Exploring Pyrazoline-Thiophene Hybrids as CDK2 Inhibitors: Synthesis, Mechanistic, Biological Studies and Computational Insights

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Preparation of thiocarbohydrazide (TCH, 1)

A hydrazine hydrate (5 mmol) was added to a stirred solution of methanol (50 mL) and then carbon disulfide (2 mmol) was added dropwise with continuous stirring in an ice bath for 15 min, then, reaction flask left overnight at 100 °C, the resulting white precipitate was filtered, washed with methanol, and recrystallized from water then dried to give white needle crystalline product (**1**), yield (8.0 g, 95%), M.p. 171-173 °C as reported [29].

General procedure for synthesis of thiophene-based chalcones (2a-d)

To an ethanolic solution, a mixture of equimolar amounts of thiophene-2-carbaldehyde (113 mg) and acetophenone derivatives or 2-acetylthiophene (1 mmol of each) was slowly added a solution of aqueous sodium hydroxide (100 mg in 2 mL of water). The mixture was stirred at room temperature (r.t.) for 12 hours, and the resulting precipitate was filtered, washed with water, and recrystallized from ethanol to afford **2a** (89%, M.p. 68-70 °C as reported [30]), **2b** (83%, M.p. 126.128 °C as reported [31]), **2c** (76%, M.p. 78-80 °C as reported [20]) and **2d** (70%, M.p. 97-99 °C as reported [31]).

General procedure for synthesis of 3-aryl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (3a-d)

A thiocarbohydrazide (TCH, **1**) (5 mmol) was refluxed with different derivatives of chalcone (**2a-d**) (2 mmol) and NaOH (4 mmol) in *absolute* ethanol for 24 h. TLC was used to see the reaction progression. After the reaction was completed, the formed precipitate was filtered and washed several times with hot distilled water, followed by hot ethanol, and then recrystallized from diethyl ether to provide the key intermediates (**3a-d**).

3-Phenyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (3a)

A yellow powder, yield (245 mg, 81%), M.p. 199-201 °C. ^{1}H NMR δ (ppm): 3.28 (dd, 1H, 4-H_a of pyrazoline moiety, J = 18.0 Hz, 3.5 Hz), 3.78 (dd, 1H, 4-H_b of pyrazoline moiety, J = 18.0 Hz, 11.0 Hz), 4.75 (s, 2H, NH_2), 6.13 (dd, 1H, 5-H_X of pyrazoline moiety, J = 11.0 Hz, 3.0 Hz), 6.88 (t, 1H, 4-H_1 of thiophene moiety, J = 4.5 Hz), 6.95 (d, 1H, 3-H_1 of thiophene moiety, J = 3.5 Hz), 7.32 (d, 1H, 5-H_1 of thiophene moiety, J = 5.0 Hz), 7.41-7.43 (m, 3H, 3,4,5-H_3 of phenyl moiety), 7.90 (d, 2H, 2,6-H_2 of phenyl moiety, J = 7.5 Hz), 9.77 (s, 1H, NH). ^{13}C NMR δ (ppm): 41.67 (C4 pyrazoline), 60.28 (C5 pyrazoline), 125.24, 127.08, 129.32, 129.44, 130.43, 135.65, 145.76, 154.07, 175.52 (C=S). Anal. Calcd. for $C_{14}H_{14}N_4S_2$: C, 55.60; H, 4.67; N, 18.53; S, 21.20. Found: C, 55.89; H, 4.65; N, 18.48; S, 21.24 %.

3-(4-Chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (3b)

A yellow powder, yield (260 mg, 77%), M.p. 195-197 °C. ^{1}H NMR δ (ppm): 3.28 (dd, 1H, 4-H_a of pyrazoline moiety, *J* = 18.0 Hz, 3.0 Hz), 3.77 (dd, 1H, 4-H_b of pyrazoline moiety, *J* = 18.0 Hz, 11.0 Hz), 4.81 (s, 2H, NH_2), 6.13 (dd, 1H, 5-H_X of pyrazoline moiety, *J* = 11.0 Hz, 3.0 Hz), 6.88 (t, 1H, 4-H_1 of thiophene moiety, *J* = 4.5 Hz), 6.95 (d, 1H, 5-H_1 of thiophene moiety, *J* = 3.5 Hz), 7.32 (d, 1H, 3-H_1 of thiophene moiety, *J* = 5.0 Hz), 7.50 (d, 2H, 2,6-H_2 of chlorophenyl moiety, *J* = 8.5 Hz), 7.92 (d, 2H, 3,5-H_2 of chlorophenyl moiety, *J* = 8.5 Hz), 9.85 (s, 1H, NH). ^{13}C NMR δ (ppm): 41.67 (C4 pyrazoline), 60.28 (C5 pyrazoline), 125.24, 127.08, 129.31, 129.44, 130.42, 135.65, 145.75, 154.06, 175.52 (C=S). Anal. Calcd. for $C_{14}H_{13}ClN_4S_2$: C, 49.92; H, 3.89; N, 16.63; S, 19.03. Found: C, 50.14; H, 3.90; N, 16.67; S, 18.99 %.

5-(Thiophen-2-yl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (3c)

A yellow powder, yield (255 mg, 80%), M.p. 200-202 °C. ^{1}H NMR δ (ppm): 2.31 (s, 3H, CH_3), 3.26 (dd, 1H, 4-H_a of pyrazoline moiety, *J* = 18.0 Hz, 3.0 Hz), 3.75 (dd, 1H, 4-H_b of pyrazoline moiety, *J* = 18.0 Hz, 11.0 Hz), 4.74 (s, 2H, NH_2), 6.11 (dd, 1H, 5-H_X of pyrazoline moiety, *J* = 11.0 Hz, 3.0 Hz), 6.88 (t, 1H, 4-H_1 of thiophene moiety, *J* = 4.5 Hz), 6.94 (d, 1H, 5-H_1 of thiophene moiety, *J* = 3.5 Hz), 7.23 (d, 2H, 3,5-H_2 of methylphenyl moiety, *J* = 8.0 Hz), 7.31 (d, 1H, 3-H_1 of thiophene moiety, *J* = 5.0 Hz), 7.79 (d, 2H, 2,6-H_2 of methylphenyl moiety, *J* = 8.0 Hz), 9.71 (s, 1H, NH). ^{13}C NMR δ (ppm): 21.63 (CH_3), 41.82 (C4 pyrazoline), 60.01 (C5 pyrazoline), 125.17, 127.06, 127.70, 128.66, 129.36, 129.83, 130.89, 134.46, 141.02, 145.91, 155.26, 175.52 (C=S). Anal. Calcd. for $C_{15}H_{16}N_4S_2$: C, 56.93; H, 5.10; N, 17.71; S, 20.26. Found: C, 57.17; H, 5.11; N, 17.76; S, 20.19 %.

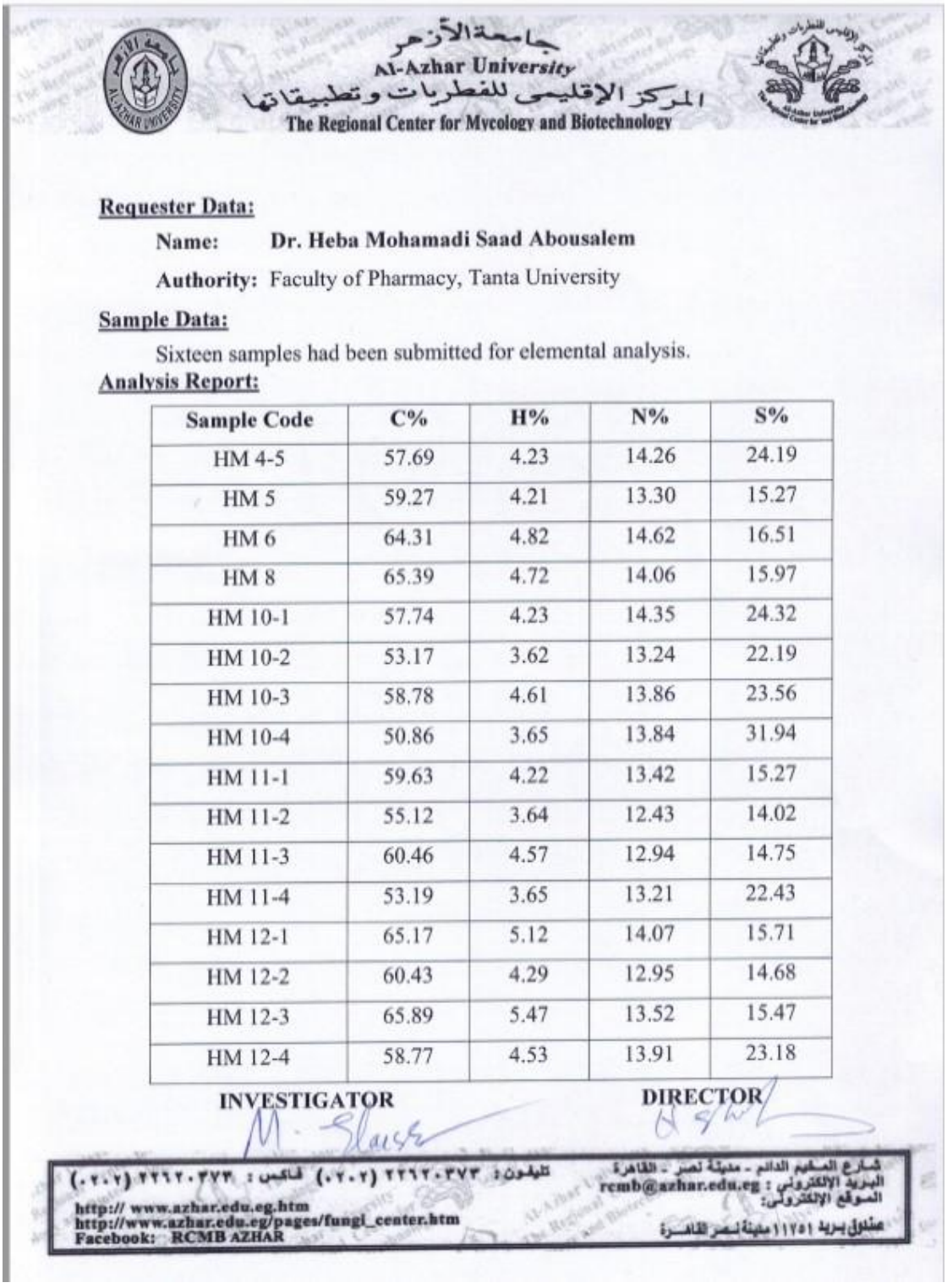
3,5-Di(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothiohydrazide (3d)

A yellow powder, yield (287 mg, 93%), M.p. 195-197 °C. ^{1}H NMR δ (ppm): 3.28 (dd, 1H, 4-H_a of pyrazoline moiety, *J* = 18.0 Hz, 3.0 Hz), 3.81 (dd, 1H, 4-H_b of pyrazoline moiety, *J* = 17.5 Hz, 11.0 Hz), 4.76 (s, 2H, NH_2), 6.14 (dd, 1H, 5-H_X of pyrazoline moiety, *J* = 11.0 Hz, 3.0 Hz), 6.89 (t, 1H, 4-H_1 of 3-thiophen-2-yl moiety, *J* = 4.5 Hz), 6.95 (d, 1H, 5-H_1 of 3-thiophen-2-yl moiety, *J* = 3.5 Hz), 7.13 (t, 1H, 4-H_1 of 5-thiophen-2-yl moiety, *J* = 4.5 Hz), 7.33 (d, 1H, 3-H_1 of 3-thiophen-2-yl moiety, *J* = 5.0), 7.51 (d, 1H, 5-H_1 of 5-thiophen-2-yl moiety *J* = 3.5 Hz), 7.74 (d, 1H, 3-H_1 of 5-thiophen-2-yl moiety, *J* = 5.0 Hz), 9.47 (s, 1H, NH). ^{13}C NMR δ (ppm): 42.57 (C4 pyrazoline), 60.14 (C5 pyrazoline), 125.23, 127.11, 128.66, 130.99, 131.51, 131.58, 134.40, 145.57, 151.27, 175.02 (C=S). Anal. Calcd. for $C_{12}H_{12}N_4S_3$: C, 46.73; H, 3.92; N, 18.17; S, 31.18. Found: C, 46.49; H, 3.90; N, 18.21; S, 31.13 %.

Yields and elemental analysis

Table S1. Yields and elemental analysis of targeted compounds (**3a-d**) and (**4a-p**)

Code	Yield (%)	Elemental analysis							
		Calculated				Found			
		C%	H%	N%	S%	C%	H%	N%	S%
3a	81	55.60	4.67	18.53	21.20	55.89	4.65	18.48	21.54
3b	77	49.92	3.89	16.63	19.03	50.14	3.90	16.67	18.99
3c	80	56.93	5.10	17.71	20.26	57.17	5.11	17.76	20.19
3d	93	46.73	3.92	18.17	31.18	46.49	3.90	18.21	31.13
4a	61	64.59	4.65	14.35	16.42	64.31	4.82	14.62	16.51
4b	64	59.35	4.03	13.18	15.09	59.27	4.21	13.30	15.27
4c	64	65.32	4.98	13.85	15.85	65.39	4.72	14.06	15.97
4d	59	57.55	4.07	14.13	24.25	57.69	4.23	14.26	24.19
4e	53	59.35	4.03	13.18	15.09	59.63	4.22	13.42	15.27
4f	53	54.90	3.51	12.20	13.96	55.12	3.64	12.43	14.02
4g	50	60.19	4.36	12.76	14.61	60.46	4.57	12.94	14.57
4h	56	52.95	3.51	13.00	22.32	53.19	3.65	13.21	22.43
4i	53	65.32	4.98	13.85	15.85	65.17	5.12	14.07	15.71
4j	56	60.19	4.36	12.76	14.61	60.43	4.29	12.95	14.68
4k	50	66.00	5.30	13.39	15.32	65.89	5.47	13.52	15.47
4l	61	58.51	4.42	13.65	23.43	58.77	4.53	13.91	23.18
4m	54	57.55	4.07	14.13	24.25	57.74	4.23	14.35	24.32
4n	53	52.95	3.51	13.00	22.32	53.17	3.62	13.24	22.19
4o	52	58.51	4.42	13.65	23.43	58.78	4.61	13.86	23.56
4p	54	50.72	3.51	13.92	31.86	50.86	3.65	13.84	31.94



جامعة الأزهر
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The Regional Center for Mycology and Biotechnology

Requester Data:

Name: **Dr. Heba Mohamadi Saad Abousalem**

Authority: Faculty of Pharmacy, Tanta University

Sample Data:

Sixteen samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%	S%
HM 4-5	57.69	4.23	14.26	24.19
HM 5	59.27	4.21	13.30	15.27
HM 6	64.31	4.82	14.62	16.51
HM 8	65.39	4.72	14.06	15.97
HM 10-1	57.74	4.23	14.35	24.32
HM 10-2	53.17	3.62	13.24	22.19
HM 10-3	58.78	4.61	13.86	23.56
HM 10-4	50.86	3.65	13.84	31.94
HM 11-1	59.63	4.22	13.42	15.27
HM 11-2	55.12	3.64	12.43	14.02
HM 11-3	60.46	4.57	12.94	14.75
HM 11-4	53.19	3.65	13.21	22.43
HM 12-1	65.17	5.12	14.07	15.71
HM 12-2	60.43	4.29	12.95	14.68
HM 12-3	65.89	5.47	13.52	15.47
HM 12-4	58.77	4.53	13.91	23.18

INVESTIGATOR **DIRECTOR**

تليفون: ٢٢٦٢٠٣٧٣ (٠٢٠٢) فاكس: ٢٢٦٢٠٣٧٣ (٠٢٠٢)
شارع المعلم الدائم - مدينة نصر - القاهرة
البريد الإلكتروني: rcmb@azhar.edu.eg
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http://www.azhar.edu.eg/pages/fungi_center.htm
Facebook: RCMB AZHAR
صندوق بريد ١١٧٥١ مدينة نصر القاهرة

Figure S1. Elemental analysis of targeted compounds (**4a-p**)

^{1}H NMR spectral data of the intermediate compounds (3a-d)

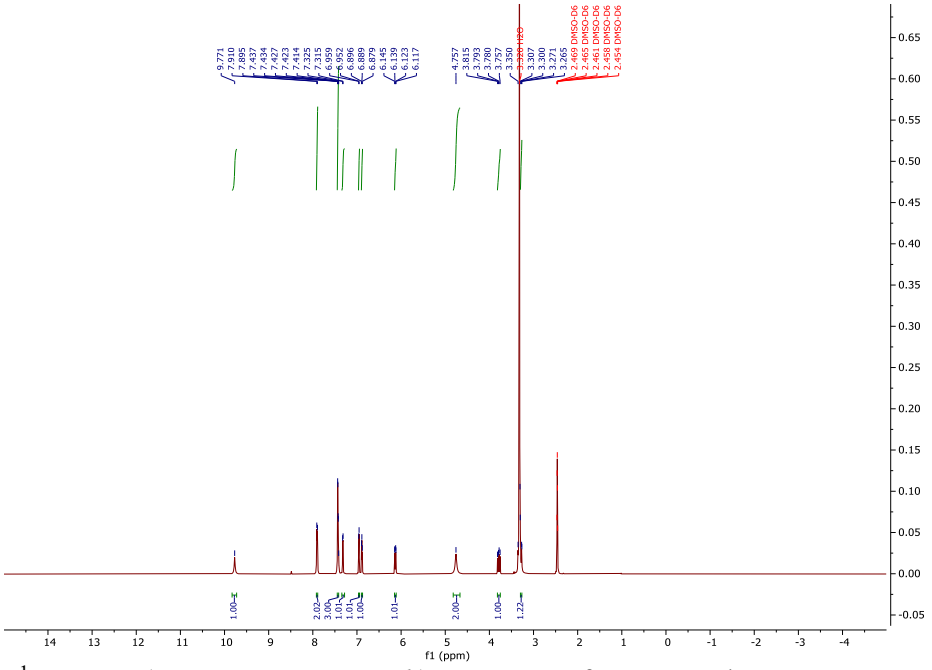


Figure S2. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3a**

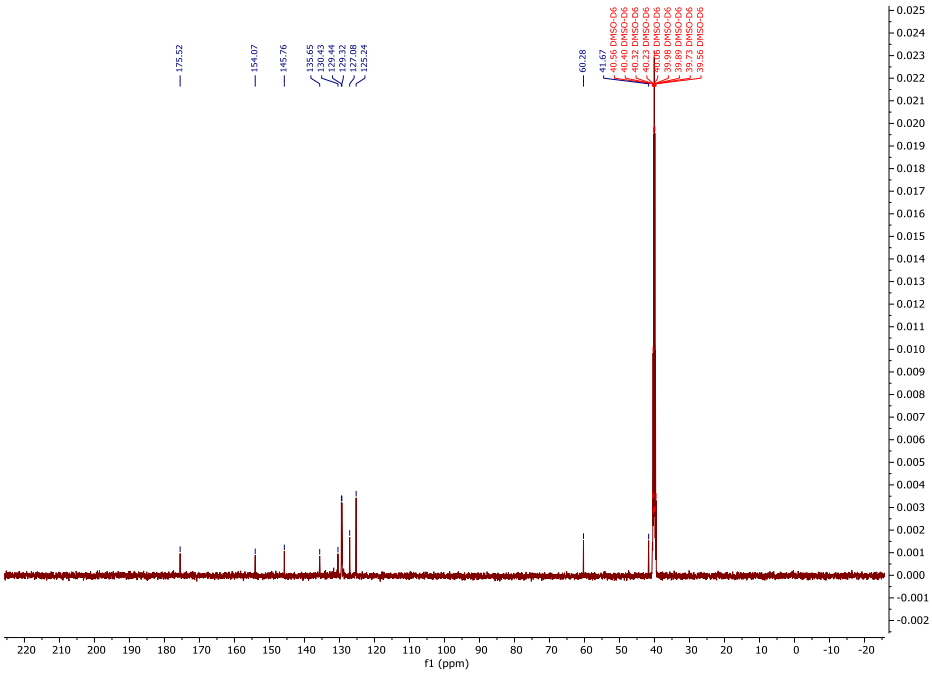


Figure S3. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3a**

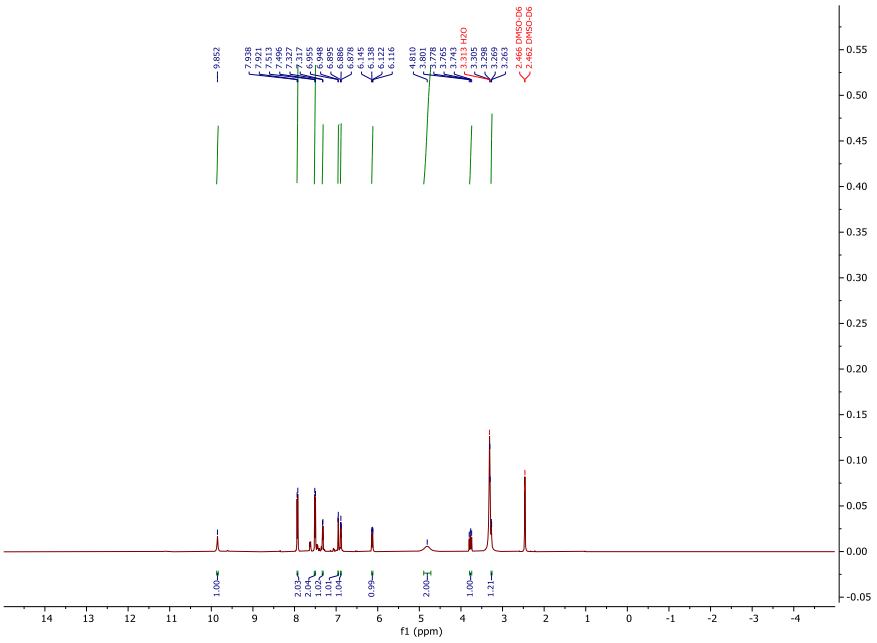


Figure S4. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3b**

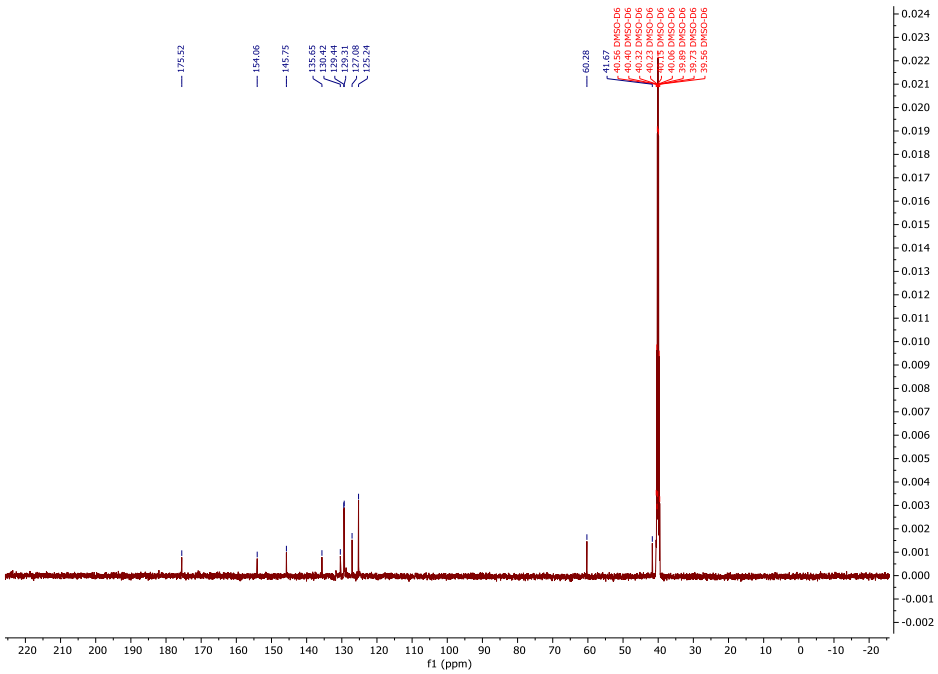


Figure S5. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3b**

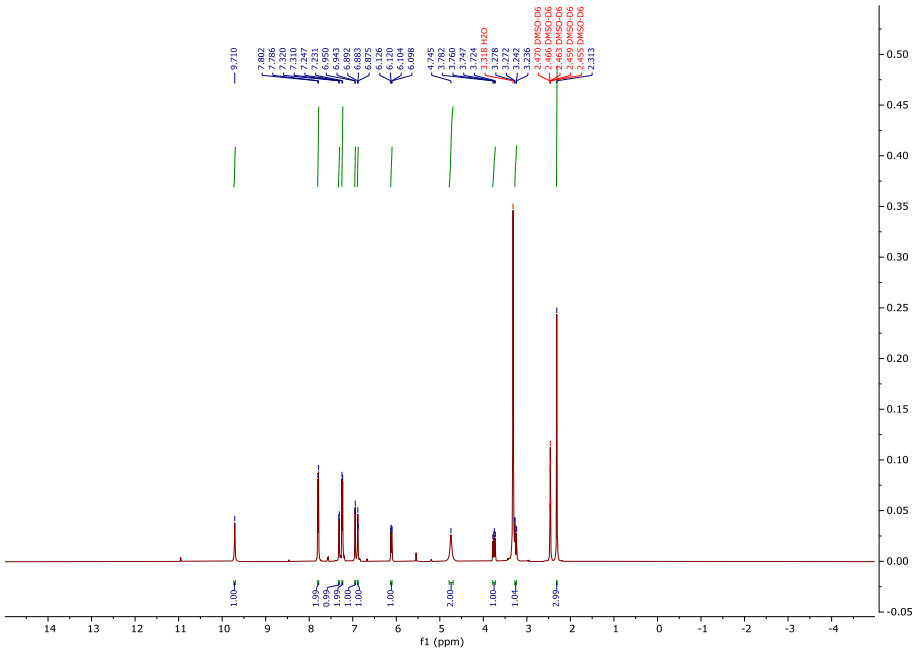


Figure S6. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3c**

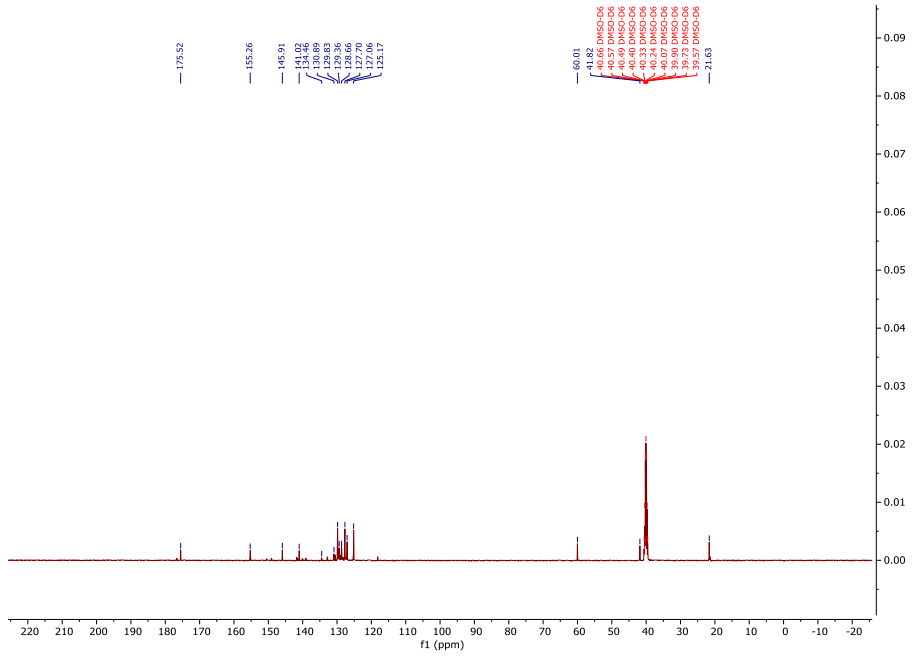


Figure S7. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3c**

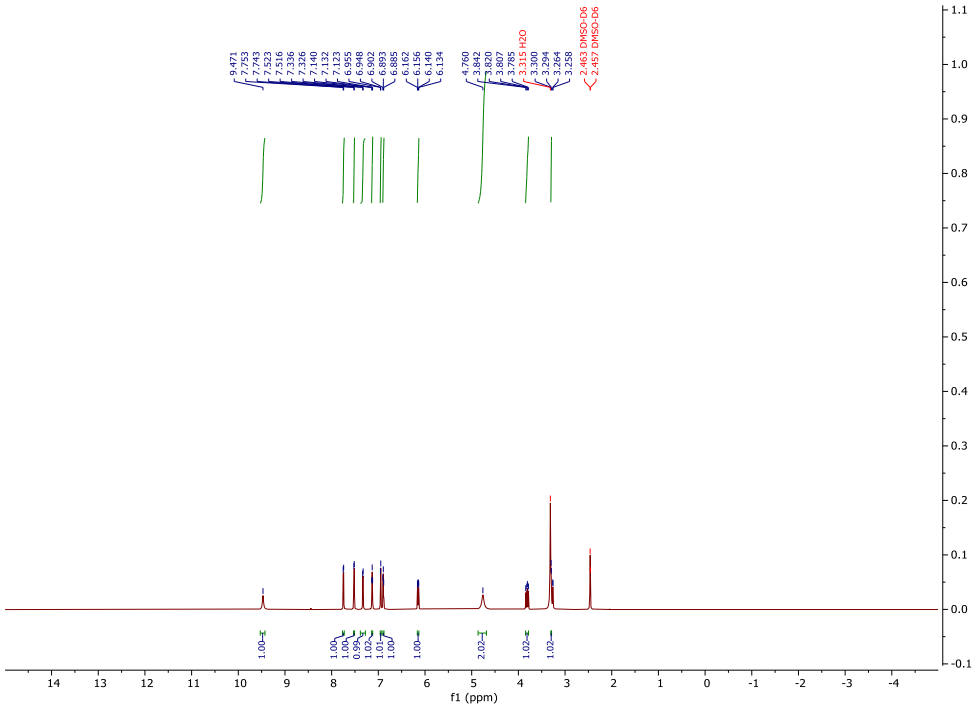


Figure S8. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3d**

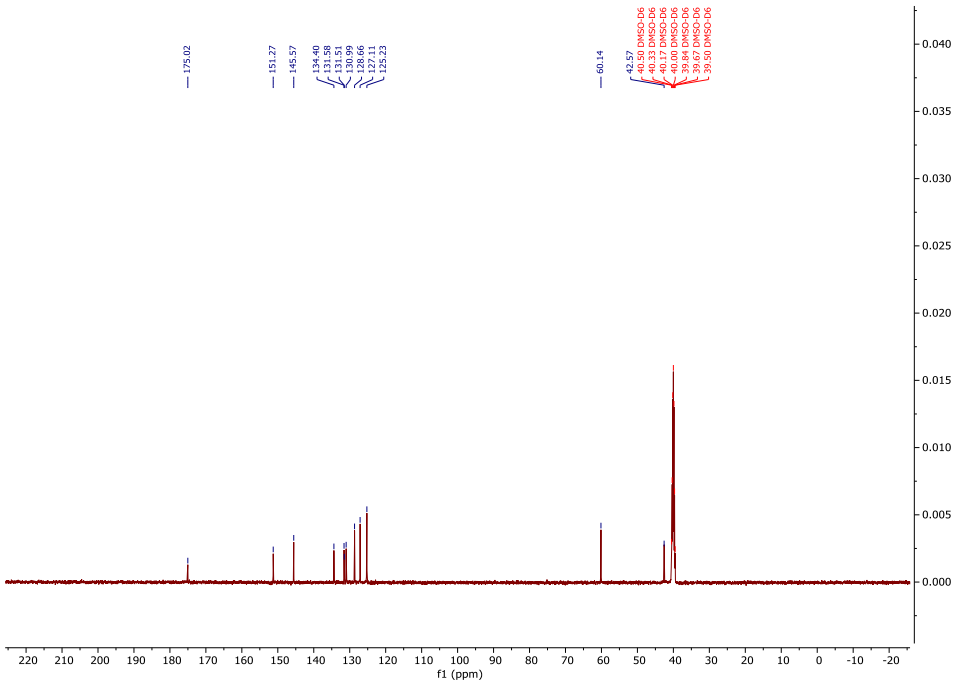


Figure S9. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3d**

^{1}H NMR and ^{13}C NMR spectral data of the target compounds (4a-p)

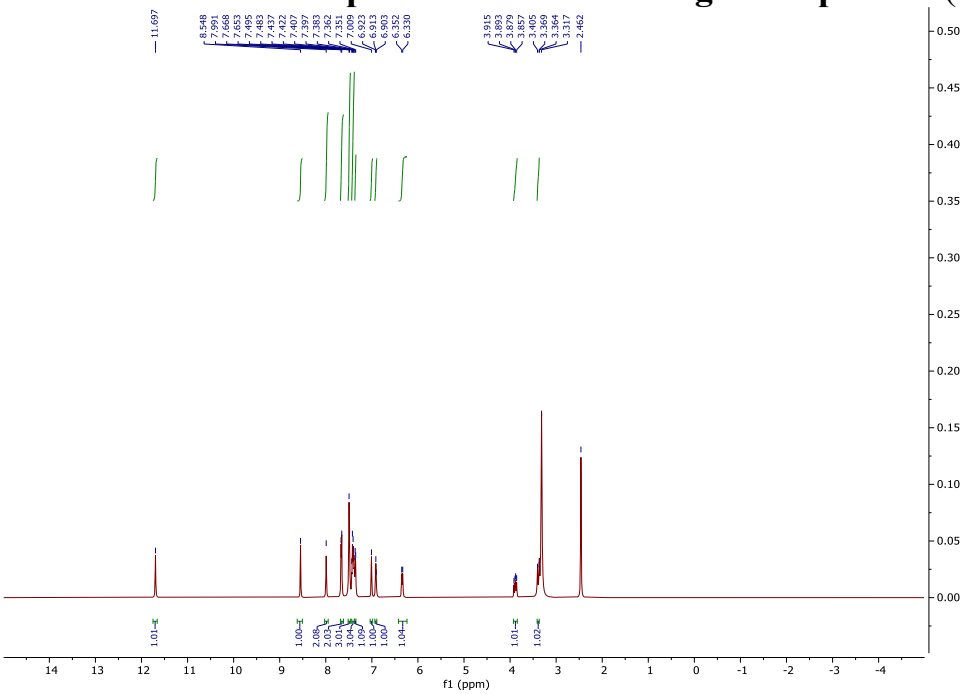


Figure S10. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4a**

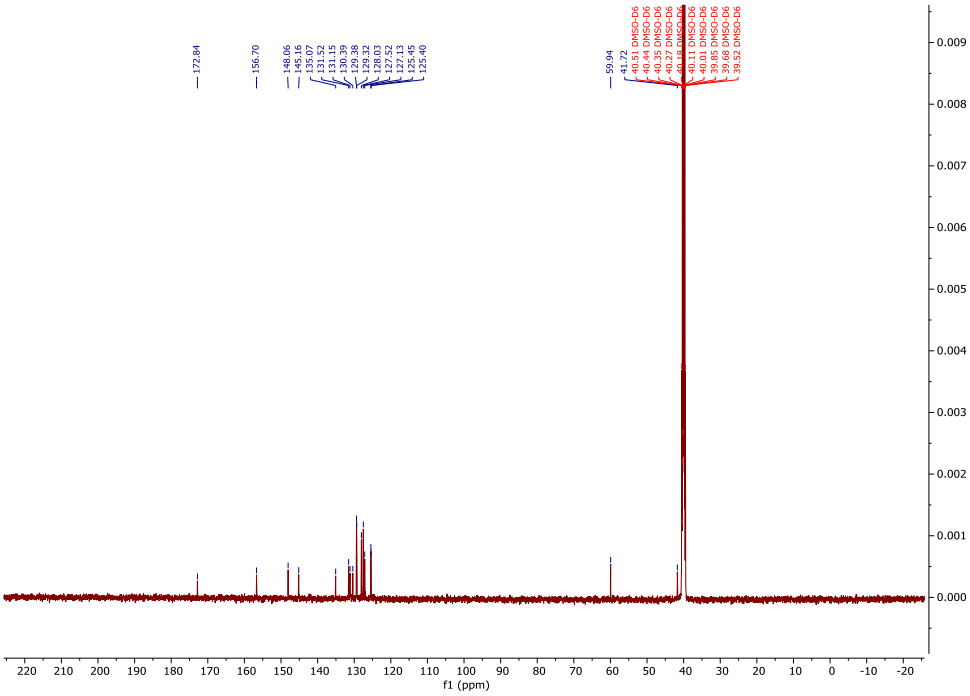


Figure S11. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4a**

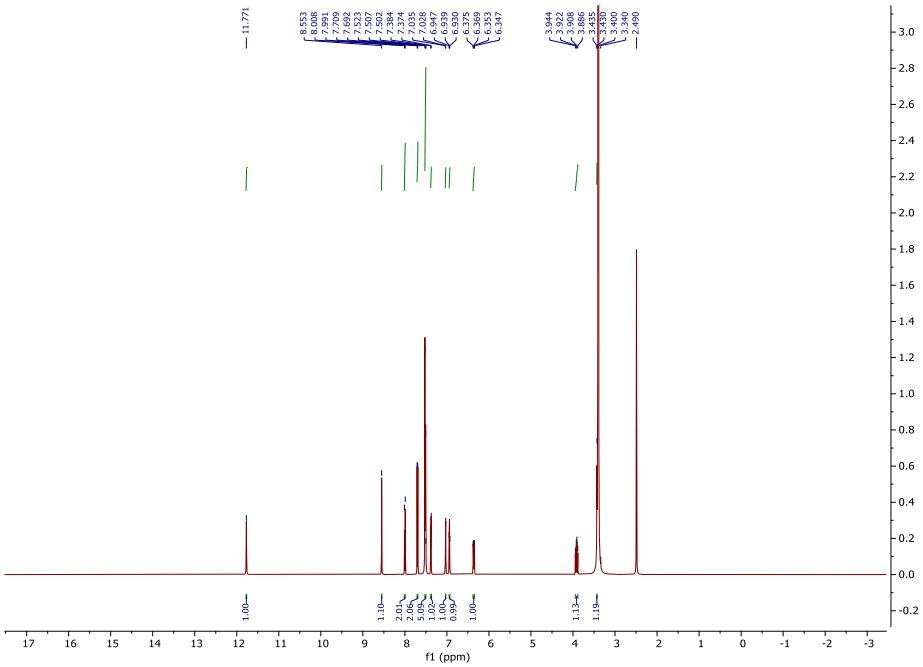


Figure S12. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4b**

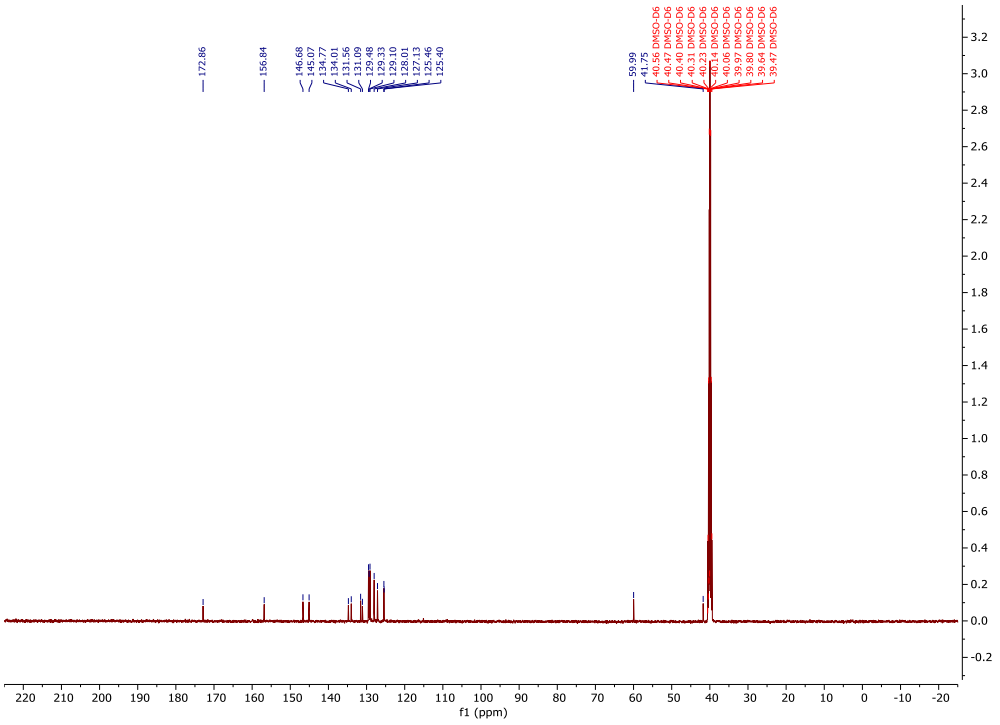


Figure S13. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4b**

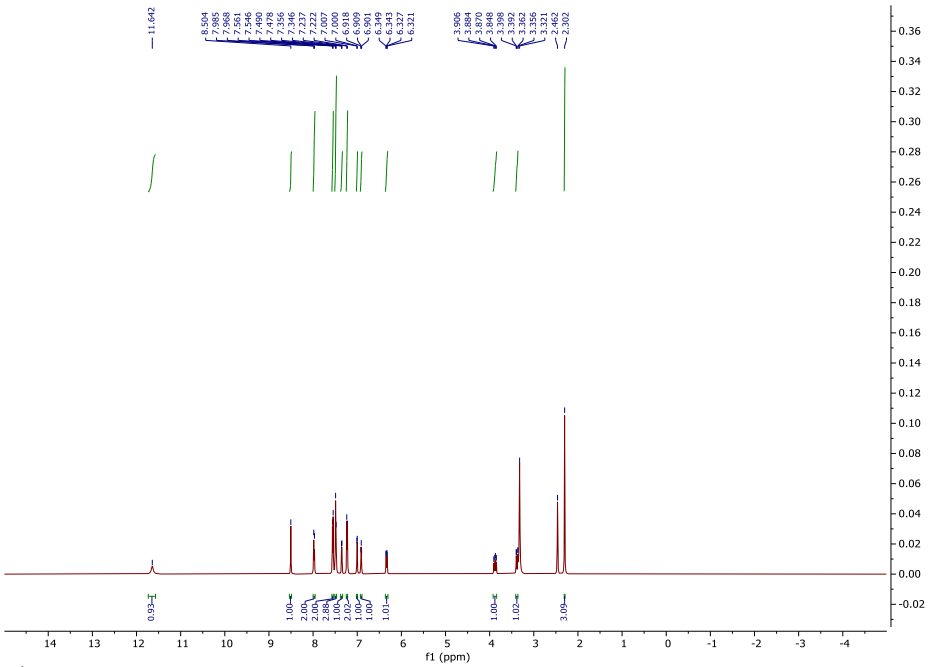


Figure S14. [1]H NMR (500 MHz, DMSO-d_6) spectrum of compound **4c**

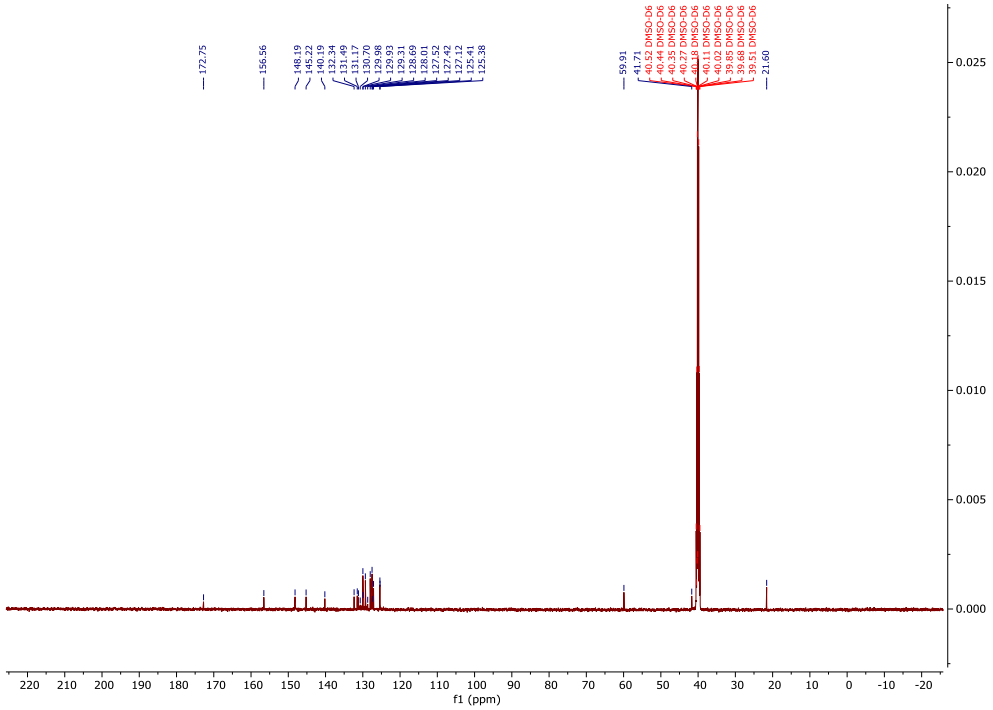


Figure S15. [13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **4c**

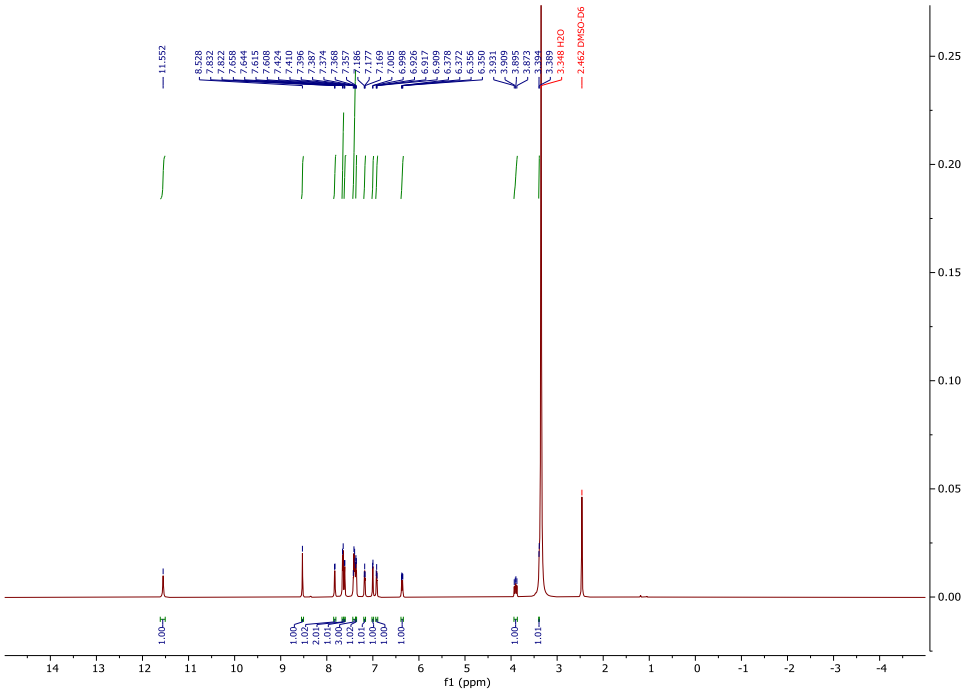


Figure S16. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4d**

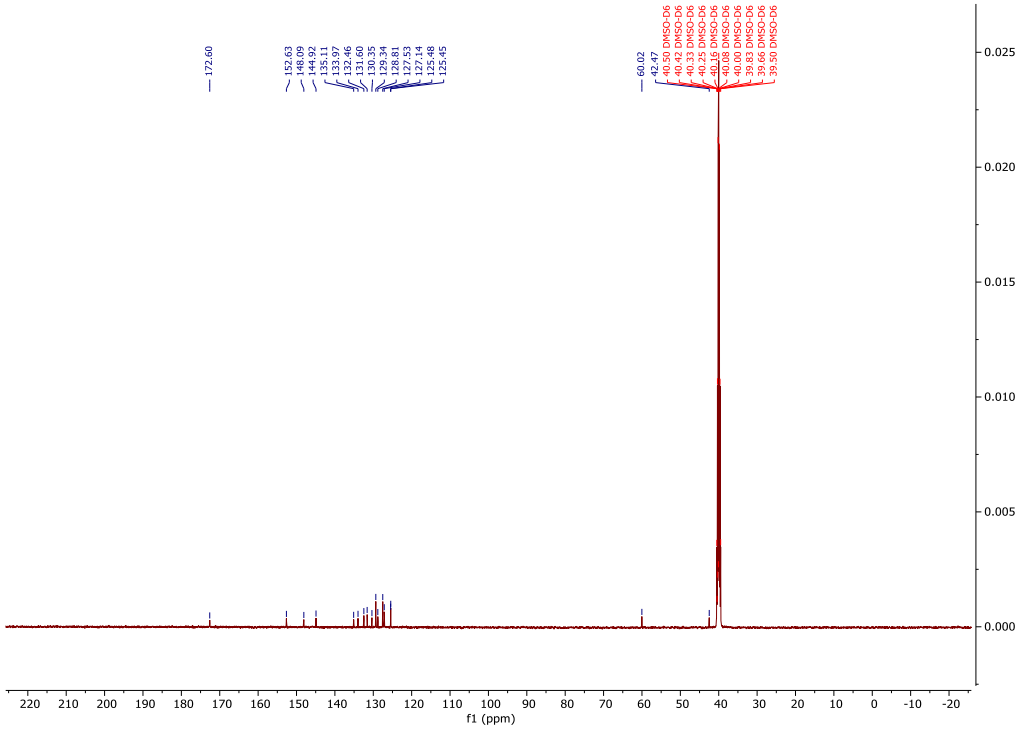


Figure S17. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4d**

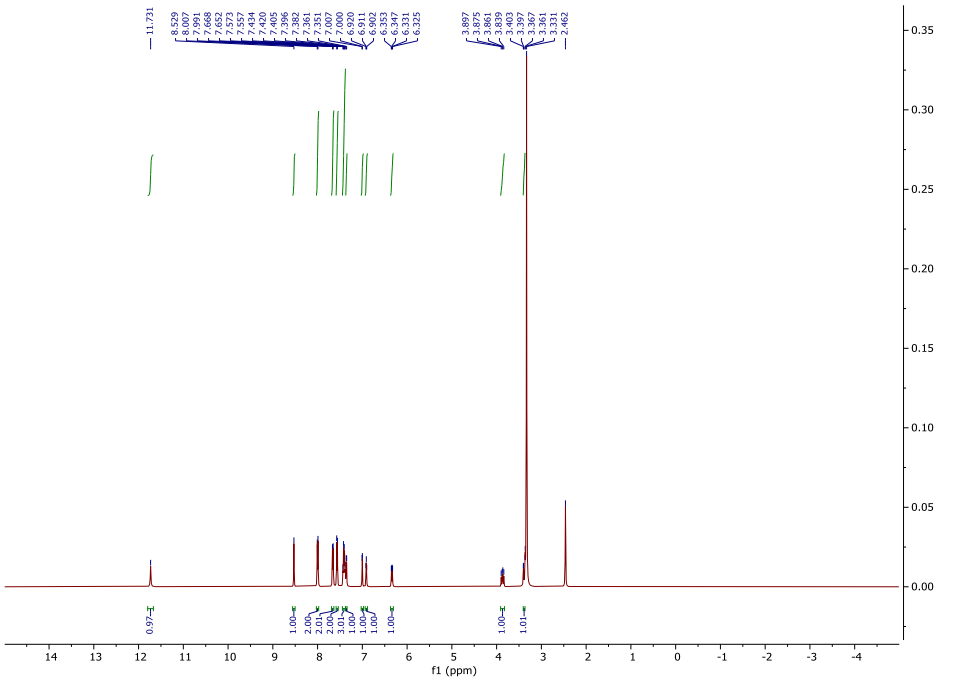


Figure S18. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4e**

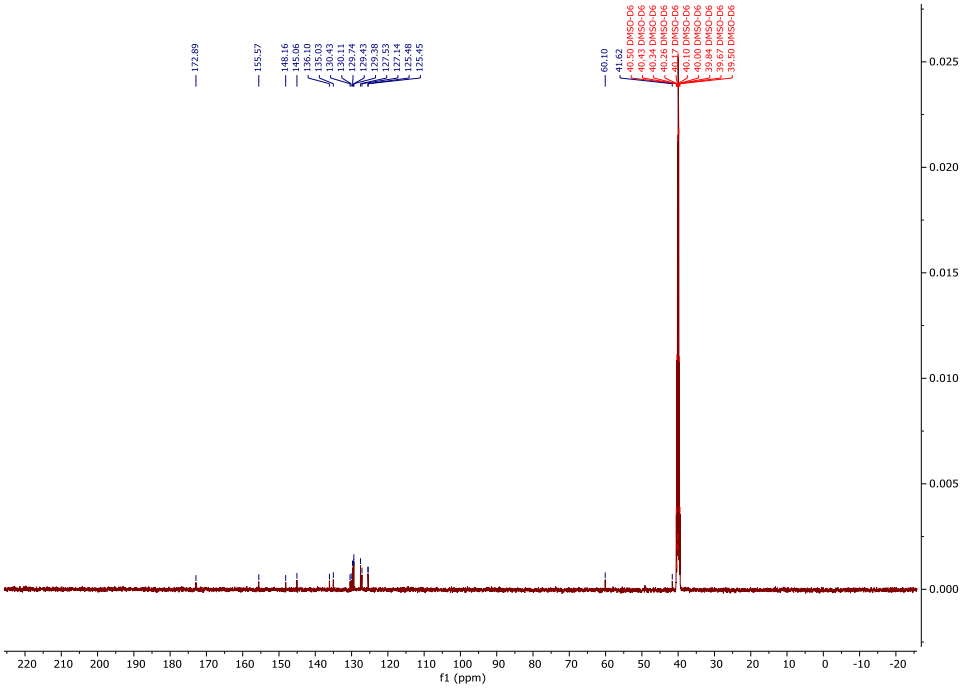


Figure S19. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4e**

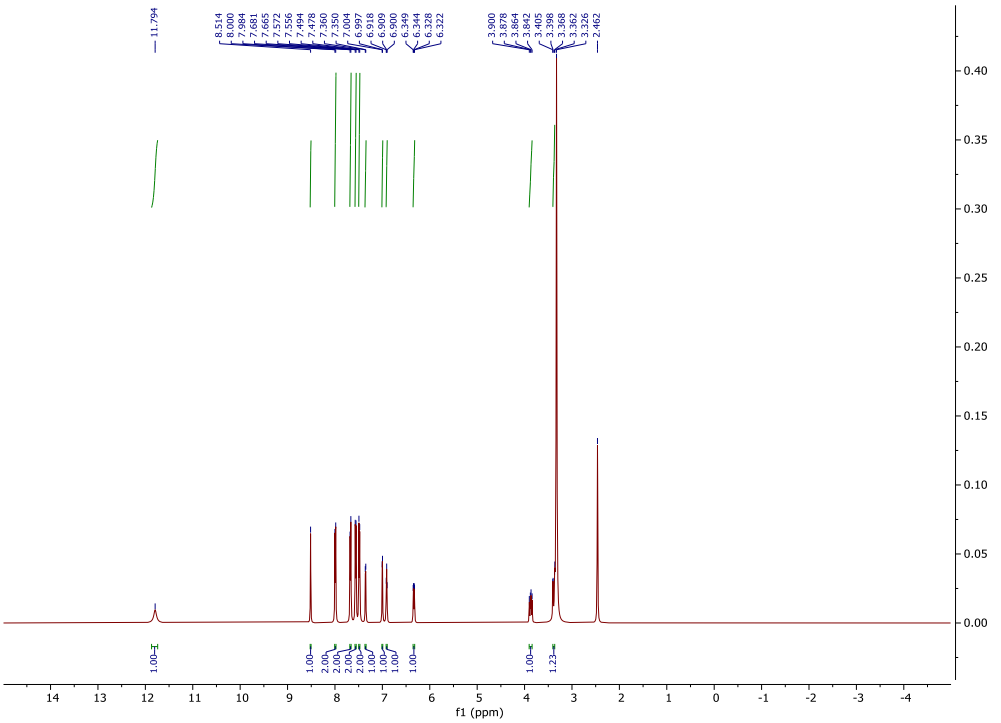


Figure S20. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4f**

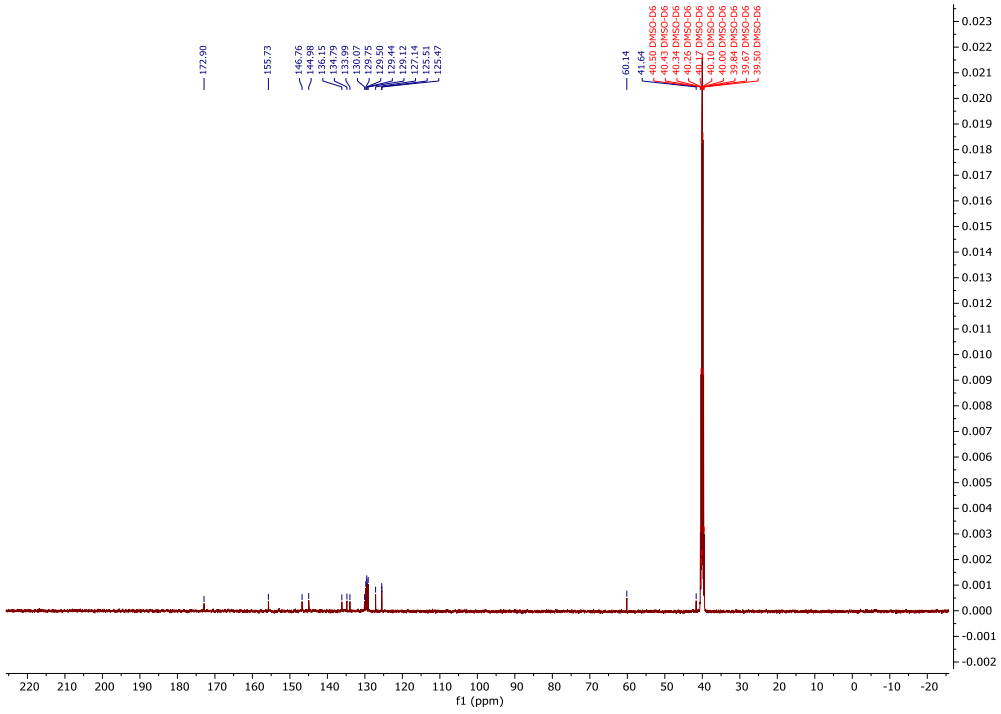


Figure S21. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4f**

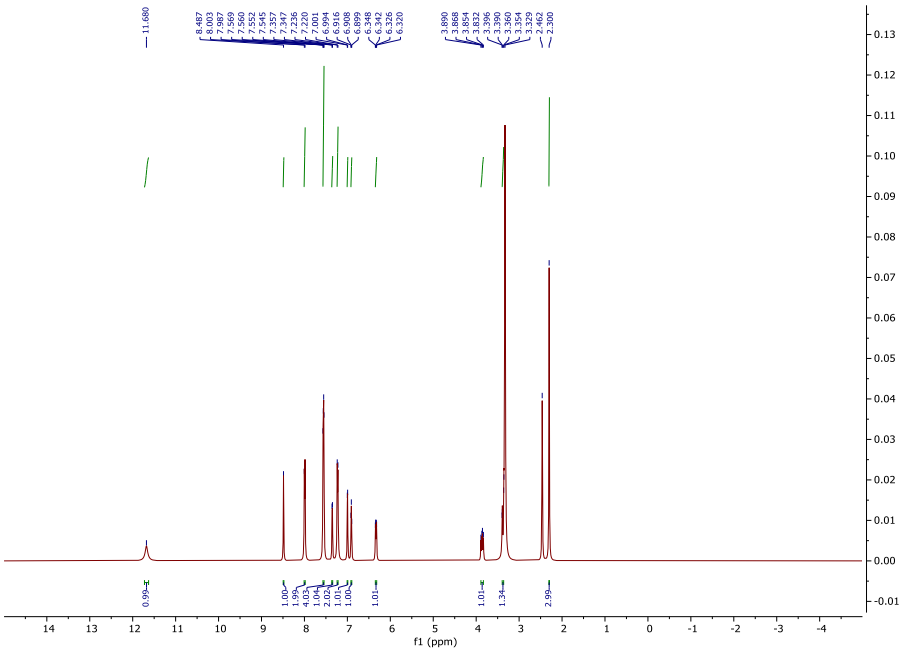


Figure S22. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4g**

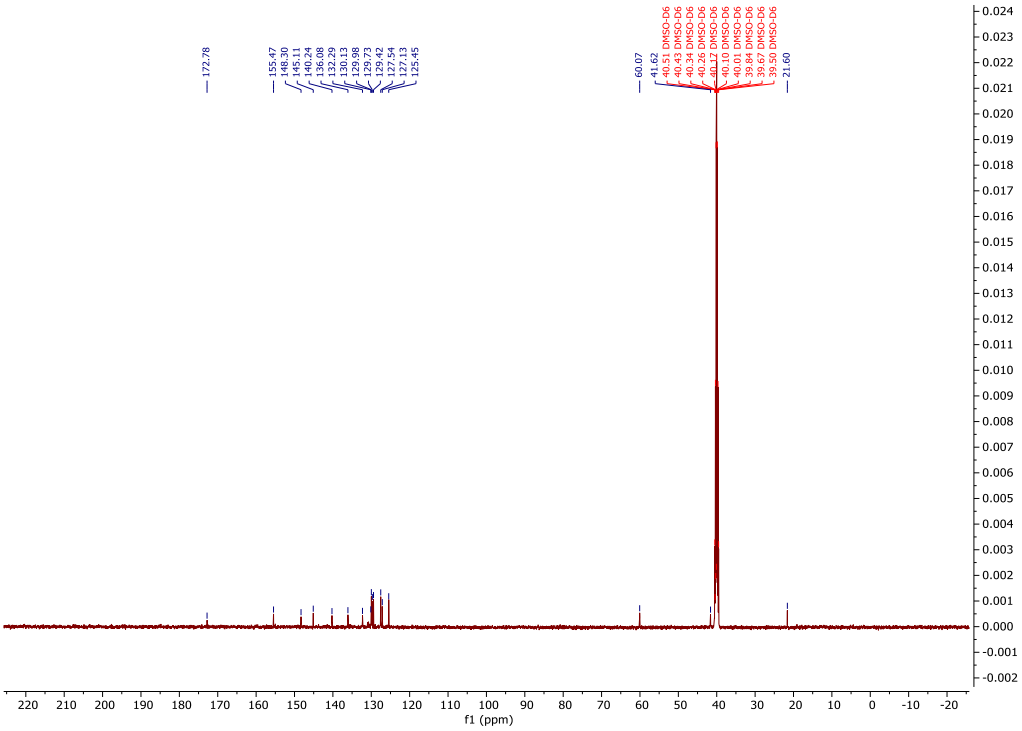


Figure S23. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4g**

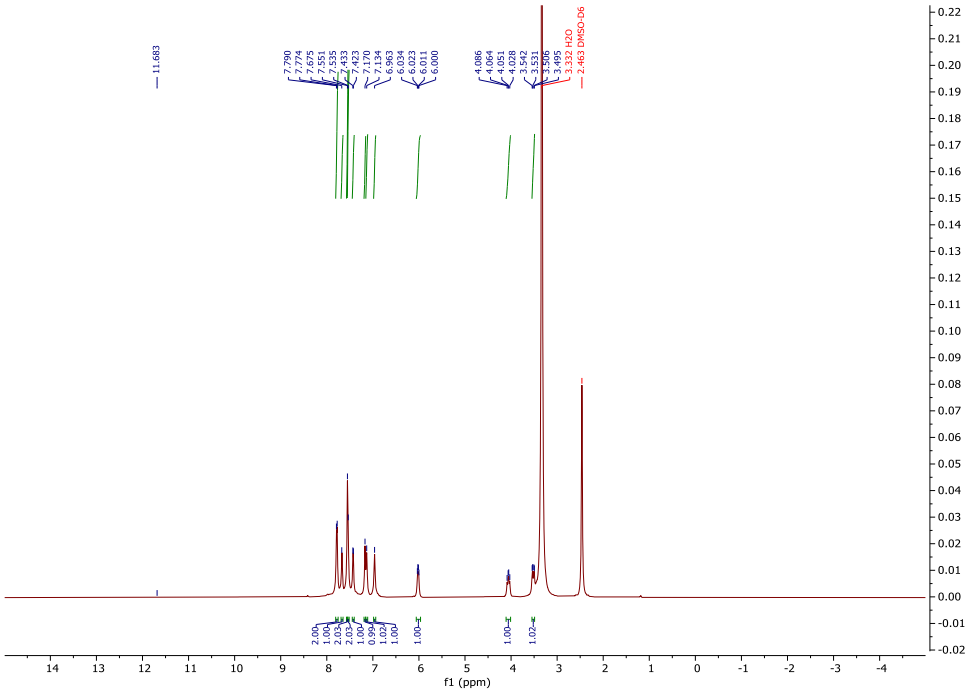


Figure S24. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4h**

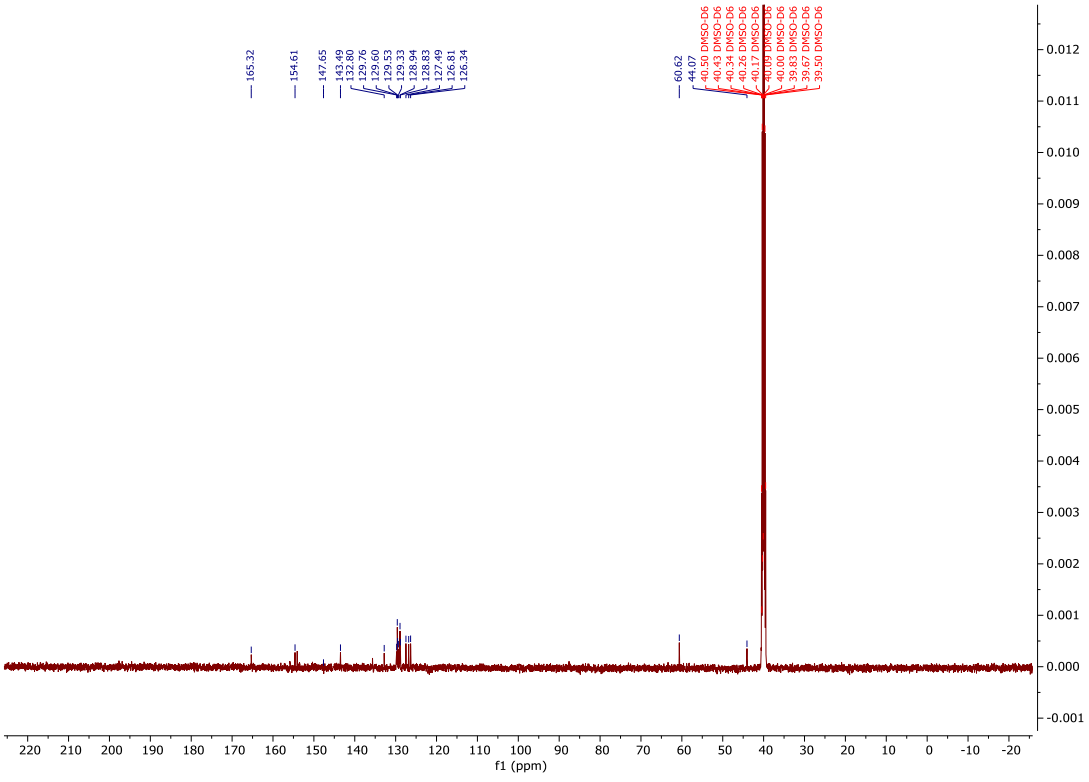


Figure S25. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4h**

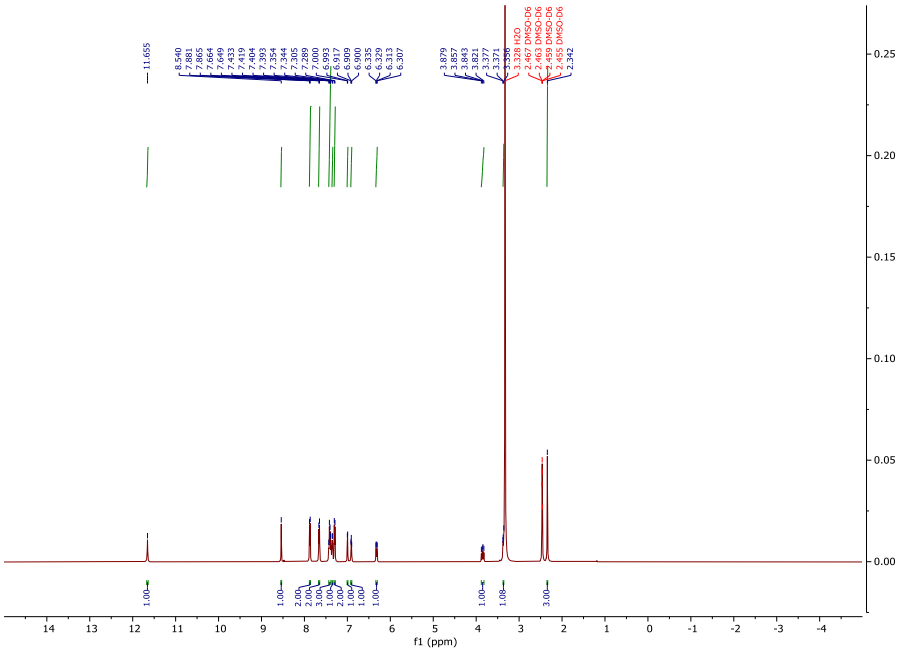


Figure S26. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4i**

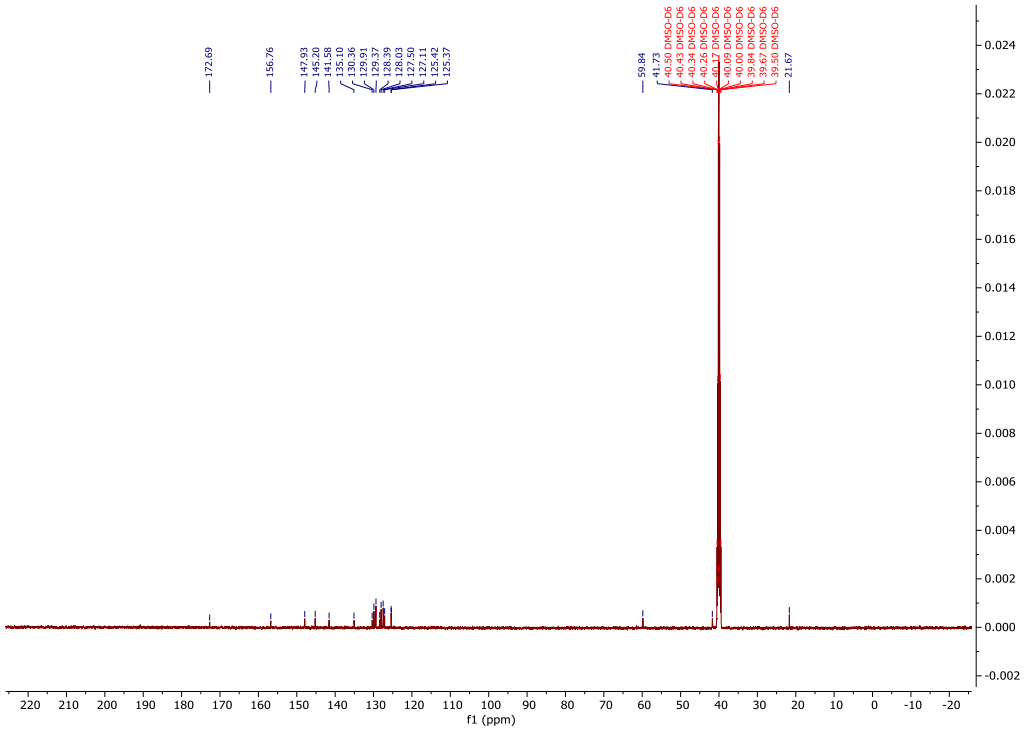


Figure S27. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4i**

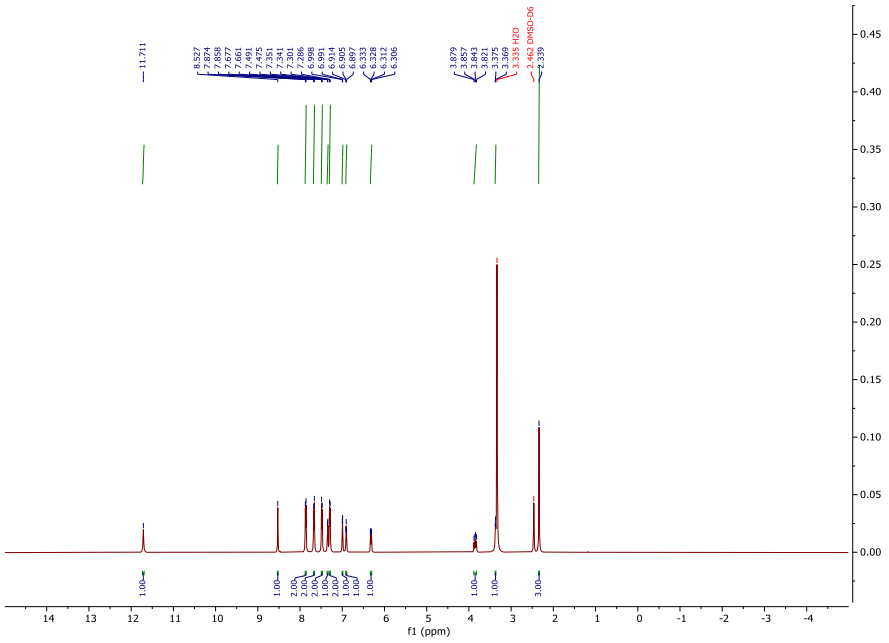


Figure S28. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4j**

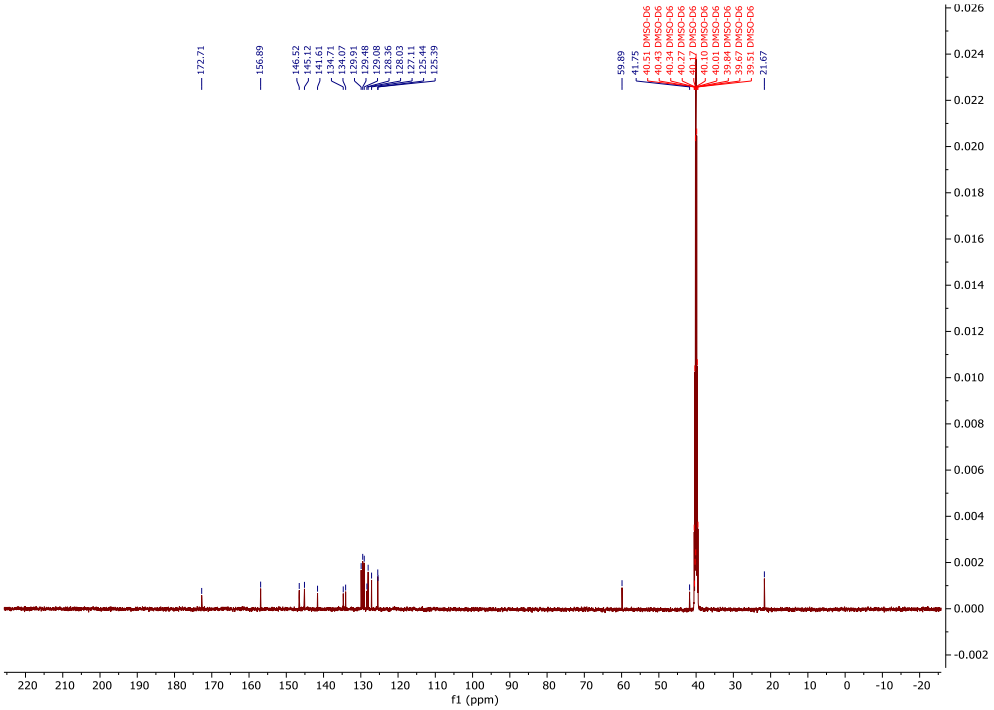


Figure S29. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4j**

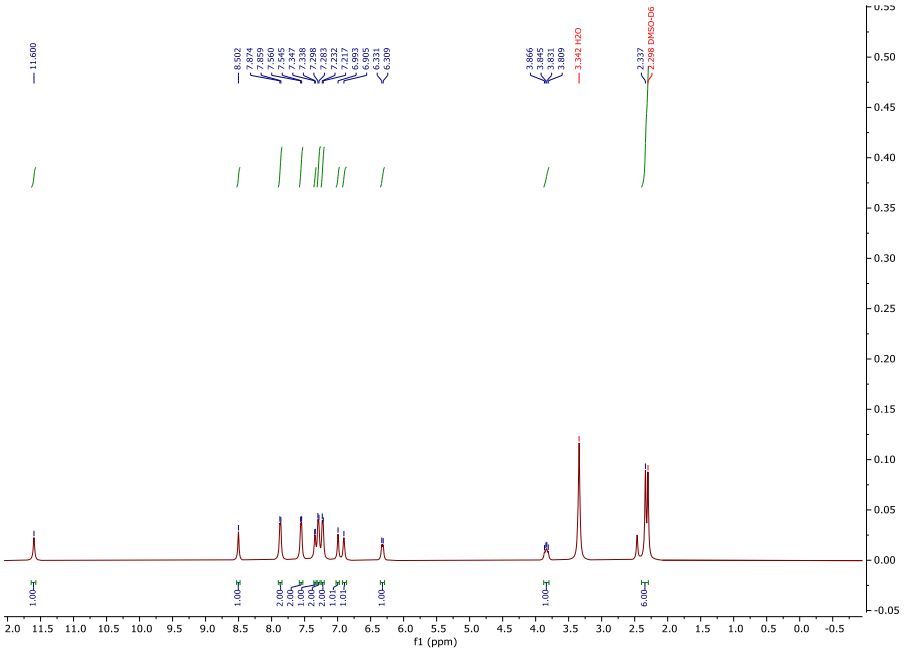


Figure S30. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4k**

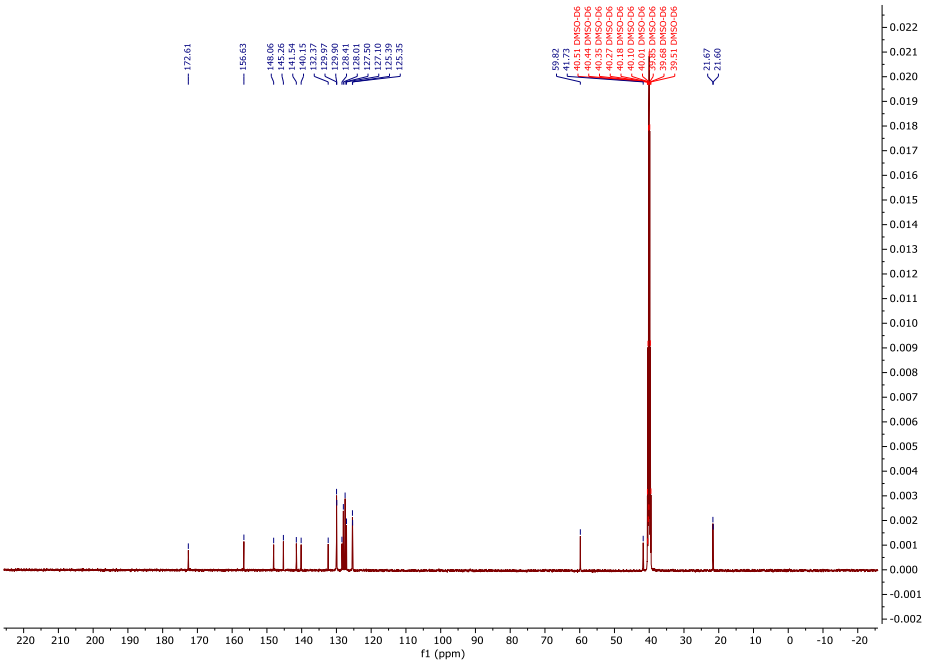


Figure S31. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4k**

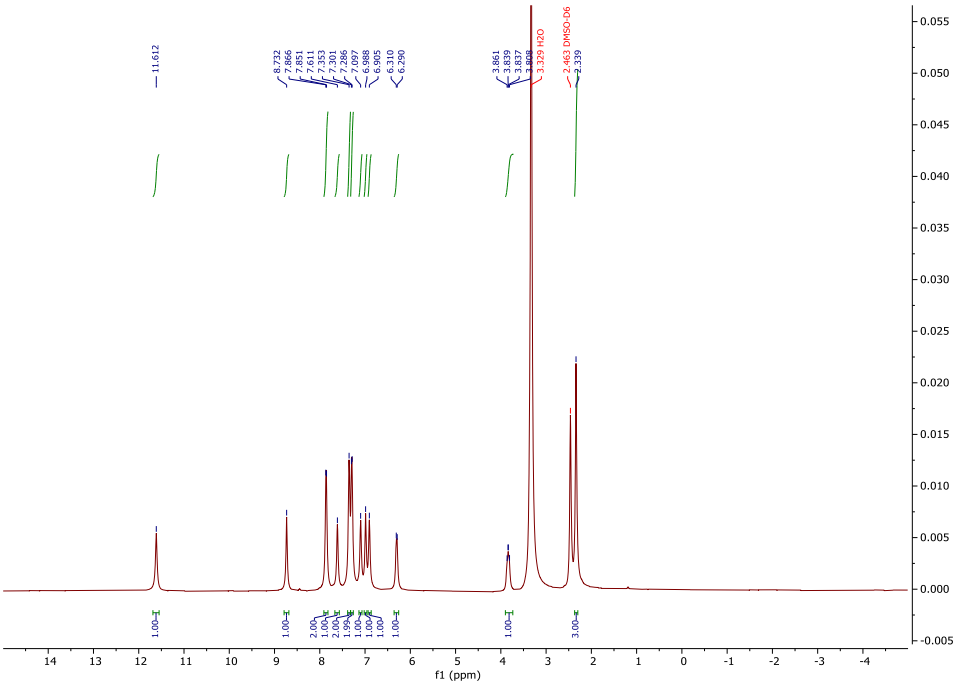


Figure S32. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4l**

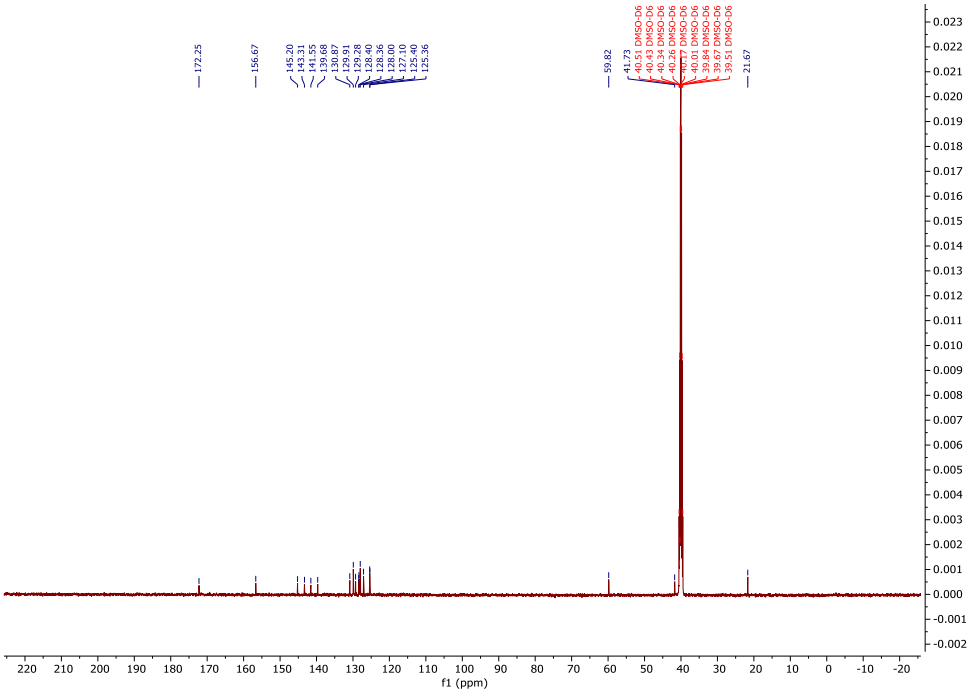


Figure S33. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4l**

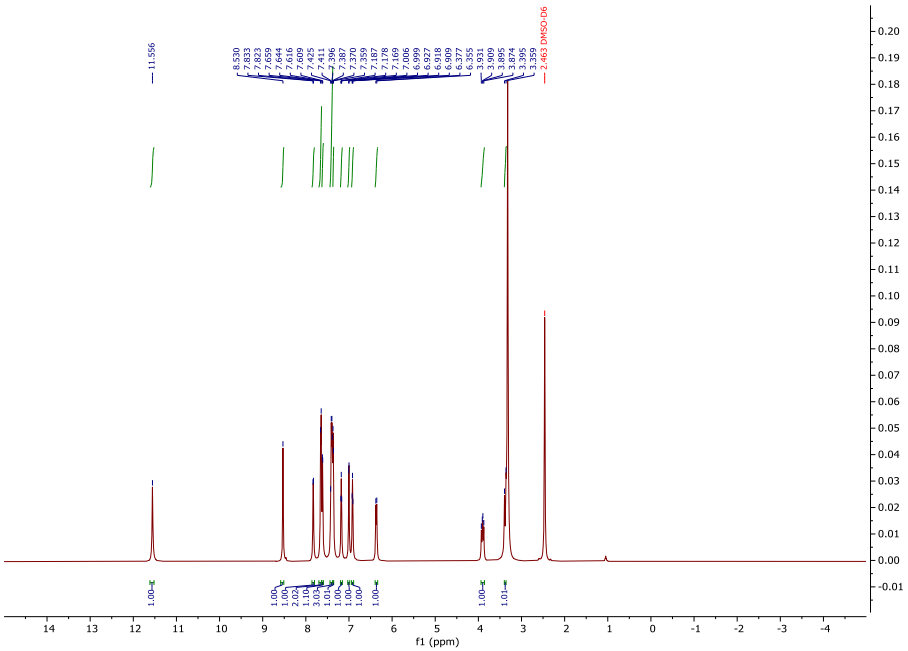


Figure S34. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4m**

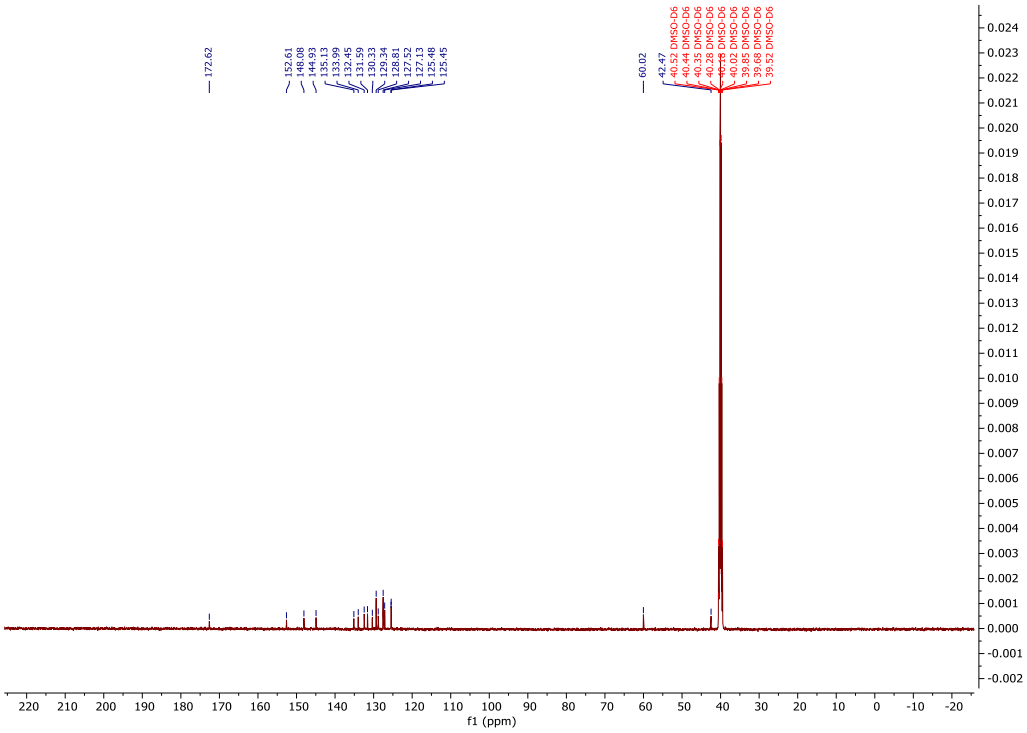


Figure S35. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4m**

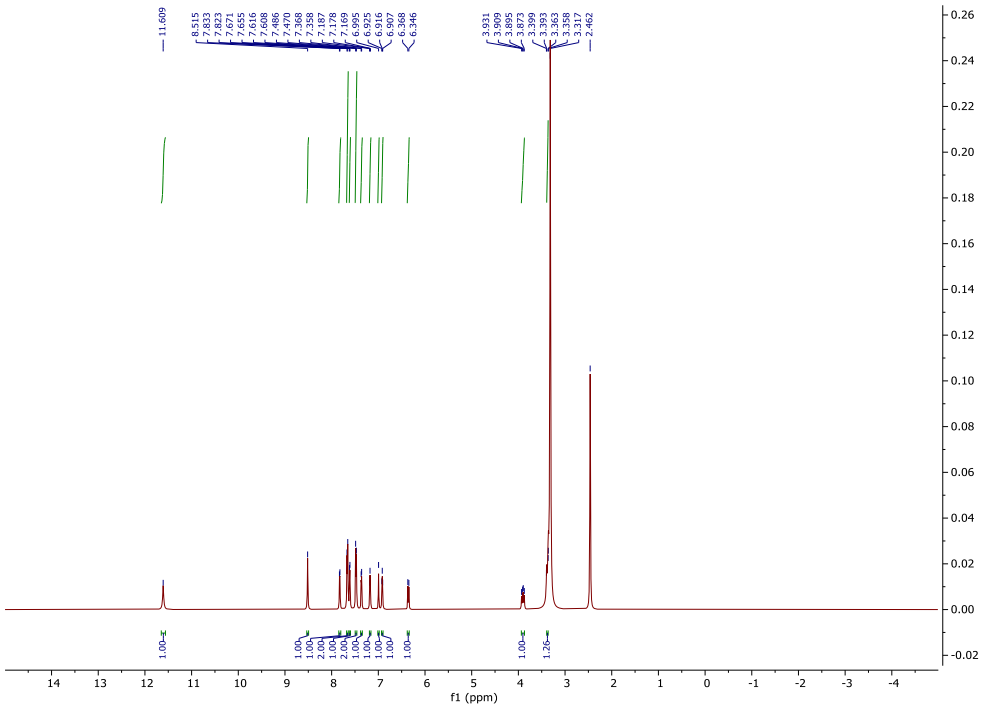


Figure S36. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4n**

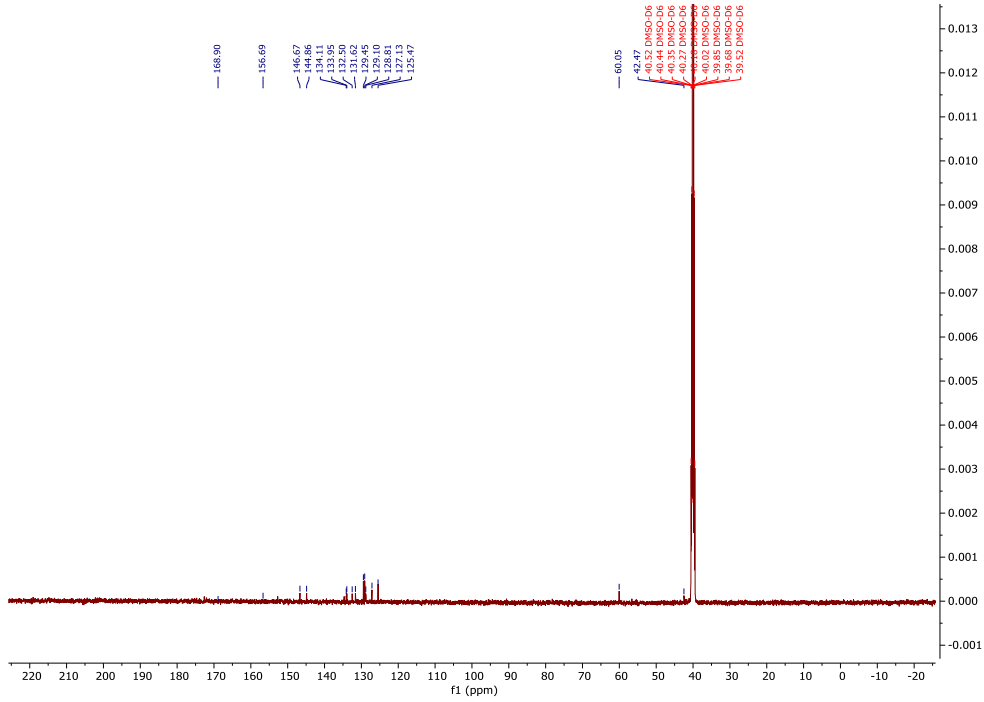


Figure S37. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4n**

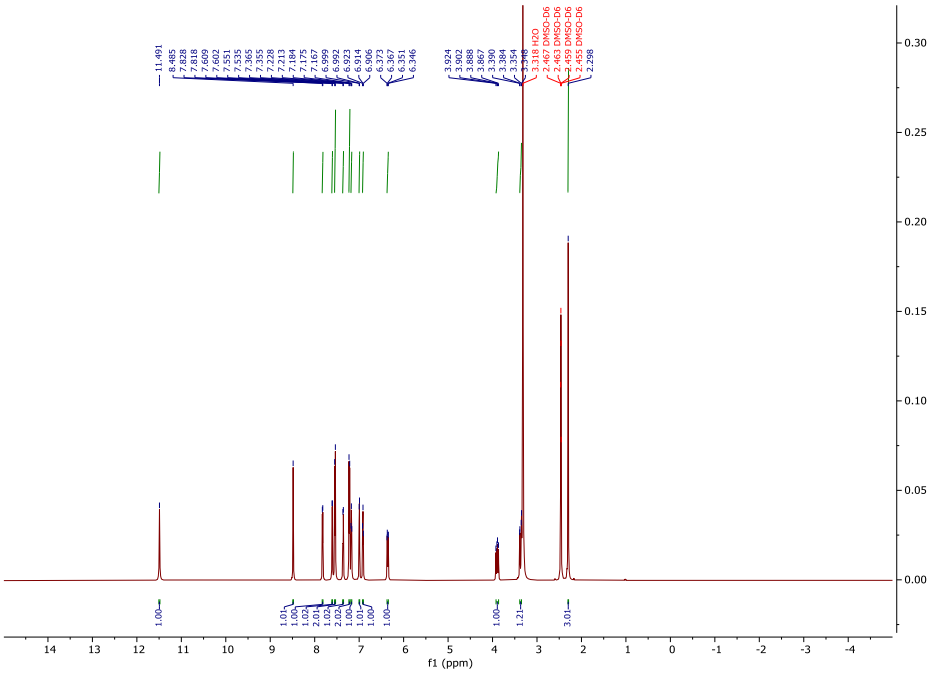


Figure S38. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4o**

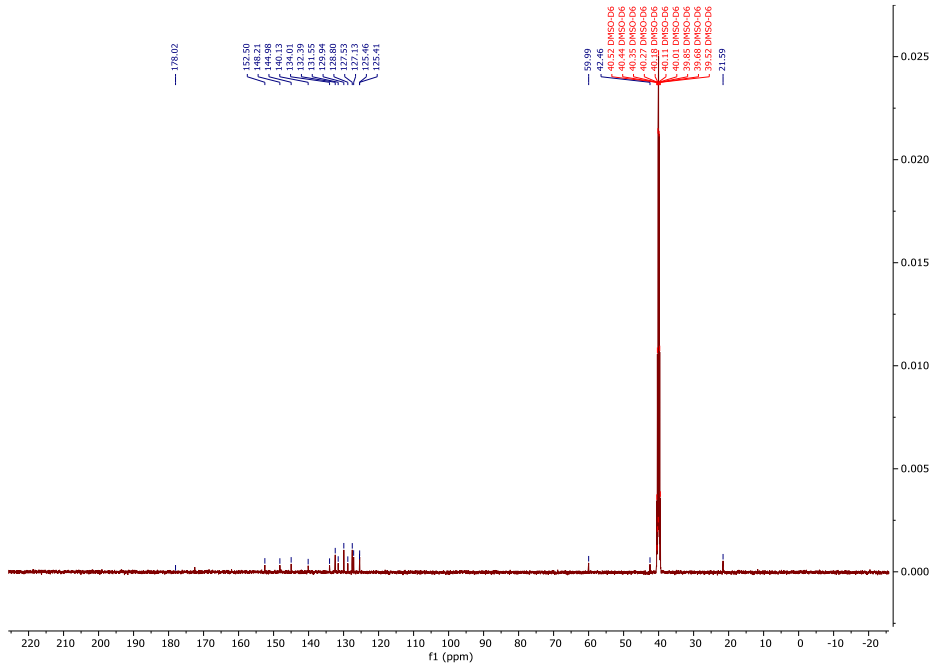


Figure S39. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4o**

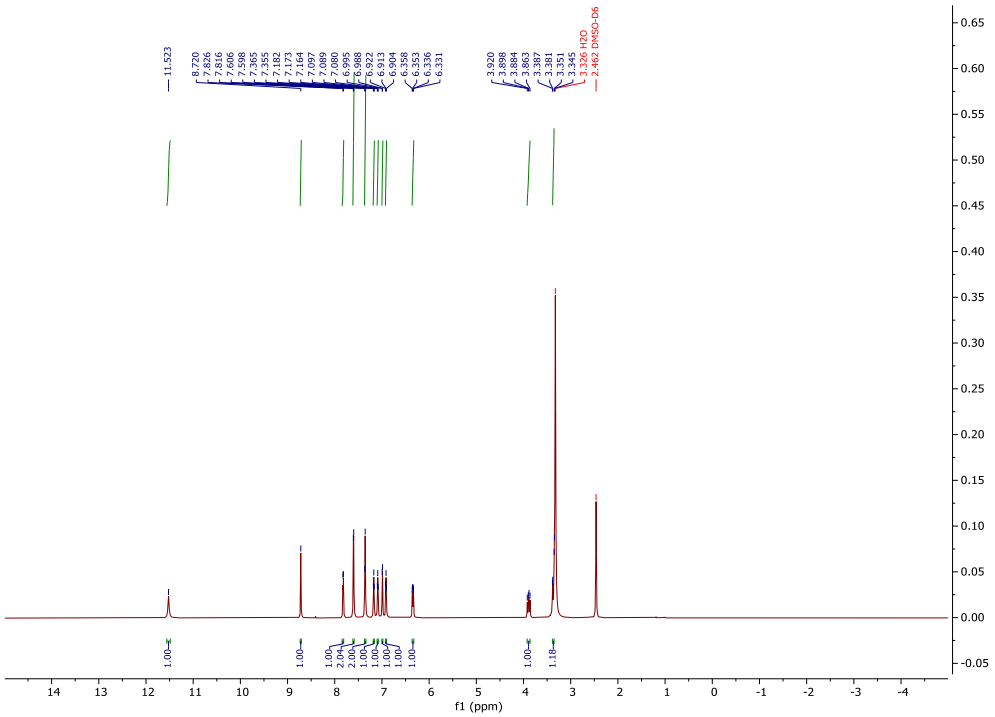


Figure S40. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4p**

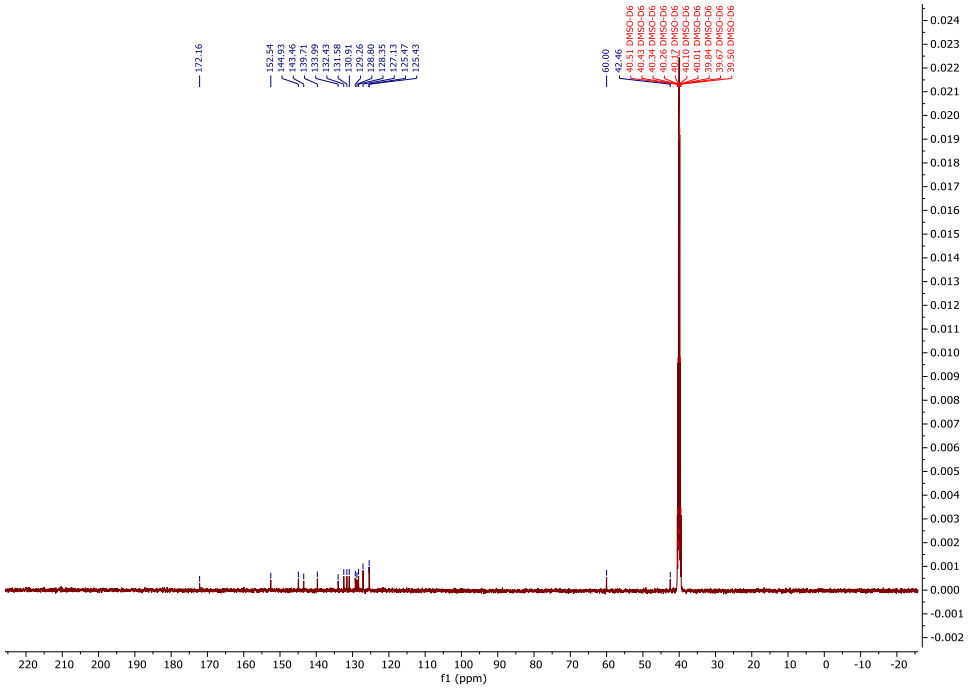


Figure S41. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4p**

Mass spectral data of the target compounds (4a-p)

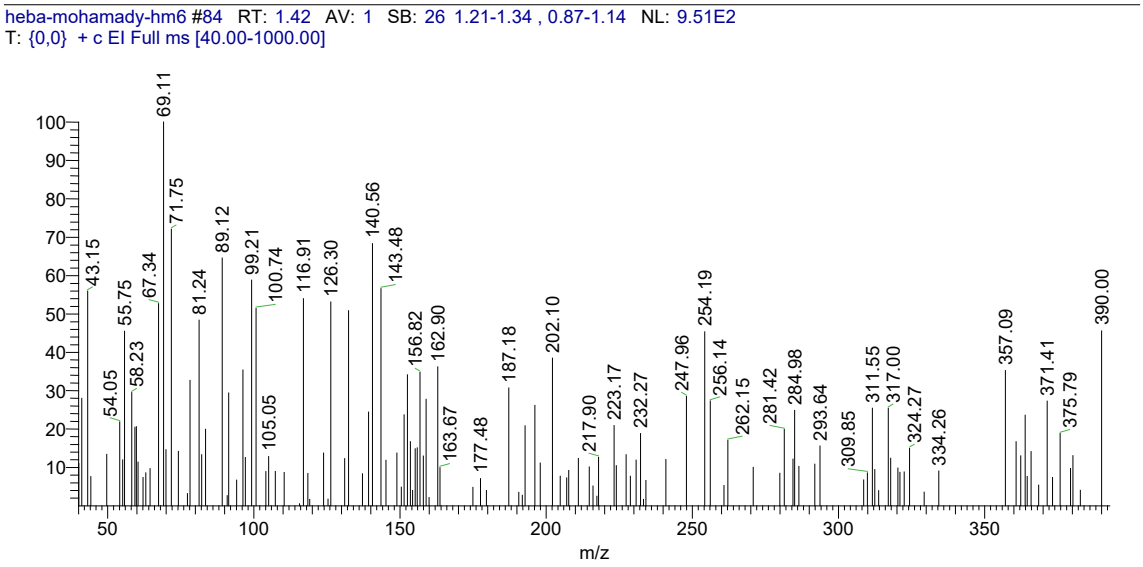


Figure S42. Mass spectrum of compound **4a**

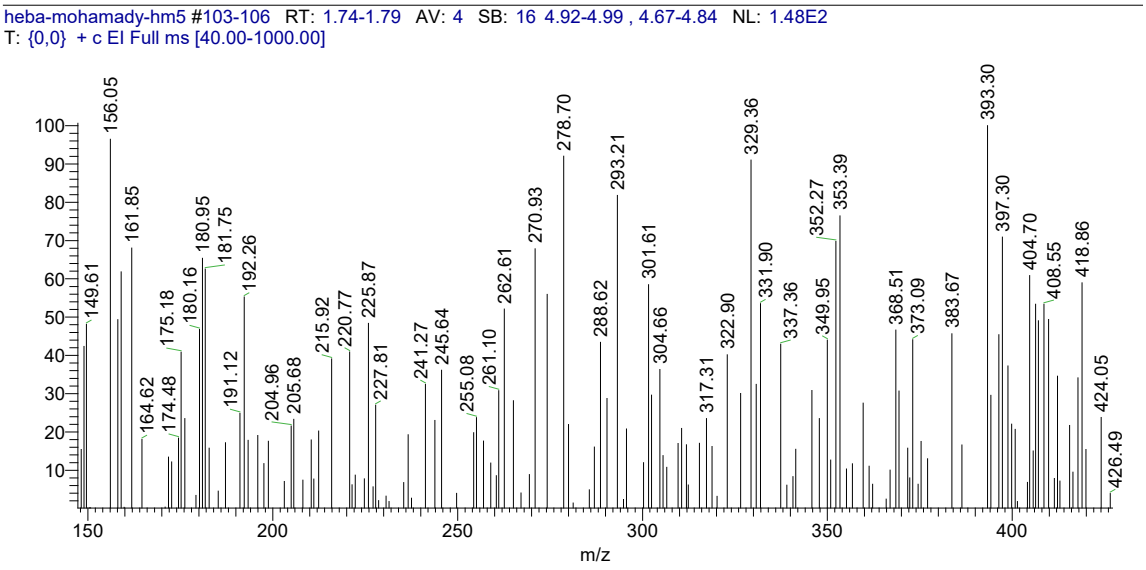


Figure S43. Mass spectrum of compound **4b**

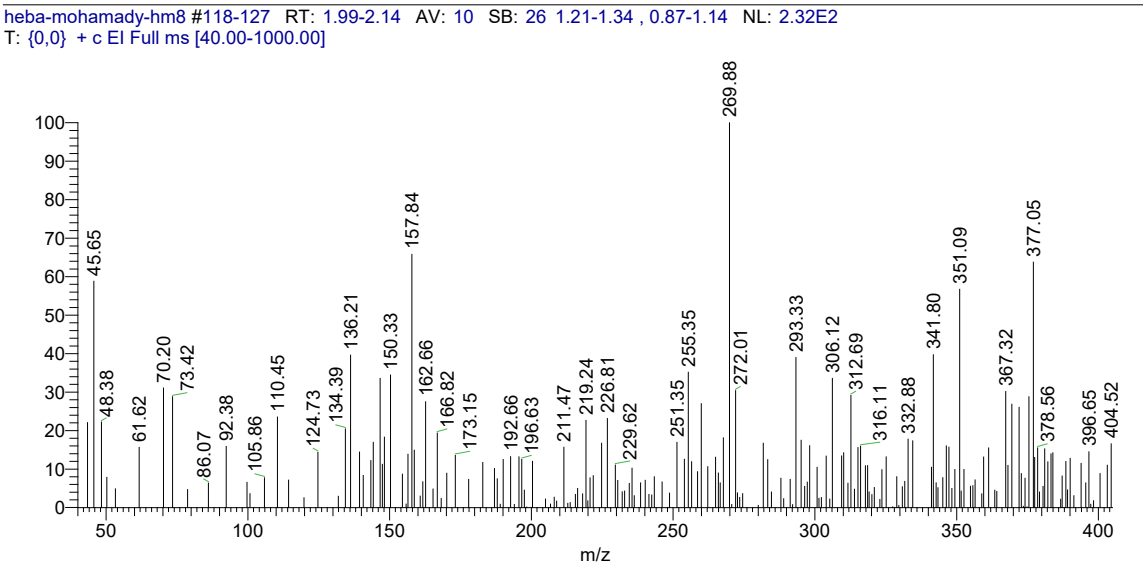


Figure S44. Mass spectrum of compound **4c**

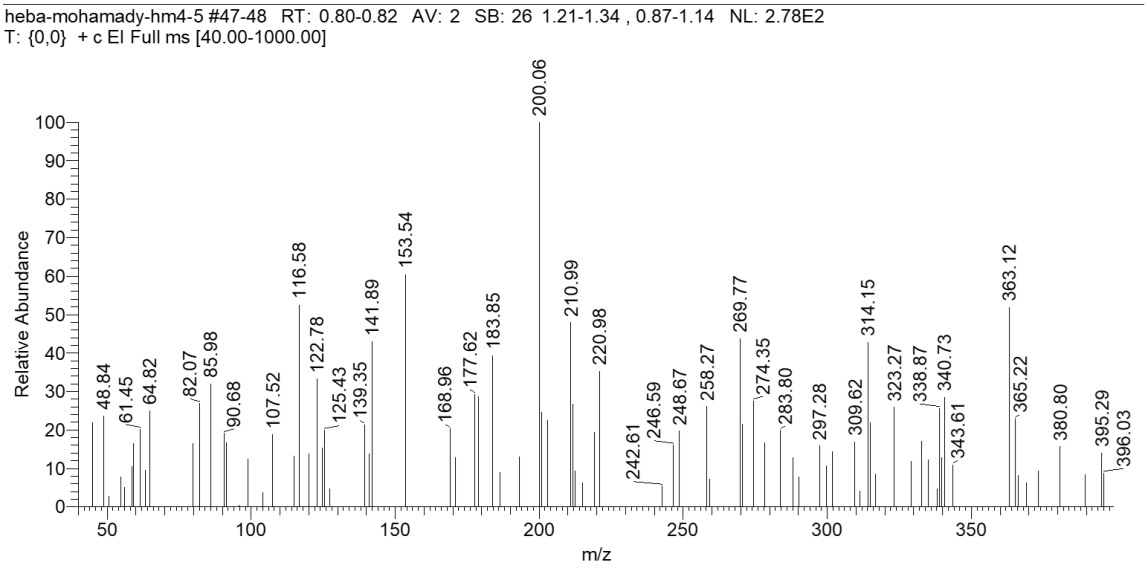


Figure S45. Mass spectrum of compound **4d**

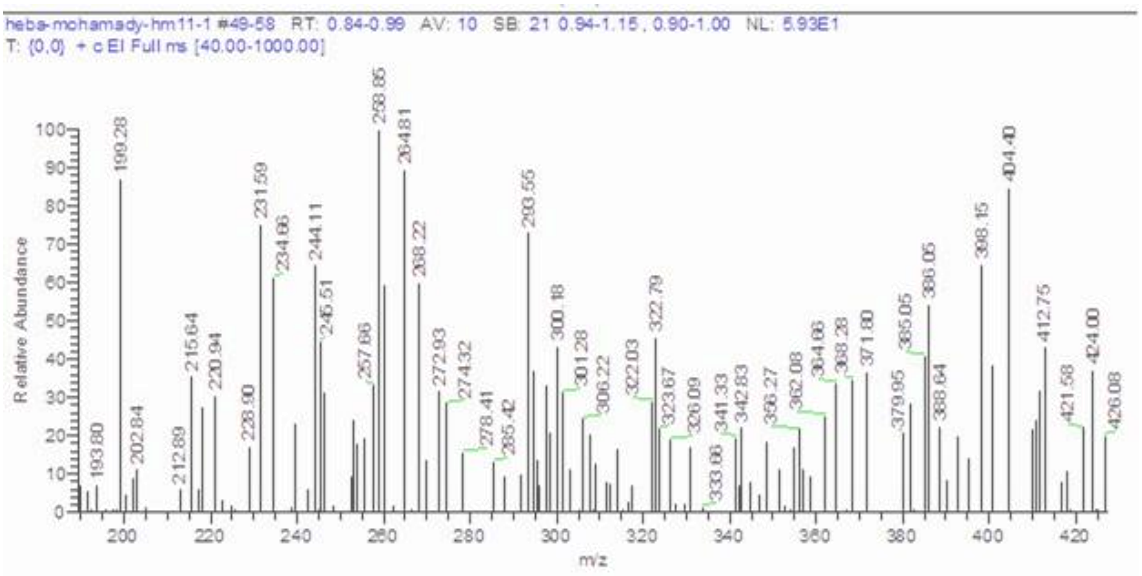


Figure S46. Mass spectrum of compound **4e**

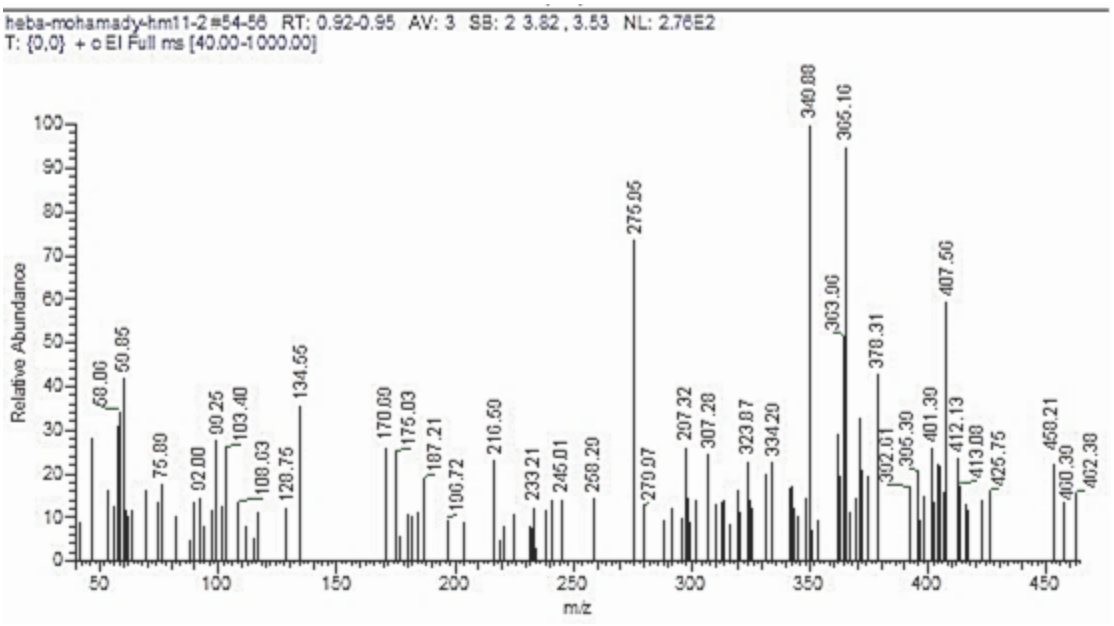


Figure S47. Mass spectrum of compound **4f**

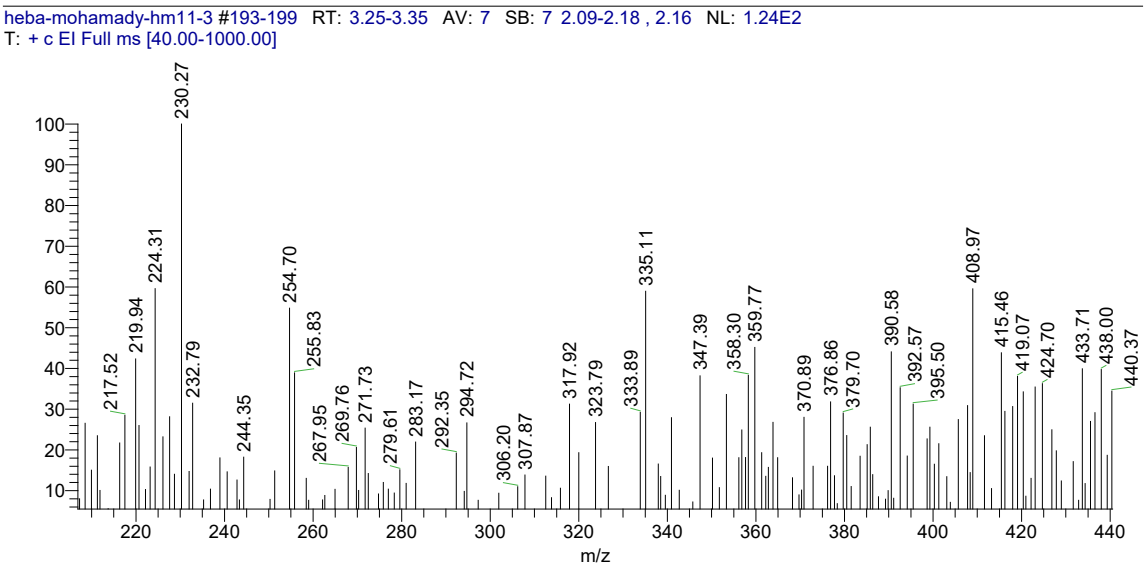


Figure S48. Mass spectrum of compound **4g**

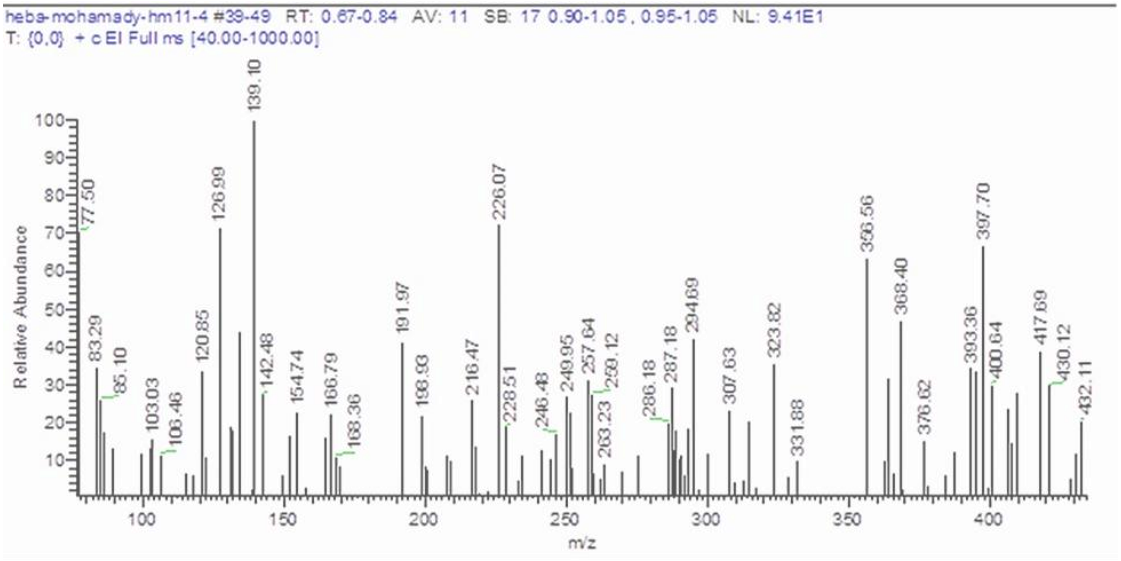


Figure S49. Mass spectrum of compound **4h**

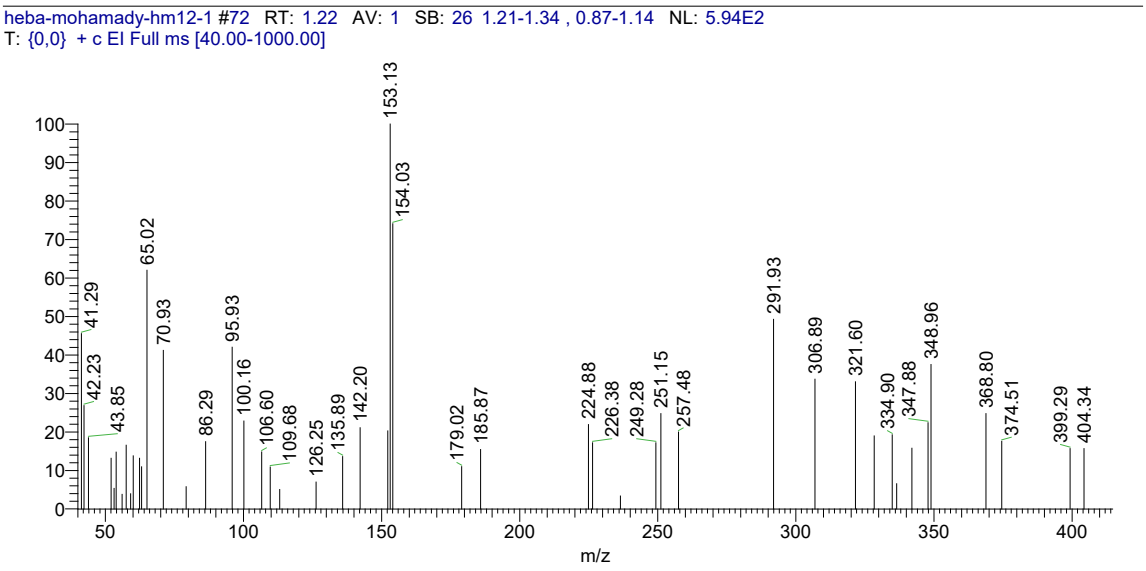


Figure S50. Mass spectrum of compound **4i**

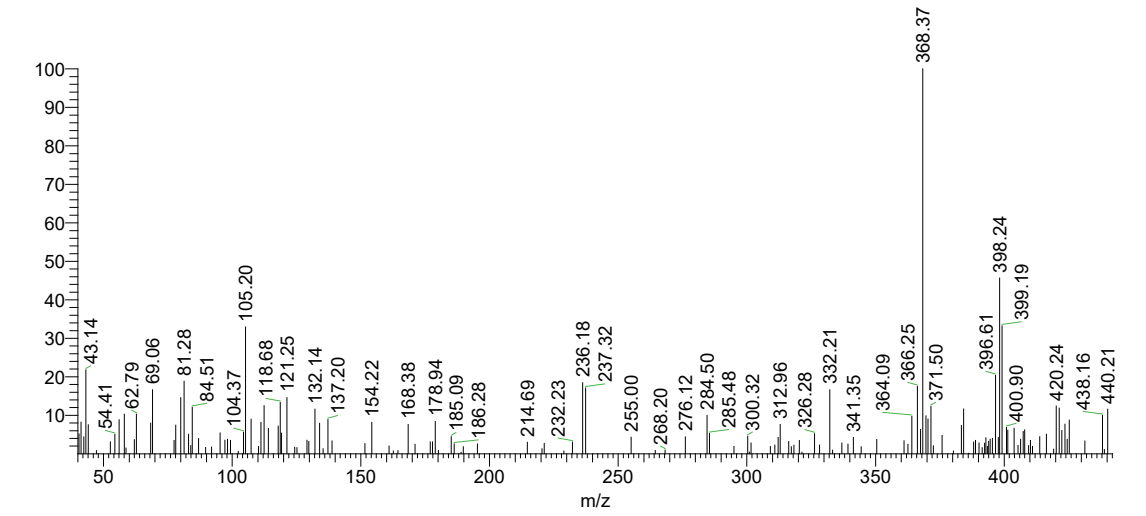


Figure S51. Mass spectrum of compound **4j**

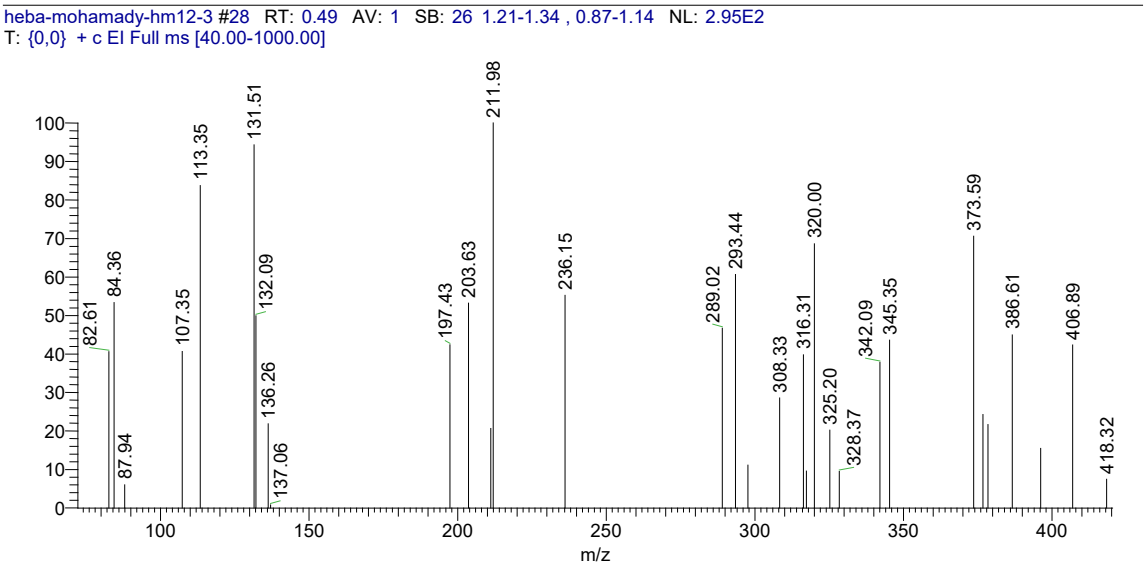


Figure S52. Mass spectrum of compound **4k**

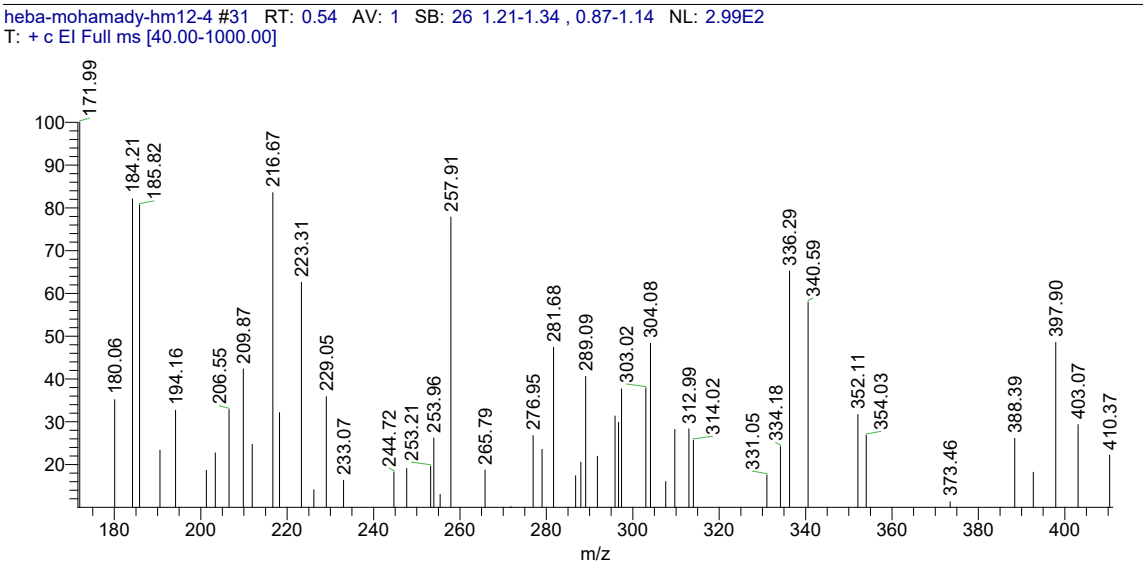


Figure S53. Mass spectrum of compound **4l**

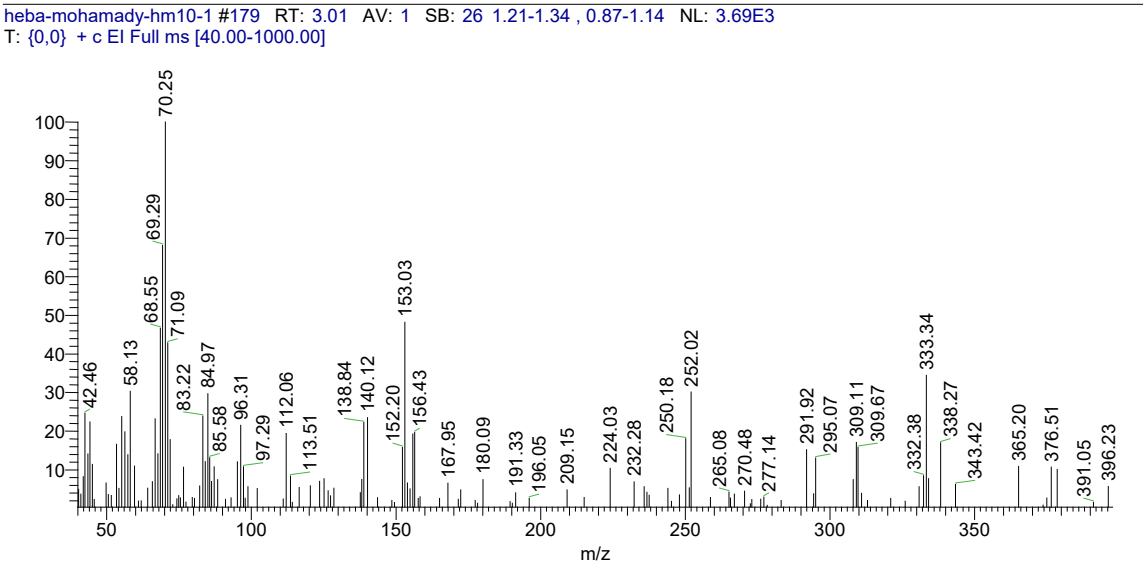


Figure S54. Mass spectrum of compound **4m**

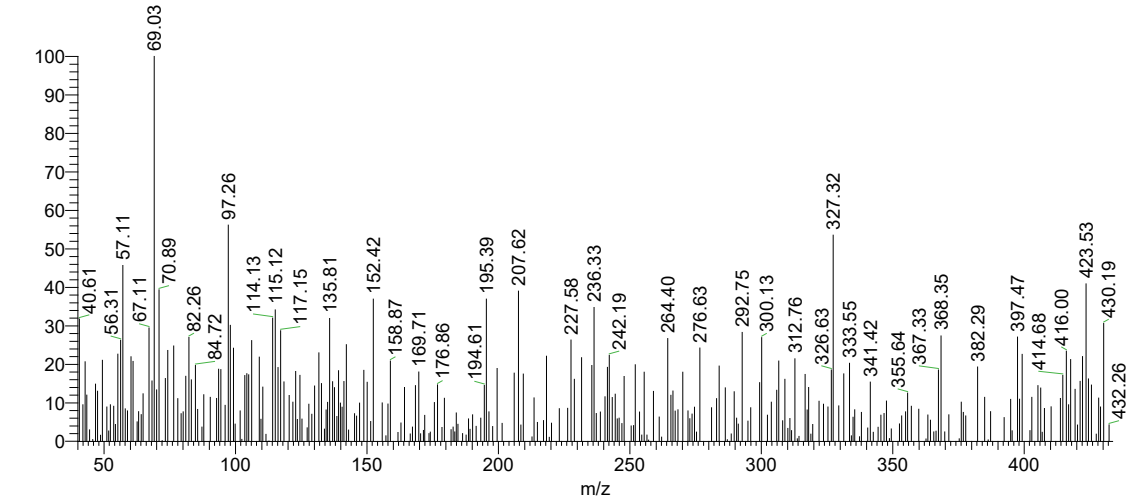


Figure S55. Mass spectrum of compound **4n**

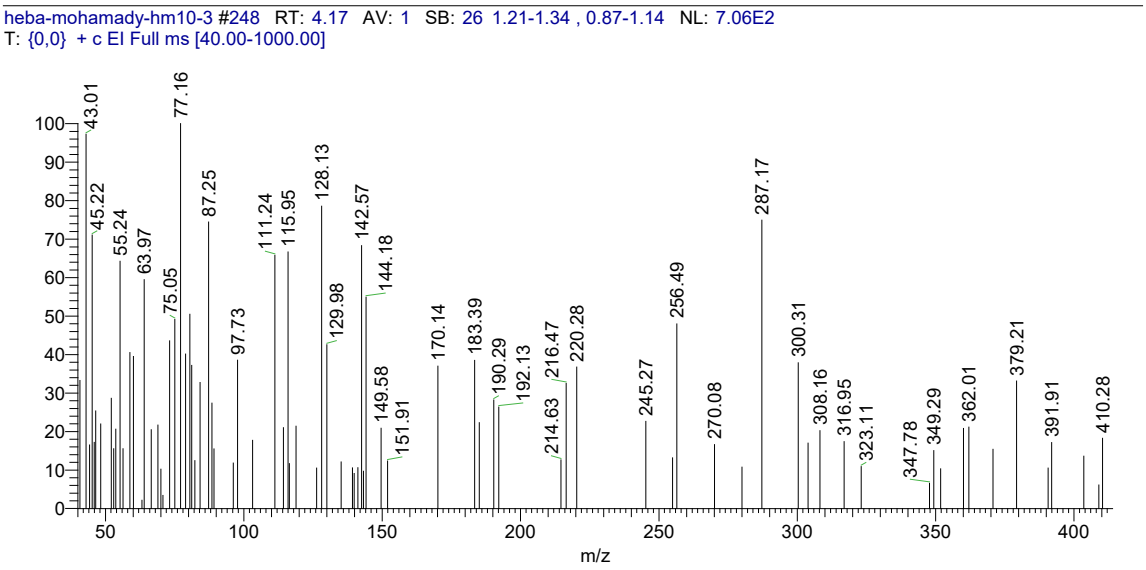


Figure S56. Mass spectrum of compound **4o**

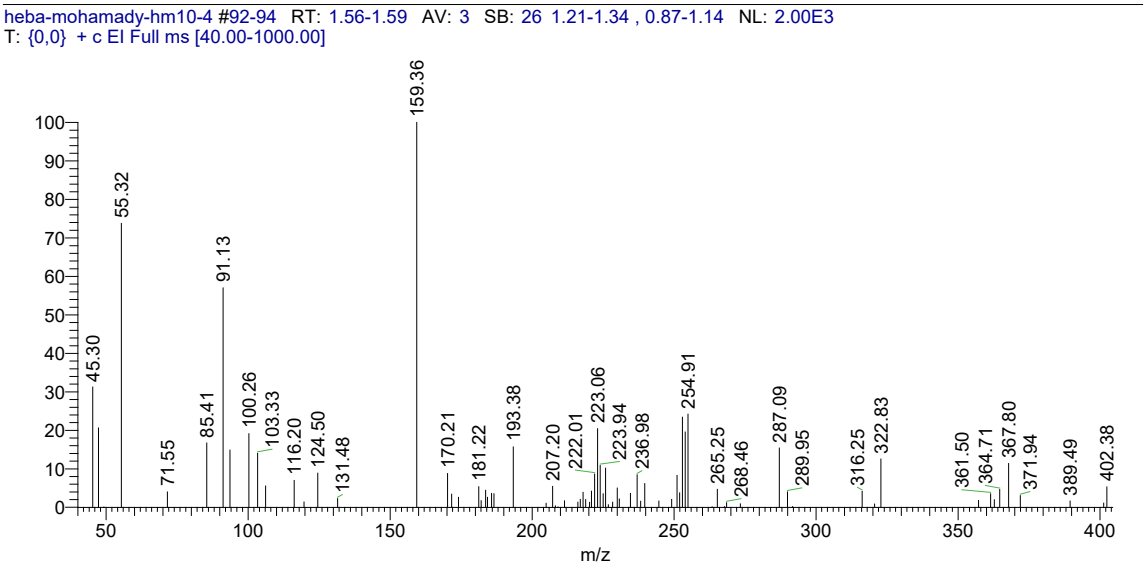
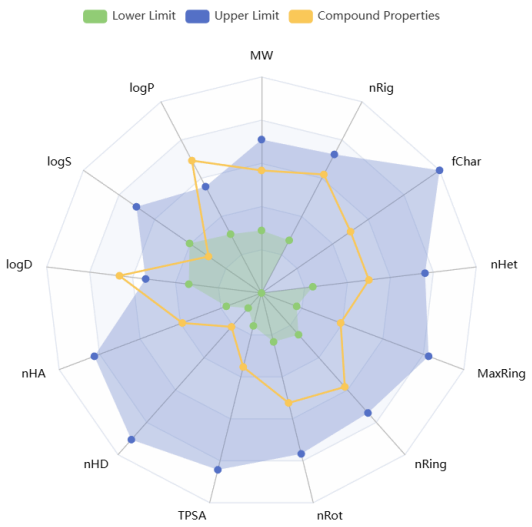
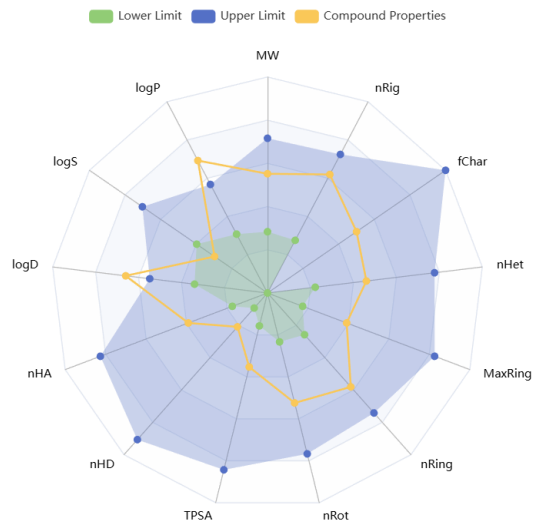


Figure S57. Mass spectrum of compound **4p**



Compound 4n



Compound 4o

Figure S58. Pharmacokinetic radar chart of compounds **4n** and **4o**.