

Supplementary Material

Design, synthesis, and anti-hepatocellular carcinoma of thiopyrimidine/chalcone hybrids as dual STAT3/STAT5 inhibitors

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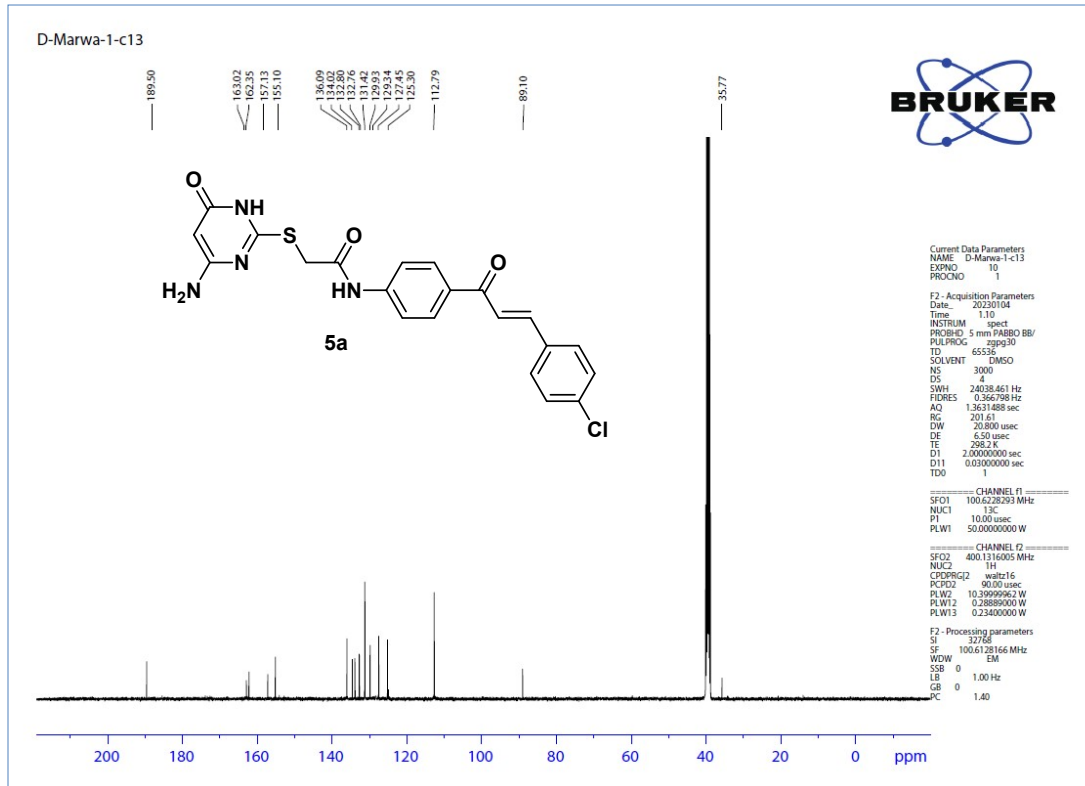
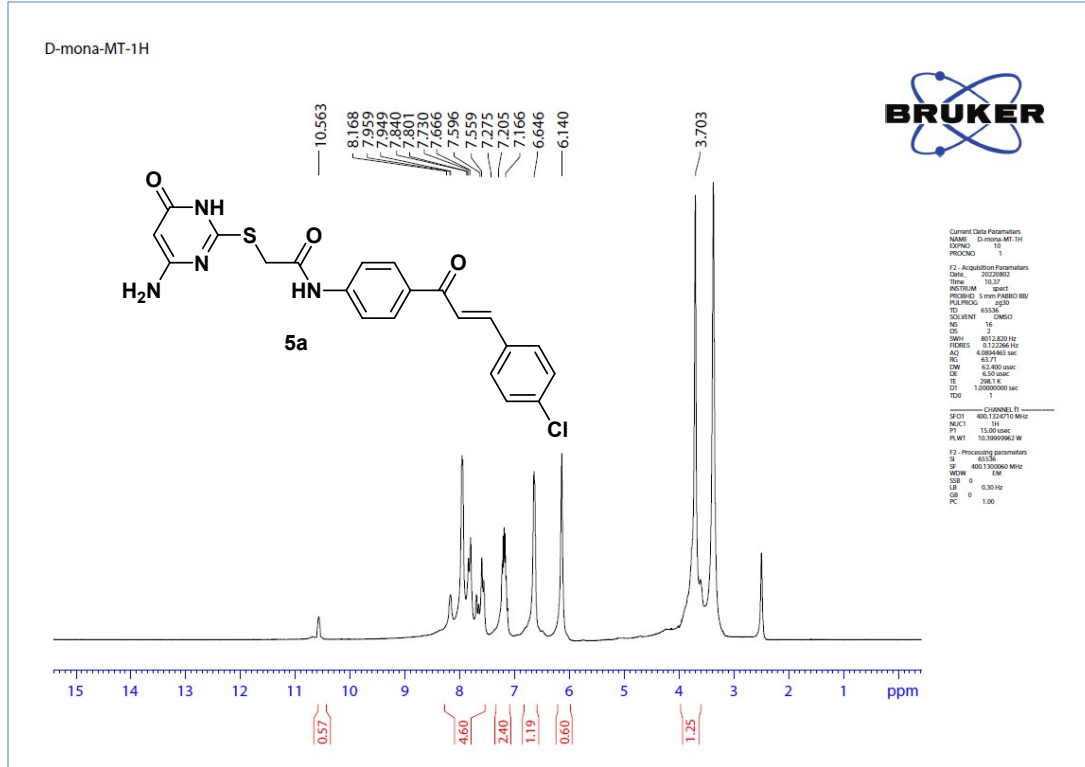
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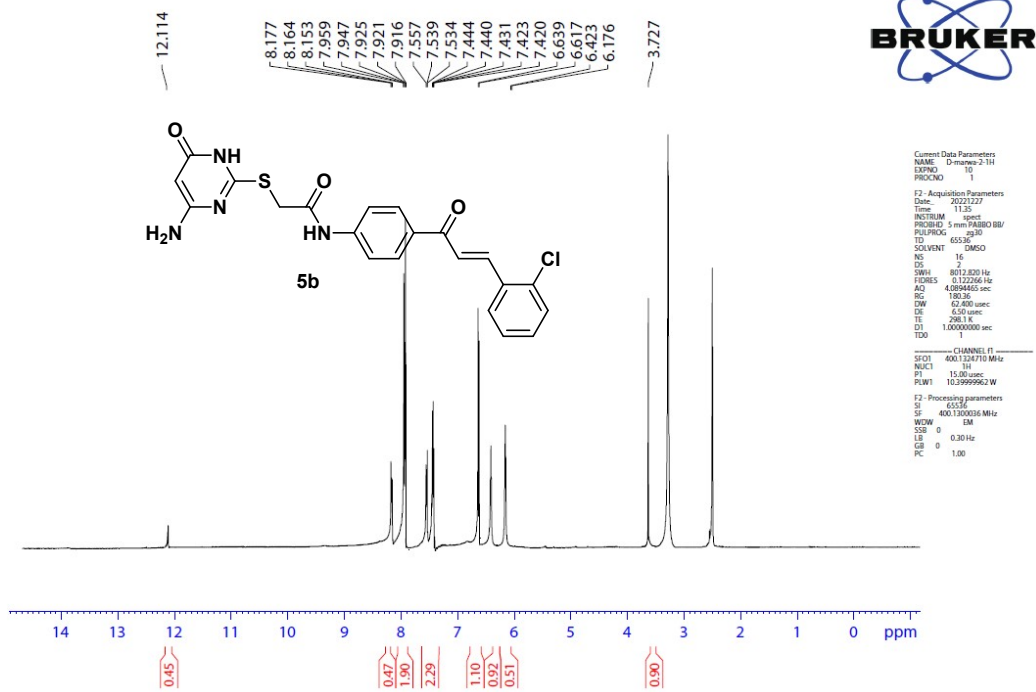
1- Chemistry Materials

Reagents and solvents were bought from standard commercial sources and used without any extra purification. The reported yields are for products that have been cleaned. Thin-layer chromatography (TLC) on 0.25 mm thick Merck Silica Gel 60 F254 was used to check all reactions regularly and a UV lamp was used to see what was happening. The Stuart SMP3 device was used to measure the melting points of open capillary tubes. IR spectra were measured with a Shimadzu FT/IR 1650 (Perkin Elmer) spectrometer (KBr). The ^1H and ^{13}C NMR spectra were recorded in DMSO- d_6 using a Brüker Advance-400 instrument (400 MHz for ^1H and 100 MHz for ^{13}C) (400 MHz for ^1H and 100 MHz for ^{13}C). Chemical shifts (δ) are given in parts per million (ppm) relative to TMS as an internal standard or to the solvent in which the spectrum was recorded. At the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo, a mass spectrum was done on a direct inlet part to mass analyzer in a Thermo Scientific GCMS model ISQ. Mass spectroscopy was done using the Electron Impact mode. We used a PerkinElmer 240 elemental analyzer to look at the C, H, and N elements in each derived congener.

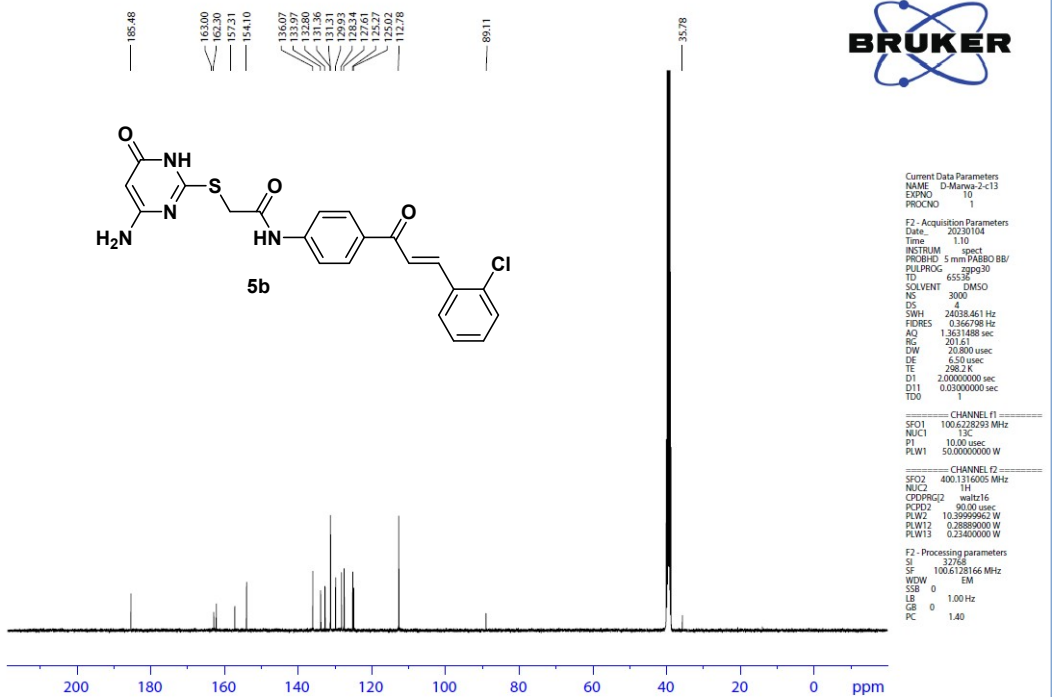
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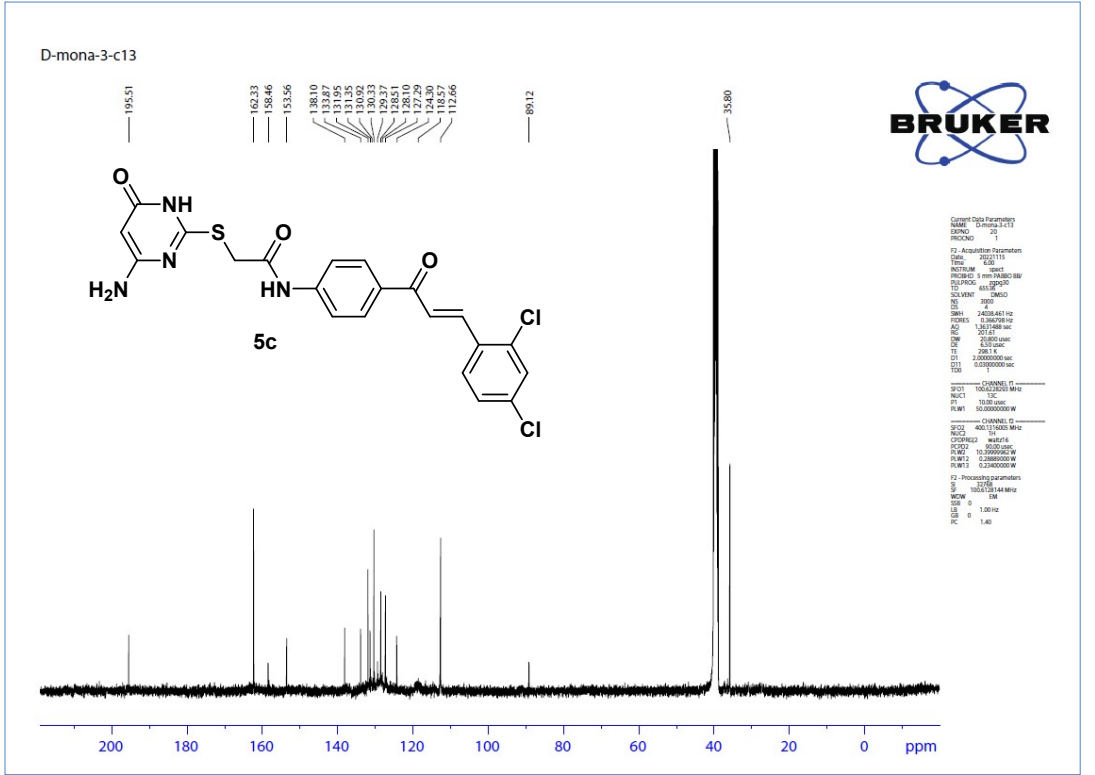
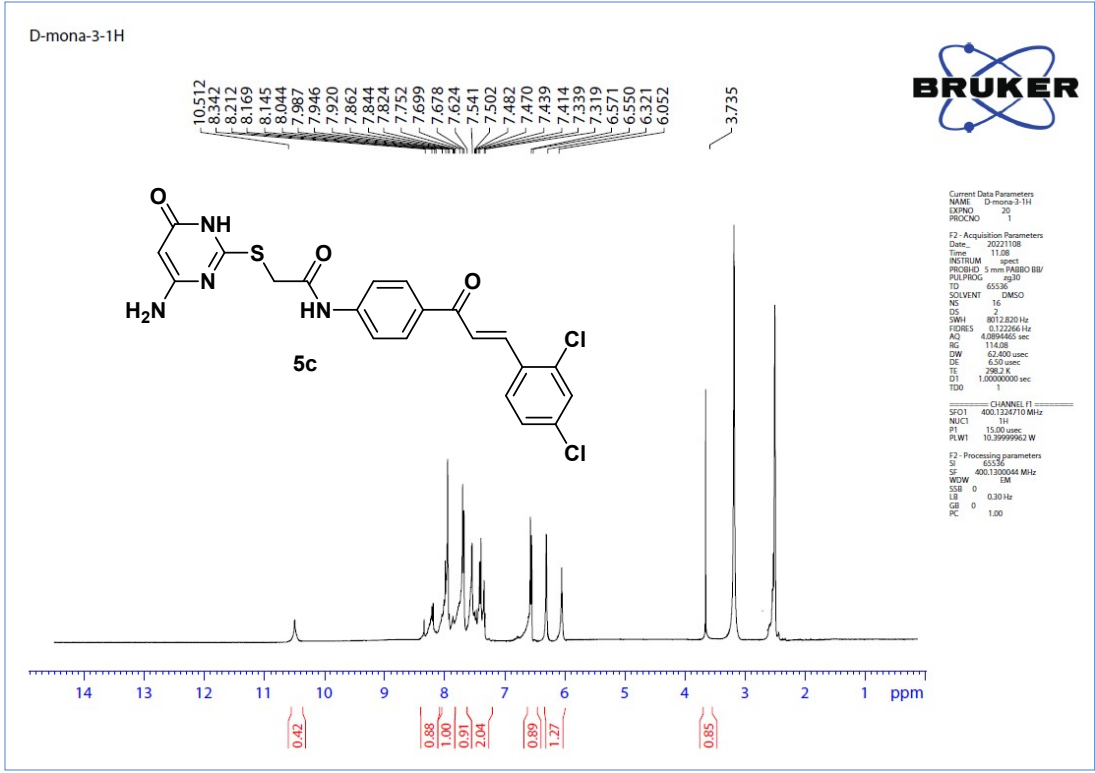


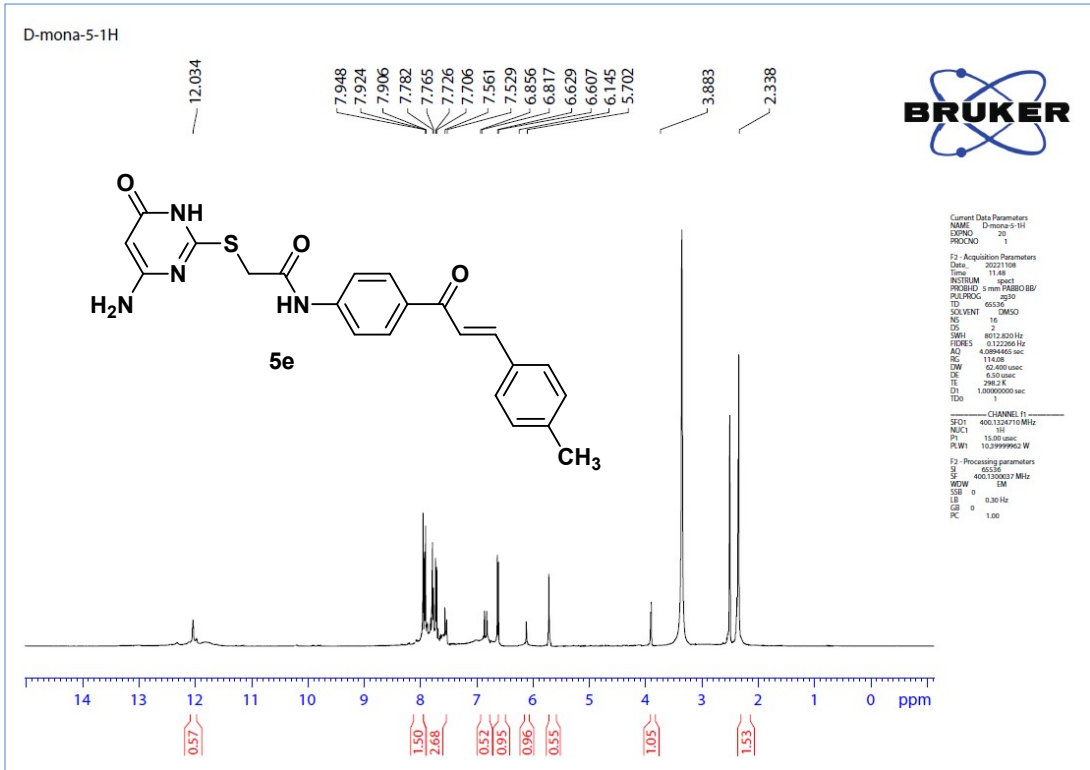
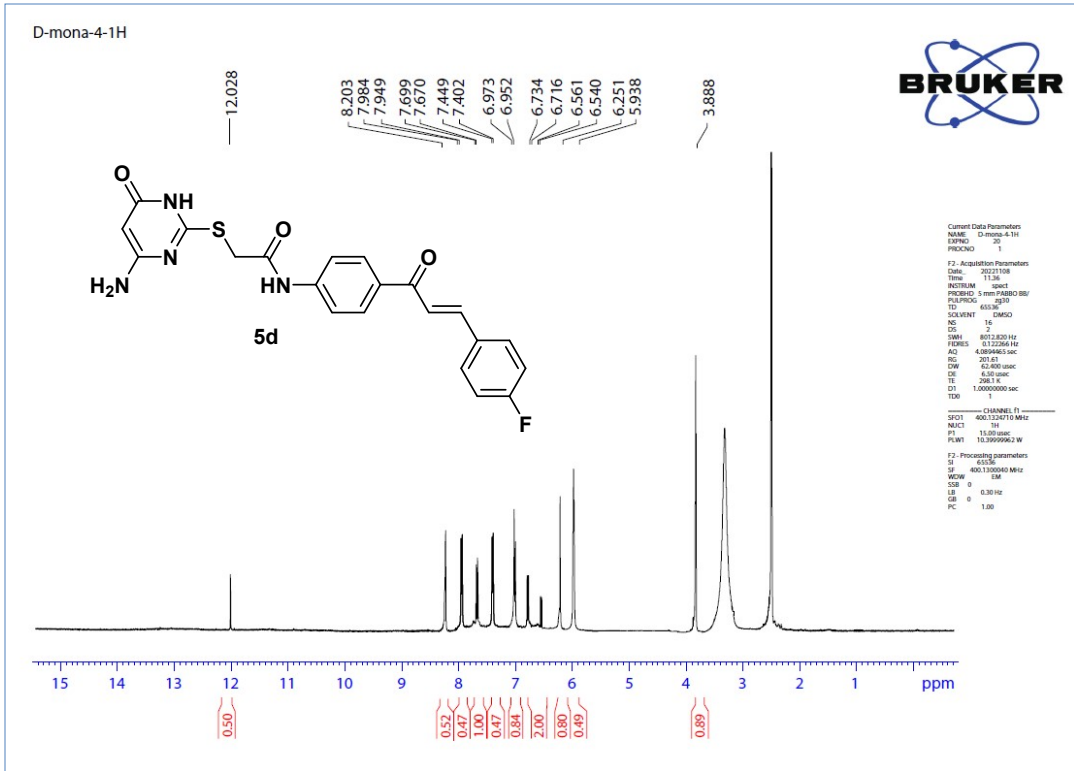
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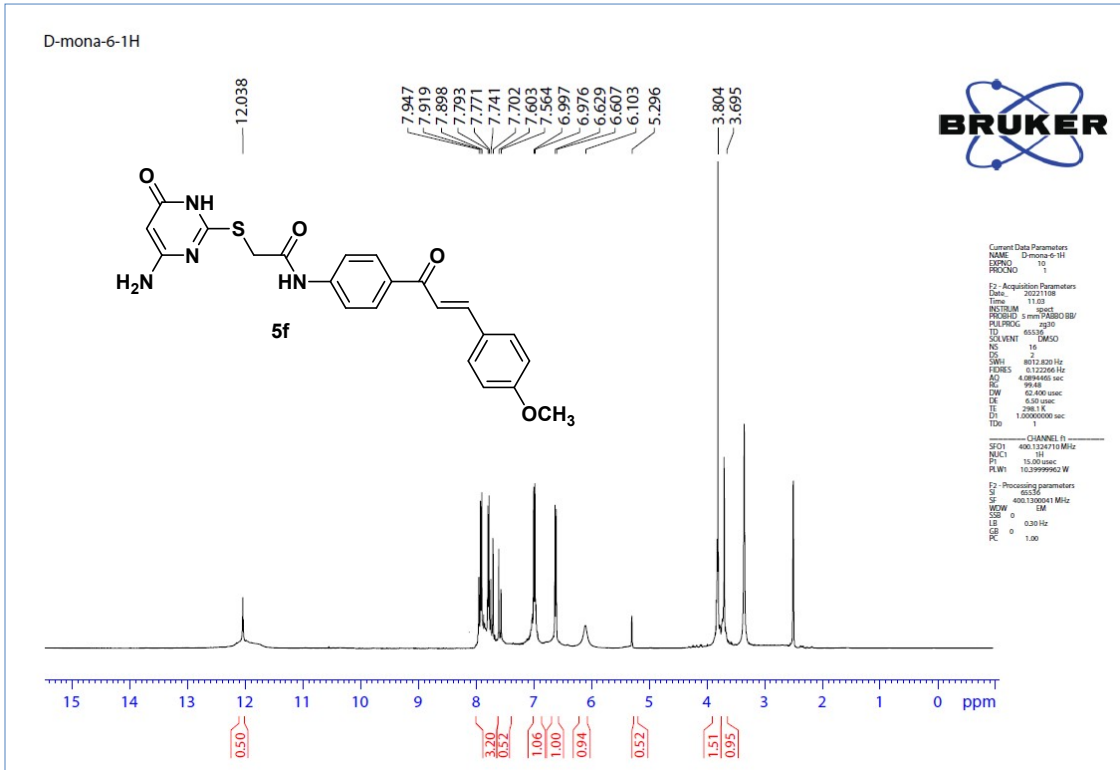
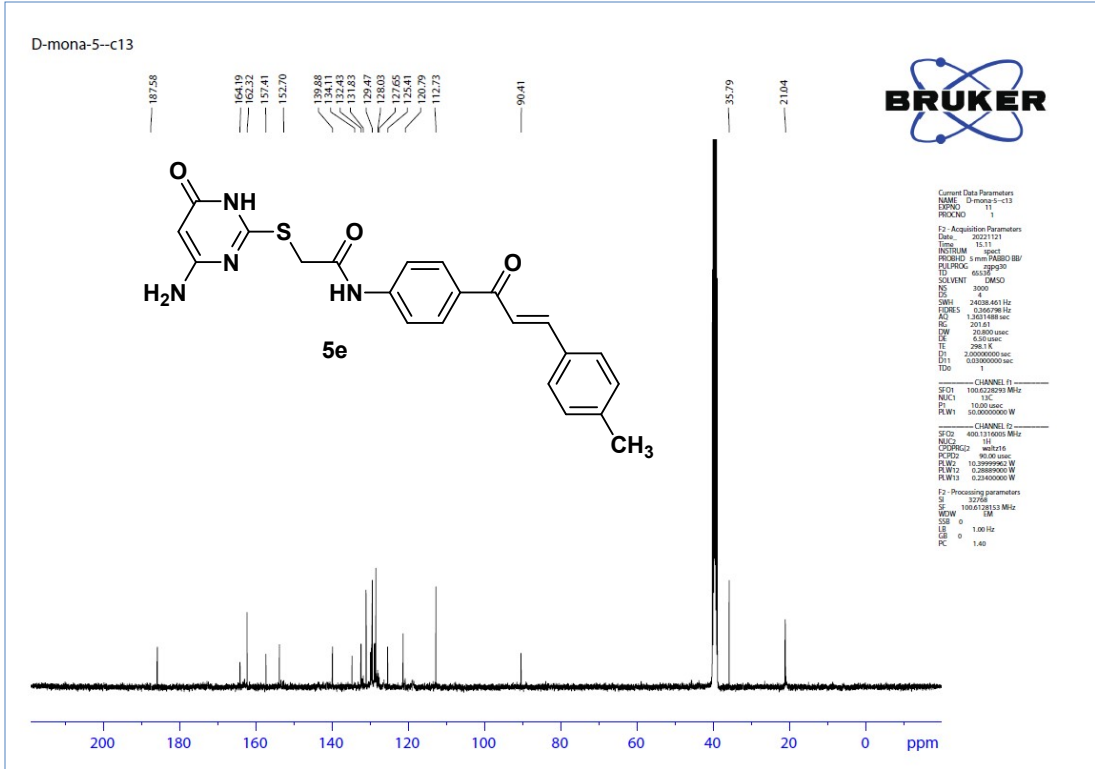


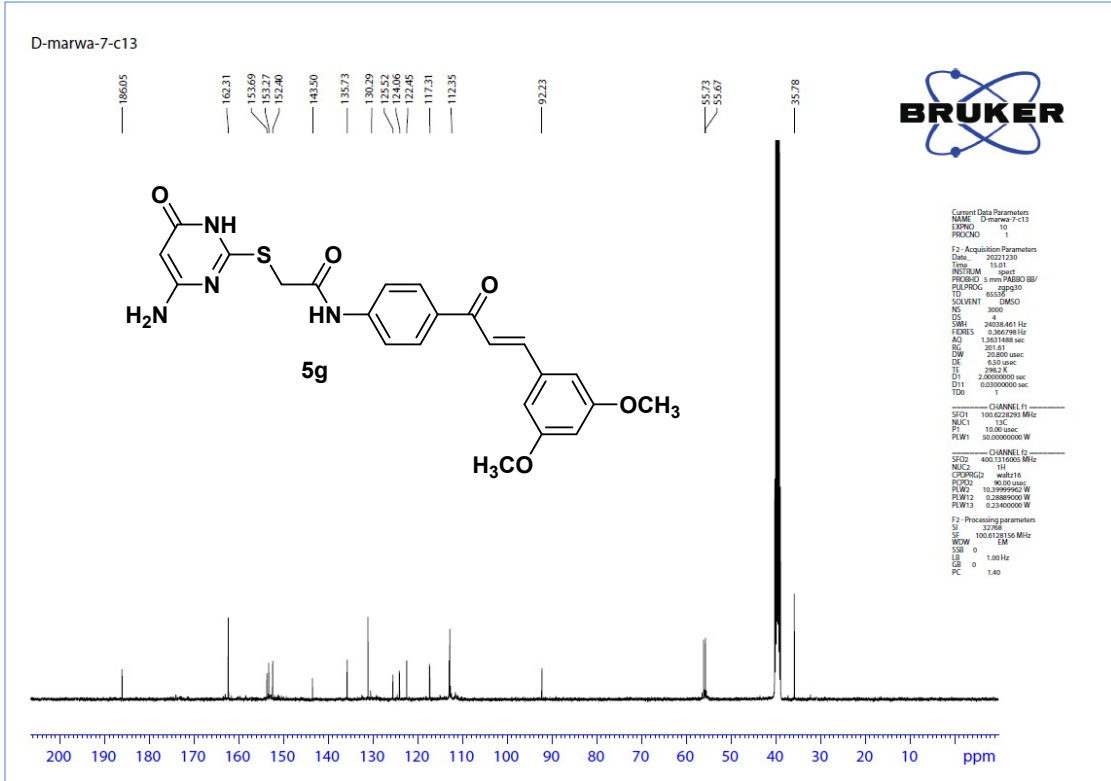
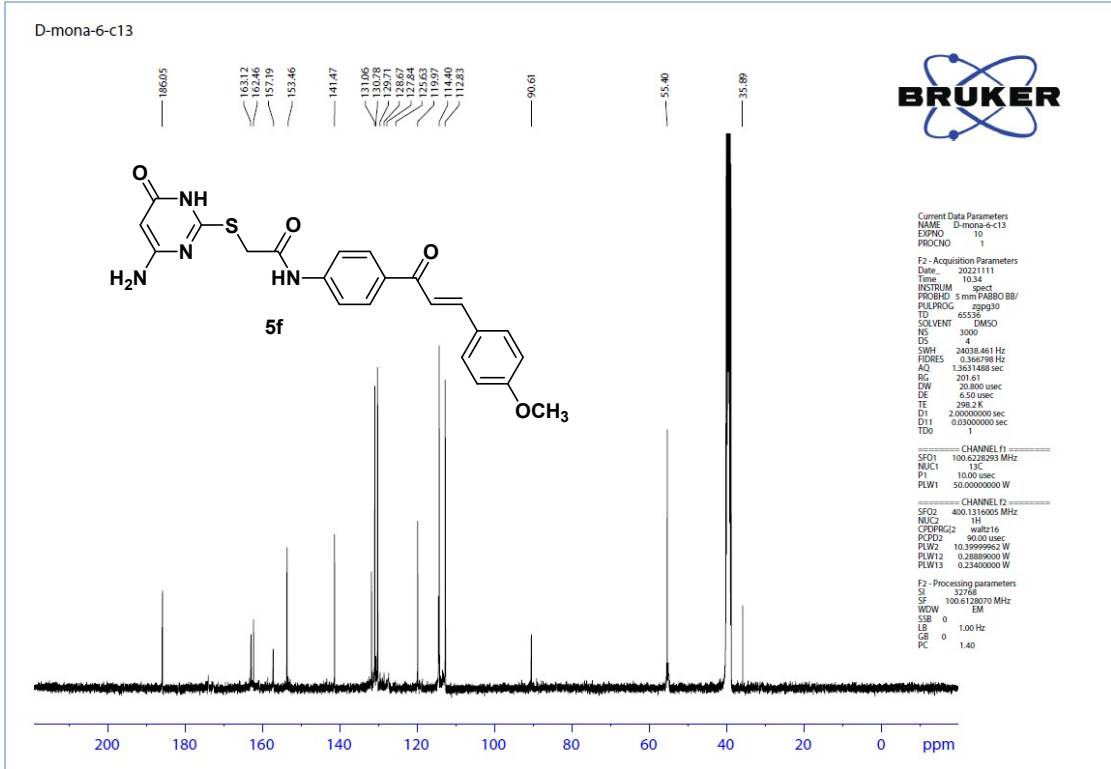
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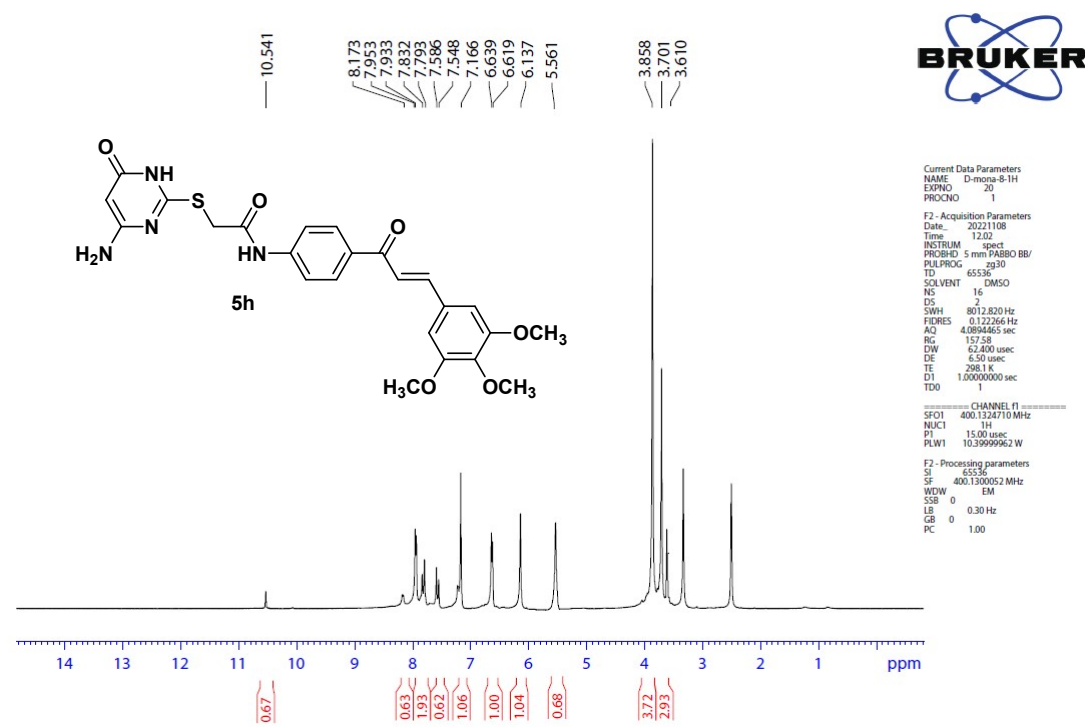




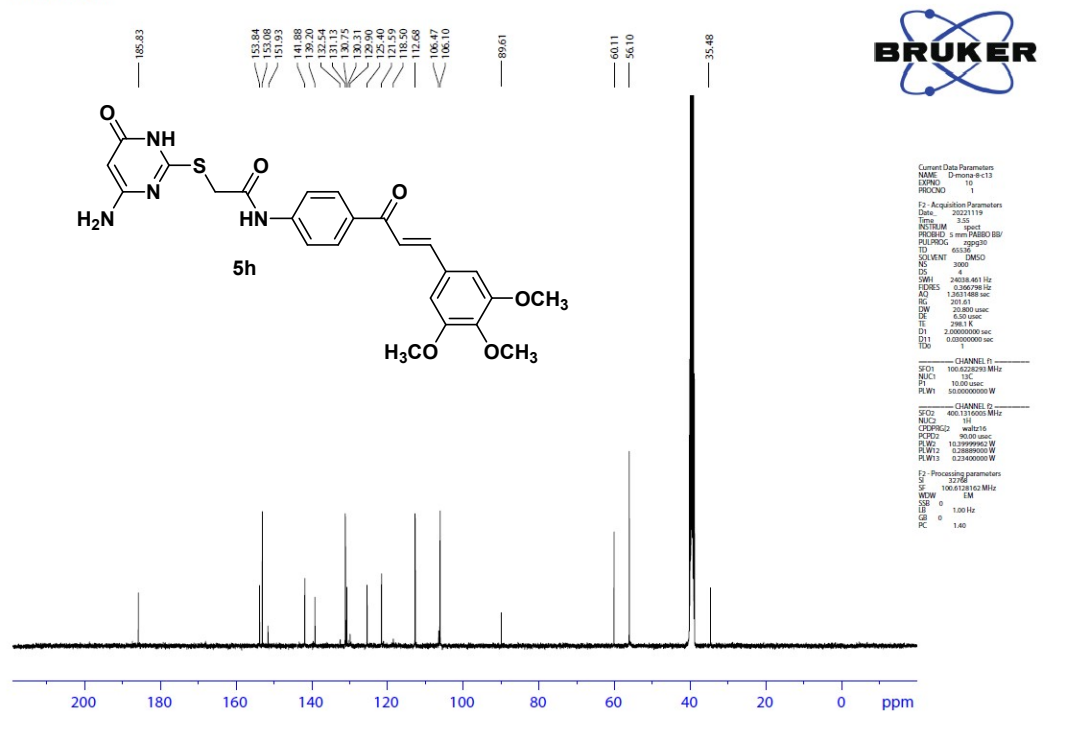




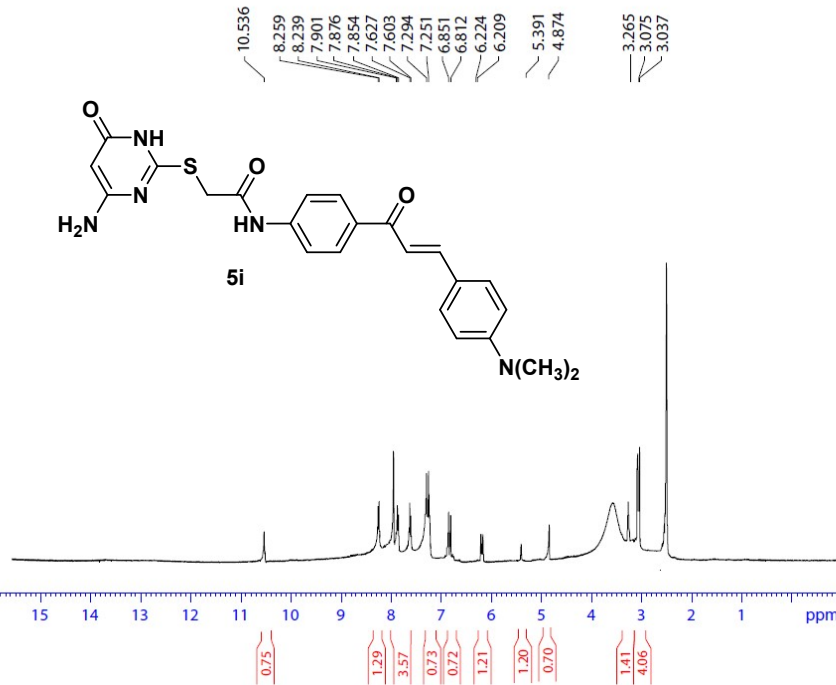
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D-mona-8-c13



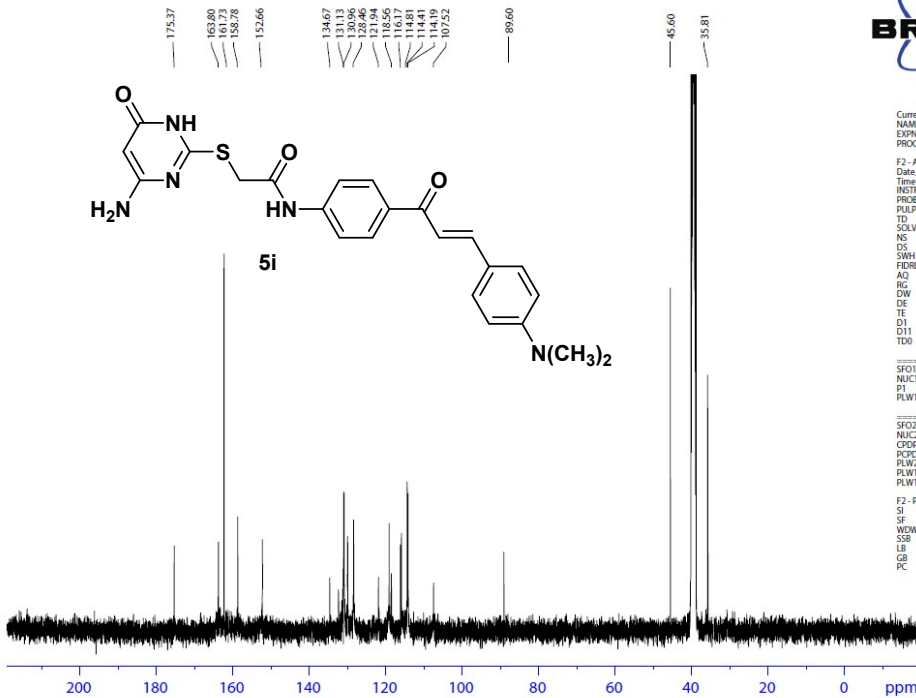
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PROCNO 1
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Time 10:47
INSTRUM spect
PROBHD 5 mm F4BBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 157.58
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
D1 1.0000000 sec
TDO 1

===== CHANNEL f1 =====
SFO1 400.1324710 MHz
NUC1 1H
P1 15.00 usec
PLW1 10.3999962 W
F2 - Processing parameters
SI 65536
SF 400.130036 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

D-mona-9-c13



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PROCNO 1
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TD 65536
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NS 3000
DS 4
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FIDRES 0.360798 Hz
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TDO 1

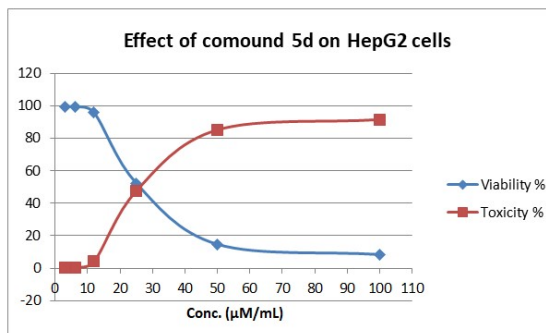
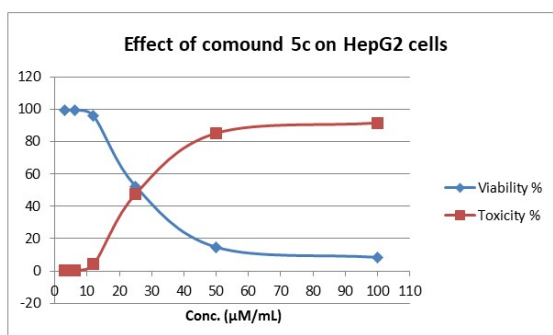
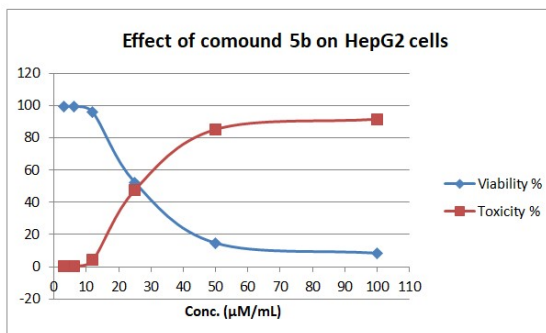
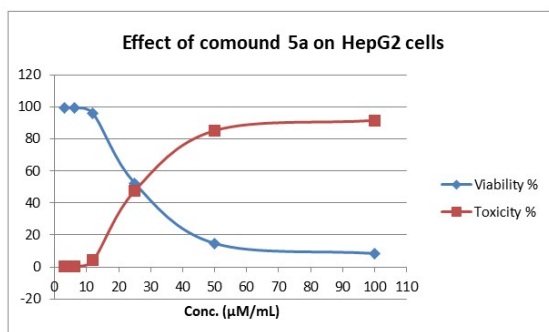
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NUC1 13C
P1 10.00 usec
PLW1 50.0000000 W
===== CHANNEL f2 =====
SFO2 400.1316095 MHz
NUC2 1H
CPCPRG2 waltz16
PCPRD2 90.00 usec
PLW2 10.3999962 W
PLW12 0.2888000 W
PLW13 0.2340000 W
F2 - Processing parameters
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SF 100.618136 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

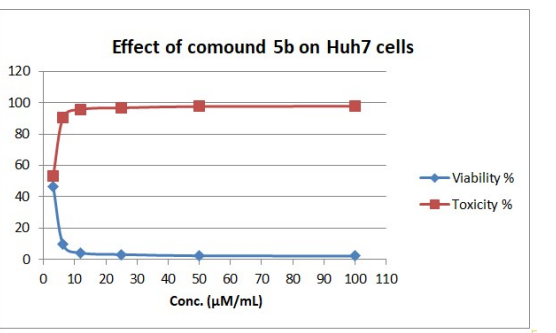
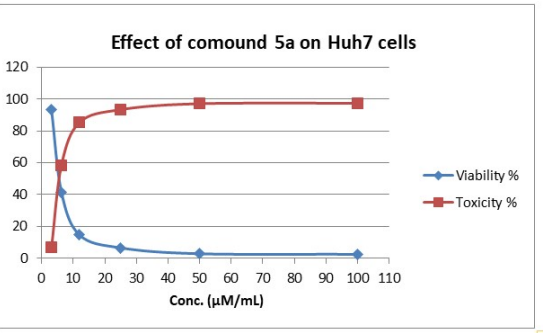
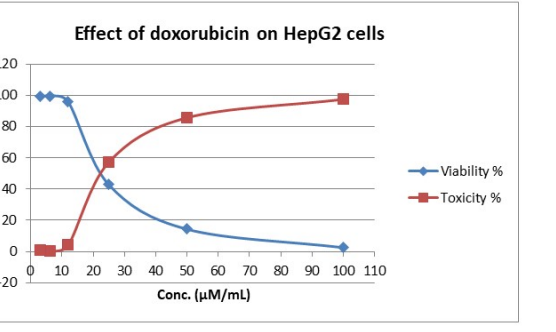
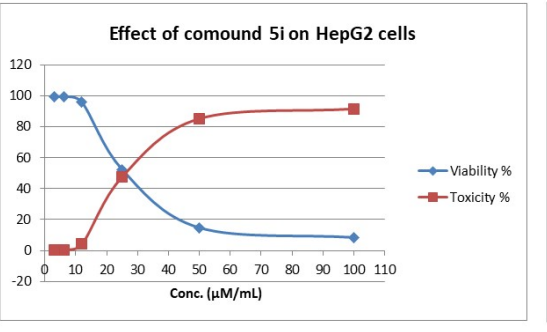
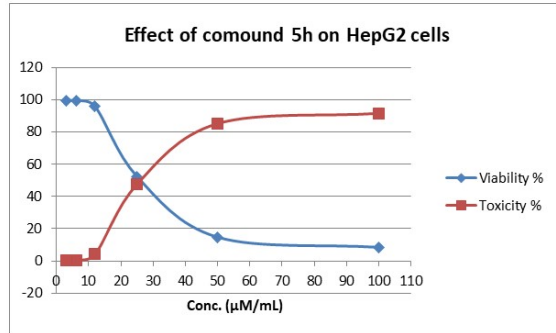
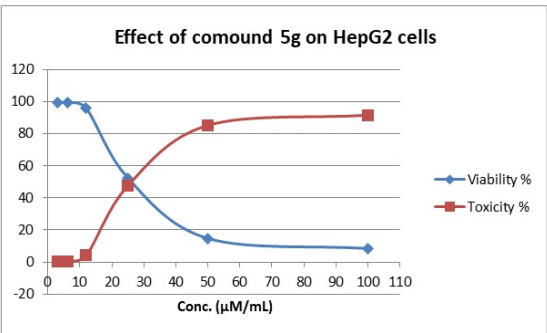
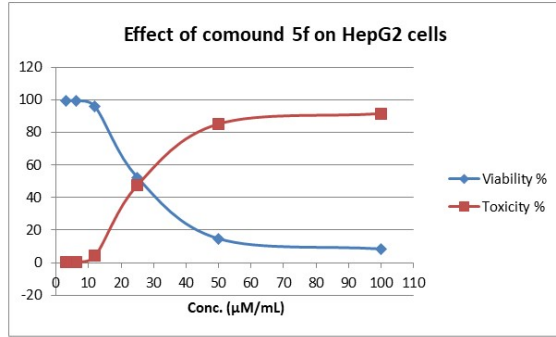
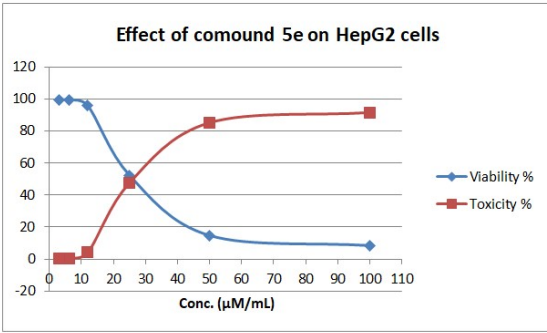
3- Biological activity

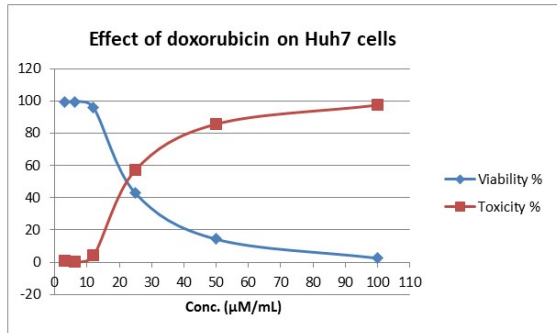
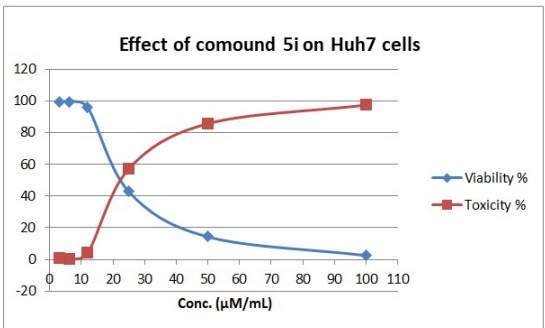
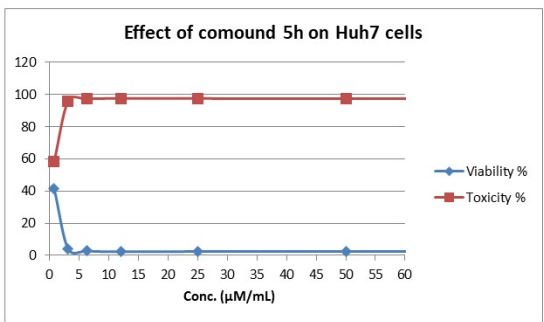
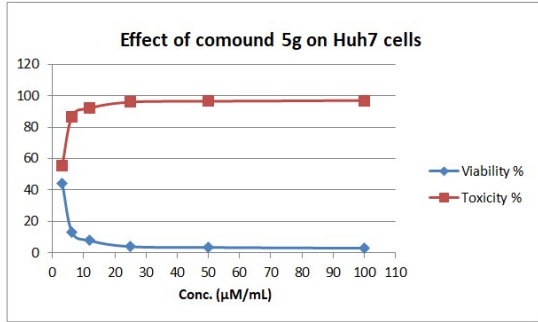
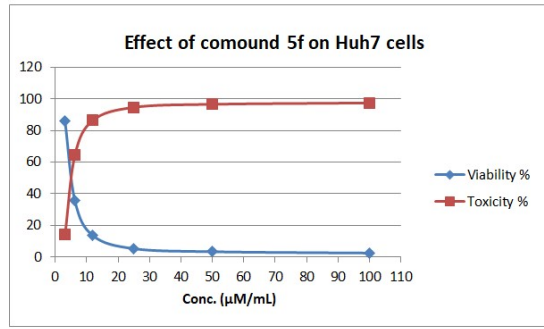
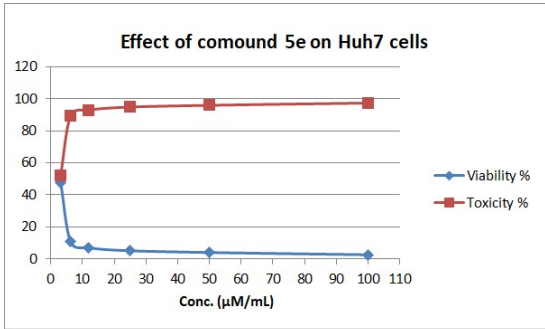
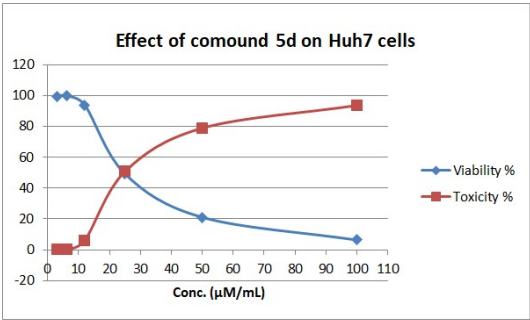
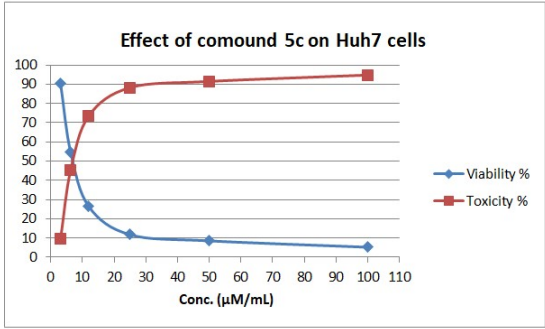
3.1. *In vitro* Cytotoxicity

Using a Sigma *in vitro* MTT-based assay package, the MTT test was utilized to evaluate the cytotoxic activity of target compounds. Prostate cancer cell lines PC-3 and DU-145 were obtained from the American Type Culture Collection and grown in DMEM media with FBS, penicillin, and streptomycin at 37 °C and 5% CO₂. Before being trypsinized, plated, and incubated overnight at 37 °C, the cells were washed in PBS. Cells were grown for 48 hours at 37 °C after being treated with varied concentrations of study chemicals. The cells were treated with MTT reagent for 4 hours at 37 °C in the dark. A plate reader was used to quantify absorbance at 590 nm, and cell viability was assessed. IC₅₀ values were then estimated and the SD was measured after three independent tests.

3.2. Cytotoxicity IC₅₀ curves







3.3. STAT3/5 assay

1. STAT3 Assay

Detailed Results:

standard	conc %
st1	50
st2	25
st3	12.5
st4	6.25
st5	3.13
st6	1.56
st7	0.78

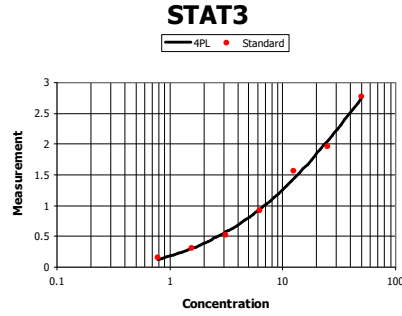


Plate map:

	1	2	3	4	5	6	7	8	9	10	11	12
A	st1	5h	--	--	--	--	--	--	--	--	--	--
B	st2	5h	--	--	--	--	--	--	--	--	--	--
C	st3	5e	--	--	--	--	--	--	--	--	--	--
D	st4	5e	--	--	--	--	--	--	--	--	--	--
E	st5	cont	--	--	--	--	--	--	--	--	--	--
F	st6	cont	--	--	--	--	--	--	--	--	--	--
G	st7	--	--	--	--	--	--	--	--	--	--	--
H	B	--	--	--	--	--	--	--	--	--	--	--

OD

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.795	0.608	0	0	0	0	0	0	0	0	0	0
B	1.981	0.592	0	0	0	0	0	0	0	0	0	0
C	1.577	0.793	0	0	0	0	0	0	0	0	0	0
D	0.941	0.828	0	0	0	0	0	0	0	0	0	0
E	0.546	1.567	0	0	0	0	0	0	0	0	0	0
F	0.321	1.601	0	0	0	0	0	0	0	0	0	0
G	0.169	0	0	0	0	0	0	0	0	0	0	0
H	0.022	0	0	0	0	0	0	0	0	0	0	0

Calibrator	Wells	Conc.	Raw (Corrected)	Backfit	Recovery %
Standard1	A1	50	2.77	51.23	102.5
Standard2	B1	25	1.96	22.64	90.54
Standard3	C1	12.5	1.56	14.41	115.3
Standard4	D1	6.25	0.919	6.041	96.65
Standard5	E1	3.125	0.524	2.832	90.61

Standard6	F1	1.563	0.299	1.537	98.39
Standard7	G1	0.7813	0.147	0.8648	110.7

Sample	Wells	Raw	Background Corrected	Conc.	Conc. (Average)	%CV	SD	SEM
5h	A2	0.608	0.578	3.253	3.197	2.47	0.0789	0.0558
	B2	0.592		3.142				
5e	C2	0.793	0.789	4.688	4.839	4.42	0.214	0.151
	D2	0.828		4.991				
cont	E2	1.57	1.56	14.24	14.53	2.84	0.413	0.292
	F2	1.6		14.82				
Blank	H1	0.022	0	0.371	0.371	-	-	0

2. STAT5 Assay

Detailed results

STANDARDS	ng/ml
St.1	25
St.2	12.5
St.3	6.25
St.4	3.12
St.5	1.56
St.6	0.78
St.7	0.39

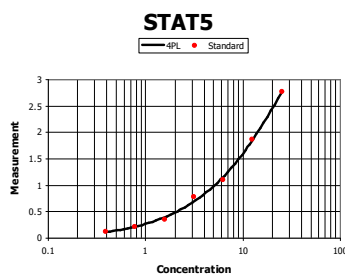


Plate map

	1	2	3	4	5	6	7	8	9	10	11	12
A	st 1	5h	---	--	--	--	--	--	--	--	--	--
B	st 2	5h	---	--	--	--	--	--	--	--	--	--
C	st 3	5e	---	--	--	--	--	--	--	--	--	--
D	st 4	5e	---	--	--	--	--	--	--	--	--	--
E	st 5	cont	---	--	--	--	--	--	--	--	--	--
F	st 6	cont	---	--	--	--	--	--	--	--	--	--
G	st 7	--	---	--	--	--	--	--	--	--	--	--
H	B	--	---	--	--	--	--	--	--	--	--	--

Samples OD results

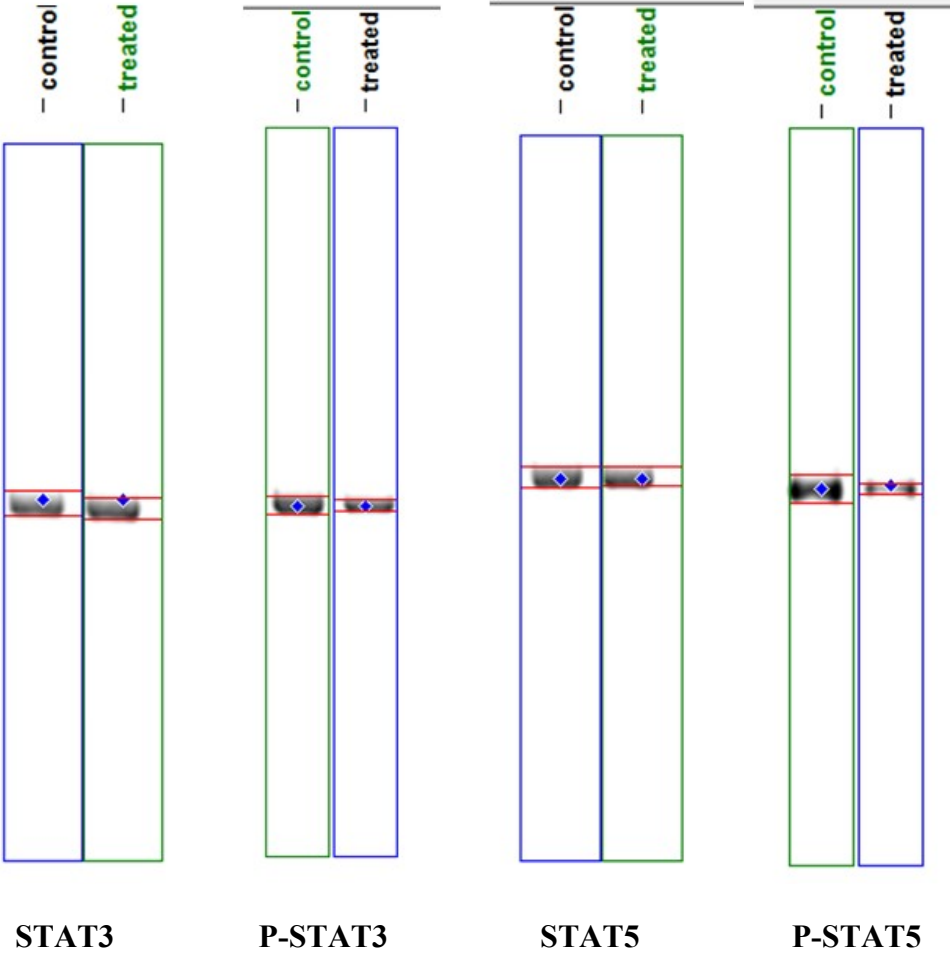
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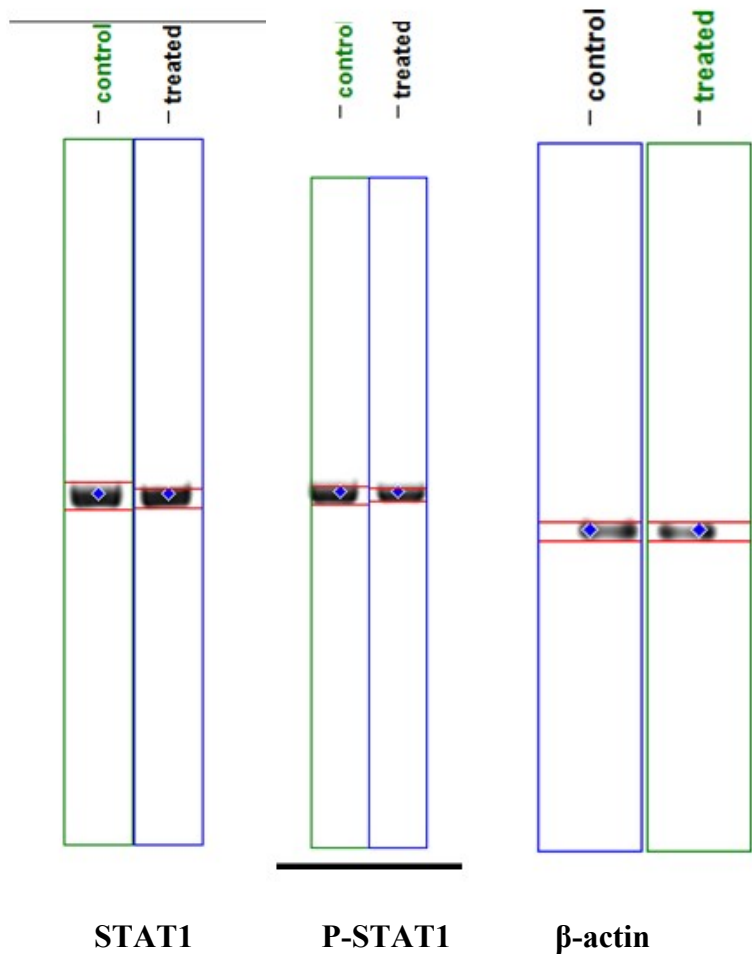
A	2.792	0.566	0	0	0	0	0	0	0	0	0	0
B	1.886	0.549	0	0	0	0	0	0	0	0	0	0
C	1.119	0.889	0	0	0	0	0	0	0	0	0	0
D	0.791	0.951	0	0	0	0	0	0	0	0	0	0
E	0.365	1.803	0	0	0	0	0	0	0	0	0	0
F	0.228	1.668	0	0	0	0	0	0	0	0	0	0
G	0.147	0	0	0	0	0	0	0	0	0	0	0
H	0.018	0	0	0	0	0	0	0	0	0	0	0

Calibrator	Wells	Conc.	Raw (Corrected)	Backfit	Recovery %
Standard1	A1	25	2.77	24.96	99.83
Standard2	B1	12.5	1.87	12.7	101.6
Standard3	C1	6.25	1.1	5.832	93.31
Standard4	D1	3.125	0.773	3.624	116
Standard5	E1	1.563	0.347	1.335	85.45
Standard6	F1	0.7813	0.21	0.7469	95.6
Standard7	G1	0.3906	0.129	0.4405	112.8

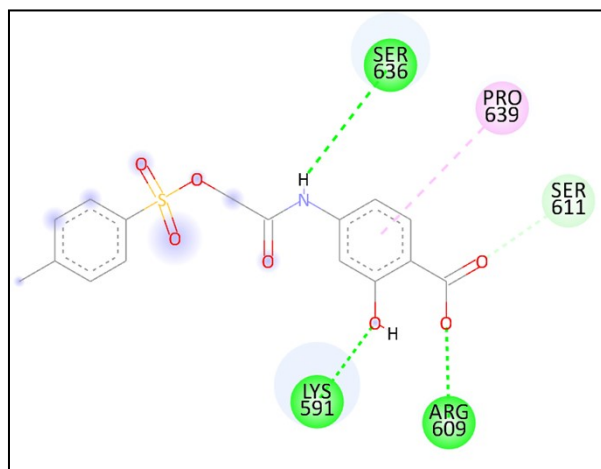
Sample	Wells	Raw	Background Corrected	Conc.	Conc. (Average)	%CV	SD	SEM
5h	A2	0.566	0.54	2.333	2.288	2.79	0.0638	0.0451
	B2	0.549		2.243				
5e	C2	0.889	0.902	4.242	4.447	6.51	0.289	0.205
	D2	0.951		4.652				
cont	E2	1.8	1.72	11.82	11.15	8.55	0.953	0.674
	F2	1.67		10.48				
Blank	H1	0.018	0	0.04569	0.04569	-	-	0

3.4. Western blot analysis

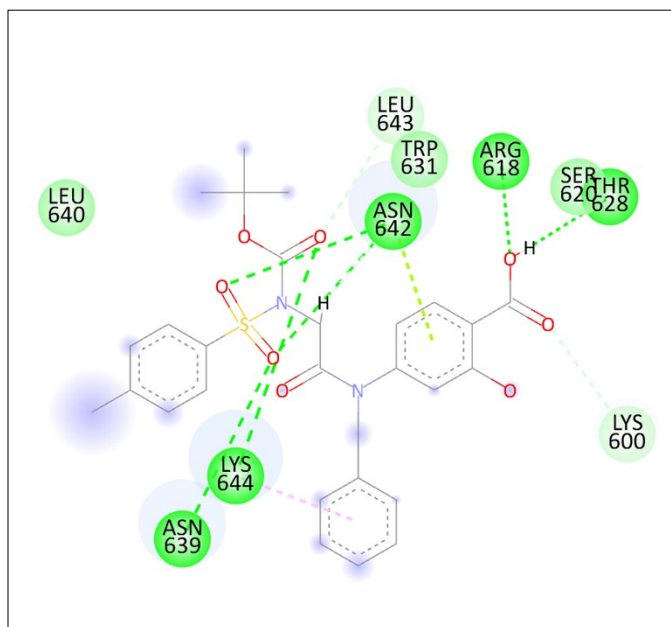




4. Docking Study



Binding poses of **S3I-201** inside STAT3 active site.



Binding pose of **SF-1-088** inside STAT5 active site