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ChemistrySELECT

Supporting Information

Antimicrobial Activity of Thiocarbohydrazones: Experimental Studies and Alignment-Independent 3D QSAR Models

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Table S1. Statistics of the PCA model generated after 2 FFD cycle (out of 590 variables, 346 were retained).

Component	SSX	SSX_{acc}	VarX	$VarX_{acc}$
1	44.76	44.76	40.98	40.98
2	14.77	59.53	12.61	53.60
3	10.94	70.48	9.88	63.48
4	6.00	76.47	4.94	68.42
5	4.62	81.09	3.84	72.26

Table S2. Statistics of 3LV PLS model applying different CV procedures. Settings: FFD-LV = 3, 20% of dummy variables, 20 randomization cycles, 4 random groups. 2 cycles of FFD were applied.

Component	SSX_{acc}	SDEC	SDEP	r^2_{acc}	q^2_{acc}
3 LV (LOO)	60.69	2.19	4.55	0.92	0.67
3LV (LTO)	59.76	2.26	4.83	0.92	0.62
3 LV (RG)	61.48	2.37	5.36	0.91	0.54

Table S3. Statistics of the PCA model generated after 2 FFD cycle (out of 600 variables, 316 were retained).

Component	SSX	SSX_{acc}	VarX	$VarX_{acc}$
1	44.93	44.93	41.78	41.78
2	12.94	57.87	10.98	52.76
3	9.04	66.92	7.75	60.51
4	6.87	73.78	6.06	66.57
5	4.51	78.30	3.72	70.30

Table S4. Statistics of 2LV PLS model applying different CV procedures. Settings: FFD-LV = 3, 20% of dummy variables, 20 randomization cycles, 4 random groups. 2 cycles of FFD were applied.

Component	SSX_{acc}	SDEC	SDEP	r^2_{acc}	q^2_{acc}
2LV (LOO)	54.51	3.09	4.51	0.81	0.59
2LV (LTO)	54.27	3.19	4.78	0.79	0.54
2LV (RG)	54.28	3.15	4.82	0.80	0.53

Experimental Section

Materials

Salicylaldehyde, methyl-2-pyridyl ketone, methyl-3-pyridyl ketone, methyl 4-pyridyl ketone, 2-pyridinecarboxaldehyde, 3-pyridinecarboxaldehyde, 4-pyridinecarboxaldehyde, 2-quinolinecarboxaldehyde, 6-methyl-2-pyridinecarboxaldehyde and thiocarbohydrazide were obtained from Sigma. 8-quinolinecarboxaldehyde (98 %) and 8-hydroxy-2-quinolinecarboxaldehyde (98 %) were obtained from Acros Organics. All used solvents were of spectroscopic quality.

Methods

Elemental analyses (C, H, N) were performed by the standard micromethods using the ELEMENTARVario ELIII C.H.N.S=O analyzer. Fourier-transform infrared (FTIR) spectra were obtained using FTIR BOMEM MB 100 in the form of KBr pellets. FTIR spectra were recorded in the transmission mode between 400 and 4000 cm^{-1} with a resolution of 4 cm^{-1}. Abbreviations used for IR spectra: vs, very strong; s, strong; m, medium; w, weak. All NMR spectral measurements were performed on a Bruker Avance III 500 spectrometer equipped with a broad-band direct probe. The spectra were recorded at room temperature in DMSO-d_6. Chemical shifts are given on δ scale relative to tetramethylsilane (TMS), as internal standard for ^{1}H and ^{13}C. Coupling constants (J) were expressed in Hz. Abbreviations used for NMR spectra: s, singlet; dd, doublet of doublets; ddd, double double doublet.

General procedure for synthesis of m-TCHs 1-11

A mixture of thiocarbohydrazide (dhS, **1 mmol**) and various *N*-heteroaromatic aldehydes and ketone (**0.5 mmol**) was refluxed for about 3 h in 30 mL mixture of ethanol/water (1:3, v/v) with a one drop of hydrochloric acid. After completion of the reaction, the crude product was filtered and recrystallized from appropriate solvent to obtained pure compound. The compounds **1** [1], **2** and **5** [2], **7** [3], **8-10** [4], and **11** [5], are known compounds which have been previously synthesized. All these components are synthesized according to our procedures and their purity was verified through melting point and elemental analysis. Synthesis for compound **6** has been published before [6], but without spectral characterization. Here, we presented detailed characterization of **6.**

*Methyl 3-pyridyl ketone thiocarbonohydrazone (**3**)*. Yellow crystal; Yield 88 %; M.p. 174-175 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3273s (NH_2), 3169m (NH), 3018w (CH_{aryl}), 1507vs (amide II), 1224s (C=S); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2,30 (s, 3H, H-C8); 4,96 (s, 2H, H–N5);); 7,39 (dd, 1H, H–C5, $^{3}J_{5,4}$ = 8,0 Hz, $^{3}J_{5,6}$ = 4,8 Hz); 8,36 (dd, 1H, H–C4, $^{3}J_{4,5}$ =8,0 Hz); 8,55 (dd, 1H, H–C6, $^{3}J_{6,5}$ = 4,8 Hz,); 9,13 (d, 1H, H–C2); 9,90 (s, 1H, H-N4); 10,31 (s, 1H, H–N3); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm, TMS): 13,61 (C8); 123,15 (C5); 133,20 (C3); 133,90 (C4); 145,60 (C7); 147,89 (C2); 149,65 (C6); 176,20 (C9); Elemental Analysis: Calculated:: %C, 45.91; %H, 5.30; %N, 33.47; %S, 15,32 %, Found: %C, 45.88; %H, 5.20; %N, 33.35; %S, 15,44 %. Solvent for crystallization: methanol (Figs. S1-S2).

*Methyl 4-pyridyl ketone thiocarbonohydrazone (**4**).* Yellow solid; Yield 74 %; M.p. 201-202^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3297s (NH_2), 3167m (NH), 3024w (CH_{aryl}), 1501vs (amide II), 1220s (C=S); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): δ: 2,27 (s, 3H, H-C8); 4,98 (s, 2H, H–N5); 7,93 (d,d 2H, H–C3, H–C5, $^{3}J_{3,2}$ = $^{3}J_{5,6}$ = 4,7 Hz); 8,57 (d,d 2H, H–C2 = H–C6, $^{3}J_{2,3}$ = $^{3}J_{6,5}$ = 4,7 Hz); 9,97 (s, 1H, H–N4); 10,40 (s, 1H, H–N3); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm, TMS): 13,24 (C8); 120,72 (C3,C5); 144,70 (C7); 145,09 (C4); 149,72 (C2,C6); 176,19 (C9); Elemental Analysis: Calculated: %C, 45.91; %H, 5.30; %N, 33.47, %S, 15,32 %, Found: %C, 45.95; %H, 5.22; %N, 33.28, %S, 15,36. Solvent for crystallization: ethanol (Figs. S3-S4).

*3-pyridylaldehyde thiocarbohydrazones (**6**)* Yelow solid; Yield 74 %; M.p. 200-201 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3263s (NH_2), 3164m (NH), 2961w (CH_{aryl}), 1503vs (amide II), 1272s (C=S); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 4,90 (s, 2H, H-N5); 7,41 (dd, 1H, H–C5, $^{3}J_{5,4}$ = 7,9 Hz, $^{3}J_{5,6}$ = 4,75 Hz); 8,02 (s, 1H, H-C7); 8,30 (d, 1H, H–C4, $^{3}J_{4,5}$ =7,9 Hz); 8,54 (dd, 1H, H–C6, $^{3}J_{6,5}$ = 4,75); 8,93 (d, 1H, H–C2); 9,97 (s, 1H, H–N4); 11,57 (s, 1H, H–N3). 13C NMR (126 MHz, DMSO-d_6, δ ppm, TMS): 123,69 (C5); 130,28 (C3); 133,86 (C4); 139,05 (C7); 148,83 (C2); 150,15 (C6); 175,85 (C8); Elemental Analysis: Calculated: %C, 43.06; %H, 4.65; %N, 35.87; %S, 16.42. Found: %C, 42.98; %H, 4.61; %N, 35.72 %; %S, 16.33. Solvent for crystallization: ethanol (Figs. S5-S6).

General procedure for synthesis of b-TCHs 12-22

Solution of *N*-heteroaromatic aldehydes and ketone (**2,1 mmol**) in ethanol (10 mL) was added into hot solution of dhS (**1 mmol**) in ethanol (40 mL). After the catalytic amount of conc. hydrochloric acid was added, reaction mixture was stirred and refluxed for 3 h at 80 °C. The resulting precipitate was collected by filtration and recrystallized from appropriate solvent. The

syntheses of compounds **12** [7, 8], **13**, **14**,[10] **15-18** [8], **19-21** [4], and **22** [9] have been published earlier. All these components are synthesized according to our procedures and their purity was verified through melting point and elemental analysis.

Antimicrobial activity

Agar diffusion method

Antibacterial activity was evaluated using four different strains of Gram-positive bacteria: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Clostridium sporogenes* (ATCC 19404) and *Kocuria rhizophila* (ATCC 9341), and four different strains of Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus hauseri* (ATCC 13315) and *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076). Antibacterial activity was determined by the well diffusion method. [11] To each Petri dish (90 mm diameter), 22 mL of nutrient agar (HiMedia, Mumbai, India) and 100 µL of bacterial suspension (10^6 colony forming units (CFU)) were added. A well with a diameter of 8 mm was then punched carefully using a sterile cork borer and 100 µL test substance (1 mg/100 µL DMSO) were added to each labeled well. Amikacin (30 µg/100 µL H_2O) was used as a positive control, whereas 100 µL water and DMSO served as negative controls. The same procedure was repeated for different microorganisms. After the inoculation of the organisms, compounds and controls, the plates were incubated for 24 h at 37 °C. Zones of bacterial growth inhibition were recorded in millimeters. The fungi tested were: *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763) and *Aspergillus brasiliensis* (ATCC 16404). Sabouraud dextrose agar (Torlak, Belgrade, Serbia) was prepared according to the manufacturer's instructions. Into each sterile Petri dish (90 mm diameter), 22 mL of agar suspension was poured and 100 µL of fungi suspension (10^5 CFU) were added. A wells with a diameter of 8 mm were punched in agar using a sterile cork borer. Into each well 100 µL of test substance (1 mg/100 µL DMSO) were added. Nystatin (30 µg/100 µL DMSO) was used as a positive control, whereas 100 µL of DMSO served as a negative control. The plates were incubated for 48 h at 24 °C. Antifungal activity was determined by measuring the diameter of the inhibition zone.

Broth microdilution antimicrobial assay

Minimum inhibitory (MIC) and bactericidal concentrations (MBC) of the compounds were determined using broth microdilution method according to CLSI 2005 [12,13]. Antimicrobial activity was tested against two bacterial and one fungal strain of the American Type Culture Collection

(ATCC): *Staphylococcus aureus* 6538, *Pseudomonas aeruginosa* 9027, and *C. albicans* 10231. The antimicrobial assay was performed in 96-well microtiter plates (Sarstedt, Gerrmany). Mueller Hinton broth was used for bacterial strains and malt broth for candida (BioLife, Milan, Italy). The concentration of microbial suspensions was set to 10^5 CFU. The compounds were dissolved in 5% DMSO aqueous solution, covering the range of concentrations from 0.0024 to 5 mg/mL. Triphenyltetrazolium chloride (TTC, 0.0075%) was added to bacterial suspension as a growth indicator. Positive growth control was 5% DMSO in appropriate medium. Bacterial strains were incubated for 24 h at 37 °C, while candida was incubated for 48 h at 25 °C. The concentration of compound at which there was no visible microbial growth was taken as a MIC value. MBC was determined by serial sub-cultivation of the samples taken from each well that showed no visible microbial growth into microtiter plates containing appropriate medium. The lowest concentration of each compound that achieved the loss of any visible growth after repeated incubation was considered as MBC. All determinations were performed in triplicate.

Alignment-independent 3D QSAR models

3D QSAR models were made using Pentacle 1.06 software. [14] Pentacle calculates alignment-independent descriptors (GRIND-2 descriptors) from molecular interaction fields (MIFs). [15] GRIND-2 descriptors are designed to overcome the major problem of 3D QSAR studies - structure alignment. MIFs were calculated using four chemical probes: **DRY**, which represents hydrophobic interactions; **O** is sp^2 carbonyl probe which mapped H-bond donor features of molecules; **N1** is neutral flat –NH probing H-bond acceptors in molecules, and **TIP** probe which is introducing the shape of molecules in model building. Important positions around molecules (hot spots) are extracted from MIFs using AMANDA discretization algorithm. [16] Encoding of the filtered MIFs into GRIND variables was performed by the maximal auto- and cross-correlation (MACC2) algorithm. Obtained hot spots should represent 3D position of favorable interaction points of molecules, and should shape the virtual receptor site (VRS). All other parameters for MIF computation were as default. Since the number of calculated descriptors largely exceeds the number of compounds, matrix of descriptors is analyzed with principal component analysis (PCA) and partial least squares (PLS) regression analysis. [17] Initial conformation of compounds **1**-**22** was generated in OMEGA 2.4.3, [18] using MMFF94s force field. [19] Database of lowest energy conformers was further optimized using semiempirical PM6 method implemented in MOPAC 2016. [20] VEGA ZZ 3.1.0 was used as a GUI. [21]

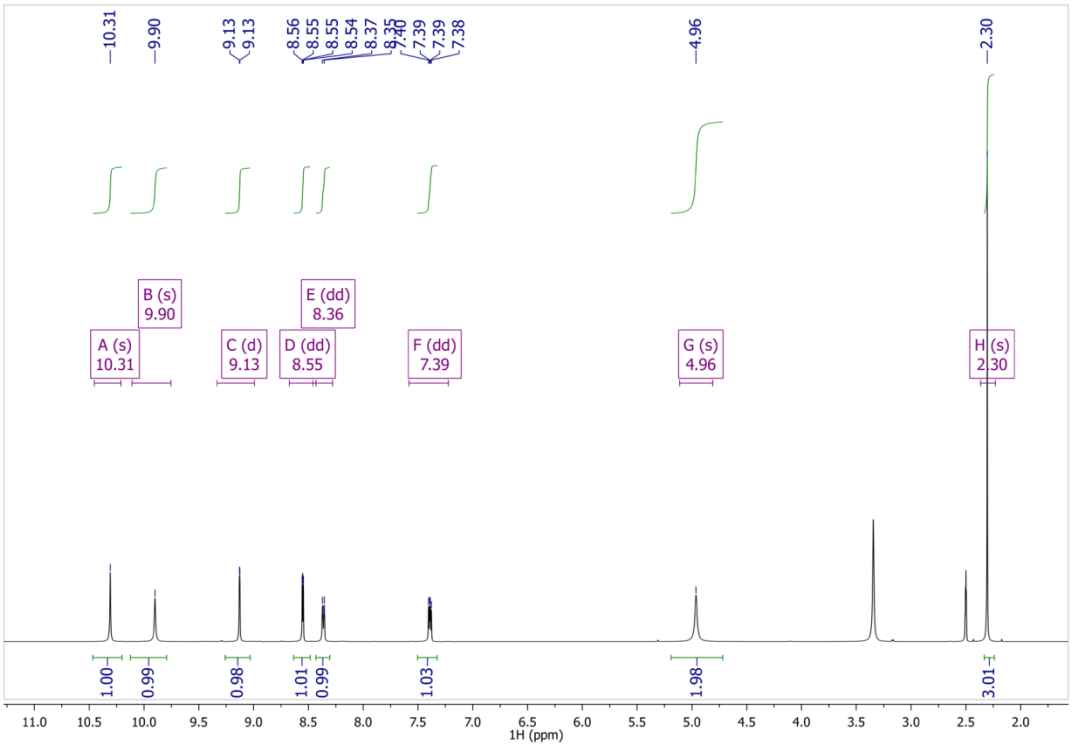

Figure S1. ^{1}H NMR spectrum of **3** in DMSO-*d6.*

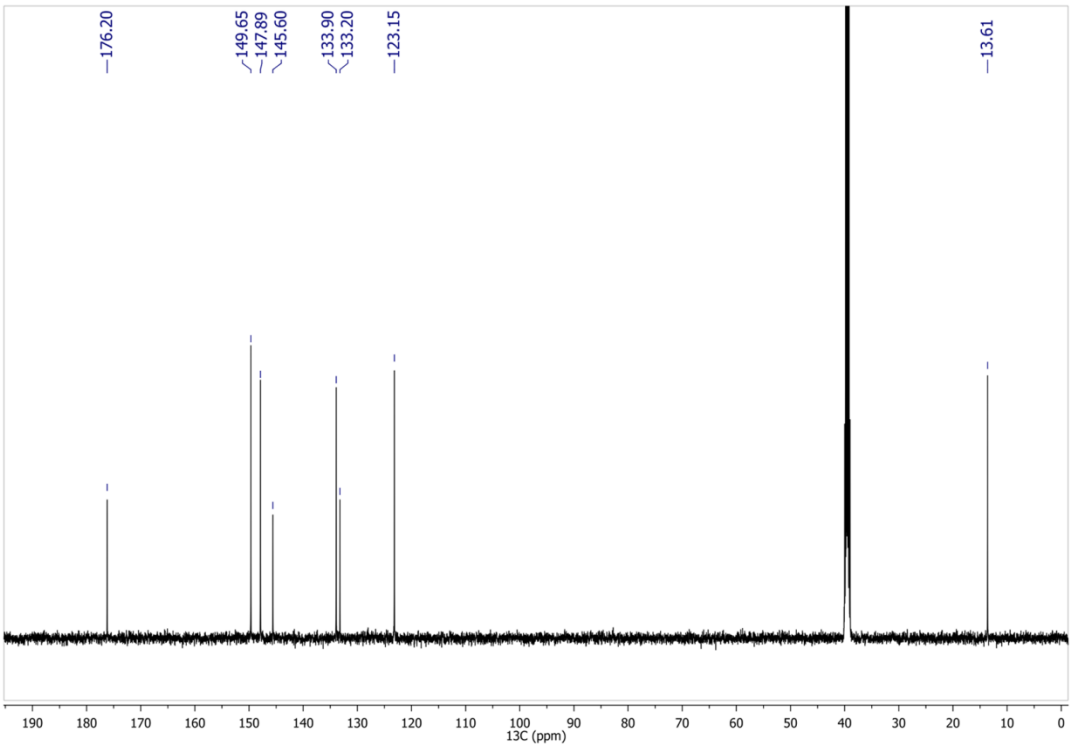

Figure S2.^{13}C NMR spectrum of **3** in DMSO-*d6.*

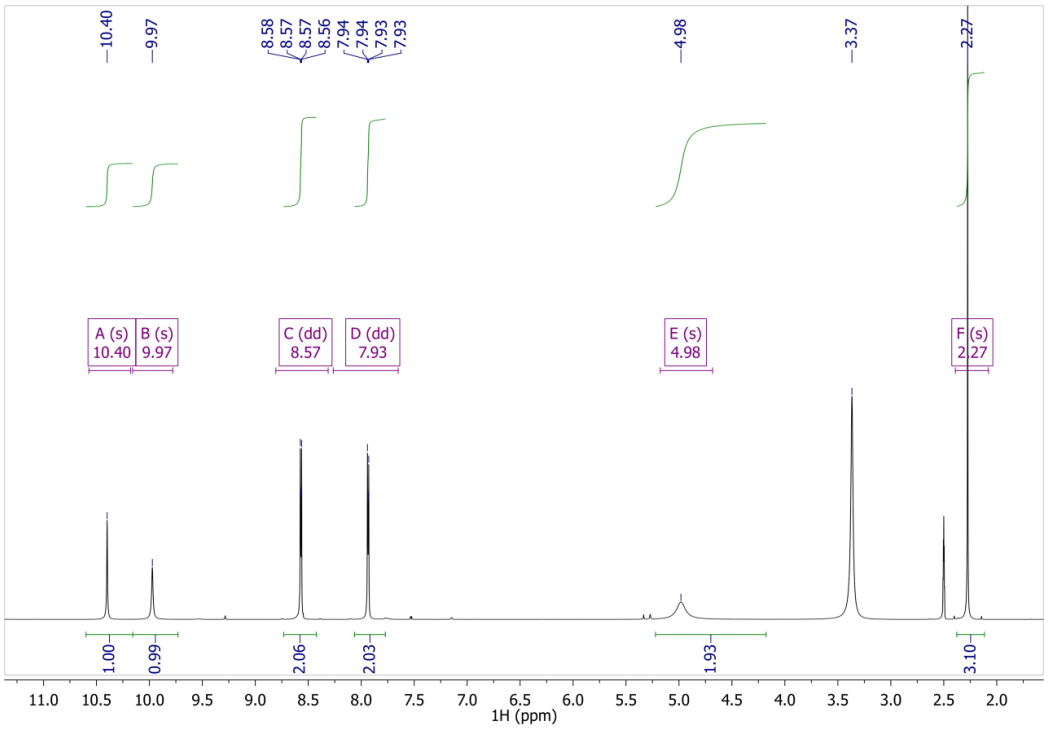

Figure S3. ^{1}H NMR spectrum of **4** in DMSO-*d6.*

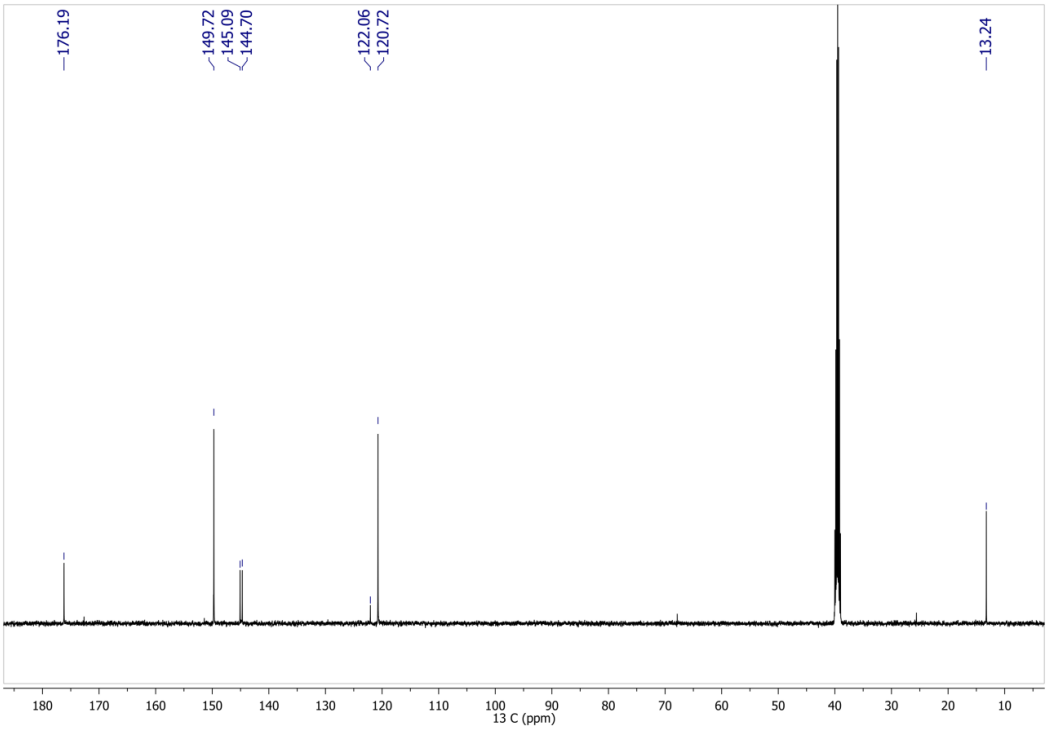

Figure S4.^{13}C NMR spectrum of **4** in DMSO-*d6.*

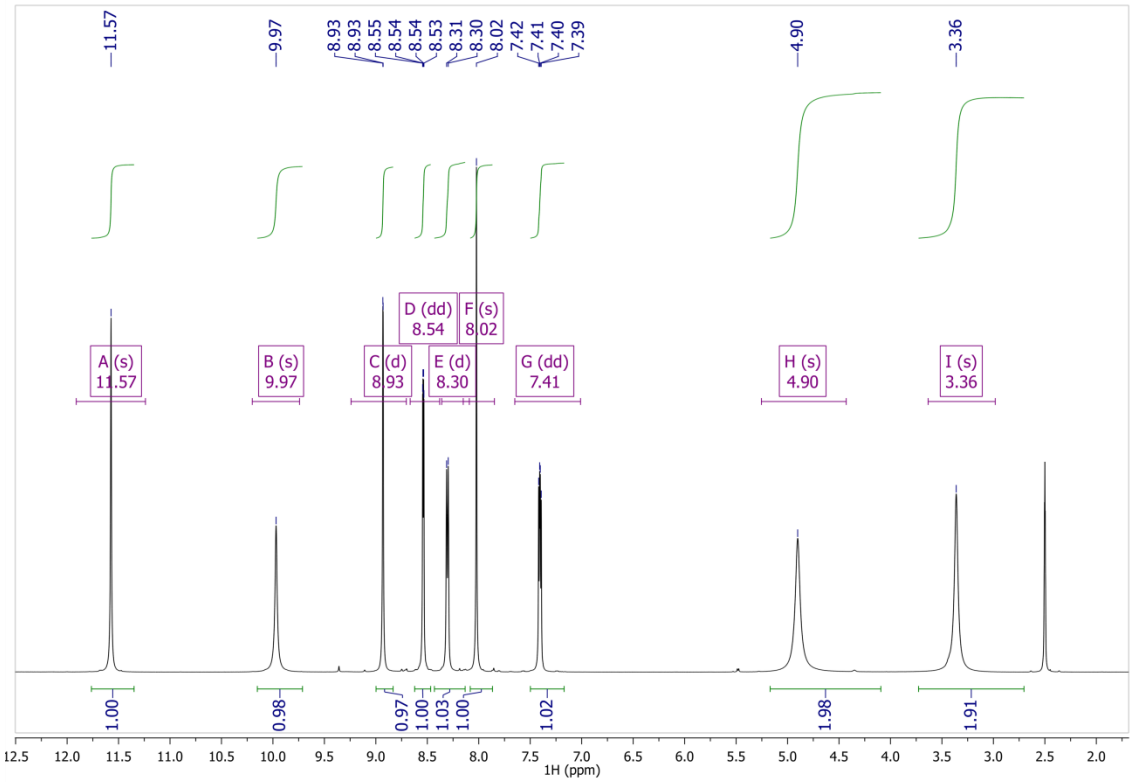

Figure S5. ^{1}H NMR spectrum of **6** in DMSO-*d6.*

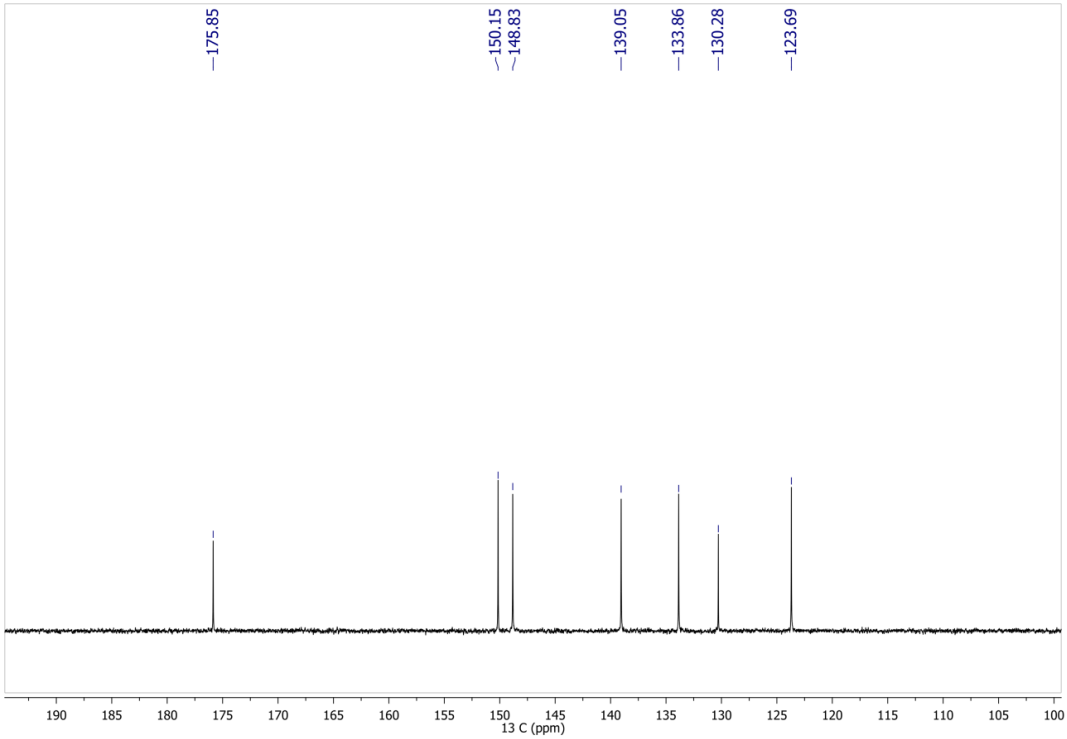

Figure S6.^{13}C NMR spectrum of **6** in DMSO-*d6.*

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