Electronic Supplementary Material (ESI) for RSC Advances.

Synthesis and study of antibiofilm and antivirulence properties of flavonol analogues generated by palladium catalyzed ligand free Suzuki-Miyaura coupling against *Pseudomonas aeruginosa* PAO1

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[e] Department of Integrative Biology, School of Bioscience and Technology, Vellore Institute of Technology VIT, Vellore-632014

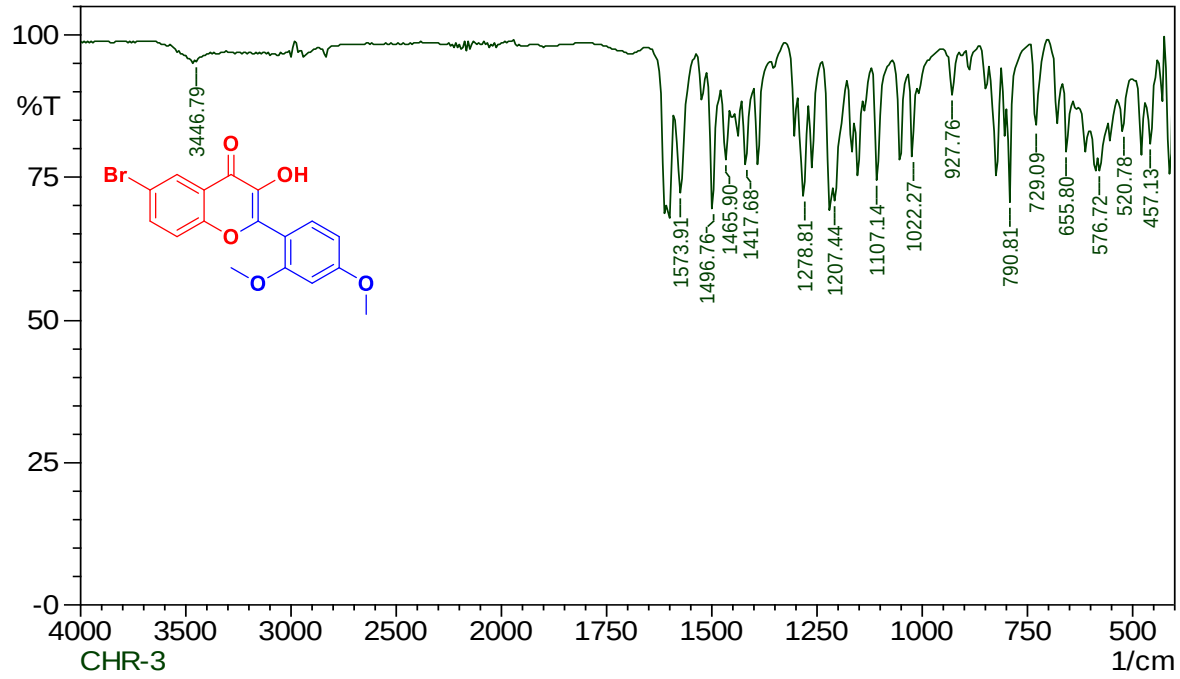

Figure S1 IR spectrum of compound 3a

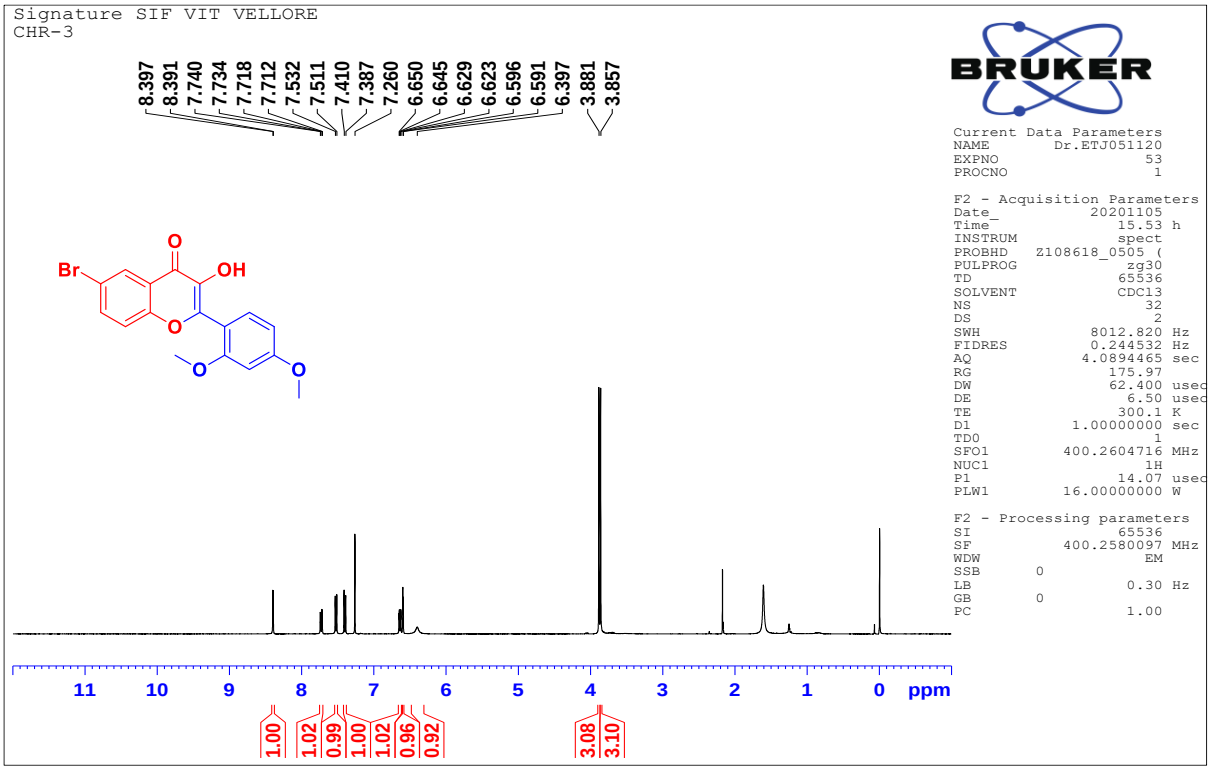

Figure S2 ^{1}H NMR spectrum of compound 3a

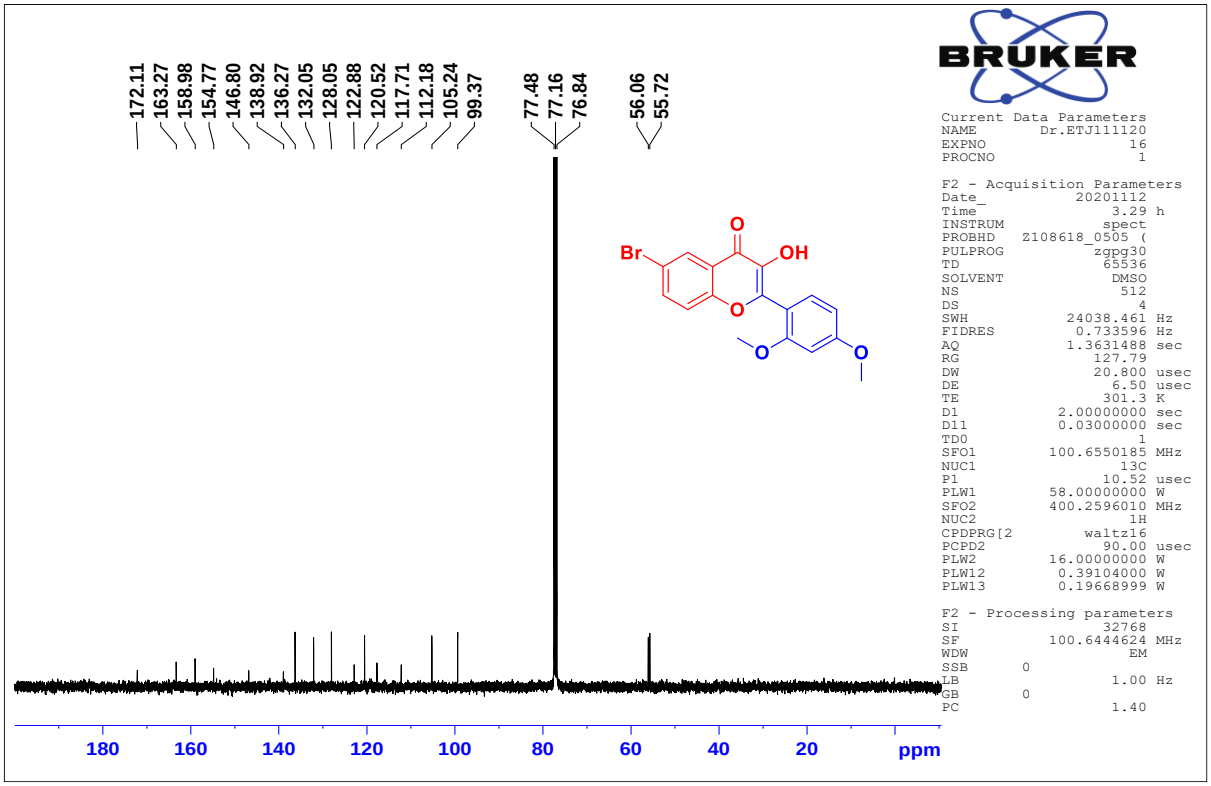

Figure S3 ^{13}C NMR spectrum of compound 3a

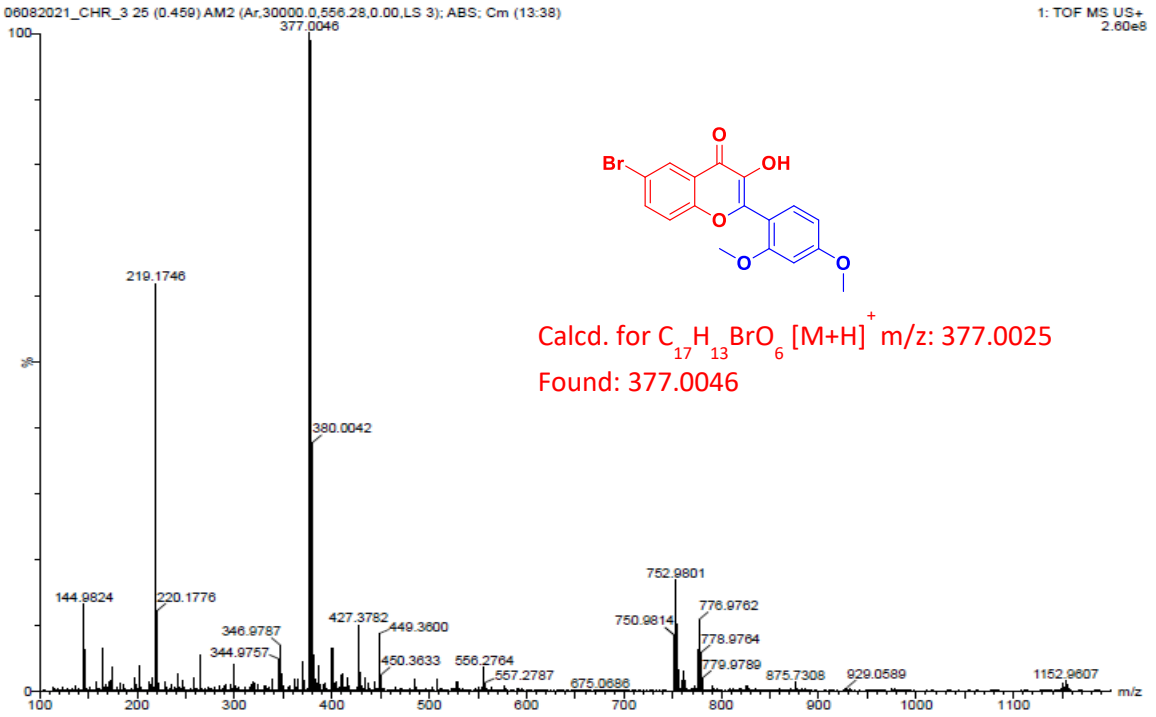

Figure S4 HRMS NMR spectrum of compound 3a

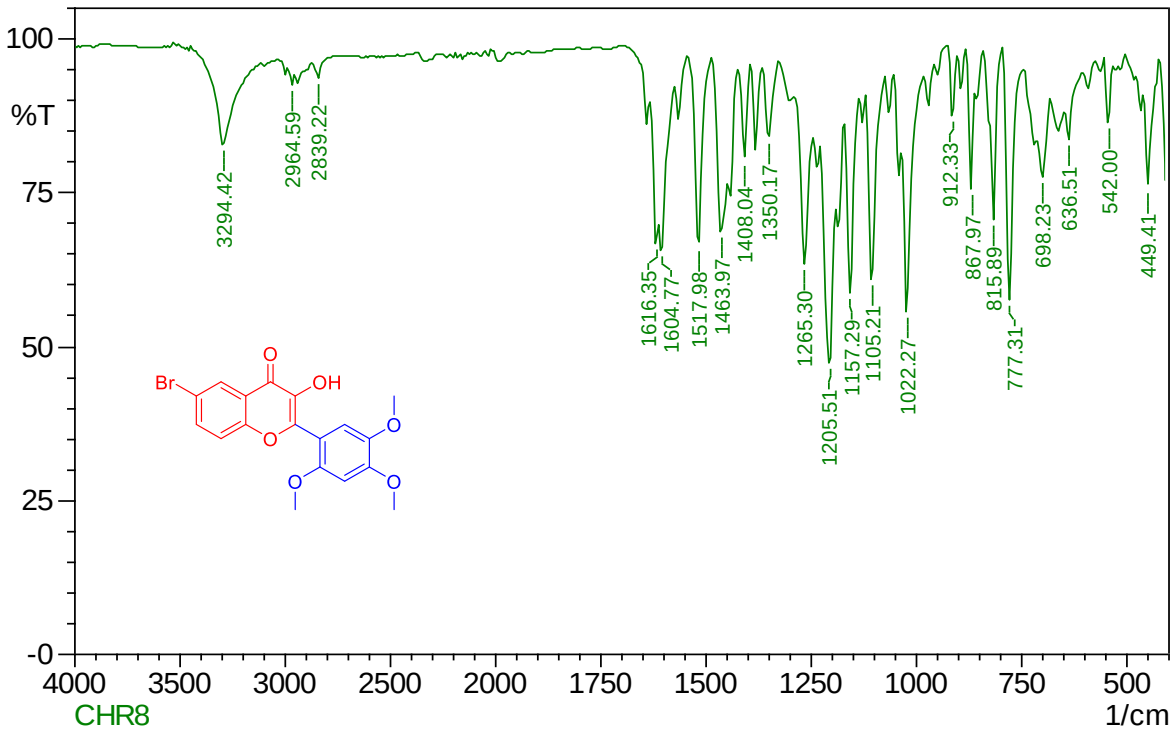

Figure S5 IR spectrum of compound 3b

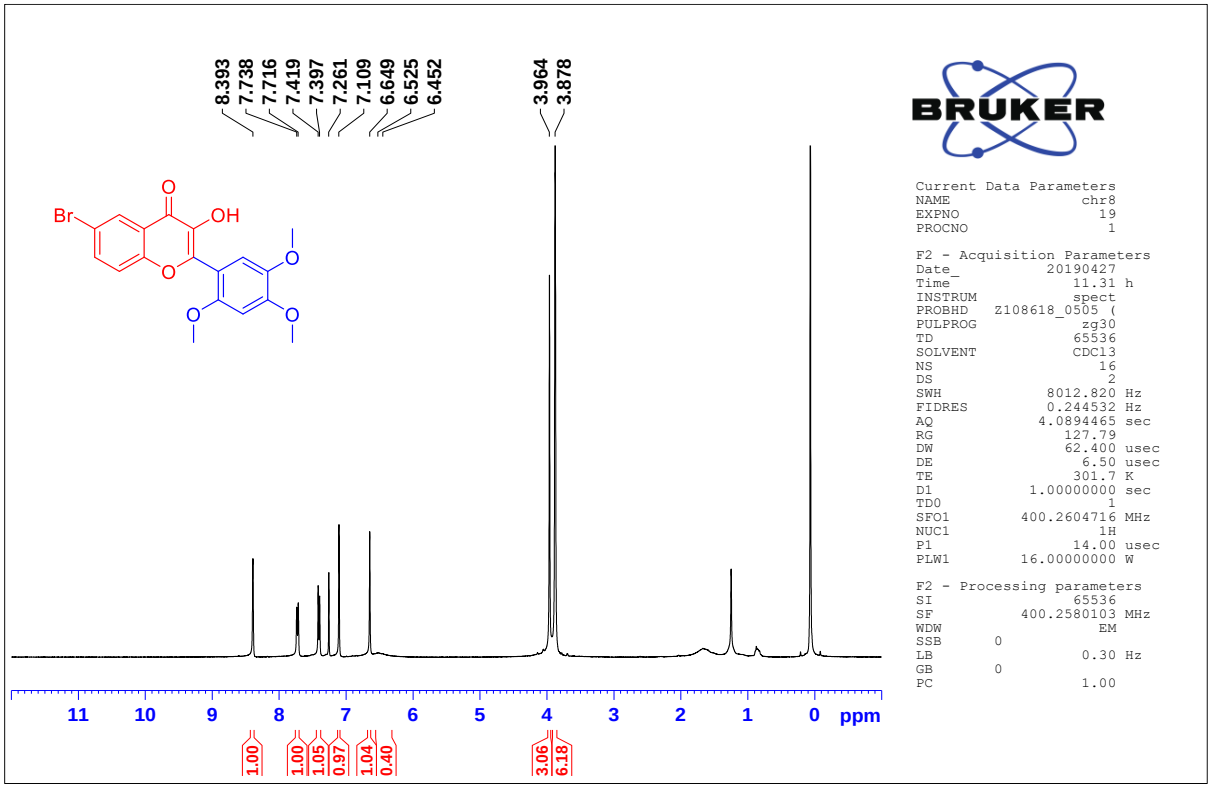

Figure S6 ^{1}H NMR spectrum of compound 3b

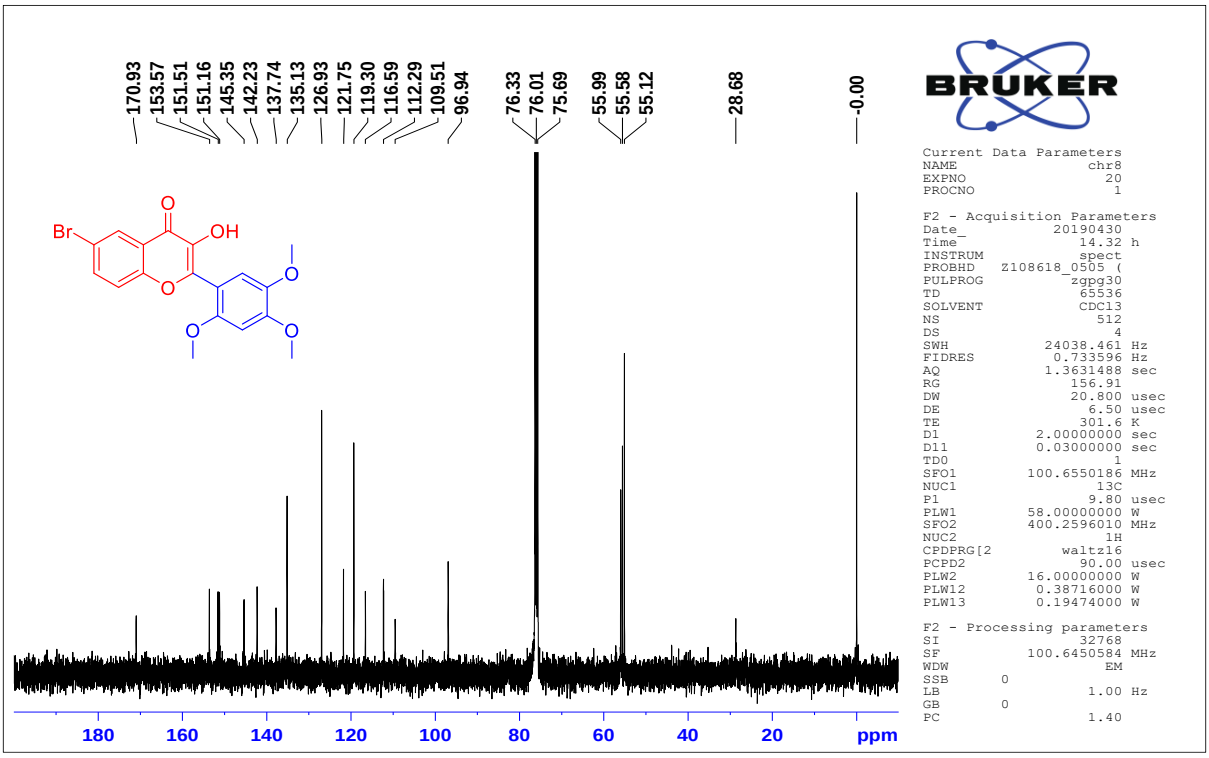

Figure S7 ^{13}C NMR spectrum of compound 3b

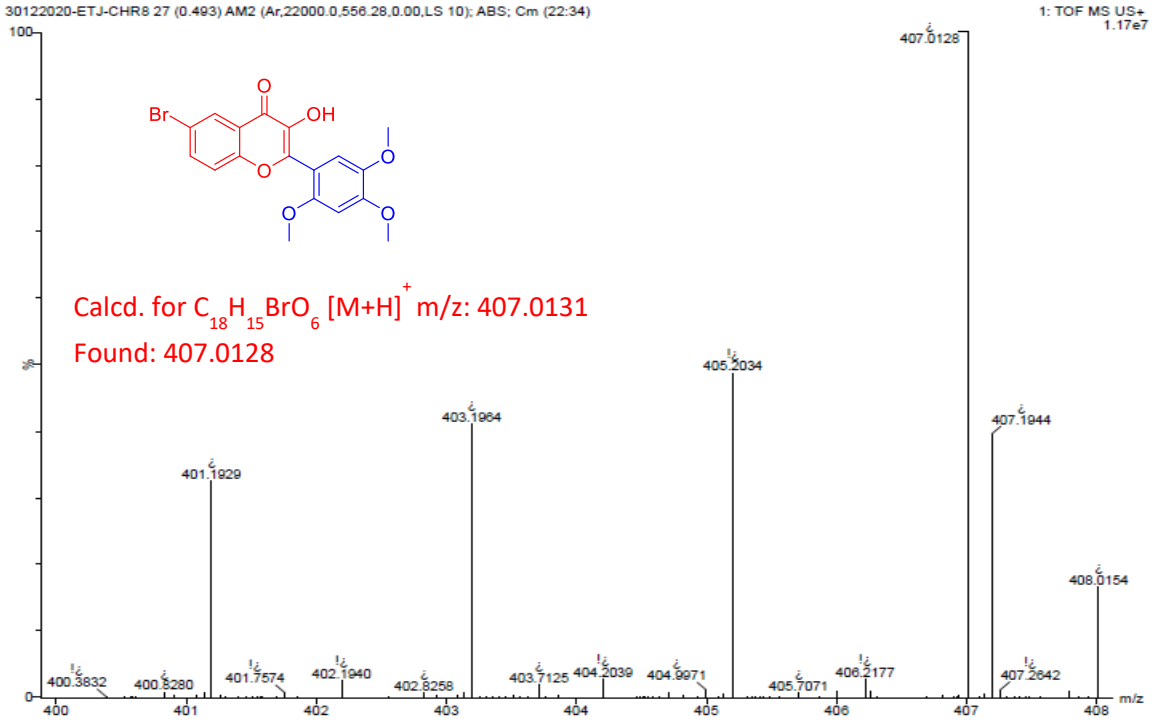

Figure S8 HRMS spectrum of compound 3b

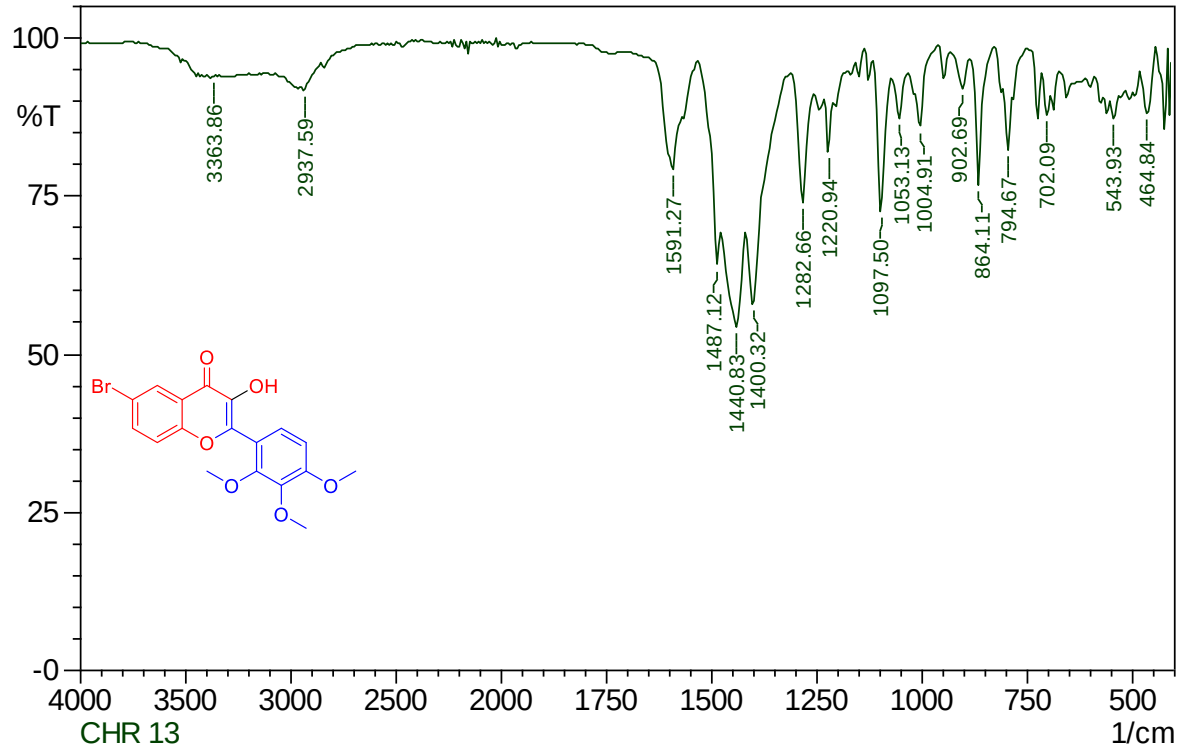

Figure S9 IR spectrum of compound 3c

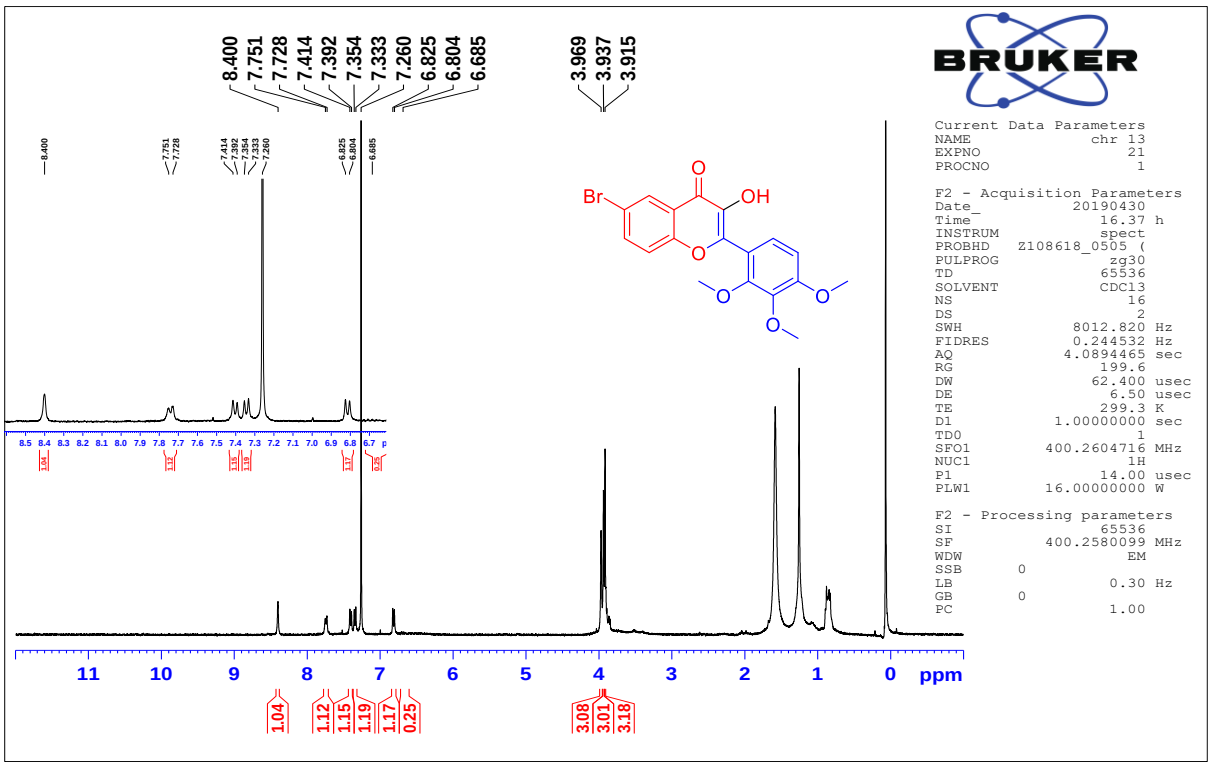

Figure S10 ^{1}H NMR spectrum of compound 3c

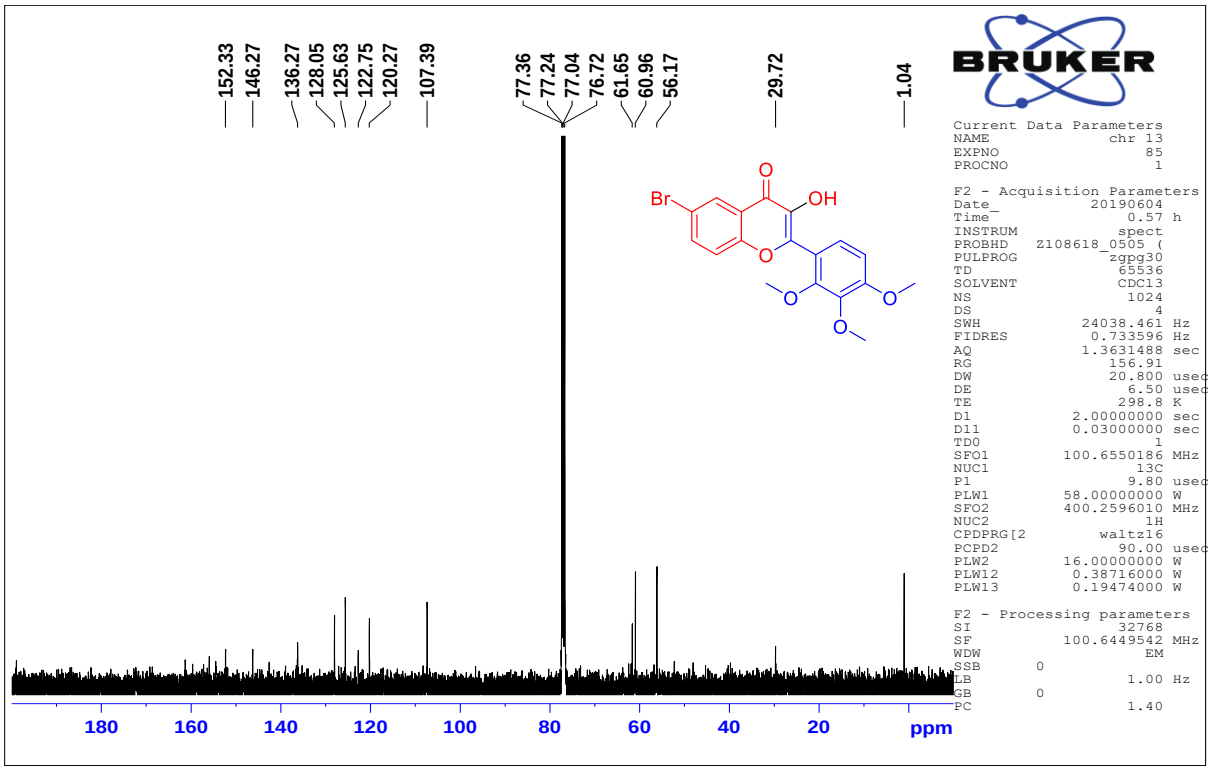

Figure S11 ^{13}C NMR spectrum of compound 3c

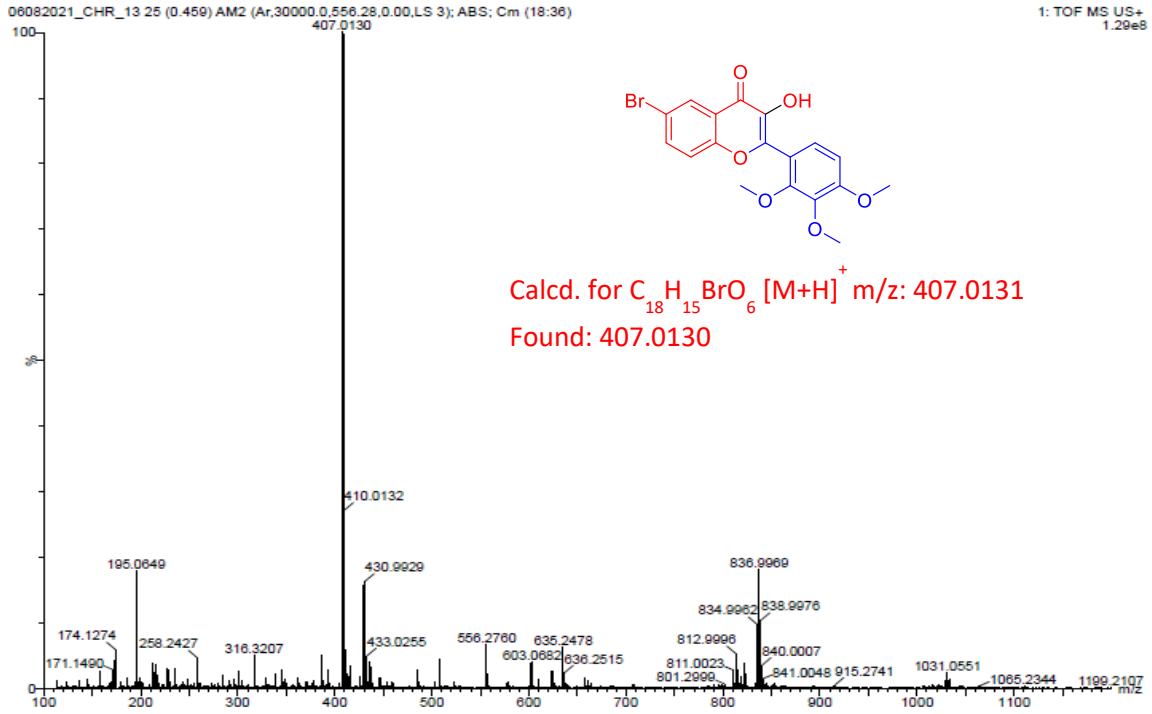

Figure S12 HRMS spectrum of compound 3c

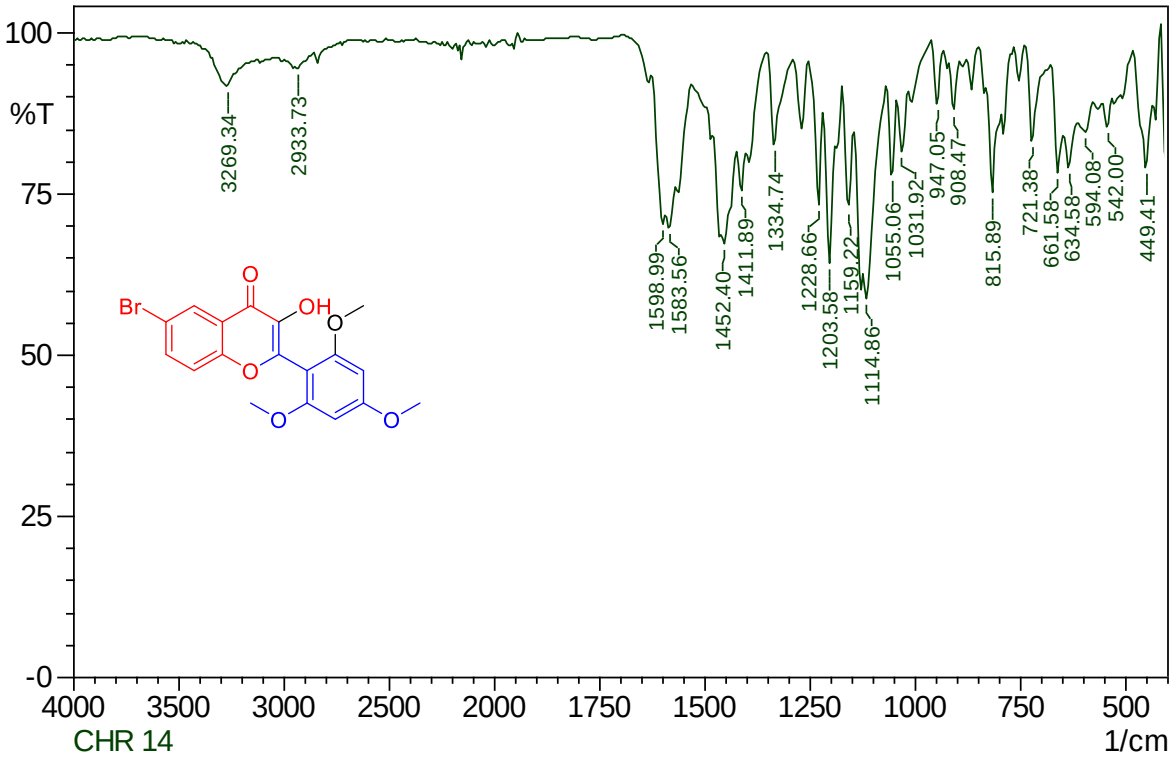

Figure S13 IR spectrum of compound 3d

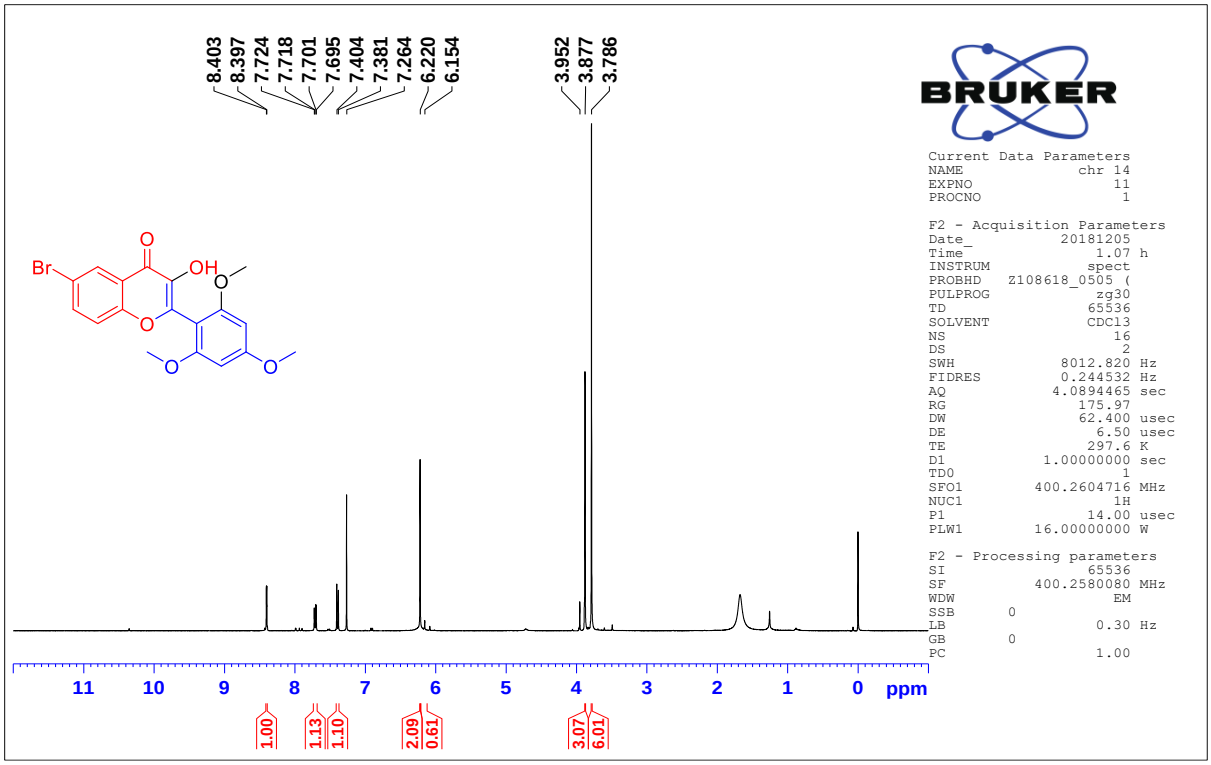

Figure S14 ^{1}H NMR spectrum of compound 3d

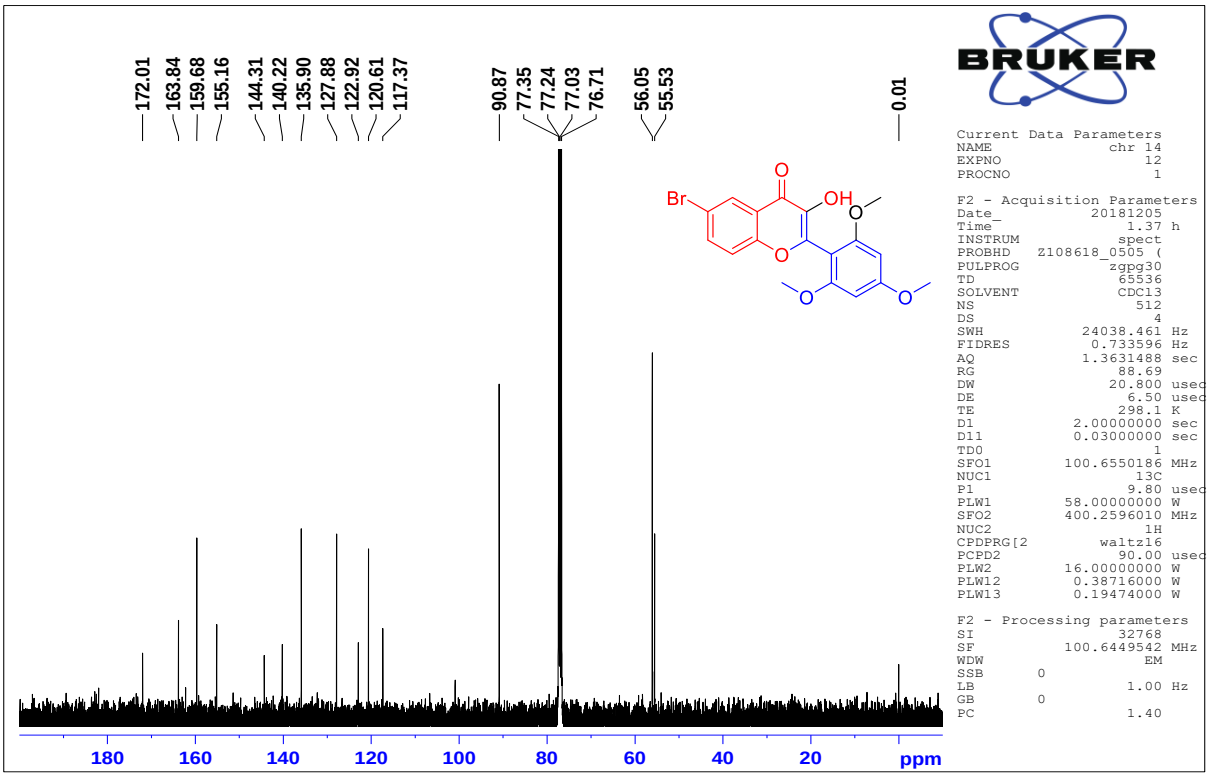

Figure S15 ^{13}C NMR spectrum of compound 3d

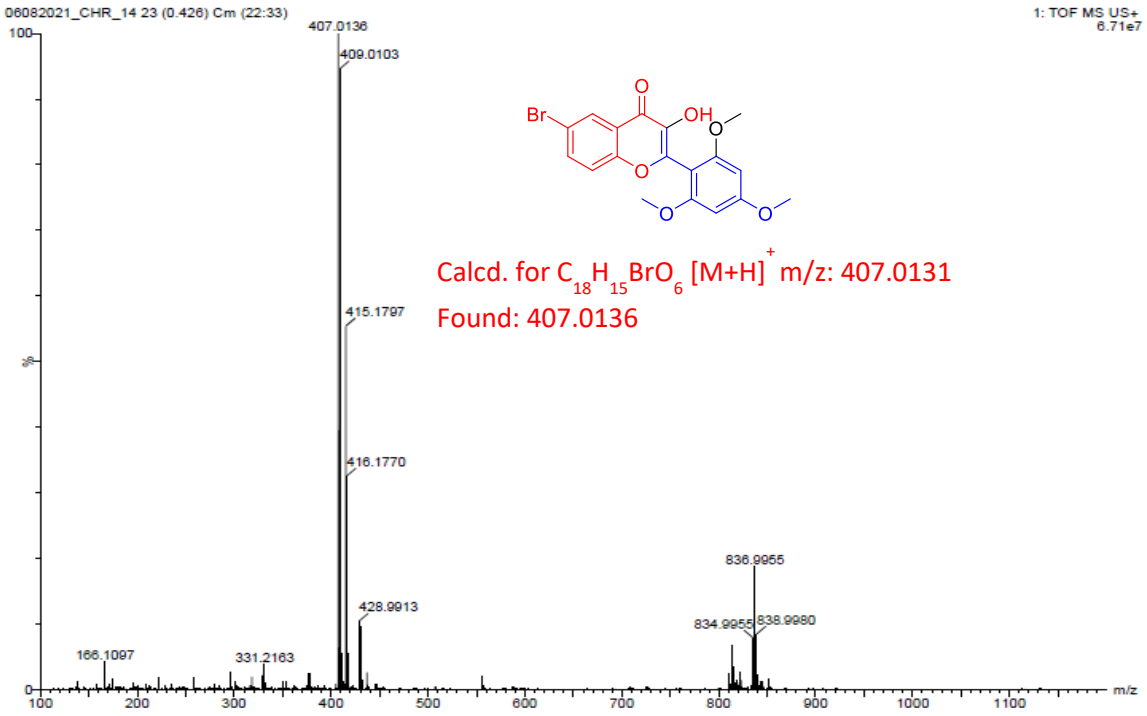

Figure S16 HRMS spectrum of compound 3d

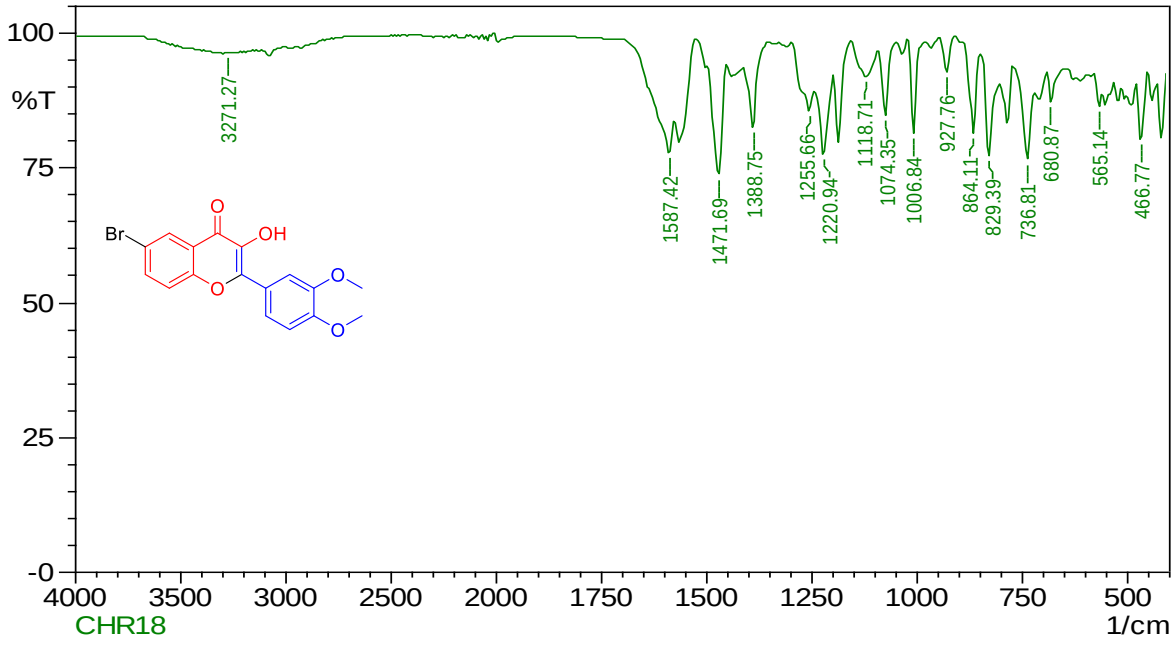

Figure S17 IR spectrum of compound 3e

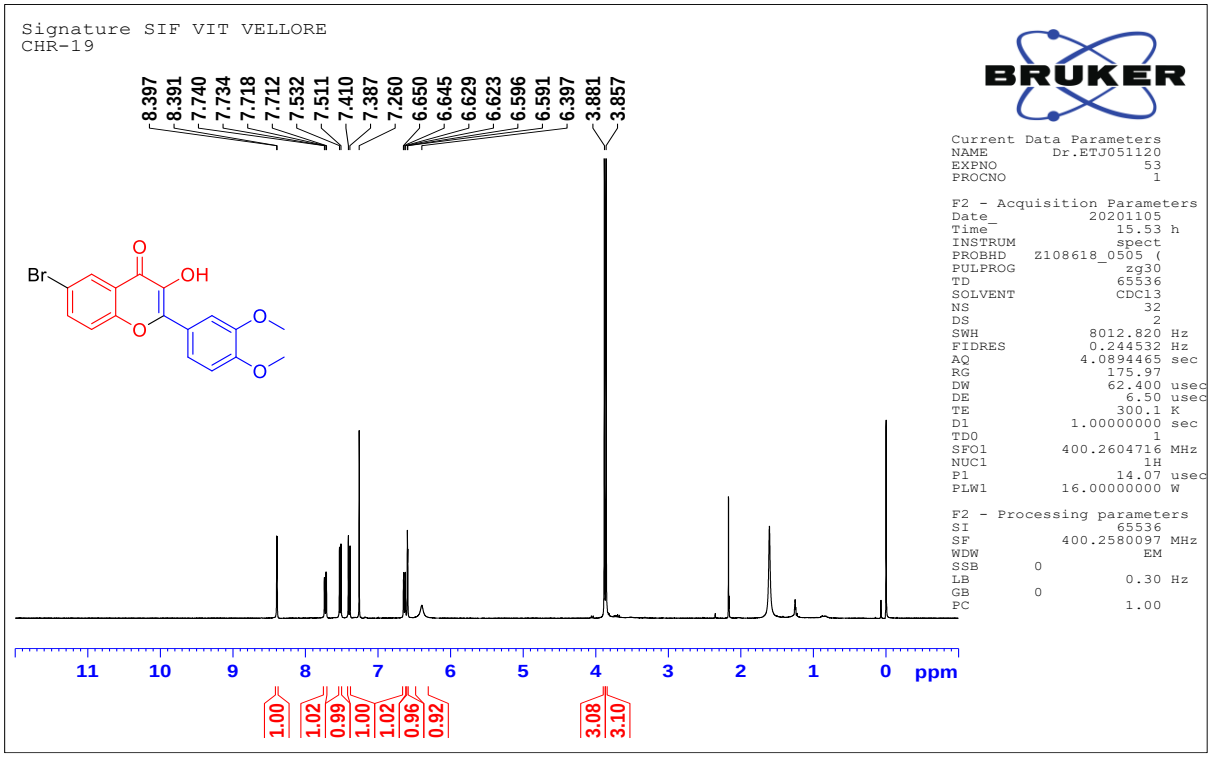

Figure S18 ^{1}H NMR spectrum of compound 3e

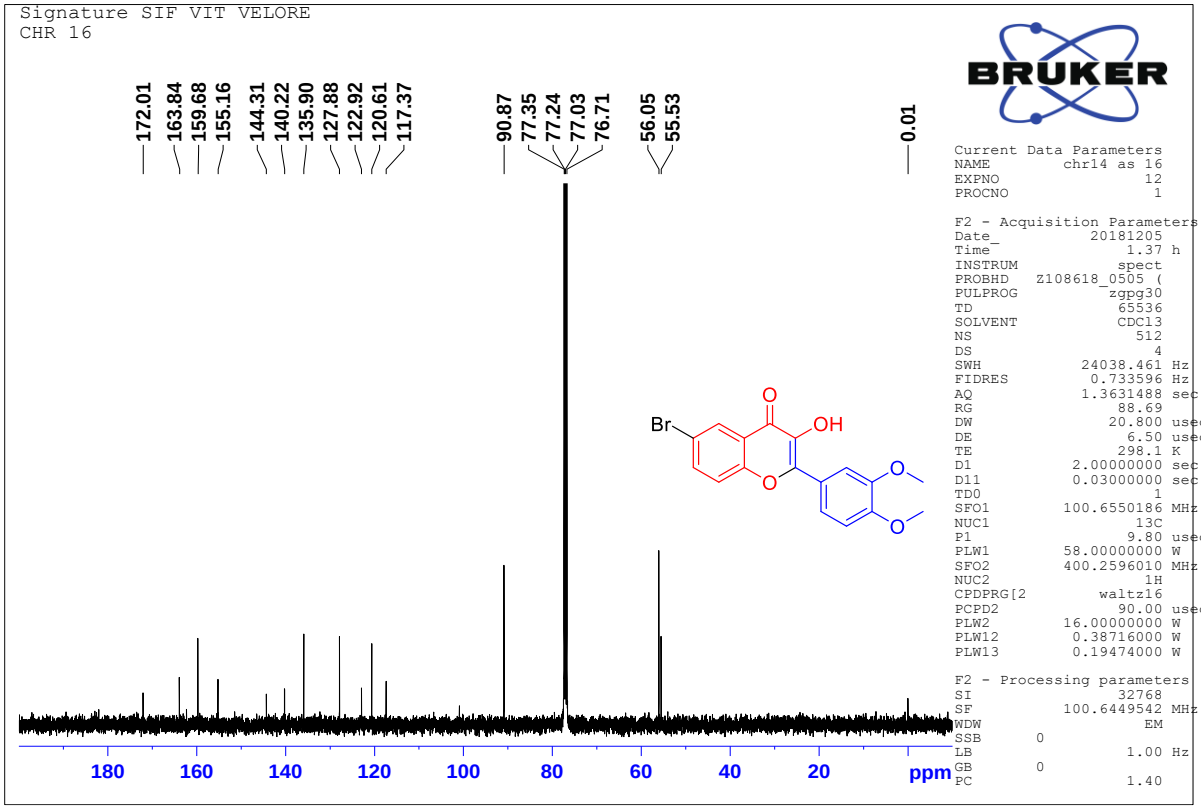

Figure S19 ^{13}C NMR spectrum of compound 3e

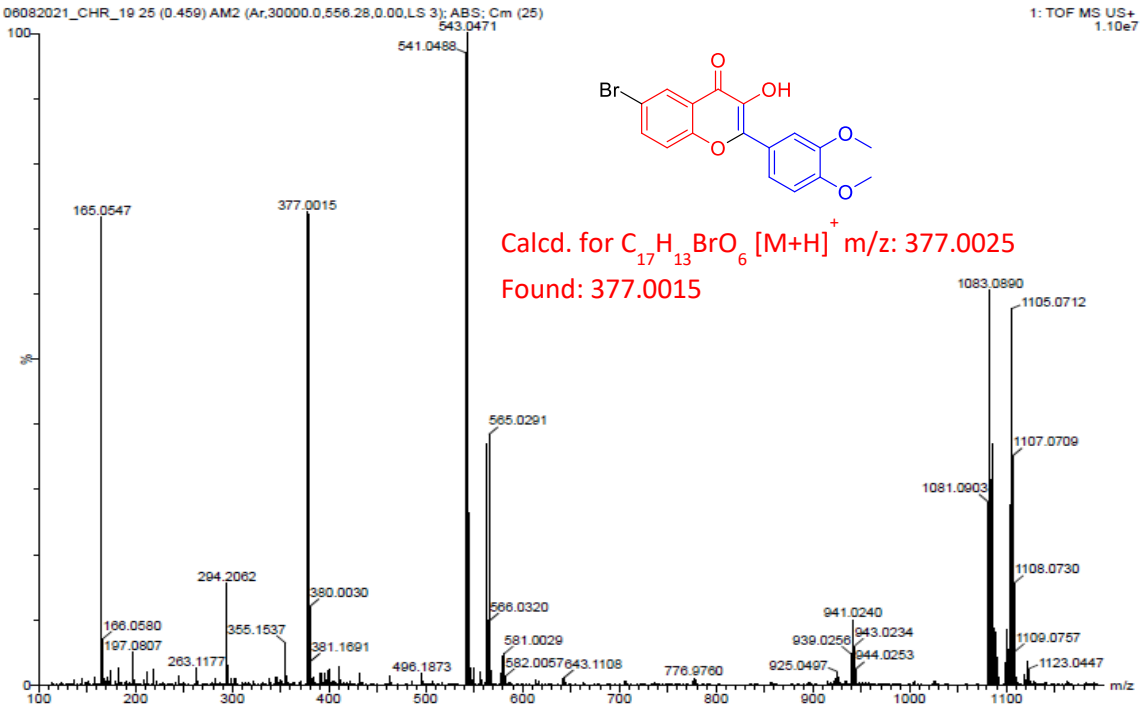

Figure S20 HRMS spectrum of compound 3e

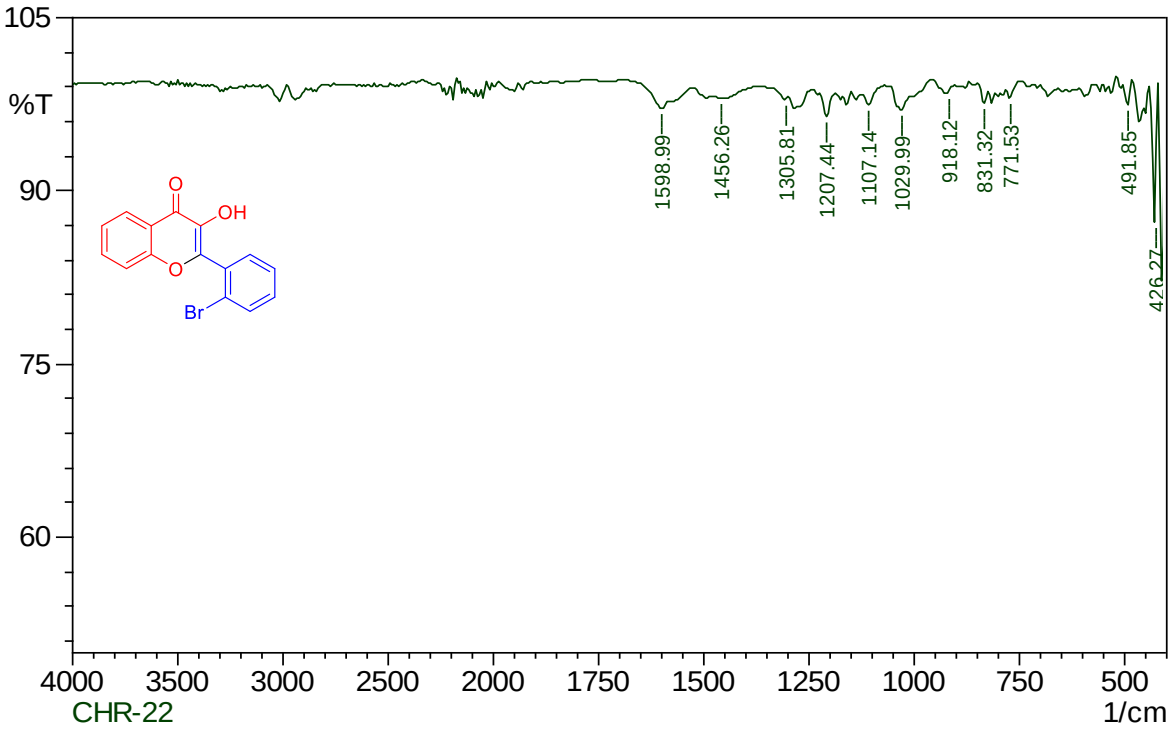

Figure S21 IR spectrum of compound 3f

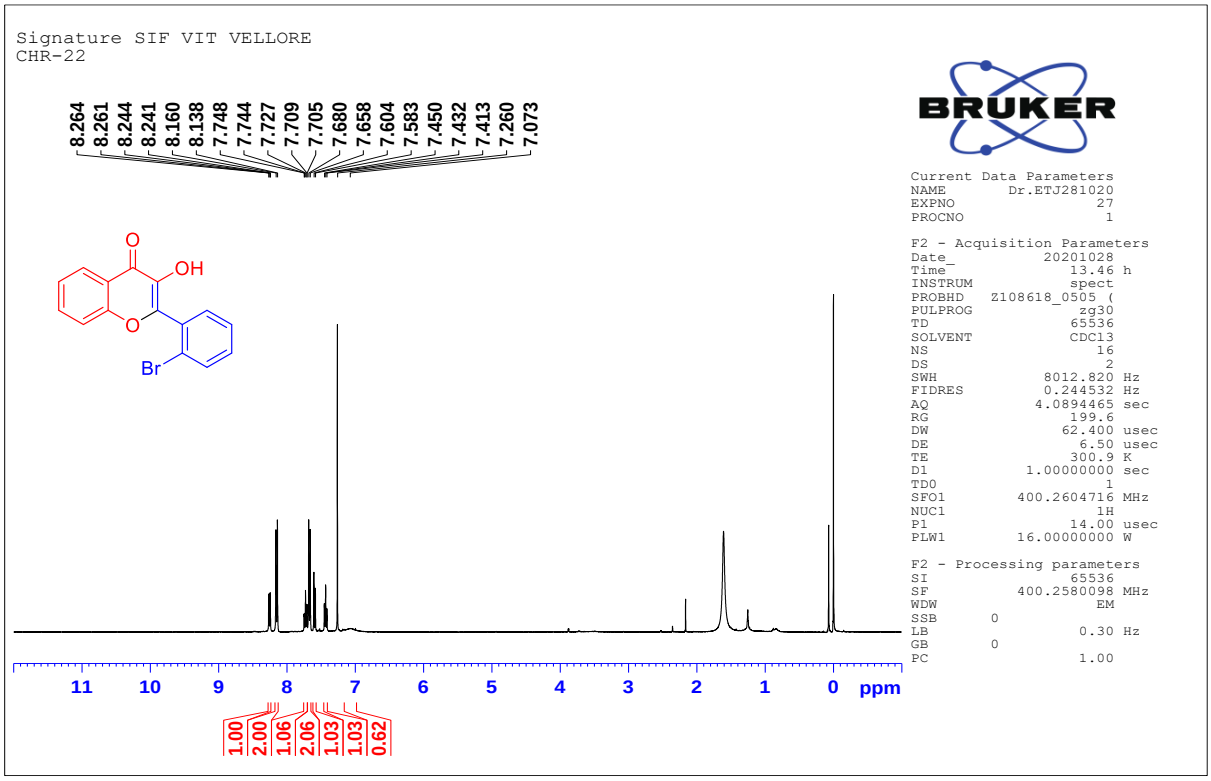

Figure S22 IR spectrum of compound 3f

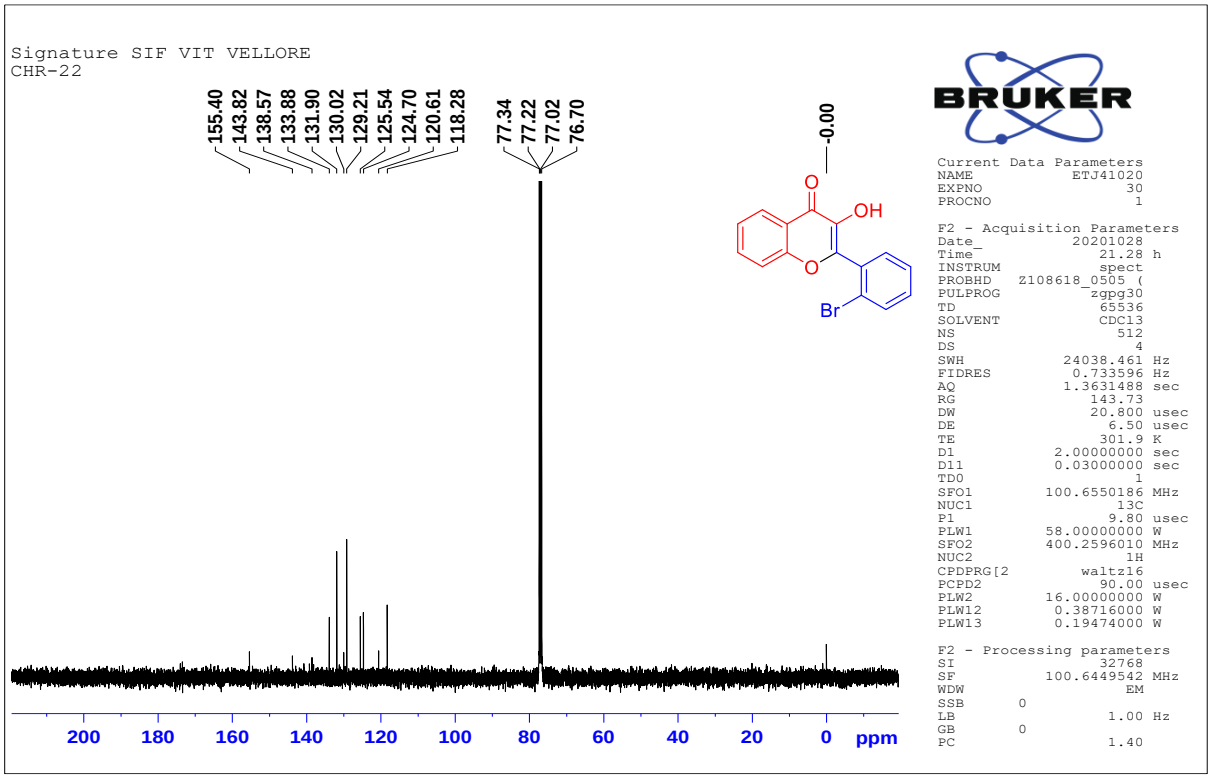

Figure S23 ^{13}C NMR spectrum of compound 3f

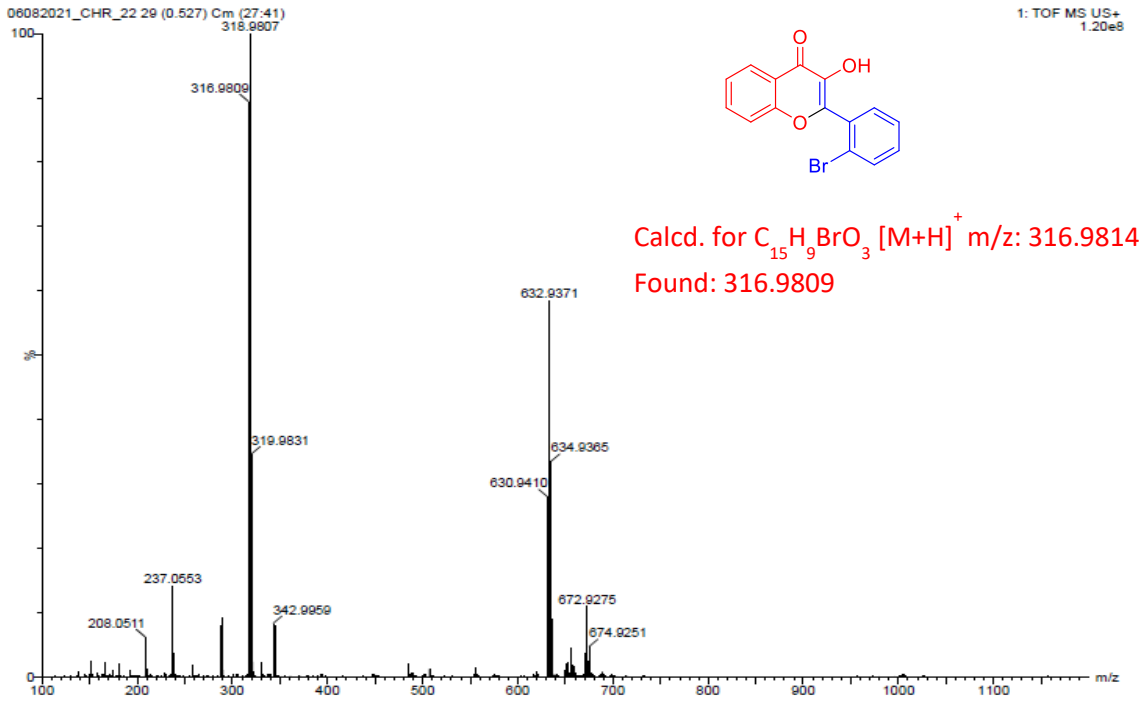

Figure S24 HRMS spectrum of compound 3f

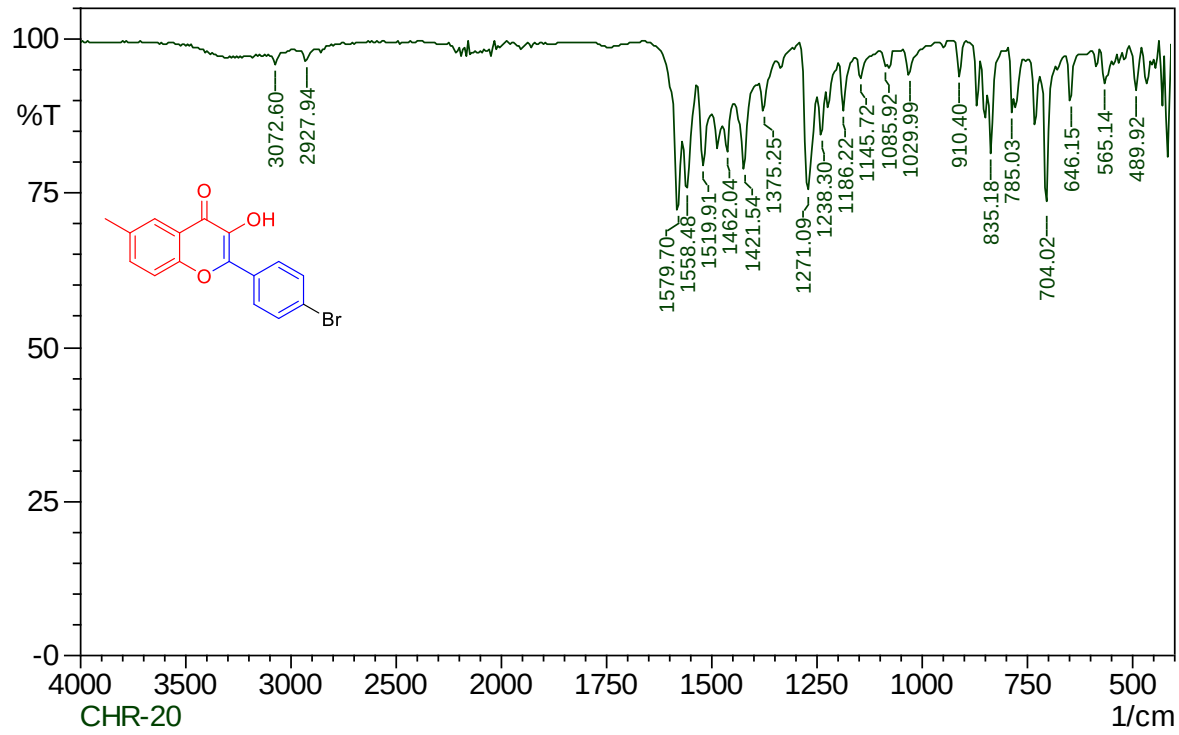

Figure S25 IR spectrum of compound 3g

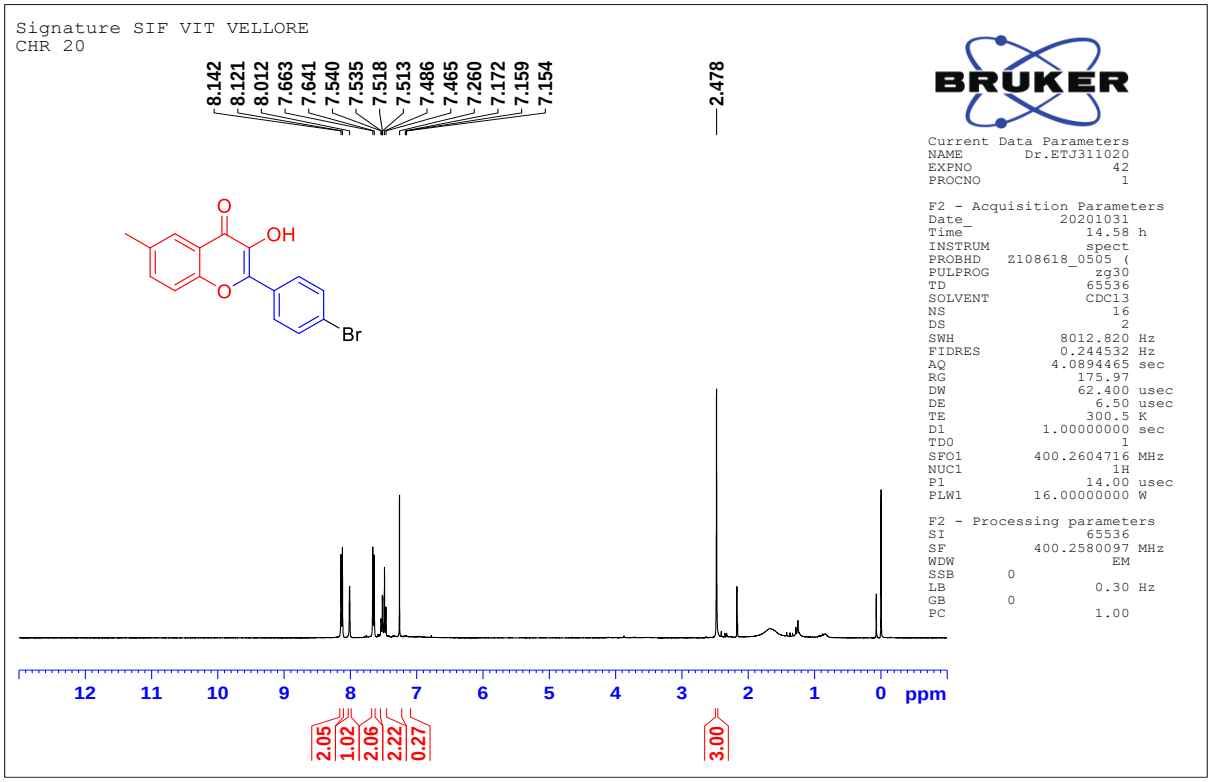

Figure S26 ^{1}H NMR spectrum of compound 3g

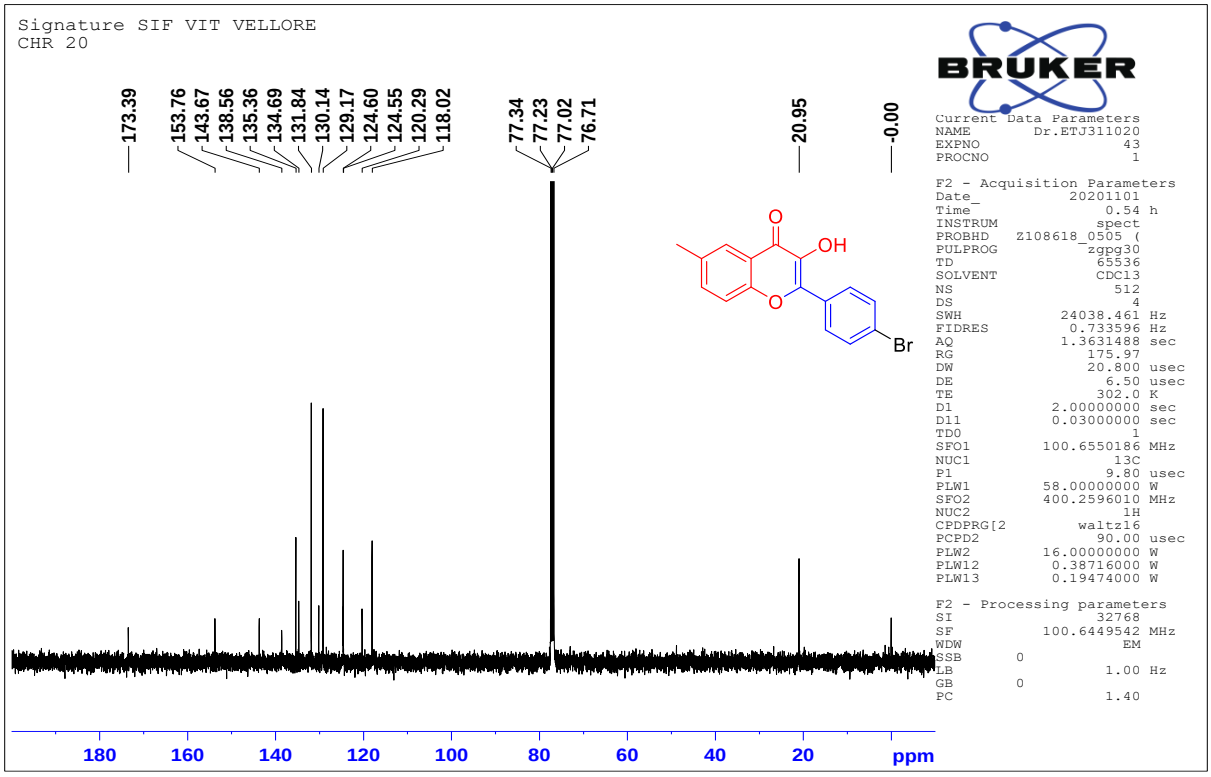

Figure S27 ^{13}C NMR spectrum of compound 3g

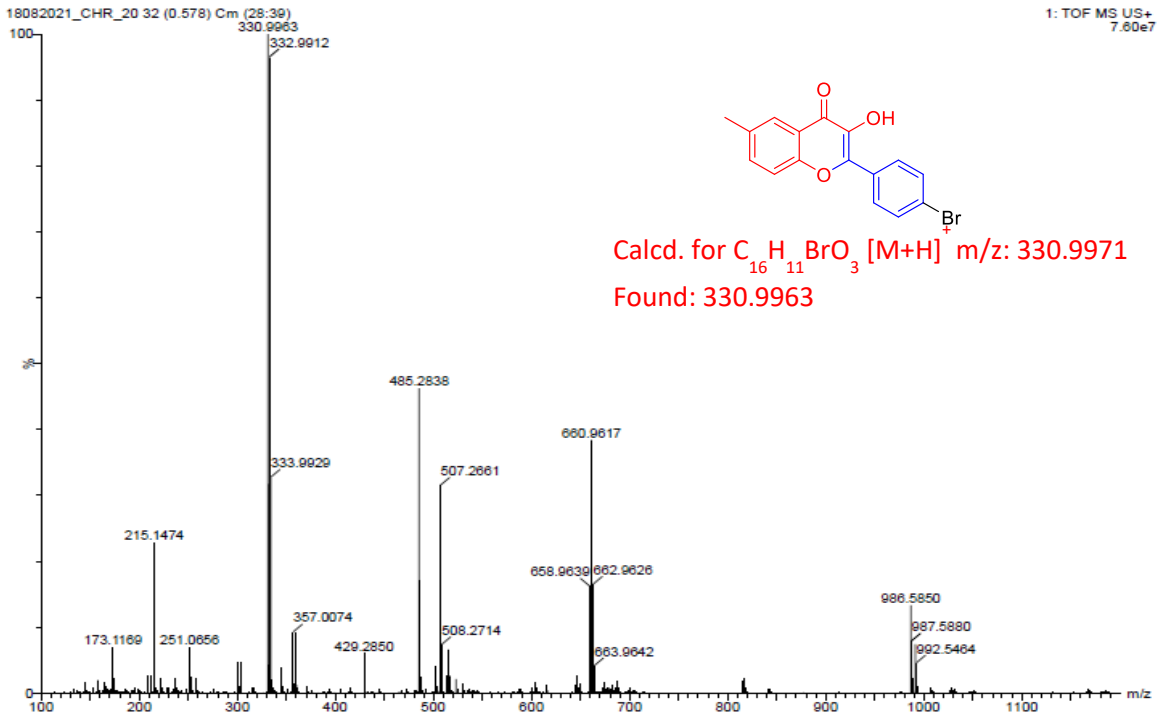

Figure S28 HRMS spectrum of compound 3g

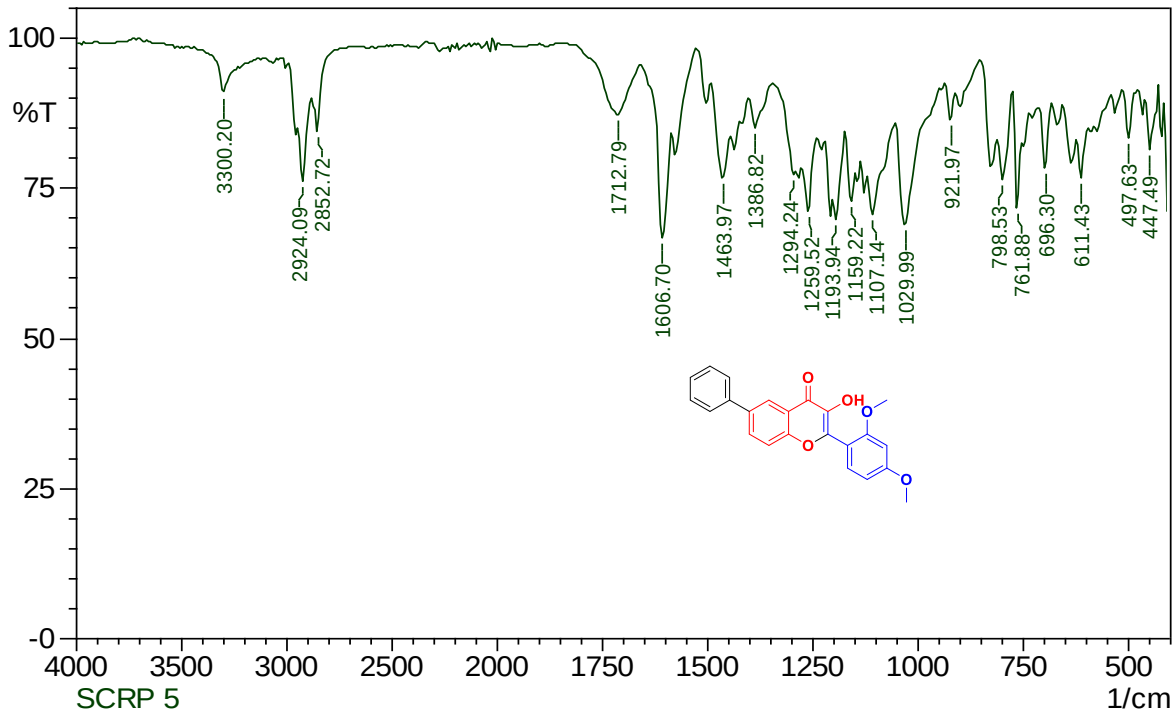

Figure S29 IR spectrum of compound 5a

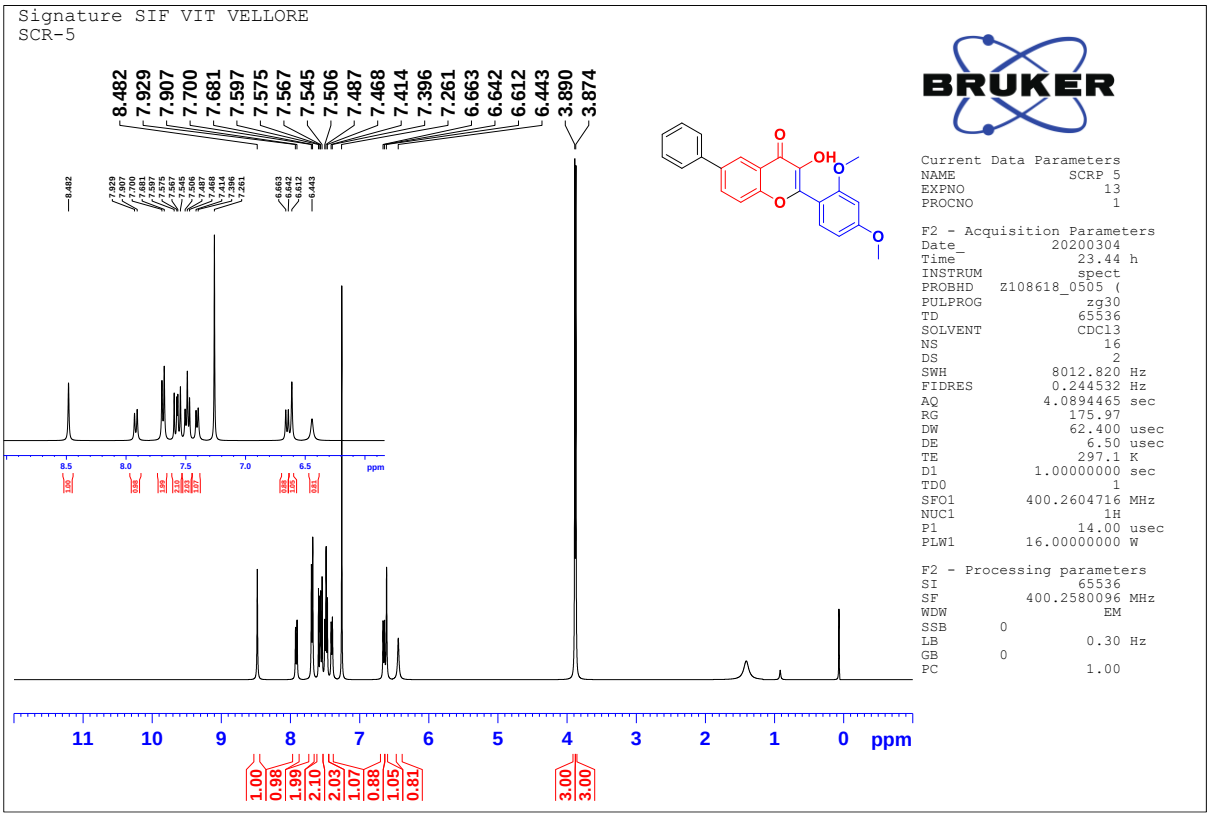

Figure S30 ^{1}H NMR spectrum of compound 5a

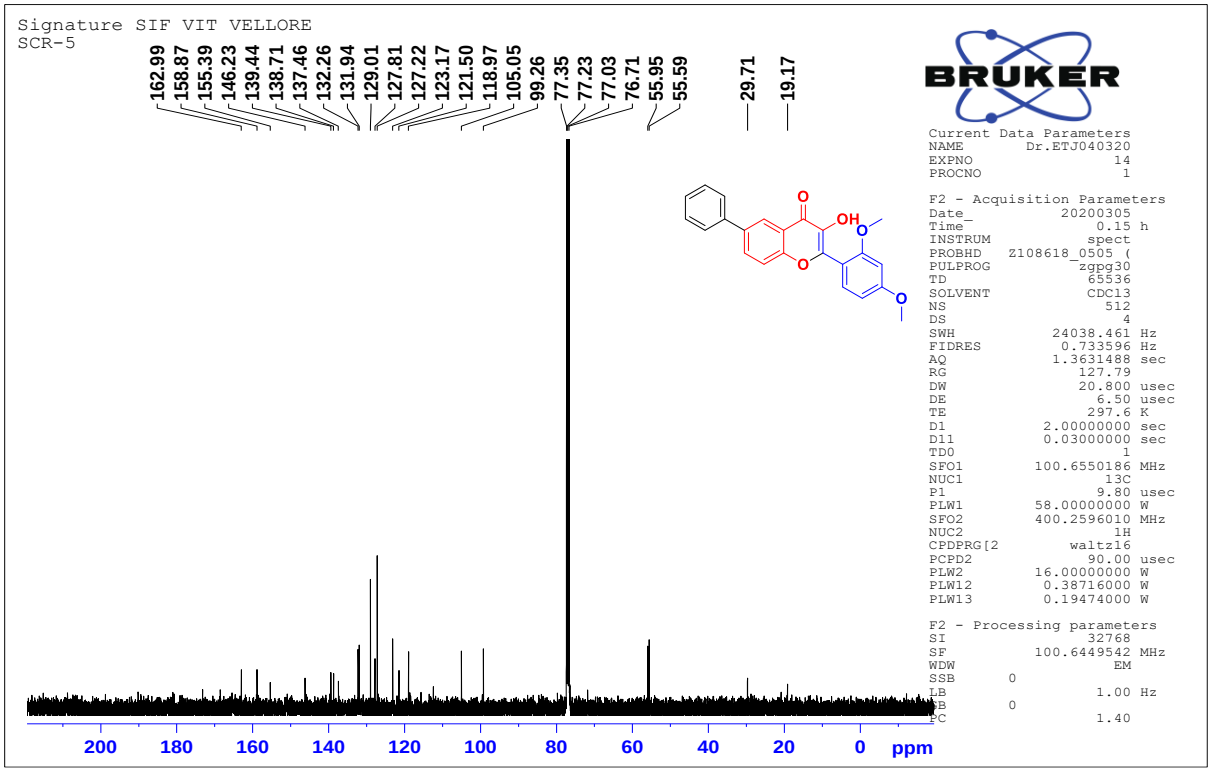

Figure S31 ^{13}C NMR spectrum of compound 5a

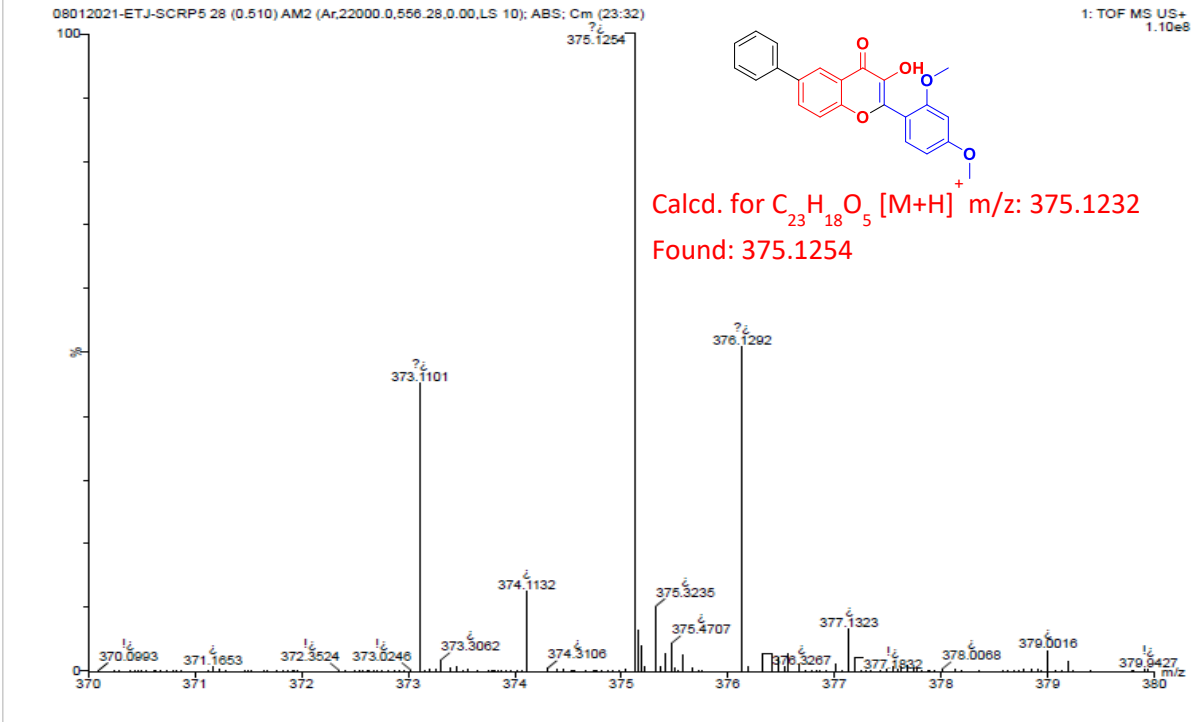

Figure S32 HRMS spectrum of compound 5a

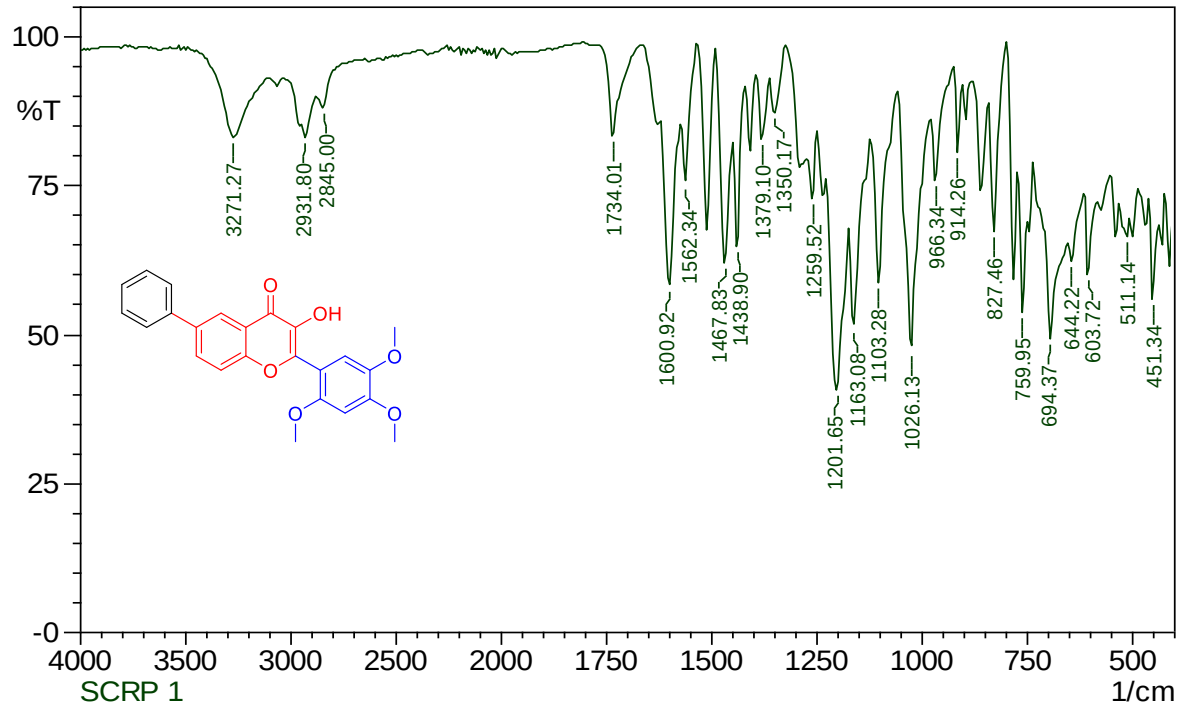

Figure S33 IR spectrum of compound 5b

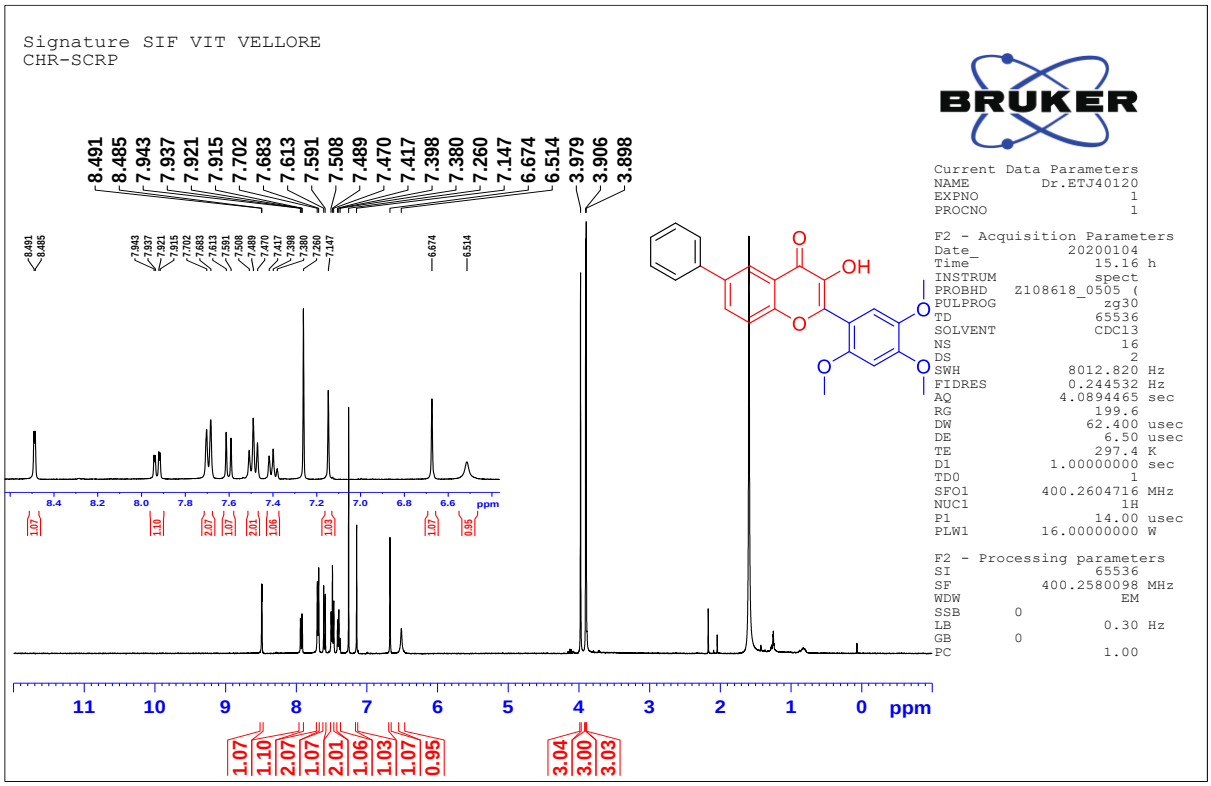

Figure S34 ^{1}H NMR spectrum of compound 5b

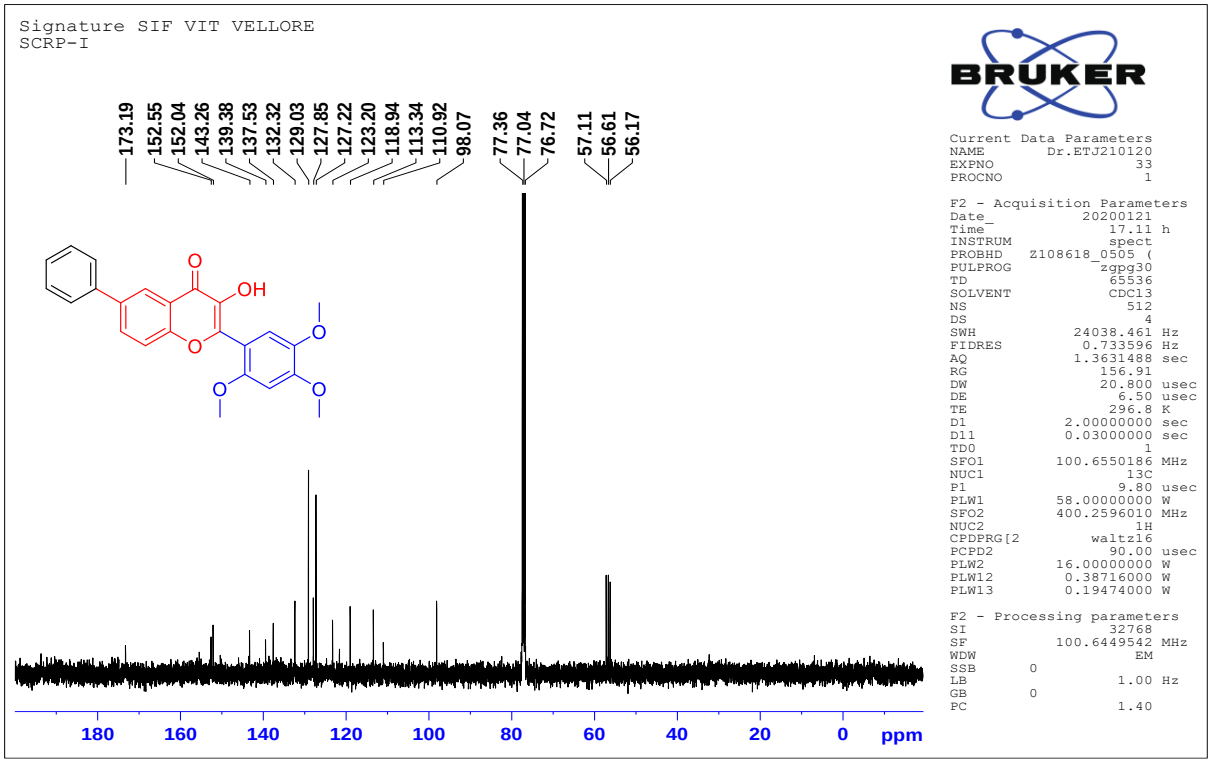

Figure S35 ^{13}C NMR spectrum of compound 5b

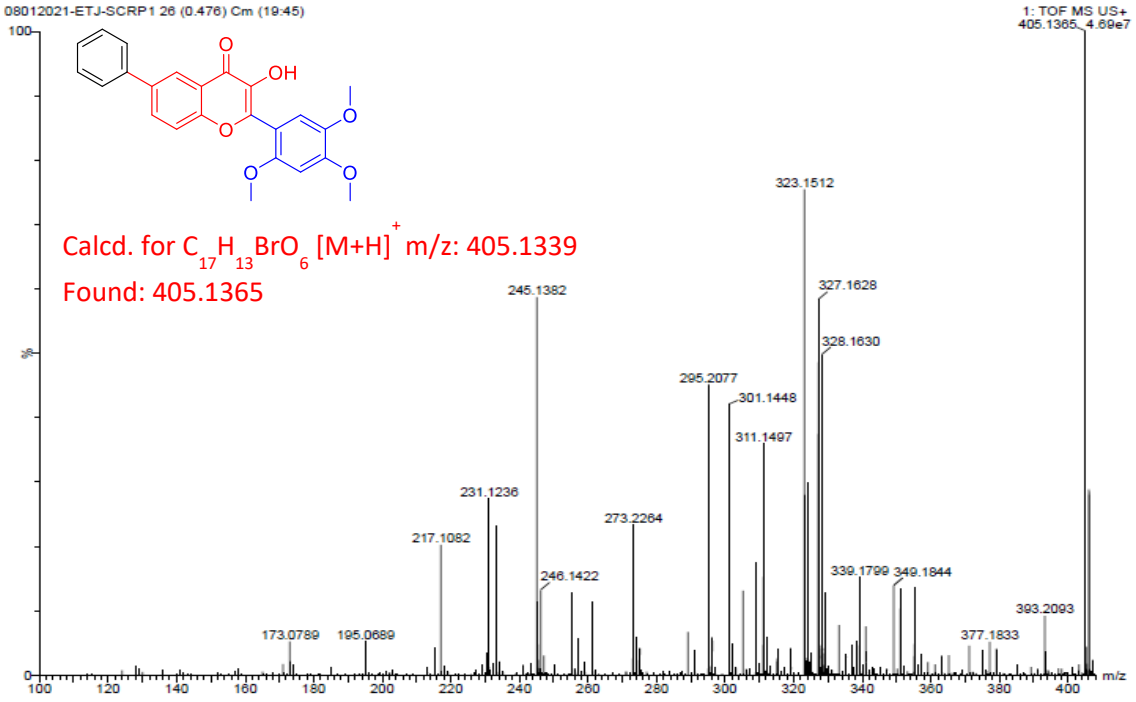

Figure S36 HRMS spectrum of compound 5b

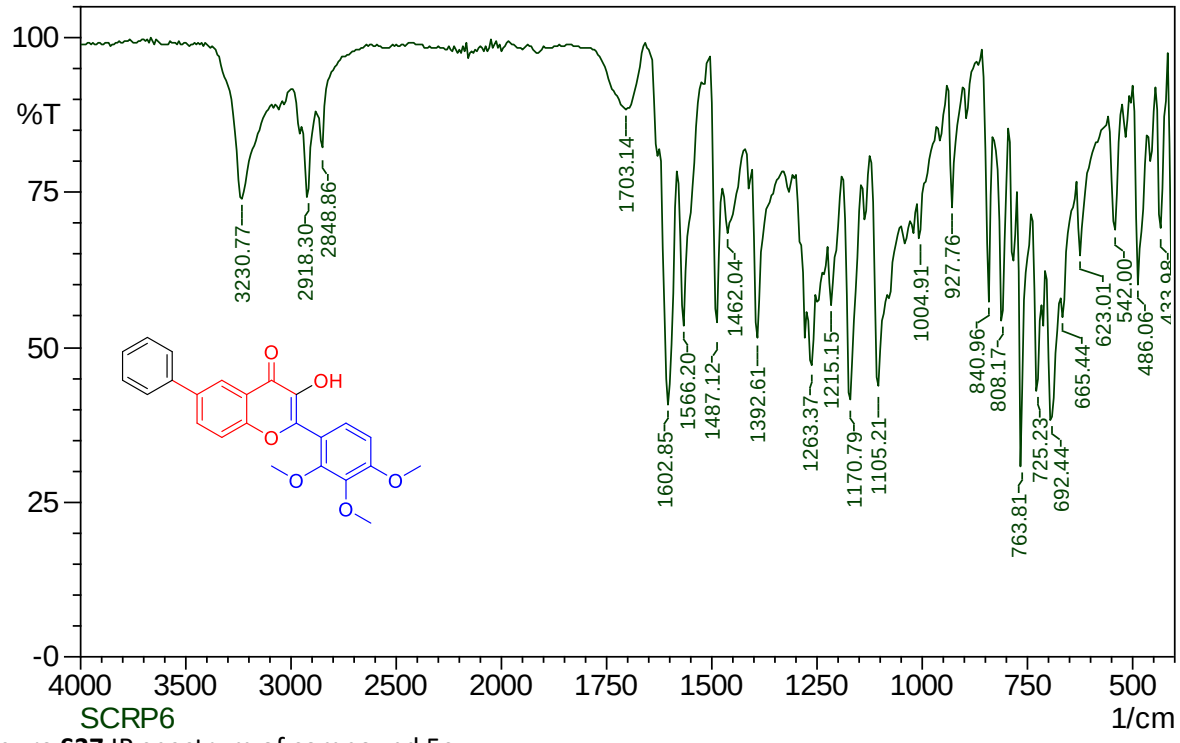

Figure S37 IR spectrum of compound 5c

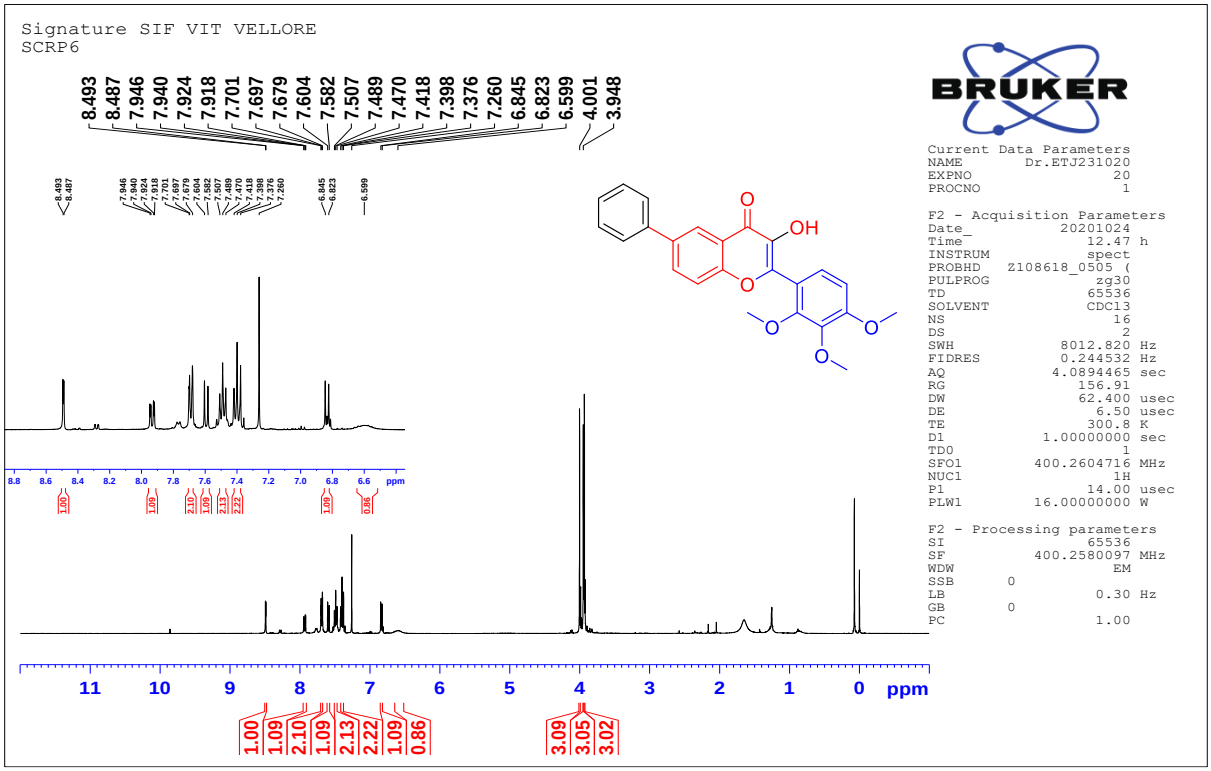

Figure S38 [1]H NMR spectrum of compound 5c

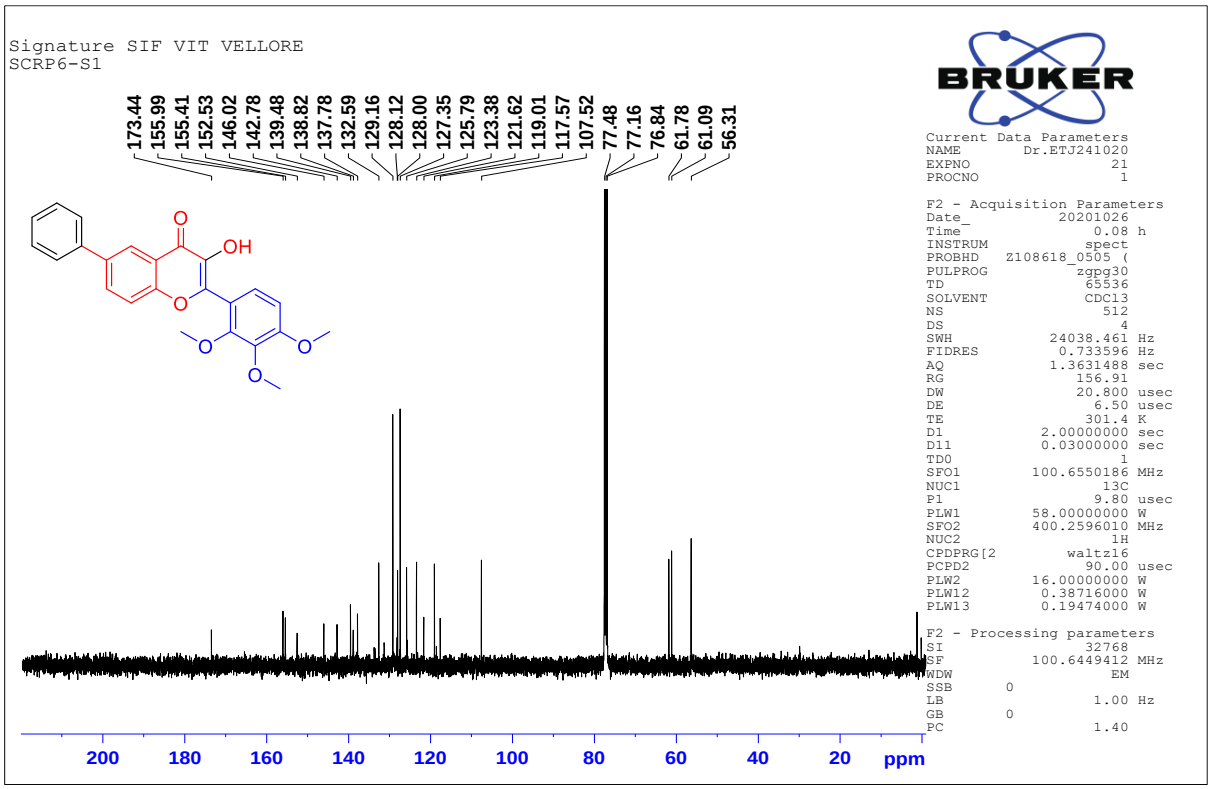

Figure S39 [13] C NMR spectrum of compound 5c

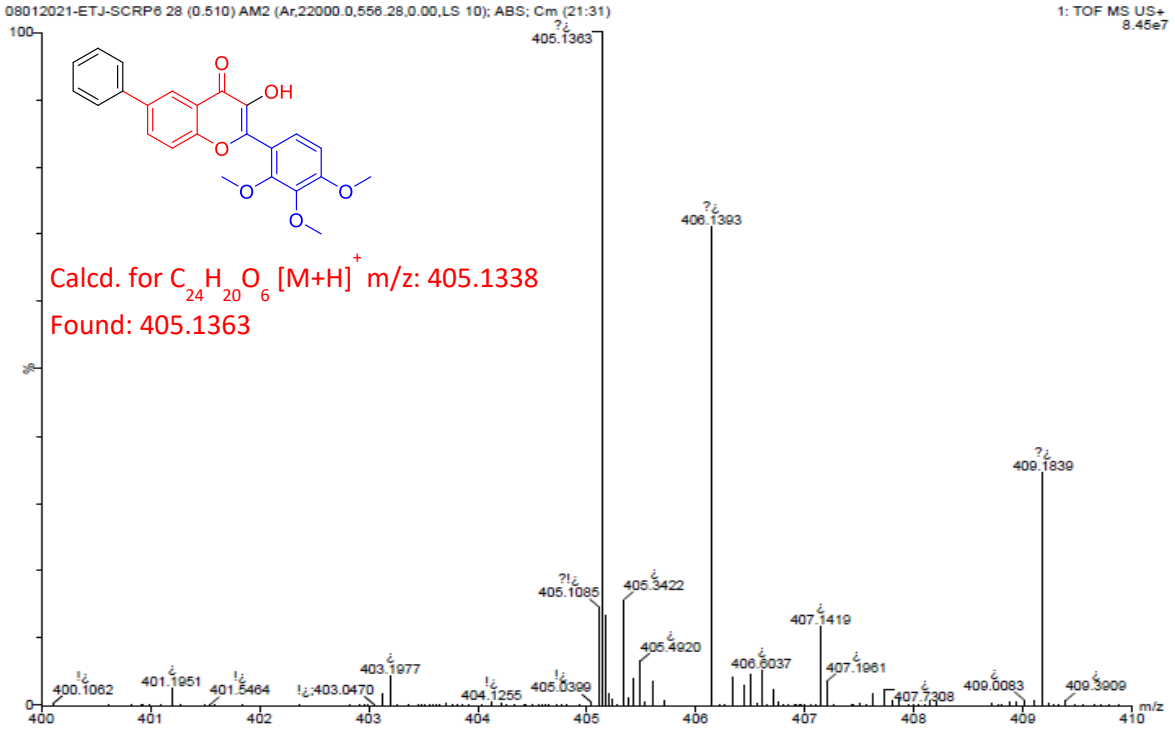

Figure S40 HRMS spectrum of compound 5c

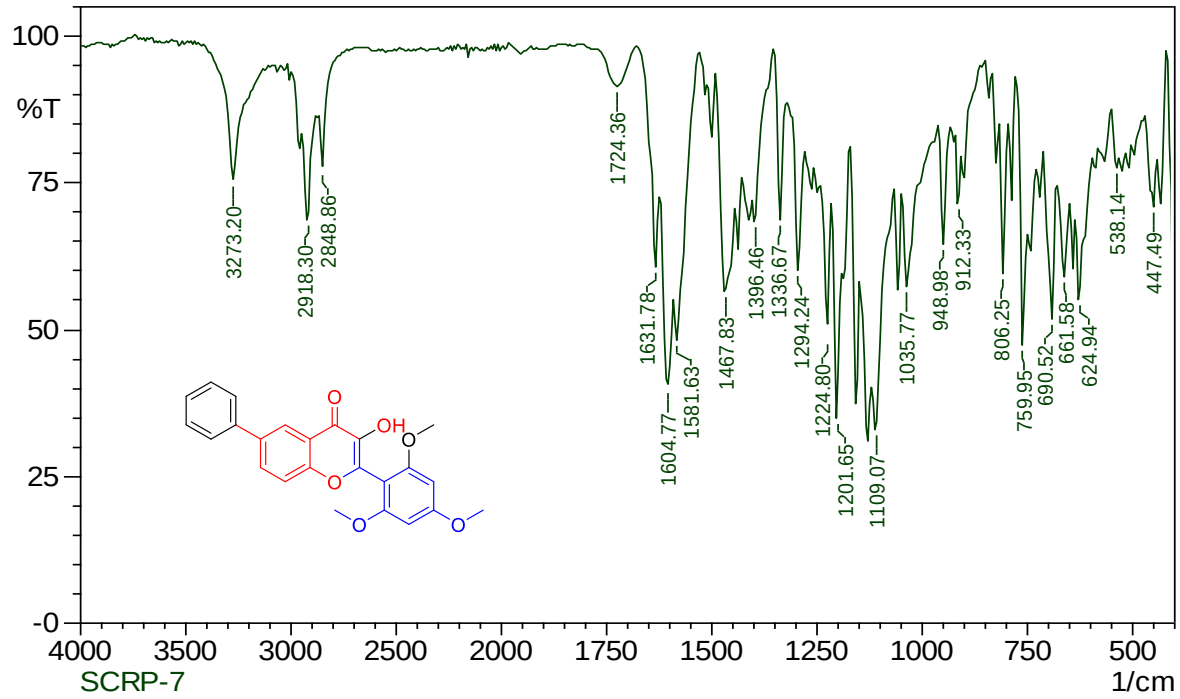

Figure S41 IR spectrum of compound 5d

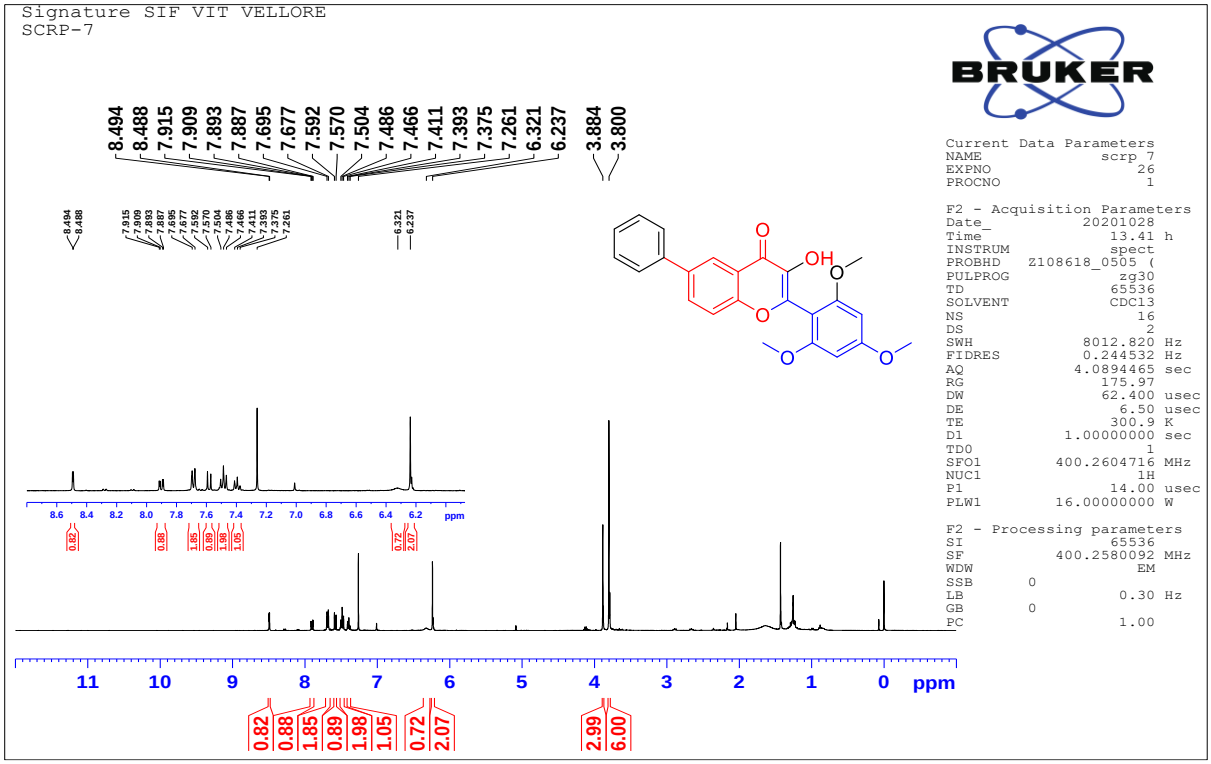

Figure S42 ^{1}H NMR spectrum of compound 5d

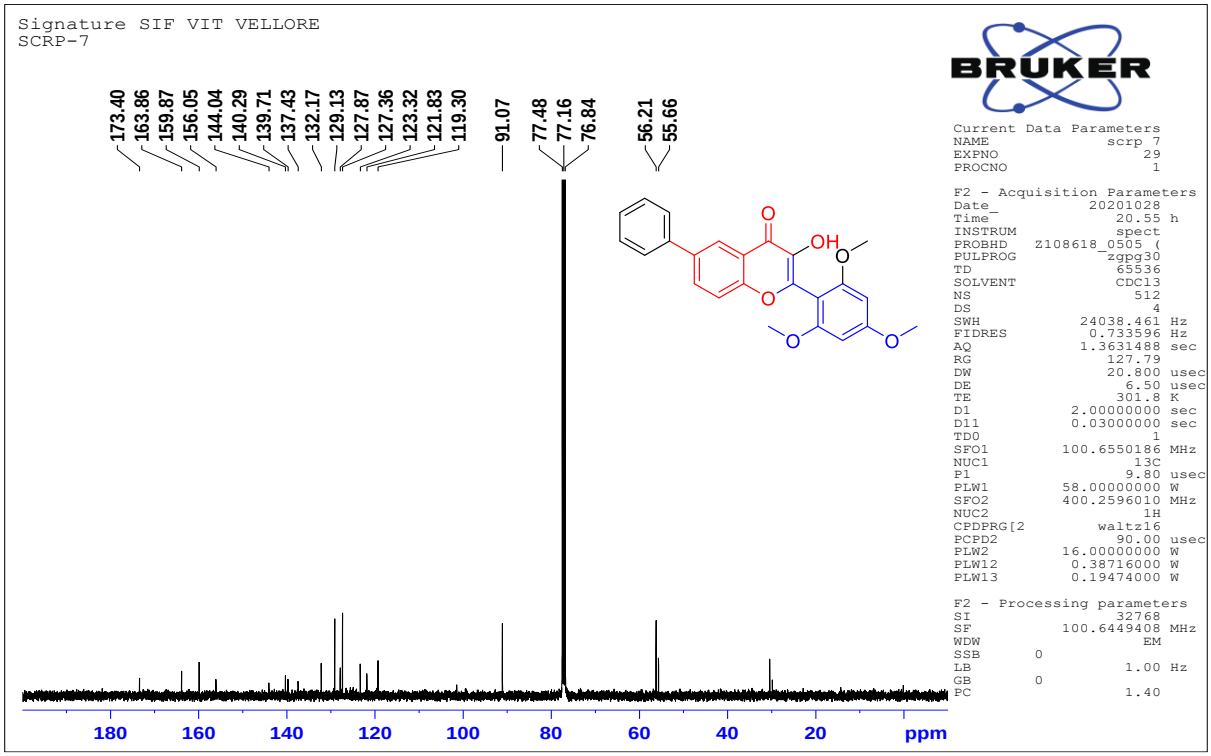

Figure S43 ^{13}C NMR spectrum of compound 5d

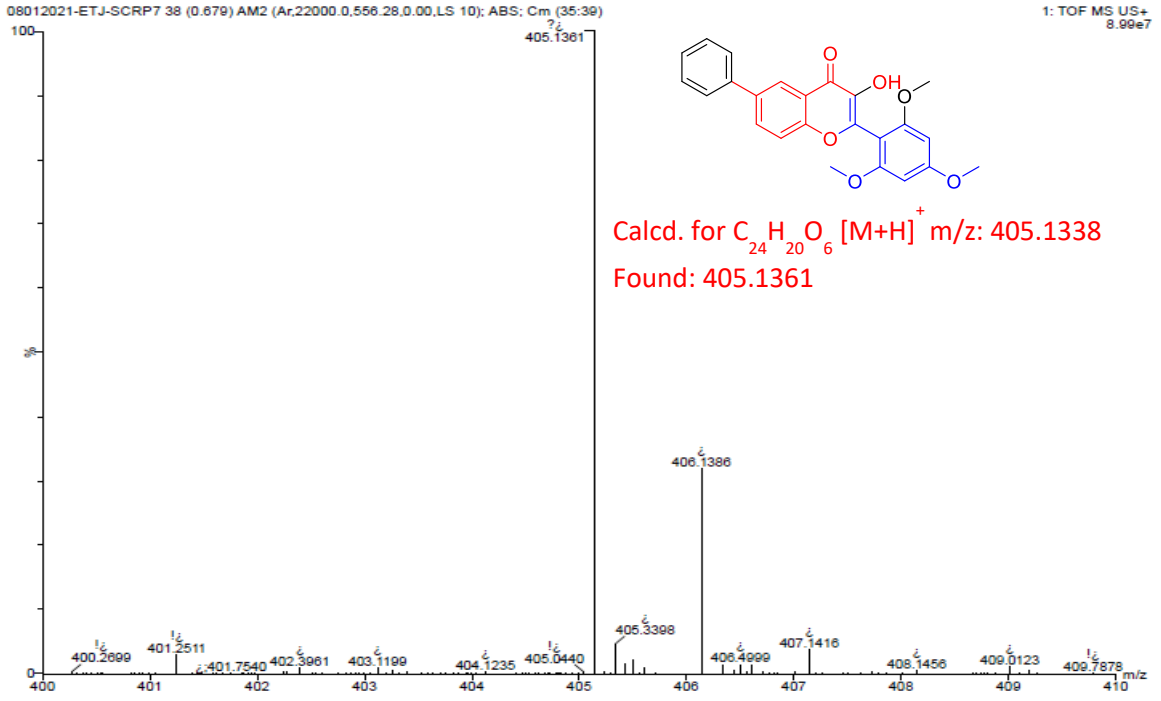

Figure S44 HRMS spectrum of compound 5d

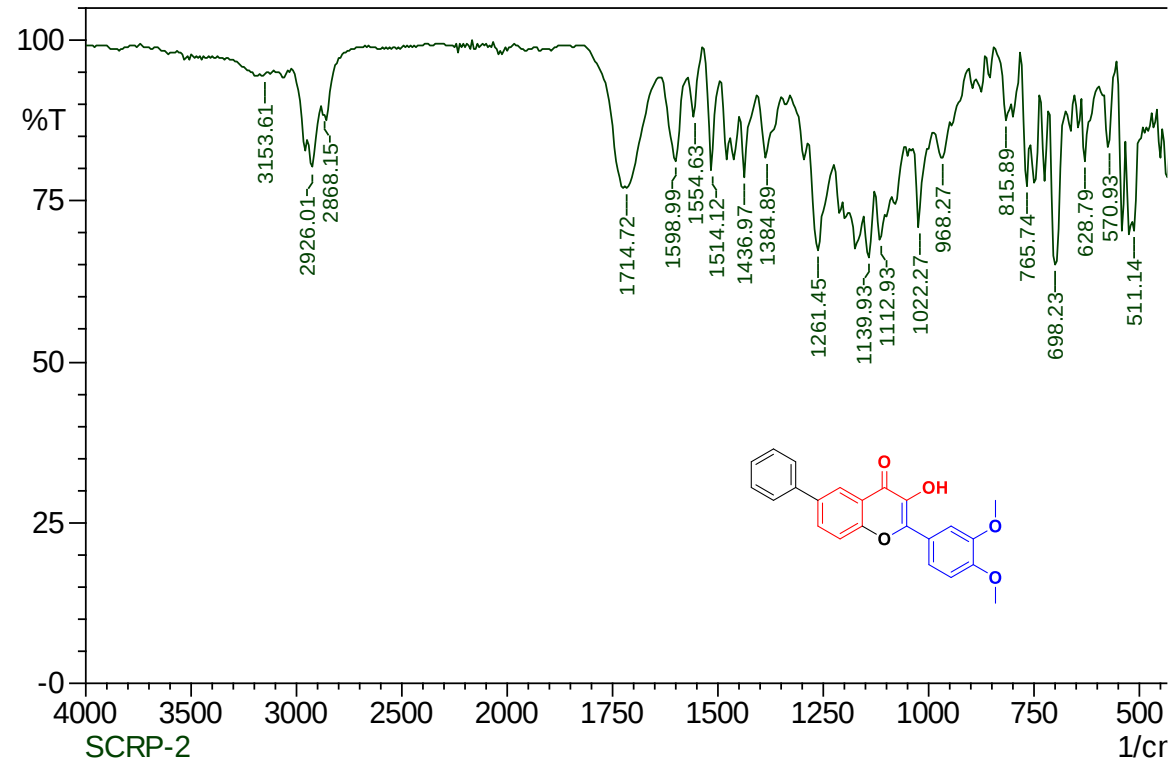

Figure S45 IR spectrum of compound 5e

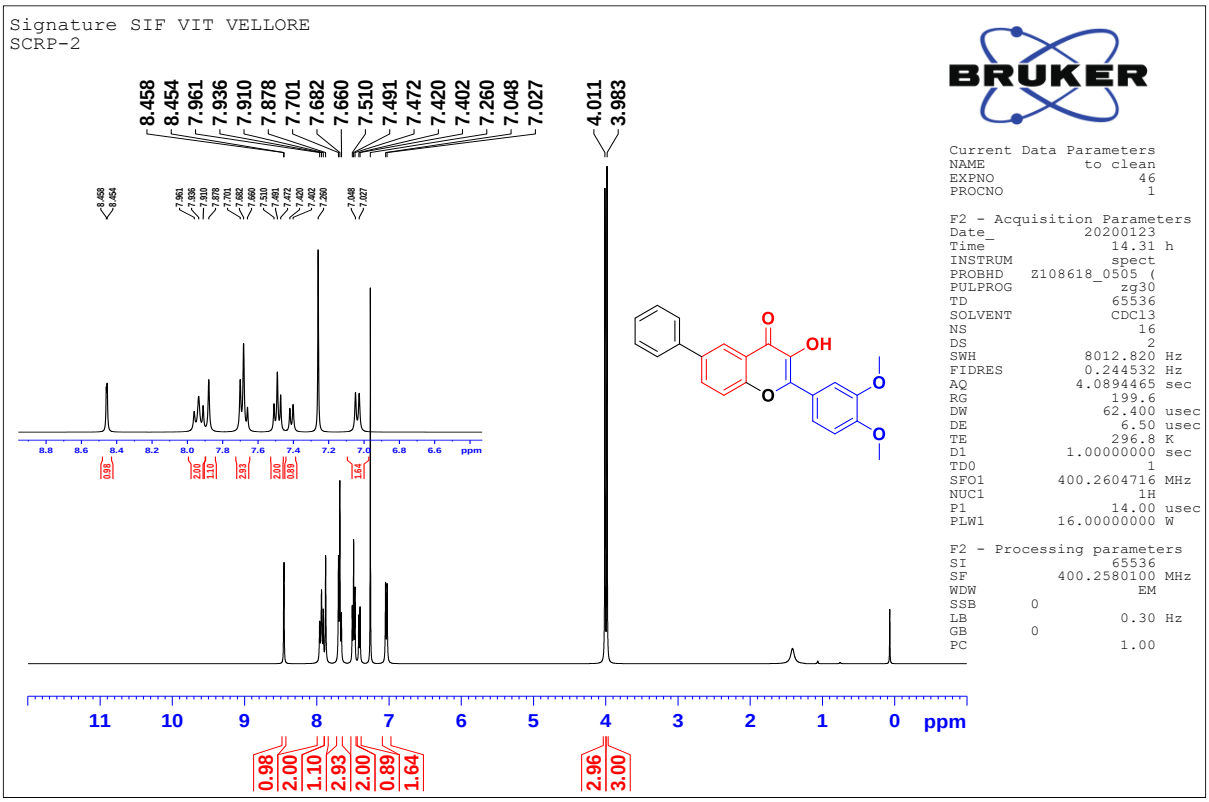

Figure S46 ^{1}H NMR spectrum of compound 5e

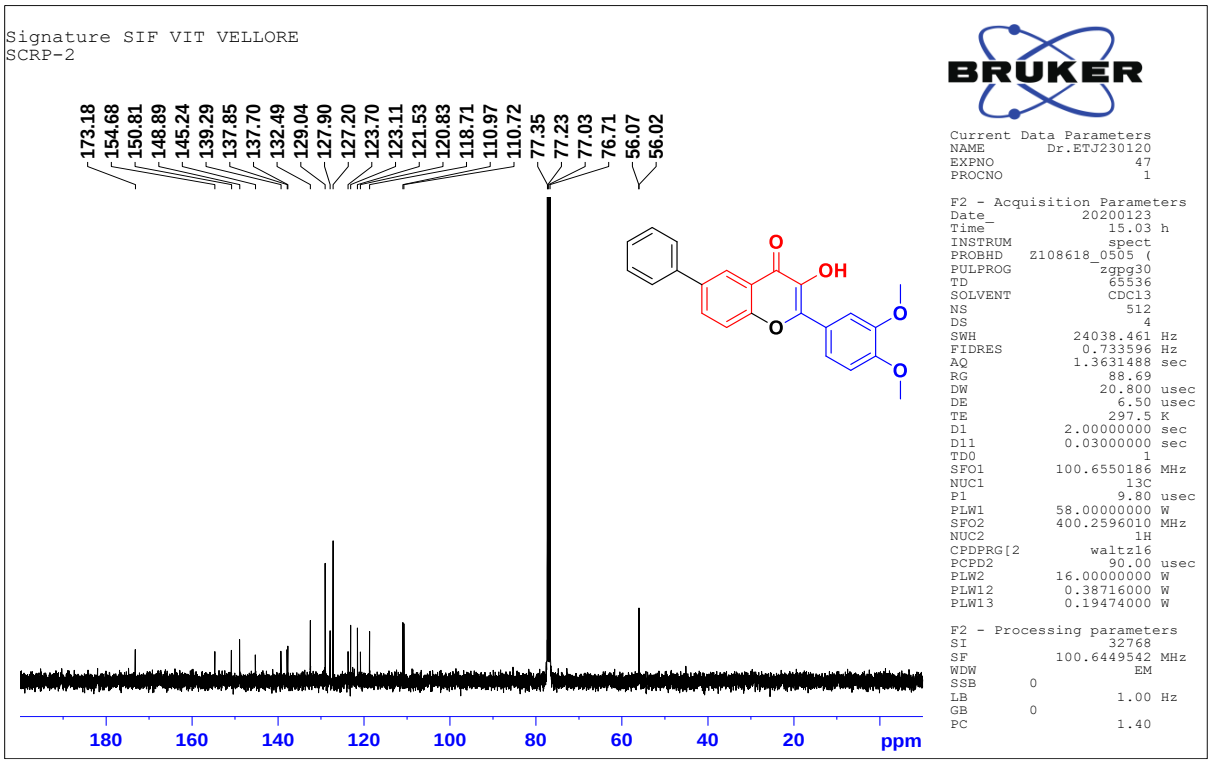

Figure S47 ^{13}C NMR spectrum of compound 5e

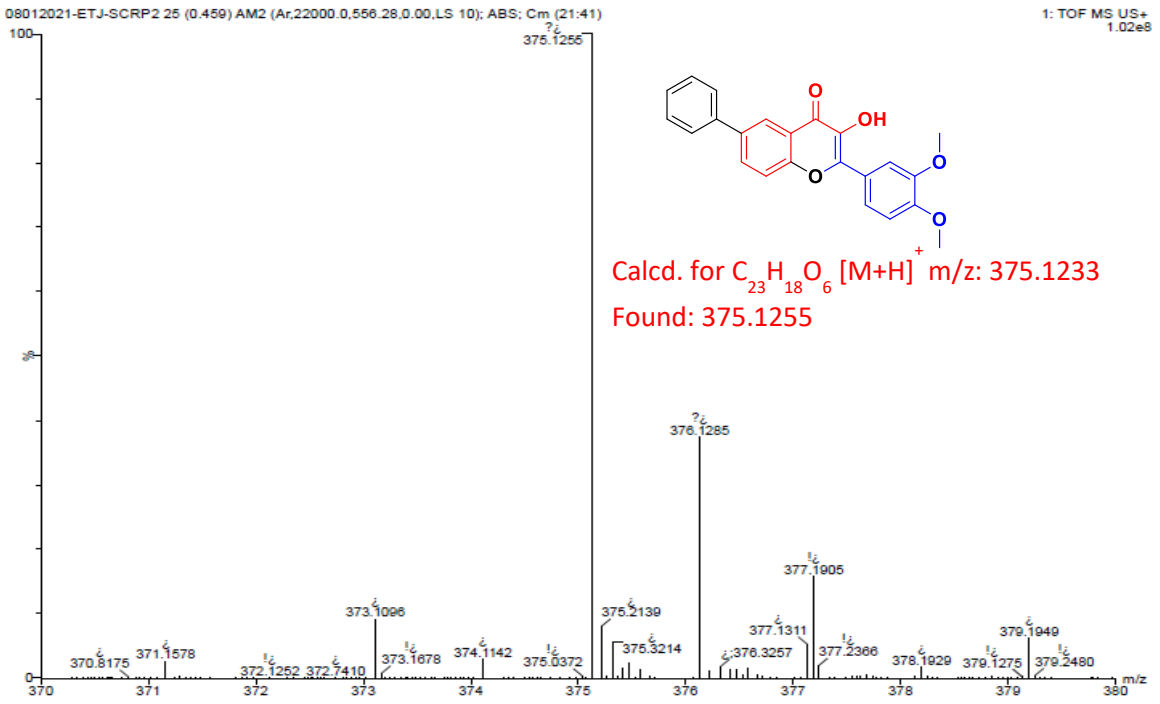

Figure S48 HRMS spectrum of compound 5e

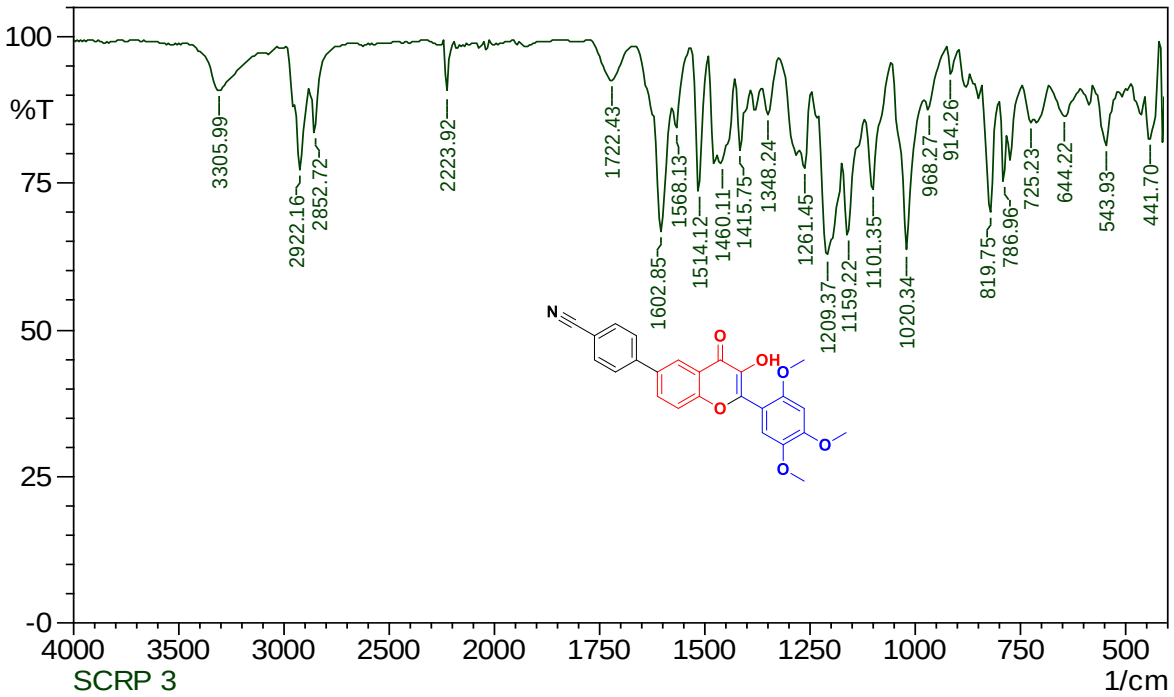

Figure S49 IR spectrum of compound 5f

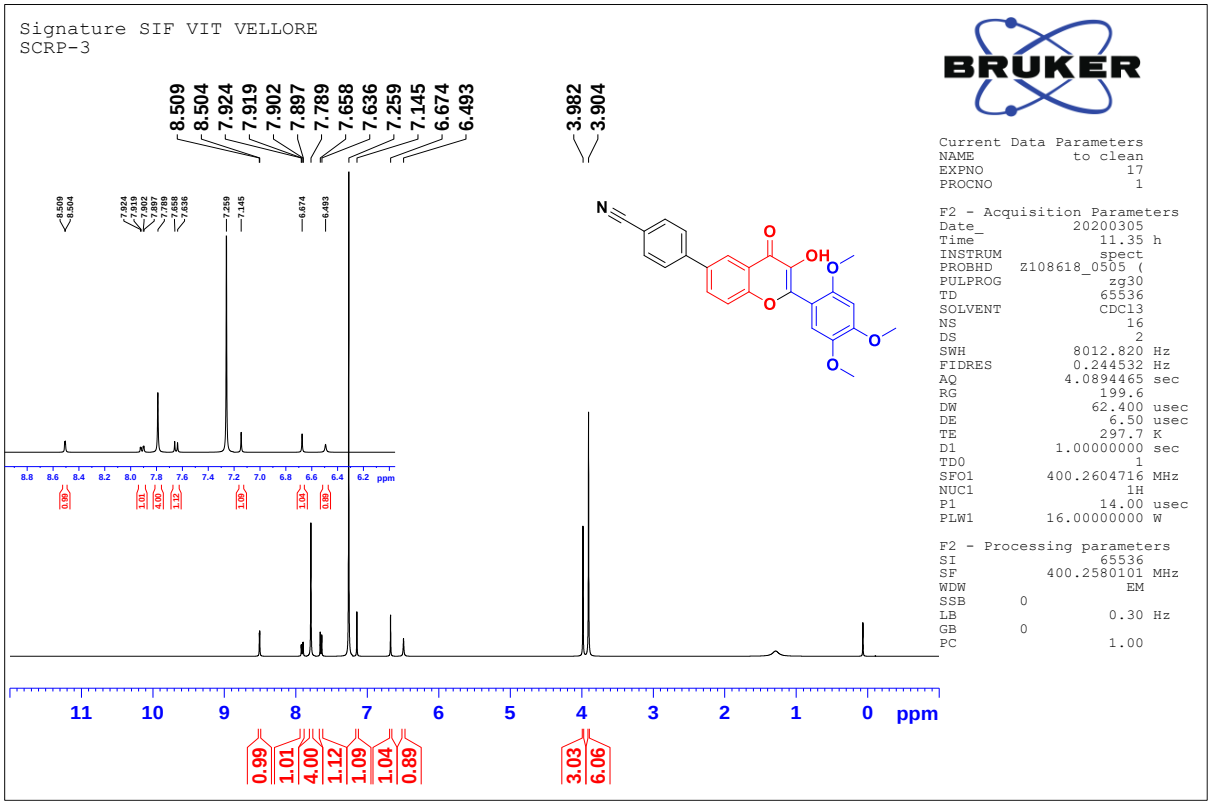

Figure S50 ^{1}H NMR spectrum of compound 5f

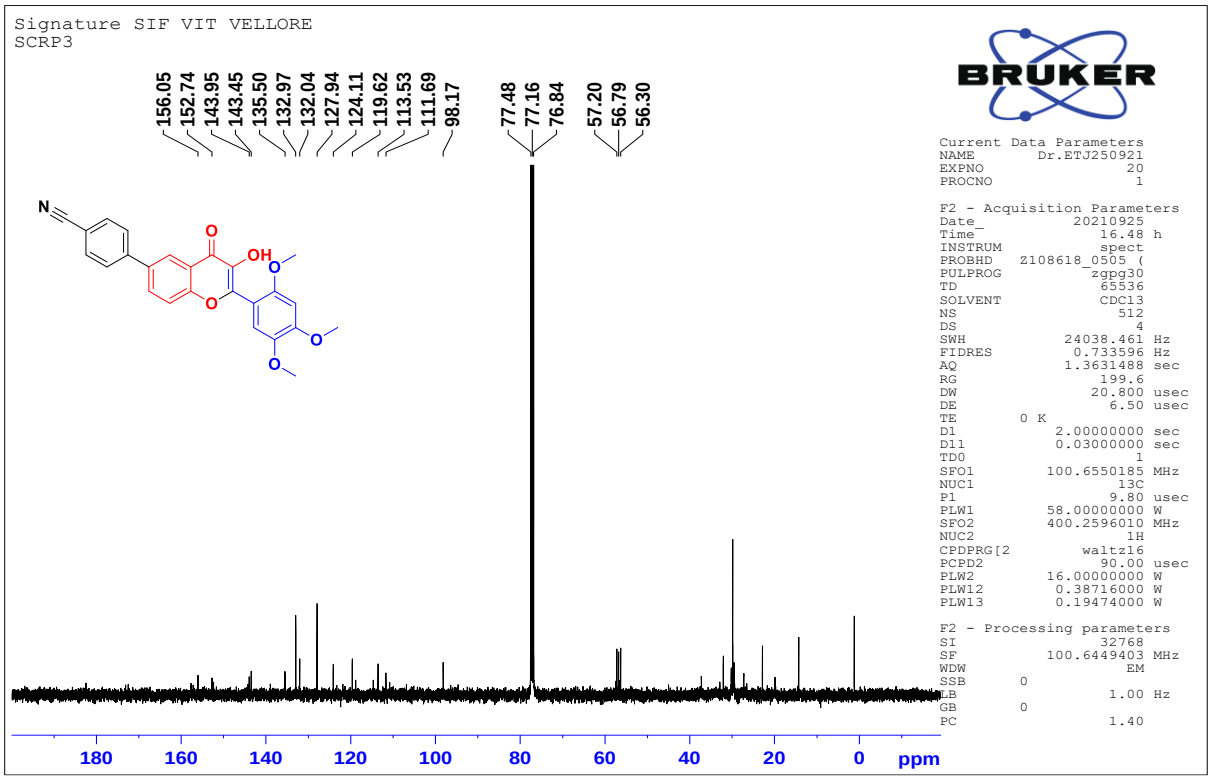

Figure S51 ^{13}C NMR spectrum of compound 5f

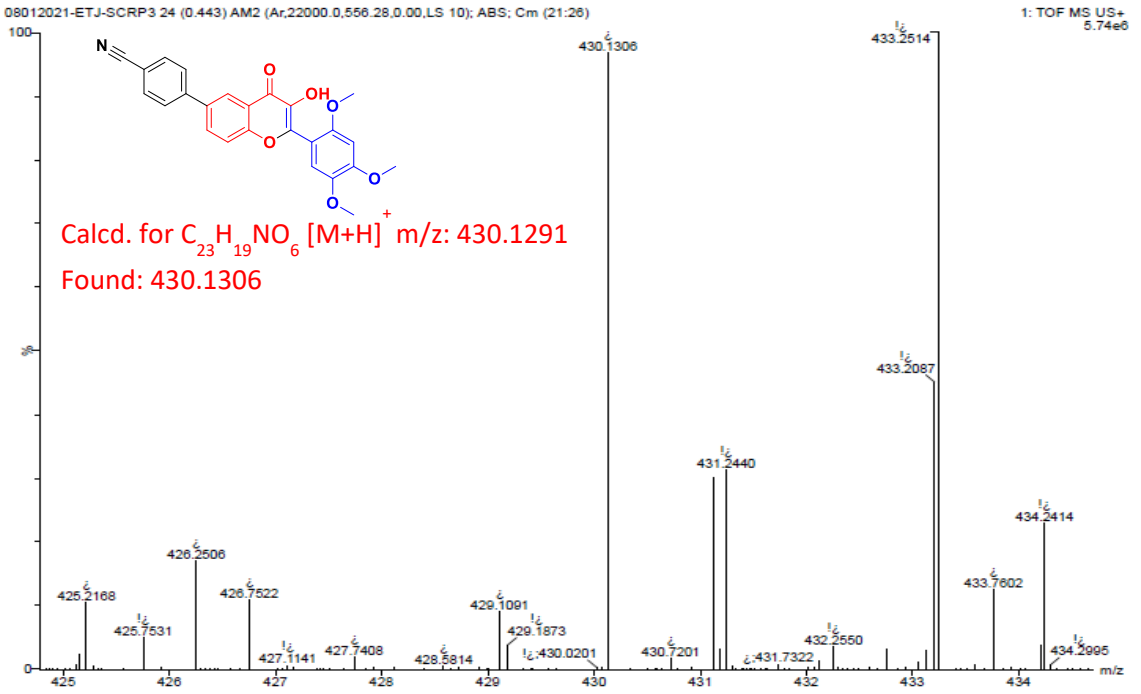

Figure S52 HRMS spectrum of compound 5f

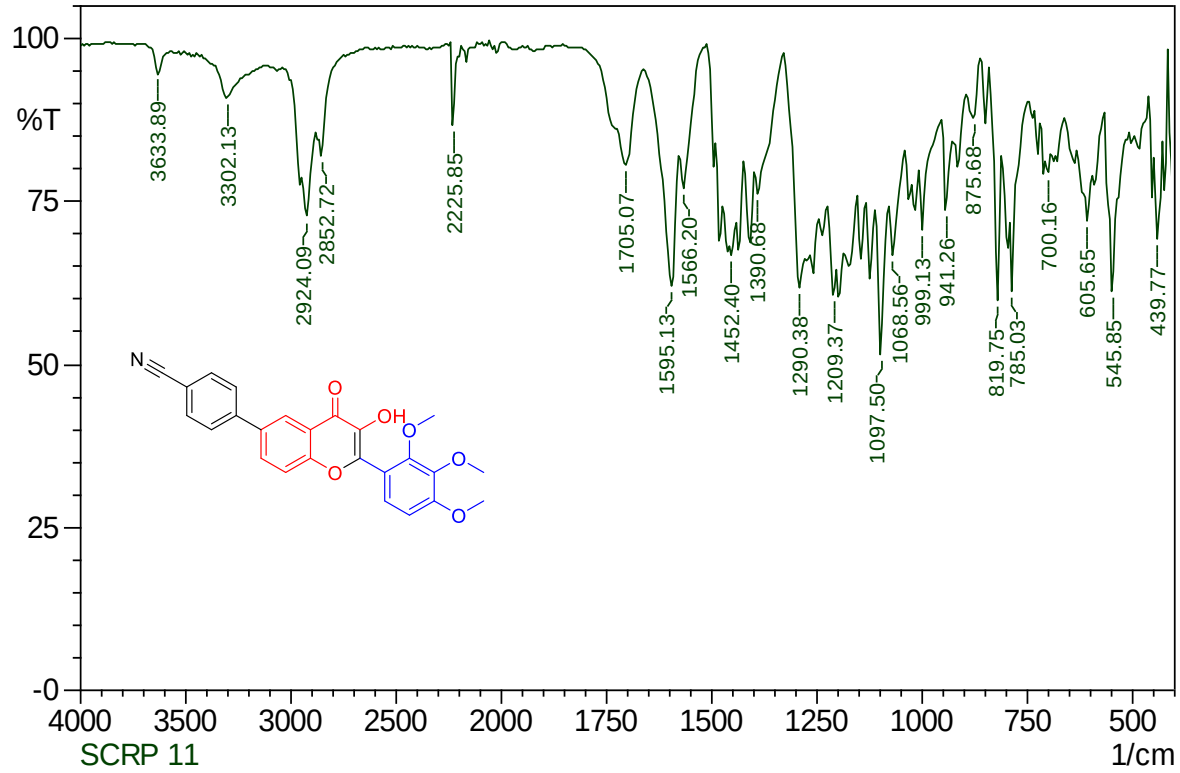

Figure S53 IR spectrum of compound 5g

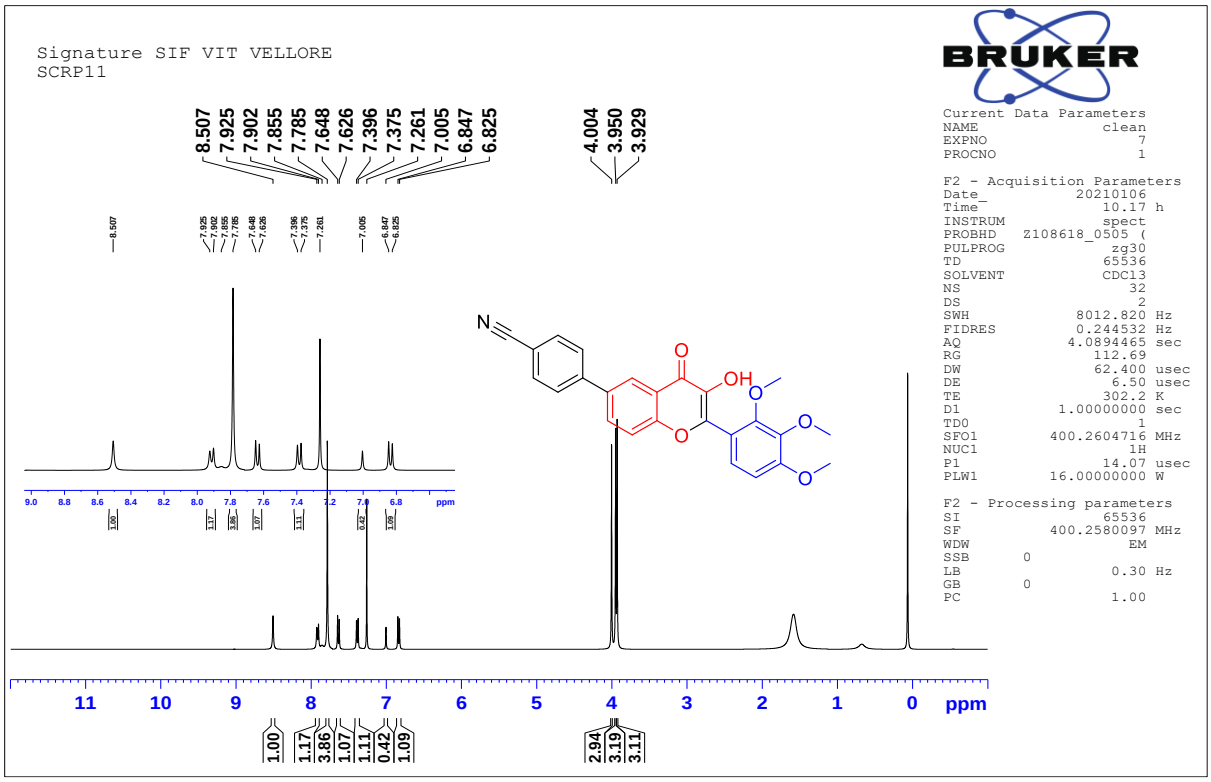

Figure 54 ^{1}H NMR spectrum of compound 5g

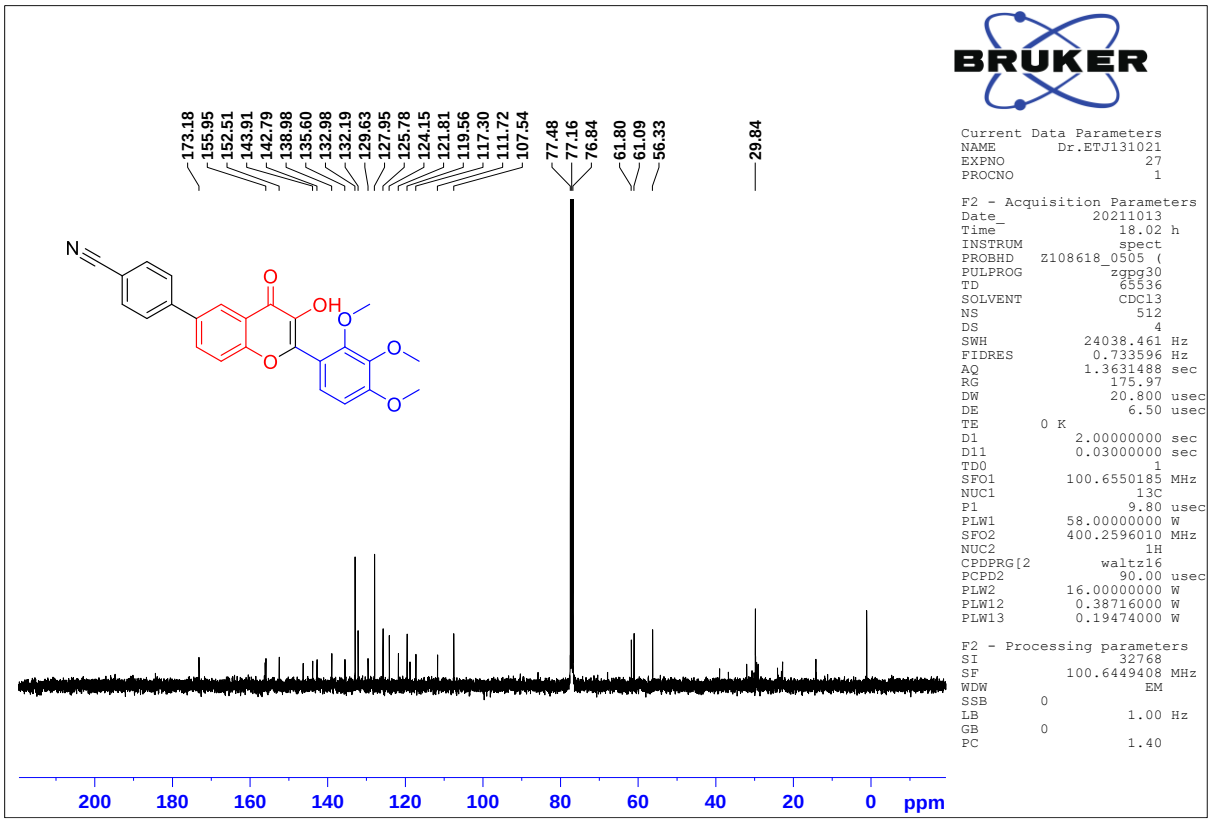

Figure S55 ^{13}C NMR spectrum of compound 5g

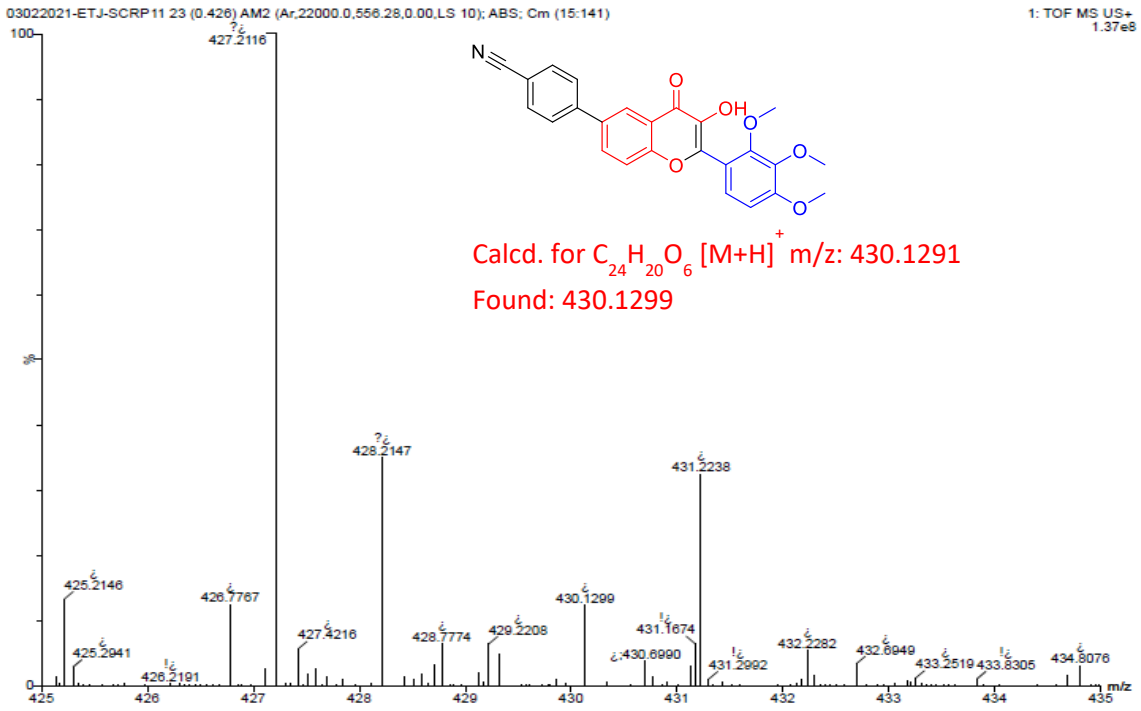

Figure S56 HRMS spectrum of compound 5g

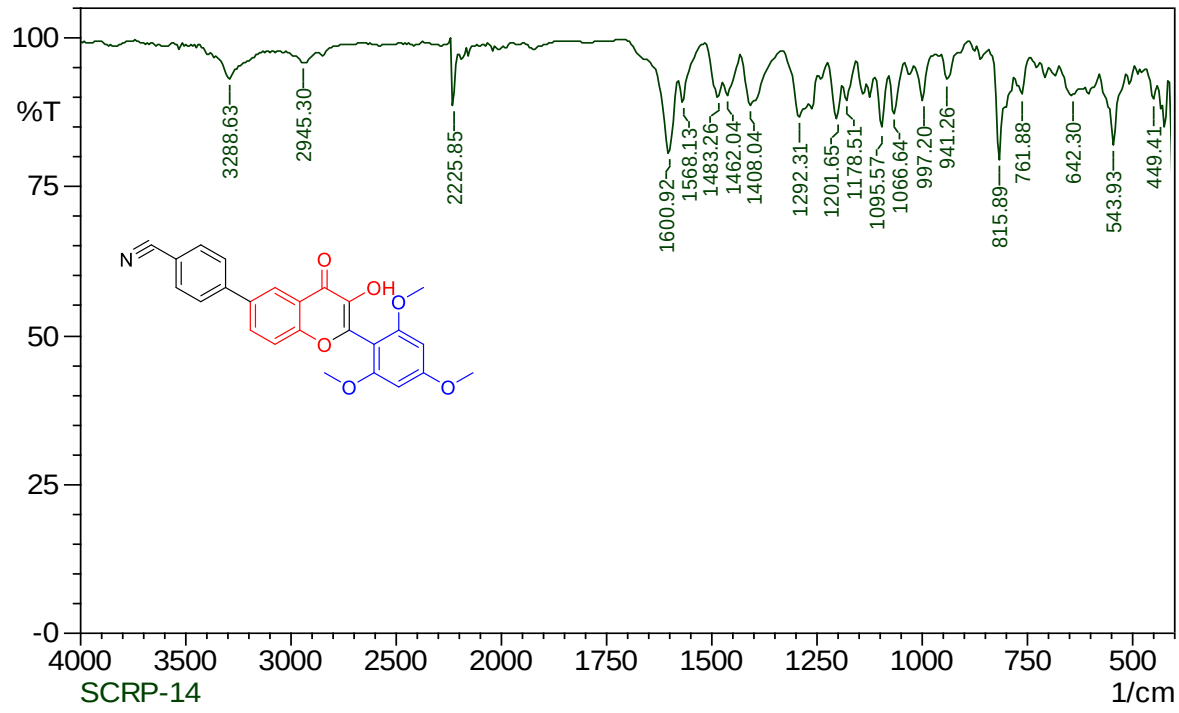

Figure S57 IR spectrum of compound 5h

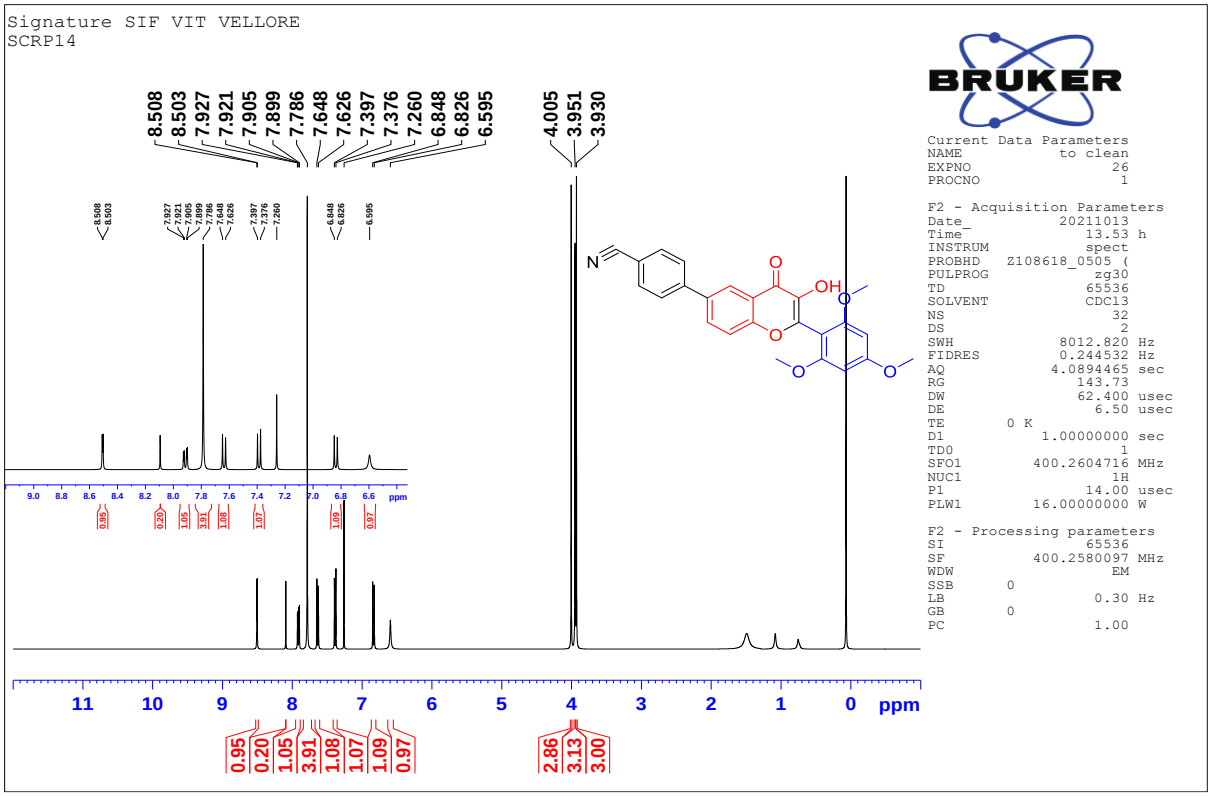

Figure S58 ^{1}H NMR spectrum of compound 5h

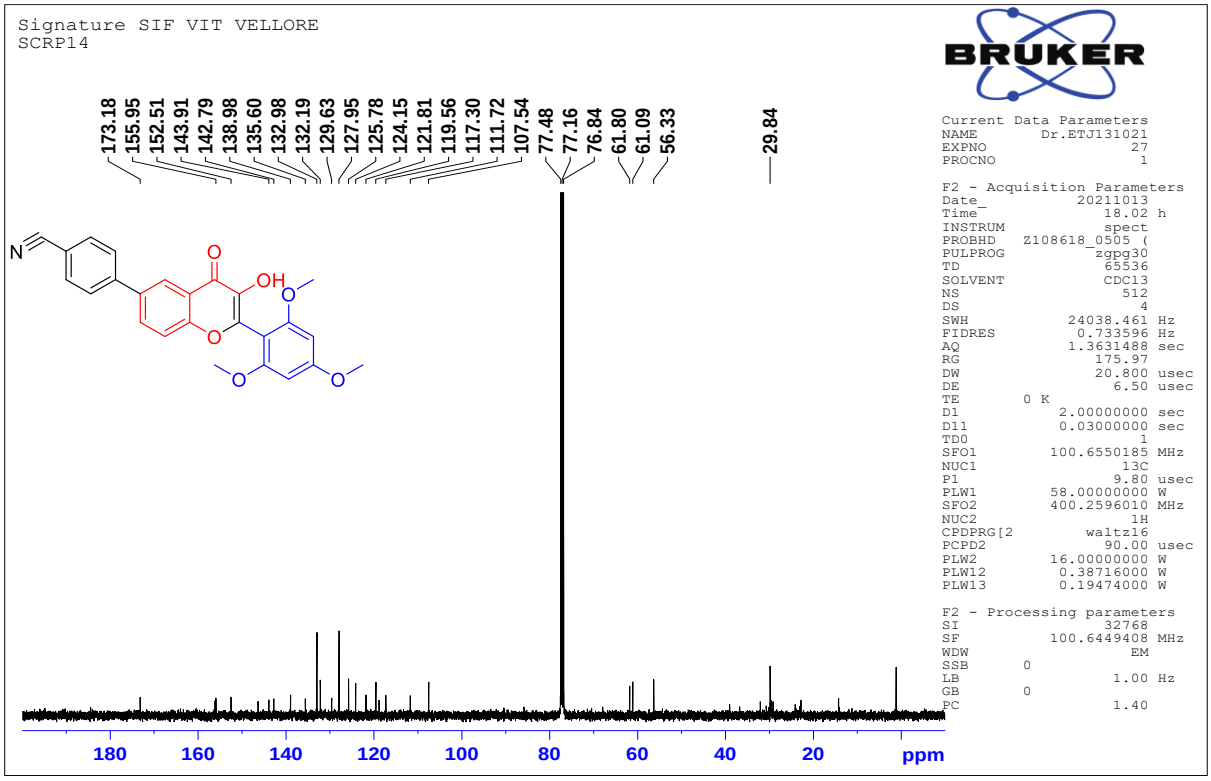

Figure S59 ^{13} C NMR spectrum of compound 5h

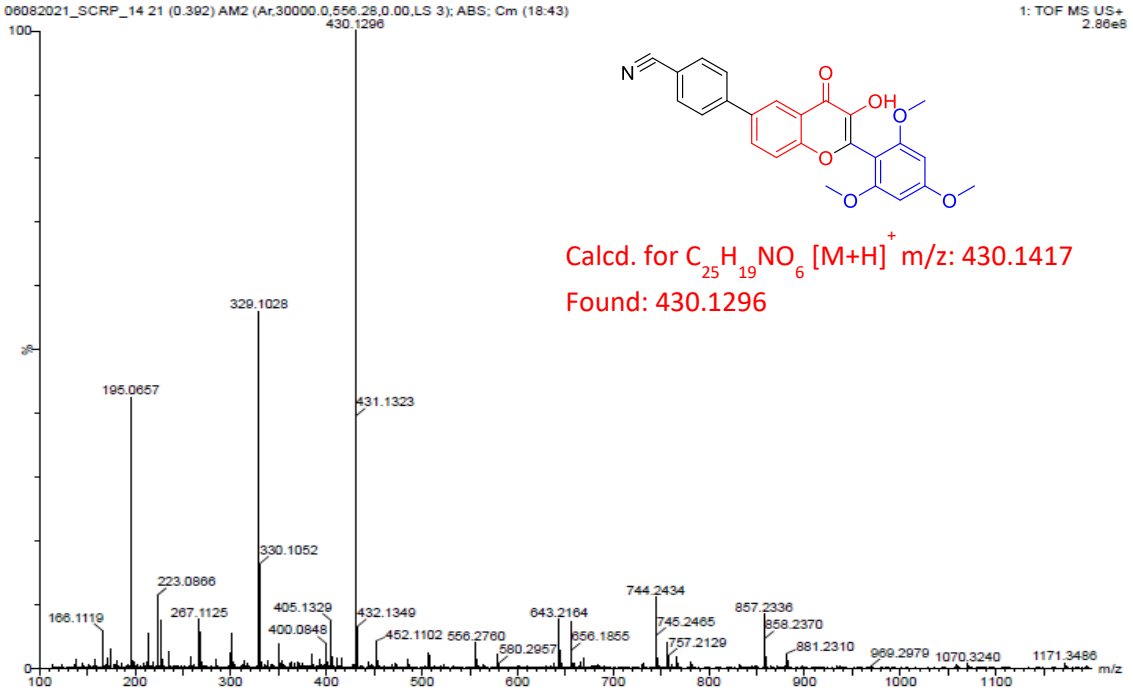

Figure S60 HRMS spectrum of compound 5h

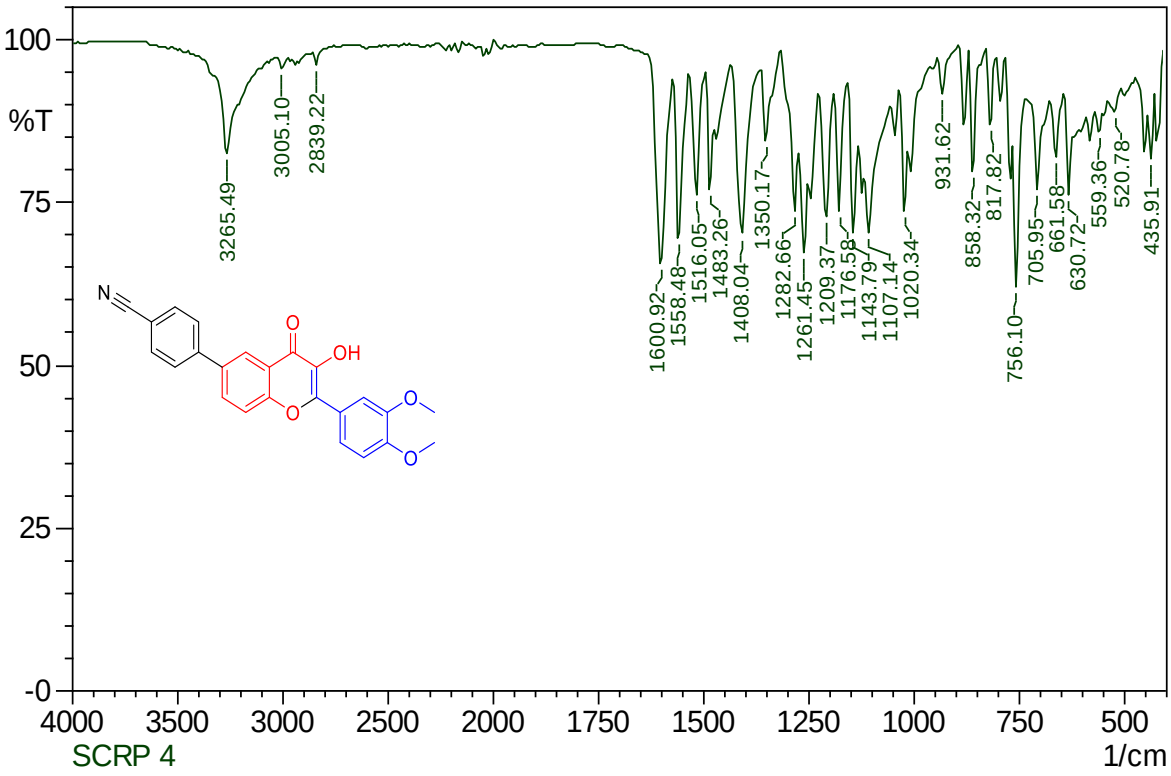

Figure S61 IR spectrum of compound 5i

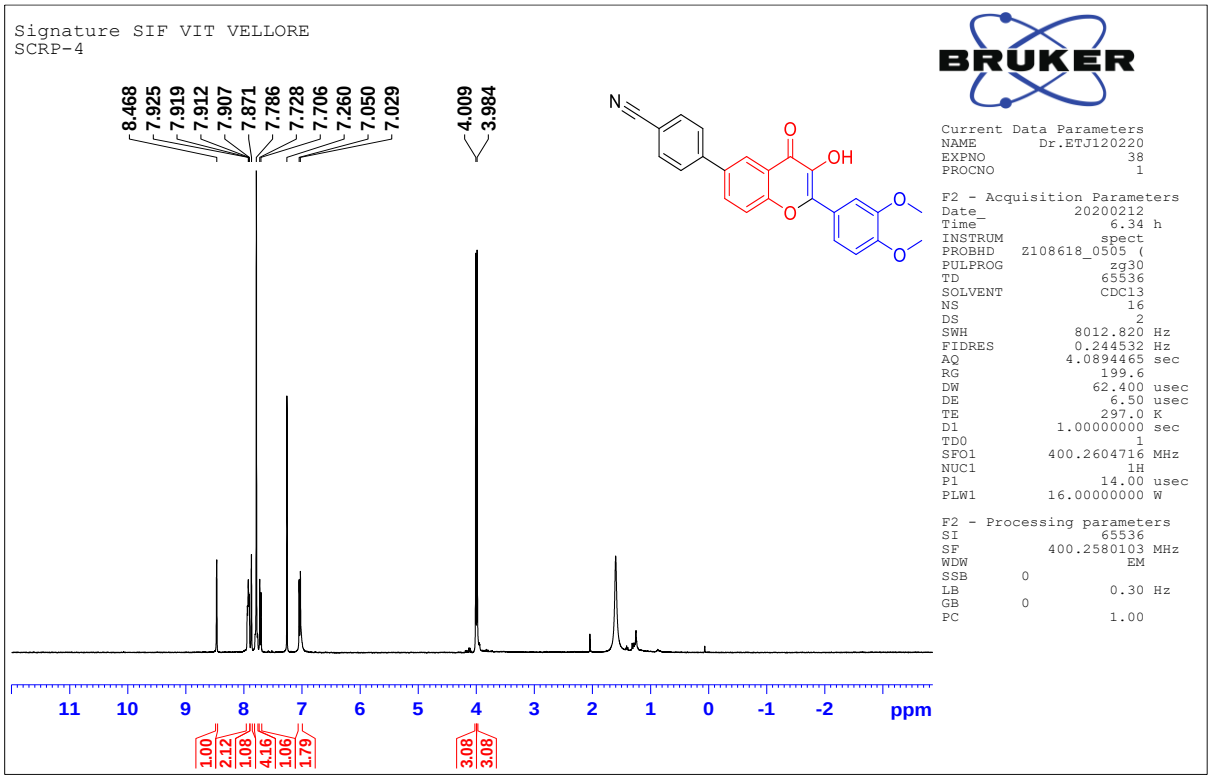

Figure S62 ^{1}H NMR spectrum of compound 5i

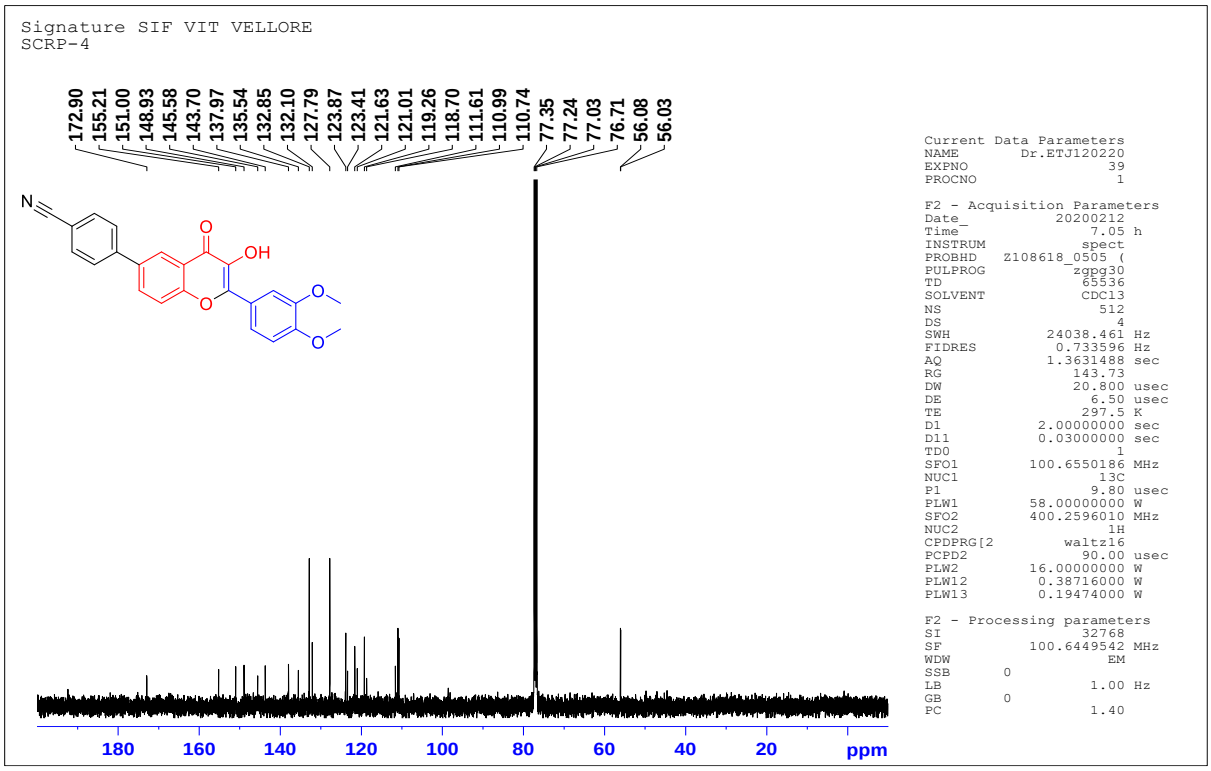

Figure S63 ^{13} C NMR spectrum of compound 5i

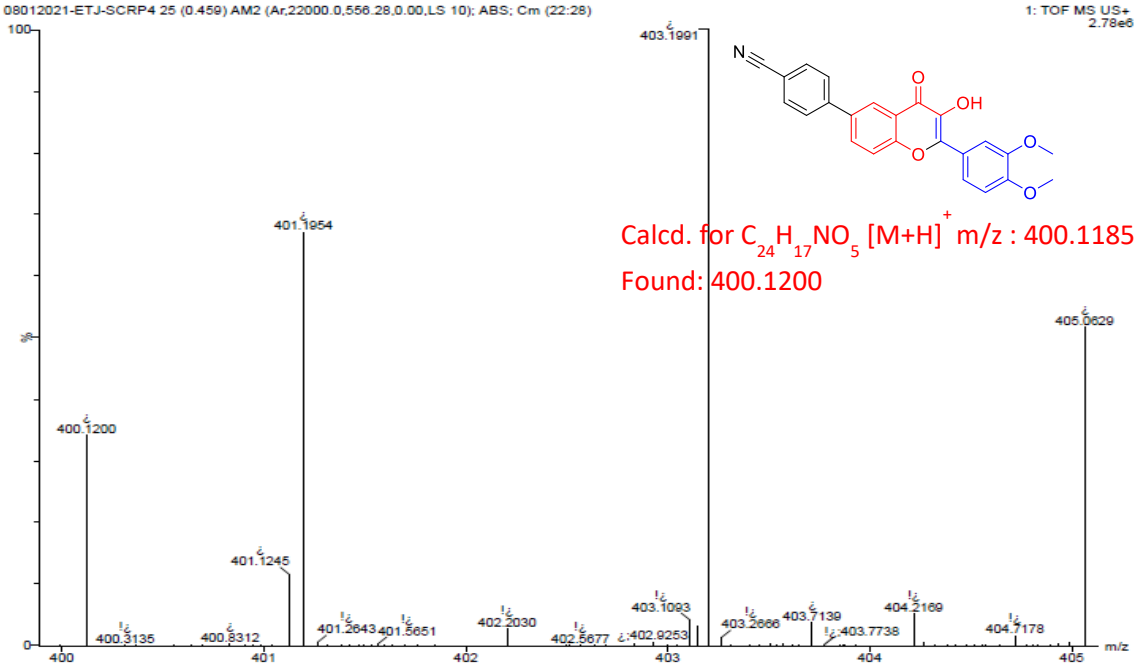

Figure S64 HRMS spectrum of compound 5i

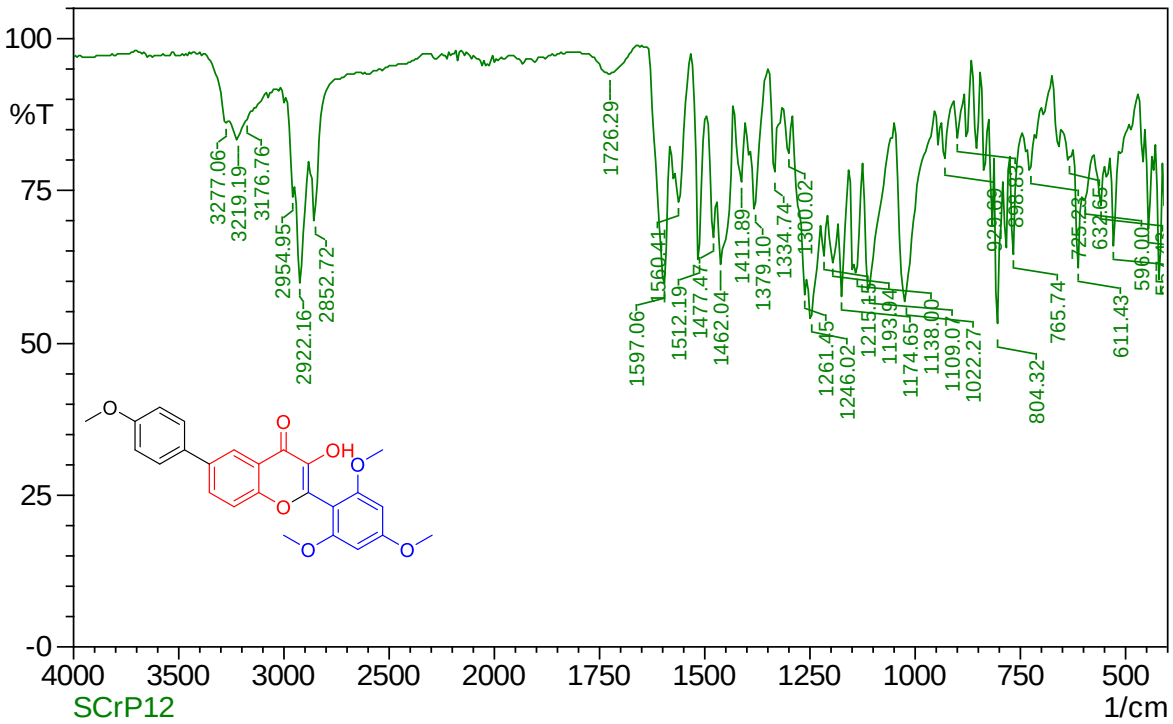

Figure S65 IR spectrum of compound 5j

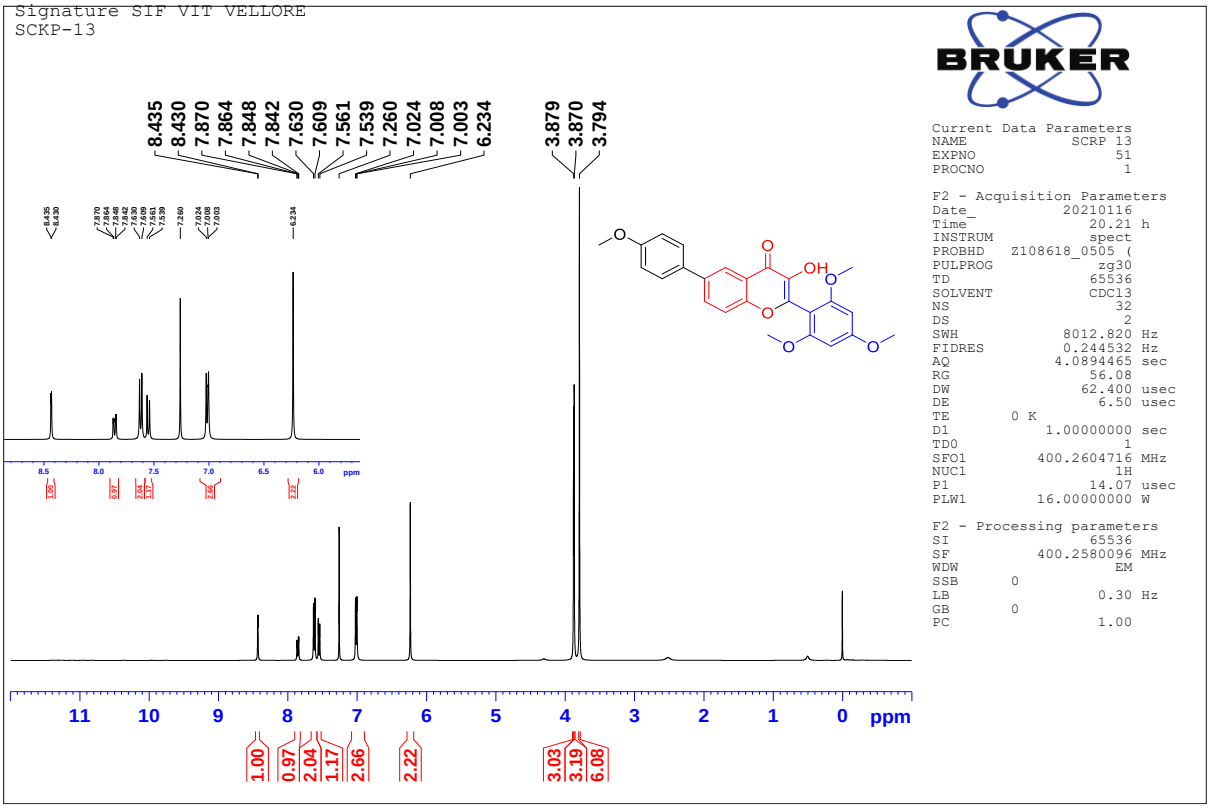

Figure S66 ^{1}H NMR spectrum of compound 5j

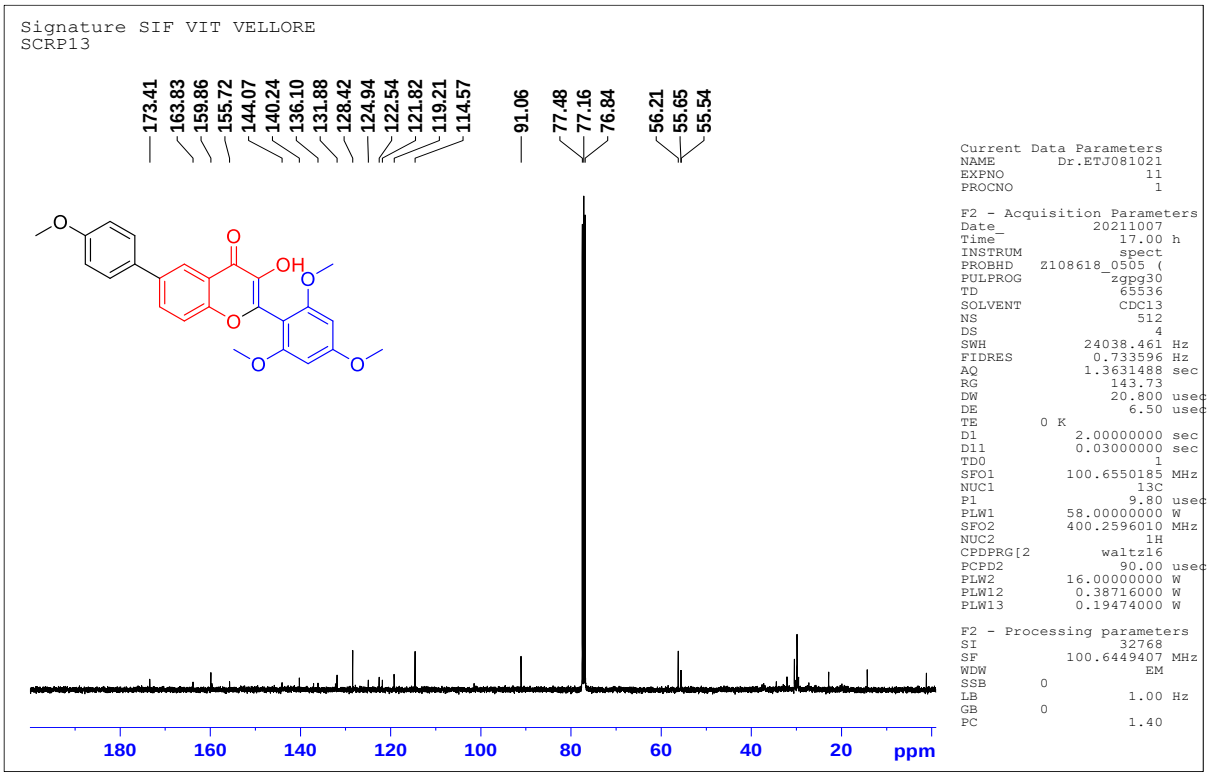

Figure S67 ^{13}C NMR spectrum of compound 5j

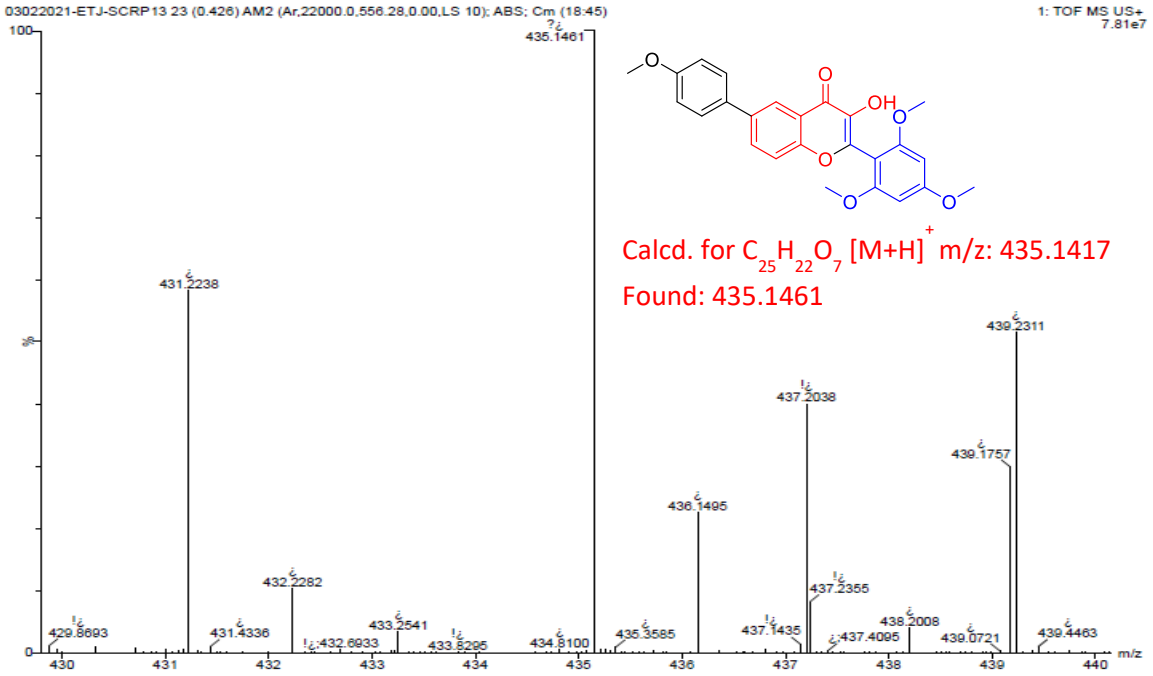

Figure S68 HRMS spectrum of compound 5j

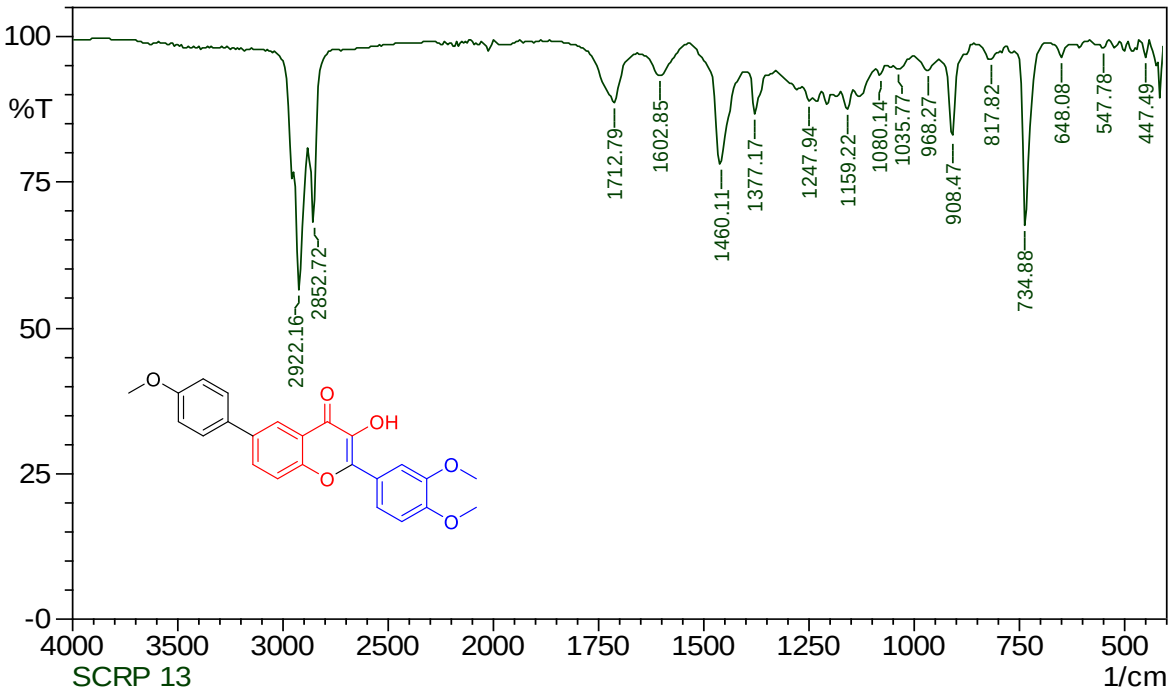

Figure S69 IR spectrum of compound 5k

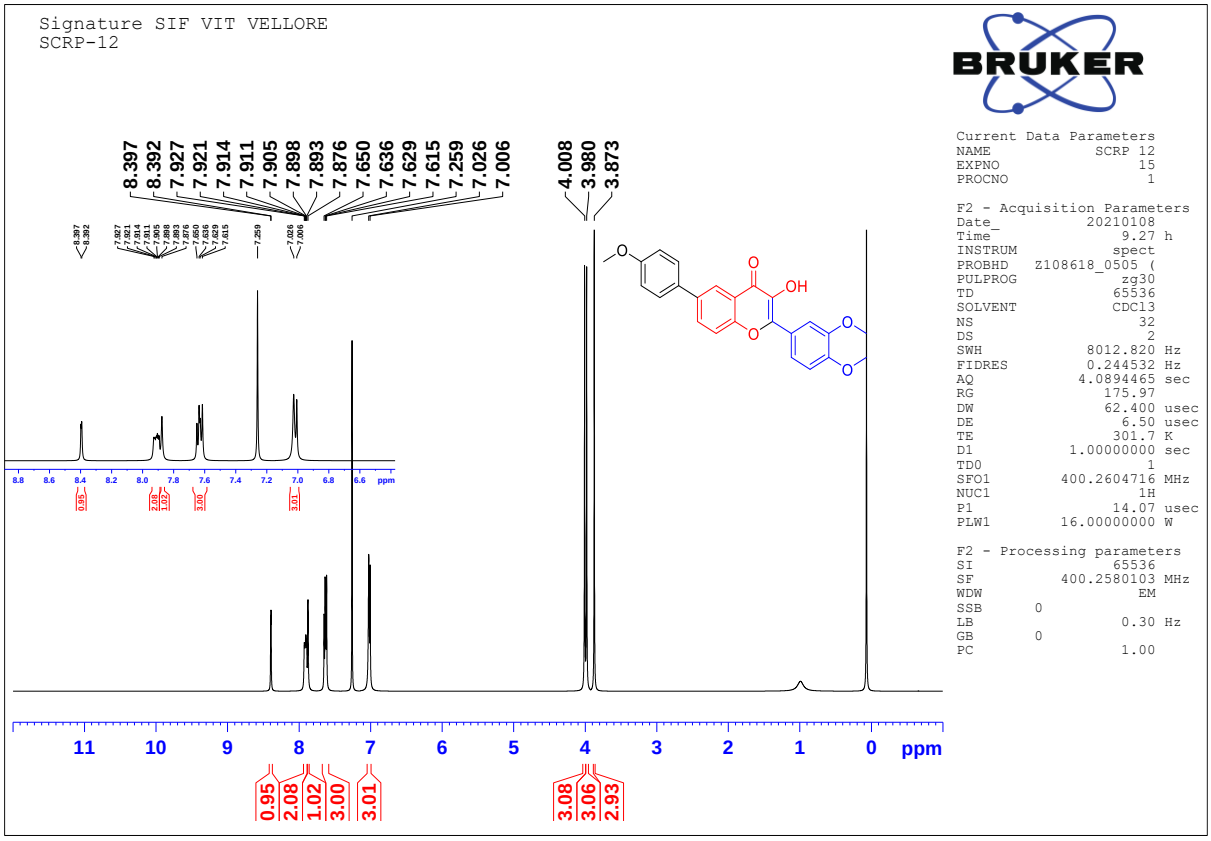

Figure S70 ^{1}H NMR spectrum of compound 5k

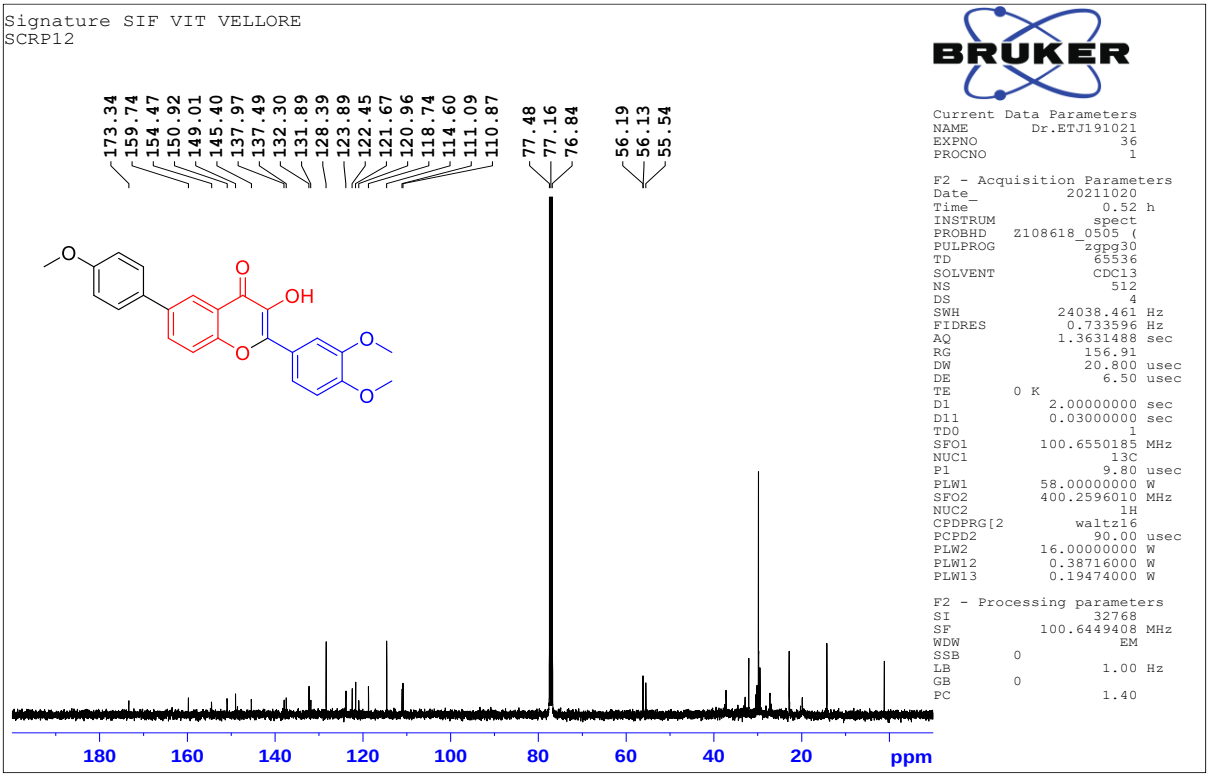

Figure S71 ^{1}H NMR spectrum of compound 5k

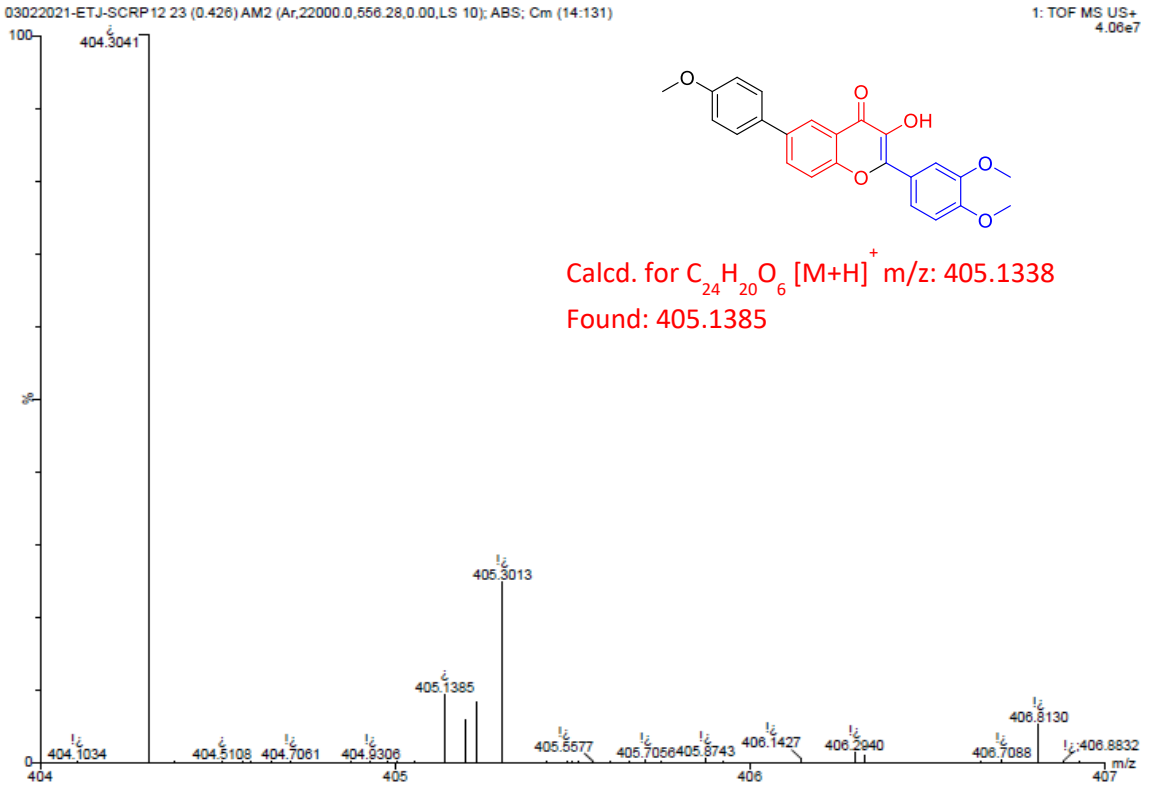

Figure S72 HRMS spectrum of compound 5k

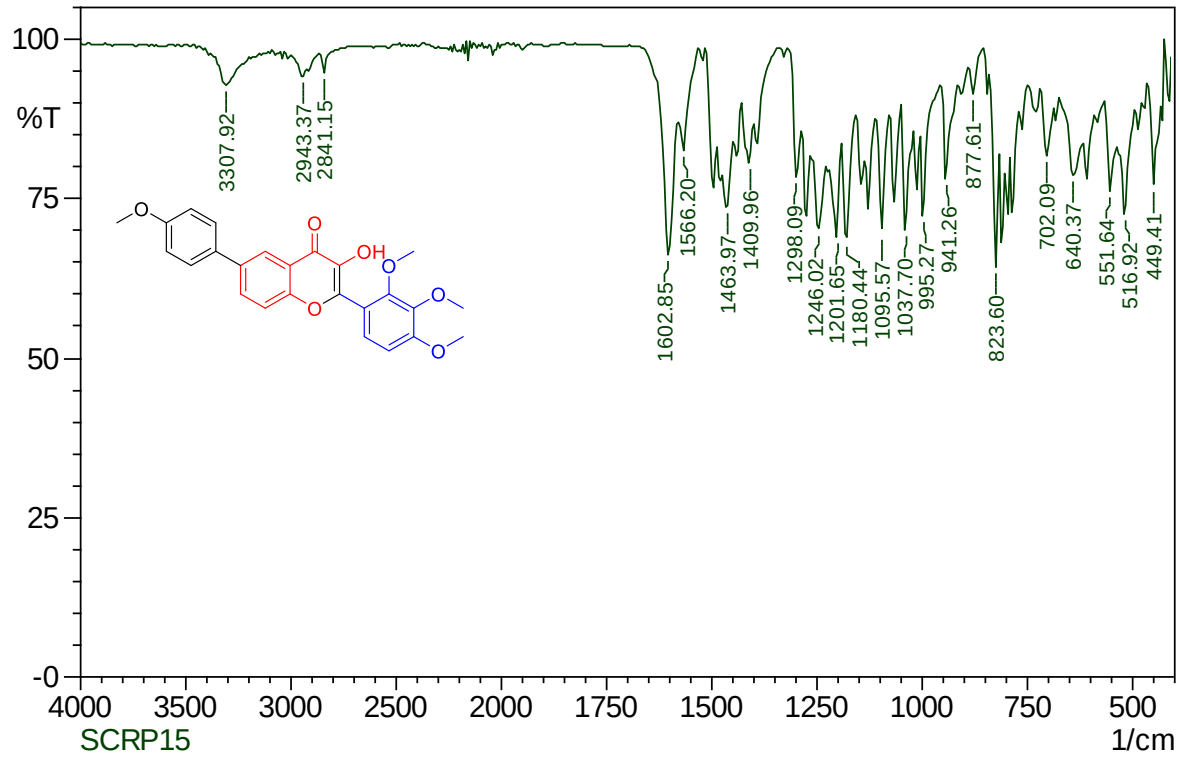

Figure S73 IR spectrum of compound 5l

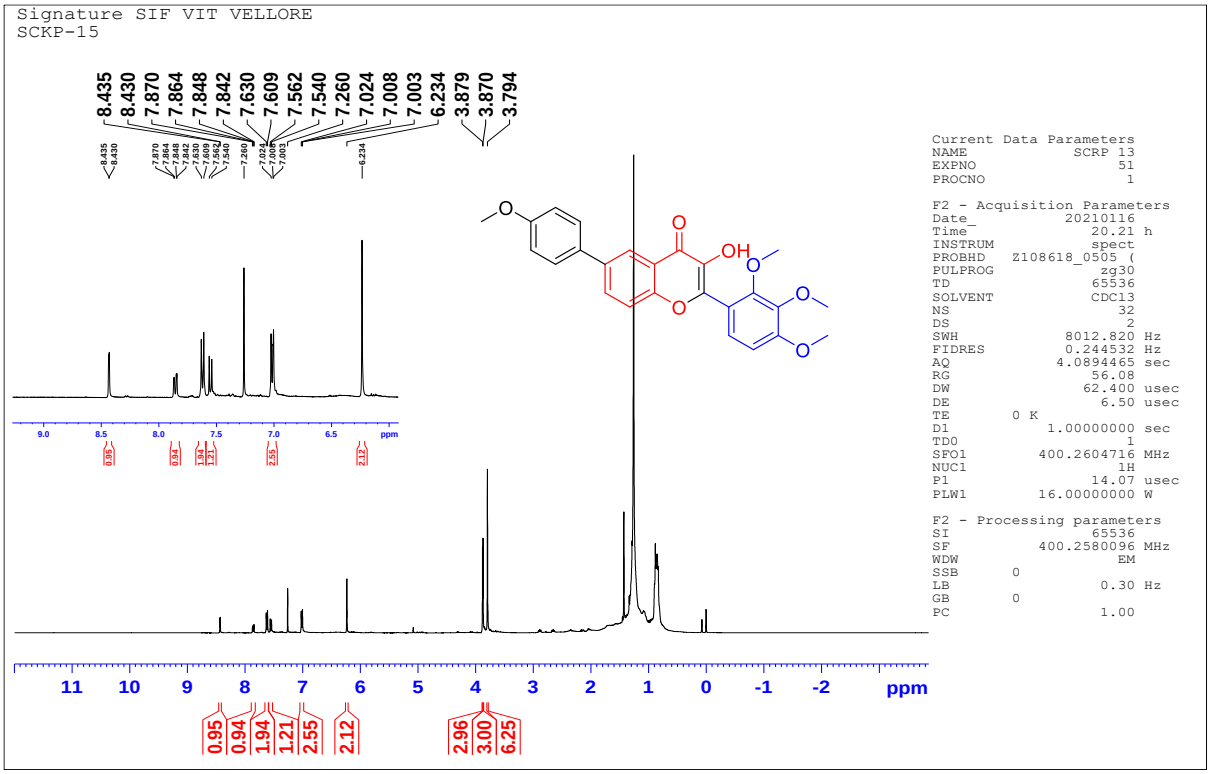

Figure S74 ^{1}H NMR spectrum of compound 5l

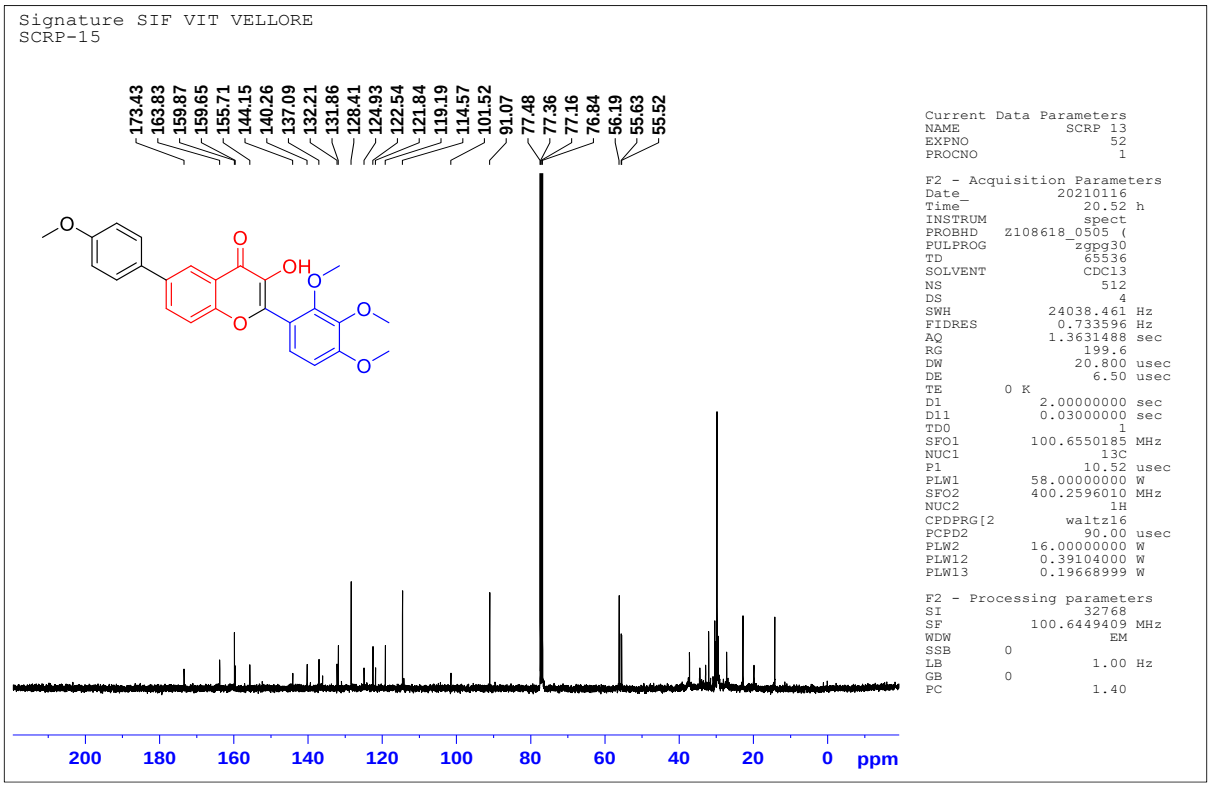

Figure S75 ^{13}C NMR spectrum of compound 5l

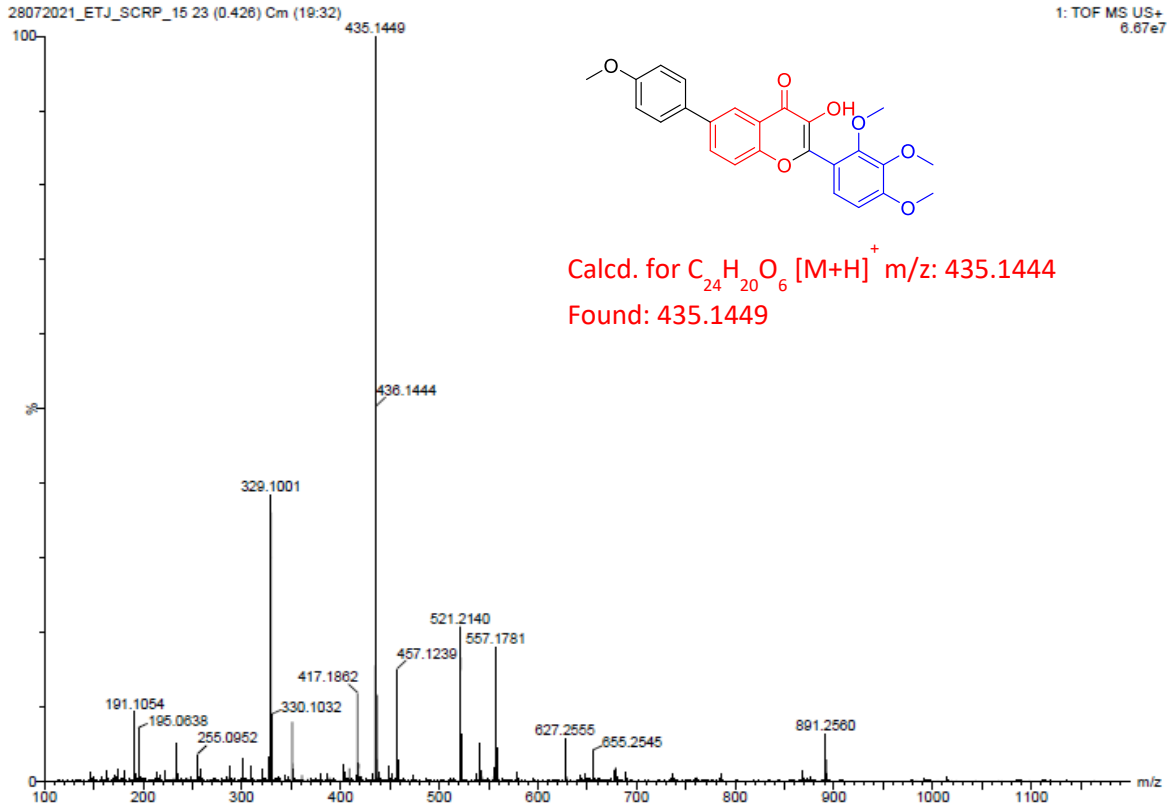

Figure S76 HRMS spectrum of compound 5l

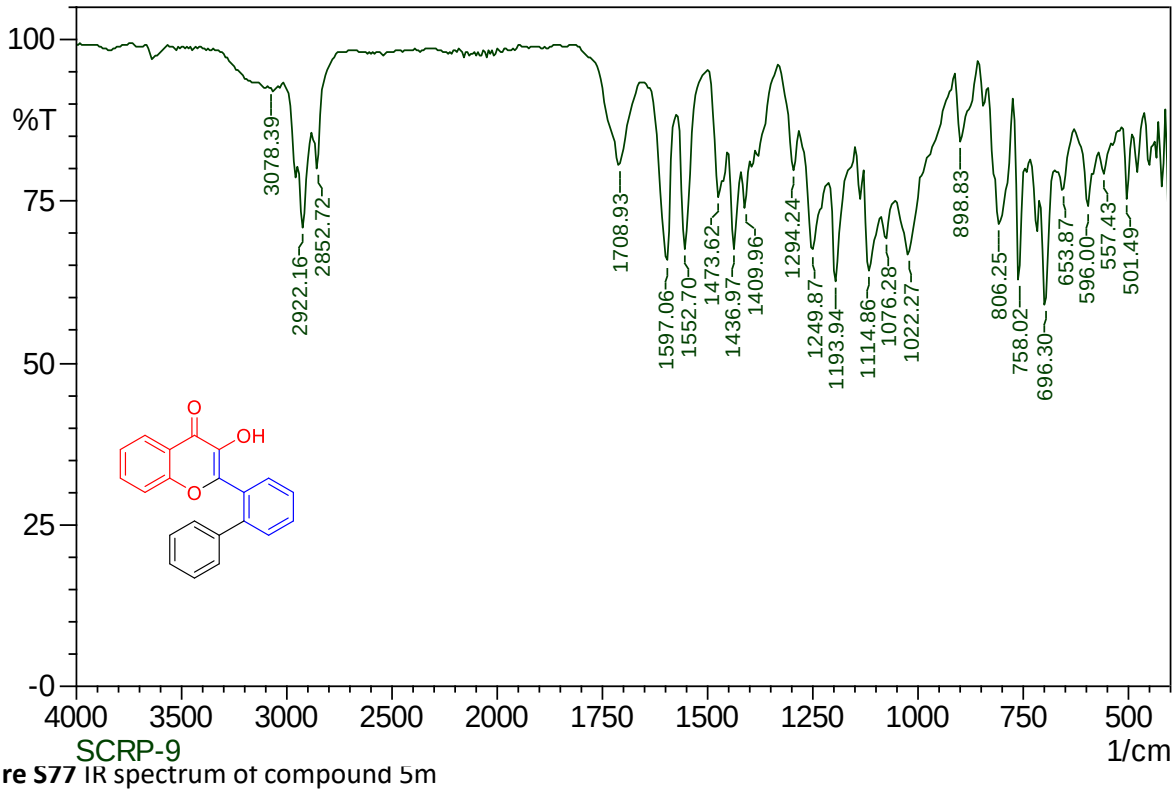

Figure S77 IR spectrum of compound 5m

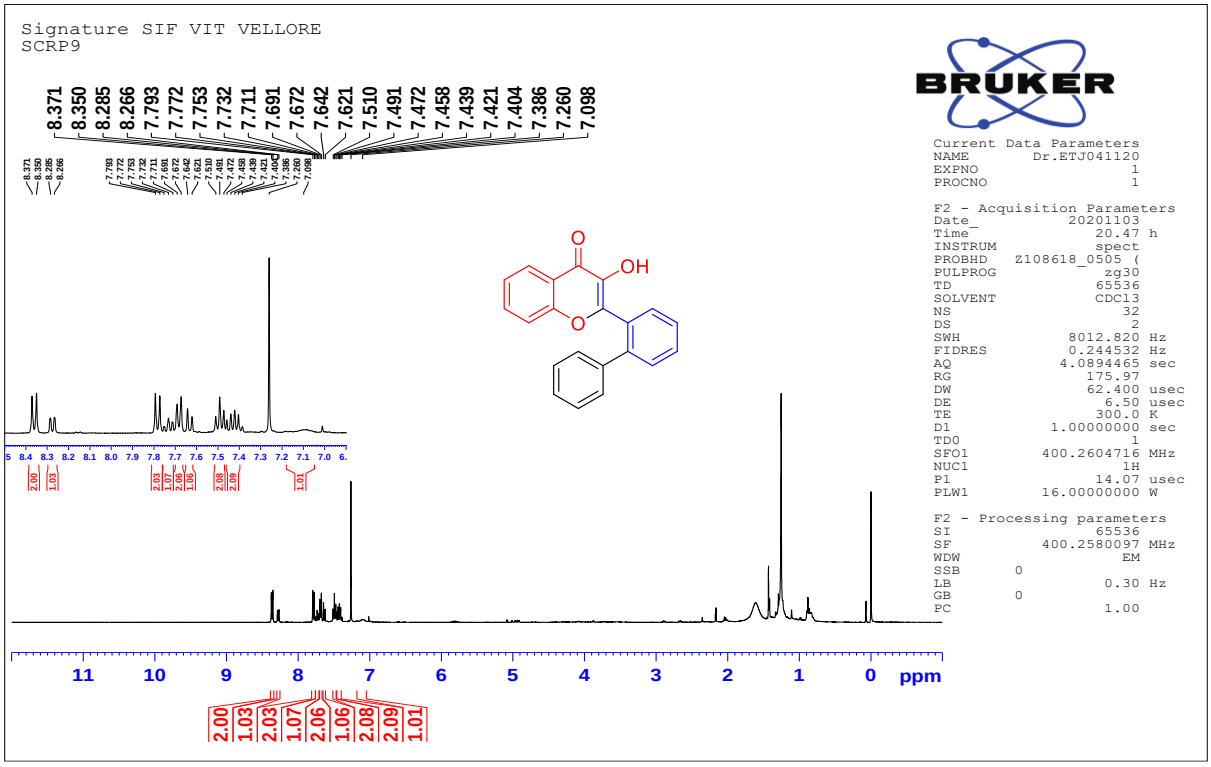

Figure S78 ^{1}H NMR spectrum of compound 5m

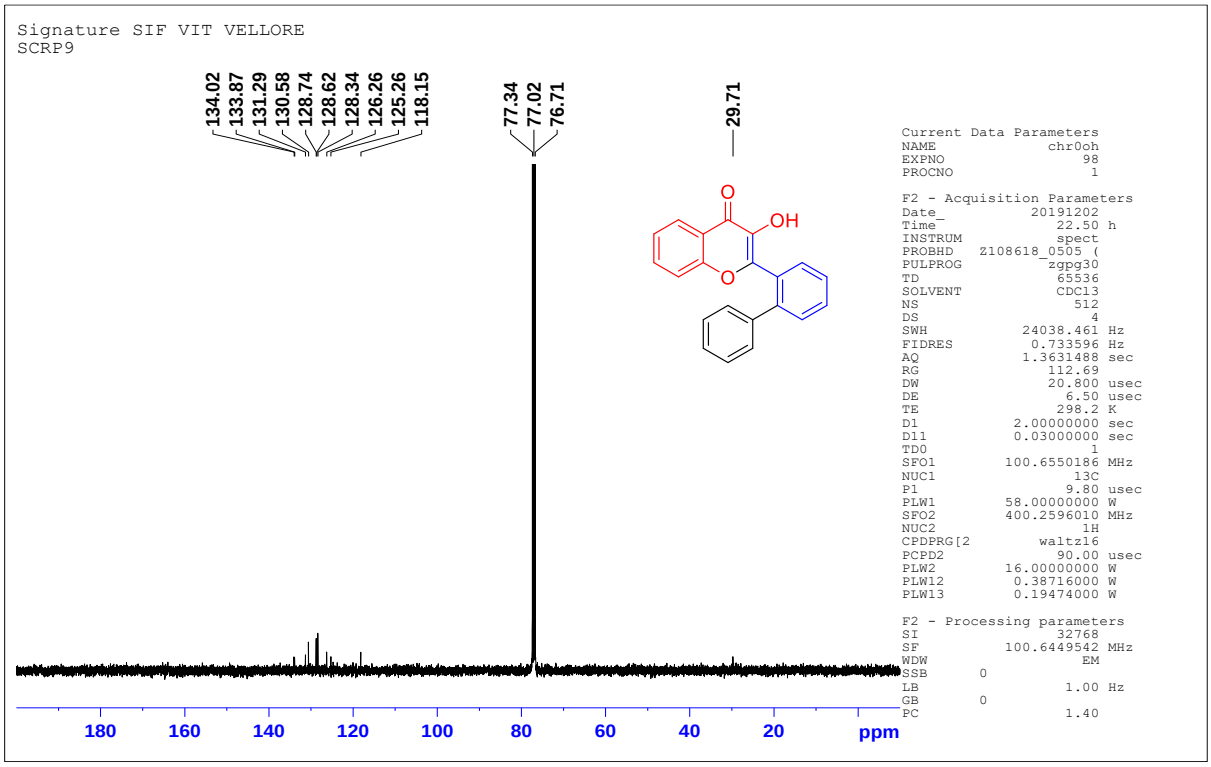

Figure S79 ^{13}C NMR spectrum of compound 5m

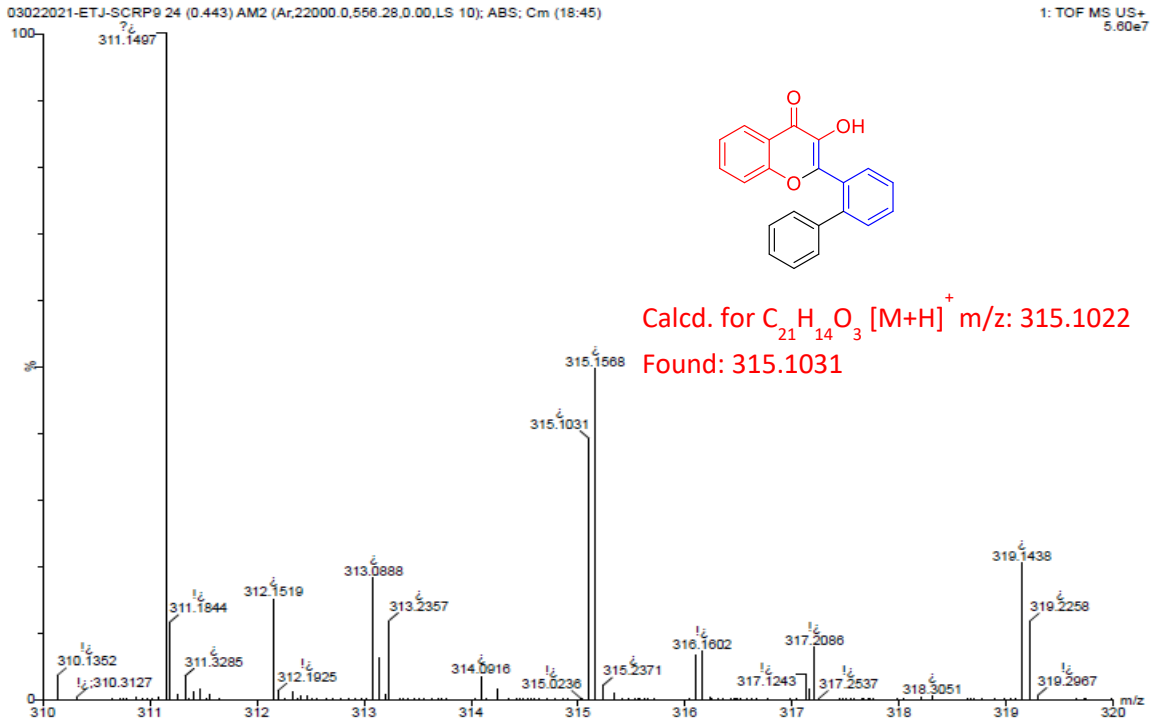

Figure S80 HRMS spectrum of compound 5m

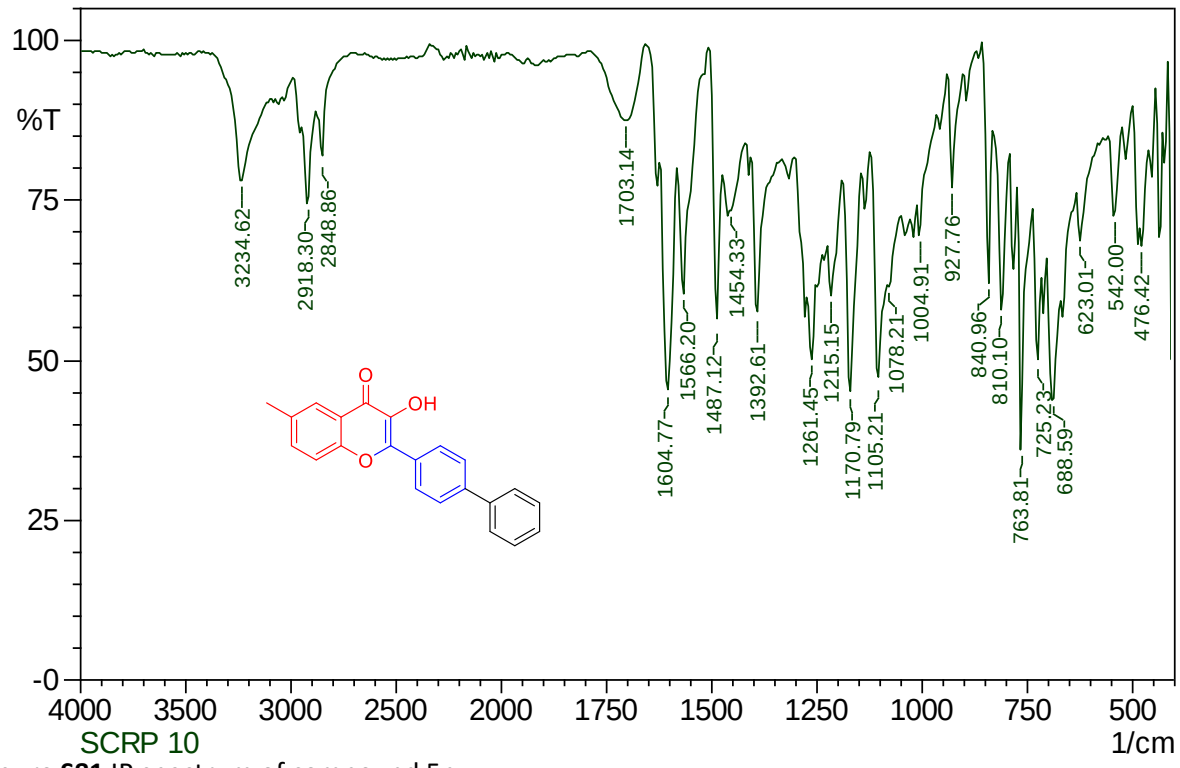

Figure S81 IR spectrum of compound 5n

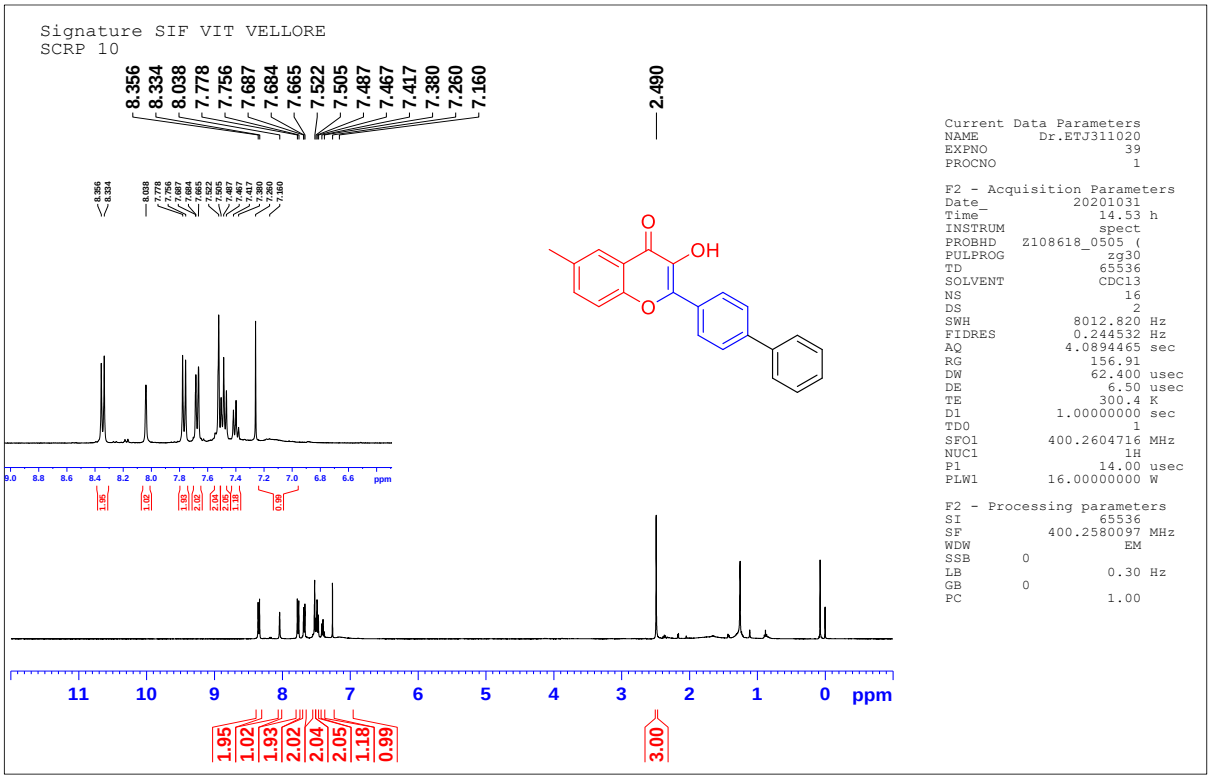

Figure S82 ^{1}H NMR spectrum of compound 5n

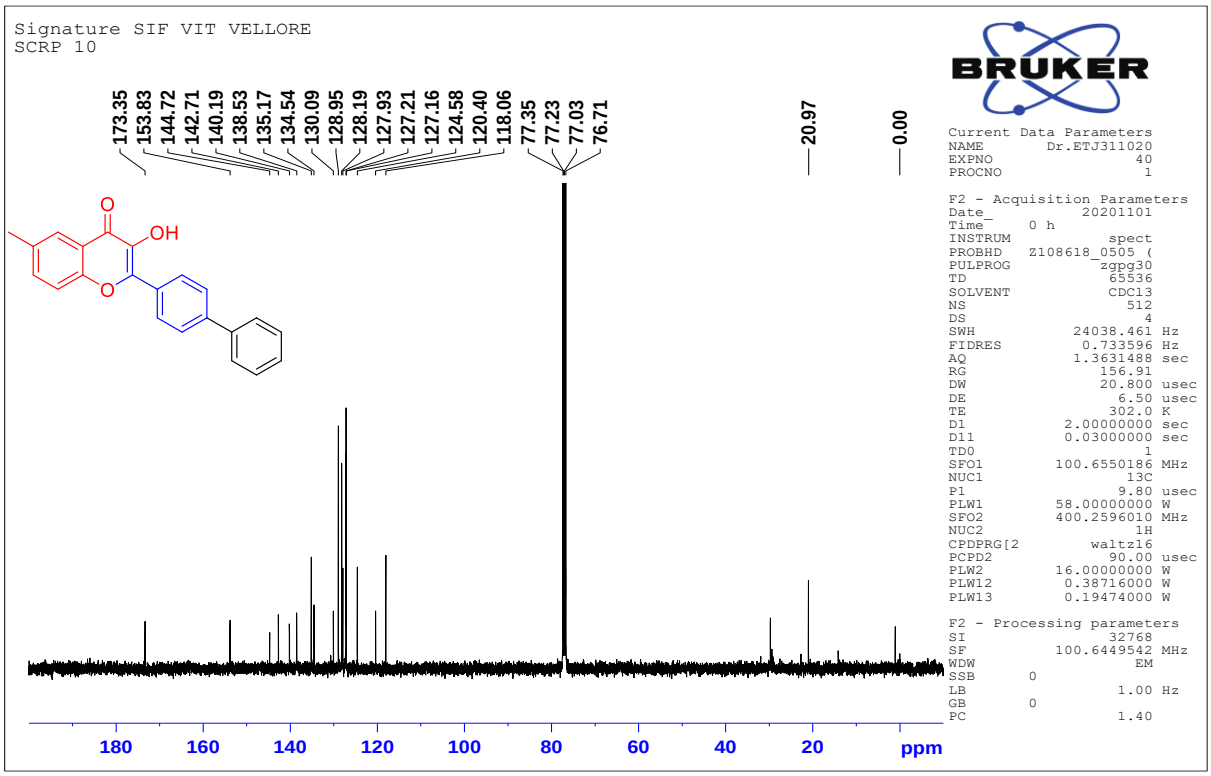

Figure S83^{13}C NMR spectrum of compound 5n

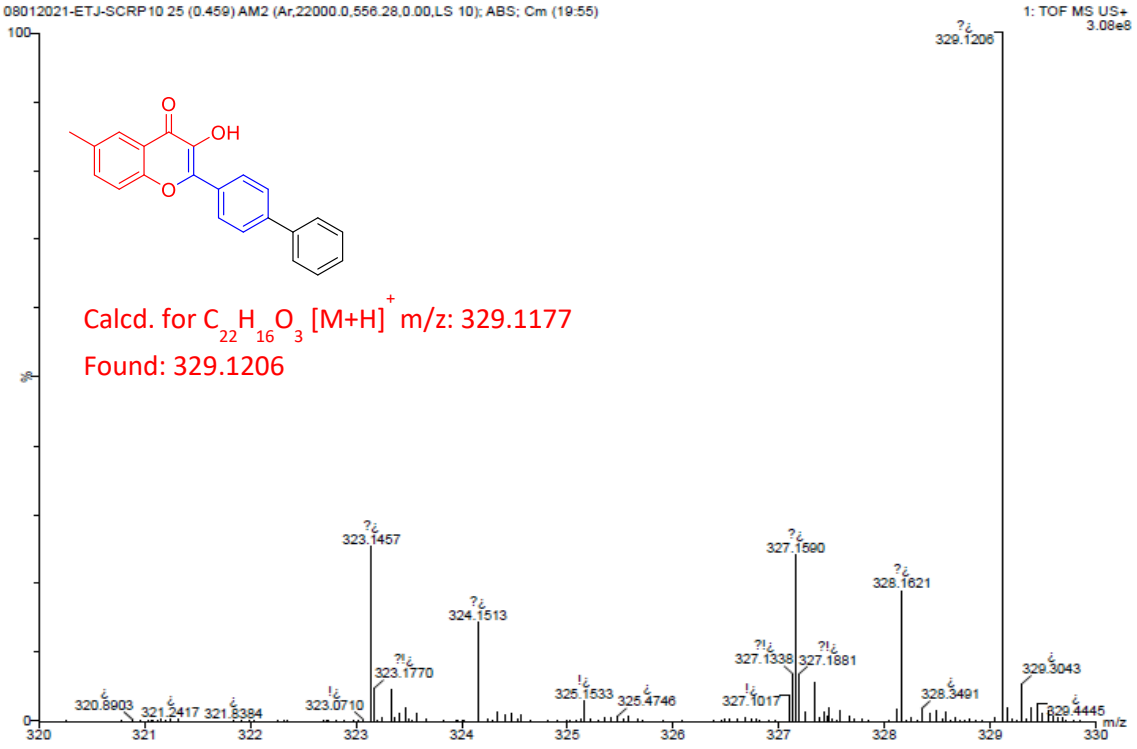

Figure S84HRMS spectrum of compound 5n

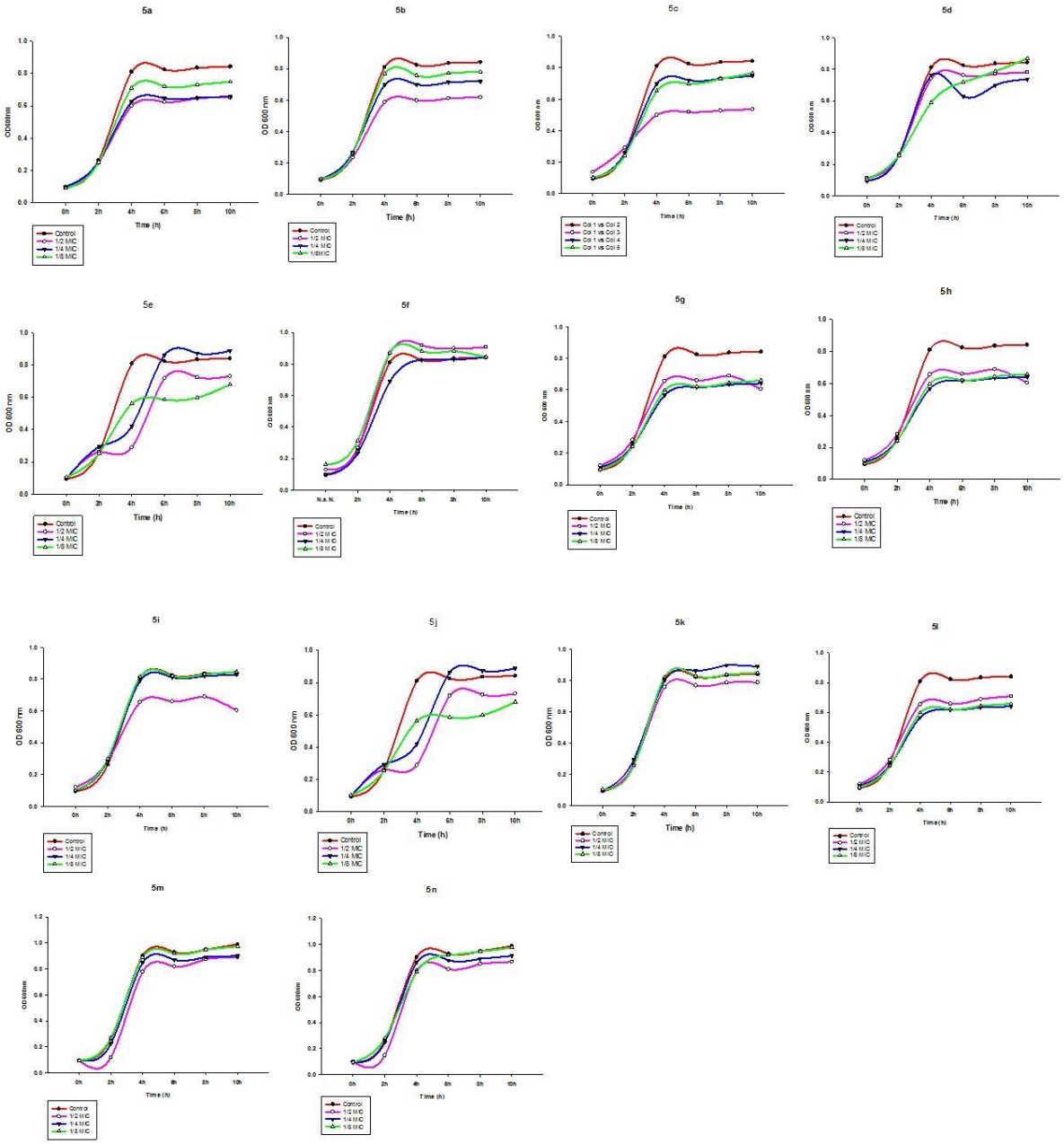

Figure S85 Growth curve analysis of *P. aeruginosa* in ½ MIC, ¼ MIC, 1/8 MIC MIC dosages of compounds 5a-5n. Comparisons were performed with different flavonols and that of the untreated control. The graphs are plotted with average OD 600 of triplicates.

Table S1 Percentage Inhibition of biofilm at different sub-MIC concentrations of compounds

compound	Percentage Inhibition of biofilm at different sub-MIC concentrations		
	½ MIC	¼ MIC	1/8 MIC
5a	66	51	41
5b	70	61	52
5c	65	53	38
5d	62	50	34
5e	81	67	55
5f	92	95	86
5g	84	59	48
5h	75	62	49
5i	71	64	56
5j	82	67	49
5k	69	57	42
5l	63	60	55
5m	89	83	74
5n	77	66	51

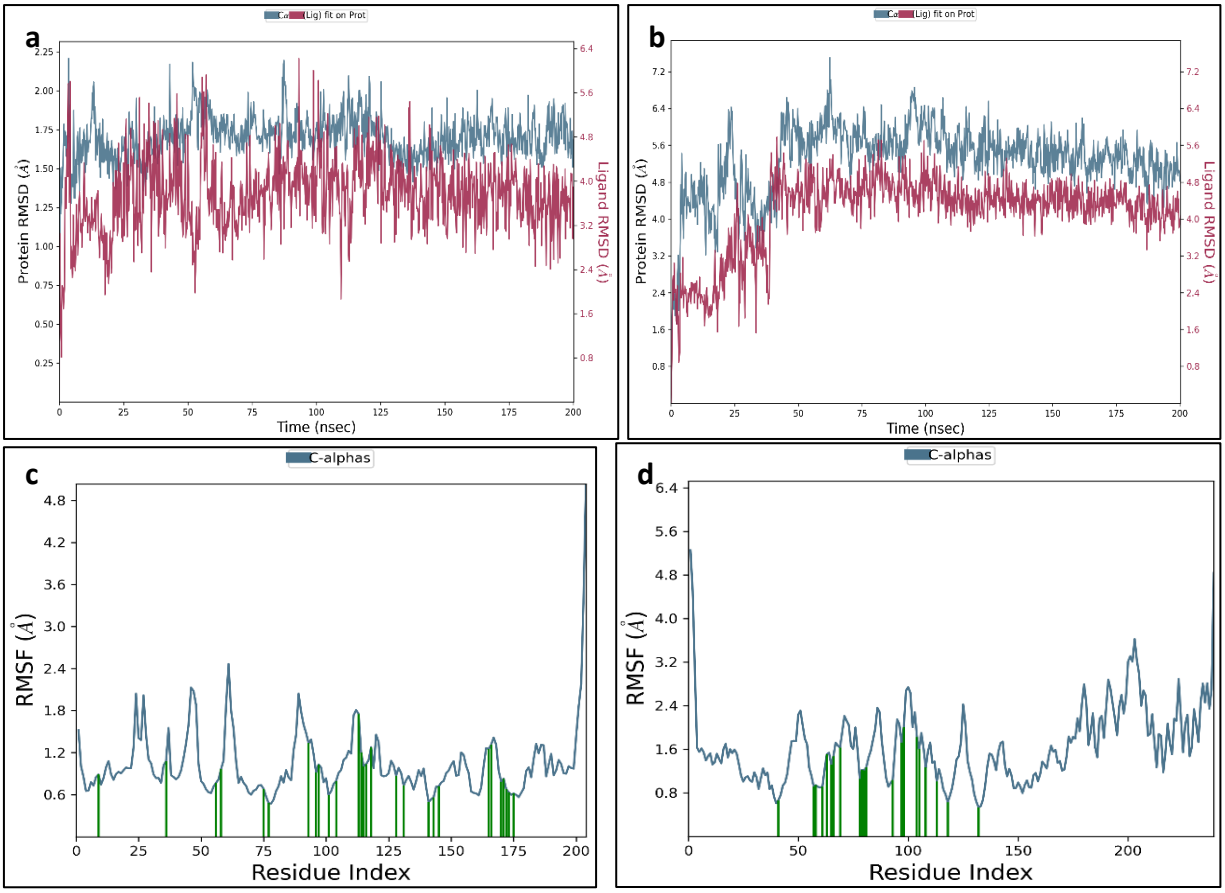

Figure S86 (a) 4JVI-5m complex RMSD **(b)** 8DQ0-5m complex RMSD **(c)** RMSF of 4JVI protein with binding site marked in green lines **(d)** RMSF of 8DQ0 protein with binding site marked in green lines

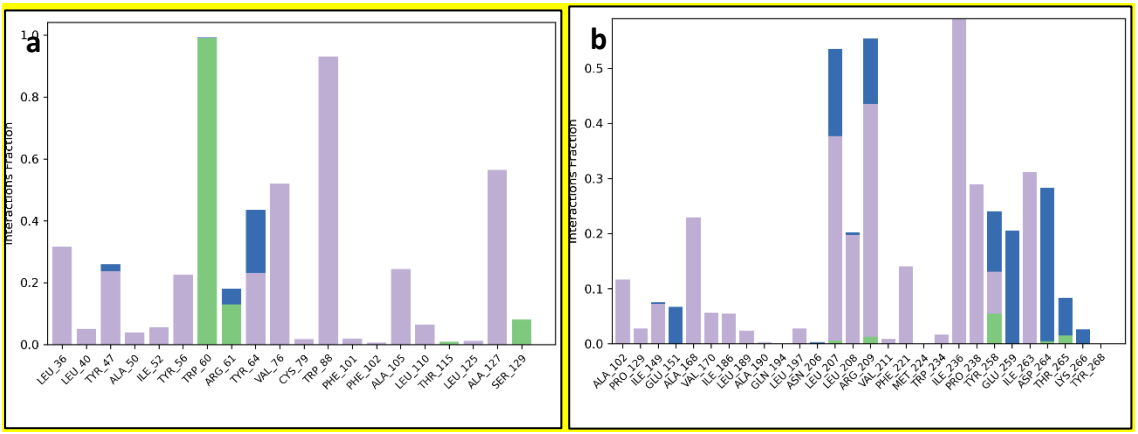

Figure S87 (a) 2UV0-5m complex interaction histogram **(b)** 4JVI-5m complex interaction histogram

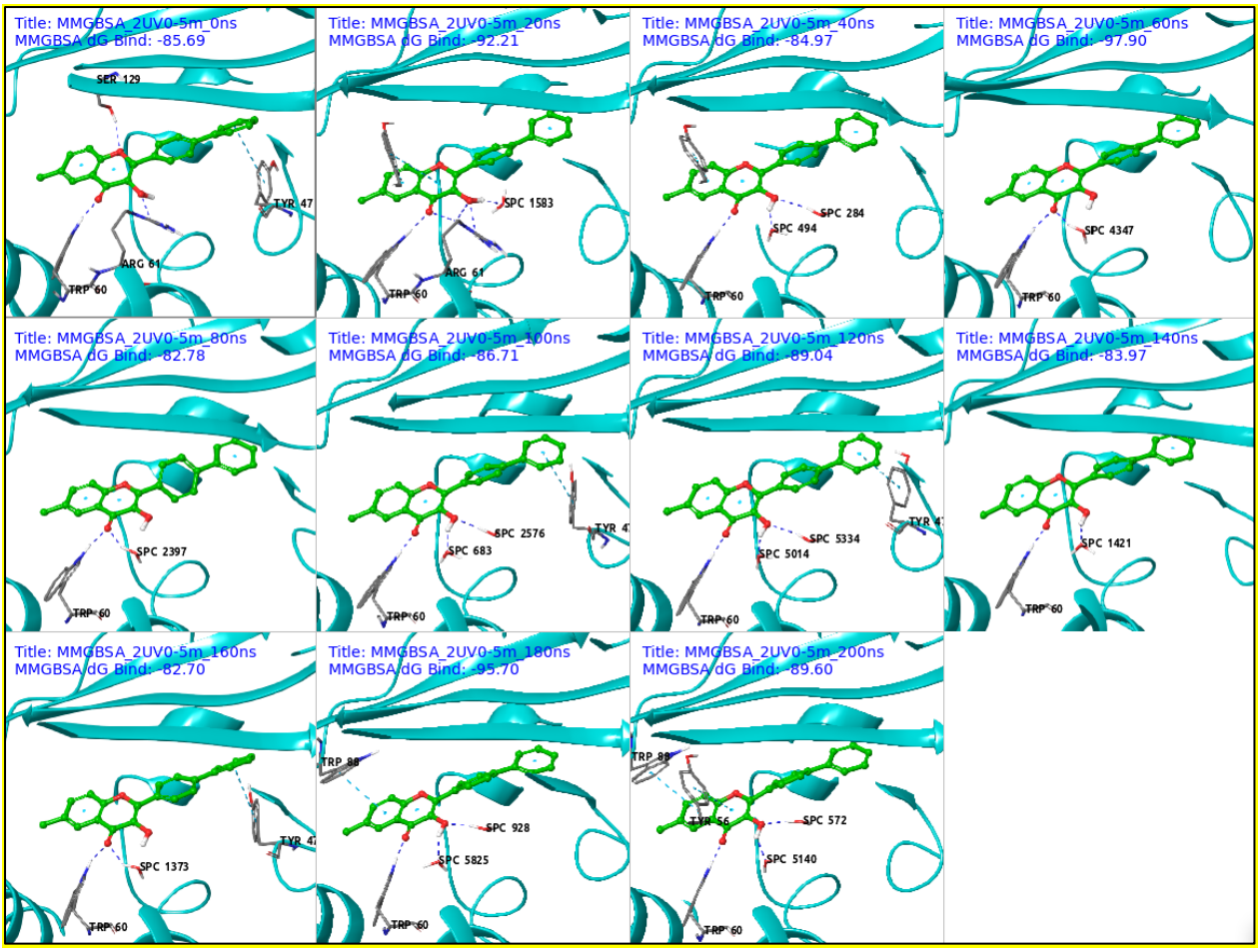

Figure S88 Interactions of 5m ligand with transcriptional activator protein LasR (PDB ID: 2UV0) in 20ns interval frames of 200ns simulation study.

Table S2: 2UV0-5m complex binding free energy distribution for the selected frames

Complex	Frame	ΔG Coulomb	ΔG Covalent	ΔG H-bond	ΔG Lipo	ΔG Packing	ΔG Solv_GB	ΔG vdW	ΔG bind
2UV0-5m	0ns	-28.57	4.82	-2.22	-30.29	-4.49	29.59	-54.51	-85.69
2UV0-5m	20ns	-30.57	5.97	-2.20	-30.79	-4.67	28.03	-57.96	-92.21
2UV0-5m	40ns	-23.66	6.20	-1.11	-29.08	-3.81	20.70	-54.19	-84.97
2UV0-5m	60ns	-23.86	4.98	-1.61	-31.21	-6.34	17.92	-57.76	-97.90
2UV0-5m	80ns	-25313	7.54	-1.23	-27.77	-4.57	22.81	-54.40	-82.78
2UV0-5m	100ns	-28.99	1.74	-2.22	-28.16	-5.33	22.44	-46.18	-86.71
2UV0-5m	120ns	-30.60	8.13	-1.23	-29.16	-3.85	22.68	-54.99	-89.04
2UV0-5m	140ns	-23.24	5.45	-1.04	-29.32	-3.98	19.83	-51.65	-83.97

2UV0-5m	160ns	-22.21	6.16	-1.64	-26.53	-3.93	19.55	-54.09	-82.70
2UV0-5m	180ns	-37.16	5.10	-1.60	-29.71	-5.26	16.90	-43.96	-95.70
2UV0-5m	200ns	-28.20	6.80	-1.61	-30.73	-5.21	22.30	-52.95	-89.60

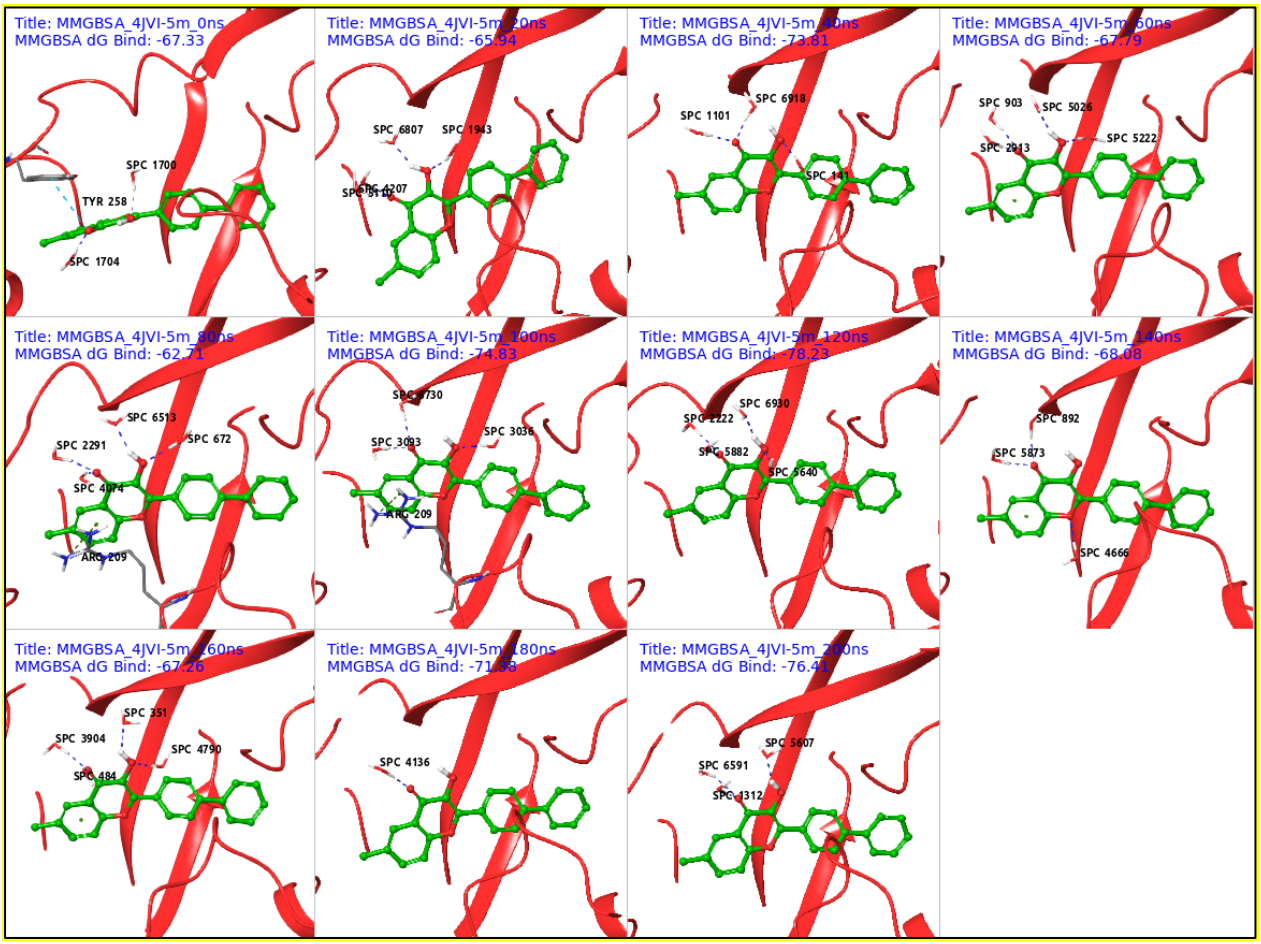

Figure S89 Interactions of 5m ligand with PqsR co-inducer binding domain of Pseudomonas aeruginosa (PDB ID: 4JVI) in 20ns interval frames of 200ns simulation study.

Table S3: 4JVI-5m complex binding free energy distribution for the selected frames

Complex	Frame	ΔG Coulomb	ΔG Covalent	ΔG H-bond	ΔG Lipo	ΔG Packing	ΔG Solv_GB	ΔG vdW	ΔG bind
4JVI-5m	0ns	-23.58	3.00	0.07	-27.20	-3.20	21.83	-38.27	-67.33
4JVI-5m	20ns	-15.27	2.38	0.63	-24.82	-3.67	25.60	-50.78	-65.94
4JVI-5m	40ns	-10.86	3.78	0.02	-28.76	-3.57	20.93	-55.35	-73.81
4JVI-5m	60ns	-4.03	1.99	-0.46	-28.10	-3.63	20.68	-54.21	-67.79
4JVI-5m	80ns	6.80	2.79	0.21	-28.70	-3.34	9.17	-49.66	-62.71
4JVI-5m	100ns	-12.44	2.93	-2.24	-28.42	-3.73	21.85	-52.78	-74.83
4JVI-5m	120ns	-15.75	6.84	-0.55	-31.18	-3.33	18.76	-53.01	-78.23

4JVI-5m	140ns	-11.00	6.23	-1.88	-29.03	-0.13	21.87	-54.14	-68.08
4JVI-5m	160ns	-18.93	4.80	-0.54	-30.20	-0.49	19.62	-41.51	-67.26
4JVI-5m	180ns	-0.80	-1.80	-1.13	-29.26	-1.99	13.77	-50.14	-71.38
4JVI-5m	200ns	-23.82	3.16	-2.11	-27.72	-3.43	19.97	-42.44	-76.41

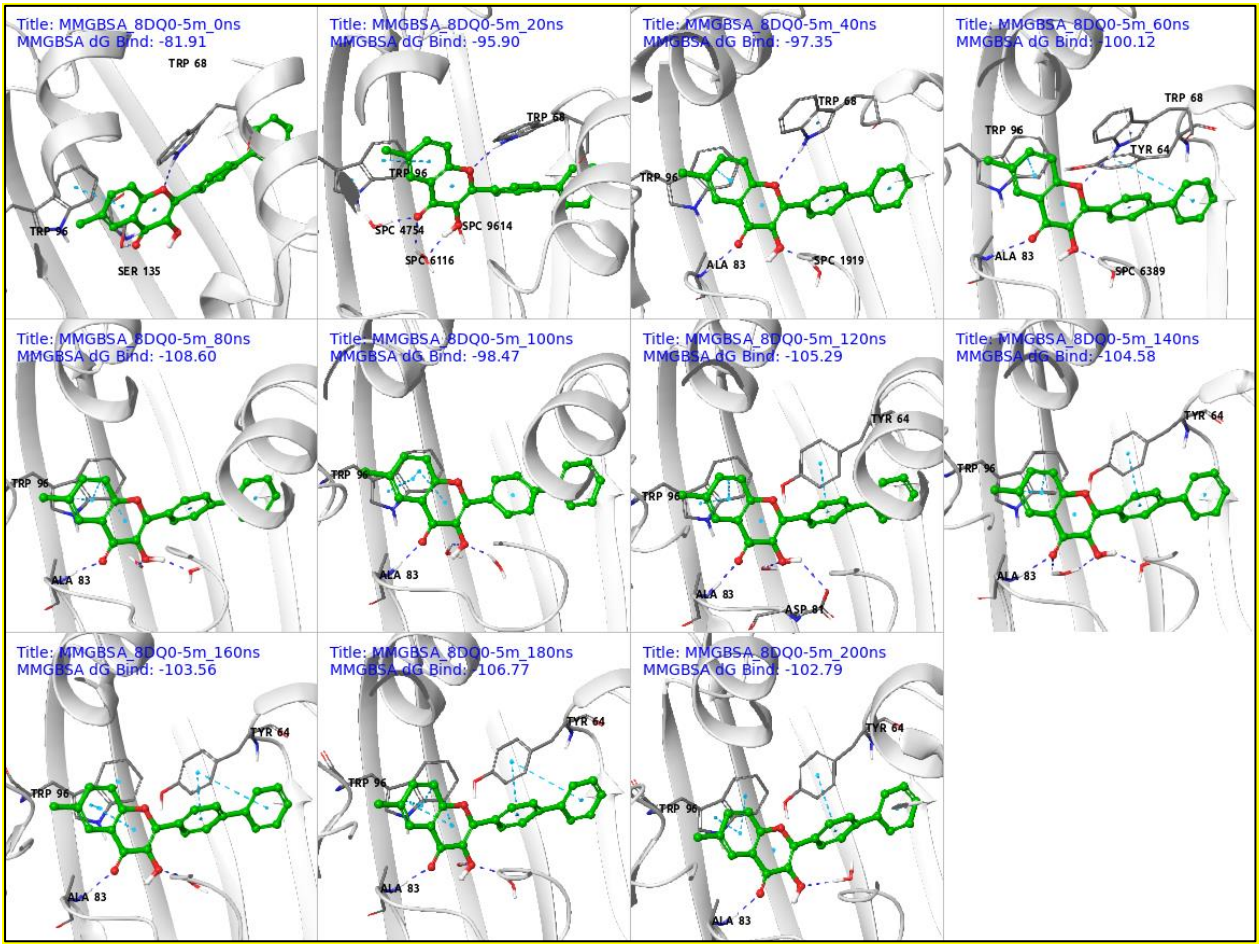

Figure S90 Interactions of 5m ligand with quorum-sensing receptor RhlR of Pseudomonas aeruginosa (PDB ID: 8DQ0) in 20ns interval frames of 200ns simulation study.