

Supporting Information

**Probing the Formation of a Seleninic Acid in Living Cells by the
Fluorescence Switching of a Glutathione Peroxidase Mimetic**

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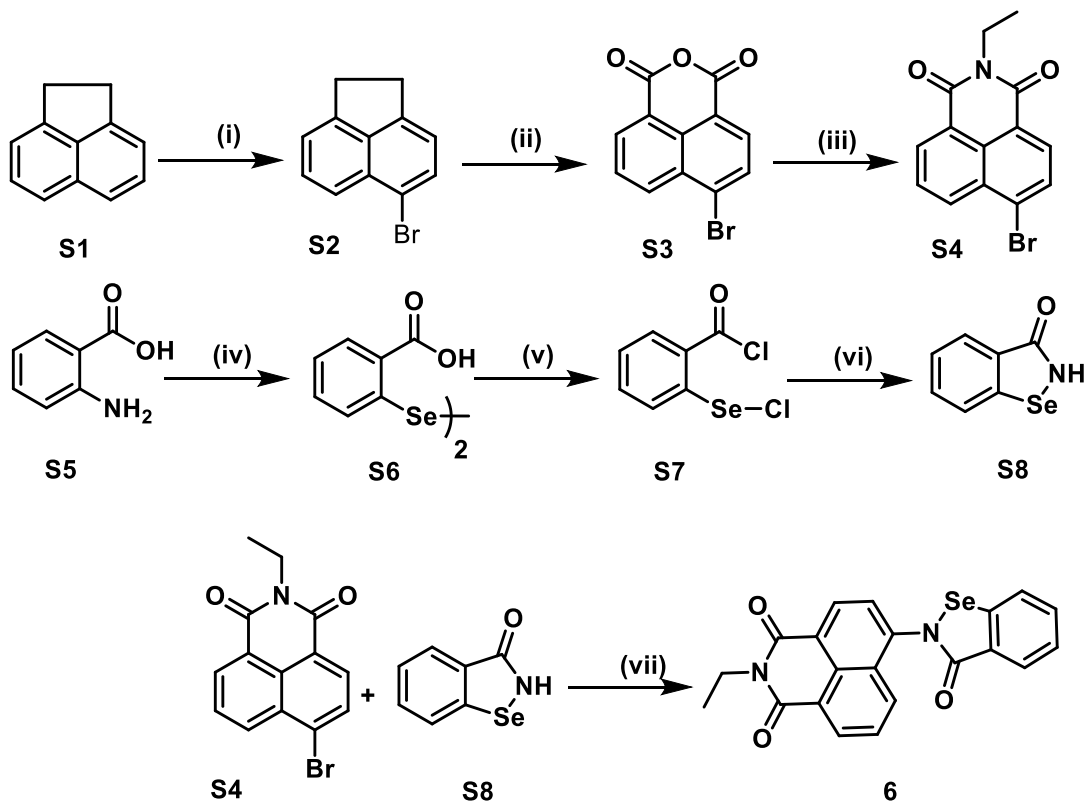
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1. Materials and General Experimental Conditions

K₂Cr₂O₇, bromine, methyl iodide, NaBH₄ and K₂CO₃ were purchased from SD Fine-Chem Limited, India. NaNO₂ and Se powder were obtained from Sigma Aldrich, USA, and the commercial materials were used without any further purification. All reactions have been carried out with distilled and dried solvents under an atmosphere of N₂, and oven-dried glassware with standard vacuum-line techniques. Dry DMF was obtained from distilled over CaH₂ under anhydrous condition. All workups and purifications were carried out with reagent-grade solvents in air. Thin-layer chromatography analyses were carried out on pre-coated silica gel plates (Merck), and spots were visualized by short/long wavelength UV irradiation. Column chromatography was performed on glass columns loaded with silica gel (230–400 or 100–200 mesh) using EtOAc/CHCl₃/Pet ether as eluent. Liquid state NMR spectra were recorded in CDCl₃ or d₆-DMSO as a solvent. ¹H (400 MHz), ¹³C (100.56 MHz) and ⁷⁷Se (76.29 MHz) NMR spectra were obtained using a Bruker 400 MHz NMR spectrometer. Chemical shifts are reported in ppm with respect to tetra methylsilane (SiMe₄) for ¹H and ¹³C and with respects to Me₂Se (external standard) for ⁷⁷Se NMR experiments. The solvent resonance was used as internal standard (CDCl₃, δ 7.26; d₆-DMSO, δ 2.50 for ¹H NMR and CDCl₃; δ 77.00, d₆-DMSO; δ 39.52 for ¹³C NMR). For ¹H NMR, data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz) and integration. Electronic absorption spectra were recorded on a Perkin Elmer LAMBDA 750 UV/visible spectrophotometer. Fluorescence emission studies were carried out on a Horiba JOBIN YVON Fluoromax-4 spectrometer. High-performance liquid chromatography (HPLC) experiments were carried out on a Waters Alliance System (Milford, MA, USA) consisting of a 2695 separation module and a 2996 photodiode-array detector. The assays were performed in 1.8 mL sample vials, and a built-in auto-sampler was used for sample injection. The HPLC system was controlled with EMPOWER software (Waters Corporation, Milford, MA, USA). Samples were analysed by reverse-phase HPLC method (Chromasol ONYX C-18 column, 0.4x250mm, 5µm) 1mL/min gradient elution method. The optimized elution parameters were mobile phase A (15 mM ammonium acetate-acetic acid buffer pH 4) and mobile phase C (0.1% TFA in acetonitrile) 10-90%, 0-25 min. Mass spectral studies were carried out on a Q-TOF micro mass spectrometer with ESI-MS mode analysis.

2. Experimental Procedure

2.1.1. Synthetic route to compound 6



Scheme S1: Synthesis of compd. **6** (i) NBS, DMF, 2 h (ii) K₂Cr₂O₇, AcOH, 120 °C, 5 h (iii) Et-NH₂, 1,4-dioxane, 110 °C, 12 h (iv) NaNO₂, HCl, Se powder, NaBH₄ (v) SOCl₂, (cat.) DMF, 75 °C, 6 h (vi) NH₃ sol., dry ACN, rt, 1 h (vii) K₂CO₃, dry DMF, 85 °C, 36 h.

2.1.3. Synthesis of 4-bromo acenaphthalene (**S2**)^[1]

A solution of NBS (17.79 g, 0.1 mol) was dissolved in 50 mL of DMF. To this was added a suspension of acenaphthene (**S1**) (15.4 g, 0.1 mol) in 50 mL of DMF. Exothermic reaction was observed, and the reaction mixture was continuously stirred at room temperature for 2 h. The completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured into 1.5 L of cold water. The precipitate was filtered through suction and washed with water (3 × 100 mL). The precipitate was dried in air dried oven. The compound was purified by column chromatography using 100% pet ether. The product was obtained as a pale-yellow solid (15.8 g, 68% yield). The NMR spectrum of **S2** was recorded in CDCl₃ solvent. ¹H NMR (400 MHz, CDCl₃): δ(ppm) 7.79-7.77 (d, J=8.4 Hz, 1H), 7.66-7.65 (d, J=7.3 Hz, 1H), 7.57-7.53 (t, J=7.2 Hz, 1H), 7.33-7.31 (d, J=6.8 Hz, 1H), 7.13-7.11 (d, J=7.2 Hz, 1H), 3.42-3.39 (m, 2H), 3.34-3.33

(m, 2H). ¹³C NMR (100.56 MHz, CDCl₃): δ(ppm) 146.7, 146.4, 140.7, 137.3, 129.5, 122.2, 120.6, 120.5, 117.2, 31.1, 30.3.

2.1.4. Synthesis of 4-bromo naphthalicanhydride (S3)^[2]

To a solution of sodium dichromate dihydrate (31.9 g, 0.1 mol) in 150 mL of glacial acetic acid, 4-bromo acenaphthalene (**S2**) (10 g, 0.04 mol) was added with continuous stirring. The reaction mixture was refluxed for 6 h. The completion of the reaction was monitored by TLC. The reaction mixture was cooled to 0 °C and then the dark green solution was further diluted with cold water (100 mL). The dark green liquid was filtered through suction, and a yellow-orange solid was obtained. The precipitate was dissolved in 4% aqueous solution of sodium hydroxide with heating at 55 °C and then cooled to rt. The yellow colour suspension was filtered through suction and then the filtrate was neutralized with a 5% aqueous solution of hydrochloric acid. The precipitate was dried in oven and then the crude product was purified by column chromatography on silica gel (60-120 mesh) using 50:50 ethyl acetate / petroleum ether to afford a pale-orange solid (5.70 g, 48% yield). The NMR spectrum of **S3** was recorded in DMSO-d₆ solvent. ¹H NMR (400 MHz, DMSO-d₆): δ(ppm) 8.57-8.55 (d, J=8.1Hz, 2H), 8.32-8.30 (d, J=7.8Hz, 1H), 8.22-8.20 (d, J=7.8Hz, 1H), 8.02-7.98 (t, J=7.8Hz, 1H). ¹³C NMR (100.56 MHz, DMSO-d₆): δ(ppm) 161.0, 161.0, 134.5, 134.1, 133.4, 132.5, 131.3, 131.1, 130.8, 130.0, 120.7, 119.9.

2.1.5. Synthesis of 4-bromo-1,8-naphthalimide (S4)^[3]

4-Bromo-1,8-naphthalic anhydride **S3** (4 g, 14.5 mmol) and ethyl amine (1.05 mL, 17.3 mmol) were dissolved in 80mL of 1,4-dioxane. The reaction mixture was refluxed for 7 h. Then the reaction mixture was cooled to room temperature. Consecutively, a second aliquot of ethyl amine (1.05 mL, 17.3 mmol) was added. The reaction mixture was refluxed for another 7 h. The reaction mixture was cooled to room temperature and poured into ice cold water. The precipitate was collected by suction filtration and then washed three times with water. The crude product was purified by column chromatography on silica gel (60-120 mesh) using 10:90 petroleum ether/ethyl acetate to obtain a white solid (3.42 g, 78% yield). The NMR spectrum of **S4** was recorded in CDCl₃ solvent. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.81-8.79 (d, J=8.7Hz, 1H), 8.72-8.70 (d, J=7.4Hz, 1H), 8.67-8.65 (d, J=8.1Hz, 1H), 8.39-8.37 (d, J=8Hz, 1H), 7.98-7.94 (q, J=8.9Hz, 1H), 4.26-4.21 (q, J=14.2Hz, 2H), 1.35-1.31 (t, J=6.8Hz, 3H). ¹³C NMR (100.56 MHz, CDCl₃): δ 163.5, 162.7, 149.9, 132.8, 130.3, 130.1, 129.7, 129.5, 127.5, 124.3, 124.1, 123.5, 36.4, 13.7.

2.1.6. Synthesis of 2, 2'-diselanediylidibenzoic acid (S6)^[4]

Diazotization reaction was carried out first wherein NaNO₂ (3g, 43.7 mmol) was dissolved in 15 mL of water and was then added drop-wise to a continuously stirring solution of anthranilic acid (6 g, 43.7 mmol) in 60 mL of 1 M HCl solution taken in a round bottom flask and temperature range of 0 °C to 5 °C was maintained.

The stirring was continued for another 10 min and then 2 N NaOH was added to the solution till the solution became basic and a colour change of solution to golden-orange was observed.

Next, selenium powder (3.5 g, 43.7 mmol) was suspended in 15 mL of 2 N NaOH solution in another round-bottom flask kept in an ice bath. Then, NaBH₄ (1.6 g, 43.7 mmol) was added to a mixture of 5 mL, 2 N NaOH and 5 mL of H₂O and this freshly prepared solution was added drop-wise with continuous stirring to selenium for its reduction. The stirring was continued for another 30 min. This reaction yielded a change from grey colour to a deep reddish-brown on partial reaction and further changed to white as Na₂Se₂ was formed on completion of the reaction. Consecutively, the prepared solution of diazonium salt was taken and was added progressively to the alkaline solution of disodium diselenide under vigorous stirring. The temperature was initially maintained at 0 °C to 5 °C for 5 min and then the reaction mixture was stirred at room temperature for the next 3 h. On completion, the reaction mixture was acidified with HCl to attain a pH around 2 and filtered. A pale-yellow solid was obtained.

2.1.7. Synthesis of 2, 2'-diselanediyldibenzoyl chloride (**S7**)^[5]

For the synthesis of 2, 2'-diselanediyldibenzoyl chloride, the diselenide substrate **S6** was taken and an excess of thionyl chloride (15.88 mL, 0.22 mol) was added drop-wise in the presence of catalytic amounts of DMF (200 μL, 10 mol %) with continuous stirring. A CaCl₂ guard tube was fixed to the condenser and the reaction mixture was then refluxed at 75 °C for 6 h. Thionyl chloride was then removed under reduced pressure and then the desired product was obtained by dissolving the reaction mixture in petroleum ether and filtering it. The filtrate was then collected, and the solvent was evaporated to obtain the product as yellow colour solid.

2.1.8. Synthesis of benzo-[d]-[1,2] selenazol-3(2H)-one (**S8**)^[6]

The substrate **S7** (1 g, 3.92 mmol) was taken in 20 mL of dry acetonitrile and 25% aqueous solution of ammonia (1.1 mL, 13.0 mmol) was added drop-wise to it with continuous stirring over a period of 10 min at 0 °C under nitrogen atmosphere. The stirring was then continued for 1 h followed by evaporation of solvent under reduced pressure. Then, 50 mL of water was added to the reaction mixture and it was stirred for another 2 h at room temperature. The reaction mixture was diluted with 100 mL of cold water and filtered through suction. The crude product was purified by active neutral alumina column chromatograph using 90:10 EtOAc / methanol as eluent to afford pale-yellow crystalline solid **S8** (51%). ⁷⁷Se NMR (76.29 MHz, DMSO-d₆): δ (ppm) 794.4, ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 9.17 (bs, 1H), 8.06-8.04 (d, J=8.1 Hz, 1H), 7.82-7.80 (d, J=7.8 Hz, 1H), 7.62-7.58 (m, 1H), 7.42-7.38 (m, 1H). ¹³C NMR (100.56 MHz, d₆-DMSO): δ (ppm) 169.5, 142.3, 132.3, 128.5, 128.1, 127.1, 126.4.

2.1.9. Synthesis of compd. 6

4-Bromo-N-ethyl naphthalimide (277 mg, 2.0 mmol), benzo [d] [1,2]-selenazol-3(2*H*)-one (297 mg, 3.0 mmol) and potassium carbonate (362.7 mg, 5.0 mmol) were dissolved in dry DMF. The reaction mixture was heated at 85 °C under nitrogen atmosphere for 36h. The progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and then crude reaction mixture was extracted with ethyl acetate (3 × 25 mL) and washed with saturated brine solution (3 × 50 mL). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by washing with ethyl acetate and methanol several times to afford **6** as a yellow crystalline solid (207 mg, 54% yield). ⁷⁷Se NMR (76.29 MHz, DMSO-*d*₆): δ (ppm) 945.4 ¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 8.54-8.49 (m, 2H), 8.28-8.26 (d, J=8Hz, 1H), 8.13-8.11 (d, J=8.7Hz, 1H), 7.95-7.93 (d, J=7.7Hz, 1H), 7.87-7.82 (m, 2H), 7.75-7.70 (m, 1H), 7.54-7.50 (t, J=7.5Hz, 1H), 4.11-4.05 (q, J=7.1Hz, 2H), 1.24-1.20 (t, J=6.9Hz, 3H). ¹³C NMR (100.56 MHz, *d*₆-DMSO): δ (ppm) 167.3, 164, 163.6, 142.8, 141.6, 133.4, 132.0, 131.6, 131.2, 129.6, 129.2, 129.1, 128.8, 128.5, 127.7, 127.4, 127.0, 123.5, 122.5, 35.8, 14.0. ESI-MS analysis of compd. **6**, m/z calcd for C₂₁H₁₄N₂O₃Se: 423.0248 [M+H]⁺; found: 423.1504.

2.1.10. Single crystal X-ray crystallography:

Single crystals were obtained through slow evaporation method using ACN / DMSO solvent system. The single crystal X-ray diffraction data were collected on a Bruker SMART APEX CCD diffractometer utilizing SMART/SAINT software.^[7] Intensity data were collected using graphite-monochromatized Mo-K α radiation of wavelength 0.71073 Å at room temperature. Single crystals were mounted at room temperature on a loop and the data collected at 100K. All the structures were solved by SHELX-97 program incorporated in WinGX. Empirical absorption corrections were applied with SADABS.^[8,9] The structures were solved by direct methods (SIR-92) and refined by full-matrix least-squares procedures on F² for all reflections (SHELX-97). CCDC number:1905225

2.1.11. Generation of Reactive Oxygen species (ROS)

Synthesis of Peroxynitrite^[10]: A solution of 30% H₂O₂ (5.7 mL) was diluted in 50 mL of cold water. To this, 30 mL of 5N NaOH and 0.04 M DTPA in 0.05 N NaOH were added with gentle stirring, and the resulting mixture was diluted to a volume of 100 mL. The conc. of H₂O₂ in the final solution was 0.5 M, with pH ranging from 12.5 to 13.0. The buffered H₂O₂ was stirred vigorously with an equimolar amount of isoamyl nitrite (0.5M or 6.7 mL) for 3-4 h at room temperature. The reaction was monitored by withdrawing aliquots at an interval of 15 or 30 min and assaying for peroxynitrite at 302 nm by UV-spectrometer. When the required concentration of peroxynitrite was reached, the aqueous phase was washed with (3 × 100 mL) volume of dichloromethane, chloroform, and hexane in a separating funnel to remove the unreacted isoamyl alcohol and isoamyl nitrate. The unreacted H₂O₂ was removed by passing the aqueous solution through a

column filled with 25 g of granular MnO₂. The concentration of the stock solution of peroxyxynitrite was measured after 500 times dilution with 0.1 N NaOH solution and then assaying for peroxyxynitrite at 302 nm ($\epsilon = 1670\text{M}^{-1}\text{cm}^{-1}$) by UV-spectrometric method.

Generation Hydroxy radicals: The preparation of 10 mM of •OH (stock solution) was carried out by following the literature method.^[11] Briefly, 1.52 g of anhydrous FeSO₄ was dissolved in 0.5 mL of ultrapure water. To this, 3.36 mg of EDTA and 5.68 μL of an 8.8 M solution of H₂O₂ were added. The total volume of the final solution was increased to 1 mL by addition of ultrapure water. From this 10 mM stock solution, appropriate volume was used to get 10 μM •OH as the final concentration.

Generation of Superoxide: The preparation of 10 mM of O₂⁻ (stock solution) was carried out by following the literature procedure using anhydrous potassium superoxide (0.71 mg) was dissolved in aprotic solvent (1 mL of DMSO) in the presence of 18-crown-6 (26.4 mg).^[12] From this 10 mM stock solution, appropriate volume was used to get 10 μM O₂⁻ as the final concentration.

2.2. Cell lines and culture condition

HepG2-hepatocellular carcinoma (HB8065) was obtained from ATCC, Washington DC, USA. The cells were grown in MEM supplemented with 2.2 g/L sodium bicarbonate, 10% supplemental FBS, penicillin G (100 unites/mL) streptomycin (100 mg/mL). The cells were cultured till 80 - 90% confluence and further sub-cultured in different sized cell culture dishes/plates depending on the type of experiment in a humidified incubator at 37 °C with 5% CO₂. For fluorescence reader assays, the cells were cultured on black walled 96-well plates with clear film bottom.

2.3. Isolation and culture of primary HUVEC

HUVECs were isolated and cultured as described previously.^[13] Human umbilical cords were collected from a hospital with informed consent from the volunteers according to the approved guidelines of Institutional Human Ethics Committee (IHEC No: 1-15092017), Indian Institute of Science, Bangalore. The cords which were stored in M199 medium supplemented with antibiotics transported to the lab and the isolation was created out within 4 – 6 hours. As a first step, the cord vein was cannulated with three way stop cock on both the ends and tightly locked with cable tie. The vein was flushed with PBS buffer containing antibiotics slowly via three way stop cock to remove blood from the vein. After washing, the vein was filled with 0.25% Trypsin/EDTA and incubated for 20 minutes at 37 °C in a water bath. The dissociated cells of the vein were collected and centrifuged at 3000 rpm for 10 minutes. The cell pellet was re-suspended in M199 medium supplemented with 20% FBS, 1 X penicillin /streptomycin and seeded on T25 culture flask pre-coated with gelatin and incubated the culture flask in CO₂ incubator at 37 °C. On the following day the medium was replaced with M199 medium supplemented with 20% FBS, antibiotics and endothelial cell growth factor (ECGS) (Sigma E2759). The culture medium was changed once in two days till the cells attain 70% to 80%

confluence which usually takes about 6 to 7 days from the day of isolation. The morphology of HUVECs was observed through inverted phase contrast microscope which had “polygonal cobblestone” like appearance. The primary HUVECs were further sub-cultured in different sized cell culture dishes depending on the type of experiment in humidified incubator at 37 °C with 5% CO₂.

2.4. Cell viability assay with MTT

The viability of the cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay as described previously.^[13] The cells were plated on 96-well microtiter cell culture plates at 20,000 cells per well for 24 h before they were incubated for 30 minutes, 1, 6, 12 and 24 h with various concentrations of compd. **6**. After the indicated treatment, the cells were incubated for 3 h at 37 °C in a culture medium containing 10 μmol L⁻¹ MTT in phosphate-buffered saline (PBS). The blue MTT formazan precipitate was then dissolved in 100 μL of DMSO, and the absorbance was measured at 570 nm with a multi-well plate reader. The cell viability was expressed as the percentage of the absorption values in the treated cells relative to the non-treated (control) cells. The data are presented as the means ± the standard error of the triplicated experiments.

2.5. Intracellular fluorescence intensity assay

After cells were seeded in to 96-well cell culture grade microtiter plates at 10,000 -20,000 cells/well and cultured for 24 h, the cells were treated with compounds and inhibitors for various time points according to the experimental condition, then washed 3 times with PBS buffer. After washing, the MEM medium was added and the fluorescence intensity in the cells was measured by GloMax® Microplate Reader (Promega).

2.6. Confocal microscopy

The cells were cultured on glass coverslips in 12-well culture plates (0.2 X 10⁶ per well). The cells were allowed to adhere and proliferate for 24 hours. Cells were then incubated with compounds or inhibitors at different time points depending on the experimental condition. After the treatment, the cells were washed with PBS and fixed with 4% PFA (paraformaldehyde) followed by wash with PBS buffer. Finally, the cover slips containing fixed cells were mounted on microscopic slides using fluoromount (F4680, Sigma). Images were captured with Zeiss LSM880 (Airyscan) confocal microscopy using excitation wavelength of 405 nm laser for naphthalimide based compounds, 570 nm (red laser) for Amplex Red and 520 nm (green laser) for DHE. The images were further cropped and extracted using Zen v2.3 (blue edition) software. Identical exposure times and processing were used in all experiments.

2.7. HPLC analysis of compd. 6 and its intermediates from cell lysates

HepG2 cells were cultured on 6-well culture plates (1×10^6 per well) and allowed to adhere and proliferate for 24 hours. The cells were pre-treated with either 250 μM H_2O_2 (A) or 50 μM BSO (B) or 20 mM 3-AT (C) for 30 min. After pre-treatment, the cells were washed thoroughly three times with PBS and further the cells incubated with 25 μM compd. **6** for 5 min. Cells were washed again with PBS for three times and finally the cells were harvested from culture plate by using 0.25% Trypsin/EDTA treatment. The collected cells (cells from 2 X 6-well plates were pooled with approximately of 18-20 million cells) were centrifuged and re-suspended the cell pellet with 250 μL of lysis buffer (150 mM NaCl, 1 mM EDTA, 1% Triton-X, 10 mM Tris (pH 7.4) and 1 mM EGTA). The cells were lysed by three cycles of rapid freeze (in liquid nitrogen) and thawing and sonication for 30 sec using 35% vibration amplitude. Finally, the cell lysate was injected to HPLC and chromatogram was extracted at 345 nm.

2.8. Statistical analysis

Results are represented as mean (\pm standard deviation SD) of three independent experiments. Data were analysed using GraphPad Prism 6 software (GraphPad Prism, Inc., San Diego, CA, USA). Un-paired 't' test were used for statistical analysis of differences in two groups and one-way analysis of variance (ANOVA) with a post-hoc Tukey's test for multiple comparisons. $p < 0.05$ was considered as statistically significant difference.

3. Results

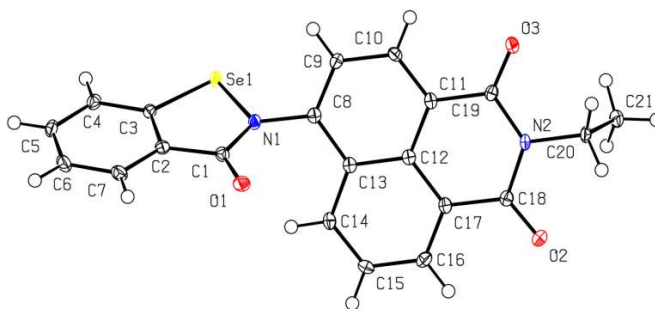


Figure S01: ORTEP diagram of compd. **6**. Thermal ellipsoids are drawn at 50% probability.

Table 1. Crystal data and structure refinement for Compd. **6**

Parameters	Compd. 6	Parameters	Compd. 6
Empirical formula	C ₂₁ H ₁₄ N ₂ O ₃ Se	Index ranges	-10 ≤ h ≤ 10, -8 ≤ k ≤ 8, -19 ≤ l ≤ 20
Formula weight	421.30	Reflections collected	14443
Temperature	100(2) K	Independent reflections	3623 [R(int) = 0.0467]
Wavelength	0.71073 Å	Completeness to theta = 25.242°	100.0 %
Crystal system	Monoclinic	Absorption correction	Semi-empirical from equivalents
Space group	P n	Max. and min. transmission	0.7456 and 0.5985
Unit cell dimensions	a = 8.3796(12) Å, b = 6.4686(8) Å, c = 15.425(2) Å α = γ = 90°; β = 104.496(6)°	Refinement method	Full-matrix least-squares on F ²
Volume	809.51(19) Å ³	Data / restraints / parameters	3623 / 2 / 246
Z	2	Goodness-of-fit on F ²	1.040
Density (calculated)	1.728 Mg/m ³	Final R indices [I > 2σ(I)]	R1 ^a = 0.0210, wR2 ^{b,c} = 0.0489
Absorption coefficient	2.346 mm ⁻¹	R indices (all data)	R1 = 0.0216, wR2 = 0.0491
F(000)	424	Absolute structure parameter	0.025(8)
Crystal size	0.120 x 0.090 x 0.050 mm ³	Largest diff. peak and hole	0.380 and -0.306 e.Å ⁻³
Theta range for data collection	2.539 to 27.549°.		

^aR = $\sum |F_o| - |F_c| / \sum |F_o|$; ^bR_w = $\{\sum [w(|F_o| - |F_c|)^2] / \sum [wF_o^2]\}^{1/2}$; ^cw = 1/σ²(F_o).

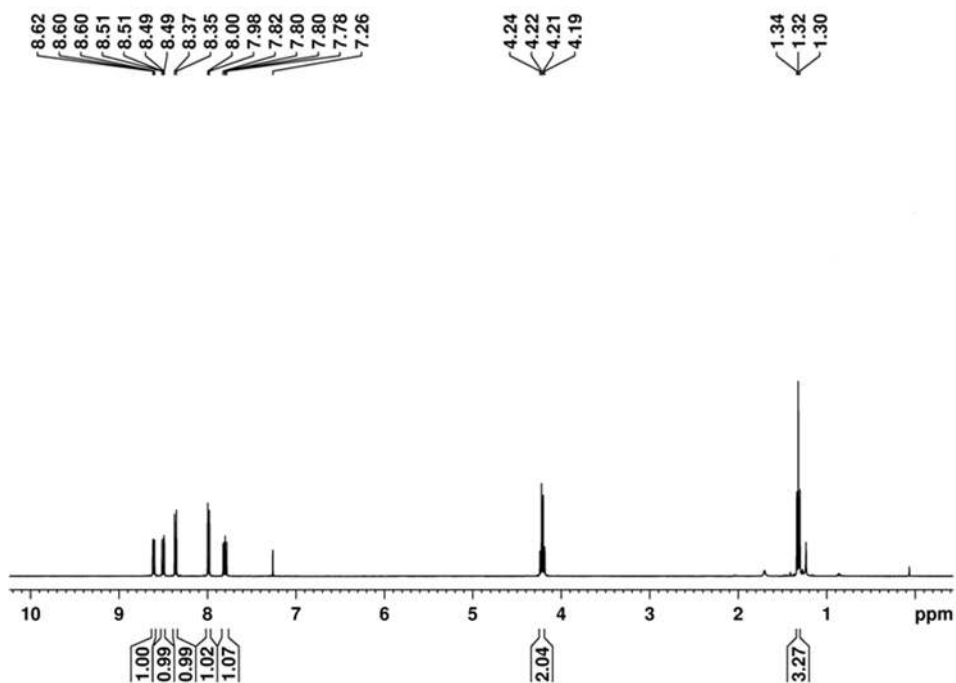


Figure S02: ^1H NMR spectrum (CDCl_3) of 4-bromo-1,8-naphthalimide.

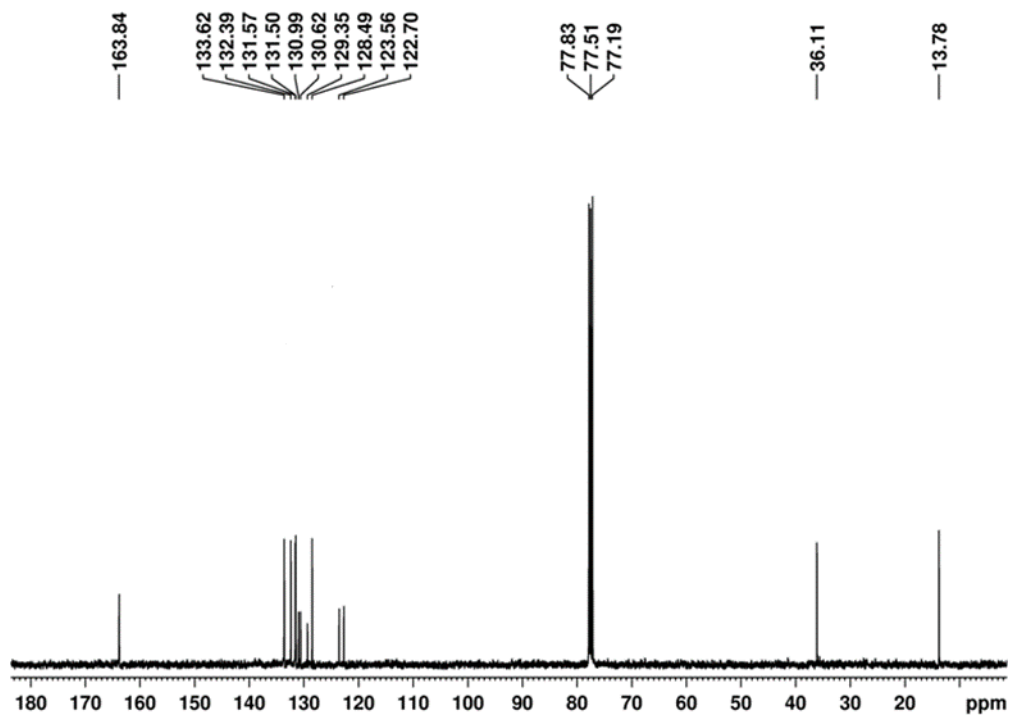


Figure S03: ^{13}C NMR spectrum (CDCl_3) of 4-bromo-1,8-naphthalimide.

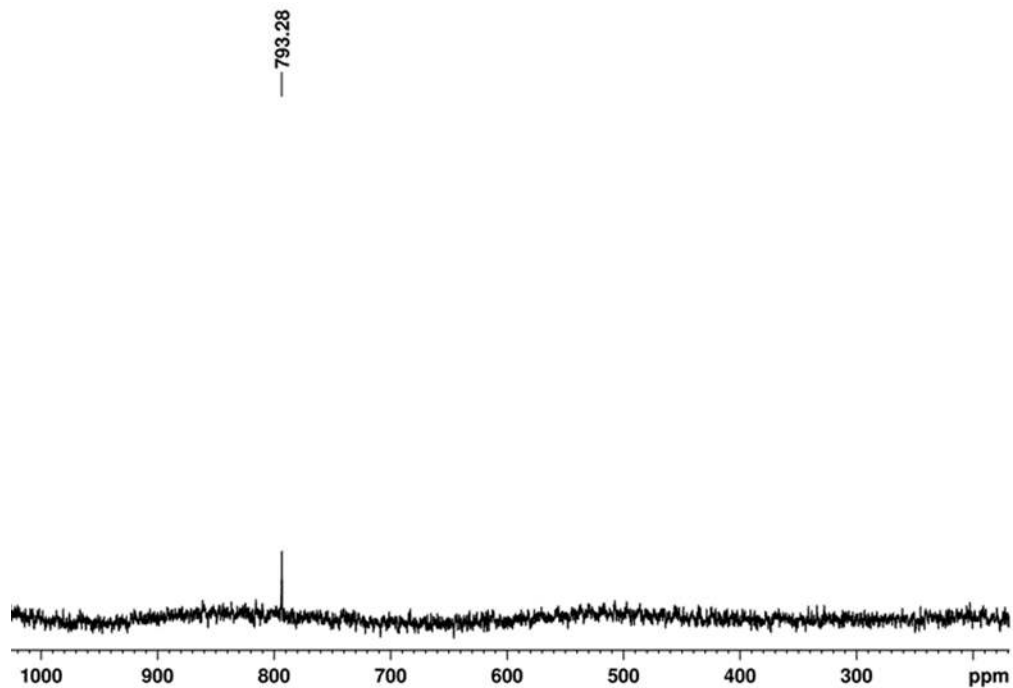


Figure S04: ^{77}Se NMR spectrum (d_6 -DMSO) of benzo[*d*][1,2]-selenazol-3(2*H*)-one.

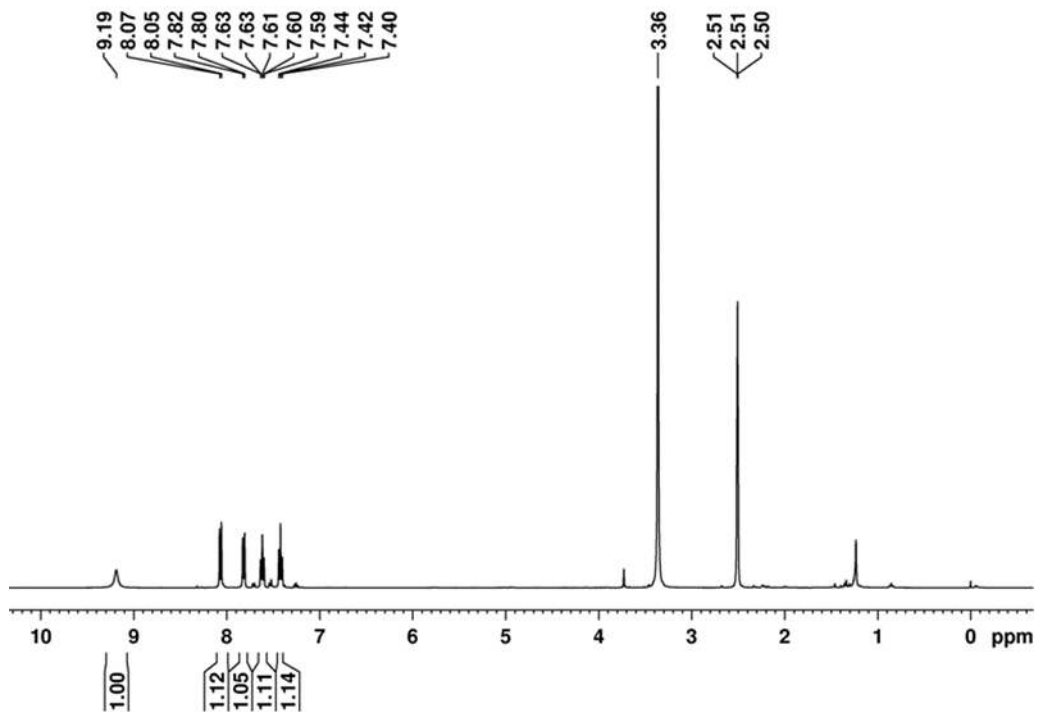


Figure S05: ^1H NMR spectrum (d_6 -DMSO) of benzo[*d*][1,2] selenazol-3(2*H*)-one.

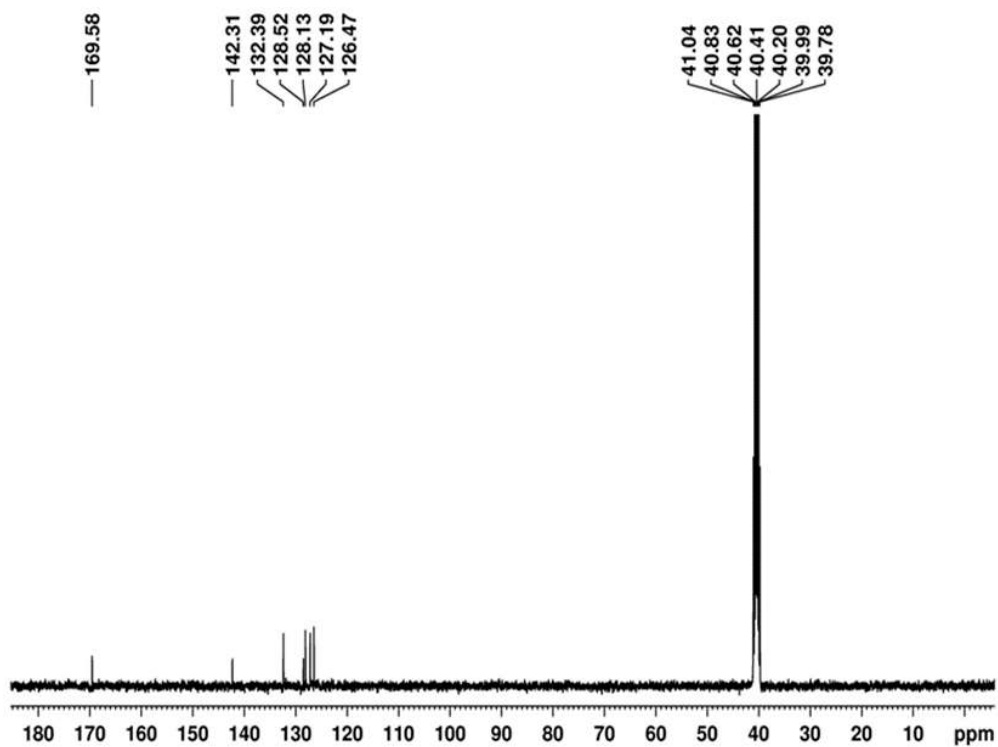


Figure S06: ^{13}C NMR spectrum (d_6 -DMSO) of benzo[d][1,2]-selenazol-3(2H)-one.

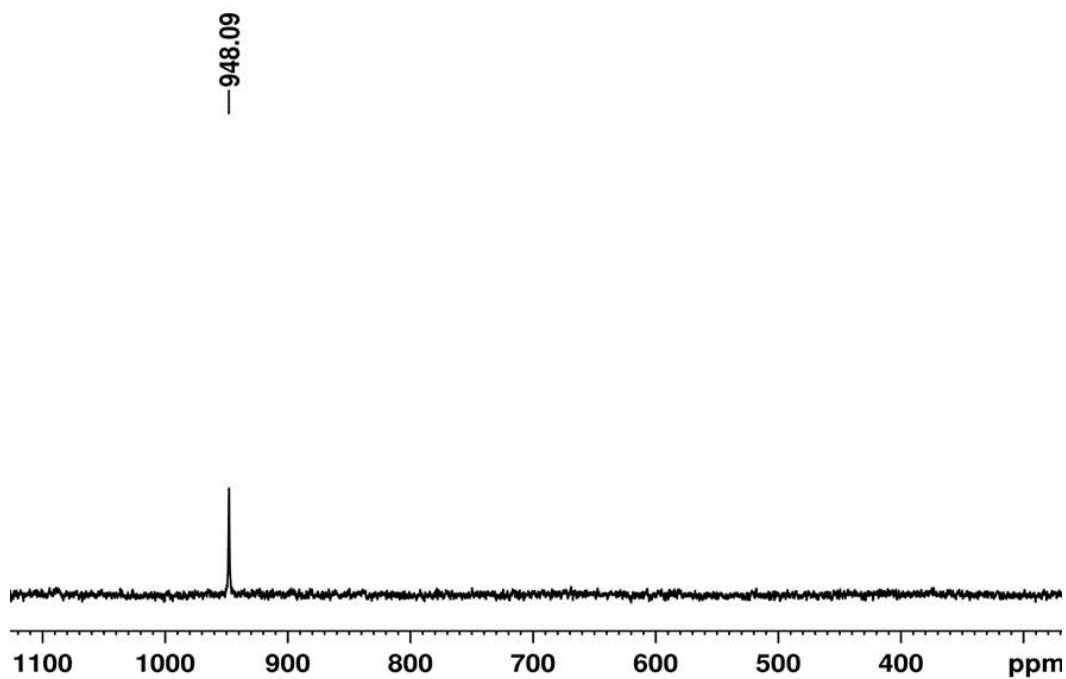


Figure S07: ^{77}Se NMR spectrum (d_6 -DMSO) of compd. 6.

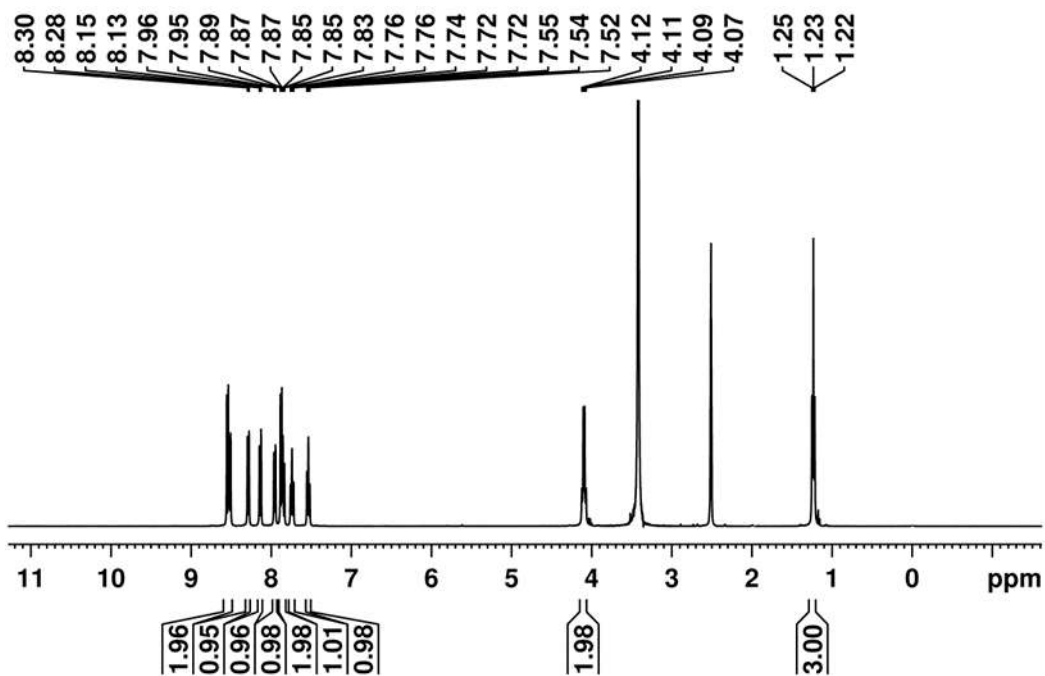


Figure S08: ^1H NMR spectrum (d_6 -DMSO) of compd. 6.

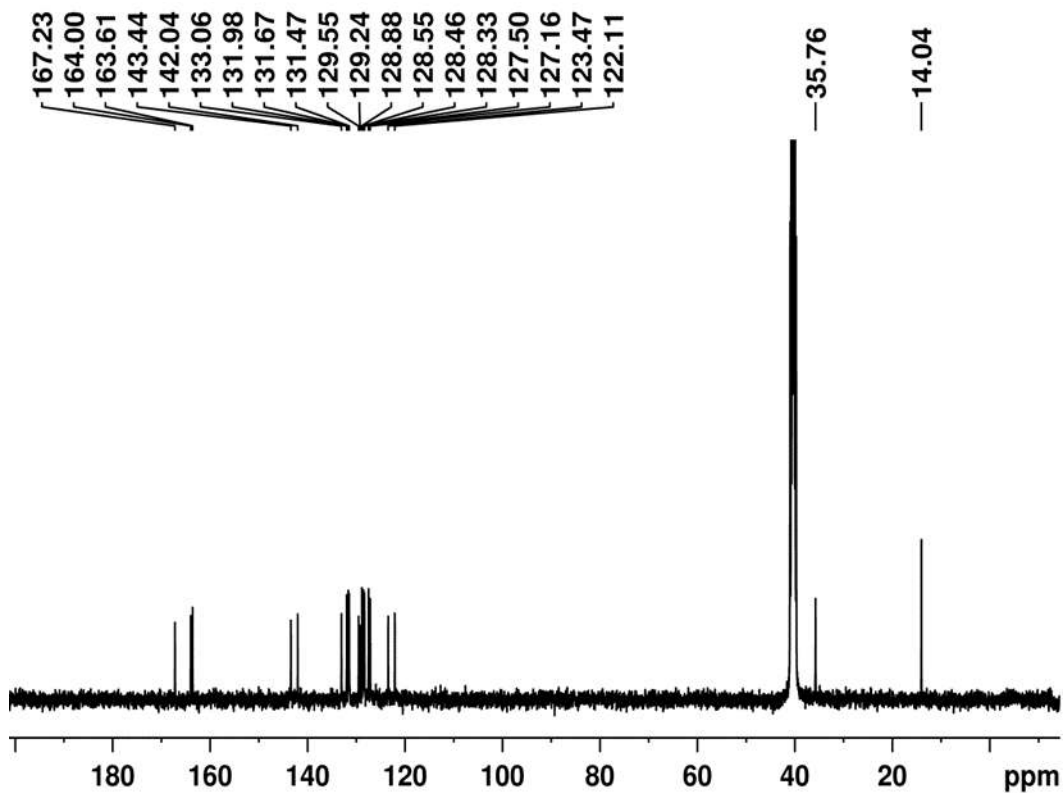


Figure S09: ^{13}C NMR spectrum (d_6 -DMSO) of compd. 6.

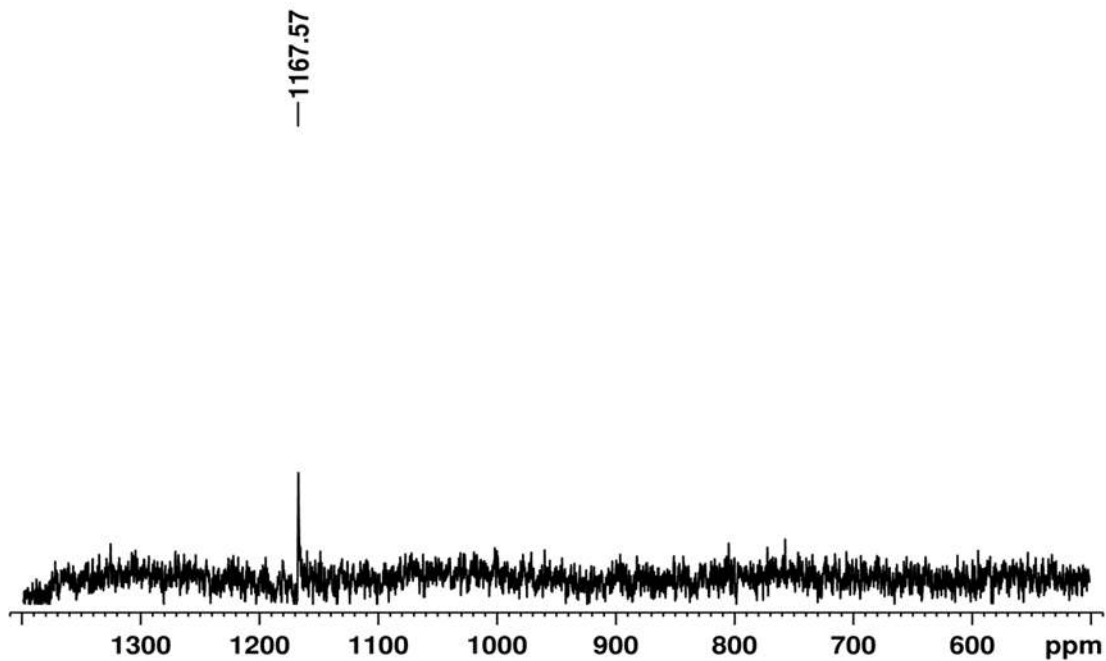


Figure 10: ^{77}Se NMR spectrum (d_6 -DMSO) of compd. 10.

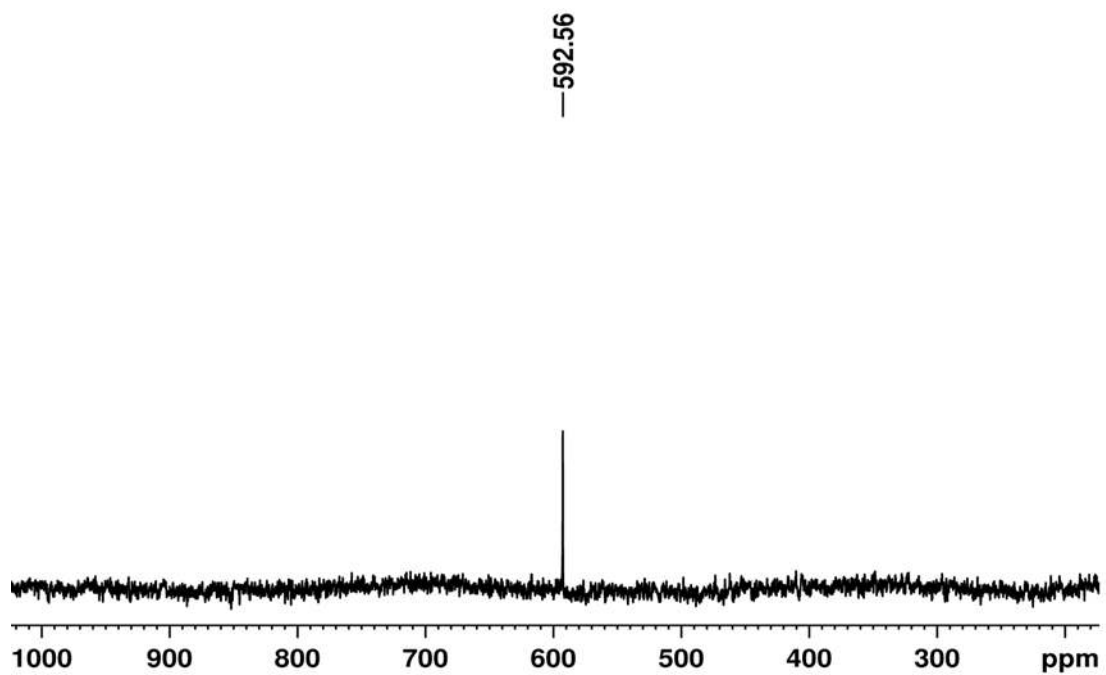


Figure S11: ^{77}Se NMR spectrum (d_6 -DMSO) of compd. 11

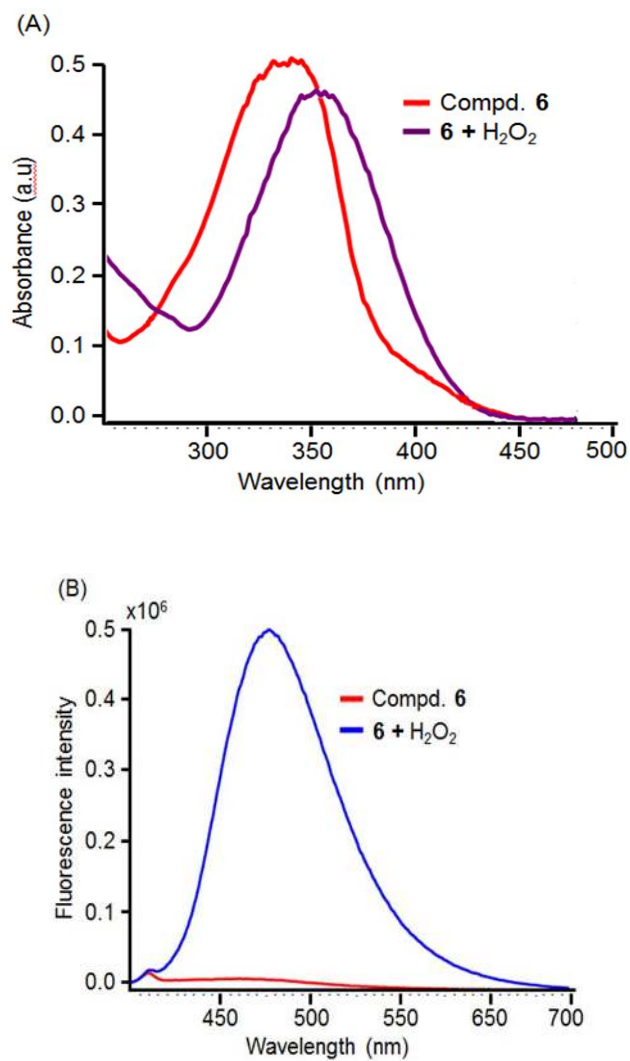


Figure S12: Photophysical properties of compd. **6**. The UV-Vis absorption spectra **(A)** and fluorescence emission spectra **(B)** of compd. **6** (10 μ M, pH = 7.4, λ_{ex} = 350 nm λ_{em} = 475 nm) with the addition of 10 μ M H₂O₂ and incubation of 10 min.

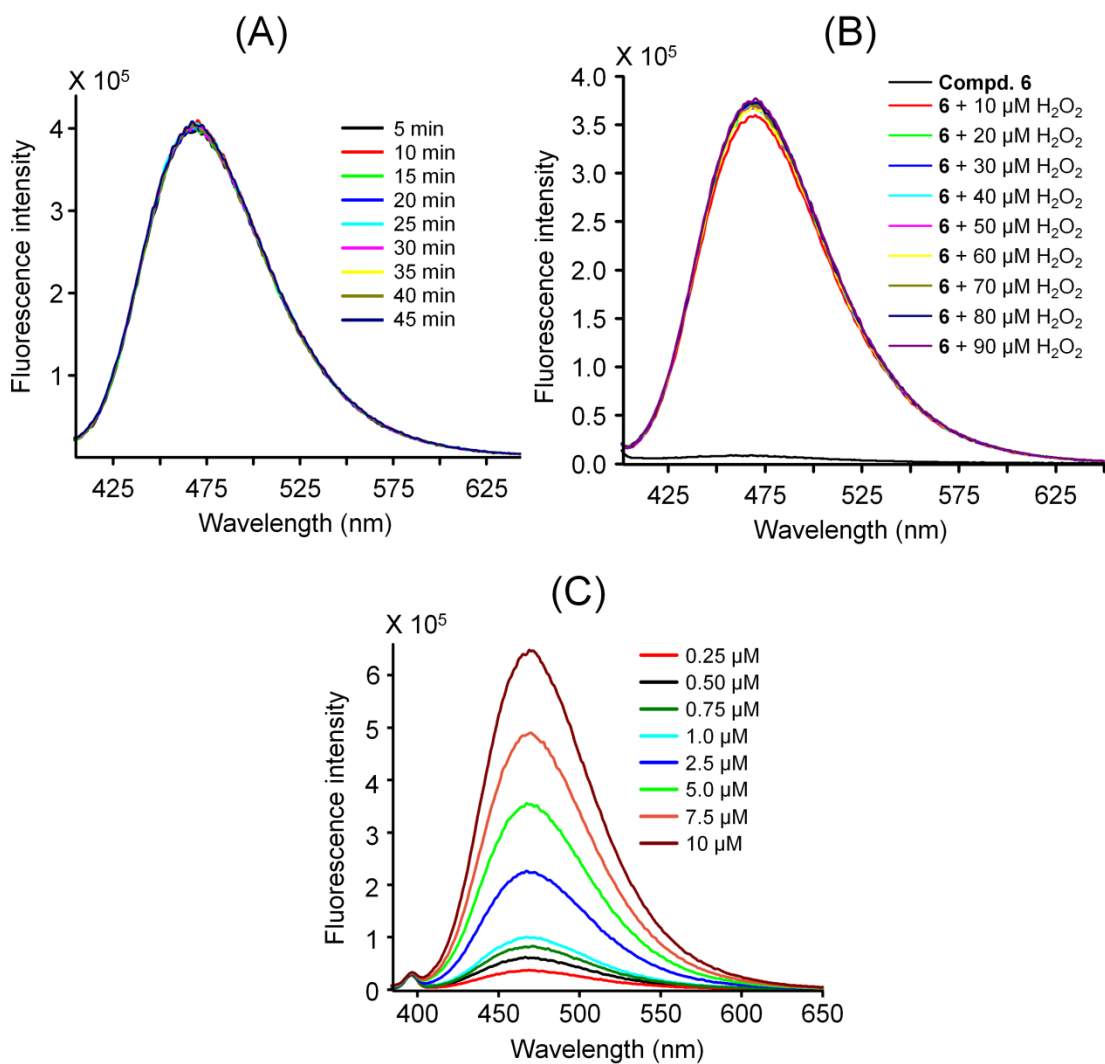


Figure S13. (A) Fluorescence spectra of compd. **6** (5 μM , pH = 7.4, λ_{ex} = 350 nm λ_{em} = 475 nm) with after addition of 10 μM H_2O_2 at different time points. (B) Fluorescence spectra of compd. **6** (5 μM , pH = 7.4, λ_{ex} = 350 nm λ_{em} = 475 nm) after addition of different amounts of H_2O_2 and 5 min incubation time. (C) Fluorescence spectra obtained for different concentrations of compd. **6** (pH = 7.4, λ_{ex} = 350 nm λ_{em} = 475 nm) after addition of 10 μM H_2O_2 and 5 min incubation time.

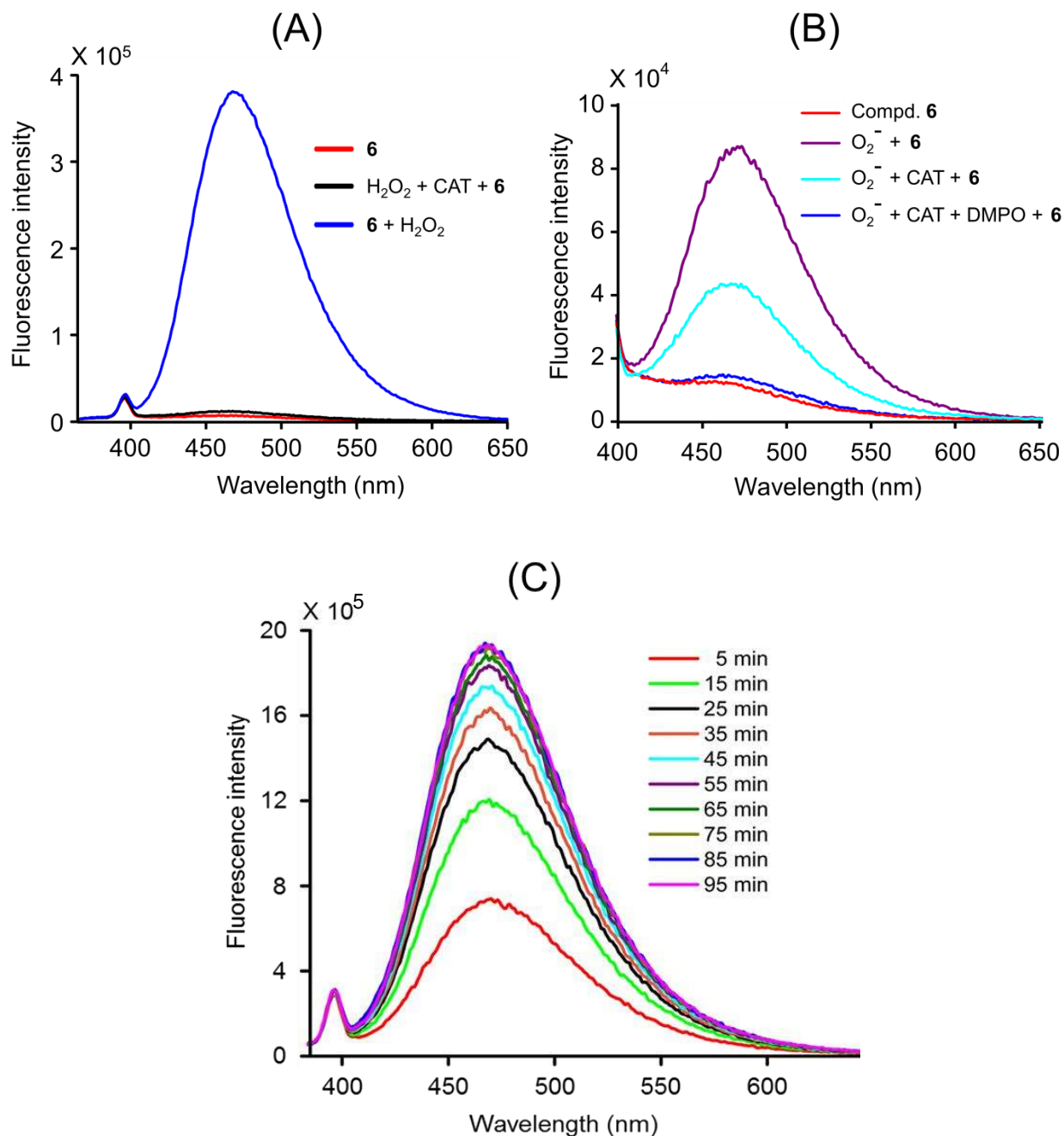


Figure S14: (A) Fluorescence spectra of compd. **6** ($5 \mu\text{M}$, $\text{pH} = 7.4$, $\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 475 \text{ nm}$) after the addition of $10 \mu\text{M}$ H_2O_2 in the presence and absence of catalase (CAT) (540 U/mL) and 5 min incubation time. (B) Fluorescence spectra of compd. **6** ($5 \mu\text{M}$, $\text{pH} = 7.4$, $\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 475 \text{ nm}$) after the addition of $10 \mu\text{M}$ O_2^- with CAT (540 U/mL), 0.25 mM EDTA, $25 \mu\text{M}$ DETAPAC, 0.1 M DMPO in phosphate buffer and 5 min incubation time. (C) Fluorescence spectra of compd. **6** ($5 \mu\text{M}$, $\text{pH} = 7.4$, $\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 475 \text{ nm}$) after the addition of $10 \mu\text{M}$ O_2^- at different time points.

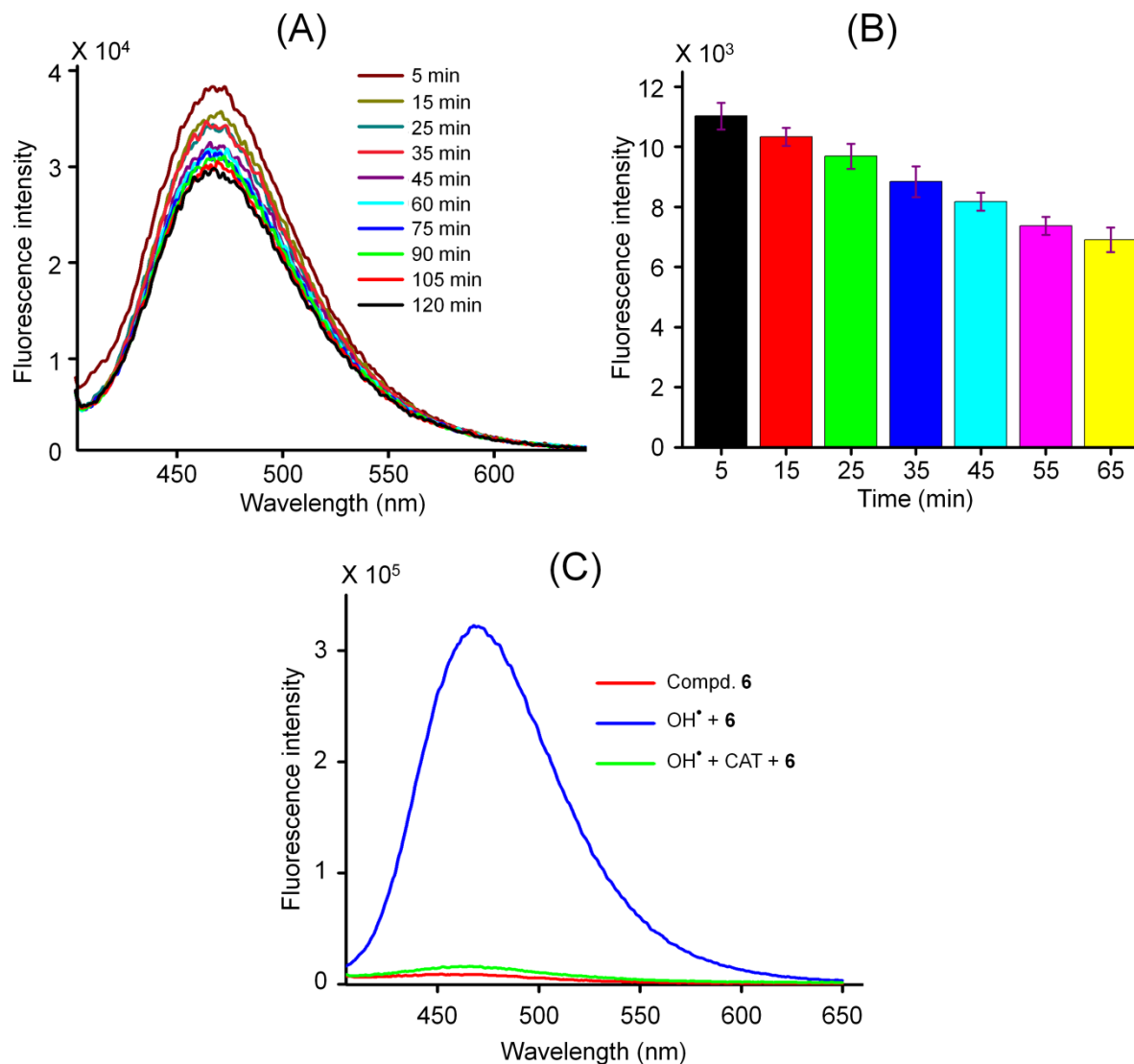


Figure S15: (A) Fluorescence spectra of compd. 6 (5 μM , pH = 7.4, $\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 475 \text{ nm}$) after the addition of 10 μM O_2^- and CAT (540 U/mL) at different time points. (B) Fluorescence spectra of DHE (5 μM , pH = 7.4, $\lambda_{\text{ex}} = 520 \text{ nm}$, $\lambda_{\text{em}} = 610 \text{ nm}$) after the addition of 10 μM O_2^- without catalase at different time points. (C) Fluorescence spectra of compd. 6 (5 μM , pH = 7.4, $\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 475 \text{ nm}$) after the addition of 10 μM $\cdot\text{OH}$ in the presence or absence of catalase and 5 min incubation time.

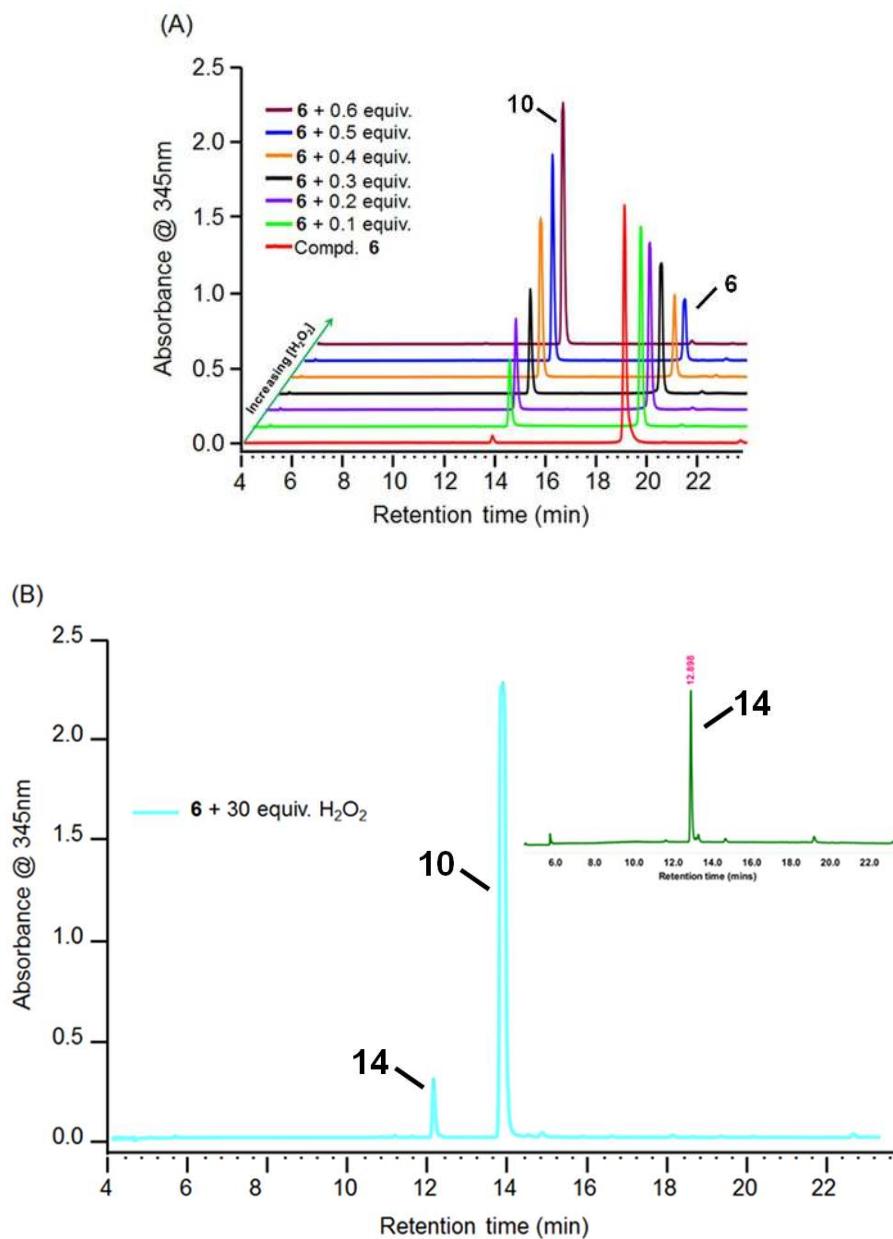


Figure S16: (A) HPLC chromatograms obtained after treating compd. **6** with various amounts of H_2O_2 , indicating the formation of the seleninic acid **10**. (B) Reaction of **6** with 30 equiv. of H_2O_2 . Only a minor amount of the selenonic acid **14** was observed after 30 min. Inset: HPLC chromatogram of **6** with 30 equiv. of H_2O_2 , indicating the formation of selenonic acid after prolonged reaction time.

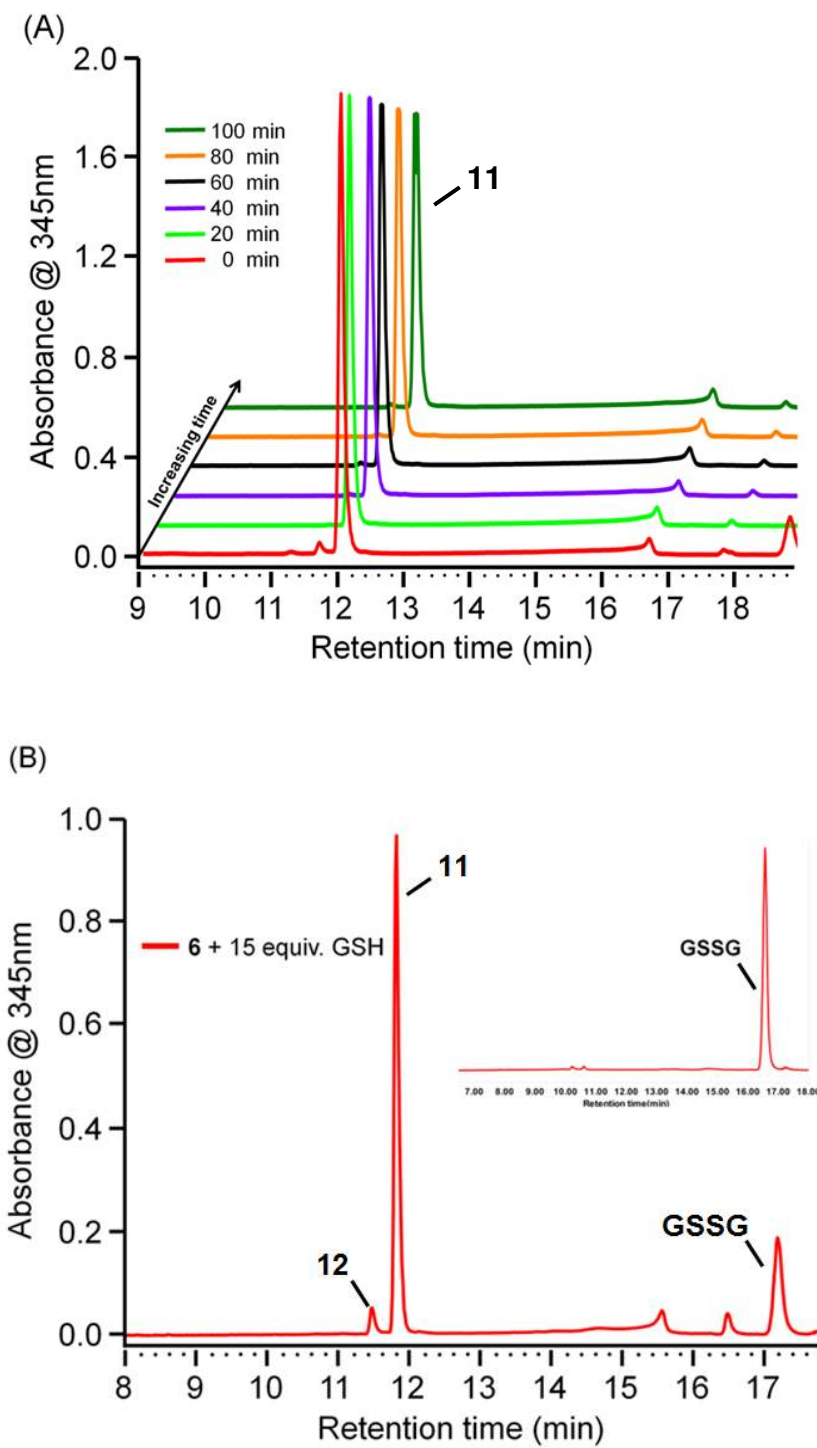


Figure S17: (A) HPLC chromatograms showing a slow time-dependent decrease in the concentration of compd. 11 upon addition of GSH (10 equiv.). (B) HPLC chromatogram for the reaction of compd. 6 with 15 equiv. of GSH. A small amount of selenol 12 was observed at 11.6 min. Inset: pure sample of GSSG.

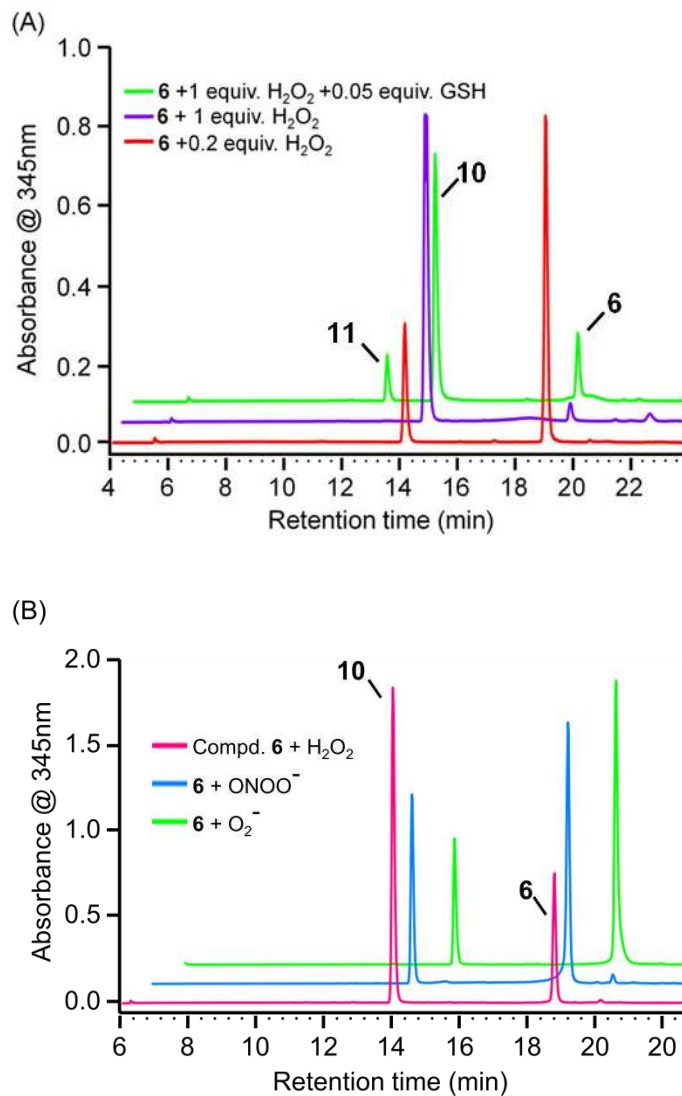


Figure S18: (A) HPLC chromatograms indicating the regeneration of compd. **6** after the addition of a very small amount of GSH to the seleninic acid (compd. **10**). (B) HPLC chromatograms obtained for the reactions of compd. **6** with different ROS (H_2O_2 , ONOO^- , and O_2^-). All of them produce the seleninic acid (compd. **10**) as the final product.

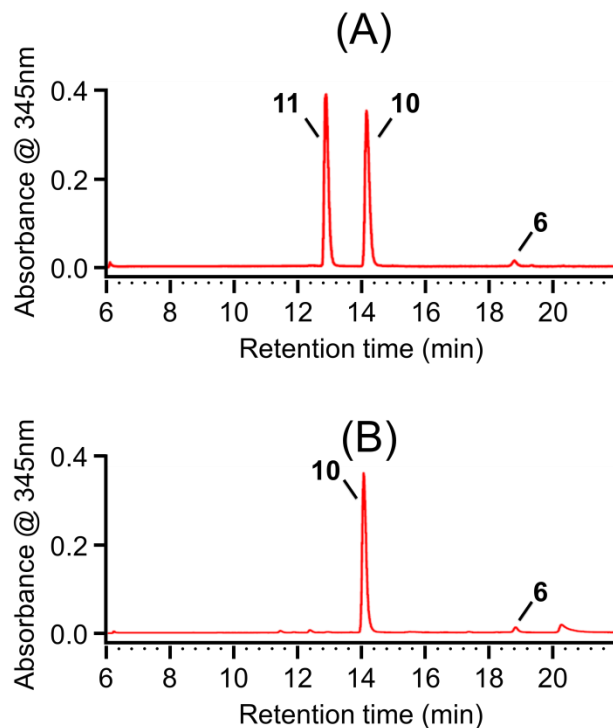


Figure S19: (A) HPLC chromatogram obtained for compounds isolated from the cell lysate (HepG2 cells) after treating the cells with compd. **6** and BSO. The authentic sample of compd. **11** was co-eluted to confirm the intracellular formation of comp. **11**. (B) HPLC chromatogram obtained for compounds isolated from the cell lysate (HepG2 cells) after treating compd. **6** with 3-AT (catalase inhibitor). The inhibition of catalase increases the intracellular H_2O_2 concentration. The authentic sample of compd. **10** was co-eluted to confirm the intracellular formation of comp. **10**.

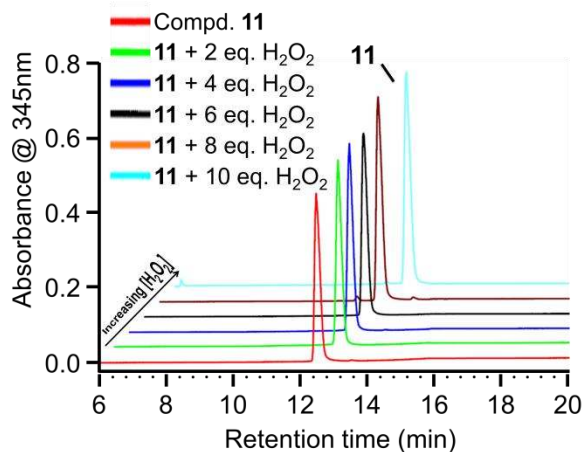


Figure S20: HPLC chromatograms obtained after treatment of compd. **11** with different concentration of H_2O_2 and 30 min incubation time. No reaction of **11** with H_2O_2 was observed.

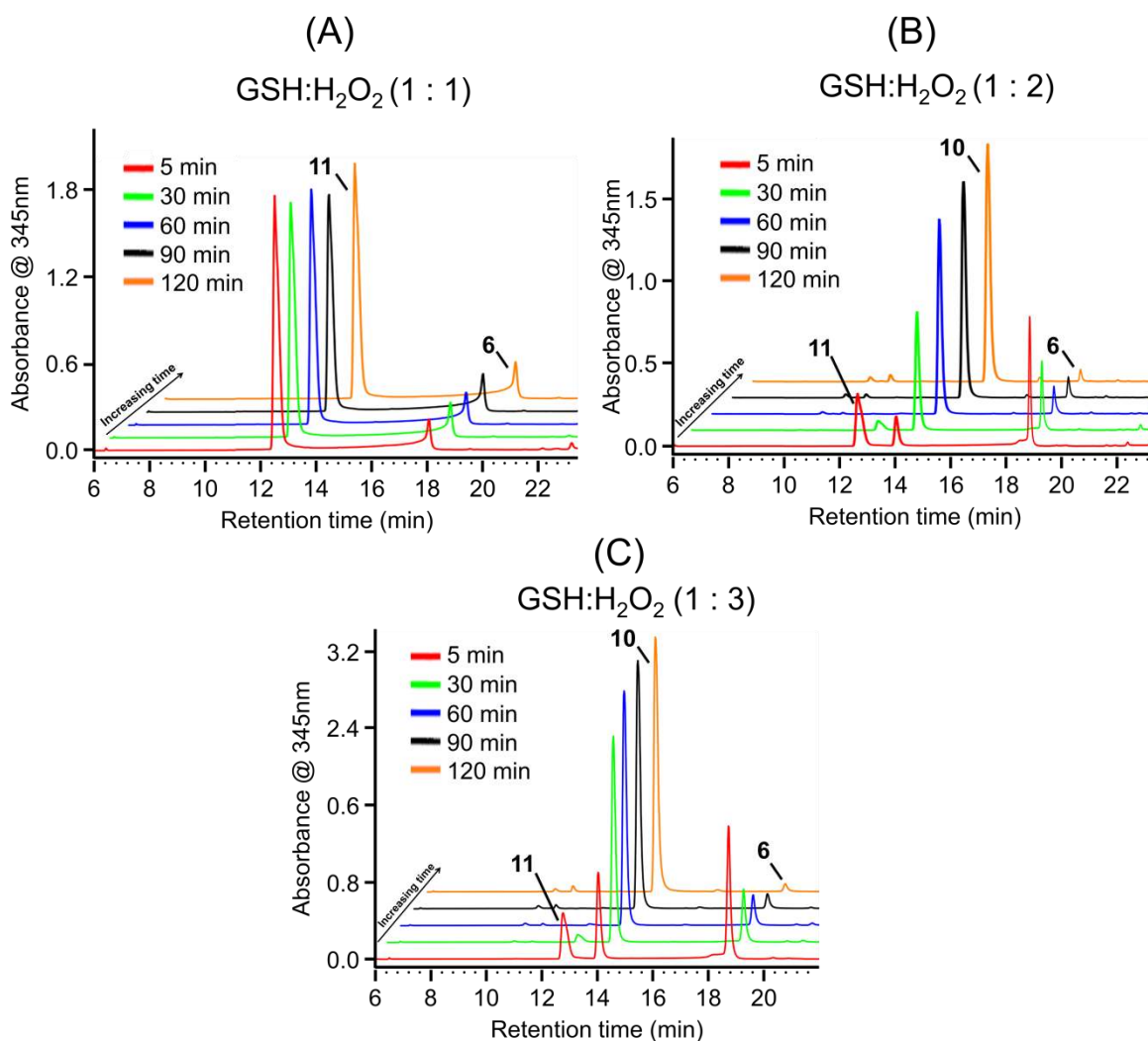


Figure S21: (A) HPLC chromatograms obtained after treatment of compd. **6** with GSH and H₂O₂ (1:1) at different time points. (B) HPLC chromatograms obtained after treatment of compd. **6** with GSH and H₂O₂ in 1:2 molar ratio at different time point. (C) HPLC chromatograms obtained after treatment of compd. **6** with GSH and H₂O₂ in 1:3 molar ratio at different time point.

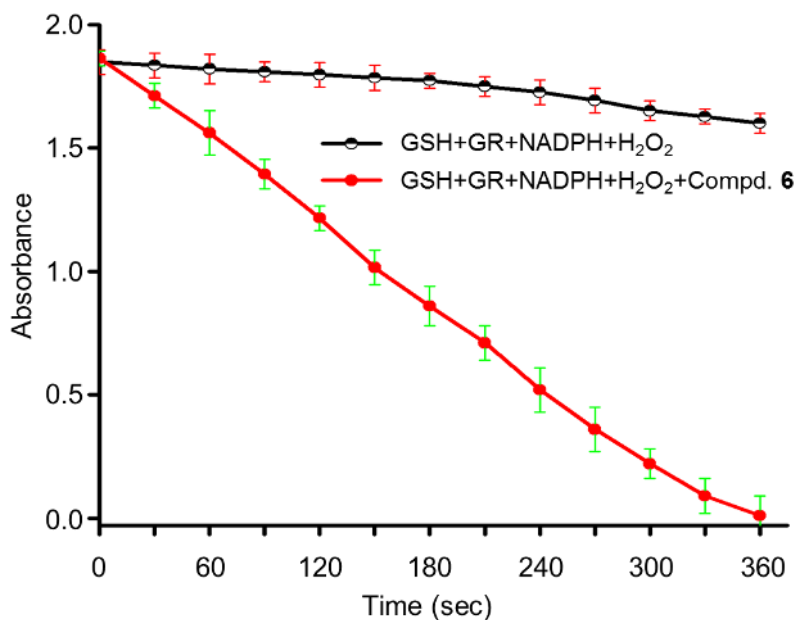


Figure S22: Absorbance vs time (sec) plot for the glutathione peroxidase (GPx)-like activity of compd.6. Assay conditions: Compd. 6: 10 μ M, GSH: 2 mM, NADPH: 0.2 mM, glutathione reductase (GR): 1.7 units, H₂O₂: 1.6 mM, phosphate buffer, pH 7.4, 25 °C. The decrease in the absorbance due to a decrease in the concentration of NADPH was monitored spectrophotometrically at 340 nm. The activity in the presence of **6** was found to be higher than that of the control rate, indicating that compd. **6** undergoes a redox reaction in the presence of GSH and H₂O₂.

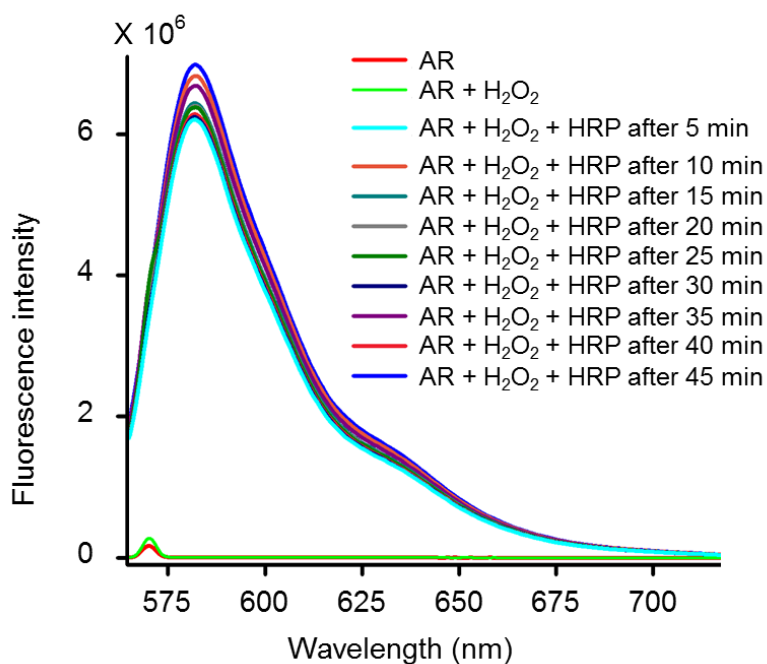


Figure S23: The fluorescence spectra of Amplex Red (AR) ($2 \mu\text{M}$, $\text{pH} = 7.4$, $\lambda_{\text{ex}} = 570 \text{ nm}$, $\lambda_{\text{em}} = 585 \text{ nm}$) obtained after the addition of $2 \mu\text{M}$ H_2O_2 and 0.15 U/mL HRP. The spectra were recorded after different incubation time as indicated in the Figure.

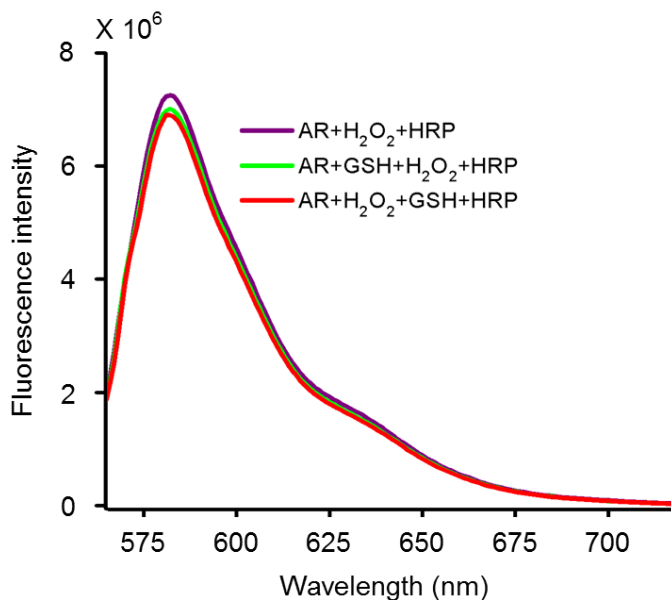


Figure S24: The fluorescence spectra of Amplex Red (AR) ($2 \mu\text{M}$, $\text{pH} = 7.4$, $\lambda_{\text{ex}} = 570 \text{ nm}$, $\lambda_{\text{em}} = 585 \text{ nm}$) obtained after simultaneous treatment of AR with $2 \mu\text{M}$ H_2O_2 , $2 \mu\text{M}$ GSH and 0.15 U/mL HRP and 10 min incubation time.

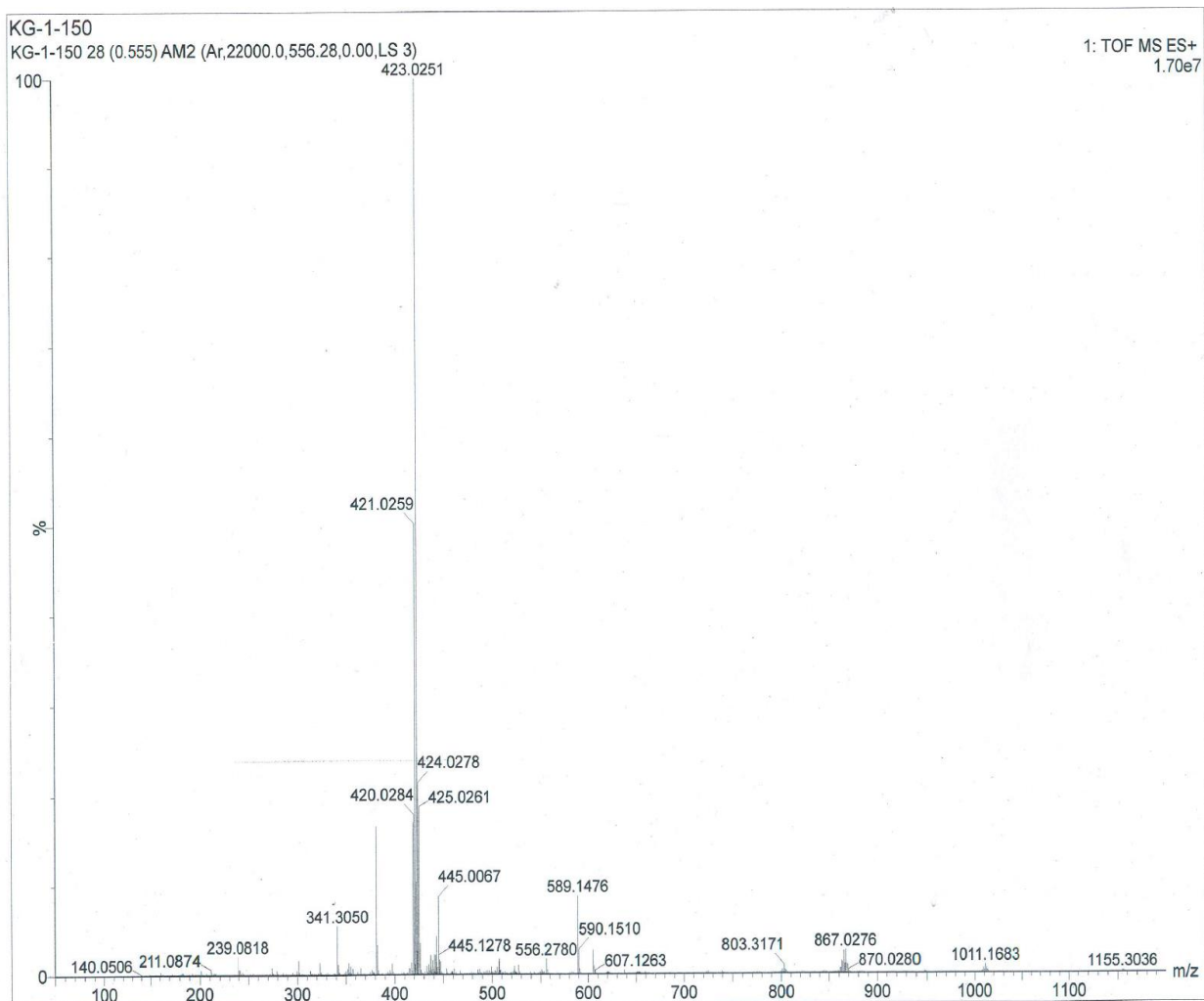


Figure S25: ESI-MS spectrum of compd. **6**. The m/z calcd for $C_{21}H_{14}N_2O_3Se_1$: 423.0248 $[M+H]^+$; found: 423.0251.

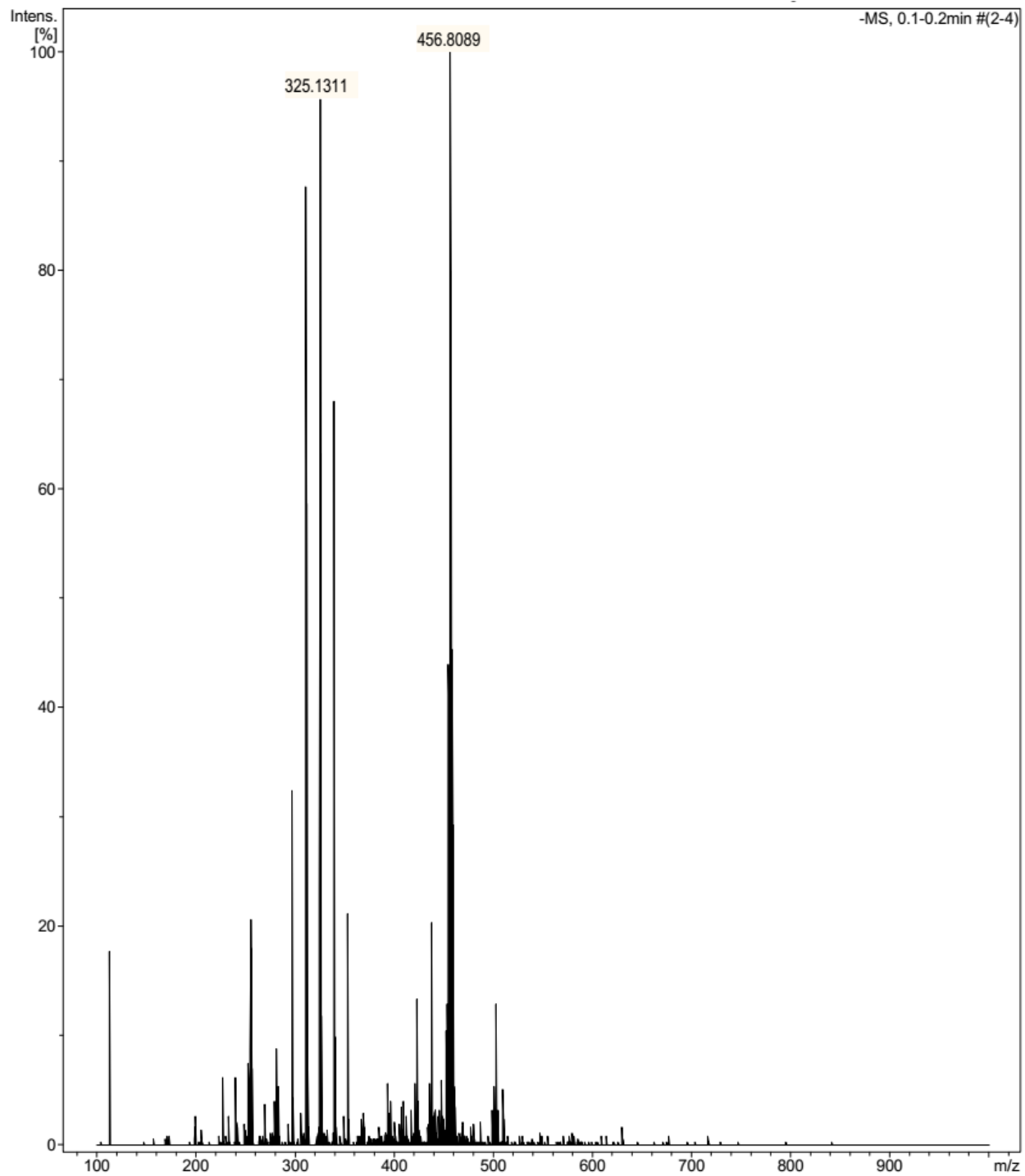


Figure S26: ESI-MS spectrum of compd. **10**. The m/z calcd for $C_{21}H_{16}N_2O_5Se_1$: 457.0302 $[M+H]^+$; found: 456.8089.

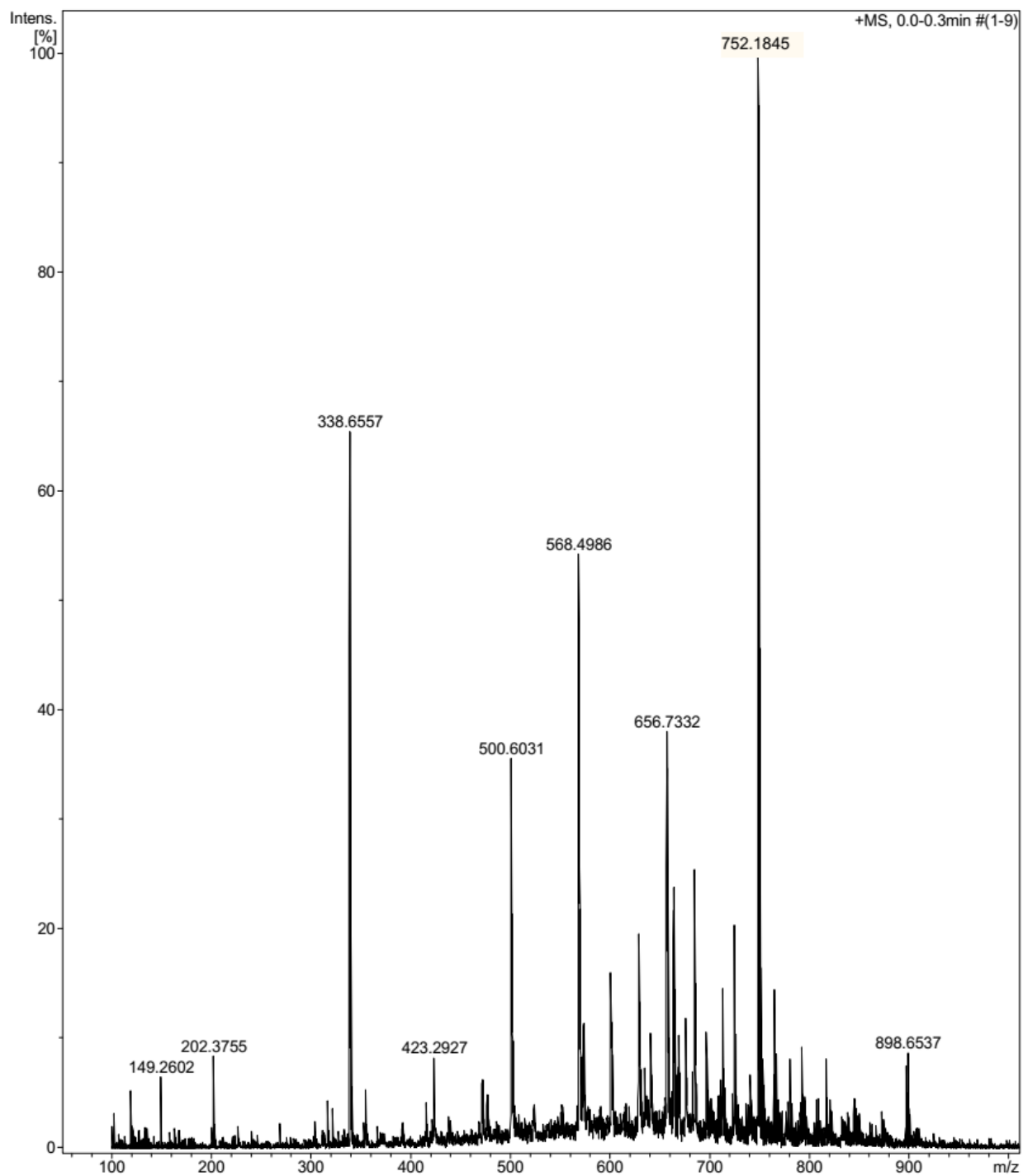


Figure S27: ESI-MS spectrum of compd. **11**. The m/z calcd for $C_{31}H_{31}N_5O_9S_1Se_1$: 752.0905 $[M+Na]^+$; found: 752.1845.

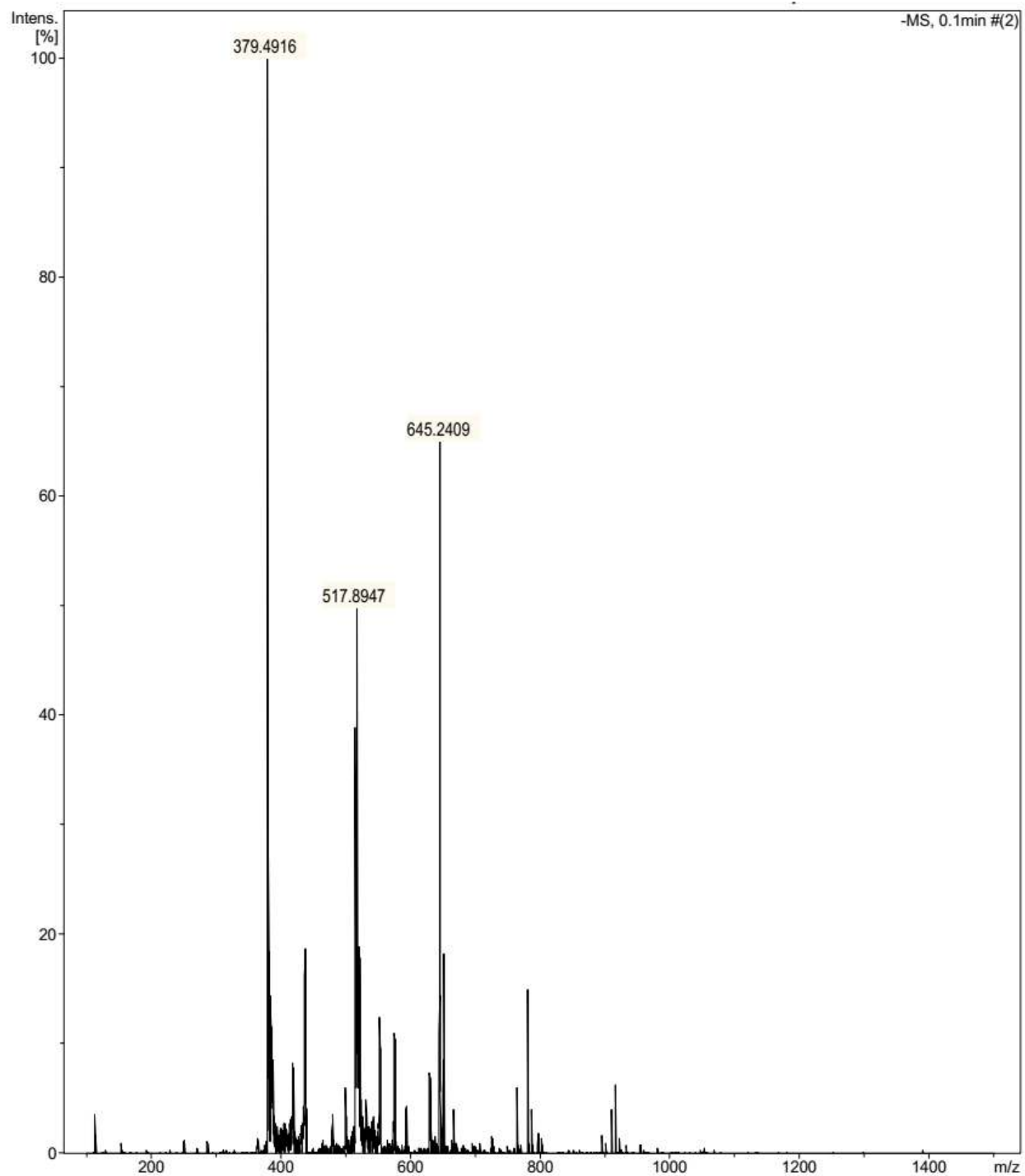


Figure S28: ESI-MS spectrum of compd. **14**. The m/z calcd for $C_{21}H_{16}N_2O_6Se_1$: 517.9969 $[M+2Na]^+$; found: 517.8947.

3.12. Computational Methods

All computational calculations were performed using Gaussian09 suite of quantum chemical programs. The hybrid Beck 3-Lee-Yang-Parr (B3LYP) exchange correlations functional were applied for DFT calculations to predict the molecular geometries having minimum energy. Geometries were fully optimized at B3LYP level using 6-311G** basis set was used. Natural Bond Orbital (NBO) calculations were performed using 6-311G** basis set.^[14-16]

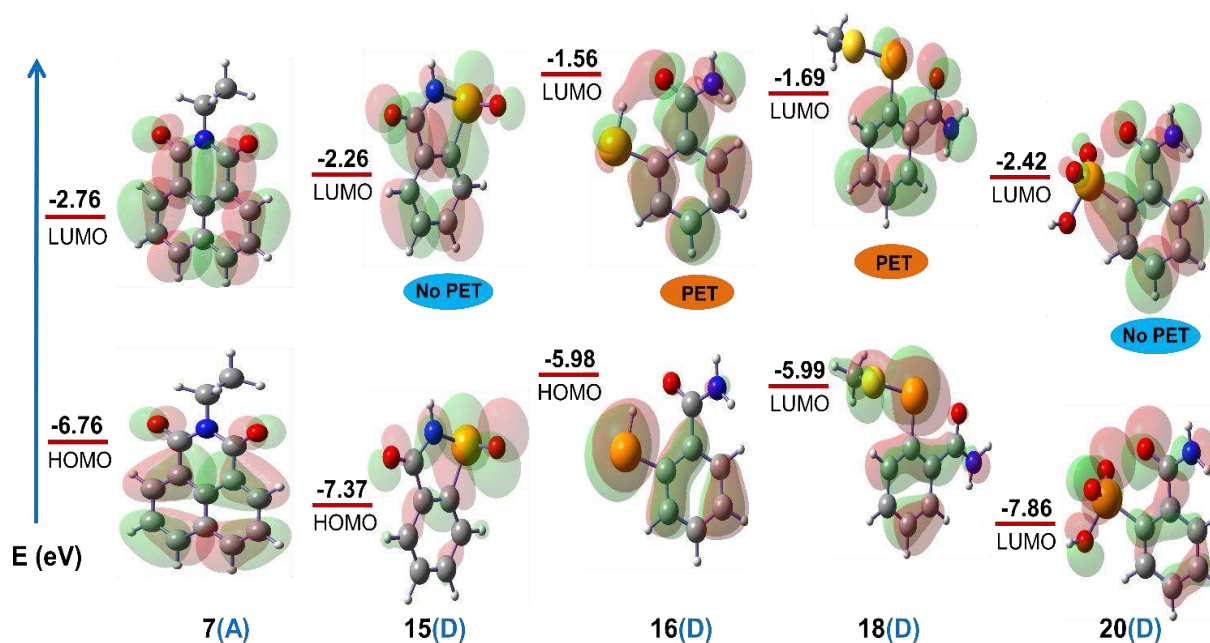


Figure S29: The HOMO-LUMO energy levels of compound 7(A), 15(D), 16(D), 18(D) and 20(D) obtained from DFT calculations.



Compound 7

8	1.805420000	-2.284186000	-0.228581000
8	1.805439000	2.284103000	-0.228671000
7	1.804717000	-0.000083000	-0.265837000
6	-3.125334000	1.248069000	0.140911000
6	-2.422598000	2.437065000	0.087759000
1	-2.952258000	3.385270000	0.129137000
6	-1.014300000	2.431073000	-0.020509000
1	-0.452966000	3.360152000	-0.062483000
6	-0.321209000	1.232330000	-0.076243000
6	-1.022543000	0.000003000	-0.022784000
6	-2.447688000	0.000044000	0.088464000
6	-3.125403000	-1.247943000	0.140908000
1	-4.210447000	-1.254101000	0.223945000
6	-2.422731000	-2.436976000	0.087765000
1	-2.952442000	-3.385153000	0.129130000
6	-1.014427000	-2.431062000	-0.020469000
1	-0.453142000	-3.360171000	-0.062408000
6	-0.321268000	-1.232360000	-0.076211000
6	1.159894000	-1.246135000	-0.193073000
6	1.159938000	1.246033000	-0.193141000
6	3.277766000	-0.000182000	-0.398179000

1	3.540856000	0.891184000	-0.971214000
1	3.540735000	-0.891976000	-0.970615000
6	3.989314000	0.000191000	0.956076000
1	3.729377000	0.892960000	1.535196000
1	5.075029000	0.000084000	0.800806000
1	3.729274000	-0.892198000	1.535739000
1	-4.210376000	1.254289000	0.223994000



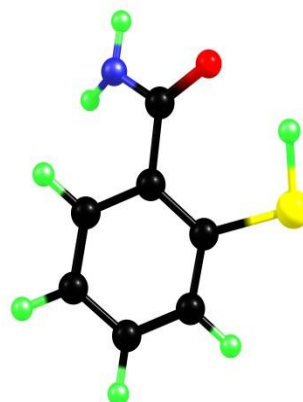
Compound 8

8	-0.396392000	2.902136000	0.000796000
7	-1.633111000	0.968159000	-0.001125000
6	-0.450140000	1.679312000	0.000327000
6	0.687748000	0.727945000	-0.000207000
6	0.353507000	-0.632999000	0.000394000
6	1.340198000	-1.622516000	0.000497000
1	1.082265000	-2.678591000	0.000575000
6	2.676981000	-1.222802000	0.000397000
1	3.458338000	-1.979068000	0.000520000
6	3.025025000	0.139074000	0.000175000
1	4.072684000	0.428060000	0.000184000
6	2.032149000	1.116056000	-0.000020000
1	2.270042000	2.176841000	-0.000107000
34	-1.522096000	-0.896995000	-0.000332000
1	-2.521960000	1.451967000	0.002231000



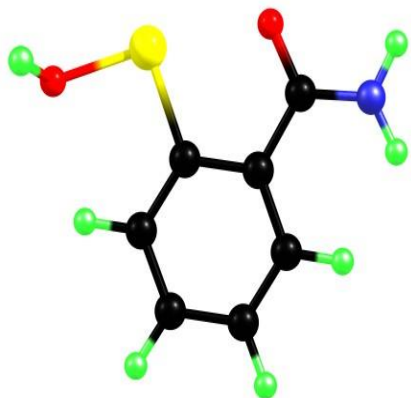
Compound15

8	-0.389179000	3.004935000	0.121816000
7	1.221173000	1.376012000	-0.099856000
6	-0.077575000	1.832410000	0.022852000
6	-1.016986000	0.669356000	0.001229000
6	-0.426008000	-0.586456000	-0.124096000
6	-1.171885000	-1.758381000	-0.136722000
1	-0.698055000	-2.732962000	-0.224124000
6	-2.562986000	-1.648687000	-0.013691000
1	-3.174246000	-2.547656000	-0.013285000
6	-3.171379000	-0.392188000	0.110247000
1	-4.252496000	-0.326278000	0.201609000
6	-2.402414000	0.775618000	0.122060000
1	-2.855109000	1.758476000	0.223827000
34	1.518279000	-0.489532000	-0.279064000
8	2.113917000	-1.071020000	1.157054000
1	1.987705000	2.039073000	-0.123109000



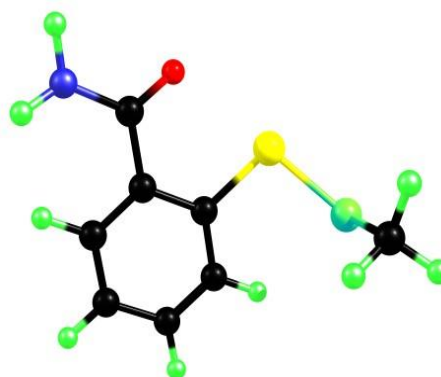
Compound 16

34	-1.981991000	-0.262313000	0.082201000
8	-0.296320000	2.329280000	-0.637621000
6	0.675663000	1.805412000	-0.097505000
6	0.926443000	0.323282000	-0.078413000
6	-0.113889000	-0.631564000	-0.000387000
6	0.223549000	-1.995681000	0.043970000
1	-0.573006000	-2.733928000	0.104821000
6	1.547362000	-2.422741000	-0.005094000
1	1.770444000	-3.486654000	0.022705000
6	2.578719000	-1.484456000	-0.111716000
1	3.615449000	-1.803337000	-0.177938000
6	2.259367000	-0.129813000	-0.142340000
1	3.057630000	0.599849000	-0.252819000
7	1.634289000	2.576740000	0.508289000
1	1.441233000	3.566893000	0.566096000
1	2.279943000	2.182599000	1.174713000
1	-1.856758000	1.135154000	-0.340574000



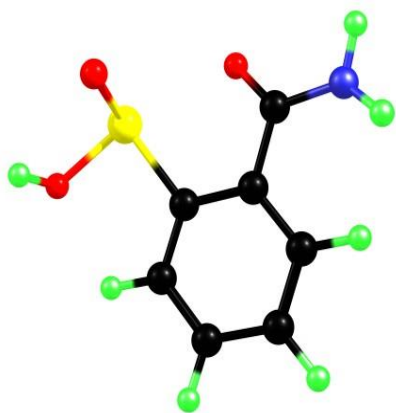
Compound 17

8	-0.029272000	-2.155797000	-0.001927000
7	-2.274822000	-2.384369000	0.023062000
6	-1.151706000	-1.619897000	0.006847000
6	-1.236951000	-0.148346000	0.007965000
6	-0.010998000	0.555163000	-0.002601000
6	-0.024331000	1.959470000	-0.019824000
1	0.921258000	2.490960000	-0.048679000
6	-1.234656000	2.646650000	-0.012805000
1	-1.228953000	3.734341000	-0.026611000
6	-2.456326000	1.955853000	0.006775000
1	-3.397571000	2.498385000	0.012780000
6	-2.447780000	0.566456000	0.014501000
1	-3.399554000	0.039691000	0.028635000
1	-3.202725000	-1.999612000	-0.031936000
8	2.743645000	1.079744000	-0.049505000
1	3.089950000	1.182517000	0.846724000
34	1.616573000	-0.433922000	-0.015178000
1	-2.160627000	-3.386004000	-0.020007000



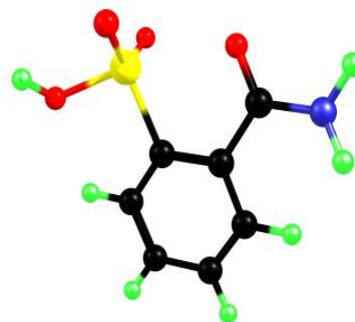
Compound 18

34	0.978165000	-0.931589000	0.323048000
8	-1.265006000	-2.192953000	0.130627000
6	-2.081556000	-1.309764000	-0.142979000
6	-1.710887000	0.126197000	-0.026799000
6	-0.351629000	0.468167000	0.173109000
6	-0.004175000	1.821450000	0.284843000
1	1.036482000	2.087954000	0.447410000
6	-0.971663000	2.819967000	0.200989000
1	-0.673494000	3.861622000	0.297029000
6	-2.318586000	2.489097000	0.013426000
1	-3.079039000	3.263772000	-0.034629000
6	-2.674284000	1.149333000	-0.098844000
1	-3.726122000	0.897626000	-0.210080000
7	-3.349776000	-1.635697000	-0.539870000
1	-3.526037000	-2.617947000	-0.695460000
1	-3.944442000	-0.975664000	-1.014257000
16	2.881394000	0.261411000	0.311789000
6	3.173820000	0.490947000	-1.488584000
1	2.406695000	1.131844000	-1.933020000
1	4.150724000	0.977764000	-1.585246000
1	3.197551000	-0.474383000	-2.000898000



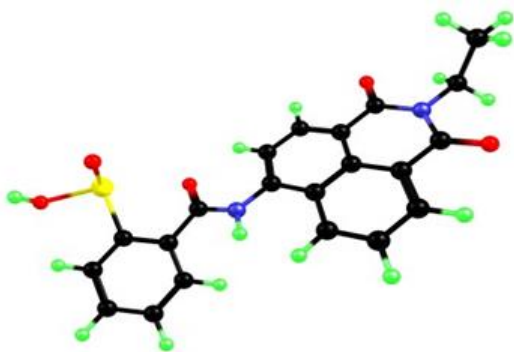
Compound 19

8	-0.049881000	-2.149475000	-0.497875000
7	-2.179440000	-2.537263000	0.151982000
6	-1.143478000	-1.705163000	-0.141950000
6	-1.362217000	-0.227406000	-0.056729000
6	-0.237693000	0.616000000	-0.072458000
6	-0.391500000	1.999816000	-0.023966000
1	0.490765000	2.631342000	-0.064222000
6	-1.672574000	2.555313000	0.071526000
1	-1.788523000	3.635696000	0.113397000
6	-2.797462000	1.728225000	0.096157000
1	-3.794067000	2.157926000	0.154538000
6	-2.640219000	0.342675000	0.031823000
1	-3.527150000	-0.286041000	0.014795000
1	-2.982631000	-2.218293000	0.670188000
8	1.880660000	-0.700646000	1.339221000
8	2.436066000	1.456313000	-0.207371000
1	2.848833000	1.483508000	0.672051000
34	1.568044000	-0.200713000	-0.214675000
1	-1.968534000	-3.525343000	0.176104000



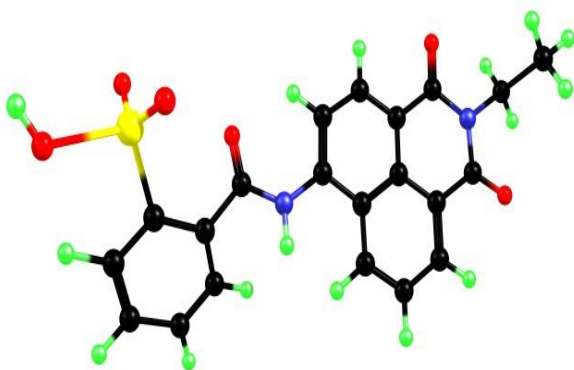
Compound 20

8	-0.027803000	2.128221000	0.248740000
7	-2.191936000	2.642536000	-0.168748000
6	-1.175999000	1.757454000	0.029970000
6	-1.488385000	0.290883000	0.007461000
6	-0.422325000	-0.621116000	-0.012091000
6	-0.641425000	-1.997107000	-0.004712000
1	0.201985000	-2.677047000	-0.020815000
6	-1.954057000	-2.480361000	0.008156000
1	-2.127874000	-3.553227000	0.012184000
6	-3.029638000	-1.592097000	0.028498000
1	-4.049718000	-1.966035000	0.051957000
6	-2.795015000	-0.216042000	0.026890000
1	-3.643783000	0.460736000	0.075057000
1	-3.070788000	2.367107000	-0.576545000
8	1.891276000	0.788034000	-1.346536000
8	2.169661000	-1.639486000	-0.256453000
1	2.705592000	-1.520354000	-1.057228000
34	1.436729000	0.020885000	0.026542000
1	-1.932084000	3.617307000	-0.229029000
8	1.898428000	0.374980000	1.544025000



Compound 10

6	-3.849338000	2.644865000	0.570612000	1	-6.897990000	-1.886971000	-1.051414000
6	-4.025906000	1.320318000	0.227659000	1	-6.734665000	-2.516511000	1.367832000
6	-2.912344000	0.495542000	-0.065016000	1	-7.399642000	-0.930448000	1.825844000
6	-1.591466000	1.035964000	0.000066000	1	-8.398620000	-2.093038000	0.943407000
6	-1.456775000	2.404371000	0.345548000	7	-5.546569000	-0.564675000	-0.207720000
6	-2.555501000	3.187421000	0.625797000	7	0.808072000	0.703927000	-0.220580000
1	-4.722939000	3.245820000	0.789186000	1	0.876221000	1.622294000	0.185234000
6	-3.115368000	-0.859322000	-0.418522000	6	2.013387000	0.112762000	-0.508494000
6	-0.486520000	0.162977000	-0.294920000	6	3.218513000	0.993809000	-0.375176000
1	-0.481962000	2.876428000	0.393273000	6	4.460159000	0.386001000	-0.136509000
1	-2.420522000	4.229884000	0.888110000	6	3.168887000	2.379679000	-0.562295000
6	-0.718689000	-1.159191000	-0.641135000	6	4.329720000	3.145962000	-0.494756000
6	-2.027333000	-1.658312000	-0.700886000	6	5.618064000	1.148212000	-0.068479000
1	0.118083000	-1.803947000	-0.854684000	6	5.552130000	2.531733000	-0.243783000
1	-2.200517000	-2.693390000	-0.967599000	34	4.550602000	-1.599566000	0.160299000
6	-5.409471000	0.774921000	0.168093000	8	2.142199000	-1.054027000	-0.854893000
6	-4.478907000	-1.432705000	-0.491675000	1	2.231845000	2.866770000	-0.809924000
6	-6.914958000	-1.117220000	-0.282476000	1	4.277931000	4.216689000	-0.652857000
6	-7.384509000	-1.698626000	1.050137000	1	6.458892000	3.123636000	-0.193164000
8	-6.384085000	1.456661000	0.429467000	1	6.563455000	0.655410000	0.120604000
				8	6.347488000	-1.581643000	0.637040000
				1	6.326412000	-1.522284000	1.605204000
				8	3.807325000	-1.784134000	1.613660000
				8	-4.686063000	-2.595983000	-0.783866000
				1	-7.564544000	-0.301815000	-0.595114000



Compound 14

6	-4.017174000	2.631307000	0.626660000	8	-4.811435000	-2.589420000	-0.828046000
6	-4.183215000	1.311585000	0.260310000	1	-7.715508000	-0.340780000	-0.532823000
6	-3.064829000	0.507405000	-0.068548000	1	-7.036188000	-1.906913000	-1.033821000
6	-1.750168000	1.063250000	-0.013635000	1	-6.817959000	-2.582252000	1.368997000
6	-1.626194000	2.426954000	0.353455000	1	-7.496699000	-1.015516000	1.871129000
6	-2.729677000	3.190222000	0.667743000	1	-8.495471000	-2.174855000	0.984096000
1	-4.894322000	3.216281000	0.872704000	7	-5.687008000	-0.583574000	-0.188504000
6	-3.256175000	-0.841529000	-0.450223000	7	0.650842000	0.762323000	-0.262191000
6	-0.639953000	0.210098000	-0.341231000	1	0.723295000	1.643780000	0.218947000
1	-0.656338000	2.910496000	0.384722000	6	1.851898000	0.195301000	-0.619482000
1	-2.603818000	4.229999000	0.944946000	6	3.046930000	1.092951000	-0.442118000
6	-0.859505000	-1.105489000	-0.717841000	6	4.286707000	0.549036000	-0.082804000
6	-2.163160000	-1.619382000	-0.770260000	6	2.990685000	2.457933000	-0.739111000
1	-0.018064000	-1.734510000	-0.959997000	6	4.132203000	3.253301000	-0.667730000
1	-2.327429000	-2.650000000	-1.058869000	6	5.429484000	1.332555000	0.004716000
6	-5.560510000	0.749170000	0.214029000	6	5.347724000	2.693278000	-0.293211000
6	-4.613625000	-1.430797000	-0.512503000	34	4.428868000	-1.356865000	0.389733000
6	-7.049111000	-1.153192000	-0.249096000	8	1.993299000	-0.928346000	-1.062261000
6	-7.485272000	-1.767812000	1.079943000	1	2.056713000	2.894822000	-1.073952000
8	-6.538796000	1.412098000	0.508218000	1	4.069650000	4.306307000	-0.915198000
				1	6.238347000	3.307048000	-0.231178000
				1	6.362971000	0.889239000	0.319928000
				8	6.007970000	-1.198346000	1.279193000
				1	6.545707000	-1.927898000	0.935258000
				8	4.813470000	-2.321886000	-0.852975000
				8	3.312289000	-1.706597000	1.502708000

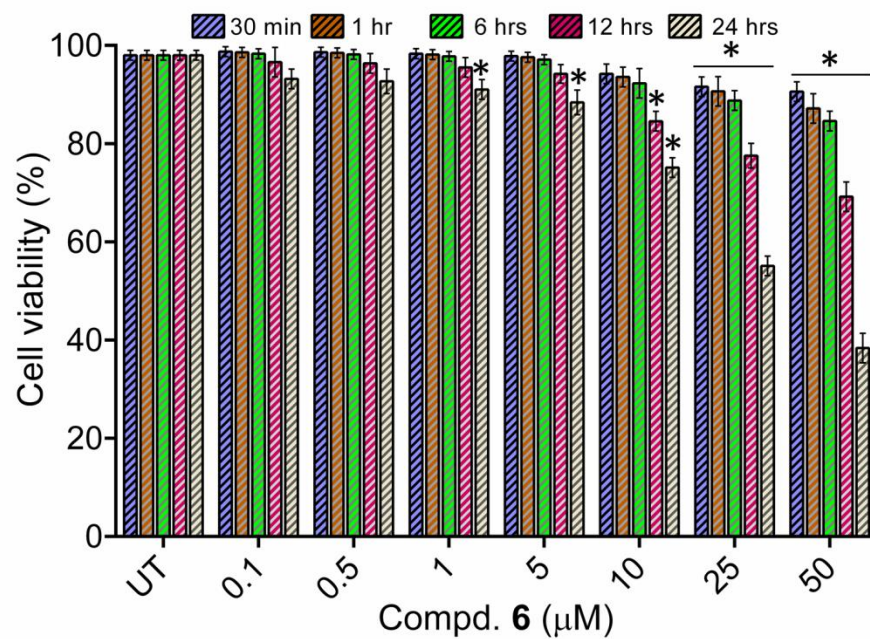


Figure S30: Cell viability of HepG2 cells evaluated by MTT assay after incubation of various concentrations of compd. **6** and analyzed at different time points. Data are expressed as mean \pm SD, * indicates $p < 0.05$ compared to untreated controls.

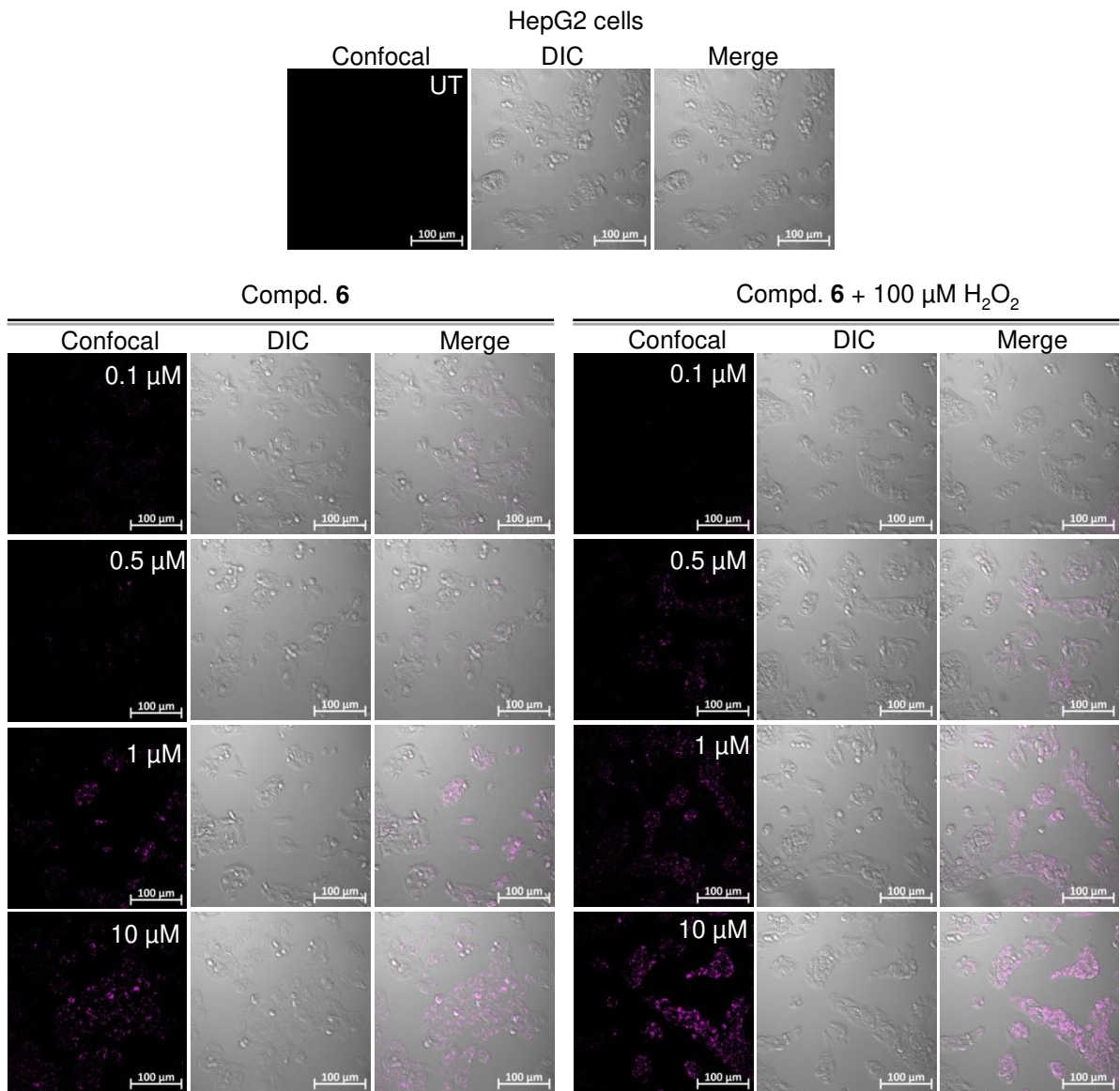


Figure S31: Confocal microscopy images of HepG2 treated with various concentrations of compd. 6 in the absence and presence of 100 μM of H₂O₂ for 30 minutes.

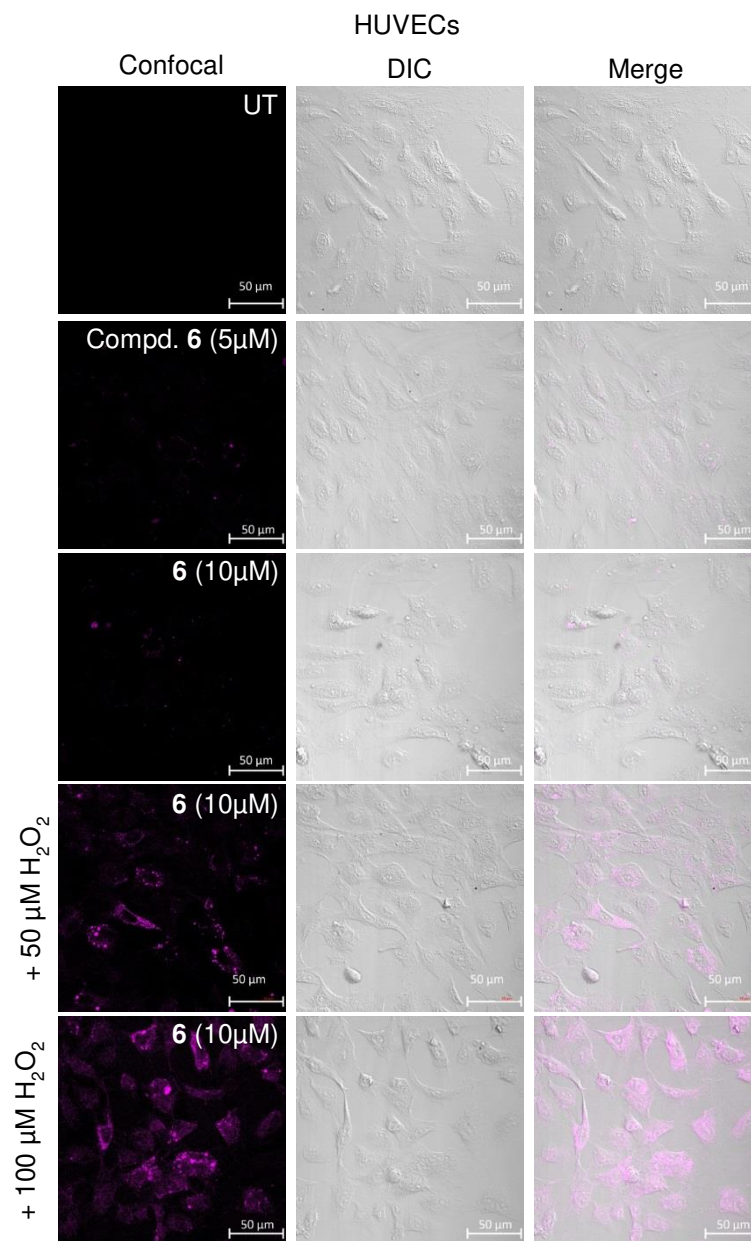


Figure S32: Confocal microscopy images of HUVEC treated with different concentrations of compd. **6** in the absence and presence of H_2O_2 for 30 minutes.

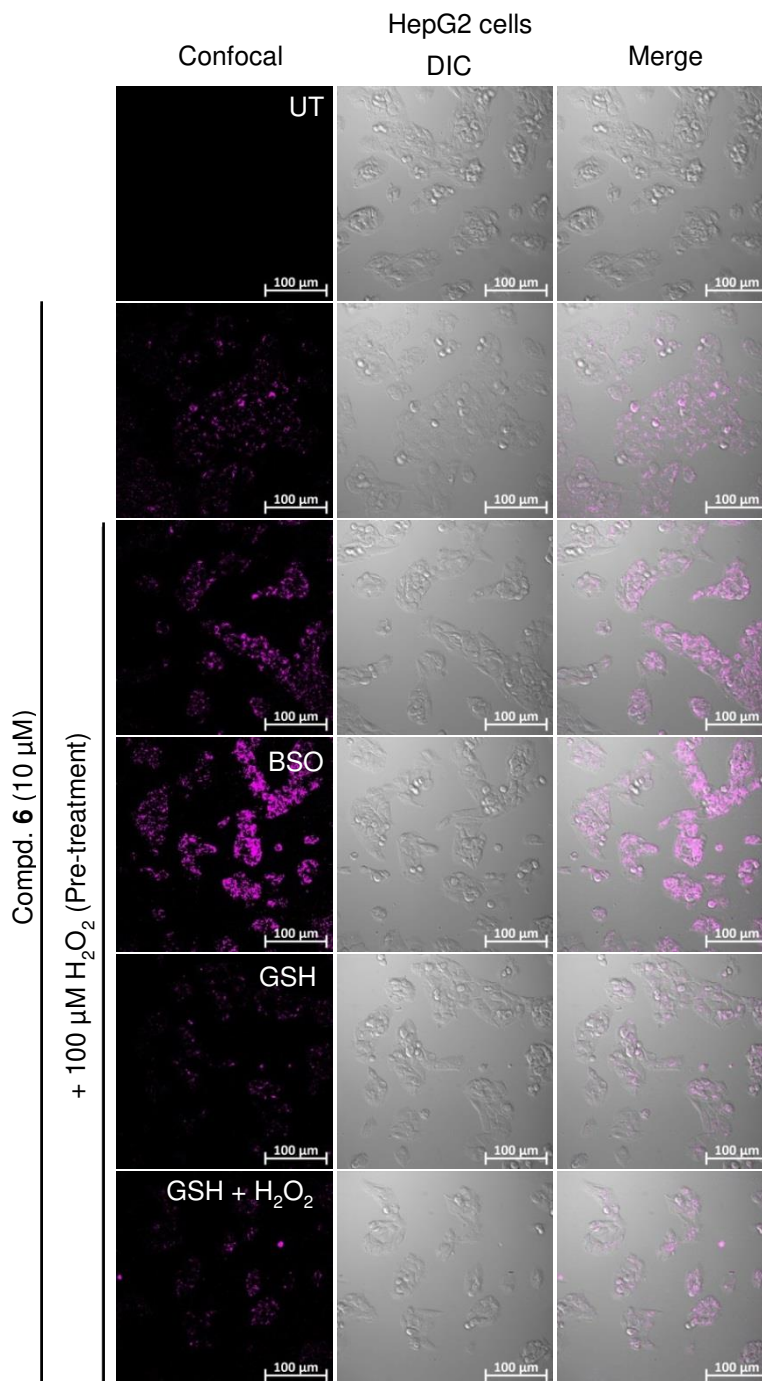


Figure S33: Fluorescence response of compd. **6** in HepG2 cells with GSH or GSH synthesis inhibitor (BSO). HepG2 cells were first pre-treated with 100 μ M H₂O₂ for 15 minutes. For depletion of GSH, 50 μ M BSO (GSH inhibitor) was incubated with the cells pre-treated with H₂O₂. To replenish GSH levels, the cells were treated with 200 μ M GSH for 5 minutes. To measure H₂O₂ scavenging ability of compd. **6**, 10 μ M compd. **6** was used to treat the cells for 5 minutes. The fluorescent intensity was measured immediately after the treatment using a fluorescence plate reader.

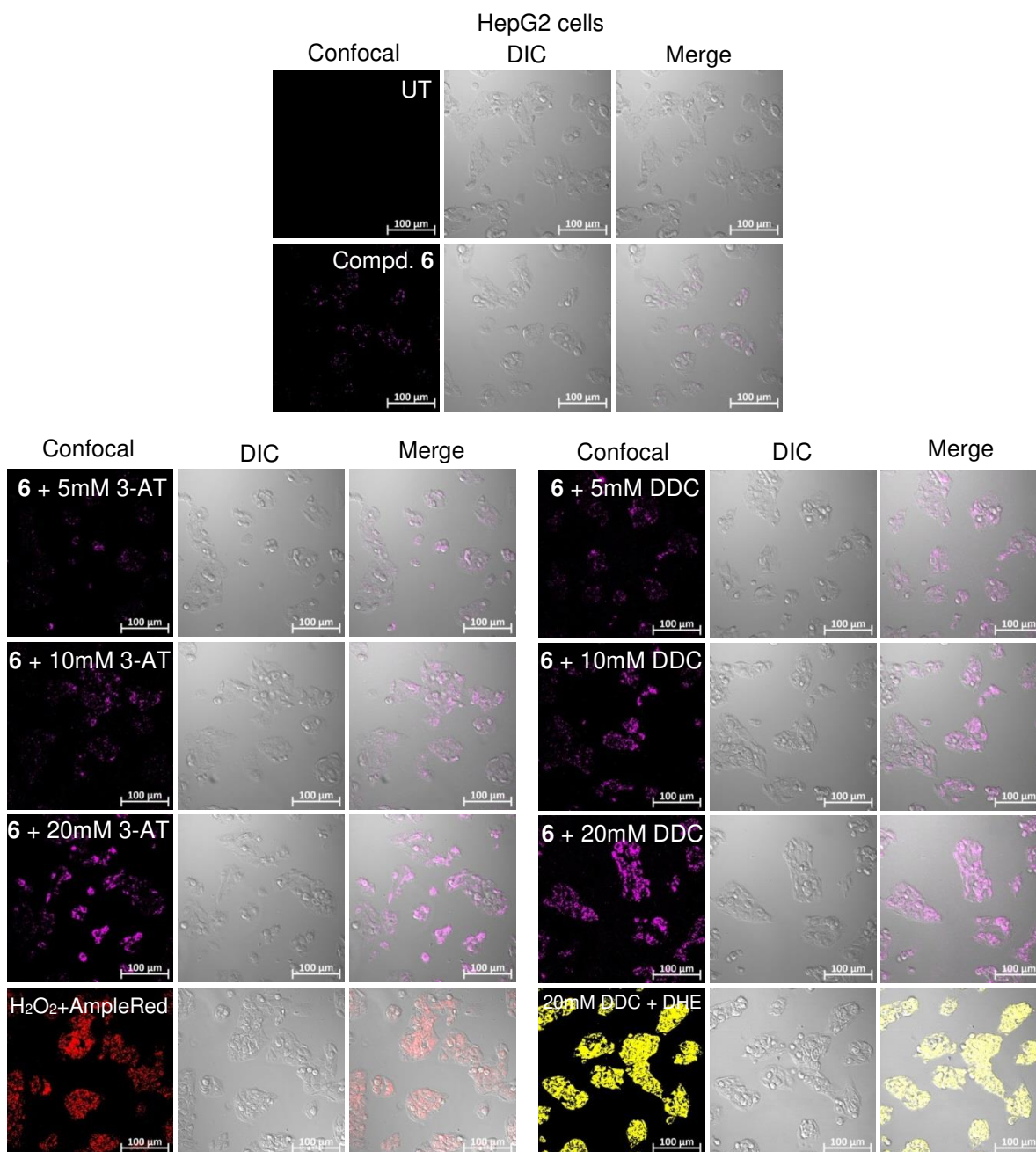


Figure S34: Fluorescence response of compd. 6 in HepG2 cells with inhibitors of catalase (or) SOD. HepG2 cells were pre-treated either with different concentrations of 3-AT or DDC for 15 minutes, followed by treatment with 10 μ M concentration of compd. 6 for 30 minutes. For AmplexRed-based H₂O₂ detection, the cells were first treated with 20 mM 3-AT for 15 minutes, followed by treatment with 50 μ M Amplex® Red reagent containing 0.2 U/ml of HRP along with 100 μ M H₂O₂ for 30 minutes. For endogenous superoxide detection by DHE, the cells were pre-treated with 20 mM DDC for 15 minutes, followed by treatment with 10 μ M of DHE for 30 minutes.

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