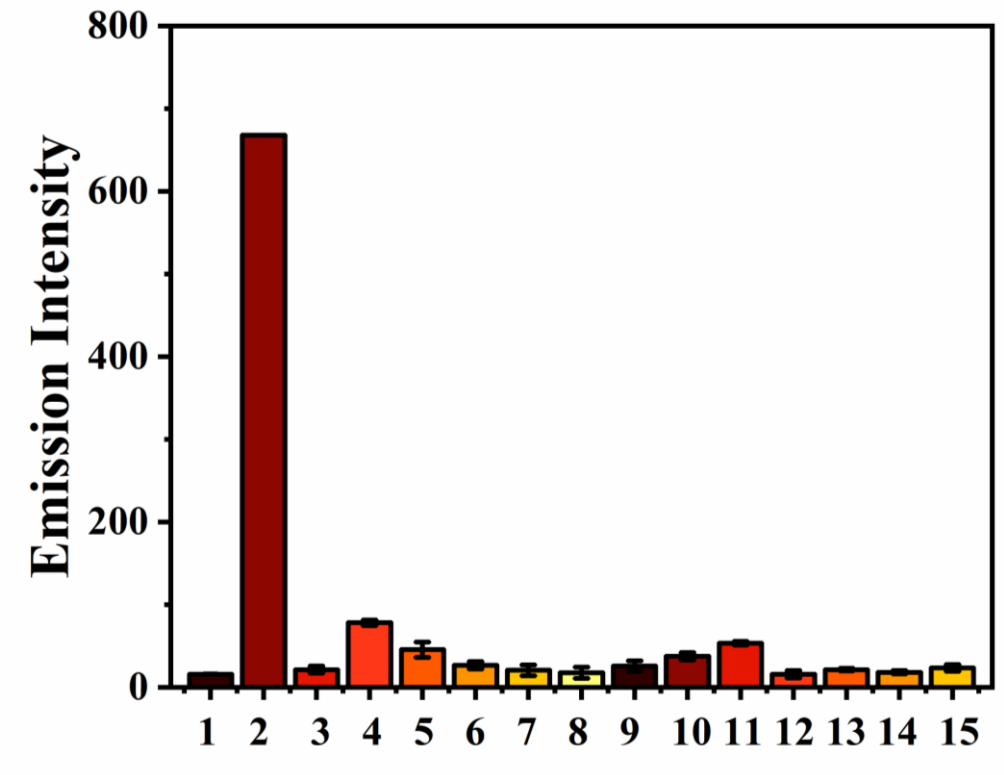


Figure:-S16. Selectivity study bar graph for fluorescence emission of the probe (5 μM) with (1 = probe, 2 = KO_2, 3 = Ca^+, 4 = Na^+, 5 = Co^{2+}, 6 = Al^{3+}, 7 = Pb^{2+}, 8 = Fe^{3+}, 9 = Mn^{2+}, 10 = Cd^{2+}, 11 = Ag^+, 12 = Fe^{2+}, 13 = Zn^{2+}, 14 = K^+, 15 = Hg^{2+}) 667 μM incubated for 40 min at rt (λ_{ex} = 352 nm, λ_{em} = 511 nm), slit width 5/5 nm.

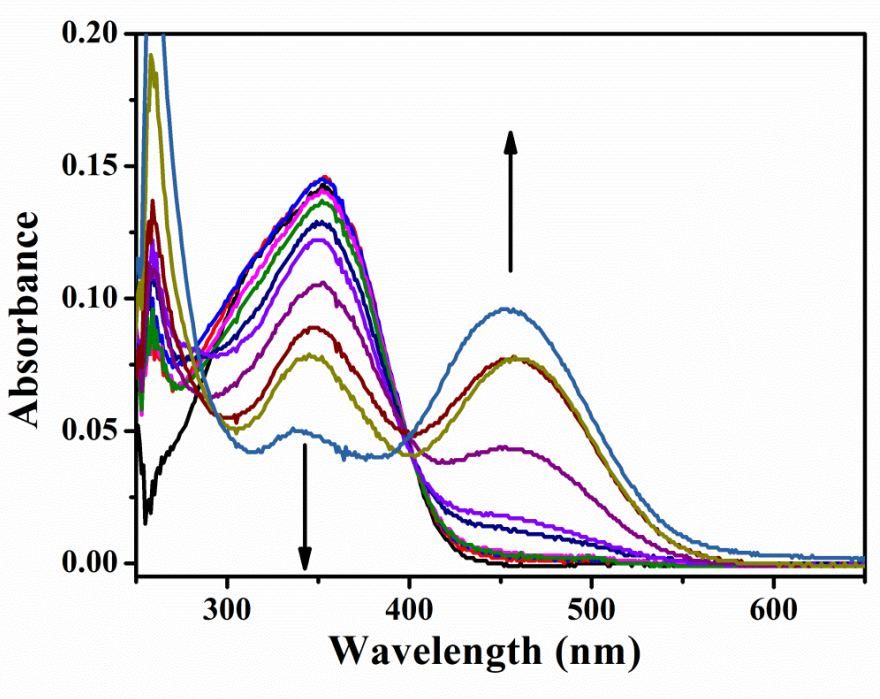


Figure:-S17. UV–vis spectral changes of titration of the probe (5 μM) with superoxide (0-13 μM) incubated for 40 min at rt.

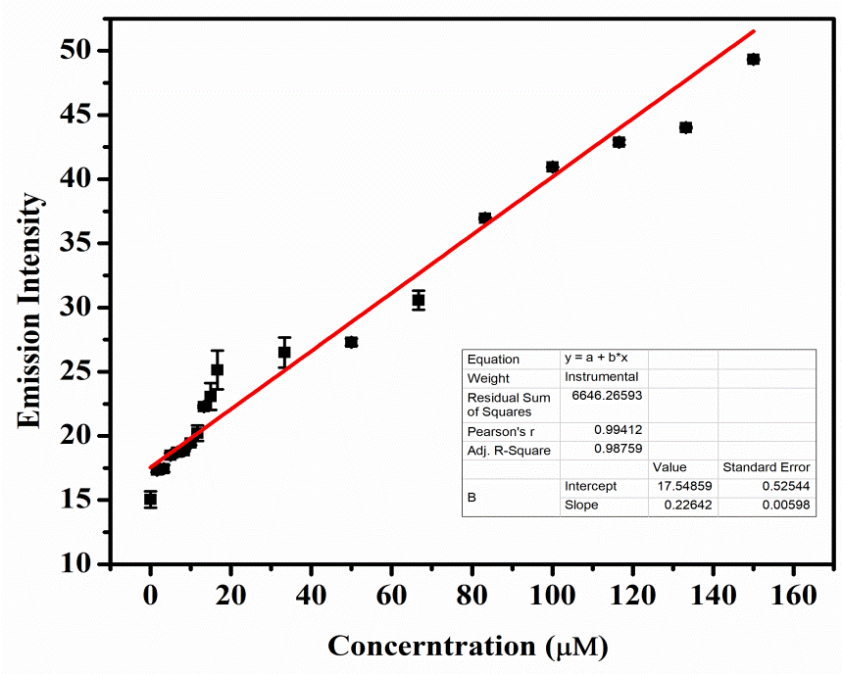


Figure:-S18. Plot for the calculation of detection limit of the probe (5 μM) with increasing concentration of NaOCl (0-150 μM) incubated for 40 min at rt (λ_{ex} = 352 nm, λ_{em} = 511 nm), slit width 5/5 nm.

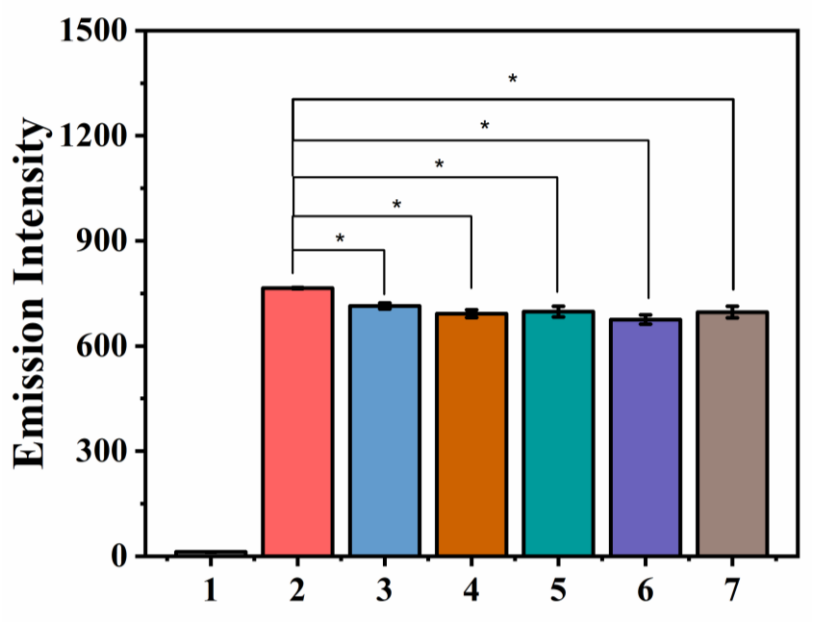


Figure:-S19. Emission spectra of the probe (5 μM) with biothiols (HCY, CYS, GSH, NAC, D-Meth) (667 μM) and KO_2 (667 μM) incubated for 40 min at rt (λ_{ex} = 352 nm, λ_{em} = 511 nm), slit width 5/5 nm respectively in DMSO (1 = probe, 2 = probe + KO_2, 3 = probe + KO_2 + HCY, 4 = probe + KO_2 + CYS, 5 = probe + KO_2 + GSH, 6 = probe + KO_2 + NAC, 7 = probe + KO_2+ D-Meth). Asterisks denote statistical significance *p<0.05.

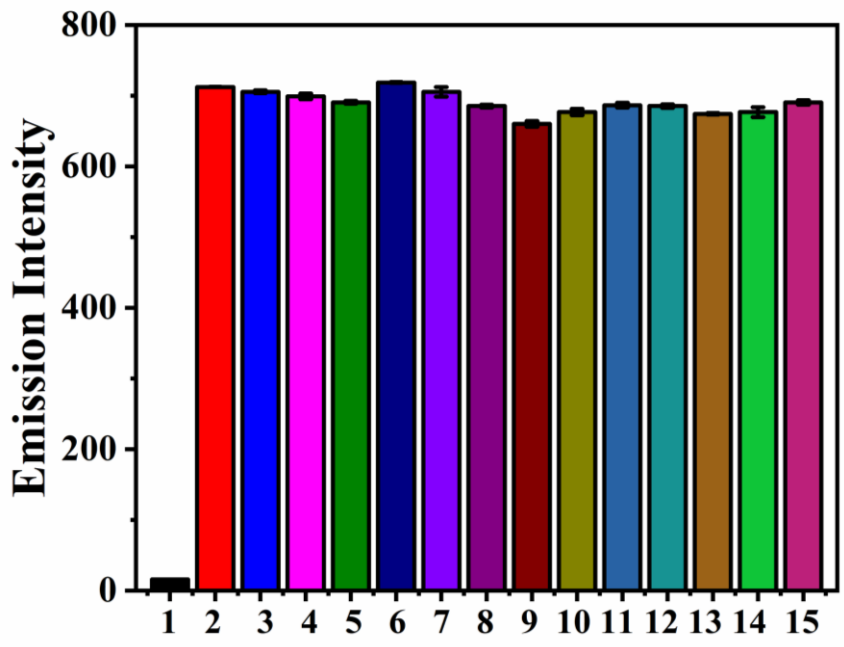


Figure:-S20. Emission spectra of the probe (5 μM) with biothiols ($O_2^{\bullet-}$, Ca^+, Na^+, Co^{2+}, Al^{3+}, Pb^{2+}, Fe^{3+}, Mn^{2+}, Cd^{2+}, Ag^+, Fe^{2+}, Zn^{2+}, K^+, Hg^{2+}) and KO_2 (667 μM) incubated for 40 min at rt (λ_{ex} = 352 nm, λ_{em} = 511 nm), slit width 5/5 nm respectively in DMSO (1 = probe, 2 = probe + KO_2, 3 = probe + KO_2 + Ca^+, 4 = probe + KO_2 + Na^+, 5 = probe + KO_2 + Co^{2+}, 6 = probe + KO_2 + Al^{3+}, 7 = probe + KO_2+ Pb^{2+}, 8 = probe + KO_2 + Fe^{3+}, 9 = probe + KO_2 + Mn^{2+}, 10 = probe + KO_2 + Cd^{2+}, 11 = probe + KO_2 + Ag^+, 12 = probe + KO_2+ Fe^{2+}, 13 = probe + KO_2 + Zn^{2+}, 14 = probe + KO_2 + K^+, 15 = probe + KO_2 + Hg^{2+}).

MTT Assay

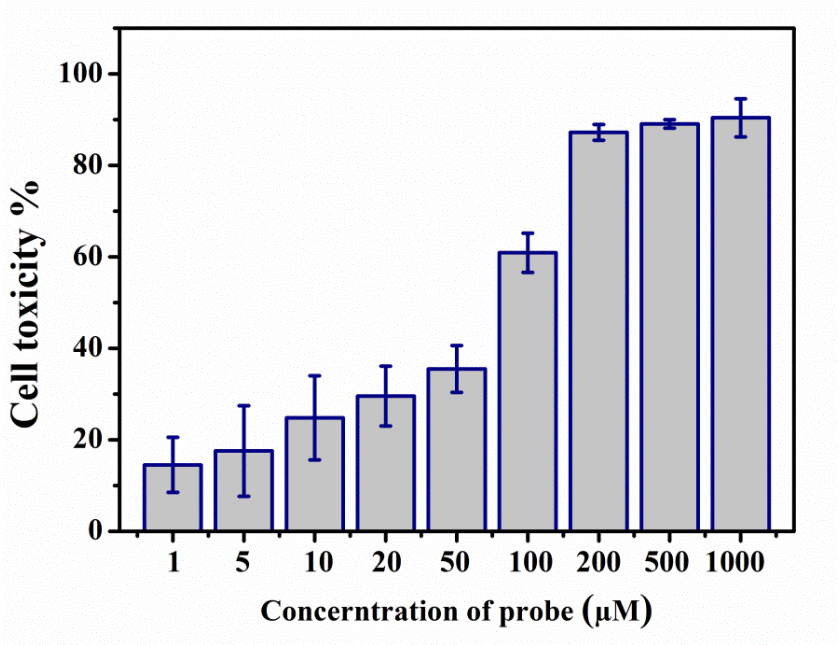


Figure:-S21. Concentration dependent cell toxicity assay for Madin-Darby canine kidney cells (MDCK) incubated with the probe.

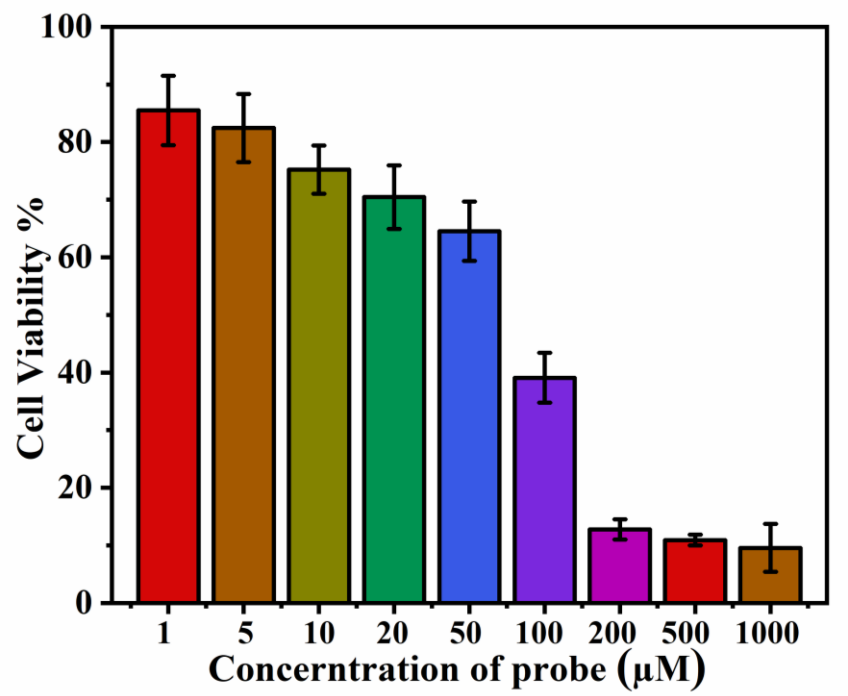


Figure:-S22. Concentration dependent cell viability assay for Madin-Darby canine kidney cells (MDCK) incubated with the probe.

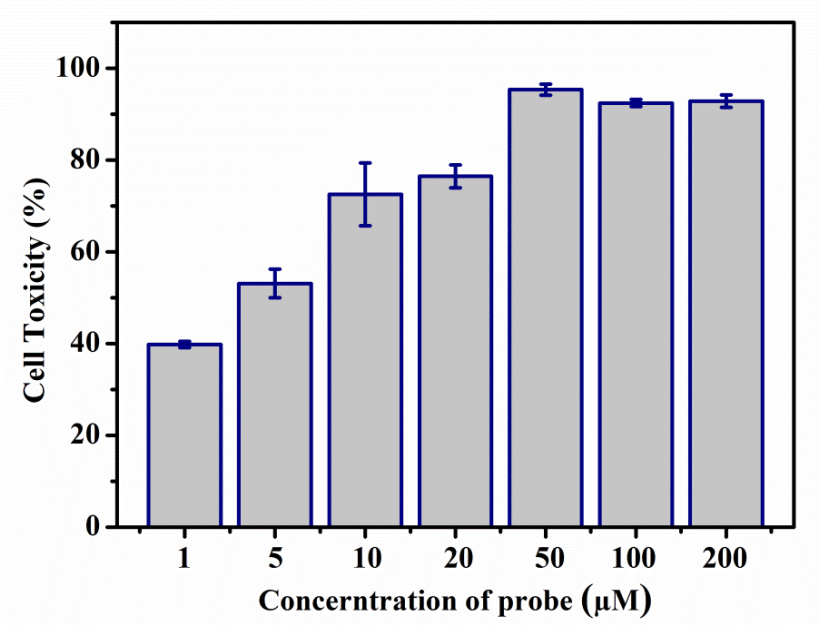


Figure:-S23. Concentration dependent cell toxicity assay for Adenocarcinomic human alveolar basal epithelial cells (A549) incubated with the probe.

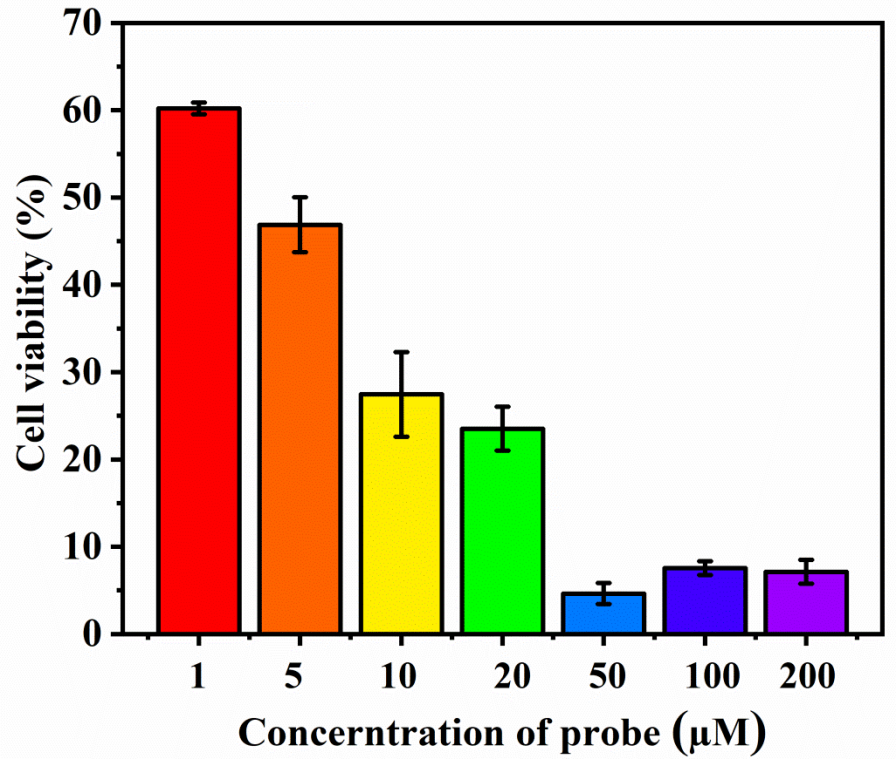


Figure:-S24. Concentration dependent cell vaibility assay for Adenocarcinomic human alveolar basal epithelial cells (A549) incubated with the probe.

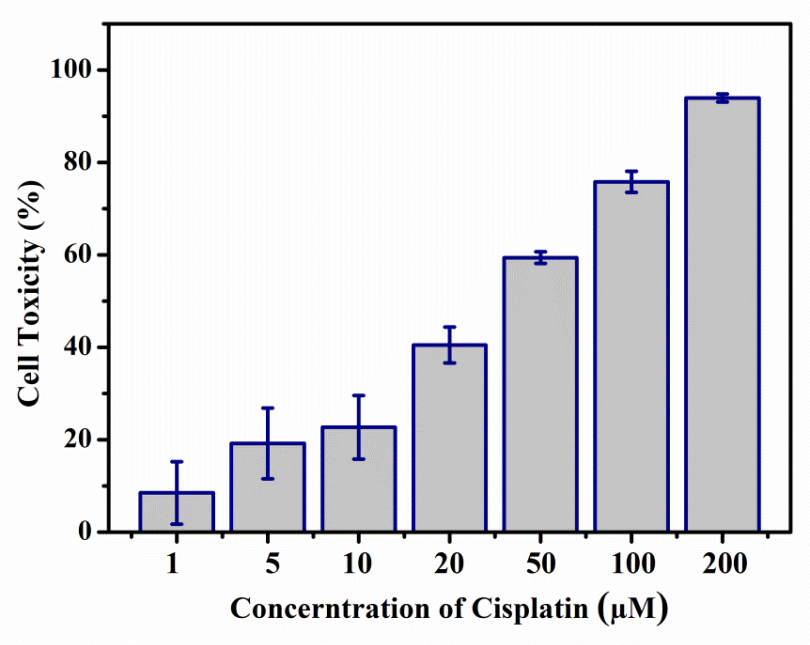


Figure:-S25. Concentration dependent cell toxicity assay Adenocarcinomic human alveolar basal epithelial cells (A549) incubated with cisplatin.

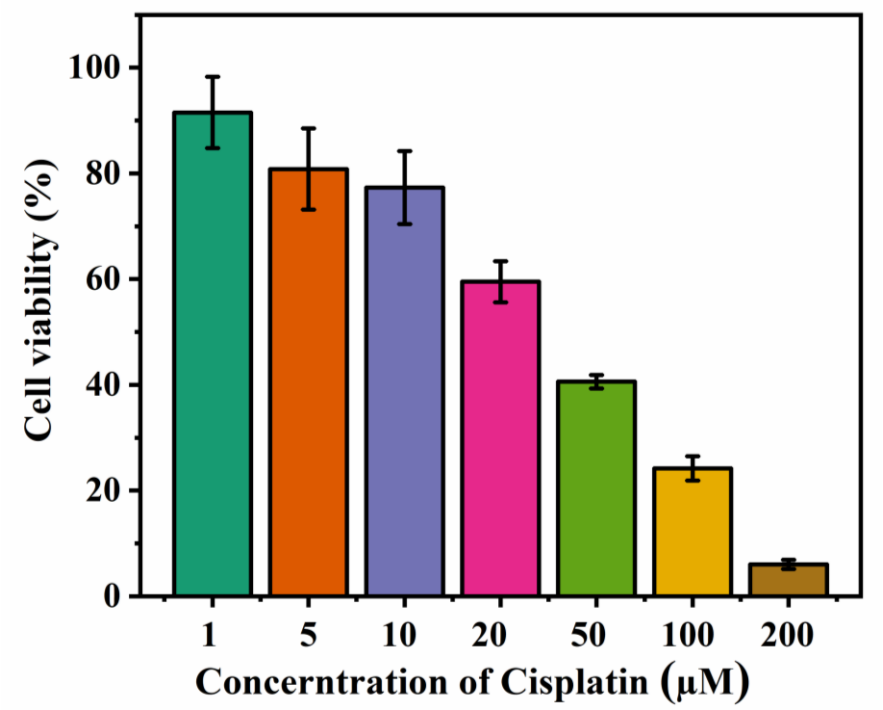


Figure:-S26. Concentration dependent cell viability assay Adenocarcinomic human alveolar basal epithelial cells (A549) incubated with cisplatin.

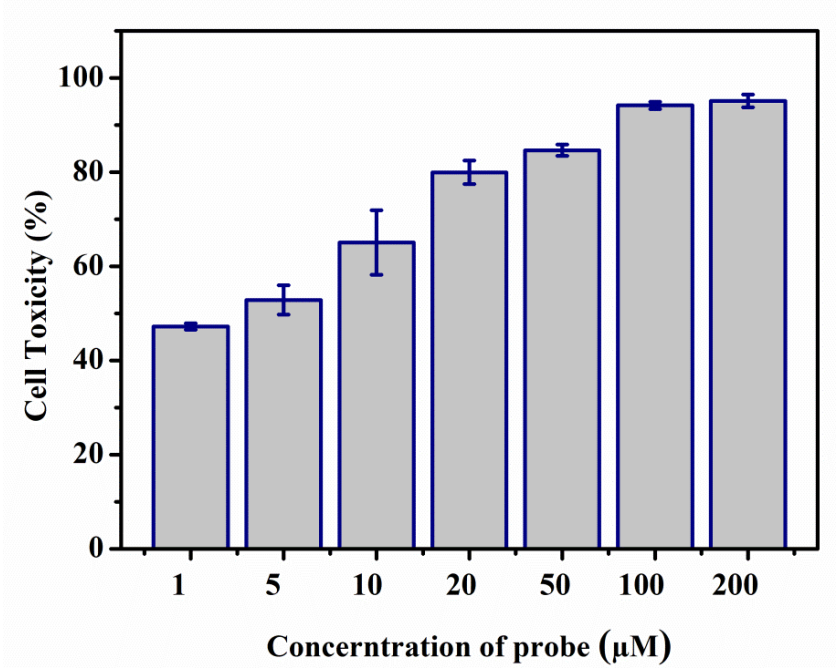


Figure:-S27. Concentration dependent cell toxicity assay for breast cancer cells (MCF-7) incubated with the probe.

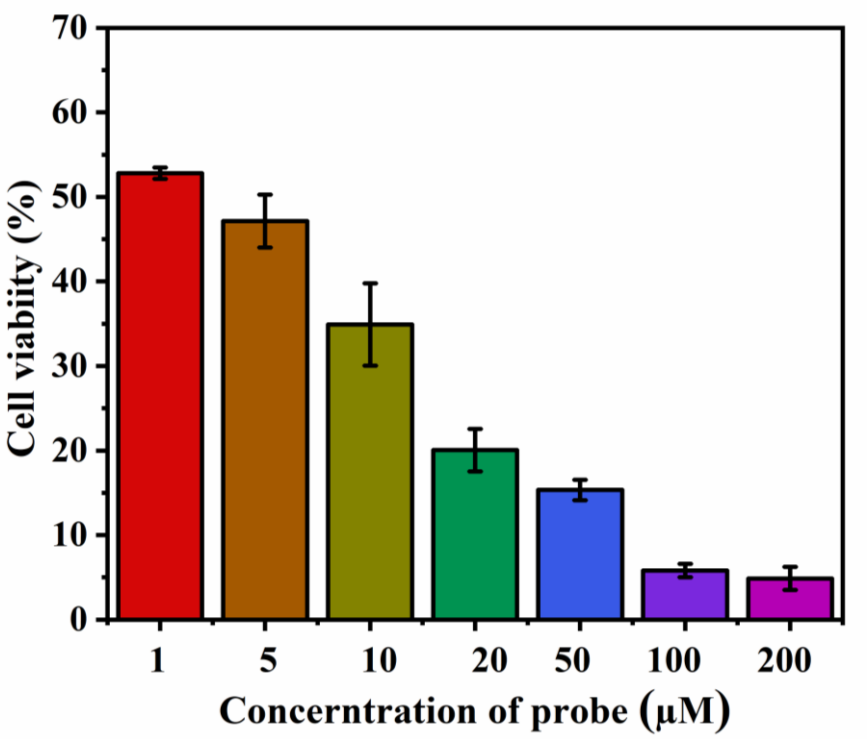


Figure:-S28. Concentration dependent cell viability assay for breast cancer cells (MCF-7) incubated with the probe.

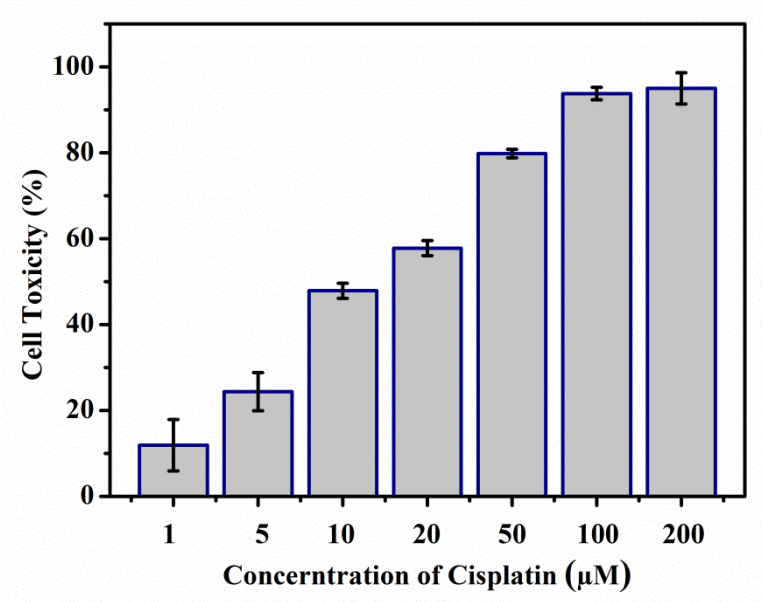


Figure:-S29. Concentration dependent cell toxicity assay for breast cancer cells (MCF-7) incubated with cisplatin.

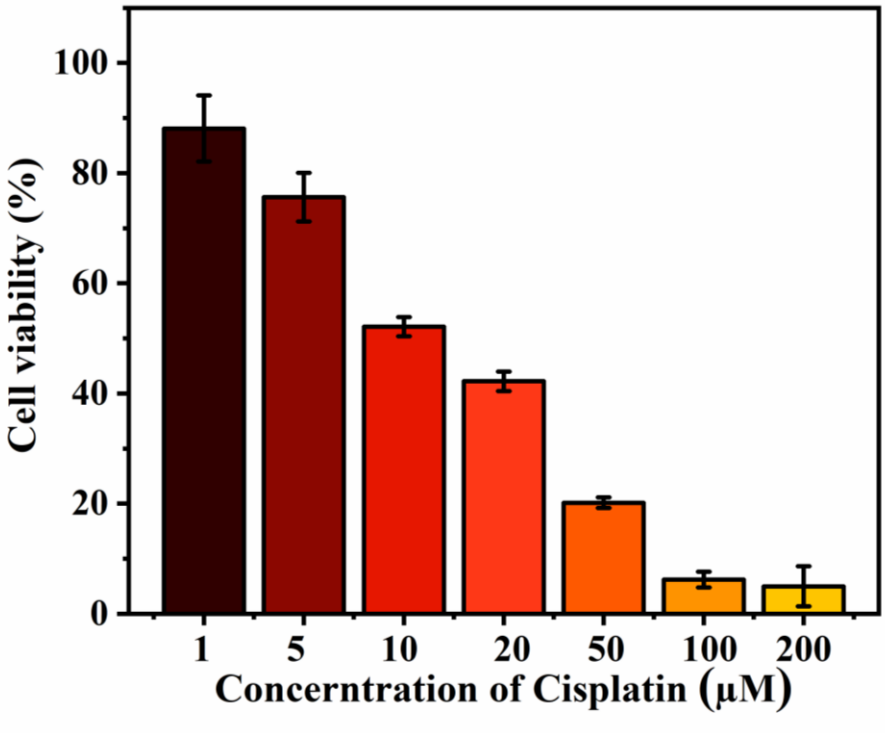


Figure:-S30. Concentration dependent cell viability assay for breast cancer cells (MCF-7) incubated with cisplatin.

Microscopic Photographs

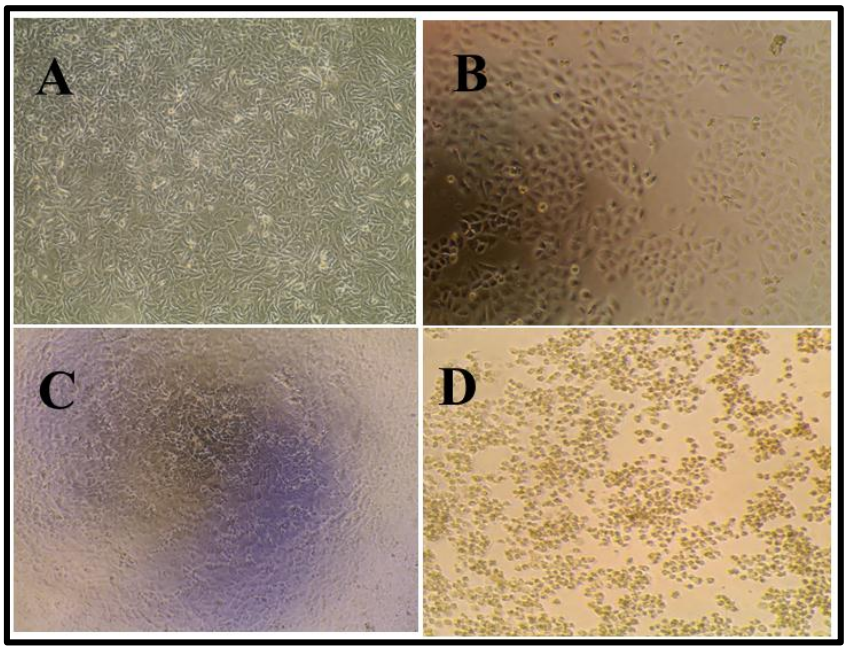


Figure:-S31. Microscopic photographs of A549 cells treated with cisplatin at concentrations of (A) cell control (B) 1 μM, (C) 10 μM and (D) 100 μM. Image credit EVOS™ XL Core Imaging System (400X).

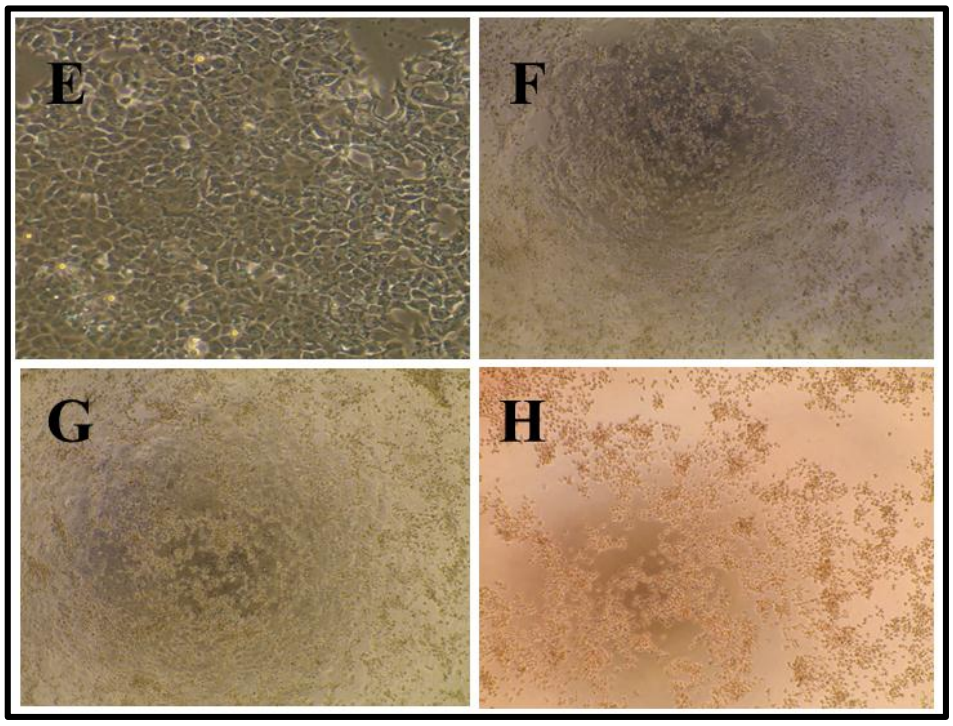


Figure:-S32. Microscopic photographs of MCF-7 cells treated with cisplatin at concentrations of (E) cell control (F) 10 μM, (G) 20 μM and (H) 100 μM. Image credit EVOS™ XL Core Imaging System (400X).

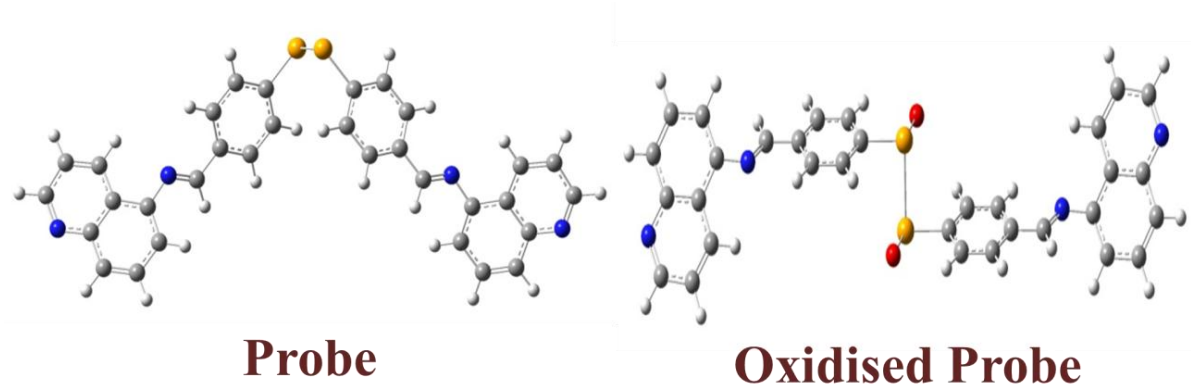


Figure:-S33. DFT-optimized structures of the probe and the oxidised probe at B3LYP/6-31G(d) level of theory.

Table S1:- Selected bond distances (R), bond angles (A) and dihedral angles in the probe and oxidised probe.

The Probe	Oxidised Probe
R(Se-Se) = 2.351 Å	R(Se-Se) = 2.559 Å
R(Se-C) = 1.925 Å	R(Se-C) = 1.943 Å
A(Se-Se-C) = 100.3°	A(Se-Se-C) = 89.5°
D(C-Se-Se-C) = 81.7°	D(C-Se-Se-C) = 180.0°
	R(Se-O) = 1.667 Å
	A(Se-Se-O) = 103.1°

Reference

1 S. Panda, S. S. Zade, H. B. Singh and G. Wolmershäuser, *J. Organomet. Chem.,* 2005, **690**, 3142-3148.