Supporting Information

for

Regioselective convergent synthesis of 2-arylidene thiazolo[3,2-*a*]pyrimidines as potential anti-chikungunya agents.

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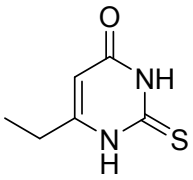
General methods and material

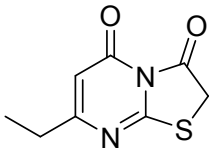
All reagents and solvents were purified and dried by standard techniques. Melting points were measured with a Stuart apparatus and were uncorrected. Reactions were monitored by TLC analysis using silica gel GF/UV 254. NMR spectra were recorded on Varian Gemini-300BB 500 MHz FT-NMR spectrometers (Varian Inc., Palo Alto, CA). ^{1}H spectra were run at 500 MHz and ^{13}C spectra were run at 126 MHz, in deuterated dimethylsulfoxide (DMSO-d_6), $(CD_3)_2CO$ and $CDCl_3$. 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. Electrospray (ESI single quadrupole) mass spectra have their ion mass to charge values (m/z) stated with their relative abundances as a percentage in parentheses. Peaks assigned to the molecular ion are denoted as [M+H] or [M+Na]. Column chromatography was performed using silica gel 60 (0.063-0.200 mm). All reagents and solvents were purified and dried by standard techniques.

6-Ethyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (3):



After preparing a sodium ethoxide solution by dissolving sodium (4.6 gm, 0.1 mole) in absolute ethanol (150 mL), the thiourea **2** (3.8 gm, 0.05 mole) was added with stirring with complete dissolution. The ethyl 3-oxopentanoate ester **1** was then added (7.21 gm, 0.05 mole) and the reaction mixture was heated at reflux for 4 hours. After cooling the reaction mixture, water was added dropwise till completer dissolution of the white formed precipitate. Ice was added, and neutralization of the alkaline solution was accomplished using HCl (5M). The reaction flask left overnight in the fridge and the white formed precipitate was filtered, washed thoroughly 3 times with water and 2 timed with diethyl ether and dried to give **3** as a white powder (5.38 gm, 69%). In some cases, when the glacial acetic acid used instead of HCl as neutralizing agent, **3** was obtained as pink colour. m.p: 230 °C; ^{1}H NMR ($(CD_3)_2SO_2$) δ: 1.07 (t, J = 7.5 Hz, 3H, CH_3), 2.33 (q, J = 7.5 Hz, 2H, CH_2), 5.33 (s, 1H, pyridine-H), 12.30 (br s, 2H, 2 x -NH); ^{13}C NMR (CD_3OD): 13.4, 27.8, 103.9, 162.0, 165.8, 179.4; MS (ESI): 157 (20%, M+H), 179 (100%, M+Na).

7-Ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (5):



A previously dried anhydrous sodium acetate (82 mg, 1 mmole) was added to a suspension of 6-ethyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **3** (156 mg, 1

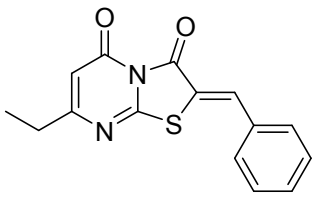
mmole) and bromoacetic acid (153 mg,1.1 mmole) in 2 mL glacial acetic acid and 1 mL acetic anhydride. The reaction flask was heated gently at no more than 60 °C for 3h. The formed precipitate was filtered while hot and washed thoroughly with water (3x10 mL) and diethyl ether (2 x 5 mL) and dried to afford (79 mg ,40%) of **5**. ^{1}H NMR ($(CD_3)_2CO$) δ: 1.19 (t, *J* = 7.5 Hz, 3H, CH_3), 2.49 (q, *J* = 7.5 Hz, 2H, CH_2), 4.02 (s, 2H, CH_2), 6.02 (s, 1H, pyridine-H); ^{13}C NMR ($(CD_3)_2CO$): 11.5, 30.0, 32.4, 105.7, 147.3, 163.6, 169.0, 169.3; MS (ESI): 197 (25%, M+H), 219 (100%, M+Na); HRMS (ESI) calcd for $C_8H_9N_2O_2S$: 197.0381 found 197.0385.

General procedures for the synthesis of 2-aryl-7-ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (6-20):

Method A: A previously dried anhydrous sodium acetate (164 mg, 2 mmole) was added to a solution of 6-ethyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **3** (156 mg, 1 mmole), corresponding aldehyde (1.1 mmole) and bromoacetic acid (153 mg,1.1 mmole) in 2 mL glacial acetic acid and 1 mL acetic anhydride. After gentle heating at no more than 60 ºC, the reaction was monitored using TLC analysis till consumption of the stating material or until conversion was observed to stall. Heating was then discontinued, and the reaction mixture was filtered while hot and the residue washed with water (3 × 10 mL), and diethyl ether (2 × 10 mL) then dried to give the desired thiazolopyrimidine **6-20**.

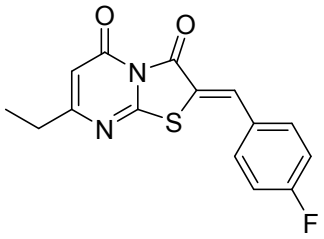
Method B: A previously dried anhydrous sodium acetate (82 mg, 1 mmole) was added to a suspension of 7-ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (196 mg, 1 mmole) **5** in glacial acetic acid, 1.1 mmole of the appropriate aldehyde was added and the mixture was gently heated at less than 60 ºC. The reaction was monitored by TLC and the workup was similar to method A.

(*Z*)-2-benzylidene-7-ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (6): The compound was



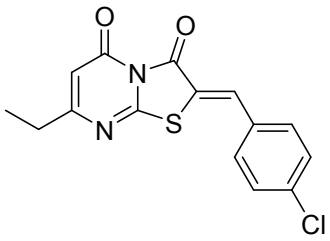
prepared according to method A (yield = 39%) and method B (yield = 24% over 2 steps); m.p: 170 °C; ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 2.54 (q, *J* = 7.5 Hz, 2H, CH_2), 6.10 (s, 1H, pyridine-H), 7.50-7.54 (m, 3H, ArH), 7.58 (d, *J* = 7.5 Hz, 2H, ArH), 8.07 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$): 11.7, 30.5, 108.9, 118.6, 129.6, 130.8, 131.4, 133.1, 137.4, 158.2, 158.9, 163.2, 168.4; MS (ESI): 285 (60%, M+H), 307 (100%, M+Na), 323 (70%, M+K$^+$); HRMS (ESI) calcd for $C_{15}H_{12}N_2O_2SNa$: 307.0502, found: 307.0517.

(*Z*)-7-ethyl-2-(4-fluorobenzylidene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (7): The compound



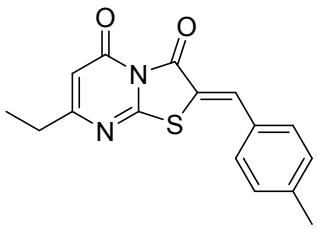
was prepared according to method A (yield = 40%) m.p: 210 °C; ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.5 Hz, 3H, CH_3), 2.56 (q, *J* = 7.5 Hz, 2H, CH_2), 6.11 (s, 1H, pyridine-H), 7.21 (t, *J* = 8.5 Hz, 2H, Ar-H), 7.56 (dd, *J* = 8.5, 5.3 Hz, 2H, Ar-H), 8.01 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.6, 30.3, 108.8, 116.8 (d, J^{2}_{C-F} 21 Hz), 118.1, 129.3, 132.8 (d, J^{3}_{C-F} 9 Hz), 135.9, 157.7, 158.7, 163.0, 163.0 (d, J^{1}_{C-F} 267 Hz), 168.3; MS (ESI): 303 (10%, M+H), 325 (65%, M+Na), 627 (100%, 2M+Na); HRMS (ESI) calcd for $C_{15}H_{12}FN_2O_2S$: 303.0618, found: 303.0604.

(*Z*)-2-(4-chlorobenzylidene)-7-ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (8): The compound

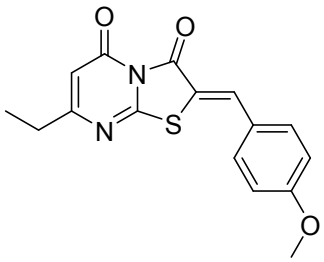


was prepared according to method A (yield = 48%); m.p: 184 °C; ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.5 Hz, 3H, CH_3), 2.55 (q, *J* = 7.5 Hz, 2H, CH_2), 6.12 (s, 1H, pyridine-H), 7.50 (m, 4H, ArH), 8.02 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$): 11.6, 30.4, 108.9, 119.1, 129.8, 131.4, 131.8, 135.7, 137.5, 157.6, 158.6, 162.9, 168.3; MS (ESI): 319 (20%, M+H), 341 (100%, M+Na), 357 (25%, M+K); HRMS (ESI) calcd for $C_{15}H_{11}ClN_2O_2SNa$: 319.0302 found: 319.0308.

(*Z*)-7-ethyl-2-(4-methylbenzylidene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (9): The compound



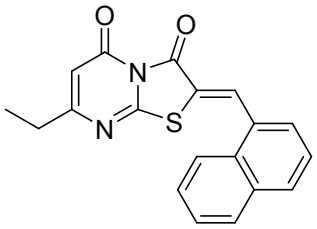
was prepared according to method A (yield = 37%) m.p: 180 °C; ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.56 (q, *J* = 7.5 Hz, 2H, CH_2), 6.09 (s, 1H, pyridine-H), 7.31 (d, *J* = 8 Hz, 2H, Ar-H), 7.46 (d, *J* = 8 Hz, 2H, Ar-H), 8.04 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.6, 21.7, 30.4, 108.7, 117.1, 130.2, 130.2, 130.9, 137.5, 142.4, 158.2, 158.8, 163.2, 168.2; MS (ESI): 299 (7%, M+H), 321 (35%, M+Na), 619 (100%, 2M+Na); HRMS (ESI) calcd for $C_{16}H_{14}N_2O_2SNa$: 321.0688, found: 321.0674.



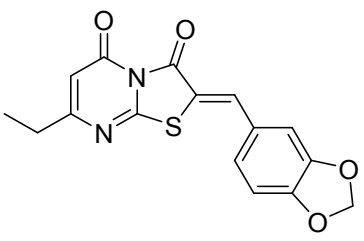
(*Z*)-7-ethyl-2-(4-methoxybenzylidene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (10): The compound was prepared according to method A

(yield = 44%) m.p: 168 °C; ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.5 Hz, 3H, CH_3), 2.56 (q, *J* = 7.5 Hz, 2H, CH_2), 6.08 (s, 1H, pyridine-H), 7.02 (d, *J* = 9.0 Hz, 2H, ArH), 7.53 (d, *J* = 9.0 Hz, 2H, ArH), 8.01 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$): 11.6, 30.3, 55.6, 108.5, 115.0, 115.1, 125.6, 132.9, 137.2, 158.3, 158.9, 162.2, 163.2, 168.2; MS (ESI): 315 (10%, M+H), 337 (25%, M+Na), 651 (100%, 2M+Na); HRMS (ESI) calcd for $C_{16}H_{14}N_2O_3SNa$: 337.0606 found: 337.0623.

(*Z*)-7-ethyl-2-(naphthalen-1-ylmethylene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (11): The compound was prepared according to method A (yield = 70%) m.p: 175 °C; ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 2.56 (q, *J* = 7.5 Hz, 2H, CH_2), 6.26 (s, 1H, pyridine-H), 7.57-7.67 (m, 3H, Ar-H), 7.73 (d, *J* = 7.0 Hz, 1H, Ar-H), 7.92 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.98 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.14 (d, *J* = 8.5 Hz, 1H, Ar-H), 8.82 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.7, 30.5, 108.8, 121.6, 123.5, 125.4, 127.2 (2C), 127.9, 129.2, 130.3, 131.9, 132.1, 133.9, 134.4, 158.5, 159.0, 162.8, 168.4; MS (ESI): 335 (20%, M+H), 691 (100%, 2M+Na); HRMS (ESI) calcd for $C_{19}H_{15}N_2O_2S$: 335.0849, found: 335.0854.



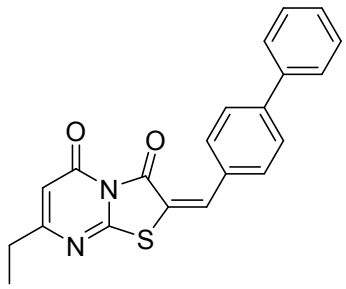
(*Z*)-4-((7-ethyl-3,5-dioxo-5*H*-thiazolo[3,2-*a*]pyrimidin-2(3*H*)-ylidene)methyl)-2-methoxyphenyl acetate (12): The compound was prepared according to method A (yield = 59%) m.p: 208 °C; ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 2.34 (s, 3H, $COCH_3$), 2.56 (q, *J* = 7.5 Hz, 2H, CH_2), 3.90 (s, 3H, OCH_3), 6.10 (s, 1H, pyridine-H), 7.13 (s, 1H, Ar-H), 7.18 (s, 2H, Ar-H), 8.01 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.0, 20.0, 29.8, 55.4, 108.2, 113.4, 117.9, 123.1, 123.3, 131.2, 135.9, 141.5, 151.2, 157.2, 158.0, 162.3, 167.6, 167.9; MS (ESI): 373 (15%, M+H), 767 (100%, 2M+Na); HRMS (ESI) calcd for $C_{18}H_{17}N_2O_5S$: 373.0862, found: 373.0858.



(***Z*)-2-(benzo[*d*][1,3]dioxol-5-ylmethylene)-7-ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2H)-dione (13):** The compound was prepared according to method A (yield = 67%) m.p: 206 °C; ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.5 Hz, 3H, CH_3), 2.55 (q, *J* = 7.5 Hz, 2H, CH_2), 6.07-6.10 (m, 3H, pyridine-H + OCH_2O), 6.93 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 7.12 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.95 (s, 1H,

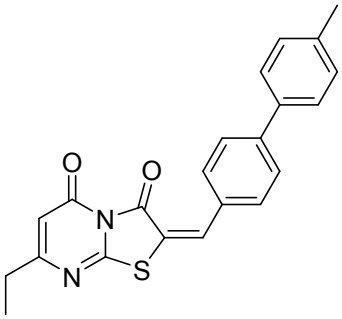
Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.7, 30.5, 120.3, 108.7, 109.4, 109.5, 115.9, 127.4, 127.8, 137.3, 148.9, 150.6, 158.2, 158.9, 163.2, 168.3; MS (ESI): 351 (70%, M+Na), 679 (100%, 2M+Na); HRMS (ESI) calcd for $C_{16}H_{12}N_2O_4SNa$: 351.0405, found: 351.0415.

(*Z*)-2-([1,1'-biphenyl]-4-ylmethylene)-7-ethyl-5H-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (14): The



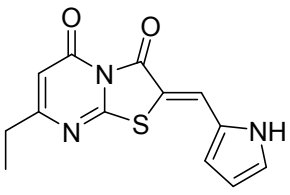
compound was prepared according to method A (yield = 77%) m.p: 172 °C; ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.5 Hz, 3H, CH_3), 2.55 (q, *J* = 7.5 Hz, 2H, CH_2), 6.08 (s, 1H, pyridine-H), 7.39-7.42 (m, 1H, ArH), 7.47 (t, *J* = 7.5 Hz, 2H, ArH), 7.62-7.64 (m, 4H, ArH), 7.74 (d, *J* = 8 Hz, 2H, ArH), 8.07 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.7, 30.4, 108.8, 118.1, 127.2, 128.0, 128.5, 129.2, 131.4, 131.8, 136.9, 139.5, 144.0, 158.1, 158.8, 163.1, 168.3; MS (ESI): 383 (70%, M+Na), 743 (100%, 2M+Na); HRMS (ESI) calcd for $C_{21}H_{16}N_2O_2SNa$: 383.0837, found: 383.0830.

(*Z*)-7-ethyl-2-((4'-methyl-[1,1'-biphenyl]-4-yl)methylene)-5H-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-



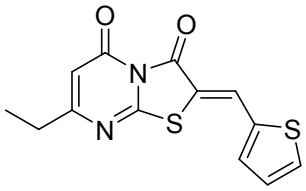
dione (15): The compound was prepared according to method A (yield = 69%) m.p: 218 °C; ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 2.56 (q, *J* = 7.5 Hz, 2H, CH_2), 7.28 (d, *J* = 7.8 Hz, 2H, ArH), 7.54 (d, *J* = 7.8 Hz, 2H, ArH), 7.62 (d, *J* = 8.1 Hz, 2H, ArH), 7.73 (d, *J* = 8.1 Hz, 2H, ArH), 8.08 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.7, 21.3, 30.5, 108.8, 117.9, 127.1, 127.8, 129.9, 131.5, 131.6, 136.6, 137.0, 138.6, 144.1, 158.2, 158.9, 163.2, 168.3; MS (ESI): 375 (65%, M+H), 397 (75%, M+Na), 771 (100%, 2M+Na); HRMS (ESI) calcd for $C_{22}H_{18}N_2O_2SNa$: 397.0996, found: 397.0987.

(*Z*)-2-((1*H*-pyrrol-2-yl)methylene)-7-ethyl-5H-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (16):The



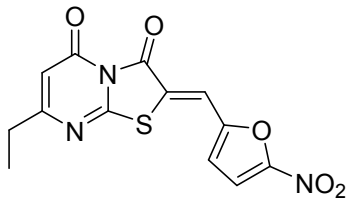
compound was prepared according to method A (yield = 27%) m.p: 220 °C; ^{1}H NMR (DMSO-d_6) δ: 1.15 (t, *J* = 7.5 Hz, 3H, CH_3), 2.48 (q, *J* = 7.5 Hz, 2H, CH_2), 6.05 (s, 1H, pyridine-H), 6.44 (s, 1H, ArH), 6.66 (s, 1H, ArH), 7.32 (s, 1H, ArH), 7.89 (s, 1H, Arylidene H), 11.86 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 12.3, 30.1, 108.6, 110.8, 113.5, 116.1, 126.1, 126.8, 128.0, 159.0, 159.3, 163.5, 167.9; MS (ESI): 274 (10%, M+H), 296 (40%, M+Na), 569 (15%, 2M+Na); HRMS (ESI) calcd for $C_{13}H_{11}N_3O_2SNa$: 296.0484, found: 296.0470.

(*Z*)-7-ethyl-2-(thiophen-2-ylmethylene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (17): The



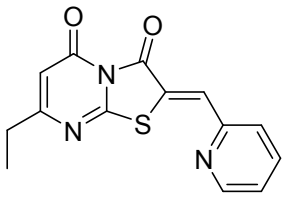
compound was prepared according to method A (yield = 43%) m.p: 216 °C; ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.5 Hz, 3H, CH_3), 2.57 (q, *J* = 7.5 Hz, 2H, CH_2), 6.11 (s, 1H, pyridine-H), 7.24 (m, 1H, ArH), 7.51 (d, *J* = 3.0 Hz, 1H, ArH), 7.75, (d, *J* = 5.0 Hz, 1H, ArH), 8.01 (s, 1H, Arylidene H); ^{13}C NMR ($CDCl_3$) δ: 11.6, 30.4, 108.8, 116.3, 129.1, 129.6, 133.3, 134.7, 137.7, 157.8, 158.9, 162.9, 168.3; MS (ESI): 291 (15%, M+H), 313 (75%, M+Na), 603 (100%, 2M+Na); HRMS (ESI) calcd for $C_{13}H_{10}N_2O_2S_2Na$: 313.0081, found: 313.0071.

(*Z*)-7-ethyl-2-((5-nitrofuran-2-yl)methylene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (18): The



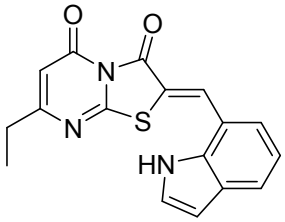
compound was prepared according to method A (yield = 46%) m.p: 202 °C; ^{1}H NMR (DMSO-d_6) δ: 1.14 (t, *J* = 7.5 Hz, 3H, CH_3), 2.48 (q, *J* = 7.5 Hz, 2H, CH_2), 6.14 (s, 1H, pyridine-H), 7.40 (d, *J* = 3.8 Hz, 1H, ArH), 7.84 (d, *J* = 3.8 Hz, 1H, Ar-H), 7.94 (s, 1H, Arylidene H); ^{13}C NMR (DMSO-d_6) δ: 11.5, 29.3, 108.5, 114.7, 118.6, 120.4, 123.7, 151.1, 152.8, 157.8, 158.3, 162.1, 167.2; MS (ESI): 320 (5%, M+H), 661 (100%, 2M+Na); HRMS (ESI) calcd for $C_{13}H_{10}N_3O_5S$: 320.0335, found: 320.0341.

(*Z*)-7-ethyl-2-(pyridin-2-ylmethylene)-5*H*-thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione (19): The



compound was prepared according to method A (yield = 22%) m.p: 225 °C; ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 2.56 (q, *J* = 7.5 Hz, 2H, CH2), 6.08 (s, 1H, pyrimidine H), 7.32 (dd, *J* = 7.4, 4.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.81 (dd, *J* = 7.4, 6.7 Hz, 1H), 7.98 (s, 1H, Arylidene H), 8.78 (d, J = 4.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 11.6, 30.4, 108.5, 124.0, 124.3, 157.5, 132.0, 137.1, 149.2, 151.3, 159.1, 162.1, 163.5, 168.5; MS (ESI): 286 (5%, M+H), 308 (10%, M+Na); HRMS (ESI) calcd for $C_{14}H_{12}N_3O_2S$: 286.0649, found: 286.0650.

(*Z*)-2-((1*H*-indol-7-yl)methylene)-7-ethyl-5*H*-thiazolo[3,2-*a*]pyrimidine-



3,5(2*H*)-dione (20): The compound was prepared according to method A (yield

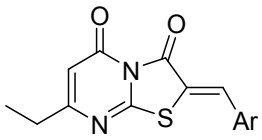
= 37%) m.p: 190 °C; ^{1}H NMR ($CDCl_3$) δ: 1.27 (t, *J* = 7.5 Hz, 3H, CH_3), 2.57 (q, *J* = 7.5 Hz, 2H, CH_2), 6.08 (s, 1H, pyrimidine H), 6.64 (s, 1H, ArH), 7.18 (t, *J* = 7.5 Hz, 1H, ArH), 7.34 (d, *J* = 7.5 Hz, 1H, ArH), 7.46 (s, 1H, ArH), 7.72 (d, *J* = 7.5 Hz, 1H, ArH), 8.78 (s, 1H, Arylidene H), 9.67 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 11.7, 30.4, 103.2, 108.4, 110.0, 117.2, 118.4, 119.9, 122.1, 125.0, 129.4, 133.4, 135.5, 158.8, 159.2, 163.5, 168.4; MS (ESI): 324 (30%, M+H), 346 (100%, M+Na); HRMS (ESI) calcd for $C_{17}H_{14}N_3O_2S$: 324.0799, found: 324.0807.

Biological evaluation

Anti-viral assay

CHIKV Indian Ocean strain 899 (Genbank FJ959103.1) was generously provided by Prof. S. Günther (Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany) (Panning M et al., Emerging Infectious Diseases 2008). BGM cells were maintained in cell growth medium composed of minimum essential medium (MEM Rega-3, Gibco, Belgium) supplemented with 10% Foetal Bovine Serum (FBS, Integro, The Netherlands), 1% L-glutamine (Gibco), and 1% sodium bicarbonate (Gibco). The antiviral assays were performed in virus growth medium which is the respective cell growth medium supplemented with 2% (instead of 10%) FBS. Cell cultures were maintained at 37 °C in an atmosphere of 5% CO2 and 95-99% humidity. BGM cells were seeded in 96-well tissue culture plates (Becton Dickinson, Aalst, Belgium) at a density of 2.5 x 104 cells/well in 100 µl assay medium and were allowed to adhere overnight. Next, a compound dilution series was prepared in the medium on top of the cells after which the cultures were infected with 0.001 MOI of CHIKV 899 inoculum in 100 µl assay medium. On day 5 post-infection (p.i.), the plates were processed using the MTS/PMS method as described by the manufacturer (Promega, The Netherlands). The 50% effective concentration (EC_{50}), which is defined as the compound concentration that is required to inhibit virus-induced cell death by 50%, was determined using logarithmic interpolation. All assay wells were checked microscopically for minor signs of virus induced CPE or possible alterations to the cell or monolayer morphology caused by the compound.

Table S1. The observed %inhibition of CHIKV activity of the novel compounds **6**-**20**.



6-20

Compound	Ar	% inhibition at 20 µg/mL	EC_{50}(µM)
6	-C_6H_5	19	ND
7	4-FC_6H_4	ND	ND
8	4-ClC_6H_4	ND	ND
9	4-$CH_3C_6H_4$	ND	ND
10	4-$OCH_3C_6H_4$	4.3	ND
11	1-Naphthyl	29	ND
12	4-OAc-3-OCH_3 C_6H_3	5.5	ND
13	3,4-$(CH_2O_2)C_6H_3$	ND	ND
14	4-C_6H_5-C_6H_4	5.0	ND
15	4(4-$CH_3C_6H_4)C_6H_4$	58	42
16	2-Pyrrolo	ND	ND

ND: not determined; EC_{50}: concentration of compound that inhibits virus-induced cell death with 50%.

X-ray Crystallographic data for Compound 12:

Crystal data. Compound **12**. $C_{18}H_{16}N_2O_5S$, *M*= 372.40, *T*=150 K, Triclinic, $P\bar{1}$, *Z*=2, *a*= 7.8375 (3), *b*= 8.2458 (3), *c*= 14.5394 (7) Å, *α* = 87.176 (4)°, *β* = 74.562 (4)°, *γ* = 73.335 (4)° ,*V*= 867.31 (7) Å^3, D_x= 1.426 g cm^{-3}, Cu *Kα* radiation, *λ*=1.54184 Å, 16946 reflections measured (*ϑ* = 6–74˚), merged to 3491 unique data, *R*=0.034 [for 3396 data with *I* > 2σ*(I)*], *R*w= 0.089 [all data], *S* = 1.00

Structure determination of compound **12**. Images were measured on an Agilent SuperNova diffractometer (Cu *K*α radiation, mirror monochromator, λ=1.54184 Å) and data extracted using the CrysAlis PRO package.[1] Structure solution was by direct methods (SIR92).[2] The structure was refined using the CRYSTALS program package.[3] CDCC 1968317.

Computational Methods

Structures were built intuitively in Avogadro and initially optimized with Molecular Mechanics (Universal force field). These structures were used for subsequent DFT calculations utilizing Gaussian16 with initial optimization at B3LYP/def2SVP for minima structures and M06-2X/6-31G(d) for transition states. All final structures and thermodynamics were calculated with M06-2X/aug-cc-pVDZ and solvation in acetic acid modelled (SMD). Minima were confirmed by analysis of the normal modes. The transition states were confirmed saddle points by observation of one imaginary frequency with mode analysis indicating molecular displacement corresponding to the transition of interest. All structures energies were also corrected for dispersion (D3) and geometric counterpoise (gCP).

Thermodynamic changes in the Gibbs free energy of reaction $\Delta_r G^0\{298K\}$ from the starting materials, compound **5** and benzaldehyde, were calculated as per equation (E1), that is $\Delta_r G^0\{298K\}$ for the product of interest is equal to the sum of electronic energy of the product – the sum of electronic energy for the starting materials, with all values having the free energy correction applied.

$$\Delta_r G°(298\ K) = \sum(\varepsilon_0 + G_{corr})_{products} - \sum(\varepsilon_0 + G_{corr})_{reactants} \qquad \text{(E1)}$$

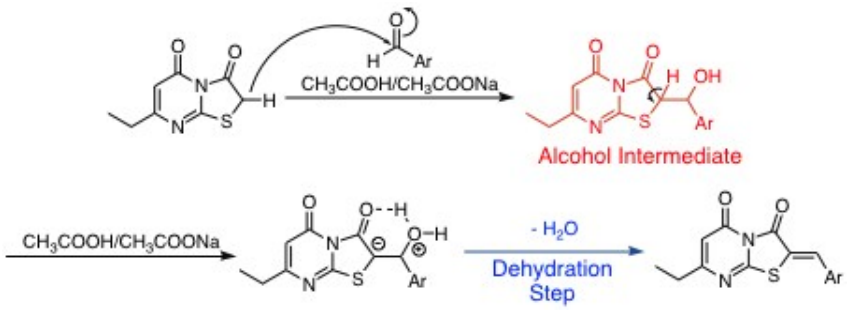
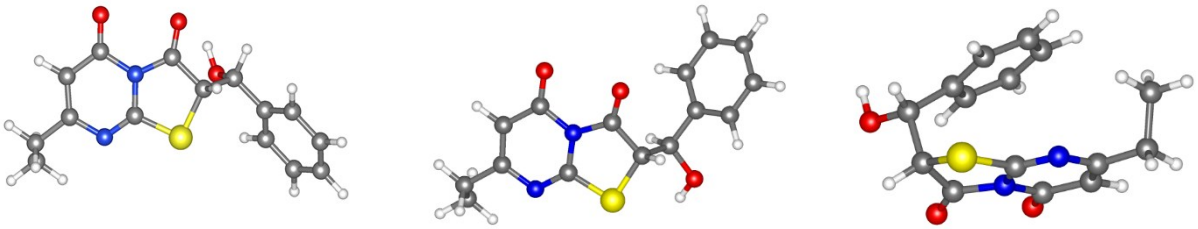


Figure S1: Proposed mechanism for the synthesis of isomers *Z* and *E*, The alcohol intermediate of interest (red) and the dehydration step (blue) are highlighted. Note the *Z* isomer is the product of the reaction in this example.



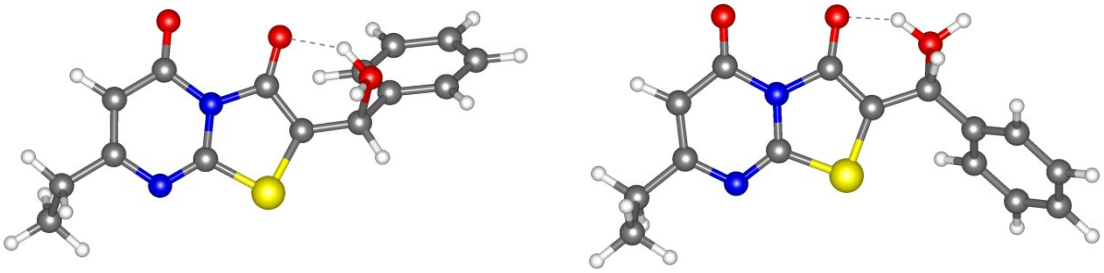
C1
-2.30(M06-2X)
-1.43 (M06-2X-D3-gCP)

C2
+0.35 (M06-2X)
+1.20 (M06-2X-D3-gCP)

C3
-1.30 (M06-2X)
-0.01 (M06-2X-D3-gCP)

Figure S2: Structures of three alcohol intermediate conformers (**C1** – **C3**) investigated. $\Delta_r G^0${298 K} energies calculated relative to the starting materials, Compound **5** and benzaldehyde, in kcal.mol^{-1}. All methods employed the aug-cc-pVDZ basis set.

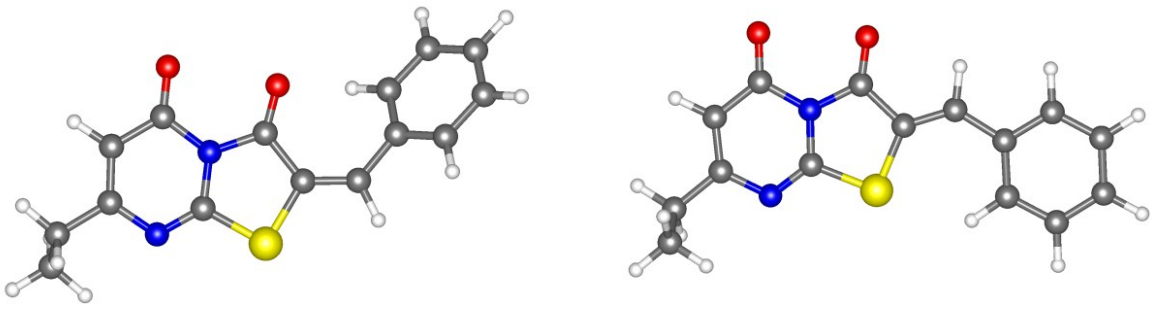
a)



T1
+32.58 (M06-2X)
+33.51 (M06-2X-D3-gCP)

T2
+30.05 (M06-2X)
+30.90 (M06-2X-D3-gCP)

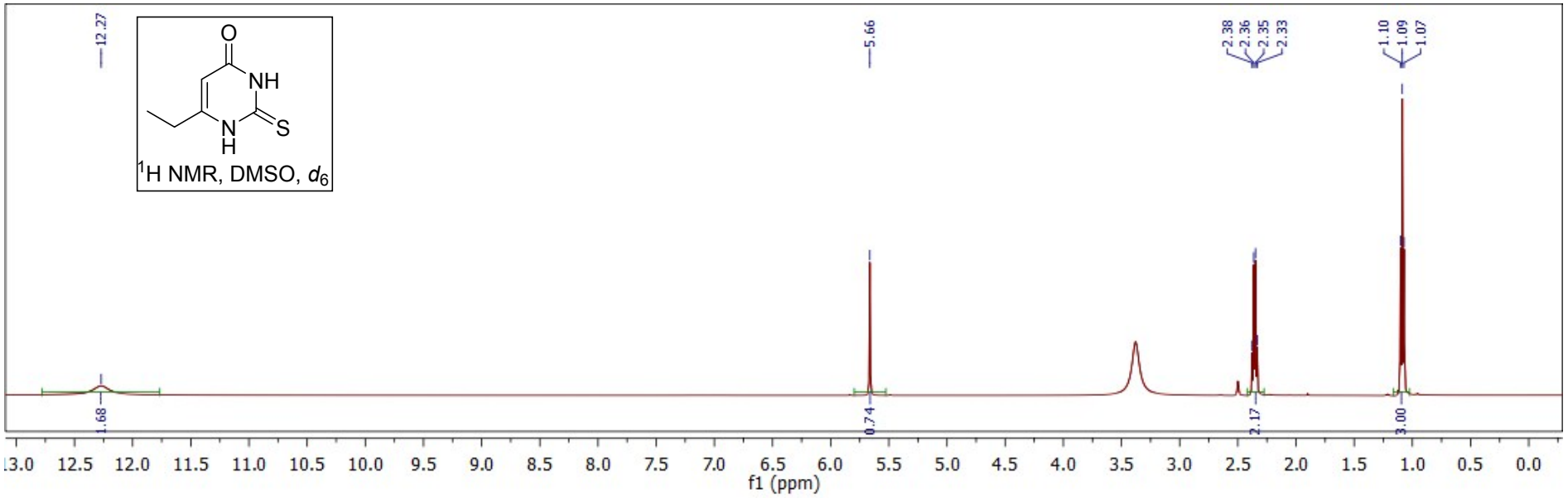
b)

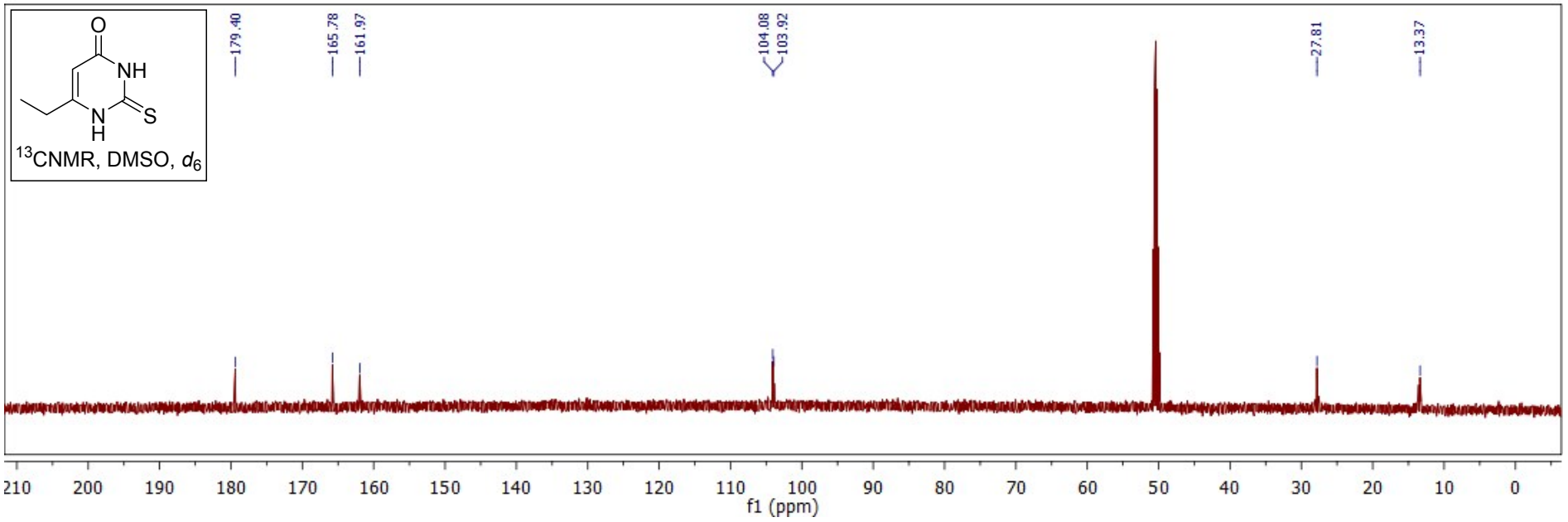


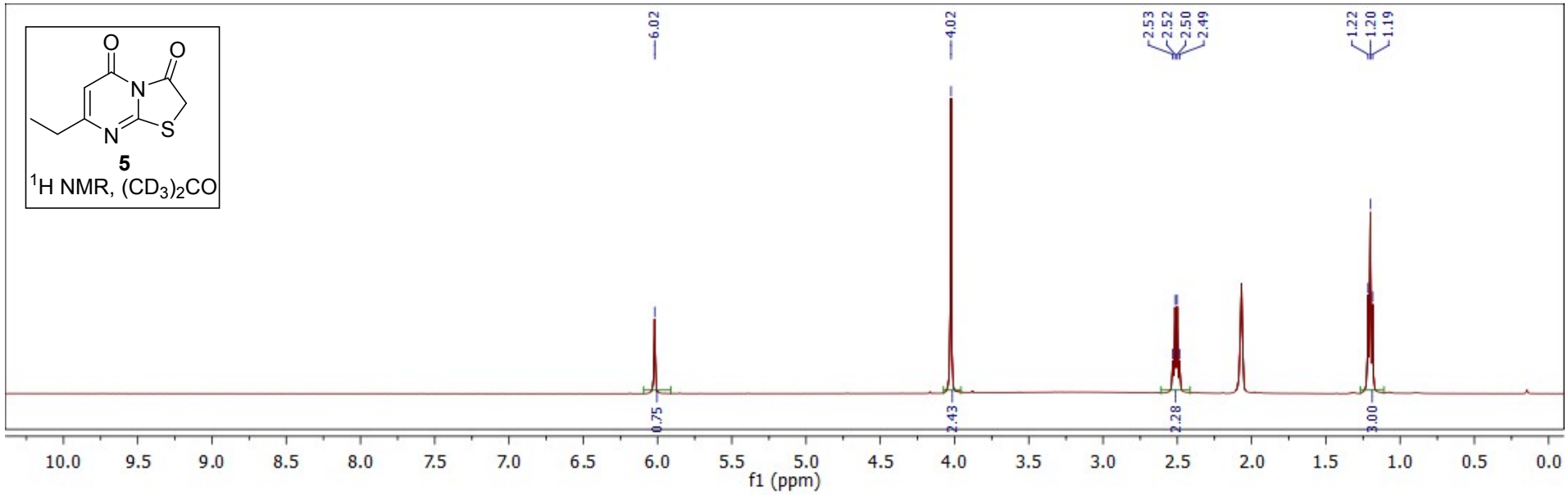
6(*E*)
-2.40 (M06-2X)
-2.43 (M06-2X-D3-gCP)

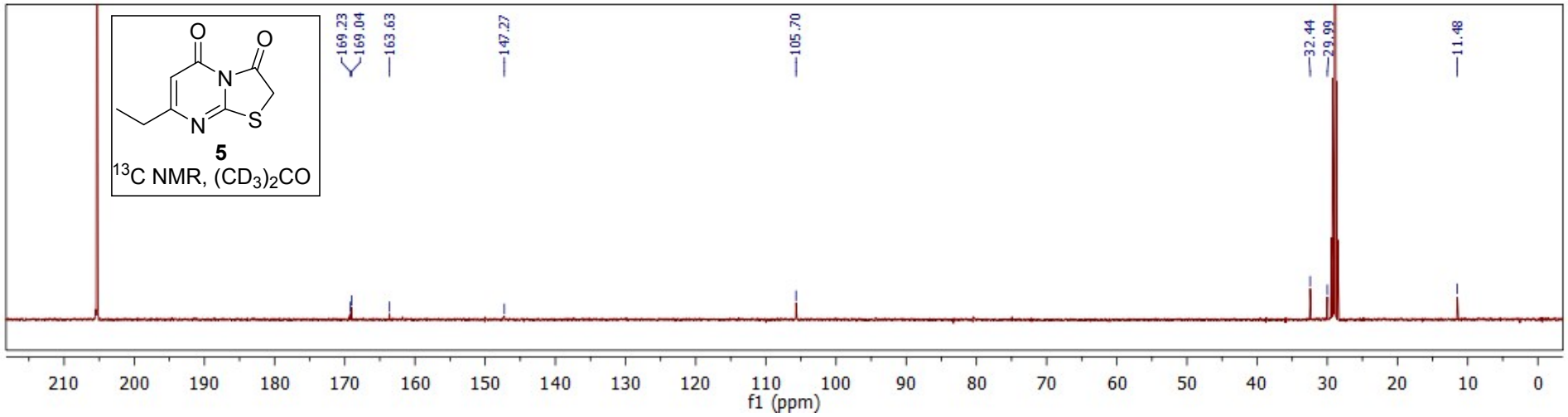
6(*Z*)
-6.78 (M06-2X)
-6.85 (M06-2X-D3-gCP)

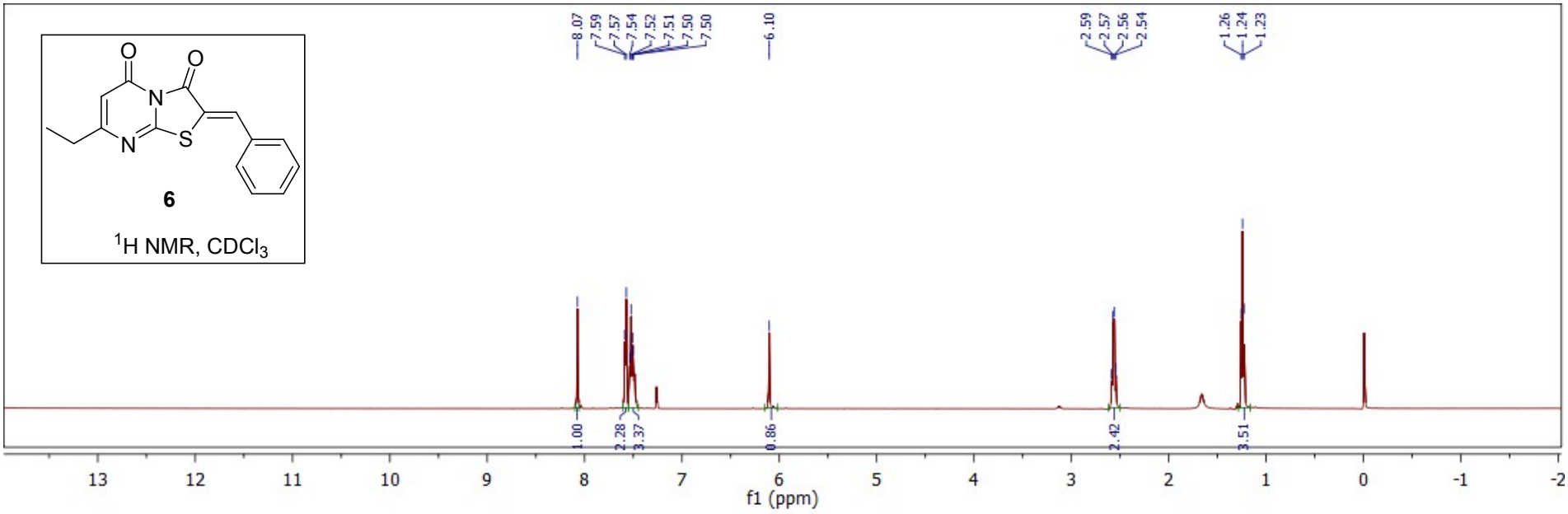
Figure S3: a) Calculated transition states for the formation of the *E* (**T1**) and *Z* (**T2**) isomers, corresponding to water leaving the structure. **b)** Optimized *E* and *Z* isomers of compound **6** calculated. $\Delta_r G^0${298 K} energies relative to the starting materials, compound **5** and benzaldehyde.

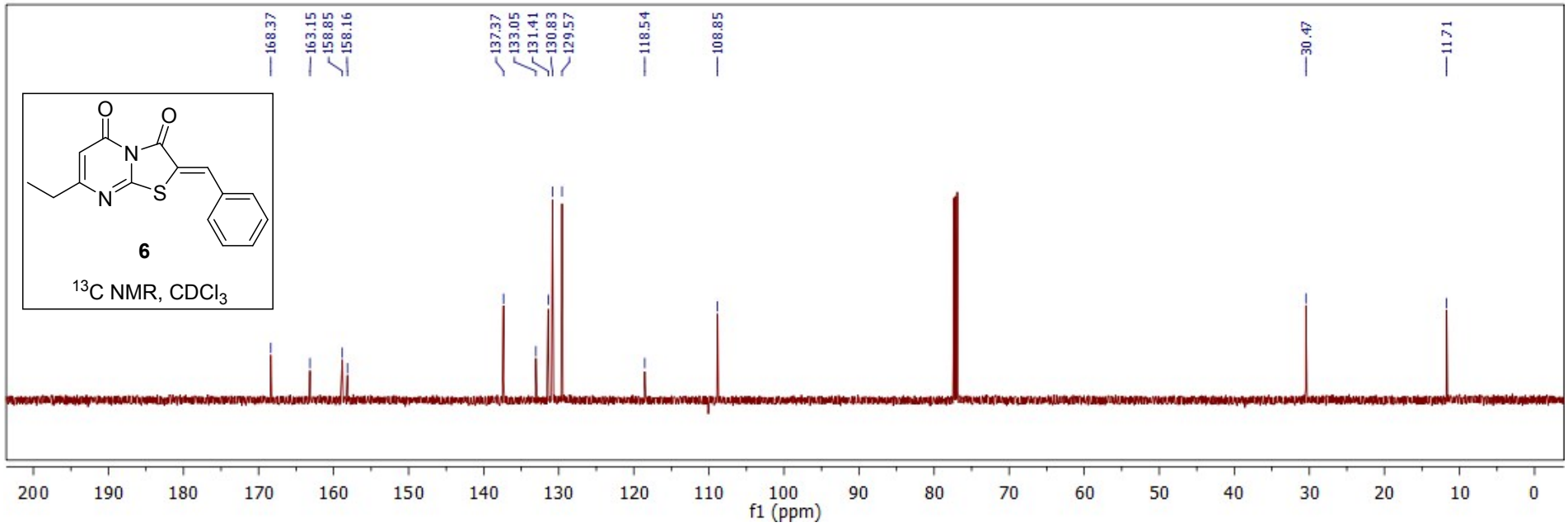

12.27
5.66
2.38
2.36
2.35
2.33
1.10
1.09
1.07
1.68
0.74
2.17
3.00
f1 (ppm)
^{1}H NMR, DMSO, d_6

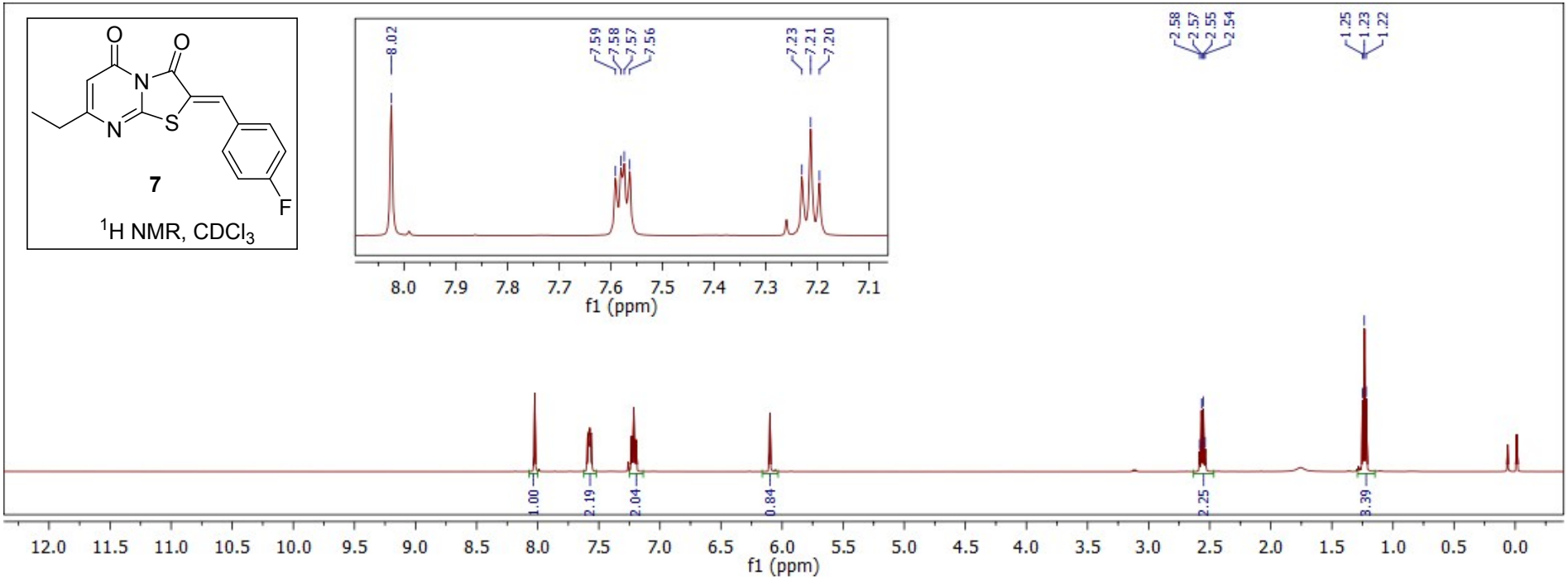

179.40
165.78
161.97
104.08
103.92
27.81
13.37
f1 (ppm)
^{13}CNMR, DMSO, d_6

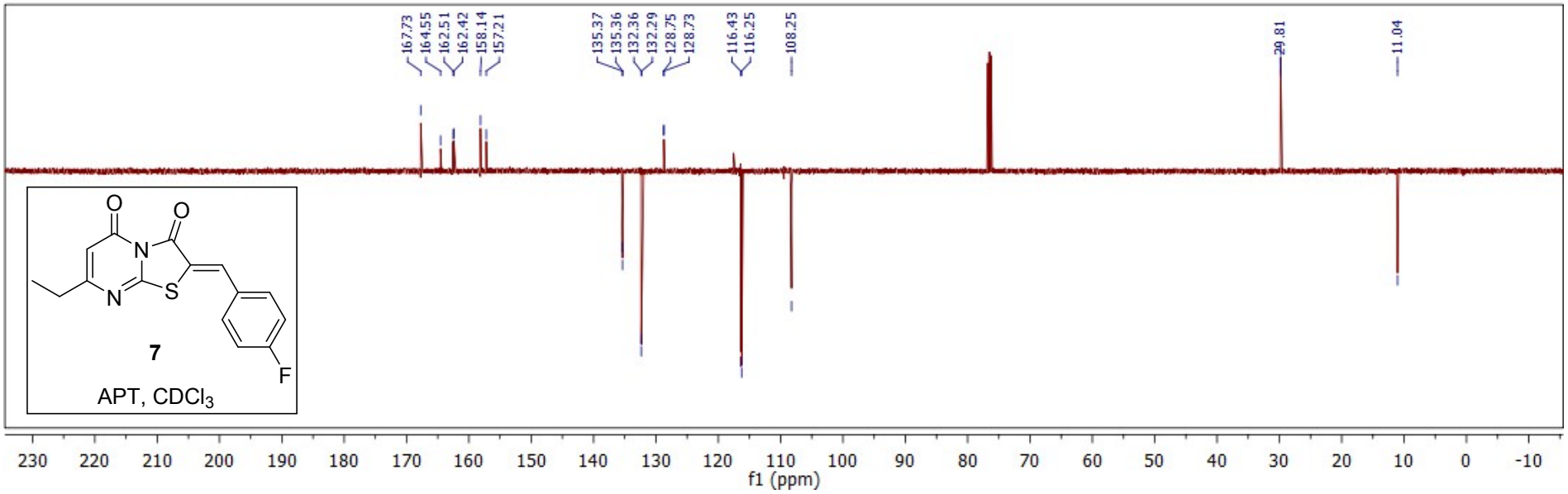

5
^{1}H NMR, $(CD_3)_2CO$
6.02
4.02
2.53
2.52
2.50
2.49
1.22
1.20
1.19
0.75
2.43
2.28
3.00
f1 (ppm)

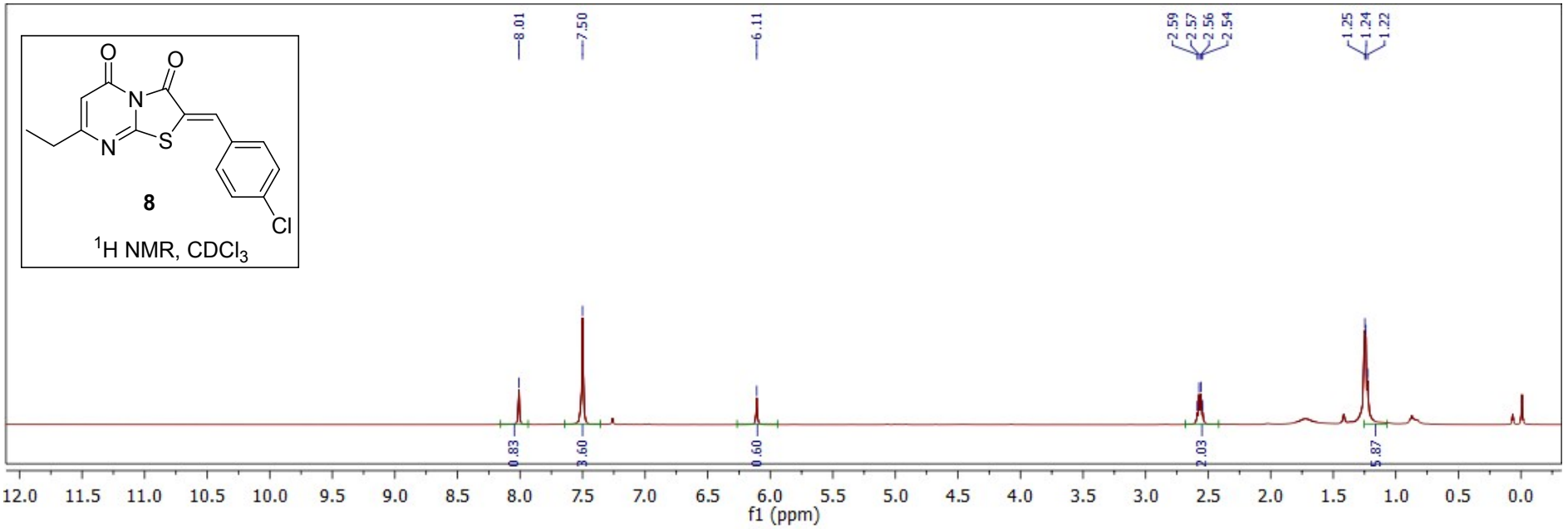

5
^{13}C NMR, $(CD_3)_2CO$
169.23
169.04
163.63
147.27
105.70
32.44
29.99
11.48
f1 (ppm)

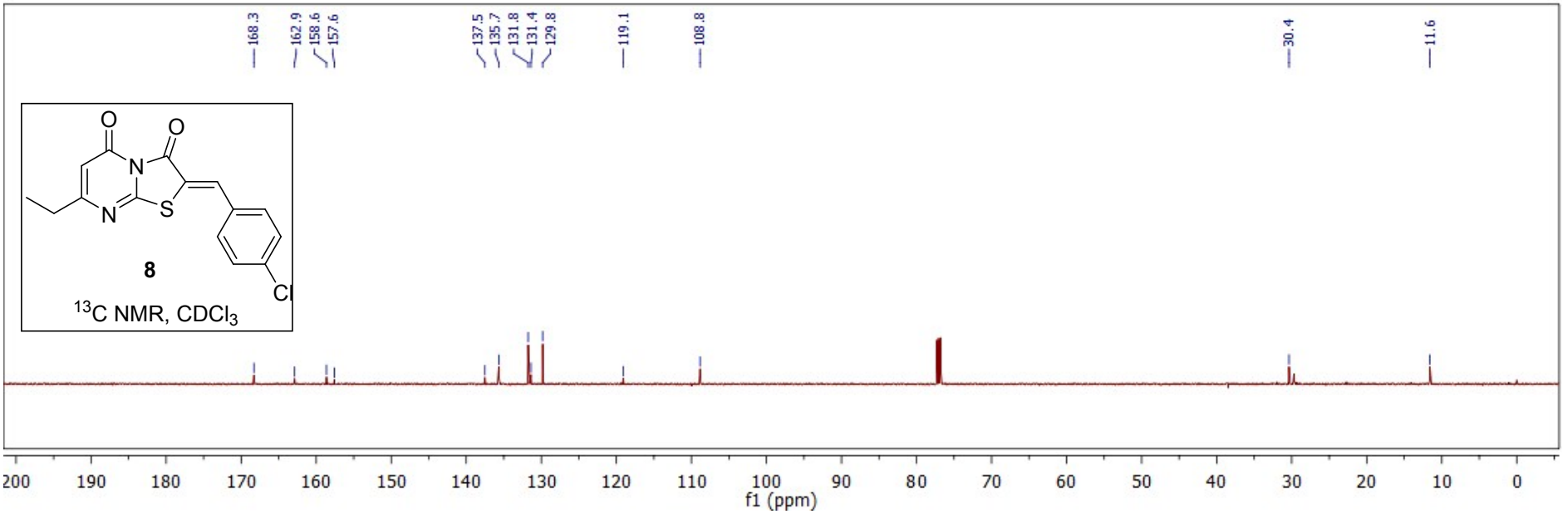

6
^{1}H NMR, $CDCl_3$
8.07
7.59
7.57
7.54
7.52
7.51
7.50
7.50
6.10
2.59
2.57
2.56
2.54
1.26
1.24
1.23
1.00
2.28
3.37
0.86
2.42
3.51
f1 (ppm)

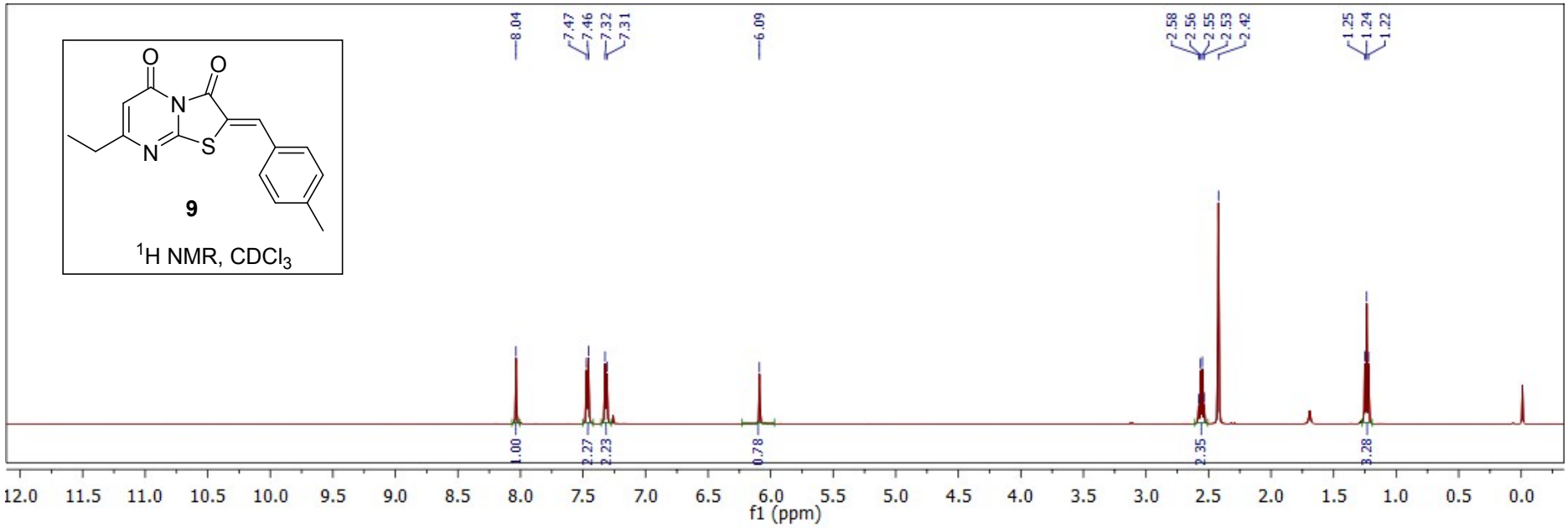

6
^{13}C NMR, $CDCl_3$
168.37
163.15
158.85
158.16
137.37
133.05
131.41
130.83
129.57
118.54
108.85
30.47
11.71
f1 (ppm)

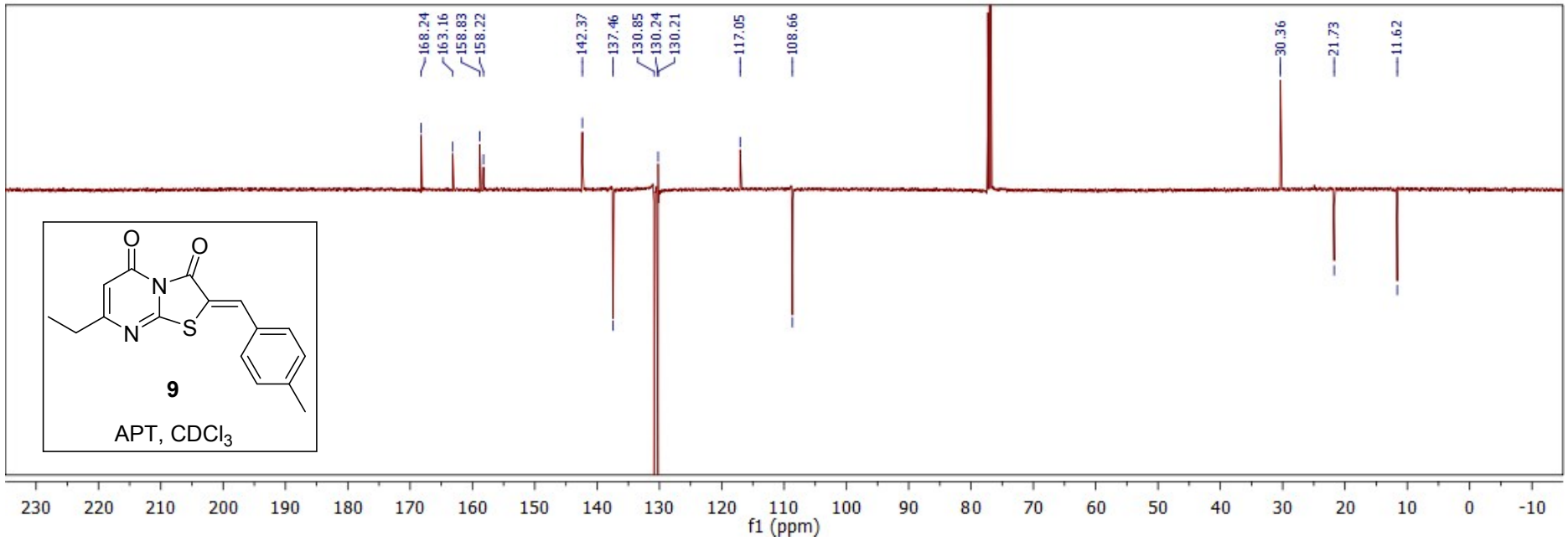

7
^{1}H NMR, $CDCl_3$
8.02
7.59
7.58
7.57
7.56
7.23
7.21
7.20
2.58
2.57
2.55
2.54
1.25
1.23
1.22
1.00
2.19
2.04
0.84
2.25
3.39
f1 (ppm)

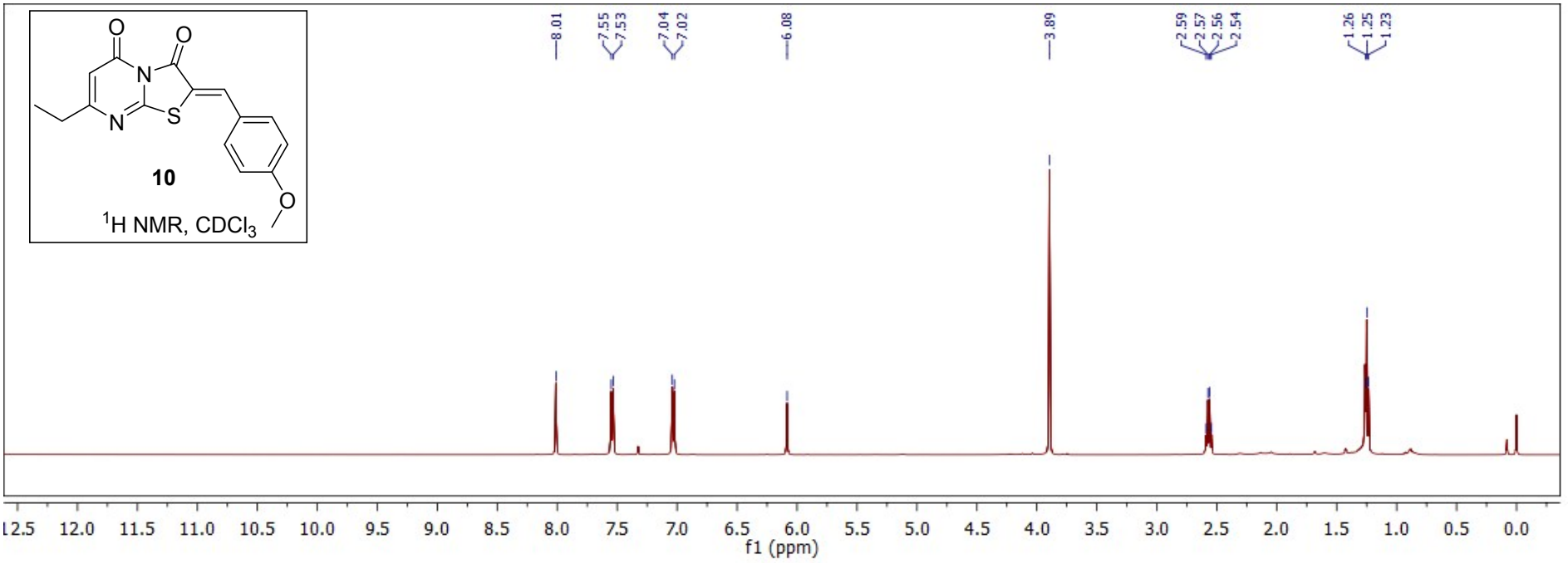

7
APT, $CDCl_3$
167.73
164.55
162.51
162.42
158.14
157.21
135.37
135.36
132.36
132.29
128.75
128.73
116.43
116.25
108.25
29.81
11.04
f1 (ppm)

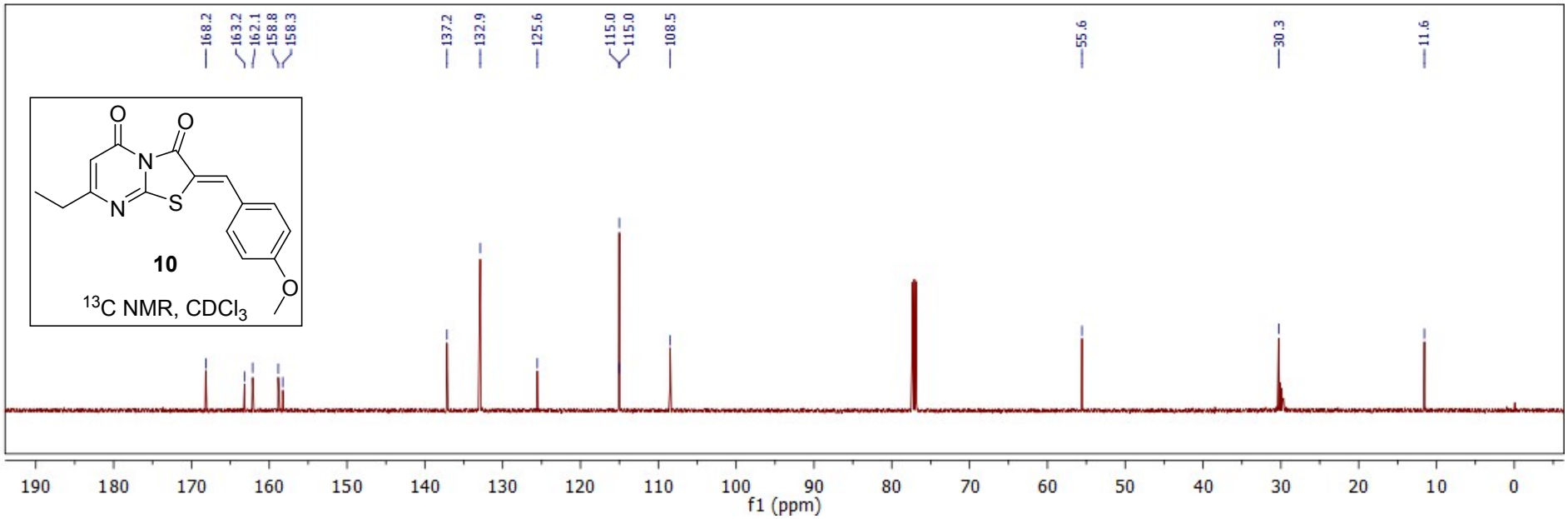

8.01
7.50
6.11
2.59
2.57
2.56
2.54
1.25
1.24
1.22
0.83
3.60
0.60
2.03
5.87
8
^{1}H NMR, $CDCl_3$
f1 (ppm)

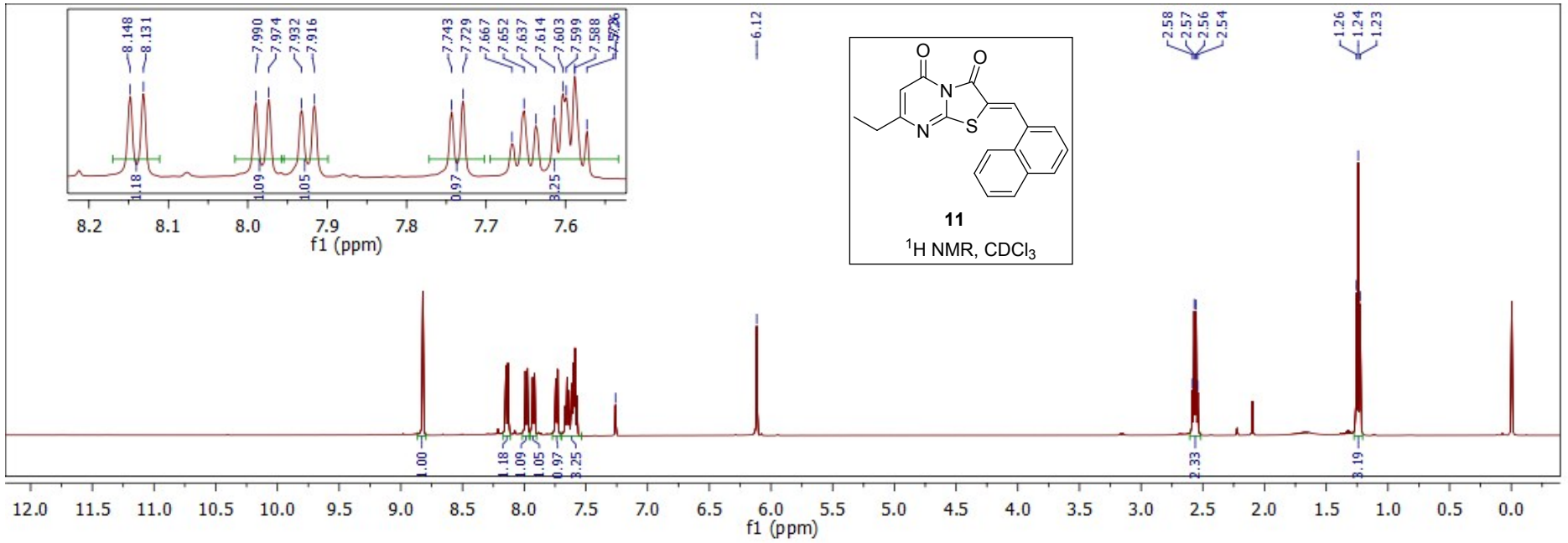

168.3
162.9
158.6
157.6
137.5
135.7
131.8
131.4
129.8
119.1
108.8
30.4
11.6
8
^{13}C NMR, $CDCl_3$
f1 (ppm)

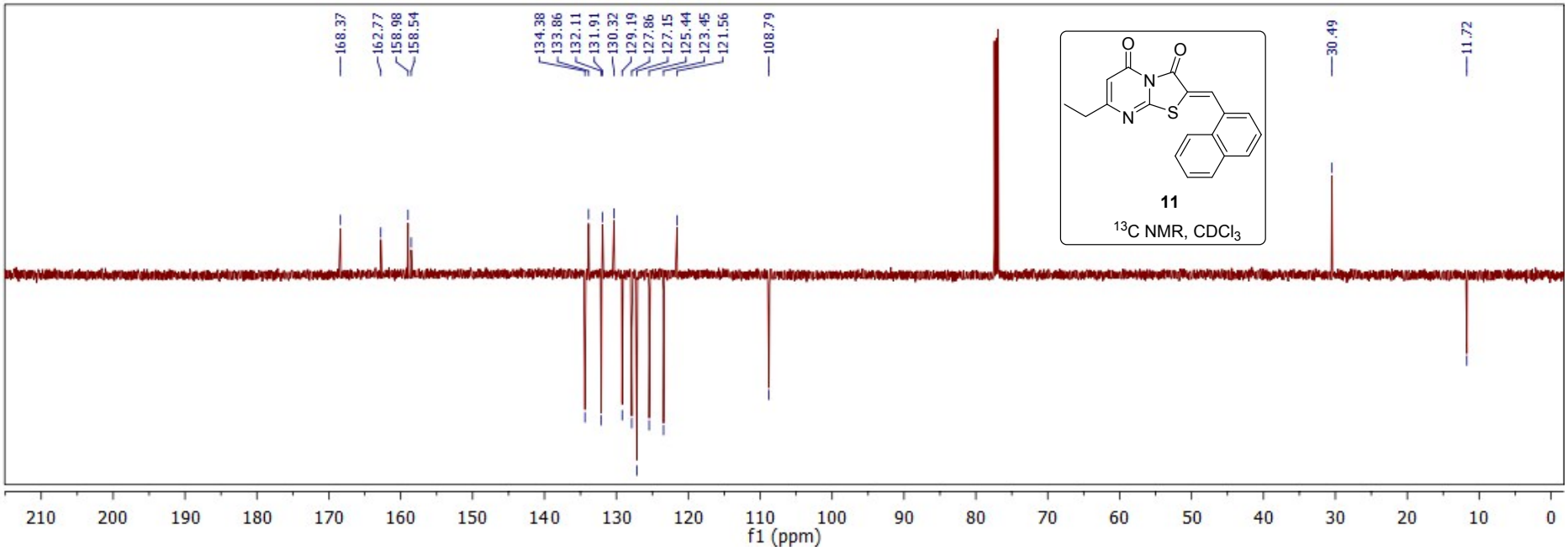

9
^{1}H NMR, $CDCl_3$
8.04
7.47
7.46
7.32
7.31
6.09
2.58
2.56
2.55
2.53
2.42
1.25
1.24
1.22
1.00
2.27
2.23
0.78
2.35
3.28
f1 (ppm)

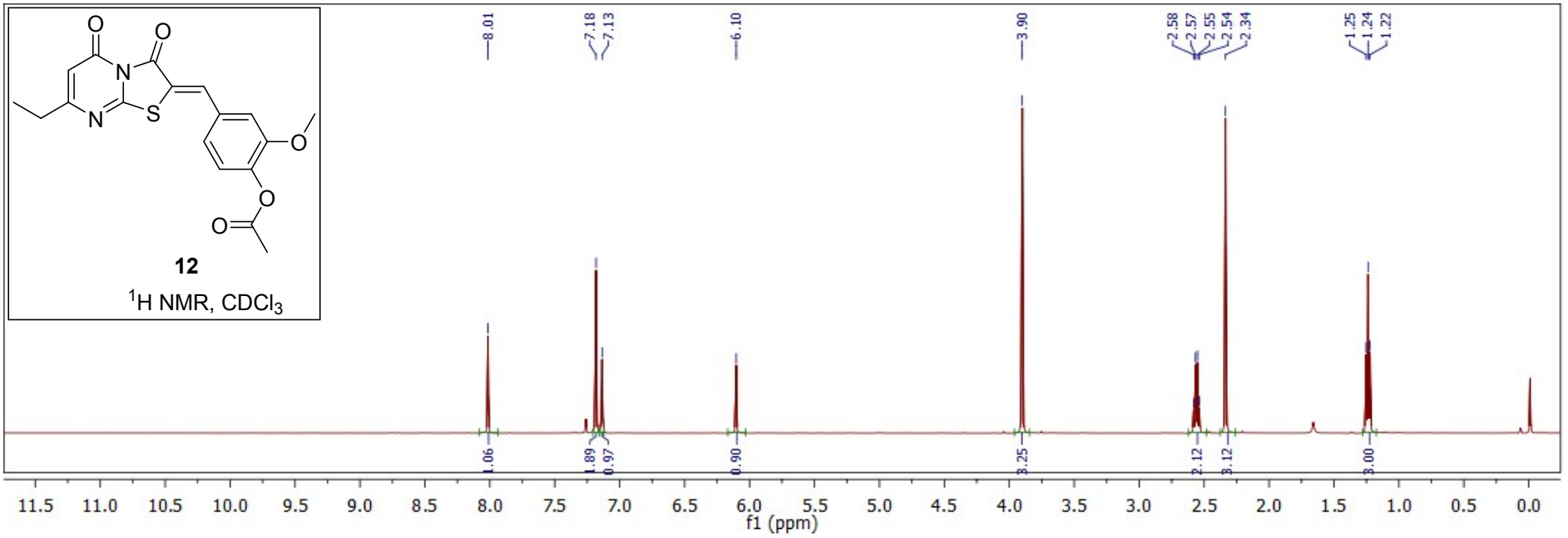

9
APT, $CDCl_3$
168.24
163.16
158.83
158.22
142.37
137.46
130.85
130.24
130.21
117.05
108.66
30.36
21.73
11.62
f1 (ppm)

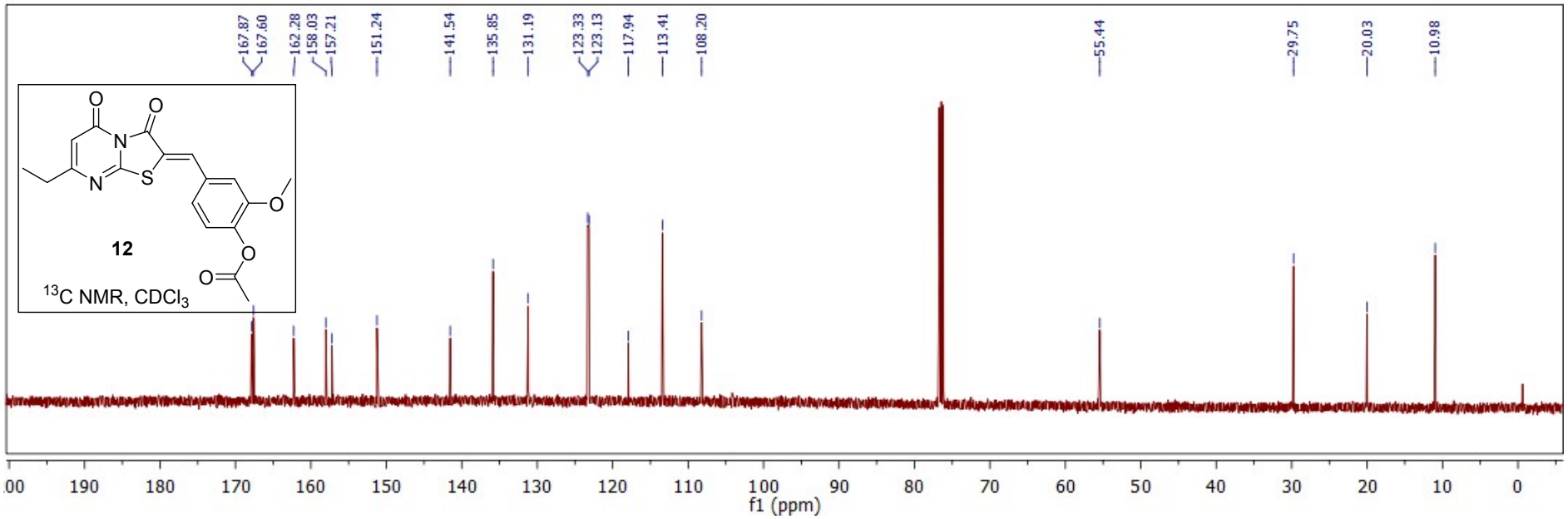

10
^{1}H NMR, CDCl$_3$
8.01
7.55
7.53
7.04
7.02
6.08
3.89
2.59
2.57
2.56
2.54
1.26
1.25
1.23
f1 (ppm)

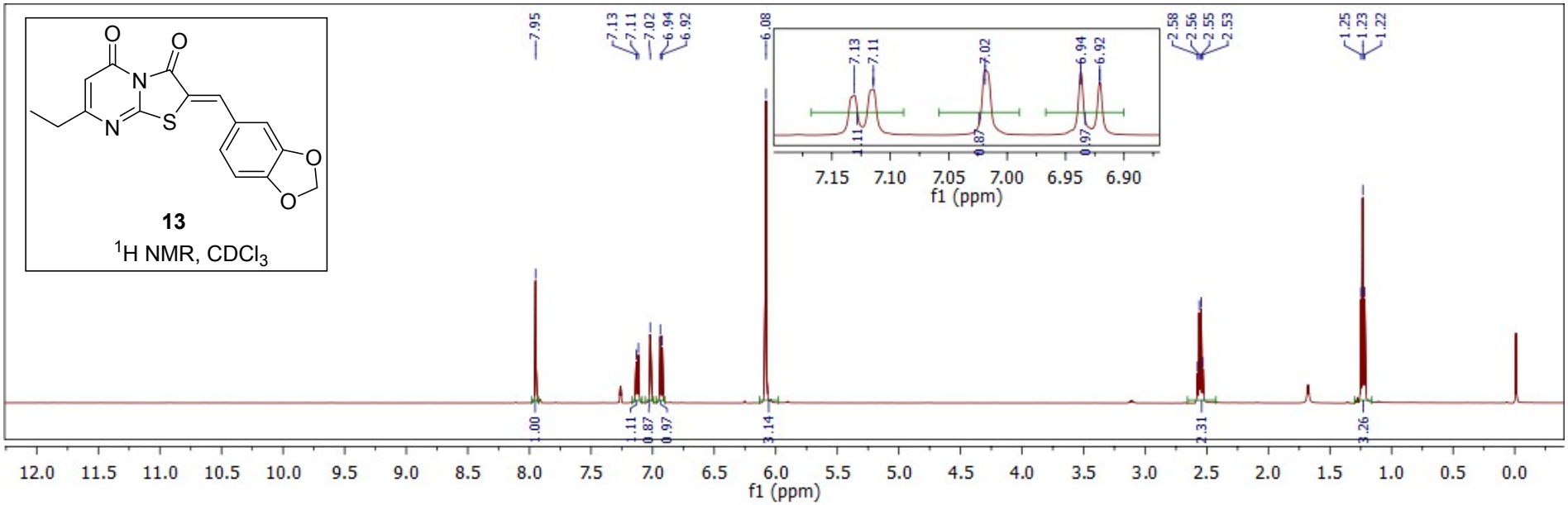

10
^{13}C NMR, CDCl$_3$
168.2
163.2
162.1
158.8
158.3
137.2
132.9
125.6
115.0
115.0
108.5
55.6
30.3
11.6
f1 (ppm)

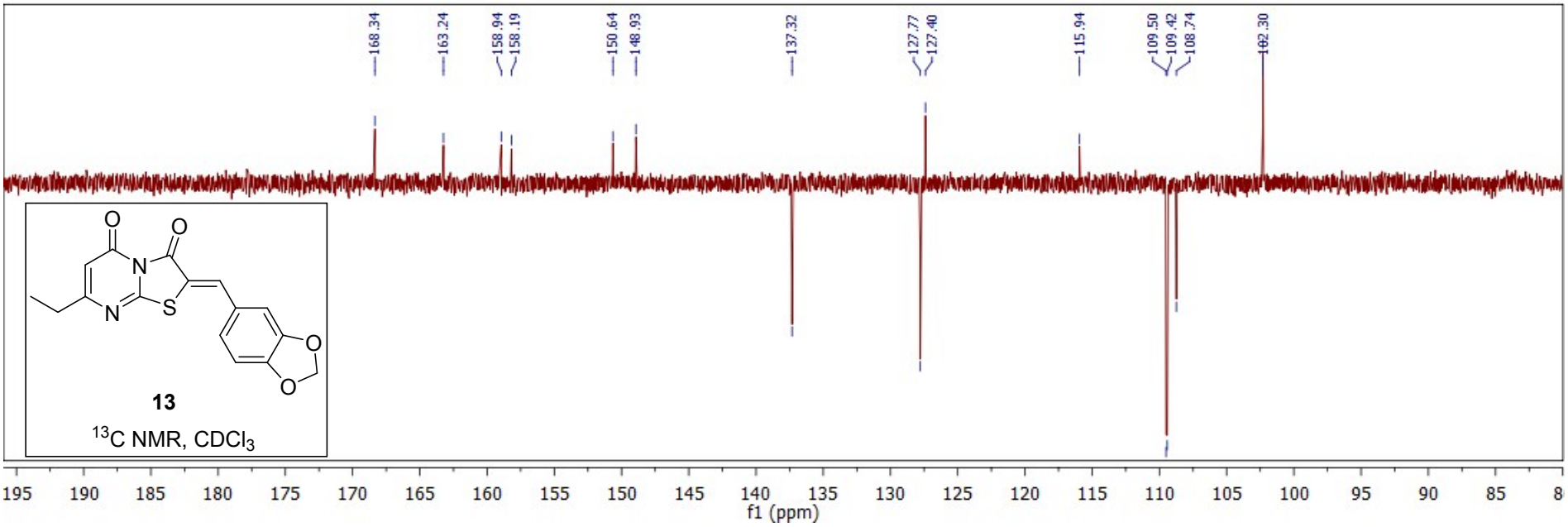

11
^{1}H NMR, CDCl$_3$
f1 (ppm)

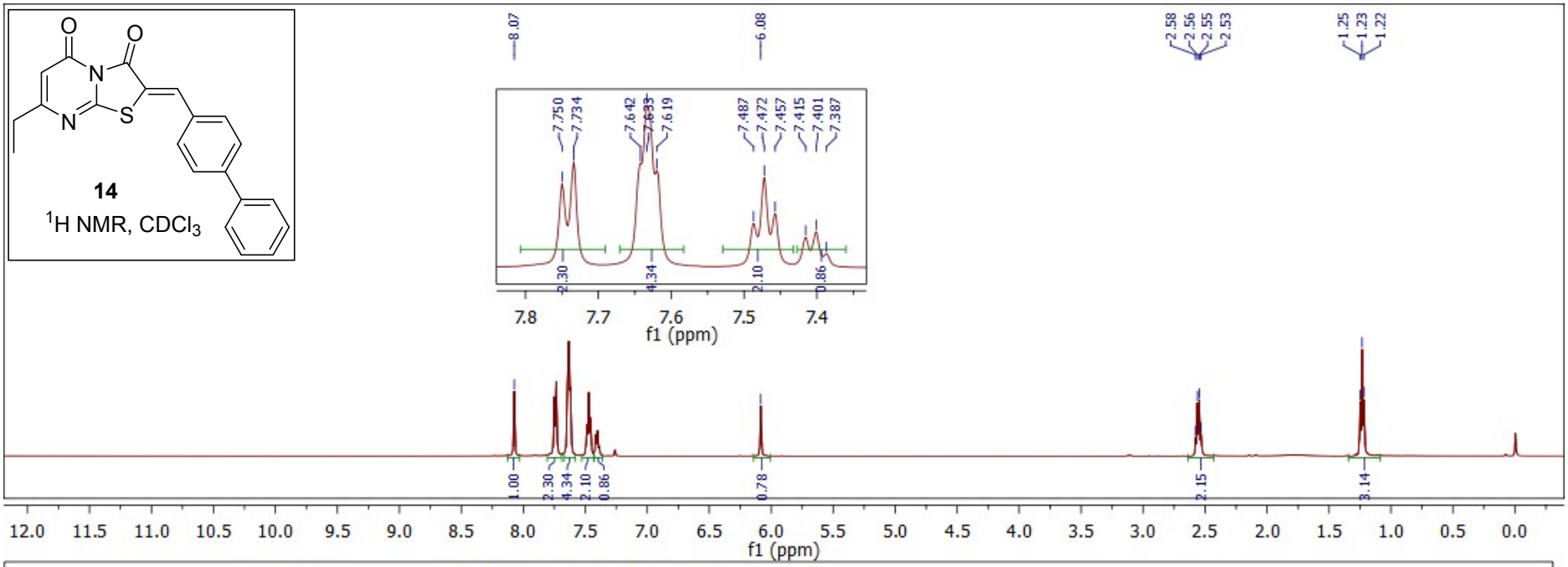

11
^{13}C NMR, CDCl$_3$
f1 (ppm)

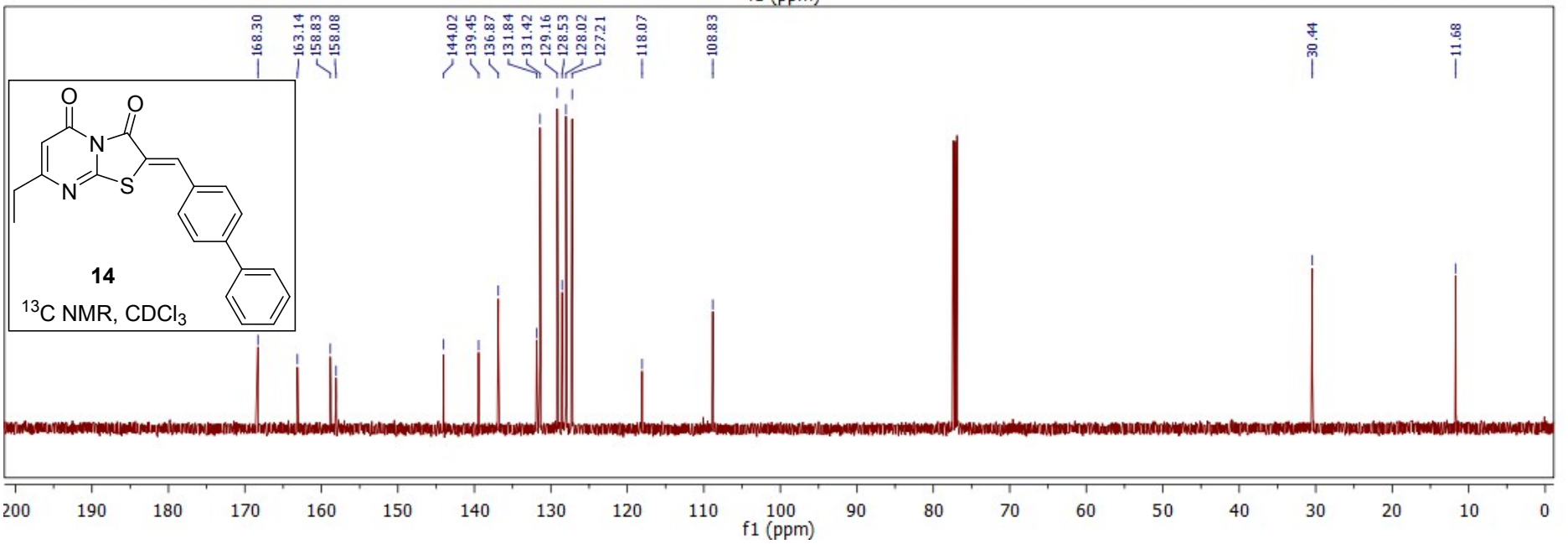

12
^{1}H NMR, $CDCl_3$
8.01
7.18
7.13
6.10
3.90
2.58
2.57
2.55
2.54
2.34
1.25
1.24
1.22
1.06
1.89
0.97
0.90
3.25
2.12
3.12
3.00
f1 (ppm)

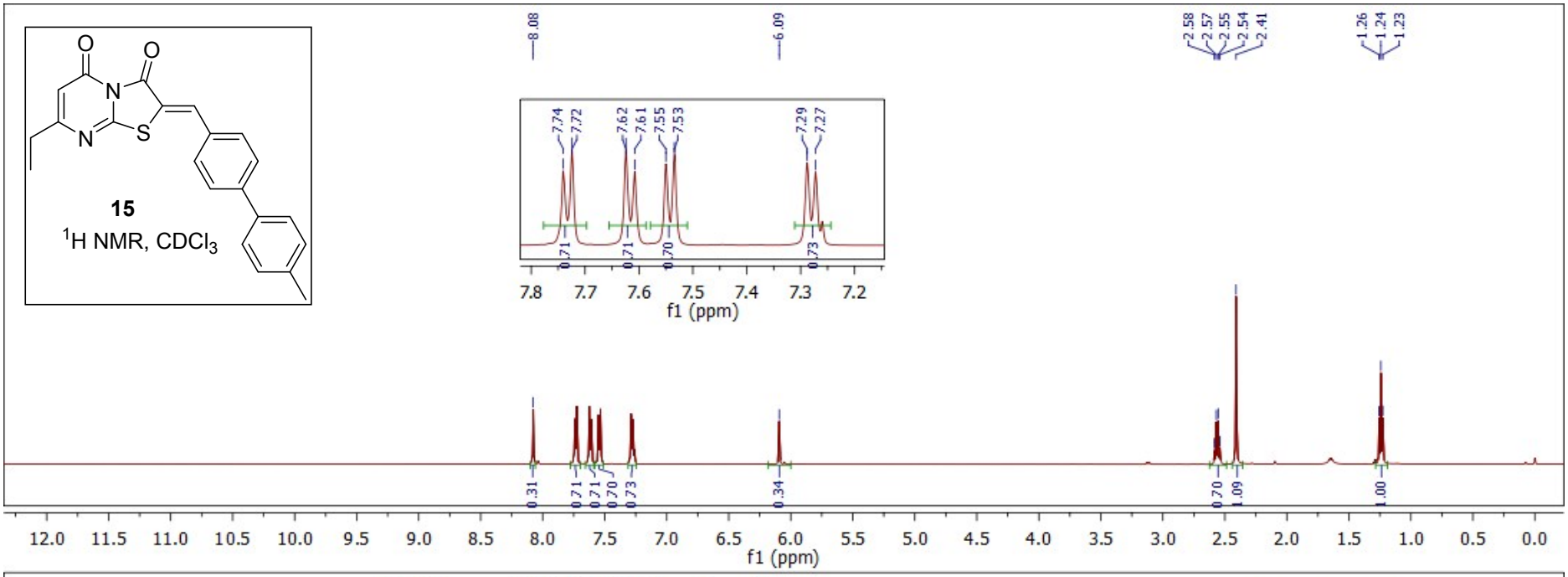

12
^{13}C NMR, $CDCl_3$
167.87
167.60
162.28
158.03
157.21
151.24
141.54
135.85
131.19
123.33
123.13
117.94
113.41
108.20
55.44
29.75
20.03
10.98
f1 (ppm)

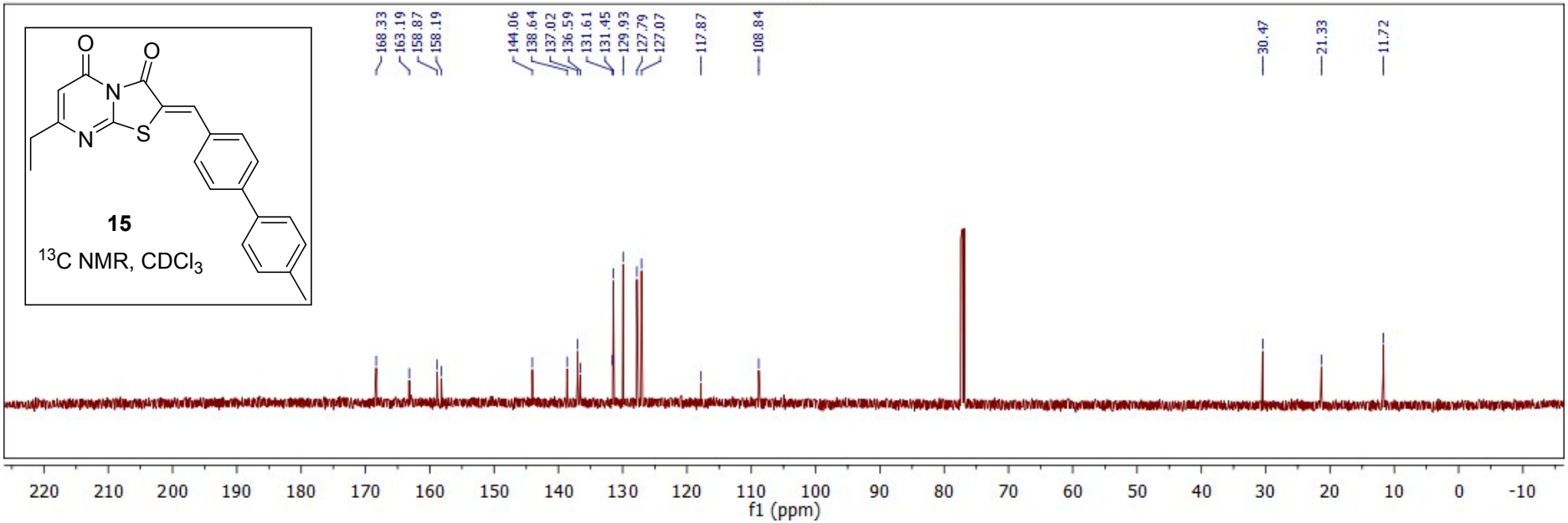

13
1H NMR, CDCl3
f1 (ppm)

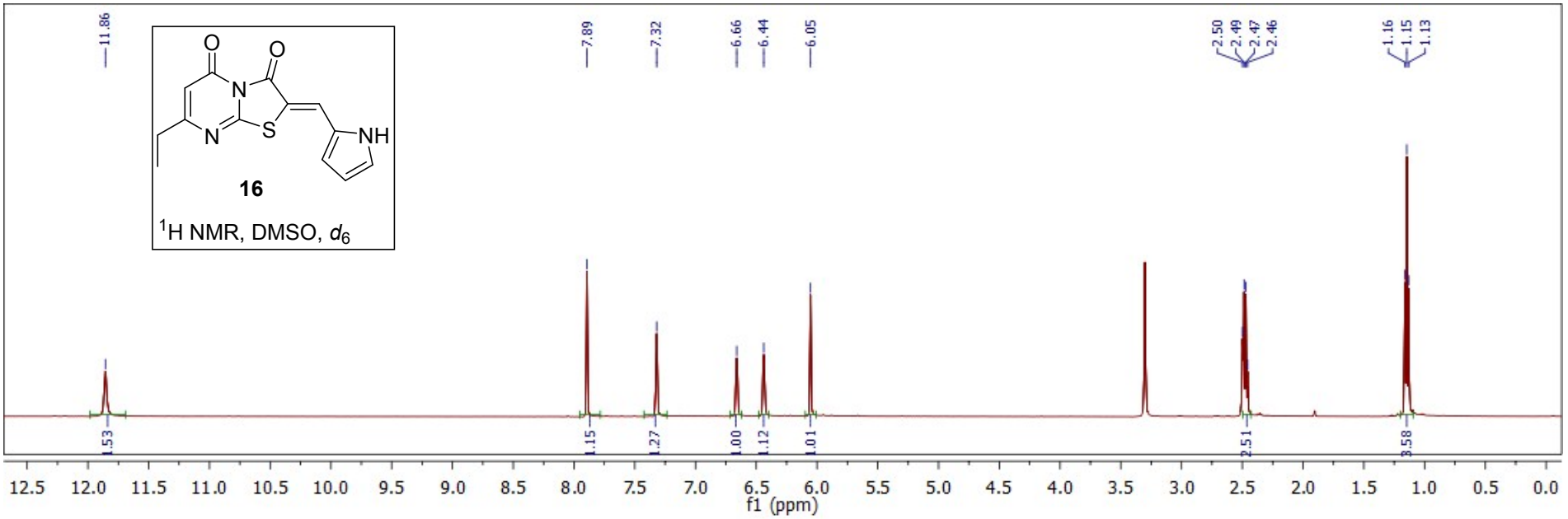

13
13C NMR, CDCl3
f1 (ppm)

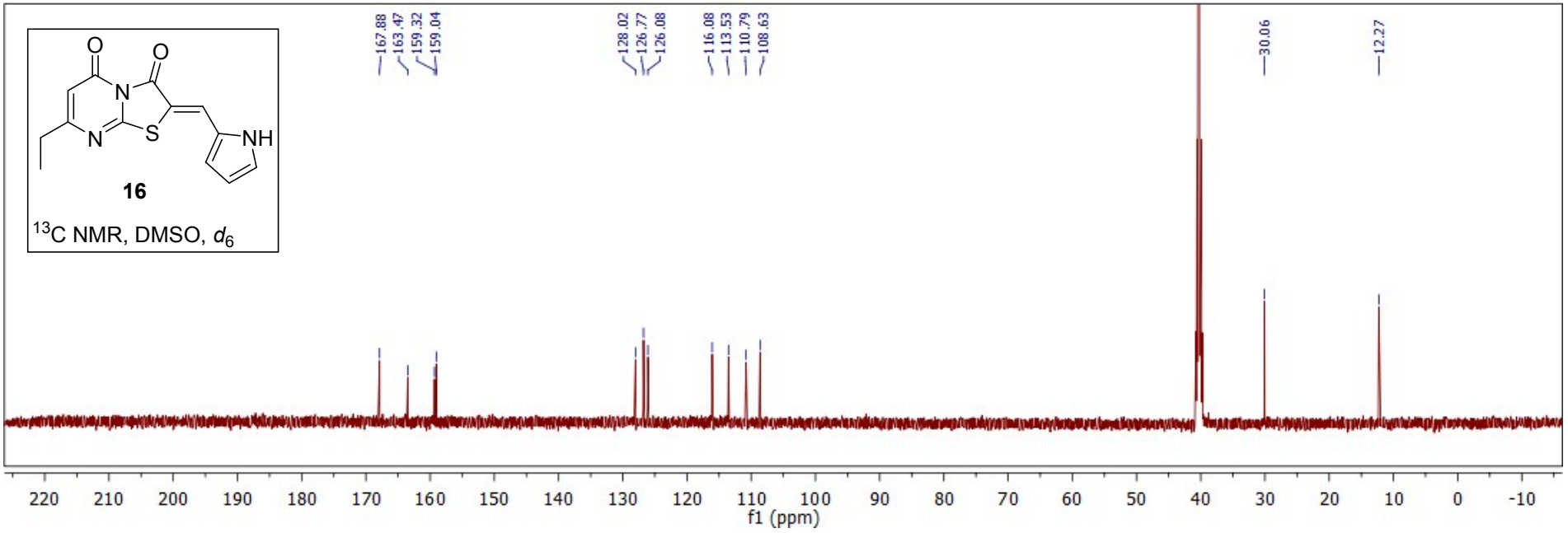

14
1H NMR, CDCl3
8.07
6.08
2.58
2.56
2.55
2.53
1.25
1.23
1.22
7.750
7.734
7.642
7.633
7.619
7.487
7.472
7.457
7.415
7.401
7.387
2.30
4.34
2.10
0.86
7.8
7.7
7.6
7.5
7.4
f1 (ppm)
1.00
2.30
4.34
2.10
0.86
0.78
2.15
3.14
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

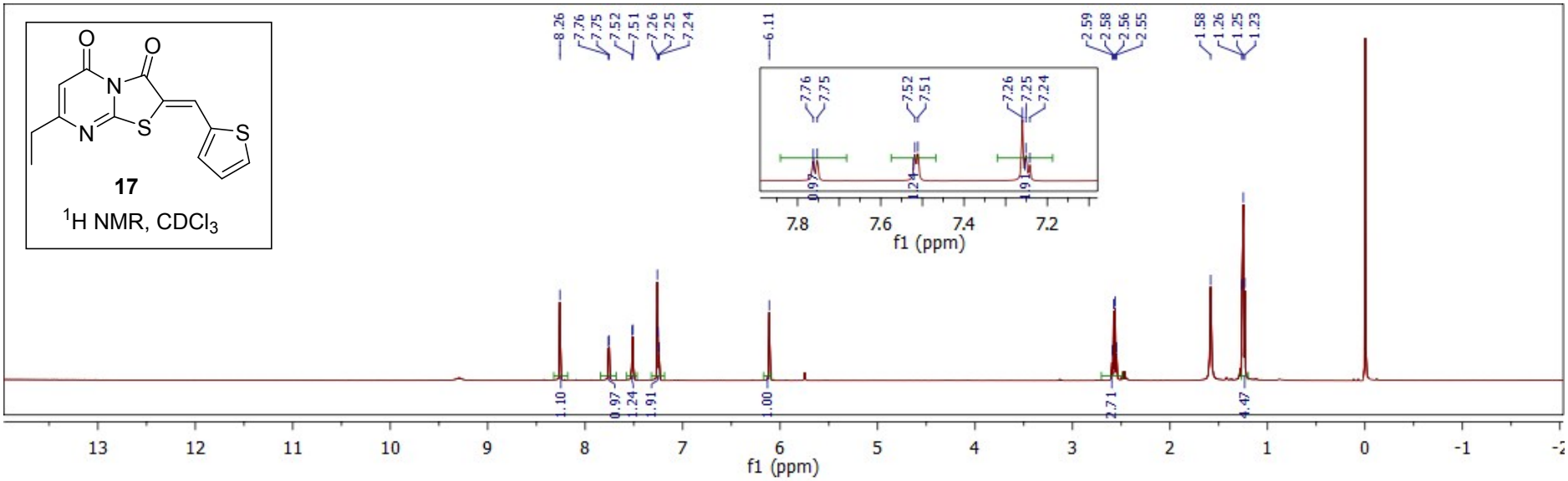

14
13C NMR, CDCl3
168.30
163.14
158.83
158.08
144.02
139.45
136.87
131.84
131.42
129.16
128.53
128.02
127.21
118.07
108.83
30.44
11.68
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

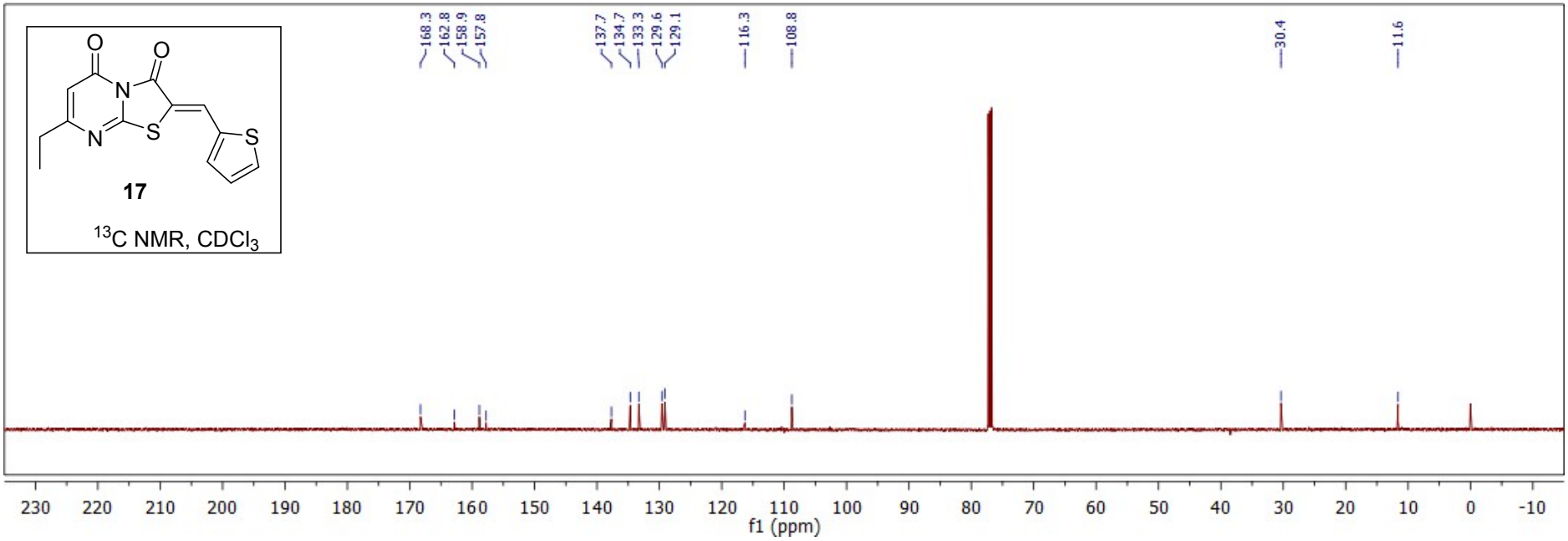

15
1H NMR, CDCl3
8.08
6.09
2.58
2.57
2.55
2.54
2.41
1.26
1.24
1.23
7.74
7.72
7.62
7.61
7.55
7.53
7.29
7.27
0.71
0.71
0.70
0.73
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
0.31
0.71
0.71
0.70
0.73
0.34
0.70
1.09
1.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

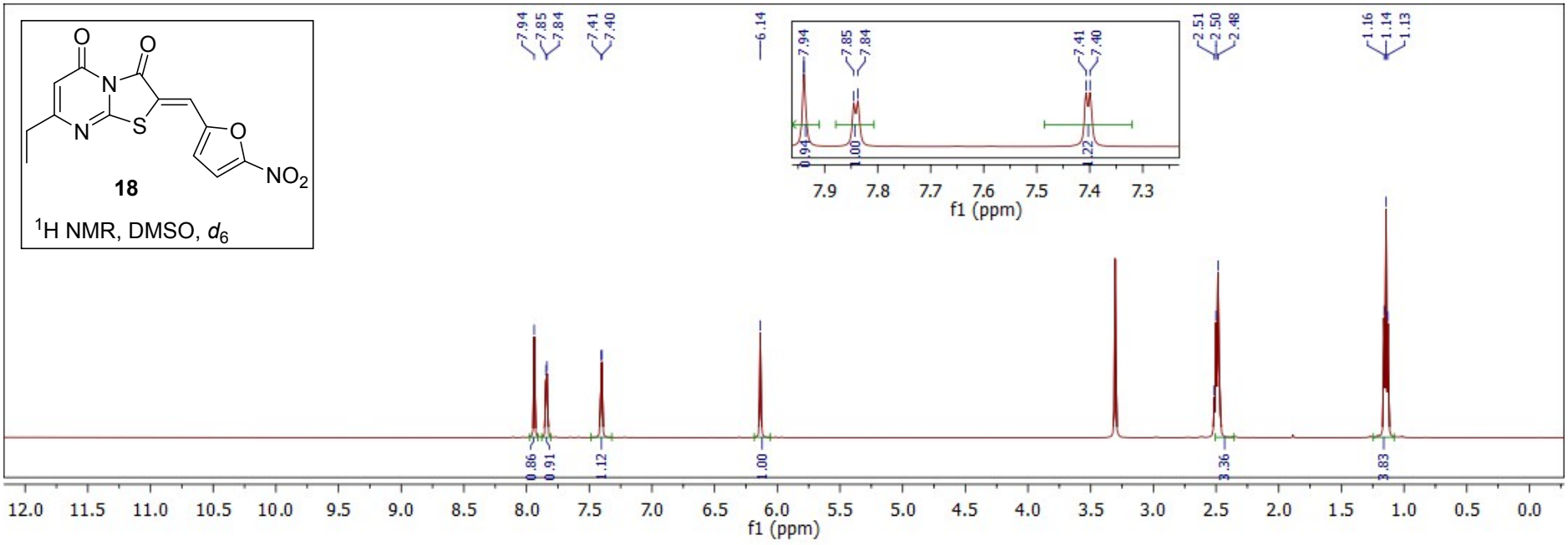

15
13C NMR, CDCl3
168.33
163.19
158.87
158.19
144.06
138.64
137.02
136.59
131.61
131.45
129.93
127.79
127.07
117.87
108.84
30.47
21.33
11.72
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

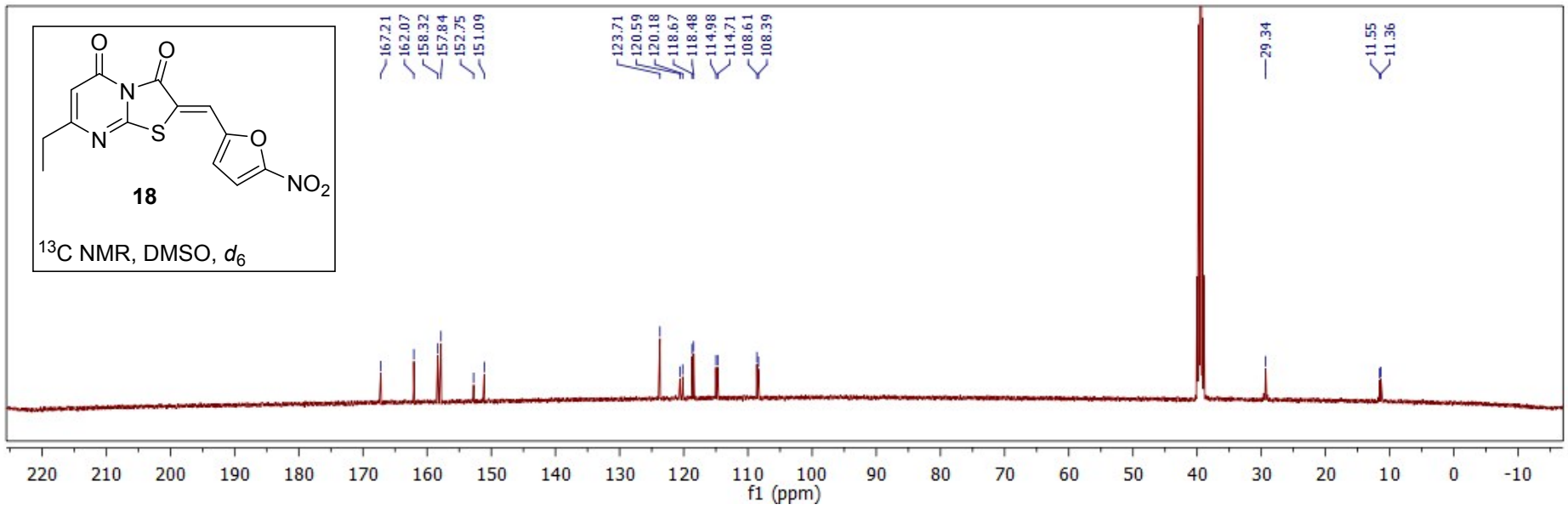

11.86
7.89
7.32
6.66
6.44
6.05
2.50
2.49
2.47
2.46
1.16
1.15
1.13
1.53
1.15
1.27
1.00
1.12
1.01
2.51
3.58
16
1H NMR, DMSO, d6
f1 (ppm)

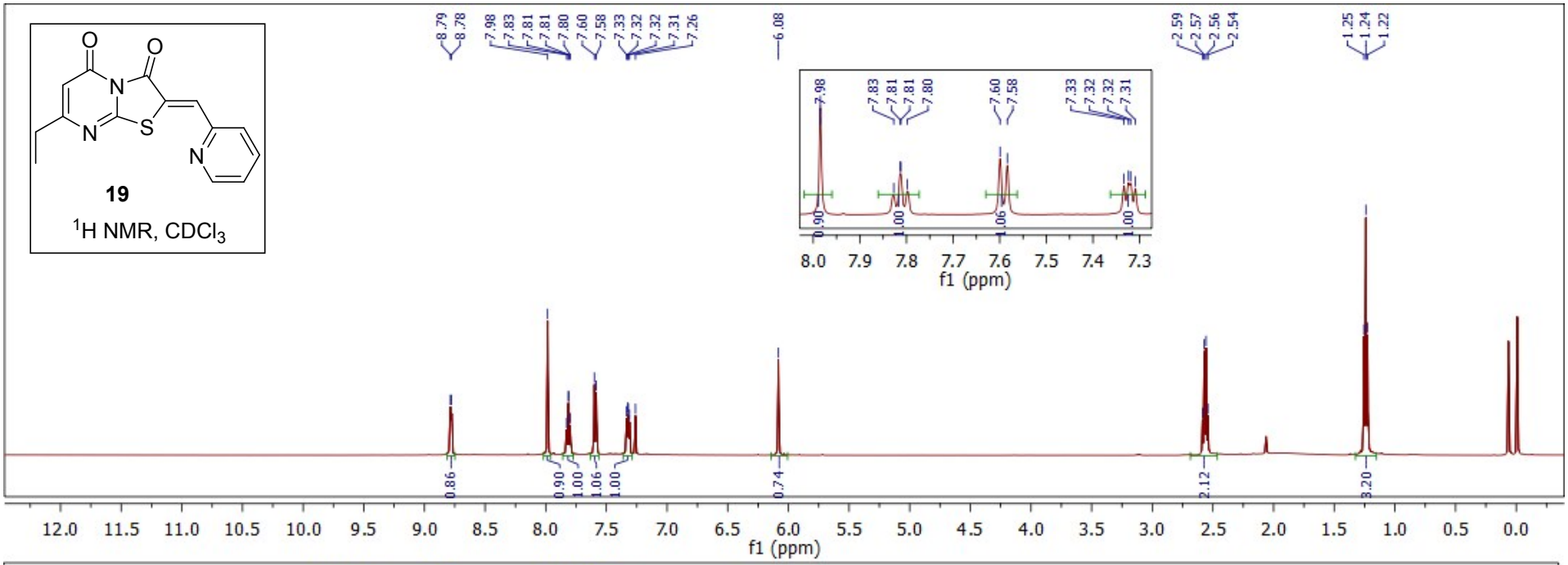

167.88
163.47
159.32
159.04
128.02
126.77
126.08
116.08
113.53
110.79
108.63
30.06
12.27
16
13C NMR, DMSO, d6
f1 (ppm)

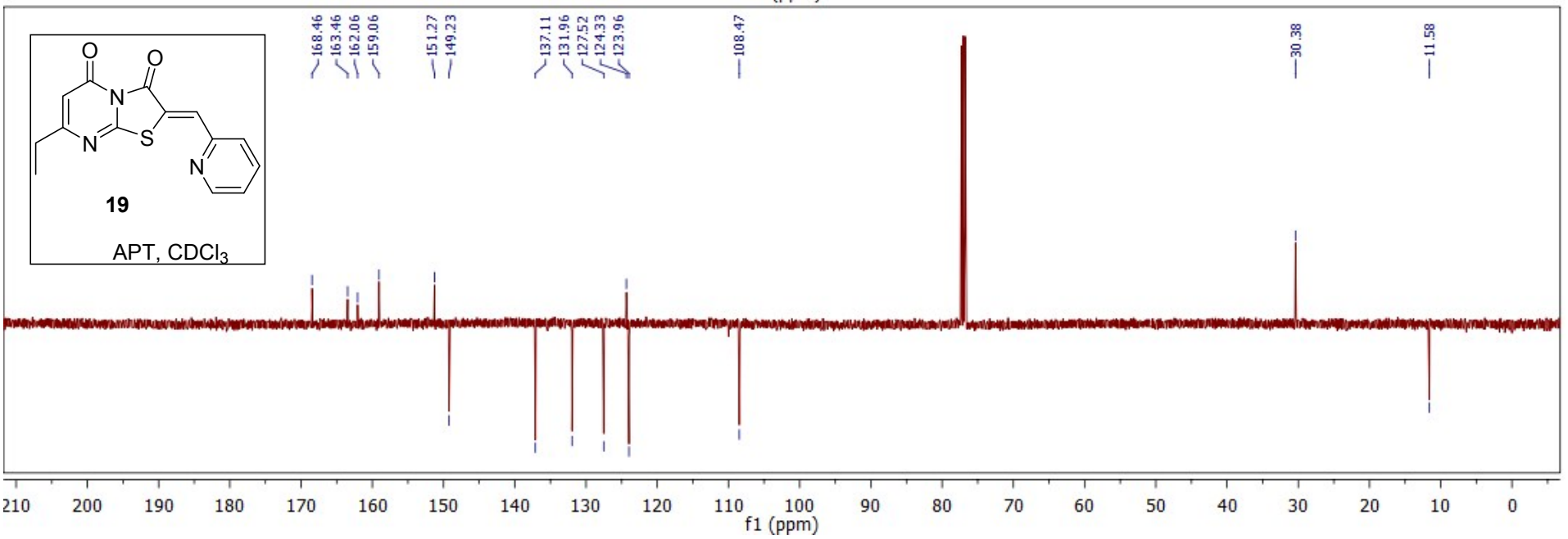

17
1H NMR, CDCl3
8.26
7.76
7.75
7.52
7.51
7.26
7.25
7.24
6.11
2.59
2.58
2.56
2.55
1.58
1.26
1.25
1.23
1.10
0.97
1.24
1.91
1.00
2.71
4.47
f1 (ppm)

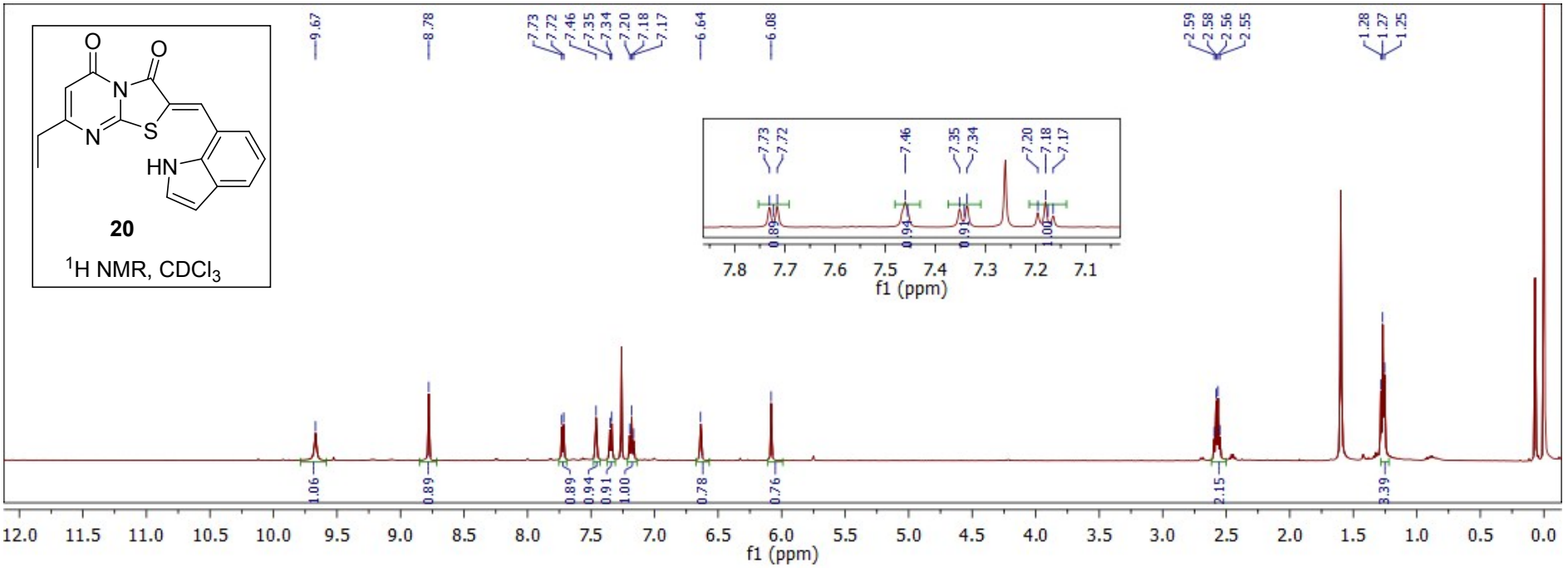

17
13C NMR, CDCl3
168.3
162.8
158.9
157.8
137.7
134.7
133.3
129.6
129.1
116.3
108.8
30.4
11.6
f1 (ppm)

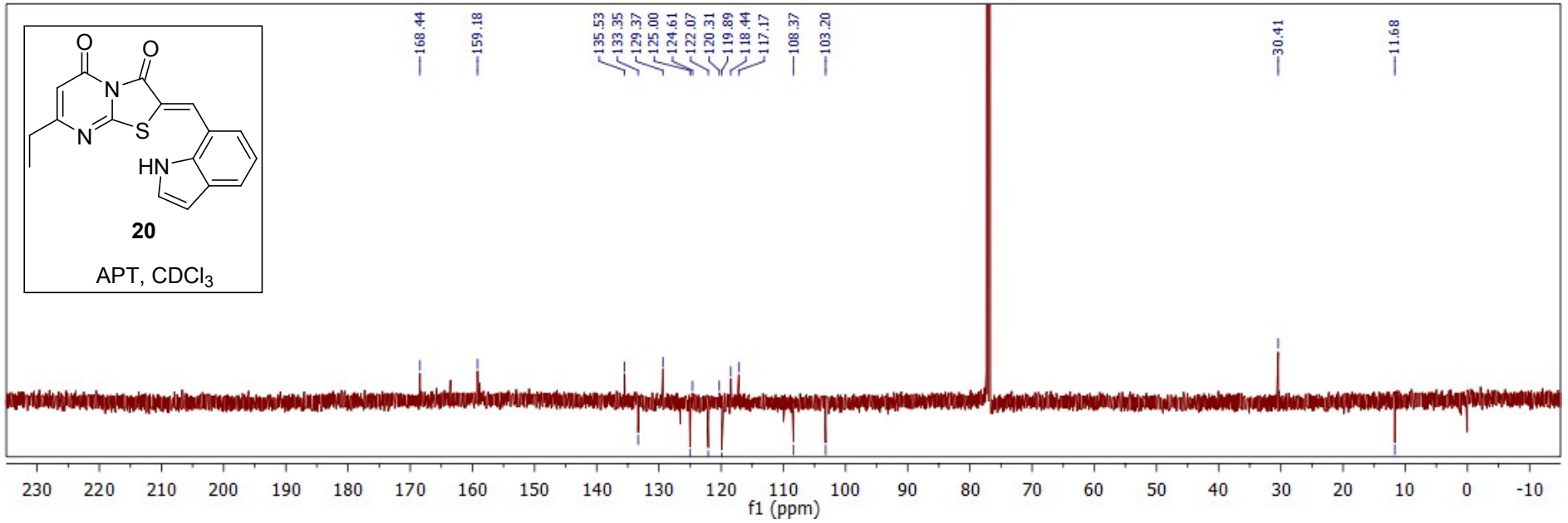

18
^{1}H NMR, DMSO, d_6
f1 (ppm)


18
^{13}C NMR, DMSO, d_6
f1 (ppm)


19
^{1}H NMR, $CDCl_3$
f1 (ppm)


19
APT, $CDCl_3$
f1 (ppm)


20
^{1}H NMR, $CDCl_3$
f1 (ppm)


20
APT, $CDCl_3$
f1 (ppm)

References:

1- CrysAlis PRO, Agilent Technologies (2013), Version 1.171.37.21t, Yarnton, Oxfordshire, England.

2- A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori and M. Camalli, *J. Appl. Crystallogr*., 1994, **27**, 435.

3- P. W. Betteridge, J. R. Carruthers, R. I. Cooper, K. Prout and D. J. Watkin. *J. Appl. Crystallogr*. 2003, **36**, 1487.