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Supporting Information

Small Molecule Inhibitors of Interferon-Induced JAK-STAT Signalling

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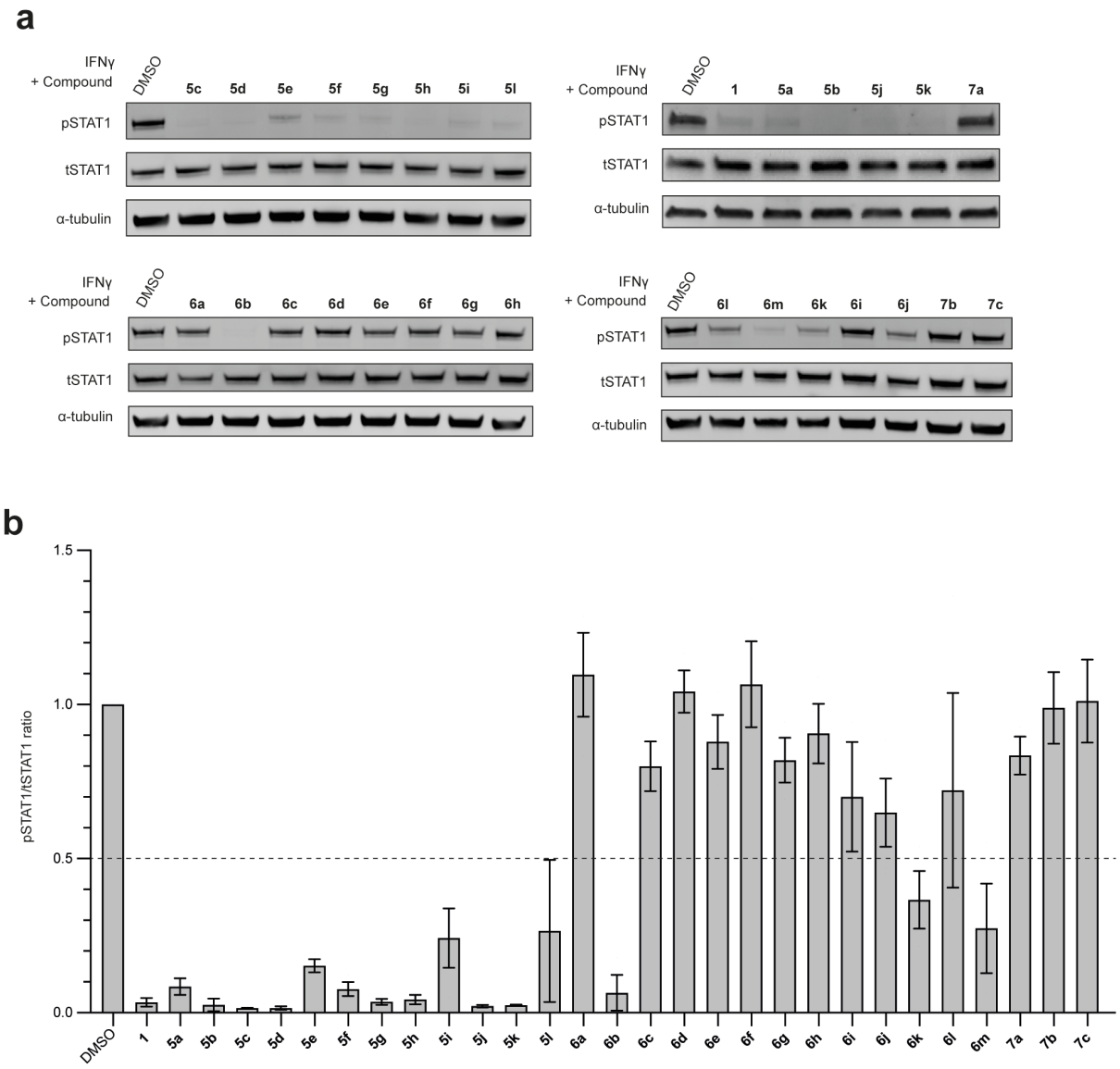
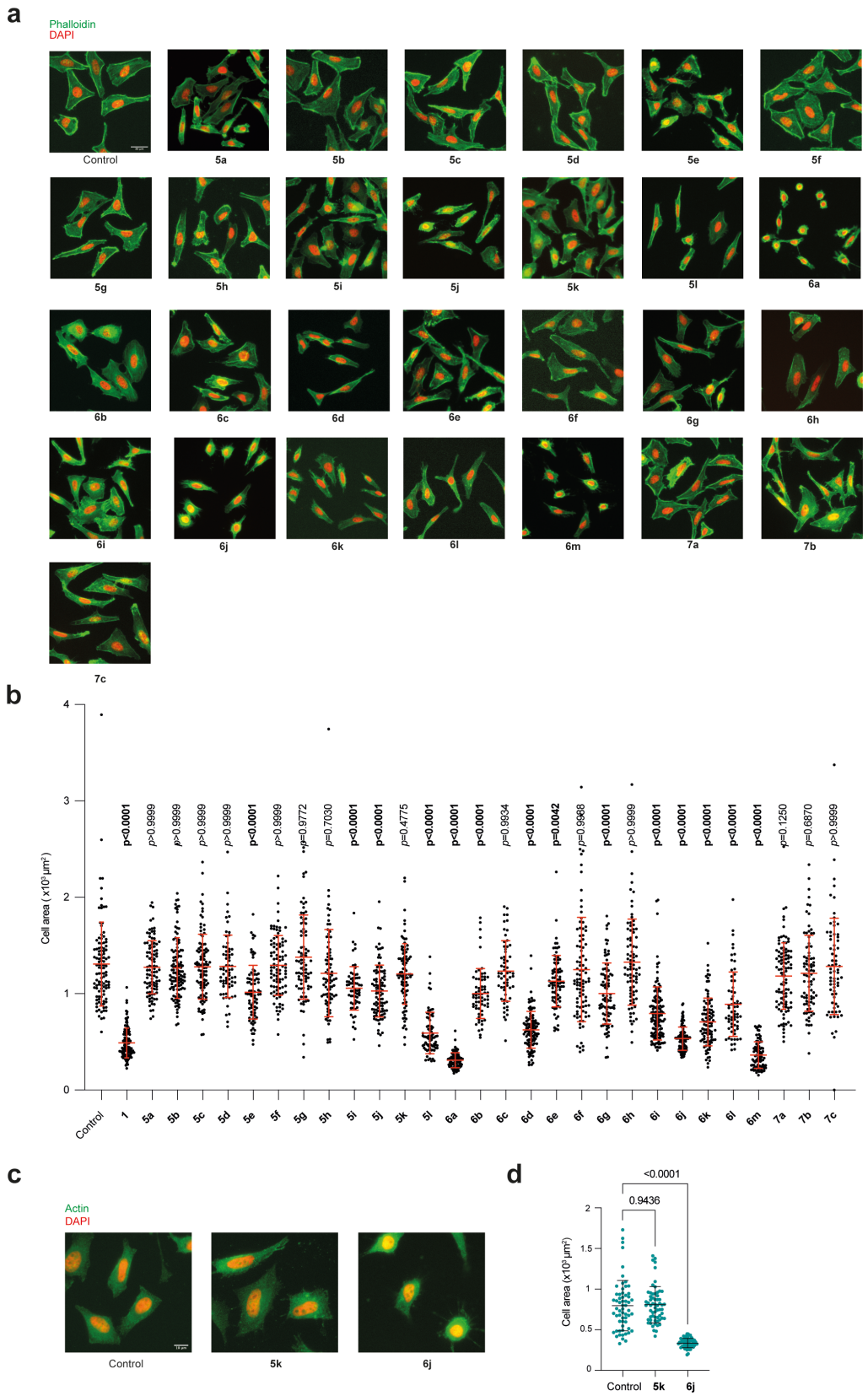
Supporting Information

Contents

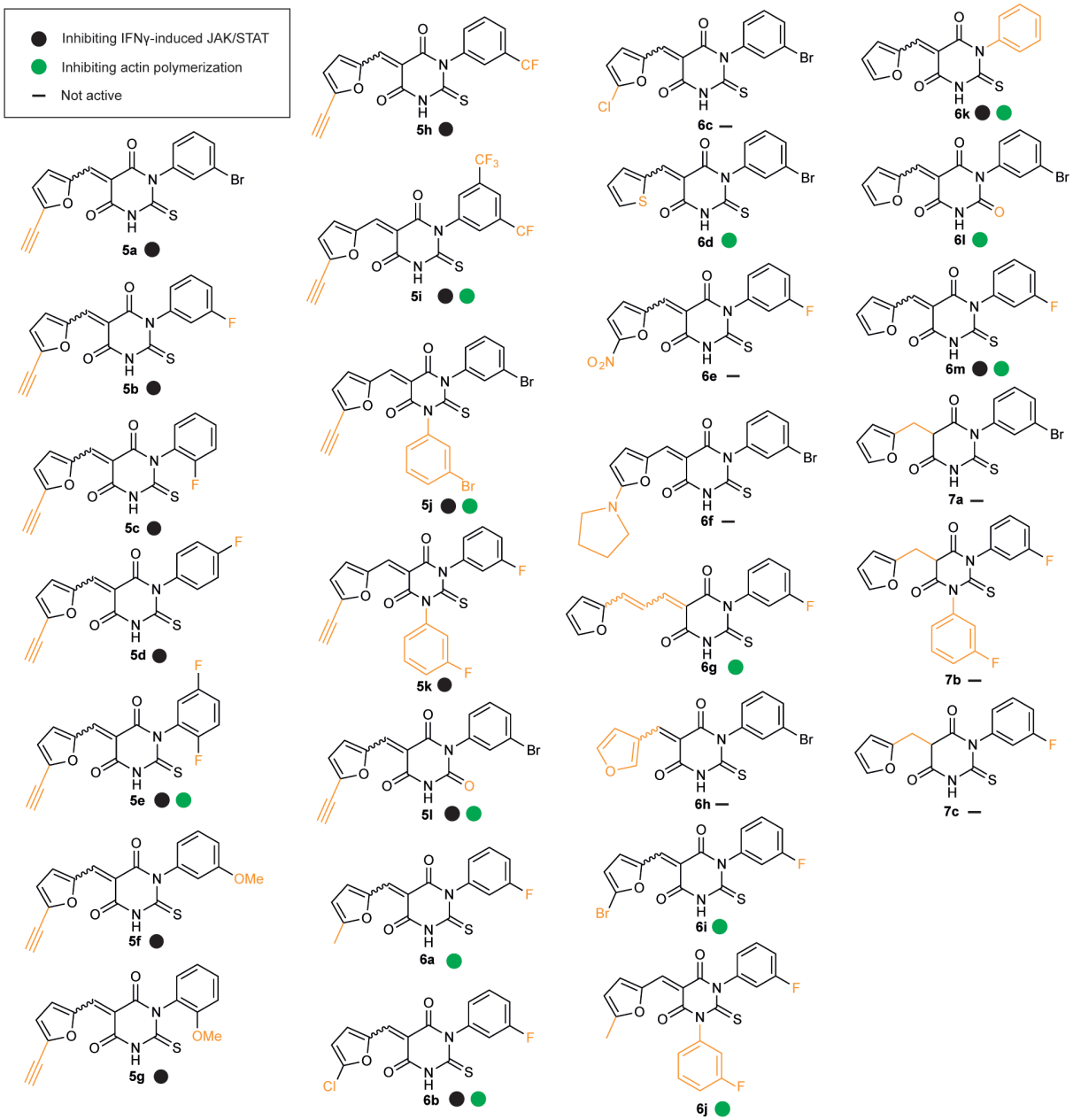


Supplementary Figure 1 (S1). a) Anti-IFNγ activity screening performed using immunoblot analysis of pSTAT1 and tSTAT1 levels in HeLa cells treated with IFNγ (1000 $U.ml^{-1}$, 20 min), preincubated or not with compounds (40 µM) for 20 min. Immunoblots on top right (screening of **1, 5a, 5b, 5j, 5k, 7a**) are duplicates of Figure 2b. b) Quantification of pSTAT1/tSTAT1 of the immunoblot in **a**. *n* = 3 independent experiments.

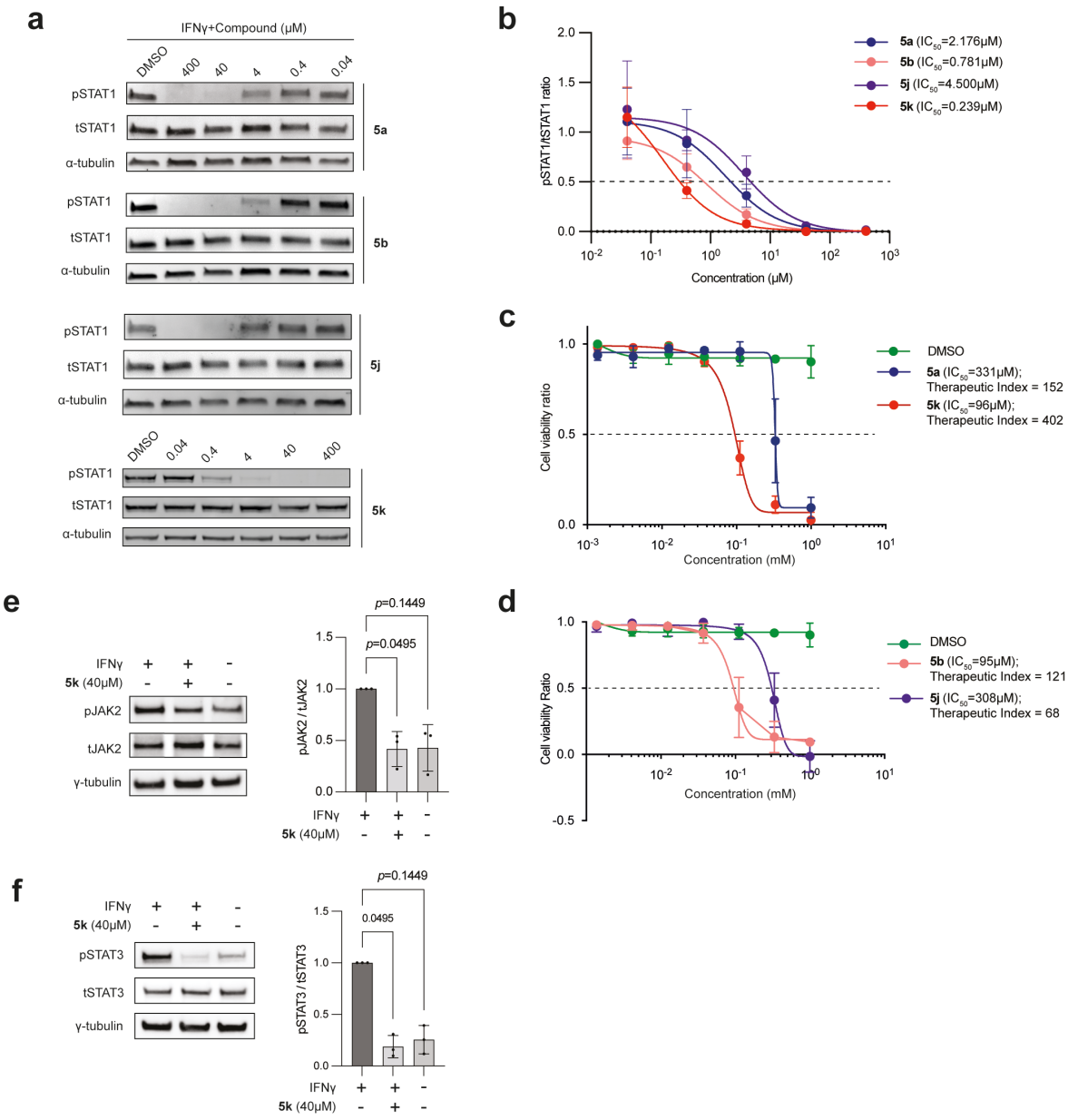


Supplementary Figure 2 (S2). a) Actin polymerization activity performed using fluorescence microscopy. Images of HeLa cells treated with small molecules (40 µM) for 20 min and stained for actin with phalloidin Alexa-488. Scale bar = 30 µm. Images of Control**, 5a, 5b, 5j, 5k, 7a** and **6j** are duplicates of Figure 2c. b) Quantification of cell area. Mean value ± SD. Statistical analysis with one-way ANOVA. *n* = 3 independent experiments. c) Anti-actin polymerization activity screening (actin antibody) performed using fluorescence microscopy images of HeLa cells treated with compounds (40 µM) for 20

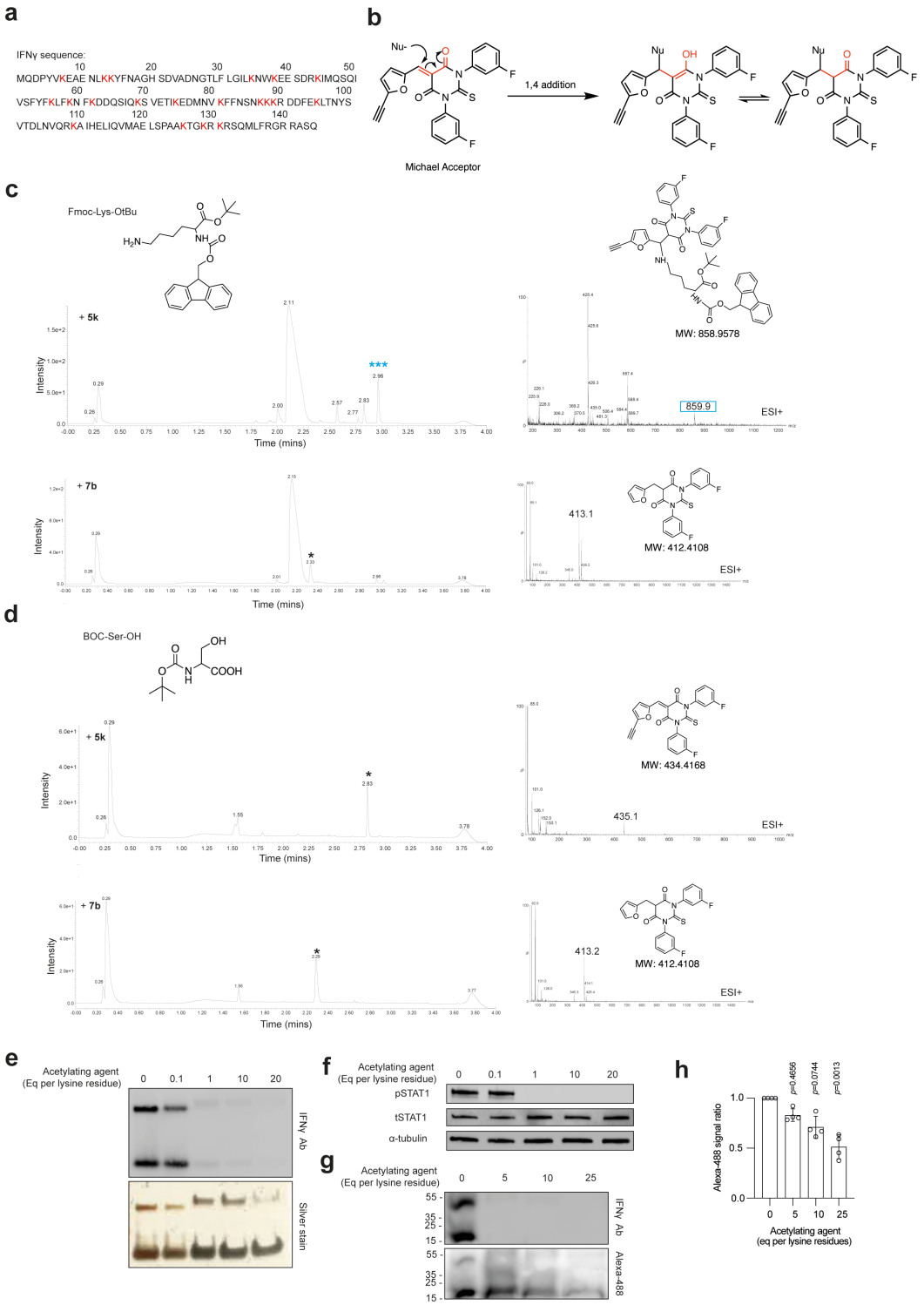
min. Scale bar = 10 µm. d) Quantification of cell area from **c**. Mean value ± SD. Statistical analysis with one-way ANOVA. *n* = 3 independent experiments.



Supplementary Figure 3 (S3). Structure of derivatives of **1** (SMIFH2) along with their activities as indicated.

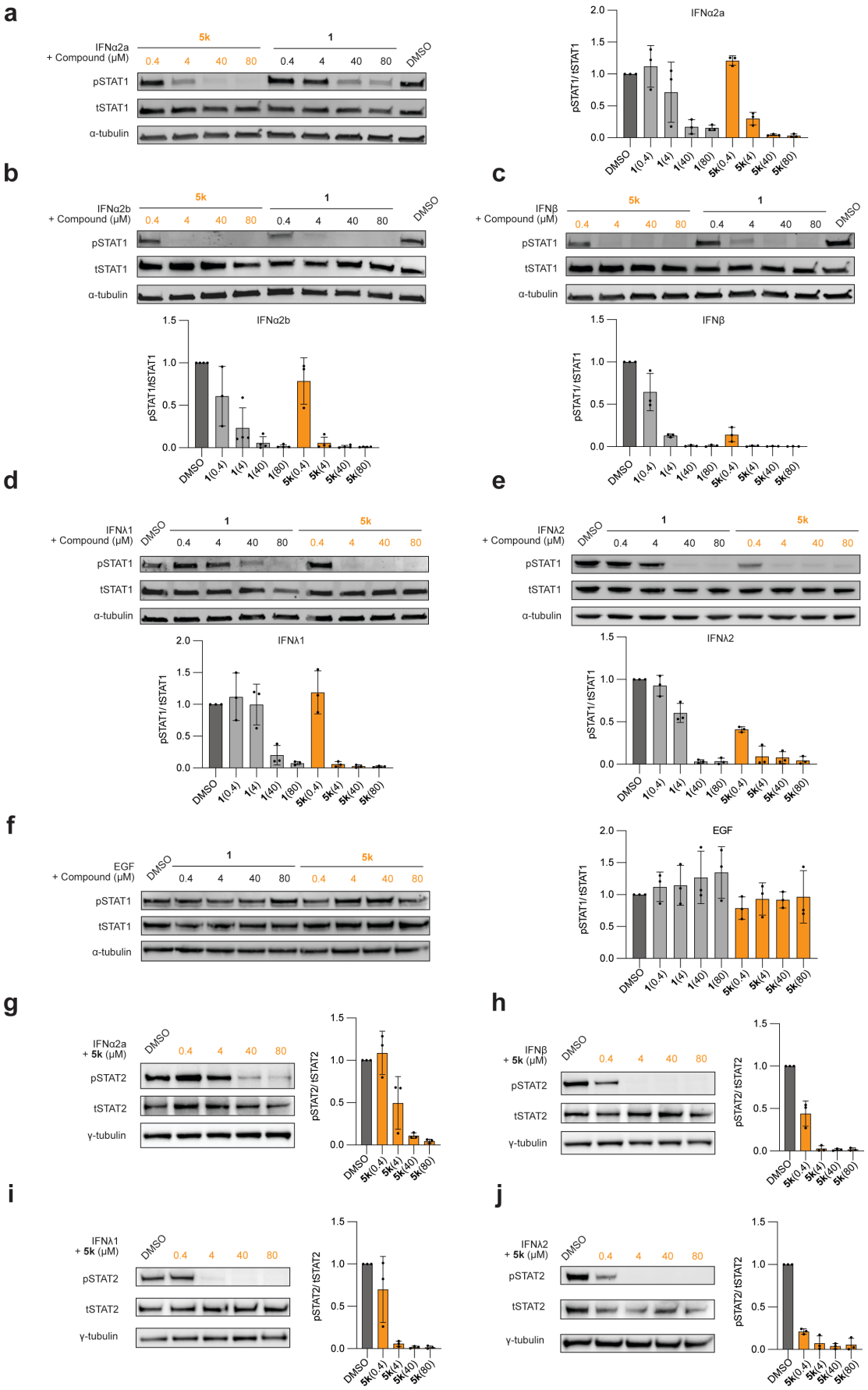


Supplementary Figure 4 (S4). a) Immunoblot analysis of pSTAT1 and tSTAT1 levels in HeLa cells treated for 20 min with IFNγ (1000 U.ml^{-1}), preincubated or not with different concentrations of indicated compounds for 20 min. Tubulin serves as a loading control. b) Quantification of pSTAT1/tSTAT1 of immunoblot in **a**. IC_{50} towards IFNγ are determined by non-linear curve fit. *n* = 3 independent experiments. c) Dose-response viability curves of HeLa cells treated for 24 h with specified compounds. IC_{50} are determined by non-linear curve fit. *n* = 3 independent experiments. Therapeutic Index= (IC_{50} of Cell Viability/ IC_{50} of pSTAT1 inhibition). d) Dose-response viability curves of HeLa cells treated for 24 h with specified compounds. IC_{50} are determined by non-linear curve fit. *n* = 3 independent experiments. Therapeutic Index= (IC_{50} of Cell Viability/ IC_{50} of pSTAT1 inhibition). e) Immunoblot analysis of phosphorylation status of JAK2 (pJAK2) and total JAK2 (tJAK2) levels in HeLa cells treated for 20 min by IFNγ (1000 U.ml^{-1}), preincubated or not with **5k** (40 μM) for 20 min as indicated. Tubulin serves as a loading control. The corresponding quantification as ratio of pJAK2/tJAK2. Mean value ± SD. Statistical analysis with Kruskal-Wallis test with Dunn's post-test. *n* = 3 independent experiments. f) Immunoblot analysis of pSTAT3 and tSTAT3 levels in HeLa cells treated for 20 min by IFNγ (1000 U.ml^{-1}), preincubated or not with **5k** (40 μM) for 20 min as indicated. Tubulin serves as a loading control. The corresponding quantification as ratio of pSTAT3/tSTAT3. Mean value ± SD. Statistical analysis with Kruskal-Wallis test with Dunn's post-test. *n* = 3 independent experiments.



Supplementary Figure 5 (S5). a) IFNγ amino acid sequence. Lysine residues are highlighted in red. b) Reaction showing the nucleophilic attack on the Michael acceptor region (highlighted in red) of **5k** resulting in 1,4 Michael addition product. c) Chromatogram of UPLC-MS analysis of **5k** (up) and **7b** (down) incubated at 37°C in PBS for 12 h with Fmoc-Lysine-OtBu respectively and the corresponding ESI+ spectrum of the peak marked by asterisks (*). 1,4 Michael addition product is observed with **5k** and Fmoc-Lysine-OtBu indicated by ***. d) Chromatogram of UPLC-MS analysis of **5k** (up) and **7b** (down) incubated at 37°C in PBS for 24h with Boc-Serine-OH respectively and the corresponding ESI+ spectrum of the peak marked by asterisk (*). No 1,4 Michael addition product is observed with **5k**. e) Immunoblot showing the effect of acetylating agent on IFNγ. Top: IFNγ antibody. Bottom: silver staining. f) Immunoblot showing the effect of acetylated IFNγ on JAK-STAT signalling. g) Immunoblot analysis of IFNγ preincubated with analogue **5k** and acetylating agent as indicated. *n* = 4 independent

experiments. h) Alexa-488 signal ratio of immunoblot in **g**. Mean value ± SD. Statistical analysis with Kruskal-Wallis test with Dunn's post-test.

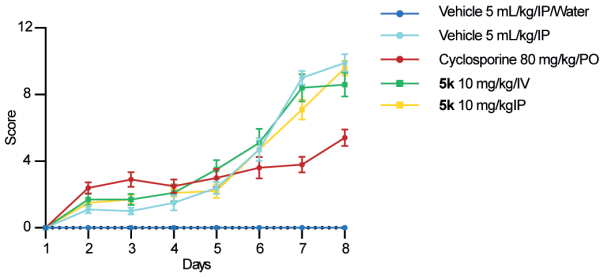


Supplementary Figure 6 (S6). Immunoblot analysis of pSTAT1 and tSTAT1 levels in HeLa cells treated for 20 min with a) IFNα2a (1000 U.ml^{-1}), b) IFNα2b (1000 U.ml^{-1}), c) IFNβ (1000 U.ml^{-1}), d) IFNλ1 (1000 U.ml^{-1}), e) IFNλ2 (1000 U.ml^{-1}) and f) EGF (15.6 nM) preincubated with concentrations of **1** and **5k** for 20 min as indicated and corresponding pSTAT1/tSTAT1 ratio. *n* = 3 independent experiments. Immunoblot analysis of pSTAT2 and tSTAT2 levels in HeLa cells treated for 20 min with g) IFNα2a (1000 U.ml^{-1}), h) IFNβ (1000 U.ml^{-1}), i) IFNλ1 (1000 U.ml^{-1}) and j) IFNλ2 (1000 U.ml^{-1}) preincubated with concentrations of **5k** for 20 min as indicated and corresponding pSTAT2/tSTAT2. *n* = 3 independent experiments.

a

Route		IV	IP
Target Dose	(mg/kg)	2	10
$t_{½}$	(hr)	3.63	4.10
T_{max}	(hr)		2.00
C_{max}	(ng/mL)	3710	1442
C_0	(ng/mL)	4860	
$AUC_{0\text{-}last}$	(hr*ng/mL)	2072	8249
$AUC_{0\text{-}last}$/Dose	(hr*ng/mL)	1036	825
$AUC_{0\text{-}inf}$	(hr*ng/mL)	2085	8421
CL	mL/min/kg	16.0	
V_{ss}	L/kg	2.84	
relative F	%		79.6

b



Supplementary Figure 7 (S7). a) Pharmacokinetic profiling of compound in the C57BL/6N male mice following intravenous (IV) and intraperitoneal (IP) administration. b) Acute model of DSS-induced colitis in C57BL/6 mice. Disease clinical index (mean +/- SEM).

Material and methods:
A) Chemical synthesis

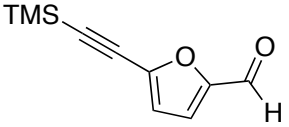
General information:

All solvents and chemicals were purchased from commercially sources and used without further purification. Solvents were dried under standard conditions. Reactions were monitored by thin layer chromatography (TLC) using precoated silica on aluminium sheets from Merck (60 F_{254}). TLC sheets were visualized with UV light. Products were purified on column chromatography with Silica gel 60 from Alfa Aesar (0.036-0.071 mm; 215-400 mesh), a Combiflash RF+ Teledyne Isco system fitted with pre-packed silica gel columns (Interchim, 4-300 g columns, 50 µm particle size), a preparative HPLC Quaternary Gradient 2545 equipped with a Photodiode Array detector (Waters) fitted with a reverse phase column (XBridge Prep C_{18} 5 µm OBD 30×150 mm) in acetonitrile and water system.

NMR spectroscopy was performed on Bruker spectrometers. Spectra were run at 298 K unless stated otherwise. ^{1}H-NMR were recorded at 400 or 500 MHz, and chemical shifts δ are expressed in ppm using the residual non-deuterated solvent signal as internal standard and the coupling constants *J* are specified in Hz and E/Z to denote the signal from isomers. The following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; td, triplet of doublets; q, quadruplet; m, multiplet; bs, broad signal. We only reported labile protons that could be clearly identified in the spectra, namely for compounds **2c**, **2f**, **7a, 7b** and **7c**. ^{13}C-NMR were recorded at 101 or 126 MHz, and chemical shifts δ ppm are expressed in ppm using deuterated solvent signal as internal standard and the coupling constants J with Fluorine are specified in Hz. ^{19}F-NMR were recorded at 376 or 471 MHz, and chemical shifts δ are expressed in ppm. Molecular structures were characterized using a comprehensive dataset including ^{1}H- and ^{13}C-NMR spectra (1D and 2D experiments including COSY, HMBC, and HSQC). For the final compounds which are in isomeric mixtures, the signals corresponding to either E or Z are separated by "/" and denoted by (E/Z). MestReNova V-10.0.1 was used to process the spectrum.
The purity of final compounds, determined to be >95% by UPLC-MS, and low-resolution mass spectra (LRMS) were recorded on a Waters Acquity H-class equipped with a Photodiode array detector and SQ Detector 2 fitted with a reverse phase column (Aquity UPLC® BEH C_{18} 1.7 µm, 2.1x50 mm). Gradient used was H_2O/ACN-95/5 till 0.2 min, 95/5 to 0/100 till 2.5 min, 0/100 till 3 min, 0/100 to 95/5 till 3.10 min and 95/5 till 4 min. High resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific Q-Exactive Plus equipped with a Robotic TriVersa NanoMate Advion.

Abbreviations used. ACN, acetonitrile; AcOH, acetic acid; aq., aqueous; DCM, dichloromethane; DMSO, dimethylsulfoxide; eq, equivalent(s); cHex, cyclohexane ; EtOAc, ethyl acetate; Et_3N, triethylamine; ESI, electrospray ionization; HPLC, high pressure liquid chromatography; HRMS, high resolution mass spectroscopy; LRMS, low resolution mass spectroscopy; MeOH, methanol; min, minutes; h, hour ; MS, mass spectrometry; NMR, nuclear magnetic resonance; r.t., room temperature; TMS, trimethylsilane; THF, tetrahydrofuran; TLC, thin-layer chromatography.

1. Synthesis of 5-((trimethylsilyl)ethynyl)furan-2-carbaldehyde **1a**



5-((trimethylsilyl)ethynyl)furan-2-carbaldehyde **1a** was synthesized according to published procedure.[1] Trimethylsilylacetylene (835 µL, 1.05 eq) and triethylamine anhydrous (2 mL) were dissolved in THF (5 mL). The mixture was degassed three times and stirred under argon. 5-bromofuran-2-carbaldehyde (1.00 g, 5.71 mmoles) and $PdCl_2(PPh_3)_2$ (80 mg, 2 mol%) were added. After 1 min of stirring, CuI (43.5 mg, 4 mol%) was added, then the mixture was stirred at r.t. for 16 h then concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (cHex/EtOAc, 6/4) to give of designed product, pale yellow crystals. Percentage yield: 626.5 mg, 57%.

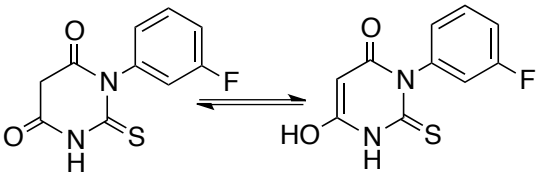
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.61 (s, 1H), 7.19 (d, *J* = 3.7 Hz, 1H), 6.71 (d, *J* = 3.7 Hz, 1H), 0.27 (s, 9H). HRMS(ESI+) *m/z*: calculated for $C_{10}H_{13}O_2Si^+$ $(M+H)^+$: 193.0607; found: 193.0680

2. Synthesis of thiobarbituric acid derivatives

Analogues of thiobarbituric acids were synthesized according to published procedures[2,3]. To a suspension of thiourea derivative (1 eq) in propanol (5 mL), diethyl malonate (2.5 eq) and sodium methoxide (25% wt in MeOH or powder, 2.5 eq) were added under Argon atmosphere. The reaction mixture was stirred under reflux (105°C) overnight. Completion of the reaction was checked by TLC analysis and UPLC-MS. The crude was then cooled to r.t., and quenched with acetic acid to reach about pH=6-7. The solvent was evaporated *in vacuo*, and the product was purified from the crude by flash chromatography on silica gel or using a CombiFlash system (DCM/MeOH, 8/2).

The Keto-Enol Tautomers are observed in the NMR spectra and their corresponding forms are stated in the description. In case of MeOD-d_4 as solvent, due to exchange with MeOD-d_4, the protons involved in Keto-Enol tautomerization cannot be observed, which is concordant with the literature[3].

1-(3-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **2a**

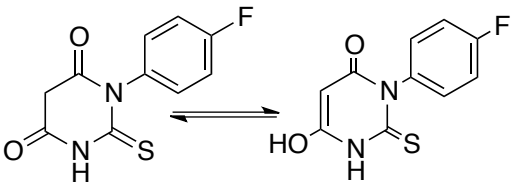


From 1-(3-fluorophenyl)thiourea (400 mg, 2.35 mmoles), Diethyl malonate (896 µL), sodium methoxide (25% solution in MeOH) (1343 µL).

Light Yellow powder, Percentage Yield =89.6%

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.66 (bs, 1H), 7.48 – 7.25 (m, 1H), 7.20 – 7.03 (m, 1H), 6.96 – 6.82 (m, 2H), 4.24 (s, 1H) *Enol Form*. ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 176.5, 163.4, 162.3 (d, J_{C-F} = 242.4 Hz,), 162.9, 143.4 (d, J_{C-F} = 10.7 Hz), 129.8 (d, J_{C-F} = 8.7 Hz), 126.6, 117.5 (d, J_{C-F} = 22.1 Hz), 114.1 (d, J_{C-F} = 20.9 Hz), 79.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm: -114.65. HRMS(ESI+) *m/z*: calculated for $C_{10}H_8FN_2O_2S^+$ $(M+H)^+$: 239.0212; found: 239.0285

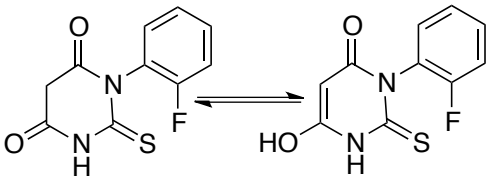
1-(4-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **2b**



From 1-(4-fluorophenyl)thiourea (250 mg, 1.47 mmoles), Diethyl malonate (558 µL), sodium methoxide (25% solution in MeOH) (671 µL). Yellow powder, Percentage Yield =248 mg, 71%.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.69 (bs, 1H), 7.16 (t, $J_{C\text{-}F}$ = 8.8 Hz, 2H), 7.05 (dd, $J_{C\text{-}F}$ = 8.7 Hz, J = 5.0 Hz, 2H), 4.27 (s, 1H, 82% *Enol*), 3.17 (d, J = 4.5 Hz, 1H, 17%*Keto*). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 176.8, 163.6, 163.0, 161.3 (d, $J_{C\text{-}F}$ = 242.1 Hz), 137.9, 131.9 (d, $J_{C\text{-}F}$ = 8.7 Hz, 2C), 115.3 (d, $J_{C\text{-}F}$ = 22.6 Hz, 2C), 79.9, 49.0. HRMS(ESI+) *m/z*: calculated for $C_{10}H_8FN_2O_2S^+$ $(M+H)^+$: 239.0212; found: 239.0285

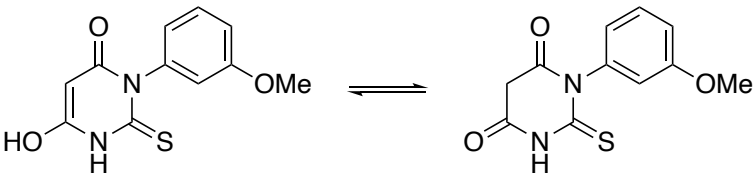
1-(2-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **2c**



From 1-(2-fluorophenyl)thiourea (250mg, 1.47 mmoles), Diethyl malonate (558 µL), sodium methoxide (25% solution in MeOH) (671 µL). Yellow powder, Percentage Yield=219 mg, 63%.

^{1}H NMR (500 MHz, MeOD-d_4) δ ppm: 7.35 – 7.23 (m, 1H), 7.19 – 7.01 (m, 3H), 3.25 (s, 1H, *Keto form, 1H exchanged with MeOD-d_4*). ^{13}C NMR (126 MHz, MeOD-d_4) δ ppm: 177.0, 165.4, 164.8, 158.2 (d, $J_{C\text{-}F}$ = 249.7 Hz), 131.0, 129.6 (d, $J_{C\text{-}F}$ = 7.9 Hz), 127.6 (d, $J_{C\text{-}F}$ = 13.4 Hz), 123.9, 115.55 (d, $J_{C\text{-}F}$ = 20.0 Hz), 48.5 *(Keto form).* ^{19}F NMR (376 MHz, MeOD-d_4) δ ppm: -123.64. HRMS(ESI+) *m/z*: calculated for $C_{10}H_8FN_2O_2S^+$ $(M+H)^+$: 239.0212; found: 239.0285

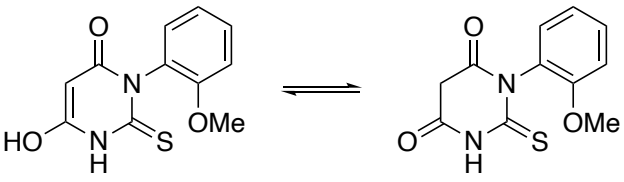
1-(3-methoxyphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione) **2d**



From 1-(3-methoxyphenyl)thiourea (250mg, 1.37 mmoles), Diethyl malonate (525 µL), sodium methoxide (25% solution in MeOH) (630 µL). Pale white powder, Percentage Yield: 283 mg, 83%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.61 (bs, 1H), 7.25 (t, J=8.0 Hz, 2H), 6.84 (ddd, J = 8.3, 2.5, 0.9 Hz, 2H), 6.64 – 6.59 (m, 2H), 6.57 (t, J = 2.2 Hz, 2H), 4.26 (s, 1H, 50% *Enol*), 3.74 (s, 6H), 3.17 (s, 2H, 50%*Keto*). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 176.5, 163.6, 162.9, 159.7, 142.8, 129.1, 122.5, 115.9, 112.8, 79.9(*Enol*), 55.6, 49.0 (*Keto*). HRMS(ESI+) *m/z*: calculated for $C_{11}H_{11}N_2O_3S^+$ $(M+H)^+$: 251.0412; found: 251.0485

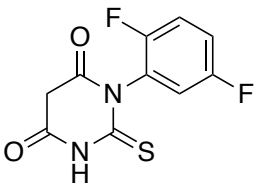
1-(2-methoxyphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione) **2e**



From 1-(2-methoxyphenyl)thiourea (250 mg, 1.37 mmoles), Diethyl malonate (525 µL), sodium methoxide (25% solution in MeOH) (630 µL). Pale white powder, Percentage Yield: 311 mg, 91%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.62 (bs, 1H), 7.25 (ddd, *J*=8.3, 6.9, 2.2 Hz, 1H), 7.00 (d, *J*=7.8 Hz, 1H), 6.97 – 6.87 (m, 2H), 4.24 (s, 1H, 68% *Enol*), 3.69 (s, 3H), 3.17 (s, 1H, 32% *Keto*). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 176.7, 163.4, 162.9, 155.5, 131.1, 130.4, 128.8, 120.4, 112.3, 79.9 (*Enol*), 55.9, 49.0 (*Keto*). HRMS(ESI+) *m/z*: calculated for $C_{11}H_{11}N_2O_3S^+$ $(M+H)^+$: 251.0412; found: 251.0485

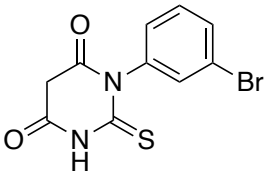
1-(2,5-difluorophenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **2f**



From 1-(2,5-difluorophenyl)thiourea (500 mg, 2.66 mmoles), Diethyl malonate (403 µL), sodium methoxide (25% solution in MeOH) (1518 µL). Light yellow powder, Percentage Yield: 572 mg, 84%.

^{1}H NMR (500 MHz, MeOD-d_4) δ ppm: 7.06 (2H, ddd, *J* =15.1, 8.2, 4.0 Hz), 6.97 – 6.84 (1H, m), 3.25 (1H, s, *Keto form, 1H exchanged with MeOD-d_4*). ^{13}C NMR (126 MHz, MeOD-d_4) δ ppm: 176.9, 165.3, 165.0, 158.4 (d, J_{C-F} = 241.1 Hz), 154.8 (d, J_{C-F} = 244.4 Hz), 128.6 (dd, J_{C-F} = 15.3, 11.6 Hz), 117.9 (d, J_{C-F}=25.4 Hz), 116.3 (dd, J_{C-F}=22.9, 9.4 Hz), 115.9 (dd, J_{C-F}=24.1, 8.1 Hz), 48.5. ^{19}F NMR (376 MHz, MeOD-d_4) δ ppm: -120.90 (d, J_{F-F}=16.0 Hz), -128.75 (d, J_{F-F}=16.1 Hz). HRMS(ESI+) *m/z*: calculated for $C_{10}H_7F_2N_2O_2S^+$ $(M+H)^+$: 257.0118; found: 257.0189

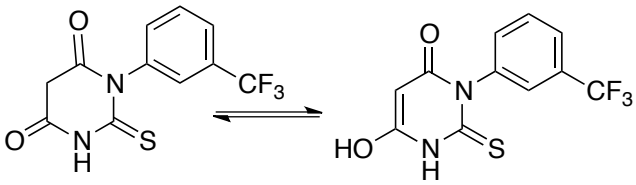
1-(3-bromophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **2g**



From 1-(3-bromophenyl)thiourea (200 mg, 0.87 mmoles), Diethyl malonate (129 µL), sodium methoxide (25% solution in MeOH) (495 µL). Light pale yellow powder. Percentage Yield: 115 mg, 52%.

^{1}H NMR (400 MHz, CD_3CN) δ ppm: 10.73 (bs, 1H), 7.53 (m,1H), 7.40 (t, *J* = 1.9 Hz, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.20 (m, 1H), 3.30 (s, 2H), 1.99 (s, 1H , Acetic Acid). ^{13}C NMR (101 MHz, CD_3CN) δ ppm 177.7, 174.0 (Acetic Acid), 165.3, 165.1, 142.9, 133.1, 131.0, 130.7, 129.3, 121.5, 49.5, 20.7 (Acetic Acid). HRMS(ESI+) *m/z*: calculated for $C_{10}H_8BrN_2O_2S^+$ $(M+H)^+$: 298.9412; found: 300.9464.

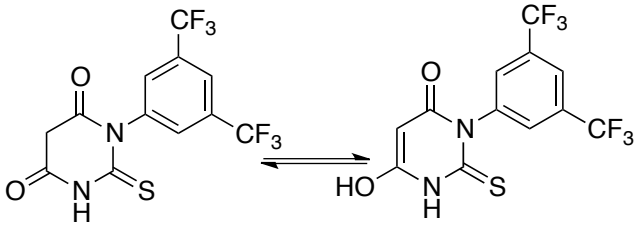
2-thioxo-1-(3-(trifluoromethyl)phenyl)dihydropyrimidine-4,6(1*H*,5*H*)-dione **2h**



From 1-(3-(trifluoromethyl)phenyl)thiourea (200 mg, 0.91 mmoles), Diethyl malonate (207 µL), sodium methoxide (25% solution in MeOH) (520 µL). Light yellow powder. Percentage Yield: 259 mg, 99%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.80 (bs, 1H), 7.69 – 7.55 (m, 2H), 7.46 – 7.32 (m, 2H), 4.27 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 176.6, 163.4, 162.9, 142.5, 134.6, 129.8, 129.4 (q, J_{C-F} = 31.9 Hz), 126.98/ 126.94, 124.6 (q, J_{C-F} = 272.0 Hz), 124.1, 79.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm: -60.84. HRMS(ESI+) *m/z*: calculated for $C_{11}H_8F_3N_2O_2S^+$ $(M+H)^+$: 289.0180; found: 289.0253.

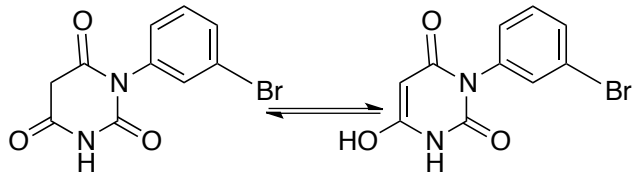
1-(3,5-bis(trifluoromethyl)phenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **2i**



From 1-(3,5-bis(trifluoromethyl)phenyl)thiourea (200 mg, 0.69 mmoles), Diethyl malonate (116 µL), sodium methoxide (94 mg). Light yellow powder. Percentage Yield: 64 mg, 26%

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 11.01 (bs, 1H), 8.09 (s, 1H), 7.87 (d, J = 1.5 Hz, 2H), 4.35 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 176.5, 163.1, 162.9, 143.8, 131.7(2C), 130.6 (d, $J_{C\text{-}F}$ = 33.0 Hz, 2C), 123.7 (d, $J_{C\text{-}F}$ = 272.8 Hz, 2C), 121.2, 79.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm: -61.12. HRMS(ESI+) *m/z*: calculated for $C_{12}H_7F_6N_2O_2S^+$ $(M+H)^+$: 357.0054; found: 357.0127.

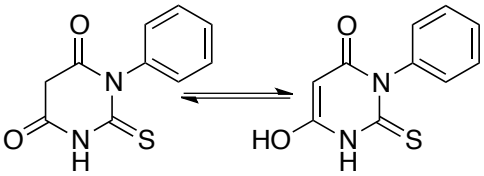
1-(3-bromophenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione **2j**



From 1-(3-bromophenyl)urea (200 mg, 0.93 mmoles), Diethyl malonate (353 µL), sodium methoxide (25% solution in MeOH) (531 µL). Light pale white powder; Yield: 169.7 mg, 65%. *Product not very pure*, however engaged in next step.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 11.50 (s, 1H), 7.66 – 7.56 (m, 1H), 7.53 – 7.39 (m, 2H), 7.26 (d, J = 7.8 Hz, 1H), 4.13 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 168.56 (2C), 167.5, 158.5, 151.9, 132.4, 131.1, 129.0, 121.4, 66.5. HRMS(ESI+) *m/z*: calculated for $C_{10}H_8BrN_2O_3^+$ $(M+H)^+$: 282.9640; found: 282.9713.

1-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **2k**



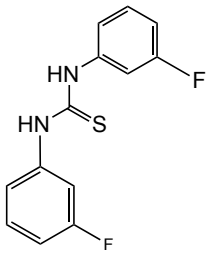
From 1-phenylthiourea (500 mg, 3.28 mmoles), Diethyl malonate (1250 µL), sodium methoxide (25% solution in MeOH) (1500 µL). Light pale white powder; Yield: 423 mg, 60%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.50 (bs, 1H), 7.39 – 7.31 (m, 2H), 7.29 – 7.21 (m, 1H), 7.07 – 6.92 (m, 2H), 4.22 (s, 1H, *Enol form)*. ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 176.7, 163.5, 162.8, 141.9, 130.1 (2C), 128.5 (2C), 127.1, 79.8. HRMS(ESI+) *m/z*: calculated for $C_{10}H_9N_2O_2S^+$ $(M+H)^+$: 221.0306; found: 221.0376.

3. Synthesis of Bis-phenyl-thiourea

The protocol was adapted from previously described procedure[4]. Fluoro/Bromo-3-isothiocyanatobenzene and 3-Fluoro/Bromoaniline in 1:1 ratio were mixed mechanically using pestle and mortar for about 15-20 minutes until cream white colour paste is formed. The crude was washed with DCM and a white colour solid powder product is obtained.

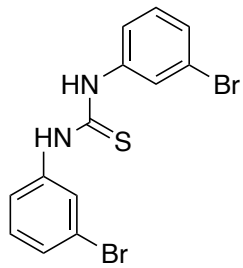
1,3-bis(3-fluorophenyl)thiourea **3a**



1-fluoro-3-isothiocyanatobenzene (9 g, 7.09 mL, 58.75 mmoles), 3-fluoroaniline (5.65 mL,1 eq); Yield : 14.38 g, 74%.

^{1}H NMR (400 MHz, Acetone-d_6,) δ ppm: 9.34 (s, 2H), 7.68 – 7.49 (m, 2H), 7.46 – 7.34 (m, 2H), 7.36 – 7.26 (m, 2H), 6.95 (tdd, J = 8.2, 2.6, 1.0 Hz, 2H). ^{13}C NMR (101 MHz, Acetone-d_6,) δ ppm: 180.24, 162.54 (d, J_{C-F} = 242.9 Hz, 2C), 141.01 (d, J_{C-F} = 10.6 Hz, 2C), 130.07 (d, J_{C-F} =9.5 Hz, 2C), 119.56 (d, J_{C-F} = 2.9 Hz, 2C), 112.40 – 109.72 (m, 4C). ^{19}F NMR (376 MHz, Acetone-d_6) δ ppm: -113.90 . HRMS(ESI+) *m/z*: calculated for $C_{13}H_{11}F_2N_2S^+$ $(M+H)^+$: 265.0533; found: 265.0606

1,3-bis(3-bromophenyl)thiourea **3b**



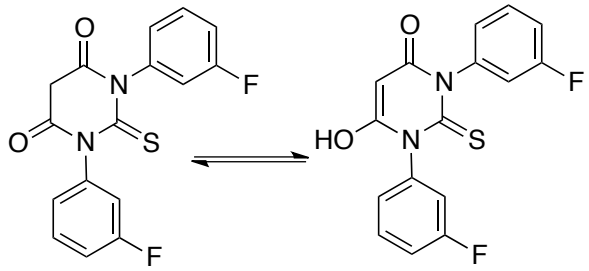
1-bromo-3-isothiocyanatobenzene (100 mg, 0.47 mmoles), 3-bromoaniline (48 µL, 1 eq). Yield: 110 mg, 61%.

^{1}H NMR (500 MHz, Acetone-d_6) δ ppm: 9.30 (s, 2H), 7.88 (t, J = 2.0 Hz, 2H), 7.58 – 7.48 (m, 2H), 7.43 – 7.18 (m, 4H). ^{13}C NMR (126 MHz), Acetone-d_6) δ ppm: 180.53, 140.80 (2C), 130.31 (2C), 127.92 (2C), 127.01 (2C), 123.04 (2C), 121.42 (2C). LRMS(ESI+) *m/z*: calculated for $C_{13}H_{10}Br_2N_2S^+$ $(M+H)^+$: 384.9; found: 384.9

4. Synthesis of Diphenyl-Thiobarbituric acid

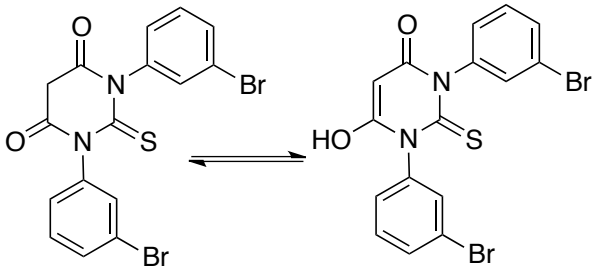
1,3-bis(3-fluoro/bromophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione was synthesized as per previously described protocol[3]. Briefly, to 1,3-bis(3-fluorophenyl/bromo)thiourea (1 eq) in anhydrous $CHCl_3$, 1.2-1.3 eq of Malonic acid and 2 eq of Phosphonyl trichloride were added. The reaction mixture was put on reflux at 55°C for about 48 h. The crude was then washed with H_2O and recrystallized in ethanol to obtain crystals.

1,3-bis(3-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **4a**



3a (1 g, 3.79 mmoles), Malonic acid (473 mg, 1.2 eq) and Phosphoryl trichloride (691 µL, 2 eq). Light yellow tinge crystals; Yield= 755 mg, 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.39 (q, J = 7.7 Hz, 2H), 7.12 (t, J = 7.5 Hz, 2H), 7.05 – 6.91 (m, 4H), 4.48 (s, 1H, *Enol*), 3.18 (s, 1H, *Keto*). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 178.7, 162.5 (d, J_{C-F} = 242.4 Hz, 2C), 162.5 (2C), 144.0 (2C), 123.0 (d, J_{C-F} = 8.8 Hz, 2C), 126.5 (2C), 117.4 (d, J_{C-F} = 22.4 Hz, 2C), 114.2 (d, J_{C-F} = 20.7 Hz, 2C), 80.0 (*Enol*), 49.07(*Keto*). ^{19}F NMR (471 MHz, DMSO-d_6) δ ppm: -114.51. HRMS(ESI+) *m/z*: calculated for $C_{16}H_{11}F_2N_2O_2S^+$ $(M+H)^+$: 333.0431; found: 333.0504.

1,3-bis(3-bromophenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **4b**



3b (62 mg, 0.16 mmoles), Malonic acid (22 mg, 1.3 eq) and Phosphoryl trichloride (35 µL, 2 eq). Yellow crystals; Yield= 51.5 mg, 70%.

^{1}H NMR (400 MHz, Acetone-d_6) δ ppm: 7.45 – 7.39 (m, 2H), 7.35 (dt, *J* = 8.9, 1.9 Hz, 2H), 7.29 (t, *J* = 7.9 Hz, 2H), 7.17 (t, *J* = 7.2 Hz, 2H), 4.61 (s, 1H). ^{13}C NMR (101 MHz, Acetone-d_6) δ ppm: 179.2, 162.8 (2C), 144.3 (2C), 133.0 (2C), 129.6 (2C), 129.4 (2C), 129.1 (2C), 120.6 (2C), 79.8. HRMS(ESI+) *m/z*: calculated for $C_{16}H_{11}Br_2N_2O_2S^+$ $(M+H)^+$: 452.8830; found: 454.8882.

5. Synthesis of analogues with alkyne group by Knoevenagel condensation

The following protocols were adapted from literature[5,6].

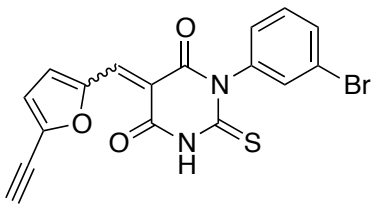
Step 1: Two methods were used:

Method A[5] : The thiobarbituric acid derivatives (1 eq) and the 5-((trimethylsilyl)ethynyl)furan-2-carbaldehyde (1 eq) were dissolved in dry MeOH. To the reaction mixture was added catalytic amount of pyridine (1-2 drops) and the reaction was stirred at 60 °C for 2 hours. Then, the solvent and pyridine were removed under reduced pressure to obtain the crude product.

Method B[6] : The thiobarbituric acid derivatives (1 eq) and the 5-((trimethylsilyl)ethynyl)furan-2-carbaldehyde (1 eq) were dissolved in distilled water and refluxed for 1 hour. Then, the water was removed under reduced pressure to obtain the crude product.

Step 2: The crude product from step 1 was dissolved in dry MeOH (2 ml) and K_2CO_3 (2 eq) was added. The reaction was stirred for 2 hours at r.t. and the solvent was removed under reduced pressure. The crude product was purified by preparative HPLC using acetonitrile and water and the solvents were removed under reduced pressure at 30-33°C as freeze drying leads to degradation of the product. In some analogues, flash chromatography on silica gel using a CombiFlash system were used for the purification.

1-(3-bromophenyl)-5-((5-ethynylfuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **5a**



Using Method B of Step 1; **2g** (46 mg, 0.15 mmoles) and **1a** (41 mg, 1.02 eq) were dissolved in distilled water (2 mL), Brown powder, Yield= 4.9 mg, 7.9% (E/Z mixture; 50:50).

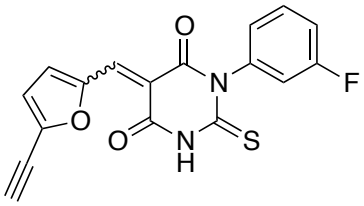
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 12.04 (bs, 0.5H)/11.98 (bs, 0.5H) (E/Z), 8.74 (d, *J* = 3.9 Hz, 0.5H)/8.55 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.25 (s, 0.5H)/ 8.18 (s, 0.5H) (E/Z), 7.59 (t, *J* = 7.1 Hz, 1H), 7.48 (dt, *J* = 13.7, 2.0 Hz, 1H), 7.39 (m, 1H), 7.25 (m, 1H), 7.06 (d, *J* = 3.9 Hz, 0.5H)/7.01 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 4.48(s, 0.5H)/4.47(s, 0.5H) (E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.37, 161.36/159.79/159.71/158.60 (E/Z, 2C), 151.45/151.30 (E/Z), 142.43, 140.70/140.45 (E/Z), 137.49/137.16 (E/Z), 132.39/132.25 (E/Z), 131.23, 130.07,

128.39/128.21 (E/Z), 127.59/127.54 (E/Z), 121.52/121.50 (E/Z), 120.13/120.11 (E/Z), 114.49/114.29 (E/Z), 87.54, 72.91.

HRMS(ESI+) *m/z*: calculated for $C_{17}H_{10}BrN_2O_3S^+$ $(M+H)^+$: 402.2340; found: 402.9570

5-((5-ethynylfuran-2-yl)methylene)-1-(3-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **5b**



Using Method A of Step 1: **2a** (100 mg, 0.42 mmoles) and **1a** (81 mg, 1.01 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 25.5 mg, 17.8% (E/Z mixture; 50:50).

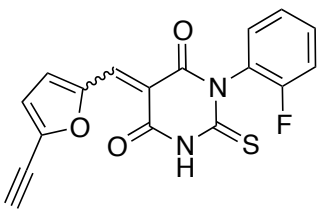
^{1}H NMR (500 MHz, THF-d_8) δ ppm : 12.04 (bs, 0.5H)/11.98 (bs, 0.5H) (E/Z), 8.74 (d, *J* = 3.9 Hz, 0.5H)/8.54 (d, *J* = 4.0 Hz, 0.5H) (E/Z), 8.25 (s, 0.5H)/8.19 (s, 0.5H) (E/Z), 7.50 – 7.44 (m, 1H), 7.22 – 7.16 (m, 1H), 7.12 – 7.00 (m, 3H), 4.48 (s, 0.5H)/4.47 (s, 0.5H) (E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.34/179.25 (E/Z), 162.87 (d, J_{C-F} = 245.1 Hz), 161.34/159.77 (E/Z), 159.73/158.62 (E/Z), 151.45/151.31 (E/Z), 142.41, 140.78 (d, J_{C-F} = 10.4 Hz)/ 140.53 (d, J_{C-F} = 10.3 Hz) (E/Z), 137.48/137.12 (E/Z), 129.82 (d, J_{C-F} =8.7 Hz), 127.52, 125.32 (d, J_{C-F}=21.1 Hz), 120.12, 116.73 (d, J_{C-F} = 23.7 Hz)/ 116.58 (d, J_{C-F} = 23.4 Hz) (E/Z), 115.13/114.96 (E/Z), 114.43 (d, *J*=21.8 Hz), 87.54, 72.92.

^{19}F NMR (471 MHz, THF- d_8) δ ppm: -114.11.

HRMS(ESI+) *m/z*: calculated for $C_{17}H_{10}FN_2O_3S^+$ $(M+H)^+$: 341.0318; found: 341.0391.

5-((5-ethynylfuran-2-yl)methylene)-1-(2-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **5c**



Using Method A of Step 1: **2c** (50 mg, 0.21 mmoles) and **1a** (41 mg, 1.02 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 5.4 mg, 7.6% (E/Z mixture; 50:50).

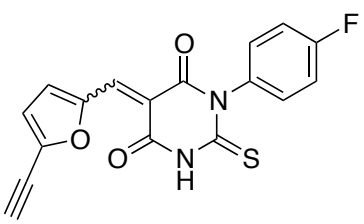
^{1}H NMR (500 MHz, THF-d_8) δ ppm : 12.13 (bs, 0.5H)/12.06 (bs, 0.5H) (E/Z), 8.76 (d, *J* = 3.9 Hz, 0.5H)/8.55 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.28 (s, 0.5H)/8.20 (s, 0.5H) (E/Z), 7.46 (t, *J* = 6.9 Hz, 1H), 7.37 – 7.30 (m, 1H), 7.29 – 7.22 (m, 2H), 7.04 (dd, *J* = 26.9 (E/Z), 3.9 Hz, 1H), 4.48 (s, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 178.73, 160.89/159.27 (E/Z), 159.6, 158.23 (d, J_{C-F}=233.0 Hz), 151.46/151.31 (E/Z), 142.55, 137.90/137.60 (E/Z), 131.06 (d, J_{C-F}=20.7 Hz), 130.37 (d, J_{C-F}=6.7 Hz), 127.75, 124.21, 120.15, 115.79 (d, J_{C-F}=19.8 Hz), 114.46, 114.01/113.80 (E/Z), 87.59, 72.91.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -122.65/-122.77 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{17}H_{10}FN_2O_3S^+$ $(M+H)^+$: 341.0318; found: 341.0391.

5-((5-ethynylfuran-2-yl)methylene)-1-(4-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **5d**



Using Method A of Step 1: **2b** (50 mg, 0.21 mmoles) and **1a** (41 mg, 1.01 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 6.7 mg, 9.3% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, THF-d_8) δ ppm : 12.02 (bs, 0.5H)/11.96 (bs, 0.5H) (E/Z), 8.73 (d, *J* = 3.9 Hz, 0.5H)/8.54 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.24

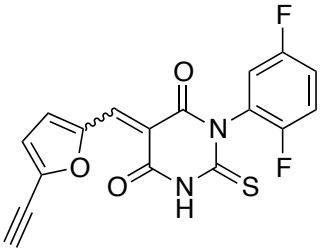
(s, 0.5H)/8.18 (s, 0.5H) (E/Z), 7.29 – 7.24 (m, 2H), 7.22 – 7.18 (m, 2H), 7.06 (d, *J* = 3.9 Hz, 0.5H)/7.01 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 4.47 (s, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.70/179.60 (E/Z), 162.37 (d, J_{C-F} = 245.7 Hz), 161.52/158.61 (E/Z), 159.96/159.71 (E /Z), 151.47/151.33 (E/Z), 142.35, 137.47/137.05 (E/Z), 135.47/135.19 (E/Z), 131.10 (d, J_{C-F} = 8.9 Hz), 130.93 (d, J_{C-F}=8.7 Hz), 127.42, 120.09, 115.39 (d, J_{C-F} = 23.1 Hz, 2C), 114.61/114.45 (E/Z), 87.49, 72.92.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -115.05/-115.06 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{17}H_{10}FN_2O_3S^+$ $(M+H)^+$: 341.0318; found: 341.0391.

1-(2,5-difluorophenyl)-5-((5-ethynylfuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **5e**



Using Method A of Step 1: **2f** (100 mg, 0.39 mmoles) and **1a** (75 mg, 1.01 eq) was dissolved in anhydrous MeOH (3 mL). Purified by Flash chromatography in (cHex/EtOAc). Brown powder, Yield: 14.3 mg, 10.2%. Traces of grease in sample due to cHex (E/Z mixture; 50:50).

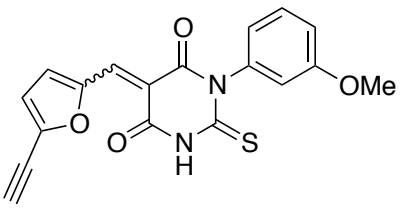
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 12.19 (bs, 0.5H)/12.13 (bs, 0.5H) (E/Z), 8.76 (d, *J* = 3.9 Hz, 0.5H)/8.56 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.29 (s, 0.5H)/8.21 (s, 0.5H) (E/Z), 7.33 – 7.23 (m, 2H), 7.22 – 7.14 (m, 1H), 7.08 (d, *J* = 3.9 Hz, 0.5H)/7.03 (d, *J* = 3.9 Hz, 0.5H)(E/Z), 4.49 (s, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 178.58/ 178.45 (E/Z), 160.83/159.64 (E/Z), 158.41 (d, J_{C-F}=242.6 Hz), 159.17/158.54 (E/Z), 154.85 (d, *J* = 248.1 Hz)/ 154.72 (d, *J* = 245.7 Hz)(E/Z), 151.41/151.25 (E/Z), 142.76/ 142.71 (E/Z), 138.05/137.83 (E/Z), 128.03/127.96 (E/Z), 127.57 – 127.00 (m), 120.22, 118.02 (d, *J* = 17.8 Hz)/ 117.82 (d, *J* = 17.7 Hz)(E/Z), 117.09-116.60 (m, 2C), 113.76/113.54 (E/Z), 87.76/87.73 (E/Z), 72.88.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -119.29 (d, *J*= 2.9 Hz)/-119.32 (d, *J* =2.7 Hz) (E/Z), -127.66 (d, J_{F-F}=16.2 Hz)/-127.80 (d, J_{F-F}=16.2 Hz) (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{17}H_9F_2N_2O_3S^+$ $(M+H)^+$: 358.0224; found: 359.0296.

5-((5-ethynylfuran-2-yl)methylene)-1-(3-methoxyphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **5f**



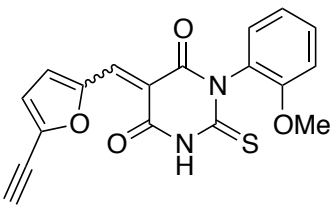
Using Method A of Step 1: **2d** (43 mg, 0.17 mmoles) and **1a** (33 mg, 1 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 5.4 mg, 8.9% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.96 (bs, 0.5H)/11.90 (bs, 0.5H) (E/Z), 8.73 (d, *J* = 3.9 Hz, 0.5H)/8.55 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.24 (s, 0.5H)/8.17 (s, 0.5H) (E/Z), 7.33 (td, *J* = 8.0, 5.3 Hz, 1H), 7.05 (d, *J* = 3.9 Hz, 0.5H)/7.00 (d, *J* = 4.0 Hz, 0.5H) (E/Z), 6.99 – 6.94 (m, 1H), 6.85 – 6.78 (m, 2H), 4.46 (s, 1H), 3.80 (s, 3H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.31, 161.24/158.62 (E/Z), 160.38, 159.72, 151.51/ 151.37 (E/Z), 142.22, 140.35/140.06 (E/Z), 137.33/136.90 (E/Z), 129.02, 127.31/127.24(E/Z), 121.25/ 121.09 (E/Z), 120.04, 114.85/114.73 (E/Z),114.56, 113.77, 87.38, 72.95, 54.53.

HRMS(ESI+) *m/z*: calculated for $C_{18}H_{13}N_2O_4S^+$ $(M+H)^+$: 353.0518; found: 353.0591

5-((5-ethynylfuran-2-yl)methylene)-1-(2-methoxyphenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **5g**



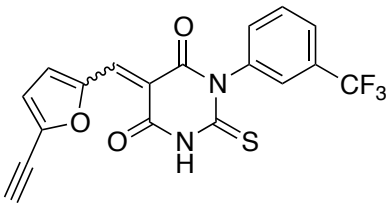
Using Method A of Step 1: **2e** (30 mg, 0.12 mmoles) and 1a (23 mg, 1 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 5.9 mg, 13.9% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.97 (bs, 0.5H)/11.90 (bs, 0.5H) (E/Z), 8.74 (d, *J* = 4.0 Hz, 0.5H)/8.54 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.25 (s, 0.5H)/8.17 (s, 0.5H) (E/Z), 7.38 (q, *J* = 7.1, 6.5 Hz, 1H), 7.20 – 7.13 (m, 1H), 7.12 – 7.04 (m, 1H), 7.03 – 6.96 (m, 2H), 4.46 (s, 1H), 3.78 (s, 3H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.08 /179.00 (E/Z), 160.73/159.71 (E/Z), 159.22/158.62 (E/Z), 155.27/155.12 (E/Z), 151.54/151.40 (E/Z), 142.29/142.25 (E/Z), 137.69/137.27 (E/Z), 130.13/129.99 (E/Z), 129.61, 127.88/127.58 (E/Z), 127.42/127.33 (E/Z), 120.13, 120.05/120.01 (E/Z), 114.32/114.15 (E/Z), 111.67, 87.38, 72.95, 54.97.

HRMS(ESI+) *m/z*: calculated for $C_{18}H_{13}N_2O_4S^+$ $(M+H)^+$: 353.0518; found: 353.0591.

5-((5-ethynylfuran-2-yl)methylene)-2-thioxo-1-(3-(trifluoromethyl)phenyl)dihydropyrimidine-4,6(1*H*,5*H*)-dione **5h**



Using Method A of Step 1: **2h** (30 mg, 0.11 mmoles) and **1a** (23 mg, 1 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 5.9 mg, 13.9% (E/Z mixture; 50:50).

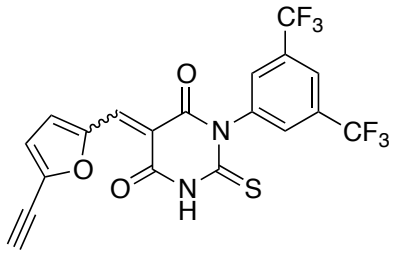
^{1}H NMR (500 MHz Acetone-d_6) δ ppm: 11.80 (bs, 0.5H)/11.75 (bs, 0.5H) (E/Z), 8.72 (d, *J* = 3.9 Hz, 0.5H)/8.50 (d, *J* = 4.0 Hz, 0.5H) (E/Z), 8.21 (s, 0.5H)/8.15 (s, 0.5H)(E/Z), 7.88 – 7.77 (m, 3H), 7.72 (ddt, *J*=13.4, 8.0, 1.6 Hz, 1H), 7.21 (dd, *J* = 4.0, 0.8 Hz, 0.5H)/7.15 (dd, *J* = 4.0, 0.8 Hz, 0.5H) (E/Z), 4.67 (s, 0.5H)/4.67 (s, 0.5H) (E/Z).

^{13}C NMR (126 MHz), Acetone-d_6) δ ppm: 179.63/179.52 (E/Z), 161.95/158.69 (E/Z), 159.98/160.16 (E/Z), 151.27/151.13 (E/Z), 142.35, 140.35/140.11 (E/Z), 137.90/137.50 (E/Z), 133.66/133.49 (E/Z), 131.02 – 130.53 (m), 130.21/130.16 (E/Z), 128.11/128.02 (E/Z), 126.50/126.36 (E/Z), 125.34, 124.12 (d, $J_{C\text{-}F}$ = 271.5 Hz), 120.76, 114.67/114.55 (E/Z), 88.21, 72.85.

^{19}F NMR (471 MHz, Acetone-d_6) δ ppm: -63.02/ -63.04 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{18}H_{10}F_3N_2O_3S^+$ $(M+H)^+$: 391.0286; found: 391.0359.

1-(3,5-bis(trifluoromethyl)phenyl)-5-((5-ethynylfuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **5i**



Using Method A of Step 1: **2i** (41 mg, 0.12 mmoles and **1a** (22 mg, 1 eq) was dissolved in anhydrous MeOH (2 mL). Brown powder, Yield: 5.2 mg, 9.8% (E/Z mixture; 50:50).

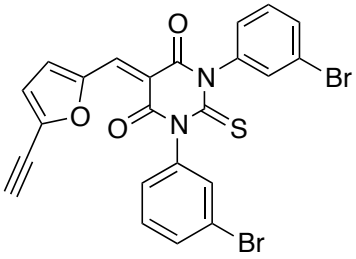
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 12.24 (bs, 0.5H)/12.18 (bs, 0.5H) (E/Z), 8.77 (d, J = 4.0 Hz, 0.5H)/8.55 (d, J = 3.9 Hz, 0.5H) (E/Z), 8.31 (s, 0.5H)/8.22 (s, 0.5H) (E/Z), 8.15 (d, J = 7.1 Hz, 1H), 8.00 (s, 1H), 7.97 (s, 1H), 7.10 (d, J = 3.9 Hz, 0.5H)/7.04 (d, J = 3.9 Hz, 0.5H) (E/Z), 4.52 (s, 0.5H)/4.51 (s, 0.5H)(E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.36/179.23 (E/Z), 161.57/158.57 (E/Z), 159.90/159.69 (E/Z), 151.33/151.17 (E/Z), 142.86, 140.97, 137.91/137.73 (E/Z), 132.02 (d, $J_{C\text{-}F}$ = 34.2 Hz, 2C), 130.65/130.49 (E/Z), 128.19, 123.26 (d, $J_{C\text{-}F}$ = 272.4 Hz, 2C), 122.28, 120.32/120.28 (E/Z), 113.97/113.75 (E/Z), 87.88, 72.84.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -63.57, -63.59.

HRMS(ESI+) *m/z*: calculated for $C_{19}H_9F_6N_2O_3S^+$ $(M+H)^+$: 459.0160; found: 459.0233.

1,3-bis(3-bromophenyl)-5-((5-ethynylfuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **5j**



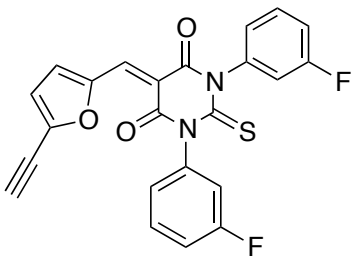
Using Method B of Step 1: **4b** (30 mg, 0.07 mmoles) and **1a** (12 mg, 1 eq) was dissolved in distilled water (2 mL). Brown powder, Yield: 4.8 mg, 13.06%.

^{1}H NMR (500 MHz, THF-d_8) δ ppm: 8.62 (d, J=4.0 Hz, 1H), 8.31 (s, 1H), 7.65 – 7.56 (m, 2H), 7.50 (dt, J=13.4, 2.0 Hz, 2H), 7.45 – 7.38 (m, 2H), 7.32 – 7.22 (m, 2H), 7.05 (d, J=3.9 Hz, 1H), 4.50 (s, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 180.2, 160.5, 159.0, 151.4, 142.7, 141.5, 141.2, 138.4, 132.2, 132.1, 131.2 (2C), 130.3 (2C), 128.2 (2C), 128.0, 121.7 (2C), 120.3, 114.2, 87.9, 72.9.

HRMS(ESI+) *m/z*: calculated for $C_{23}H_{13}Br_2N_2O_3S^+$ $(M+H)^+$: 557.2280; found: 556.8988.

5-((5-ethynylfuran-2-yl)methylene)-1,3-bis(3-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **5k**



Using Method B of Step 1: **4a** (296 mg, 0.89 mmoles) and **1a** (171 mg, 1 eq) was dissolved in distilled water (5mL). Orange-brown powder, Yield: 54.3 mg, 14.1%.

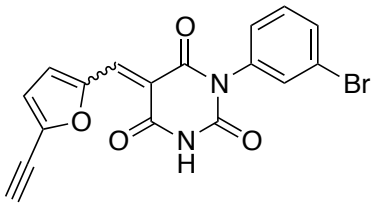
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 8.61 (d, J = 4.0 Hz, 1H), 8.31 (s, 1H), 7.54 – 7.41 (m, 2H), 7.23 – 7.15 (m, 2H), 7.15 – 7.06 (m, 4H), 7.05 (d, J = 3.9 Hz, 1H), 4.50 (s, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 180.1, 163.0 (d, $J_{C\text{-}F}$ = 245.3 Hz, 2C), 160.5, 159.0, 151.4, 142.8, 141.6 (d, $J_{C\text{-}F}$ = 10.6 Hz), 141.3 (d, $J_{C\text{-}F}$ = 10.4 Hz), 138.3, 130.0 (d, $J_{C\text{-}F}$ = 8.8 Hz, 2C), 128.0, 125.1 (d, $J_{C\text{-}F}$ = 21.9 Hz, 2C), 120.3, 116.6 (d, $J_{C\text{-}F}$ = 18.8 Hz), 116.4 (d, $J_{C\text{-}F}$ = 19.0 Hz), 115.0 (d, $J_{C\text{-}F}$ = 21.1 Hz, 2C), 114.4, 87.8, 72.9.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -113.91, -113.94.

HRMS(ESI+) *m/z*: calculated for $C_{23}H_{13}F_2N_2O_3S^+$ $(M+H)^+$: 435.0537; found: 435.0609.

1-(3-bromophenyl)-5-((5-ethynylfuran-2-yl)methylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione **5l**



Using Method A of Step 1: **4j** (67.8 mg, 0.24 mmoles) and **1a** (46.5 mg, 1 eq) was dissolved in anhydrous MeOH (2mL). Brown powder powder, Yield: 8.5mg, 9.2% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, Acetone-d_6) δ ppm: 10.55 (bs, 0.5H)/10.48 (bs, 0.5H) (E/Z), 8.63 (d, *J* = 3.9 Hz, 0.5H)/8.46 (d, *J* = 3.8 Hz, 0.5H) (E/Z), 8.20 (s, 0.5H)/ 8.16 (s, 0.5H)(E/Z), 7.76 – 7.63 (m, 2H), 7.54 – 7.42 (m, 2H), 7.14 (dd, *J* = 4.0, 0.8 Hz, 0.5H)/7.09 (dd, *J* = 4.0, 0.8 Hz, 0.5H)(E/Z), 4.52 (s, 0.5H)/4.52 (s, 0.5H)(E/Z).

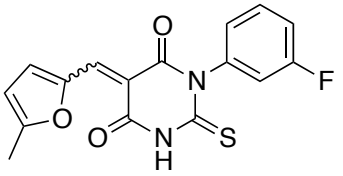
^{13}C NMR (126 MHz, Acetone-d_6) δ ppm: 161.68, 161.41, 160.81, 151.11/151.00 (E/Z), 149.72/149.63 (E/Z), 141.77, 137.32/137.00 (E/Z), 132.41/132.25 (E/Z), 131.46, 130.51, 128.57/128.40 (E/Z), 127.10/126.96 (E/Z), 121.31/121.25 (E/Z), 120.29, 114.56/114.47 (E/Z), 87.43, 72.84.

HRMS(ESI+) *m/z*: calculated for $C_{17}H_{10}BrN_2O_4^+$ $(M+H)^+$: 384.9746; found: 384.9818.

6. Synthesis of analogues by Knoevenagel condensation in acidic condition.[7]

1 equivalent of thiourea derivatives was dissolved in 2 mL of acetic acid, the solution became yellow in most cases. Furan derivative (1eq) was added, and the solution mixture was stirred at rt for 15 minutes to 1 hour. Completion of the reaction was followed by TLC in 2 solvents systems (Hex/EA and DCM/MeOH to make sure the starting material was converted) and by UPLC-MS. When the reaction was completed, the solvent was evaporated *in vacuo*, and the crude solid was mixed with 10 mL of a mixture of cHex/EtOAc (4:6) and sonicated. The resultant solution was filtered with the same cHex/EtOAc mixture as before. The liquid was then evaporated to obtain a pure product. Further purifications are carried out for impure product and purification methods are written against the analogues.

1-(3-fluorophenyl)-5-((5-methylfuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6a**



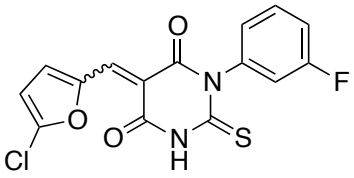
50 mg (0.21 mmoles) of **2a** and 5-methylfuran-2-carbaldehyde (23.5 mg, 1eq) were dissolved in 2 mL of acetic acid. Reaction time = 15 minutes. Yellow-orange powder, Yield: 9.2 mg, 13.2% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, Acetone-d_6) δ ppm: 11.58 (bs, 0.5H)/11.52 (bs, 0.5H) (E/Z), 8.77 (d, *J* = 3.8 Hz, 0.5H)/8.57 (d, *J* = 3.8 Hz, 0.5H) (E/Z), 8.25 (s, 0.5H)/ 8.18 (s, 0.5H)(E/Z), 7.67 – 7.48 (m, 1H), 7.39 – 7.12 (m, 3H), 6.69 (dt, *J* = 3.8, 0.8 Hz, 0.5H)/6.64 (dt, *J* = 3.8, 0.8 Hz, 0.5H) (E/Z), 2.53 (s, 3H).

^{13}C NMR (126 MHz, Acetone-d_6) δ ppm: 179.42/179.33 (E/Z), 164.44/ 166.40 (E/Z), 163.74/163.69 (E/Z), 162.74 (d, J_{C-F} =243.9Hz), 161.80/160.22 (E/Z), 160.40/158.92 (E/Z), 150.35/150.20 (E/Z), 141.08 (d, J_{C-F} = 10.5Hz), 138.91/138.46 (E/Z), 130.33 (d, J_{C-F} = 8.9 Hz), 125.78/125.62 (E/Z), 116.78 (d, J_{C-F} = 23.5 Hz), 115.26 (d, J_{C-F} = 21.1 Hz), 113.59, 110.83/110.73 (E/Z), 13.69.

HRMS(ESI+) *m/z*: calculated for $C_{16}H_{12}FN_2O_3S^+$ $(M+H)^+$:331.0474; found: 331.0547

5-((5-chlorofuran-2-yl)methylene)-1-(3-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6b**



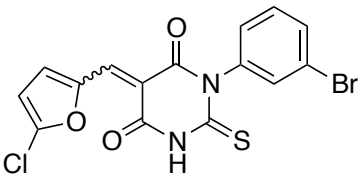
50 mg (0.21 mmoles) of **2a** and 5-methylfuran-2-carbaldehyde (27.5 mg, 1eq) were dissolved in 2 mL of acetic acid. Reaction time = 25 minutes. Yellow-orange powder, Yield: 8.2 mg, 11.1% (E/Z mixture; 50:50).

^{1}H NMR (Acetone-d_6, 500 MHz) δ ppm: 11.71 (bs, 0.5H)/ /11.66 (bs, 0.5H)(E/Z), 8.75 (d, J = 3.9 Hz, 0.5H)/8.56 (d, J = 4.0 Hz, 0.5H) (E/Z), 8.18 (s, 0.5H)/8.12 (s, 0.5H) (E/Z), 7.64 – 7.53 (m, 1H), 7.37 – 7.16 (m, 3H), 6.94 (dd, J = 3.9, 0.7 Hz, 0.5H)/6.88 (dd, J = 4.0, 0.7 Hz, 0.5H) (E/Z).

^{13}C NMR (126 MHz, Acetone-d_6) δ ppm: 179.46/179.38 (E/Z), 162.72 (d, $J_{C\text{-}F}$ = 246.2 Hz), 161.75/159.95 (E/Z), 160.10/158.77 (E/Z), 151.05/150.93 (E/Z), 145.22, 141.00 (d, $J_{C\text{-}F}$ = 10.0 Hz)/ 140.73 (d, $J_{C\text{-}F}$ = 10.4 Hz) (E/Z), 137.72/137.29 (E/Z), 130.45 (d, $J_{C\text{-}F}$ = 5.8 Hz)/130.38 (d, $J_{C\text{-}F}$ = 5.6 Hz) (E/Z), 130.23/130.14 (E/Z), 125.70/125.54 (E/Z), 116.80 (d, $J_{C\text{-}F}$ =19.8 Hz)/116.61 (d, $J_{C\text{-}F}$ = 19.9 Hz) (E/Z), 115.40 (d, $J_{C\text{-}F}$ = 21.0 Hz), 113.55/113.45 (E/Z), 113.00.

HRMS(ESI+) *m/z*: calculated for $C_{15}H_9ClFN_2O_3S^+$ $(M+H)^+$: 350.9928; found: 351.0001.

5-((5-chlorofuran-2-yl)methylene)-1-(3-bromophenyl)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6c**



60 mg (0.20 mmoles) of **2g** and 5-chlorofuran-2-carbaldehyde (20 mg,1 eq) were dissolved in 2 mL of acetic acid. Reaction time = 45 minutes. Yellow powder, Yield: 52.1 mg, 63.1% (E/Z mixture; 50:50).

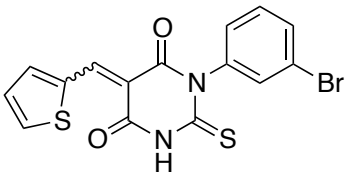
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 12.04 (bs, 0.5H)/11.98 (bs, 0.5H) (E/Z), 8.79 (d, J = 3.9 Hz, 0.5H)/8.60 (d, J = 3.9 Hz, 0.5H) (E/Z), 8.24 (s, 0.5H)/8.17 (s, 0.5H) (E/Z), 7.59 (t, J = 6.2, 1H), 7.48 (d, J = 14.0 Hz, 1H), 7.44 – 7.35 (m, 1H), 7.29 – 7.19 (m, 1H), 6.81 (d, J = 3.9 Hz, 0.5H)/6.76 (d, J = 3.9 Hz, 0.5H) (E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.37/179.27 (E/Z), 161.34/159.90 (E/Z), 159.69/158.72 (E/Z), 151.30/151.18 (E/Z), 145.03/144.98 (E/Z), 140.69/140.43 (E/Z), 137.35/137.01 (E/Z), 132.39/ 132.25 (E/Z), 131.24, 130.08, 129.75/129.68 (E/Z), 128.39/128.21 (E/Z), 121.53, 113.51/113.33 (E/Z), 112.48.

HRMS(ESI+) *m/z*: calculated for $C_{15}H_9BrClN_2O_3S^+$ $(M+H)^+$: 412.6540; found: 412.9180.

1-(3-bromophenyl)-5-(thiophen-2-ylmethylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **6d**

150 mg (0.50 mmoles) of **2g** and thiophene-2-carbaldehyde (49 µL, 1.05 eq) were dissolved in 1.5 mL of acetic acid. Reaction time = 40 minutes. Yellow powder, Yield: 32 mg, 16.2% (E/Z mixture; 50:50).

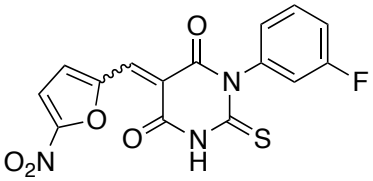


^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.93 (bs, 1H), 8.79 (s, 0.5H)/8.71 (s, 0.5H) (E/Z), 8.25 – 8.16 (m, 1H), 8.15 – 8.08 (m, 1H), 7.63 – 7.56 (m, 1H), 7.49 (dt, J = 17.8, 2.0 Hz, 1H), 7.39 (q, J = 8.1 Hz, 1H), 7.34 (q, J = 4.8 Hz, 1H), 7.29 – 7.19 (m, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.52/179.44 (E/Z), 161.78, 160.70, 147.78/147.44 (E/Z), 145.37, 142.34, 140.87/140.37 (E/Z), 137.48/137.23 (E/Z), 132.49/132.33 (E/Z), 131.20/131.14 (E/Z),

130.05/130.01 (E/Z), 128.47/128.28 (E/Z), 128.13, 121.52 /121.46 (E/Z), 112.14/111.98 (E/Z). HRMS(ESI+) *m/z*: calculated for $C_{15}H_{10}BrN_2O_2S_2^+$ $(M+H)^+$: 392.9289; found: 392.9362.

1-(3-fluorophenyl)-5-((5-nitrofuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **6e**



50mg (0.21 mmoles) of **2a** and 5-nitrofuran-2-carbaldehyde (23 mg, 1eq) were dissolved in 2 mL of acetic acid. Reaction time = 25 minutes. Purified by flash chromatography on silica using EtOAc-cHex. Yellow powder, Yield: 4 mg, 5.2% (E/Z mixture; 50:50).

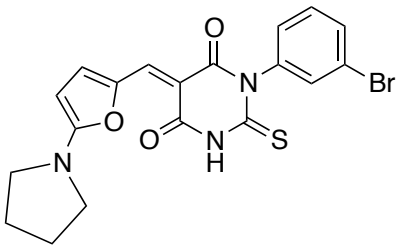
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 12.23 (bs, 0.5H)/12.17 (bs, 0.5H) (E/Z), 8.70 (d, *J* = 4.1 Hz, 0.5H)/8.52 (d, *J* = 4.1 Hz, 0.5H) (E/Z), 8.27 (s, 0.5H)/8.21 (s, 0.5H) (E/Z), 7.67 (d, *J* = 4.1 Hz, 0.5H)/7.64 (d, *J* = 4.1 Hz, 0.5H)(E/Z), 7.56 – 7.40 (m, 1H), 7.31 – 7.17 (m, 1H), 7.14 – 7.00 (m, 2H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.32/179.23 (E/Z), 162.87 (d, $J_{C\text{-}F}$ = 242.8 Hz), 160.69/159.63 (E/Z), 159.03/158.38 (E/Z), 154.03, 150.89/150.76 (E/Z), 140.34 (d, $J_{C\text{-}F}$ = 21.9 Hz), 136.41 (d, $J_{C\text{-}F}$ = 41.0 Hz), 130.02/129.95 (E/Z), 126.30, 125.21 (d, $J_{C\text{-}F}$ = 20.6 Hz), 119.78/119.58 (E/Z), 116.63 (d, $J_{C\text{-}F}$ = 17.3 Hz)/116.44 (d, $J_{C\text{-}F}$ = 16.8 Hz) (E/Z), 115.28 (d, $J_{C\text{-}F}$ = 21.1 Hz), 112.74.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -113.85/ -113.88 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{15}H_9FN_3O_5S^+$ $(M+H)^+$: 362.0169; found: 362.0241

1-(3-bromophenyl)-5-((5-(pyrrolidin-1-yl)furan-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6f**



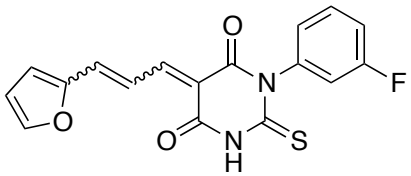
50 mg (0.17 mmoles) of **2g** and 5-(pyrrolidin-1-yl)furan-2-carbaldehyde (28 mg, 1.05 eq) were dissolved in 2 mL of acetic acid. Reaction time = 40 minutes. Purified by flash chromatography on silica using EtOAc-cHex. Yellow-Orange powder, Yield: 20.2 mg, 27.1%.

^{1}H NMR (500 MHz, CD_2Cl_2) δ ppm: 9.20 (s, 1H), 7.90 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.48 – 7.37 (m, 2H), 7.24 (d, *J* = 7.9 Hz, 1H), 6.03 (d, *J* = 4.9 Hz, 1H), 3.70 (m, 4H), 2.14 (t, *J* = 4.1 Hz, 4H).

^{13}C NMR (126 MHz, CD_2Cl_2) δ ppm: 177.5, 165.5 (3C), 147.4, 143.0, 141.1, 132.3, 131.4, 130.3, 128.2 (2C), 121.9, 102.0, 96.5, (49.68, 49.51, 49.34, 49.17, 49.00, 48.83, 48.66) (2C), 25.2 (2C).

HRMS(ESI+) *m/z*: calculated for $C_{19}H_{17}BrN_3O_3S^+$ $(M+H)^+$: 446.0096; found: 446.0169.

(1-(3-fluorophenyl)-5-(3-(furan-2-yl)allylidene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6g**



50 mg (0.21 mmoles) of **2a** and 3-(furan-2-yl)acrylaldehyde (26 mg, 1 eq) were dissolved in 2 mL of acetic acid. Reaction time = 45 min; Orange Red powder, Yield: 11.3 mg, 15.72% (E/Z mixture; 50:50).

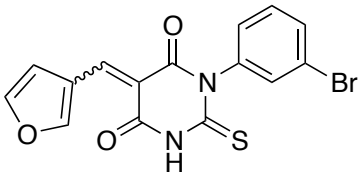
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.81 (bs, 0.5H)/11.79 (bs, 0.5H) (E/Z), 8.52 (dd, *J* = 15.2, 12.3 Hz, 0.5H)/8.38 (dd, *J* = 15.1, 12.4 Hz, 0.5H) (E/Z), 8.17 (d, *J* = 12.4 Hz, 0.5H)/8.12 (d, *J* = 12.4 Hz, 0.5H)

(E/Z), 7.83 (d, *J* = 1.8 Hz, 0.5H)/ 7.78 (d, *J* = 1.8 Hz, 0.5H) (E/Z), 7.52 – 7.38 (m, 2H), 7.20 – 7.13 (m, 1H), 7.10 – 7.02 (m, 2H), 6.99 (t, *J* = 3.3 Hz, 1H), 6.67 (dd, *J* = 3.5, 1.8 Hz, 0.5H)/6.64 (dd, *J* = 3.6, 1.8 Hz, 0.5H) (E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.53, 162.81 (d, J_{C-F} = 245.8 Hz), 161.34/159.43 (E/Z), 160.50/159.81 (E/Z), 155.39/154.81 (E/Z), 152.68, 147.26/147.21 (E/Z), 1140.90 (d, J_{C-F} = 10.4 Hz)/ 140.68 (d, J_{C-F} = 10.5 Hz) (E/Z), 138.48/138.41 (E/Z), 129.66 (d, J_{C-F} = 8.8 Hz), 125.45/125.34 (E/Z), 123.26/ 123.06 (E/Z), 117.96/117.83 (E/Z), 116.78 (d, *J* = 23.8 Hz)/116.67 (d, J_{C-F} = 23.4 Hz)(E/Z), 115.46/115.39 (E/Z), 114.85 (d, J_{C-F} = 21.2 Hz), 113.30.

HRMS(ESI+) *m/z*: calculated for $C_{17}H_{12}FN_2O_3S^+$ $(M+H)^+$: 343.0474; found: 343.0546.

1-(3-bromophenyl)-5-(furan-3-ylmethylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6h**

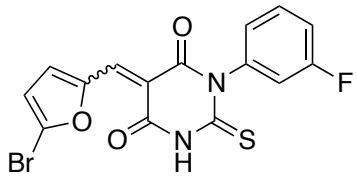


70 mg (0.23 mmoles) of **2g** and furan-3-carbaldehyde (19.5 µL,1.05 eq) were dissolved in 1.5 mL of acetic acid. Reaction time = 45 minutes. Yellow powder, Yield: 42.8 mg, 48.4% (E/Z mixture; 50:50). ^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.95 (bs, 0.5H)/11.92 (bs, 0.5H) (E/Z), 8.94 (s, 0.5H)/8.86 (s, 0.5H) (E/Z), 8.44 (s, 0.5H)/8.37 (s, 0.5H) (E/Z), 7.72 (s, 0.5H)/7.69 (s, 0.5H) (E/Z), 7.63 – 7.56 (m, 1H), 7.52 – 7.32 (m, 3H), 7.29 – 7.19 (m, 1H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.57/179.48 (E/Z), 161.83/158.87 (E/Z), 160.14/160.06 (E/Z), 155.04, 146.31/146.06 (E/Z), 144.55, 140.82/140.56 (E/Z), 132.43/132.30 (E/Z), 131.18, 130.05, 128.42/128.25 (E/Z), 122.17/121.95 (E/Z), 121.52/121.48 (E/Z), 116.23/116.04 (E/Z), 113.74/113.68 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{15}H_{10}BrN_2O_3S^+$ $(M+H)^+$: 376.9517; found: 376.9590.

5-((5-bromofuran-2-yl)methylene)-1-(3-fluorophenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **6i**



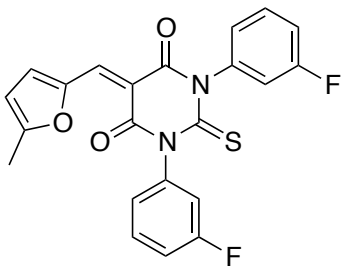
50 mg (0.21 mmoles) of **2a** and 5-bromofuran-2-carbaldehyde (37 mg, 1 eq) were dissolved in 2 mL of acetic acid. Reaction time = 25 minutes. Yellow powder, Yield: 8.9 mg; 10.7% (E/Z mixture; 50:50). ^{1}H NMR (500 MHz, THF-d_8) δ ppm: 12.02 (bs, 0.5H)/11.96 (bs, 0.5H) (E/Z), 8.72 (d, *J* = 3.9 Hz, 0.5H)/8.53 (d, *J* = 3.9 Hz, 0.5H) (E/Z), 8.27 (s, 0.5H)/8.20 (s, 0.5H) (E/Z), 7.55 – 7.39 (m, 1H), 7.27 – 7.14 (m, 1H), 7.14 – 7.02 (m, 2H), 6.92 (d, *J* = 3.9 Hz, 0.5H)/6.87 (d, *J* = 3.9 Hz, 0.5H) (E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 179.35, 162.82 (d, J_{C-F} = 256.0 Hz), 161.31/158.73 (E/Z), 159.87/159.70 (E/Z), 153.60/153.47 (E/Z), 140.85/140.58 (E/Z), 137.25/136.89 (E/Z), 132.80/132.85, 129.79 (d, J_{C-F} = 8.8 Hz), 129.48/129.46 (E/Z), 125.32 (d, J_{C-F} = 19.2 Hz),117.39, 116.72 (d, J_{C-F} = 23 Hz)/116.58(d, J_{C-F} = 23.4 Hz) (E/Z), 115.01 (d, J_{C-F} = 20.6 Hz), 113.31/ 113.15 (E/Z).

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -114.16/ -114.16 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{15}H_9BrFN_2O_3S^+$ $(M+H)^+$: 396.2024; found: 396.9475.

1,3-bis(3-fluorophenyl)-5-((5-methylfuran-2-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6j**



100 mg (0.30 mmoles) of 4a and 5-methylfuran-2-carbaldehyde (29.6 µL, 1 eq) were dissolved in 2 mL of acetic acid. Reaction time = 30 minutes. Purified by flash chromatography on silica using EtOAc-cHex. Yellow-orange powder, Yield: 28 mg, 21.8%.

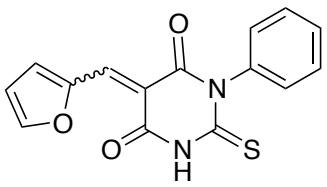
^{1}H NMR (500 MHz, THF-d_8) δ ppm: 8.64 (d, *J*=3.8 Hz, 1H), 8.33 (s, 1H), 7.53 – 7.32 (m, 2H), 7.28 – 6.95 (m, 6H), 6.52 (d, *J*=3.7 Hz, 1H), 2.46 (s, 3H).

^{13}C NMR (126 MHz, THF-d_8) δ ppm: 180.2, 164.2, 163.0 (d, J_{C-F} = 244.8 Hz, 2C), 160.9, 159.2, 150.6, 141.8 (d, J_{C-F} = 10.3 Hz), 141.5 (d, J_{C-F} = 10.6 Hz), 139.4, 131.3, 129.9 (d, J_{C-F} = 8.9 Hz, 2C), 125.3 (d, J_{C-F} = 23.0 Hz, 2C), 116.7 (d, J_{C-F} = 19.7 Hz), 116.5 (d, J_{C-F} = 20.3 Hz), 114.8 (d, J_{C-F} = 21.0 Hz, 2C), 113.2, 110.8, 13.4.

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -114.09, -114.11.

HRMS(ESI+) *m/z*: calculated for $C_{22}H_{15}F_2N_2O_3S^+$ $(M+H)^+$: 425.0693; found: 425.0766.

5-(furan-2-ylmethylene)-1-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6k**



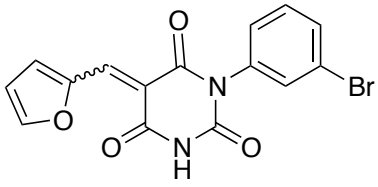
100 mg (0.45 mmoles) of **2k** and furan-2-carbaldehyde (39.6 µL, 1 eq) were dissolved in 2 mL of acetic acid. Reaction time = 45 minutes. Brown powder, Yield: 25 mg, 18% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.93 (s, 1H)/11.87 (s, 1H) (E/Z), 8.79 (d, *J* = 3.8 Hz, 1H)/8.59 (d, *J* = 3.8 Hz, 1H) (E/Z), 8.36 (s, 1H)/8.28 (s, 1H) (E/Z), 8.11 (d, *J* = 1.6 Hz, 1H)/8.09 (d, *J* = 1.6 Hz, 1H) (E/Z), 7.50 – 7.35 (m, 6H), 7.30 – 7.18 (m, 4H), 6.86 (d, *J* = 3.0 Hz, 1H)/ 6.80 (d, *J* = 3.1 Hz, 1H) (E/Z).

^{13}C NMR (126 MHz, THF-d_8) δ ppm : 179.65/179.57 (E/Z), 161.62/158.76 (E/Z), 159.96, 151.45/151.30 (E/Z), 151.15/151.07 (E/Z), 139.58/139.31(E/Z), 138.91/138.48(E/Z), 129.18, 129.01, 128.51, 128.48, 127.89, 127.52/127.45 (E/Z), 114.96, 113.32/ 113.15 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{15}H_{11}N_2O_3S^+$ $(M+H)^+$: 299.0412; found :299.0485

1-(3-bromophenyl)-5-(furan-2-ylmethylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione **6l**



100 mg (0.35 mmoles) of **2j** and furan-2-carbaldehyde (31 µL, 1.05 eq) were dissolved in 2 mL of acetic acid. Reaction time = 15 minutes. Brown powder, Yield: 18 mg, 14% (E/Z mixture; 50:50).

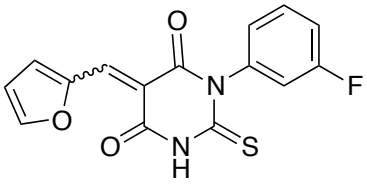
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm : 11.77 (s, 0.5H)/11.69 (s, 0.5H) (E/Z), 8.55 (d, *J* = 3.8 Hz, 0.5H)/8.37 (d, *J* = 3.8 Hz, 0.5H) (E/Z), 8.31 (s, 0.5H)/8.29 (s, 0.5H) (E/Z), 8.16 (s, 0.5H)/8.09 (s, 0.5H) (E/Z), 7.72 – 7.56 (m, 2H), 7.52 – 7.43 (m, 1H), 7.42 – 7.32 (m, 1H), 6.96 (s, 0.5H)/6.89 (s, 0.5H) (E/Z).

^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 162.92, 162.34, 161.36/161.26 (E/Z), 151.64/ 151.60 (E/Z), 150.22/150.10/149.99 (E/Z), 137.57/137.39 (E/Z), 137.00/136.72 (E/Z), 132.13/131.98 (E/Z), 131.29,

130.69/130.64 (E/Z), 128.62/128.46 (E/Z), 127.11/126.93 (E/Z), 121.01/120.94 (E/Z), 115.41/115.38 (E/Z), 112.97/ 112.84 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{15}H_{10}BrN_2O_4^+$ $(M+H)^+$: 360.9746; found :360.9818

1-(3-fluorophenyl)-5-(furan-2-ylmethylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione **6m**



30 mg (0.13 mmoles) of **2g** and furan-2-carbaldehyde (12 µL, 1.1 eq) were dissolved in 1.5 mL of acetic acid. Reaction time = 45 minutes. Yellow powder, Yield: 10 mg, 25% (E/Z mixture; 50:50).

^{1}H NMR (500 MHz, THF-d_8) δ ppm: 11.99 (bs, 0.5H)/11.93 (bs, 0.5H) (E/Z), 8.79 (dt, *J* = 3.8, 0.5 Hz, 0.5H)/8.60 (dt, *J* = 3.9, 0.5 Hz, 0.5H) (E/Z), 8.36 (s, 0.5H)/ 8.29 (s, 0.5H) (E/Z), 8.12 (dd, *J* = 1.7, 0.6 Hz, 0.5H)/8.11 (dd, *J* = 1.7, 0.6 Hz, 0.5H) (E/Z), 7.55 – 7.41 (m, 1H), 7.24 – 7.14 (m, 1H), 7.13 – 7.03 (m, 2H), 6.87 (ddd, *J* = 3.8, 1.7, 0.8 Hz, 0.5H)/6.81 (ddd, *J* = 3.9, 1.7, 0.9 Hz, 0.5H)(E/Z).

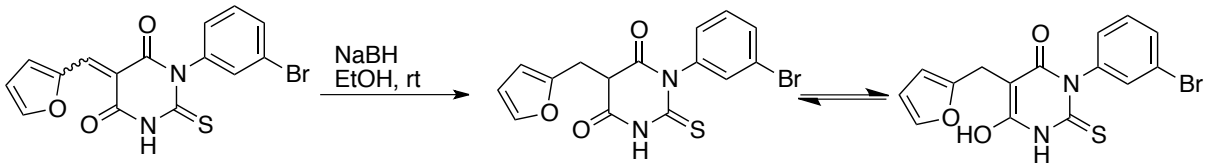
^{13}C NMR (126 MHz, THF-d_8) δ ppm : 180.44/180.35 (E/Z), 163.90 (d, J_{C-F} = 245.3 Hz)/ 163.86 (d, J_{C-F} = 245.3 Hz)(E/Z), 162.58/160.97 (E/Z) , 160.90/159.77 (E/Z), 152.43/152.28 (E/Z), 152.39/152.32 (E/Z), 141.83 (d, J_{C-F} = 30.6 Hz)/ 141.74 (d, J_{C-F} = 30.8 Hz) (E/Z), 140.05/139.70 (E/Z), 130.81 (d, J_{C-F}=8.4 Hz), 128.74, 126.46/126.29 (E/Z), 117.81 (d, *J* = 19.6 Hz)/117.62 (d, *J* = 19.6 Hz)(E/Z), 116.09, 115.92, 114.04 (d, *J* = 22.7 Hz).

^{19}F NMR (471 MHz, THF-d_8) δ ppm: -114.19/-114.20 (E/Z).

HRMS(ESI+) *m/z*: calculated for $C_{15}H_{10}FN_2O_3S^+$ $(M+H)^+$: 317.0318; found: 317.0393.

7. Reduction of some analogues[7] :

3-(3-bromophenyl)-5-(furan-2-ylmethyl)-6-hydroxy-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **7a**



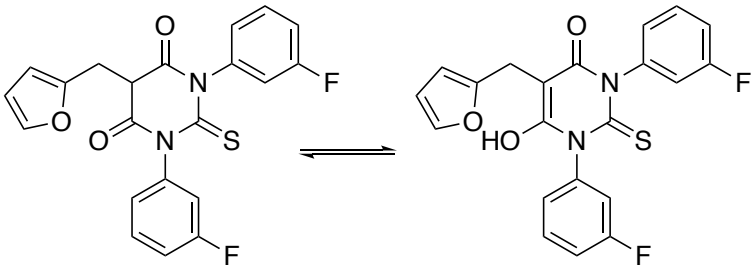
To SMIFH2 (23.1 mg, 0.06 mmoles) in EtOH (2mL) at 0°C, $NaBH_4$ (9.3 mg, 4 eq) was added, and the reaction was brought to r.t. gradually for 1.5 hours and quenched it with acetic acid. The solvents were evaporated under reduced pressure and the prep-HPLC in water/ACN was performed for purification. Colourless product was formed. Yield: 6 mg, 26.1%.

^{1}H NMR (500 MHz,MeOD-d_4) δ ppm: 7.55 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.43 (t, *J* = 1.9 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.27 – 7.21 (m, 2H), 6.21 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.99 (d, *J* = 3.1 Hz, 1H), 3.75 (s, 2H), 3.37 (s, 1H, exchanged with Tautomer).

^{13}C NMR (126 MHz, MeOD-d_4) δ ppm: 175.5, 164.7 (2C), 155.6, 141.9, 139.9, 132.3, 130.6, 129.9, 128.2, 121.4, 109.7, 104.1, 90.8, 21.9.

HRMS(ESI+) *m/z*: calculated for $C_{15}H_{12}BrN_2O_3S^+$ $(M+H)^+$: 380.2284; found: 380.9726

1,3-bis(3-fluorophenyl)-5-(furan-2-ylmethyl)-6-hydroxy-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **7b**



4a (100 mg, 0.12 mmoles) and furan-2-carbaldehyde (24.6 µL, 1 eq) were solubilized in 2 mL Acetic Acid and stirred for 30 minutes at rt. The crude mixture was dried under vacuum and purified by flash chromatography on silica using cHex-EtOAc. The product obtained (50 mg, 1 eq) was dissolved in EtOH (2mL) at 0°C, then $NaBH_4$(19 mg, 4 eq) was added, and the reaction was brought to r.t. gradually for 1.5 hours and then quenched with acetic acid. The solvents were evaporated under reduced pressure and the flash chromatography on silica gel, DCM MeOH (8/2). Colourless product was formed. Yield: 13.9 mg; 28%.

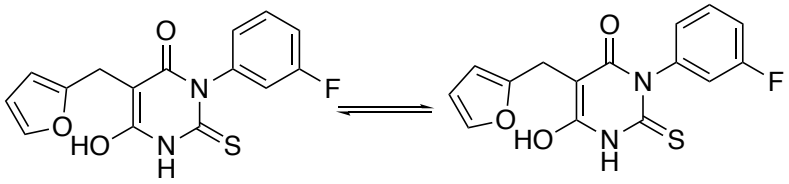
^{1}H NMR (500 MHz, MeOD-*d*$_4$) δ ppm: 7.46 – 7.38 (m, 2H), 7.28 (dd, *J* = 1.8, 0.9 Hz, 1H), 7.12 – 6.96 (m, 6H), 6.24 (dd, *J* = 3.1, 1.9 Hz, 1H), 6.01 (dd, *J* = 3.1, 1.0 Hz, 1H), 3.71 (s, 2H).

^{13}C NMR (126 MHz, MeOD-*d*$_4$) δ ppm: 177.6, 163.4 (2C), 163.0 (d, J_{C-F} = 244.3 Hz, 2C), 155.8, 143.0 (d, J_{C-F} = 10.3 Hz, 2C), 139.8, 129.5 (d, J_{C-F} = 8.8 Hz, 2C), 125.2, 116.6 (d, J_{C-F} = 23.0 Hz, 2C), 113.9 (d, J_{C-F} = 21.2 Hz, 2C), 109.7, 104.1, 90.4, 22.4.

^{19}F NMR (376 MHz, MeOD-*d*$_4$) δ ppm: -115.66, -115.68.

HRMS(ESI+) *m/z*: calculated for $C_{21}H_{15}F_2N_2O_3S^+$ $(M+H)^+$: 413.0693; found: 413.0763.

3-(3-fluorophenyl)-5-(furan-2-ylmethyl)-6-hydroxy-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **7c**



To **6m** (30.1 mg, 0.09 mmoles) in EtOH (2mL) at 0°C, $NaBH_4$ (11 mg, 3 eq) was added, and the reaction was brought to r.t. gradually for 1.5 hours and then quenched with acetic acid. The solvents were evaporated under reduced pressure and the flash chromatography on silica, DCM/MeOH (8/2). Colourless product was formed. Yield: 9.2 mg; 30.5%.

^{1}H NMR (500 MHz, MeOD-*d*$_4$) δ ppm: 7.52 – 7.39 (m, 1H), 7.27 (s, 1H), 7.11 (td, *J* = 8.6, 2.5 Hz, 1H), 7.01 (d, *J* = 7.9 Hz, 1H), 6.96 (dt, *J* = 9.6, 2.3 Hz, 1H), 6.23 (t, *J* = 2.5 Hz, 1H), 5.96 (d, *J*=3.1 Hz, 1H), 3.66 (s, 2H).

^{13}C NMR (126 MHz, MeOD-*d*$_4$) δ ppm: 175.3, 163.2 (2C), 162.9 (d, J_{C-F} = 260.5 Hz), 155.7, 142.1 (d, J_{C-F} = 10.2 Hz), 139.8, 129.5 (d, J_{C-F} = 9.2 Hz), 125.2, 116.6 (d, J_{C-F} = 22.9 Hz), 114.2 (d, J_{C-F} = 21.3 Hz), 109.6, 103.9, 90.0, 21.7.

^{19}F NMR (376 MHz, MeOD-*d*$_4$) δ ppm: -115.60.

HRMS(ESI+) *m/z*: calculated for $C_{15}H_{12}FN_2O_3S^+$ $(M+H)^+$: 319.044; found: 319.0547.

9. Nucleophilic amino acids and **5k/7b** reaction:

Nucleophilic amino acids: Fmoc-Lysine-OtBu.HCl and Boc-Serine-OH 20 mM stock solution in MeOH and 1.75 mM stock solution in DMSO of **5k, 7b** were prepared. In eppendorf tubes, 200 µL of DPBS (1x) (Gibco), 50 µL of **5k/7b** and 50µL of an amino acid were added and left at 37°C with sand bath for 12 hours. The samples were analysed by UPLC-MS after 2 and 12 hours.

B) CPMG filter NMR experiments

Compounds (1.75 mM stock solution) were made in deuterated DMSO and stored at -20°C. NMR samples were prepared in 558 µL D_2O, 12 µL (9 µM) of Compound **5k** and **6b** and 18.2 µL of DPBS (1x) or 18.2 µL (35 µM) of IFNγ (300 µM in PBS). Both samples were preincubated at 37°C for 20 minutes before running the NMR. All the ^{19}F NMR experiments were recorded at 37°C with a Bruker 500 spectrometer operating at a ^{19}F Larmor frequency of 470.5 MHz. The protocol was adapted from literature[8]. The R2 filter experiments were recorded with the Carr-Purcell-Meibom-Gill scheme with a time interval of 40 ms between the 180° pulses and a length of 0.16 s. The spectra were acquired with proton decoupling using the Waltz-16 composite pulse sequence with a 90° pulse of 80 µs. The data were collected with a spectral width from 75 to 135 ppm and 384 scans recorded for each spectrum.

C) Biological experiments

Reagents: DMEM Glutamax (Gibco, Life Technologies), Alexa-488 and Alexa-568 labelled-Phalloidin (ThermoFisher Scientific), DAPI (Sigma-Aldrich) , FluoromountG (ThermoFisher Scientific) , Bovine Serum Albumin (BSA) (Euromedex) , SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific), Mini-PROTEAN® TGX™ Precast Gels (BioRad), Cell Titer-Blue® reagent (Promega), Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 488 dye (ThermoFisher Scientific), Recombinant Human IFNγ (Biolegend), DPBS (1x) (Gibco, Life Technologies) , bis(sulfosuccinimidyl)suberate (BS3) (ThermoFisher Scientific), Pierce™ Streptavidin Magnetic Beads (ThermoFisher Scientific), Pierce™ Silver Stain for Mass Spectrometry (ThermoFisher Scientific), Recombinant Human EGF (Miltenyi Biotec), Recombinant Human IFNα2a (Miltenyi Biotec), Recombinant Human IFNα2b (ThermoFisher Scientific), Recombinant Human IFNβ-1a (Miltenyi Biotec), Recombinant Human IFNλ1 (BioLegend), Recombinant Human IFNλ2 (BioLegend).

Cell culture: HeLa cells were grown at 37°C under 5% CO_2 in DMEM high glucose Glutamax (Gibco, Life Technologies) complemented with 10% FBS (v/v) (Gibco, Life Technologies) and supplemented with 5 mM pyruvate (v/v) (Gibco, Life Technologies) and 1% penicillin- streptomycin (v/v) (Gibco, Life Technologies).

In vivo studies: Animal work was conducted at Selvita Ltd according to 2010/63/EU and National legislation regulating the use of laboratory animals in scientific research and for other purposes (Official Gazette 47/11). An institutional Committee on Animal Research Ethics (CARE-Zg) oversaw that animal-related procedures were not compromising the animal welfare. The accreditation number of the project is: KLASA:UP/1-322-01/19-01/82, URBROJ: 525-10/1241-20-4.

Primary antibodies (Western blot): phospho-STAT1 Tyr701 (BD Transduction Laboratories, 612132, 1:1000), STAT1 (Cell Signaling, 9172, 1:1000), alpha-tubulin (Sigma, clone B512, T5168, 1:5000); IFNγ (Abcam, EPR1108, ab133566, 1:1000); phospho-JAK2 Tyr1007/1008 (Cell Signaling, 3776, 1:1000); JAK2 (Cell Signaling, 3230, 1:1000); phospho-STAT2 Tyr689 (Merk, 07-224, 1:1000), STAT2 (Abcam, ab32367, 1:1000); phospho-STAT3 Tyr705 (Cell Signaling, 9145, 1:1000); STAT3 (Cell Signaling, 9132, 1:1000); Primary antibodies (Immunofluorescence): beta actin (Abcam, 8226, 1:200); Secondary

antibodies: anti-mouse-Alexa-488 (Invitrogen, A21202), anti-mouse-HRP (Jackson ImmunoResearch, 715-035-151) and anti-rabbit-HRP (Jackson ImmunoResearch, 715-035-152) were used at 1:5000 for western blot and anti-mouse-Alexa-488 (Invitrogen, A21202) was used at 1:200 for immunofluorescence.

JAK-STAT signalling activation assay

20 mM stock solution of compounds in DMSO were prepared and stored at -20°C.

JAK-STAT stimulation on HeLa cells were done using previously described protocol.[9] For phosphorylation status of STAT1 (pSTAT1) in different cytokines: 1000 U.ml^{-1} of IFNγ, IFNα2a, IFNα2b, IFNβ, IFNλ1, IFNλ2 or 15.6 nM EGF in DMEM containing 0.2% BSA with different concentrations (0.4, 4, 40, 80 µM) of compound **1** (SMIFH2) and **5k**, and DMSO (as control) were incubated for 20 min at 37°C and added to HeLa cells for 20 min at 37°C.

In case of screening compounds, 40 µM compounds were preincubated with 1000 U.ml^{-1} of IFNγ in DMEM containing 0.2% BSA for 20 min at 37°C before adding it to HeLa cells. 10-fold 7 or 5 serial dilutions from 400 µM of compounds were made in DMEM containing 1000 U.ml^{-1} of IFNγ and 0.2% BSA, and incubated for 20 min before stimulating JAK-STAT for finding IC_{50} towards IFNγ. Similarly, for cross-linked IFNγ, HeLa cells were treated with the preincubated mixture at 37°C for 20 min for JAK-STAT signalling.

For comparing pSTAT1 status in different conditions of **5k** treatment, JAK-STAT stimulation on HeLa cells were made by adding mixture containing 1000 U.ml^{-1} IFNγ in DMEM with 0.2% BSA and 40 µM compound preincubated for 20 min at 37°C; similar mixture without preincubation; 40 µM compound in DMEM for 20 min at 37°C, then followed by addition of 1000 UI.ml^{-1} IFNγ to the media and stimulated the JAK-STAT signalling for 20 min further.

Immunoblotting

Cells were lysed in sample buffer (62.5 mM Tris/HCl, pH 6.0, 2% v/v SDS, 10% glycerol v/v, 40 mM dithiothreitol, and 0.03% w/v phenol red). Samples were analysed by SDS-PAGE on 4-15% Mini-PROTEAN® TGX™ Precast Gels or on 4-15% Mini-PROTEAN® TGX™ Stain Free Gel (Bio-Rad) and immuno-blotted with the indicated primary antibodies and horseradish peroxidase or Alexa-488 conjugated secondary antibodies. Chemiluminescence signal was revealed using Pierce ECL Western Blotting Substrate, SuperSignal West Dura Extended Duration Substrate or SuperSignal West Femto Substrate (Thermo Scientific Life Technologies). Acquisition and quantification were performed with the ChemiDoc MP Imaging System (Bio-Rad). Phosphorylated protein over total ratio was determined on the same blot.

***In cellulo* actin nucleation assay**

HeLa cells grown on coverslips were treated with DMEM containing 0.2% BSA and the compounds (40 µM) for 20 min at 37°C, washed with cold PBS (two times) and then fixed with 4% paraformaldehyde for 30 min at room temperature, quenched in 50 mM NH_4Cl for 10 min and permeabilised with 0.05% saponin in 0.2% BSA in PBS for 20 min. Cells were incubated with 165 nM phalloidin for 1 h at room

temperature. DAPI containing fluoromount-G was used to mount coverslips onto glass slide. Cell areas were measured with ImageJ software (NIH).

For anti-actin immunofluorescence assay, after cell permeabilization, primary antibody (1µL in 200µL of 0.05% saponin in PBS) was added for 1 h. It was washed with PBS for 5 min for 3 times and secondary antibody (1µL in 200µL of 0.05% saponin in PBS) was added for 1 h. Washed with PBS for 5 min for 3 times and DAPI containing fluoromount-G was used to mount coverslips onto glass slide for further analysis.

Cell viability assay

Cell viability assay was carried out by plating 10,000 cells/well in 96-well plates. 3-Fold 8 serial dilutions of the compounds from 3 mM were made in DMEM. HeLa cells were treated for 24 h with the compounds of different concentrations made. In case of preincubation with IFNγ, 6000 $U.ml^{-1}$ of IFNγ per concentration of compounds were used and incubated for 20 min. According to manufacturer's protocol, Cell Titer-Blue® reagent was added after 24 h treatment and cells were incubated for 3 h before recording fluorescence intensities (λex. 560/20 nm; λem. 590/10 nm) using a Perkin Elmer Wallac 1420 Victor2 Microplate Reader.

Acetylation of IFNγ

Acetylation of Lysine and N-terminal amino groups were done according to previously described protocol.[10] Briefly, 1 µg of IFNγ was dissolved in 20 µL of NH_4HCO_3 (50 mM). Acetic anhydride (5 or 10 or 25 eq per lysine residues (14 Lysine)) were added along with 20 eq of **5k** and incubated at 37°C for 30 min before adding the click reagents for further immunoblot analysis. Quantification was done using ImageJ software (NIH).

Click reaction for Native Gel

The protocol was adapted from previously described protocol on protein electrophoresis and in-gel fluorescence scanning.[11] 6 µL of IFNγ from stock solution (71 µM final) were added on 120 µL of NH_4HCO_3 (50 mM) and divided into 6 parts (20 µL each). Parts 2 and 7 (control) were treated with 20 eq of **5k** (0.6 µL of 2 mM stock in DMSO), Part 3 and 7 with 20 eq of **7b** (0.6 µL of 2 mM stock in DMSO), Part 4 was co-treated with 20 eq of **5k** and **7b** (2 mM stock in DMSO), acetic anhydride (10 eq per lysine residues, 0.8 µL) was added to part 5 and 6, and incubated at 37°C for 30 min. Then, all parts were added with 30 µL of click reaction cocktail (prepared using the manufacturer's protocol of Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 488 dye; 430 µL of 1x Click-iT reaction buffer with 20 µL of $CuSO_4$ solution, 1.2 µL of Alexa-488-azide, 50 µL of reaction buffer additive (sodium ascorbate)) and incubated for 45 min. Corresponding amount of Sample Solubilizing Buffer (1%(w/v) SDS, 100 mM Tris-HCl (pH 9.5)) to reach up to 100 µL were added and 50 µL were loaded on native gel (Mini-PROTEAN® TGX™ Precast Gel) using running buffer 1x (250 mM Tris, 1.92 M glycine, pH 9.2) to perform western blot analysis. Proteins were transferred on nitrocellulose membrane and scanned for Alexa-488 signal using ChemiDoc MP Imaging System (Bio-Rad). Then, further incubation with primary, then secondary antibodies was done for IFNγ immunoblotting.

Cross-linking of IFNγ

The cross-linking reactions were performed as previously described in literature.[12] Briefly, 1 µg of IFNγ in 10 µL of PBS was mixed with 2 µL of 10 mM solution of BS3 and the mixture was kept on ice for 1 h. The reaction was stopped by addition of 10 µL of 1 M Tris-HCl, pH 6.8. The reaction product was analysed by SDS-PAGE and western blot analysis. To test the activity of the cross-linked IFNγ, 1.1 µL of the reaction mixture was added to 1 mL DMEM (1000 $U.ml^{-1}$ final) containing 0.2% BSA, incubated at 37°C with or without **5k** (40 µM final) and activation of JAK-STAT signalling was determined in HeLa cells stimulated for 20 min by lysate immunoblotting as mentioned above.

Pharmacokinetic profiling in the C57BL/6N mice

Each group of mice (C57BL/6 male, n = 9, Charles River Germany) were given with **5k** via intravenous (IV) at dose of 2 mg/kg and intraperitoneal (IP) administration at dose of 10mg/kg in the formulation of DMSO/cremaphor/PBS (10/10/80; v/v/v). Blood samples were analysed by LC-MS/MS (Shimadzu LC30AD/Shimadzu SIL30ACMP/Shimadzu CTO30A) at the interval of 0.05, 0.25, 0.5, 1, 2, 4, 8, 24 h.

Acute model of DSS-induced colitis in C57BL/6 mice

Mice (C57BL/6 male, n = 50, Charles River Germany) were grouped into 5. Groups 2-5 received 3% Dextran Sulfate Sodium (DSS) solution in drinking water for 7 full days and group 1 received water only. Animals in group 3 were administered with Cyclosporine A (pharmacological control) at 80 mg/kg per os (PO), once per day (QD). Animals in group 4 were administered intravenously (IV)/QD with 10 mg/kg of **5k**, while animals in group 5 received 10 mg/kg of **5k** given via intraperitoneal (IP) route once per day (QD). Animals in groups 1 and 2 were administered IP/QD with 5 mL/kg of DMSO/cremophor/PBS (10/10/80 v/v/v) vehicle solution. The efficacy of the therapeutic treatments was assessed daily through clinical monitoring consisting of body weight changes, stool consistency, colorectal bleeding condition. Colorectal bleeding score was defined with 0 for no presence of blood in stool or anal region, 2 for visible blood in stool pellets and 4 for gross bleeding and/or blood around anus region. Disease activity index (DAI) was calculated as combined score of a) weight loss b) stool consistency and c) colorectal bleeding daily with maximum score of 12. Mice were sacrificed by ketamine/xylazine overdose at day 8.

Statistical analysis

All analyses were performed using GraphPad Prism version 7.0 and 9.1.1, (GraphPad Software, La Jolla, CA, USA). One-way ANOVA, Two-way ANOVA with Dunnett's multiple comparison test and Kruskal-Wallis test with Dunn's post-test were performed. Significance of mean comparison is marked on the graphs by asterisks. Error bars denote SEM or SD.

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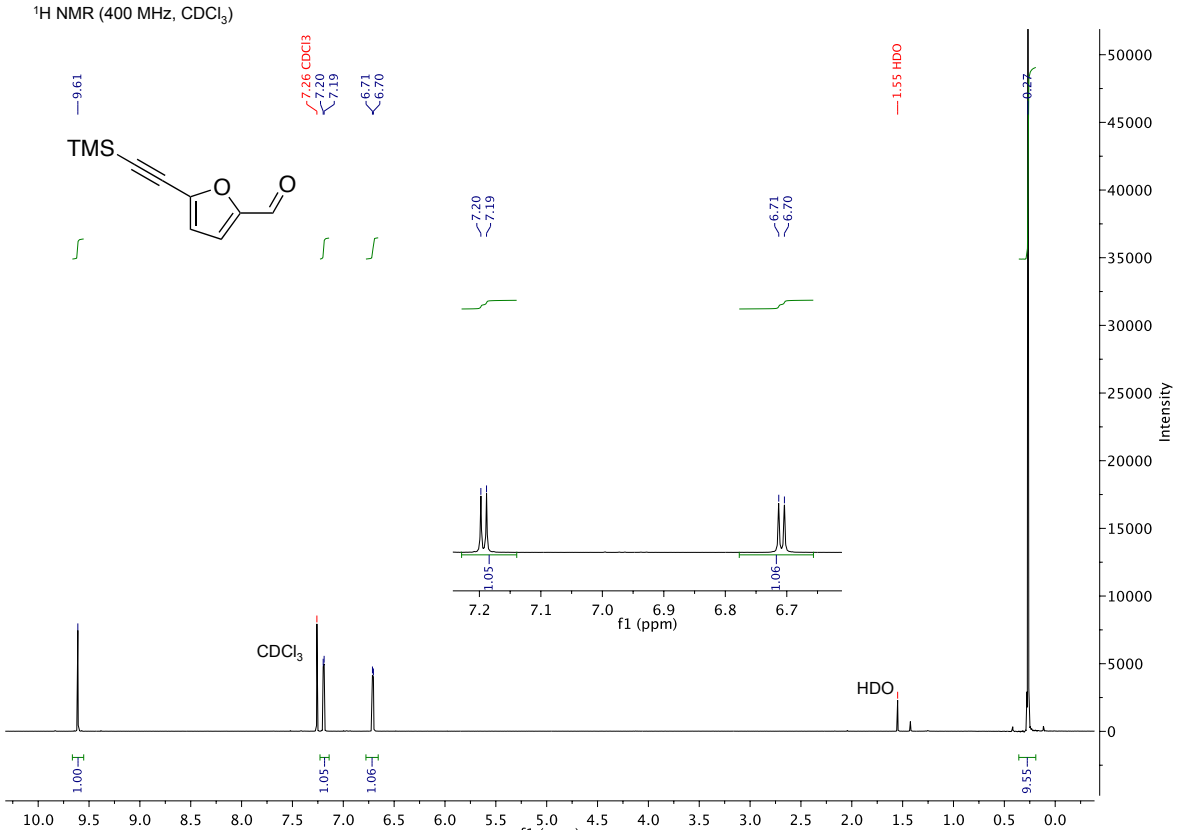
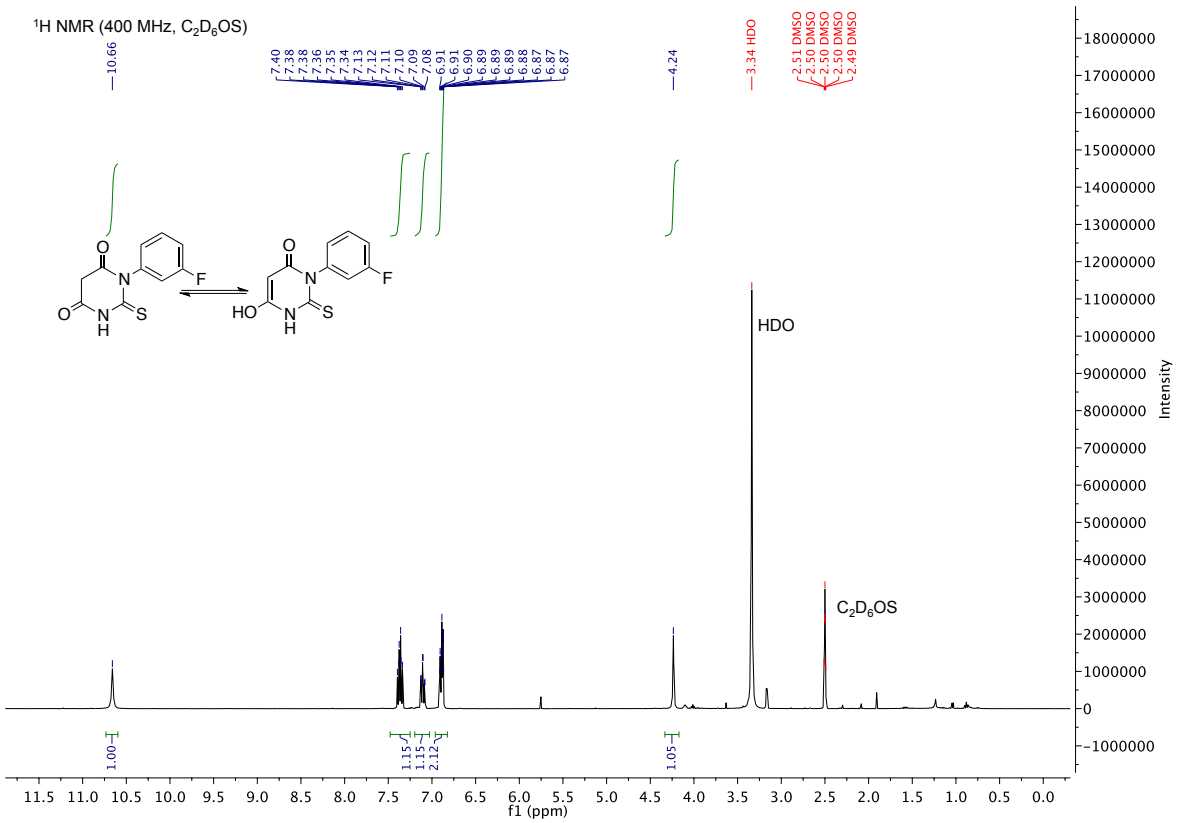
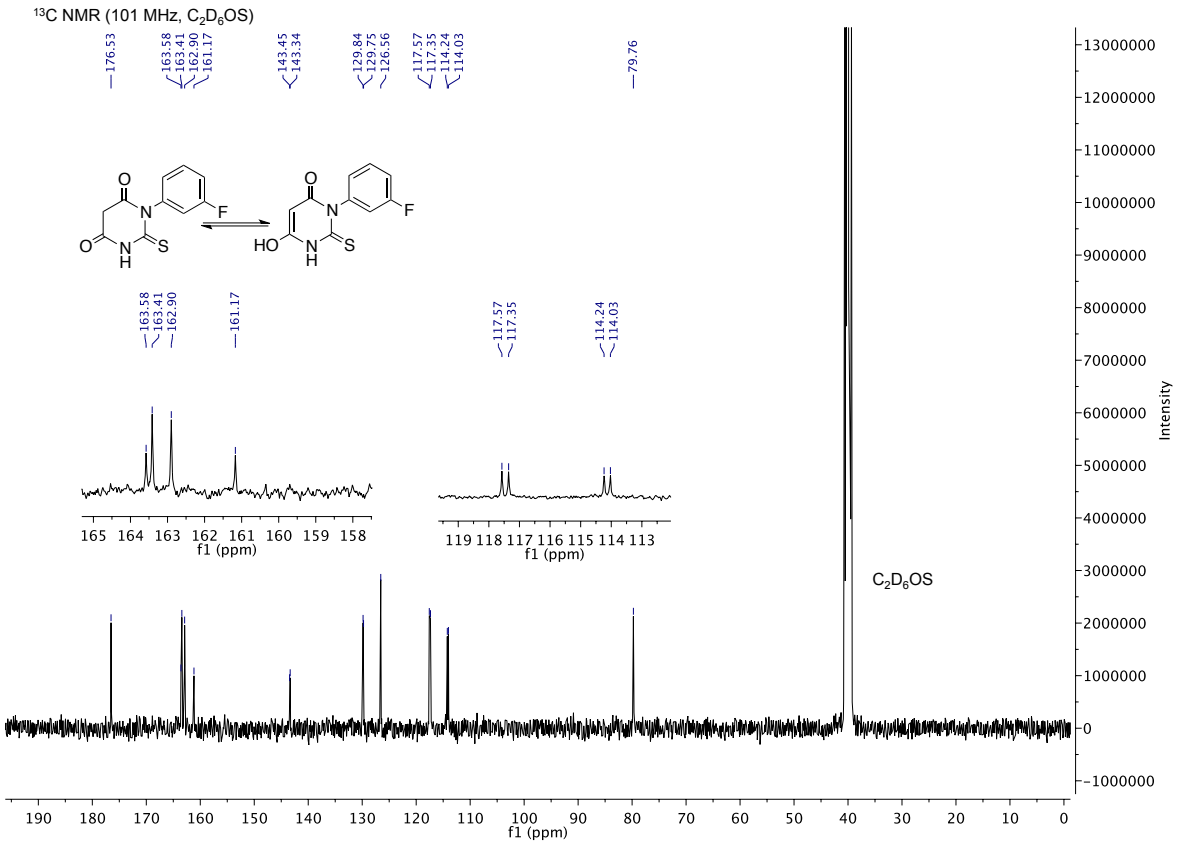
NMR spectra of all compounds (^{1}H, ^{13}C, ^{19}F spectra)

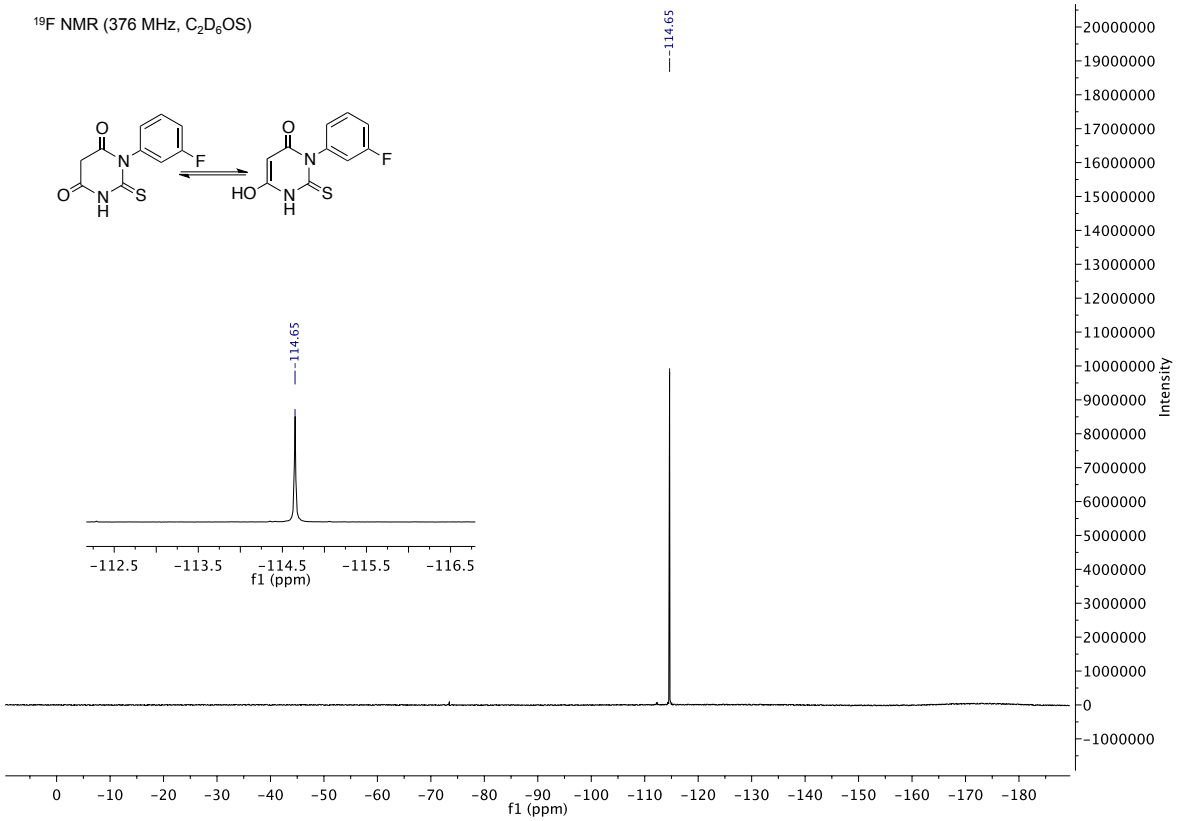
Compound **1a**



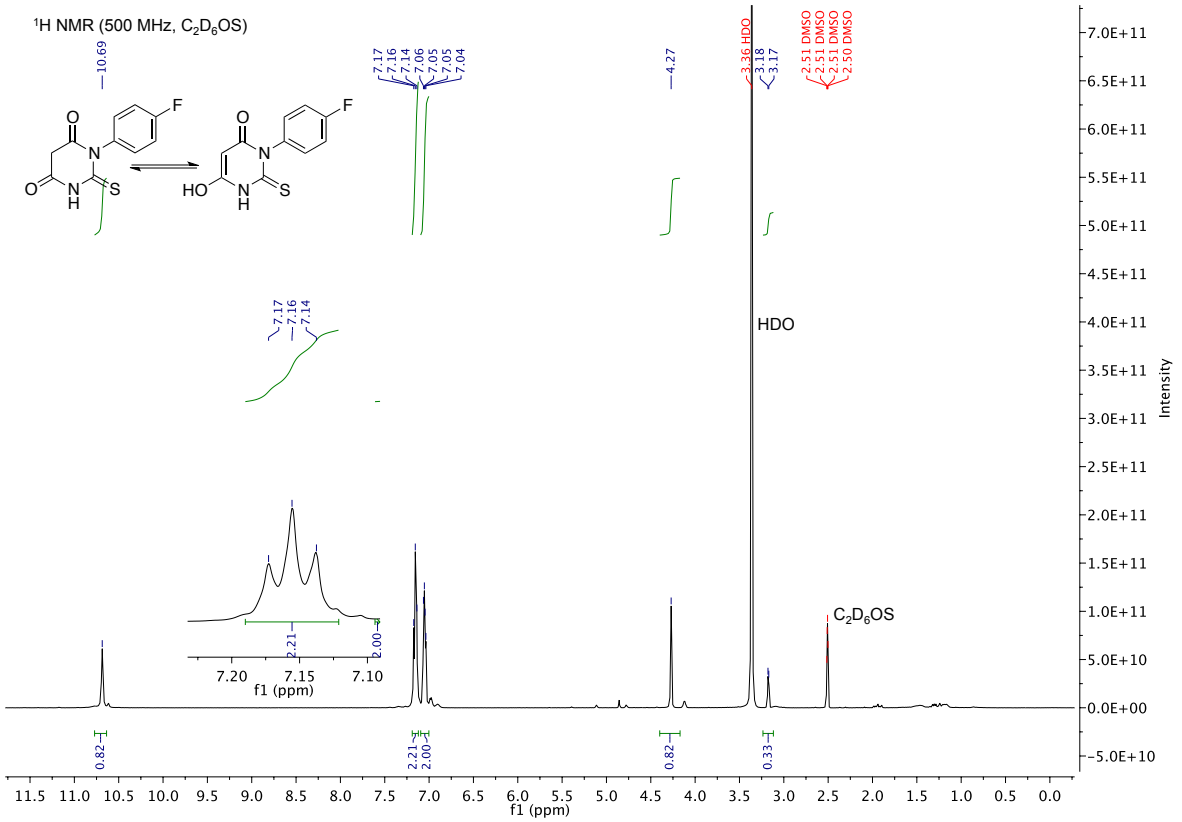
Compound **2a**

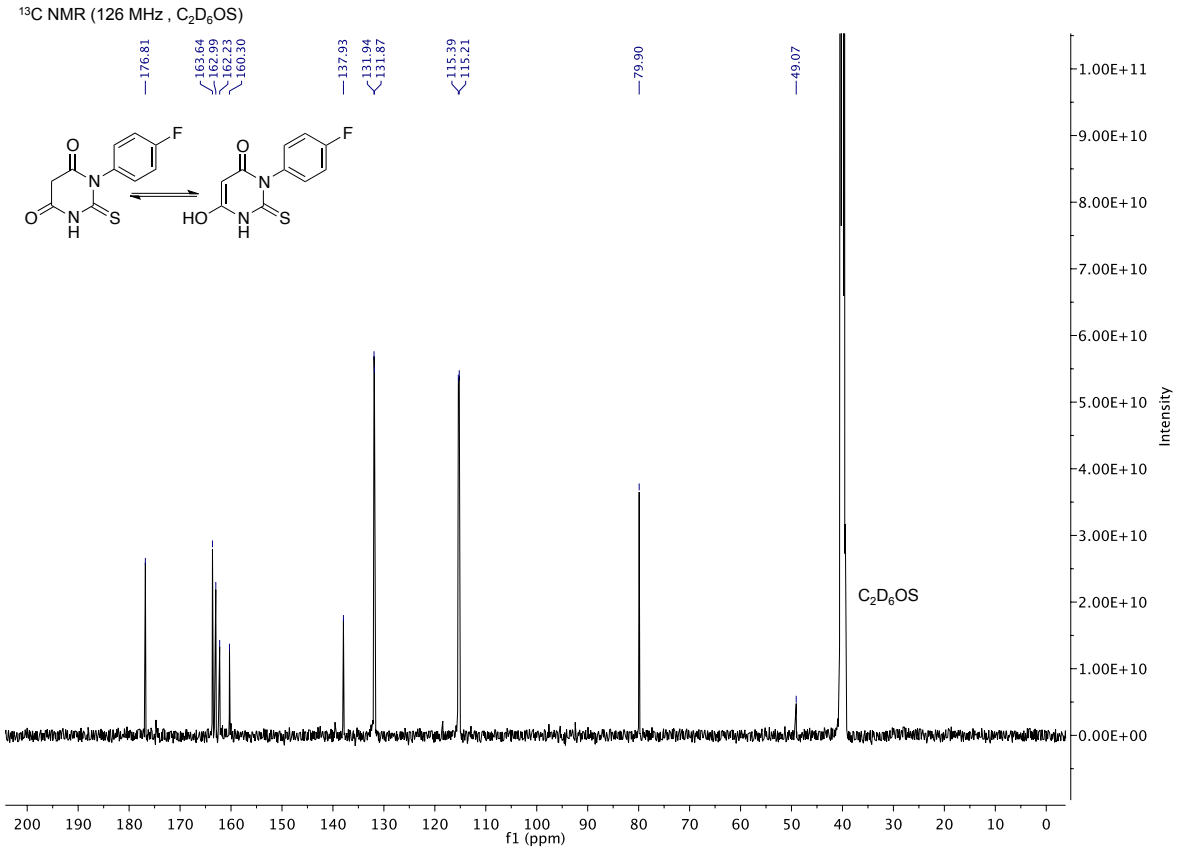

^{1}H NMR (400 MHz, C_2D_6OS)
10.66
7.40
7.38
7.38
7.36
7.35
7.34
7.13
7.12
7.11
7.10
7.09
7.08
6.91
6.91
6.90
6.89
6.89
6.89
6.88
6.87
6.87
6.87
4.24
3.34 HDO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
HDO
C_2D_6OS
Intensity
f1 (ppm)


^{13}C NMR (101 MHz, C_2D_6OS)
176.53
163.58
163.41
162.90
161.17
143.45
143.34
129.84
129.75
126.56
117.57
117.35
114.24
114.03
79.76
C_2D_6OS
Intensity
f1 (ppm)

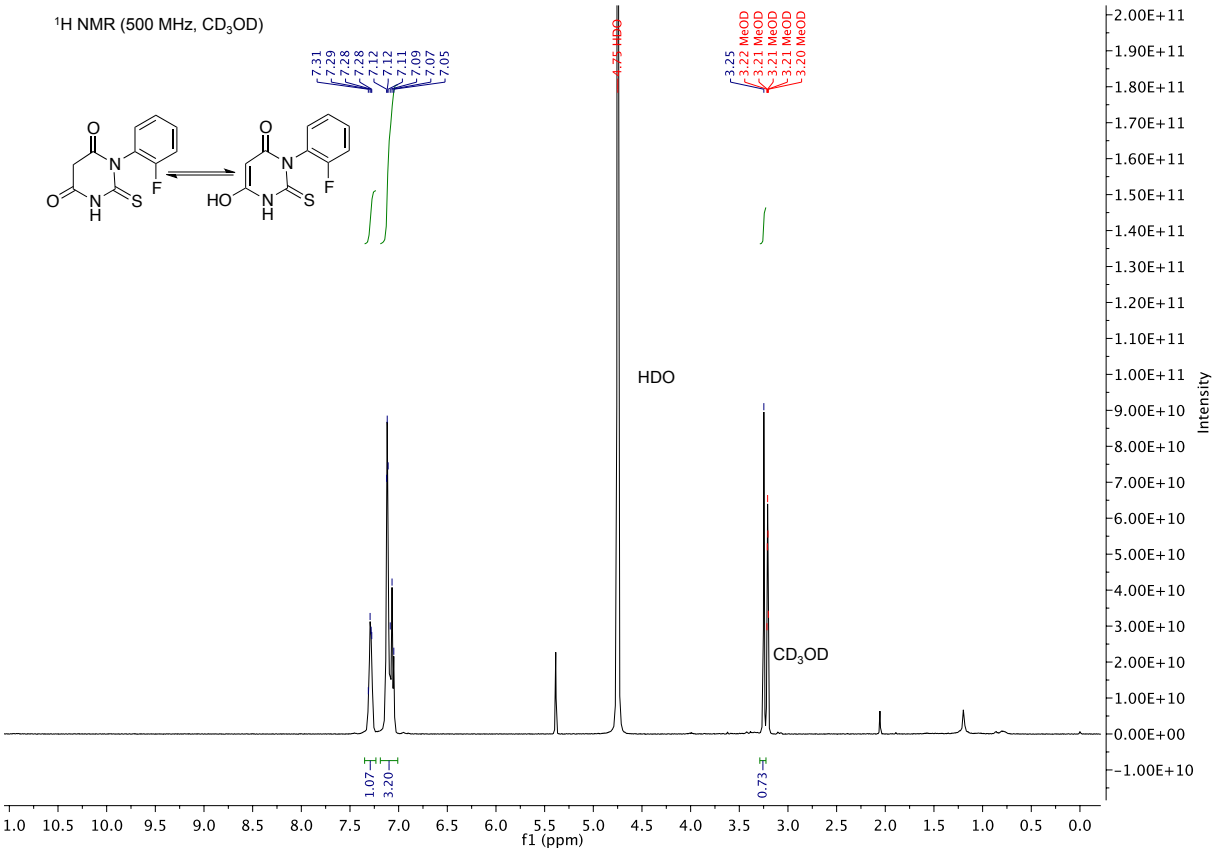

19F NMR (376 MHz, C2D6OS)
-114.65
-114.65
-112.5
-113.5
-114.5
-115.5
-116.5
f1 (ppm)
Intensity
f1 (ppm)

Compound **2b**

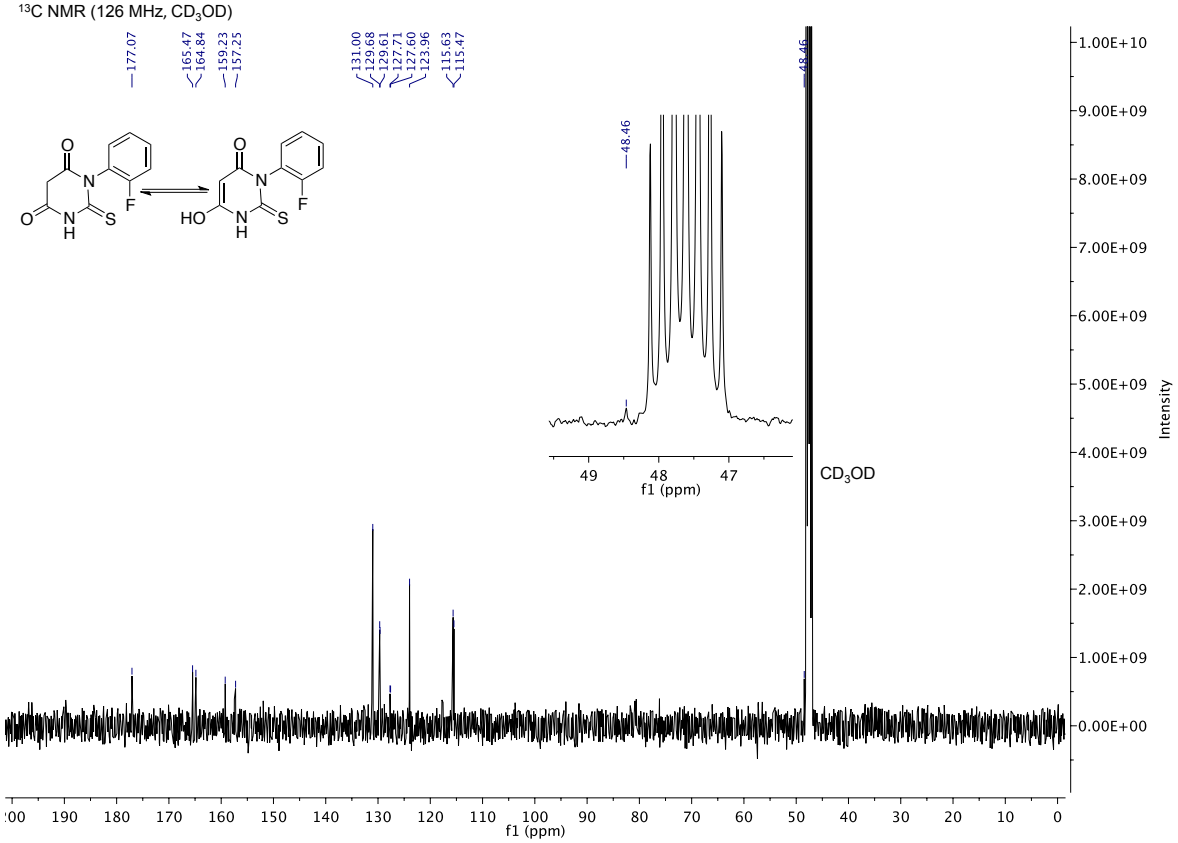

1H NMR (500 MHz, C2D6OS)
10.69
7.17
7.16
7.14
7.06
7.05
7.05
7.04
4.27
3.36 HDO
3.18
3.17
2.51 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
HDO
C2D6OS
0.82
2.21
2.00
0.82
0.33
7.20
7.15
7.10
f1 (ppm)
Intensity
f1 (ppm)


13C NMR (126 MHz , C2D6OS)
176.81
163.64
162.99
162.23
160.30
137.93
131.94
131.87
115.39
115.21
79.90
49.07
C2D6OS
Intensity
f1 (ppm)

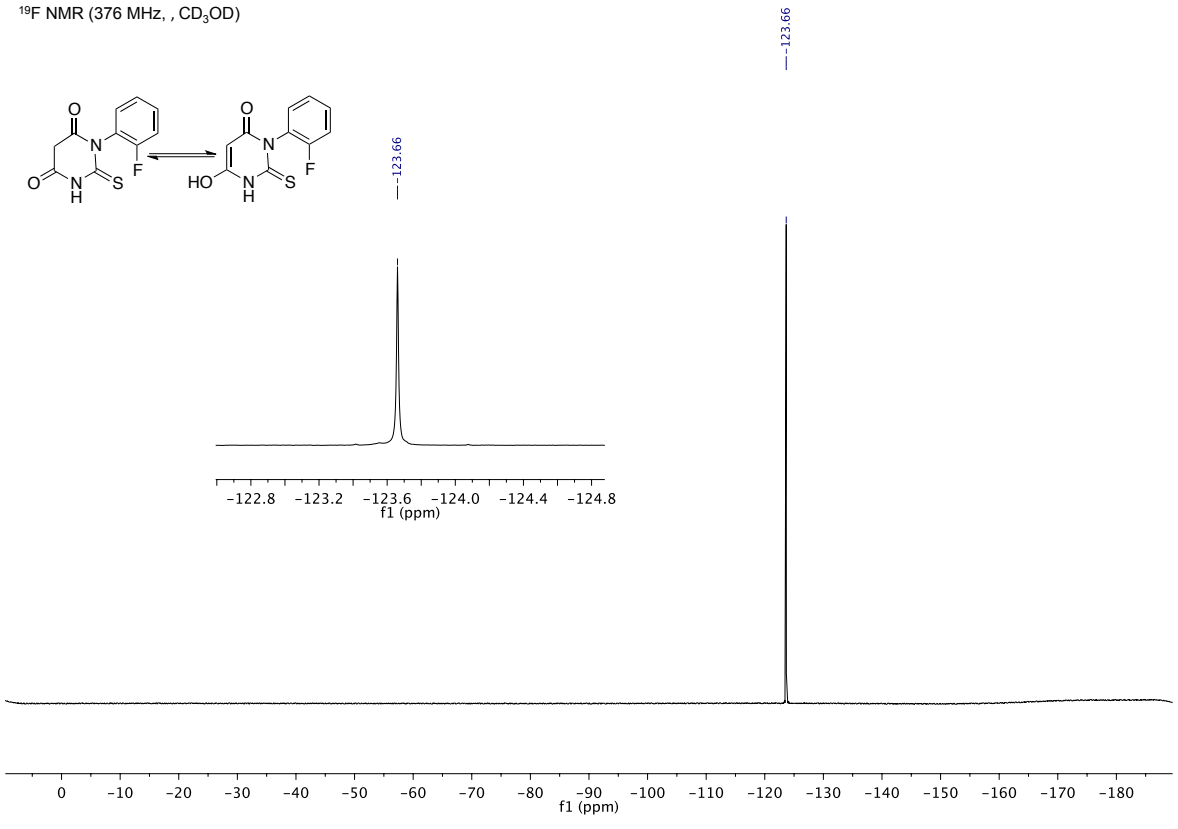
Compound **2c**


1H NMR (500 MHz, CD3OD)
7.31
7.29
7.28
7.28
7.12
7.12
7.11
7.09
7.07
7.05
4.75 HDO
3.25
3.22 MeOD
3.21 MeOD
3.21 MeOD
3.21 MeOD
3.20 MeOD
HDO
CD3OD
1.07
3.20
0.73
Intensity
f1 (ppm)

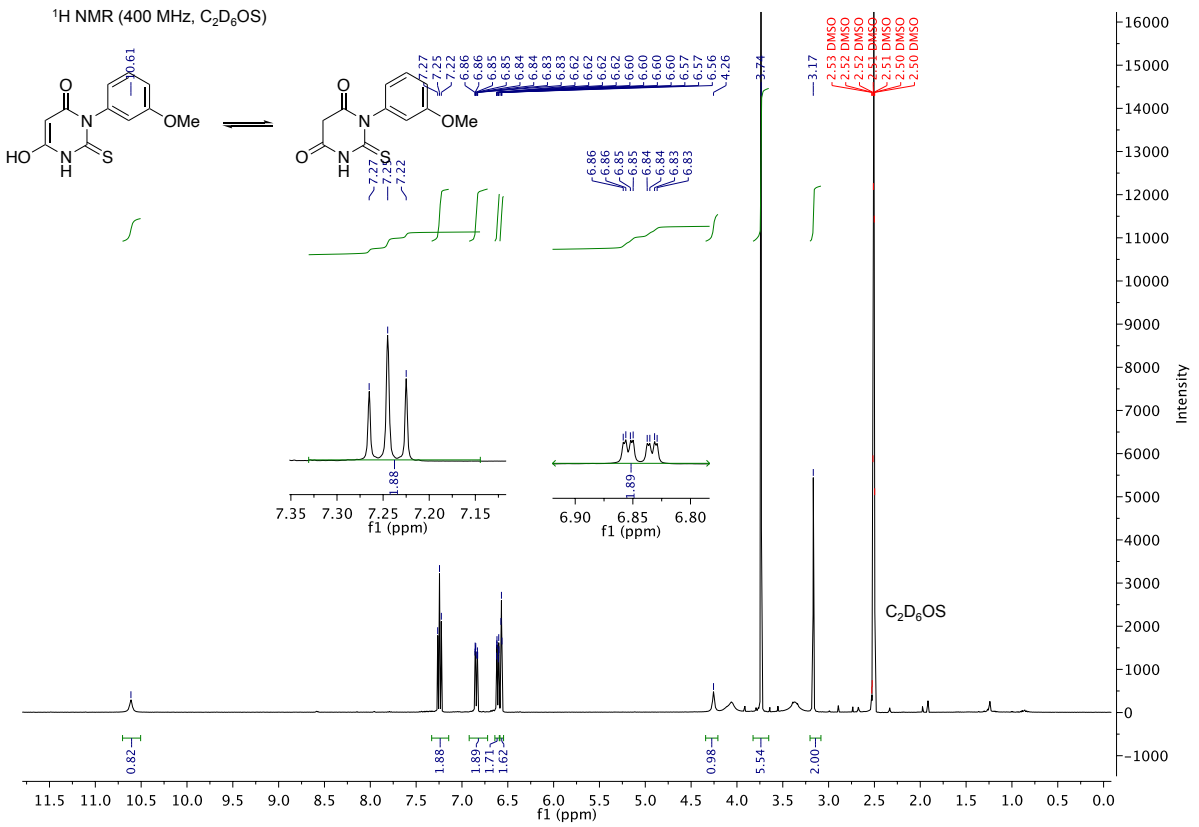
^{13}C NMR (126 MHz, CD_3OD)

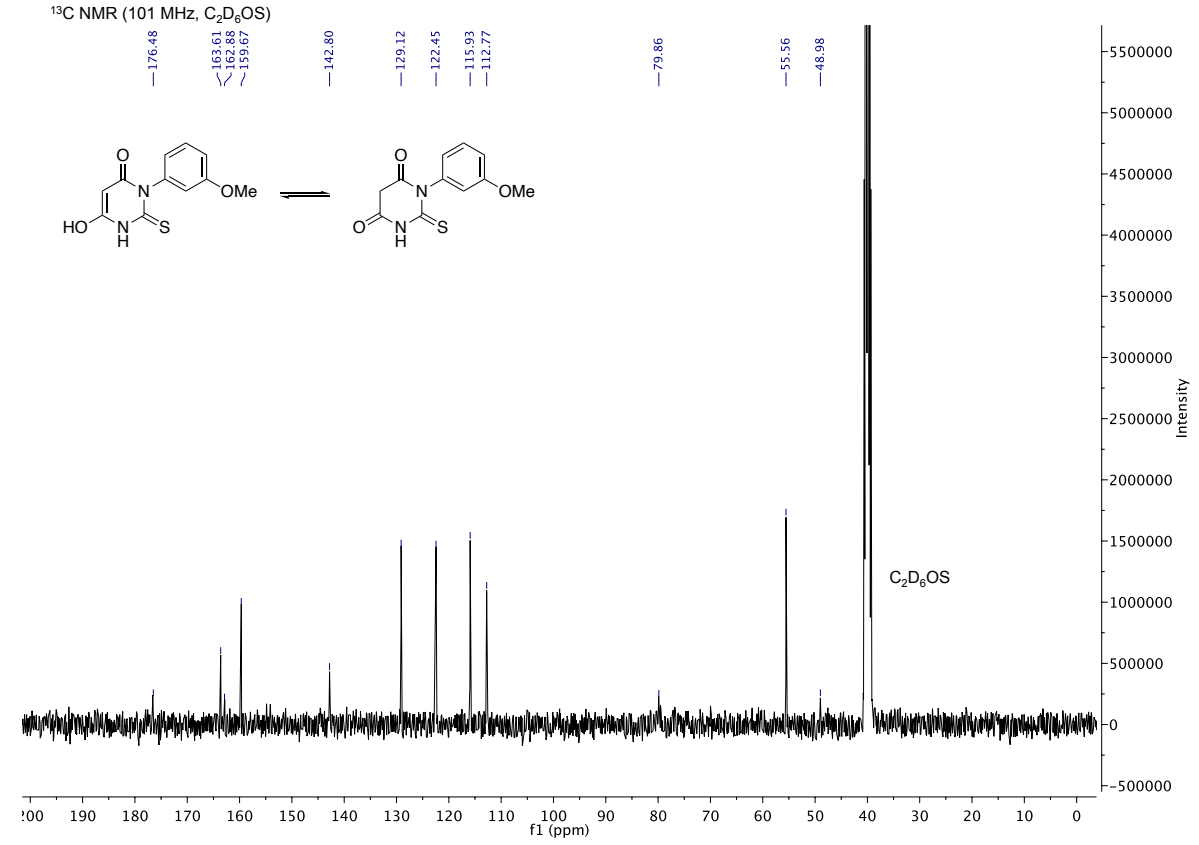


^{19}F NMR (376 MHz, , CD_3OD)

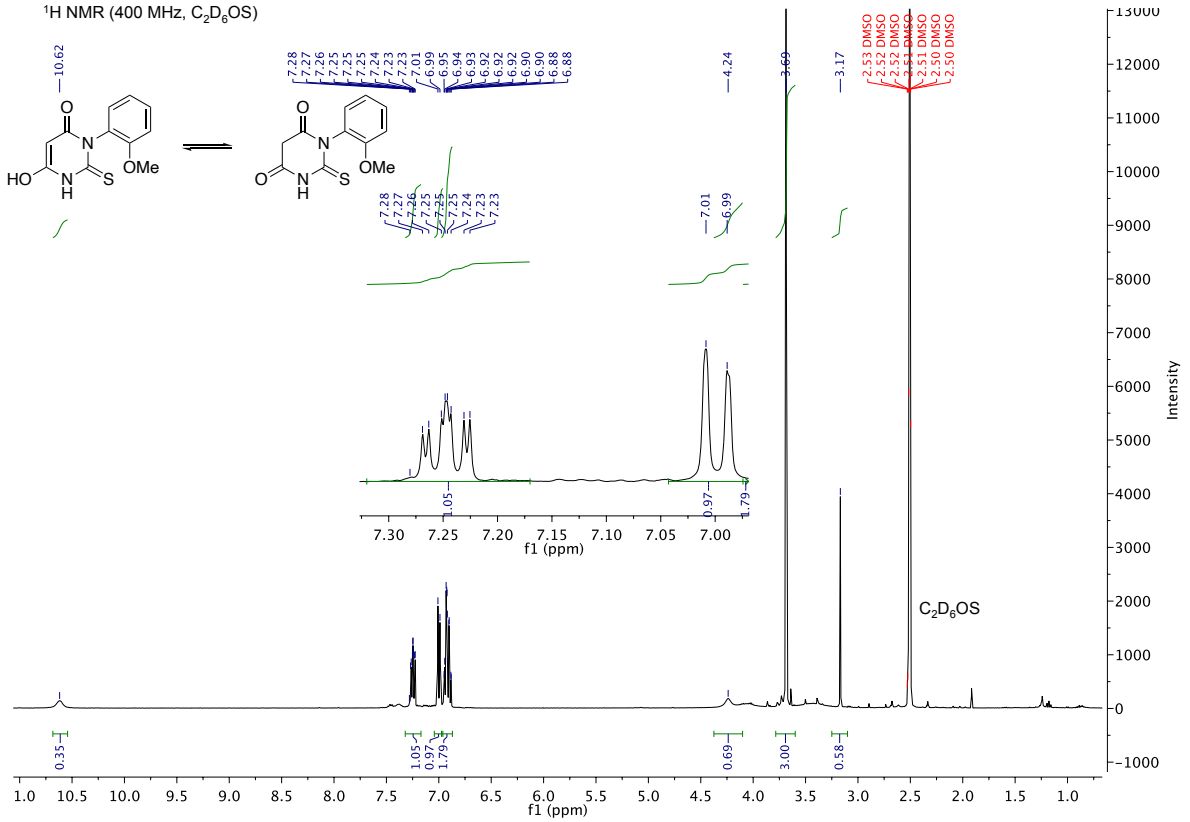


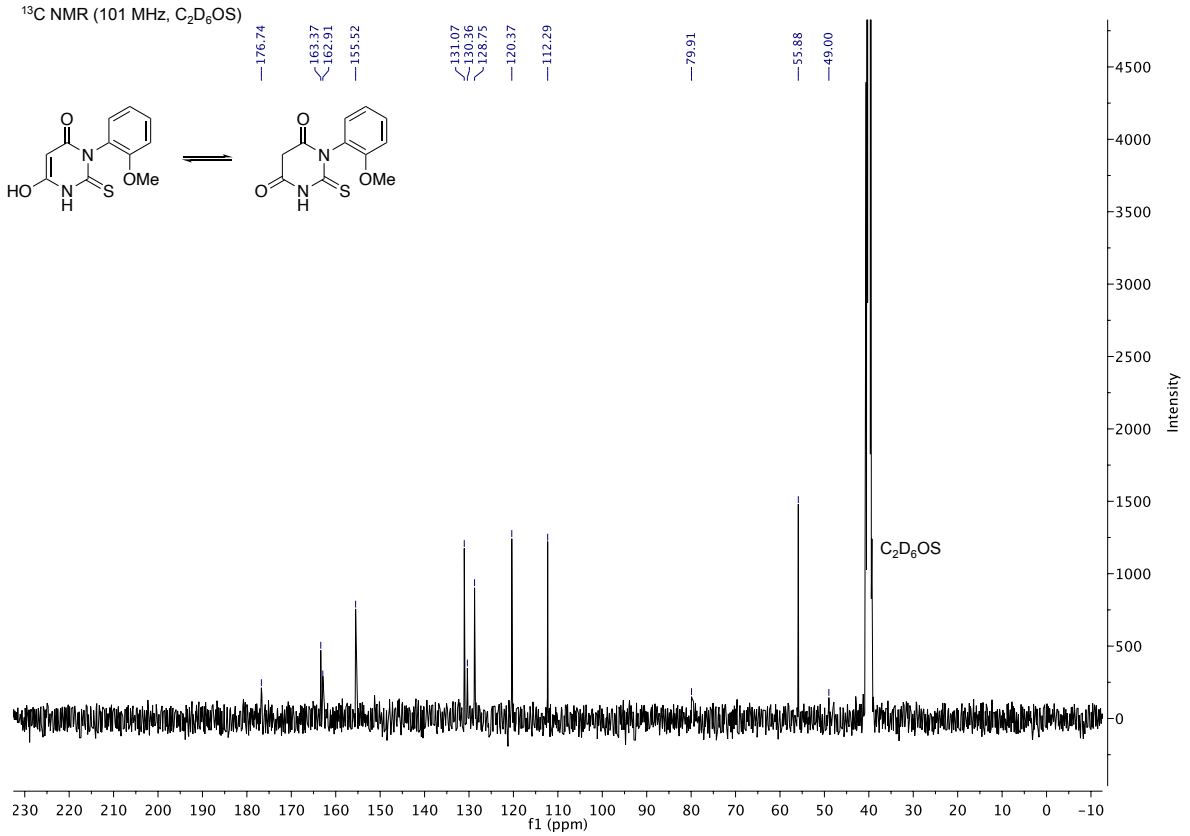
Compound **2d**


¹H NMR (400 MHz, C2D6OS)
OMe
HO
N
H
S
O
10.61
7.27
7.25
7.22
6.86
6.85
6.84
6.83
6.62
6.60
6.57
6.56
4.26
3.74
3.17
2.53 DMSO
2.52 DMSO
2.51 DMSO
2.50 DMSO
1.88
1.89
f1 (ppm)
C2D6OS
Intensity
0.82
1.88
1.89
1.71
1.62
0.98
5.54
2.00

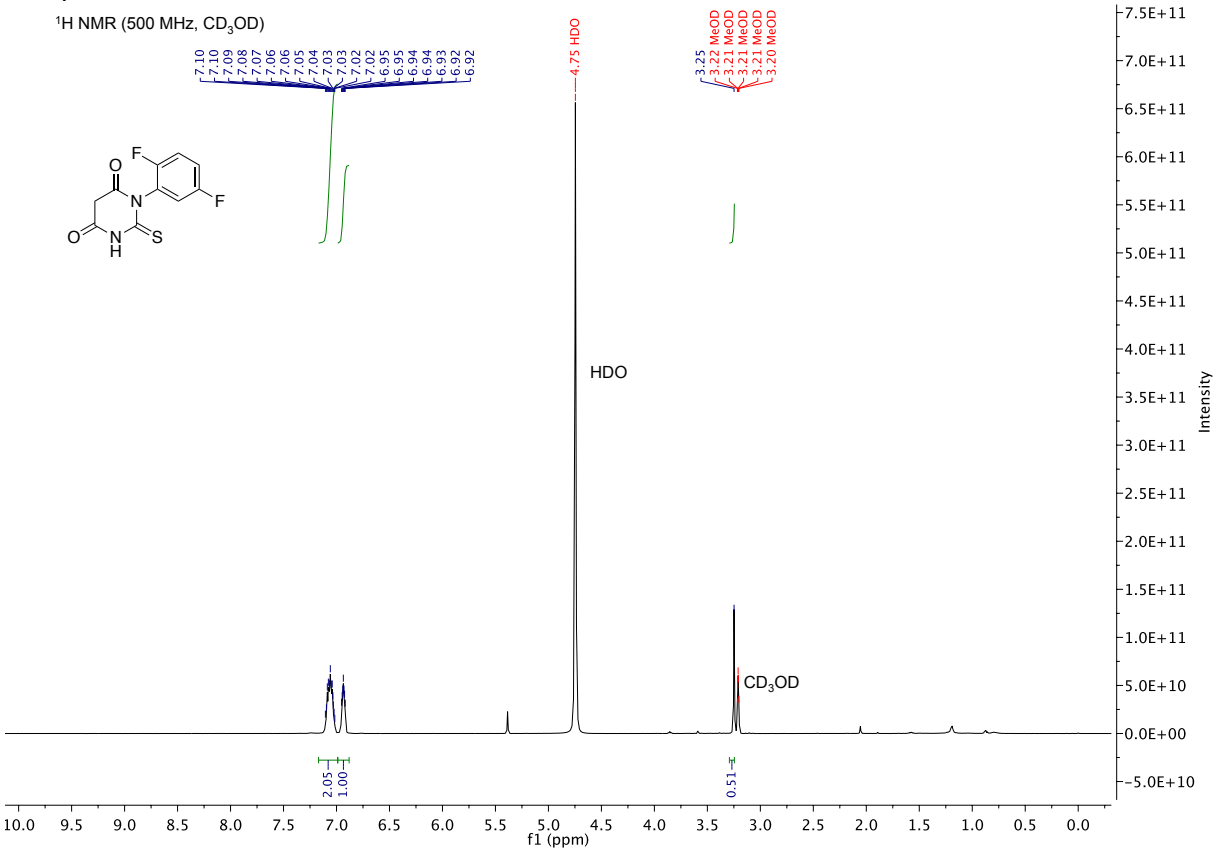

¹³C NMR (101 MHz, C2D6OS)
176.48
163.61
162.88
159.67
142.80
129.12
122.45
115.93
112.77
79.86
55.56
48.98
OMe
HO
N
H
S
O
f1 (ppm)
C2D6OS
Intensity

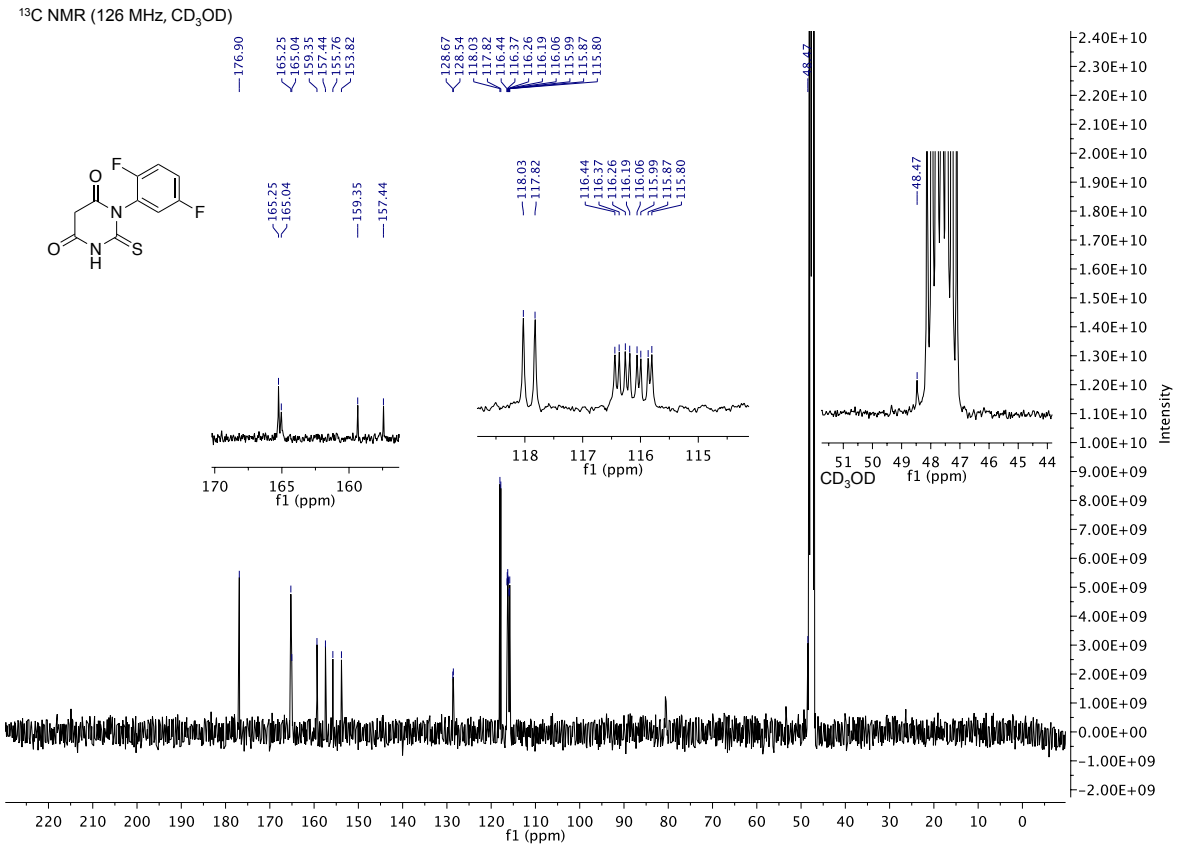
Compound **2e**

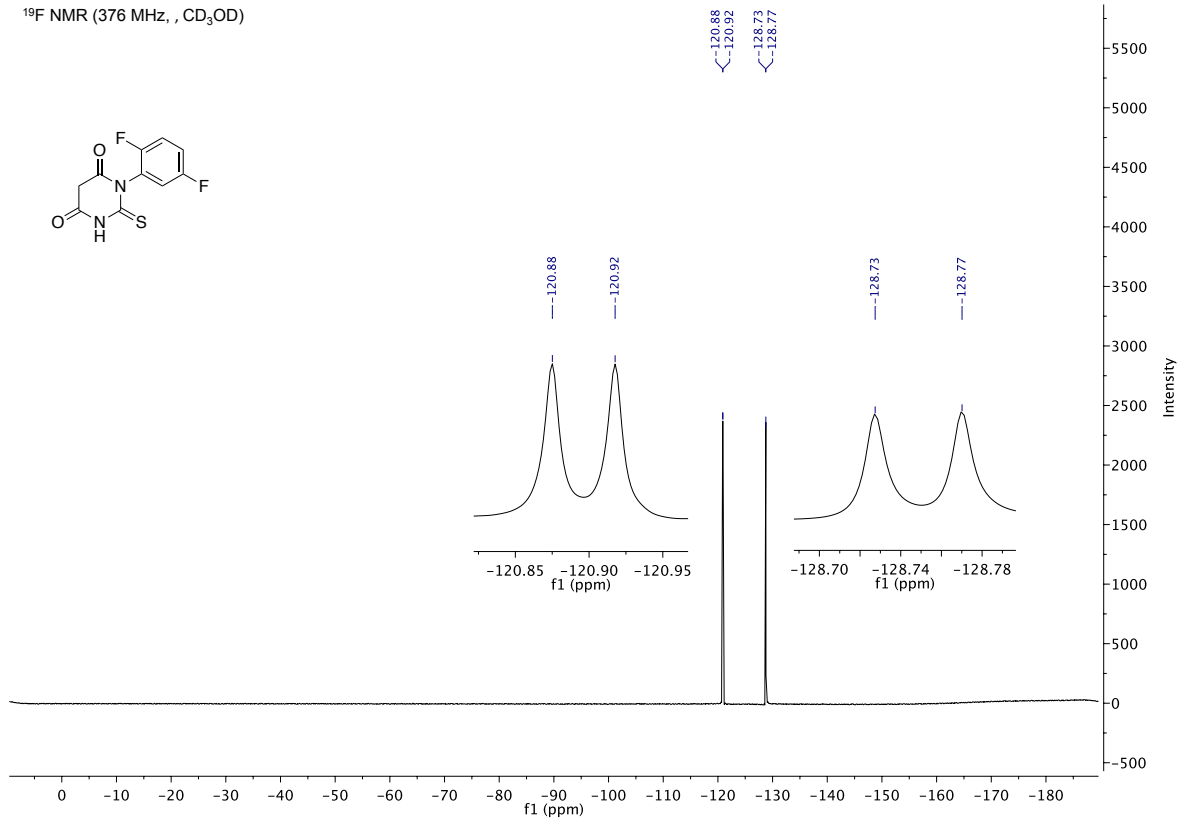

^{1}H NMR (400 MHz, C_2D_6OS)
10.62
7.28
7.27
7.26
7.25
7.25
7.25
7.24
7.23
7.23
7.01
6.99
6.95
6.94
6.93
6.92
6.92
6.92
6.90
6.90
6.88
6.88
4.24
3.69
3.17
2.53 DMSO
2.52 DMSO
2.52 DMSO
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
HO
OMe
O
N
H
S
1.05
0.97
1.79
7.30 7.25 7.20 7.15 7.10 7.05 7.00
f1 (ppm)
C_2D_6OS
Intensity
0.35
1.05
0.97
1.79
0.69
3.00
0.58
f1 (ppm)


^{13}C NMR (101 MHz, C_2D_6OS)
176.74
163.37
162.91
155.52
131.07
130.36
128.75
120.37
112.29
79.91
55.88
49.00
HO
OMe
O
N
H
S
C_2D_6OS
Intensity
f1 (ppm)

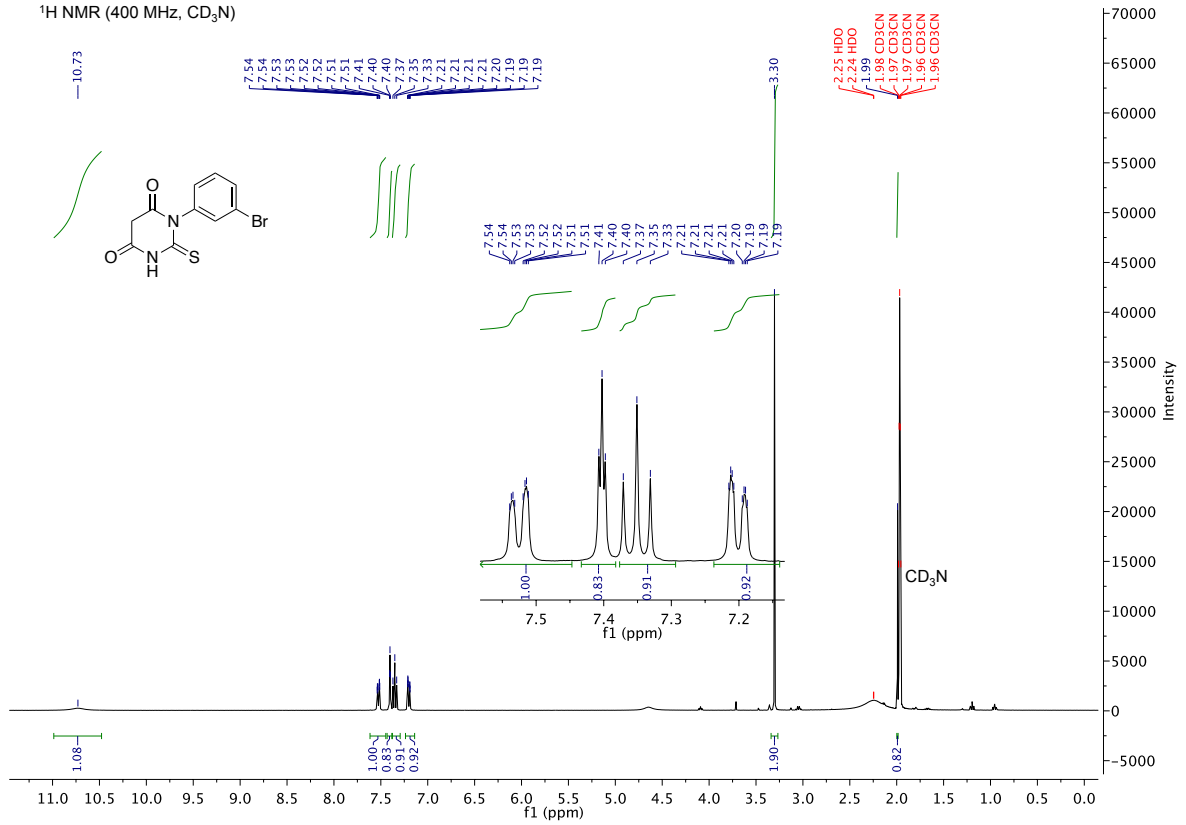
Compound **2f**

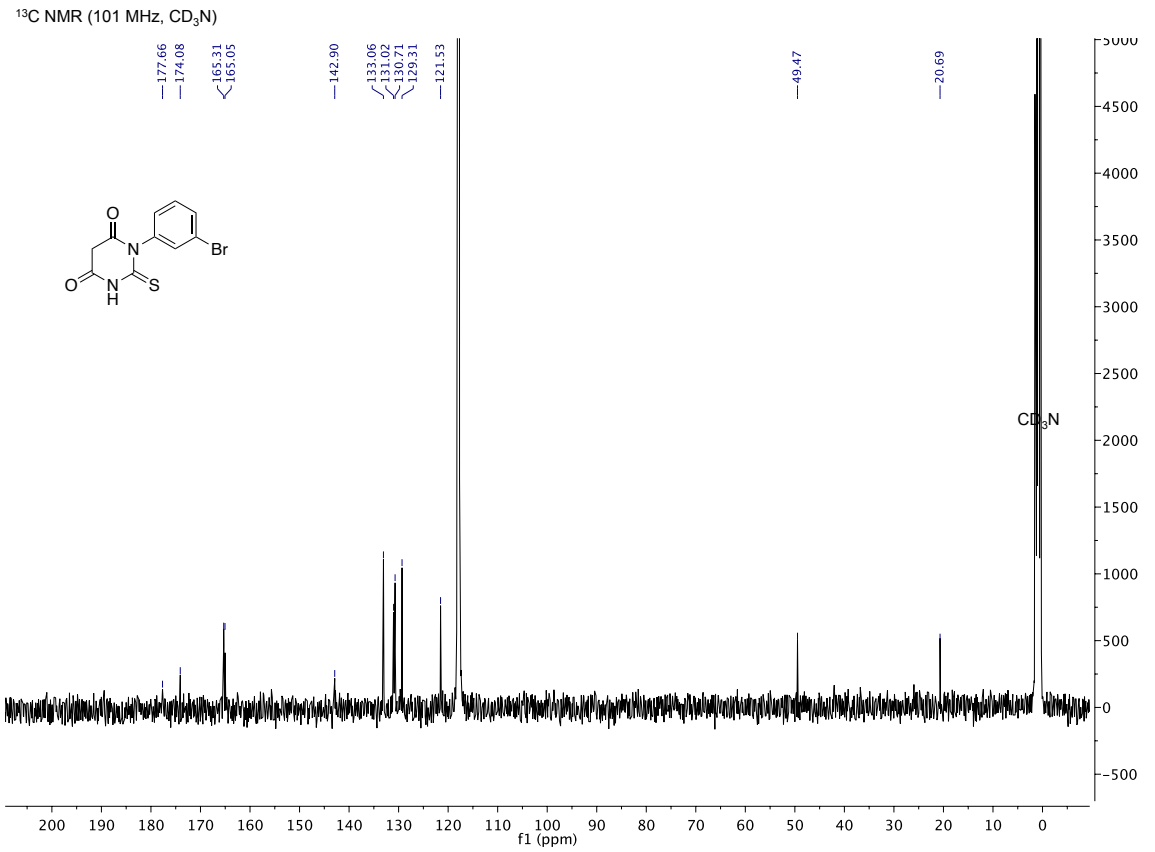

^{1}H NMR (500 MHz, CD_3OD)
HDO
CD_3OD
Intensity
f1 (ppm)


^{13}C NMR (126 MHz, CD_3OD)
CD_3OD
Intensity
f1 (ppm)

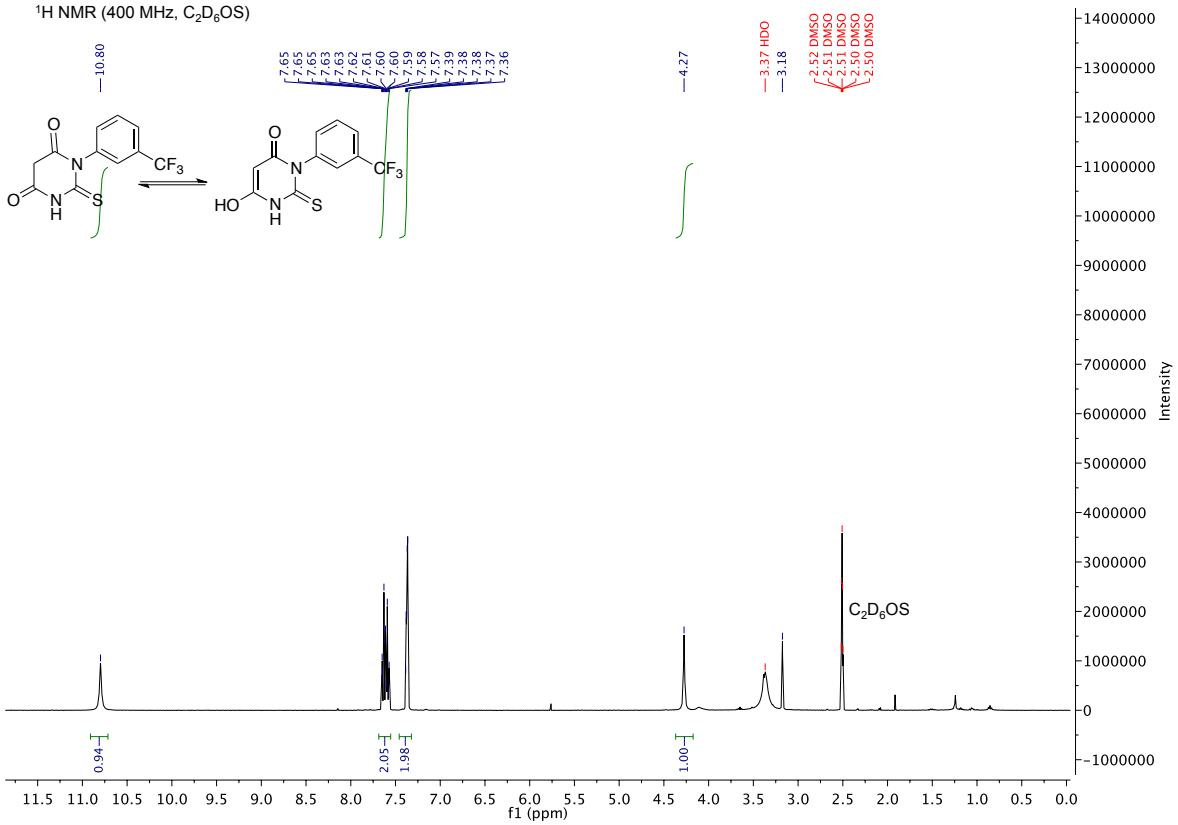

19F NMR (376 MHz, , CD3OD)
-120.88
-120.92
-128.73
-128.77
f1 (ppm)
Intensity

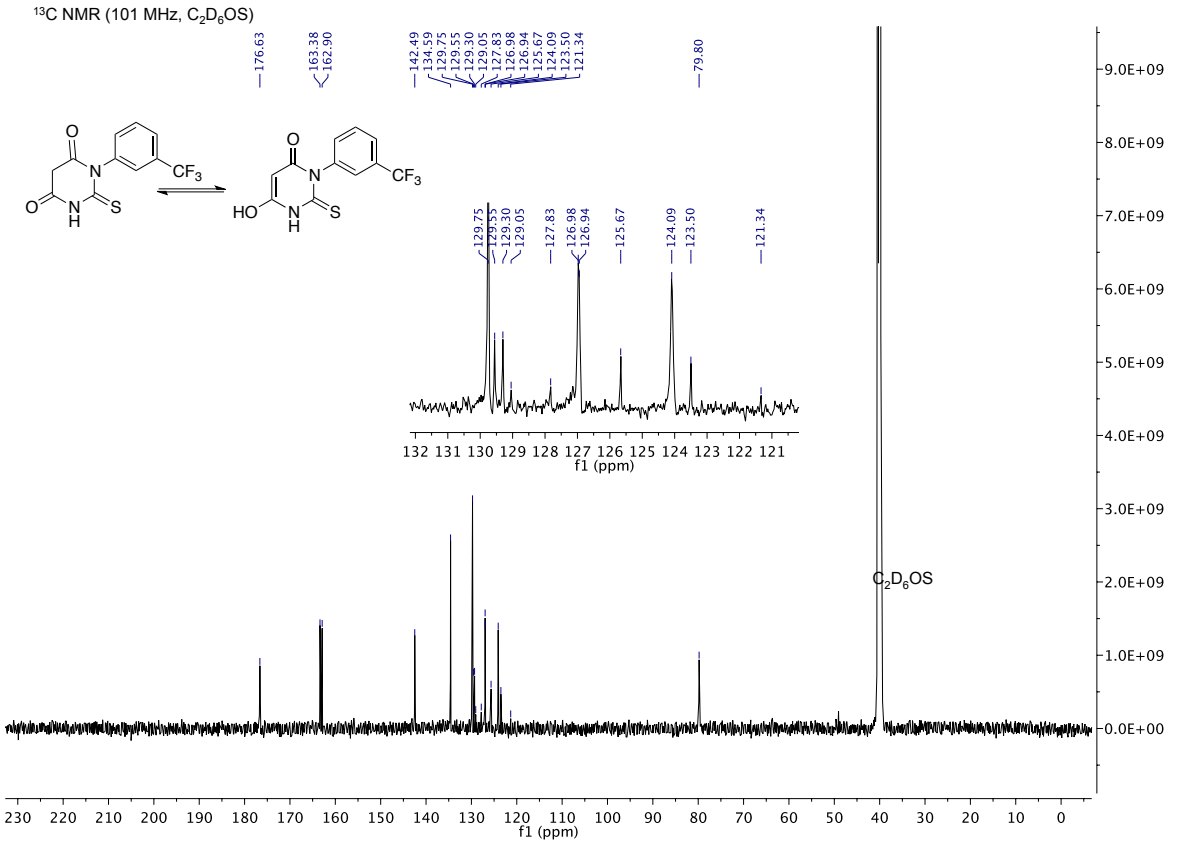
Compound **2g**

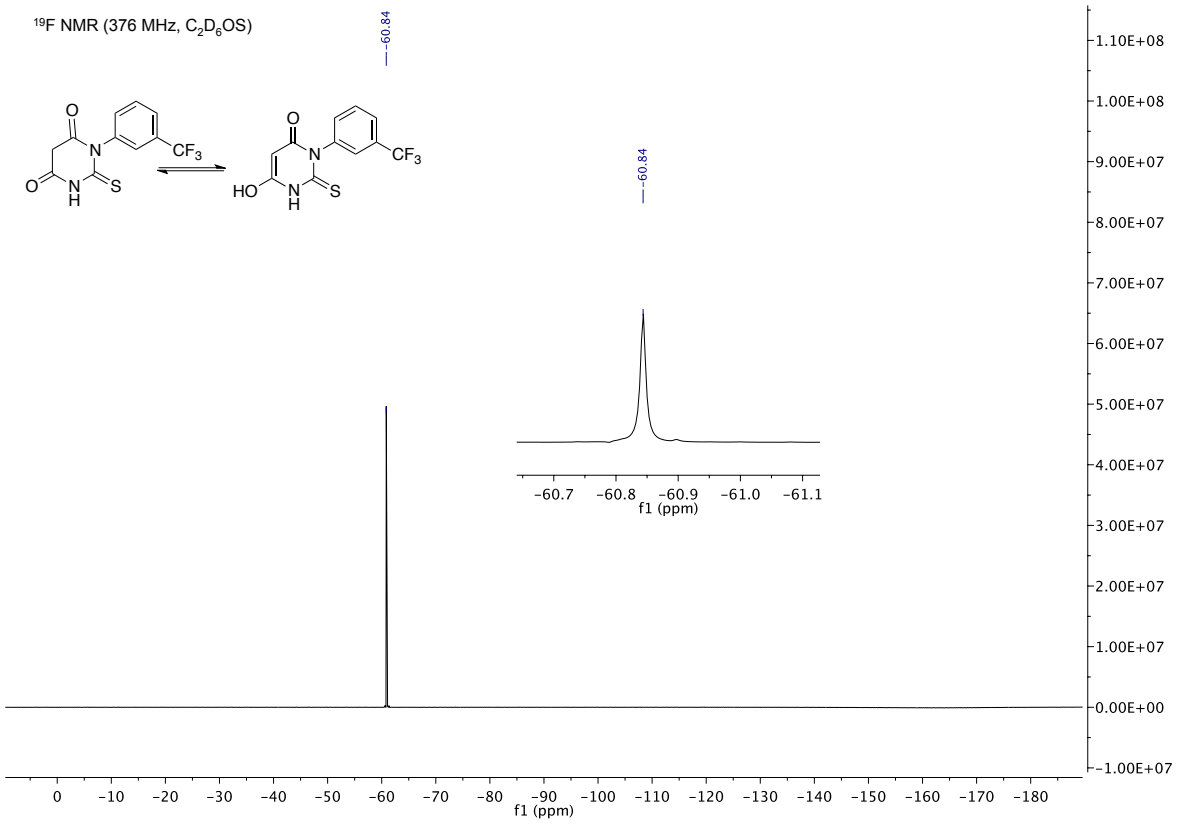

1H NMR (400 MHz, CD3N)
10.73
7.54
7.53
7.52
7.51
7.41
7.40
7.37
7.35
7.33
7.21
7.20
7.19
3.30
2.25 HDO
2.24 HDO
1.99
1.98 CD3CN
1.97 CD3CN
1.96 CD3CN
CD3N
f1 (ppm)
Intensity


13C NMR (101 MHz, CD3N)
177.66
174.08
165.31
165.05
142.90
133.06
131.02
130.71
129.31
121.53
49.47
20.69
CD3N
Br
f1 (ppm)

Compound **2h**

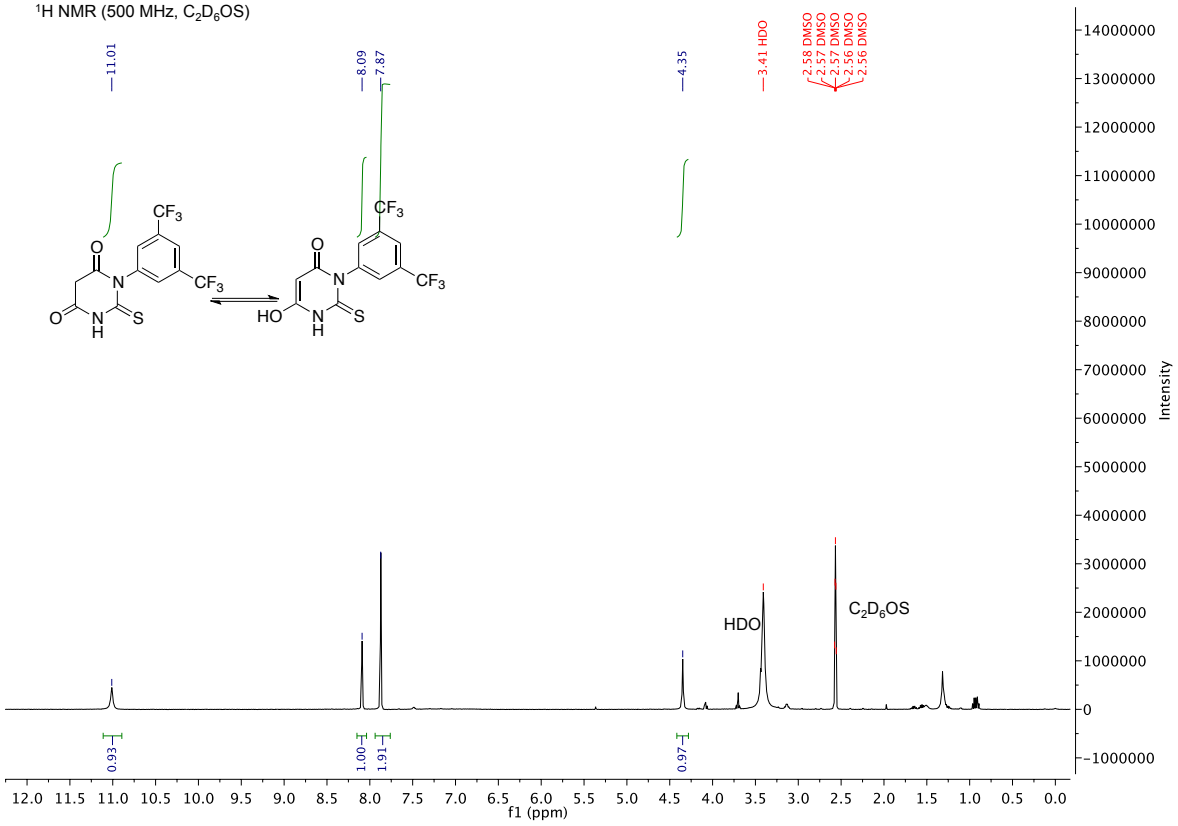

1H NMR (400 MHz, C2D6OS)
10.80
7.65
7.65
7.65
7.63
7.63
7.62
7.61
7.60
7.60
7.59
7.58
7.57
7.39
7.38
7.38
7.37
7.36
4.27
3.37 HDO
3.18
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
CF3
HO
C2D6OS
0.94
2.05
1.98
1.00
f1 (ppm)
Intensity


13C NMR (101 MHz, C2D6OS)
176.63
163.38
162.90
142.49
134.59
129.75
129.55
129.30
129.05
127.83
126.98
126.94
125.67
124.09
123.50
121.34
79.80
C2D6OS
f1 (ppm)

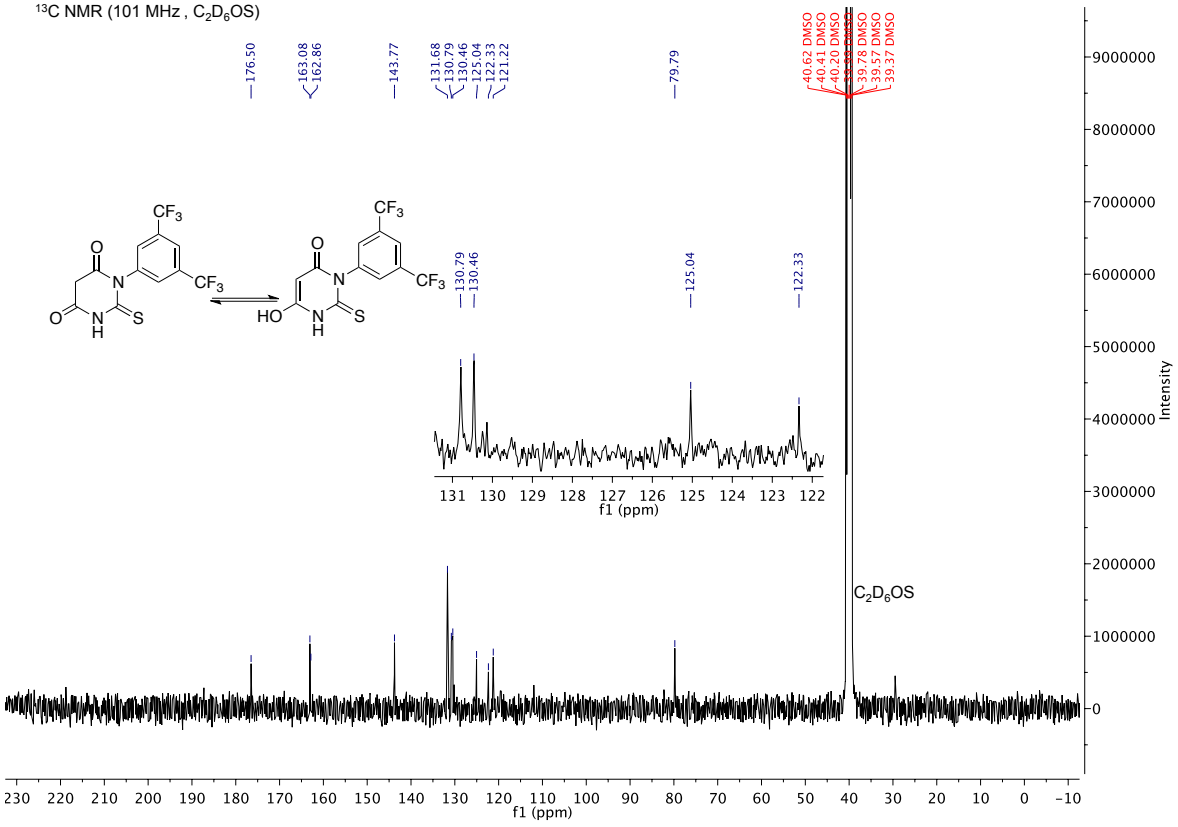

19F NMR (376 MHz, C2D6OS)
-60.84
f1 (ppm)

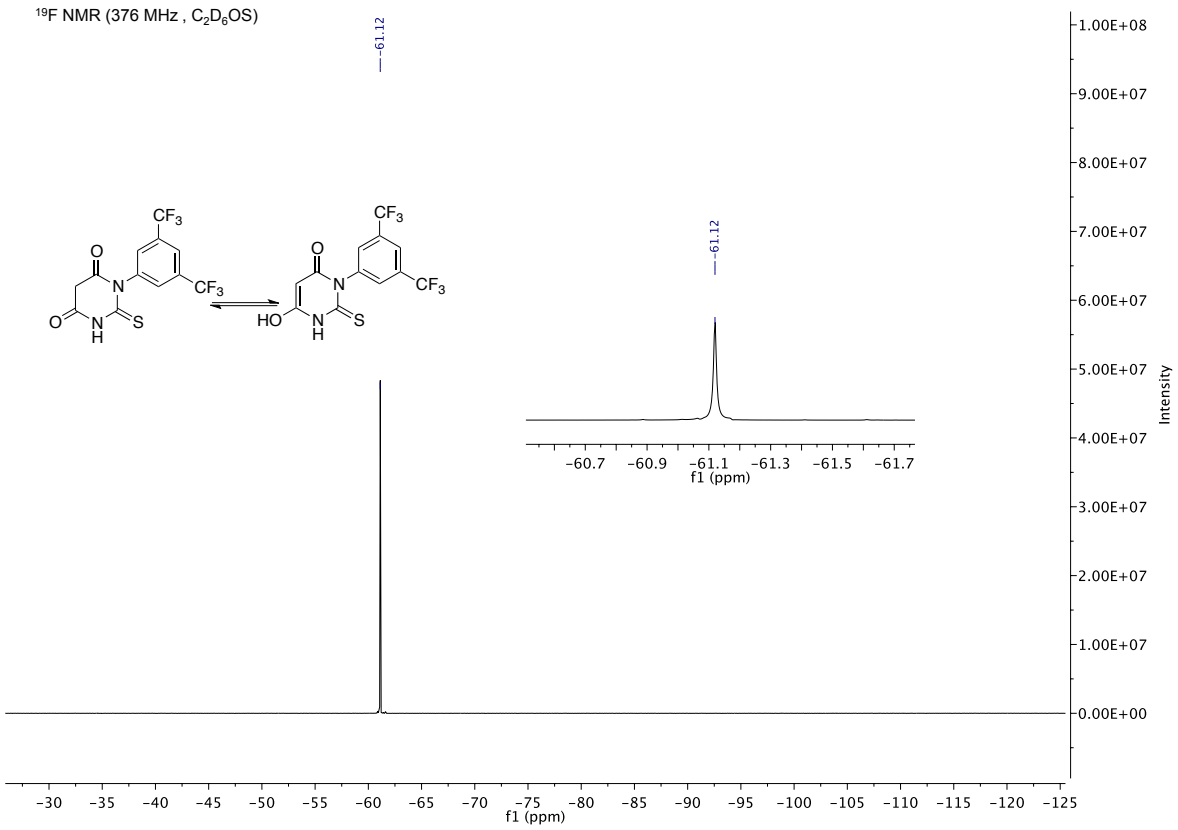
Compound **2i**

^{1}H NMR (500 MHz, C_2D_6OS)

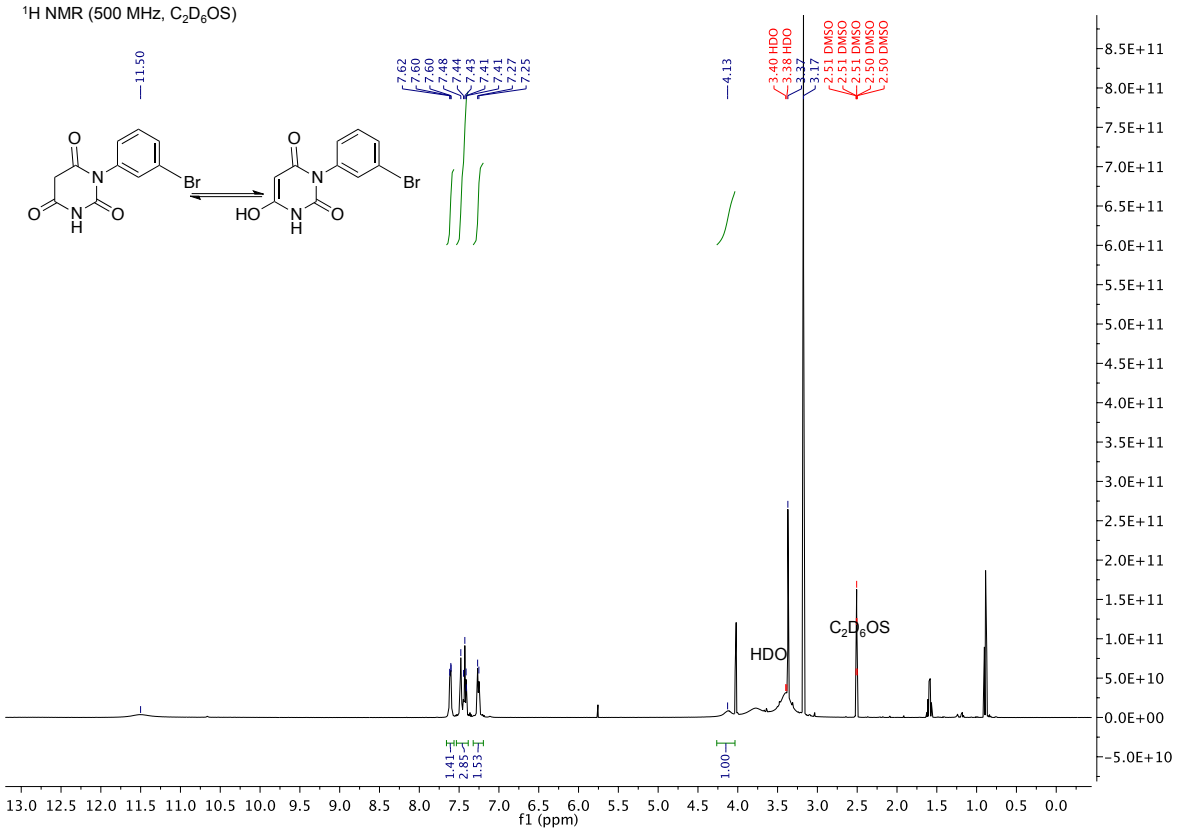


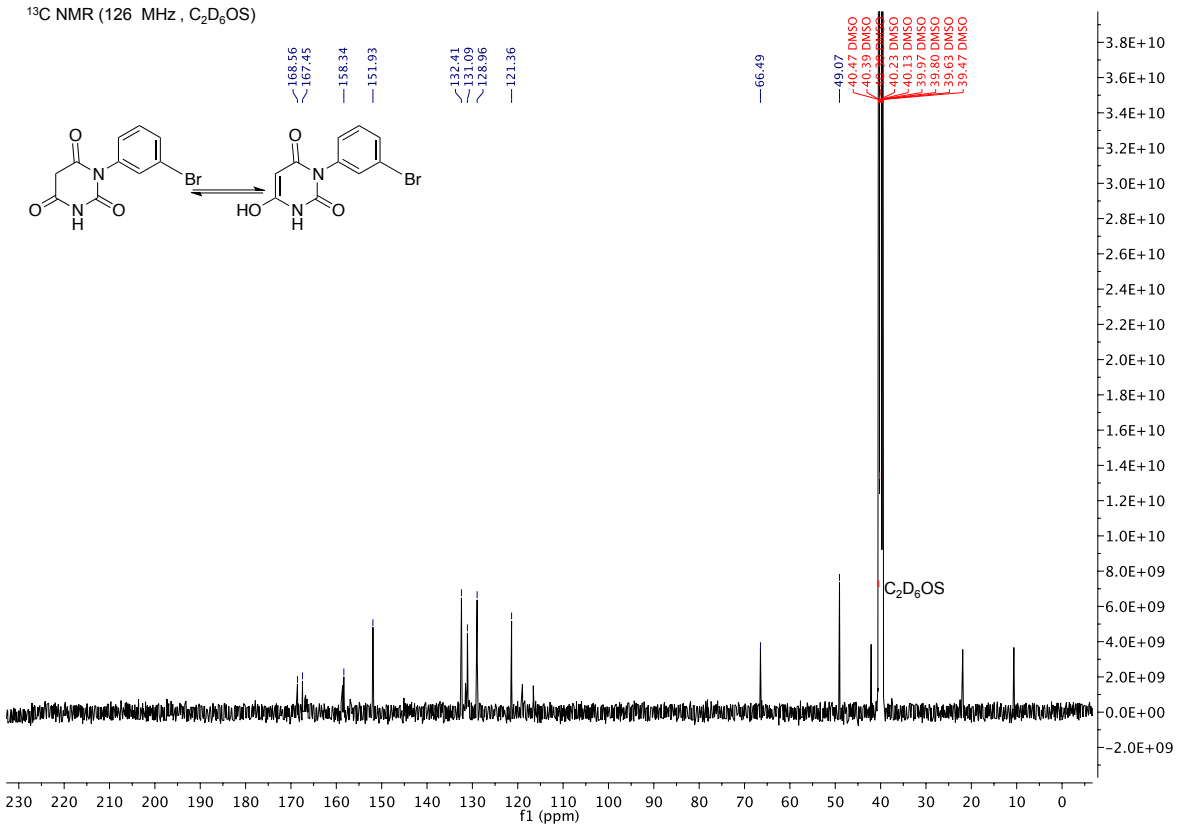
^{13}C NMR (101 MHz , C_2D_6OS)



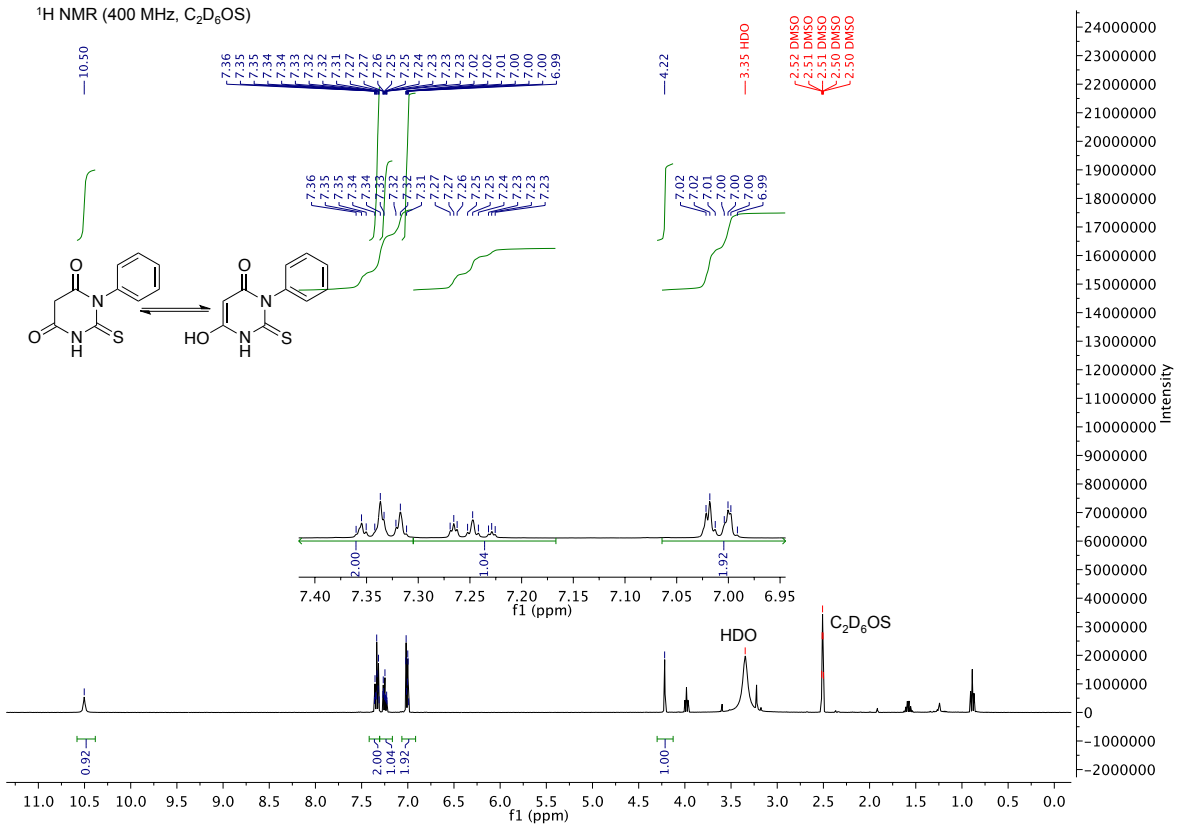


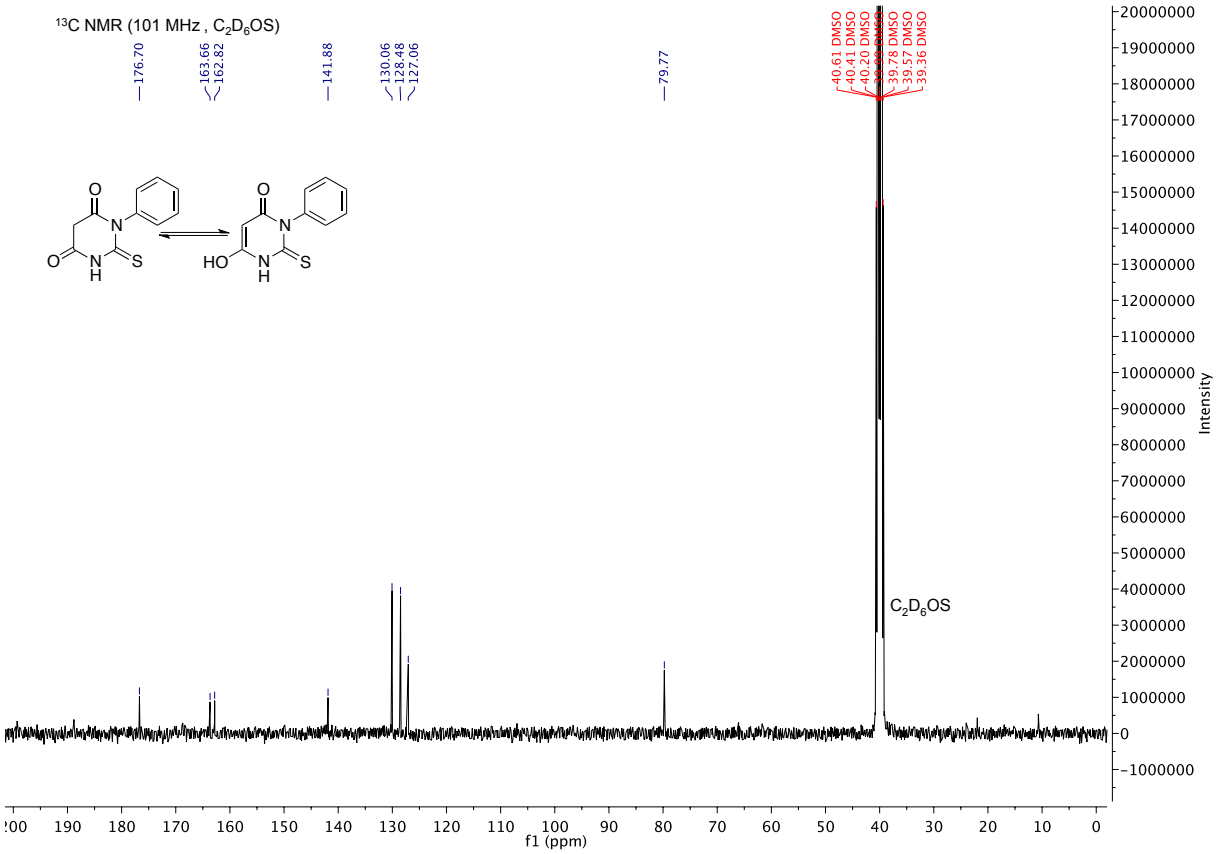
Compound **2j** (Not pure)



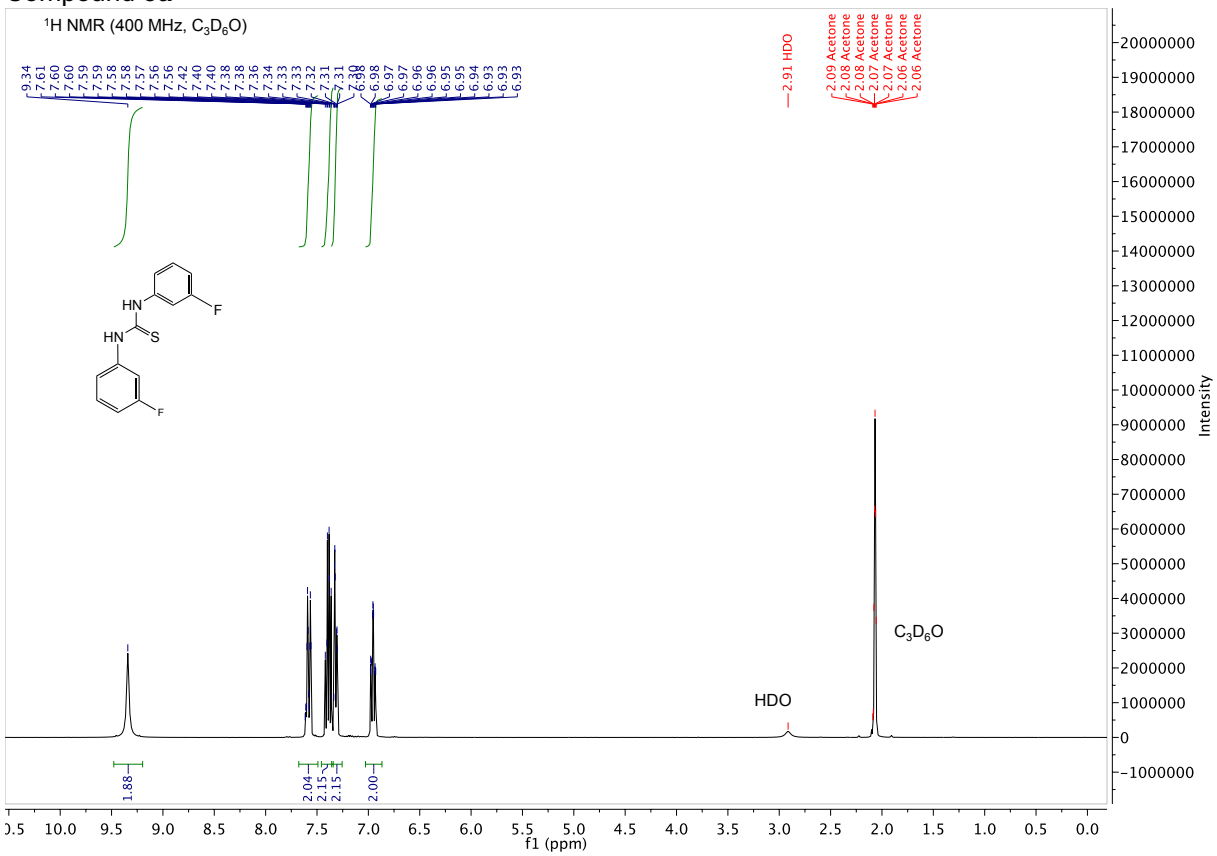

13C NMR (126 MHz , C2D6OS)
168.56
167.45
158.34
151.93
132.41
131.09
128.96
121.36
66.49
49.07
40.47 DMSO
40.39 DMSO
40.23 DMSO
40.13 DMSO
39.97 DMSO
39.80 DMSO
39.63 DMSO
39.47 DMSO
C2D6OS
f1 (ppm)

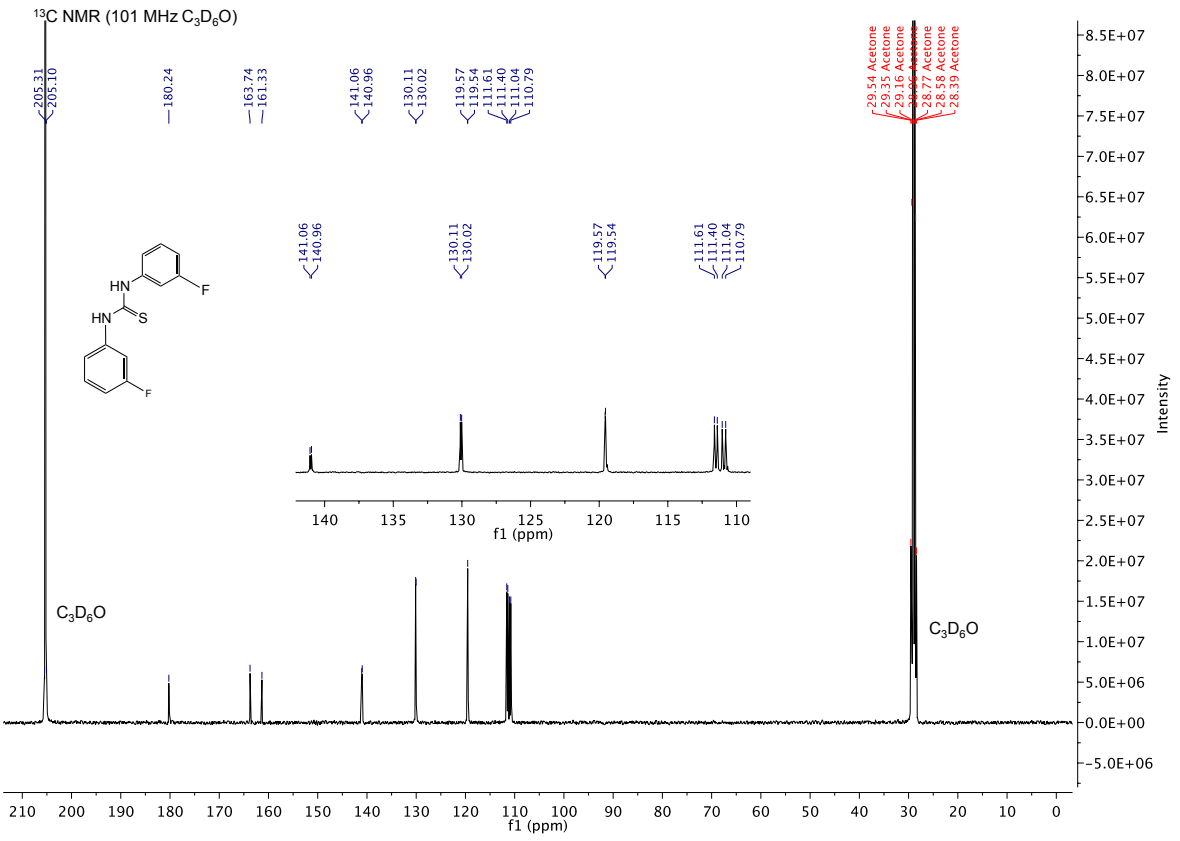
Compound **2k**

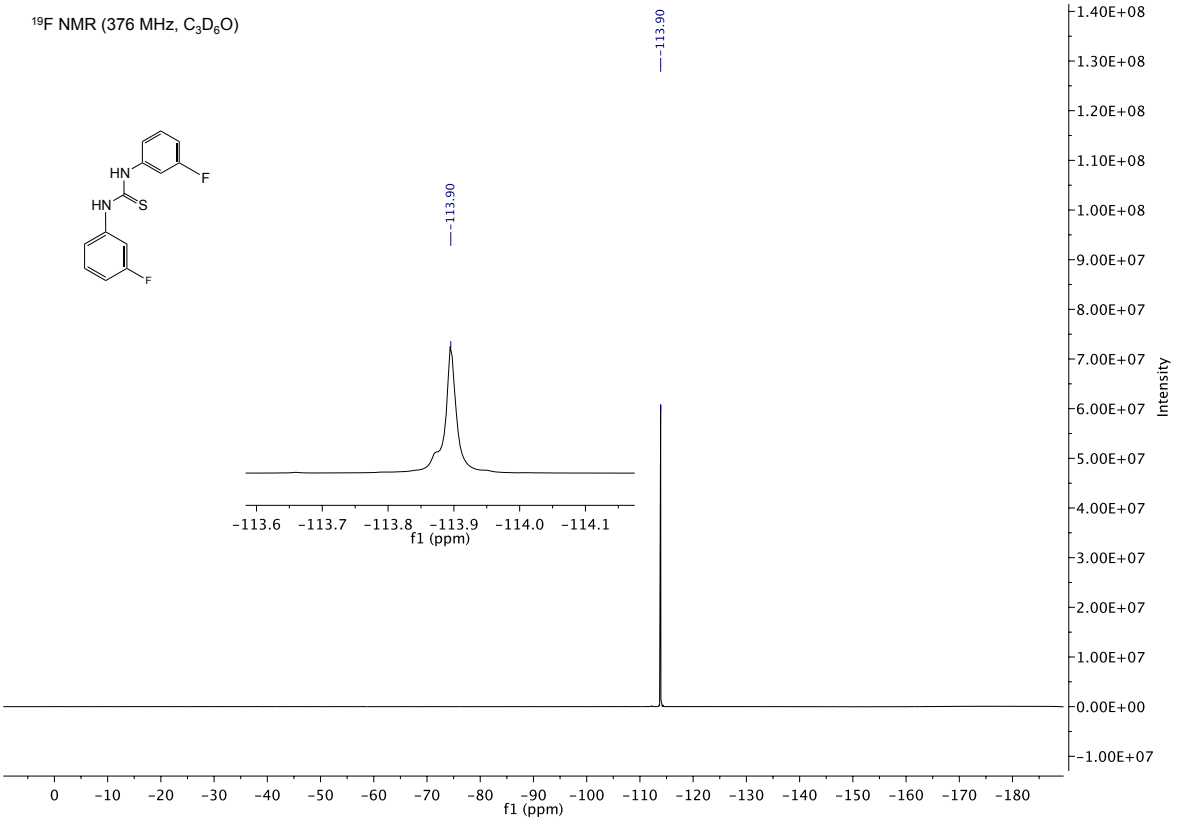

1H NMR (400 MHz, C2D6OS)
10.50
7.36
7.35
7.34
7.33
7.32
7.31
7.27
7.26
7.25
7.24
7.23
7.02
7.01
7.00
6.99
4.22
3.35 HDO
2.52 DMSO
2.51 DMSO
2.50 DMSO
2.00
1.04
1.92
HDO
C2D6OS
0.92
2.00
1.04
1.92
1.00
f1 (ppm)
Intensity


13C NMR (101 MHz , C2D6OS)
176.70
163.66
162.82
141.88
130.06
128.48
127.06
79.77
40.61 DMSO
40.41 DMSO
40.20 DMSO
39.78 DMSO
39.57 DMSO
39.36 DMSO
C2D6OS
Intensity
f1 (ppm)

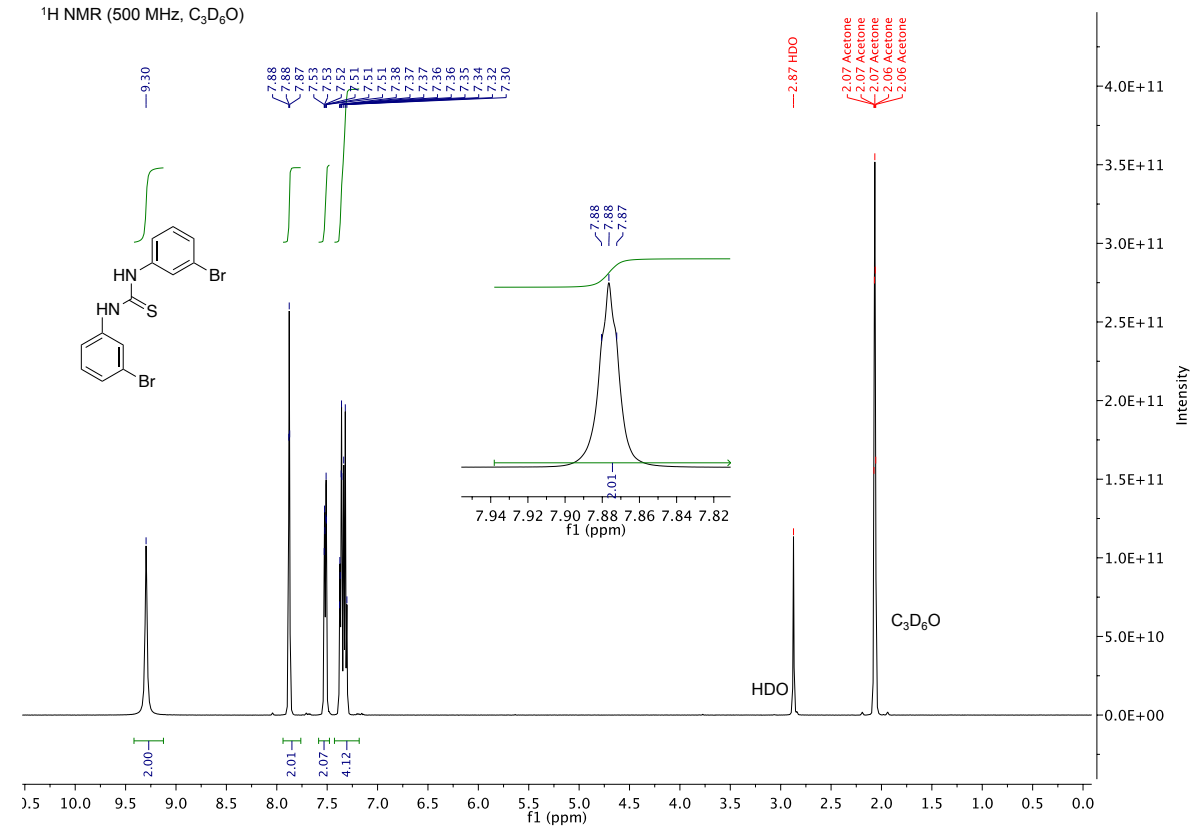
Compound **3a**

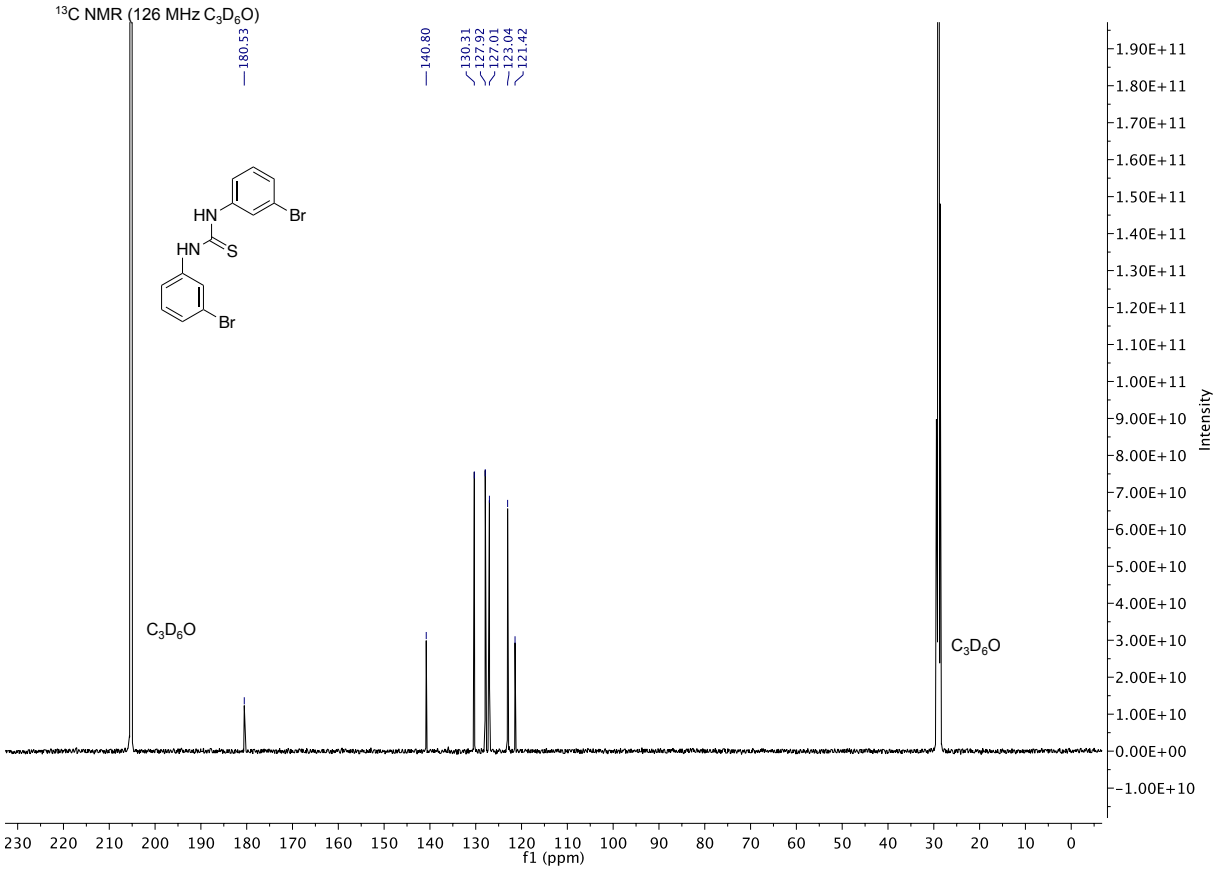

1H NMR (400 MHz, C3D6O)
2.91 HDO
2.09 Acetone
2.08 Acetone
2.08 Acetone
2.07 Acetone
2.07 Acetone
2.06 Acetone
2.06 Acetone
HDO
C3D6O
1.88
2.04
2.15
2.15
2.00
Intensity
f1 (ppm)


13C NMR (101 MHz C3D6O)
205.31
205.10
180.24
163.74
161.33
141.06
140.96
130.11
130.02
119.57
119.54
111.61
111.40
111.04
110.79
29.54 Acetone
29.35 Acetone
29.16 Acetone
28.77 Acetone
28.58 Acetone
28.39 Acetone
HN
HN
S
F
F
C3D6O
C3D6O
f1 (ppm)
Intensity

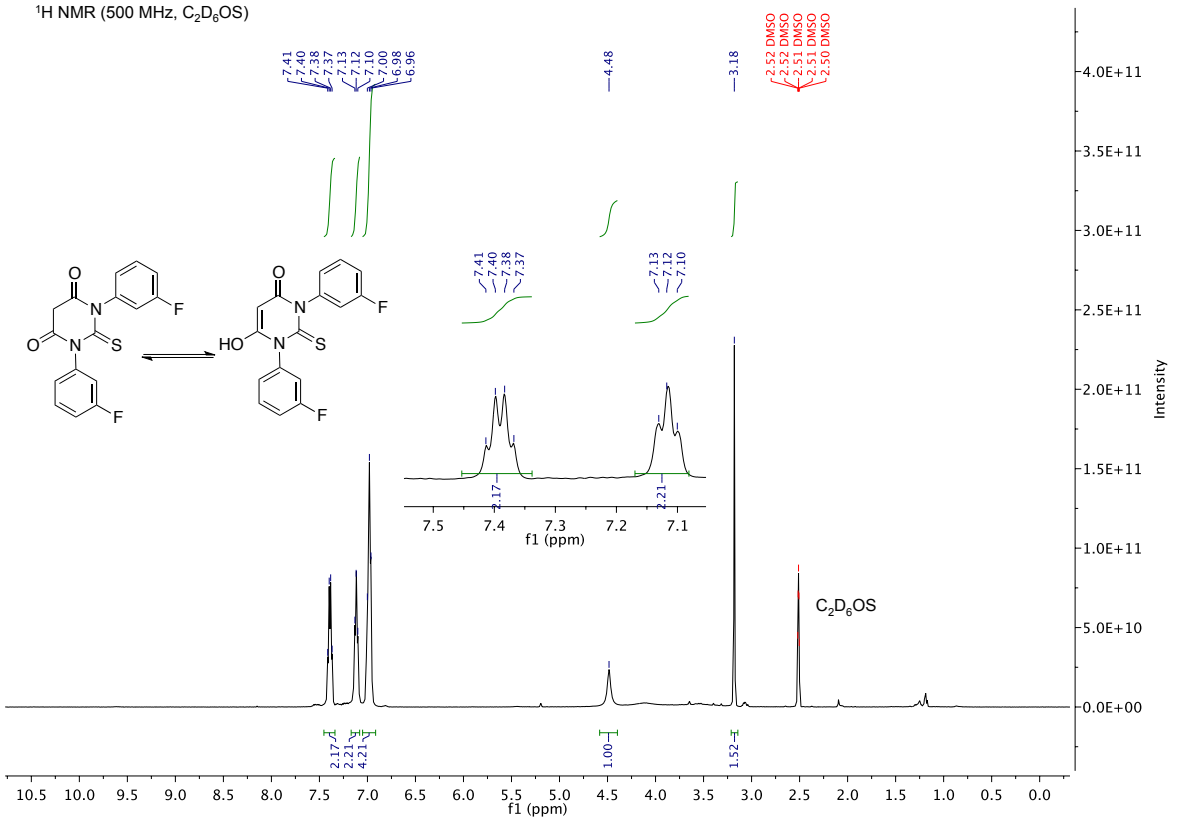

19F NMR (376 MHz, C3D6O)
-113.90
-113.90
HN
HN
S
F
F
f1 (ppm)
Intensity

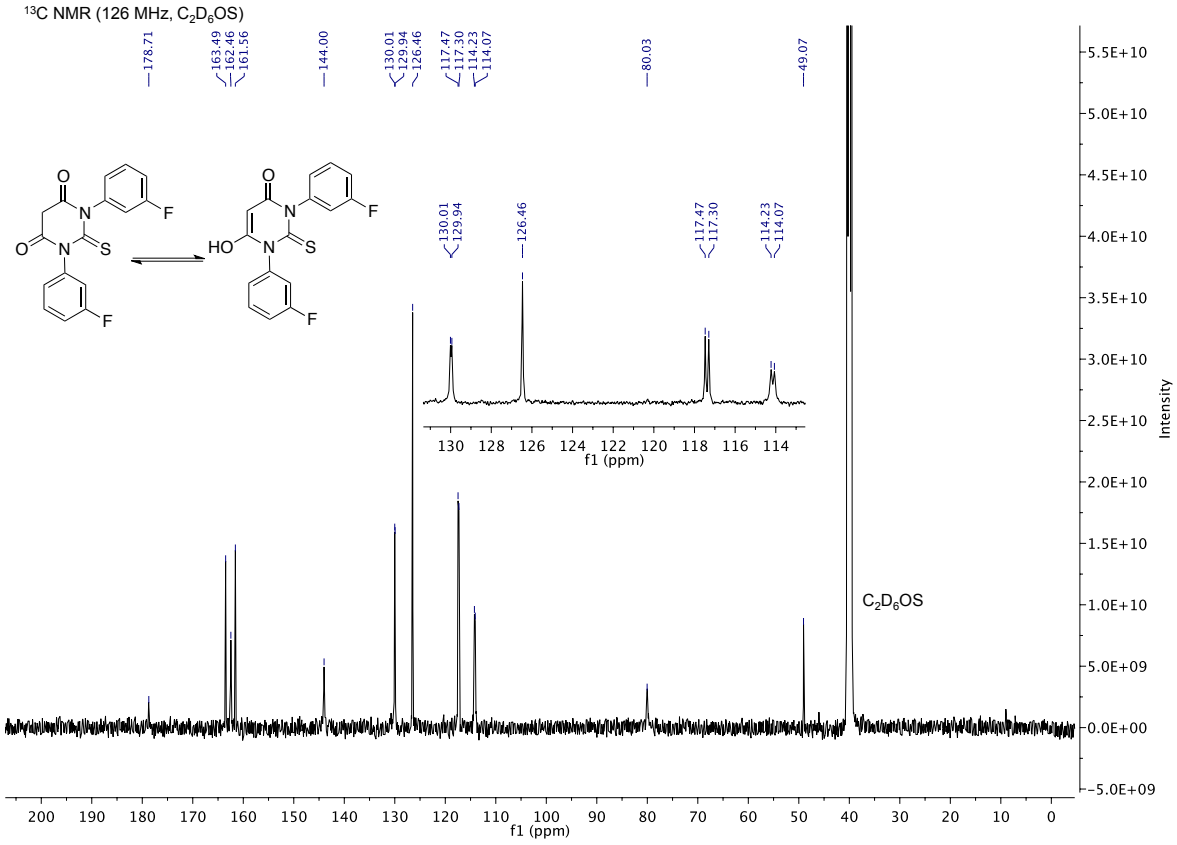
Compound **3b**

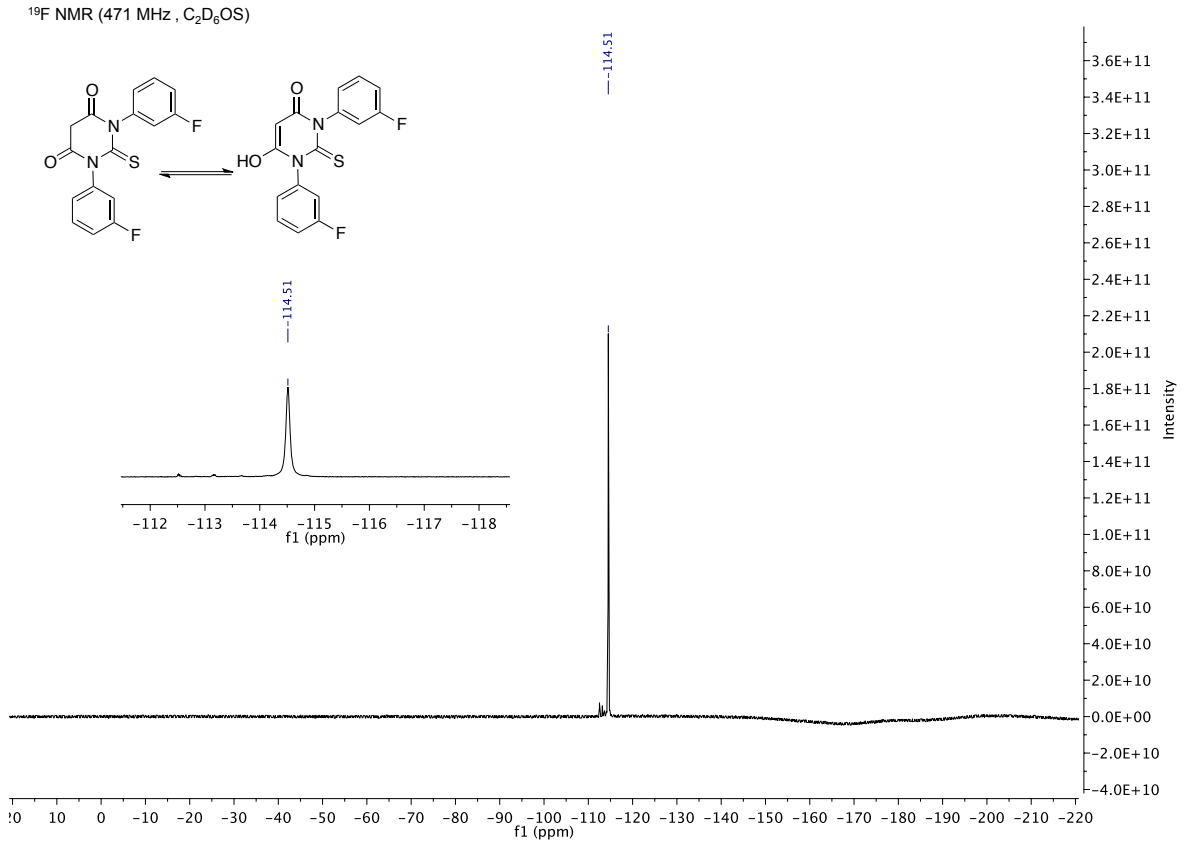

1H NMR (500 MHz, C3D6O)
9.30
7.88
7.88
7.87
7.87
7.53
7.52
7.51
7.51
7.51
7.38
7.37
7.37
7.36
7.36
7.35
7.34
7.32
7.30
2.87 HDO
2.07 Acetone
2.07 Acetone
2.07 Acetone
2.06 Acetone
2.06 Acetone
2.06 Acetone
HN
Br
HN
S
Br
7.94 7.92 7.90 7.88 7.86 7.84 7.82
f1 (ppm)
2.01
C3D6O
HDO
Intensity
2.00
2.01
2.07
4.12
f1 (ppm)


13C NMR (126 MHz C3D6O)
180.53
140.80
130.31
127.92
127.01
123.04
121.42
HN
Br
HN
S
Br
C3D6O
C3D6O
Intensity
f1 (ppm)

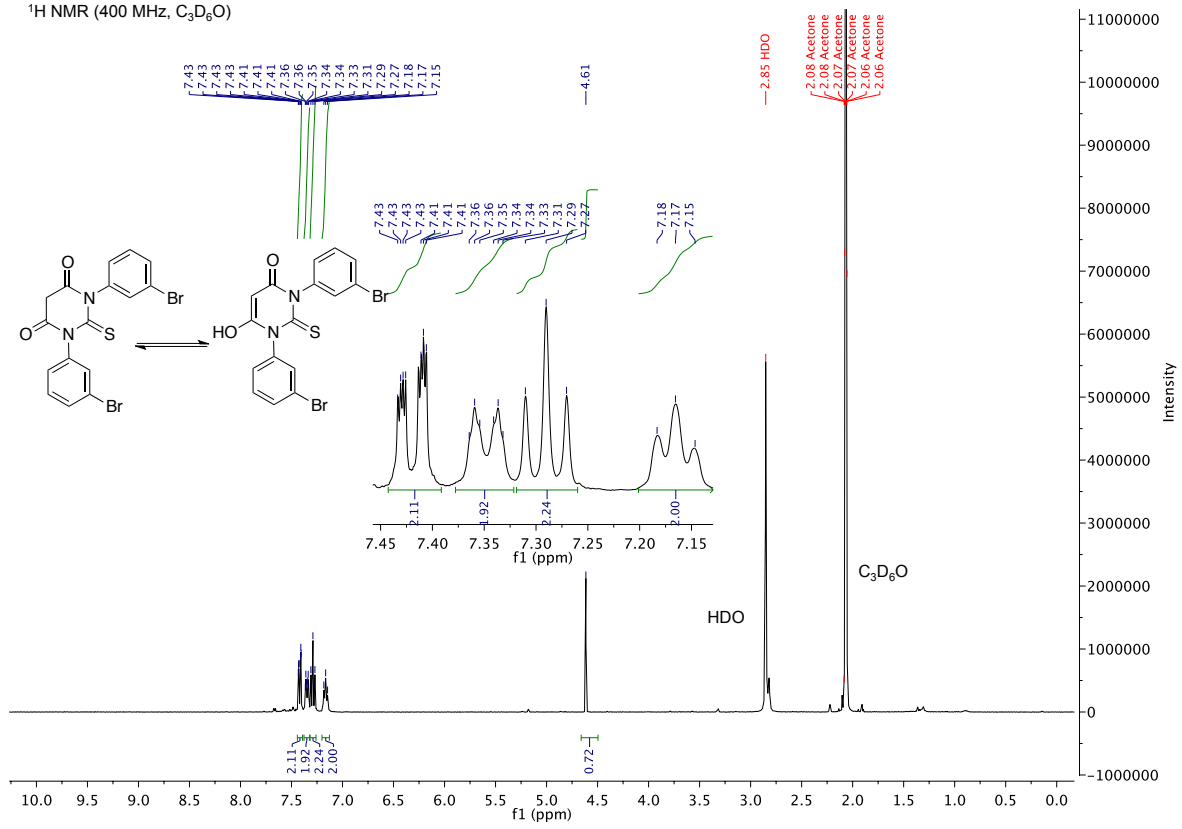
Compound **4a**

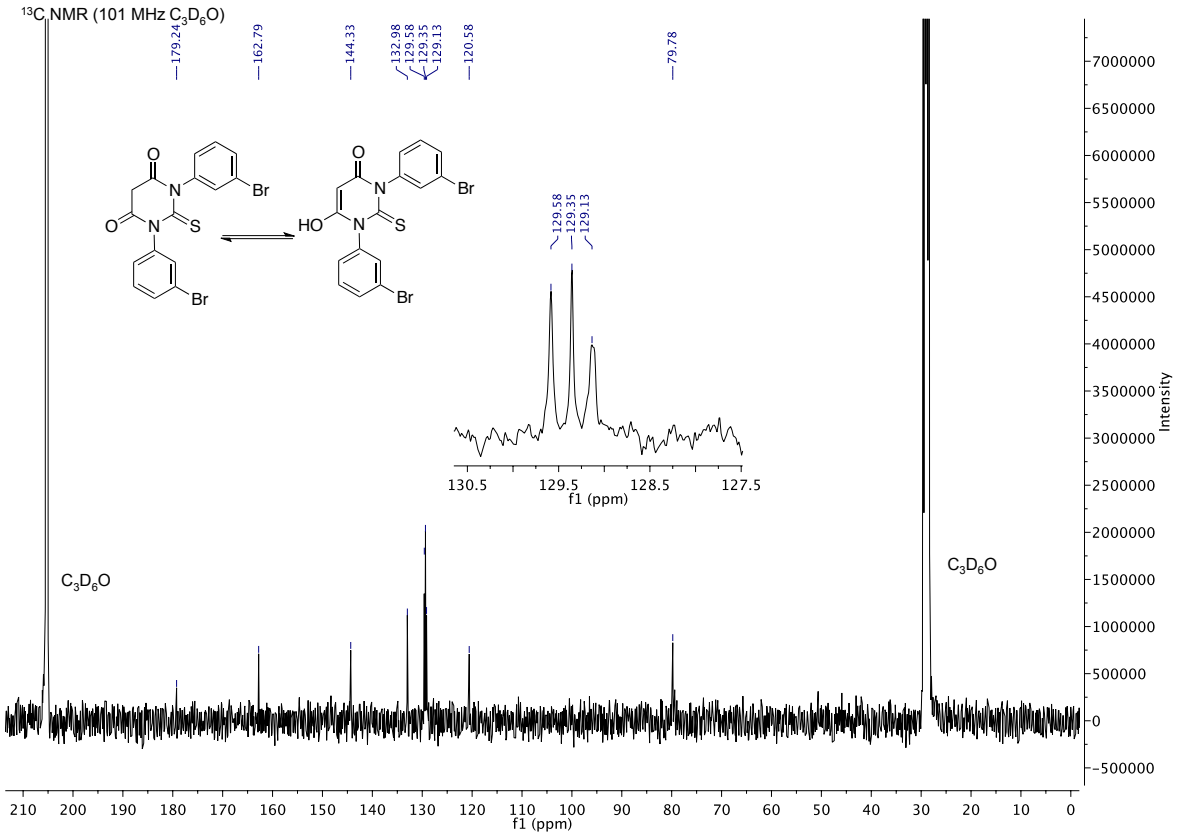

^{1}H NMR (500 MHz, C_2D_6OS)
7.41
7.40
7.38
7.37
7.13
7.12
7.10
7.00
6.98
6.96
4.48
3.18
2.52 DMSO
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
HO
F
S
2.17
2.21
4.21
1.00
1.52
C_2D_6OS
Intensity
f1 (ppm)


^{13}C NMR (126 MHz, C_2D_6OS)
178.71
163.49
162.46
161.56
144.00
130.01
129.94
126.46
117.47
117.30
114.23
114.07
80.03
49.07
HO
F
S
C_2D_6OS
Intensity
f1 (ppm)

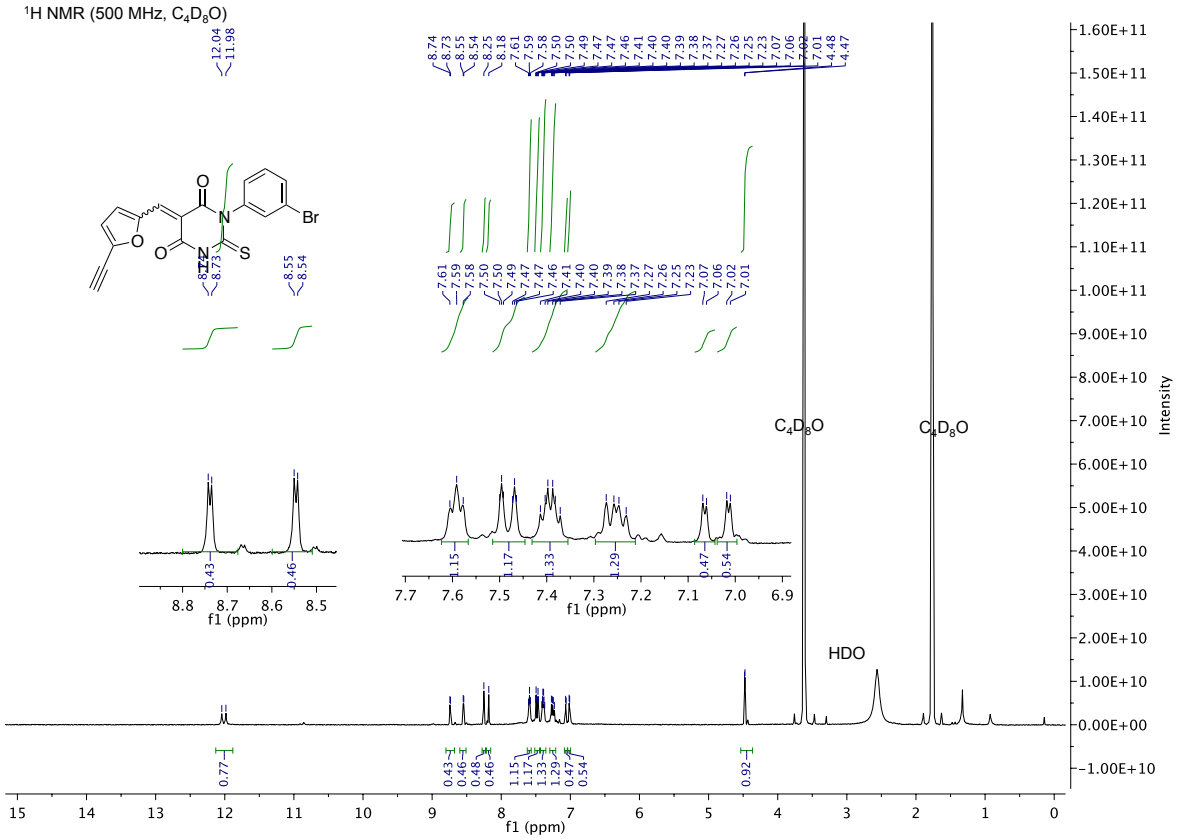

19F NMR (471 MHz , C2D6OS)
-114.51
f1 (ppm)
Intensity

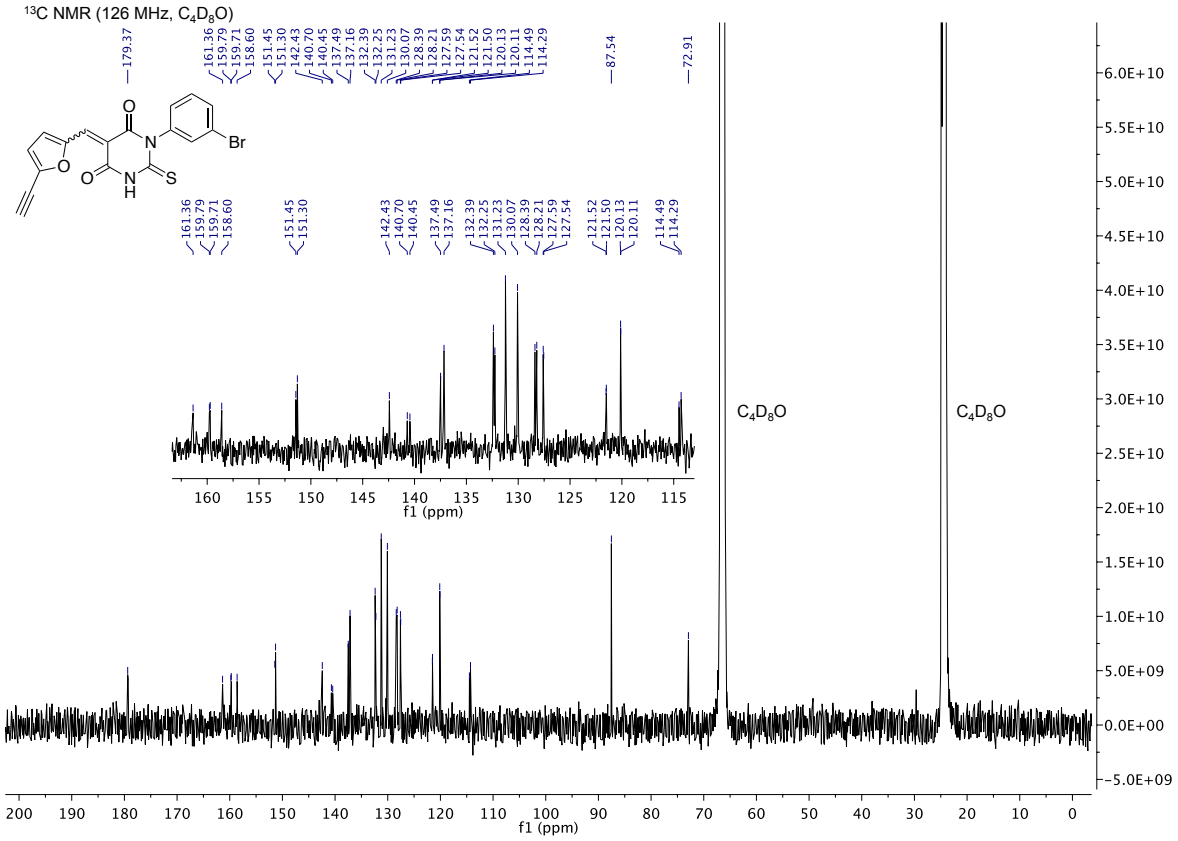
Compound **4b**


1H NMR (400 MHz, C3D6O)
2.85 HDO
2.08 Acetone
2.07 Acetone
2.06 Acetone
4.61
HDO
C3D6O
f1 (ppm)
Intensity

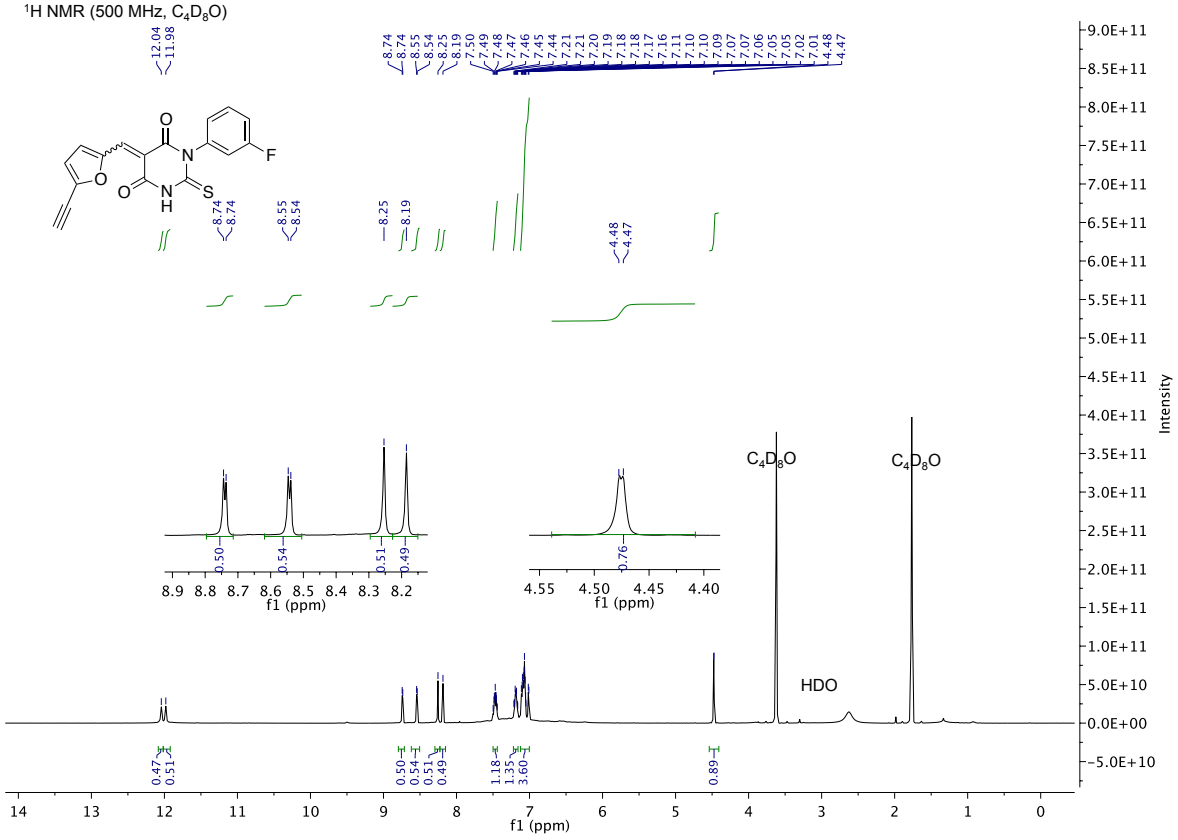

13C NMR (101 MHz C3D6O)
179.24
162.79
144.33
132.98
129.58
129.35
129.13
120.58
79.78
C3D6O
C3D6O
f1 (ppm)
Intensity

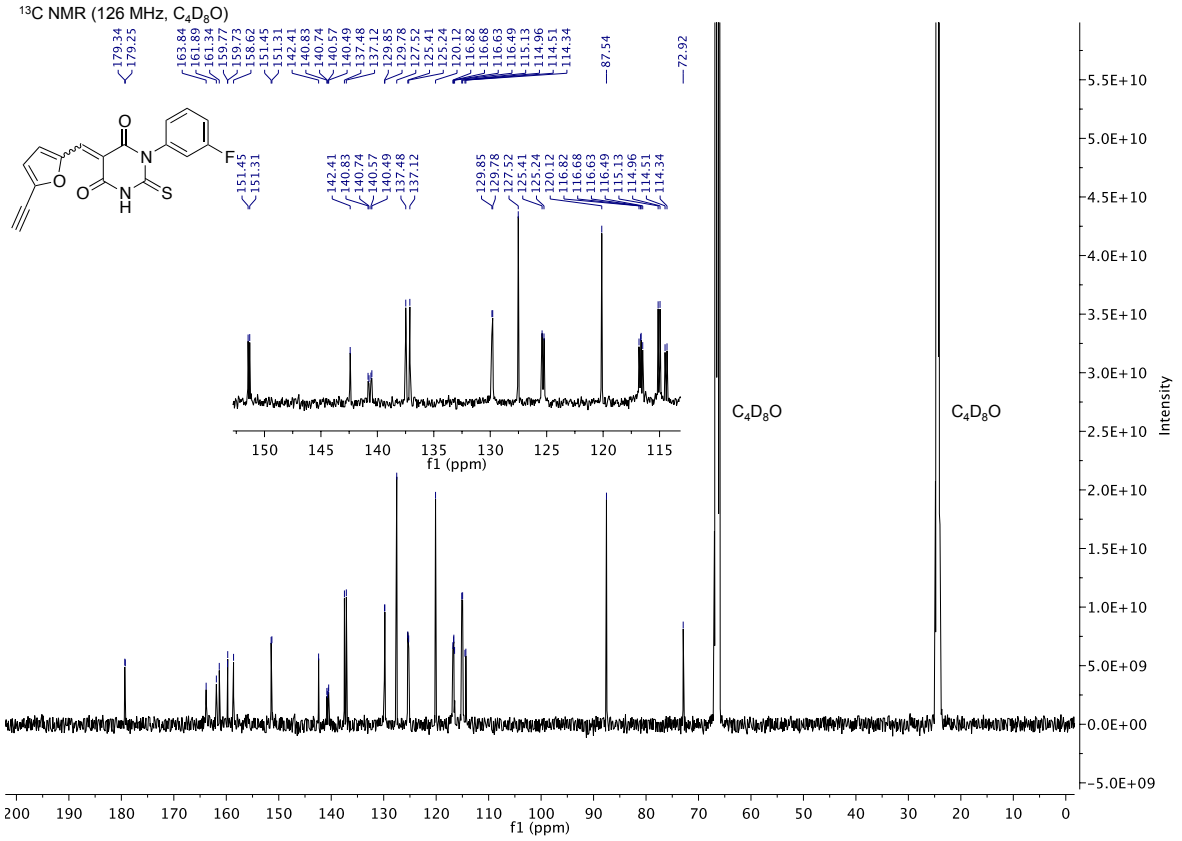
Compound **5a**

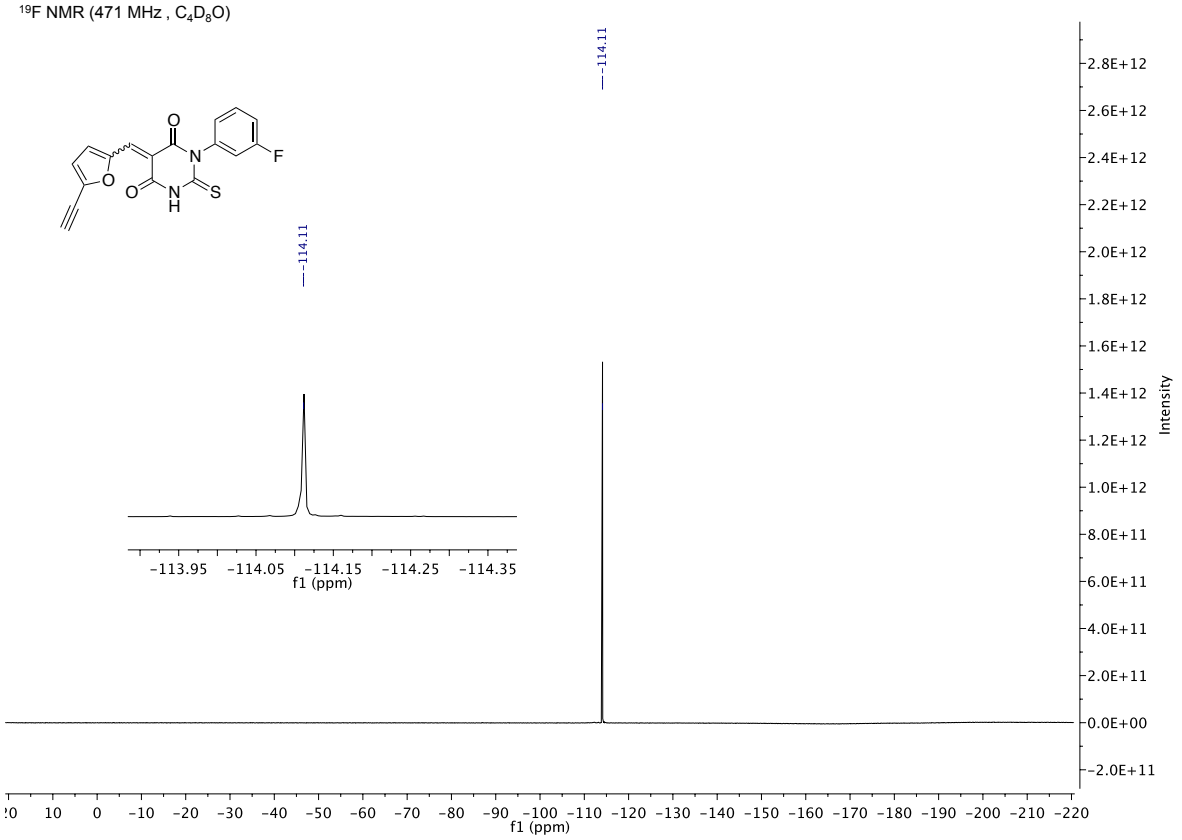

1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
f1 (ppm)
Intensity


13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
f1 (ppm)

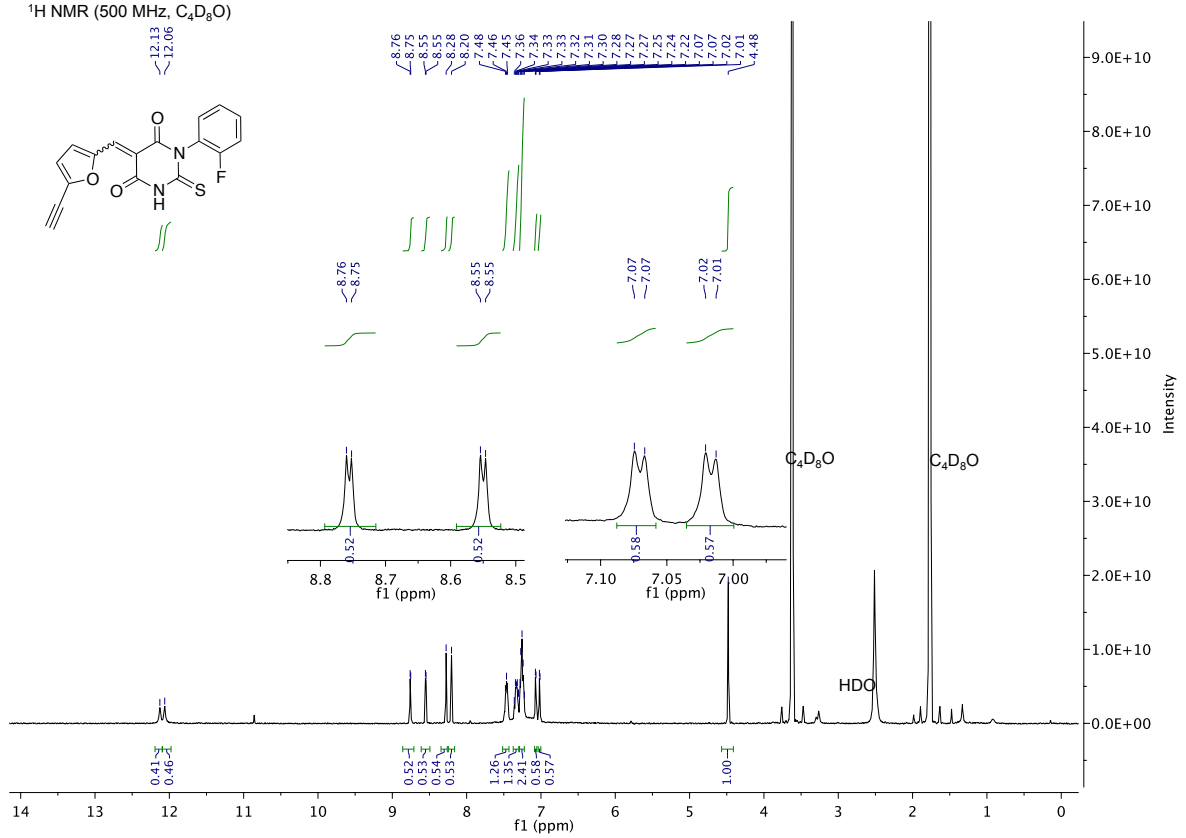
Compound **5b**

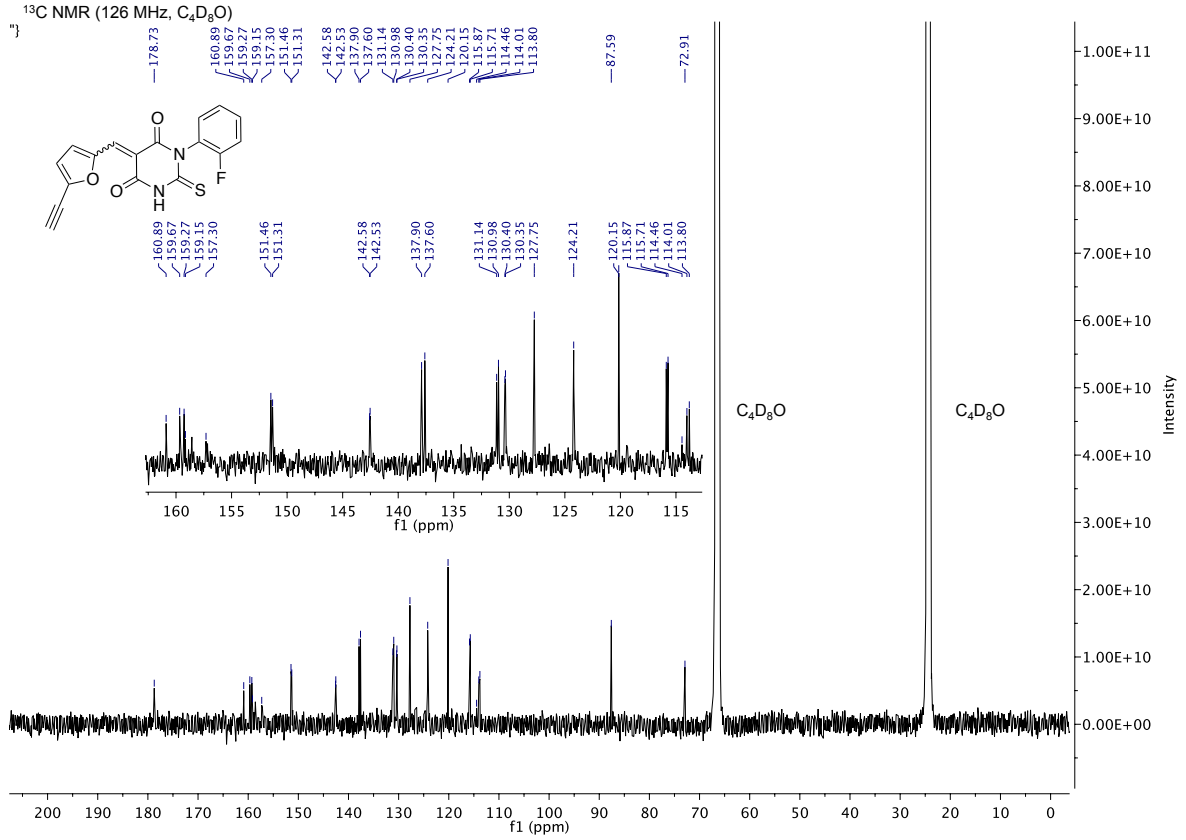

1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
f1 (ppm)
Intensity

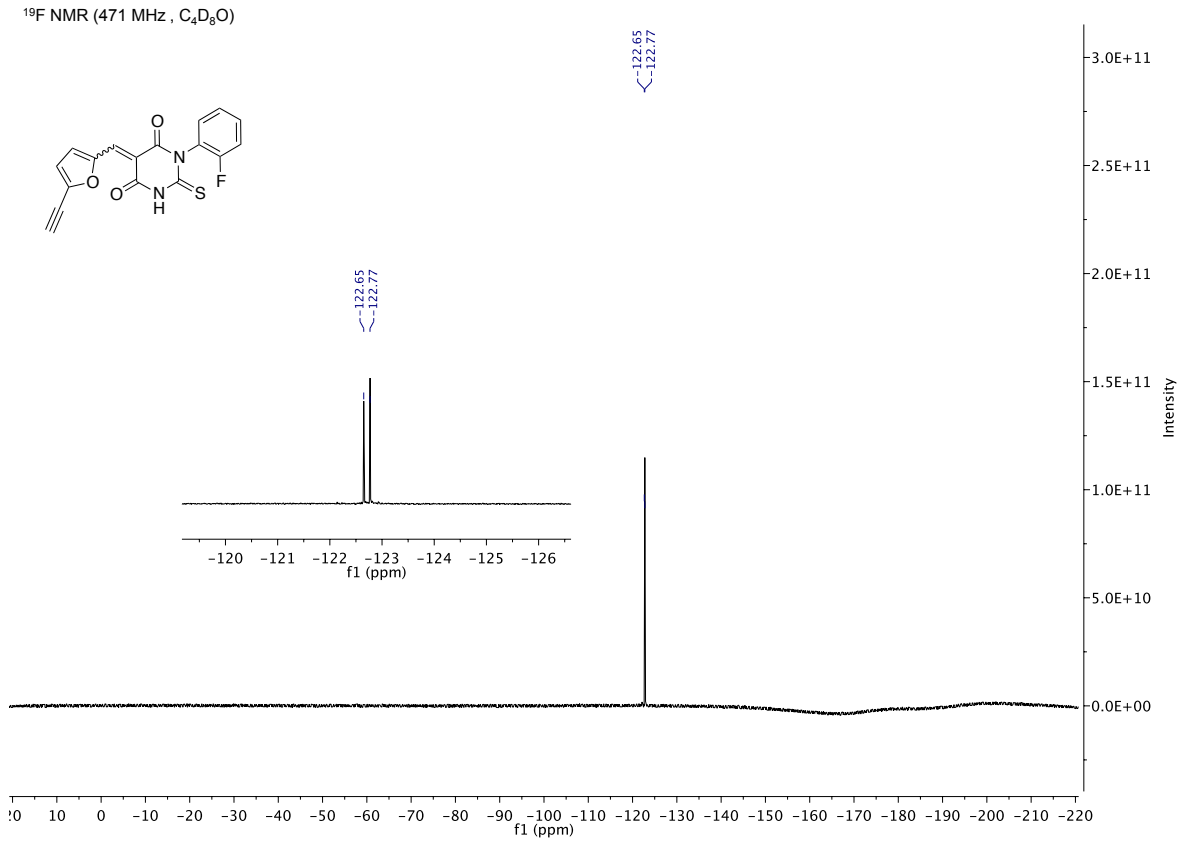

13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
f1 (ppm)
Intensity


19F NMR (471 MHz , C4D8O)
-114.11
f1 (ppm)
Intensity

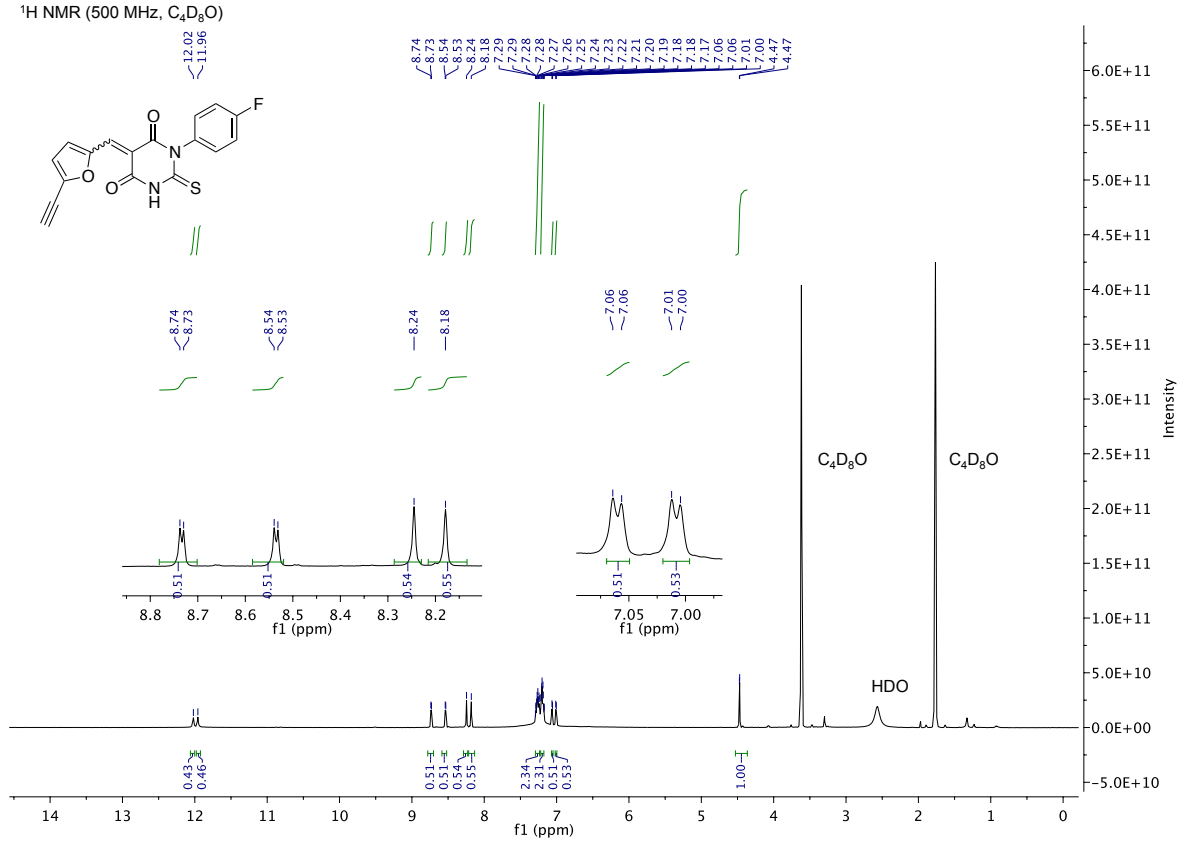
Compound **5c**

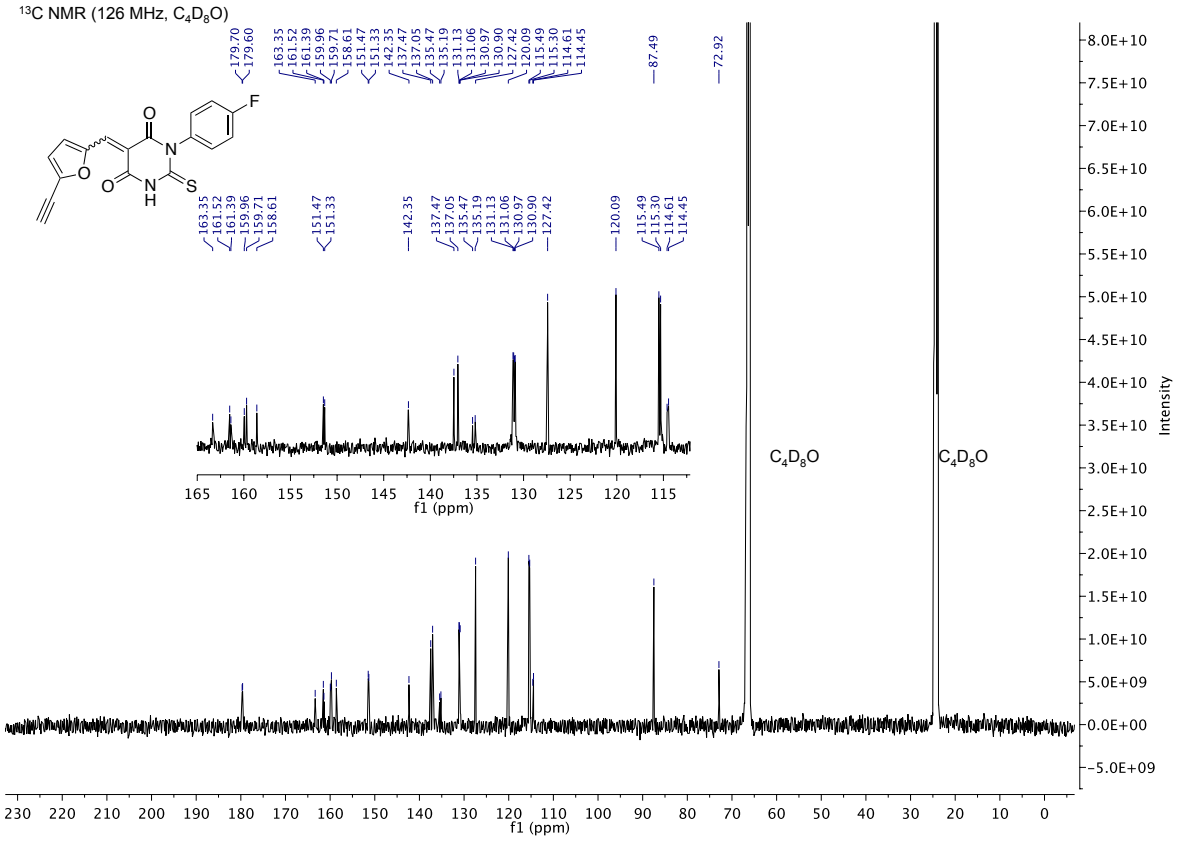

^{1}H NMR (500 MHz, C_4D_8O)
C_4D_8O
C_4D_8O
HDO
f1 (ppm)
Intensity

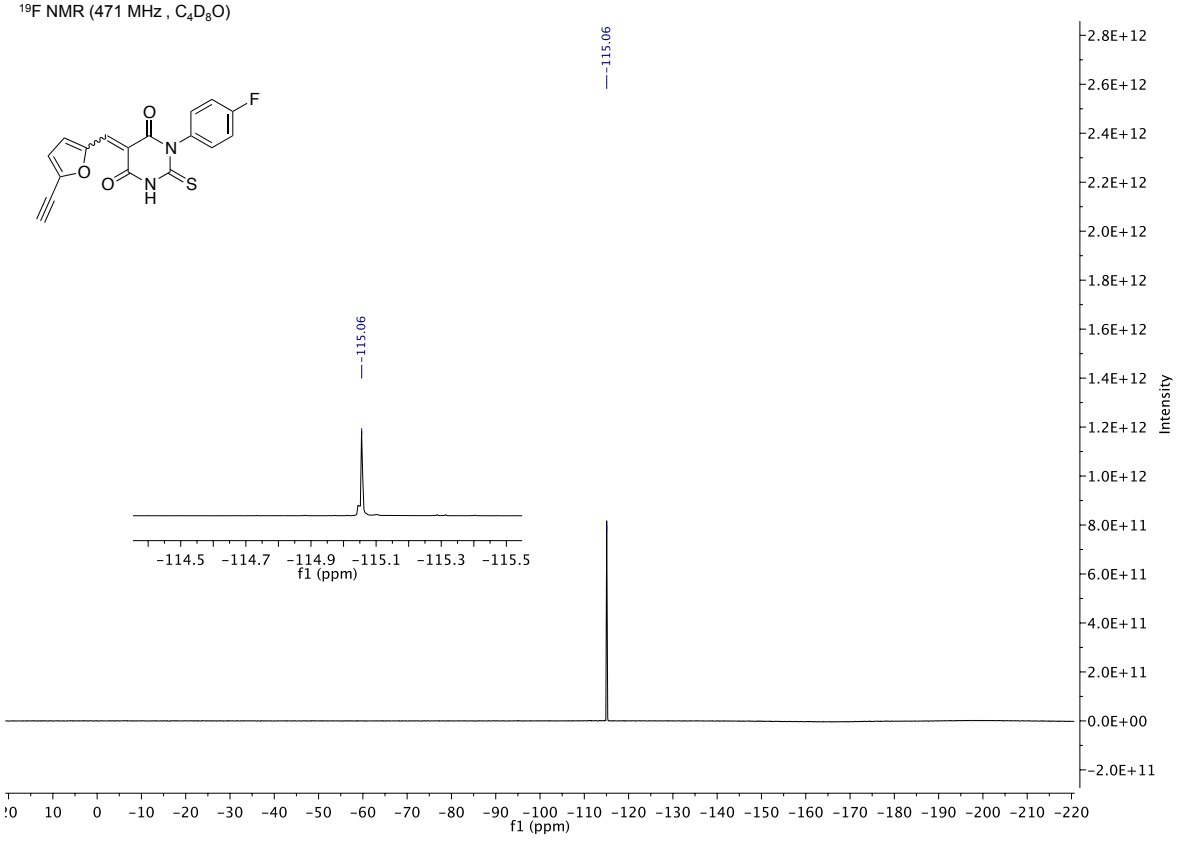

^{13}C NMR (126 MHz, C_4D_8O)
C_4D_8O
C_4D_8O
f1 (ppm)
Intensity


19F NMR (471 MHz , C4D8O)
-122.65
-122.77
f1 (ppm)
Intensity

Compound **5d**

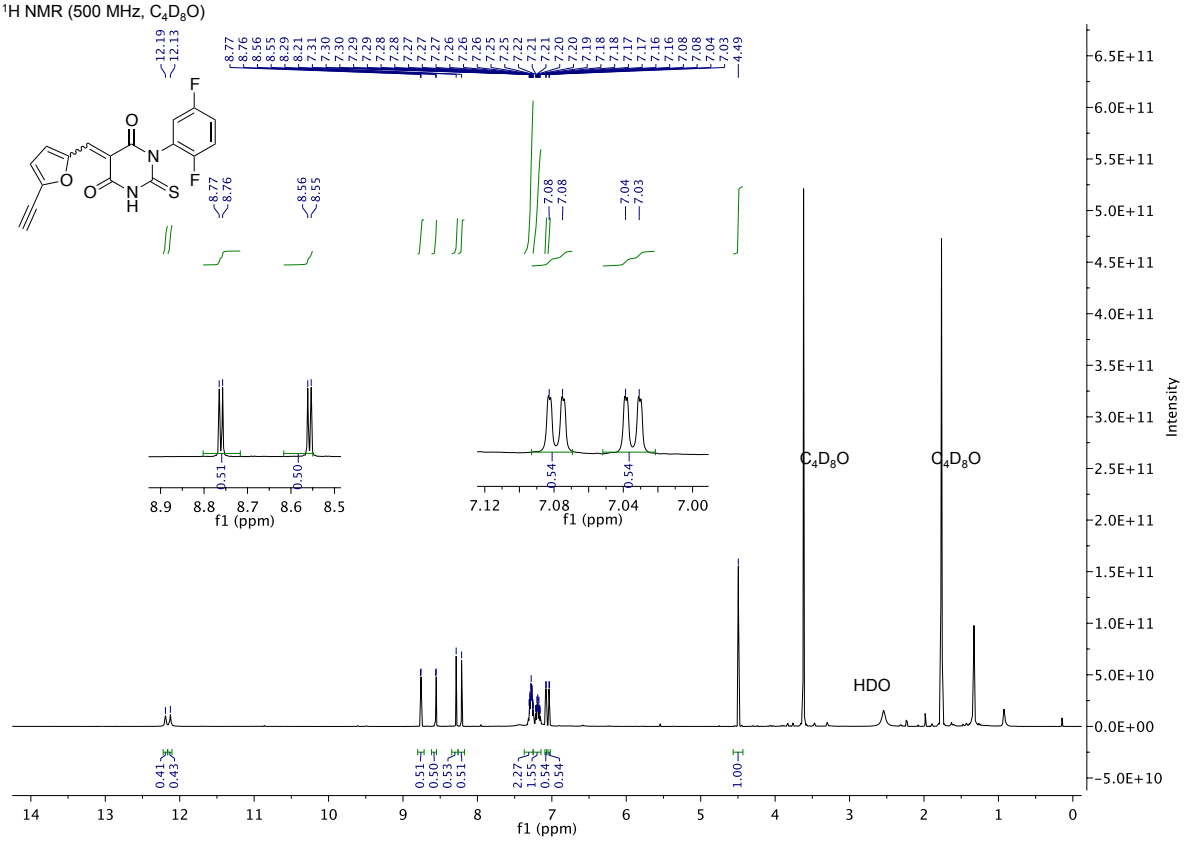

1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
f1 (ppm)
Intensity


13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
f1 (ppm)
Intensity

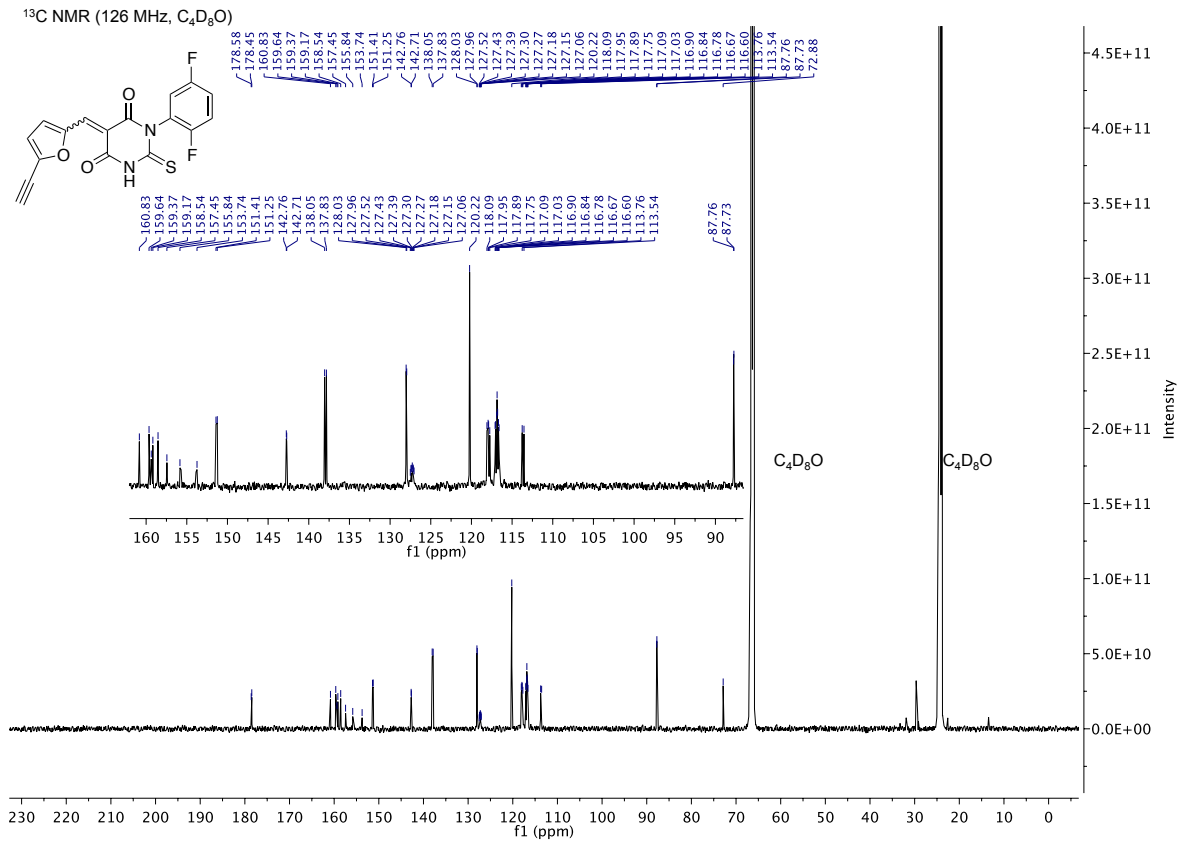

19F NMR (471 MHz , C4D8O)
-115.06
f1 (ppm)
Intensity

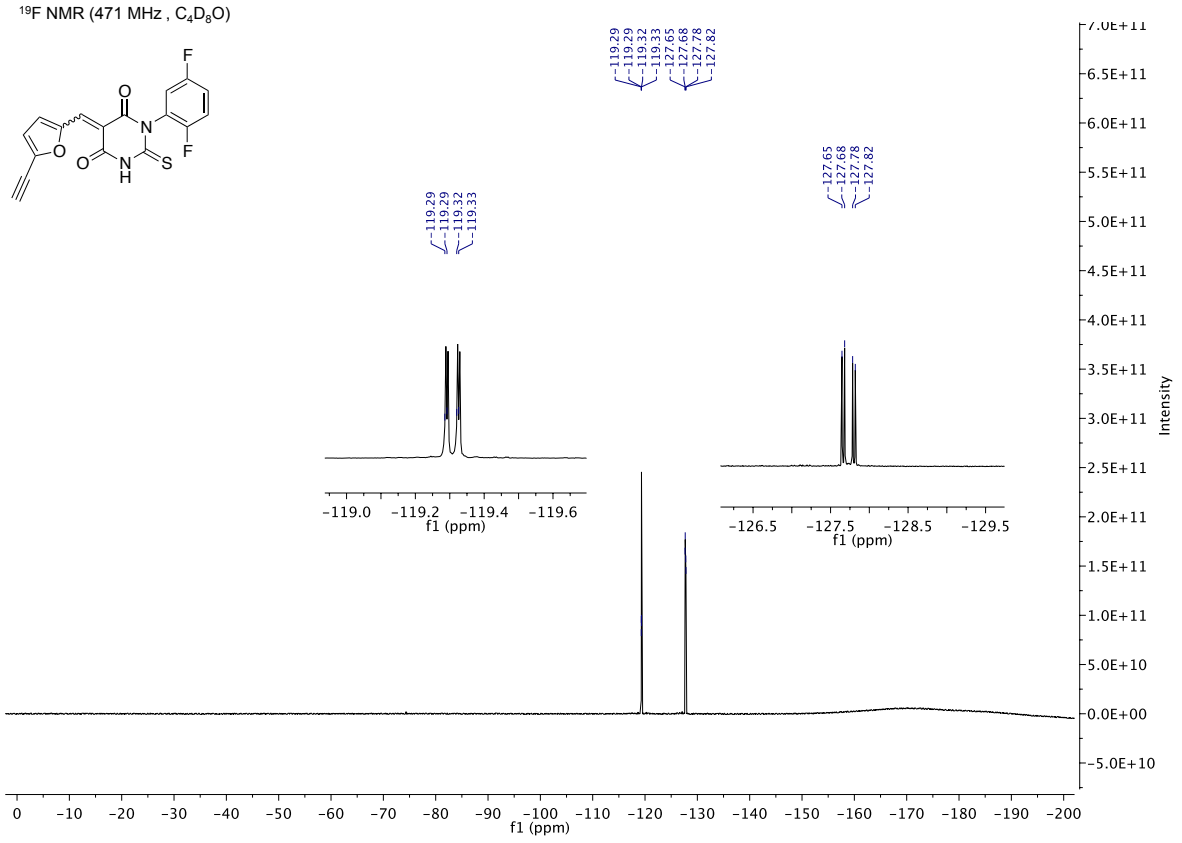
Compound **5e**

^{1}H NMR (500 MHz, C_4D_8O)

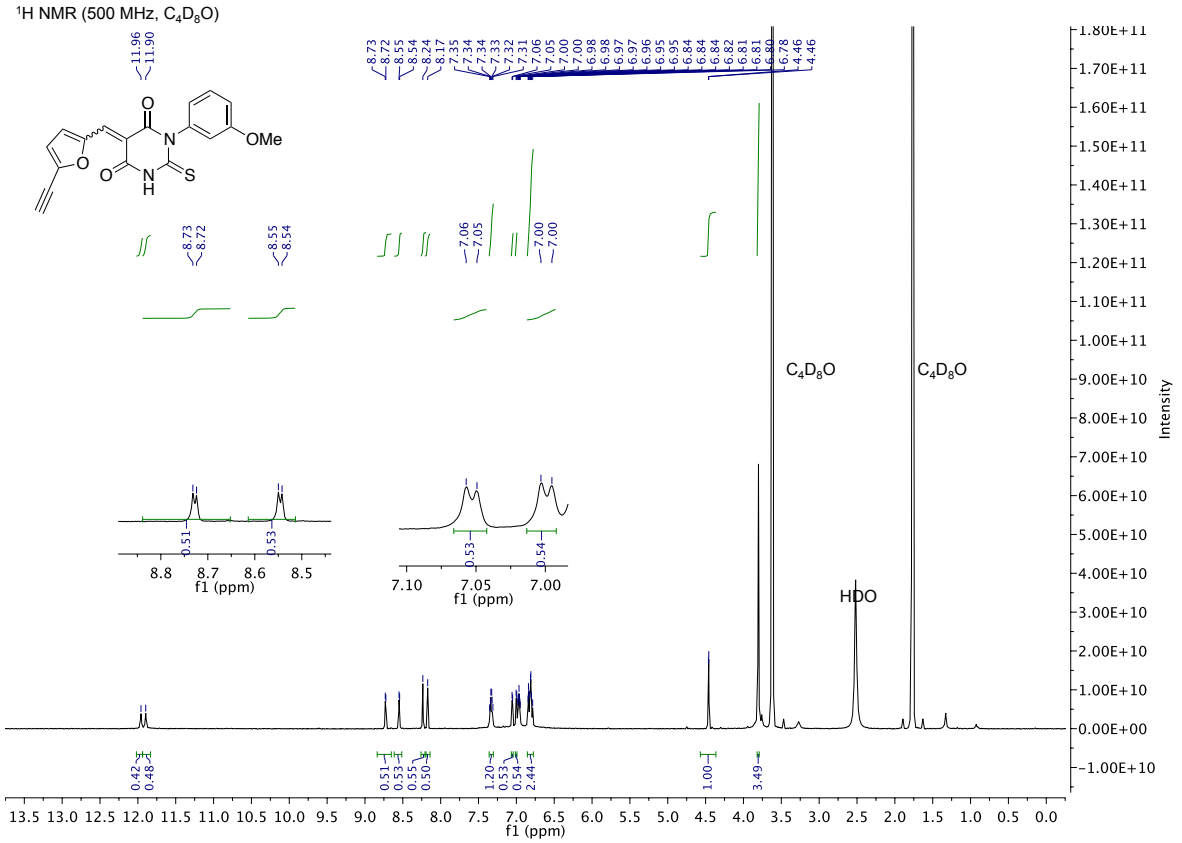


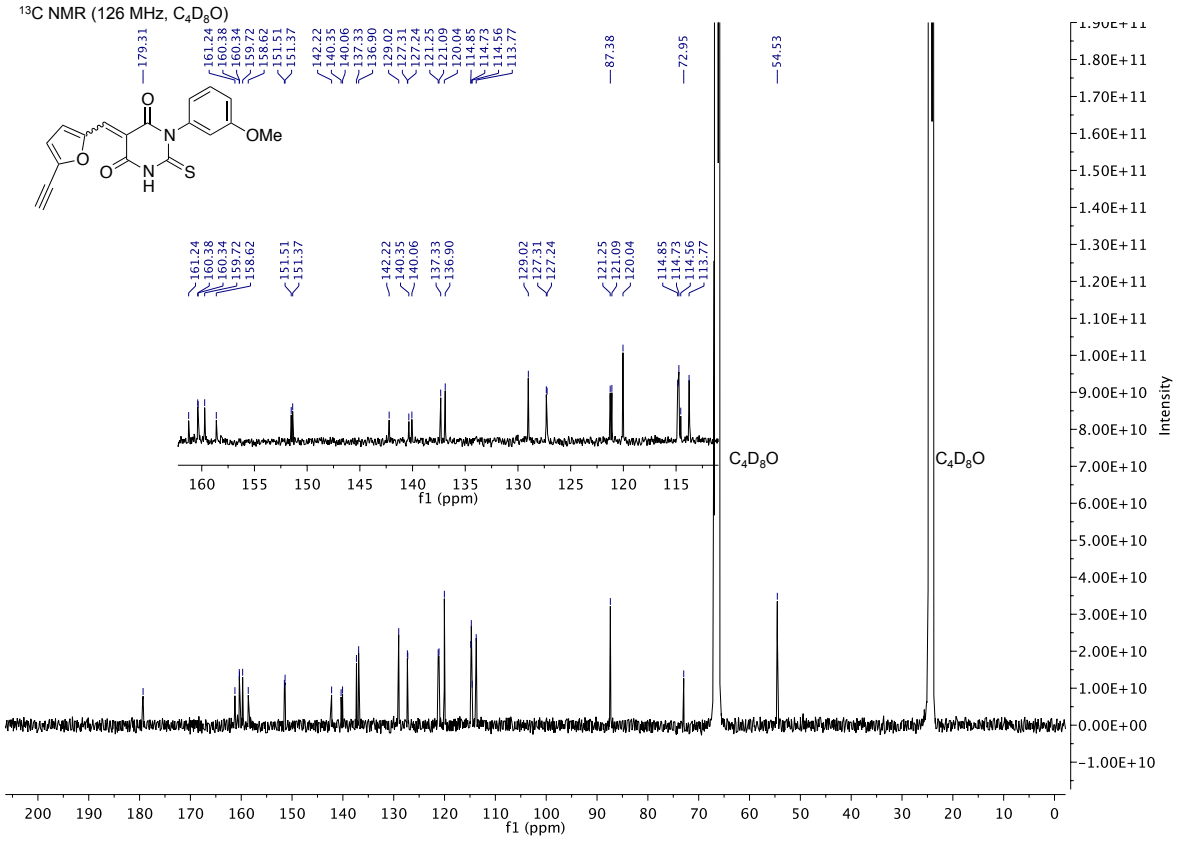
^{13}C NMR (126 MHz, C_4D_8O)



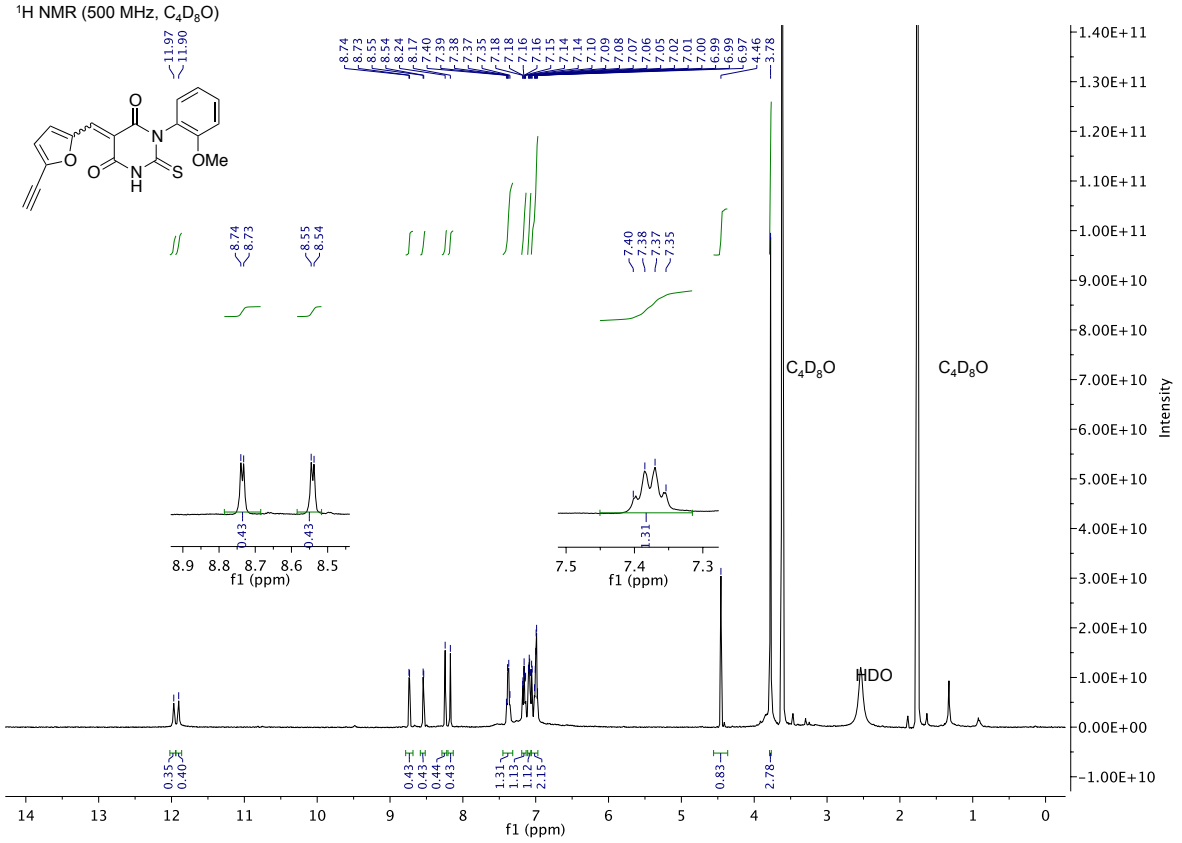

19F NMR (471 MHz , C4D8O)
F
O
N
F
O
O
N
H
S
119.29
119.29
119.32
119.33
127.65
127.68
127.78
127.82
−119.0 −119.2 −119.4 −119.6
f1 (ppm)
−126.5 −127.5 −128.5 −129.5
f1 (ppm)
Intensity
f1 (ppm)

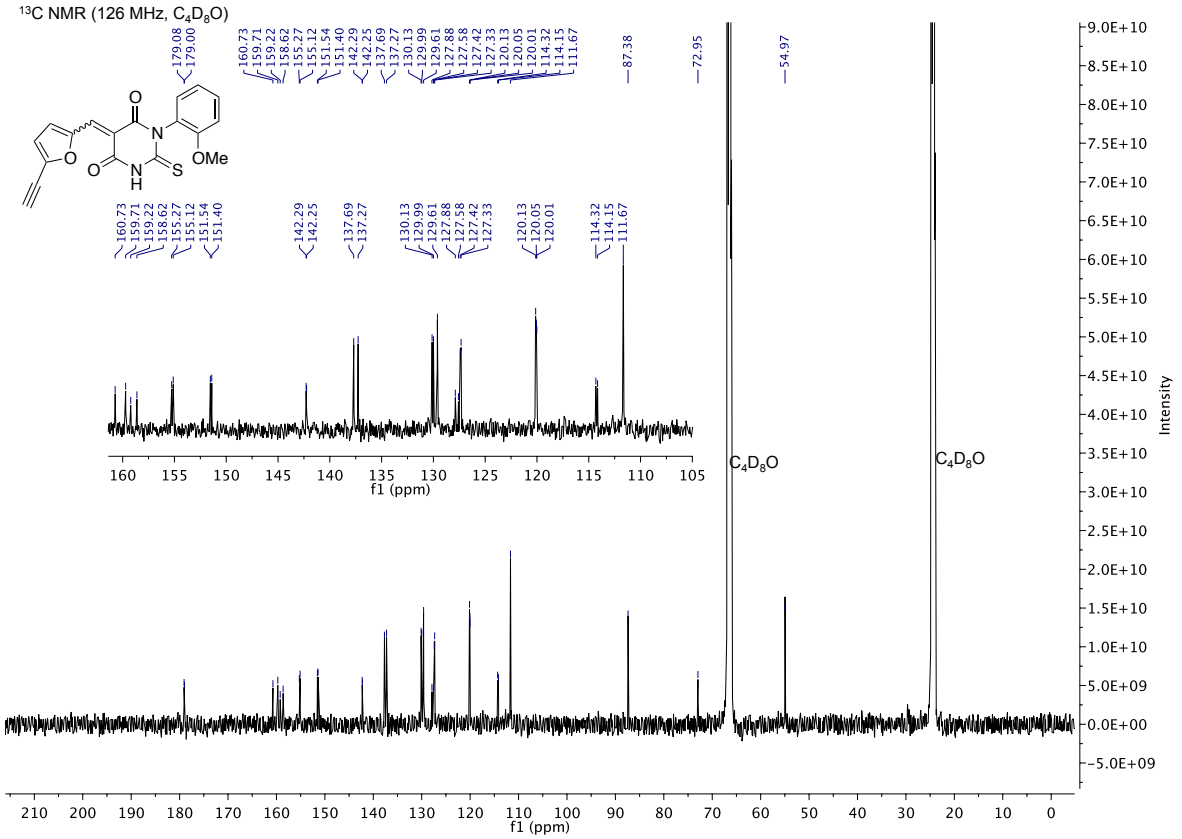
Compound **5f**


1H NMR (500 MHz, C4D8O)
11.96
11.90
OMe
8.73
8.72
8.55
8.54
8.24
8.17
7.35
7.34
7.34
7.33
7.32
7.31
7.06
7.05
7.00
7.00
6.98
6.98
6.97
6.97
6.96
6.95
6.95
6.84
6.84
6.84
6.82
6.81
6.81
6.80
6.78
4.46
4.46
C4D8O
C4D8O
HDO
0.51
0.53
8.8 8.7 8.6 8.5
f1 (ppm)
0.53
0.54
7.10 7.05 7.00
f1 (ppm)
0.42
0.48
0.51
0.53
0.55
0.50
1.20
0.53
0.54
2.44
1.00
3.49
f1 (ppm)
Intensity

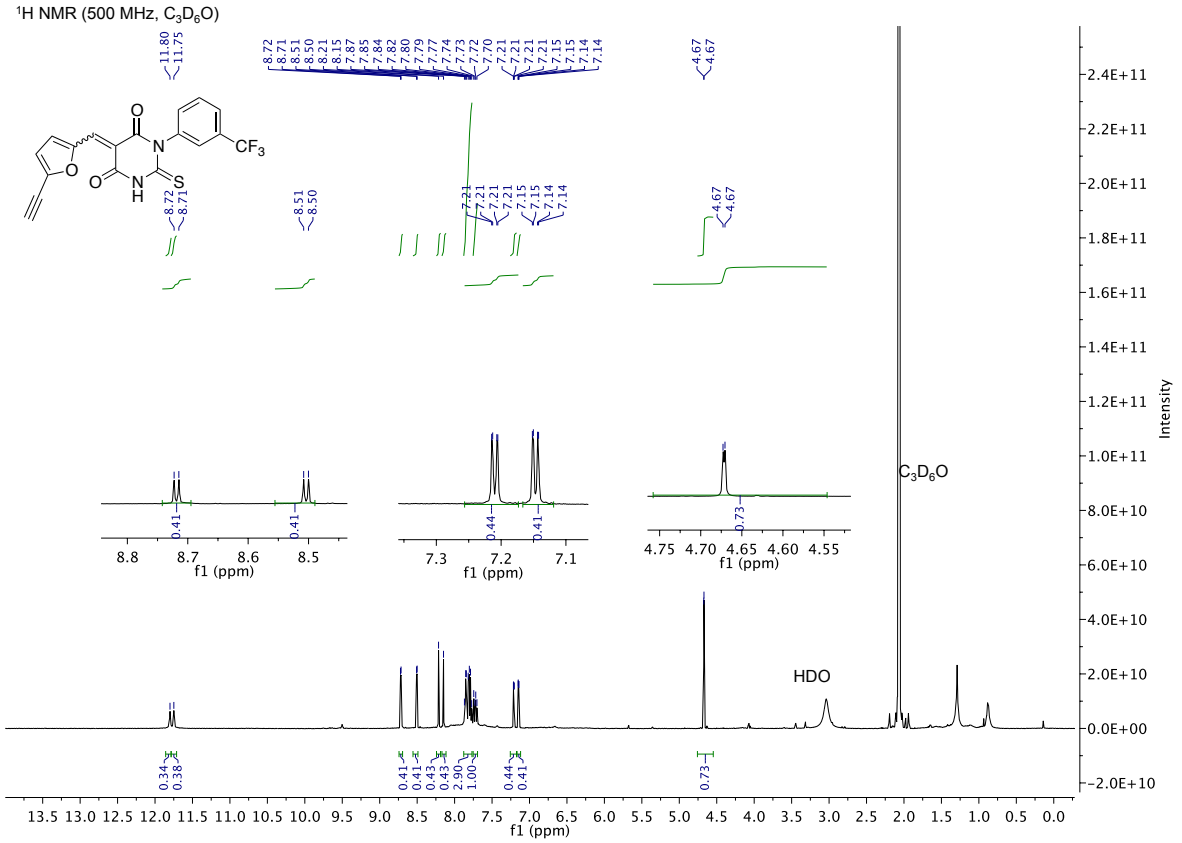

13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
f1 (ppm)
Intensity

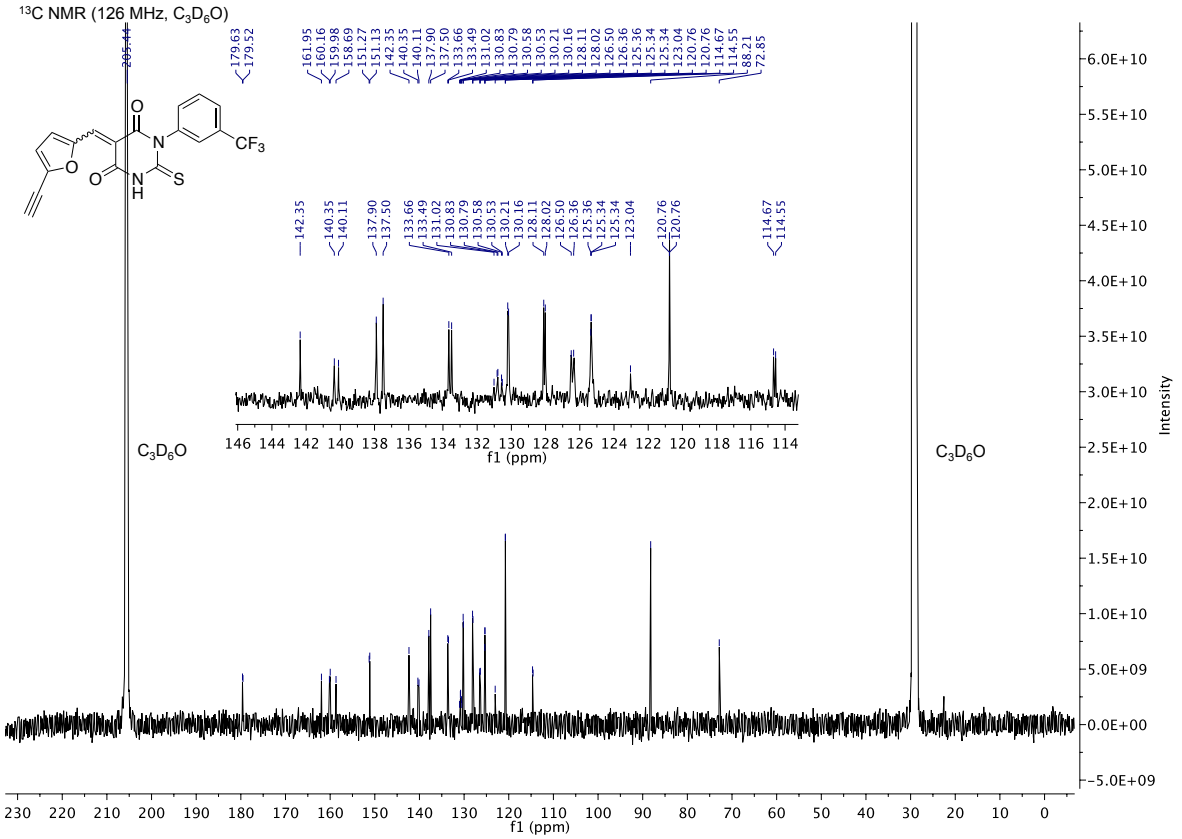
Compound **5g**

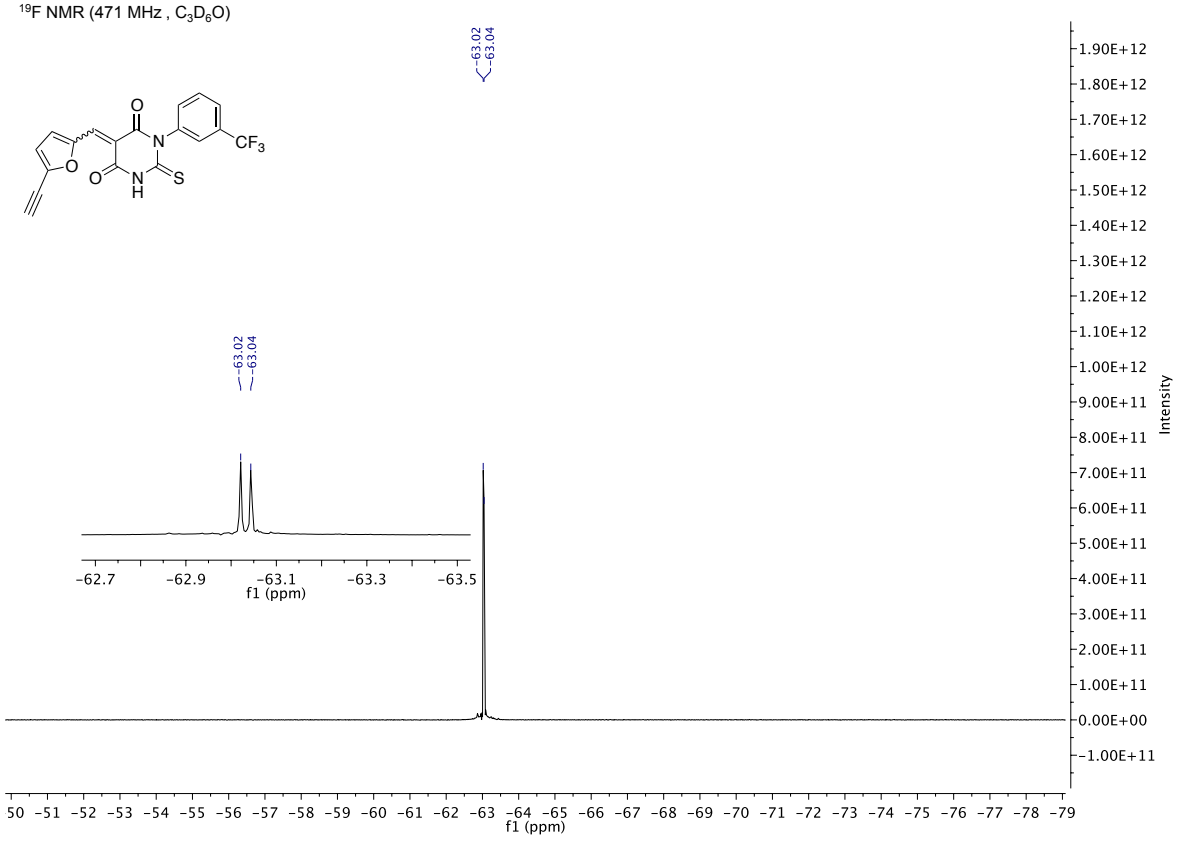

1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
f1 (ppm)
Intensity


13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
f1 (ppm)
Intensity

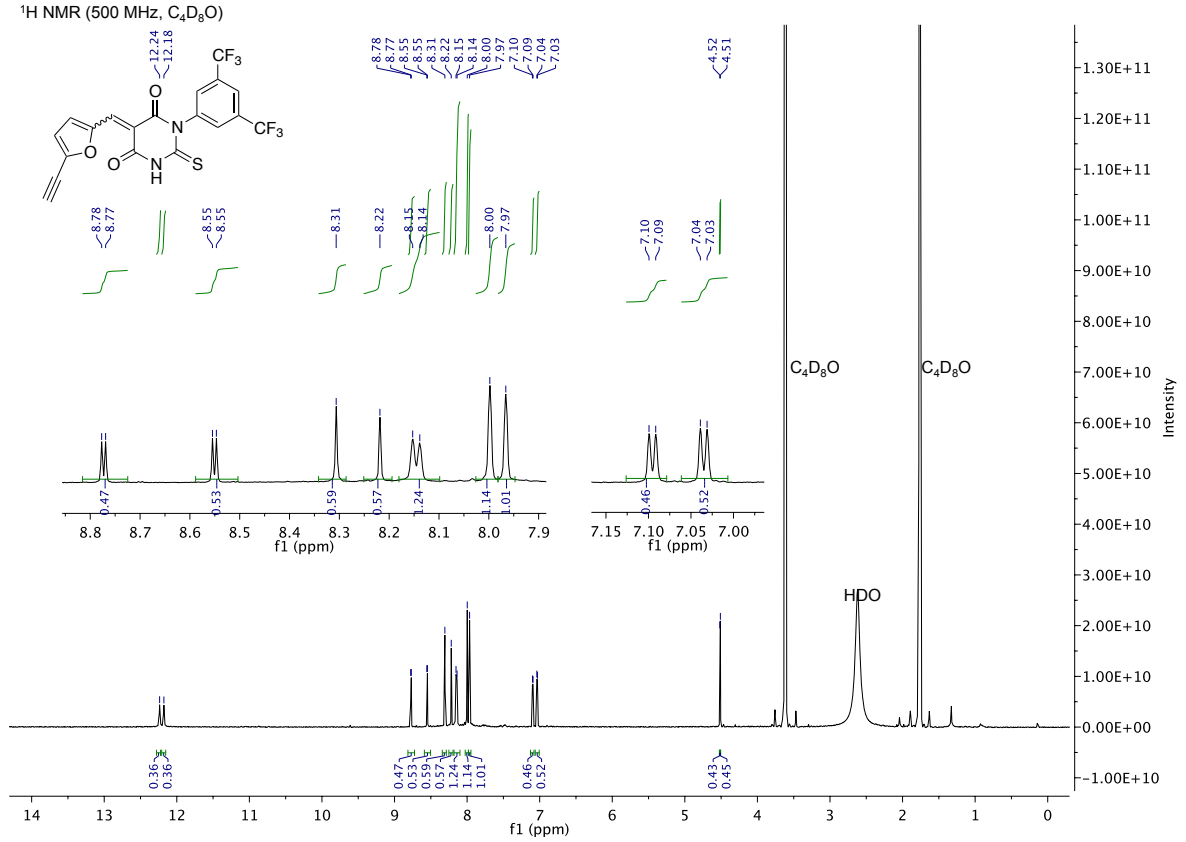
Compound **5h**

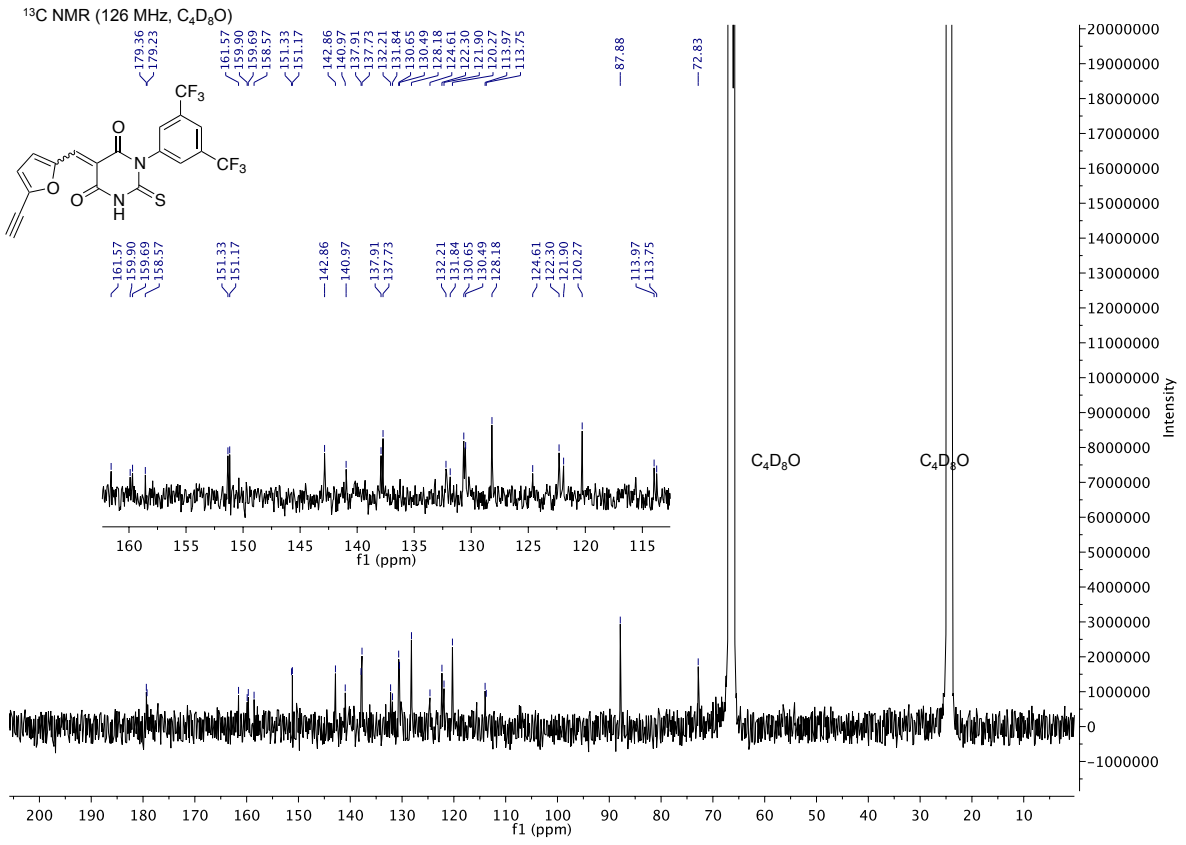

1H NMR (500 MHz, C3D6O)
C3D6O
HDO
f1 (ppm)
Intensity

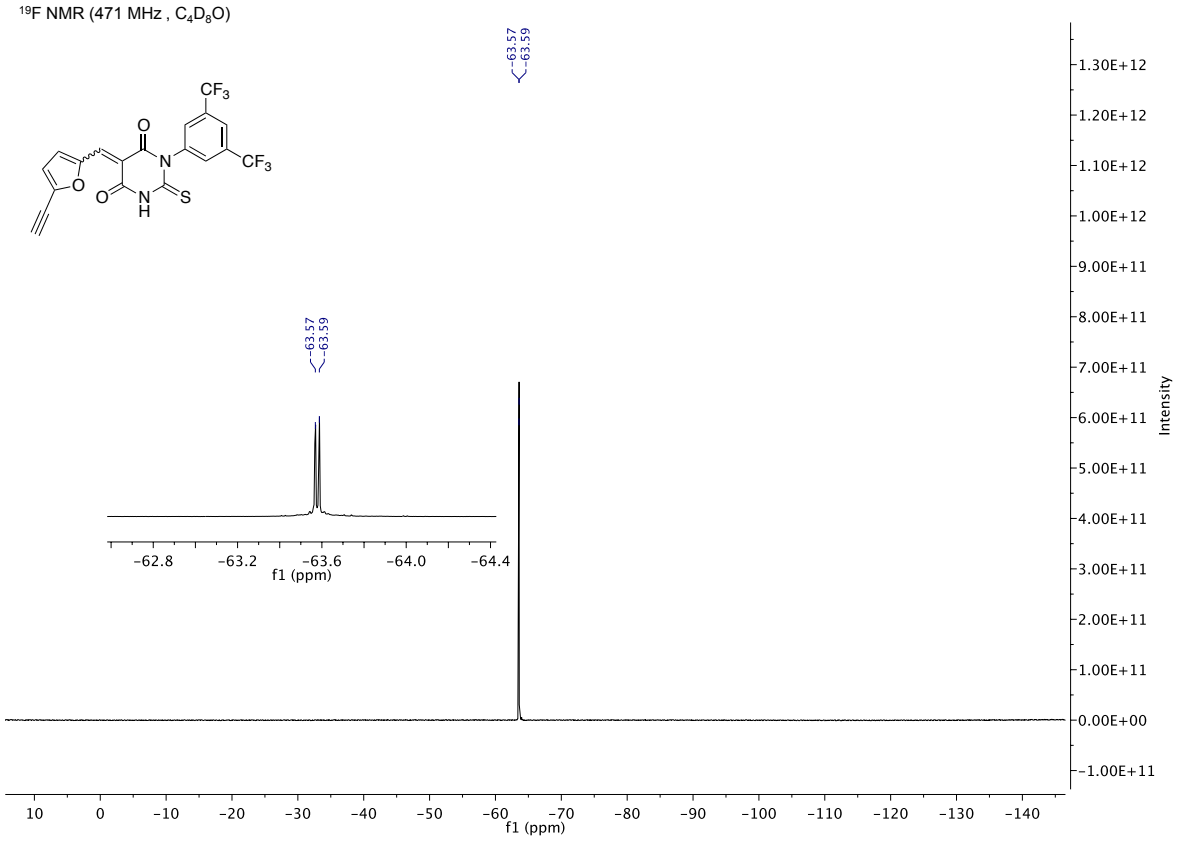

13C NMR (126 MHz, C3D6O)
C3D6O
C3D6O
f1 (ppm)
Intensity


19F NMR (471 MHz , C3D6O)
f1 (ppm)
Intensity

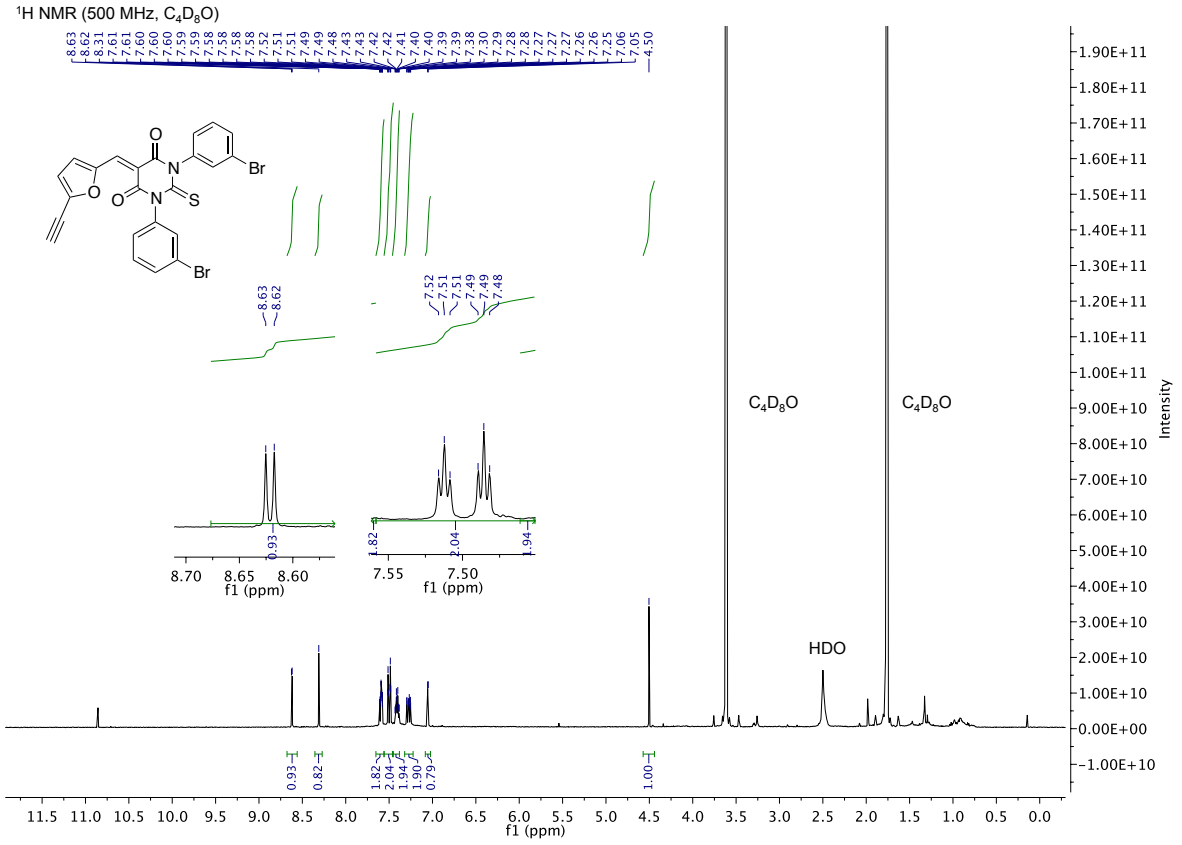
Compound **5i**

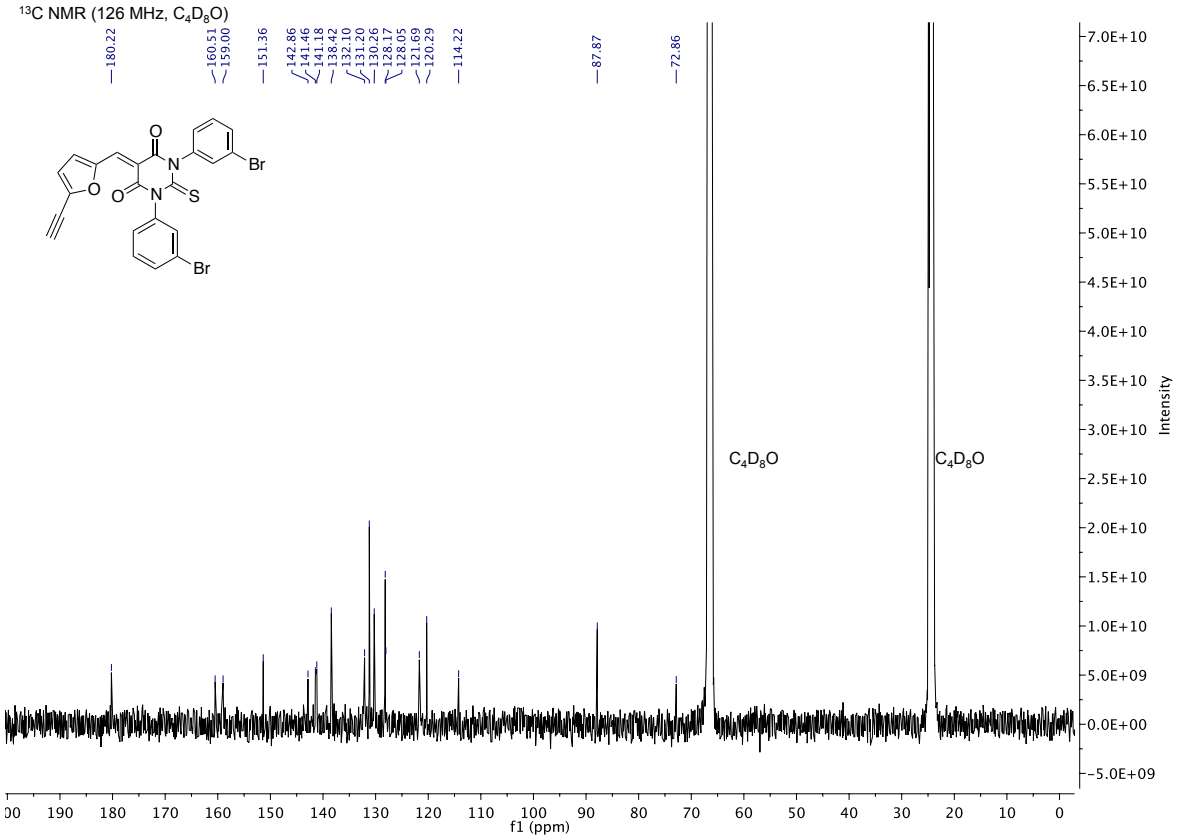

^{1}H NMR (500 MHz, C_4D_8O)
C_4D_8O
C_4D_8O
HDO
f1 (ppm)
Intensity


^{13}C NMR (126 MHz, C_4D_8O)
C_4D_8O
C_4D_8O
f1 (ppm)
Intensity

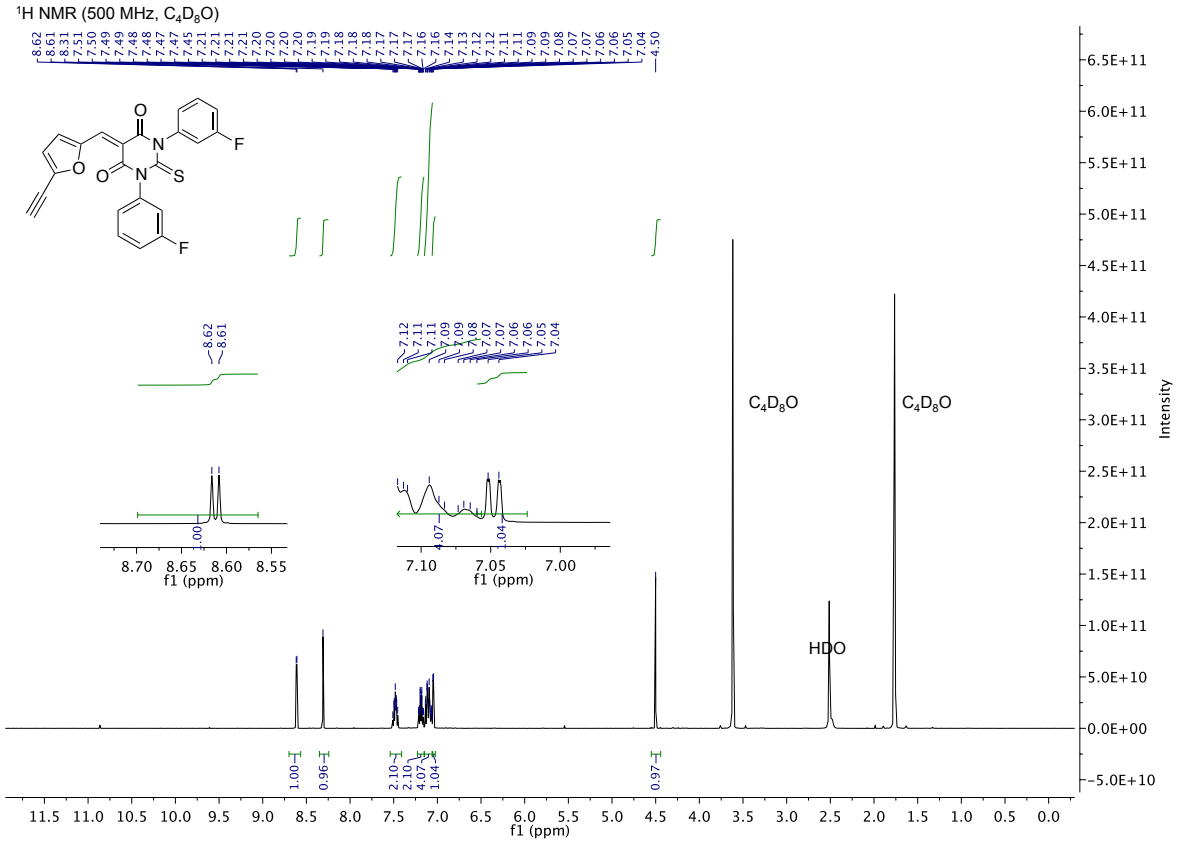

19F NMR (471 MHz , C4D8O)
CF3
CF3
O
N
O
N
H
S
O
-63.57
-63.59
-62.8
-63.2
-63.6
-64.0
-64.4
f1 (ppm)
Intensity
f1 (ppm)

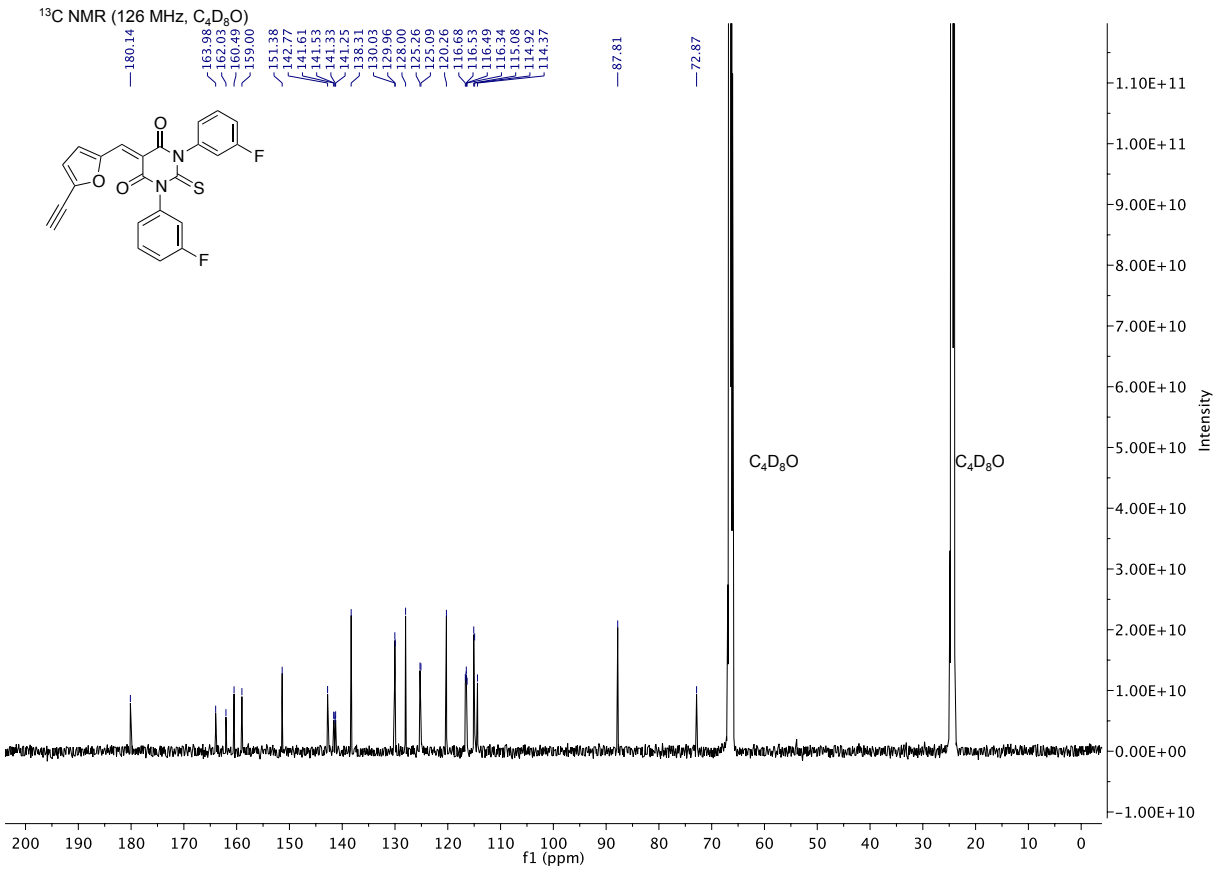
Compound **5l**

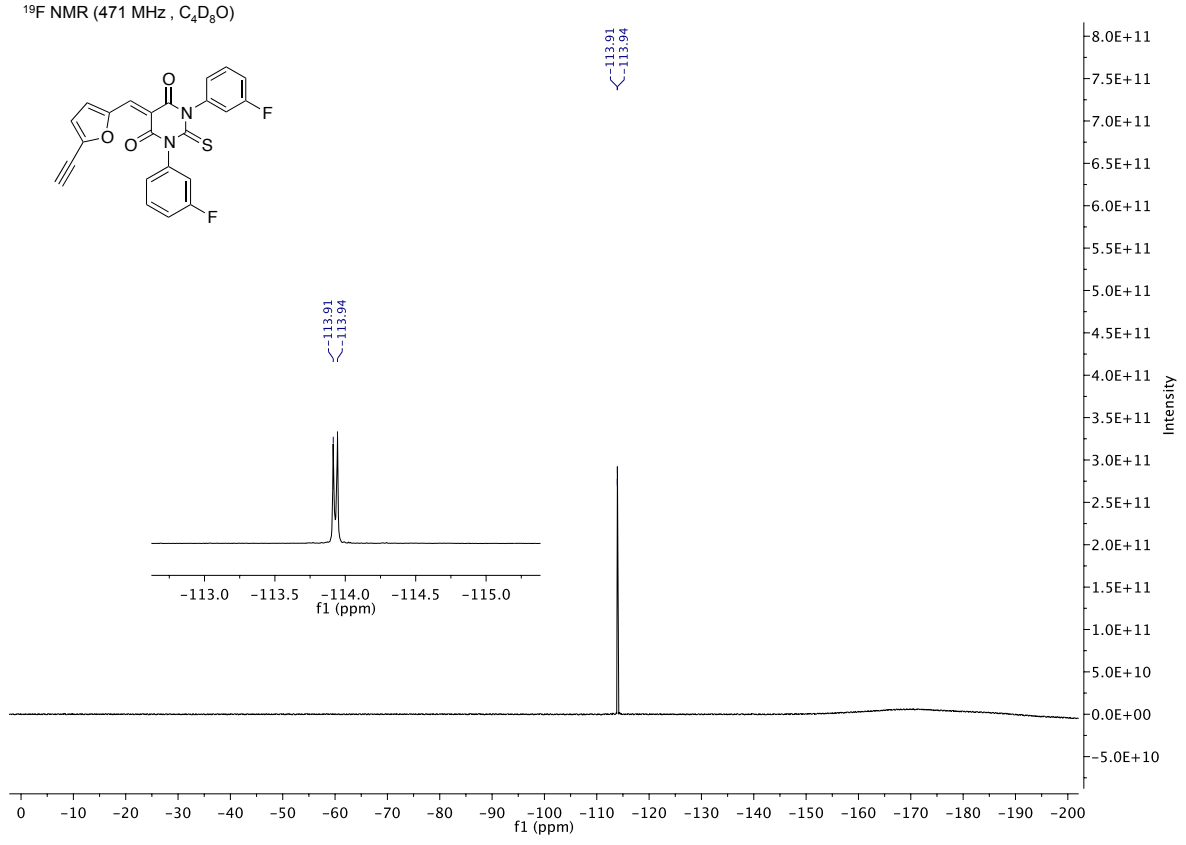

1H NMR (500 MHz, C4D8O)
Br
Br
C4D8O
C4D8O
HDO
8.70
8.65
8.60
7.55
7.50
f1 (ppm)
Intensity
f1 (ppm)


13C NMR (126 MHz, C4D8O)
180.22
160.51
159.00
151.36
142.86
141.46
141.18
138.42
132.10
131.20
130.26
128.17
128.05
121.69
120.29
114.22
87.87
72.86
Br
Br
C4D8O
C4D8O
Intensity
f1 (ppm)

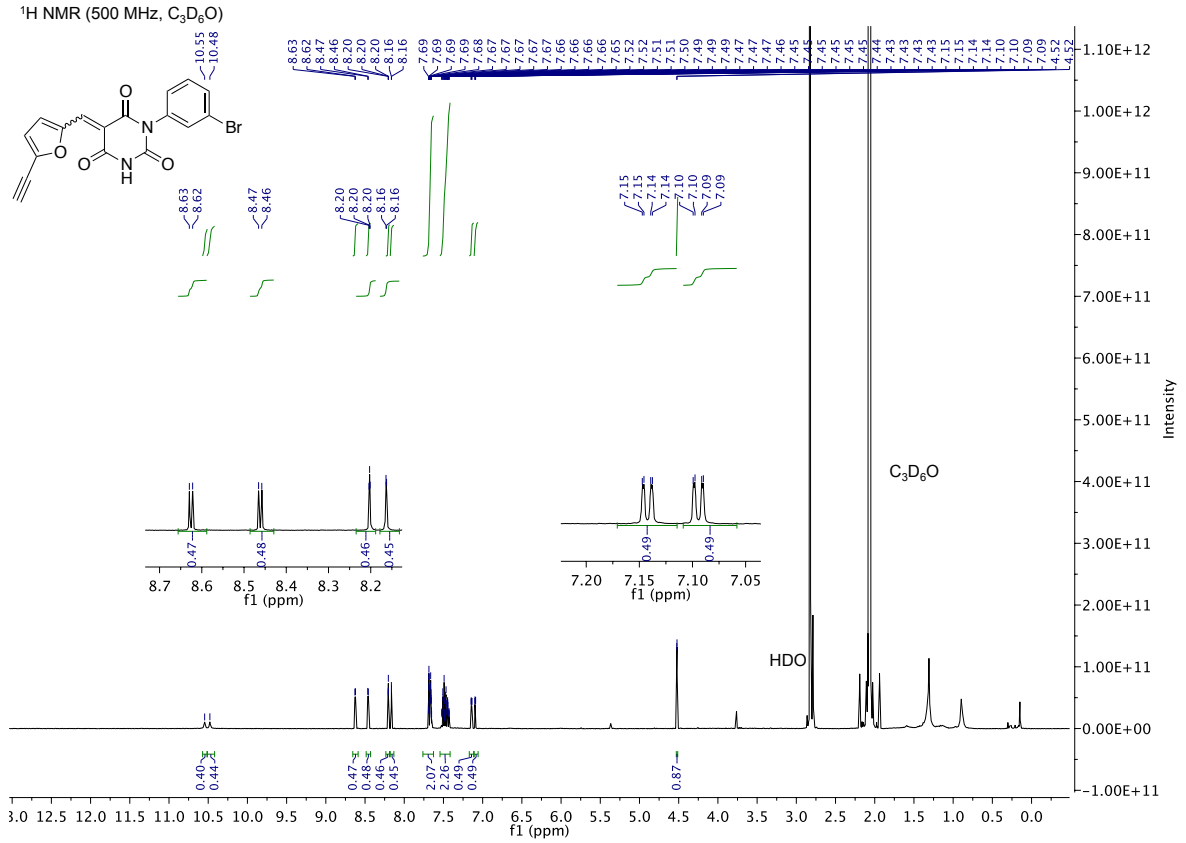
Compound **5k**

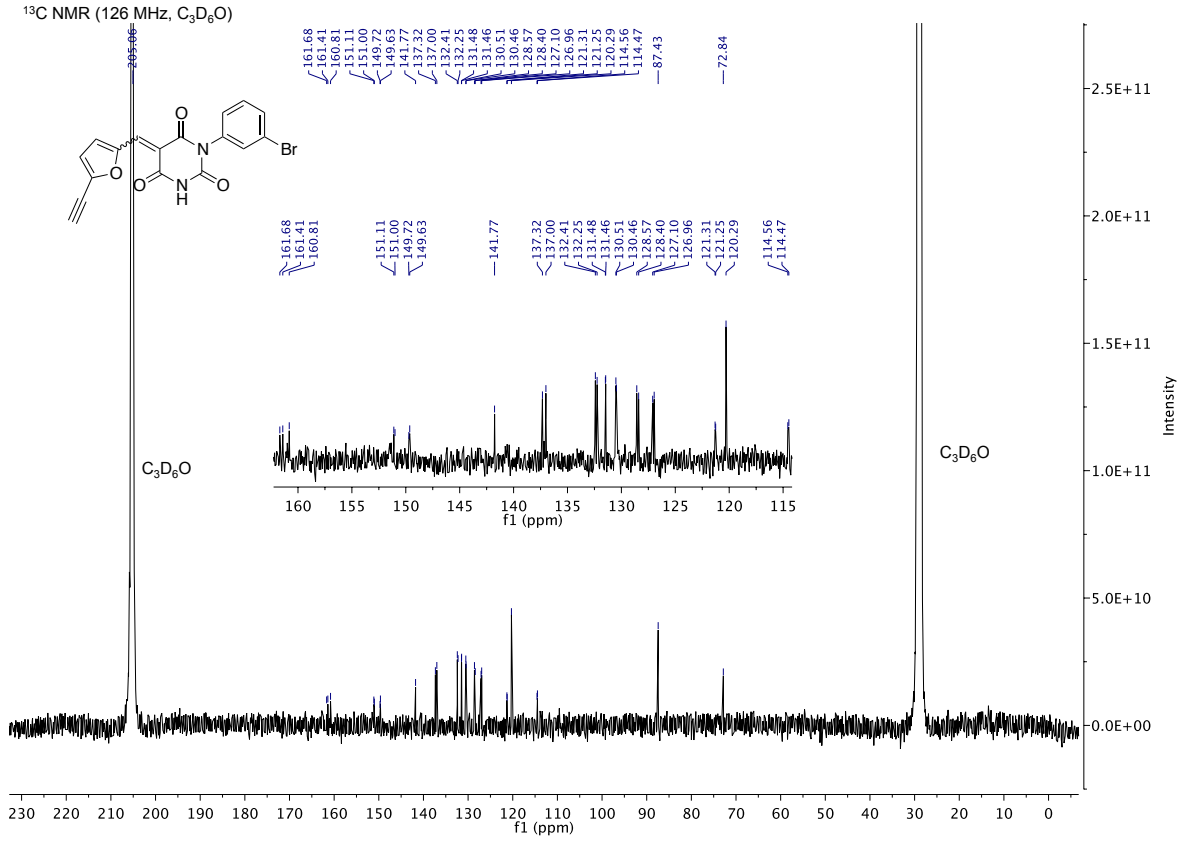

1H NMR (500 MHz, C4D8O)
F
F
C4D8O
C4D8O
HDO
Intensity
f1 (ppm)


^{13}C NMR (126 MHz, C_4D_8O)
180.14
163.98
162.03
160.49
159.00
151.38
142.77
141.61
141.53
141.33
141.25
138.31
130.03
129.96
128.00
125.26
125.09
120.26
116.68
116.53
116.49
116.34
115.08
114.92
114.37
87.81
72.87
C_4D_8O
C_4D_8O
Intensity
f1 (ppm)

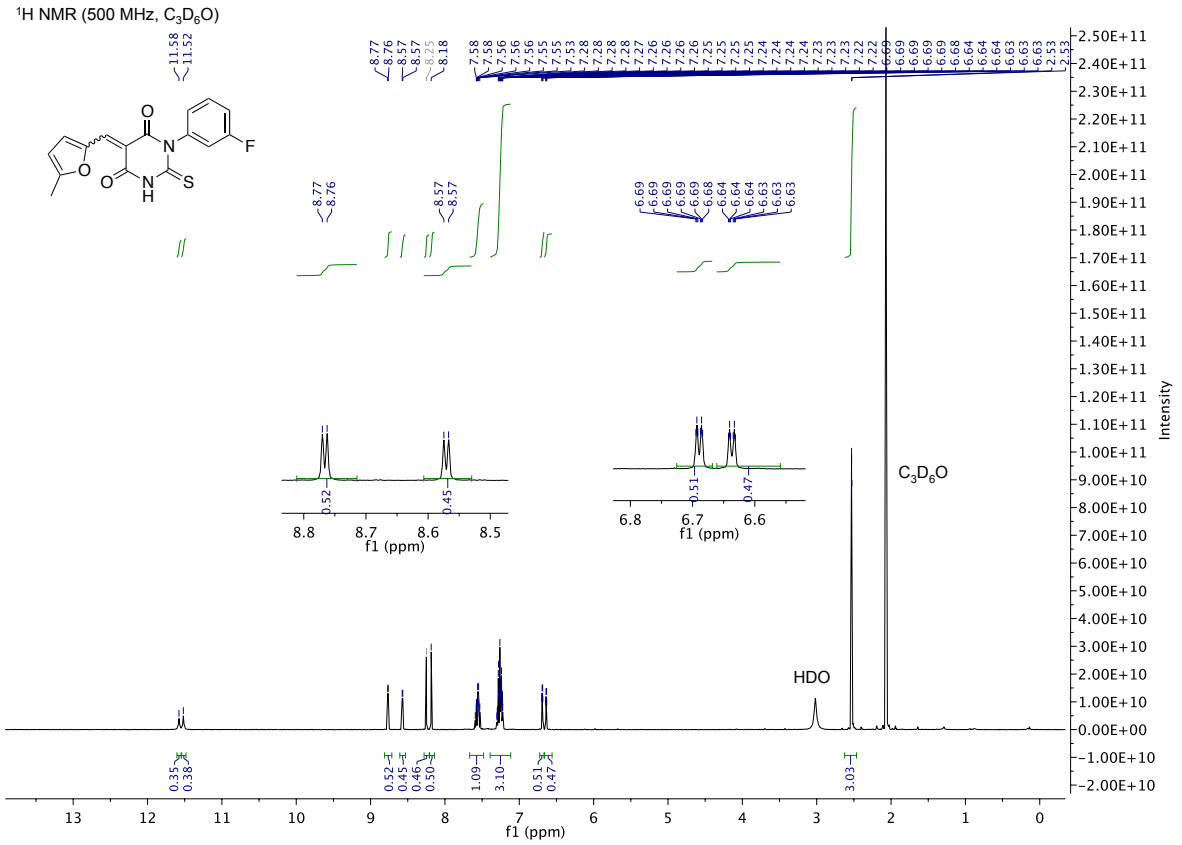

^{19}F NMR (471 MHz , C_4D_8O)
-113.91
-113.94
-113.91
-113.94
Intensity
f1 (ppm)
f1 (ppm)

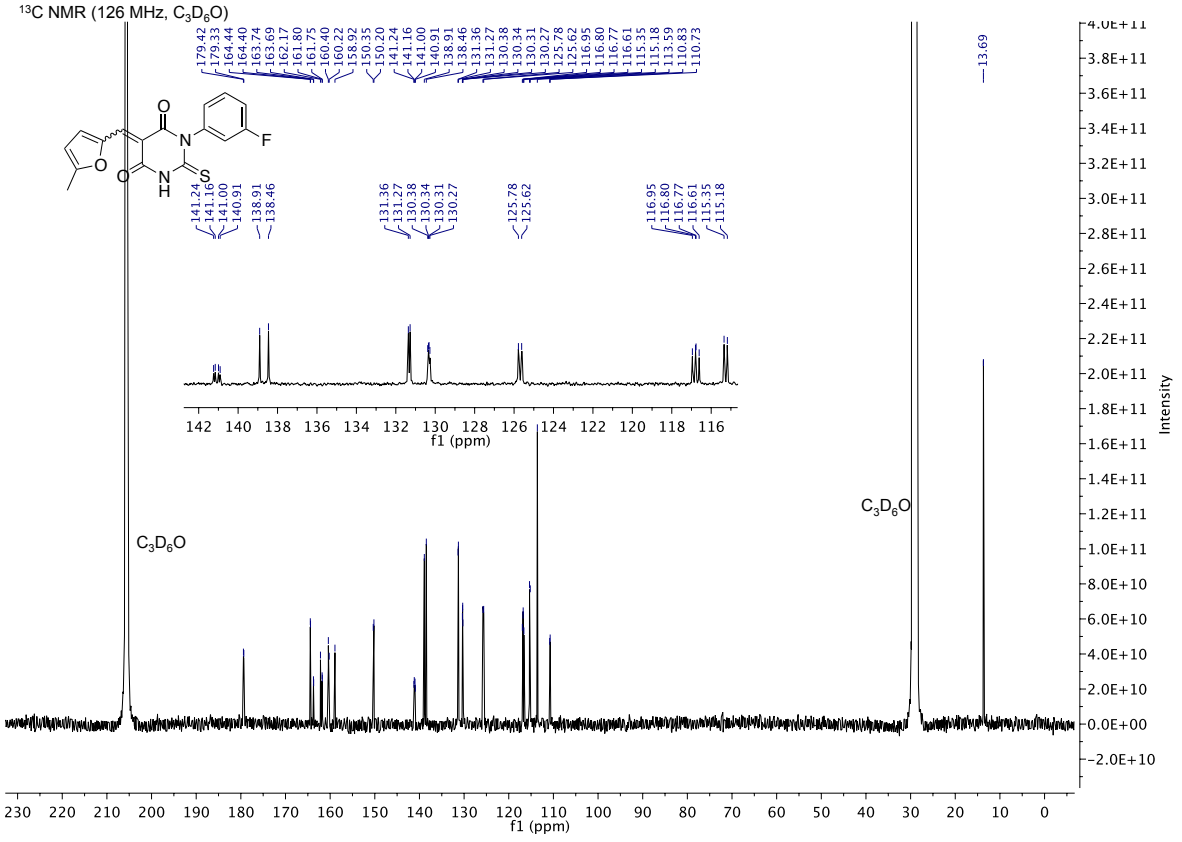
Compound **5l**


^{1}H NMR (500 MHz, C_3D_6O)
HDO
C_3D_6O
f1 (ppm)
Intensity

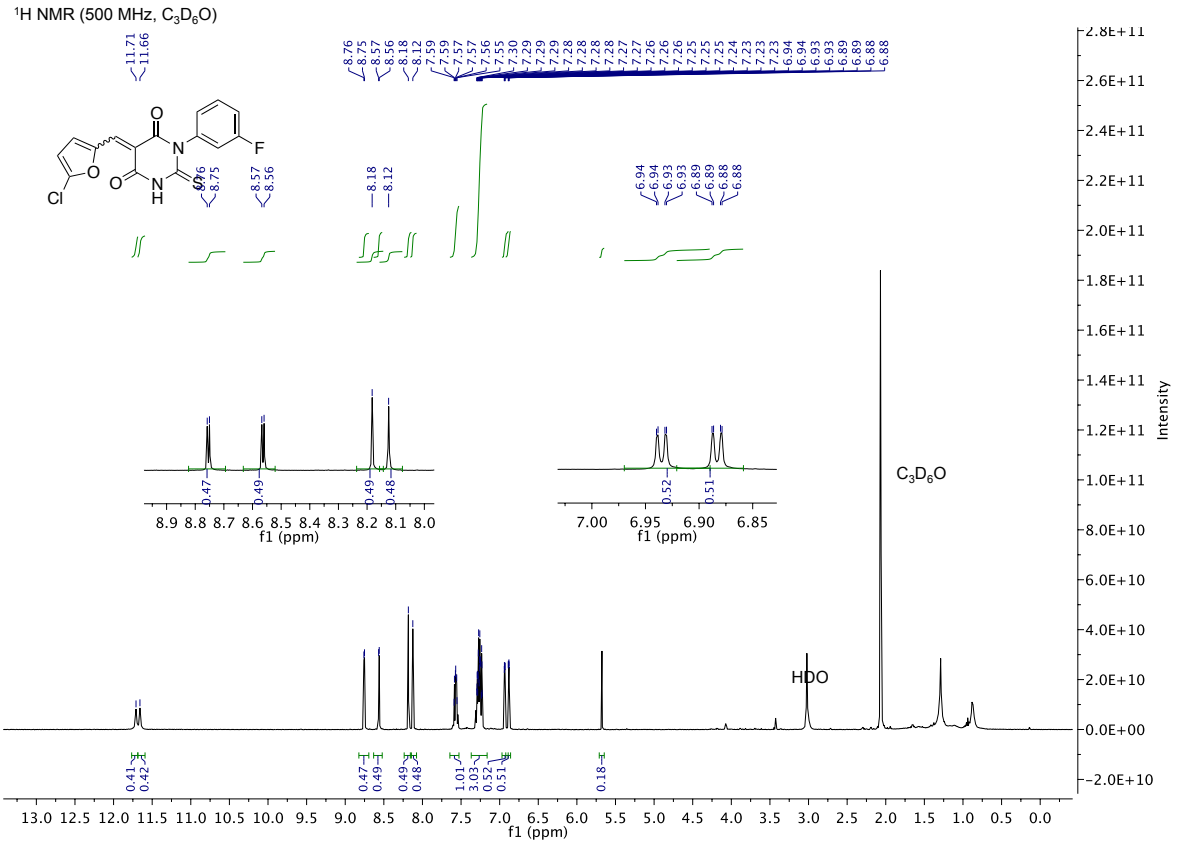

^{13}C NMR (126 MHz, C_3D_6O)
C_3D_6O
C_3D_6O
f1 (ppm)
Intensity

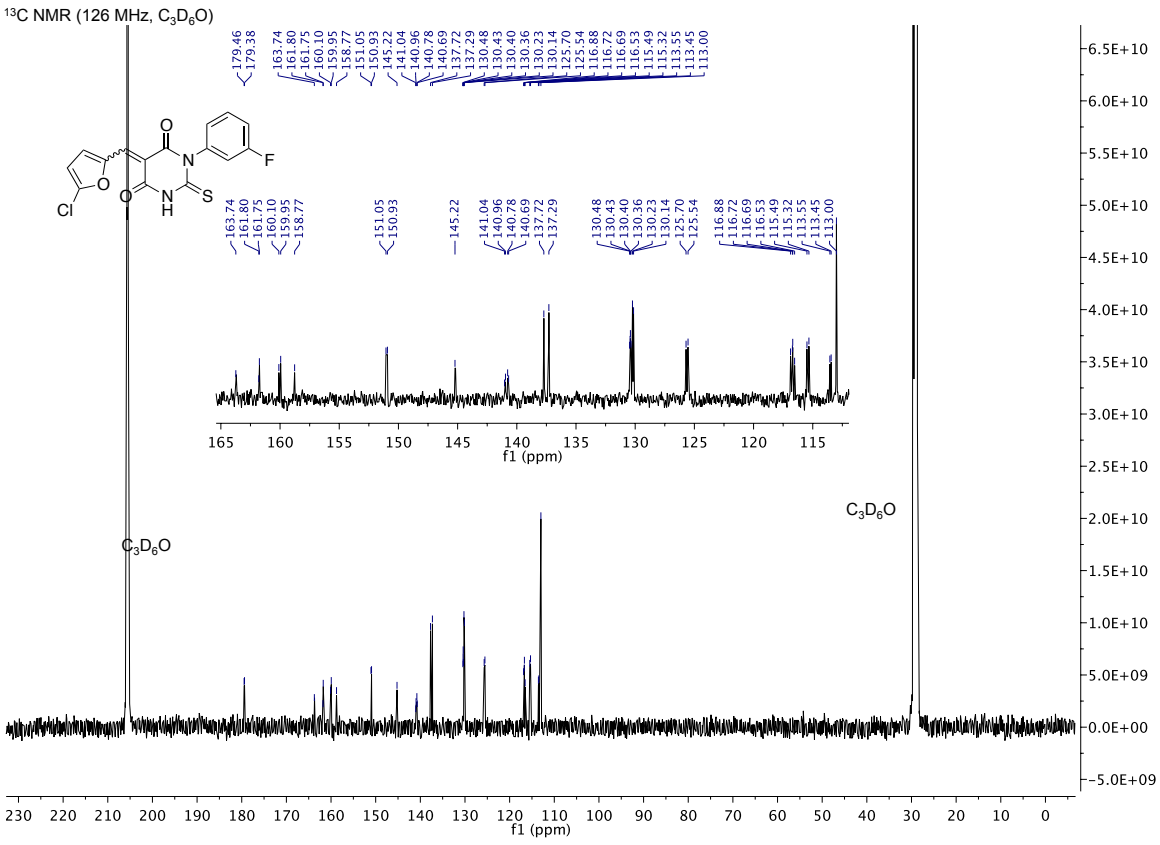
Compound **6a**


^{1}H NMR (500 MHz, C_3D_6O)
HDO
C_3D_6O
f1 (ppm)
Intensity

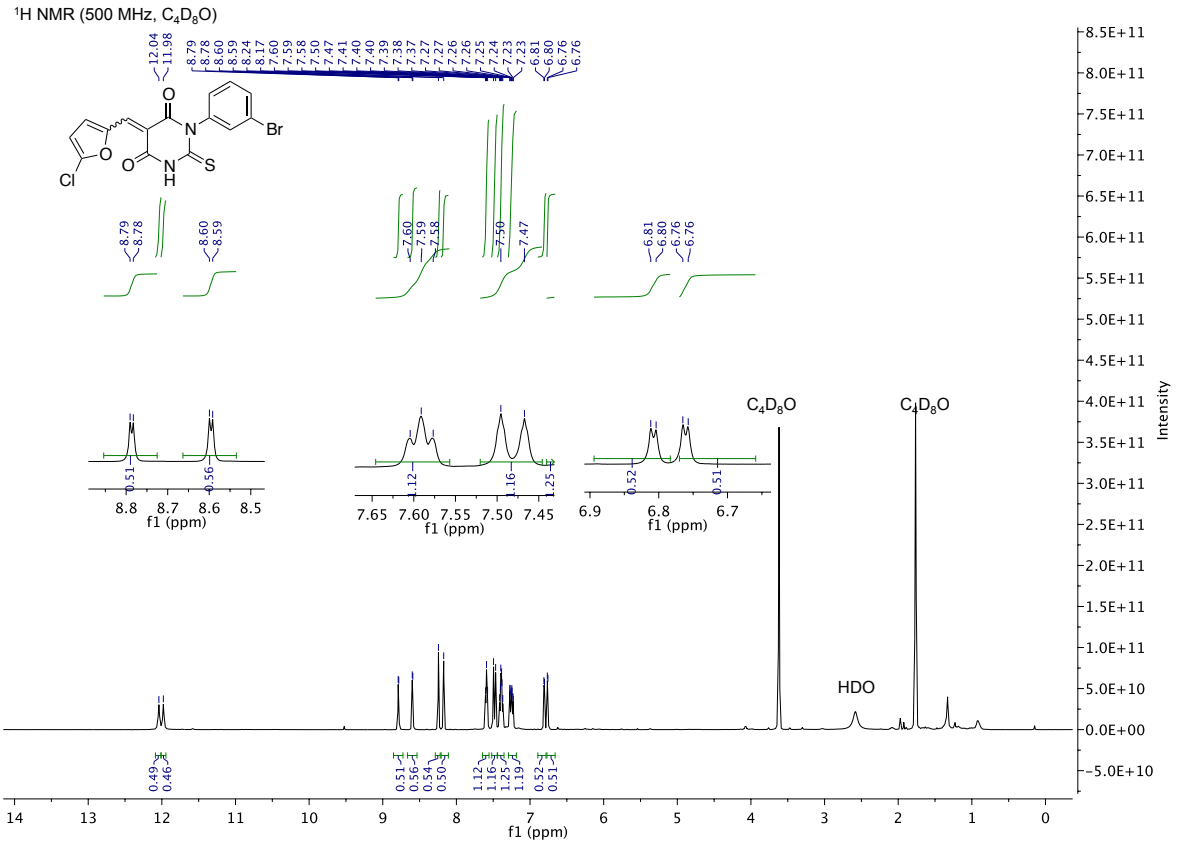

^{13}C NMR (126 MHz, C_3D_6O)
C_3D_6O
C_3D_6O
f1 (ppm)
Intensity

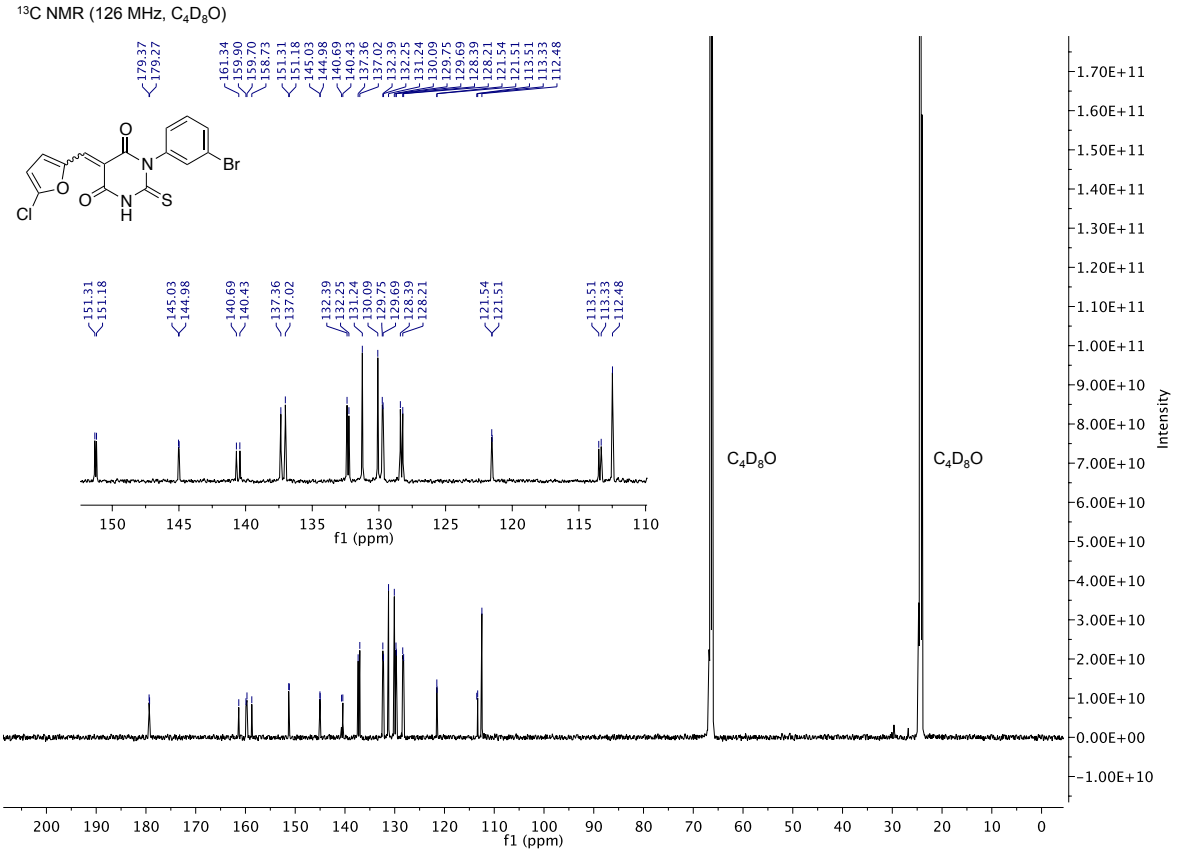
Compound **6b**


^{1}H NMR (500 MHz, C_3D_6O)
HDO
C_3D_6O
f1 (ppm)
Intensity


^{13}C NMR (126 MHz, C_3D_6O)
C_3D_6O
C_3D_6O
f1 (ppm)

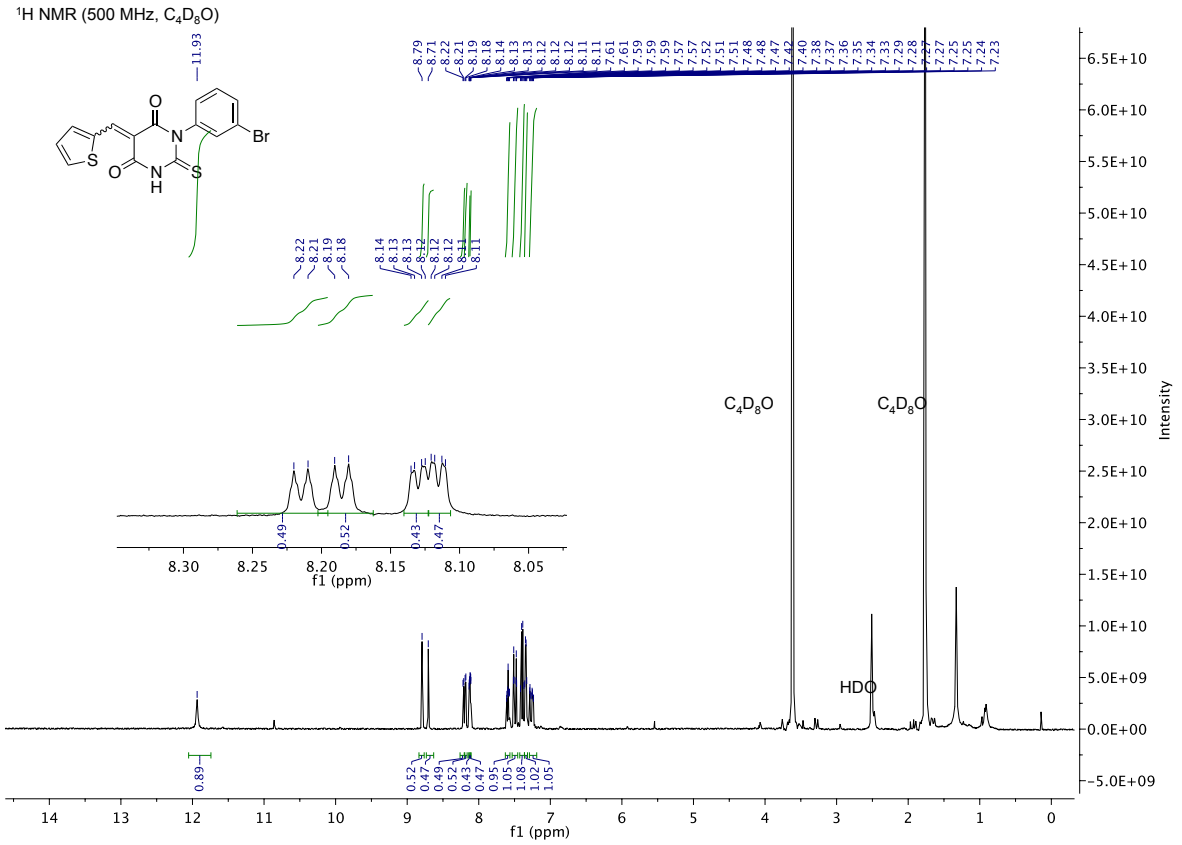
Compound **6c**


1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
Intensity
f1 (ppm)

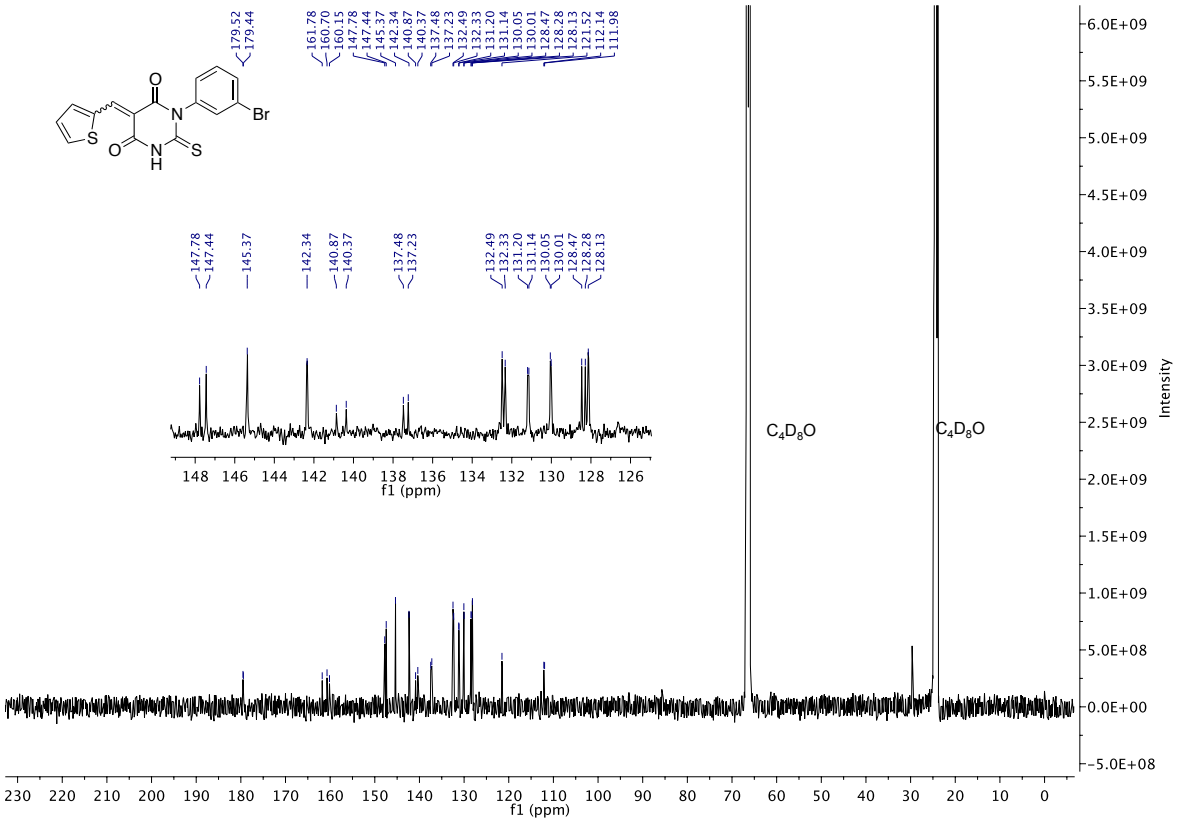

13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
Intensity
f1 (ppm)

Compound **6d**

^{1}H NMR (500 MHz, C_4D_8O)

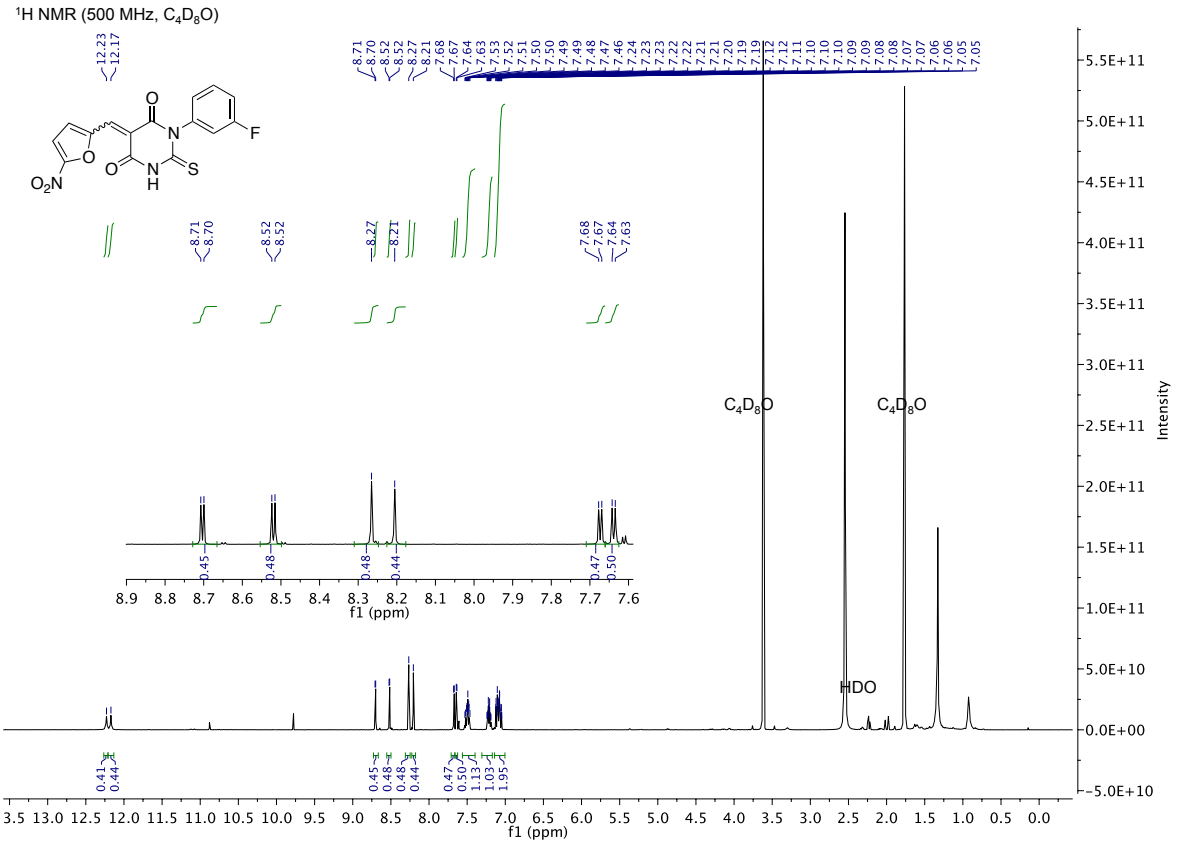


^{13}C NMR (126 MHz, C_4D_8O)

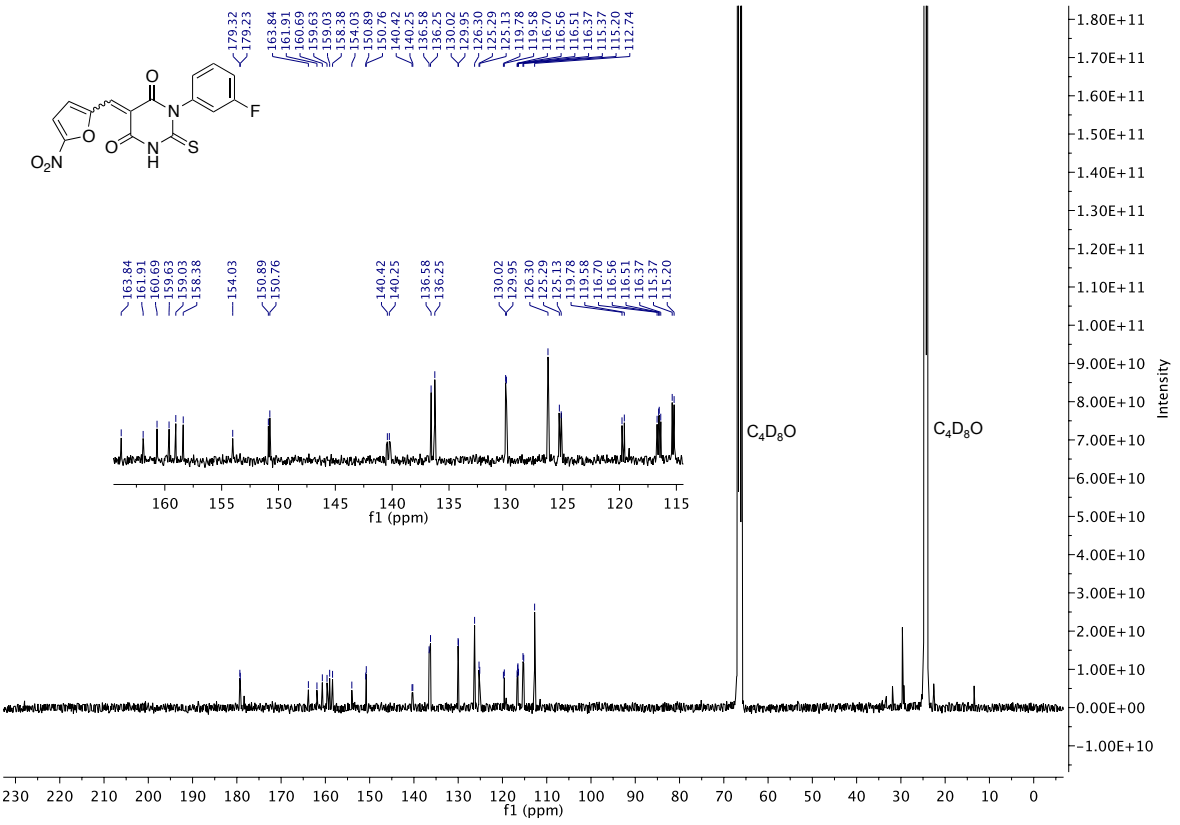


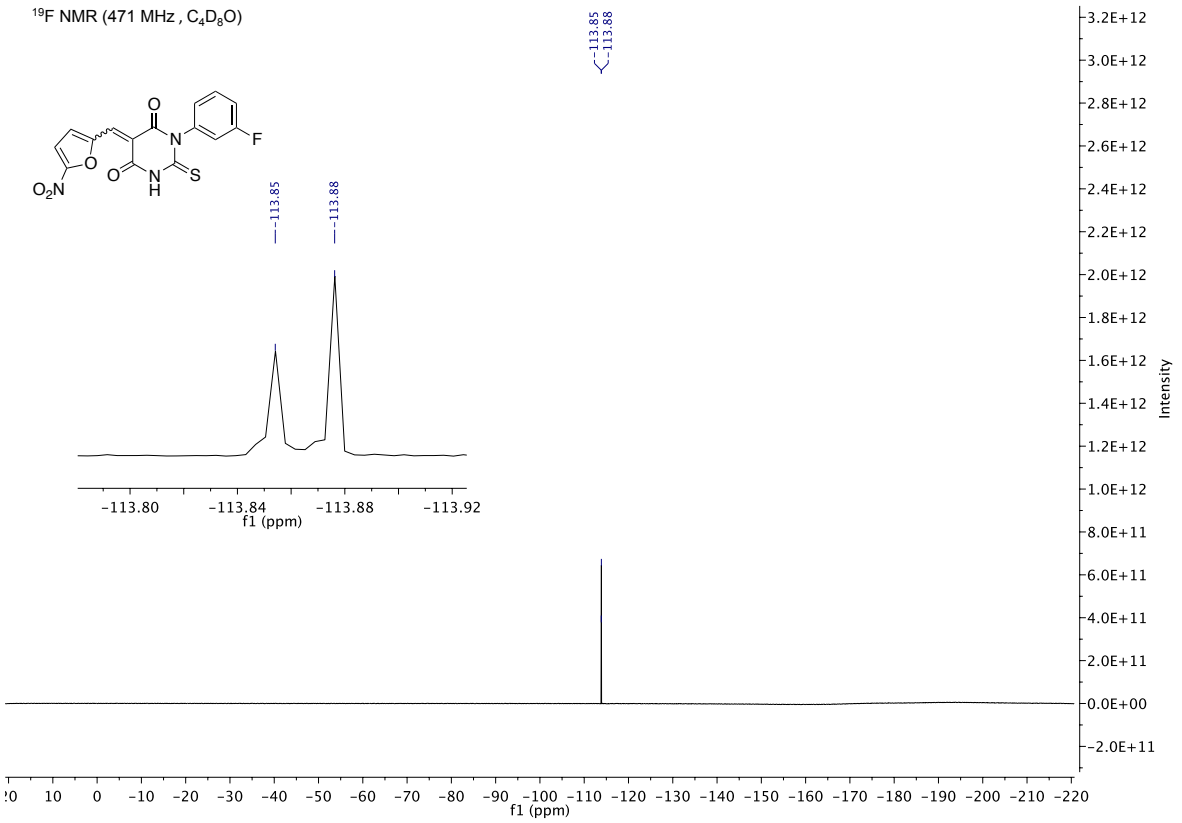
Compound **6e**

^{1}H NMR (500 MHz, C_4D_8O)

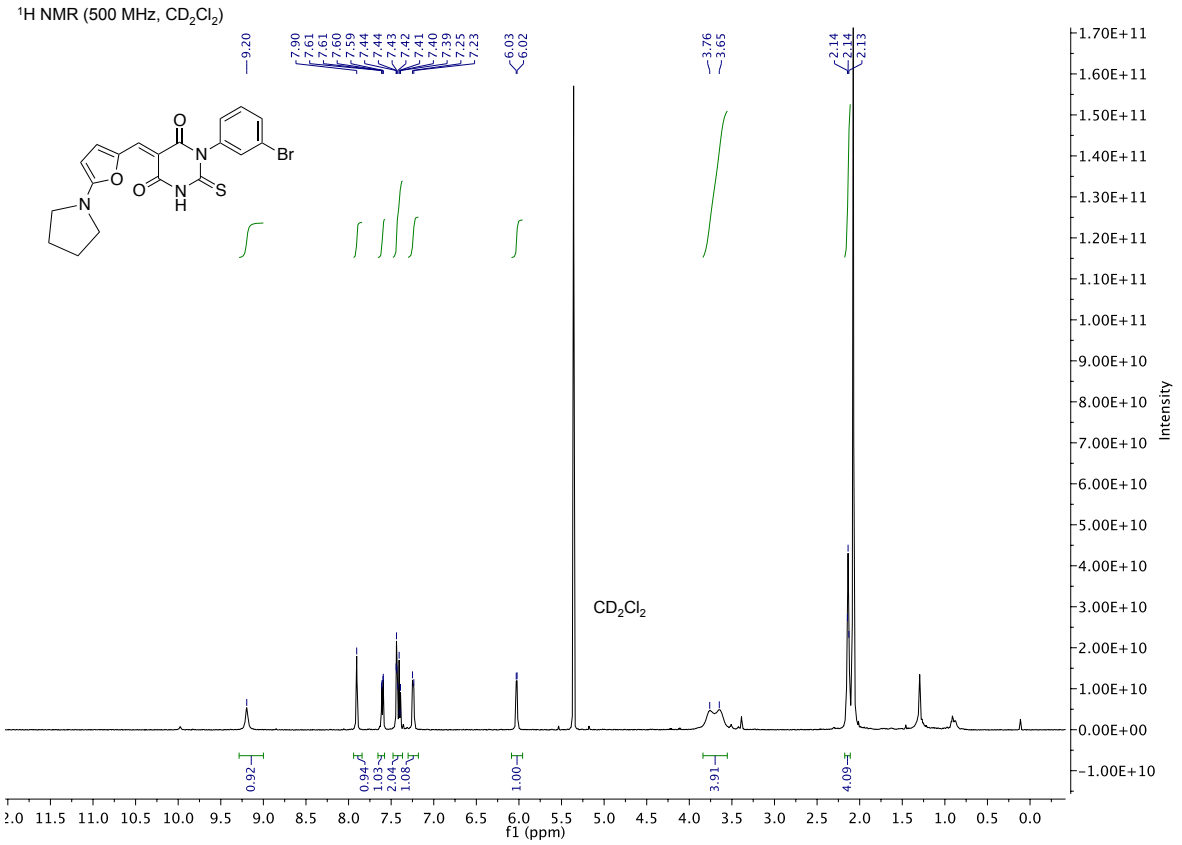


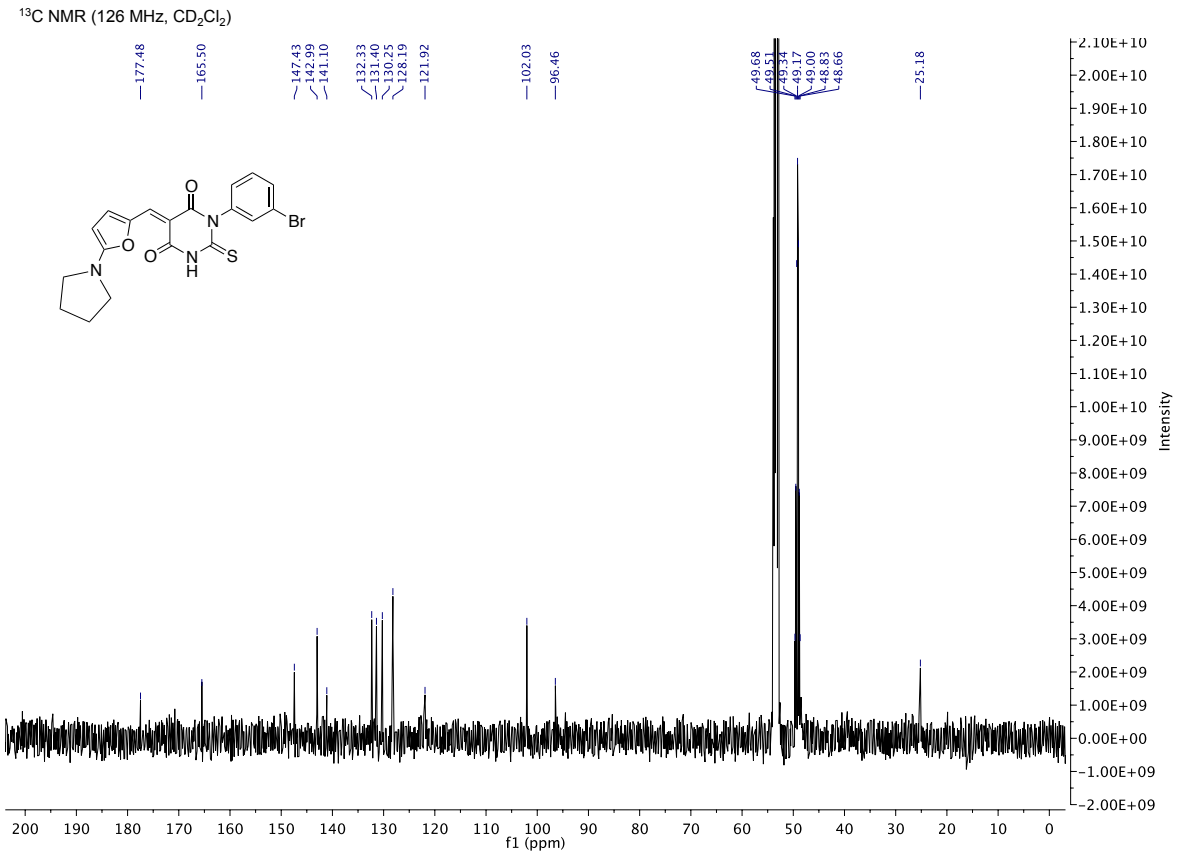
^{13}C NMR (126 MHz, C_4D_8O)



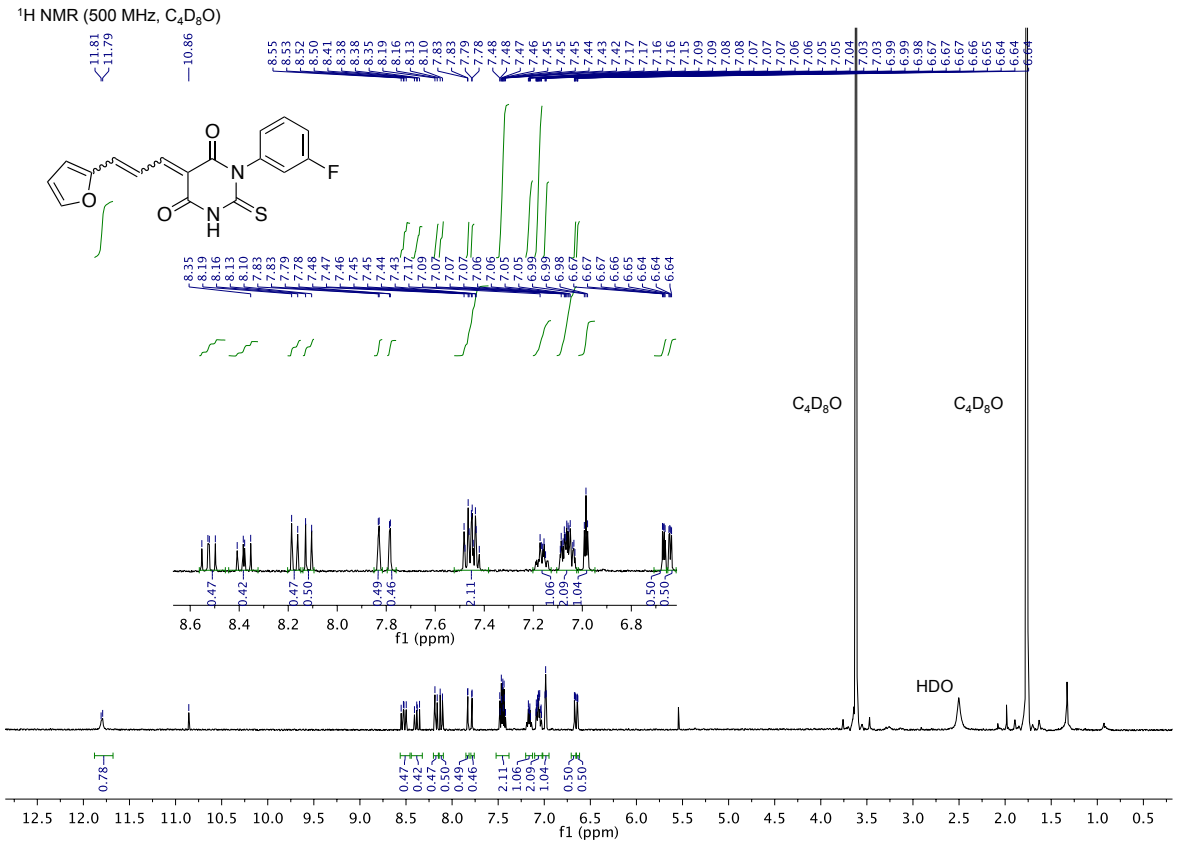

19F NMR (471 MHz , C4D8O)
-113.85
-113.88
-113.80
-113.84
-113.88
-113.92
f1 (ppm)
Intensity
f1 (ppm)

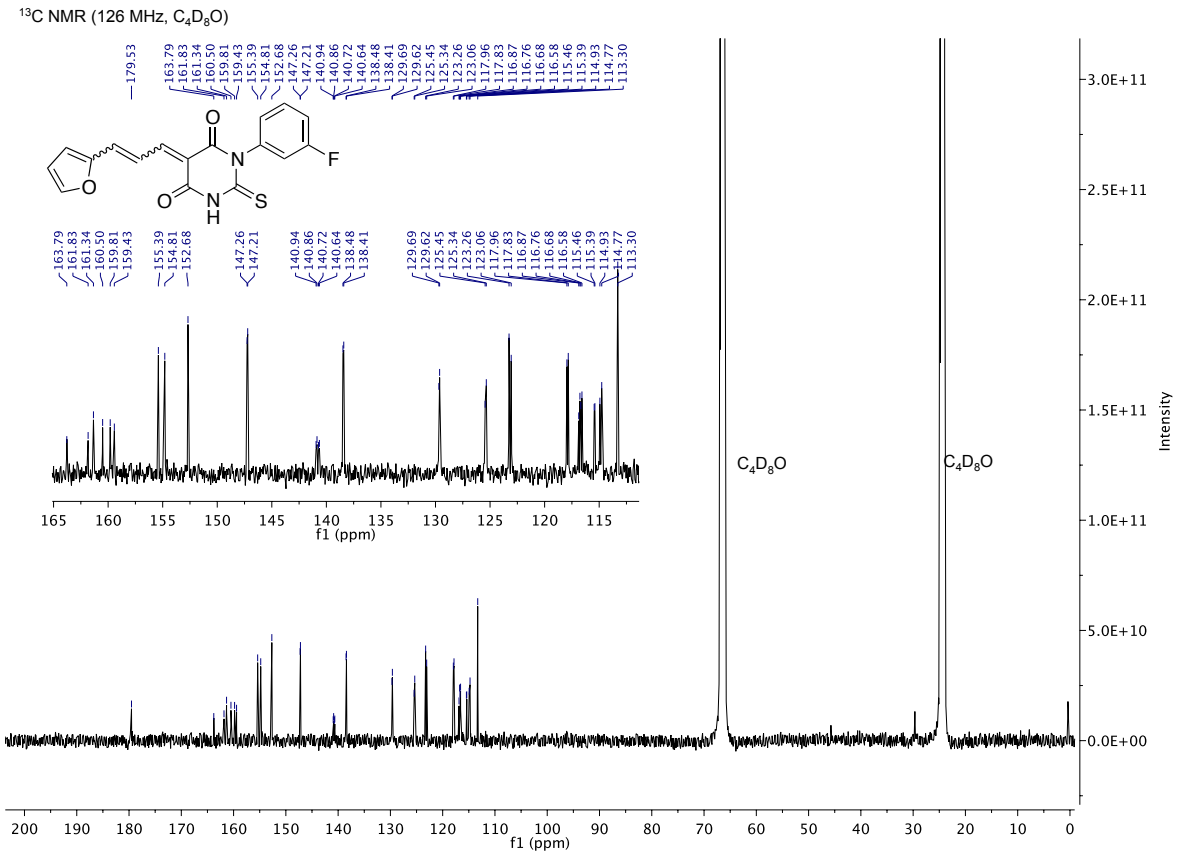
Compound **6f**


1H NMR (500 MHz, CD2Cl2)
9.20
7.90
7.61
7.61
7.60
7.59
7.44
7.44
7.43
7.42
7.41
7.40
7.39
7.25
7.23
6.03
6.02
3.76
3.65
2.14
2.14
2.13
CD2Cl2
0.92
0.94
1.03
2.04
1.08
1.00
3.91
4.09
f1 (ppm)
Intensity

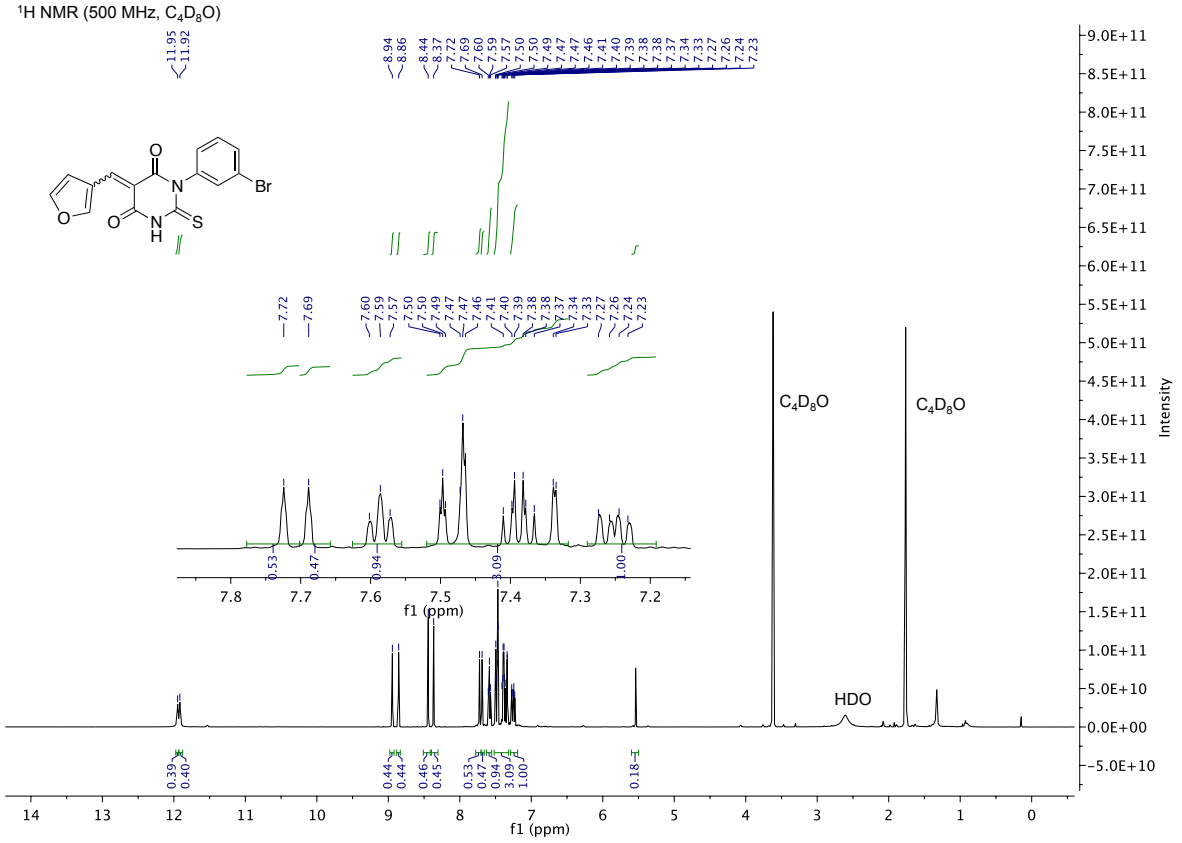

13C NMR (126 MHz, CD2Cl2)
177.48
165.50
147.43
142.99
141.10
132.33
131.40
130.25
128.19
121.92
102.03
96.46
49.68
49.51
49.34
49.17
49.00
48.83
48.66
25.18
Br
Intensity
f1 (ppm)

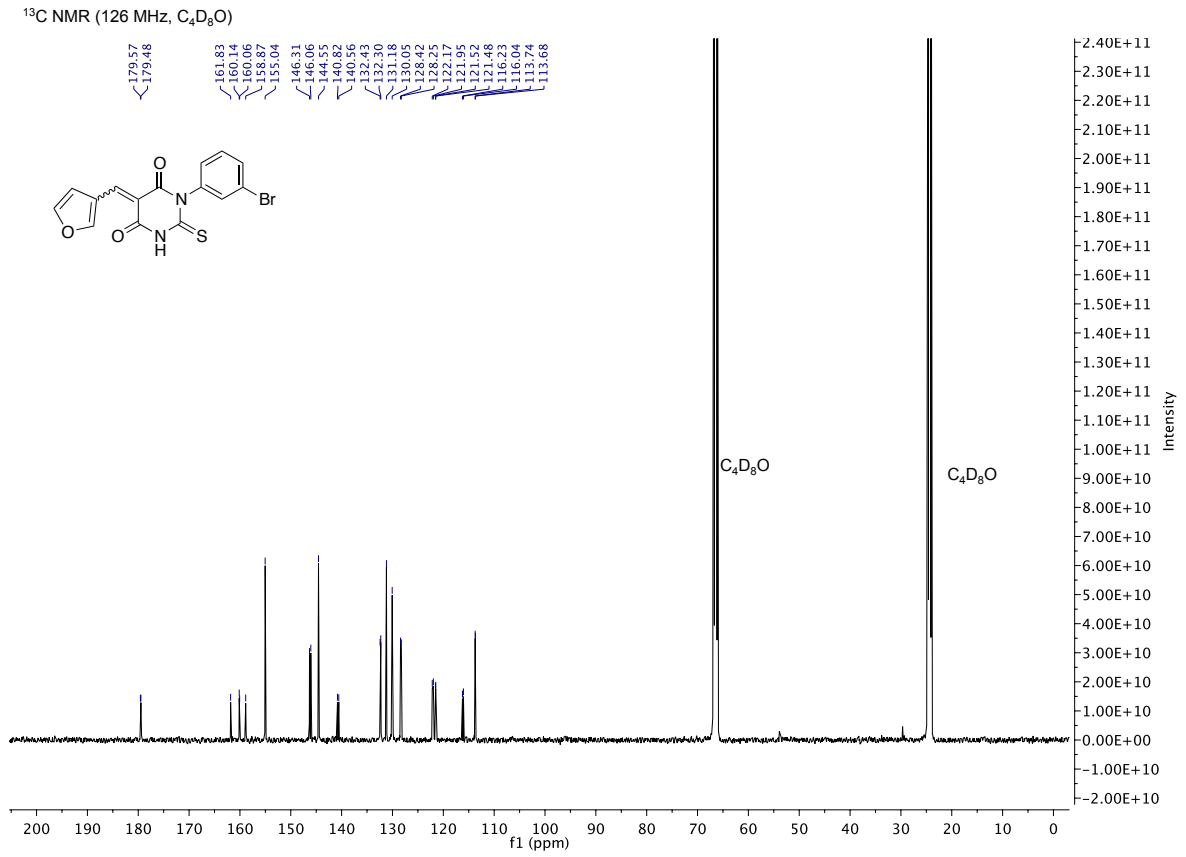
Compound **6g**


1H NMR (500 MHz, C4D8O)
11.81
11.79
10.86
F
C4D8O
C4D8O
HDO
f1 (ppm)

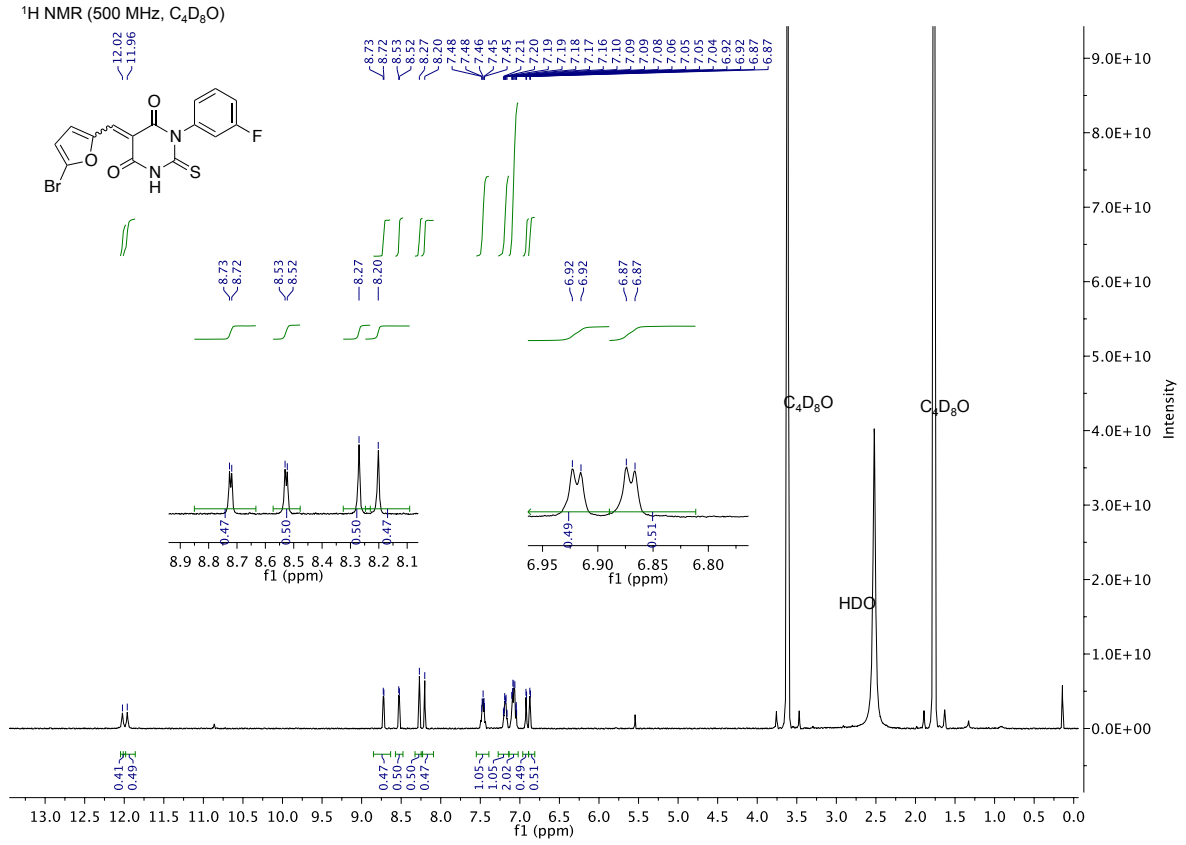

13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
Intensity
f1 (ppm)

Compound **6h**

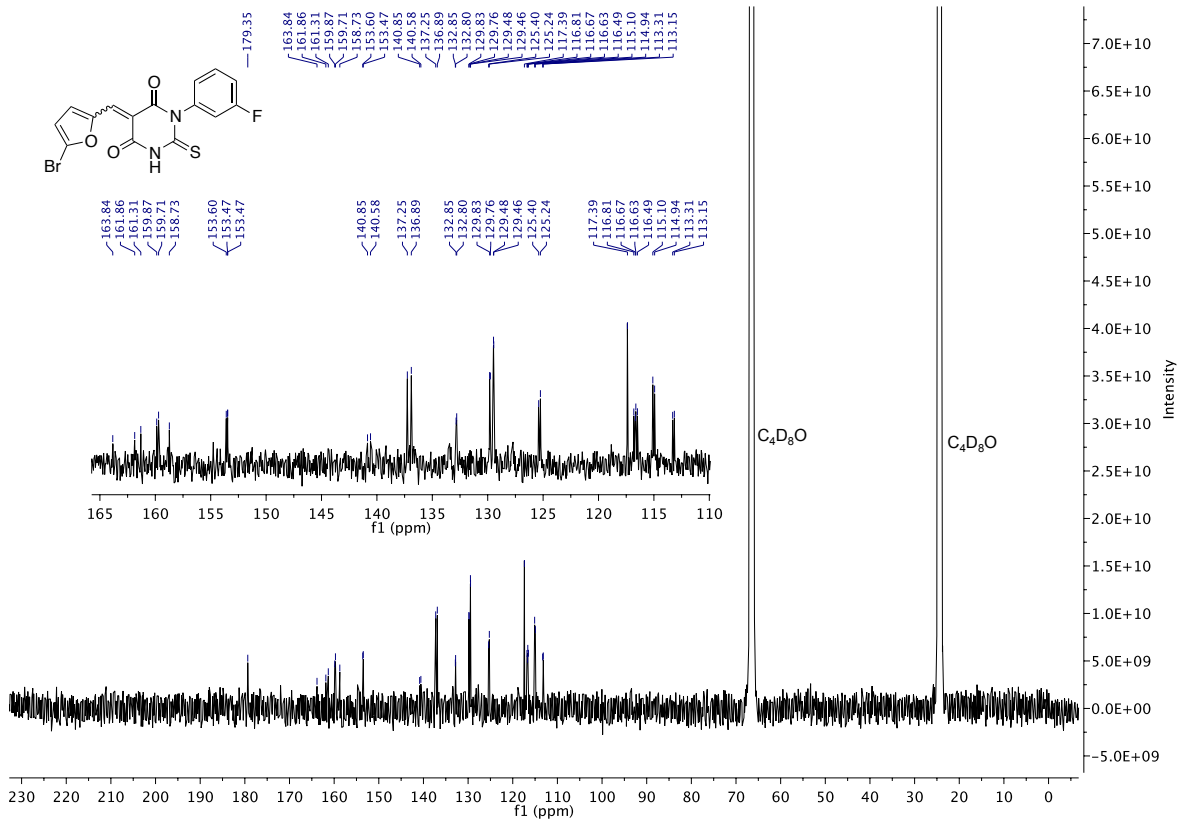

1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
Intensity
f1 (ppm)


13C NMR (126 MHz, C4D8O)
C4D8O
C4D8O
Intensity
f1 (ppm)

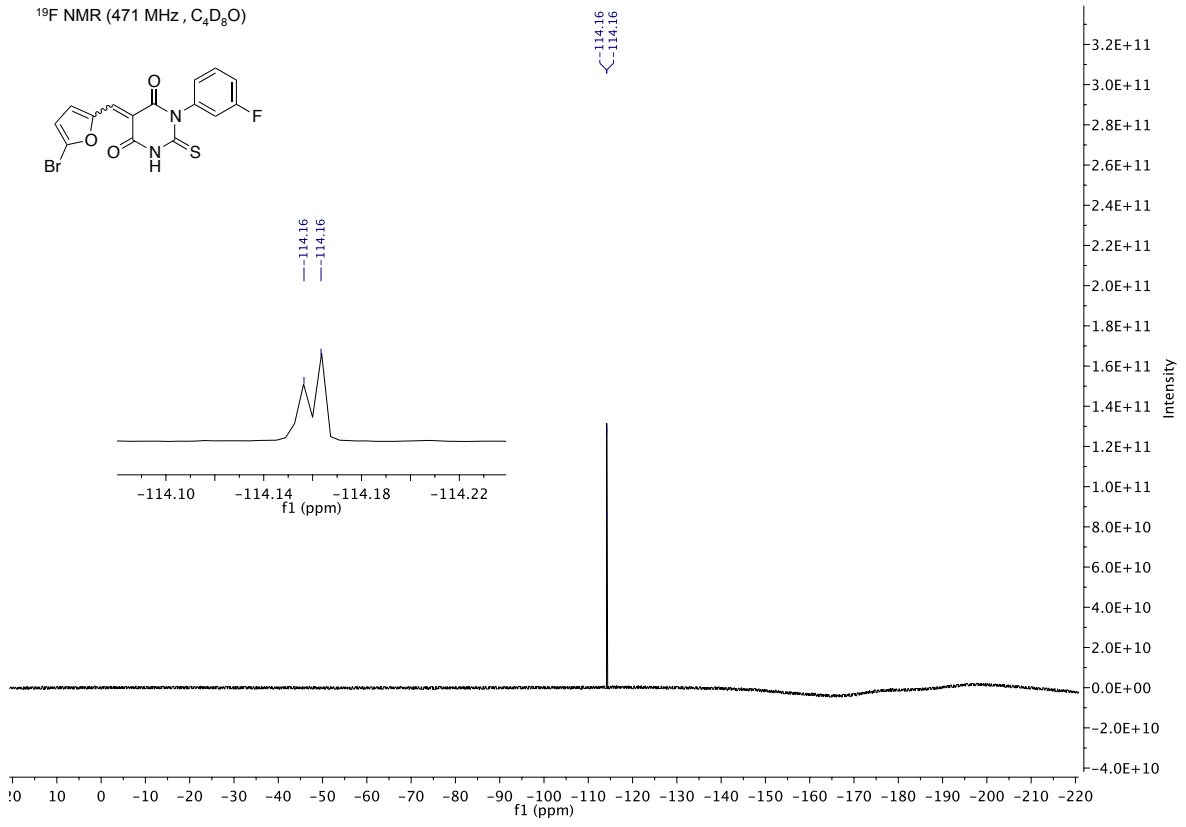
Compound **6i**


1H NMR (500 MHz, C4D8O)
C4D8O
C4D8O
HDO
Intensity
f1 (ppm)

^{13}C NMR (126 MHz, C_4D_8O)

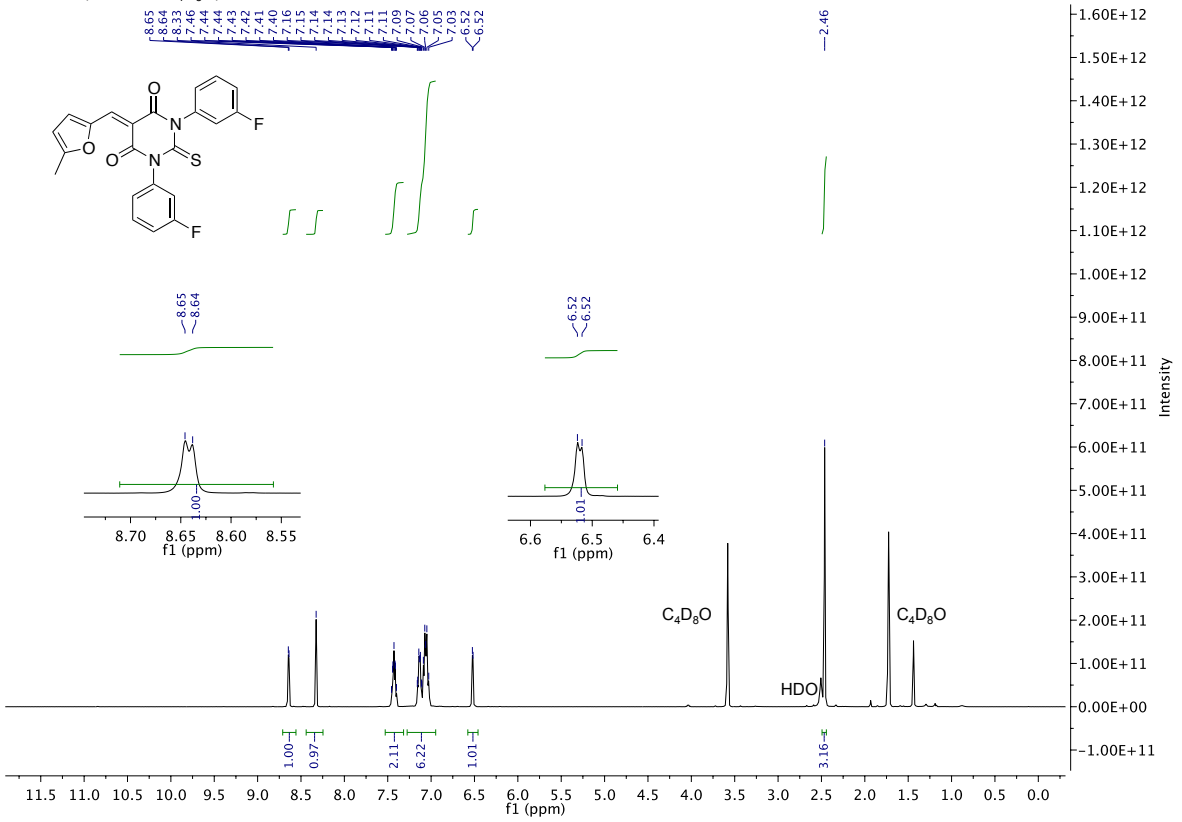


^{19}F NMR (471 MHz , C_4D_8O)

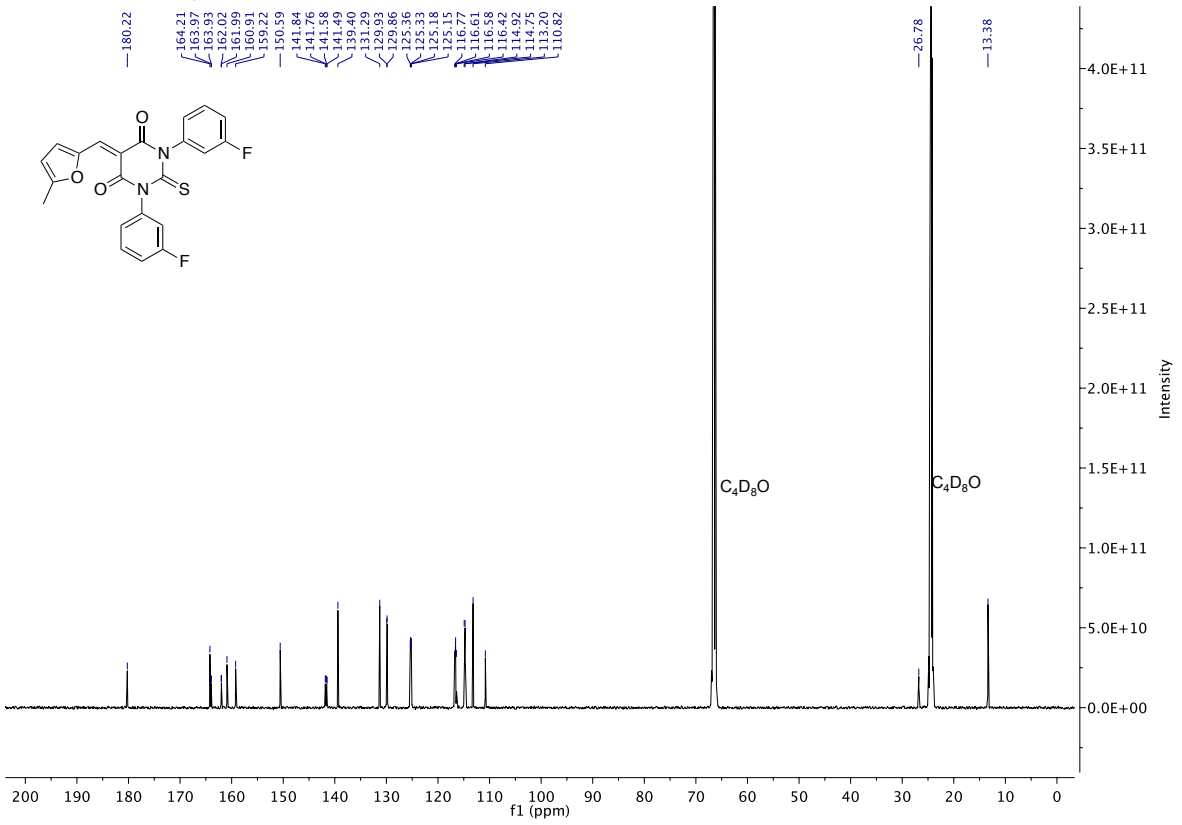


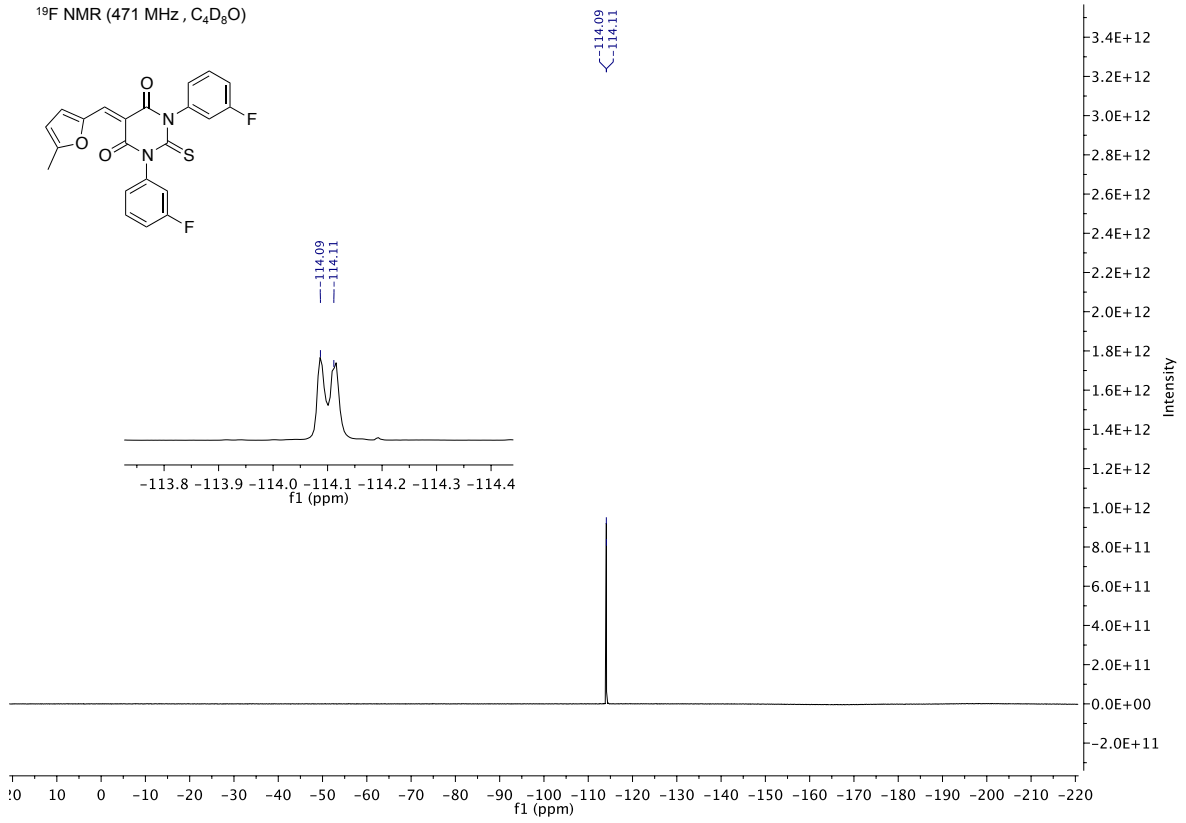
Compound **6j**

^{1}H NMR (500 MHz, C_4D_8O)

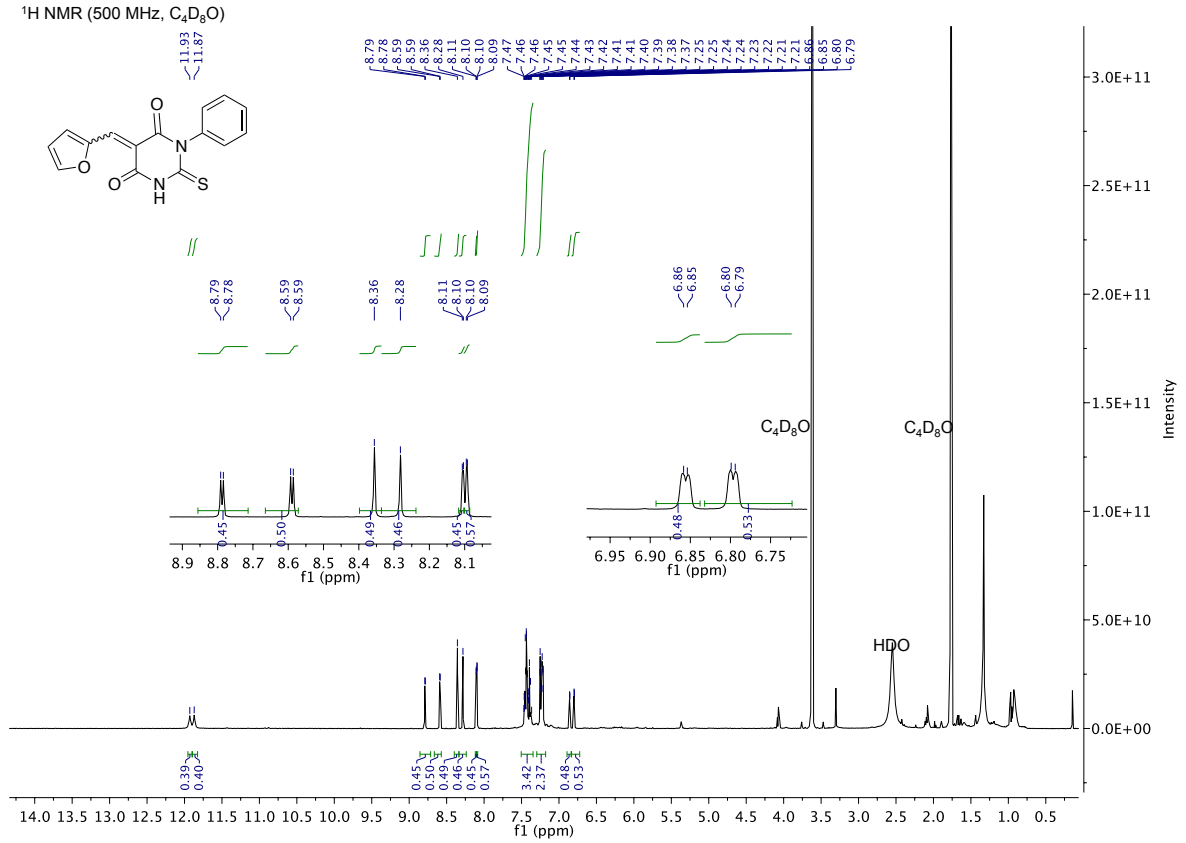


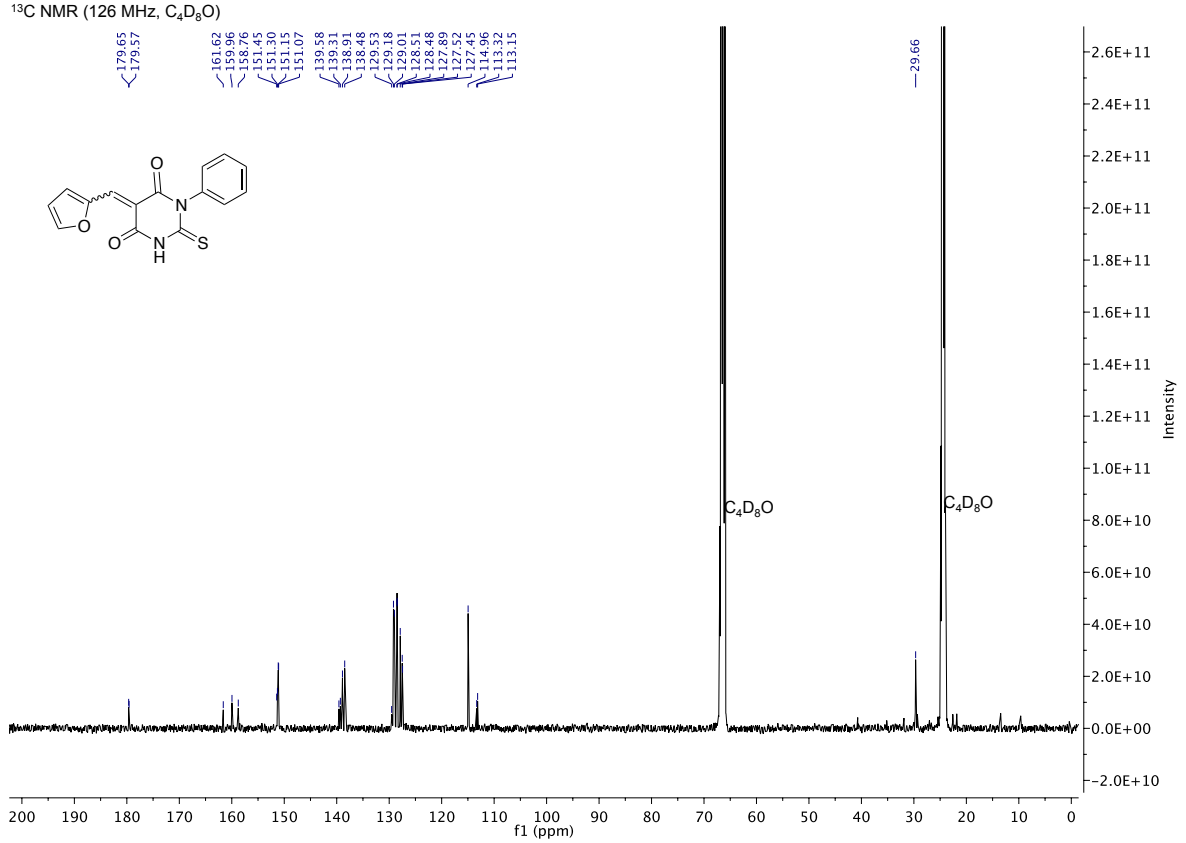
^{13}C NMR (126 MHz, C_4D_8O)



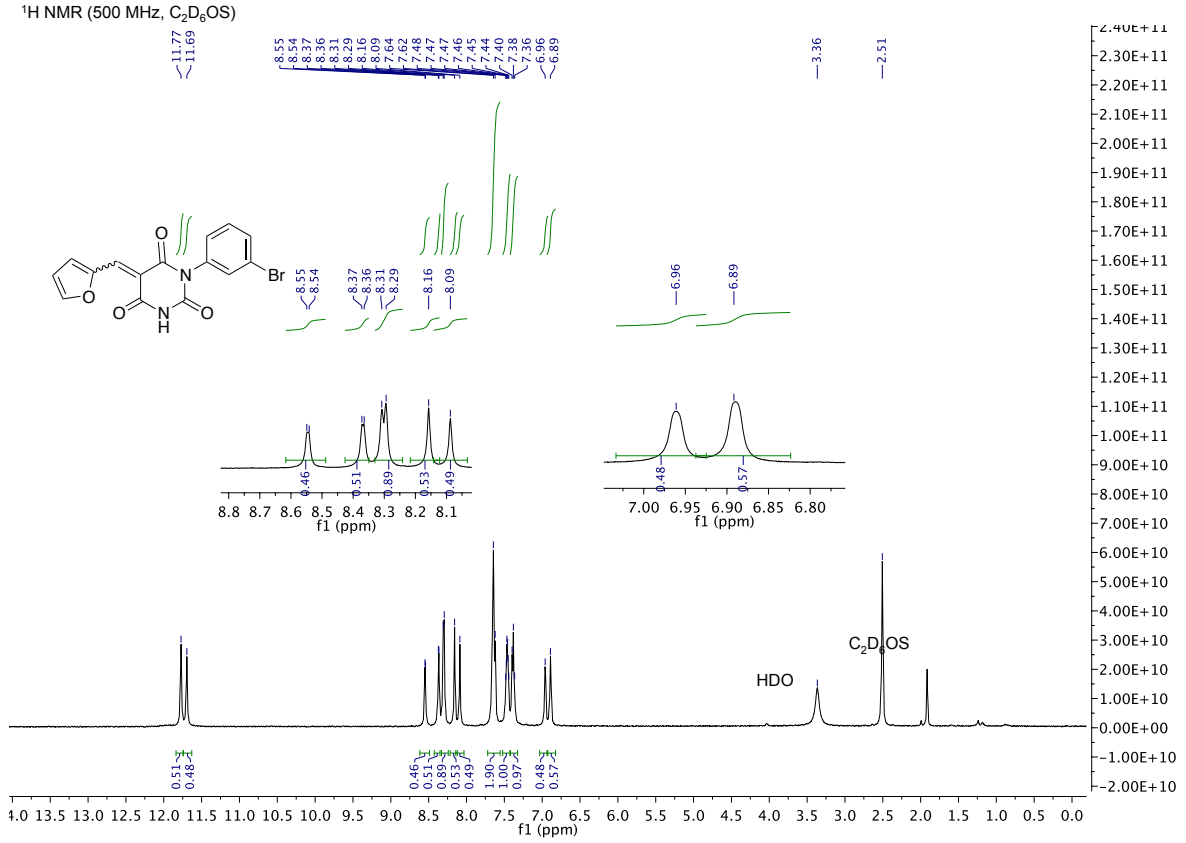

^{19}F NMR (471 MHz , C_4D_8O)
-114.09
-114.11
-114.09
-114.11
-113.8 -113.9 -114.0 -114.1 -114.2 -114.3 -114.4
f1 (ppm)
Intensity
f1 (ppm)

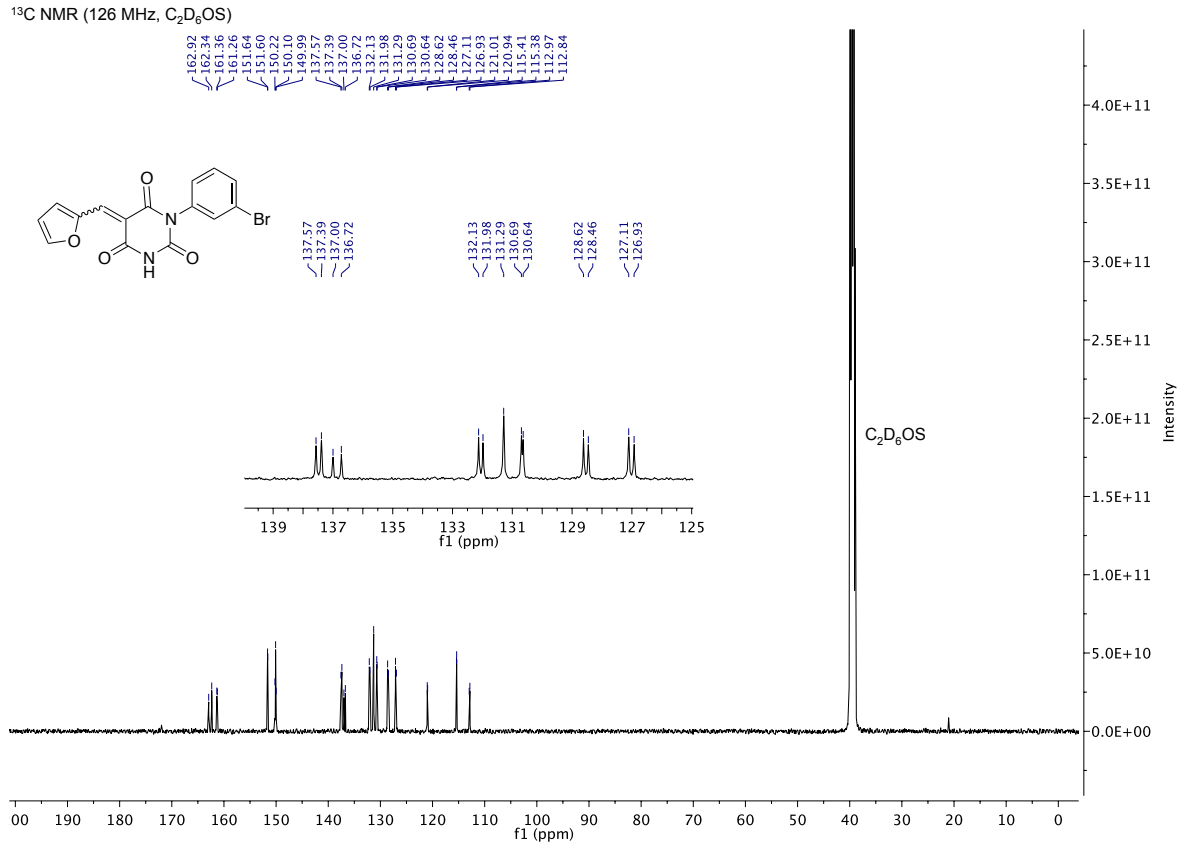
Compound **6k**


^{1}H NMR (500 MHz, C_4D_8O)
11.93
11.87
8.79 8.78 8.59 8.59 8.36 8.28 8.11 8.10 8.10 8.09 7.47 7.46 7.46 7.45 7.45 7.44 7.43 7.42 7.41 7.41 7.40 7.39 7.38 7.37 7.25 7.25 7.24 7.24 7.23 7.22 7.21 7.21 6.86 6.85 6.80 6.79
8.79 8.78
8.59 8.59
8.36
8.28
8.11 8.10 8.10 8.09
6.86 6.85
6.80 6.79
C_4D_8O
C_4D_8O
HDO
0.45 0.50 0.49 0.46 0.45 0.57
0.48 0.53
f1 (ppm)
0.39 0.40 0.45 0.50 0.49 0.46 0.45 0.57 3.42 2.37 0.48 0.53
f1 (ppm)
Intensity

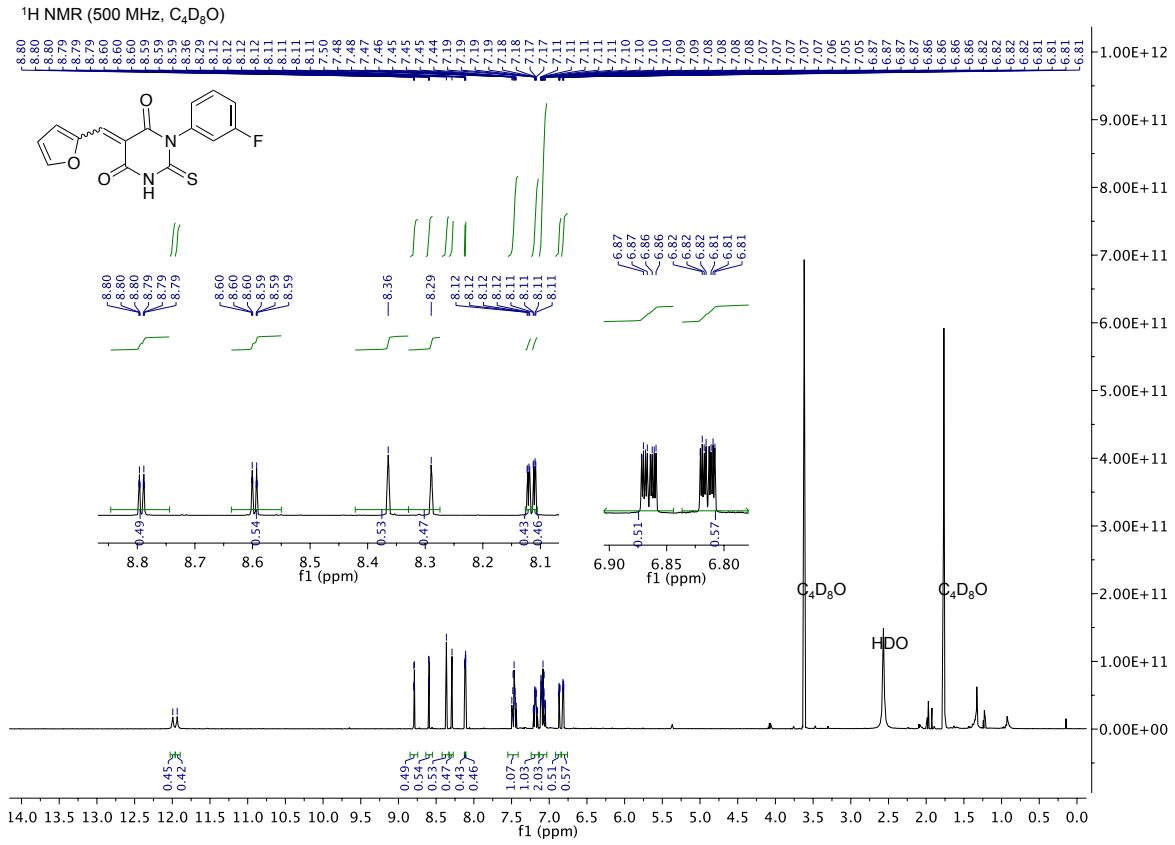

13C NMR (126 MHz, C4D8O)
179.65
179.57
161.62
159.96
158.76
151.45
151.30
151.15
151.07
139.58
139.31
138.91
138.48
129.53
129.18
129.01
128.51
128.48
127.89
127.52
127.45
114.96
113.32
113.15
29.66
C4D8O
C4D8O
Intensity
f1 (ppm)

Compound **6l**

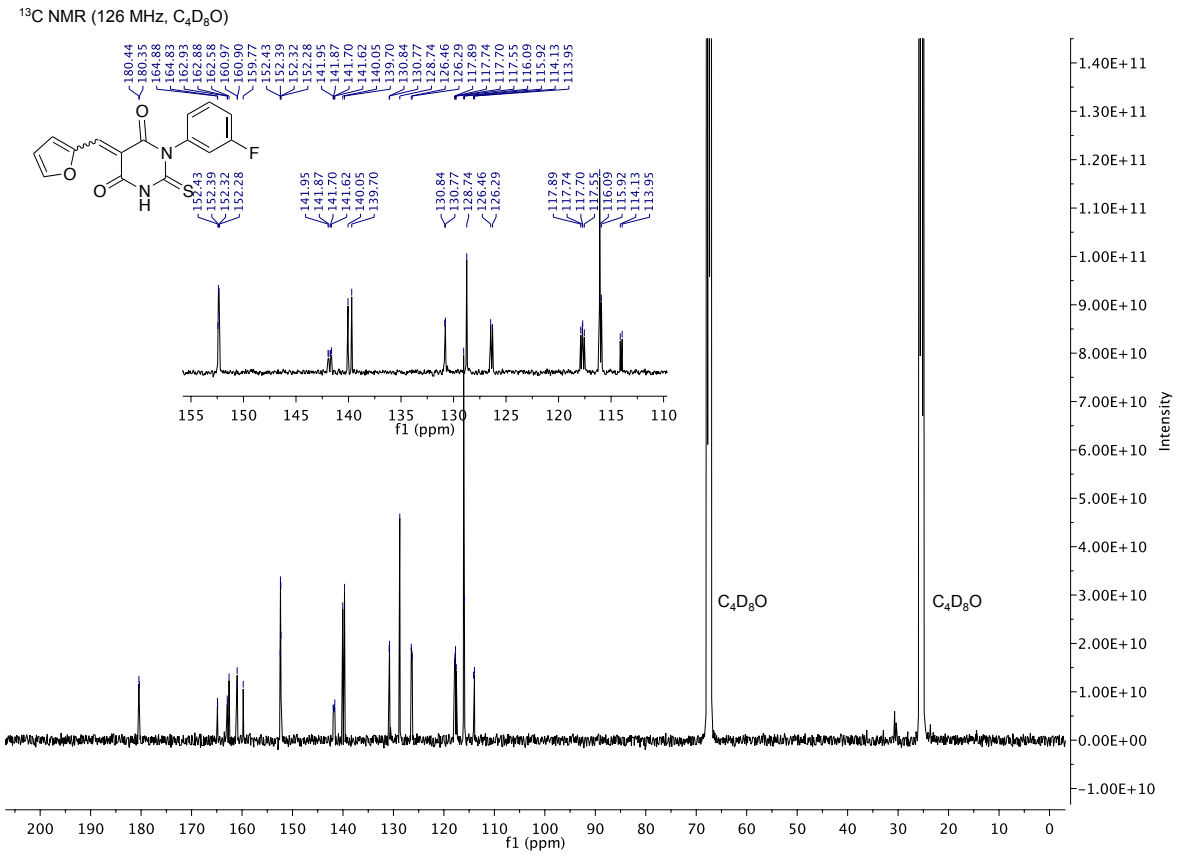

1H NMR (500 MHz, C2D6OS)
11.77
11.69
8.55
8.54
8.37
8.36
8.31
8.29
8.16
8.09
7.64
7.62
7.48
7.47
7.47
7.46
7.45
7.44
7.40
7.38
7.36
6.96
6.89
3.36
2.51
Br
0.46
0.51
0.89
0.53
0.49
0.48
0.57
f1 (ppm)
C2D6OS
HDO
f1 (ppm)


13C NMR (126 MHz, C2D6OS)
C2D6OS
f1 (ppm)
Intensity

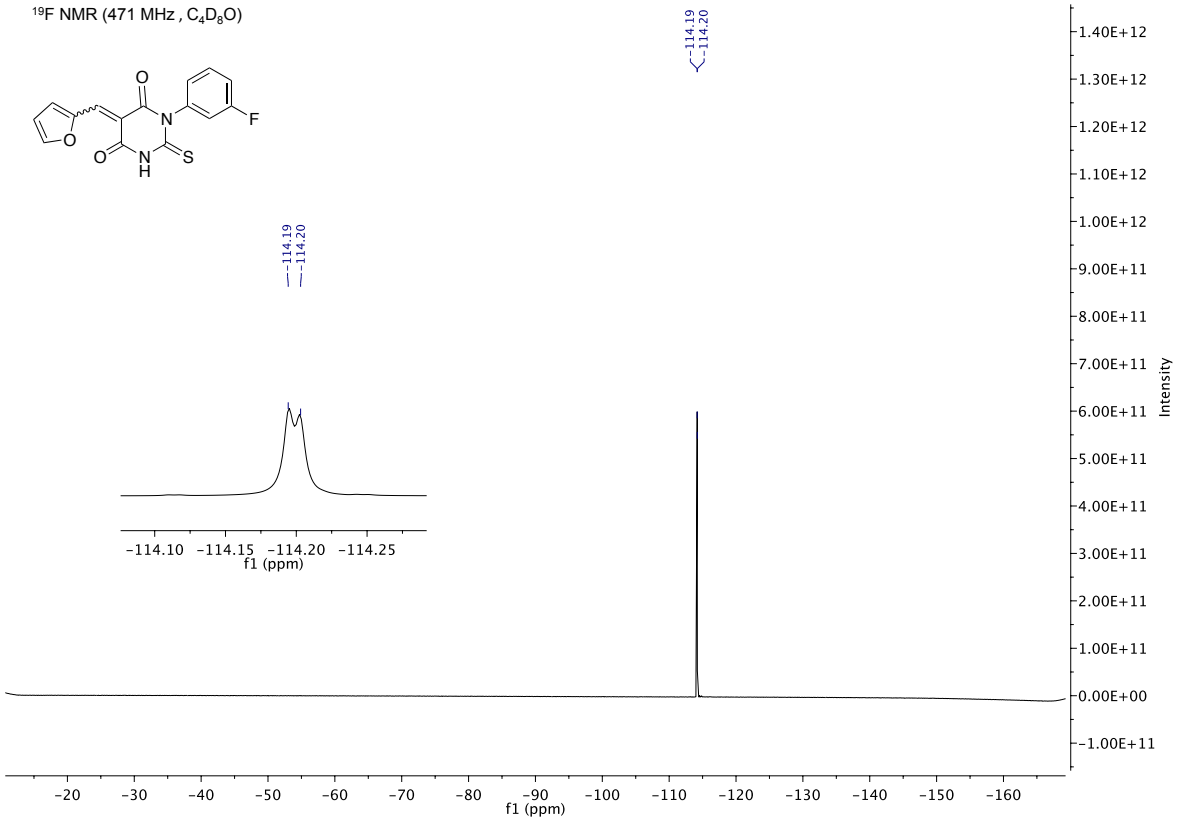
Compound **6m**


1H NMR (500 MHz, C4D8O)
C4D8O
HDO
C4D8O
f1 (ppm)

^{13}C NMR (126 MHz, C_4D_8O)

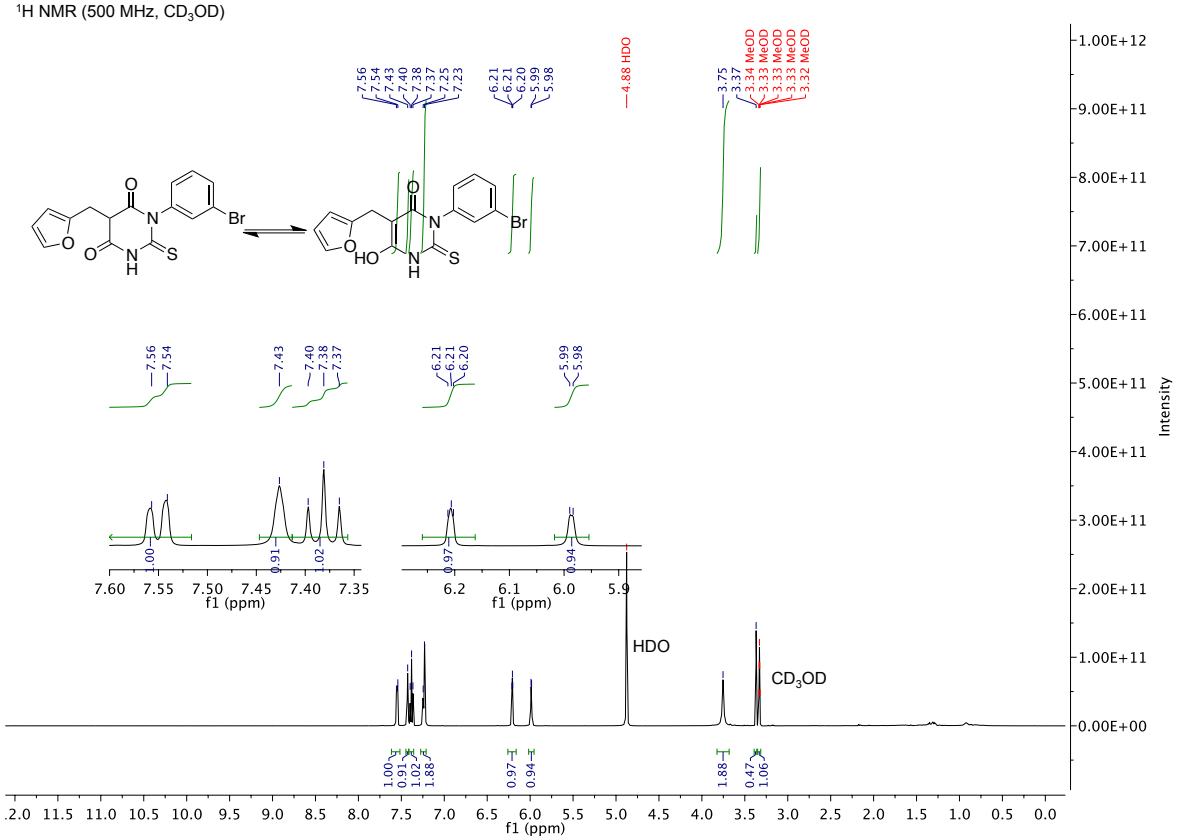


^{19}F NMR (471 MHz , C_4D_8O)

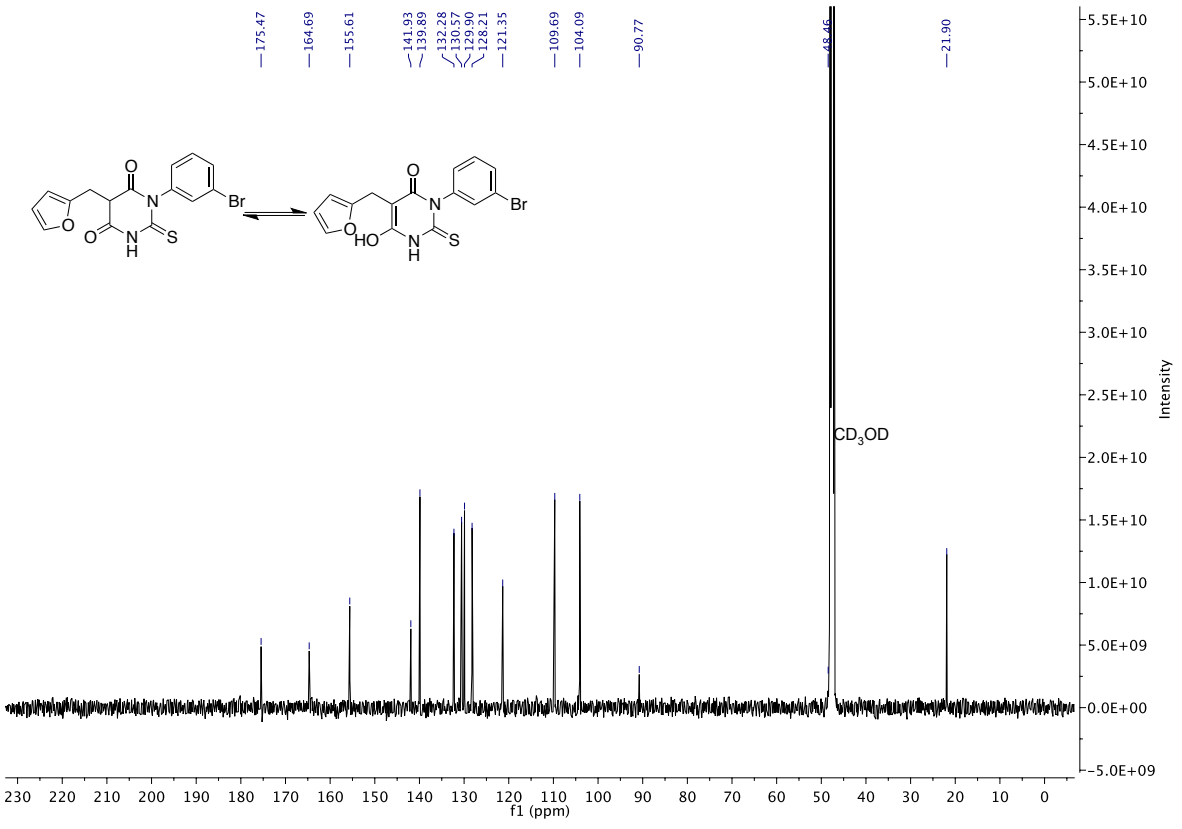


Compound **7a**

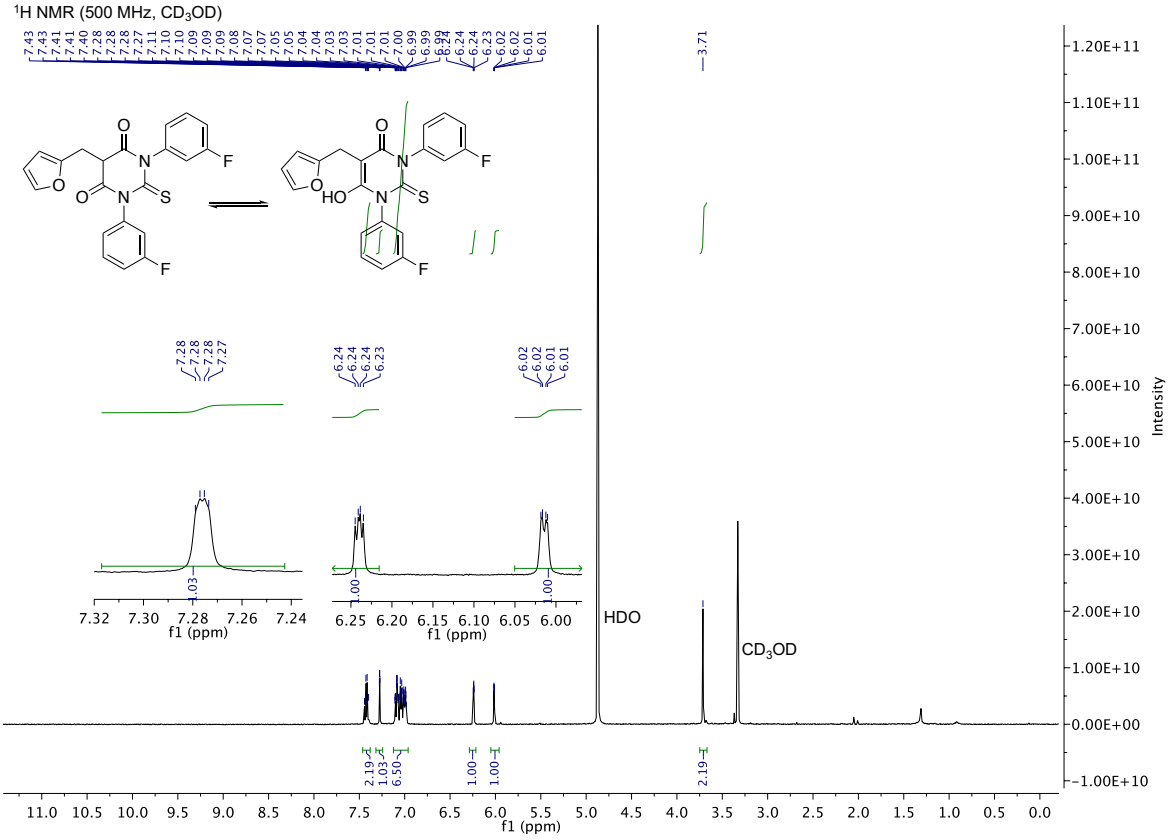
^{1}H NMR (500 MHz, CD_3OD)

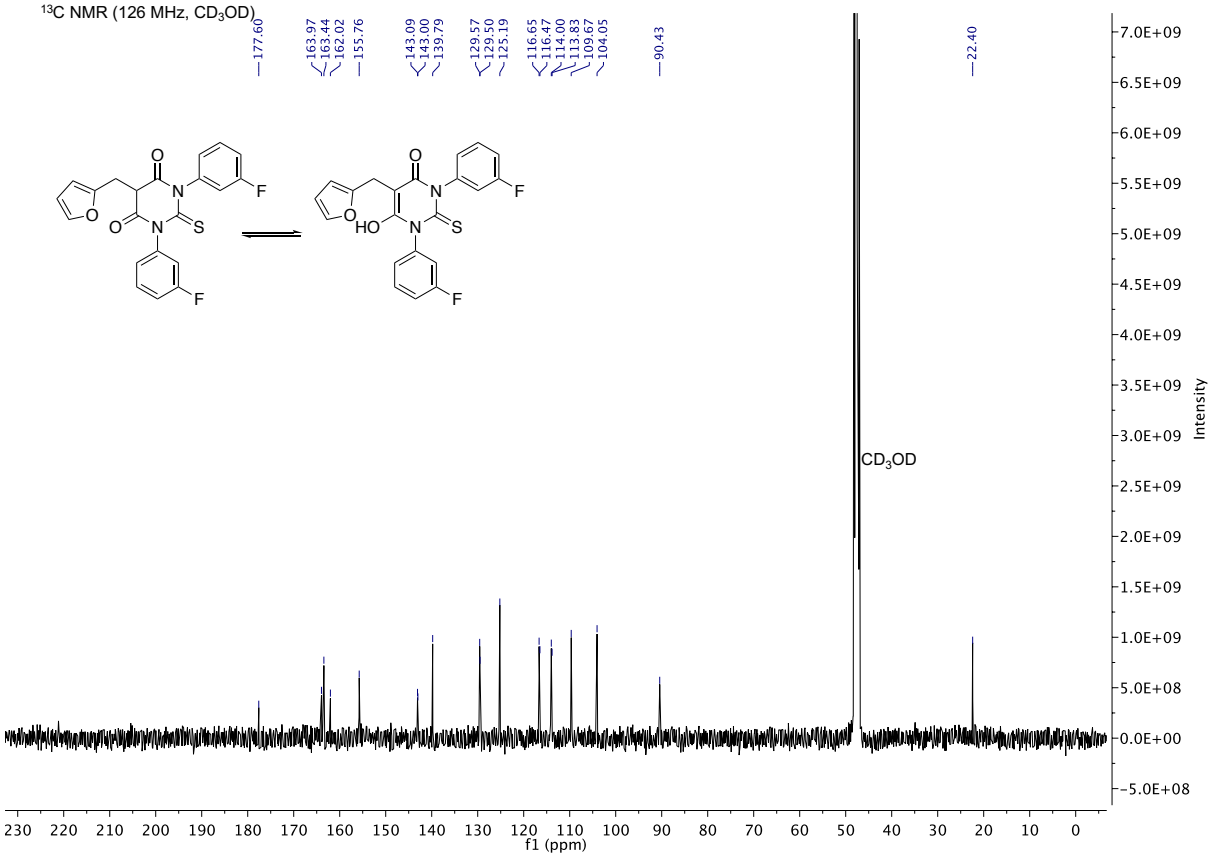


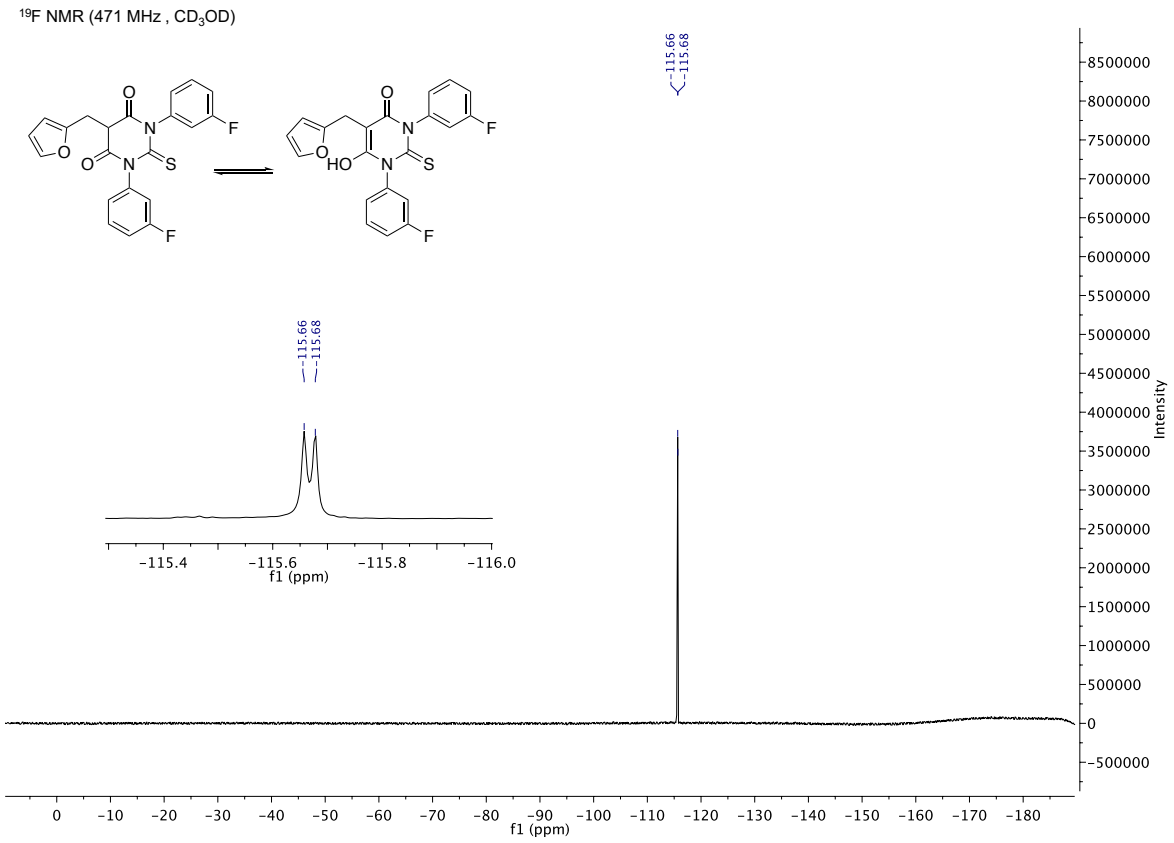
^{13}C NMR (126 MHz, CD_3OD)



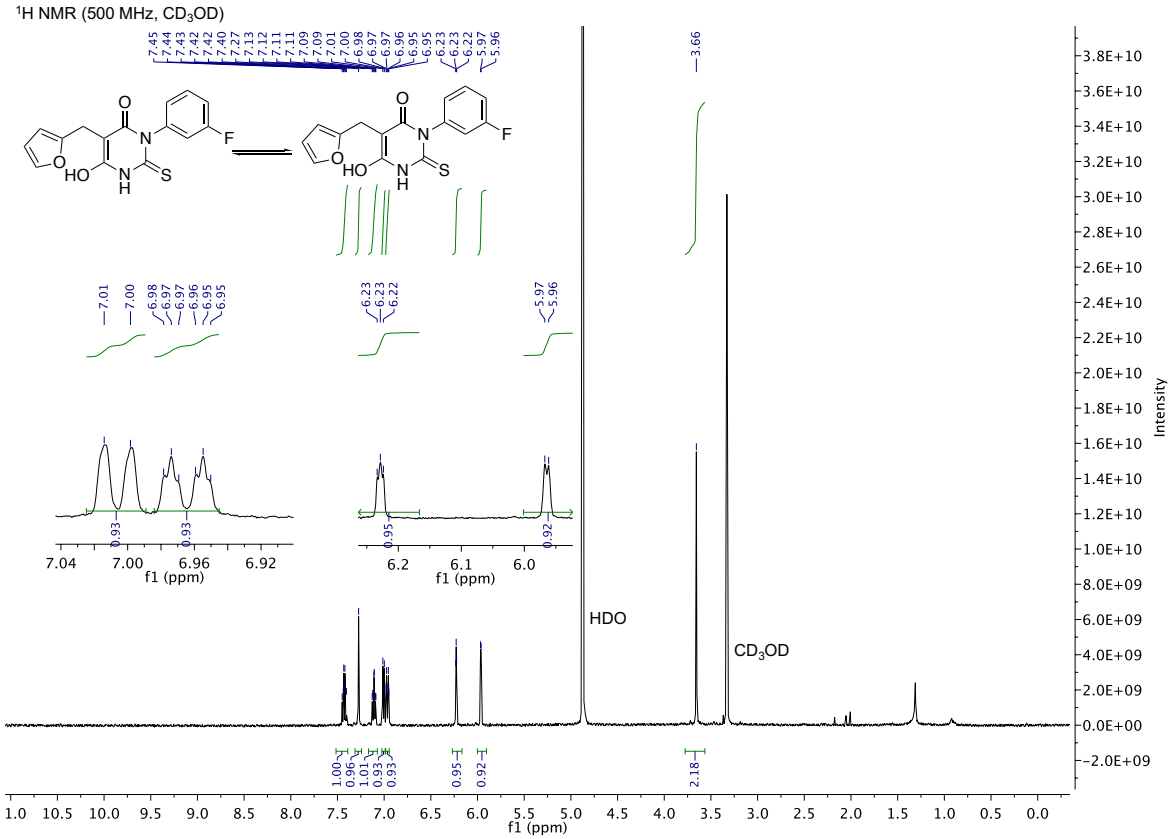
Compound **7b**


1H NMR (500 MHz, CD3OD)
HDO
CD3OD
f1 (ppm)
Intensity

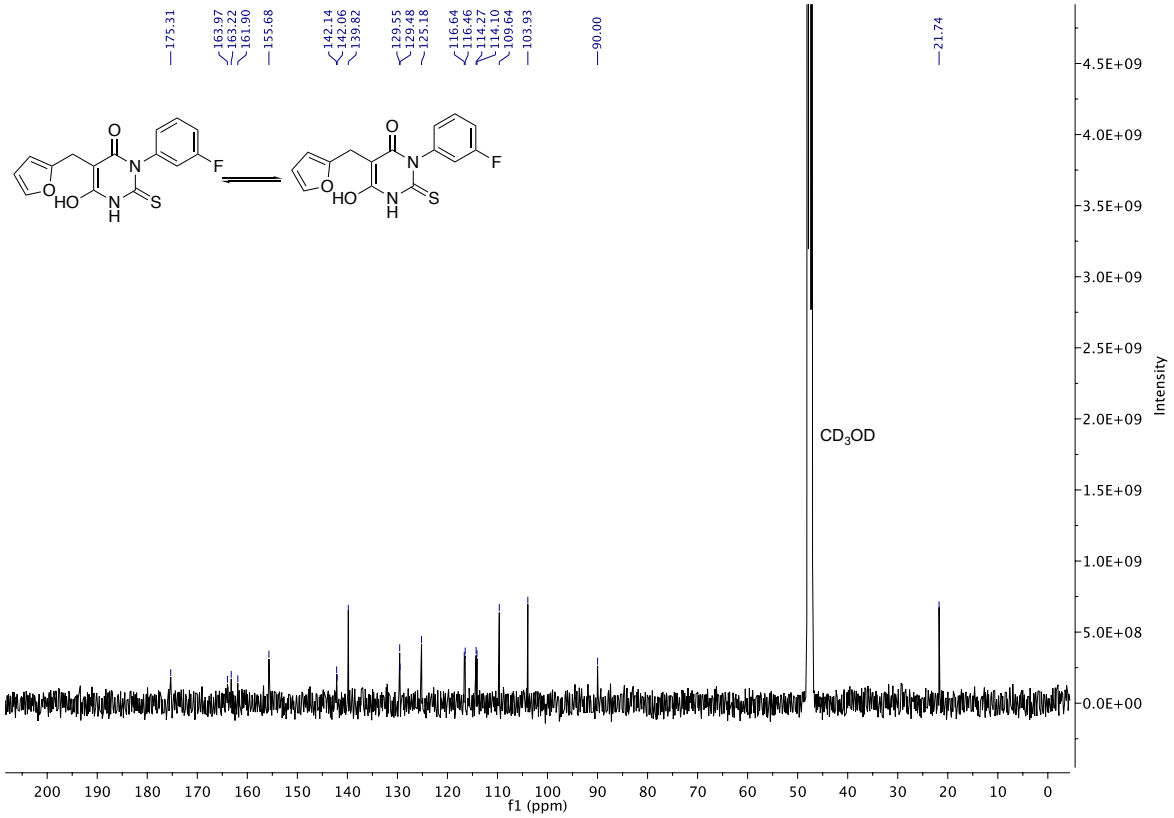

13C NMR (126 MHz, CD3OD)
CD3OD
f1 (ppm)
Intensity


19F NMR (471 MHz , CD3OD)
-115.66
-115.68
f1 (ppm)
Intensity

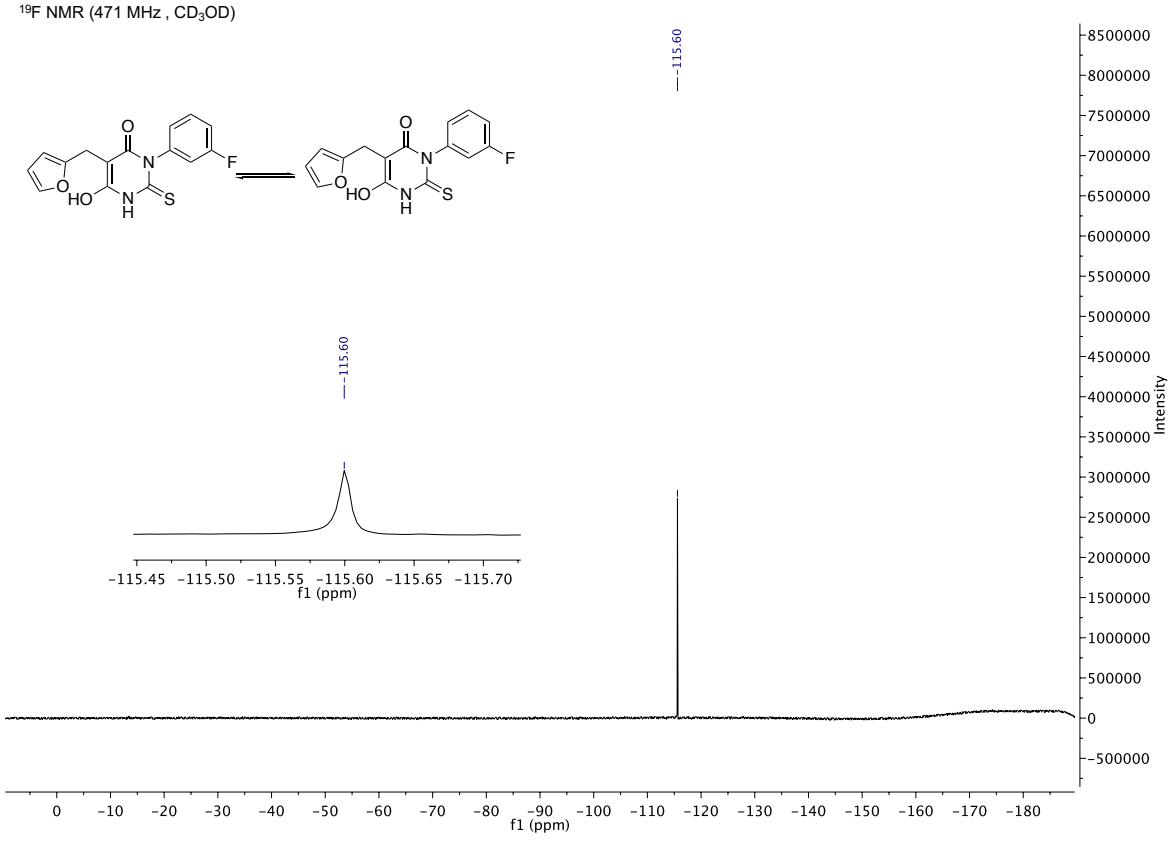
Compound **7c**


1H NMR (500 MHz, CD3OD)
HDO
CD3OD
f1 (ppm)
Intensity

^{13}C NMR (126 MHz, CD_3OD)

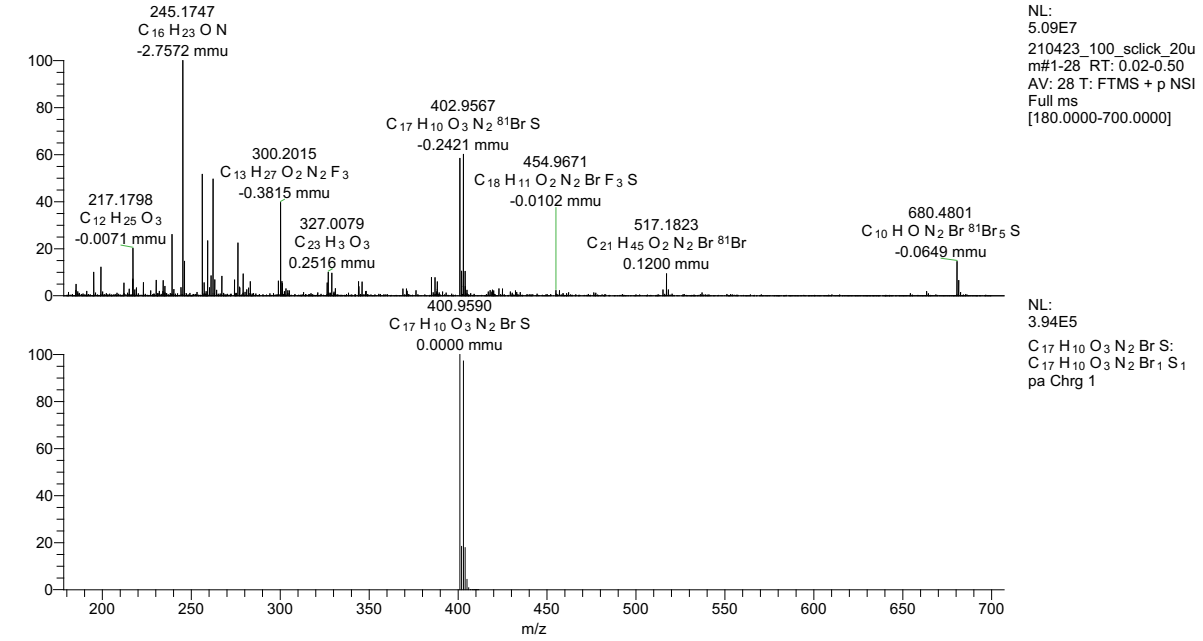


^{19}F NMR (471 MHz , CD_3OD)

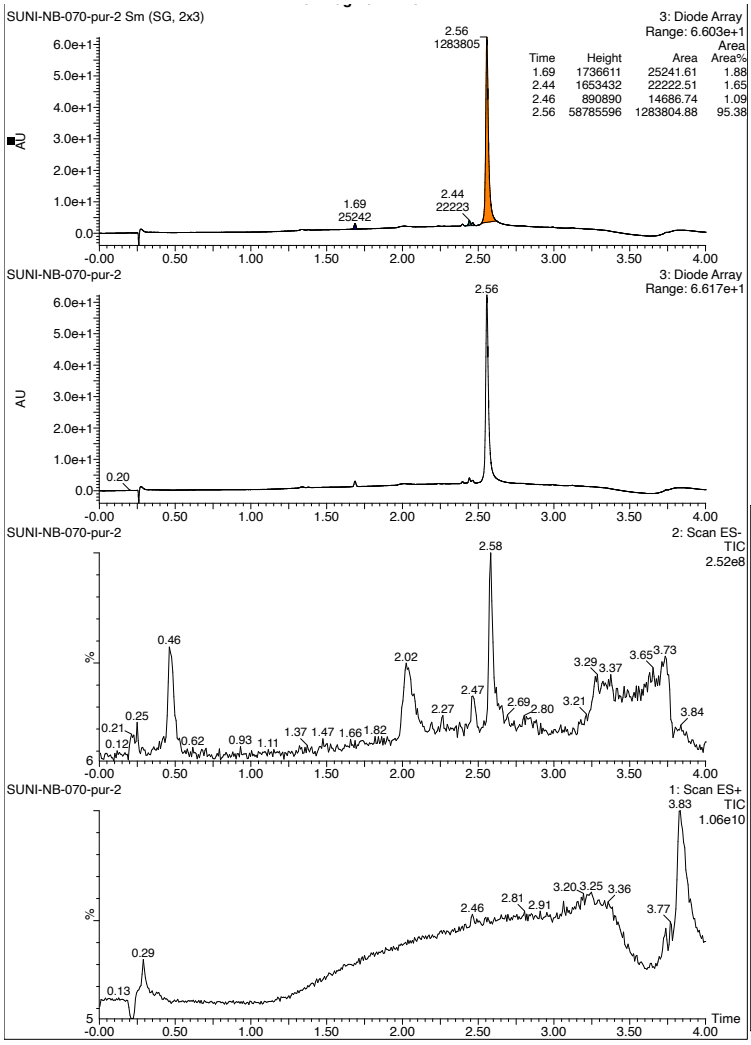


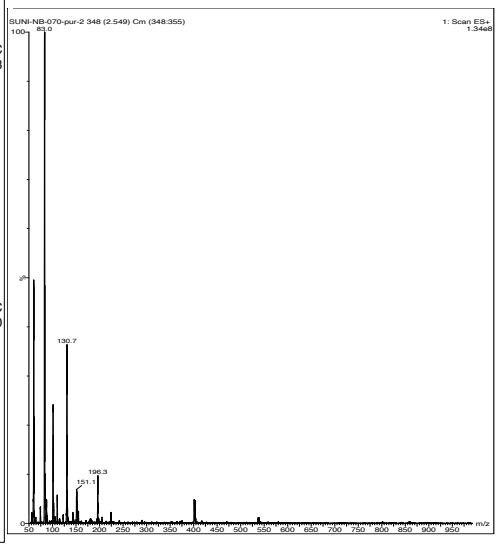
HRMS spectra and UPLC-MS chromatogram of final compounds

Compound **5a**

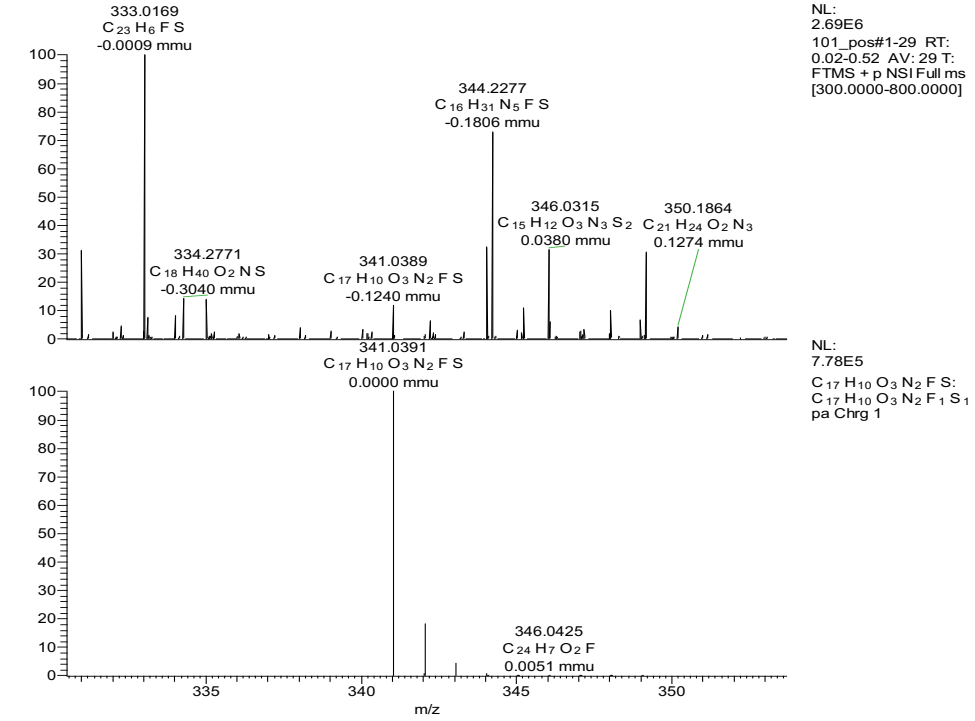


UPLC-MS

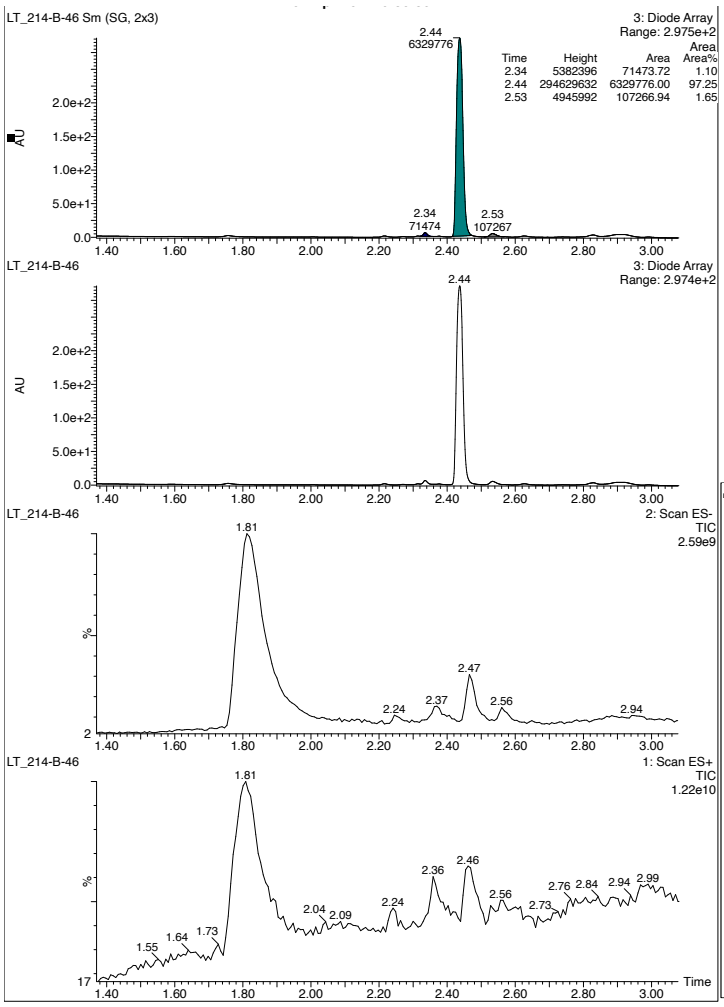


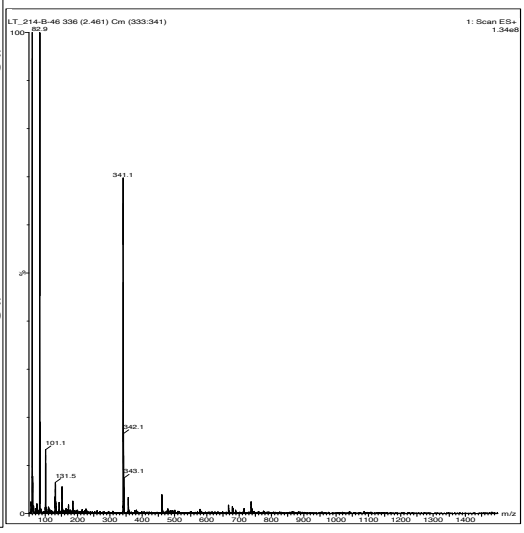


Compound **5b**

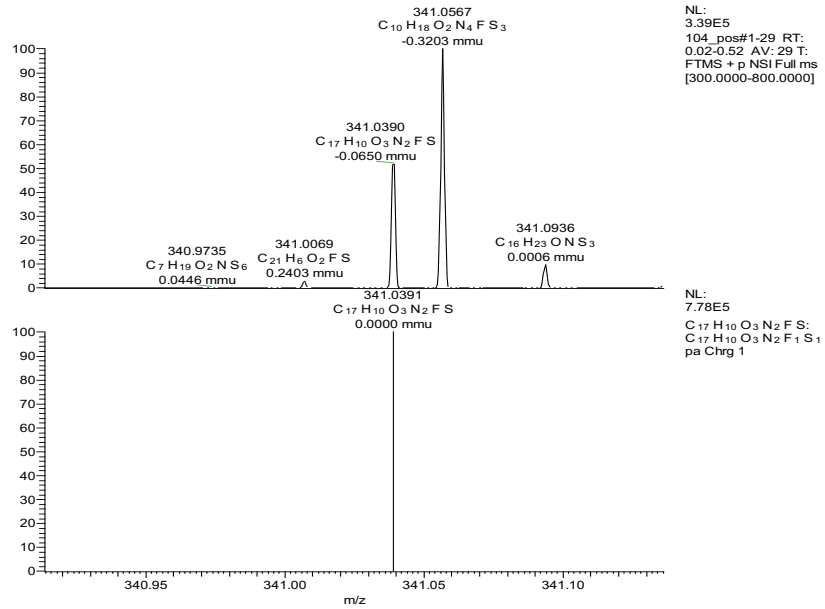


UPLC-MS

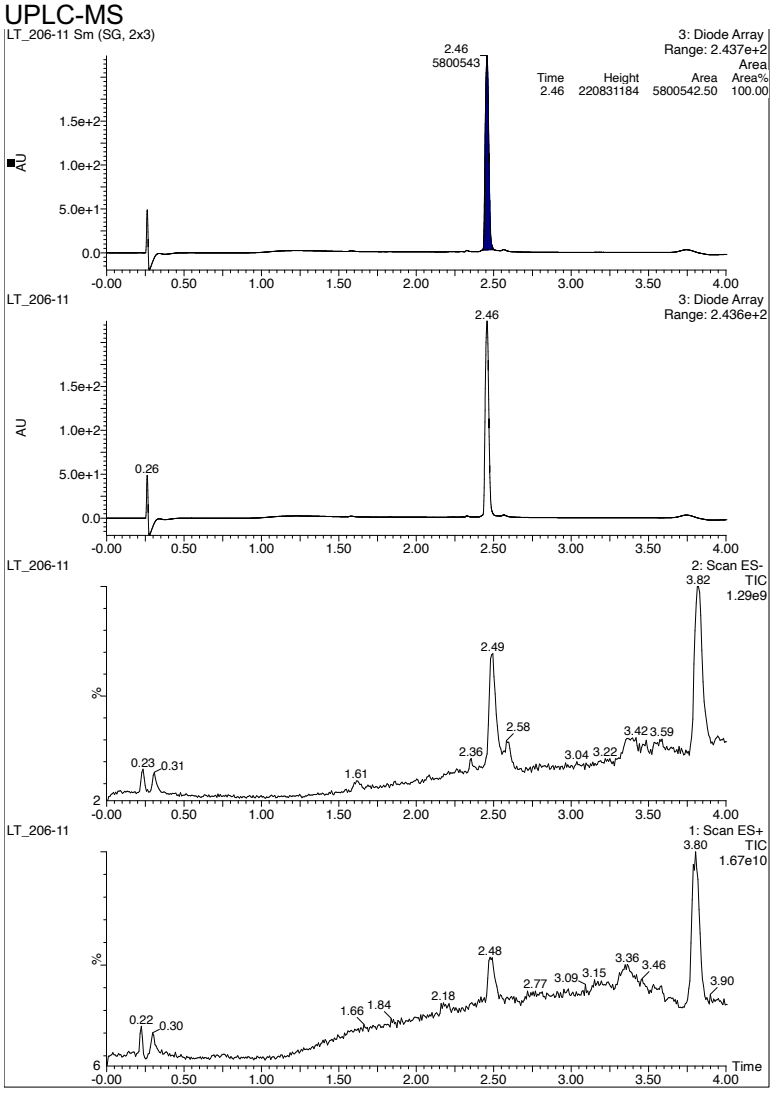


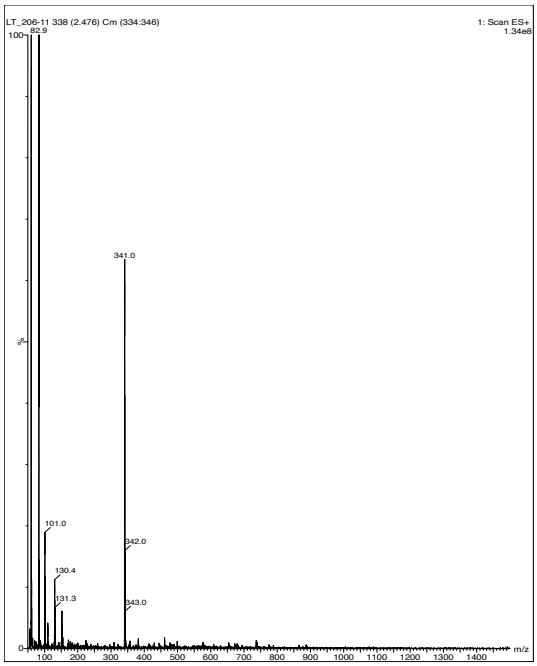


Compound **5c**

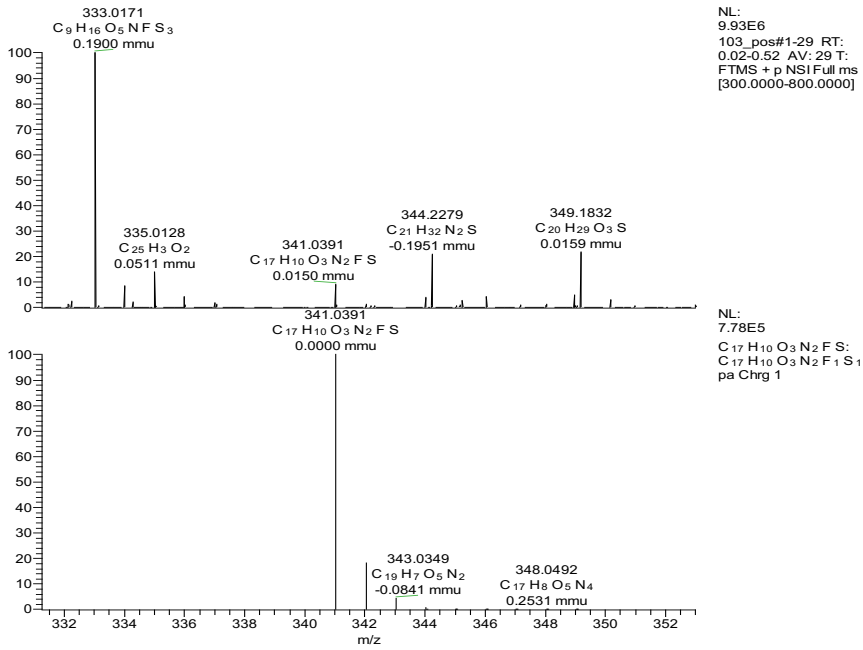


UPLC-MS

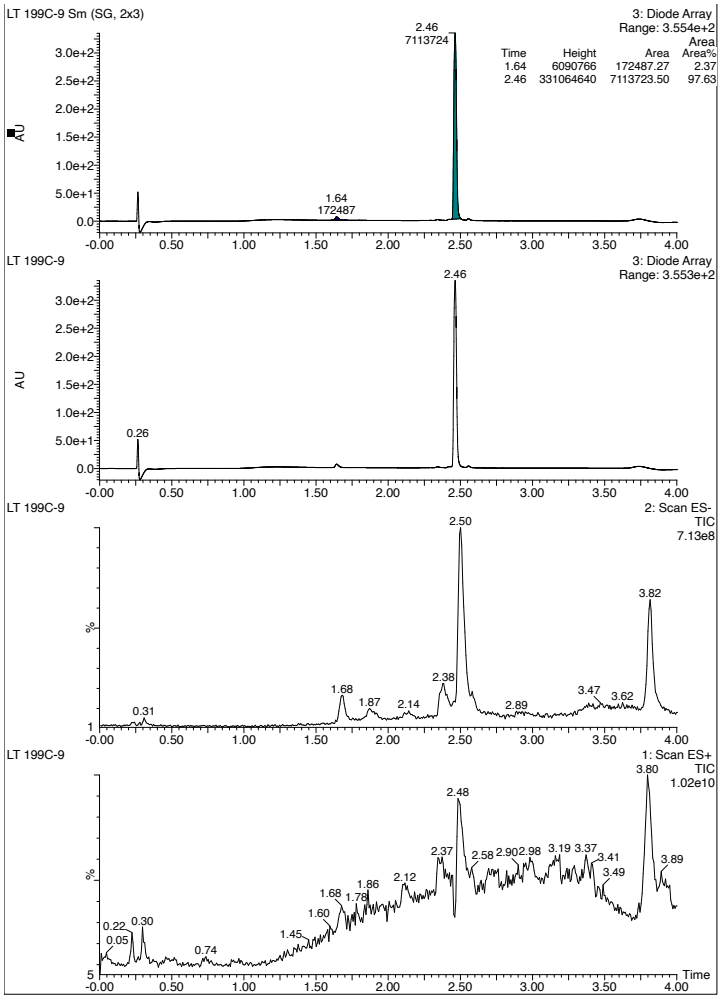


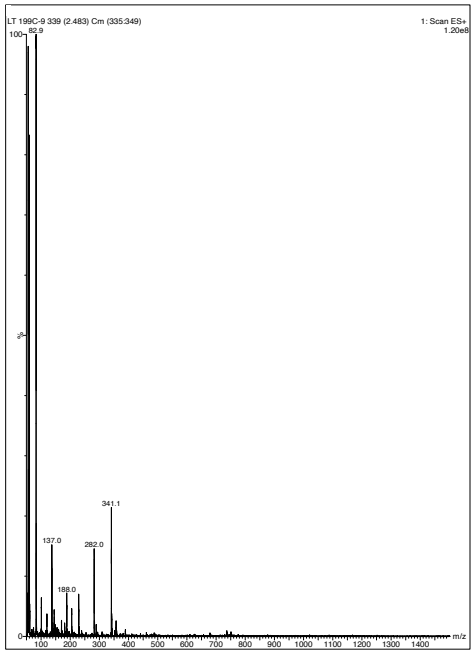


Compound **5d**

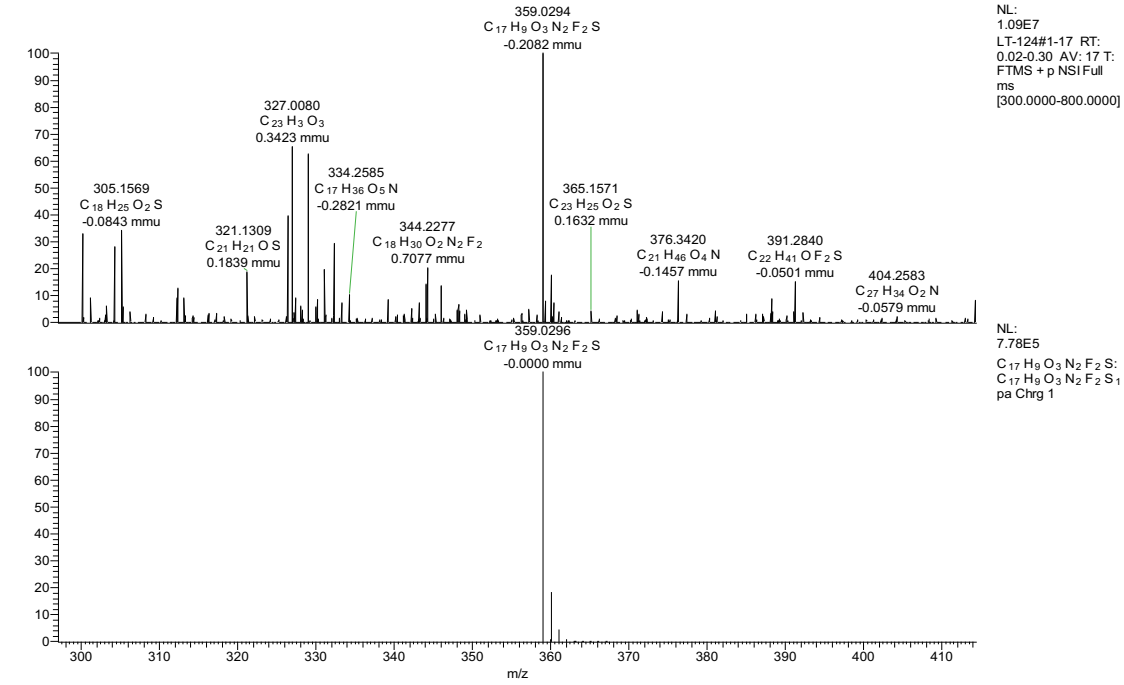


UPLC-MS

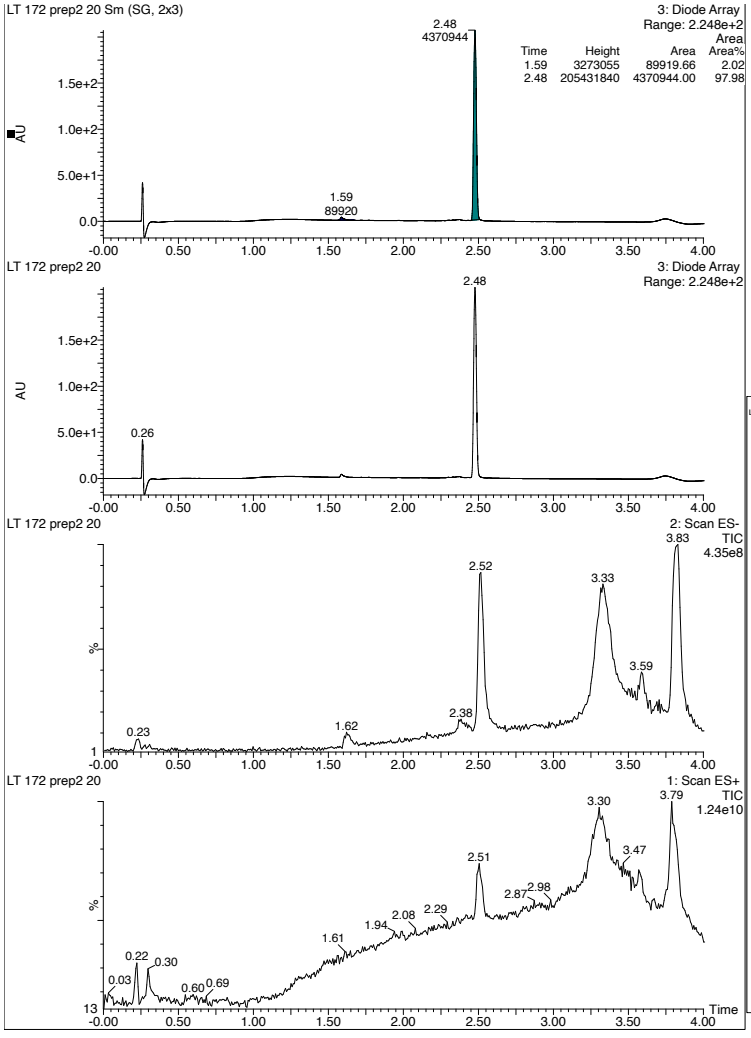


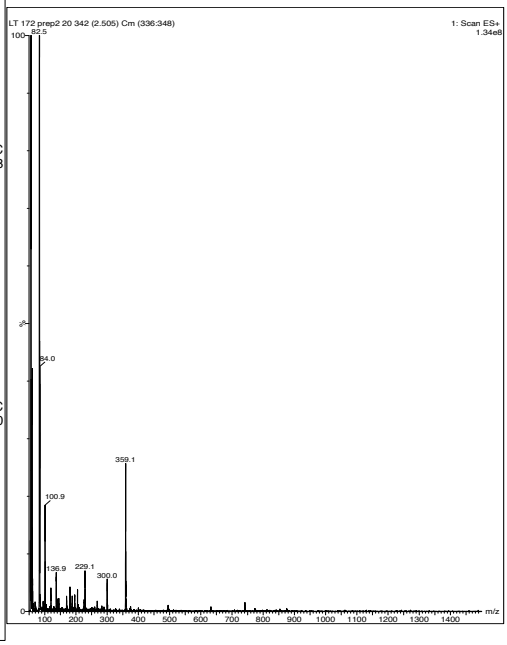


Compound **5e**

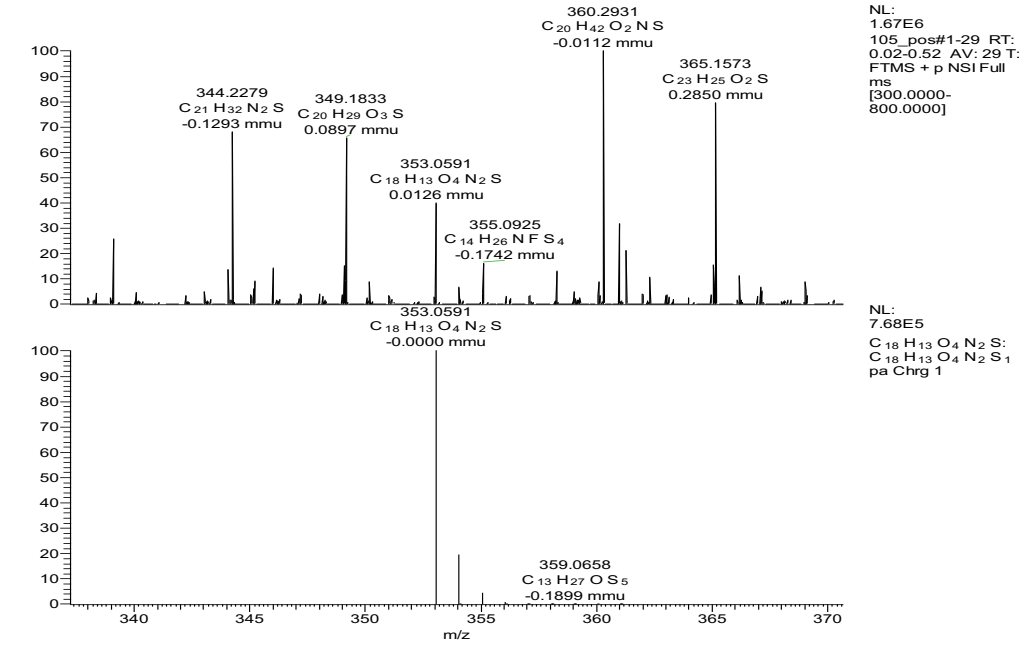


UPLC-MS

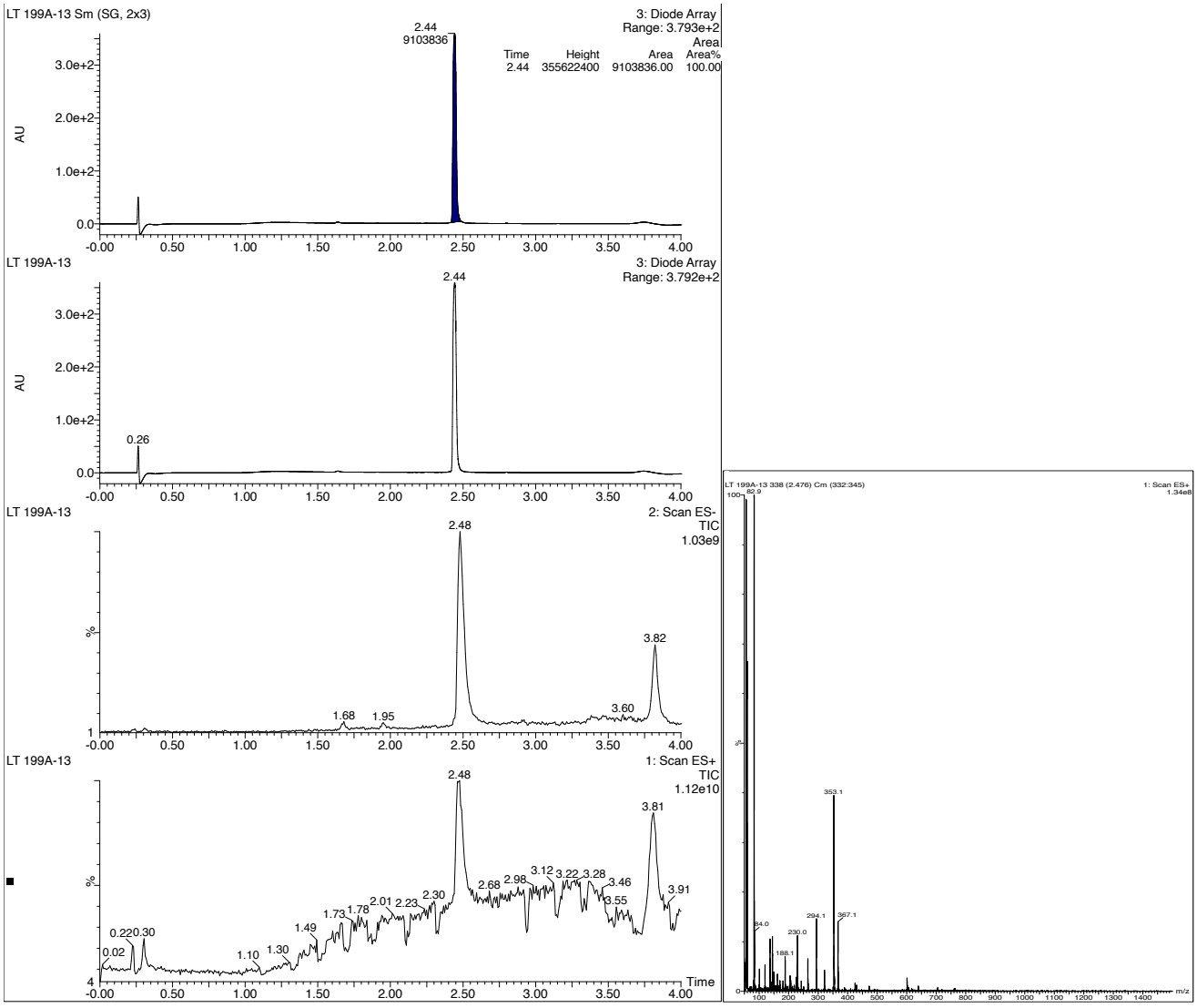




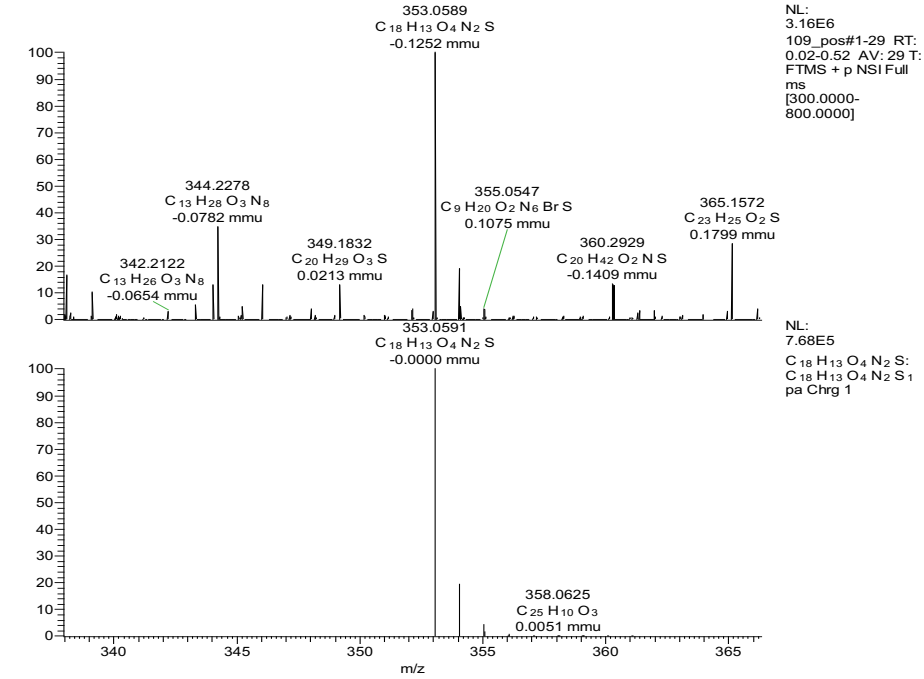
Compound **5f**



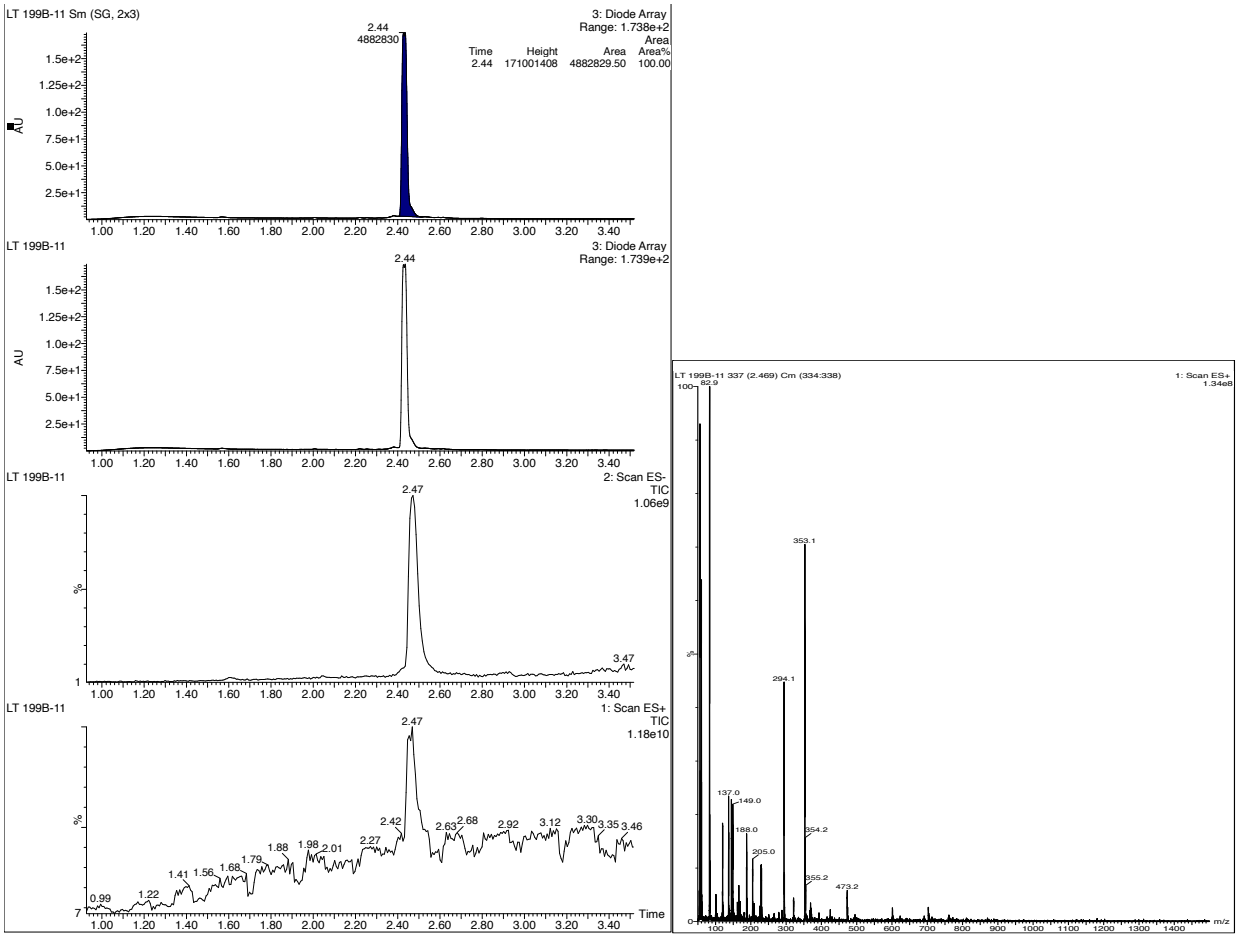
UPLC-MS



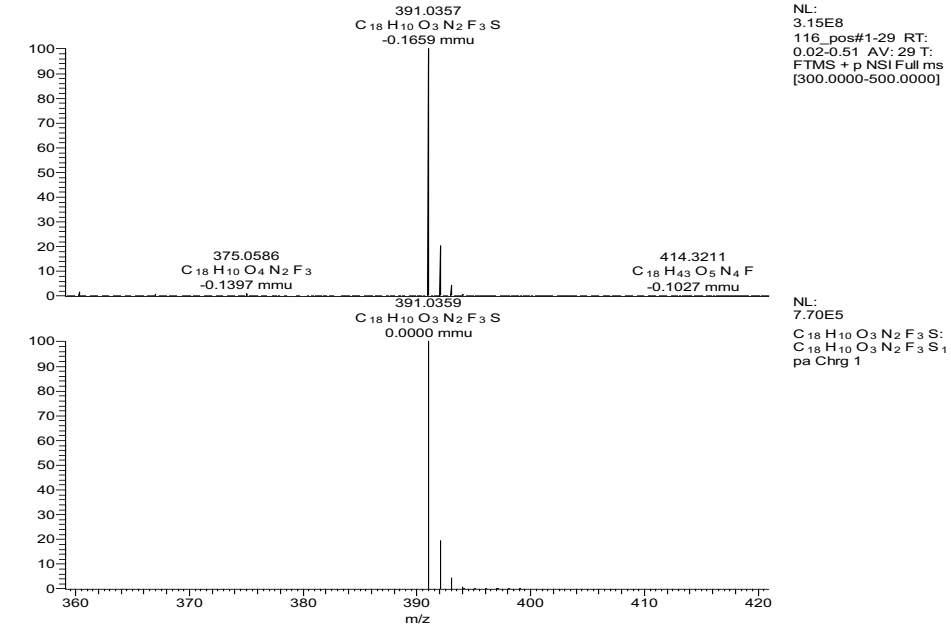
Compound **5g**



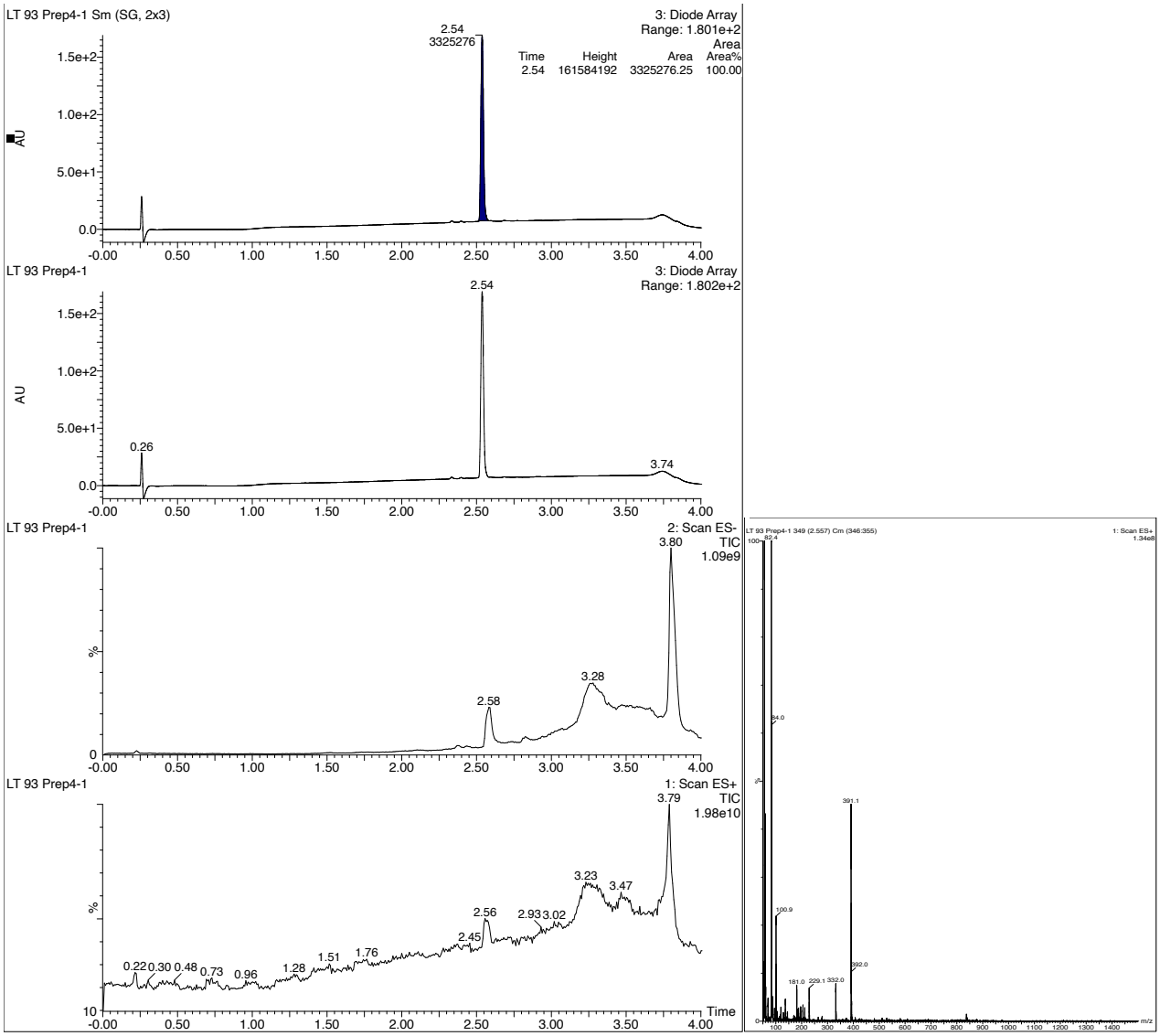
UPLC-MS



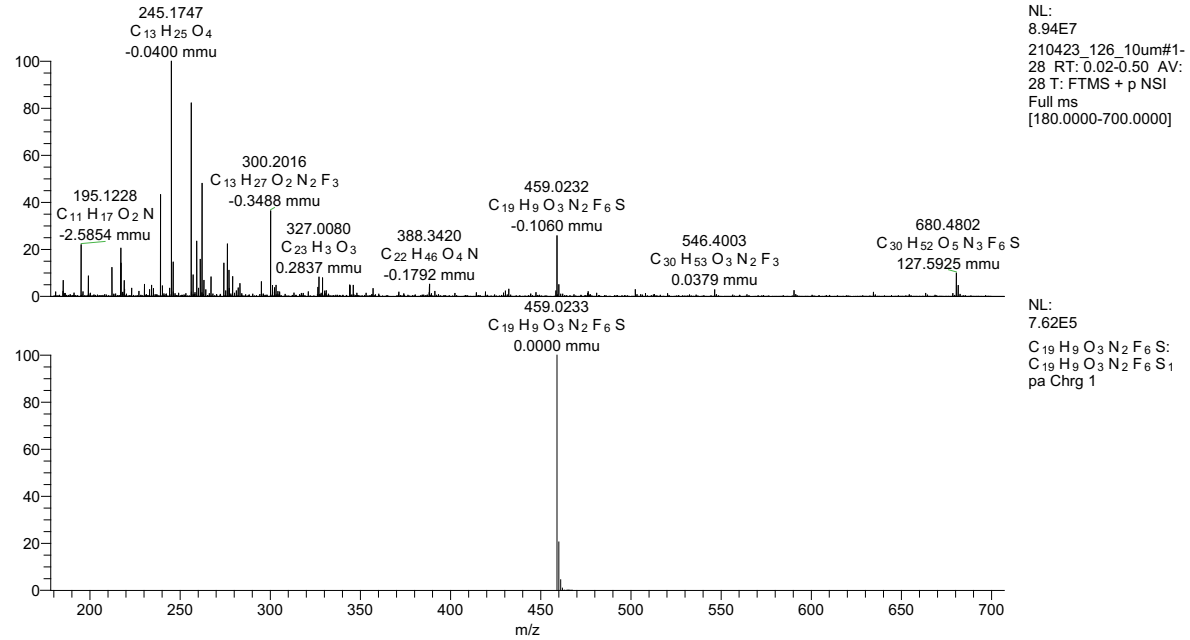
Compound **5h**



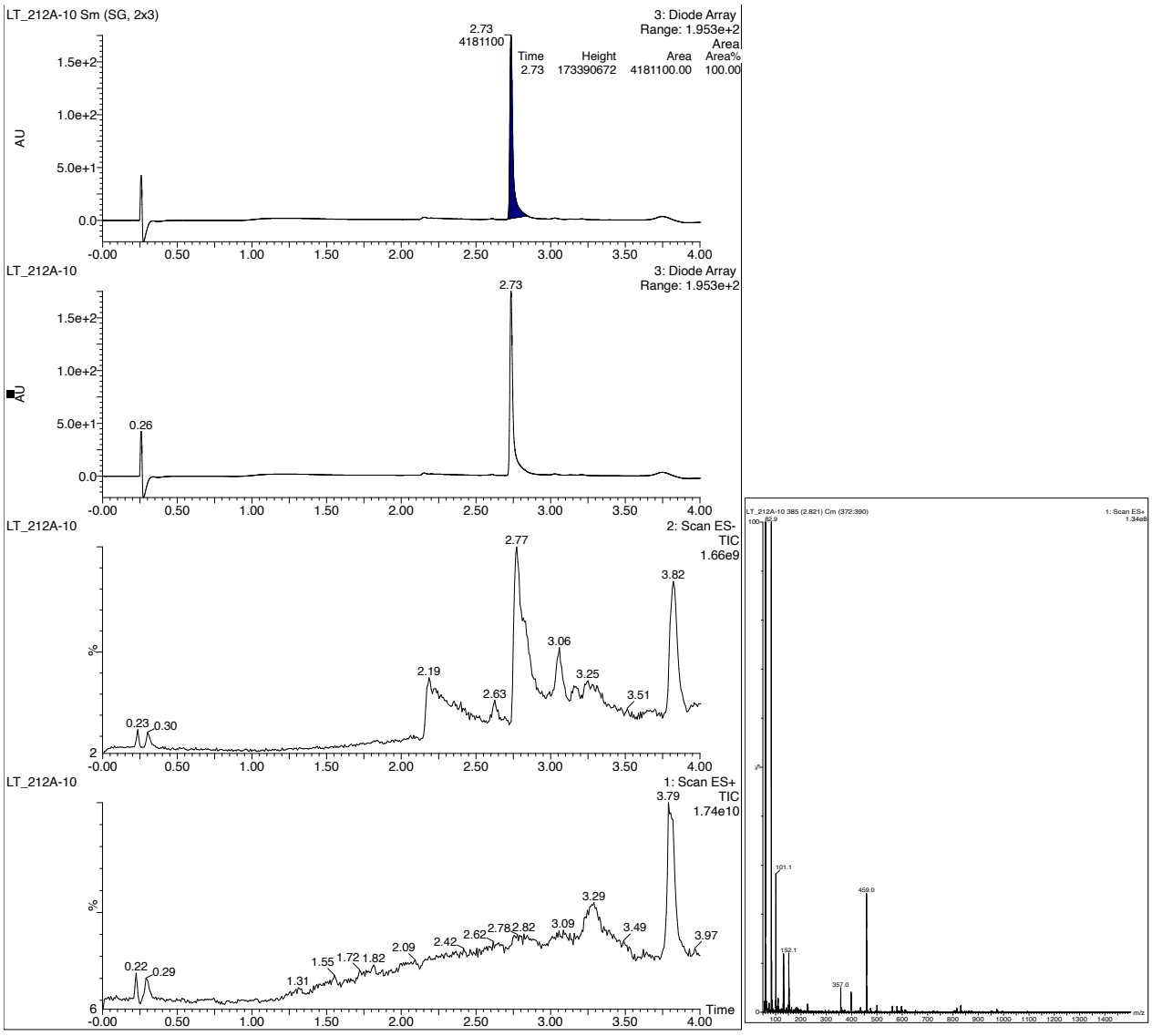
UPLC-MS



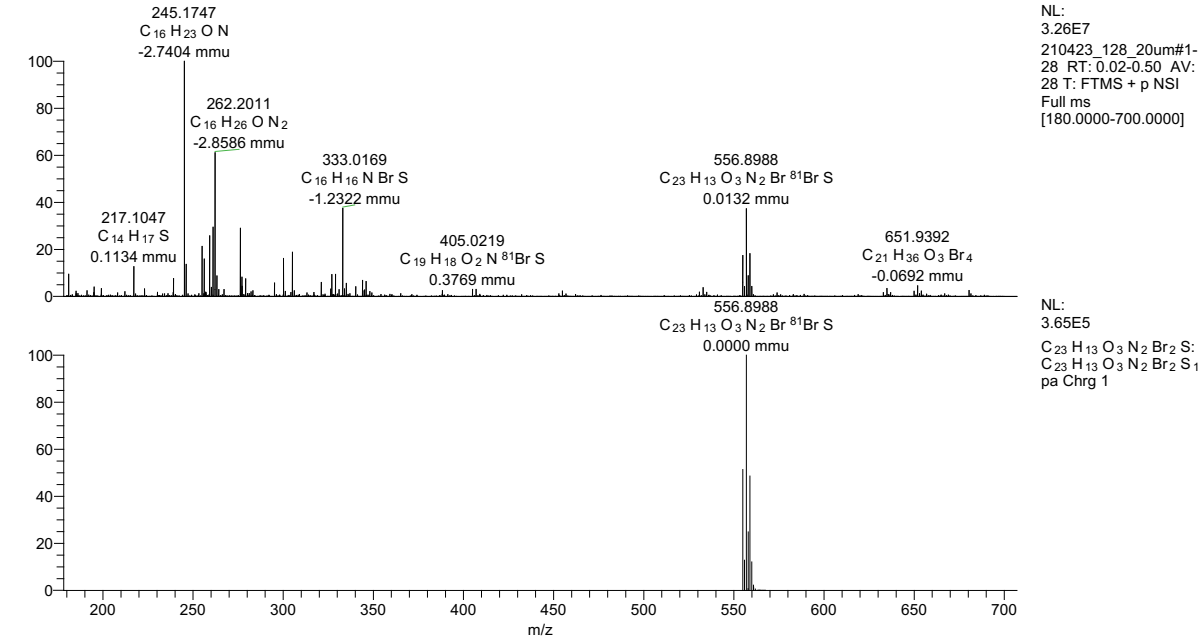
Compound **5i**



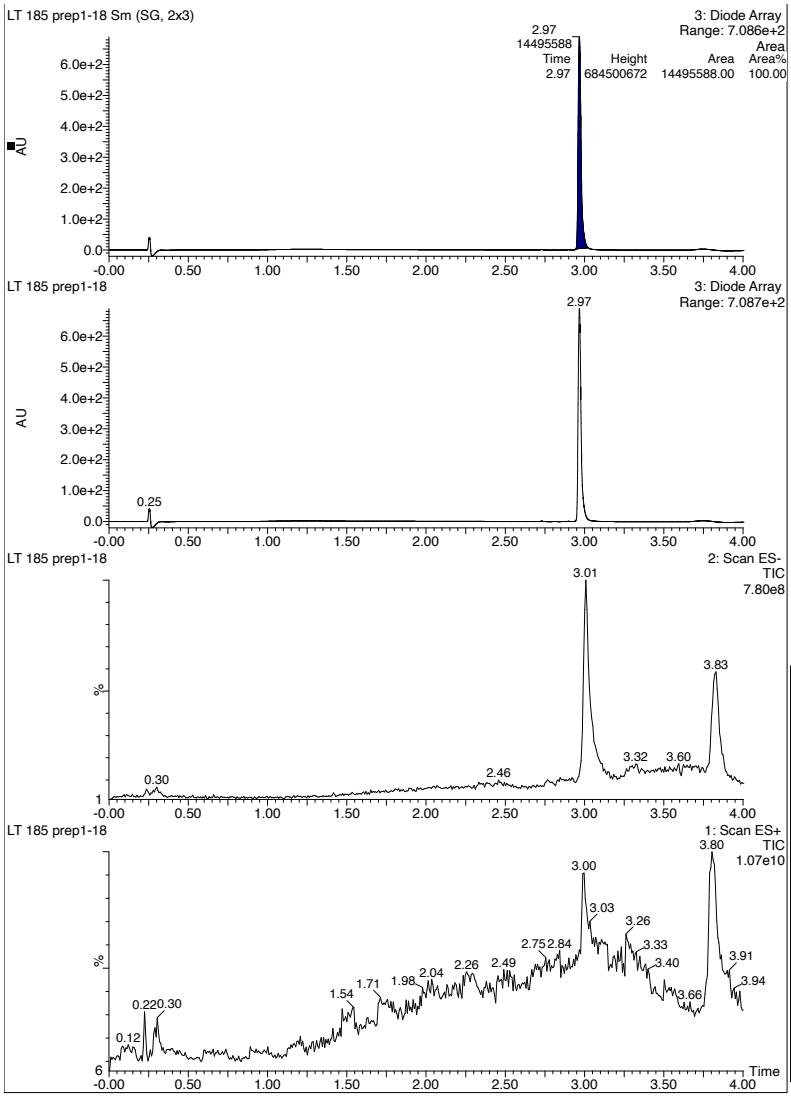
UPLC-MS



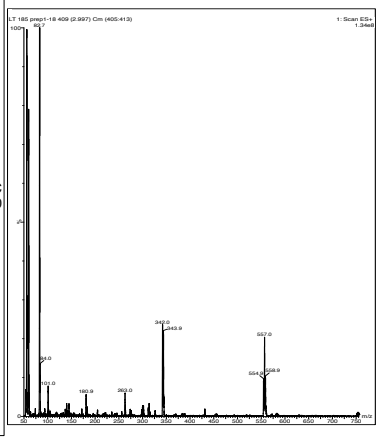
Compound **5j**

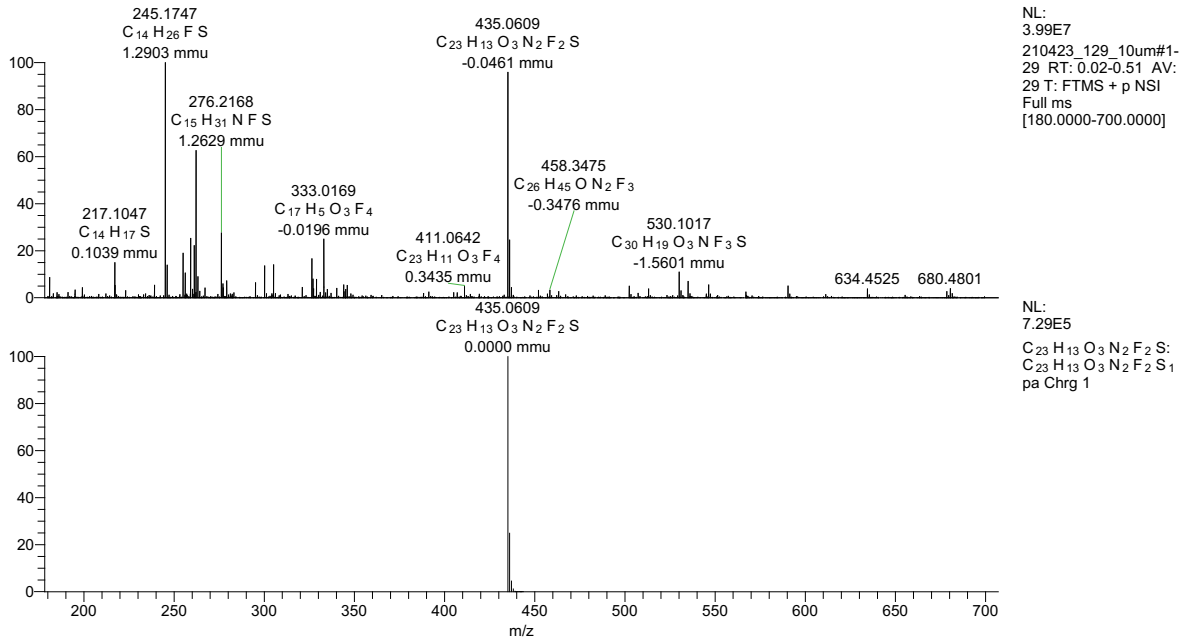


UPLC-MS

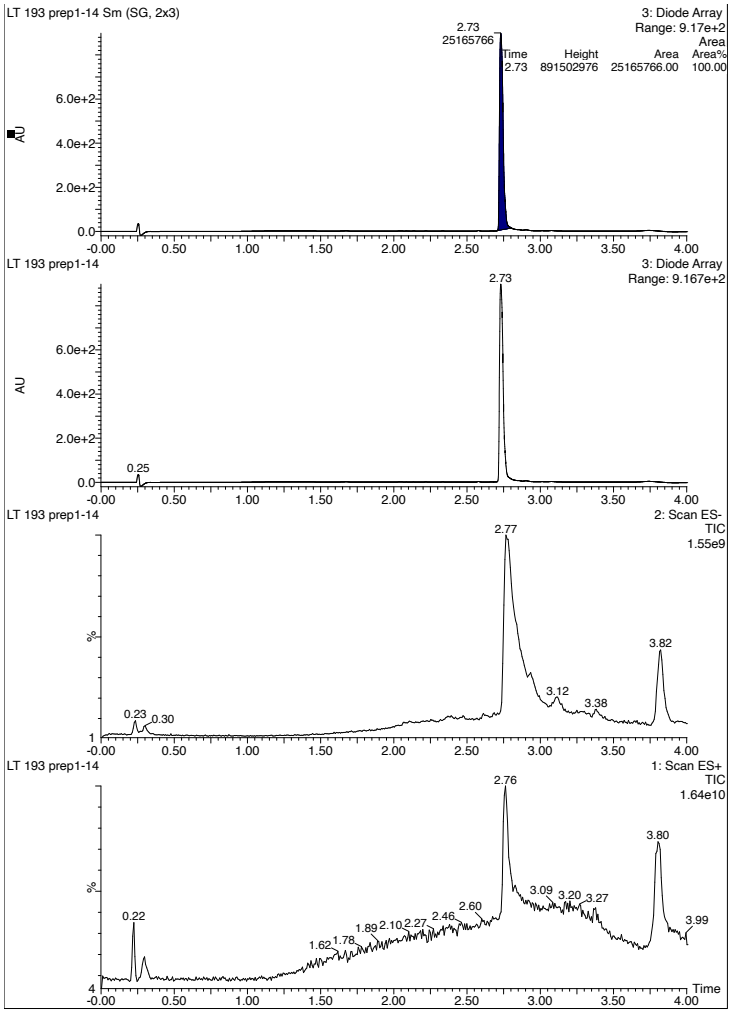


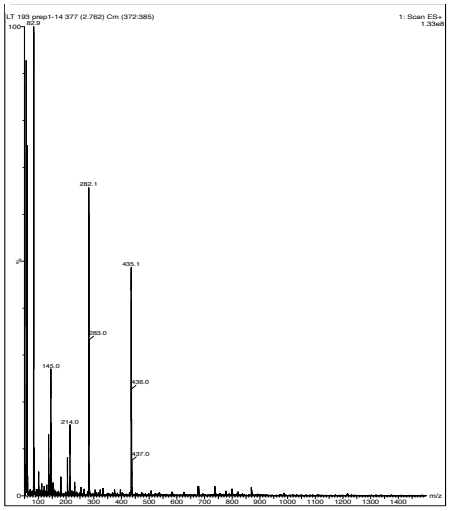
Compound **5k**



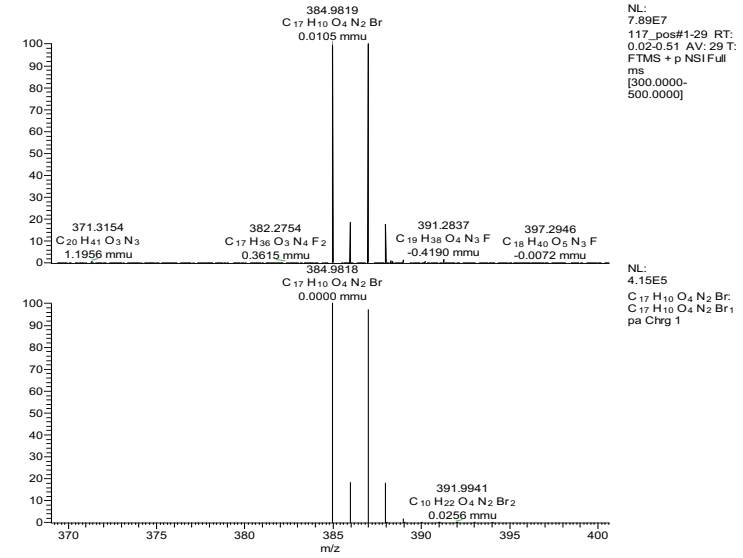

245.1747
C14 H26 F S
1.2903 mmu
435.0609
C23 H13 O3 N2 F2 S
-0.0461 mmu
276.2168
C15 H31 N F S
1.2629 mmu
458.3475
C26 H45 O N2 F3
-0.3476 mmu
333.0169
C17 H5 O3 F4
-0.0196 mmu
217.1047
C14 H17 S
0.1039 mmu
411.0642
C23 H11 O3 F4
0.3435 mmu
530.1017
C30 H19 O3 N F3 S
-1.5601 mmu
634.4525
680.4801
NL:
3.99E7
210423_129_10um#1-29 RT: 0.02-0.51 AV: 29 T: FTMS + p NSI Full ms [180.0000-700.0000]
435.0609
C23 H13 O3 N2 F2 S
0.0000 mmu
NL:
7.29E5
C23 H13 O3 N2 F2 S: C23 H13 O3 N2 F2 S1 pa Chrg 1
m/z

UPLC-MS

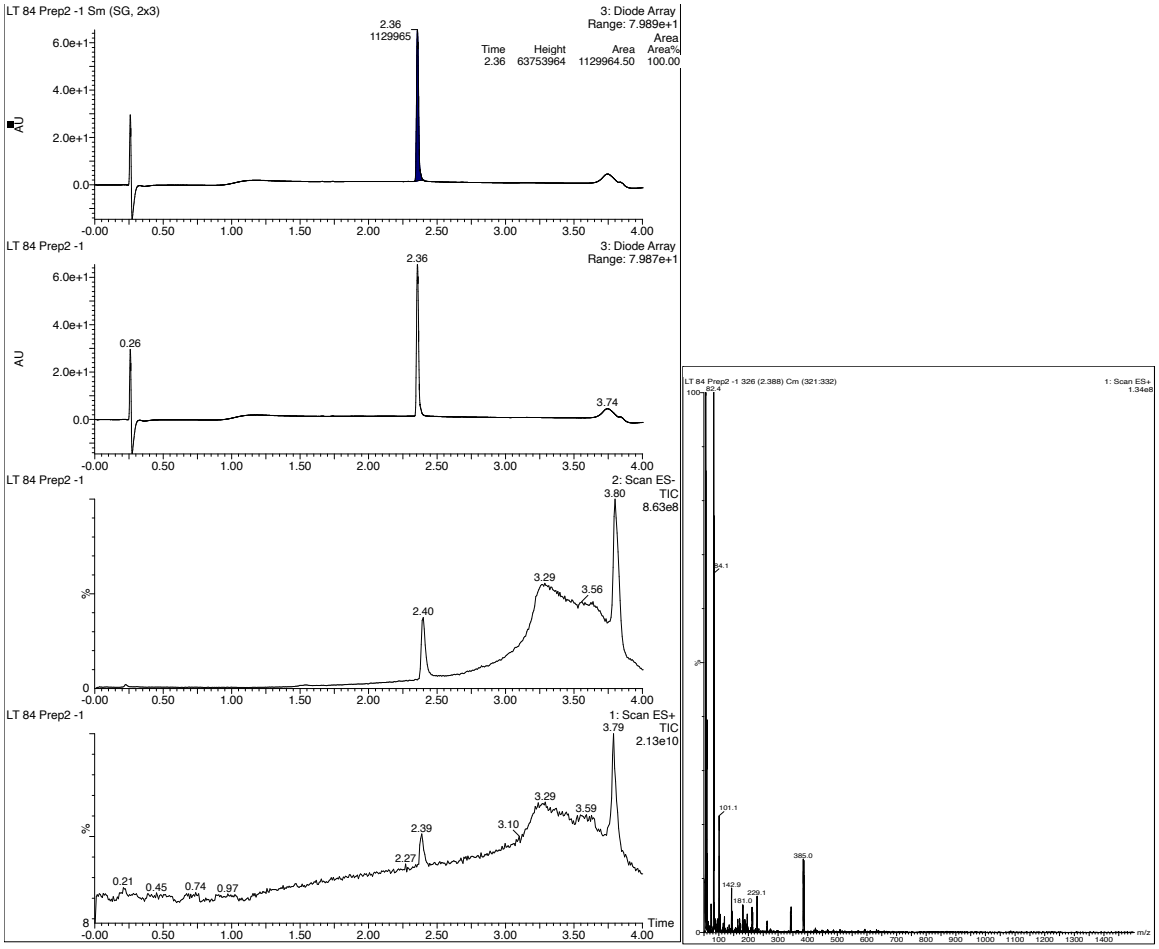

LT 193 prep1-14 Sm (SG, 2x3)
3: Diode Array
Range: 9.17e+2
2.73
25165766
Time Height Area Area%
2.73 891502976 25165766.00 100.00
AU
LT 193 prep1-14
3: Diode Array
Range: 9.167e+2
0.25
LT 193 prep1-14
2: Scan ES-
TIC
1.55e9
0.23 0.30 2.77 3.12 3.38 3.82
LT 193 prep1-14
1: Scan ES+
TIC
1.64e10
0.22 1.62 1.78 1.89 2.10 2.27 2.46 2.60 2.76 3.09 3.20 3.27 3.80 3.99
Time



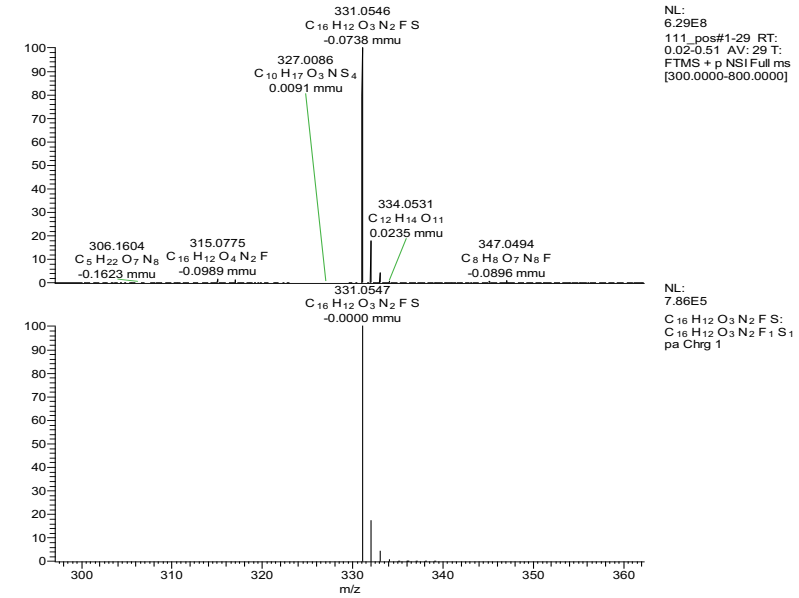
Compound **5l**



UPLC-MS



Compound **6a**



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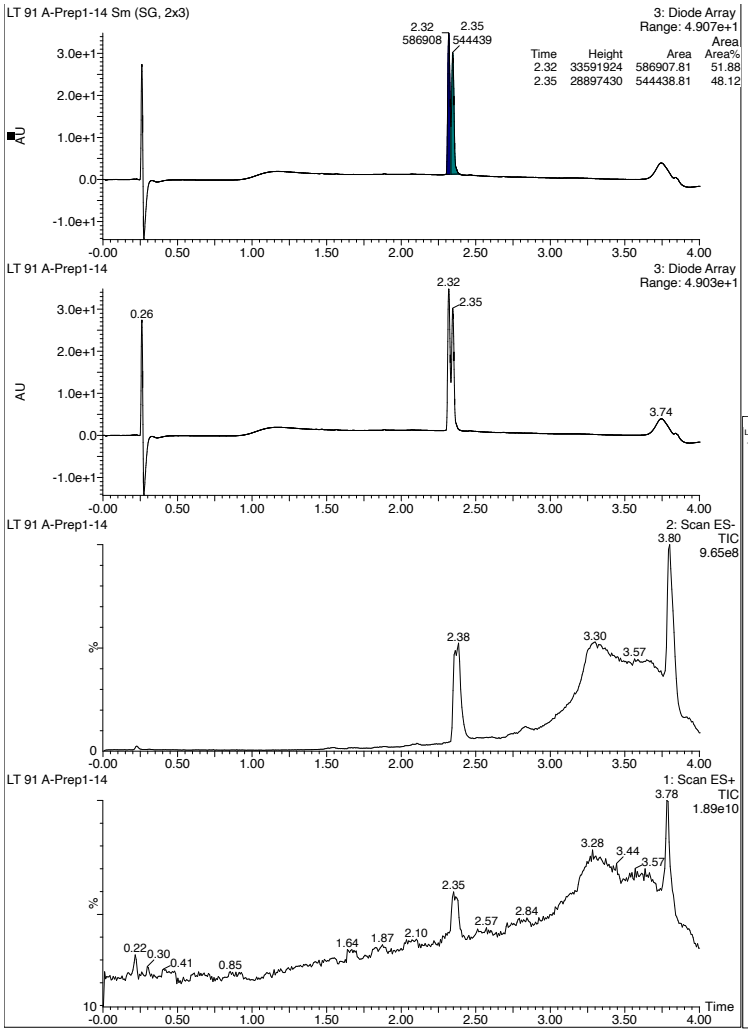

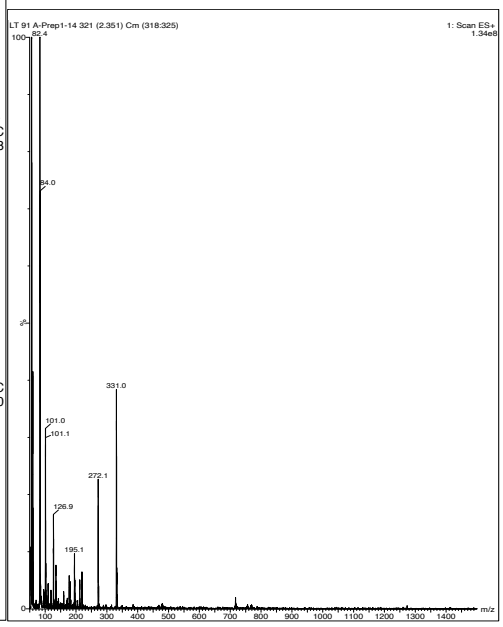

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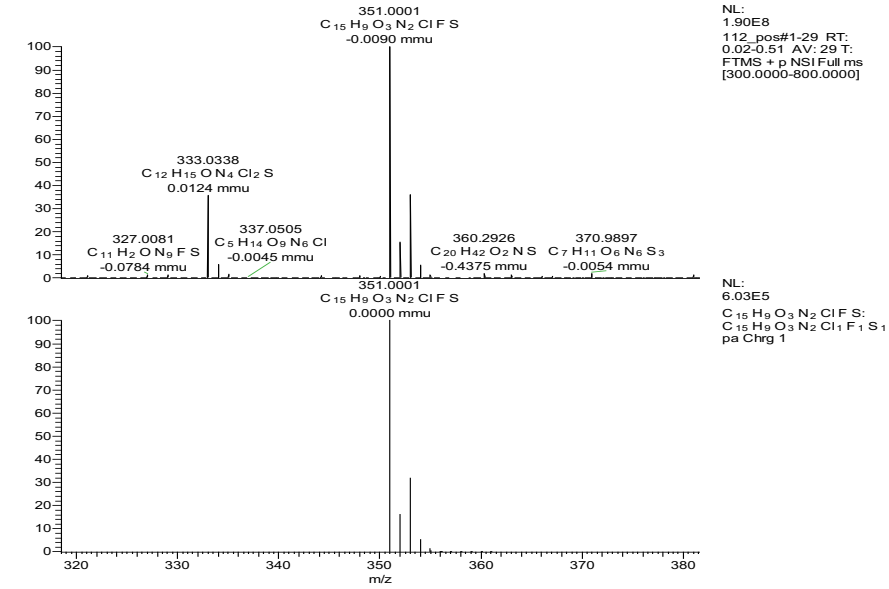

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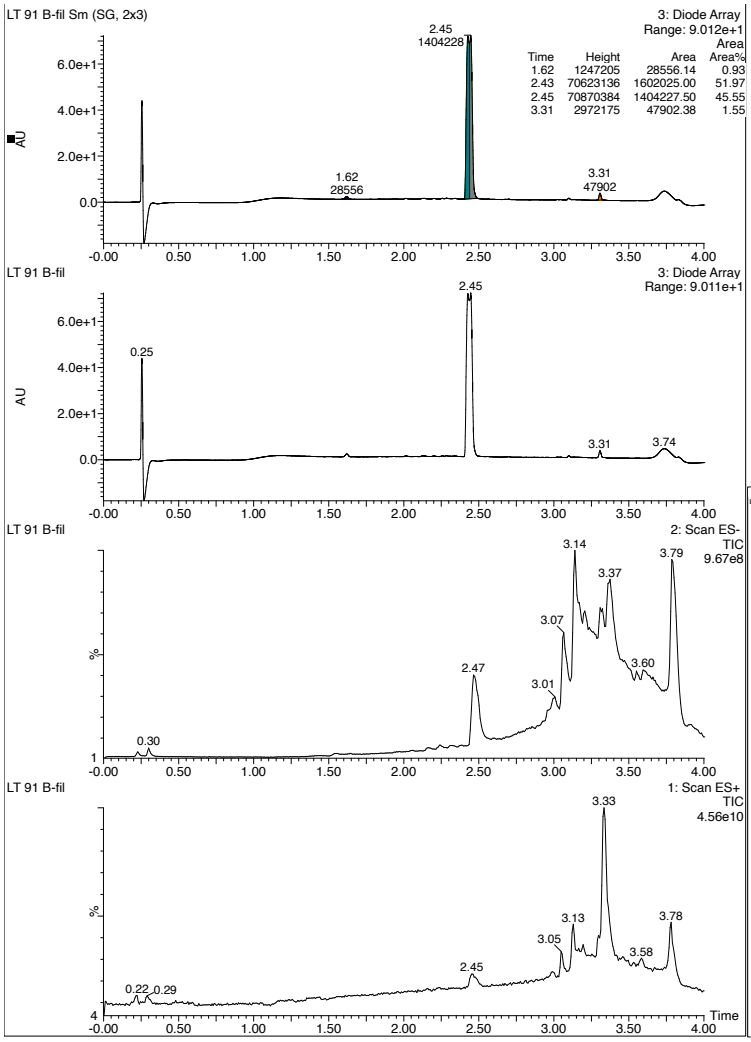

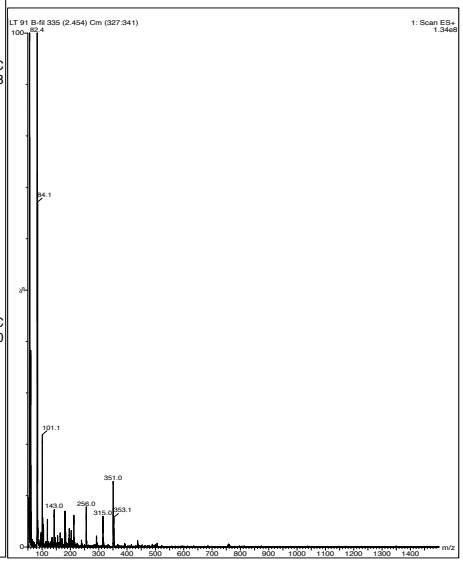

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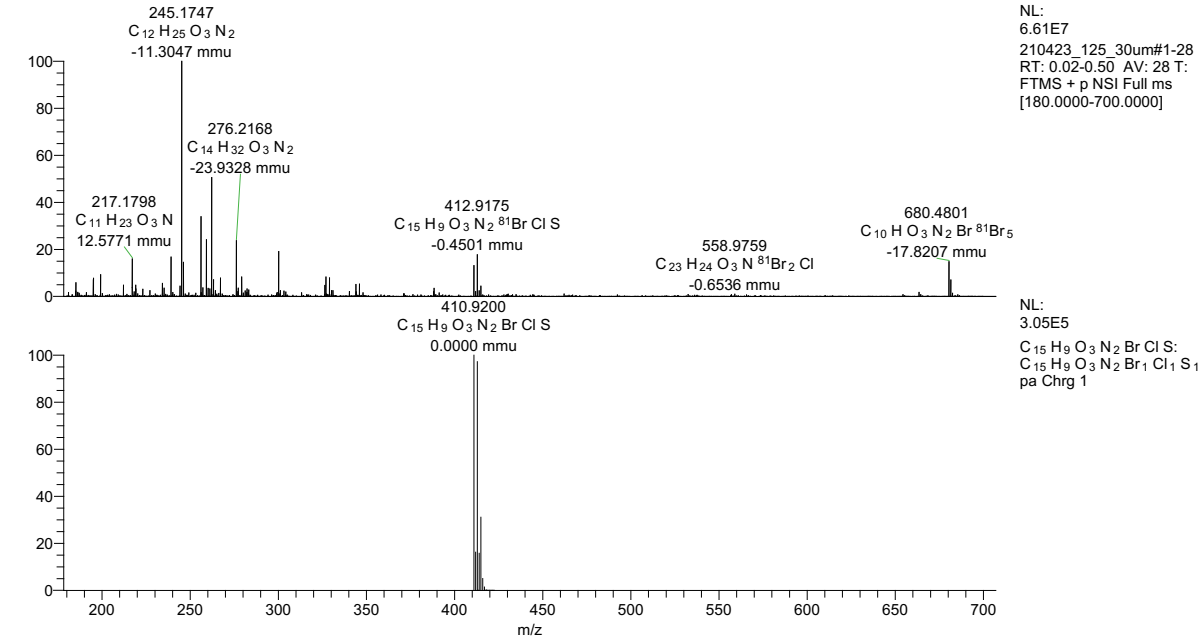

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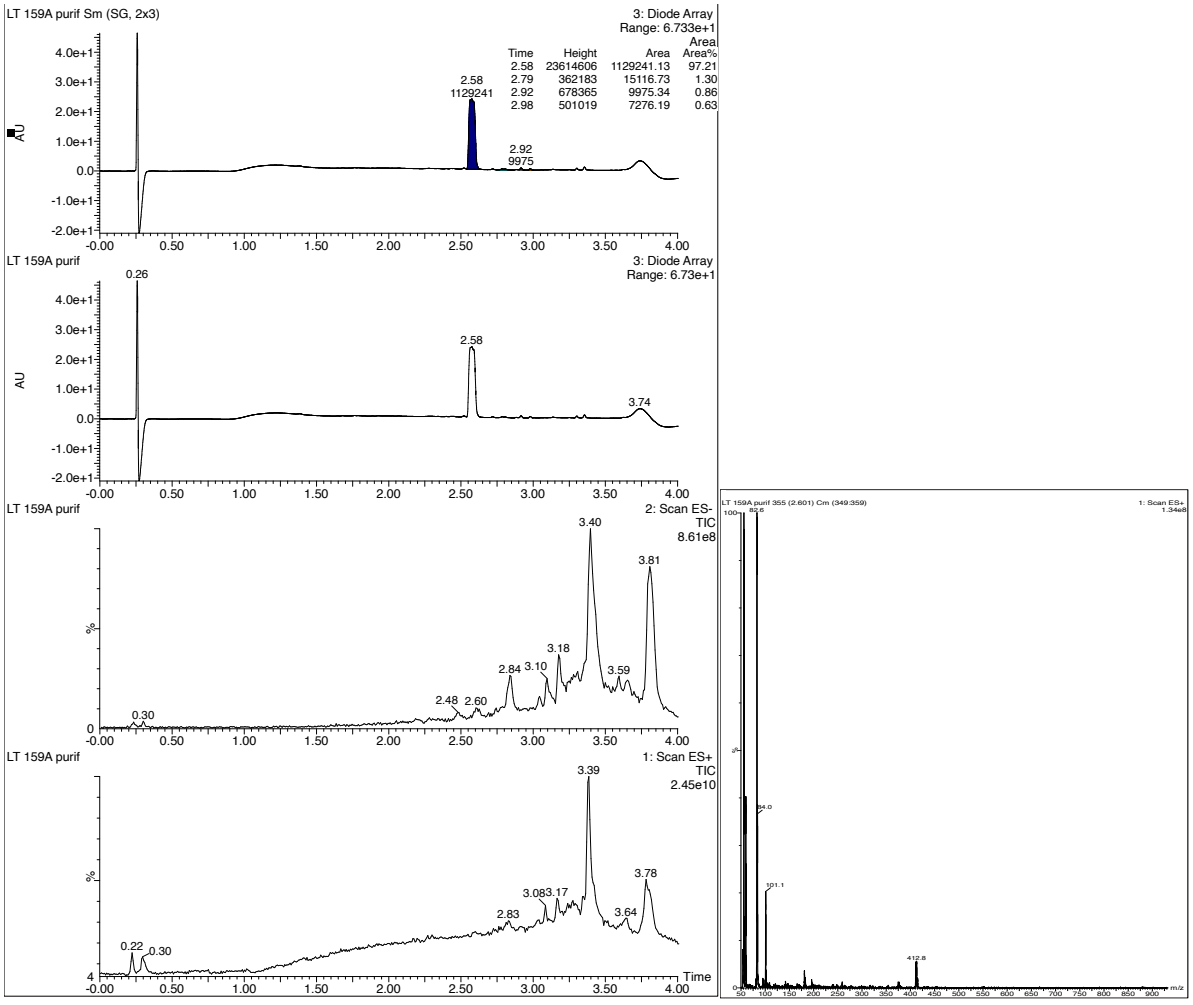

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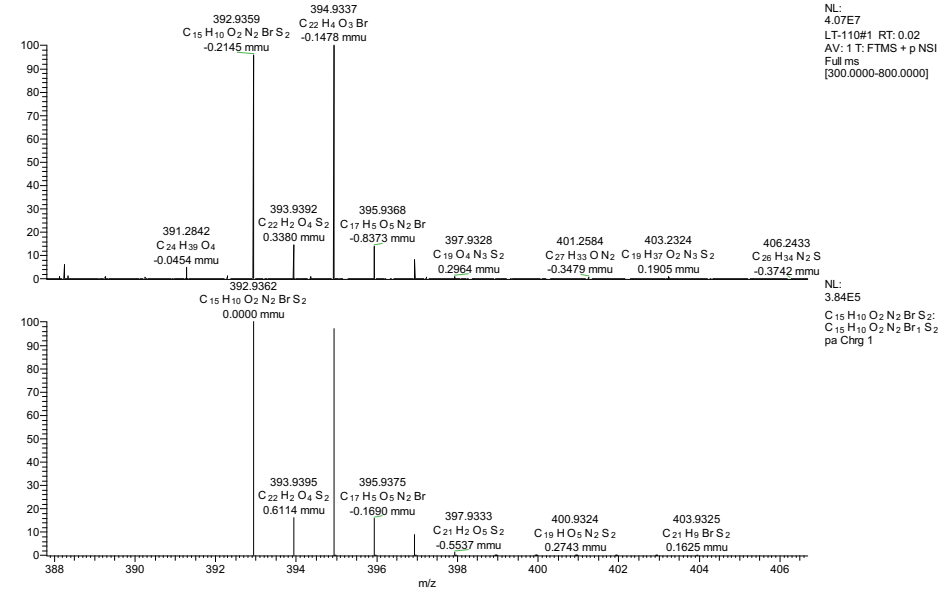

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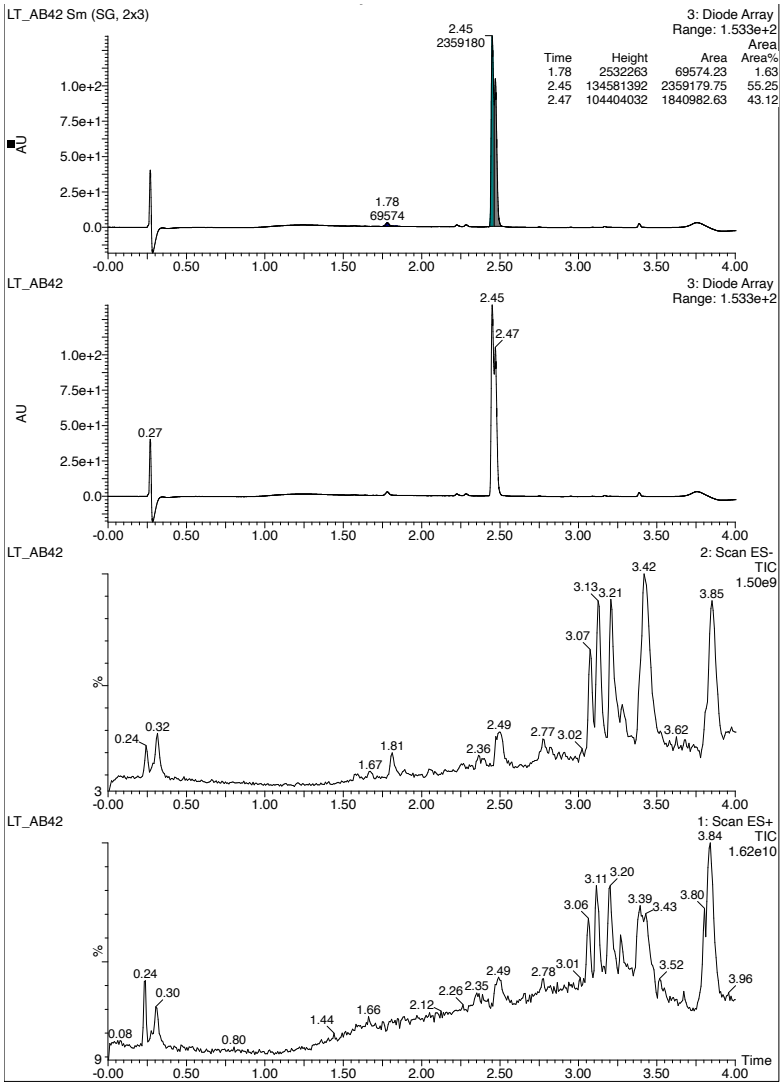

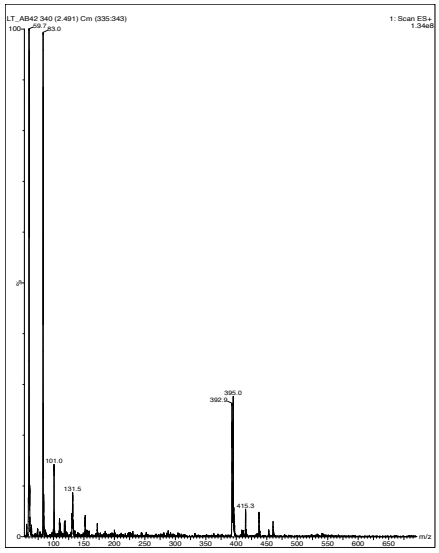

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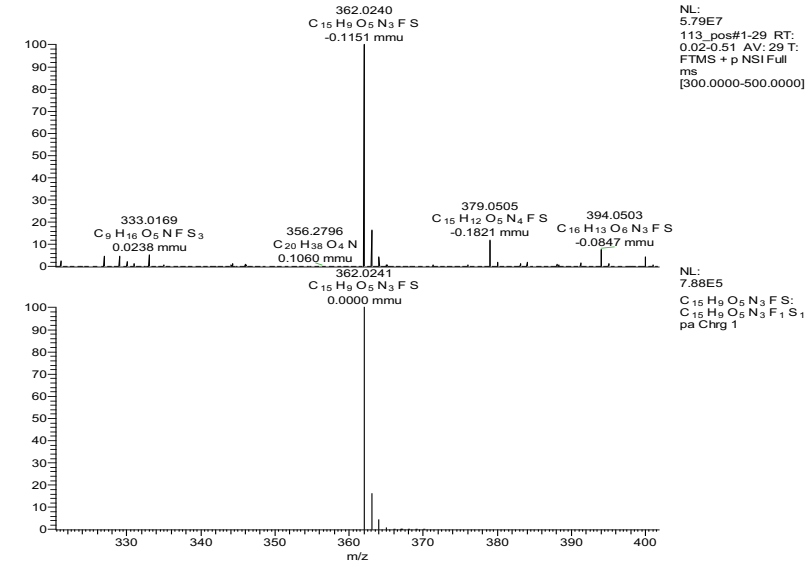

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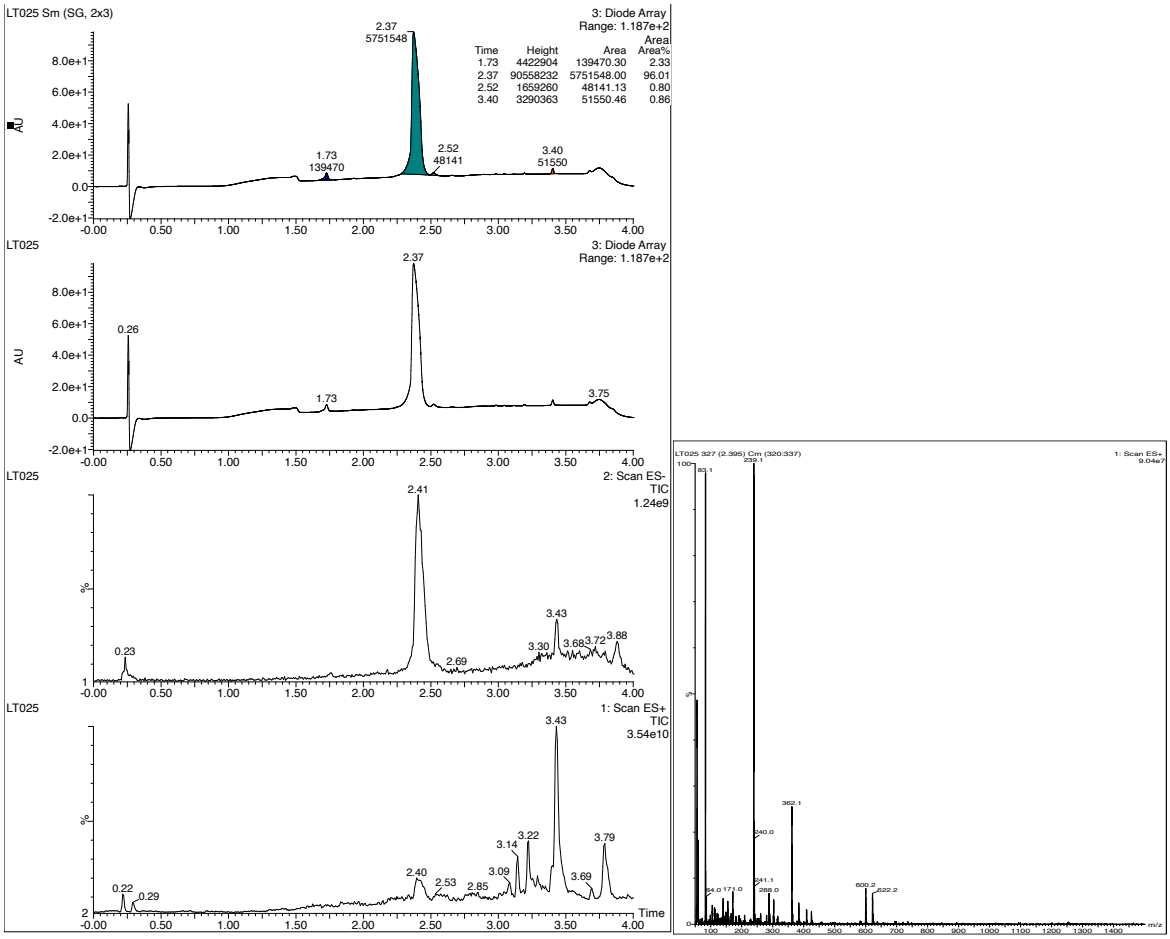

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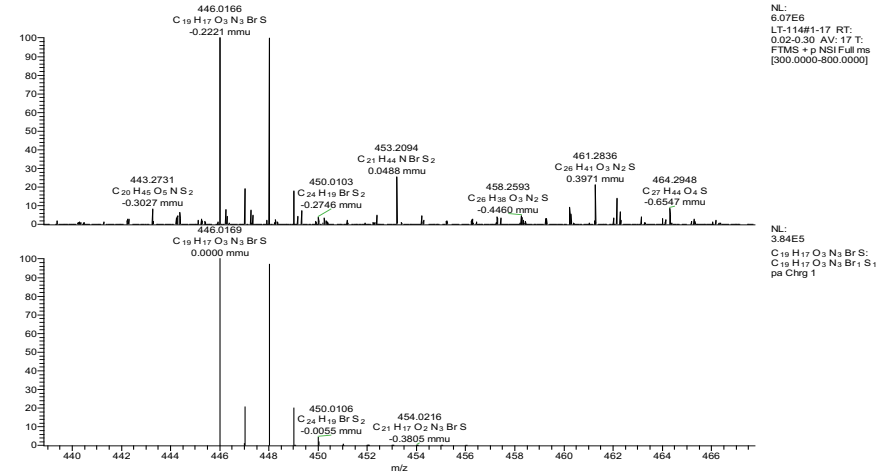

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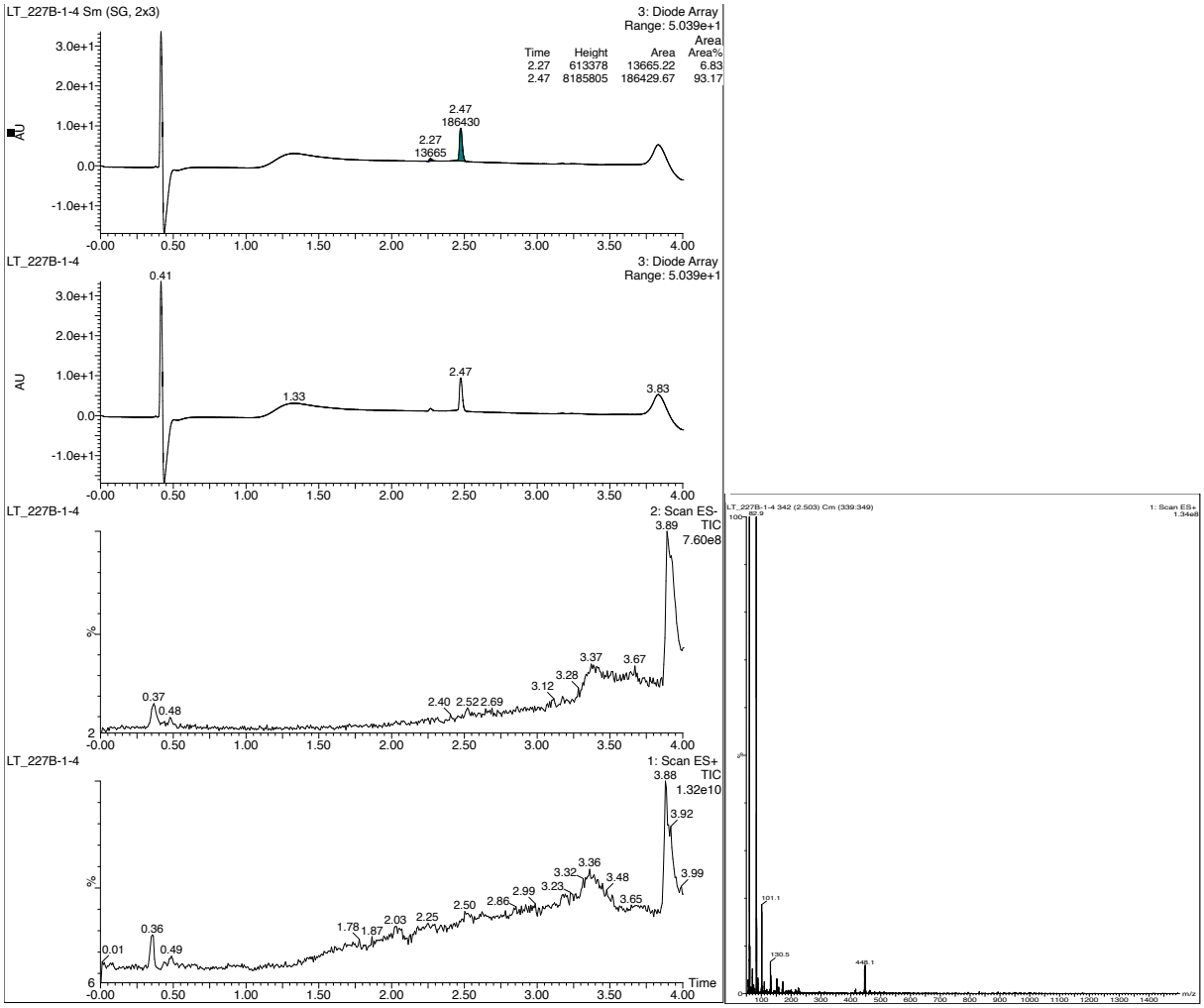
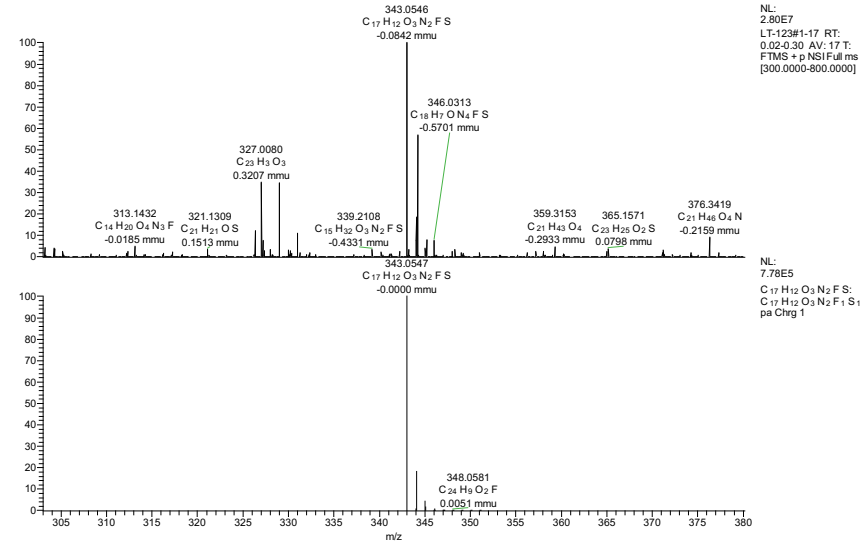


Compound **6g**



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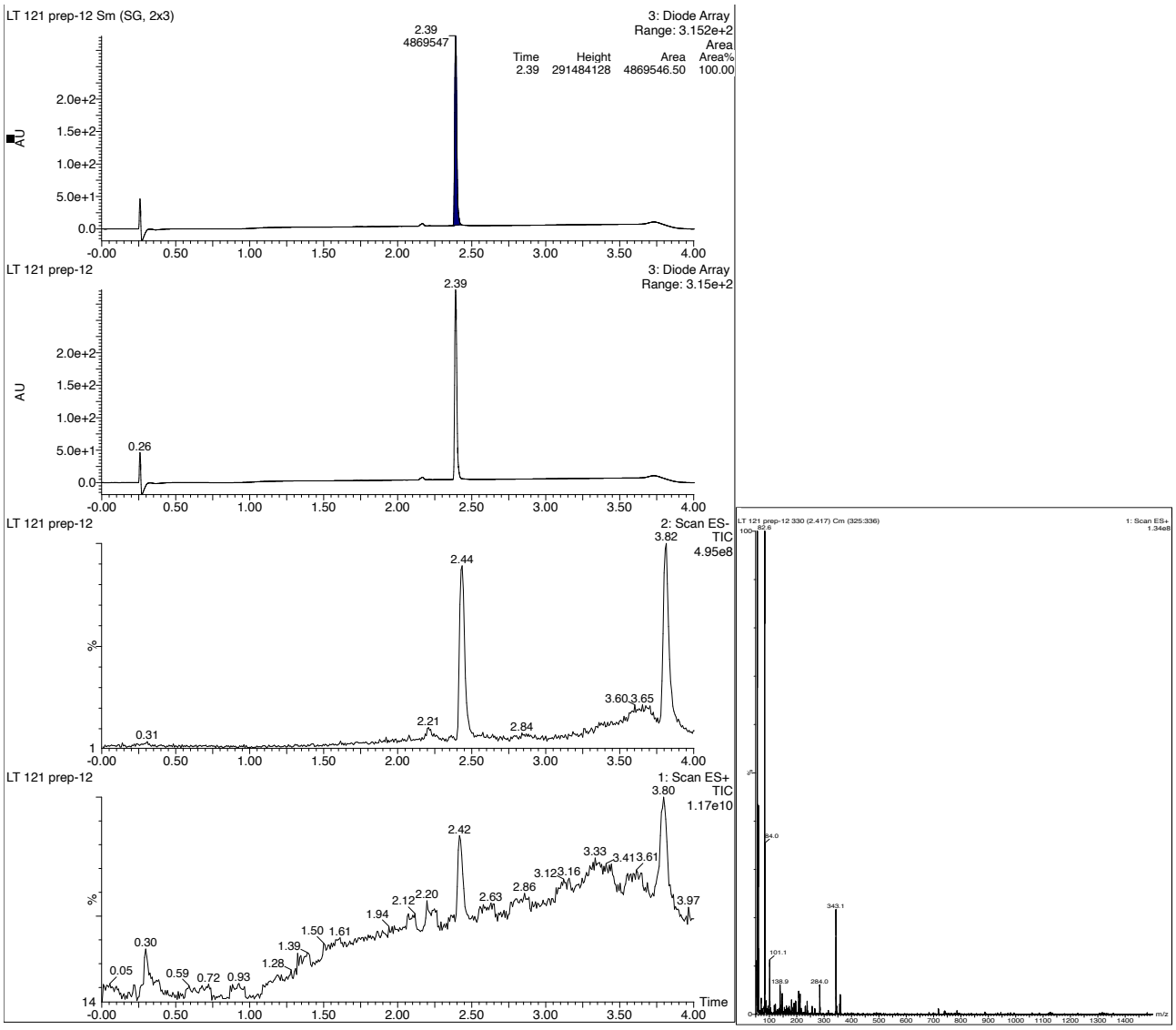

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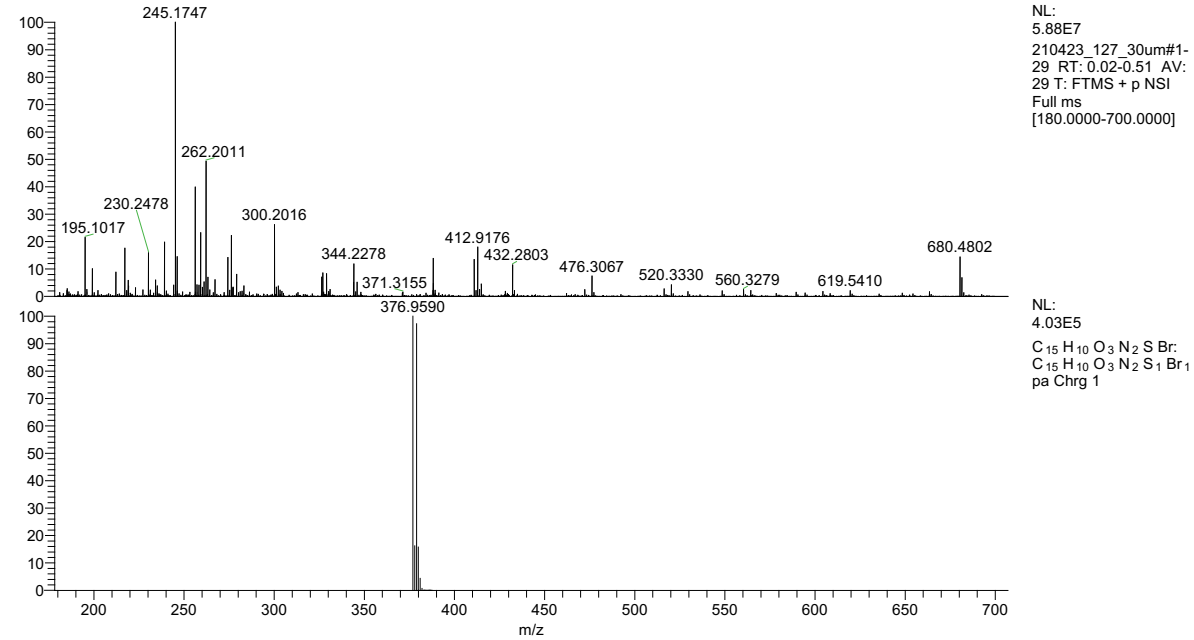

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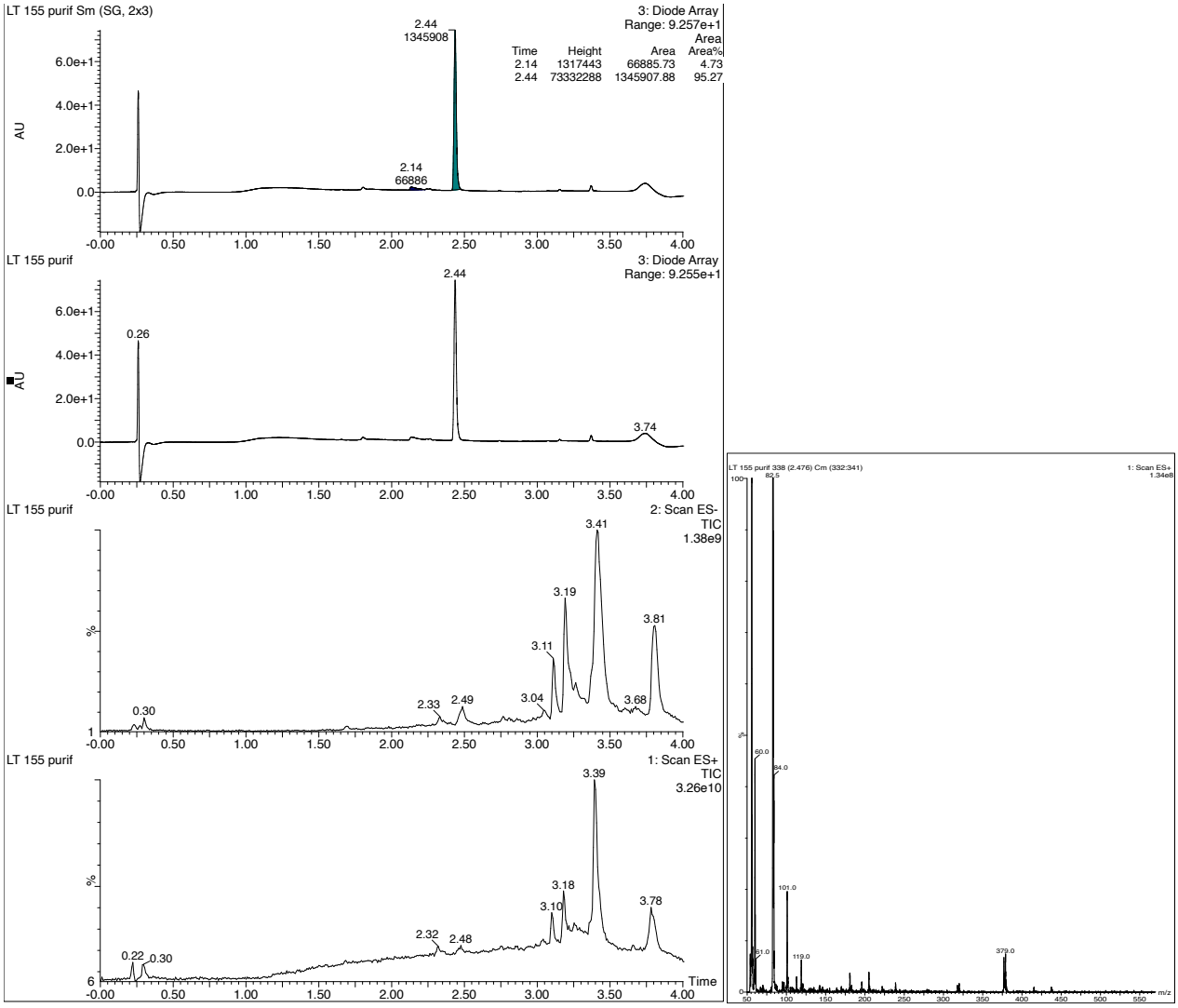
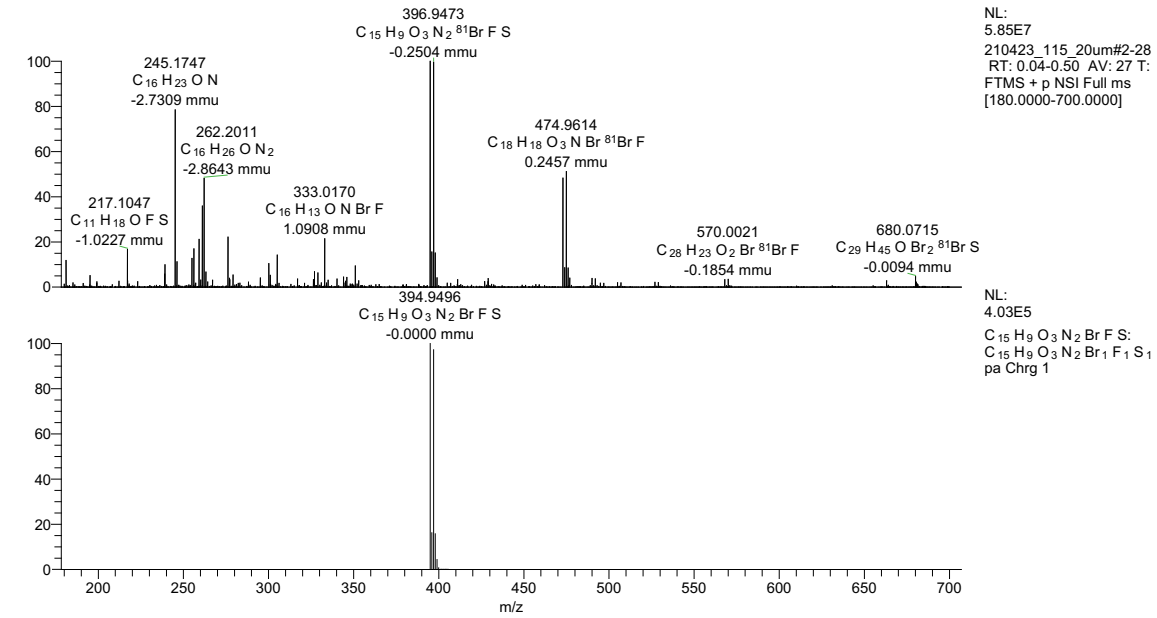


Compound **6i**



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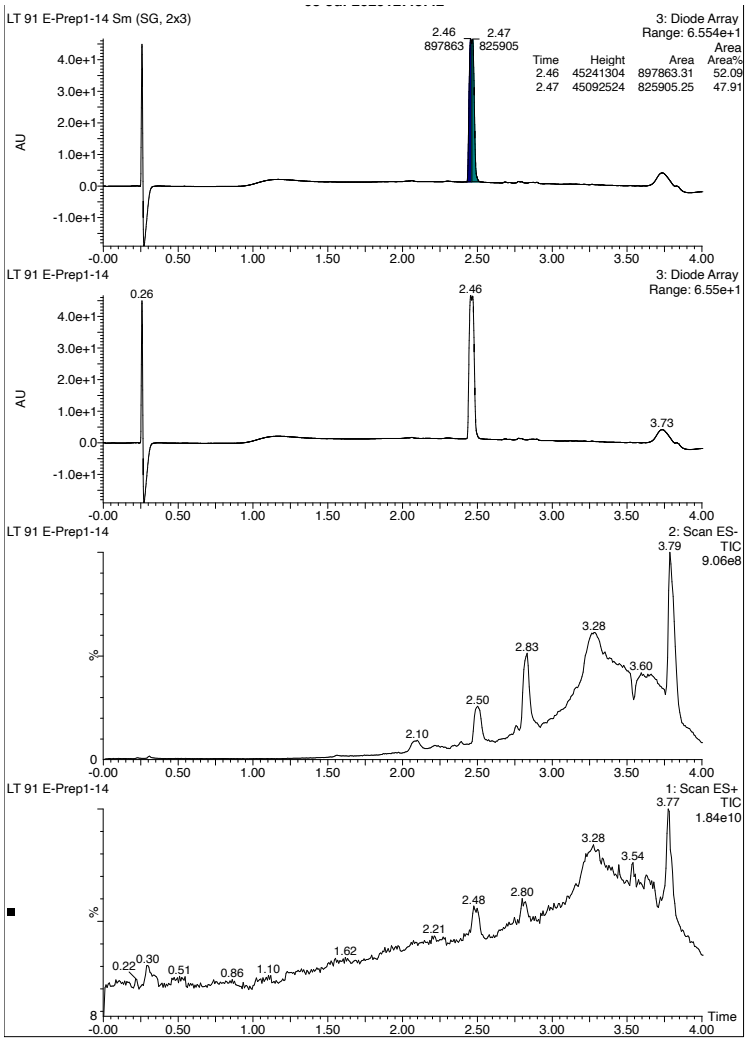

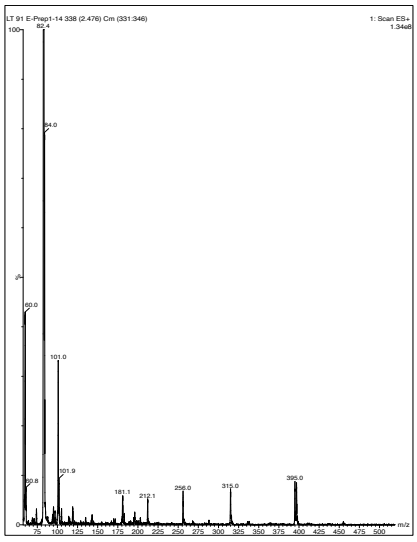

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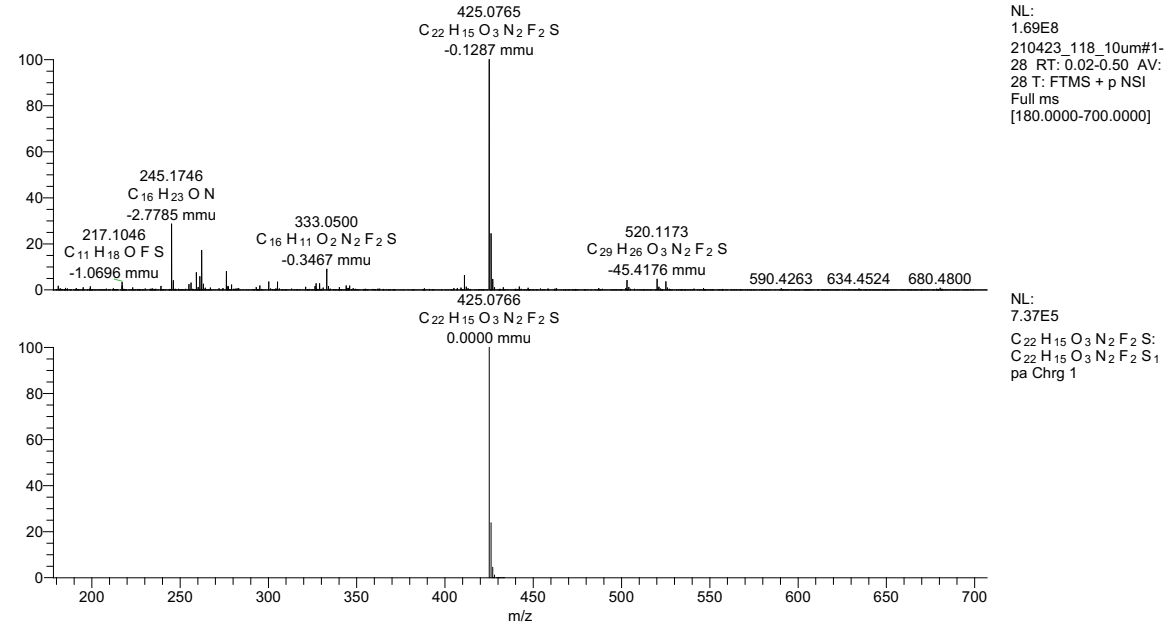

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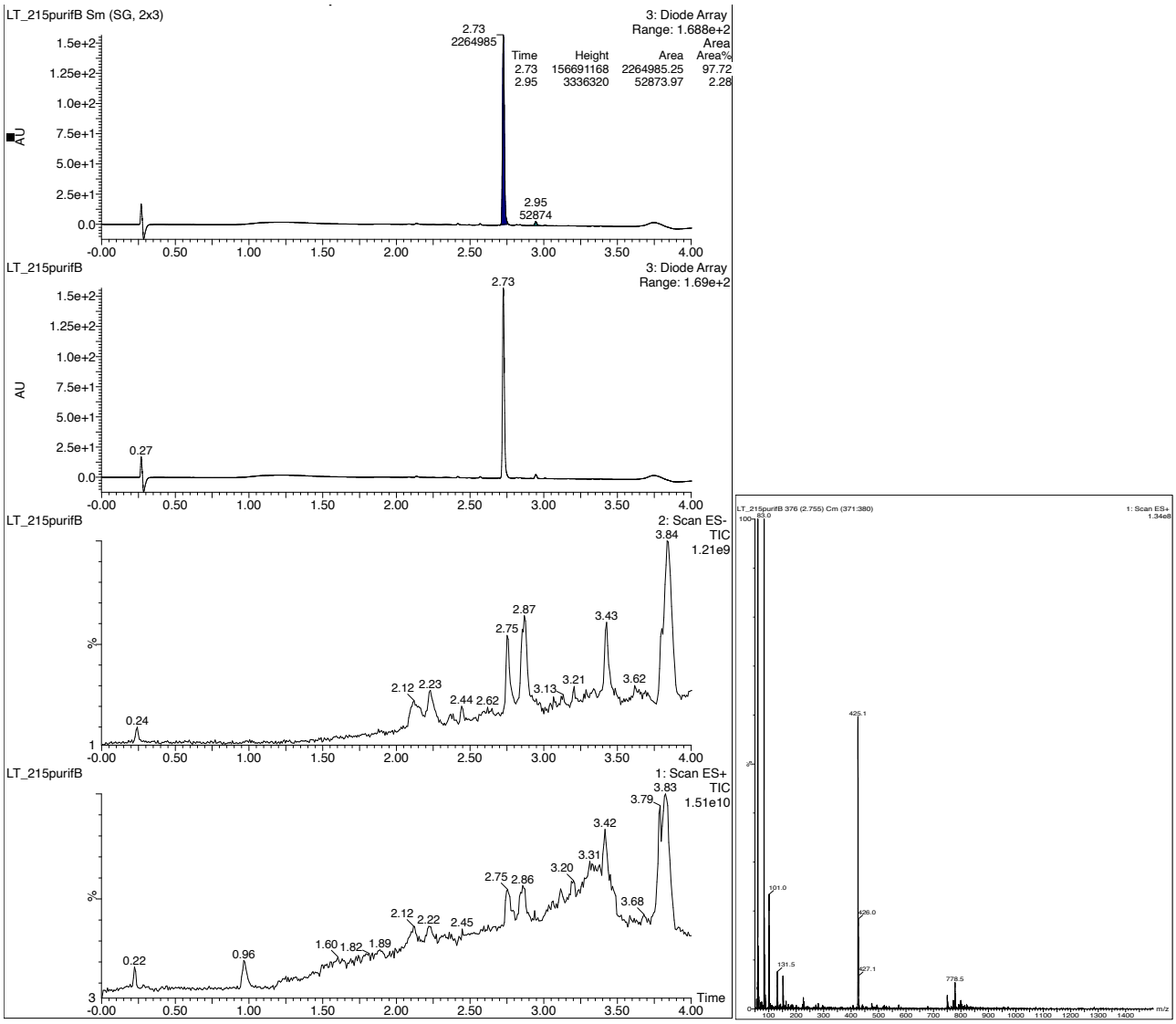
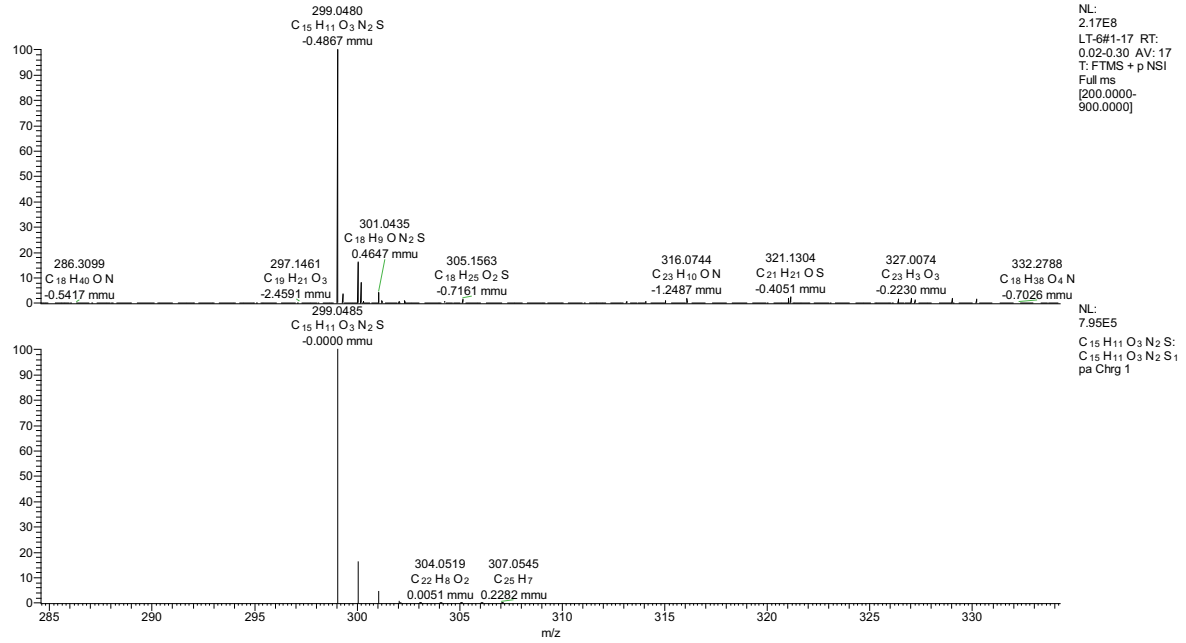


Compound **6k**



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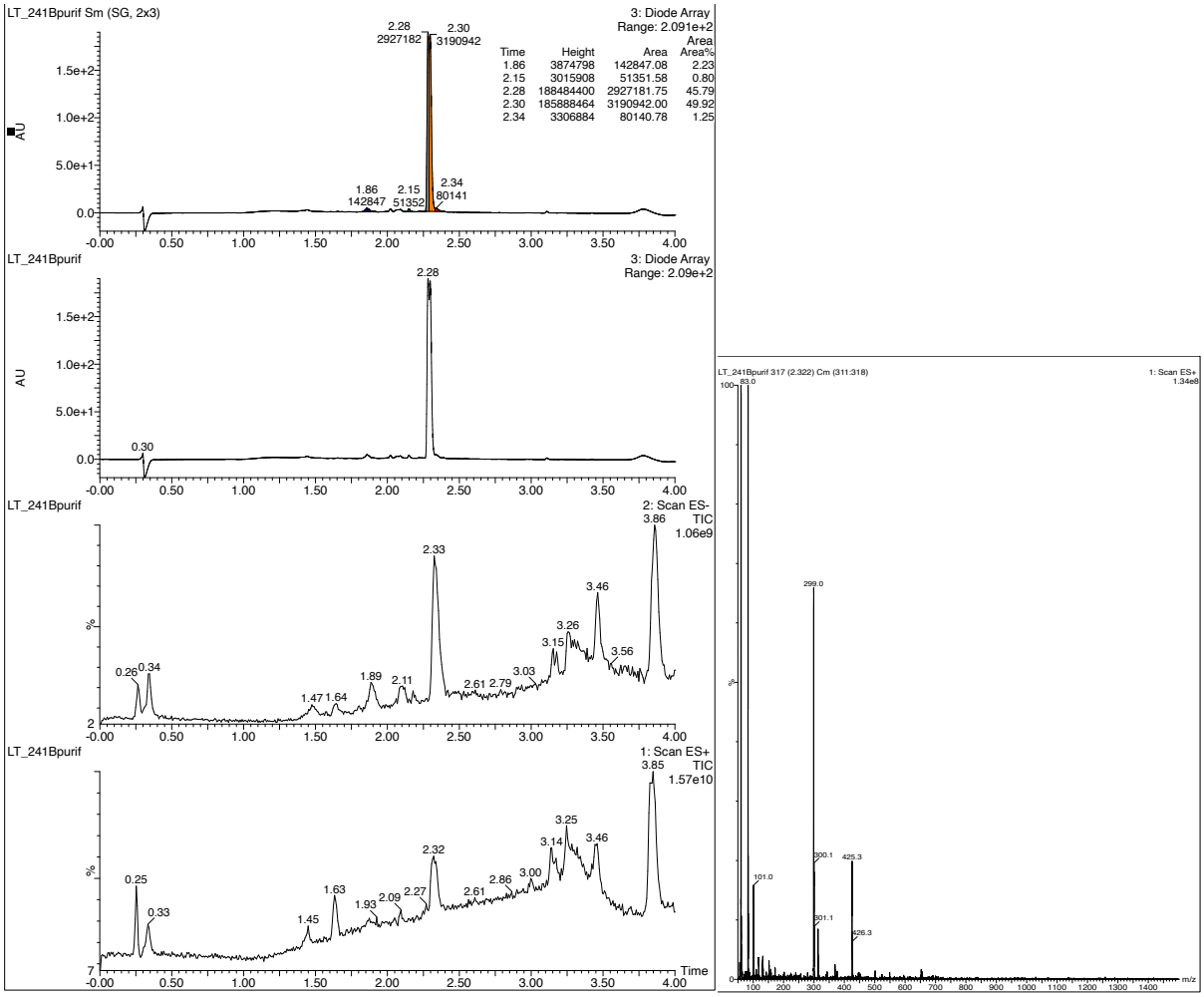

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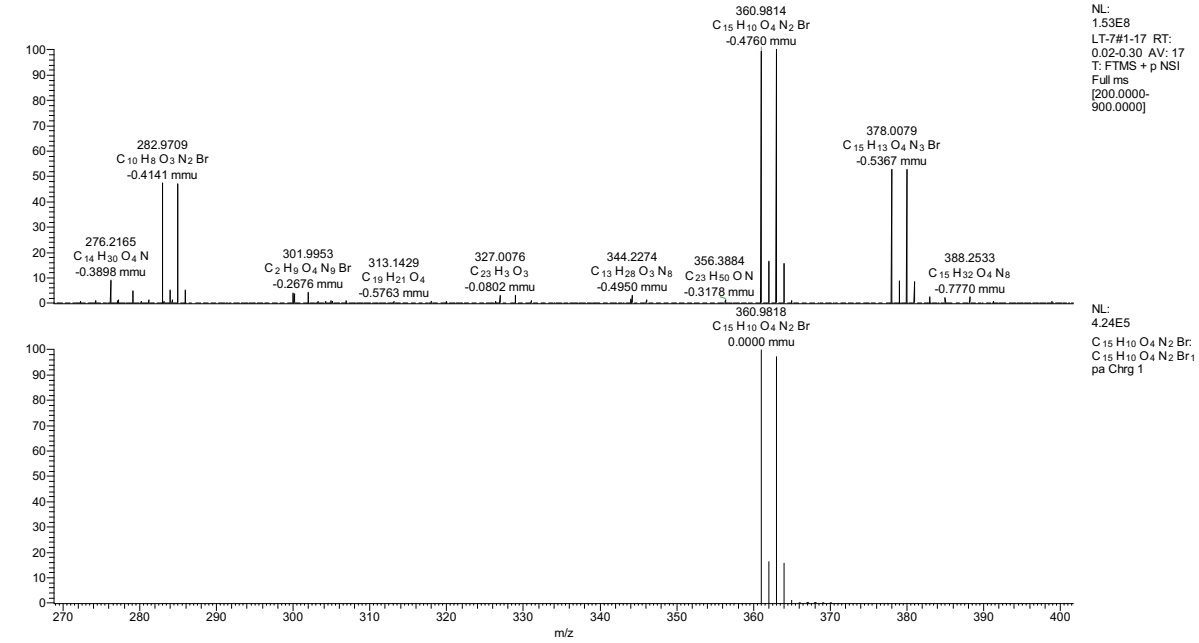

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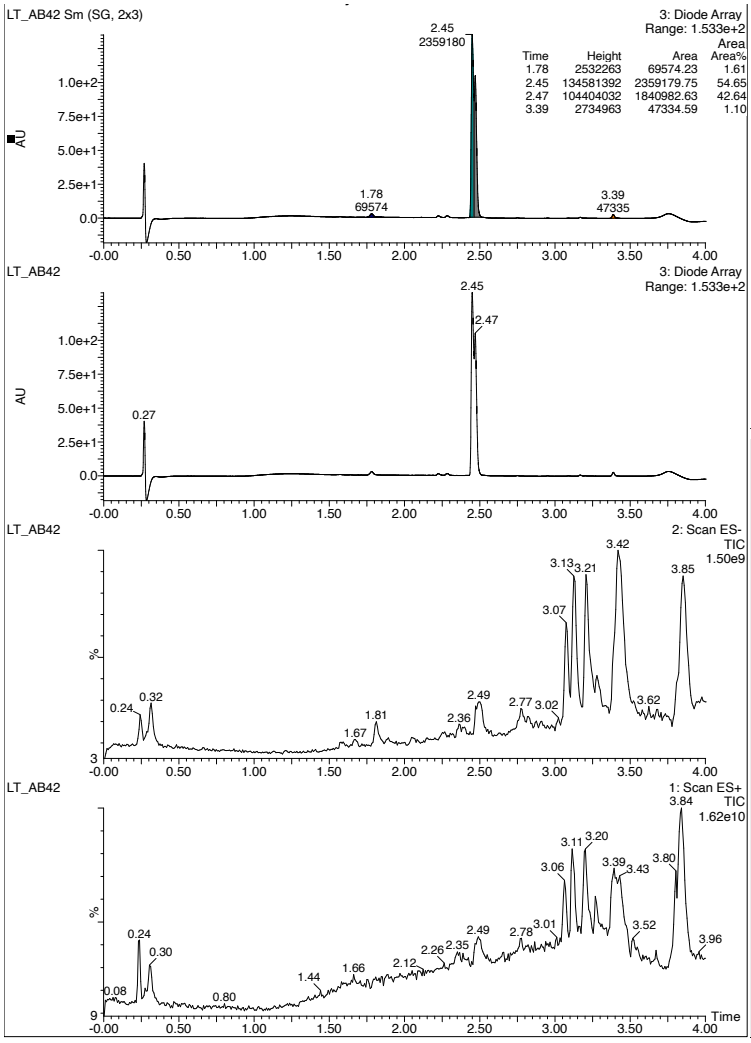

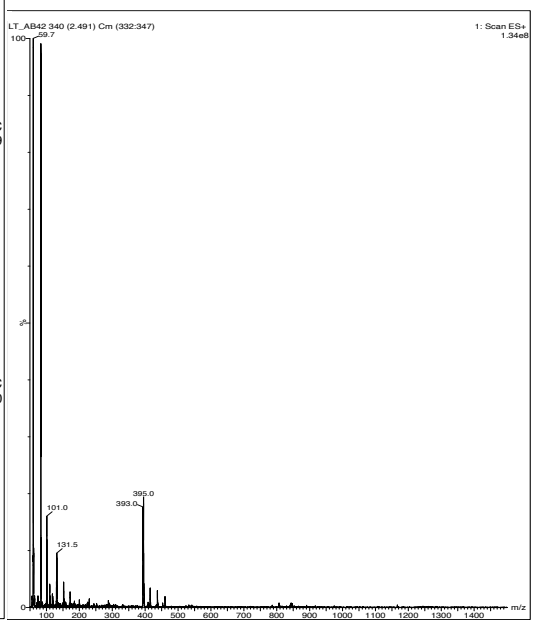

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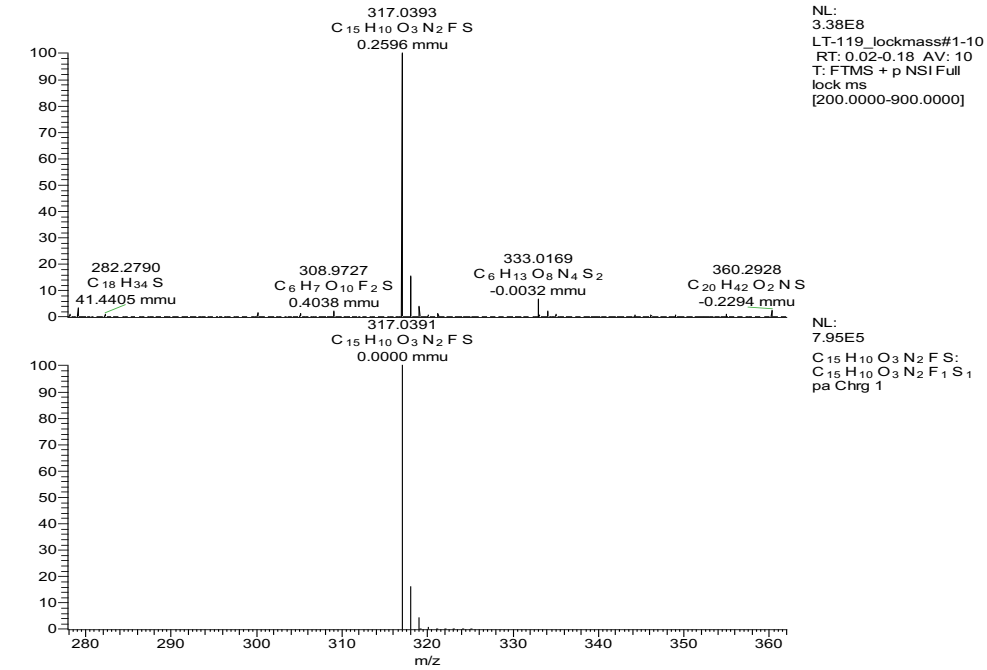

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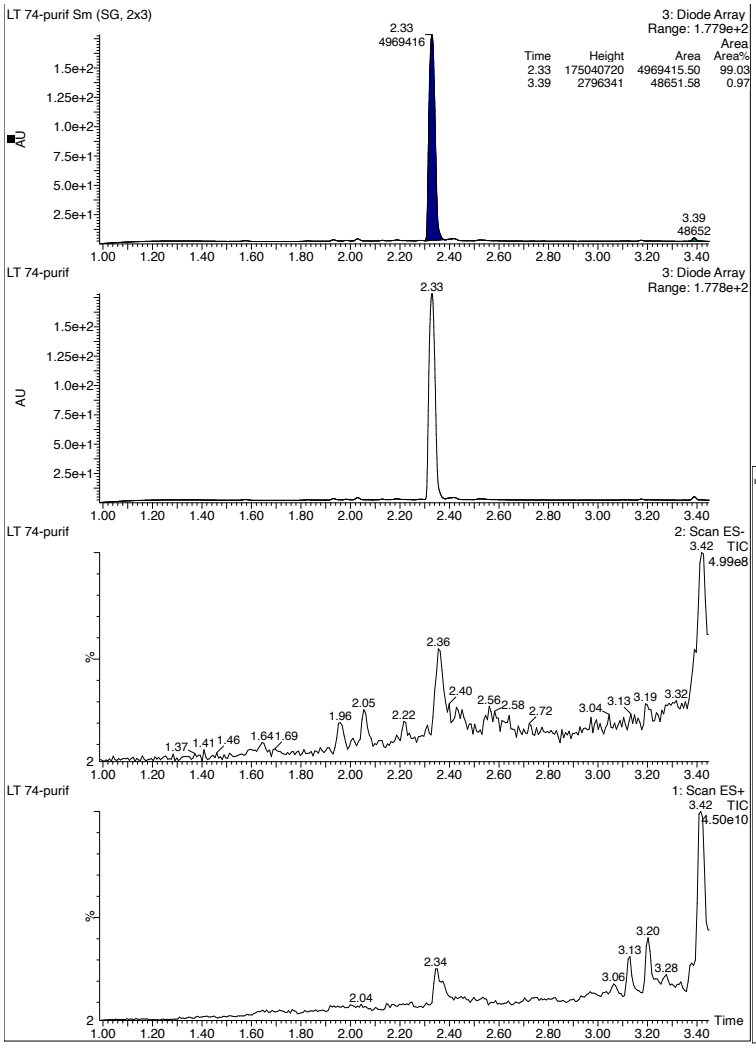
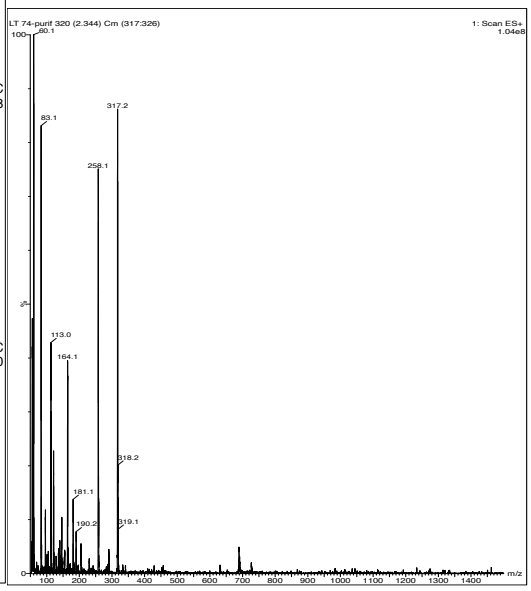
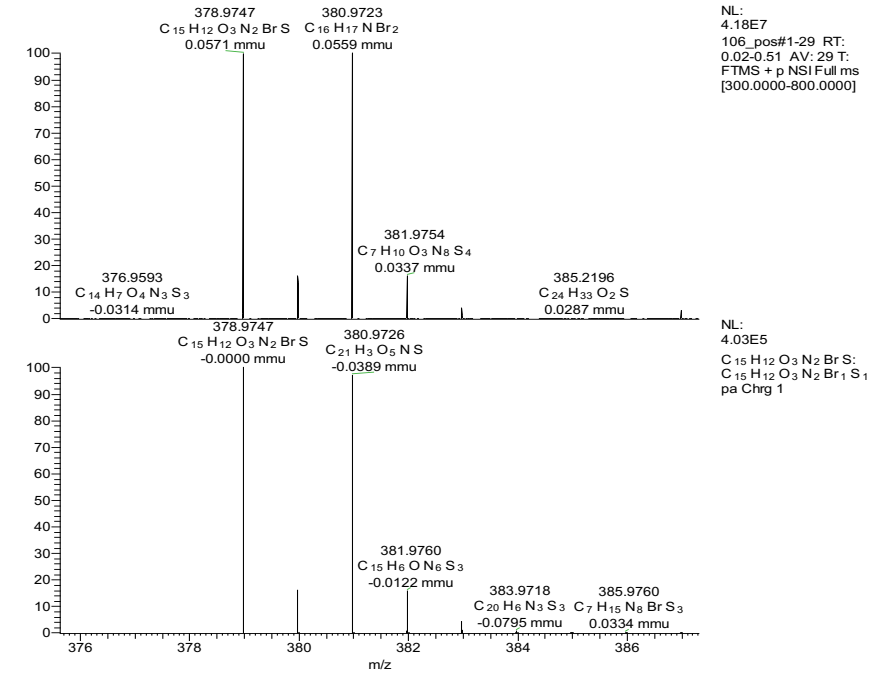




Compound **7a**



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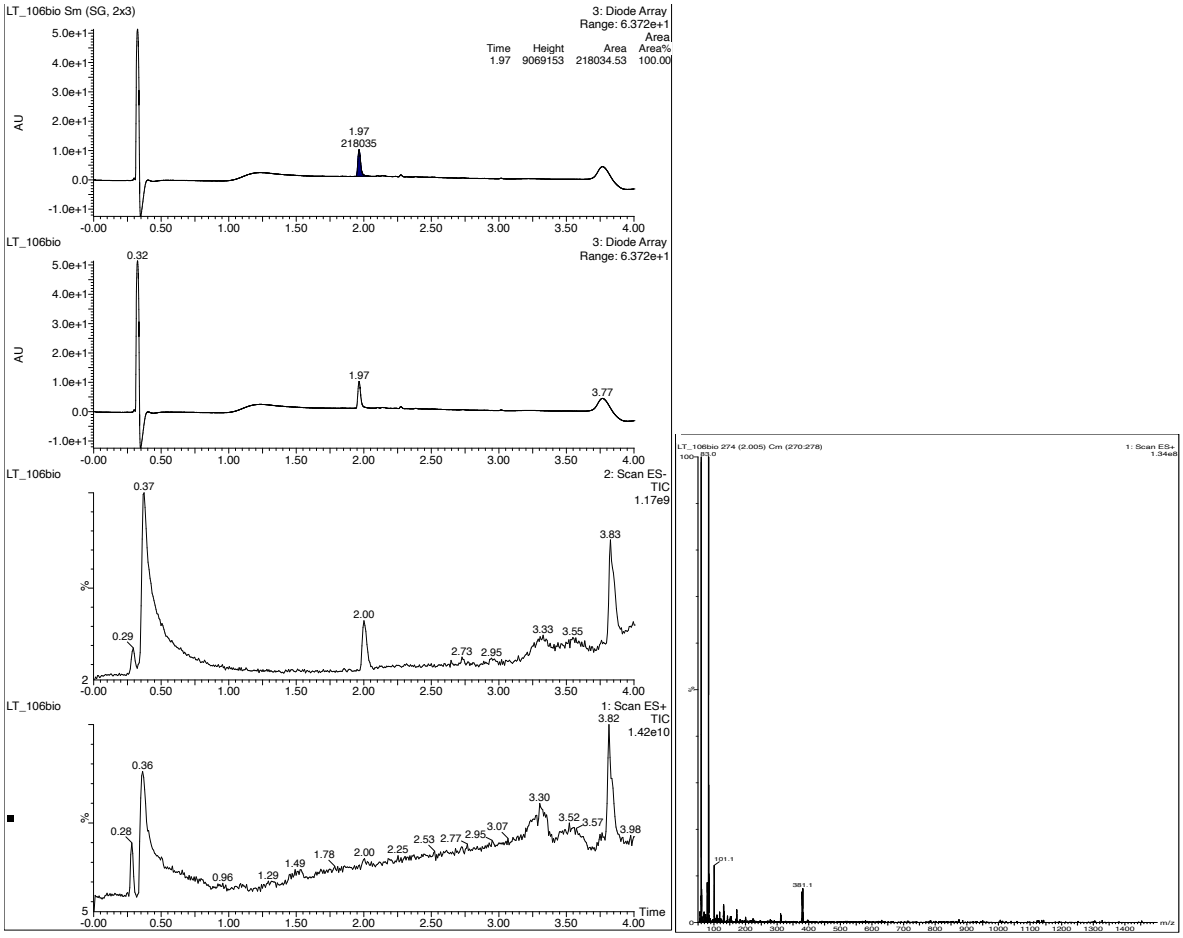
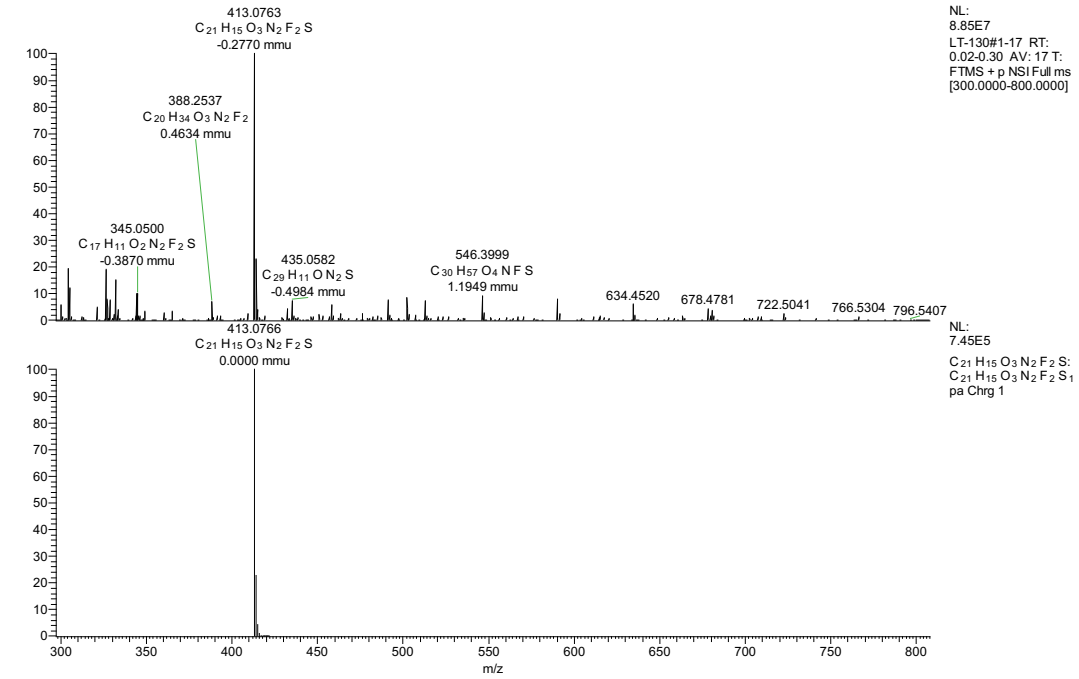


Compound **7b**



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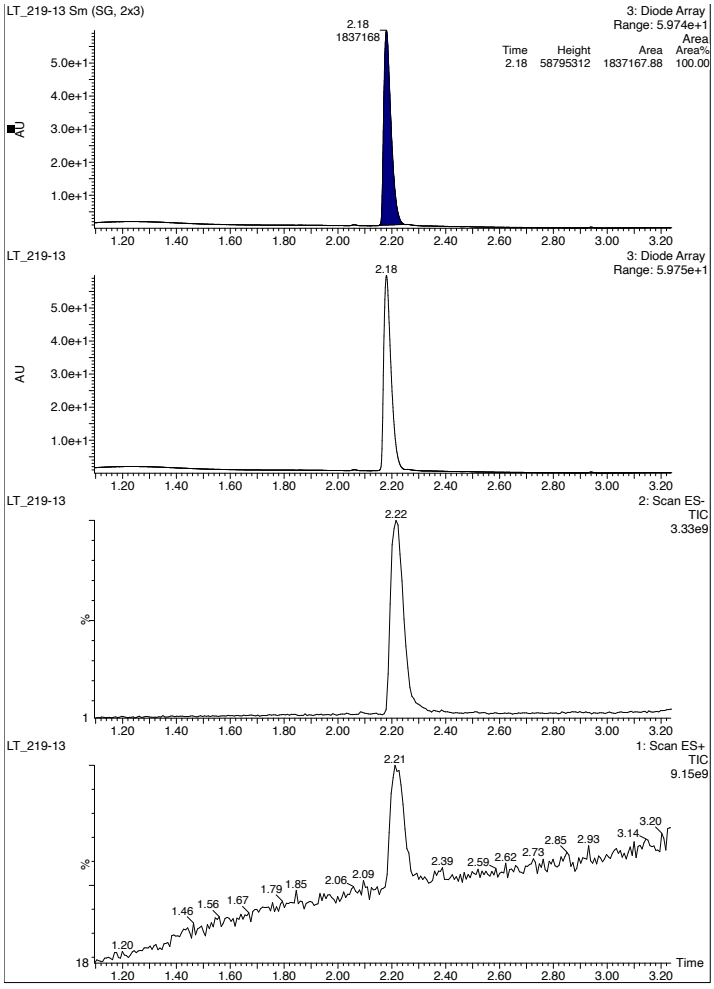
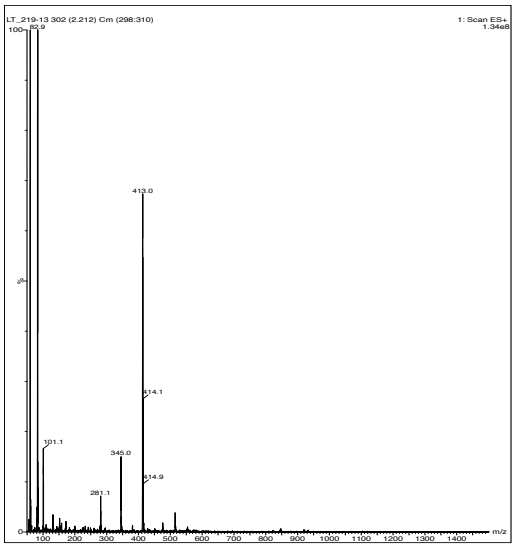
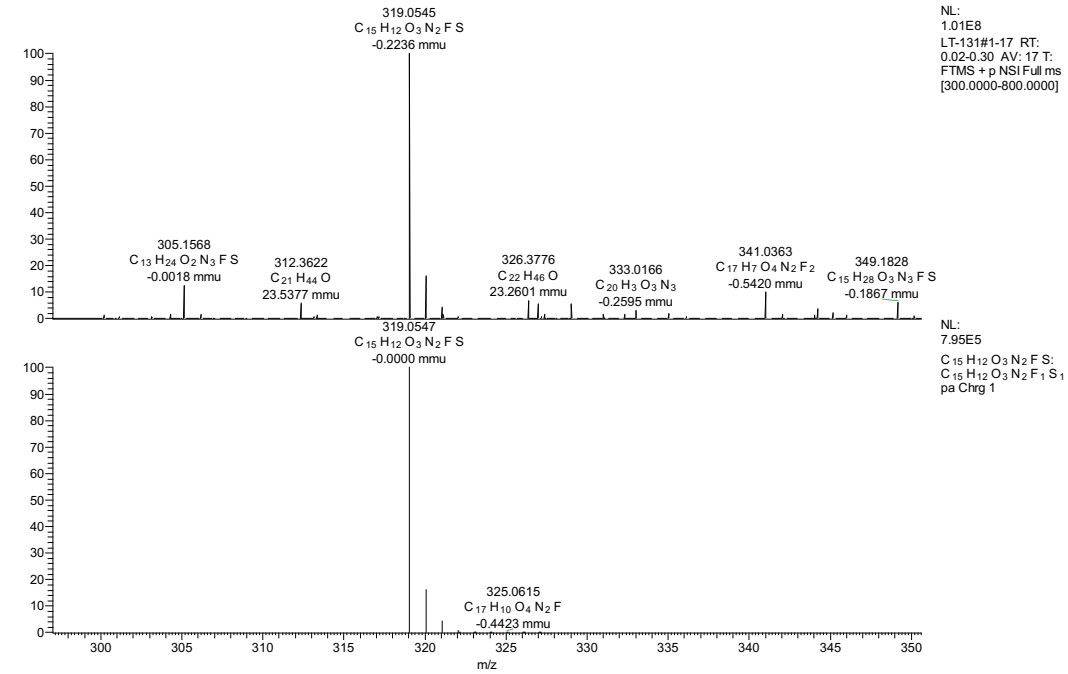




Compound **7c**



UPLC-MS