

Supplementary Material

Flavonoid derivatives as new potent inhibitors of cysteine proteases: An important step toward the design of new compounds for the treatment of leishmaniasis

Estela Mariana Guimarães Lourenço¹, Juliana Fortes Di Iório², Fernanda da Silva³, Felipe Leonardo Bley Fialho¹, Melquisedeque Mateus Monteiro⁴, Adilson Beatriz¹, Renata Trentin Perdomo⁴, Euzébio Guimarães Barbosa⁵, Jean Pierre Oses,⁶ Carla Cardozo Pinto de Arruda³, Wagner Alves de Souza Júdice^{2,*}, Jamal Rafique^{1,*}, Dênis Pires de Lima^{1,*}

- ¹ Laboratory of Synthesis and Transformation of Organic Molecules - SINTMOL, Institute of Chemistry, Universidade Federal de Mato Grosso do Sul, Av. Senador Filinto Muller, 1555, Campo Grande, MS, Brazil
- ² Centro Interdisciplinar de Investigação Bioquímica (CIIB), Universidade de Mogi das Cruzes (UMC), Mogi das Cruzes, SP, Brazil
- ³ Laboratório de Parasitologia Humana, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
- ⁴ Laboratory of Molecular Biology and Cell Culture, School of Pharmaceutical Sciences, Food Technology, and Nutrition, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
- ⁵ Laboratório de Química Farmacêutica Computacional, Departamento de Farmácia, Universidade Federal do Rio Grande do Norte, Natal, Brazil
- ⁶ Laboratório de Neurociências, Instituto de Biociências, Universidade Federal do Rio Grande, Rio Grande, RS, Brazil.

* Correspondence:

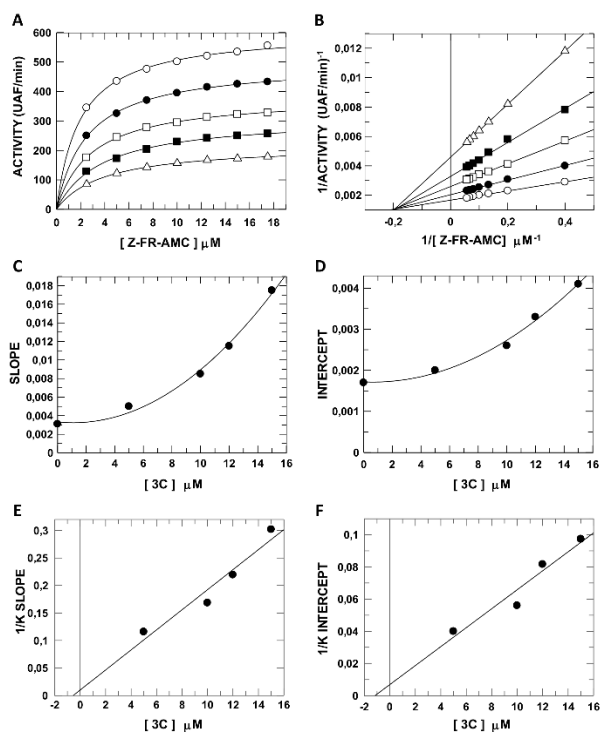
wagnerjudice@gmail.com (WASJ)

jamal.chm@gmail.com; jamal.rafique@ufms.br (JR)

denis.lima@ufms.br (DPL)

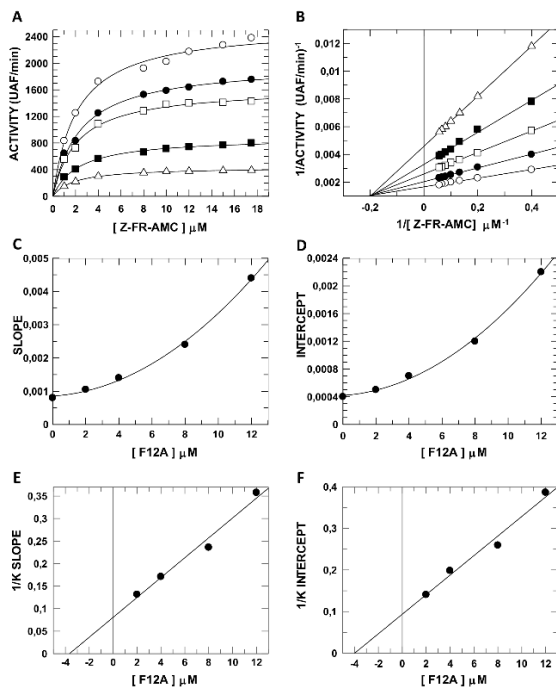
Summary

Figure S1: Determination of the affinity constants of the 3c compound in the inhibition of rCPB2.8.....	3
Figure S2: Determination of the affinity constants of the f12a compound in the inhibition of rCPB2.8.....	3
Figure S3: Determination of the affinity constants of the f12b compound in the inhibition of rCPB2.8.....	4
Figure S4: Determination of the affinity constants of the 3c compound in the inhibition of rCPB3.....	4
Figure S5: Determination of the affinity constants of the f12a compound in the inhibition of rCPB3.....	5
Figure S6: Determination of the affinity constants of the f12b compound in the inhibition of rCPB3.....	5
Figure S7: Determination of the affinity constants of the 3c compound in the inhibition of rH84Y.....	6
Figure S8: Determination of the affinity constants of the f12a compound in the inhibition of rH84Y.....	6
Figure S9: Determination of the affinity constants of the f12b compound in the inhibition of rH84Y.....	7
Table S1: Differences of amino acid residues at the active site of rCPB2.8, rCPB3 and rH84Y.....	Error! Bookmark not defined.
Table S2: Potential energy values for the biding positions of compound 3c at the active site of rCPB2.8	9
Table S3: Potential energy values for the biding positions of compound f12a at the active site of rCPB3a.....	9
Figure S10: Cytotoxicity of all flavonoid derivatives against NHI-3T3 cells.....	9
NMR spectra section	10



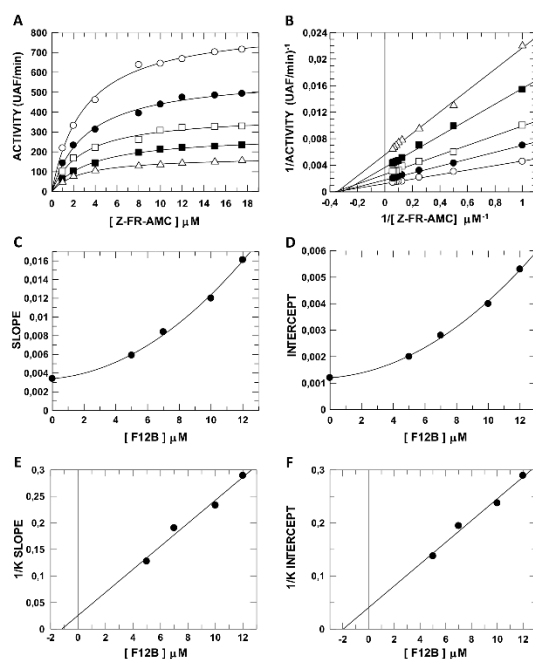
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{Slope}}$ replot; F: $1/K_{\text{Intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 5 \mu\text{M}$; $\bullet \rightarrow 10 \mu\text{M}$; $\blacksquare \rightarrow 12 \mu\text{M}$; $\triangle \rightarrow 15 \mu\text{M}$

Figure S1: Determination of the affinity constants of the **3c** compound in the inhibition of rCPB2.8



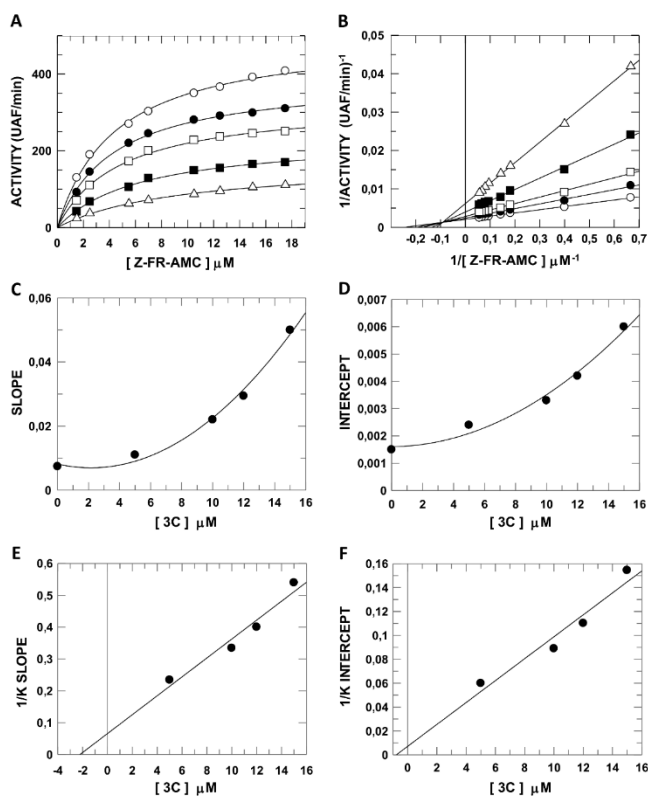
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{Slope}}$ replot; F: $1/K_{\text{Intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 2 \mu\text{M}$; $\bullet \rightarrow 4 \mu\text{M}$; $\blacksquare \rightarrow 8 \mu\text{M}$; $\triangle \rightarrow 12 \mu\text{M}$

Figure S2: Determination of the affinity constants of the **f12a** compound in the inhibition of rCPB2.8.



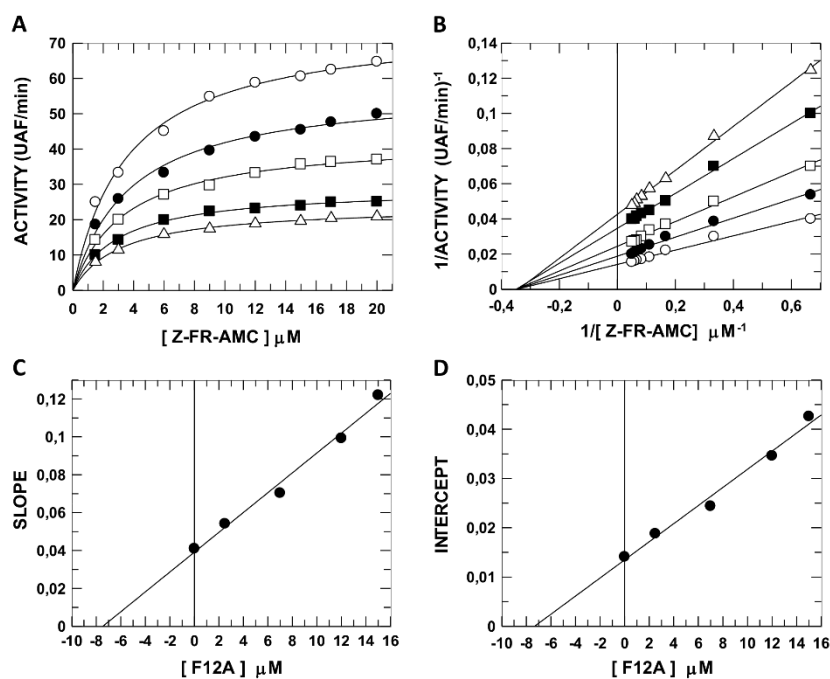
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{slope}}$ replot; F: $1/K_{\text{intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 5 \mu\text{M}$; $\bullet \rightarrow 7 \mu\text{M}$; $\blacksquare \rightarrow 10 \mu\text{M}$; $\triangle \rightarrow 12 \mu\text{M}$

Figure S3: Determination of the affinity constants of the **f12b** compound in the inhibition of rCPB2.8.



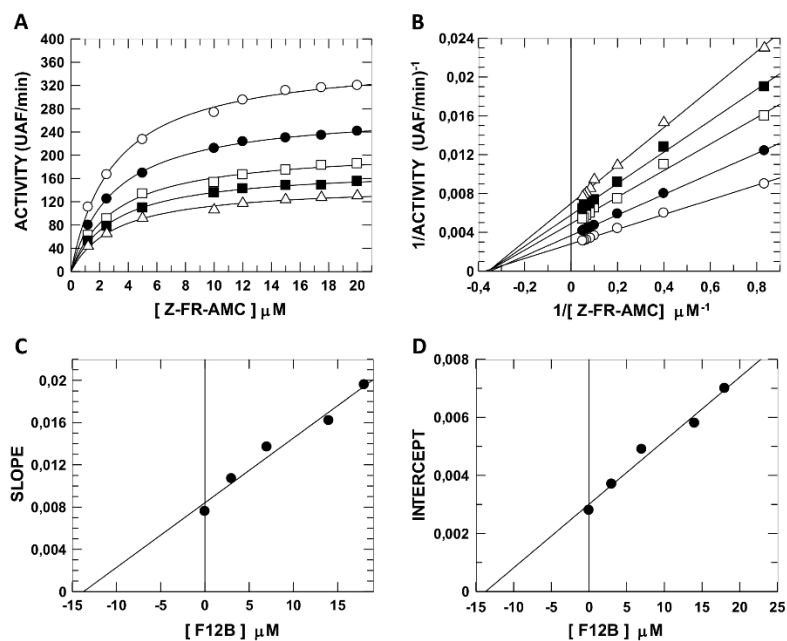
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{slope}}$ replot; F: $1/K_{\text{intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 5 \mu\text{M}$; $\bullet \rightarrow 10 \mu\text{M}$; $\blacksquare \rightarrow 12 \mu\text{M}$; $\triangle \rightarrow 15 \mu\text{M}$

Figure S4: Determination of the affinity constants of the **3c** compound in the inhibition of rCPB3.



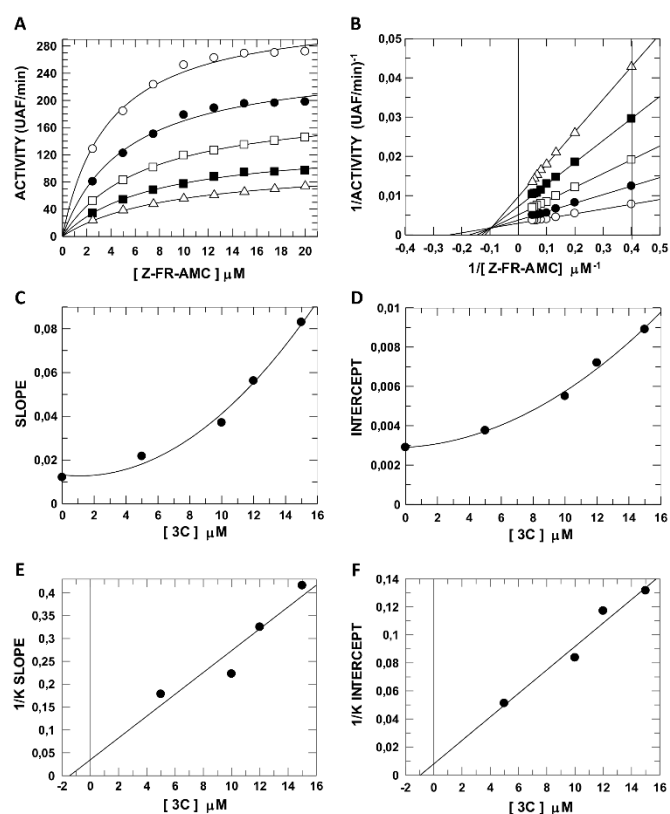
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{Slope}}$ replot; F: $1/K_{\text{Intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow$ 2.5 μM ; $\bullet \rightarrow$ 7 μM ; $\blacksquare \rightarrow$ 12 μM ; $\triangle \rightarrow$ 15 μM

Figure S5: Determination of the affinity constants of the **f12a** compound in the inhibition of rCPB3.



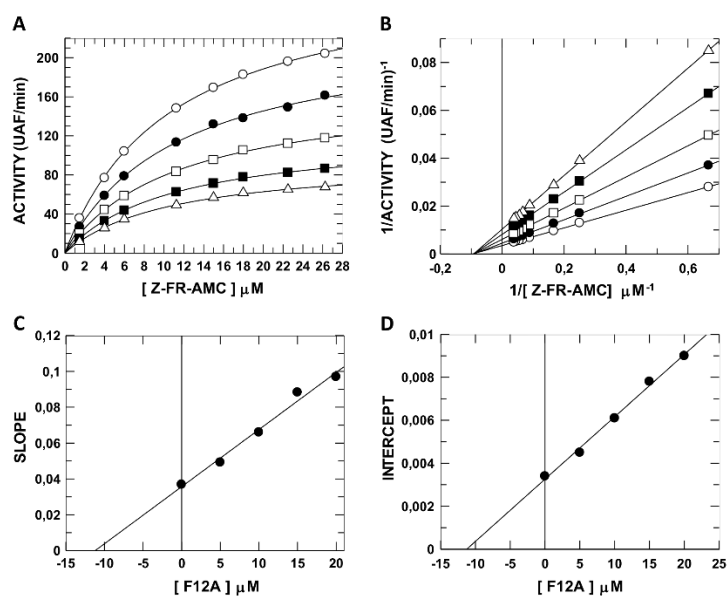
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{Slope}}$ replot; F: $1/K_{\text{Intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow$ 3 μM ; $\bullet \rightarrow$ 7 μM ; $\blacksquare \rightarrow$ 14 μM ; $\triangle \rightarrow$ 18 μM

Figure S6: Determination of the affinity constants of the **f12b** compound in the inhibition of rCPB3.



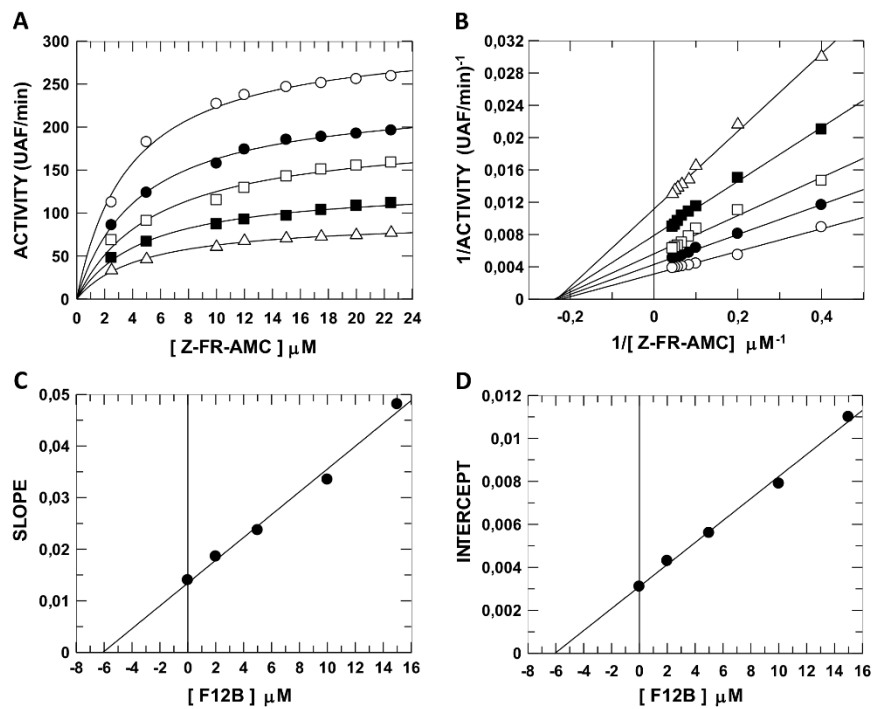
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{slope}}$ replot; F: $1/K_{\text{intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 5 \mu\text{M}$; $\bullet \rightarrow 10 \mu\text{M}$; $\blacksquare \rightarrow 12 \mu\text{M}$; $\triangle \rightarrow 15 \mu\text{M}$

Figure S7: Determination of the affinity constants of the **3c** compound in the inhibition of rH84Y.



A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{slope}}$ replot; F: $1/K_{\text{intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 5 \mu\text{M}$; $\bullet \rightarrow 10 \mu\text{M}$; $\blacksquare \rightarrow 15 \mu\text{M}$; $\triangle \rightarrow 20 \mu\text{M}$

Figure S8: Determination of the affinity constants of the **f12a** compound in the inhibition of rH84Y.



A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{\text{slope}}$ replot; F: $1/K_{\text{intercept}}$ replot. $\circ \rightarrow$ control; $\bullet \rightarrow 2 \mu\text{M}$; $\bullet \rightarrow 5 \mu\text{M}$; $\blacksquare \rightarrow 10 \mu\text{M}$; $\triangle \rightarrow 15 \mu\text{M}$

Figure S9: Determination of the affinity constants of the **f12b** compound in the inhibition of rH84Y.

Table S1: Differences of amino acid residues at the active site of rCPB2.8, rCPB3 and rH84Y

Enzyme ¹	Amino acid residues of active site ²				
	143	185	186	189	209
rCPB2.8	ASN	ASP	ASP	ASP	HIS
rCPB3	ASP	ASN	SER	SER	HIS
rH84Y	ASP	ASN	SER	SER	TYR

¹The number of amino acid residue may change depends on the crystal structure. ²All of three isoforms are recombinant enzymes (r) lacking the C-terminal region.

Table S2: Potential energy values for the binding positions of compound **3c** at the active site of rCPB2.8^a

Positions	Potential energy	Δ Energy
1	-58282	4751
2	-105794	0
3	-90651	1514
4	-101704	409
5	-98316	747
6	-52679	5312
7	-50644	5515
8	-53940	51854

a: All values are expressed in kcal mol⁻¹

Table S3: Potential energy values for the binding positions of compound **f12a** at the active site of rCPB3^a

Positions	Potential energy	Δ Energy
1	-106212	586
2	-62477	44321
3	-106398	400
4	-97614	9184
5	-106798	0
6	-103814	2984
7	-103849	2949
8	-106176	622

a: All values are expressed in kcal mol⁻¹

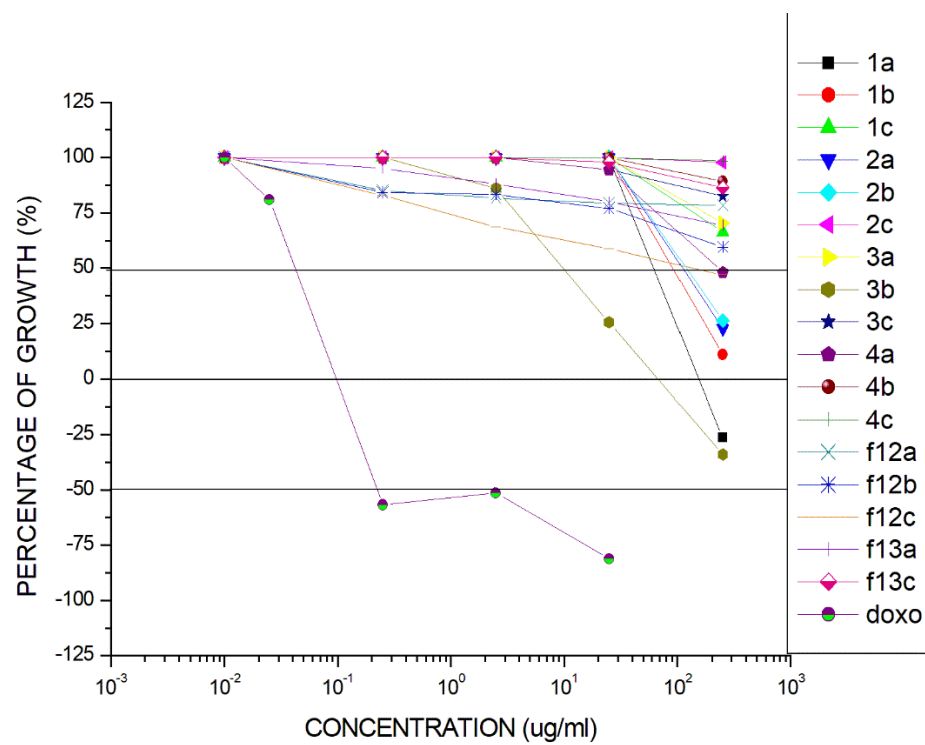
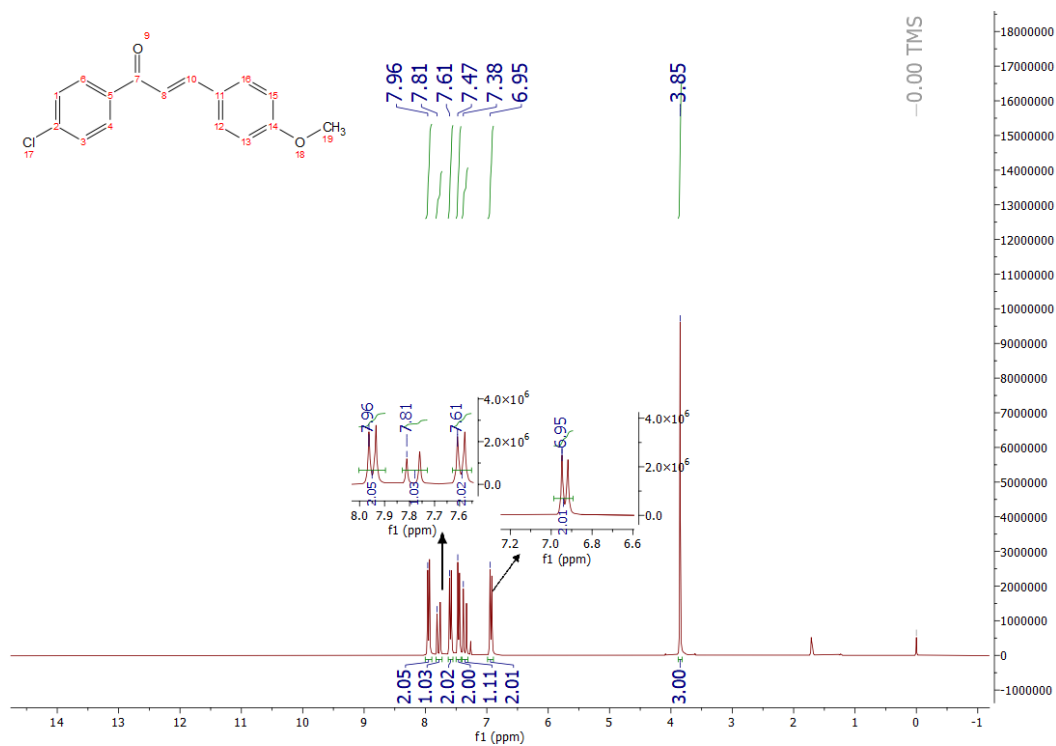


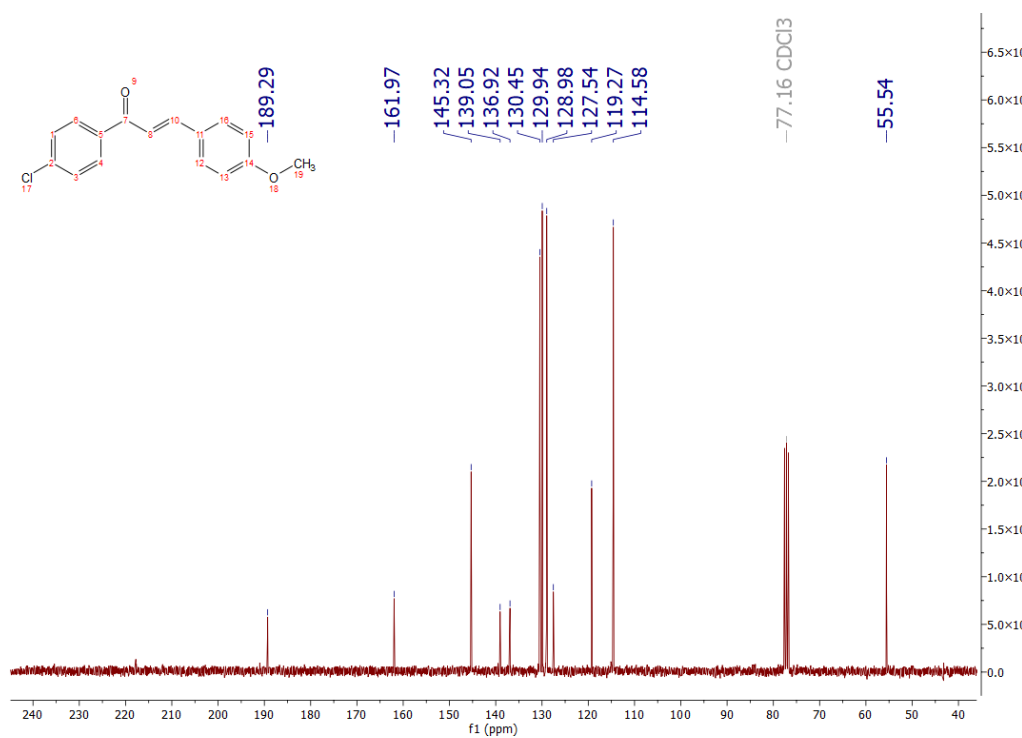
Figure S10: Cytotoxicity of all flavonoid derivatives against NHI-3T3 cells

NMR spectra section

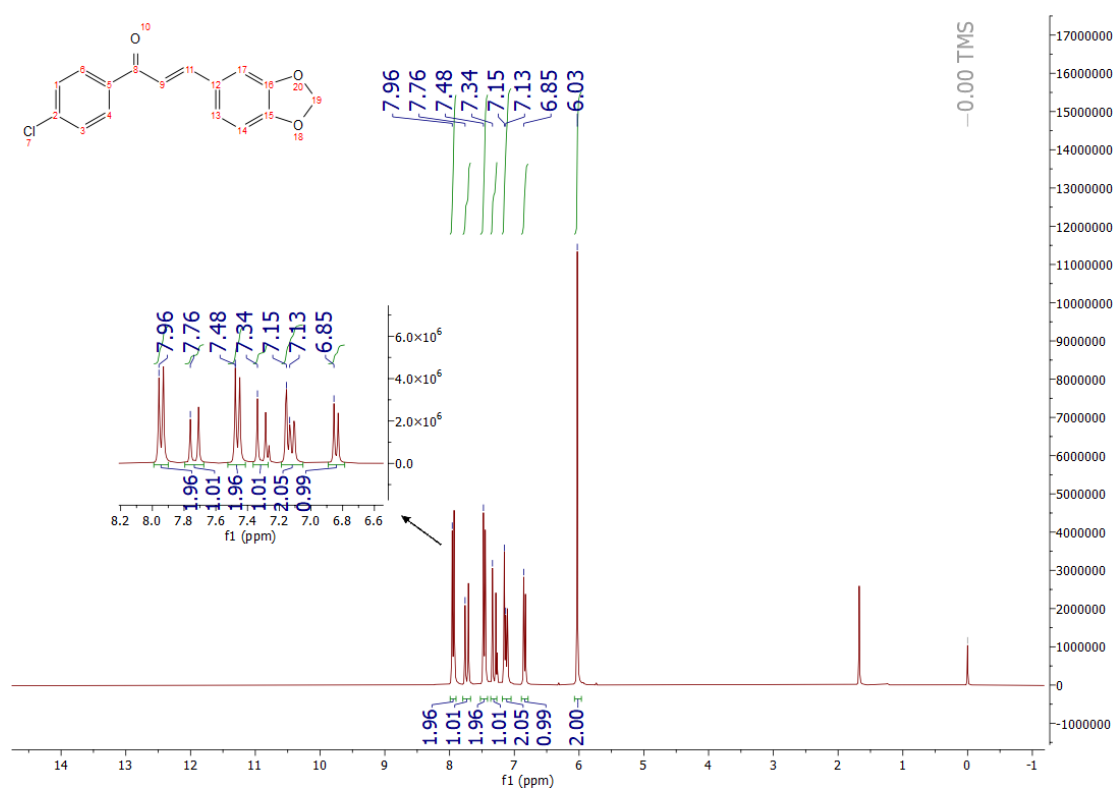
Spectra 1: ^1H NMR of compound 1a	11
Spectra 2: ^{13}C NMR of compound 1a	11
Spectra 3: ^1H NMR of compound 1b	12
Spectra 4: ^{13}C NMR of compound 1b	12
Spectra 5: ^1H NMR of compound 1c	13
Spectra 6: ^{13}C NMR of compound 1c	13
Spectra 7: ^1H NMR of compound 2a	14
Spectra 8: ^{13}C NMR of compound 2a	14
Spectra 9: ^1H NMR of compound 2b	15
Spectra 10: ^{13}C NMR of compound 2b	15
Spectra 11: ^1H NMR of compound 2c	16
Spectra 12: ^{13}C NMR of compound 2c	16
Spectra 13: ^1H NMR of compound 3a	17
Spectra 14: ^{13}C NMR of compound 3a	17
Spectra 15: ^1H NMR of compound 3b	18
Spectra 16: ^{13}C NMR of compound 3b	18
Spectra 17: ^1H NMR of compound 3c	19
Spectra 18: ^{13}C NMR of compound 3c	19
Spectra 19: ^1H NMR of compound 4a	20
Spectra 20: ^{13}C NMR of compound 4a	20
Spectra 21: ^1H NMR of compound 4b	21
Spectra 22: ^{13}C NMR of compound 4b	21
Spectra 23: ^1H NMR of compound 4c	22
Spectra 24: ^{13}C NMR of compound 4c	22
Spectra 25: ^1H NMR of compound f12a	23
Spectra 26: ^{13}C NMR of compound f12a	23
Spectra 27: ^1H NMR of compound f1b	24
Spectra 28: ^{13}C NMR of compound f12b	24
Spectra 29: ^1H NMR of compound f1c	25
Spectra 30: ^{13}C NMR of compound f12c	25
Spectra 31: ^1H NMR of compound f3a	26
Spectra 32: ^{13}C NMR of compound f13a	26
Spectra 33: ^1H NMR of compound f3c	27
Spectra 34: ^{13}C NMR of compound f13c	27



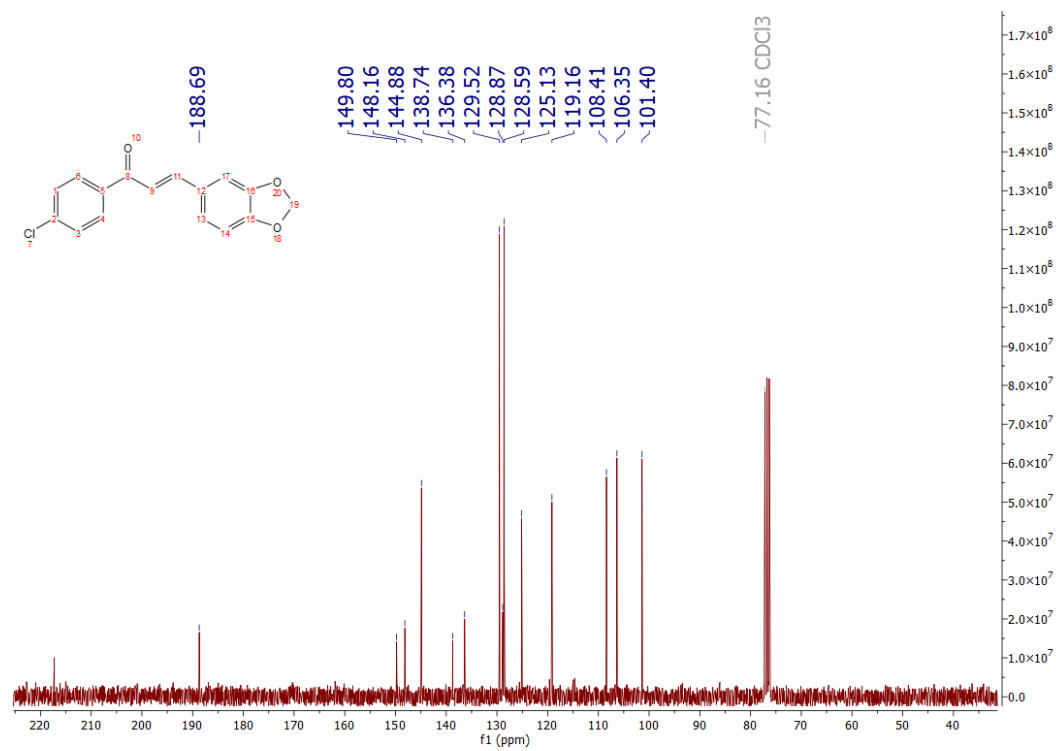
Spectra 1: ¹H NMR of compound 1a



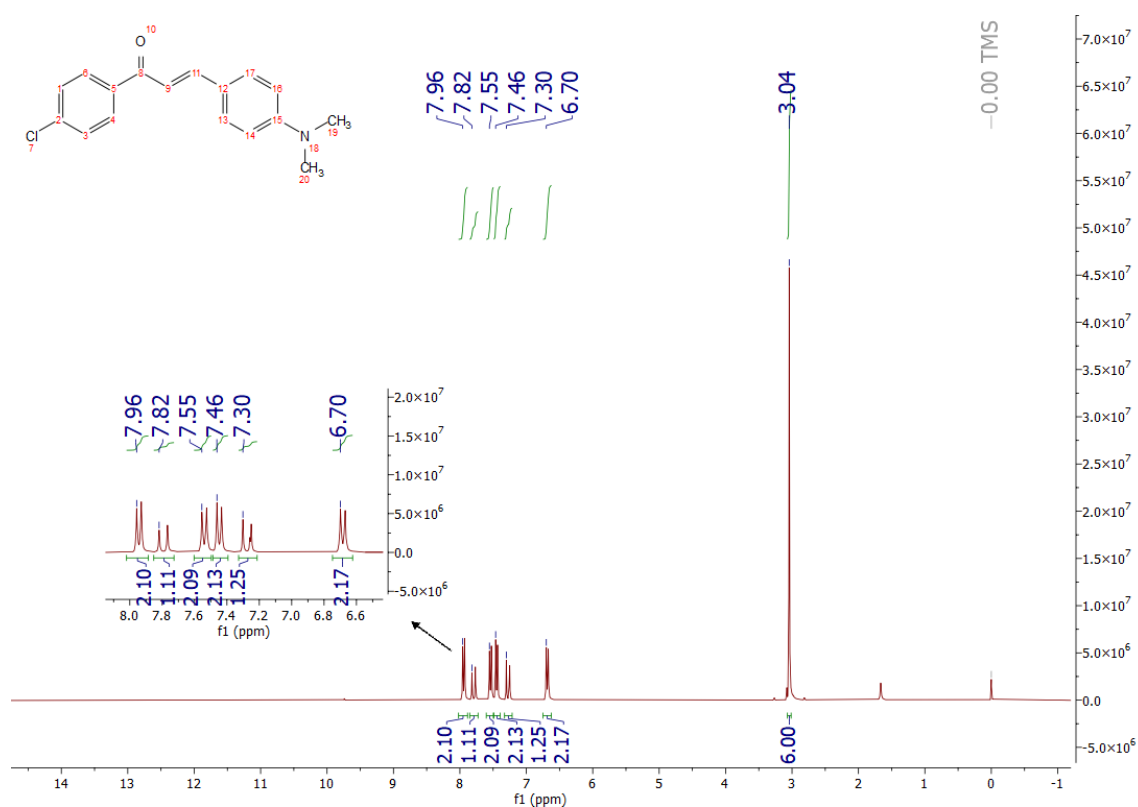
Spectra 2: ¹³C NMR of compound 1a



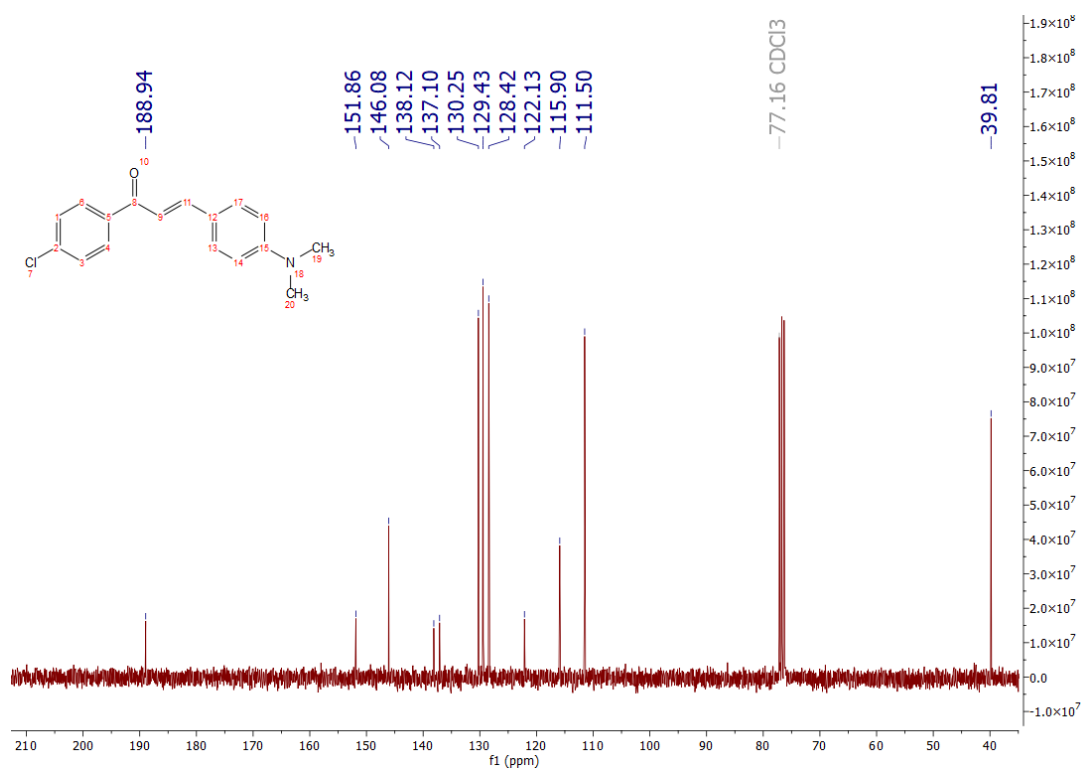
Spectra 3: ¹H NMR of compound **1b**



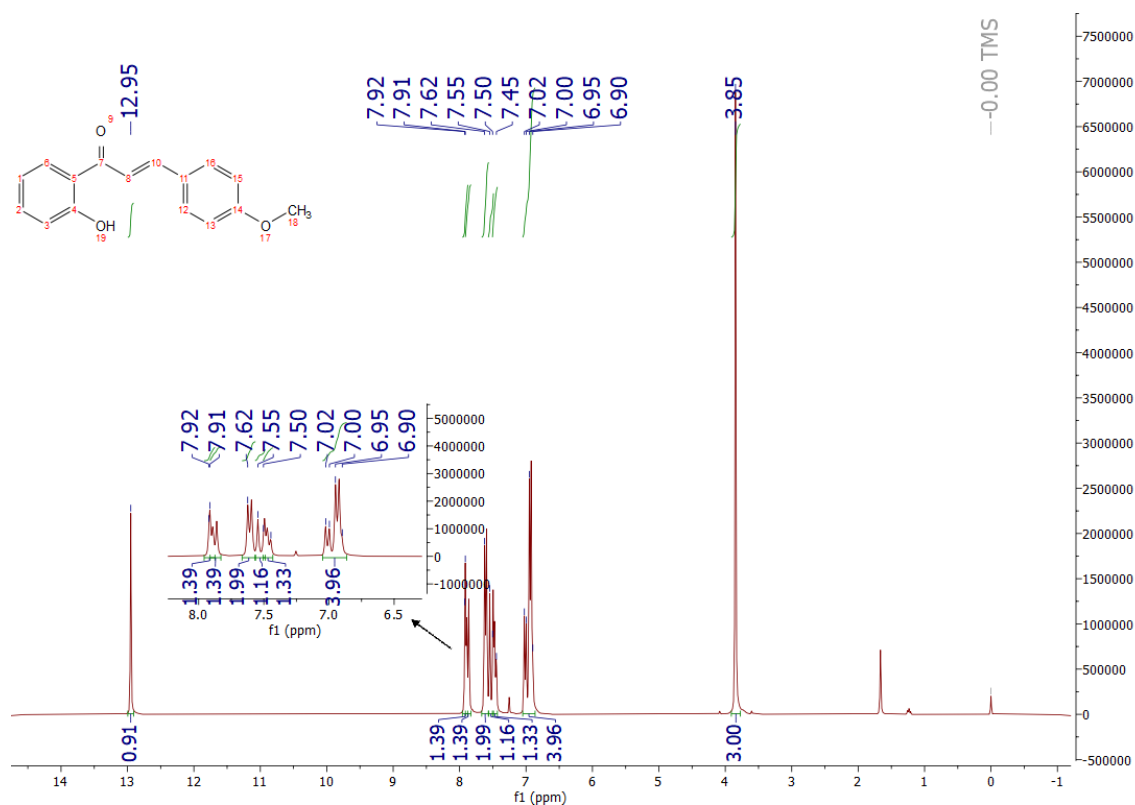
Spectra 4: ¹³C NMR of compound **1b**



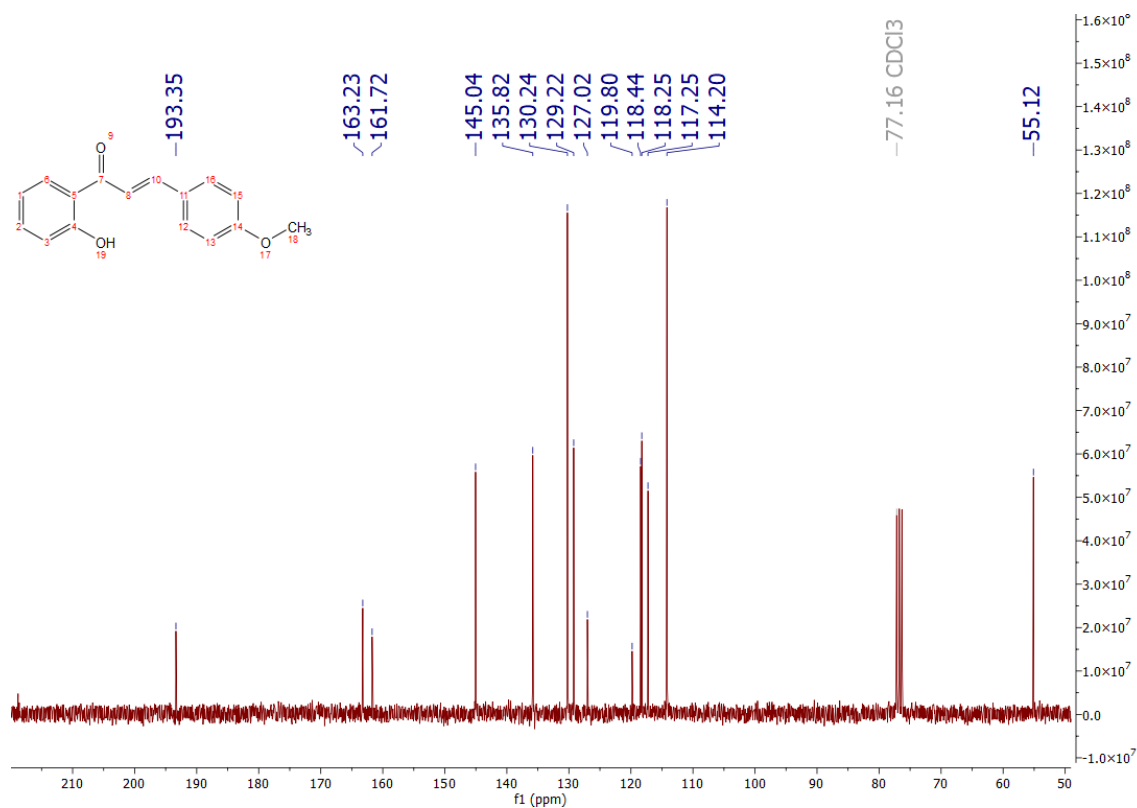
Spectra 5: ¹H NMR of compound **1c**



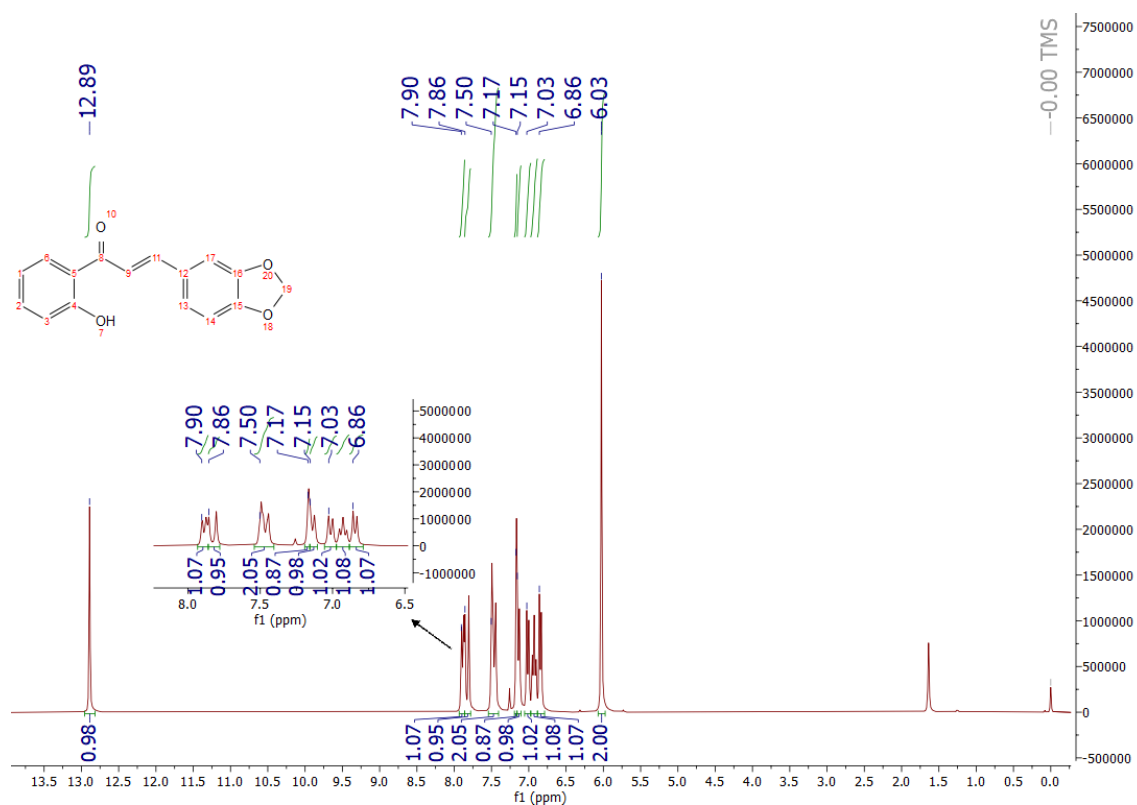
Spectra 6: ¹³C NMR of compound **1c**



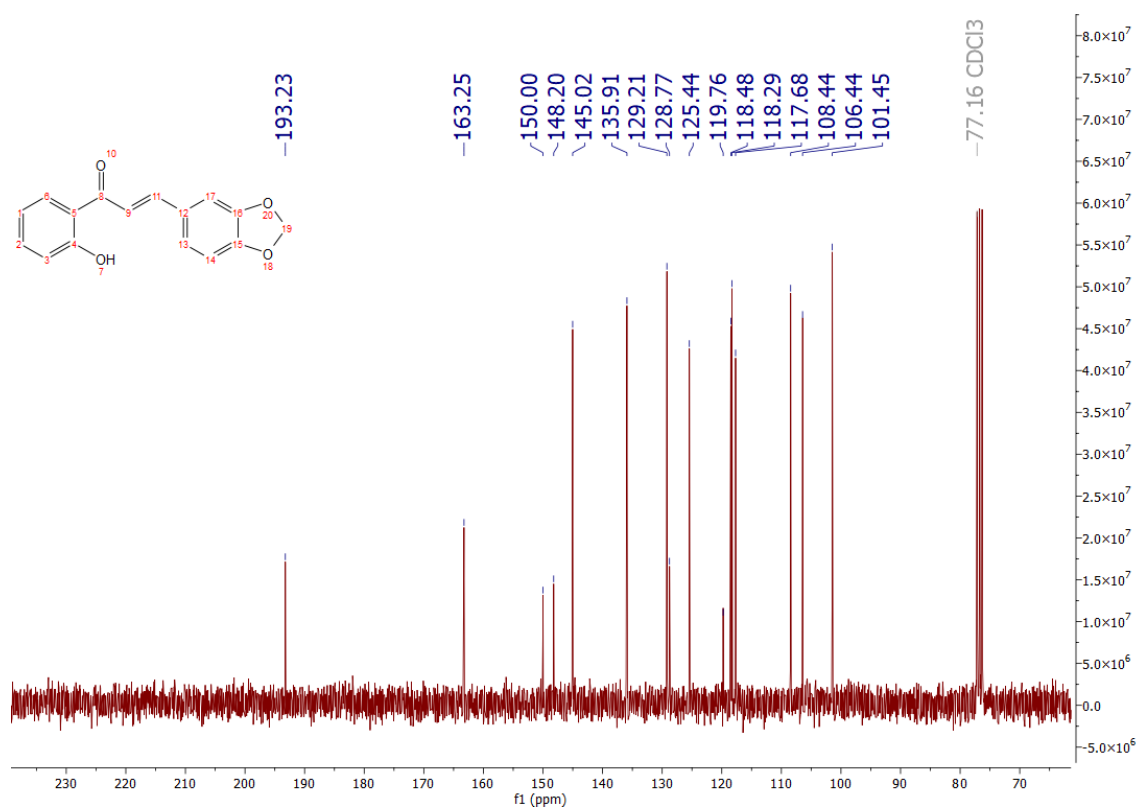
Spectra 7: ¹H NMR of compound **2a**



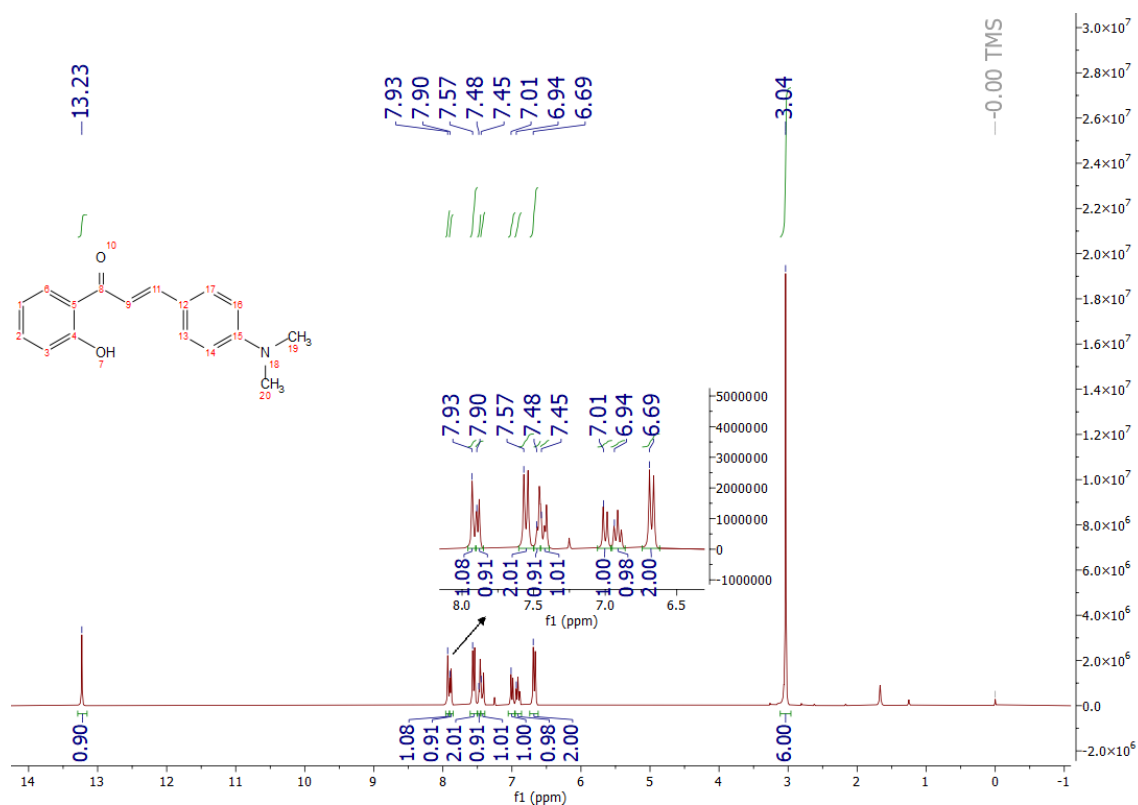
Spectra 8: ¹³C NMR of compound **2a**



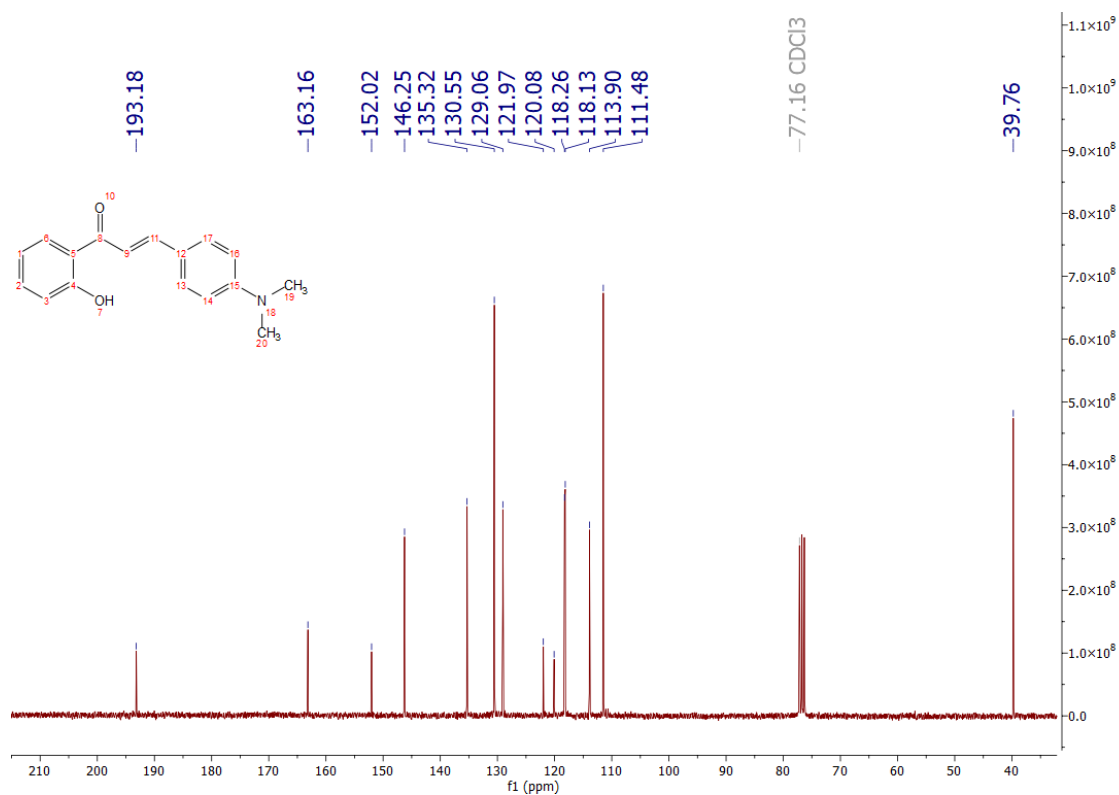
Spectra 9: ¹H NMR of compound **2b**



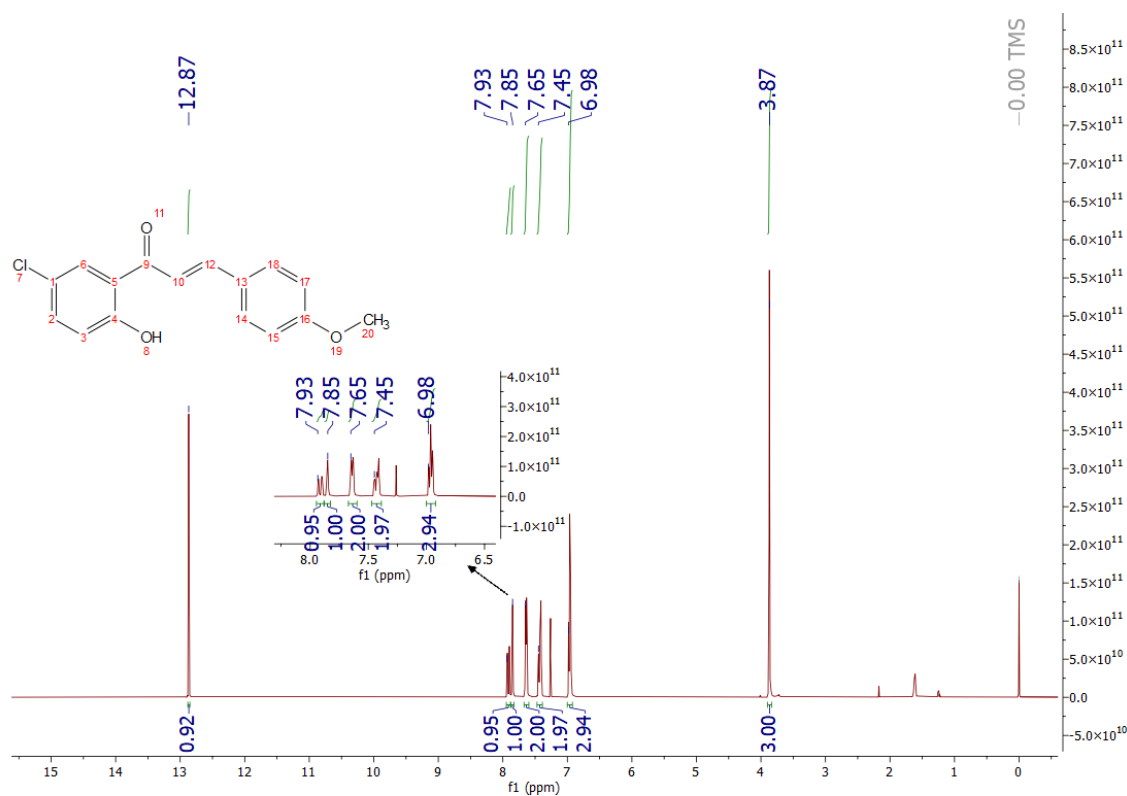
Spectra 10: ¹³C NMR of compound **2b**



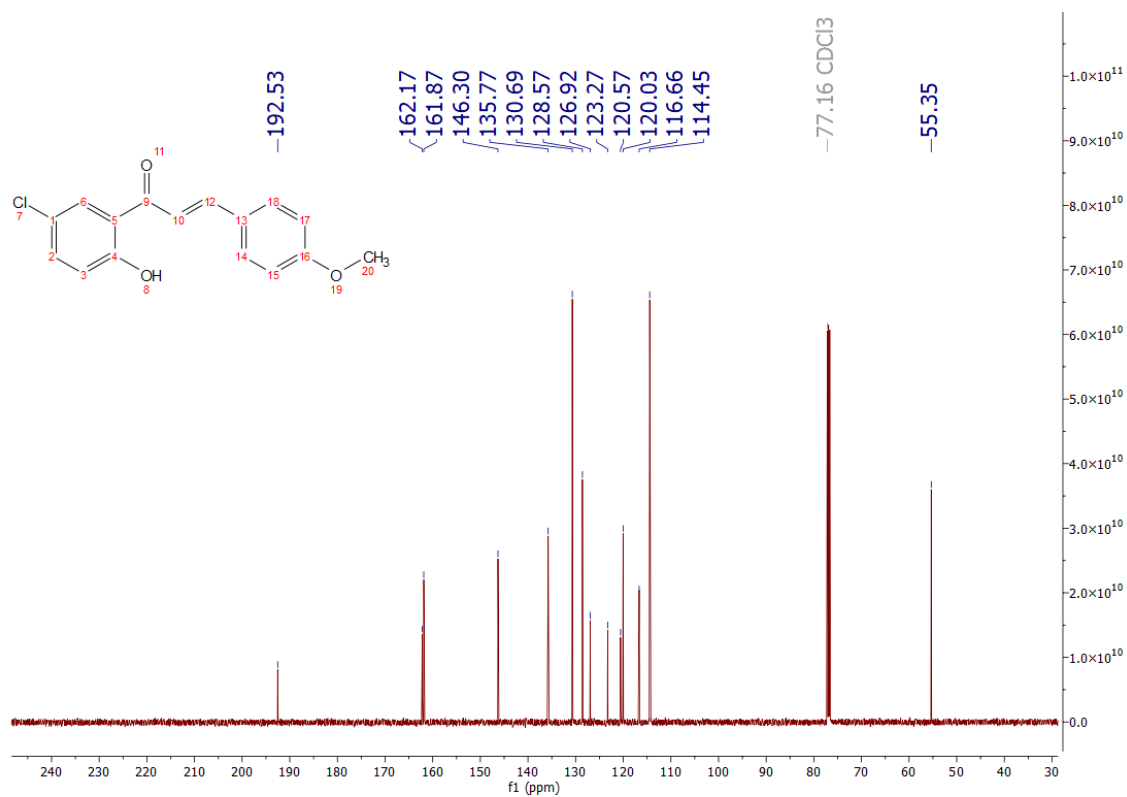
Spectra 11: ¹H NMR of compound **2c**



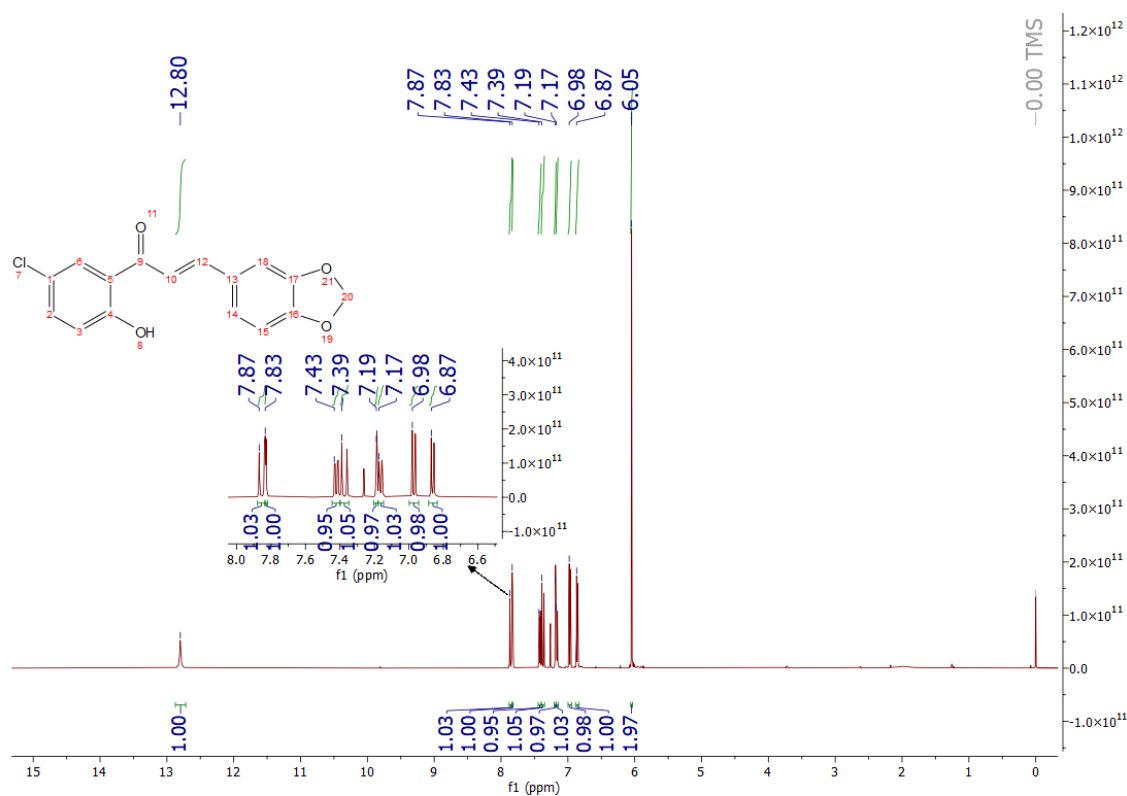
Spectra 12: ¹³C NMR of compound **2c**



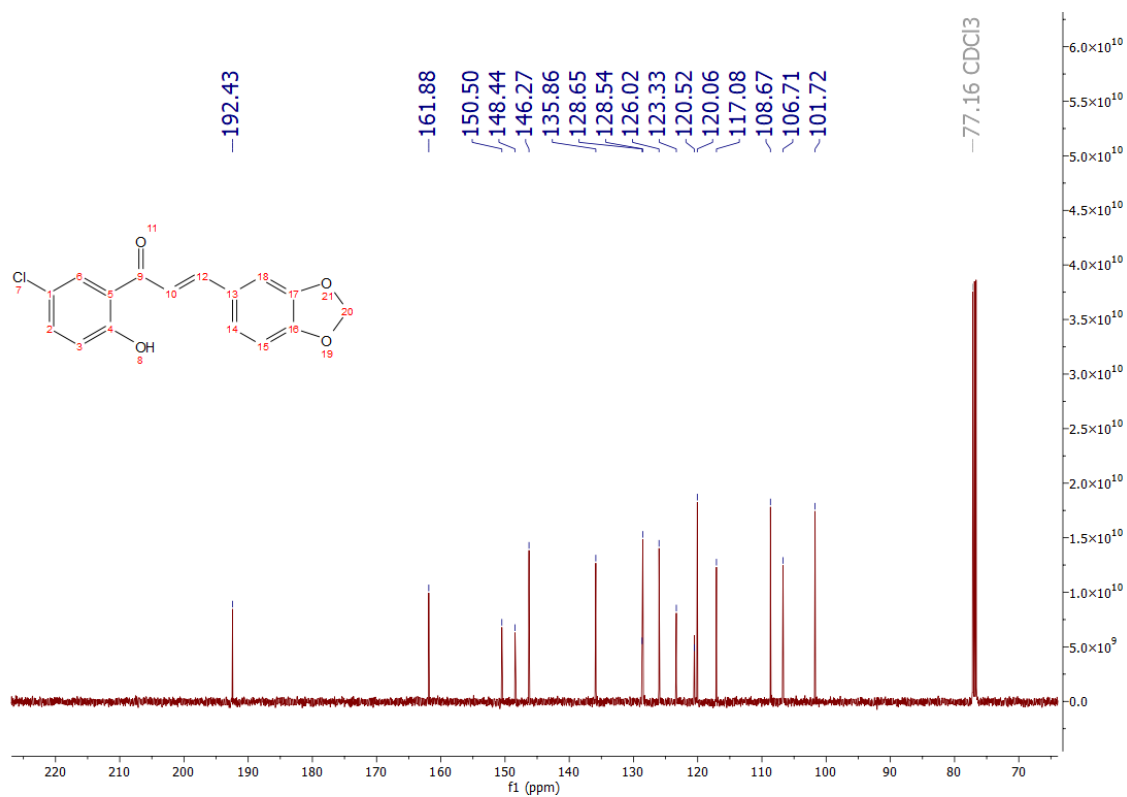
Spectra 13: ¹H NMR of compound 3a



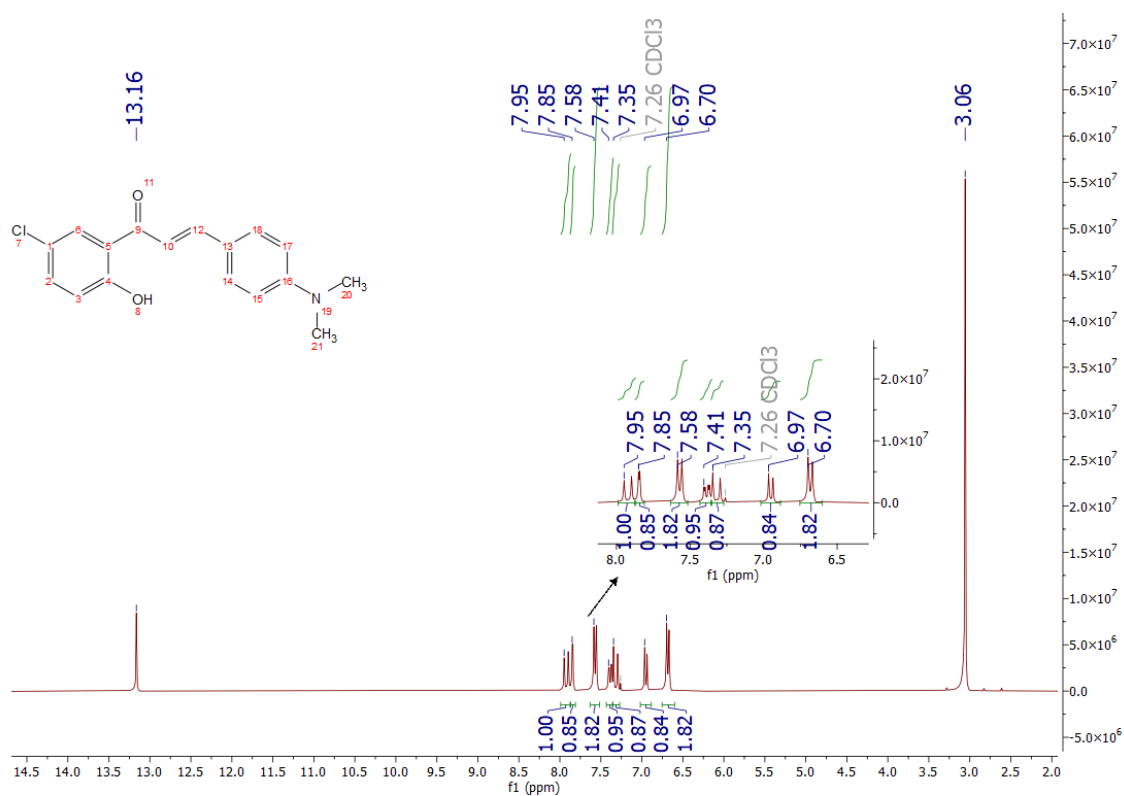
Spectra 14: ¹³C NMR of compound 3a



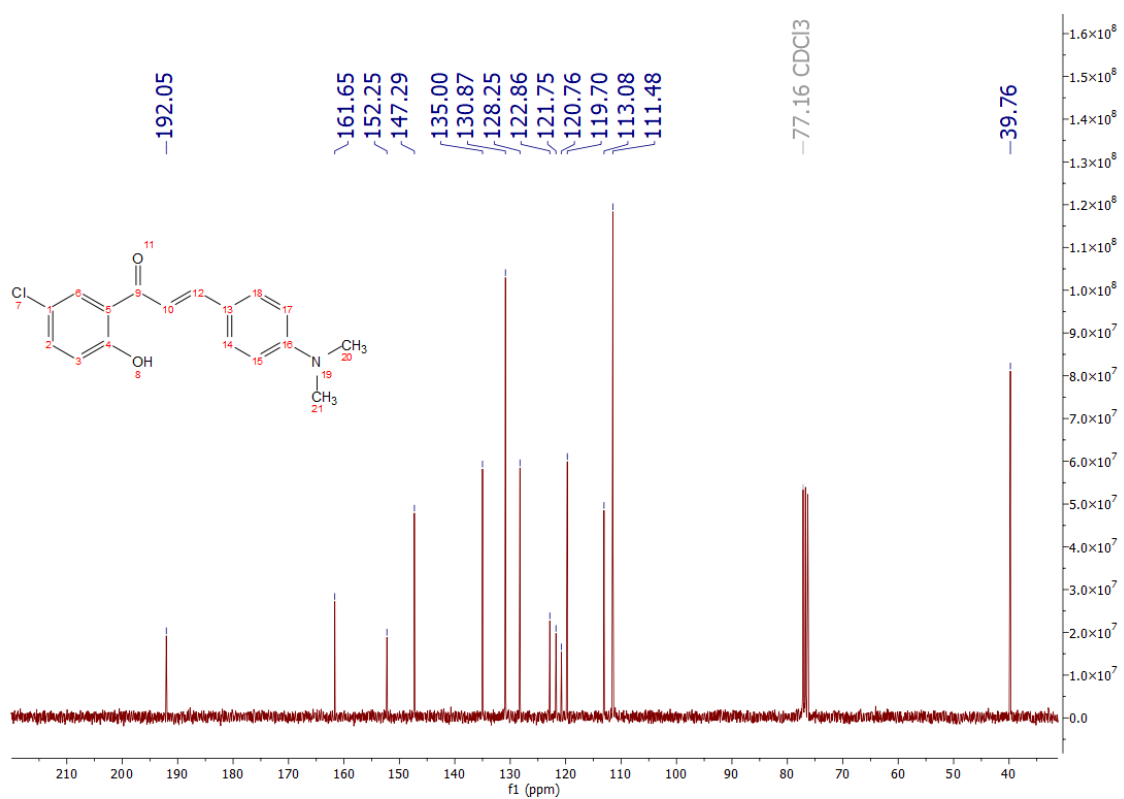
Spectra 15: ¹H NMR of compound **3b**



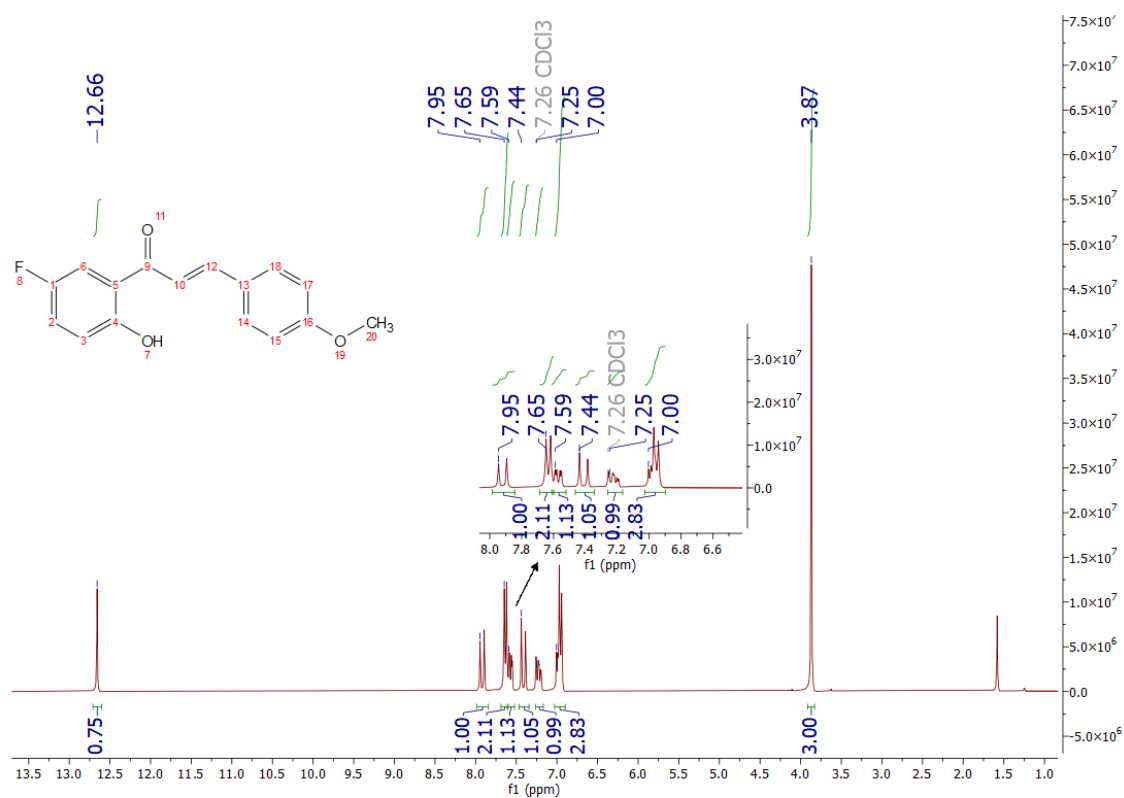
Spectra 16: ¹³C NMR of compound **3b**



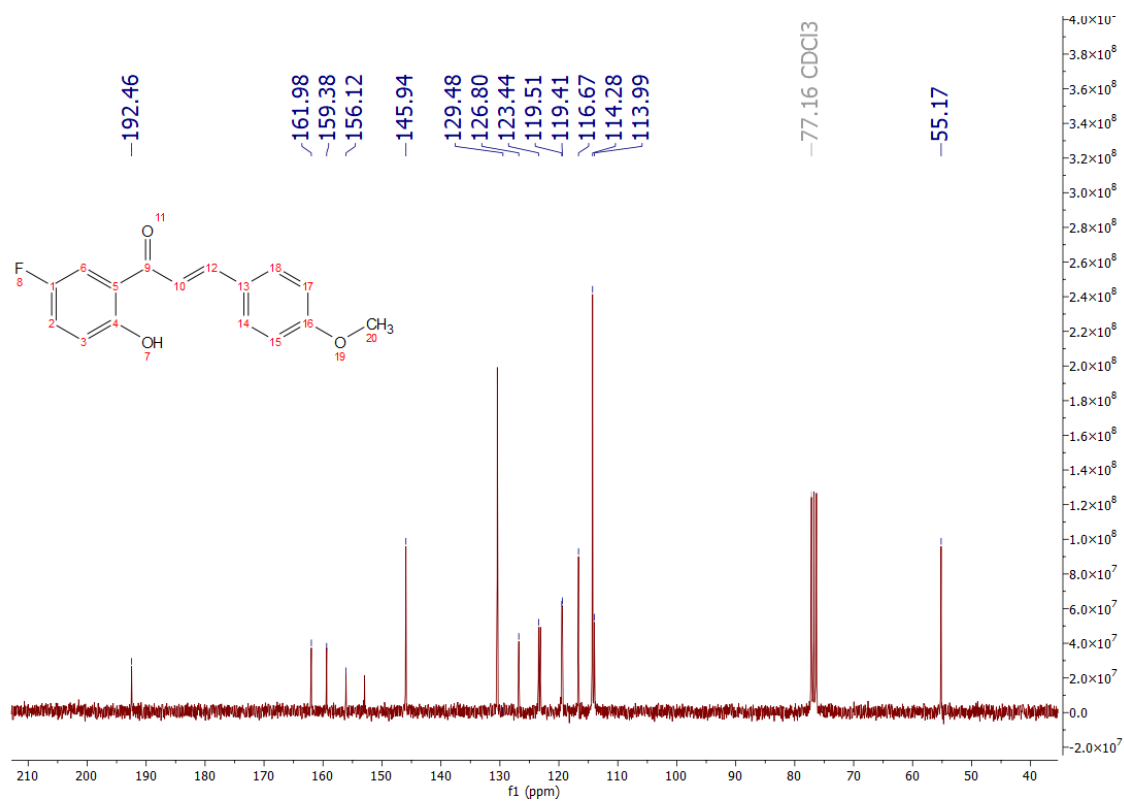
Spectra 17: ¹H NMR of compound 3c



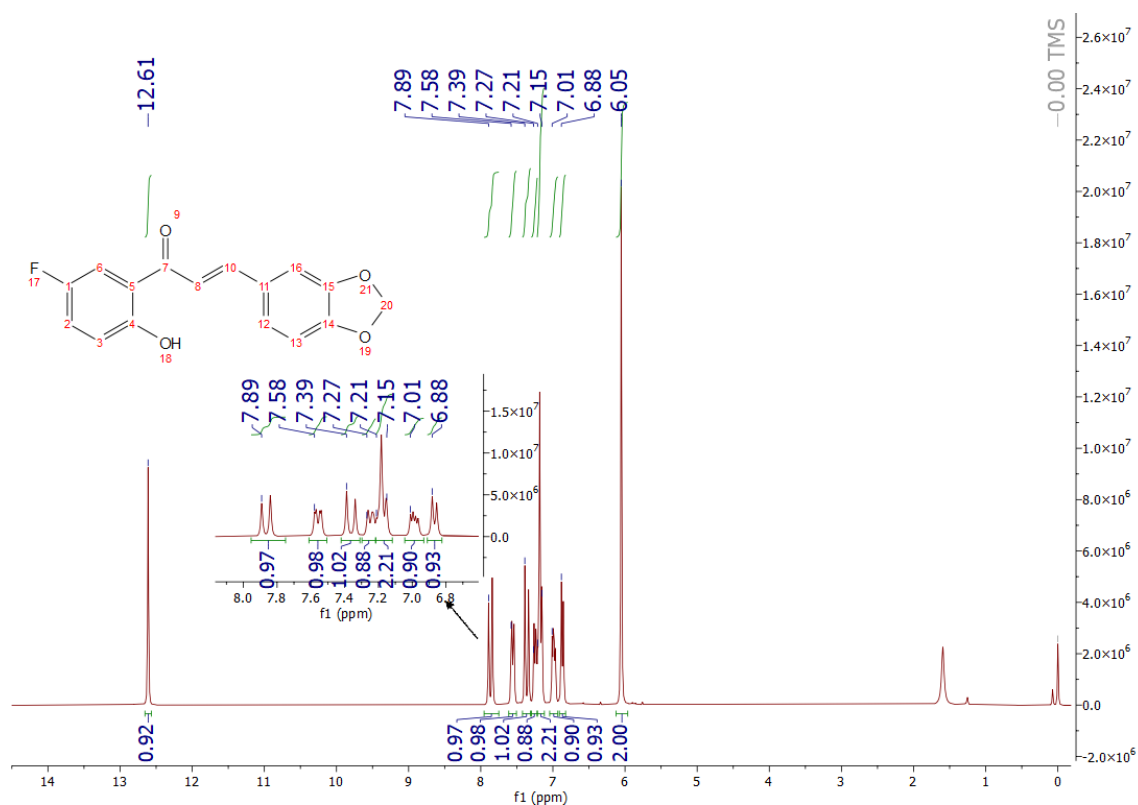
Spectra 18: ¹³C NMR of compound 3c



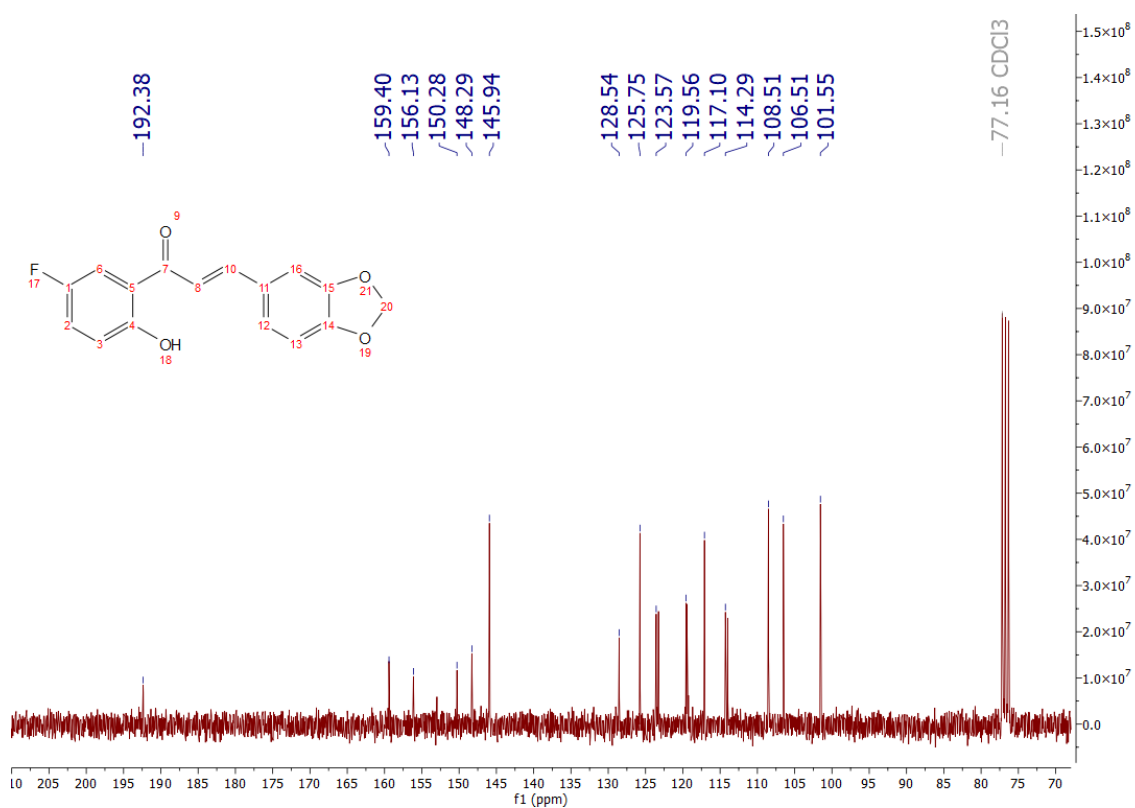
Spectra 19: ¹H NMR of compound 4a



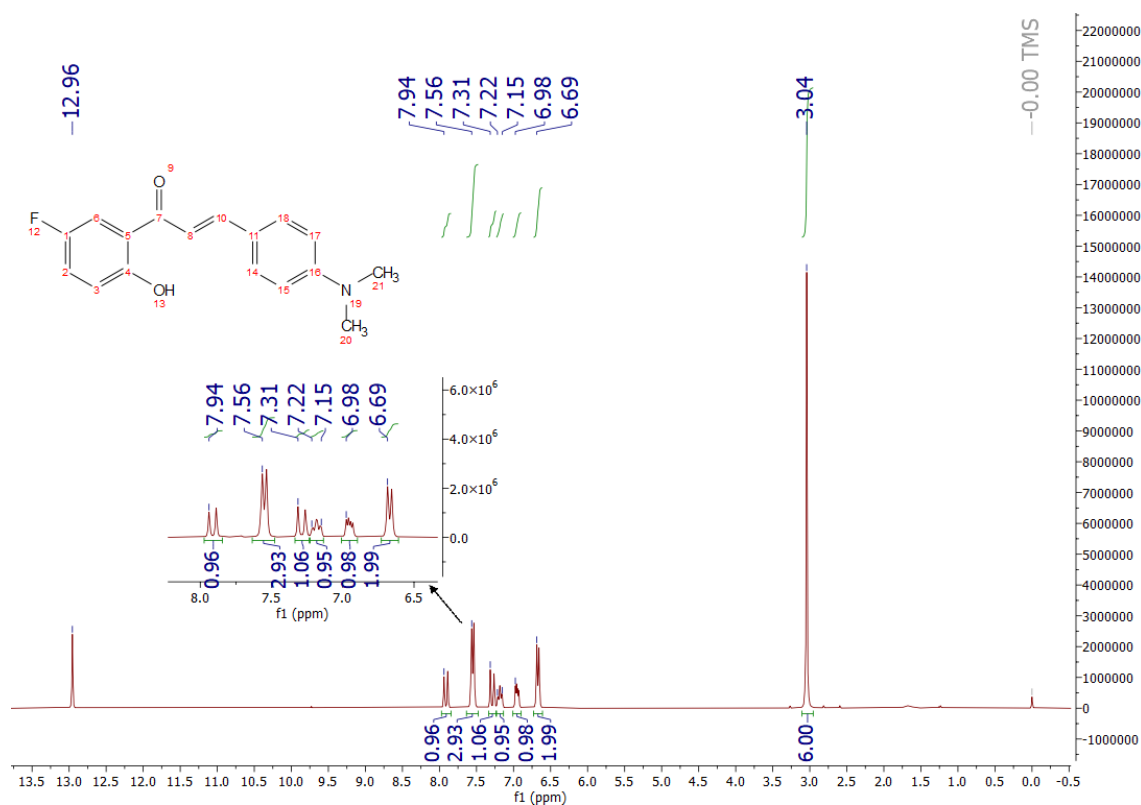
Spectra 20: ¹³C NMR of compound 4a



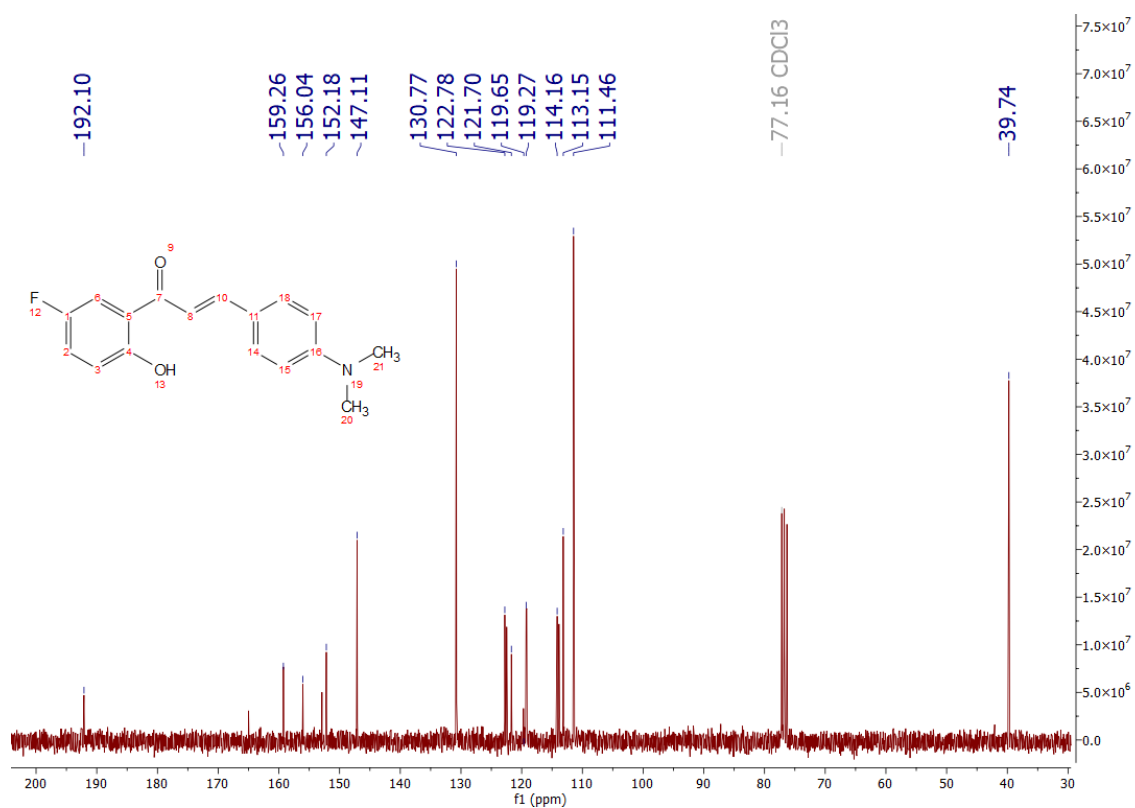
Spectra 21: ¹H NMR of compound **4b**



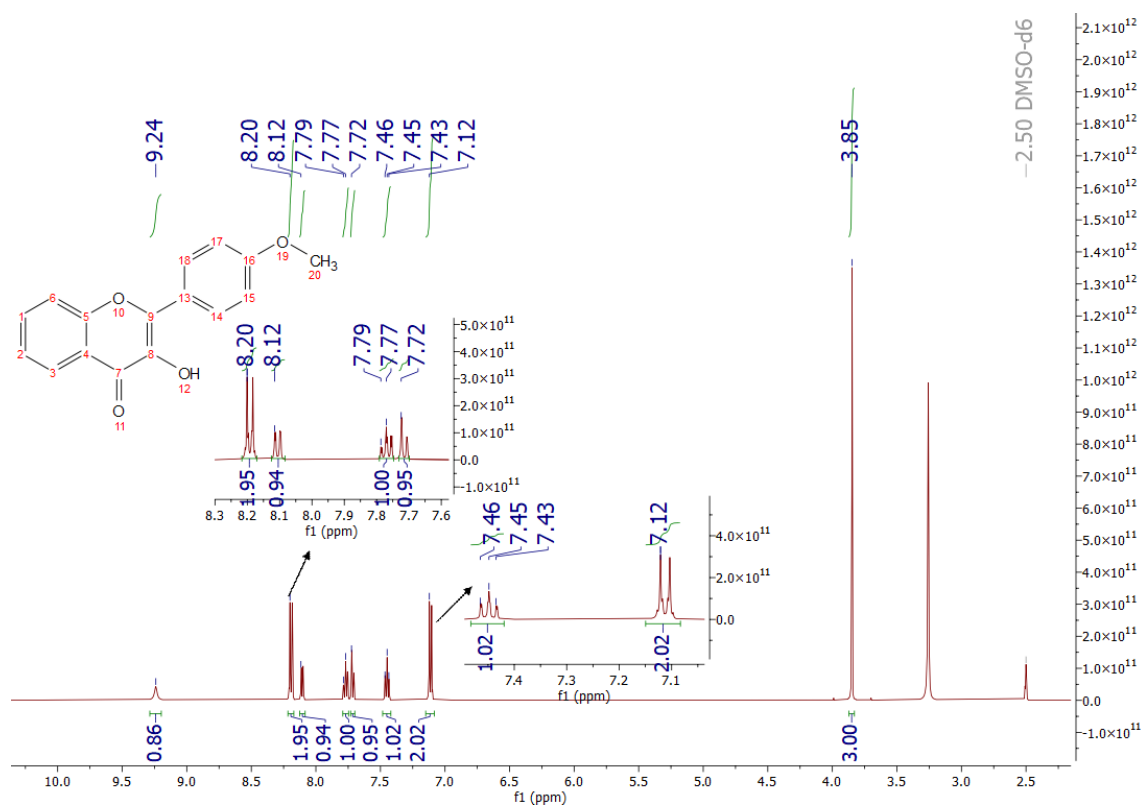
Spectra 22: ¹³C NMR of compound **4b**



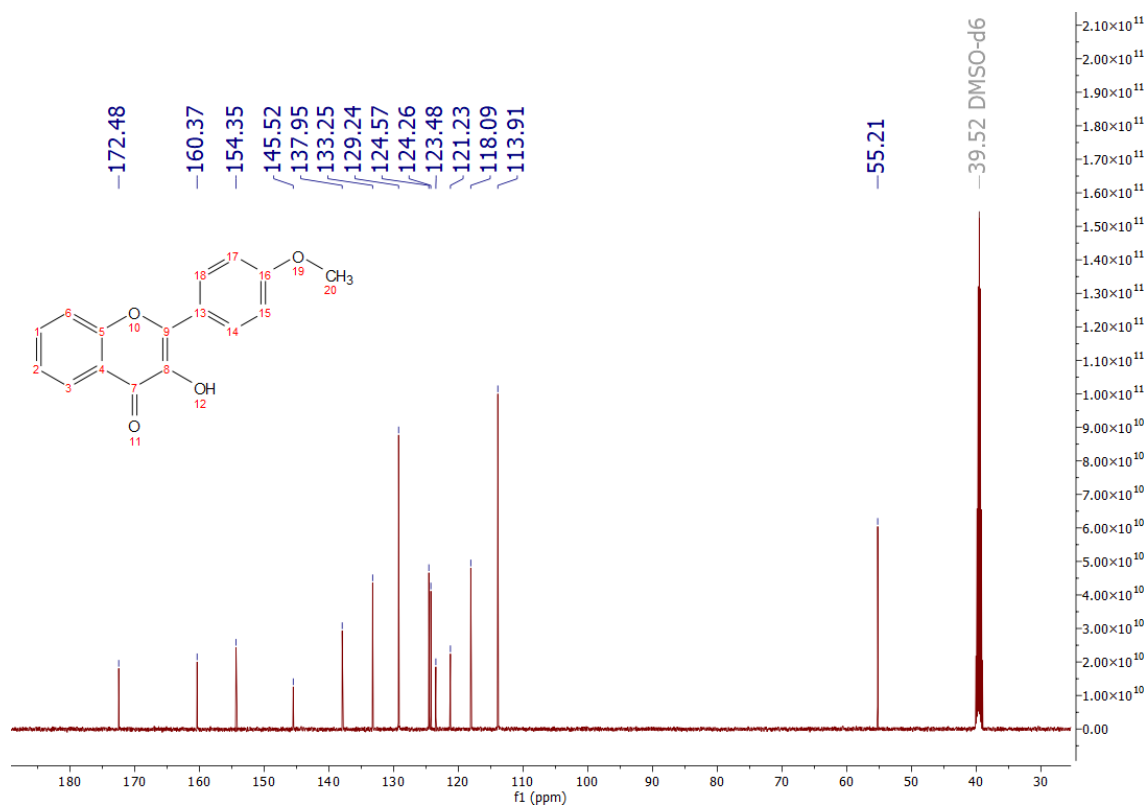
Spectra 23: ¹H NMR of compound **4c**



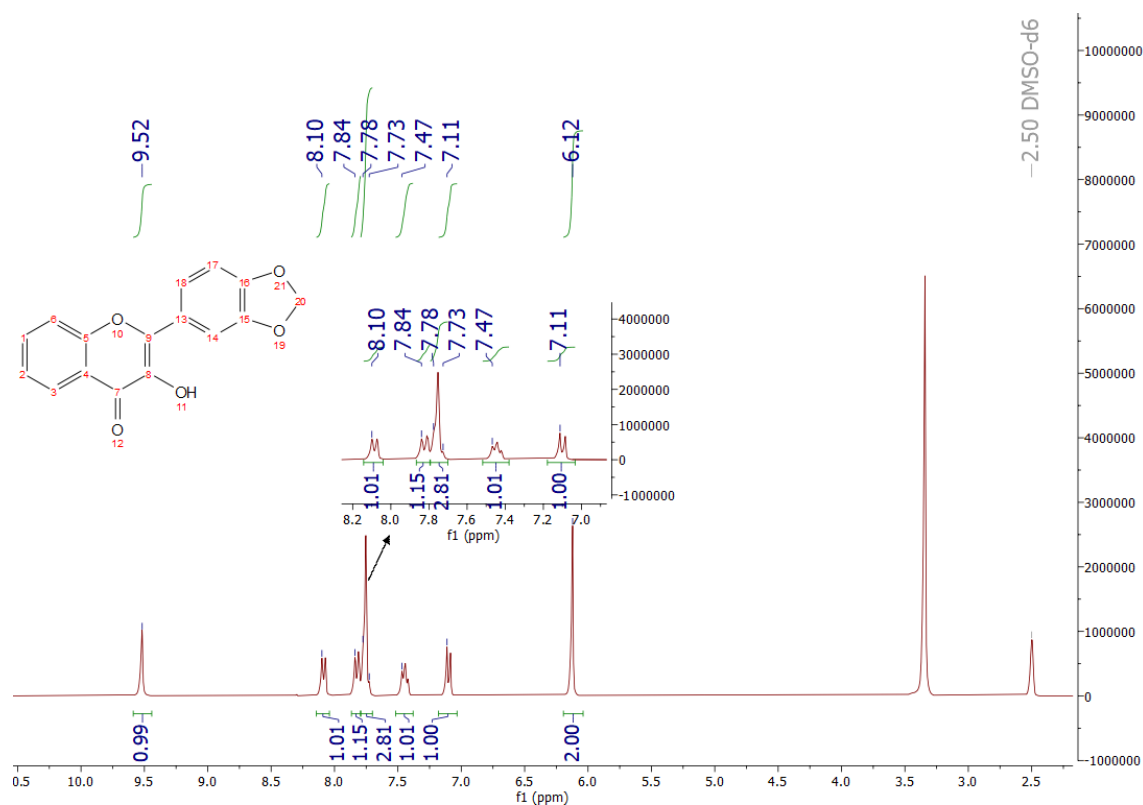
Spectra 24: ¹³C NMR of compound **4c**



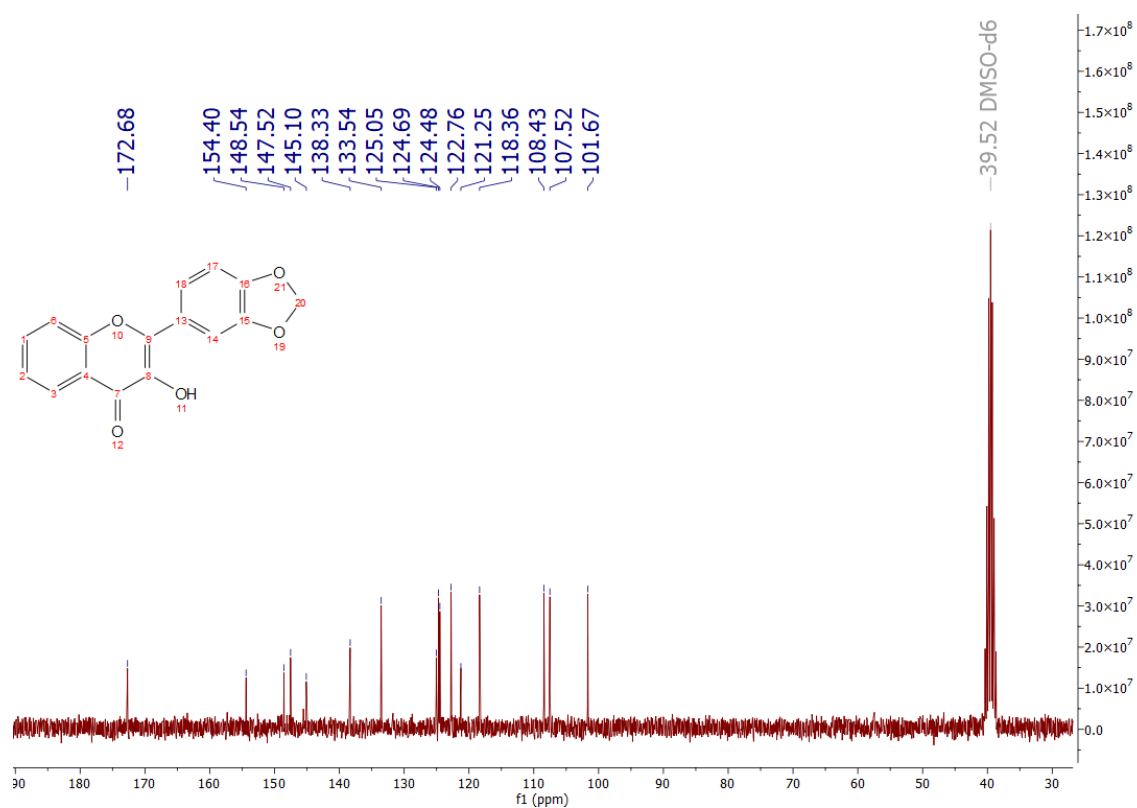
Spectra 25: ¹H NMR of compound **f12a**



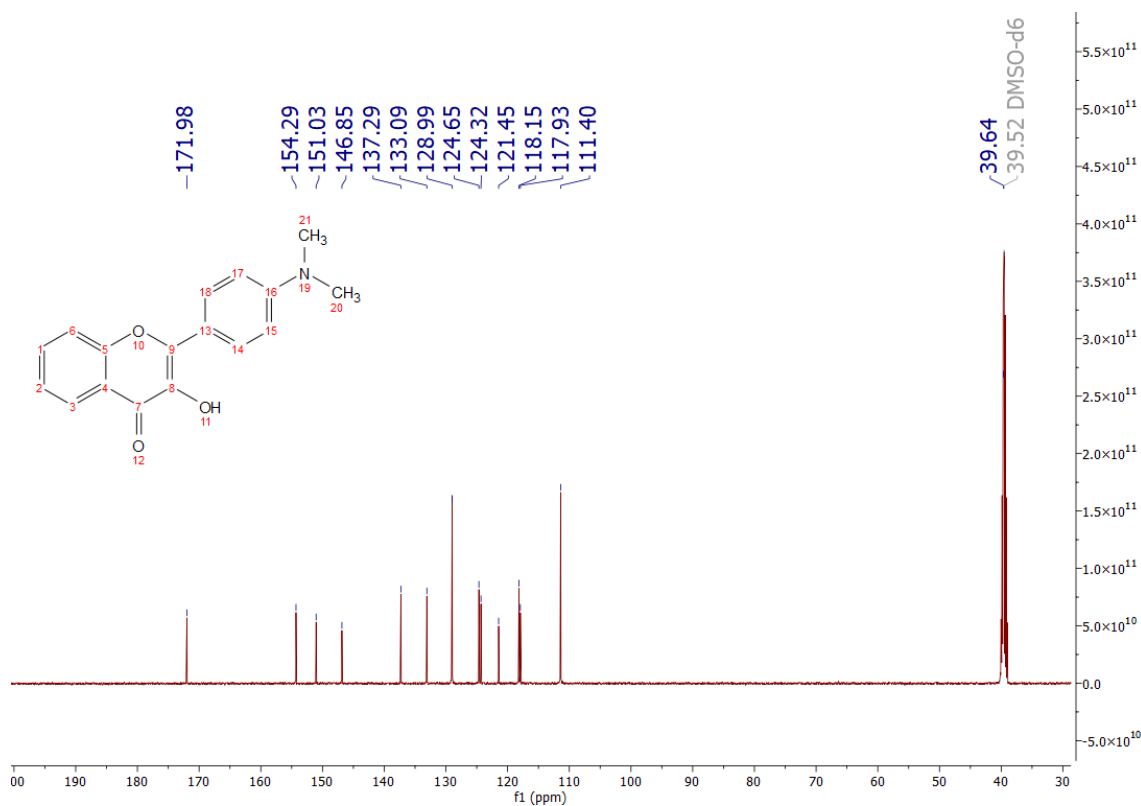
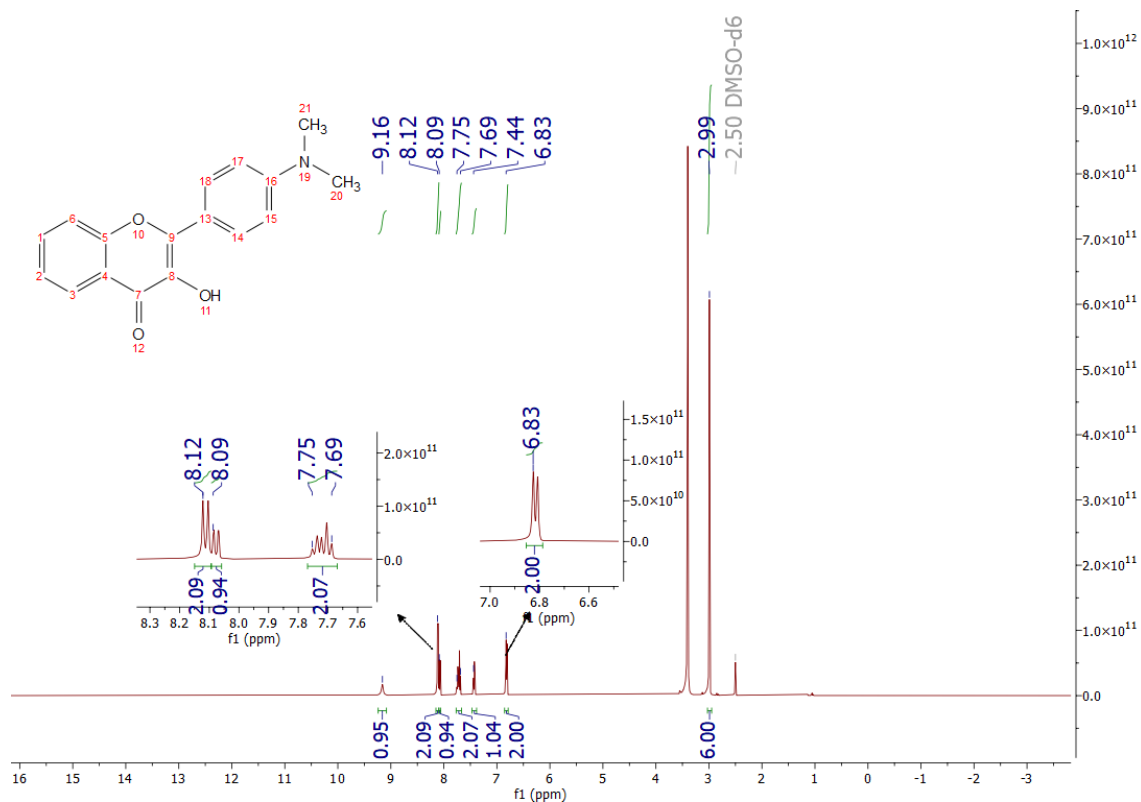
Spectra 26: ¹³C NMR of compound **f12a**

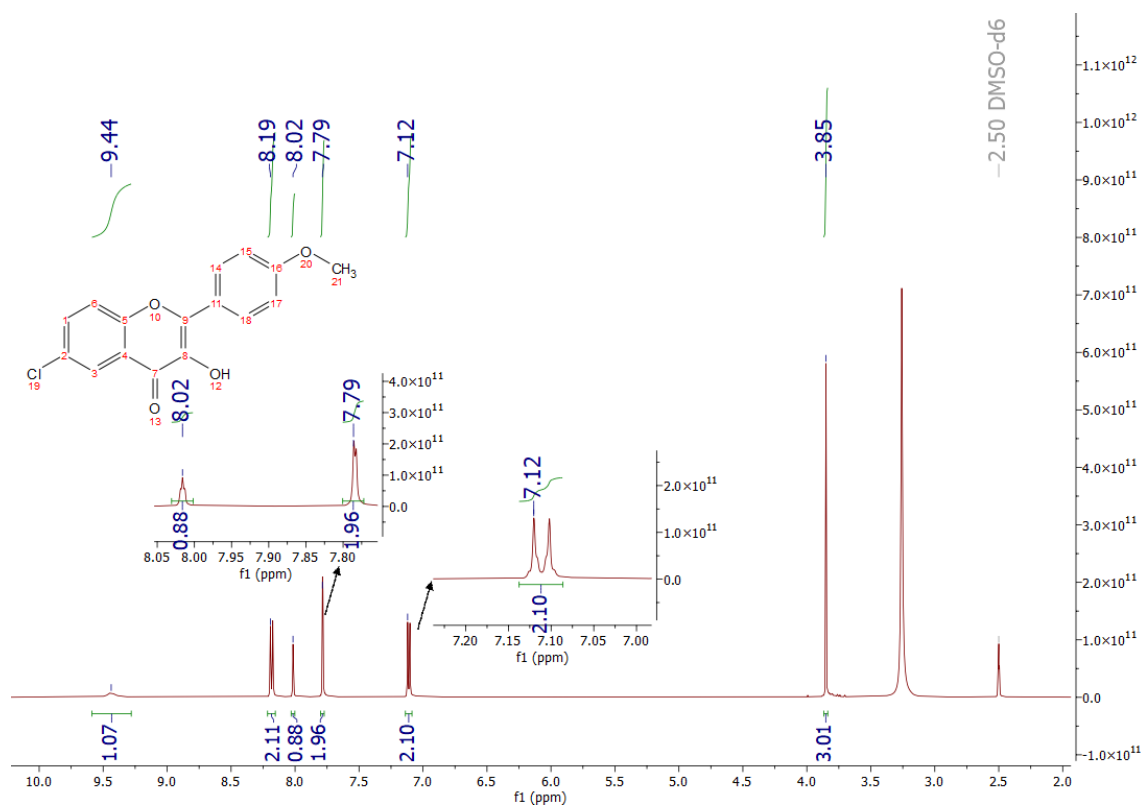


Spectra 27: ¹H NMR of compound **f1b**

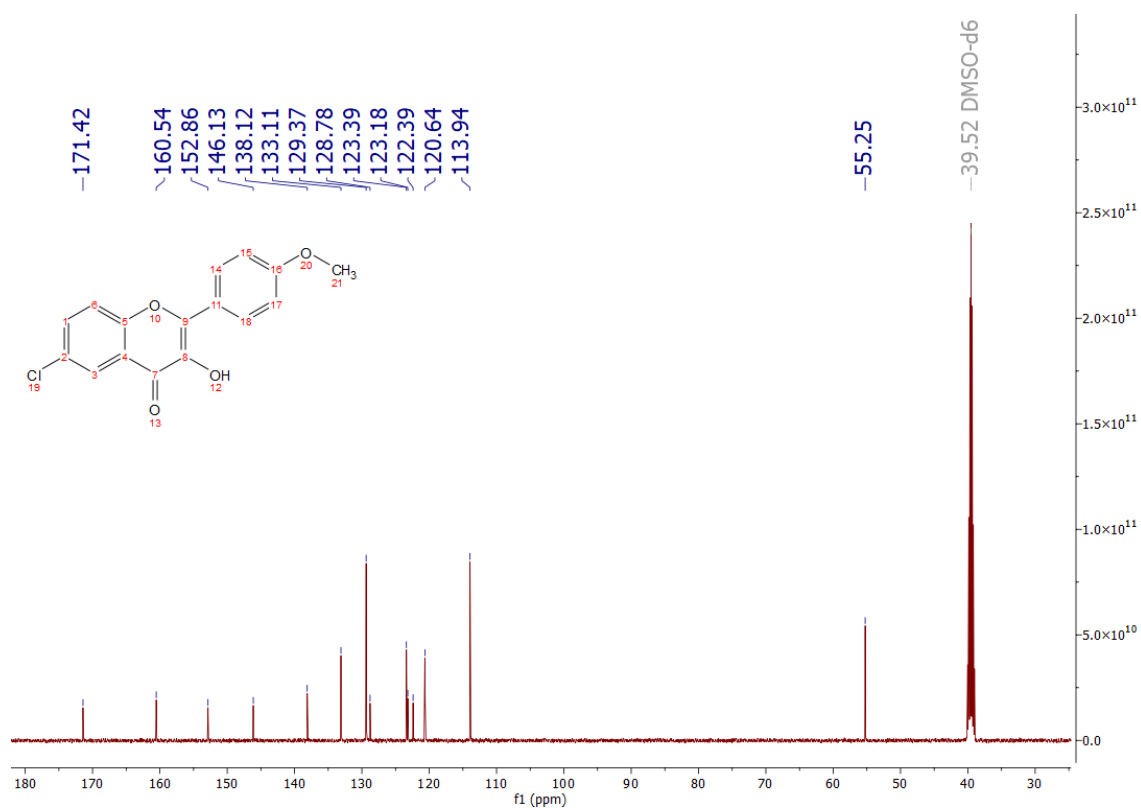


Spectra 28: ¹³C NMR of compound **f1b**

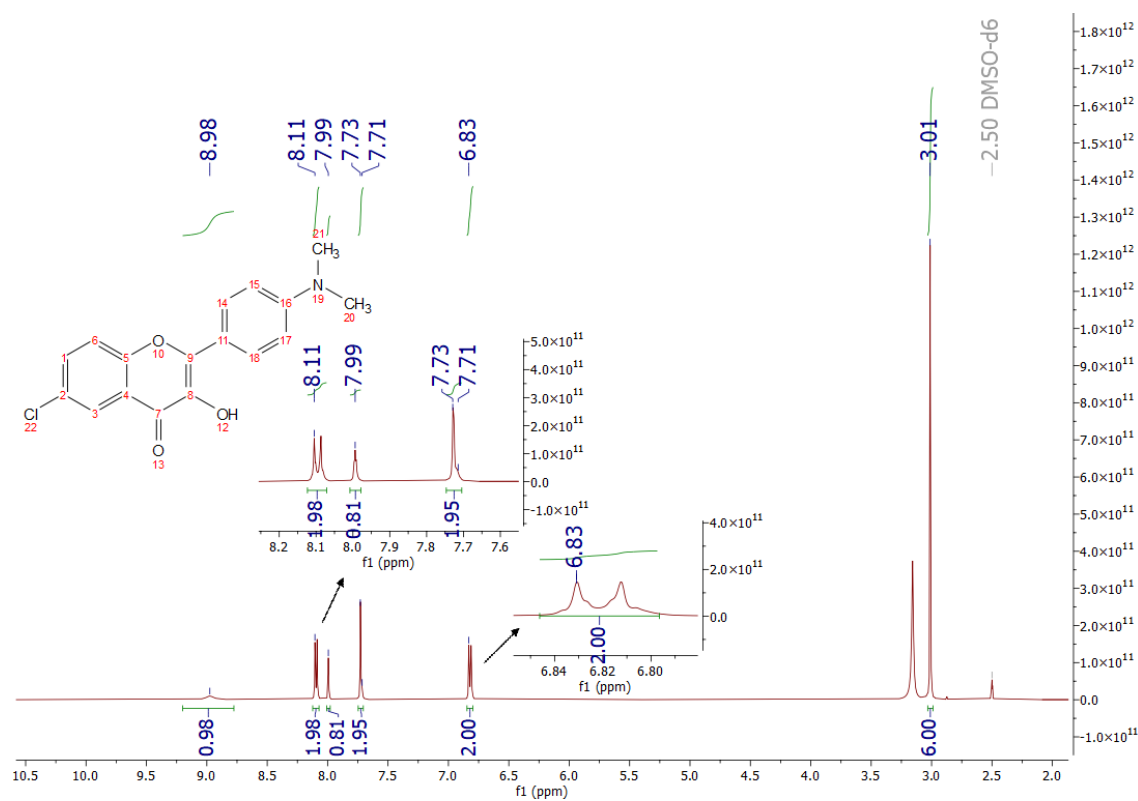




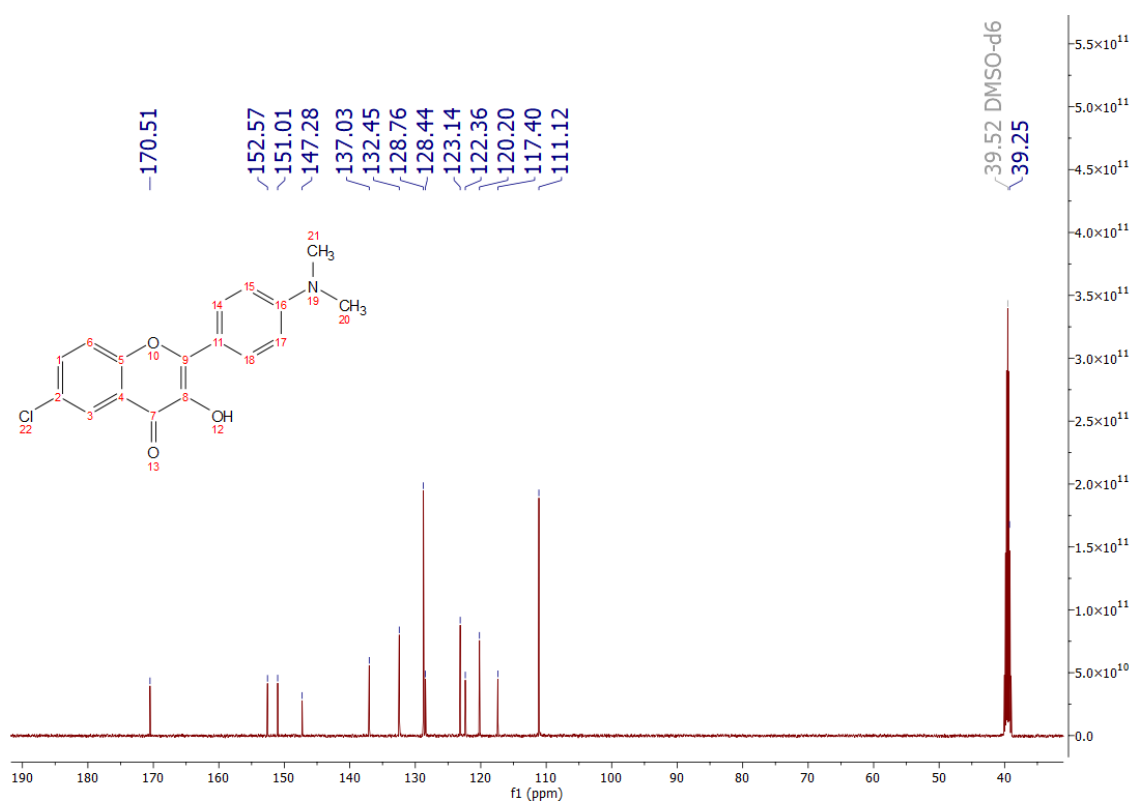
Spectra 31: ¹H NMR of compound **f3a**



Spectra 32: ¹³C NMR of compound **f13a**



Spectra 33: ¹H NMR of compound **f3c**



Spectra 34: ¹³C NMR of compound **f13c**