Supporting Information

Synthesis and Single Crystal X-ray Study of Phenylselenyl Embedded Coumarin-Based Sensors for Selective Detection of Superoxide

*Gauri S. Malankar,[a] Divyesh S. Shelar,[a] R. J. Butcher,[b] Sudesh T. Manjare[a]**

[a]Department of Chemistry, University of Mumbai, Mumbai, 400098, India

[b]Howard University, Washington DC, USA

Corresponding Author

* Email id: sudeshmanjare@chemistry.mu.ac.in

TABLE OF CONTENTS

EXPERIMETAL SECTION

Generation of ROS

ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$) of 0.1 M concentration were prepared using distilled water.

Generation of $O_2^{\bullet-}$: Commercial solid potassium superoxide was used as source of the superoxide radical anion.

Generation of ^{-}OCl: The source of NaOCl was commercial bleach and was diluted with deionized water.

Generation of H_2O_2: The commercial available hydrogen peroxide solution was diluted with deionized water.

Generation of $^{t}BuO_2H$: The commercial available *tert*-Butyl hydroperoxide solution was diluted with deionized water.

Generation of $^{\bullet}OH$: Hydroxyl radical (•OH) was generated by Fenton reaction. Ferrous sulphate was added to generate •OH in the presence of 10 equiv of H_2O_2. The $^{\bullet}OH$ concentration was equivalent to that of Fe(II) concentration.

Generation of $^{t}BuO^{\bullet}$: tert-Butoxide radical (^{t}BuO•) was also generated by Fenton reaction. It was generated by using tert-Butyl hydroperoxide in presence of ferrous sulphate.

Quantum yield

Quantum yield was calculated according to the formula (1),

$$\phi = (\phi_R)\ (I/I_R)\ (A_R/A)\ (\eta^2/\eta_R^2) \qquad (1)$$

Where, ϕ is the quantum yield, I is integrated area under the corrected emission spectra, A is absorbance at excitation wavelength, η is refractive index. The subscript R refers to the reference fluorophore of known quantum yield.[1] Anthracene diluted with ethanol used as a standard, which has quantum yield of 0.27. The excitation and emission slit width used for the experiment is 3 nm/3 nm.

pH study

The pH dependent experiment was carried out in 1 mM PBS buffer of pH range 4-12 with both the probes and the probes with superoxide. The solutions of probe **4** were incubated for 10 min and solutions of probe **5** were incubated for 30 min. Further, fluorescence measurements were recorded.

Time dependent study

The time dependent study of probe **4** was performed using 10 μM of probe **4** solution and 167 μM of superoxide solution. The spectrum was recorded for 30 min. The excitation used for the experiment was 388 nm with slit width 5 nm/5 nm. Time dependent study of probe **5** was carried out using probe **5** solution (20 μM) and 167 μM of superoxide solution. The spectrum was recorded for 1 h at an excitation maxima 380 nm using slit width 5 nm/5 nm.

Detection limit

Increasing concentration study was performed to calculate detection limit of both the probes. To the solution of probe **4**, 0 - 7 equivalent of superoxide solution was added while in the solution of probe **5**, 0 - 5 equivalent of superoxide solution was added in increasing fashion. The solutions of probes **4** and **5** were incubated for 10 and 30 min, respectively. Detection limits were calculated using the equation (2),

$$\text{Detection limit} = 3\sigma/k \qquad (2)$$

Where σ is standard deviation

k is slope.

Standard deviation was calculated by taking 10 readings of the probes solutions.

Interference study

For this experiment, in the solutions of probes **4** (10 μM) and **5** (20 μM) with 167 μM of superoxide, the other ROS ($^{-}$OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$, 167 μM) were added. The spectra were recorded after incubation.

Reversibility study

To check the reversibility of the oxidized probes in presence of biothiols, the probes were reacted with 167 μM of superoxide and then 167 μM of biothiols (DL-homocysteine, L-cysteine, glutathione (GSH) and N-acetyl-L-cysteine) were added to the solution and incubated for another 10 min, then the spectra were recorded. Further to check the redox

cycles of the probes **4** and **5** the same experiment was performed continuously with superoxide and GSH/NAC.

CHARACTERIZATION

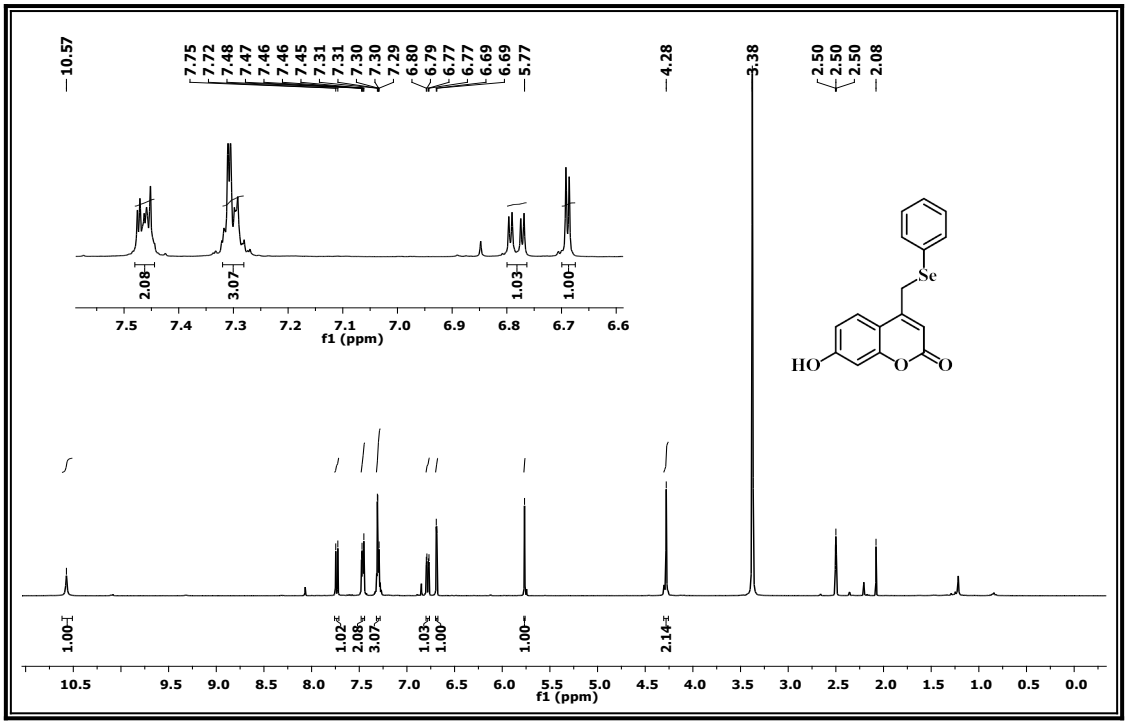


Figure S1. ^{1}H NMR spectrum of probe **4** in DMSO-d_6.

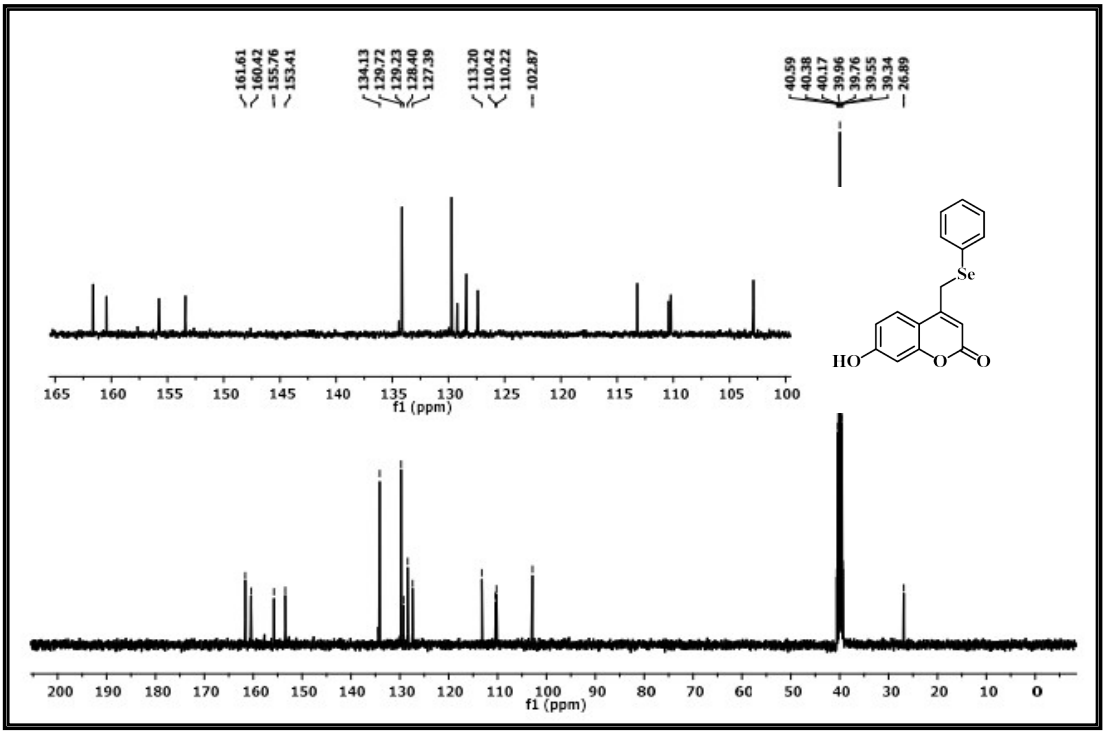


Figure S2.^{13}C NMR spectrum of probe **4** in DMSO-d_6.

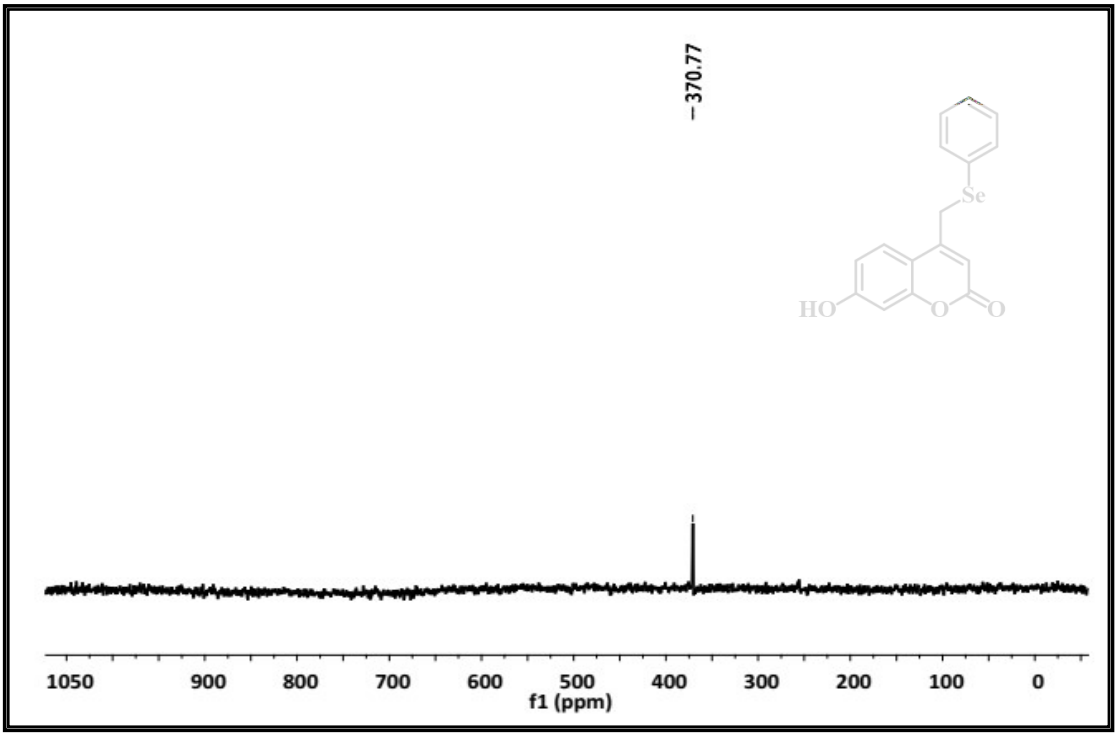


Figure S3. ^{77}Se NMR spectrum of probe **4** in DMSO-d_6.

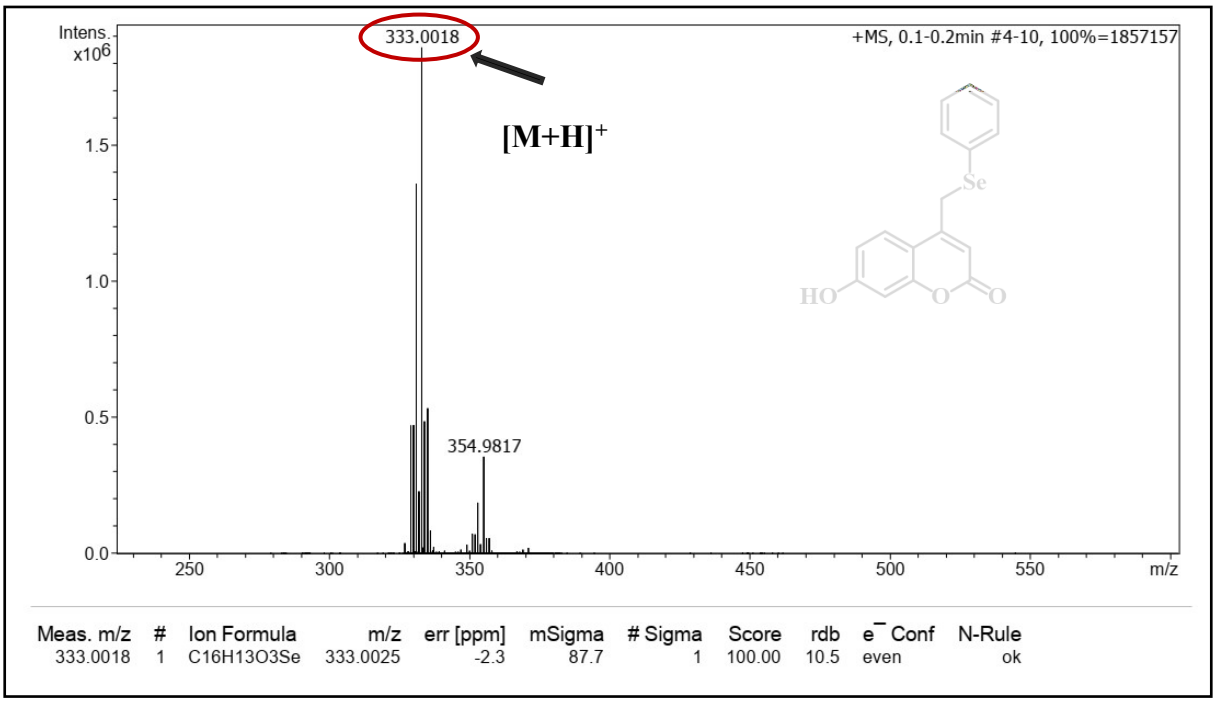


Figure S4. Mass spectrum of probe **4**.

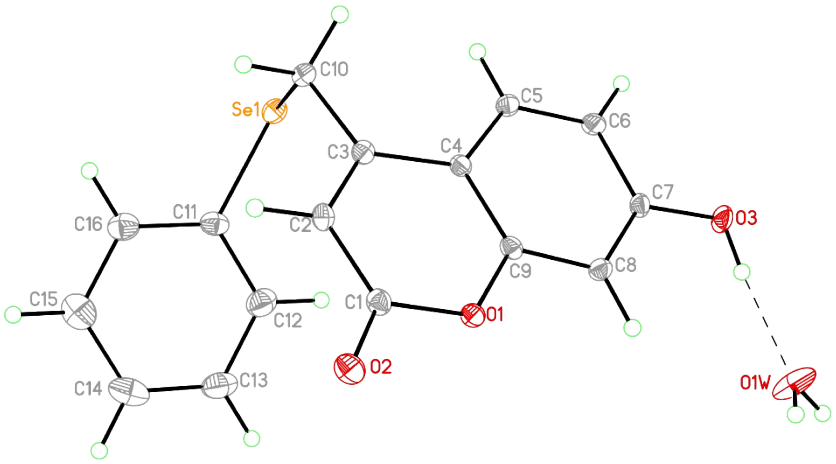


Figure S5. Crystal diagram of probe **4**.

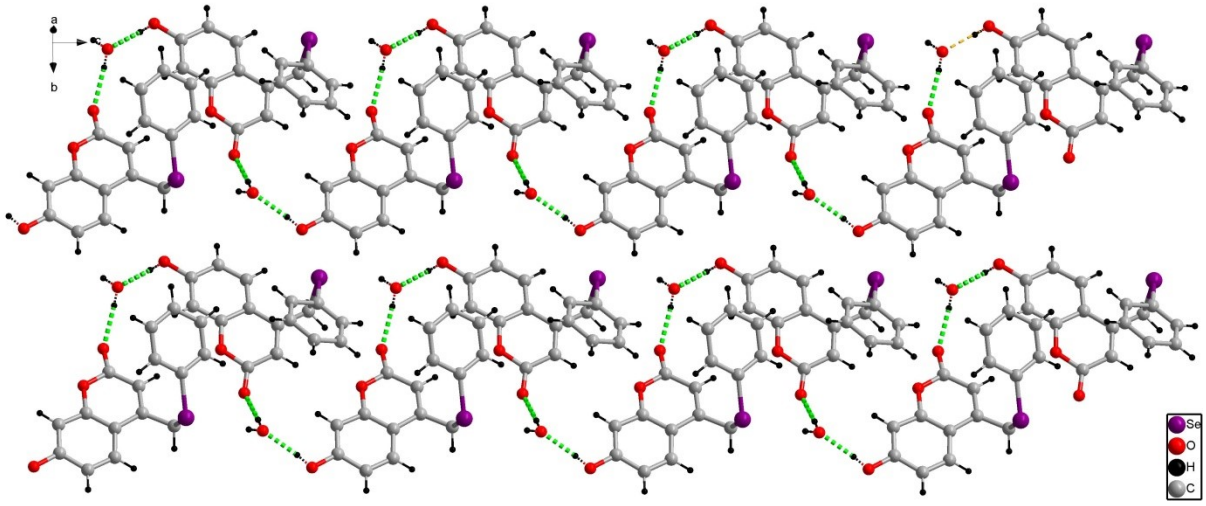


Figure S6. Crystal packing diagram of probe **4** showing hydrogen bonding interactions.

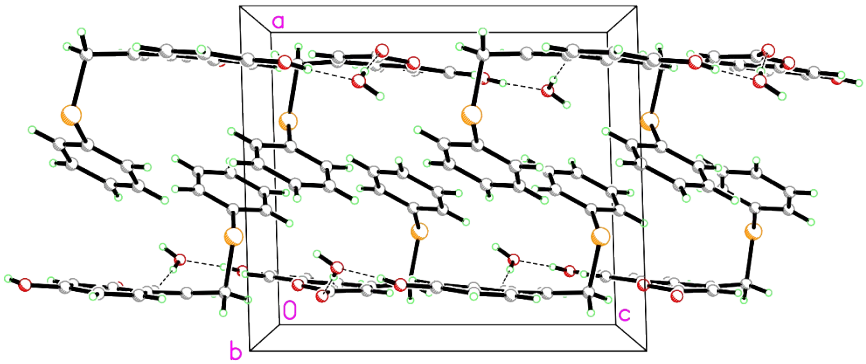


Figure S7. Crystal packing diagram of probe **4**.

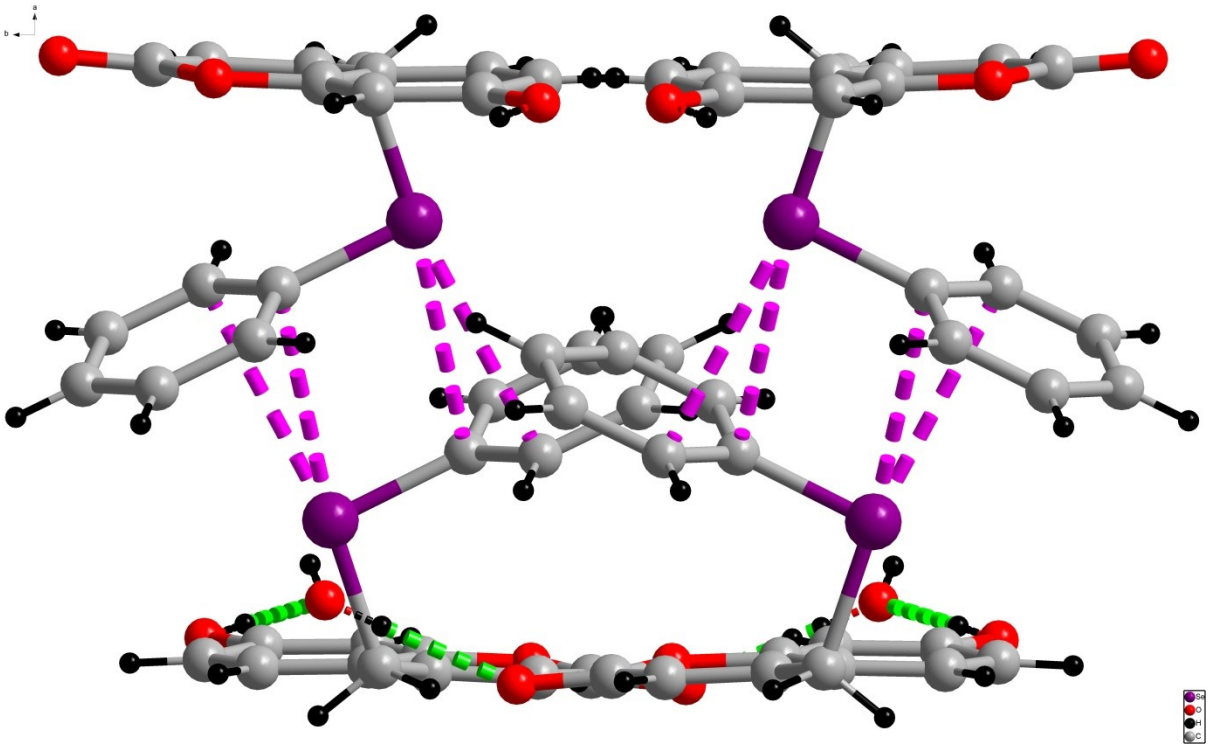


Figure S8. Crystal packing diagram of probe **4** showing intermolecular interactions.

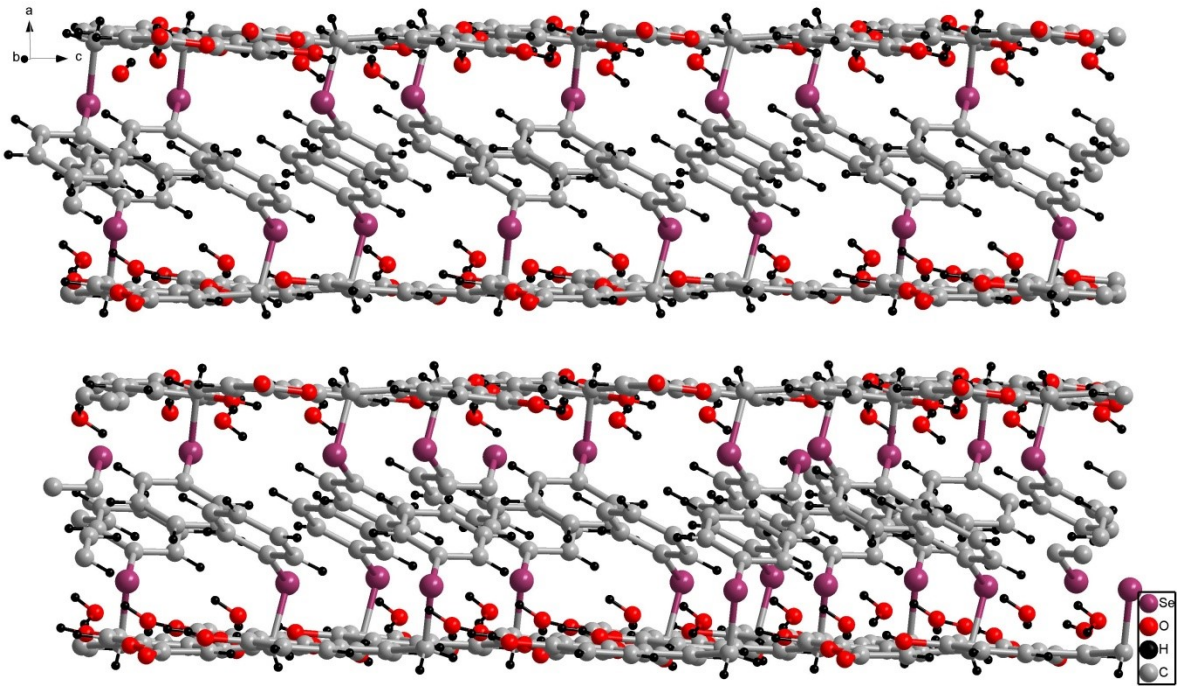


Figure S9. Crystal packing diagram of probe **4** showing the arrangement of molecular layers**.**

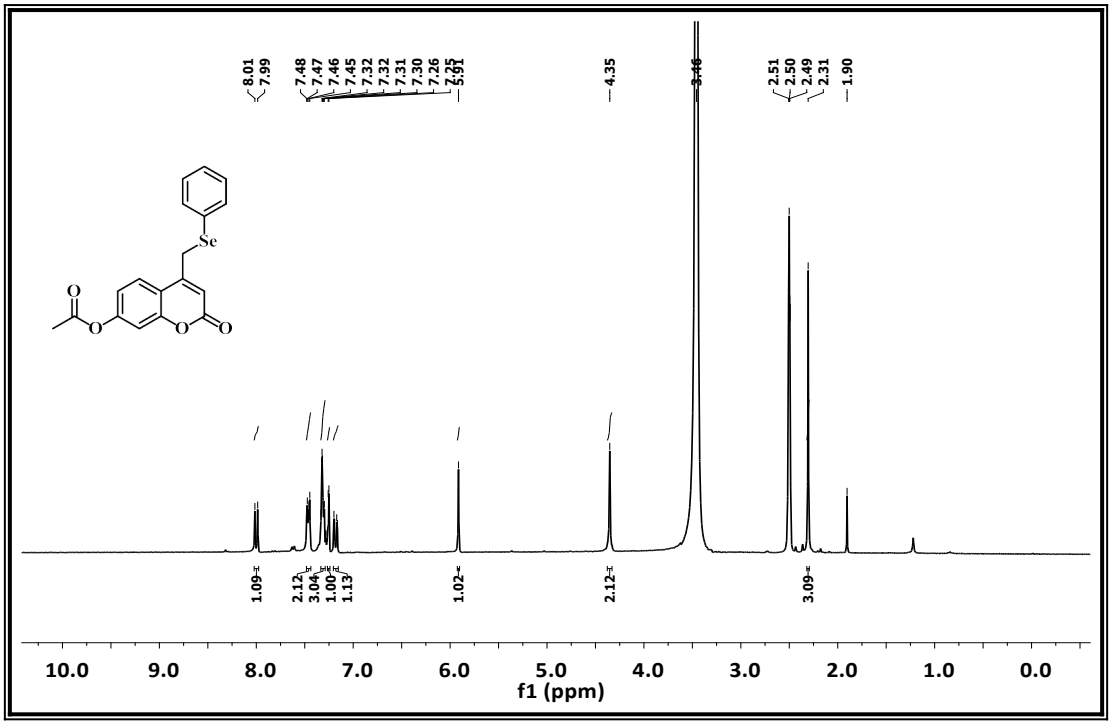


Figure S10. ^{1}H NMR spectrum of probe **5** in DMSO-d_6.

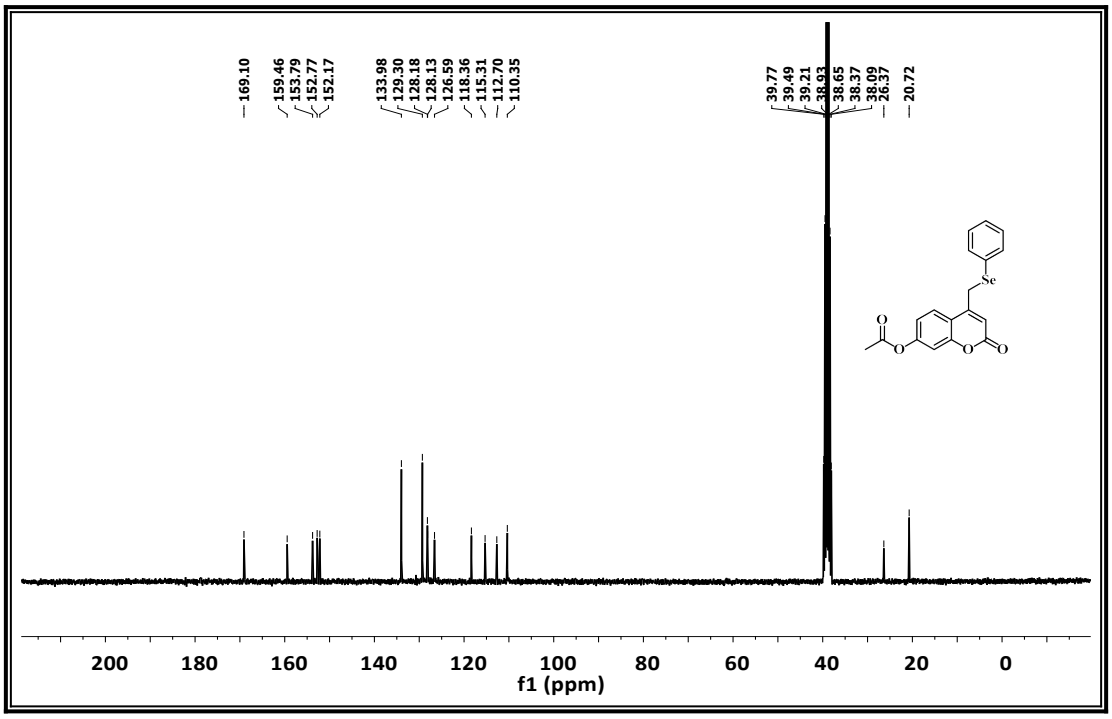


Figure S11. ^{13}C NMR spectrum of probe **5** in DMSO-d_6.

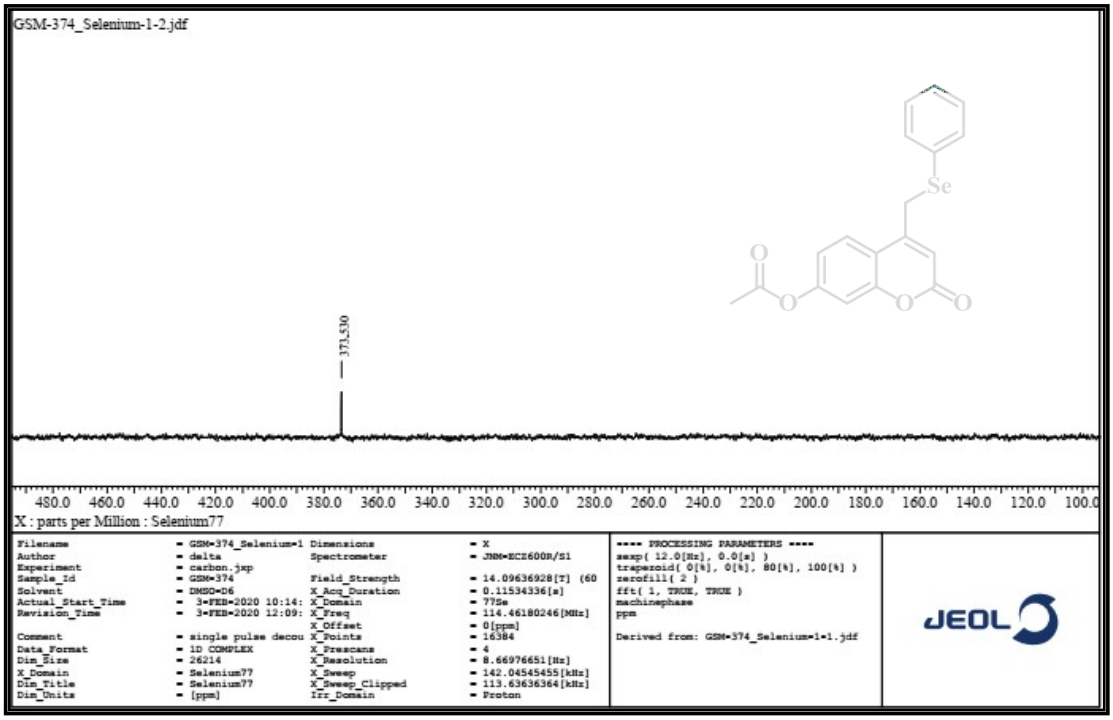


Figure S12. ^{77}Se NMR spectrum of probe **5** in DMSO-d_6.

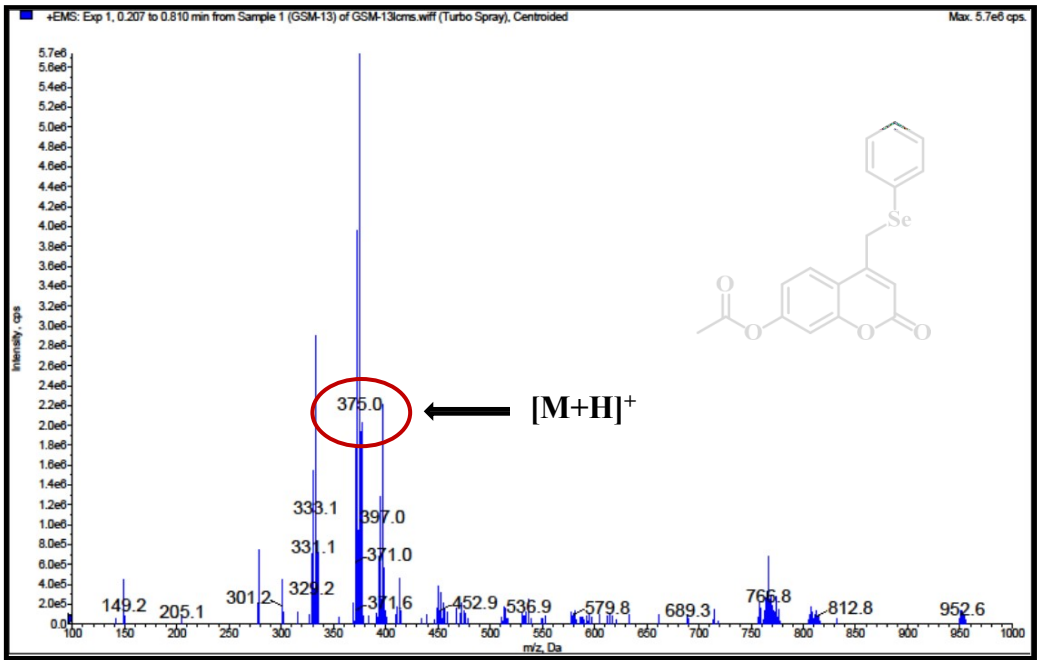


Figure S13. Mass spectrum of probe **5**.

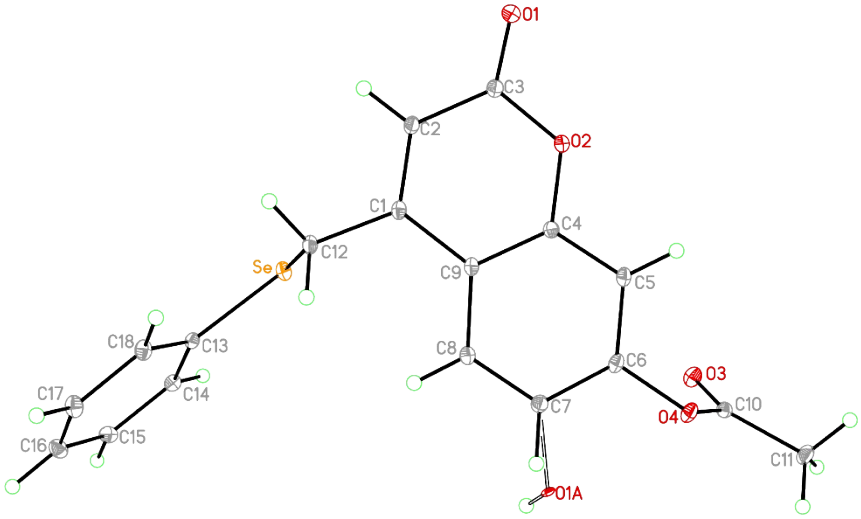


Figure S14. Crystal diagram of probe **5.**

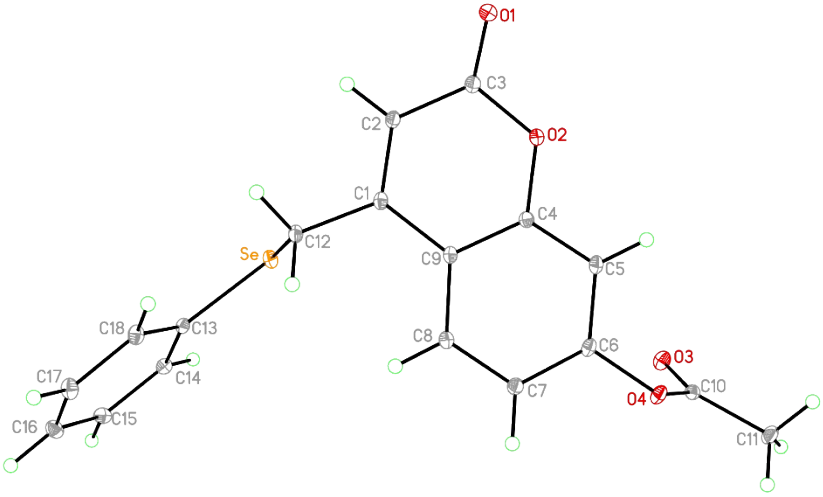


Figure S15. Crystal diagram of probe **5.**

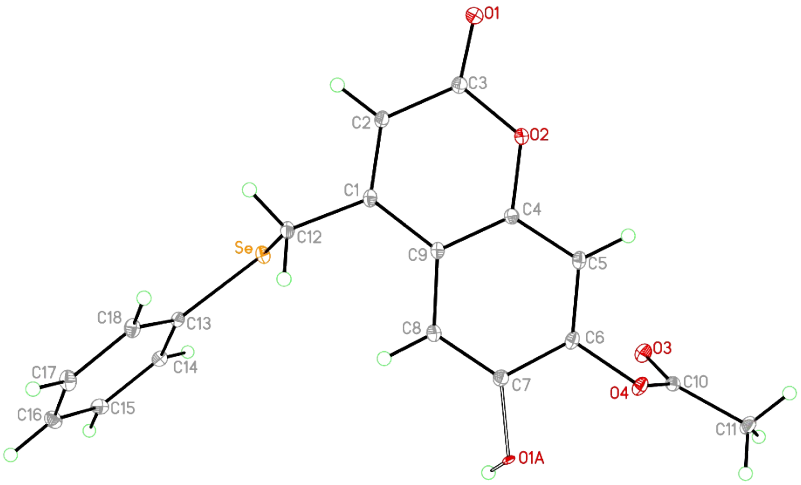


Figure S16. Crystal diagram of probe **5.**

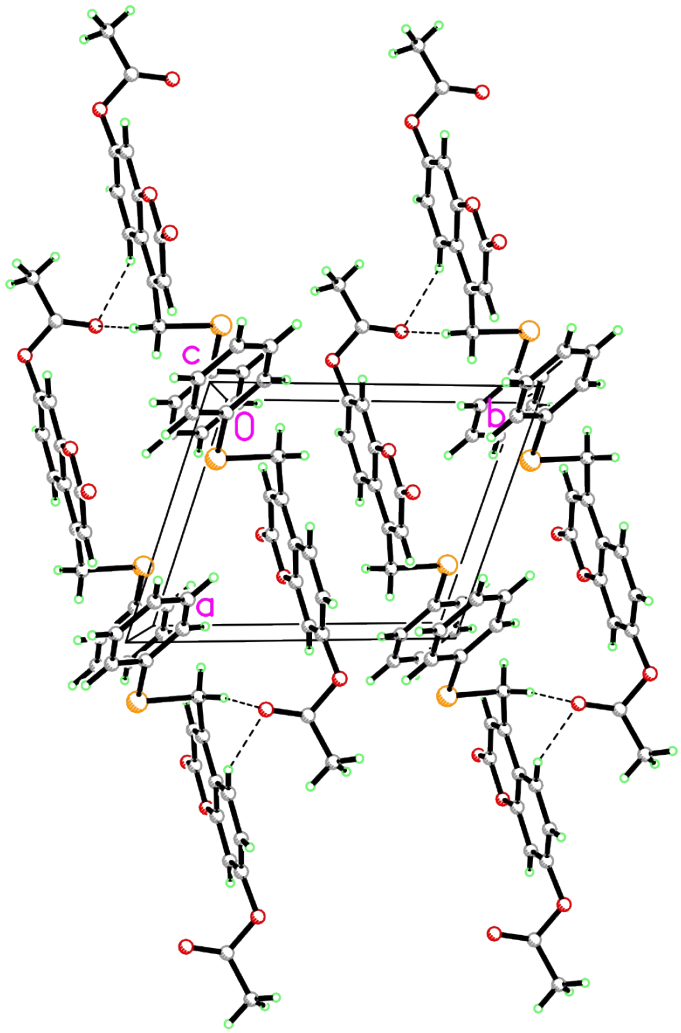


Figure S17. Crystal packing diagram of probe **5.**

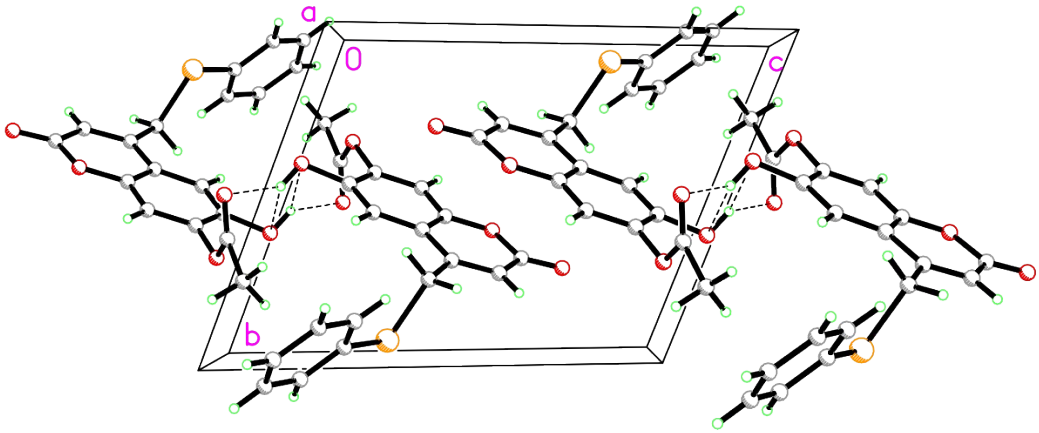


Figure S18. Crystal packing diagram of probe **5.**

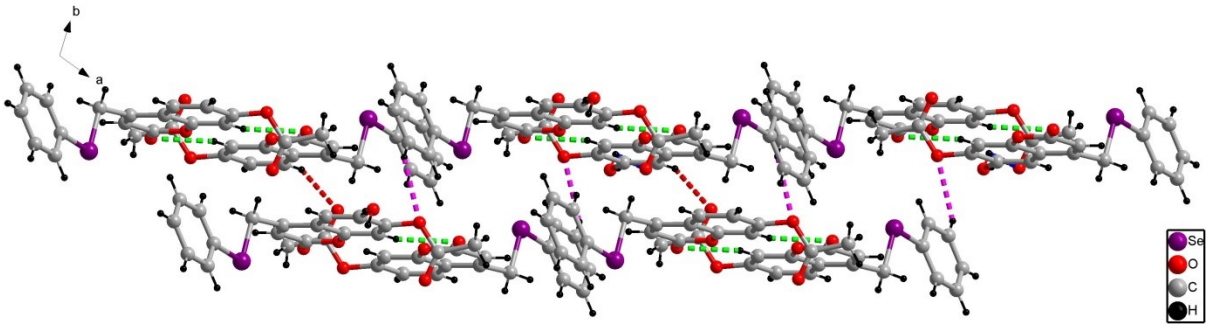


Figure S19. Crystal packing diagram of probe **5** showing intermolecular interactions.

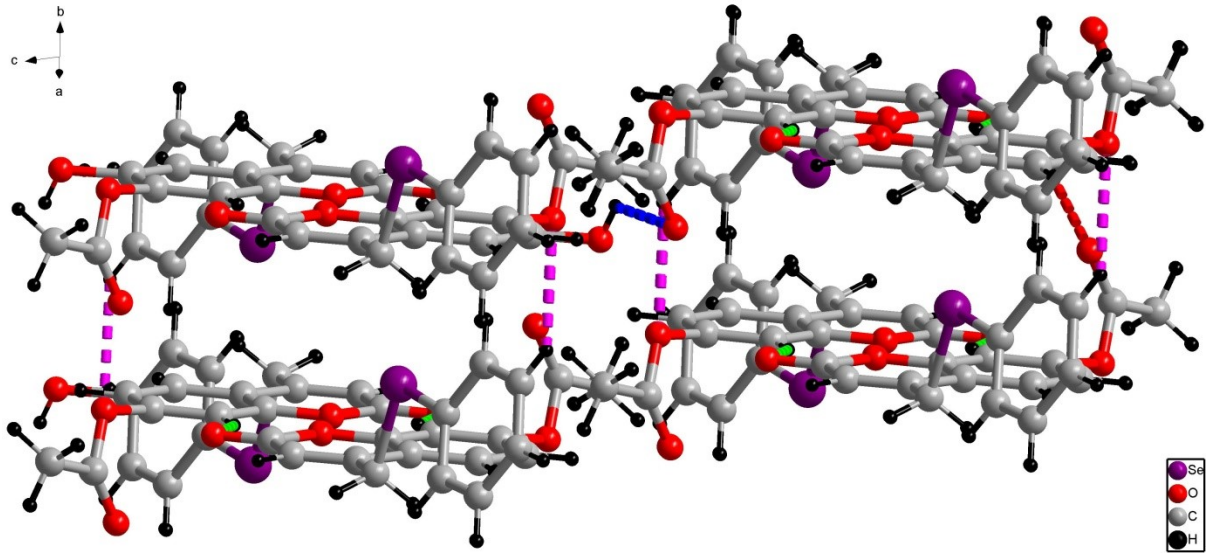


Figure S20. Crystal packing diagram of probe **5** showing step like structure.

Table S1. Refinement details of X-ray structure of the probes **4** and **5**.

Compound	**Probe 4 (CCDC# 2156613)**	**Probe 5 (CCDC# 2156614)**
Formula	$C_{16}H_{12}O_3Se, H_2O$	$C_{18}H_{14}O_{4.08}Se$
Crystal System	Monoclinic	Triclinic
Space Group	P 21/c	P-1
T/K	100(2) K	100(2) K
a [A^0]	10.3926(7)	7.6073(5)
b [A^0]	11.6371(7)	9.4274(9)
c [A^0]	11.9617(6)	12.1570(11)
α [^{0}]	90°	106.478(3)
β [^{0}]	91.690(4)°	104.683(2)
γ [^{0}]	90°	103.015(2)
V [Å^3]	1446.02(15)	766.15(11)
Z	4	2
ρ_{cal}Mg/m^3	1.604	1.624
μ(mm^{-1})	2.609	2.469
F(000)	704	377
Crystal Size [mm^3]	0.24 x 0.17 x 0.09	0.21 x 0.16 x 0.08
GOF	1.032	1.027
***2θ* range (deg)**	2.628 to 29.655	2.378 to 32.563
Reflections collected	4066	5560
Independent reflections	4066	5560
Parameters	200	220
R_{int}	0.0534	0.0345
R_1,wR2[I>2σ(I)]	R1 = 0.0514, wR2 = 0.1099	R1 = 0.0295, wR2 = 0.0639
R_1,wR2[I>2σ(I)]	R1 = 0.0879, wR2 = 0.1233	R1 = 0.0382, wR2 = 0.0669

PHOTOPHYSICAL MEASUREMENTS

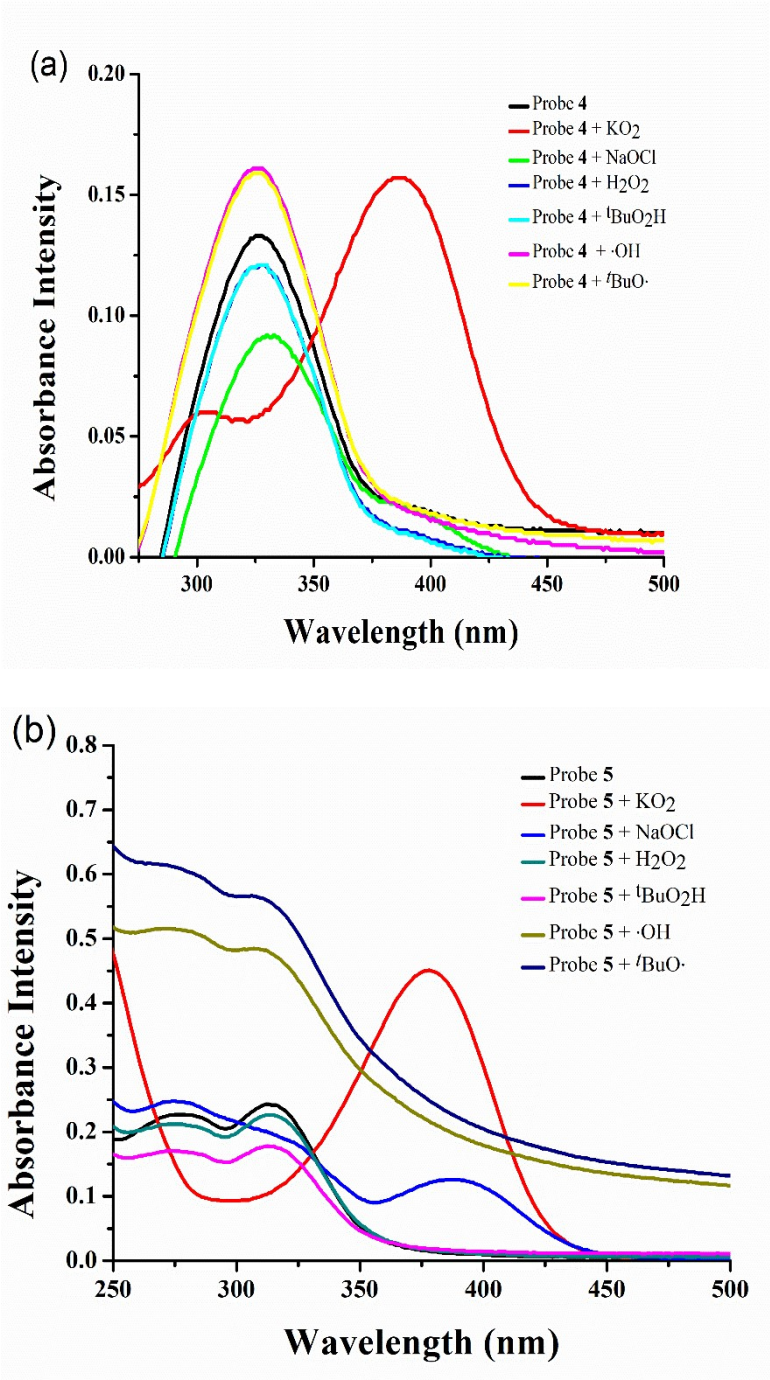


Figure S21. (a) UV-visible absorption spectra of probe **4** (10 μM, water/acetonitrile: v/v = 80:20) with 167 μM of ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$) incubated for 10 min at rt (λ_{ex} = 388 nm); (b) UV-visible absorption spectra of probe **5** (20 μM, water/DMSO: v/v = 80:20) with 167 μM of ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$) incubated for 30 min at rt (λ_{ex} = 380 nm).

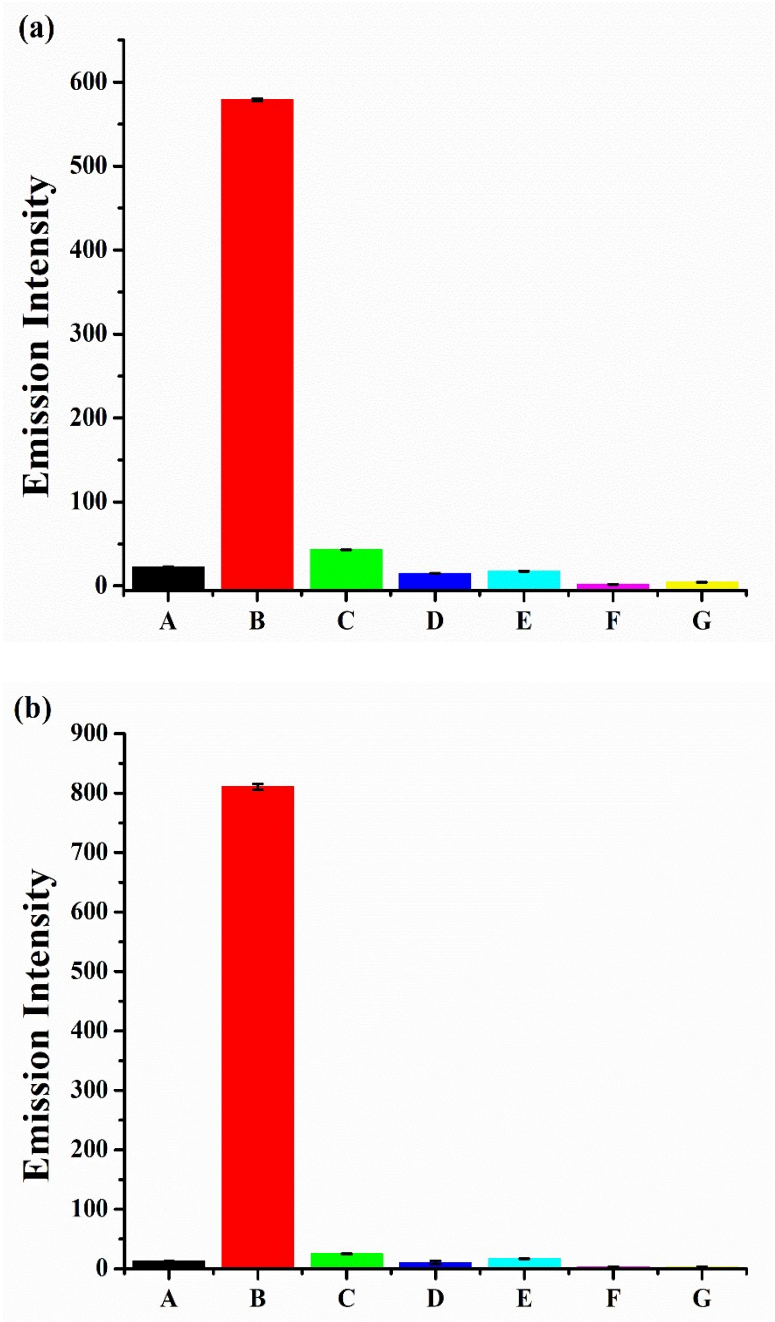
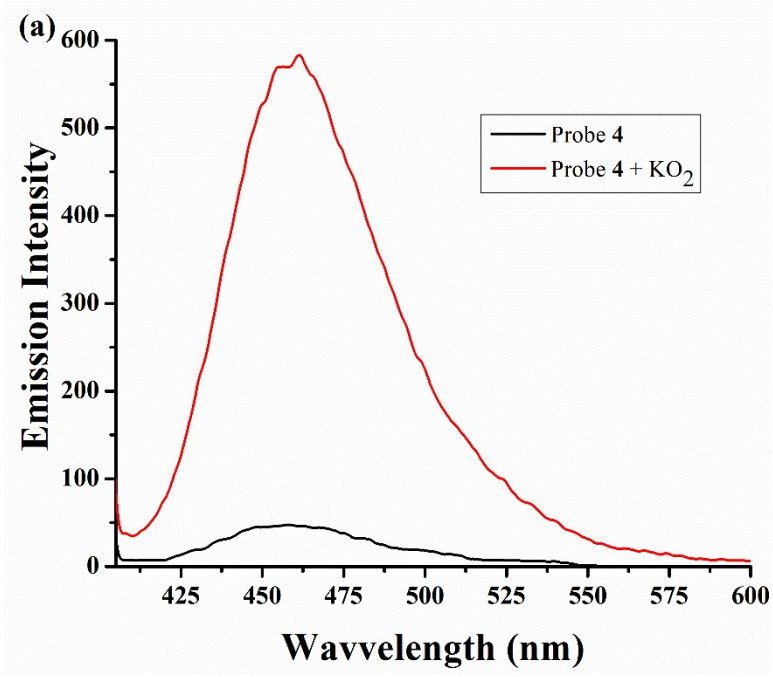


Figure S22. (a) Selectivity study bar graph for fluorescence of probe **4** (10 μM), water/acetonitrile: v/v = 80:20 with various 167 μM of ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$), incubated for 10 min at rt. λ_{ex} = 388 nm, λ_{em} = 469 nm. Ex and em slit width 5 nm/5 nm. (A = probe **4**, B = probe **4** + KO_2, C = probe **4** + NaOCl, D = probe **4** + H_2O_2, E = probe **4** + $^{t}BuO_2H$, F = probe **4** + $^{\bullet}OH$, G = probe **4** + $^{t}BuO^{\bullet}$); (b) Selectivity study bar graph for fluorescence of probe **5** (20 μM), water/DMSO: v/v = 80:20 with various 167 μM of ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$), incubated for 30 min at rt. λ_{ex} = 380 nm, λ_{em} = 458 nm. Ex and em slit width 5 nm/5 nm. (A = probe **5**, B = probe **5** + KO_2, C = probe **5** +

NaOCl, D = probe **5** + H_2O_2, E = probe **5** + $^tBuO_2H$, F = probe **5** + $^{\bullet}OH$, G = probe **5** + $^tBuO^{\bullet}$).



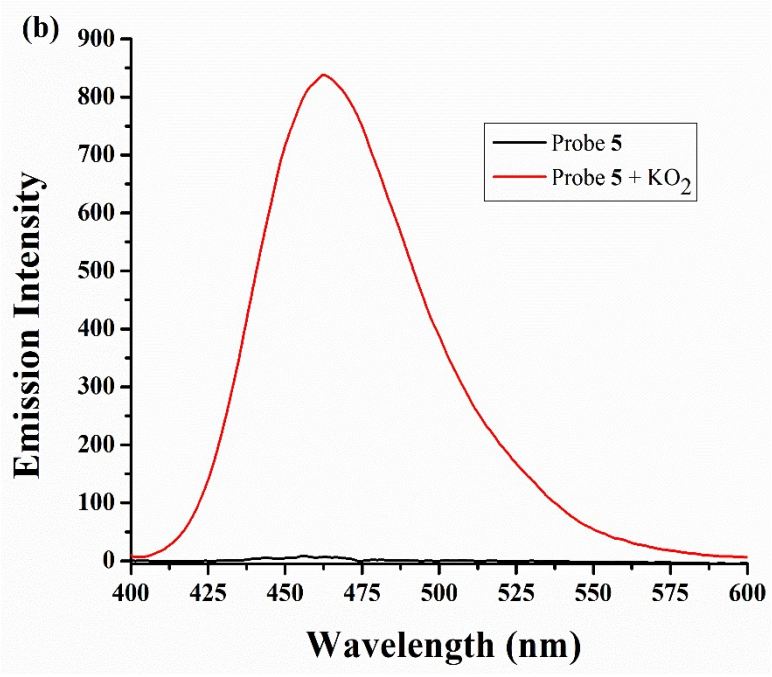


Figure S23. (a) Emission spectra of probe **4** (10 μM), water/acetonitrile: v/v = 80:20 with 167 μM of $O_2^{\bullet-}$ (in DMSO) incubated for 10 min at rt. λ_{ex} = 388 nm, λ_{em} = 462 nm. Ex and em slit width 5 nm/5 nm; (b) Emission spectra of probe **5** (20 μM),

water/DMSO: v/v = 80:20 with 167 μM of $O_2^{\bullet-}$ (in DMSO), incubated for 30 min at rt. λ_{ex} = 380 nm, λ_{em} = 458 nm. Ex and em slit width 5 nm/5 nm.

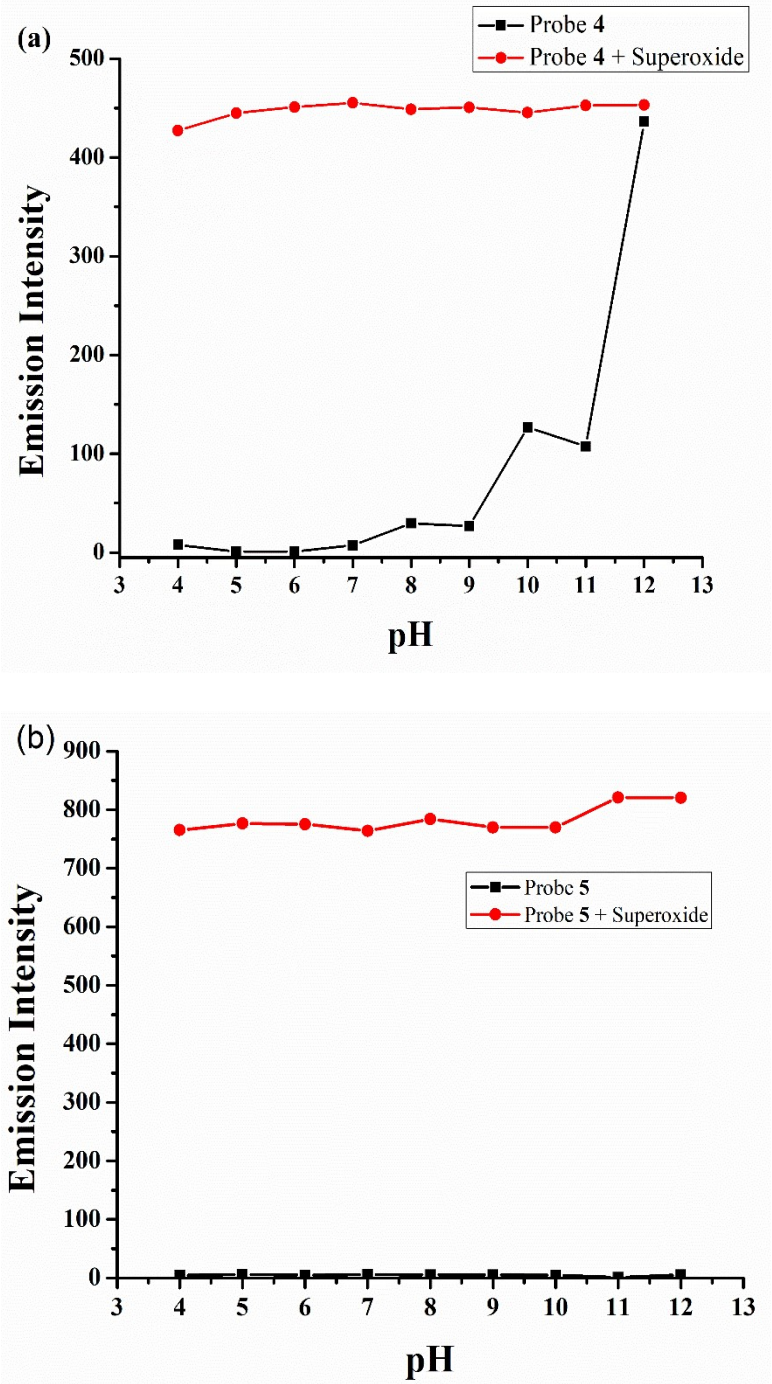


Figure S24. (a) Fluorescence intensity changes of probe **4** (10 μM, black) and probe **4** (10 μM) with 167 μM of superoxide, (red), (λ_{ex} = 388 nm, λ_{em}= 469 nm) slit width 5 nm/5 nm under different pH range; (b) Fluorescence intensity changes of probe **5** (20 μM, black) and

probe **5** (20 µM) with 167 µM of superoxide (red), (λ_{ex} = 380 nm, λ_{em}= 458 nm) slit width 5 nm/5 nm under different pH range.

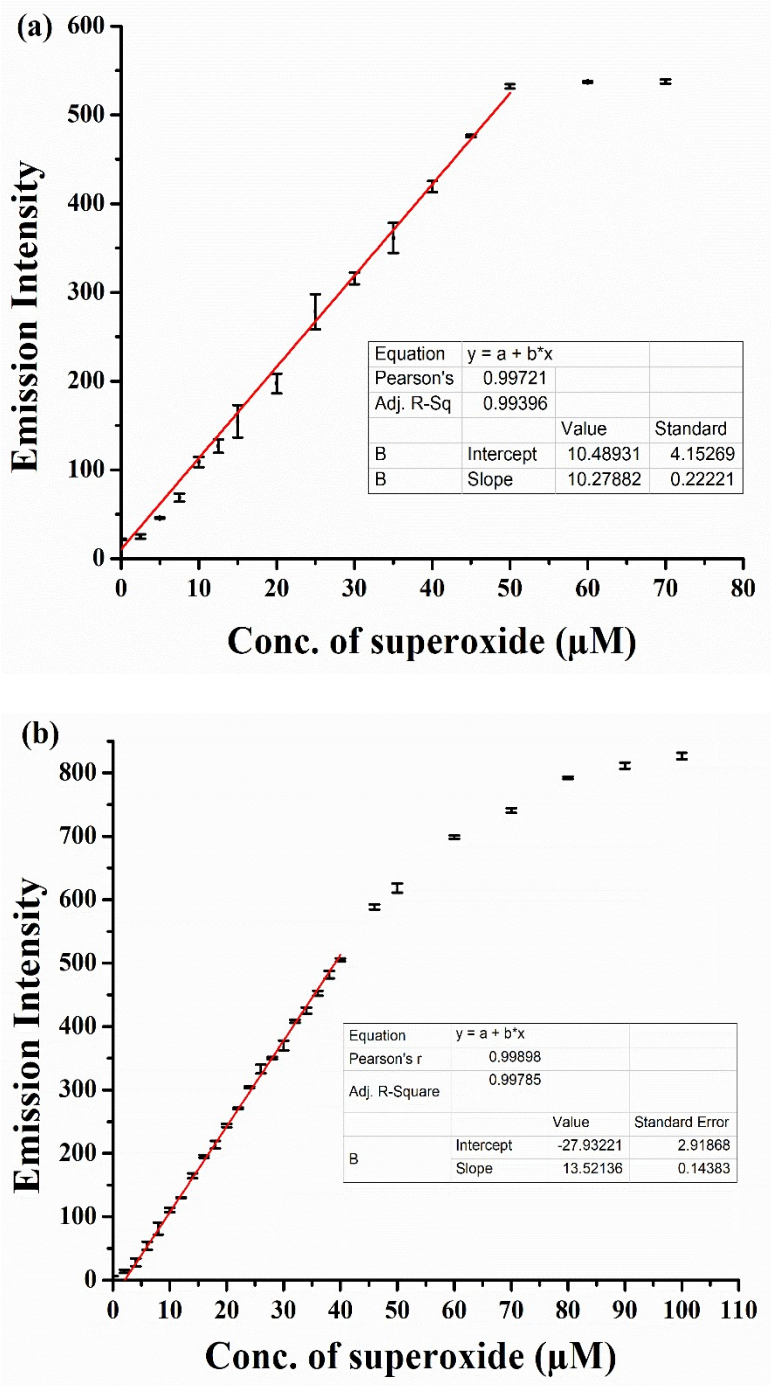


Figure S25. (a) Plot for the calculation of detection limit of probe **4** (10 µM, water/acetonitrile: v/v = 80:20) with increasing concentration of superoxide (0 - 7 equiv) incubated for 10 min at rt, (λ_{ex} = 388 nm, λ_{em}= 469 nm). Ex. and Em. slit width 5 nm/5 nm; (b) Plot for the calculation of detection limit of probe **5** (20 µM, water/DMSO: v/v = 80:20)

with increasing concentration of superoxide (0 - 5 equiv) incubated for 30 min at rt (λ_{ex} = 380 nm, λ_{em}= 458 nm). Ex. and Em. slit width 5 nm/5 nm.

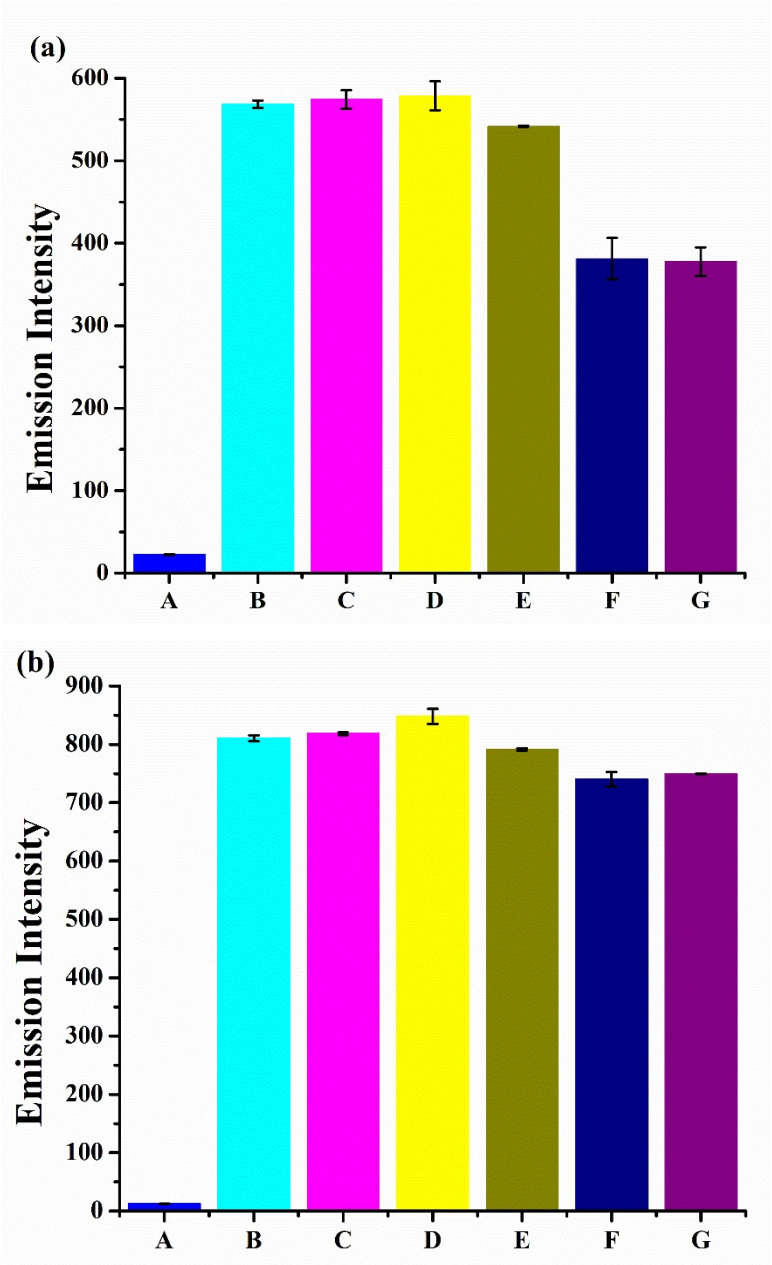


Figure S26. (a) Fluorescence spectra after addition of ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$, 167 μM) to probe **4** (10 μM, water/acetonitrile: v/v = 80:20) and 167 μM of $O_2^{\bullet-}$, incubated for 10 min at rt, (λ_{ex} = 388 nm, λ_{em}= 469 nm), slit width 5 nm/5 nm. (A = probe **4**, B = probe **4** + KO_2, C = probe **4** + KO_2 + NaOCl, D = probe **4** + KO_2 + H_2O_2, E = probe **4** + KO_2 + $^{t}BuO_2H$, F = probe **4** + KO_2 + $^{\bullet}OH$, G = probe **4** + KO_2 + $^{t}BuO^{\bullet}$); (b) Fluorescence spectra after addition of ROS ($O_2^{\bullet-}$, ^{-}OCl, H_2O_2, $^{t}BuO_2H$, $^{\bullet}OH$ and $^{t}BuO^{\bullet}$, 167 μM) to probe **5** (20 μM, water/DMSO: v/v = 80:20) and 167 μM of $O_2^{\bullet-}$, incubated for 30 min at rt, (λ_{ex} = 380 nm, λ_{em}= 458 nm), slit width 5 nm/5 nm. (A = probe **5**, B = probe **5** + KO_2, C = probe **5**

+ KO_2+ NaOCl, D = probe **5** + KO_2 + H_2O_2, E = probe **5** + KO_2 + $^tBuO_2H$, F = probe **5** + KO_2 + $^{\bullet}OH$, G = probe **5** + KO_2+ $^tBuO^{\bullet}$).

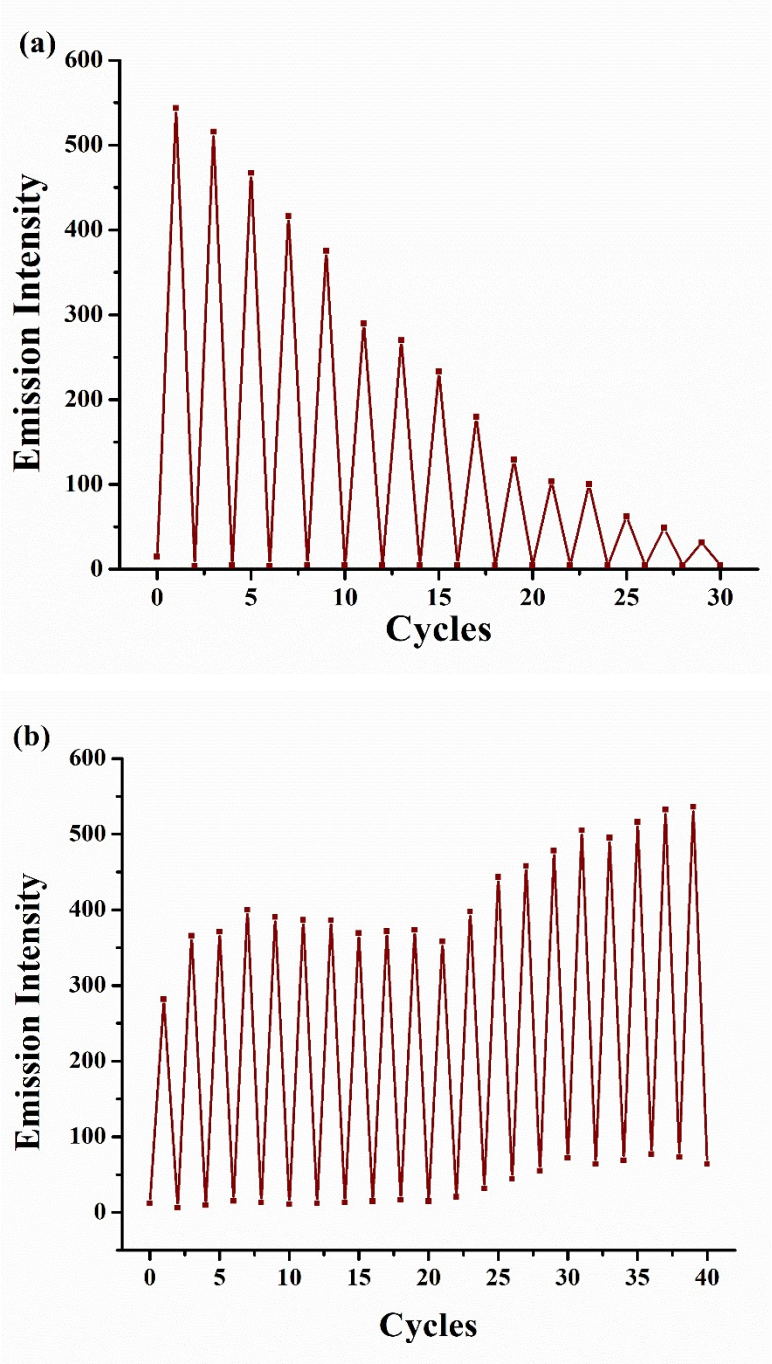


Figure S27. (a) Redox cycles of probe **4** (10 μM) with 167 μM of superoxide and 167 μM of GSH (λ_{ex} = 388 nm, λ_{em}= 469 nm), slit width 5 nm/5 nm; (b) Redox cycles of probe **5** (20 μM) with 167 μM of superoxide and 167 μM of GSH (λ_{ex} = 380 nm, λ_{em}= 458 nm), slit width 5 nm/5 nm.

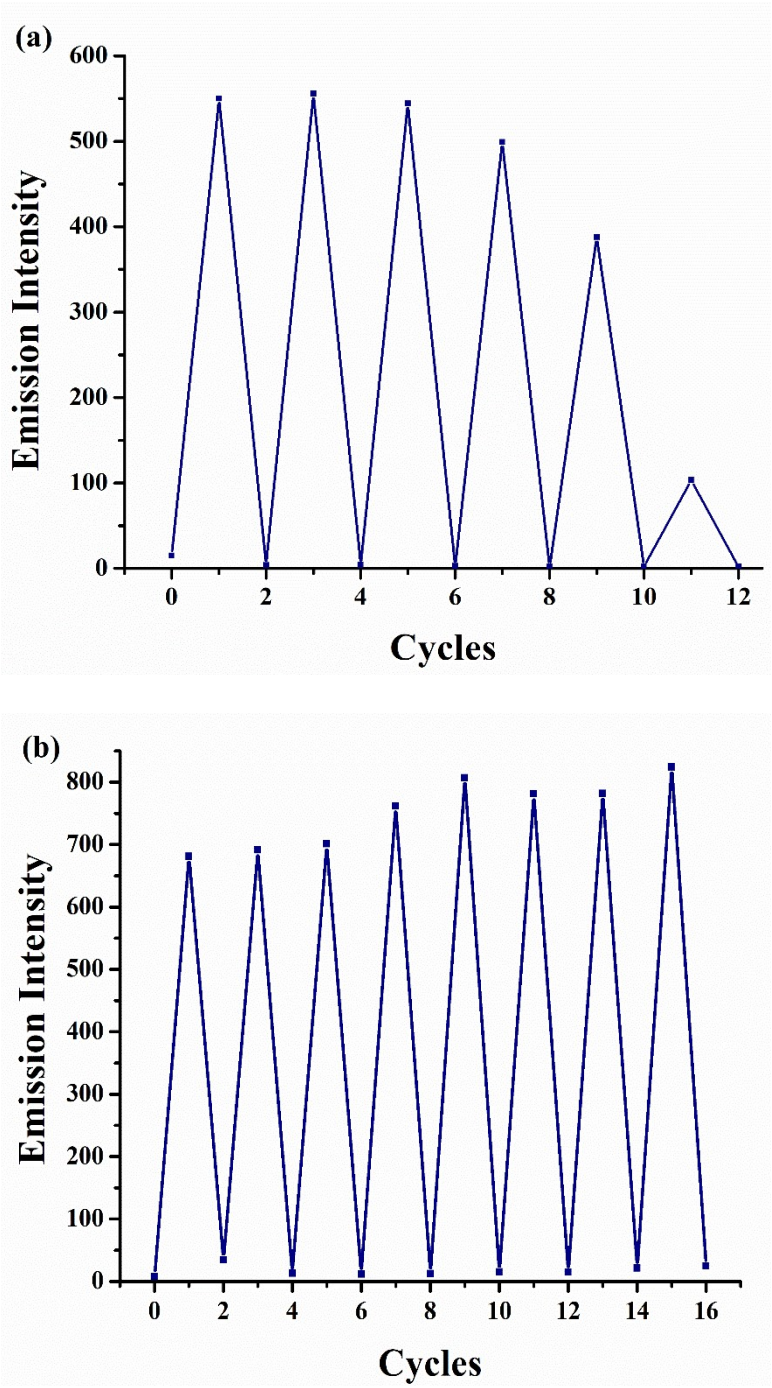


Figure S28. (a) Redox cycles of probe **4** (10 μM) with 167 μM of superoxide and 167 μM of N-acetyl-L-cysteine; (b) Redox cycles of probe **5** (20 μM) with 167 μM of superoxide and 167 μM of N-acetyl-L-cysteine.

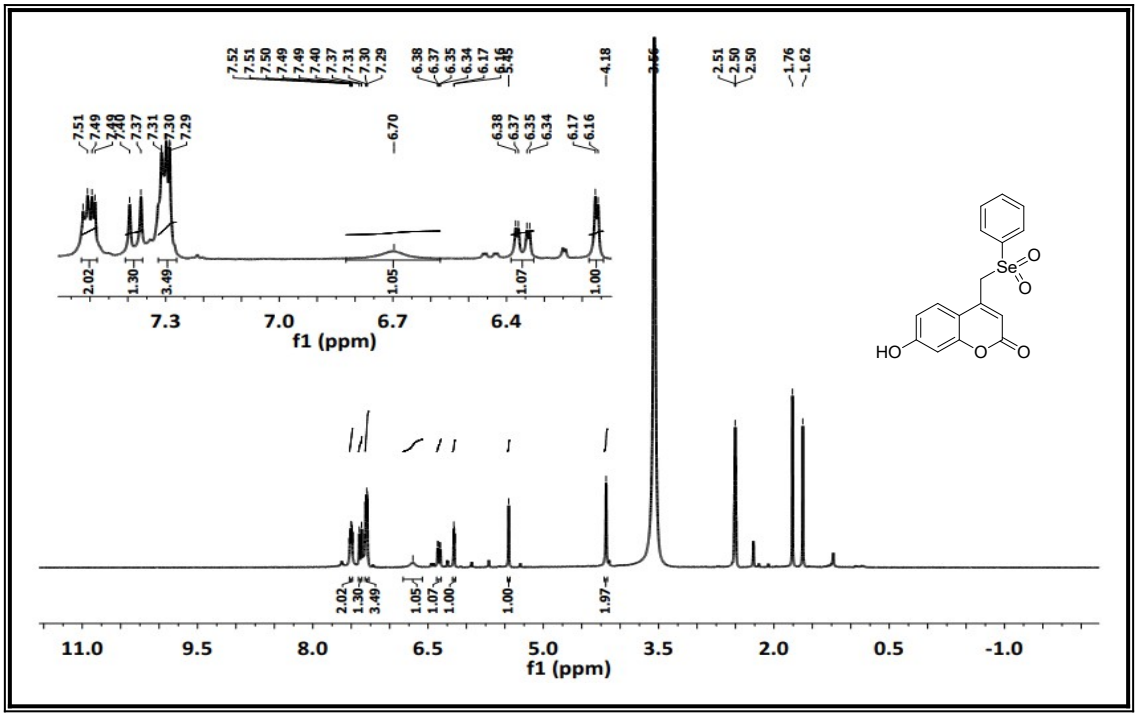


Figure S29. ^{1}H NMR spectrum of compound **6** in DMSO-d_6.

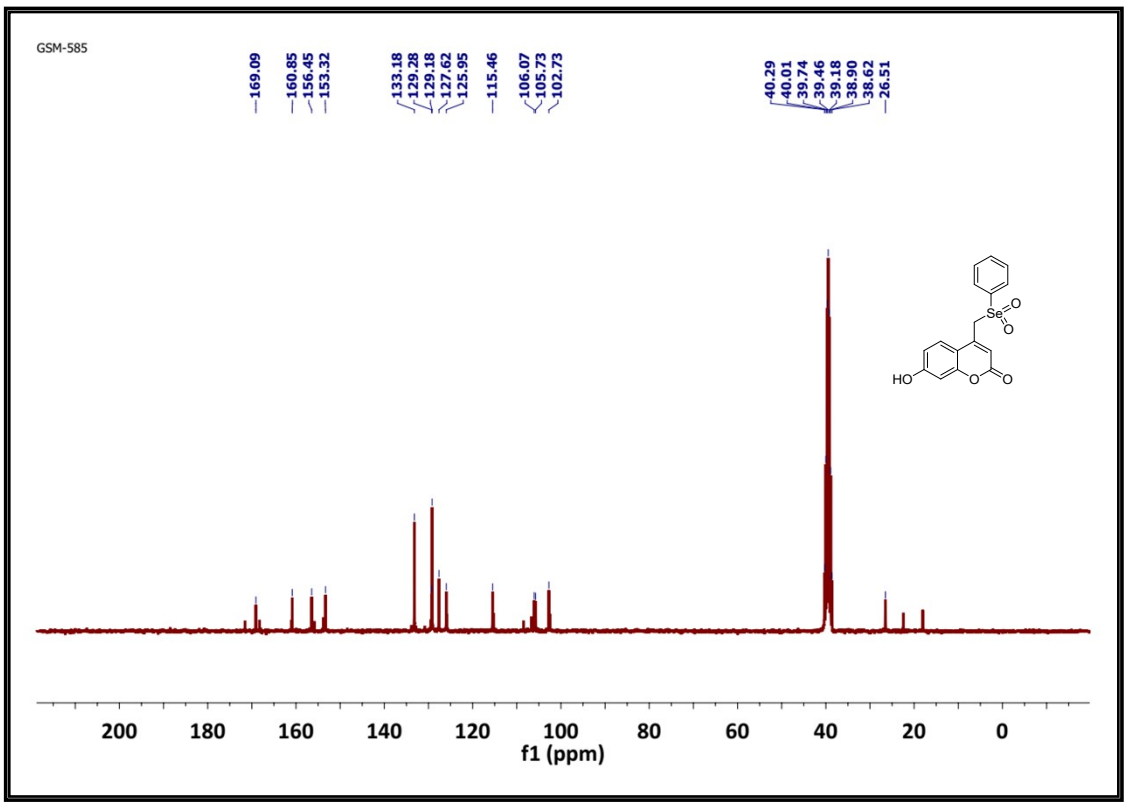


Figure S30. ^{13}C NMR spectrum of compound **6** in DMSO-d_6.

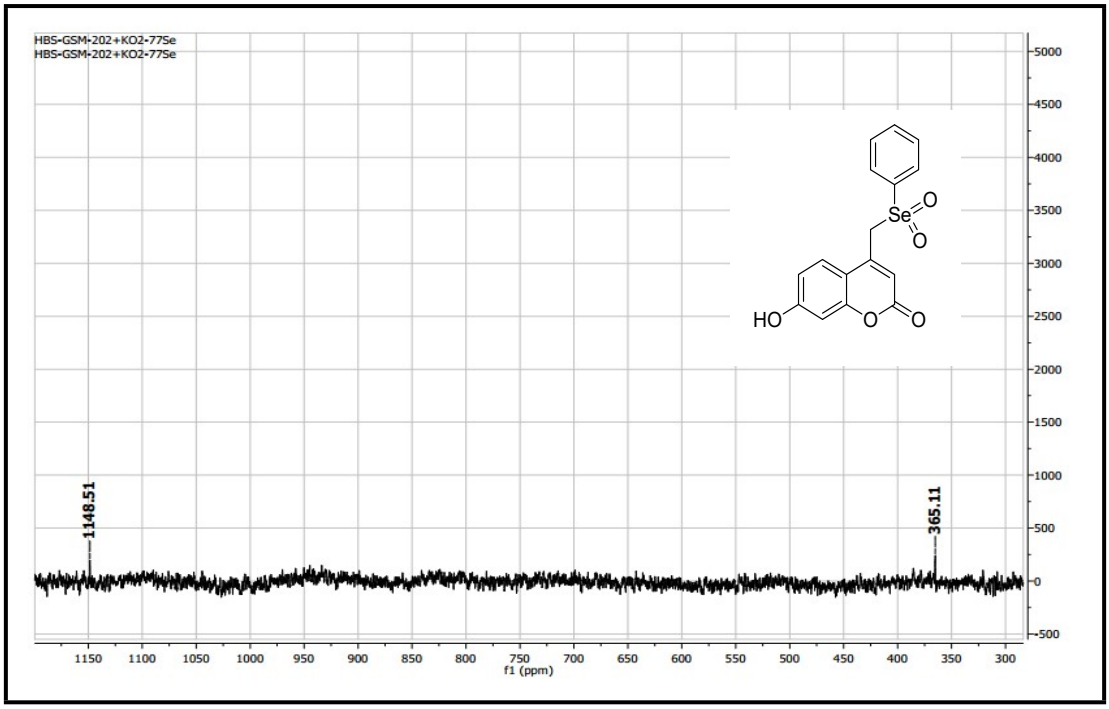


Figure S31. ^{77}Se NMR spectrum of compound **6** in DMSO-d_6.

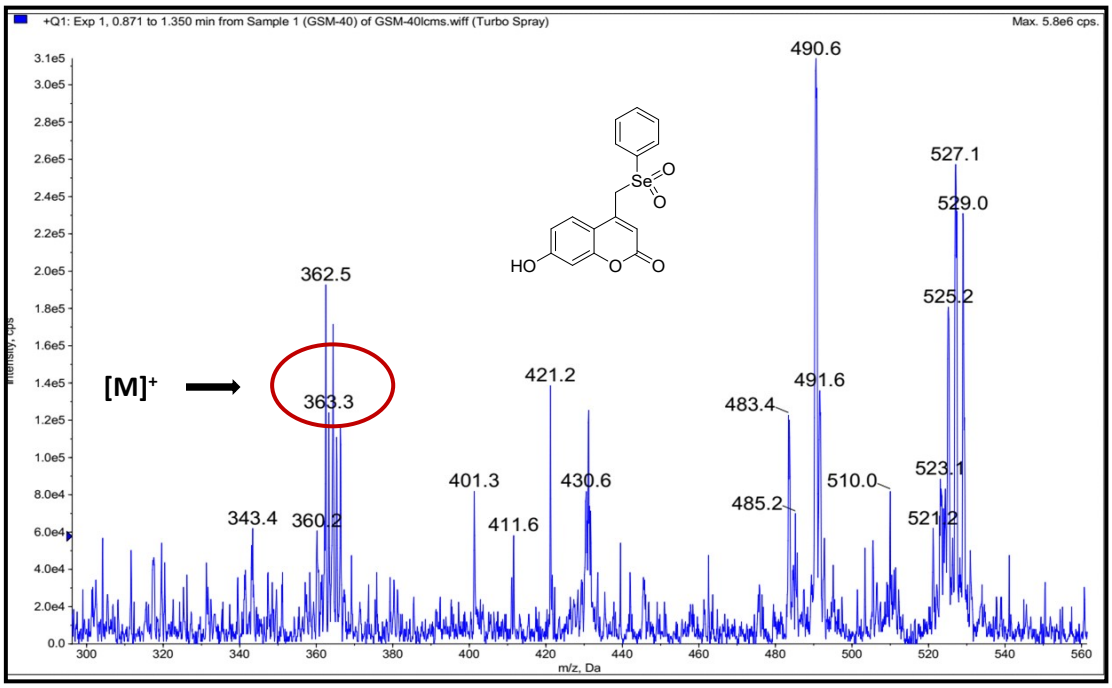


Figure S32. Mass spectrum of compound **6**.

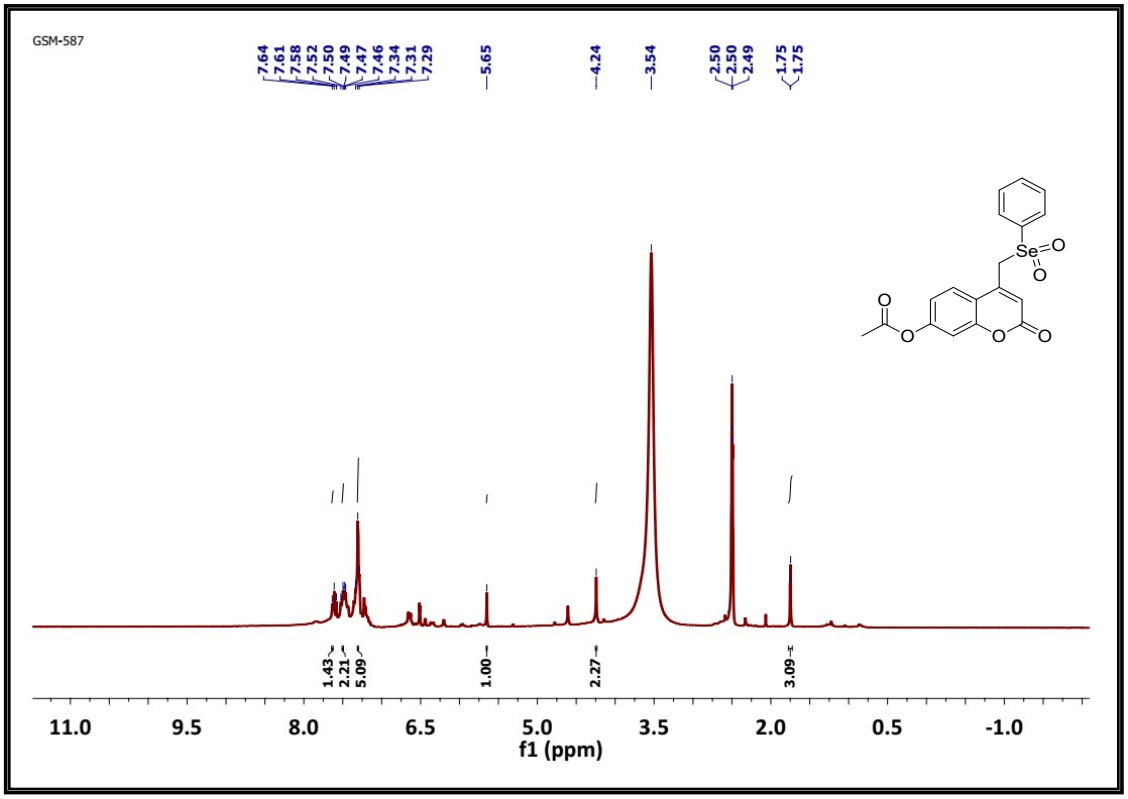


Figure S33. ^{1}H NMR spectrum of compound **7** in DMSO-d_6.

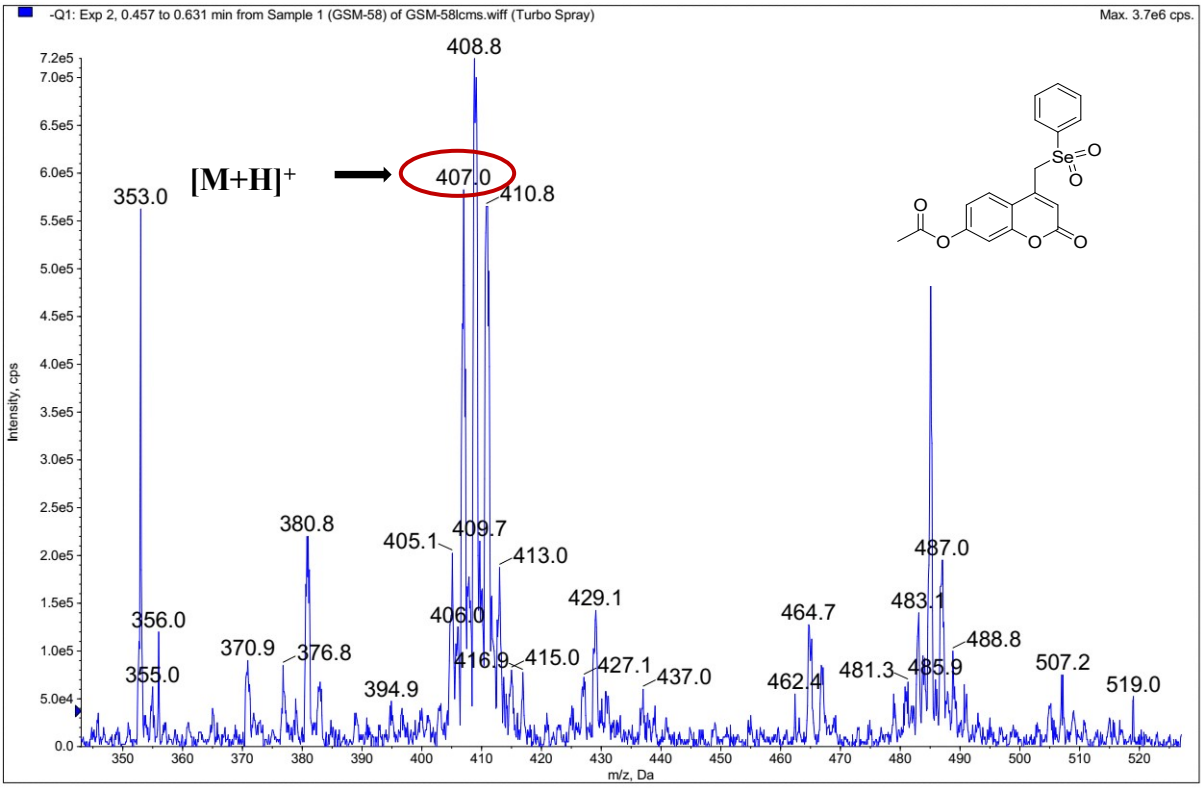


Figure S34. Mass spectrum of compound **7**.

REFERENCES

1. J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd Ed., Kluwer Academic/Plenum Publishers, New York, London, Moscow, Dordrecht, 1999.