ESI

Pegylation Approach Applied to Erlotinib-Carbonic Anhydrase Inhibitors Hybrids Towards Anticancer Agents

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Selectivity index (SI) values for the first ERL-clicked derivatives

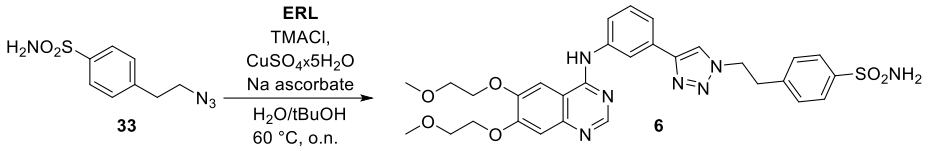
Table S1. Selectivity index (SI) values of hCAs IX and XII over the other tested isoforms for compounds **1a-d**, **2-3a-b**, **4**, **5**, **AZT**, **ERL**, and **AAZ**.

cpd	SI							
	I/IX	II/IX	Va/IX	VI/IX	I/XII	II/XII	Va/XII	VI/XII
1a	3.78	0.11	0.71	19.46	-	-	-	-
1b	4.72	1.22	0.64	9.61	-	-	-	-
1c	191.42	0.27	-	3.28	125	0.18	-	2.15
1d	0.47	0.23	0.65	2.57	1.55	0.75	2.17	8.55
2a	0.01	0.02	0.02	0.09	0.47	1.49	1.19	5.67
2b	0.97	0.02	0.03	0.25	-	-	-	-
3a	0.88	0.14	2.45	65.03	-	-	-	-
3b	0.06	0.01	0.19	0.34	-	-	-	-
4	0.01	0.01	0.01	0.07	-	-	-	-
5	0.00	0.00	0.10	0.07	-	-	-	-
AZT	-	-	-	-	-	1.16	-	0.86
ERL	-	-	-	-	-	-	-	-
AAZ	9.73	0.47	2.45	0.43	43.86	2.12	11.05	1.93

SI values are calculated as the ratio between the K_I values of the physiologically relevant CA isoform I or II (as indicated) and the CA isoform of interest (hCA IX or hCA XII, as indicated). The higher the SI value, the higher the isoform preference.

Synthesis of compound 6

Compound **6** was synthesized by directly connecting the phenethylsulfonamide (**33**) to **ERL** in the same reaction conditions previously illustrated (**Scheme S1**).



Scheme S1. Synthesis of **ERL**-clicked derivative **6**.

Procedure for the synthesis of compound 6
4-(2-(4-(3-((6,7-bis(2-methoxyethoxy)quinazolin-4-yl)amino)phenyl)-1H-1,2,3-triazol-1-yl)ethyl)benzenesulfonamide

Erlotinib hydrochloride (**ERL**) (1 equiv), Na ascorbate (1.6 equiv), $CuSO_4x5H_2O$ (0.8 equiv) tetramethylammonium chloride (4.5 equiv) and commercially available **33** (1.1 equiv) were dissolved in H_2O/*tert*-butanol (1:1) and stirred overnight at 60 °C. Then, the reaction mixture was filtered through a cake of Celite 521© and the filtrate was treated with slush. The crude was extracted with DCM thrice. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The crude material was purified by flash column chromatography (MeOH/DCM: 4:96), to yield compounds **6**. Brown powder. Yield: 25%. **^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm):** 8.58 (1H, s), 8.54 (1H, s), 8.23 (1H, s), 7.98 (1H, s), 7.93 (1H, s), 7.88 (1H, t, *J* = 7.9 Hz), 7.72 (2H, d, *J* = 8.6 Hz), 7.52 (1H, d, *J* = 8.3 Hz), 7.46 (1H, d, *J* = 7.9 Hz), 7.42 (2H, d, *J* = 8.7 Hz), 7.28 (2H, bs, SO_2NH_2, exchange with D_2O), 7.24 (1H, s), 4.72 (4H, t, *J* = 7.2 Hz), 4.30 (4H, t, *J* = 3.4 Hz), 3.77 (4H, m), 3.38 (3H, s), 3.36 (3H, s). **^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):** 157.3, 154.6, 149.2, 147.2, 143.6, 142.8, 141.1, 132.1, 130.2, 130.0, 126.8, 122.8, 122.4, 121.3, 119.8, 109.6, 104.4, 71.2, 71.1, 69.5, 69.1, 59.4, 59.4, 51.2, 36.2; MS (ESI positive) *m/z*: 620.3 $[M+H]^+$. Elemental analysis: calculated C, 58.15; H, 5.37; N, 15.82; found C, 58.16; H, 5.39; N, 15.81.

Antiproliferative effect of the first series of ERL clicked derivatives 1a-d, ERL, and SLC-0111

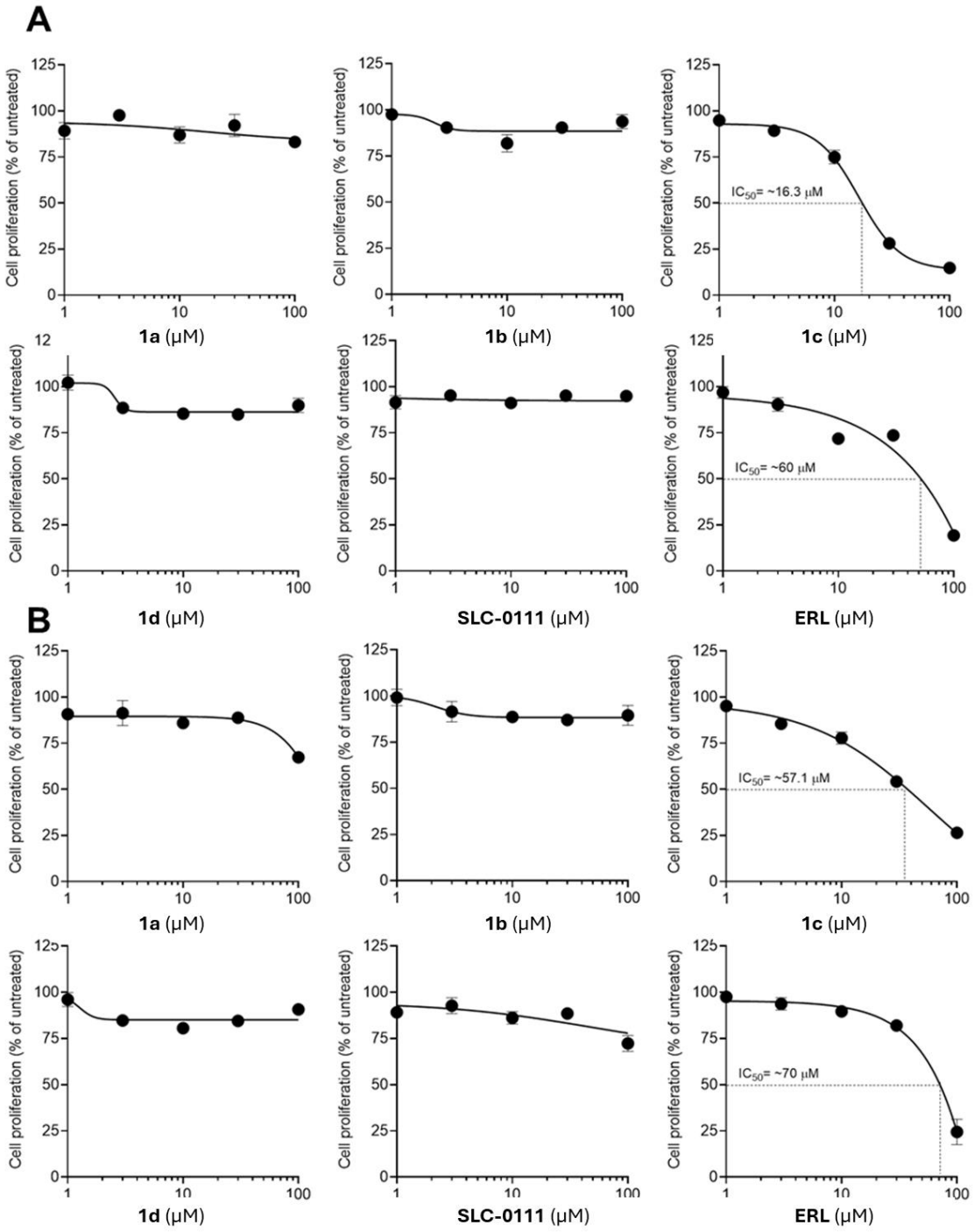
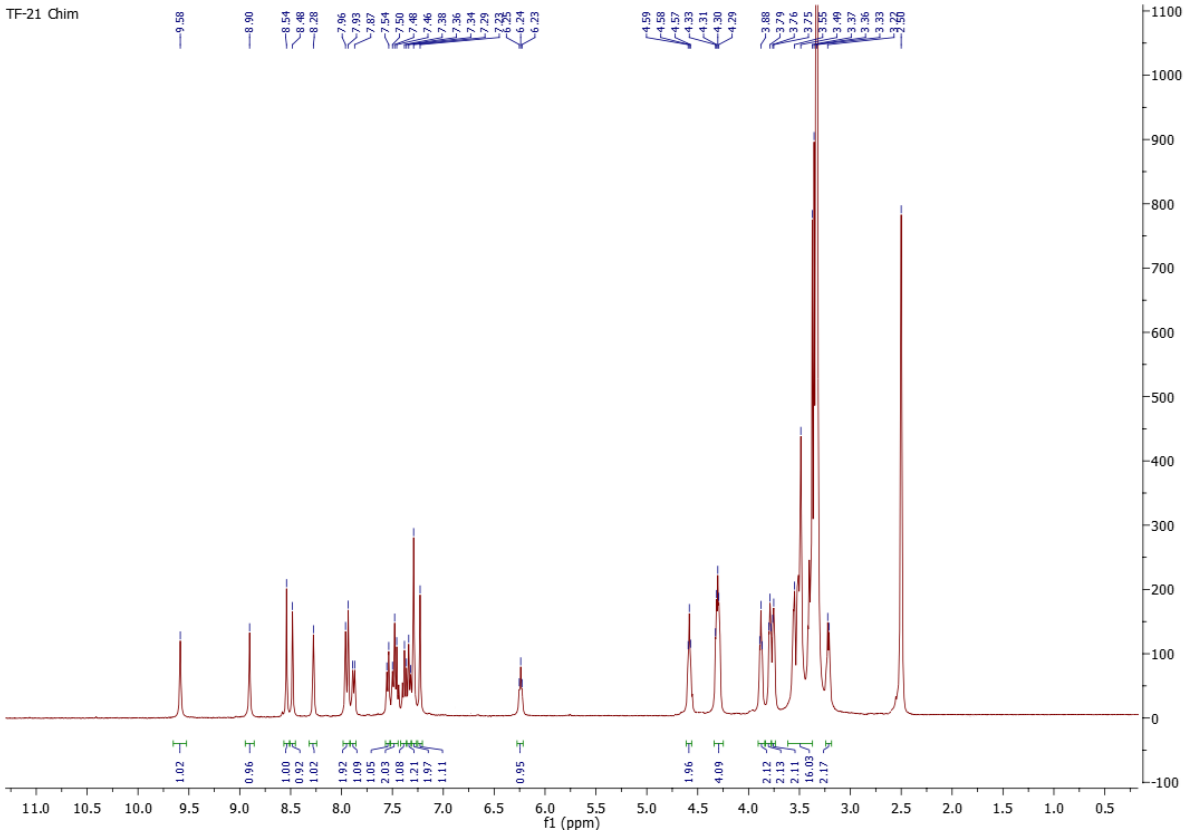
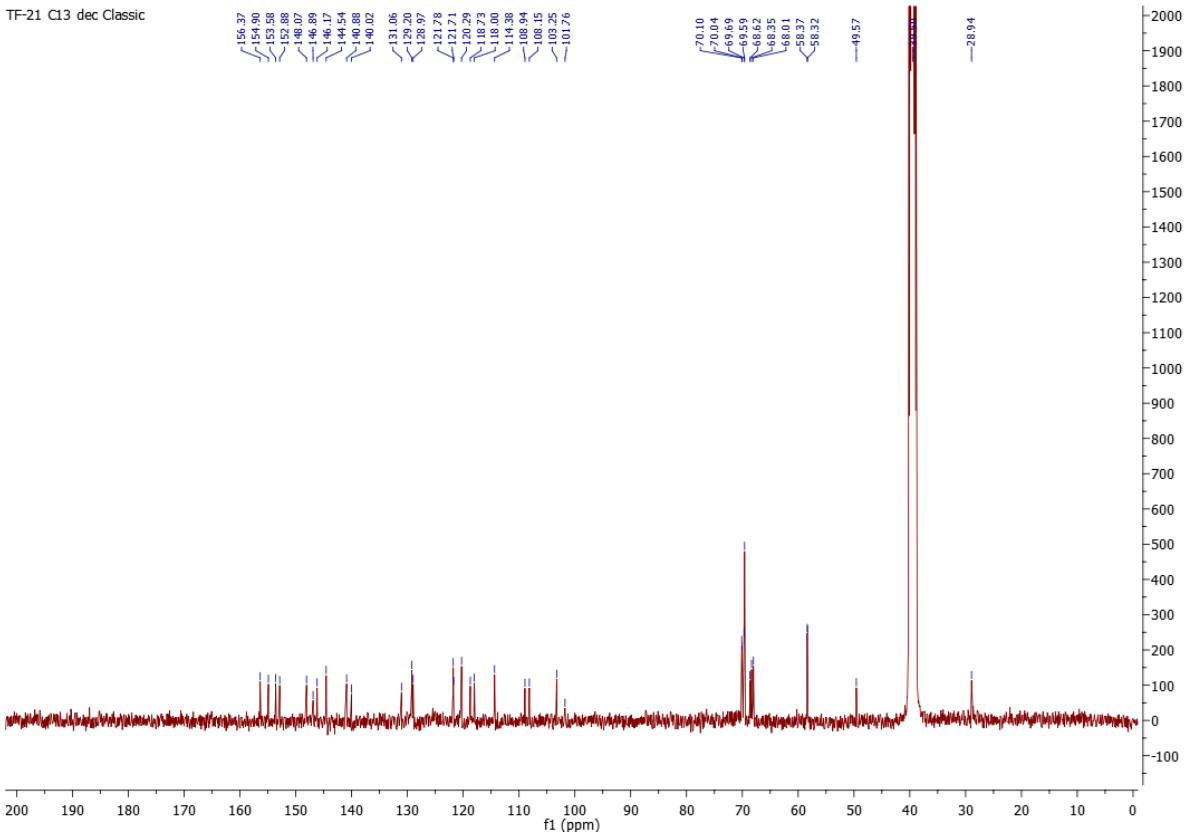


Figure S1. Cell proliferation of A549 (**A**) and PANC-1 (**B**) cells treated for 72 hours in hypoxic conditions with compounds **1a-d**, **SLC-0111**, or **ERL**. Cell proliferation has been assessed with CCK-8 assay and is referred to the untreated/control considered as 100%, mean ± SEM, and the IC_{50} are reported.

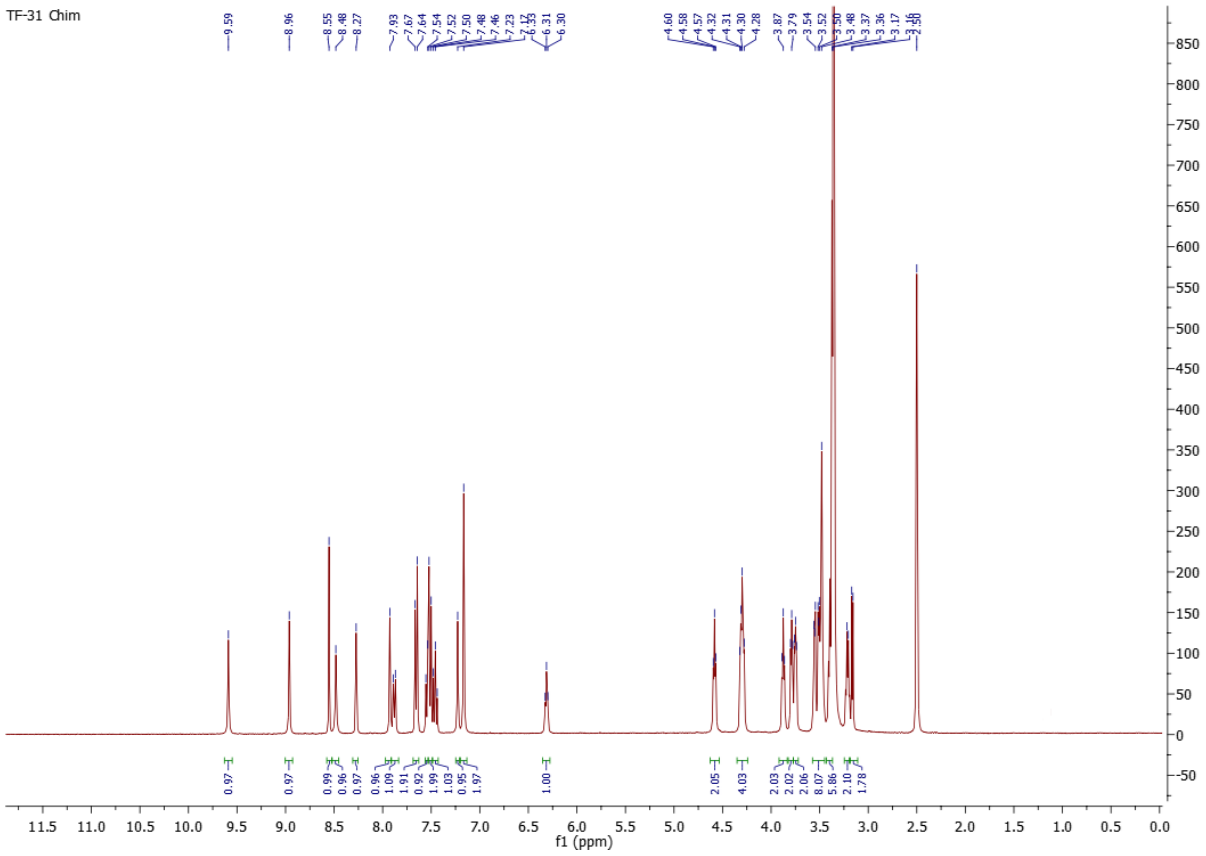
^{1}H and ^{13}C NMR of representative compounds



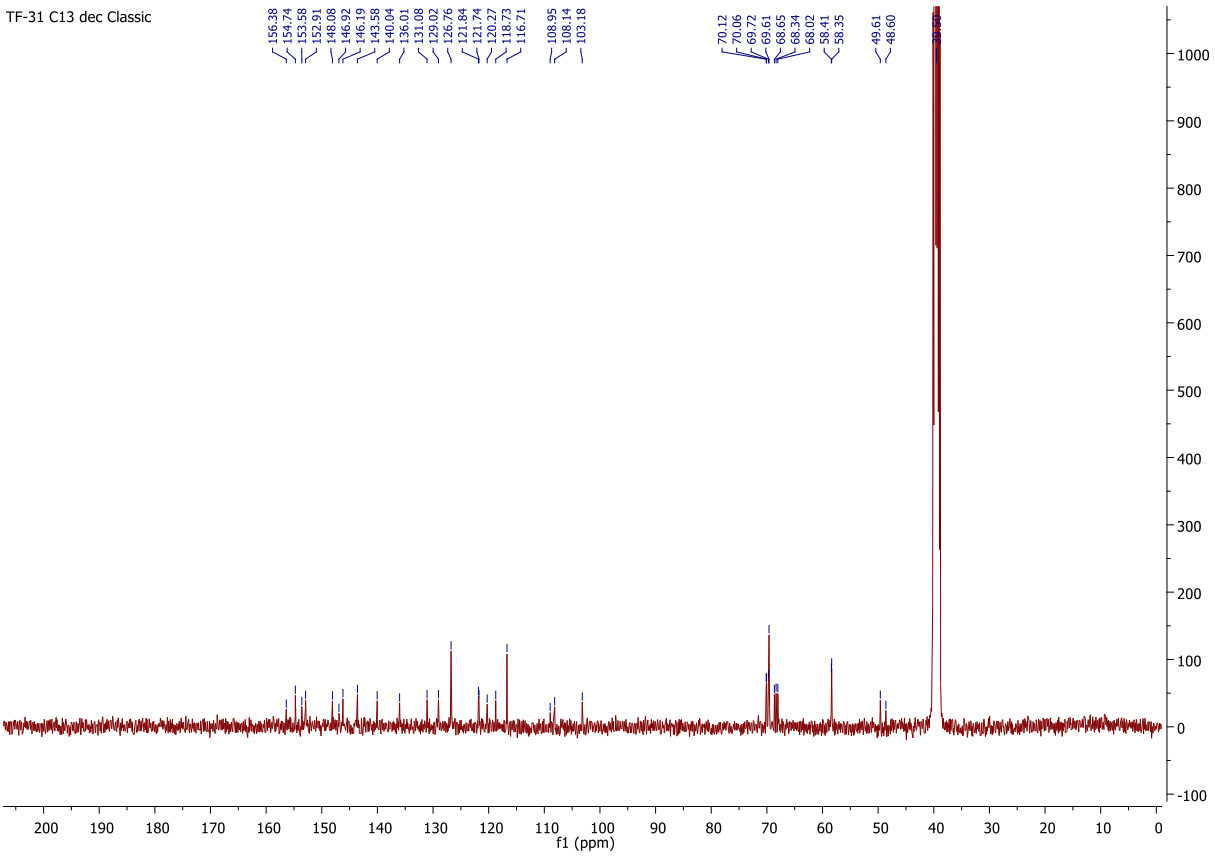
^{1}H NMR spectrum of compound **7a** (400 MHz, DMSO-d_6)



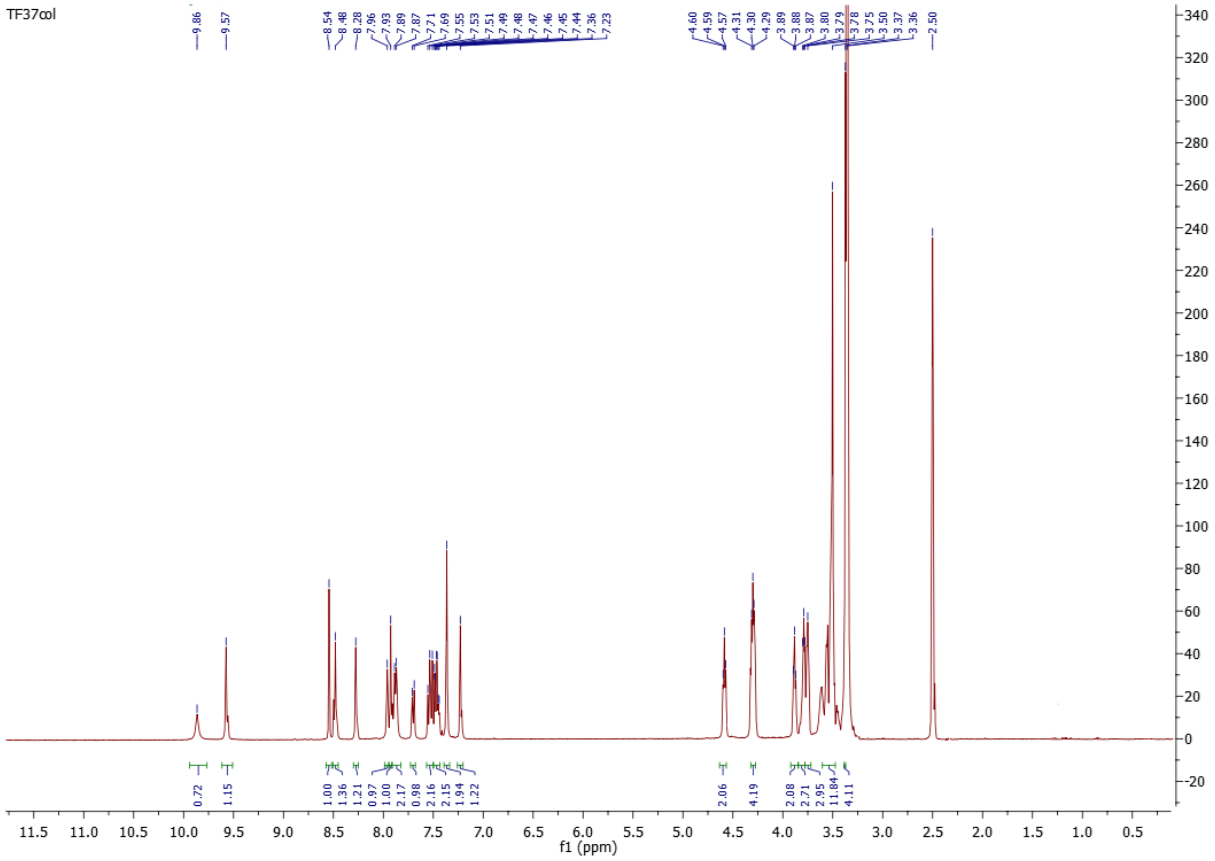
^{13}C NMR spectrum of compound **7a** (100 MHz, DMSO-d_6)



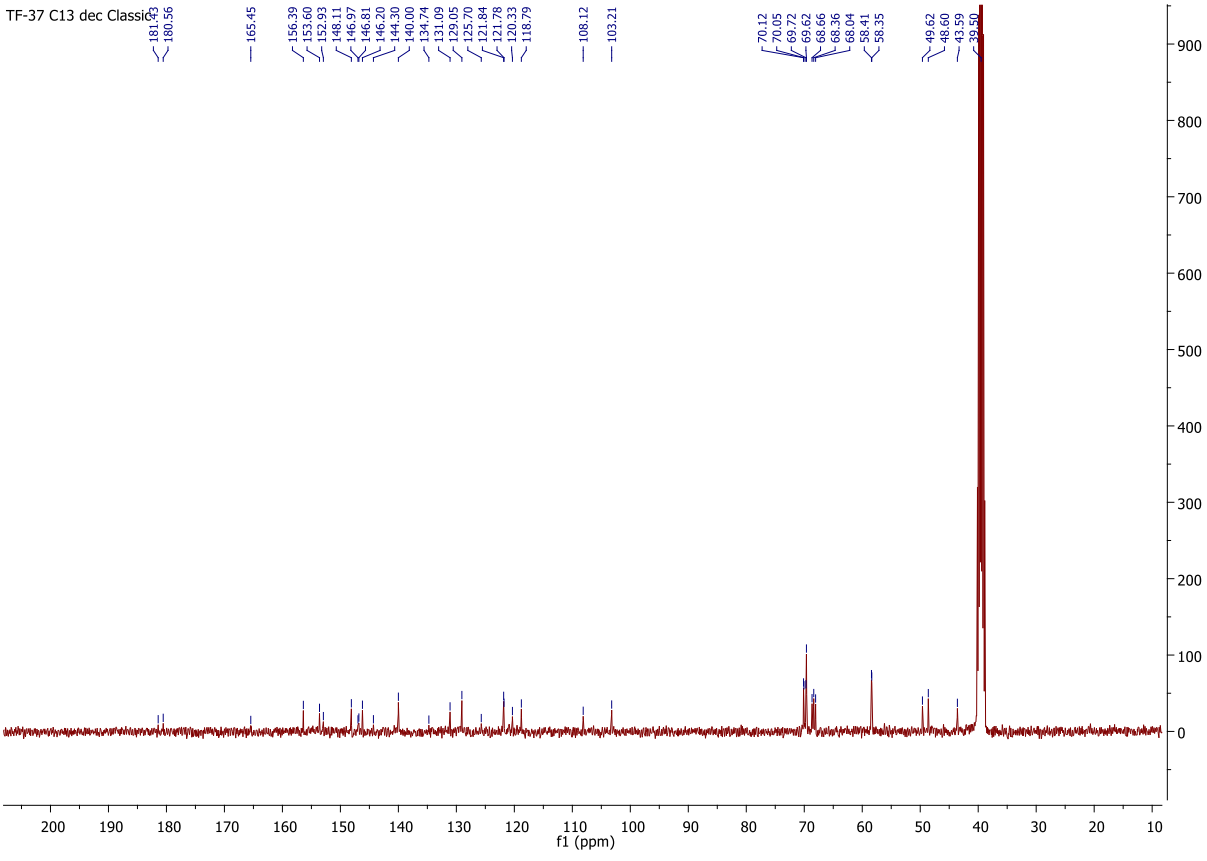
^{1}H NMR spectrum of compound **7b** (400 MHz, DMSO-d_6)



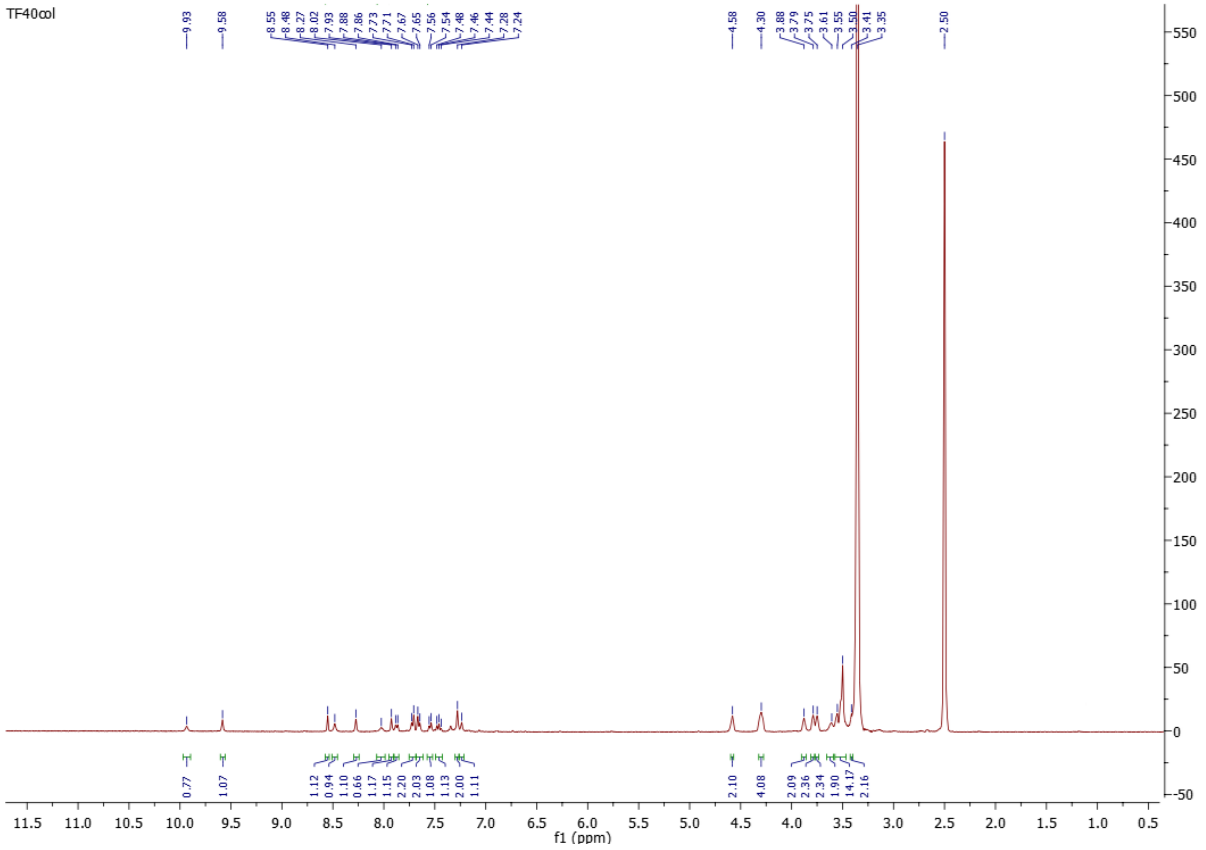
^{13}C NMR spectrum of compound **7b** (100 MHz, DMSO-d_6)



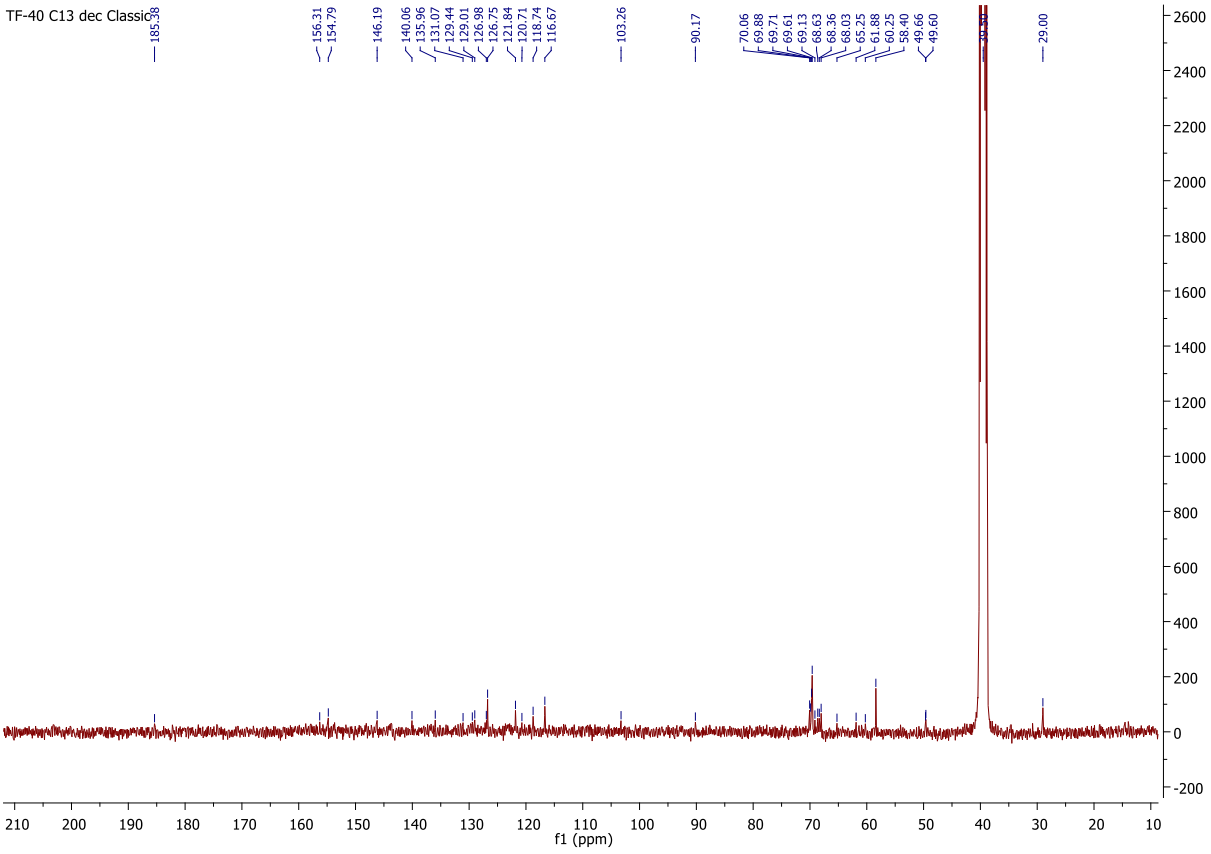
^{1}H NMR spectrum of compound **8a** (400 MHz, DMSO-d_6)



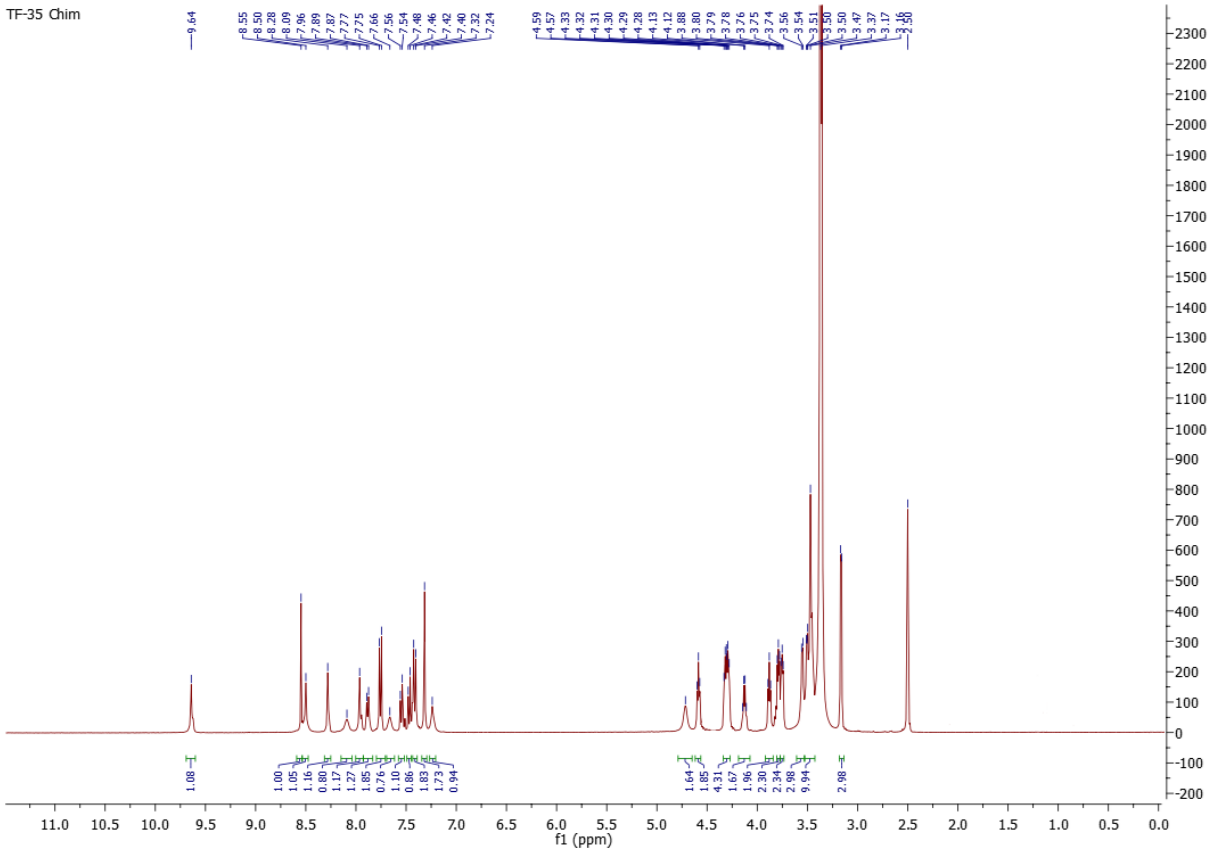
^{13}C NMR spectrum of compound **8a** (100 MHz, DMSO-d_6)



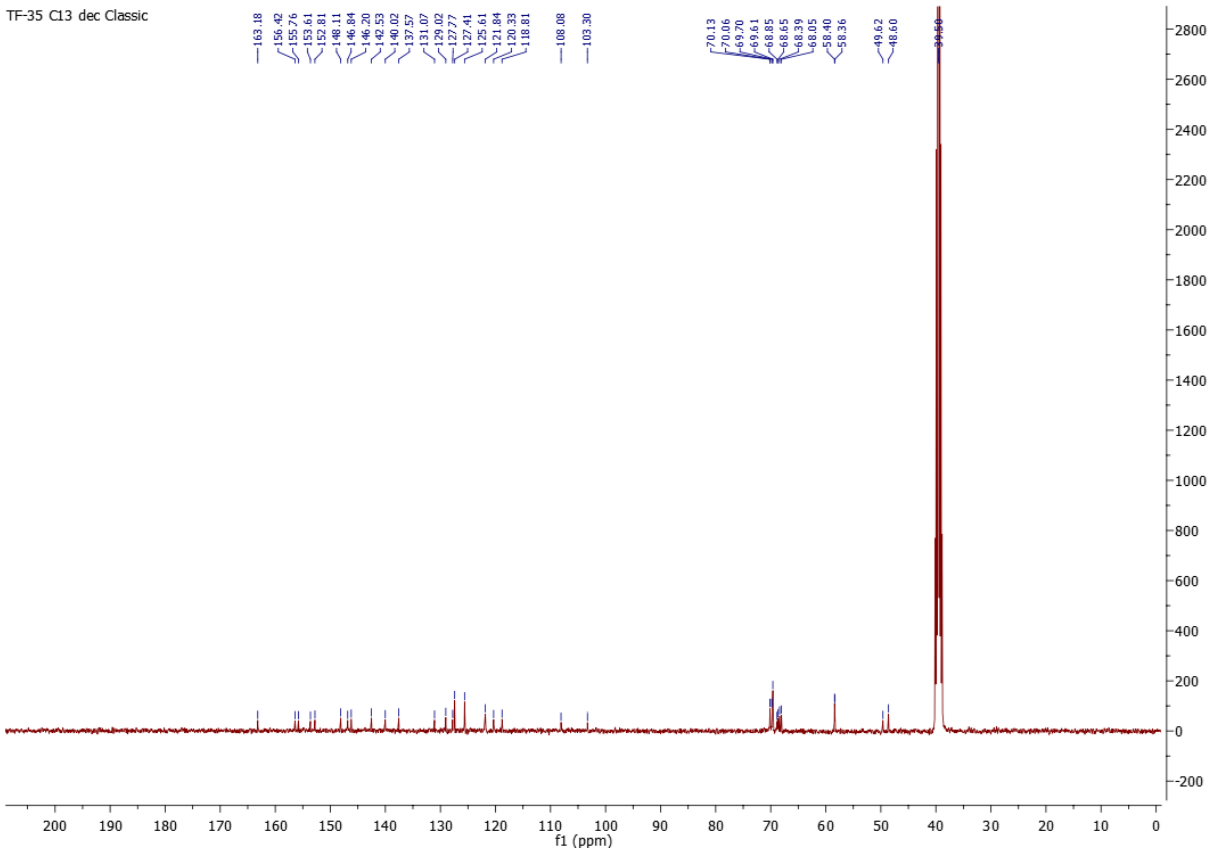
^{1}H NMR spectrum of compound **8b** (400 MHz, DMSO-d_6)



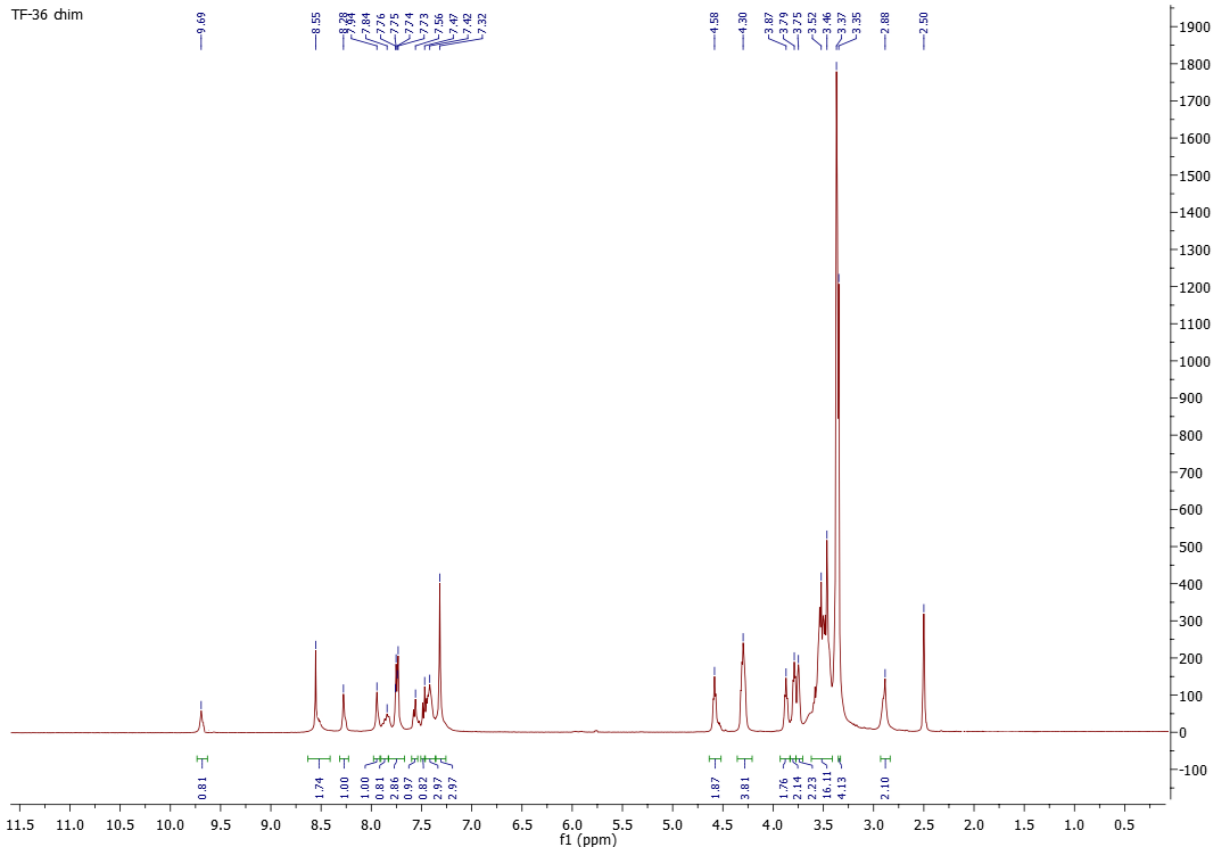
^{13}C NMR spectrum of compound **8b** (100 MHz, DMSO-d_6)



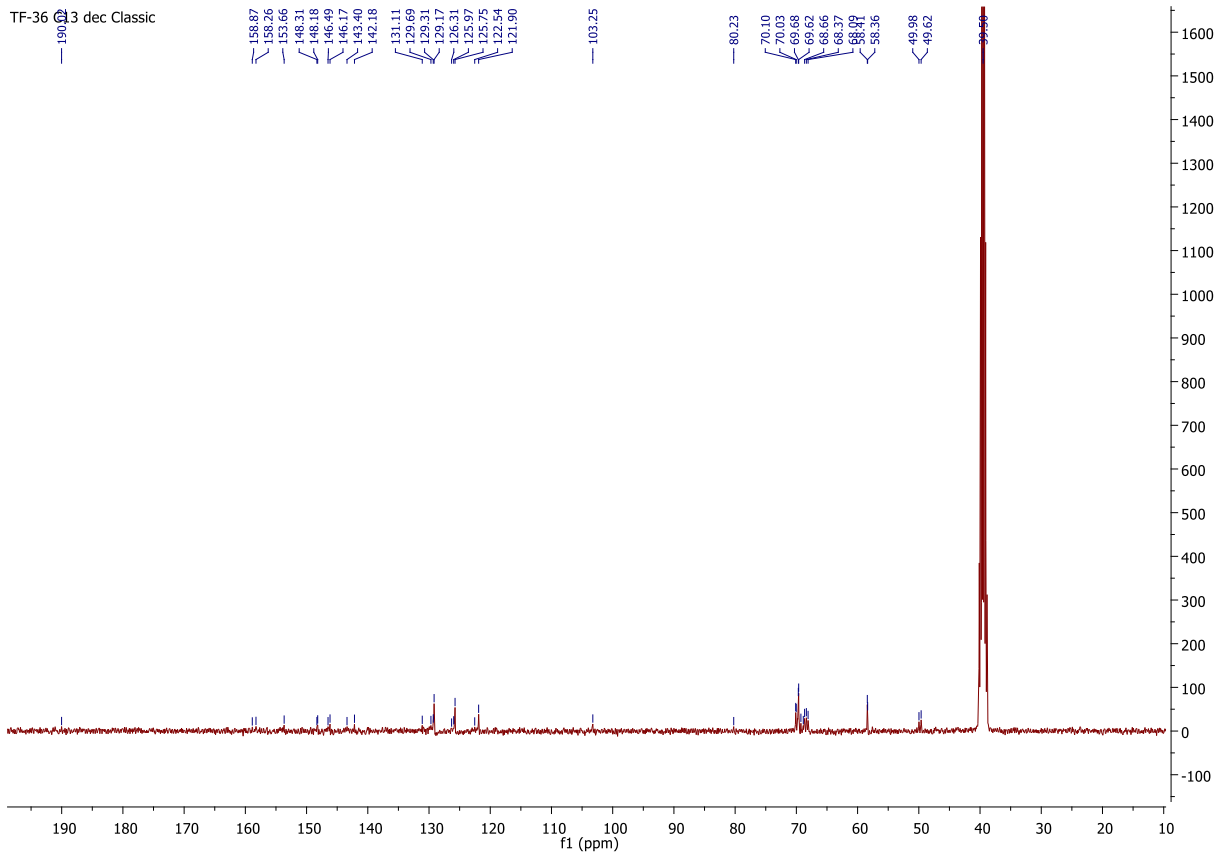
^{1}H NMR spectrum of compound **8c** (400 MHz, DMSO-d_6)



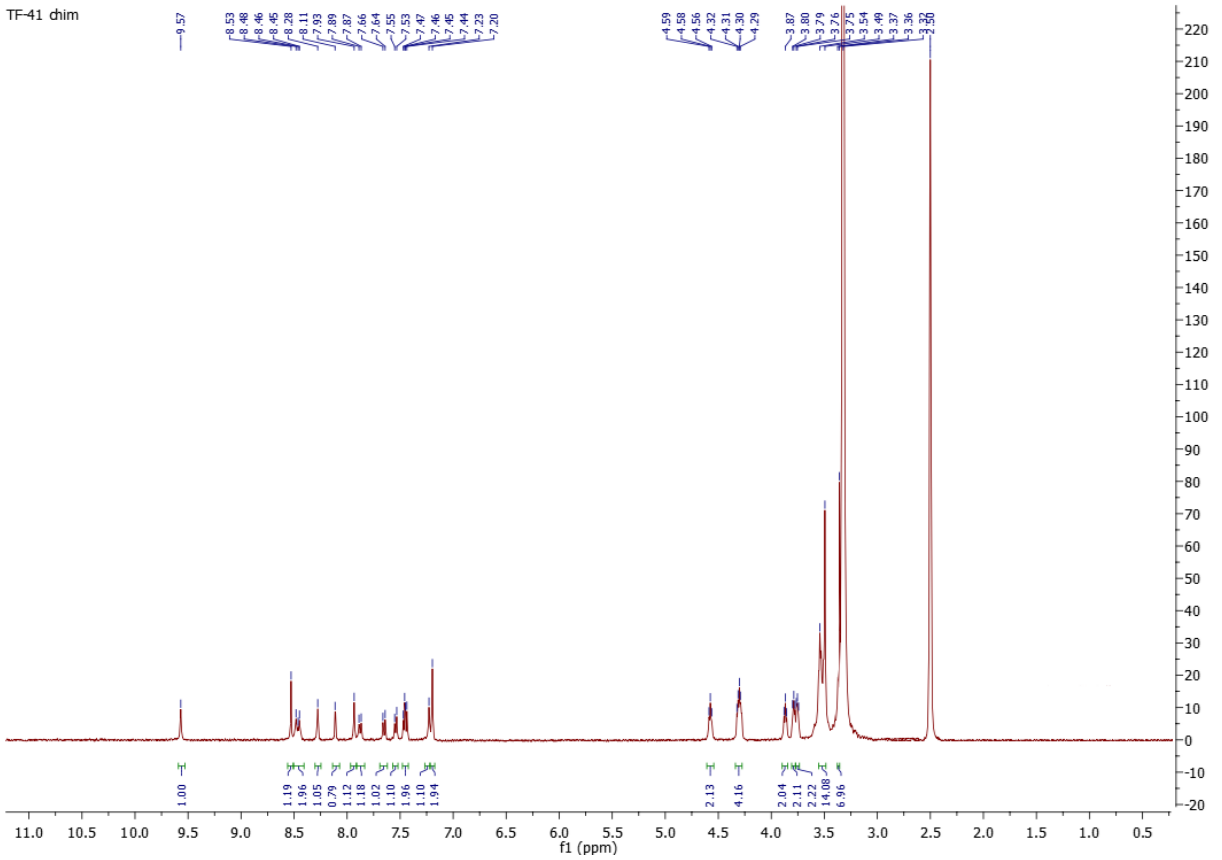
^{13}C NMR spectrum of compound **8c** (100 MHz, DMSO-d_6)



^{1}H NMR spectrum of compound **8d** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **8d** (100 MHz, DMSO-d_6)



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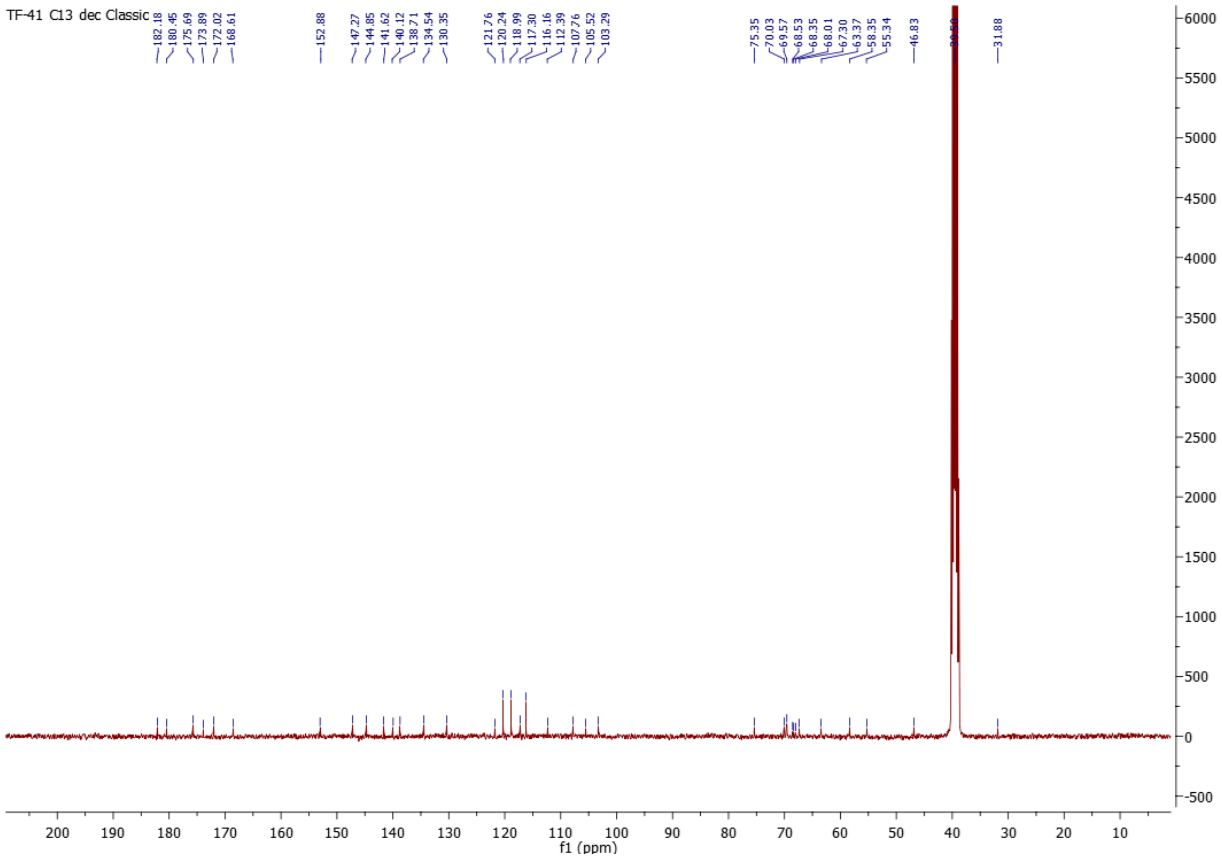
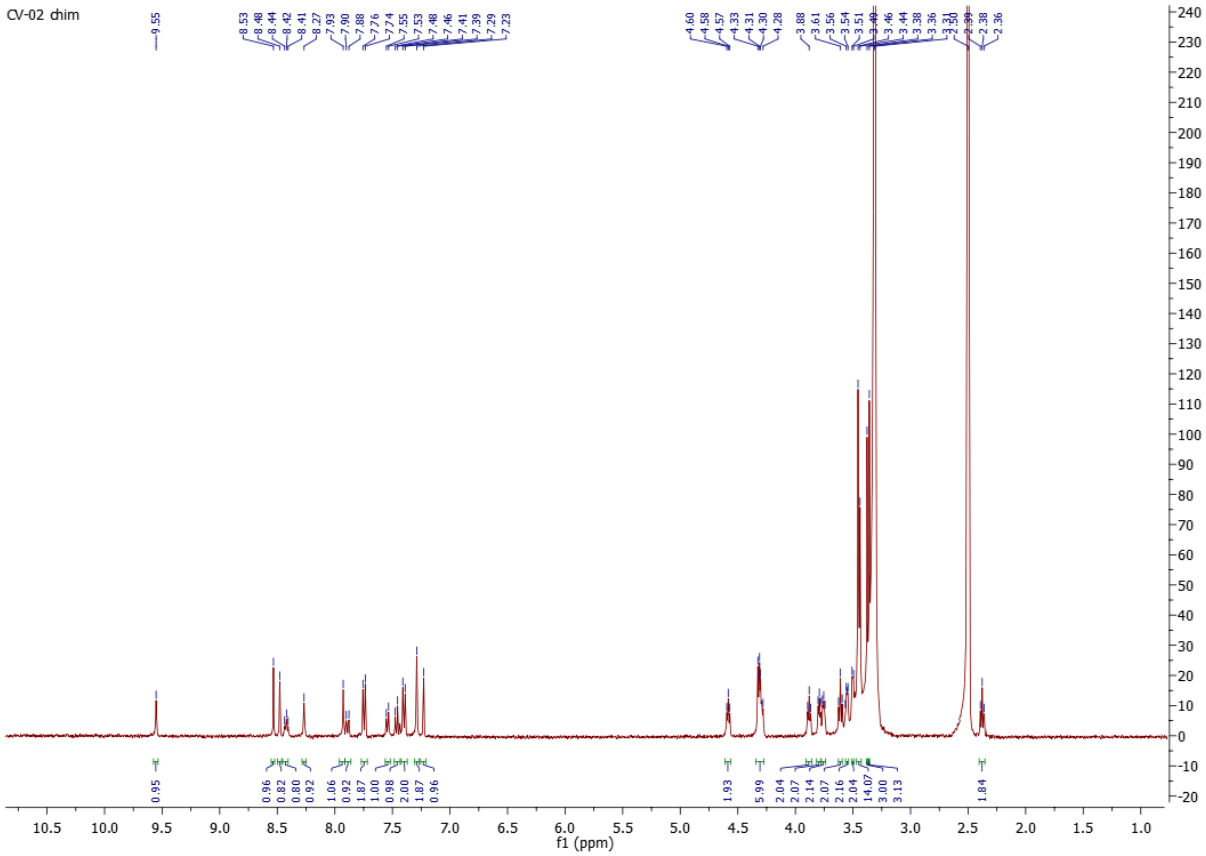
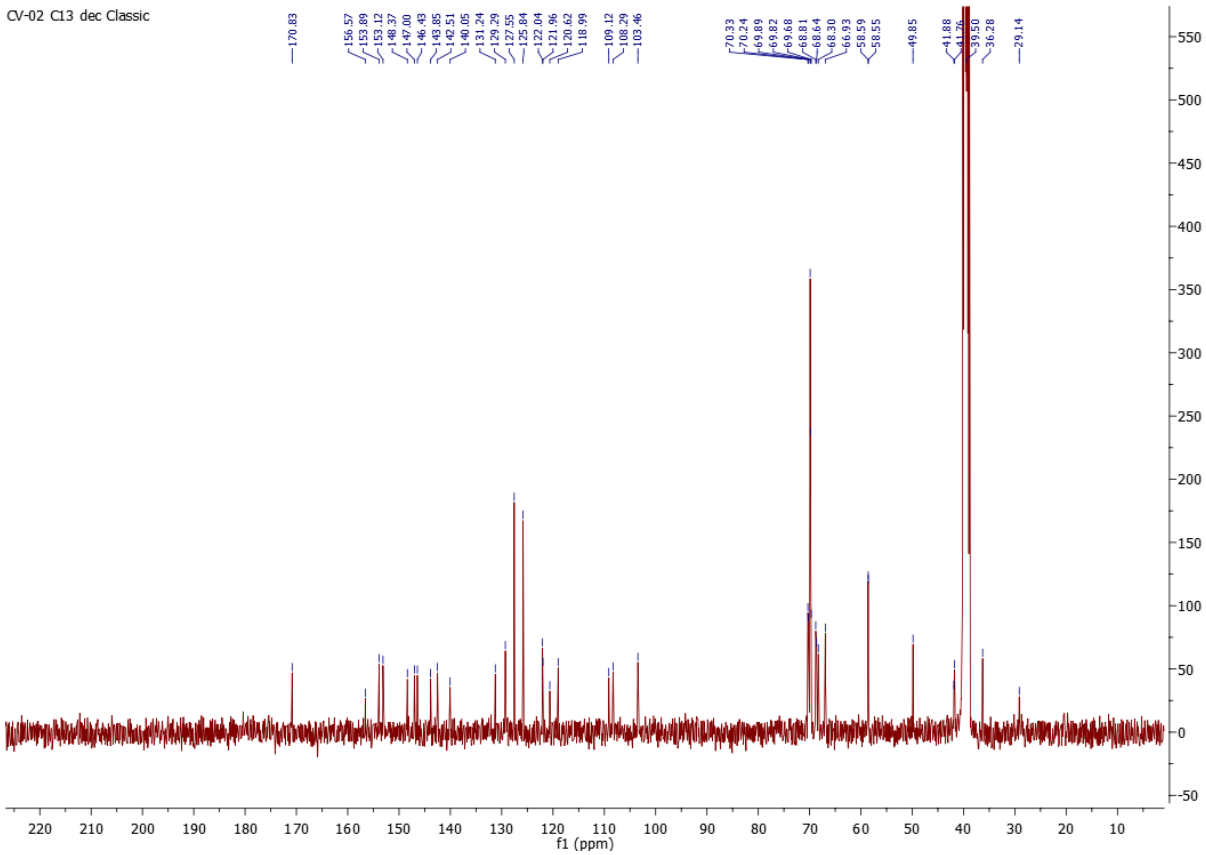


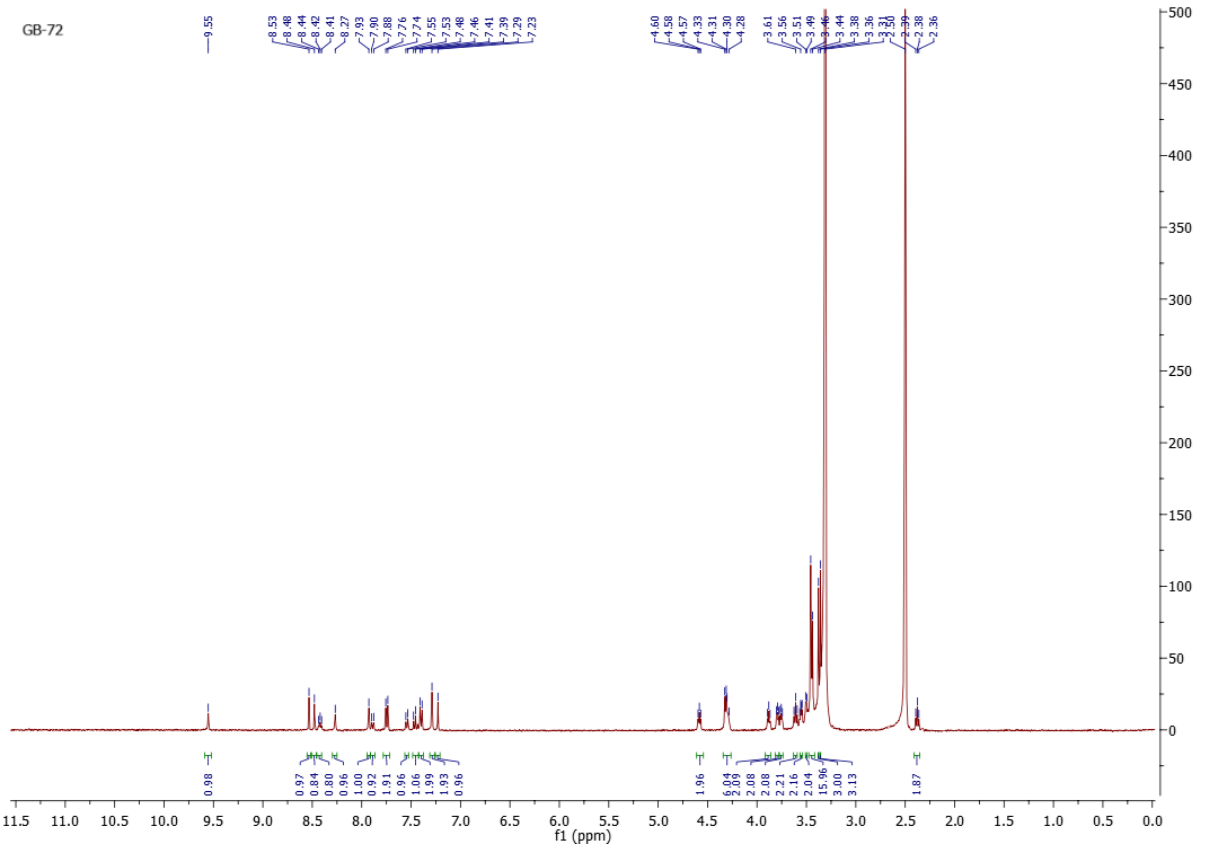
^{13}C NMR spectrum of compound **9** (100 MHz, DMSO-d_6)



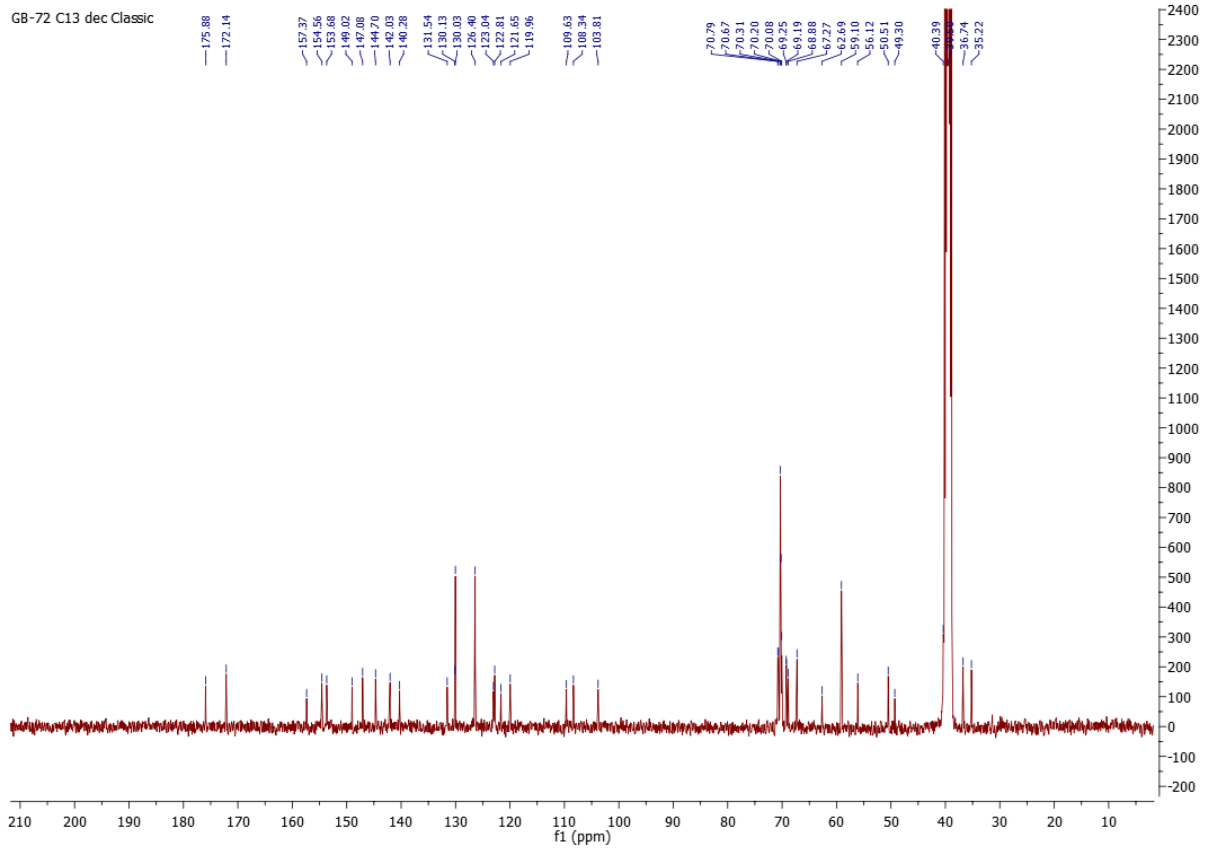
^{1}H NMR spectrum of compound **10a** (400 MHz, DMSO-d_6)



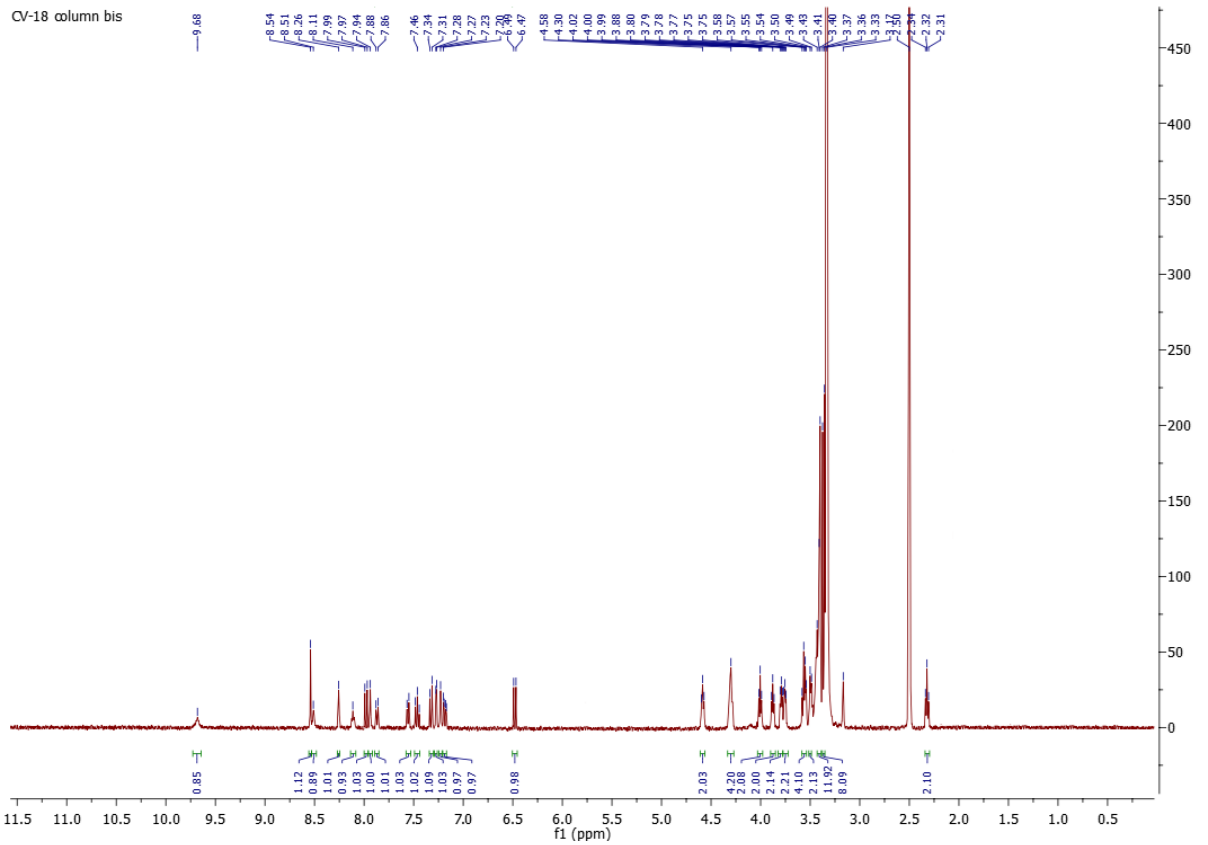
^{13}C NMR spectrum of compound **10a** (100 MHz, DMSO-d_6)



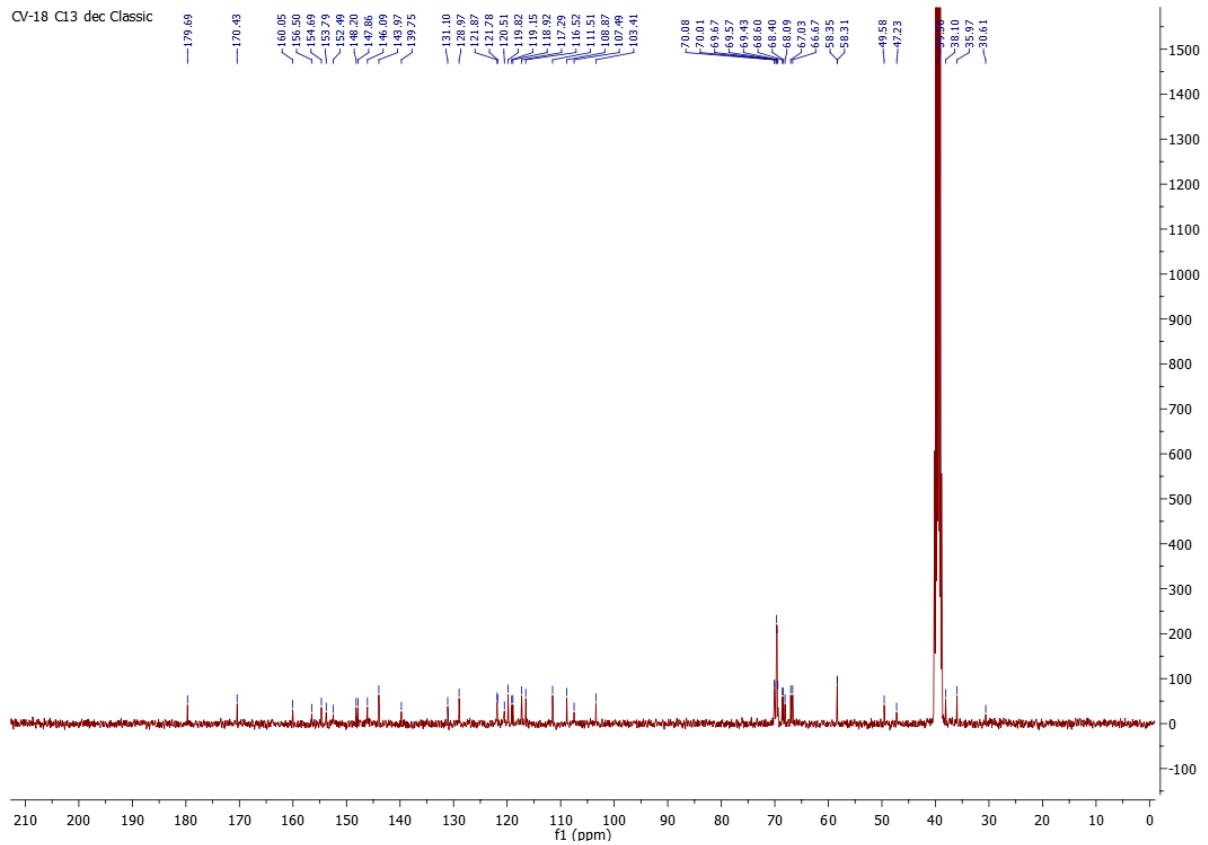
^{1}H NMR spectrum of compound **10b** (400 MHz, DMSO-d_6)



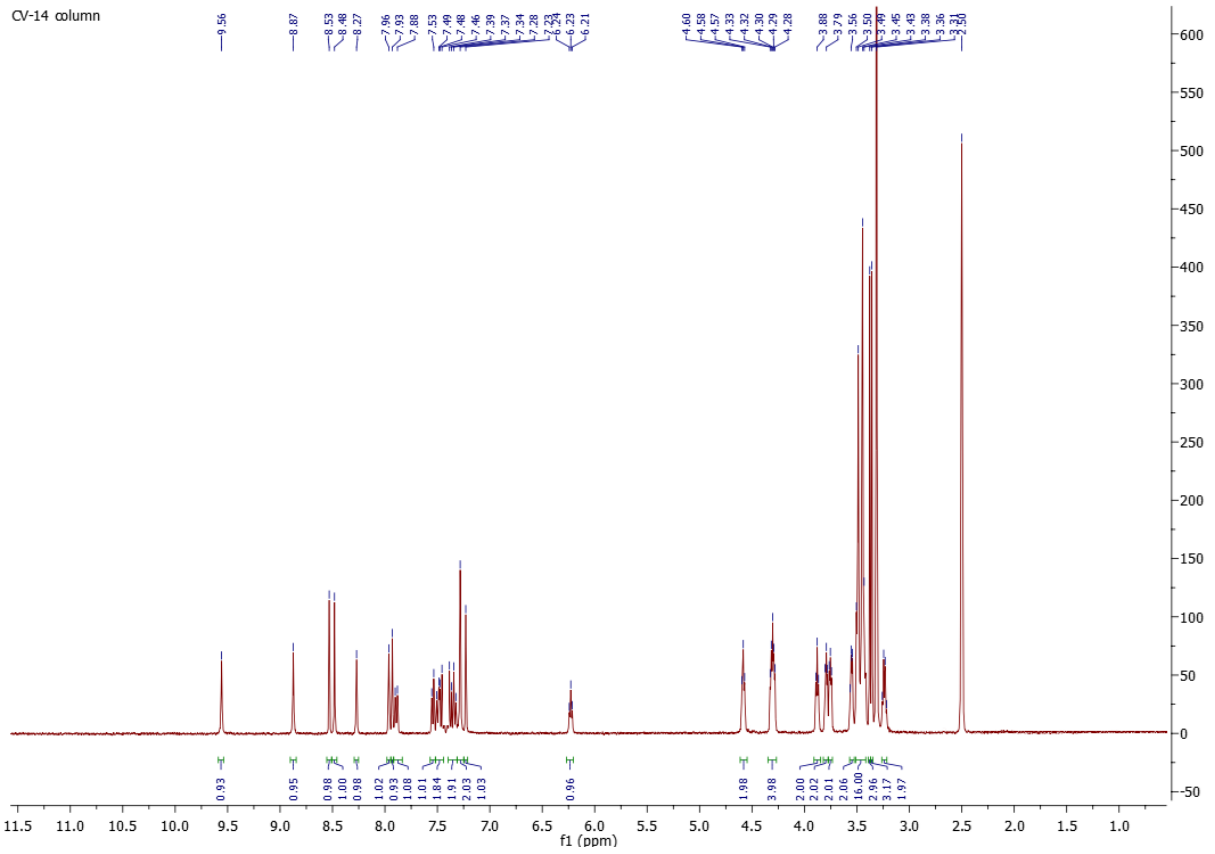
^{13}C NMR spectrum of compound **10b** (100 MHz, DMSO-d_6)



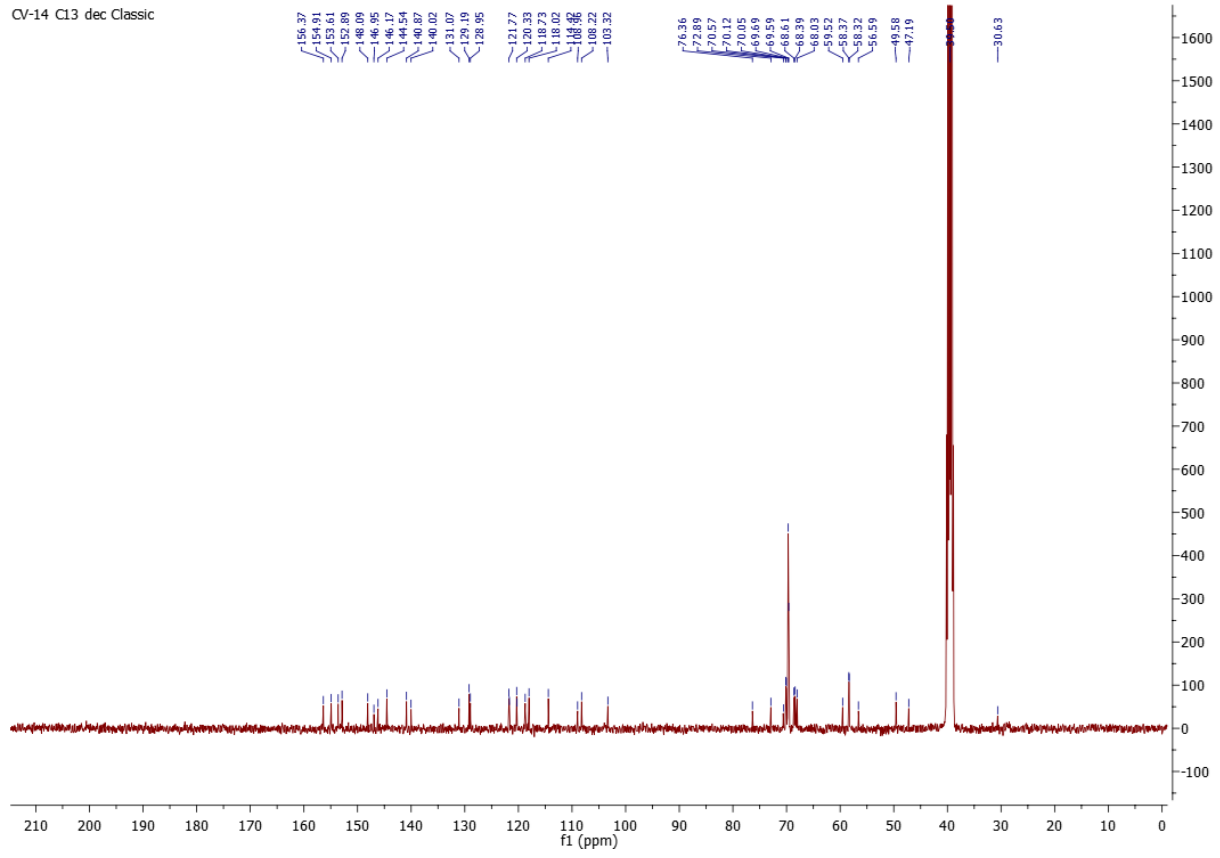
^{1}H NMR spectrum of compound **11** (400 MHz, DMSO-d_6)



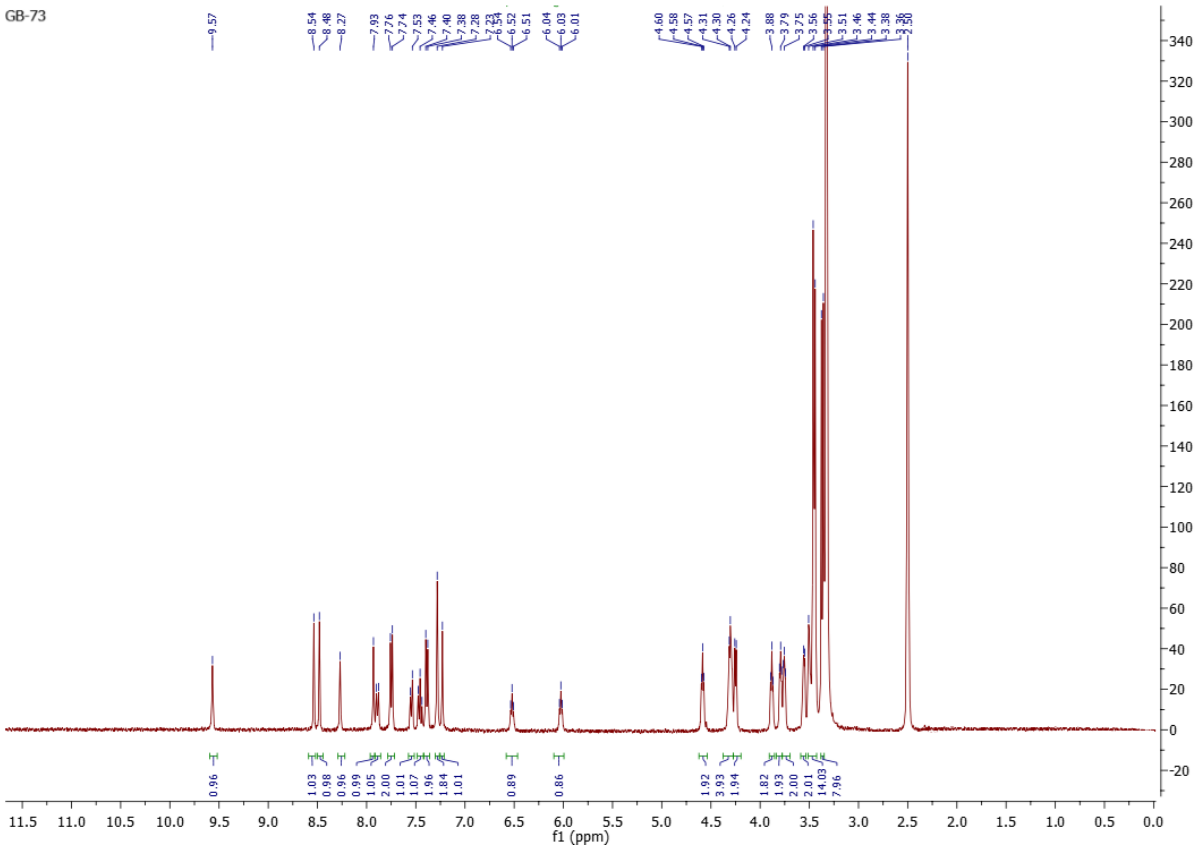
^{13}C NMR spectrum of compound **11** (100 MHz, DMSO-d_6)



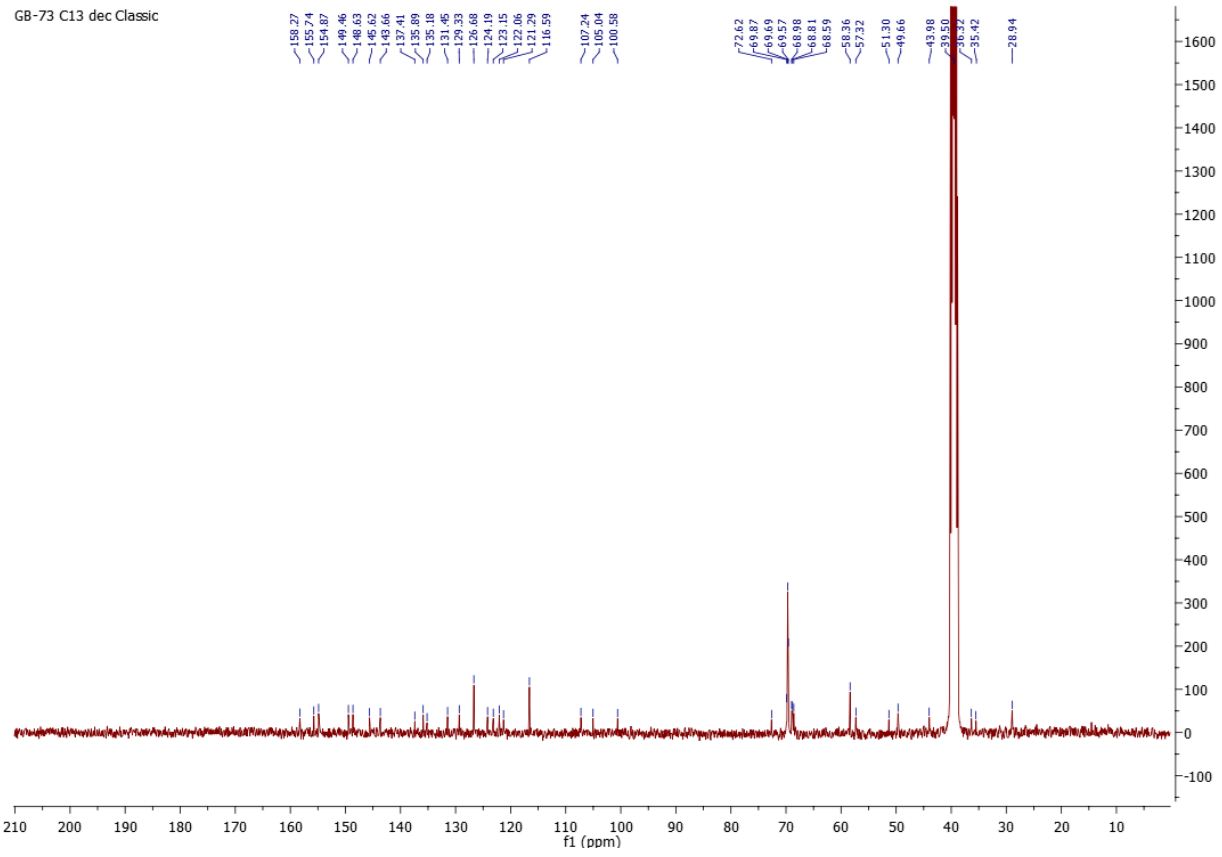
^{1}H NMR spectrum of compound **12a** (400 MHz, DMSO-d_6)



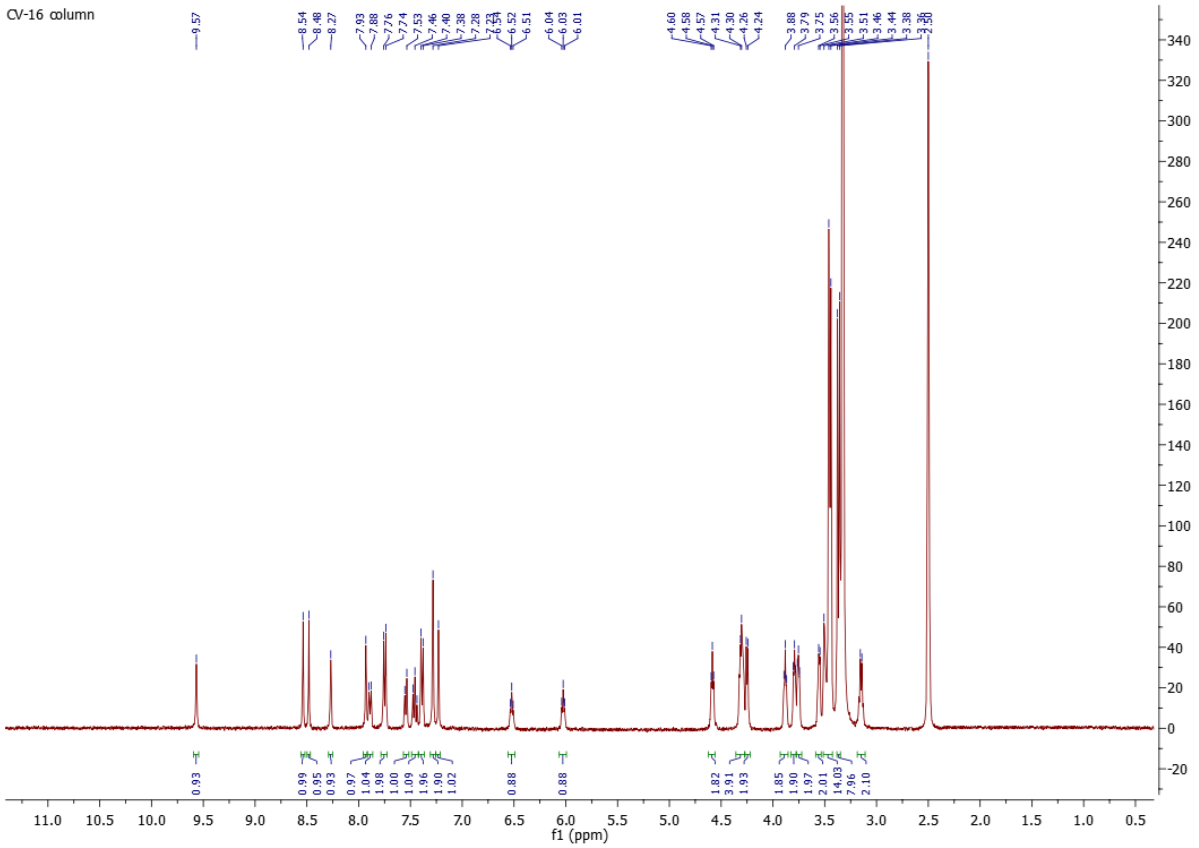
^{13}C NMR spectrum of compound **12a** (100 MHz, DMSO-d_6)



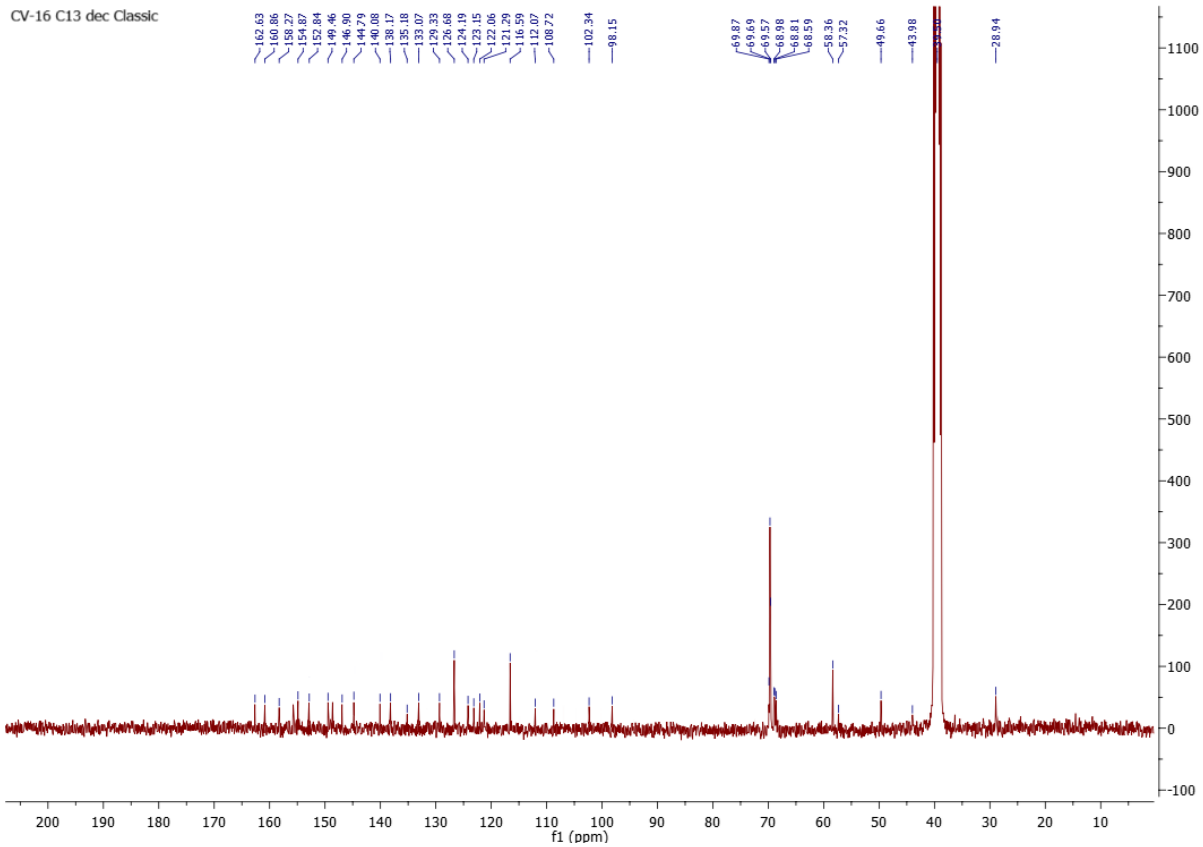
^{1}H NMR spectrum of compound **12b** (400 MHz, DMSO-d_6)



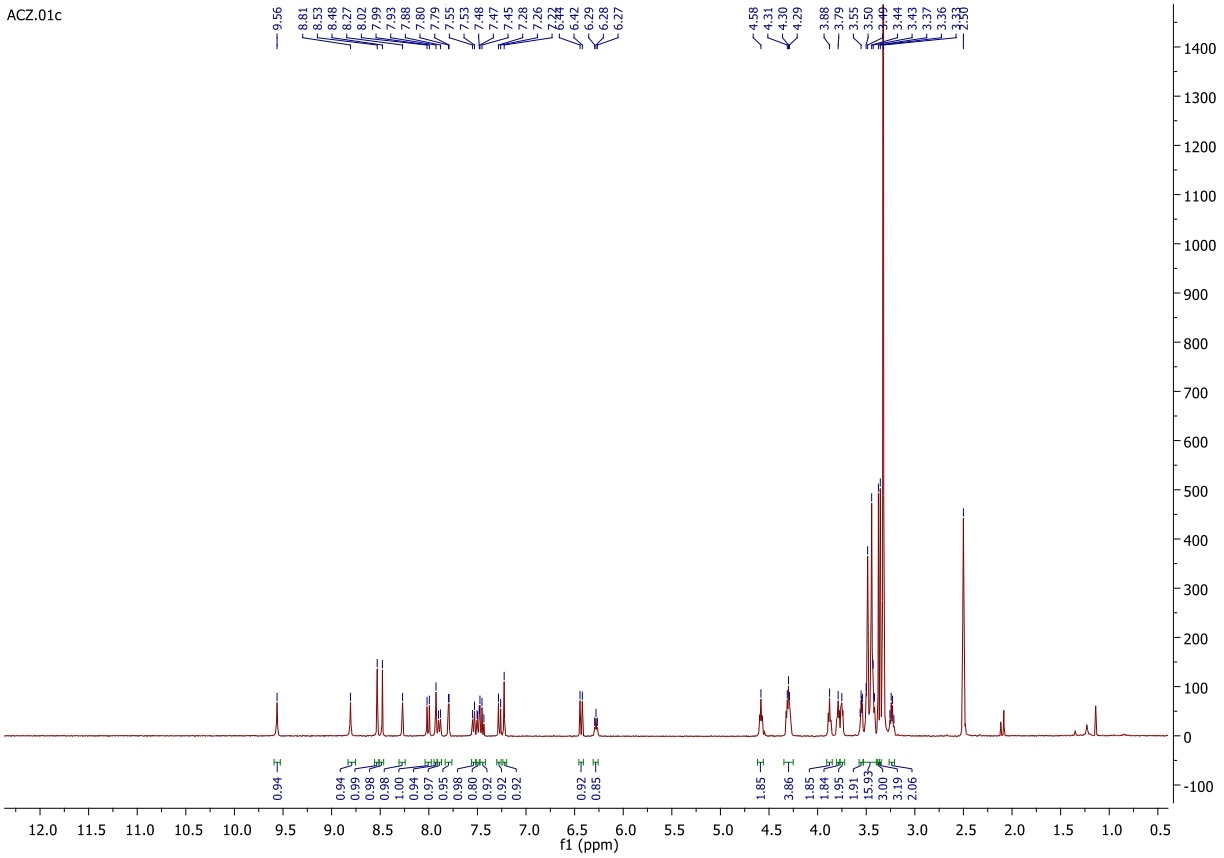
^{13}C NMR spectrum of compound **12b** (100 MHz, DMSO-d_6)



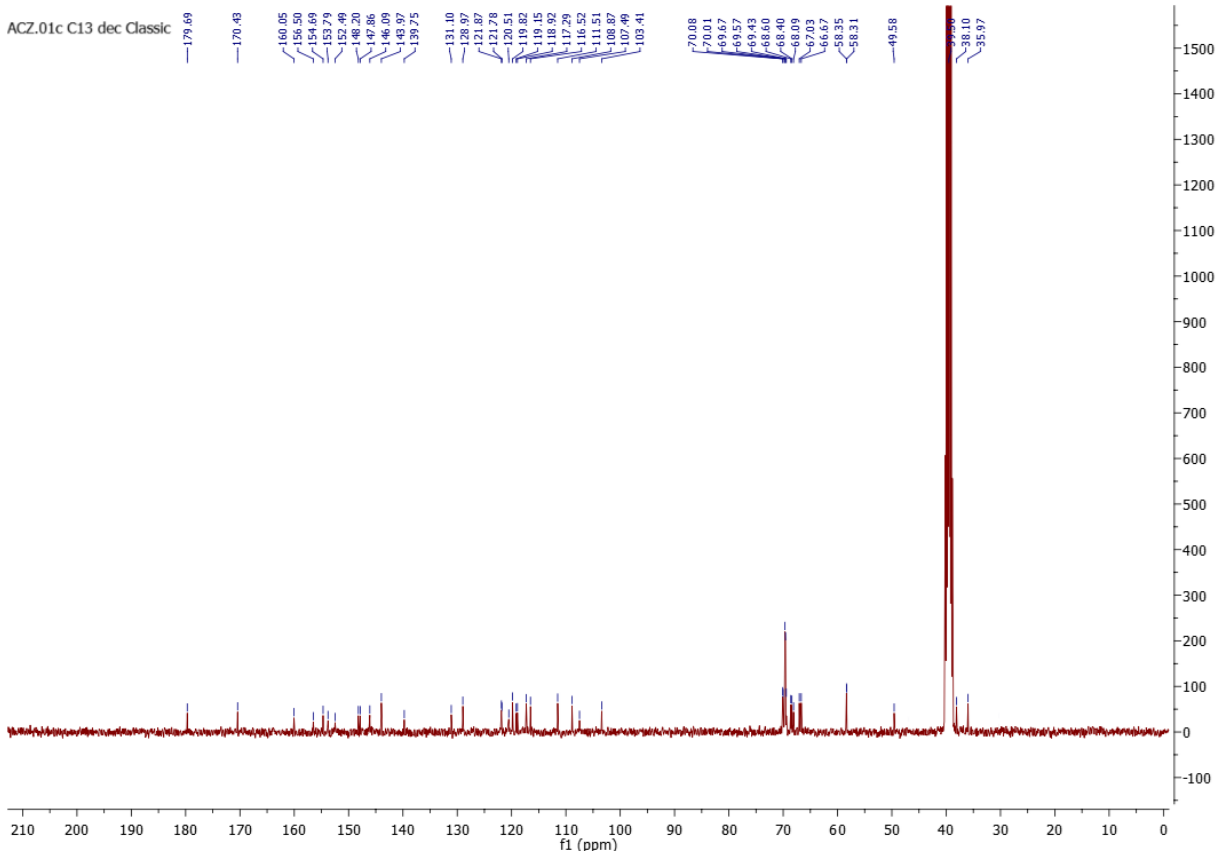
^{1}H NMR spectrum of compound **12c** (400 MHz, DMSO-d_6)



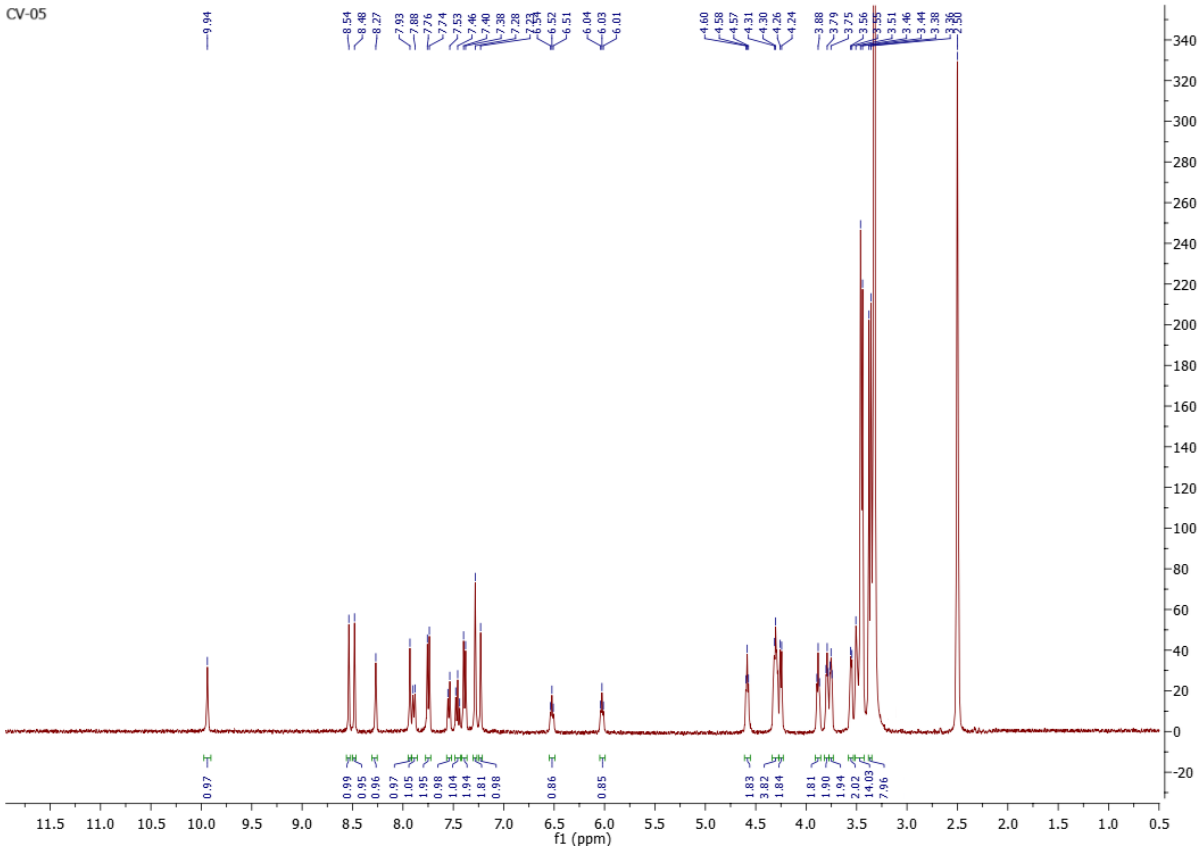
^{13}C NMR spectrum of compound **12c** (100 MHz, DMSO-d_6)



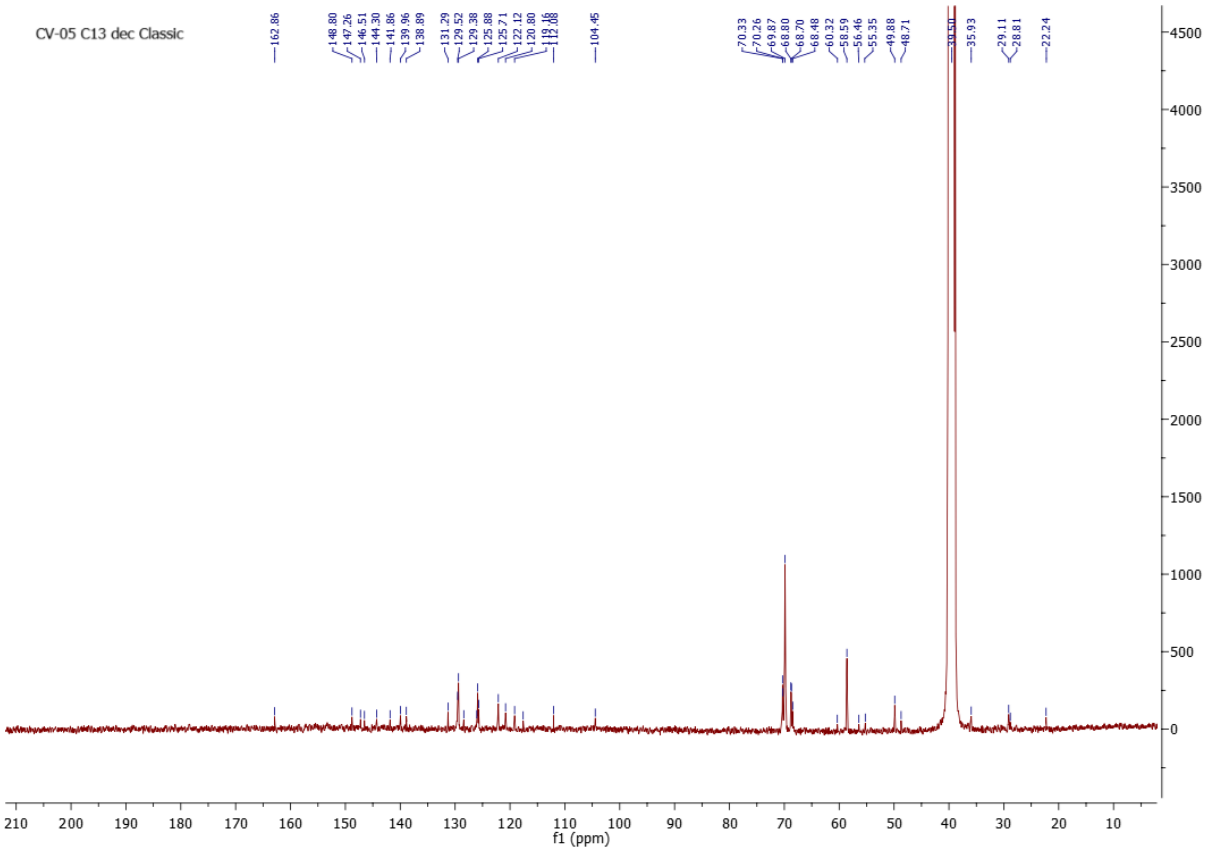
^{1}H NMR spectrum of compound **13** (400 MHz, DMSO-d_6)



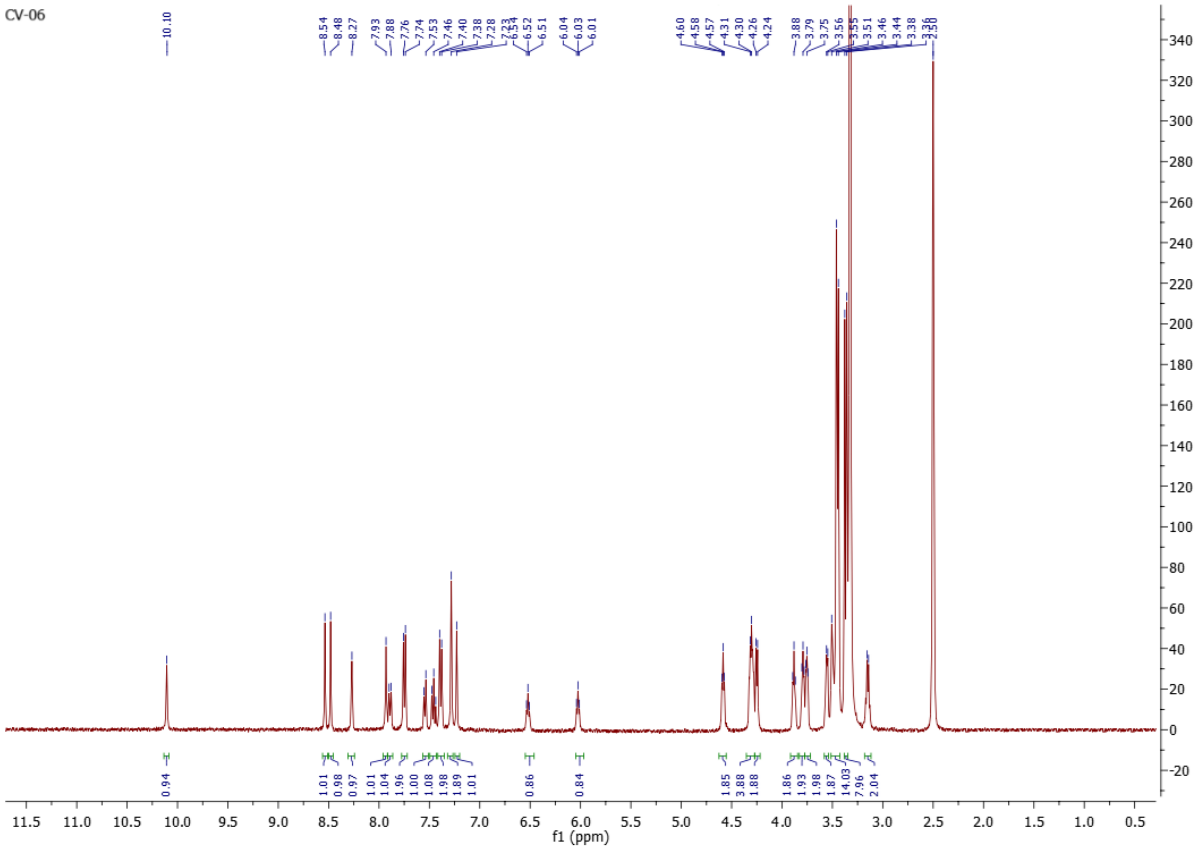
^{13}C NMR spectrum of compound **13** (100 MHz, DMSO-d_6)



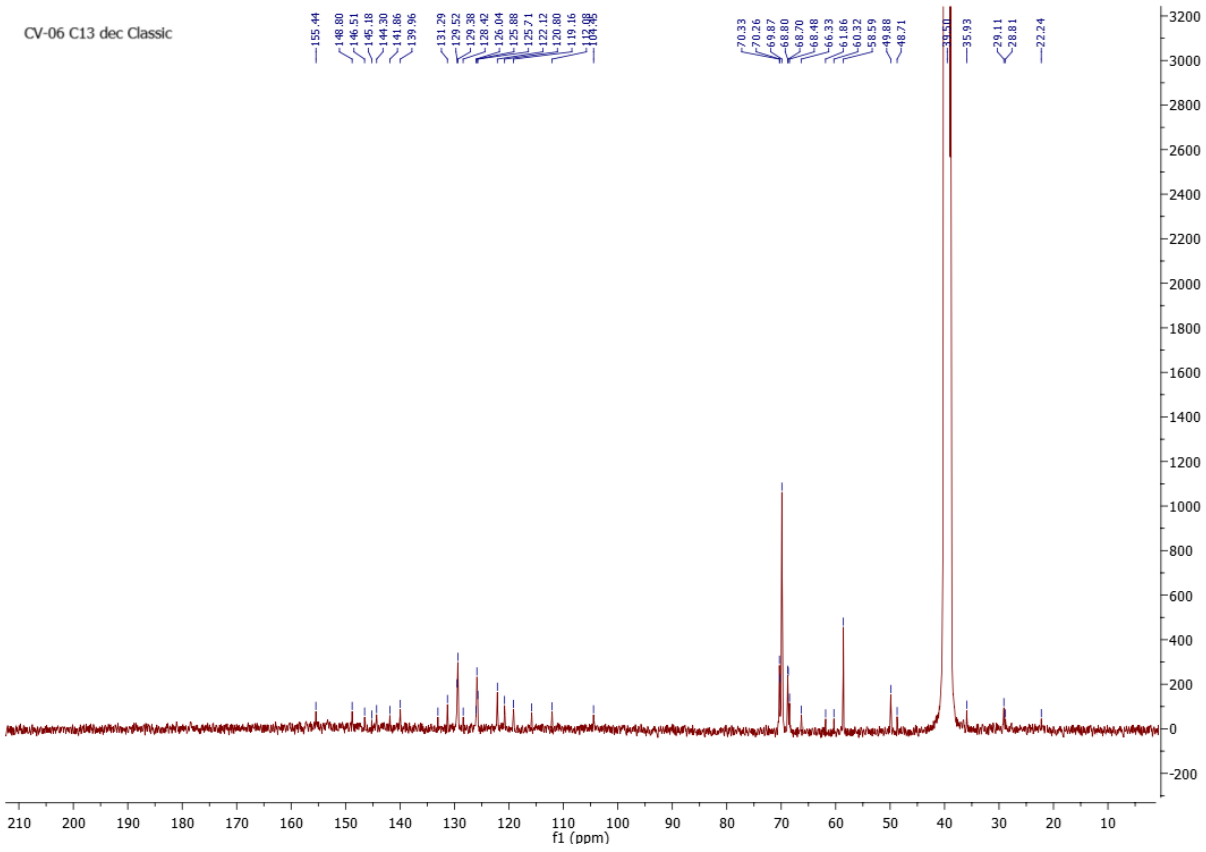
^{1}H NMR spectrum of compound **14b** (400 MHz, DMSO-d_6)



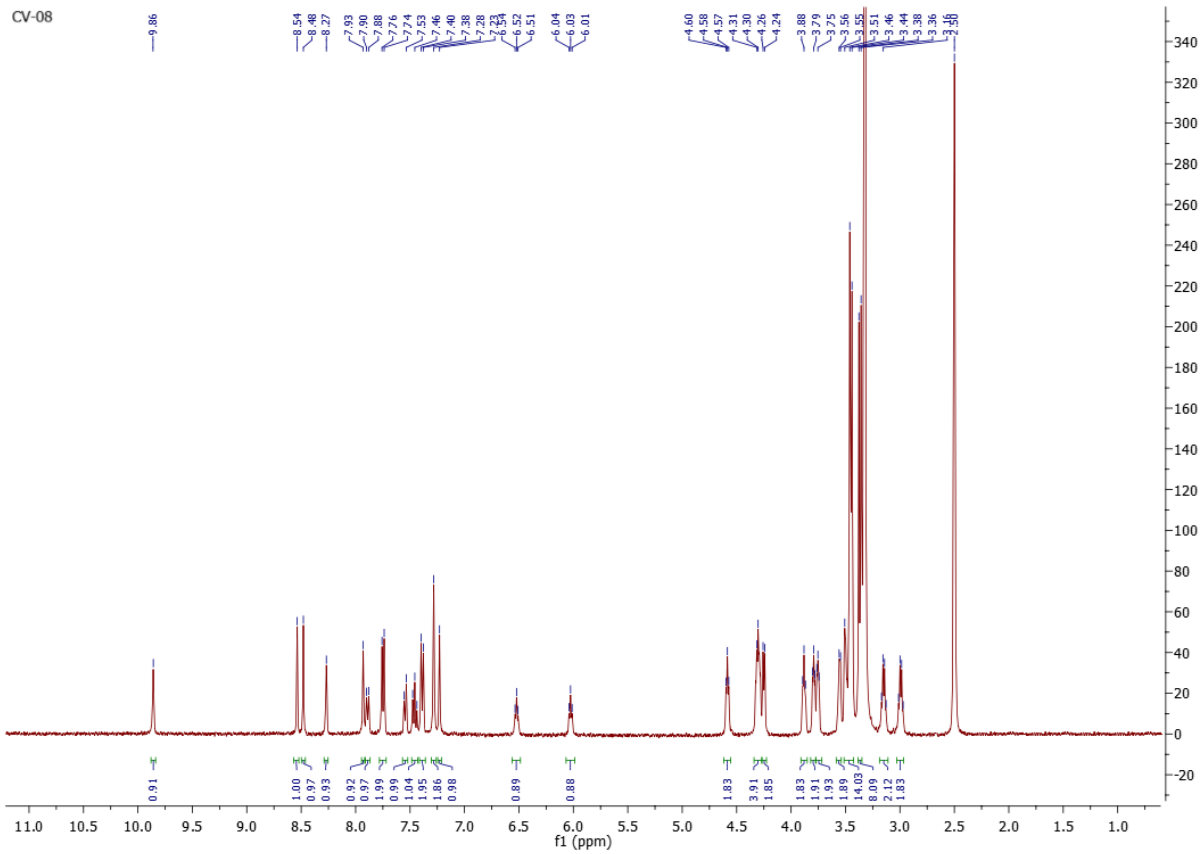
^{13}C NMR spectrum of compound **14b** (100 MHz, DMSO-d_6)



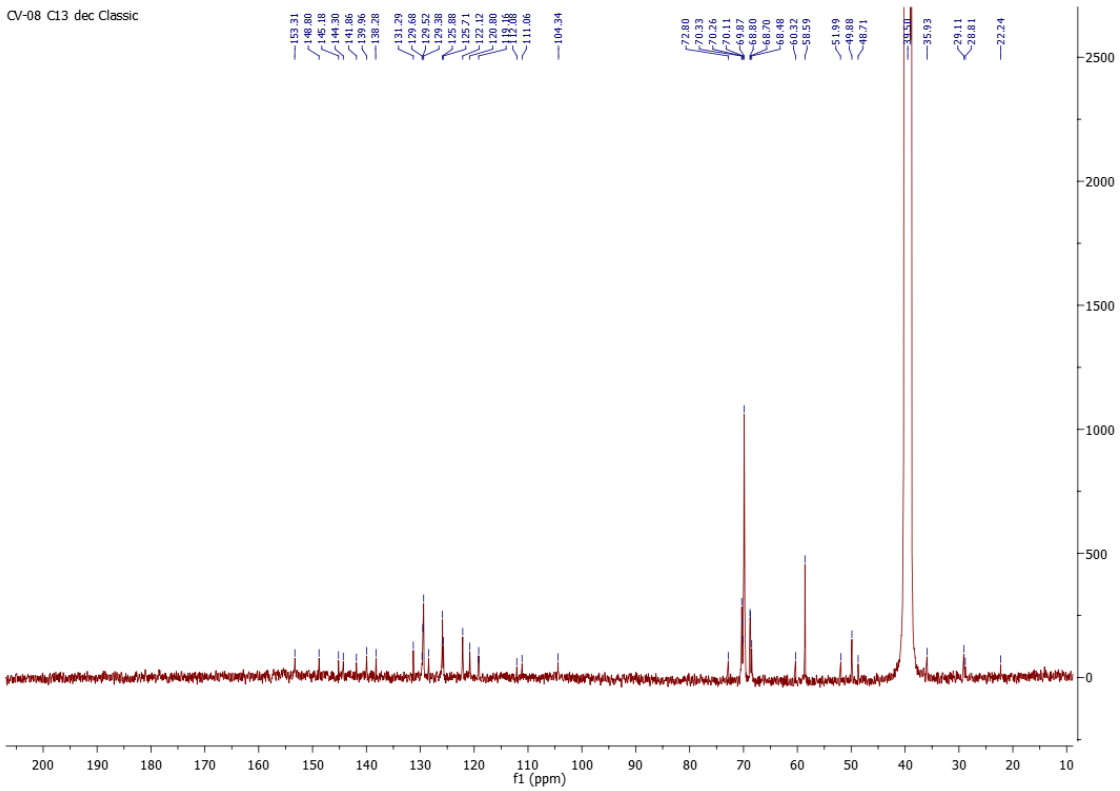
^{1}H NMR spectrum of compound **14c** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **14c** (100 MHz, DMSO-d_6)



^{1}H NMR spectrum of compound **14d** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **14d** (100 MHz, DMSO-d_6)