

Electronic Supplementary Information (ESI)
for
Photocontrolled Endogenous Reactive Oxygen Species (ROS)
Generation

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1. General methods:

All the chemicals and solvents were purchased from commercial sources and used as received unless stated otherwise. Column chromatography was performed using Silica gel Spectrochem (100-200 mesh) as stationary phase. Preparative high performance liquid chromatography (HPLC) was done using Combiflash EZ prep UV using a Kromasil®C-18 preparative column (250 mm × 21.2 mm, 5 μm). ¹H and ¹³C spectra were recorded on a JEOL 400 MHz (or 100 MHz for ¹³C) or a Bruker 400 MHz (or 100 MHz for ¹³C) spectrometer unless otherwise specified using as an internal tetramethylsilane ($\delta_{\text{H}} = 0.00$, $\delta_{\text{C}} = 0.0$). Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The following abbreviations are used: br (broad signal), m (multiplet), s (singlet), d (doublet), t (triplet) and dd (doublet of doublets). High-resolution mass spectra were obtained from HRMS-ESI-Q-Time of Flight LC/MS. FT-IR spectra were recorded using BRUKER-ALPHA FT-IR spectrometer. Analytical HPLC was performed on an Agilent1260-infinity with Phenomenex®C-18 reverse phase column (250 mm × 4.6 mm, 5 μm). Irradiation was done using 365 nm UV-LED flashlight-3W and intensity was calibrated using GENTEC-EO-UNO laser power meter. Photometric and fluorometric measurements were performed using a Thermo Scientific Varioskan microtiter plate reader.

2. Synthesis and characterization:

Compounds **1**¹, **2**¹, **8**², **5c**³, **12**⁴ and **13**⁵ were synthesized using previously reported procedures and analytical data were consistent with reported values. ¹H NMR spectra of **5c** and **12** are attached

Synthesis of 2-(4-formyl-2-methoxy-5-nitrophenoxy)ethyl nitrate (9): To a solution of 4-(2-hydroxyethoxy)-3-methoxybenzaldehyde (3.4 g, 17.33 mmol) in glacial acetic acid (10 mL) was stirred at ice cold condition and a mixture of conc. HNO₃ (10 mL) and conc. H₂SO₄ was added dropwise. The reaction mixture was stirred additionally for 1 h at r.t. After completion of reaction as monitored by TLC analysis, reaction was quenched with ice water and extracted with dichloromethane. This dichloromethane fractions were combined, dried over anhydrous sodium sulphate and concentrated under reduced pressure to get crude which was further purified by silica gel column chromatography to afford compound **9** as a yellowish solid (1.5 g, 30 %); FT-IR (ν_{max} , cm⁻¹): 2921, 1691, 1521, 1338; ¹H NMR (400 MHz, CDCl₃): δ 10.46 (s, 1H), 7.63 (s, 1H), 7.43 (s, 1H), 4.94 – 4.90 (m, 2H), 4.47 – 4.42 (m, 2H), 4.02 (s, 3H); HRMS (ESI-TOF) for [C₁₀H₁₀N₂O₈ + H]⁺ calcd: 287.0515; found: 287.0507.

Synthesis of 4-(2-azidoethoxy)-5-methoxy-2-nitrobenzaldehyde (10): To a solution of **9** (2.5 g, 8.74 mmol) in anhydrous *N,N*-Dimethylformamide (20 mL) with continuous stirring, sodium azide (1.00 g, 15.38 mmol) was added. The reaction mixture was stirred at 80 °C for 16 h in inert atmosphere. After completion of reaction as monitored by TLC analysis, DMF was evaporated under reduced pressure to obtained crude, which was purified by silica gel column chromatography to afford compound **10** as a yellowish solid (1.2 g, 52 %); FT-IR (ν_{max} , cm⁻¹): 2109, 1691, 1520, 1337; ¹H NMR (400 MHz, CDCl₃): δ 10.45 (s, 1H), 7.63 (s, 1H), 7.43 (s, 1H), 4.32 (t, *J* = 5.0 Hz, 2 H), 4.02 (s, 3H), 3.74 (t, *J* = 5.0 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 187.7, 153.7, 151.1, 143.4, 126.3, 110.2, 108.5, 68.6, 56.7, 49.8; HRMS (ESI-TOF) for [C₁₀H₁₀N₄O₅ + H]⁺ calcd: 267.0729; found: 267.0724.

Synthesis of 4-(2-azidoethoxy)-5-methoxy-2-nitrophenyl)methanol (11): To a solution of **10** (936 mg, 3.52 mmol) in anhydrous methanol (20 mL) with continuous stirring, NaBH₄ (410 mg, 10.84 mmol) was added portion wise. The reaction mixture was stirred at r.t. for 30 min in inert atmosphere. After completion of reaction as monitored by TLC analysis, reaction was quenched with saturated NH₄Cl solution and extracted with ethyl acetate. These ethyl acetate fractions were combined, dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtained crude, which was purified by silica gel column chromatography to afford compound **11** as a yellowish solid (750 mg, 80 %); FT-IR (ν_{max} , cm⁻¹): 3264, 2921, 2108, 1516, 1329; ¹H NMR (400 MHz, CDCl₃): δ 7.73 (s, 1H), 7.21 (s, 1H), 4.98 (s, 2H), 4.25 (t, *J* = 5.0 Hz, 2H), 4.00 (s, 3H), 3.70 (t, *J* = 5.0 Hz, 2H), 2.20 – 2.20 (br, 1H); ¹³C NMR (100

MHz, CDCl₃): δ 154.6, 146.7, 139.5, 133.2, 111.4, 110.2, 68.4, 62.8, 56.5, 50.0; HRMS (ESI-TOF) for [C₁₀H₁₂N₄O₅ + Na]⁺ calcd: 291.0705 ; found: 291.0702.

Synthesis of 1-(2-azidoethoxy)-4-(bromomethyl)-2-methoxy-5-nitrobenzene (5d): To a solution of **11** (1.00 g, 3.73 mmol) in anhydrous dichloromethane (40 mL) kept at ice bath and PBr₃ (1.06 mL, 11.16 mmol) was added. The reaction mixture was stirred at ice cold condition for 2 h in inert atmosphere. After completion of reaction as monitored by TLC analysis, reaction was quenched with saturated NaHCO₃ solution and extracted with dichloromethane. These organic fractions were combined, dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtained crude, which was purified by silica gel column chromatography to afford compound **5d** as a yellowish solid (450 mg, 36 %); FT-IR (ν_{max} , cm⁻¹): 2920, 2103, 1517, 1338; ¹H NMR (400 MHz, CDCl₃): δ 7.69 (s, 1H), 6.97 (s, 1H), 4.87 (s, 2H), 4.25 (t, *J* = 5.0 Hz, 2H), 3.99 (s, 3H), 3.70 (t, *J* = 5.0 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 153.8, 147.6, 140.0, 128.3, 114.1, 110.4, 68.4, 56.5, 49.9, 30.0; HRMS (ESI-TOF) for [C₁₀H₁₁BrN₄O₄ + Na]⁺ calcd: 352.9861; found: 352.9860.

Synthesis of 5-((2-nitrobenzyl)oxy)-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione (4a): A solution of **5a** (128 mg, 0.592 mmol), **1** (100 mg, 0.393 mmol) and Ag₂O (274 mg, 1.18 mmol) in anhydrous dichloromethane (5 mL) was kept at continuous stirring for 3 days at room temperature in inert atmosphere. After complete consumption of starting material which was confirmed by TLC analysis, reaction mixture was subjected to normal silica gel column chromatography using hexane: ethylacetate (100:0 to 70:30) as a eluent to obtained white solid which was further washed with acetonitrile to afford pure compound **4a** as a white solid (85 mg, 55 %); FT-IR (ν_{max} , cm⁻¹): 2924, 1682, 1526, 1342; ¹H NMR (400 MHz, CDCl₃): δ 8.40 (d, *J* = 7.8 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 7.83 (t, *J* = 7.5 Hz, 1H), 7.67 – 7.56 (m, 2H), 7.53 (t, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 6.26 – 6.11 (m, 2H), 5.63 – 5.51 (m, 2H), 3.41 – 3.30 (m, 2H), 3.29 – 3.20 (m, 2H), 1.80 – 1.69 (m, 2H), 1.49 – 1.35 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 197.9, 196.3, 156.7, 146.3, 138.7, 134.7, 134.6, 134.0, 133.5, 133.2, 129.1, 128.4, 125.6, 124.9, 119.4, 118.0, 67.9, 52.1, 50.9, 34.5, 34.0, 24.8, 24.6; HRMS (ESI-TOF) for [C₂₃H₁₉NO₅ + H]⁺ calcd: 390.1341; found: 390.1345.

Synthesis of 5-((4,5-dimethoxy-2-nitrobenzyl)oxy)-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione (4b) and 6-((4,5-dimethoxy-2-nitrobenzyl)-5-hydroxy-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione (6): To a solution of **5b** (114 mg, 0.413 mmol), **1** (149 mg, 0.586 mmol) and Ag₂O (270 mg, 1.17 mmol) in anhydrous dichloromethane (5 mL) was kept at continuous stirring for 3 days at room temperature in inert atmosphere. After complete consumption of starting material which was confirmed by TLC analysis, reaction mixture was subjected to normal silica gel column chromatography

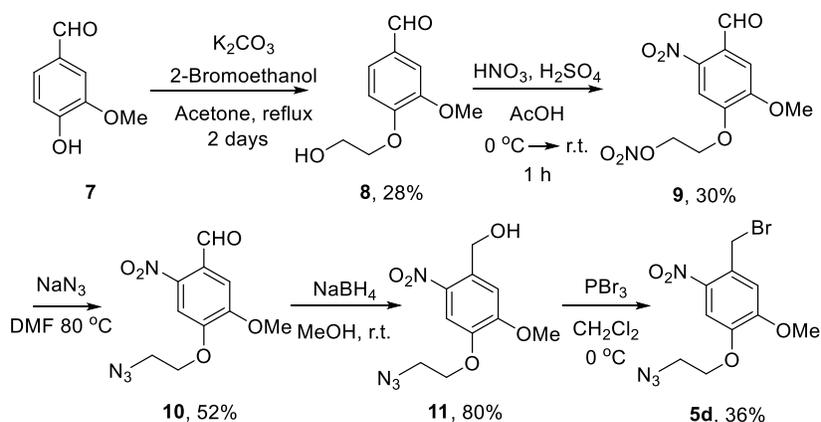
using hexane: ethylacetate (100:0 to 85:15) as an eluent to afford compound **4b** (55 mg, 30 %) and **6** (20 mg, 11 %); Characterization of **4b**; FT-IR (ν_{max} , cm^{-1}): 2923, 1737, 1682, 1517, 1376; ^1H NMR (400 MHz, CDCl_3): δ 8.25 (s, 1H), 7.80 (s, 1H), 7.69 – 7.62 (m, 1H), 7.62 – 7.55 (m, 1H), 7.44 – 7.36 (m, 1H), 6.22 – 6.11 (m, 2H), 5.56 (s, 2H), 4.26 (s, 3H), 3.99 (s, 3H), 3.35 – 3.26 (m, 2H), 3.23 (s, 2H), 1.78 – 1.70 (m, 2H), 1.45 – 1.36 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ 198.0, 196.2, 156.6, 154.7, 147.8, 138.6, 138.3, 134.8, 133.9, 133.6, 129.0, 125.2, 119.2, 117.7, 110.7, 107.7, 67.9, 57.2, 56.4, 52.1, 50.8, 34.6, 34.3, 24.8, 24.7; HRMS (ESI-TOF) for $[\text{C}_{25}\text{H}_{23}\text{NO}_7 + \text{Na}]^+$ calcd: 472.1372; found: 472.1374.

Characterization data of **6**; FT-IR (ν_{max} , cm^{-1}): 2929, 1734, 1681, 1520; ^1H NMR (400 MHz, CDCl_3): δ 13.10 (s, 1H), 7.66 (s, 1H), 7.46 (d, $J = 7.8$ Hz, 1H), 7.29 (s, 1H), 6.78 (s, 1H), 6.23-6.13(m, 2H), 4.46-4.32 (m, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.43-3.30 (m, 2H), 3.27 (m, 1H), 3.18 (m, 1H), 1.85 – 1.76 (m, 2H), 1.42 (dd, $J = 6.5, 4.7$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ 205.1, 197.0, 159.8, 153.1, 147.7, 141.5, 136.5, 134.6, 134.1, 133.8, 133.3, 128.7, 117.9, 117.5, 114.2, 108.3, 56.4, 50.3, 49.9, 36.2, 35.9, 33.2, 25.0, 24.8; HRMS (ESI-TOF) for $[\text{C}_{25}\text{H}_{23}\text{NO}_7 + \text{H}]^+$ calcd: 450.1553; found: 450.1550.

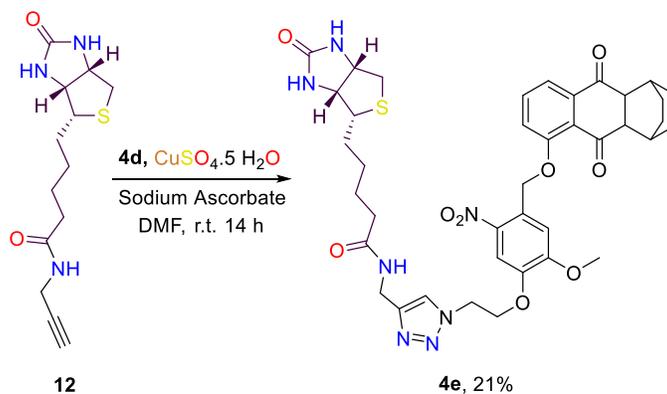
Synthesis of 5-((4-(2-azidoethoxy)-5-methoxy-2-nitrobenzyl)oxy)-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione (4d): To a solution of **5d** (400 mg, 1.21 mmol), **1** (460 mg, 1.81 mmol) and Ag_2O (1.95 g, 8.41 mmol) in anhydrous dichloromethane (30 mL) was kept at continuous stirring for 4 days at room temperature in inert atmosphere. After complete consumption of starting material which was confirmed by TLC analysis, reaction mixture was subjected to normal silica gel column chromatography using hexane: ethylacetate (100:0 to 50:50) as a eluent to afford compound **4d** as a pale yellow solid (150 mg, 25 %); FT-IR (ν_{max} , cm^{-1}): 2922, 2103, 1682, 1520; ^1H NMR (400 MHz, CDCl_3): δ 8.26 (s, 1H), 7.82 (s, 1H), 7.66 (t, $J = 7.9$ Hz, 1H), 7.61 – 7.57 (m, 1H), 7.40 (d, $J = 8.0$ Hz, 1H), 6.21 – 6.11 (m, 2H), 5.56 (s, 2H), 4.28 (t, $J = 5.0$ Hz, 2H), 4.24 (s, 3H), 3.73 (t, $J = 5.0$ Hz, 2H), 3.35 – 3.26 (m, 2H), 3.24 (s, 2H), 1.77 - 1.73 (m, 2H), 1.44 - 1.37 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ 198.0, 196.2, 156.5, 155.3, 146.4, 138.6, 138.0, 134.8, 133.9, 133.6, 129.9, 125.2, 119.2, 117.7, 111.2, 109.7, 68.3, 67.8, 57.2, 52.0, 50.8, 50.0, 34.6, 34.3, 24.8, 24.7; HRMS (ESI-TOF) for $[\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_7 + \text{Na}]^+$ calcd: 527.1543; found: 527.1542.

Synthesis of N-((1-(2-(4-(((9,10-dioxo-1,4,4a,9,9a,10-hexahydro-1,4-ethanoanthracen-5-yl)oxy)methyl)-2-methoxy-5-nitrophenoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-5-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (4e): To a solution of **4d** (105 mg, 0.208 mmol), **12** (50 mg, 0.178 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (88 mg, 0.352 mmol) and sodium ascorbate (71 mg, 0.358 mmol) in anhydrous *N,N*-Dimethylformamide (7 mL) was kept at continuous stirring for 14 h at room temperature in inert atmosphere. After complete consumption of starting material which was confirmed by TLC analysis,

reaction mixture was subjected to normal silica gel column chromatography using chloroform: methanol (100:0 to 90:10) as an eluent to obtain a pale yellow solid, which was further purified by preparative HPLC using MeOH:Acetonitrile (30:70): water as mobile phase to afford compound **4e** as a white solid (30 mg, 21 %); FT-IR (ν_{max} , cm^{-1}): 3230, 2924, 1684, 1523, 1329; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 8.31 (t, $J = 5.7$ Hz, 1H), 8.04 (s, 1H), 7.99 (s, 1H), 7.80 (s, 1H), 7.75 (t, $J = 8.0$ Hz, 1H), 7.53 (d, $J = 8.3$ Hz, 1H), 7.44 (d, $J = 7.6$ Hz, 1H), 6.40 (s, 1H), 6.35 (s, 1H), 6.19 - 6.07 (m, 2H), 5.62 - 5.48 (m, 2H), 4.78 (t, $J = 4.8$ Hz, 2H), 4.55 (t, $J = 4.9$ Hz, 2H), 4.35 - 4.24 (m, 3H), 4.15 - 4.03 (m, 4H), 3.21 - 3.13 (m, 2H), 3.11 - 2.01 (m, 1H), 2.79 (dd, $J = 12.4, 4.9$ Hz, 1H), 2.56 (d, $J = 12.7$ Hz, 1H), 2.14 - 2.06 (m, 2H), 1.76 - 1.66 (m, 2H), 1.64 - 1.38 (m, 4H), 1.34 - 1.22 (m, 4H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 197.4, 195.6, 171.8, 162.6, 156.0, 154.0, 145.8, 145.1, 138.3, 137.9, 134.9, 134.0, 133.4, 128.9, 124.7, 123.1, 118.4, 118.2, 111.1, 110.0, 67.6, 67.2, 60.9, 59.1, 56.4, 55.3, 51.3, 50.1, 48.7, 34.9, 34.0, 33.5, 33.1, 28.1, 27.9, 25.1, 24.0; HRMS (ESI-TOF) for $[\text{C}_{39}\text{H}_{43}\text{N}_7\text{O}_9\text{S} + \text{H}]^+$ calcd: 786.2921; found: 786.2916.



Scheme S1. Synthesis of Bromide **5d**.



Scheme S2. Synthesis of biotinylated adduct **4e** using copper catalyzed alkyne-azide click reaction.

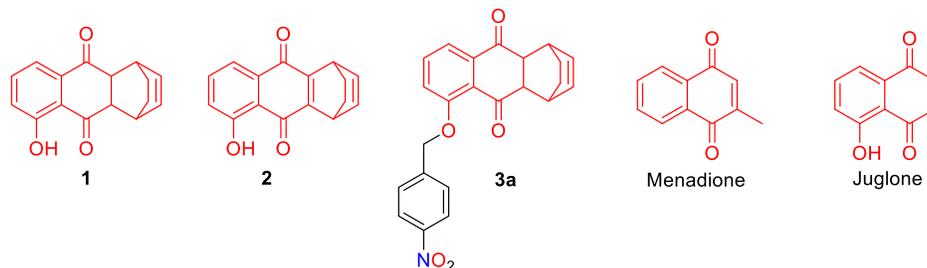


Figure S1.

3. Stock Solution Preparation:

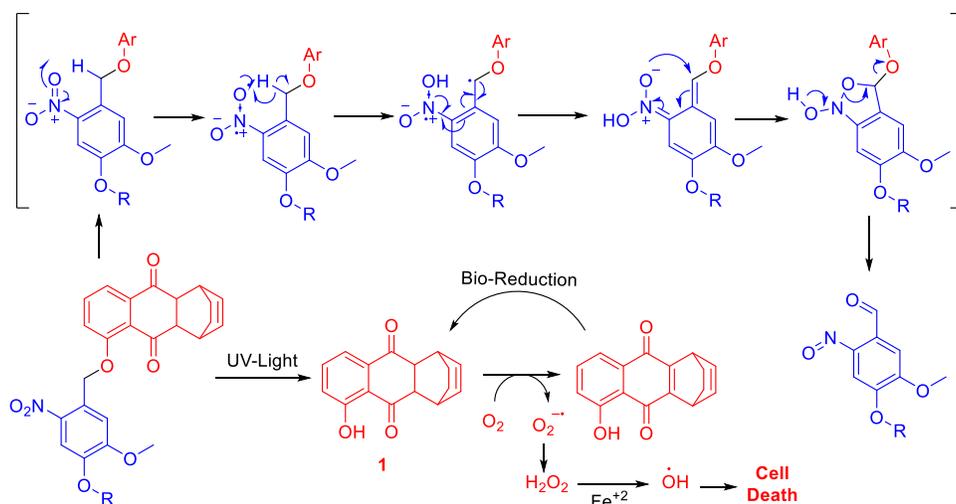
10 mM stocks of all the compounds **4a**, **4b** and **4e** were independently prepared in DMSO and stored in the dark at -4 °C. 10 mM stocks of Boronate ester probe **13**, dihydroethidium (DHE) and Amplex® red were prepared in DMSO and stored at -4 °C. 100 U/mL stocks of Superoxide dismutase (SOD) enzyme, 100 U/mL stocks of horseradish peroxidase (HRP) and 10000 U/mL stocks of catalase enzyme were prepared in phosphate buffer (50 mM) of pH 7.4 and stored at -4 °C. For making working solution for Amplex-red assay, HRP (100 µL of 100 U/mL) and Amplex® Red (50 µL of 10 mM) were mixed in phosphate buffer of pH 7.4 (4850 µL) and stored in dark at 0 °C until its use. (Working solution of Amplex® Red needs to be freshly prepared)

4. General procedure for irradiation:

A quartz cuvette containing compounds **4a**, **4b** and **4e** in phosphate buffer or phosphate buffer: acetonitrile mixture (60: 40) were independently irradiated using 365 nm (30 mW/cm²) LED flashlight at room temperature in a closed chamber. This solution was used for further analysis as described below.

5. Photo-cleavage study by HPLC after irradiation:

A quartz cuvette containing compounds **4a**, **4b** and **4e** (2.5 µL of 10 mM) in 60: 40 mixture of phosphate buffer:Acetonitrile of pH 7.4 (497 µL) was independently irradiated. Similarly, compounds **4a**, **4b** and **4e** (2.5 µL of 10 mM) in 60: 40 mixture of phosphate buffer: Acetonitrile of pH 7.4 (497 µL) was independently kept in dark for 40 min. 25 µL of aliquot from each Sample of these irradiated and non-irradiated solutions were injected in HPLC and analysis was conducted using a diode array detector (DAD) operating at 250 nm. A mobile phase of water: acetonitrile was used with a run time of 25 min. A multistep gradient was used with a flow rate of 1 mL/min starting with 50: 50 → 0 to 2 min, 50:50 to 10: 90 → 2 - 17 min, 10: 90 → 17 - 20 min, 10: 90 to 50: 50 → 20 - 22 min, 50:50 → 22 - 25 min.



Scheme S3. Mechanism of photolysis of 2-nitro benzyl derivatives to release alcohol/phenol derivatives and generation of ROS from compound **1**.

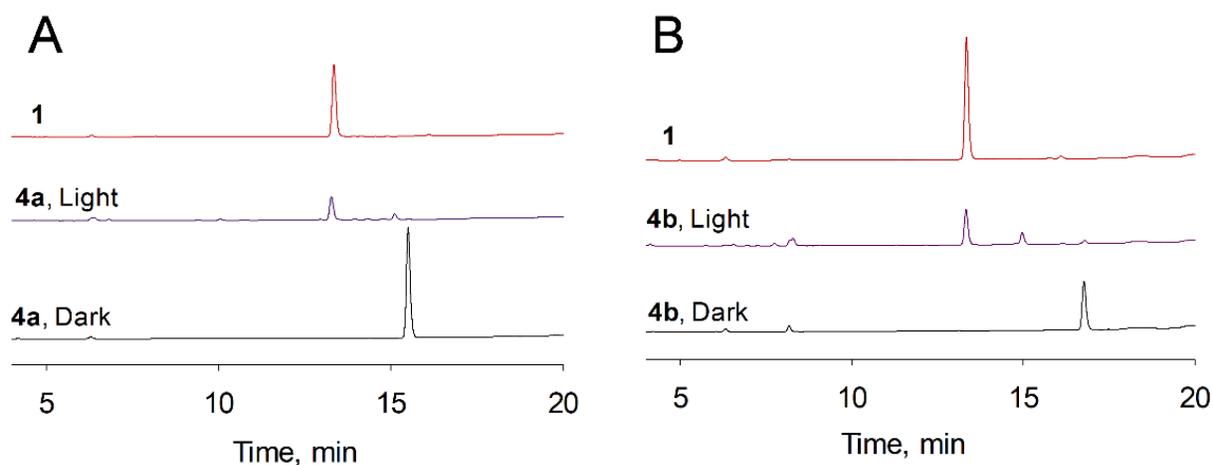
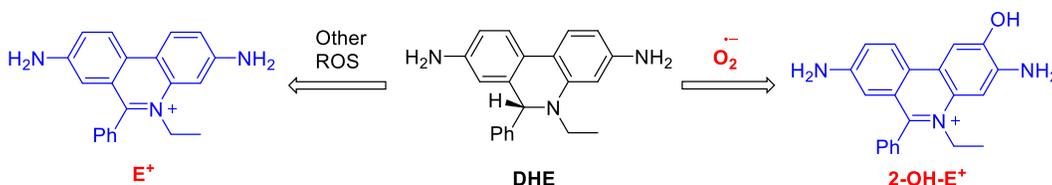


Figure S2. Photolysis of **4a** and **4b**; A & B) HPLC traces of **4a** & **4b** (50 μ M) in the presence and absence of Light, Light irradiation was performed by 365 nm light for 15 min with the 30 mW/cm² intensity.

6. Superoxide detection using DHE Assay.⁶⁻¹⁰

A quartz cuvette containing compounds **4a**, **4b** and **4e** (1.25 μ L of 10 mM) in phosphate buffer of pH 8.0 (496 μ L) was independently irradiated. Similarly, compounds **4a**, **4b** and **4e** (1.25 μ L of 10 mM) in phosphate buffer of pH 8.0 (496 μ L) was independently kept in dark. In the non-irradiated and irradiated samples, DHE (2.5 μ L of 10 mM) was added independently and incubated for 1 h at 37 $^{\circ}$ C. The reaction mixture was filtered (0.45 μ m) and injected (50 μ L) in an Agilent high performance liquid chromatograph (HPLC) attached with a fluorescence detector (excitation at 480 nm; emission at 580 nm). The column used was Agilent1260-infinity with Phenomenex[®] C-18 reverse phase column (250 mm \times 4.6 mm, 5 μ m), the mobile phase was water: acetonitrile containing 0.1% trifluoroacetic acid and a gradient starting with

90: 10 % → 0 min, 10: 90 to 44: 56 → 0 – 35 min, 0: 100 → 35 – 37 min, 0: 100 → 37 – 40 min, 10: 90 → 40 – 42 min, 10: 90 → 42 – 45 min was used with a flow rate of 0.5 mL/min. **1**, a known superoxide generator (1.25 μL of 10 mM) were mixed in phosphate buffer (pH 8.0, 50 mM) along with DHE (2.5 μL of 10 mM) for 1 h and served as a positive control. A Known scavenger of superoxide, Superoxide dismutase enzyme (5 μL of 100 U/mL) was also used as a control with the irradiated sample. Signal for superoxide was not observed in SOD treated samples.



Scheme S4. Reaction of dihydroethidium (DHE) with superoxide and other ROS (H₂O₂, $\dot{O}H$ etc.) to form 2-hydroxyethidium (2-OH-E⁺) and ethidium (E⁺) respectively.

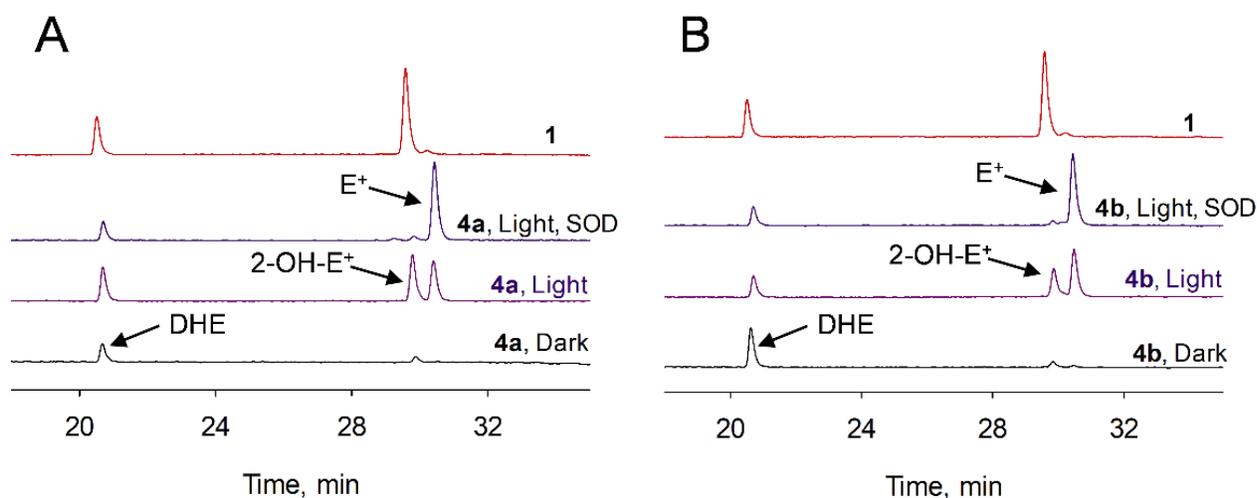


Figure S3. DHE Assay with **4a** & **4b**; A & B) HPLC traces of DHE assay for superoxide detection, **4a** & **4b** (25 μM) was irradiated in 30 mW/cm² 365 nm light for 15 min followed by addition of DHE and incubated for 1 h, as a control, SOD was used for quenching the superoxide.

7. H₂O₂ detection using Boronate-Ester Probe **13**:^{11,12}

A quartz cuvette containing compounds **4a**, **4b** and **4e** (2.0 μL of 10 mM) in phosphate buffer of pH 7.4 (790 μL) was independently irradiated. Similarly, compounds **4a**, **4b** and **4e** (2.0 μL of 10 mM) in phosphate buffer of pH 7.4 (790 μL) was independently kept in dark. In the non-irradiated and irradiated samples, **13** (8 μL of 10 mM) was added independently and incubated for 2 h at 37 °C. As a positive control, **1** (2.0 μL

of 10 mM) in phosphate buffer of pH 7.4 (790 μ L) followed by addition of probe **13** (8 μ L of 10 mM) and incubated for 2 h at 37 $^{\circ}$ C. In another control experiment, **4a**, **4b** and **4e** (2.0 μ L of 10 mM) in phosphate buffer of pH 7.4 (782 μ L) was irradiated, followed by addition of catalase enzyme (8 μ L of 10000 U/mL), which treated with probe **13** (8 μ L of 10 mM) and incubated for 2 h at 37 $^{\circ}$ C. All these irradiation experiments were done in duplicates. Further aliquots (200 μ L) of these solutions were transferred to the 96 well-plate in triplicates and the fluorescence was measured using Thermo Scientific Varioskan microtiter plate reader ($\lambda_{\text{ex}} = 320$ nm and $\lambda_{\text{em}} = 460$ nm).

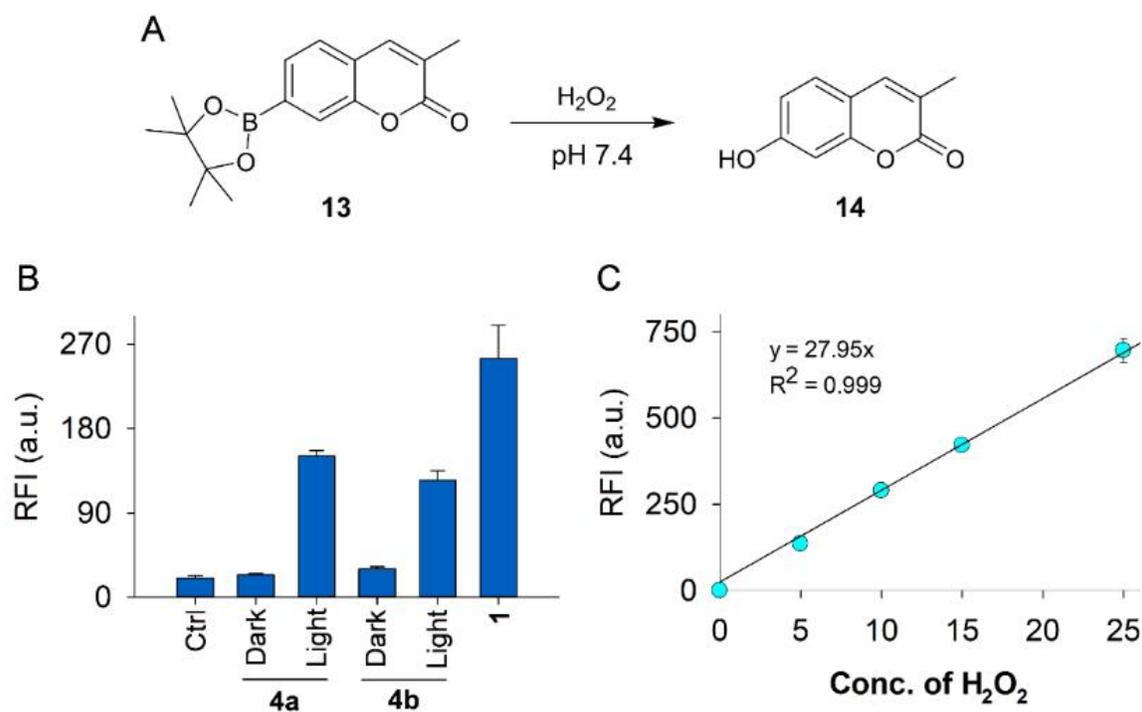


Figure S4. (A) Reaction of hydrogen peroxide with coumarin-boronate ester dye (**13**) to form umbelliferone derivative (**14**) (B) Hydrogen peroxide detection using coumarin-boronate ester **13** dye as a probe. (C) Calibration curve of hydrogen peroxide using probe **13**. Oxidation of the boronate ester to the phenol results in an increased fluorescence signal (excitation 320 nm; emission 460 nm). Data represent the mean \pm s.d. for 3 technical replicates per group.

8. H_2O_2 detection using Amplex[®] Red:

A quartz cuvette containing compounds **4e** (1.25 μ L of 10 mM) in phosphate buffer of pH 7.4 (499 μ L) was irradiated. Similarly, compounds **4e** (1.25 μ L of 10 mM) in phosphate buffer of pH 7.4 (499 μ L) was kept in dark. As a positive control, **1** (1.25 μ L of 10 mM) in phosphate buffer of pH 7.4 (499 μ L) was used in dark. In a separate control experiment, **4e** (1.25 μ L of 10 mM) in phosphate buffer of pH 7.4 (494 μ L)

was irradiated, followed by addition of catalase enzyme (5 μL of 10,000 U/mL). Aliquots (100 μL) of these solutions were transferred to the 96 well-plate in triplicates and incubated for 2 h at 37 $^{\circ}\text{C}$. The working solution of Amplex[®] Red (100 μL) was added in each well and further incubated for 30 min at 37 $^{\circ}\text{C}$. Fluorescence measurement was done using Thermo Scientific Varioskan microtiter plate reader ($\lambda_{\text{ex}} = 550$ nm and $\lambda_{\text{em}} = 590$ nm).

9. Extracellular H_2O_2 detection using Boronate-Ester Probe **13**:

Human adenocarcinomic alveolar basal epithelial cells, A549 cells were seeded at a concentration of 1×10^4 cells/well overnight in a 96-well plate in complete RPMI media. Cells were treated with 50 μM concentrations of the compound **4e** and **1**. Cells were first exposed to 365 nm light for 5 min and as a control other section of plate was covered with aluminum foil, kept in dark then incubated for 2 h at 37 $^{\circ}\text{C}$. In the non-irradiated and irradiated part of the plate, **13** (100 μM) was added and incubated for 2 h at 37 $^{\circ}\text{C}$. All these experiments were done in triplicate. Further aliquots (200 μL) from these wells were transferred to another 96 well-plate and the fluorescence was measured using Thermo Scientific Varioskan microtiter plate reader ($\lambda_{\text{ex}} = 320$ nm and $\lambda_{\text{em}} = 460$ nm).

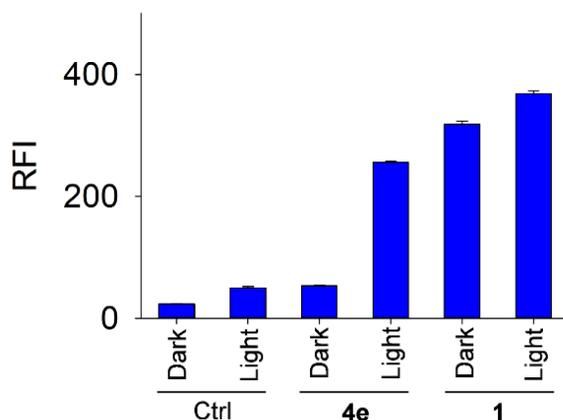


Figure S5. Extracellular hydrogen peroxide detection using coumarin-boronate ester **13** dye as a probe. Oxidation of the boronate ester to the phenol results in an increased fluorescence signal (excitation 320 nm; emission 460 nm). Data represent the mean \pm s.e.m. for 3 technical replicates per group.

10: Extracellular H_2O_2 detection using Amplex[®] Red:

Human adenocarcinomic alveolar basal epithelial cells, A549 cells were seeded at a concentration of 1×10^4 cells/well overnight in a 96-well plate in complete RPMI media. Cells were treated with 50 μM concentrations of the compound **4e** and **1**. Cells were first exposed to 365 nm light for 5 min and other as a control other section of plate was covered with aluminum foil, kept in dark then incubated for 4 h at 37

°C. Further aliquots (50 µL) from these wells were transferred to another 96 well-plate. The working solution of Amplex® Red (50 µL) was added in each well and further incubated for 30 min at 37 °C. Fluorescence measurement was done using Thermo Scientific Varioskan microtiter plate reader ($\lambda_{\text{ex}} = 550$ nm and $\lambda_{\text{em}} = 590$ nm).

11. Intracellular ROS detection using H₂DCFDA dye by Fluorescence measurement:

Human adenocarcinomic alveolar basal epithelial cells, A549 cells were seeded at a concentration of 2×10^4 cells/well overnight in two parts of 96-well plate in complete RPMI media. Cells in both parts were exposed to 50 µM of the compound **4e**. After 2 h incubation at 37 °C, old media of each wells was replaced with fresh media and one part of the plate was exposed to 365 nm light for 5 min and other part of plate was covered with aluminum foil then incubated for 1 h at 37 °C. A 10 µM solution of 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) was prepared in RPMI media and 300 µL of this solution was added to each well. After 10 min incubation, the excess dye was removed and washed twice with PBS and finally 200 µL of PBS was added. Intensity of all the wells were measured in Operetta CLS™ high-content analysis system by PerkinElmer in the GFP channel (excitation 488 nm; emission 514 nm).

12. Intracellular ROS detection by H₂DCFDA dye by Fluorescence Imaging:

Human adenocarcinomic alveolar basal epithelial cells, A549 cells were seeded at a concentration of 1×10^5 cells/well in two parts of 12-well plate in complete RPMI media and incubated for 48 h at 37 °C. Cells were treated with 50 µM of the compound **4e** and further incubated for 2 h at 37 °C. Old media of each wells were removed and washed twice with PBS. At this point, fresh media was added and one part of the plate was exposed to 365 nm light for 5 min and other part of plate was covered with aluminum foil then incubated for 1 h at 37 °C. A 10 µM solution of 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) was prepared in RPMI media and 1 mL of this solution was added to each well. After 10 min incubation, the excess dye was removed and washed twice with PBS and finally 1 mL of PBS was added. All the wells were Imaged in EVOS® FL Auto Imaging System in the GFP channel (excitation 488 nm; emission 514 nm).

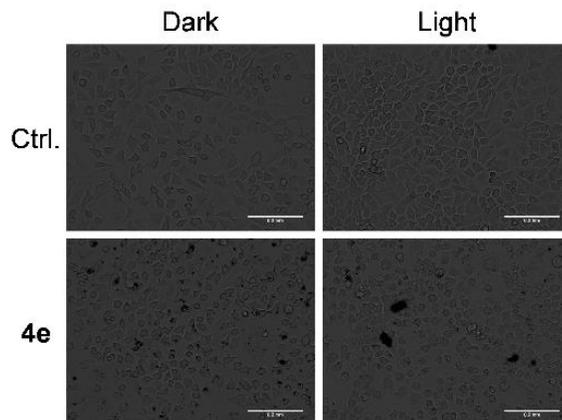


Figure S6: Trans images of Intracellular ROS detection using H₂DCF-DA as a probe (Scale bar = 0.2 mm).

13. Cell Viability assay using MTT dye:

Human adenocarcinomic alveolar basal epithelial cells, A549 cells and Human adenocarcinomic colon cells, DLD-1 were seeded at a concentration of 1×10^3 cells/well overnight in two 96-well plates in complete RPMI media. Cells in both plates were exposed to varying concentrations of the compound **4e** and **1** prepared as a DMSO stock solution so that the final concentration of DMSO was 0.5%. One of the plates was first exposed to 365 nm light for 5 min and other plate was kept in dark then incubated for 72 h at 37 °C. A solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was prepared by dissolving MTT reagent (6 mg) in 12 mL RPMI media. 100 μ L of this solution was added to each well. After 4 h incubation, the media was removed carefully and 100 μ L of DMSO was added to each well. Spectrophotometric analysis of each well was carried out at 570 nm using a Thermo Scientific Varioskan microplate reader to estimate cell viability.

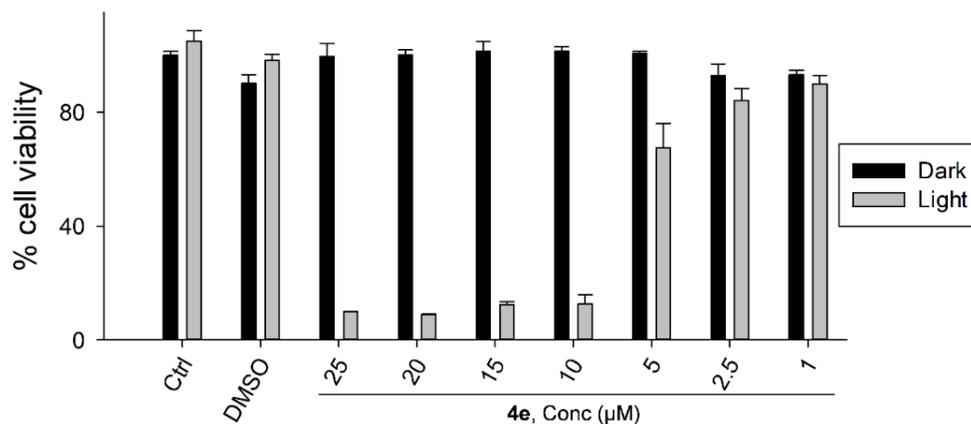


Figure S7. Cell viability assay -MTT assay with varying concentration of **4e** using A549 cell line. Data represent the mean \pm s.e.m. for 3 technical replicates per group.

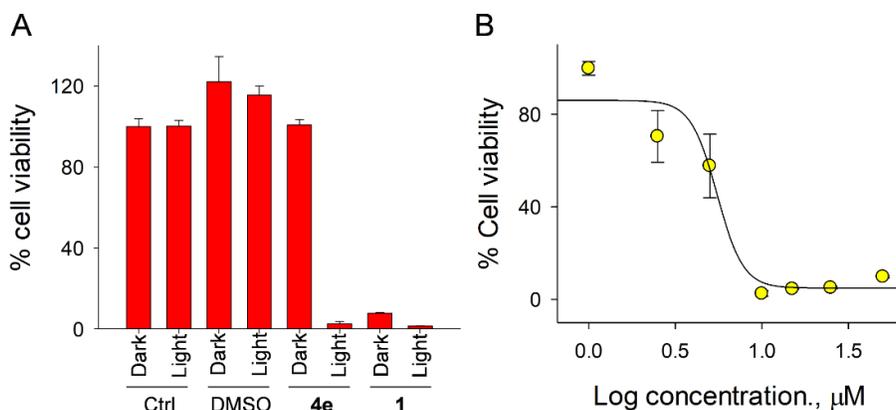


Figure S8. Cell viability assay using DLD-1 cells A) MTT assay with 10 μM compounds and B) Growth inhibition curve after irradiation with **4e** and IC₅₀ was found to be 5.3 μM; Data represent the mean±s.e.m. for 3 technical replicates per group.

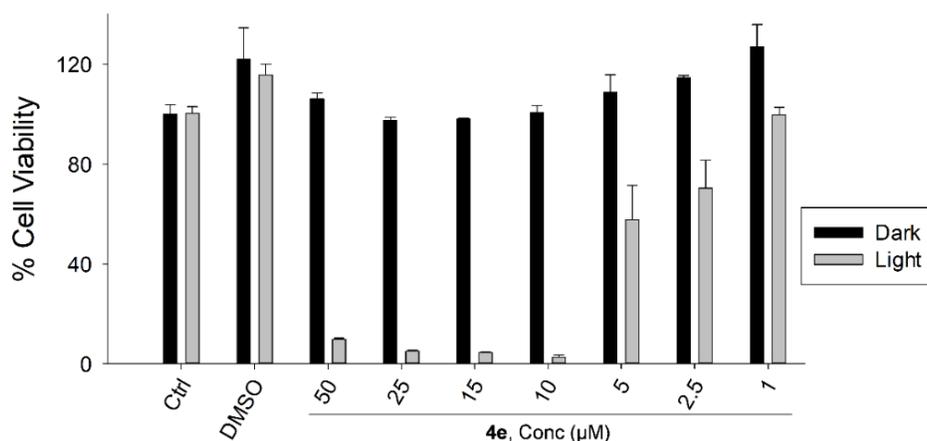


Figure S9. Cell viability assay -MTT assay with varying concentration of **4e** using DLD-1 cell line; Data represent the mean±s.e.m. for 3 technical replicates per group.

14. Thiol Reactivity of ROS generators:

Compound **1**, **2**, Juglone and Menadione (5 μL of 10 mM stock in DMSO) in the 1:1 mixture of pH 7.4 phosphate buffer:Acetonitrile (945 μL) were independently reacted with glutathione (50 μL of 10 mM) for 4 h at 37 °C. Reaction mixtures were filtered (0.45 μm) and injected (25 μL) in an Agilent high performance liquid chromatograph (HPLC) attached with a Diode array detector operating at 250 nm. A mobile phase of water: acetonitrile was used with a run time of 20 min. A multistep gradient was used with a flow rate of 1 mL/min starting with 50: 50 → 0 to 2 min, 50:50 to 10: 90 → 2 - 17 min, 10: 90 to 50:50 → 17 – 19 min, 50:50 → 19 - 20 min.

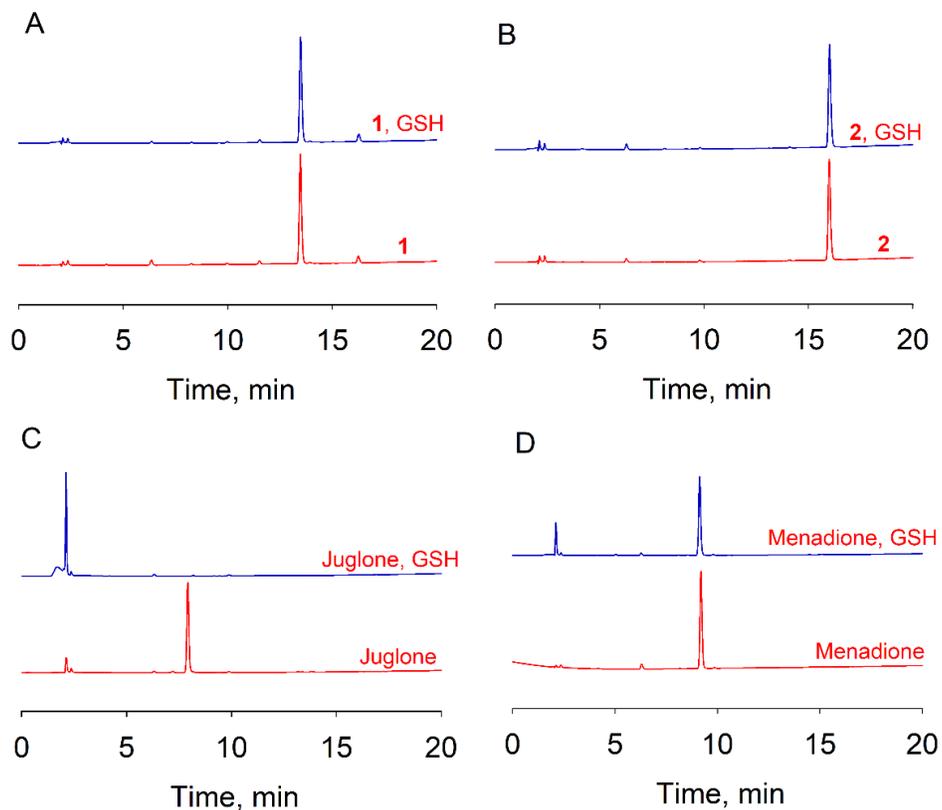


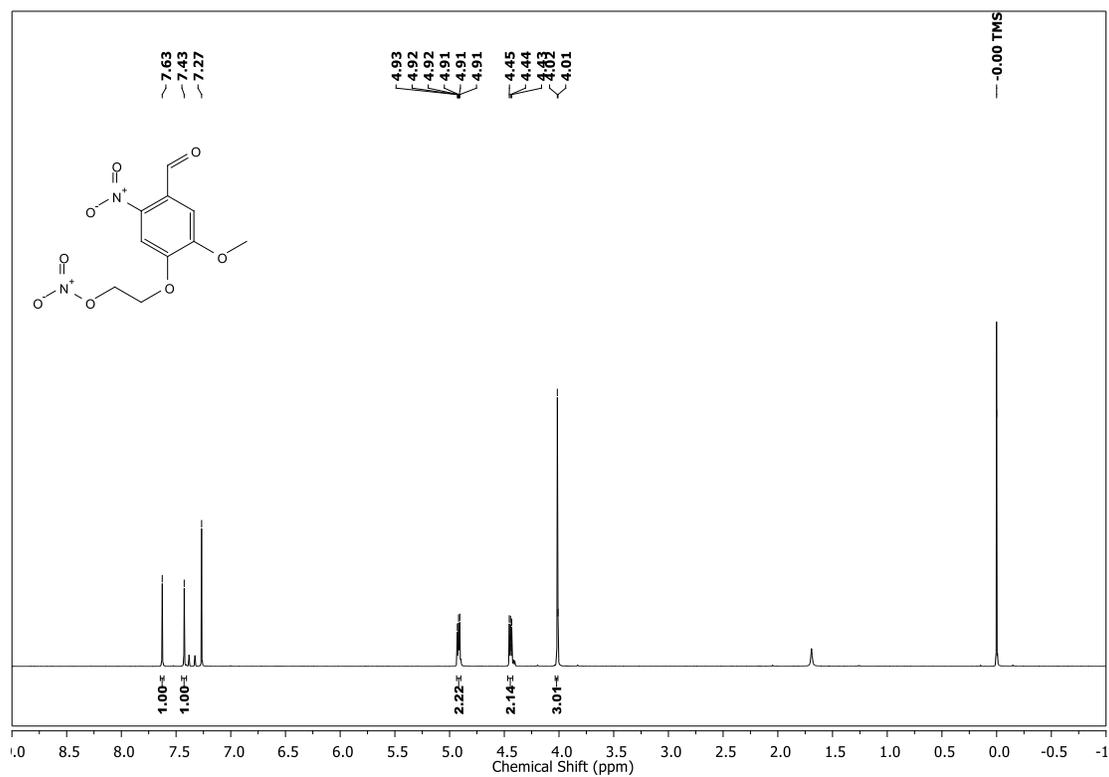
Figure S10: HPLC traces of glutathione reaction with A) **1**; B) **2**; C) juglone and D) Menadione. 50 μM of compounds were treated with 500 μM of glutathione and incubated for 4 h (GSH = Glutathione). Compound **1** does not react with GSH because of absence of α , β -unsaturated site whereas compound **2** which is a 1,4-anthraquinone derivative, is a candidate for reaction with thiols through the Michael reaction. However, in compound **2**, the α , β -unsaturated site was designed to be sterically less accessible towards attack by thiol. In 1,4-quinones such as menadione, reaction with thiols is facile.

15. References:

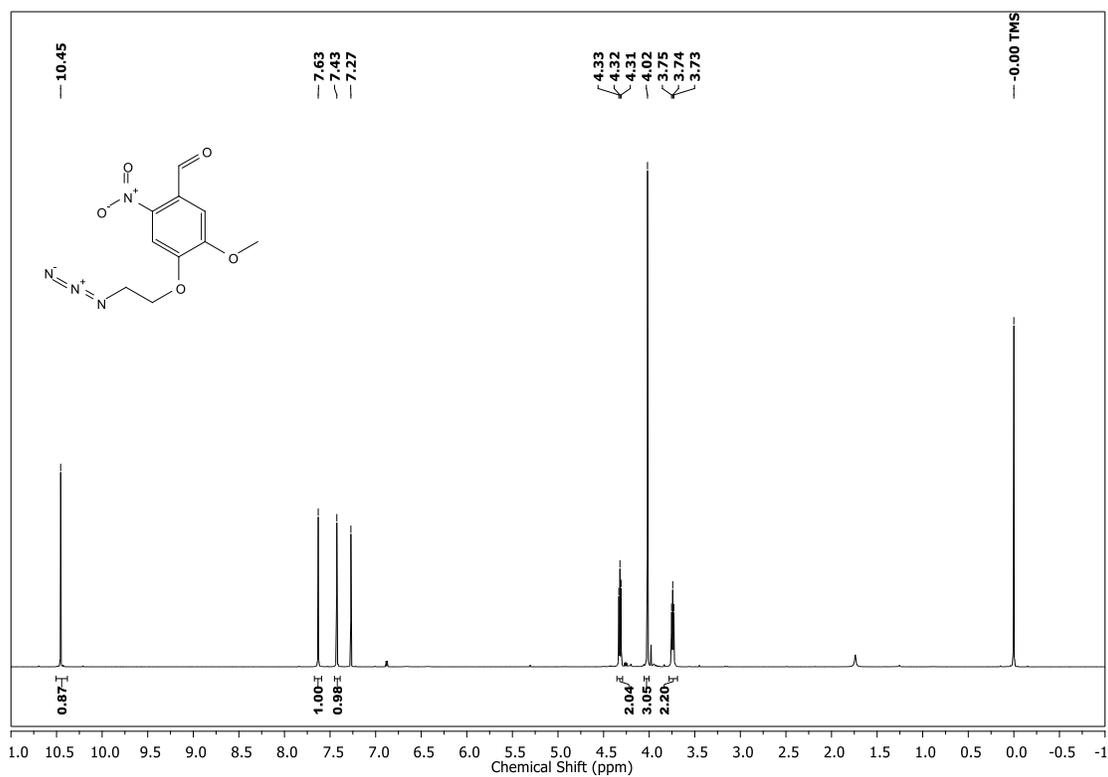
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16. NMR spectra:

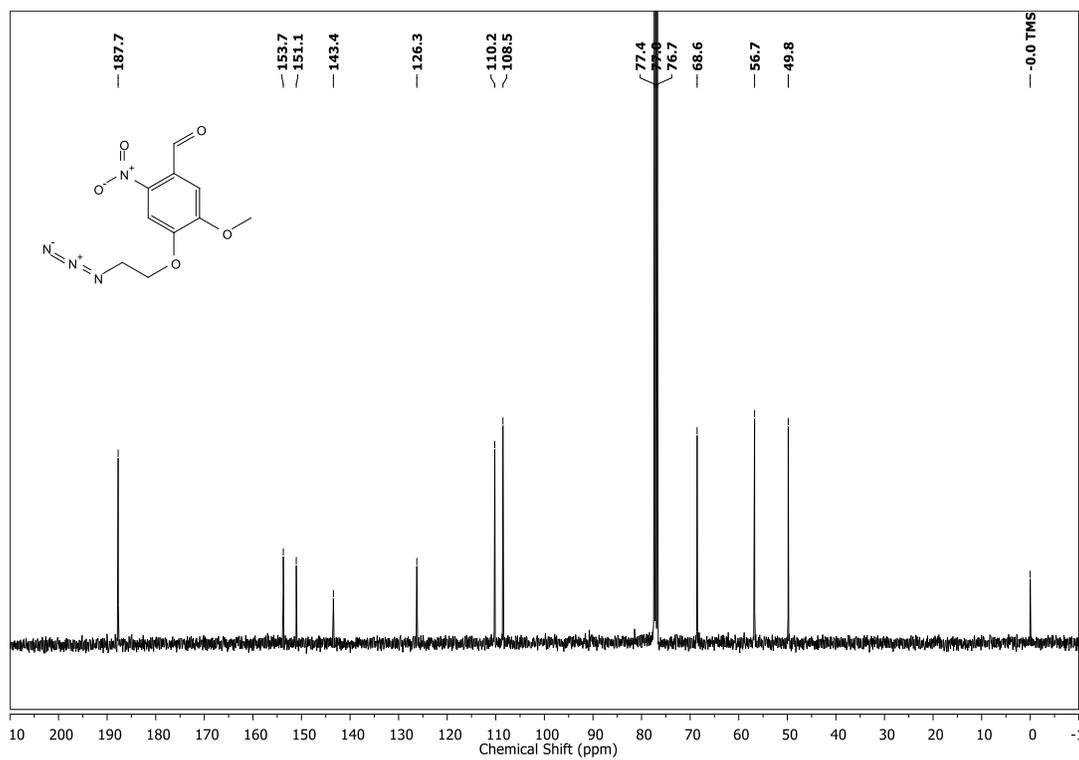
^1H NMR of **9** (CDCl_3 , 400 MHz):



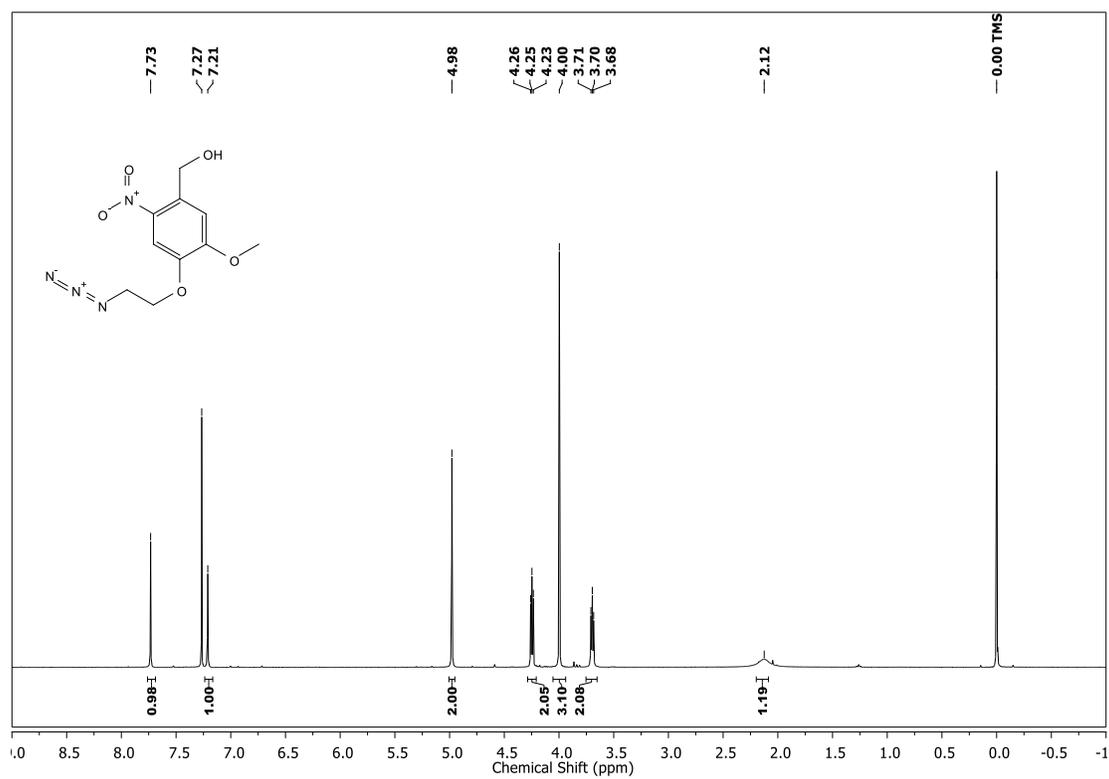
^1H NMR of **10** (CDCl_3 , 400 MHz):



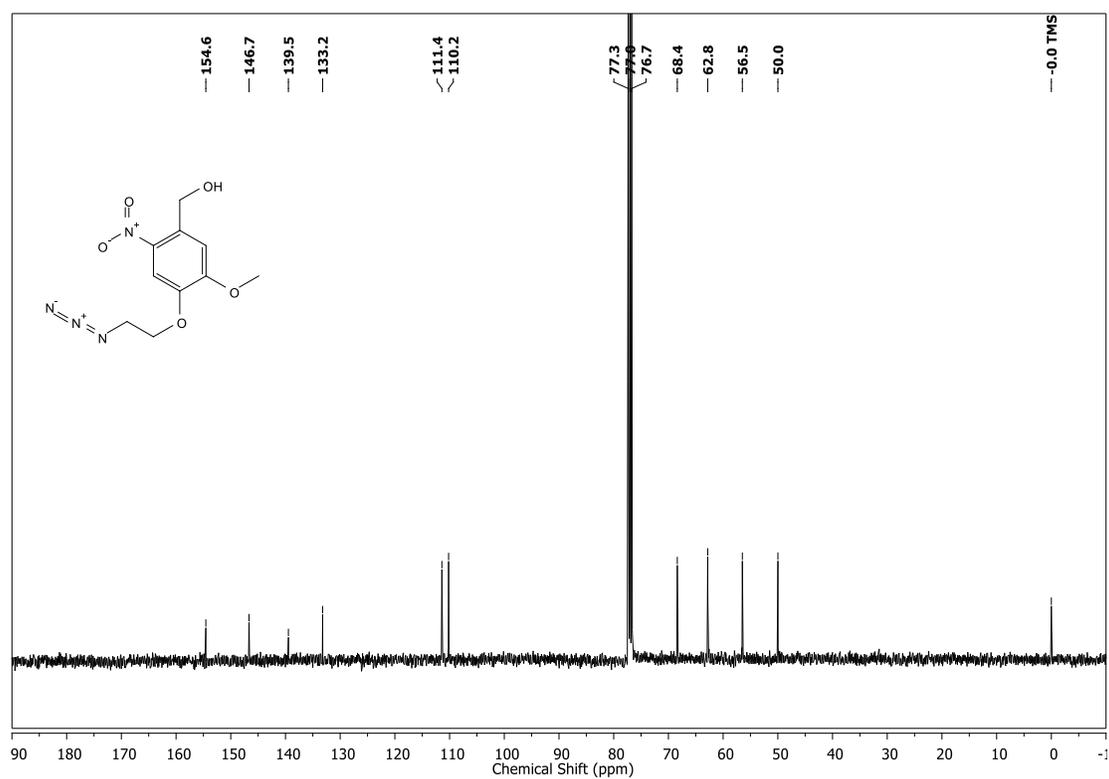
^{13}C -NMR of **10** (CDCl_3 , 100 MHz):



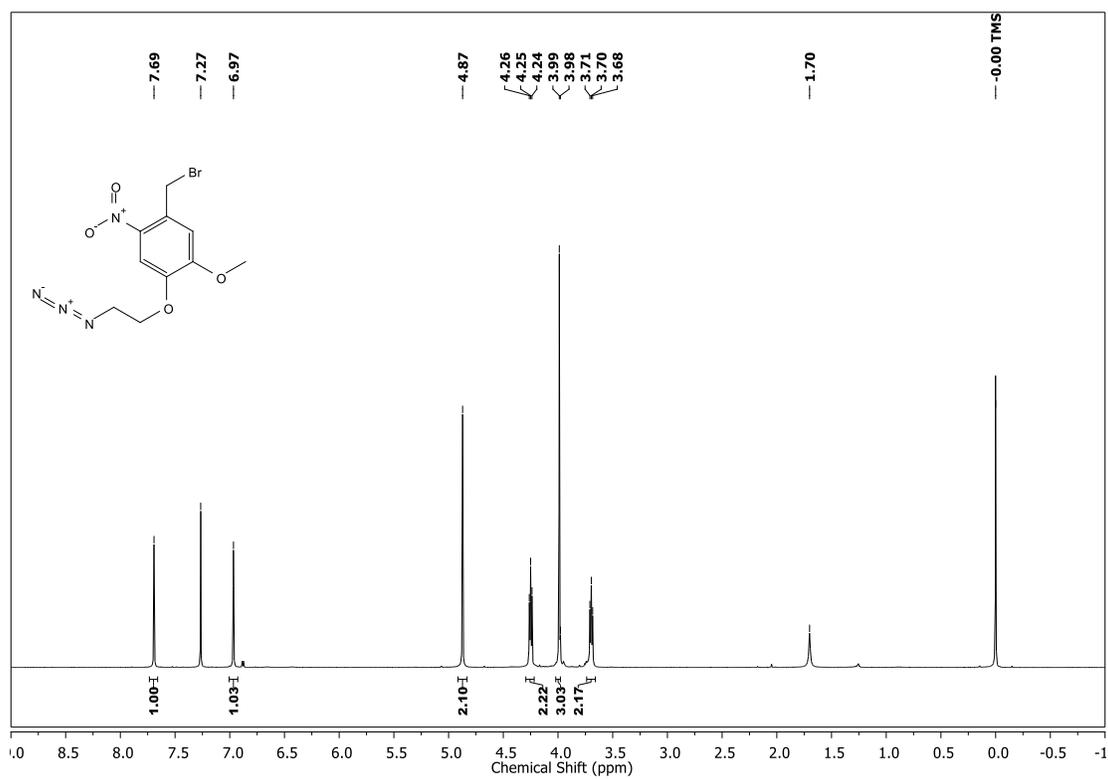
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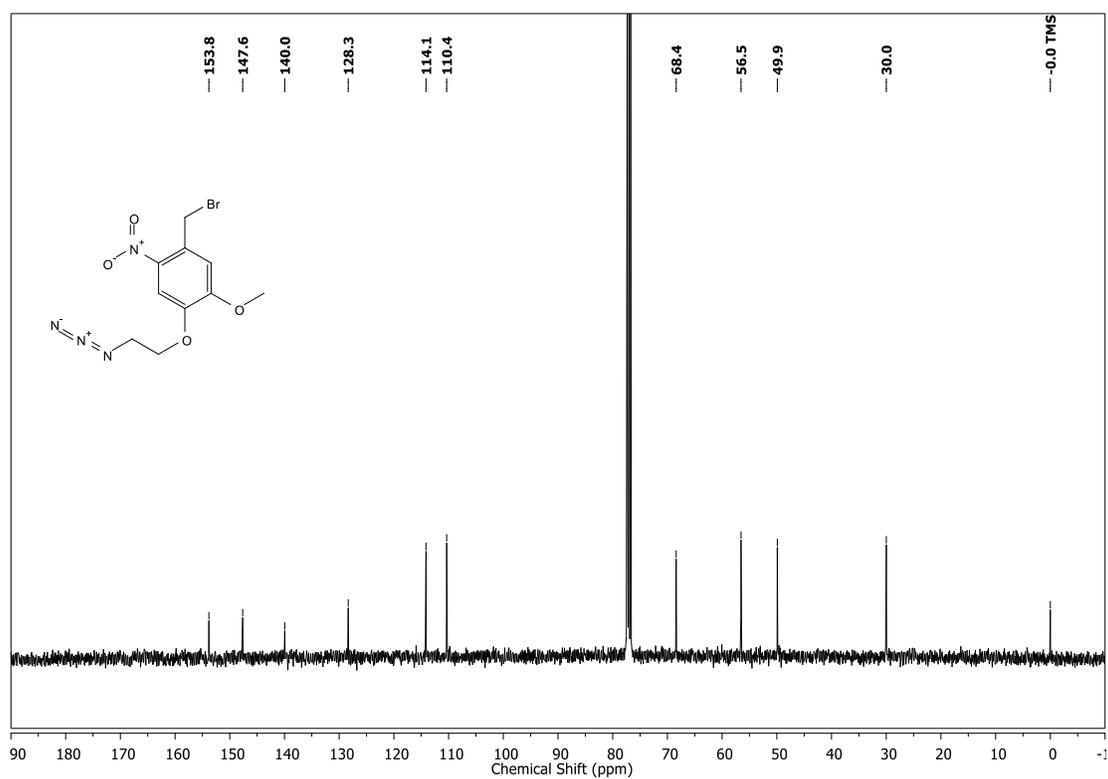
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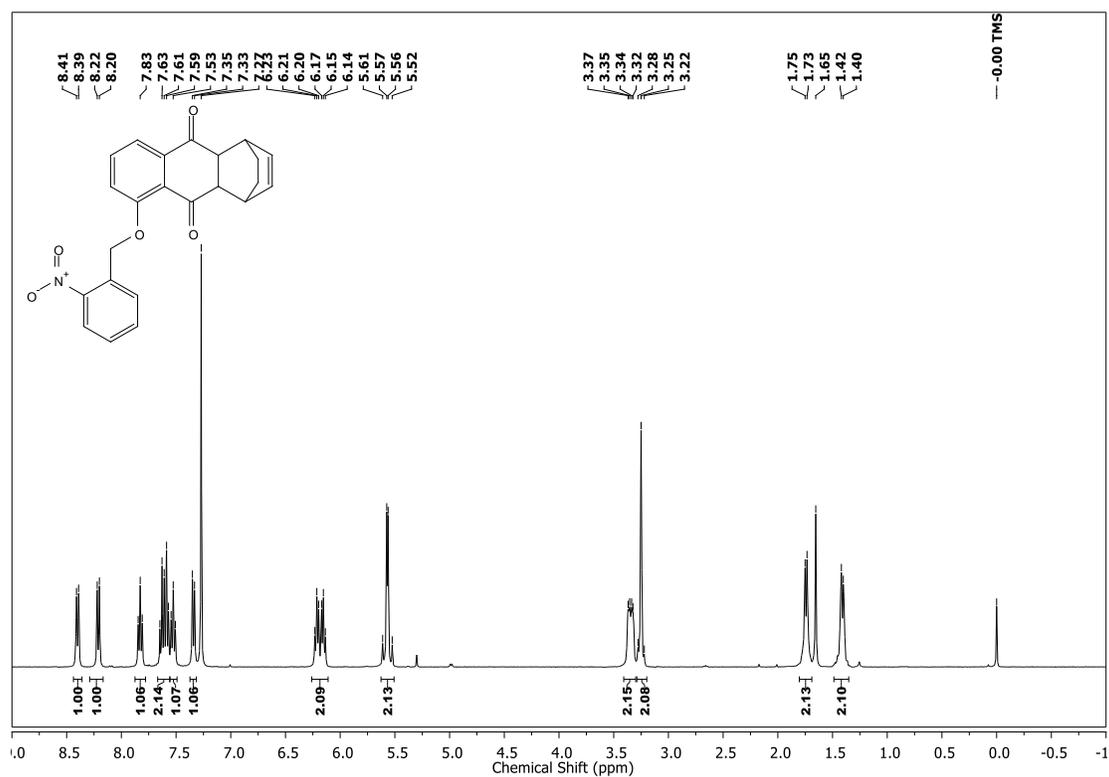
^1H NMR of **5d** (CDCl_3 , 400 MHz):



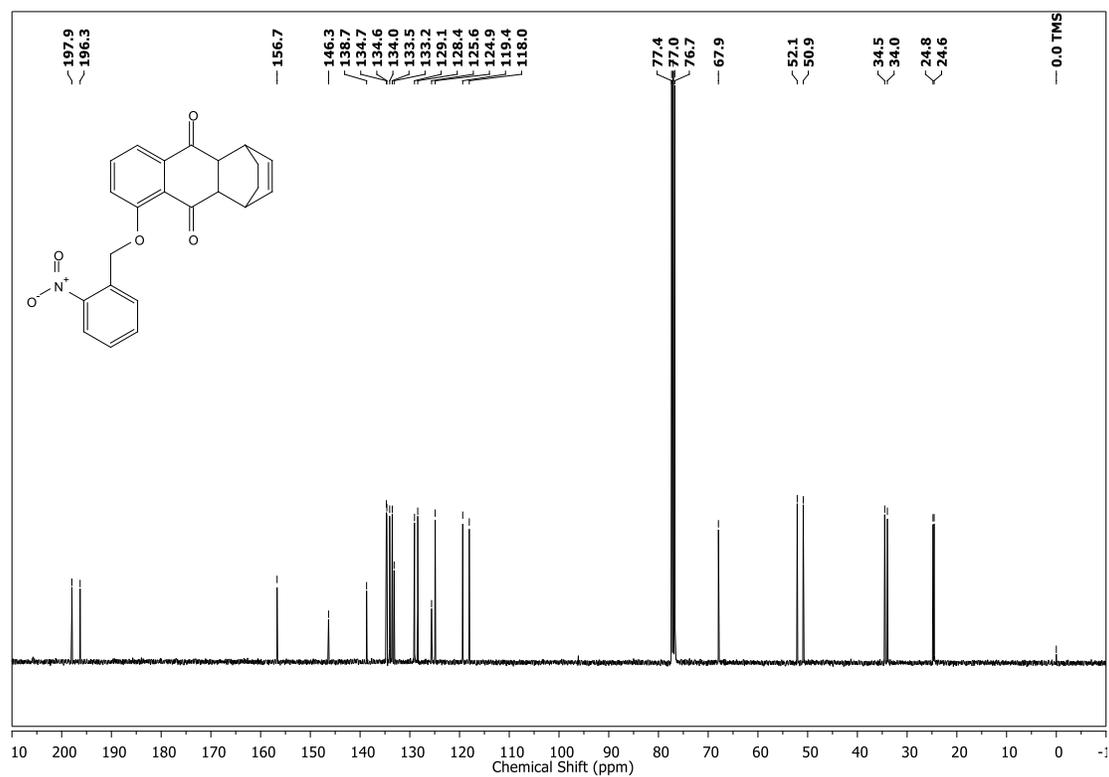
^{13}C -NMR of **5d** (CDCl_3 , 100 MHz):



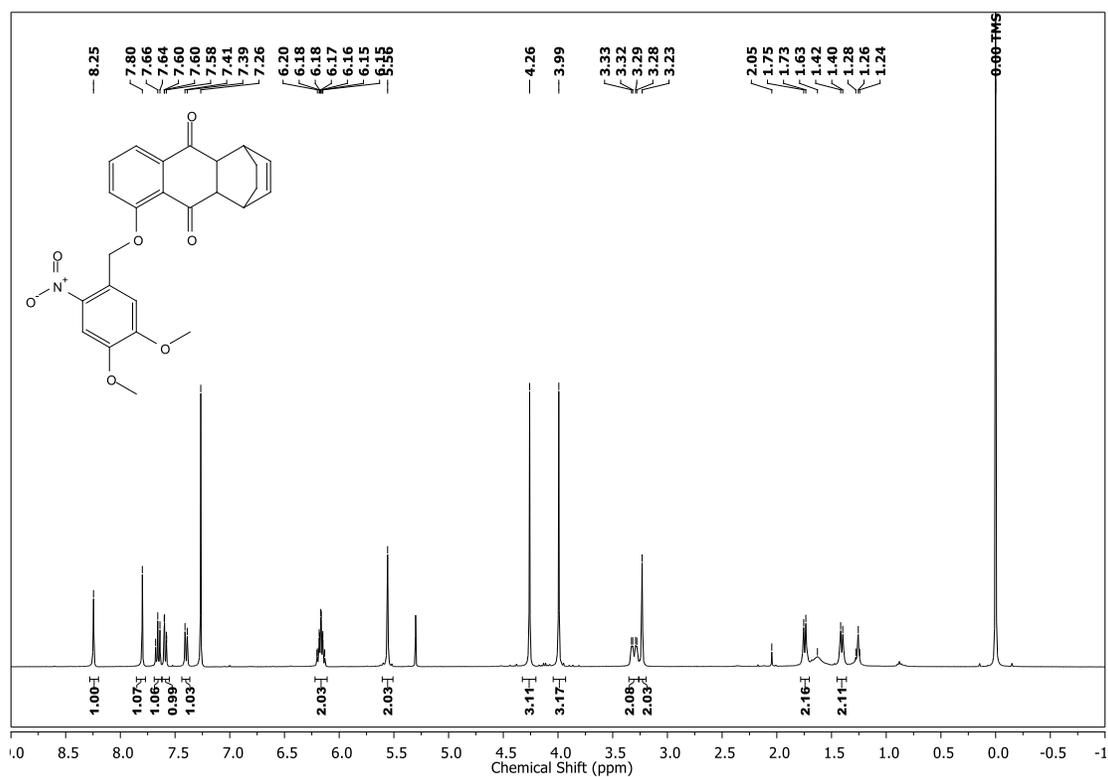
^1H NMR of **4a** (CDCl_3 , 400 MHz):



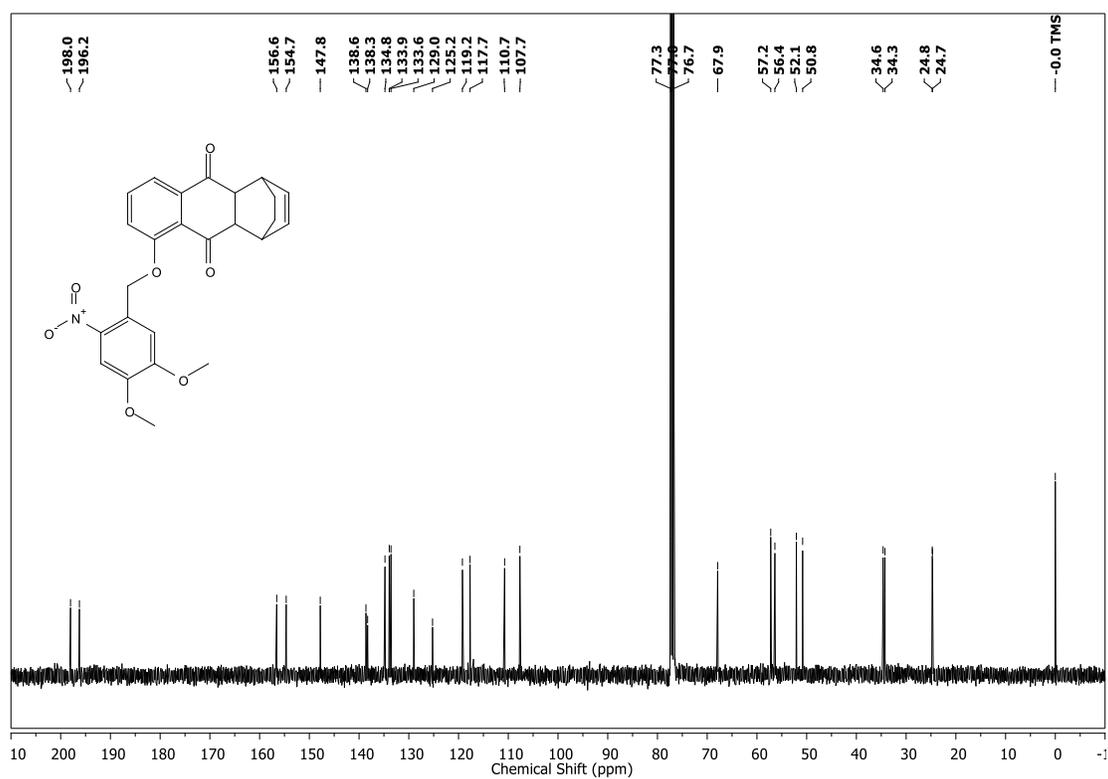
^{13}C -NMR of **4a** (CDCl_3 , 100 MHz):



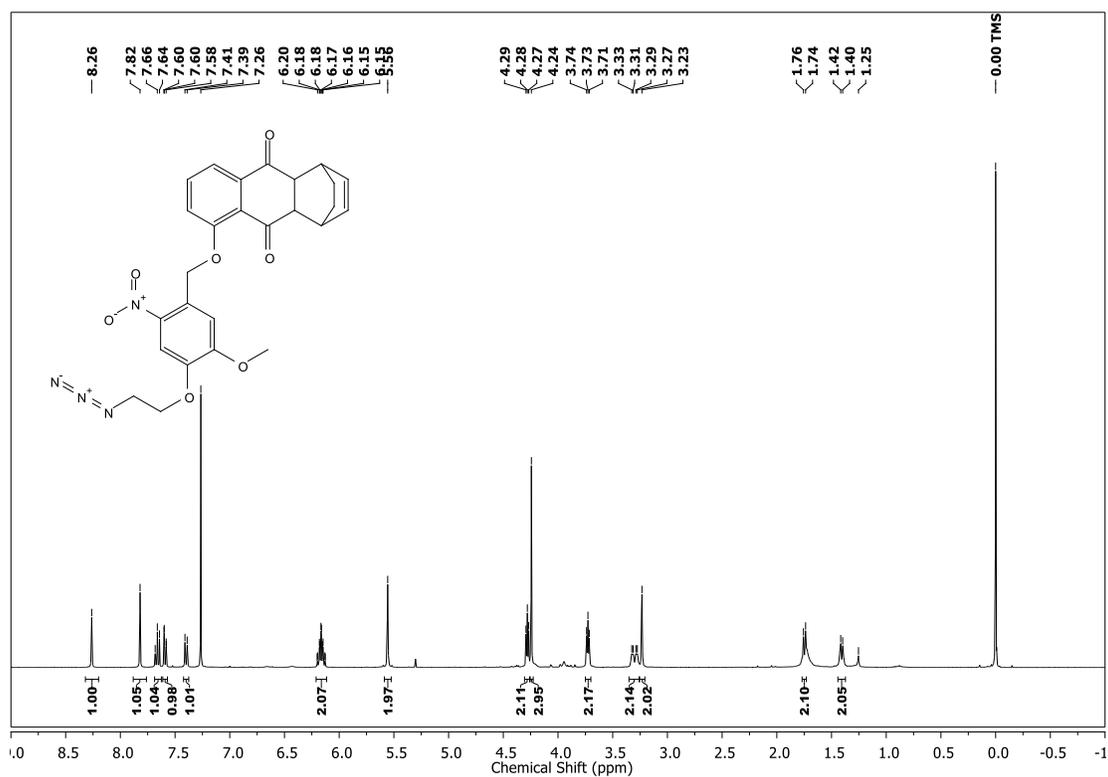
^1H NMR of **4b** (CDCl_3 , 400 MHz):



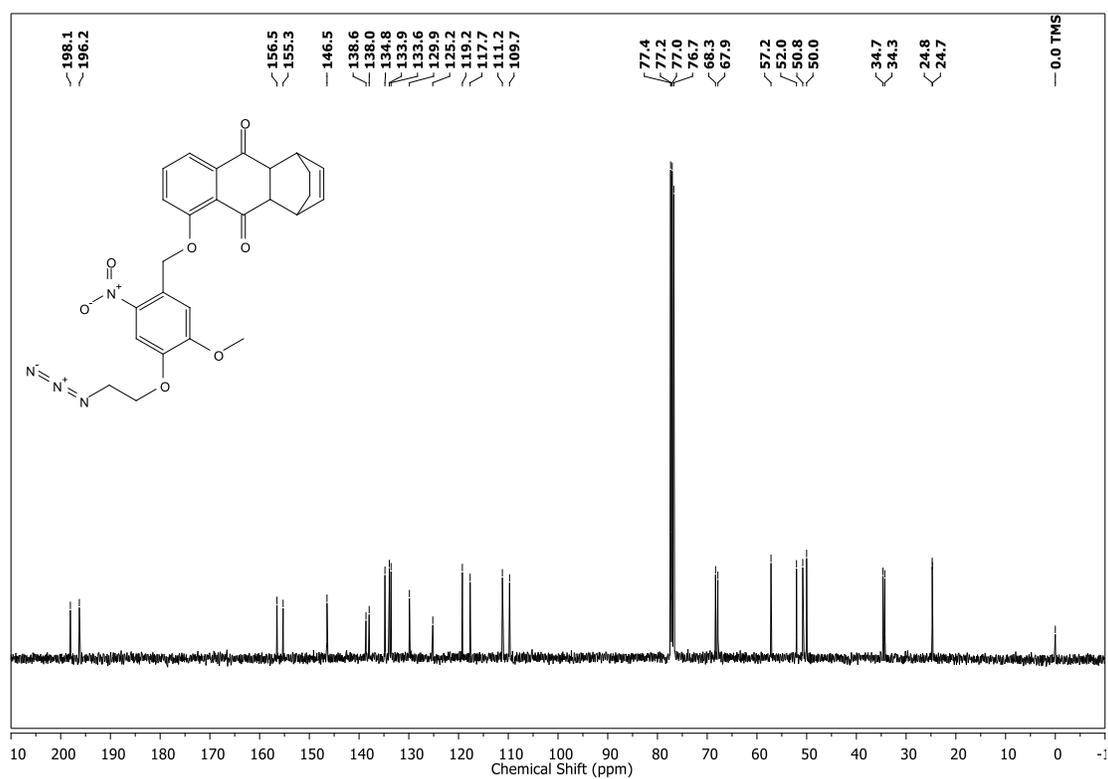
^{13}C -NMR of **4b** (CDCl_3 , 100 MHz):



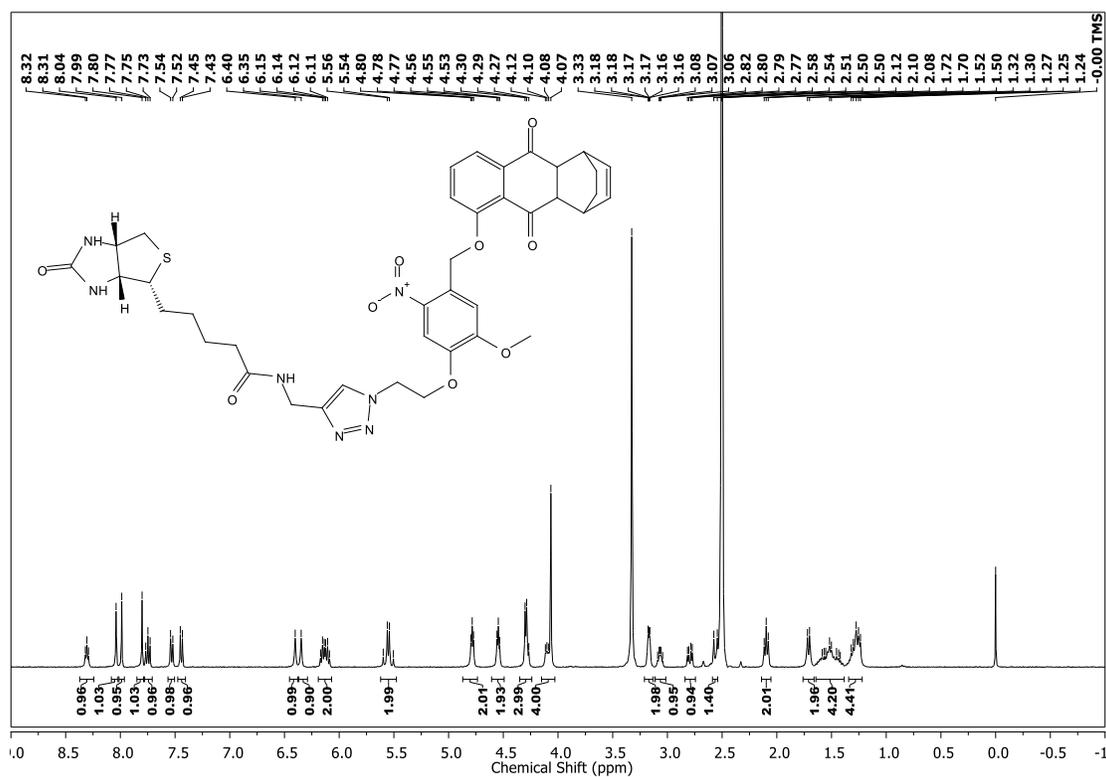
^1H NMR of **4d** (CDCl_3 , 400 MHz):



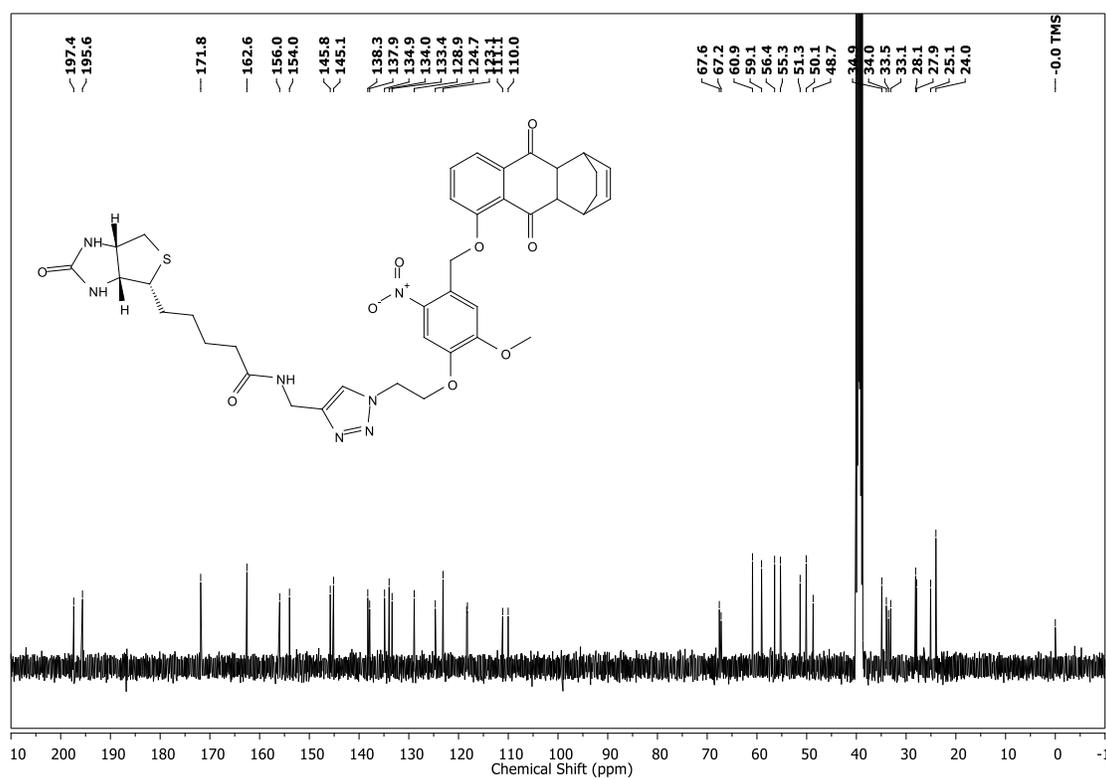
^{13}C -NMR of **4d** (CDCl_3 , 100 MHz):



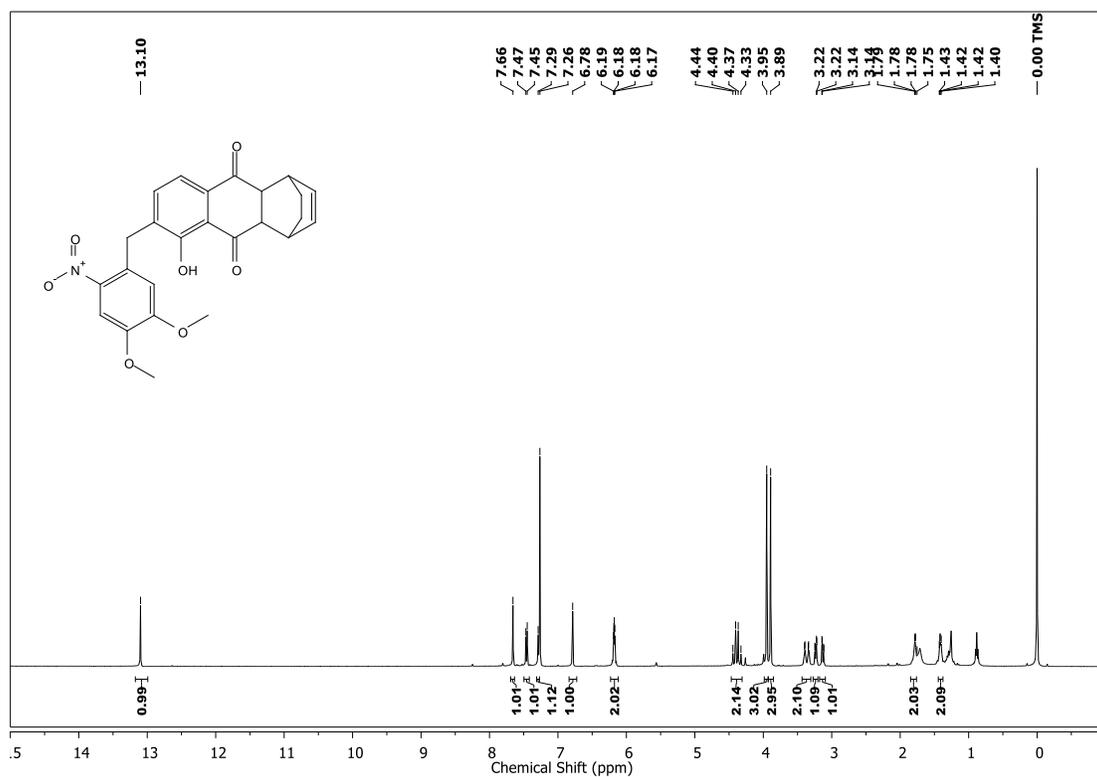
¹H NMR of **4e** (DMSO-*d*₆, 400 MHz):



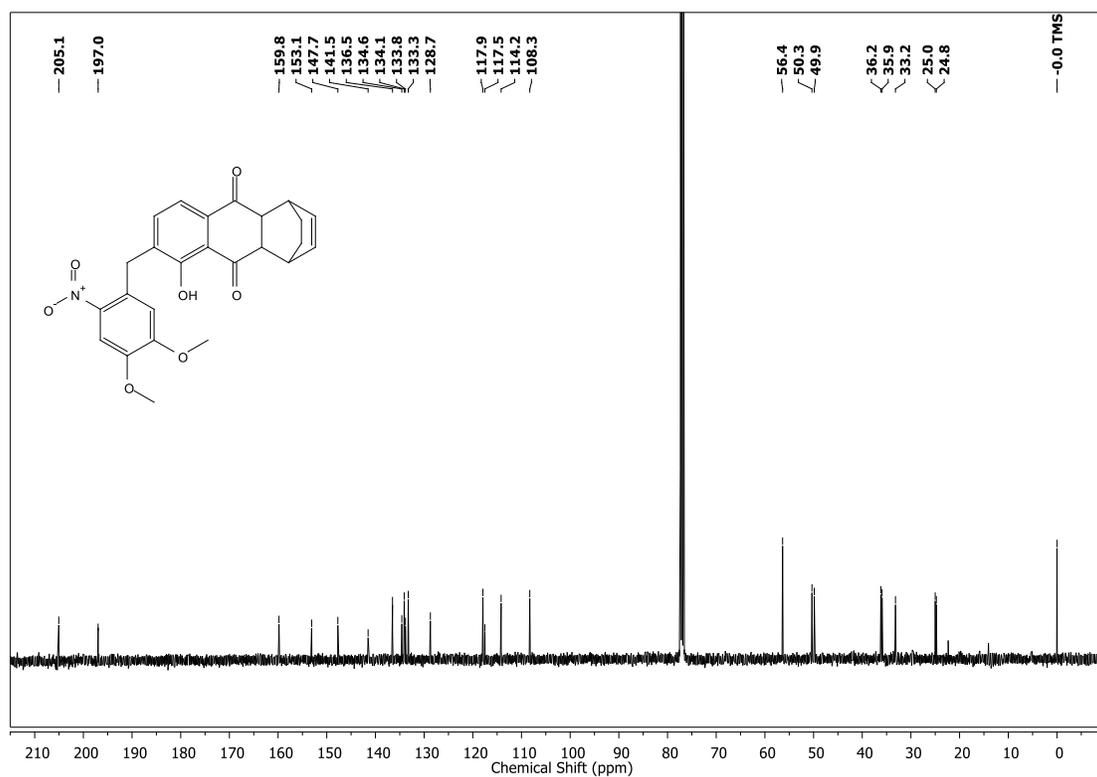
¹³C-NMR of **4e** (DMSO-*d*₆, 100 MHz):



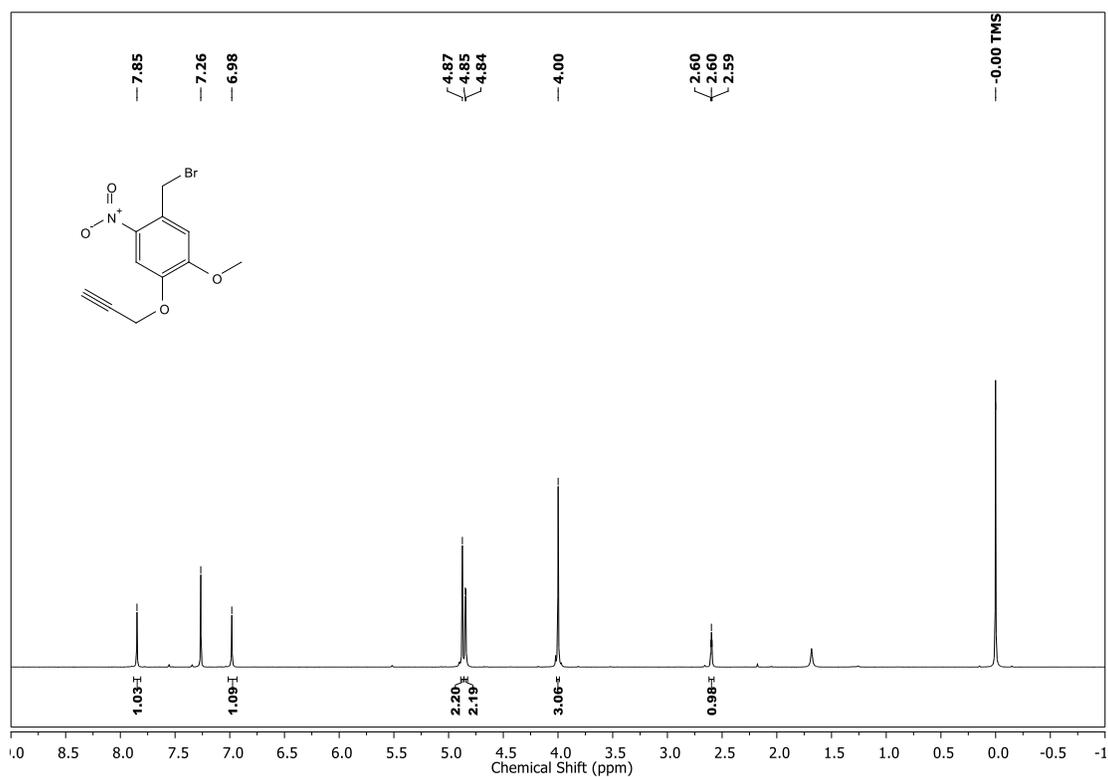
^1H NMR of **6** (CDCl_3 , 400 MHz):



^{13}C NMR of **6** (CDCl_3 , 100 MHz):



^1H NMR of **5c** (CDCl_3 , 400 MHz):



^1H -NMR of **12** ($\text{DMSO}-d_6$, 400 MHz):

