

Supplementary Material

Discovery of C-3 tethered 2-oxo-benzo[1,4]oxazines as potent antioxidants: Bio-inspired based design, synthesis, biological evaluation, cytotoxic and Insilico molecular docking studies

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Keywords: 2-Oxo-benzo[1,4]oxazines; Antioxidant; DPPH, FRAP. Ascorbic acid. BHT

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Representative characterization spectra (^1H and ^{13}C NMR) of C-3 tethered 2-oxo-benzo[1,4]oxazines (20b, 20j-t, 20x and 20z):

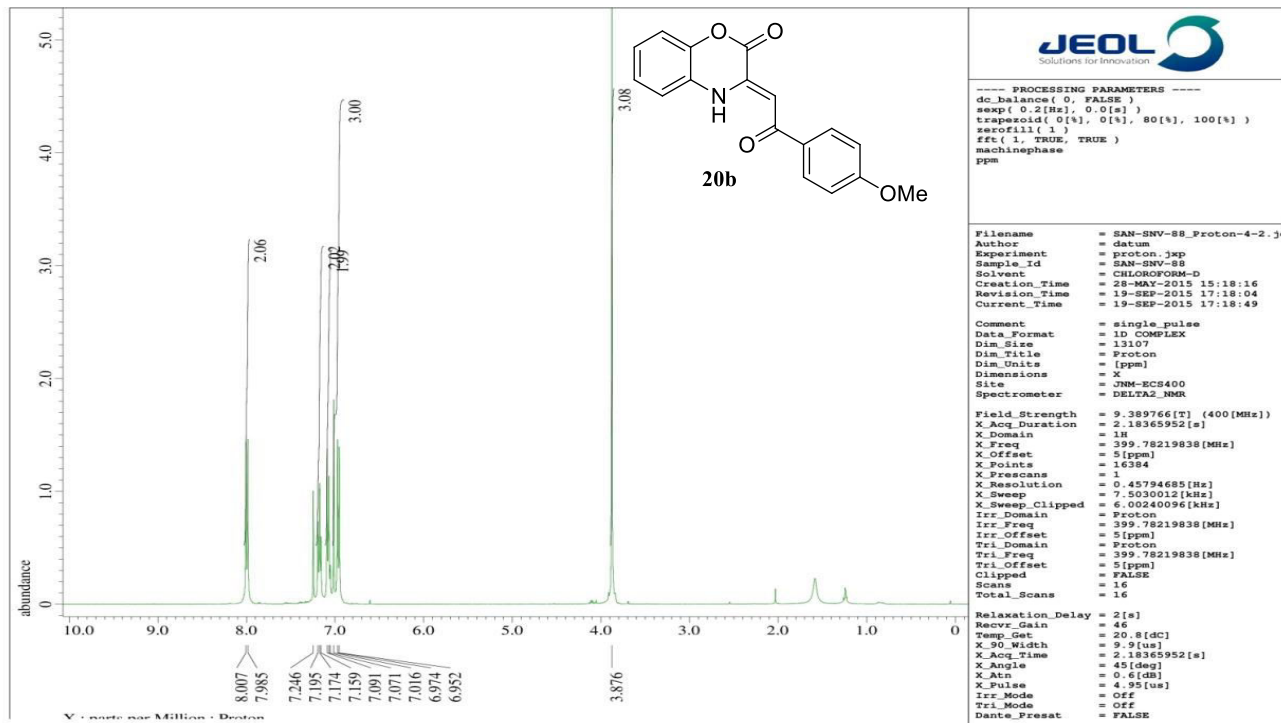


Figure 1. ^1H NMR Spectra of Compound 20b.

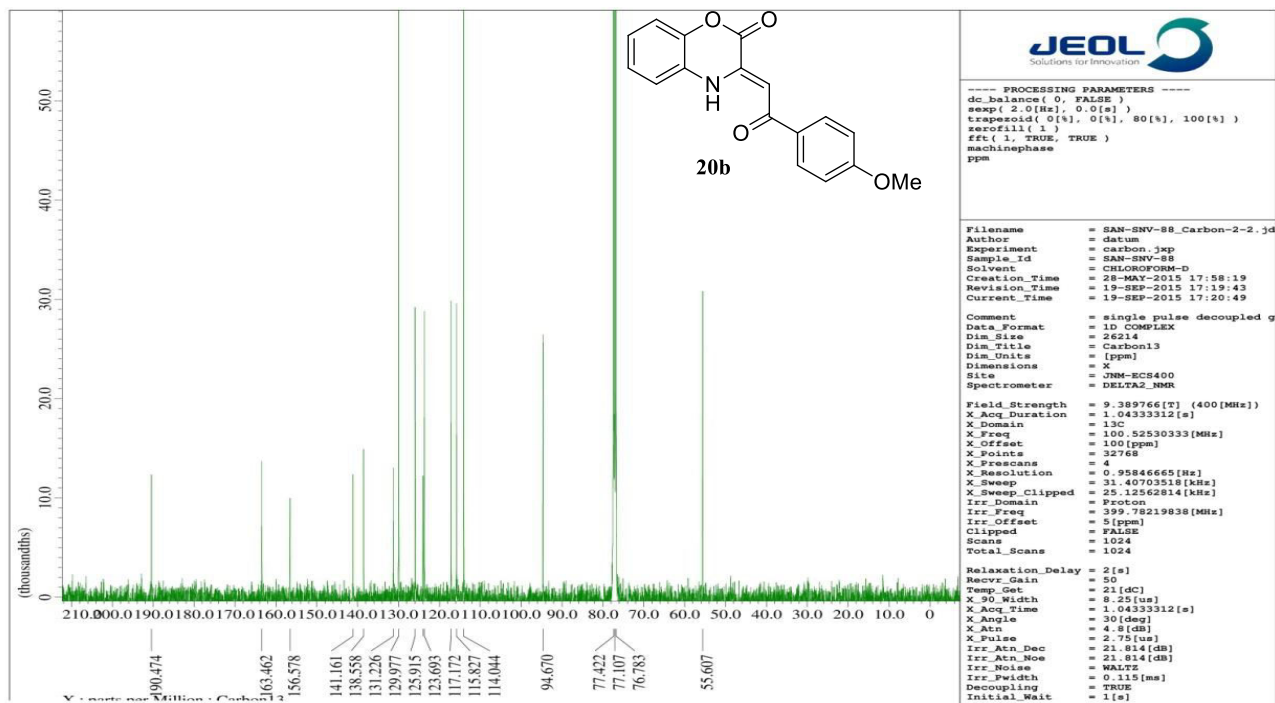


Figure 2. ^{13}C NMR Spectra of Compound 20b.

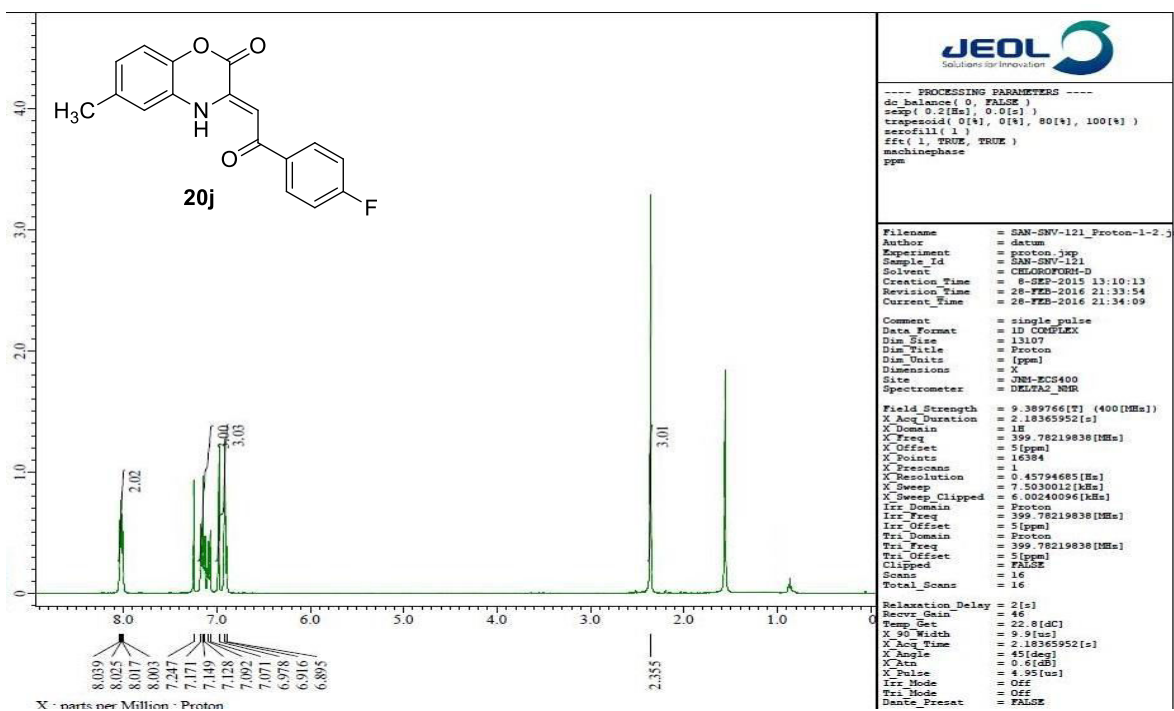


Figure 3. ¹H NMR Spectra of Compound 20j.

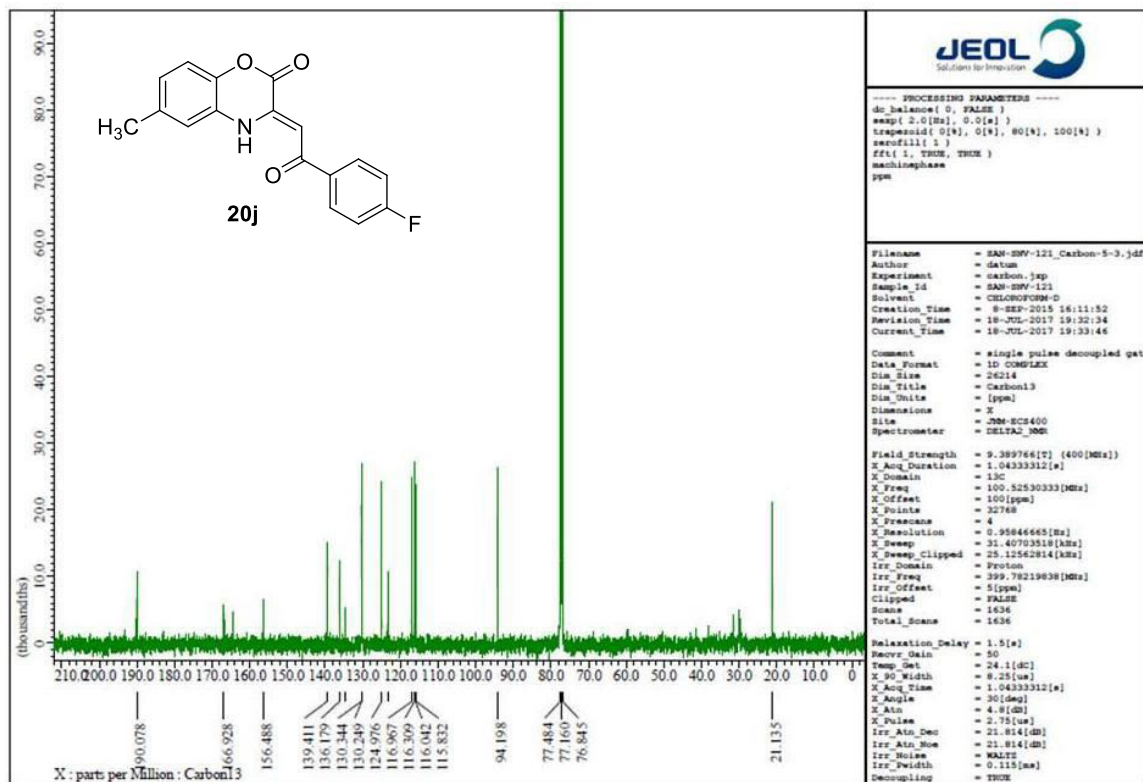
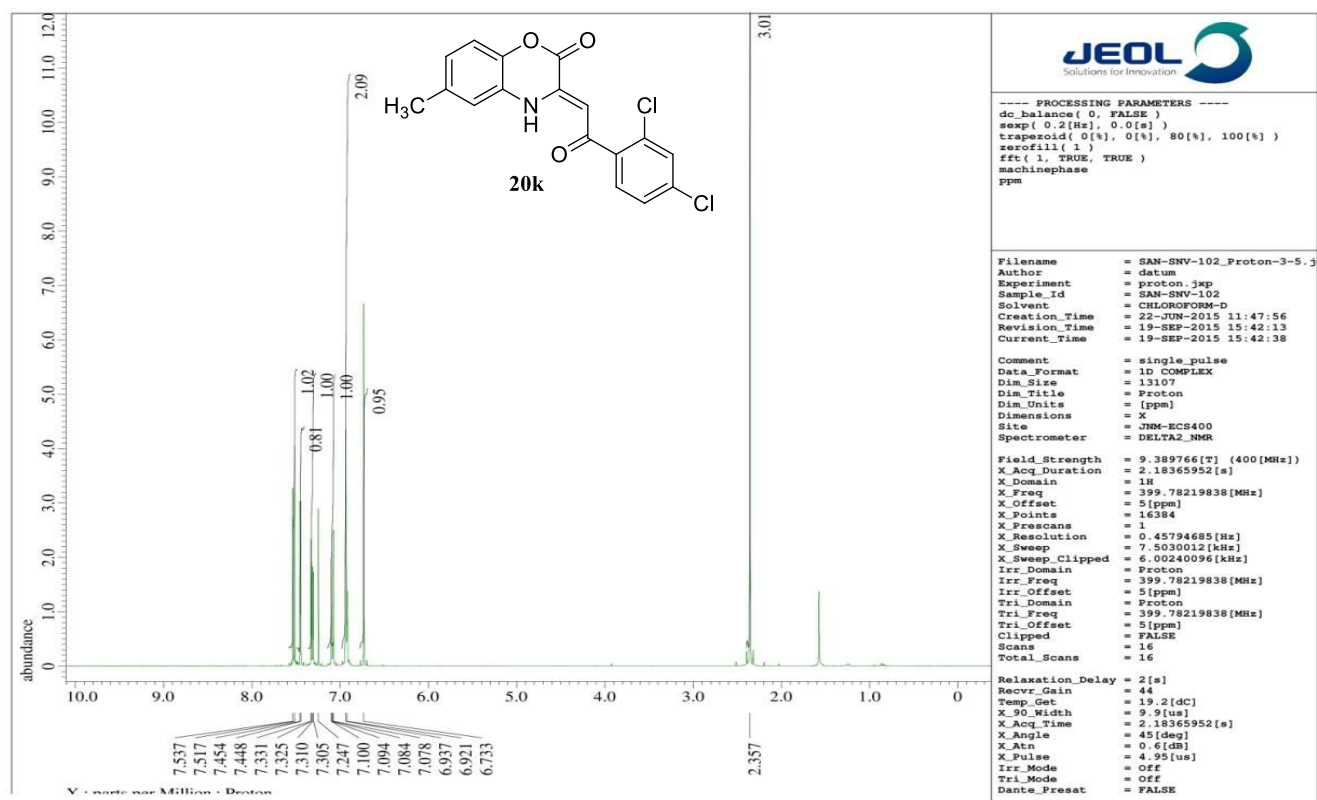
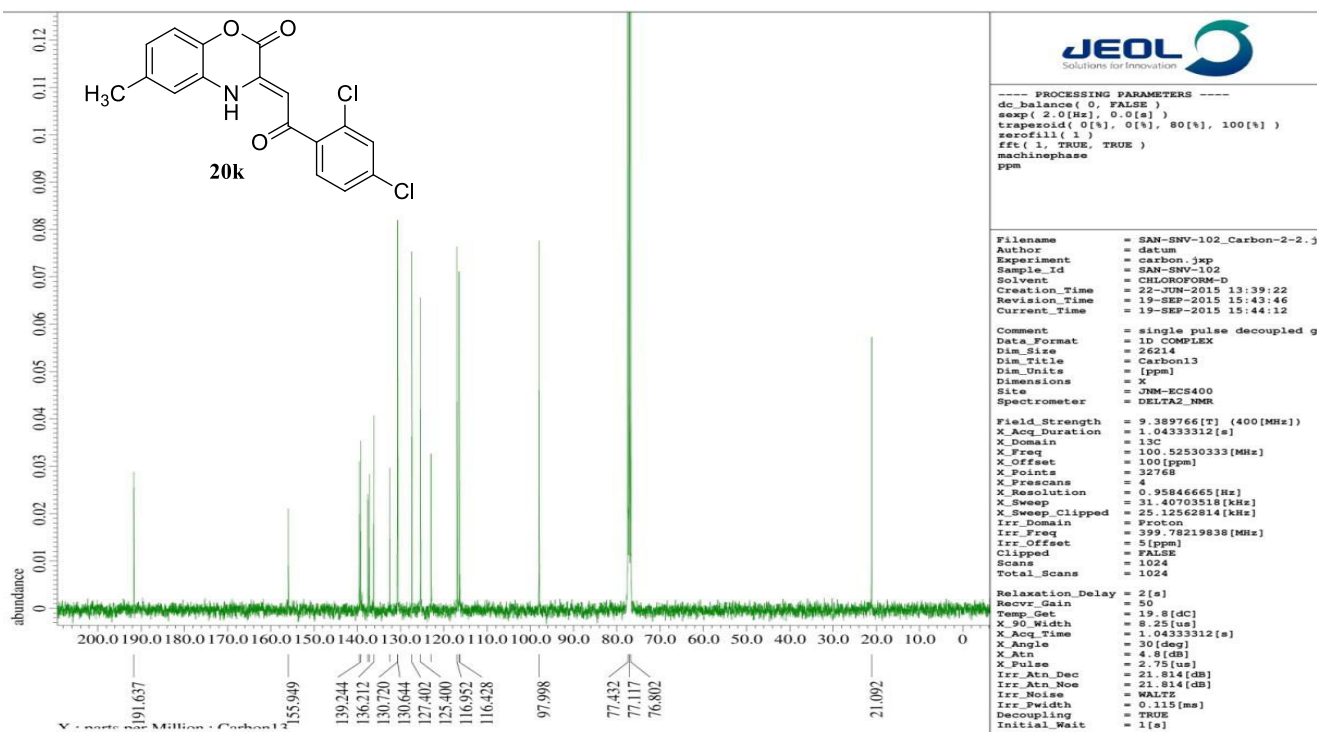


Figure 4. ¹³C NMR Spectra of Compound 20j.

Figure 5. ¹H NMR Spectra of Compound 20k.Figure 6. ¹³C NMR Spectra of Compound 20k.

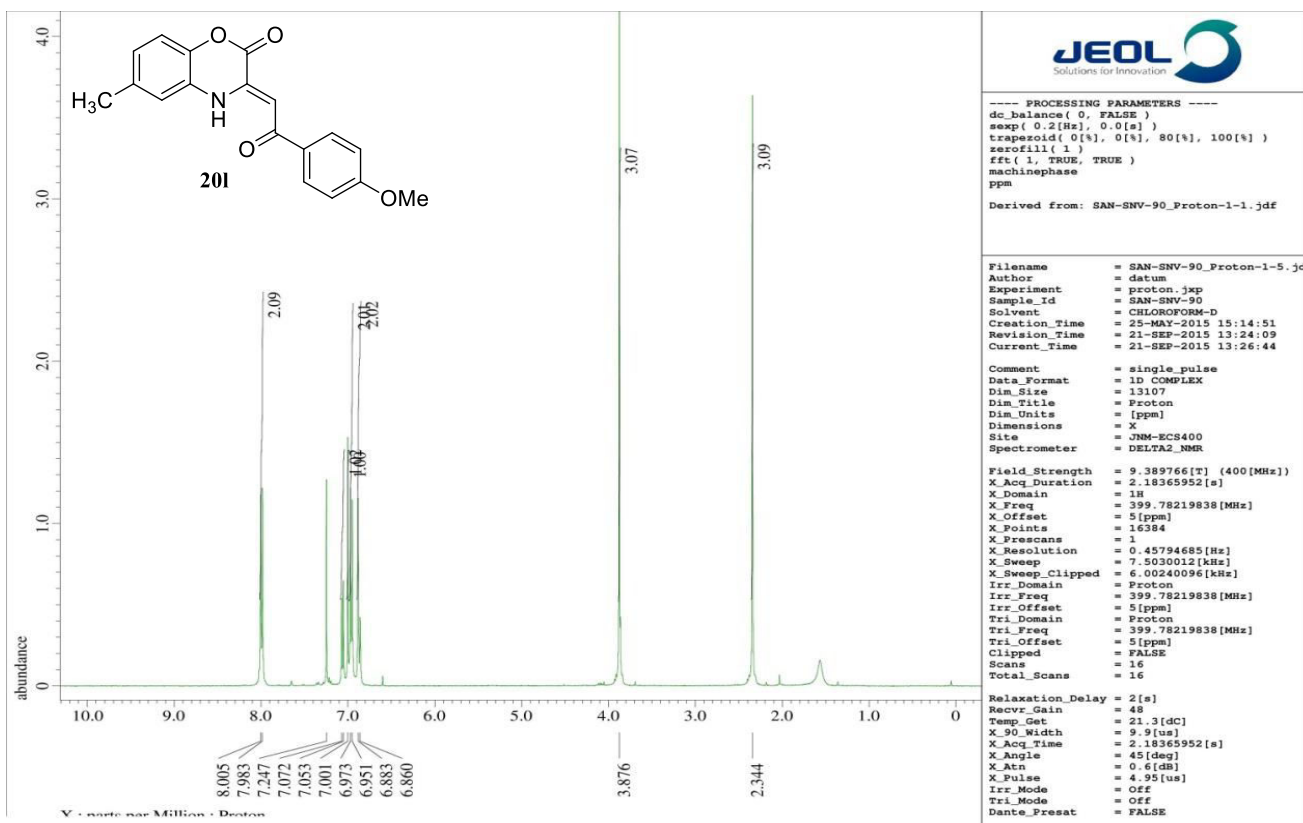


Figure 7. ¹H NMR Spectra of Compound 20l.

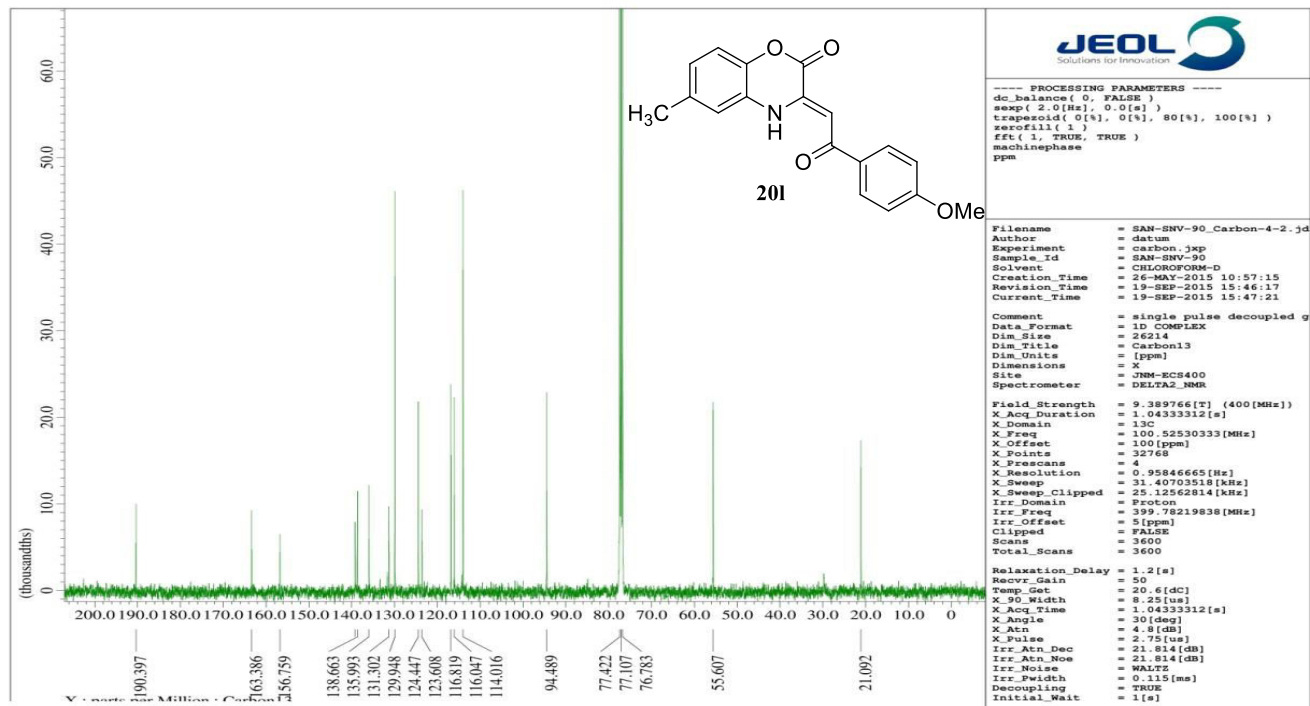


Figure 8. ¹³C NMR Spectra of Compound 20l.

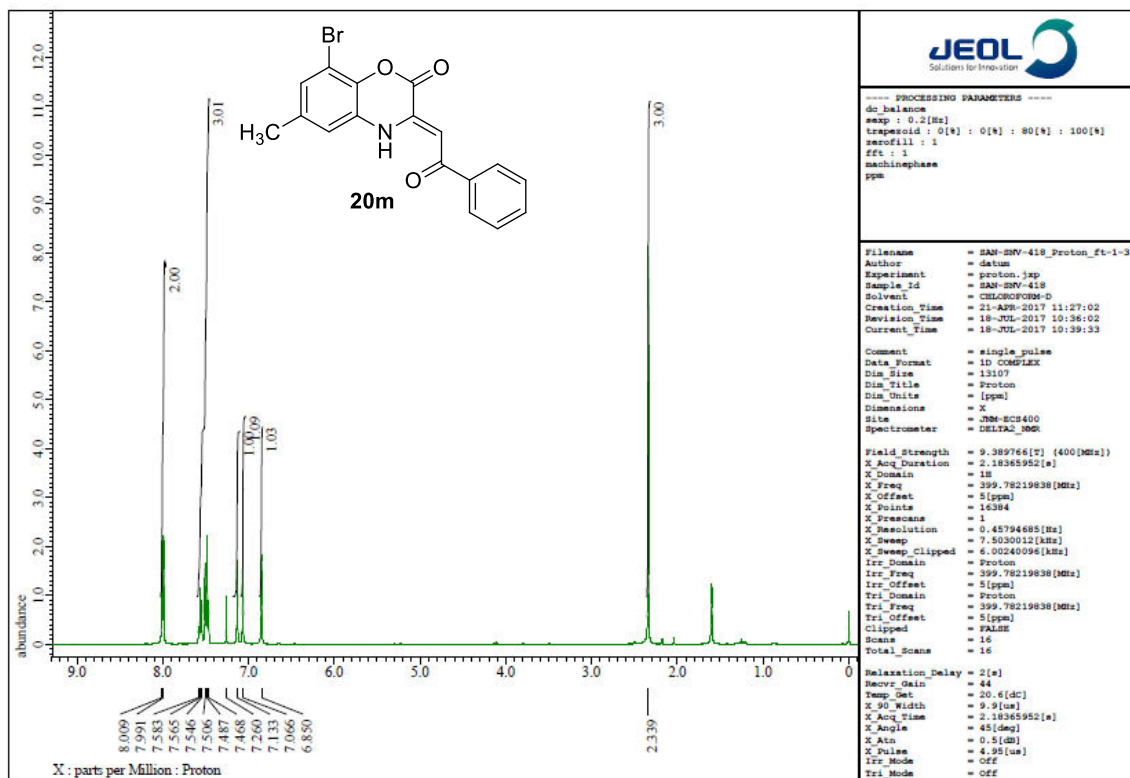


Figure 9. ¹H NMR Spectra of Compound 20m.

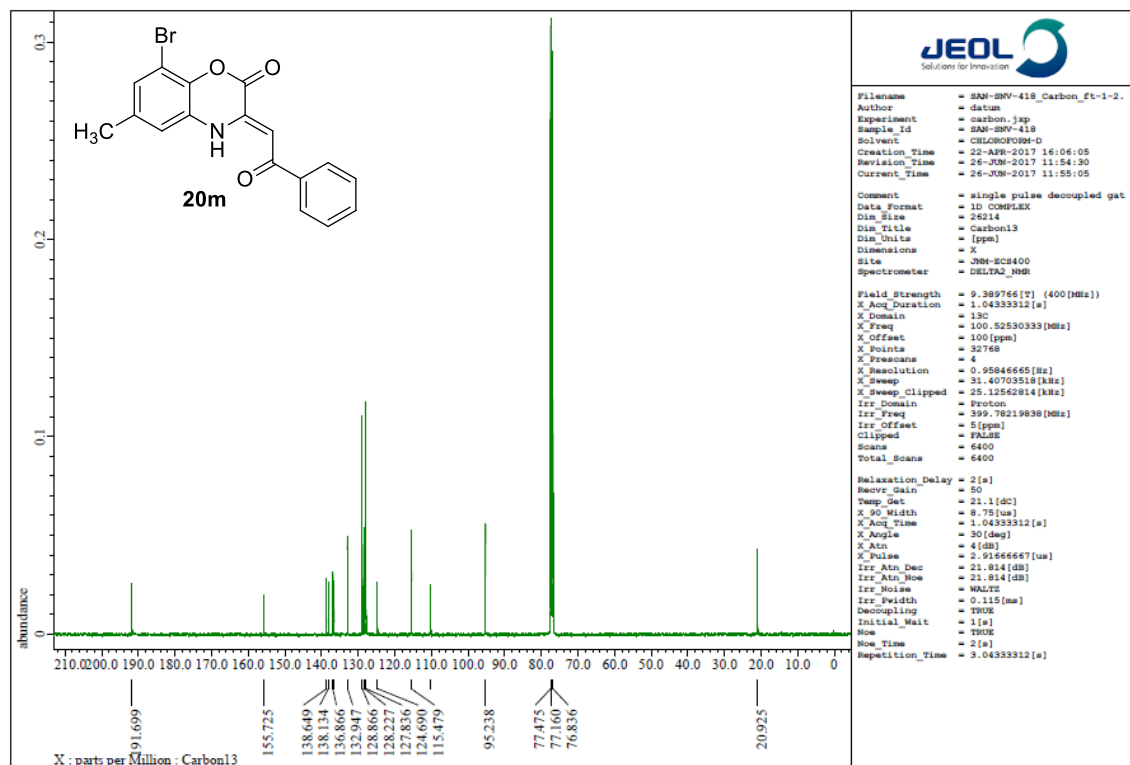


Figure 10. ¹³C NMR Spectra of Compound 20m.

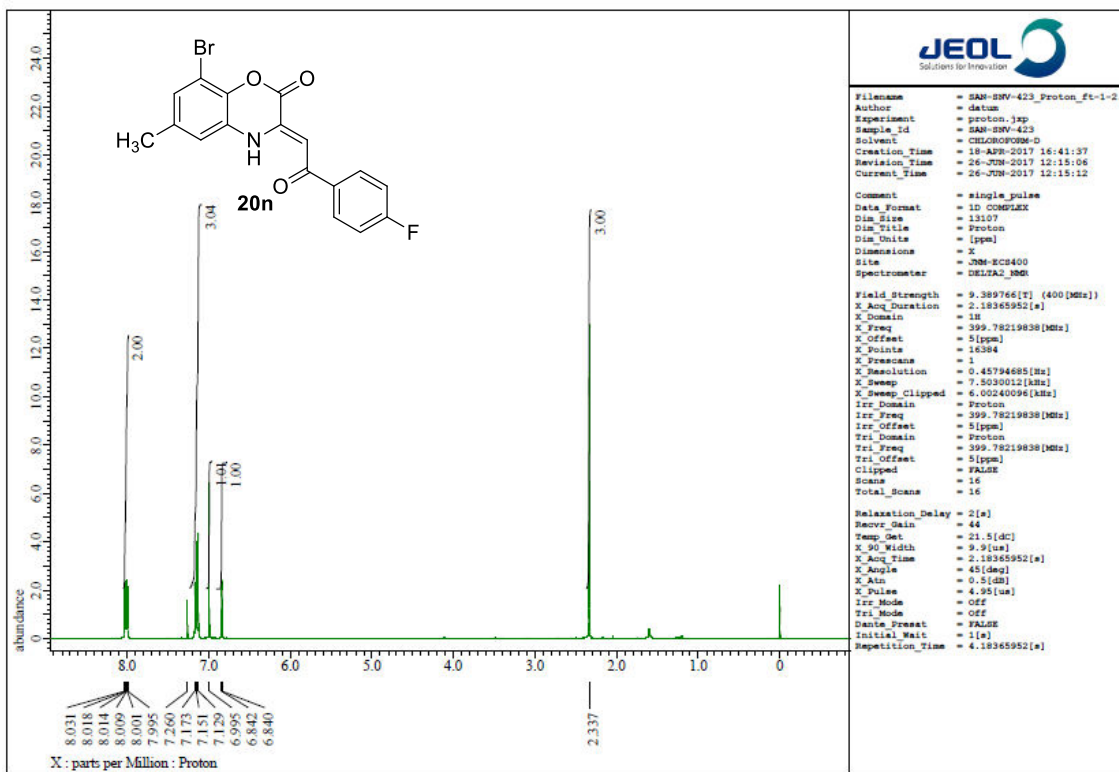


Figure 11. ¹H NMR Spectra of Compound 20n.

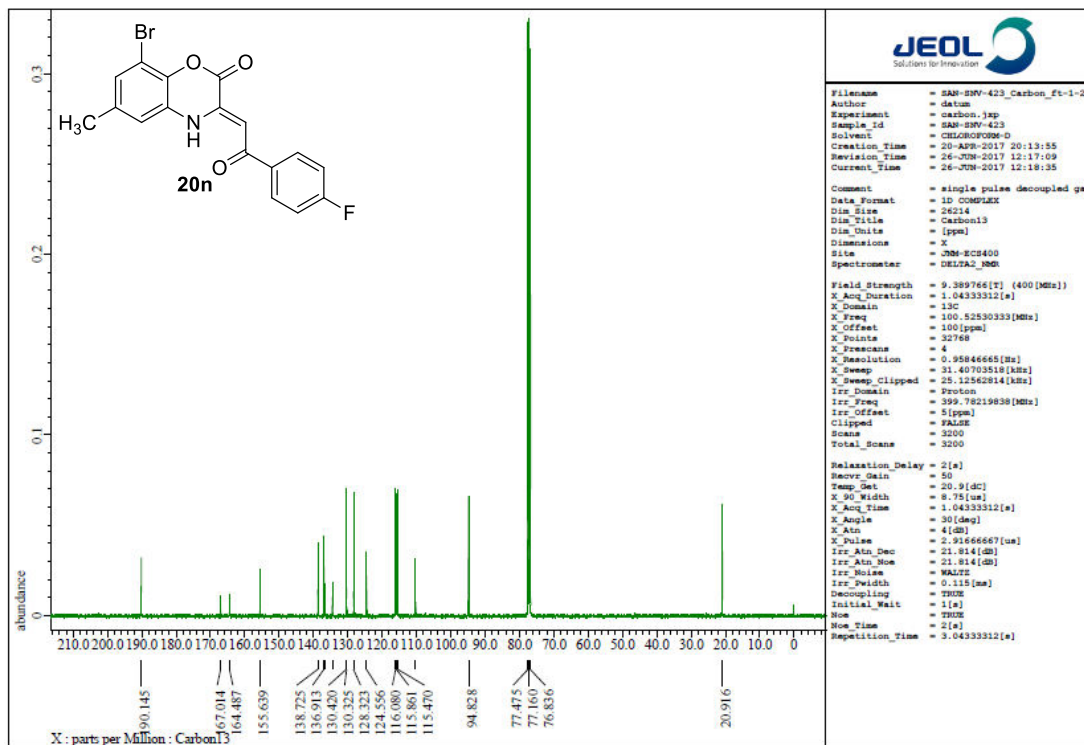


Figure 12. ¹³C NMR Spectra of Compound 20n.

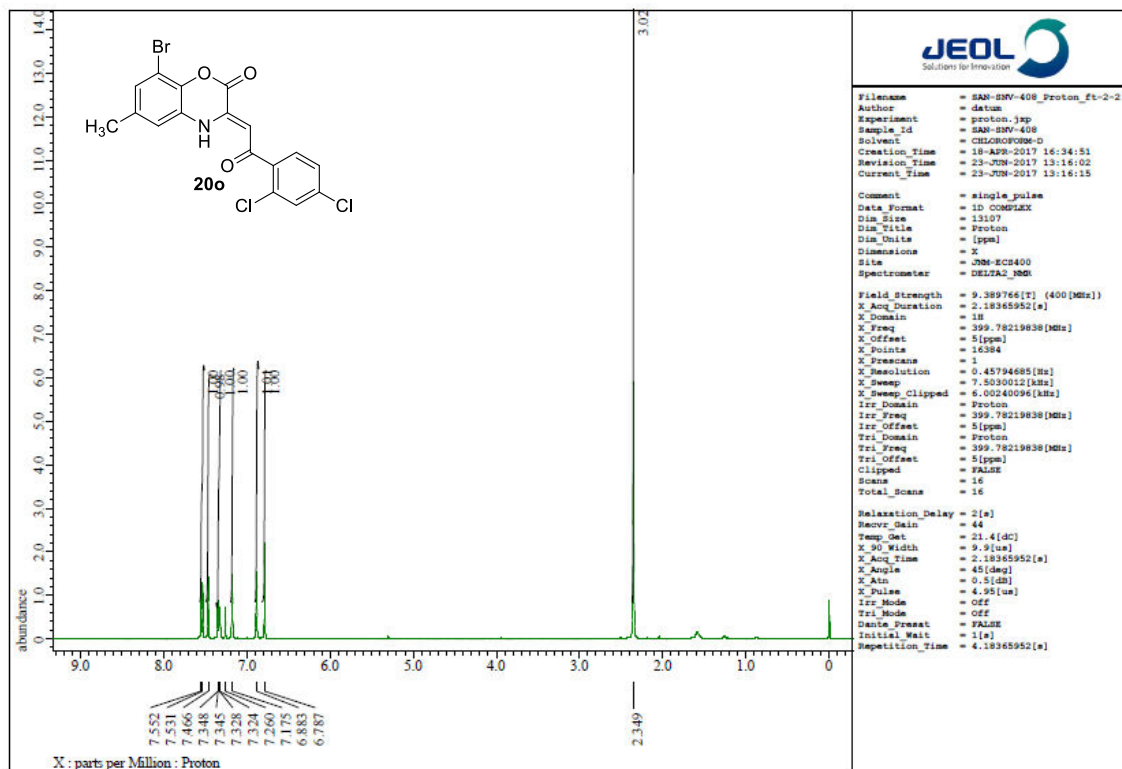


Figure 13. ¹H NMR Spectra of Compound 20o.

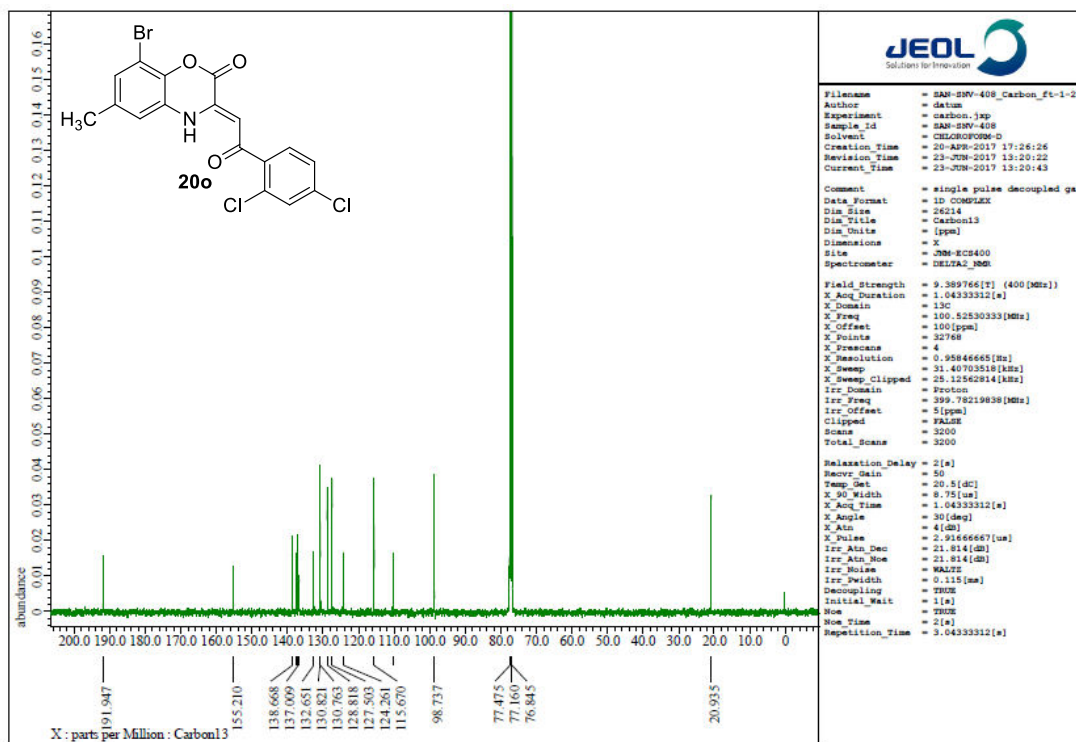


Figure 14. ¹³C NMR Spectra of Compound 20o.

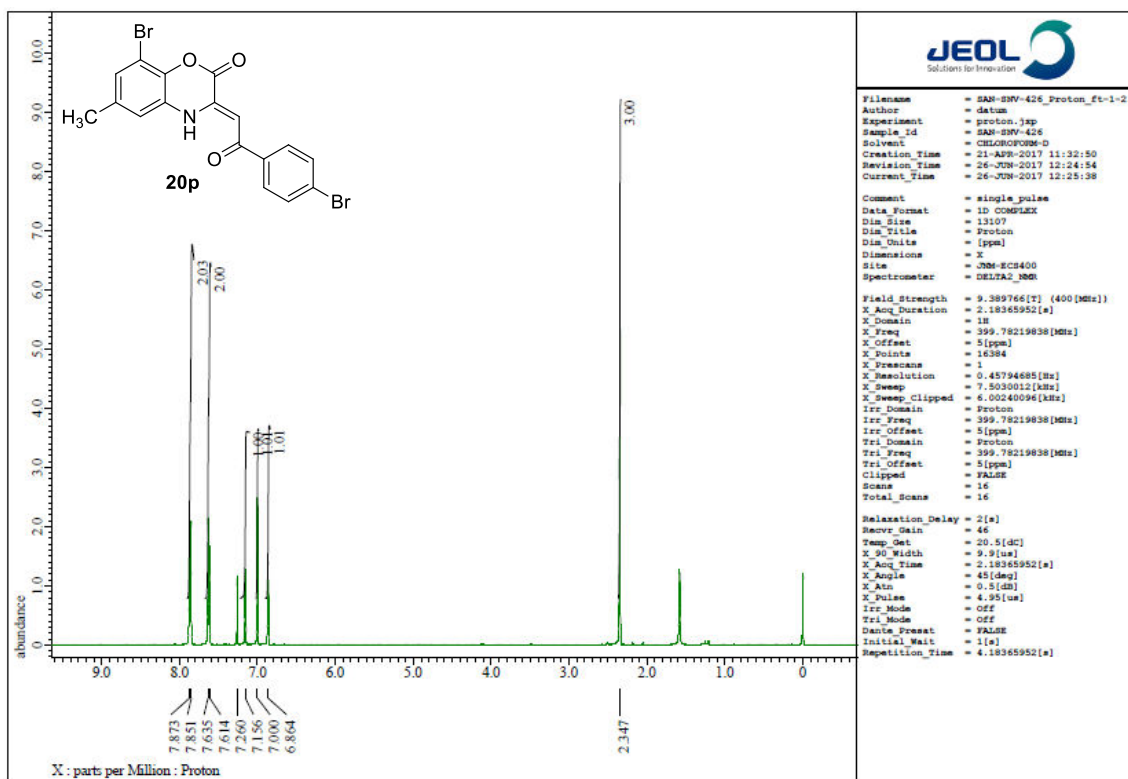


Figure 15. ¹H NMR Spectra of Compound 20p.

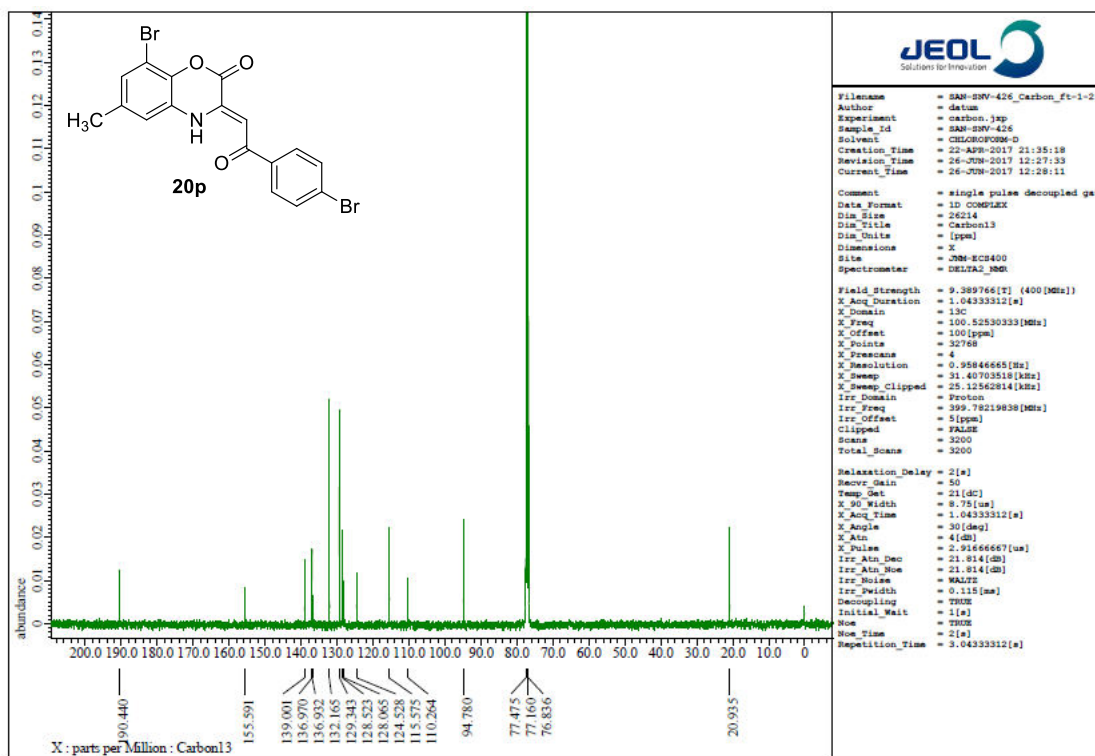


Figure 16. ¹³C NMR Spectra of Compound 20p.

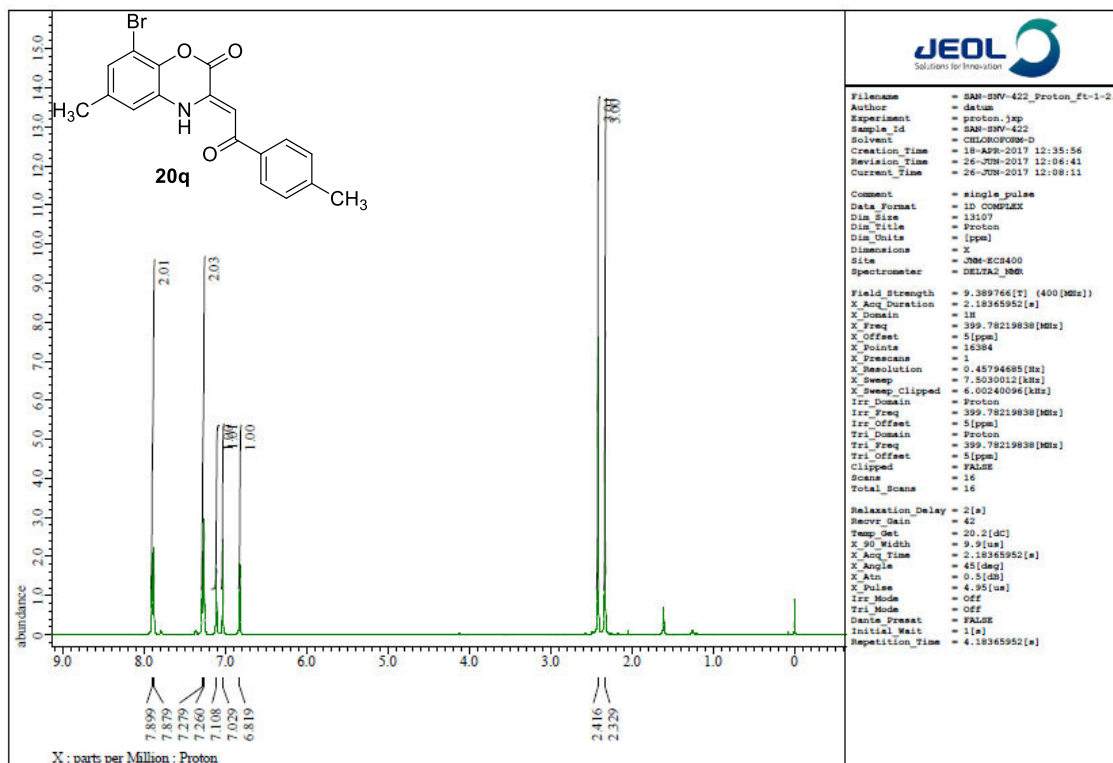


Figure 17. ¹H NMR Spectra of Compound 20q.

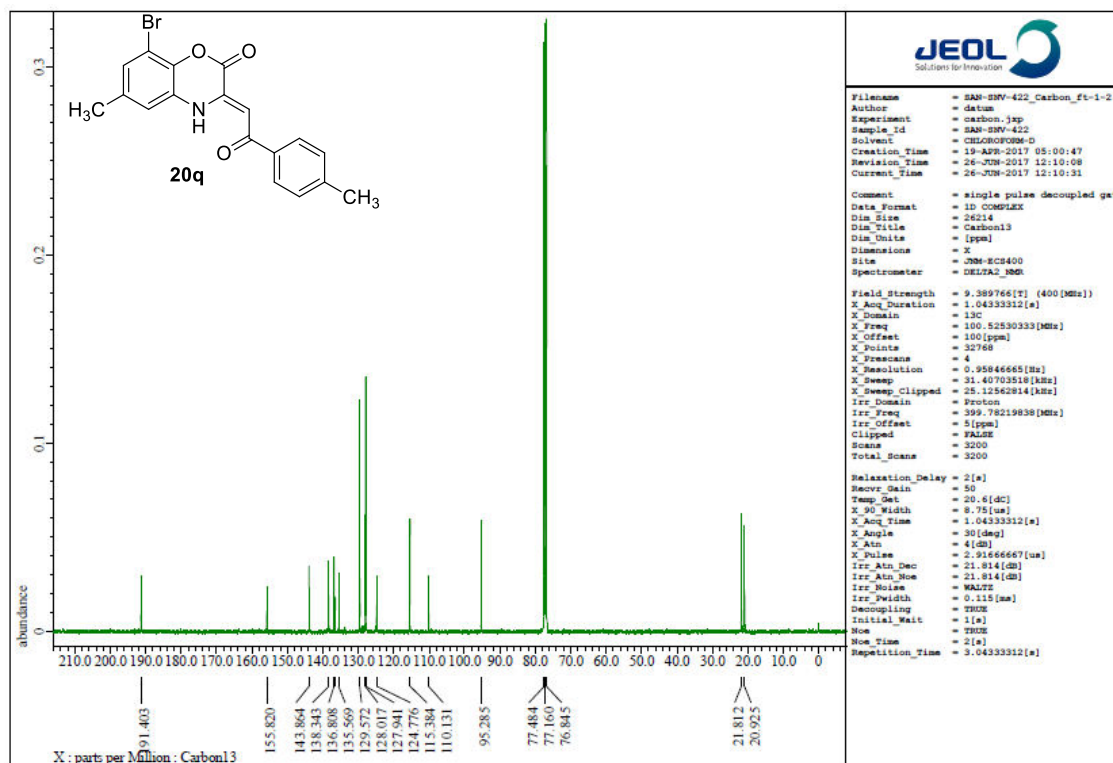


Figure 18. ¹³C NMR Spectra of Compound 20q.

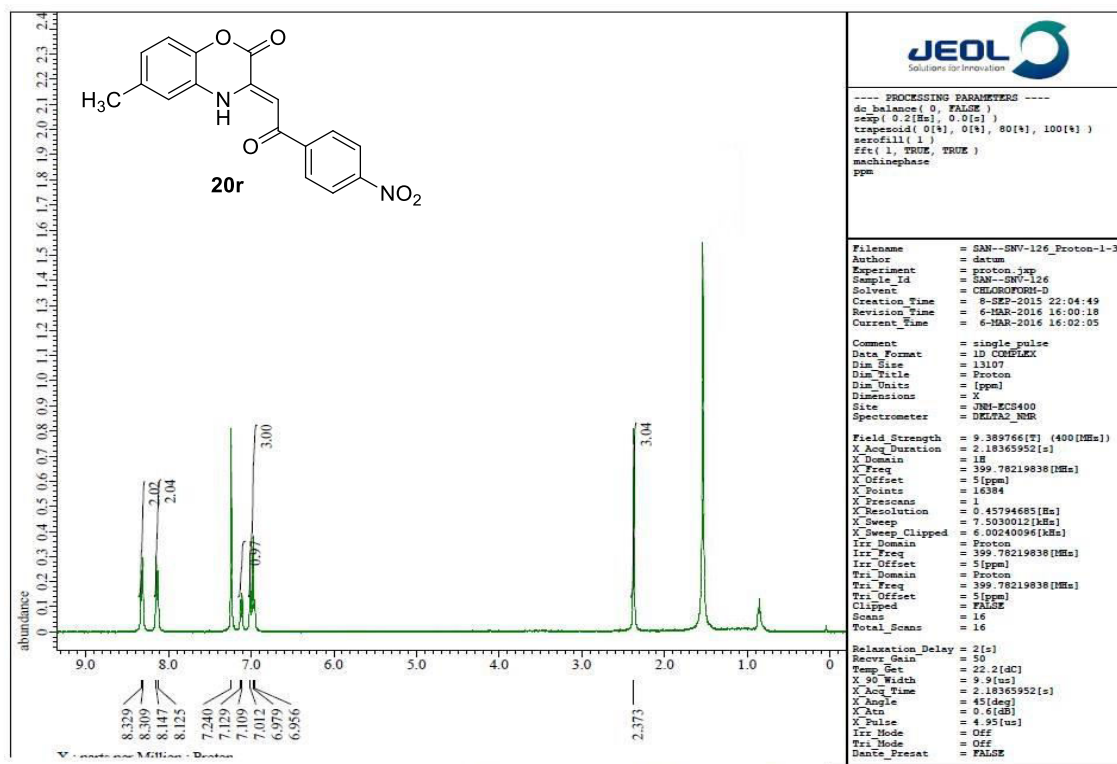


Figure 19. ¹H NMR Spectra of Compound 20r.

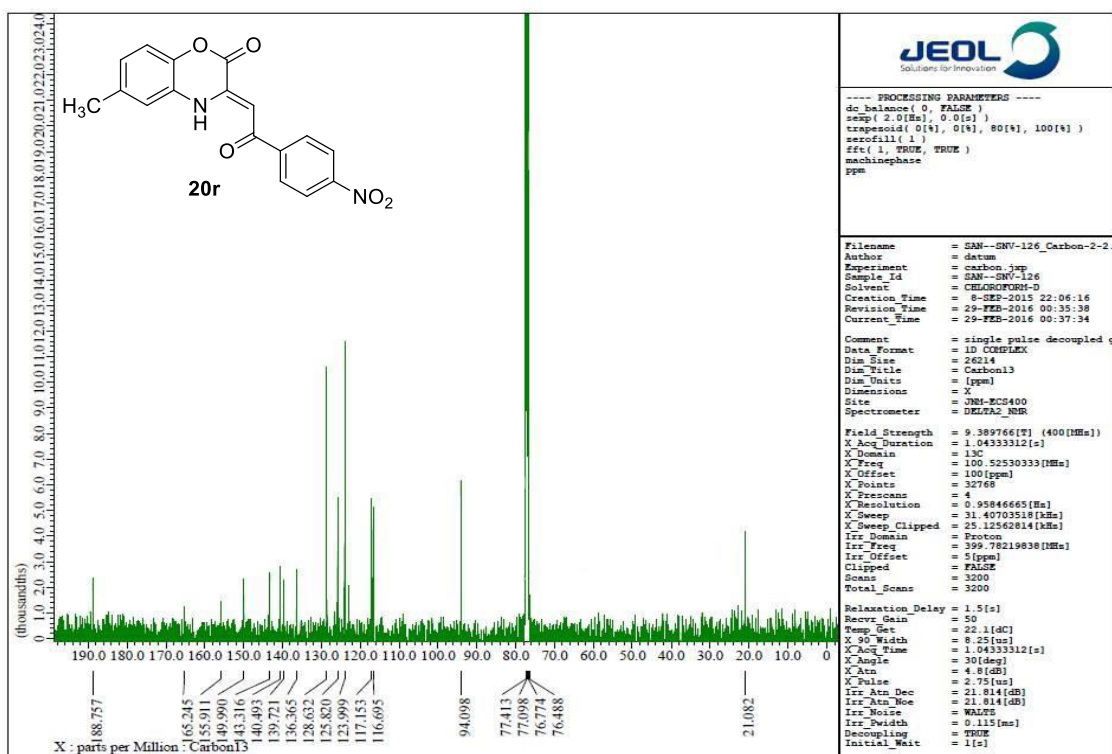
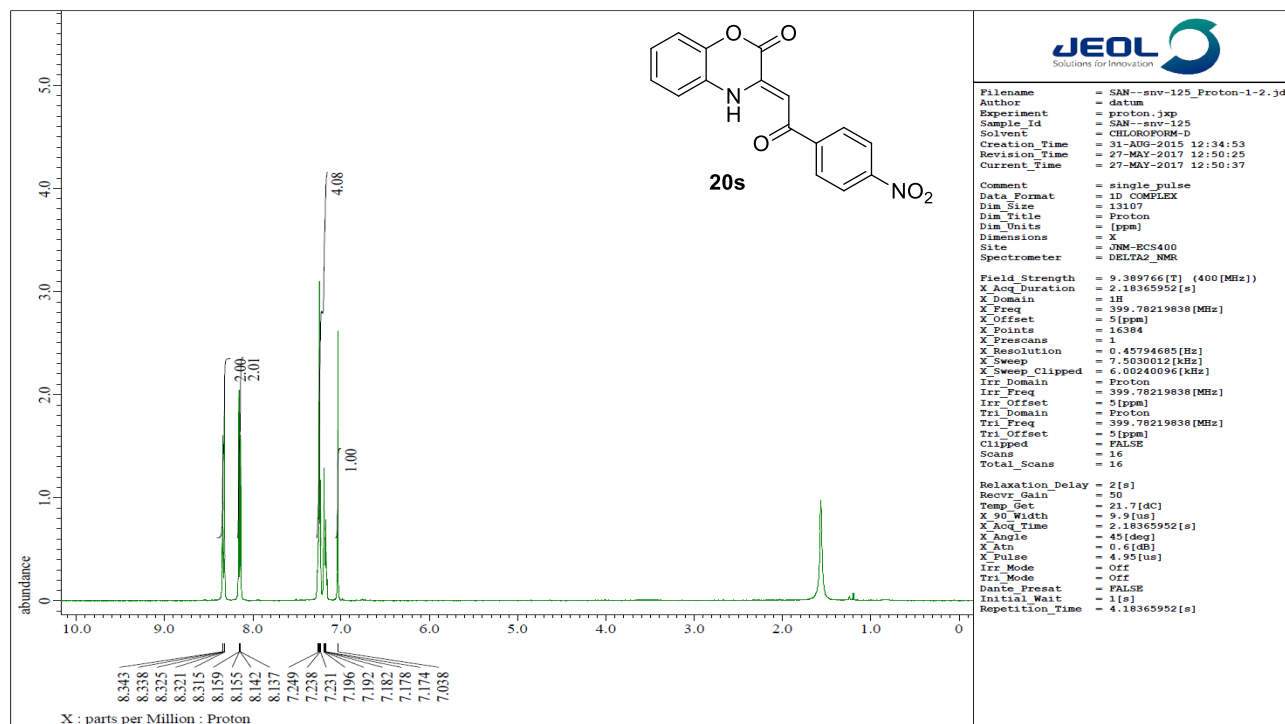
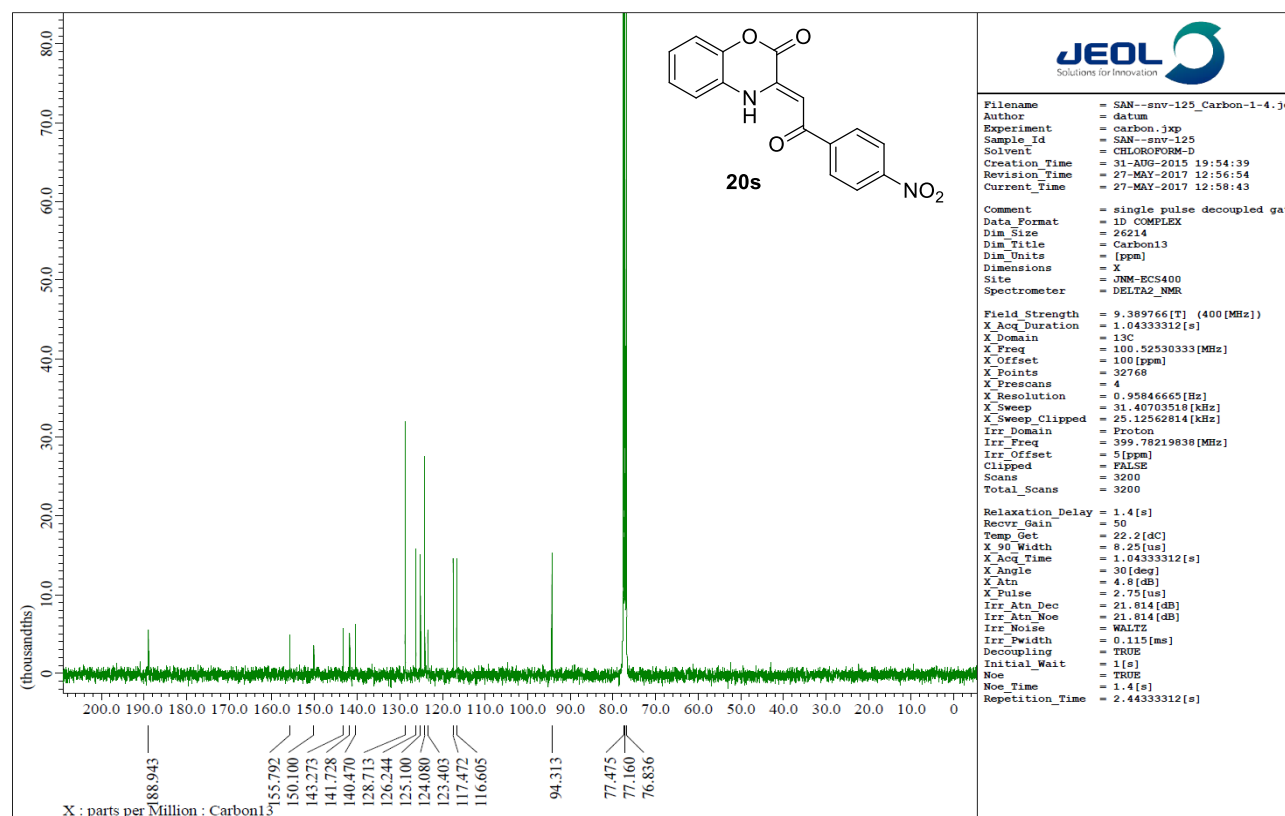
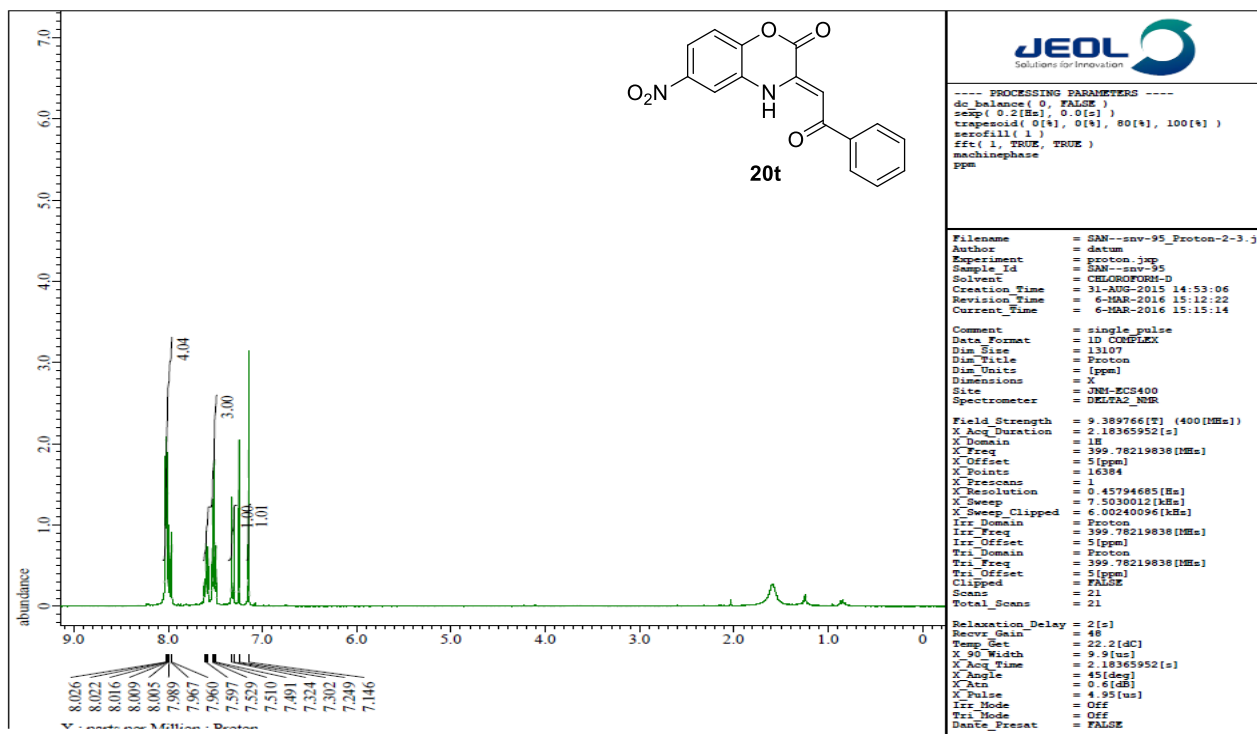


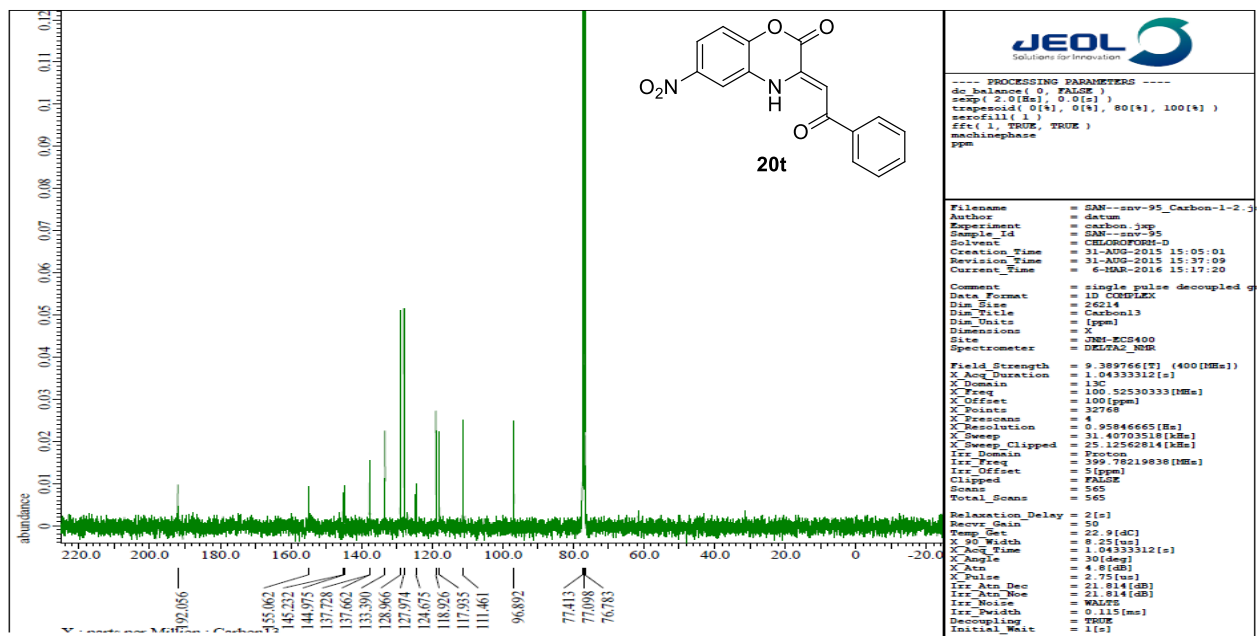
Figure 20. ¹³C NMR Spectra of Compound 20r.

Figure 21. ¹H NMR Spectra of Compound 20s.Figure 22. ¹³C NMR Spectra of Compound 20s.



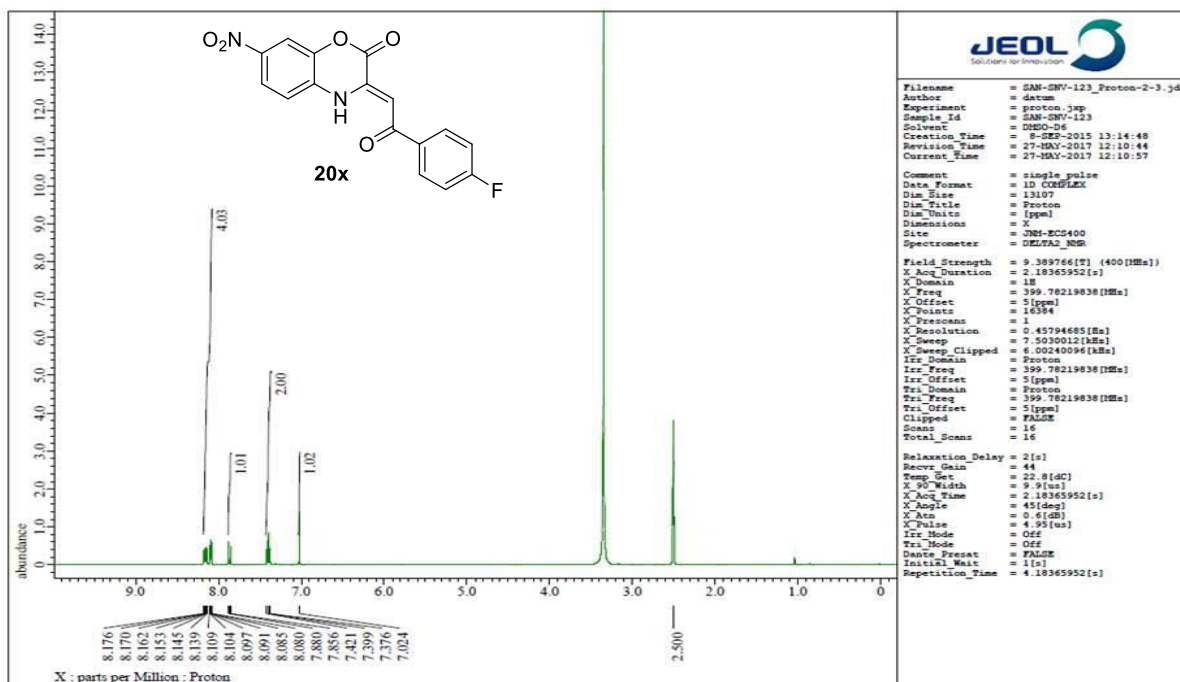
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Figure 23. ¹H NMR Spectra of Compound 20t.



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Figure 24. ¹³C NMR Spectra of Compound 20t.



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Figure 25. ^1H NMR Spectra of Compound **20x**.

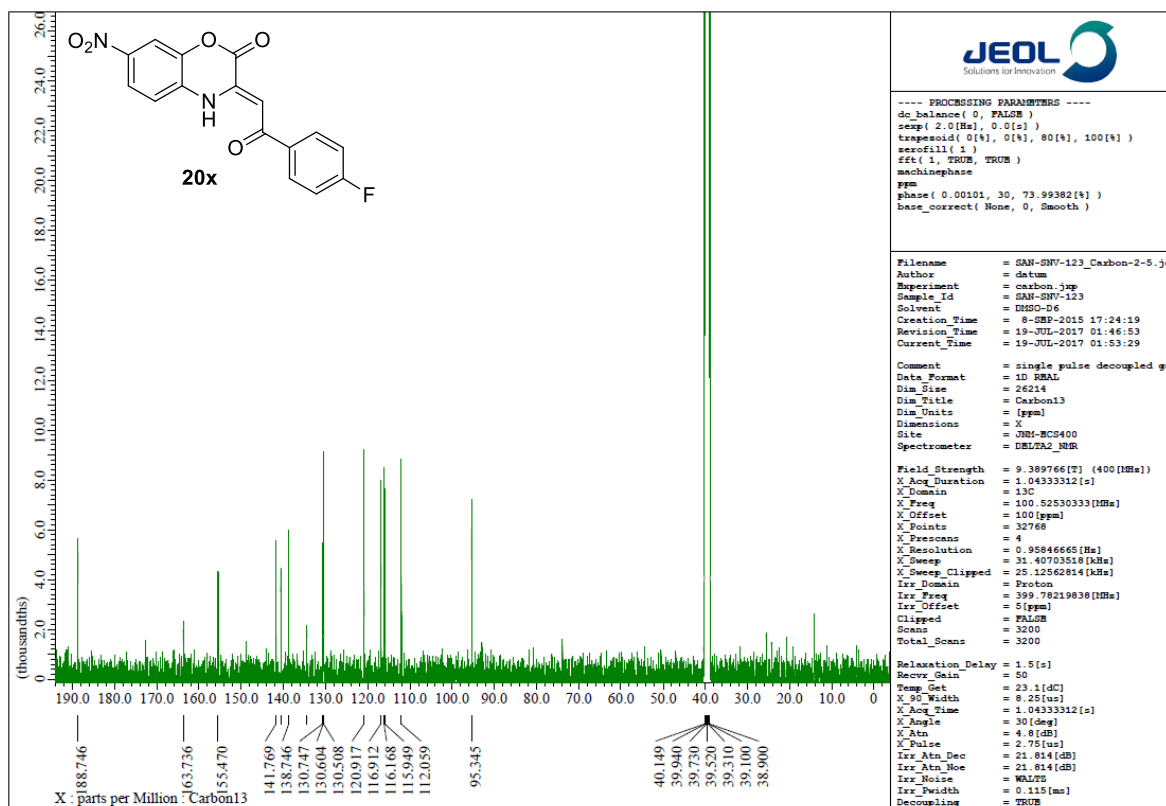
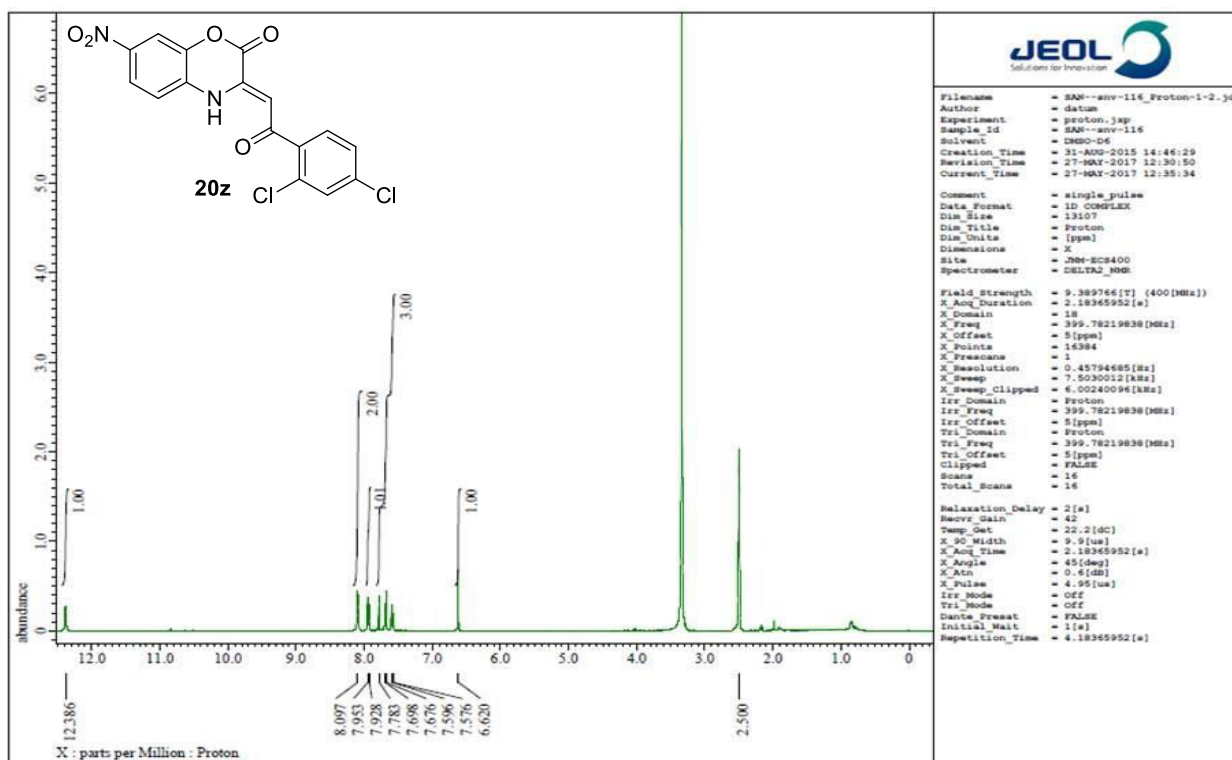


Figure 26. ^{13}C NMR Spectra of Compound **20x**.



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Figure 27. ^1H NMR Spectra of Compound **20z**.

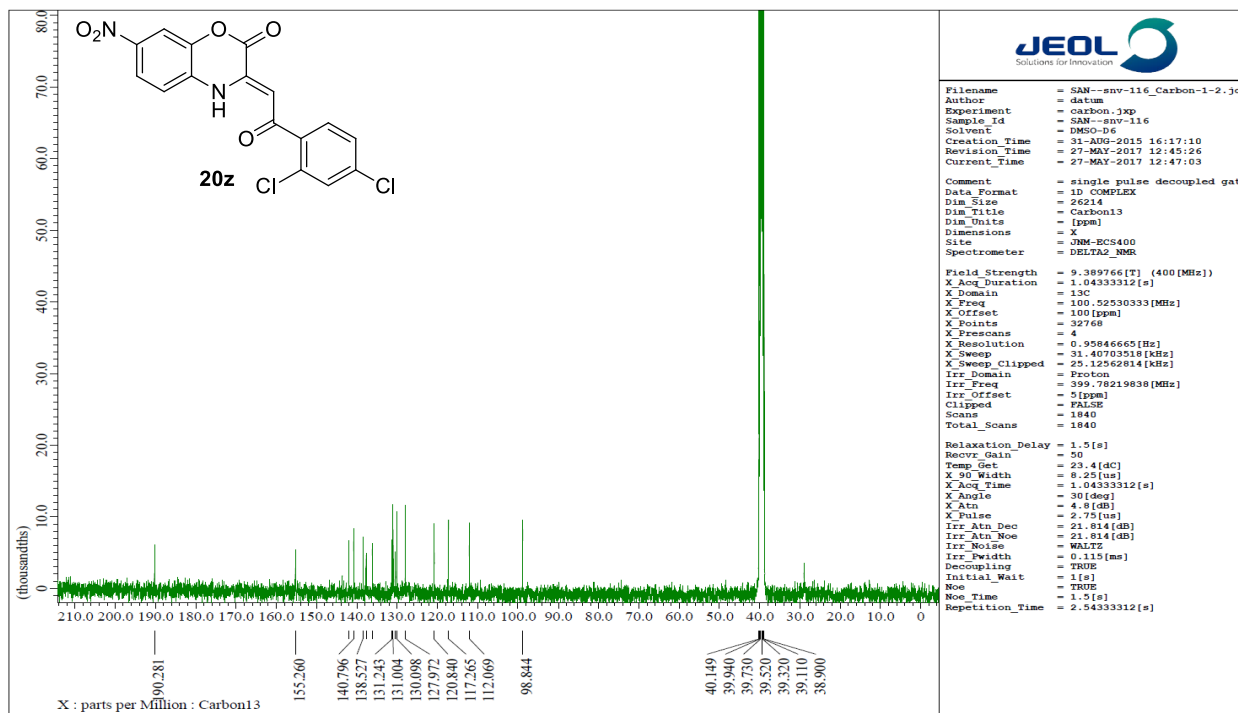


Figure 28. ^{13}C NMR Spectra of Compound **20z**.

Cytotoxicity Assay

The cytotoxic effect of the active compounds prepared on cells was detected *in vitro* using the mitochondrial cytotoxic test according to Danihelová et al. (2013) with modifications. Cell viability was evaluated using thiazolyl blue tetrazolium bromide (MTT), which indicates the metabolic activity of cells. The experiment was performed in 96-well microplates. The cells were seeded at a density of 3.5×10^3 3T3 fibroblast cells per well. Samples were dissolved in DMSO (stock solution 10 mM) and subsequently diluted in medium to the final concentration of 25 μ M to 250 μ M (concentration of DMSO 0.5%) and after 24 h they were added to the cells. Microplates were cultivated for 72 h in thermostat at 37 °C and 5% CO₂ atmosphere. After incubation thiazolyl blue tetrazolium bromide (3.33 mg/ml phosphate buffered saline, pH=7.4) was pipetted to each well and left to incubate for further two hours. Then the medium with MTT solution was removed. Formazan crystals in viable cells were dissolved in the lysis solution (4 mM HCl and 0.1% Nonidet P40 in ethanol). Microplates were shaken 15 min at 1500 rpm. Absorbance was measured at 540 nm and reference wavelength at 740 nm. Each value is the mean of 6 wells with standard deviation. Inhibition activity was expressed as percentages of control with DMSO.

