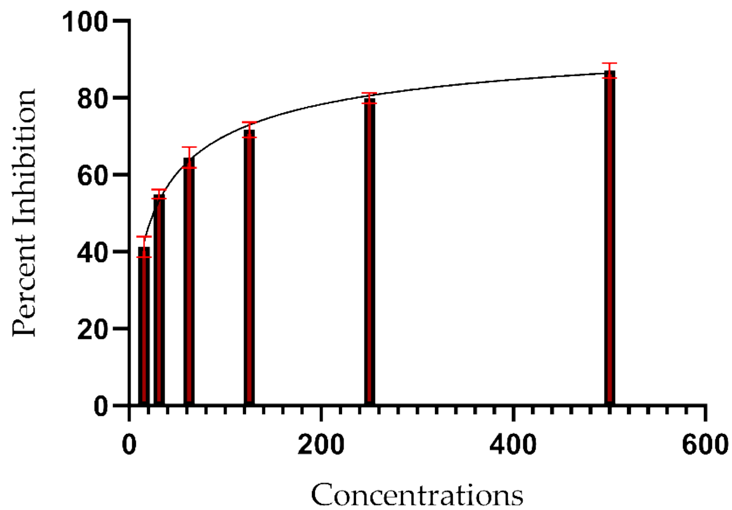
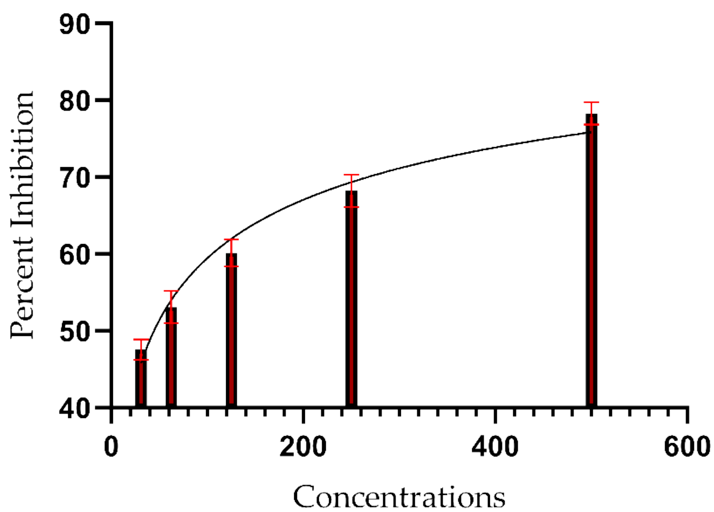
Design, synthesis, *in vitro,* and *in silico* study of *1*-benzyl-indole hybrid thiosemicarbazones as competitive tyrosinase inhibitors

Content

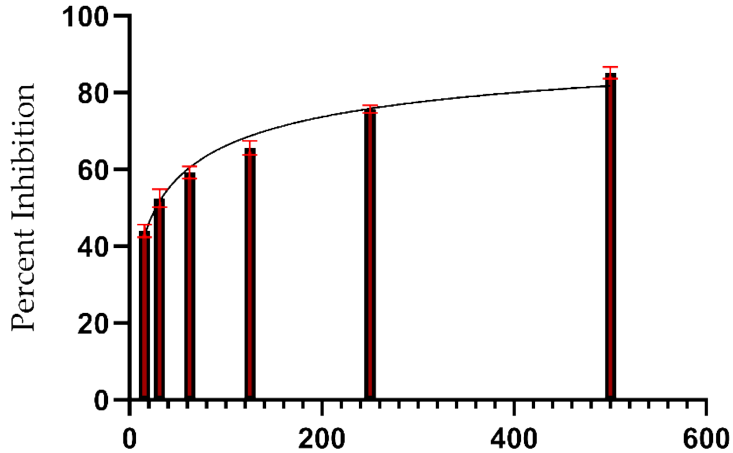
Dose Curve response for compounds 5a-5r



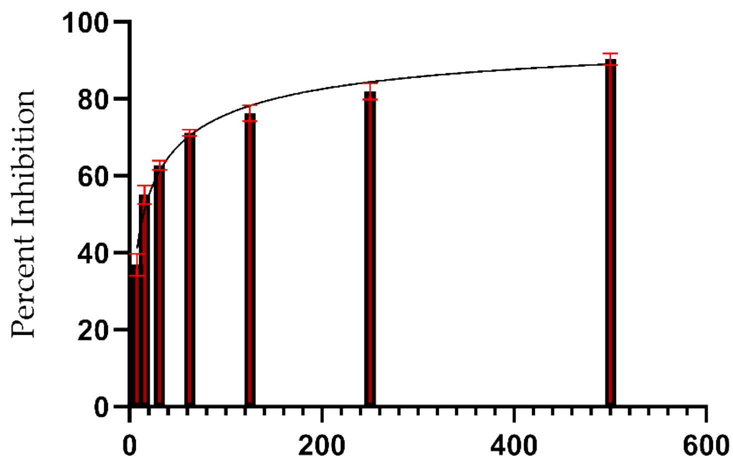
Compound **5a**



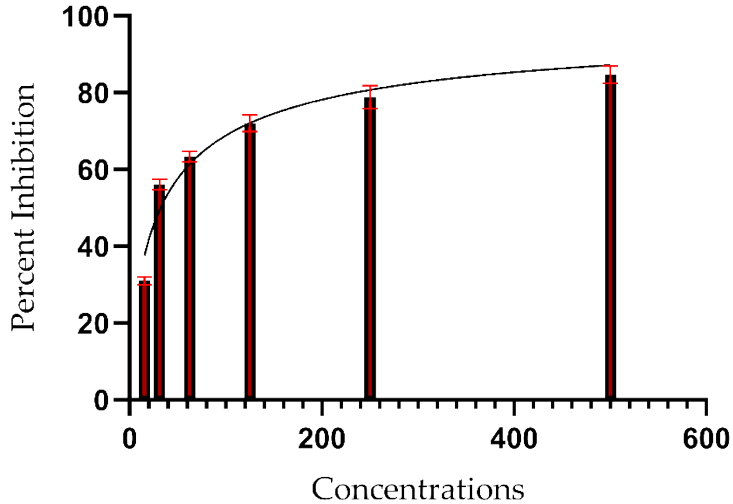
Compound **5b**



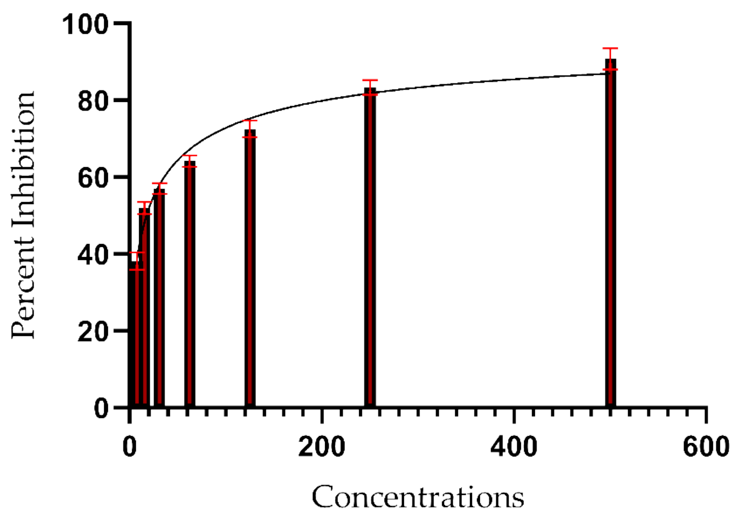
Concentrations
Compound **5c**



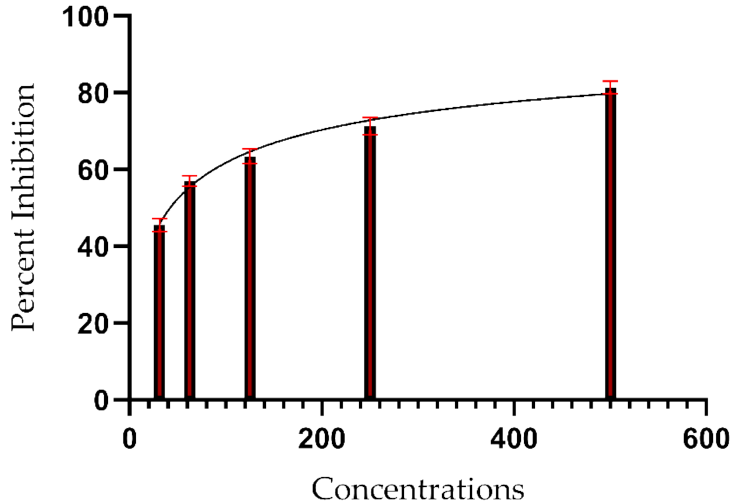
Concentrations
Compound **5d**



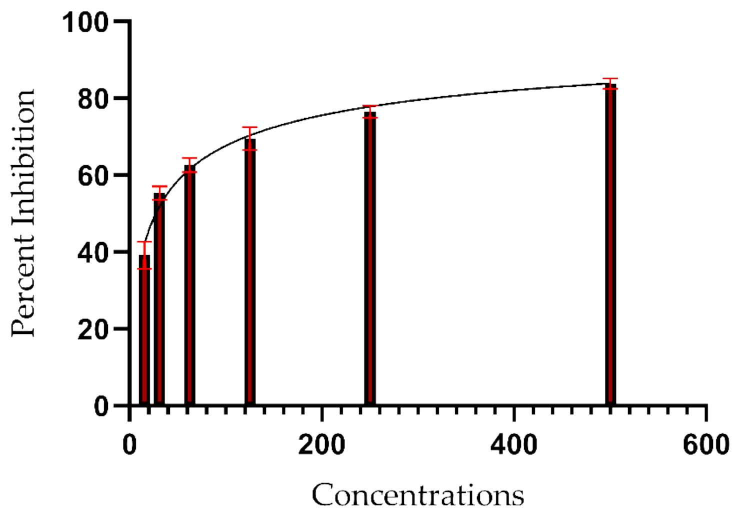
Compound **5e**



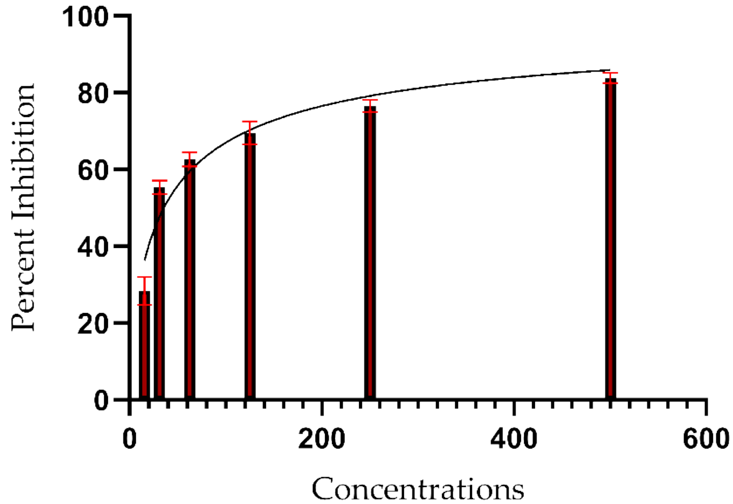
Compound **5f**



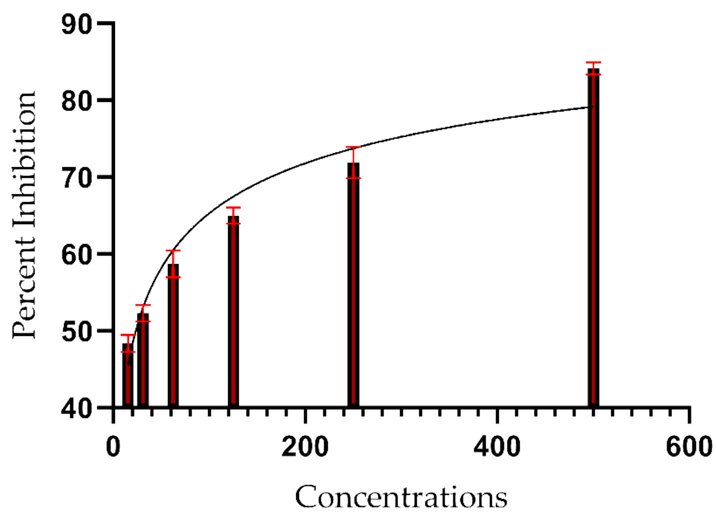
Compound **5g**



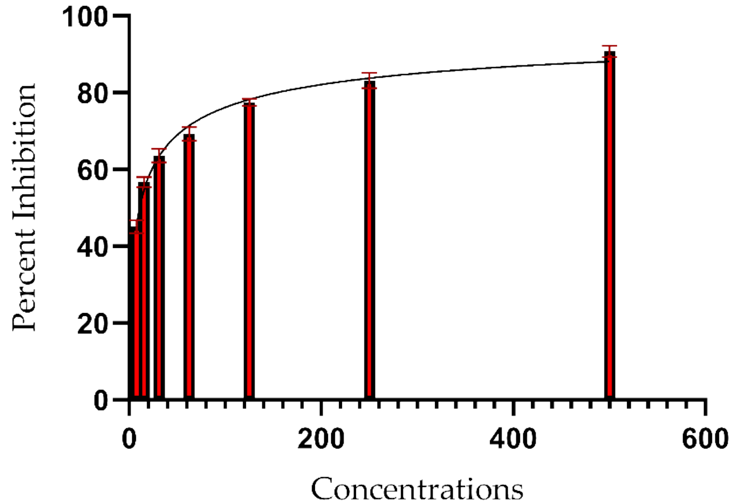
Compound **5h**



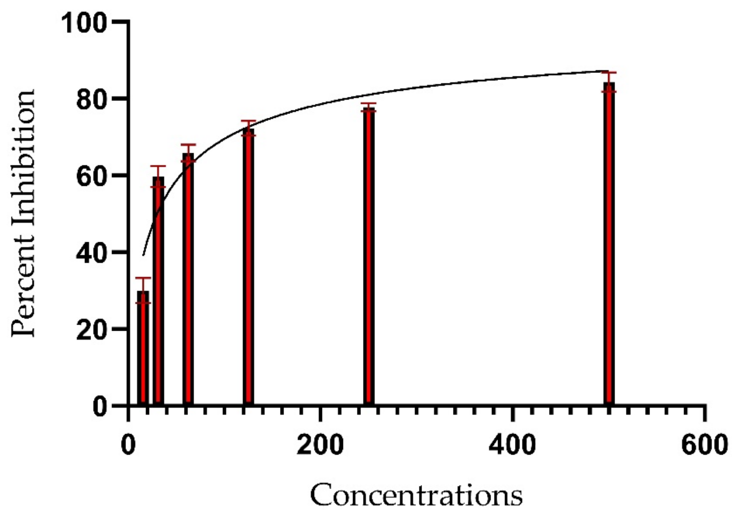
Compound **5i**



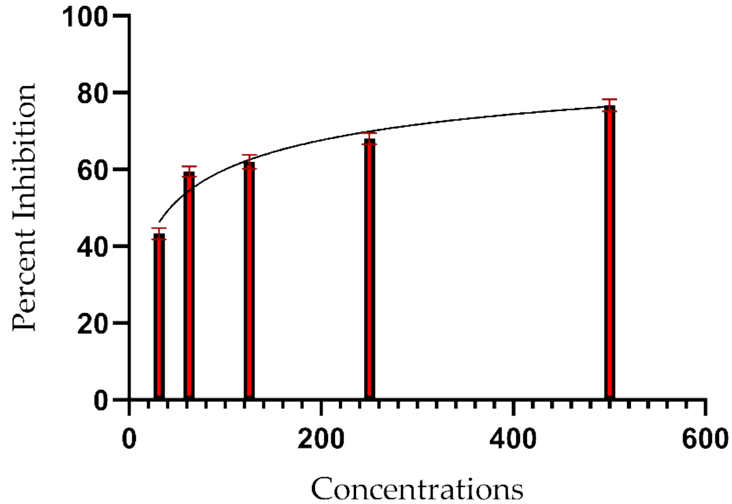
Compound **5j**



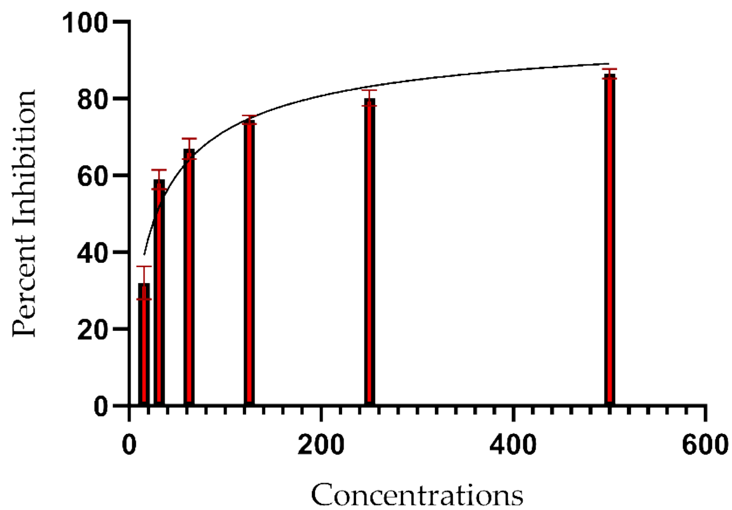
Compound **5k**



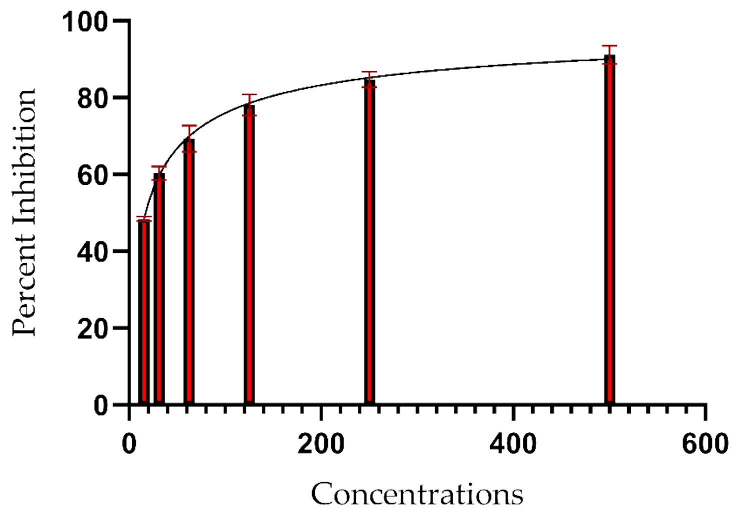
Compound **5l**



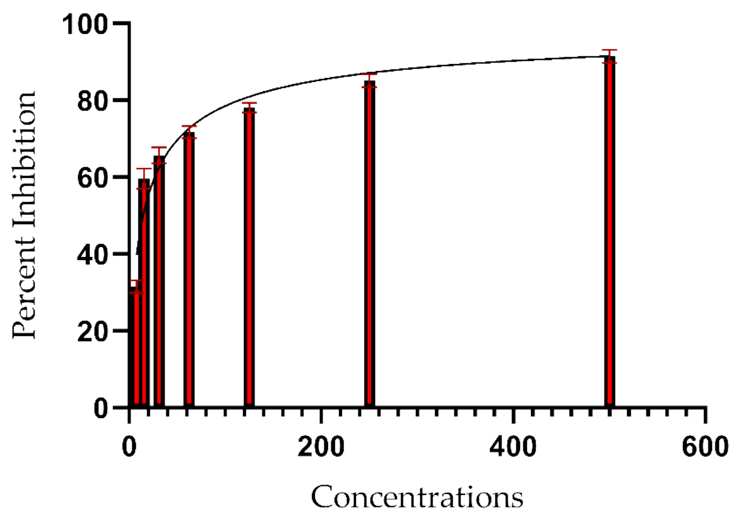
Compound **5m**



Compound **5n**



Compound **5o**



Compound **5q**

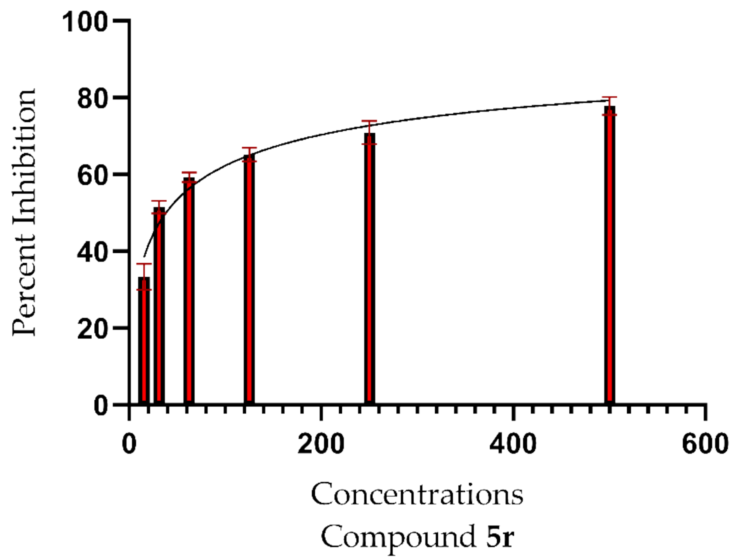


Figure S1. Dose curve response for compounds 5a- 5r against tyrosinase.

Glide molecular docking studies

The compounds 5c and 5b demonstrated strong binding interactions with tyrosinase target protein with gScores values even much better than the co-crystallized ligand and the kojic acid. Table S1 details the gScore, emodel, H-bonding, and hydrophobic interacting residues for the ligands 5c and 5b. Gln307, Thr308, and Thr360 are the common amino acid residue involved in polar contacts. Ile17, Tyr311, and Trp358 are showing common hydrophobic interacting residues as shown in **Figure S2.**

Table S1. Glide Score, H-Bonding Interactions with Distances in Å, Polar, and Hydrophobic Interacting Residues for Investigated Ligands with target protein possessing tyrosinase activity (PDB ID: 2Y9W).

Lignd	gScore (kcal/mol)	Emodel (kcal/mol)	HBI residue (distance Å)	Polar interacting amino acid residues	Hydrophobic interacting residues
5c	-5.033	-60.546	Asp357 (1.97)	Gln307, Thr308, Thr360, Ser364	Ile17, Tyr311, Trp358, Phe368, Val371
5b	-5.005	-54.341	Glu359 (1.72, 2.02)	Gln307, Thr308, Thr360, Ser380	Ile17, Tyr311, Trp358

Abbreviation: HBI, hydrogen bonding interactions.

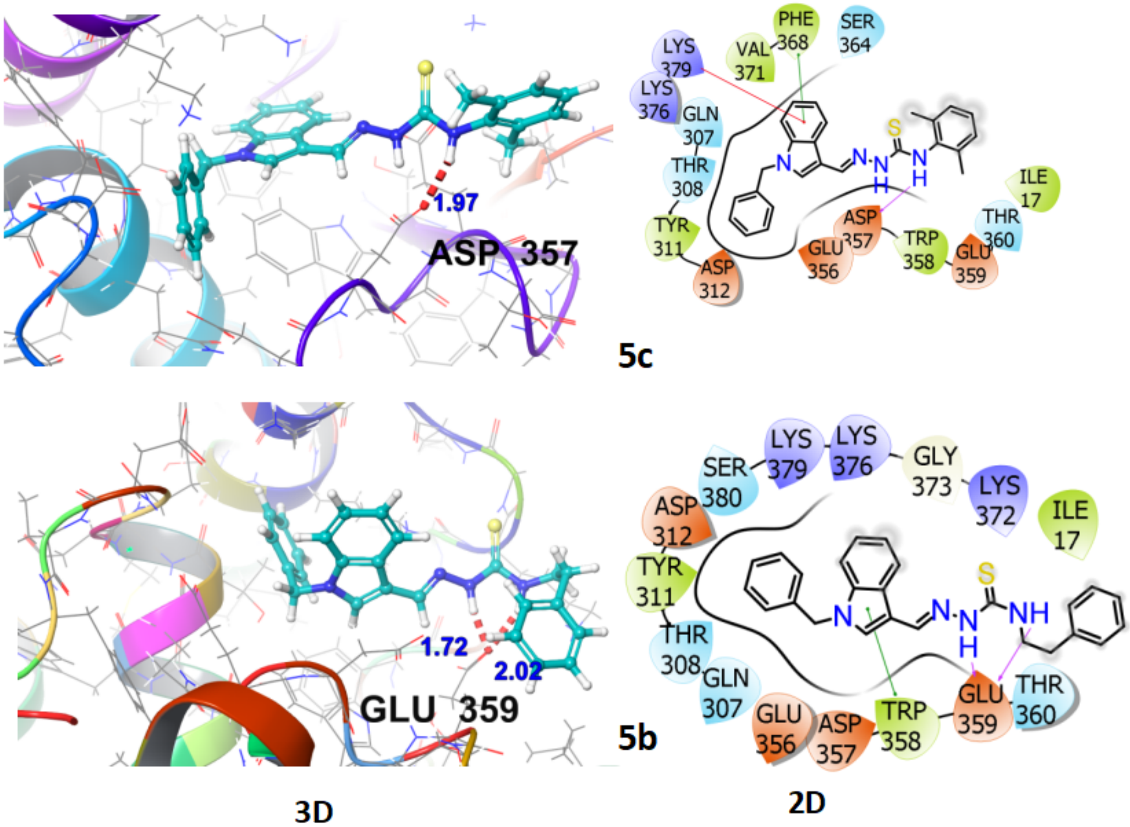


Figure S2. 3D and 2D representation of interaction with tyrosinase target protein (PDB ID: 2Y9W).

Table S2. Docking Score, glide Score, and glide emodel values of all investigated ligands with target protein possessing tyrosinase activity (PDB ID: 2Y9W).

Ligand	**docking score (kcal/mol)**	**glide gscore (kcal/mol)**	**glide emodel (kcal/mol)**
5k	-5.157	-5.157	-63.013
5c	-5.033	-5.033	-60.546
5b	-5.005	-5.005	-54.341
5a	-4.666	-4.666	-55.237
kojic acid	-4.416	-4.549	-34.051
5p	-4.313	-4.313	-45.738
5m	-4.236	-4.236	-47.798
5f	-4.129	-4.129	-54.012
5q	-4.033	-4.033	-55.055
5d	-3.876	-3.876	-42.321
5e	-3.815	-3.815	-46.426
5i	-3.778	-3.778	-49.164

5h	-3.554	-3.554	-49.496
5o	-3.454	-3.454	-48.434
5l	-3.311	-3.311	-48.872
5n	-3.183	-3.183	-46.608
5g	-3.085	-3.085	-42.012
5r	-2.983	-2.983	-42.112
5j	-2.185	-2.185	-34.915
co-crystallized-ligand-2Y9W	-0.129	-0.129	-19.187

Table S3. The ADMET Properties of all investigated ligands.

Ligand	Canonical SMILES	Formula	MW	#Heavy atoms	#Aromatic heavy atoms	Fraction Csp3	#Rotatable bonds	#H-bond acceptors	#H-bond donors	MR	TPSA	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P
5a	COc1ccc(cc1)NC(=S)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C24H22N4OS	414.52	30	21	0.08	8	2	2	127.06	82.67	3.9	4.8	4.83	3.28	5.19
5b	S=C(Nc1ccccc1)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H20N4S	384.5	28	21	0.04	7	1	2	120.56	73.44	3.59	4.83	4.82	3.63	5.12
5c	S=C(Nc1c(C)cccc1C)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C25H24N4S	412.55	30	21	0.12	7	1	2	130.5	73.44	3.95	5.56	5.44	4.04	6.18
5d	S=C(Nc1ccc(cc1)F)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H19FN4S	402.49	29	21	0.04	7	2	2	120.52	73.44	3.73	4.93	5.38	4	5.54
5e	S=C(NCc1ccc(cc1)	C24H21	432.97	30	21	0.08	8	1	2	128.84	73.44	4.2	5.4	5.19	4.04	6.16

	Cl)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	ClN4S													
5f	S=C(Nc1cccc(c1Cl)Cl)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H18Cl2N4S	453.39	30	21	0.04	7	1	2	130.58	73.44	4.05	6.09	6.13	4.58	6.4
5g	S=C(Nc1ccccc1)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H20N4S	384.5	28	21	0.04	7	1	2	120.56	73.44	3.59	4.83	4.82	3.63	5.12
5h	S=C(Nc1cc(cc1C)C)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C25H24N4S	412.55	30	21	0.12	7	1	2	130.5	73.44	3.82	5.56	5.44	4.04	6.18
5i	S=C(NCc1ccccc1)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C24H22N4S	398.52	29	21	0.08	8	1	2	123.83	73.44	3.97	4.77	4.54	3.57	5.52
5j	S=C(Nc1ccc(cc1)Br)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H19BrN4S	463.39	29	21	0.04	7	1	2	128.26	73.44	4.09	5.52	5.58	4.21	5.8
5k	S=C(NCc1ccc(cc1)C)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C25H24N4S	412.55	30	21	0.12	8	1	2	128.8	73.44	4.12	5.13	4.84	3.78	6.05
5l	COc1cccc(c1)NC(=S)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C24H22N4OS	414.52	30	21	0.08	8	2	2	127.06	82.67	3.94	4.8	4.83	3.28	5.19
5	COc1cccc	C25	444.55	32	21	0.12	9	3	2	133.55	91.9	4.02	4.78	4.84	2.94	5.26

m	(c1NC(=S)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1)OC	H24N4O2S														
5n	S=C(Nc1ccc(cc1)C)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C24H22N4S	398.52	29	21	0.08	7	1	2	125.53	73.44	3.94	5.2	5.13	3.84	5.65
5o	S=C(Nc1cccc(c1)[N+](=O)[O-])N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H19N5O2S	429.49	31	21	0.04	8	3	2	129.39	119.26	3.3	4.66	4.73	2.67	2.96
5p	CNC(=S)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C18H18N4S	322.43	23	15	0.11	6	1	2	99.35	73.44	2.91	3.27	3.12	2.46	4.04
5q	S=C(Nc1ccc(cc1)[N+](=O)[O-])N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C23H19N5O2S	429.49	31	21	0.04	8	3	2	129.39	119.26	3.27	4.66	4.73	2.67	2.96
5r	S=C(Nc1ccc2c(c1)cccc2)N/N=C/c1cn(c2c1cccc2)Cc1ccccc1	C27H22N4S	434.56	32	25	0.04	7	1	2	138.07	73.44	4.08	6.08	5.97	4.28	6.15
Kojic acid	OCc1occ(c(=O)c1)O	C6H6O4	142.11	10	6	0.17	1	4	2	33.13	70.67	1.12	-0.64	-0.31	-1.69	0.74

Ligand	Consensus Log P	ESOL Log S	ESOL Solubility	ESOL Solubility (mol/l)	ESOL Class	Ali Log S	Ali Solubility (mg/ml)	Ali Solubility	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility (mg/ml)	Silicos-IT Solubility (mol/l)	Silicos-IT class	GI absorption	BBB permeant	Pgp substrate
5a	4.4	-5.42	1.56E-03	3.77E-06	Moderately soluble	-6.27	2.24E-04	5.39E-07	Poorly soluble	-8.28	2.17E-06	5.22E-09	Poorly soluble	High	No	No

5b	4.4	-5.36	1.68E-03	4.37E-06	Moderately soluble	-6.11	3.02E-04	7.84E-07	Poorly soluble	-8.18	2.54E-06	6.61E-09	Poorly soluble	High	No	No
5c	5.03	-5.96	4.56E-04	1.11E-06	Moderately soluble	-6.86	5.65E-05	1.37E-07	Poorly soluble	-8.93	4.84E-07	1.17E-09	Poorly soluble	High	No	Yes
5d	4.72	-5.52	1.23E-03	3.05E-06	Moderately soluble	-6.21	2.49E-04	6.18E-07	Poorly soluble	-8.44	1.45E-06	3.60E-09	Poorly soluble	High	No	No
5e	5	-5.92	5.25E-04	1.21E-06	Moderately soluble	-6.7	8.70E-05	2.01E-07	Poorly soluble	-9.16	3.01E-07	6.95E-10	Poorly soluble	High	No	Yes
5f	5.45	-6.54	1.30E-04	2.86E-07	Poorly soluble	-7.41	1.75E-05	3.86E-08	Poorly soluble	-9.35	2.03E-07	4.48E-10	Poorly soluble	High	No	No
5g	4.4	-5.36	1.68E-03	4.37E-06	Moderately soluble	-6.11	3.02E-04	7.84E-07	Poorly soluble	-8.18	2.54E-06	6.61E-09	Poorly soluble	High	No	No
5h	5.01	-5.96	4.56E-04	1.11E-06	Moderately soluble	-6.86	5.65E-05	1.37E-07	Poorly soluble	-8.93	4.84E-07	1.17E-09	Poorly soluble	High	No	Yes
5i	4.47	-5.32	1.89E-03	4.74E-06	Moderately soluble	-6.04	3.61E-04	9.05E-07	Poorly soluble	-8.57	1.07E-06	2.67E-09	Poorly soluble	High	No	Yes
5j	5.04	-6.26	2.52E-04	5.44E-07	Poorly soluble	-6.82	6.99E-05	1.51E-07	Poorly soluble	-8.96	5.09E-07	1.10E-09	Poorly soluble	High	No	No
5k	4.78	-5.62	9.90E-04	2.40E-06	Moderately soluble	-6.42	1.58E-04	3.83E-07	Poorly soluble	-8.95	4.65E-07	1.13E-09	Poorly soluble	High	No	Yes
5l	4.41	-5.42	1.56E-03	3.77E-06	Moderately soluble	-6.27	2.24E-04	5.39E-07	Poorly soluble	-8.28	2.17E-06	5.22E-09	Poorly soluble	High	No	No
5m	4.37	-5.5	1.41E-03	3.17E-06	Moderately soluble	-6.44	1.61E-04	3.62E-07	Poorly soluble	-8.38	1.85E-06	4.16E-09	Poorly soluble	High	No	No
5n	4.75	-	8.70	2.18E-06	Mode	-	1.29E-04	3.24	Poor	-8.56	1.11E-06	2.78E-	Poor	High	No	No

		5.66	E-04		rately soluble	6.49		E-07	ly soluble			09	ly soluble			
5o	3.66	-5.41	1.66E-03	3.87E-06	Moderately soluble	-6.89	5.52E-05	1.28E-07	Poorly soluble	-7.52	1.30E-05	3.02E-08	Poorly soluble	Low	No	No
5p	3.16	-3.99	3.33E-02	1.03E-04	Soluble	-4.49	1.05E-02	3.26E-05	Moderately soluble	-6.09	2.60E-04	8.07E-07	Poorly soluble	High	Yes	No
5q	3.66	-5.41	1.66E-03	3.87E-06	Moderately soluble	-6.89	5.52E-05	1.28E-07	Poorly soluble	-7.52	1.30E-05	3.02E-08	Poorly soluble	Low	No	No
5r	5.31	-6.48	1.44E-04	3.31E-07	Poorly soluble	-7.4	1.72E-05	3.96E-08	Poorly soluble	-9.81	6.71E-08	1.55E-10	Poorly soluble	High	No	No
Kojic acid	-0.16	-0.7	2.86E+01	2.01E-01	Very soluble	-0.37	6.05E+01	4.25E-01	Very soluble	-1.17	9.72E+00	6.84E-02	Soluble	High	No	No

Ligand	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	**log Kp (cm/s)**	**Lipinski #violations**	**Ghose #violations**	**Veber #violations**	**Egan #violations**	**Muegge #violations**	**Bioavailability Score**	**PAINS #alerts**	**Brenk #alerts**	**Leadlikeness #violations**	**Synthetic Accessibility**
5a	Yes	Yes	Yes	Yes	Yes	-5.42	0	0	0	0	0	0.55	1	2	3	3.2
5b	Yes	Yes	Yes	Yes	Yes	-5.22	0	0	0	0	0	0.55	1	2	2	3.14
5c	Yes	Yes	Yes	Yes	Yes	-4.87	0	1	0	0	1	0.55	1	2	2	3.38
5d	Yes	Yes	Yes	Yes	Yes	-5.25	0	0	0	0	0	0.55	1	2	2	3.12
5e	Yes	Yes	Yes	Yes	Yes	-5.11	0	0	0	0	1	0.55	0	2	3	3.2
5f	No	Yes	Yes	No	Yes	-4.74	1	2	0	1	1	0.55	1	2	2	3.26
5g	Yes	Yes	Yes	Yes	Yes	-5.22	0	0	0	0	0	0.55	1	2	2	3.14
5h	Yes	Yes	Yes	Yes	Yes	-4.87	0	1	0	0	1	0.55	1	2	2	3.39
5i	Yes	Yes	Yes	Yes	Yes	-5.34	0	0	0	0	0	0.55	0	2	3	3.19
5j	Yes	Yes	Yes	Yes	Yes	-5.21	1	0	0	0	1	0.55	1	2	2	3.2
5k	Yes	Yes	Yes	Yes	Yes	-5.17	0	0	0	0	1	0.55	0	2	3	3.3
5l	Yes	Yes	Yes	Yes	Yes	-5.42	0	0	0	0	0	0.55	1	2	3	3.29
5m	No	Yes	Yes	Yes	Yes	-5.62	0	1	0	0	0	0.55	1	2	3	3.51
5n	Yes	Yes	Yes	Yes	Yes	-5.04	0	0	0	0	1	0.55	1	2	2	3.26
5o	No	Yes	Yes	No	Yes	-5.61	0	0	0	0	0	0.55	1	4	3	3.49
5p	Yes	Yes	Yes	Yes	Yes	-5.95	0	0	0	0	0	0.55	0	2	0	2.75
5q	No	Yes	Yes	No	Yes	-5.61	0	0	0	0	0	0.55	1	4	3	3.3
5r	Yes	Yes	Yes	No	Yes	-4.63	1	2	0	1	1	0.55	1	2	2	3.37

Kojic acid	No	No	No	No	No	-7.62	0	3	0	0	1	0.55	0	0	1	2.53

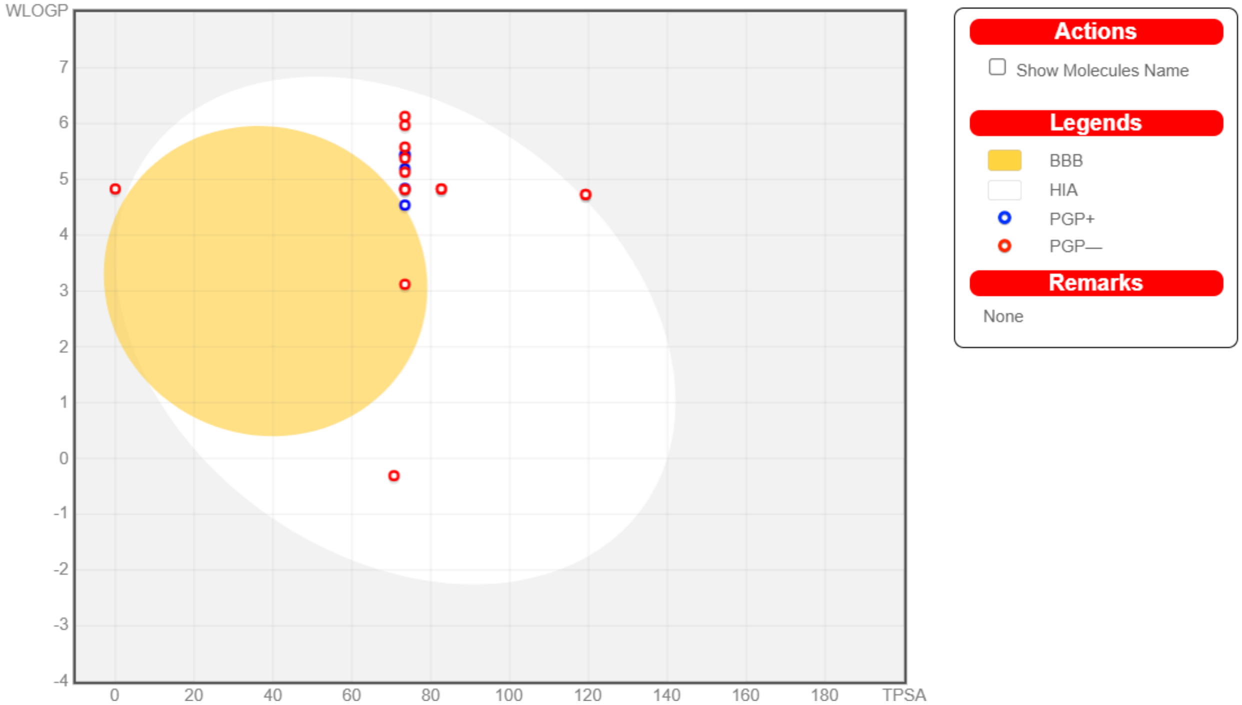
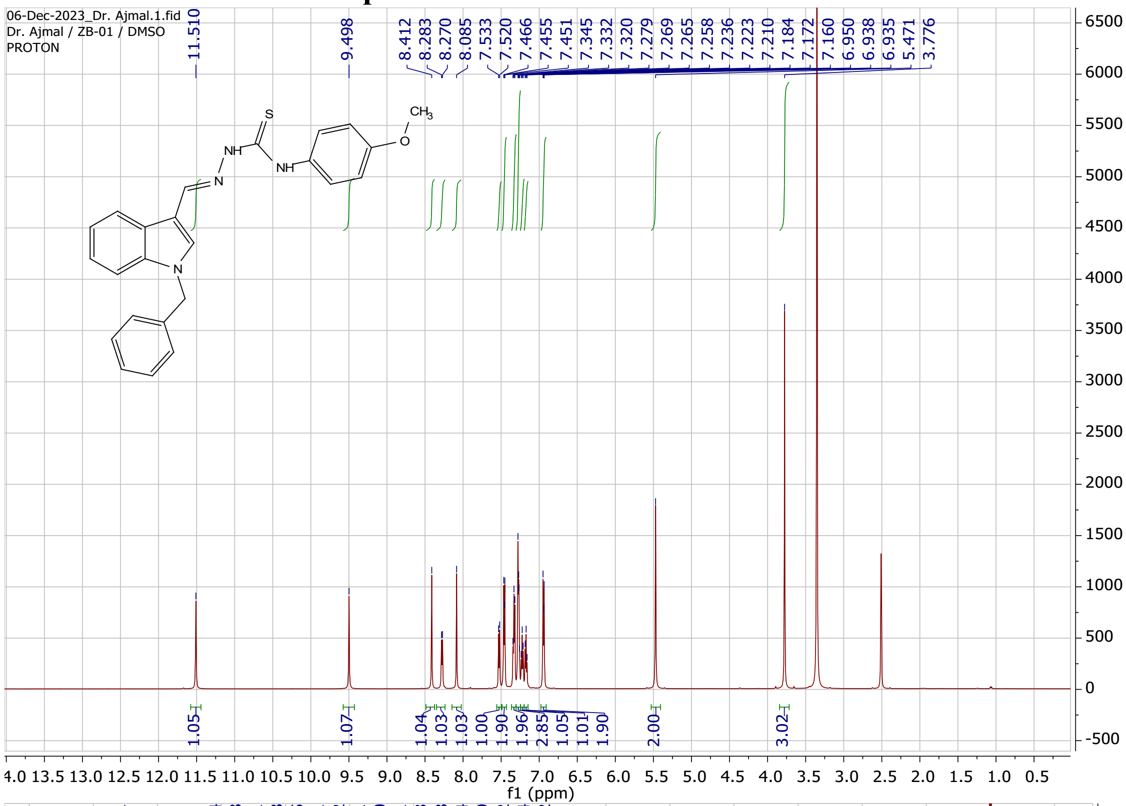
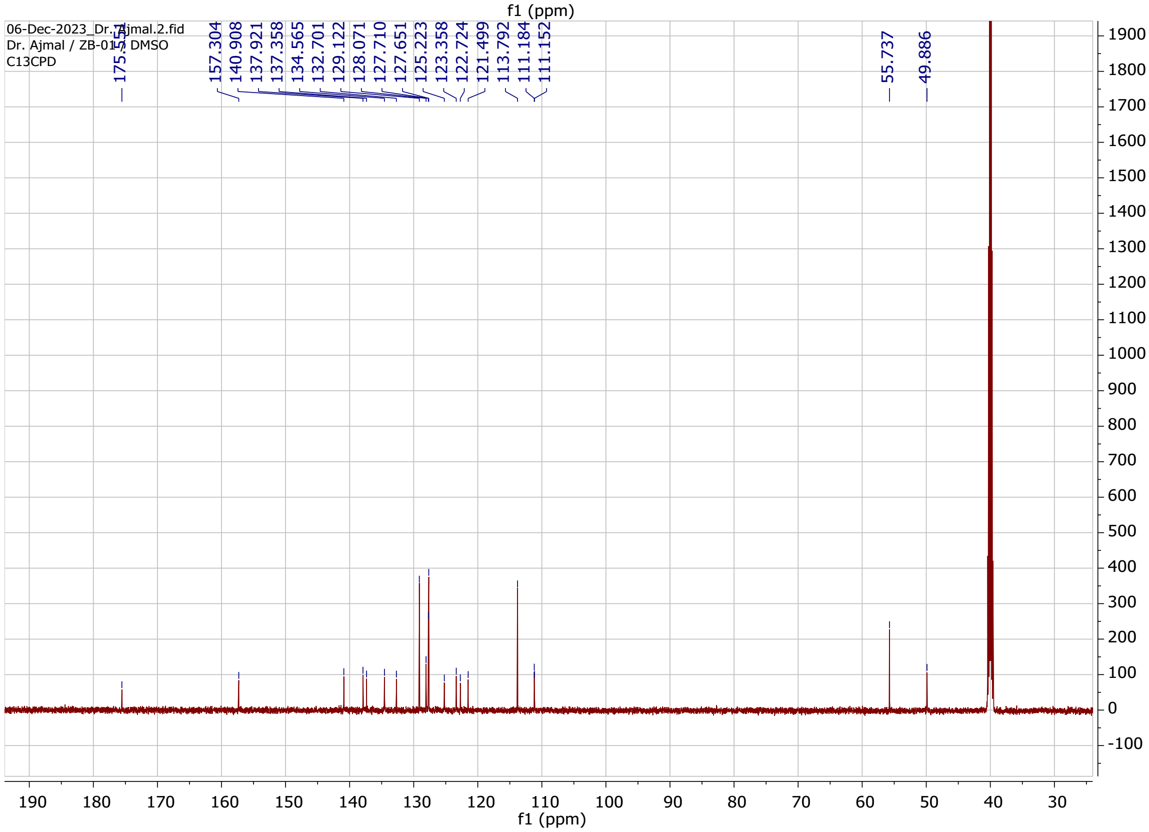


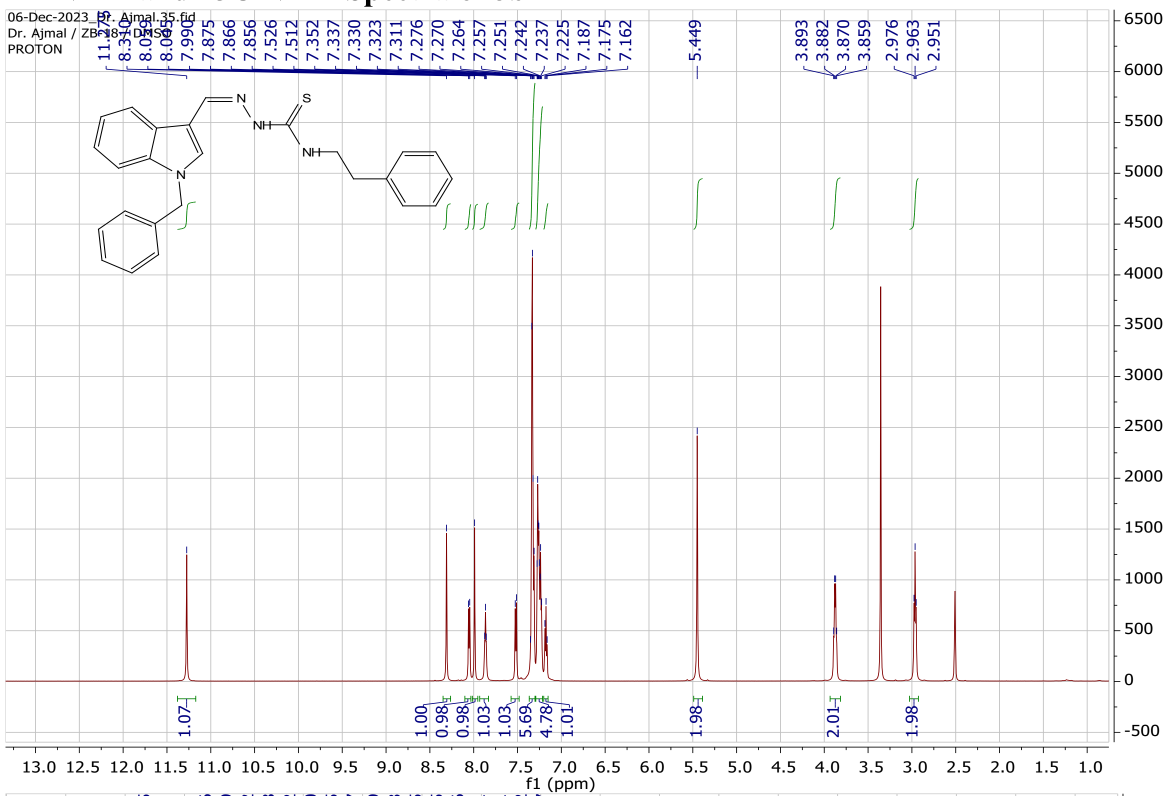
Figure S3. The boiled-egg plot (plot of WLOGP against TPSA) of all investigated compounds from SwissADME web tool.

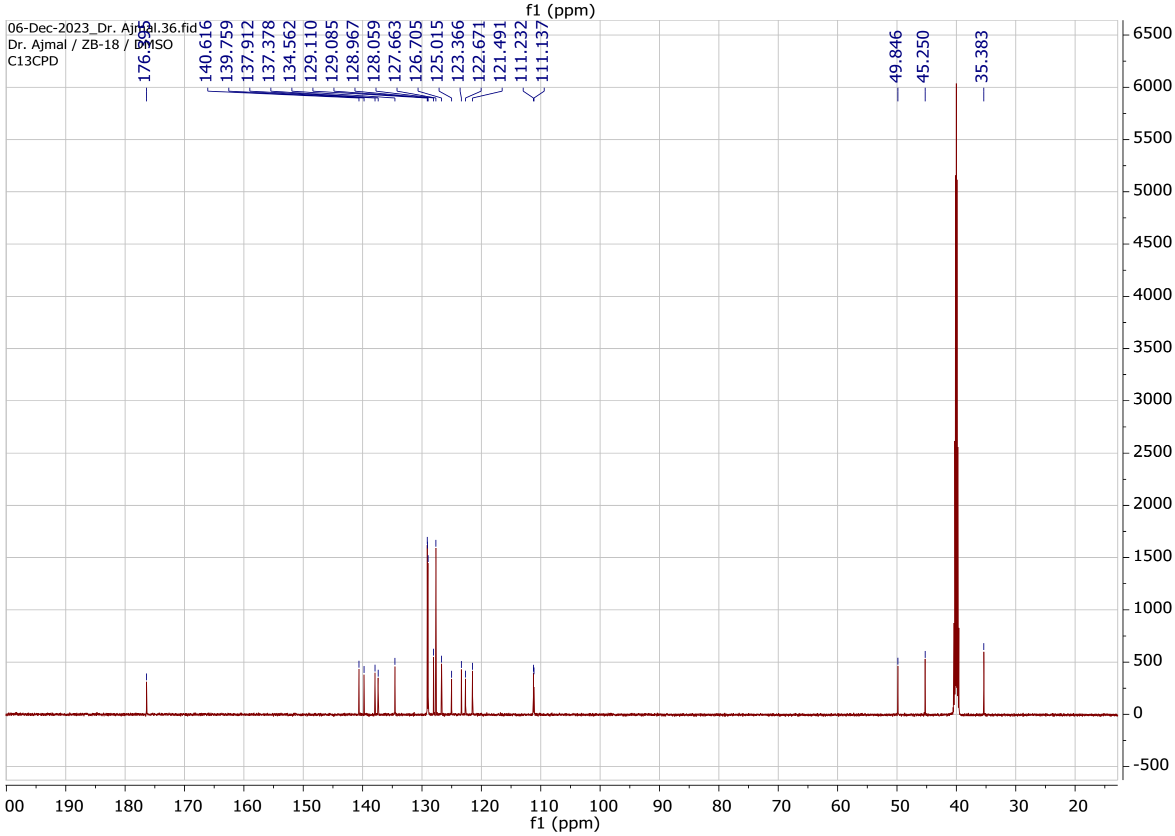
1H NMR and 13C NMR Spectra of 5a



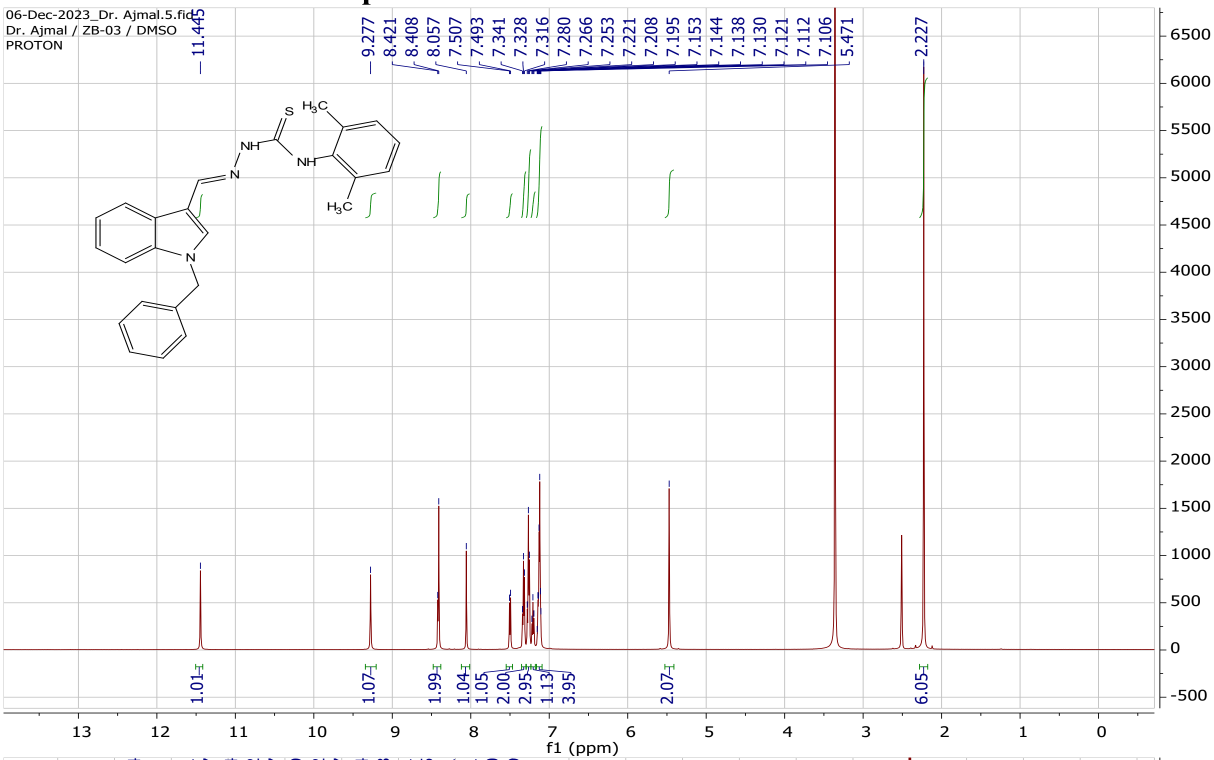


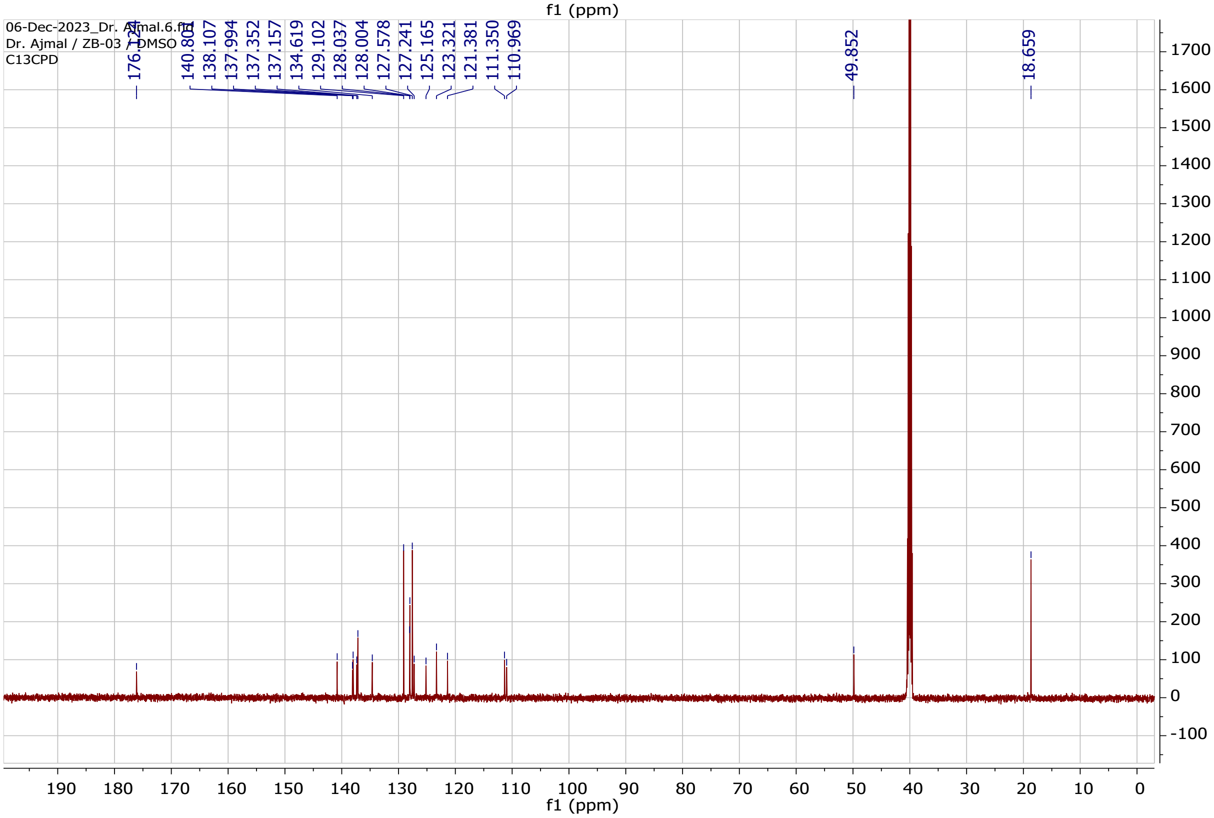
1H NMR and 13C NMR Spectra of 5b



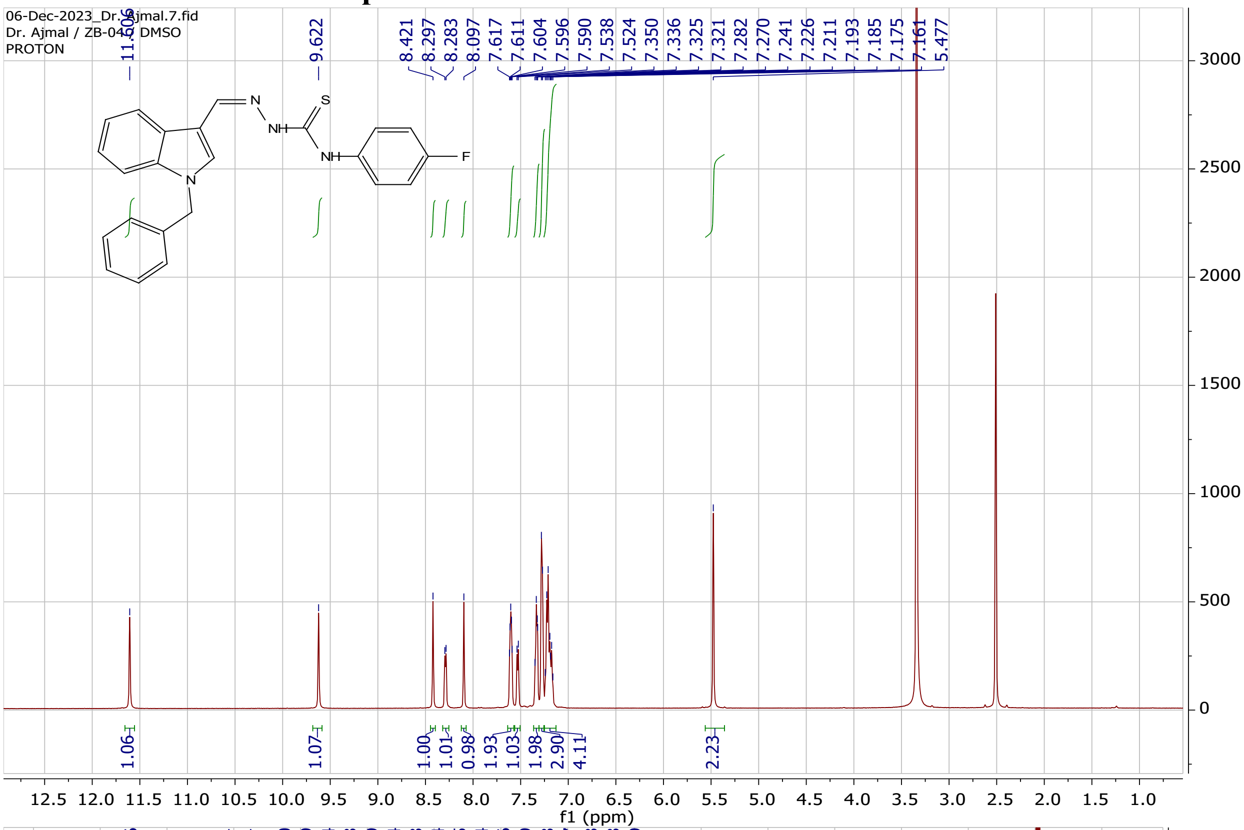


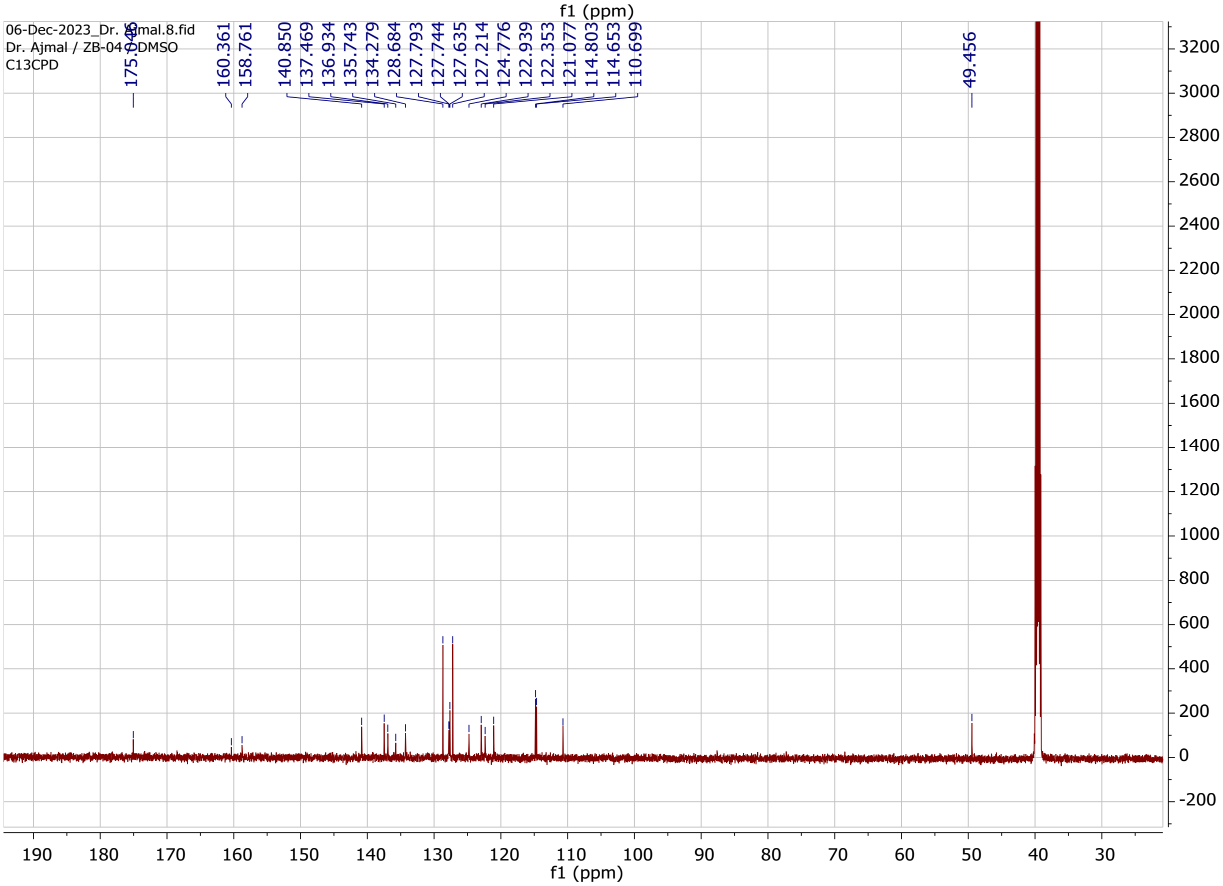
1H NMR and 13C NMR Spectra of 5c



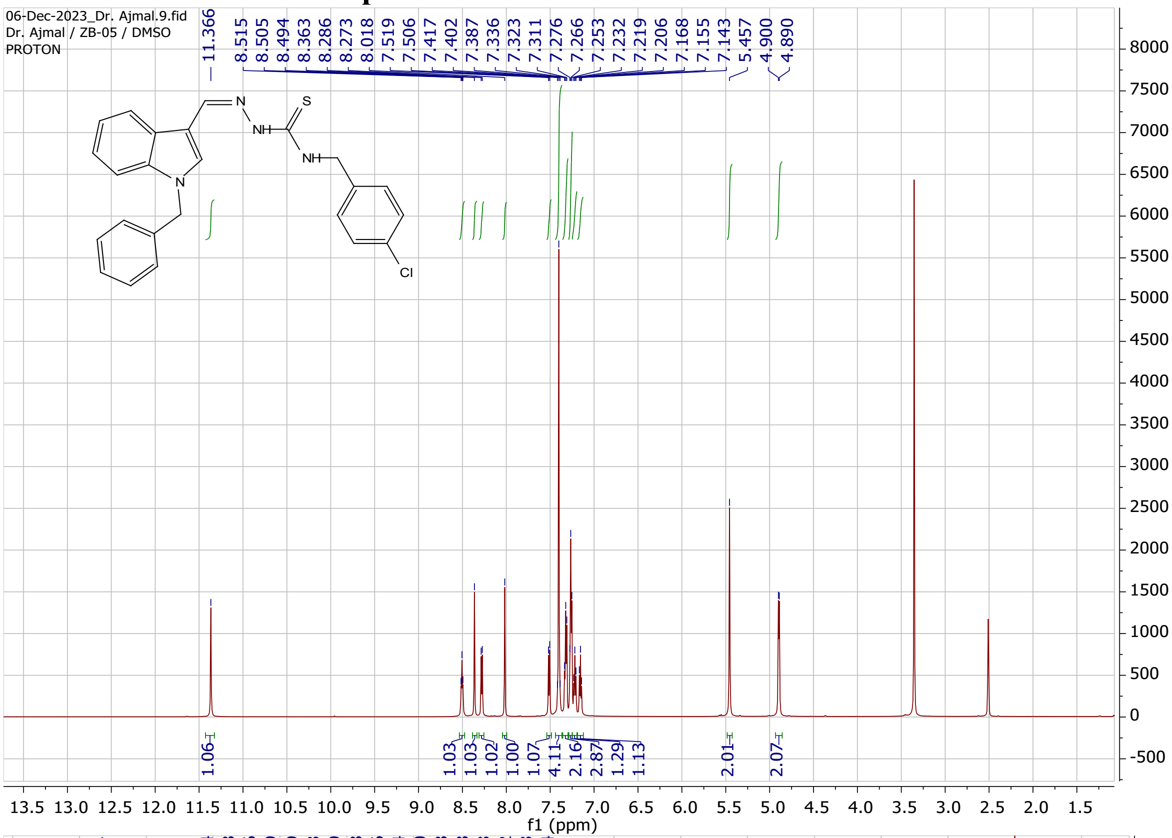


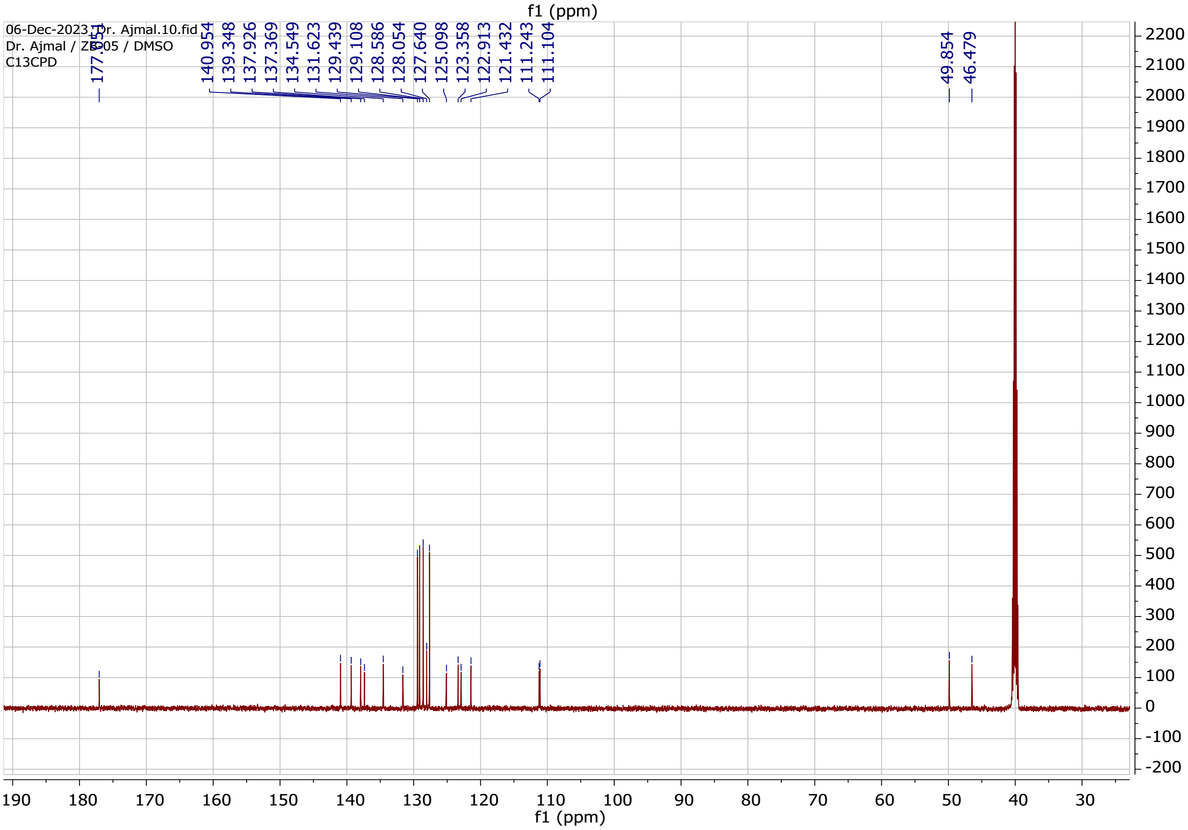
1H NMR and 13C NMR Spectra of 5d



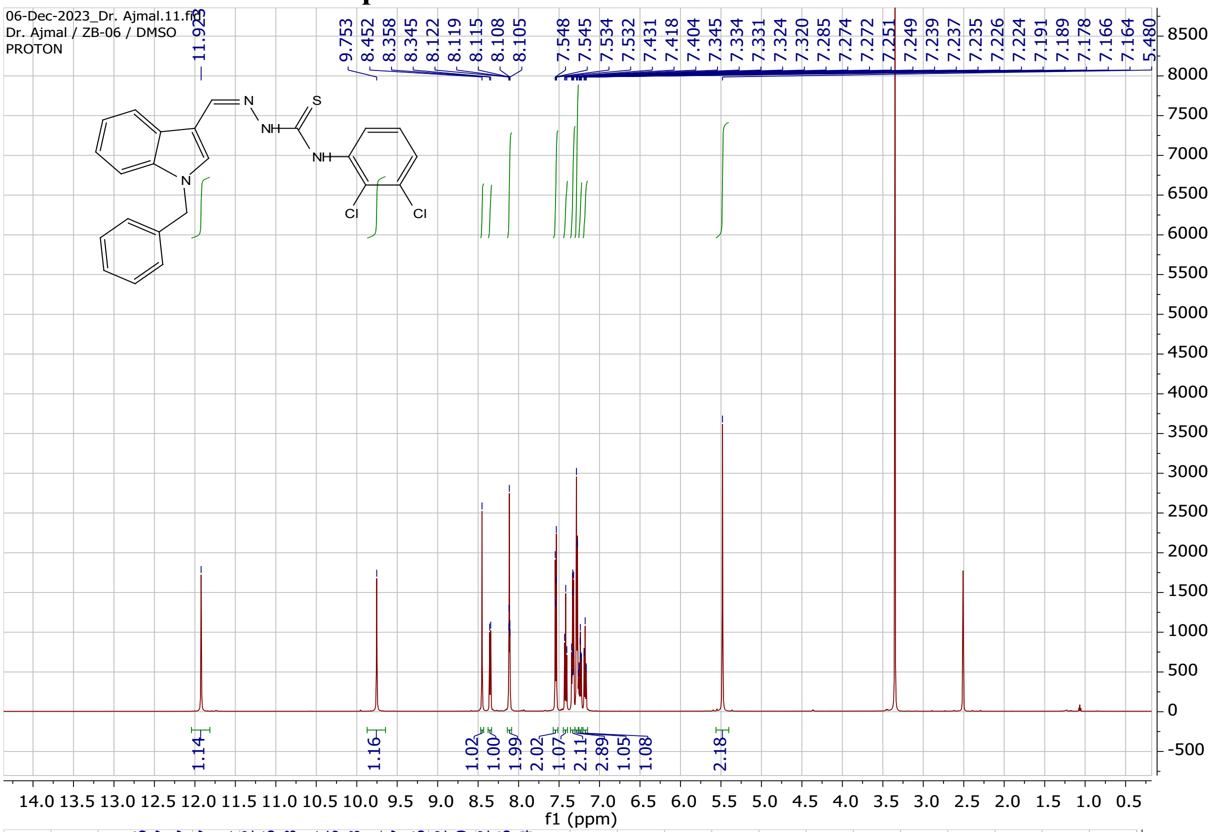


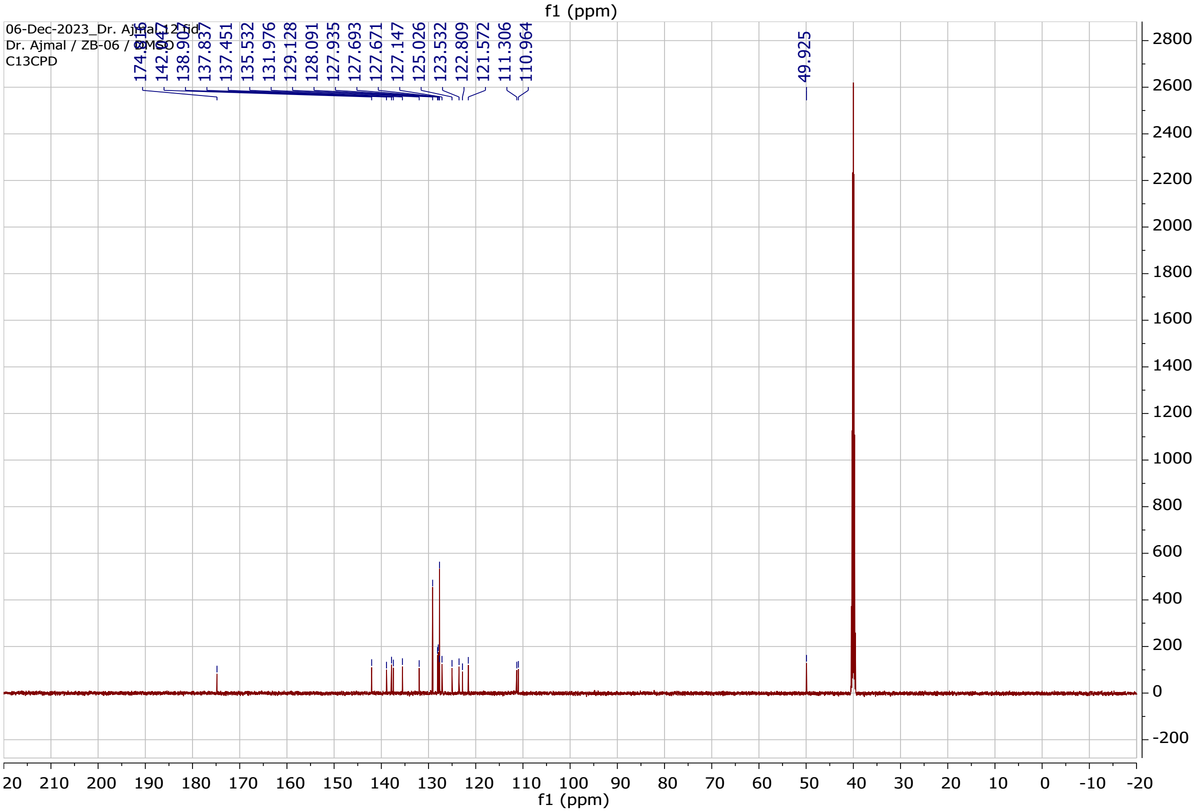
1H NMR and 13C NMR Spectra of 5e



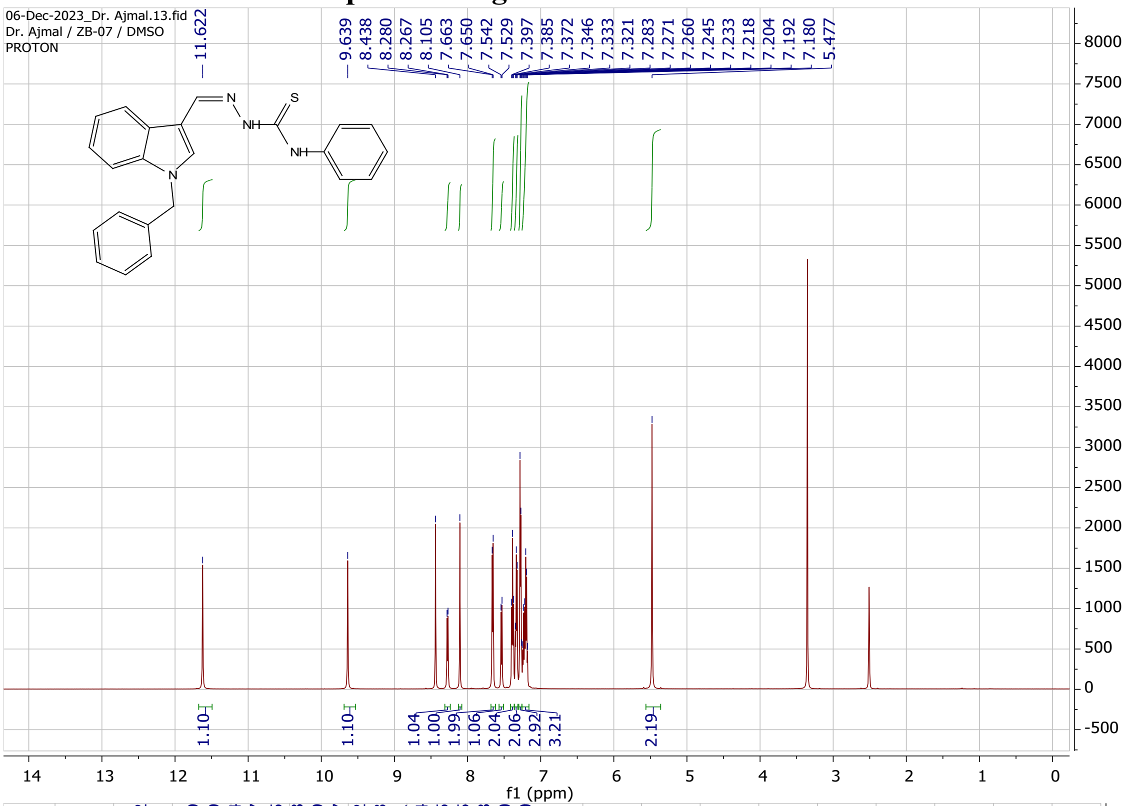


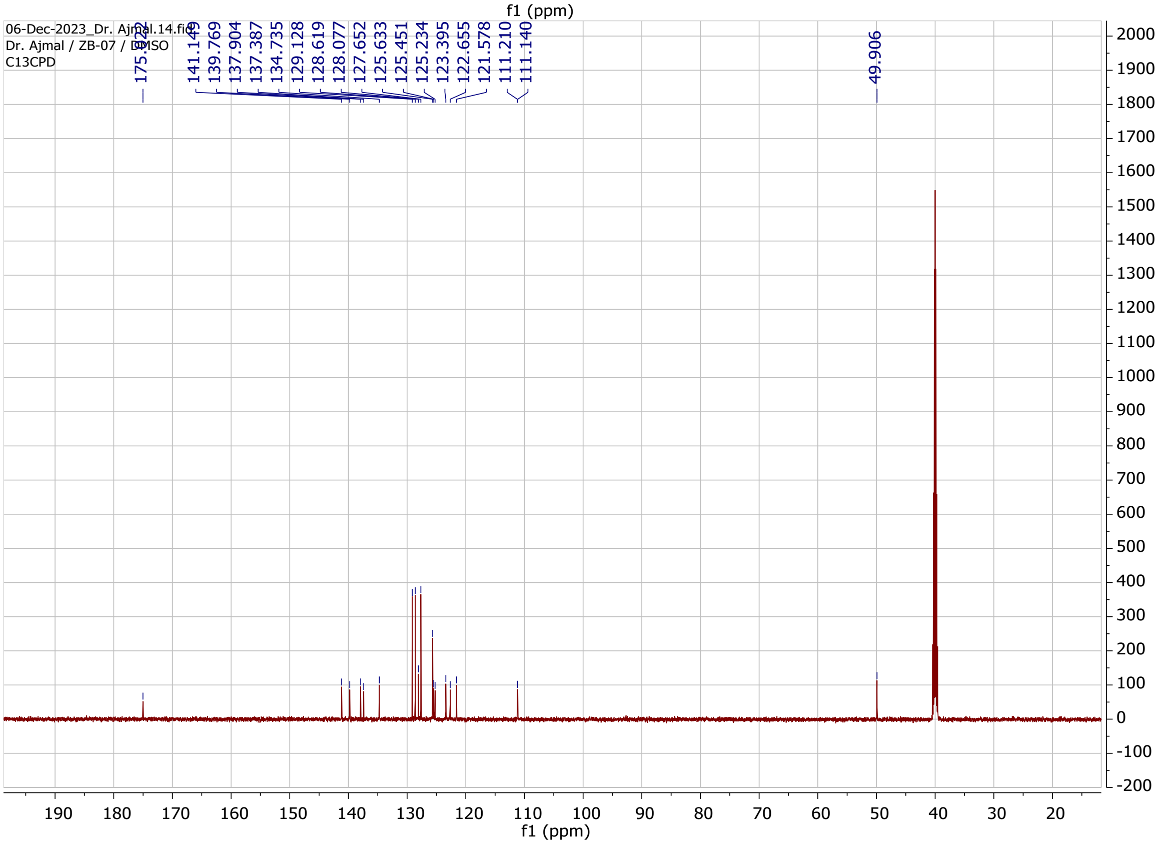
1H NMR and 13C NMR Spectra of 5f



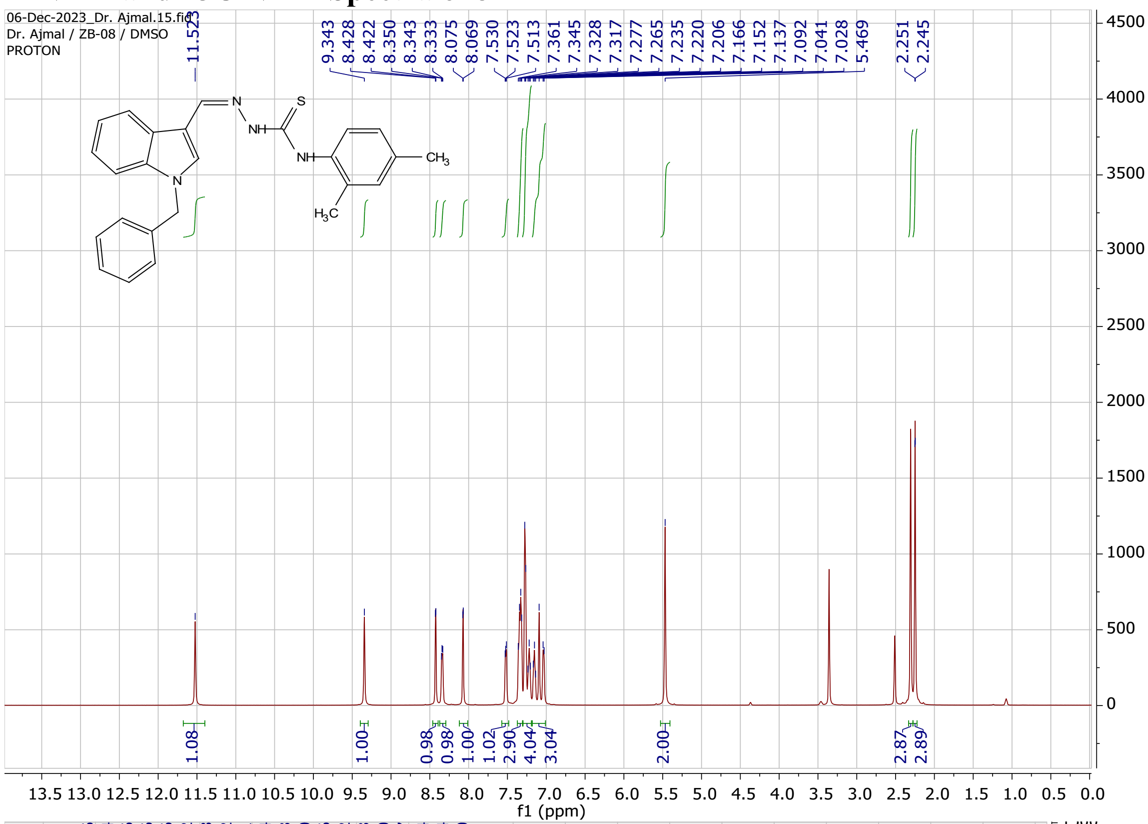


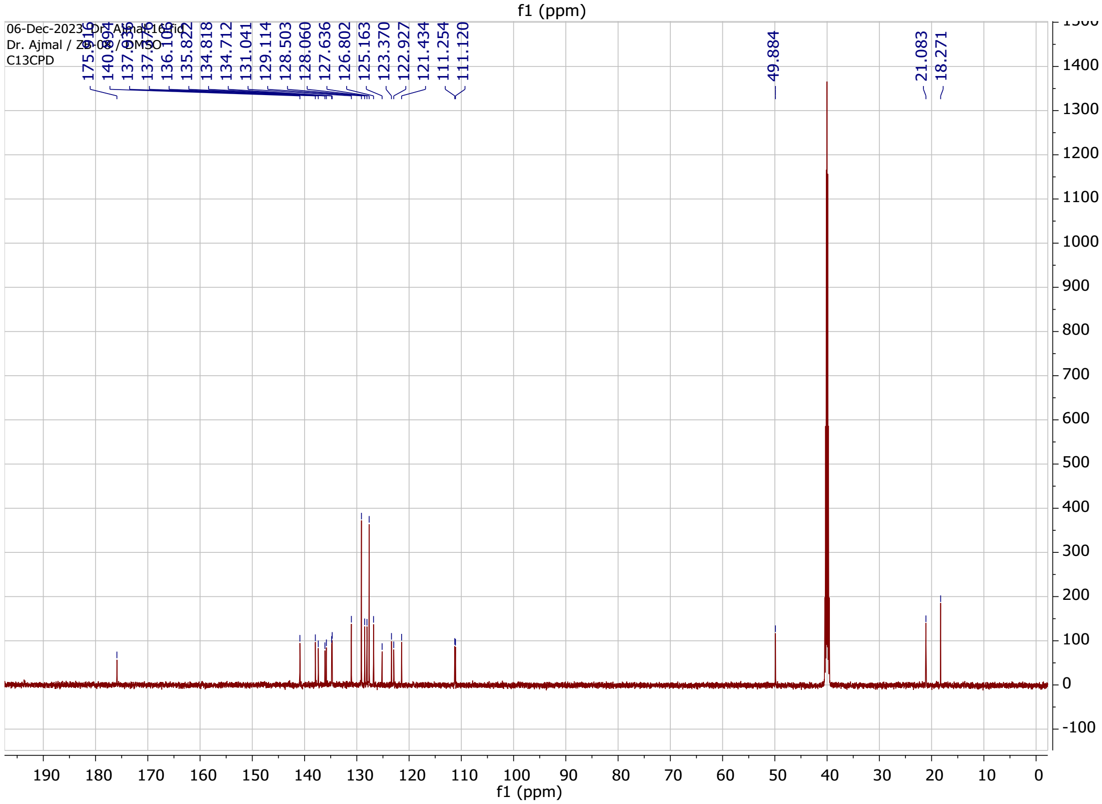
H NMR and 13C NMR Spectra of 5g



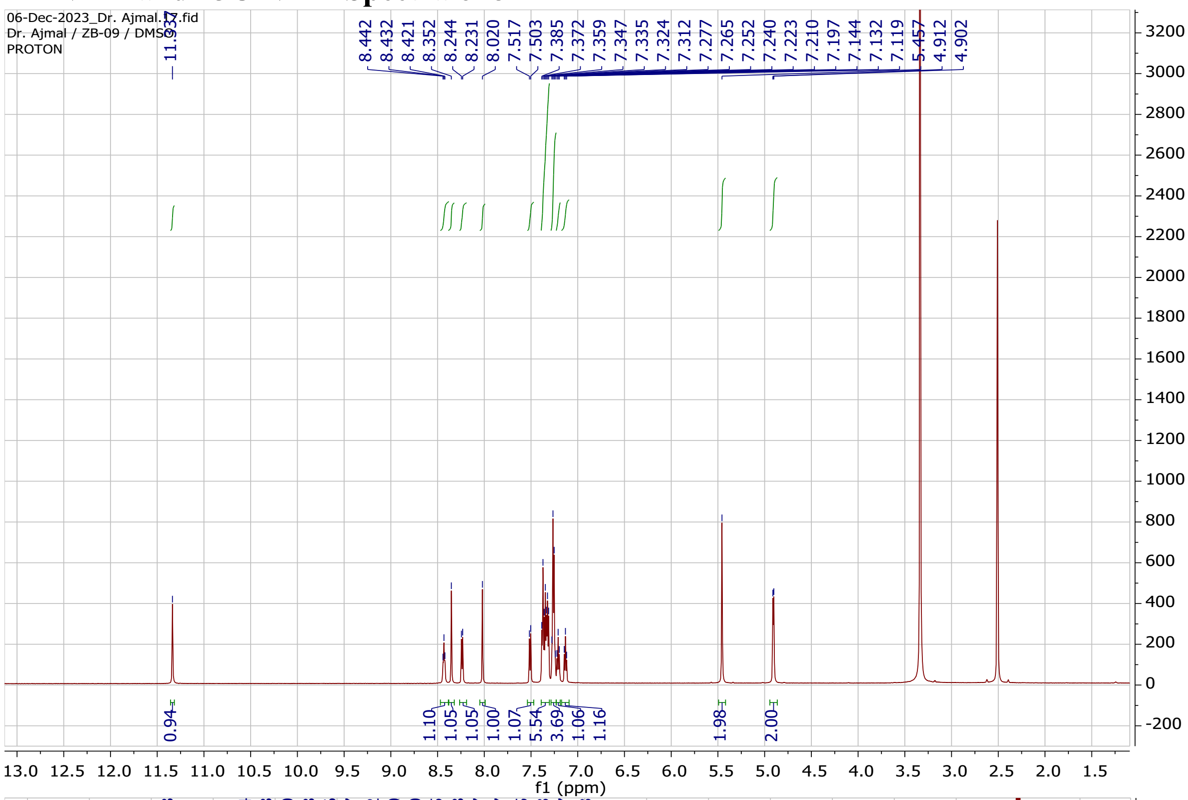


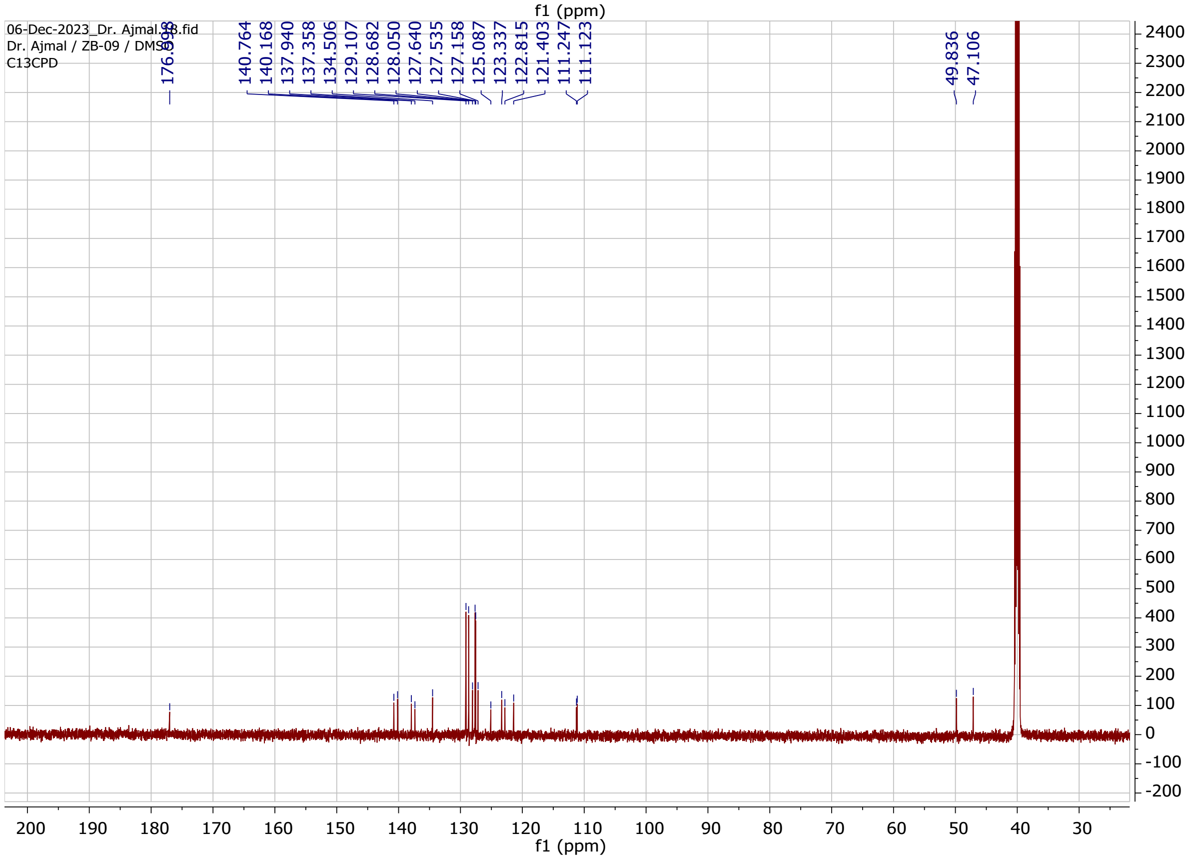
H NMR and 13C NMR Spectra of 5h



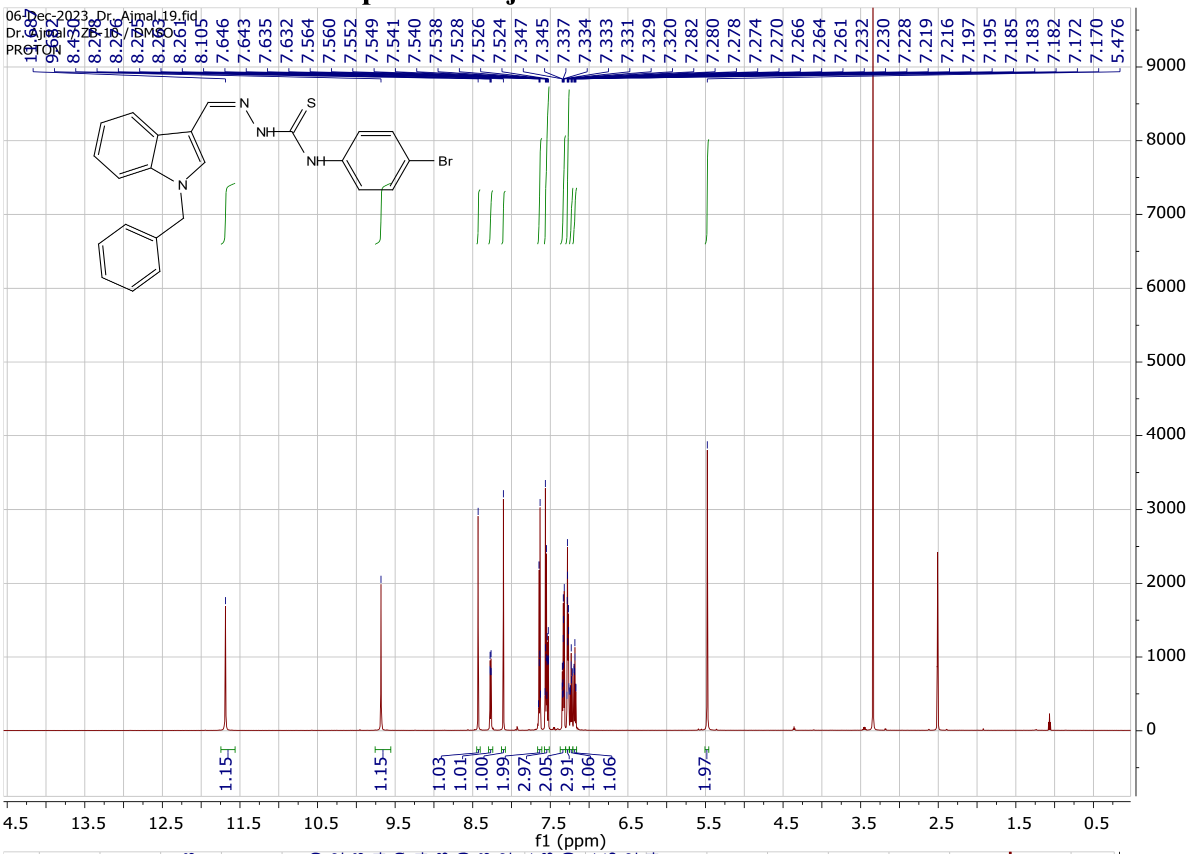


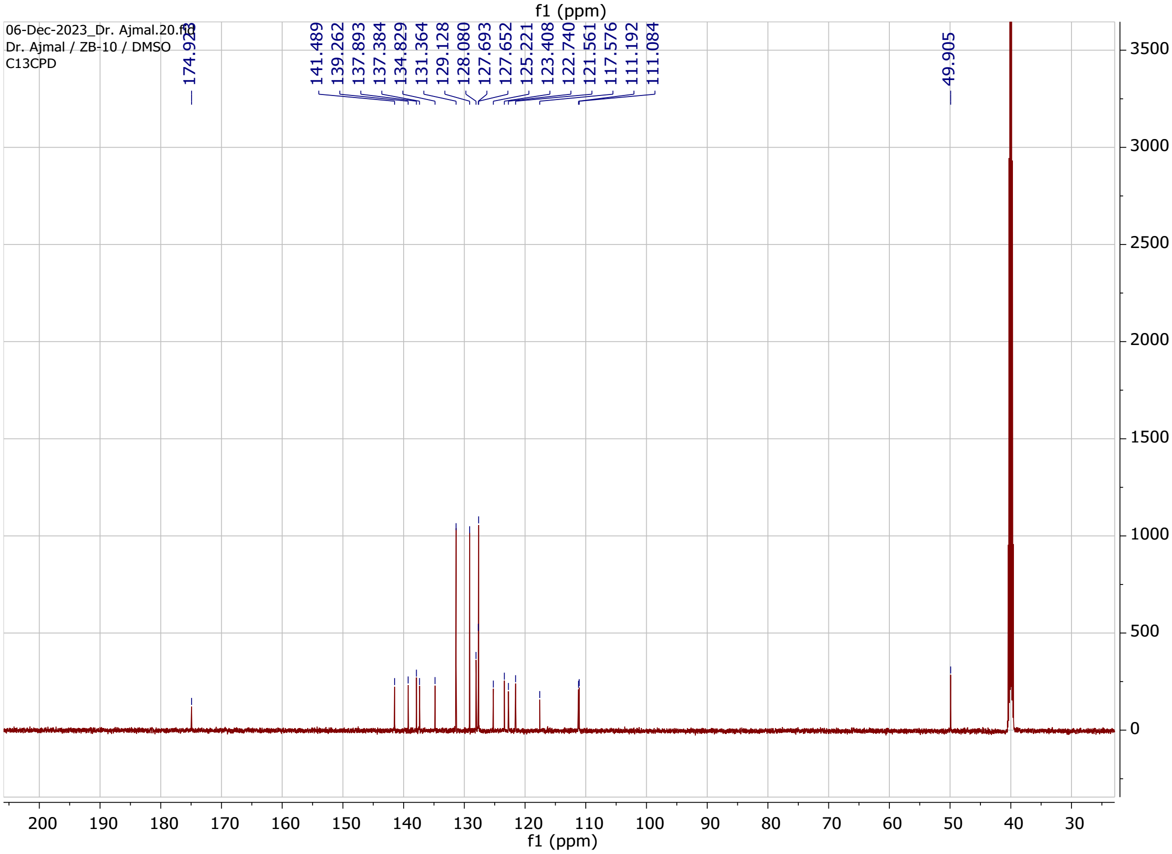
1H NMR and 13C NMR Spectra of 5i



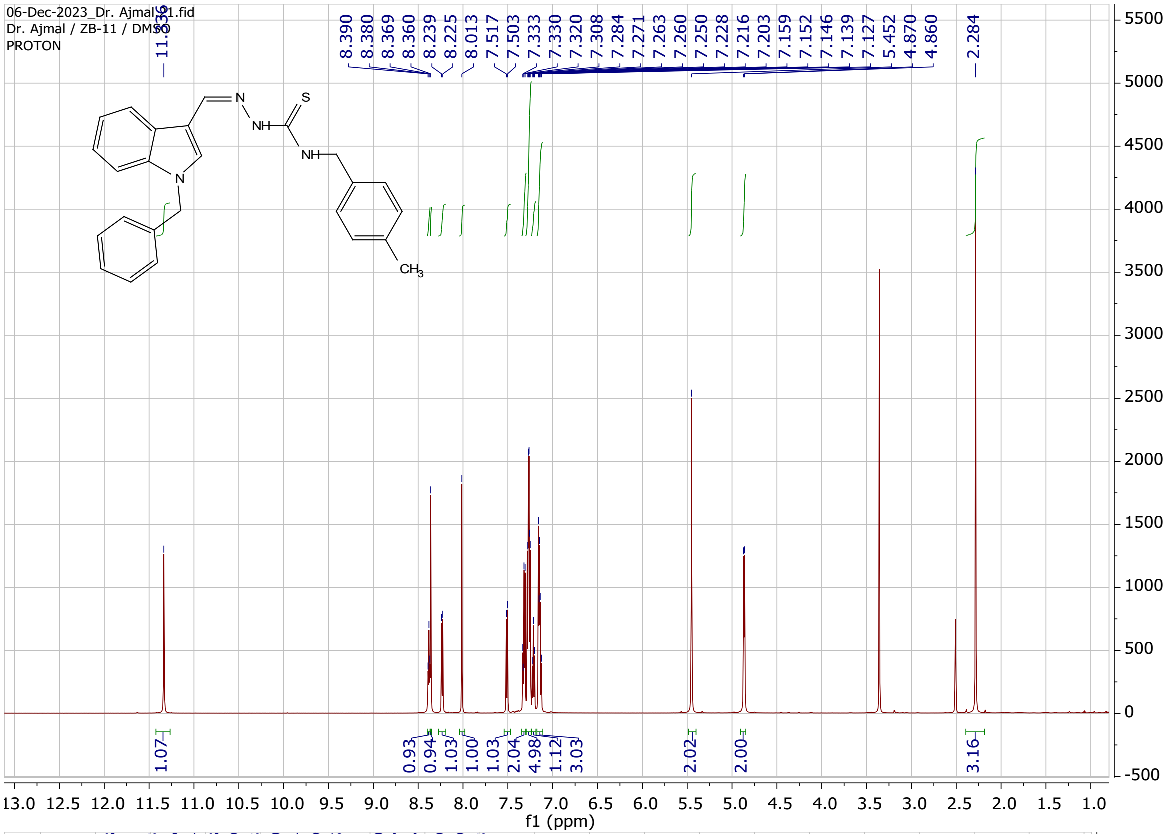


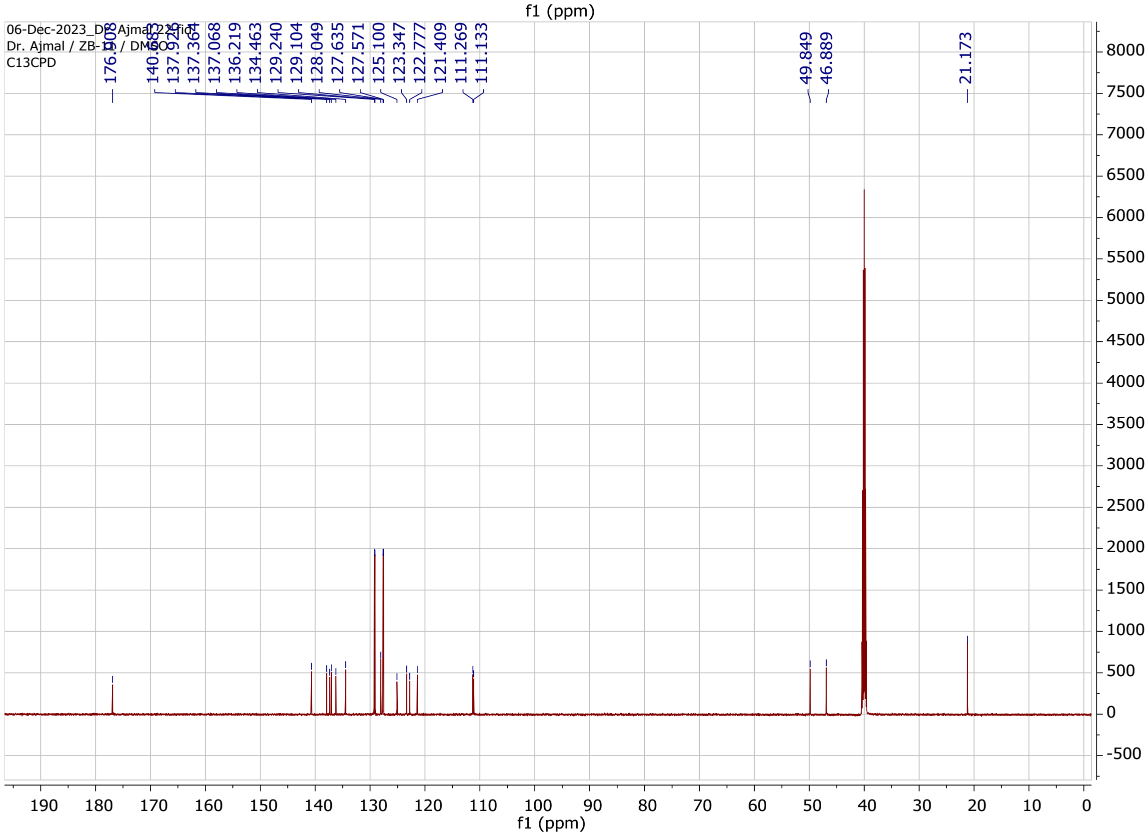
1H NMR and 13C NMR Spectra of 5j



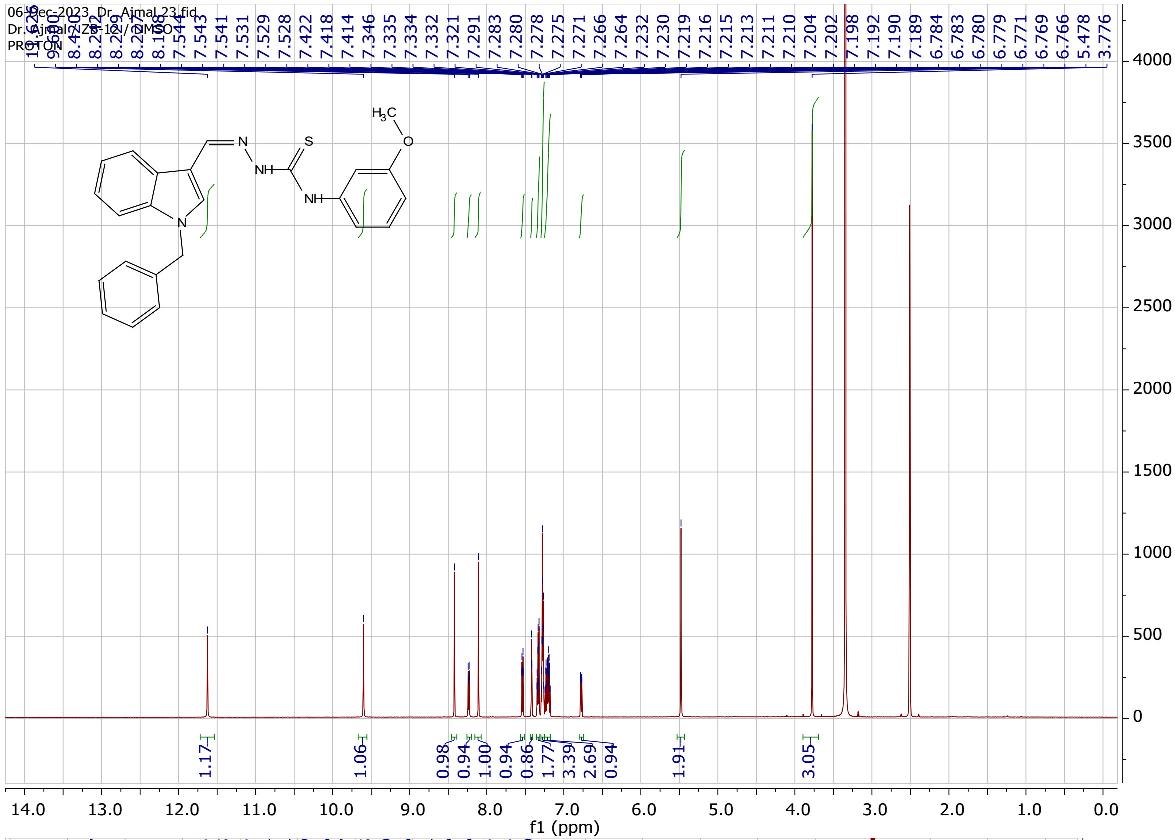


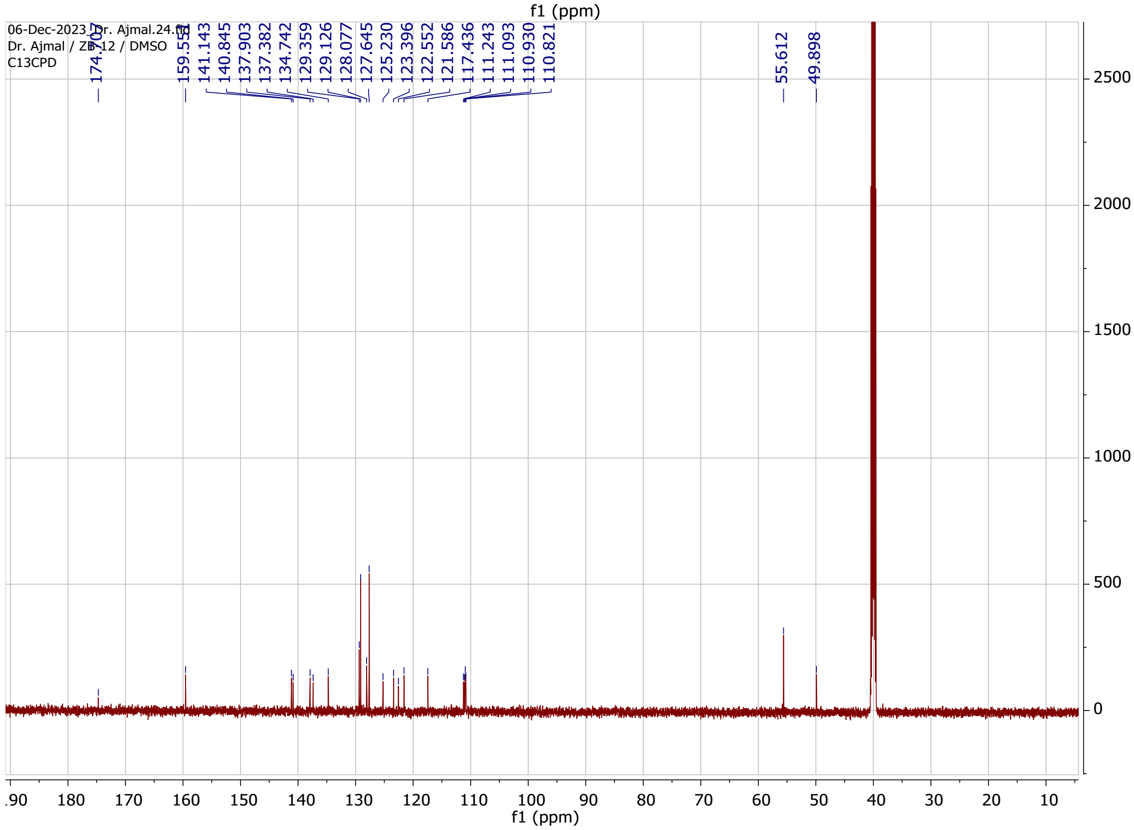
^{1}H NMR and ^{13}C NMR Spectra of 5k



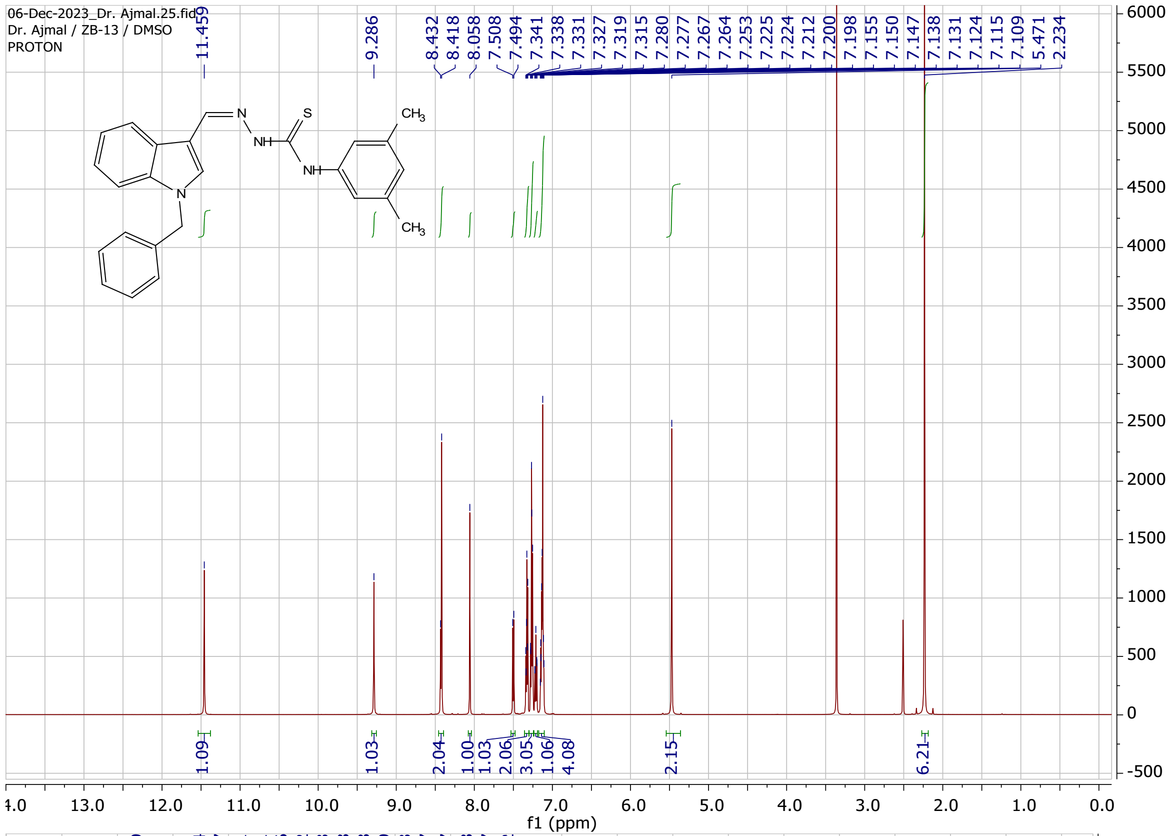


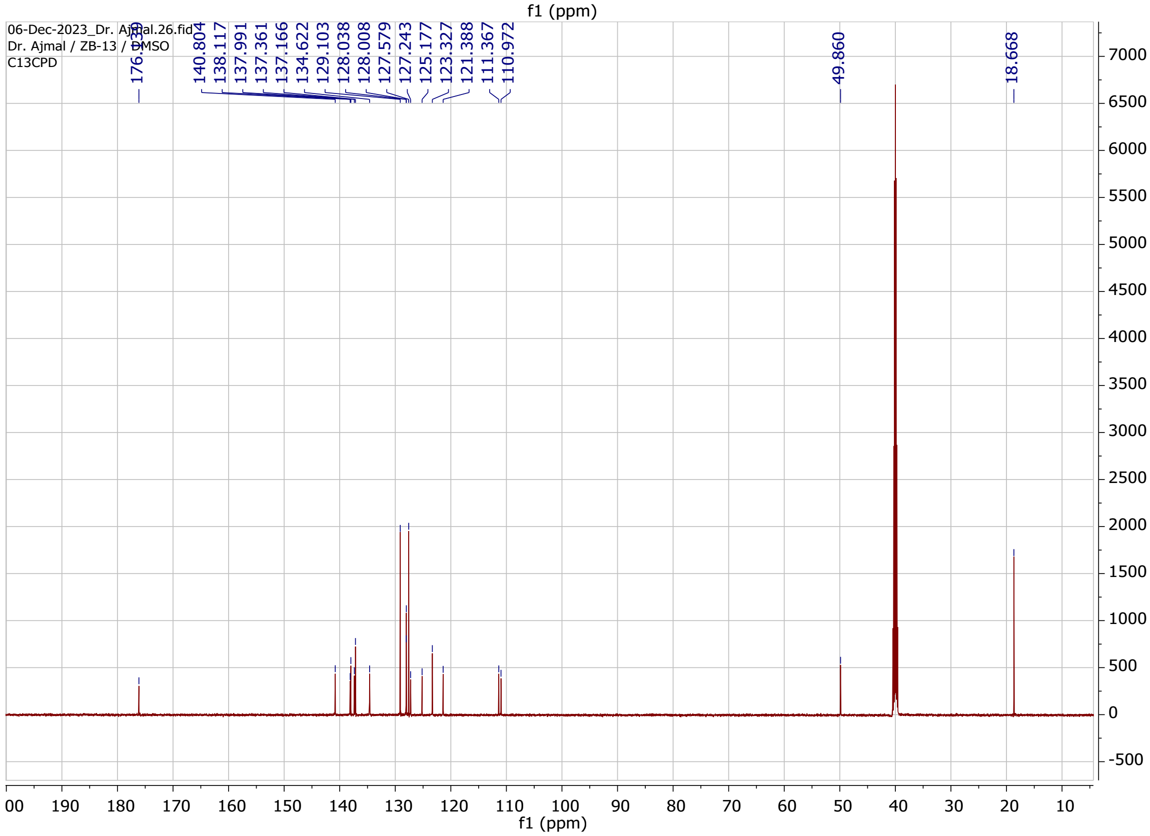
1H NMR and 13C NMR Spectra of 5l



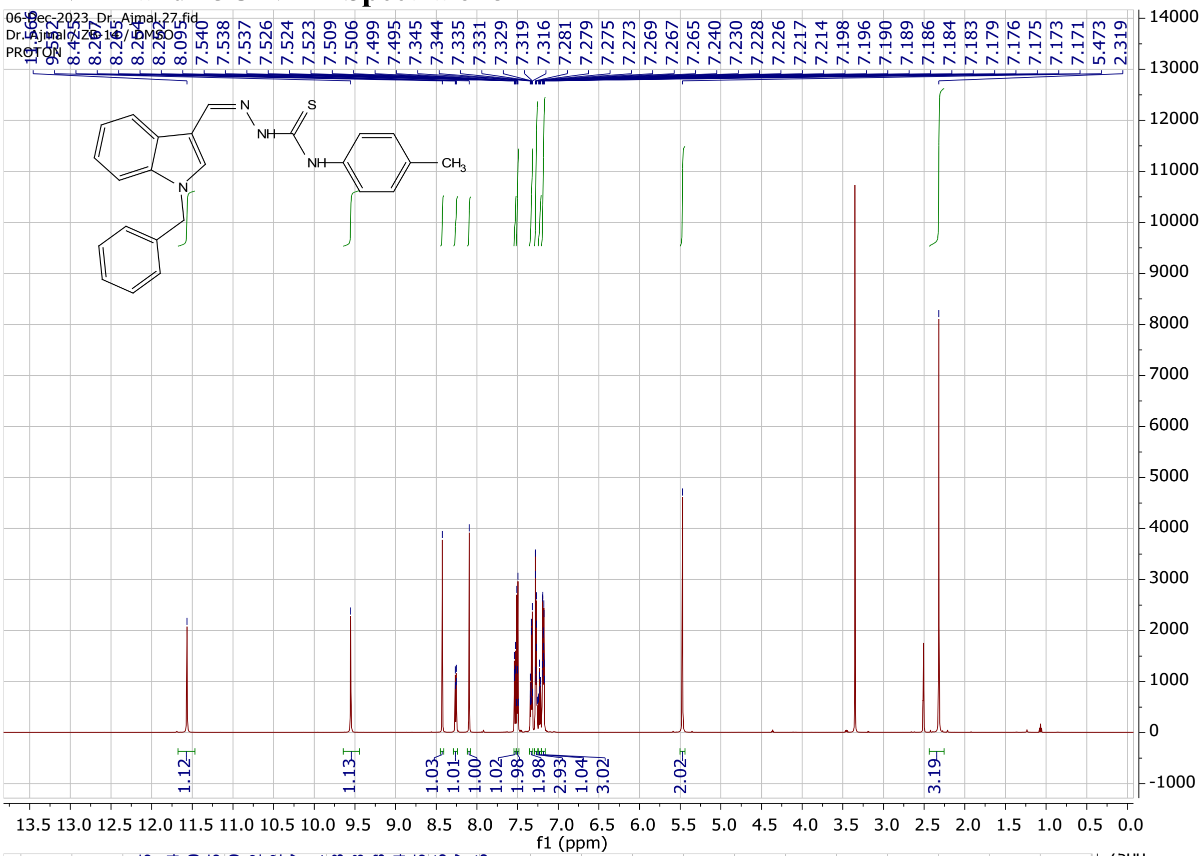


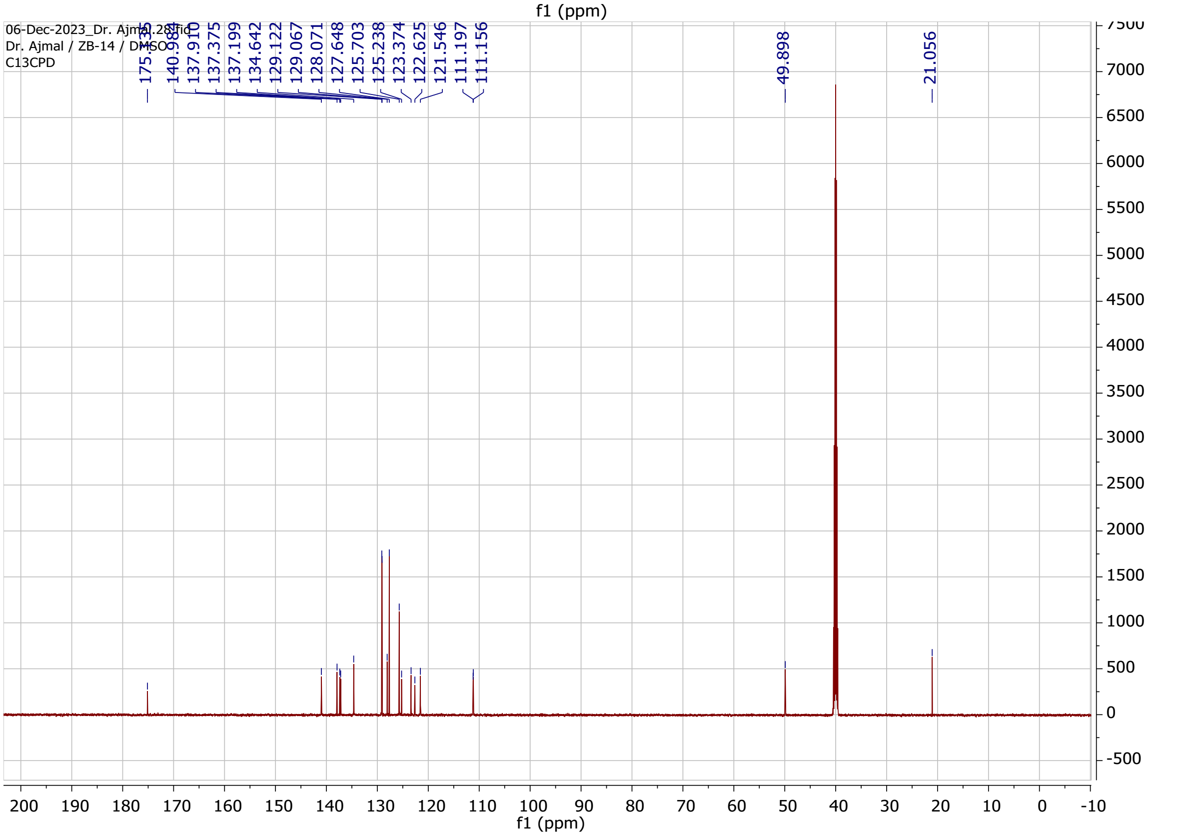
1H NMR and 13C NMR Spectra of 5m



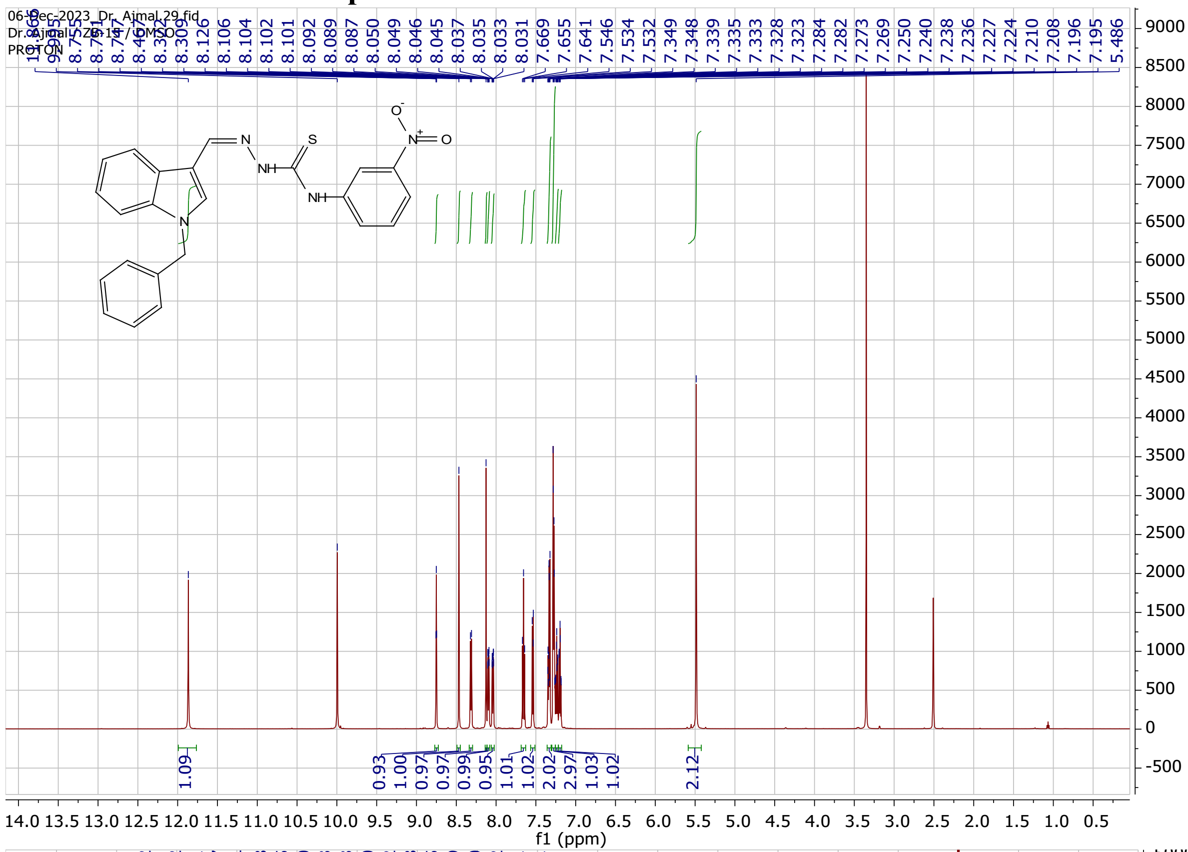


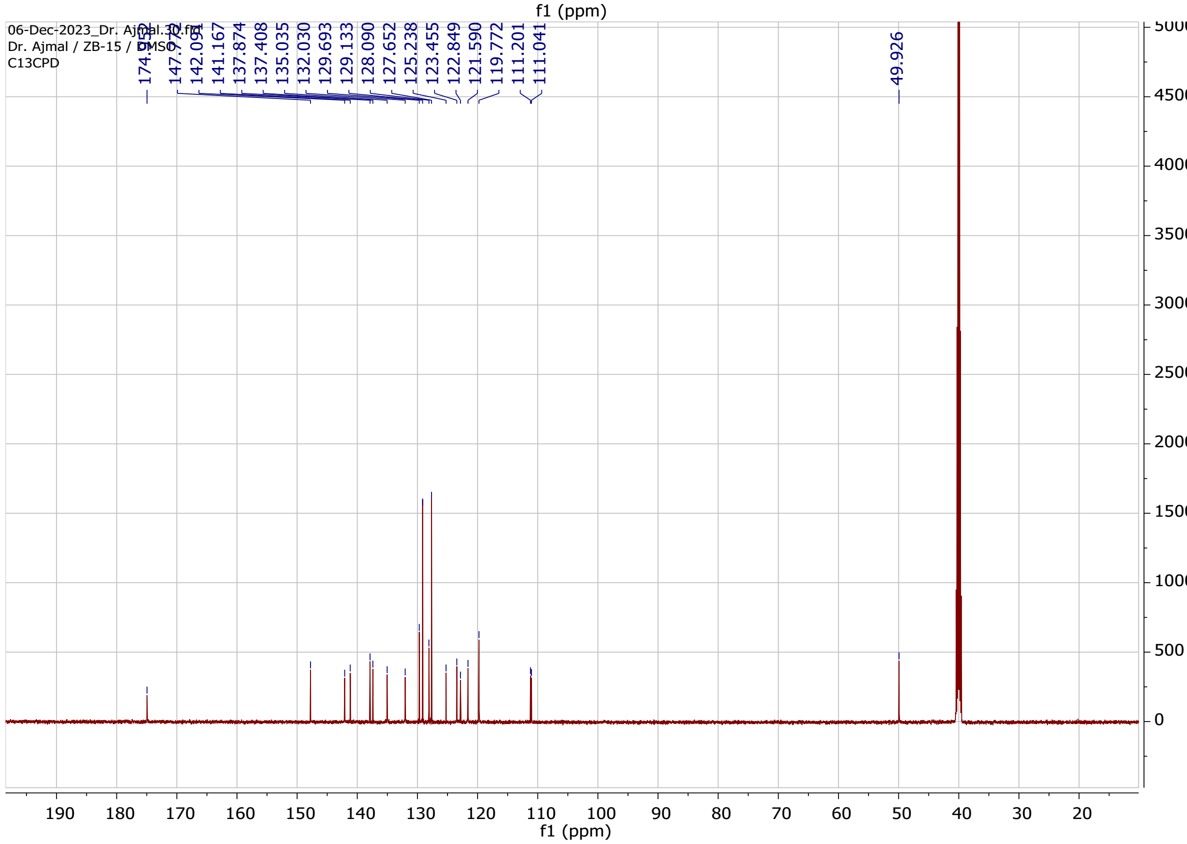
1H NMR and 13C NMR Spectra of 5n



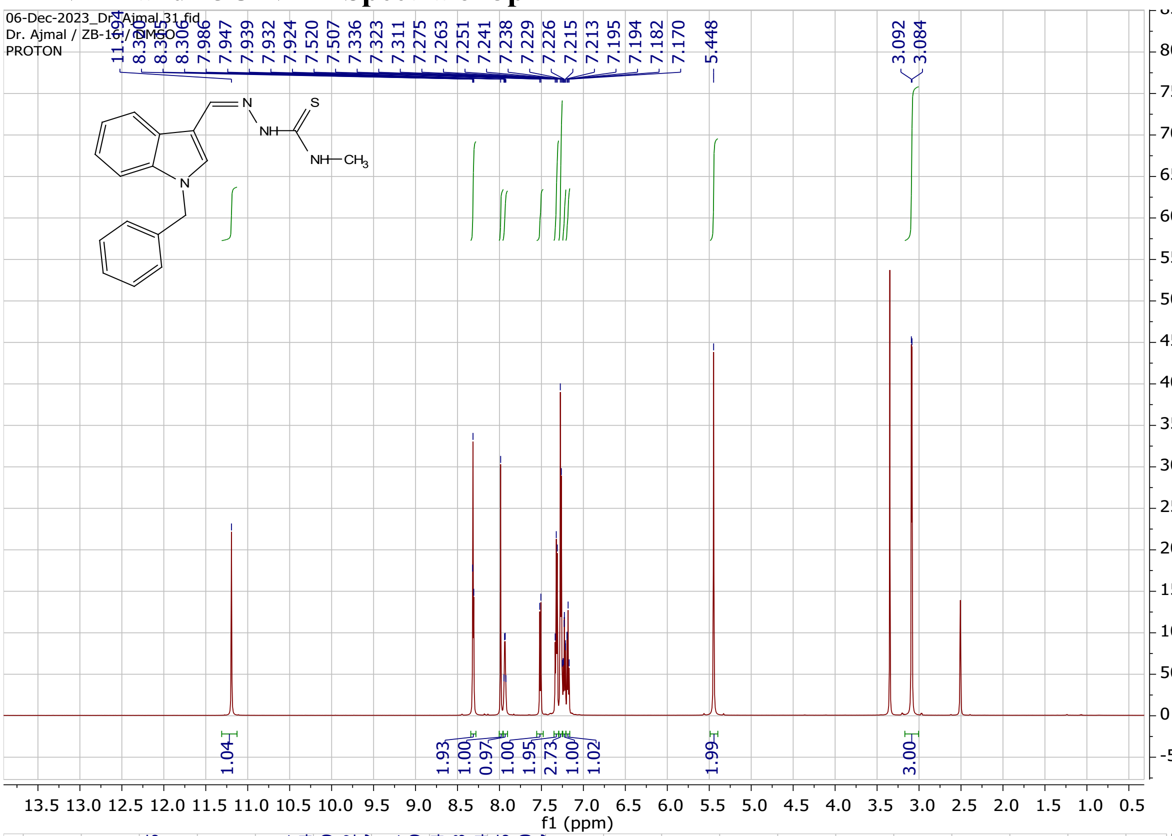


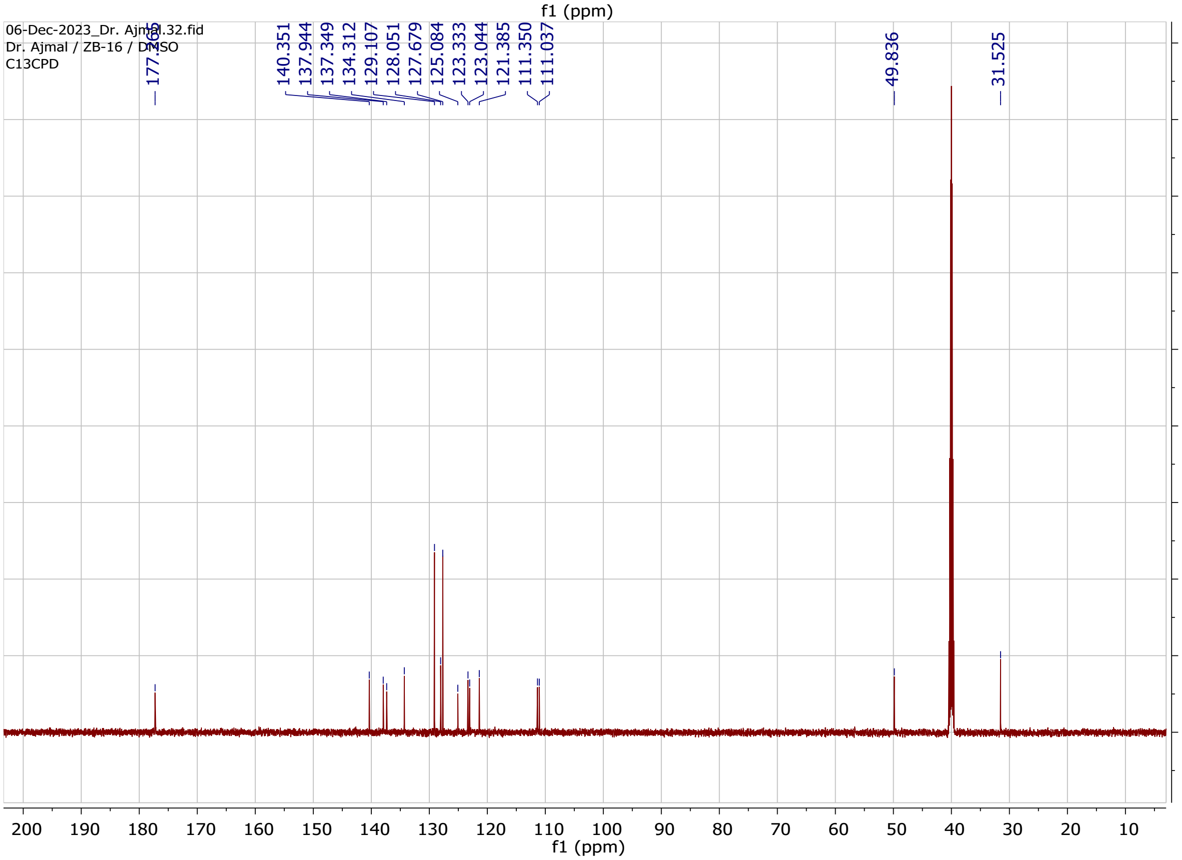
^{1}H NMR and ^{13}C NMR Spectra of 5o



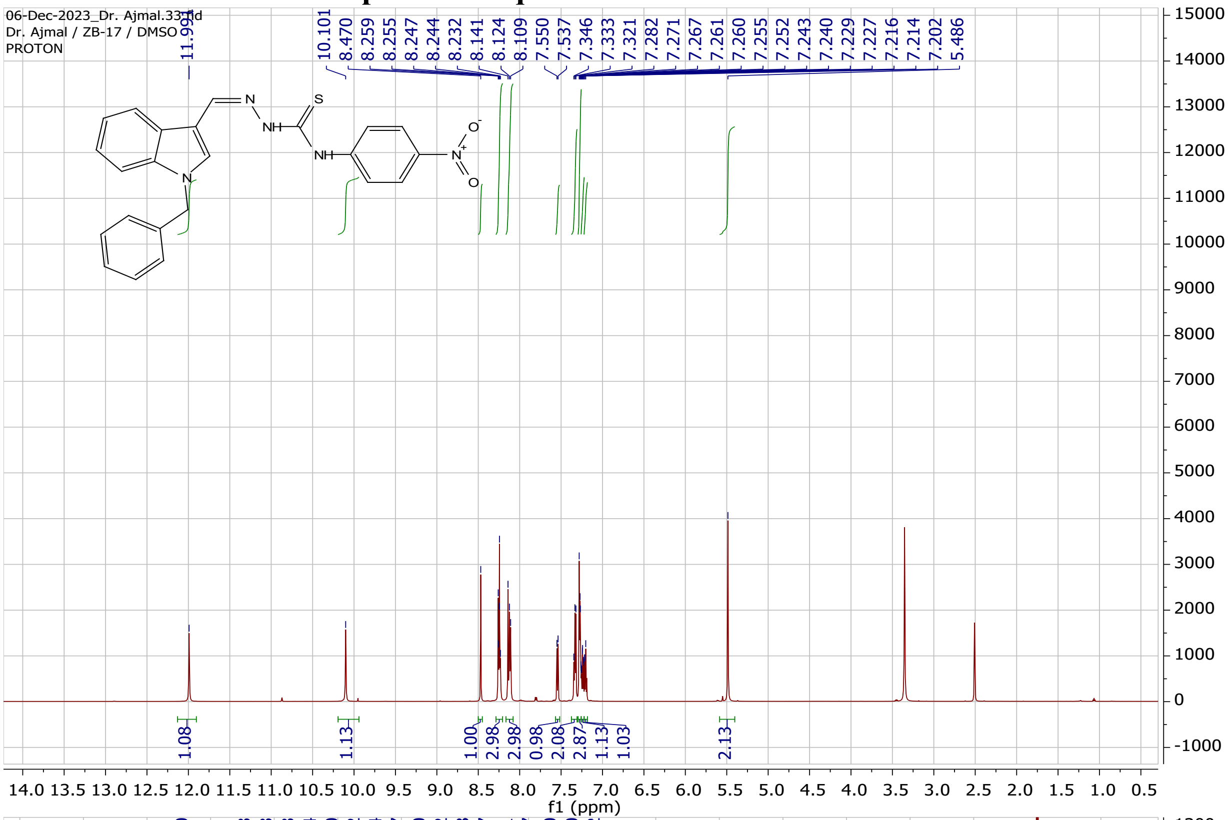


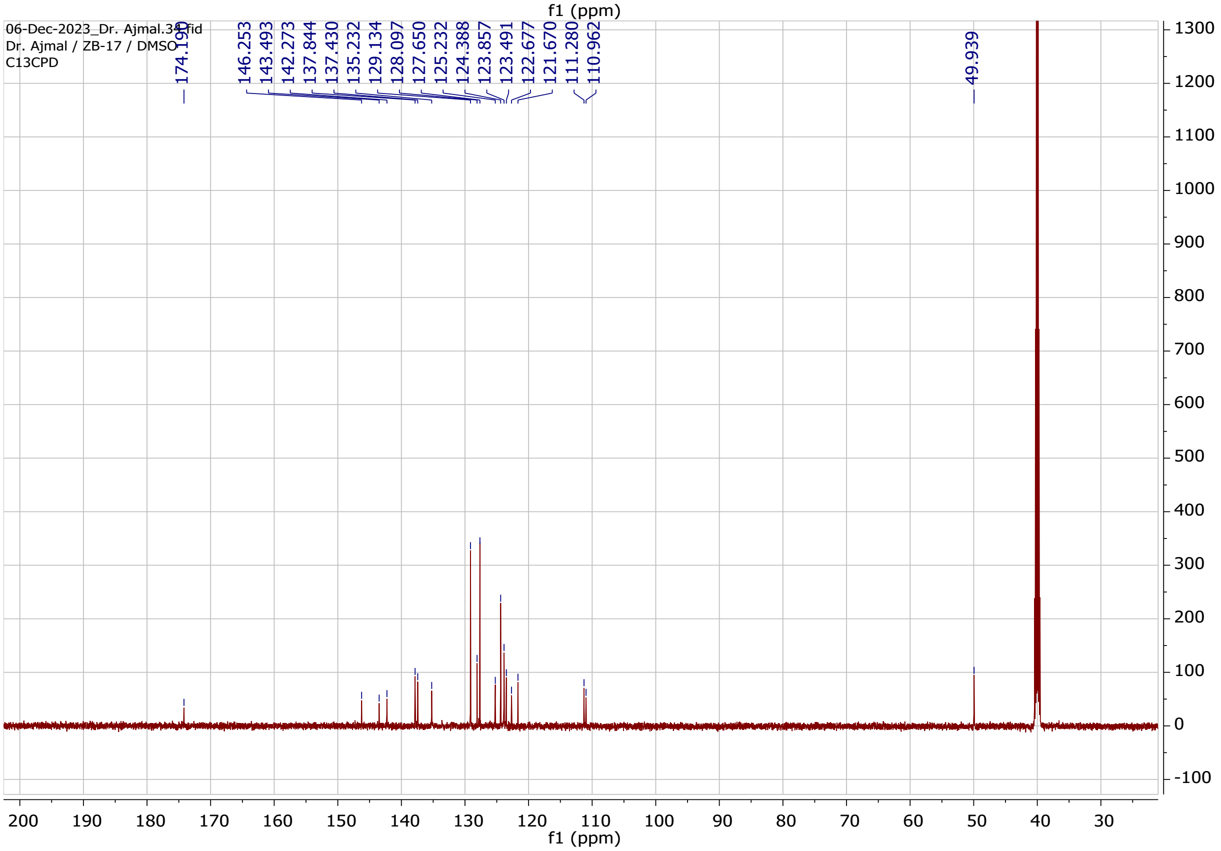
1H NMR and 13C NMR Spectra of 5p



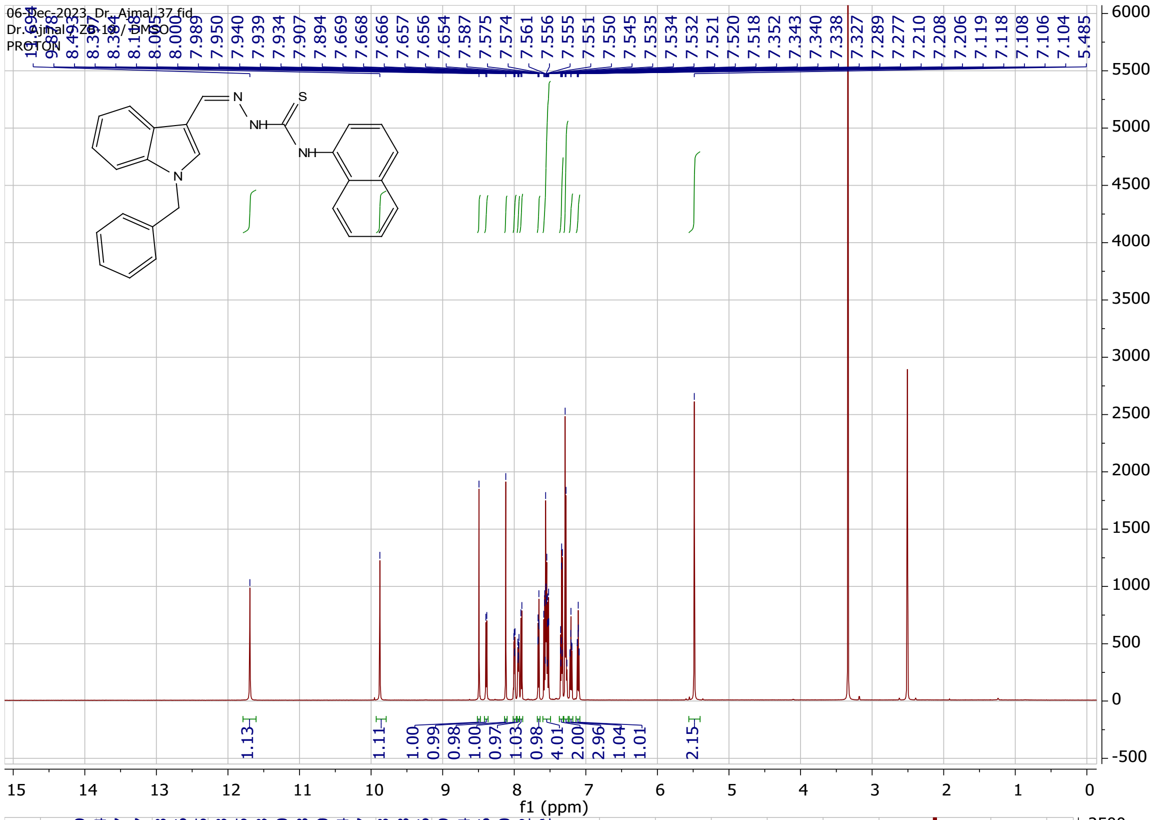


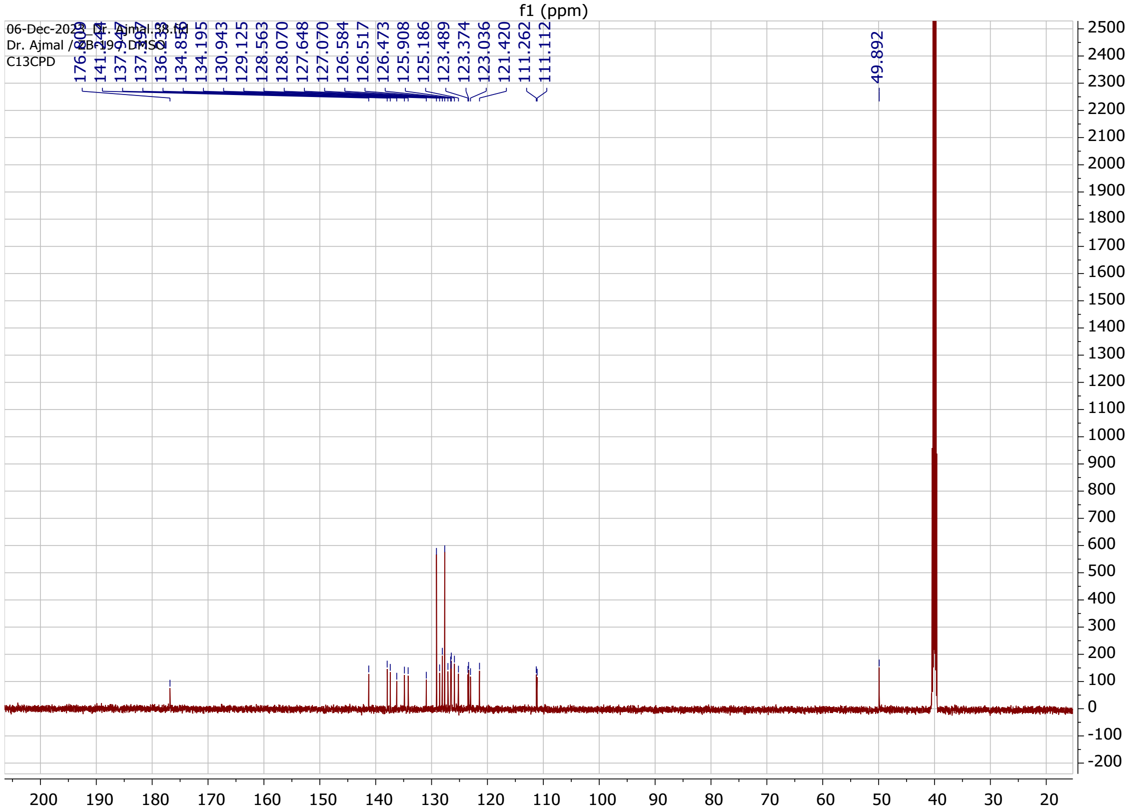
1H NMR and 13C NMR Spectra of 5q



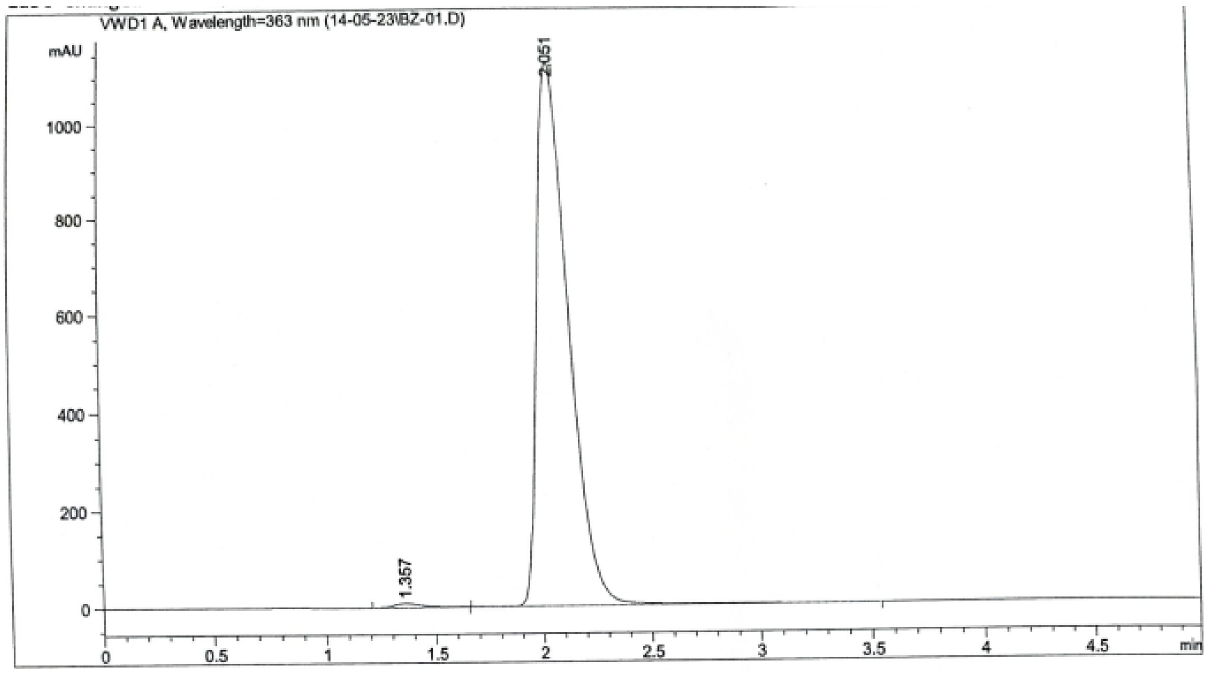


1H NMR and 13C NMR Spectra of 5r

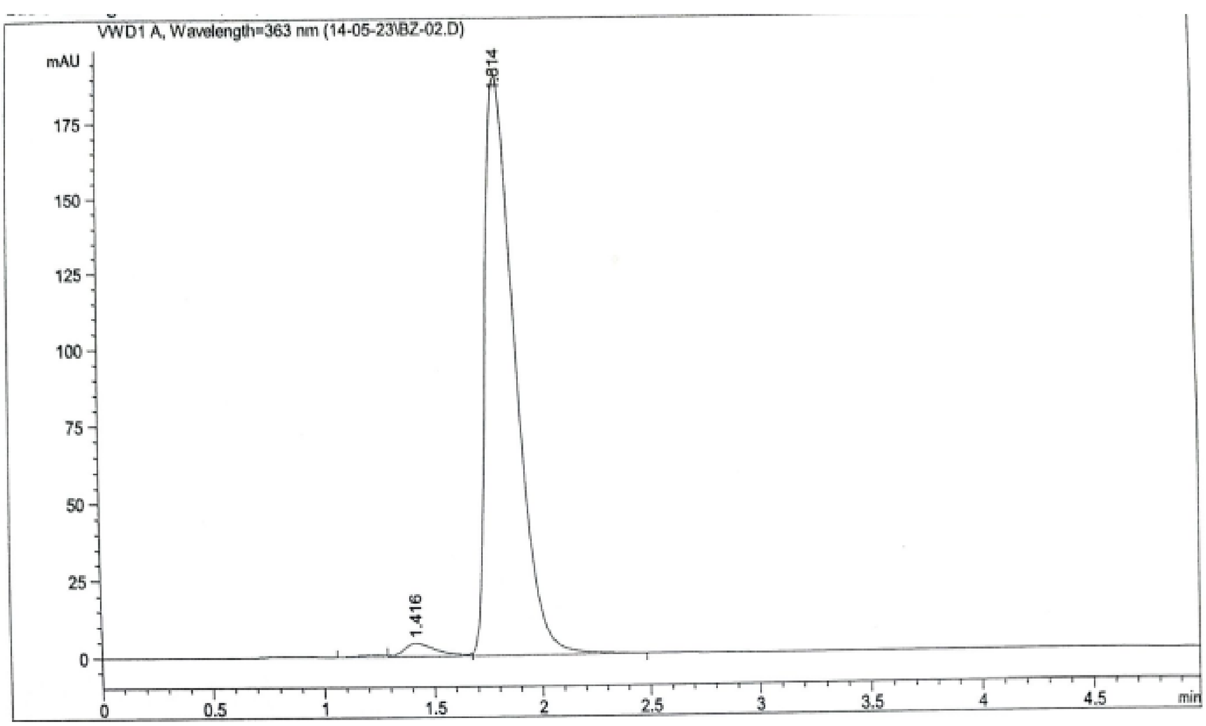




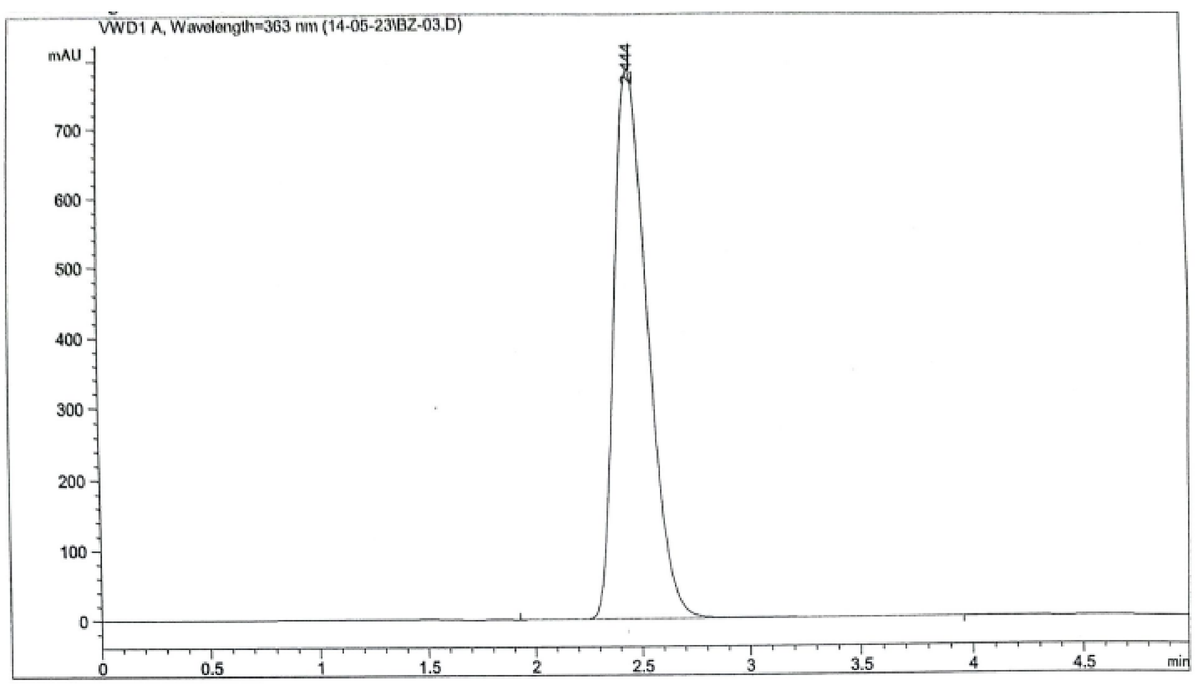
HPLC chromatogram of 5a



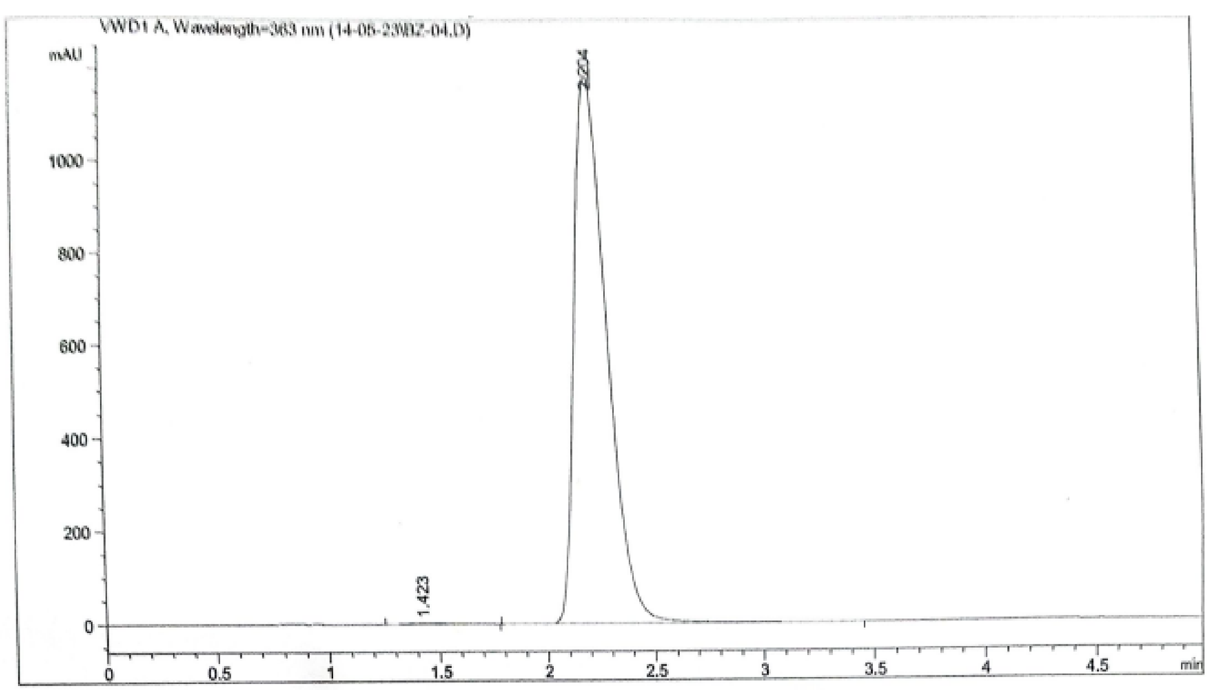
HPLC chromatogram of 5b



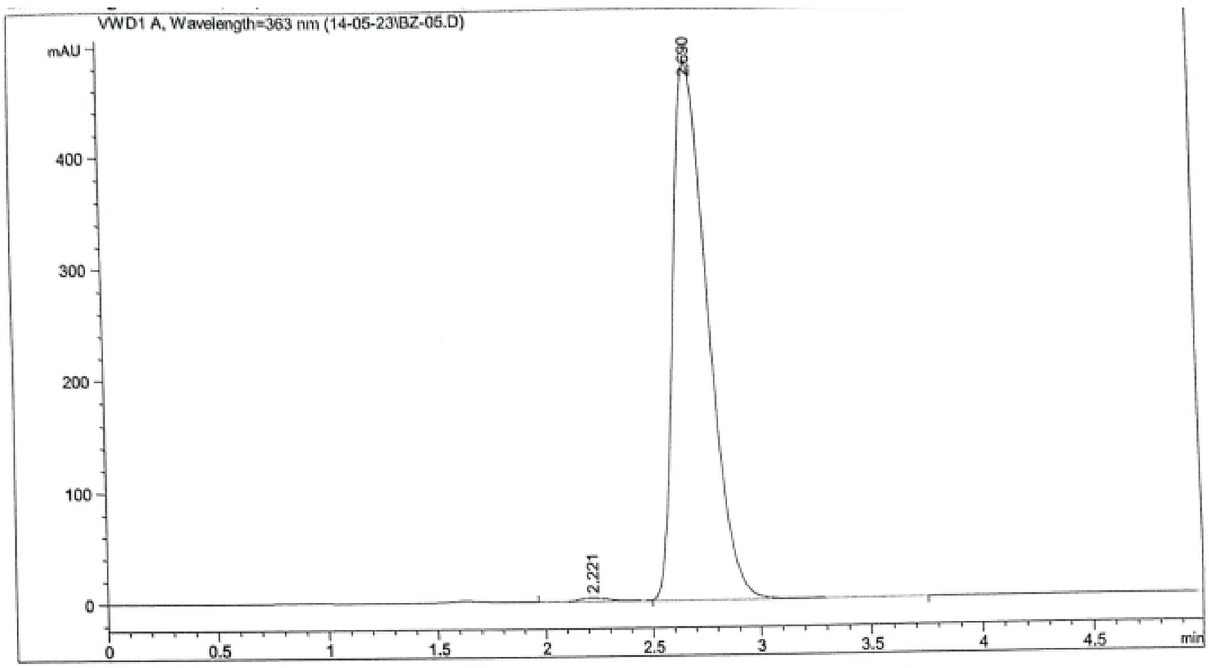
HPLC chromatogram of 5c



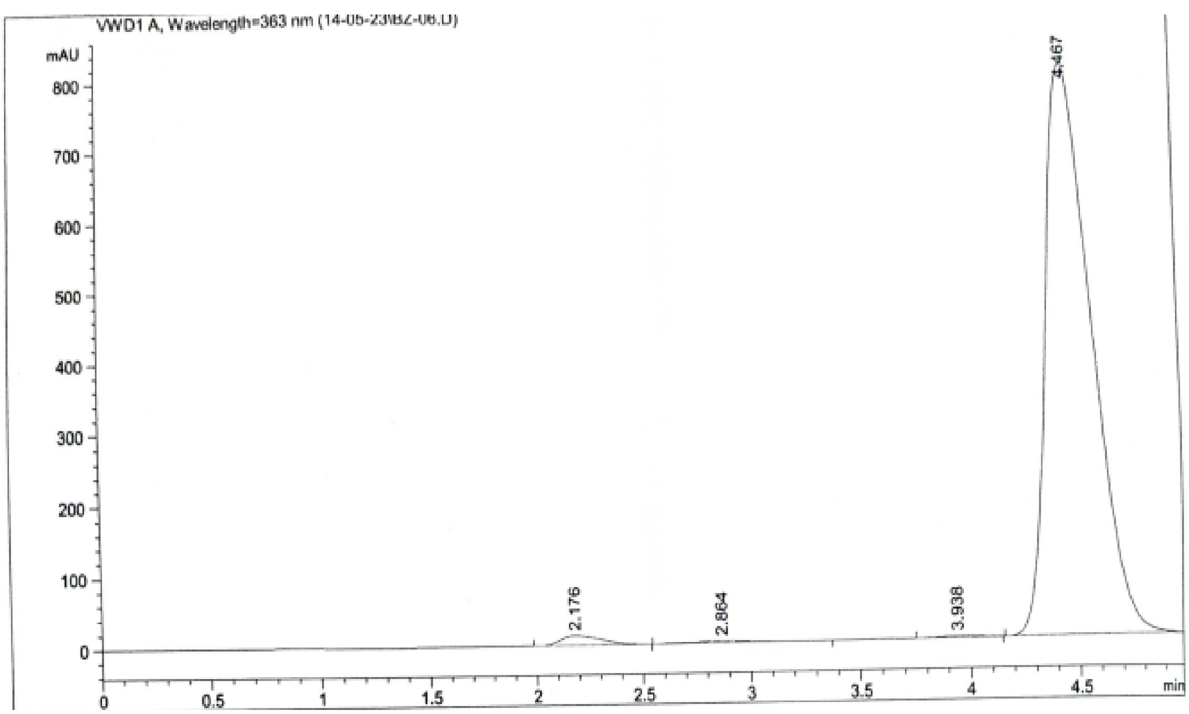
HPLC chromatogram of 5d



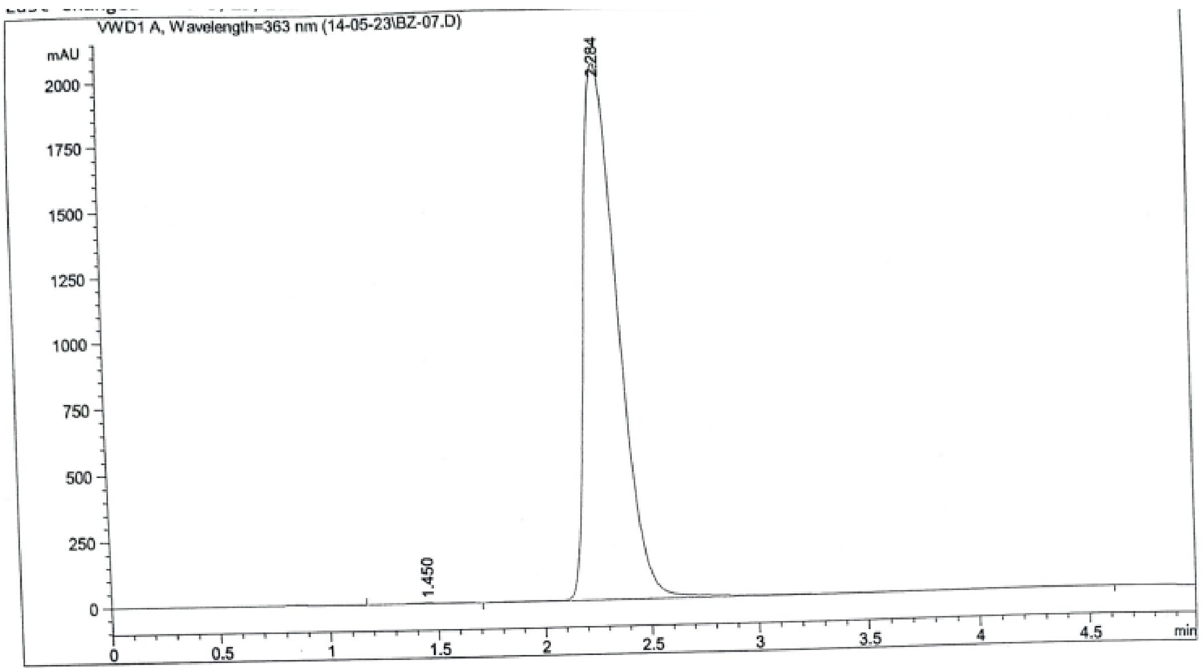
HPLC chromatogram of 5e



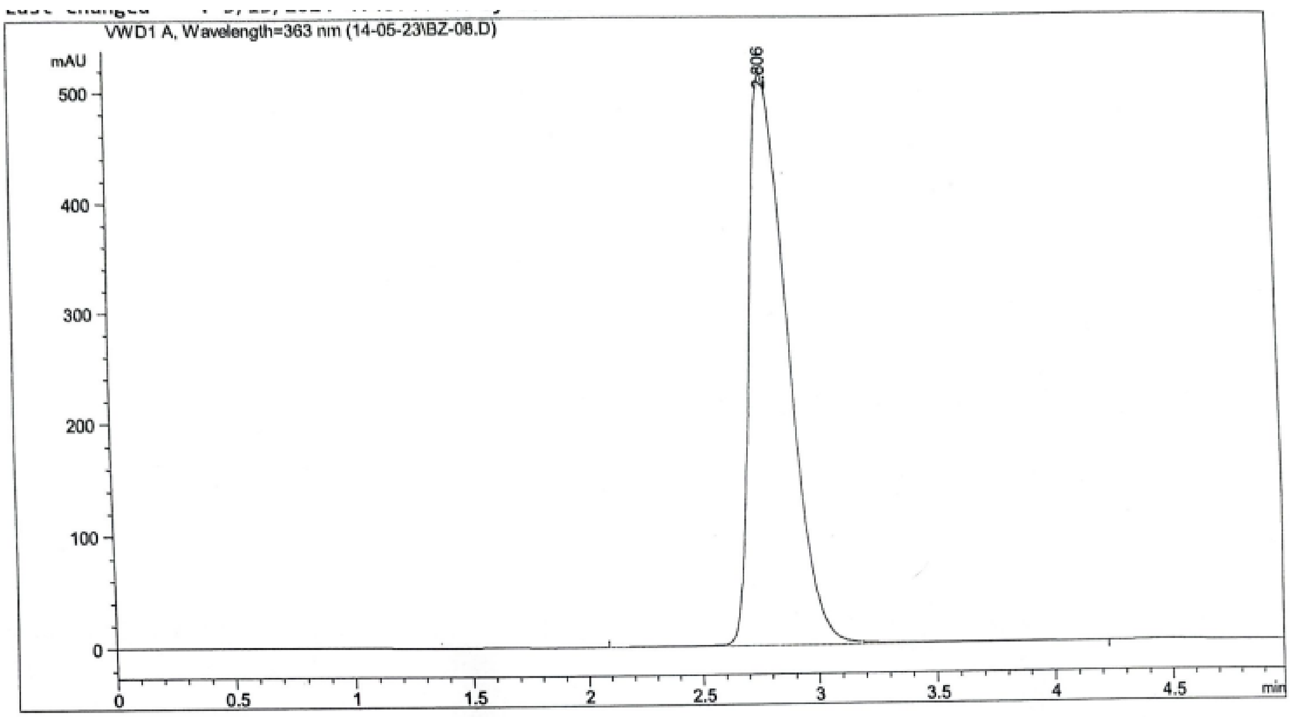
HPLC chromatogram of 5f



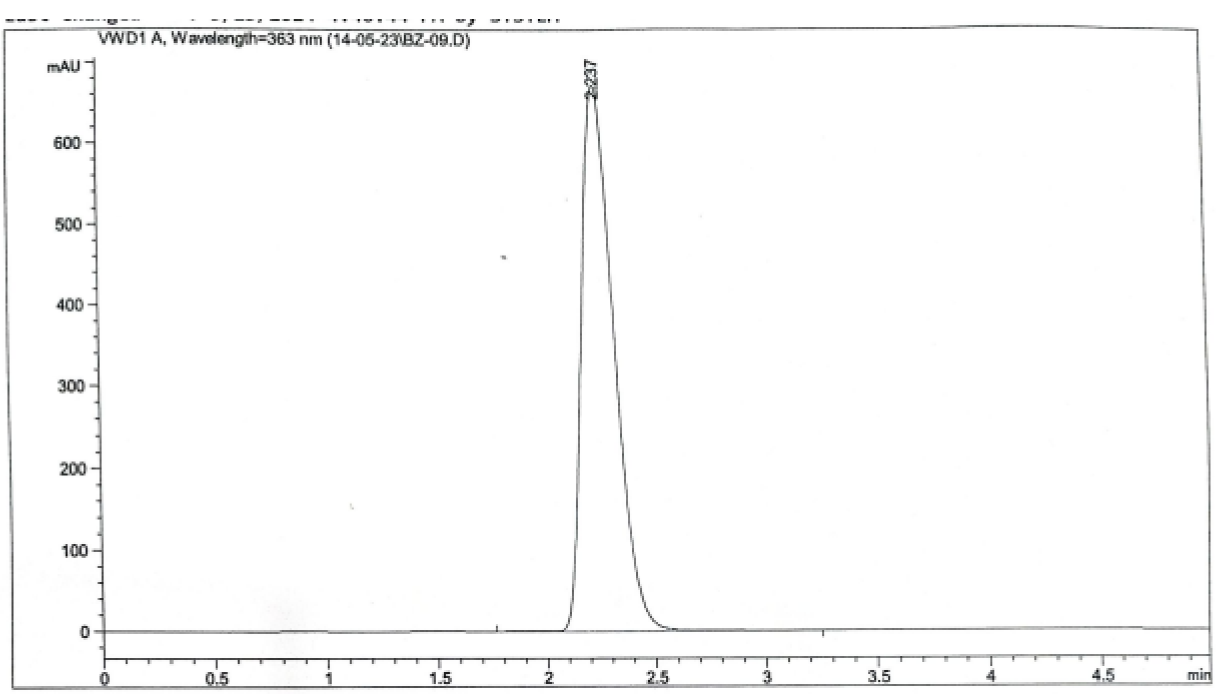
HPLC chromatogram of 5g



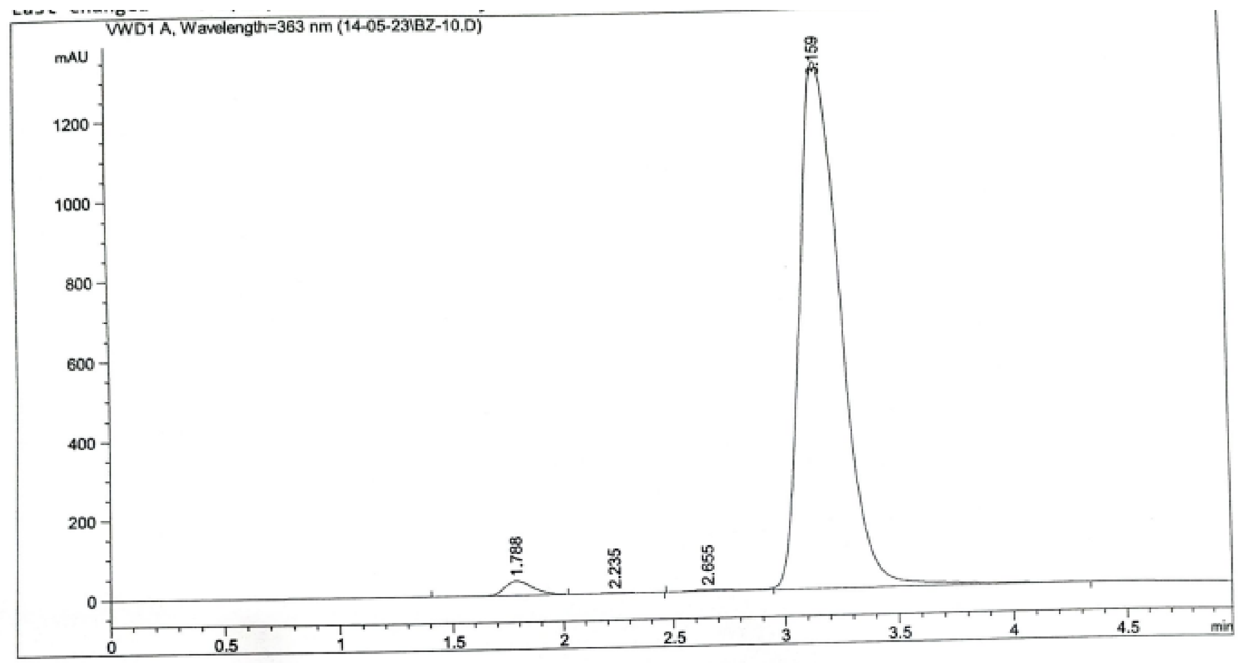
HPLC chromatogram of 5h



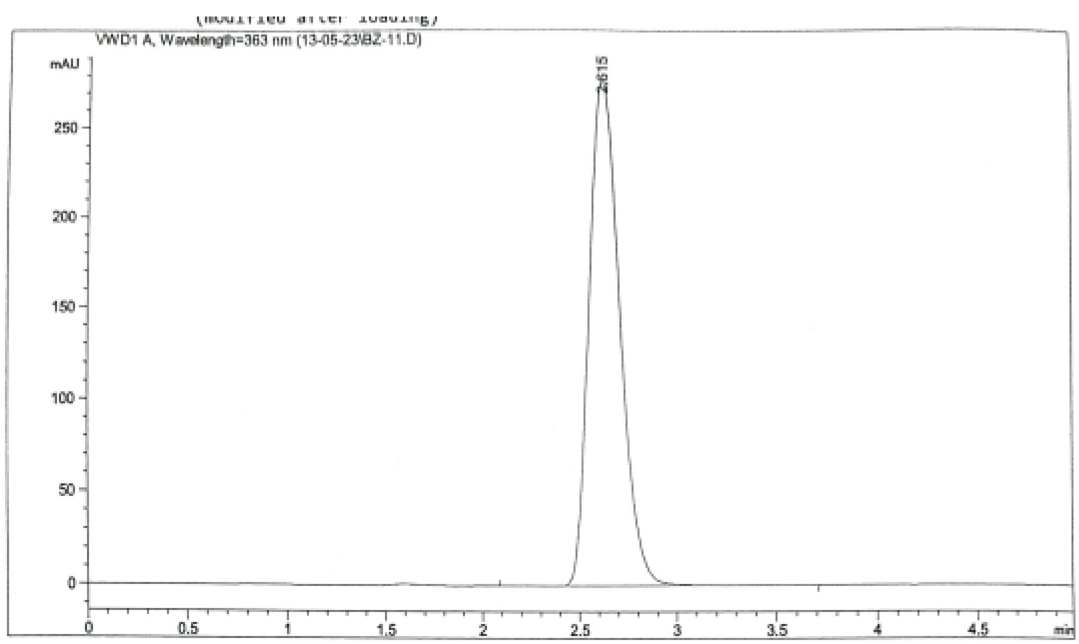
HPLC chromatogram of 5i



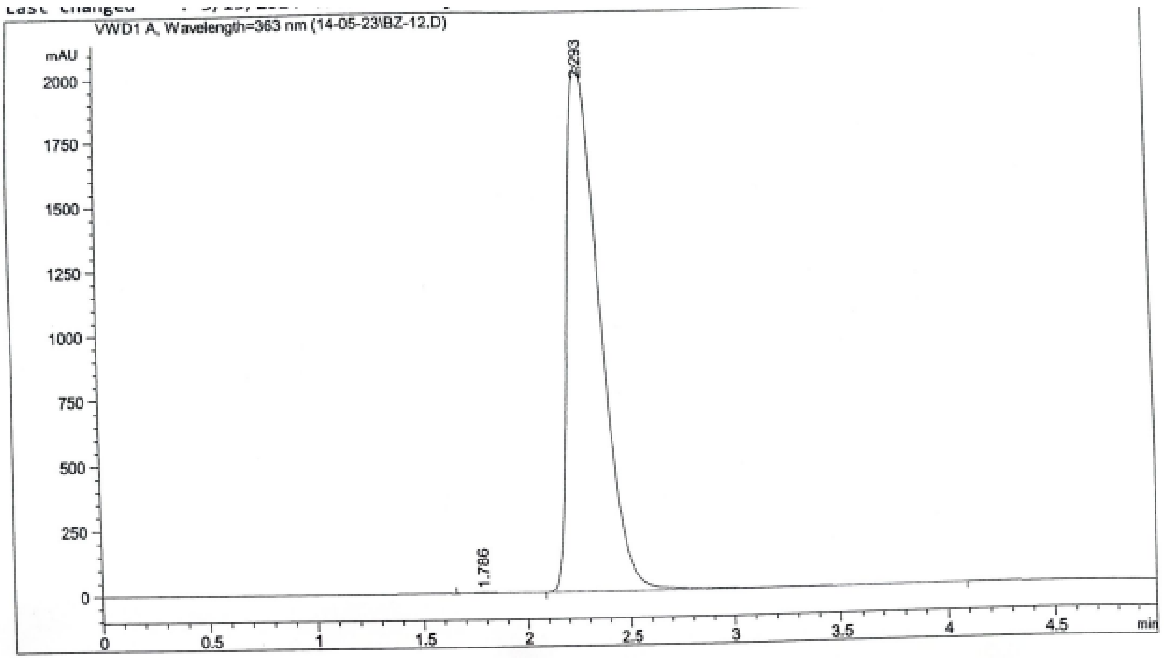
HPLC chromatogram of 5j



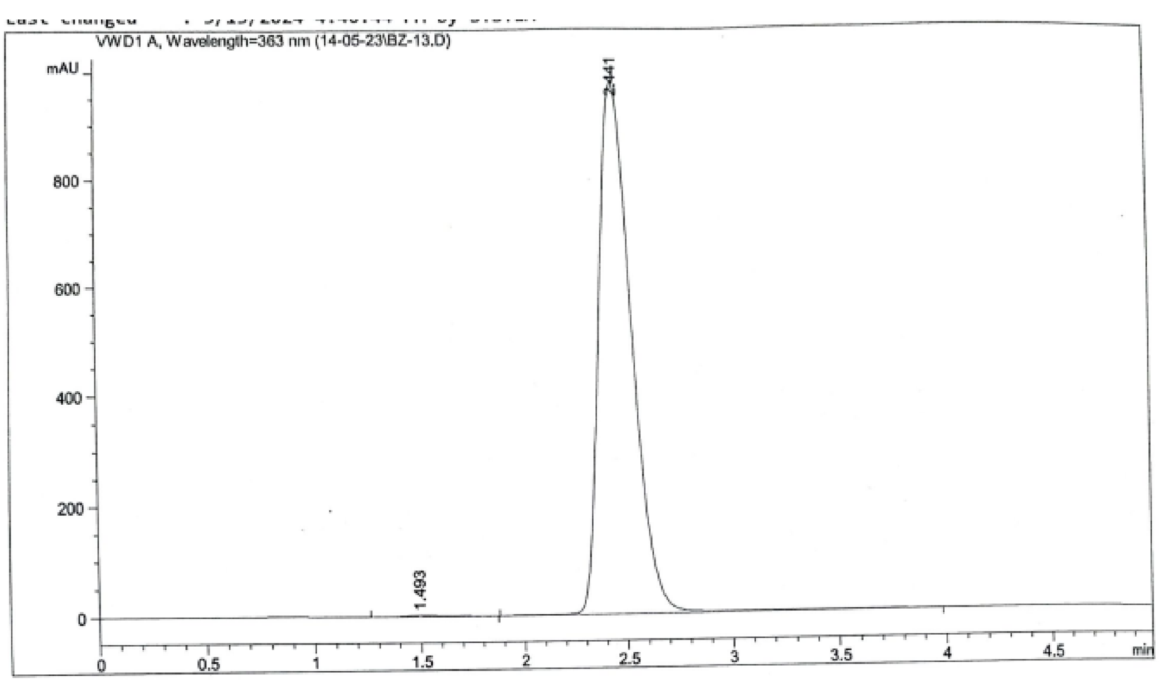
HPLC chromatogram of 5k



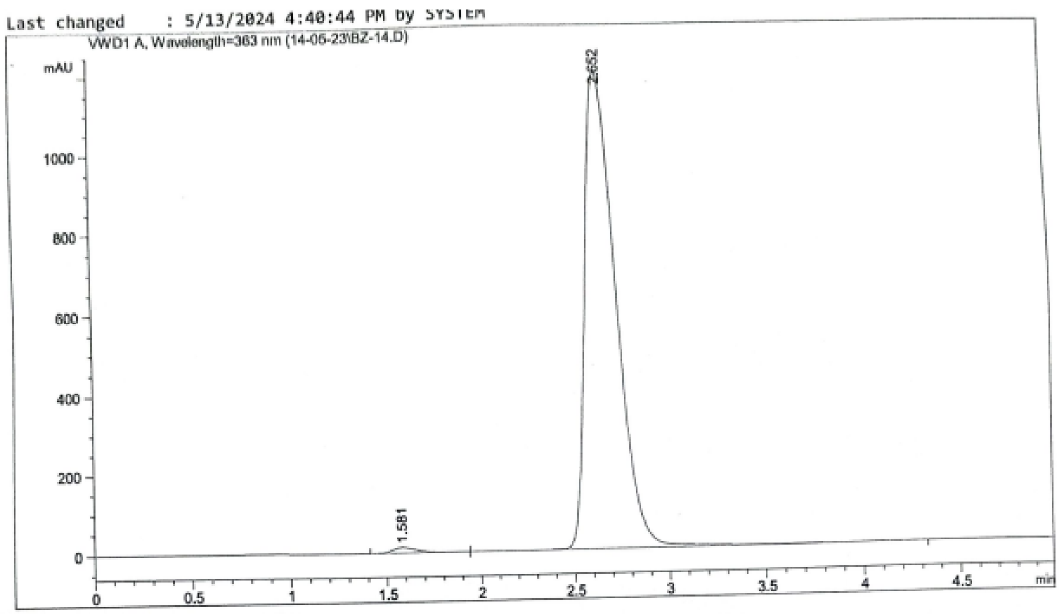
HPLC chromatogram of 5l



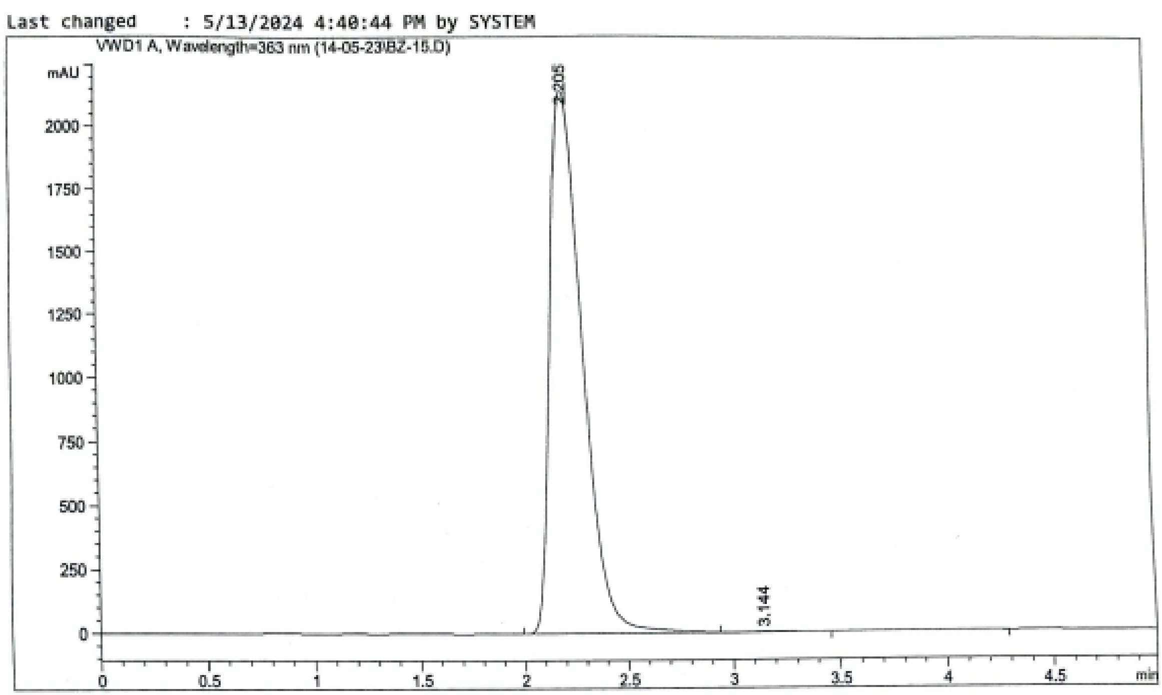
HPLC chromatogram of 5m



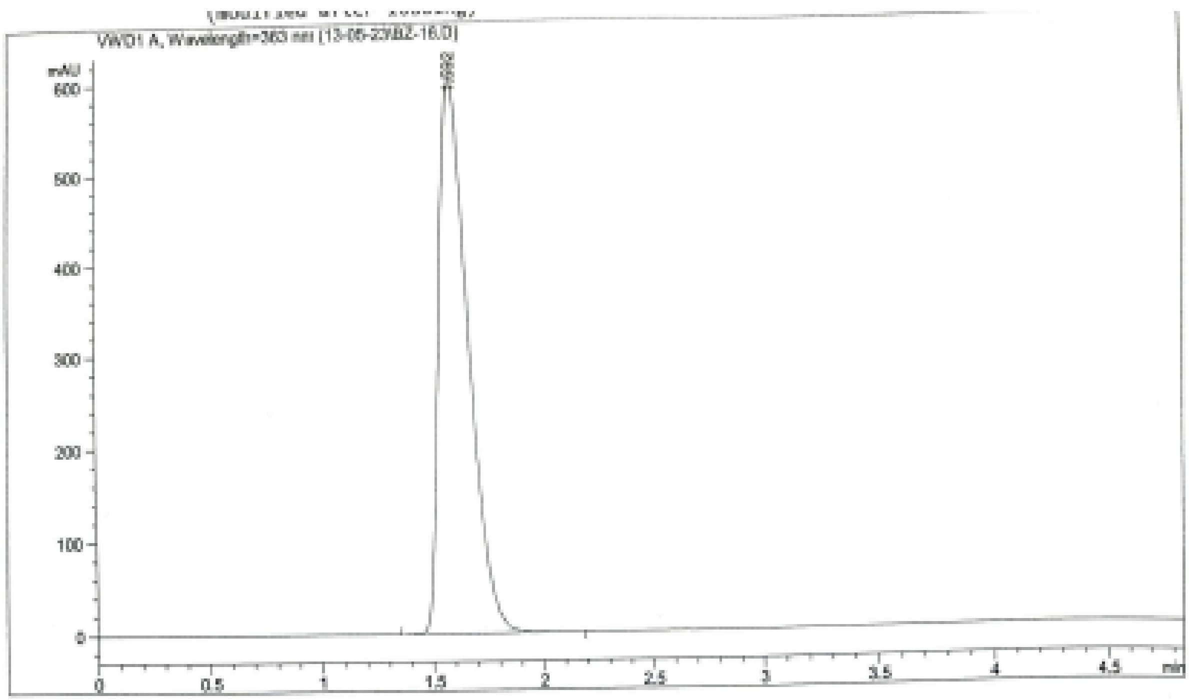
HPLC chromatogram of 5n



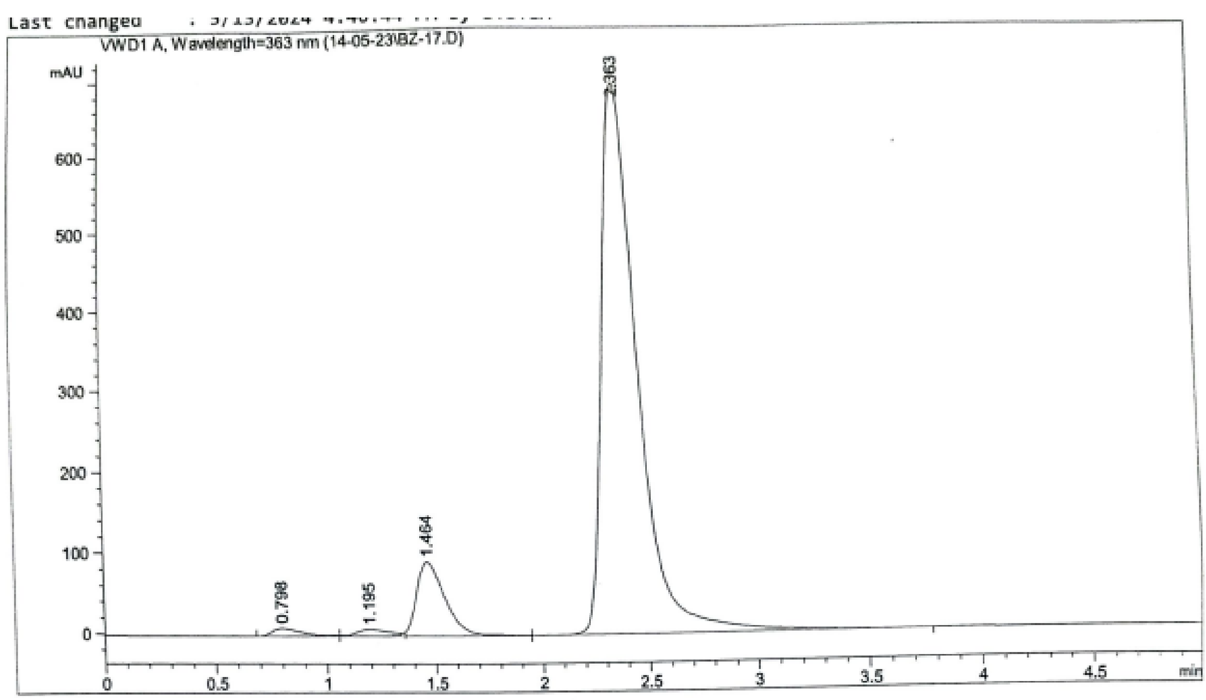
HPLC chromatogram of 5o



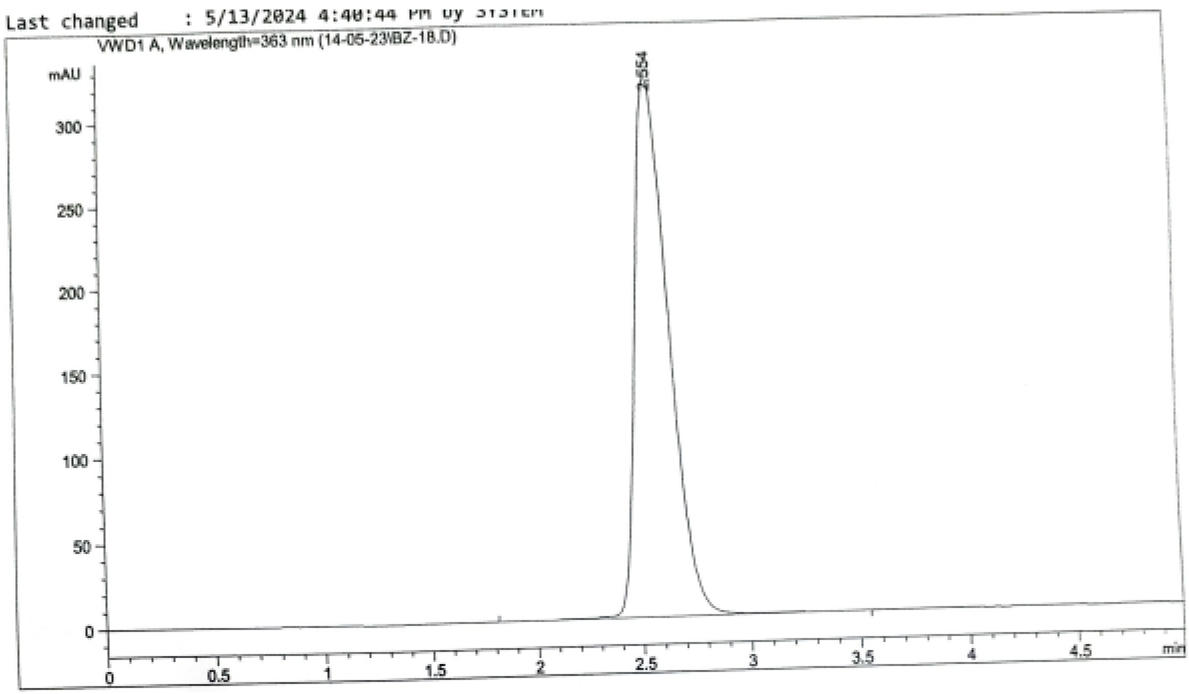
HPLC chromatogram of 5p



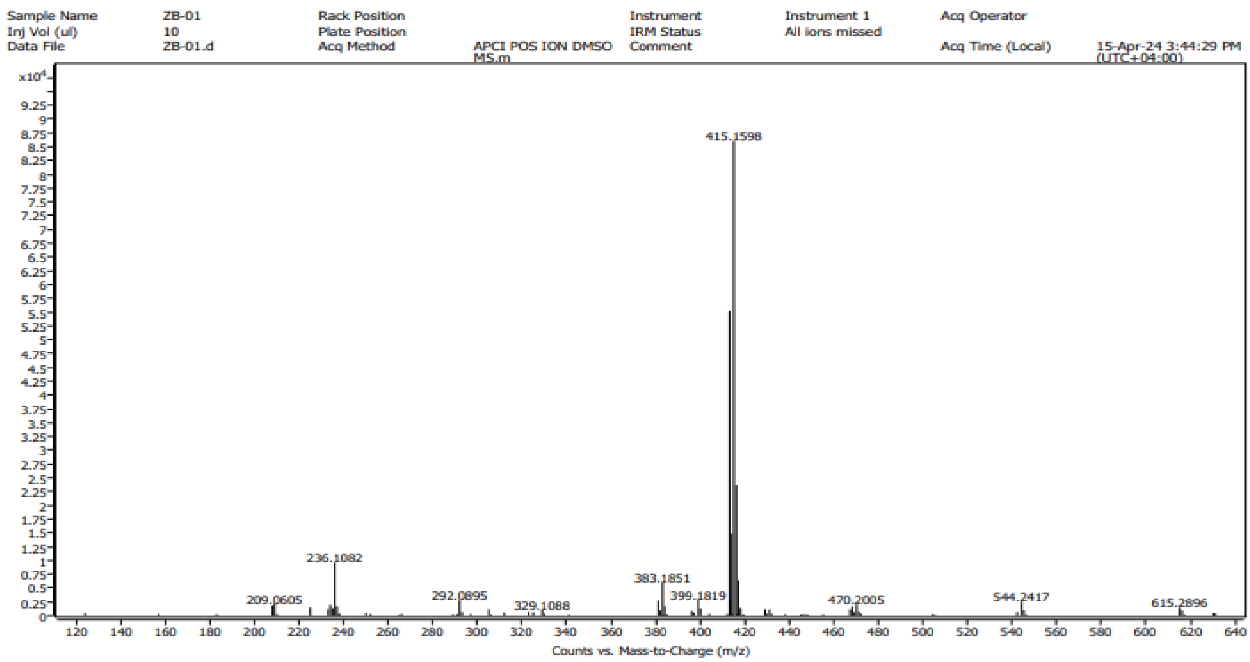
HPLC chromatogram of 5q



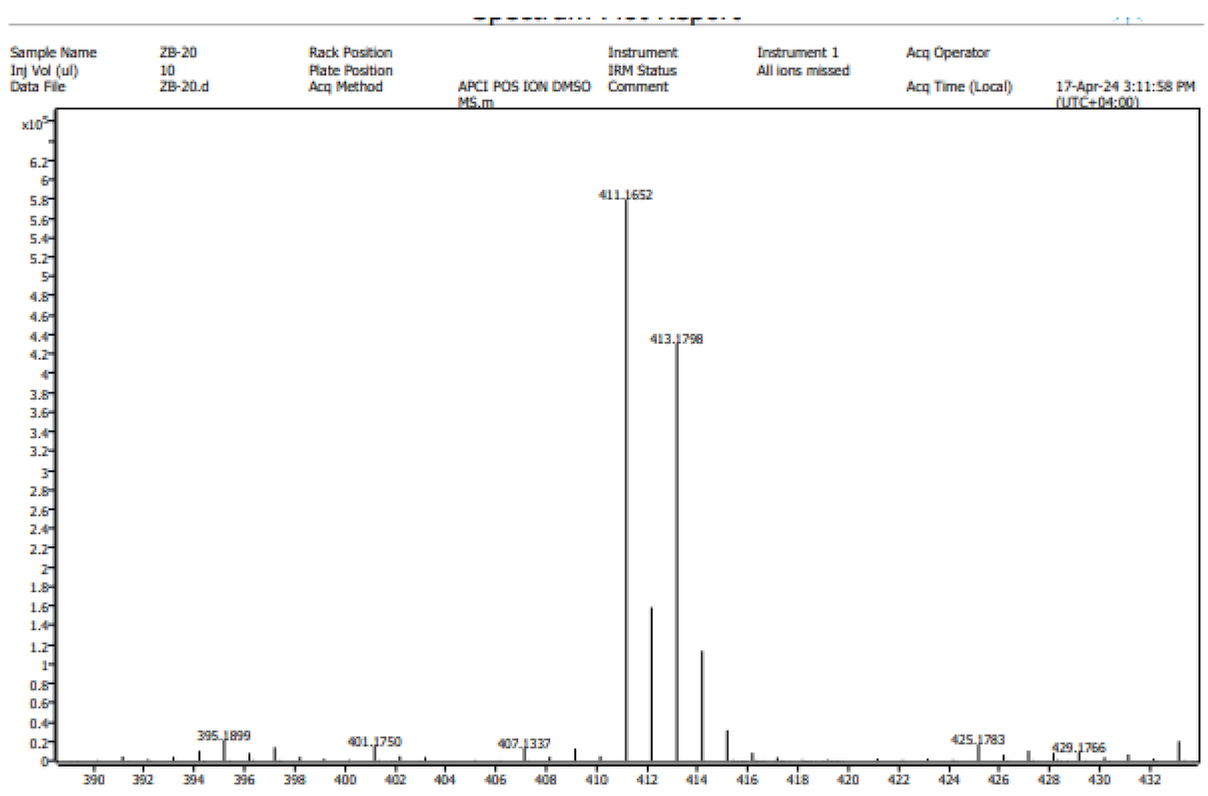
HPLC chromatogram of 5r



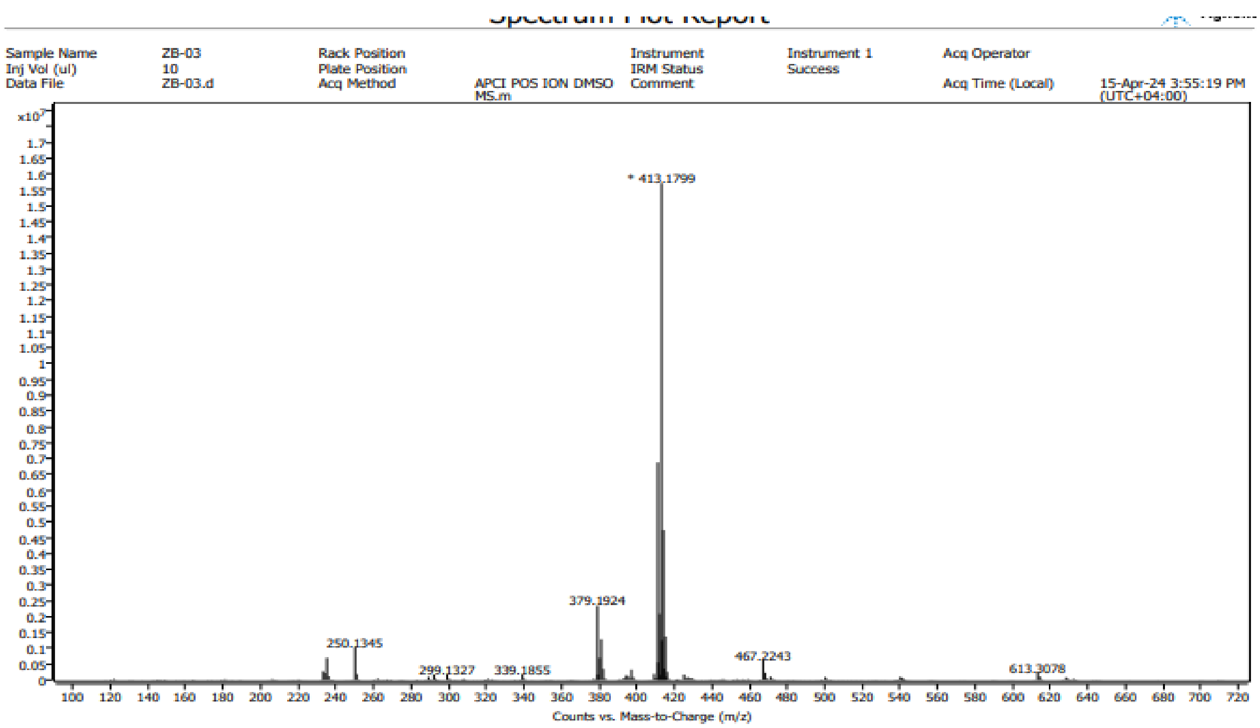
Mass spectrum of 5a



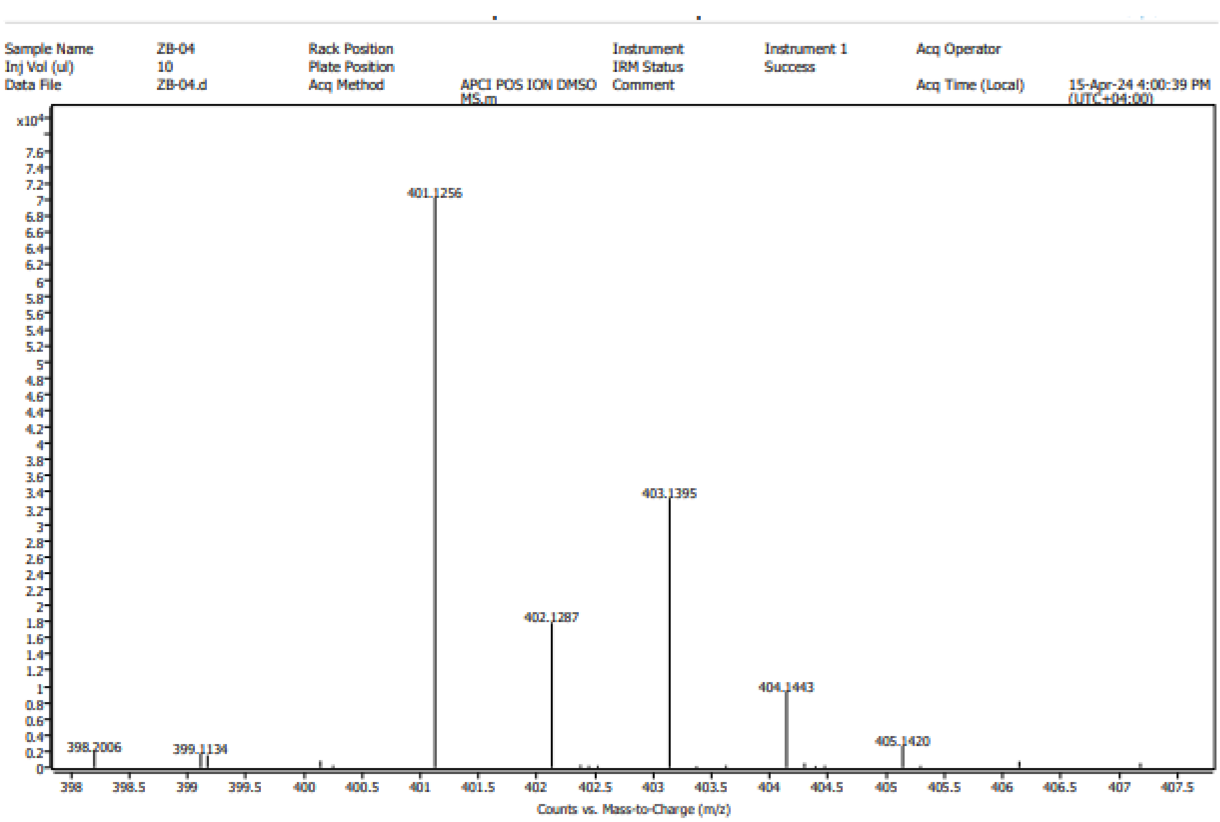
Mass spectrum of 5b



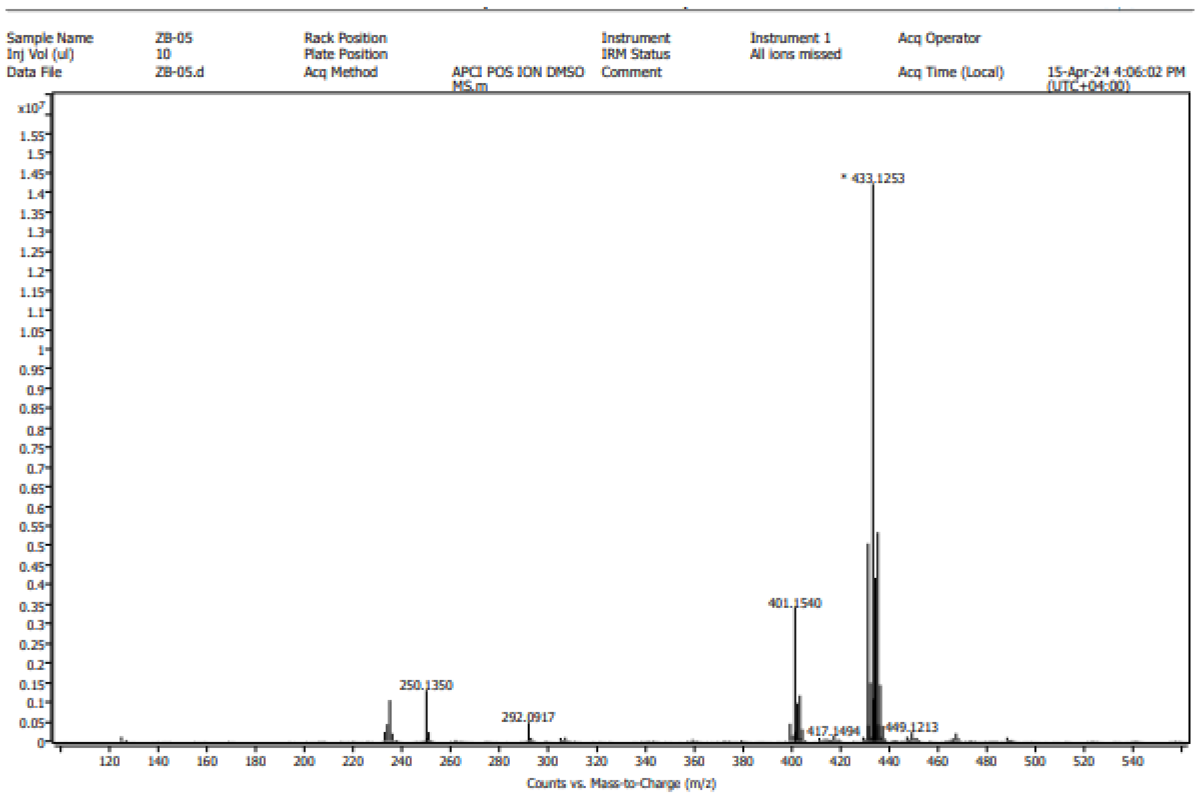
Mass spectrum of 5c



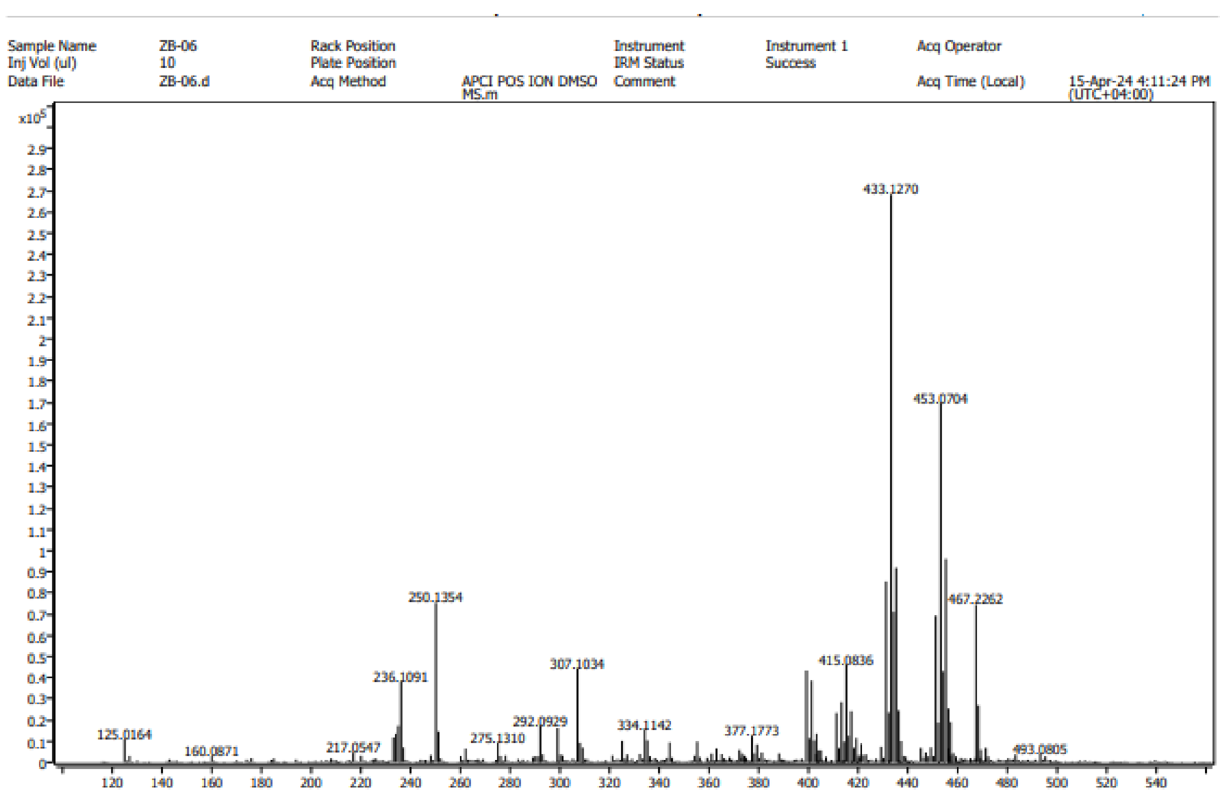
Mass spectrum of 5d



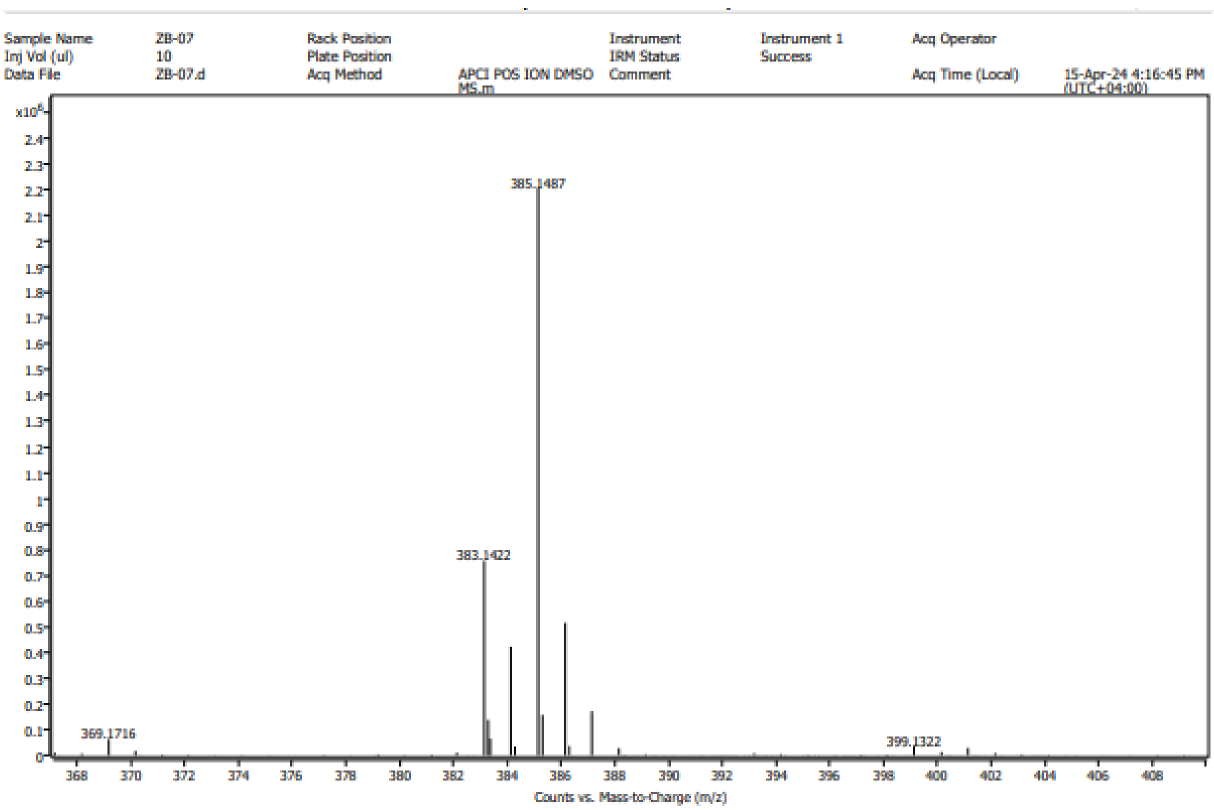
Mass spectrum of 5e



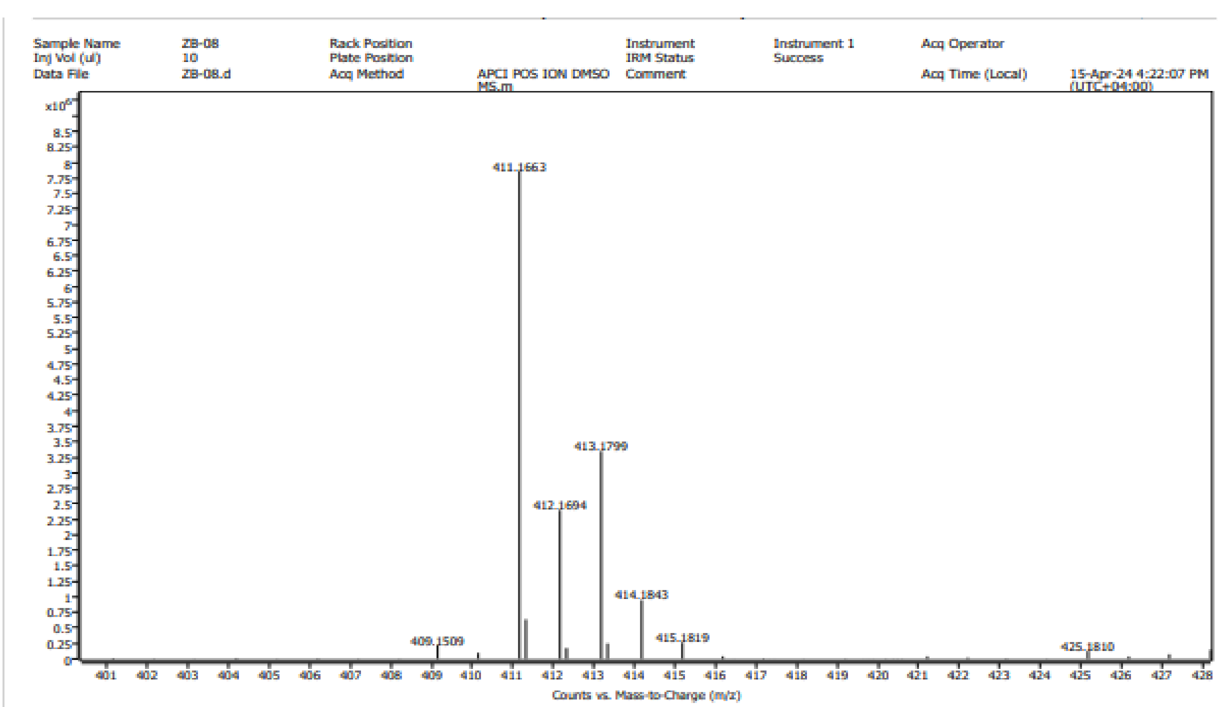
Mass spectrum of 5f



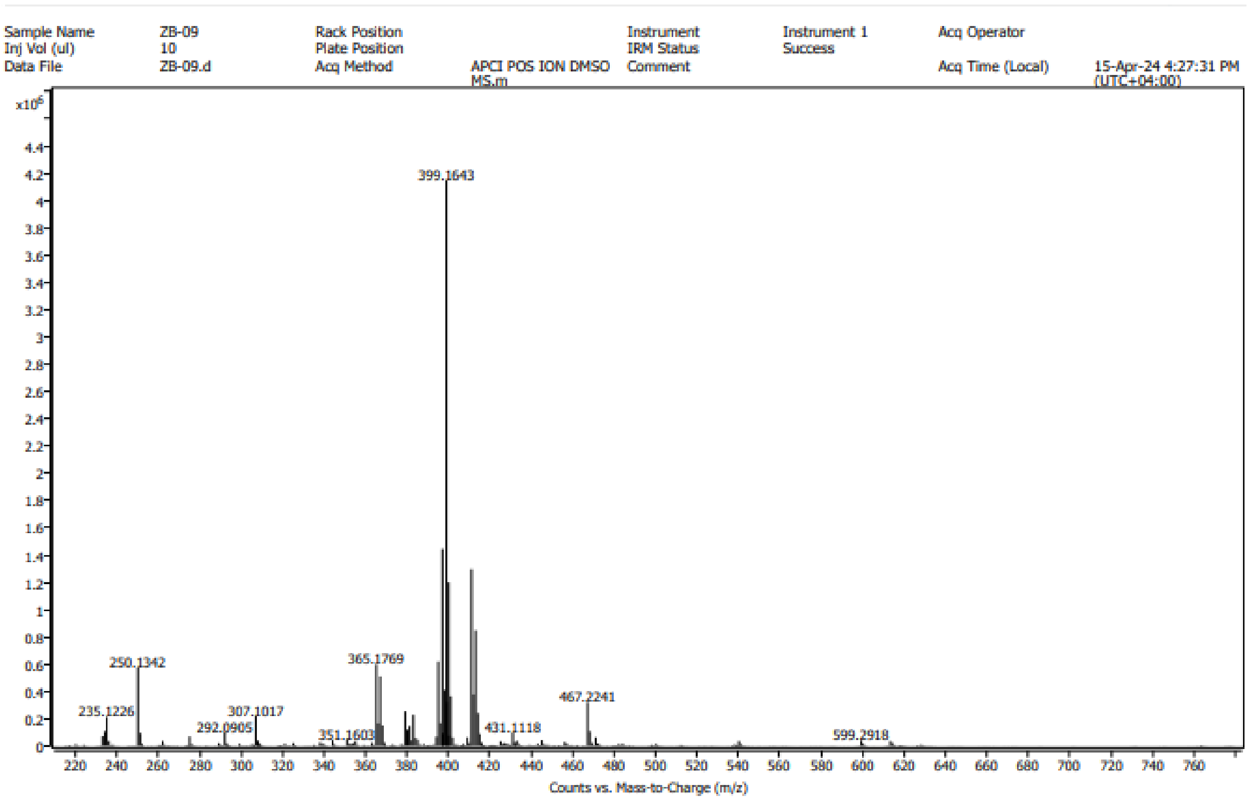
Mass spectrum of 5g



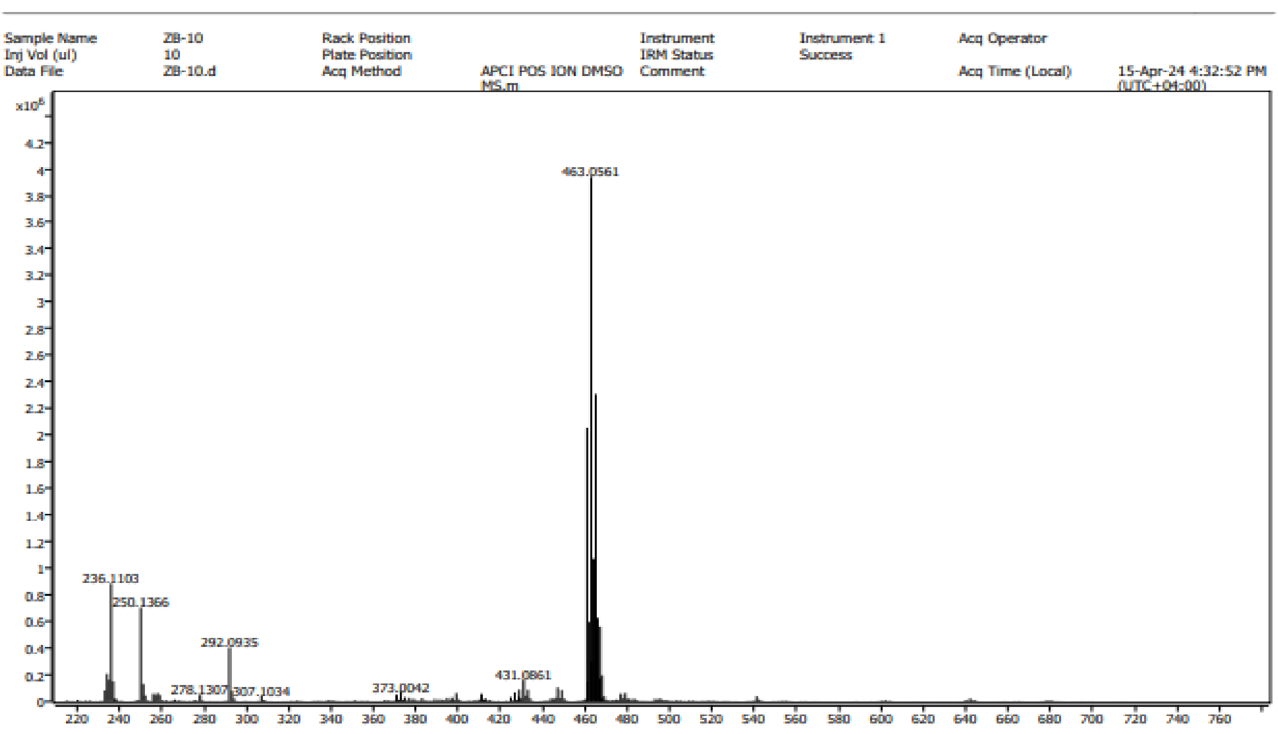
Mass spectrum of 5h



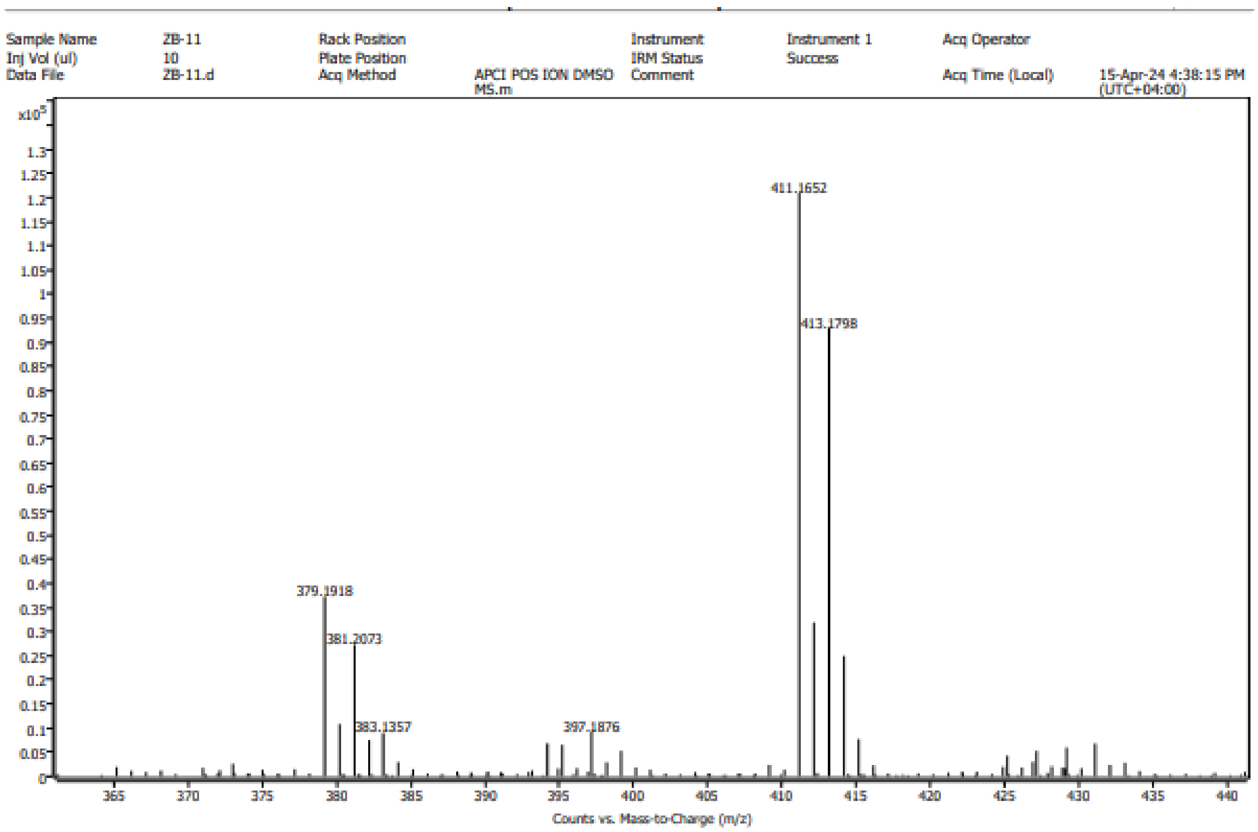
Mass spectrum of 5i



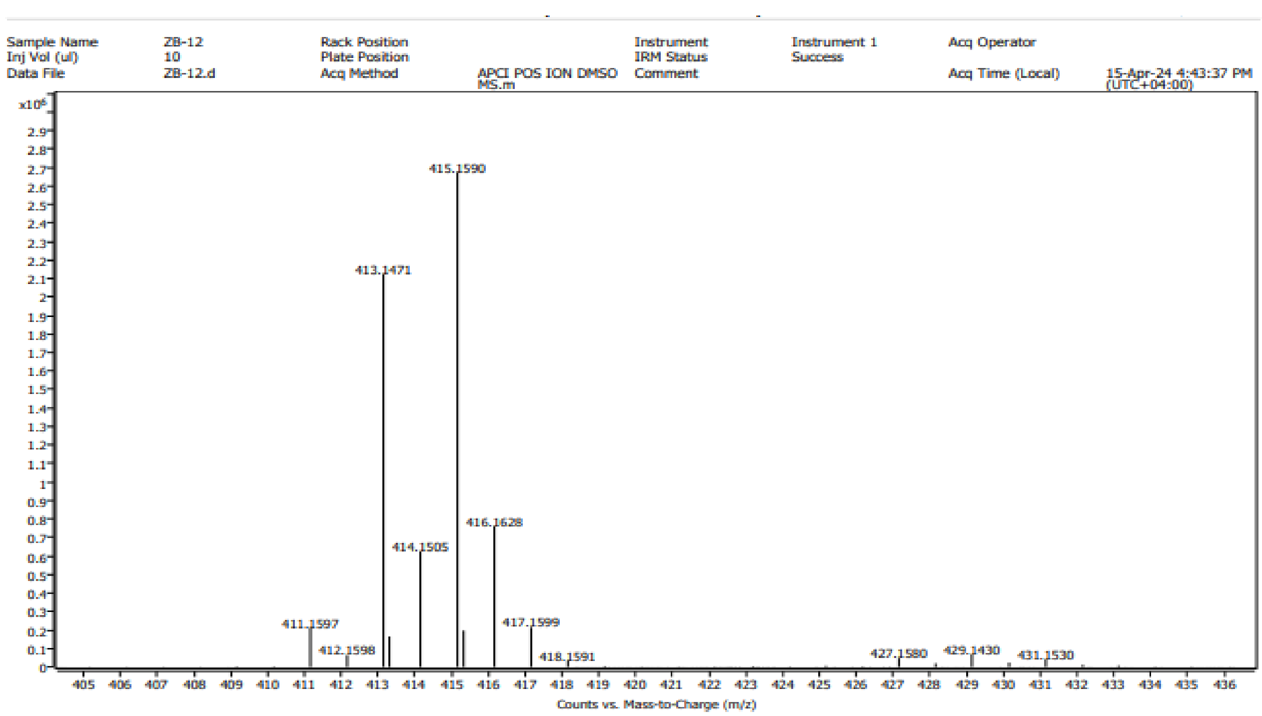
Mass spectrum of 5j



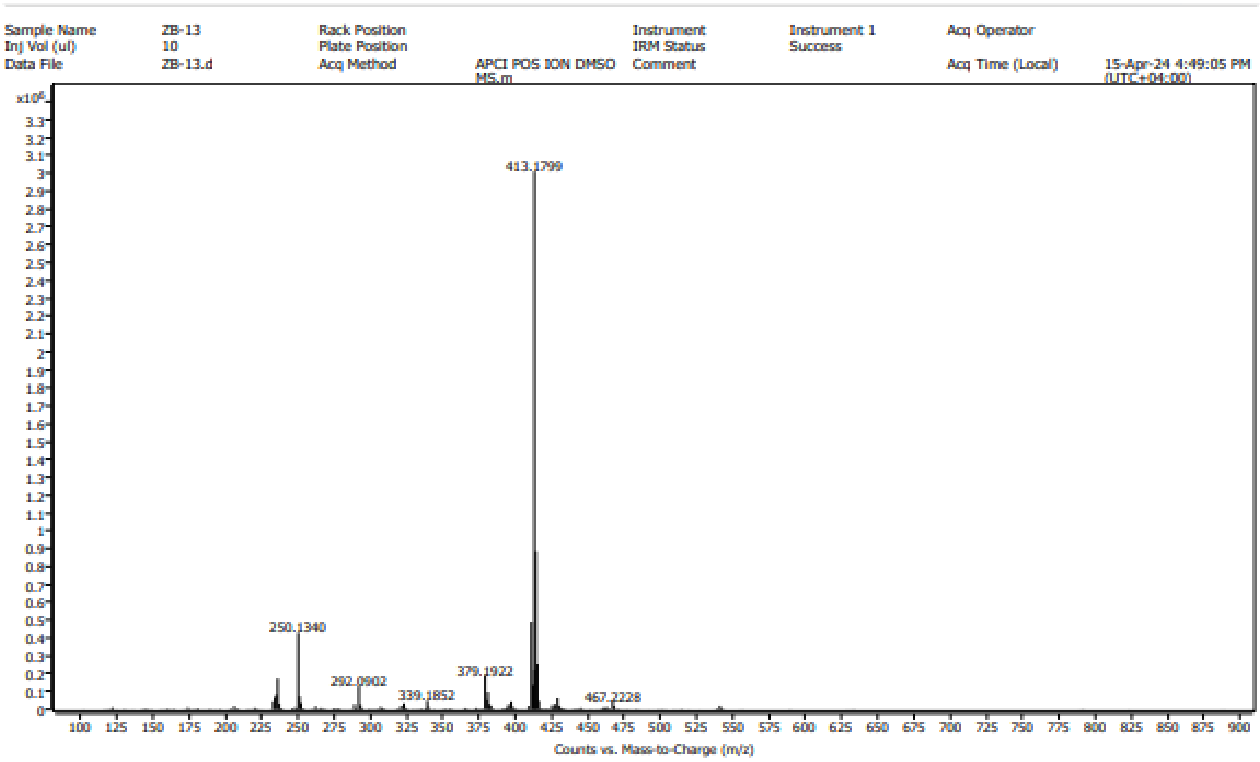
Mass spectrum of 5k



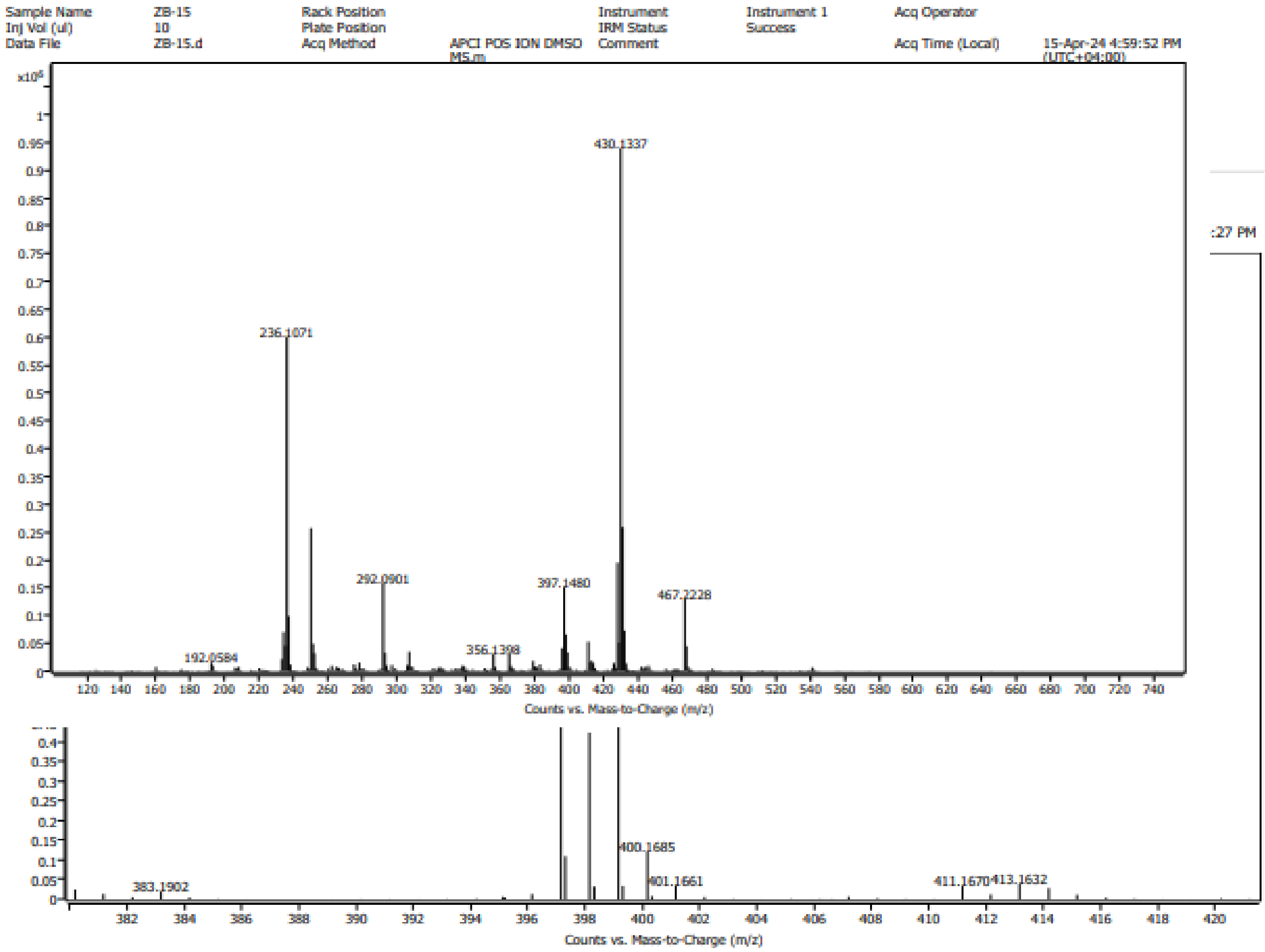
Mass spectrum of 5l



Mass spectrum of 5m

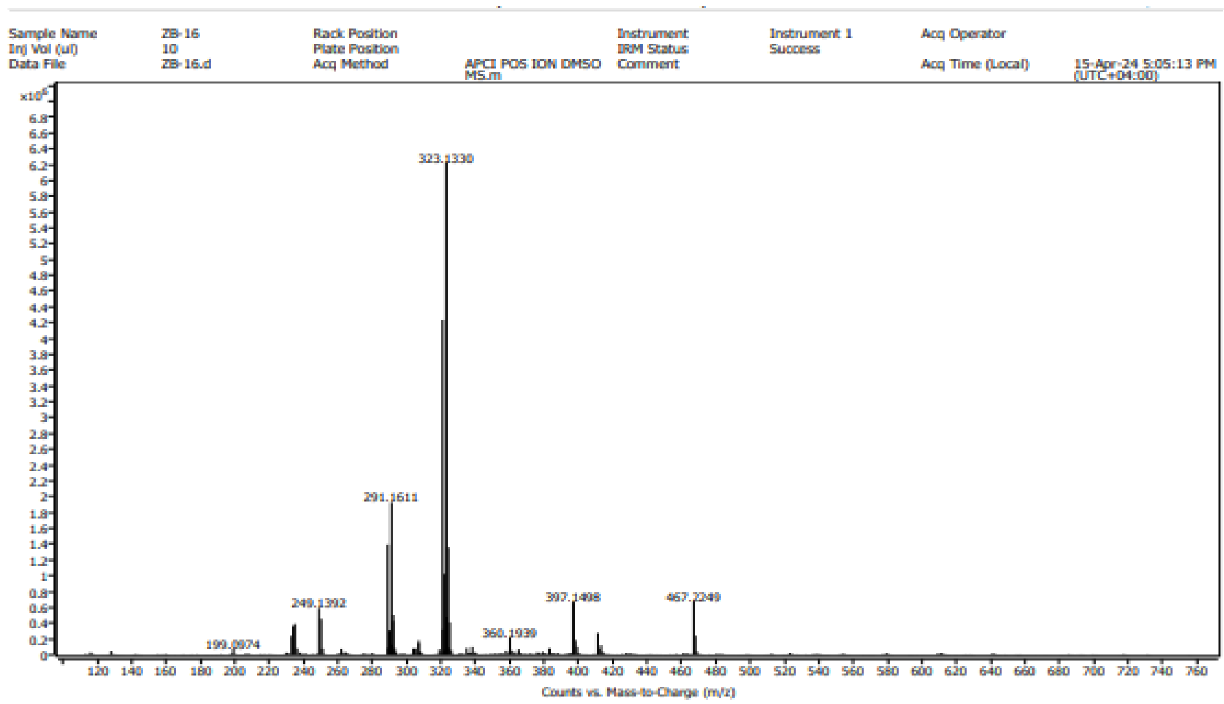


Mass spectrum of 5n

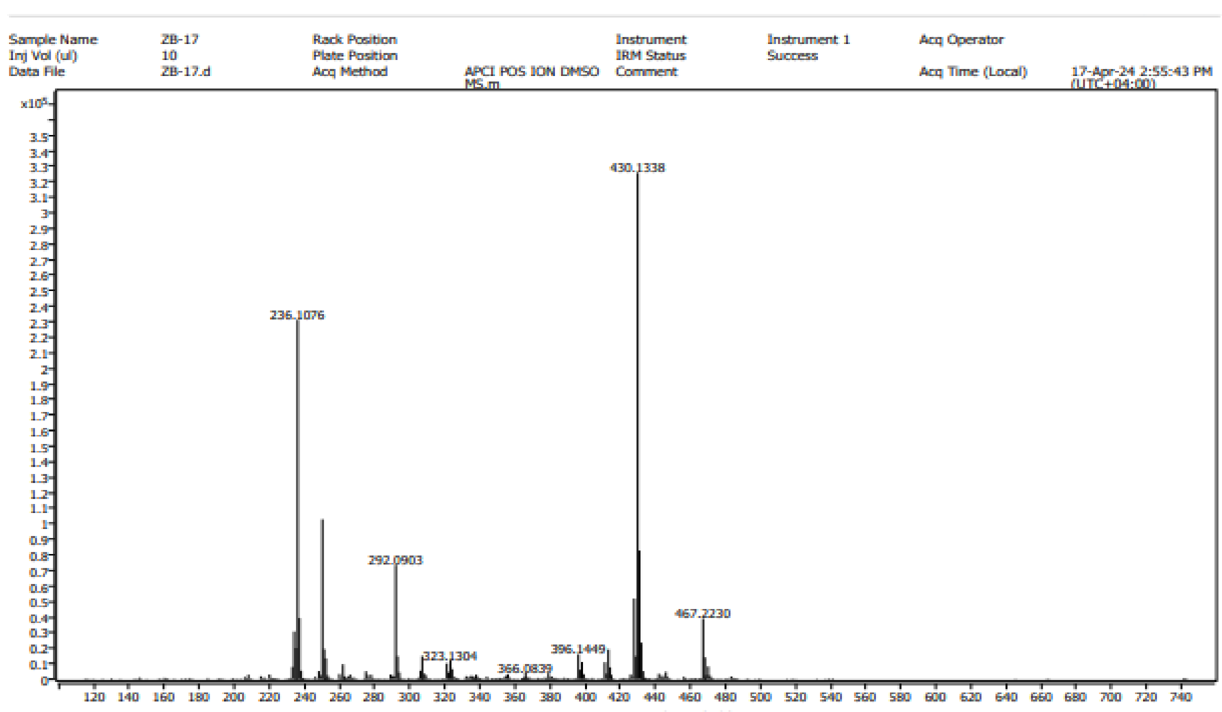


Mass spectrum of 5o

Mass spectrum of 5p



Mass spectrum of 5q



Mass spectrum of 5r