Supporting Information

for

An improved synthesis of pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones and their antimicrobial activity

Mohamed Fares [a,b,*], Soha R. Abd El Hadi [b], Radwa A. Eladwy [c], Aly A. Shoun [d], Marwa M. Abdel-Aziz [e], Wagdy M. Eldehna [f], Hatem A. Abdel-Aziz [g], Paul A. Keller [a,*]

[a] *School of Chemistry, University of Wollongong, Wollongong 2522, New South Wales, Australia*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo, 11829, Egypt*
[c] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo 11829, Egypt*
[d] *Department of Microbiology & Immunology, Faculty of Pharmacy, Badr University in Cairo, Cairo, Egypt*
[e] *The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo 11759, Egypt*
[f] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh, Egypt*
[g] *Department of Applied Organic Chemistry, National Research Center, Dokki, Cairo 12622, Egypt*

Table of Contents

General methods and material **SI3**

General procedure for the preparation of propenones (3a-j) **SI3**

General procedure A for the preparation of 10a-j **SI4**

Procedure B for the preparation of 10a **SI4**

Procedure C for the preparation of 10a **SI4**

Typical procedure for the preparation of 11 ..... **SI8**

Typical procedure for the preparation of 12 ..... **SI8**

Pharmacokinetic properties calculation **SI9**

Biological evaluation **SI9**

***In vitro* cytotoxicity** **SI10**

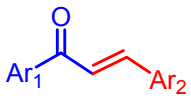
References **SI10**

NMR data of compounds 10a-j, 11 and 12 ..... **SI11**

General methods and material

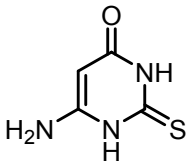
Melting points were measured with a Stuart apparatus and were uncorrected. Microwave reactions were carried out on a Discover SP - Microwave Synthesizer (CEM corporation, USA) with an emission frequency of 2455 MHz and 300 watts ±10%. Reactions were monitored by TLC analysis using silica gel GF/UV 254. NMR spectra were recorded on Varian Gemini-300BB 300, 400 and 500 MHz FT-NMR spectrometers (Varian Inc., Palo Alto, CA) and Bruker 400 MHz FT-NMR spectrometer. ^{1}H spectra were run at 300, 400 and 500 MHz and ^{13}C spectra were run at 100 and 125 MHz, in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as internal standard and coupling constant (J) values are reported in Hertz. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Microanalyses were carried out using Perkin Elmer PE 2400 CHN Elemental Analyzer and the results were within ± 0.4%. All reagents and solvents were purified and dried by standard techniques.

General procedure for the preparation of propenones (3a-j)[1]



To a mixture of *p*-chloroacetophenone **1a** (154 mg, 1 mmole) or 2-acetyl thiophene **1b** (126 mg, 1 mmol) and the corresponding aldehyde **2a-e** (1 mmol) in methanol (5.0 mL), was slowly added a solution of potassium hydroxide (5%, 1.2 mL). The mixture was stirred at room temperature for 12 h and the resulting precipitate filtered, washed with water, air dried, and recrystallized from ethanol. Yield: 62 - 75%; Melting points were consistent with that reported.[1]

Synthesis of 2-Amino-2-thiouracil 6[2]

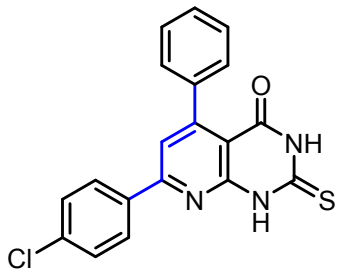


A sodium ethoxide solution was prepared by dissolving sodium (2.3 g, 0.1 mole) in absolute ethanol (150 mL). Thiourea **5** (3.8 g, 0.05 mole) was added with stirring until complete dissolution followed by the addition of ethyl cyanoacetate **4** (5.7 g, 0.05 mole) and the reaction mixture was heated at reflux for 4 h. After cooling, water was added dropwise till complete dissolution of the white precipitate. Ice was added and neutralization of the alkaline solution was accomplished by adding HCl (5 M) dropwise. The reaction flask left overnight at 0 ºC and the resulting white precipitate was filtered, washed with water (3 x 30 mL), diethyl ether (2 x 30 mL) then air dried to give **6** (4.86 g, 68%) as a white powder. m.p >300 °C as reported.[2]

General procedure A: Preparation of 5-aryl-7-(thiophen-2-yl / 4-chlorophenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10a-j

A solution of 6-amino-2-thiouracil **6** (143 mg, 1 mmol) and the appropriate chalcone **3a-j** (1 mmol) in dry DMF (3 mL) was heated at reflux for 15-20 h and the reaction monitored by TLC analysis. The mixture was cooled overnight and the resulting solid filtered, dried and recrystallized from DMF to give the pyrido[2,3-*d*]pyrimidine derivatives **10a-j**.

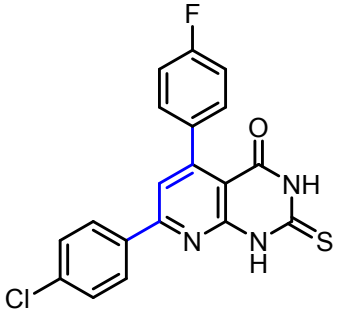
7-(4-Chlorophenyl)-5-phenyl-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10a[3]



Using general procedure A and 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one **3a** (243 mg, 1 mmole), compound **10a** (161 mg, 44%) was isolated as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.42-7.45 (m, 5H, ArH), 7.58 (d, 2H, J = 8.5 Hz, ArH), 7.66 (s, 1H, ArH), 8.26 (d, 2H, J = 8.5 Hz, ArH), 12.37 (s, 1H, NH); 13.08 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 108.2, 119.1, 127.4, 128.2, 128.7, 128.9, 129.4, 135.3, 135.7, 138.2, 152.6, 153.8, 157.9, 158.6, 175.4; MS (ESI$^-$), *m/z* 364 (100%) [M-H]$^-$, 380 (70%) [M + Cl]; Anal. Calcd for $C_{19}H_{12}ClN_3OS$: C, 62.38; H, 3.31; N, 11.49. Found: C, 62.56; H, 3.51; N, 11.26.

Procedure B: A solution of 6-amino-2-thiouracil **6** (143 mg, 1 mmol) and 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one **3a** (242 mg, 1 mmol) in TFA (3 mL) was heated at reflux for 12 h and the reaction monitored by TLC analysis. The mixture was cooled, left overnight and the resulting solid filtered, washed with water (3 x 5 mL) then diethyl ether (2 x 5 mL) and dried to give **10a** (102 mg, 28%).

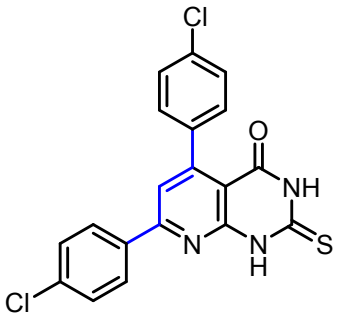
Procedure C: A solution of 6-amino-2-thiouracil **6** (72 mg, 0.5 mmol) and 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one **3a** (121 mg, 0.5 mmol), iodine pellets (6.4 mg, 0.05 mmol) in dry DMF (2 mL) was subjected to microwave radiation in a sealed tube and the reaction monitored by TLC analysis. The mixture was cooled, the lid was removed, left overnight and the resulting solid filtered and dried (108 mg, 59%).



7-(4-Chlorophenyl)-5-(4-fluorophenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10b

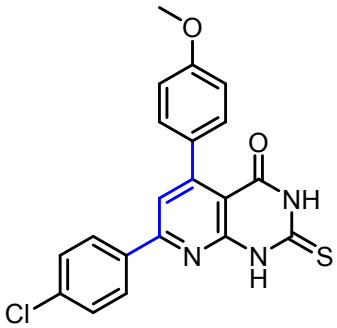
Using general procedure A and 1-(4-chlorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one **3b** (261 mg, 1 mmole), compound

10b (199 mg, 52%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.22 (t, 2H, J = 8.8 Hz ArH), 7.49 (dd, 2H, J = 6.0, 5.5 Hz, ArH), 7.58 (d, 2H, J = 8.8 Hz, ArH), 7.67 (s, 1H, pyridine H), 8.25 (d, 2H, J = 8.8 Hz, ArH), 12.38 (s, 1H, -NH, D_2O exchangeable); 13.08 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 108.3, 114.2 ($^2J_{F\text{-}C}$ = 20.9 Hz), 119.1, 128.9, 129.3, 131.0 ($^3J_{F\text{-}C}$ = 8.6 Hz), 134.4, 135.3, 135.8, 152.6, 152.7, 158.0, 158.7, 161.3 ($^1J_{F\text{-}C}$ = 243.1 Hz), 175.4; MS (ESI$^-$), *m/z* 382 (100%) [^{35}Cl, M-H]$^-$, 384 (33%) [^{37}Cl, M-H]$^-$; Anal. Calcd for $C_{19}H_{11}ClFN_3OS$: C, 59.46; H, 2.89; N, 10.95. Found: C, 59.70; H, 3.11; N, 11.26.



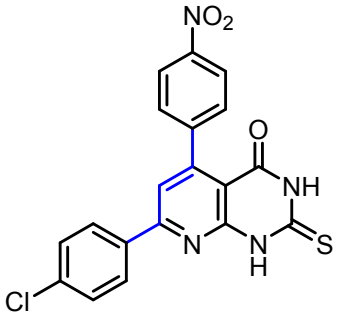
5,7-Bis(4-chlorophenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10c

Using general procedure A and 1,3-bis(4-chlorophenyl)prop-2-en-1-one **3c** (277 mg, 1 mmole), compound **10c** (200 mg, 50%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.46-7.50 (m, 4H, ArH), 7.58 (d, 2H, J = 8.3 Hz, ArH), 7.68 (s, 1H, pyridine H), 8.25 (d, 2H, J = 8.3 Hz, ArH), 12.41 (s, 1H, -NH, D_2O exchangeable), 13.10 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 108.2, 118.9, 127.4, 128.9, 129.3, 130.6, 133.1, 135.2, 135.8, 137.1, 152.4, 152.6, 158.0, 158.7, 175.4; MS (ESI$^-$), *m/*z 398 (100%) [^{35}Cl, ^{35}Cl, M-H]$^-$, 400 (70%) [^{37}Cl, ^{35}Cl, M-H]$^-$; Anal. Calcd for $C_{19}H_{11}Cl_2N_3OS$: C, 57.01; H, 2.77; N, 10.50. Found: C, 57.32; H, 2.85; N, 10.36.



7-(4-Chlorophenyl)-5-(4-methoxyphenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10d

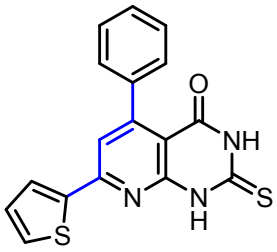
Using general procedure A and 1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **3d** (273 mg, 1 mmole), compound **10d** (181 mg, 46%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 3.81 (s, 3H, OCH_3), 6.94 (d, 2H, J = 8.0 Hz, ArH), 7.39 (d, 2H, J = 8.0 Hz, ArH), 7.41 (d, 2H, J = 8.0 Hz, ArH), 7.54 (s, 1H, pyridine H), 8.20 (d, 2H, J = 8.0 Hz, ArH), 12.37 (s, 1H, -NH, D_2O exchangeable), 12.02 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.7, 108.6, 113.4, 119.6, 129.4, 129.8, 130.7, 131.0, 135.9, 136.1, 153.2, 154.1, 158.3, 159.2, 160.1, 175.8; MS (EI), *m/z* 395 (82%) [^{35}Cl, M]$^+$, 397 (17%) [^{37}Cl, M]$^+$; Anal. Calcd for $C_{20}H_{14}ClN_3O_2S$: C, 60.68; H, 3.56; N, 10.62. Found: C, 60.93; H, 3.78; N, 10.98.



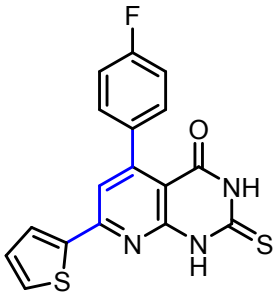
7-(4-Chlorophenyl)-5-(4-nitrophenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10e

Using general procedure A and 1-(4-chlorophenyl)-3-(4-nitrophenyl)prop-2-en-1-one **3e** (288 mg, 1 mmole), compound **10e** (250 mg, 61%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.62 (d, 2H, J = 8.8 Hz, ArH), 7.73 (d, 2H, J = 8.8 Hz, ArH), 7.76 (s, 1H, pyridine H), 8.27-8.35 (m, 4H, ArH), 12.48 (s, 1H, -NH, D_2O exchangeable), 13.19 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 108.7, 119.0, 123.0, 129.5, 129.9, 130.6, 135.6, 136.4, 145.7, 147.7, 151.8, 153.0, 158.8, 159.2, 176.0; MS (EI), *m/z* 410 (100%) [^{35}Cl, M$^+$], 412 (39%) [^{37}Cl, M]$^+$.

5-Phenyl-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10f[4]



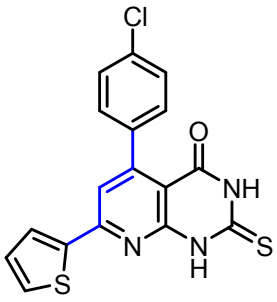
Using general procedure A and 3-phenyl-1-(thiophen-2-yl)prop-2-en-1-one **3f** (214 mg, 1 mmole), compound **10f** (219 mg, 65%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.18-7.19 (m, 1H, H4 thiophene), 7.38-7.52 (m, 5H, ArH), 7.59-7.70 (m, 1H, H5 thiophene), 7.92 (s, 1H, pyridine H), 8.07-8.15 (m, 1H, H3 thiophene), 12.29 (br. s, 1H, NH, D_2O exchangeable), 13.05 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 108.0, 118.1, 127.9, 128.6, 129.0, 129.4, 129.6, 132.5, 138.8, 142.8, 153.1, 154.0, 155.4, 158.9, 175.9; MS (EI), *m/z* 337 (100%) [M$^+$], 338 (30%) [M+1]$^+$; Anal. Calcd for $C_{17}H_{11}N_3OS_2$: C, 60.52; H, 3.29; N, 12.45. Found: C, 60.30; H, 3.26; N, 12.59.



5-(4-Fluorophenyl)-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10g

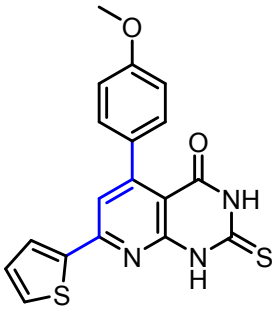
Using general procedure A and 3-(4-fluorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3g** (232 mg, 1 mmole), compound **10g** (202 MG, 57%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.06-7.13 (m, 3H, 2ArH and H4 thiophene), 7.33 (t, 2H, J = 6.0 H, ArH), 7.46 (s, 1H, pyridine H), 7.68 (d, 1H, J = 4.0 Hz, H5 thiophene), 7.93 (d, 1H, J = 4.0 Hz, H3 thiophene), 12.22 (br. s, 1H, NH, D_2O exchangeable), 12.90 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 108.0, 114.7 ($^2J_{F\text{-}C}$ = 20.9 Hz), 118.1, 129.4, 129.7, 131.3 ($^3J_{F\text{-}C}$ = 9.0 Hz), 132.3, 135.0, 142.8, 152.9, 153.1, 155.5, 159.0, 161.5 ($^1J_{F\text{-}C}$ = 240.0 Hz), 175.9; MS (EI), *m/z* 355 (100%) [M$^+$], 356 (17%) [M+1]$^+$; Anal. Calcd for $C_{17}H_{10}FN_3OS_2$: C, 57.45; H, 2.84; N, 11.82. Found: C, 57.64; H, 2.61; N, 12.09.

5-(4-Chlorophenyl)-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10h



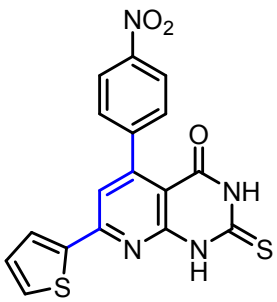
Using general procedure A and 3-(4-chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3h** (249 mg, 1 mmole), compound **10h** (159 mg, 43%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.20 (d, J = 3.9 Hz, 1H, H4 thiophene), 7.23 (d, J = 8.7 Hz, 2H, ArH), 7.45-7.59 (m, 3H, 2ArH and H5 thiophene), 7.81 (d, J = 4.8 Hz, 1H, H3 thiophene), 8.07 (s, 1H, pyridine H), 12.23 (br. s, 1H, NH, D_2O exchangeable), 12.95 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 108.0, 117.9, 127.9, 129.4, 131.0, 132.4, 133.5, 137.6, 142.8, 152.6, 153.1, 155.5, 159.0, 162.1, 175.9; MS (EI), *m/z* 371 (100%) [^{35}Cl, M$^+$], 373 (39) [^{37}Cl, M]$^+$; Anal. Calcd for $C_{17}H_{10}ClN_3OS_2$: C, 54.91; H, 2.71; N, 11.30. Found: C, 54.72; H, 2.56; N, 11.56.

5-(4-Methoxyphenyl)-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10i



Using general procedure A and 3-(4-methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3i** (244 mg, 1 mmole), compound **10i** (146 mg, 40%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H, OCH_3), 6.96 (d, J = 8.7 Hz, 2H, ArH), 7.20 (dd, J = 3.9, 4.8 Hz, 1H, H4 thiophene), 7.37 (d, J = 8.7 Hz, 2H, ArH), 7.56 (s, 1H, pyridine H), 7.81 (d, J = 4.8 Hz, 1H, H5 thiophene), 8.06-8.08 (m, 1H, H3 thiophene), 12.23 (br. s, 1H, NH, D_2O exchangeable), 12.90 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.7, 107.9, 113.4, 118.1, 129.4, 129.5, 130.7, 130.8, 132.1, 142.9, 153.2, 153.8, 155.2, 159.0, 160.0, 175.8; MS (EI), *m/z* 367 (100%) [M$^+$]; Anal. Calcd for $C_{18}H_{13}N_3O_2S_2$: C, 58.84; H, 3.57; N, 11.44. Found: C, 59.02; H, 3.74; N, 11.30.

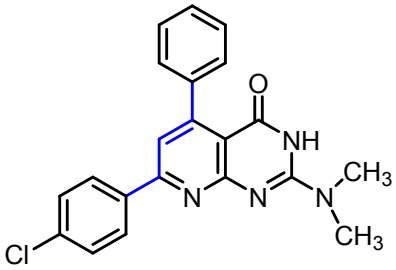
5-(4-Nitrophenyl)-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one 10j



Using general procedure A and 3-(4-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3j** (259 mg, 1 mmole), compound **10j** (191 mg, 50%) was synthesized as a buff solid. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.21 (dd, J = 3.9, 1.2 Hz, 1H, H4 thiophene), 7.66-7.67 (m, 1H, H5 thiophene), 7.68 (d, J = 8.7 Hz, 2H, ArH), 7.83 (d, J = 5.1 Hz, 1H, H3 thiophene), 8.07 (s, 1H, pyridine H), 8.26 (d, J = 8.7 Hz, 2H, ArH), 12.34 (br. s, 1H, NH, D_2O exchangeable), 13.05 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6)

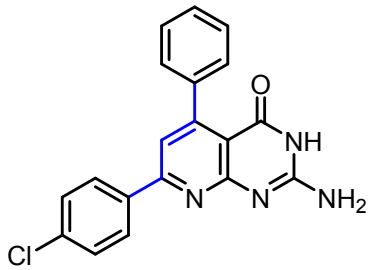
δ: 107.9, 117.5, 123.0, 124.4, 126.4, 129.5, 130.4, 130.5, 132.6, 147.7, 151.5, 153.0, 155.8, 159.1, 176.0; MS (EI), *m/z* 382 (100%) [M^+], 383 (37%) [M+1]$^+$; Anal. Calcd for $C_{18}H_{13}N_3O_2S_2$: C, 58.84; H, 3.57; N, 11.44. Found: C, 58.98; H, 3.86; N, 11.62.

7-(4-Chlorophenyl)-2-(dimethylamino)-5-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one 11



A solution of 6-amino-2-thiouracil **6** (72 mg, 0.5 mmol), 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one **3a** (121 mg, 0.5 mmol) and iodine (25 mg, 40 mol%) in dry DMF (2 mL) was subjected to microwave radiation for 1 h in a sealed tube and the reaction monitored by TLC analysis. The mixture was cooled, the lid was removed, left overnight and the resulting solid was subjected to flash chromatography (methanol/dichloromethane, 1:10) to give **11** (73 mg, 39%) as a yellow powder, M.p. >300 ºC; ^{1}H NMR (DMSO,d_6) δ: 3.14 (s, 6H, 2CH_3), 7.39-7.45 (m, 6H, 5ArH and pyridine H), 7.54 (d, *J* = 8.3 Hz, 2H, ArH), 8.22 (d, *J* = 8.3 Hz, 2H, ArH), 11.20 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 38.0, 107.7, 117.5, 126.0, 127.8, 128.2, 129.0, 129.2, 129.7, 135.5, 137.0, 140.2, 153.6, 153.8, 158.7, 163.1; MS (ESI$^+$), *m/z* 377 (100%) [^{35}Cl, M+H]$^+$, 379 32% [^{37}Cl, M+H]; HRMS (ESI$^+$) calcd for $C_{21}H_{17}ClN_4O$+H: 377.1187; found 377.1169.

2-Amino-7-(4-chlorophenyl)-5-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one 12



A solution of 6-amino-2-thiouracil **6** (72 mg, 0.5 mmol), 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one **3a** (121 mg, 0.5 mmol) and iodine (25 mg, 40 mol%) in formamide (2 mL) was subjected to microwave radiation for 1 h in a sealed tube and the reaction monitored by TLC analysis. The mixture was cooled, the lid was removed, left overnight and the resulting solid was subjected to flash chromatography (methanol/dichloromethane, 1:10) to give **12** (60 mg, 34%) as a brown powder. M.p. >300 ºC; ^{1}H NMR (DMSO-d_6) δ: 6.69 (s, 2H, NH_2), 7.39-7.54 (m, 6H, 5ArH and pyridine H), 7.54 (d, *J* = 8.3 Hz, 2H, ArH), 8.21 (d, *J* = 8.3 Hz, 2H, ArH), 10.95 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 108.5, 117.3, 127.7, 128.0, 129.0, 129.2, 129.5, 135.4, 137.1, 140.4, 153.7, 154.7, 158.4, 163.4, 163.5; MS (ESI$^+$), *m/z* 349 (100%) [^{35}Cl, M+H]$^+$, 351 (35%) [^{37}Cl, M+H]$^+$; HRMS (ESI$^+$) calcd for $C_{19}H_{13}{}^{35}ClN_4O$ + Na: 371.0669; found 371.0676.

Pharmacokinetic properties calculation

The drug likeness, HBD and HBA scores of all the compounds **10a-j** were calculated using the MolSoft online calculation kits. Prediction of the lipophilicity was performed using ALOGPS

2.1 program [5], while calculation of TPSA, the number of rotatable bonds, used the Molinspiration property calculation kit.[6] Reduced molecular flexibility is measured by the number of rotatable bonds and is greatly linked to oral bioavailability. The number of rotatable bonds less or equal to ten potentially increases the oral bioavailability. %ABS is inversely proportional to the molecular volume and TPSA and is calculated using the formula %ABS = 109 ± 0.345 x TPSA.

Biological evaluation

Antimicrobial activity

All strains were provided by the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Antibacterial and antifungal activities were expressed as the diameter of inhibition zones from an agar well diffusion method. Holes (1 cm diameter) were created in the agar using a sterile cork borer in either sterile malt agar plates (fungi) or sterile nutrient agar plates (bacteria), which had previously been uniformly seeded with microorganisms. The holes were filled with fungal filtrates (100 µL). Plates were left in an incubator at 4 ºC for one hour for diffusion and then incubated at 37 ºC for bacteria and 28 ºC for fungi. Inhibition zones were measured after 24 h of incubation for bacteria and 48 h of incubation for fungi. Amphotericin B was the antifungal standard, whereas ampicillin, gentamycin and vancomycin served as standards for Gram +ve, Gram -ve, and methicillin resistant *Staphylococcus aureus*, respectively. The experiments were performed in triplicate and the average zone of inhibition was calculated.

Minimum inhibitory concentration

MIC was performed using a serial dilution technique starting with 100 mmol concentration of compounds dissolved in DMSO (1 mL) and then reduced by successive twofold dilutions of stock solution using a calibrated micropipette. Amphotericin B was used as the antifungal standard and ampicillin, gentamycin and vancomycin served as standards for Gram +ve, Gram -ve, and methicillin resistant *Staphylococcus aureus*, respectively. The final solution concentrations were 62.50, 31.25, 15.63, 7.81, 3.90, 1.95, 0.98, 0.49, 0.24 and 0.12 µmol/mL. The microtiter plates were incubated at 37 ºC for bacteria and 28 ºC for fungi and were read using a microplate reader after 24 h for bacteria and after 48 h for fungi. In each case, triplicate tests were performed and the average was taken as final reading. MIC was expressed as the lowest concentration inhibiting test organism's growth.

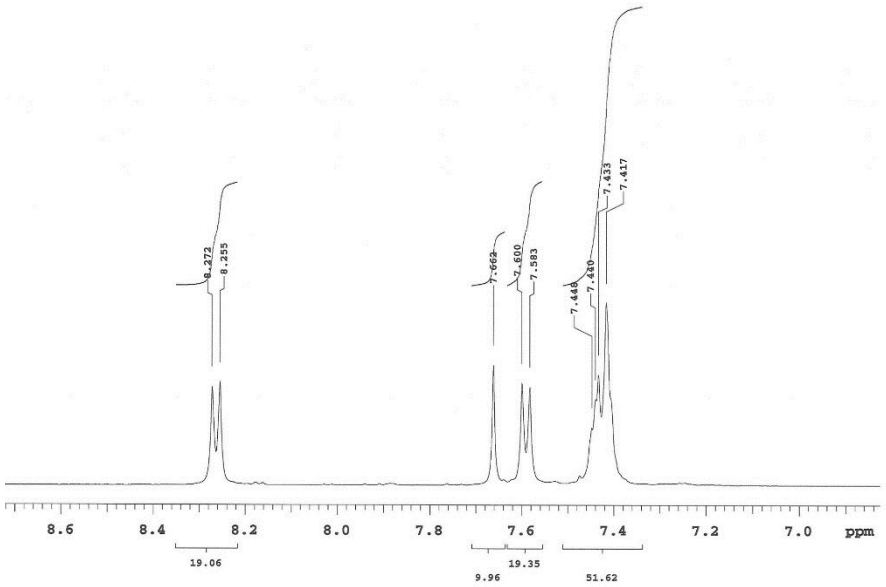
In vitro cytotoxicity

CCD-33Co (normal colon cells) were obtained from the American Type Culture Collection. Cells were propagated in DMEM supplemented with 10% heat-inactivated FBS (Hyclone), 10 µg/mL

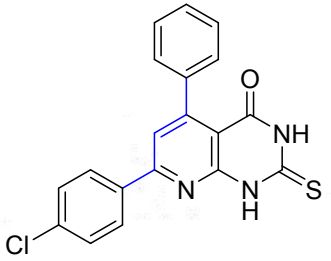
of insulin (Sigma), and 1% penicillin-streptomycin. All other chemicals and reagents were purchased from Sigma, or Invitrogen. Cytotoxicity was determined using a MTT assay following a reported procedure.[7] The 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA). The data presented are the mean of three separate experiments.

References:

1. Y. Qian, G.-Y. Ma, Y. Yang, K. Cheng, Q.-Z. Zheng, W.-J. Mao, L. Shi, J. Zhao and H.-L. Zhu, *Biorg. Med. Chem.*, 2010, **18**, 4310-4316.
2. T. Elsaman, M. Fares, H. A. Abdel-Aziz, M. I. Attia, H. A. Ghabbour and K. M. Dawood, *J. Chem.*, 2013, **2013**.
3. J. Quiroga, B. Insuasty, A. Sanchez, M. Nogueras and H. Meier, *J. Heterocycl. Chem.*, 1992, **29**, 1045-1048.
4. M. Fares, S. M. Abou-Seri, H. A. Abdel-Aziz, S. E. Abbas, M. M. Youssef and R. A. Eladwy, *Eur. J. Med. Chem.*, 2014, **83**, 155-166.
5. I. V. Tetko and V. Y. Tachuk, *http://www.vcclab.org/lab/alogps/*.
6. M. Cheminformatics, *http://www.molinspiration.com/cgi-bin/properties*.
7. F. Denizot and R. Lang, *J. Immunol. Methods*, 1986, **89**, 271-277.



Agilent Technologies



10a

Pulse 45.0 degrees
Acq. time 2.048 sec
Width 7998.4 Hz
Single scan
OBSERVE H1, 499.9072837 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min 3 sec

13.084
12.367
8.272
8.255
7.662
7.600
7.583
7.448
7.440
7.433
7.417
3.322
2.490

13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

6.61
7.11
16.45
16.70
8.54

mm160728_H_4_Cl_Acetophenone_PP_CARBON

Sample Name:
mm160728_H_4_Cl_Acetophenone_PP
Data Collected on:
ernst.sci.uow.edu.au-inova500
Archive directory:

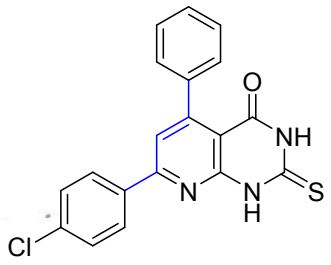
Sample directory:

FidFile: CARBON

Pulse Sequence: CARBON (s2pul)
Solvent: dmso
Data collected on: Jul 28 2016

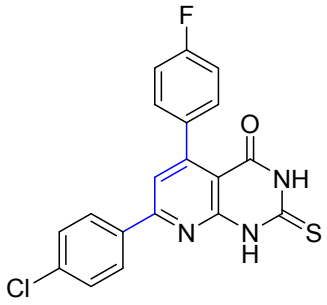
Temp. 22.5 C / 295.6 K
Operator: uowvnmrs

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31421.8 Hz
192 repetitions
OBSERVE C13, 125.7018460 MHz
DECOUPLE H1, 499.9097792 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 59 min

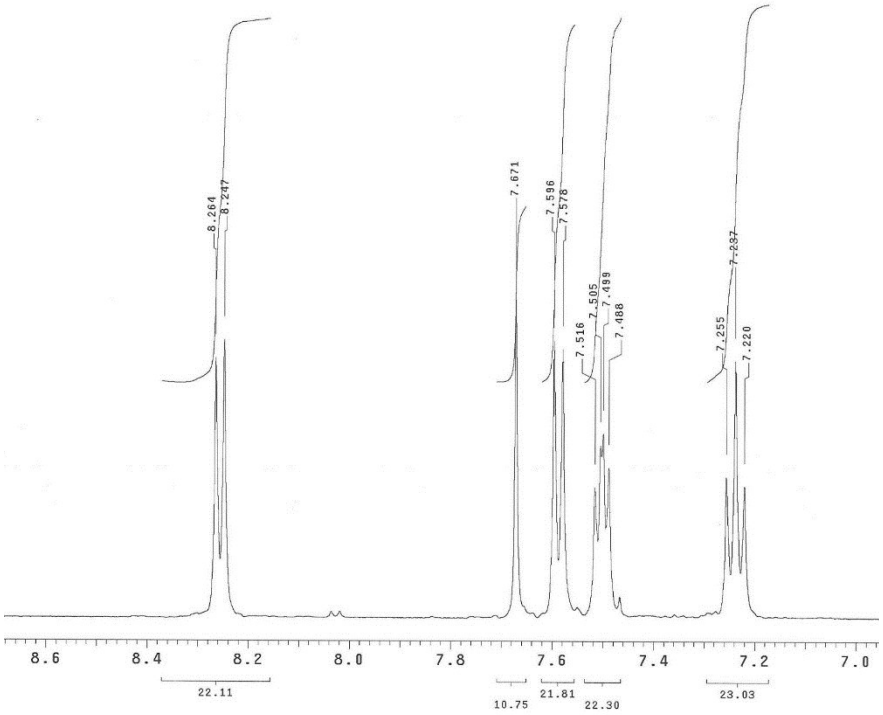


10a

Agilent Technologies



10b



File: Proton

Pulse Sequence: s2pul

Solvent: dmso
Temp. 25.0 C / 298.1 K
Operator: uowvnmrs
VNMRS-500 "pyne06.domain.com"

Relax. delay 0.001 sec
Pulse 45.0 degrees
Acq. time 2.045 sec
Width 8012.8 Hz
Single scan
OBSERVE H1, 499.7437645 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 0 min, 2 sec

mm160726_P_F_Bnezaldehyde_P_Cl_Acetophenone_CARBON

Sample Name:
mm160726_P_F_Bnezaldehyde_P_Cl_Acetophenone
Data Collected on:
ernst.sci.uow.edu.au-inova500
Archive directory:

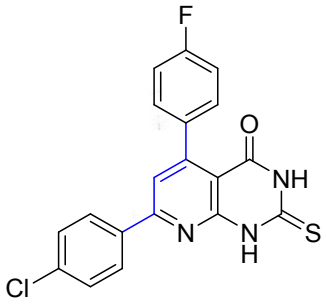
Sample directory:

FidFile: CARBON

Pulse Sequence: CARBON (s2pul)
Solvent: dmso
Data collected on: Jul 26 2016

Temp. 23.3 C / 296.4 K
Operator: uowvnmrs

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31421.8 Hz
2816 repetitions
OBSERVE C13, 125.7018460 MHz
DECOUPLE H1, 499.9097792 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 59 min



10b

Agilent Technologies

mm160726_4_CL_Benzald_4_Cl_Acetophenone_PROTON

Sample Name:

Data Collected on:
ernst.sci.uow.edu.au-inova500
Archive directory:

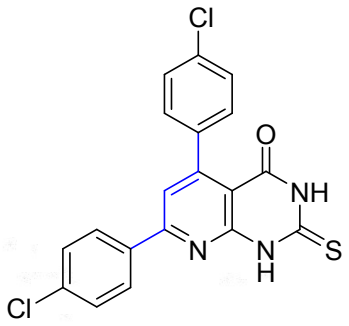
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Jul 26 2016

Operator: uowvnmrs

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.048 sec
Width 7998.4 Hz
Single scan
OBSERVE H1, 499.9072847 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min 3 sec



10c

Agilent Technologies

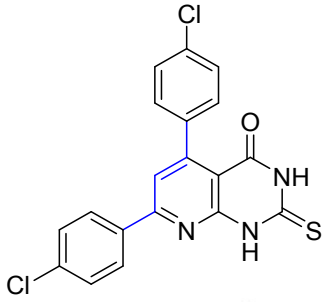
mm160726_4_Cl_Benzald_4_Cl_Acetophenone_Carbon

File: Carbon

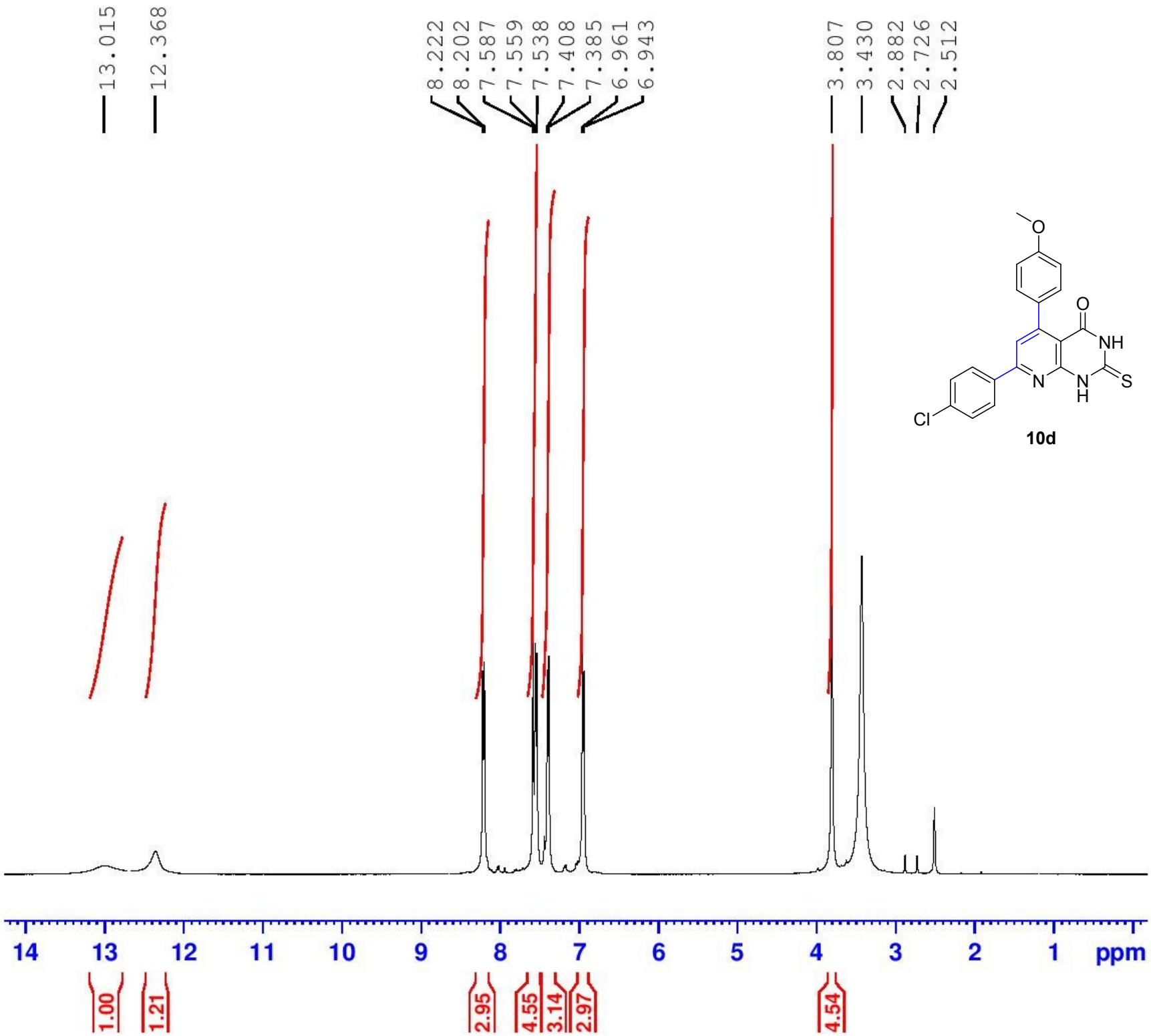
Pulse Sequence: s2pul

Solvent: dmso
Temp. 25.0 C / 298.1 K
Operator: uowvnmrs
VNMRS-500 "pyne06.domain.com"

Relax. delay 0.500 sec
Pulse 45.0 degrees
Acq. time 1.500 sec
Width 30487.8 Hz
1280 repetitions
OBSERVE C13, 125.6607298 MHz
DECOUPLE H1, 499.7462675 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 59 min, 59 sec



10c

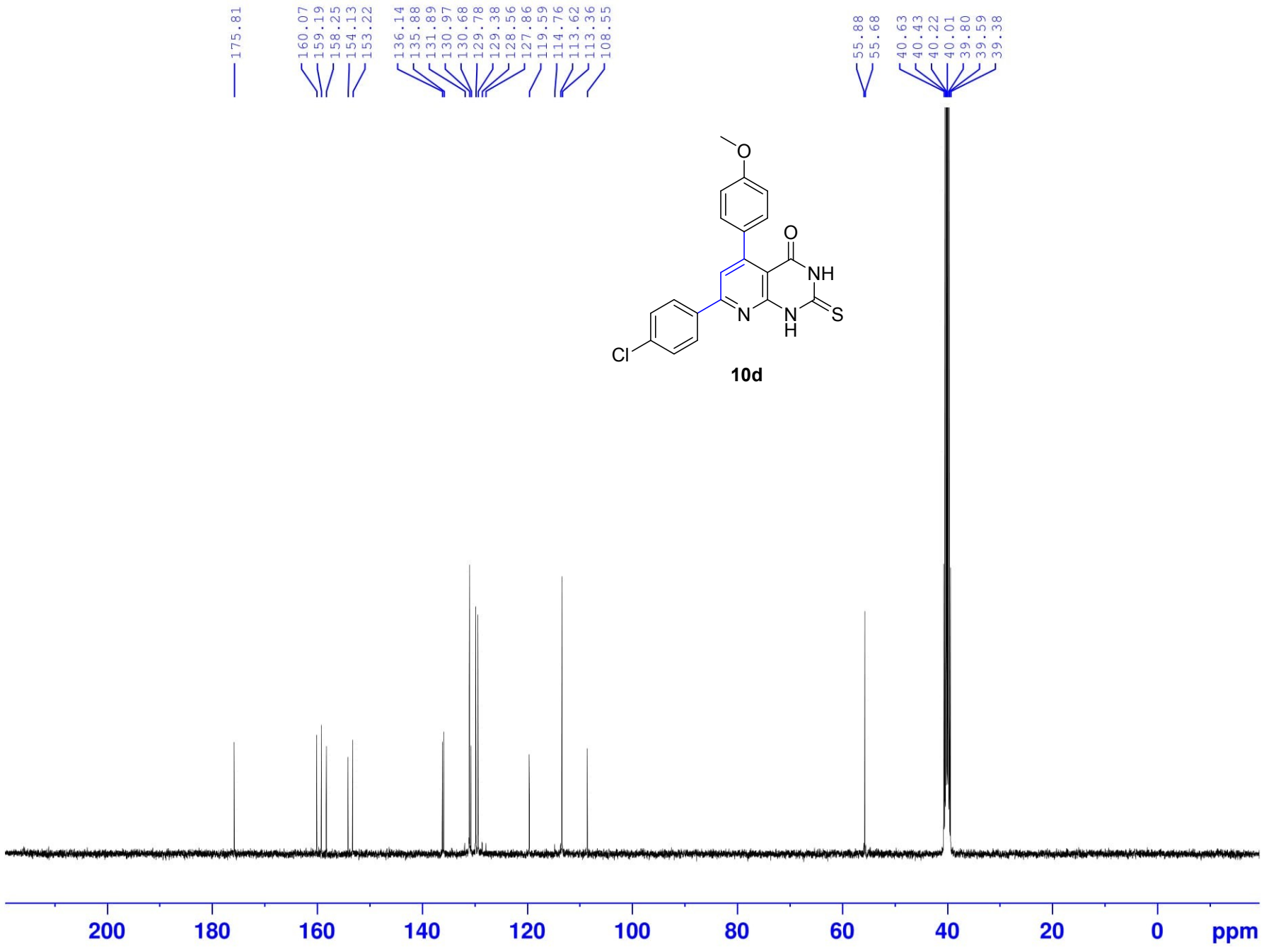


10d

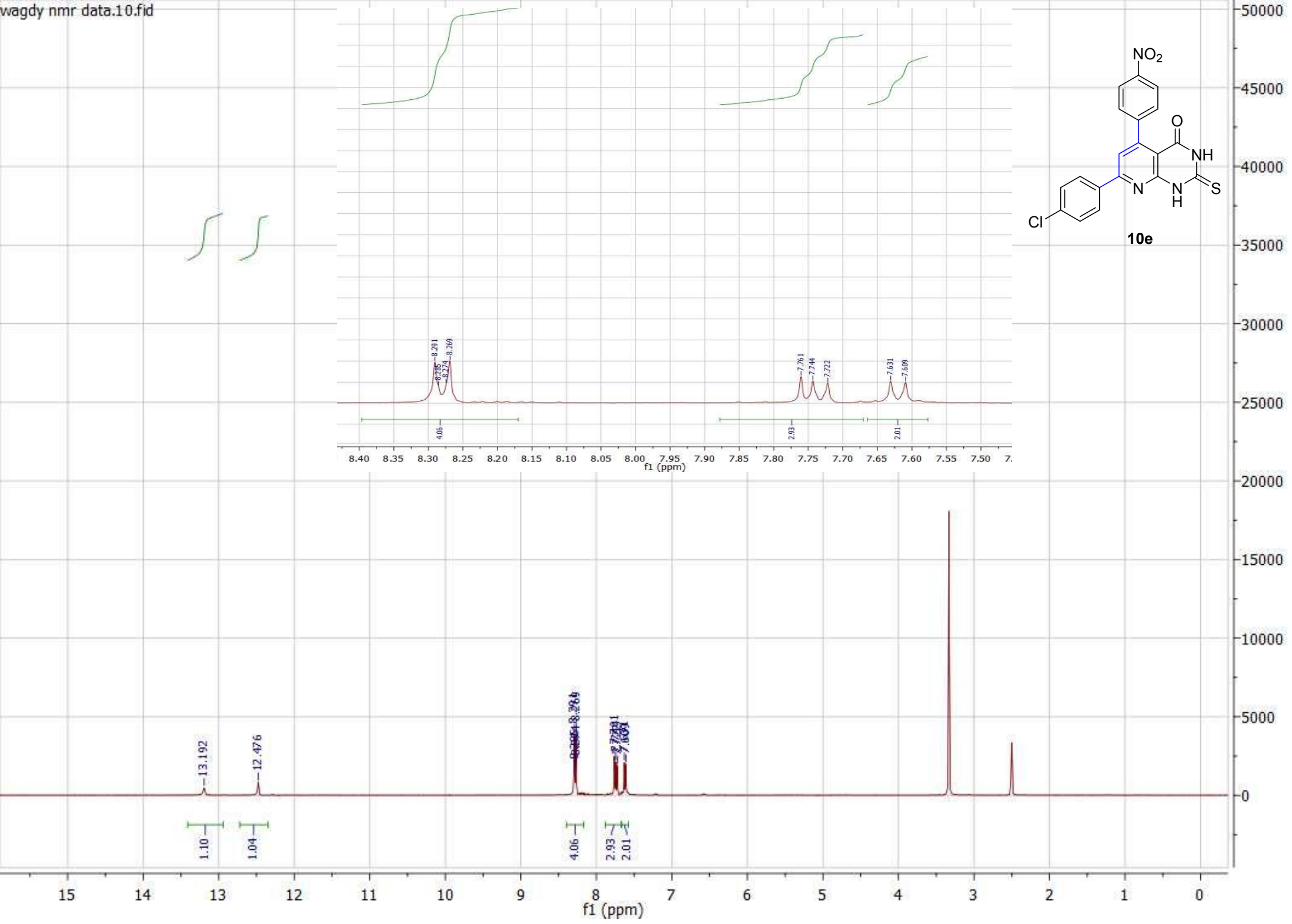
BRUKER

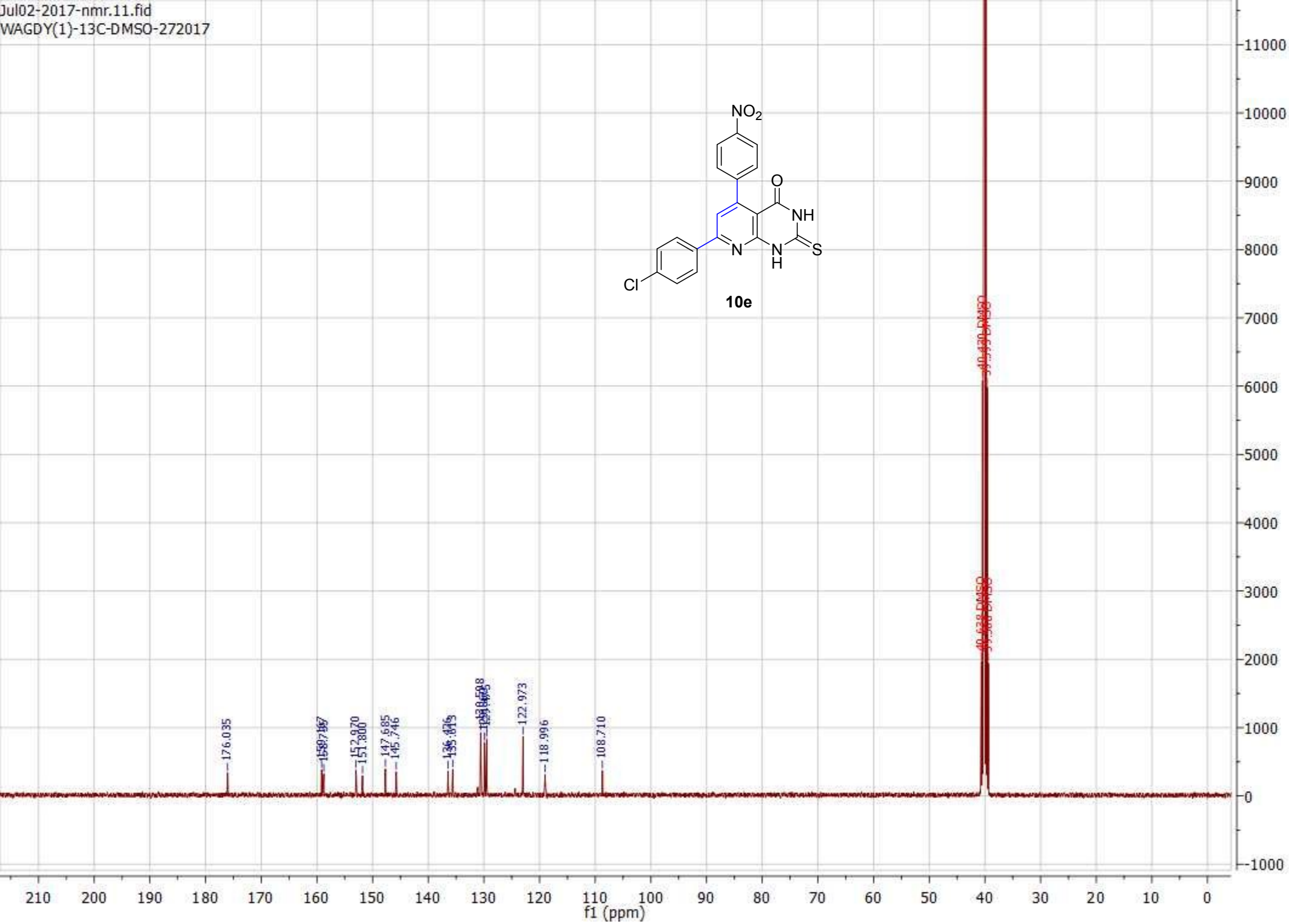
```
Current Data Parameters
NAME                  soha-5
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20170207
Time                 10.09
INSTRUM              spect
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                     64
DS                      2
SWH               8012.820 Hz
FIDRES            0.122266 Hz
AQ                4.0894465 sec
RG                   205.37
DW                  62.400 usec
DE                   6.50 usec
TE                  298.0 K
D1             1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1          400.1524711 MHz
NUC1                     1H
P1                   12.00 usec
PLW1          18.00000000 W

F2 - Processing parameters
SI                    65536
SF            400.1500000 MHz
WDW                     EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

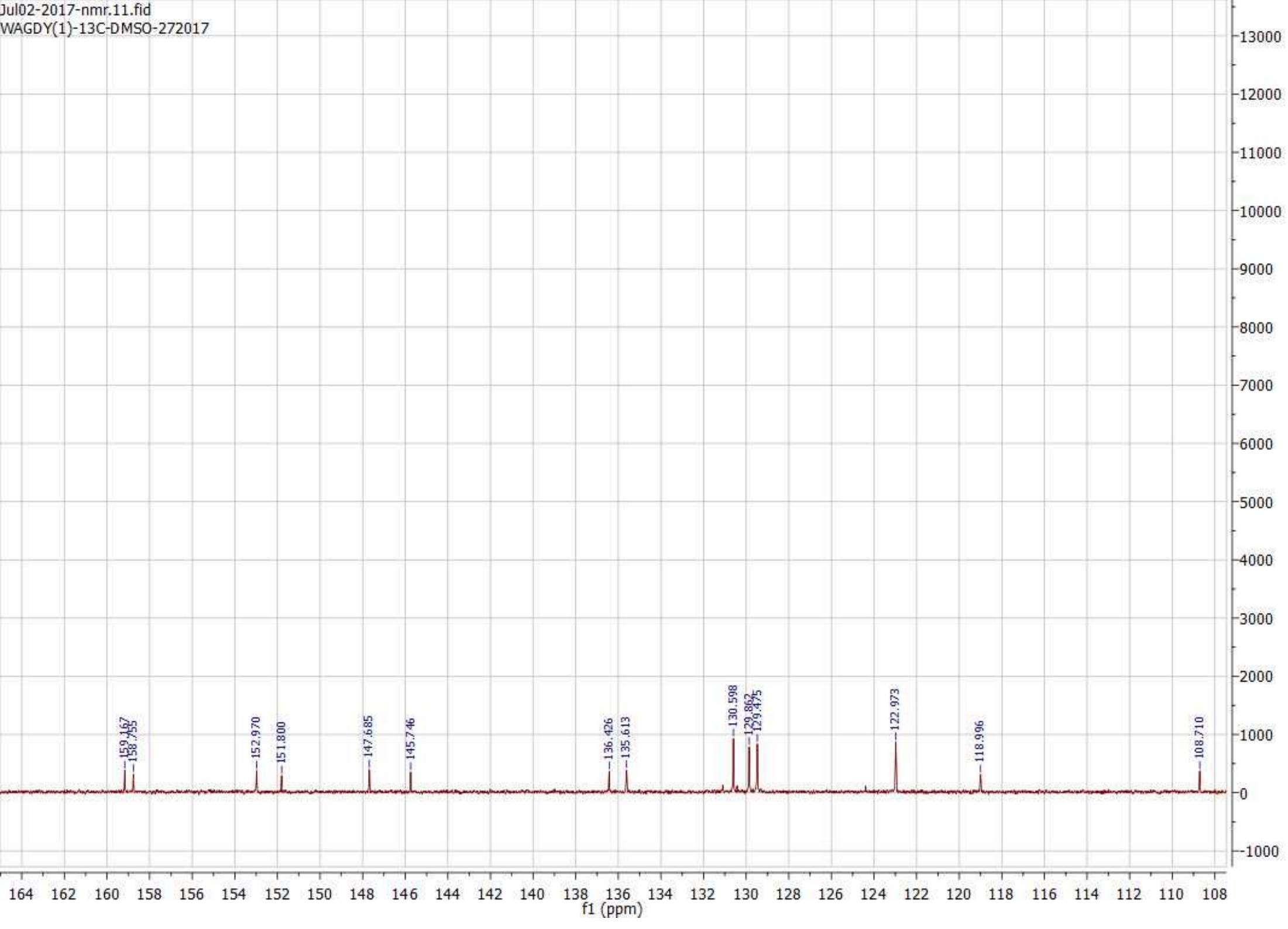
WAGDY(5)-13C-DMSO-272017

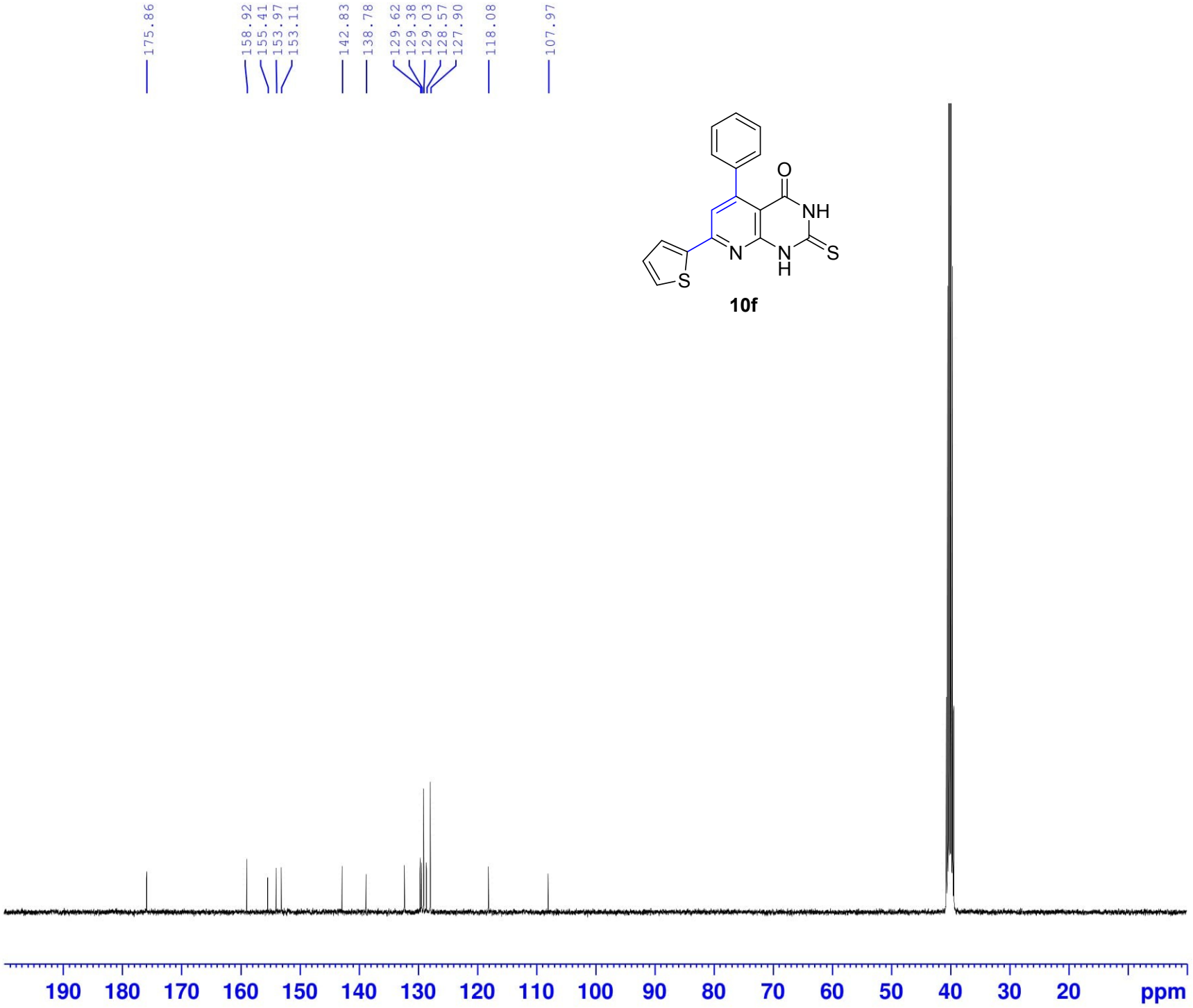



```
Current Data Parameters
NAME      Jul02-2017-nmr
EXPNO                 20
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170702
Time               13.42
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                194.81
DW                20.800 usec
DE                 6.50 usec
TE                300.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         100.6328883 MHz
NUC1                 13C
P1                10.00 usec
PLW1         66.00000000 W

======== CHANNEL f2 ========
SFO2         400.1716007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             90.00 usec
PLW2         16.50000000 W
PLW12         0.20370001 W
PLW13         0.16500001 W

F2 - Processing parameters
SI                 32768
SF           100.6228270 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```


wagdy nmr data.10.fid
8.291
8.285
8.274
8.269
7.761
7.744
7.722
7.631
7.609
4.06
2.93
2.01
f1 (ppm)
NO2
O
NH
N
N
H
S
Cl
10e
13.192
12.476
1.10
1.04
4.06
2.93
2.01
f1 (ppm)

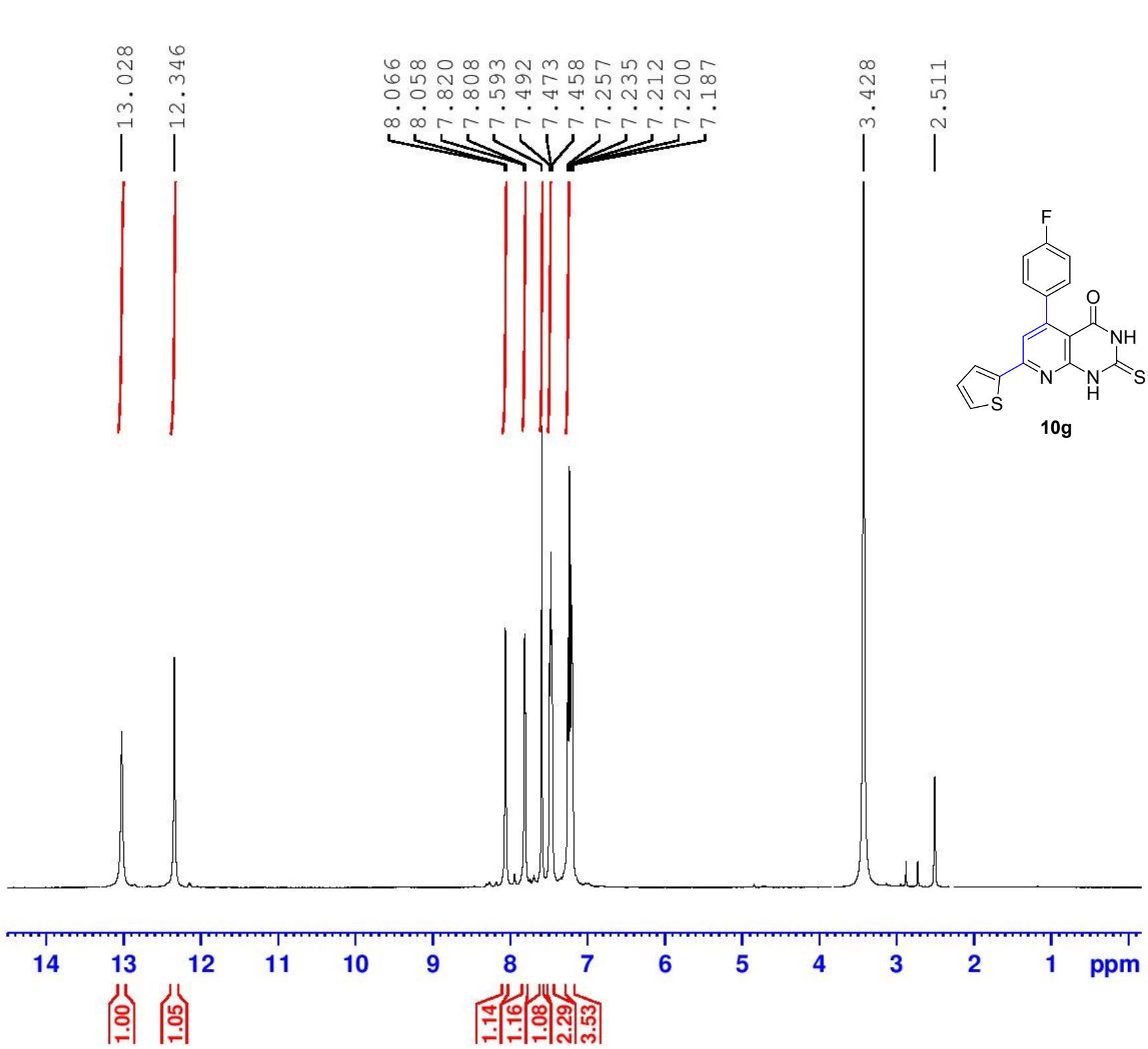

Jul02-2017-nmr.11.fid
WAGDY(1)-13C-DMSO-272017
NO2
O
NH
N
N
H
S
Cl
10e
176.035
152.970
151.800
147.685
145.746
122.973
118.996
108.710
40.430 DMSO
40.638 DMSO
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Jul02-2017-nmr.11.fid
WAGDY(1)-13C-DMSO-272017
159.167
158.755
152.970
151.800
147.685
145.746
136.426
135.613
130.598
129.862
129.475
122.973
118.996
108.710
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108
f1 (ppm)



BRUKER

```
Current Data Parameters
NAME      Jul02-2017-nmr
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_           20170702
Time               22.44
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                194.81
DW                20.800 usec
DE                 6.50 usec
TE                300.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        100.6328883 MHz
NUC1                13C
P1                10.00 usec
PLW1        66.00000000 W

======== CHANNEL f2 ========
SFO2        400.1716007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        16.50000000 W
PLW12        0.20370001 W
PLW13        0.16500001 W

F2 - Processing parameters
SI                 32768
SF          100.6228270 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



10g

Current Data Parameters

NAME	soha-7
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

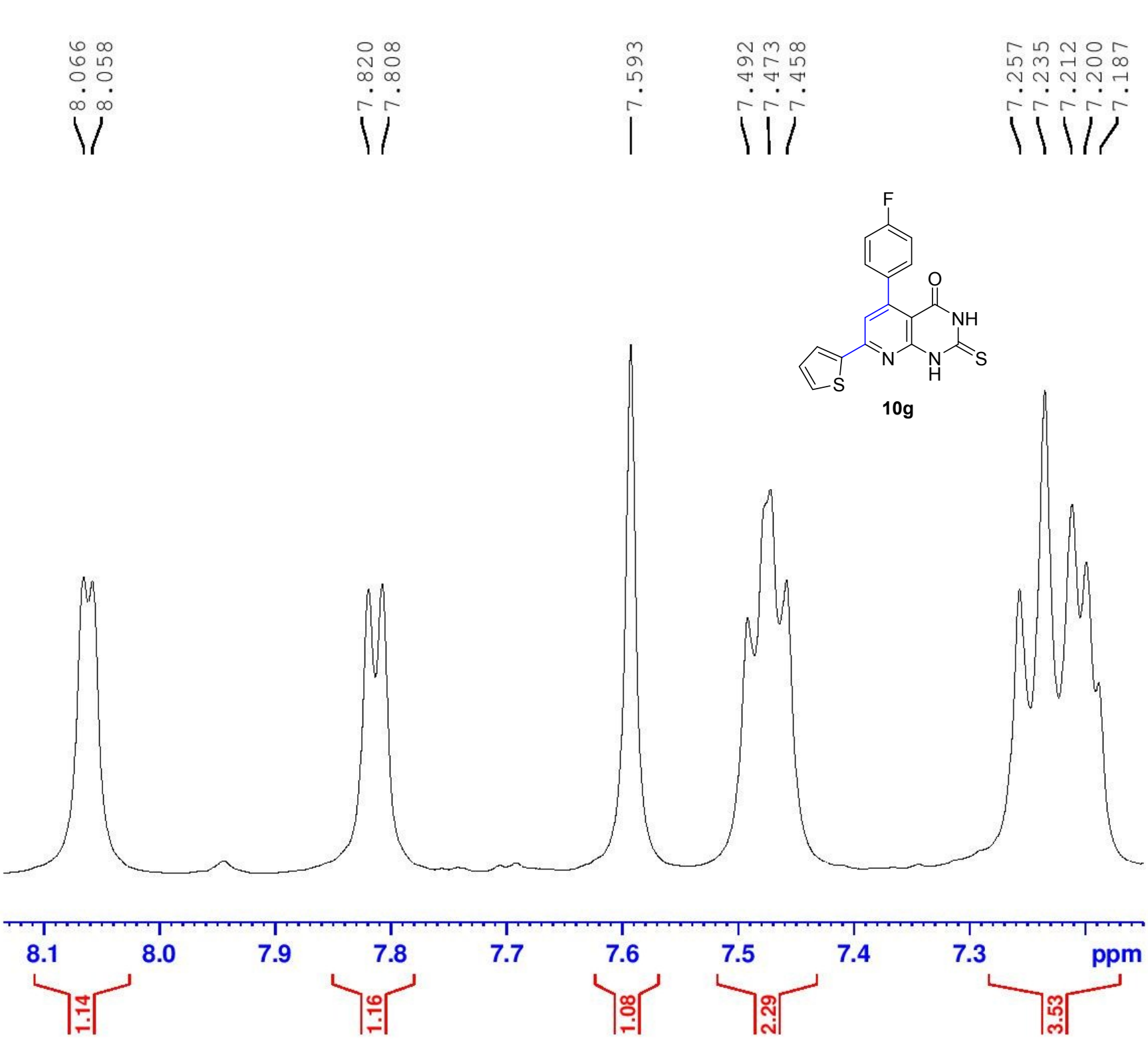
Date_	20170207
Time	10.18
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	64
DS	2
SWH	8012.820 Hz
FIDRES	0.122266 Hz
AQ	4.0894465 sec
RG	205.37
DW	62.400 usec
DE	6.50 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

SFO1	400.1524711 MHz
NUC1	1H
P1	12.00 usec
PLW1	18.00000000 W

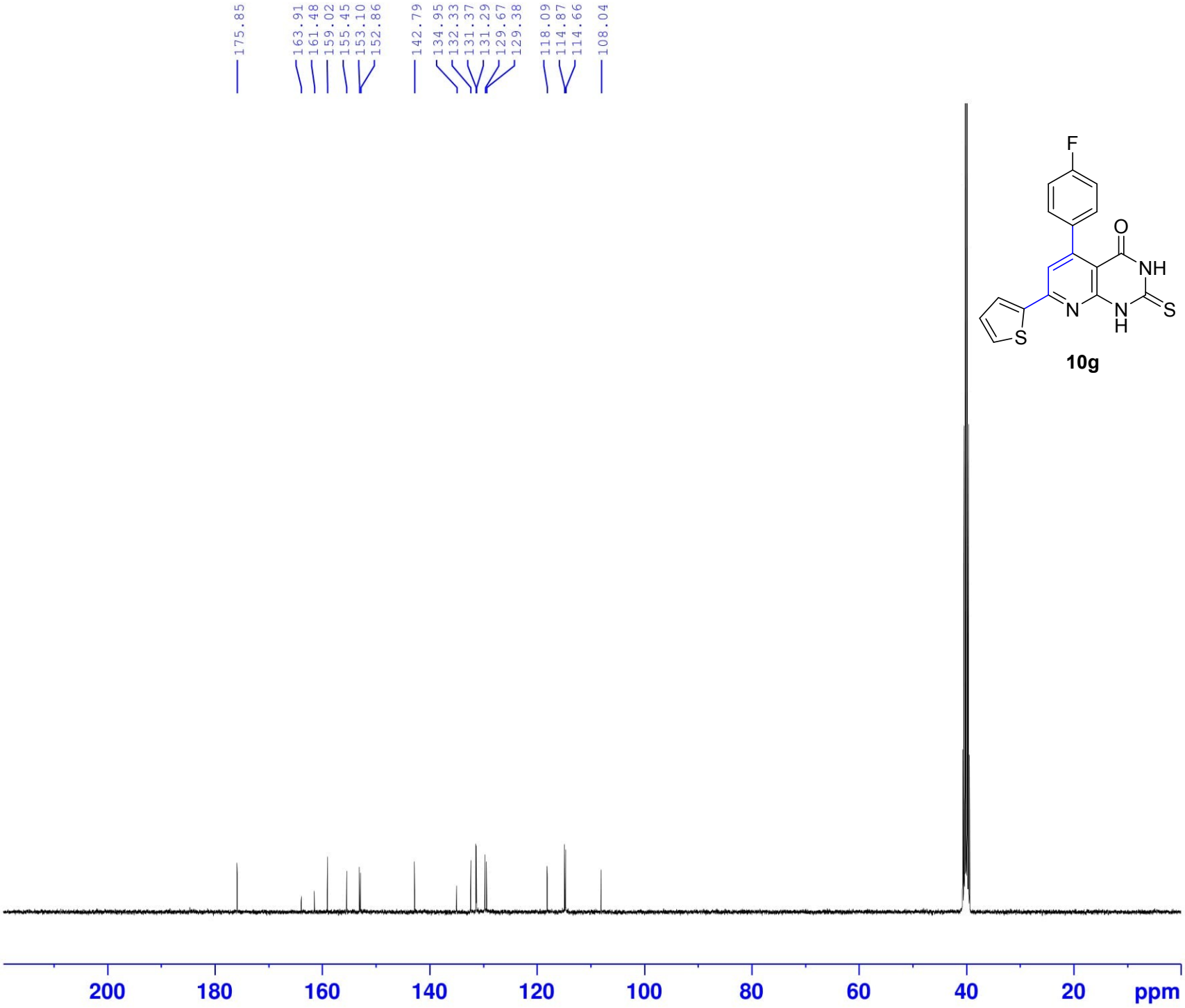
F2 - Processing parameters

SI	65536
SF	400.1500000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



BRUKER

```
Current Data Parameters
NAME                  soha-7
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20170207
Time                  10.18
INSTRUM               spect
PROBHD    5 mm PABBO BB/
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                        64
DS                         2
SWH               8012.820 Hz
FIDRES            0.122266 Hz
AQ                4.0894465 sec
RG                   205.37
DW                   62.400 usec
DE                    6.50 usec
TE                   298.0 K
D1              1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1           400.1524711 MHz
NUC1                     1H
P1                   12.00 usec
PLW1           18.00000000 W

F2 - Processing parameters
SI                    65536
SF             400.1500000 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```



10g

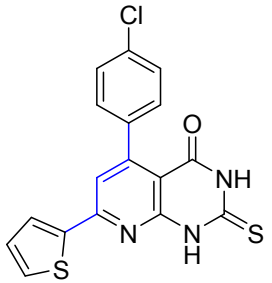
Current Data Parameters
NAME Jul02-2017-nmr
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20170702
Time 23.46
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 194.81
DW 20.800 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

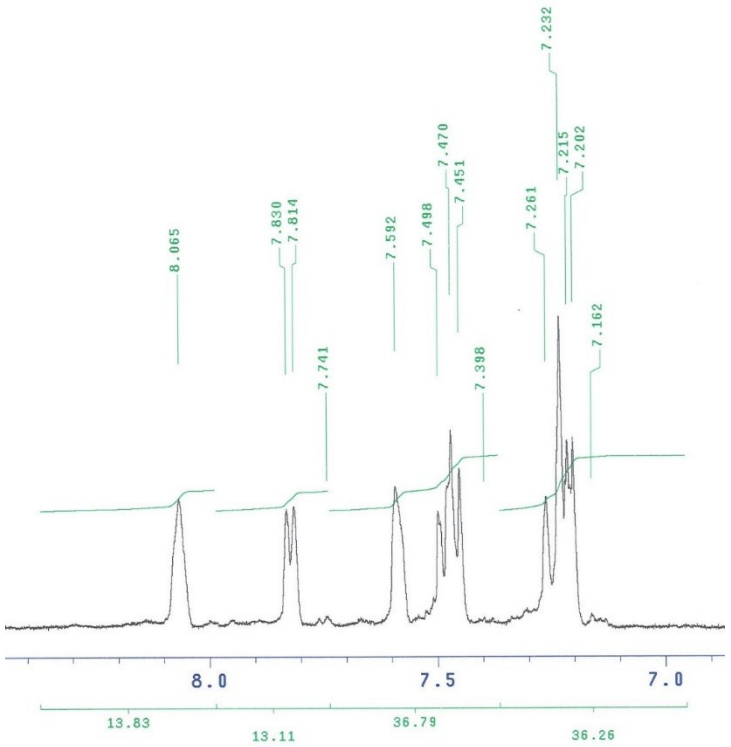
======== CHANNEL f1 ========
SFO1 100.6328883 MHz
NUC1 13C
P1 10.00 usec
PLW1 66.00000000 W

======== CHANNEL f2 ========
SFO2 400.1716007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 16.50000000 W
PLW12 0.20370001 W
PLW13 0.16500001 W

F2 - Processing parameters
SI 32768
SF 100.6228270 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



10h

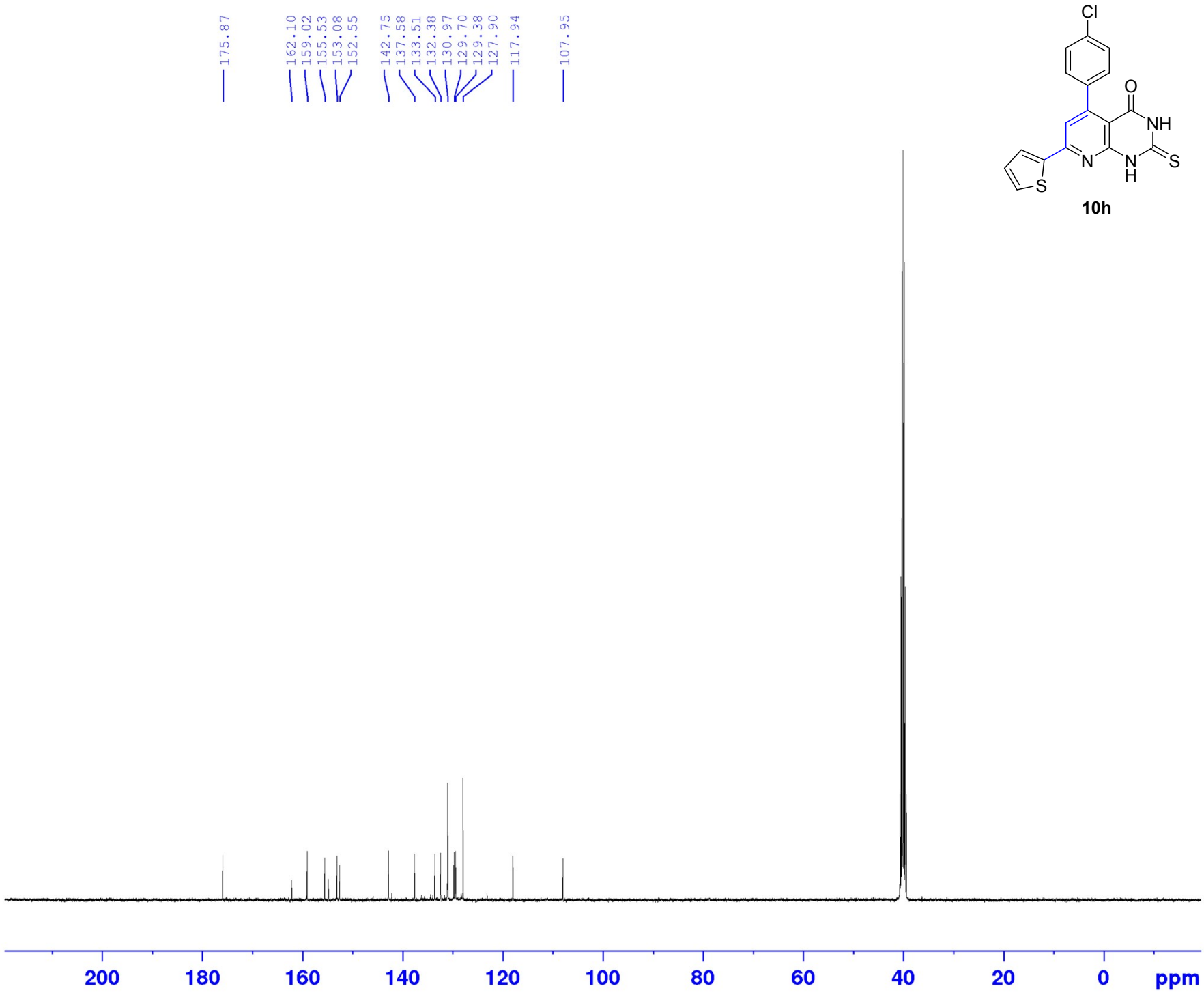


Dr.MohamedFars-8PP-H1-DMSO-Main.Defence.Chemical.Laboratory

Pulse Sequence: s2pul

Solvent: DMSO
Temp. 25.0 C / 298.1 K
GEMINI-300BB "NMR"

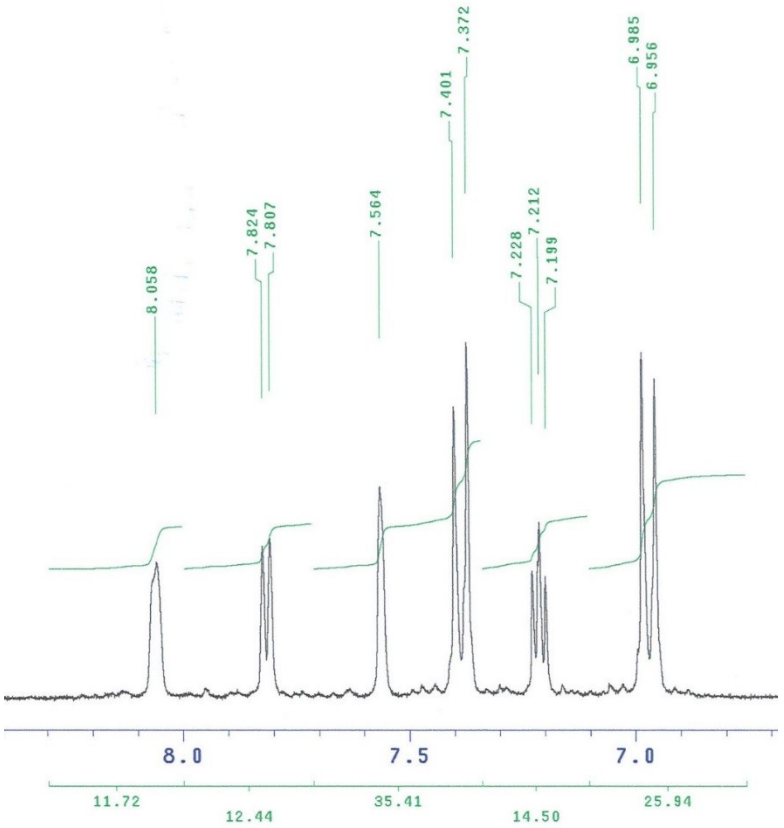
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.127 sec
Width 6000.2 Hz
20 repetitions
OBSERVE H1, 300.0117460 MHz
DATA PROCESSING
FT size 65536
Total time 5 min, 5 sec

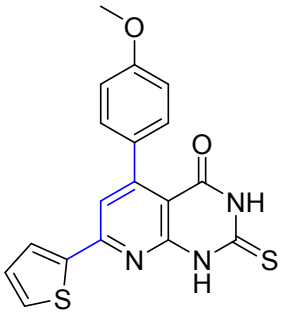


10h

BRUKER

```
Current Data Parameters
NAME      Jul02-2017-nmr
EXPNO                  8
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170703
Time                0.49
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT            DMSO
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG               194.81
DW               20.800 usec
DE                6.50 usec
TE               300.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6328883 MHz
NUC1                 13C
P1                10.00 usec
PLW1         66.00000000 W

======== CHANNEL f2 ========
SFO2         400.1716007 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         16.50000000 W
PLW12         0.20370001 W
PLW13         0.16500001 W

F2 - Processing parameters
SI                32768
SF           100.6228270 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```





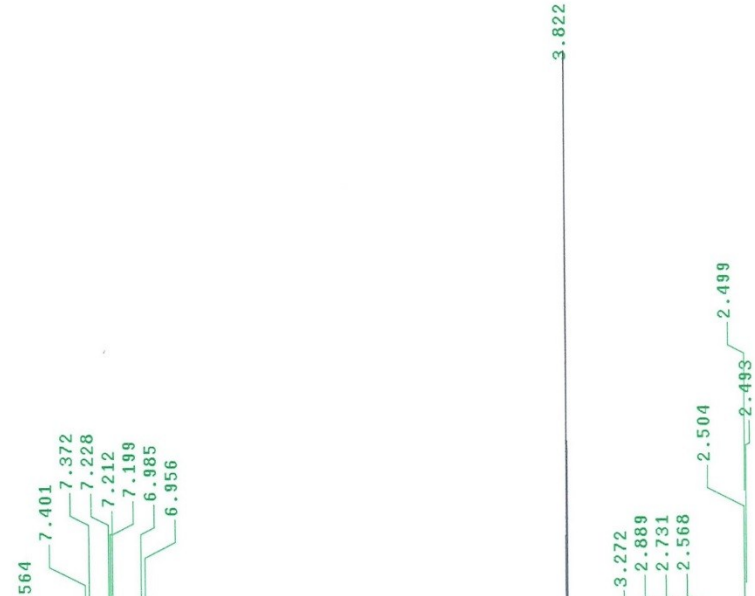
10i

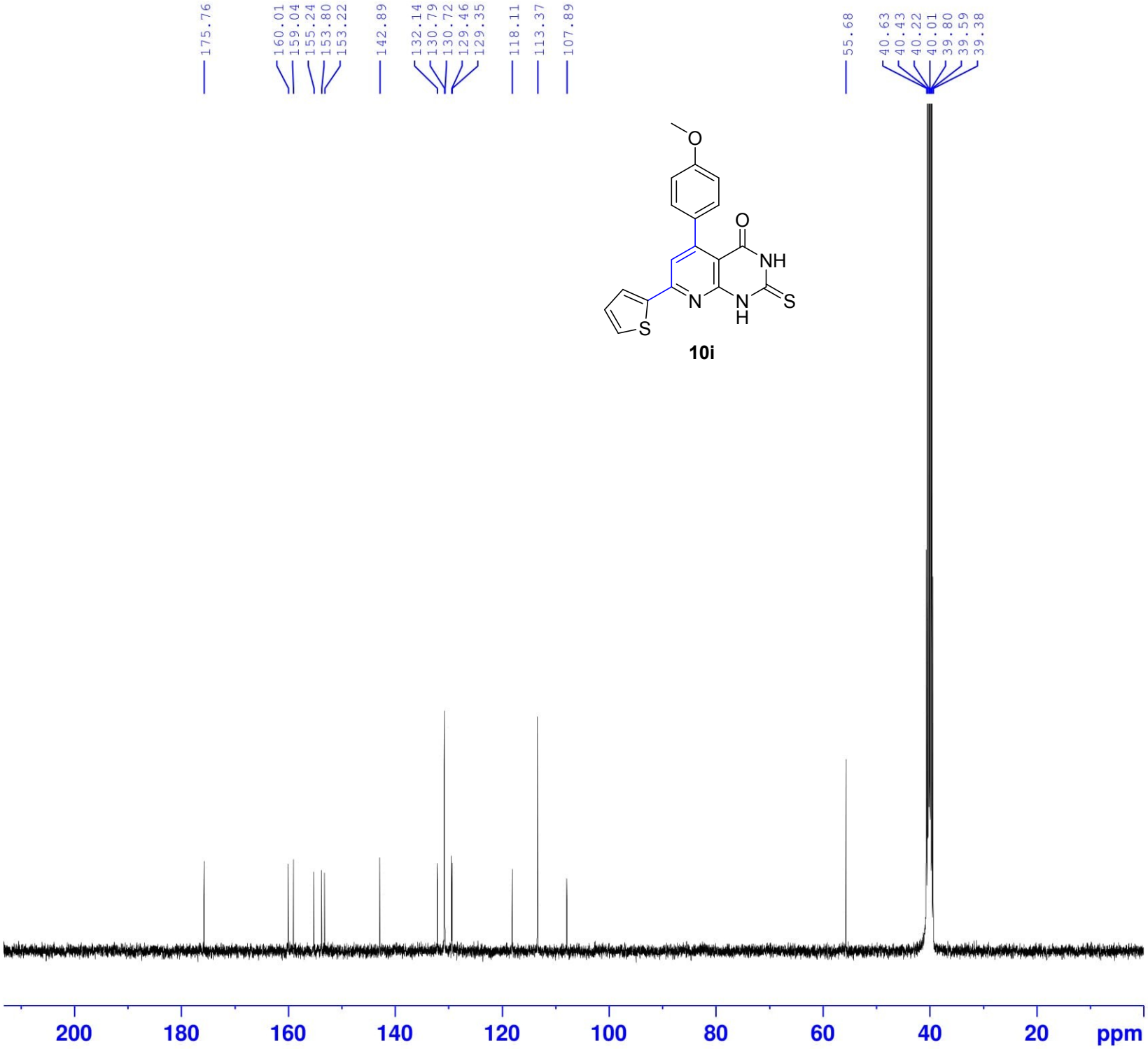
Dr.MohamedFars-9PP-H1-DMSO-Main.Defence.Chemical.Laboratory

Pulse Sequence: s2pul

Solvent: DMSO
Temp. 25.0 C / 298.1 K
File: Dr.MohamedFars-9PP-H1-DMSO-Main.Defence.Chemical.Laboratory
GEMINI-300BB "NMR"

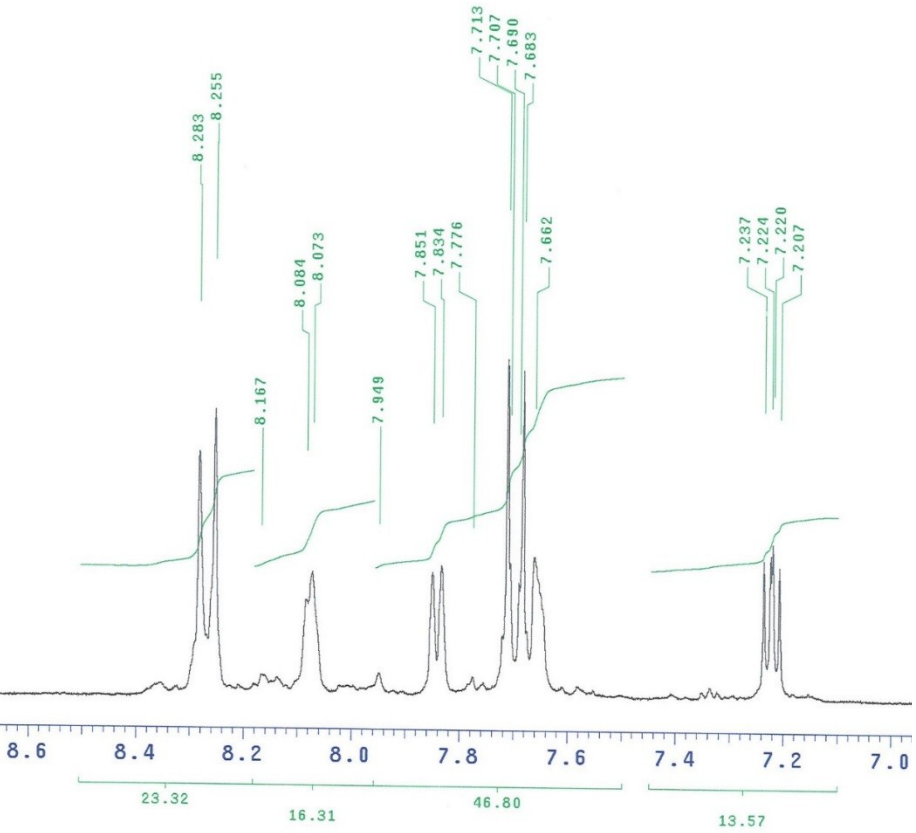
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.127 sec
Width 6000.2 Hz
20 repetitions
OBSERVE H1, 300.0117460 MHz
DATA PROCESSING
FT size 65536
Total time 5 min, 5 sec

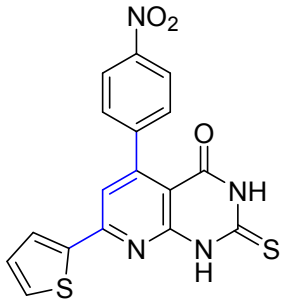




BRUKER

```
Current Data Parameters
NAME      Jul02-2017-nmr
EXPNO                   9
PROCNO                  1

F2 - Acquisition Parameters
Date_           20170703
Time                1.51
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                  1024
DS                     4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG               194.81
DW               20.800 usec
DE                6.50 usec
TE               300.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         100.6328883 MHz
NUC1                 13C
P1                10.00 usec
PLW1         66.00000000 W

======== CHANNEL f2 ========
SFO2         400.1716007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             90.00 usec
PLW2         16.50000000 W
PLW12         0.20370001 W
PLW13         0.16500001 W

F2 - Processing parameters
SI                  32768
SF           100.6228270 MHz
WDW                    EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```





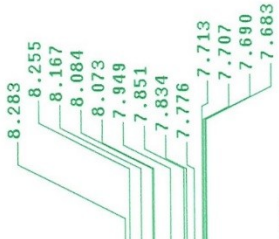
10j

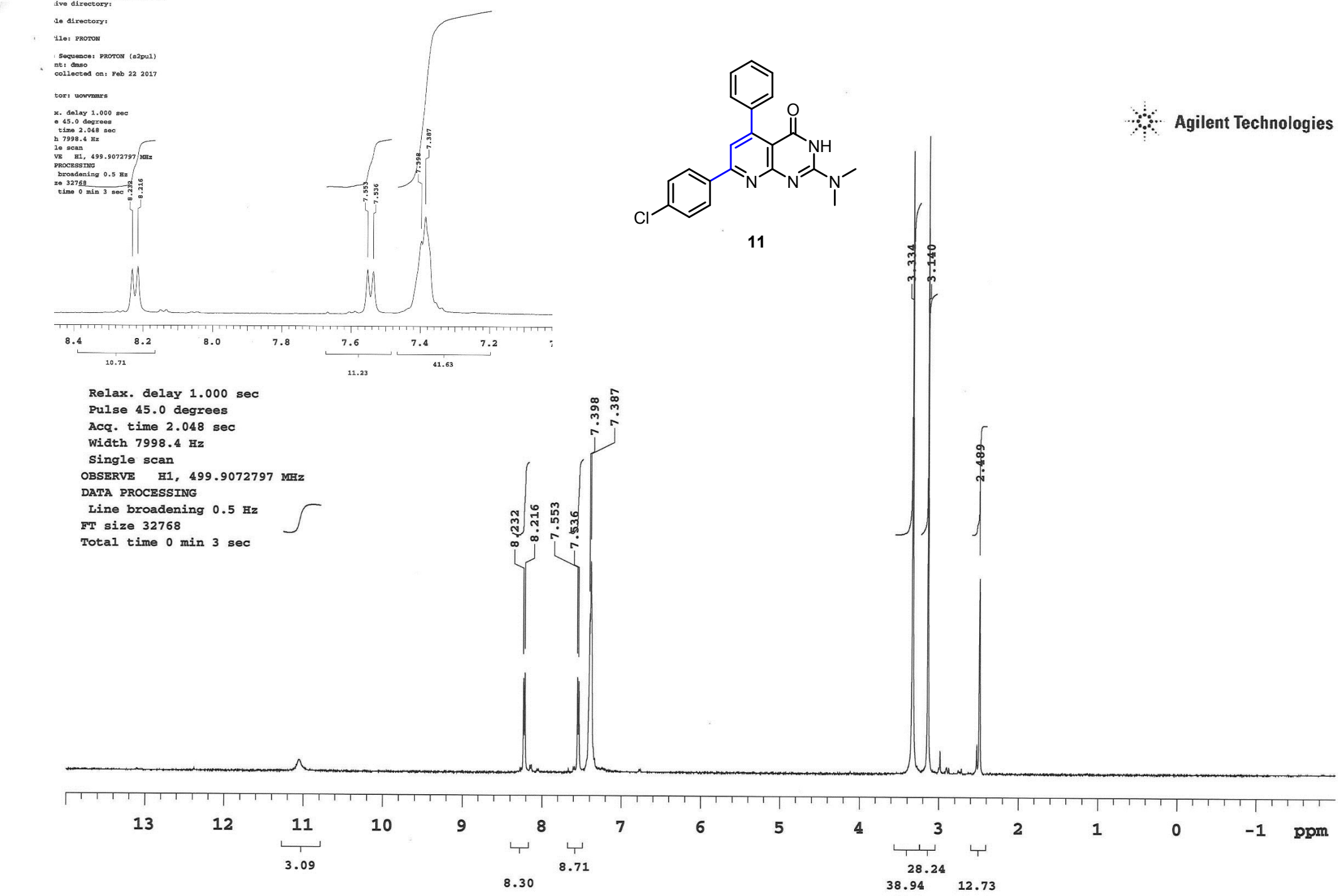
Dr.MohamedFars-10PP-H1-DMSO-Main.Defence.Chemical.Laboratory

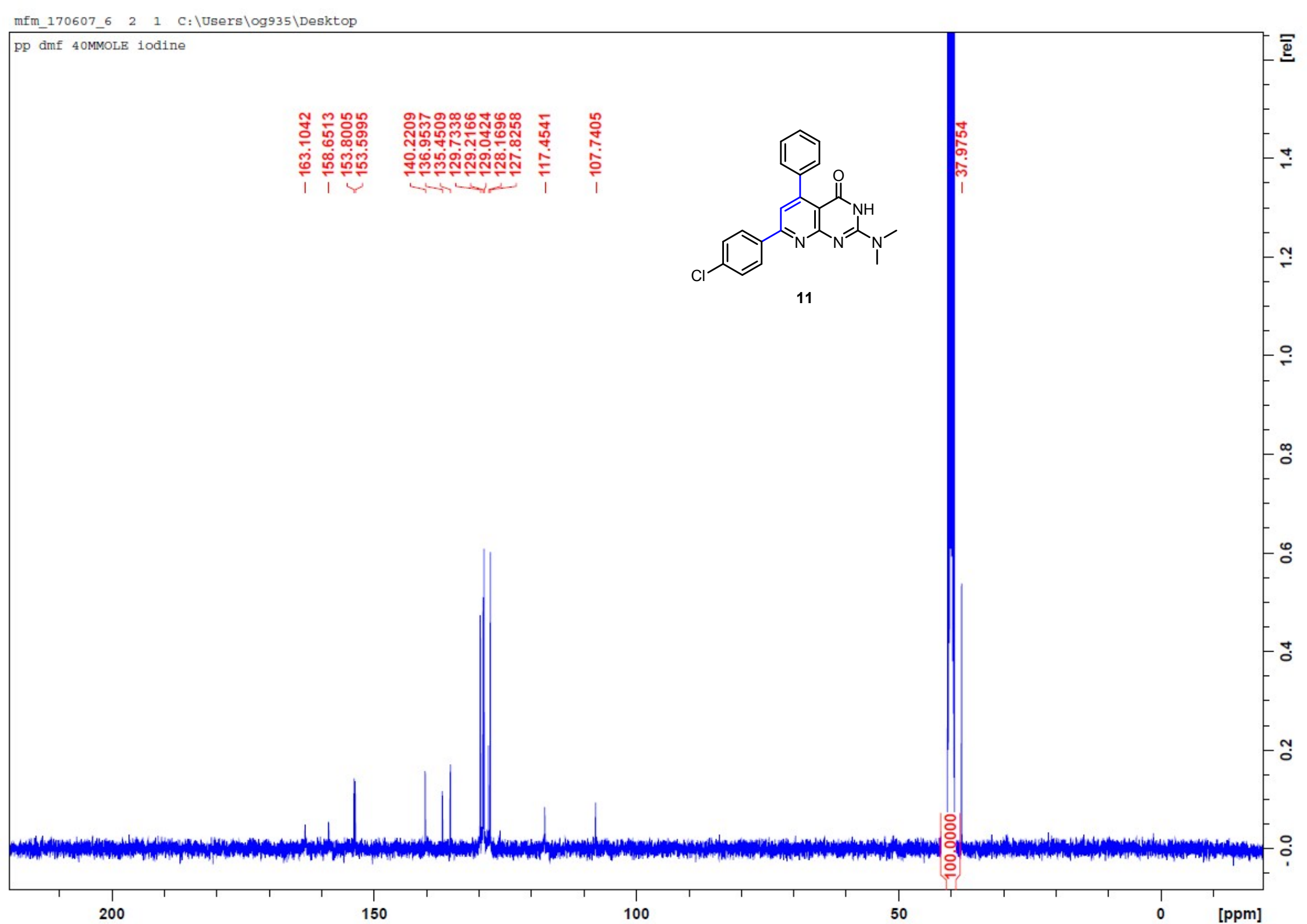
Pulse Sequence: s2pul

Solvent: DMSO
Temp. 25.0 C / 298.1 K
GEMINI-300BB "NMR"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.127 sec
Width 6000.2 Hz
24 repetitions
OBSERVE H1, 300.0117460 MHz
DATA PROCESSING
FT size 65536
Total time 5 min, 5 sec




ive directory:
le directory:
ile: PROTON
Sequence: PROTON (s2pul)
nt: dmso
collected on: Feb 22 2017
tor: uowvmmrs
x. delay 1.000 sec
e 45.0 degrees
time 2.048 sec
h 7998.4 Hz
le scan
VE H1, 499.9072797 MHz
PROCESSING
broadening 0.5 Hz
ze 32768
time 0 min 3 sec
8.232
8.216
7.553
7.536
7.398
7.387
8.4
8.2
8.0
7.8
7.6
7.4
7.2
10.71
11.23
41.63
O
NH
N
N
N
Cl
11
Agilent Technologies
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.048 sec
Width 7998.4 Hz
Single scan
OBSERVE H1, 499.9072797 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min 3 sec
3.334
3.140
2.489
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
ppm
3.09
8.30
8.71
28.24
38.94
12.73


mfm_170607_6 2 1 C:\Users\og935\Desktop
pp dmf 40MMOLE iodine
163.1042
158.6513
153.8005
153.5995
140.2209
136.9537
135.4509
129.7338
129.2166
129.0424
128.1696
127.8258
117.4541
107.7405
37.9754
O
NH
N
N
N
Cl
11
100.0000
200
150
100
50
0
[ppm]
[rel]
1.4
1.2
1.0
0.8
0.6
0.4
0.2
-0.0

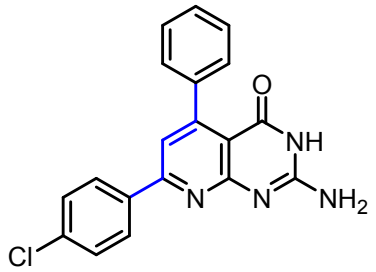
mm170228_PP_Cl_Aceto_and_benzaldehyde_Formamide_I2_Proton

File: Proton

Pulse Sequence: s2pul

Solvent: dmso
Temp. 40.0 C / 313.1 K
Operator: uowvnmrs
VNMRS-500 "pyne06.domain.com"

Relax. delay 0.001 sec
Pulse 45.0 degrees
Acq. time 2.045 sec
Width 8012.8 Hz
Single scan
OBSERVE H1, 499.7437571 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 0 min, 2 sec



12

mm170607_PP_Formamide_NH2_CARBON

Sample Name:
mm170607_PP_Formamide_NH2
Data Collected on:
ernst.sci.uow.edu.au-inova500
Archive directory:

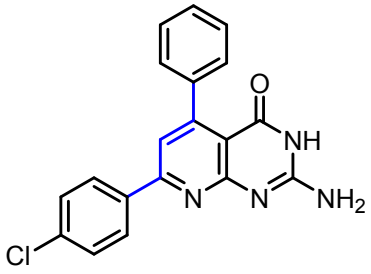
Sample directory:

FidFile: mm170607_PP_Formamide_NH2_CARBON01

Pulse Sequence: CARBON (s2pul)
Solvent: dmso
Data collected on: Jun 7 2017

Temp. 25.0 C / 298.1 K
Operator: uowvnmrs

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.043 sec
Width 31421.8 Hz
17460 repetitions
OBSERVE C13, 125.7017855 MHz
DECOUPLE H1, 499.9097792 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 9 hr, 57 min



12

Agilent Technologies