Supplementary data

Unravelling the potency of 4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile scaffold with S-arylamide hybrids as PIM-1 kinase Inhibitors: synthesis, biological activity and *in silico* studies

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Keywords: PIM kinase, cell cycle, Apoptosis, ATPase activity, Molecular Dynamics, Docking

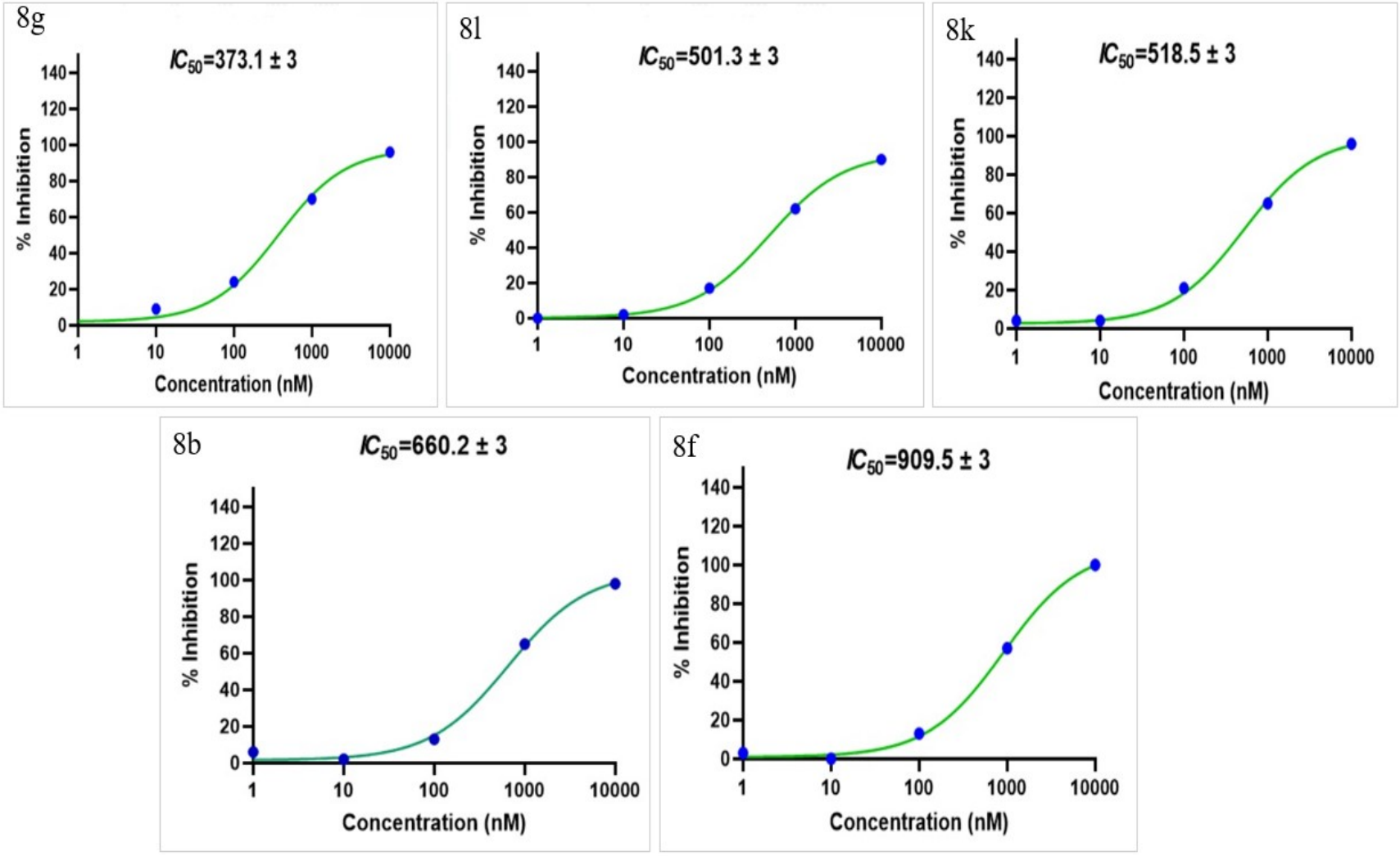


Figure S1: Some inhibitory concentration curves (IC_{50}) of targeted compounds on the PIM-1 kinase activity (Without error bar)

Table 1S: Antiproliferative assessment of the newly synthesized derivatives **(8a-n)** compared to both Doxorubicin and quercetin against different cancerous cell lines (MCF-7and PC-3) and compared to WI-38 normal cell line. Corresponding SI, Selectivity Index is calculated as IC_{50} compound (WI-38)/ IC_{50} compound (cancer cell line). Data represent mean ± SEM, n = 3.

Compounds	*IC_{50} (µg/mL) ± SEM and corresponding SI						
	WI-38	MCF-7		DU-145		PC-3	
	IC_{50}	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
8a	76.91±6.5	23.35±3.6	3.29	79.77±6.2	0.96	3.745±1.1	20.54
8b	68.30±5.8	11.78±1.9	5.80	2.940±1.9	23.23	22.66±0.89	3.01
8c	51.99±5.9	4.409±3.1	11.79	2.676±1.3	19.43	16.78±2.6	3.10
8d	63.91±5.5	8.794±6.8	7.27	45.97±4.6	1.39	8.346±1.6	7.66
8e	77.20±7.6	34.84±4.3	2.22	9.560±6.8	8.08	6.274±1.4	12.30
8f	76.95±4.7	15.12±2.4	5.09	2.217±1.0	34.71	7.713±2.1	9.98
8g	57.24±3.5	3.188±2.6	17.95	2.730±3.2	20.97	31.56±2.1	1.81
8h	60.73±3.8	5.799±5.9	10.47	28.67±2.6	2.12	14.10±0.9	4.31
8i	49.96±5.1	10.68±2.5	4.68	2.617±1.7	19.09	74.09±1.9	0.67
8j	60.67±6.0	55.86±4.6	1.09	2.517±2.1	24.10	48.49±8.9	1.25
8k	73.46± 6.5	2.221±1.7	33.08	3.320±3.6	22.13	25.63±2.1	2.87
8l	62.44±5.6	3.510±2.7	17.79	2.599±1.5	24.02	24.29±3.1	2.57
8m	71.73±2.9	30.26±3.5	2.37	25.16±1.2	2.85	7.504±3.2	9.56
8n	52.43±4.7	21.50±3.2	2.44	50.30±3.6	1.04	49.19±3.7	1.07
Standards							
Doxorubicin	32.43±2.3	18.76±1.9	1.73	59.47±4.3	0.55	34.40±1.1	0.94
Quercetin	12.60±1.7	32.83±4.7	0.38	37.93±5.1	0.33	37.83±4.2	0.33

Table 2S: Apoptosis assay measuring the percentage of viable, apoptotic, late apoptotic, and necrotic cells by AV/PI assay using flow cytometry. The assay was performed after the treatment of both PC-3 (prostate cancer) and MCF-7 (breast cancer) for 24 h with Doxorubicin (positive control), **8g**, **8b**, **8c**, **8j**, **8n**, **8f** and **8m** compared to 0.1% DMSO negative control.

Comp No	Apoptosis Analysis of Cancer Cell Line#			
	% Viable cells (LL)	% Early apoptotic cells (LR)	% Late apoptotic cells (UL)	% Necrotic cells (UR)
PC-3 cancer cell line				
Negative Control	81.55±7.4	7.51±5.2	3.24±0.65	7.70±1.3
8b	32.17±2.8**	4.08±0.8	38.53±2.4***	25.22±2.3**
8c	35.35±3.6**	5.49±1.1	36.97±2.8***	22.19±2.2**
8f	47.85±4.2**	6.23±2.1	31.43±2.1***	14.49±1.4*
8g	31.57±3.5**	1.08±0.1	58.19±2.3***	9.17±2.4
8j	32.66±4.1**	10.78±2.6	18.66±1.3**	37.91±2.6***
8m	48.03±3.7**	10.10±1.3	23.02±1.7***	18.85±1.7*
8n	43.96±3.8**	5.43±1.1	34.74±1.4***	15.87±1.3*
Doxorubicin	18.82±1.1***	3.46±2.2	54.70±4.3***	23.02±1.7**
Quercetin	15.46±1.3***	53.16±1.1***	0.33±0.01	31.05±1.1***
MCF-7 cancer cell line				
Negative Control	88.79±7.7	1.45±0.54	6.90±2.3	2.86±0.54
8b	34.51±2.6**	1.87±0.49	47.99±3.8***	15.64±2.7*
8c	38.13±2.5**	2.02±0.9	43.85±3.6***	16.00±4.1*
8f	49.25±4.5**	4.45±1.0	37.64±2.9***	8.67±2.4
8g	36.92±3.2**	2.92±1.6	51.25±4.3***	8.91±2.9
8j	38.92±2.7**	3.24±1.4	44.97±4.1***	12.87±3.8*
8m	52.90±5.1**	4.18±0.88	31.97±3.5***	10.95±3.7
8n	50.77±4.3**	1.92±0.63	42.79±2.8***	4.52±1.1
Doxorubicin	17.08±1.1***	3.79±1.2	50.01±5.1***	29.12±3.1**
Quercetin	20.72±2.8***	75.26±5.4 ***	0.1±0.01	3.92±0.7

Data represented as mean ± standard error of the mean (SEM), n = 3.

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. p values indicate (either increase or decrease) the significance compared to untreated control cells (0.1%DMSO solvent only). # LL, lower lift; UL, upper lift; LR, lower right; UR, upper right quadrants.

Table 3S: Cell cycle analysis of both PC-3 (prostate cancer) and MCF-7 (breast cancer) treated for 24h with Doxorubicin (positive control), **8g**, **8b**, 8c, **8j**, **8n**, **8f** and **8m** compared to 0.1% DMSO negative control showing the DNA content at different cycle phases.

Comp No	Cell cycle analysis of Cancer Cell Line			
	% $SubG_0$-G_1- phase	% G_0-G_1- phase	% S-phase	%G_2M
		PC-3 cancer cell line		
Control	8.20±0.6	44.94±4.5	12.90±1.3	33.46±1.3
8b	0.75±0.1	43.21±3.4	12.26±1.4	43.15±3.7
8c	1.13±0.2	44.80±3.8	13.57±1.6	39.75±2.9
8f	2.88±0.9	45.28±4.8	16.64±2.4	34.27±1.9
8g	1.57±0.7	40.10±4.0	15.24±2.0	42.27±3.2
8j	2.13±1.0	43.23±6.2	17.91±1.8	35.74±2.6
8m	1.67±0.45	47.85±4.7	14.22±4.1	35.53±4.2
8n	2.31±0.7	41.42±3.5	13.49±2.2	41.90±4.3
Doxorubicin	27.33±1.6***	33.45±3.5	15.19±2.3	23.86±1.4
Quercetin	1.59±0.2	28.09±1.4	27.79±3.8	41.25±3.3
		MCF-7 cancer cell line		
Control	7.86±0.6	67.69±5.3	16.51±3.3	7.90±4.9
8b	3.23±1.3	5.72±3.7***	6.64±4.1	84.04±5.6***
8c	14.58±2.4*	16.83±3.8***	9.57±0.99	59.03±4.9***
8f	31.50±3.1*	11.89±1.9***	8.28±3.2	48.02±5.2***
8g	5.62±2.8	7.71±4.6***	7.35±2.4	79.03±3.6***
8j	7.71±3.6	6.94±3.3***	10.22±1.4	74.51±7.5***
8m	29.24±2.8**	11.48±3.6***	10.25±0.87	48.96±4.7***
8n	23.19±2.3*	12.40±4.8***	8.39±2.6	55.81±6.1***
Doxorubicin	9.15±0.4	39.72±2.8**	18.17±2.2	32.25±2.2***
Quercetin	2.12±0.9	8.79±4.2***	16.76±2.1	71.65±6.7***

*p< 0.05, **p< 0.01 and ***p< 0.001. *P* values indicate (either increase or decrease) the significance compared to untreated control cells (0.1%DMSO solvent only)

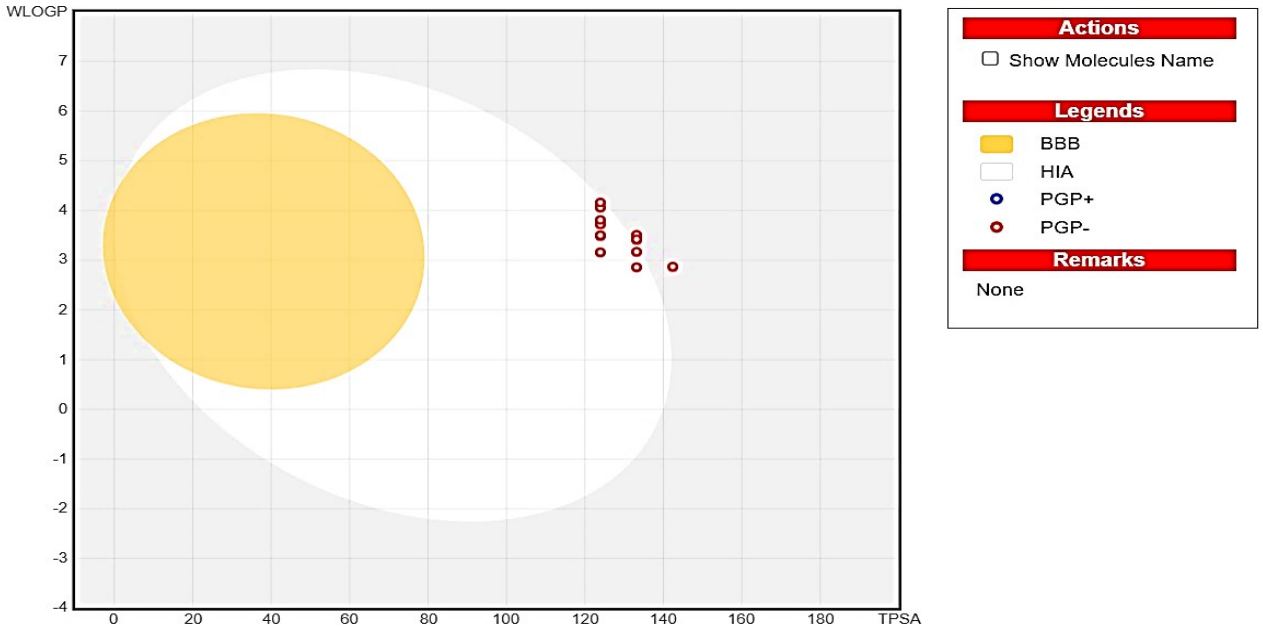


Figure S2: Human intestinal absorption (HIA) and Blood Brain Barrier (BBB) plot for the newly synthesized compounds.

^{1}HNMR and ^{13}CNMR spectra

Figure S3: ***1HNMR*** *of 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide* ***(8a)***

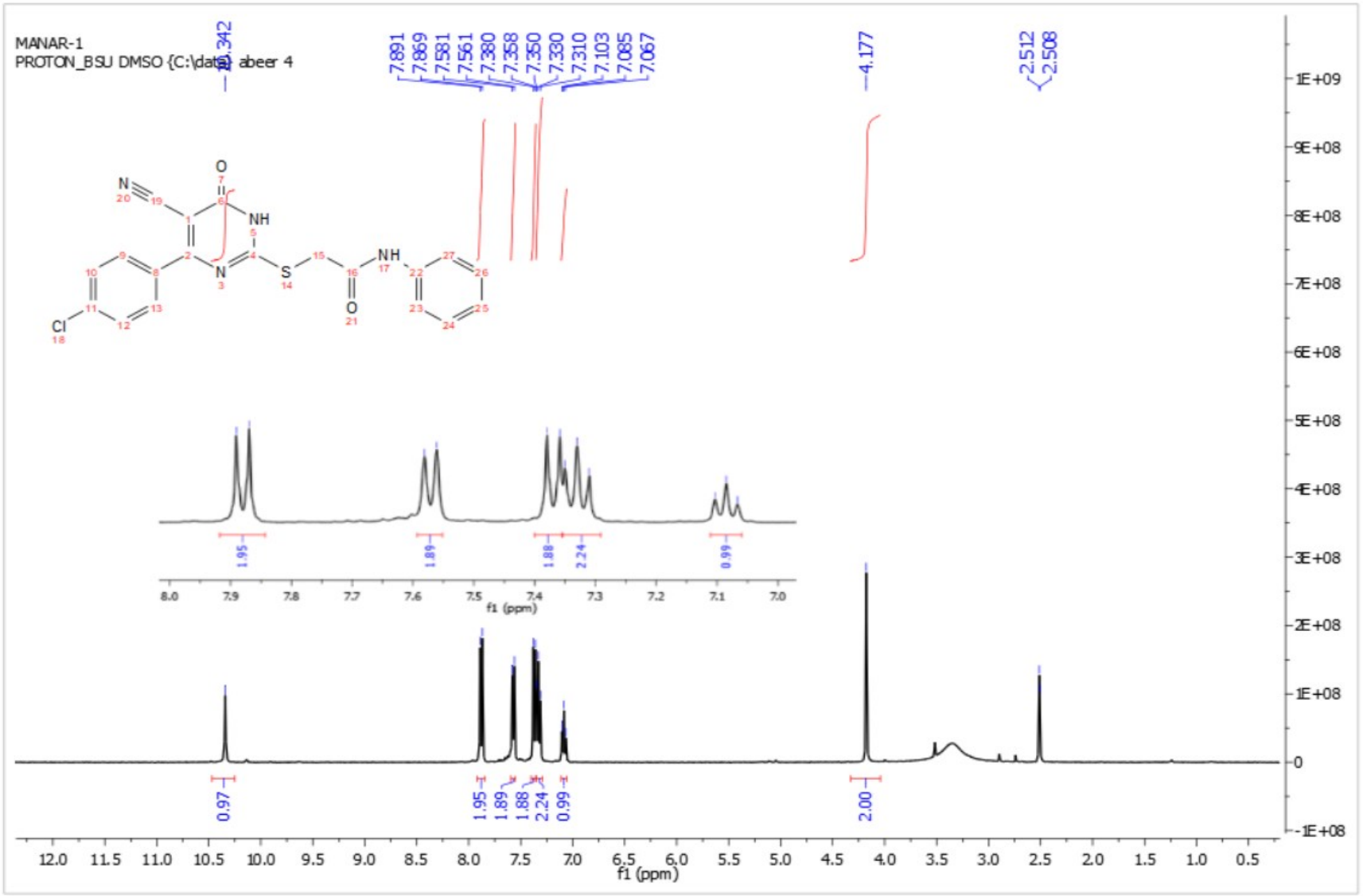


Figure S4: ***13CNMR*** *of 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide* ***(8a)***

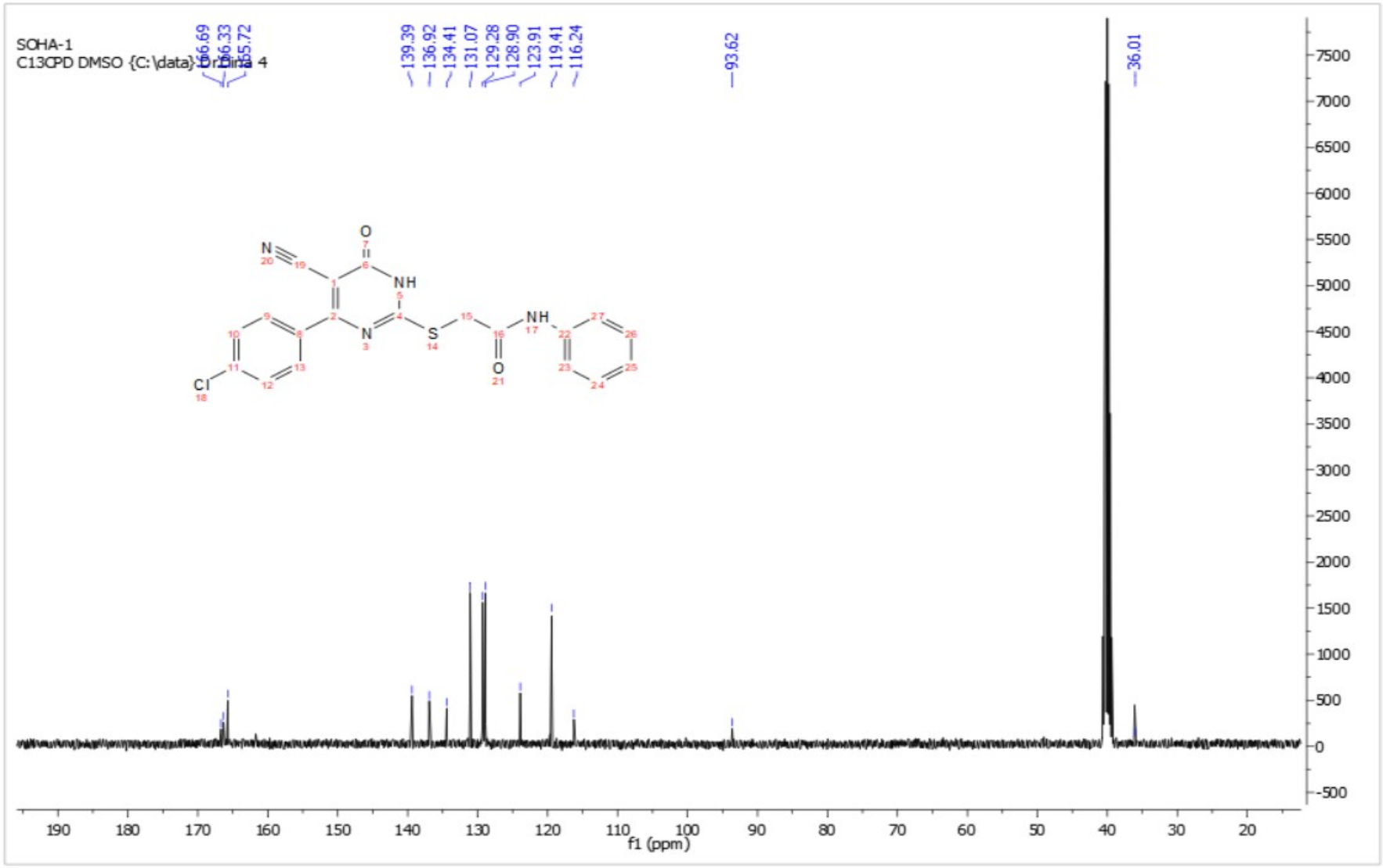


Figure S5: **1HNMR** *2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamide(8b)*

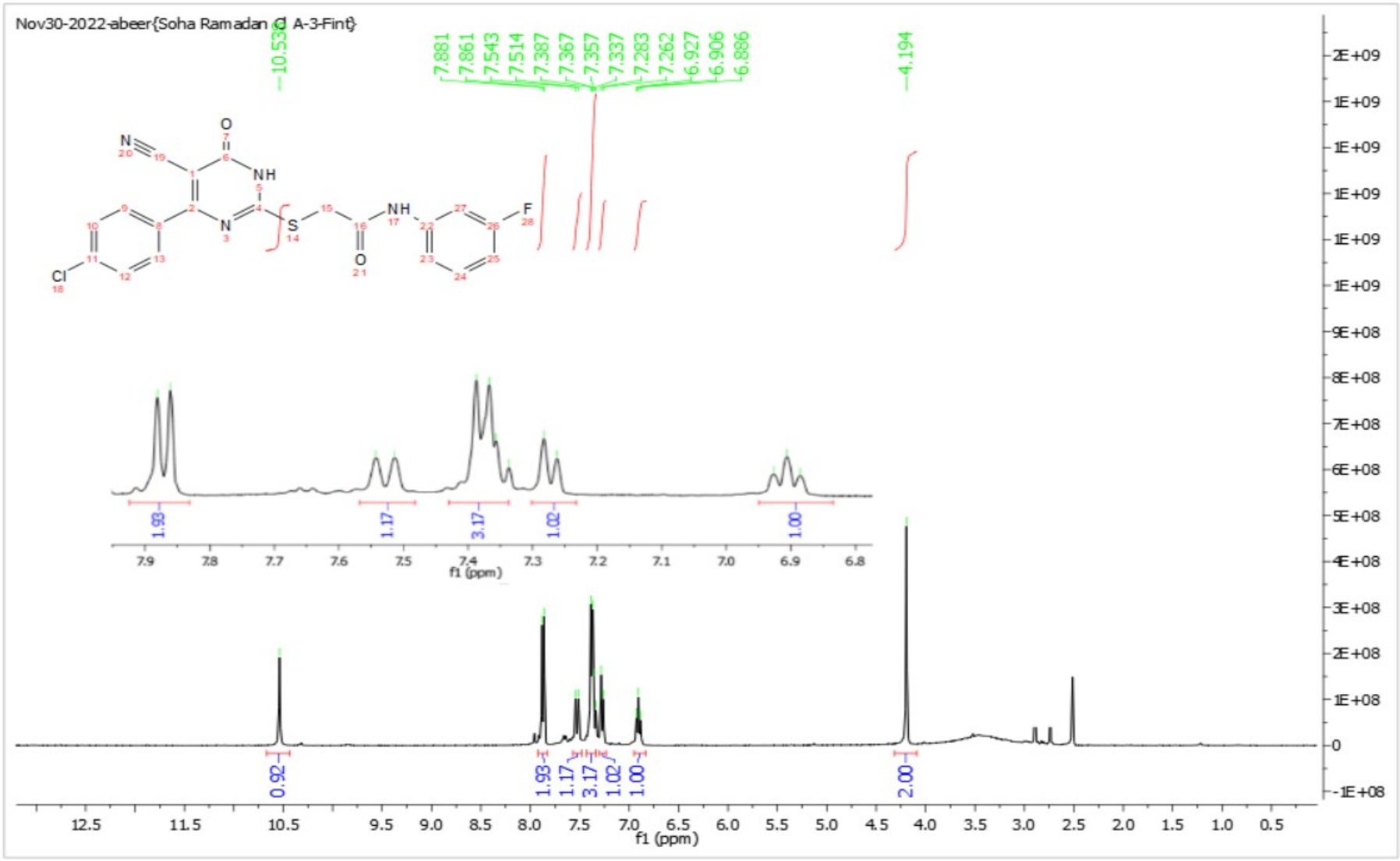


Figure S6: D_2O of **1HNMR** *2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamide(8b)*

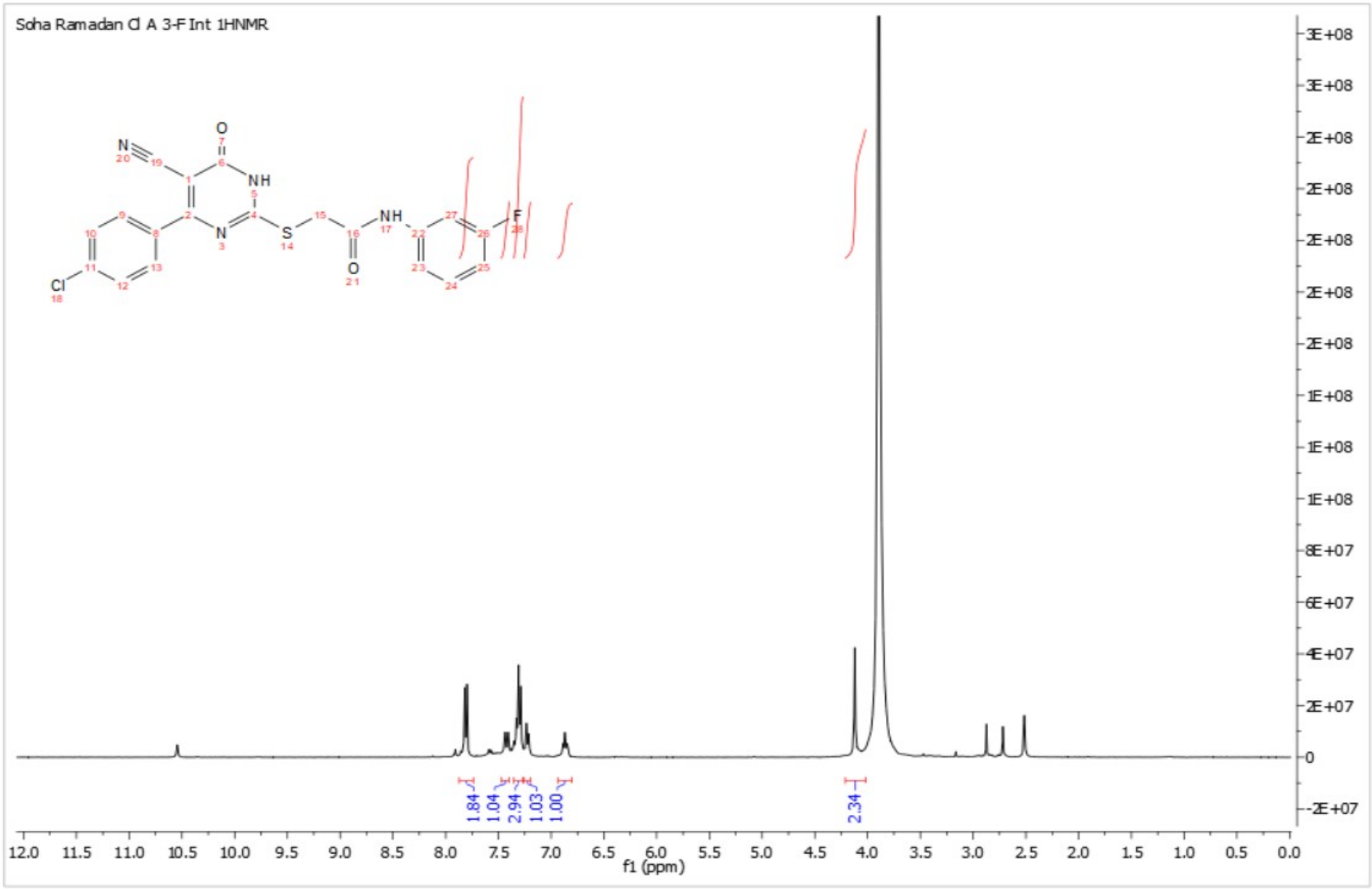


Figure S7: ***[13]CNMR*** *of 2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamide* ***(8a)***

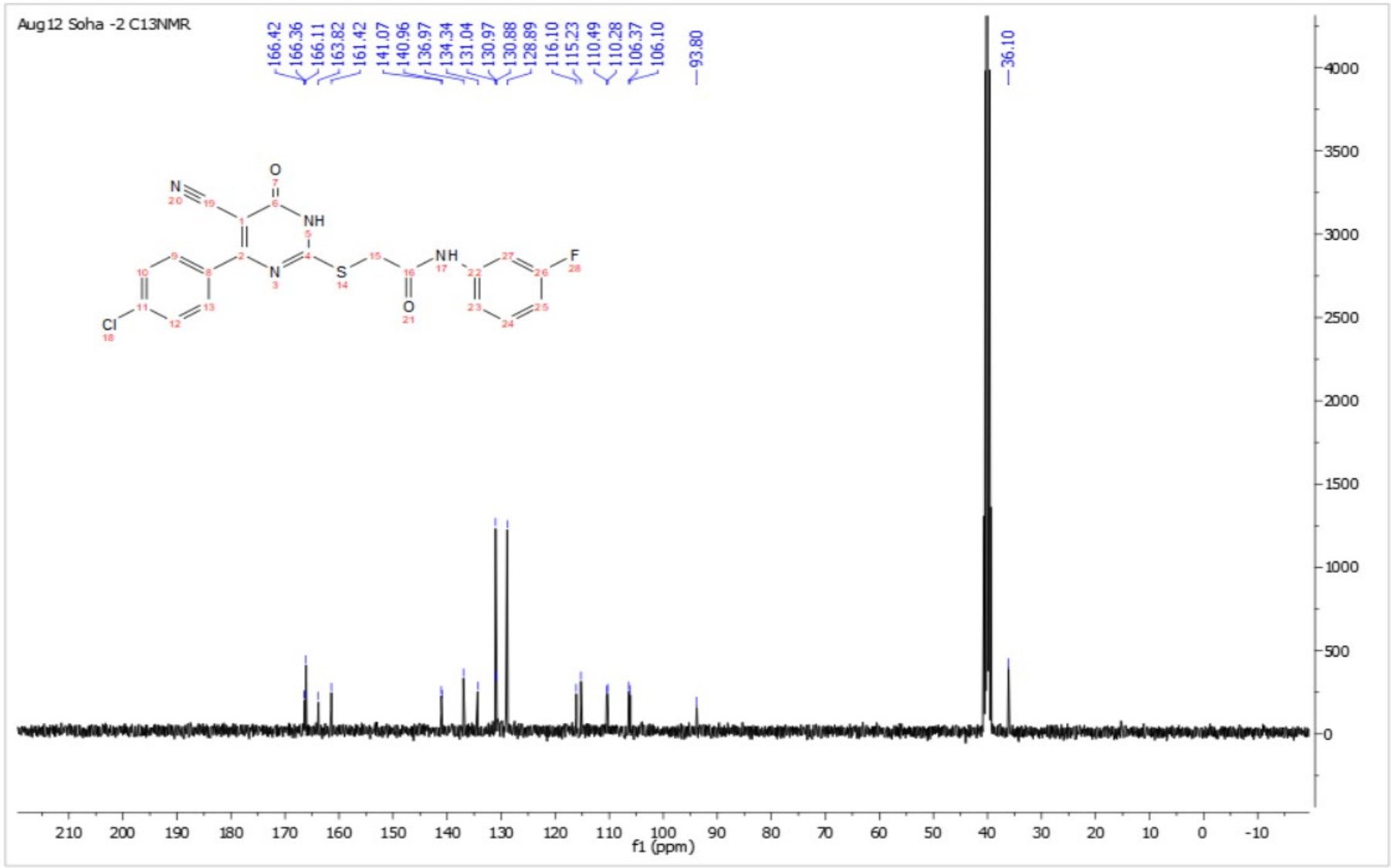


Figure S8: **^{1}HNMR** ***2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8f)***

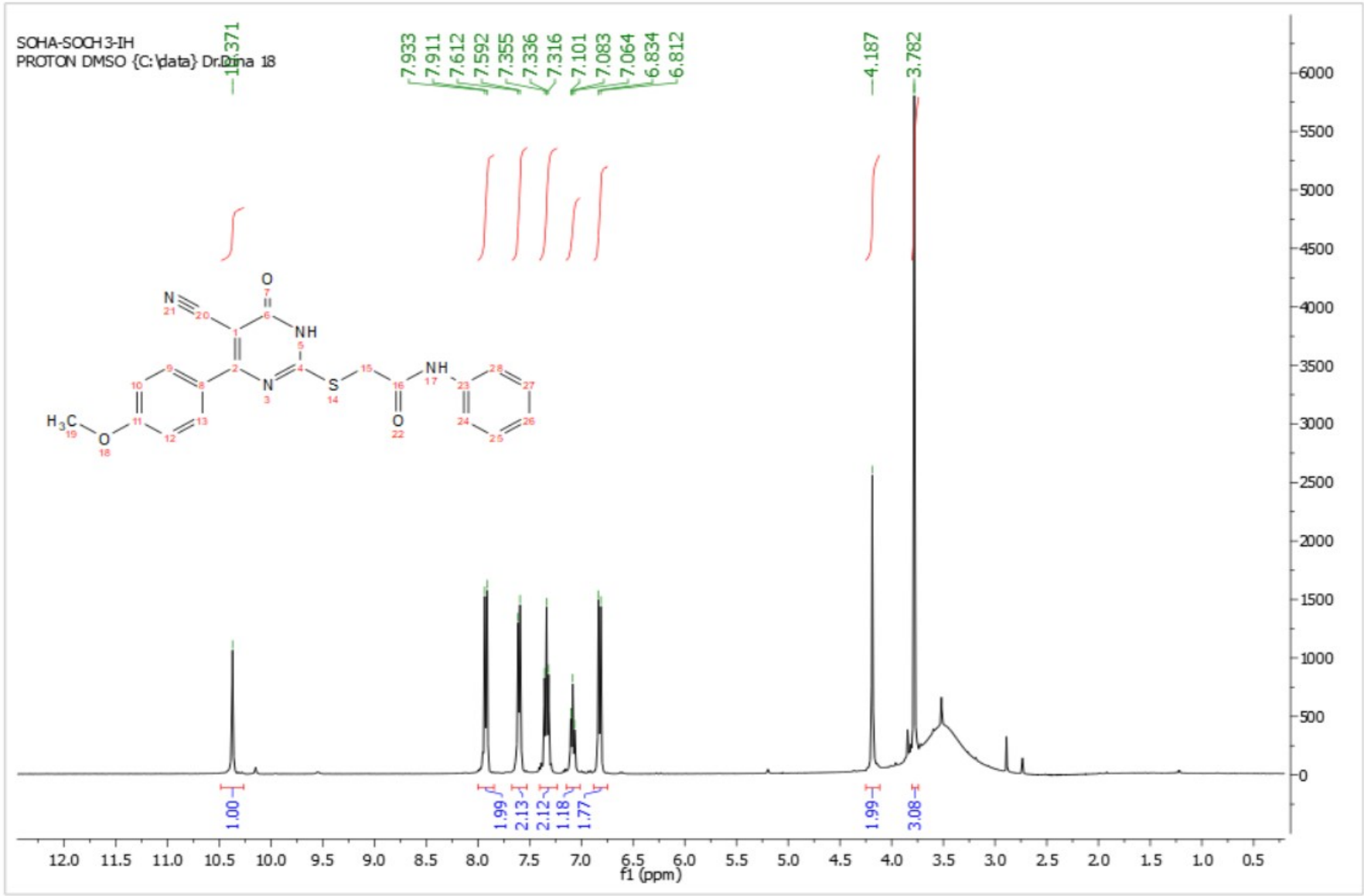


Figure S9: **D_2O ^{1}HNMR** ***2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8f)***

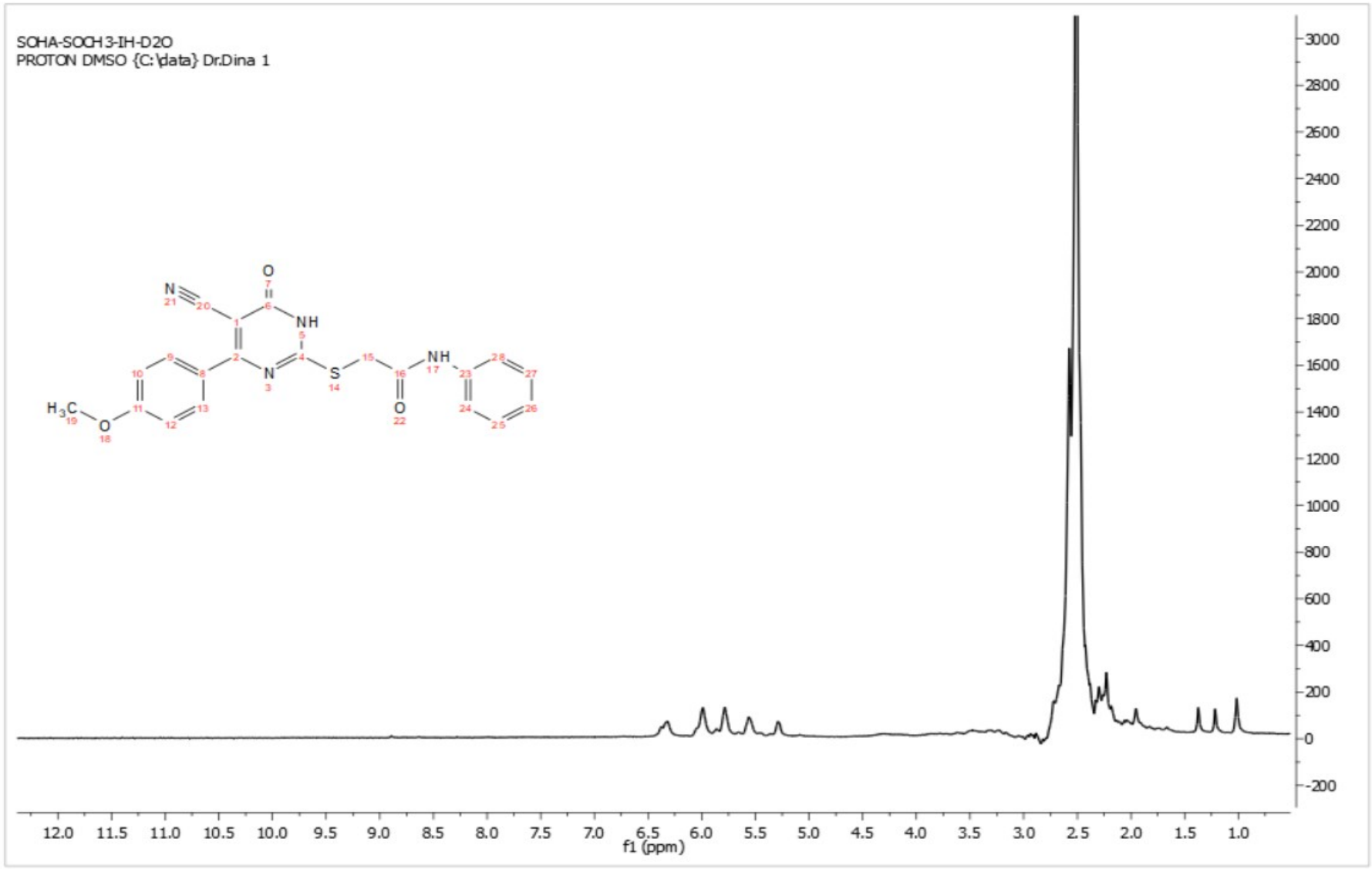


Figure S10: **^{13}CNMR** *2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8f)*

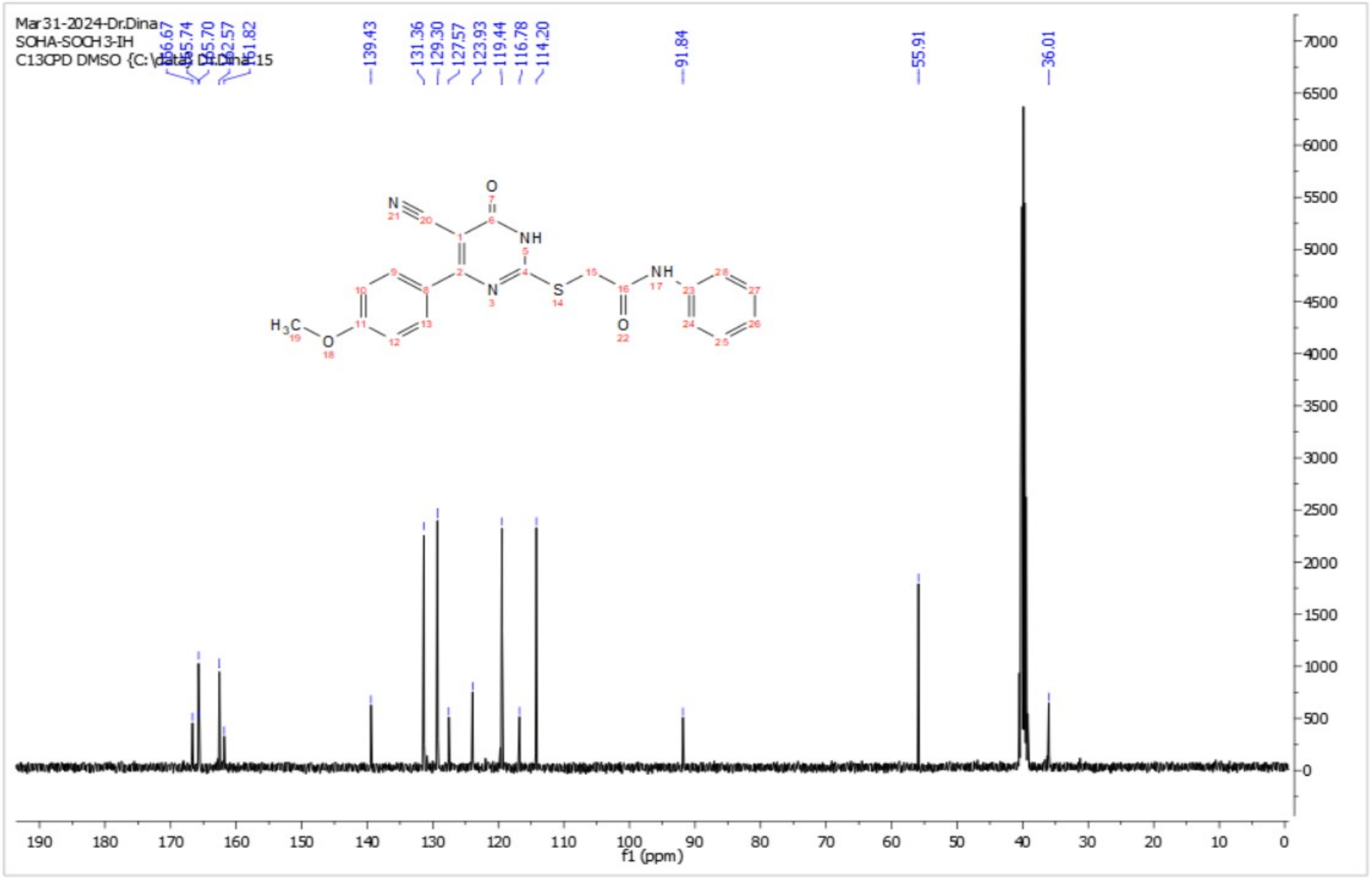


Figure S11: **^{1}HNMR** ***2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamide (8g)***

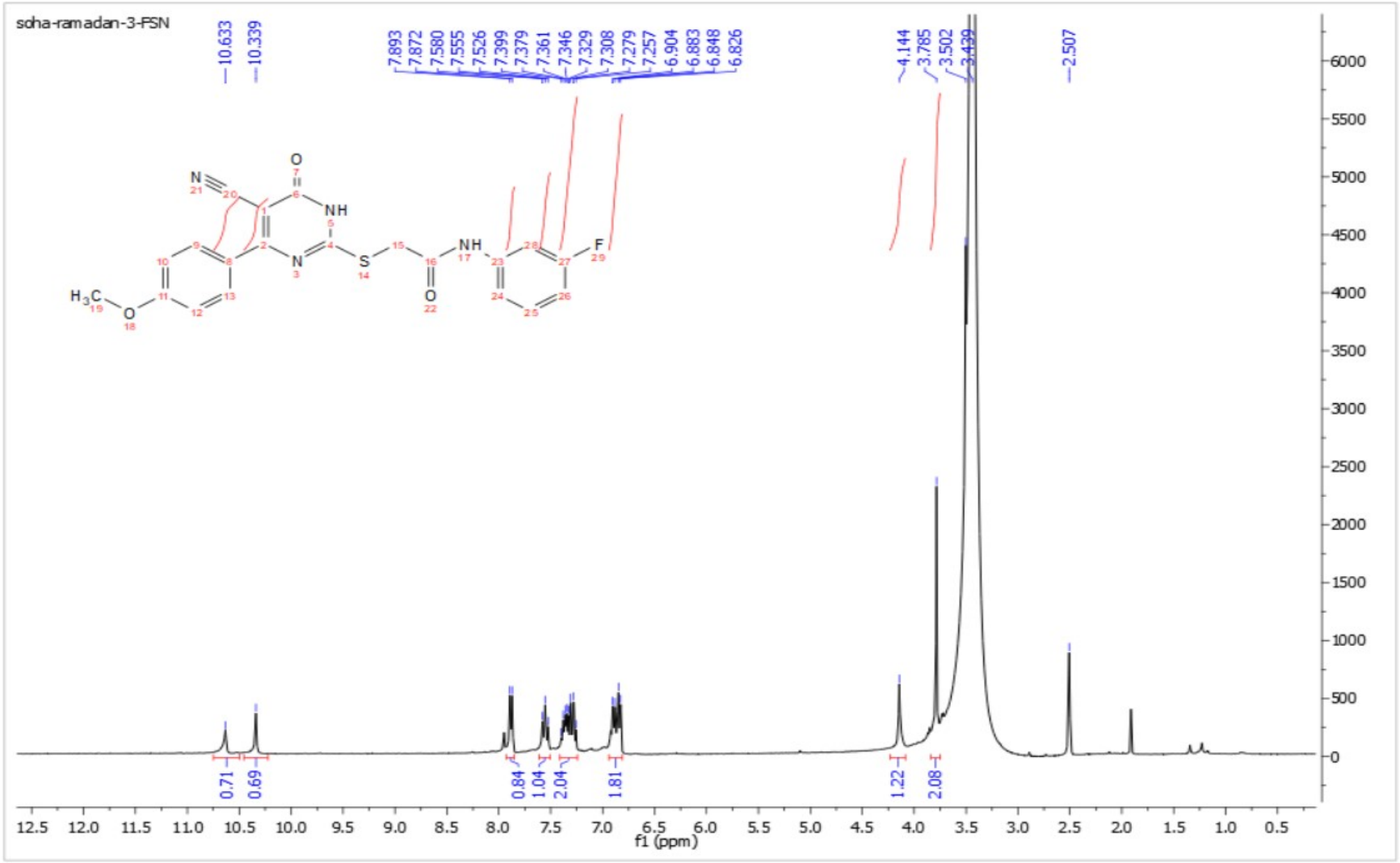


Figure S12: **^{13}CNMR** ***2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamide (8g)***

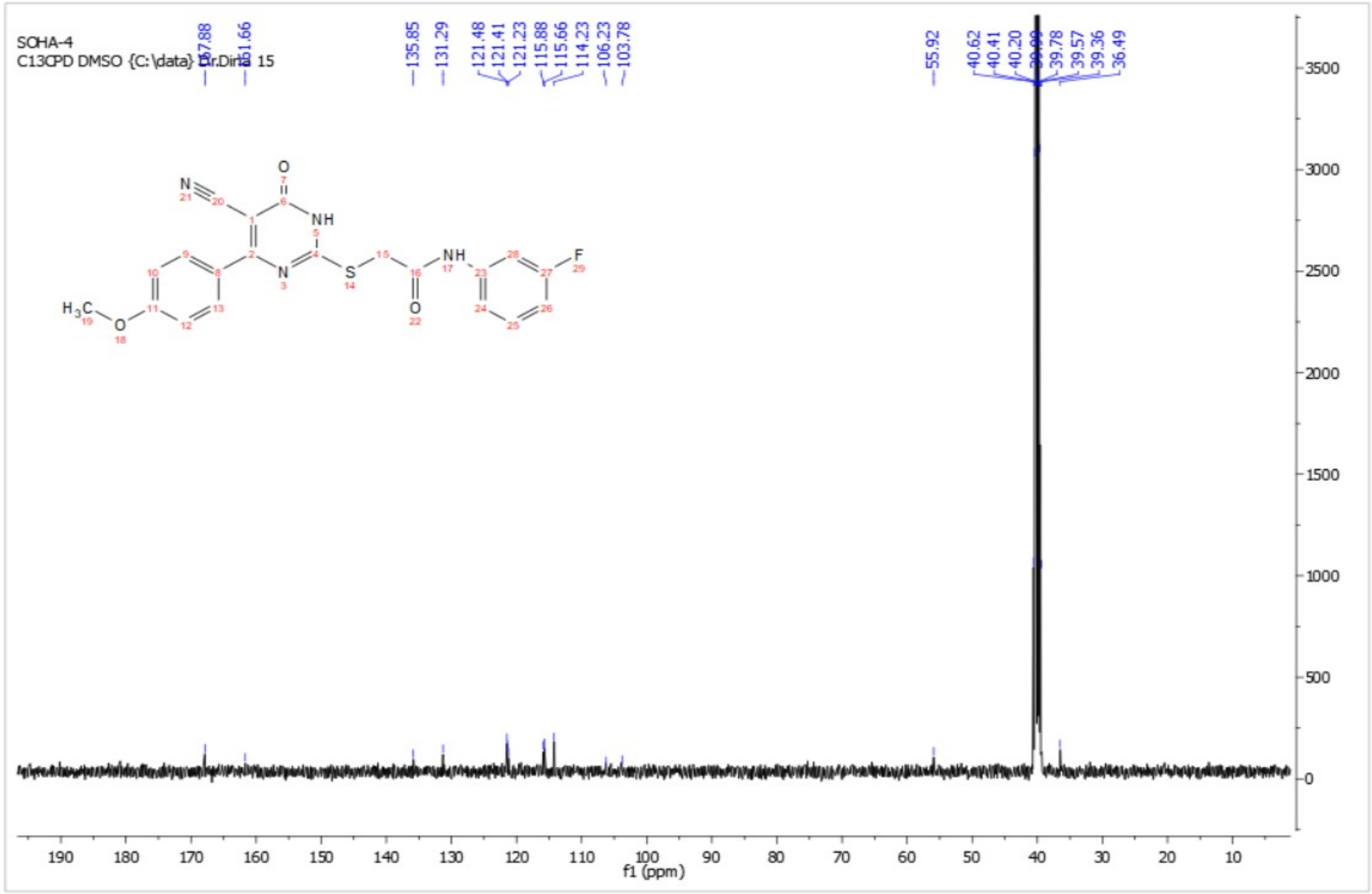


Figure S13: [1]HNMR *2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-fluorophenyl)acetamide (8h)*

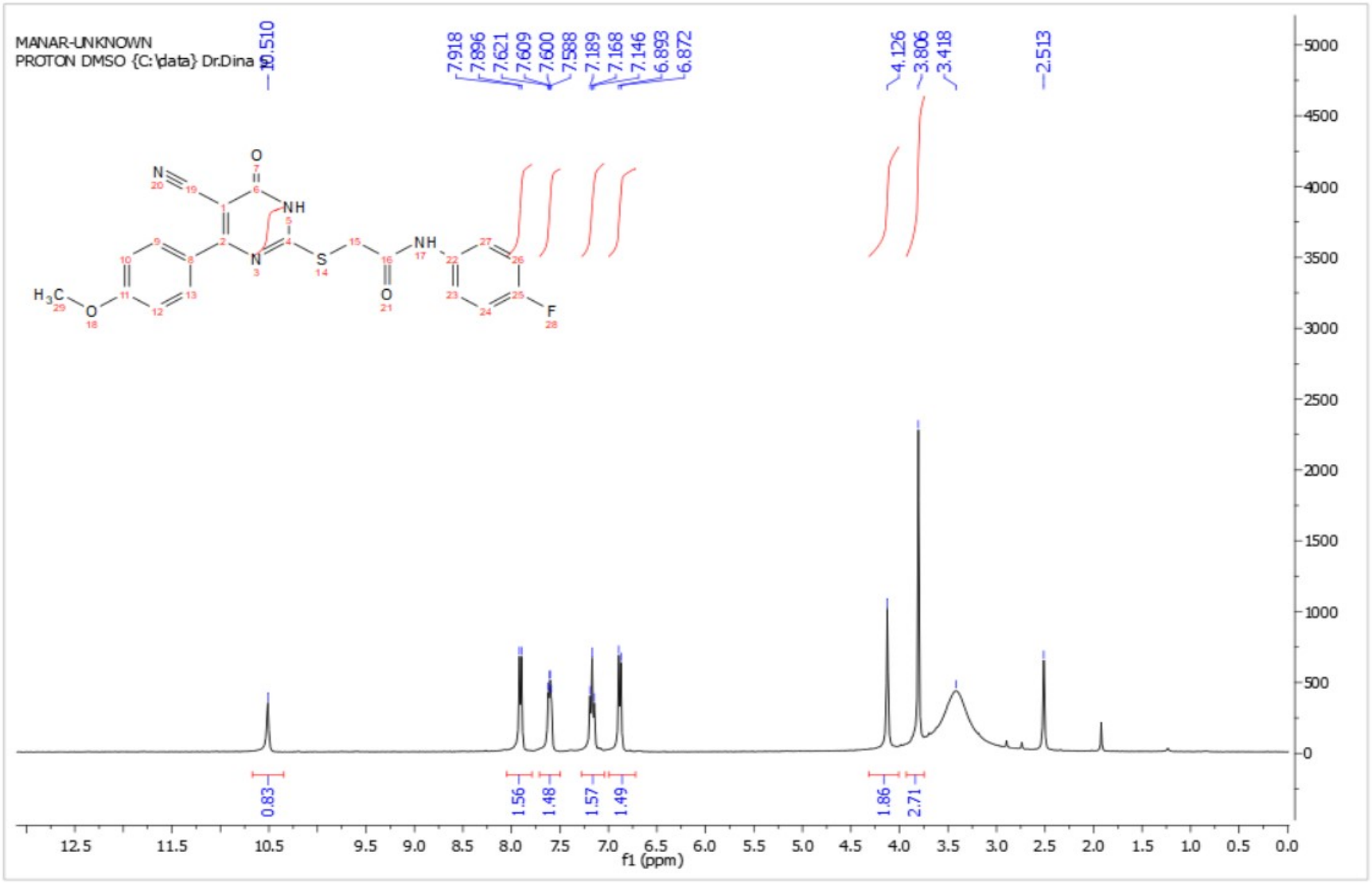


Figure S14: [1]HNMR *2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-methoxyphenyl)acetamide (8j)*

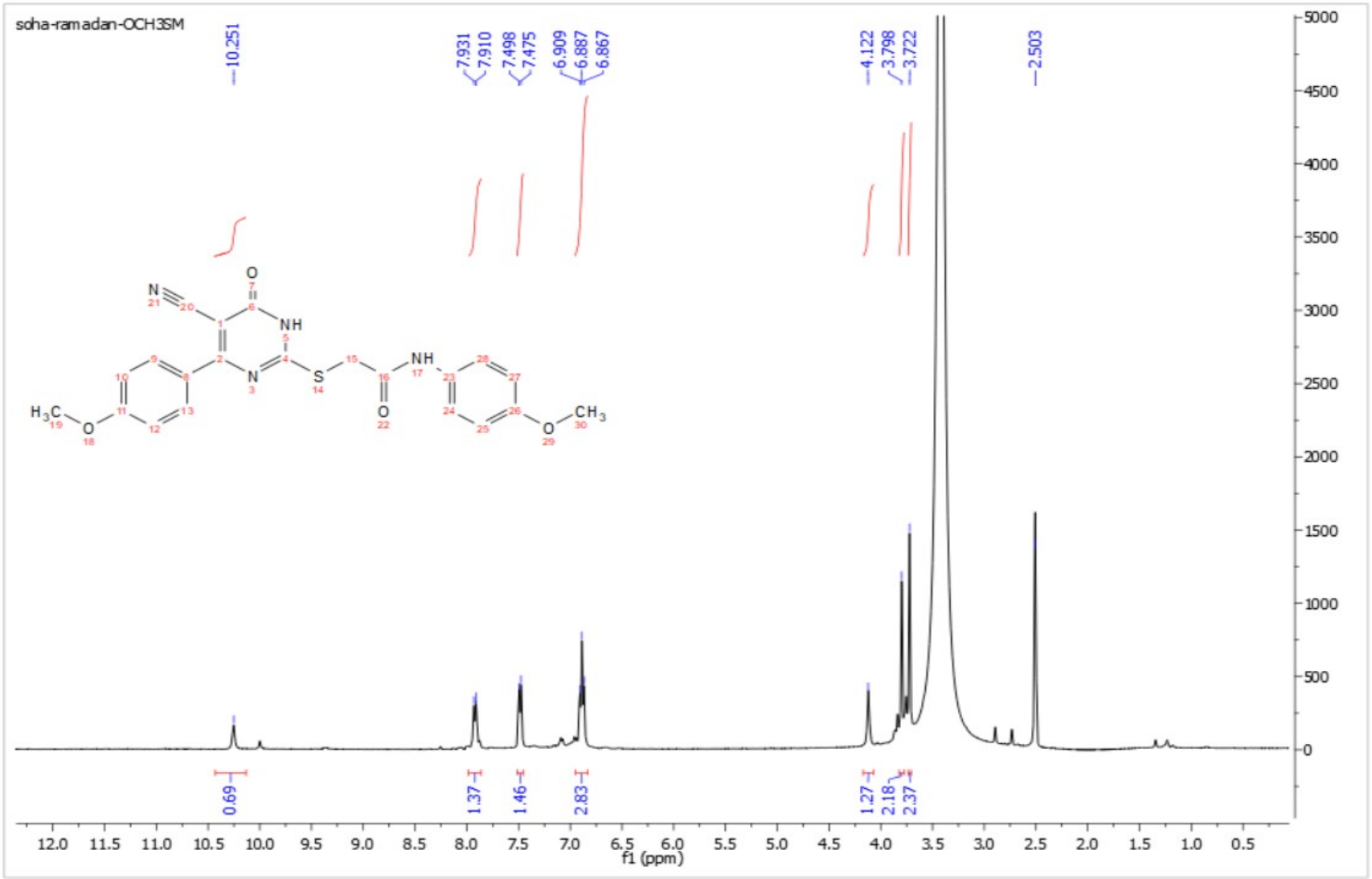


Figure S15: [1]HNMR *2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8k)*

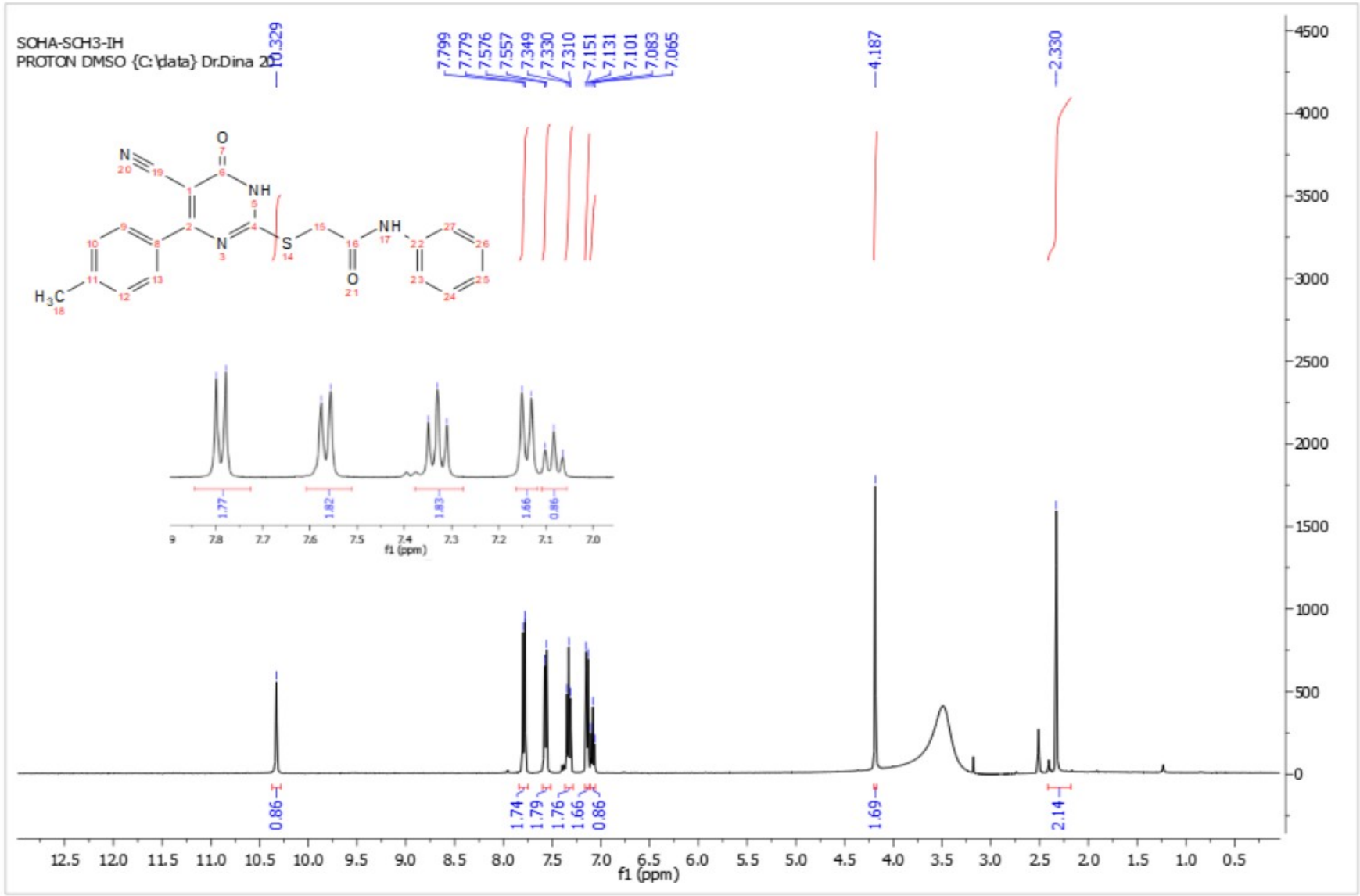


Figure S16: D_2O ^{1}HNMR *2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8k)*

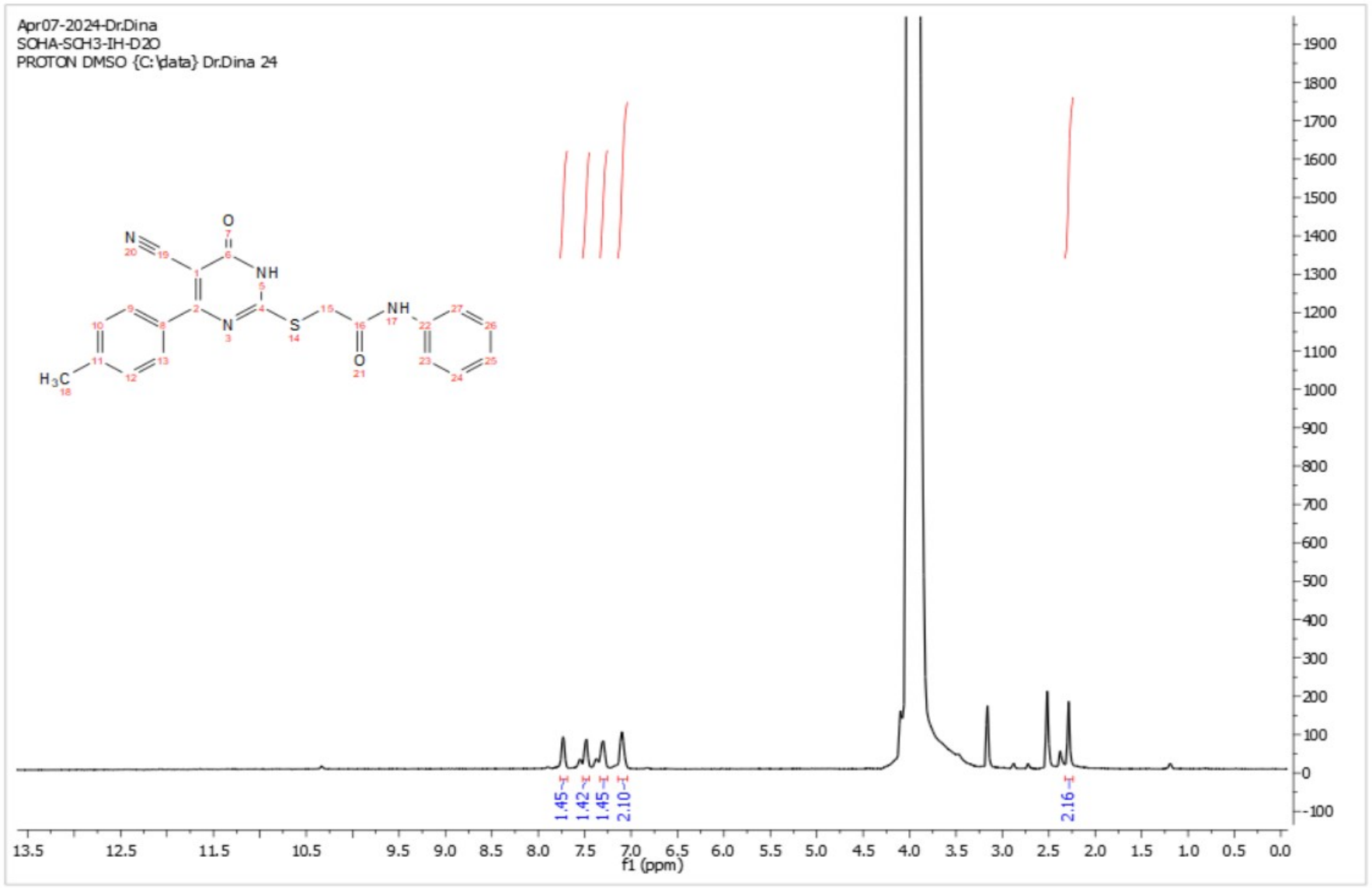


Figure S17: ^{13}CNMR *2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8k)*

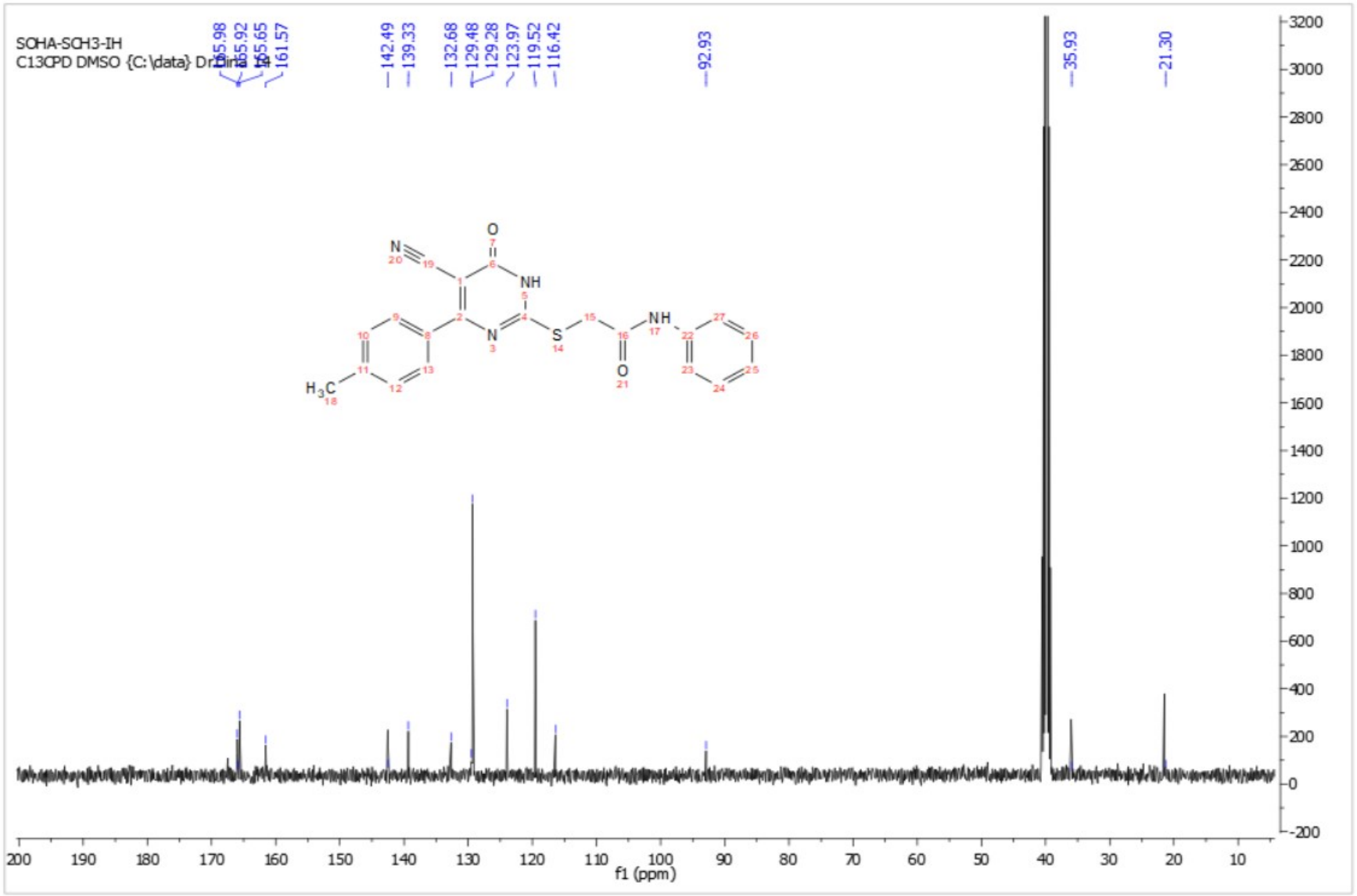


Figure S18: ^{1}HNMR *2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamid (8l)*

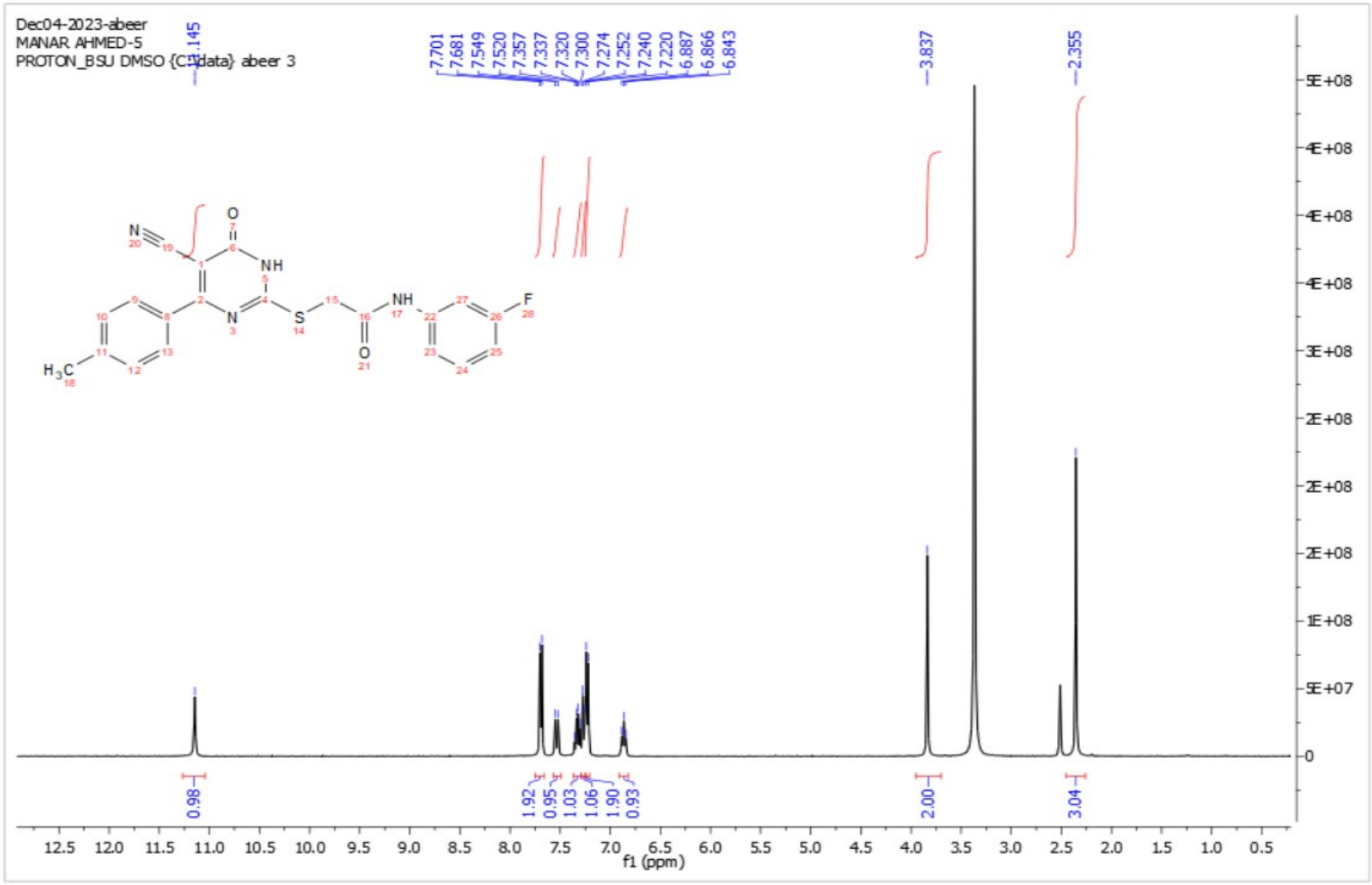
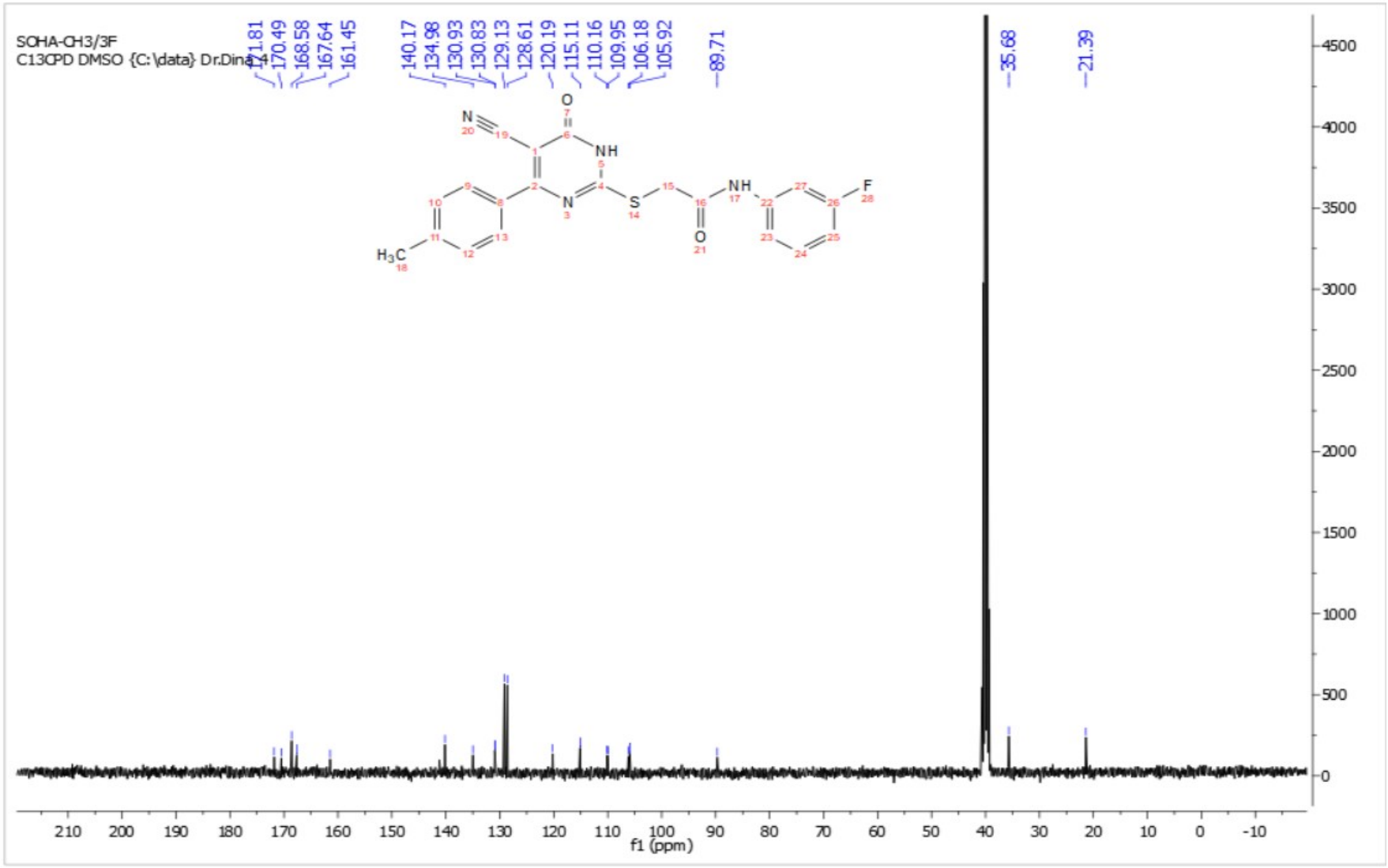


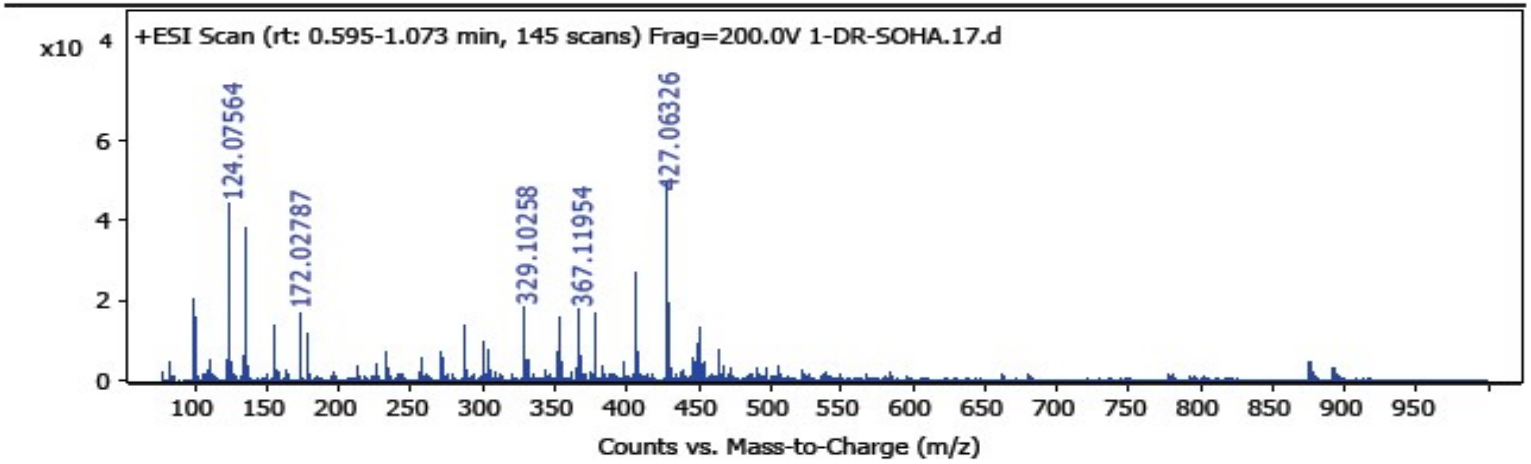
Figure S19: ^{13}**CNMR** ***2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamid (8l)***



Some representable examples of HRMS and mass spectra

Figure S20: HRMS of *2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-methoxyphenyl)acetamide (8e)*

Qualitative Analysis Report



Peak List

m/z	Abund
98.97559	20347.59
124.07564	43863.68
136.07568	37823.59
172.02787	16588.99
329.10258	18104.87
367.11954	17629.47
378.18144	16515.86
406.17648	26772.67
427.06326	48998.59
429.0607	19336.94

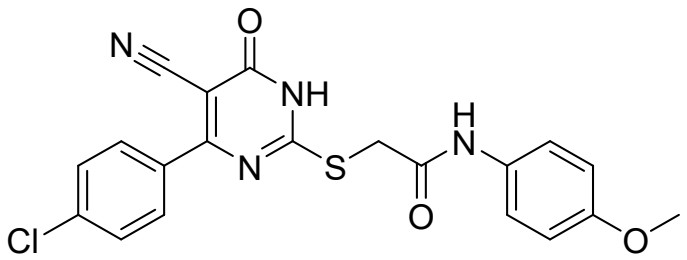


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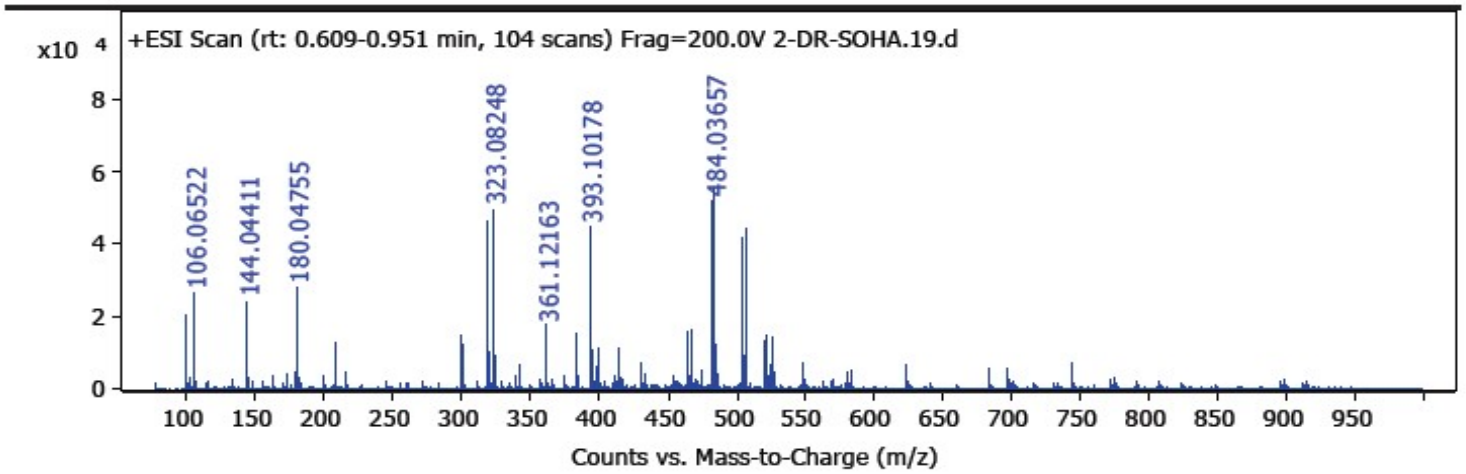
Figure S21: HRMS of 2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8f)

Qualitative Analysis Report



Peak List

m/z	Abund
106.06522	26770.48
144.04411	24213.65
180.04755	28115.76
319.11896	46851.45
323.08248	49783.42
393.10178	44851.18
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484.03657	55359.37
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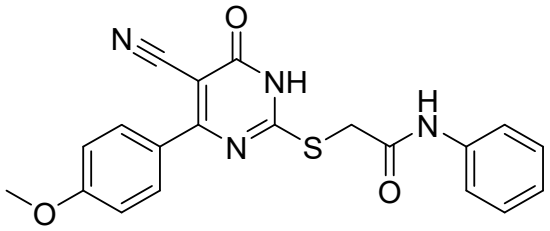

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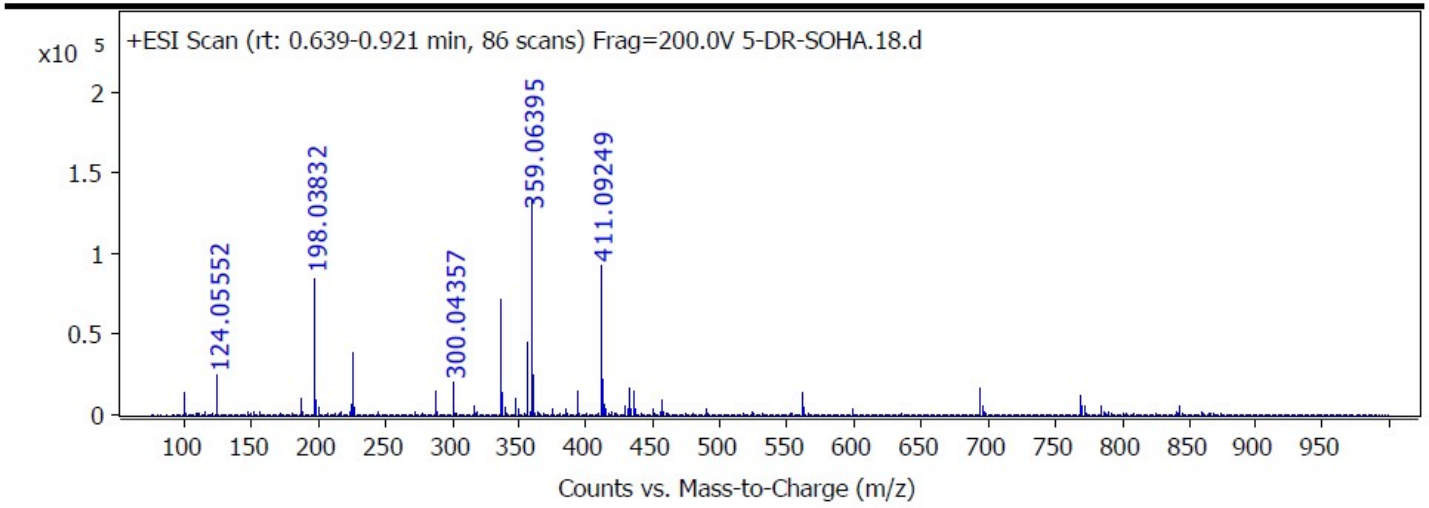
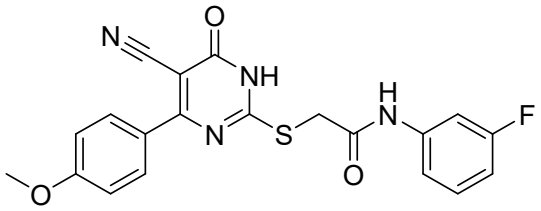
Figure S22: HRMS of 2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamide (8g)

Qualitative Analysis Report



Peak List

m/z	z	Abund
124.05552		24208.49
198.03832		84743.58
226.03308		39133.63
300.04357		19883.13
337.0822		72210.18
356.0556	2	44517.43
359.06395	1	132726.17
360.06673	1	25337.77
411.09249	1	92506.82
412.09518	1	22063.23



--- End Of Report ---

Figure S23: MS of 2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-(3-fluorophenyl)acetamid (8l)

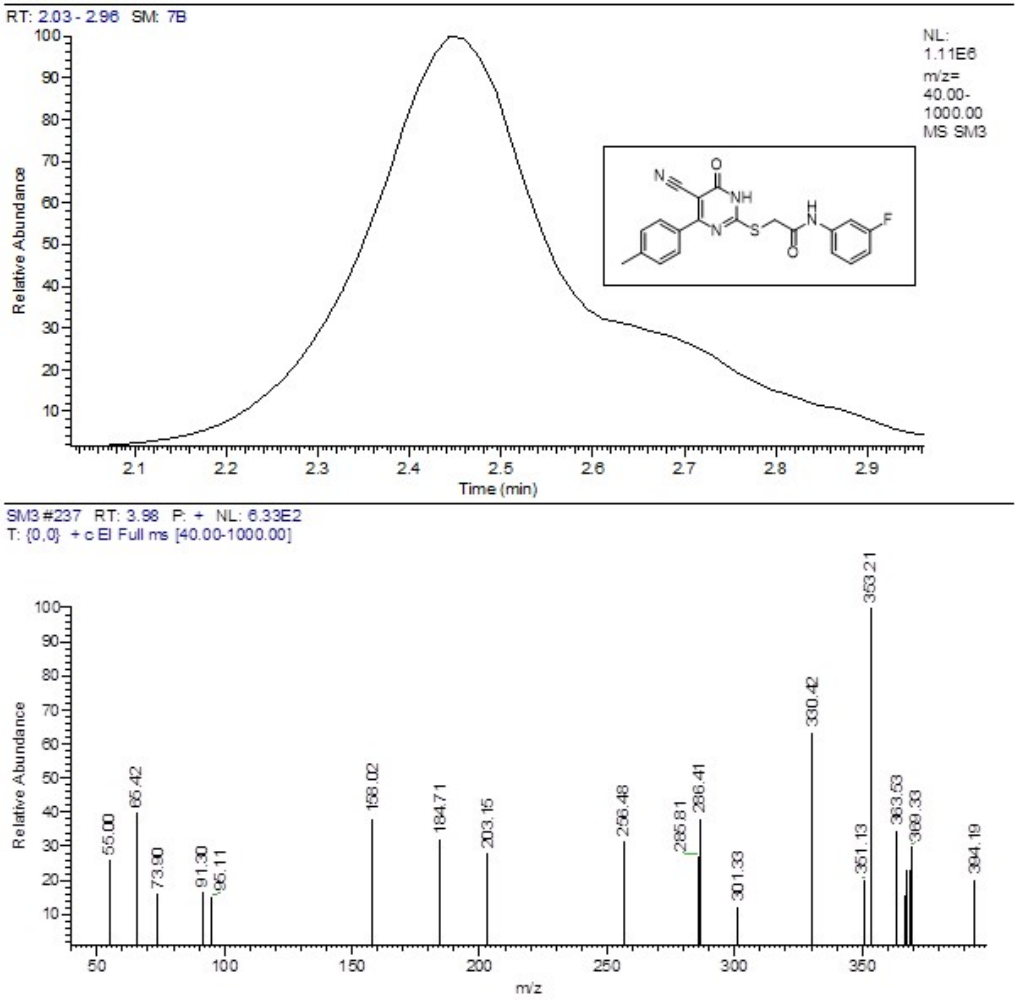


Figure S24: MS of ***2-((5-Cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)-N-phenylacetamide (8k)***

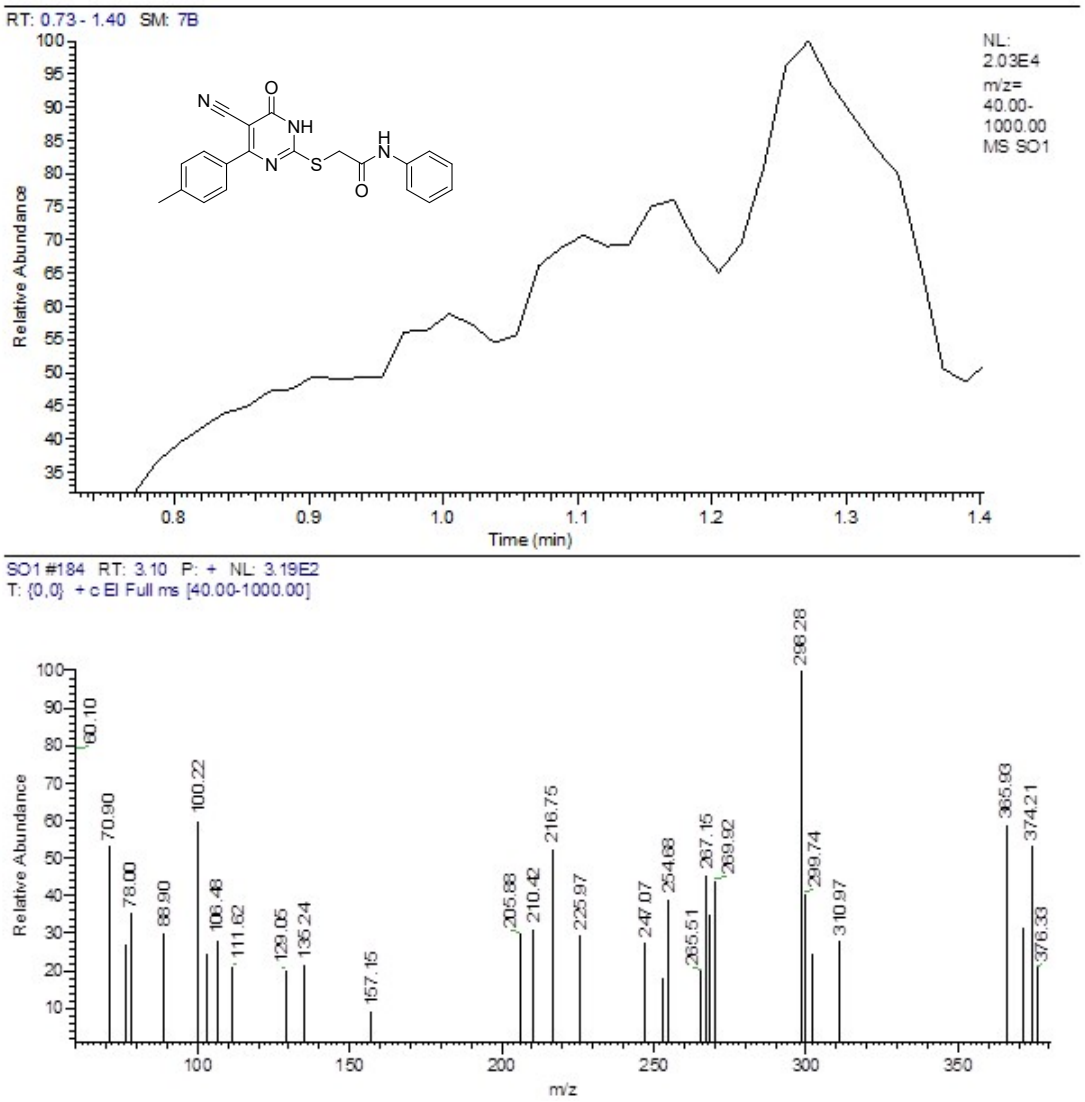


Figure S25: MS of ***N-(4-Chlorophenyl)-2-((5-cyano-6-oxo-4-(p-tolyl)-1,6-dihydropyrimidin-2-yl)thio)acetamide (8m)***

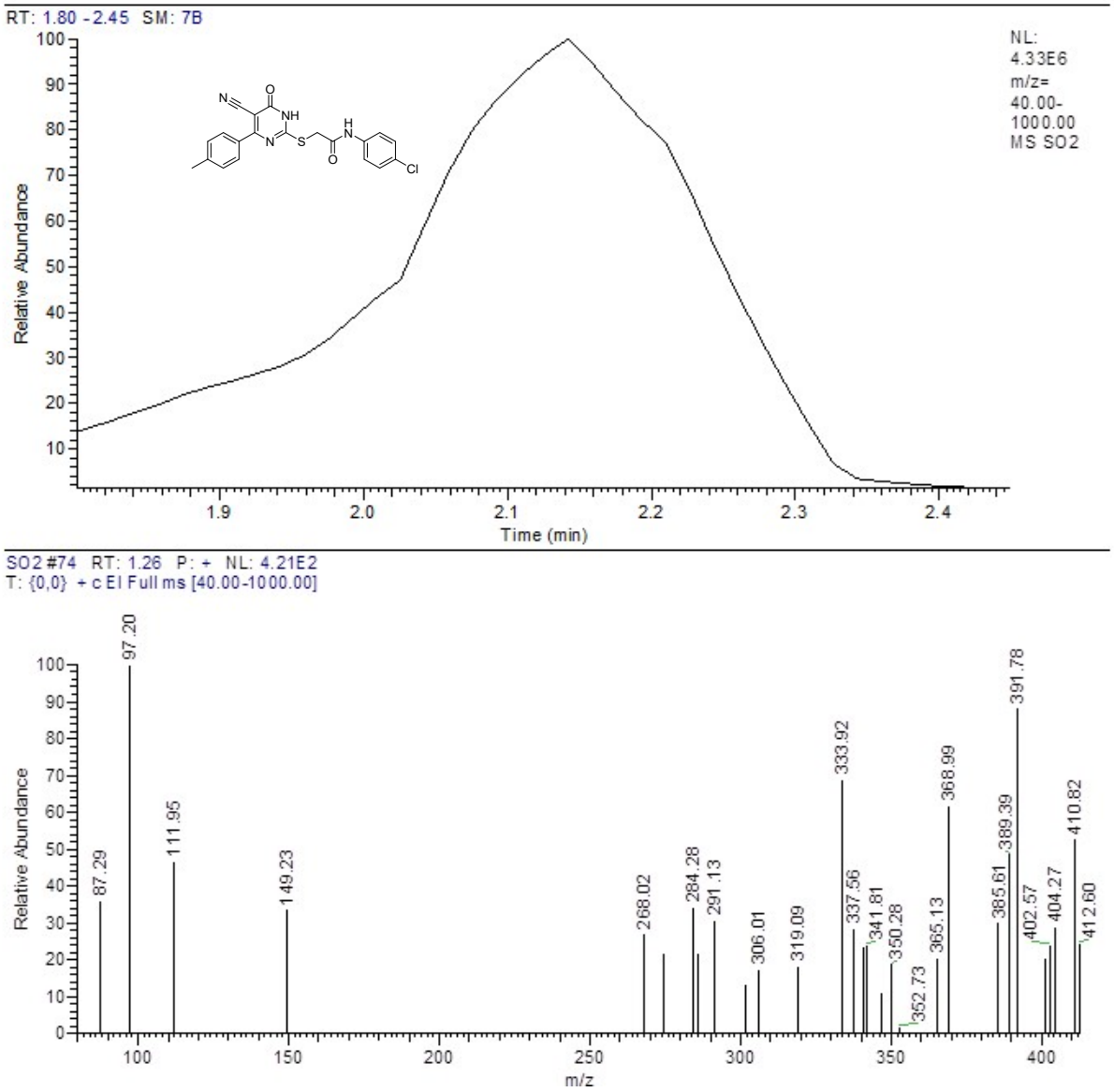
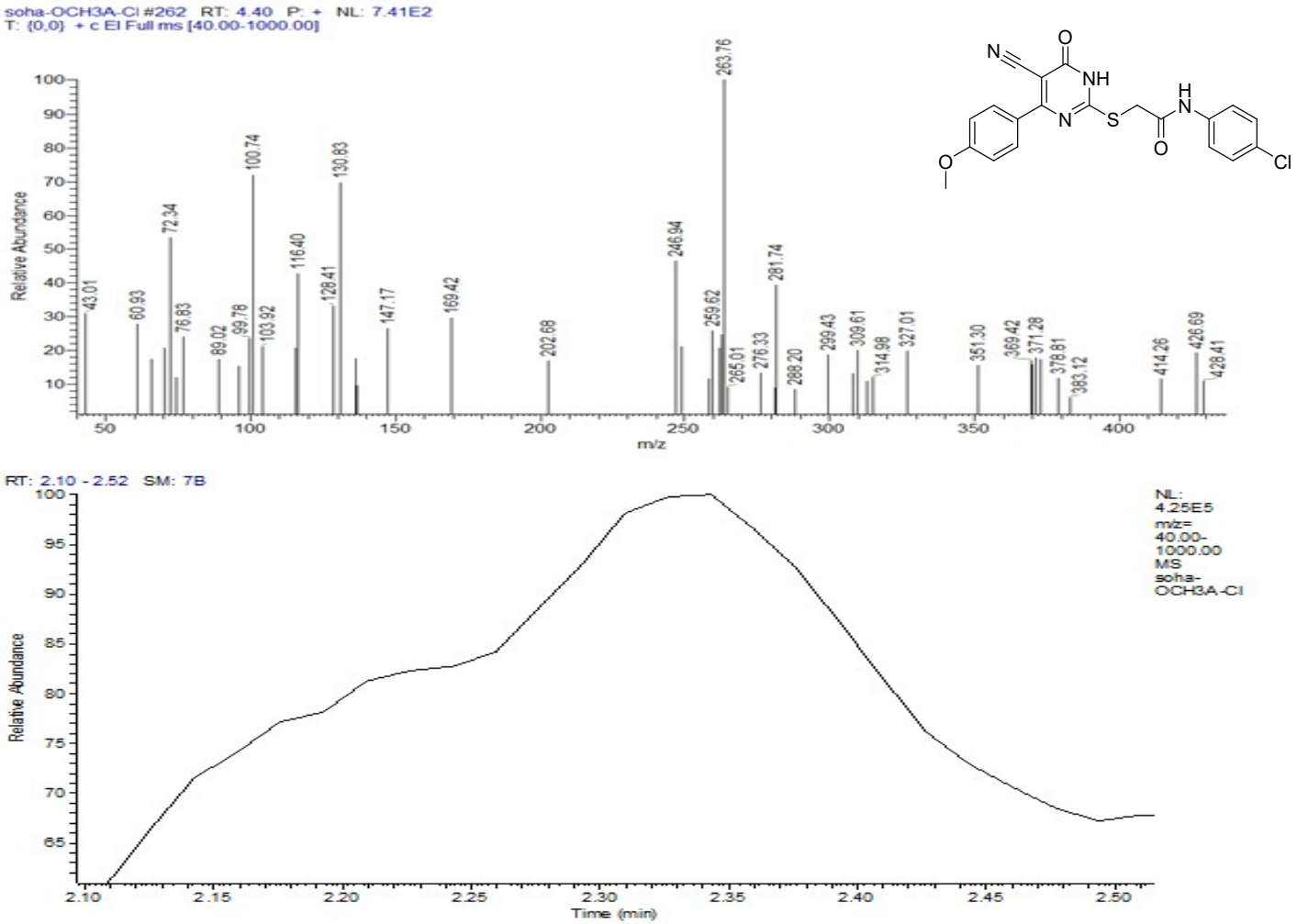


Figure S26: N-(4-Chlorophenyl)-2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (8i)



4.2 Biological Evaluation

4.2.1 In vitro PIM-1 kinase enzyme inhibition:

Assay Theory

The Z′-LYTE biochemical assay employs a fluorescence-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage (Figure 1). The peptide substrate is labeled with two fluorophores—one at each end—that make up a FRET pair. In the primary reaction, the kinase transfers the gamma-phosphate of ATP to a single tyrosine, serine or threonine residue in a synthetic FRET-peptide. In the secondary reaction, a site-specific protease recognizes and cleaves non-phosphorylated FRET-peptides. Phosphorylation of FRET-peptides suppresses cleavage by the Development Reagent. Cleavage disrupts FRET between the donor (i.e., coumarin) and acceptor (i.e., fluorescein) fluorophores on the FRET-peptide, whereas uncleaved, phosphorylated FRET-peptides maintain FRET. A ratiometric method, which calculates the ratio (the Emission Ratio) of donor emission to acceptor emission after excitation of the donor fluorophore at 400 nm, is used to quantitate reaction progress, as shown in the equation below.

$$\text{Emission Ratio} = \frac{\text{Coumarin Emission (445 nm)}}{\text{Fluorescein Emission (520 nm)}}$$

A significant benefit of this ratio metric method for quantitating reaction progress is the elimination of well to-well variations in FRET-peptide concentration and signal intensities. As a result, the assay yields very high Z′-factor values (>0.7) at a low percent phosphorylation. Both cleaved and uncleaved FRET-peptides contribute to the fluorescence signals and therefore to the Emission Ratio. The extent of phosphorylation of the FRET-peptide can be calculated from the Emission Ratio. The Emission Ratio will remain low if the FRET-peptide is phosphorylated (i.e., no kinase inhibition) and will be high if the FRET-peptide is non-phosphorylated (i.e., kinase inhibition).

Z'-LYTE Assay Conditions

Test Compounds

The Test Compounds are screened in 1% DMSO (final) in the well. For 10-point titrations, 3-fold serial dilutions were performed.

Peptide/Kinase Mixtures

All Peptide/Kinase Mixtures are diluted to a 2X working concentration in the appropriate Kinase Buffer.

ATP Solution

All ATP Solutions are diluted to a 4X working concentration in Kinase Buffer (50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA).

ATP Km apparent is previously determined using a Z′-LYTE assay.

Development Reagent Solution

The Development Reagent is diluted in Development Buffer.

Assay Protocol

Bar-coded Corning, low volume NBS, black 384-well plate (Corning Cat. #4514)

1. 100 nL – 100X Test Compound in 100% DMSO
2. 2.4 µL – Kinase buffer
3. 5 µL – 2X Peptide/Kinase Mixture
4. 2.5 µL – 4X ATP Solution
5. 30-second plate shake
6. 60-minute Kinase Reaction incubation at room temperature
7. 5 µL – Development Reagent Solution
8. 30-second plate shake
9. 60-minute Development Reaction incubation at room temperature
10. Read on fluorescence plate reader and analyze the data

Kinase-Specific Assay Conditions:

PIM1

The 2X PIM1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 1.19 ng PIM1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

Assay for anti-proliferative activity

Doxorubicin was used as the typical positive control, and all synthetic compounds (**8g**, **8b**, **8c**, **8j**, **8n**, **8f** and **8m**) were also evaluated. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Serva) colorimetric test was used to evaluate the antiproliferation and cytotoxicity. In 96-well plates, each cancer cell line was seeded at a density of 20,000 cells per well, and the cells were left to adhere for the whole night. Five successive dilutions (100, 50, 5, 0.5, and 0.1 μM) were applied to the connected cells in triplicate. Serum-free culture medium were used to dilute the stock solutions in order to create these concentrations. The cells treated with 0.1% DMSO solvent alone served as the negative control group. For a whole day, the treated cells were handled and kept under regular culture growth conditions. Following the incubation time, 20 μL of the MTT working reagent (final concentration 0.5 mg/mL) was applied to each well, and MTT powder was prepared as a stock solution (5 mg/mL). Subsequently, the MTT reagent was incubated for 4 hours at 37°C with 5% CO_2. After that, 150 μL of DMSO solubilizing solvent was added, and the mixture was incubated for 20 minutes. Using a Biotek 800 TS microplate reader, the absorbance of solubilized violet formazan crystals was determined at 570 nm. The concentration of compound that resulted in 50% inhibition of cell growth was determined to be IC_{50}. The cytotoxicity testing was conducted using WI-38 normal human fibroblast cells. The SI, or IC_{50} ccompound (WI-38)/IC_{50} compound (cancer cell line) ratio, was calculated. Increasing SI value over 1 implies a more effective and safer medicine as an anticancer compared to normal tissues.

Molecular dynamic simulations

The receptor and ligand topologies were generated by PDB2gmx (embedded in GROMACS) , both under CHARMM36 force field. After rejoining ligands and receptor topologies to generate four systems, the typical molecular dynamics scheme of GROMACS was applied for all the systems. This include, solvation, neutralization, energy minimization under CHARMM36 force field and two stages of equilibration (NVT and NPT). Finally, unrestricted production stage of 100ps was applied.The stability of the complexes was judged using RMSD and RMSF values calculated from the MDS trajectories from the production step.

The relative data of kinase selectivity assay (PIM-1 assay).

Thermo Fisher Scientific's SelectScreen™ Profiling Service: Single Point Results

SelectScreen Scientist:	David Bayer	Date:	12-May-2023	SSBK-Z'-LYTE (Madison, WI USA)	
Quality Assurance Review:	Meera Kumar	Date:	12-May-2023	Legend	
				< 40% Inhibition	
% Phosphorylation	Pass			40% - 80% Inhibition	
Z' Determination	Pass			≥ 80% Inhibition	

Project #	Compound Name	1X Test Compound Concentration (nM)	[ATP] Tested (μM)	Kinase Tested	% Inhibition Point 1	% Inhibition Point 2	% Inhibition mean	Difference Between Data Points |Point 1 - Point 2|	Development Reaction Interference	Test Compound Interference Coumarin	Test Compound Interference Fluorescein	Z'	Kinase Part# / Lot#
SSBK12643_64931	1	10000	10	PIM1	101	99	100	2	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	2	10000	10	PIM1	58	53	56	5	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	3	10000	10	PIM1	72	71	72	1	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	4	10000	10	PIM1	85	83	84	2	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	5	10000	10	PIM1	64	58	61	6	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	6	10000	10	PIM1	87	87	87	1	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	7	10000	10	PIM1	33	41	37	8	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	8	10000	10	PIM1	32	35	33	3	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	9	10000	10	PIM1	76	78	77	2	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	10	10000	10	PIM1	9	13	11	4	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	11	10000	10	PIM1	5	7	6	2	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	12	10000	10	PIM1	96	99	97	2	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	13	10000	10	PIM1	8	10	9	3	Pass	Pass	Pass	0.87	PV3503/2516446
SSBK12643_64931	14	10000	10	PIM1	3	3	3	0	Pass	Pass	Pass	0.87	PV3503/2516446

The relative data of kinase selectivity assay (PIM-1 assay)

Thermo Fisher Scientific's SelectScreen™ Profiling Service: Single Point Results

SelectScreen Scientist:	Kat Smith	Date:	26-Sep-2023	SSBK-Z'-LYTE (Madison, WI USA)
Quality Assurance Review:	Meera Kumar	Date:	26-Sep-2023	

Legend
< 40% Inhibition
40% - 80% Inhibition
≥ 80% Inhibition

% Phosphorylation	Pass
Z' Determination	Pass

Project #	Compound Name	1X Test Compound Concentration (nM)	[ATP] Tested (µM)	Kinase Tested	% Inhibition Point 1	% Inhibition Point 2	% Inhibition mean	Difference Between Data Points |Point 1 - Point 2|	Development Reaction Interference	Test Compound Interference Coumarin	Test Compound Interference Fluorescein	Z'	Kinase Part# / Lot#
SSBK12643_65894	1	10000	10	PIM1	93	98	96	4	Pass	Pass	Pass	0.84	PV3503/2516446
SSBK12643_65894	2	10000	10	PIM1	21	22	21	1	Pass	Pass	Pass	0.89	PV3503/2516446
SSBK12643_65894	3	10000	10	PIM1	94	98	96	4	Pass	Pass	Pass	0.89	PV3503/2516446
SSBK12643_65894	4	10000	10	PIM1	92	89	90	3	Pass	Pass	Pass	0.84	PV3503/2516446
SSBK12643_65894	5	10000	10	PIM1	89	89	89	0	Pass	Pass	Pass	0.89	PV3503/2516446
SSBK12643_65894	6	10000	10	PIM1	52	52	52	1	Pass	Pass	Pass	0.89	PV3503/2516446
SSBK12643_65894	7	10000	10	PIM1	46	38	42	8	Pass	Pass	Pass	0.84	PV3503/2516446
SSBK12643_65894	8	10000	10	PIM1	82	81	82	0	Pass	Pass	Pass	0.93	PV3503/2516446
SSBK12643_65894	9	10000	10	PIM1	18	22	20	4	Pass	Pass	Pass	0.89	PV3503/2516446
SSBK12643_65894	10	10000	10	PIM1	38	36	37	2	Pass	Pass	Pass	0.89	PV3503/2516446

The relative data of kinase selectivity assay (PIM-1 assay)

Thermo Fisher Scientific's SelectScreen™ Profiling Service: Single Point Results

SelectScreen Scientist:	Meera Kumar	Date:	10-Nov-2023	SSBK-Z'-LYTE (Madison, WI USA)
Quality Assurance Review:	Kat Smith	Date:	10-Nov-2023	

% Phosphorylation	Pass
Z' Determination	Pass

Legend

- < 40% Inhibition
- 40% - 80% Inhibition
- ≥ 80% Inhibition

Project #	Compound Name	1X Test Compound Concentration (nM)	[ATP] Tested (µM)	Kinase Tested	% Inhibition Point 1	% Inhibition Point 2	% Inhibition mean	Difference Between Data Points | Point 1 - Point 2 |	Development Reaction Interference	Test Compound Interference Coumarin	Test Compound Interference Fluorescein	Z'	Kinase Part# / Lot#
SSBK12643_66152	12-64931	1000	10	PIM1	66	65	65	1	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	12-64931	100	10	PIM1	15	12	13	3	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	12-64931	10	10	PIM1	0	4	2	4	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	12-64931	1	10	PIM1	6	5	6	1	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-64931	1000	10	PIM1	54	60	57	5	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-64931	100	10	PIM1	13	12	13	2	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-64931	10	10	PIM1	1	-1	0	2	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-64931	1	10	PIM1	3	3	3	0	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-65894	1000	10	PIM1	67	64	65	3	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-65894	100	10	PIM1	20	23	21	3	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-65894	10	10	PIM1	4	4	4	0	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	1-65894	1	10	PIM1	7	1	4	6	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	3-65894	1000	10	PIM1	68	72	70	4	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	3-65894	100	10	PIM1	25	24	24	1	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	3-65894	10	10	PIM1	7	12	9	5	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	3-65894	1	10	PIM1	0	-2	-1	2	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	4-65894	1000	10	PIM1	61	63	62	2	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	4-65894	100	10	PIM1	16	19	17	3	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	4-65894	10	10	PIM1	4	1	2	3	Pass	Pass	Pass	0.86	PV3503/2516446
SSBK12643_66152	4-65894	1	10	PIM1	-2	1	0	3	Pass	Pass	Pass	0.86	PV3503/2516446