Supplementary Information

Dual Function of Cyanoacetic Acid under Visible Light and Dark Conditions: Templating and Catalyzing the Formation of Unexplored Pyrimidine Derivatives and Evaluation of their Antibacterial Activity

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Contents

1. General information

1.1 Materials and methods

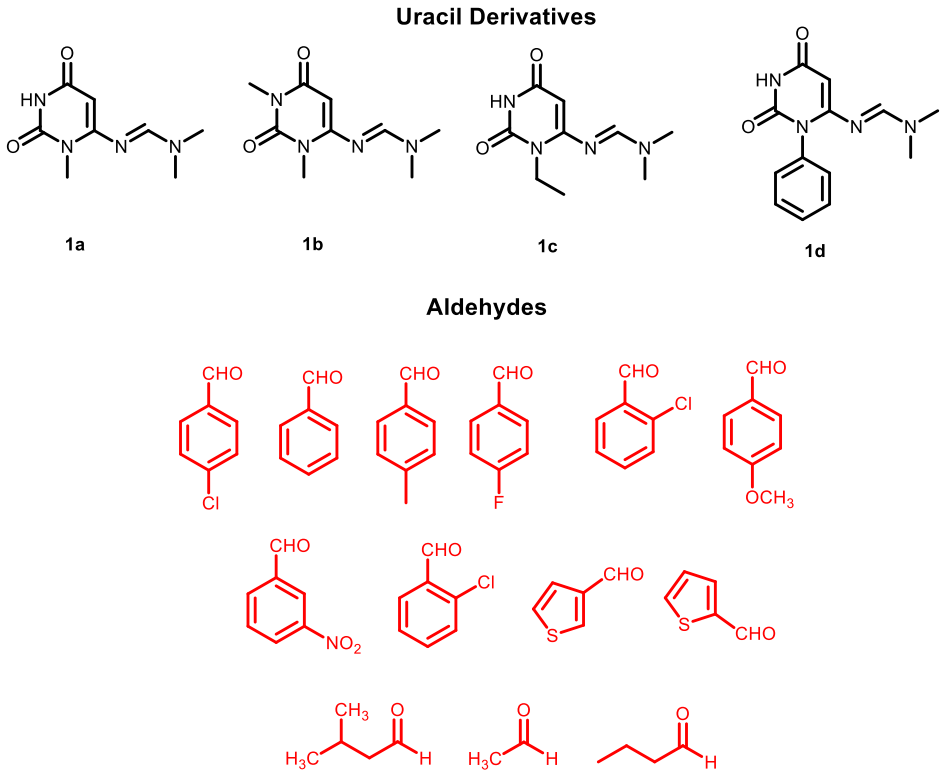
All chemicals were purchased from companies as follows: water: Milli-Q; HPLC grade acetonitrile: Merck; Cyanoacetic acid: Sigma; Aldehydes: Avra and CDH; Hexane, Ethyl acetate: SRL; DMSO-d_6: Cambridge isotope laboratories. All experiments were performed in oven-dried glassware under an air atmosphere. Reagents were used directly without purification. All the solvents were purified before use. Starting materials **1a, 1b, 1c,** and **1d** were prepared using reported procedures. Analytical thin-layer chromatography (TLC) was conducted on plates (GF-254) Merck silica gel 60 F_{254} and visualization was by ultraviolet fluorescence (λ = 254 nm).

1.2 Instrumentation

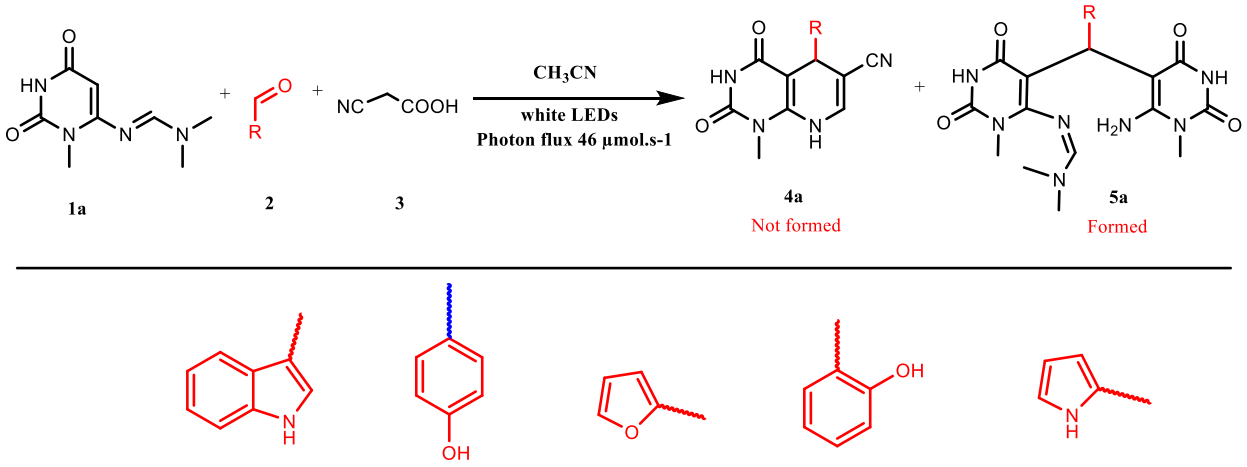
NMR spectra: ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker 500 MHz & 400 MHz FT-NMR spectrometer in $CDCl_3$ and DMSO-d_6 solvents. Chemical shifts are reported in parts per million (ppm). Data are reported as follows: chemical shift δ/ppm, coupling constants J in Hz, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Detailed information about all the instruments used is mentioned in the following table.

	Equipment	Model	Suppliers
1)	**Rotavapor**	Rotavapor R-100	BUCHI
2)	**400 MHz NMR spectrometer**	ECZ500R/S1	JEOL
3)	**500 MHz NMR spectrometer**	ECZ500R	JEOL
4)	**UV-VIS Spectrophotometer**	UV-1780	Shimadzu
5)	**High-Resolution Mass Spectrometer (HRMS)**	G2-XS QTQF	XEVO
6)	**Single crystal-XRD**	D8 Venture Diffractometer	Bruker

2. Structure of starting materials



3. Unsuccessful aldehydes for the synthesis of pyridopyrimidines



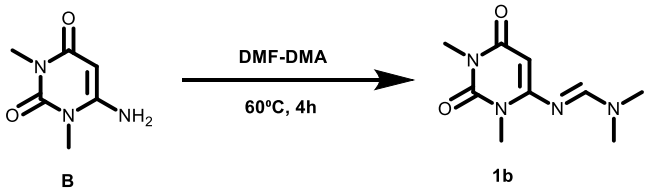
4. Preparation and characterization data of starting materials

4.1 Synthesis of N, N-dimethyl-N'-(3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4 yl)formimidamide (1a):

A mixture of 1-methyl-6-aminouracil (**A**, 10 mmol, 1 equiv.) and DMF-DMA (20 mmol, 2 equiv.) was dissolved in 50 mL of DMF and heated at 40-50ºC for 24h in an oil bath. Then, excess DMF and DMF-DMA were filtered off and washed with diethyl ether, and the crude product was dried in a vacuum oven.[1]

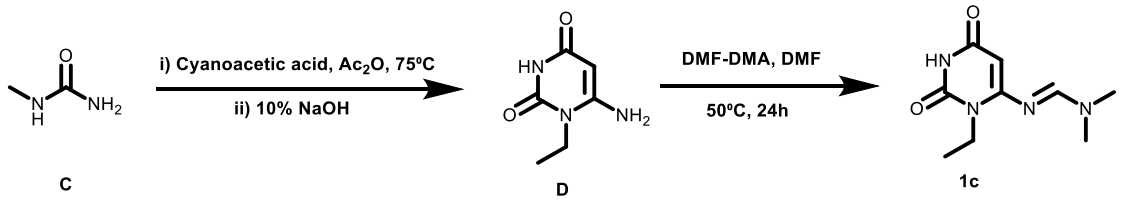
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.62 (s, 1H), 8.02 (s, 1H), 4.95 (s, 1H), 3.20 (s, 3H), 3.09 (s, 3H), 2.97 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 163.61, 161.24, 156.26, 152.33, 82.71, 40.76, 34.86, 29.10.

4.2 Synthesis of N'-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (1b):



A 25 mL round bottom flask was filled with 1,3-dimethyl-6-aminouracil (**B**, 10 mmol), and the flask was immersed in a hot oil bath at 60ºC. Then, 10 ml of DMF-DMA was added dropwise for 30 minutes duration and allowed for another 4 hours at 60ºC. Then it was cooled, and the obtained residue was recrystallized in ethanol.[2]

4.3 Synthesis of N'-(3-ethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (1c):



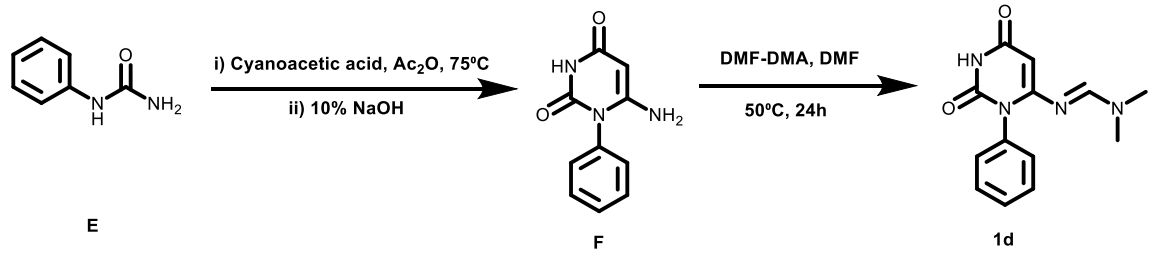
Procedure for D: A mixture of N-ethylurea (**C**, 10 mmol), cyanoacetic acid (10.1 mmol), and 5 ml of acetic anhydride was heated at 75ºC for two hours. The resulting mixture was cooled, and 50 mL of diethyl ether was added and allowed to stand for 2 hours in an ice bath. The resulting precipitate was filtered off and gradually washed with diethyl ether. The resultant air-dried precipitate was dissolved in water/ethanol (2:1),

and the 10% aqueous NaOH solution was continuously added. The mixture was continuously stirred at 85ºC, and the aminouracil precipitate gradually separated. Finally, the obtained crude product (**D**) was filtered off, washed with water, and dried in a vacuum oven.[3]

Procedure for 1c: A mixture of 1-ethyl-6-aminouracil (**D**, 5 mmol, 1equiv.) and DMF-DMA (7.5 mmol, 1.5equiv.) was dissolved in 25 mL of DMF. The mixture was heated at 45-50ºC for 24h in an oil bath. Following this time, excess DMF was dried in a vacuum oven, and diethyl ether was added. The obtained white precipitate (**1c**) was filtered off, washed with diethyl ether, and dried in a vacuum oven.[4]

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 10.59 (s, 1H), 8.06 (s, 1H), 4.96 (s, 1H), 3.90 (q, *J* = 7.0 Hz, 2H), 3.11 (s, 3H), 2.99 (s, 3H), 1.08 (t, *J* = 6.9 Hz, 3H). **^{13}C-NMR** (126 MHz, DMSO-d_6) δ: 163.68, 160.88, 156.42, 151.93, 82.83, 40.77, 36.91, 34.86, 14.30.

4.4 Synthesis of N'-(2,6-dioxo-3-phenyl-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (1d):



Procedure for F: A mixture of N-phenylurea (**E**, 10 mmol), cyanoacetic acid (10.1 mmol), and 5 ml of acetic anhydride was heated at 75ºC for two hours. The resulting mixture was cooled, 50 ml of diethyl ether was added, and it was allowed to stand for 2 hours in an ice bath. The resulting precipitate was filtered off and gradually washed with diethyl ether. The resultant air-dried precipitate was dissolved in water/ethanol (2:1), and the 10% aqueous NaOH solution was continuously added. The mixture was continuously stirred at 85ºC, and the aminouracil precipitate gradually separated. Finally, the obtained crude product (**F**) was filtered off, washed with water, and dried in a vacuum oven.[3]

Procedure for 1d: A mixture of 1-phenyl-6-aminouracil (**F**, 5 mmol, 1 equiv.) and DMF-DMA (7.5 mmol, 1.5 equiv.) was dissolved in 25 mL of DMF. The mixture was heated at 40-50ºC for 24h in an oil bath. Following this time, excess DMF was dried in a vacuum oven, and diethyl was added. The obtained white precipitate (**1d**) was filtered off, washed with diethyl ether, and dried in a vacuum oven.[2]

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 10.74 (s, 1H), 7.92 (s, 1H), 7.32 (dt, *J* = 7.9 Hz, 3H), 7.13 (t, *J* = 5.4 Hz, 2H), 5.06 (s, 1H), 2.95 (s, 3H), 2.48 (s, 3H). **^{13}C-NMR** (126 MHz, DMSO-d_6) δ: 164.08, 161.42, 155.65, 151.98, 137.57, 129.97, 128.70, 127.95, 82.70, 34.34.

5. Falling film looping photo-reactor set up for the photo-induced reactions

The reactions were carried out in a falling film looping photoreactor, designed and developed by our group.[5] It was designed to perform small-scale laboratory experiments in looping falling film batch mode or in only batch mode. It was photonically characterised and allows researchers to optimize a lab-scale reaction to various space velocities and/or photon fluxes (for falling film looping batch operations/batch operations). Thus, also confirms the scalability of the process by using different proportionally scaled versions of the reactor module (1A, 2A, and 4A) and maintaining a constant photon-to-reactant ratio. In this case, we used

only 1A reactor module in batch operation for all the reactions. The reactor mainly contains six different modules: the irradiation module, reactor module, pump module, controller module, cooling module, and case module. In our published research article, we provide all the

Figure S1. Falling film looping photoreactor consisting of a) pump module, b) irradiation module, c) controller module, and d) reactor module.

Table S1. Optical properties of 1A, 2A and 4A irradiation modules with respect to different reactor modules.

Reactor module	Wetted surface A / cm^2	Number of LEDs	Electrical current / A	Current per LED / mA	Total photon flux / µmol · s^{-1}	Total radiant power P/ W	Irradiance E / mW · cm^2
1×A	78.5	40	1	71-77	21	4.71	60.1
		40	2	143-154	38	8.56	109.0
		40	2.5	179-192	46	10.32	131.4
2×A	179.1	91	5	177-183	102	22.81	127.4
4×A	314.2	160	10	185-189	185	41.27	131.4

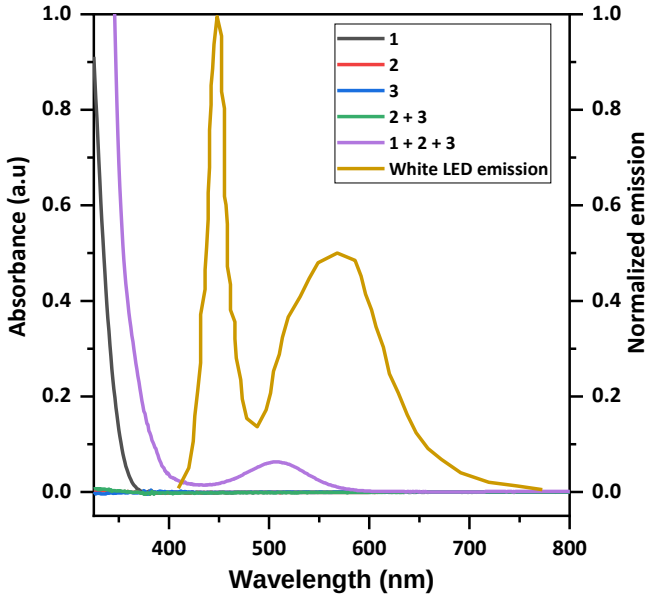


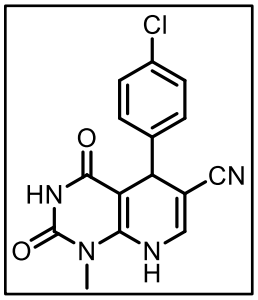
Figure S2. Absorption spectra of starting materials and reaction mixture in acetonitrile solvents, and normalized spectra of the used LED.

6. General procedure and characterization data for the synthesis of pyridopyrimidines/series 4:

Standard procedure: An oven-dried 15 mL glass vial equipped with a magnetic stir bar was charged sequentially with, a mixture of uracil derivatives **1** (1 mmol), aldehyde **2** (1 mmol), and cyanoacetic acid **3** (1 mmol) in CH_3CN (2 mL). The reaction mixture was irradiated with constant 46 µmol.s^{-1} photon flux for 8 hours at room temperature. After the complete consumption of the starting material, (indicated by TLC), the reaction is stopped and further removed under vacuum. The obtained crude product was purified by silica gel column chromatography using an ethyl acetate/hexane mixture (1:1), and separated yields were measured.

5-(4-chlorophenyl)-1-methyl-2,4-dioxo-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4aa)

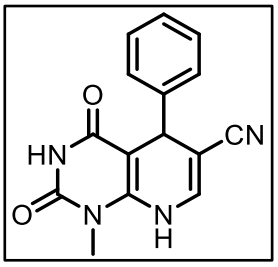
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with 4-chlorobenzaldehyde **2a** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (hexane : EtOAc = 1:1) as a white solid (59 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.04 (s, 1H), 9.82 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.20 (s, 1H), 4.56 (s, 1H), 3.28 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.87, 150.51, 145.56, 144.40, 137.69, 132.00, 129.85, 128.77, 119.41, 89.10, 87.28, 37.96, 29.34. Confirmed by single crystal-XRD.

1-methyl-2,4-dioxo-5-phenyl-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ab)

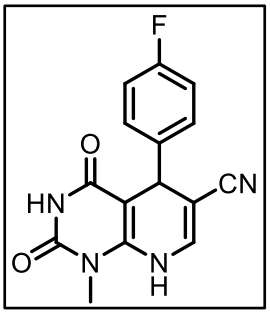
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with benzaldehyde **2b** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and w: as isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (57 % yield).



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.00 (s, 1H), 9.78 (s, 1H), 7.33 – 7.17 (m, 5H), 4.53 (s, 1H), 3.29 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.88, 150.54, 145.50, 145.44, 137.47, 128.83, 127.90, 127.40, 119.59, 89.56, 87.59, 38.38, 29.32. HRMS(ESI): Calculated for $C_{15}H_{13}N_4O_2$ $[M+H]^+$: 281.1039, found: 281.1045.

5-(4-fluorophenyl)-1-methyl-2,4-dioxo-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ac)

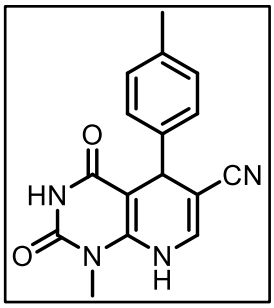
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with 4-fluorobenzaldehyde **2c** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (55 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.02 (s, 1H), 9.80 (s, 1H), 7.32 – 7.29 (m, 2H), 7.19 (s, 1H), 7.13 (t, *J* = 8.8 Hz, 2H), 4.55 (s, 1H), 3.28 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.87, 161.16, 150.53, 150.08, 137.52, 129.85, 129.81, 119.50, 115.61, 87.55, 85.10, 37.73, 29.32. HRMS(ESI): Calculated for $C_{15}H_{12}FN_4O_2$ $[M+H]^+$: 299.0944, found: 299.0941.

1-methyl-2,4-dioxo-5-(p-tolyl)-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ad)

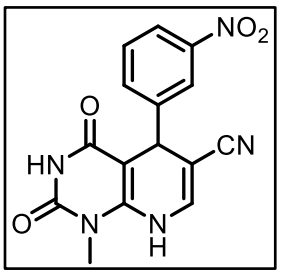
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with 4-methylbenzaldehyde **2d** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (49 % yield).



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 10.98 (s, 1H), 9.75 (s, 1H), 7.16-7.10 (m, 5H), 4.47 (s, 1H), 3.28 (s, 3H), 2.26 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 166.13, 161.88, 150.54, 145.38, 142.64, 137.28, 136.50, 129.36, 127.81, 119.64, 89.70, 87.72, 37.99, 29.30, 25.37, 21.11.

1-methyl-5-(3-nitrophenyl)-2,4-dioxo-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ae)

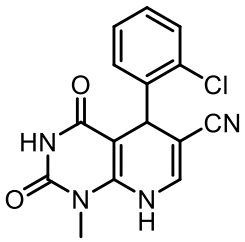
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with 3-methylbenzaldehyde **2e** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (61 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.08 (s, 1H), 9.92 (s, 1H), 8.12 (d, J = 6.7 Hz, 2H), 7.80 (d, J = 7.8 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.29 (s, 1H), 4.80 (s, 1H), 3.29 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.90, 150.51, 148.34, 147.40, 145.78, 138.41, 135.00, 130.45, 122.58, 122.44, 119.23, 88.42, 86.96, 38.34, 29.42. HRMS(ESI): Calculated for $C_{15}H_{12}N_5O_4$ $[M+H]^+$: 326.0889, found: 326.0892.

1-methyl-5-(2-nitrophenyl)-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4af)

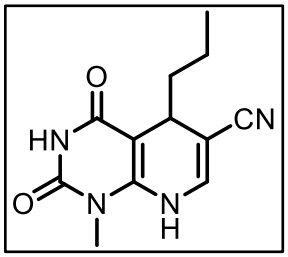
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with 2-nitrobenzaldehyde **2f** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (57 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.00 (s, 1H), 7.40-7.39 (m, 1H), 7.38-7.37 (m, 1H), 7.32-7.29 (m, 1H), 7.25 – 7.22 (m, 1H), 7.14 (s, 1H), 5.09 (s, 1H), 3.29 (s, 3H); **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.74, 150.62, 146.28, 138.14, 132.00, 131.19, 129.59, 129.06, 128.27, 88.29, 87.35, 29.41.

1-methyl-2,4-dioxo-5-propyl-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ag)

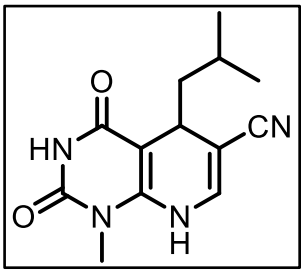
The title compound was synthesized from the reaction of uracil derivatives **1a** (1 mmol, 1 equiv.) with butyraldehyde **2g** (1 mmol, 1 equiv.) and cyanoacetic acid **3** (1 mmol, 1 equiv.) according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (29 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.03 (s, 1H), 9.00 (s, 1H), 7.16 (s, 1H), 3.94 (t, 1H), 3.22 (s, 3H), 1.64 – 1.57 (m, 2H), 1.33 – 1.22 (m, 2H), 0.86 (t, J = 7.1 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 165.86, 162.15, 150.49, 146.07, 139.23, 120.01, 117.76, 87.87, 86.63, 37.72, 32.14, 29.20, 26.43, 17.96, 14.48. HRMS(ESI): Calculated for $C_{12}H_{15}N_4O_2$ $[M+H]^+$: 247.1195, found: 247.1191.

5-isobutyl-1-methyl-2,4-dioxo-1,2,3,4,5,6,7,8-octahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ah)

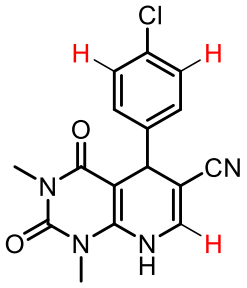
The title compound was synthesized from the reaction of uracil derivatives **1a** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (30 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.01 (s, 1H), 9.68 (s, 1H), 7.12 (s, 1H), 3.47 (t, J = 5.0 Hz, 1H), 3.21 (s, 3H), 1.78 (tt, J = 13.0, 6.5 Hz, 1H), 1.46 – 1.36 (m, 1H), 1.26 – 1.19 (m, 1H), 0.90 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 6.6 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 166.12, 162.07, 150.48, 145.70, 139.06, 120.28, 116.29, 88.48, 87.75, 46.89, 30.02, 29.15, 25.27, 24.20, 24.05, 22.62.

5-(4-chlorophenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ba)

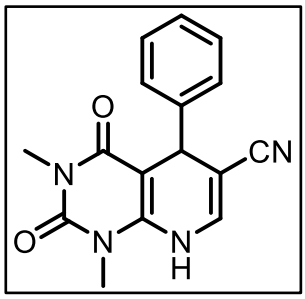
The title compound was synthesized from the reaction of uracil derivatives **1b** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (64 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.15 (s, 1H), 7.55 (d, J = 8.4 Hz, 3H, merge two types of protons (red colour)), 7.34 (d, J = 6.7 Hz, 2H), 3.62 (s, 3H), 3.15 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ: 161.09, 150.90, 144.37, 139.27, 137.59, 129.92, 129.05, 128.76, 127.98, 119.35, 89.27, 86.85, 35.42, 30.37, 28.05. HRMS(ESI): Calculated for $C_{16}H_{14}ClN_4O_2$ $[M+H]^+$: 329.0805, found: 329.0807.

1,3-dimethyl-2,4-dioxo-5-phenyl-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4bb).

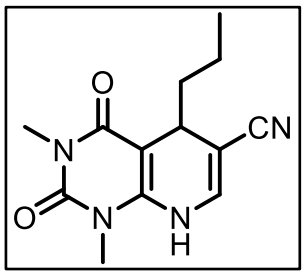
The title compound was synthesized from the reaction of uracil derivatives **1b** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (59 % yield).



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 9.88 (s, 1H), 7.32 – 7.25 (m, 5H), 7.16 (s, 1H), 4.55 (s, 1H), 3.35 (s, 3H), 3.05 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 150.95, 148.39, 143.02, 135.14, 134.25, 130.51, 122.68, 122.60, 121.91, 88.66, 86.60, 35.84, 30.51, 28.15.

1,3-dimethyl-2,4-dioxo-5-propyl-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4bc)

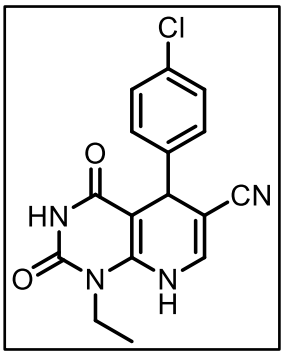
The title compound was synthesized from the reaction of uracil derivatives **1b** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (31 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.59 (s, 1H), 7.16 (s, 1H), 3.58 – 3.55 (m, 1H), 3.29 (s, 3H), 3.14 (s, 3H), 1.65 – 1.58 (m, 2H), 1.38 – 1.25 (m, 2H), 0.86 (t, J = 7.2 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.41, 150.91, 144.83, 139.14, 120.00, 88.10, 86.21, 37.70, 32.61, 30.26, 28.23, 18.01, 14.52. HRMS(ESI): Calculated for $C_{13}H_{17}N_4O_2$ $[M+H]^+$: 261.1352, found: 261.1355.

5-(4-chlorophenyl)-1-ethyl-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ca)

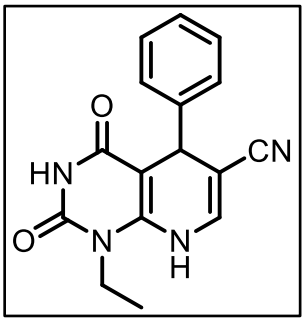
The title compound was synthesized from the reaction of uracil derivatives **1c** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (57 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.14 (s, 1H), 7.54 (d, *J* = 8.5 Hz, 3H), 7.36 (d, *J* = 8.5 Hz, 2H), 4.55 (s, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 1.22 (t, *J* = 7.0 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 159.88, 156.47, 155.26, 150.21, 134.09, 130.11, 129.60, 128.51, 116.34, 109.86, 106.09, 37.70, 13.33.

1-ethyl-2,4-dioxo-5-phenyl-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4cb)

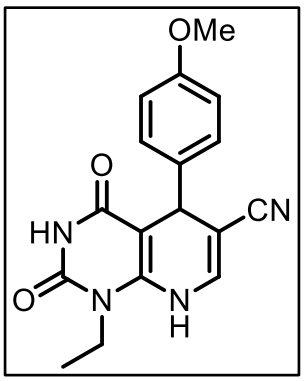
The title compound was synthesized from the reaction of uracil derivatives **1c** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (58 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.03 (s, 1H), 9.78 (s, 1H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.26 – 7.17 (m, 4H), 4.52 (s, 1H), 3.87 (q, J = 9.6 Hz, 2H), 1.14 (t, J = 6.9 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.88, 150.21, 145.41, 144.57, 137.49, 128.88, 127.84, 127.42, 119.54, 89.44, 87.87, 38.35, 37.18, 14.08. HRMS(ESI): Calculated for $C_{16}H_{15}N_4O_2$ $[M+H]^+$: 295.1195, found: 295.1194.

1-ethyl-5-(4-methoxyphenyl)-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4cc)

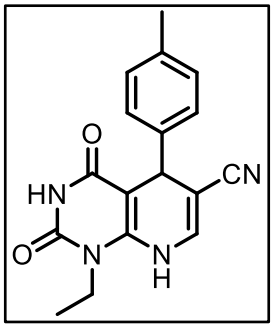
The title compound was synthesized from the reaction of uracil derivatives **1c** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (51 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.00 (s, 1H), 9.74 (s, 1H), 7.16 (d, *J* = 1.4 Hz, 2H), 7.14 (s,1H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.46 (s, 1H), 3.87 (dd, *J* = 11.6, 7.1 Hz, 2H), 3.72 (s, 3H), 1.13 (t, *J* = 7.0 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.99, 160.20, 159.86, 158.75, 144.39, 130.02, 128.99, 128.07, 119.70, 89.73, 88.25, 55.55, 37.52, 37.23, 14.08. HRMS(ESI): Calculated for $C_{17}H_{17}N_4O_3$ $[M+H]^+$: 325.1301, found: 325.1306.

1-ethyl-2,4-dioxo-5-(p-tolyl)-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4cd)

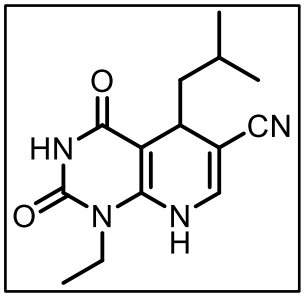
The title compound was synthesized from the reaction of uracil derivatives **1c** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (54 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.01 (s, 1H), 9.77 (s, 1H), 7.28 – 7.12 (m, 5H), 4.47 (s, 1H), 3.86 (q, J = 7.5 Hz, 2H), 2.26 (s, 3H), 1.13 (t, J = 6.9 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.87, 150.21, 144.43, 142.58, 137.30, 129.42, 128.87, 127.75, 119.59, 89.58, 88.03, 37.95, 37.15, 21.10, 14.07. HRMS(ESI): Calculated for $C_{17}H_{17}N_4O_2$ $[M+H]^+$: 309.1352, found: 309.1355.

1-ethyl-5-isobutyl-2,4-dioxo-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4ce)

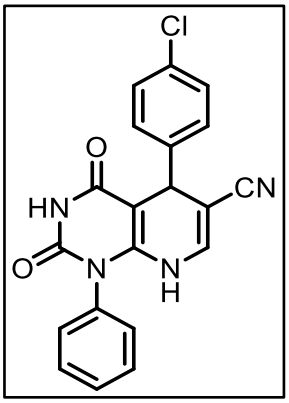
The title compound was synthesized from the reaction of uracil derivatives **1c** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (28 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.03 (s, 1H), 9.00 (s, 1H), 7.12 (s, 1H), 3.82 (q, J = 7.0 Hz, 2H), 3.49 (dd, J = 7.7 Hz, 1H), 1.23-1.17 (m, 6H), 1.06 (t, J = 7.0 Hz, 2H), 0.97-0.89 (m, 2H), 0.85 (d, J = 6.5 Hz, 2H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 162.06, 150.18, 144.90, 139.24, 120.30, 88.23, 87.99, 46.70, 36.97, 30.02, 24.02, 22.62, 14.04.

5-(4-chlorophenyl)-2,4-dioxo-1-phenyl-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4da)

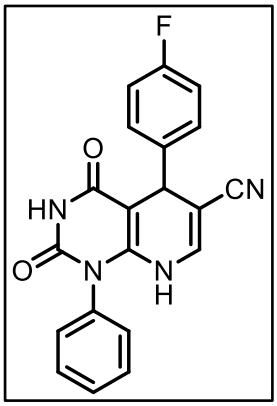
The title compound was synthesized from the reaction of uracil derivatives **1d** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (57 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 8.52 (s, 1H), 7.55 (m, 5H), 7.42-7.36 (m, 4H), 6.89 (d, *J* = 5.3 Hz, 1H), 4.62 (s, 1H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 162.32, 150.25, 144.94, 144.24, 137.72, 133.60, 132.06, 130.41, 129.98, 128.83, 119.39, 88.77, 87.21, 38.20. HRMS(ESI): Calculated for $C_{20}H_{14}ClN_4O_2$ $[M+H]^+$: 377.0805, found: 377.0807.

5-(4-fluorophenyl)-2,4-dioxo-1-phenyl-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4db)

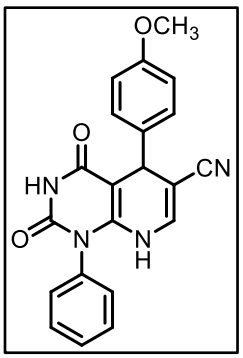
The title compound was synthesized from the reaction of uracil derivatives **1d** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (58 % yield).



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.18 (s, 1H), 8.50 (s, 1H), 7.58 – 7.42 (m, 5H), 7.39 – 7.35 (m, 2H), 7.16 (t, *J* = 8.8 Hz, 2H), 6.87 (s, 1H), 4.62 (s, 1H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 162.41, 150.31, 144.93, 142.36, 137.57, 133.61, 130.46, 130.28, 130.06, 129.99, 119.52, 115.71, 115.54, 89.14, 87.51, 38.01.

5-(4-methoxyphenyl)-2,4-dioxo-1-phenyl-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine-6-carbonitrile (4dc)

The title compound was synthesized from the reaction of uracil derivatives **1d** according to the general procedure **A** and was isolated by column chromatography eluting with hexane and ethyl acetate (EtOAc: hexane = 1:1) as a white solid (48 % yield).



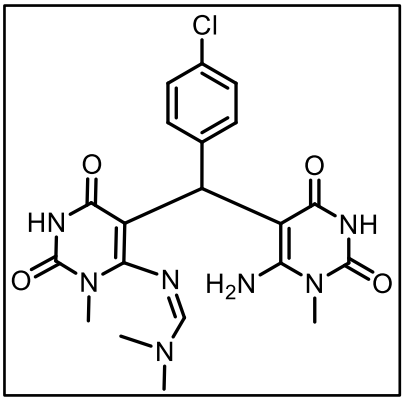
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.21 (s, 1H), 8.53 (s, 1H), 7.57-7.34 (m, 9H), 6.88 (s, 1H), 4.58 (s, 1H), 3.80 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 162.35, 150.28, 145.30, 144.89, 137.48, 133.61, 130.44, 128.91, 128.01, 127.48, 119.58, 116.39, 89.24, 87.48, 38.63. HRMS(ESI): Calculated for $C_{21}H_{17}N_4O_3$ $[M+H]^+$: 373.1301, found: 373.1303.

7. General procedure and characterization data of series 5

A mixture of Uracil derivatives **1** (1 mmol), Aldehydes **2** (1 mmol), and Cyanoacetic acid **3** (2 mmol) were mixed in a minimum amount of CH_3CN to get a paste-like mixture with constant stirring at room temperature under dark conditions for 10 hours. After completion of the reaction (monitored by TLC), the excess solvent was removed under vacuum, and the obtained product was purified by column chromatography.

N'-(5-((6-amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-chlorophenyl)methyl)-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5aa).

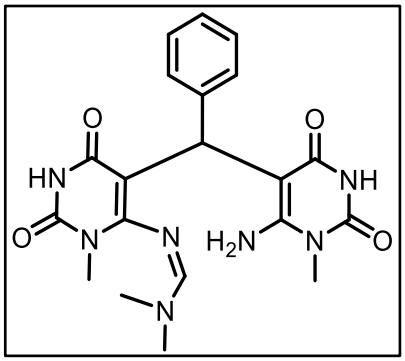
Standard procedure B: The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.,), 4-chlorobenzaldehyde (**2a**, 1 mmol, 1.00 equiv.,) and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.,). The obtained crude product was purified by silica gel column chromatography (9.5:0.5) (Ethyl acetate: hexane) afforded **5aa** (65%) as a white solid.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.08 (s, 1H), 10.51 (s, 1H), 7.26 (br, 3H, merge), 7.19 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.3 Hz, 2H), 5.41 (s, 1H), 3.24 (s, 3H), 3.15 (s, 3H), 2.88 (s, 6H). **^{13}C-NMR** (126 MHz, DMSO-d_6) δ: 166.16, 165.89, 162.92, 155.57, 154.39, 150.85, 150.64, 142.22, 137.55, 135.14, 130.34, 130.14, 129.81, 129.73, 129.23, 128.99, 128.26, 128.10, 99.97, 87.37, 65.45, 35.89, 33.78, 25.30. HRMS(ESI): Calculated for $C_{20}H_{23}ClN_7O_4$ $[M+H]^+$: 460.1500, found: 460.1527.

N'-(5-((6-amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methyl)-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5ab).

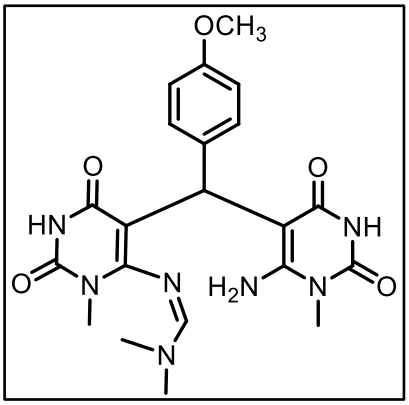
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.,), benzaldehyde (**2b**, 1 mmol, 1.00 equiv.,), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.,). The obtained crude product was purified by silica gel column chromatography (9.5:0.5) (Ethyl acetate: hexane) afforded **5ab** (67 %) as a white solid.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.02 (s, 1H), 10.47 (s, 1H), 7.25 (s, 3H, merge), 7.15 (t, *J* = 7.6 Hz, 2H), 7.04 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 8.2 Hz, 2H), 5.46 (s, 1H), 3.25 (s, 3H), 3.16 (s, 3H), 2.87 (s, 3H), 2.87 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 163.66, 162.52, 161.30, 156.31, 153.46, 152.39, 151.01, 145.18, 126.79, 124.63, 123.70, 86.11, 82.79, 40.82, 34.92, 29.32, 29.16.

N'-(5-((6-amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4 methoxyphenyl)methyl)-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5ac).

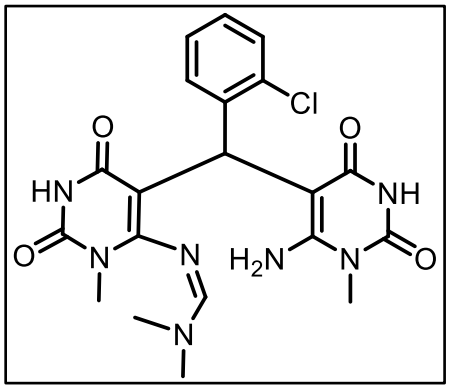
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.), 4-methoxybenzaldehyde (**2d**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). The obtained crude product was purified by silica gel column chromatography (9.5:0.5) (Ethyl acetate: hexane) afforded **5ac** (61 %) as a white solid.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.50 (s, 1H), 11.28 (s, 1H), 7.32 (br, 3H, merge), 6.92 (d, *J* = 9.5 Hz, 2H), 6.74 (d, *J* = 9.1 Hz, 2H), 5.30 (s, 1H), 3.69 (s, 3H), 3.15 (s, 3H), 3.05 (m, 3H), 2.89 (m, 3H), 2.78 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 165.81, 163.96, 162.04, 159.95, 157.71, 155.11, 146.22, 133.91, 128.91, 127.60, 113.99, 108.68, 81.38, 55.50, 34.22, 34.13, 31.27, 30.64. HRMS(ESI): Calculated for $C_{21}H_{26}N_7O_5$ $[M+H]^+$: 456.1995, found: 456.1992.

N'-(5-((6-amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(2-chlorophenyl)methyl)-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5ad).

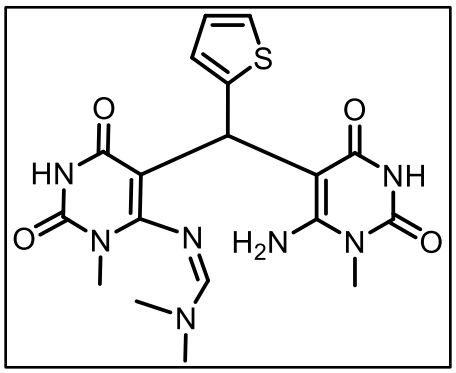
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.), 2-chlorobenzaldehyde (**2f**, 1 mmol, 1.00 equiv.,), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.,). The obtained crude product was purified by silica gel column chromatography (9.5:0.5) (Ethyl acetate: hexane) afforded **5ad** (60 %) as a white solid.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.94 (s, 1H), 10.51 (s, 1H), 7.23 – 7.09 (m, 7H, merge), 5.37 (s, 1H), 3.27 (s, 3H), 3.13 (s, 3H), 2.87 (s, 3H), 2.85 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 165.58, 163.22, 162.28, 155.40, 155.04, 151.07, 150.88, 142.28, 129.69, 129.64, 128.90, 128.00, 99.63, 87.89, 36.07, 34.11, 30.37, 29.15, 28.27.

N'-(5-((6-amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(thiophen-2-yl)methyl)-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5ae).

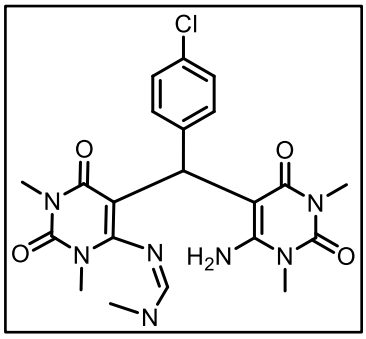
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.), thiophene-2-carbaldehyde (**2g**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). The obtained crude product was purified by silica gel column chromatography (9.5:0.5) (Ethyl acetate: hexane) afforded **5ae** (55 %) as a white solid.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.41 (s, 1H), 11.04 (s, 1H), 7.52 (s, 1H), 7.41 (s, 2H), 7.25 (d, *J* = 4.9 Hz, 1H), 6.86 – 6.83 (m, 1H), 6.71 (d, *J* = 3.3 Hz, 1H), 5.54 (s, 1H), 3.15 (s, 3H), 3.09 (s, 3H), 2.93 (s, 3H), 2.86 (s, 3H).

N'-(5-((6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-chlorophenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5ba).

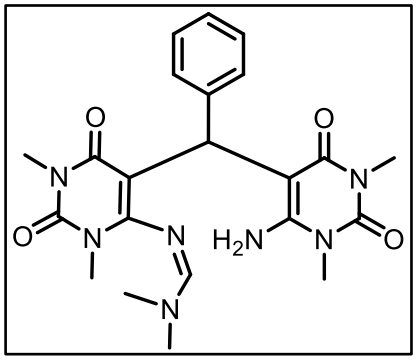
The reaction was performed according to the general procedure employing amide (**1b**, 1 mmol, 1.00 equiv.), 4-chlorobenzaldehyde (**2a**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification of the resulting reaction mixture by silica gel column chromatography (7:3) (EtOAc: hexane) afforded **5ba** (68 %) as a solid white precipitate.



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.44 (s, 1H), 7.23 – 7.21 (m, 4H), 7.07 (d, *J* = 8.4 Hz, 2H), 5.43 (s, 1H), 3.23 (s, 3H), 3.22 (s, 3H), 3.21 (s, 3H), 3.14 (s, 3H), 2.90 (s, 3H), 2.76 (s, 3H).

N'-(5-((6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5bb).

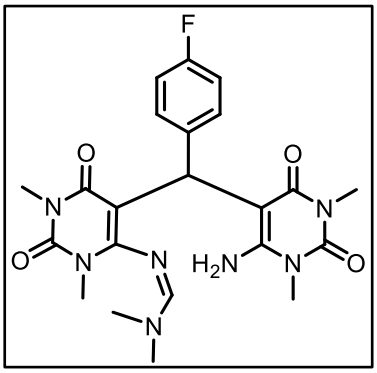
The reaction was performed according to the general procedure employing amide (**1b**, 1 mmol, 1.00 equiv.), benzaldehyde (**2b**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification of the resulting reaction mixture by silica gel column chromatography (7:3) (EtOAc: hexane) afforded **5bb** (70 %) as a white solid.



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.25 (s, 1H), 7.21-7.18 (m, 3H), 7.14 – 7.04 (m, 4H), 5.45 (s, 1H), 3.24 (s, 3H), 3.23 (s, 3H), 3.21 (s, 3H), 3.13 (s, 3H), 2.91 (s, 3H), 2.69 (s, 3H).

N'-(5-((6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-fluorophenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5bc)

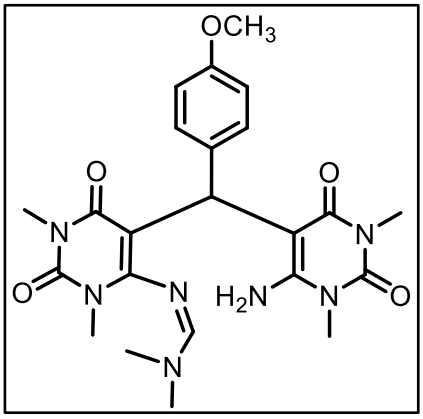
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.), 4-fluorobenzalaldehyde (**2e**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (7:3) (EtOAc: hexane) afforded **5bc** (66 %) as a white solid.



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.41 (s, 1H), 7.20 (s, 1H), 7.09 – 7.06 (m, 2H), 6.99 (t, J = 8.8 Hz, 2H), 5.42 (s, 1H), 3.23 (s, 3H), 3.22 (s, 3H), 3.21 (s, 3H), 3.13 (s, 3H), 2.91 (s, 3H), 2.74 (s, 3H).

N'-(5-((6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-methoxyphenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5bd)

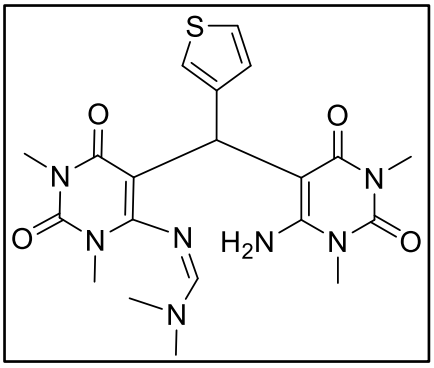
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.), 4-methoxybenzaldehyde (**2d**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (7:3) (EtOAc: hexane) afforded **5bd** (61 %) as a white solid.



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.35 (s, 1H), 7.20 (s, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 5.38 (s, 1H), 3.69 (s, 3H), 3.24 (s, 3H), 3.22 (s, 3H), 3.21 (s, 3H), 3.13 (s, 3H), 2.92 (s, 3H), 2.73 (s, 3H).

N'-(5-((6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(thiophen-3-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5be).

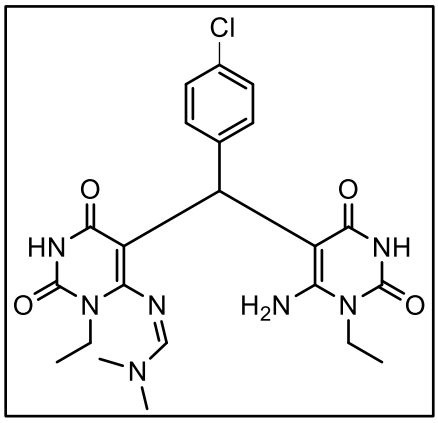
The reaction was performed according to the general procedure employing amide (**1a**, 1 mmol, 1.00 equiv.), aldehyde (**2a**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (7:3) (EtOAc: hexane) afforded **5be** (65 %) as a solid white precipitate.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.46 (s, 1H), 7.35 (dd, *J* = 4.9, 2.9 Hz, 1H), 7.30 (s, 1H), 7.09 – 7.03 (m, 1H), 6.74 (d, *J* = 4.9 Hz, 1H), 5.36 (d, *J* = 0.7 Hz, 1H), 3.24 (s, 3H), 3.22 (s, 3H), 3.20 (s, 3H), 3.15 (s, 3H), 2.94 (s, 3H), 2.79 (s, 3H).

N'-(5-((6-amino-1-ethyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-chlorophenyl)methyl)-3-ethyl-1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5ca).

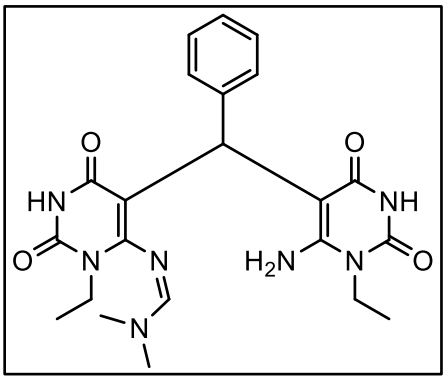
The reaction was performed according to the general procedure employing amide (**1c**, 1 mmol, 1.00 equiv.), aldehyde (**2a**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded **5ca** (59 %) as a solid white precipitate.



^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, 1H), 10.49 (s, 1H), 7.30 (s, 2H), 7.26 (s, 1H), 7.20 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.5 Hz, 2H), 5.42 (s, 1H), 3.92 – 3.87 (m, 2H), 3.79 – 3.74 (m, 2H), 2.89 (s, 3H), 2.88 (s, 3H), 1.14 (t, J = 7.0 Hz, 3H), 1.08 (t, J = 7.0 Hz, 3H).

N'-(5-((6-amino-1-ethyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methyl)-3-ethyl-1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5cb).

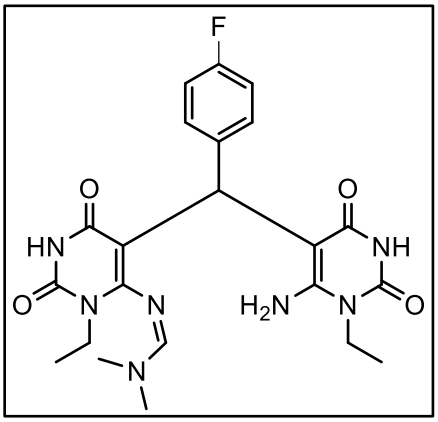
The reaction was performed according to the general procedure employing amide (**1c**, 1 mmol, 1.00 equiv.), aldehyde (**2b**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded **5cb** (61 %) as a solid white precipitate.



^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.03 (s, 1H), 10.48 (s, 1H), 7.33 (s, 2H), 7.26 (s, 1H), 7.16 (t, *J* = 7.7 Hz, 2H), 7.04 (t, *J* = 7.3 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 2H), 5.45 (s, 1H), 3.94-3.87 (m, 2H), 3.78-3.74 (m, 2H), 2.88 (s, 3H), 2.87 (s, 3H), 1.14 (t, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).

N'-(5-((6-amino-1-ethyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-fluorophenyl)methyl)-3-ethyl-1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5cc)

The reaction was performed according to the general procedure employing amide (**1c**, 1 mmol, 1.00 equiv.), aldehyde (**2c**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded **5cc** (62 %) as a solid white precipitate.

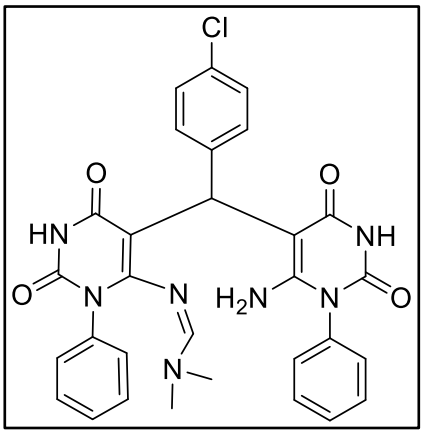


^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.02 (s, 1H), 10.47 (s, 1H), 7.30 (s, 2H), 7.26 (s, 1H), 6.94 (m, 4H), 5.41 (s, 1H), 3.93 – 3.87 (m, 2H), 3.78 – 3.74 (m, 2H), 2.89 (s, 3H), 2.88 (s, 3H), 1.14 (t, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 165.69, 163.08, 162.38, 161.53, 159.62, 155.51, 154.26, 150.70, 139.00, 128.61, 128.55, 114.88, 114.71, 99.93, 88.31, 38.16, 36.95, 36.02, 34.14, 14.15, 13.75.

N'-(5-((6-amino-2,4-dioxo-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)(4-chlorophenyl)methyl)-2,6-dioxo-3-phenyl-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5da)

The reaction was performed according to the general procedure employing amide (**1d**, 1 mmol, 1.00 equiv.), aldehyde (**2a**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded **5da** (58 %) as a solid white precipitate.

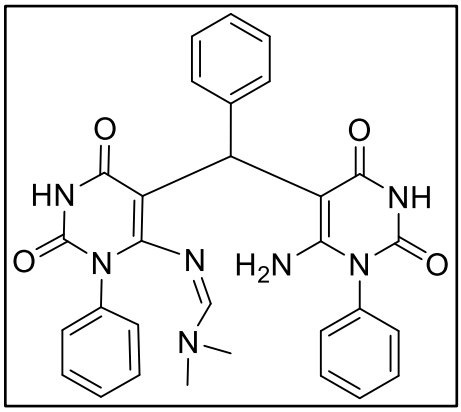
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.92 (br, 2H), 7.57-7.50 (m, 6H), 7.43 (br, 4H), 7.31-7.27 (m, 4H), 6.68 (br, 2H), 5.49 (s, 1H), 3.85 (s, 3H) **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 165.67, 163.90, 163.18, 162.38, 155.51, 154.27, 150.72, 150.69, 142.24, 134.03, 133.40, 132.84, 131.66, 131.28, 130.83, 130.22, 129.74, 129.49, 128.84, 128.13, 99.71, 87.94, 36.96, 35.18, 34.15.



N'-(5-((6-amino-2,4-dioxo-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methyl)-2,6-dioxo-3-phenyl-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5db).

The reaction was performed according to the general procedure employing amide (**1d**, 1 mmol, 1.00 equiv.), aldehyde (**2b**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (3, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded **5da** (58 %) as a solid white precipitate.

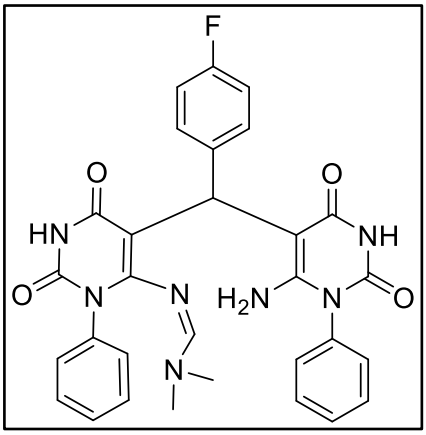
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.87 (br, 2H), 7.57-7.49 (m, 6H), 7.43 (d, *J* = 7.1 Hz. 4H), 7.29 – 7.23 (m, 5H), 7.11 (t, *J* = 7.1 Hz, 1H), 6.67 (br, 2H), 5.53 (s, 1H), 1.99 (s, 3H), 1.91 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 161.66, 160.10, 157.53, 157.38, 155.44, 154.19, 152.28, 150.36, 130.50, 130.47, 130.41, 130.10, 130.00, 129.93, 128.26, 127.24, 106.87, 70.28, 34.12, 32.37, 21.58.



N'-(5-((6-amino-2,4-dioxo-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)(4-fluorophenyl)methyl)-2,6-dioxo-3-phenyl-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5dc).

The reaction was performed according to the general procedure employing amide (**1d**, 1 mmol, 1.00 equiv.), aldehyde (**2c**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 170 mg, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (8:2) (EtOAc: hexane) afforded **5dc** (61 %) as a solid white precipitate.

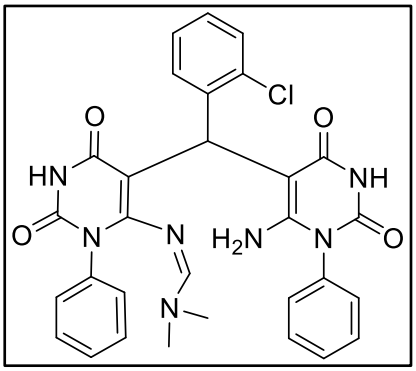
^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.16 (s, 1H), 10.63 (s, 1H), 7.56-7.46 (m, 4H), 7.44-7.38 (m, 3H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.15-7.12 (m, 4H, merge), 7.00 (t, *J* = 8.8 Hz, 2H), 6.60 (br, 2H), 5.61 (s, 1H), 2.73 (s, 3H), 2.40 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 172.72, 165.89, 162.93, 159.72, 155.55, 154.41, 150.89, 150.67, 138.93, 137.47, 135.05, 130.37, 130.30, 130.19, 130.14, 130.08, 129.80, 129.04, 128.97, 128.90, 128.82, 128.29, 100.21, 87.70, 35.68, 33.75, 21.57.



N'-(5-((6-amino-2,4-dioxo-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)(2-chlorophenyl)methyl)-2,6-dioxo-3-phenyl-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5dd).

The reaction was performed according to the general procedure employing amide (**1d**, 1 mmol, 1.00 equiv.), aldehyde (**2d**, 141mg, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 170 mg, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded 5dd (55 %) as a solid white precipitate.

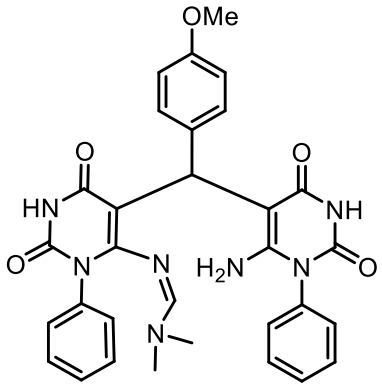
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.03 (s, 1H), 10.76 (s, 1H), 7.56 – 7.18 (m, 15H, merge), 6.32 (br, 2H), 5.49 (s, 1H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 162.77, 162.23, 159.65, 157.82, 156.39, 152.19, 151.46, 133.62, 129.40, 124.73, 113.86, 86.11, 82.17, 40.84, 34.94, 30.34, 30.11, 28.10, 27.63.



N'-(5-((6-amino-2,4-dioxo-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)(2-chloro-4-methoxyphenyl)methyl)-2,6-dioxo-3-phenyl-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (5de).

The reaction was performed according to the general procedure employing amide (**1d**, 1 mmol, 1.00 equiv.), aldehyde (**2e**, 1 mmol, 1.00 equiv.), and cyanoacetic acid (**3**, 2.00 mmol, 2.00 equiv.). Purification by silica gel column chromatography (9:1) (EtOAc: hexane) afforded **5de** (58 %) as a solid white precipitate.

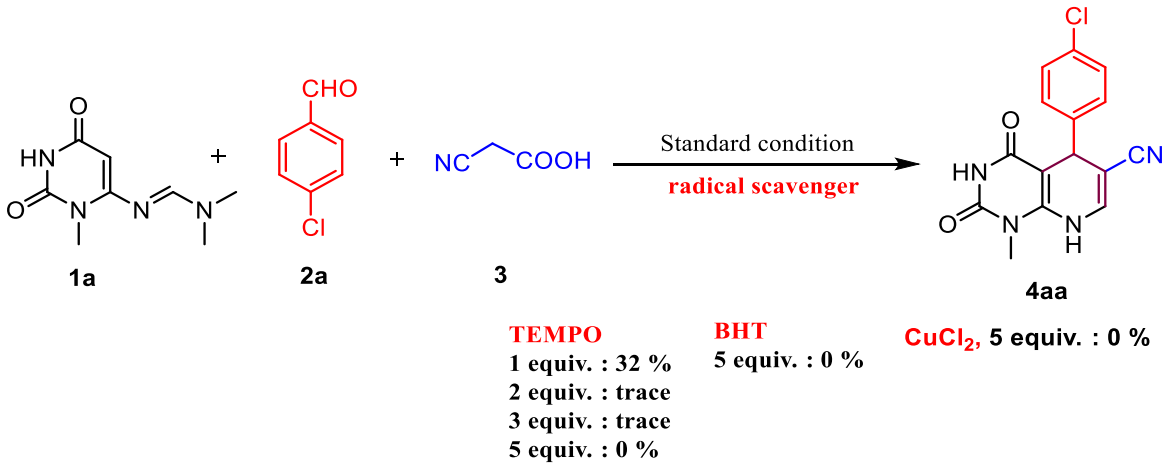
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.62 (s, 1H), 11.36 (d, 1H), 7.40 – 7.14 (m, 13H, merge), 7.02 (d, *J* = 8.4 Hz, 2H), 6.73 (d, J = 8.6Hz, 2H), 5.46 (s, 1H), 3.66 (s, 3H), 2.62 (s, 3H), 2.37 (s, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 162.76, 162.06, 159.64, 156.39, 155.10, 152.07, 142.06, 129.17, 125.64, 120.66, 85.64, 82.18, 40.84, 34.94, 30.32, 27.63.



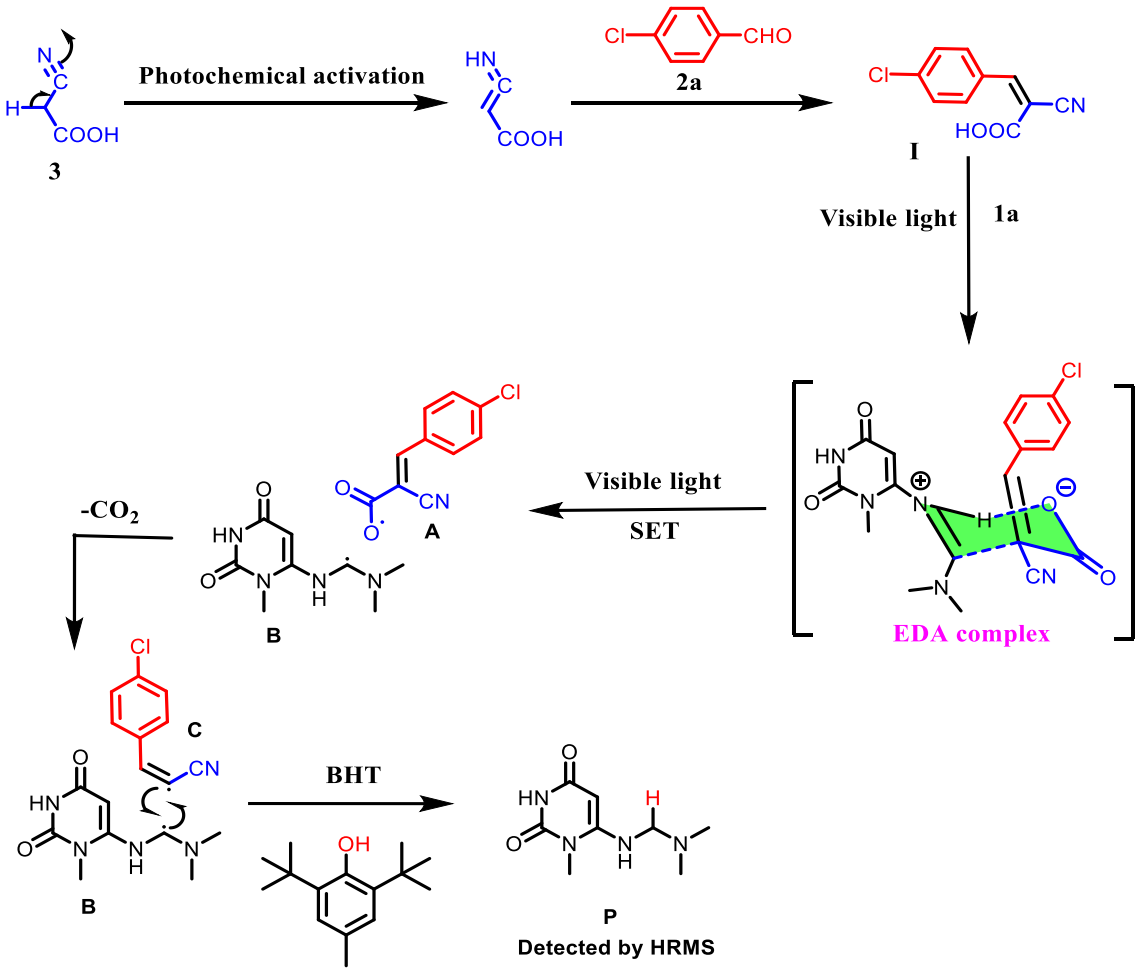
8. Controlled reaction for the mechanism study

8.1 Radical trapping reaction

To more insight into the mechanism, the reaction was further investigated under different radical trapping reagents. Initially, we carried out the reaction with 1 equivalent of TEMPO and the reaction was inhibited by 32%. However, the reaction was totally inhibited by 5 equivalents of TEMPO. The reaction was also inhibited by 5 equivalents of BHT. Therefore, the formation of the product **4aa** was initiated through a radical-mediated pathway. Further, a single electron scavenger $CuCl_2$, inhibit the reaction, which proved that reaction proceed through a SET process.



Further, BHT trapped intermediates were detected by HRMS. In the radical trapping reaction, the intermediate **B** and **C** react with BHT and generate their hydrogenated product **P** and **Q**, which inhibit the formation of product **4aa**.



Scheme S1. Radical trapping reaction with BHT.

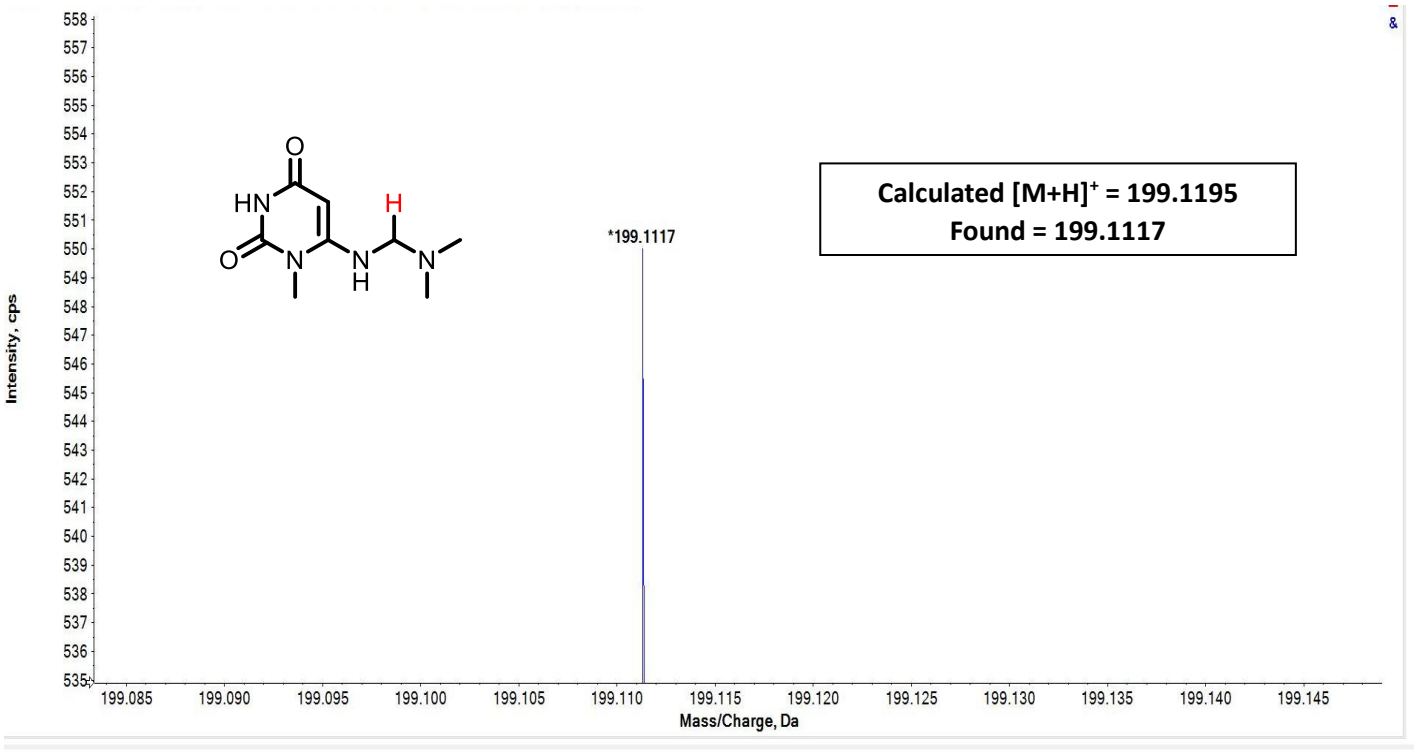


Figure S3. HRMS analysis for radical trapping reaction with BHT.

8.2 UV-visible analysis

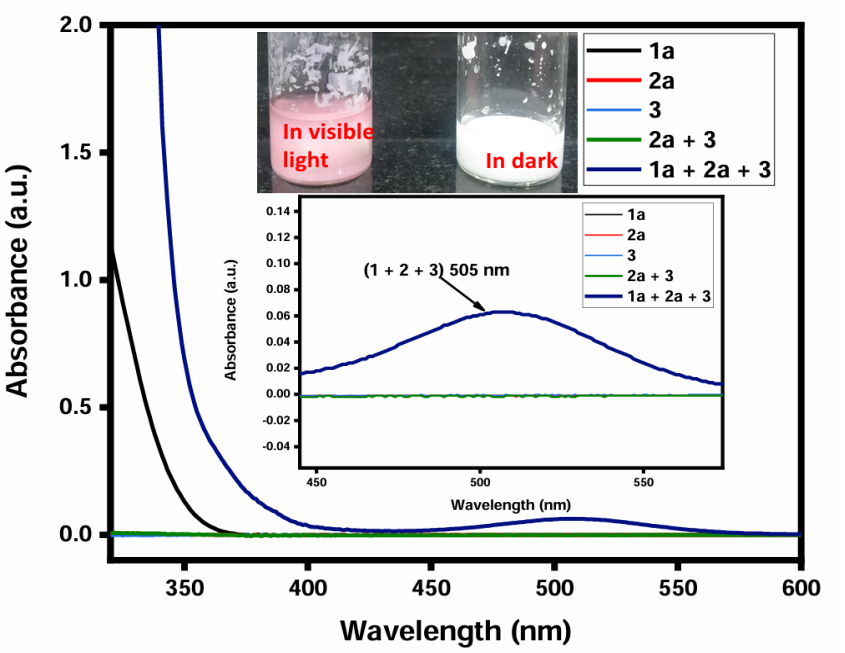
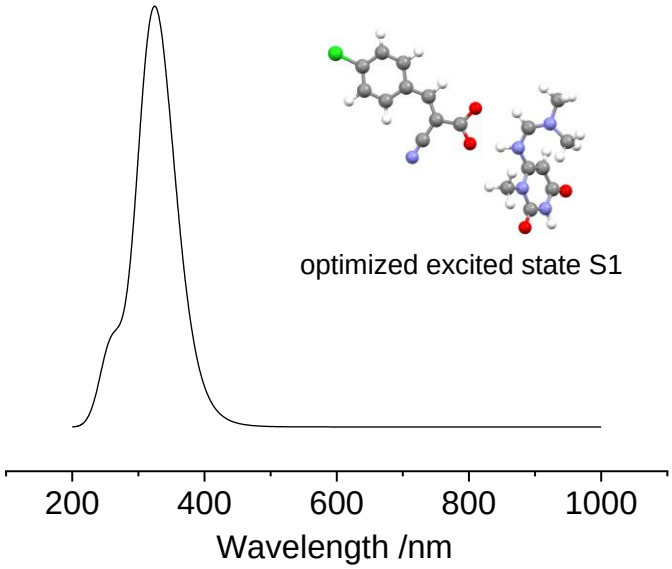
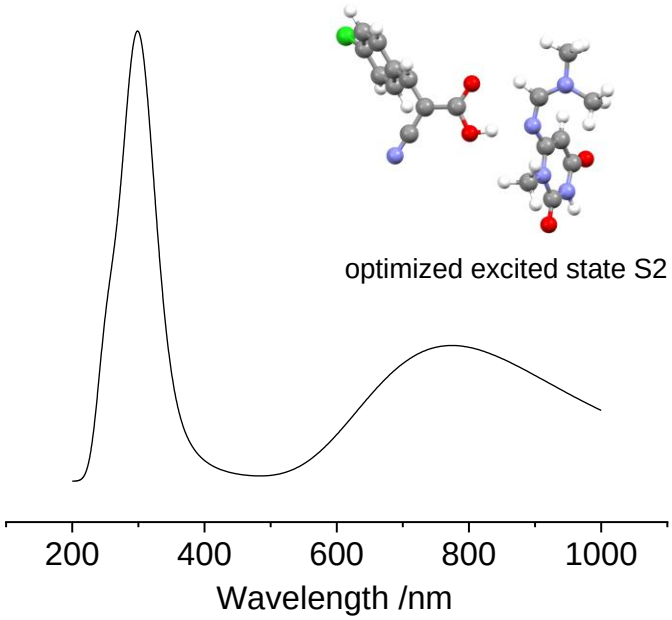
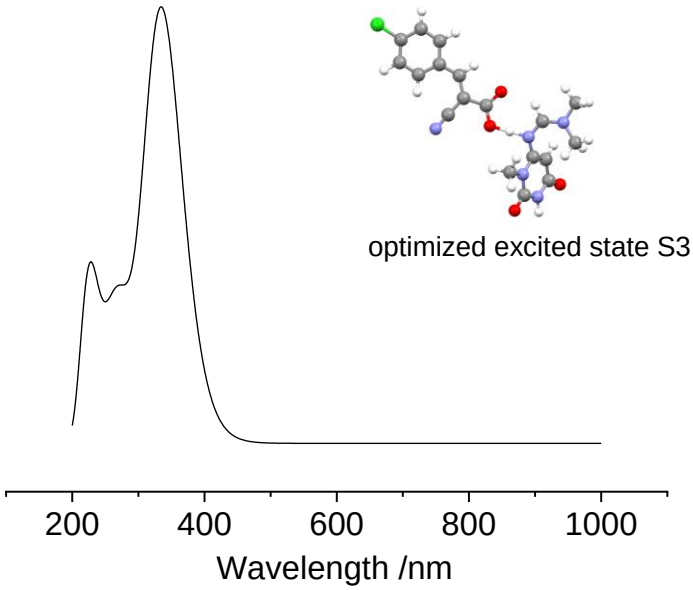


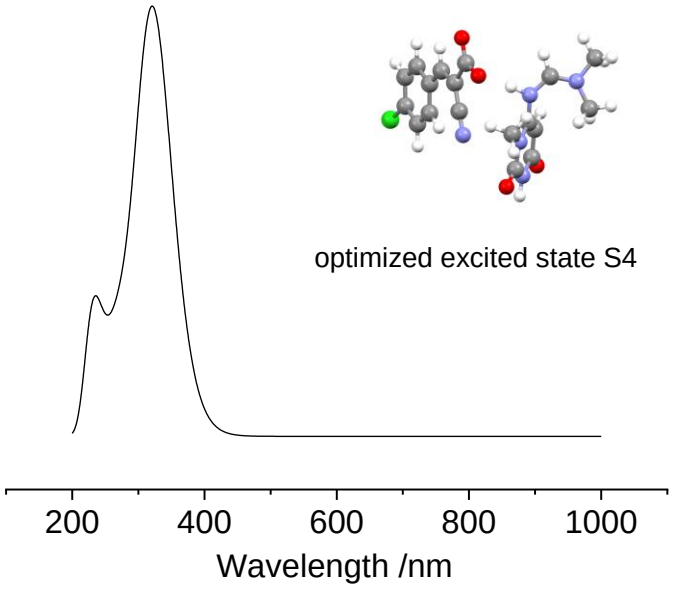
Figure S4. UV-vis absorption measurements in acetonitrile with respect to various substrates.

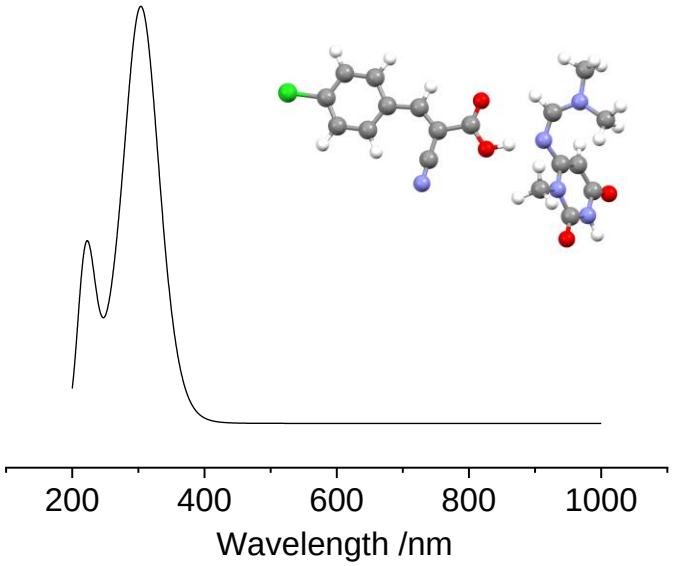
8.3 TD-DFT study






optimized excited state S3
200
400
600
800
1000
Wavelength /nm


optimized excited state S4
200
400
600
800
1000
Wavelength /nm


200
400
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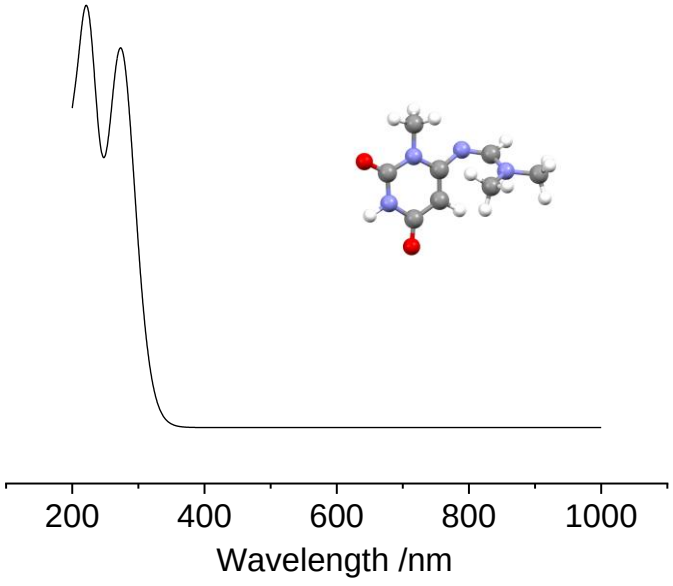
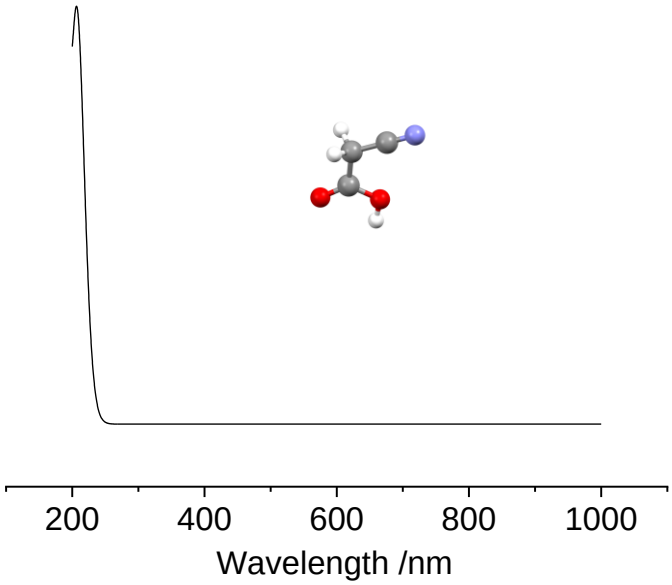
800
1000
Wavelength /nm


200
400
600
800
1000
Wavelength /nm


200
400
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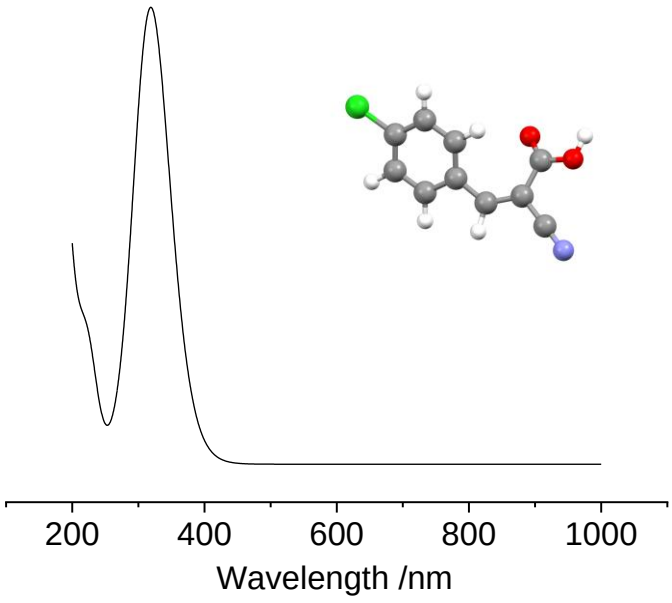
800
1000
Wavelength /nm


200
400
600
800
1000
Wavelength /nm

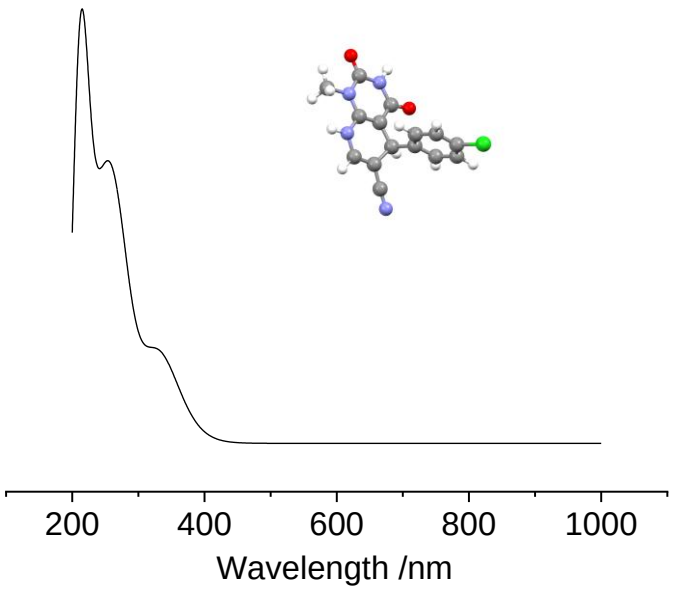
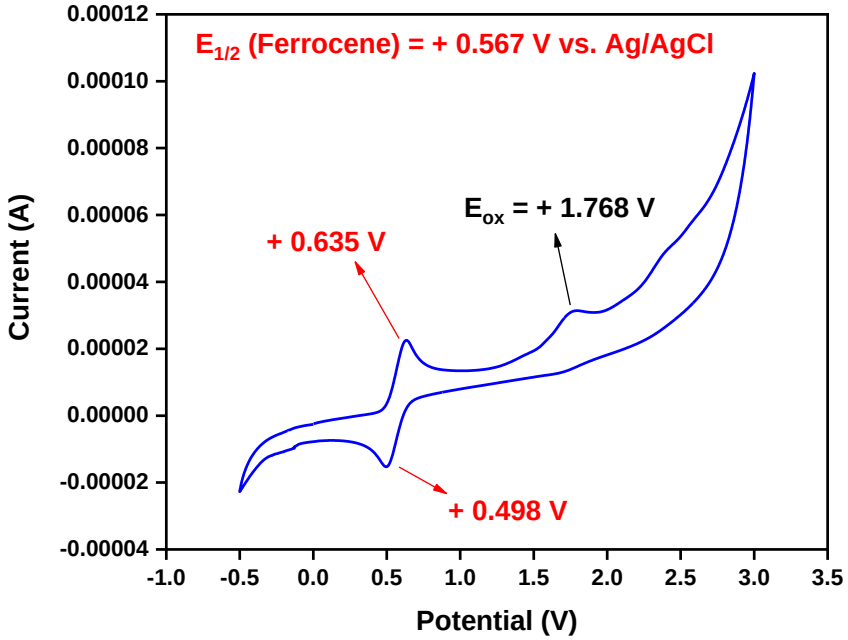


Figure S5. Theoretical UV-Visible spectra of different EDA complexes was calculated by TD-DFT at the PBE0/aug-cc-pVDZ level considering acetonitrile as a solvent.

9. Cyclic voltammetry study

Cyclic voltammetry was carried out with a Pt as counter electrode "CE", a glassy carbon electrode as working electrode "WE" and Ag/AgCl as references electrode "RE" using METROHM electrochemical workstation (PGSTAT 204) with BA.MAC.204.S module. Before use, the "WE" was cleaned with an alumina suspension (BASi) and washed with distilled water repeatedly. The RE was washed with electrolyte solution as well as distilled water and stored in 3.0M KCl solution. Purified ferrocene (recrystallized from n-hexane) was used as an internal standard. Commercially available $^{n}Bu_4N.PF_6$ was used as an electrolyte. The measurement was conducted at a concentration of 10 mM containing 0.1 mM $^{n}Bu_4N.PF_6$ in acetonitrile solvent with a scan rate 0.1 V/s.



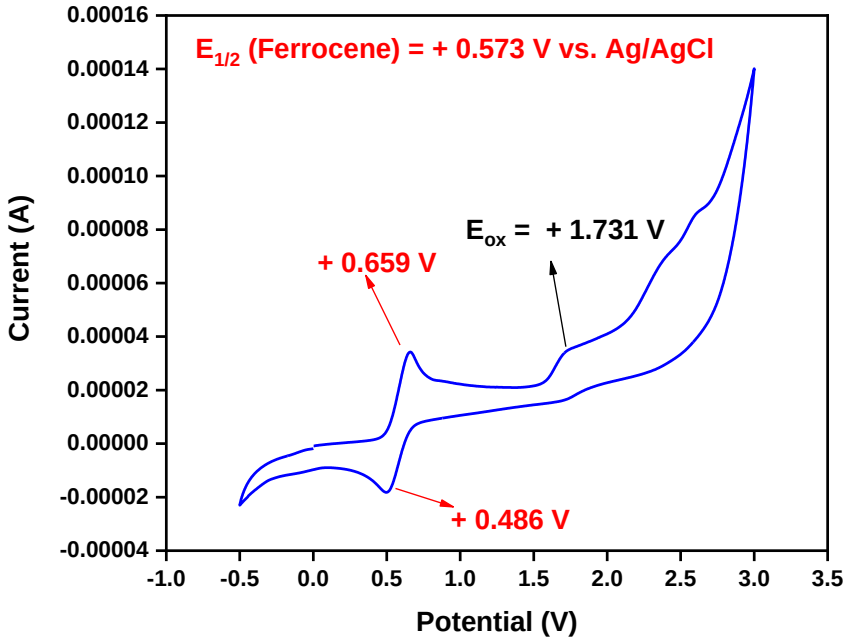
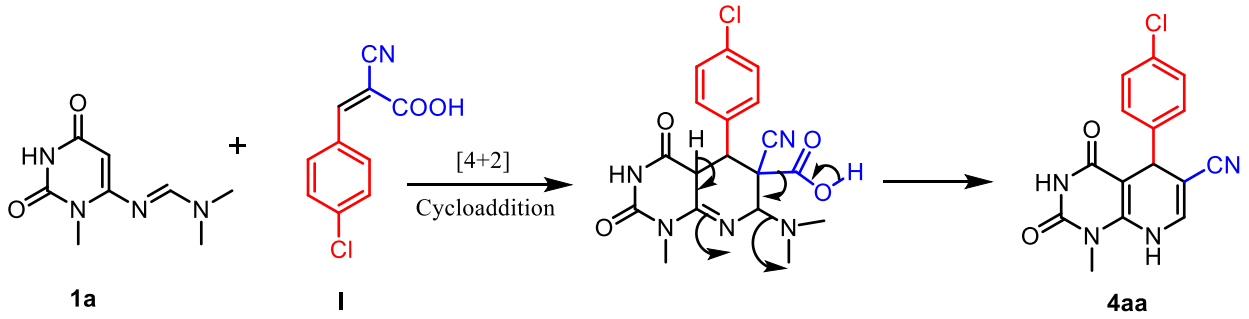


Figure S6. Cyclic voltammetry of reaction mixture at different time intervals.

10. Plausible reaction mechanism in the dark condition



Scheme S2. Plausible reaction mechanism in the dark condition.

11. HRMS data for detecting the key intermediates in the case of two equivalent of cyanoacetic acid under dark condition

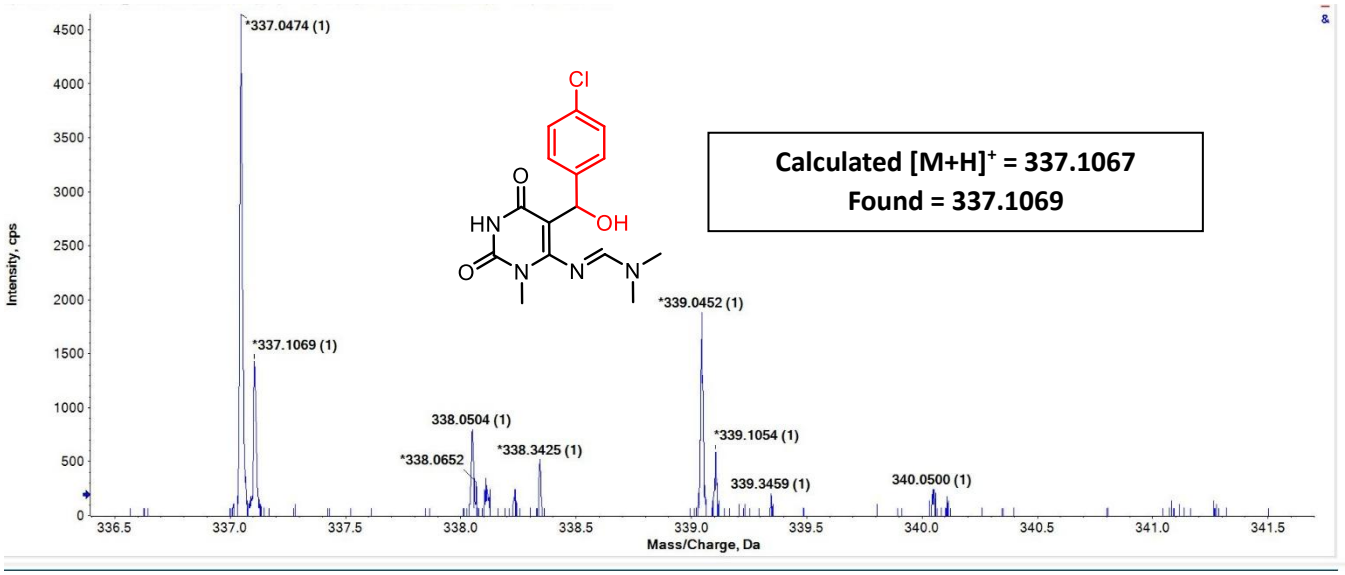


Figure S7. HRMS data of intermediate **E** as per the scheme 6 in the main manuscript.

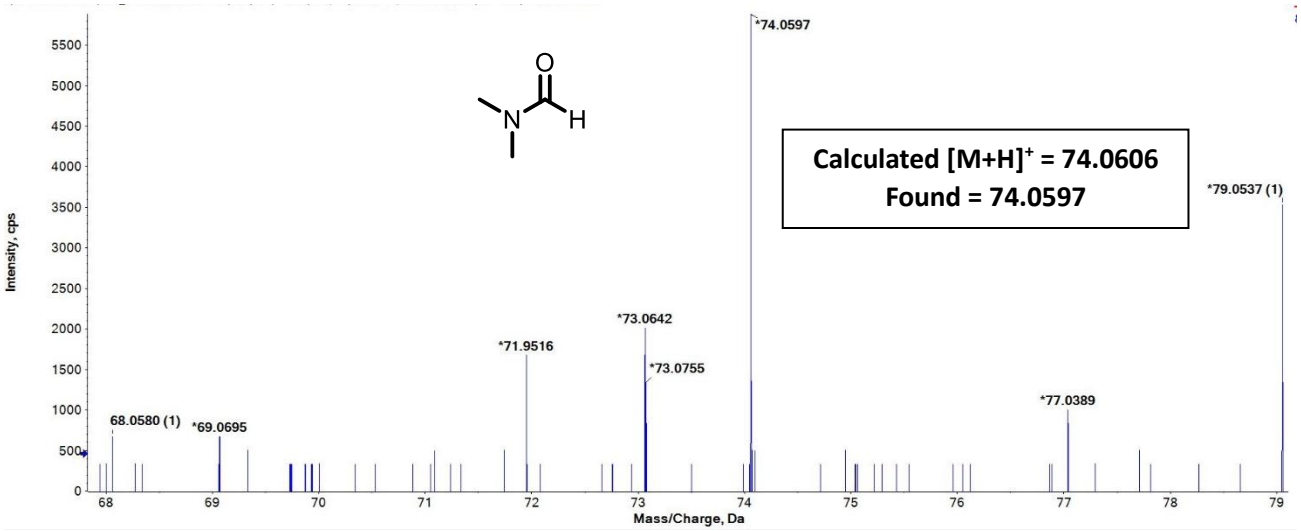
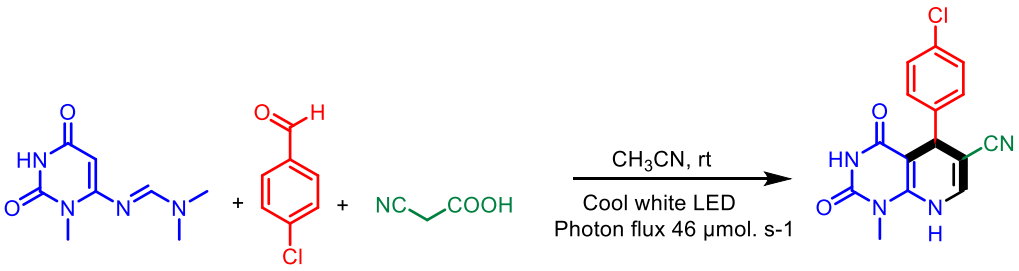


Figure S8. Detection of **DMF** as per the scheme 6 in the main manuscript.

12. Atom economy for both products

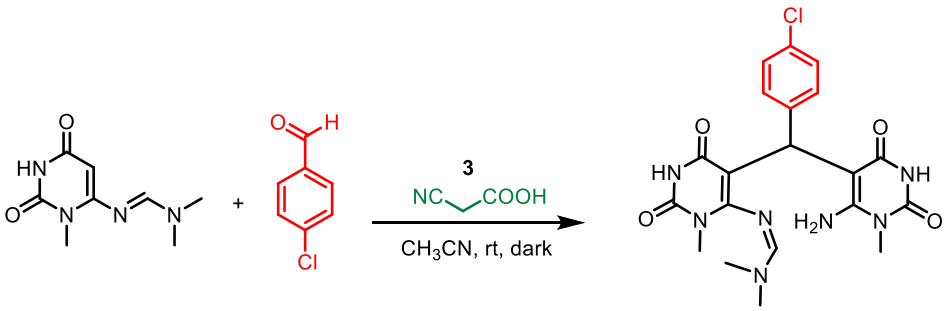


1a **2a** **3** **4a**

MW **196.09 g** **140.00 g** **85.01 g** **314.05 g**

Product	Atom Economy (%)
4aa	74.57
4ab	73.10
4ac	73.75
4ad	73.29
4ae	75.22
4af	74.57
4ag	67.07
4ah	69.70
4ai	74.66
4ba	75.39
4bb	73.31
4bc	74.21
4bd	70.84
4ca	75.39
4cb	73.31
4cc	75.17
4cd	74.21
4ce	72.91
4da	77.84

4db	76.16
4dc	77.08
4dd	77.84
4de	77.65



1a **2a** **5a**

MW	**196.09 g**	**140.00 g**	**85.01 g**	**459.14 g**

Product	Atom Economy (%)
5aa	86.27
5ab	85.34
5ac	85.74
5ad	86.17
5ae	85.85
5af	86.27
5ag	85.51
5ah	85.51
5ba	86.96
5bb	86.12
5bc	86.57
5bd	86.87
5be	86.27
5ca	86.96
5cb	86.12
5cc	86.57
5da	88.88
5db	88.26
5dc	88.59
5dd	88.86
5de	88.80

13. Single-crystal X-ray analysis

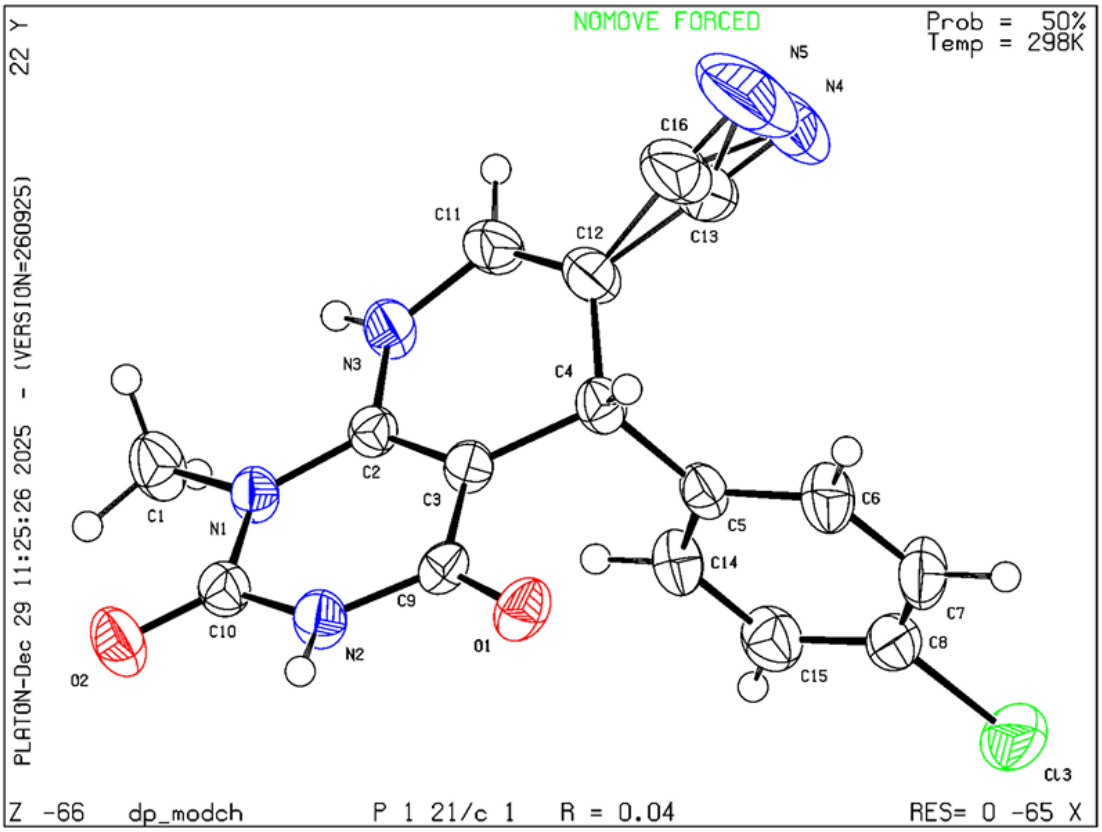
Crystals suitable for single X-ray diffraction were grown by slow evaporation of methanol-water solvent mixtures. Both crystal is stable at room temperature and in open air. Single crystal X-ray data of compound **4aa** was determined using Bruker D8 VENTURE diffractometer with PHOTON II detector and a Mo-Kα radiation (λ = 0.71073 Å). Cell refinement and data reduction was carried out using SAINT V8.40B software pakage. SADABS-2016/2 (Bruker, 2016/2) was used for absorption correction. The X-ray data for compound **5aa** was collected using Rigaku Oxford Diffraction SuperNova diffractometer with a EoS2 CCD detector and a Mo-Kα radiation (λ = 0.71073 Å). CrysAlisPro 1.171.42.49 (Rigaku OD, 2022) program package was used for cell refinement, data collection and reduction. The absorption correction was carried out using CrysAlisPro 1.171.42.49. Crystal data and cell refinement for compound **4aa** and **5aa** are given below.

Crystal data for compound 4aa (CCDC = 2519491)

- Chemical formula: $C_{15}H_{11}ClN_4O_2$
- Chemical formula weight: 314.73
- Temperature: 298 K
- Crystal system: monoclinic
- Space group: P 1 21/c 1
- a (Å): 8.1146(6)
- b (Å): 13.822(1)
- c (Å): 13.2561(8)
- α: 90
- β: 98.439(3)
- λ: 90
- Cell volume: 1470.71(18)
- Cell formula unit (Z): 4
- Dx (g/cm^3): 1.421
- μ (mm^{-1}): 0.272
- F (000): 640.0
- Tmin, Tmax: 0.679, 0.746
- R (reflection): 0.0370 (2385)
- wR2 (reflection): 0.1044 (2683)

- Data completeness: 0.999

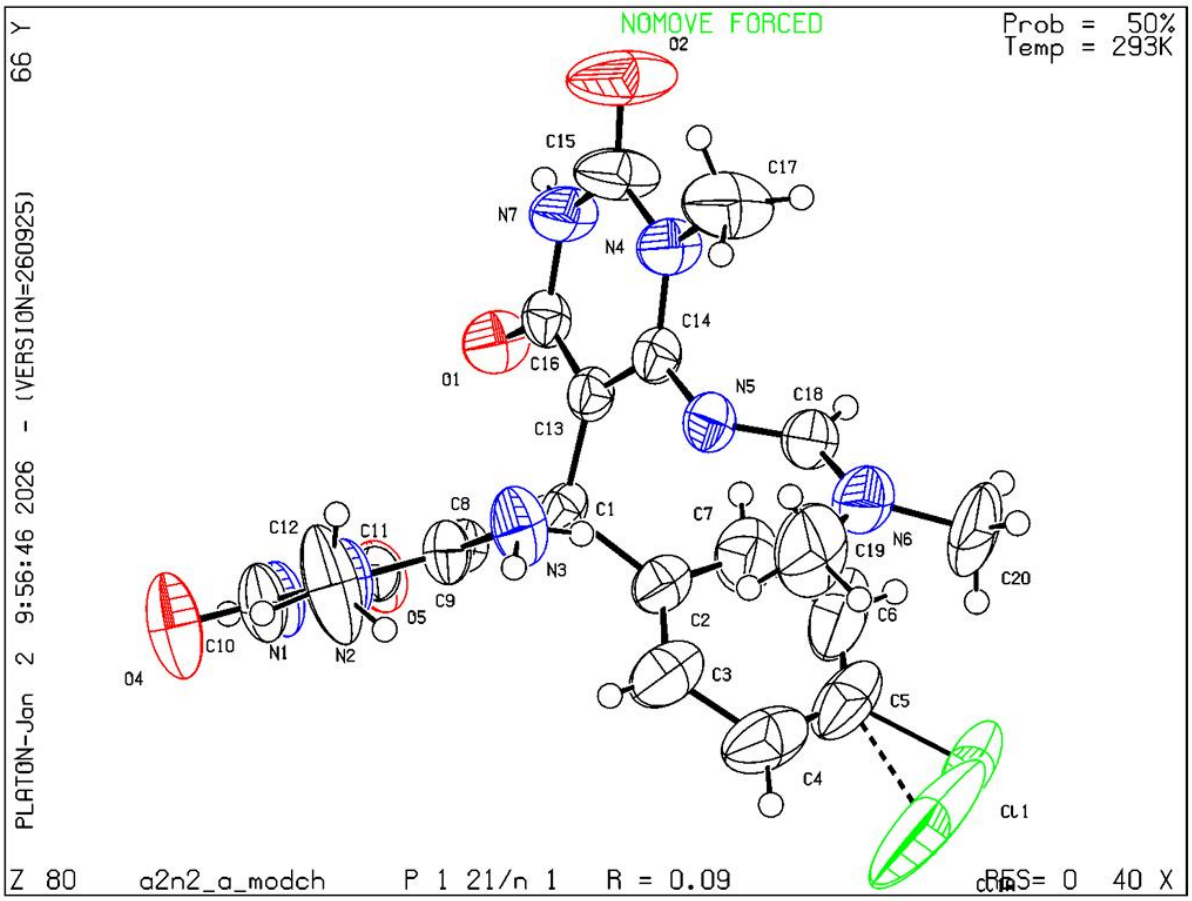
Datablock dp_modch - ellipsoid plot



Crystal data for compound 5aa (CCDC = 2519497)

- Chemical formula: $C_{20}H_{22}ClN_6O_5$
- Chemical formula weight: 488.91
- Temperature: 293 K
- Crystal system: monoclinic
- Space group: P 1 21/n 1
- a (Å): 8.0227 (11)
- b (Å): 20.042 (3)
- c (Å): 15.413 (2)
- α: 90
- β: 102.697 (14)
- λ: 90

- Cell volume: 2417.7 (6)
- Cell formula unit (Z): 4
- Dx (g/cm^3): 1.343
- μ (mm^{-1}): 0.207
- F (000): 1024.0
- Tmin, Tmax: 0.537, 1.000
- R (reflection): 0.0957 (1822)
- wR2 (reflection): 0.2961 (4084)
- Data completeness: 0.990



The crystal of compound **5aa** containing disorder, which is further supported by DFT optimized structure. The structure of **5aa** was optimized by means of the B3LYP/3-21G basis set. The FT-IR spectra of optimized structure was aligning with the experimental one.

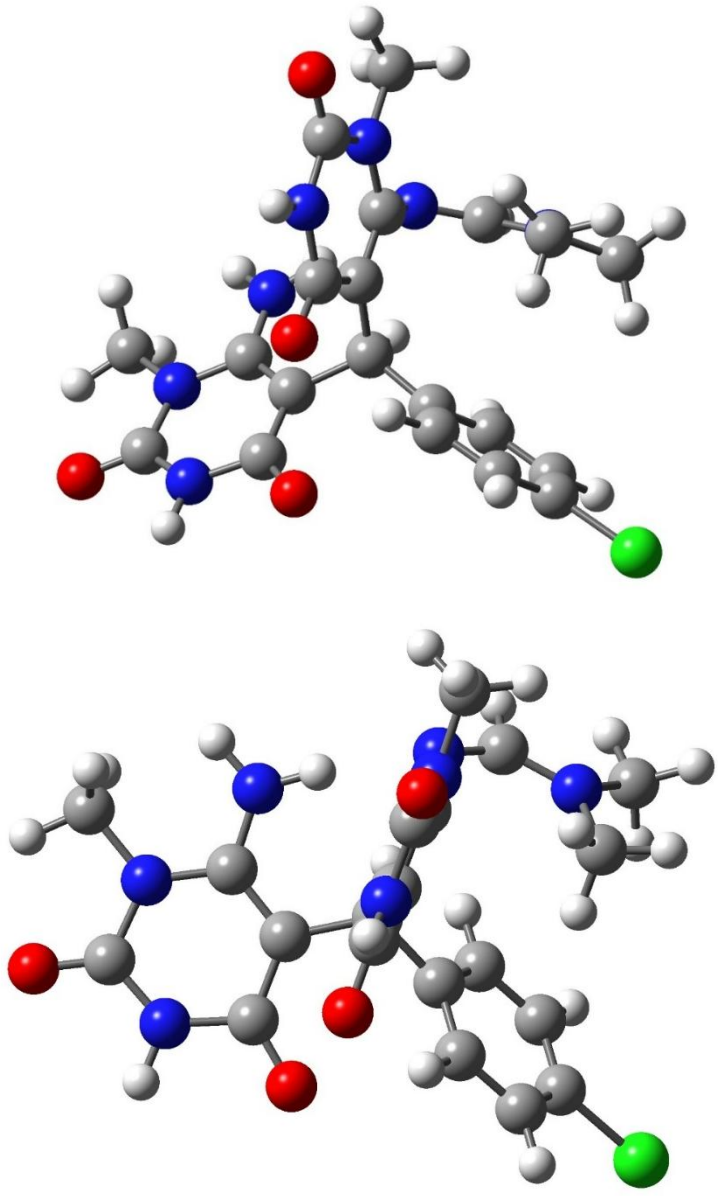


Figure S9. Optimized structure of compound **5aa** was collected from the DFT method with the basis set of B3LYP-3-21G for all the atoms.

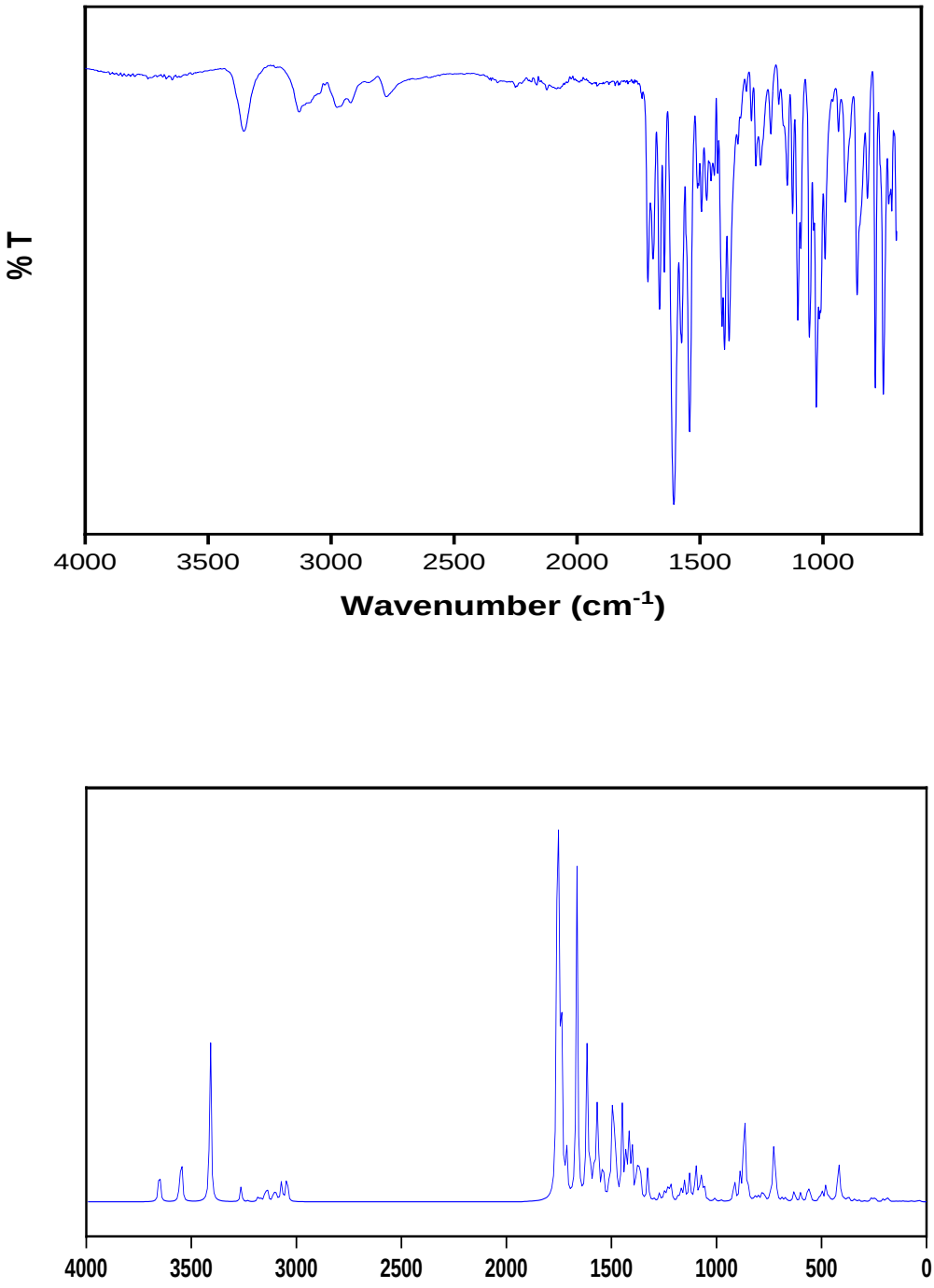


Figure S10. Experimental and theoretical FT-IR spectra of compound **5aa**.

14. Experimental antibacterial study

14.1 Experimental procedure for antibacterial activity

To sum up, various concentrations of the test substances were prepared using Milli Q-grade water. *E. coli* and *B. cereus* cells were re-cultured and purified in LB broth. The growth inhibition procedures were carried out by inoculating fixed quantities of (0, 5, 10, 25, 50, 100, 250, and 500 µg/mL in 10% DMSO) in LB broth fixed with predefined concentrations of *E. coli* and *B. cereus* cells. The cells were allowed to grow at 37°C, with the OD measured after 12 hours and as well as after 24 hours to achieve stationary growth. The OD at 24 hours was used to compute the IC_{50} for the examined compounds. For determining MBC, a fixed volume of the IC_{50} determinants was plated in LB-agar plates, and the cells were allowed to grow at 37°C for 24 hours before the number of viable cells was calculated. All the experiments were repeated in triplicate to minimize the chances of experimental error. OriginPro and Microsoft Excel were used to determine the IC_{50} value and % inhibition of the compounds.

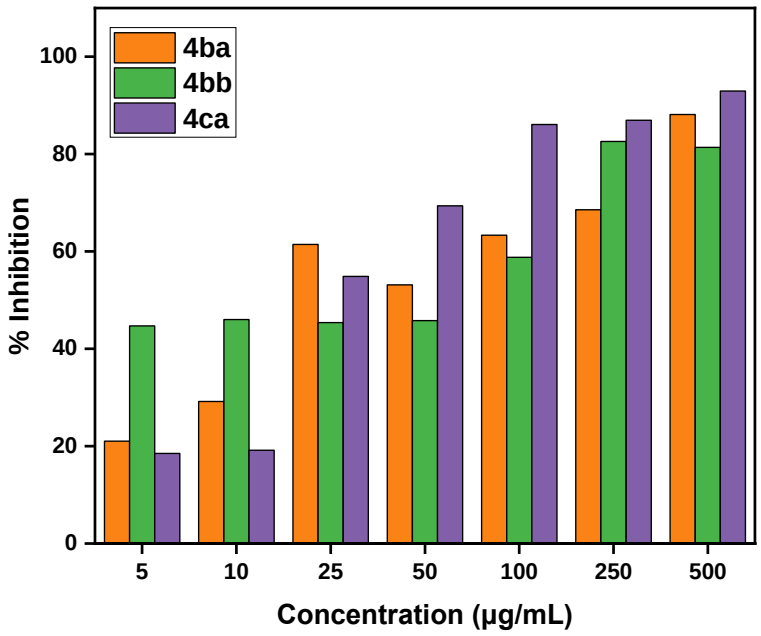


Figure S11. The percentage inhibition of the active compounds against *E. coli*.

14.2 Stability of the compound

We detected all the compound in methanol solvent using high-resolution mass spectrometry (HRMS) and single crystal X-ray diffraction (SC-XRD), indicating its stability in this solvent. However, we performed the biological analysis in 10 % DMSO-water solution. Therefore, we examined the stability of compounds **4aa** in different solvents such as methanol, water, and 10% DMSO using a UV-visible spectrophotometer (**Figure 2**). In the absorbance spectra, compound **4aa** shows the same γ_{max} value (338 nm) in the corresponding solvents. This result validated the compound stability in a 10 % DMSO-water solution.

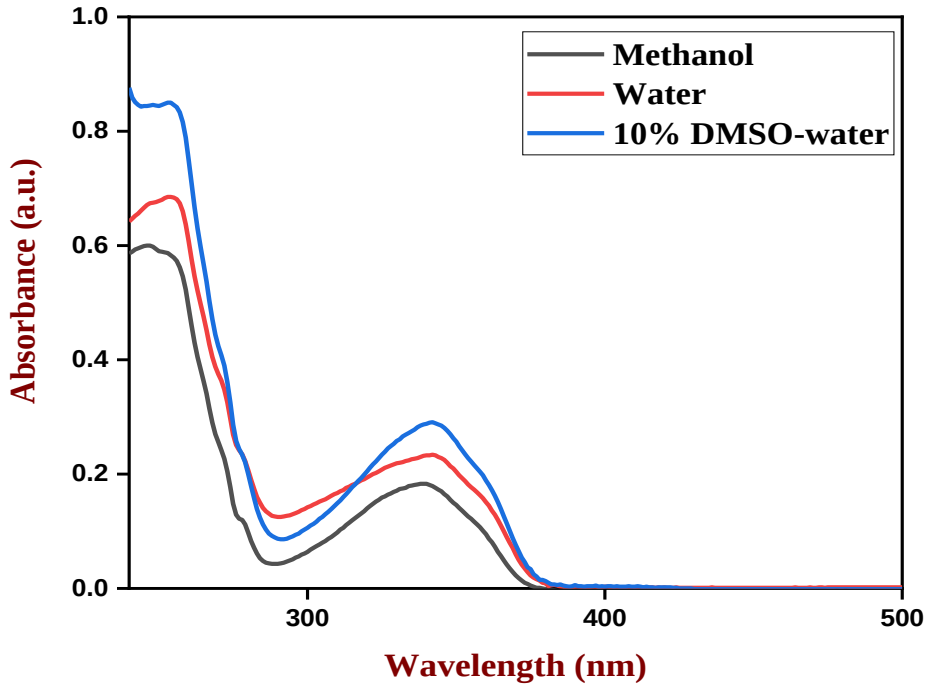


Figure S12. The absorbance spectra of compound **4aa**.

Table S2. Inhibition activity of **4aa** at different concentrations against *E. coli*.

µg/mL	SET 1	SET 2	SET 3	N Total	Mean	SD	SEM	Median	% Inhibition
0	1.005	0.993	1.109	3	1.035667	0.063791	0.03683	1.005	
5	1.017	1.021	0.994	3	1.010667	0.014572	0.008413	1.017	2.413904
10	0.985	0.991	0.976	3	0.984	0.00755	0.004359	0.985	4.988735
25	1.047	0.1001	0.995	3	0.714033	0.532317	0.307333	0.995	31.05568
50	0.514	0.599	0.613	3	0.575333	0.053575	0.030932	0.599	44.44802
100	0.471	0.521	0.393	3	0.461667	0.064508	0.037244	0.471	55.42324
250	0.245	0.269	0.311	3	0.275	0.033407	0.019287	0.269	73.44706
500	0.141	0.165	0.117	3	0.141	0.024	0.013856	0.141	86.38558

Table S3. Inhibition activity of **4ab** at different concentrations against *E. coli*.

µg/mL	SET 1	SET 2	SET 3	N Total	Mean	SD	SEM	Median	% Inhibition
0	1.125	1.049	1.131	3	1.101667	0.045709	0.02639	1.125	
5	1.017	1.005	0.996	3	1.006	0.010536	0.006083	1.005	8.683812

10	0.985	0.979	0.991	3	0.985	0.006	0.003464	0.985	10.59002
25	0.847	0.731	0.659	3	0.745667	0.094854	0.054764	0.731	32.31467
50	0.514	0.498	0.503	3	0.505	0.008185	0.004726	0.503	54.16036
100	0.471	0.467	0.442	3	0.46	0.015716	0.009074	0.467	58.24508
250	0.245	0.233	0.218	3	0.232	0.013528	0.00781	0.233	78.941
500	0.141	0.116	0.152	3	0.136333	0.018448	0.010651	0.141	87.62481

Table S4. Inhibition activity of **4ac** at different concentrations against *E. coli*.

µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	0.956	1.12	0.995	3	1.023667	0.085676	0.049465	0.995	
5	0.983	0.983	1.007	3	0.991	0.013856	0.008	0.983	3.191143
10	0.79	0.831	0.71	3	0.777	0.061539	0.035529	0.79	24.09639
25	0.713	0.79	0.687	3	0.73	0.053563	0.030925	0.713	28.68772
50	0.559	0.541	0.494	3	0.531333	0.033561	0.019376	0.541	48.09508
100	0.368	0.319	0.372	3	0.353	0.029513	0.017039	0.368	65.51612
250	0.325	0.293	0.332	3	0.316667	0.020793	0.012005	0.325	69.06545
500	0.088	0.181	0.076	3	0.115	0.057472	0.033181	0.088	88.76587

Table S5. Inhibition activity of **4ad** at different concentrations against *E. coli.*

µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	0.956	1.106	0.942	3	1.001333	0.090914	0.052489	0.956	
5	0.943	1.029	0.996	3	0.989333	0.043386	0.025049	0.996	1.198402
10	0.806	0.763	0.792	3	0.787	0.021932	0.012662	0.792	21.40479
25	0.922	0.829	0.791	3	0.847333	0.067397	0.038912	0.829	15.37949
50	0.148	0.203	0.173	3	0.174667	0.027538	0.015899	0.173	82.55659
100	0.487	0.351	0.399	3	0.412333	0.068973	0.039822	0.399	58.82157
250	0.338	0.277	0.304	3	0.306333	0.030567	0.017648	0.304	69.40746
500	0.152	0.144	0.127	3	0.141	0.012767	0.007371	0.144	85.91877

Table S6. Inhibition activity of **4ah** at different concentrations against *E. coli.*

µg/mL	SET 1	SET 2	SET 3	N Total	Mean	SD	SEM	Median	% Inhibition
0	1.12	1.108	1.151	3	1.126333	0.022189	0.012811	1.12	
5	1.129	1.106	1.131	3	1.122	0.013892	0.008021	1.129	0.384729
10	1.003	0.953	1.09	3	1.015333	0.069328	0.040026	1.003	9.854987
25	0.923	0.868	0.942	3	0.911	0.038432	0.022189	0.923	19.11808
50	0.632	0.586	0.491	3	0.569667	0.071905	0.041514	0.586	49.42291
100	0.456	0.484	0.396	3	0.445333	0.044959	0.025957	0.456	60.46168
250	0.239	0.261	0.205	3	0.235	0.028213	0.016289	0.239	79.13584
500	0.105	0.111	0.11	3	0.108667	0.003215	0.001856	0.11	90.35218

Table S7. Inhibition activity of **4ba** at different concentrations against *E. coli*.

µg/mL	SET 1	SET 2	SET 3	N Total	Mean	SD	SEM	Median	% Inhibition
0	0.971	1.105	0.993	3	1.023	0.071861	0.041489	0.993	
5	0.791	0.862	0.771	3	0.808	0.047823	0.02761	0.791	21.01662
10	0.73	0.679	0.764	3	0.724333	0.042782	0.0247	0.73	29.19518
25	0.354	0.432	0.397	3	0.394333	0.039068	0.022556	0.397	61.45324
50	0.423	0.449	0.566	3	0.479333	0.076173	0.043979	0.449	53.14435
100	0.328	0.378	0.419	3	0.375	0.045574	0.026312	0.378	63.34311
250	0.348	0.329	0.288	3	0.321667	0.030665	0.017704	0.329	68.55653
500	0.11	0.137	0.117	3	0.121333	0.014012	0.00809	0.117	88.13946

Table S8. Inhibition activity of **4bb** at different concentrations against *E. coli.*

µg/mL	SET 1	SET 2	SET 3	N Total	Mean	SD	SEM	Median	% Inhibition
0	1.319	1.311	1.298	3	1.309333	0.010599	0.006119	1.311	
5	0.752	0.721	0.699	3	0.724	0.026627	0.015373	0.721	44.70468
10	0.712	0.698	0.711	3	0.707	0.00781	0.004509	0.711	46.00305
25	0.701	0.662	0.782	3	0.715	0.061213	0.035341	0.701	45.39206

50	0.713	0.701	0.715	3	0.709667	0.007572	0.004372	0.713	45.79939
100	0.547	0.503	0.569	3	0.539667	0.033606	0.019402	0.547	58.7831
250	0.229	0.24	0.215	3	0.228	0.01253	0.007234	0.229	82.58656
500	0.255	0.279	0.198	3	0.244	0.041605	0.024021	0.255	81.36456

Table S9. Inhibition activity of **4bc** at different concentrations against *E. coli*.

µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	1.123	1.099	1.211	3	1.144333	0.058969	0.034046	1.123	
5	1.259	1.063	1.011	3	1.111	0.130782	0.075507	1.063	2.912904
10	1.09	1.18	1.066	3	1.112	0.0601	0.034699	1.09	2.825517
25	1.15	1.17	0.981	3	1.100333	0.103828	0.059945	1.15	3.845033
50	0.632	0.597	0.648	3	0.625667	0.026083	0.015059	0.632	45.32479
100	0.456	0.481	0.438	3	0.458333	0.021595	0.012468	0.456	59.94757
250	0.239	0.245	0.218	3	0.234	0.014177	0.008185	0.239	79.55141
500	0.213	0.195	0.219	3	0.209	0.01249	0.007211	0.213	81.73609

Table S10. Inhibition activity of **4ca** at different concentrations against *E. coli.*

µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	1.692	1.573	1.641	3	1.635333	0.059702	0.034469	1.641	
5	1.366	1.311	1.321	3	1.332667	0.029297	0.016915	1.321	18.50795
10	1.366	1.291	1.309	3	1.322	0.039154	0.022605	1.309	19.16021
25	0.746	0.771	0.697	3	0.738	0.037643	0.021733	0.746	54.87159
50	0.526	0.486	0.491	3	0.501	0.021794	0.012583	0.491	69.36404
100	0.243	0.221	0.219	3	0.227667	0.013317	0.007688	0.221	86.07827
250	0.232	0.188	0.221	3	0.213667	0.022898	0.01322	0.221	86.93437
500	0.126	0.119	0.101	3	0.115333	0.012897	0.007446	0.119	92.94741

Table S11. Inhibition activity of **4cb** at different concentrations against *E. coli*.

µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	0.966	1.102	0.963	3	1.010333	0.0794	0.045842	0.966	
5	1.051	0.993	0.876	3	0.973333	0.089142	0.051466	0.993	3.662158

10	0.837	0.802	0.761	3	0.8	0.038039	0.021962	0.802	20.81821
25	0.71	0.814	0.692	3	0.738667	0.065858	0.038023	0.71	26.88882
50	0.479	0.411	0.399	3	0.429667	0.043143	0.024909	0.411	57.47278
100	0.379	0.316	0.365	3	0.353333	0.033081	0.019099	0.365	65.02804
250	0.241	0.197	0.232	3	0.223333	0.023245	0.013421	0.232	77.89508
500	0.136	0.121	0.116	3	0.124333	0.010408	0.006009	0.121	87.69383

Table S12. Inhibition activity of **4cd** at different concentrations against *E. coli*.

µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	0.999	1.011	1.021	3	1.010333	0.011015	0.00636	1.011	
5	0.966	0.993	1.001	3	0.986667	0.018339	0.010588	0.993	2.342461
10	1.094	1.023	1.006	3	1.041	0.04668	0.026951	1.023	-3.0353
25	0.838	0.912	0.814	3	0.854667	0.051082	0.029492	0.838	15.40746
50	0.694	0.721	0.619	3	0.678	0.052849	0.030512	0.694	32.89343
100	0.602	0.629	0.59	3	0.607	0.019975	0.011533	0.602	39.92082
250	0.492	0.414	0.503	3	0.469667	0.048521	0.028014	0.492	53.51369
500	0.226	0.217	0.228	3	0.223667	0.005859	0.003383	0.226	77.86209

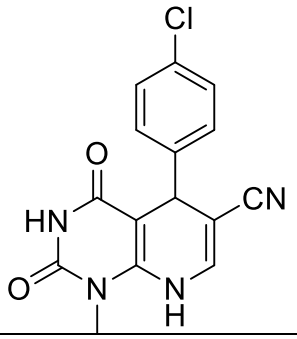
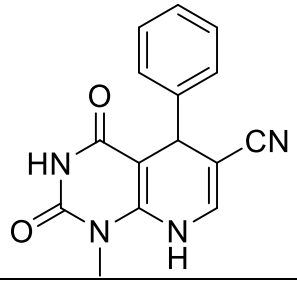
Table S13. Inhibition activity of **4da** at different concentrations against *E. coli*.

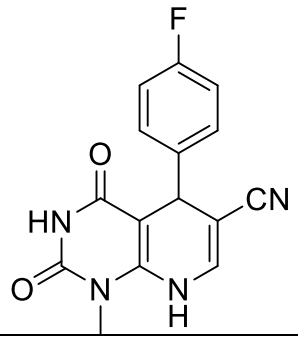
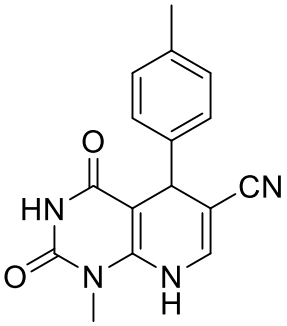
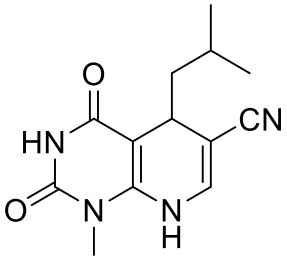
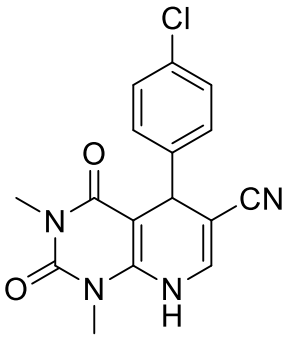
µg/mL	**SET 1**	**SET 2**	**SET 3**	**N Total**	**Mean**	**SD**	**SEM**	**Median**	**% Inhibition**
0	1.088	1.076	1.081	3	1.081667	0.006028	0.00348	1.081	
5	1.034	1.021	1.038	3	1.031	0.008888	0.005132	1.034	4.684129
10	0.729	0.729	0.816	3	0.758	0.050229	0.029	0.729	29.92296
25	0.481	0.526	0.478	3	0.495	0.026889	0.015524	0.481	54.23729
50	0.472	0.451	0.493	3	0.472	0.021	0.012124	0.472	56.36364
100	0.675	0.626	0.617	3	0.639333	0.031214	0.018022	0.626	40.89368
250	0.521	0.534	0.519	3	0.524667	0.008145	0.004702	0.521	51.49461
500	0.431	0.426	0.39	3	0.415667	0.022368	0.012914	0.426	61.57165

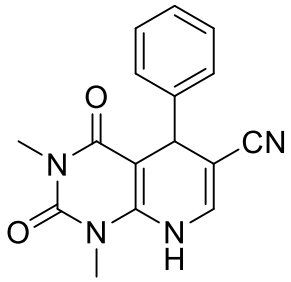
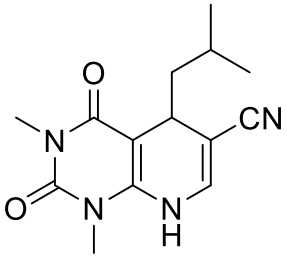
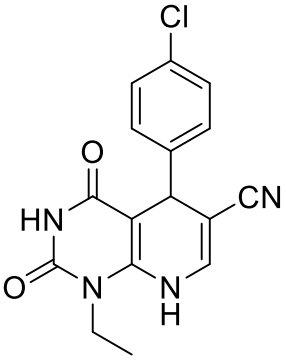
Table S14. Inhibition activity of **4db** at different concentrations against *E. coli*.

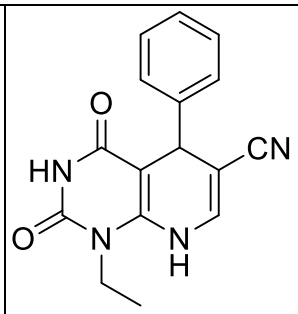
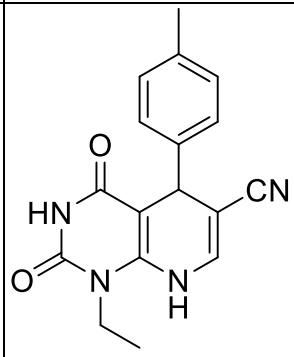
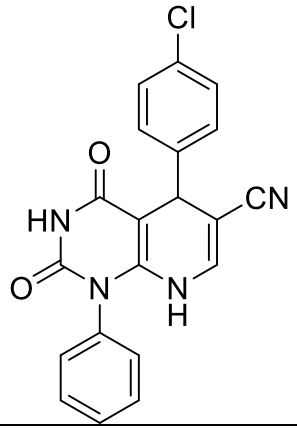
µg/mL	SET 1	SET 2	SET 3	N Total	Mean	SD	SEM	Median	% Inhibition
0	1.028	1.016	1.071	3	1.038333	0.028919	0.016697	1.028	
5	0.949	0.921	0.938	3	0.936	0.014107	0.008145	0.938	9.855538
10	0.977	0.987	0.963	3	0.975667	0.012055	0.00696	0.977	6.035313
25	0.913	0.902	0.918	3	0.911	0.008185	0.004726	0.913	12.26324
50	1.048	1.021	0.994	3	1.021	0.027	0.015588	1.021	1.669342
100	0.746	0.802	0.721	3	0.756333	0.041477	0.023947	0.746	27.15891
250	0.456	0.431	0.429	3	0.438667	0.015044	0.008686	0.431	57.75281
500	0.568	0.514	0.498	3	0.526667	0.036679	0.021177	0.514	49.27769

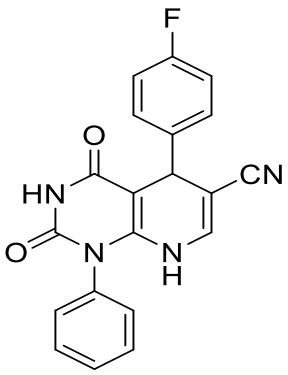
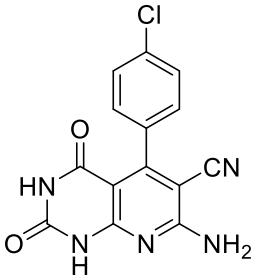
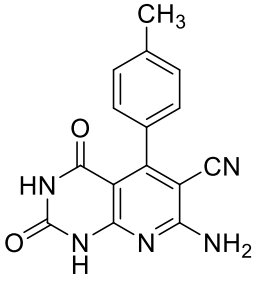
Table S15. The IC_{50} value of all the tested compounds against *E. coli* and *B. cereus*.

Test Compounds	Structure	*E. Coli* IC_{50} (µg/mL)	*B. Cereus* IC_{50} (µg/mL)
4aa		183.28	199.22
4ab		153.60	1124.65

4ac		179.82	1049.77
4ad		145.98	4479.19
4ah		170.34	3166.91
4ba		85.73	1623.75

4bb		42.57	3595.85
4bc		199.86	3330.45
4ca		43.64	2468.93

4cb		145	2352.75
4cd		260.96	3984.29
4da		254.31	589.85

4db		187.84	1789.42
Reported example Ziarani et al. (2014)		512.00	NA
Reported example Ziarani et al. (2014)		512.00	NA
Ampicillin	Known drug	34.96	267.24

Pictures of Antibacterial Study

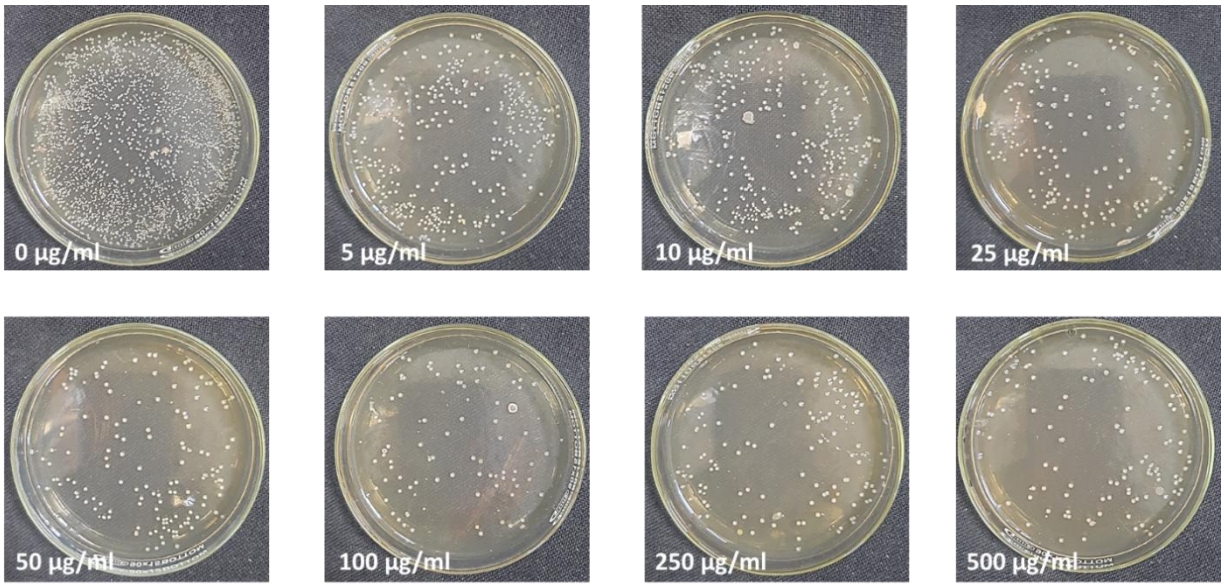


Figure S13. Antibacterial activity against *E. coli* using different concentrations of **4aa** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

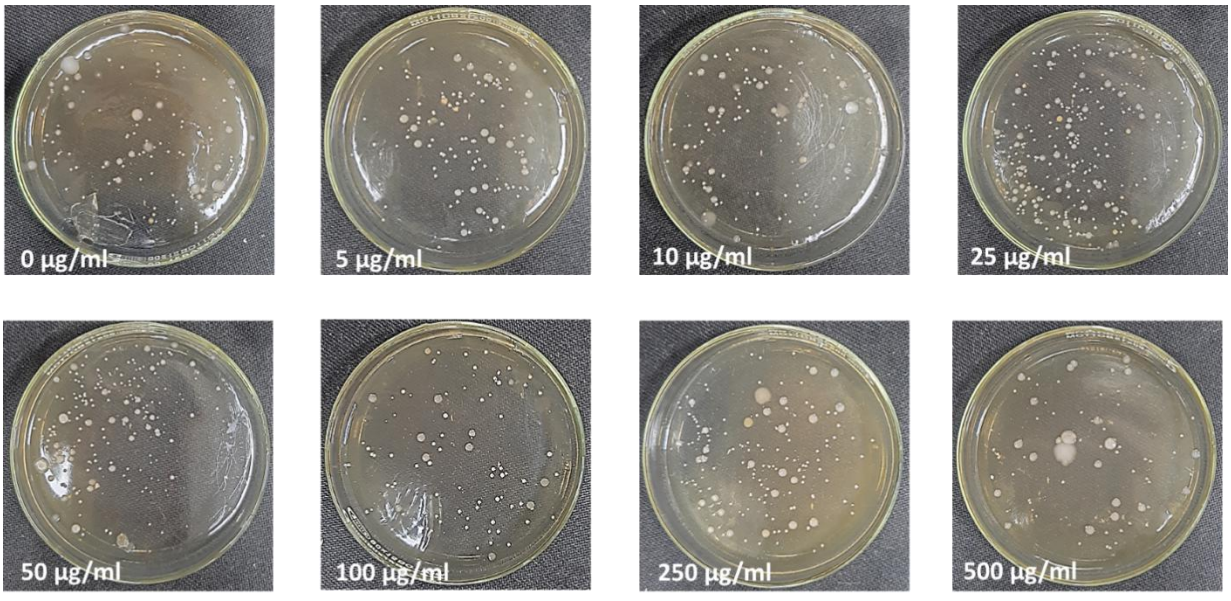


Figure S14. Antibacterial activity against *B. cereus* using different concentrations of **4aa** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

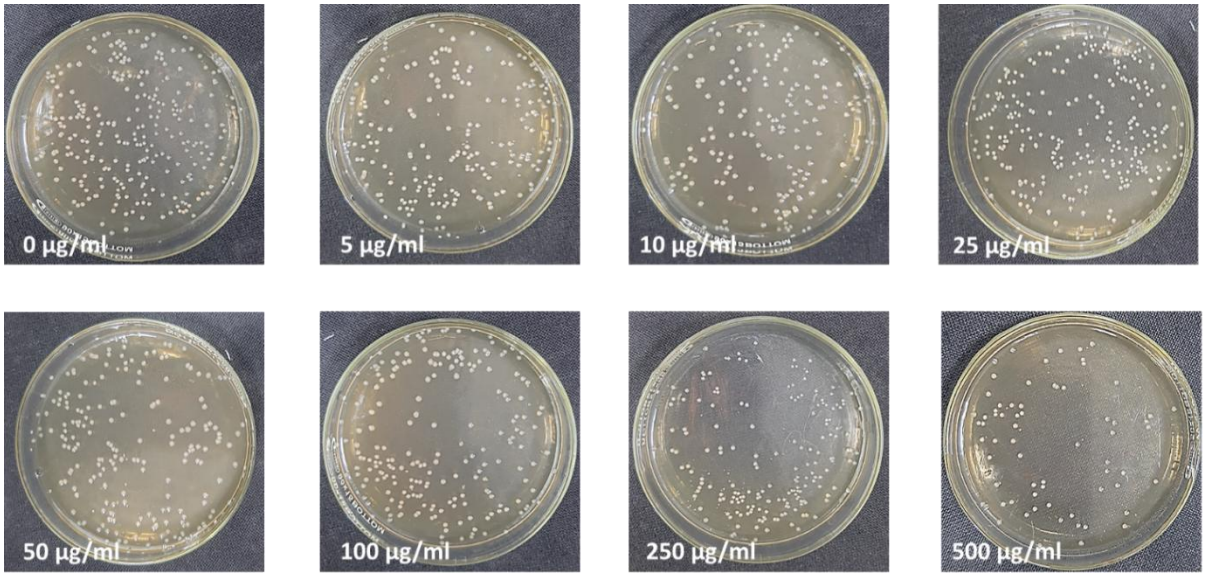


Figure S15. Antibacterial activity against *E. coli* using different concentrations of **4ab** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

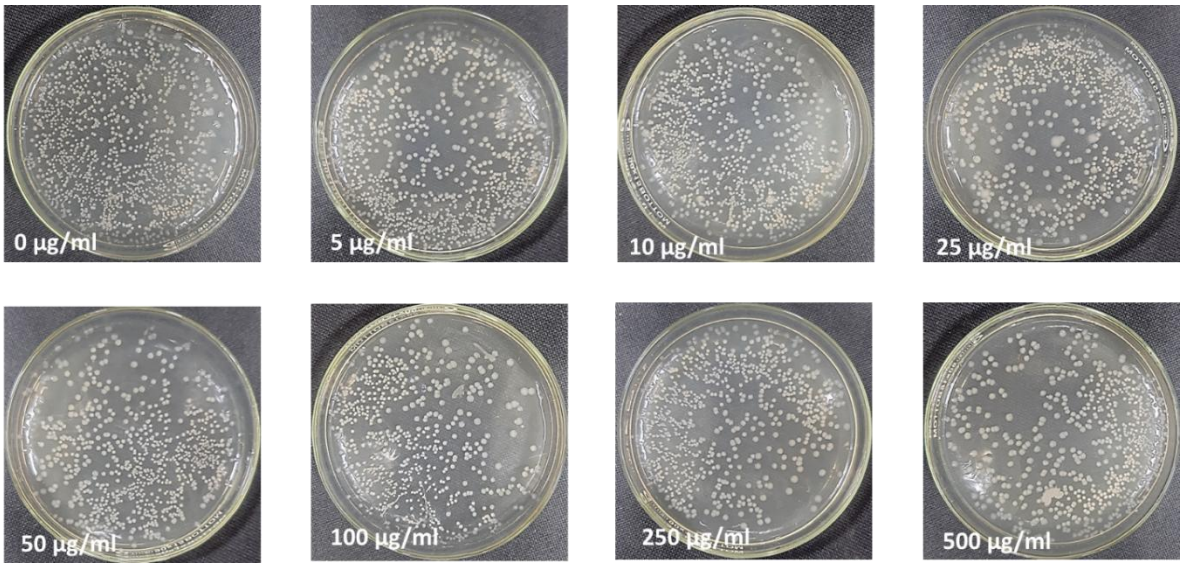


Figure S16. Antibacterial activity against *B. cereus* using different concentrations of **4ab** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

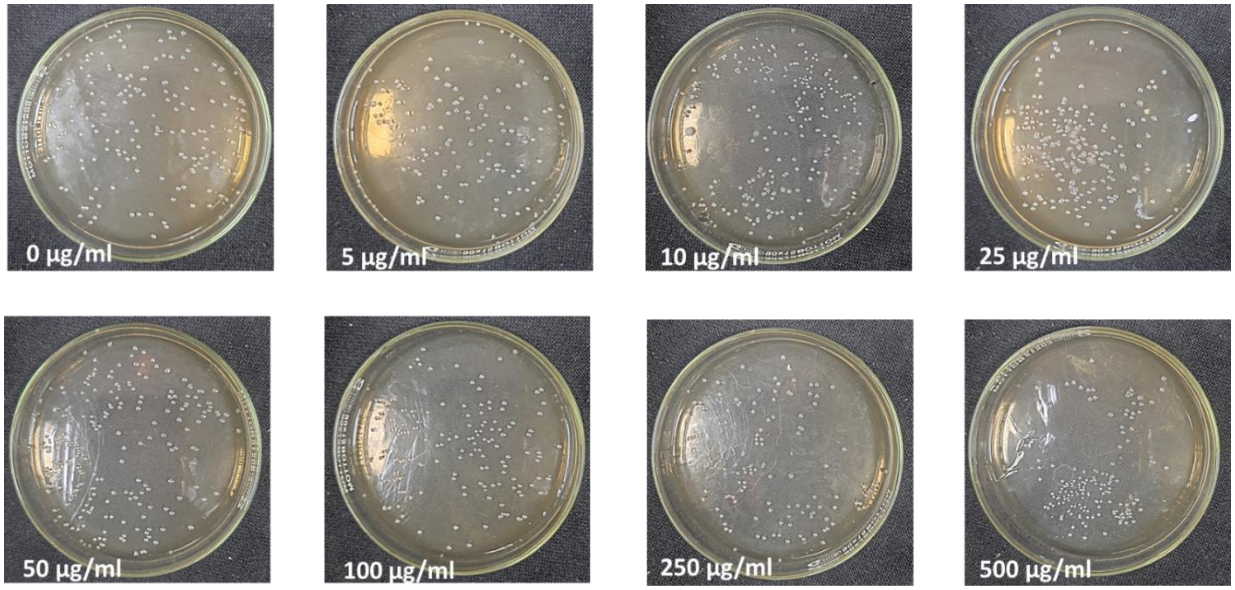


Figure S17. Antibacterial activity against *E. coli* using different concentrations of **4ad** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

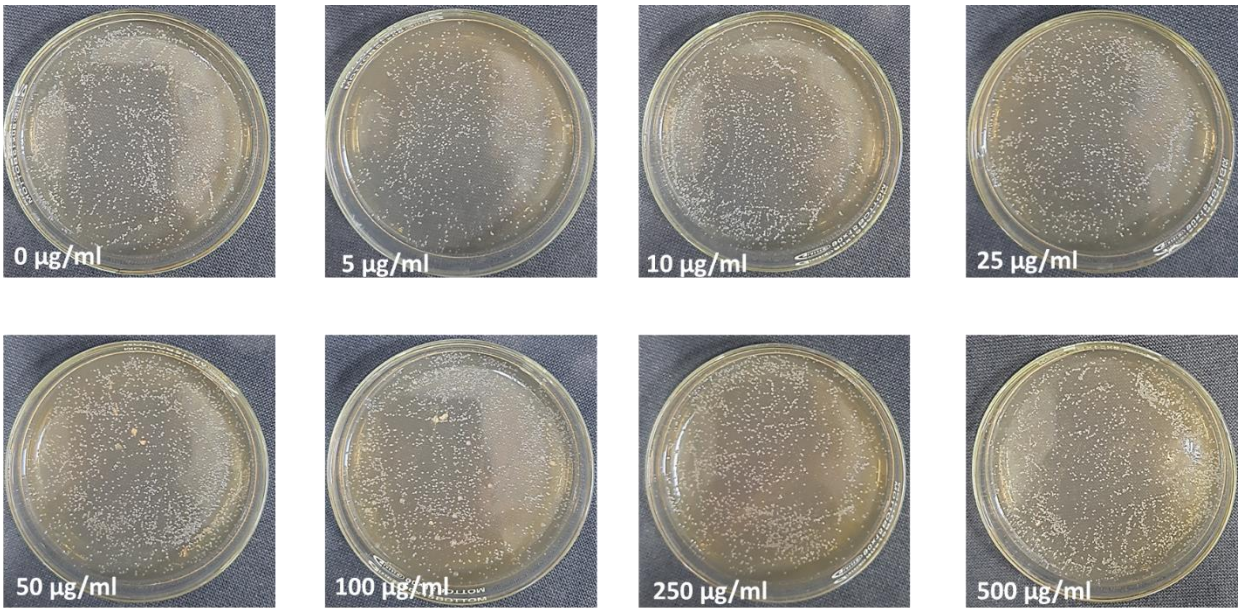


Figure S18. Antibacterial activity against *B. cereus* using different concentrations of **4ad** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

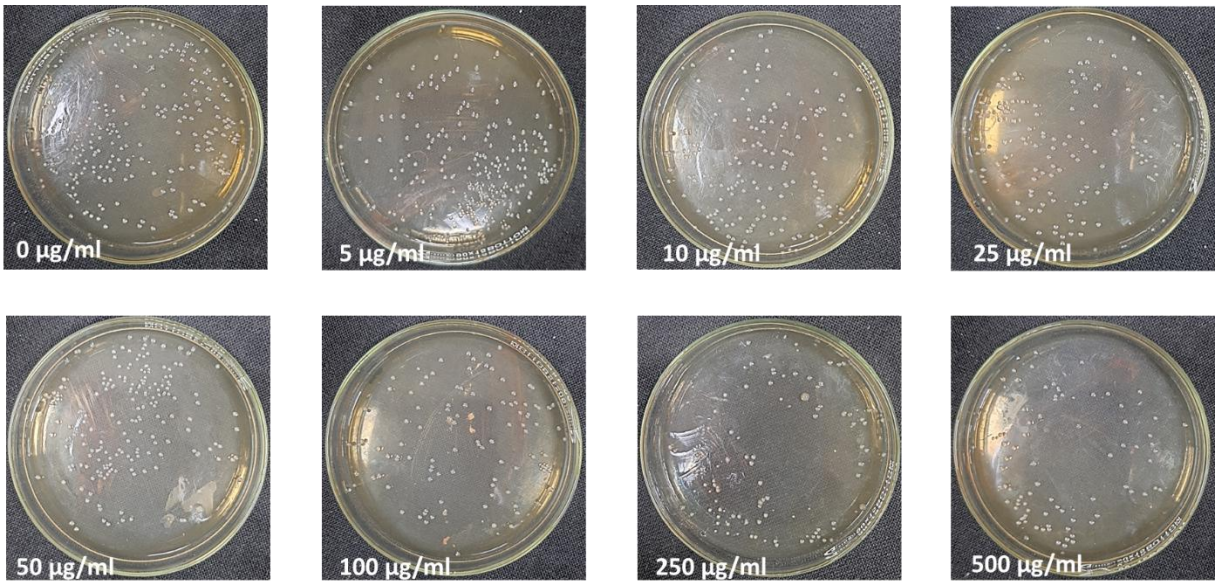


Figure S19. Antibacterial activity against *E. coli* using different concentrations of **4ac** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

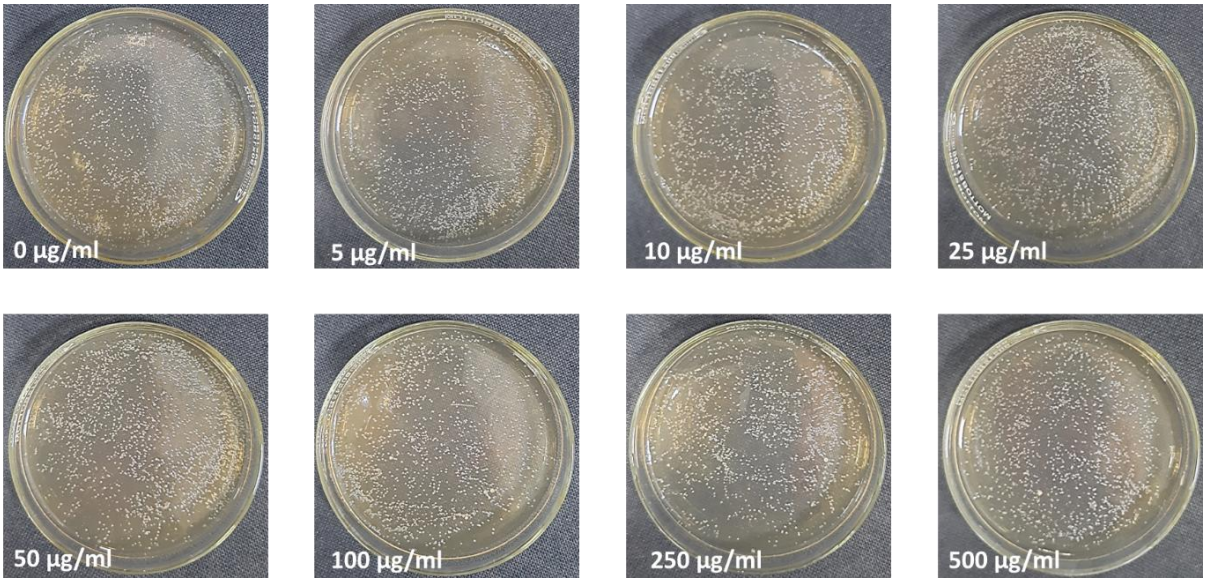


Figure S20. Antibacterial activity against *B. cereus* using different concentrations of **4ac** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

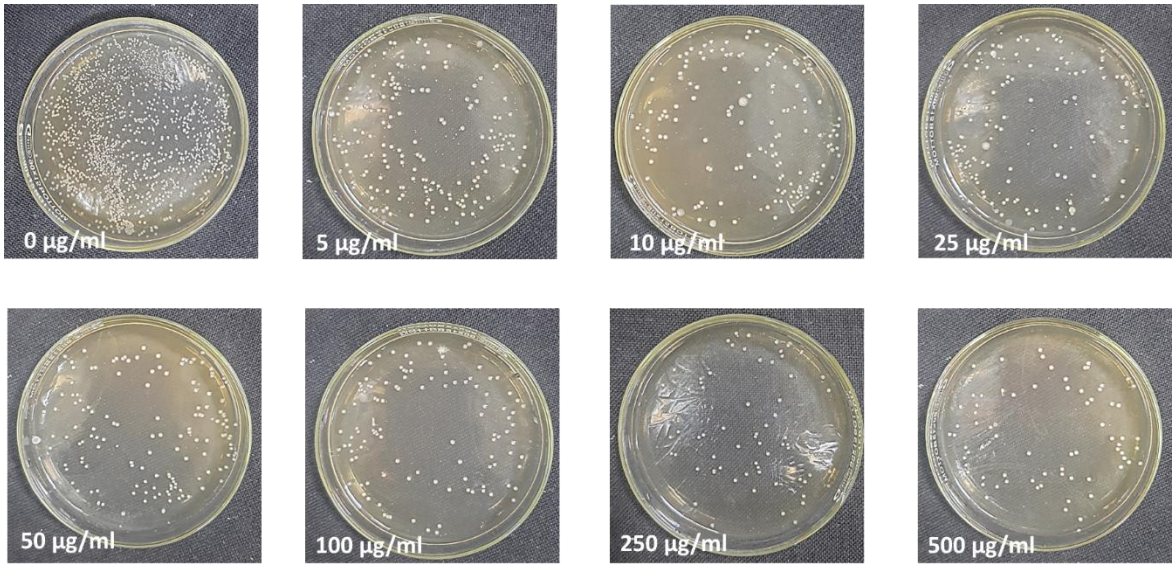


Figure S21. Antibacterial activity against *E. coli* using different concentrations of **4ah** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

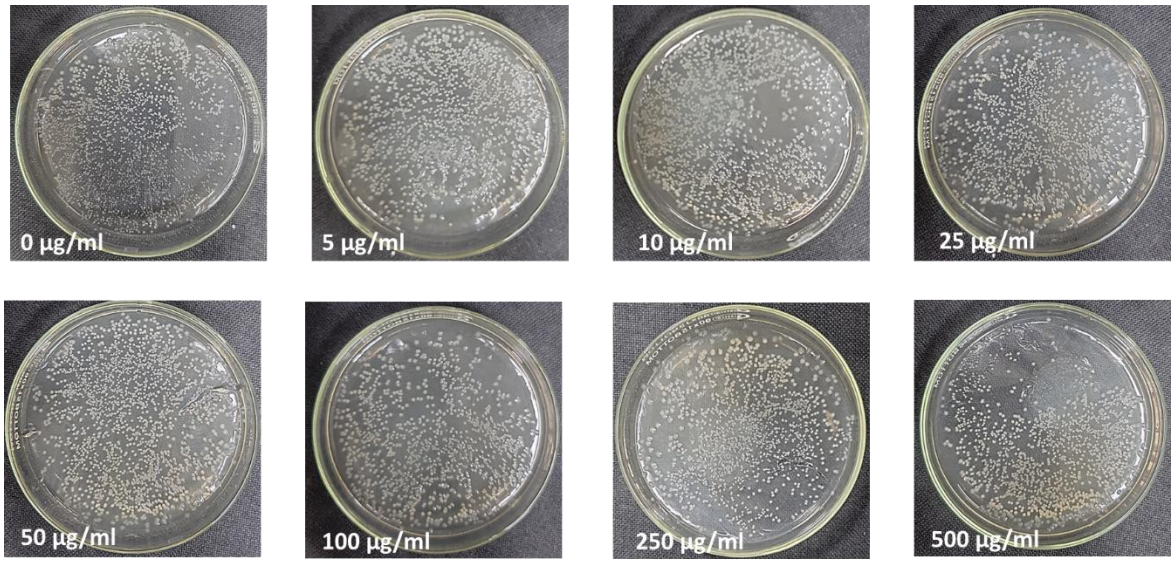


Figure S22. Antibacterial activity against *B. cereus* using different concentrations of **4ah** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

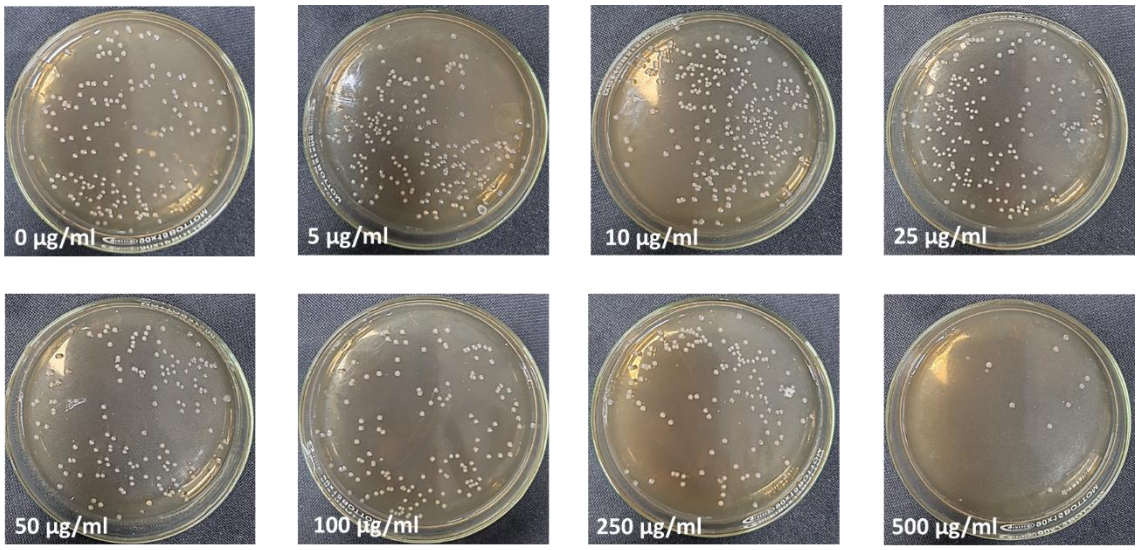


Figure S23. Antibacterial activity against *E. coli* using different concentrations of **4ba** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

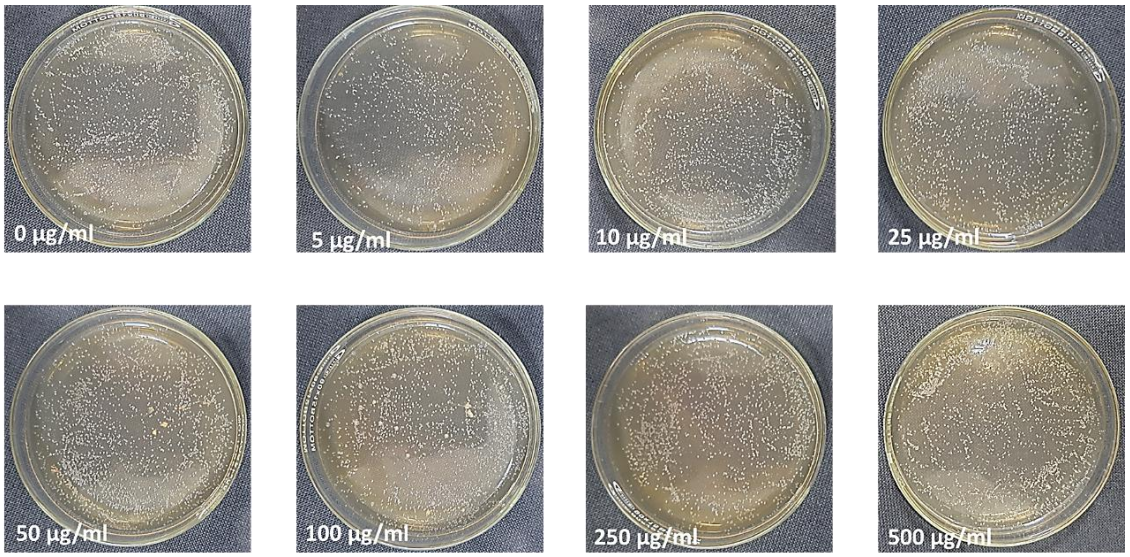


Figure S24. Antibacterial activity against *B. cereus* using different concentrations of **4ba** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

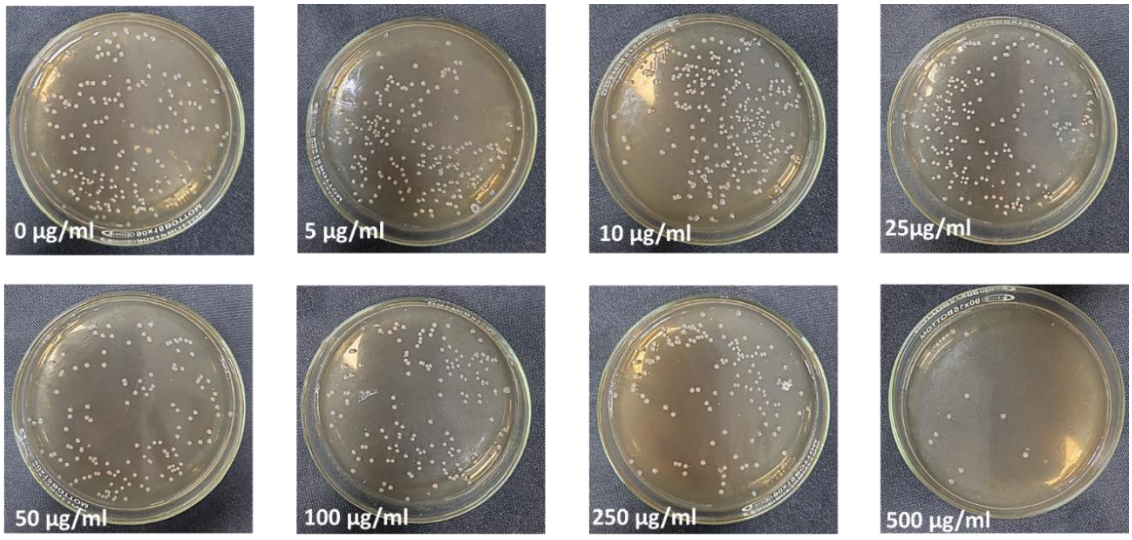


Figure S25. Antibacterial activity against *E. coli* using different concentrations of **4bb** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

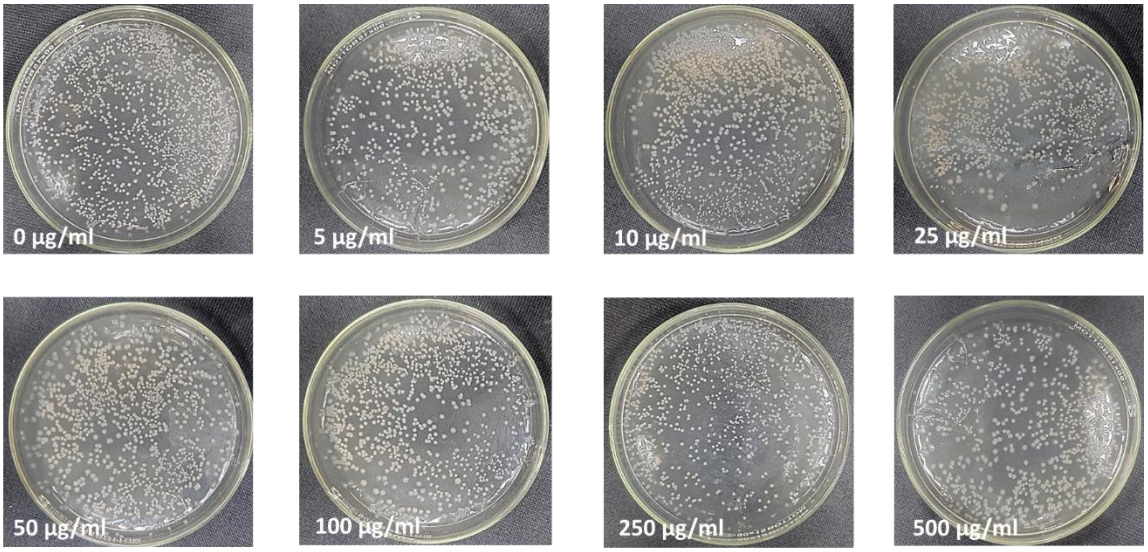


Figure S26. Antibacterial activity against *B. cereus* using different concentrations of **4bb** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

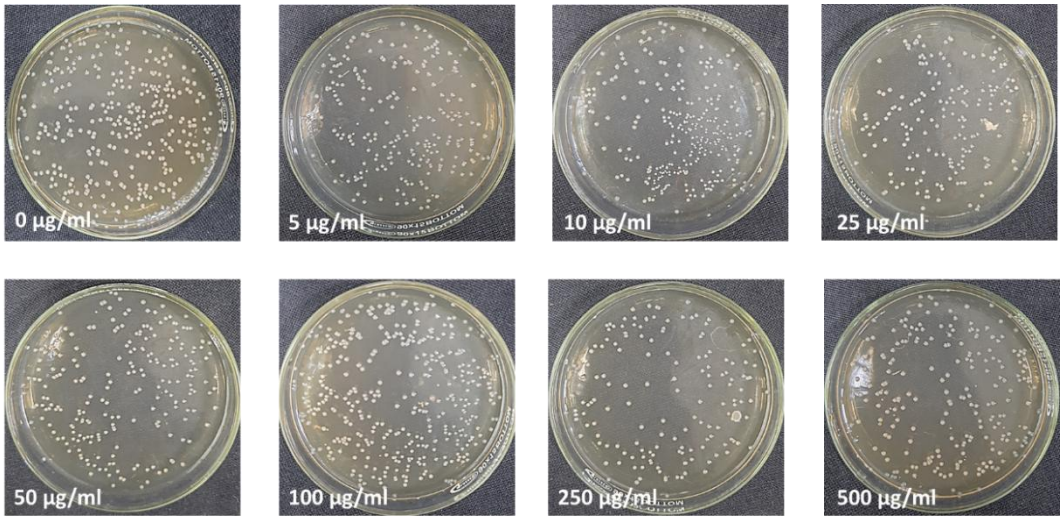


Figure S27. Antibacterial activity against *E. coli* using different concentrations of **4bc** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

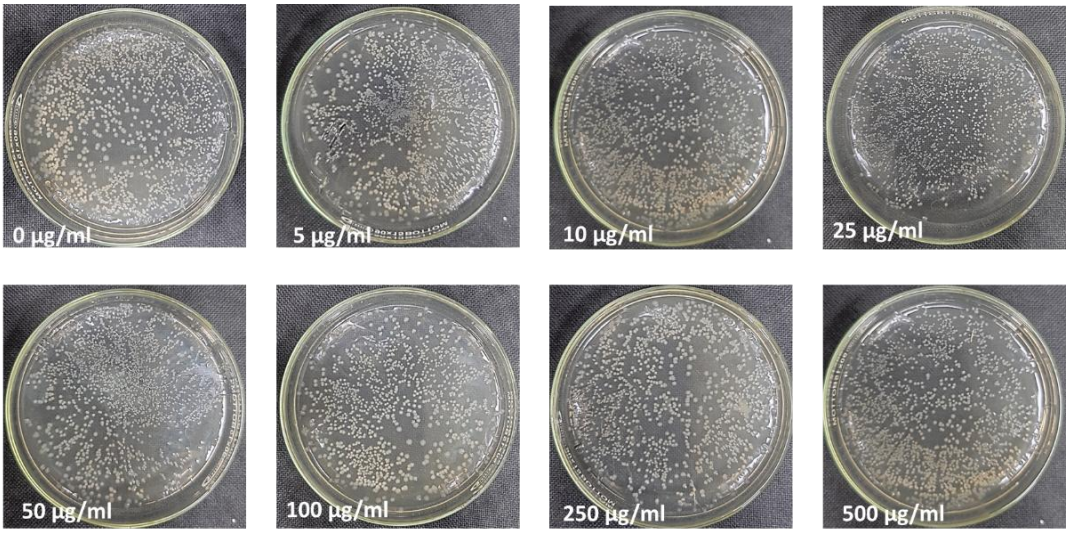


Figure S28. Antibacterial activity against *B. cereus* using different concentrations of **4bc** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

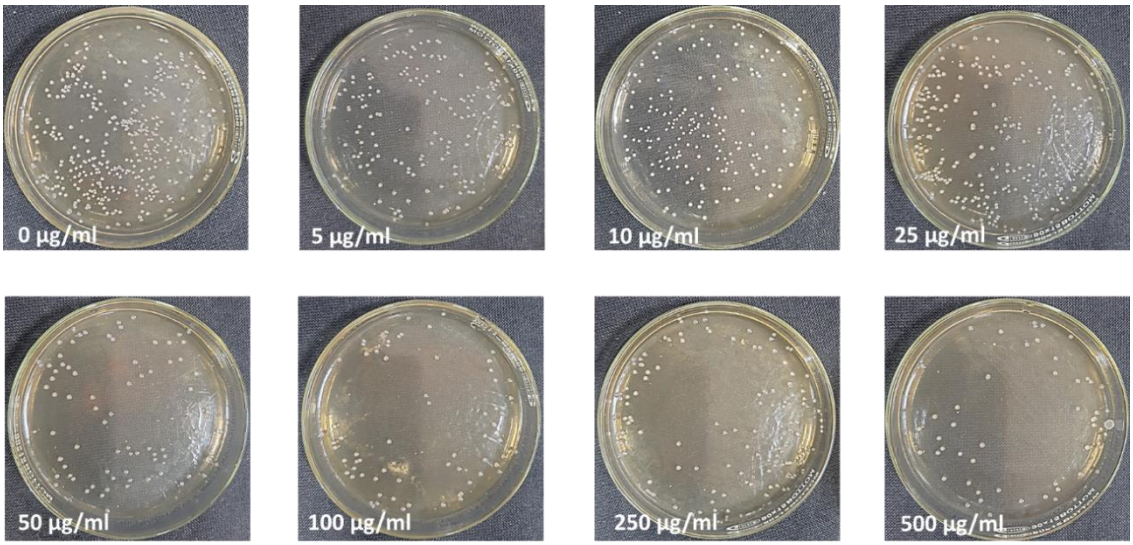


Figure S29. Antibacterial activity against *E. coli* using different concentrations of **4ca** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

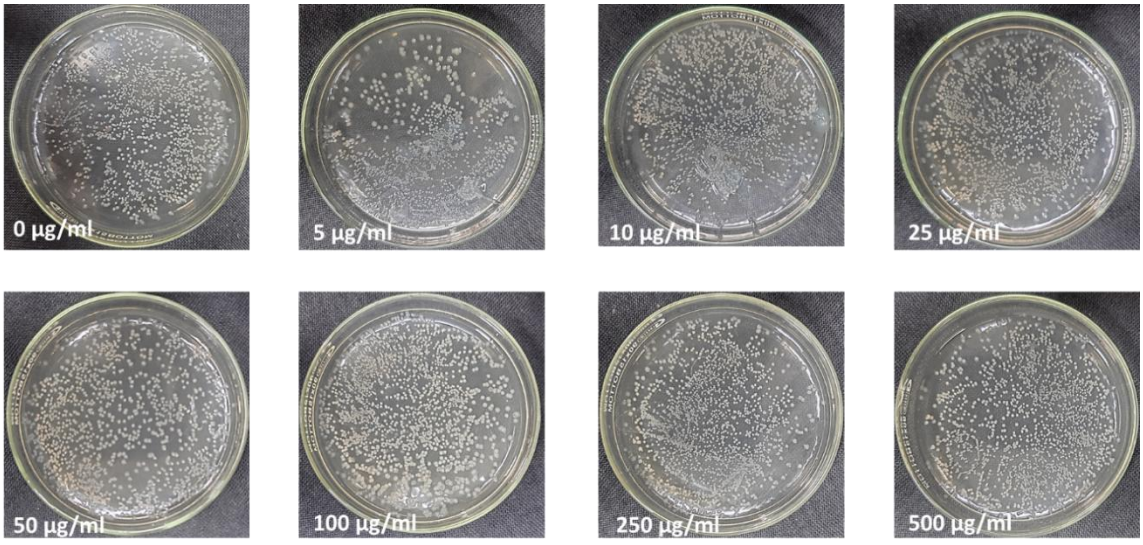


Figure S30. Antibacterial activity against *B. cereus* using different concentrations of **4ca** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

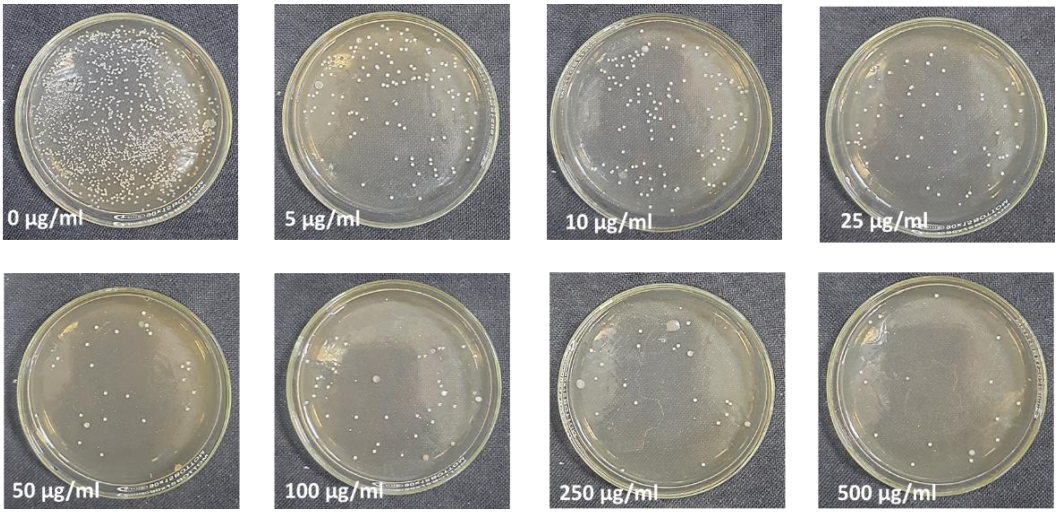


Figure S31. Antibacterial activity against *E. coli* using different concentrations of **4cb** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

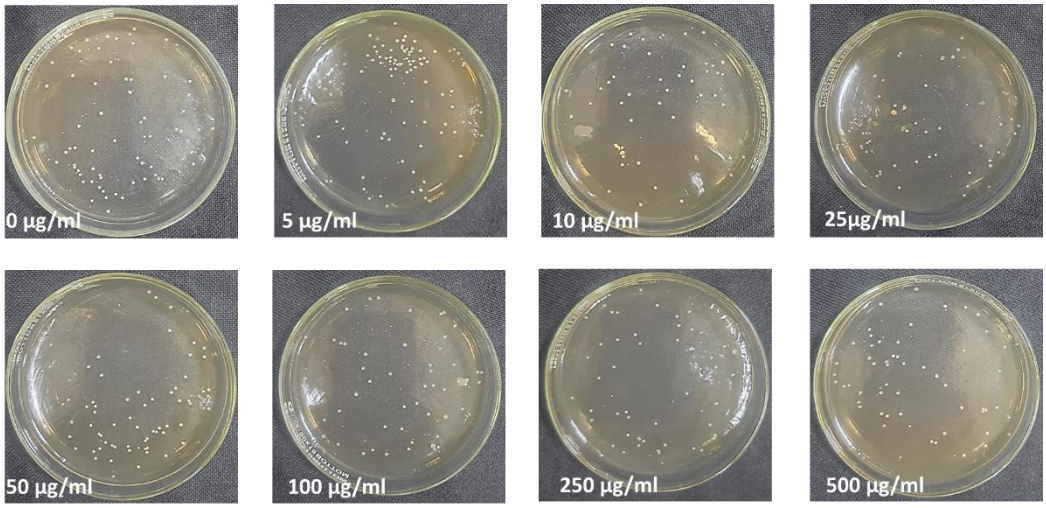


Figure S32. Antibacterial activity against *B. cereus* using different concentrations of **4cb** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

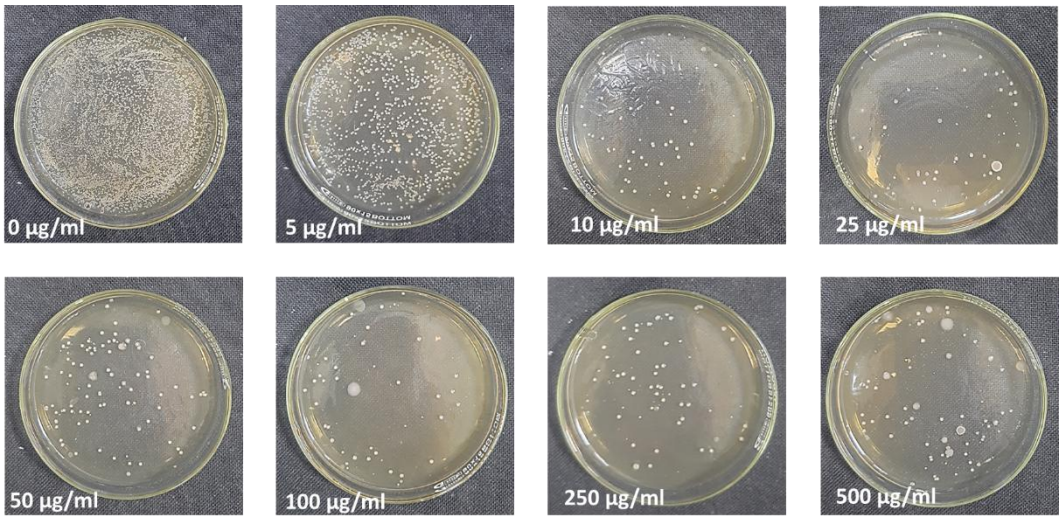


Figure S33. Antibacterial activity against *E. coli* using different concentrations of **4cd** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

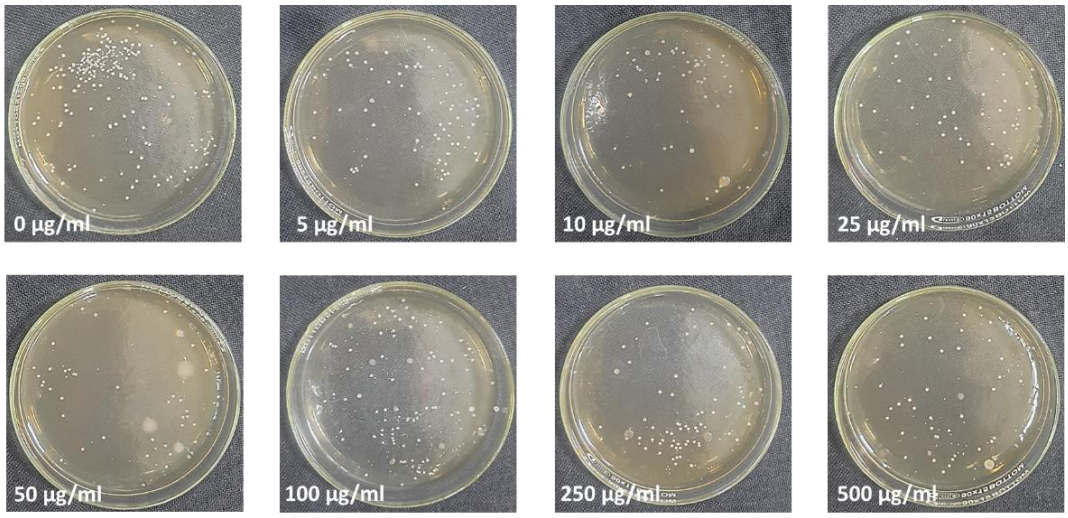


Figure S34. Antibacterial activity against *B. cereus* using different concentrations of **4cd** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

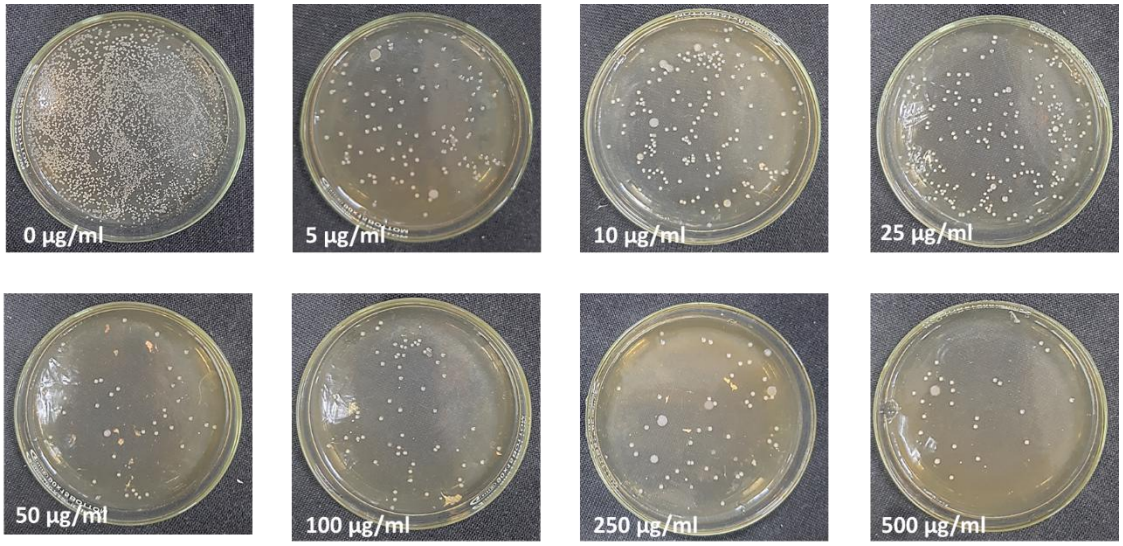


Figure S35. Antibacterial activity against *E. coli* using different concentrations of **4da** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

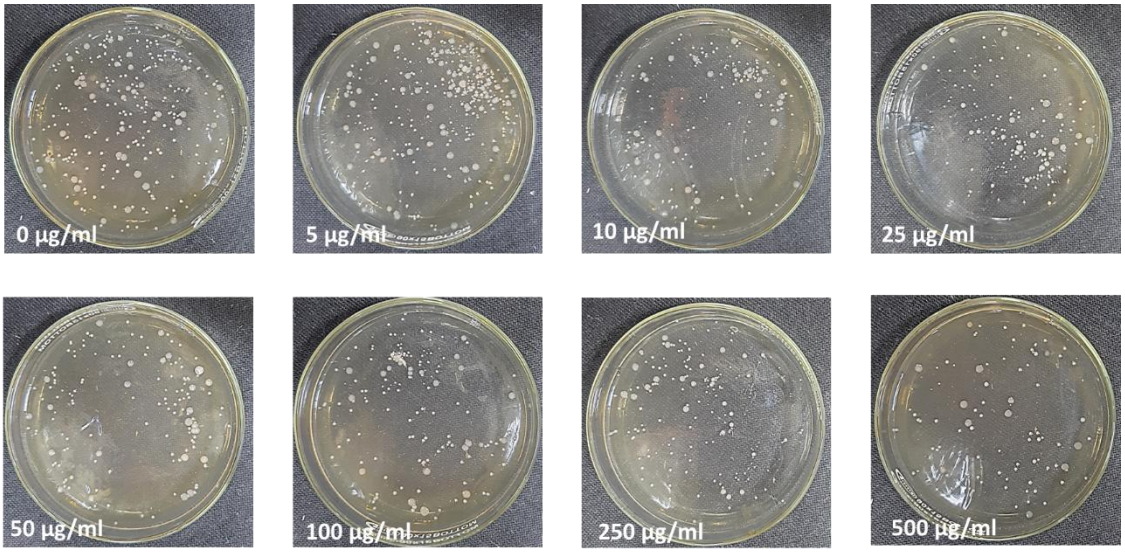


Figure S36. Antibacterial activity against *B. cereus* using different concentrations of **4da** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

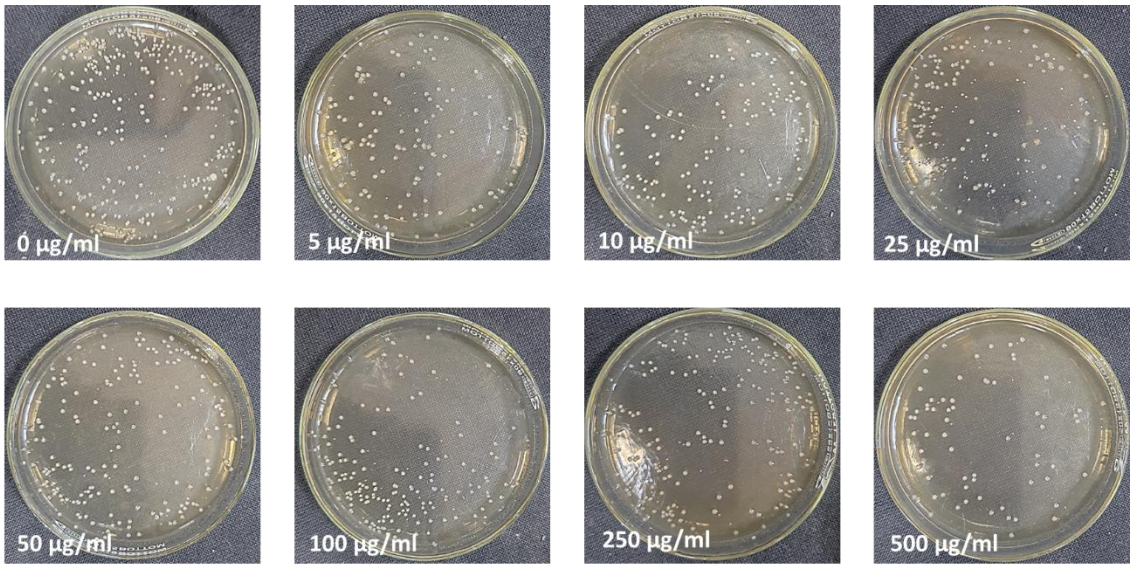


Figure S37. Antibacterial activity against *E. coli* using different concentrations of **4db** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

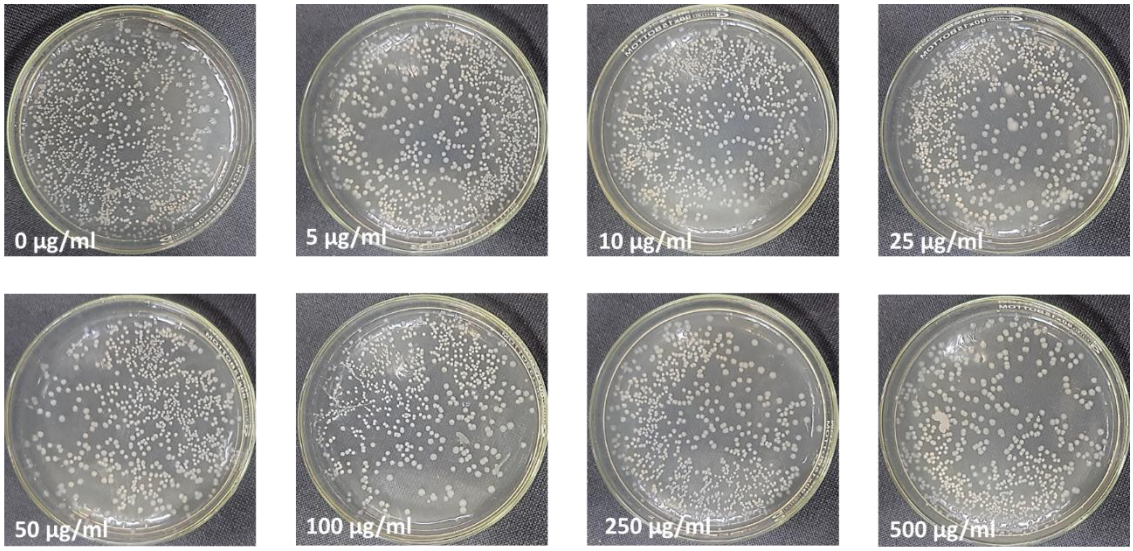


Figure S38. Antibacterial activity against *B. cereus* using different concentrations of **4db** (a) 0, (b) 5, (c) 10, (d) 25, (e) 50, (f) 100 (g) 250 (h) 500 µg/mL.

15. References

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2. A. J. Thakur, S. Das, A. K. Phukan *J. Mol. Struct.* 2009, **929**, 134-140.
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16. Copies of NMR Spectra

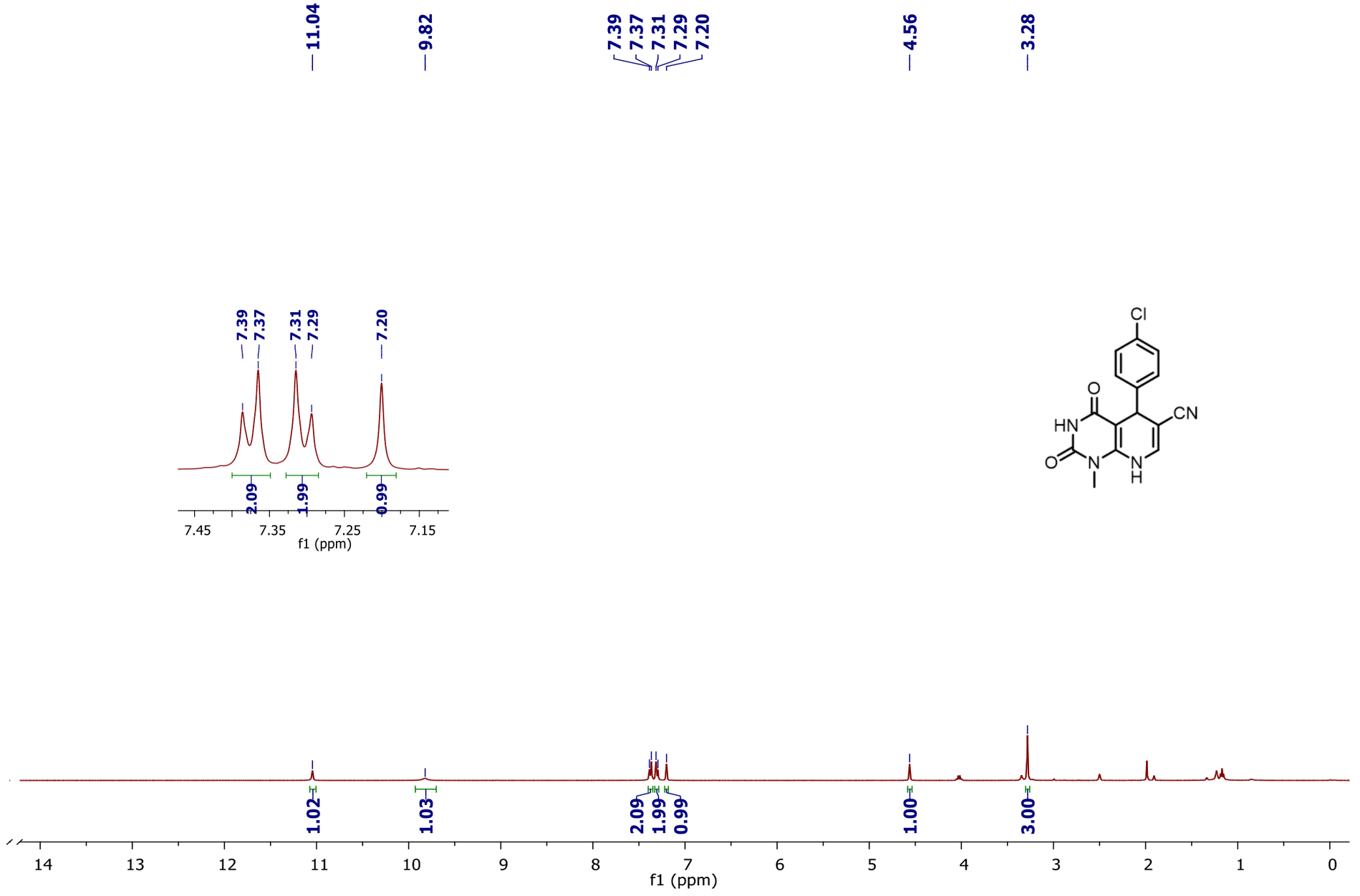

Figure S39. ^{1}H-NMR (DMSO-d_6) spectra of compound **4aa**.

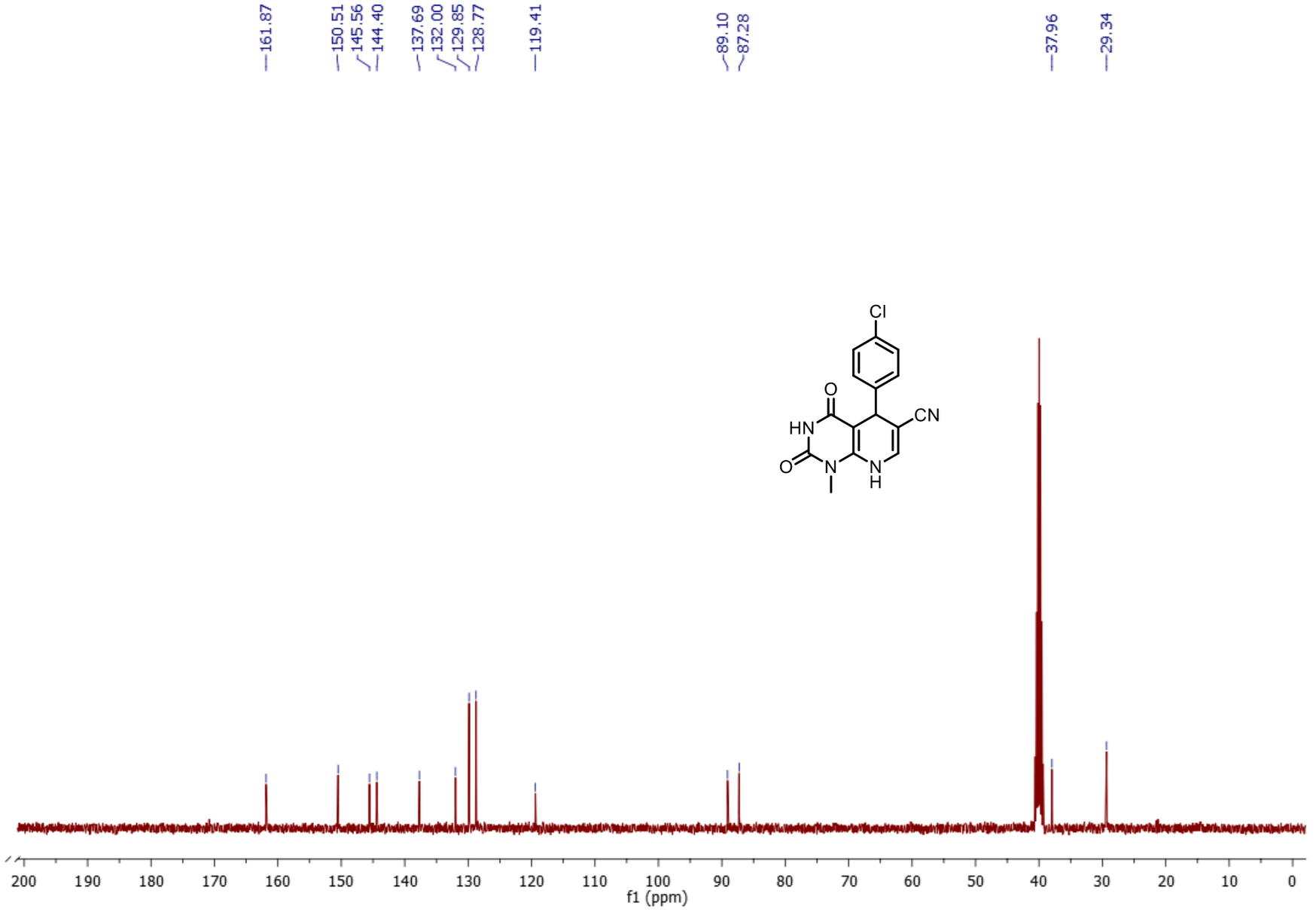

Figure S40. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **4aa**.

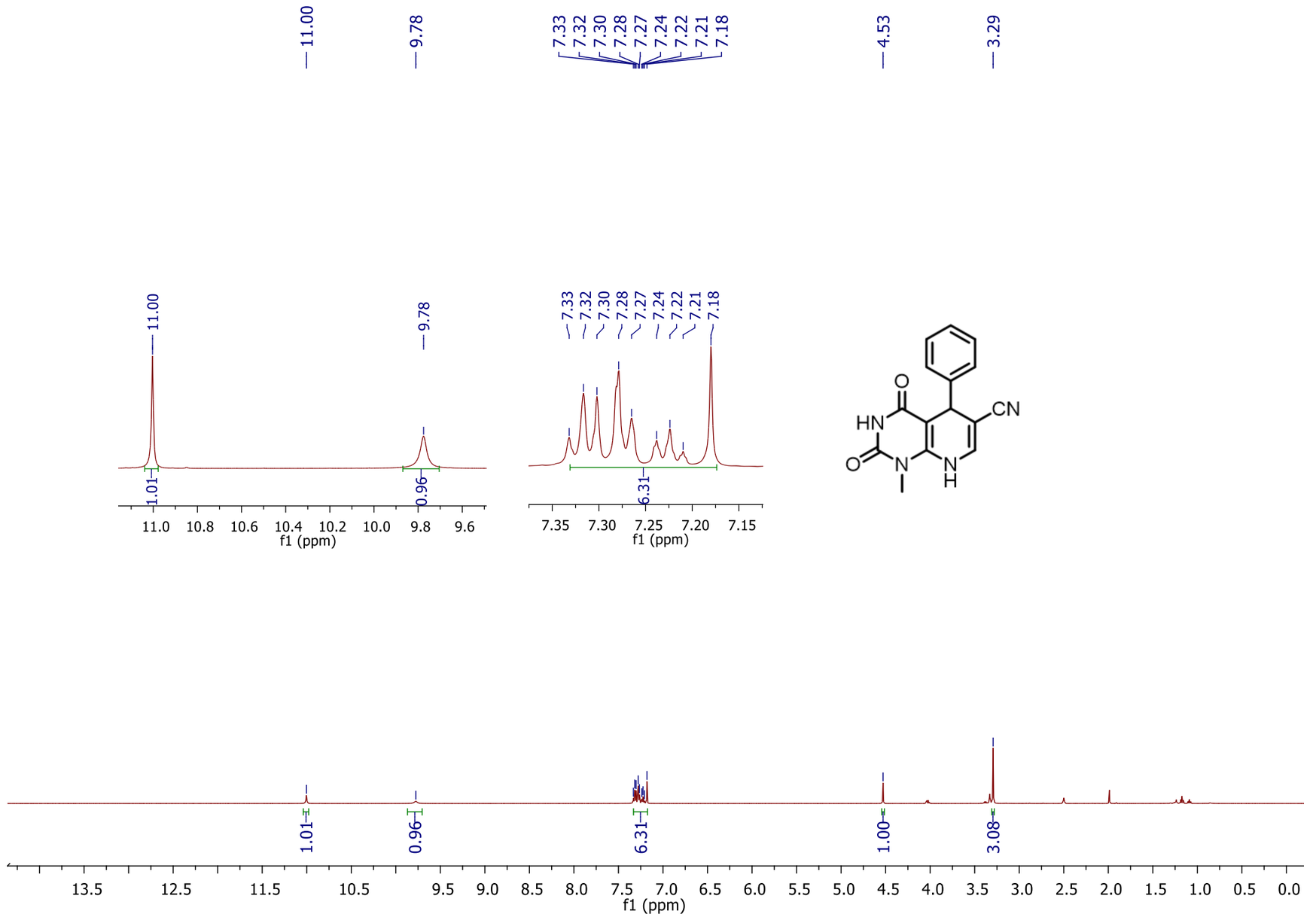

Figure S41. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ab**.

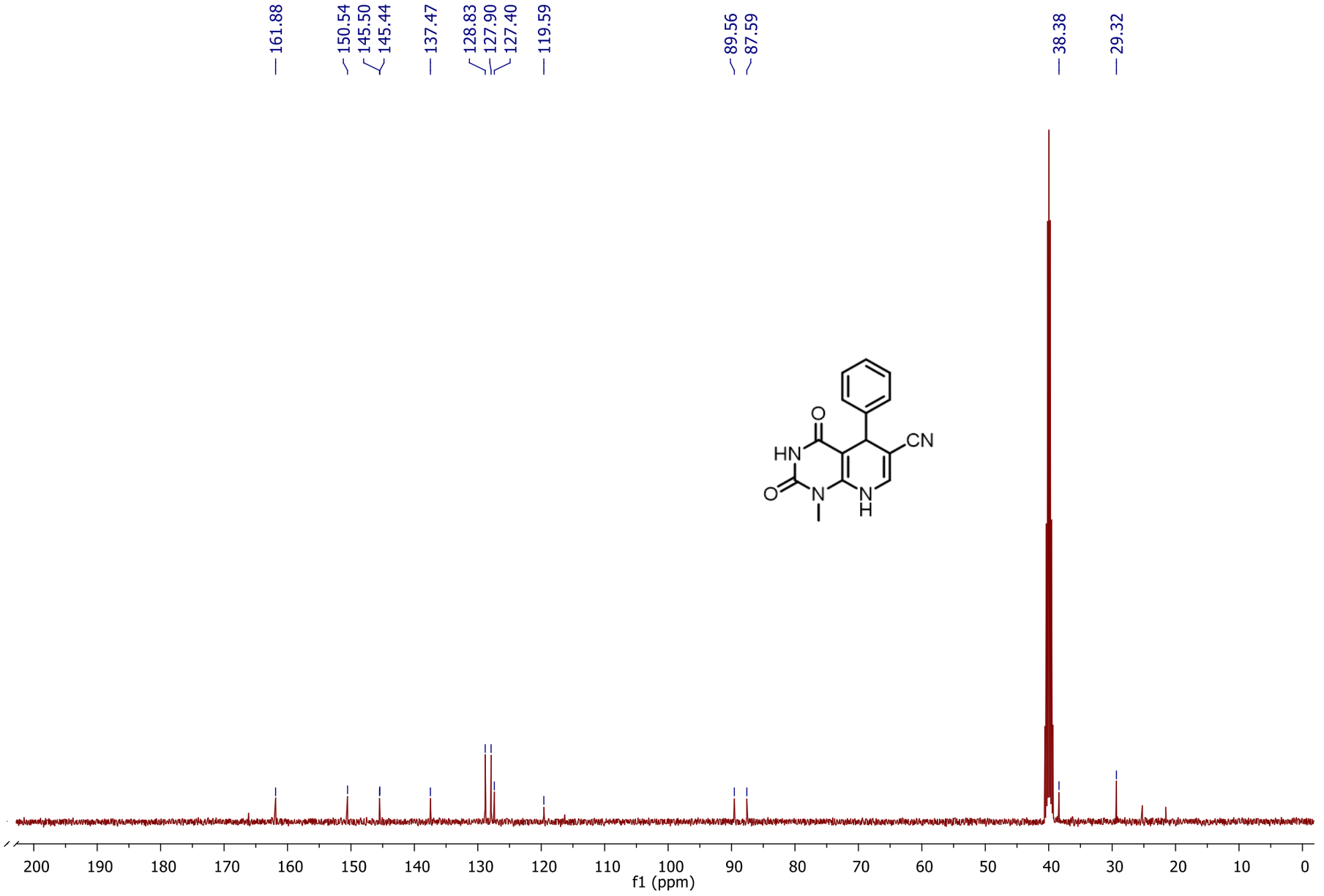

Figure S42. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **4ab**.

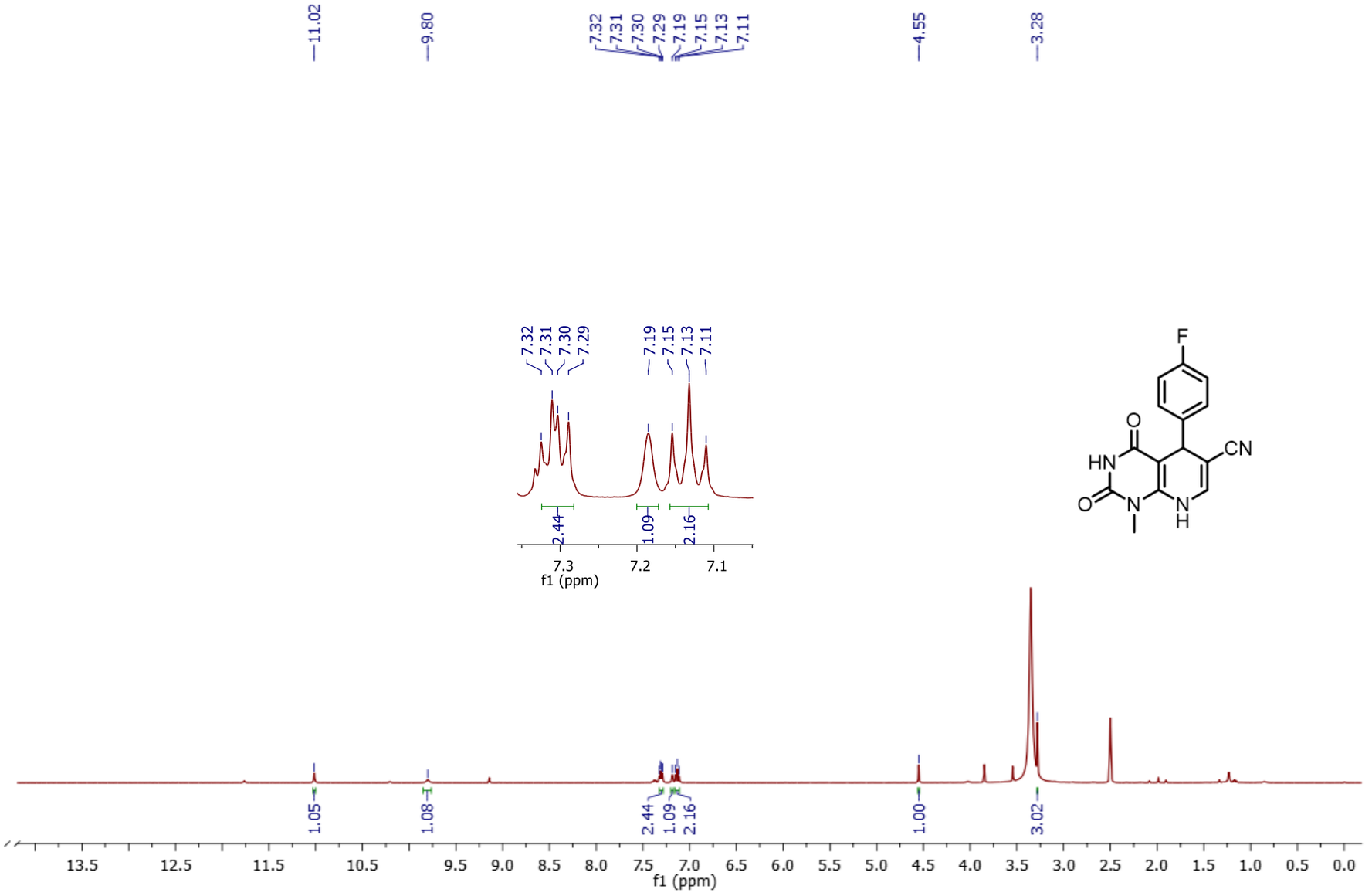

Figure S43. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ac**.

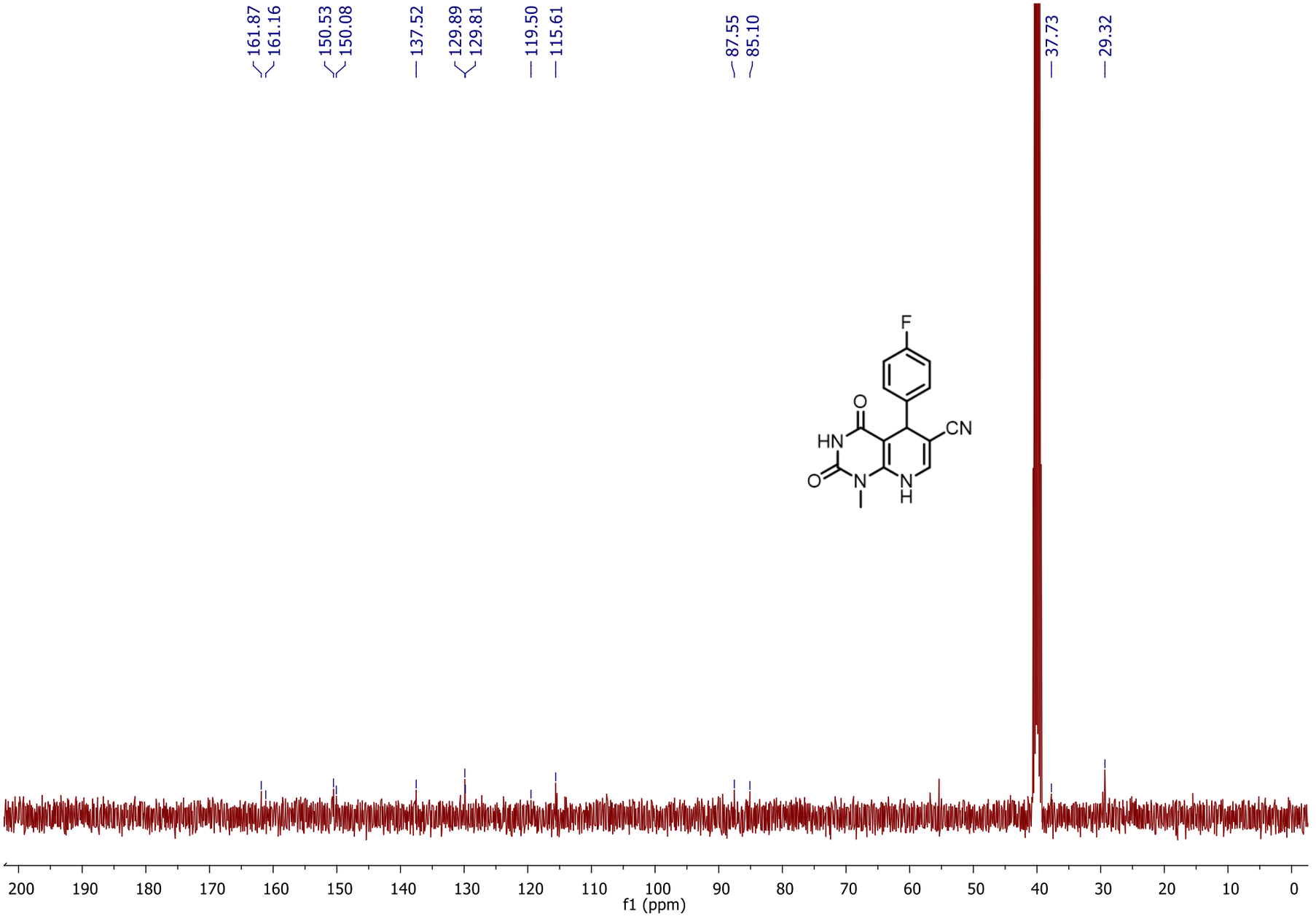

Figure S44. ^{13}C-NMR (DMSO-d_6) spectra of compound **4ac**.

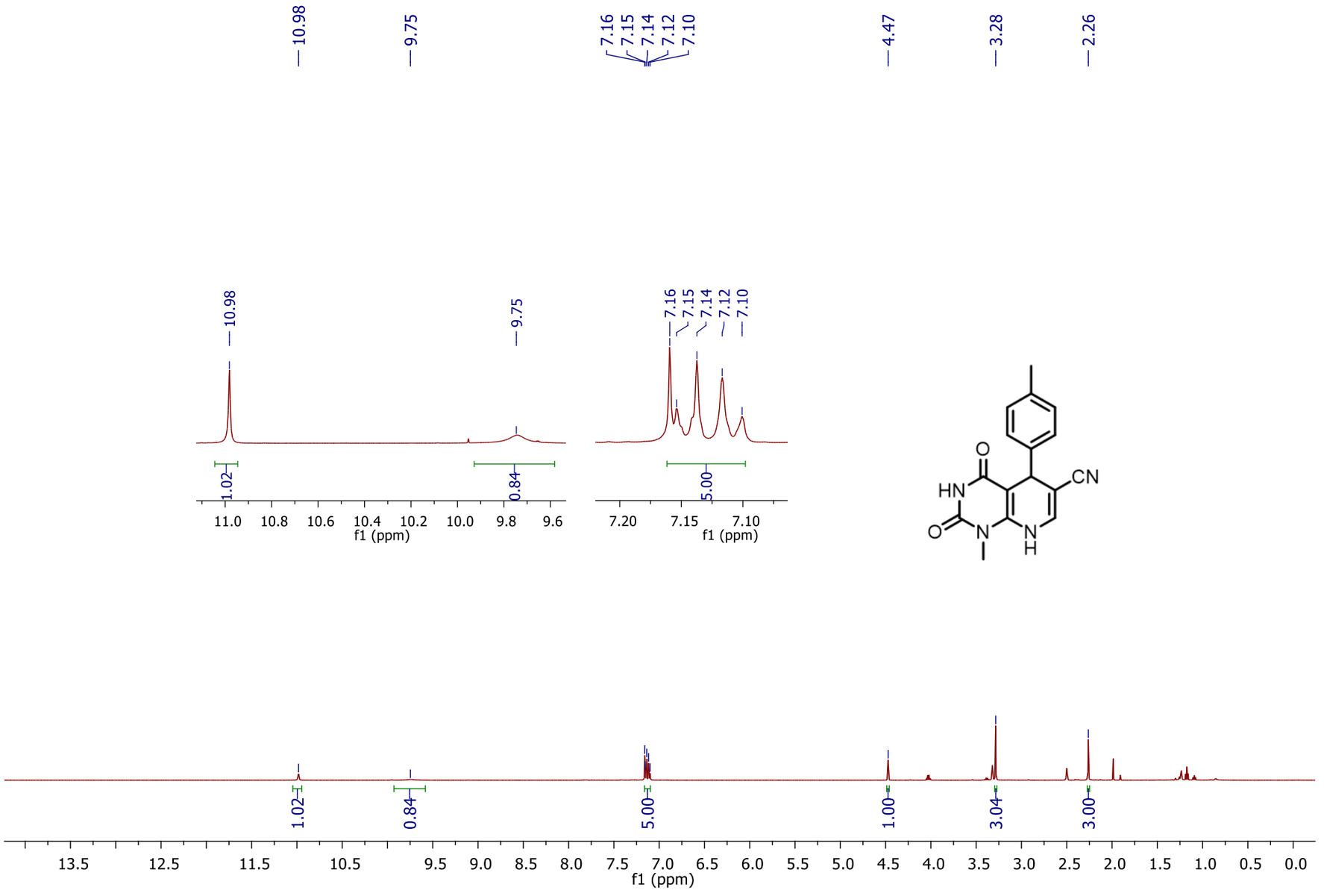

Figure S45. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ad.**

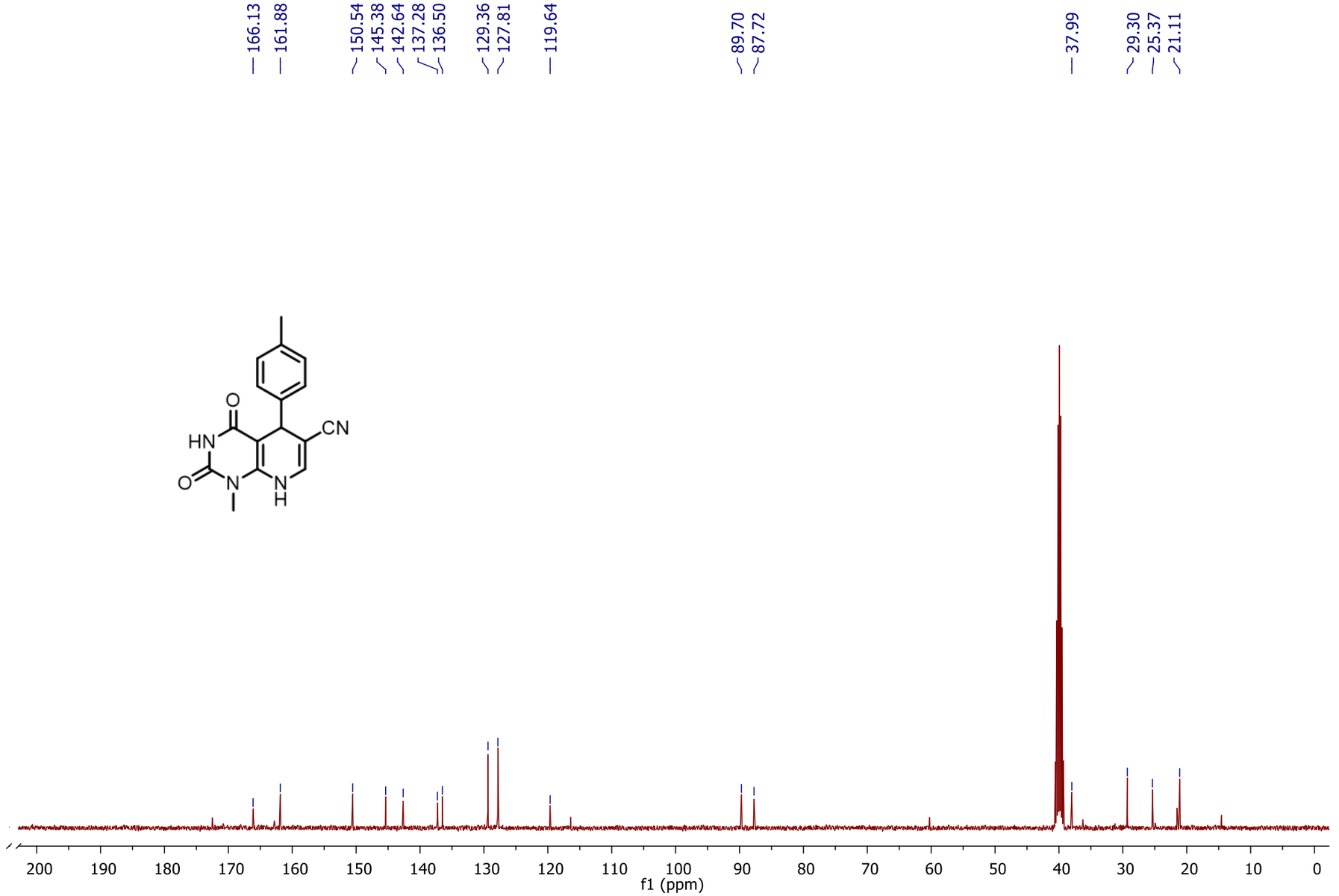

Figure S46. ^{13}C-NMR (DMSO-d_6) spectra of compound **4ad**.

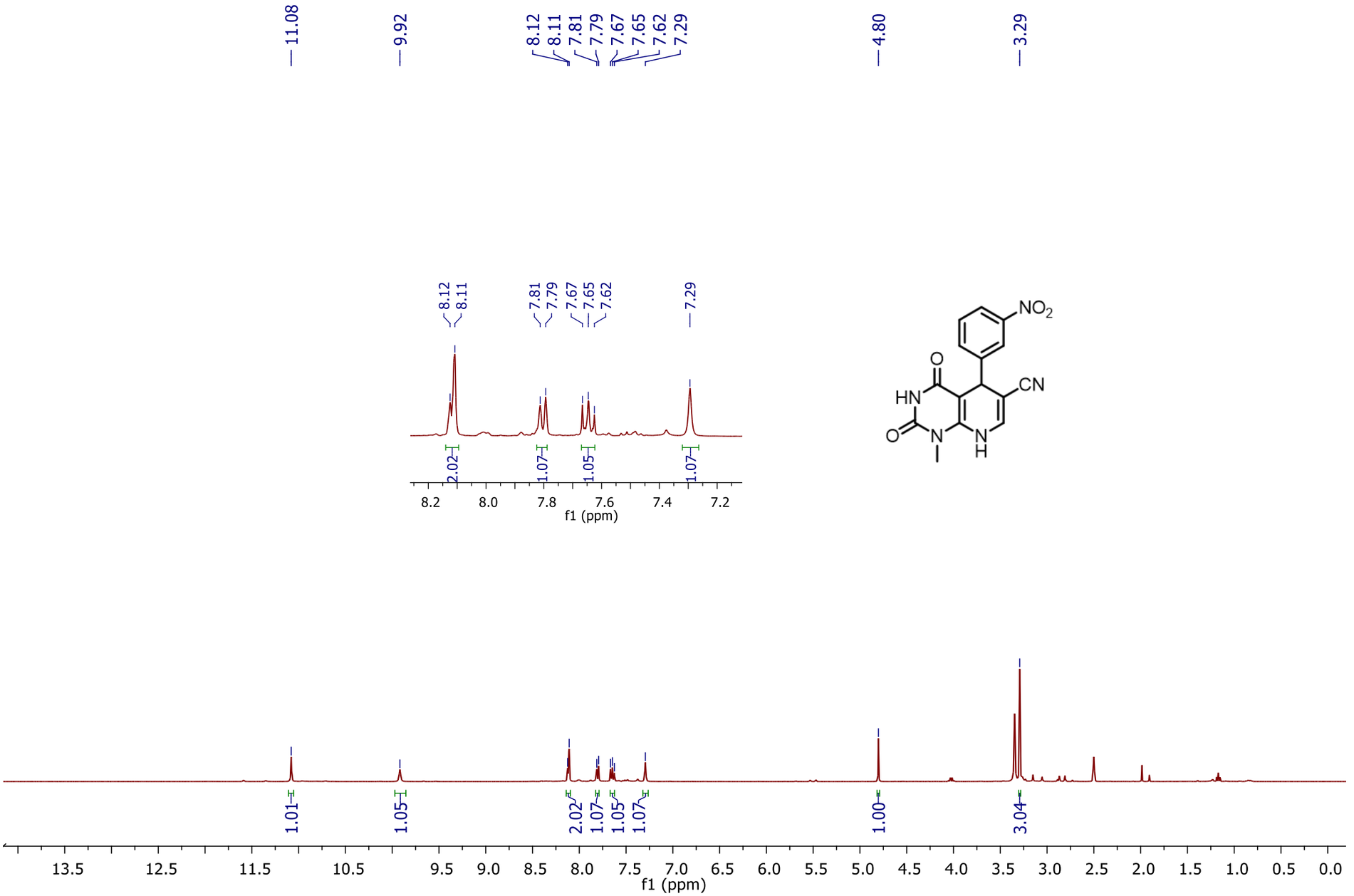

Figure S47. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ae.**

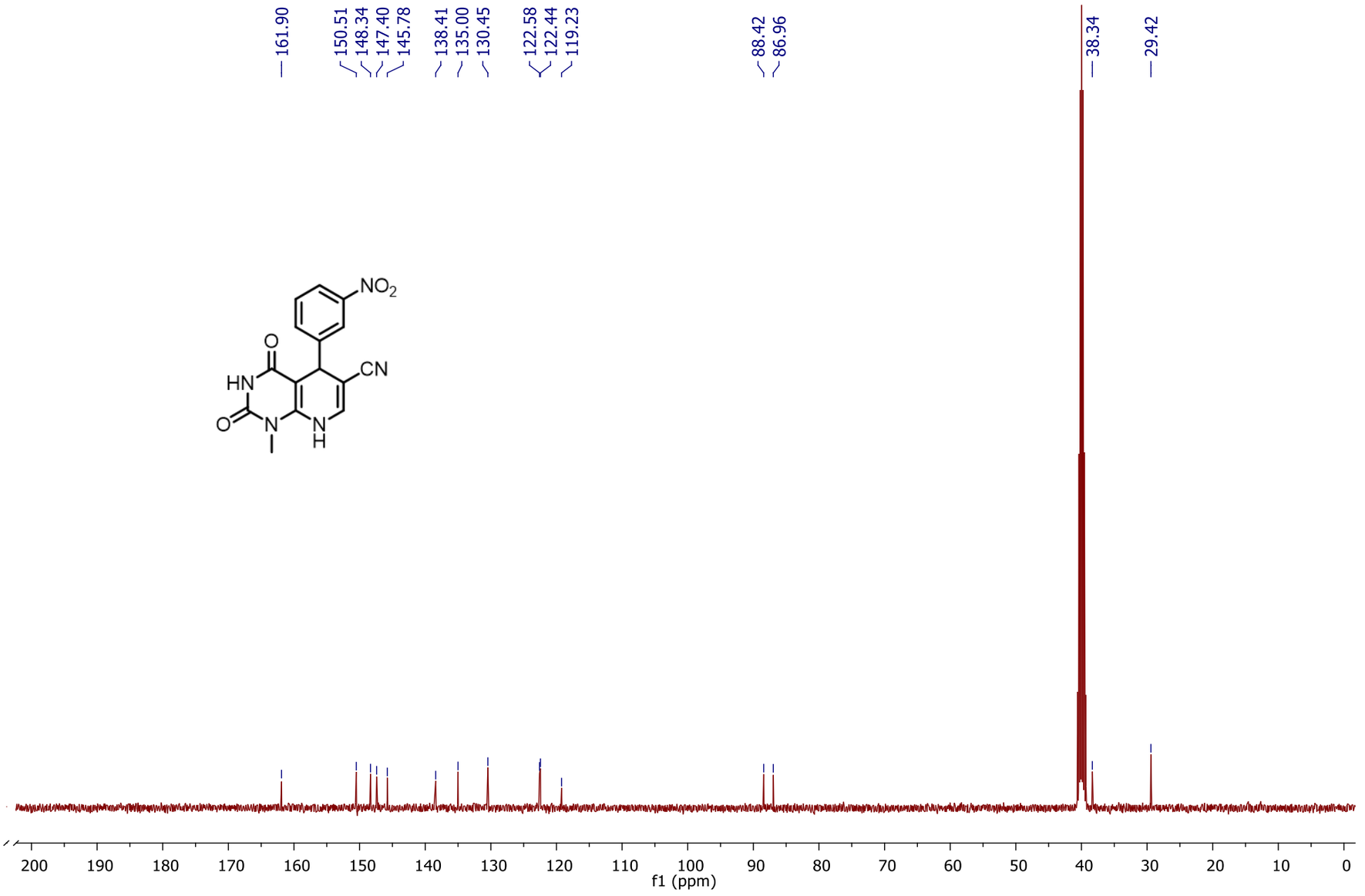

Figure S48. ^{13}C-NMR (DMSO-d_6) spectra of compound **4ae**.

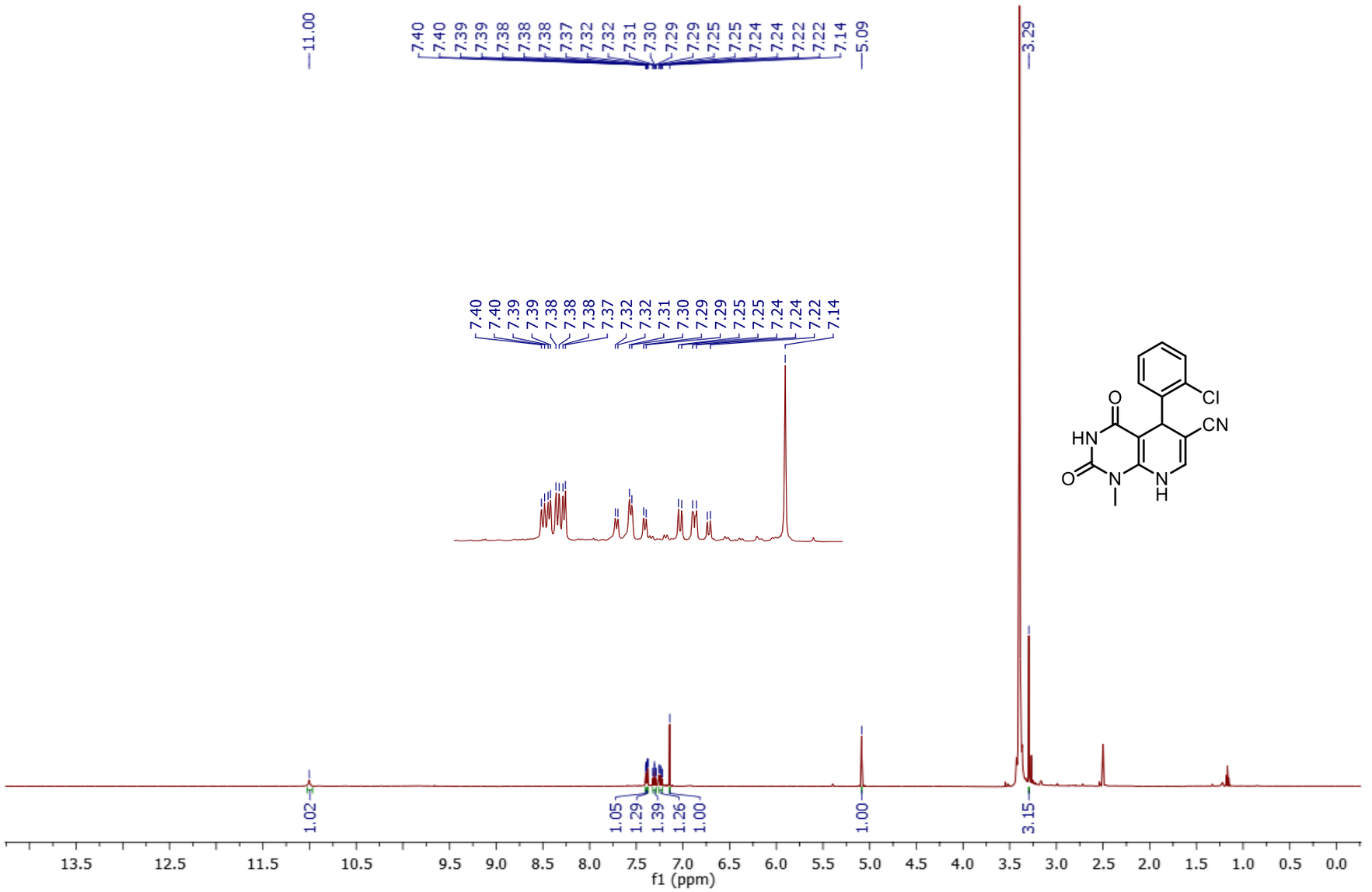

Figure S49. ^{1}H-NMR (DMSO-d_6) spectra of compound **4af.**

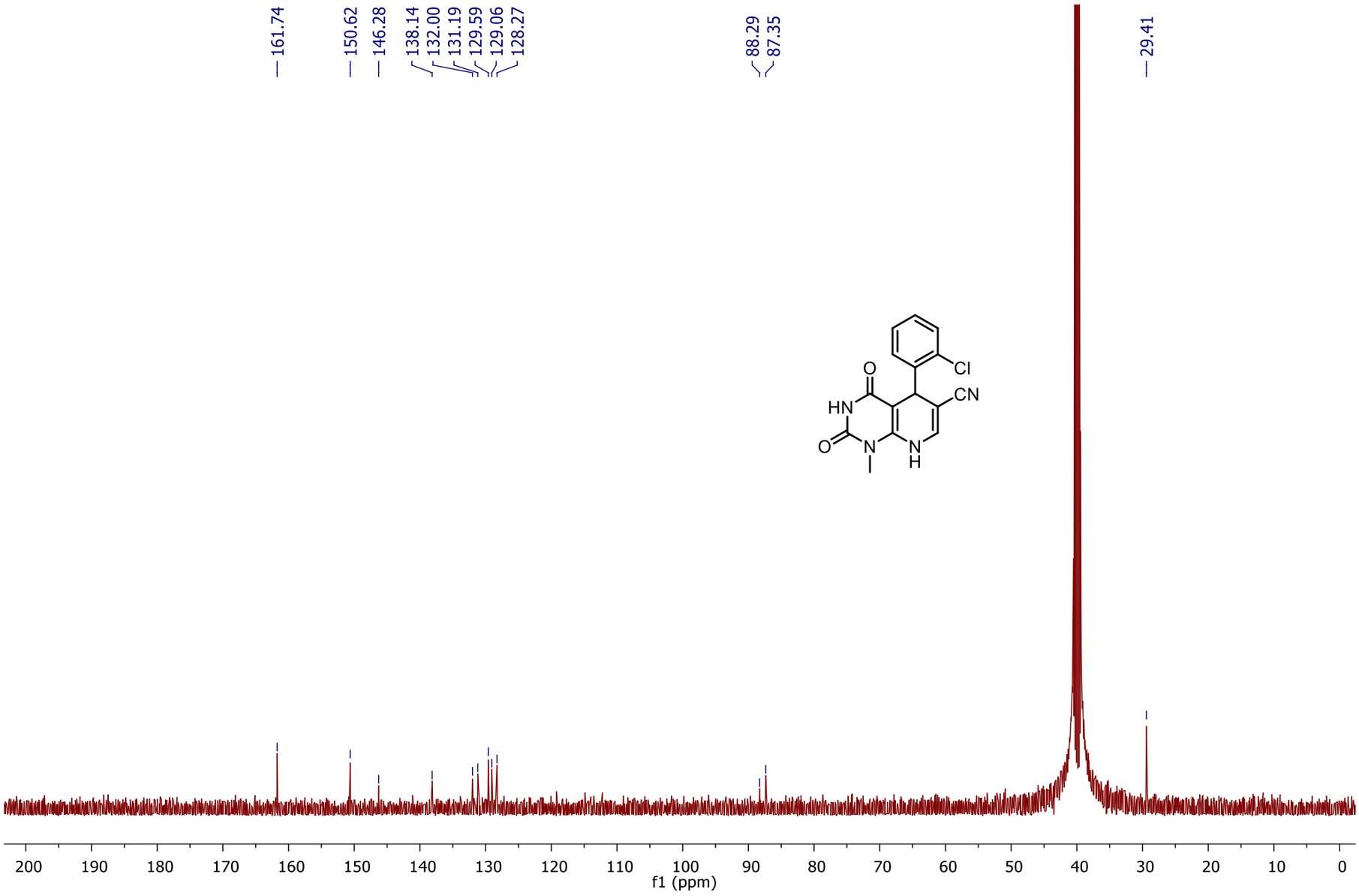

Figure S50. ^{13}C-NMR (DMSO-d_6) spectra of compound **4af**.

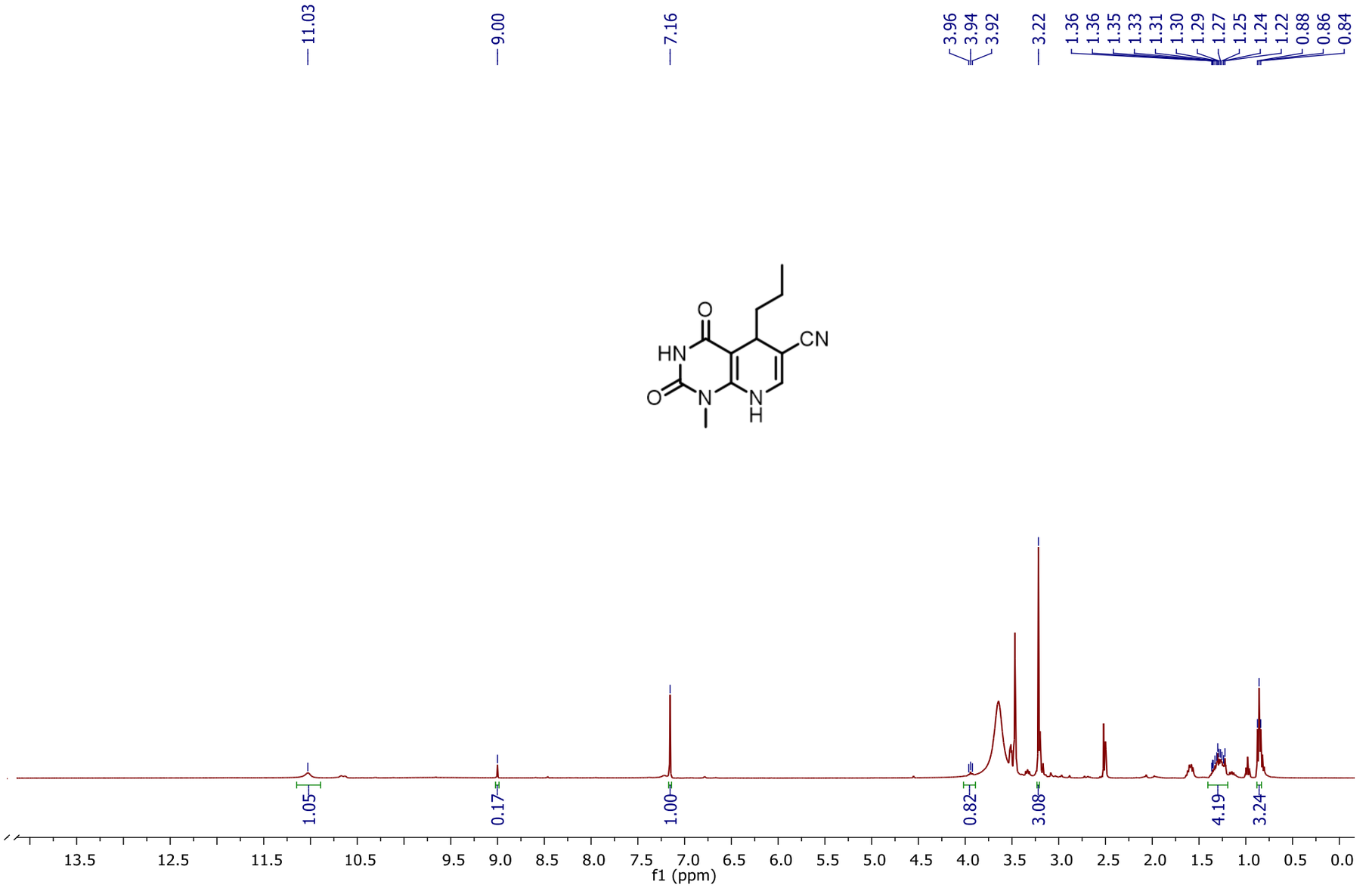

Figure S51. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ag.**

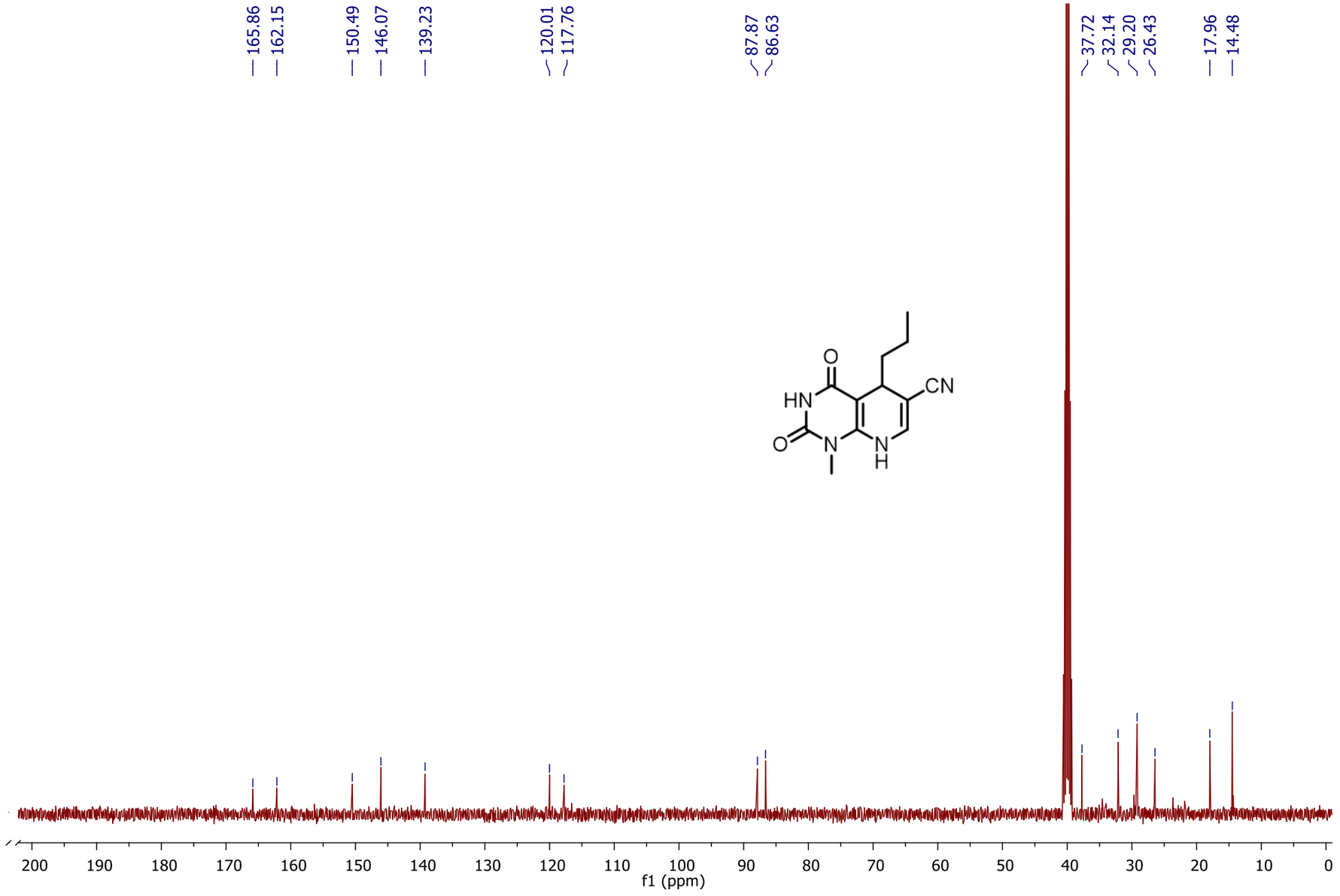

Figure S52. ^{13}C-NMR (DMSO-d_6) spectra of compound **4ag**.

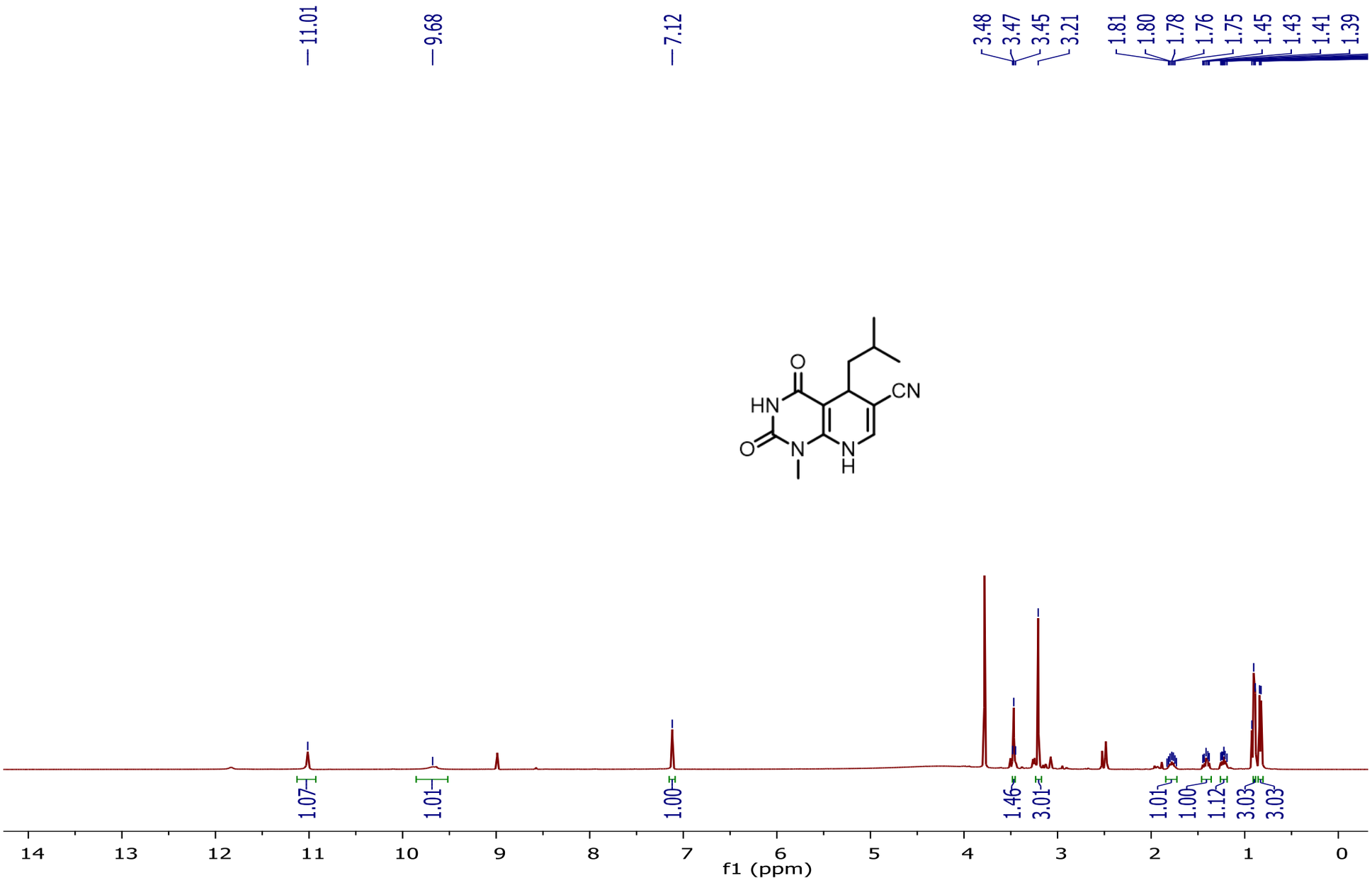

Figure S53. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ah.**

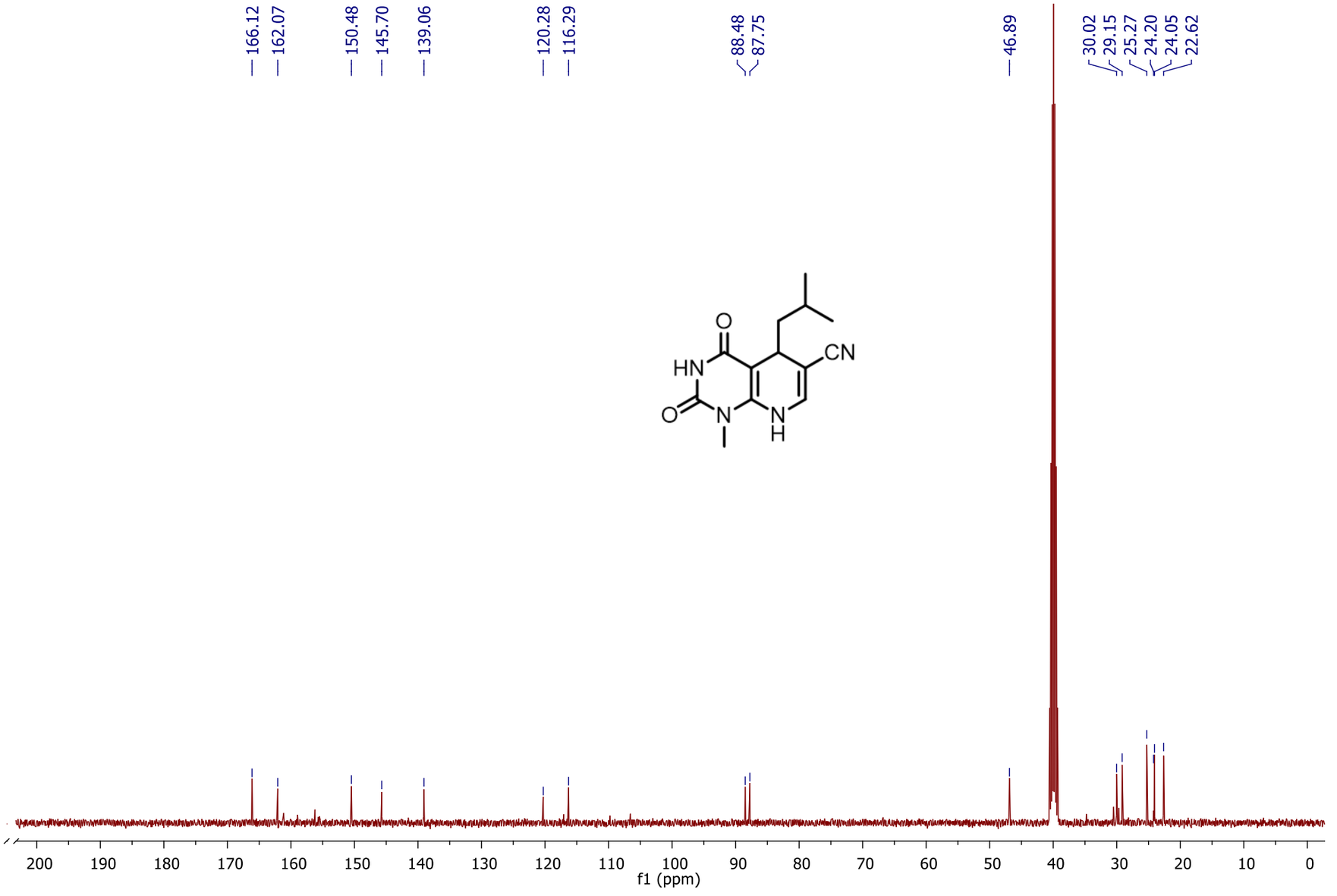

Figure S54. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **4ah**.

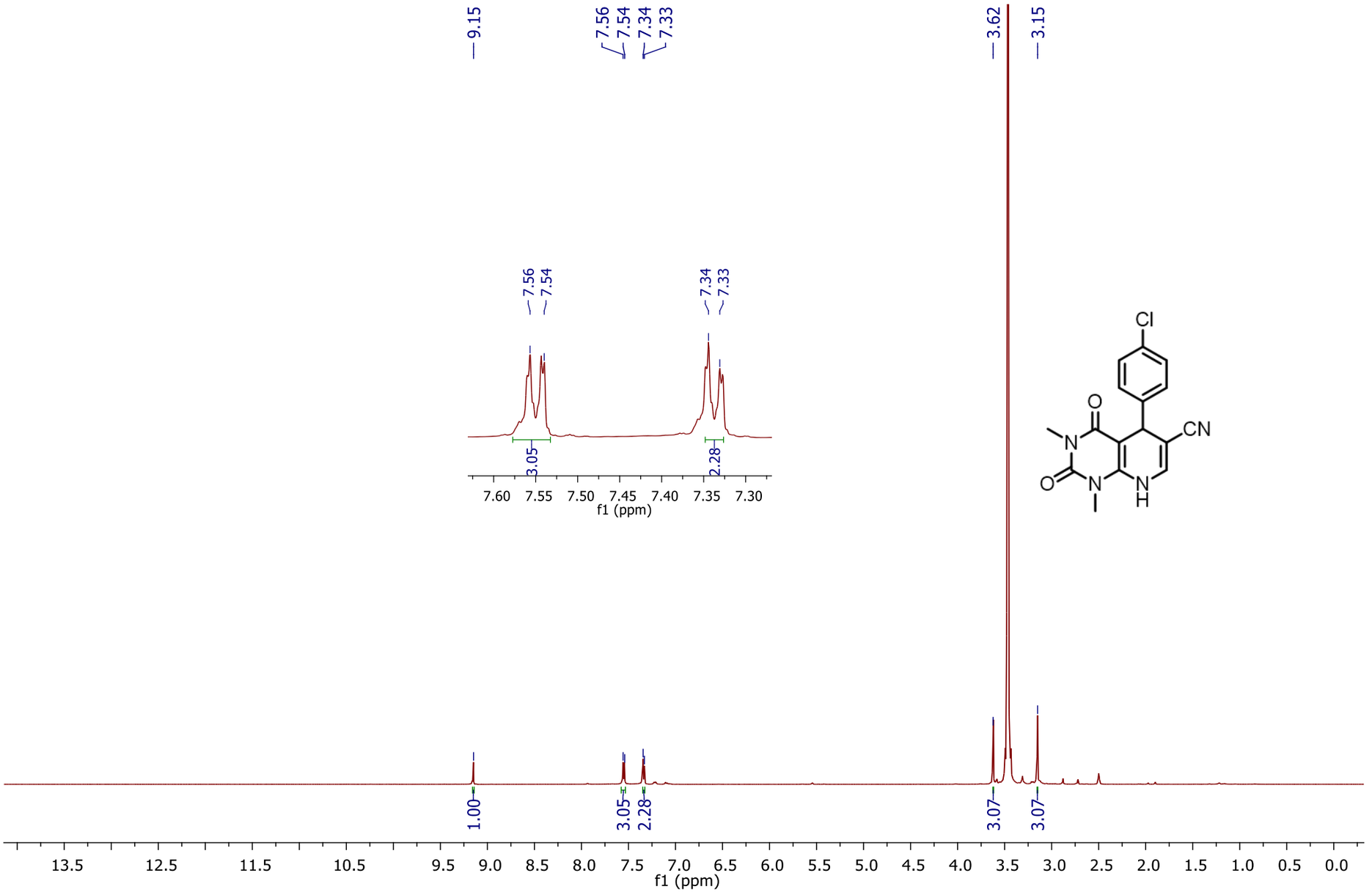

Figure S55. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ba.**

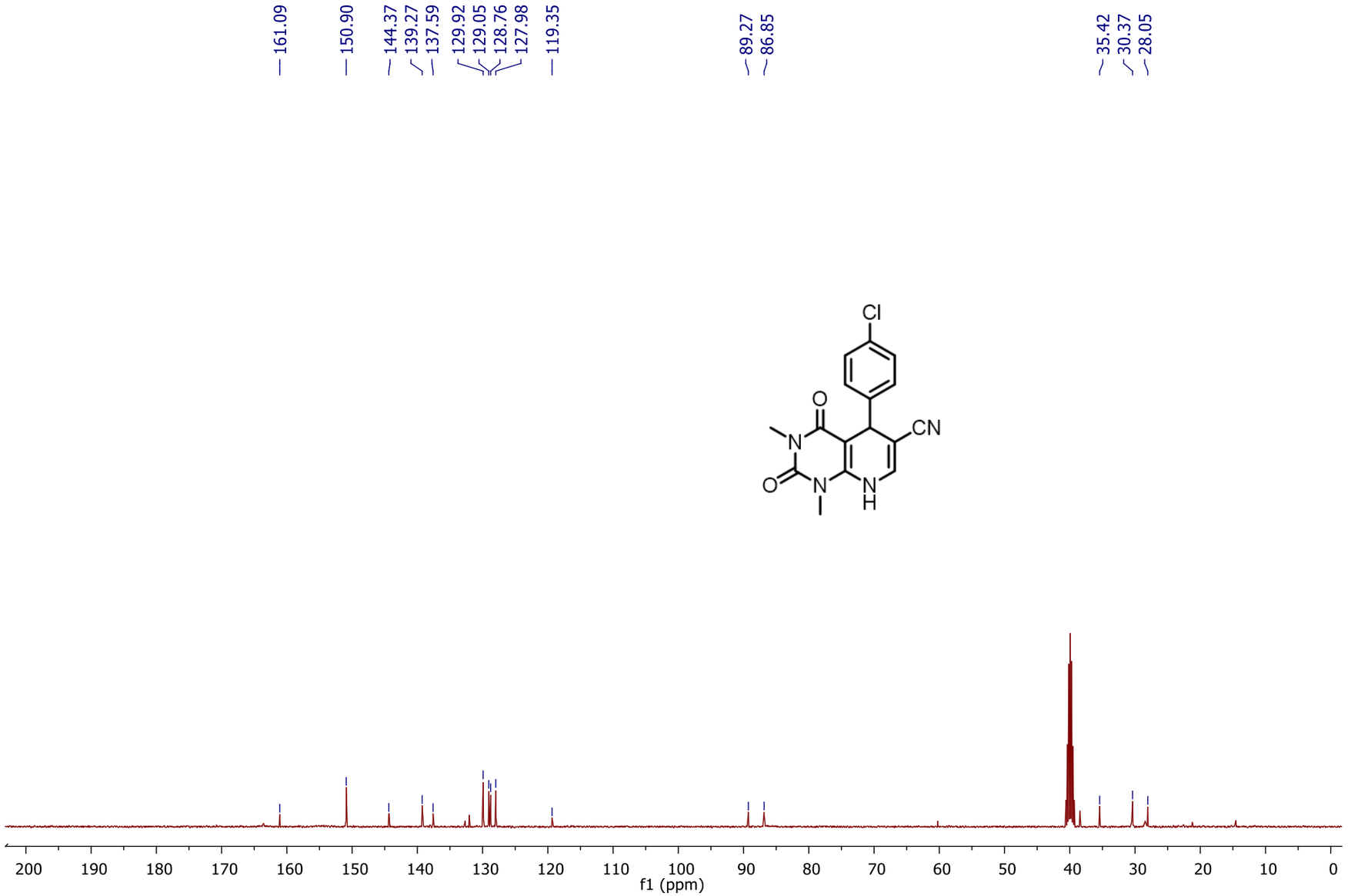

Figure S56. ^{13}C-NMR (DMSO-d_6) spectra of compound **4ba**.

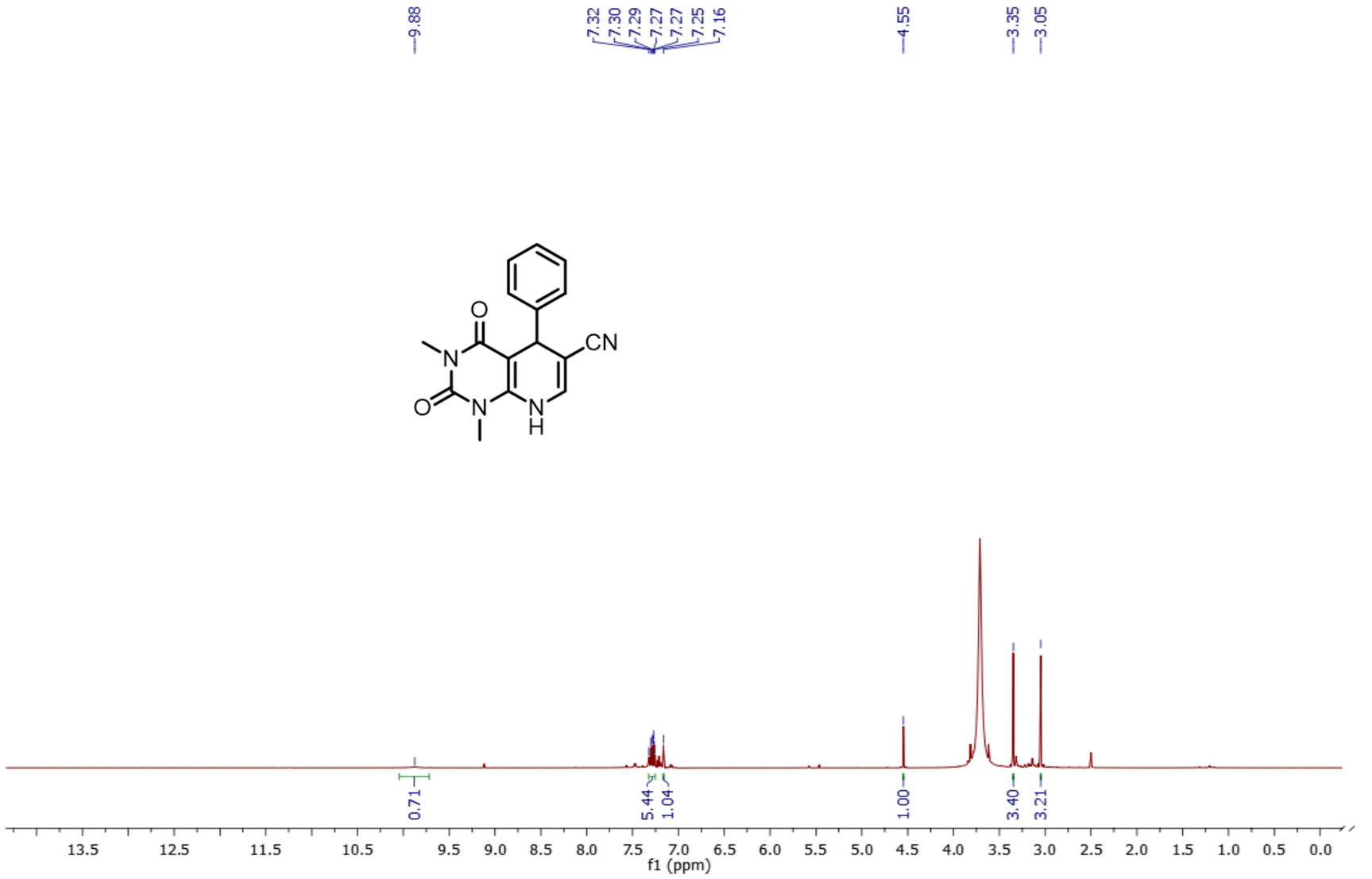

Figure S57. ^{1}H-NMR (DMSO-d_6) spectra of compound **4bb.**

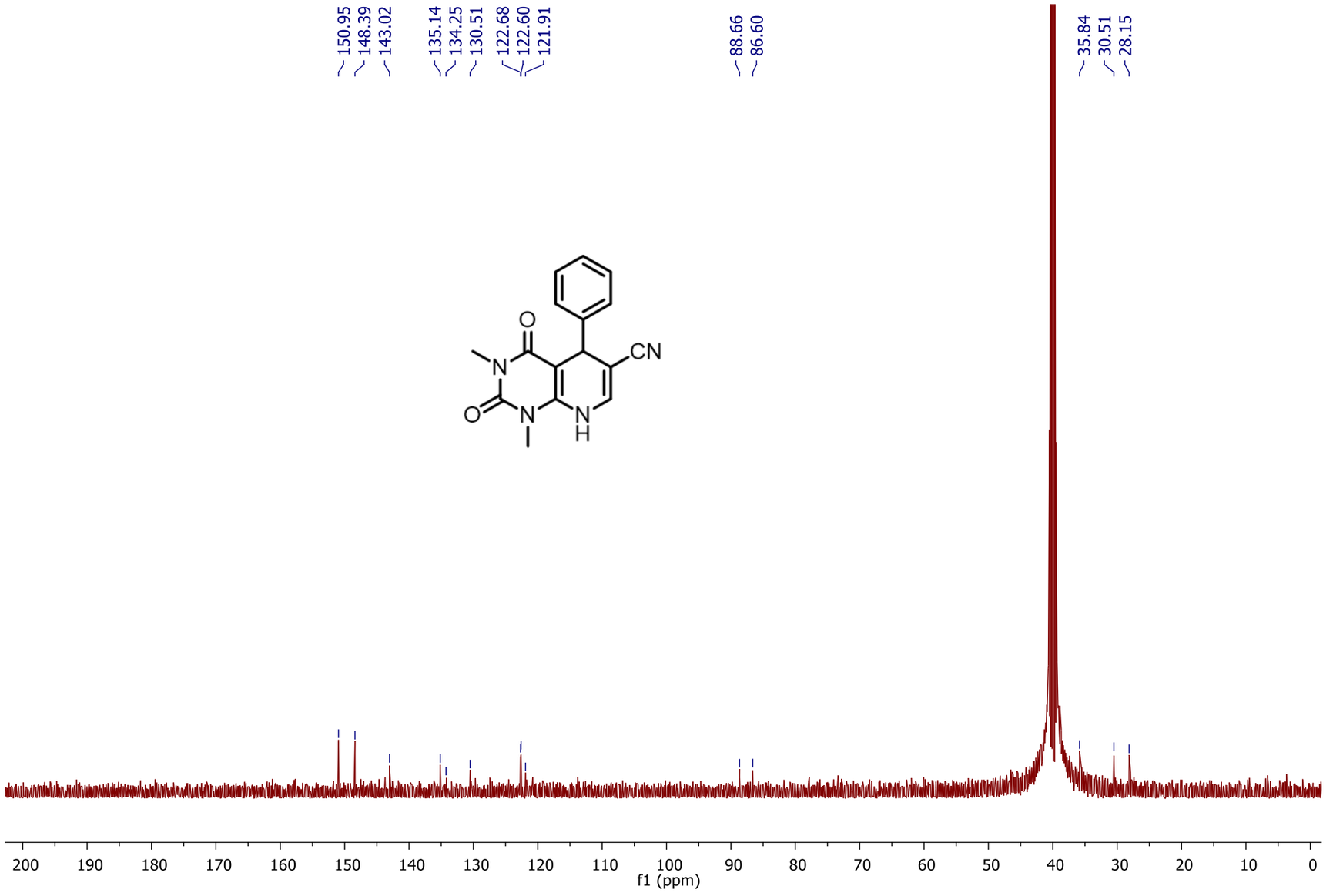

Figure S58. ^{13}C-NMR (DMSO-d_6) spectra of compound **4bb**.

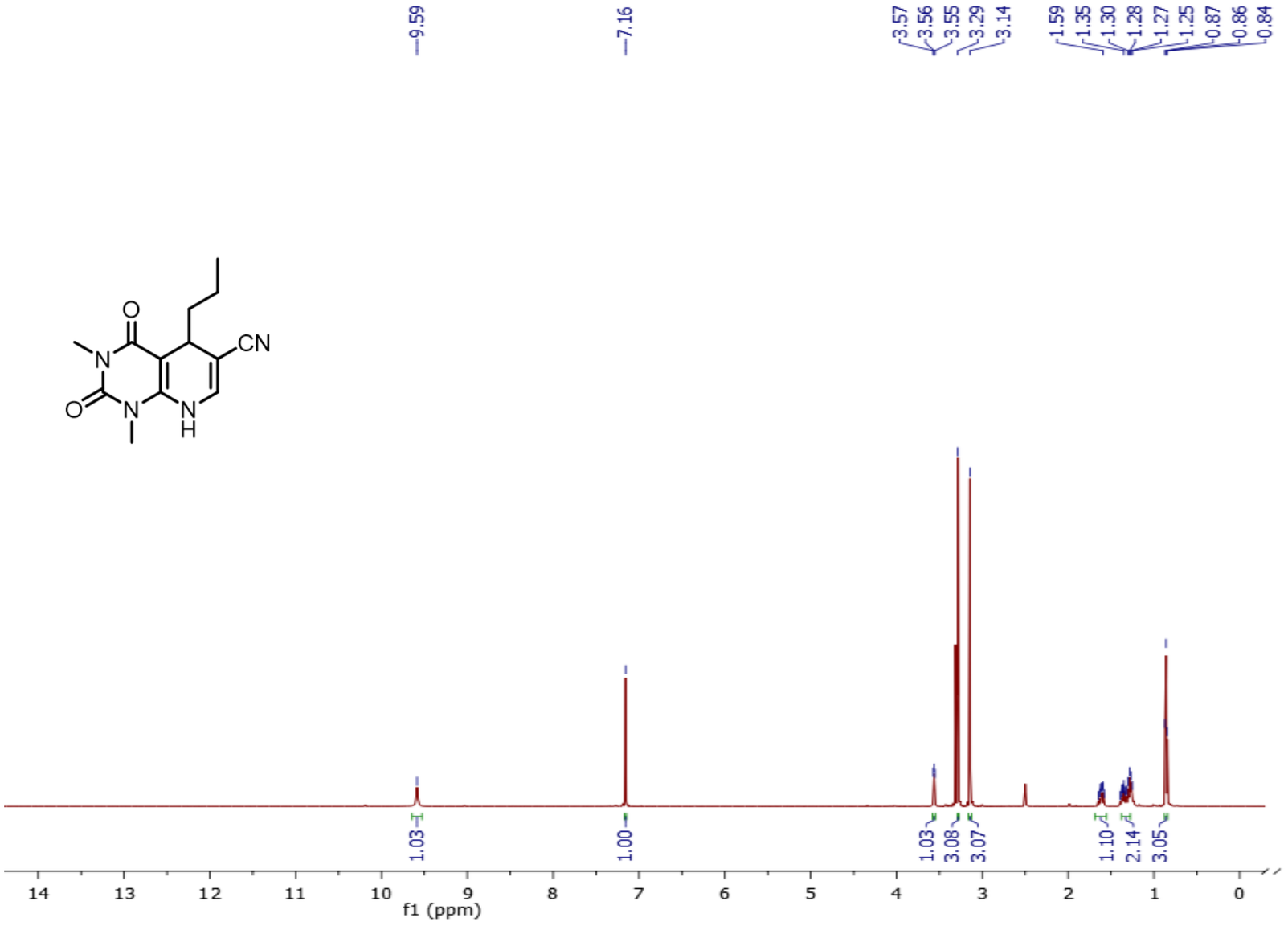

Figure S59. ^{1}H-NMR (DMSO-d_6) spectra of compound **4bc.**

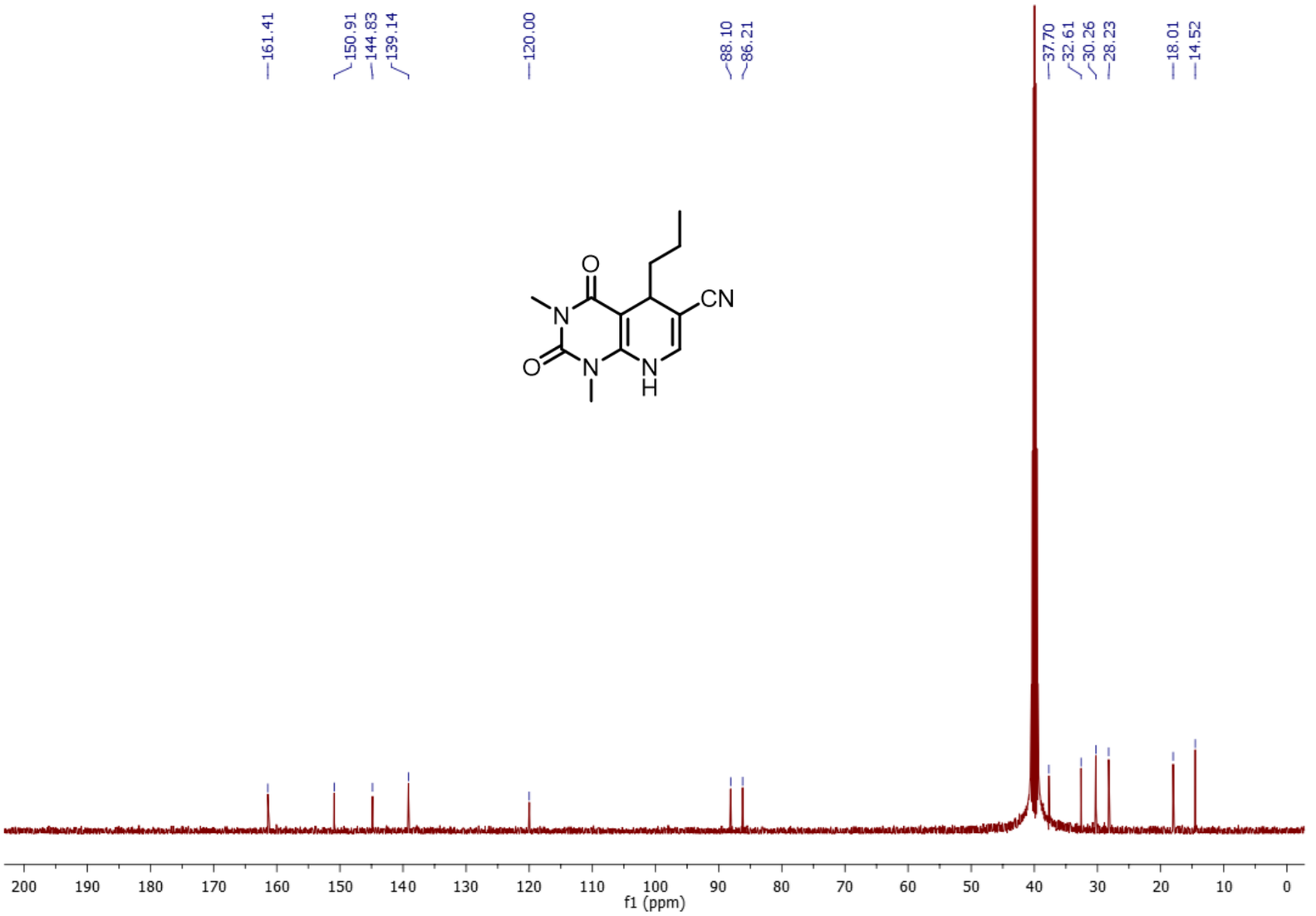

Figure S60. ^{13}C-NMR (DMSO-d_6) spectra of compound **4bc**.

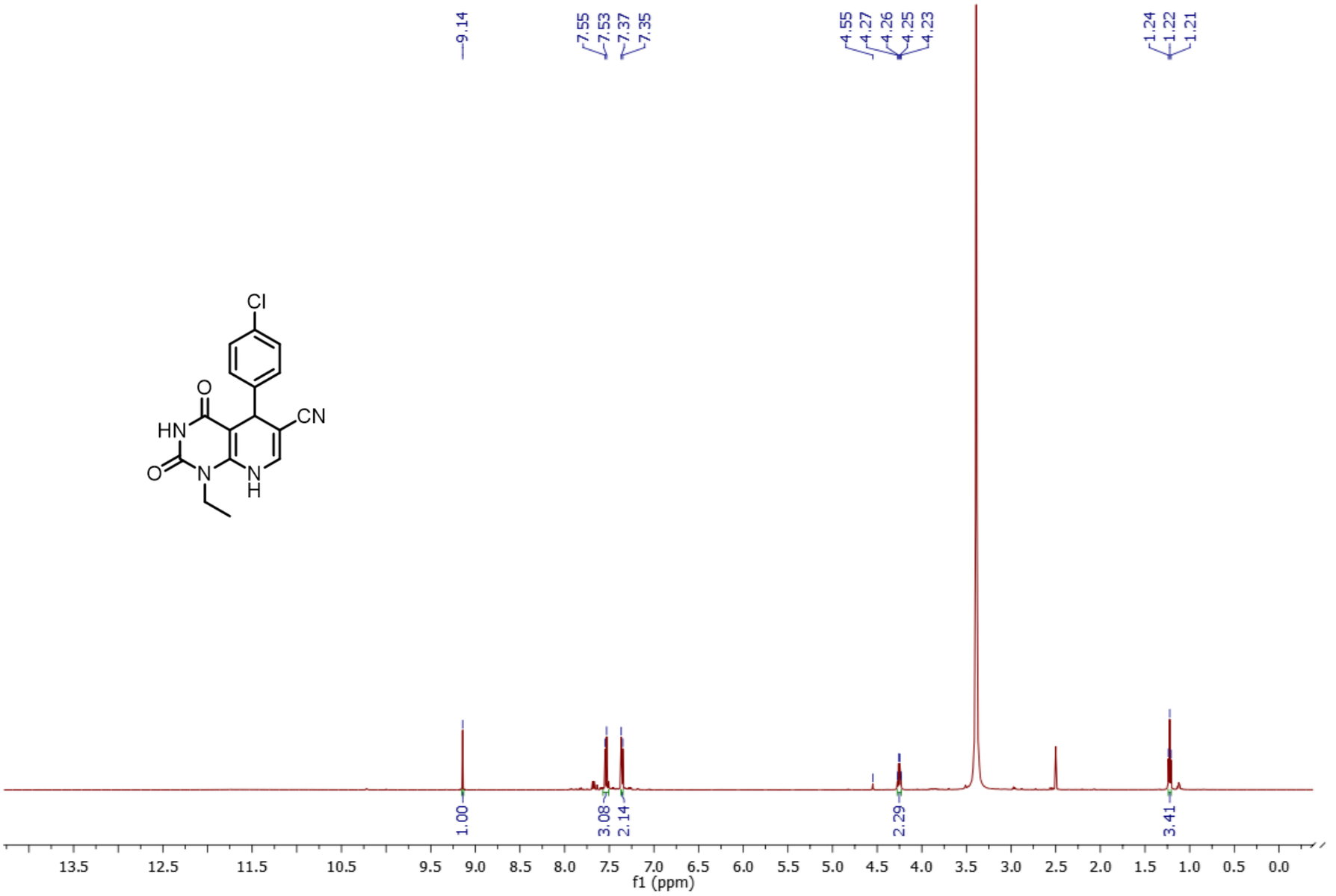

Figure S61. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ca.**

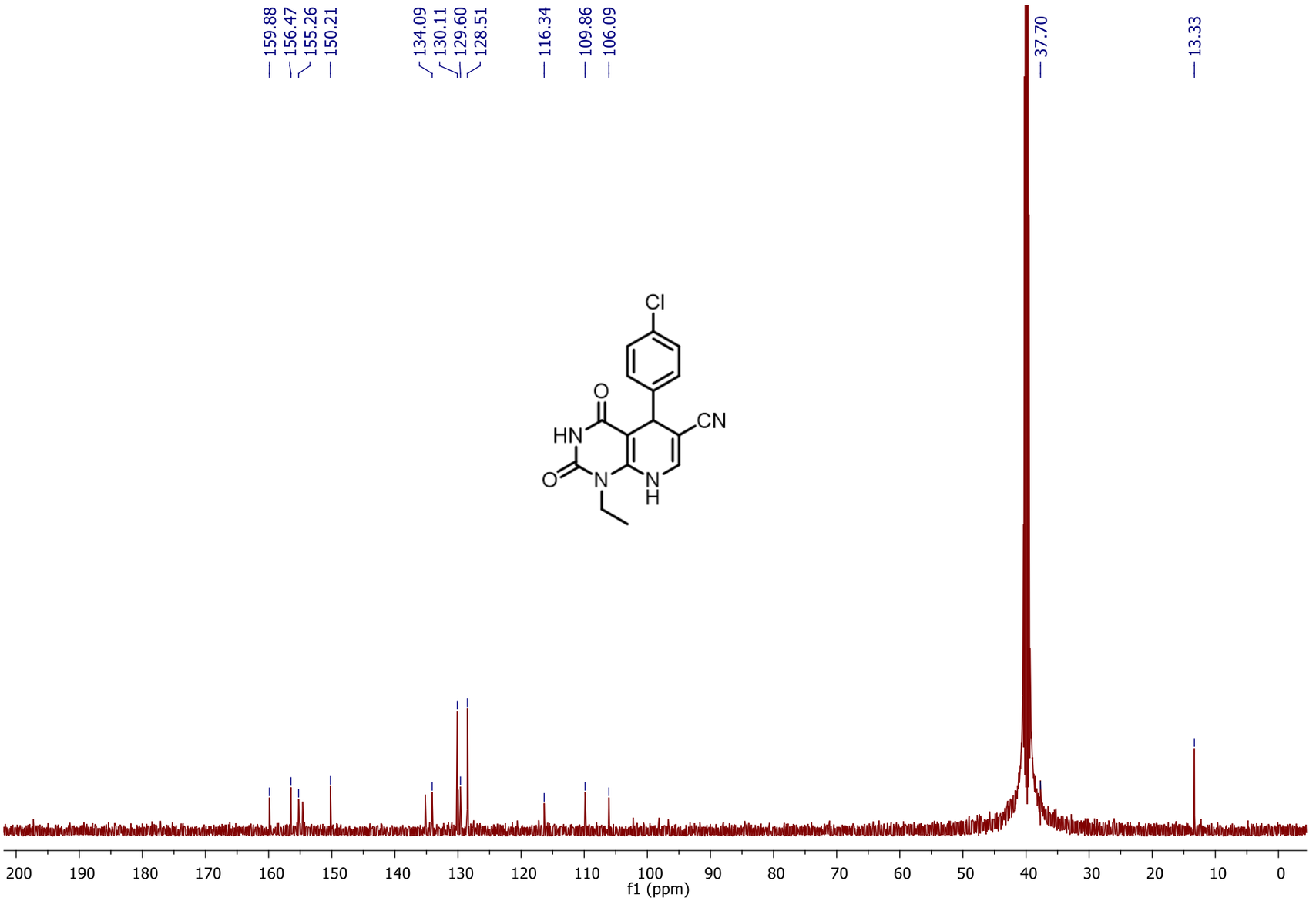

Figure S62. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **4ca**.

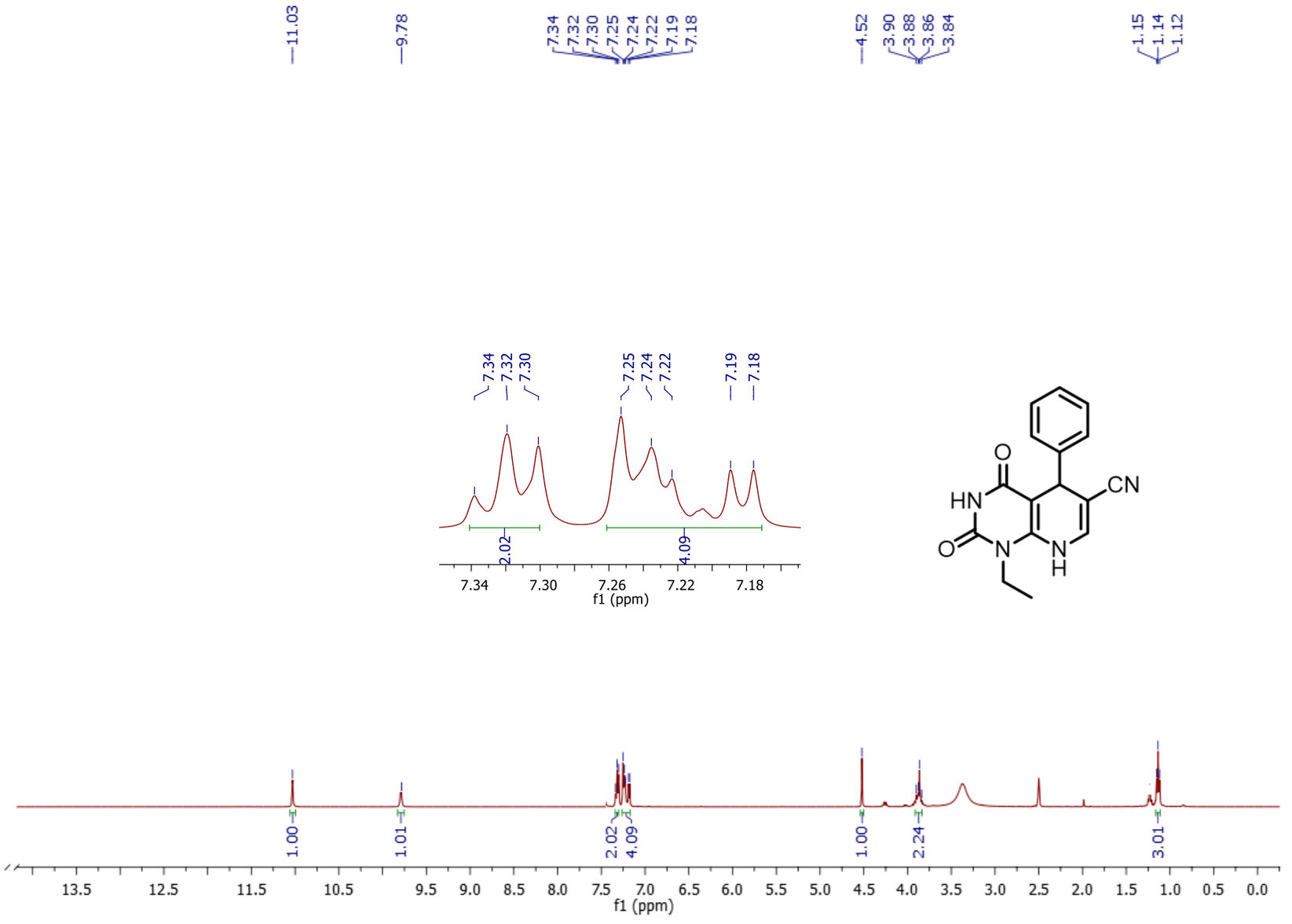

Figure S63. ^{1}H-NMR (DMSO-d_6) spectra of compound **4cb.**

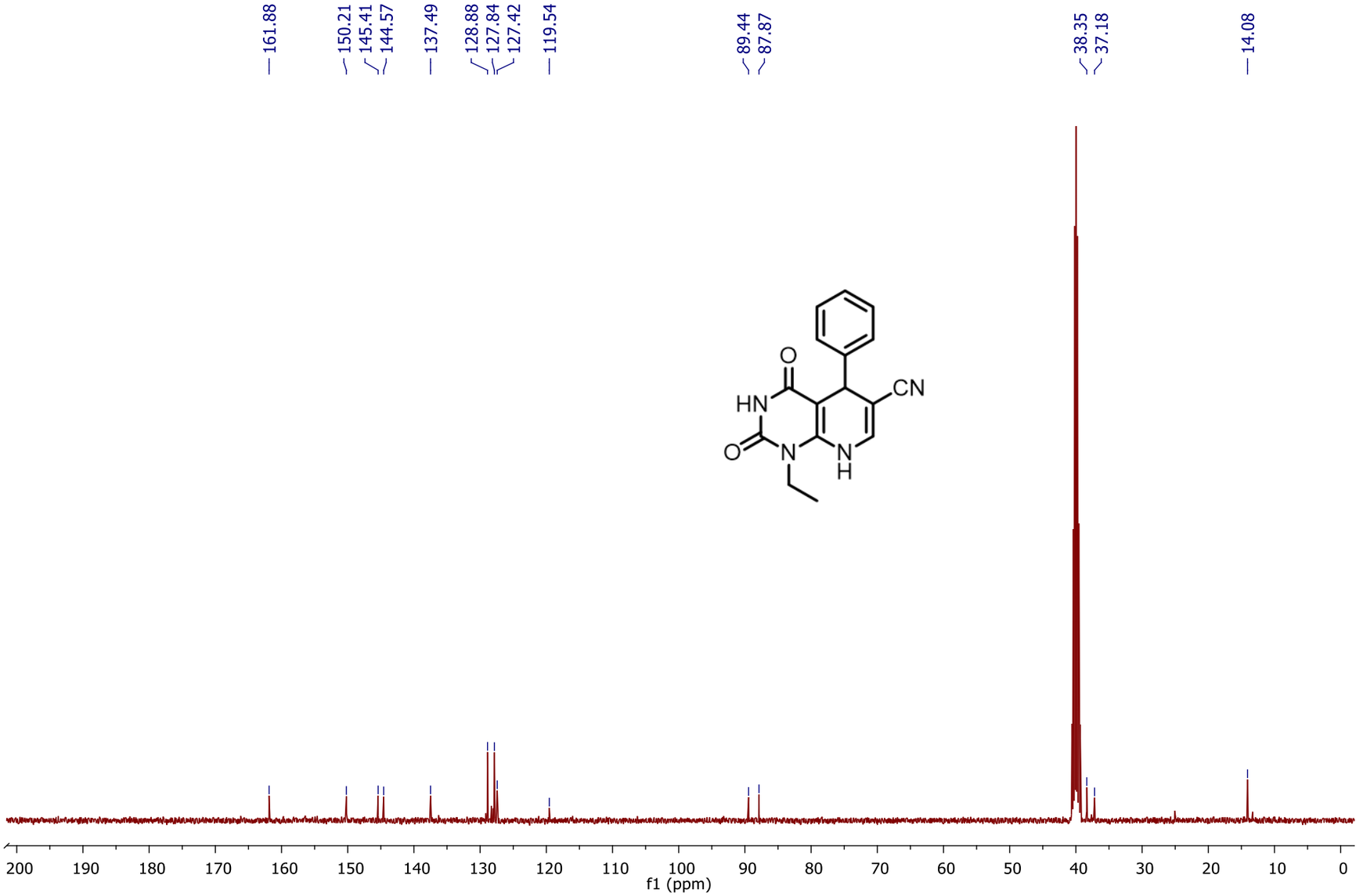

Figure S64. ^{13}C-NMR (DMSO-d_6) spectra of compound **4cb**.

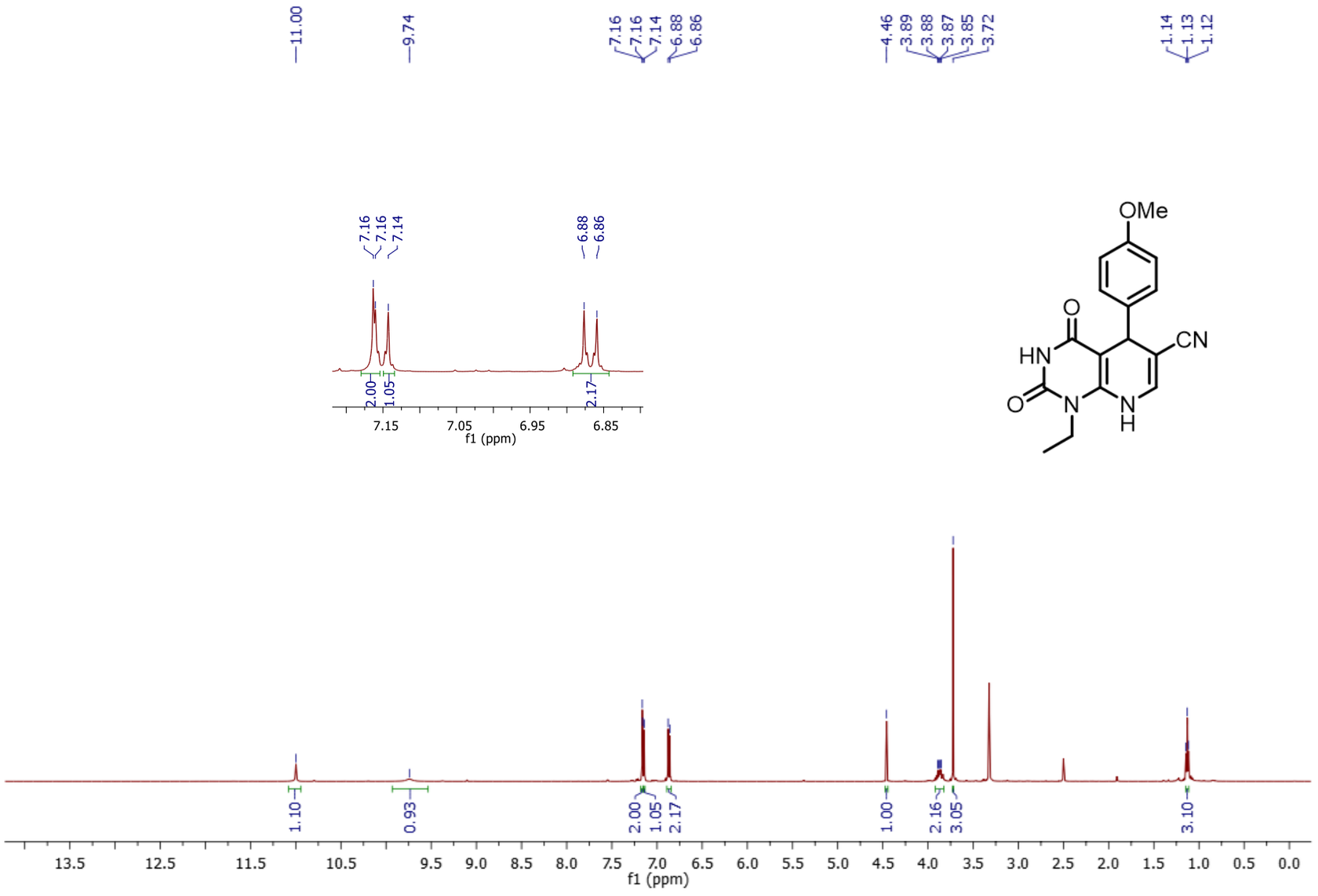

Figure S65. ^{1}H-NMR (DMSO-d_6) spectra of compound **4cc.**

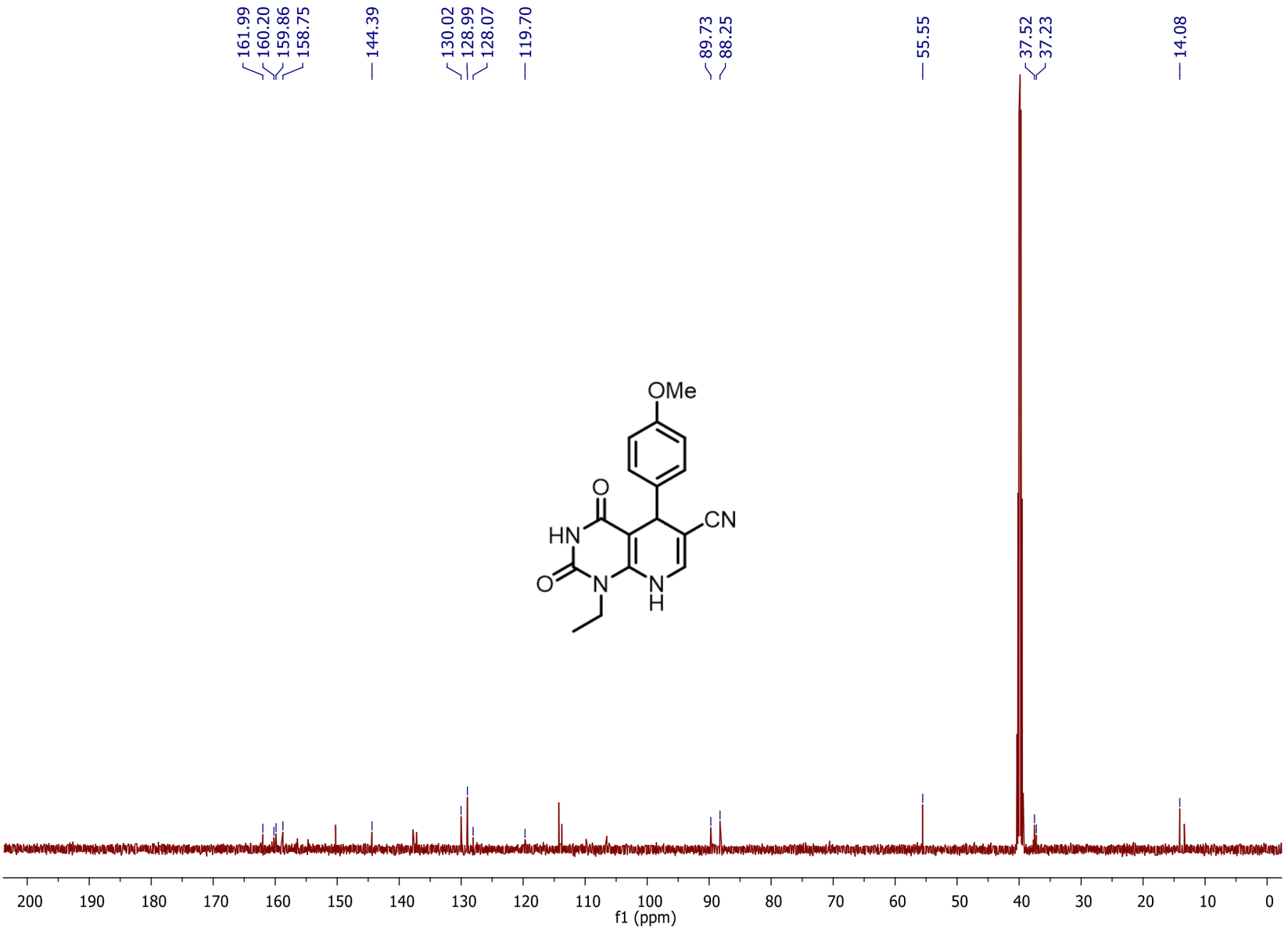

Figure S66. ^{13}C-NMR (DMSO-d_6) spectra of compound **4cc**.

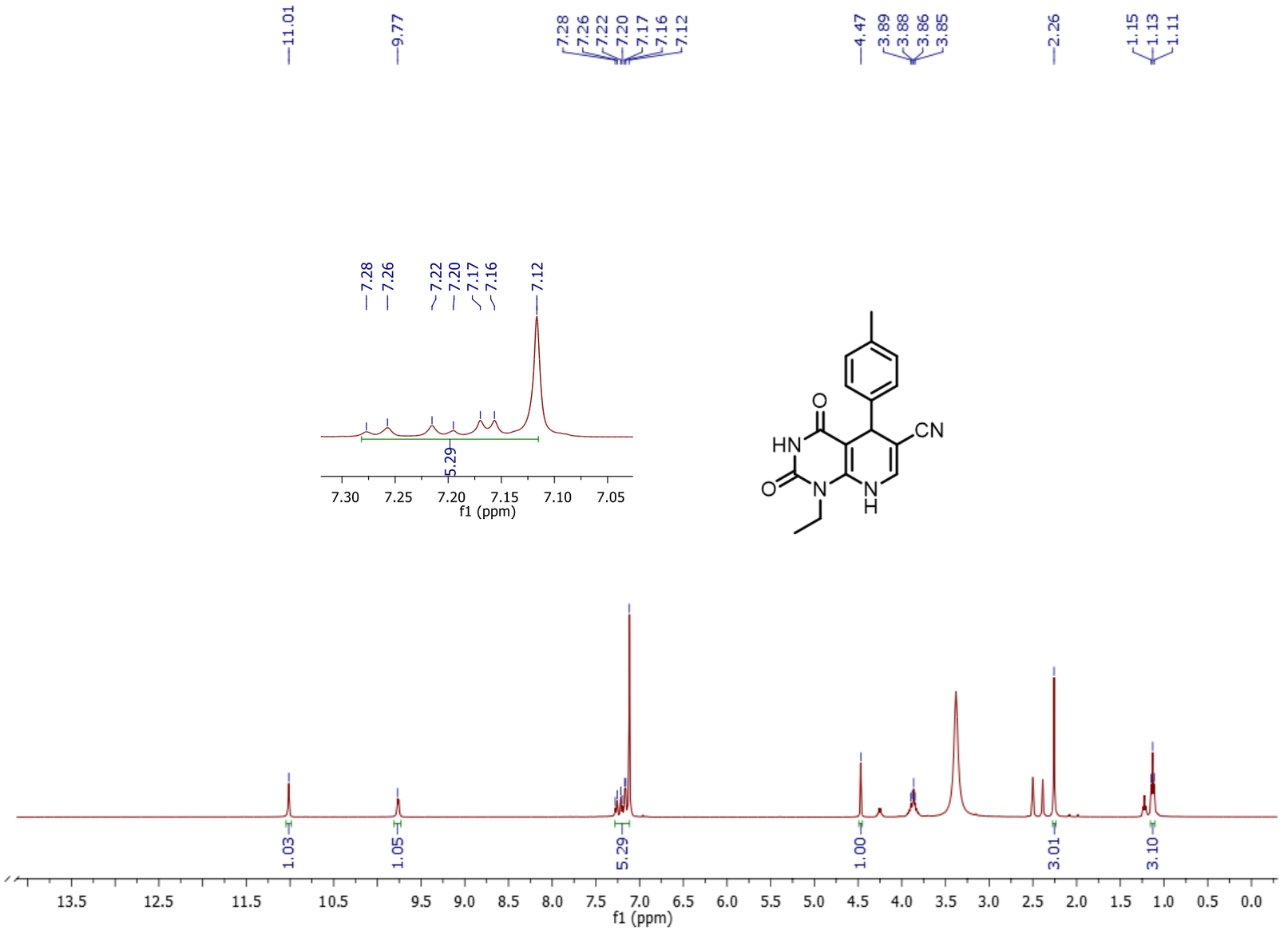

Figure S67. ^{1}H-NMR (DMSO-d_6) spectra of compound **4cd.**

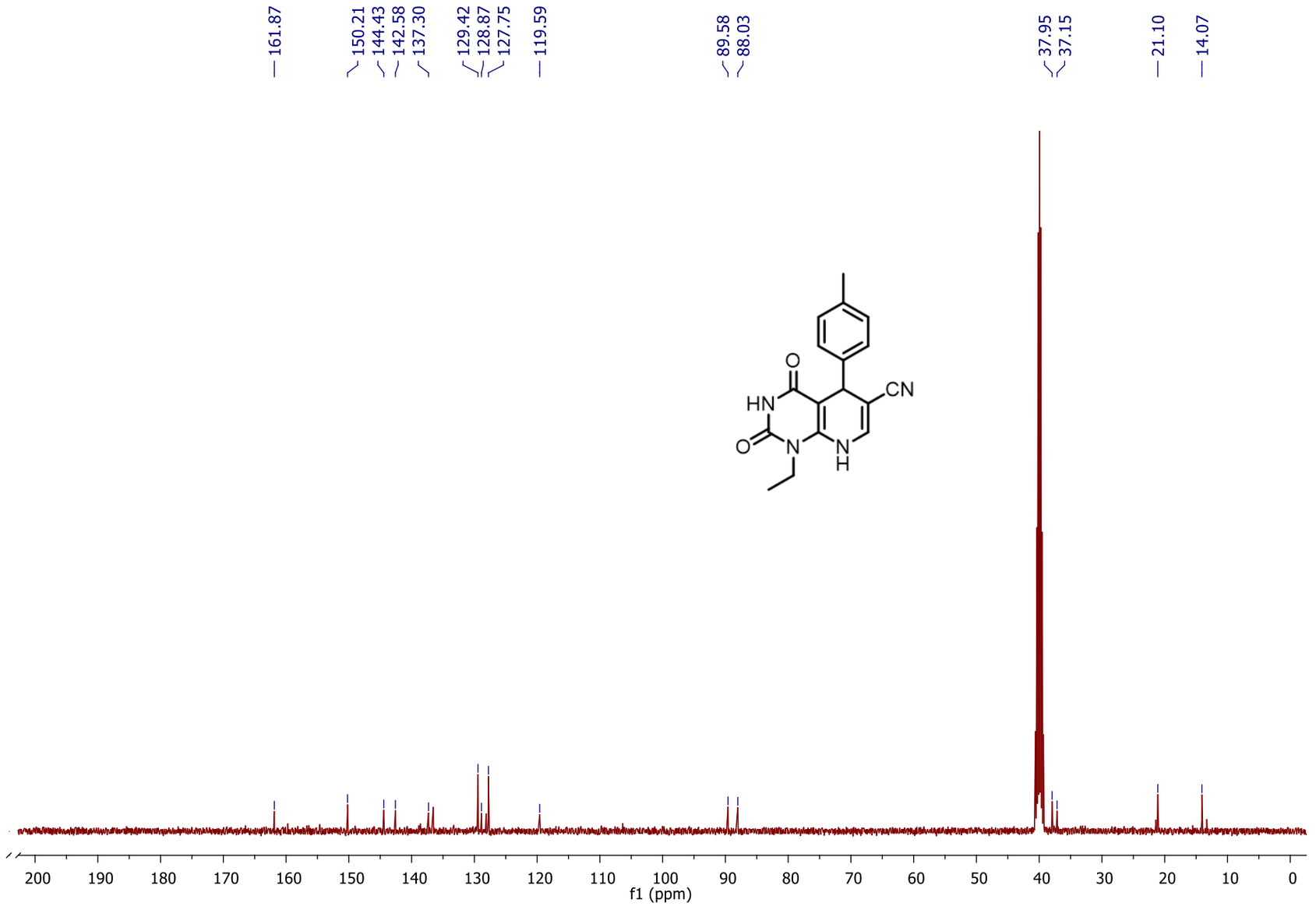

Figure S68. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **4cd**.

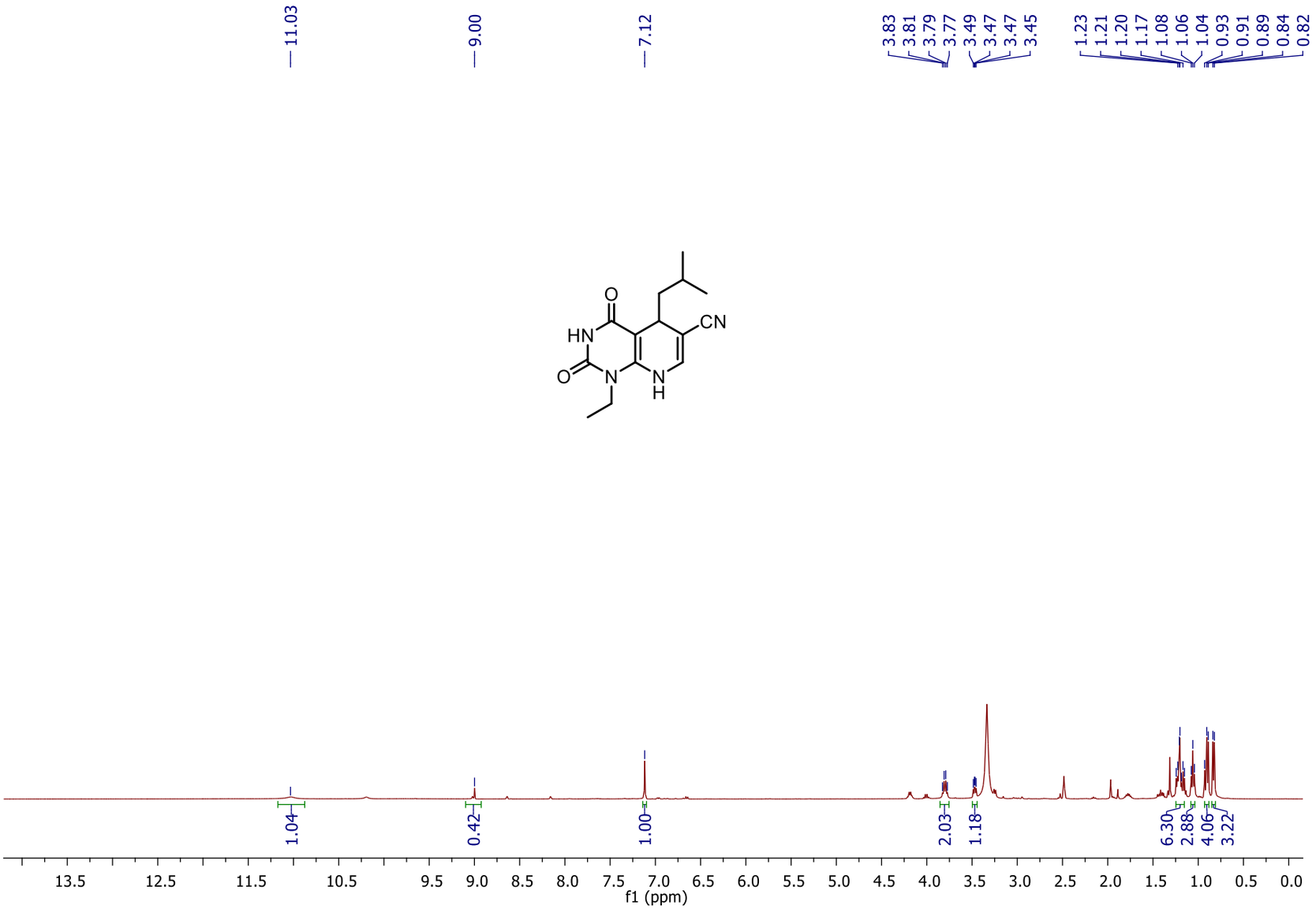

Figure S69. ^{1}H-NMR (DMSO-d_6) spectra of compound **4ce.**

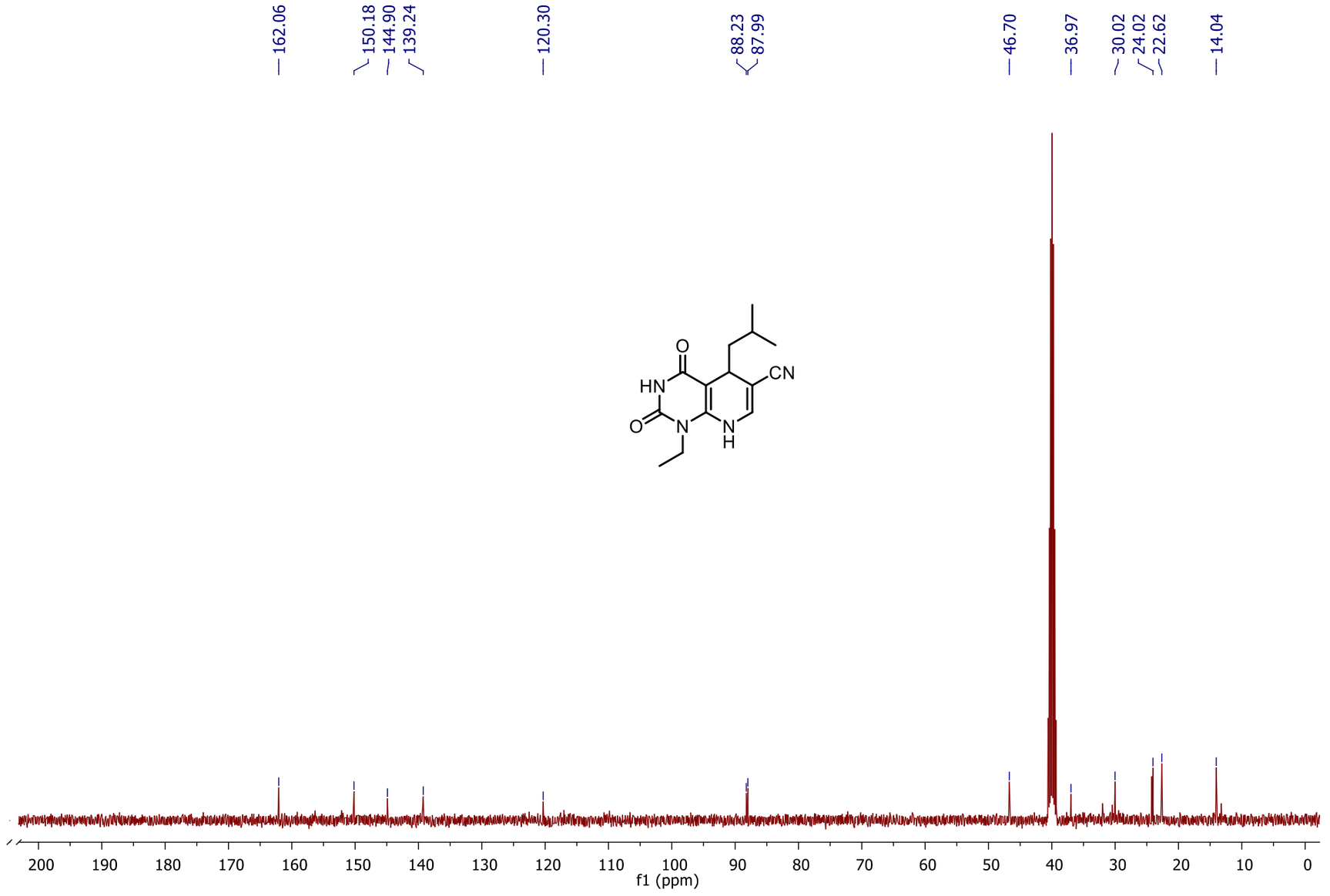

Figure S70. ^{13}C-NMR (DMSO-d_6) spectra of compound **4ce**.

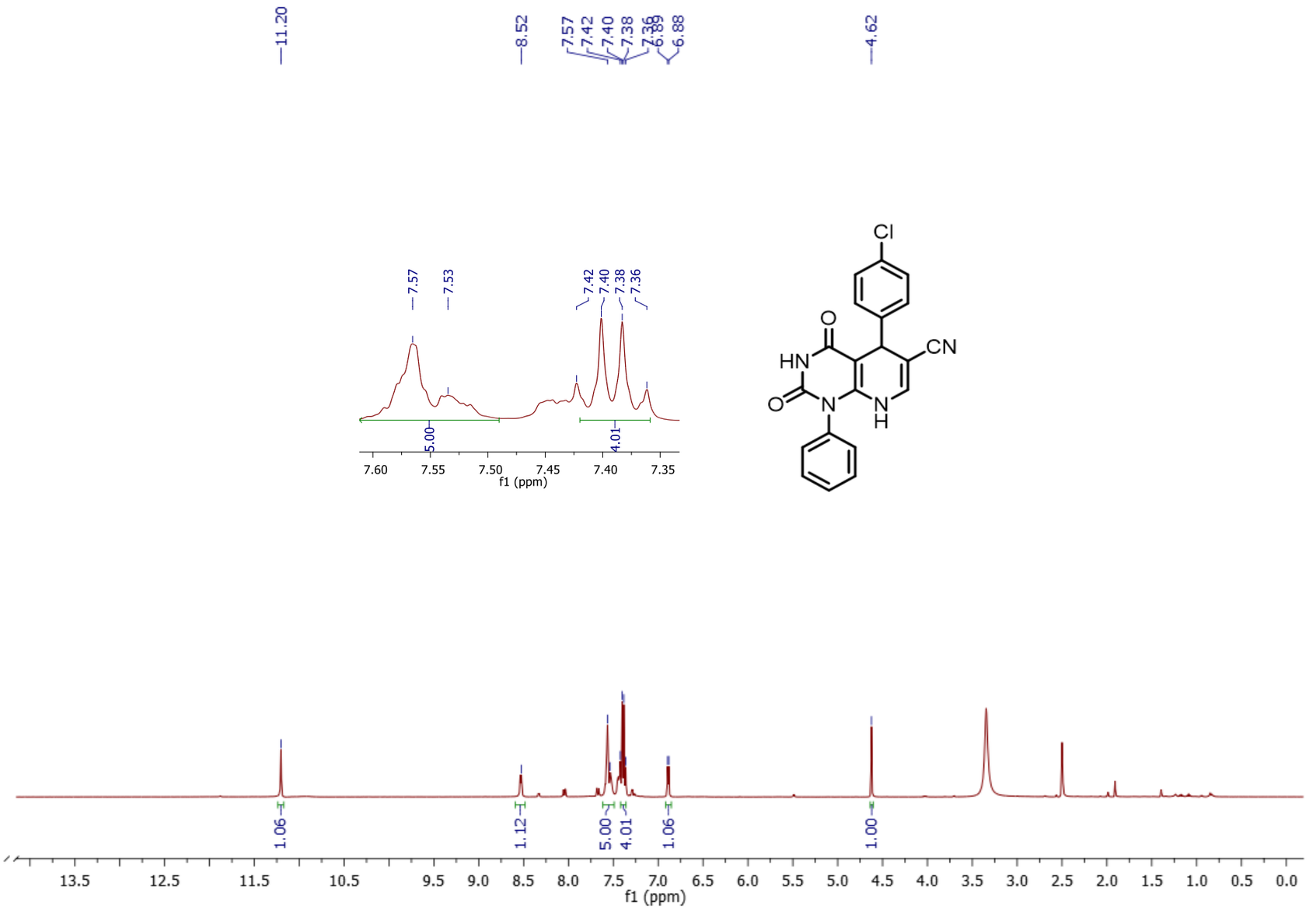

Figure S71. ^{1}H-NMR (DMSO-d_6) spectra of compound **4da.**

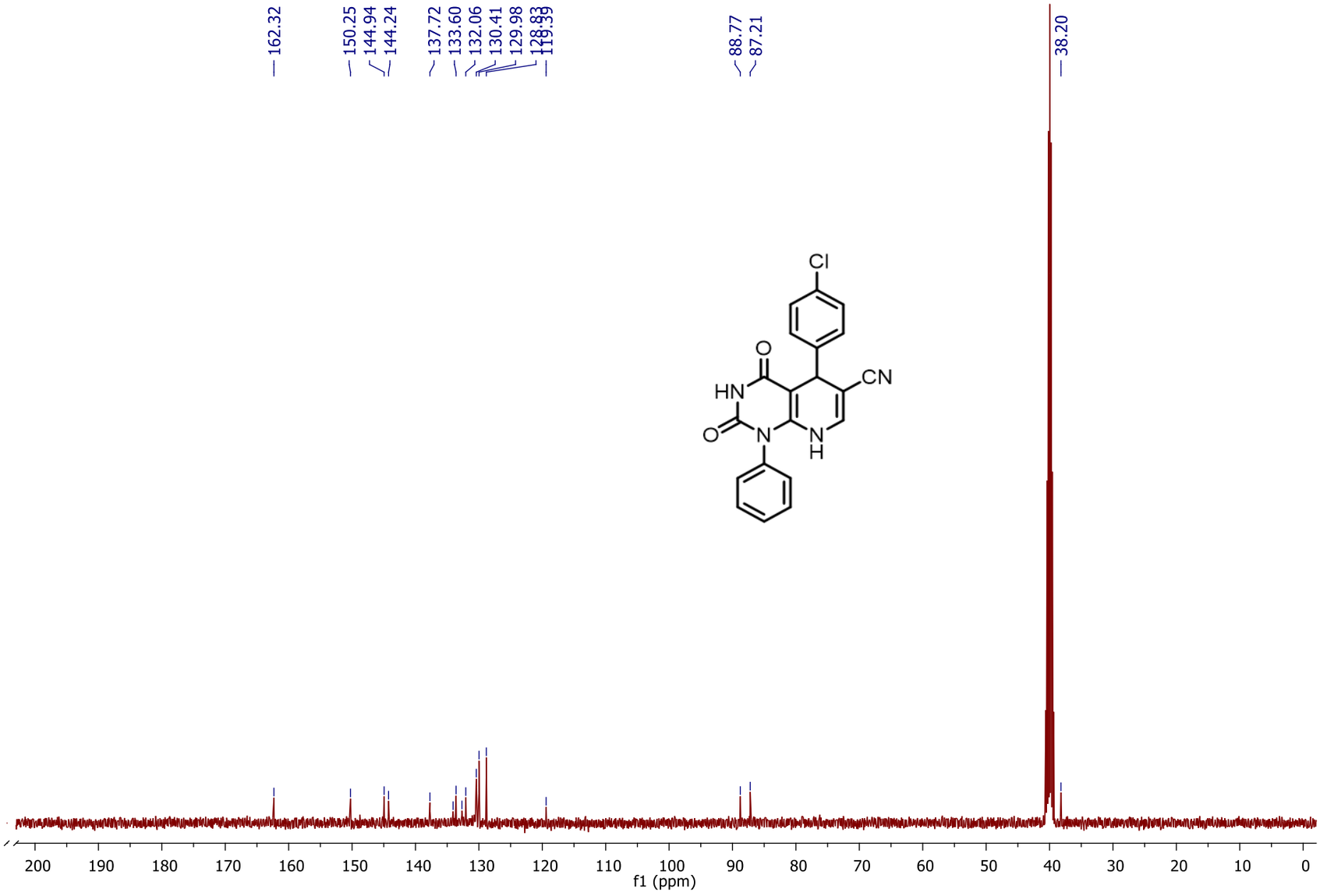

Figure S72. ^{13}C-NMR (DMSO-d_6) spectra of compound **4da.**

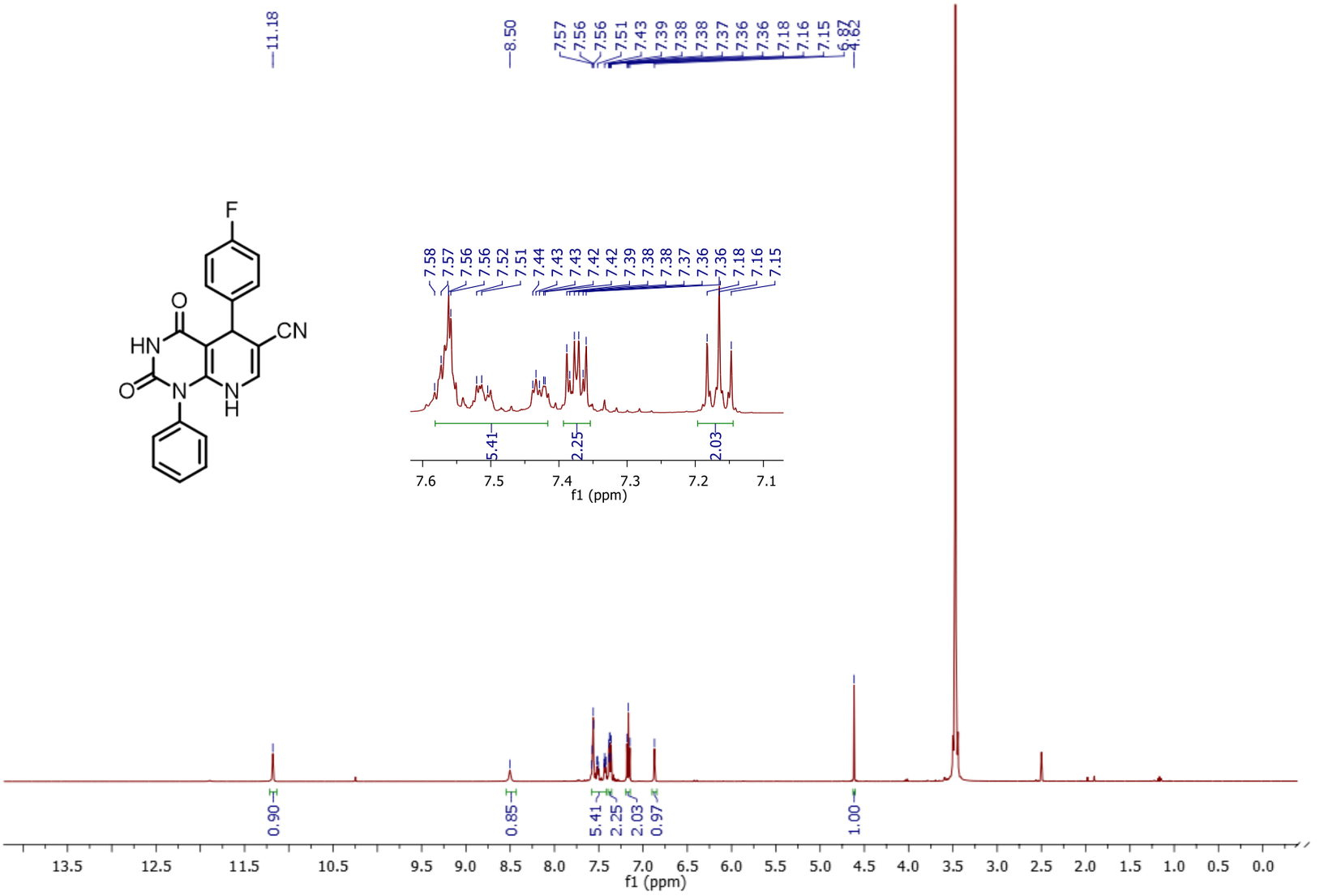

Figure S73. ^{1}H-NMR (DMSO-d_6) spectra of compound **4db.**

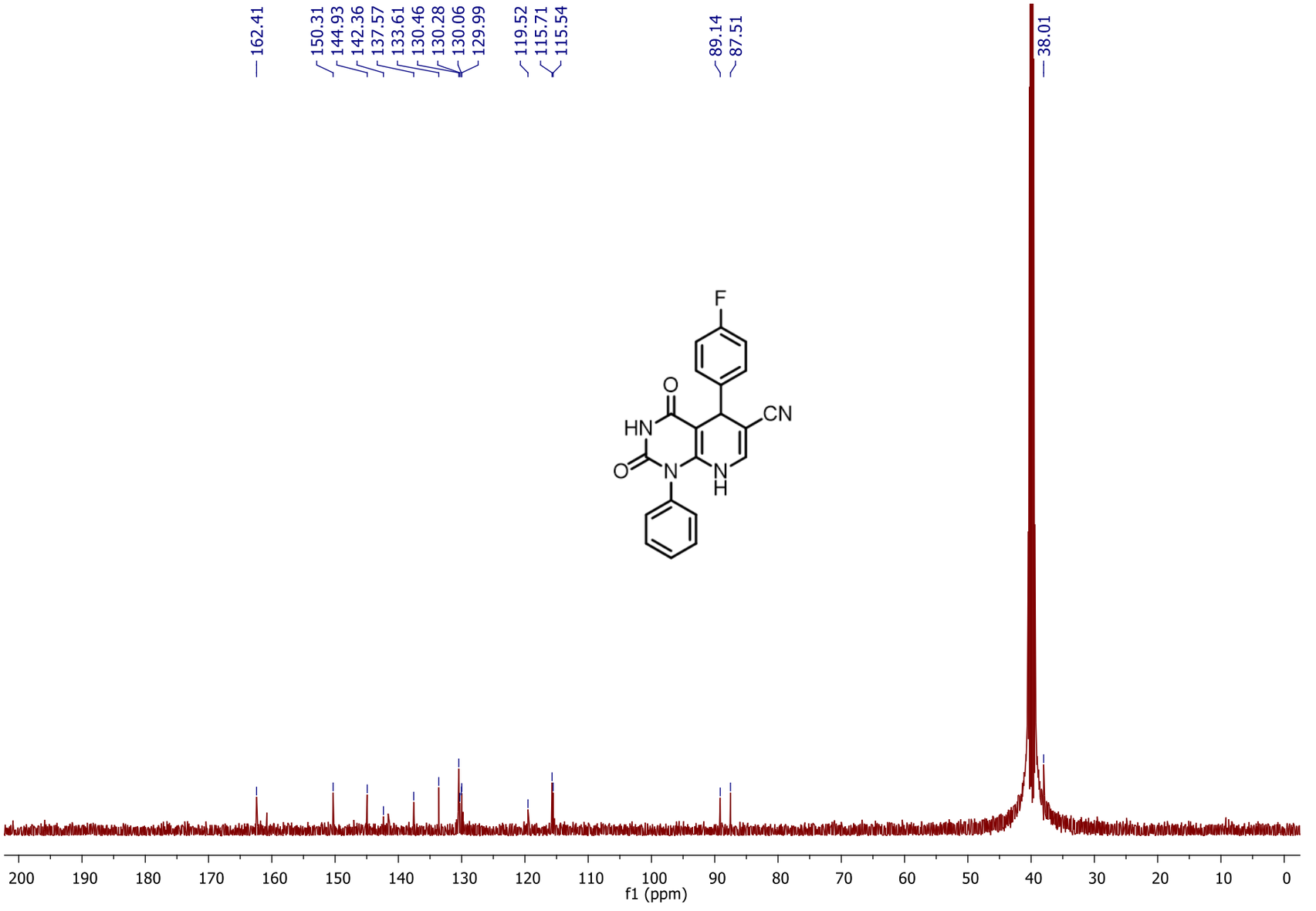

Figure S74. ^{13}C-NMR (DMSO-d_6) spectra of compound **4db.**

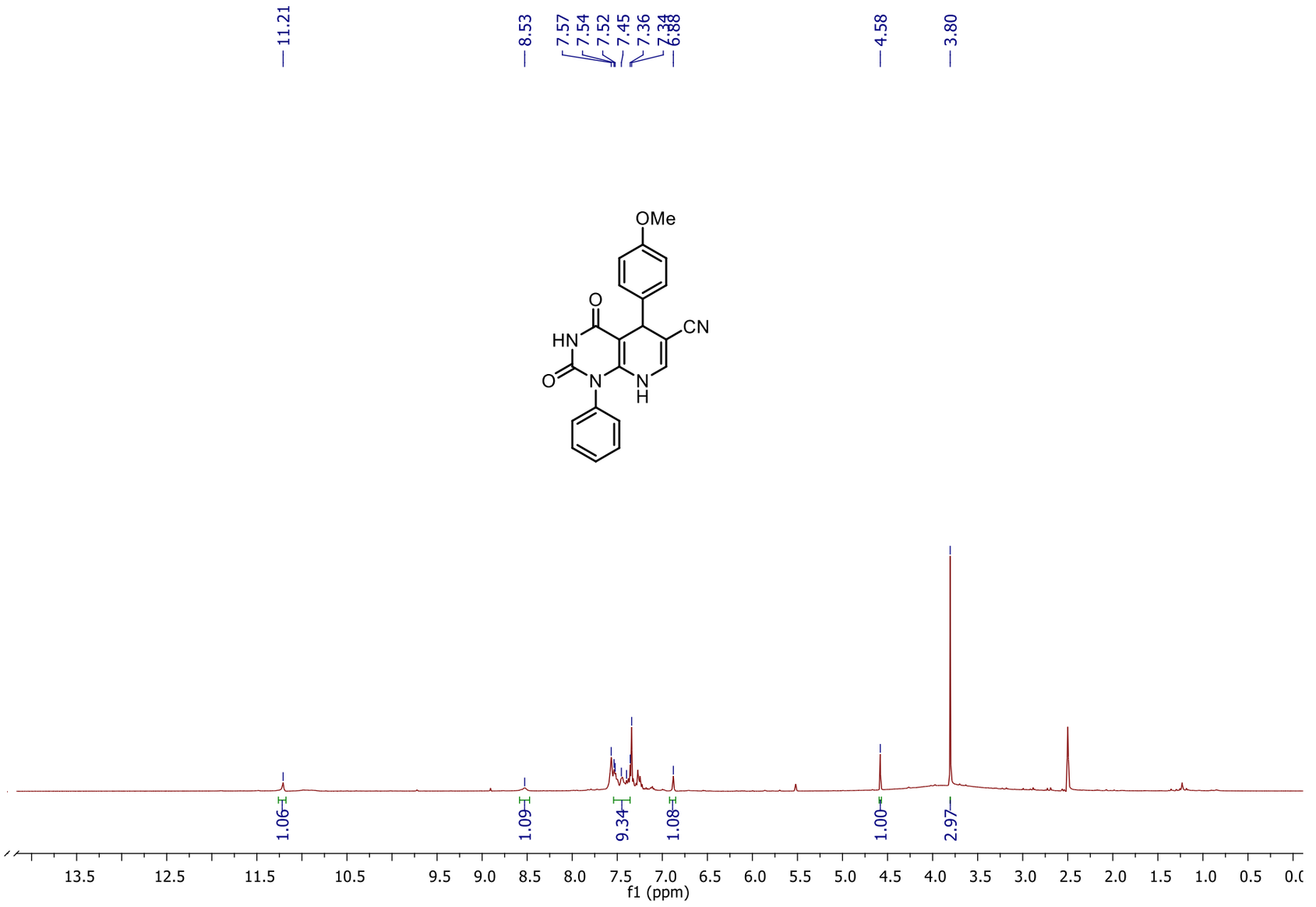

Figure S75. ^{1}H-NMR (DMSO-d_6) spectra of compound **4dc.**

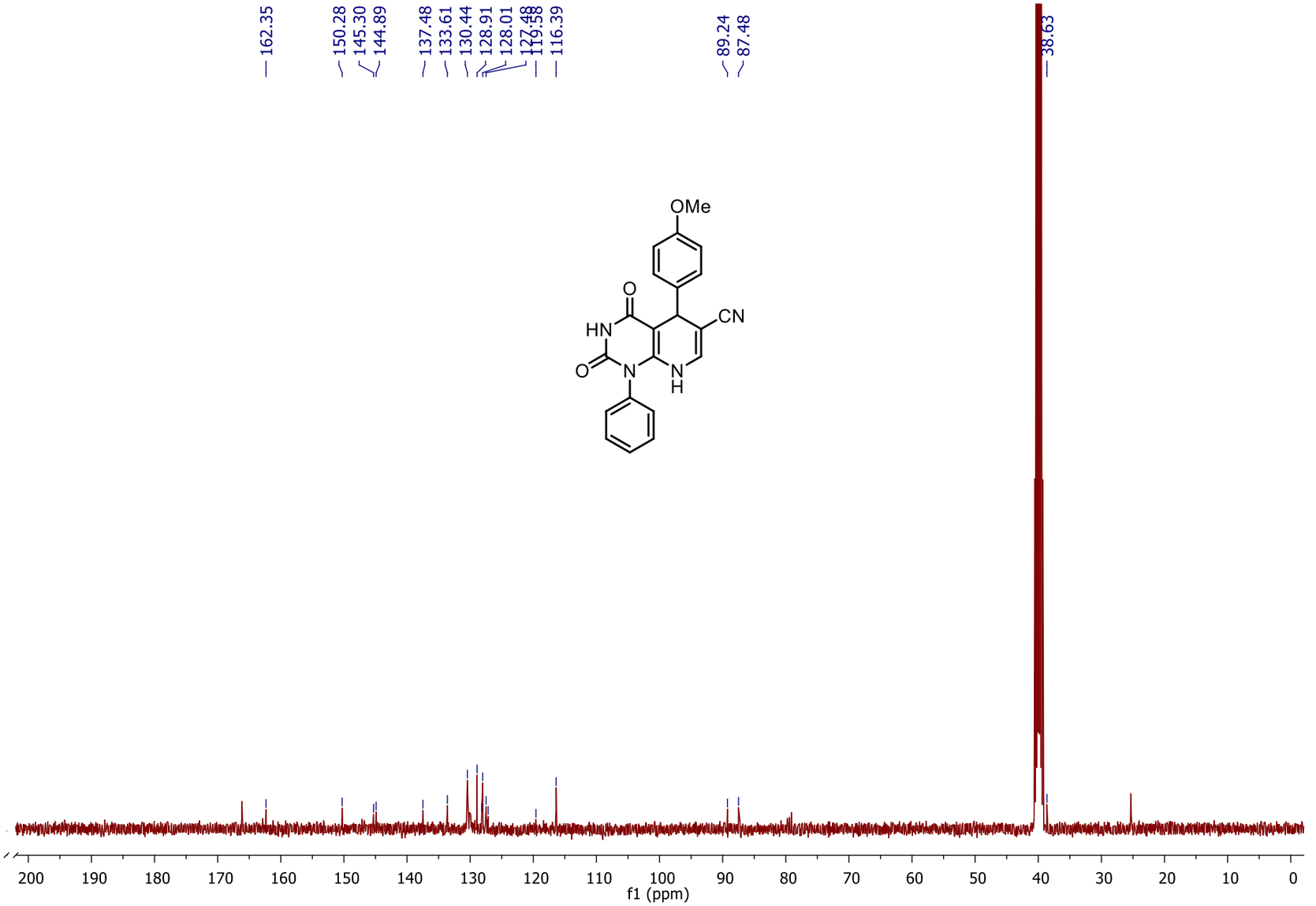

Figure S76. ^{13}C-NMR (DMSO-d_6) spectra of compound **4dc.**

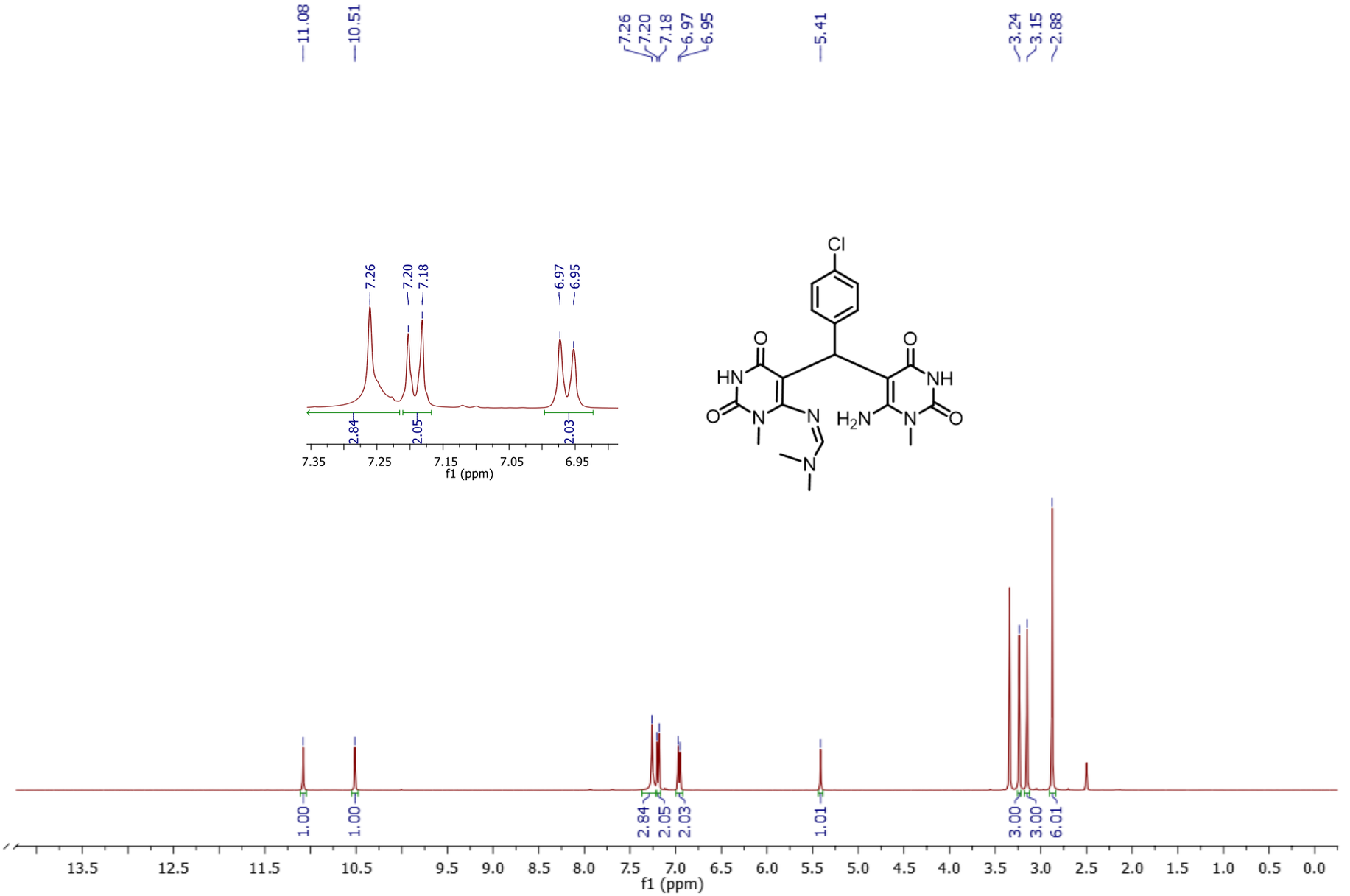

Figure S77. ^{1}H-NMR (DMSO-d_6) spectra of compound **5aa.**

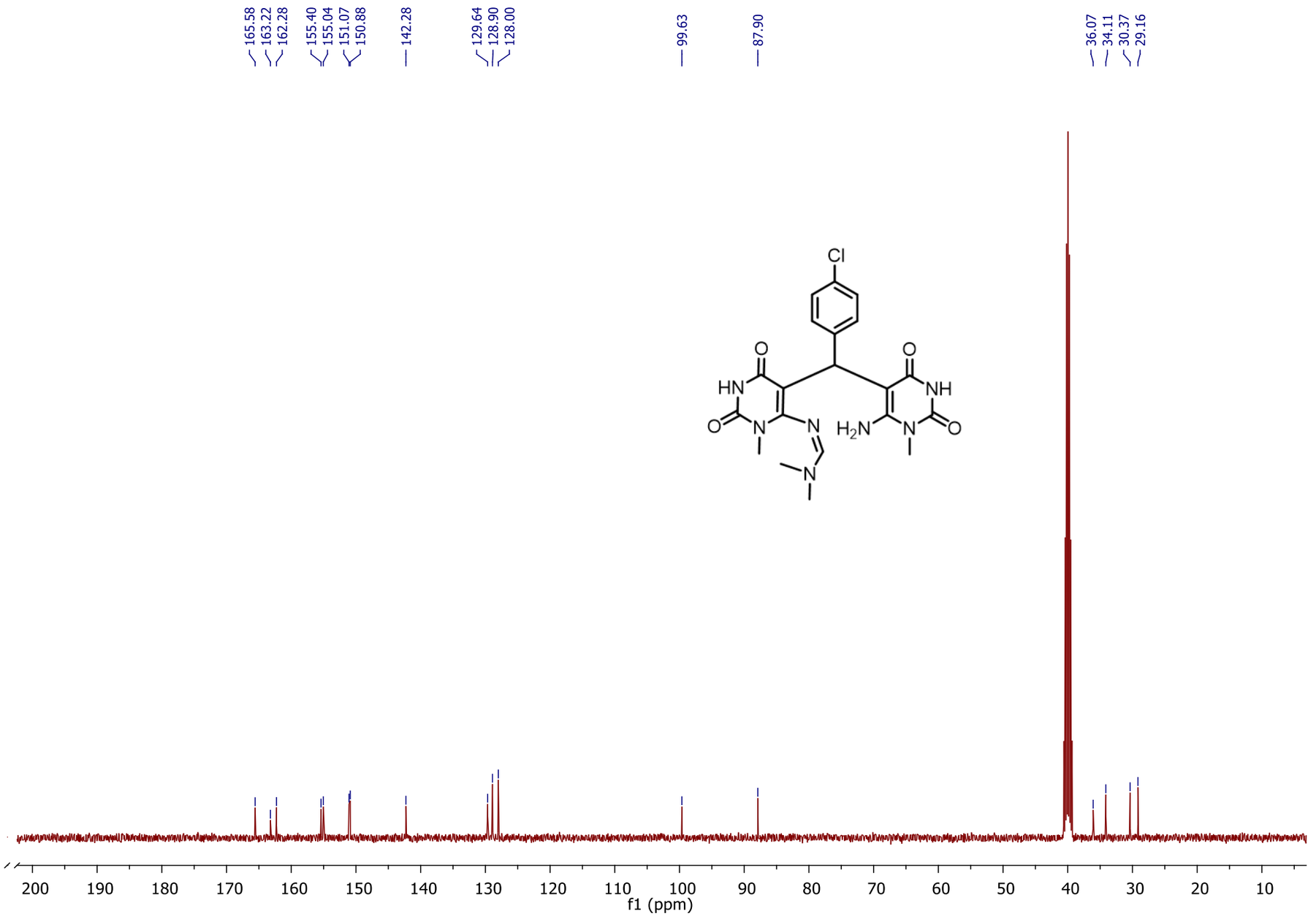

Figure S78. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **5aa.**

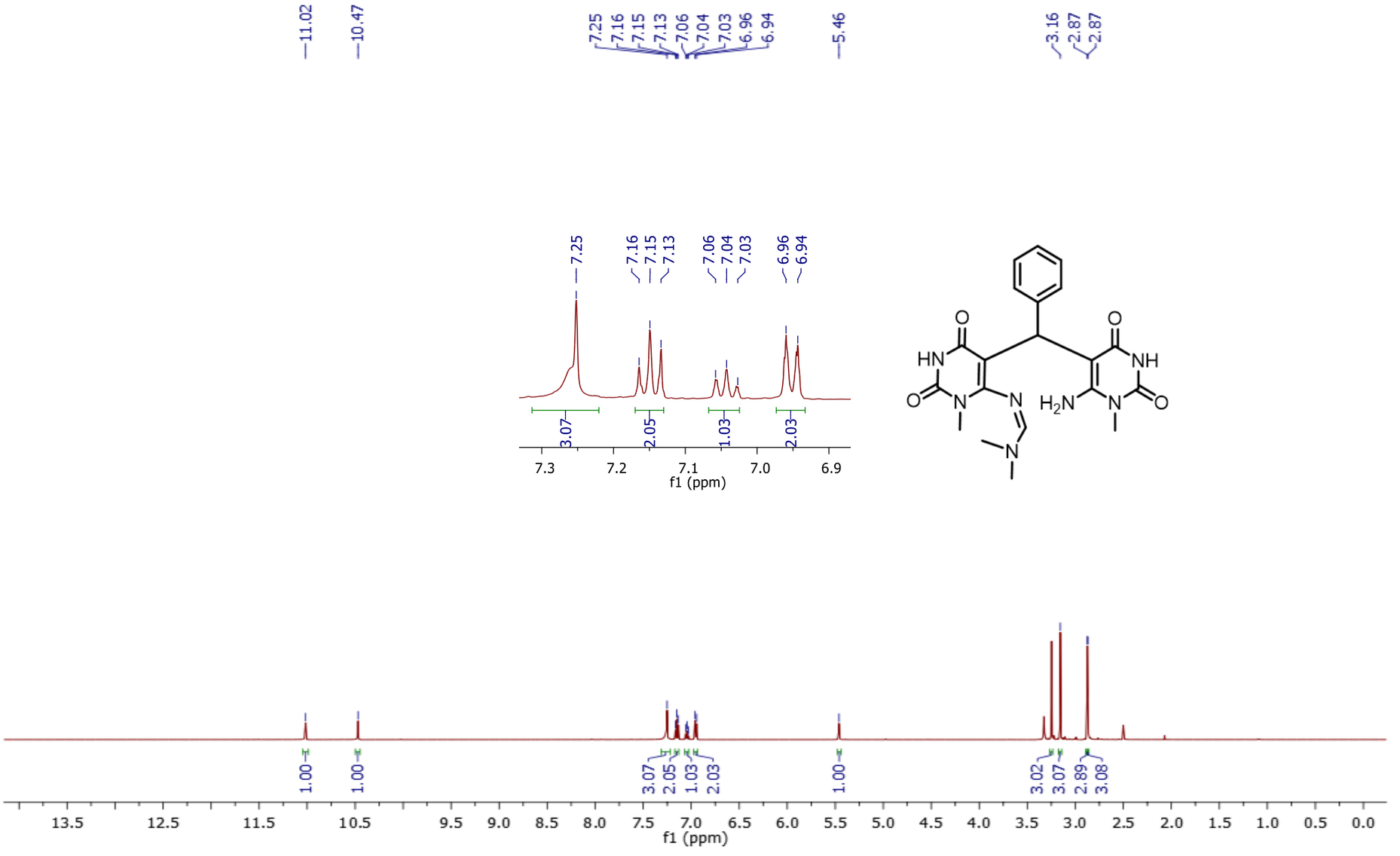

Figure S79. ^{1}H-NMR (DMSO-d_6) spectra of compound **5ab.**

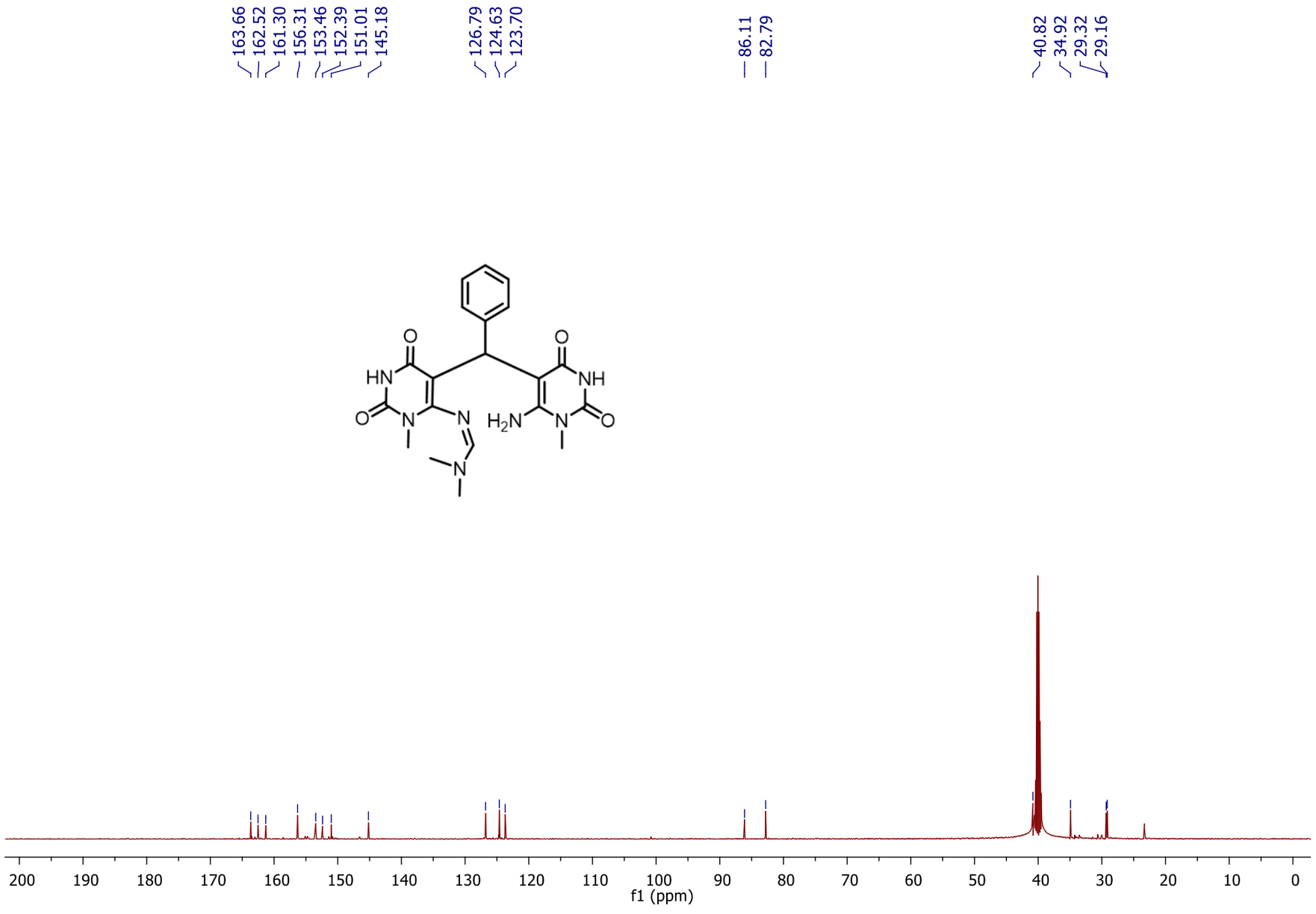

Figure S80. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **5ab.**

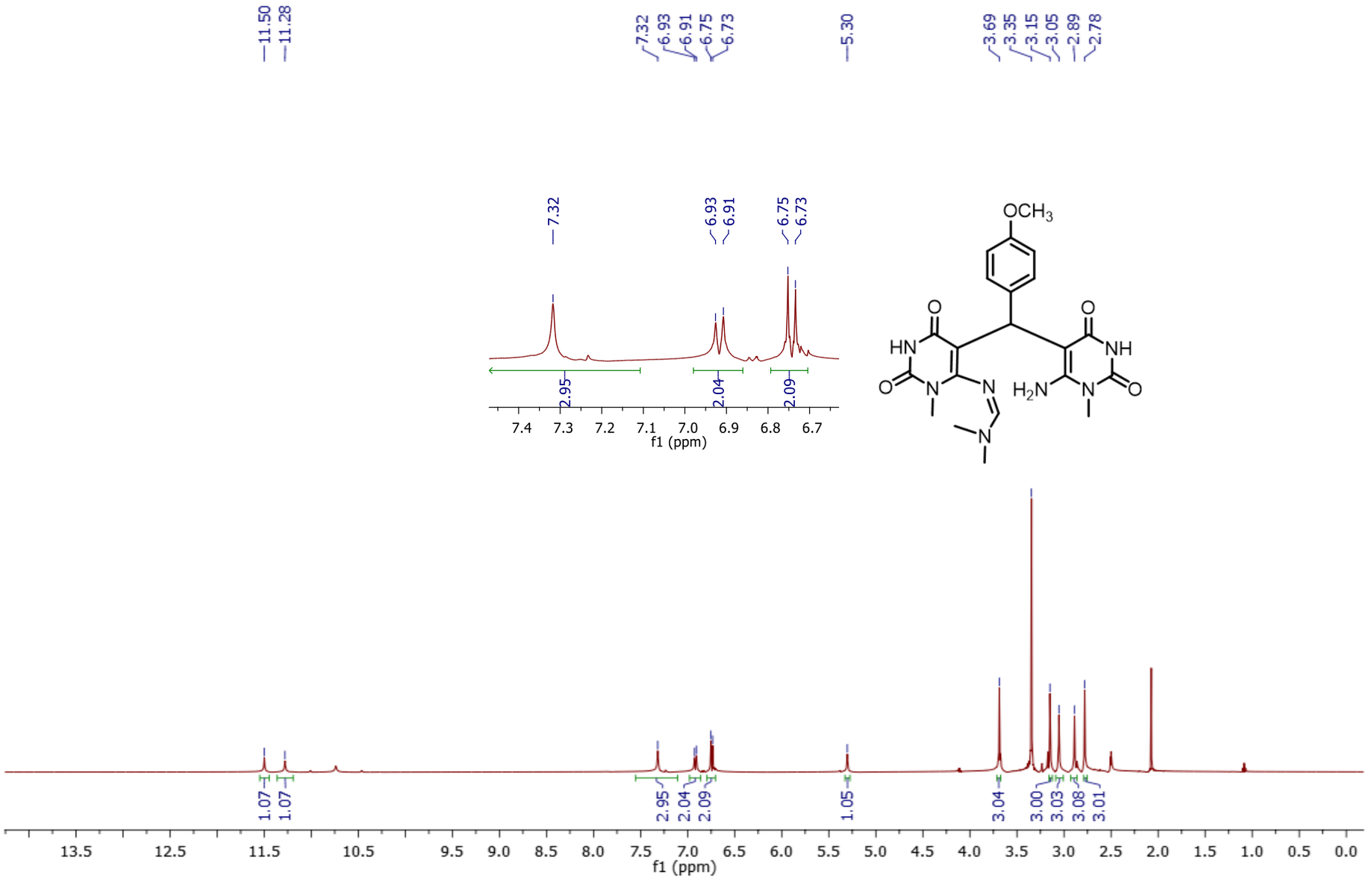

Figure S81. [1]H-NMR (DMSO-d_6) spectra of compound **5ac.**

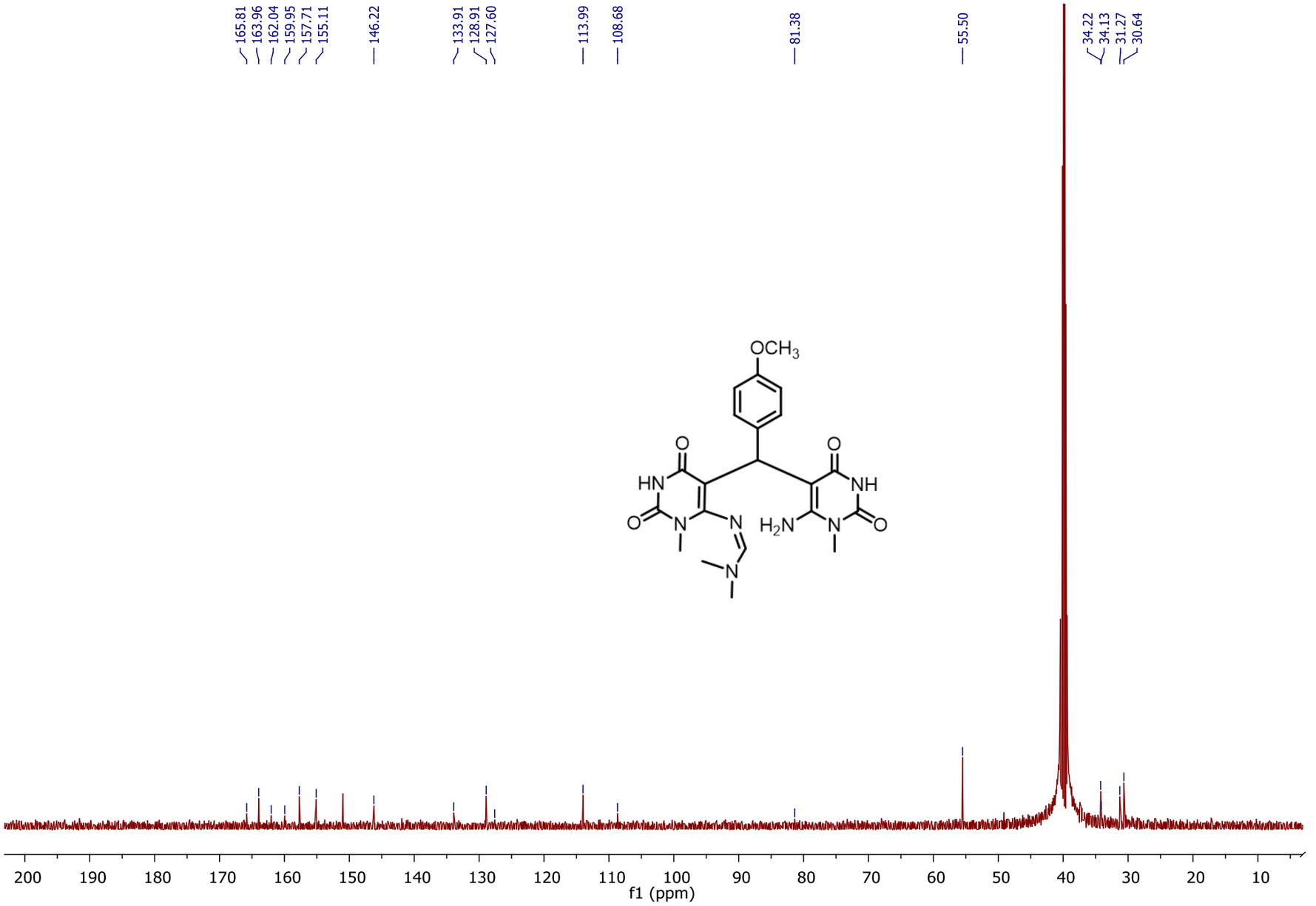

Figure S82. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **5ac.**

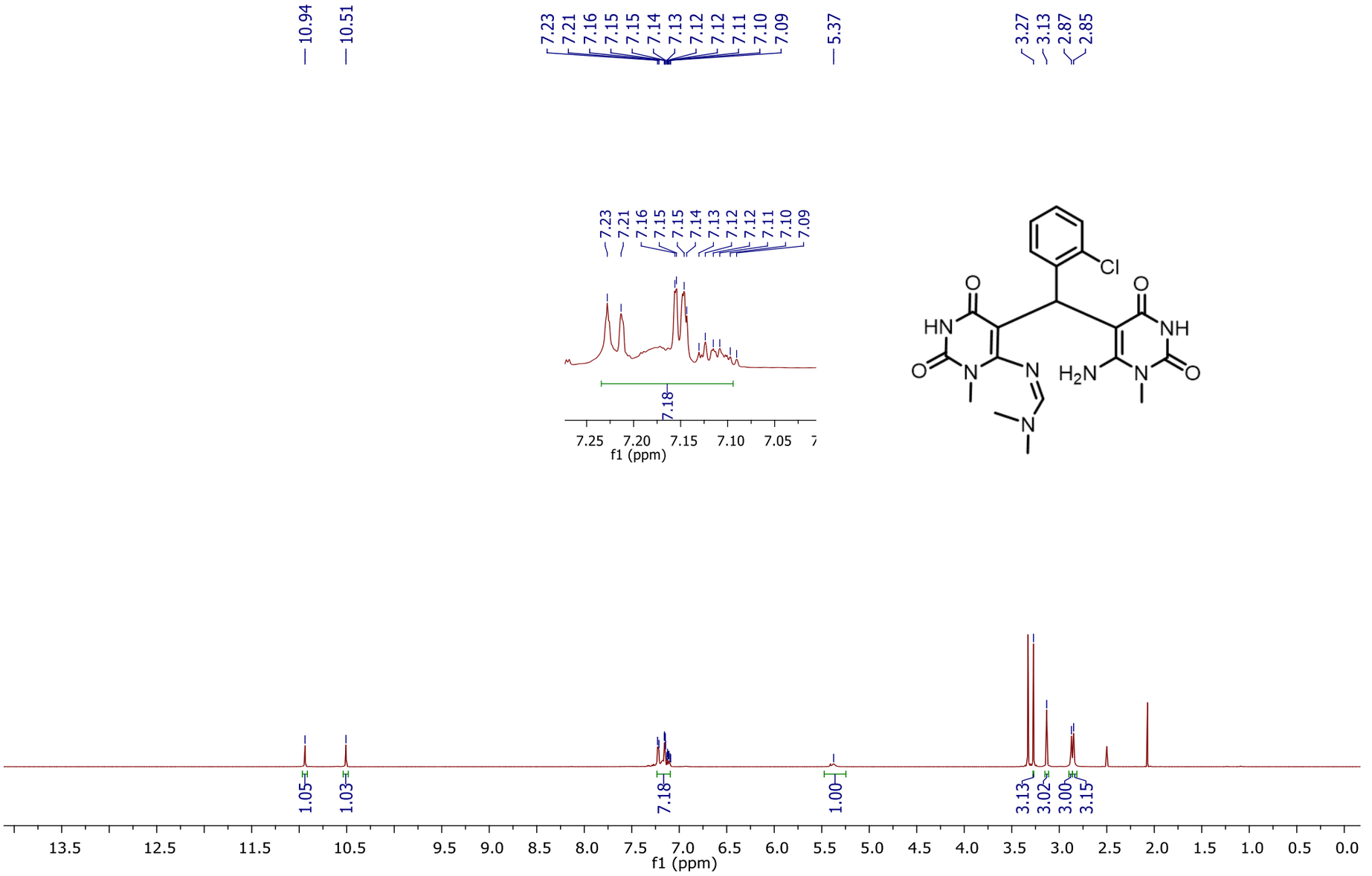

Figure S83. ^{1}H-NMR (DMSO-d_6) spectra of compound **5ad.**

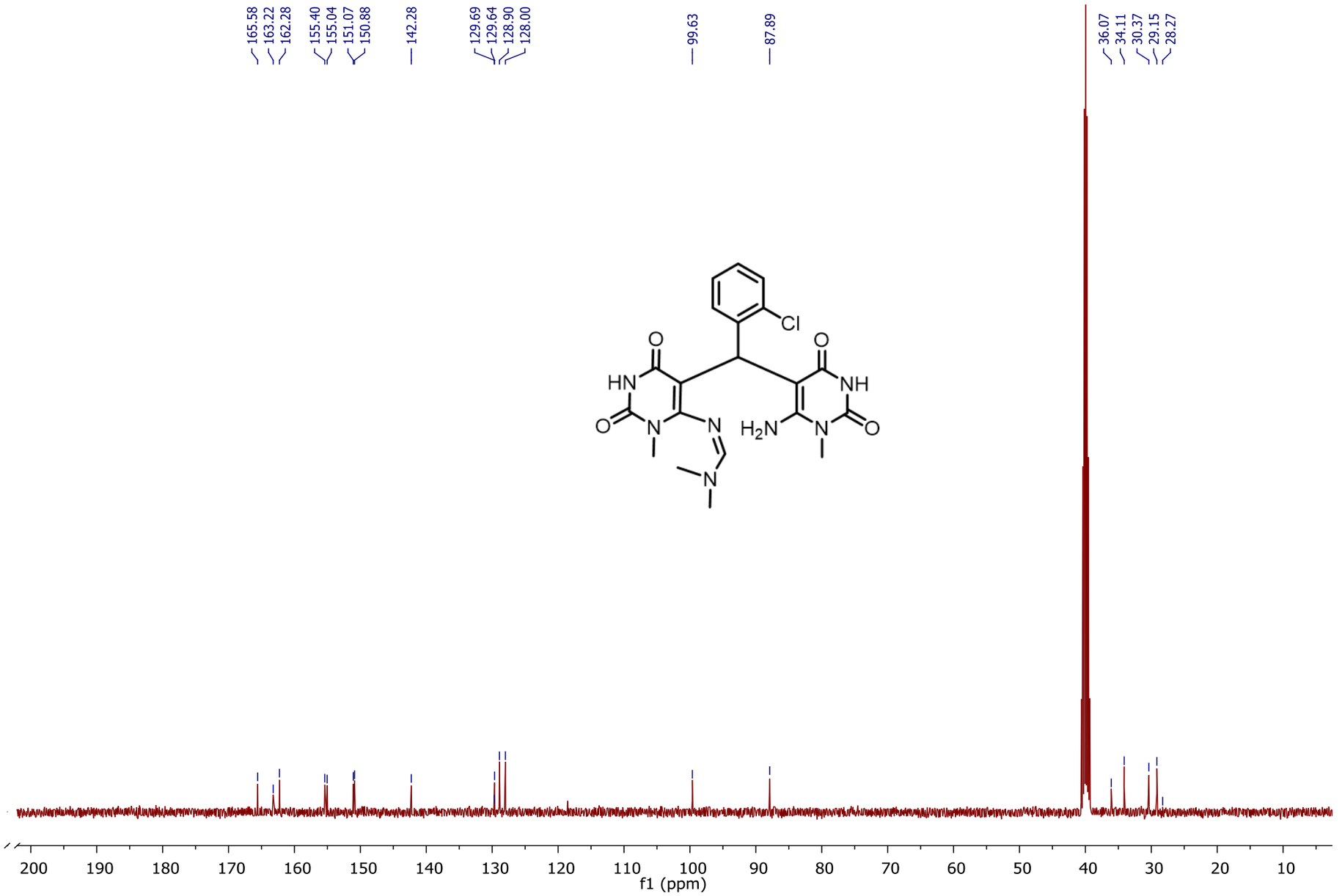

Figure S84. ^{13}C-NMR (DMSO-d_6) spectra of compound **5ad.**

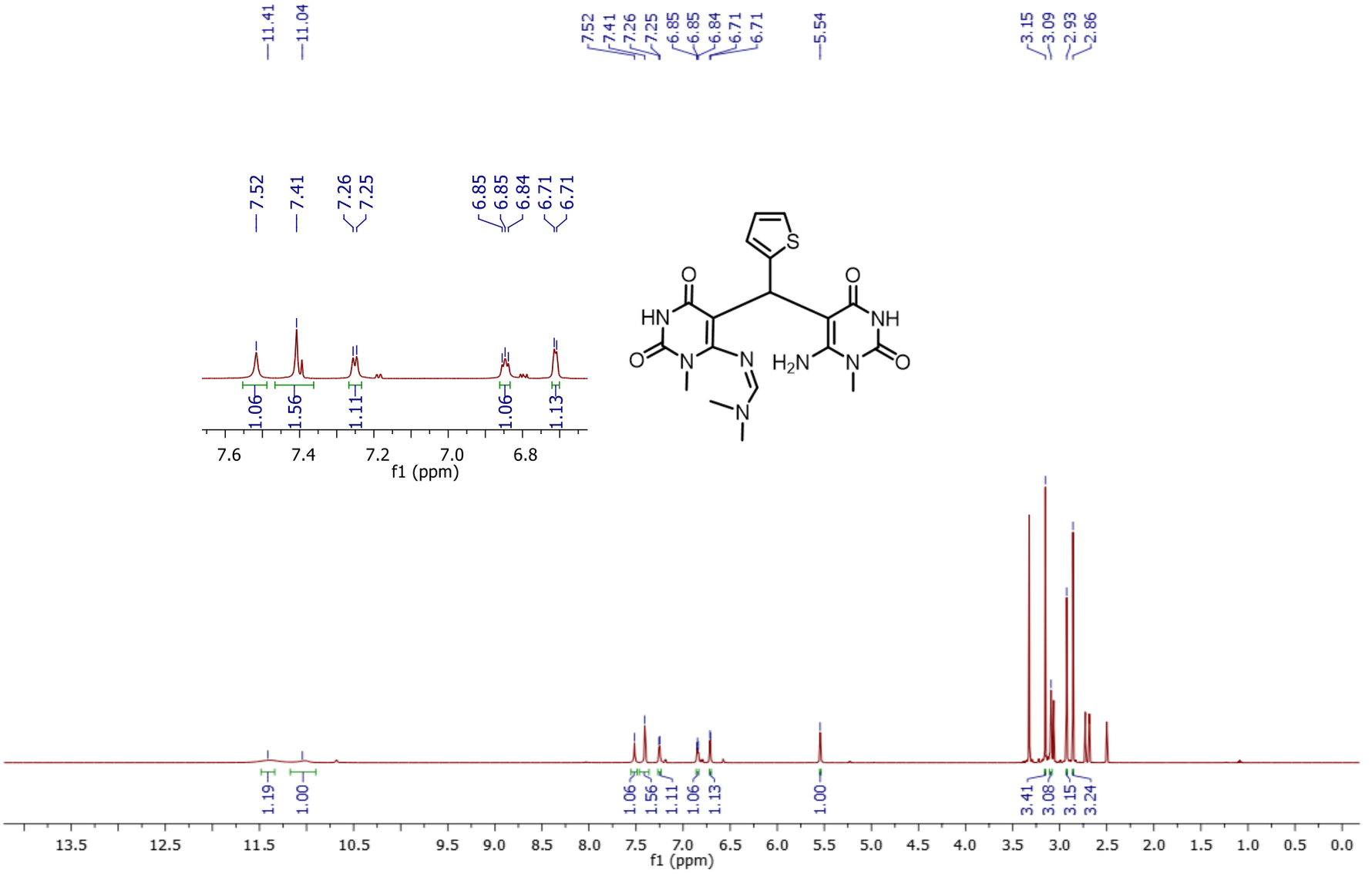

Figure S85. ^{1}H-NMR (DMSO-d_6) spectra of compound **5ae.**

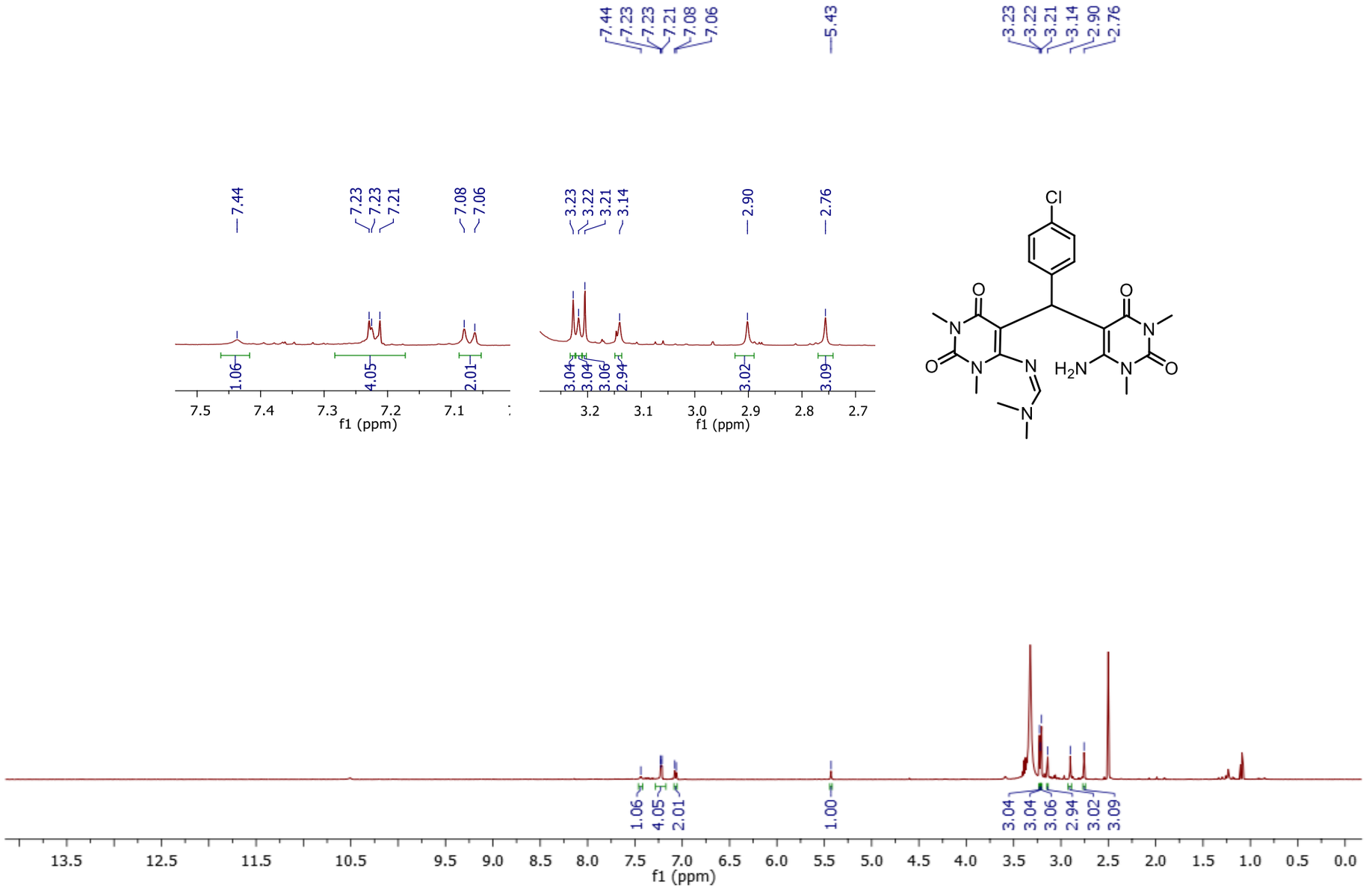

Figure S86. ^{1}H-NMR (DMSO-d_6) spectra of compound **5ba.**

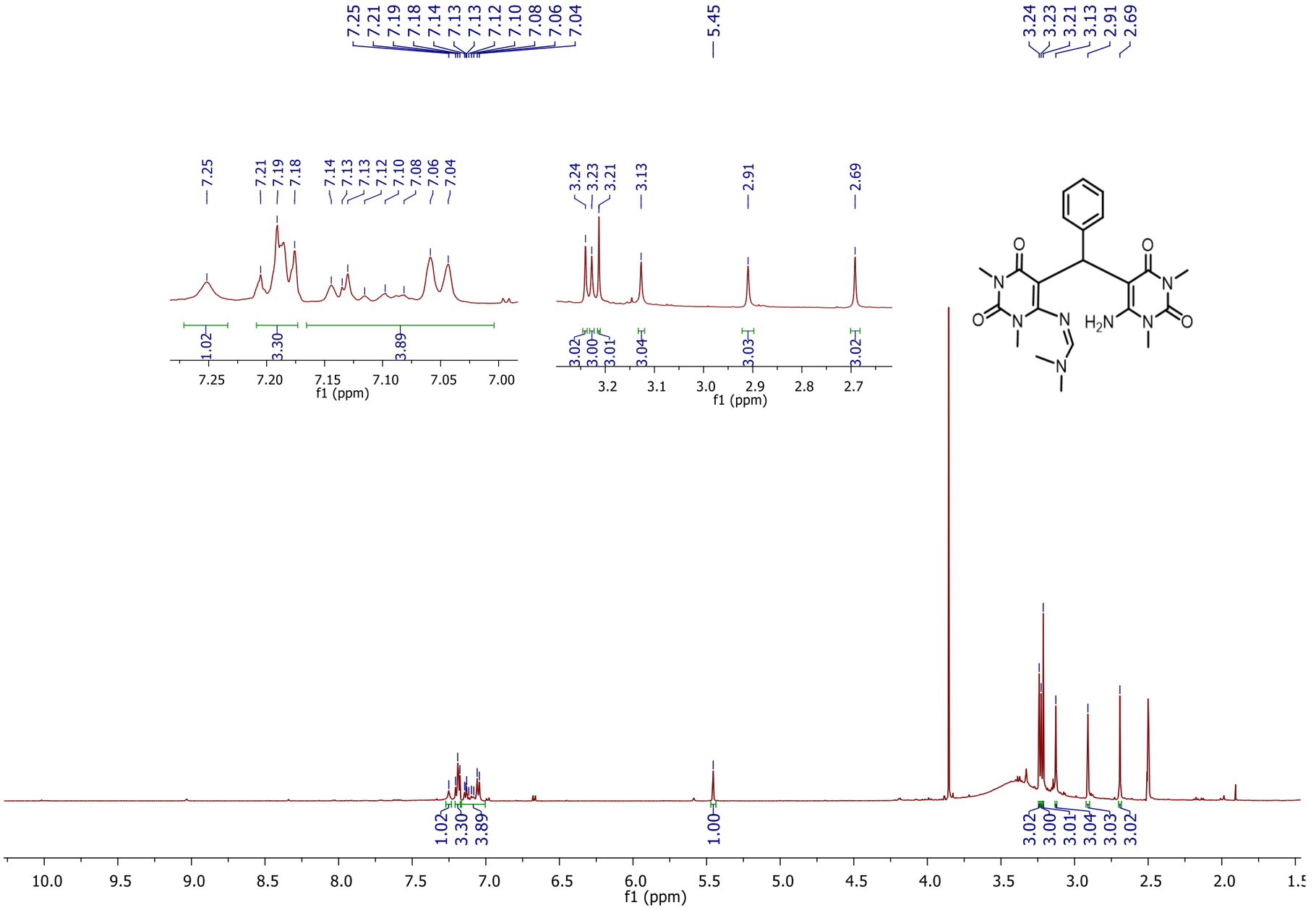

Figure S87. ^{1}H-NMR (DMSO-d_6) spectra of compound **5bb.**

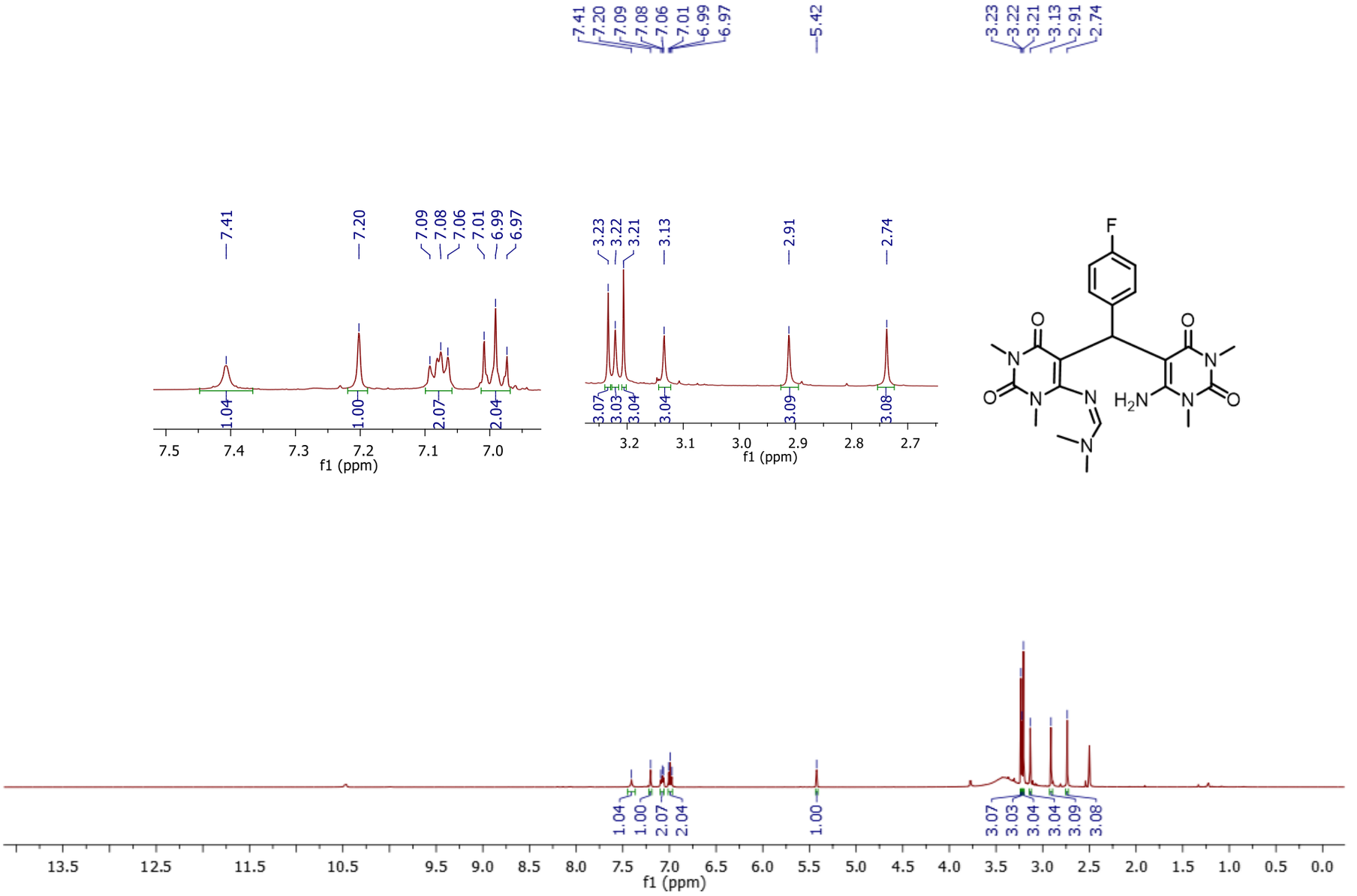

Figure S88. ^{1}H-NMR (DMSO-d_6) spectra of compound **5bc.**

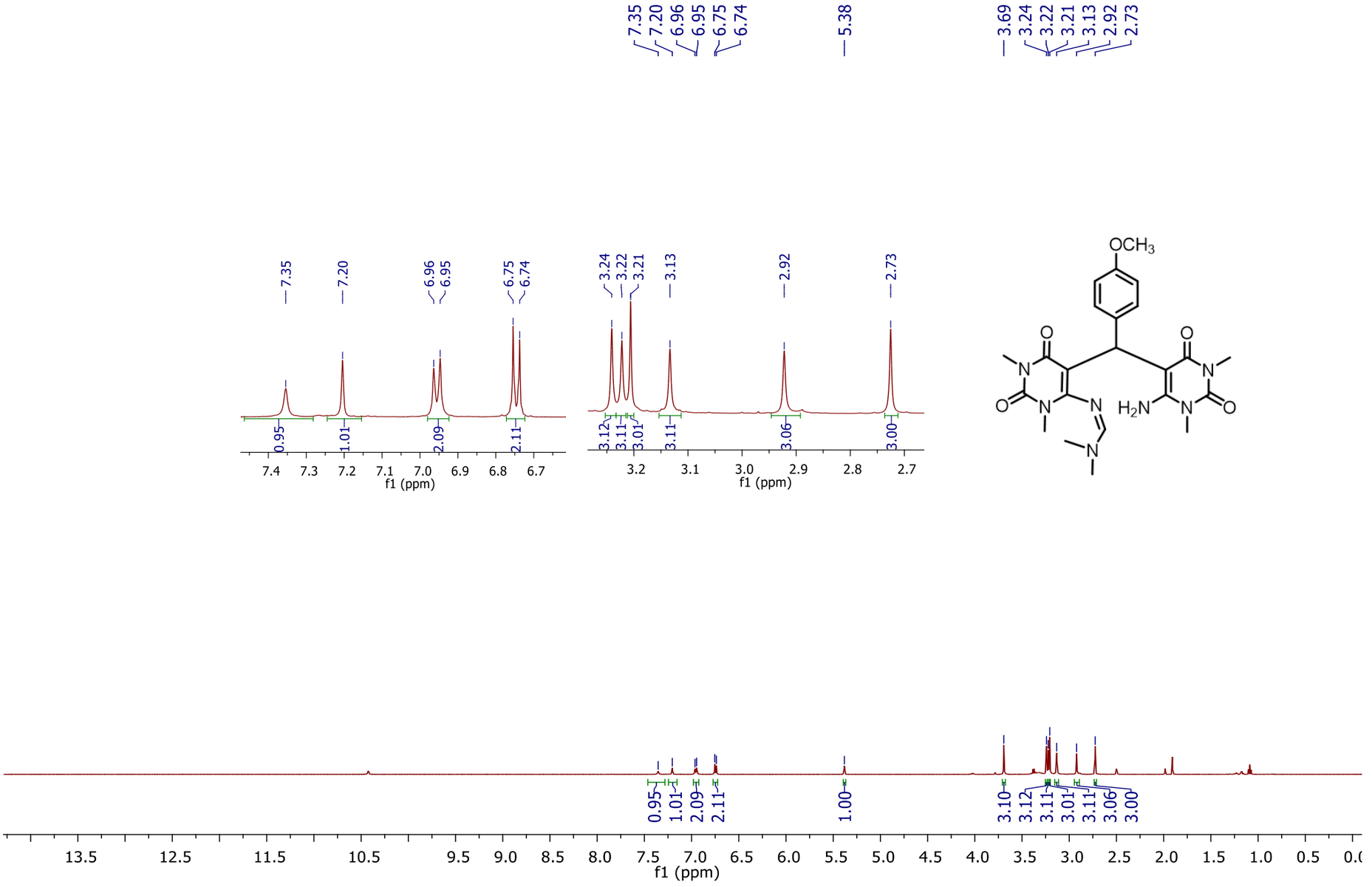

Figure S89. ^{1}H-NMR (DMSO-d_6) spectra of compound **5bd.**

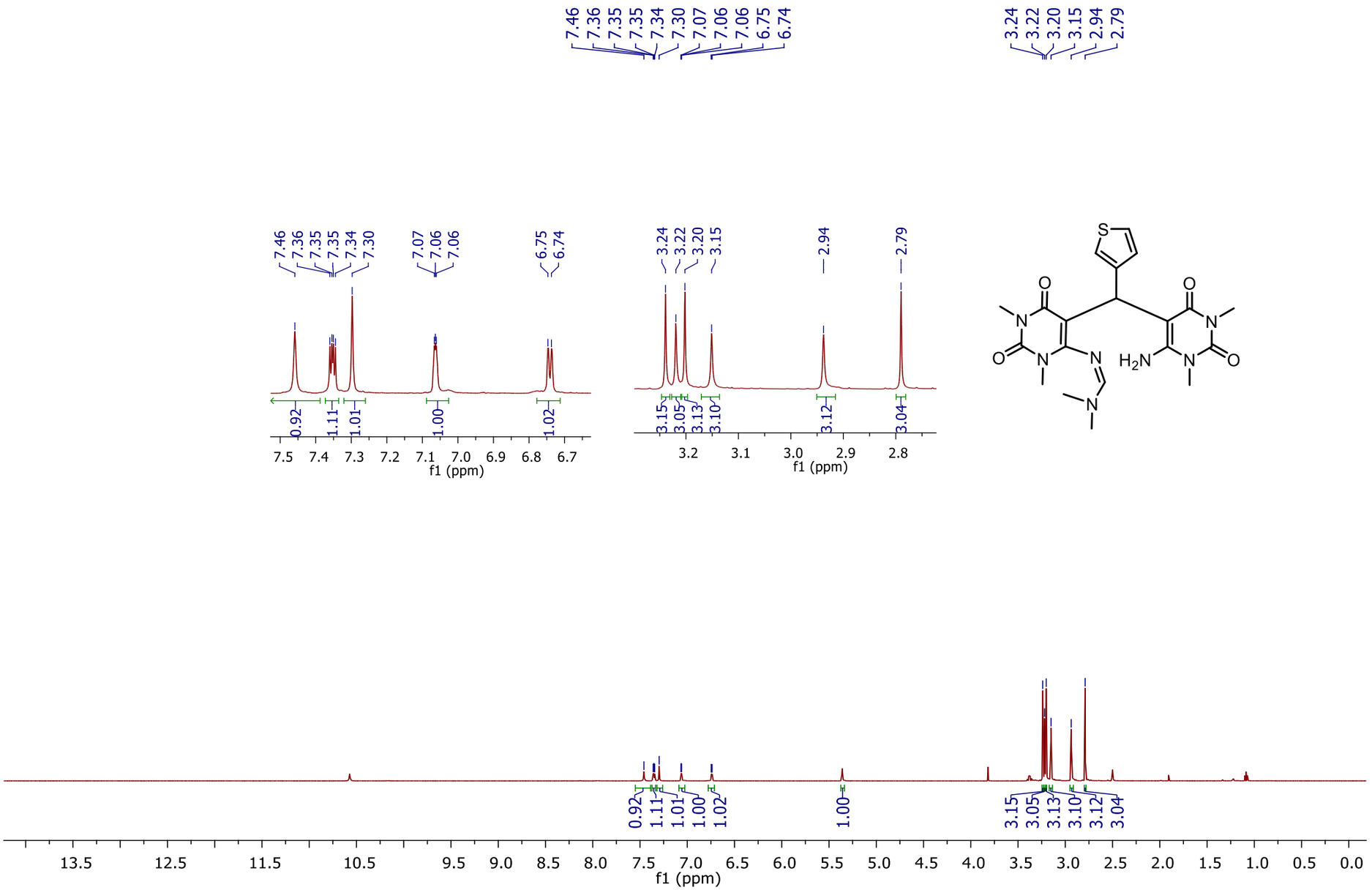

Figure S90. ^{1}H-NMR (DMSO-d_6) spectra of compound **5be.**

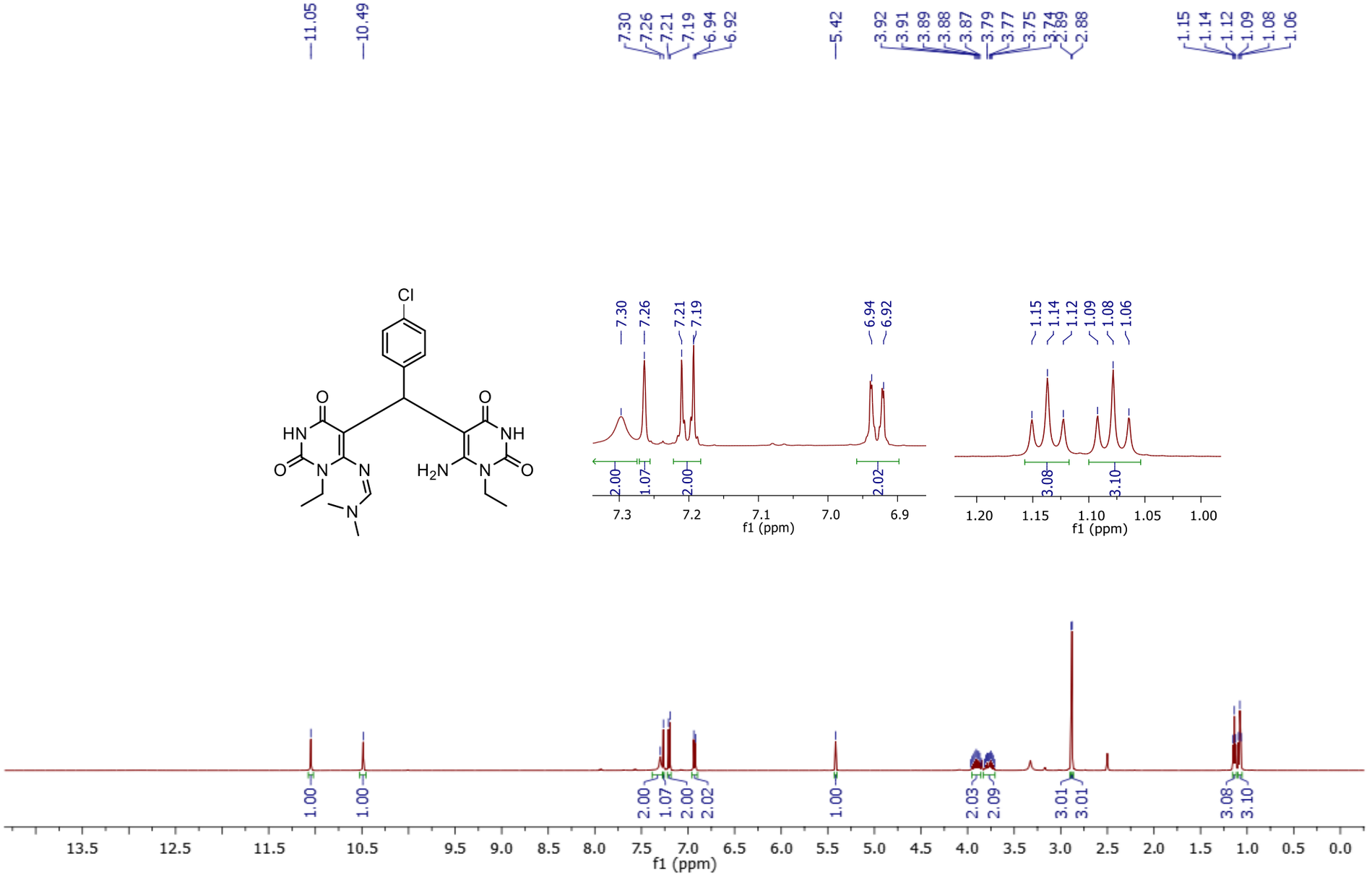

Figure S91. [1]H-NMR (DMSO-d_6) spectra of compound **5ca.**

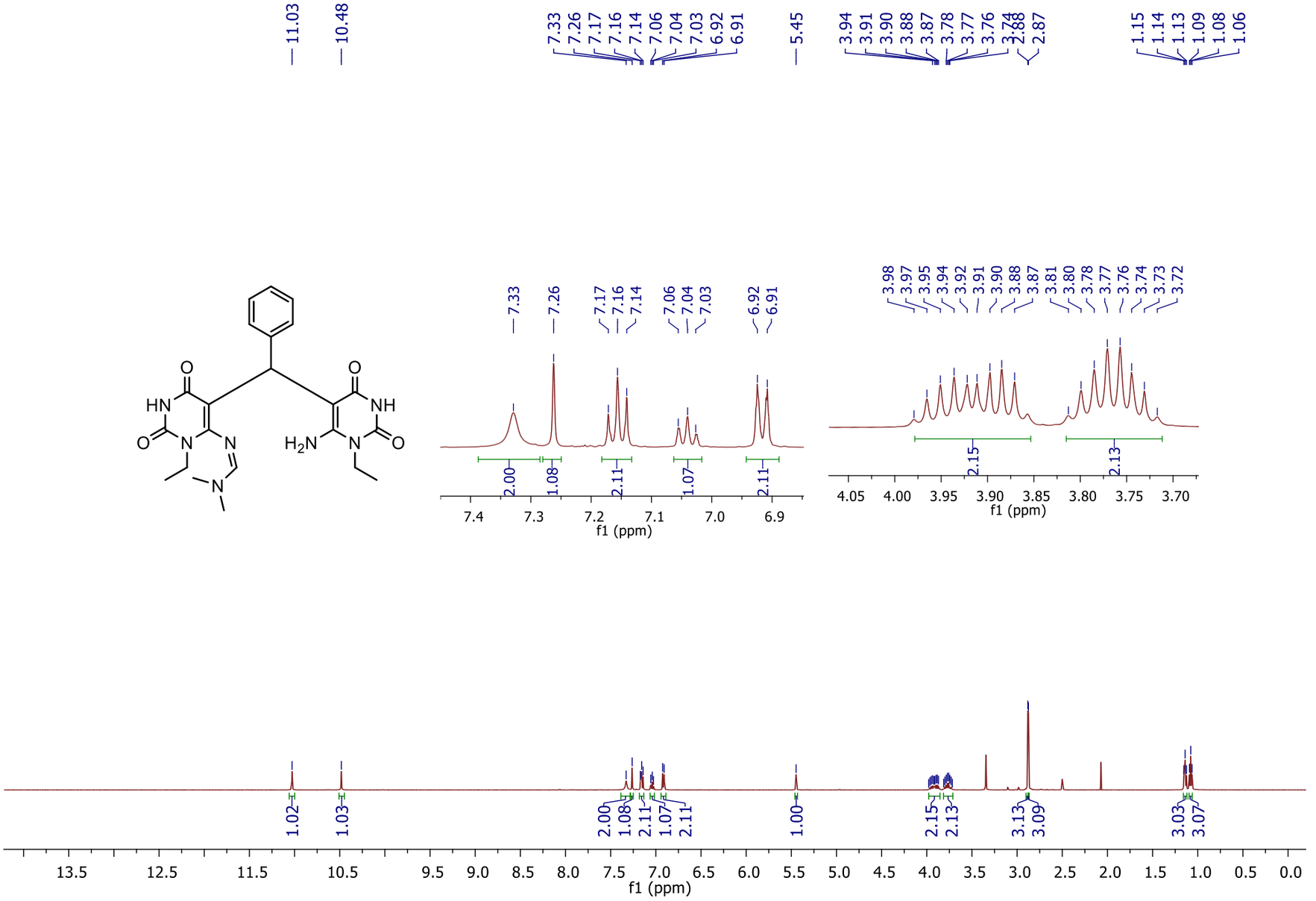

Figure S92. ^{1}H-NMR (DMSO-d_6) spectra of compound **5cb.**

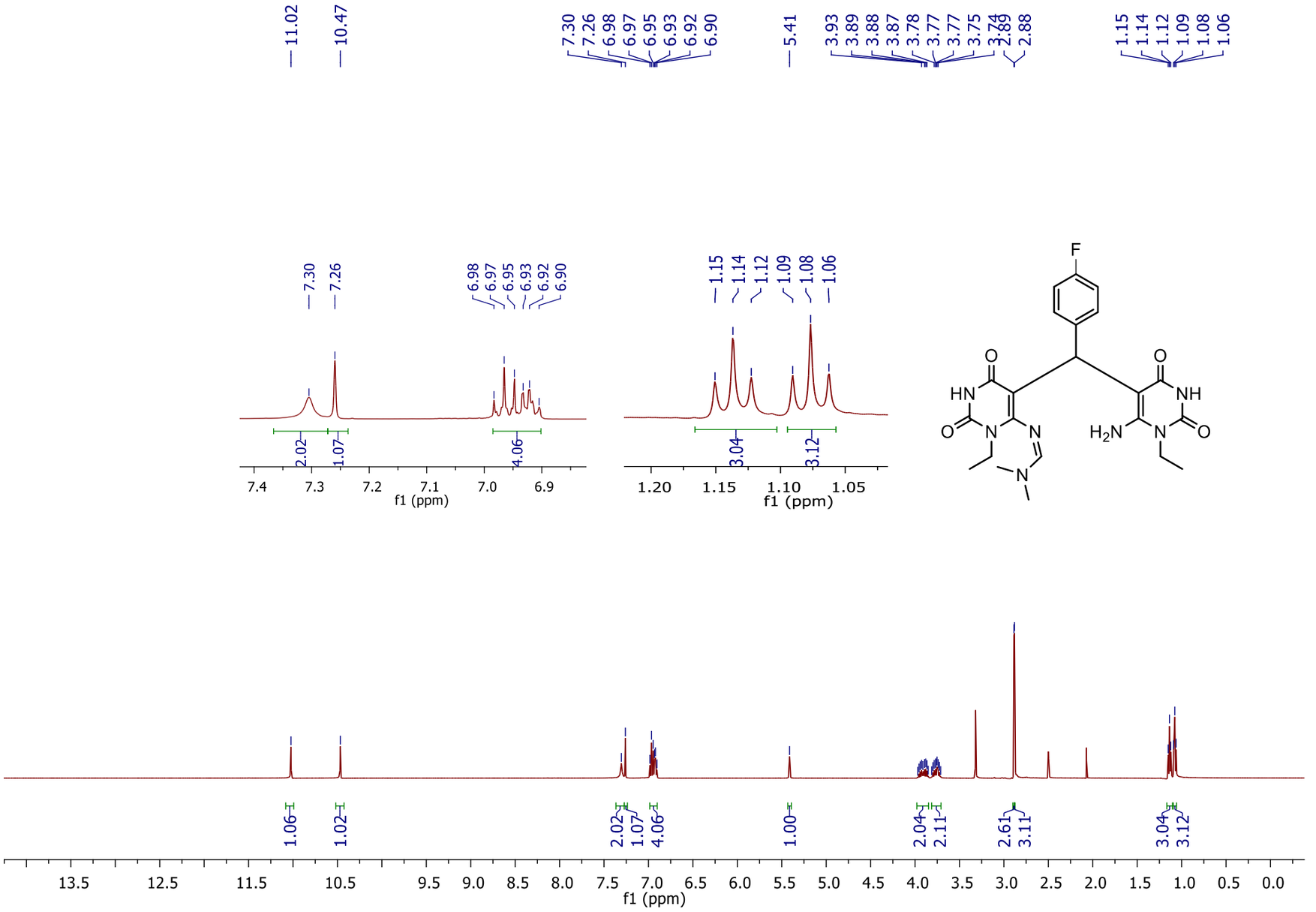

Figure S93. ^{1}H-NMR (DMSO-d_6) spectra of compound **5cc.**

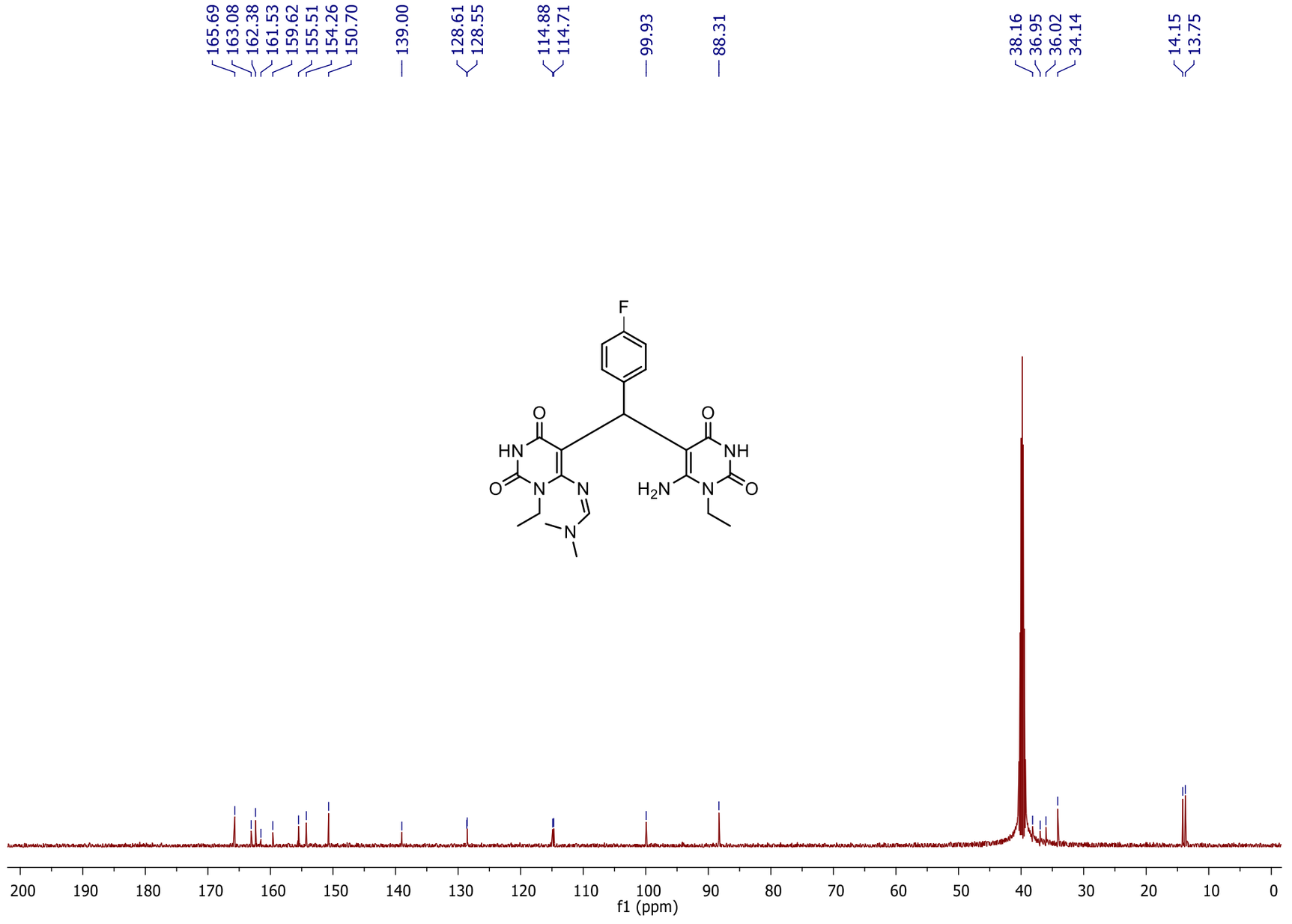

Figure S94. ^{13}C-NMR (DMSO-d_6) spectra of compound **5cc.**

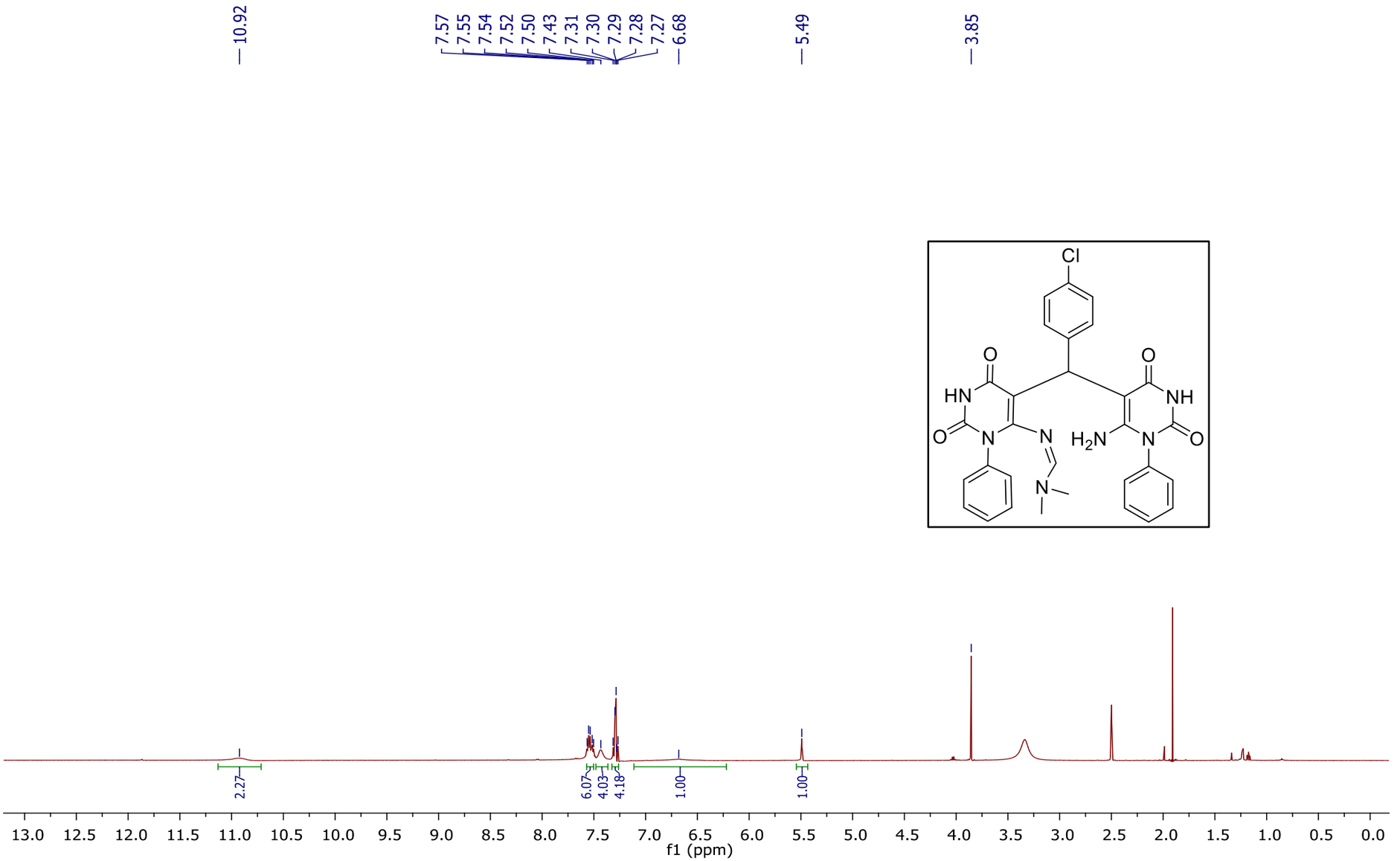

Figure S95. ^{1}H-NMR (DMSO-d_6) spectra of compound **5da.**

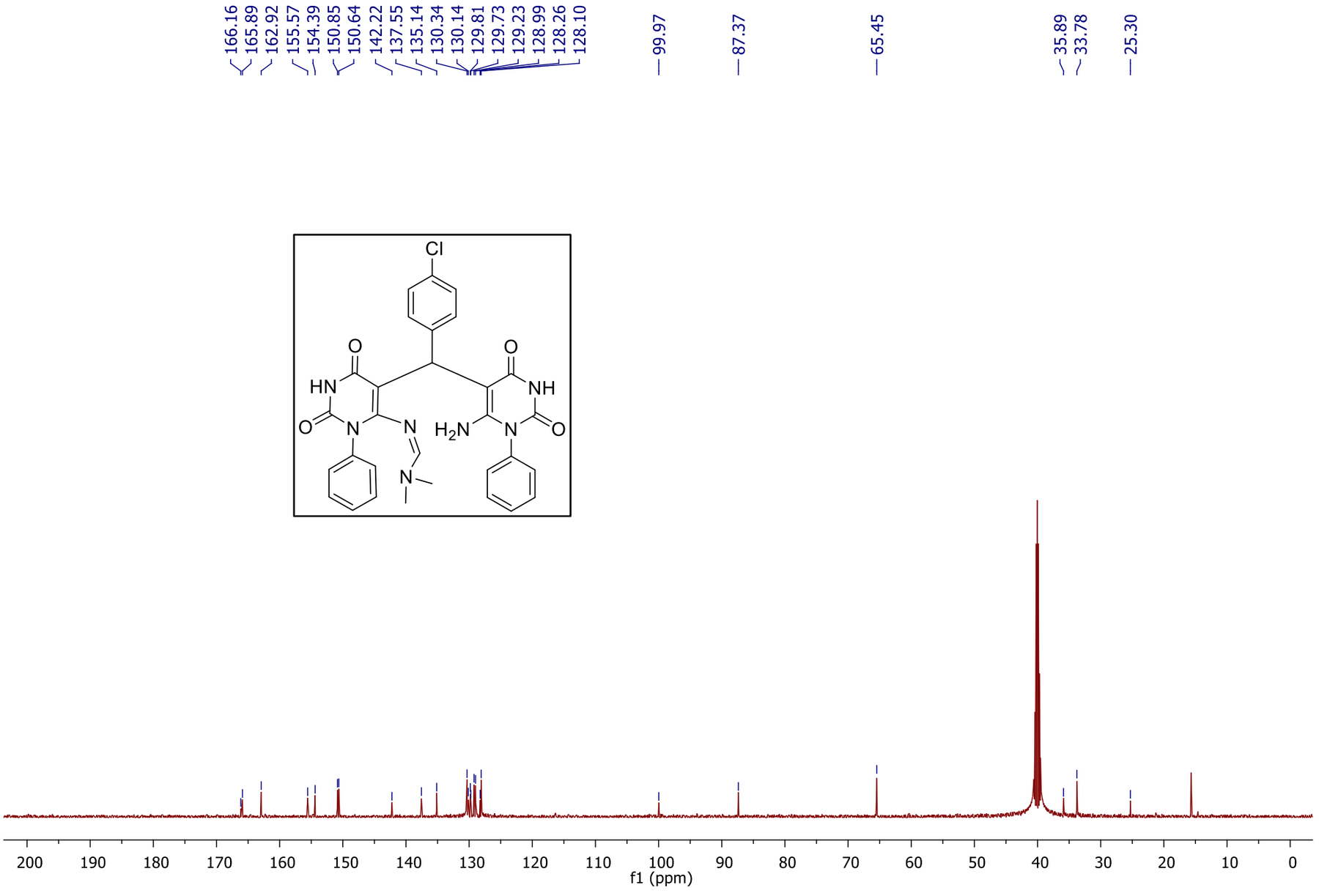

Figure S96. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **5da.**

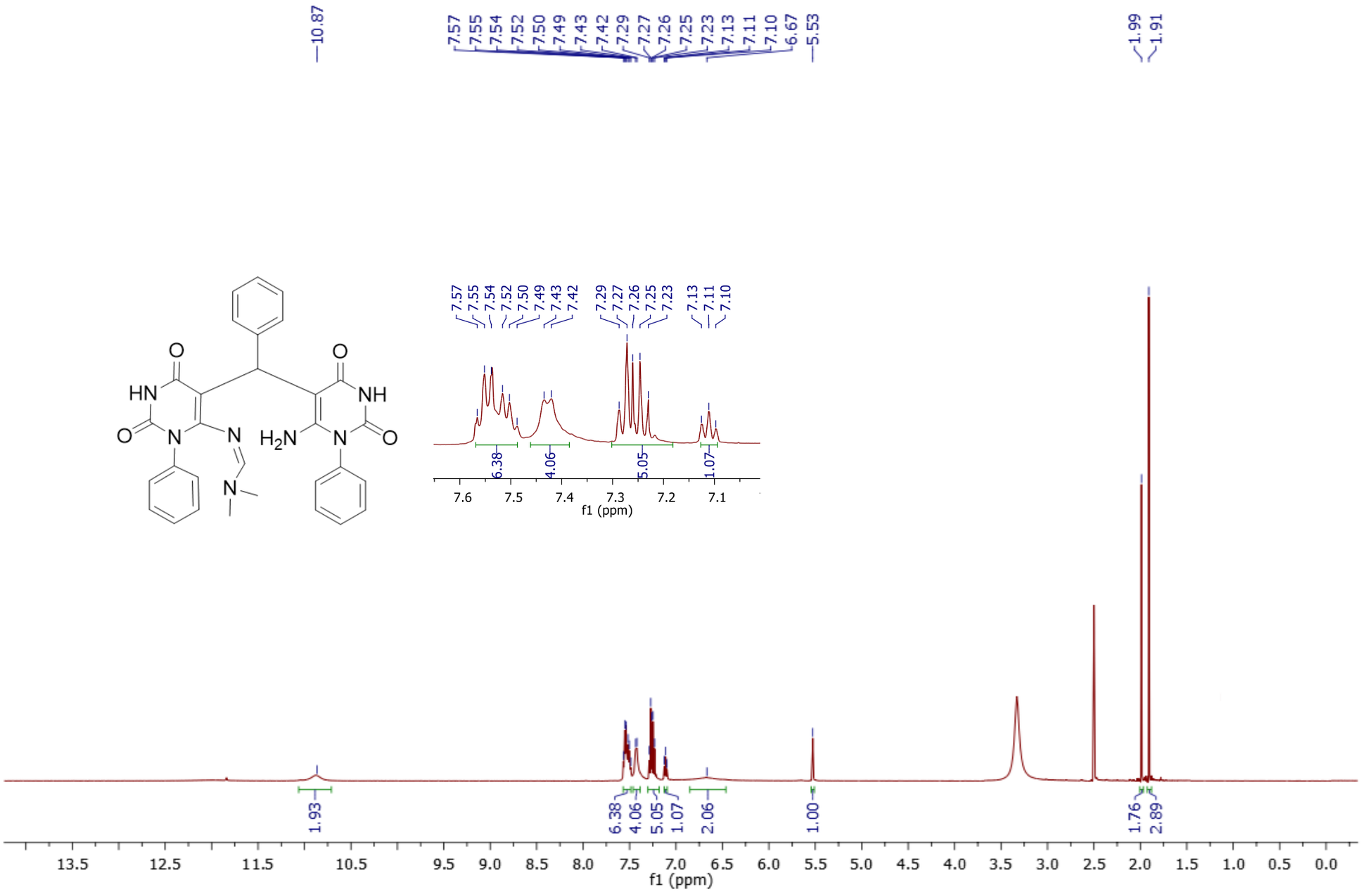

Figure S97. ^{1}H-NMR (DMSO-d_6) spectra of compound **5db.**

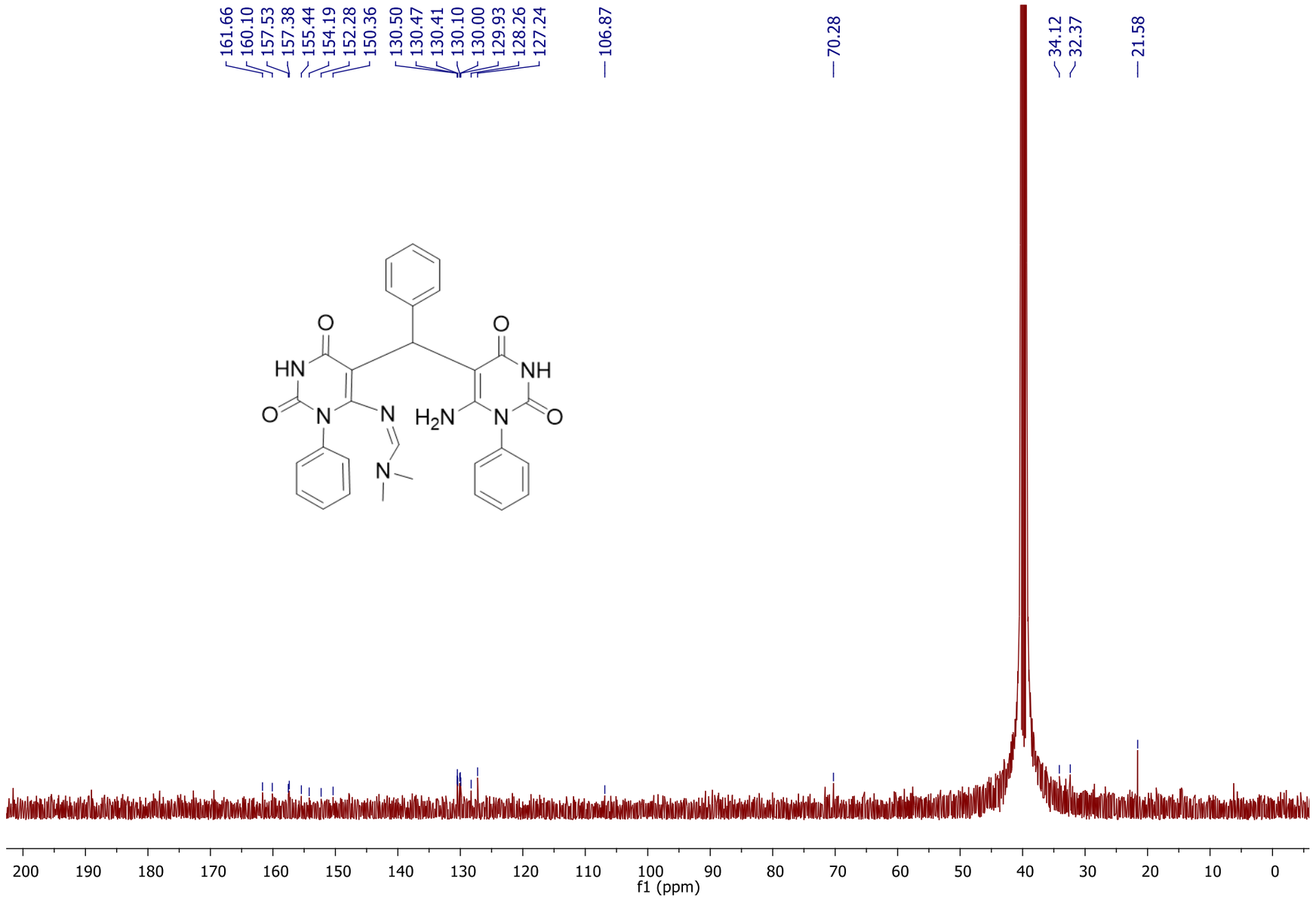

Figure S98. ^{13}C-NMR (DMSO-d_6) spectra of compound **5db.**

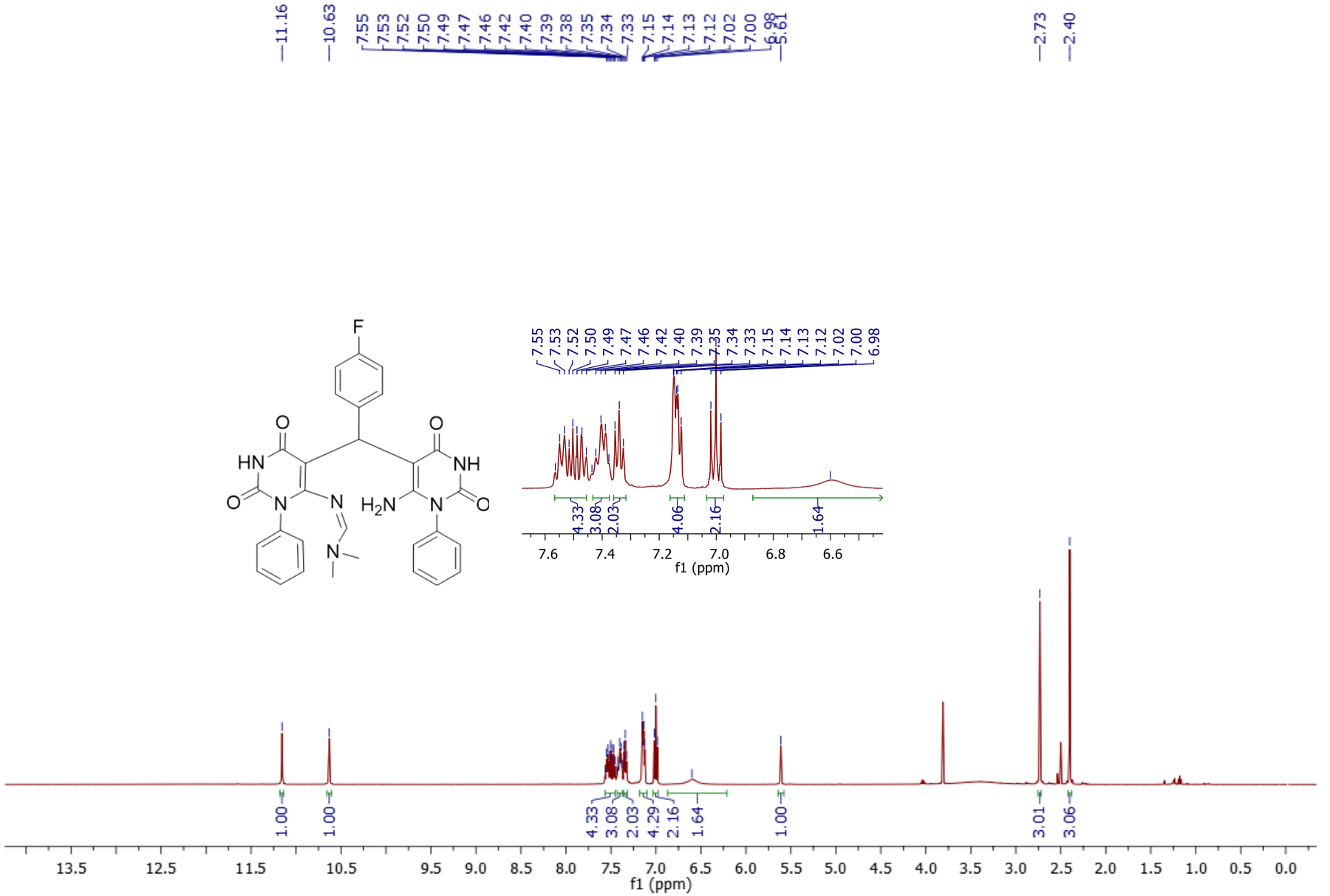

Figure S99. ^{1}H-NMR (DMSO-d_6) spectra of compound **5dc.**

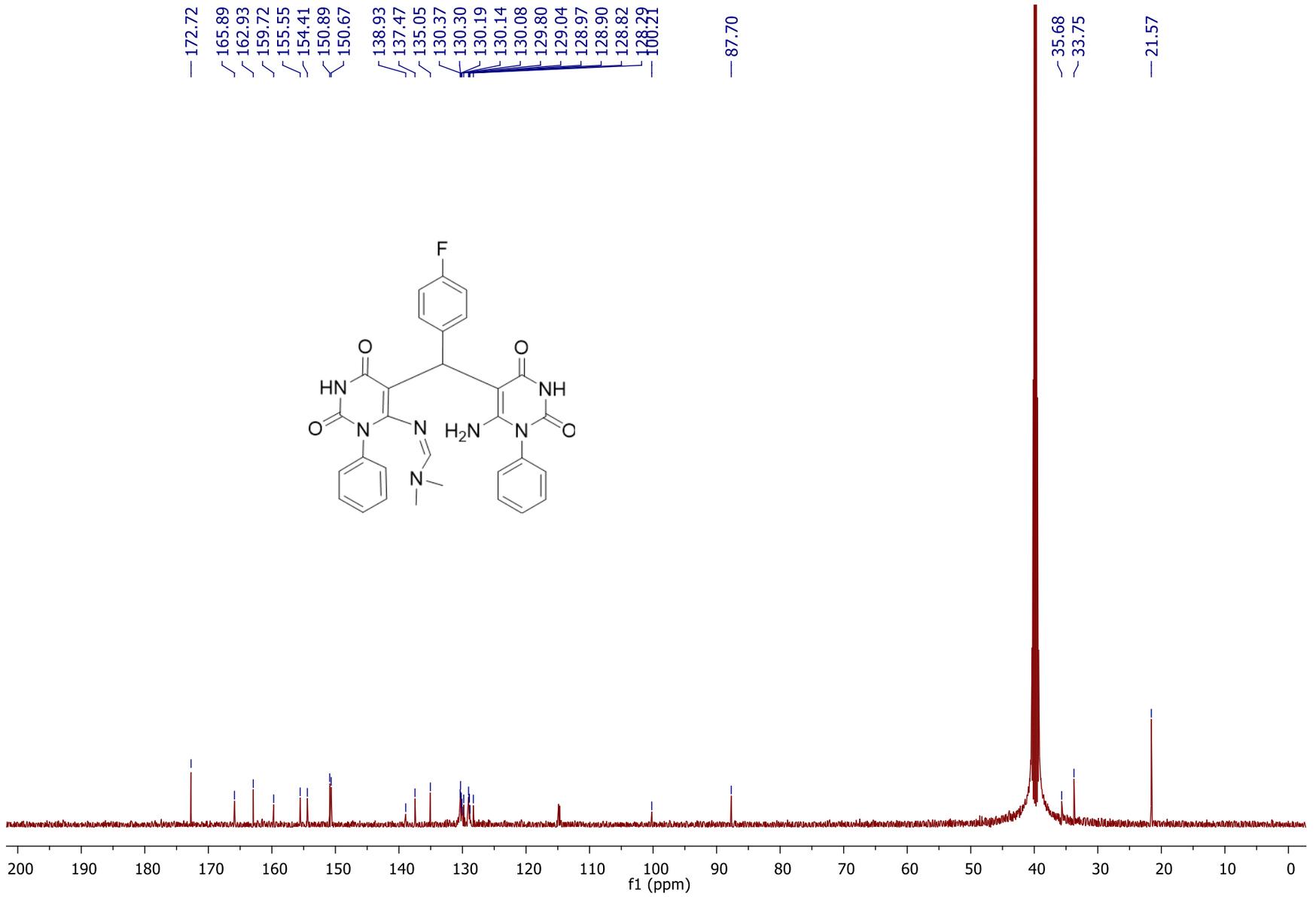

Figure S100. ^{13}C-NMR (DMSO-d_6) spectra of compound **5dc.**

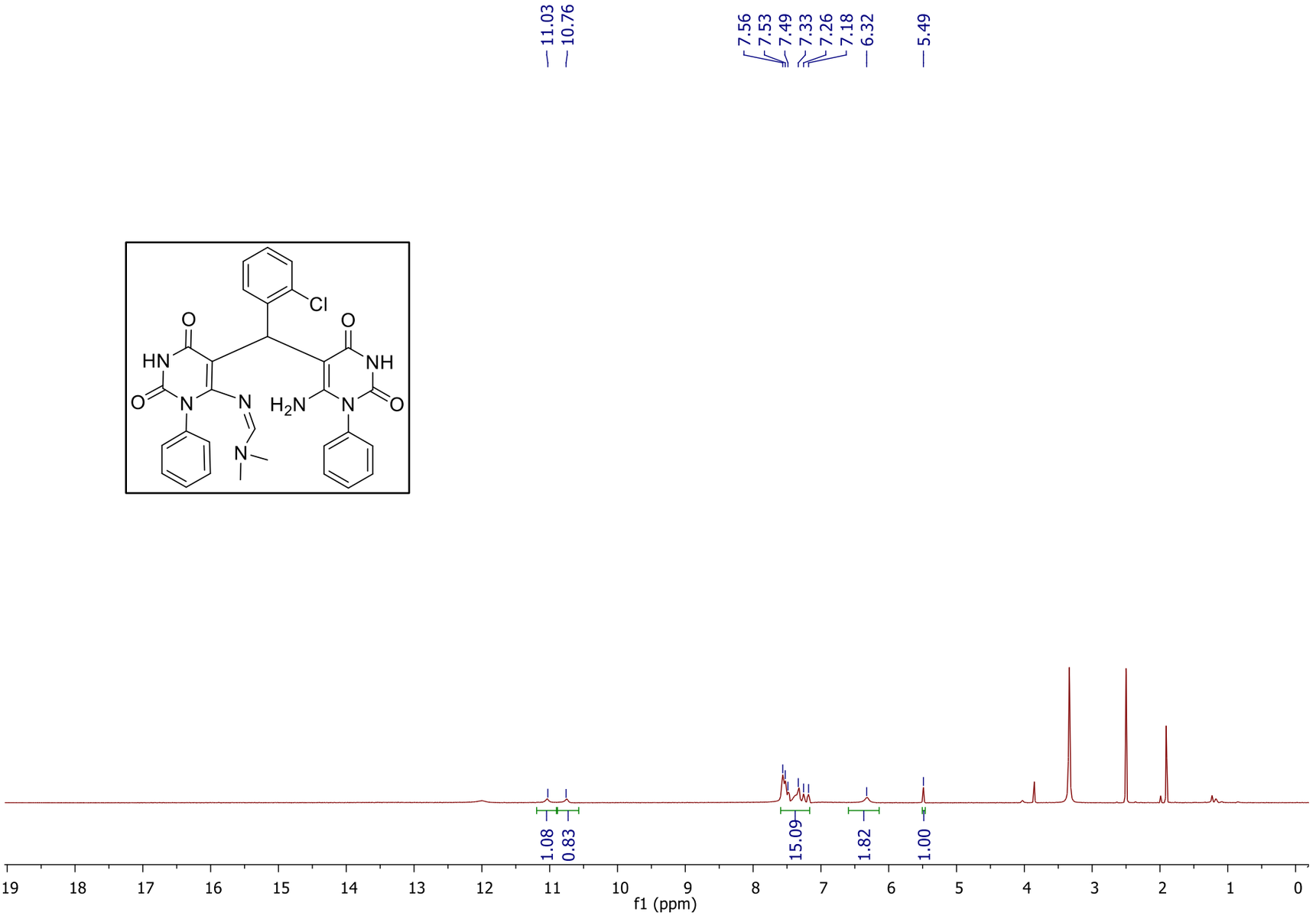

Figure S101. ^{1}H-NMR (DMSO-d_6) spectra of compound **5dd.**

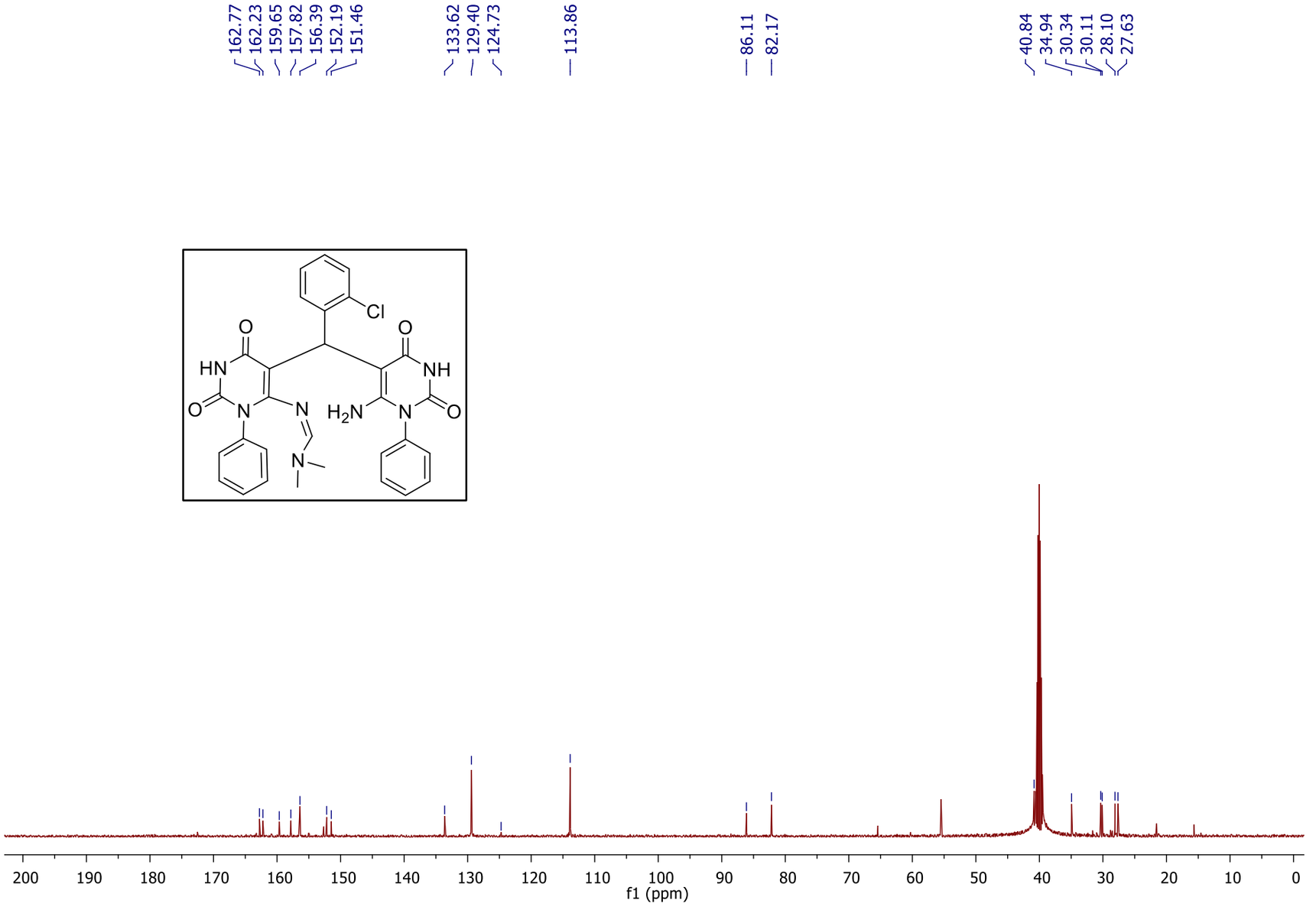

Figure S102. ^{13}C-NMR (DMSO-d$_6$) spectra of compound **5dd.**

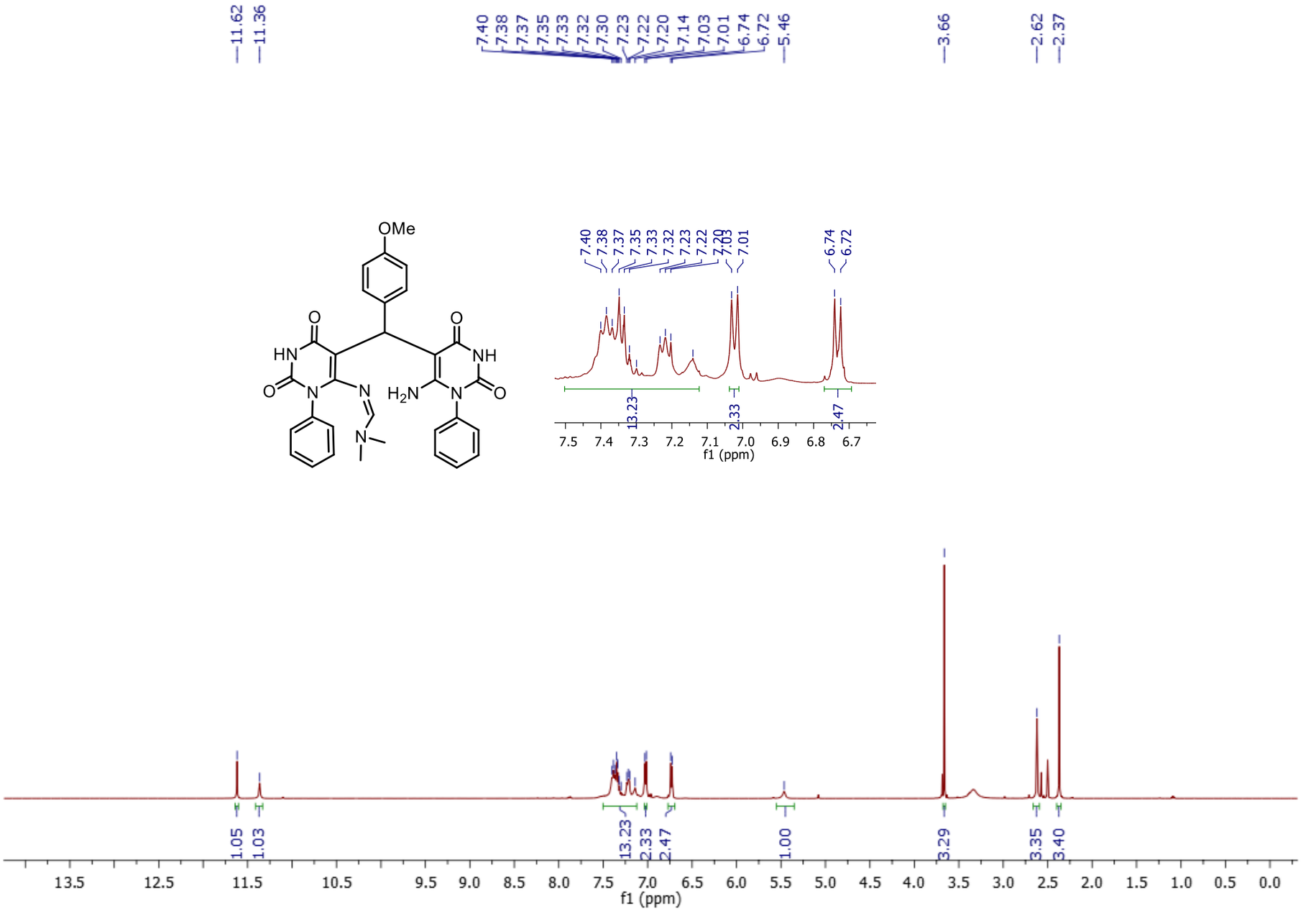

Figure S103. ^{1}H-NMR (DMSO-d_6) spectra of compound **5dd.**

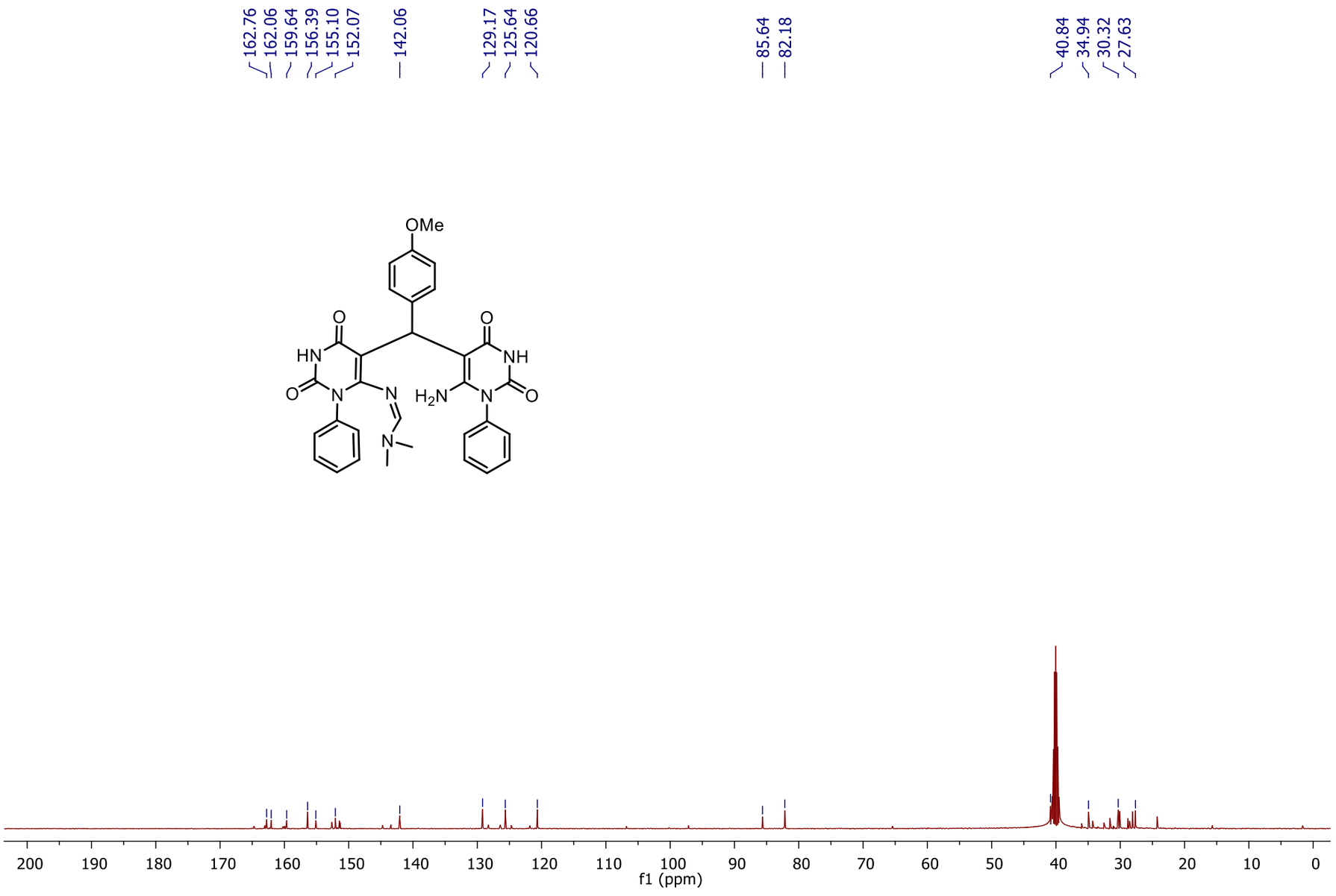

Figure S104. ^{13}C-NMR (DMSO-d_6) spectra of compound **5de.**