SUPPLEMENTARY INFORMATION

3-Hydroxypyrimidine-2,4-dione derivatives as monkeypox virus resolvase (Mpr) inhibitors

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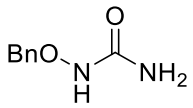
General Procedure

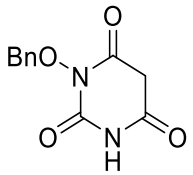
All commercial chemicals were used as supplied unless otherwise indicated. Dry solvents (THF, Et_2O, CH_2Cl_2 and DMF) were dispensed under argon from an anhydrous solvent system with two packed columns of neutral alumina or molecular sieves. Anhydrous ethanol was purchased from Sigma-Aldrich. Flash chromatography was performed on a Teledyne Combiflash RF-200 with RediSep columns (silica) with indicated mobile phase. All moisture sensitive reactions were performed under an inert atmosphere of ultra-pure argon with oven-dried glassware. A Bruker 400 MHz NMR was used to obtain the NMR spectra for compound characterization with MNova software. HRMS data were acquired using an Agilent 6230 TOF LC/MS spectrometer equipped with ESI and APCI ion modules. Glass backed plates for thin-layer chromatography (TLC) analysis were used (silica gel, Sigma, St. Louis, MO) with fluorescent indicator (254 nm). Compounds on the TLC plates were visualized with a UV lamp (254 nm). Compound purity analysis was performed using Agilent 1260 Infinity HPLC with an Eclipse C18 column (3.5 μm, 4.6 × 100 mm). HPLC conditions: flow rate, 1.0 mL/min; solvent A, 0.1% formic acid in water; solvent B, 0.1% formic acid in acetonitrile; gradient (B, %): 0–6 min (5–100), 6–8 min (100), 8–9 min (100–5). Purity was determined by total absorbance at 254 nm. The representative compounds were selected from this series for purity screening, and it has shown a purity of more than 95%.

1-(Benzyloxy)urea (6)



To a solution of N-hydroxyurea (65.7 mmol, 1 eq) in methanol (90 mL) were added potassium hydroxide (78.9 mmol, 1.2 eq) and benzyl chloride (65.7 mmol, 1 eq). The reaction mixture was refluxed for 6 h. The solvent was removed under reduced pressure and the solid formed was washed with water and adjusted their pH to neutral. The solid was filtered and undergo recrystallization with acetone and methylene chloride to afford a desired pure compound **6** as a white needle in 98% yield. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 7.47–7.27 (m, 5H), 6.35 (s, 2H), 4.73 (s, 2H); HRMS (APCI-) calcd. for $C_8H_{10}N_2O_2$ $[M-H]^-$ 165.0664, found 165.0678 (E = 8.5 ppm).

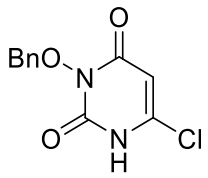
3-(Benzyloxy)-6-hydroxypyrimidine-2,4(1H,3H)-dione (7)



To a 200 mL round bottom flask were added freshly prepared sodium ethoxide (6.0 mmol, 1 eq) in anhydrous ethanol, 1-(benzyloxy)urea **6** (6.0 mmol, 1 eq) and diethyl malonate (6.0 mmol, 1 eq). The reaction mixture was stirred at 150 °C for 36 h under reflux conditions. The solvent was removed under reduced pressure. The residue was dissolved in water, then filtered to remove any precipitate. The water residue was acidified to pH 4–5 with 1N aqueous HCl to crush out the compound **7** as a light yellow solid in 95% yield. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H),

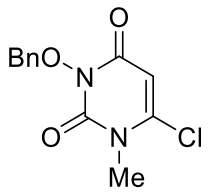
7.47–7.29 (m, 5H), 6.33 (s, 2H), 4.72 (s, 2H); HRMS (ESI-) calcd. for $C_{11}H_{10}N_2O_4$ $[M-H]^-$ 233.0562, found 233.0577 (E = 6.4 ppm).

3-(Benzyloxy)-6-chloropyrimidine-2,4(1H,3H)-dione (8)



To a solution of 3-(benzyloxy)-6-hydroxypyrimidine-2,4(1H,3H)-dione **7** (8.5 mmol, 1.0 eq) in 40 mL of phosphorus oxychloride ($POCl_3$) was added benzyl triethylammonium chloride ($BnEt_3NCl$) (17.07 mmol, 1.0 eq), and the reaction mixture was stirred at 50 °C for 36 h under reflux condition for 6 h. The reaction mixture was set to cool down to room temperature and then pour gradually onto ice. The precipitate was filtered to give compound 3-(benzyloxy)-6-chloropyrimidine-2,4(1H,3H)-dione **8** as a light yellow solid in 55% yield. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 7.47–7.29 (m, 5H), 6.33 (s, 2H), 4.72 (s, 2H); HRMS (APCI-) calcd. for $C_{11}H_9ClN_2O_3$ $[M-H]^-$ 251.0223, found 251.0251 (E = 11.2 ppm).

3-(Benzyloxy)-6-chloro-1-methylpyrimidine-2,4(1H,3H)-dione (9)



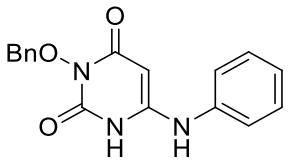
To a microwave reaction vessel were added 3-(benzyloxy)-6-chloropyrimidine-2,4(1H,3H)-dione **8** (2.0 mmol, 1.0 eq) in DMF (5 mL), followed by CS_2CO_3 (4.0 mmol, 2.0 eq) and iodomethane (4.0 mmol, 2.0 eq). The mixture was stirred at 80 °C for 3 h and the reaction was monitored by

both TLC and MS-TOF. The reaction mixture was removed in vacuum and quenched with excess of H_2O. The precipitation was filtered to provide desired compounds **9** as solid in 96%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.60–7.48 (m, 2H), 7.47–7.35 (m, 3H), 6.19 (s, 1H), 5.02 (s, 2H), 3.47 (s, 3H); HRMS (APCI+) calcd. for $C_{12}H_{11}ClN_2O_3$ $[M+H]^+$ 267.0536, found 267.0516 (E = 7.5 ppm).

General Procedure for 6-amination.

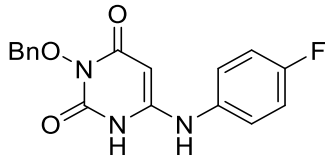
To a microwave reaction vessel were added 3-(benzyloxy)-6-chloropyrimidine-2,4(1H,3H)-dione **8** (1.2 mmol, 1.0 eq), amines (1.8 mmol, 1.5 eq) and *N*,*N*-dimethylaniline (3.6 mmol, 3.0 eq). The reaction vessel was either irradiated using microwave or heating block at 170 °C for appropriate time. The reaction was monitored by both TLC and MS-TOF. The reaction mixture was quenched with MeOH, and the precipitate was filtered to provide desired compounds **10** as solid in 27–74%.

3-(Benzyloxy)-6-(phenylamino)pyrimidine-2,4(1H,3H)-dione (10a)



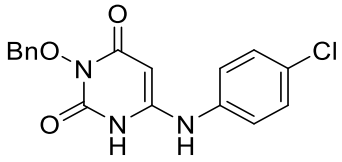
This compound was prepared following the procedure described for the preparation of **10**. Yield: 32%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.41 (s, 1H), 7.63–7.54 (m, 2H), 7.46 (dddd, J = 6.9, 5.8, 4.3, 1.7 Hz, 5H), 7.34–7.20 (m, 3H), 5.05 (s, 2H), 4.85 (s, 1H); HRMS (APCI-) calcd. for $C_{17}H_{15}N_3O_3$ $[M-H]^-$ 308.1035, found 308.1048 (E = 4.2 ppm).

3-(Benzyloxy)-6-((4-fluorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10b)



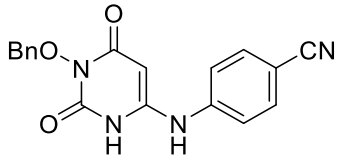
This compound was prepared following the procedure described for the preparation of **10**. Yield: 46%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 8.32 (s, 1H), 7.52 (dq, J = 6.3, 2.8, 2.4 Hz, 2H), 7.46-7.36 (m, 3H), 7.31–7.20 (m, 4H), 4.98 (s, 2H), 4.65 (s, 1H); HRMS (APCI+) calcd. for $C_{17}H_{14}FN_3O_3$ $[M+H]^+$ 328.1097, found 328.1088 (E = 2.7 ppm).

3-(Benzyloxy)-6-((4-chlorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10c)



This compound was prepared following the procedure described for the preparation of **10**. Yield: 39%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.66-8.33 (m, 1H), 7.56–7.50 (m, 2H), 7.46–7.37 (m, 5H), 7.28–7.22 (m, 2H), 4.99 (s, 2H), 4.83 (s, 1H); HRMS (ESI+) calcd. for $C_{17}H_{14}ClN_3O_3$ $[M+H]^+$ 344.0802, found 344.0799 (E = 0.9 ppm).

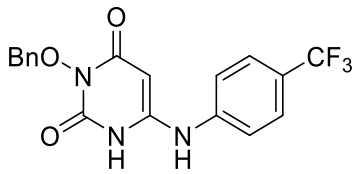
4-((1-(Benzyloxy)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)amino)benzonitrile (10d)



This compound was prepared following the procedure described for the preparation of **10**. Yield: 27%, brownish solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.09 (s, 1H), 7.84–7.75 (m,

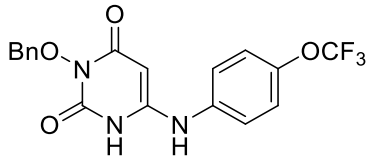
2H), 7.54 (dq, J = 6.0, 2.8, 2.4 Hz, 2H), 7.46–7.38 (m, 3H), 7.37–7.30 (m, 2H), 5.15 (s, 1H), 5.01 (s, 2H); HRMS (ESI+) calcd. for $C_{18}H_{14}N_4O_3$ $[M+H]^+$ 335.1144, found 335.1122 (E = 6.6 ppm).

3-(Benzyloxy)-6-((4-(trifluoromethyl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10e)



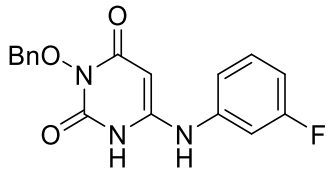
This compound was prepared following the procedure described for the preparation of **10**. Yield: 41%, light yellowish solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 8.91 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.58–7.49 (m, 2H), 7.46–7.36 (m, 5H), 5.07 (s, 1H), 5.01 (s, 2H); HRMS (ESI+) calcd. for $C_{18}H_{14}F_3N_3O_3$ $[M+H]^+$ 378.1066, found 378.1057 (E = 2.4 ppm).

3-(Benzyloxy)-6-((4-(trifluoromethoxy)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10f)



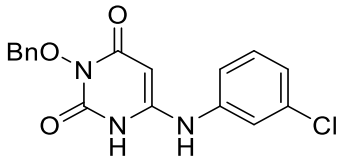
This compound was prepared following the procedure described for the preparation of **10**. Yield: 54%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.67 (s, 1H), 7.58–7.49 (m, 2H), 7.40 (ddd, J = 8.5, 5.5, 1.3 Hz, 5H), 7.36–7.31 (m, 2H), 5.00 (s, 2H), 4.85 (s, 1H); HRMS (ESI+) calcd. for $C_{18}H_{14}F_3N_3O_4$ $[M+H]^+$ 394.1015, found 394.0995 (E = 5.1 ppm).

3-(Benzyloxy)-6-((3-fluorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10g)



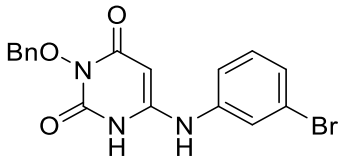
This compound was prepared following the procedure described for the preparation of **10**. Yield: 58%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 8.59 (s, 1H), 7.59–7.48 (m, 2H), 7.48–7.34 (m, 4H), 7.14–7.04 (m, 2H), 6.99 (ddt, J = 9.5, 8.6, 1.7 Hz, 1H), 5.00 (s, 2H), 4.93 (s, 1H); HRMS (ESI-) calcd. for $C_{17}H_{15}N_3O_3$ $[M-H]^-$ 326.0941, found 326.0952 (E = 3.4 ppm).

3-(Benzyloxy)-6-((3-chlorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10h)



This compound was prepared following the procedure described for the preparation of **10**. Yield: 56%, light green solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 8.67 (s, 1H), 7.65–7.53 (m, 2H), 7.50–7.43 (m, 4H), 7.34 (t, J = 2.1 Hz, 1H), 7.26 (ddt, J = 8.6, 6.7, 1.1 Hz, 2H), 5.05 (s, 2H), 4.94 (s, 1H); HRMS (APCI-) calcd. for $C_{17}H_{14}ClN_3O_3$ $[M-H]^-$ 342.0645, found 342.0660 (E = 4.4 ppm).

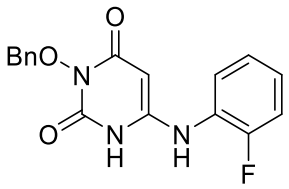
3-(Benzyloxy)-6-((3-bromophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10i)



This compound was prepared following the procedure described for the preparation of **10**. Yield: 37%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 8.58 (s, 1H), 7.61–7.47 (m,

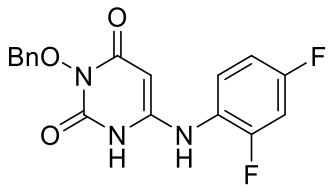
2H), 7.45–7.38 (m, 4H), 7.37–7.33 (m, 2H), 7.24 (m, 1H), 5.00 (s, 2H), 4.88 (s, 1H); HRMS (ESI+) calcd. for $C_{17}H_{14}BrN_3O_3$ $[M+H]^+$ 388.0297, found 388.0276 (E = 5.4 ppm).

3-(Benzyloxy)-6-((2-fluorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10j)



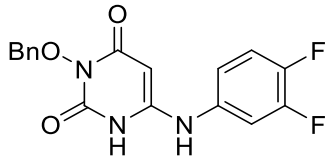
This compound was prepared following the procedure described for the preparation of **10**. Yield: 52%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.26 (s, 1H), 7.58–7.49 (m, 2H), 7.46–7.19 (m, 7H), 4.99 (s, 2H), 4.57 (s, 1H); HRMS (ESI+) calcd. for $C_{17}H_{14}FN_3O_3$ $[M+H]^+$ 328.1097, found 328.1088 (E = 2.7 ppm).

3-(Benzyloxy)-6-((2,4-difluorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10k)



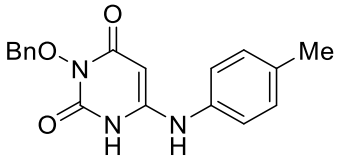
This compound was prepared following the procedure described for the preparation of **9**. Yield: 58%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.02 (d, J = 2.6 Hz, 1H), 8.22 (s, 1H), 7.52 (m, 2H), 7.48–7.37 (m, 5H), 7.15 (tdd, J = 8.6, 2.9, 1.4 Hz, 1H), 4.98 (s, 2H), 4.46 (d, J = 1.5 Hz, 1H); HRMS (ESI-) calcd. for $C_{17}H_{13}F_2N_3O_3$ $[M-H]^-$ 344.0847, found 344.0853 (E = 1.7 ppm).

3-(Benzyloxy)-6-((3,4-difluorophenyl)amino)pyrimidine-2,4(1H,3H)-dione (10l)



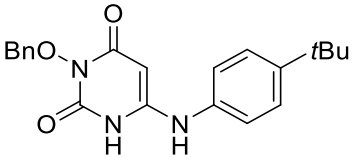
This compound was prepared following the procedure described for the preparation of **10**. Yield: 43%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.50 (s, 1H), 7.57–7.50 (m, 2H), 7.49–7.31 (m, 5H), 7.12–7.04 (m, 1H), 4.99 (s, 2H), 4.81 (s, 1H); HRMS (ESI+) calcd. for $C_{17}H_{13}F_2N_3O_3$ $[M+H]^+$ 346.1003, found 346.0983 (E = 5.8 ppm).

3-(Benzyloxy)-6-(p-tolylamino)pyrimidine-2,4(1H,3H)-dione (10m)



This compound was prepared following the procedure described for the preparation of **9**. Yield: 47%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.31 (s, 1H), 7.58–7.48 (m, 2H), 7.46–7.35 (m, 3H), 7.21 (d, J = 7.9 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 4.98 (s, 2H), 4.70 (s, 1H); HRMS (ESI+) calcd. for $C_{18}H_{17}N_3O_3$ $[M+H]^+$ 324.1348, found 324.1329 (E = 5.9 ppm).

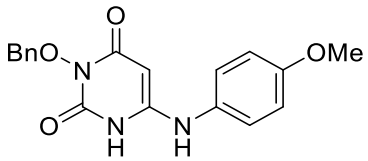
3-(Benzyloxy)-6-((4-(tert-butyl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10n)



This compound was prepared following the procedure described for the preparation of **10**. Yield: 44%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 8.24 (s, 1H), 7.57–7.50 (m,

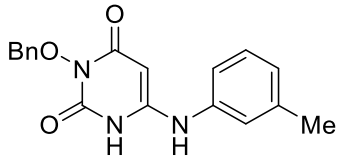
2H), 7.46–7.37 (m, 5H), 7.20–7.11 (m, 2H), 4.98 (s, 2H), 4.73 (d, *J* = 2.3 Hz, 1H), 1.29 (s, 9H); HRMS (ESI-) calcd. for $C_{21}H_{23}N_3O_3$ $[M-H]^-$ 364.1661, found 364.1678 (E = 4.7 ppm).

3-(Benzyloxy)-6-((4-methoxyphenyl)amino)pyrimidine-2,4(1H,3H)-dione (10o)



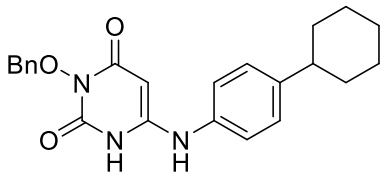
This compound was prepared following the procedure described for the preparation of **10**. Yield: 54%, light brownish solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.07 (s, 1H), 7.52 (dq, *J* = 6.1, 2.8, 2.4 Hz, 2H), 7.47–7.36 (m, 3H), 7.22–7.12 (m, 2H), 7.02–6.90 (m, 2H), 4.97 (s, 2H), 4.52 (s, 1H), 3.77 (s, 3H); HRMS (APCI-) calcd. for $C_{18}H_{17}N_3O_4$ $[M-H]^-$ 338.1141, found 338.1163 (E = 6.5 ppm).

3-(Benzyloxy)-6-(m-tolylamino)pyrimidine-2,4(1H,3H)-dione (10p)



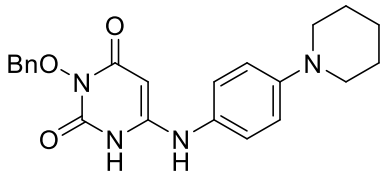
This compound was prepared following the procedure described for the preparation of **10**. Yield: 55%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.26 (s, 1H), 7.57–7.49 (m, 2H), 7.45–7.38 (m, 3H), 7.29 (t, *J* = 7.7 Hz, 1H), 7.06–6.98 (m, 3H), 4.99 (s, 2H), 4.79 (s, 1H), 2.32 (s, 3H); HRMS (APCI+) calcd. for $C_{18}H_{17}N_3O_3$ $[M+H]^+$ 324.1348, found 324.1327 (E = 6.5 ppm).

3-(Benzyloxy)-6-((4-cyclohexylphenyl)amino)pyrimidine-2,4(1H,3H)-dione (10q)



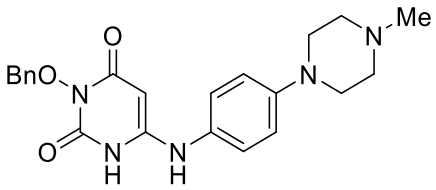
This compound was prepared following the procedure described for the preparation of **10**. Yield: 52%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.23 (s, 1H), 7.53 (dd, J = 7.5, 2.2 Hz, 2H), 7.45–7.35 (m, 3H), 7.25 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 8.2 Hz, 2H), 4.98 (s, 2H), 4.72 (s, 1H), 1.92–1.62 (m, 5H), 1.38 (td, J = 10.0, 9.3, 5.4 Hz, 5H); HRMS (ESI+) calcd. for $C_{23}H_{25}N_3O_3$ $[M+H]^+$ 392.1974, found 392.1956 (E = 4.6 ppm).

3-(Benzyloxy)-6-((4-(piperidin-1-yl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10r)



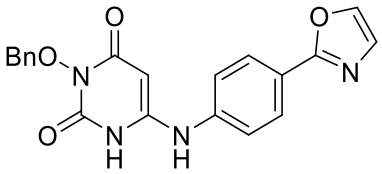
This compound was prepared following the procedure described for the preparation of **10**. Yield: 38%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 7.99 (s, 1H), 7.52 (dq, J = 6.3, 2.8, 2.4 Hz, 2H), 7.44–7.36 (m, 3H), 7.09–7.03 (m, 2H), 6.99–6.92 (m, 2H), 4.97 (s, 2H), 4.52 (s, 1H), 3.14 (t, J = 5.4 Hz, 4H), 1.66–1.50 (m, 6H); HRMS (APCI+) calcd. for $C_{22}H_{24}N_4O_3$ $[M+H]^+$ 393.1927, found 393.1918 (E = 2.3 ppm).

3-(Benzyloxy)-6-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10s)



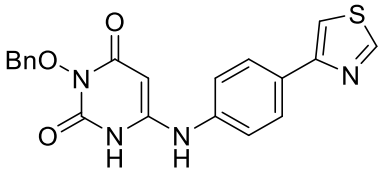
This compound was prepared following the procedure described for the preparation of **10**. Yield: 74%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.63–7.52 (m, 2H), 7.50–7.40 (m, 3H), 7.24–7.14 (m, 2H), 7.13–7.00 (m, 2H), 5.02 (s, 2H), 4.62 (s, 1H), 3.23 (s, 7H), 2.93 (s, 1H), 2.77 (s, 3H); HRMS (ESI+) calcd. for $C_{22}H_{25}N_5O_3$ $[M+H]^+$ 408.2036, found 408.2017 (E = 4.7 ppm).

3-(Benzyloxy)-6-((4-(oxazol-2-yl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10t)



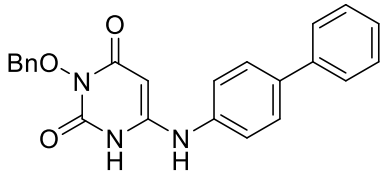
This compound was prepared following the procedure described for the preparation of **10**. Yield: 46%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 8.83 (s, 1H), 8.27 (d, J = 0.8 Hz, 1H), 8.08–8.02 (m, 2H), 7.66–7.56 (m, 2H), 7.52–7.37 (m, 6H), 5.11 (s, 1H), 5.07 (s, 2H); HRMS (ESI+) calcd. for $C_{20}H_{16}N_4O_4$ $[M+H]^+$ 377.1250, found 377.1224 (E = 6.9 ppm).

3-(Benzyloxy)-6-((4-(thiazol-4-yl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (10u)



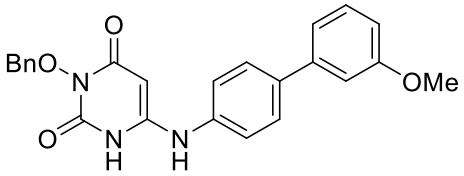
This compound was prepared following the procedure described for the preparation of **10**. Yield: 69%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.21 (d, J = 1.9 Hz, 1H), 8.51 (s, 1H), 8.14 (d, J = 1.9 Hz, 1H), 8.06–7.97 (m, 2H), 7.58–7.49 (m, 2H), 7.45–7.38 (m, 3H), 7.33–7.28 (m, 2H), 5.00 (s, 2H), 4.92 (s, 1H); HRMS (ESI+) calcd. for $C_{20}H_{16}N_4O_3S$ $[M+H]^+$ 393.1021, found 393.1012 (E = 2.3 ppm).

6-([1,1'-Biphenyl]-4-ylamino)-3-(benzyloxy)pyrimidine-2,4(1H,3H)-dione (10v)



This compound was prepared following the procedure described for the preparation of **10**. Yield: 71%, light brown solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 8.50 (s, 1H), 7.77–7.62 (m, 5H), 7.53–7.24 (m, 9H), 5.01 (s, 2H), 4.91 (s, 1H); HRMS (ESI-) calcd. for $C_{23}H_{19}N_3O_3$ $[M-H]^-$ 384.1348, found 384.1350 (E = 0.5 ppm).

3-(Benzyloxy)-6-((3'-methoxy-[1,1'-biphenyl]-4-yl)amino)pyrimidine-2,4(1H,3H)-dione (10w)



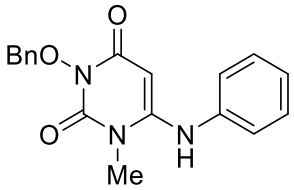
This compound was prepared following the procedure described for the preparation of **10**. Yield: 64%, light brown solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 10.04 (s, 1H), 8.41 (s, 1H), 7.77–7.64 (m, 2H), 7.38 (t, J = 7.9 Hz, 1H), 7.31–7.26 (m, 2H), 7.23 (ddd, J = 7.7, 1.8, 1.0

Hz, 1H), 7.19 (dd, J = 2.5, 1.7 Hz, 1H), 6.93 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.91 (s, 1H), 3.83 (s, 3H); HRMS (ESI+) calcd. for $C_{24}H_{21}N_3O_4$ $[M+H]^+$ 416.1610, found 416.1554 (E = 13.5 ppm).

General Procedure for the Synthesis of 3-(Benzyloxy)-1-methyl-6-(phenylamino)pyrimidine-2,4(1H,3H)-dione (11a-w)

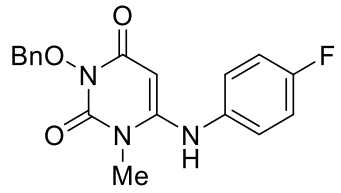
To a solution of amines (0.55 mmol, 1.0 eq) in dry THF (6 mL) at -78 °C was added LDA (1.32 mmol, 2.4 eq). The mixture was stirred for 30 min, followed by the addition of HMPA (2.0 mL) and compound **9** (1.10 mmol, 2.0 eq). The resulting mixture was stirred at -78 °C for 30 min, then room temperature for overnight. The reaction was quenched by adding 10 mL of water. The aqueous was extracted with EtOAc (10 mL × 3), and the combined organics were washed with brine and dried over Na_2SO_4. After the solvent was removed in vacuo, the residue was purified by flash column (Hexanes: EtOAc = 1:1) to give compound **11a-n** (38-73%).

3-(Benzyloxy)-1-methyl-6-(phenylamino)pyridine-2,4(1H,3H)-dione (**11a**)



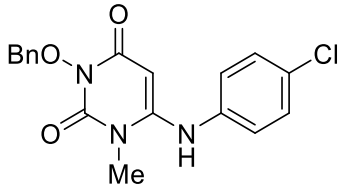
This compound was prepared following the procedure described for the preparation of **11**. Yield: 56%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.62 (s, 1H), 7.58–7.50 (m, 2H), 7.50–7.43 (m, 2H), 7.43–7.37 (m, 3H), 7.31–7.25 (m, 3H), 4.98 (s, 2H), 4.59 (s, 1H), 3.47 (s, 3H); HRMS (ESI-) calcd. for $C_{18}H_{17}N_3O_3$ $[M-H]^-$ 322.1192, found 322.1192 (E = 0 ppm).

3-(Benzyloxy)-6-((4-fluorophenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11b)



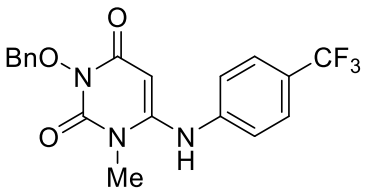
This compound was prepared following the procedure described for the preparation of **11**. Yield: 44%, light brown solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 7.53 (dq, J = 6.6, 2.4 Hz, 2H), 7.45–7.38 (m, 3H), 7.34–7.28 (m, 4H), 4.97 (s, 2H), 4.47 (s, 1H), 3.46 (s, 3H); HRMS (ESI+) calcd. for $C_{18}H_{16}FN_3O_3$ $[M+H]^+$ 342.1254, found 342.1248 (E = 1.8 ppm).

3-(Benzyloxy)-6-((4-chlorophenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11c)



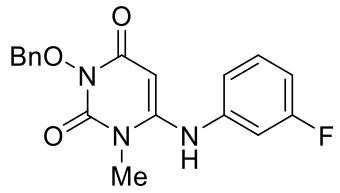
This compound was prepared following the procedure described for the preparation of **11**. Yield: 73%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.64–7.54 (m, 4H), 7.51–7.44 (m, 3H), 7.39–7.32 (m, 2H), 5.05 (s, 2H), 4.71 (s, 1H), 3.52 (s, 3H); HRMS (ESI-) calcd. for $C_{18}H_{16}ClN_3O_3$ $[M-H]^-$ 356.0802, found 356.0822 (E = 5.6 ppm).

3-(Benzyloxy)-1-methyl-6-((4-(trifluoromethyl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (11d)



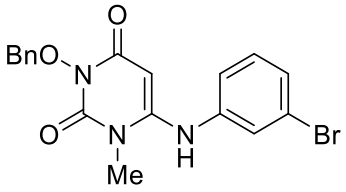
This compound was prepared following the procedure described for the preparation of **11**. Yield: 39%, light brown solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.60– 7.49 (m, 2H), 7.49–7.34 (m, 5H), 5.00 (s, 2H), 4.96 (s, 1H), 3.44 (s, 3H); HRMS (ESI-) calcd. for $C_{19}H_{16}F_3N_3O_3$ $[M-H]^-$ 390.1066, found 390.1080 (E = 3.6 ppm).

3-(Benzyloxy)-6-((3-fluorophenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11e)



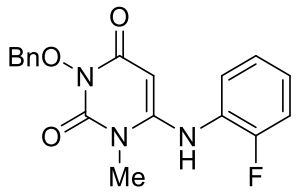
This compound was prepared following the procedure described for the preparation of **11**. Yield: 42%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 7.58–7.53 (m, 2H), 7.51–7.45 (m, 1H), 7.45–7.37 (m, 3H), 7.17–6.99 (m, 3H), 4.99 (s, 2H), 4.78 (s, 1H), 3.45 (s, 3H); HRMS (ESI-) calcd. for $C_{18}H_{16}FN_3O_3$ $[M-H]^-$ 340.1097, found 340.1113 (E = 4.7 ppm).

3-(Benzyloxy)-6-((3-bromophenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11f)



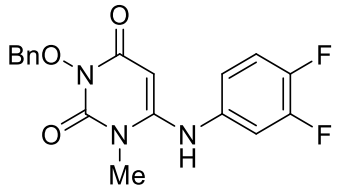
This compound was prepared following the procedure described for the preparation of **11**. Yield: 56%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 7.54 (dd, J = 7.4, 2.1 Hz, 2H), 7.48 (t, J = 1.9 Hz, 1H), 7.46–7.36 (m, 5H), 7.29 (dt, J = 7.6, 1.8 Hz, 1H), 4.99 (s, 2H), 4.73 (s, 1H), 3.44 (s, 3H); HRMS (ESI+) calcd. for $C_{18}H_{16}BrN_3O_3$ $[M+H]^+$ 402.0453, found 402.0449 (E = 1.0 ppm).

3-(Benzyloxy)-6-((2-fluorophenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11g)



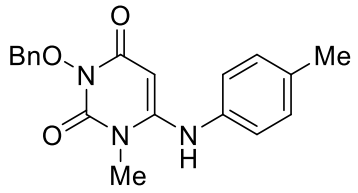
This compound was prepared following the procedure described for the preparation of **11**. Yield: 51%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 7.56–7.49 (m, 2H), 7.40 (tdd, J = 8.4, 5.4, 3.0 Hz, 6H), 7.32 (ddd, J = 8.2, 6.3, 2.2 Hz, 1H), 4.98 (s, 2H), 4.26 (d, J = 1.6 Hz, 1H), 3.48 (s, 3H); HRMS (ESI+) calcd. for $C_{18}H_{16}FN_3O_3$ $[M+H]^+$ 342.1254, found 342.1239 (E = 4.4 ppm).

3-(Benzyloxy)-6-((3,4-difluorophenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11h)



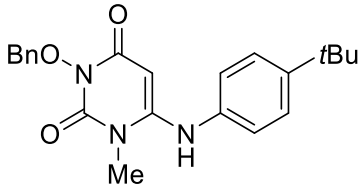
This compound was prepared following the procedure described for the preparation of **11**. Yield: 41%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H), 7.56–7.52 (m, 2H), 7.52–7.46 (m, 1H), 7.45–7.34 (m, 4H), 7.13 (ddt, J = 7.9, 3.4, 1.9 Hz, 1H), 4.98 (s, 2H), 4.65 (s, 1H), 3.44 (s, 3H); HRMS (ESI+) calcd. for $C_{18}H_{15}F_2N_3O_3$ $[M+H]^+$ 360.1160, found 360.1142 (E = 5.0 ppm).

3-(Benzyloxy)-1-methyl-6-(p-tolylamino)pyrimidine-2,4(1H,3H)-dione (11i)



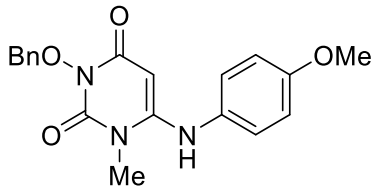
This compound was prepared following the procedure described for the preparation of **11**. Yield: 43%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 7.58–7.49 (m, 2H), 7.45–7.35 (m, 3H), 7.27 (d, J = 8.0 Hz, 2H), 7.21–7.10 (m, 2H), 4.97 (s, 2H), 4.49 (s, 1H), 3.46 (s, 3H), 2.33 (s, 3H); HRMS (ESI-) calcd. for $C_{19}H_{19}N_3O_3$ $[M-H]^-$ 336.1348, found 336.1358 (E = 3.0 ppm).

3-(Benzyloxy)-6-((4-(tert-butyl)phenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11j)



This compound was prepared following the procedure described for the preparation of **11**. Yield: 59%, light beige solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 7.56–7.51 (m, 2H), 7.50–7.45 (m, 2H), 7.44–7.37 (m, 3H), 7.22–7.15 (m, 2H), 4.97 (s, 2H), 4.54 (s, 1H), 3.46 (s, 3H), 1.31 (s, 9H); HRMS (ESI+) calcd. for $C_{22}H_{25}N_3O_3$ $[M+H]^+$ 380.1974, found 380.1956 (E = 3.0 ppm).

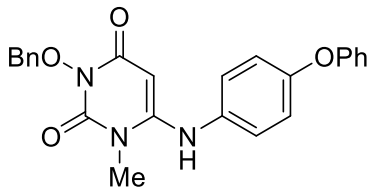
3-(Benzyloxy)-6-((4-methoxyphenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11k)



This compound was prepared following the procedure described for the preparation of **11**. Yield: 43%, light beige solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.49–7.39 (m, 2H), 7.37–

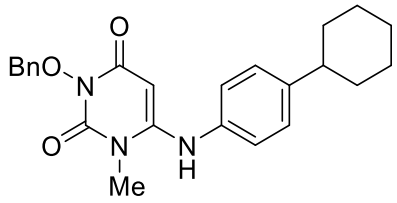
7.27 (m, 3H), 7.15–7.06 (m, 2H), 6.98–6.86 (m, 2H), 4.87 (s, 2H), 4.28 (s, 1H), 3.70 (s, 3H), 3.36 (s, 3H); HRMS (ESI-) calcd. for $C_{19}H_{19}N_3O_4$ $[M+H]^+$ 352.1297, found 352.1300 (E = 0.9 ppm).

3-(Benzyloxy)-6-((4-methoxyphenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11l)



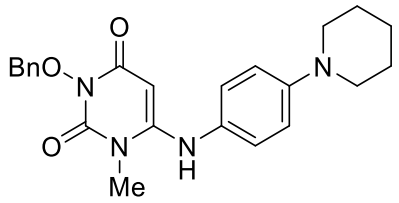
This compound was prepared following the procedure described for the preparation of **11**. Yield: 38%, white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 7.53 (dd, J = 7.4, 2.2 Hz, 2H), 7.42 (dtt, J = 11.1, 5.7, 2.0 Hz, 5H), 7.31–7.25 (m, 2H), 7.21–7.15 (m, 1H), 7.12–7.05 (m, 4H), 4.98 (s, 2H), 4.54 (s, 1H), 3.46 (s, 3H); HRMS (ESI-) calcd. for $C_{24}H_{21}N_3O_4$ $[M+H]^+$ 416.1610, found 416.1602 (E = 1.9 ppm).

3-(Benzyloxy)-6-((4-cyclohexylphenyl)amino)-1-methylpyrimidine-2,4(1H,3H)-dione (11m)



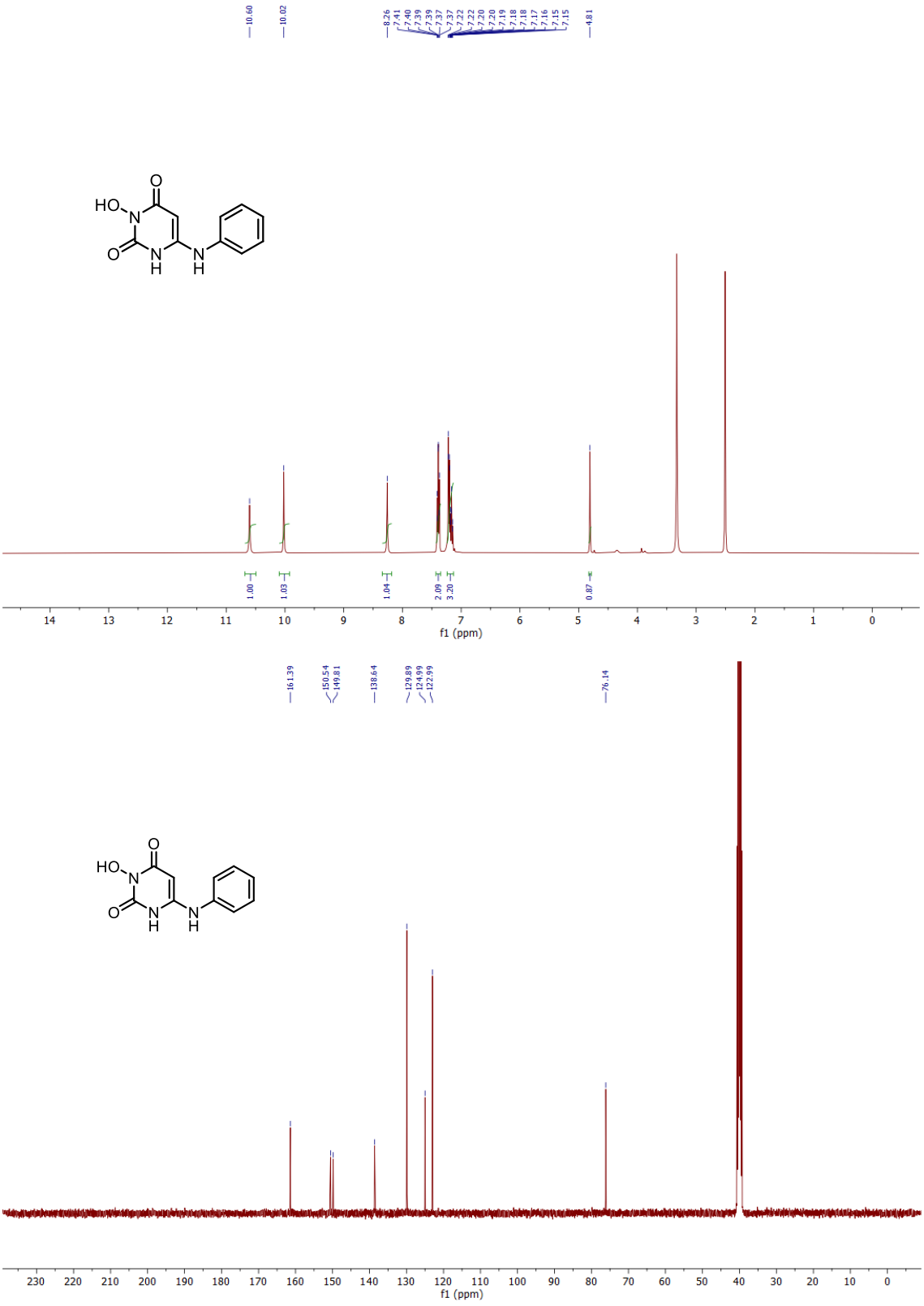
This compound was prepared following the procedure described for the preparation of **11**. Yield: 43%, light beige solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 1H), 7.59–7.48 (m, 2H), 7.45–7.38 (m, 3H), 7.34–7.26 (m, 2H), 7.22–7.13 (m, 2H), 4.97 (s, 2H), 4.52 (s, 1H), 3.46 (s, 3H), 1.81 (d, J = 6.8 Hz, 4H), 1.72 (d, J = 12.7 Hz, 1H), 1.46–1.33 (m, 3H), 1.24 (s, 2H); HRMS (ESI+) calcd. for $C_{24}H_{21}N_3O_4$ $[M+H]^+$ 406.2131, found 406.2112 (E = 4.7 ppm).

3-(Benzyloxy)-1-methyl-6-((4-(piperidin-1-yl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione (11n)



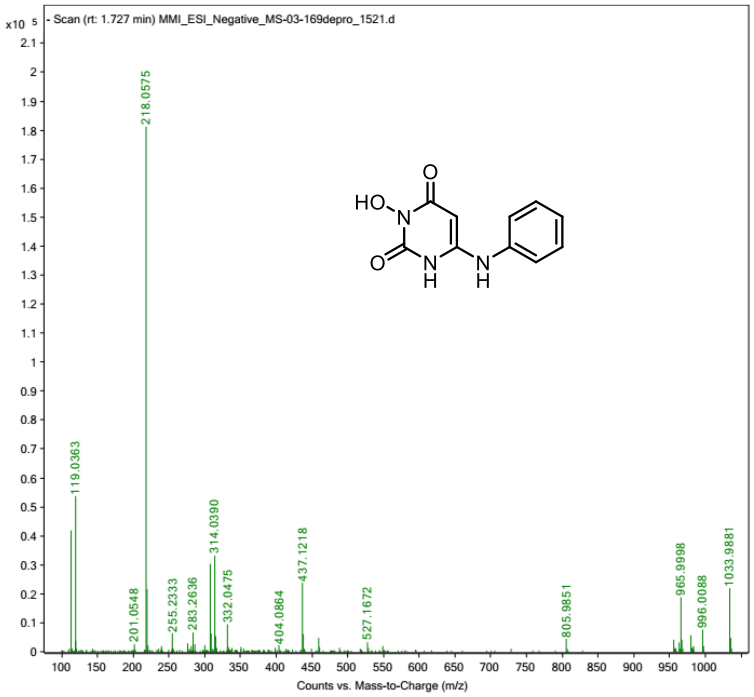
This compound was prepared following the procedure described for the preparation of **11**. Yield: 49%, light brown solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 7.57–7.49 (m, 2H), 7.45–7.37 (m, 3H), 7.12–7.04 (m, 2H), 7.02–6.96 (m, 2H), 4.96 (s, 2H), 4.38 (s, 1H), 3.44 (s, 3H), 3.20–3.13 (m, 4H), 1.69–1.50 (m, 6H); HRMS (ESI-) calcd. for $C_{23}H_{26}N_4O_3$ $[M-H]^-$ 405.1927, found 405.1949 (E = 5.4 ppm).

^{1}H NMR and ^{13}C NMR for Compound 2a in DMSO-d_6



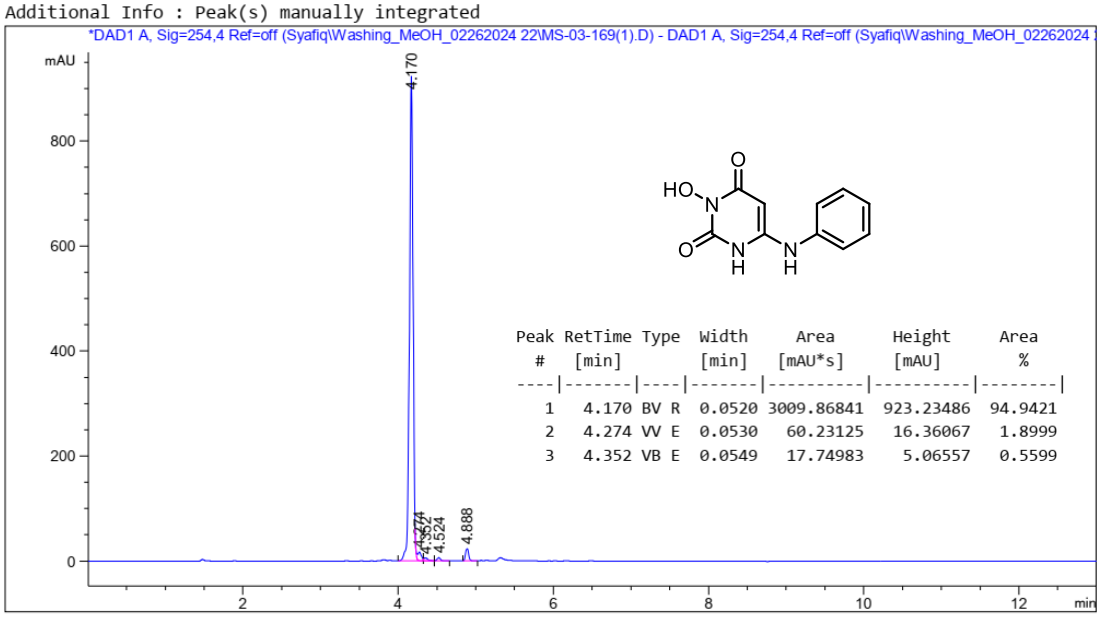
HRMS and HPLC Trace for Compound 2a

Sample Name	MS-03-169depro	**Position**	P1-D10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-169depro_1521.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	2/19/2025 7:09:58 PM (UTC-06:00)



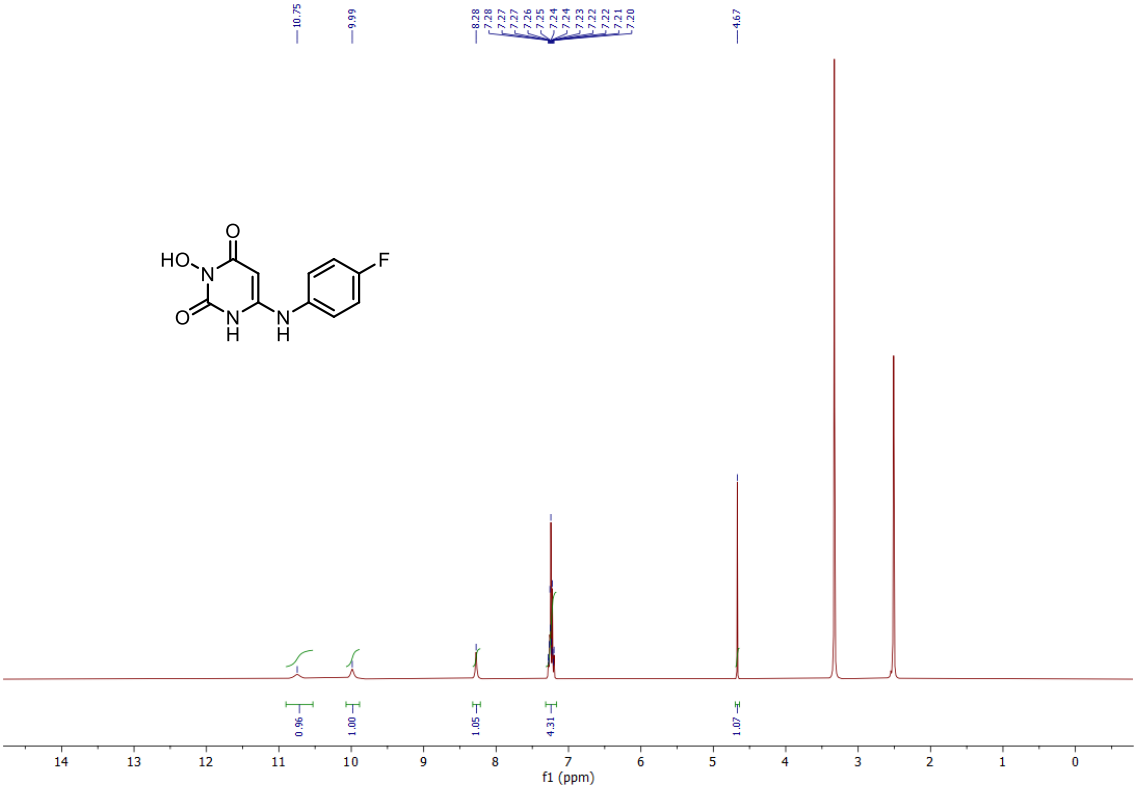
Sample Info : MS-03-169(1)

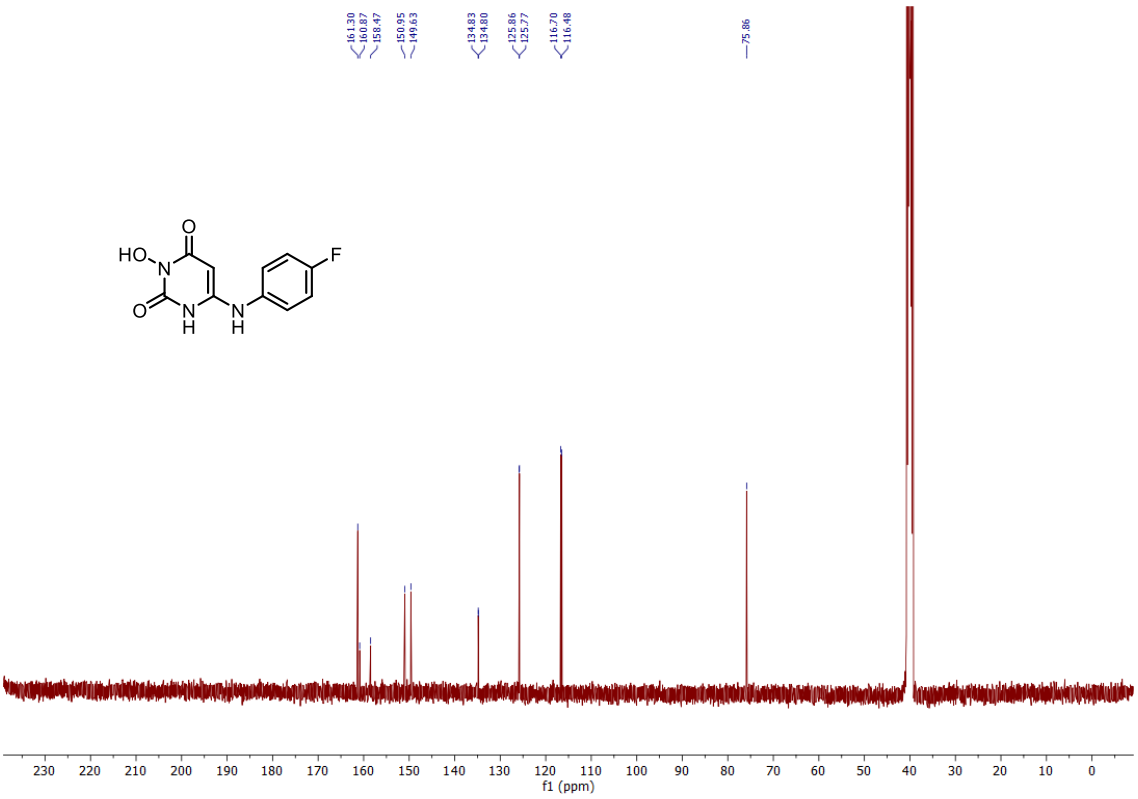
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.170	BV R	0.0520	3009.86841	923.23486	94.9421
2	4.274	VV E	0.0530	60.23125	16.36067	1.8999
3	4.352	VB E	0.0549	17.74983	5.06557	0.5599

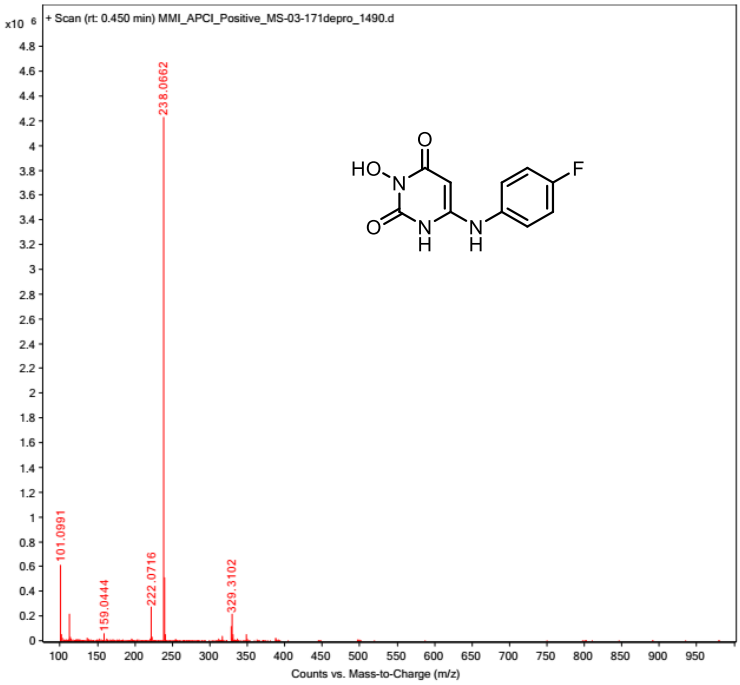
^{1}H NMR and ^{13}C NMR for Compound 2b in DMSO-d_6





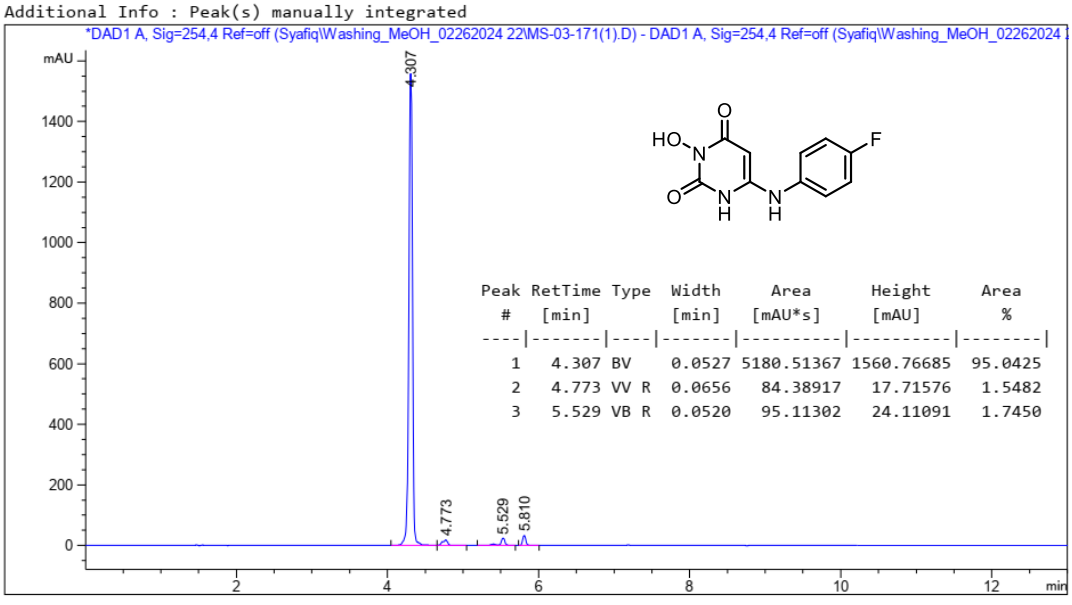
HRMS and HPLC Trace for Compound 2b

Sample Name	MS-03-171depro	**Position**	P1-C10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_APCI_Positive_MS-03-171depro_1490.d
ACQ Method	MMI_APCI_Positive.m	**Comment**		**Acquired Time**	2/19/2025 10:26:28 AM (UTC-06:00)



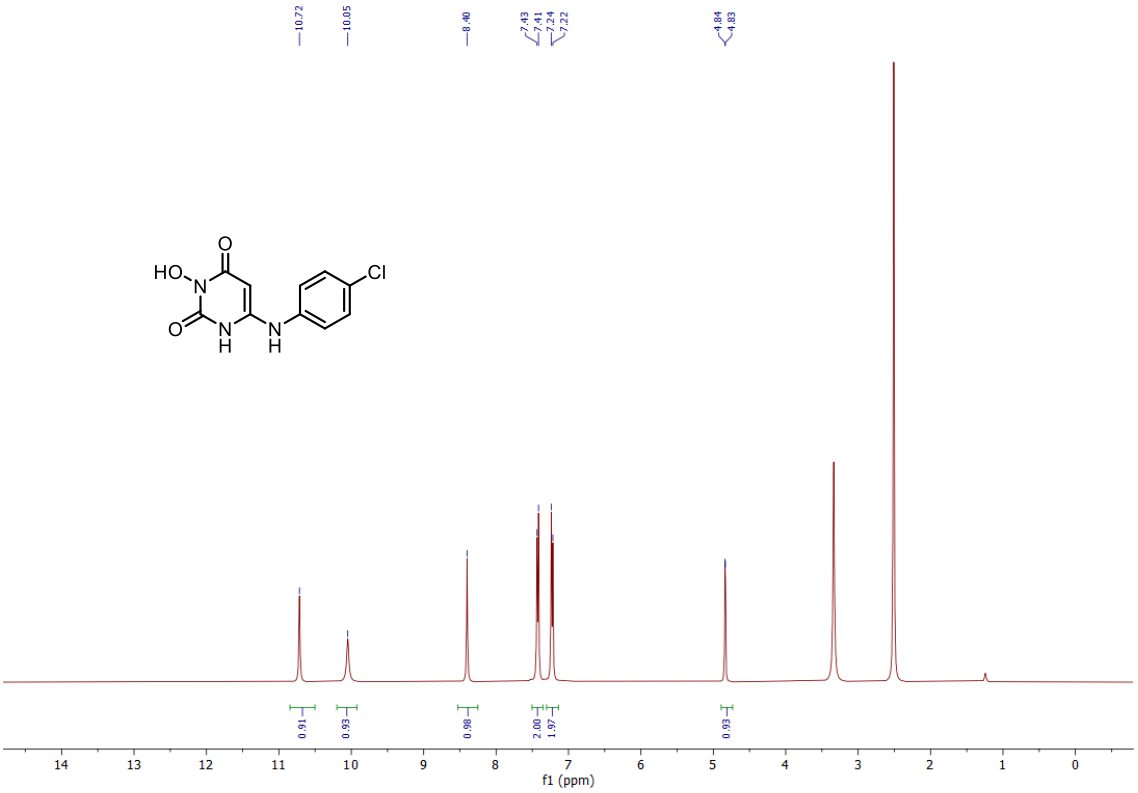
Sample Info : MS-03-171(1)

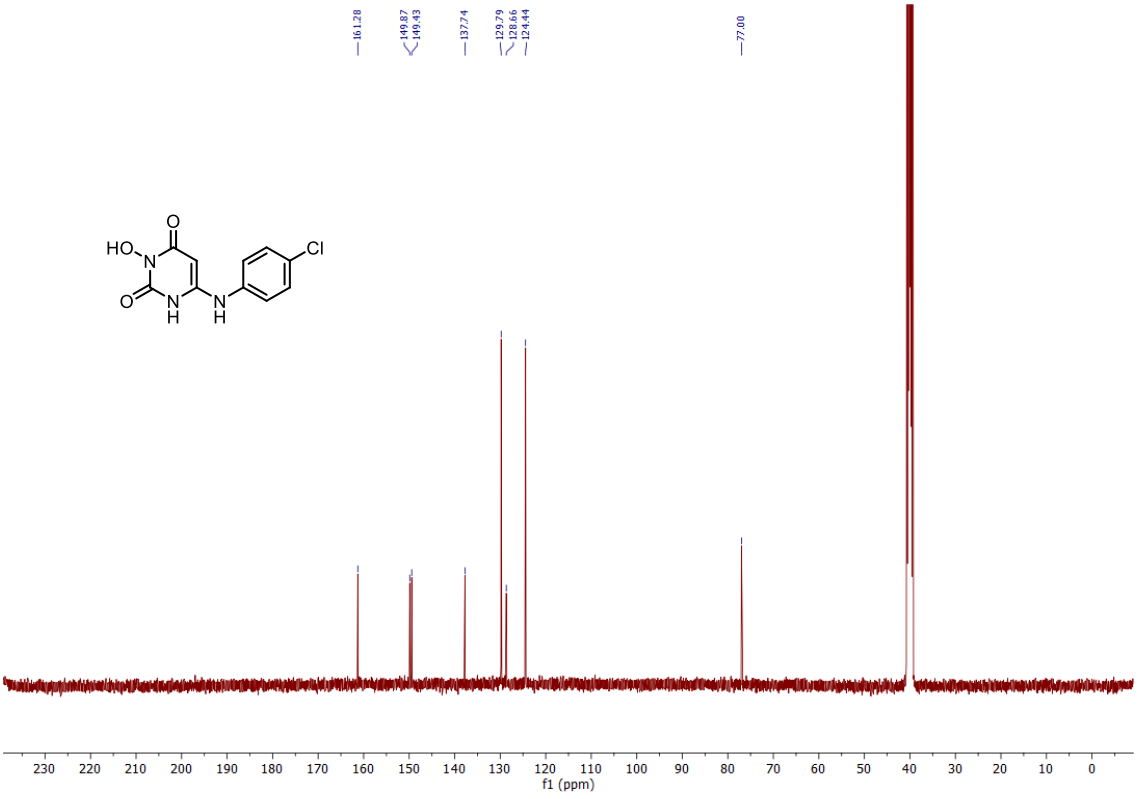
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.307	BV	0.0527	5180.51367	1560.76685	95.0425
2	4.773	VV R	0.0656	84.38917	17.71576	1.5482
3	5.529	VB R	0.0520	95.11302	24.11091	1.7450

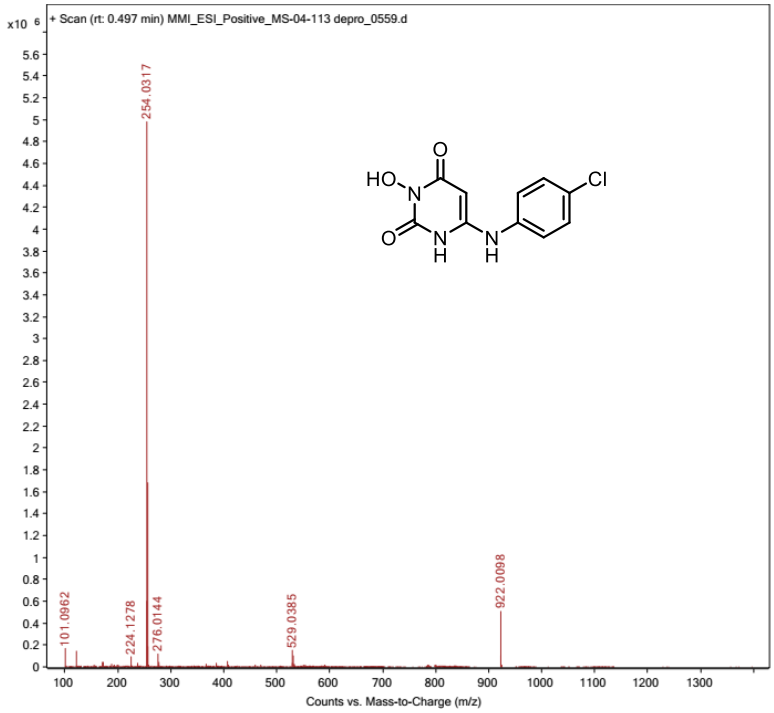
^{1}H NMR and ^{13}C NMR for Compound 2c in DMSO-d_6



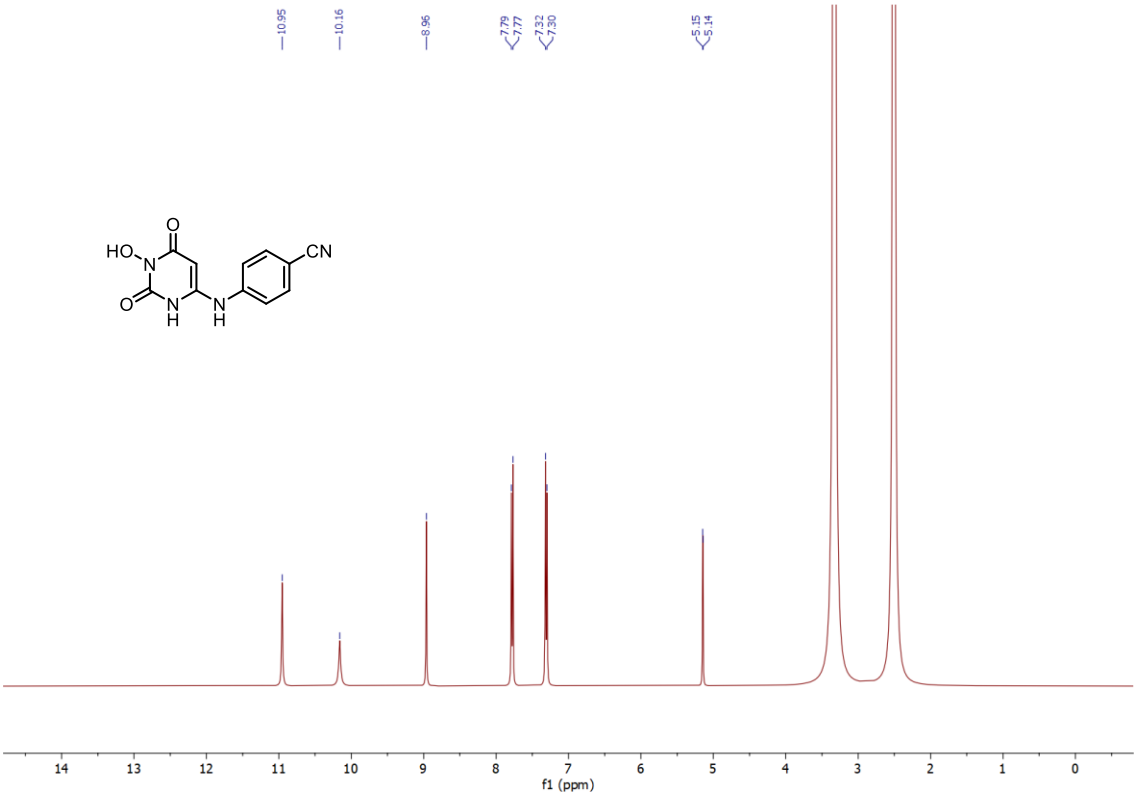


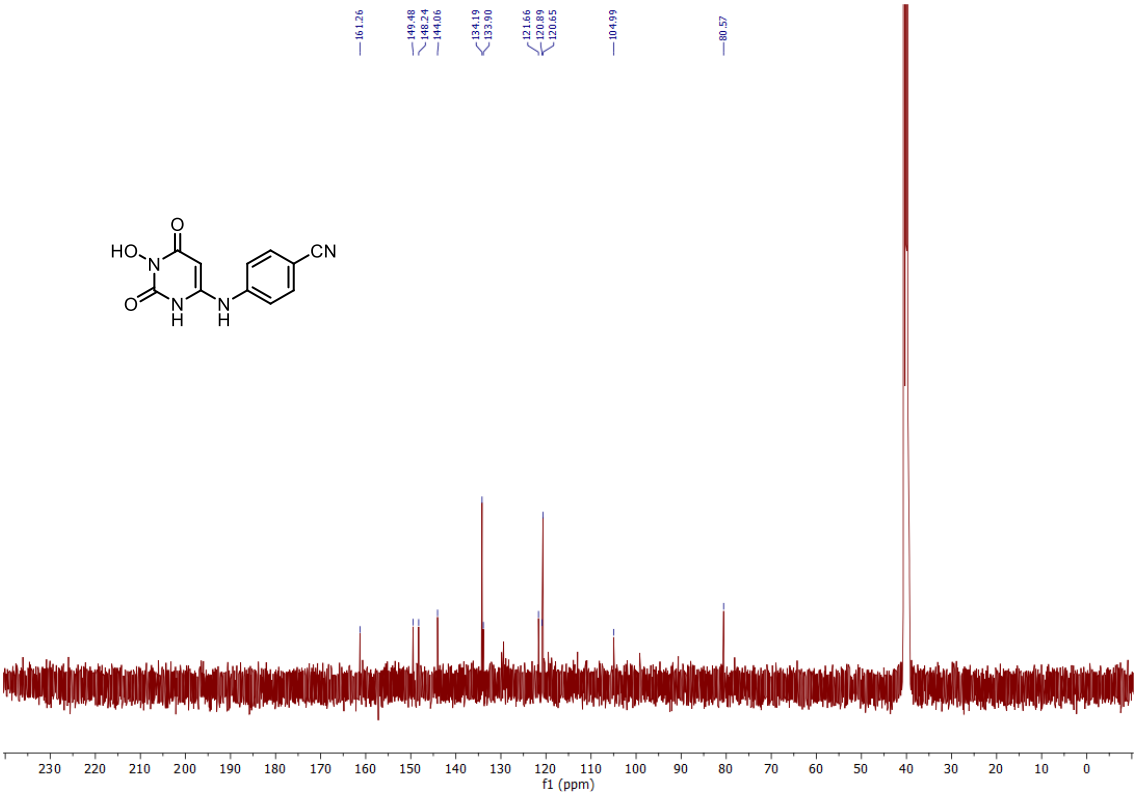
HRMS for Compound 2c

Sample Name	MS-04-113 depro	**Position**	P1-A9	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-113 depro_0559.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	12/12/2025 9:26:49 AM (UTC-06:00)



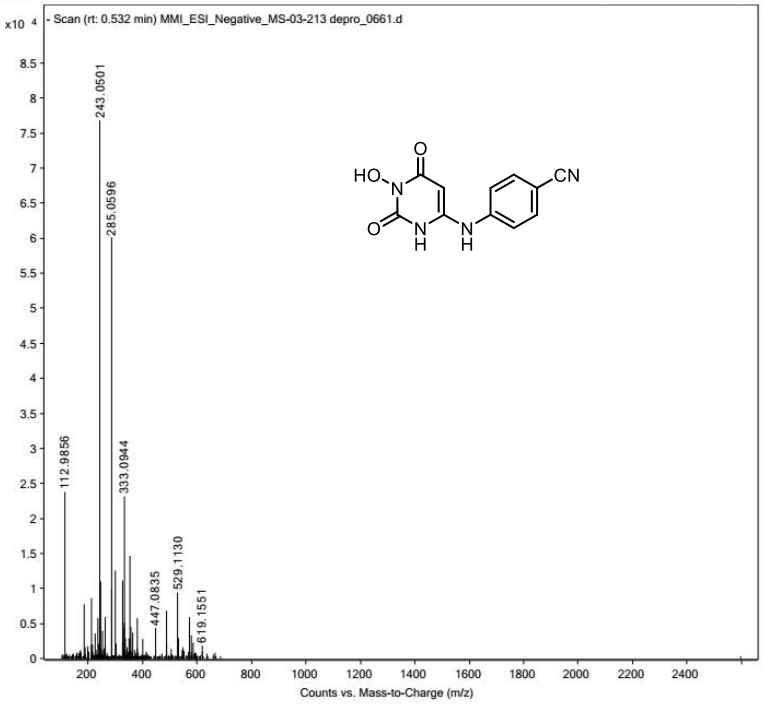
^{1}H NMR and ^{13}C NMR for Compound 2d in DMSO-*d*$_6$



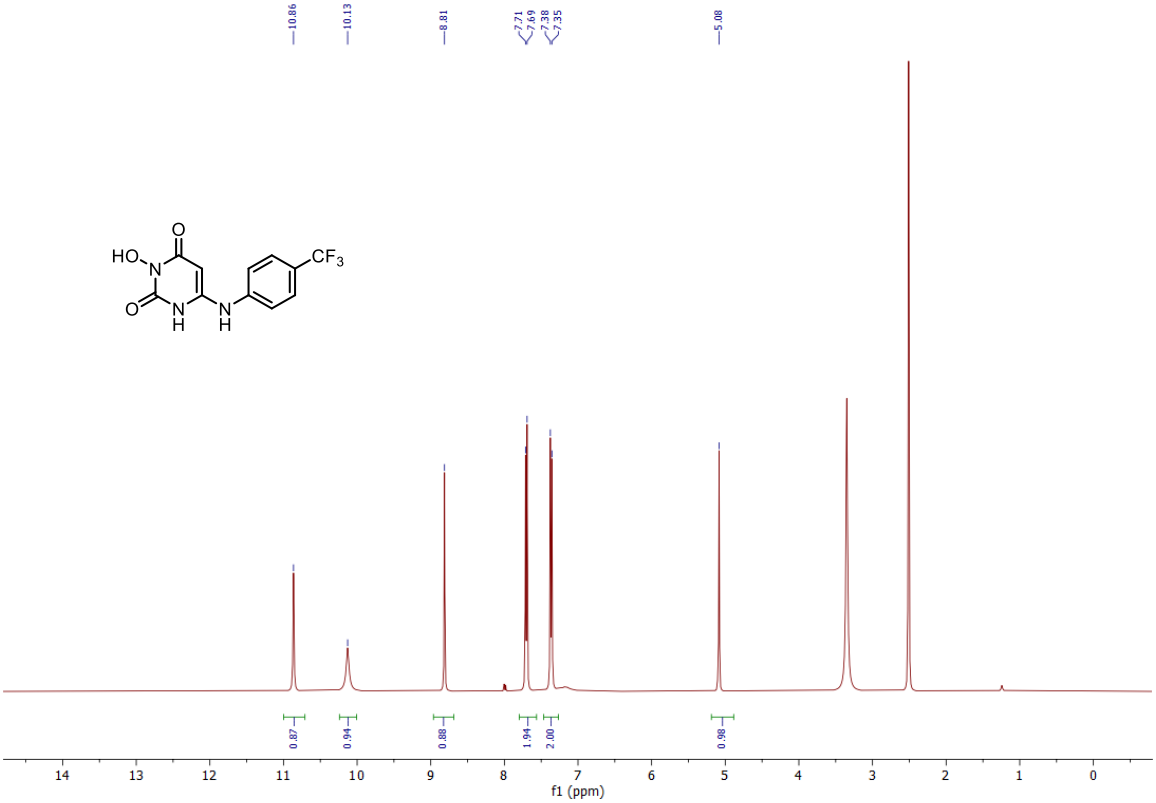


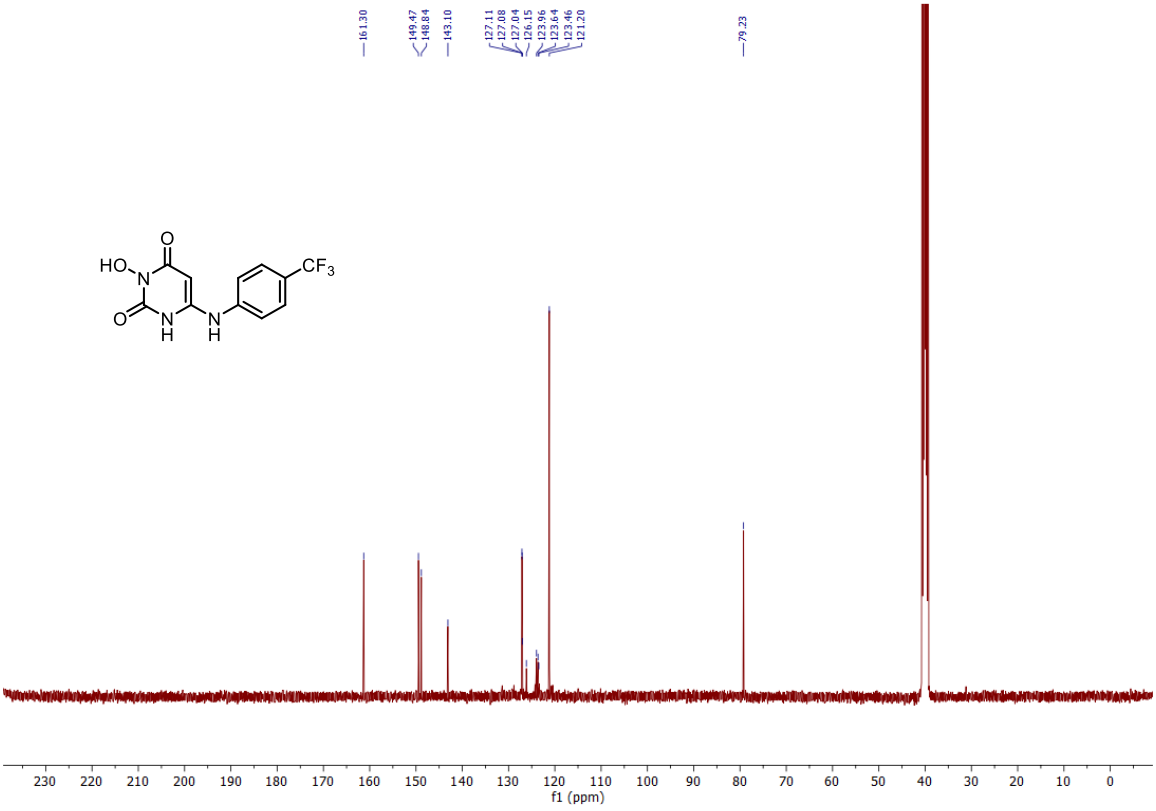
HRMS for Compound 2d

Sample Name	MS-03-213 depro	**Position**	P2-A3	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-213 depro_0661.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	12/17/2025 11:55:43 AM (UTC-06:00)



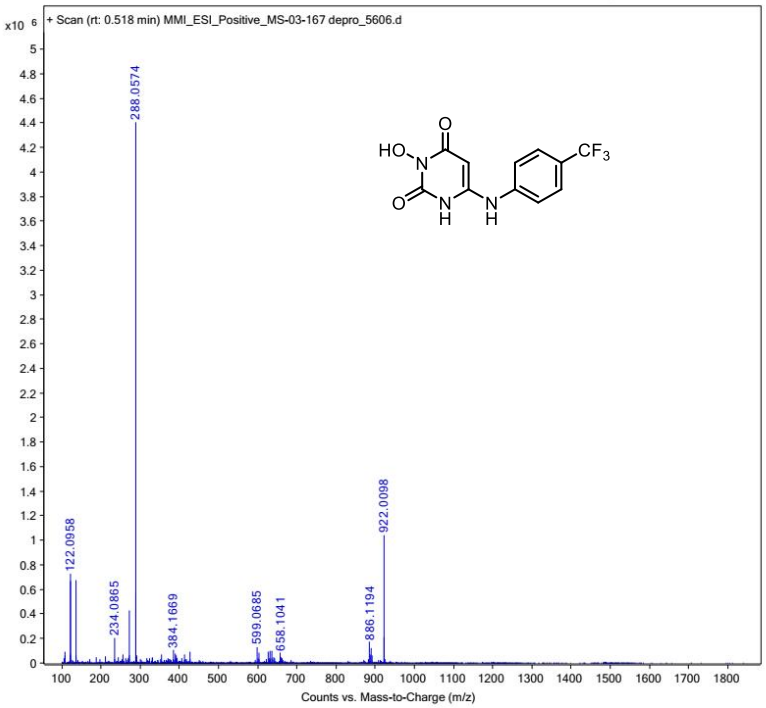
^{1}H NMR and ^{13}C NMR for Compound 2e in DMSO-d_6



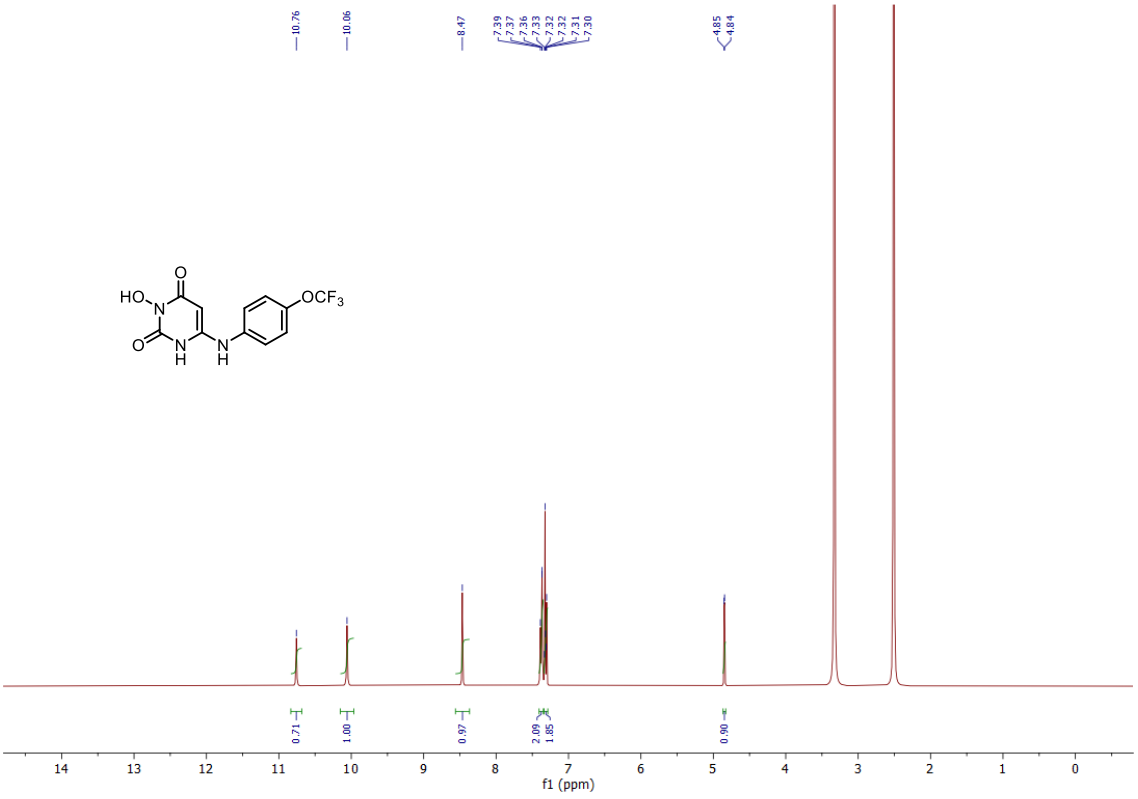


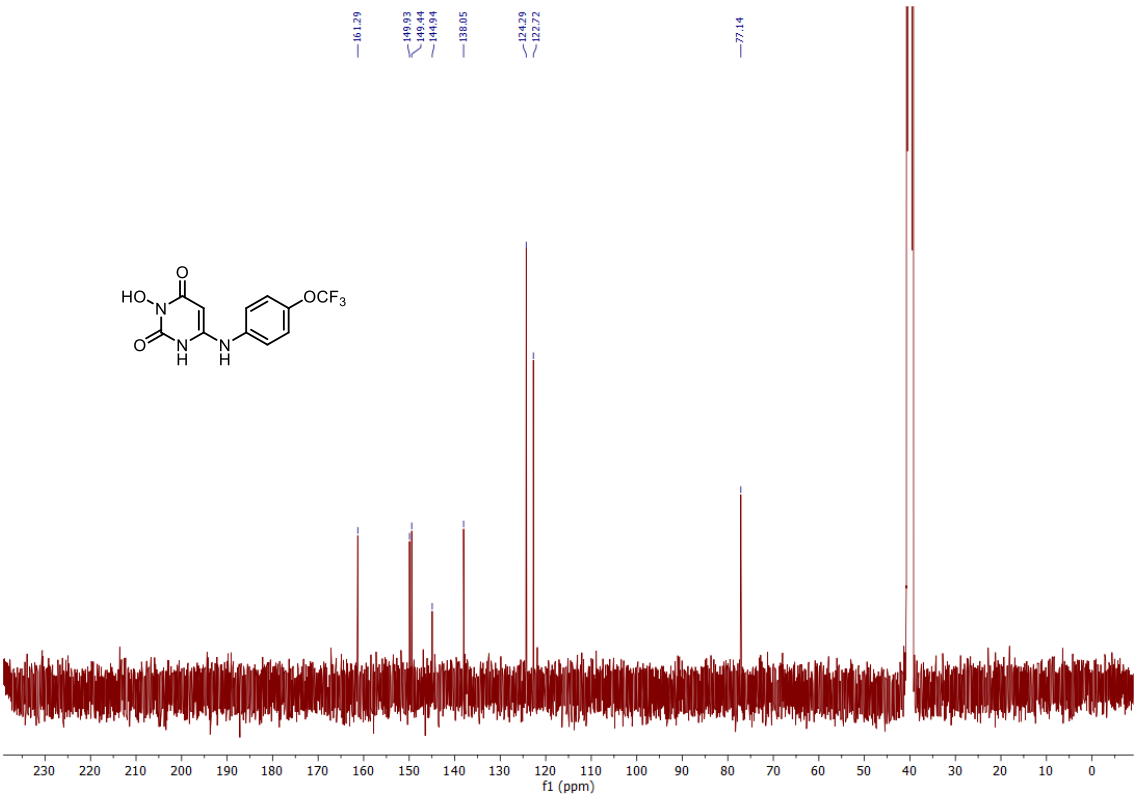
HRMS for Compound 2e

Sample Name	MS-03-167 depro	**Position**	P1-F4	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-167 depro_5606.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	7/2/2025 11:08:43 AM (UTC-05:00)



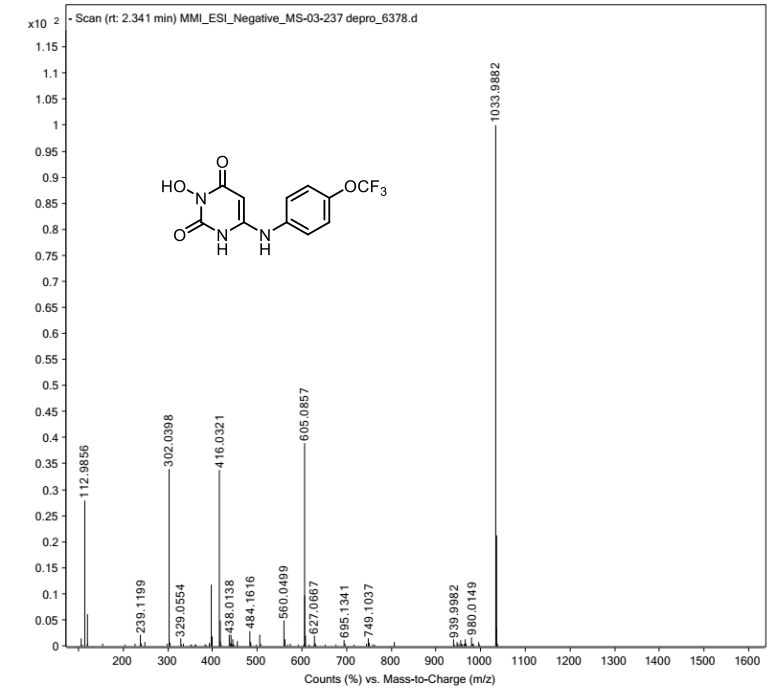
^{1}H NMR and ^{13}C NMR for Compound 2f in DMSO-d_6



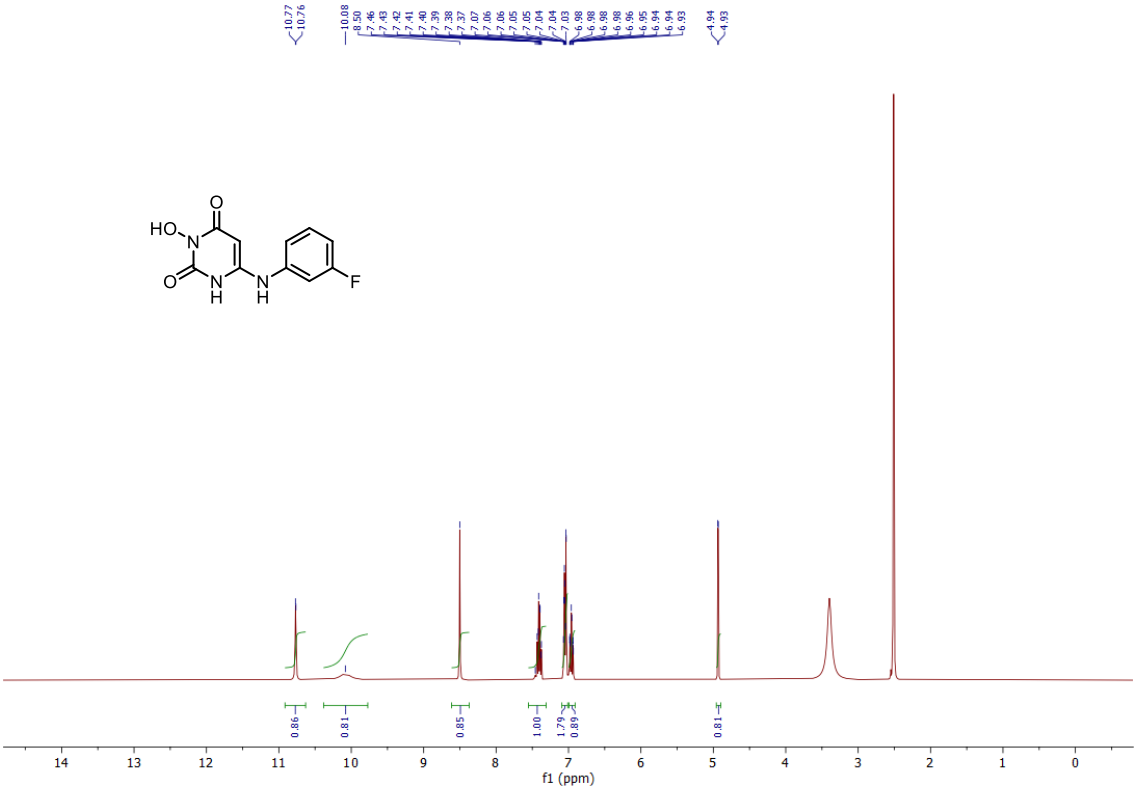


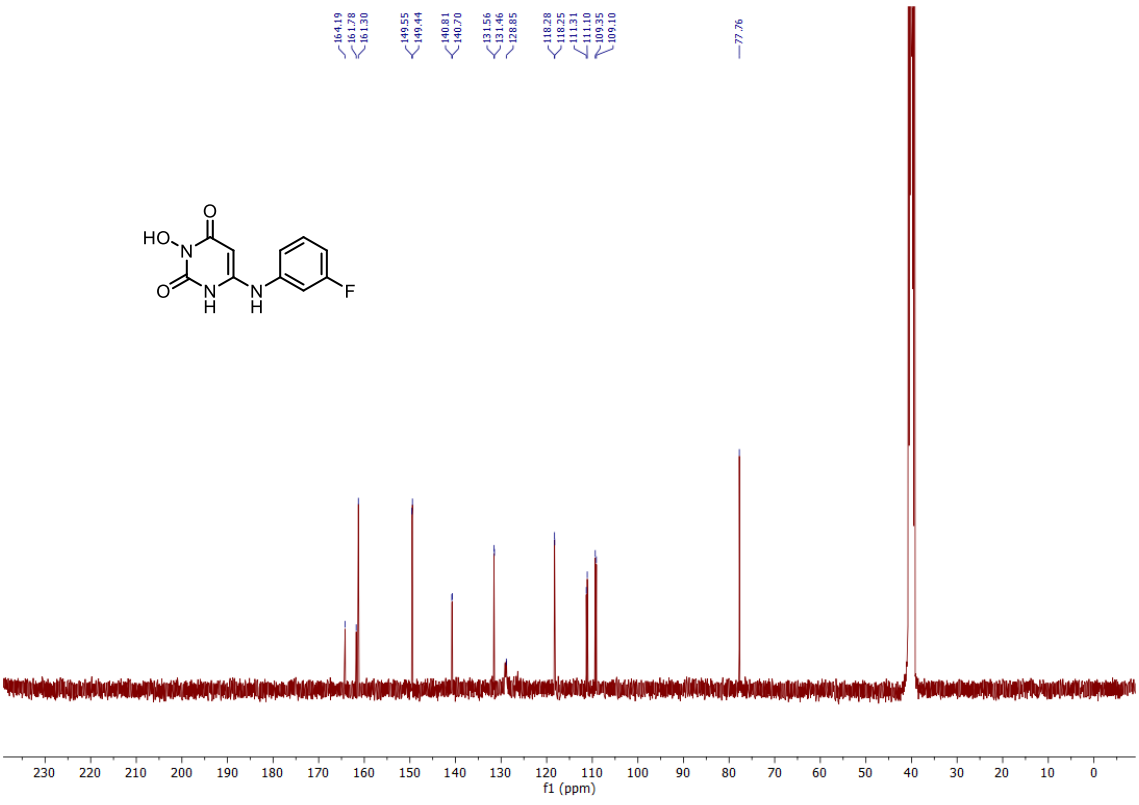
HRMS for Compound 2f

Sample Name	MS-03-237 depro	**Position**	P2-B9	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-237 depro_6378.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	7/23/2025 5:30:41 PM (UTC-05:00)



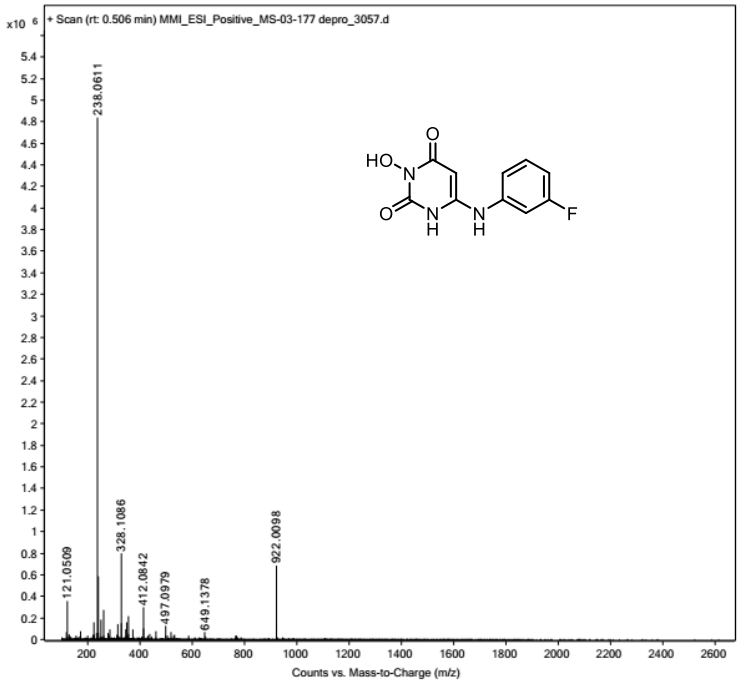
^{1}H NMR and ^{13}C NMR for Compound 2g in DMSO-d_6



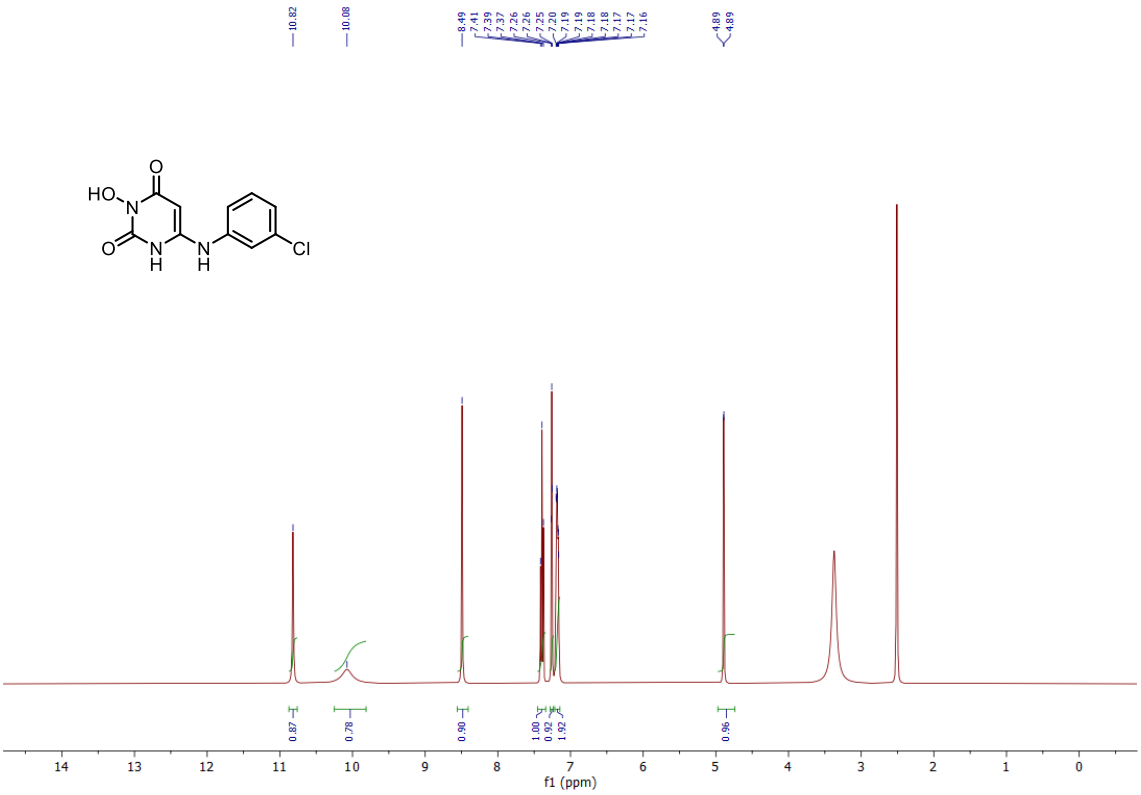


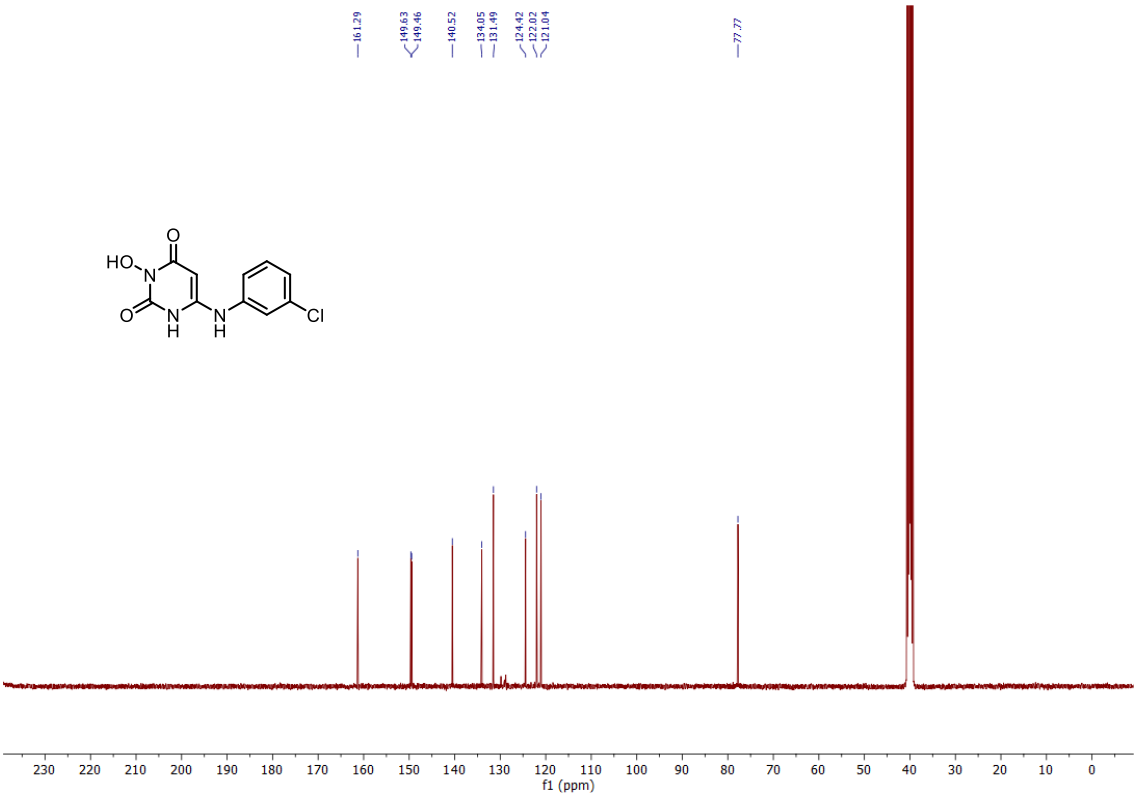
HRMS for Compound 2g

Sample Name	MS-03-177 depro	**Position**	P2-C7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-177 depro_3057.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	4/23/2025 6:30:24 PM (UTC-05:00)



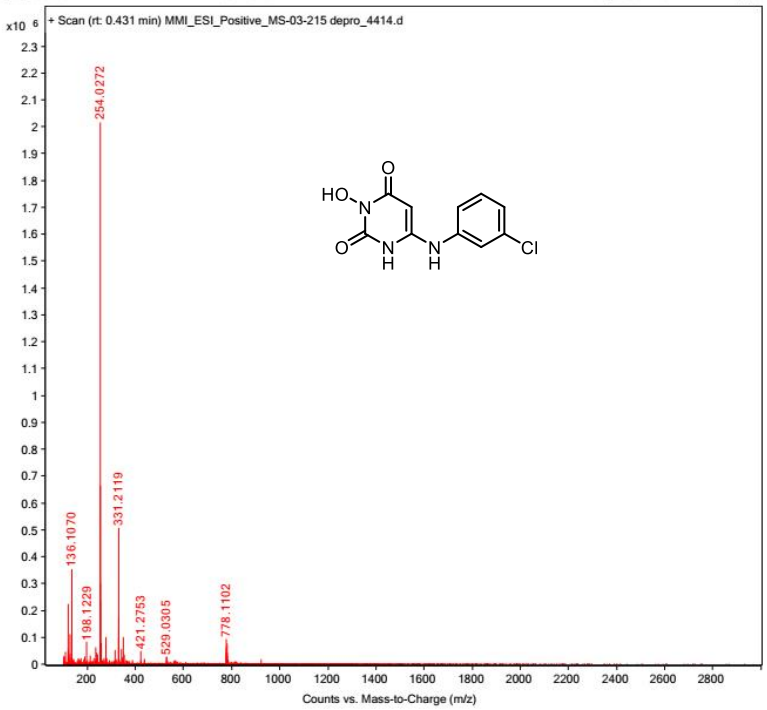
^{1}H NMR and ^{13}C NMR for Compound 2h in DMSO-d_6





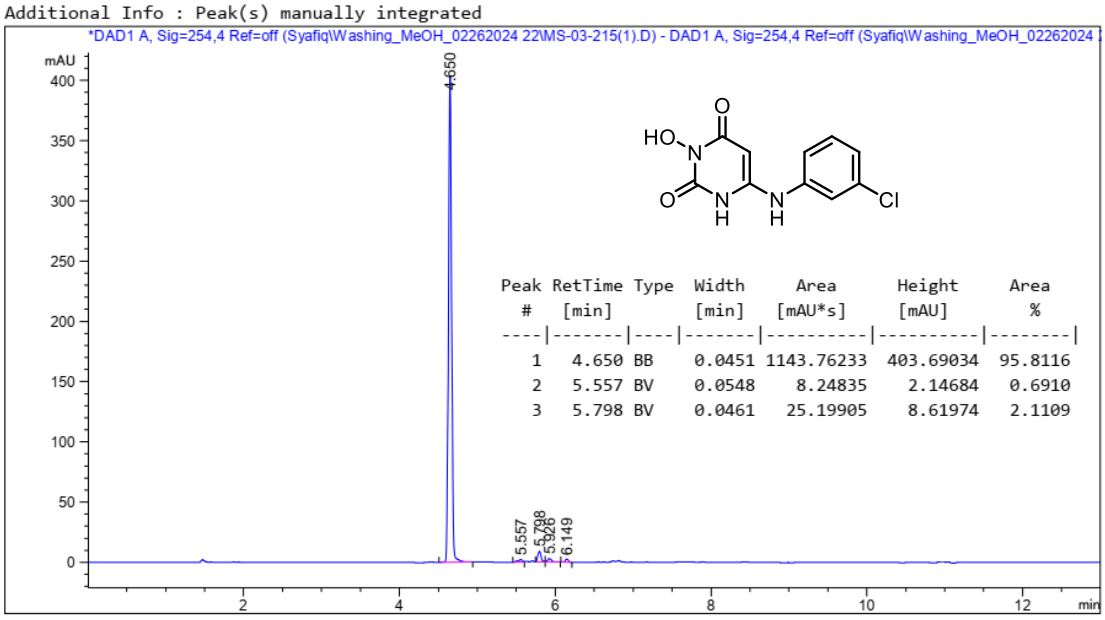
HRMS and HPLC Trace for Compound 2h

Sample Name	MS-03-215 depro	**Position**	P2-A9	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-215 depro_4414.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	6/2/2025 3:45:00 PM (UTC-05:00)



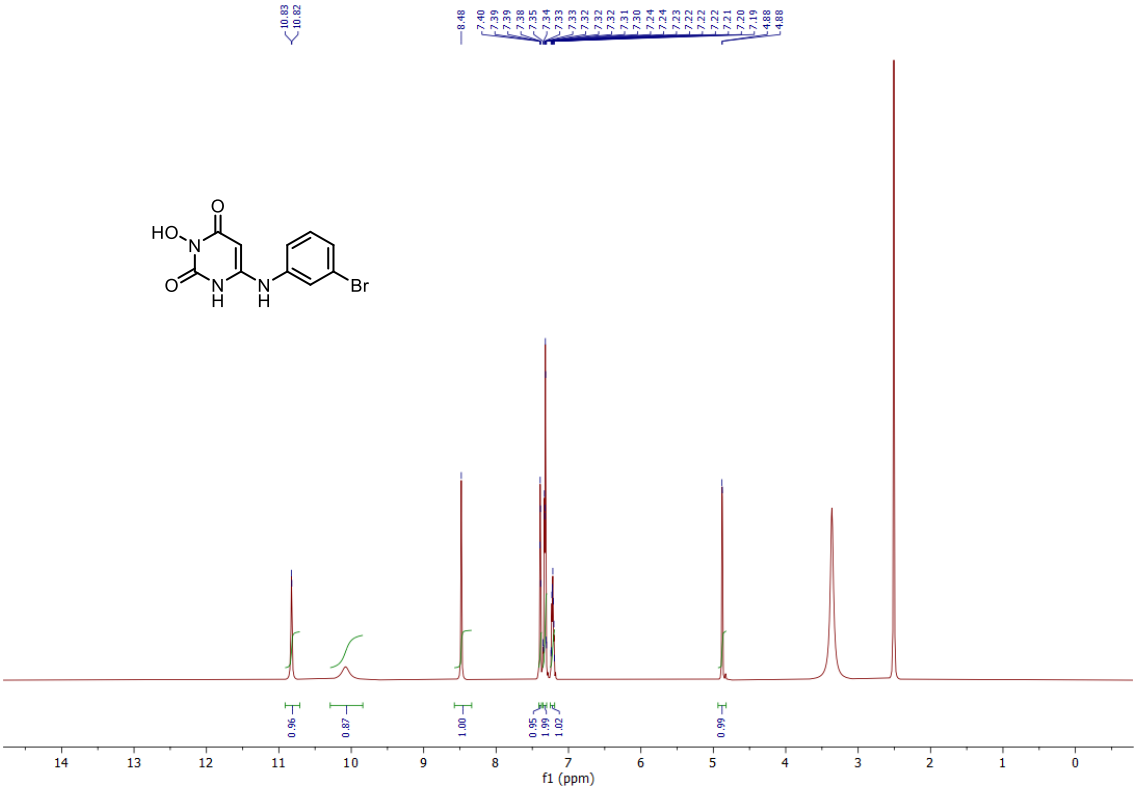
Sample Info : MS-03-215(1)

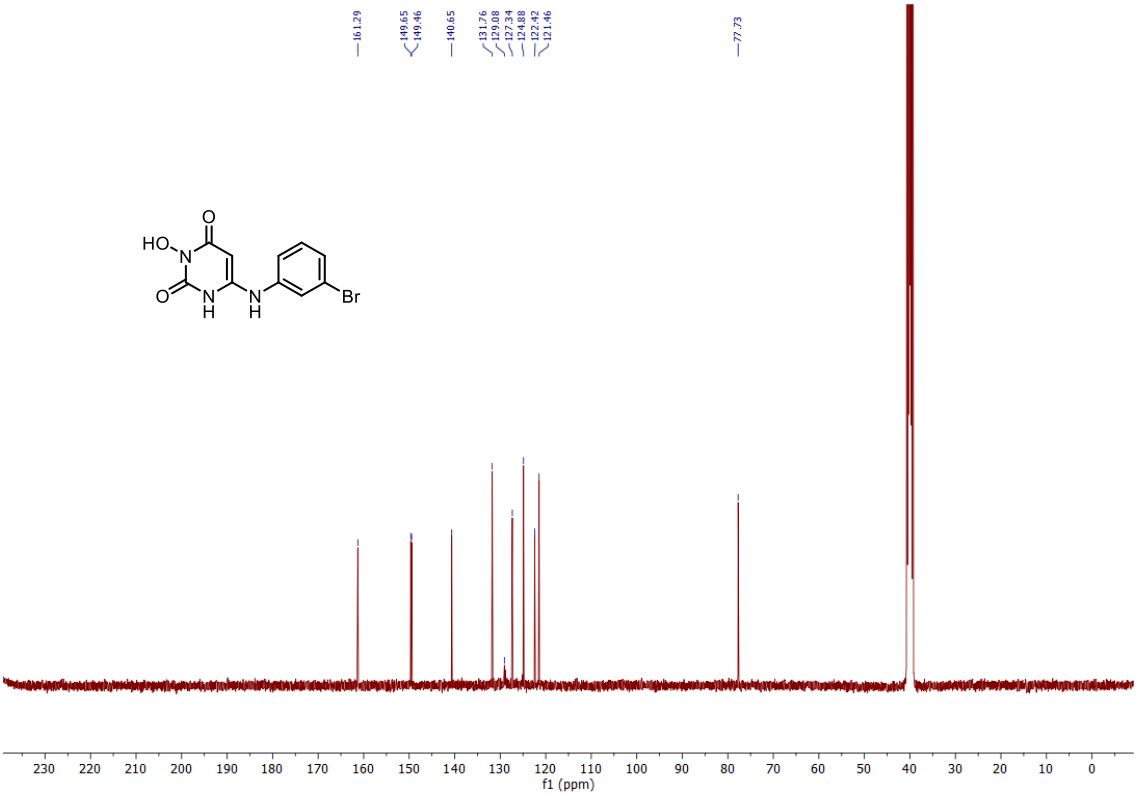
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.650	BB	0.0451	1143.76233	403.69034	95.8116
2	5.557	BV	0.0548	8.24835	2.14684	0.6910
3	5.798	BV	0.0461	25.19905	8.61974	2.1109

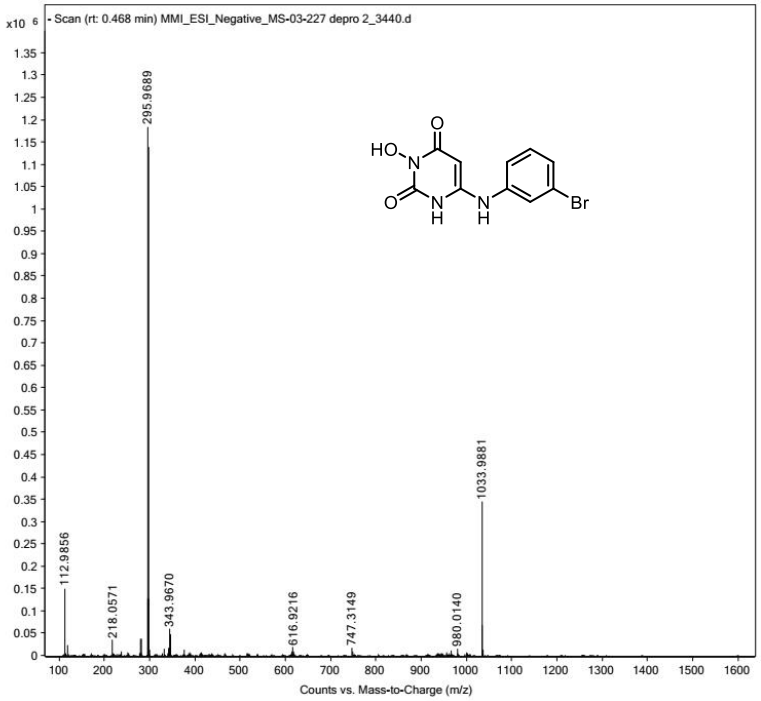
^{1}H NMR and ^{13}C NMR for Compound 2i in DMSO-d_6



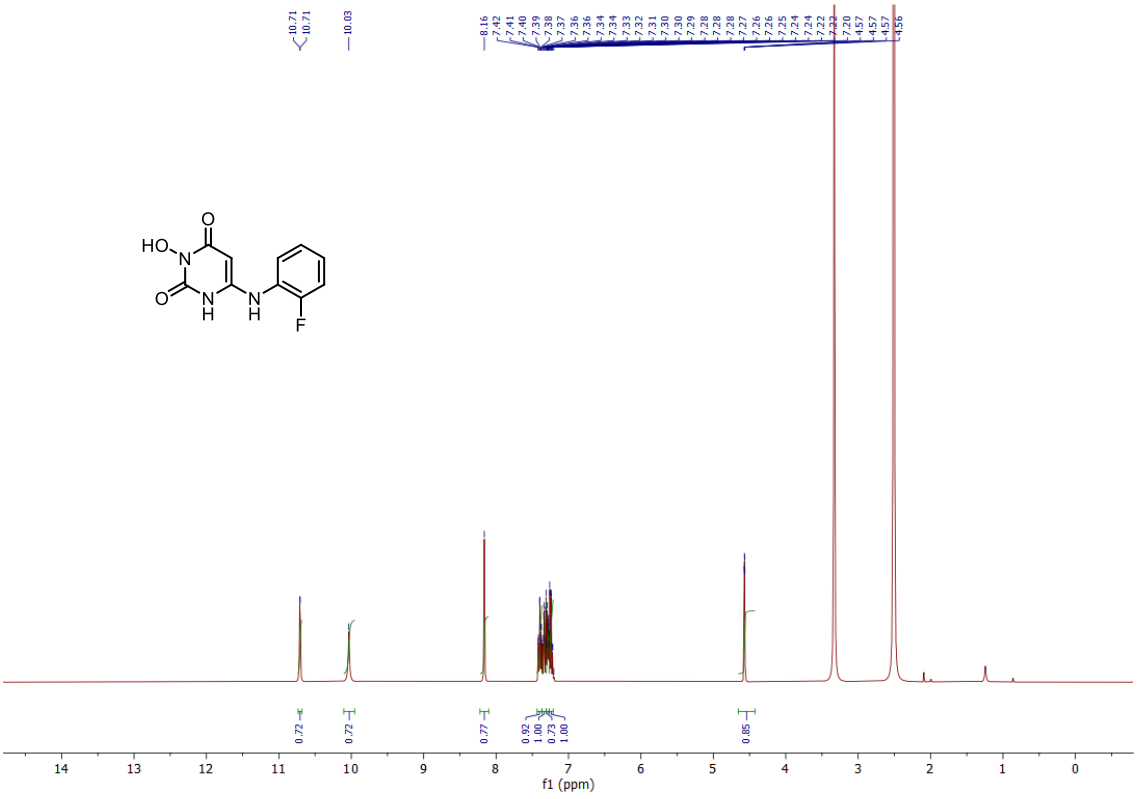


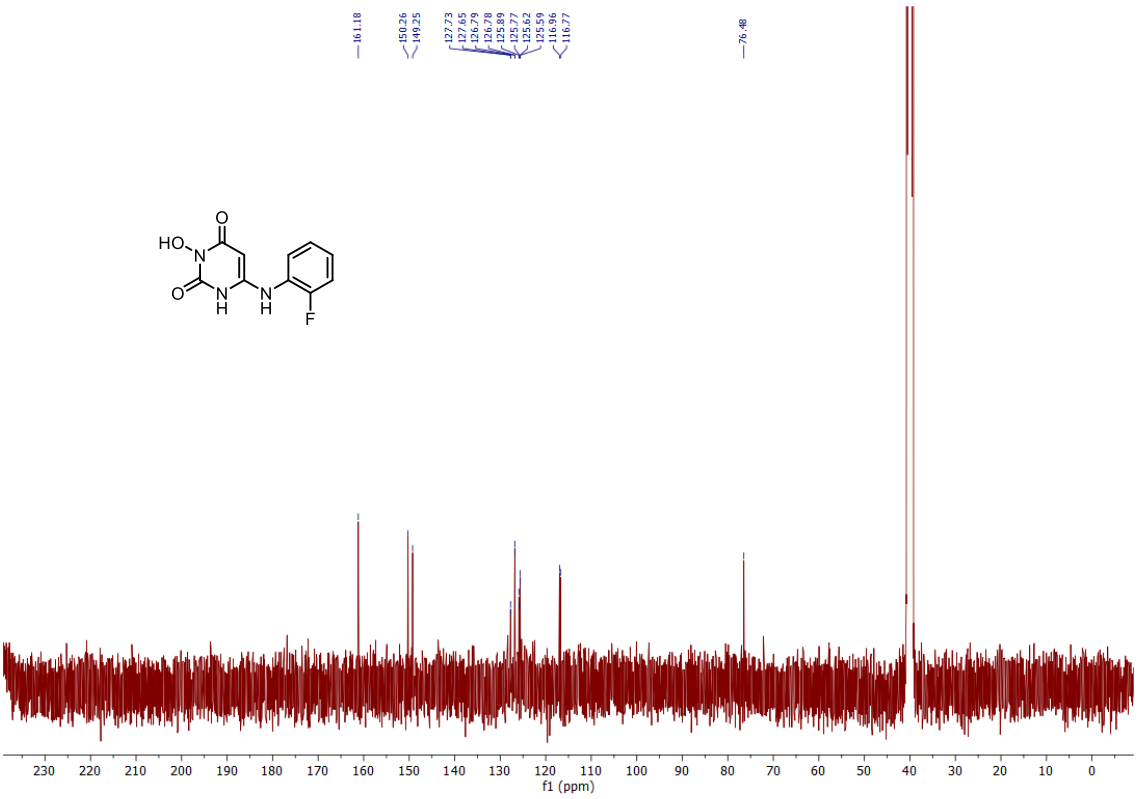
HRMS for Compound 2i

Sample Name	MS-03-227 depro 2	**Position**	P1-C7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-227 depro 2_3440.
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	5/7/2025 5:03:38 PM (UTC-05:00)



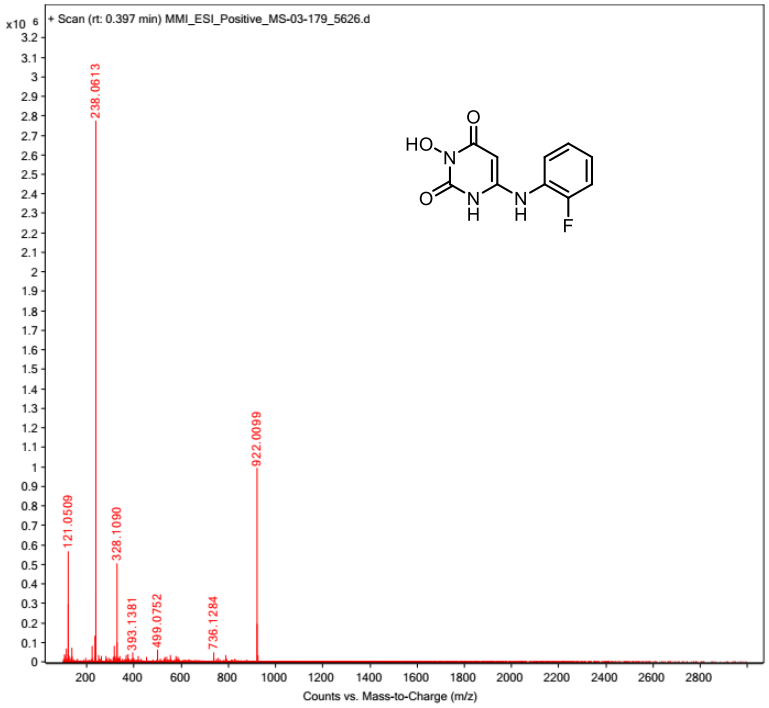
^{1}H NMR and ^{13}C NMR for Compound 2j in DMSO-d_6



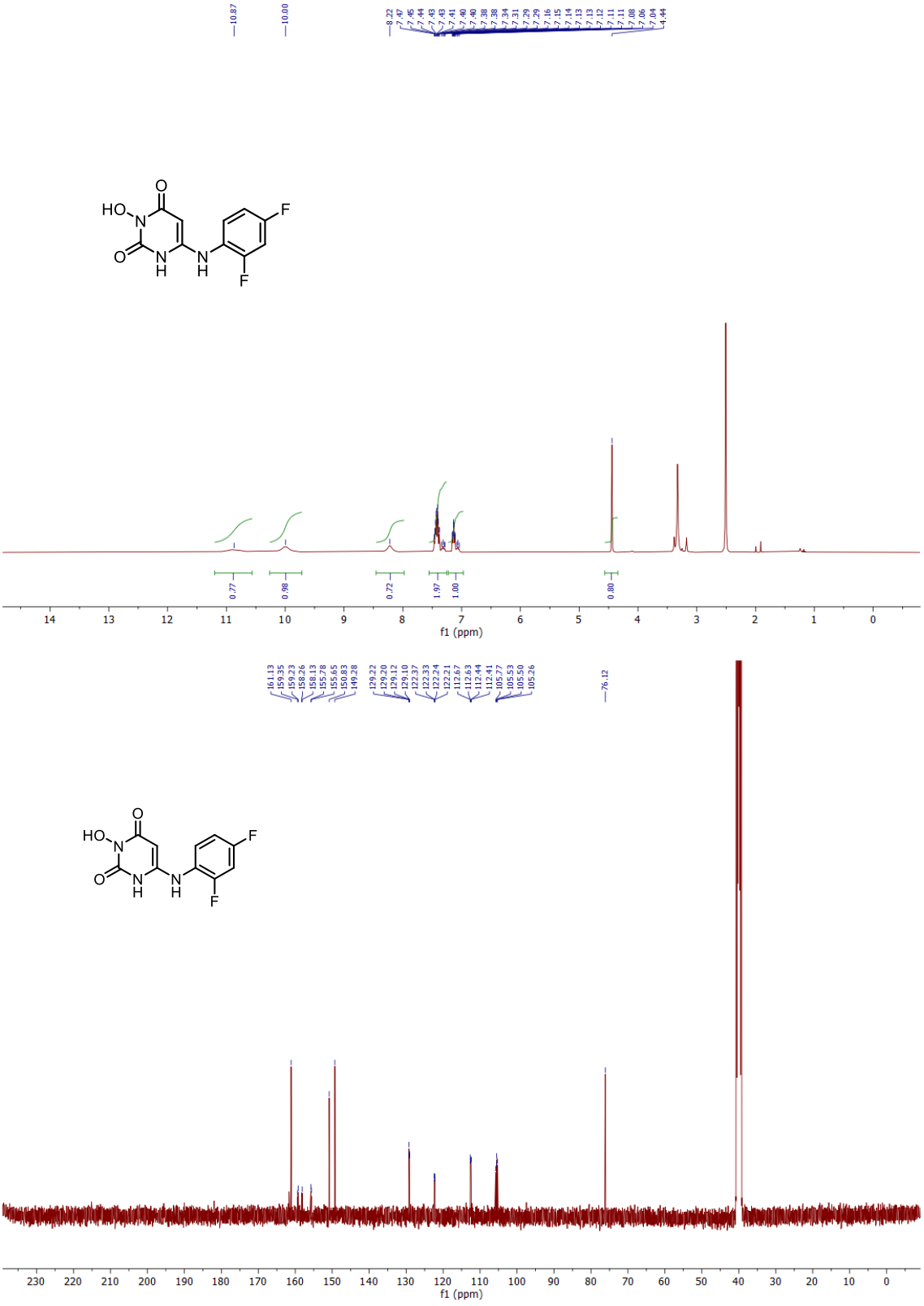


HRMS for Compound 2j

Sample Name	MS-03-179	**Position**	P2-A3	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-179_5626.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	7/2/2025 2:15:28 PM (UTC-05:00)

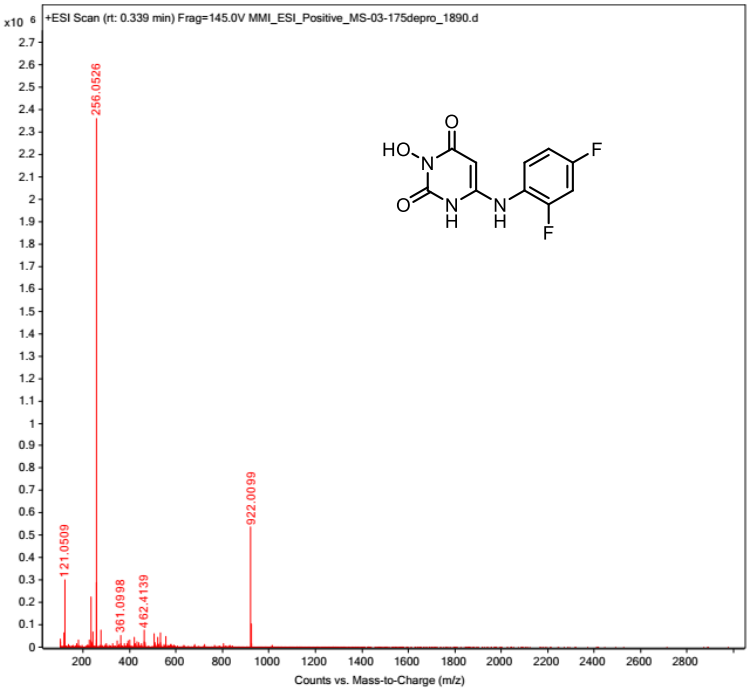


^{1}H NMR and ^{13}C NMR for Compound 2k in DMSO-d_6

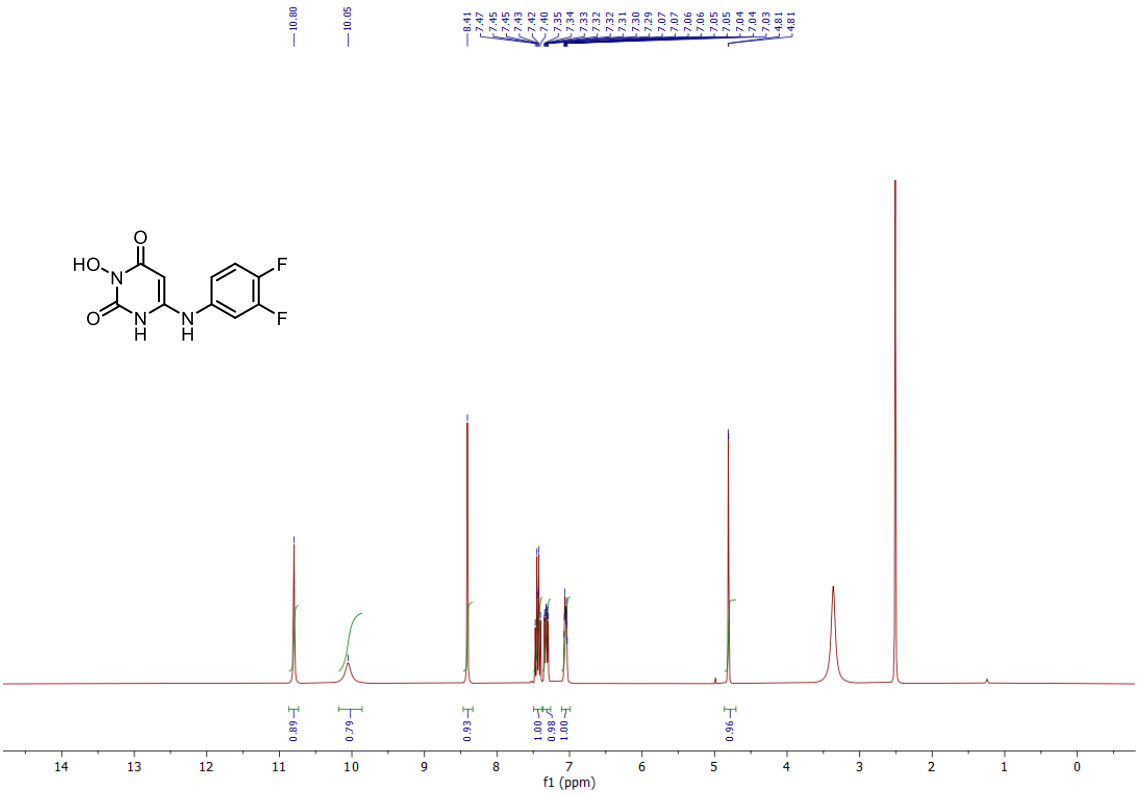


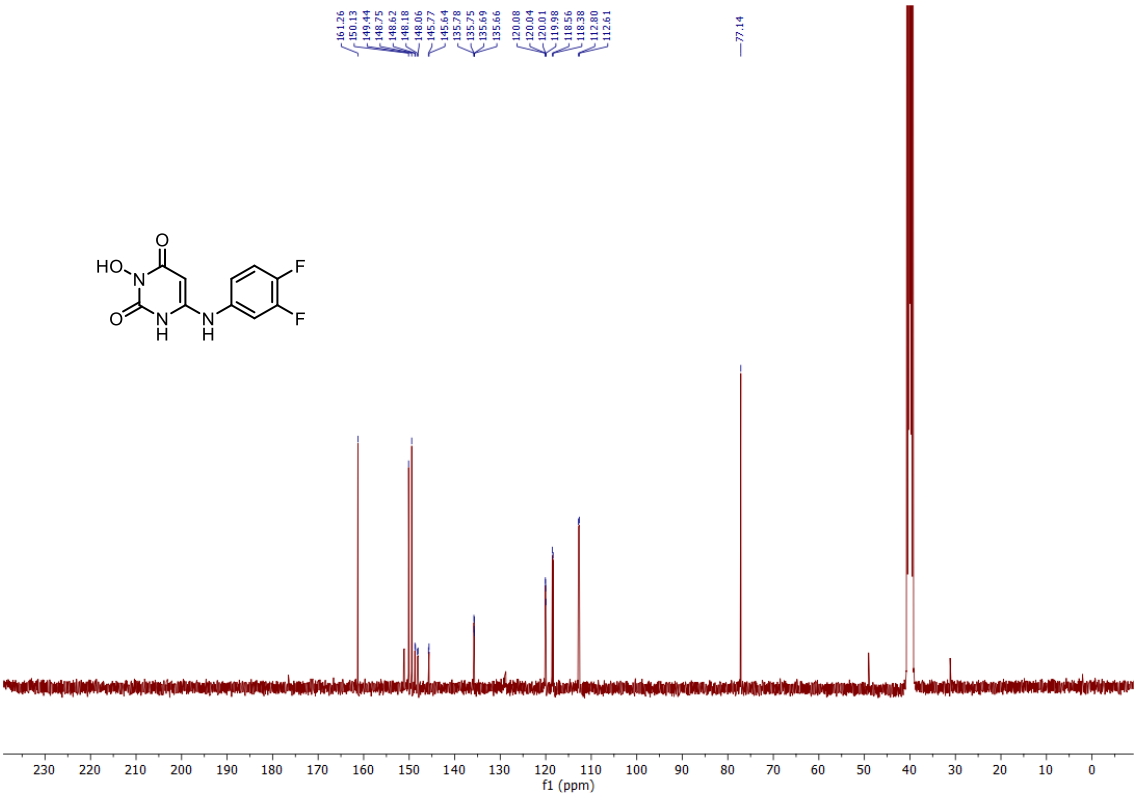
HRMS for Compound 2k

Sample Name	MS-03-175depro	**Position**	P2-B8	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-175depro_1890.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	3/7/2025 9:38:16 AM (UTC-06:00)



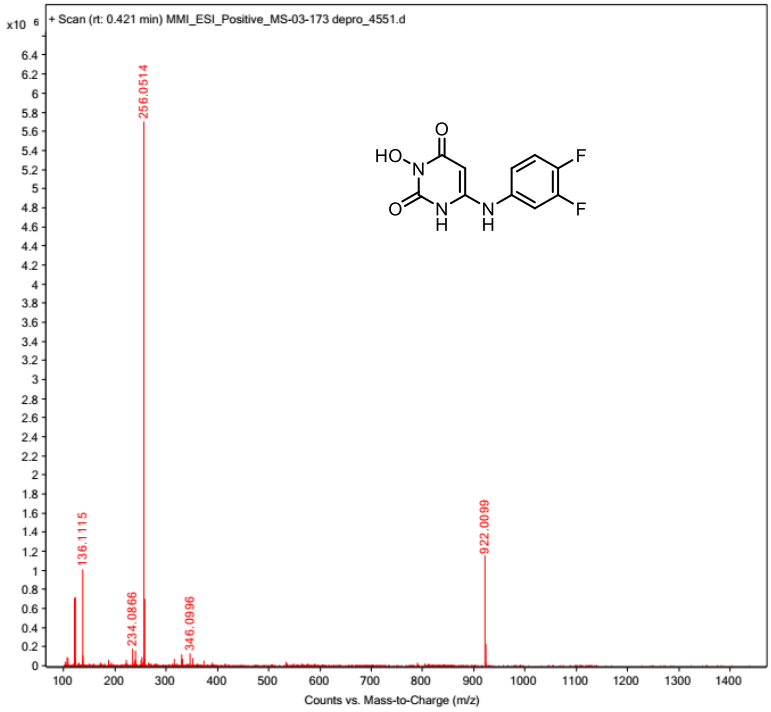
^{1}H NMR and ^{13}C NMR for Compound 2l in DMSO-d_6





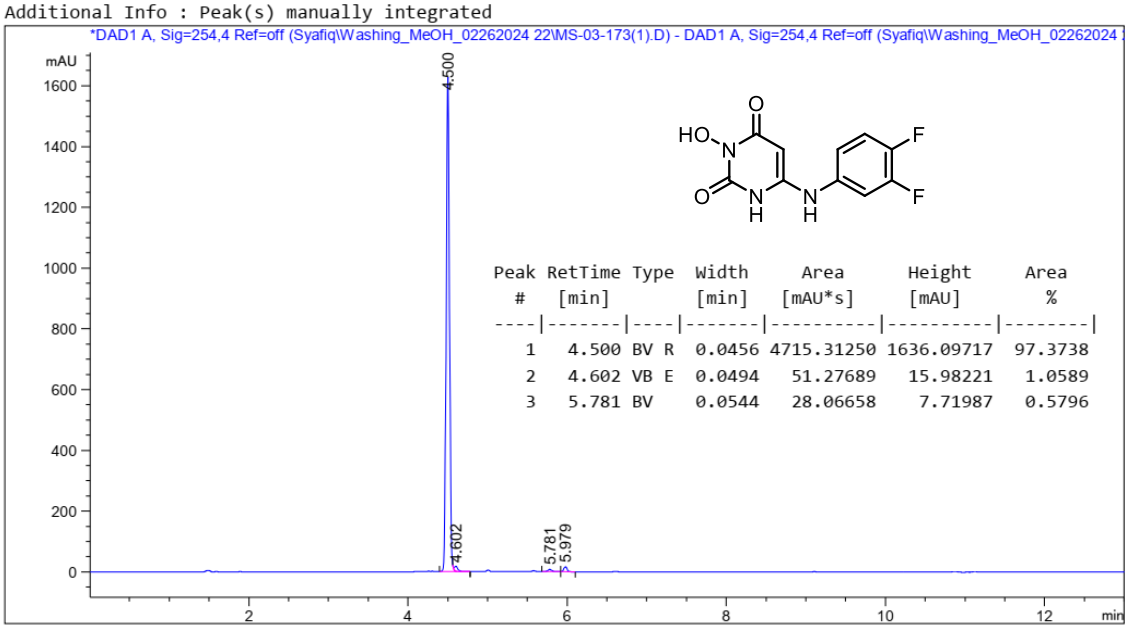
HRMS and HPLC Trace for Compound 2l

Sample Name	MS-03-173 depro	**Position**	P2-F7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-173 depro_4551.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	6/4/2025 5:34:58 PM (UTC-05:00)



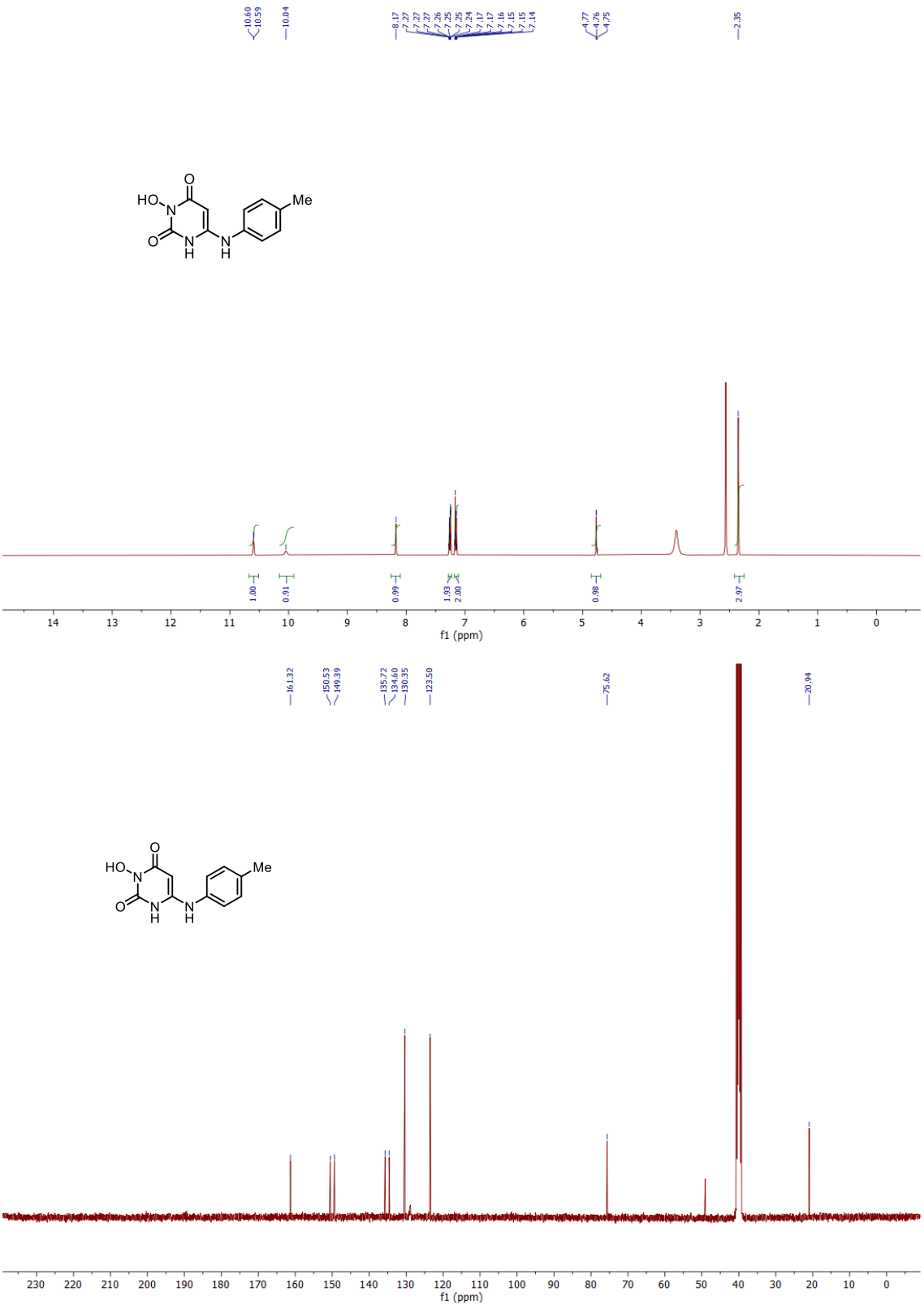
Sample Info : MS-03-173(1)

Additional Info : Peak(s) manually integrated



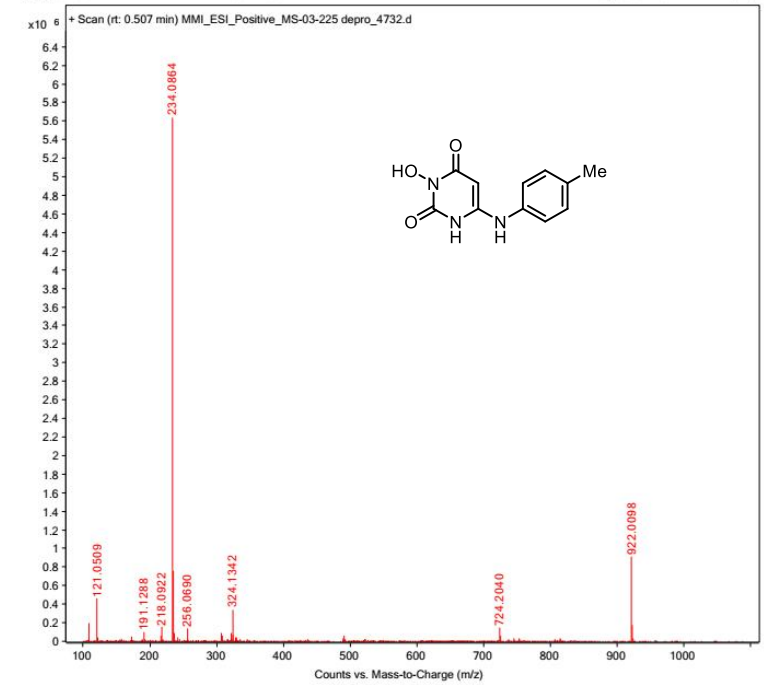
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.500	BV R	0.0456	4715.31250	1636.09717	97.3738
2	4.602	VB E	0.0494	51.27689	15.98221	1.0589
3	5.781	BV	0.0544	28.06658	7.71987	0.5796

^{1}H NMR and ^{13}C NMR for Compound 2m in DMSO-d_6

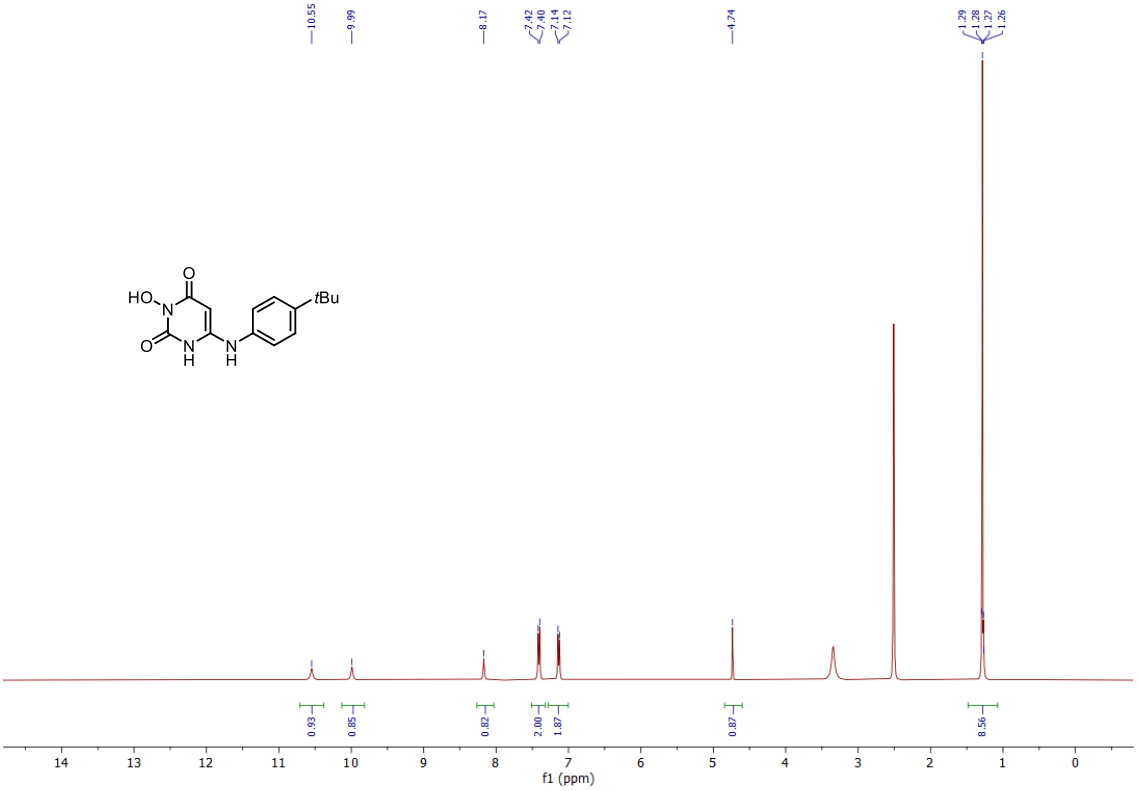


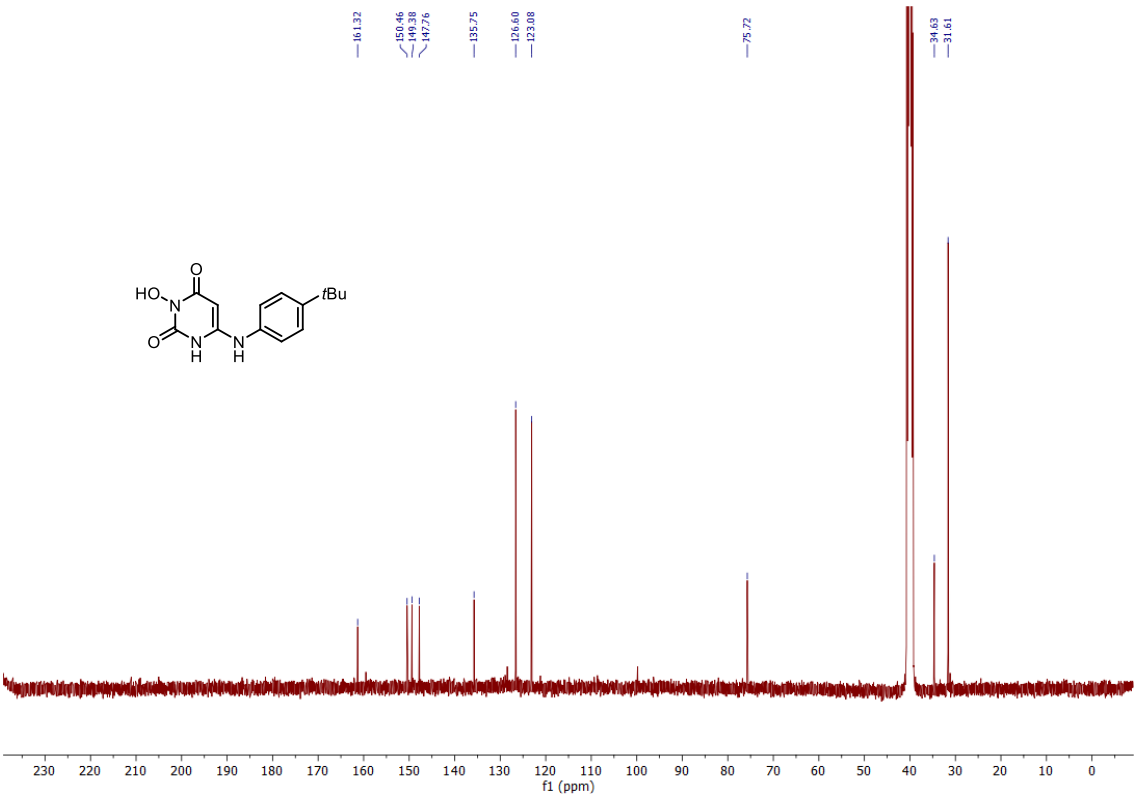
HRMS for Compound 2m

Sample Name	MS-03-225 depro	**Position**	P1-F4	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-225 depro_4732.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	6/10/2025 4:51:10 PM (UTC-05:00)



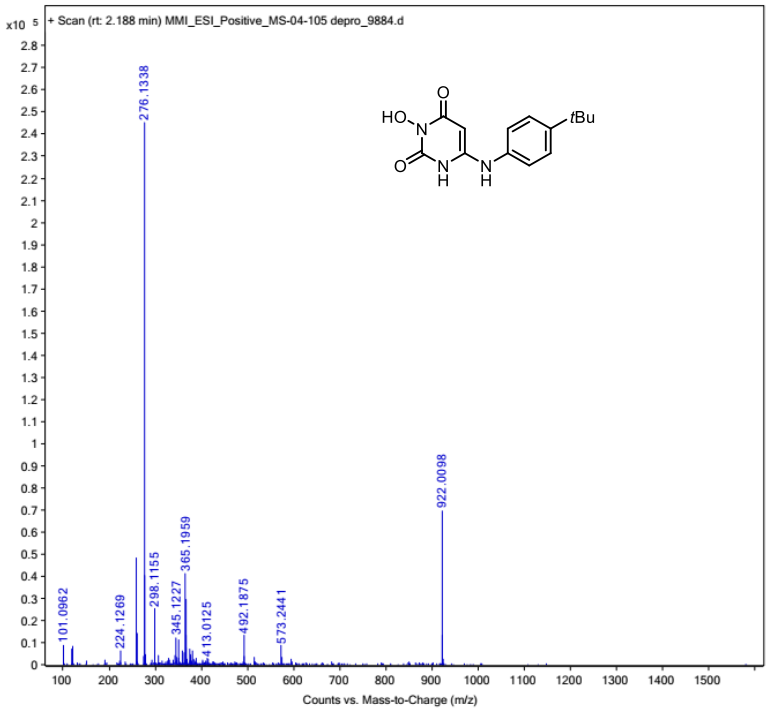
^{1}H NMR and ^{13}C NMR for Compound 2n in DMSO-d_6



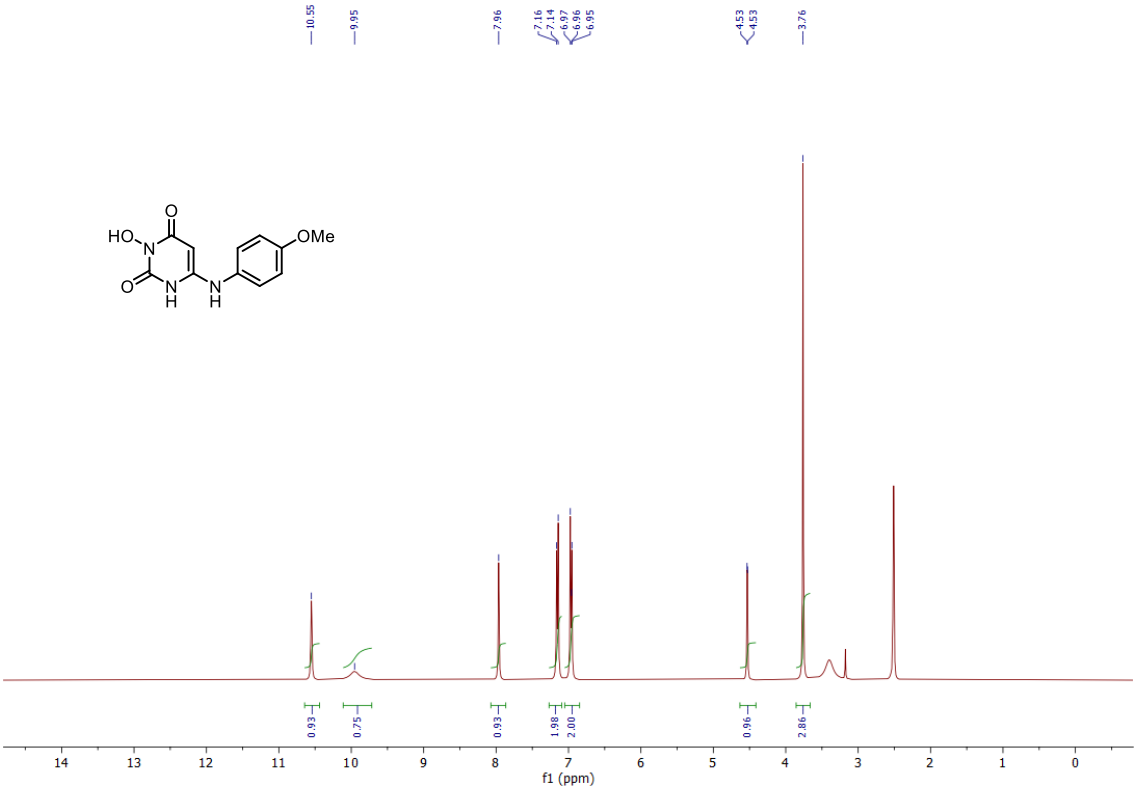


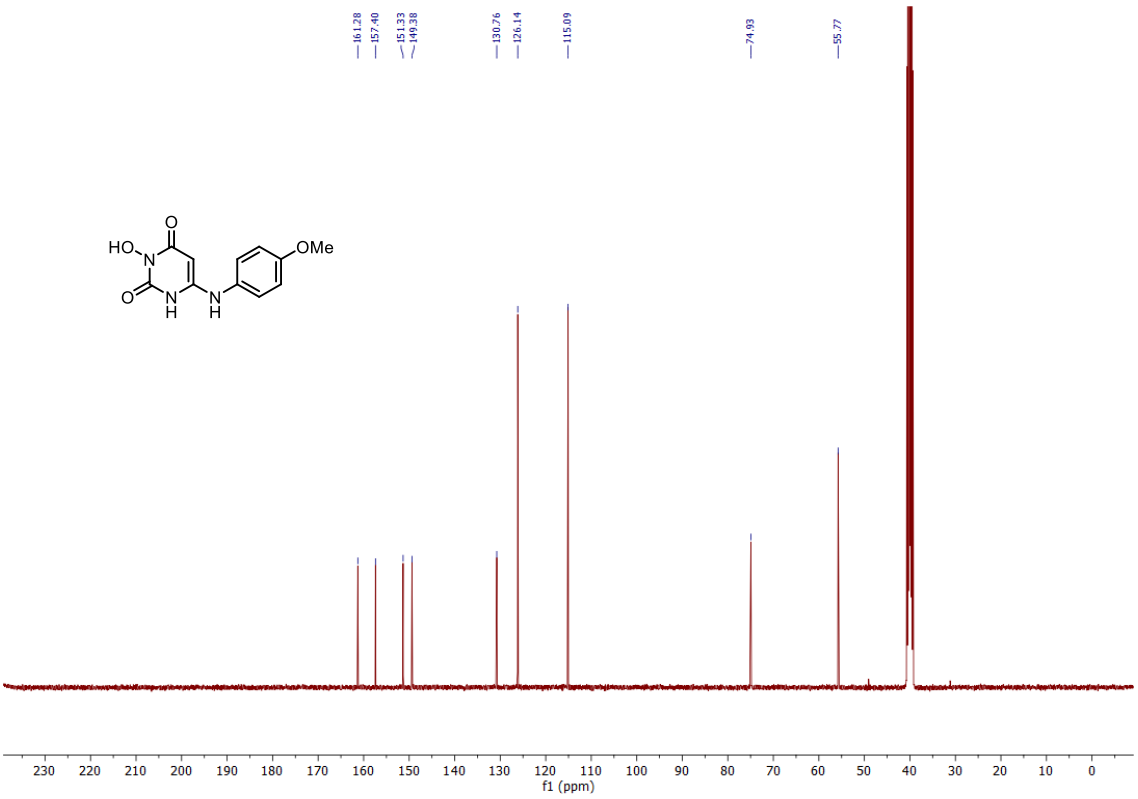
HRMS for Compound 2n

Sample Name	MS-04-105 depro	**Position**	P2-E10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-105 depro_9884.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	11/12/2025 7:15:49 PM (UTC-06:00)



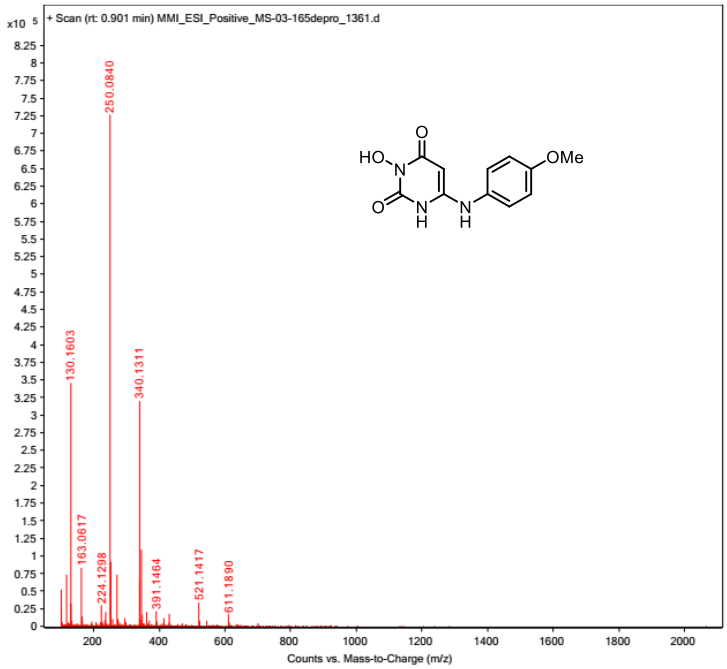
^{1}H NMR and ^{13}C NMR for Compound 2o in DMSO-d_6



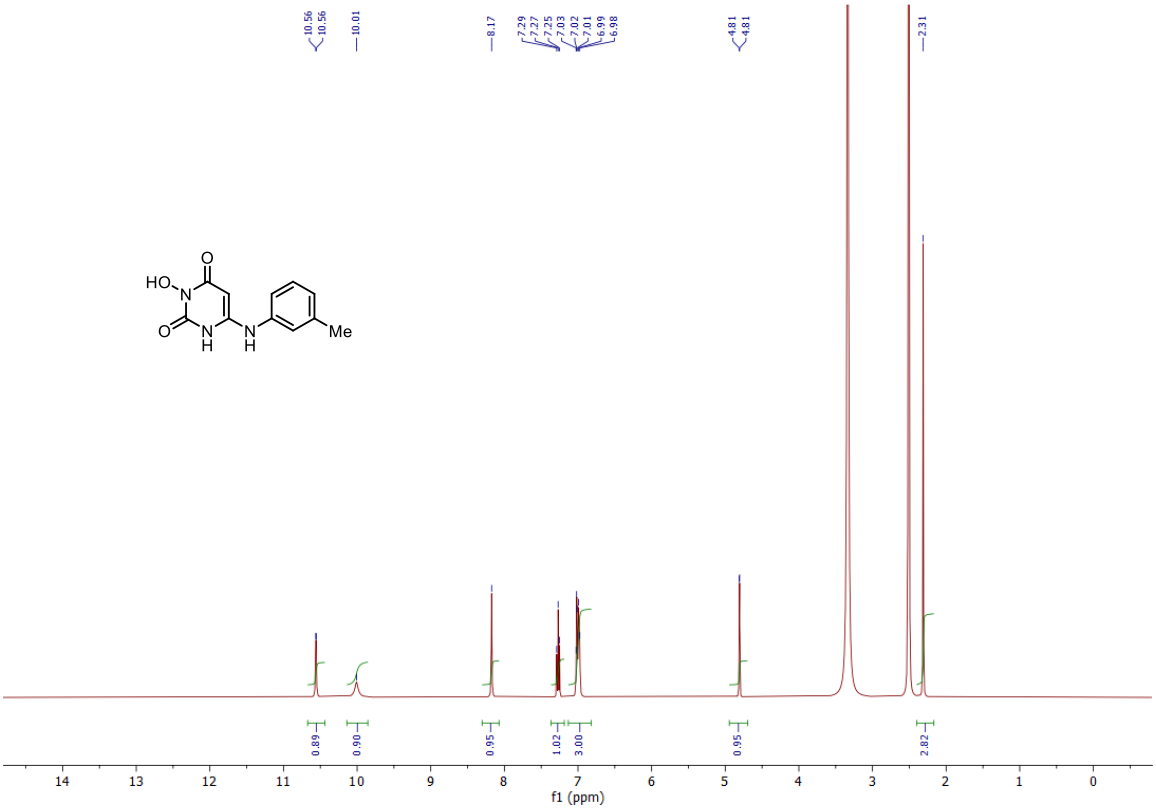


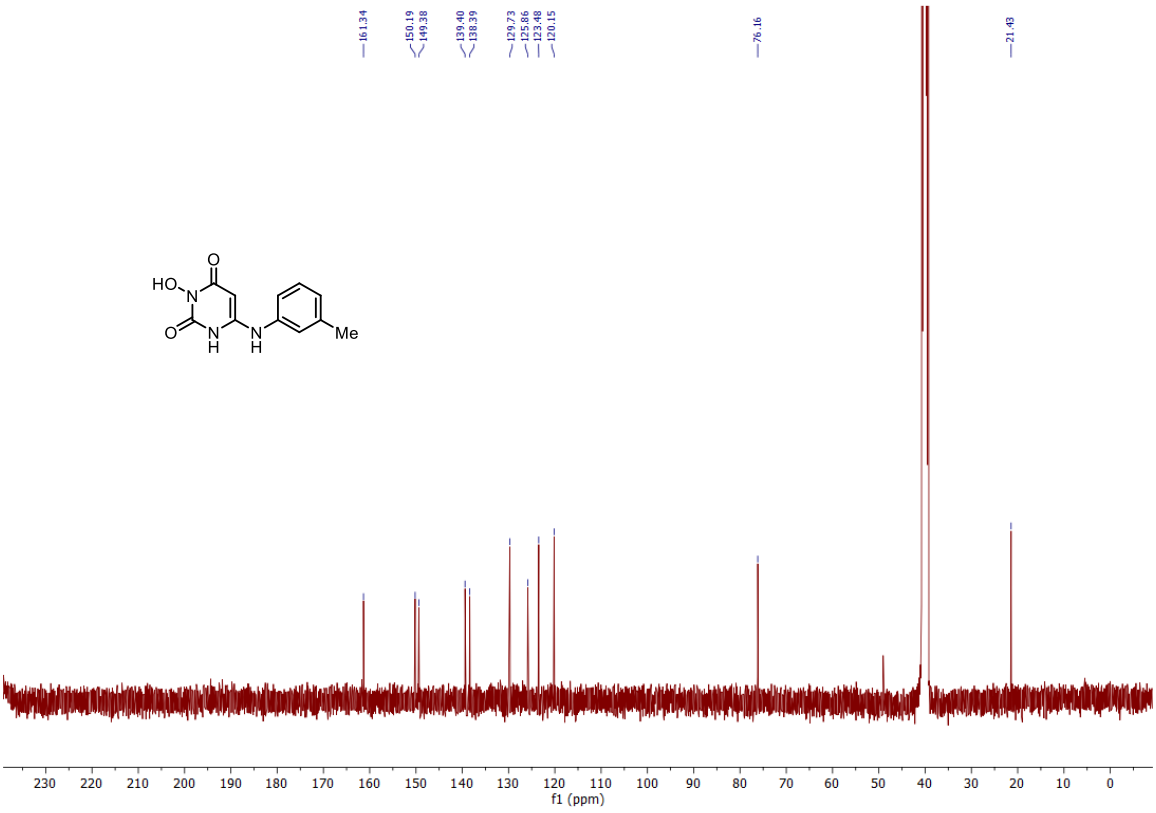
HRMS for Compound 2o

Sample Name	MS-03-165depro	**Position**	P2-E1	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-165depro_1361.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	2/13/2025 11:13:21 AM (UTC-06:00)



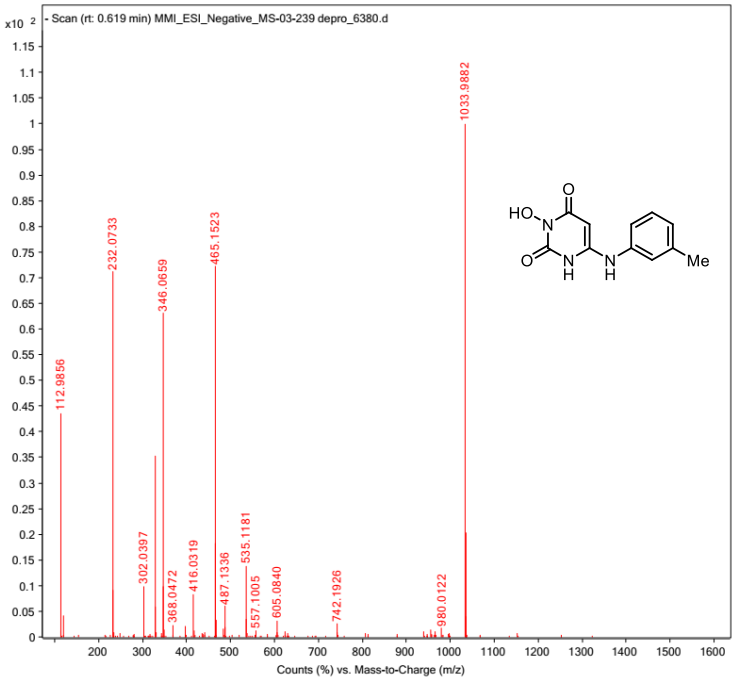
^{1}H NMR and ^{13}C NMR for Compound 2p in DMSO-d_6



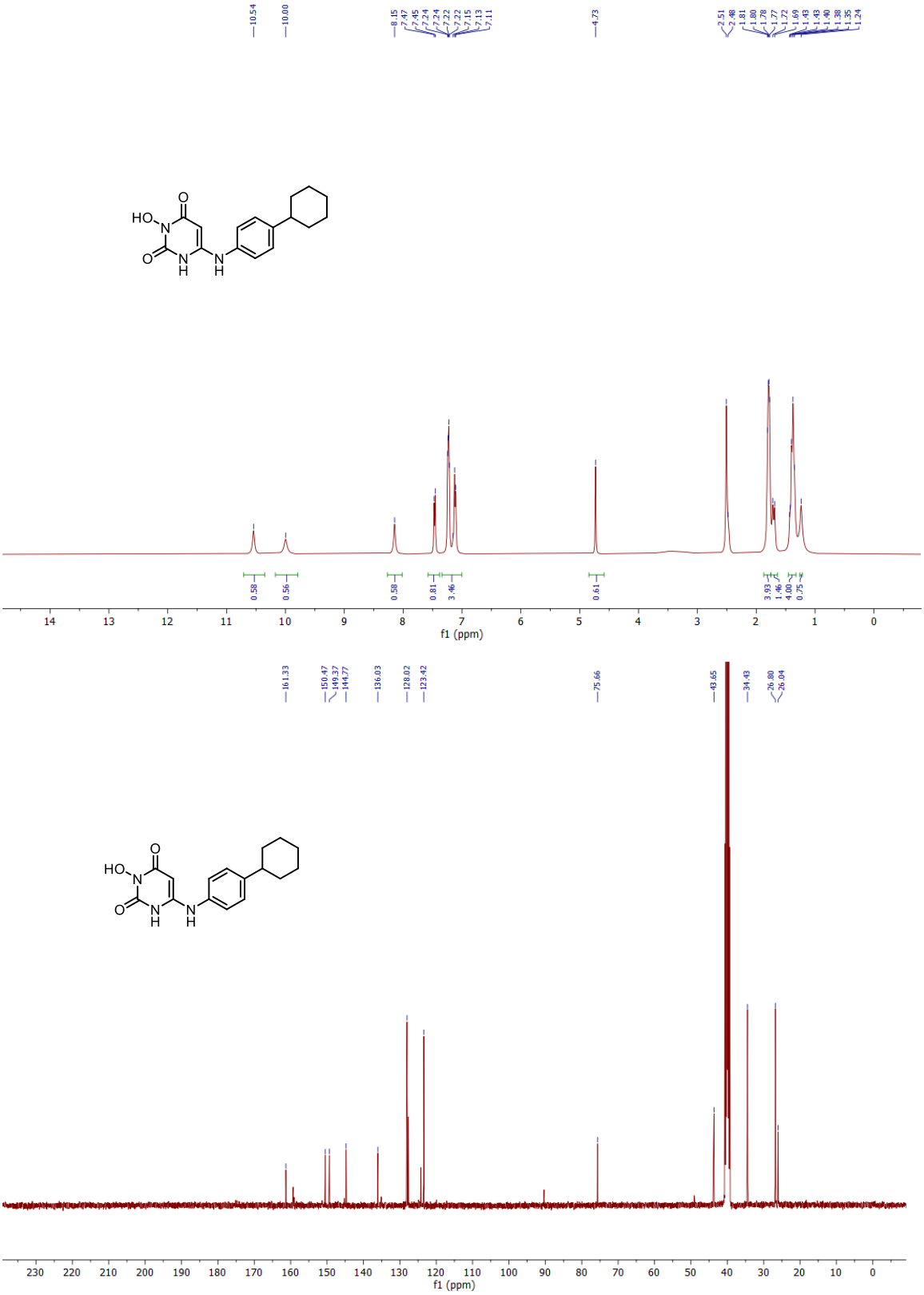


HRMS for Compound 2p

Sample Name	MS-03-239 depro	**Position**	P2-B10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-239 depro_6380.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	7/23/2025 5:42:29 PM (UTC-05:00)

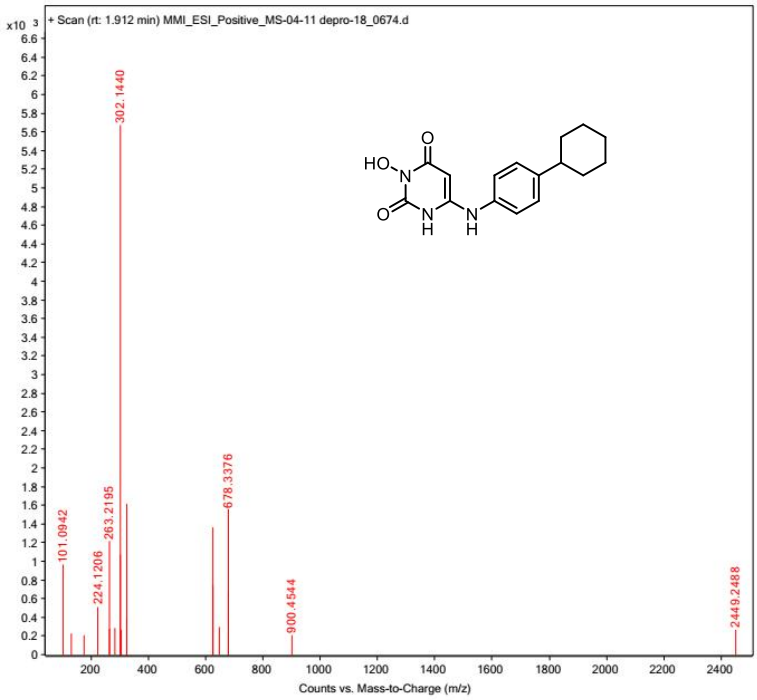


^{1}H NMR and ^{13}C NMR for Compound 2q in DMSO-d_6

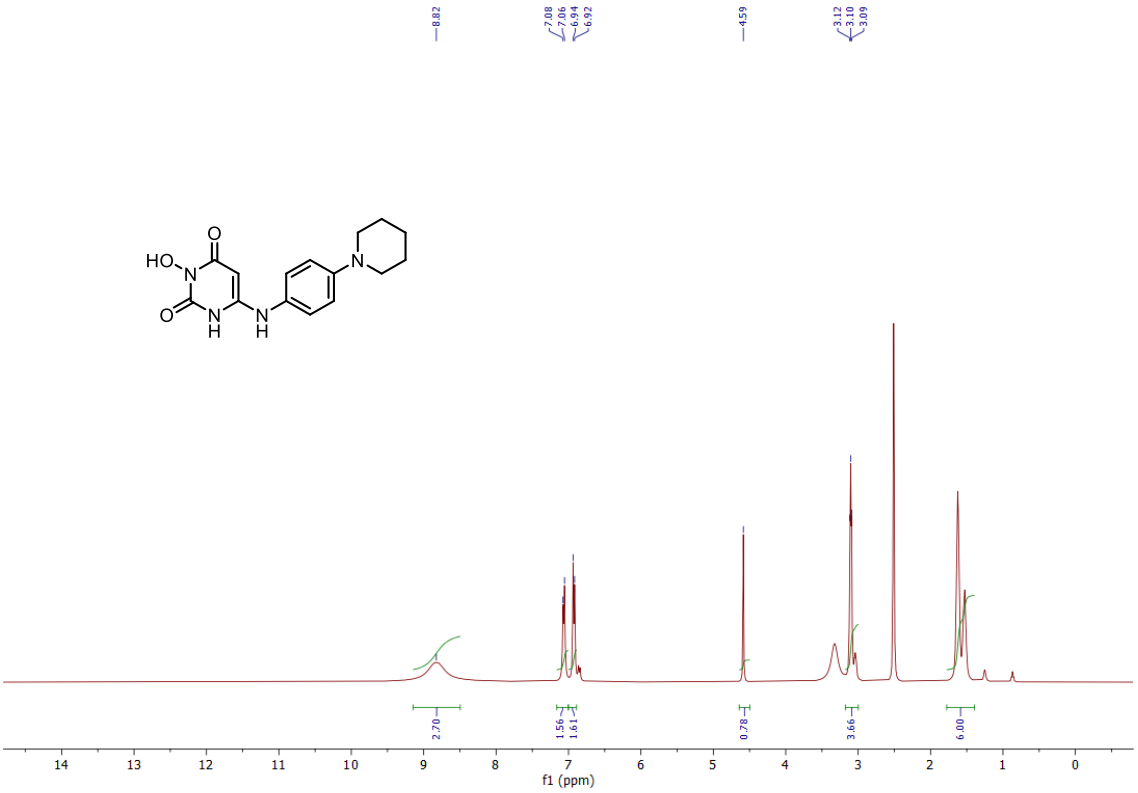


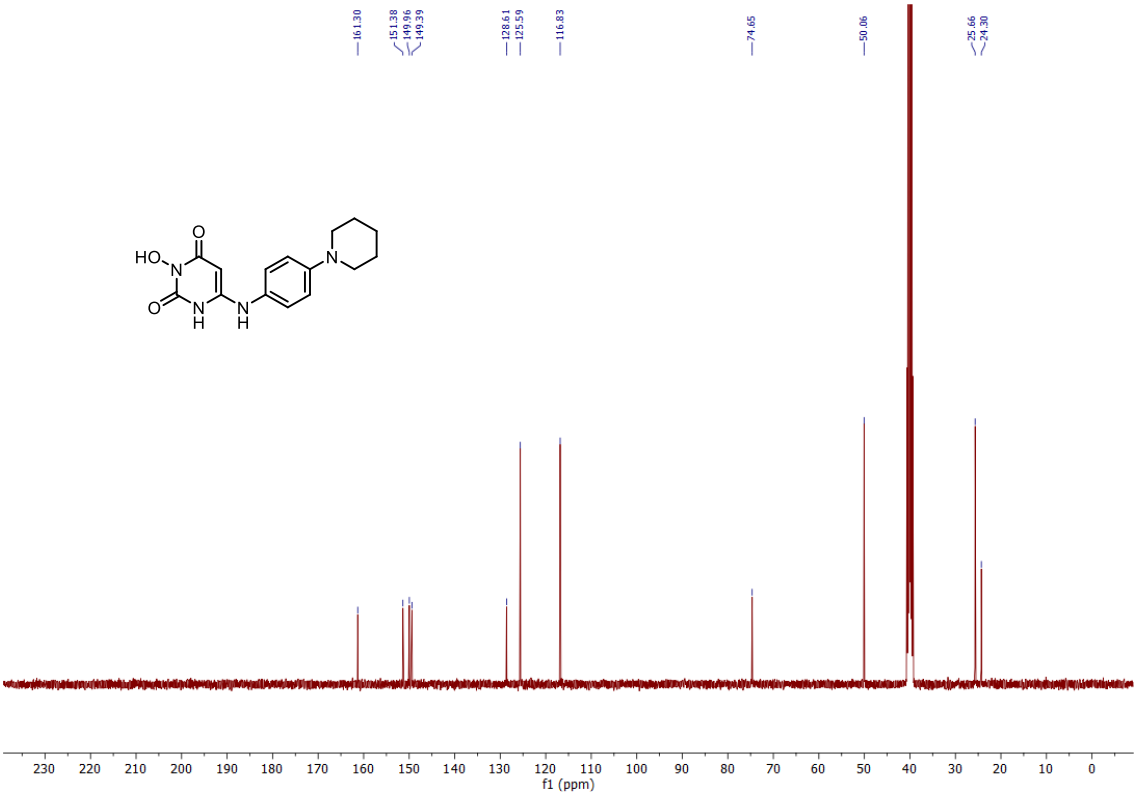
HRMS for Compound 2q

Sample Name	MS-04-11 depro-18	**Position**	P2-A7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-11 depro-18_0674.
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	12/17/2025 3:02:19 PM (UTC-06:00)



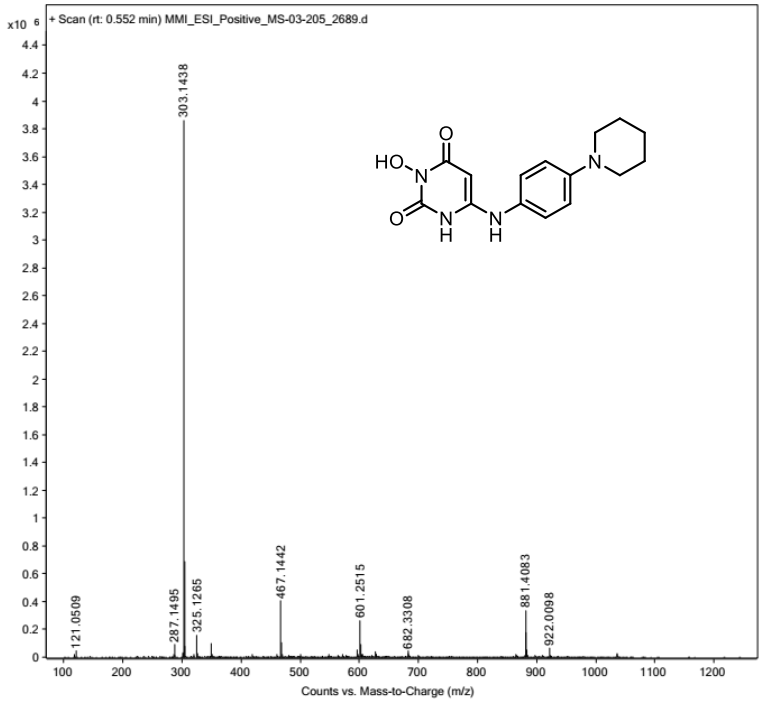
^{1}H NMR and ^{13}C NMR for Compound 2r in DMSO-d_6



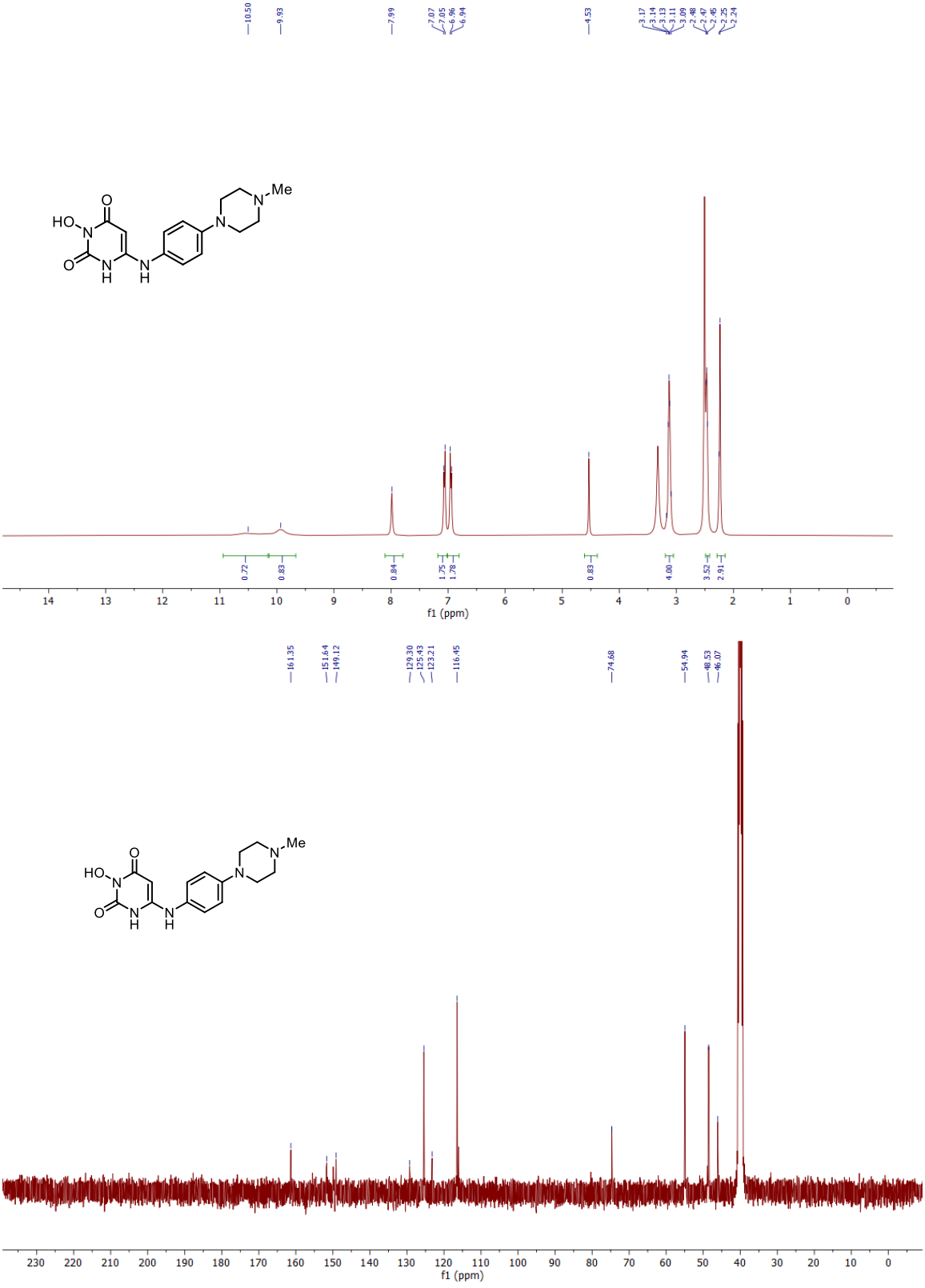


HRMS for Compound 2r

Sample Name	MS-03-205	**Position**	P1-F3	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-205_2689.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	4/14/2025 11:09:20 AM (UTC-05:00)

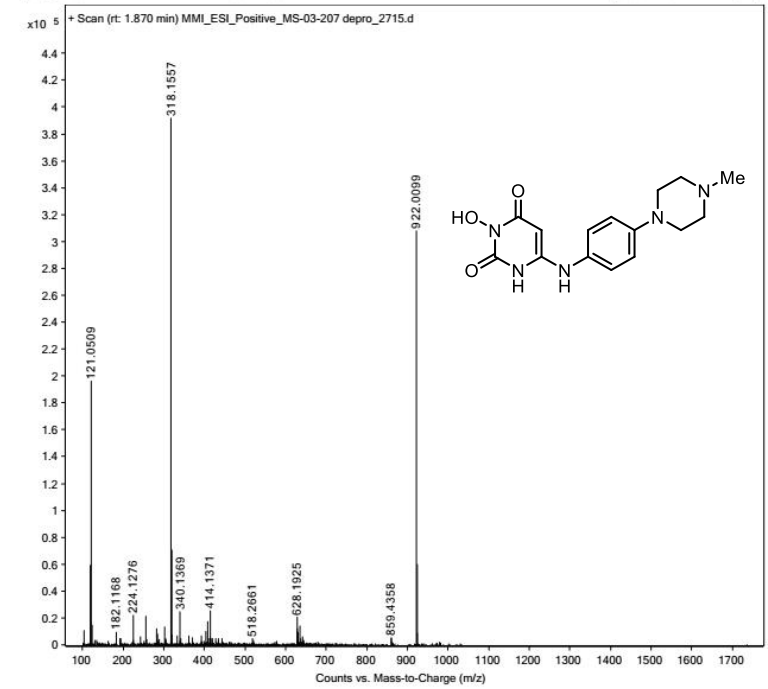


^{1}H NMR and ^{13}C NMR for Compound 2s in DMSO-d_6

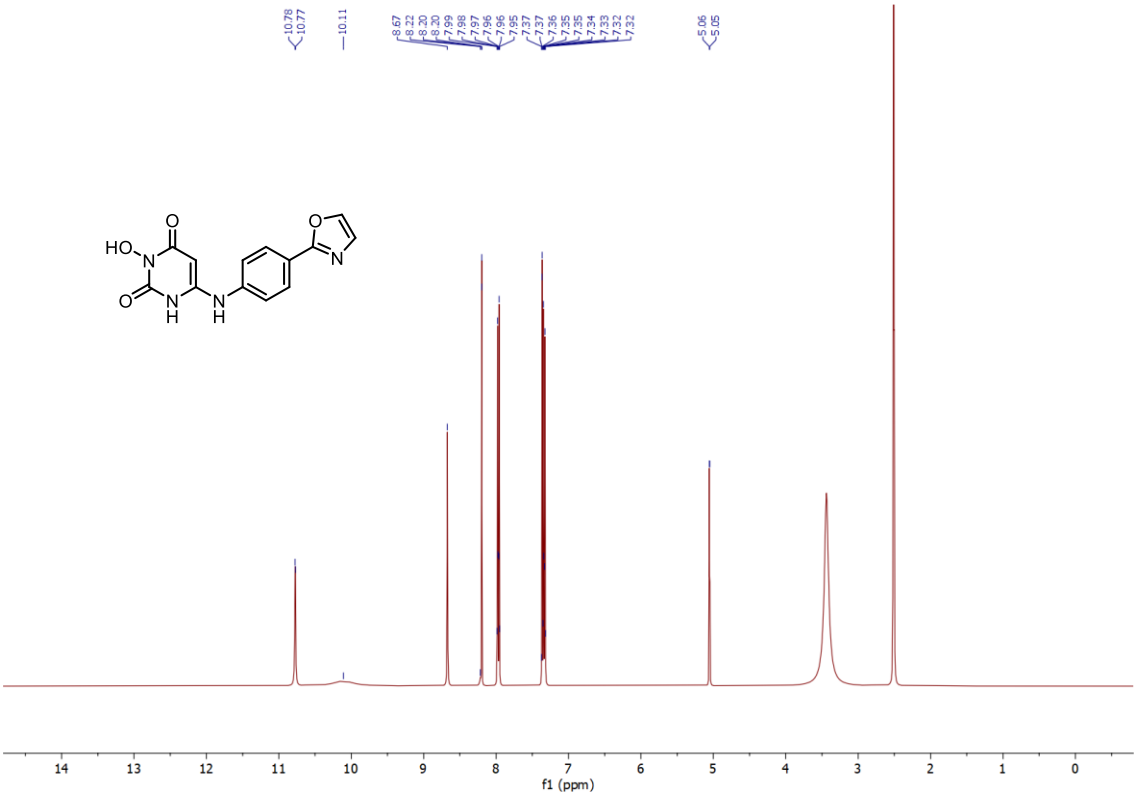


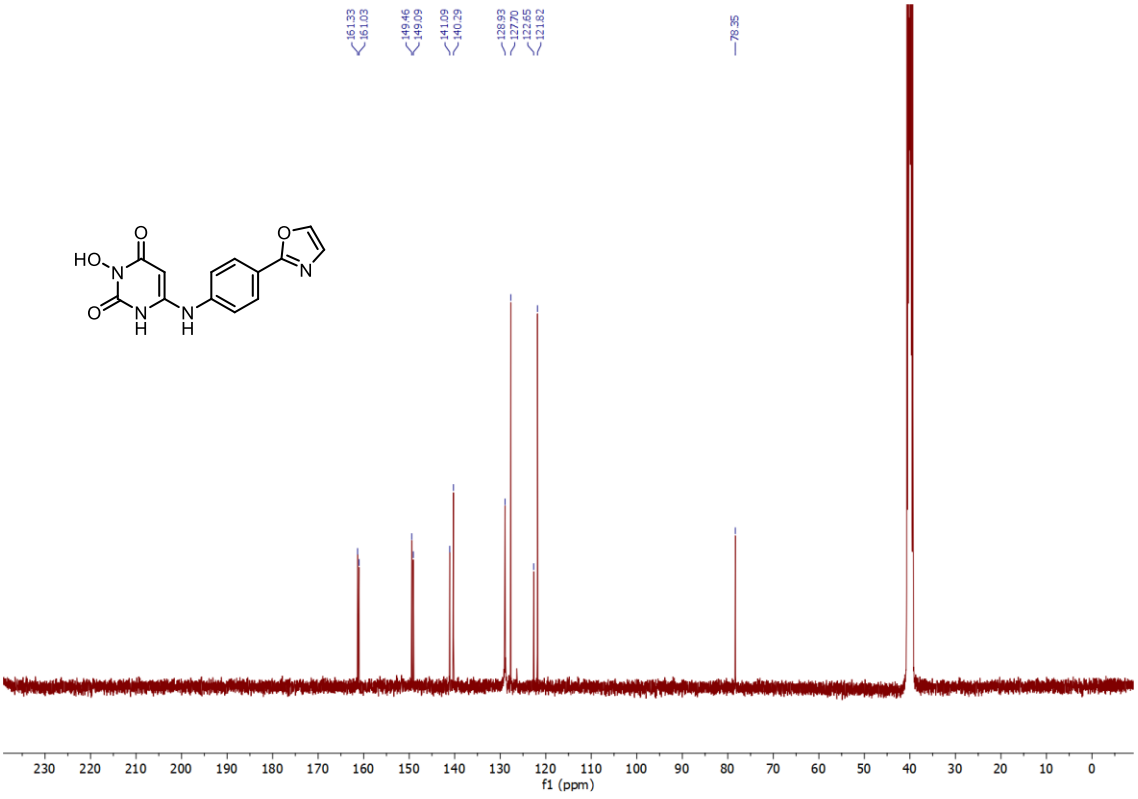
HRMS for Compound 2s

Sample Name	MS-03-207 depro	**Position**	P2-A2	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-207 depro_2715.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	4/14/2025 5:10:03 PM (UTC-05:00)



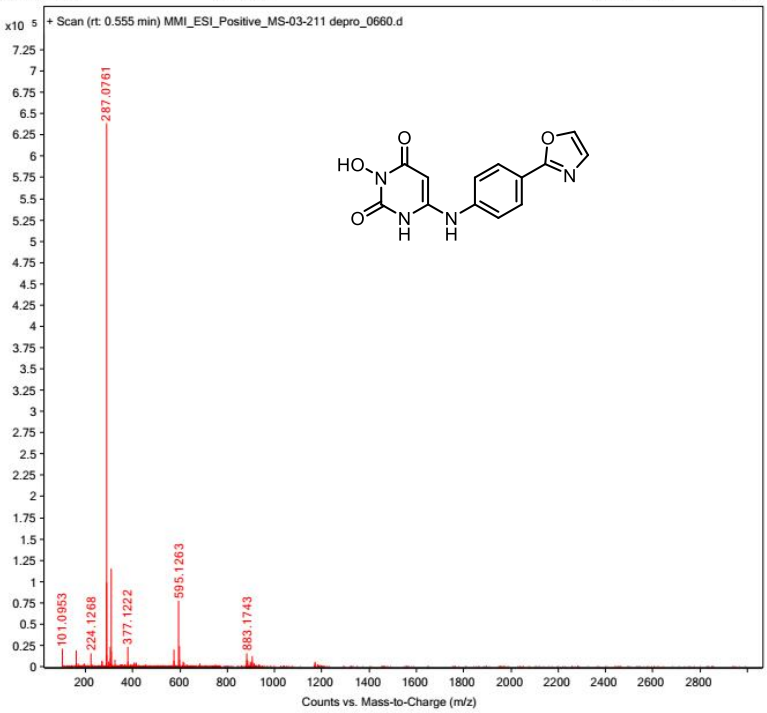
^{1}H NMR and ^{13}C NMR for Compound 2t in DMSO-d_6





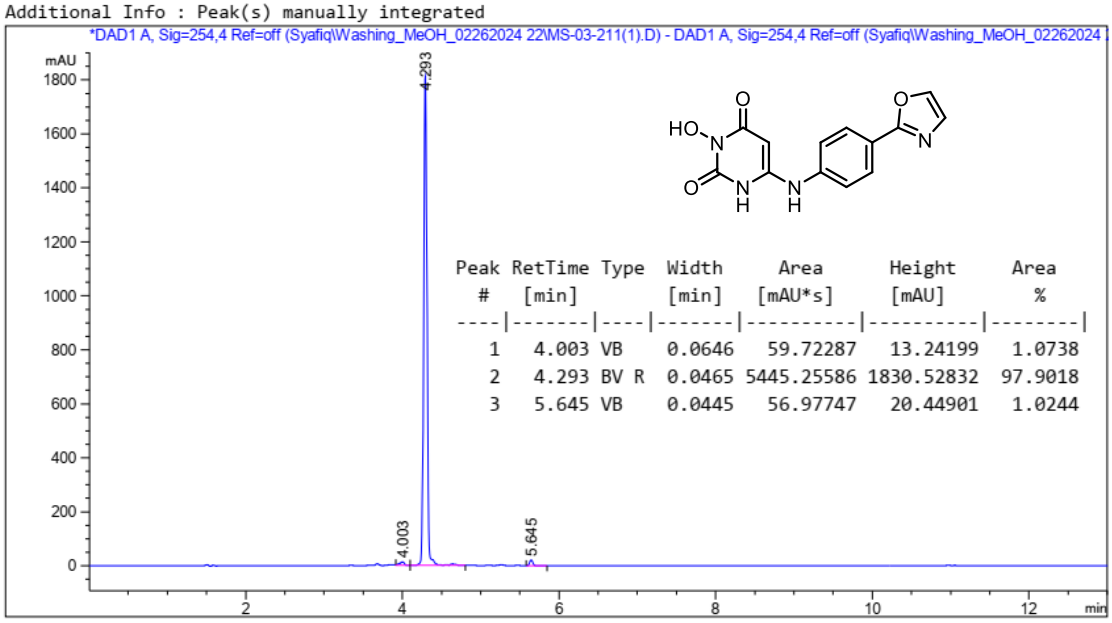
HRMS and HPLC Trace for Compound 2t

Sample Name	MS-03-211 depro	**Position**	P2-A2	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-03-211 depro_0660.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	12/17/2025 11:49:49 AM (UTC-06:00)



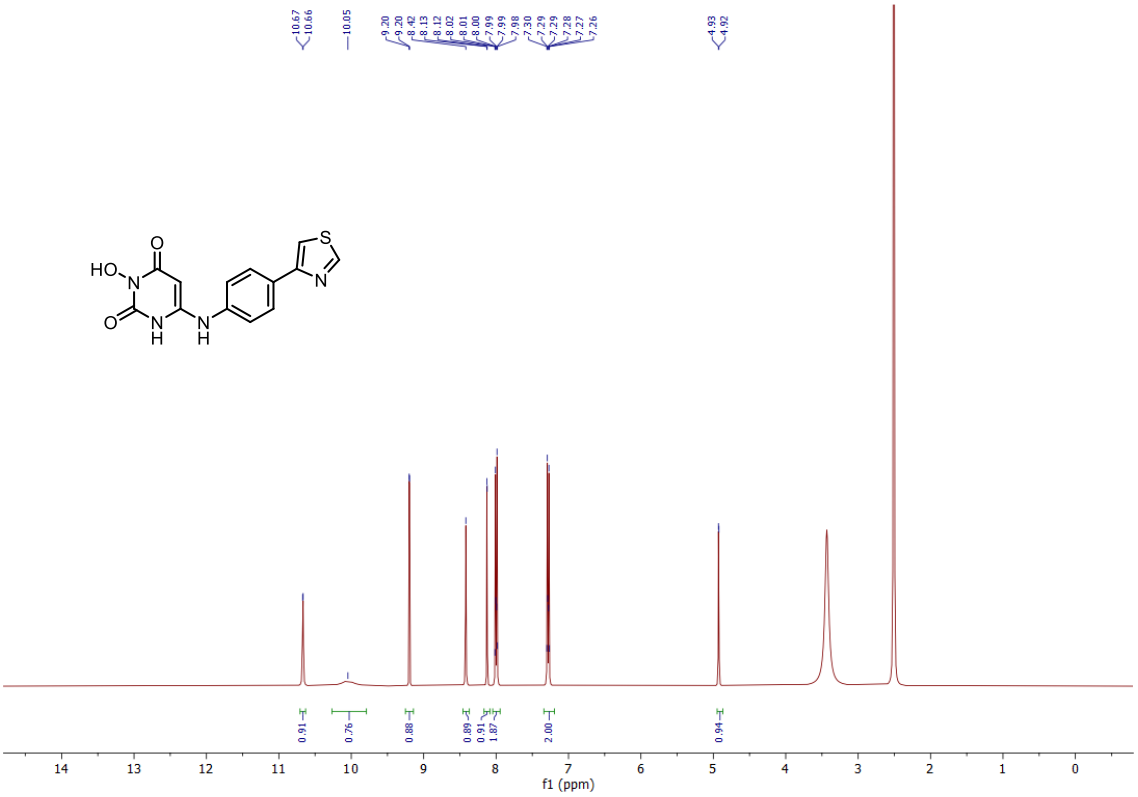
Sample Info : MS-03-211(1)

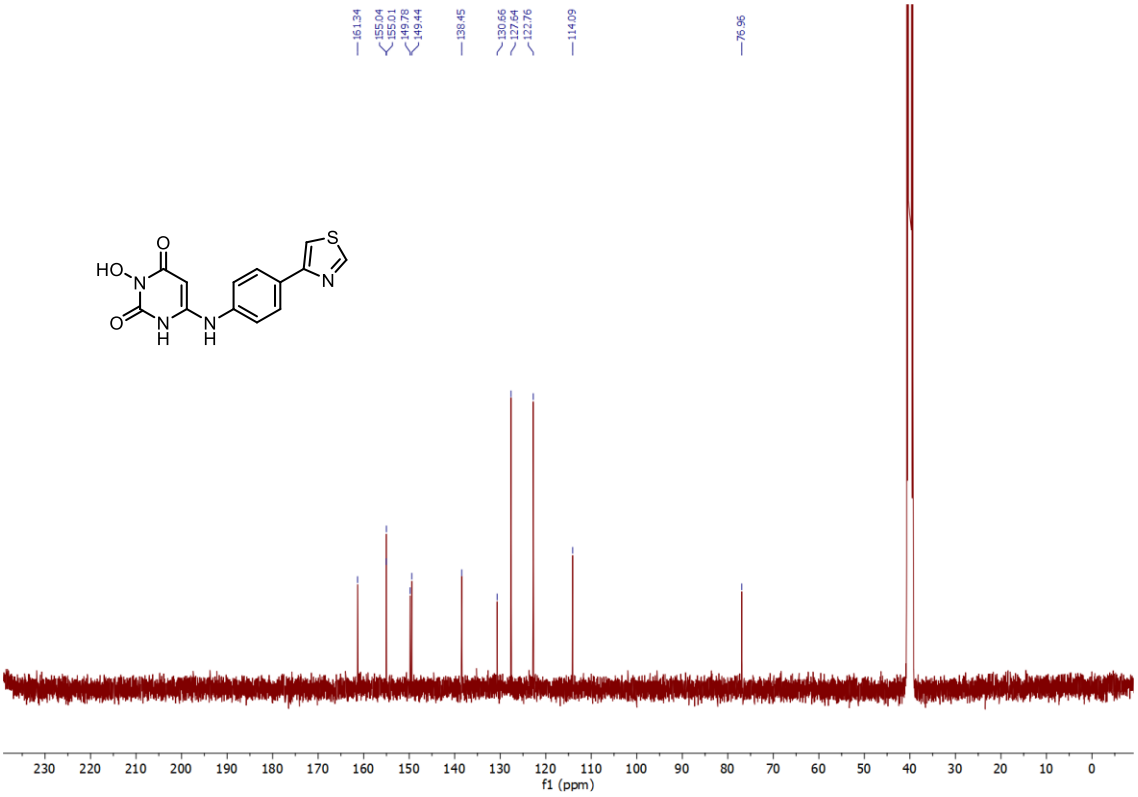
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.003	VB	0.0646	59.72287	13.24199	1.0738
2	4.293	BV R	0.0465	5445.25586	1830.52832	97.9018
3	5.645	VB	0.0445	56.97747	20.44901	1.0244

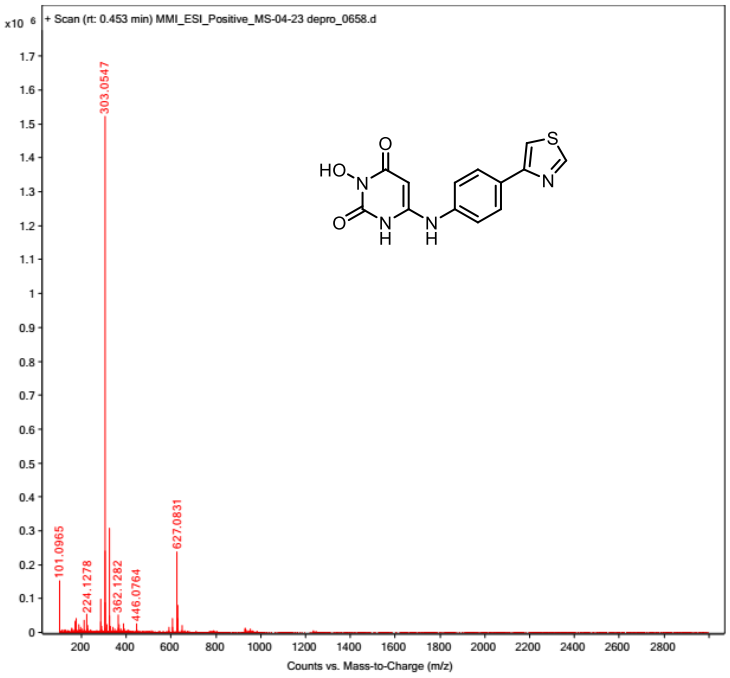
^{1}H NMR and ^{13}C NMR for Compound 2u in DMSO-d_6





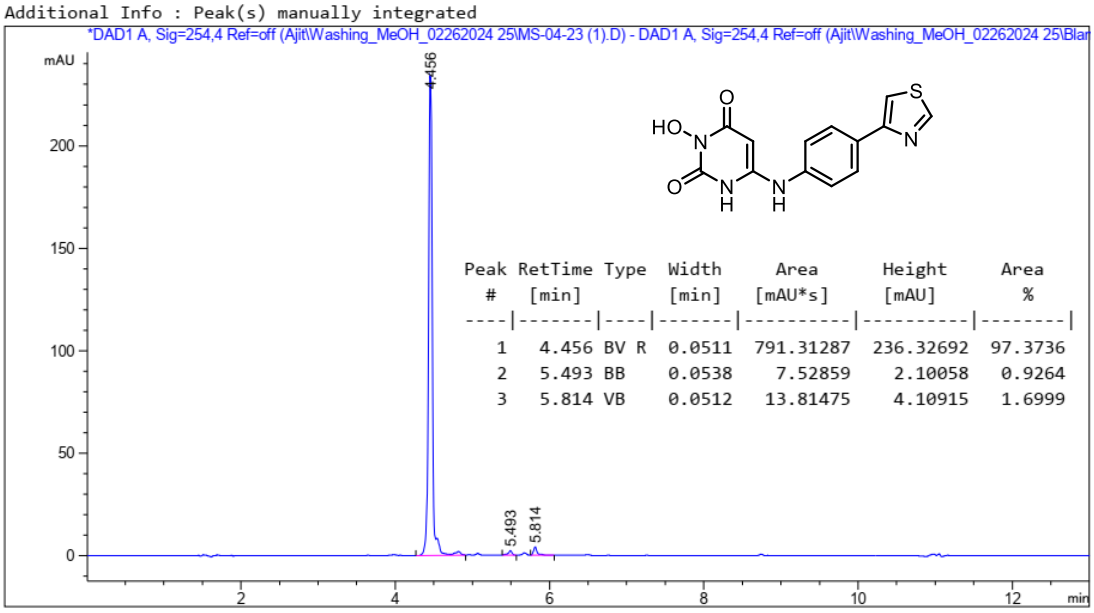
HRMS and HPLC Trace for Compound 2u

Sample Name	MS-04-23 depro	**Position**	P2-A1	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-23 depro_0658.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	12/17/2025 11:38:00 AM (UTC-06:00)



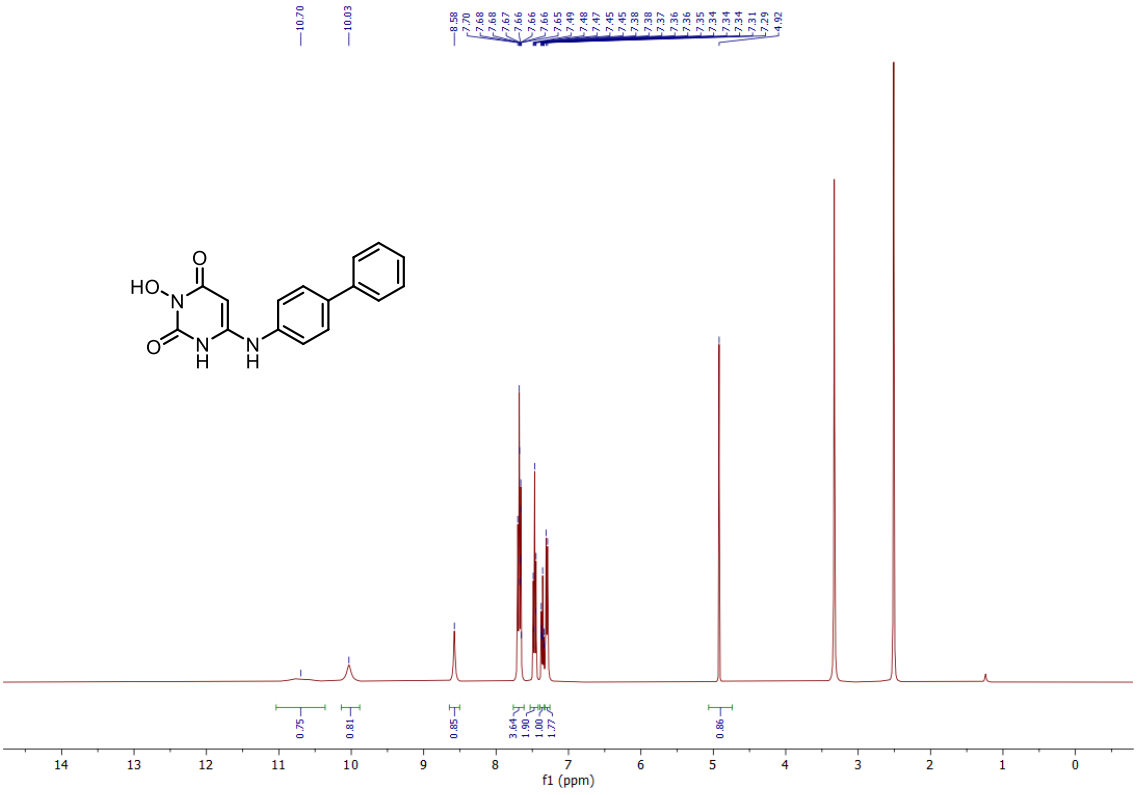
Sample Info : MS-04-23 (1)

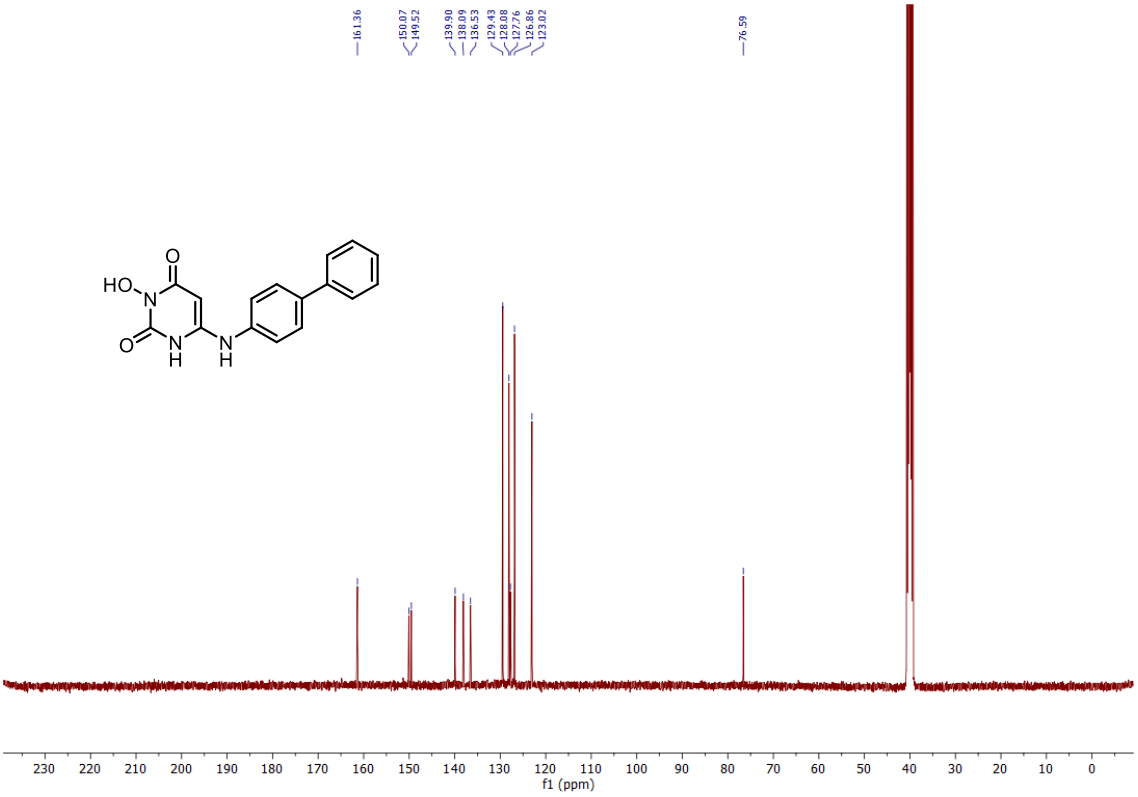
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.456	BV R	0.0511	791.31287	236.32692	97.3736
2	5.493	BB	0.0538	7.52859	2.10058	0.9264
3	5.814	VB	0.0512	13.81475	4.10915	1.6999

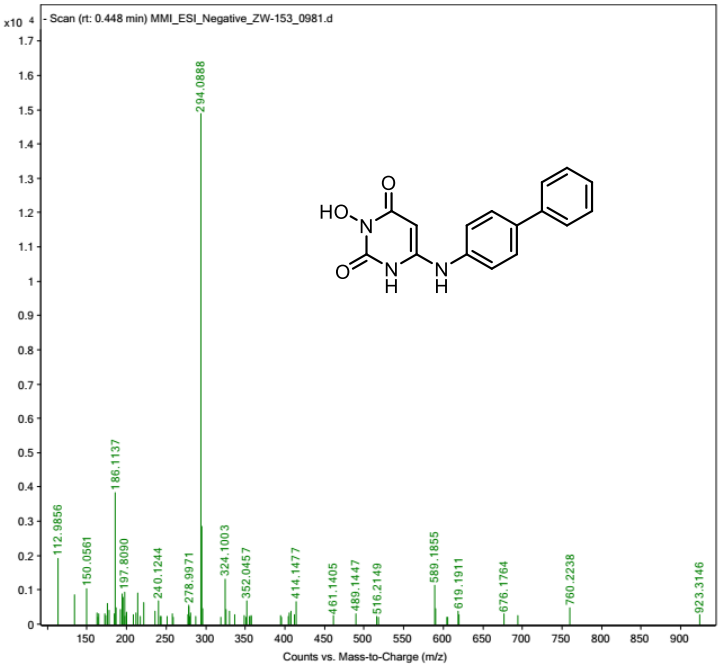
^{1}H NMR and ^{13}C NMR for Compound 2v in DMSO-d_6





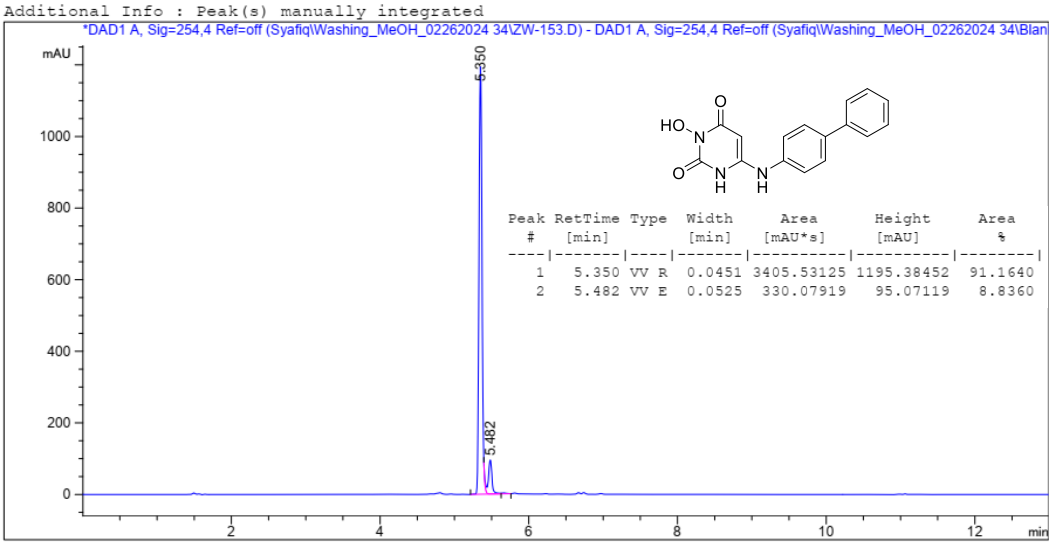
HRMS for Compound 2v

Sample Name	ZW-153	**Position**	P2-D10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_ZW-153_0981.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	1/13/2026 11:29:15 AM (UTC-06:00)



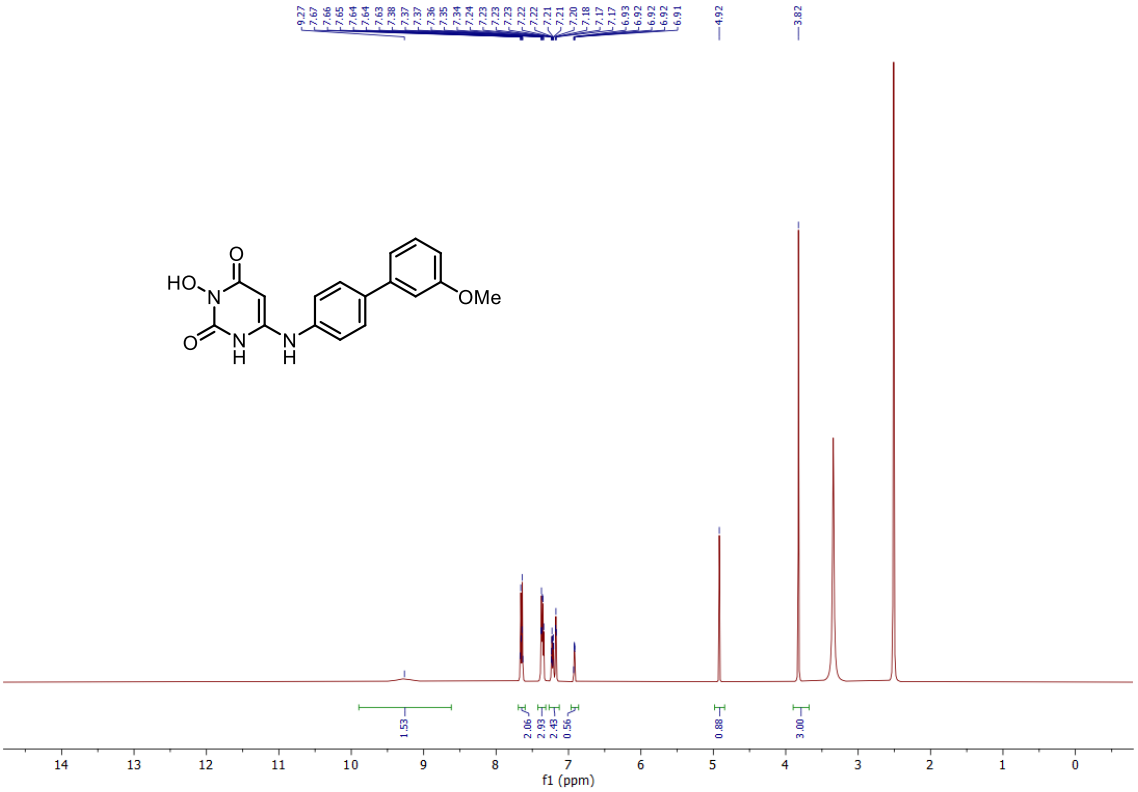
Sample Info : ZW-153

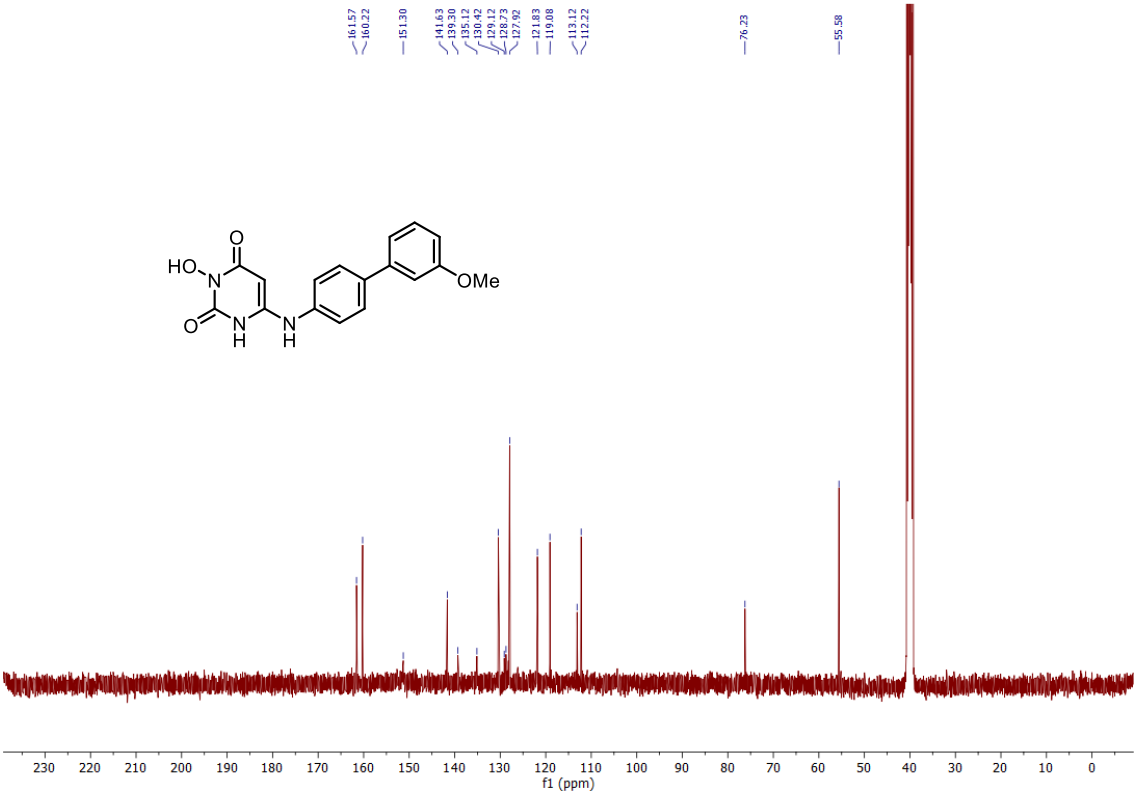
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.350	VV R	0.0451	3405.53125	1195.38452	91.1640
2	5.482	VV E	0.0525	330.07919	95.07119	8.8360

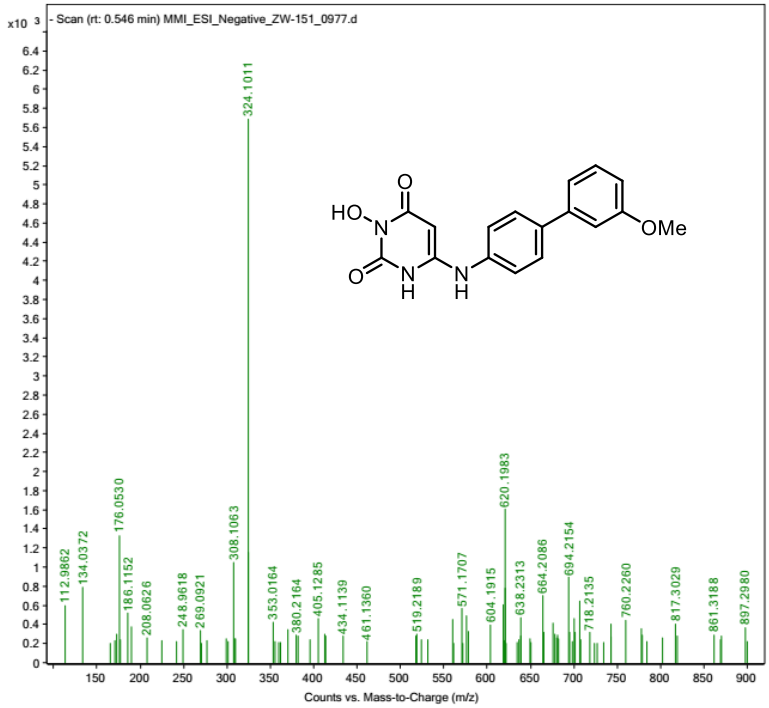
^{1}H NMR and ^{13}C NMR for Compound 2w in DMSO-*d*$_6$





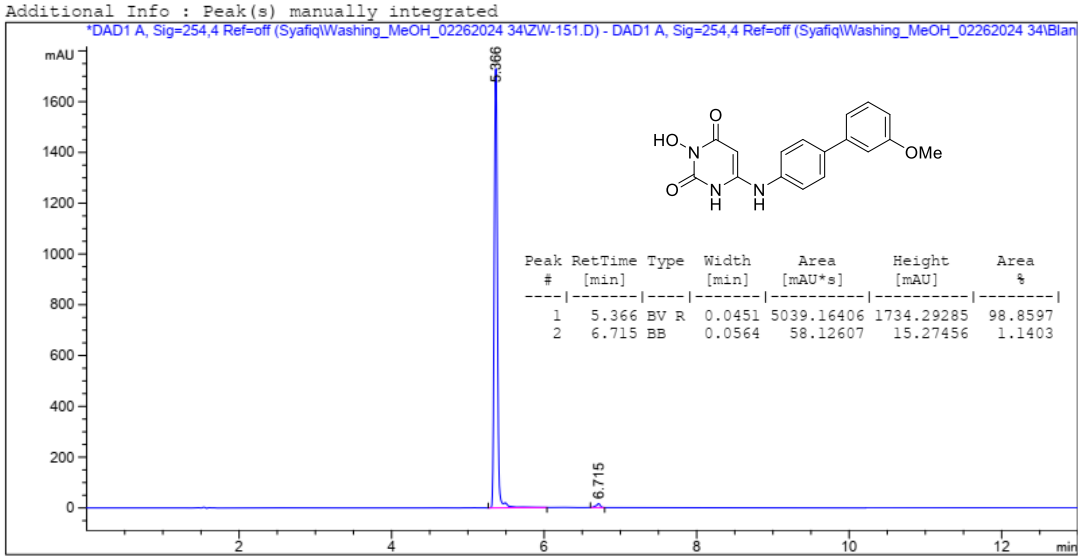
HRMS for Compound 2w

Sample Name	ZW-151	**Position**	P2-D9	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_ZW-151_0977.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	1/13/2026 11:05:37 AM (UTC-06:00)



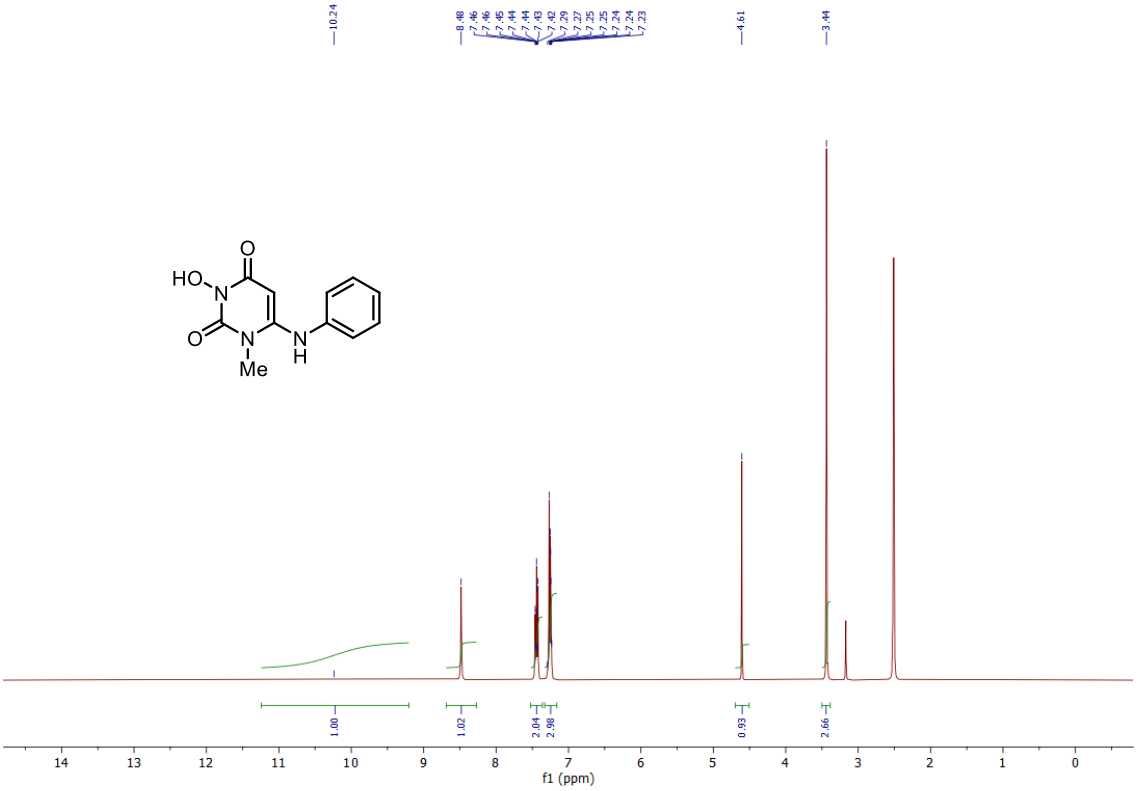
Sample Info : ZW-151

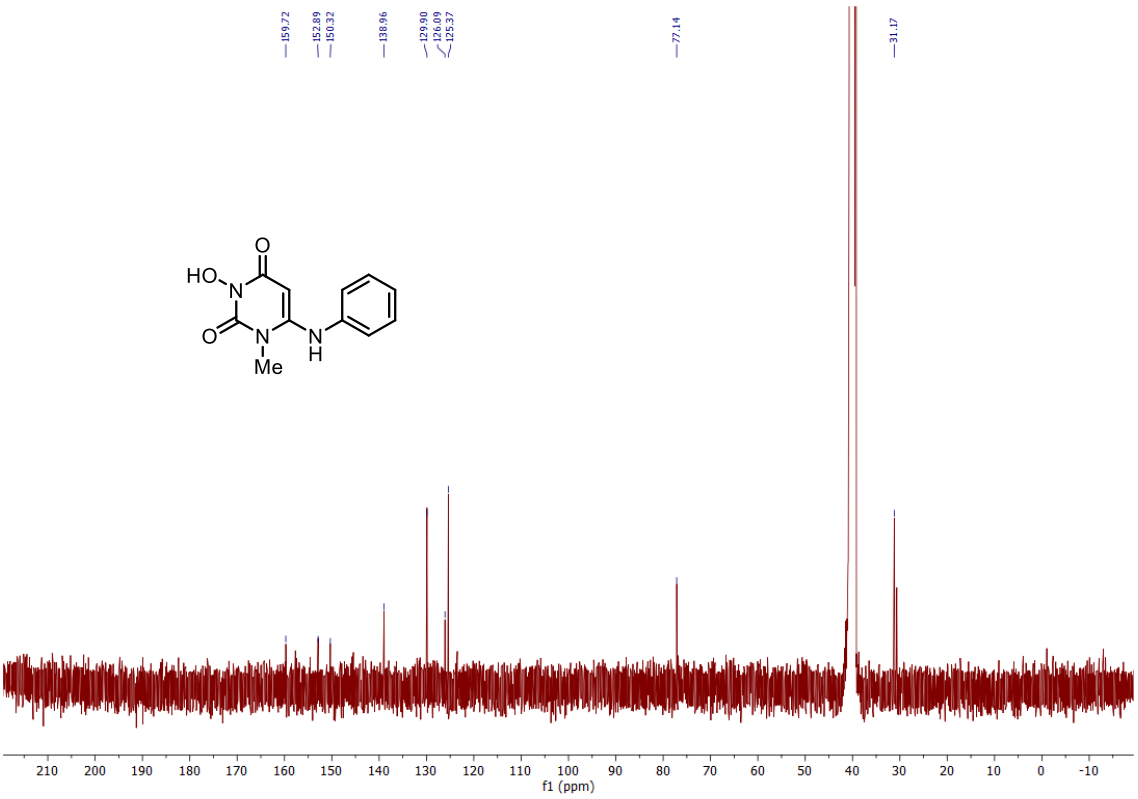
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.366	BV R	0.0451	5039.16406	1734.29285	98.8597
2	6.715	BB	0.0564	58.12607	15.27456	1.1403

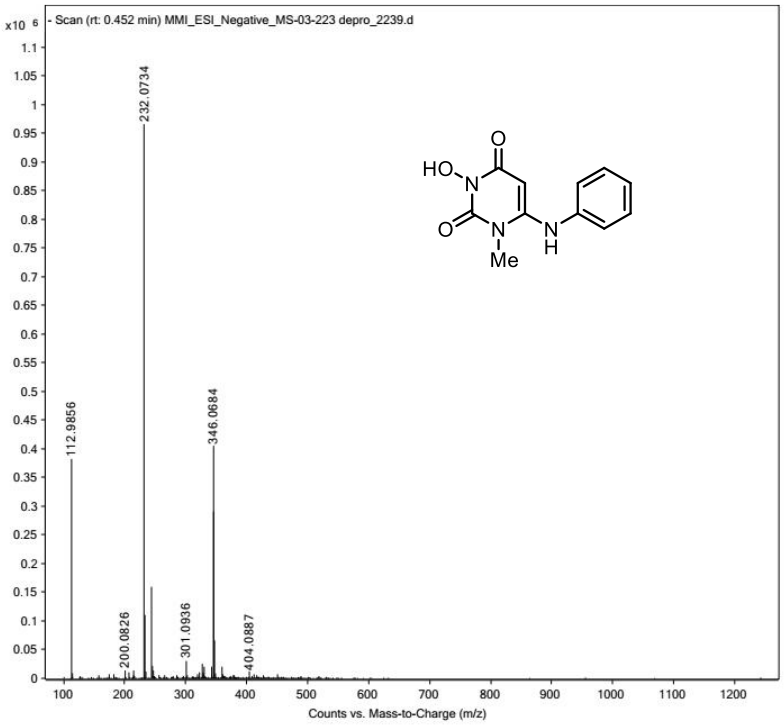
^{1}H NMR and ^{13}C NMR for Compound 3a in DMSO-d_6





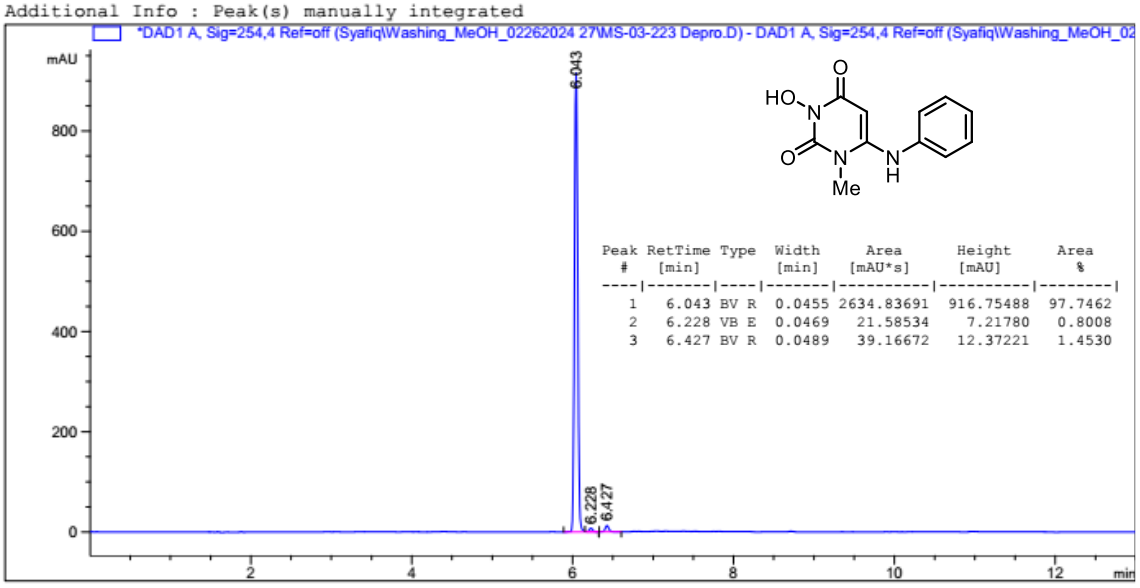
HRMS and HPLC Trace for Compound 3a

Sample Name	MS-03-223 depro	**Position**	P1-D6	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-223 depro_2239.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	3/30/2026 8:49:09 AM (UTC-05:00)



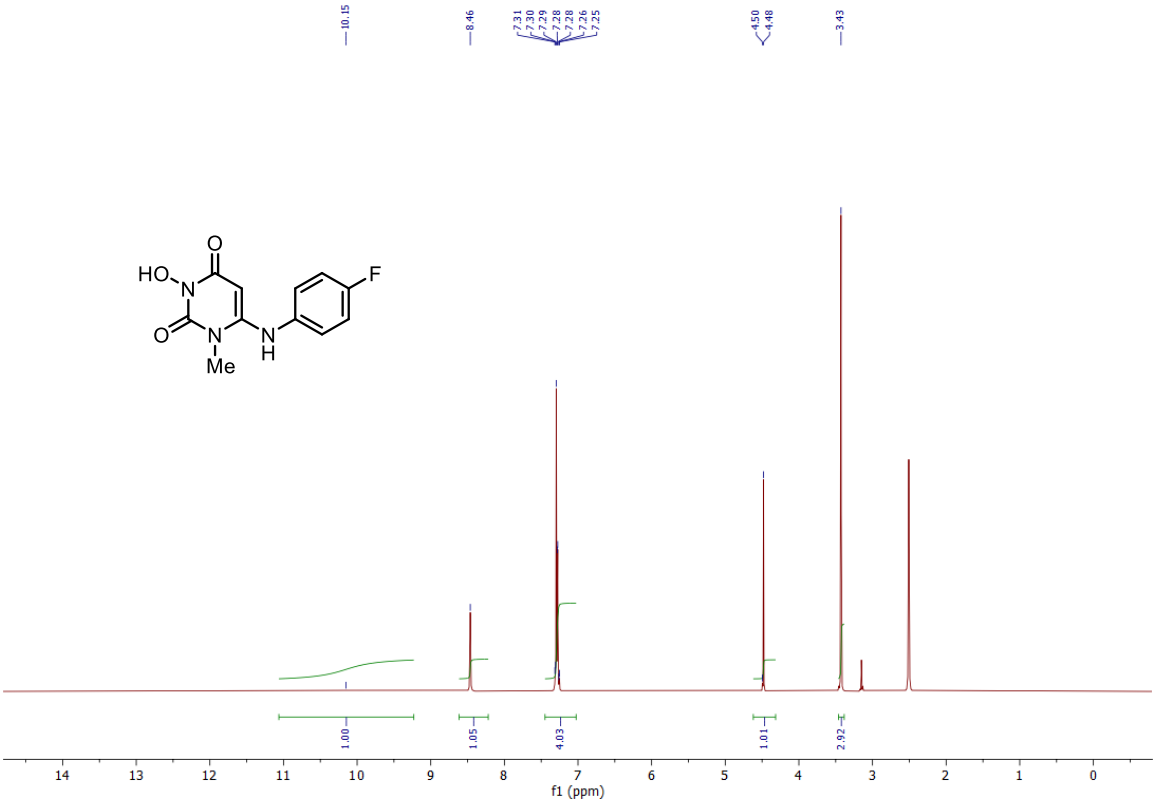
Sample Info : MS-03-223 Depro

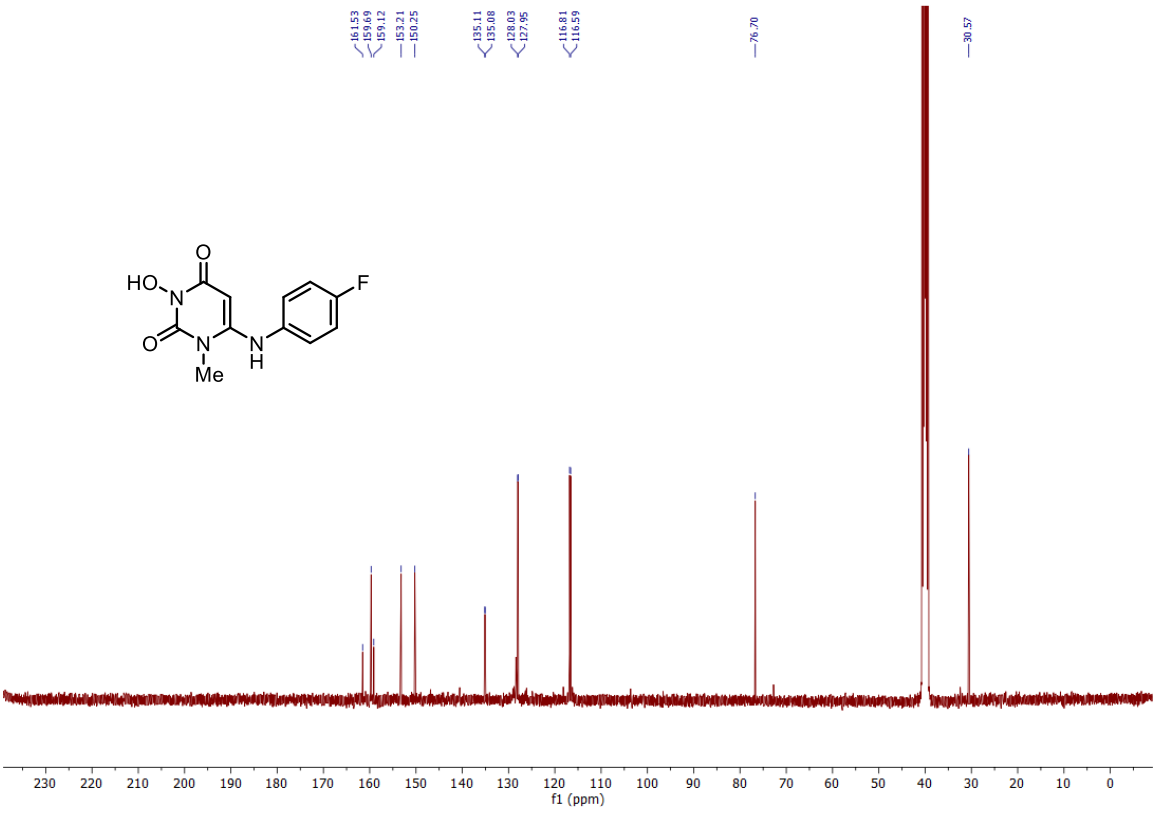
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.043	BV R	0.0455	2634.83691	916.75488	97.7462
2	6.228	VB E	0.0469	21.58534	7.21780	0.8008
3	6.427	BV R	0.0489	39.16672	12.37221	1.4530

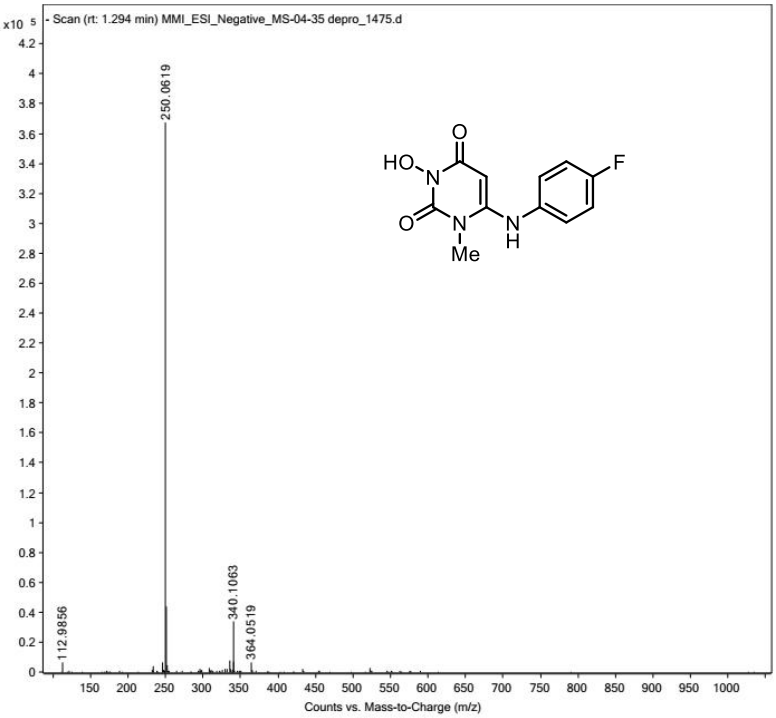
^{1}H NMR and ^{13}C NMR for Compound 3b in DMSO-*d*$_6$



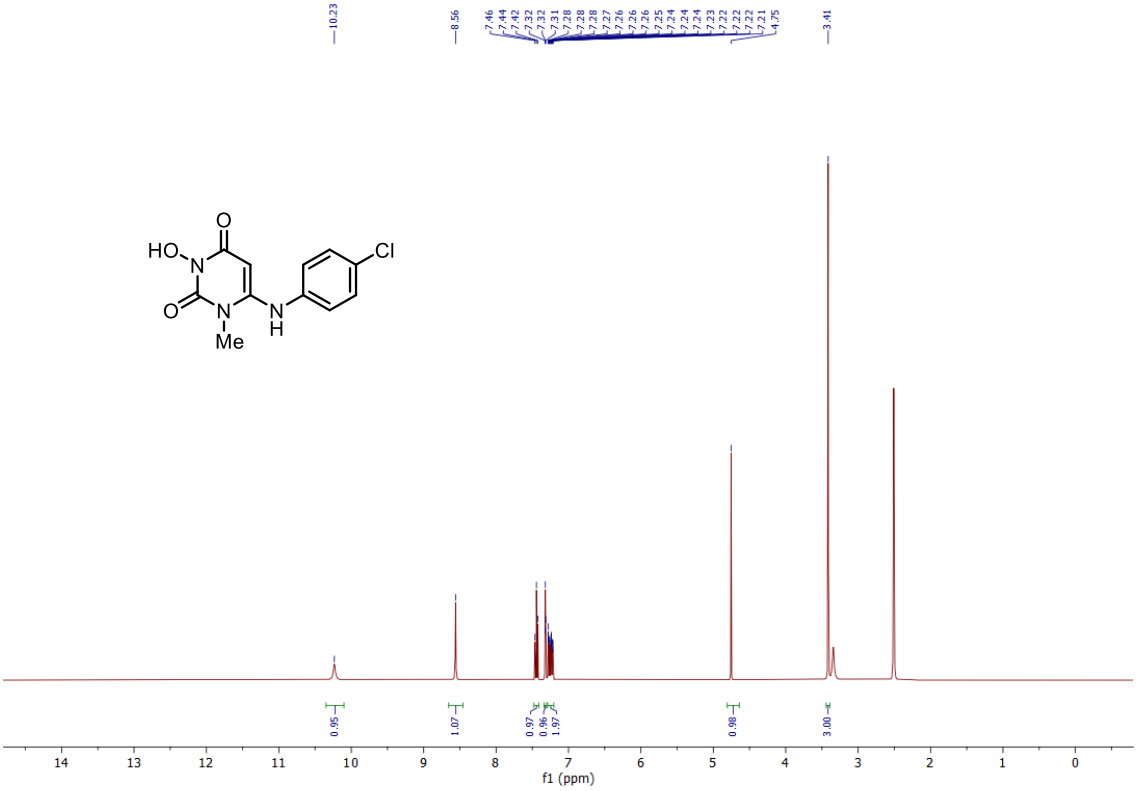


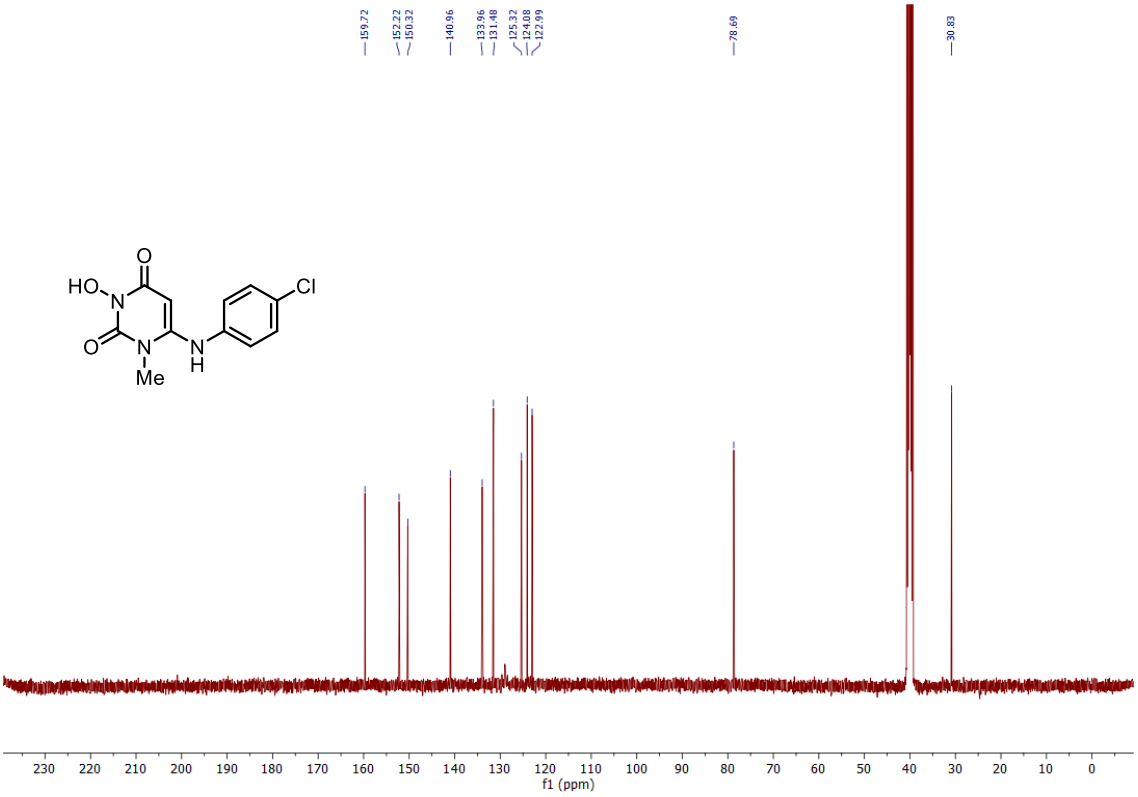
HRMS for Compound 3b

Sample Name	MS-04-35 depro	**Position**	P1-E2	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-04-35 depro_1475.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	3/10/2026 6:16:48 PM (UTC-05:00)



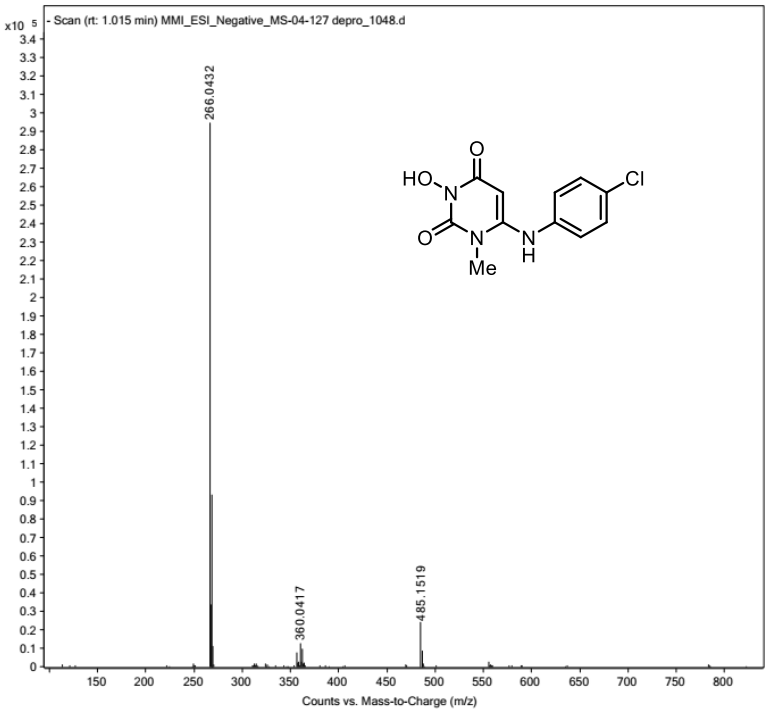
^{1}H NMR and ^{13}C NMR for Compound 3c in DMSO-d_6





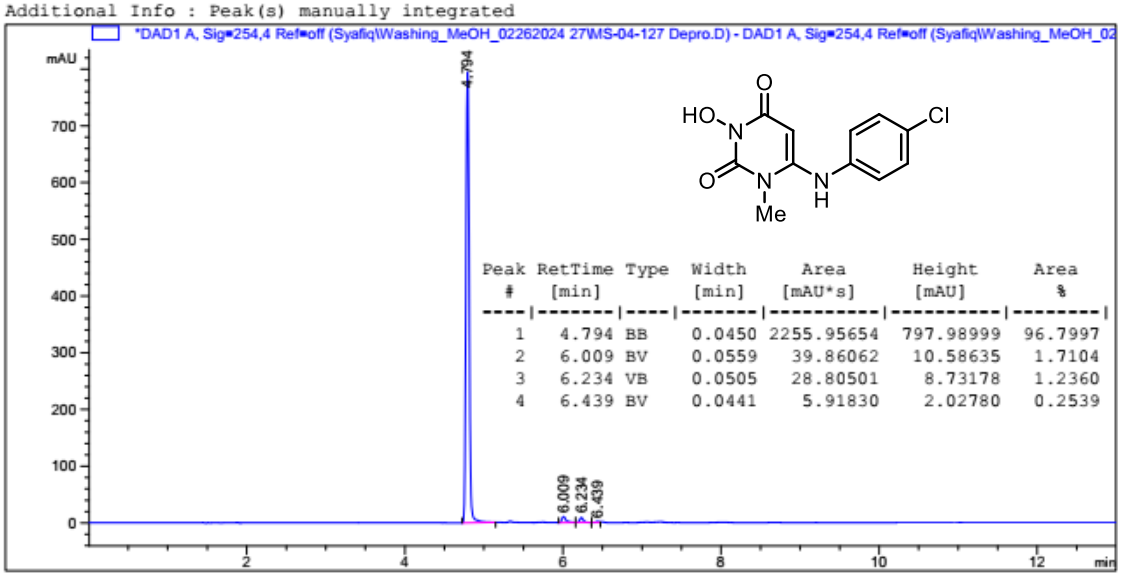
HRMS and HPLC Trace for Compound 3c

Sample Name	MS-04-127 depro	**Position**	P1-B1	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-04-127 depro_1048.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	1/16/2026 11:04:51 AM (UTC-06:00)



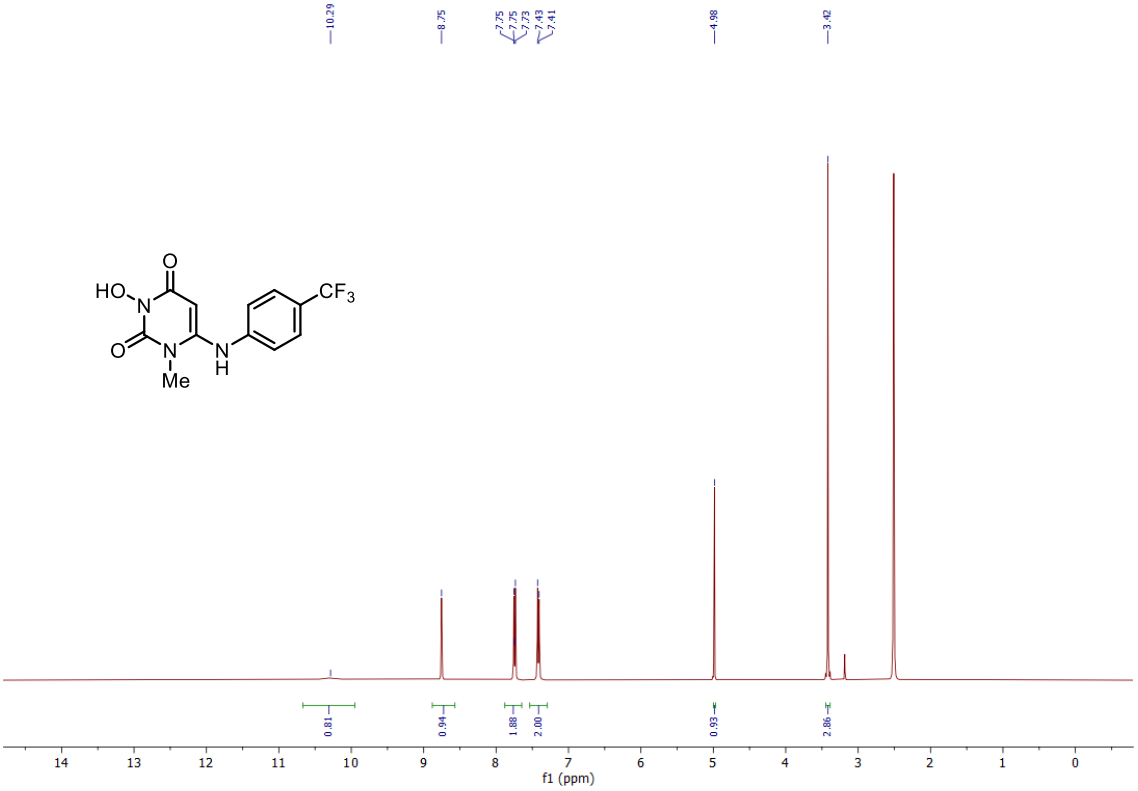
Sample Info : MS-04-127 Depro

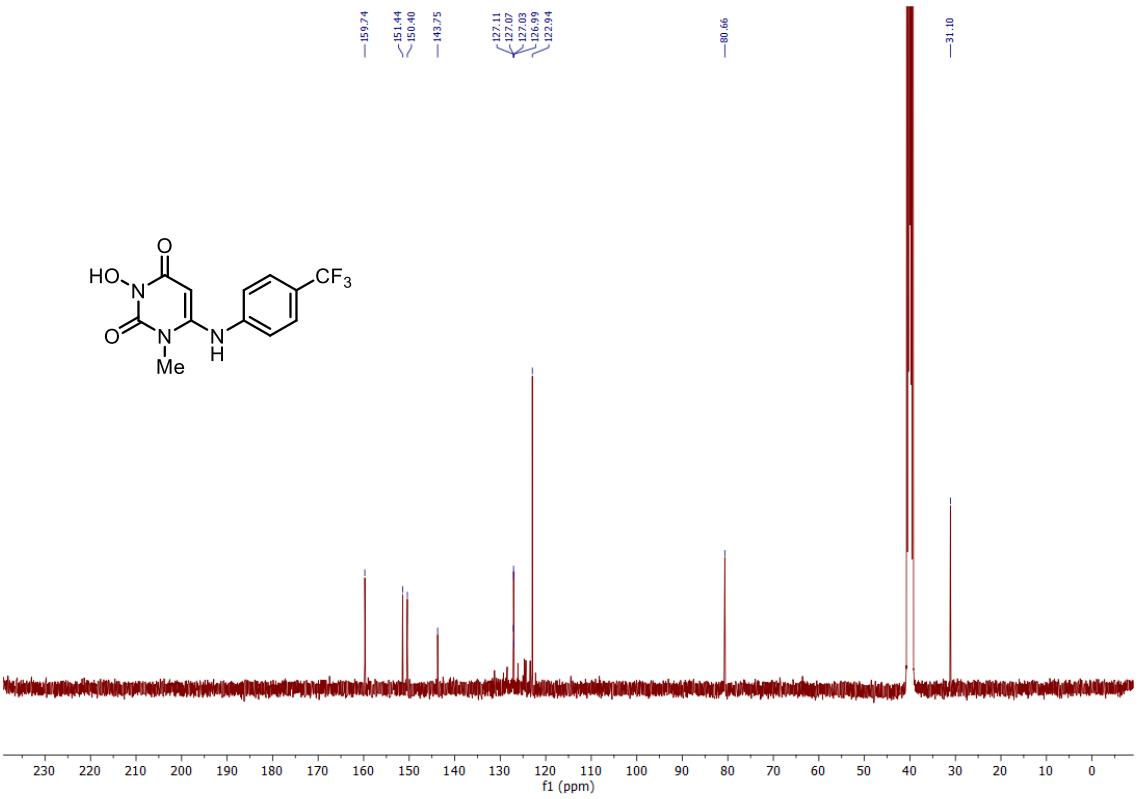
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.794	BB	0.0450	2255.95654	797.98999	96.7997
2	6.009	BV	0.0559	39.86062	10.58635	1.7104
3	6.234	VB	0.0505	28.80501	8.73178	1.2360
4	6.439	BV	0.0441	5.91830	2.02780	0.2539

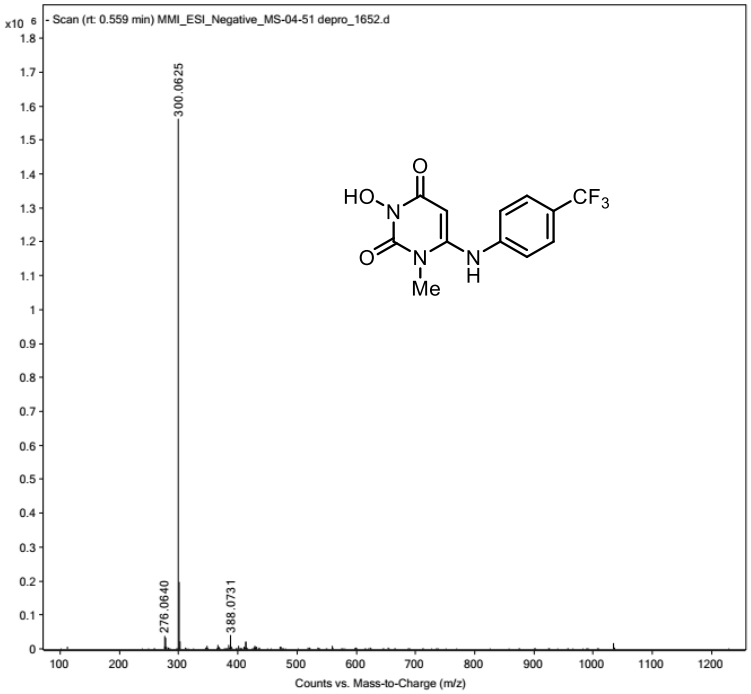
^{1}H NMR and ^{13}C NMR for Compound 3d in DMSO-d_6



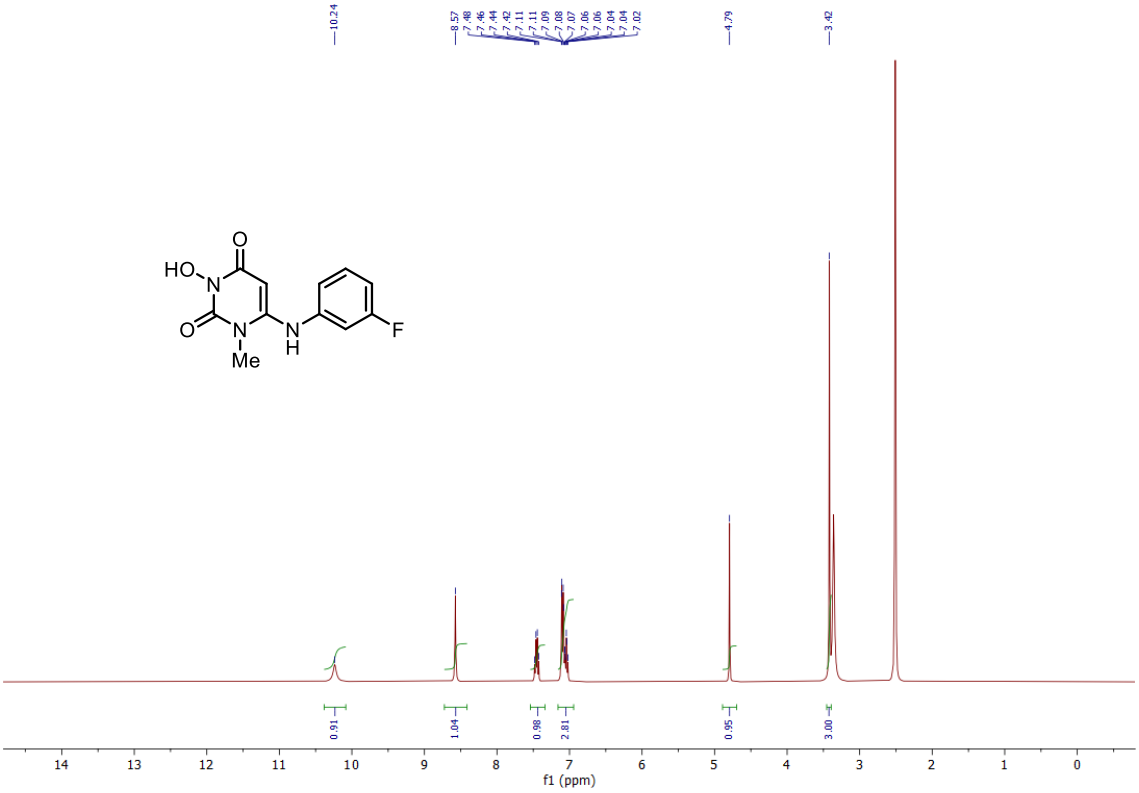


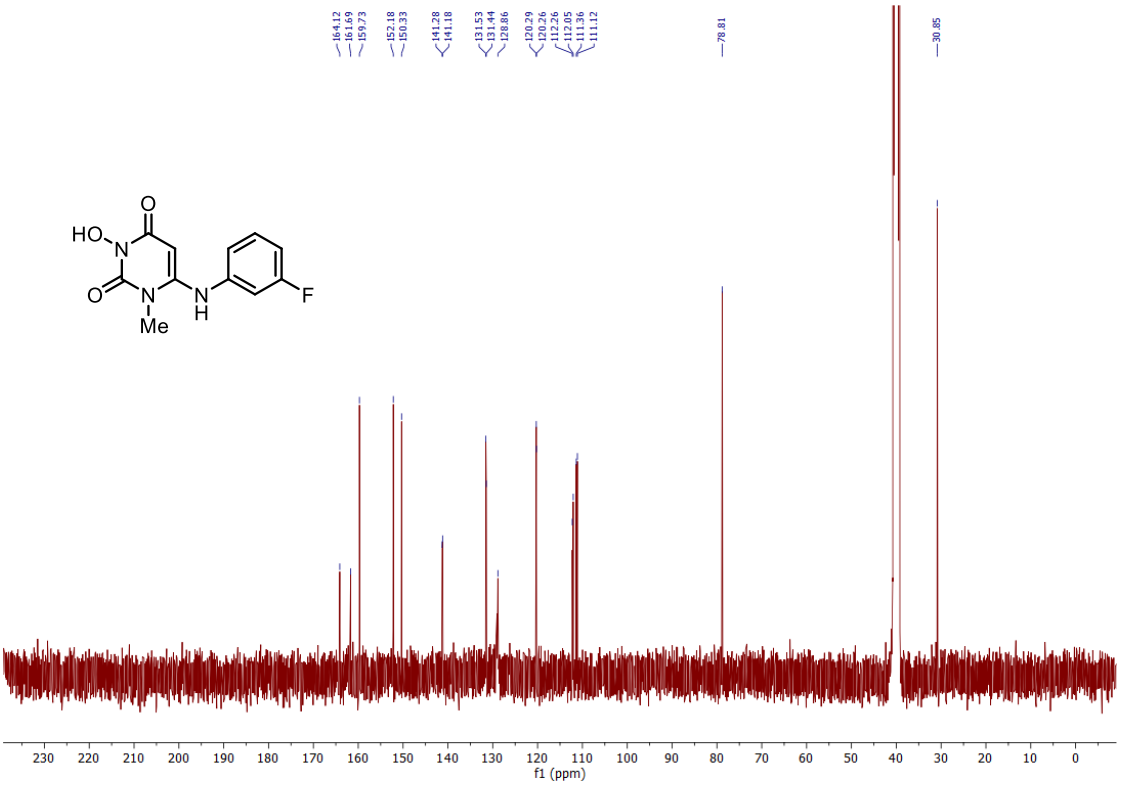
HRMS for Compound 3d

Sample Name	MS-04-51 depro	**Position**	P1-A3	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-04-51 depro_1652.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	3/13/2026 3:47:50 PM (UTC-05:00)



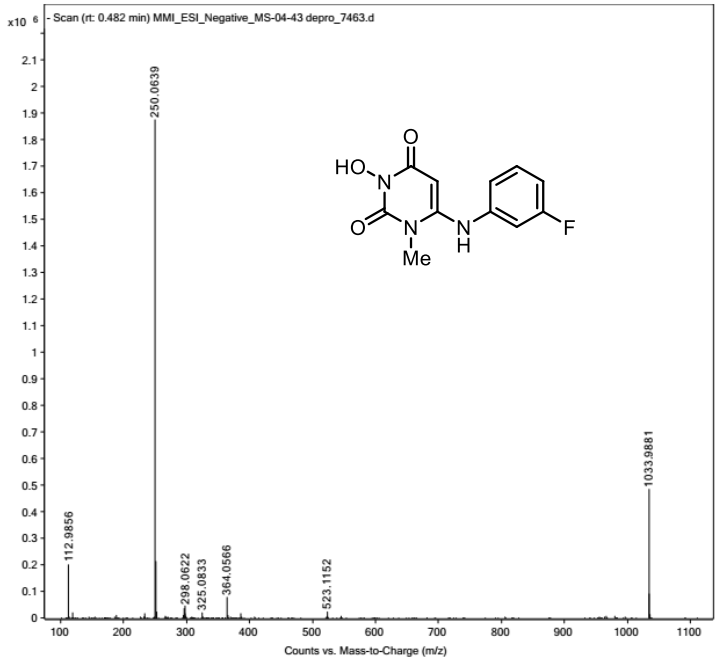
^{1}H NMR and ^{13}C NMR for Compound 3e in DMSO-d_6



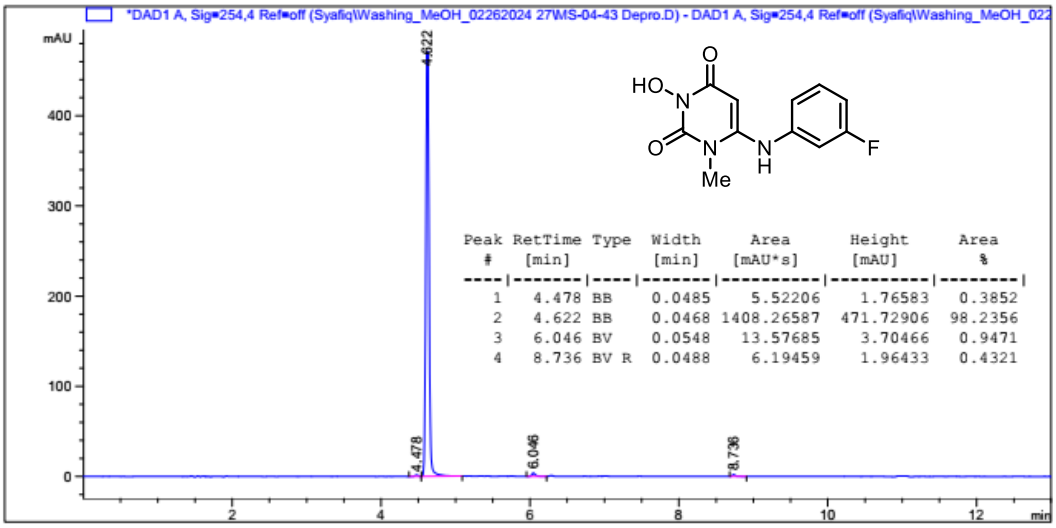


HRMS and HPLC Trace for Compound 3e

Sample Name	MS-04-43 depro	**Position**	P1-B7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-04-43 depro_7463.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	9/3/2025 12:30:02 PM (UTC-05:00)

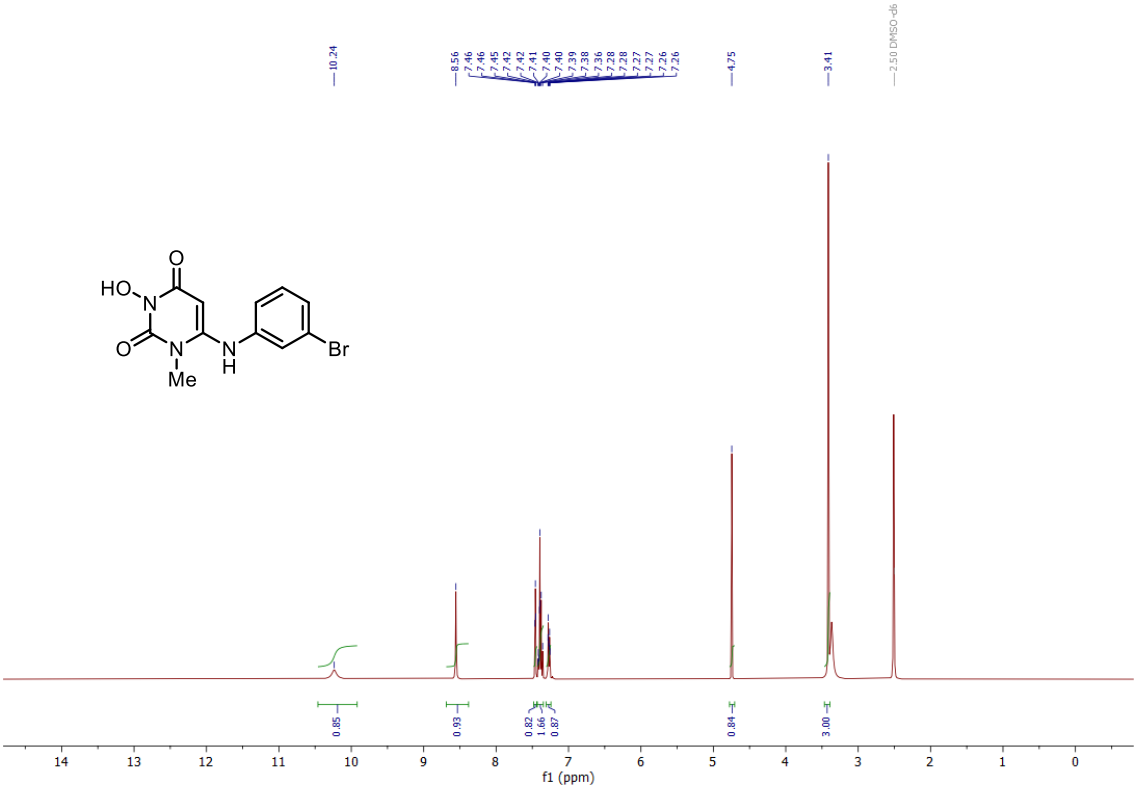


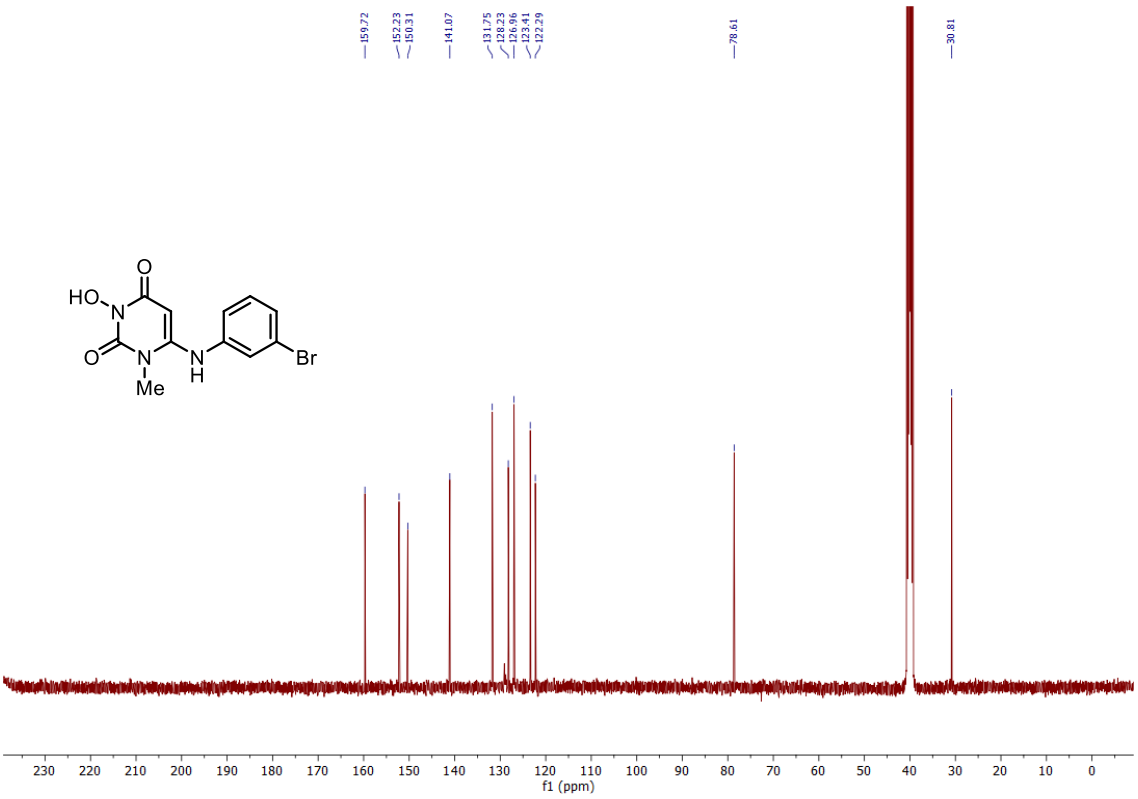
Sample Info : MS-04-43 Depro



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.478	BB	0.0485	5.52206	1.76583	0.3852
2	4.622	BB	0.0468	1408.26587	471.72906	98.2356
3	6.046	BV	0.0548	13.57685	3.70466	0.9471
4	8.736	BV R	0.0488	6.19459	1.96433	0.4321

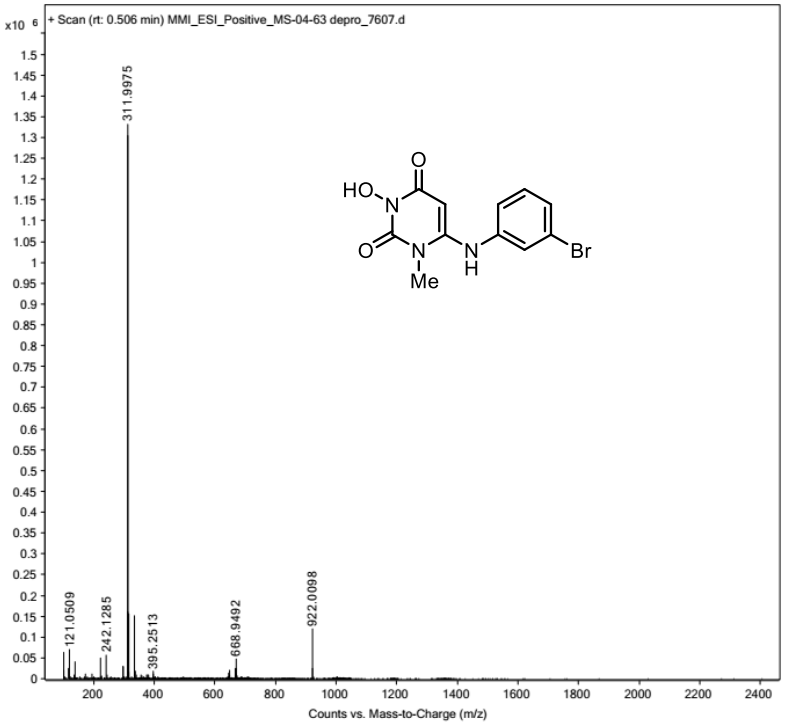
^{1}H NMR and ^{13}C NMR for Compound 3f in DMSO-d_6





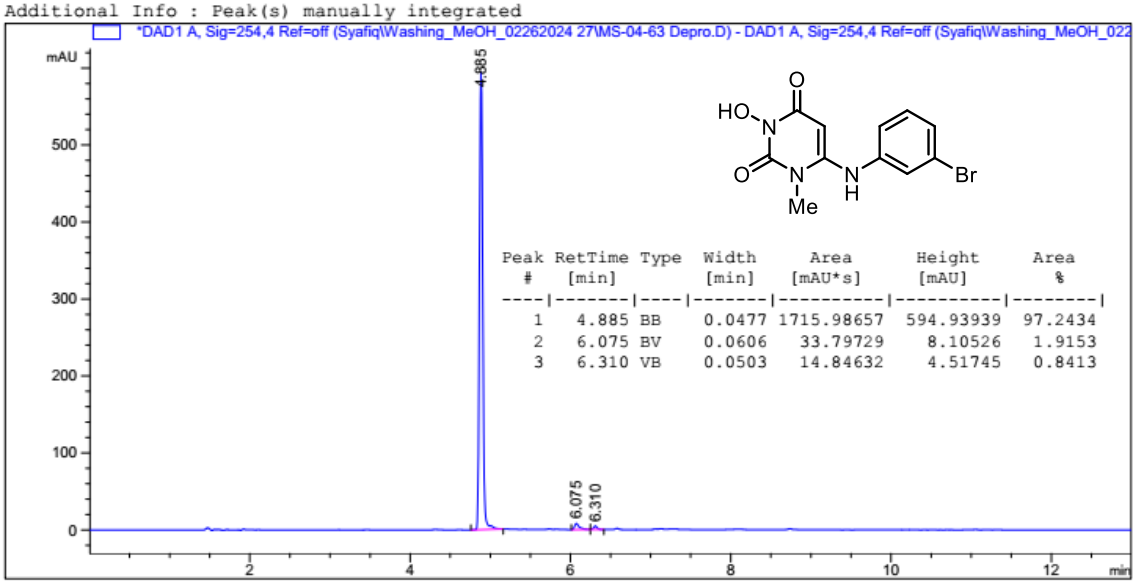
HRMS and HPLC Trace for Compound 3f

Sample Name	MS-04-63 depro	**Position**	P2-C7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-63 depro_7607.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	9/10/2025 1:50:58 PM (UTC-05:00)



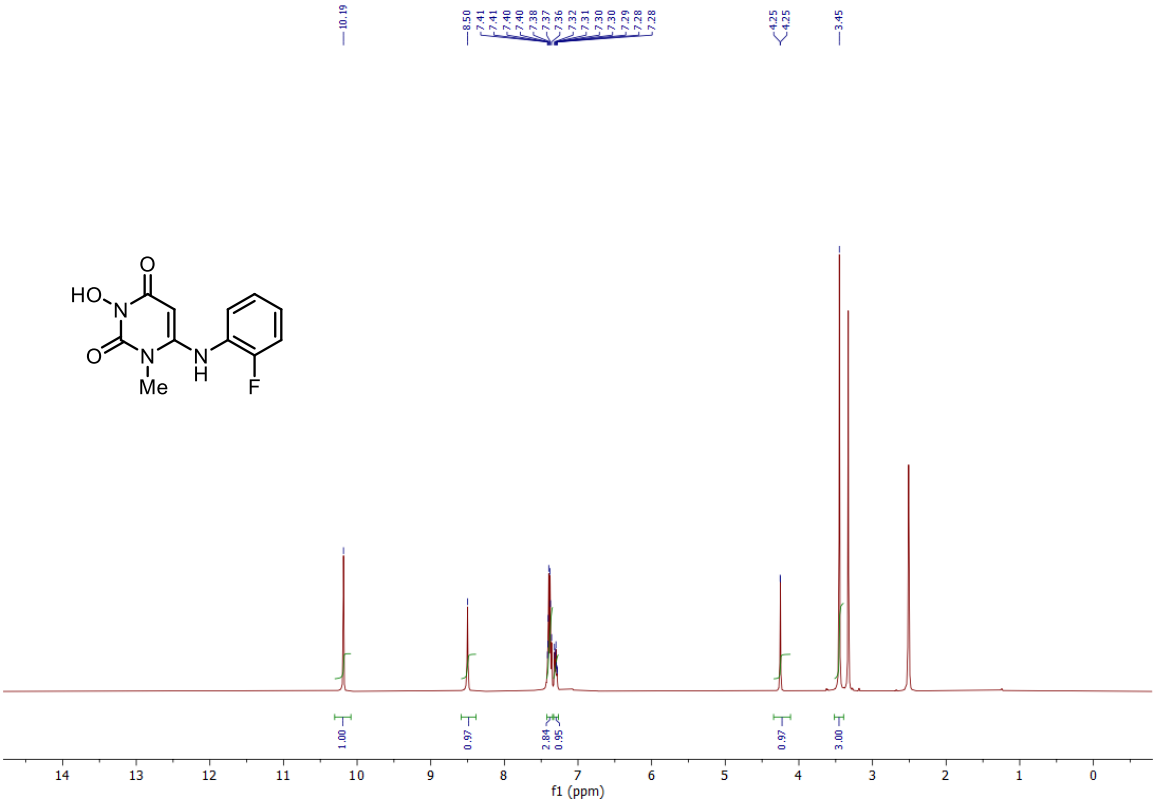
Sample Info : MS-04-63 Depro

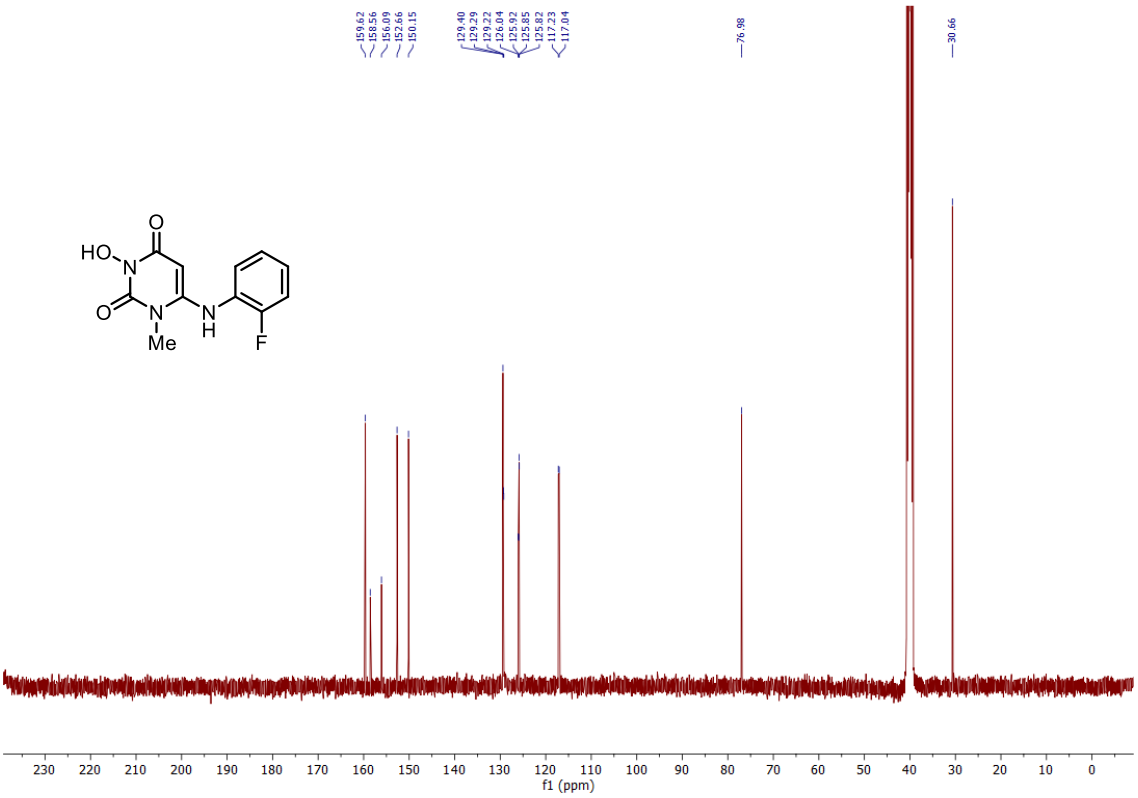
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.885	BB	0.0477	1715.98657	594.93939	97.2434
2	6.075	BV	0.0606	33.79729	8.10526	1.9153
3	6.310	VB	0.0503	14.84632	4.51745	0.8413

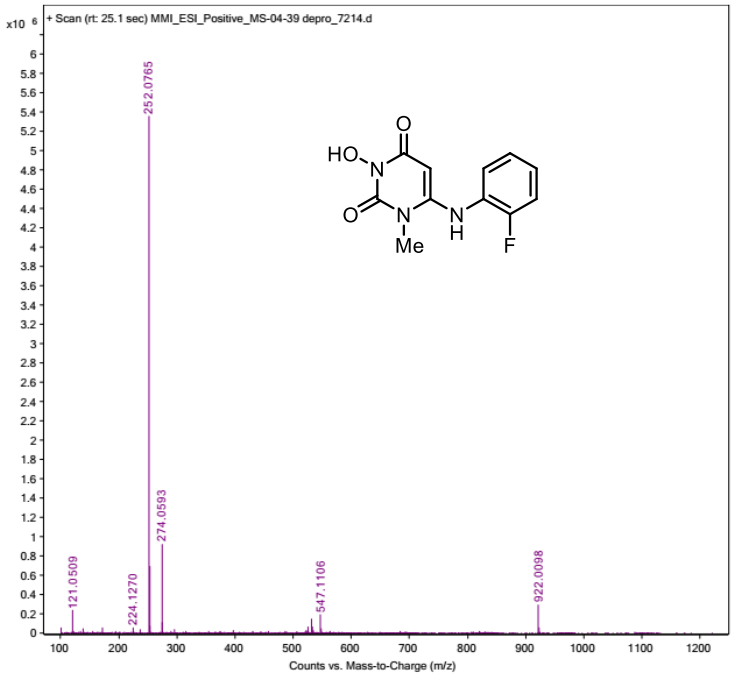
^{1}H NMR and ^{13}C NMR for Compound 3g in DMSO-*d*$_6$



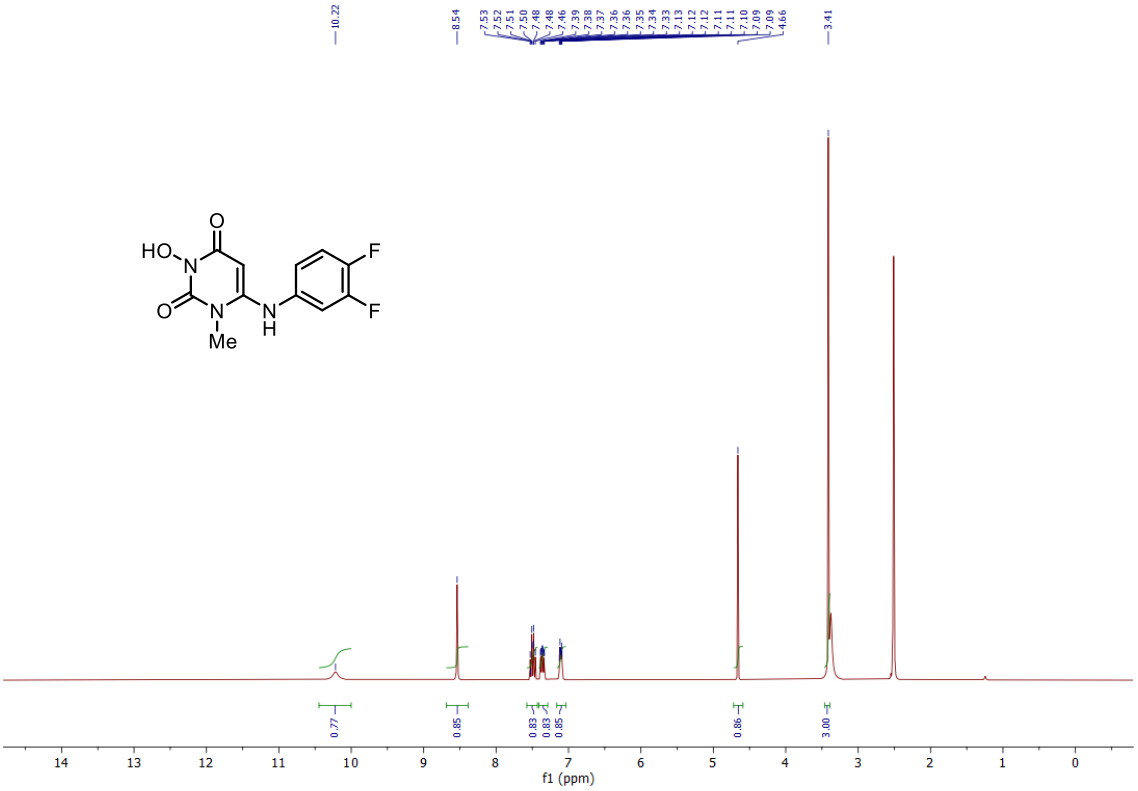


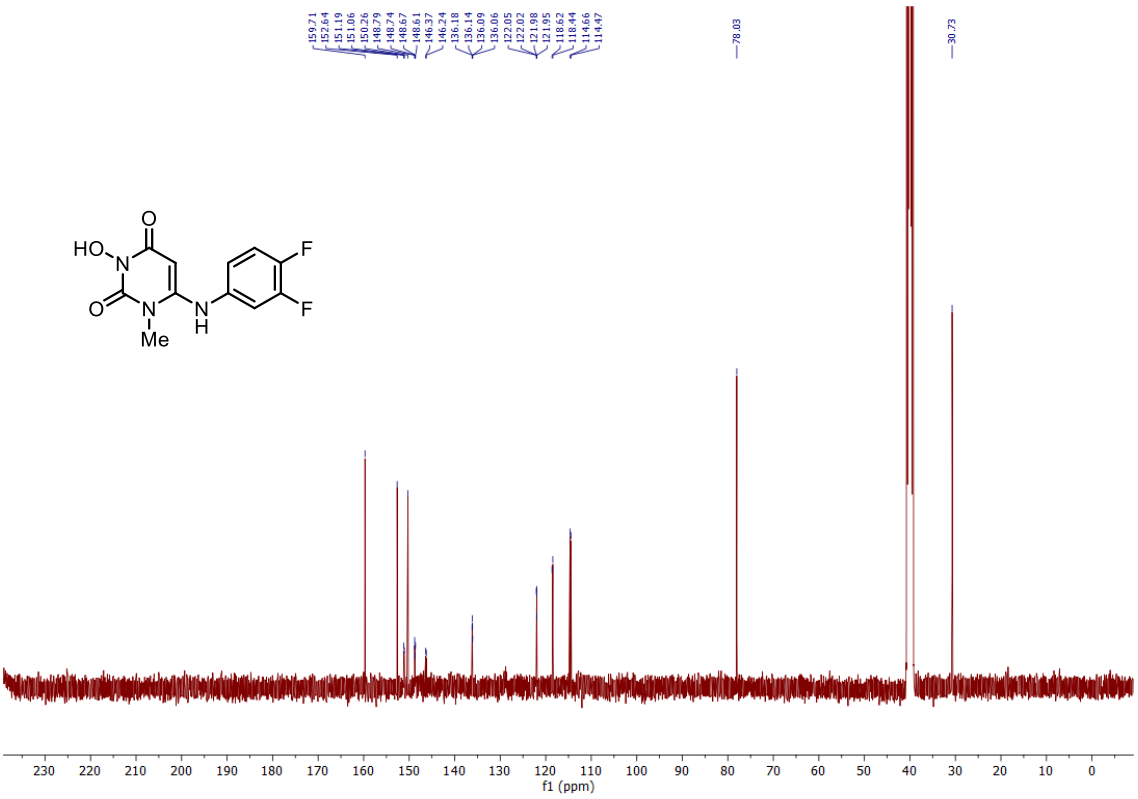
HRMS for Compound 3g

Sample Name	MS-04-39 depro	**Position**	P2-C9	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-39 depro_7214.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	8/22/2025 9:34:21 AM (UTC-05:00)



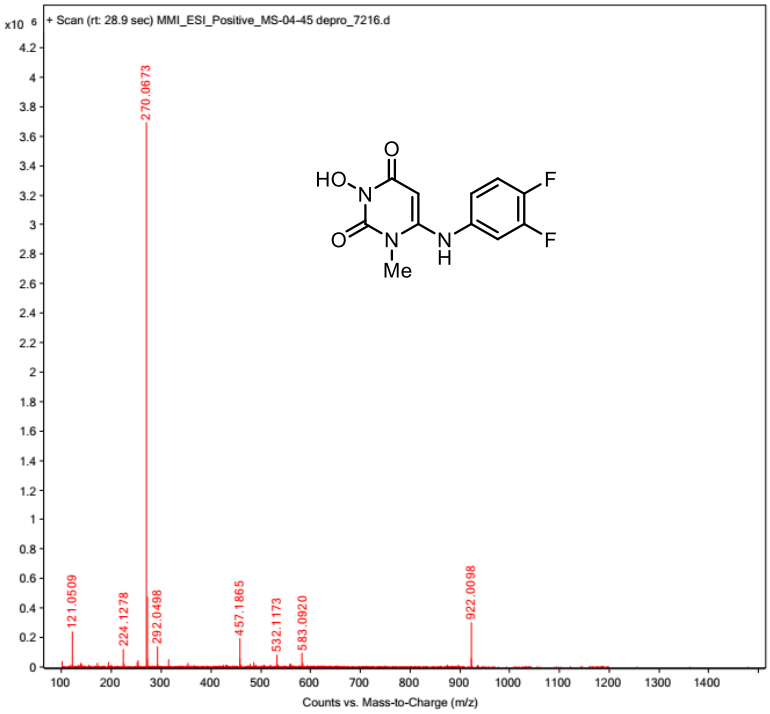
^{1}H NMR and ^{13}C NMR for Compound 3h in DMSO-d_6





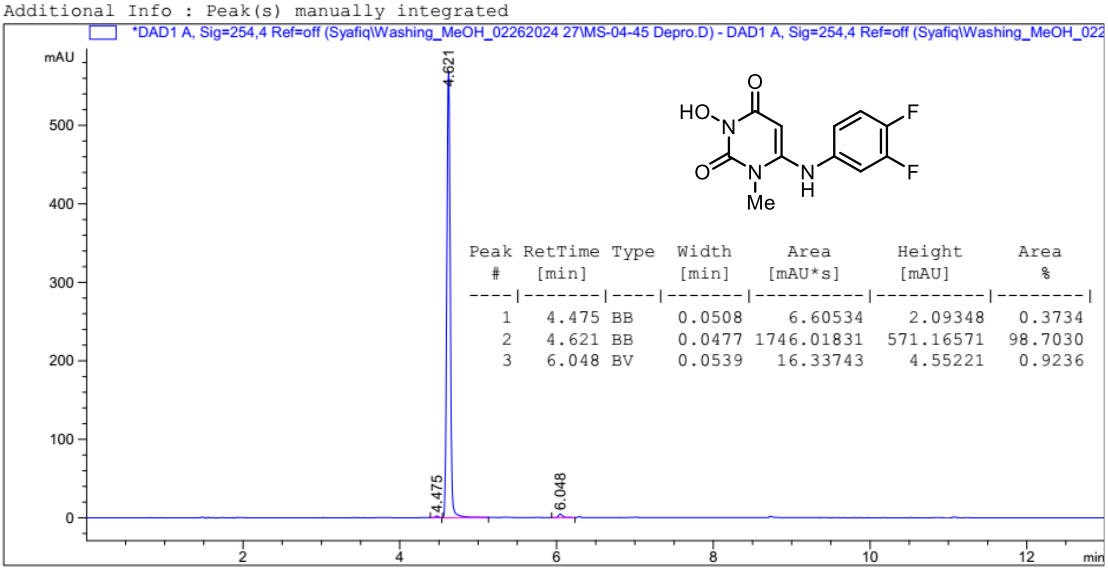
HRMS and HPLC Trace for Compound 3h

Sample Name	MS-04-45 depro	**Position**	P2-C10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-45 depro_7216.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	8/22/2025 9:46:10 AM (UTC-05:00)



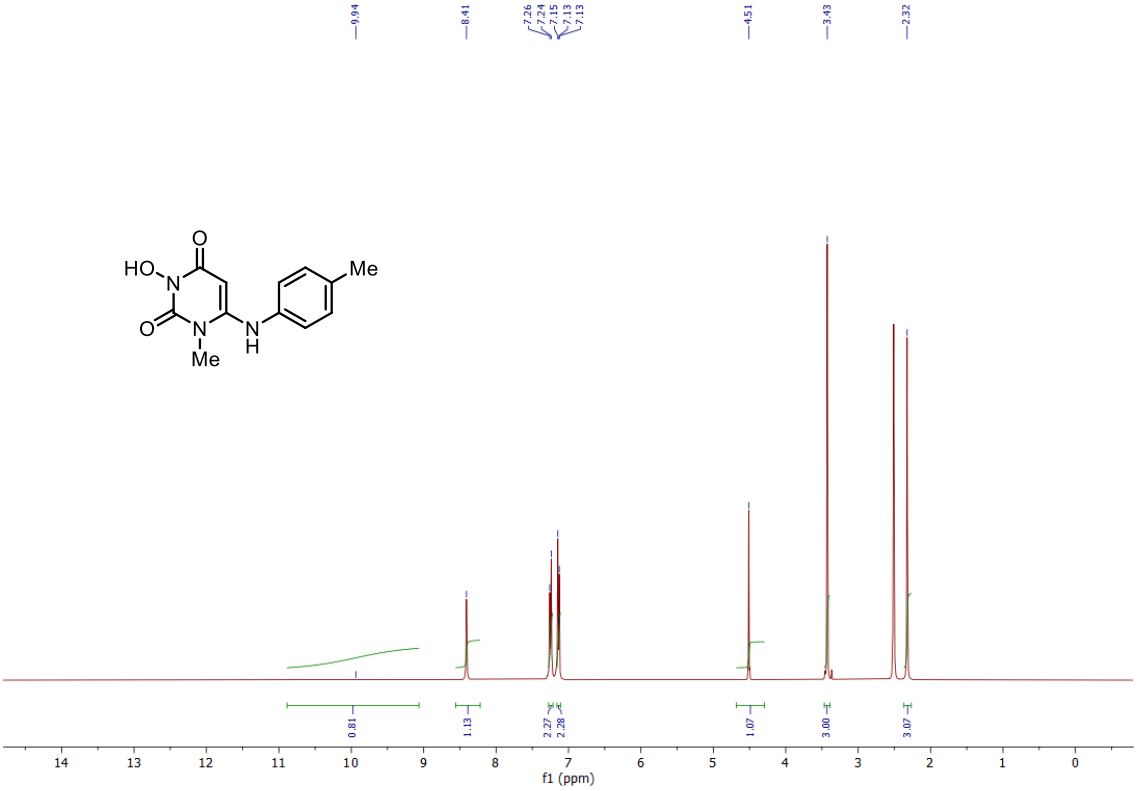
Sample Info : MS-04-45 Depro

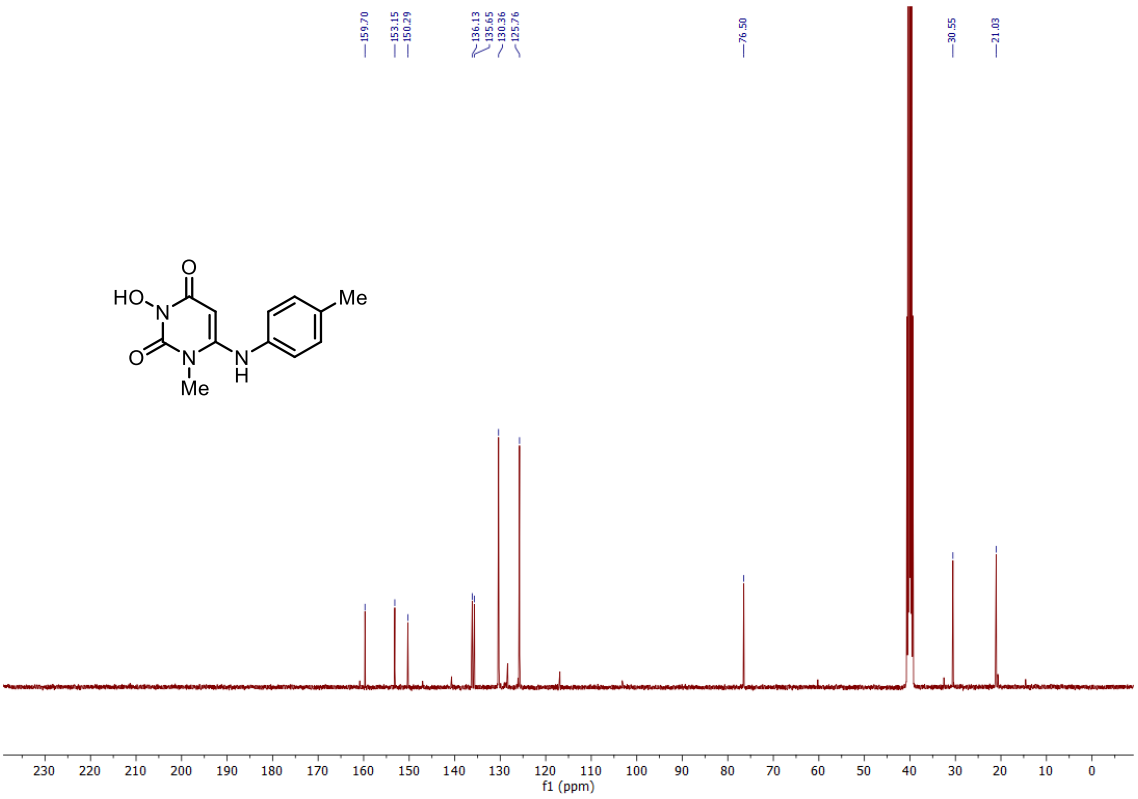
Additional Info : Peak(s) manually integrated



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.475	BB	0.0508	6.60534	2.09348	0.3734
2	4.621	BB	0.0477	1746.01831	571.16571	98.7030
3	6.048	BV	0.0539	16.33743	4.55221	0.9236

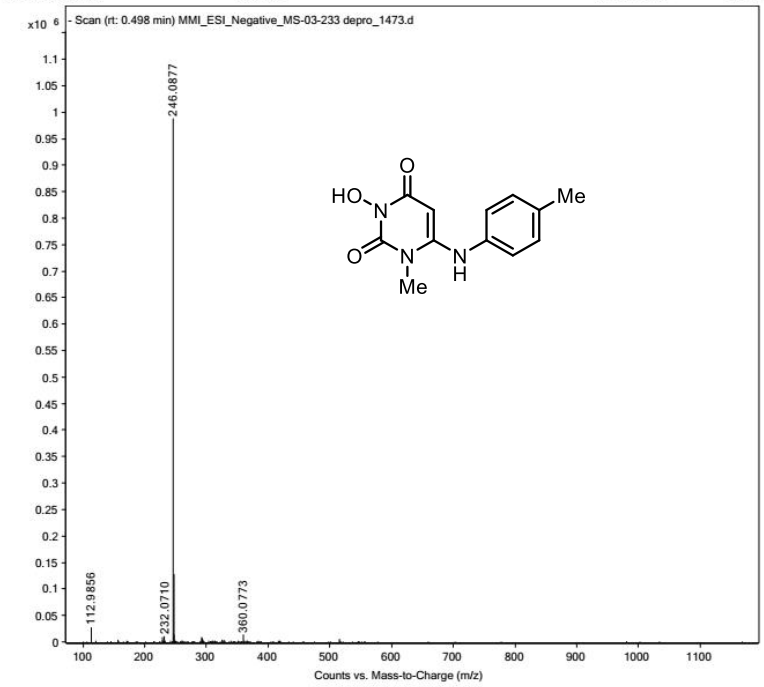
^{1}H NMR and ^{13}C NMR for Compound 3i in DMSO-d_6



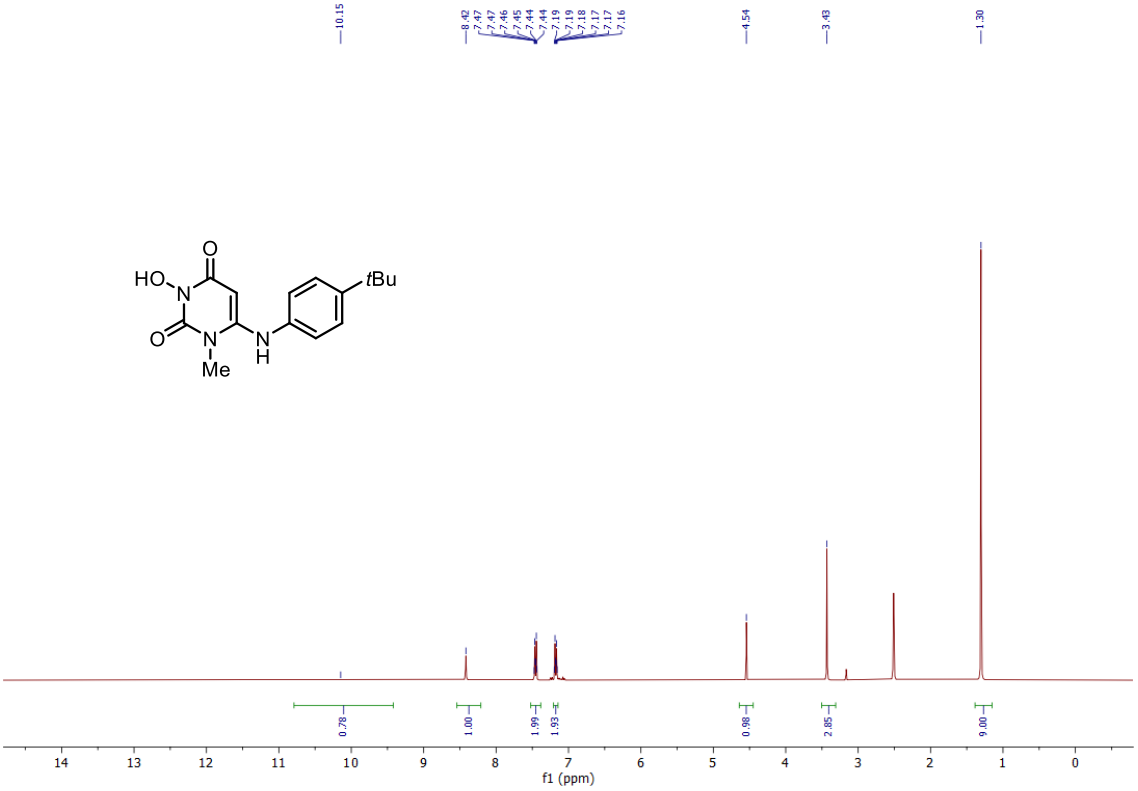


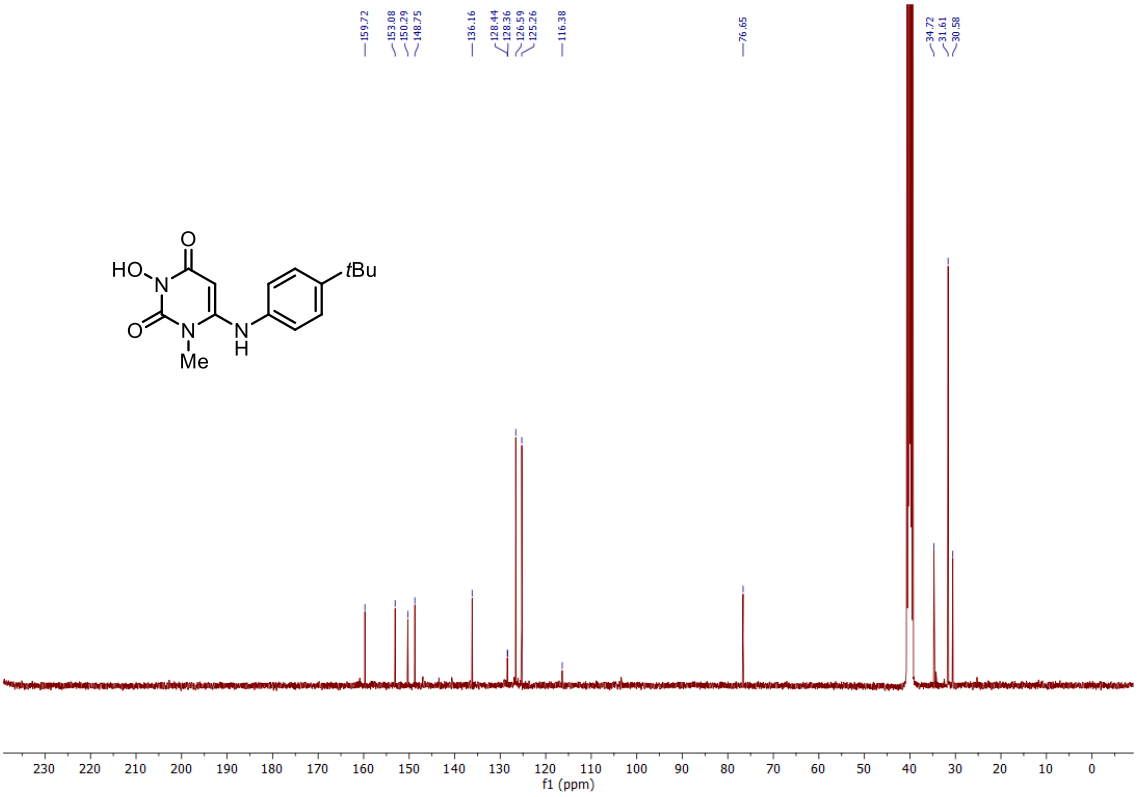
HRMS for Compound 3i

Sample Name	MS-03-233 depro	**Position**	P1-E1	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-03-233 depro_1473.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	3/10/2026 6:04:56 PM (UTC-05:00)



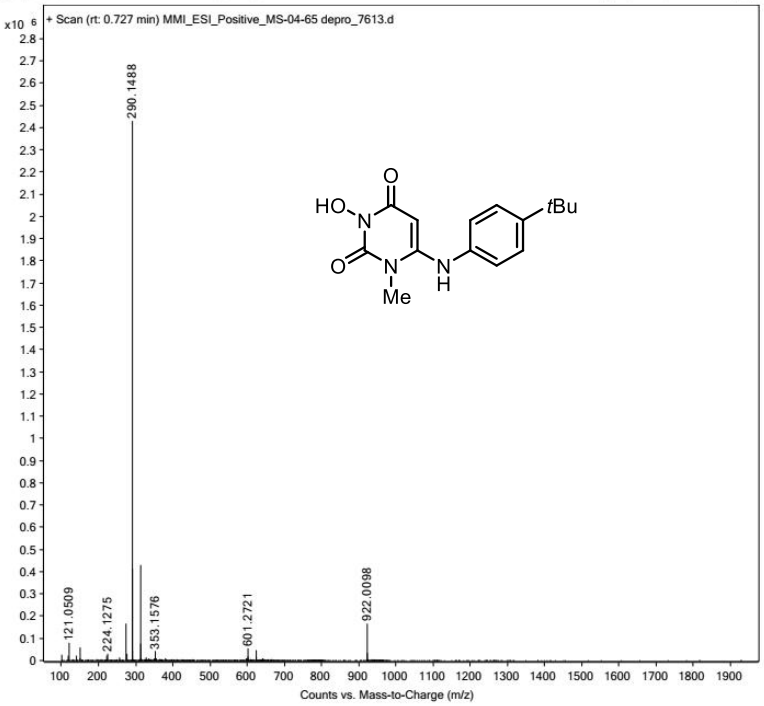
^{1}H NMR and ^{13}C NMR for Compound 3j in DMSO-d_6



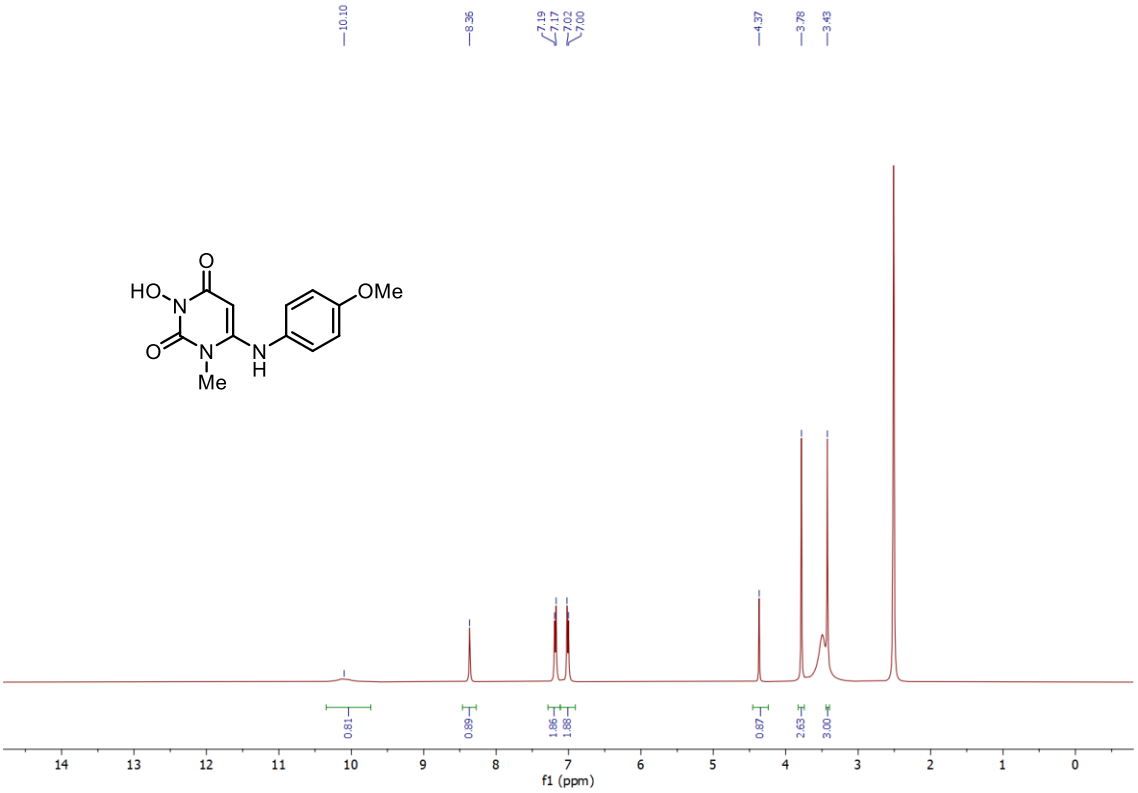


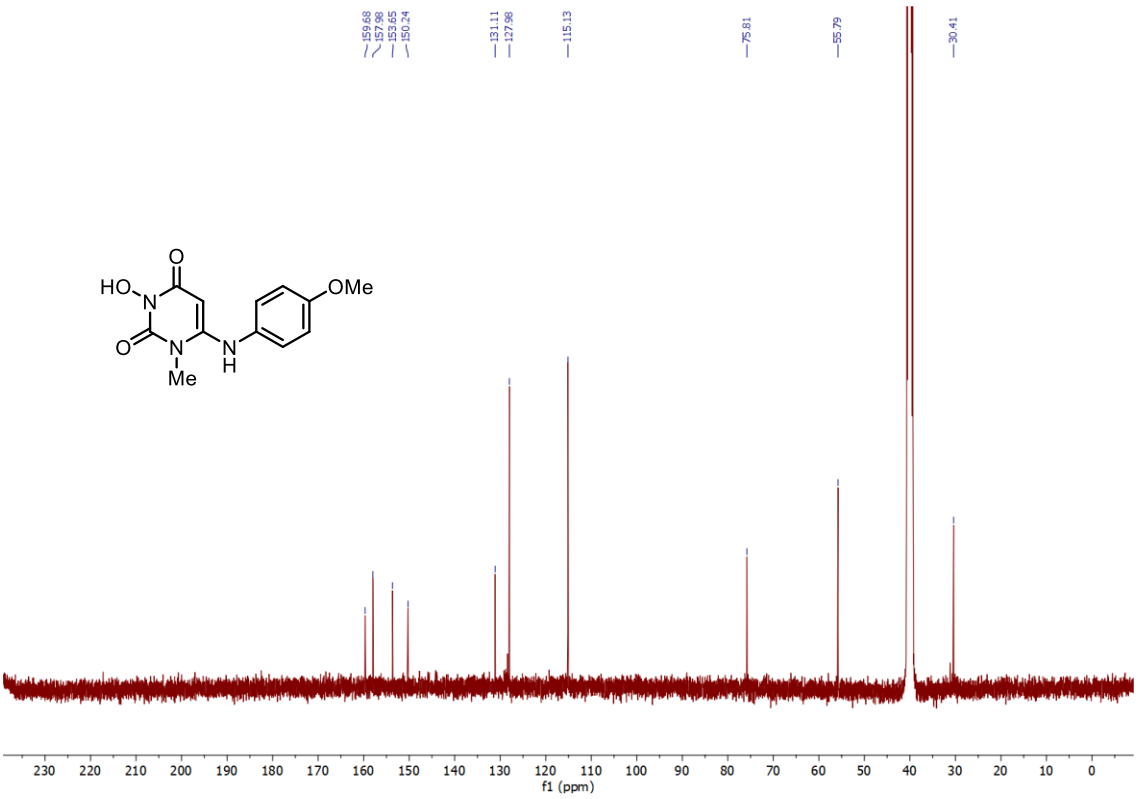
HRMS for Compound 3j

Sample Name	MS-04-65 depro	**Position**	P2-C10	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-65 depro_7613.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	9/10/2025 4:13:05 PM (UTC-05:00)



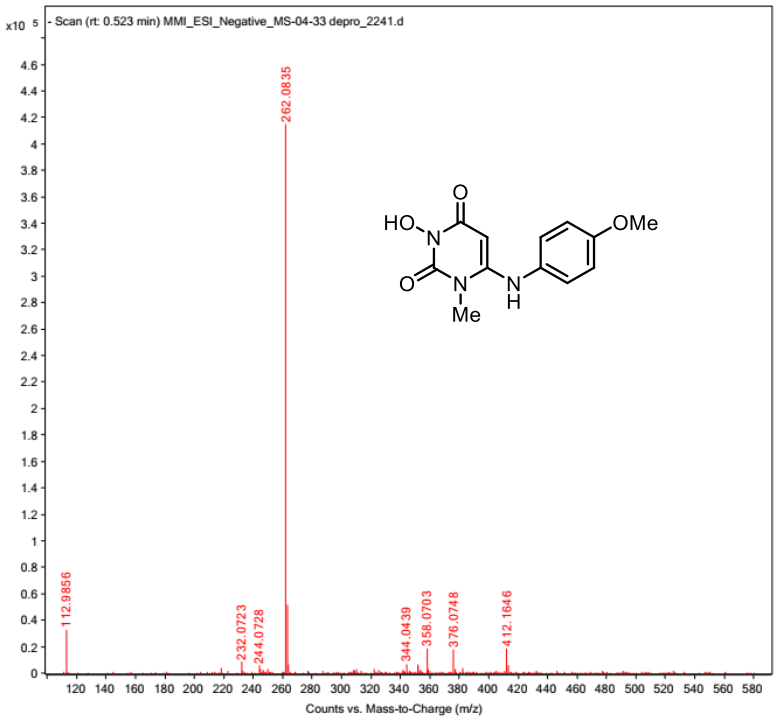
^{1}H NMR and ^{13}C NMR for Compound 3k in DMSO-d_6



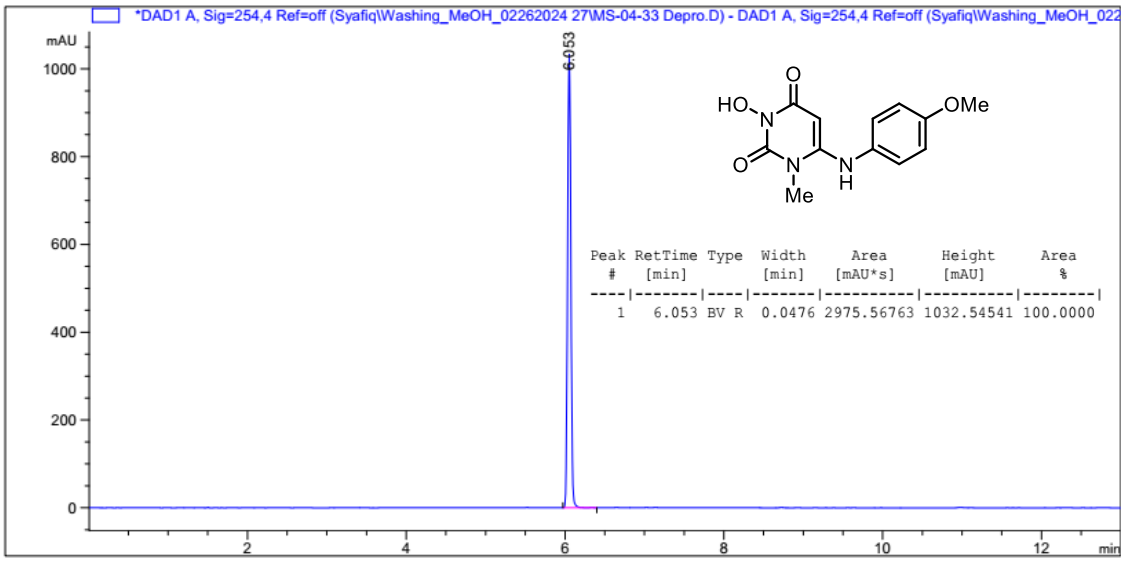


HRMS and HPLC Trace for Compound 3k

Sample Name	MS-04-33 depro	**Position**	P1-D7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Negative_MS-04-33 depro_2241.d
ACQ Method	MMI_ESI_Negative.m	**Comment**		**Acquired Time**	3/30/2026 9:01:04 AM (UTC-05:00)

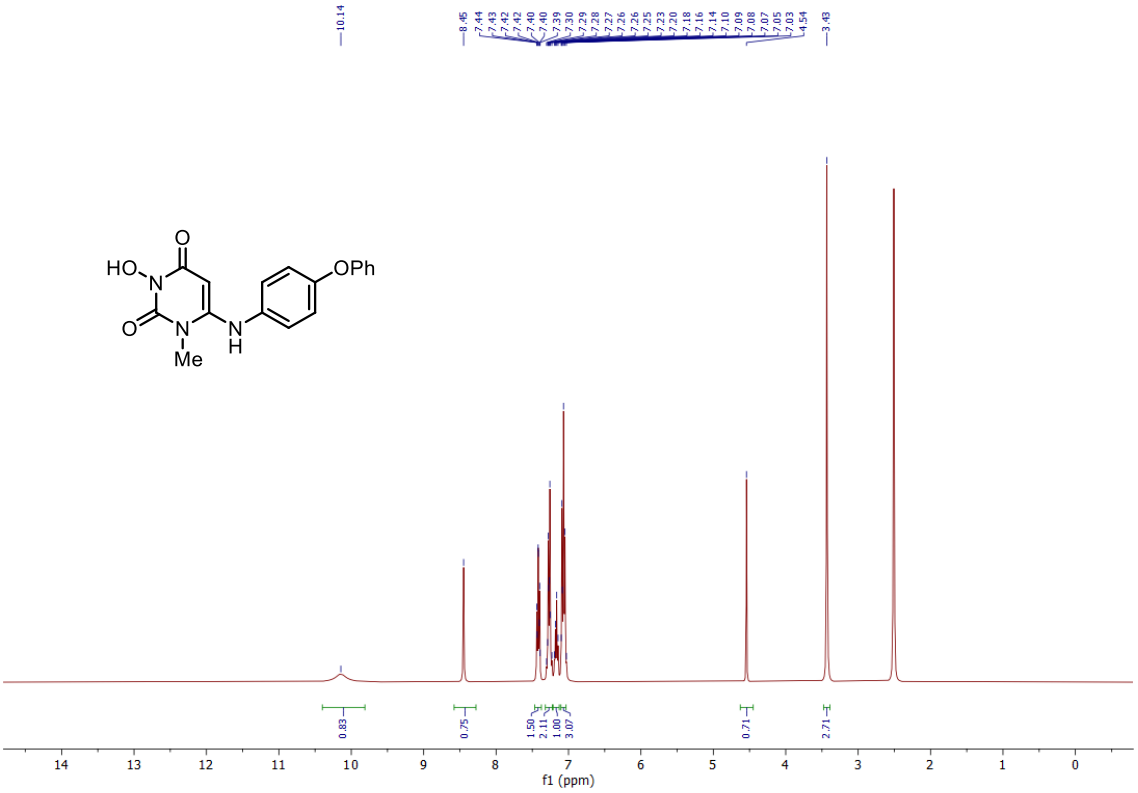


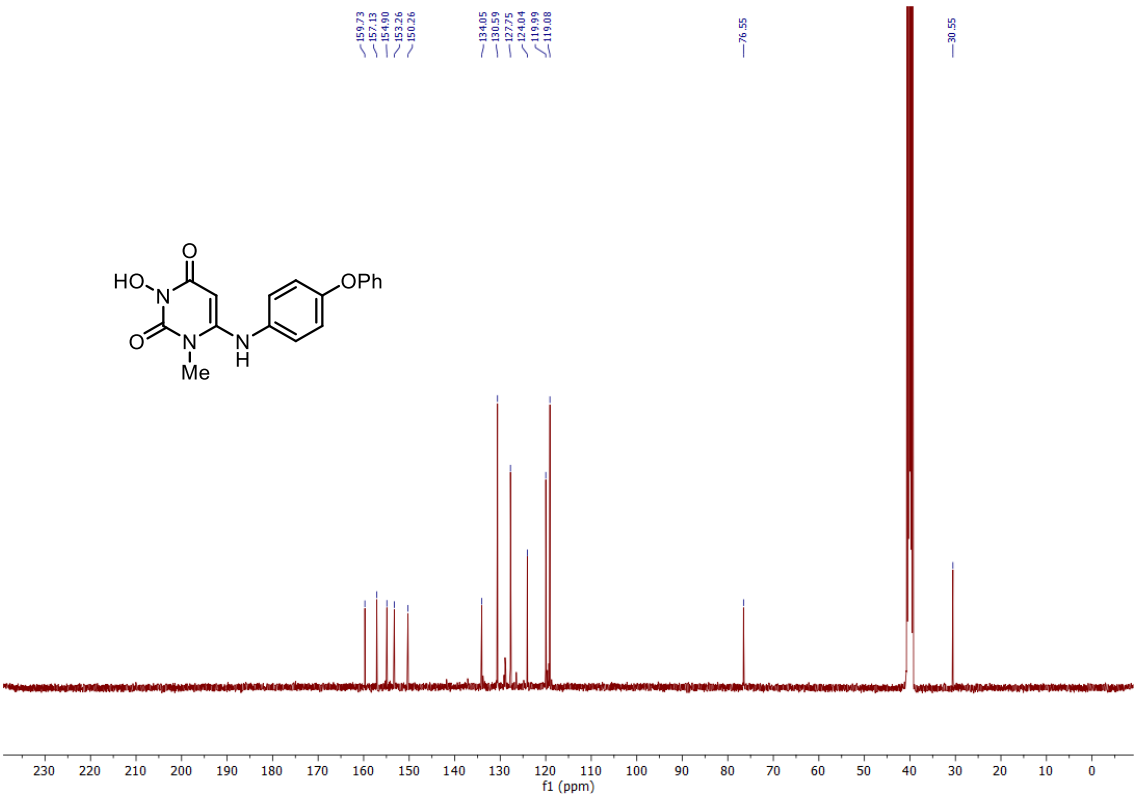
Sample Info : MS-04-33 Depro



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.053	BV R	0.0476	2975.56763	1032.54541	100.0000

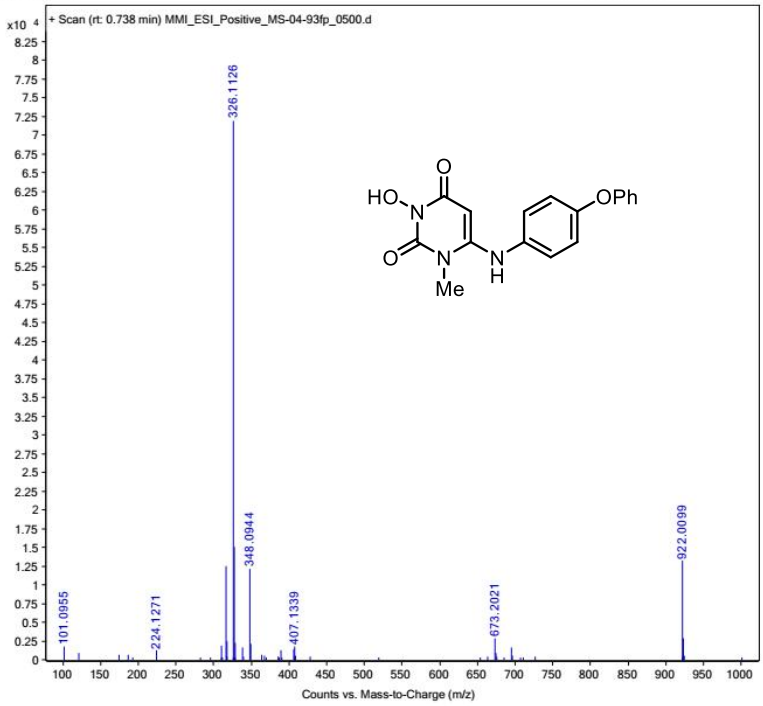
^{1}H NMR and ^{13}C NMR for Compound 3l in DMSO-d_6



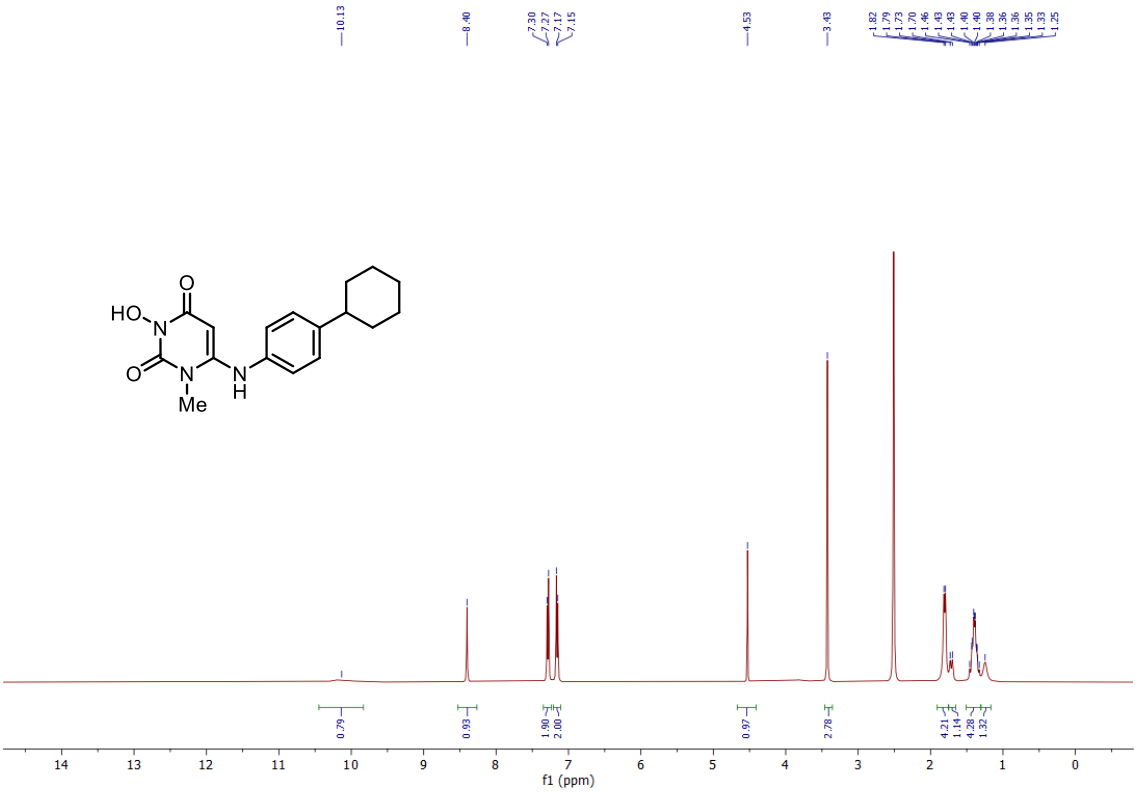


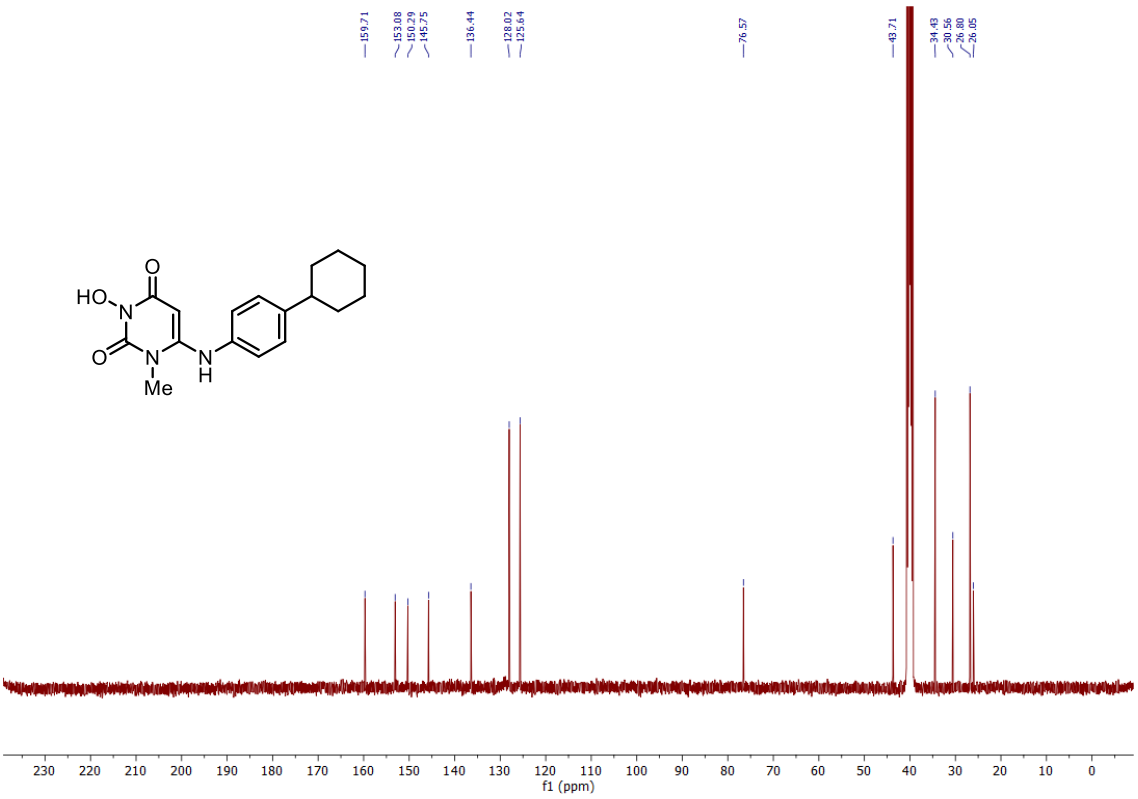
HRMS for Compound 3l

Sample Name	MS-04-93fp	**Position**	P2-D8	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-93fp_0500.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	12/10/2025 5:56:52 PM (UTC-06:00)



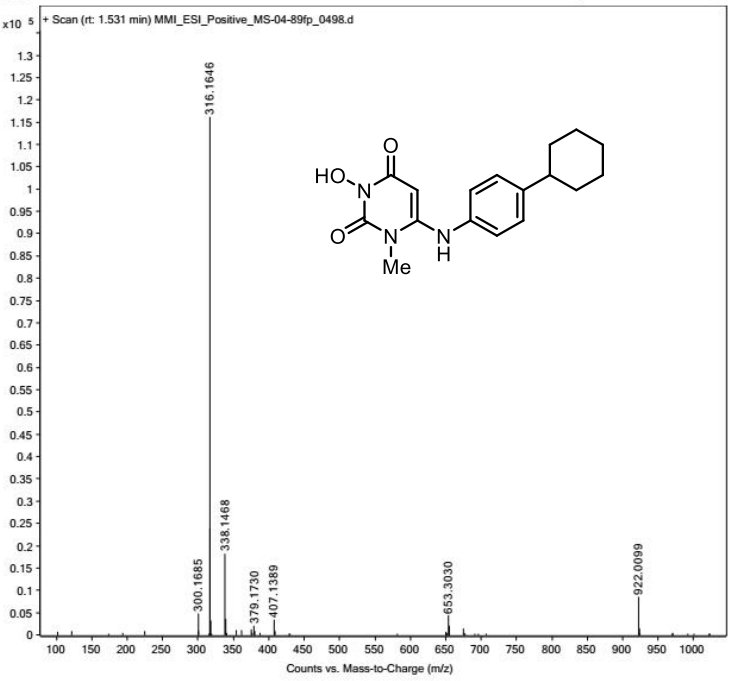
^{1}H NMR and ^{13}C NMR for Compound 3m in DMSO-d_6



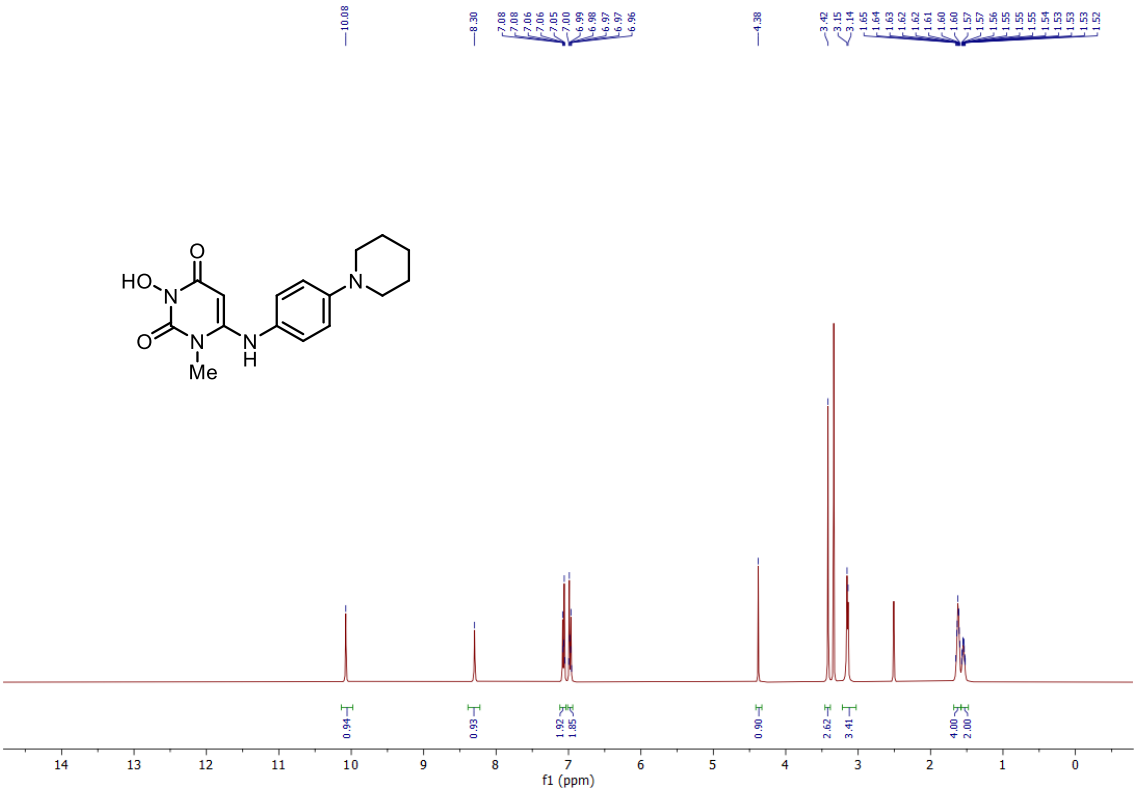


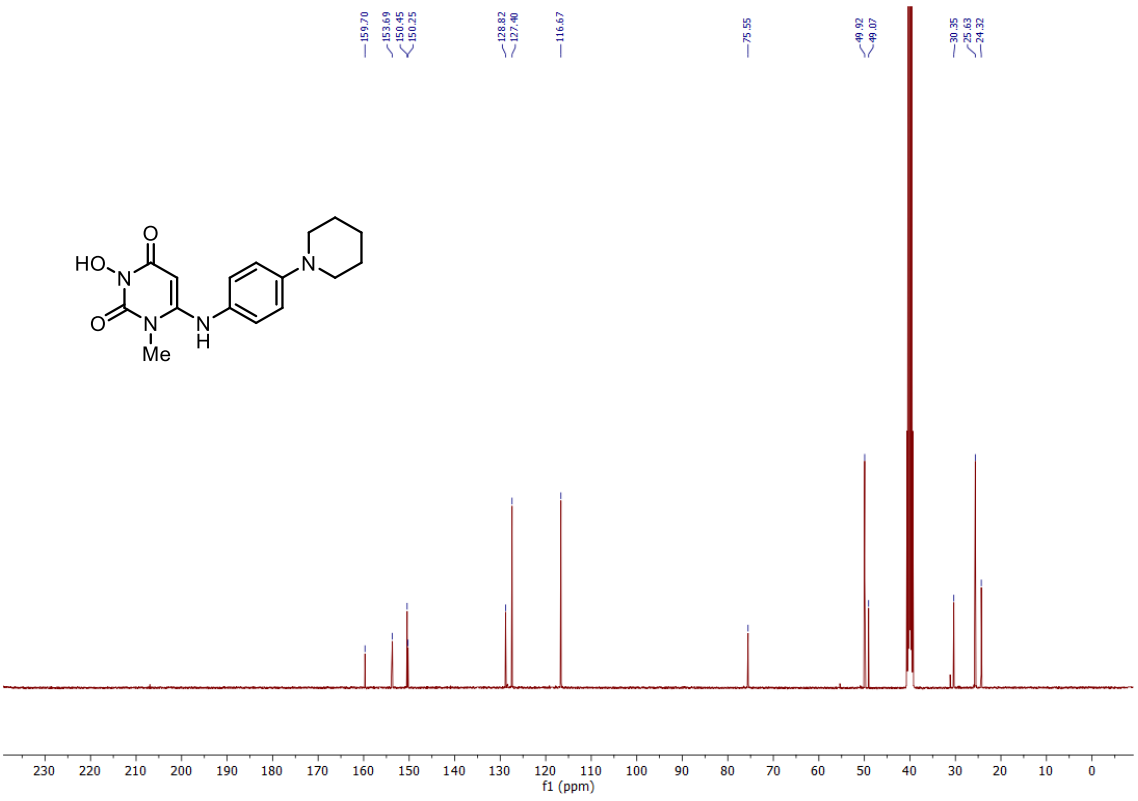
HRMS for Compound 3m

Sample Name	MS-04-89fp	**Position**	P2-D7	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-89fp_0498.d
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	12/10/2025 5:45:04 PM (UTC-06:00)



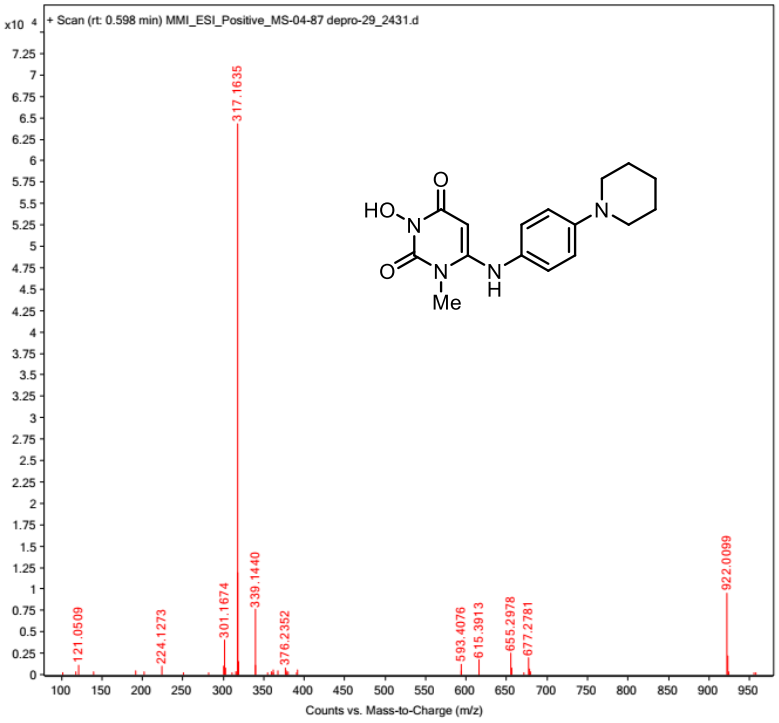
^{1}H NMR and ^{13}C NMR for Compound 3n in DMSO-d_6





HRMS for Compound 3n

Sample Name	MS-04-87 depro-29	**Position**	P1-F6	**Instrument Name**	TOF Walk Up
User Name	Syafiq	**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	MMI_ESI_Positive_MS-04-87 depro-29_2431.
ACQ Method	MMI_ESI_Positive.m	**Comment**		**Acquired Time**	4/2/2026 4:22:32 PM (UTC-05:00)



Measurement of 6-FAM fluorophore quenching by compounds

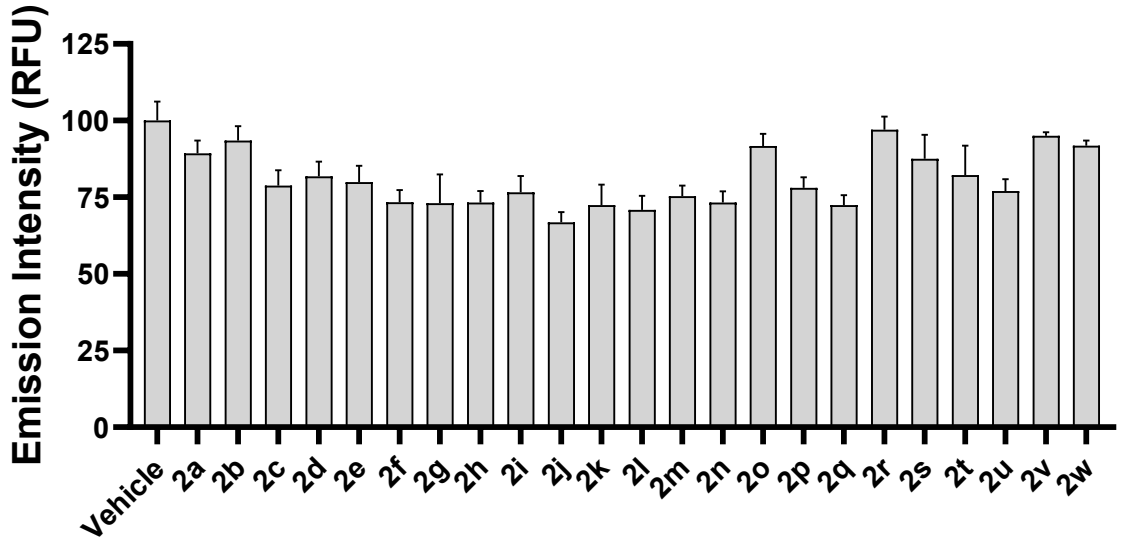


Figure S1. 6-FAM emission intensity in the presence of compounds **2a-w**

Compounds (50 μM final concentration) were mixed with the 6-FAM-labeled strand of the DNA substrate (5 nM final) in assay buffer (20 mM Tris pH 8.5, 50 mM NaCl, 10 mM $MgCl_2$, 5% glycerol, 5 mM β-ME, 0.0025% v/v Tween 20, 0.25% v/v DMSO) and incubated for 30 min at 37 °C before recording fluorescence intensity. Data are shown as mean ± SEM from n=1-2 independent experiments each performed in duplicate.

Detection of compound aggregation via centrifugation

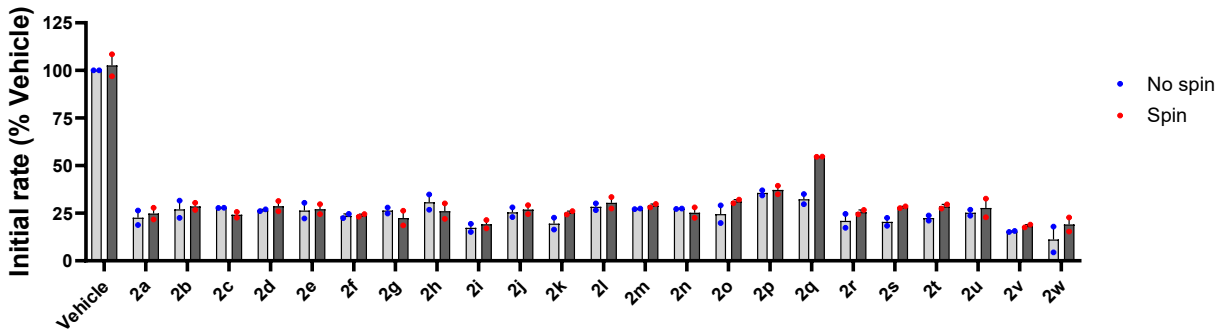
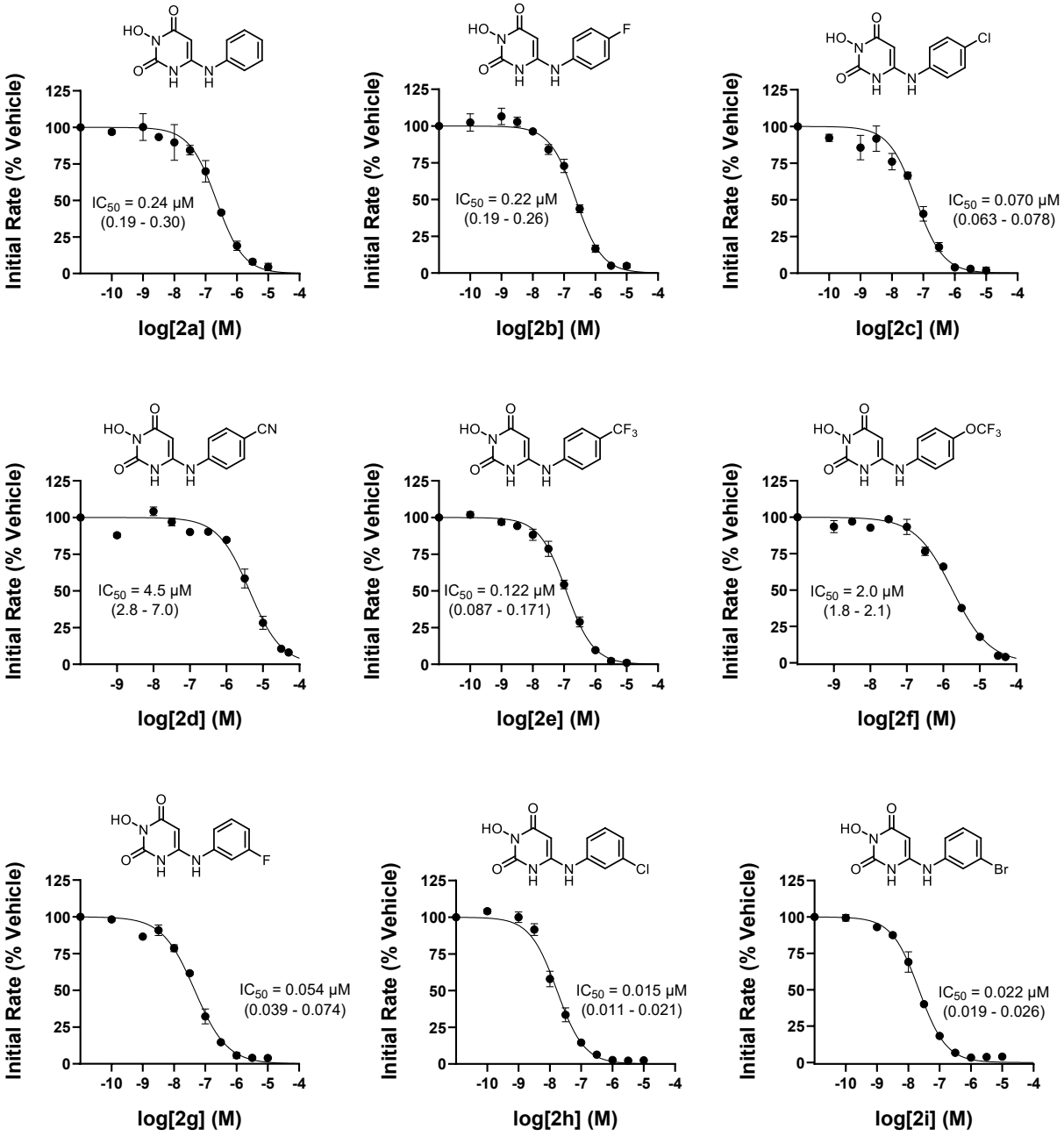
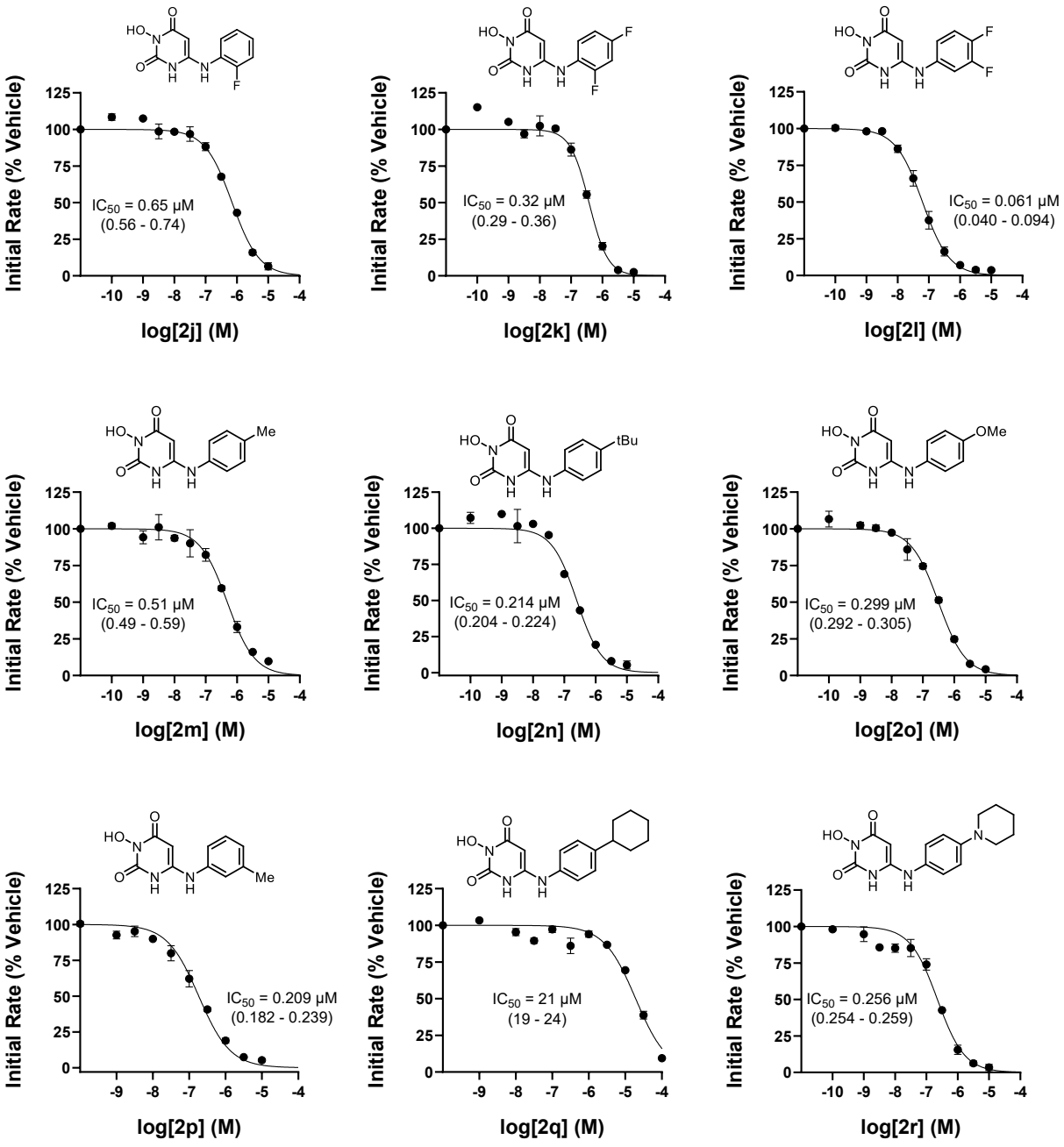


Figure S2. Effect of centrifugation on Mpr inhibition by compounds **2a-w**.

Compounds were diluted in assay buffer and split into two aliquots. One aliquot was added directly to the assay plate ("No spin") and the other aliquot was centrifuged for 20 min at 13,000 *g* prior to addition to the assay plate ("Spin"). MBP-3C-Mpr (10 nM final concentration) was pre-incubated with compound for 15 min at 37 °C before addition of substrate (5 nM final concentration) to initiate the reactions. The rate of fluorescence increase in the presence of inhibitors was expressed as a percentage of the rate in the presence of DMSO (Vehicle). Data are shown as mean ± SEM from n=2 independent experiments performed in duplicate.

Mpr dose-response curves of compounds 2a-w




Initial Rate (% Vehicle)
IC50 = 0.65 μM
(0.56 - 0.74)
log[2j] (M)
IC50 = 0.32 μM
(0.29 - 0.36)
log[2k] (M)
IC50 = 0.061 μM
(0.040 - 0.094)
log[2l] (M)
IC50 = 0.51 μM
(0.49 - 0.59)
log[2m] (M)
IC50 = 0.214 μM
(0.204 - 0.224)
log[2n] (M)
IC50 = 0.299 μM
(0.292 - 0.305)
log[2o] (M)
IC50 = 0.209 μM
(0.182 - 0.239)
log[2p] (M)
IC50 = 21 μM
(19 - 24)
log[2q] (M)
IC50 = 0.256 μM
(0.254 - 0.259)
log[2r] (M)

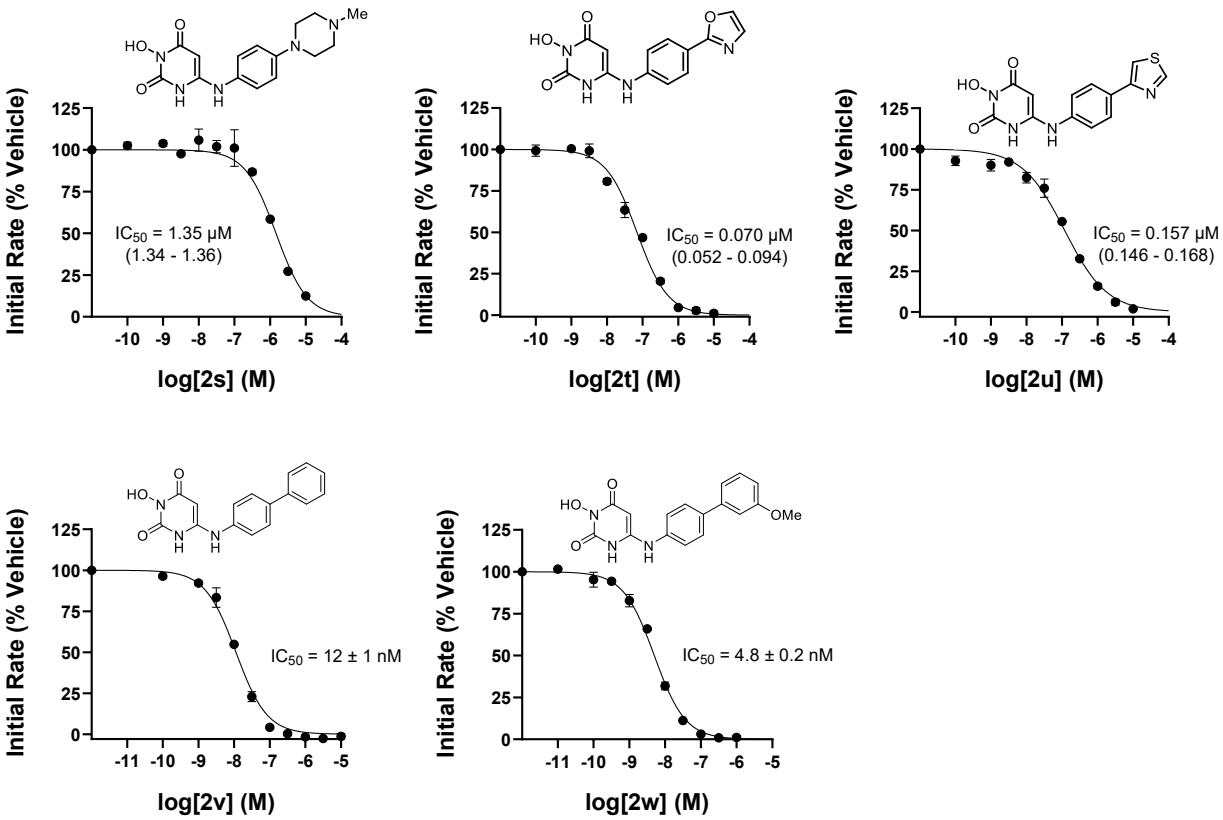


Figure S3. Concentration-response analysis of Mpr inhibitors **2a-w**.

Mpr (10 nM final concentration) was pre-incubated with compound for 15 min at 37 °C before addition of substrate (5 nM final concentration) to initiate the reaction. The rate of fluorescence increase was determined from the linear portion of each trace, and rates in the presence of inhibitors were expressed as a percentage of the rate in the presence of DMSO ("Vehicle"). Data are shown as mean ± SEM from two independent experiments, each performed in duplicate.