

Appendix A Supporting Information

Discovery of oxochroman-yl-naphthoquinone hybrids as a new chemotype of selective Enterovirus A71 inhibitors

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Materials and methods

Commercial reagents—including salicylaldehydes, the naphthoquinone **2**, and the various solvents have been purchased from Sigma-Aldrich, TCI, Fluorochem, and BLD Pharm and used as received without further purification.

¹H NMR have been recorded on Bruker Ascend-600 (600 MHz for ¹H NMR, 151 MHz for ¹³C NMR, and 564 MHz for ¹⁹F NMR) spectrometer at 300.15 K. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent signals: DMSO-d₆ at 2.50 ppm for ¹H NMR; DMSO-d₆ at 39.5 ppm for ¹³C NMR. Chemical shifts are reported to two decimal places for ¹H and ¹⁹F NMR, and one decimal place for ¹³C NMR. Coupling constants (*J*) are reported in Hertz (Hz) to the nearest 0.1 Hz. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, and combinations. Infrared spectra were recorded on a Nicolet iS10 spectrometer (Thermo Fisher Scientific Inc., Paisley, UK). The main bands are given in cm⁻¹. The main bands are given in cm⁻¹.

High-resolution mass spectrometry (**HRMS**) data have been recorded on a Bruker Daltonics MicroTOF-Q spectrometer equipped with an electrospray ionization (ESI+) source in positive mode, with a tandem Q-TOF analyser.

Melting points (**m.p.**) were determined with a SMP1 Melting Point apparatus (Stuart Scientific, Stone, UK) and they are given uncorrected in °C. All products reported showed ¹H, ¹³C and ¹⁹F NMR spectra in agreement with the assigned structures. All compounds are >95% pure by HPLC analysis. 2-oxo-2*H*-chromene-3-carboxylic acids **1a-p** have been synthesized as previously reported.[1-5] Cell lines were purchased from the American Type Culture Collection (ATCC). Cell lines supporting the multiplication of RNA and DNA viruses were Monkey kidney (Vero-76) ATCC CRL 1587 Cercopithecus Aethiops. The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Viruses were purchased from the American Type Culture Collection (ATCC). A virus representative of positive-sense, single-stranded RNAs (ssRNA+) was Picornaviridae: human enterovirus A71, strain BrCr (ATCC VR-1775), coxsackie type B5 (CVB5), strain Faulkner (ATCC VR-185). The SARS-CoV-2 PV10734, Italian strain (D614G, lineage B.1.1) was kindly provided by the Lazzaro Spallanzani Hospital (Rome, Italy). The virus representative of negative-sense, single-stranded RNAs (ssRNA-) was Pneumoviridae: human respiratory syncytial virus (RSV) strain A2 (ATCC VR-1540). Viruses were maintained in our laboratory and propagated in appropriate cell lines. The viruses were stored in small aliquots at -80 °C until use.

Biological Evaluations

Cytotoxicity Assays

Vero-76 cells were seeded in 96-well plates at an initial density of 5×10^5 cells/mL, in Dulbecco's Modified Eagle Medium (D-MEM) with L-glutamine and 25 mg/L kanamycin, supplemented with 10% FBS. Cell cultures were then incubated at 37 °C in a humidified, 5% CO₂ atmosphere, in the absence or presence of serial dilutions of test compounds. The test medium used for the cytotoxic assay, as well as for the antiviral assay, contained 1% of the appropriate serum. Cell viability was determined after 72-96 hours at 37 °C by the MTT method.

Antiviral Assay

All experimental assays involving viruses were carried out in the Biosafety Level 2 and 3 (BSL-2/3) laboratories (Microbiology and Virology Unit, Cittadella Universitaria di Monserrato). Compound's activity against EV-A71, and RSV was based on the inhibition of virus-induced cytopathogenicity in Vero-76 cells acutely infected with an m.o.i. of 0.01. The compound's activity against Sars-Cov 2 and CVB5 was determined by plaque reduction assays in infected cell monolayers, as described previously.[6-8]

Yield reduction Assay

Vero 76 cells were inoculated with EV-A71 at an m.o.i. of 0.1 in DMEM and tested **3k** at non-cytotoxic concentrations. Following a 2-hour adsorption period at 37°C and 5% CO₂, the inoculum was removed and replaced with fresh medium containing the same concentration of **3k**. After 96 (EV-A71) hours at 37°C and 5% CO₂, viral yields in the culture supernatant were determined by TCID₅₀ titration Vero-76 cells were infected with EV-A71 (m.o.i. 0.1).

Virucidal activity Assay

3k (20 μ M) was incubated with 1×10^5 PFU/mL of EV-A71 at either 4°C or 37°C for 1 hour. The mixture without the test sample was used as the control. Viral yields were determined by endpoint titration (TCID₅₀/ml).

Time of Addition Assay

The confluent monolayers of Vero-76 cells were infected for 1 hour at room temperature with EV-A71 dilutions to give a final m.o.i. of 1. Next, Vero-76 cells were treated with compound **3k** (20 μ M, approximately 10 times higher than the EC₅₀) for 1 hour during the infection period (time 0 or during infection) and at a specific time point. After each incubation period, the monolayers were washed two times with maintenance medium and incubated with fresh medium for 12 hours post-infection. Monolayers obtained from the ToA assay were frozen at -80°C to stop the infection from spreading. Following freeze-thawing, the samples were collected and centrifuged, after which the viral titers were determined by endpoint titration (TCID₅₀/ml).

Statistical Analysis

Cell-based experiments were independently repeated at least three times. The data are reported as mean $\pm$ standard deviation (SD). If not indicated, variation among samples was less than 15%. The statistical significance was calculated with an Ordinary one-way ANOVA performed in GraphPad Prism (San Diego, CA, USA), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Synthesis and Characterization of Compounds 3a-3p

To a solution of coumarin-3-carboxylic acid **1a-1p** (1.0 equiv.) in DMF (0.1 M) were added naphthoquinone **2** (1.0 equiv.) and DMAP (20 mol%). The reaction mixture was stirred at room temperature for 4 hours, before it was diluted with EtOAc, washed twice with saturated aqueous ammonium chloride solution, then extracted three times with EtOAc. The combined organic phases were combined, then dried over Na₂SO₄, and the solvent evaporated under reduced pressure to afford the crude material, which was then purified as specified in each case.

2-Hydroxy-3-(2-oxochroman-4-yl)naphthalene-1,4-dione, 3a. The title compound was obtained following the general procedure from commercially available 2-oxo-2*H*-chromene-3-carboxylic acid **1a** (38 mg, 0.20 mmol) and 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3a** was afforded as a yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in 85% isolated yield (54 mg, 0.17 mmol). M.p. 228-230°C. IR (neat, ν cm⁻¹): 3190, 1769, 1622. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 11.73 (bs, 1H, OH); 7.99 (dd, $J = 7.7, 0.9$ Hz, 1H, Ar); 7.94 (dd, $J = 7.7, 0.8$ Hz, 1H, Ar); 7.83 (dt, $J = 7.6, 1.0$ Hz, 1H, Ar); 7.78 (dt, $J = 7.4, 1.0$ Hz, 1H, Ar); 7.24 (t, $J = 7.8$ Hz, 1H, Ar); 7.16 (d, $J = 7.4$ Hz, 1H, Ar); 7.08 – 7.00 (m, 2H, Ar); 4.86 (dd, $J = 8.3, 5.3$ Hz, 1H, -CH); 3.15 (dd, $J = 16.8, 8.5$ Hz, 1H, CH); 2.95 (dd, $J = 16.8, 5.3$ Hz, 1H, CH). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 183.8, 181.0, 166.7, 156.3, 151.2, 134.6, 133.3, 131.9, 130.0, 128.1 (2C), 125.9, 125.7, 124.0,

123.1, 123.0, 116.4, 32.1, 29.3. HRMS (ESI⁺): m/z calcd for C₁₉H₁₂O₅+H⁺ [M+H]⁺ 321,0757; found 321,0753.

2-(6-Bromo-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3b. The title compound was obtained following the general procedure from commercially available 6-bromo-2-oxo-2*H*-chromene-3-carboxylic acid **1b** (54 mg, 0.20 mmol) and 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3b** was afforded as a yellow solid after flash column chromatography on silica gel (7:3 EtOAc:petroleum ether) in 68% isolated yield (54 mg, 0.14 mmol). M.p. 236-238°C. IR (neat, ν cm⁻¹): 3120, 1770, 1615. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 11.79 (bs, 1H, OH); 8.00 (dd, J = 7.5, 0.9 Hz, 1H, Ar); 7.94 (dd, J = 7.5, 0.89 Hz, 1H, Ar); 7.83 (dt, J = 7.6, 1.4 Hz, 1H, Ar); 7.79 (dt, J = 7.4, 1.3 Hz, 1H, Ar); 7.42 (dd, J = 8.6, 2.3 Hz, 1H, Ar); 7.34 (d, J = 2.1, Hz, 1H, Ar); 7.04 (d, J = 8.7 Hz, 1H, Ar); 4.85 (dd, J = 8.3, 5.8 Hz, 1H, CH); 3.12 (dd, J = 16.5, 8.4 Hz, 1H, CH); 2.95 (dd, J = 16.7, 5.7 Hz, 1H, CH). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 183.8, 181.0, 166.2, 156.8, 150.6, 134.5, 133.2, 132.0, 130.8, 130.4, 130.2, 125.9, 125.8, 125.7, 122.0, 118.7, 115.6, 31.7, 29.3. HRMS (ESI⁺): m/z calcd for C₁₉H₁₁BrO₅+H⁺ [M+H]⁺ 398,9863; found 398,9860.

2-Hydroxy-3-(6-nitro-2-oxochroman-4-yl)naphthalene-1,4-dione, 3c. The title compound was obtained following the general procedure from 6-nitro-2-oxo-2*H*-chromene-3-carboxylic acid (47 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3c** was afforded as a yellow solid after flash column chromatography on silica gel (7:3 EtOAc:petroleum ether) in an isolated yield of 71% (52 mg, 0.14 mmol). M.p. 247-248°C. IR (neat, ν cm⁻¹): 3177, 1782, 1603. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 11.92 (bs, 1H, OH); 8.12 (dd, J = 8.9, 2.4 Hz, 1H, Ar); 8.01 (d, J = 2.2 Hz, 1H, Ar); 7.97 (d, J = 7.5 Hz, 1H, Ar); 7.93 (d, J = 7.6 Hz, 1H, Ar); 7.81 (t, J = 7.3 Hz, 1H, Ar); 7.76 (d, J = 7.4 Hz, 1H, Ar); 7.30 (d, J = 9.0 Hz, 1H, Ar); 4.97 (dd, J = 9.1, 4.0 Hz, 1H, CH); 3.27 (dd, J = 16.9, 8.9 Hz, 1H, CH); 2.94 (dd, J = 17.1, 4.0 Hz, 1H, CH). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 183.3, 181.3, 165.4, 157.8, 156.0, 143.4, 134.5, 133.0, 132.2, 130.3, 125.8, 125.7, 124.8, 124.1, 123.8, 122.1, 117.7, 31.6, 29.3. HRMS (ESI⁺): m/z calcd for C₁₉H₁₁NO₇+H⁺ [M+H]⁺ 366,0608; found 366,0600.

2-(6-Chloro-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3d. The title compound was obtained following the general procedure from 6-chloro-2-oxo-2*H*-chromene-3-carboxylic acid **1d** (45 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3d** was afforded as a pale-yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH) in 72% isolated yield. (52 mg, 0.15 mmol). M.p. 227-229°C. IR (neat, ν cm⁻¹): 3141, 1770, 1611. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 11.80 (bs, 1H, OH); 8.00 (d, J = 7.5 Hz, 1H, Ar); 7.94 (d, J = 7.5 Hz, 1H, Ar); 7.83 (t, J = 7.4 Hz, 1H, Ar); 7.79 (t, J = 7.4 Hz, 1H, Ar); 7.30 (d, J = 8.7 Hz, 1H, Ar); 7.22 (s, 1H, Ar); 7.10 (d, J = 8.6 Hz, 1H, Ar); 4.85 (t, J = 6.8 Hz, 1H, CH); 3.12 (dd, J = 16.8, 8.4 Hz, 1H, CH); 2.97 (dd, J = 16.8, 5.8 Hz, 1H, CH). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 183.9, 180.9, 166.3, 156.6, 150.1, 134.5, 133.2, 131.9, 130.2, 127.9, 127.7, 127.6, 125.9, 125.7, 125.5, 121.9, 118.3, 31.6, 29.3. HRMS (ESI⁺): m/z calcd for C₁₉H₁₁ClO₅+H⁺ [M+H]⁺ 355.0368; found 355.0362.

2-(6-Fluoro-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3e. The title compound was obtained following the general procedure from commercially available 6-fluoro-2-oxo-2*H*-

chromene-3-carboxylic acid **1e** (42 mg, 0.20 mmol) and 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3e** was afforded as a yellow solid after flash column chromatography on silica gel (7:3 EtOAc:petroleum ether) in 81% isolated yield (55 mg, 0.16 mmol). M.p. 241-243°C. IR (neat, ν cm^{-1}): 3133, 1756, 1629. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 11.72 (bs, 1H, OH); 7.98 (d, J = 7.4 Hz, 1H, Ar); 7.94 (d, J = 7.6 Hz, 1H, Ar); 7.82 (t, J = 7.6 Hz, 1H, Ar); 7.77 (t, J = 7.4 Hz, 1H, Ar); 7.11 – 7.06 (m, 2H, Ar); 7.00 (d, J = 9.2 Hz, 1H, Ar); 4.85 (t, J = 7.2 Hz, 1H, CH); 3.03 (ddd, J = 23.5, 16.7, 7.3 Hz, 2H, CH_2). ^{19}F NMR (564 MHz, $\text{DMSO-}d_6$) δ : -119.47 (s). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ : 183.2, 181.4, 166.7, 158.2 (d, J = 241.1 Hz), 157.9 (d, J = 3.5 Hz), 147.5, 134.5, 132.9, 132.3, 130.3, 125.8, 125.7, 125.6, 121.3, 117.9 (d, J = 8.5 Hz), 114.6 (d, J = 23.5 Hz), 114.2 (d, J = 23.9 Hz), 31.7, 29.5. HRMS (ESI+): m/z calcd for $\text{C}_{19}\text{H}_{11}\text{FO}_5 + \text{H}^+$ $[\text{M} + \text{H}]^+$ 339.0663; found 339.0655.

2-(7-Bromo-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3f. The title compound was obtained following the general procedure from commercially available 7-bromo-2-oxo-2H-chromene-3-carboxylic acid **1f** (54 mg, 0.20 mmol) and 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3f** was afforded as a yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in a 75% isolated yield (60 mg, 0.15 mmol). M.p. 231-233°C. IR (neat, ν cm^{-1}): 3178, 1762, 1689. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 11.80 (bs, 1H, OH); 8.00 (d, J = 7.6 Hz, 1H, Ar); 7.94 (d, J = 7.6 Hz, 1H, Ar); 7.83 (t, J = 7.4 Hz, 1H, Ar); 7.79 (t, J = 7.4 Hz, 1H, Ar); 7.32 (d, J = 1.7 Hz, 1H, Ar); 7.23 (dd, J = 8.2, 1.8 Hz, 1H, Ar); 7.11 (d, J = 8.3 Hz, 1H, Ar); 4.82 (dd, J = 8.6, 5.3 Hz, 1H, CH); 3.18 (dd, J = 16.8, 8.6 Hz, 1H, CH); 2.94 (dd, J = 17.0, 5.23 Hz, 1H, CH). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ : 183.9, 180.9, 166.1, 156.3, 152.0, 134.7, 133.3, 131.8, 130.0 (2C), 126.8, 125.9, 125.7, 122.7, 122.6, 119.9, 119.2, 31.7, 29.0. HRMS (ESI+): m/z calcd for $\text{C}_{19}\text{H}_{11}\text{BrO}_5 + \text{Na}^+$ $[\text{M} + \text{Na}]^+$ 420.9682; found 420.9696 $[\text{M} + \text{Na}]^+$.

(2-Hydroxy-3-(7-hydroxy-2-oxochroman-4-yl)naphthalene-1,4-dione, 3g. The title compound was obtained following the general procedure from commercially available 7-hydroxy-2-oxo-2H-chromene-3-carboxylic acid **1g** (42 mg, 0.20 mmol) and 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3g** was afforded as a yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in an 81% isolated yield (54 mg, 0.16 mmol). M.p. 227-229°C. IR (neat, ν cm^{-1}): 3226, 1759, 1685. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 11.61 (bs, 1H, OH); 9.60 (bs, 1H, OH); 7.98 (d, J = 7.4 Hz, 1H); 7.93 (d, J = 7.4 Hz, 1H); 7.83 (t, J = 7.4 Hz, 1H); 7.78 (t, J = 7.4 Hz, 1H); 6.93 (d, J = 8.4 Hz, 1H); 6.46 (dd, J = 8.4, 2.3 Hz, 1H); 6.41 (d, J = 2.3 Hz, 1H); 4.73 (dd, J = 8.5, 4.5 Hz, 1H); 3.12 (dd, J = 16.9, 8.7 Hz, 1H); 2.87 (dd, J = 17.1, 5.4 Hz, 1H, CH). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ : 184.0, 181.1, 166.8, 157.2, 155.9, 151.8, 134.6, 133.3, 131.9, 130.0, 128.7, 125.8, 125.7, 123.7, 113.1, 111.5, 103.0, 32.3, 28.6. HRMS (ESI+): m/z calcd for $\text{C}_{19}\text{H}_{12}\text{O}_6 + \text{Na}^+$ $[\text{M} + \text{Na}]^+$ 359.0526; found 359.0539 $[\text{M} + \text{Na}]^+$.

2-Hydroxy-3-(7-methoxy-2-oxochroman-4-yl)naphthalene-1,4-dione, 3h. The title compound was obtained following the general procedure from 7-methoxy-2-oxo-2H-chromene-3-carboxylic acid **1h** (44 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3h** was afforded as an orange solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in an isolated yield of 61% (43 mg, 0.12 mmol). M.p. 224-225°C. IR (neat, ν cm^{-1}): 3133, 1758, 1618. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 11.68 (bs, 1H); 7.99 (d,

$J = 7.5$ Hz, 1H); 7.93 (d, $J = 7.5$ Hz, 1H); 7.83 (t, $J = 7.6$ Hz, 1H); 7.78 (t, $J = 7.5$ Hz, 1H); 7.04 (d, $J = 8.3$ Hz, 1H); 6.67 – 6.60 (m, 2H); 4.78 (dd, $J = 8.9, 4.5$ Hz, 1H); 3.72 (s, 3H); 3.15 (dd, $J = 16.6, 8.6$ Hz, 1H); 2.90 (dd, $J = 16.8, 4.4$ Hz, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ : 183.9, 181.1, 166.7, 159.1, 156.1, 152.0, 134.6, 133.3, 131.9, 130.0, 128.7, 125.8, 125.7, 123.5, 114.8, 110.4, 101.7, 55.4, 32.2, 28.7. HRMS (ESI+): m/z calcd for $\text{C}_{20}\text{H}_{14}\text{O}_6 + \text{H}^+$ $[\text{M} + \text{H}]^+$ 351.0863; found 351.0852.

2-(7-(Benzyloxy)-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3i. The title compound was obtained following the general procedure from 7-(benzyloxy)-2-oxo-2H-chromene-3-carboxylic acid **1i** (59 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3i** was afforded as a yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH) in 57% isolated yield (49 mg, 0.11 mmol). M.p. 224–226°C. IR (neat, ν cm^{-1}): 3160, 1756, 1634. ^1H NMR (600 MHz, DMSO- d_6) δ : 11.67 (bs, 1H); 7.98 (d, $J = 7.5$ Hz, 1H); 7.93 (d, $J = 7.6$ Hz, 1H); 7.82 (t, $J = 7.5$ Hz, 1H); 7.77 (t, $J = 7.3$ Hz, 1H); 7.45 – 7.41 (m, 2H); 7.38 (t, $J = 7.3$ Hz, 2H); 7.32 (t, $J = 7.3$ Hz, 1H); 7.05 (d, $J = 8.3$ Hz, 1H); 6.73 – 6.67 (m, 2H); 5.08 (s, 2H); 4.78 (dd, $J = 8.7, 4.7$ Hz, 1H); 3.13 (dd, $J = 16.9, 8.6$ Hz, 1H); 2.90 (dd, $J = 17.0, 4.7$ Hz, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ : 183.7, 181.2, 166.7, 158.1, 156.5, 151.9, 136.8, 134.6, 133.1, 132.0, 130.1, 128.8 (2C), 128.4, 127.8, 127.6 (2C), 125.8, 125.7, 123.3, 115.2, 111.1, 102.6, 69.4, 32.2, 28.7. HRMS (ESI+): m/z calcd for $\text{C}_{26}\text{H}_{18}\text{O}_6\text{Na}$ $[\text{M} + \text{Na}]^+$: 449.0996; found: 449.0994.

2-Hydroxy-3-(2-oxo-7-(prop-2-yn-1-yloxy)chroman-4-yl)naphthalene-1,4-dione, 3j. The title compound was obtained following the general procedure from 2-oxo-7-(prop-2-yn-1-yloxy)-2H-chromene-3-carboxylic acid **1j** (49 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3j** was afforded as an orange solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in an isolated yield of 77% (58 mg, 0.15 mmol). M.p. 230–232°C. IR (neat, ν cm^{-1}): 3138, 1764, 1610. ^1H NMR (600 MHz, DMSO- d_6) δ : 11.68 (bs, 1H, OH); 7.99 (d, $J = 7.6$ Hz, 1H, Ar); 7.94 (d, $J = 7.6$ Hz, 1H, Ar); 7.83 (t, $J = 7.4$ Hz, 1H, Ar); 7.79 (t, $J = 7.5$ Hz, 1H, Ar); 7.07 (d, $J = 8.4$ Hz, 1H, Ar); 6.71 (m, 1H, Ar, Ar), 6.68 (d, $J = 8.5$ Hz, 1H, Ar); 4.78 (m, 3H, CH_2 CH); 3.56 (m, 1H, CH); 3.16 (dd, $J = 16.8, 8.7$ Hz, 1H); 2.90 (dd, $J = 16.9, 4.3$ Hz, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ : 184.5, 181.5, 167.1, 157.4, 156.4, 152.3, 135.1, 133.8, 132.3, 130.5, 129.3, 126.3, 126.2, 123.9, 116.1, 111.7, 103.2, 79.5, 78.9, 56.1, 32.6, 29.2. HRMS (ESI+): m/z calcd for $\text{C}_{22}\text{H}_{14}\text{O}_6 + \text{H}^+$ $[\text{M} + \text{H}]^+$ 374.0790; found 397.0683 $[\text{M} + \text{Na}]^+$.

2-Hydroxy-3-(2-oxo-7-(prop-2-yn-1-yloxy)chroman-4-yl)naphthalene-1,4-dione, 3k. The title compound was obtained following the general procedure from 3-oxo-3H-benzo[*f*]chromene-2-carboxylic acid **1k** (48 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3k** was afforded as an orange solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in an isolated yield of 48% (36 mg, 0.096 mmol). M.p. 239–241°C. IR (neat, ν cm^{-1}): 3137, 1766, 1628. ^1H NMR (600 MHz, DMSO- d_6) δ : 12.01 (bs, 1H, OH); 7.91 (m, 5H, Ar); 7.77 (t, $J = 7.6$ Hz, 1H, Ar); 7.72 (t, $J = 7.5$ Hz, 1H, Ar); 7.48 (t, $J = 7.7$ Hz, 1H, Ar); 7.40 (t, $J = 7.3$ Hz, 1H, Ar); 7.29 (d, $J = 8.9$ Hz, 1H, Ar); 5.30 (d, $J = 9.7$ Hz, 1H, CH); 3.49 (dd, $J = 16.6, 9.8$ Hz, 1H, Ar); 2.83 (d, $J = 16.6$ Hz, 1H, Ar). ^{13}C NMR (151 MHz, DMSO- d_6) δ : 184.4, 181.3, 166.9, 156.3, 150.5, 135.2, 133.8, 132.2, 131.9, 130.7, 130.3, 129.7, 129.1, 127.7, 126.4, 126.2,

125.1, 124.4, 123.3, 118.1, 115.4, 33.5, 27.8. HRMS (ESI⁺): m/z calcd for C₂₃H₁₄O₅+H⁺ [M+H]⁺ 370.0841; found 393.0750 [M+Na]⁺.

2-(8-Ethoxy-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3l. The title compound was obtained following the general procedure from 8-ethoxy-2-oxo-2*H*-chromene-3-carboxylic acid **1l** (47 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3l** was afforded as a yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in 73% isolated yield (53 mg, 0.15 mmol). M.p. 236-235°C. IR (neat, ν cm⁻¹): 3162, 1760, 1620. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 11.71 (bs, 1H, OH); 7.99 (d, J = 7.5 Hz, 1H, Ar); 7.94 (d, J = 7.5 Hz, 1H, Ar); 7.83 (t, J = 7.3 Hz, 1H, Ar); 7.78 (t, J = 7.35 Hz, 1H, Ar); 6.96 – 6.91 (m, 2H, Ar); 6.70 (d, J = 7.0 Hz, 1H, Ar); 4.86 – 4.81 (m, 1H, CH); 4.10 – 4.03 (m, 2H, CH₂); 3.14 (dd, J = 16.5, 8.3 Hz, 1H, CH); 2.92 (dd, J = 16.8, 5.0 Hz, 1H, CH); 1.38 (t, J = 6.8, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 183.9, 180.9, 166.5, 156.1, 146.2, 140.5, 134.7, 133.3, 131.8, 130.0, 125.9, 125.7, 123.8, 123.8, 123.1, 119.2, 111.8, 63.9, 31.9, 29.5, 14.7. HRMS (ESI⁺): m/z calcd for C₂₁H₁₆O₆+H⁺ [M+H]⁺ 365,1020; found 365,1018.

2-(6,8-Dibromo-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3m. The title compound was obtained following the general procedure from 6,8-dibromo-2-oxo-2*H*-chromene-3-carboxylic acid **1m** (70 mg, 0.20 mmol) and 2-hydroxynaphthalene-1,4-dione (35 mg, 0.20 mmol) **2**. Product **3m** was afforded as an orange solid after flash column chromatography on silica gel (7:3 EtOAc:petroleum ether) in 63% isolated yield (61 mg, 0.13 mmol). M.p. 240-241°C. IR (neat, ν cm⁻¹): 3177, 1762, 1617. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 7.88 (d, J = 7.8 Hz, 1H, Ar); 7.79 (d, J = 7.5 Hz, 1H, Ar); 7.69 (t, J = 7.6 Hz, 1H, Ar); 7.57 (t, J = 7.4 Hz, 1H, Ar); 7.41 (m, 2H, Ar); 4.88 (t, J = 7.5 Hz, 1H, CH); 3.43 (dd, J = 16.2, 8.4 Hz, 1H, CH); 3.19 (dd, J = 16.4, 6.8 Hz, 1H, CH). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 184.8, 180.4, 172.5 (2C), 153.0, 134.4 (2C), 134.1, 133.8, 131.5, 131.1, 130.9, 125.2, 125.0, 119.5, 111.9, 108.7, 34.8, 20.7. HRMS (ESI⁺): m/z calcd for C₁₉H₁₀Br₂O₅+H⁺ [M+H]⁺ 476.8968; found 476.9010.

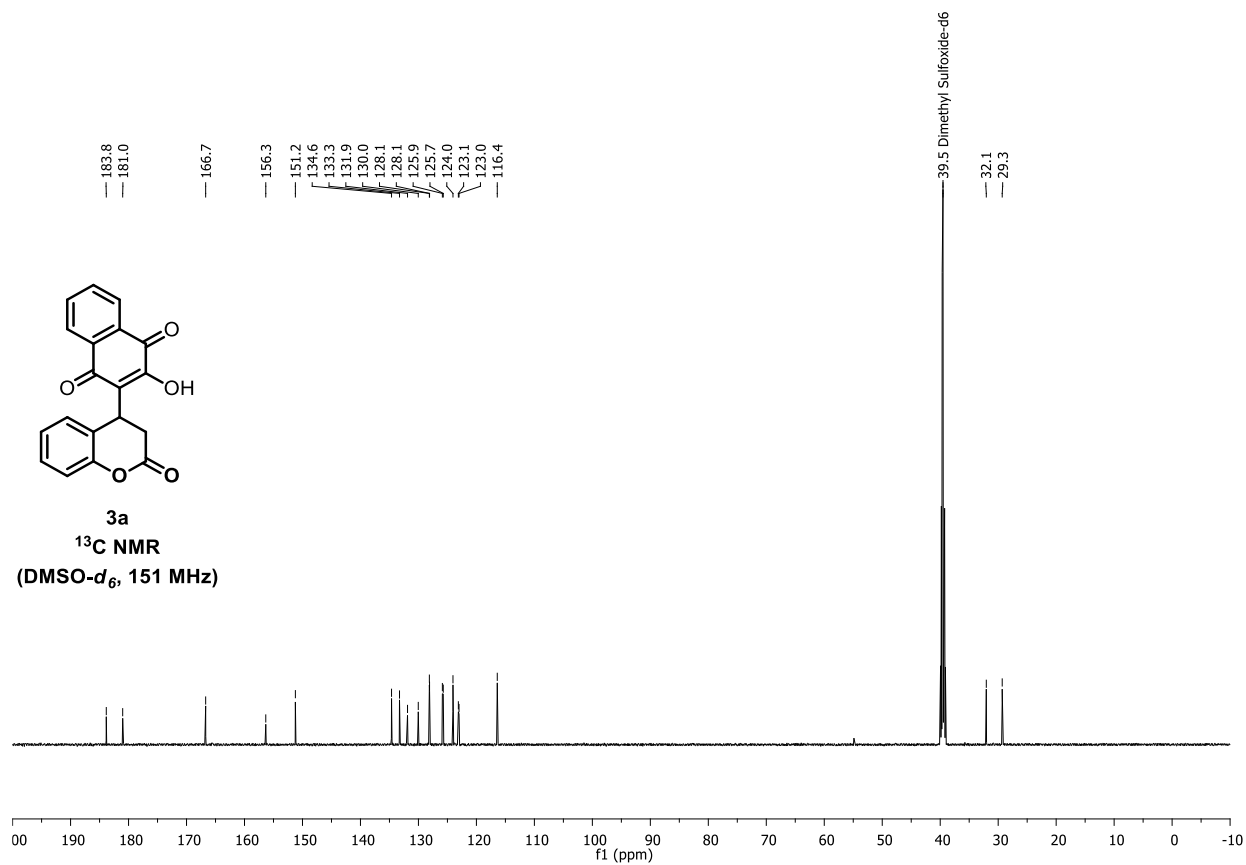
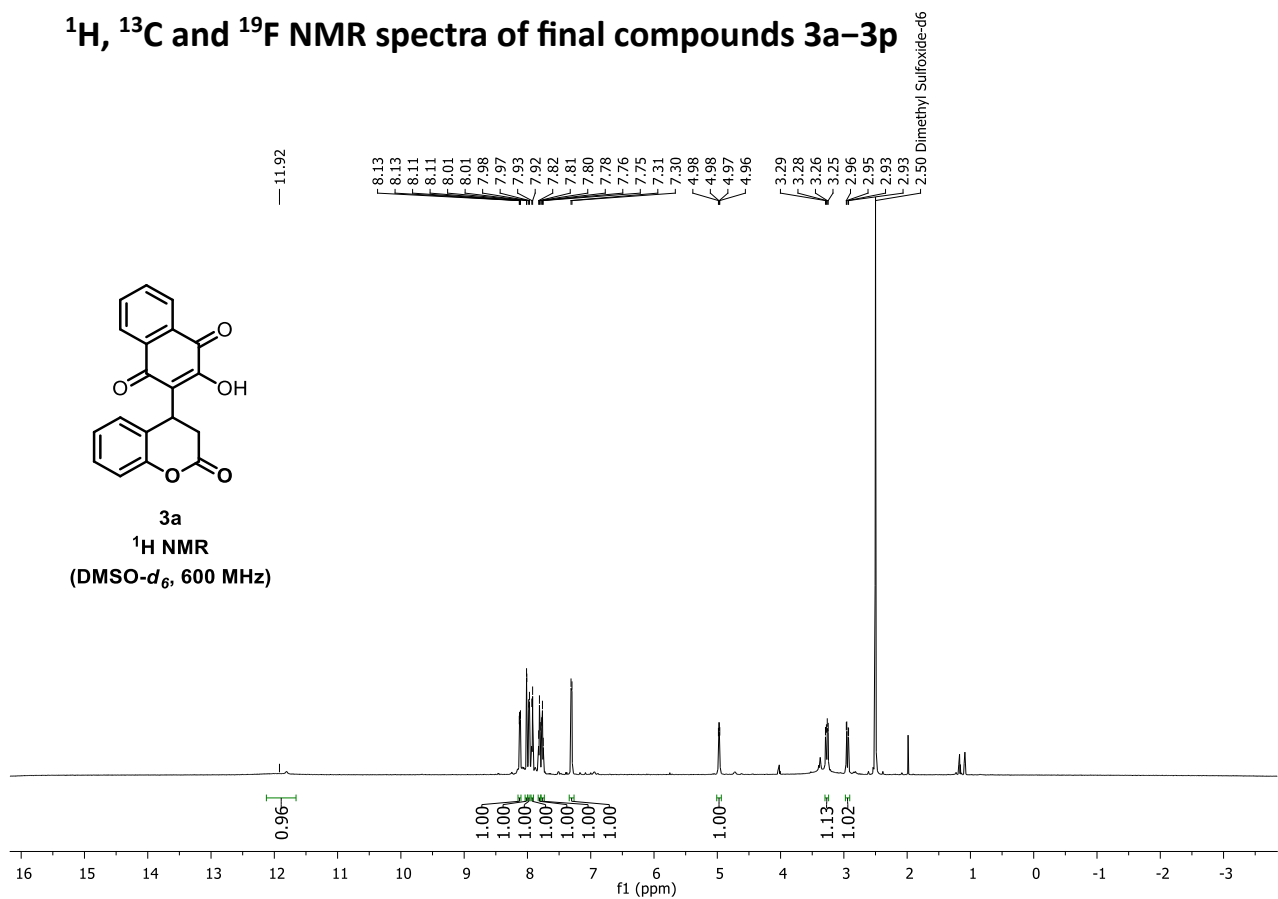
2-(6,8-Diiodo-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3n. The title compound was obtained following the general procedure from 6,8-diiodo-2-oxo-2*H*-chromene-3-carboxylic acid **1n** (88 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione **2** (35 mg, 0.20 mmol). Product **3n** was afforded as a pale-yellow solid after flash column chromatography on silica gel (98:2 DCM:MeOH) in 82% isolated yield (94 mg, 0.16 mmol). M.p. 242-244°C. IR (neat, ν cm⁻¹): 3169, 1749, 1622. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 11.82 (bs, 1H, OH); 8.01 (d, J = 7.7 Hz, 1H, Ar); 7.98 (d, J = 7.5 Hz, 1H, Ar); 7.93 (d, J = 7.6 Hz, 1H, Ar); 7.82 (t, J = 7.5 Hz, 1H, Ar); 7.77 (t, J = 7.6 Hz, 1H, Ar); 7.46 – 7.43 (m, 1H, Ar); 4.82 (dd, J = 8.5, 5.1 Hz, 1H, CH); 3.14 (dd, J = 16.5, 8.5 Hz, 1H, CH); 2.91 (dd, J = 16.5, 5.3 Hz, 1H, CH). ¹³C NMR (151 MHz, DMSO-*d*₆) δ : 183.4, 181.2, 165.8, 150.7, 144.4, 136.5 (2C), 134.5, 133.0, 132.2, 130.3, 127.0, 125.8, 125.7, 121.8, 88.7, 87.1, 32.0, 29.6. HRMS (ESI⁺): m/z calcd for C₁₉H₁₀I₂O₅+H⁺ [M+H]⁺: 572.8690; found: 572.8685.

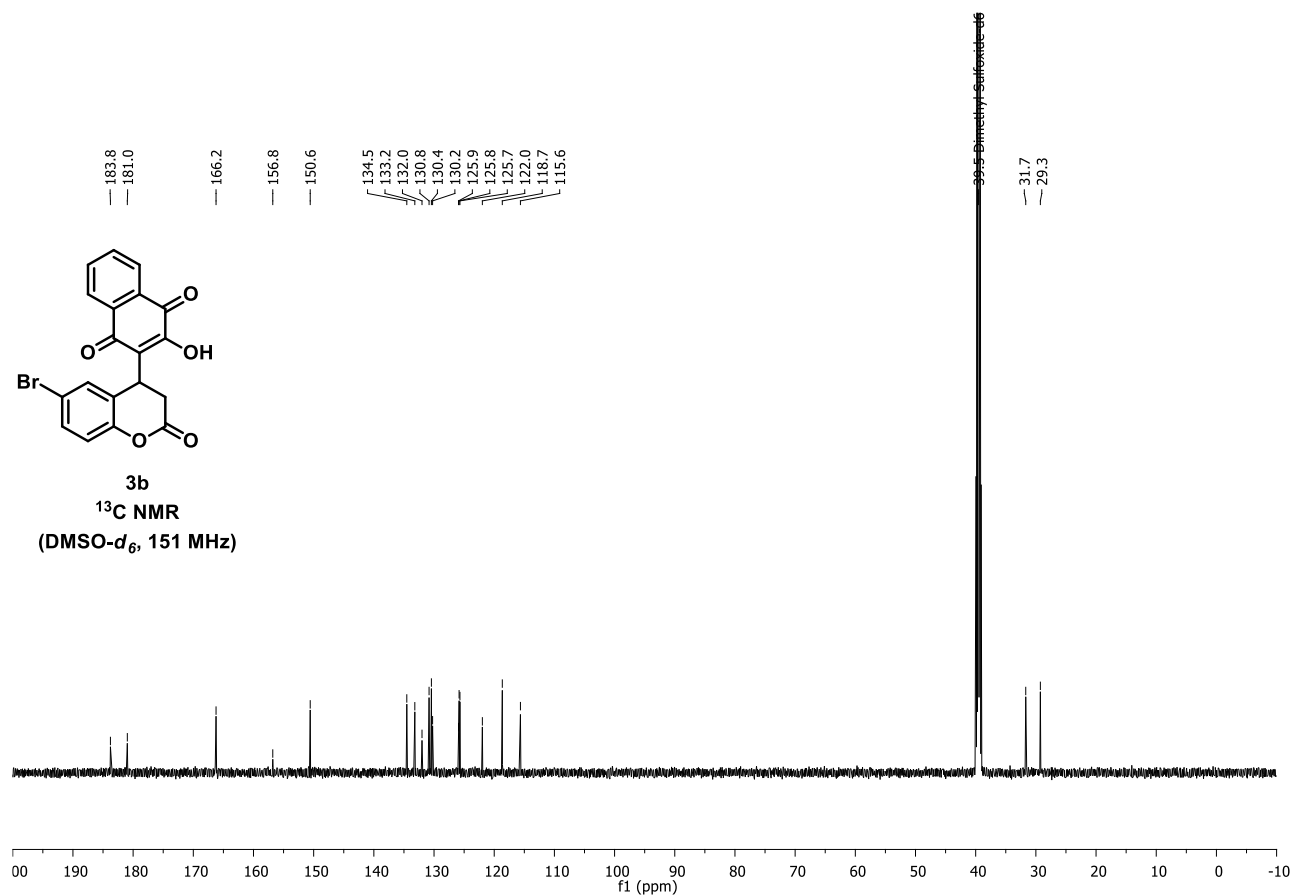
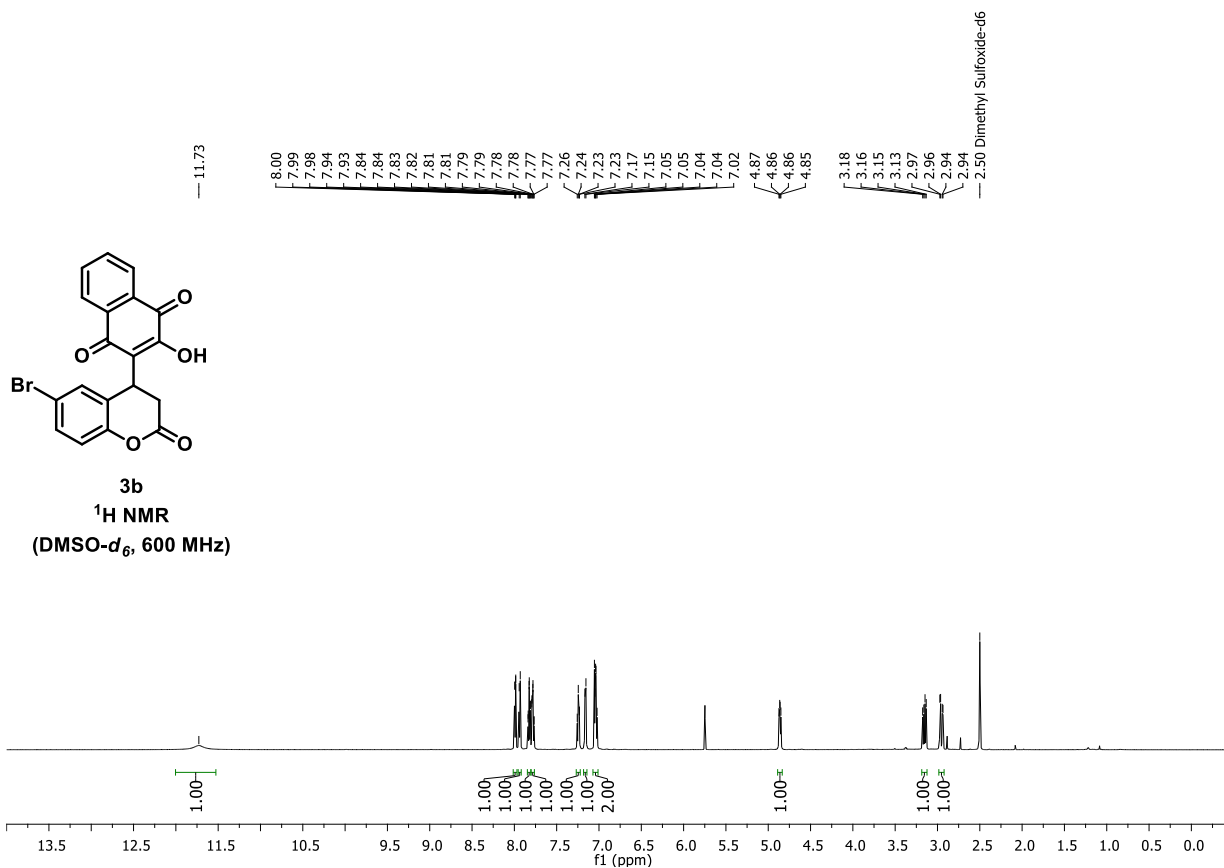
2-Hydroxy-3-(6-(4-methoxyphenyl)-2-oxochroman-4-yl)naphthalene-1,4-dione, 3o. The title compound was obtained following the general procedure from 6-chloro-2-oxo-2*H*-chromene-3-carboxylic acid **1o** (45 mg, 0.20 mmol) and commercially available 2-hydroxy-5-

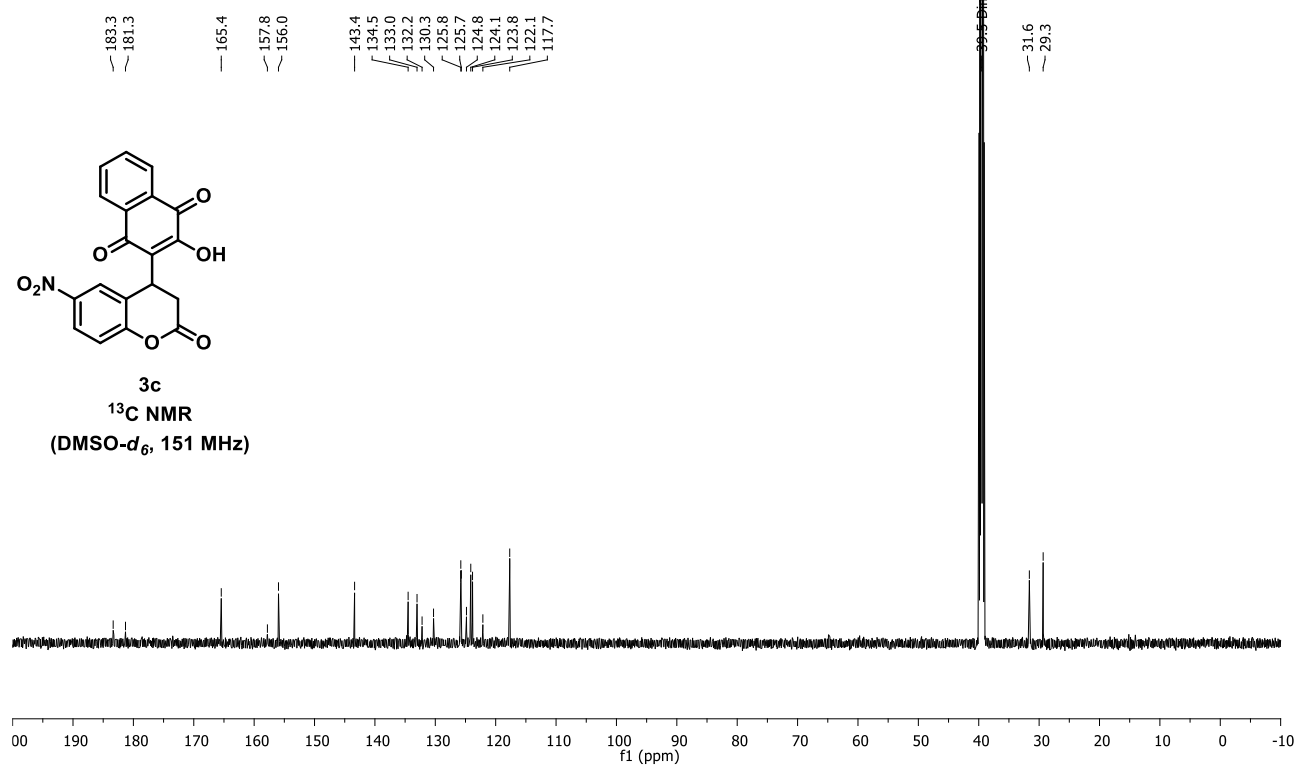
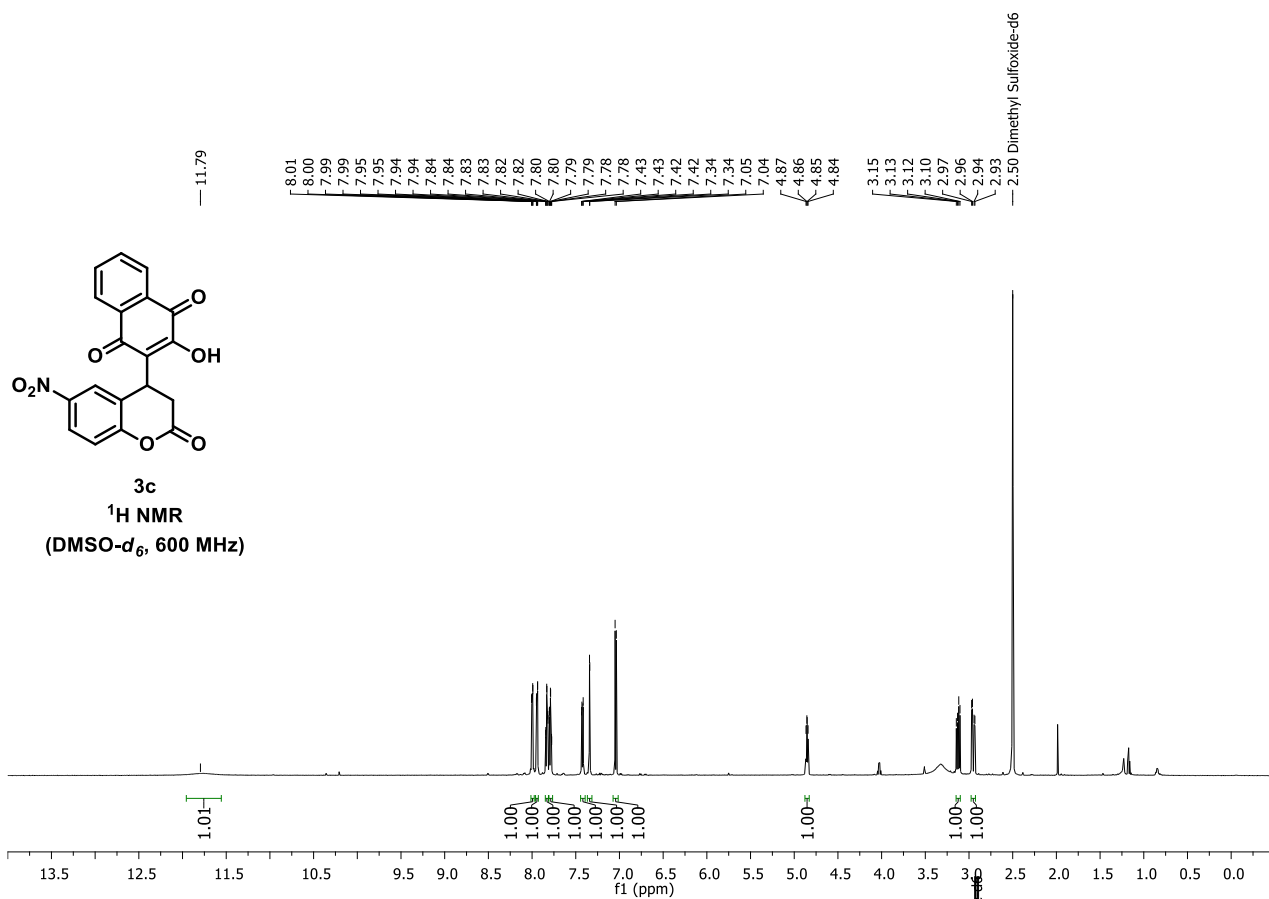
methoxynaphthalene-1,4-dione **2** (41 mg, 0.20 mmol). Product **3o** was afforded as a yellow solid after flash column chromatography on silica gel (7:3 EtOAc:petroleum ether) in 57% isolated yield (45 mg, 0.11 mmol). M.p. 251-252°C. IR (neat, ν cm^{-1}): 3137, 1770, 1717, 1626 cm^{-1} . ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 11.70 (bs, 1H, OH); 7.99 (d, J = 7.3 Hz, 1H, Ar); 7.94 (d, J = 7.5 Hz, 1H, Ar); 7.82 (t, J = 7.3 Hz, 1H, Ar); 7.78 (t, J = 7.5 Hz, 1H, Ar); 7.49 – 7.44 (m, 3H, Ar); 7.36 – 7.33 (m, 1H, Ar); 7.11 (d, J = 8.4 Hz, 1H, Ar); 6.94 (d, J = 8.7 Hz, 2H, Ar); 4.92 (dd, J = 8.6, 4.9 Hz, 1H, CH); 3.74 (s, 3H, OCH_3); 3.19 (dd, J = 16.9, 8.7 Hz, 1H, CH); 2.93 (dd, J = 16.7, 4.9 Hz, 1H, CH). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ : 184.0, 181.0, 166.7, 158.7, 156.4, 150.3, 135.9, 134.5, 133.2, 132.0, 131.7, 130.2, 127.6 (2C), 126.0 (2C), 125.8, 125.7, 123.4, 123.0, 116.8, 114.3 (2C), 55.1, 32.1, 29.5. HRMS (ESI⁺): m/z for $\text{C}_{26}\text{H}_{18}\text{O}_6 + \text{Na}^+ [\text{M} + \text{Na}]^+$ 449.0996; found 449.1009.

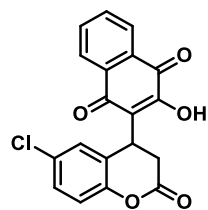
2-(7-(2,5-Dimethoxyphenyl)-2-oxochroman-4-yl)-3-hydroxynaphthalene-1,4-dione, 3p. The title compound was obtained following the general procedure 7-(2,6-dimethoxyphenyl)-2-oxo-2H-chromene-3-carboxylic acid **1p** (65 mg, 0.20 mmol) and commercially available 2-hydroxynaphthalene-1,4-dione (35 mg, 0.20 mmol). Product **3p** was afforded as an orange solid after flash column chromatography on silica gel (98:2 DCM:MeOH to 95:5 DCM:MeOH) in 45% isolated yield (41 mg, 0.09 mmol). M.p. 237-239°C. IR (neat, ν cm^{-1}): 3156, 1761, 1614. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 11.77 (bs, 1H, OH); 8.00 (d, J = 7.58 Hz, 1H, Ar); 7.96 (d, J = 7.51 Hz, 1H, Ar); 7.84 (dt, J = 7.47 1.31 Hz, 1H, Ar); 7.79 (dt, J = 7.53 1.18 Hz, 1H, Ar); 7.17 (m, 3H, Ar); 7.03 (d, J = 8.98 Hz, 1H, Ar); 6.90 (dd, J = 8.96 3.17 Hz, 1H, Ar); 6.85 (d, J = 3.11 Hz, 1H, Ar); 4.89 (dd, J = 8.65 5.11 Hz, 1H, CH); 3.73 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3); 3.18 (dd, J = 16.56 8.55 Hz, 1H, CH); 2.97 (dd, J = 16.76 5.15 Hz, 1H, CH). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ : 183.9, 181.1, 166.7, 156.5, 153.3, 150.8, 150.2, 138.0, 134.6, 133.3, 131.9, 130.1, 129.3, 127.7, 125.9, 125.7, 125.0, 123.0, 121.8, 117.0, 115.8, 113.7, 113.0, 56.0, 55.4, 32.1, 29.2. HRMS (ESI⁺): m/z calcd for $\text{C}_{27}\text{H}_{20}\text{O}_7 + \text{H}^+ [\text{M} + \text{H}]^+$ 457.1282; found 457.1265.

^1H , ^{13}C and ^{19}F NMR spectra of final compounds 3a–3p

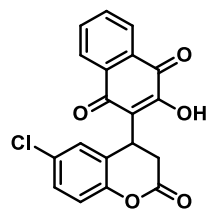




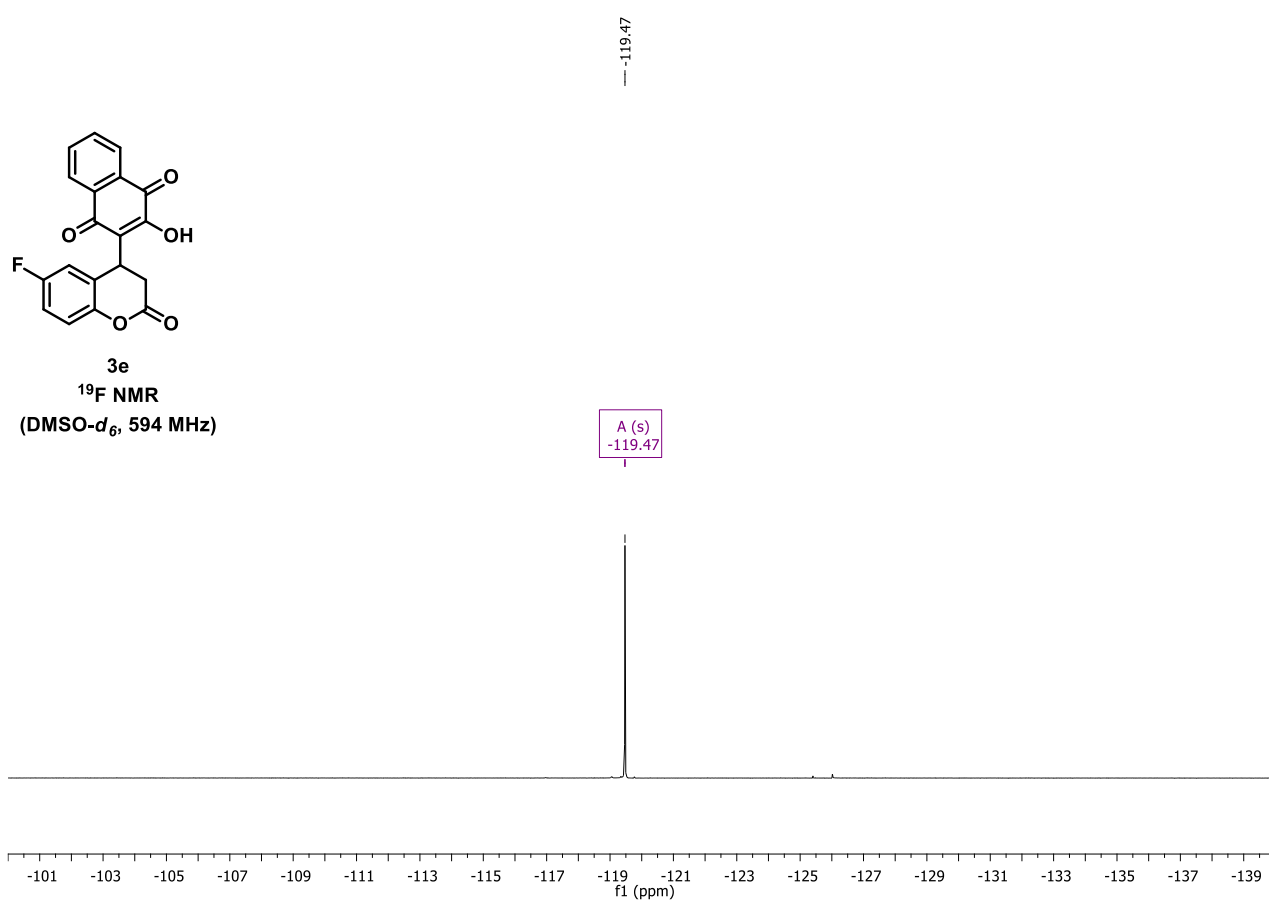
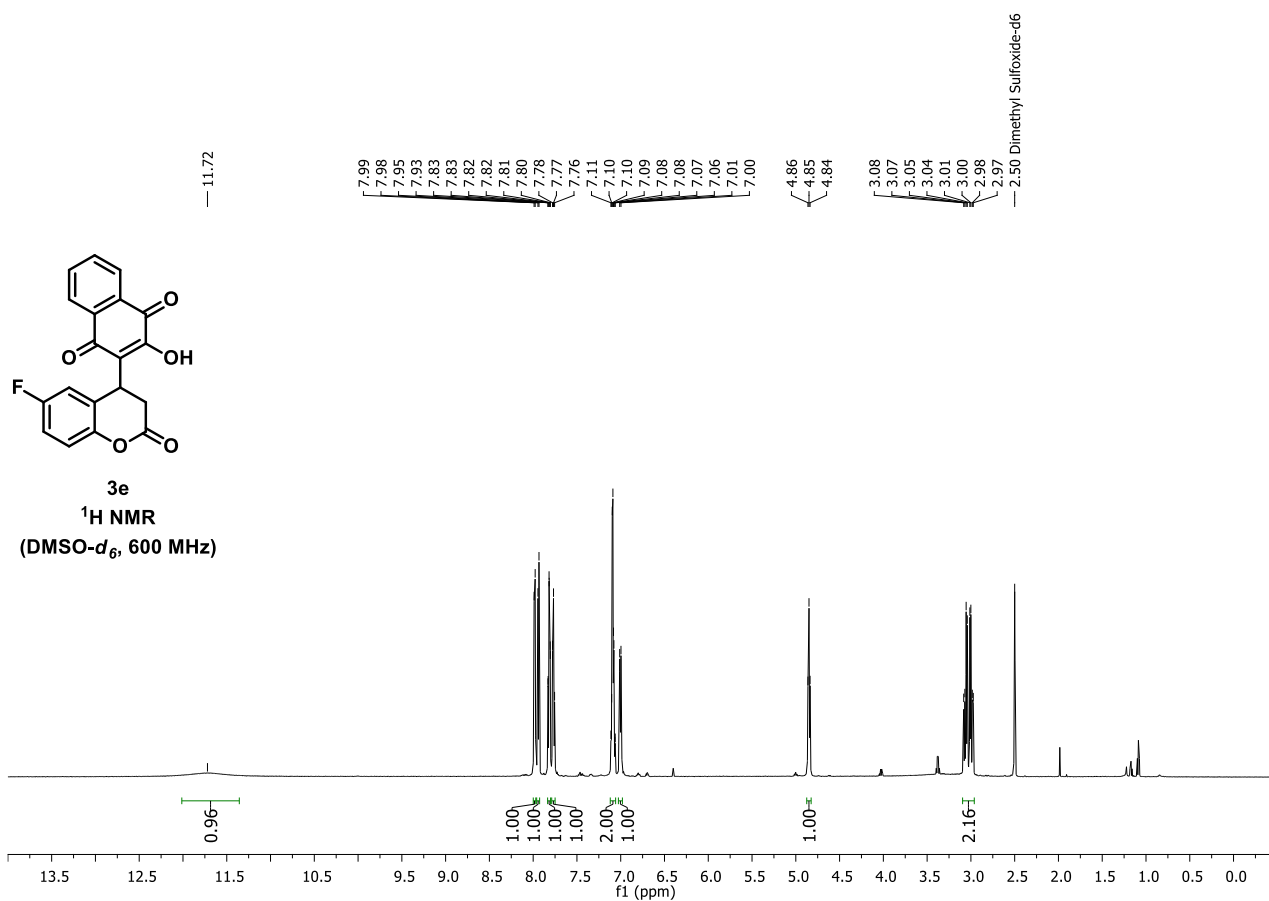


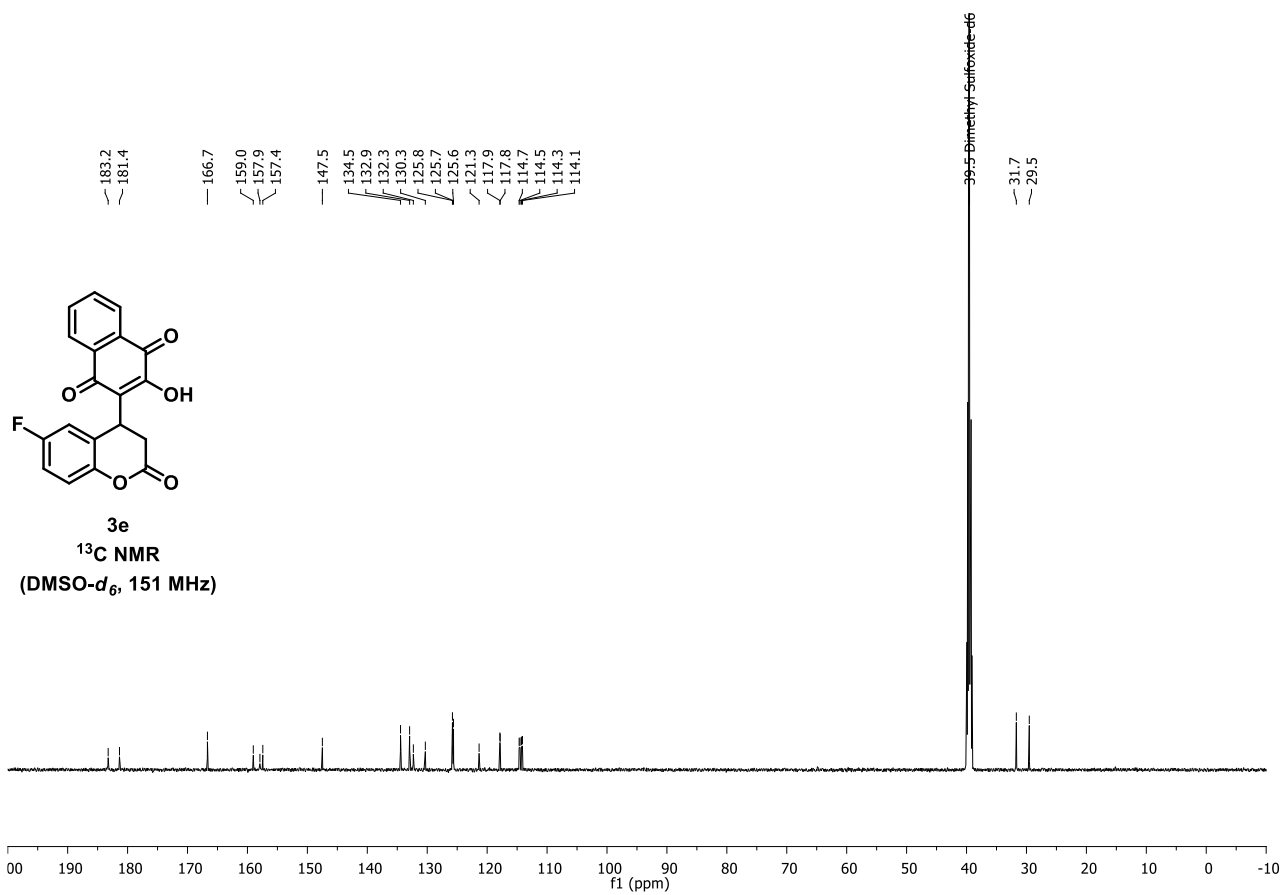


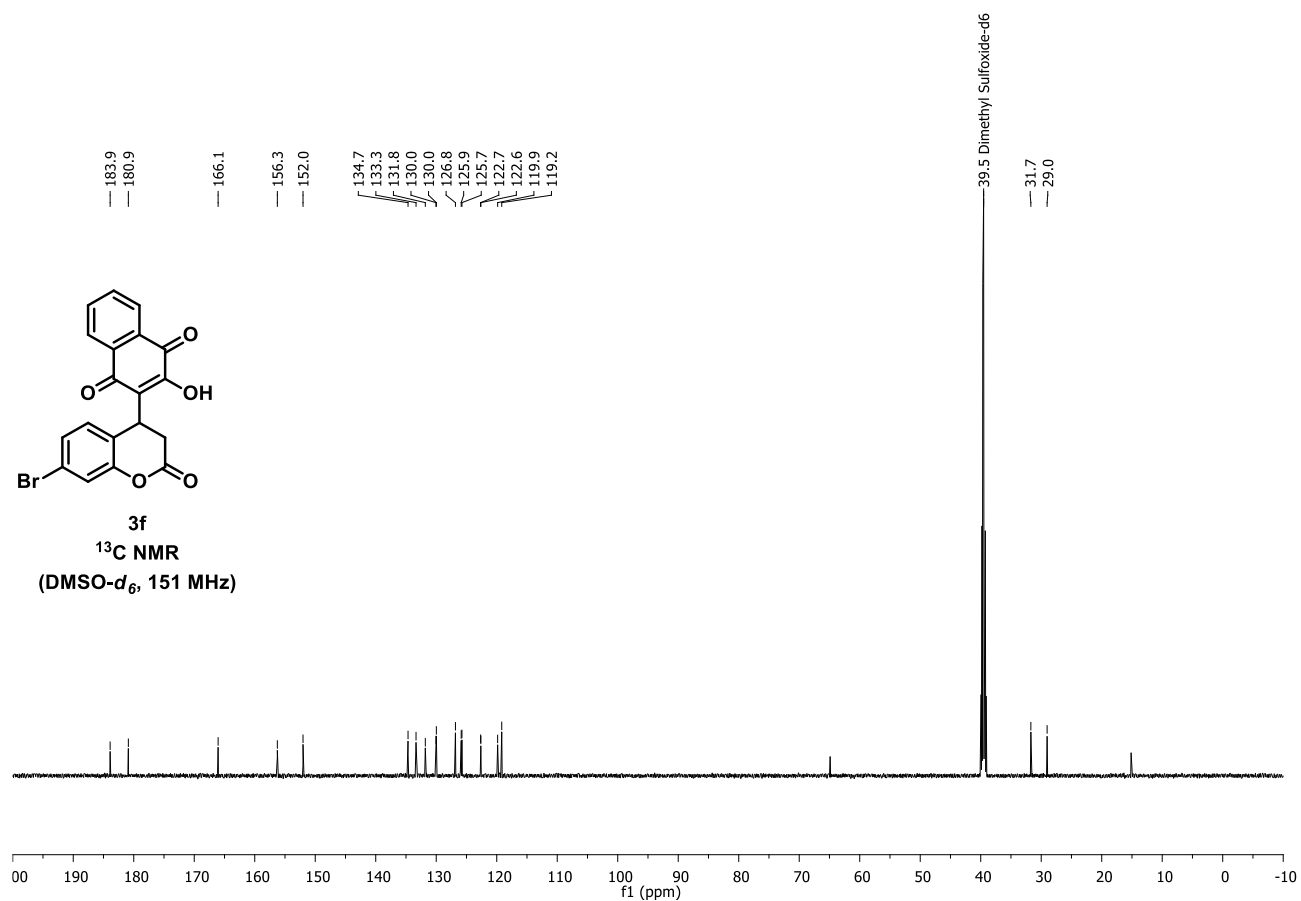
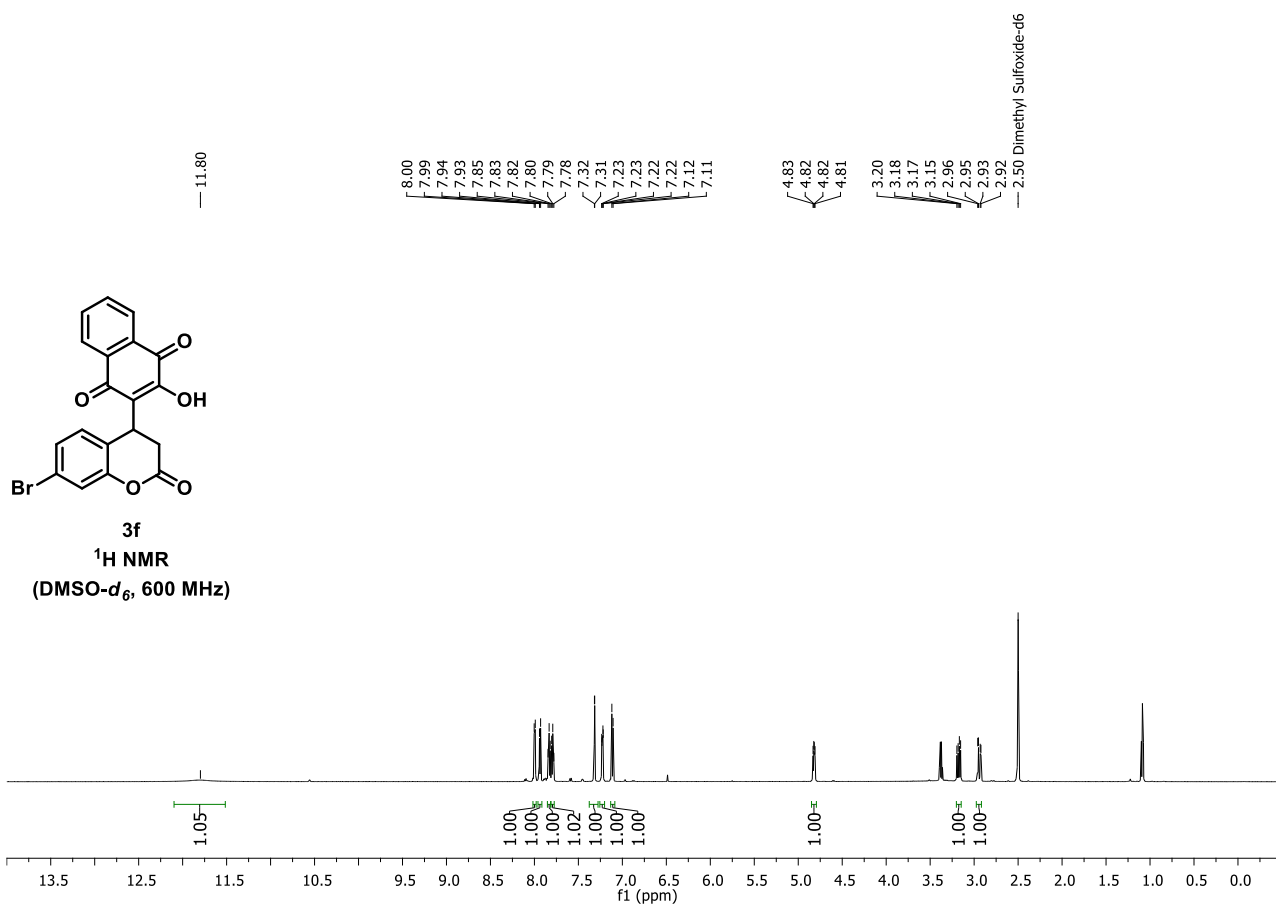
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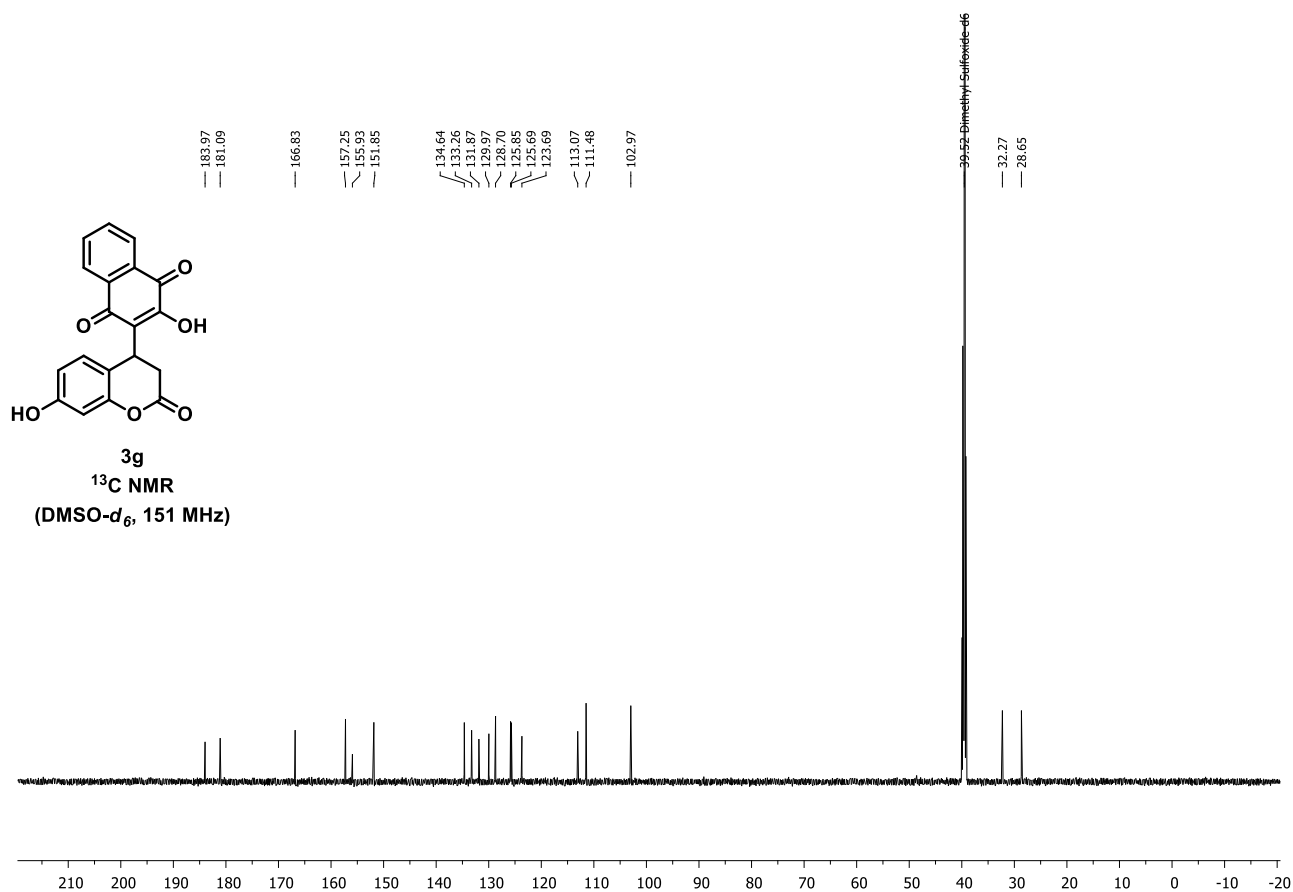
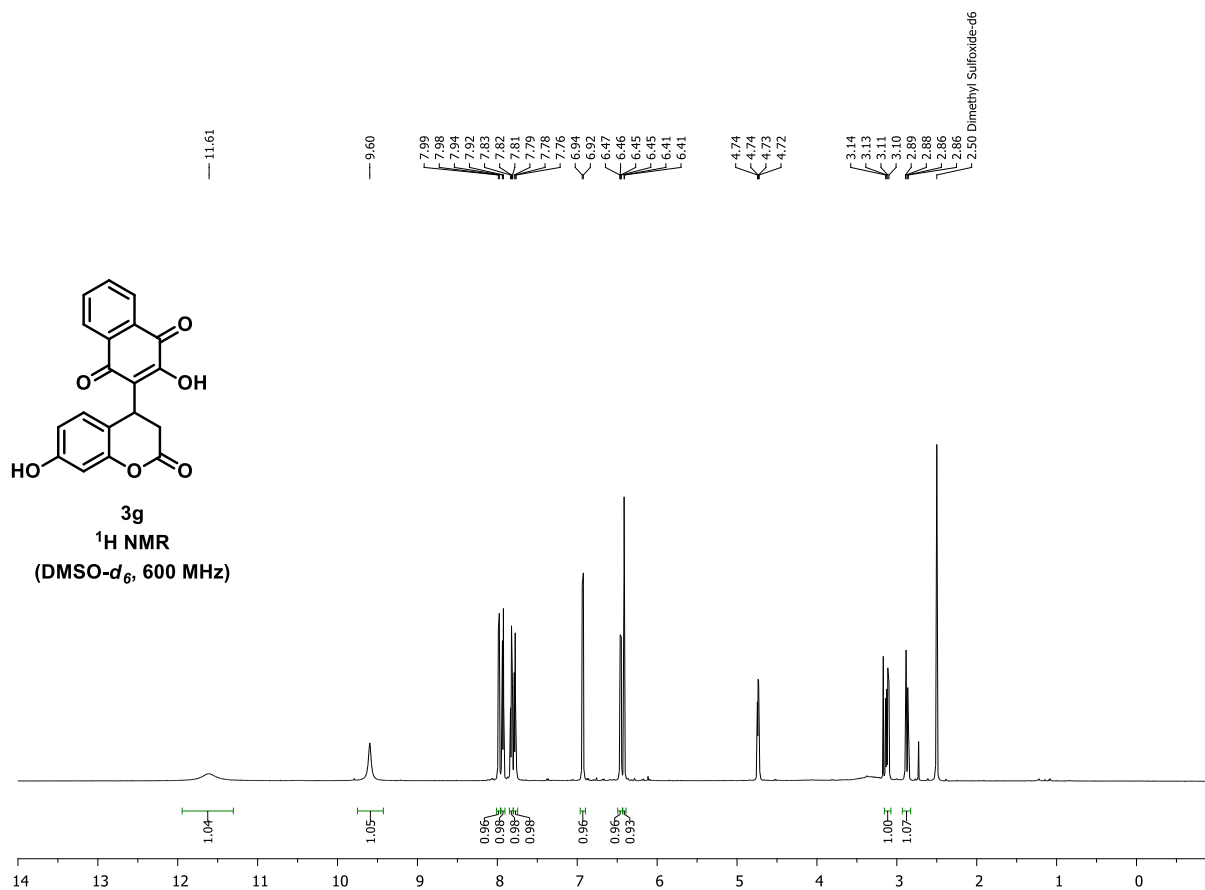


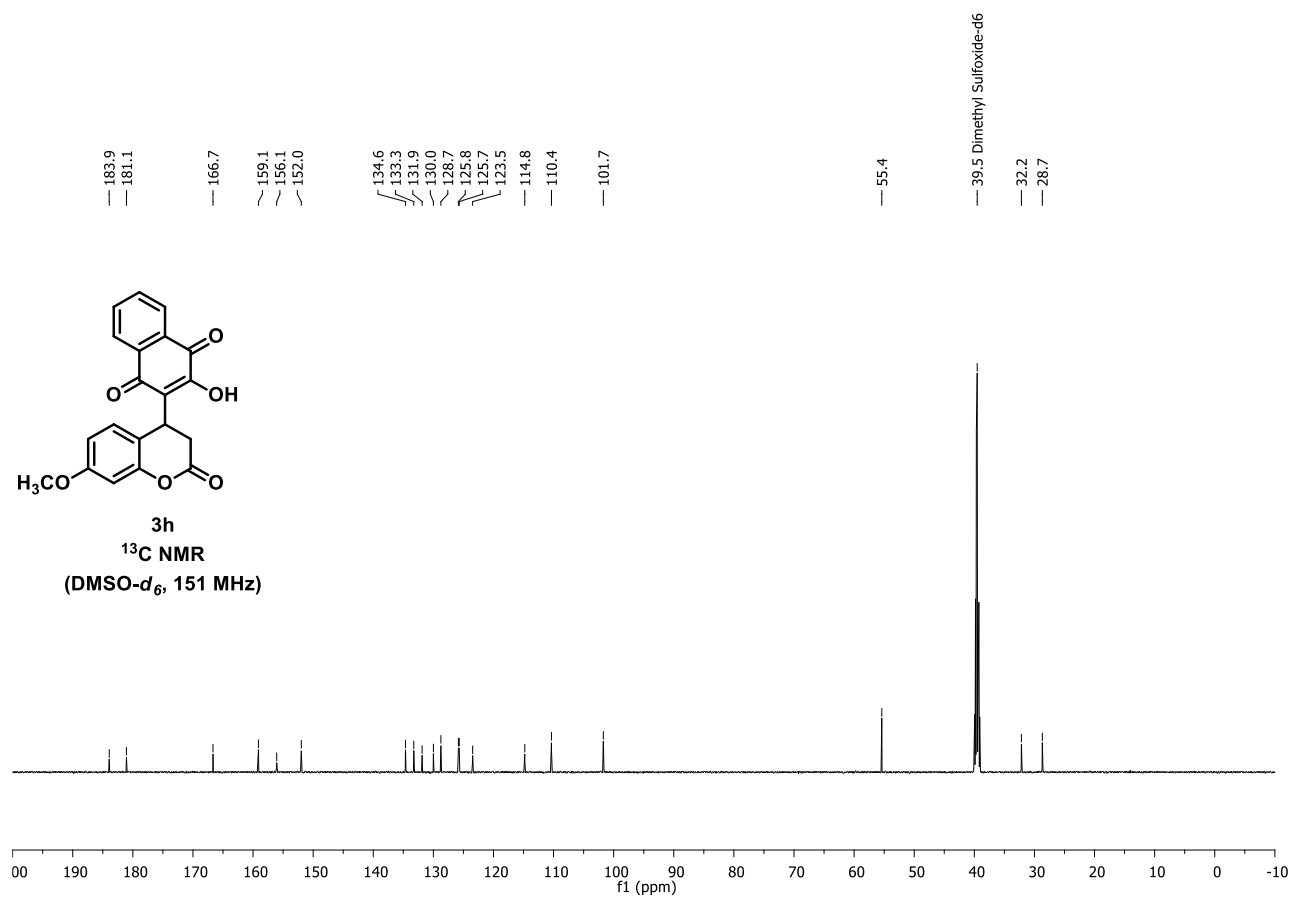
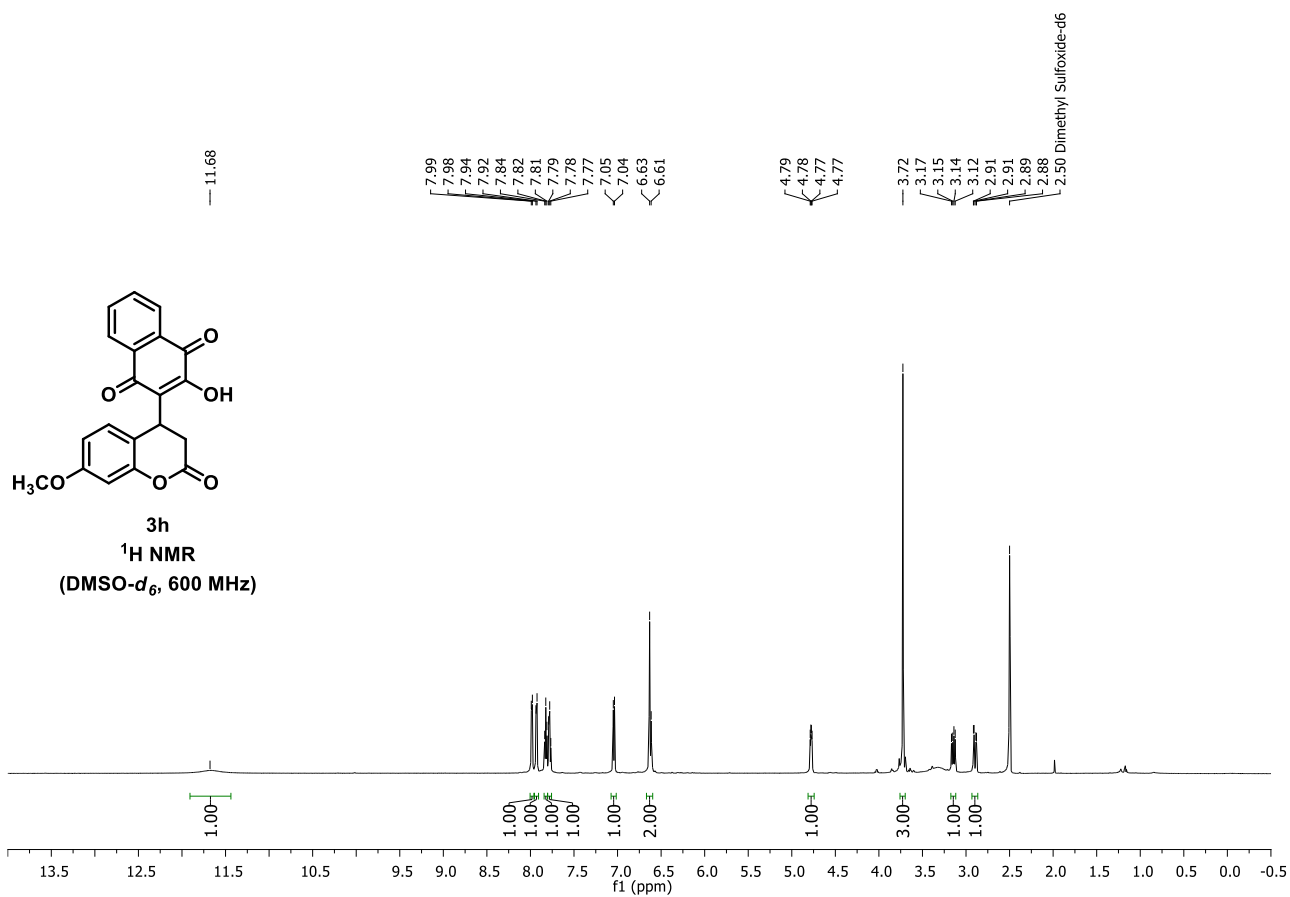
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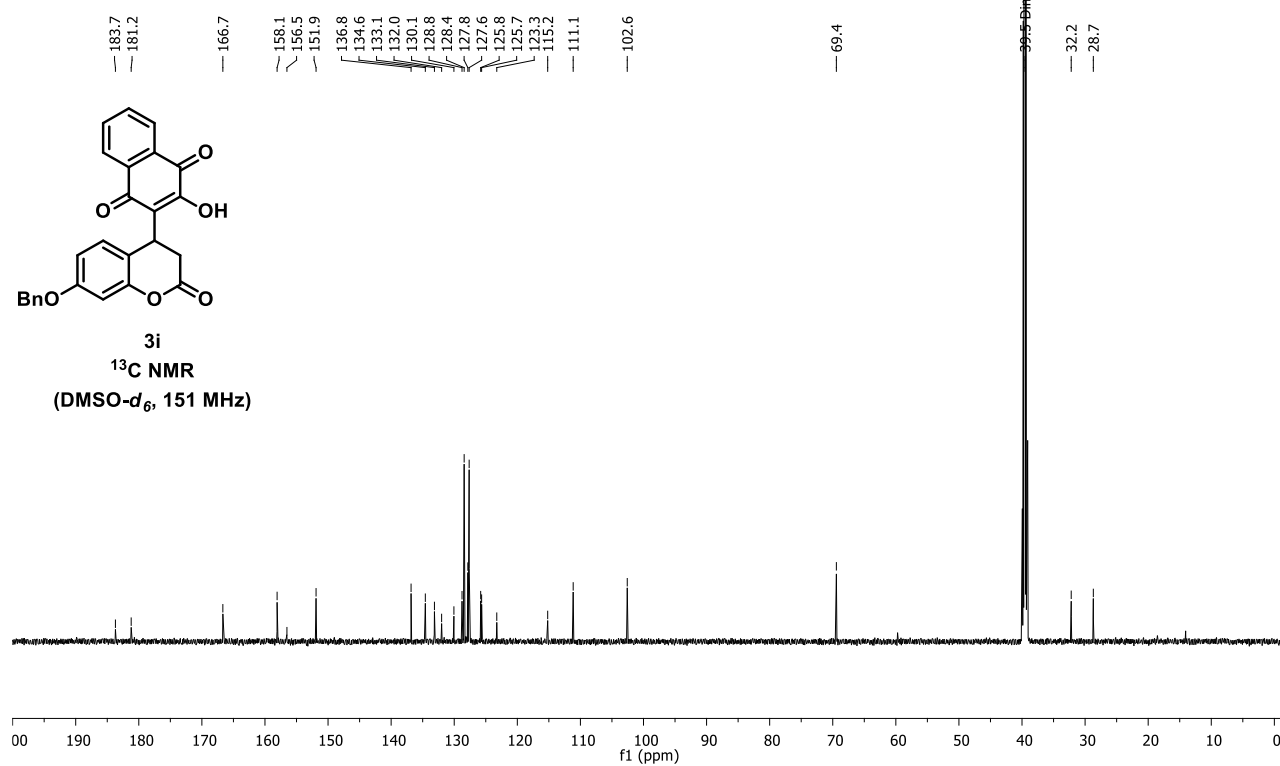
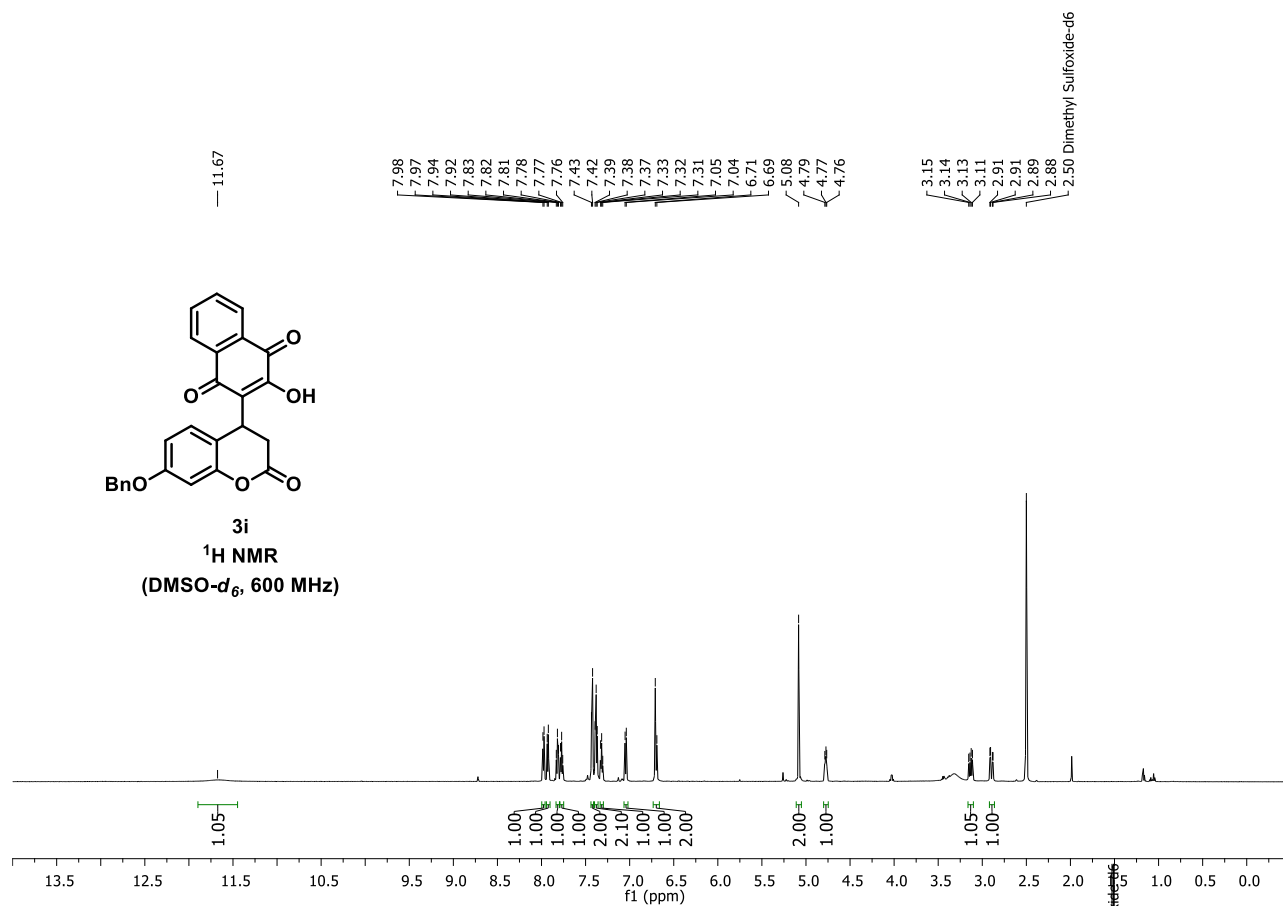


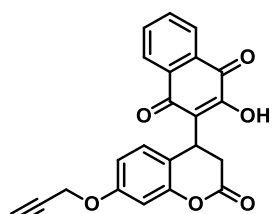




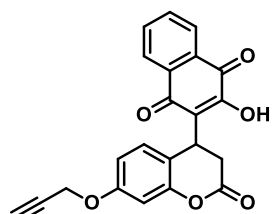
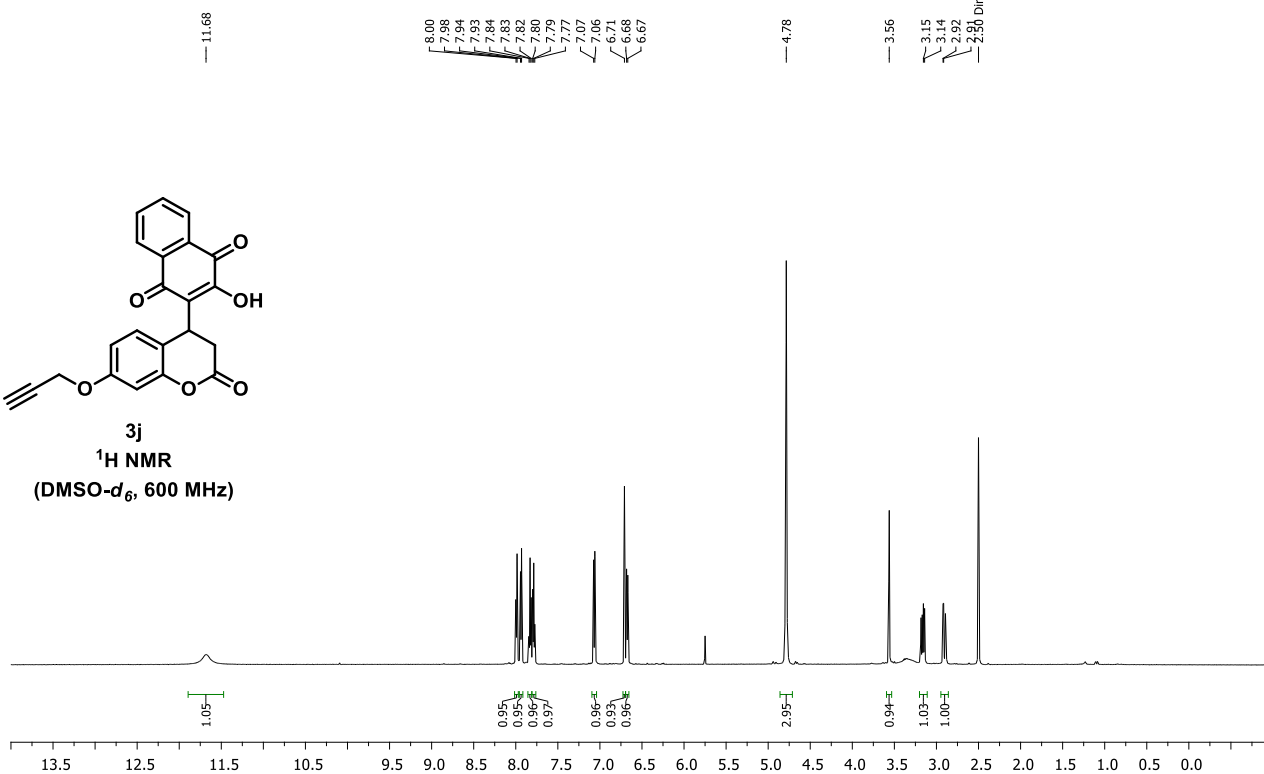




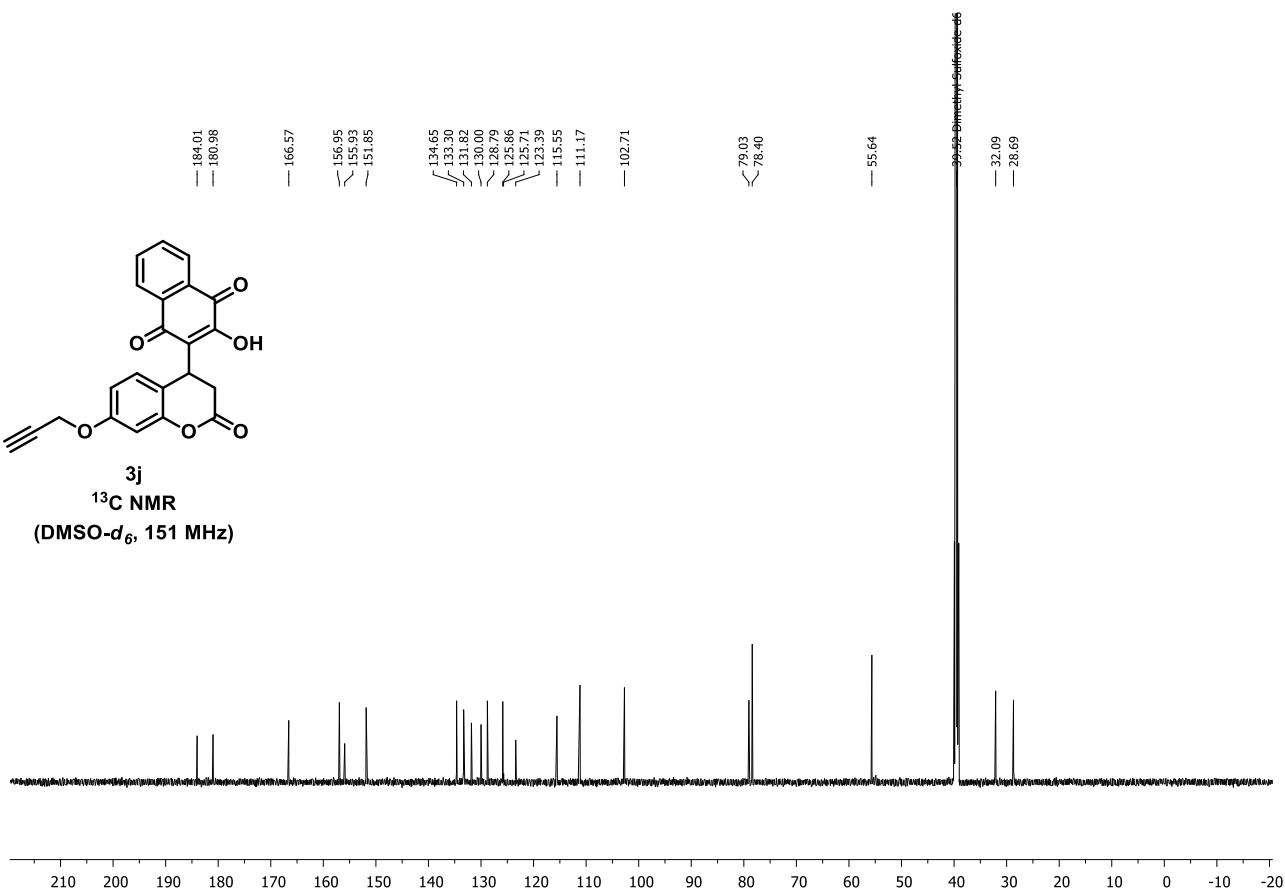


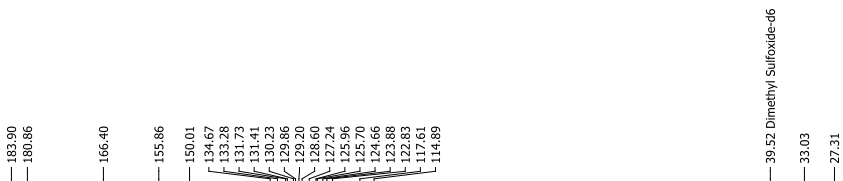
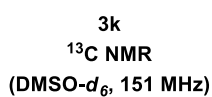
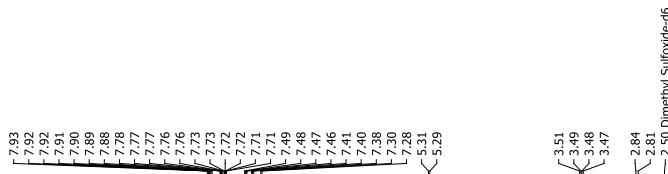
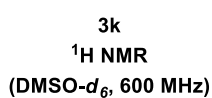


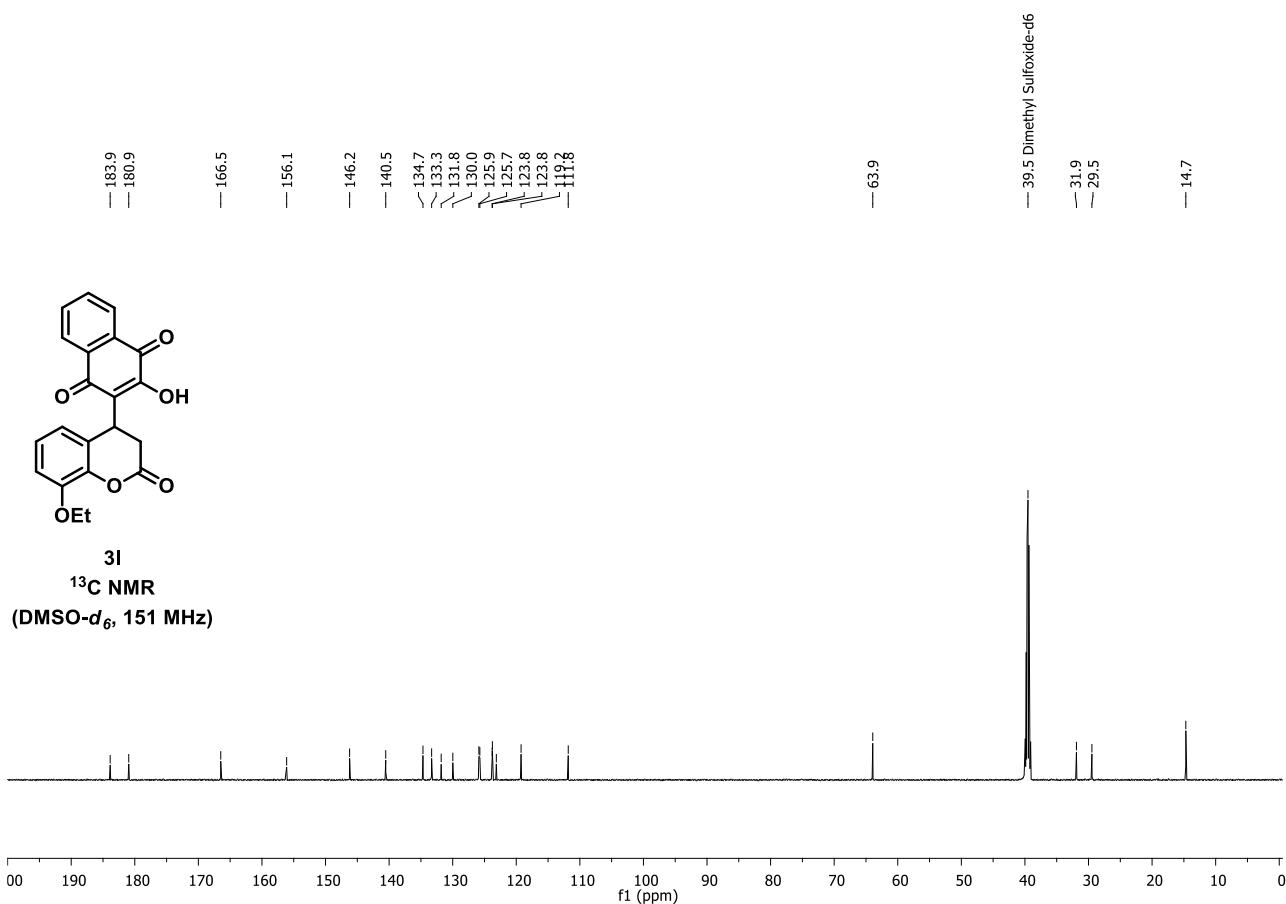
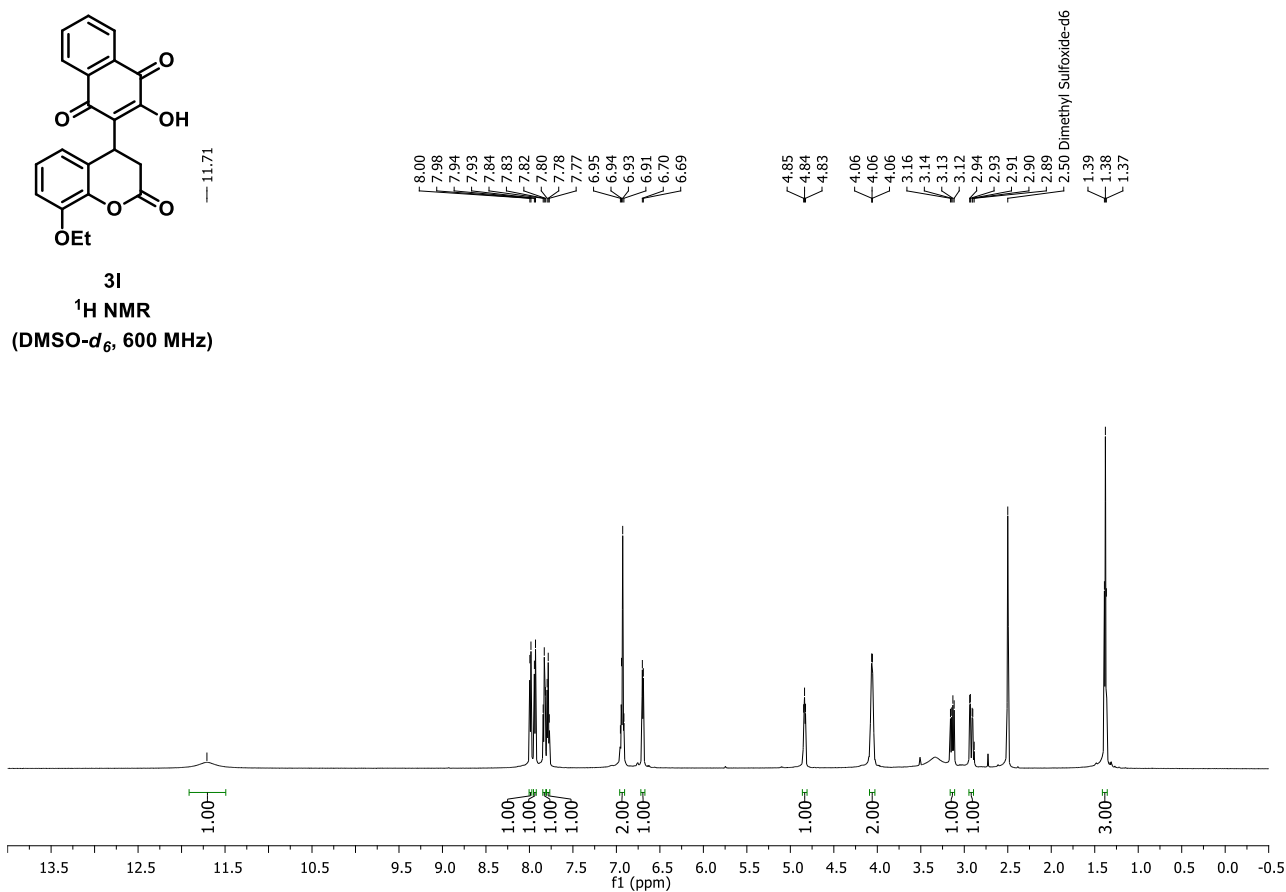
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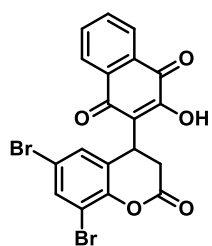


3j
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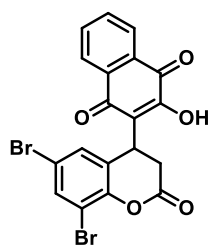
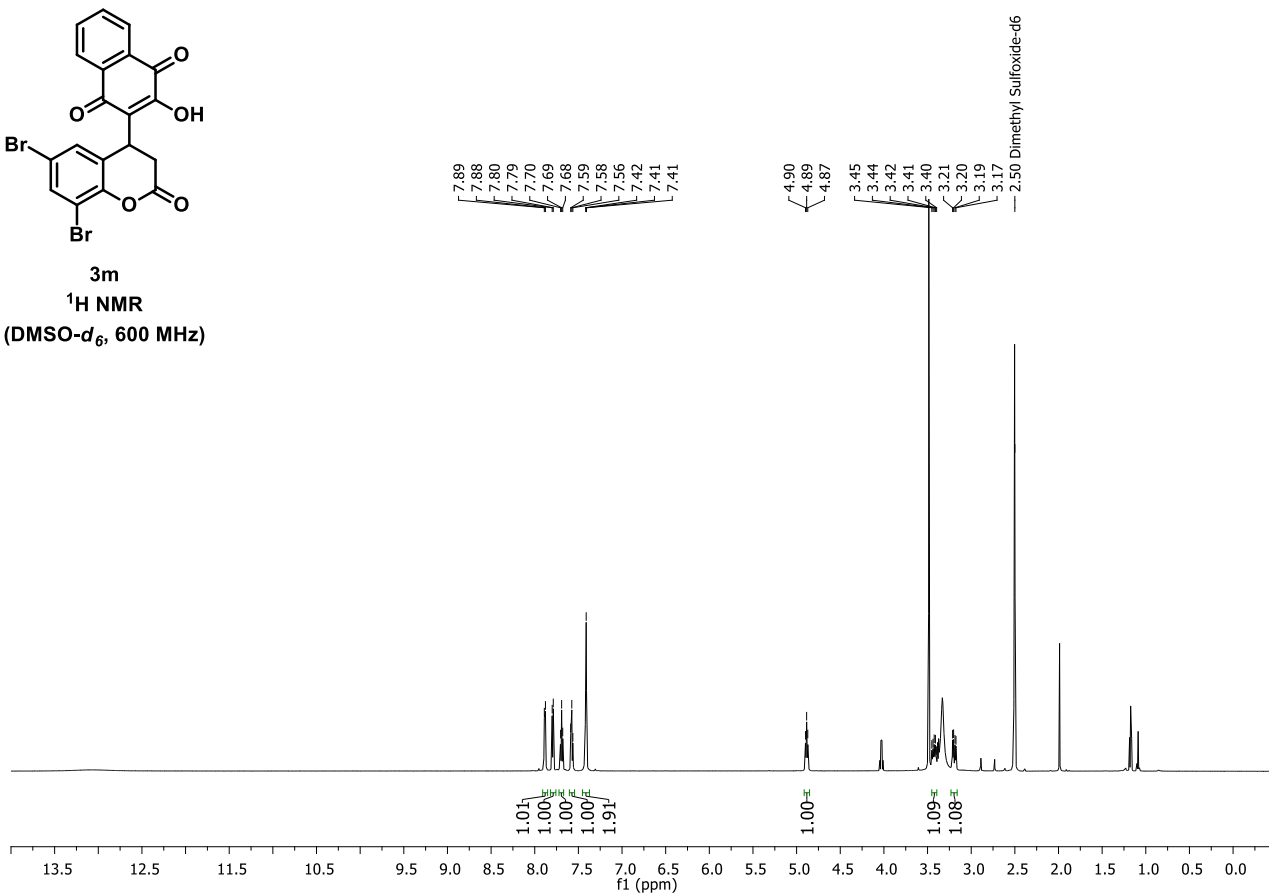




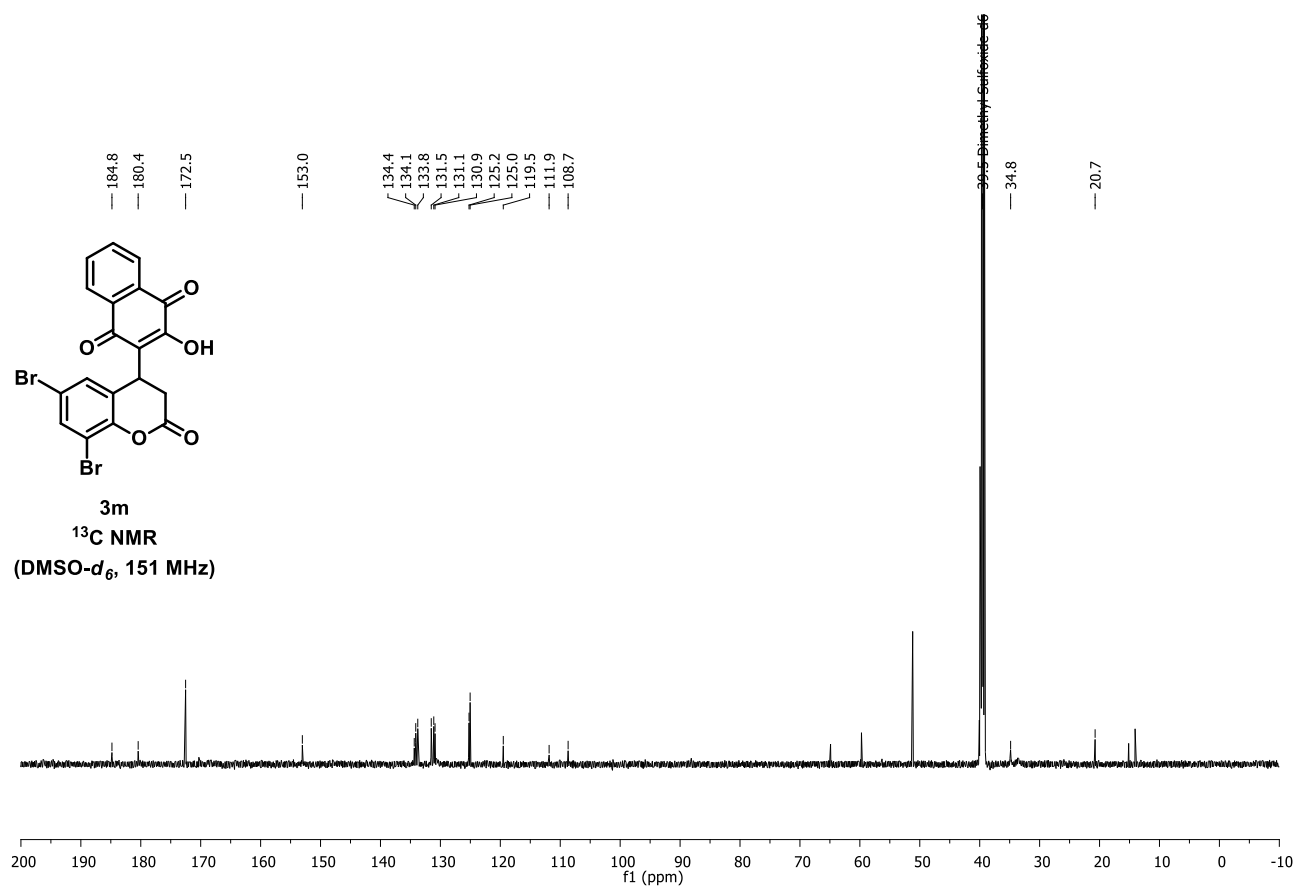


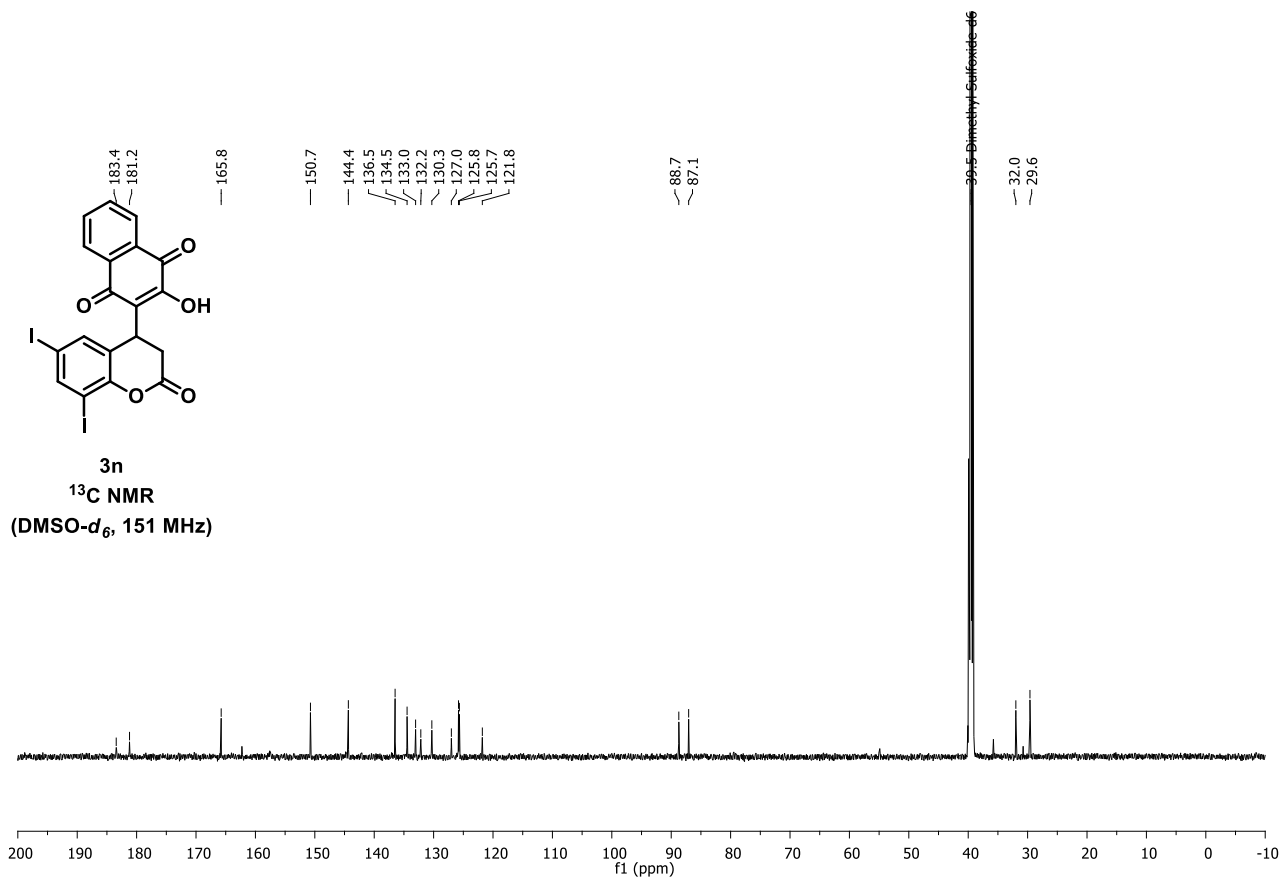
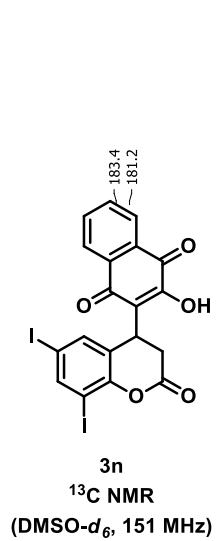
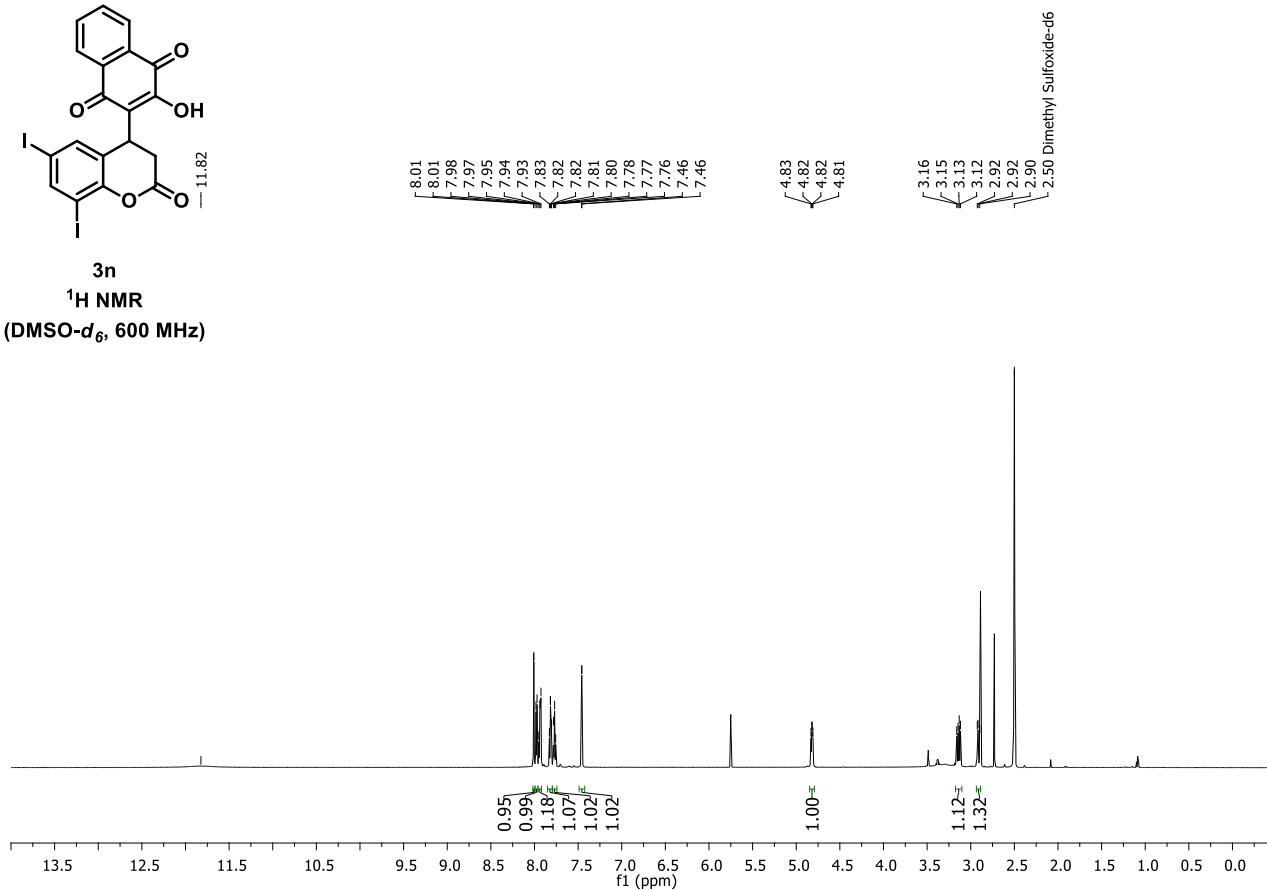
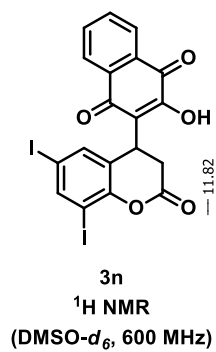


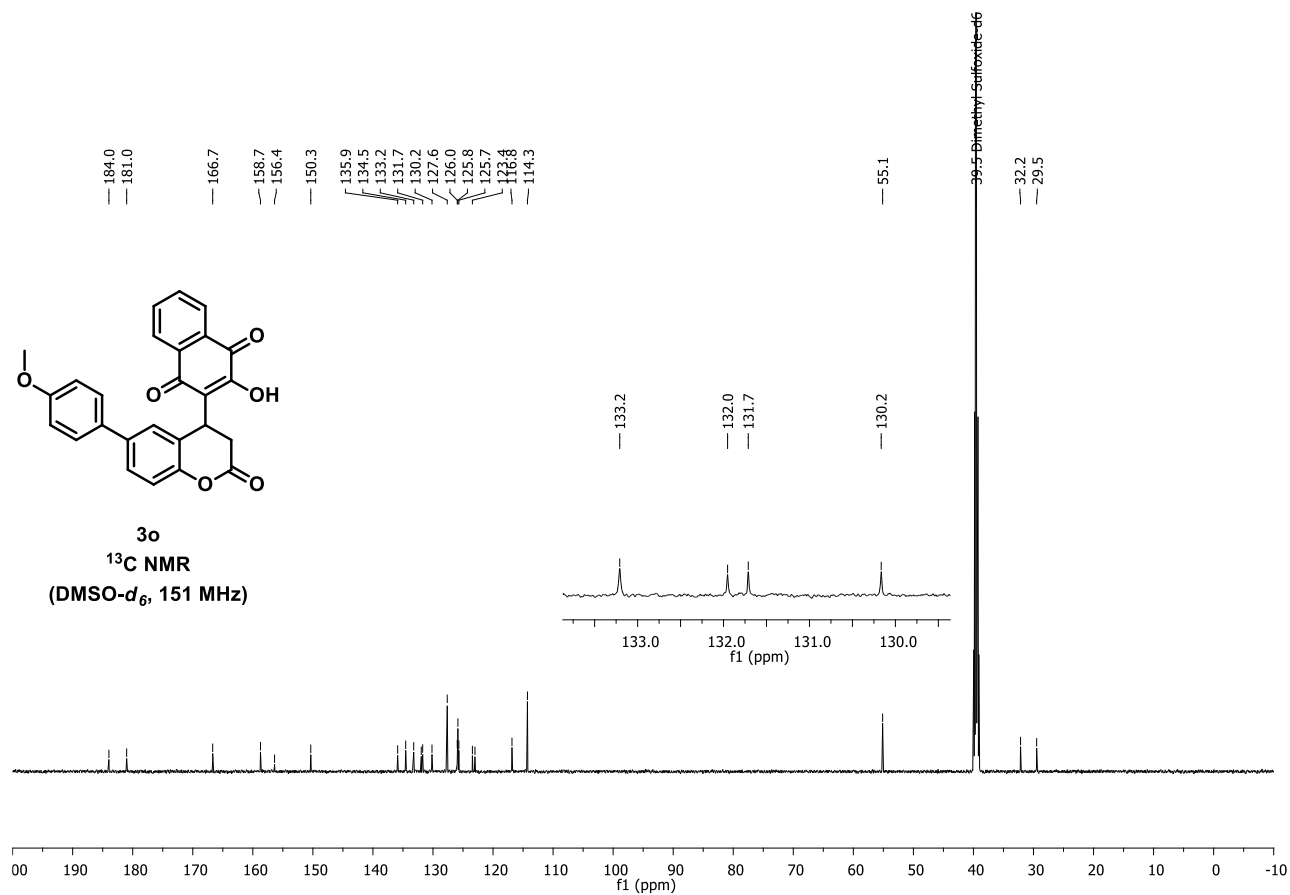
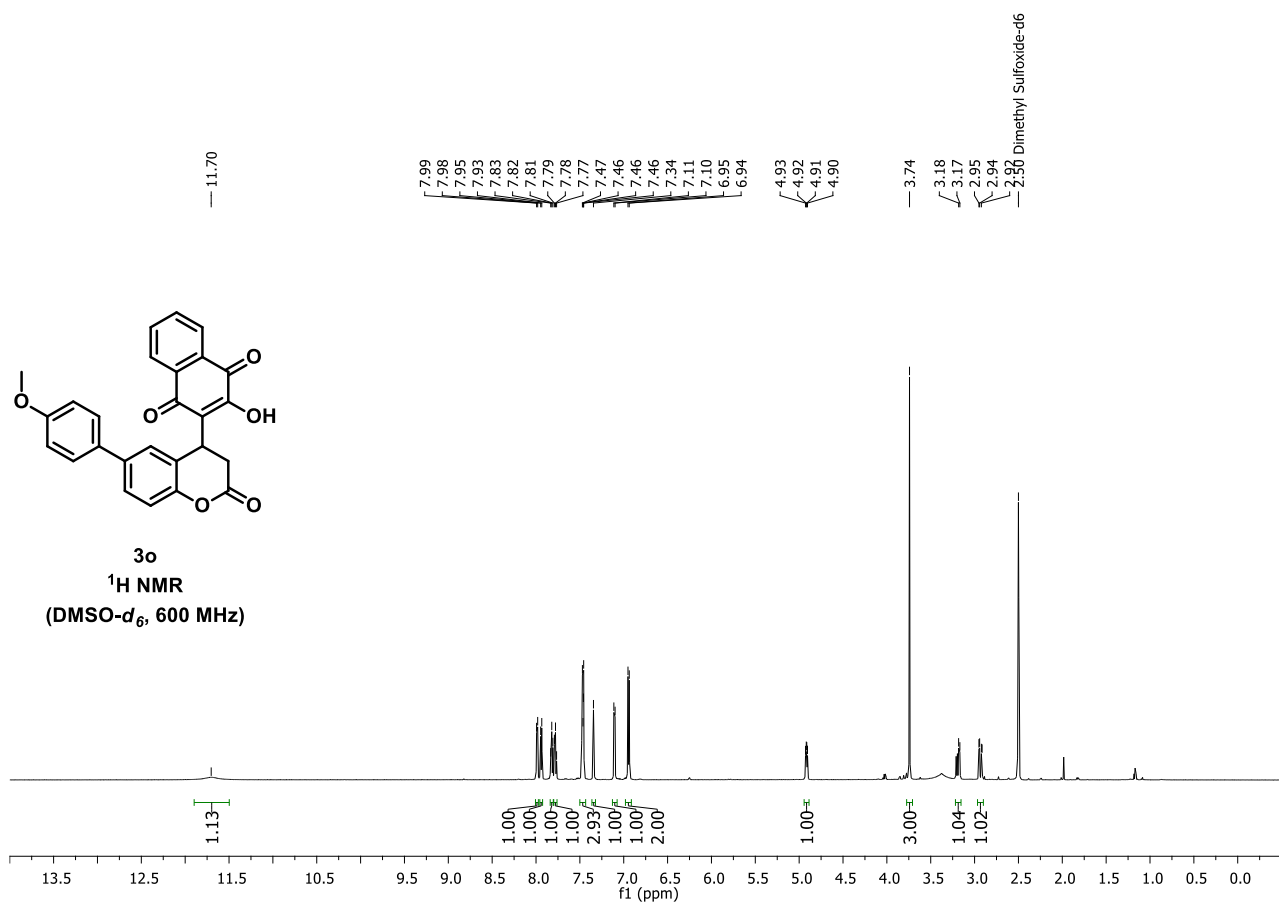
3m
¹H NMR
(DMSO-*d*₆, 600 MHz)

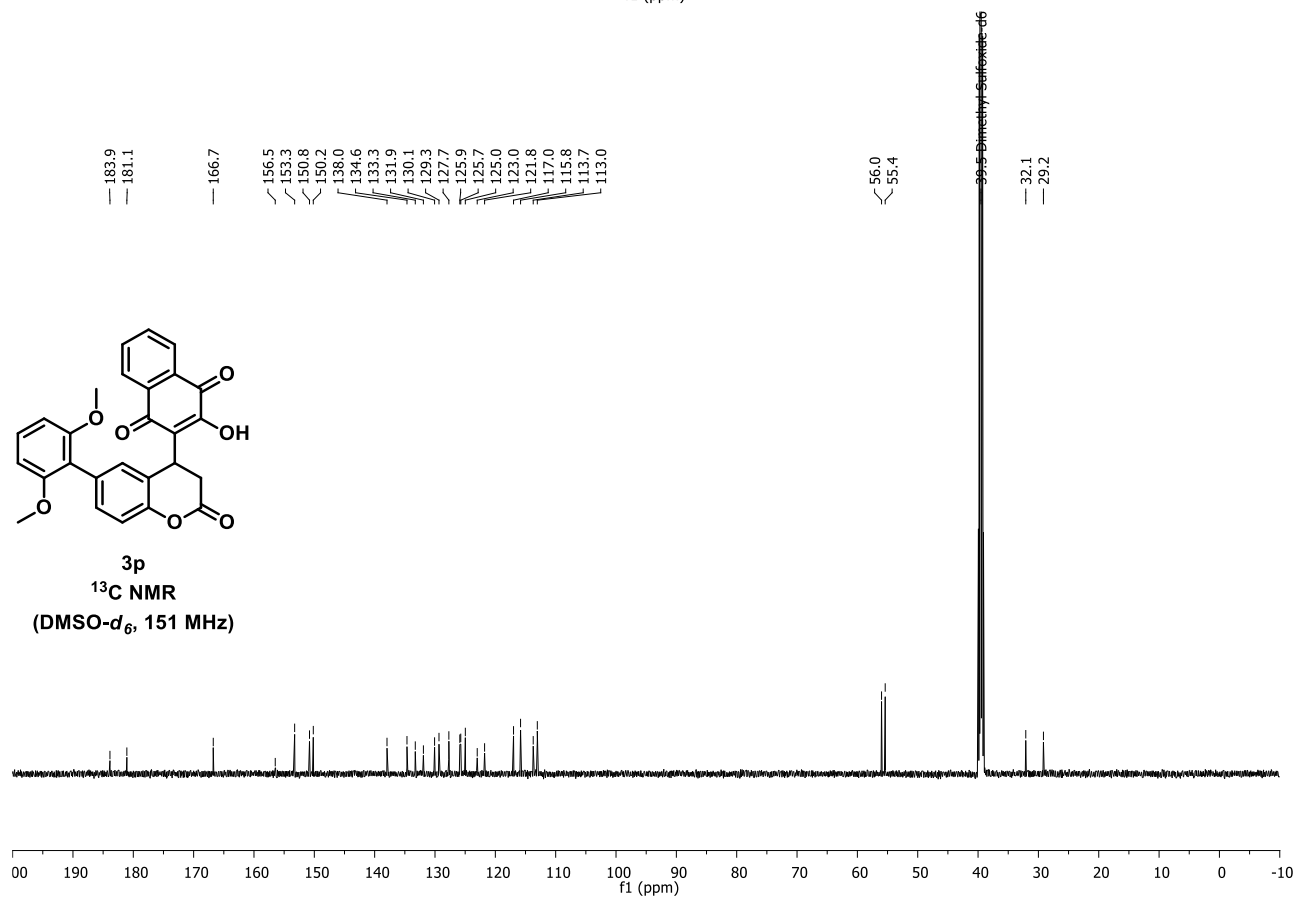
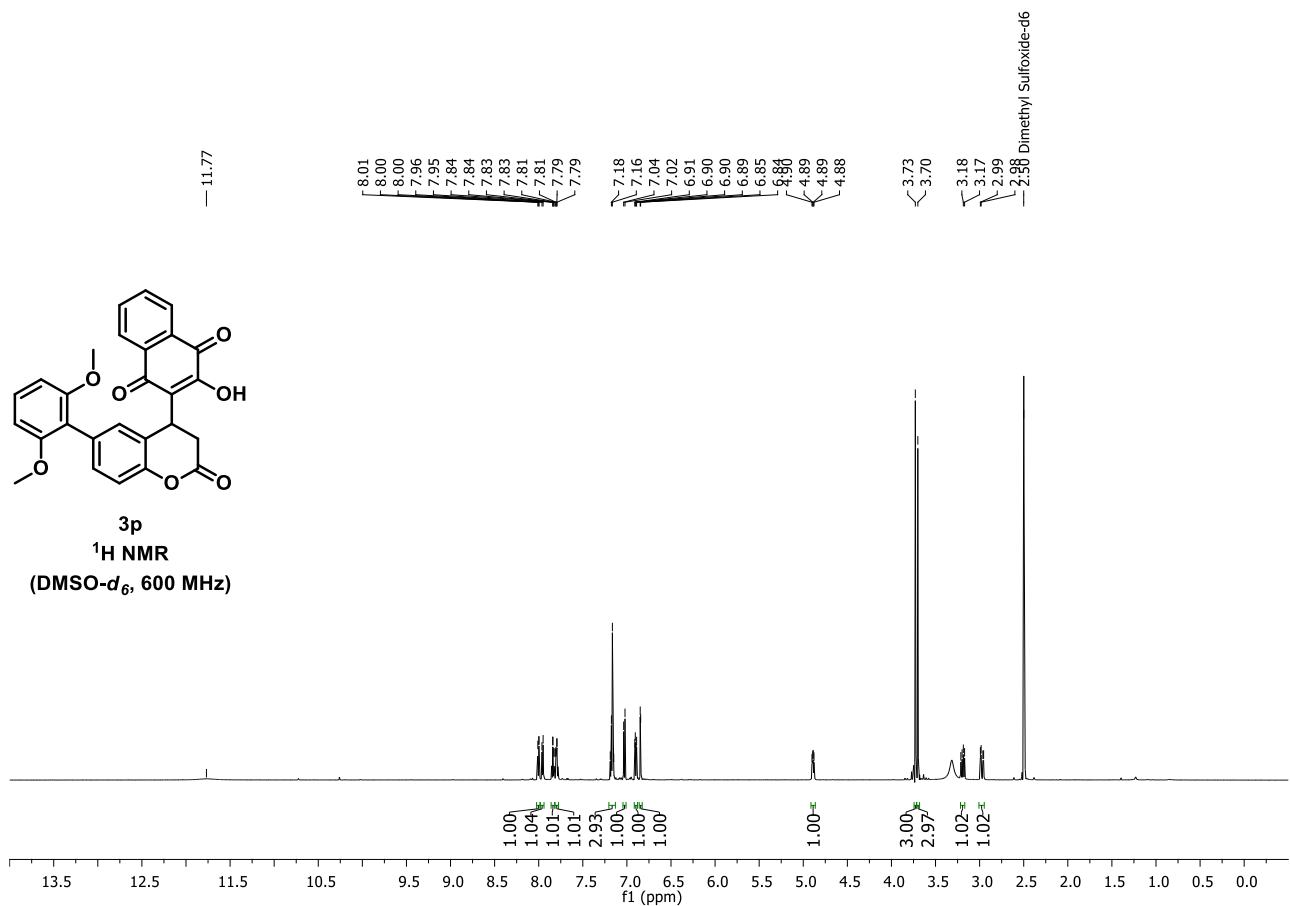


3m
¹³C NMR
(DMSO-*d*₆, 151 MHz)









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