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

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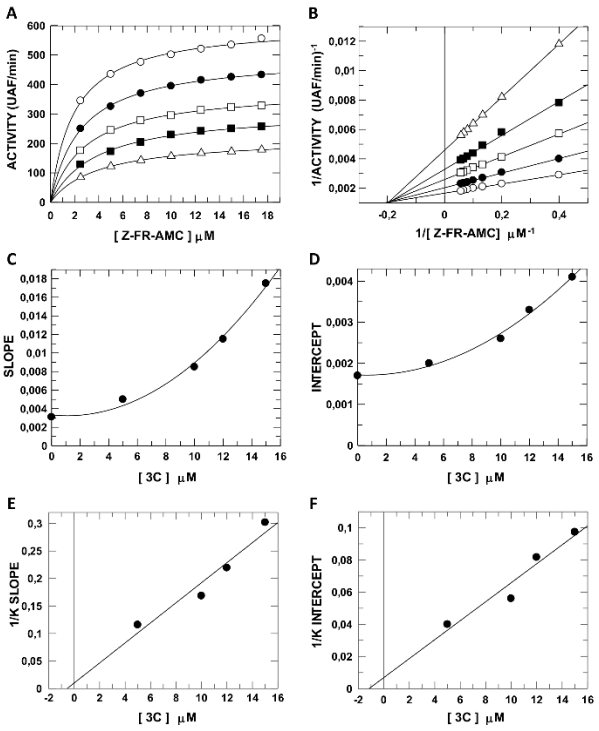
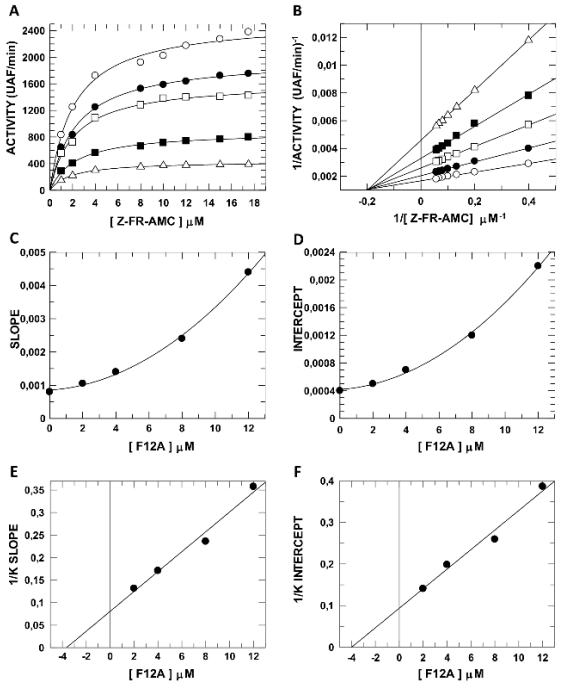
Summary



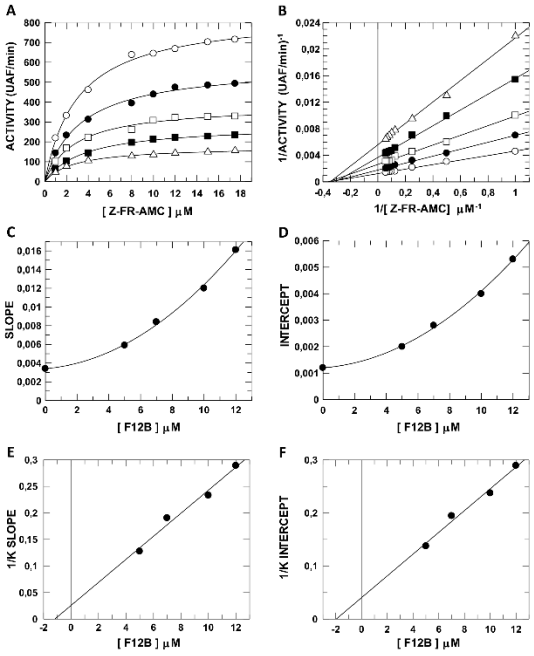
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 5 µM; ●→ 10 µM; ■→ 12 µM; △→ 15 µM

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



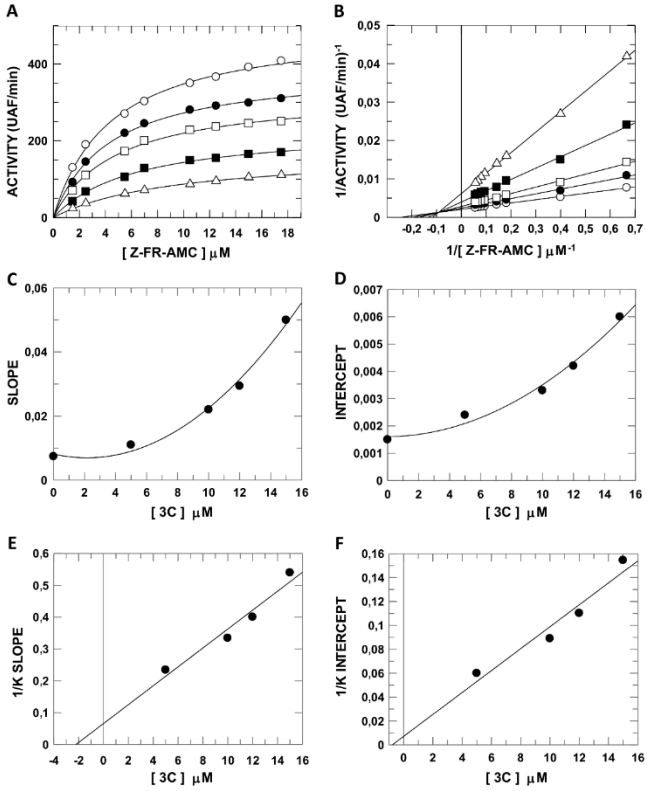
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 2 µM; ●→ 4 µM; ■→ 8 µM; △→ 12 µM

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



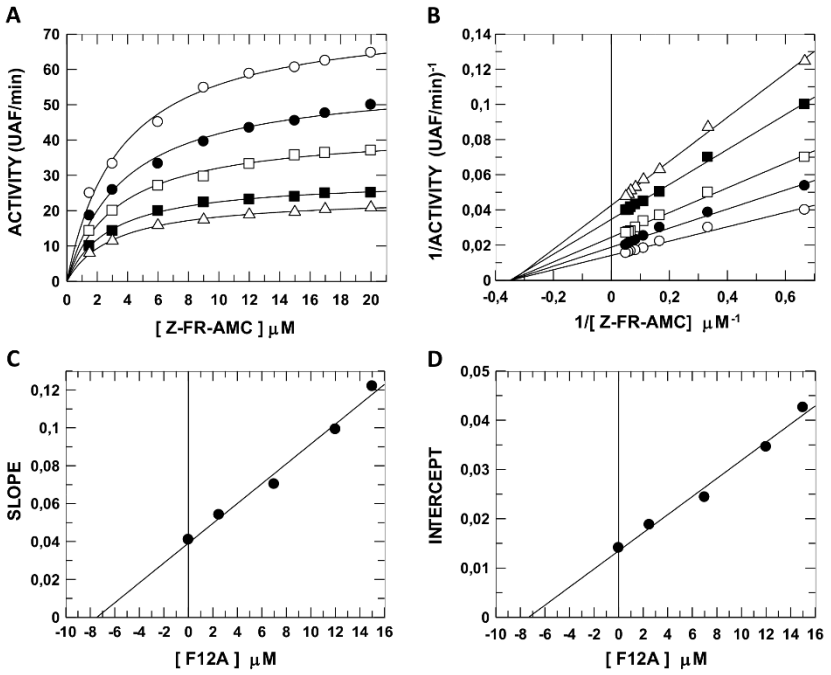
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 5 µM; ●→ 7 µM; ■→ 10 µM; △→ 12 µM

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



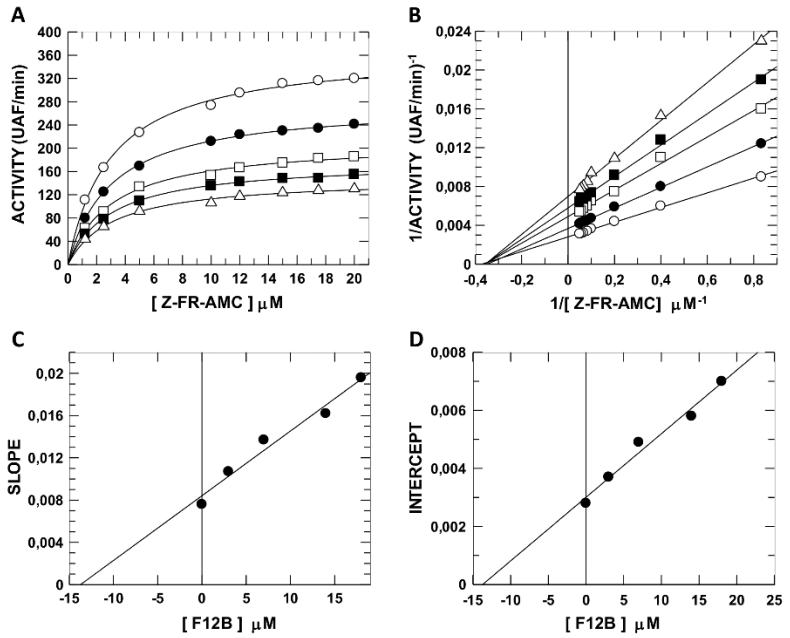
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 5 µM; ●→ 10 µM; ■→ 12 µM; △→ 15 µM

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



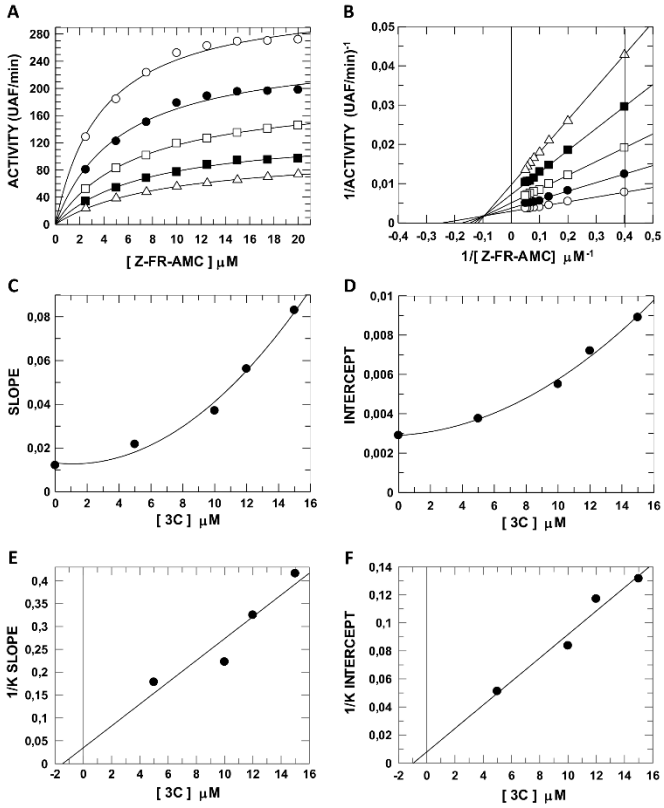
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 2.5 µM; ●→ 7 µM; ■→ 12 µM; △→ 15 µM

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



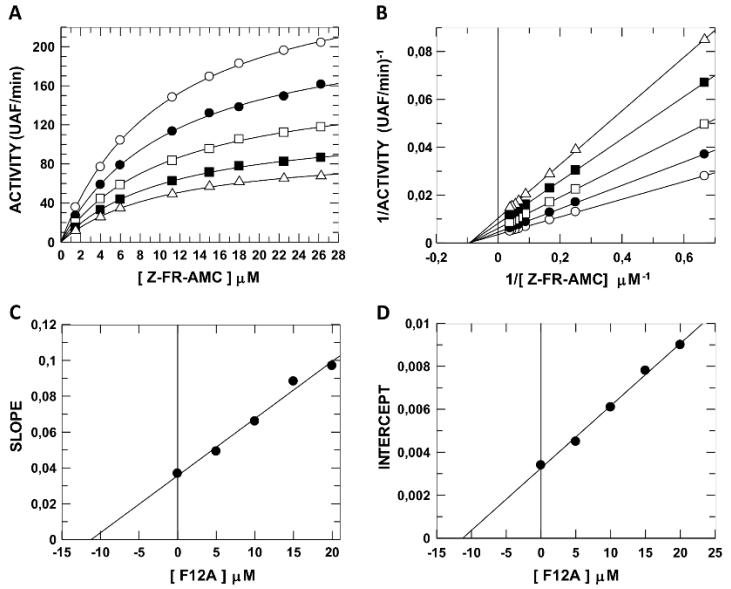
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 3 µM; ●→ 7 µM; ■→ 14 µM; △→ 18 µM

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



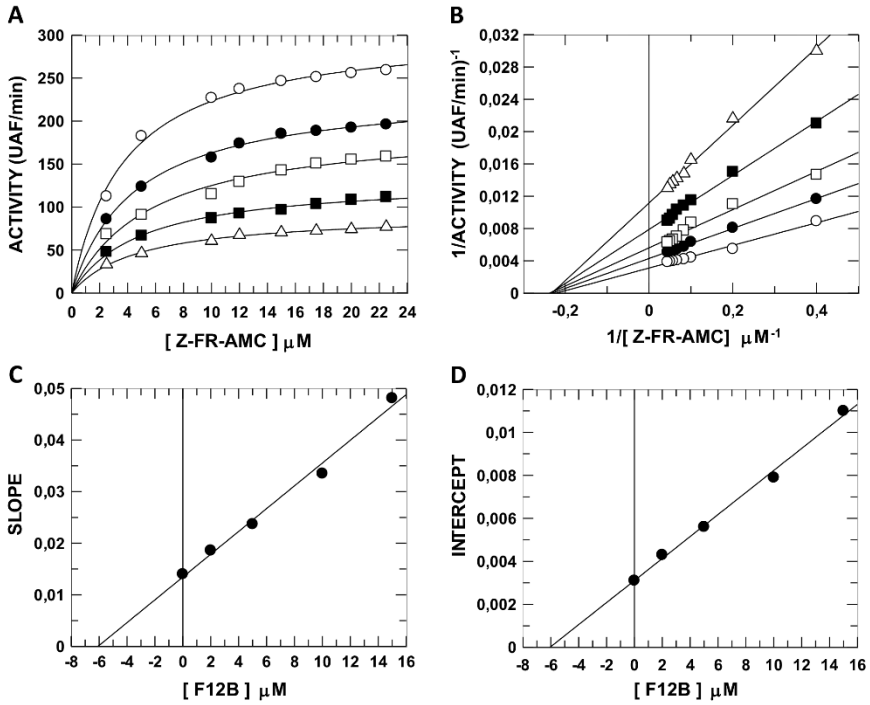
A: Michaelis-Menten plot; B: Lineweaver-Burk plot; C: Slope replot; D: Intercept replot; E: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 5 µM; ●→ 10 µM; ■→ 12 µM; △→ 15 µM

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: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 5 µM; ●→ 10 µM; ■→ 15 µM; △→ 20 µM

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: $1/K_{Slope}$ replot; F: $1/K_{Intercept}$ replot. ○→ control; ●→ 2 µM; ●→ 5 µM; ■→ 10 µM; △→ 15 µM

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

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

Table S2: Potential energy values for the biding 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 biding 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}

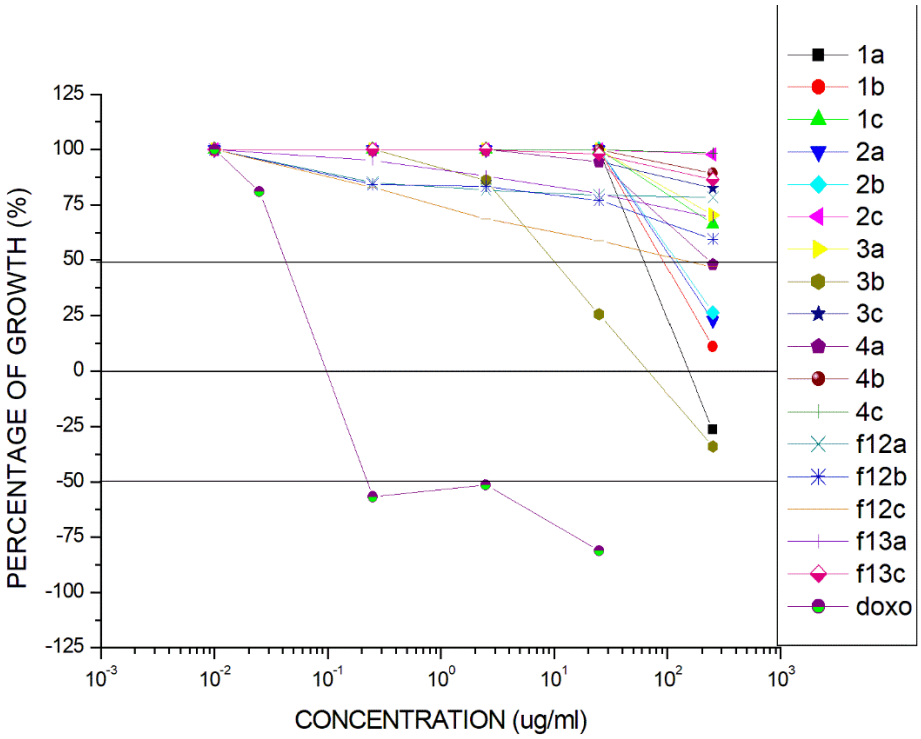
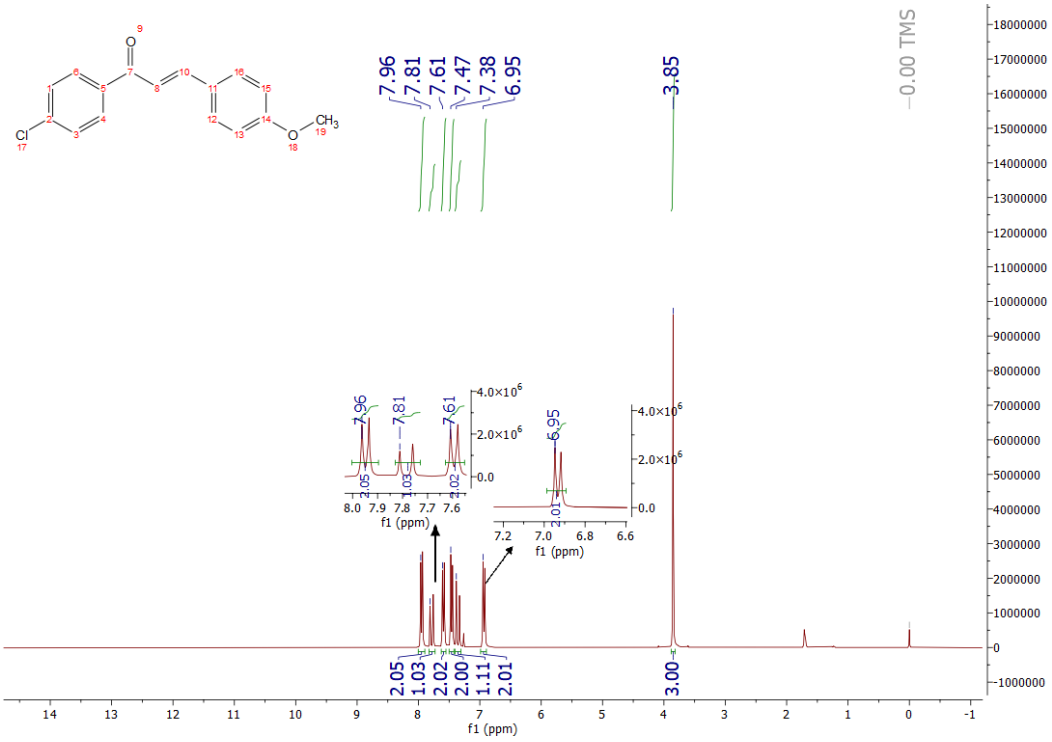
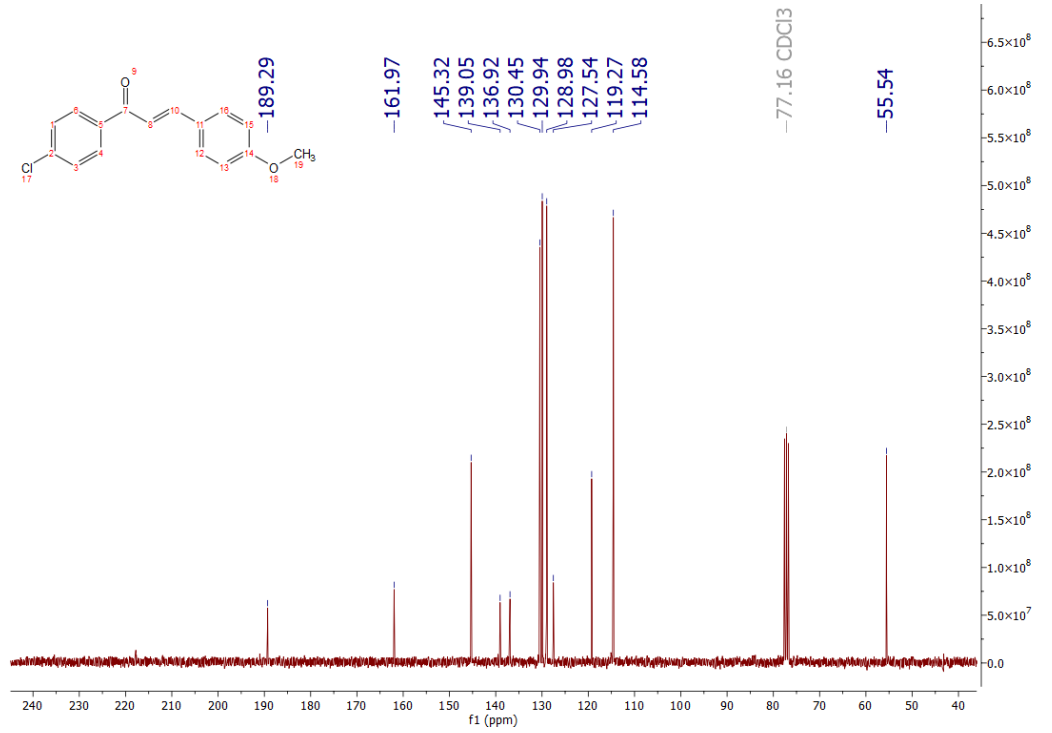


Figure S10: Cytotoxicity of all flavonoid derivatives against NHI-3T3 cells

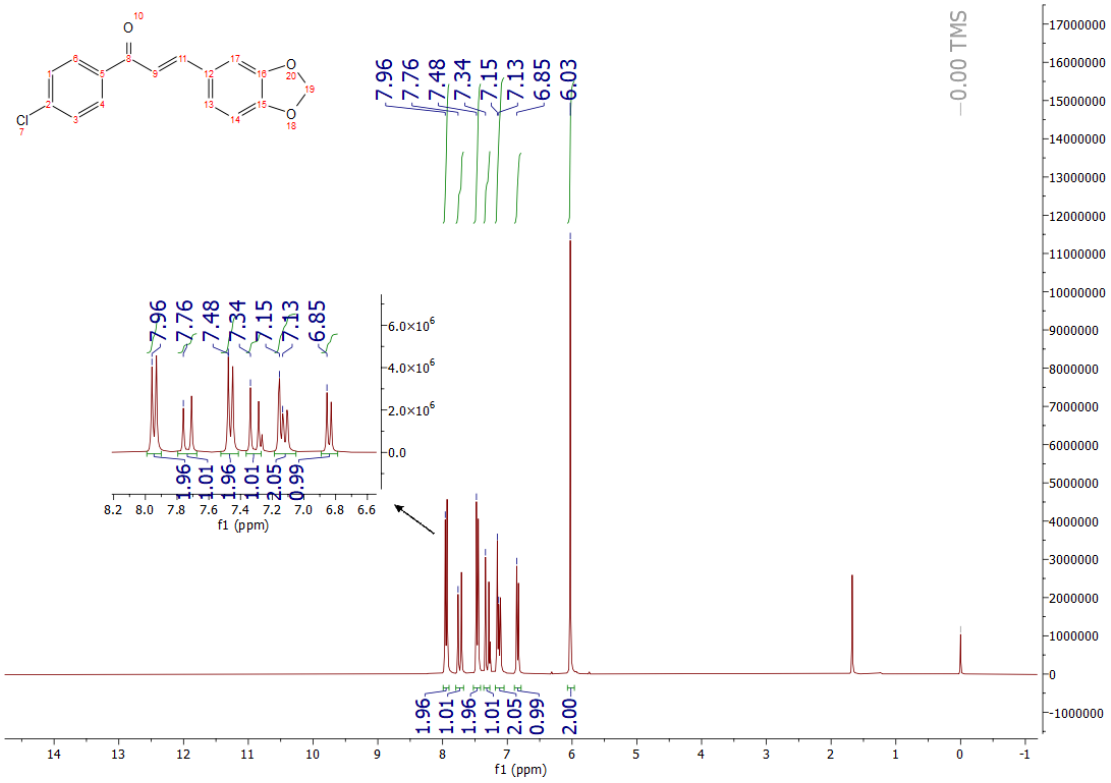
NMR spectra section



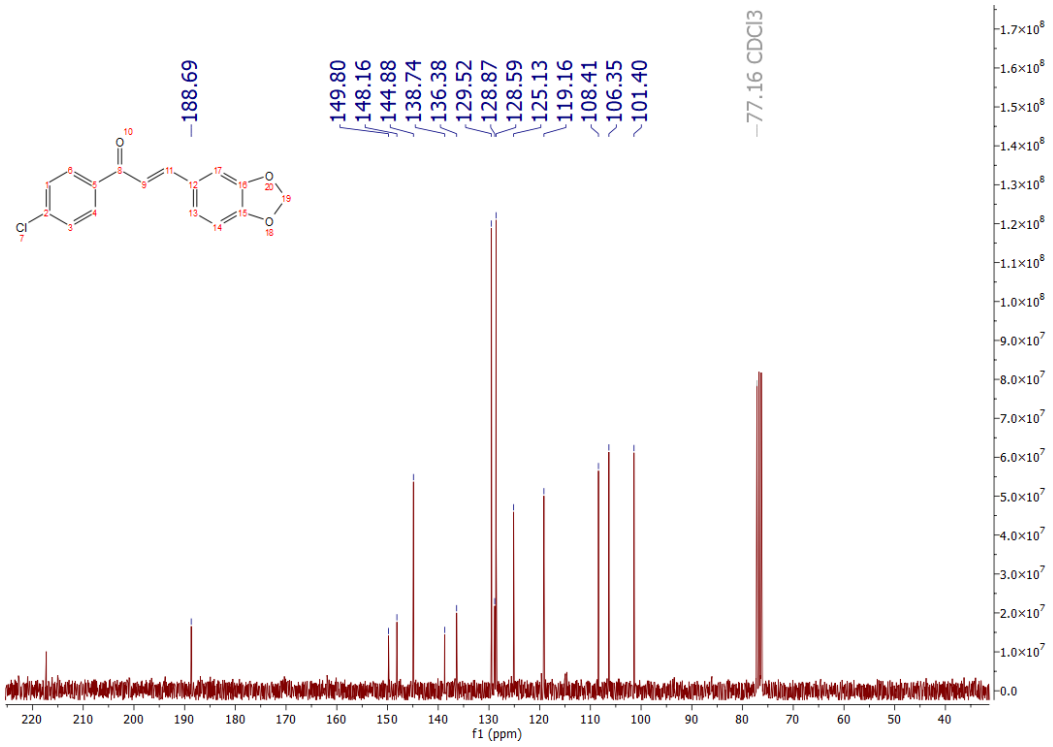
Spectra 1: ^{1}H NMR of compound **1a**



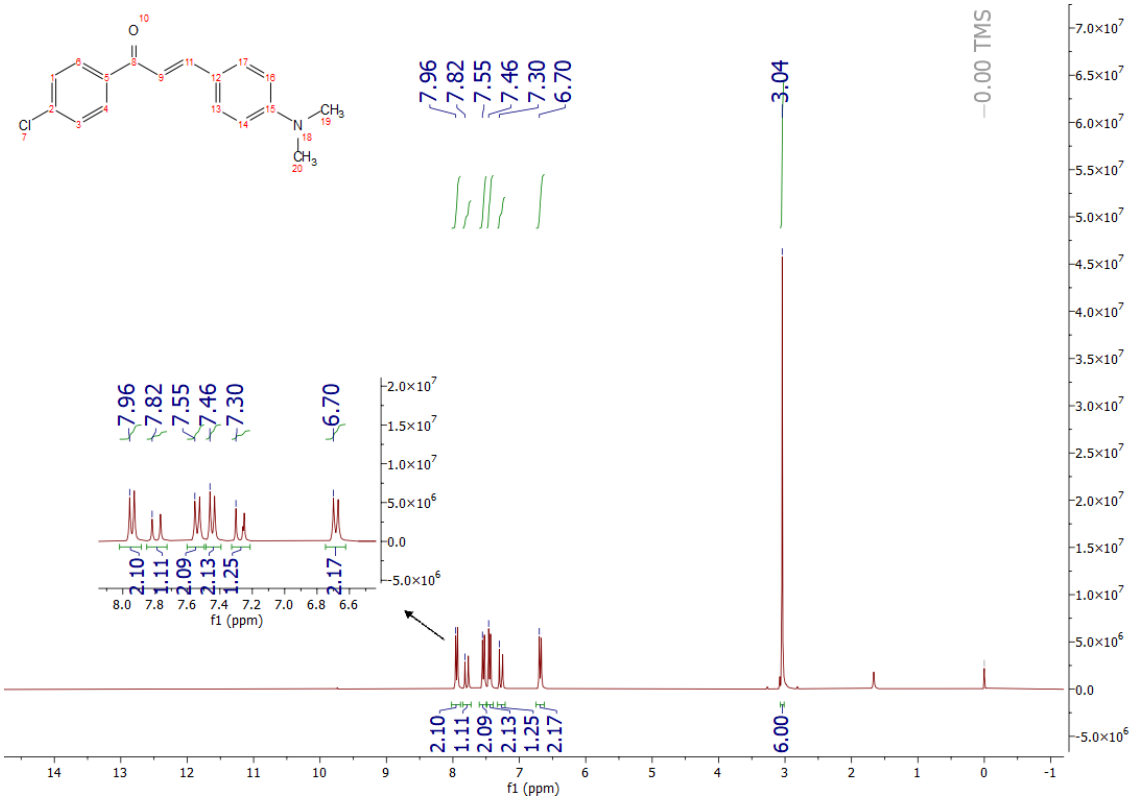
Spectra 2: ^{13}C NMR of compound **1a**



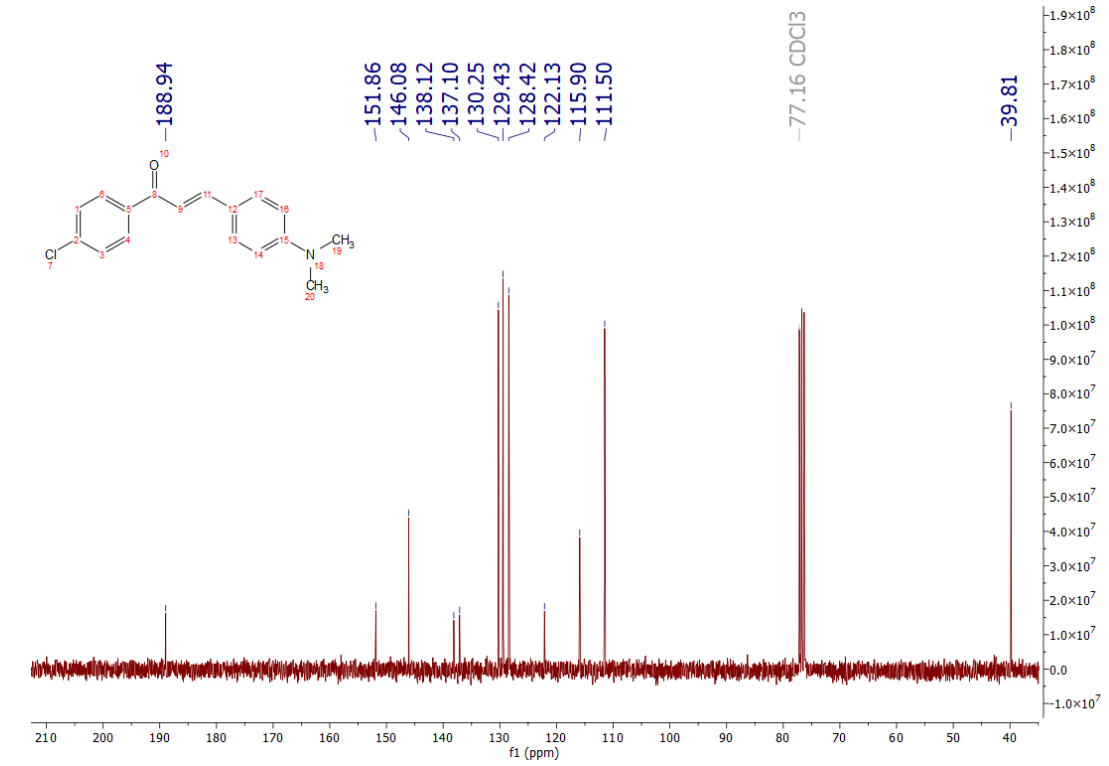
Spectra 3: ^{1}H NMR of compound **1b**



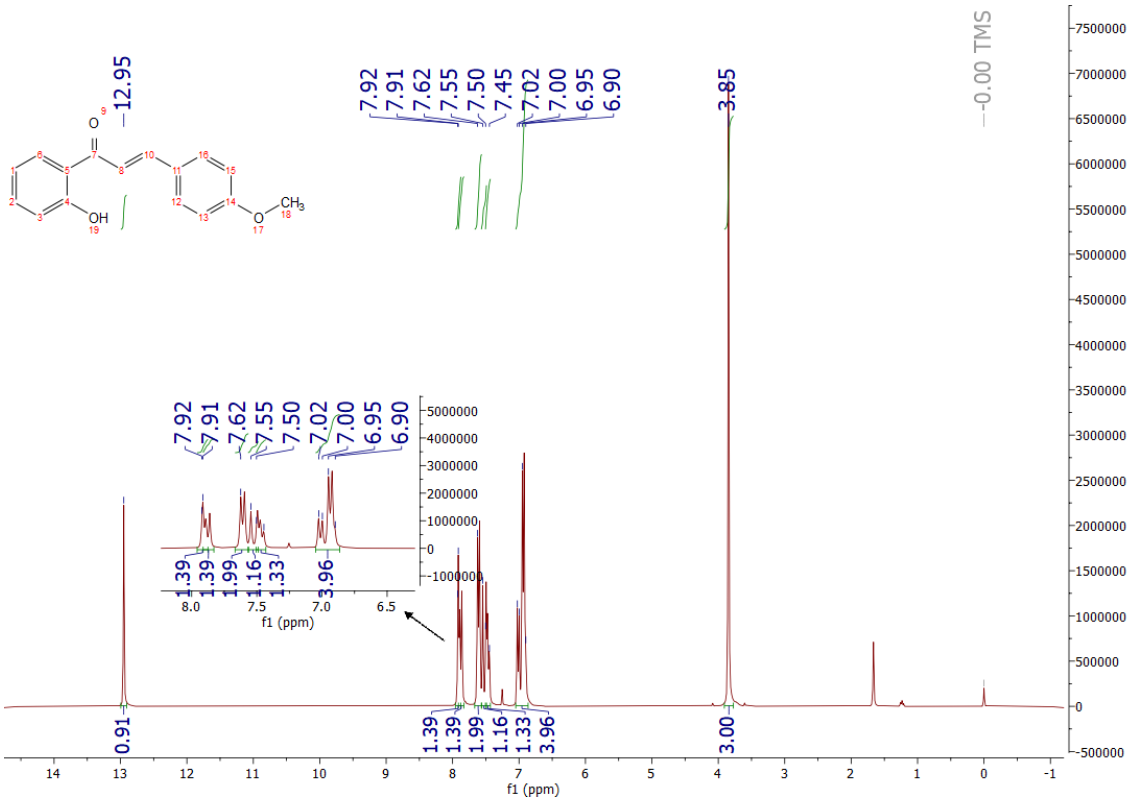
Spectra 4: ^{13}C NMR of compound **1b**



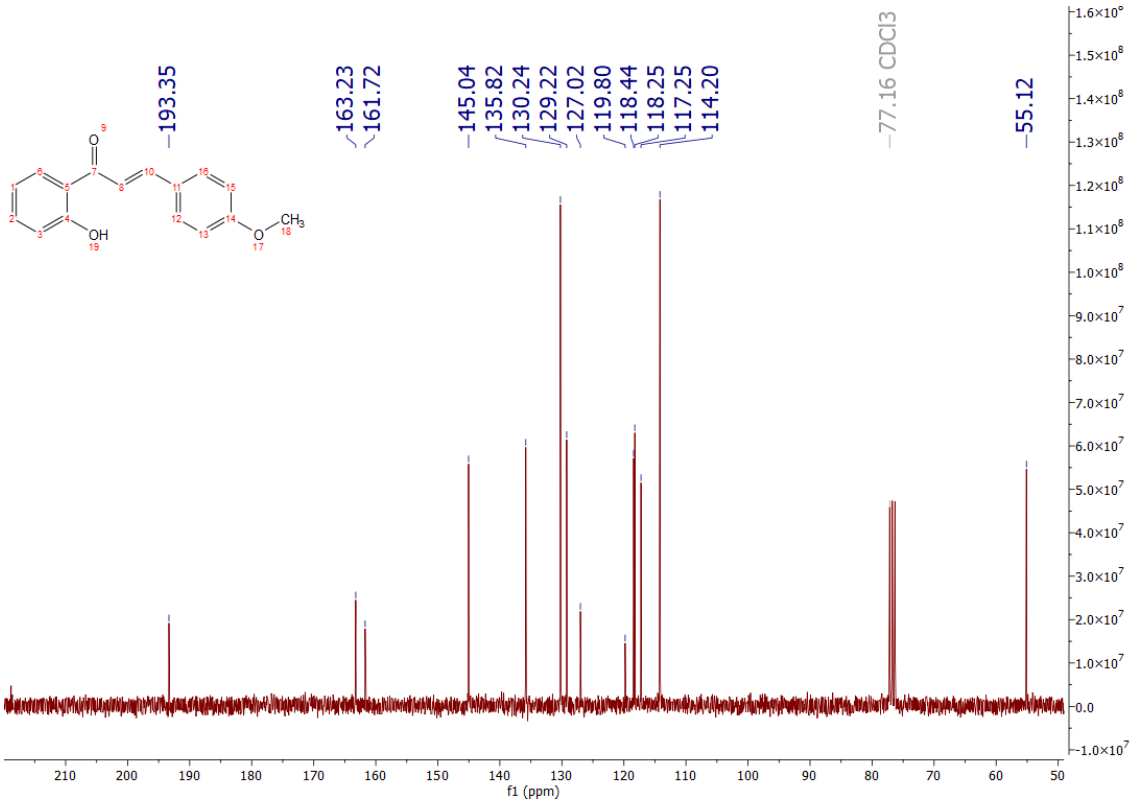
Spectra 5: ^{1}H NMR of compound **1c**



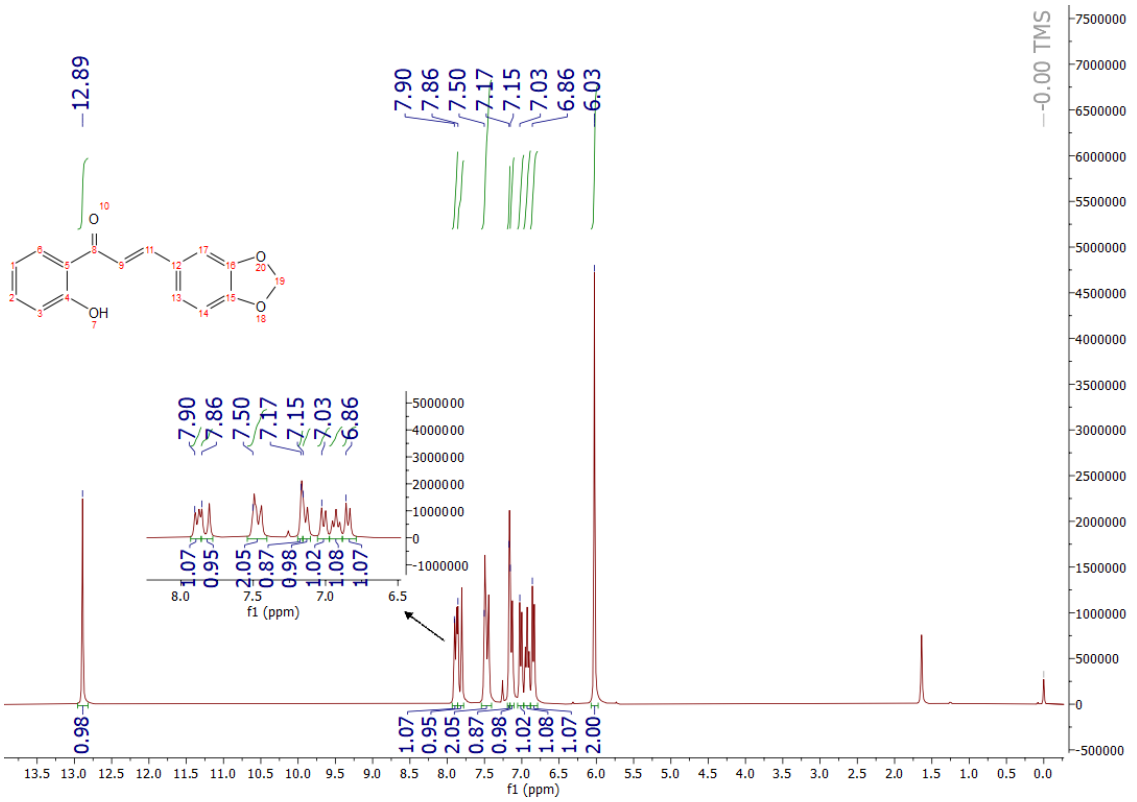
Spectra 6: ^{13}C NMR of compound **1c**



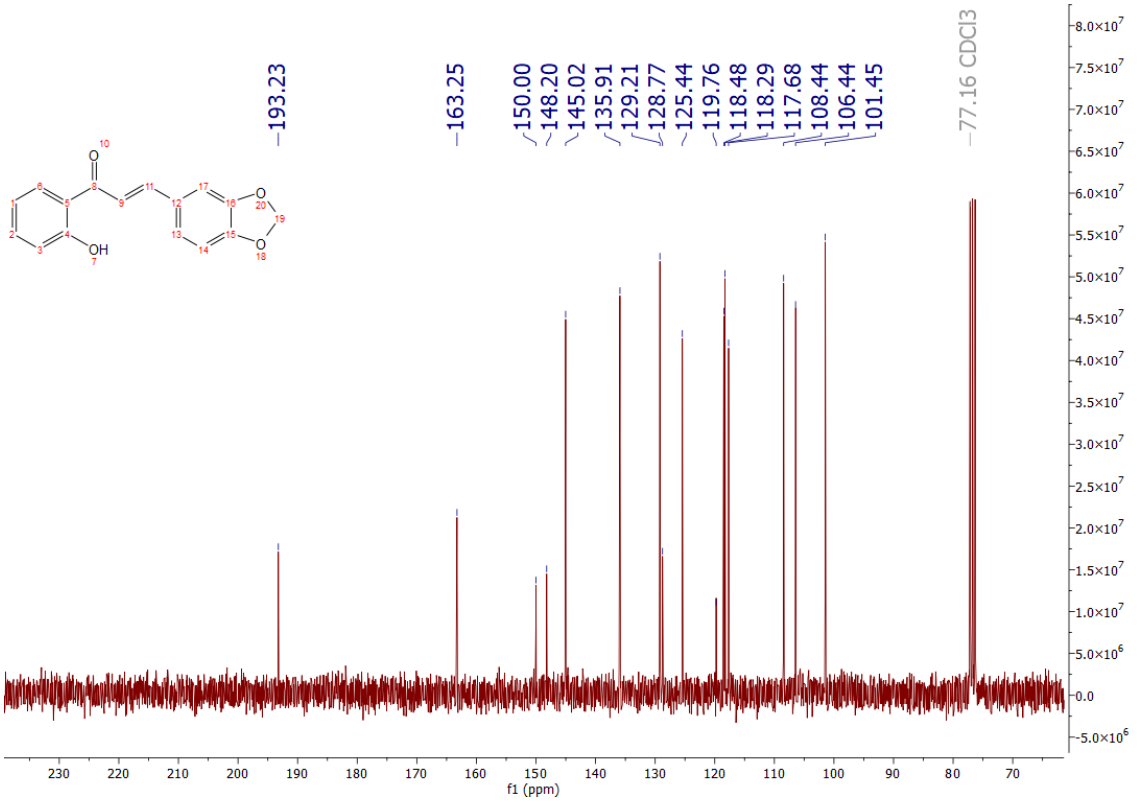
Spectra 7: ^{1}H NMR of compound **2a**



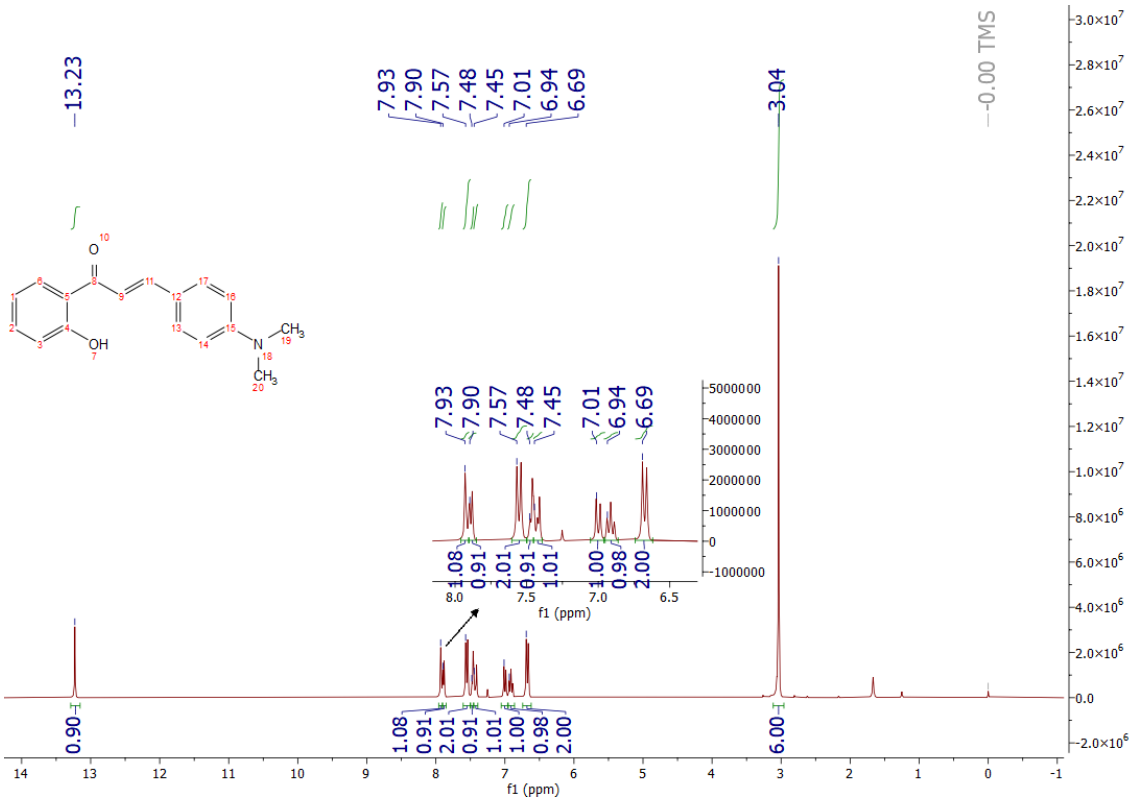
Spectra 8: ^{13}C NMR of compound **2a**



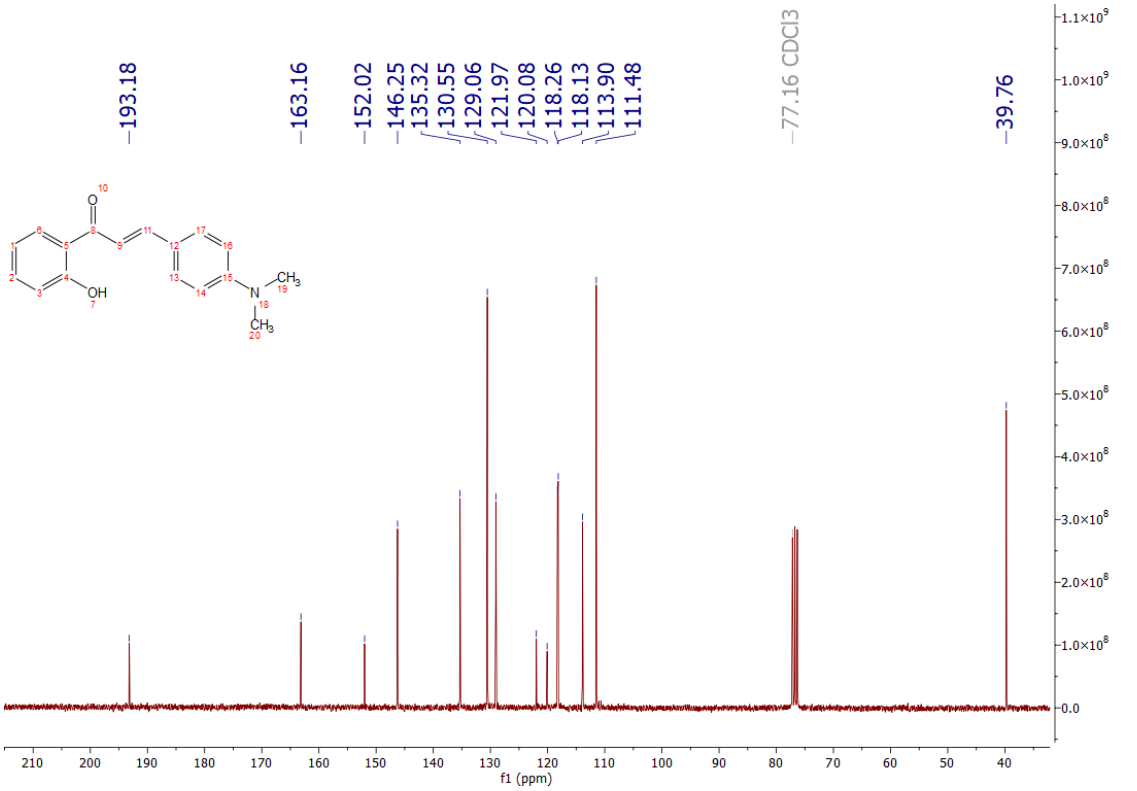
Spectra 9: ^{1}H NMR of compound **2b**



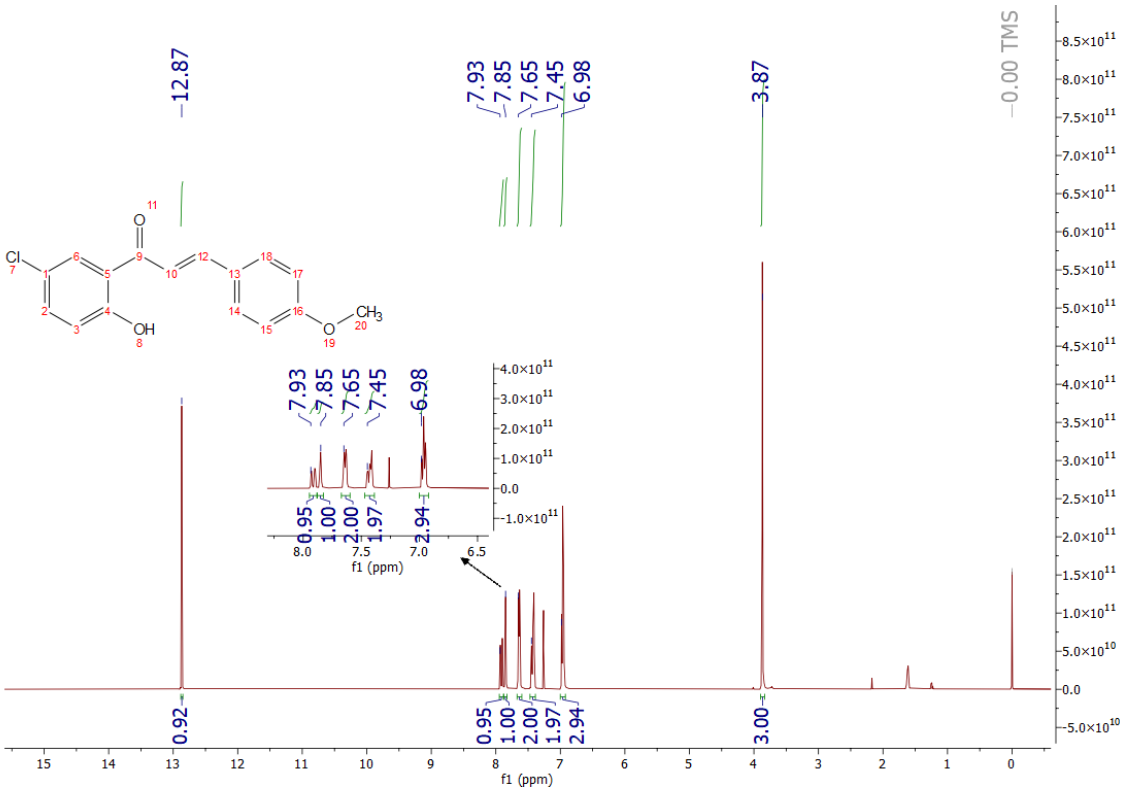
Spectra 10: ^{13}C NMR of compound **2b**



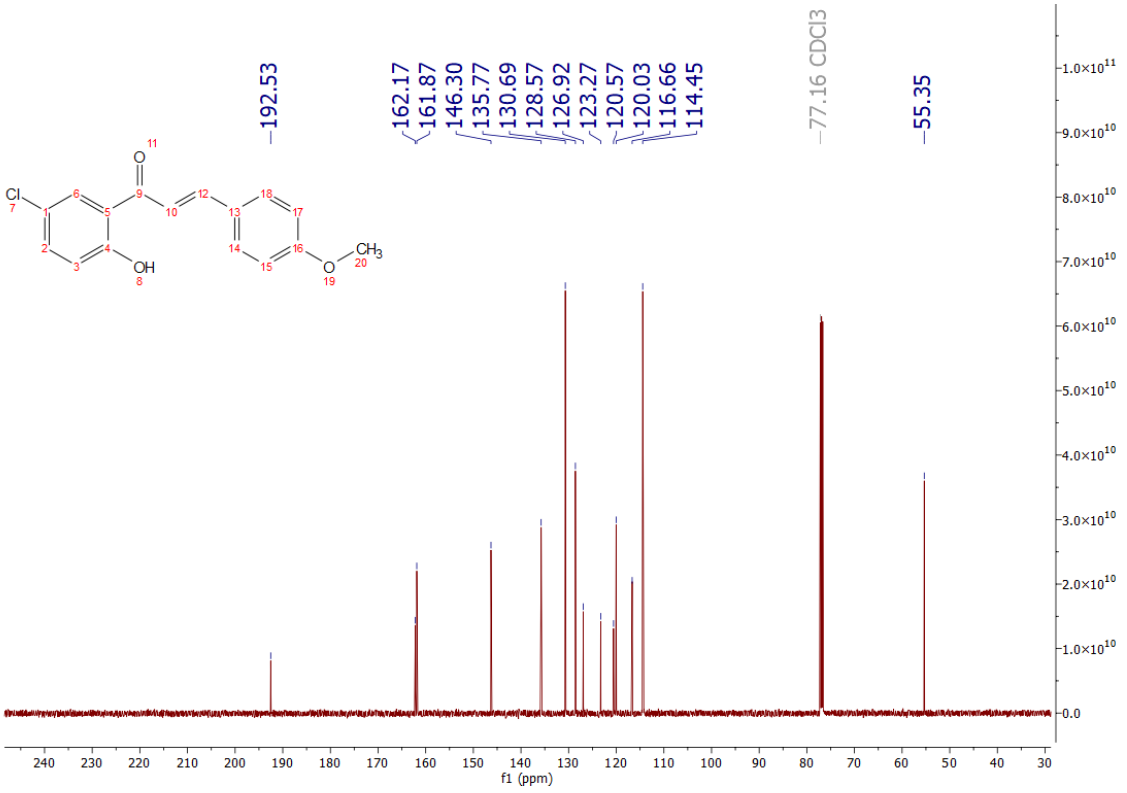
Spectra 11: ^{1}H NMR of compound **2c**



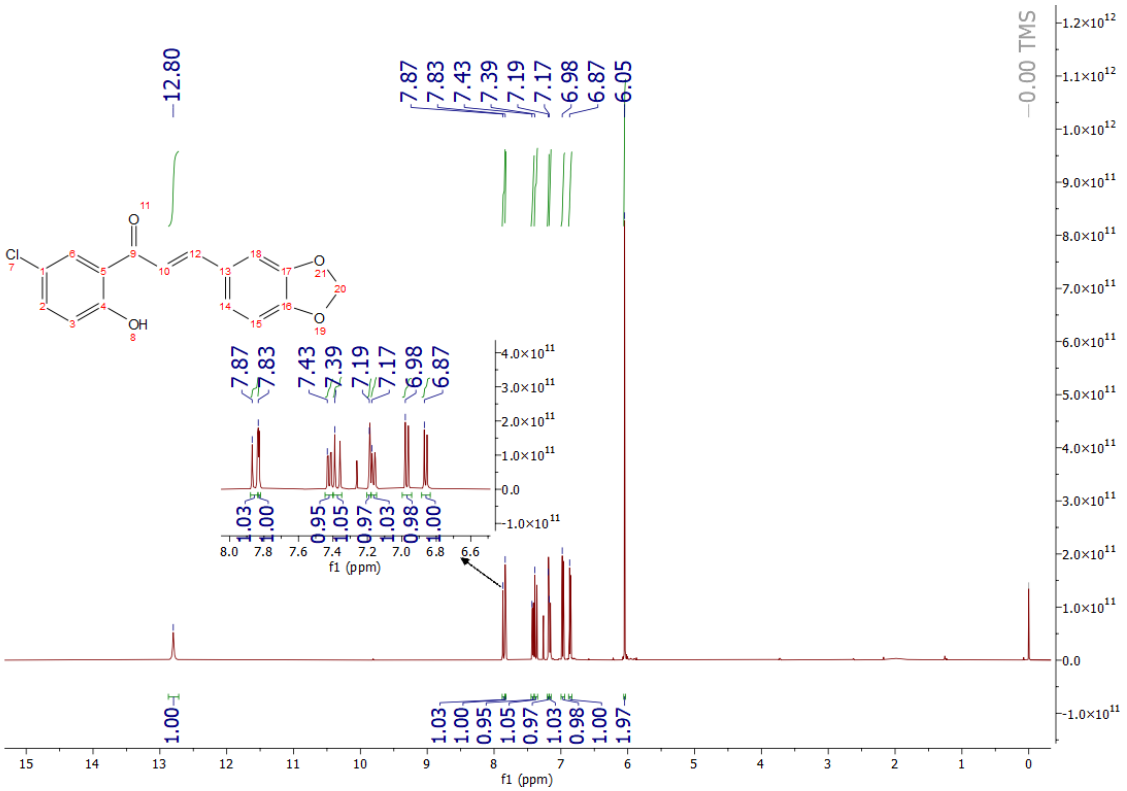
Spectra 12: ^{13}C NMR of compound **2c**



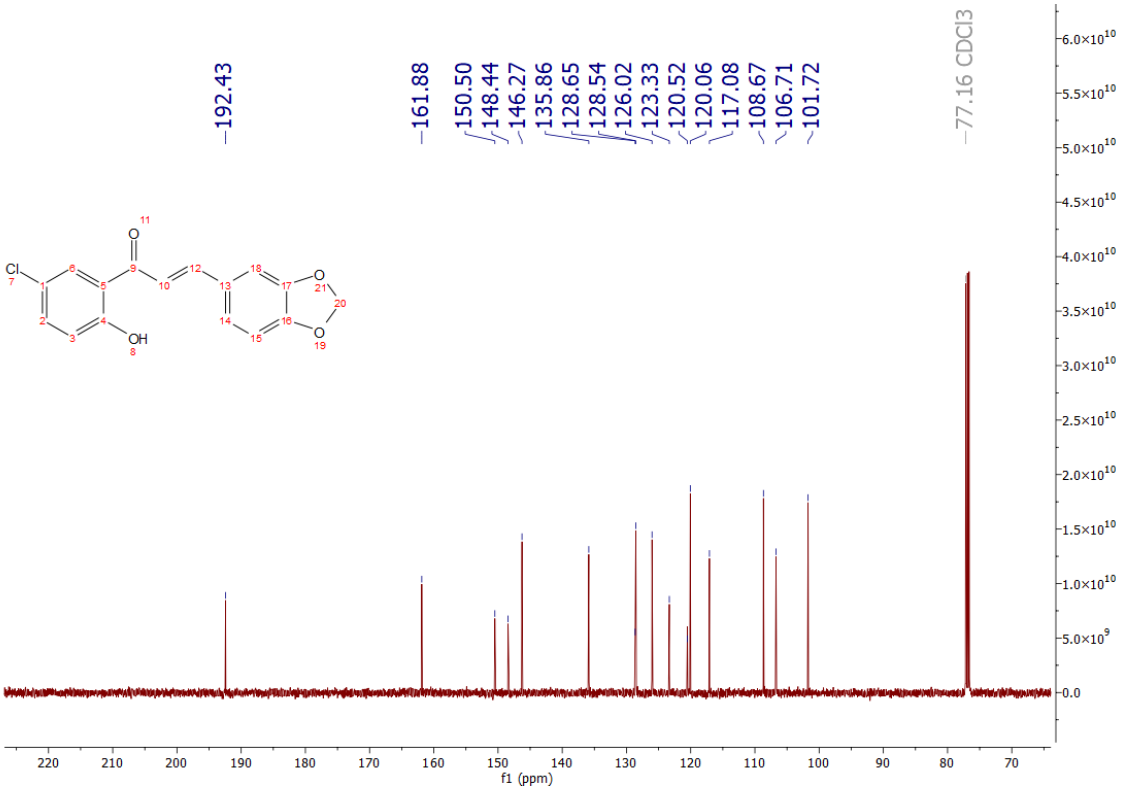
Spectra 13: ^{1}H NMR of compound **3a**



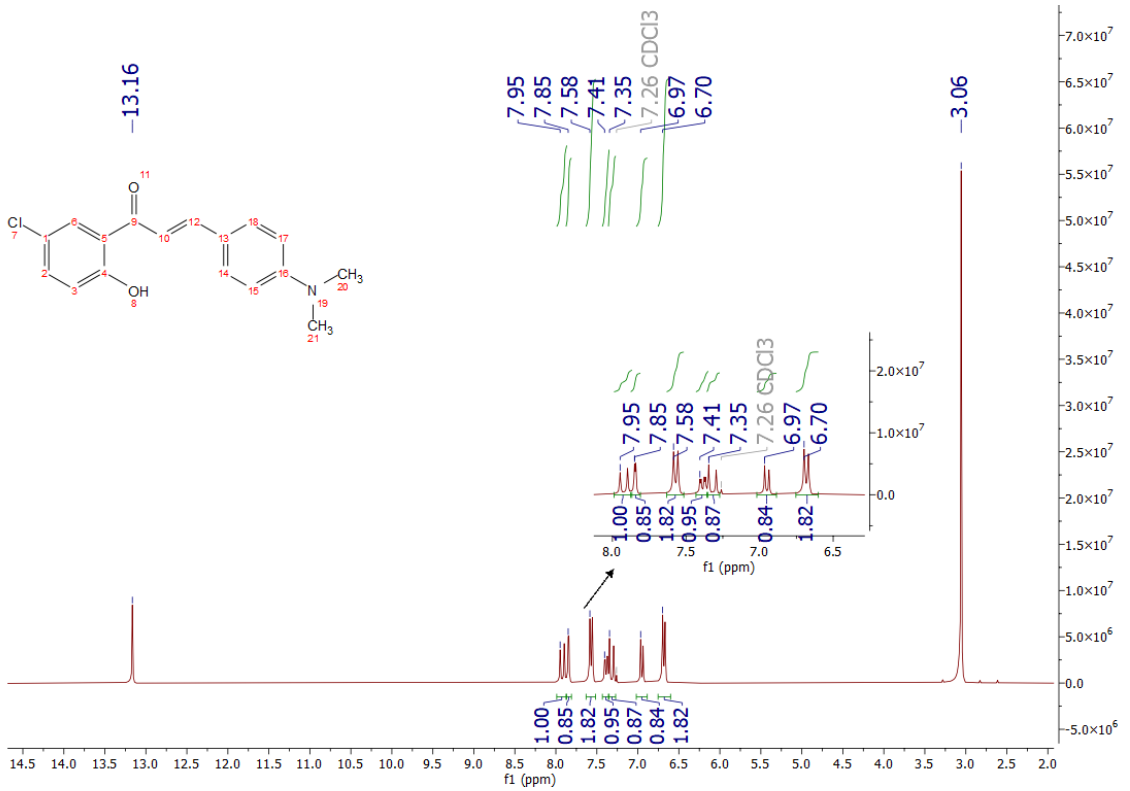
Spectra 14: ^{13}C NMR of compound **3a**



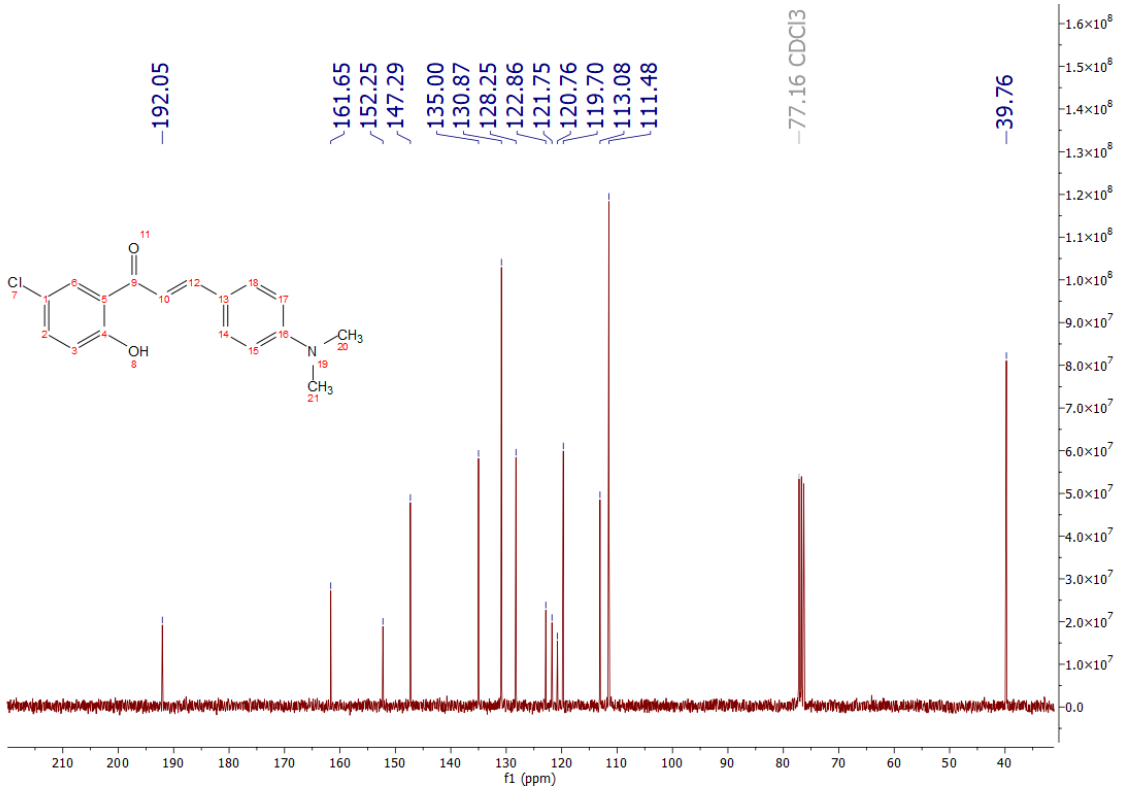
Spectra 15: ^{1}H NMR of compound **3b**



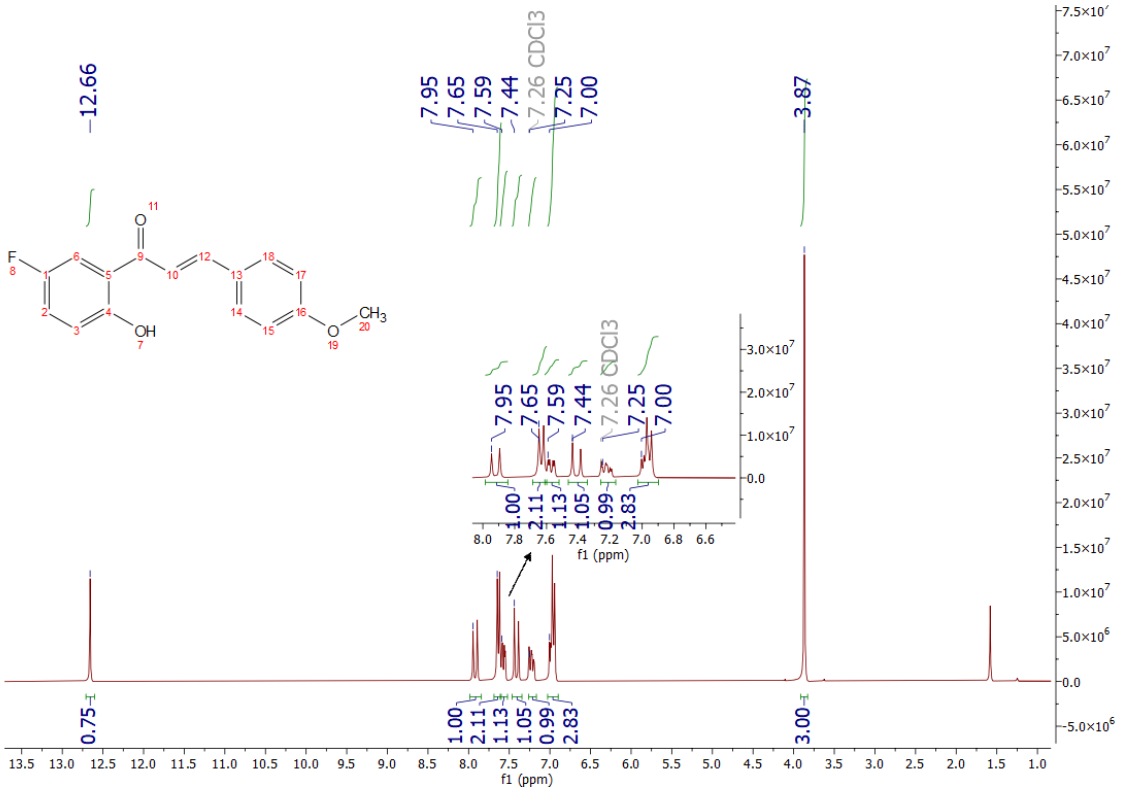
Spectra 16: ^{13}C NMR of compound **3b**



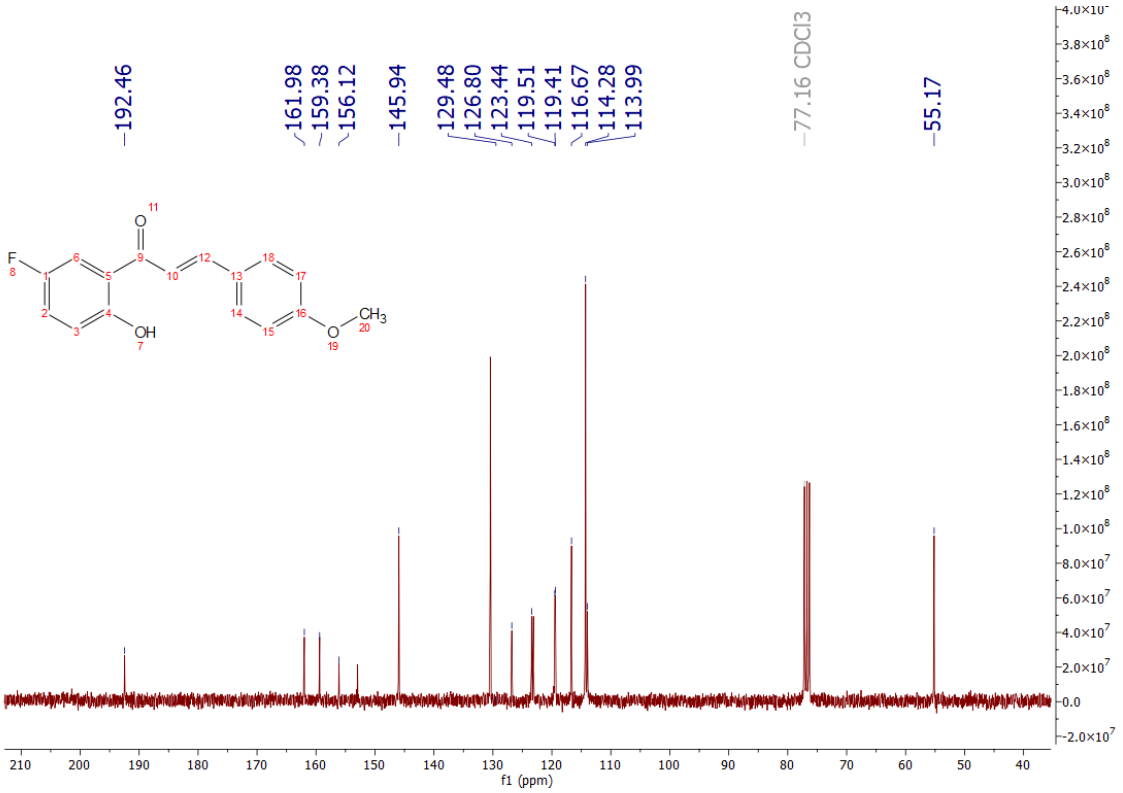
Spectra 17: ^{1}H NMR of compound **3c**



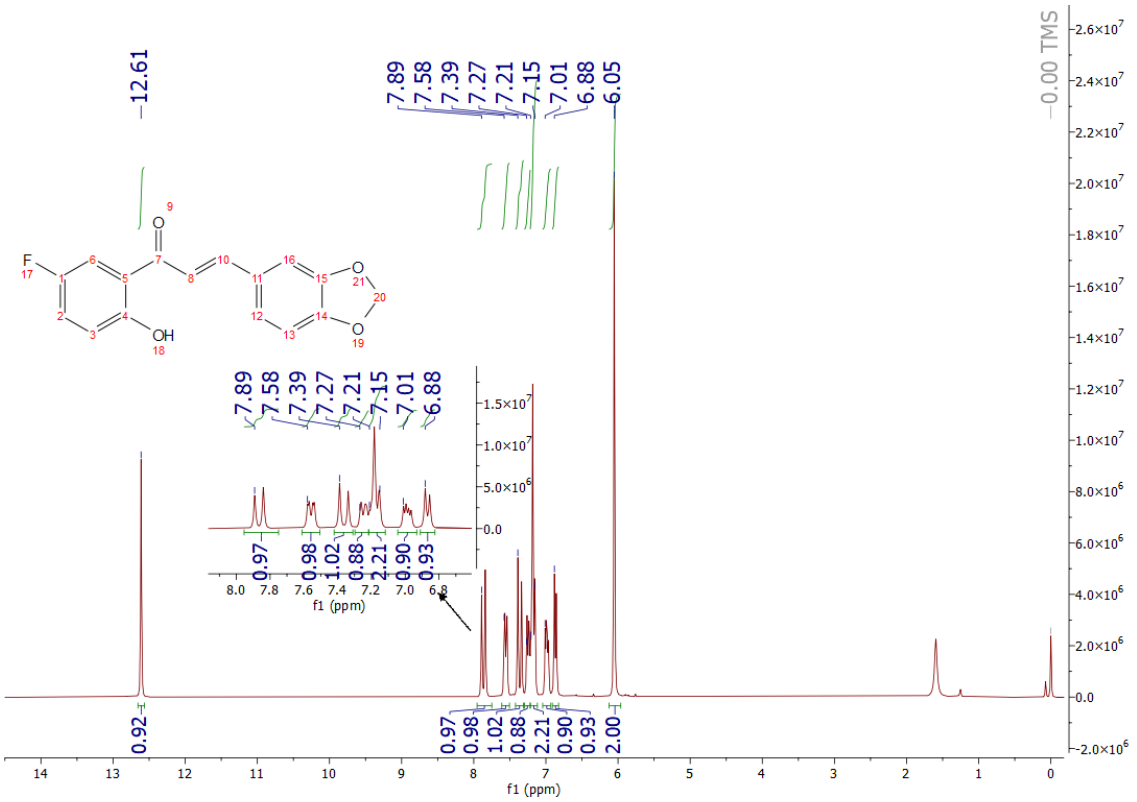
Spectra 18: ^{13}C NMR of compound **3c**



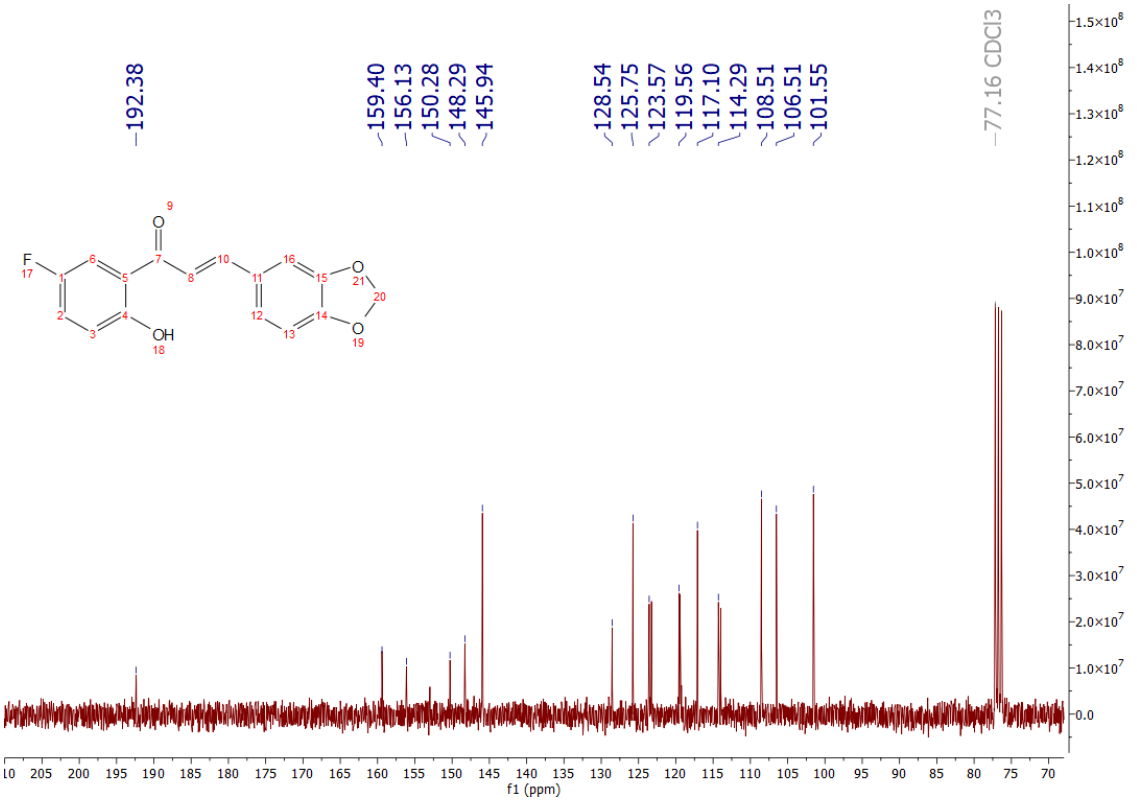
Spectra 19: ^{1}H NMR of compound **4a**



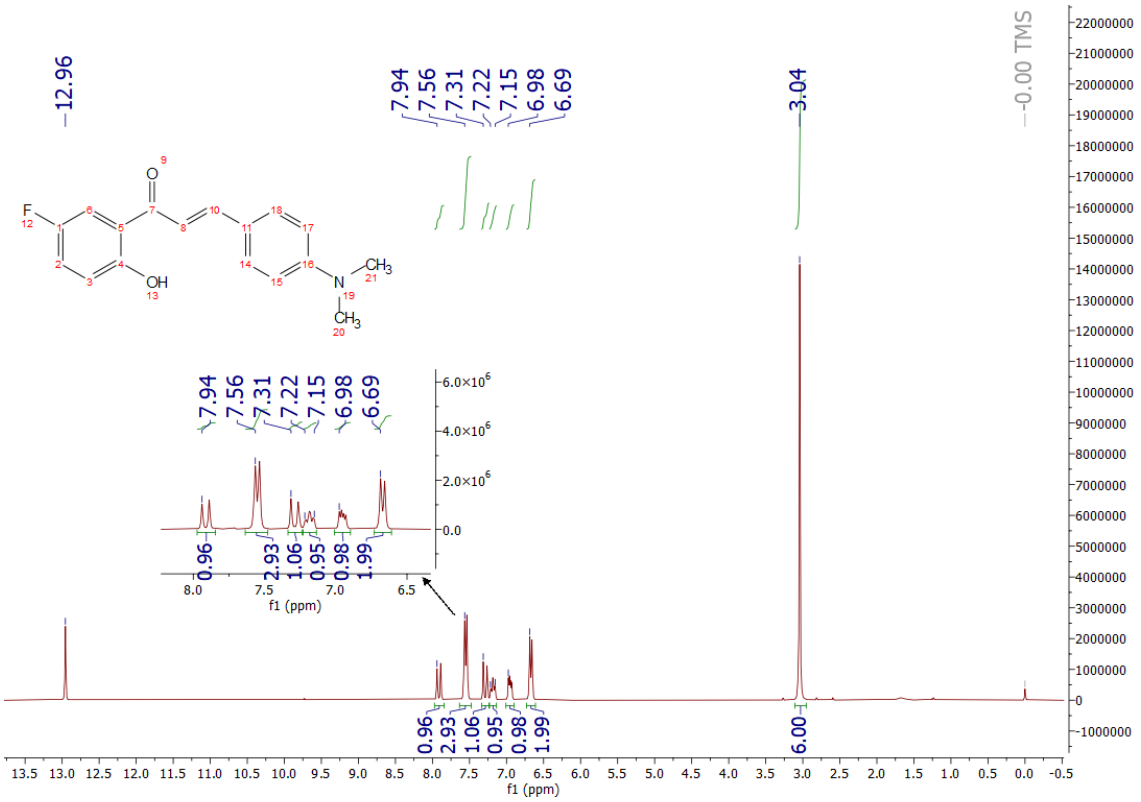
Spectra 20: ^{13}C NMR of compound **4a**



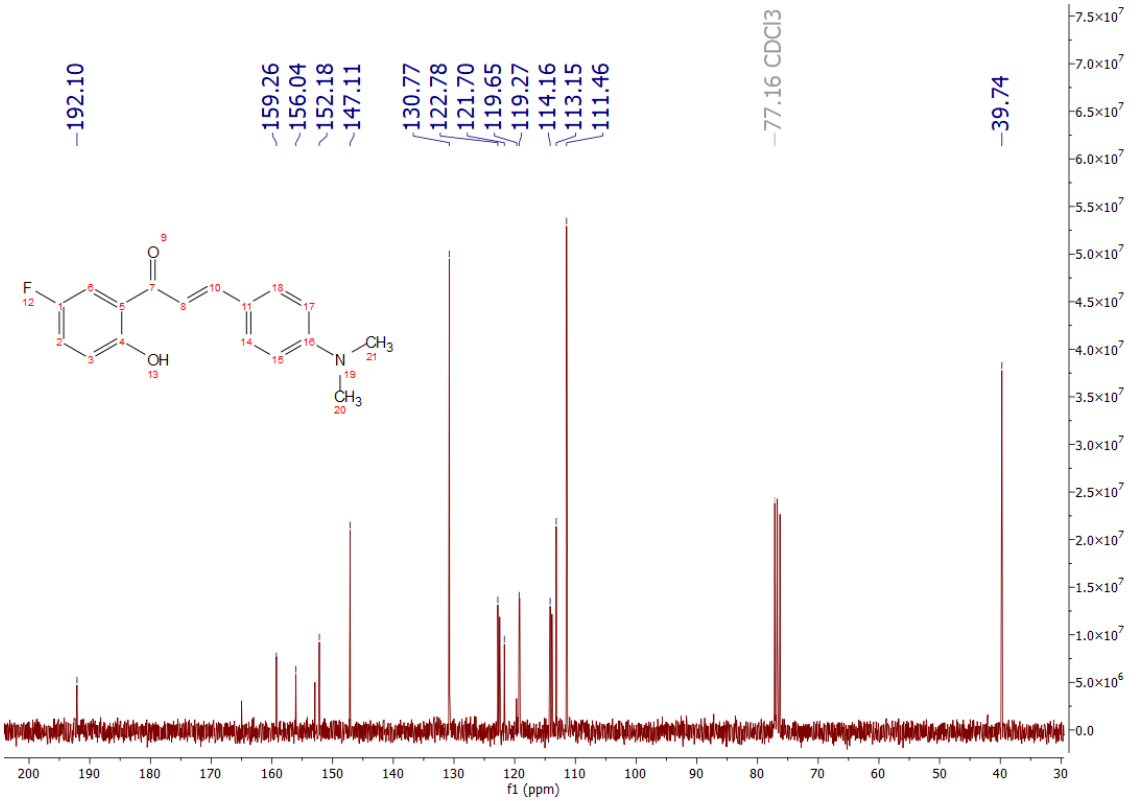
Spectra 21: ^{1}H NMR of compound **4b**



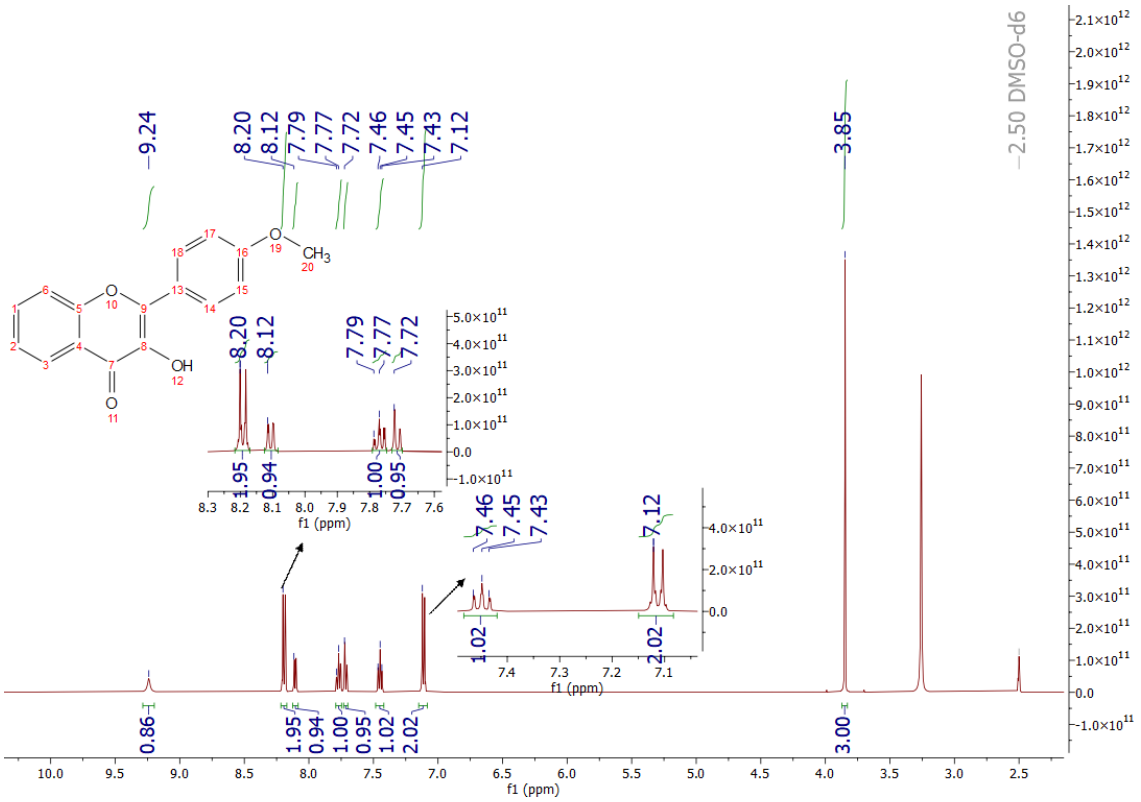
Spectra 22: ^{13}C NMR of compound **4b**



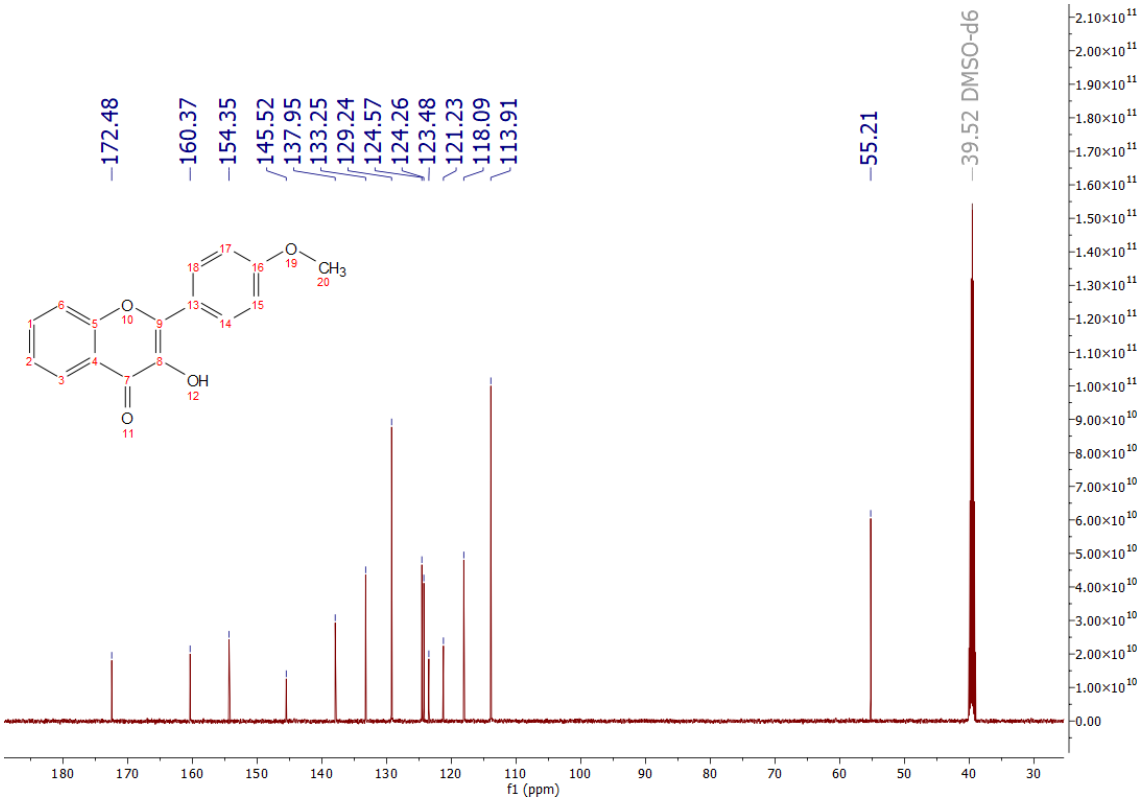
Spectra 23: ^{1}H NMR of compound **4c**



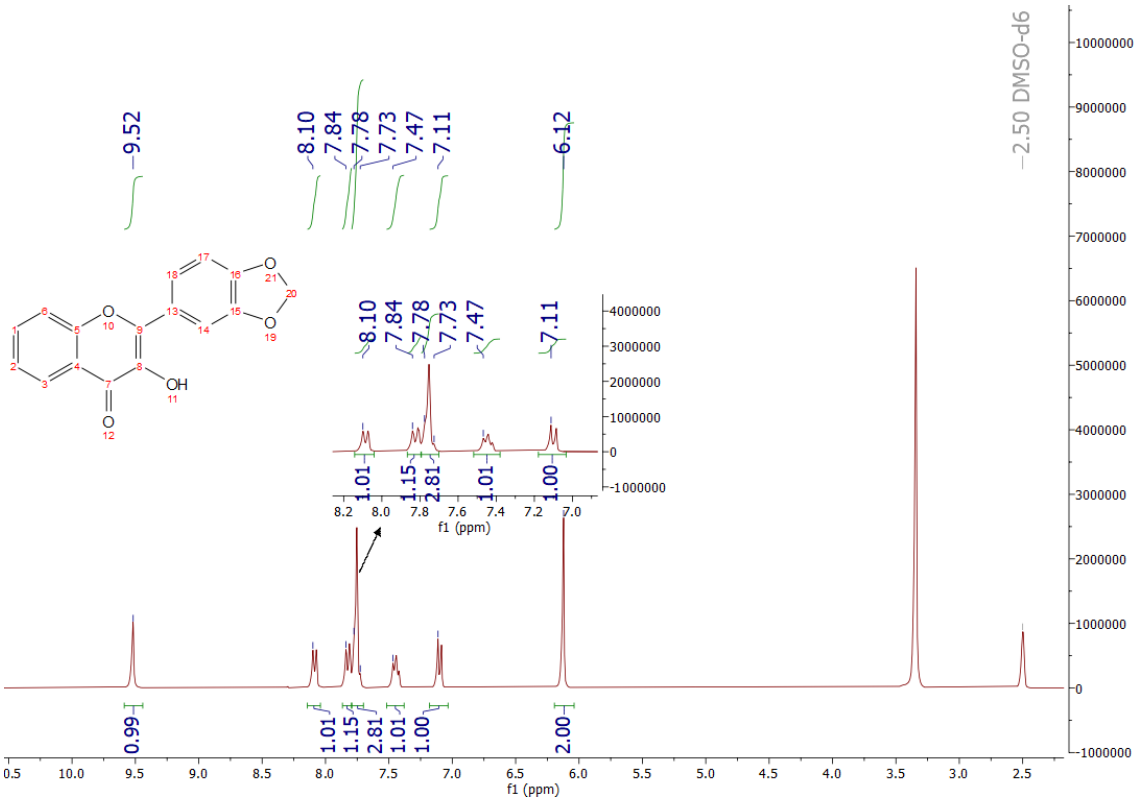
Spectra 24: ^{13}C NMR of compound **4c**



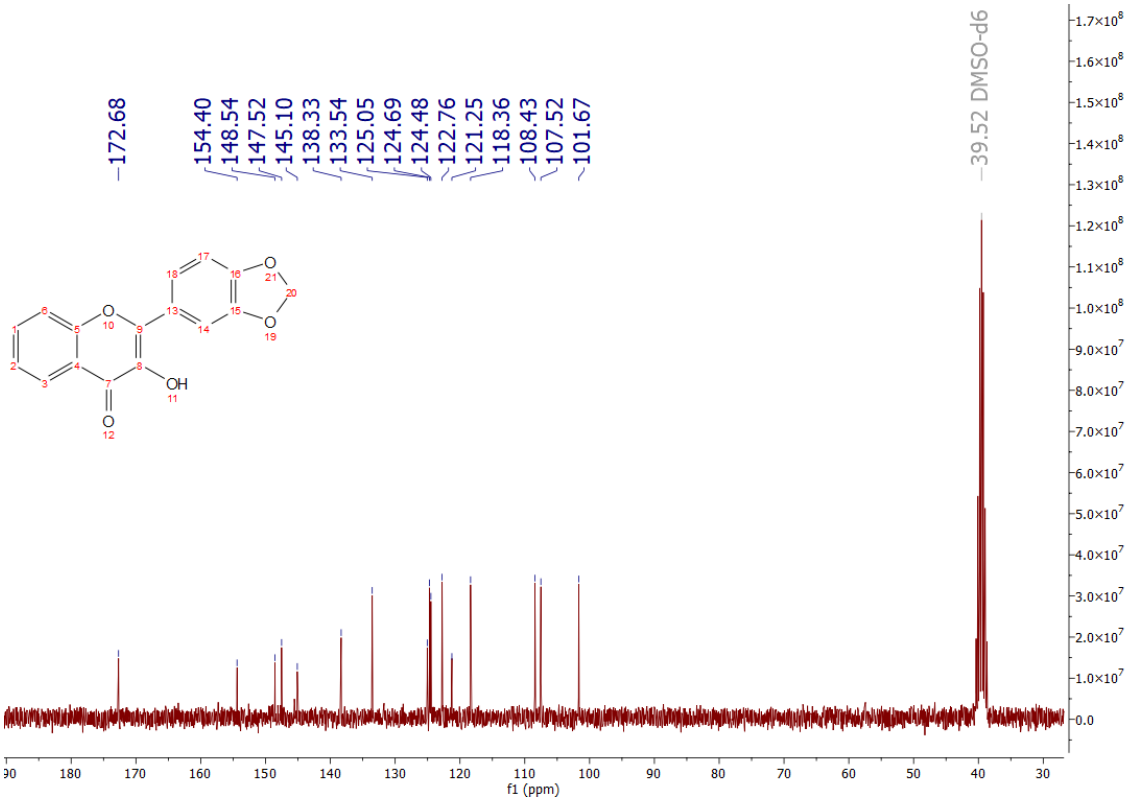
Spectra 25: ^{1}H NMR of compound **f12a**



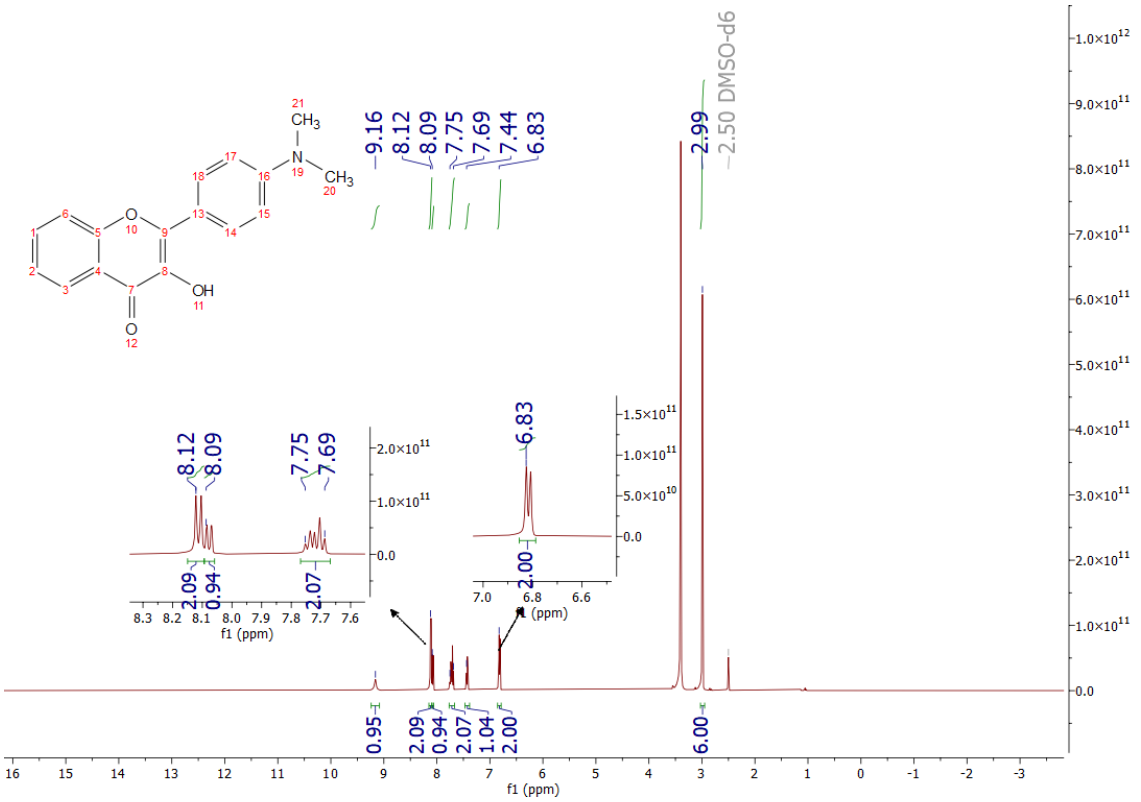
Spectra 26: ^{13}C NMR of compound **f12a**



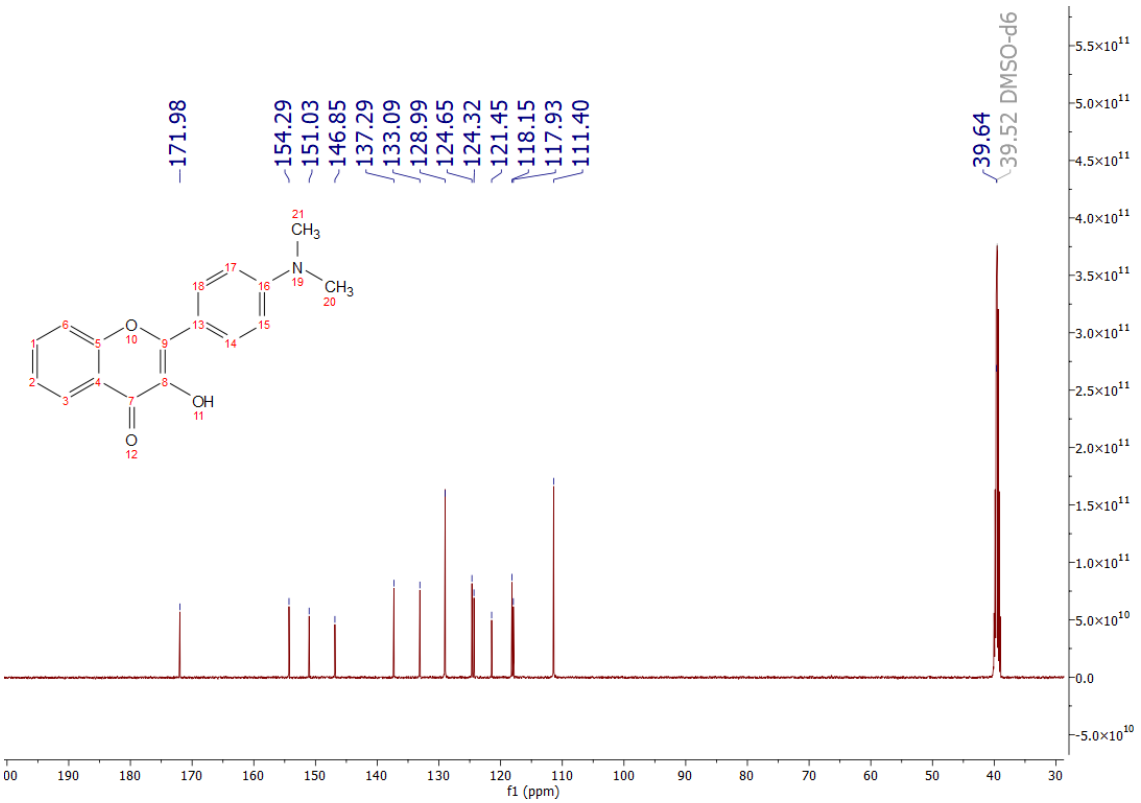
Spectra 27: ^{1}H NMR of compound **f1b**



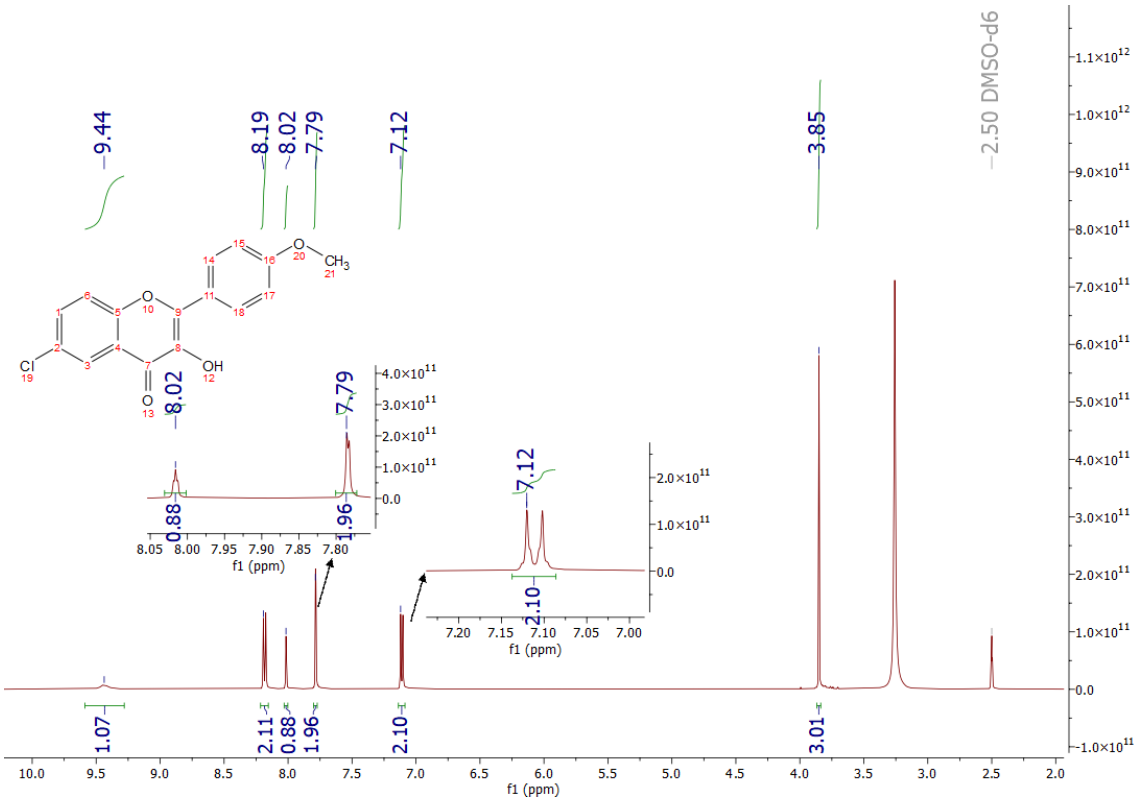
Spectra 28: ^{13}C NMR of compound **f12b**



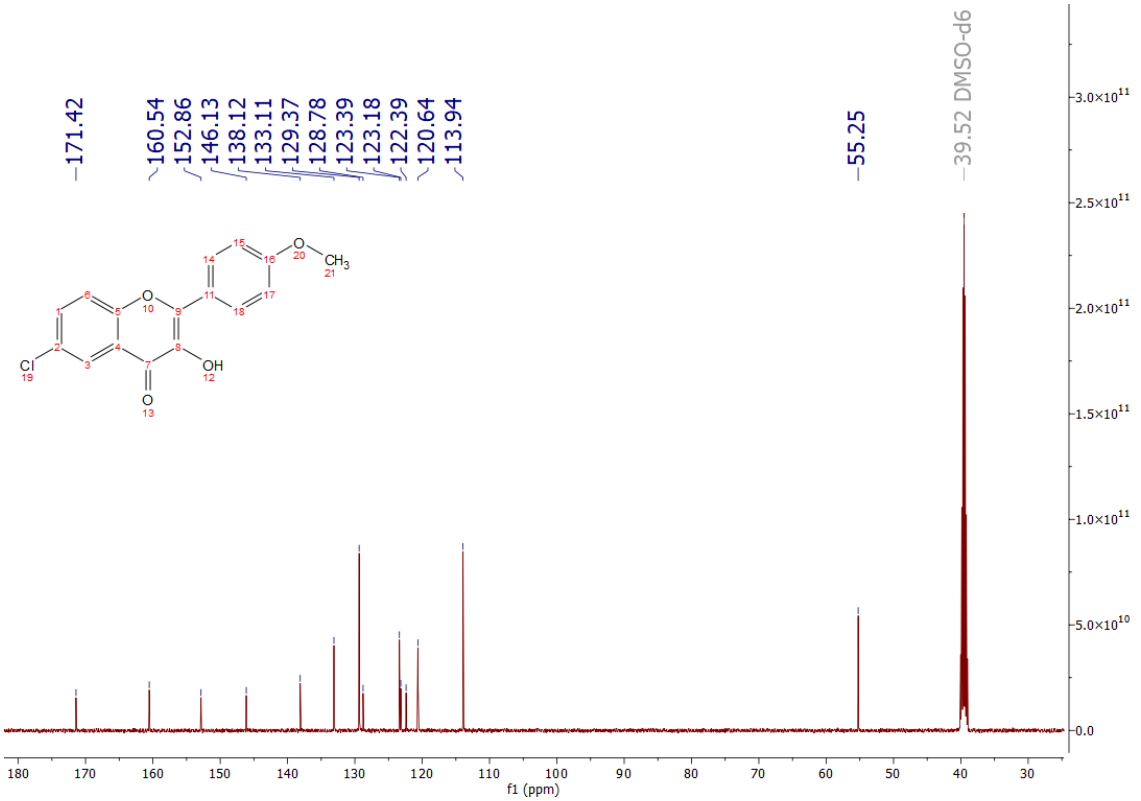
Spectra 29: ^{1}H NMR of compound **f1c**



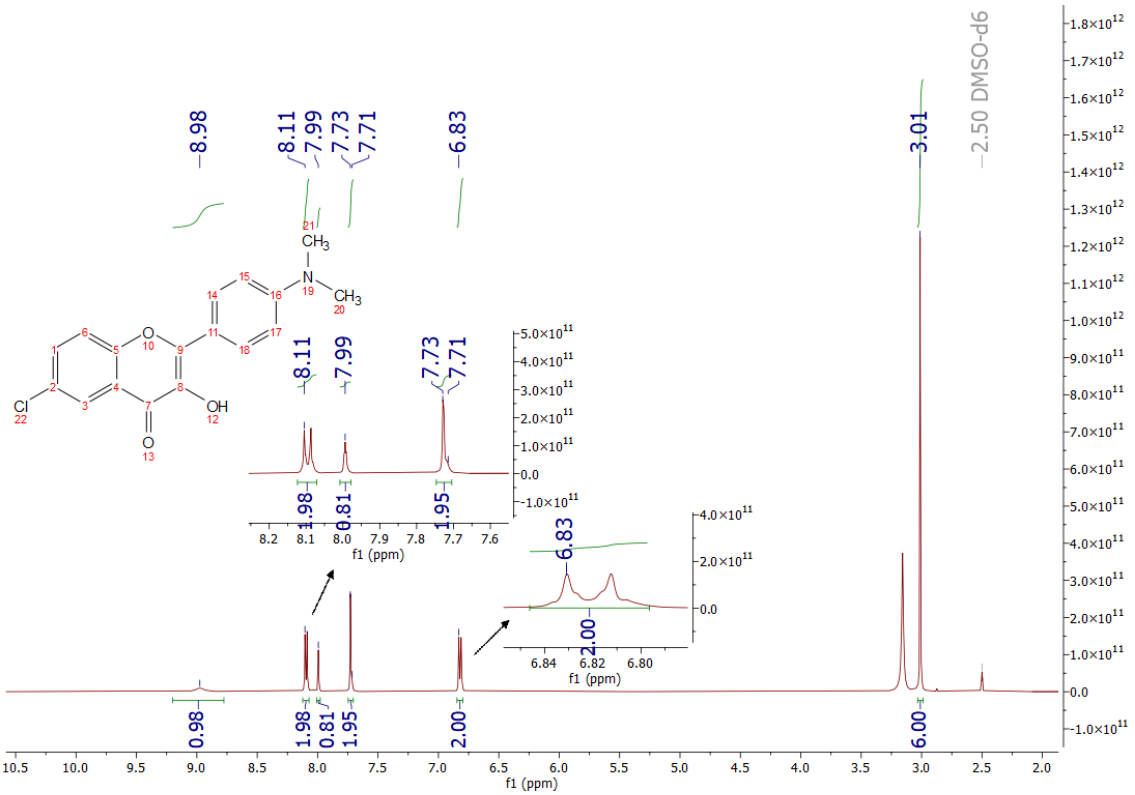
Spectra 30: ^{13}C NMR of compound **f12c**



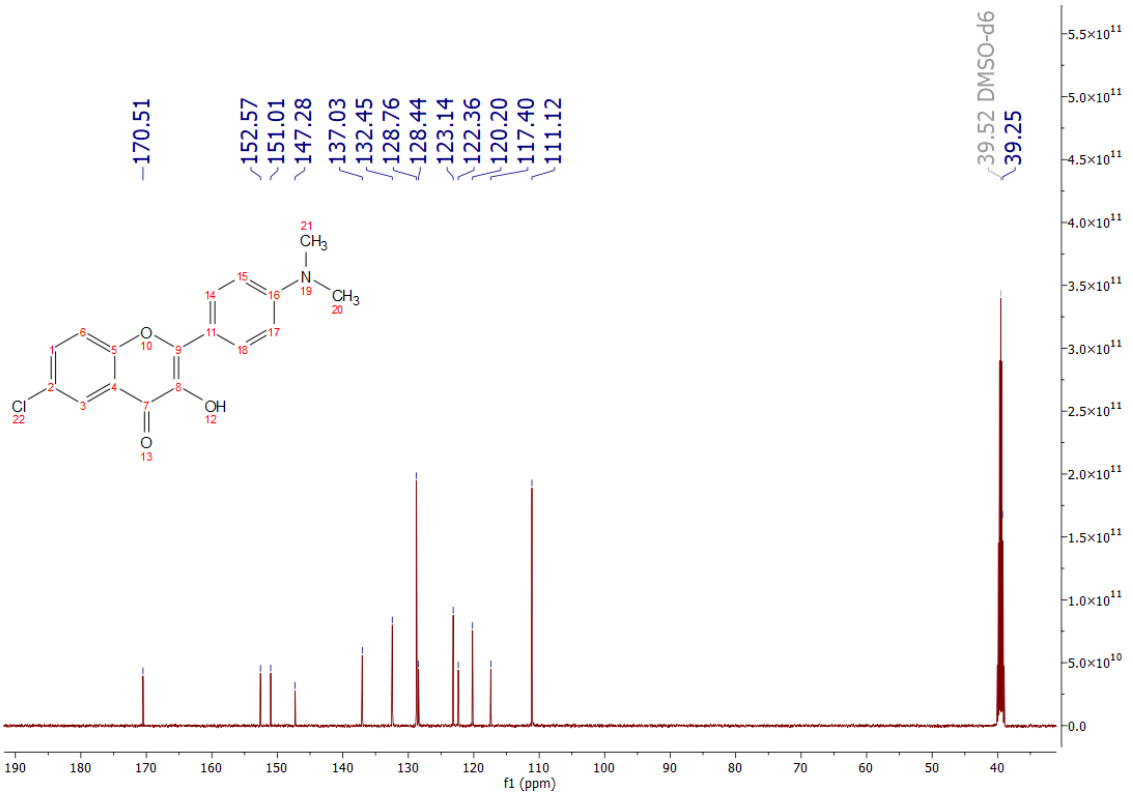
Spectra 31: ^{1}H NMR of compound **f3a**



Spectra 32: ^{13}C NMR of compound **f13a**



Spectra 33: ^{1}H NMR of compound **f3c**



Spectra 34: ^{13}C NMR of compound **f13c**