Supporting Information

Pyrazolopyrimidines as Potent Stimulators for Transient Receptor Potential Canonical 3/6/7 Channels

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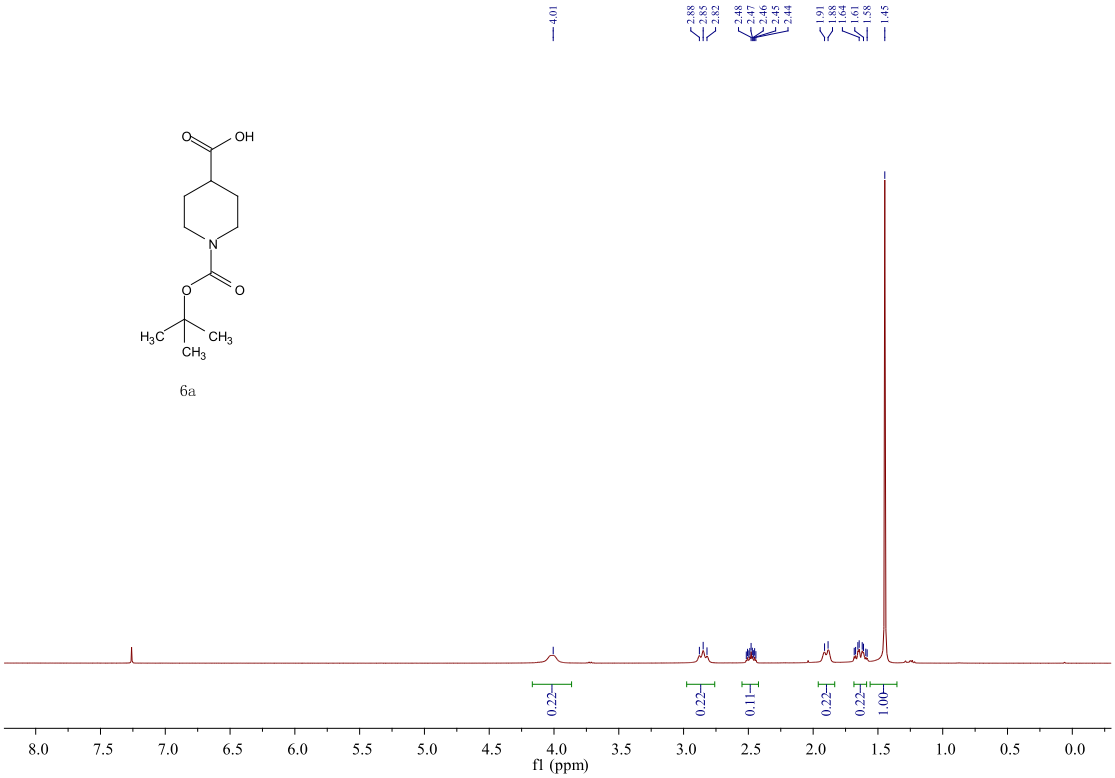
PART 1. General methods and ^{1}H NMR data of 6a-6c, 7a-7b, 8a-8d, 11a-11c

General Methods. Unless other noted, all reagents and solvents obtained were purchased from commercial suppliers and used without further purification. Melting points were determined on a Yuhua X-5 melting point apparatus. All reactions were performed under an argon atmosphere unless otherwise specified. Reaction progress was monitored using analytical thin-layer chromatography (TLC). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer (400 MHz, ^{1}H NMR; 101 MHz, ^{13}C NMR) at room temperature (rt). NMR spectra were calibrated to the solvent signals of $CDCl_3$ (δ 7.26 and 77.16 ppm), CD_3OD (δ 3.31 and 49.00ppm), or DMSO-d_6 (δ 2.50 and 39.52 ppm). The chemical shifts are provided in ppm, and the coupling constants, in Hz. The following abbreviations for multiplicities are used: s, singlet; d, doublet; dd, double doublet; ddd, three doublet; t, triplet; dt, double triplet; q, quadruplet; m, multiplet; and br, broad. High-resolution MS was carried out with a Thermo LTQ XL Orbitrap instrument. The purity of all compounds for biological testing was determined by normal and reversed phase HPLC analysis, confirming >95% purity.

^{1}H NMR for 6a



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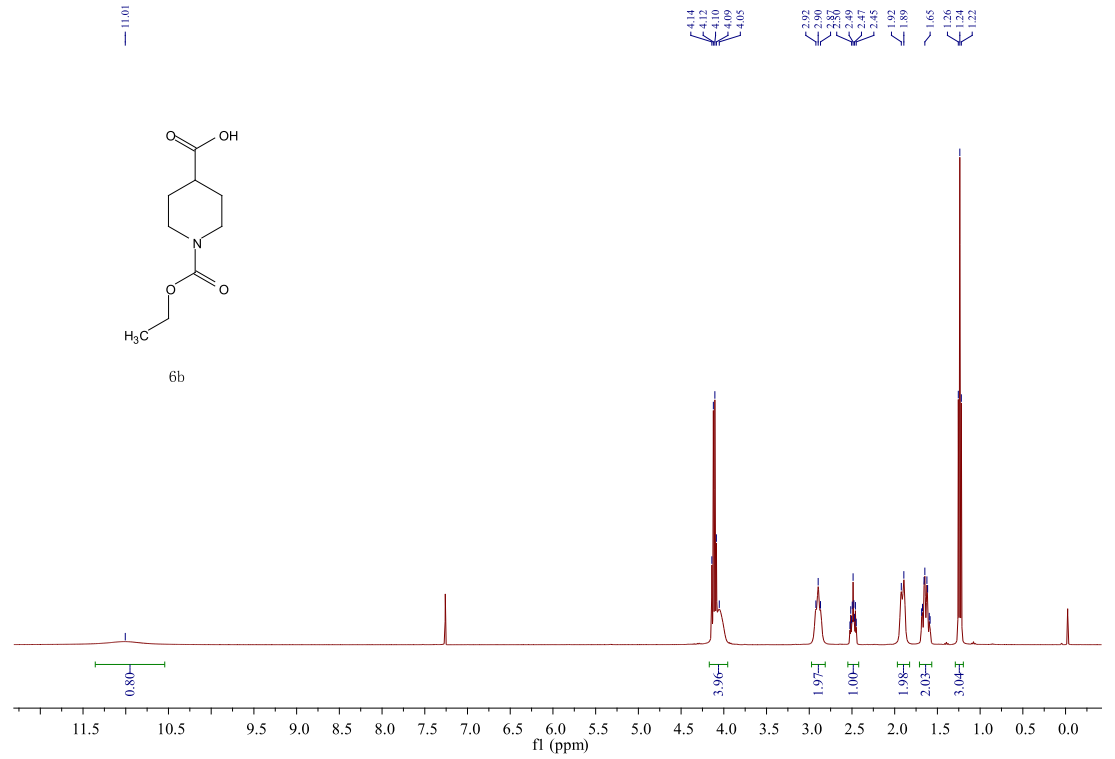

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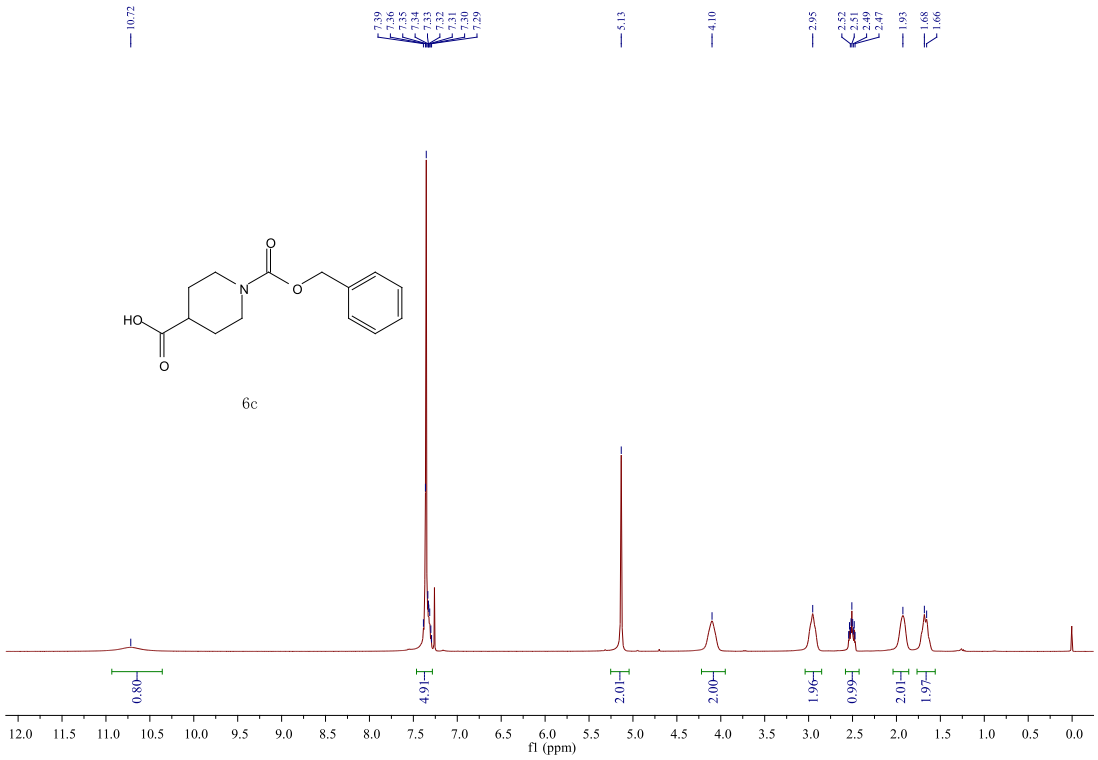

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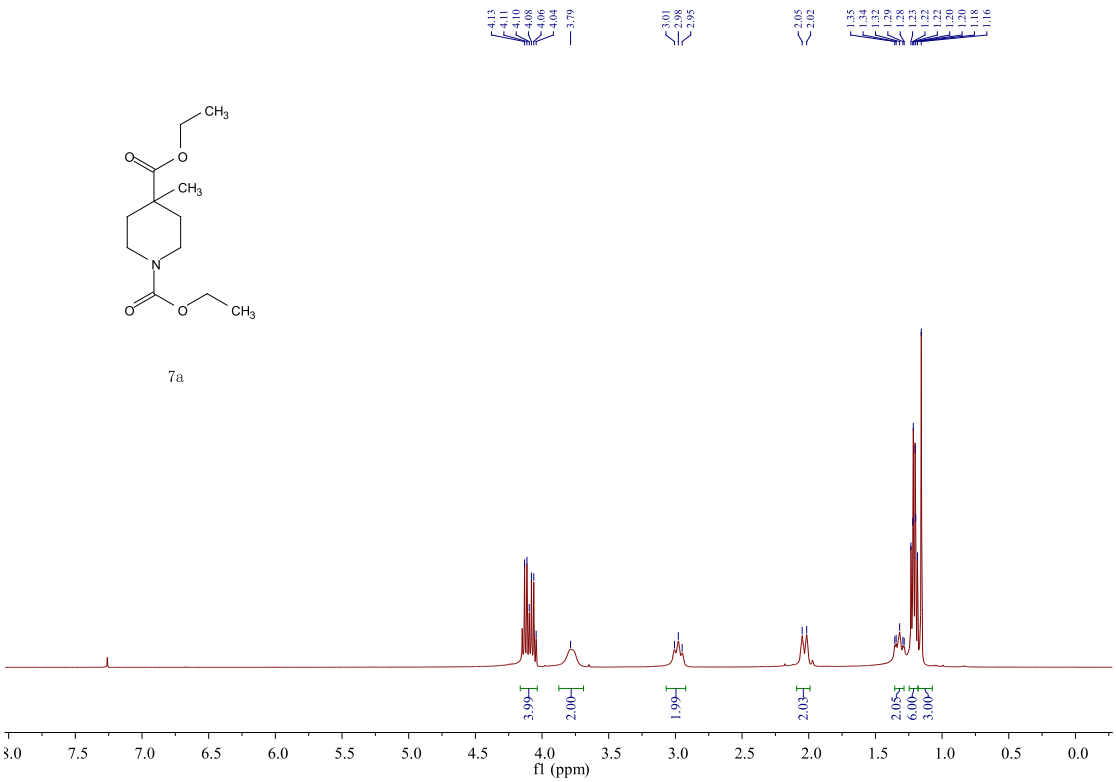

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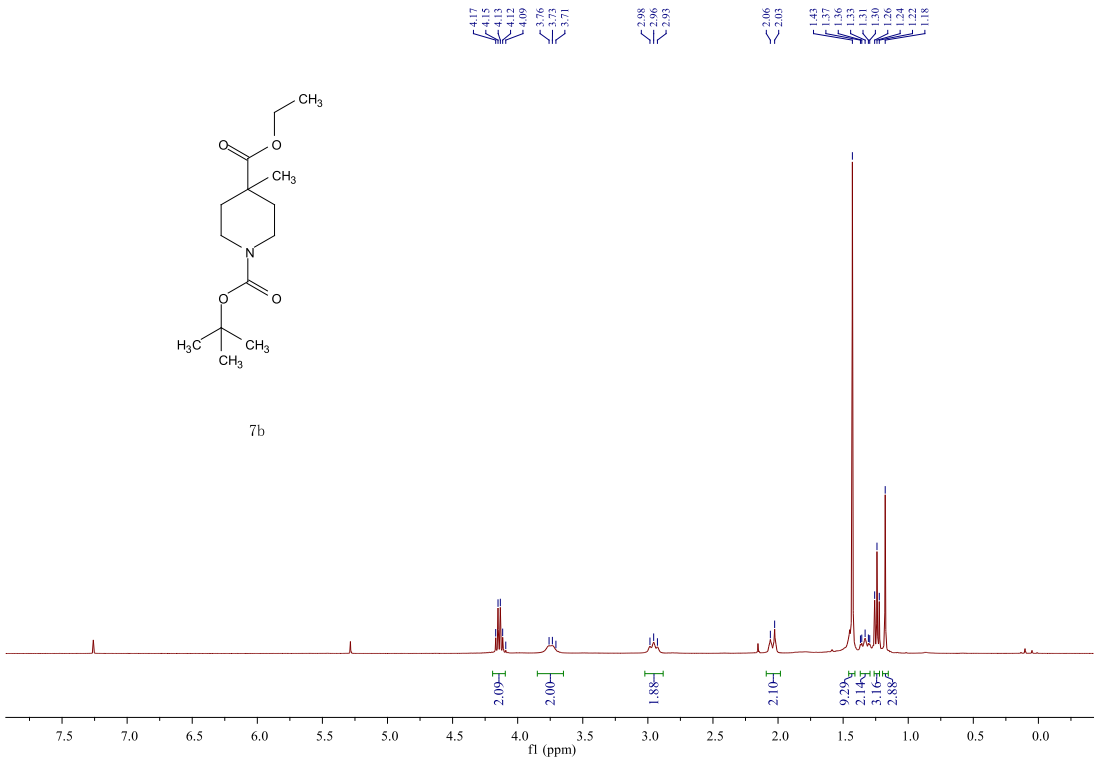
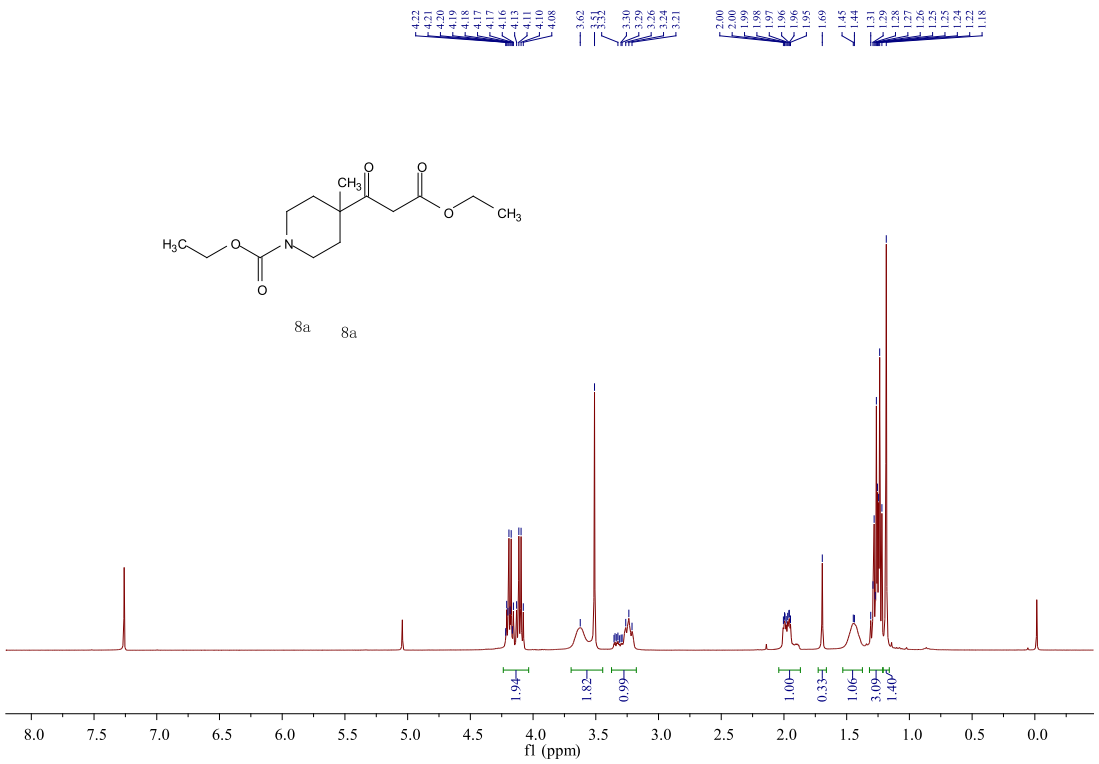


7b

^{1}H NMR for 8a



8a 8a

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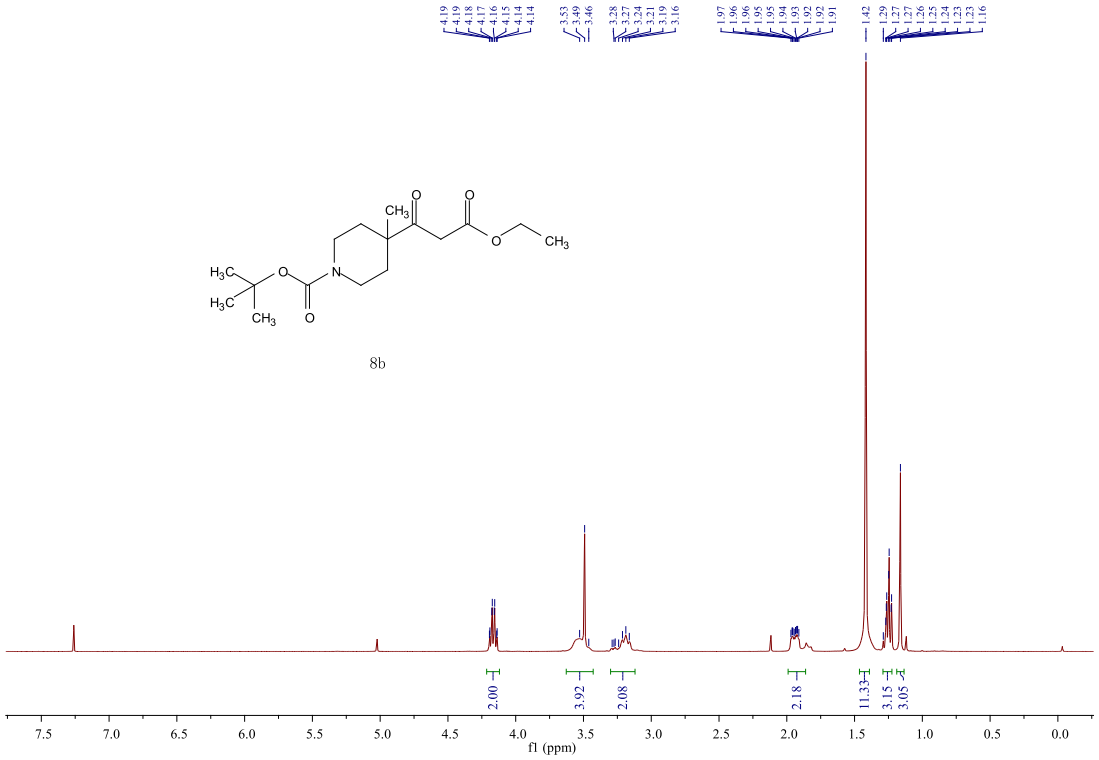

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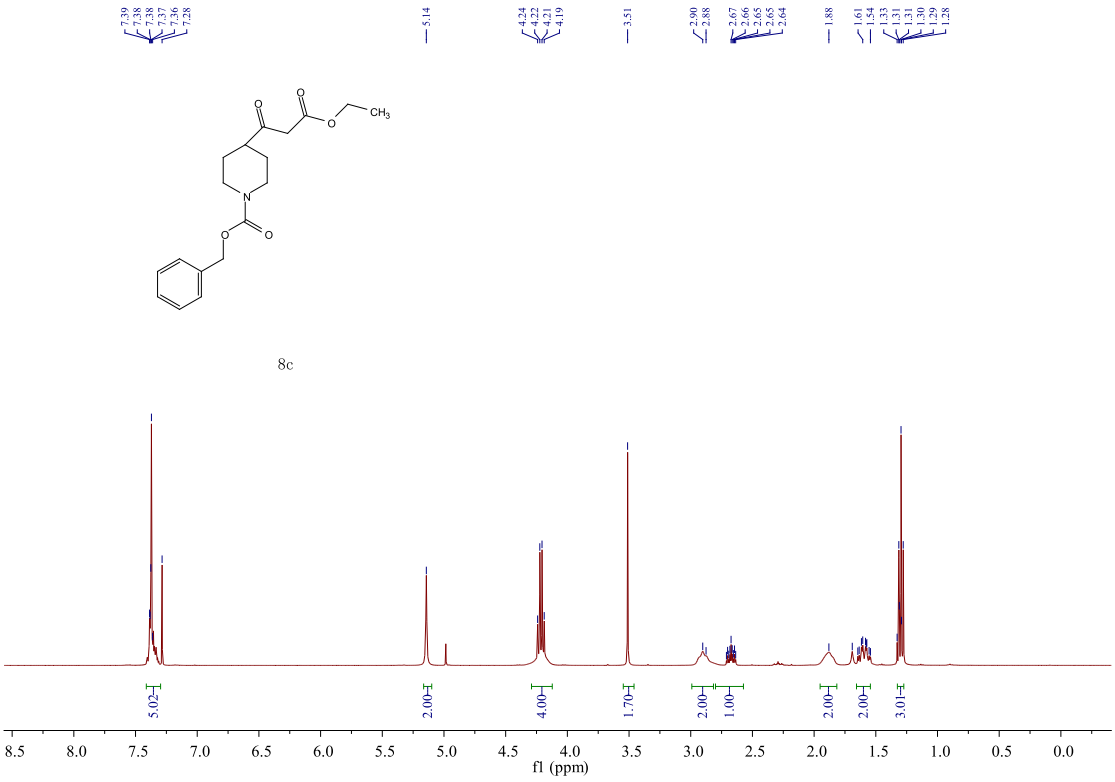

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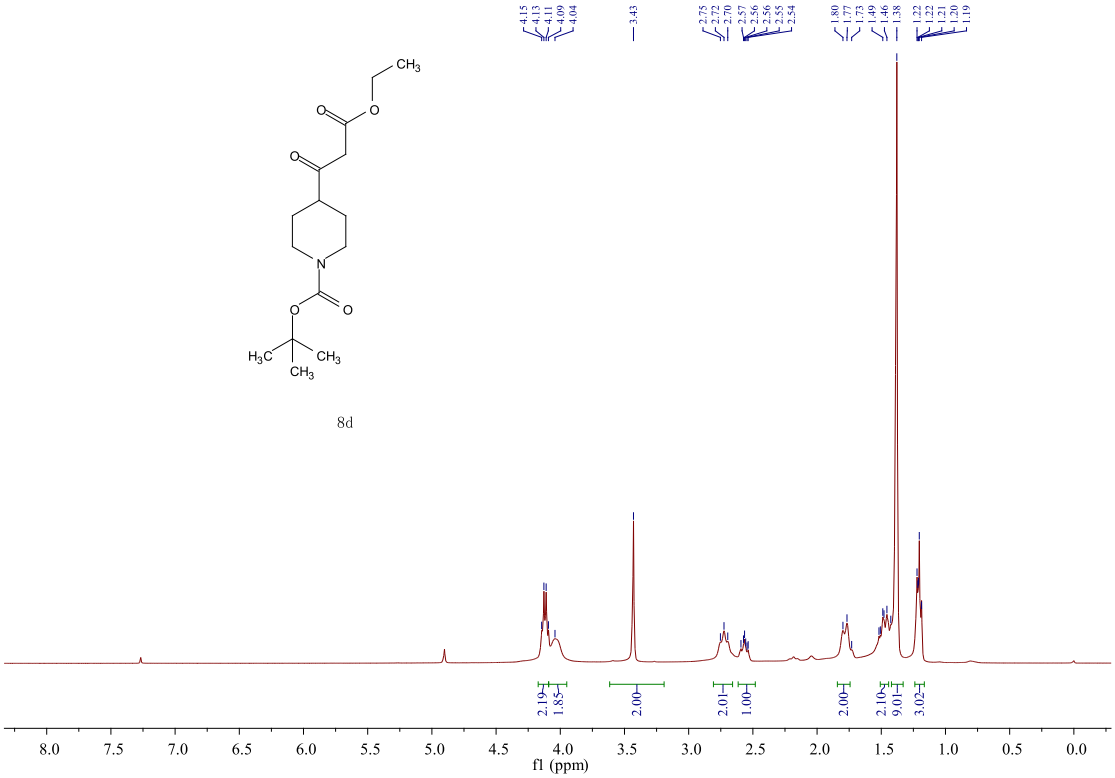

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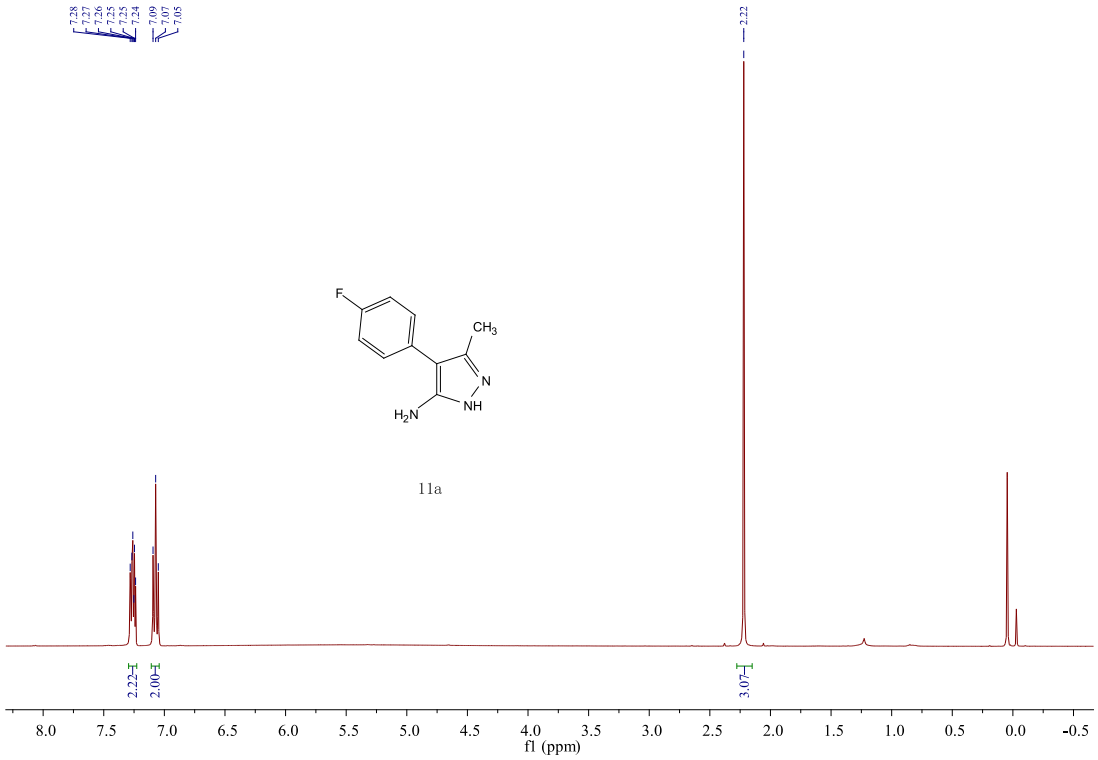

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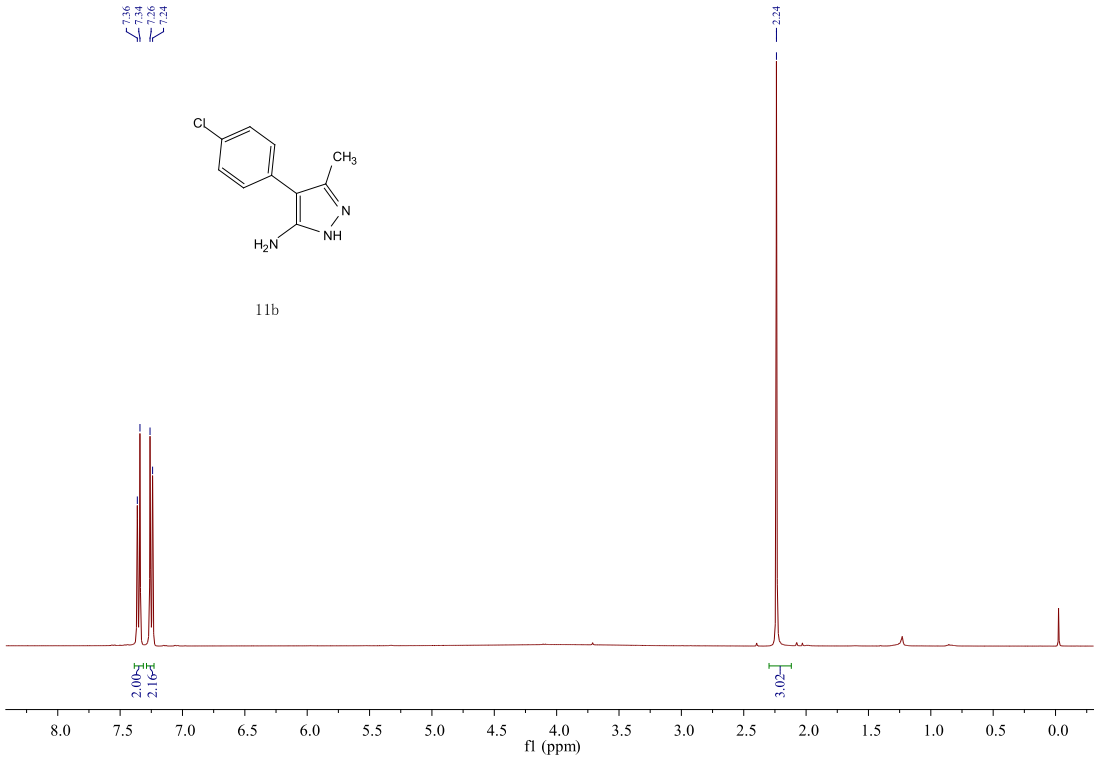

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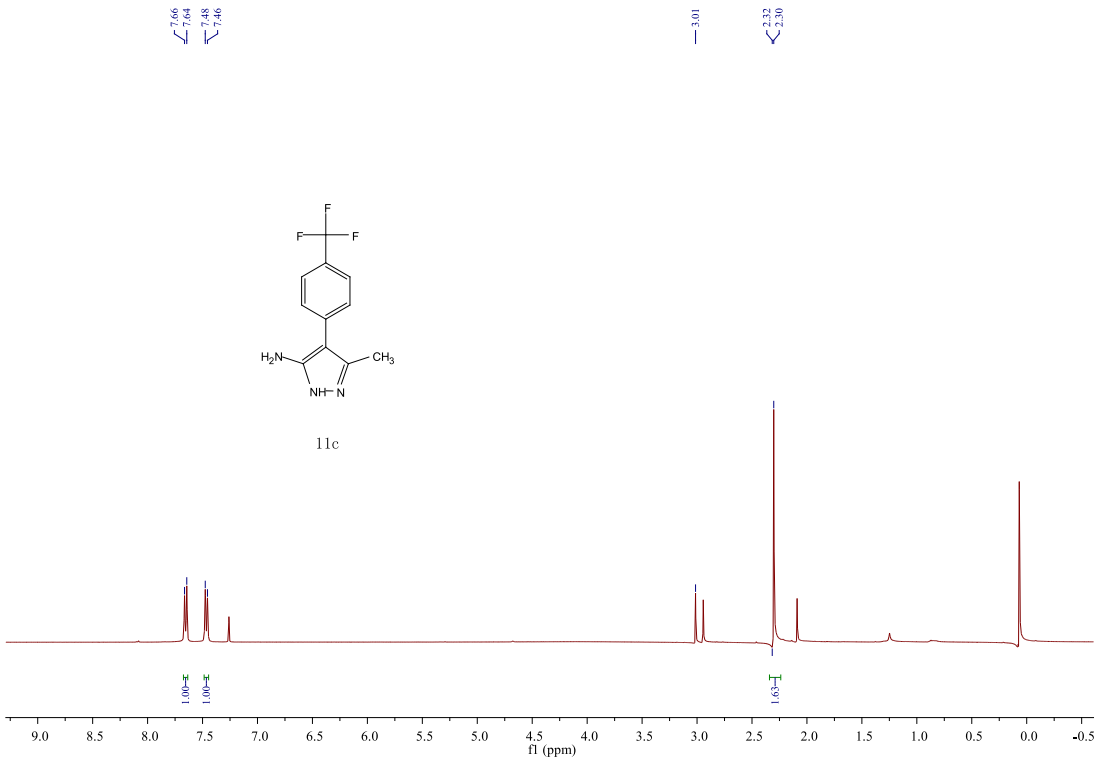

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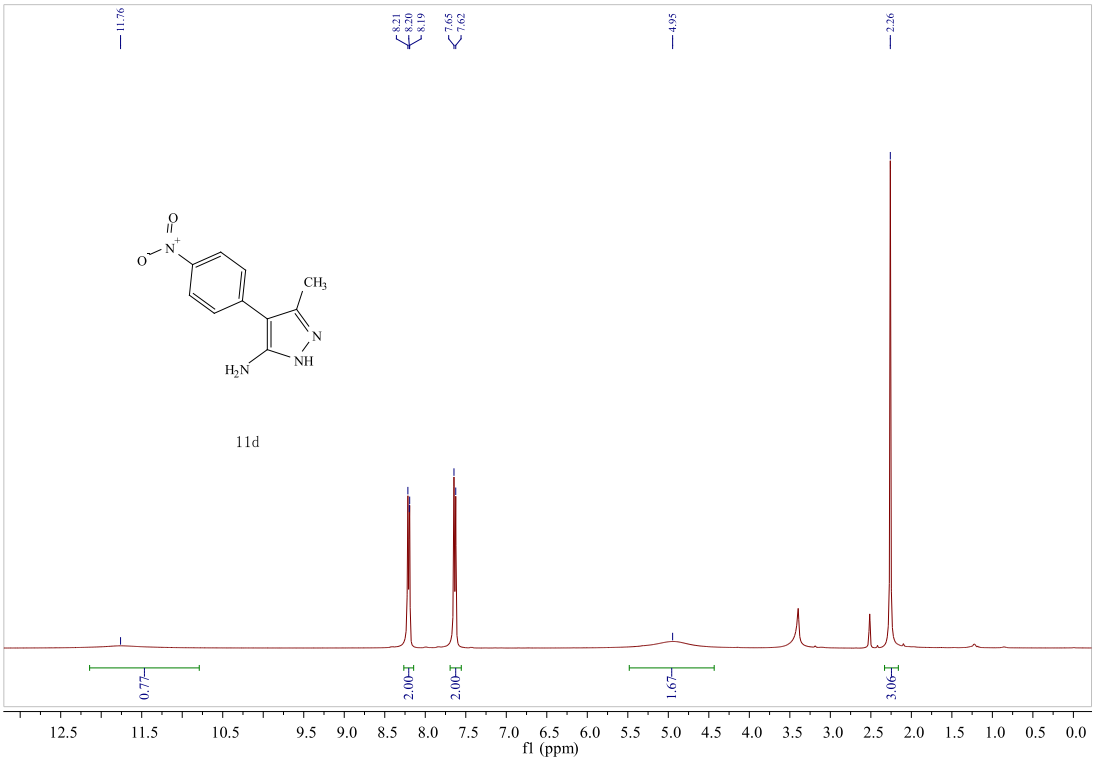
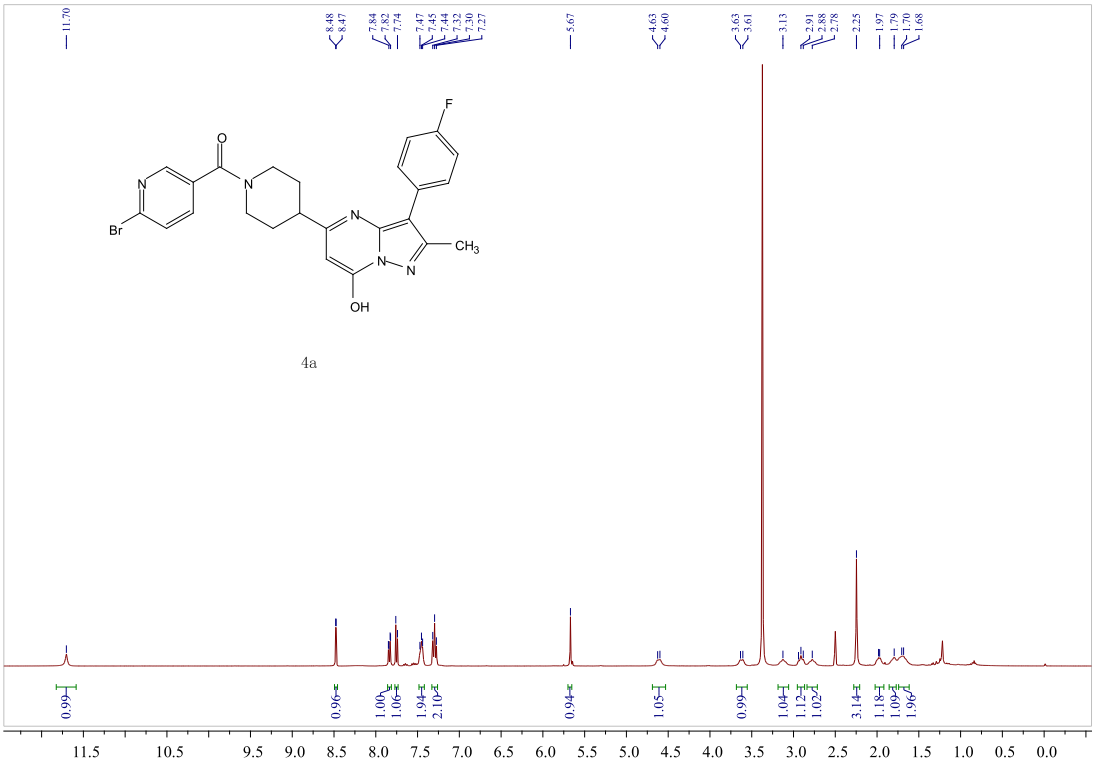
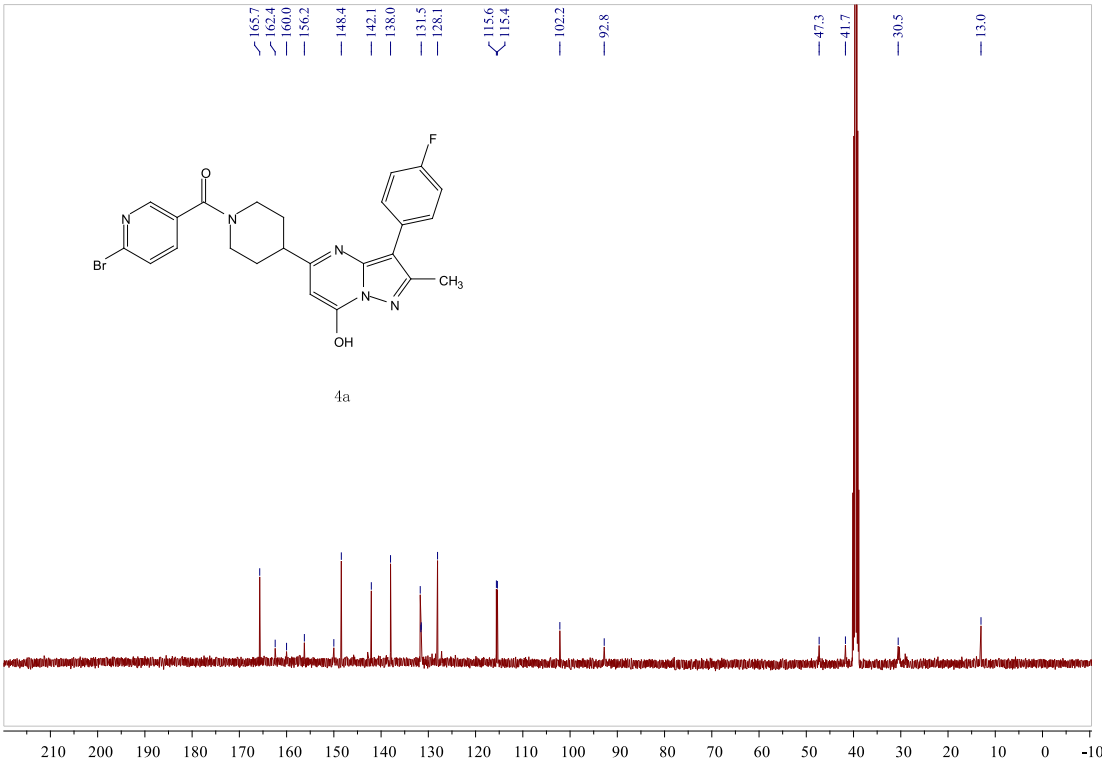


^{1}H NMR and ^{13}C NMR for 4a





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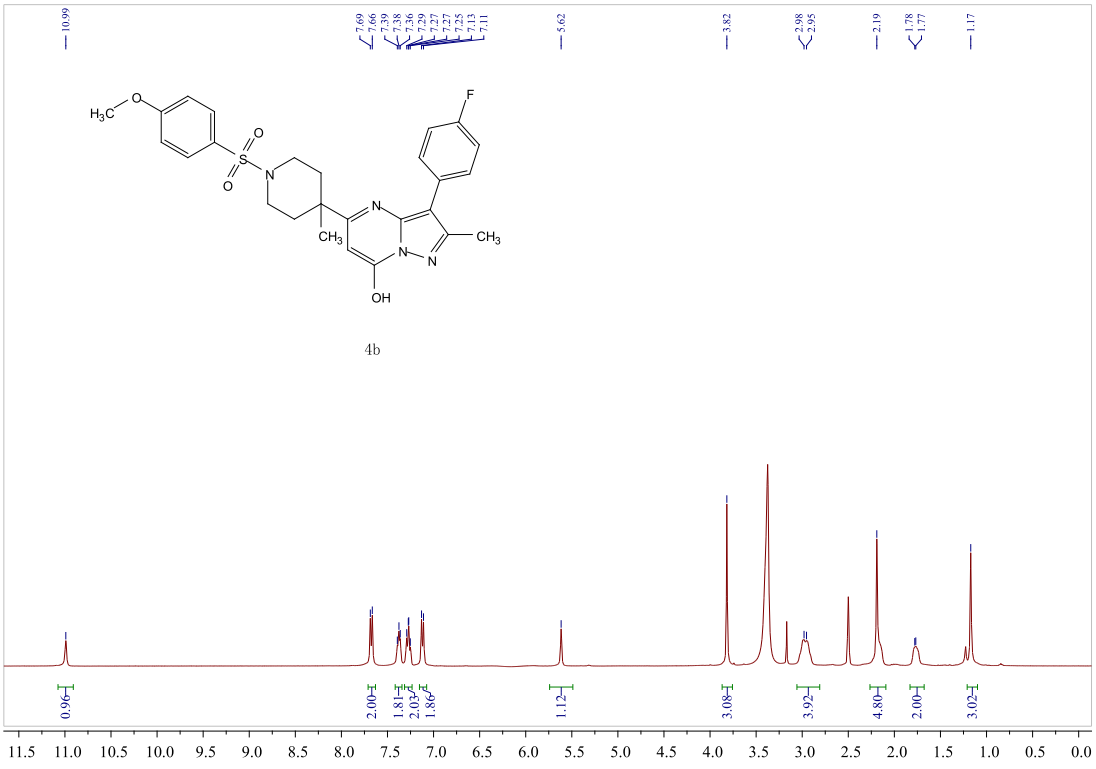

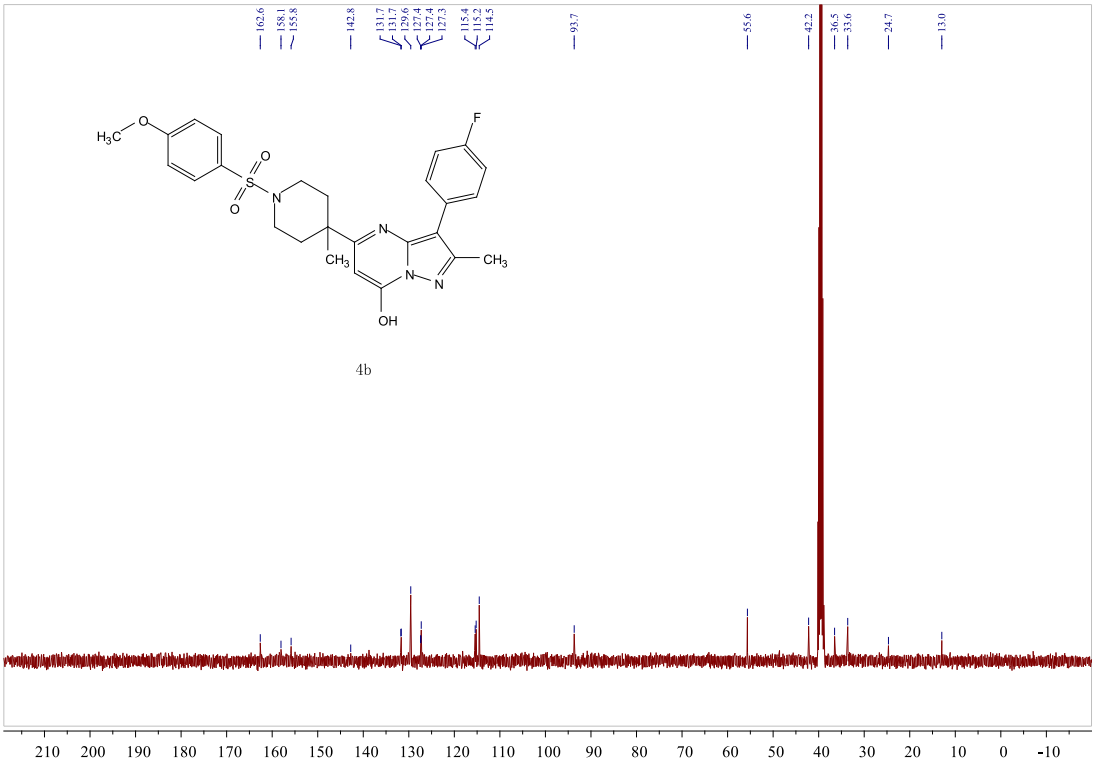

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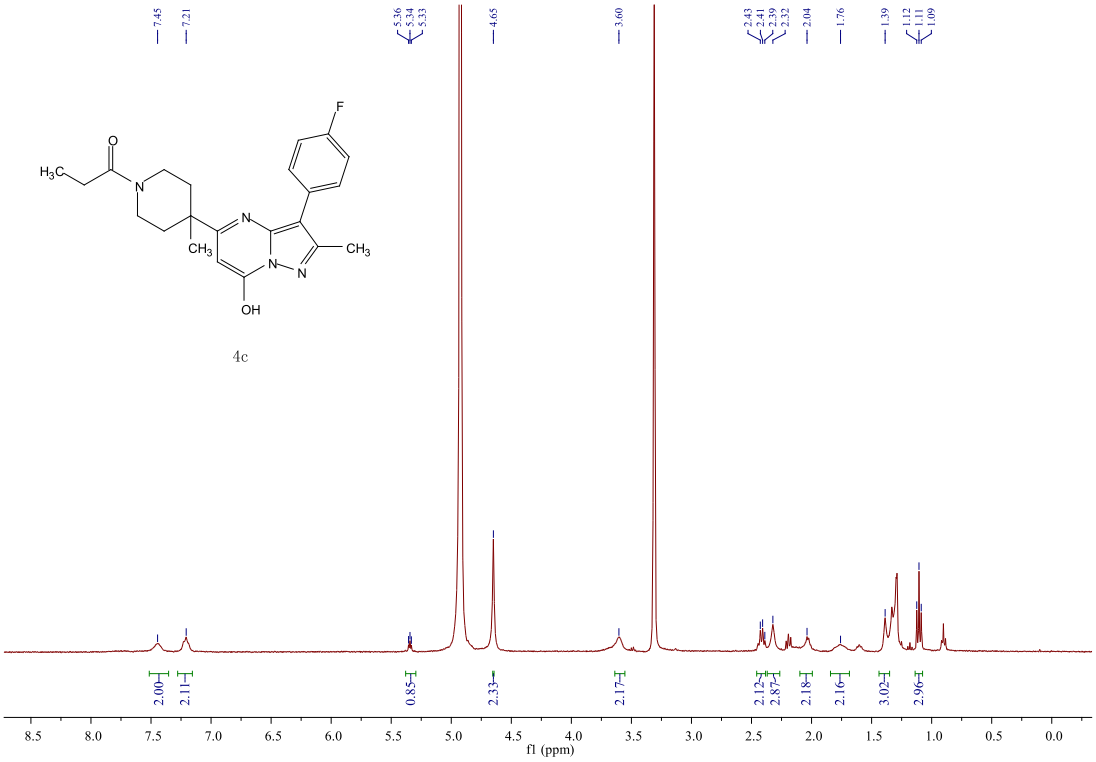

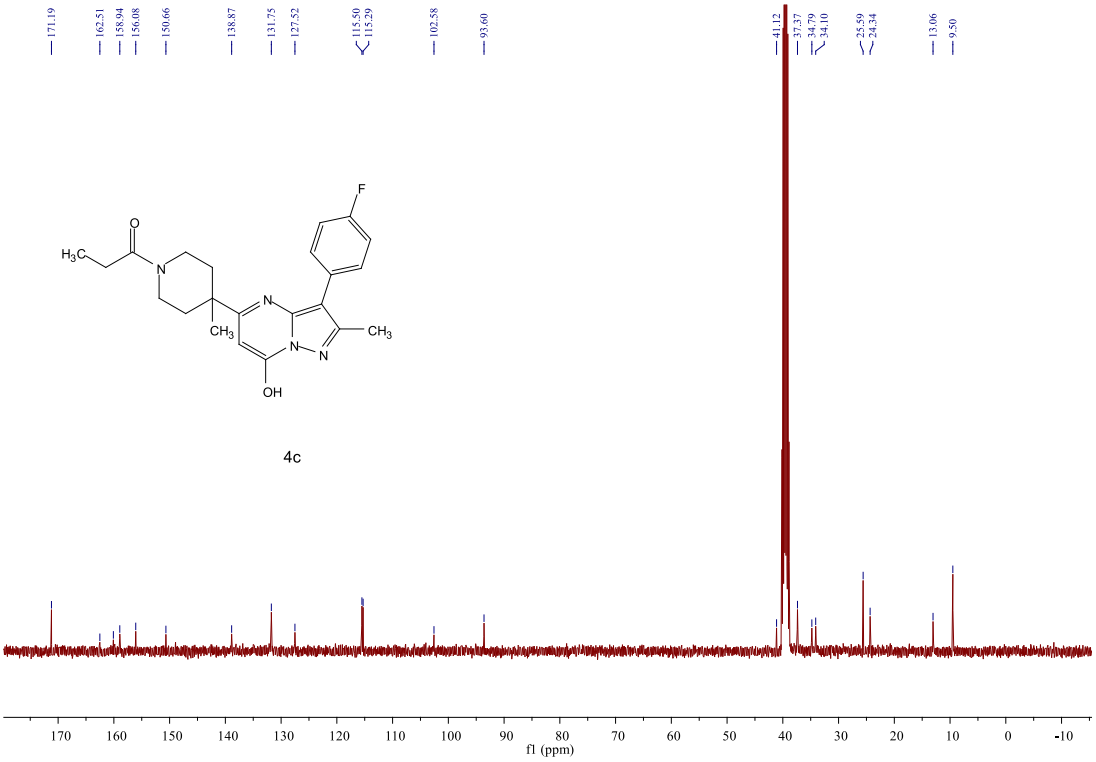

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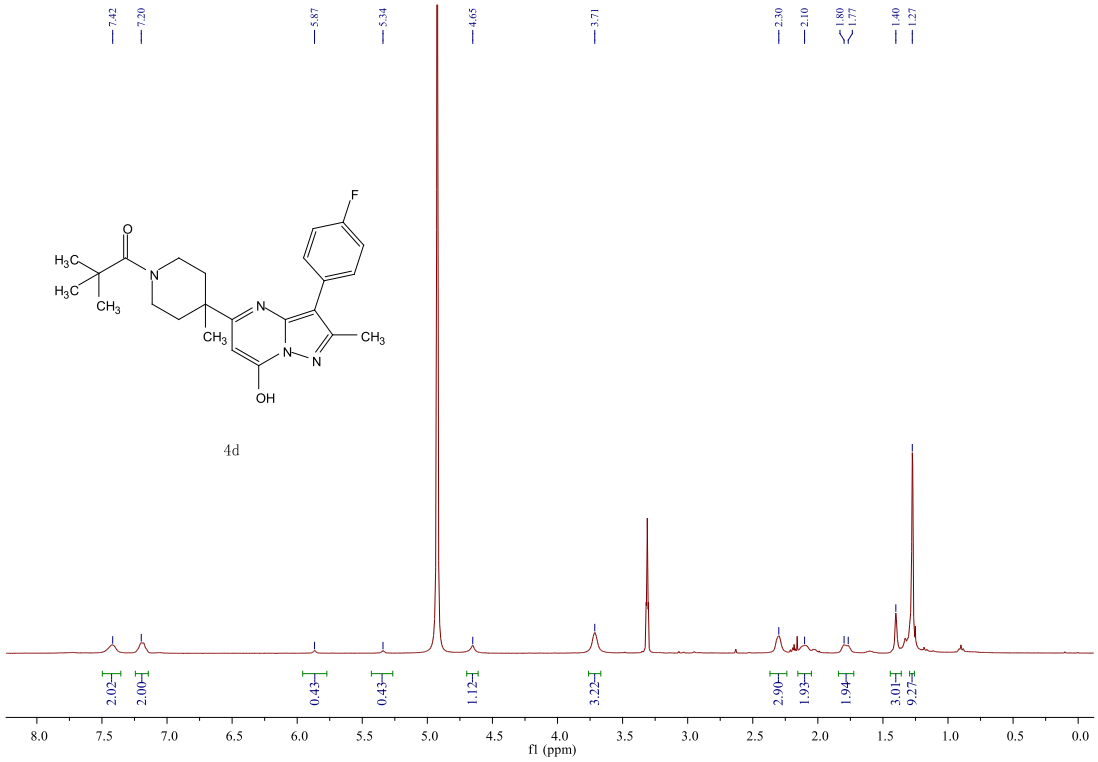
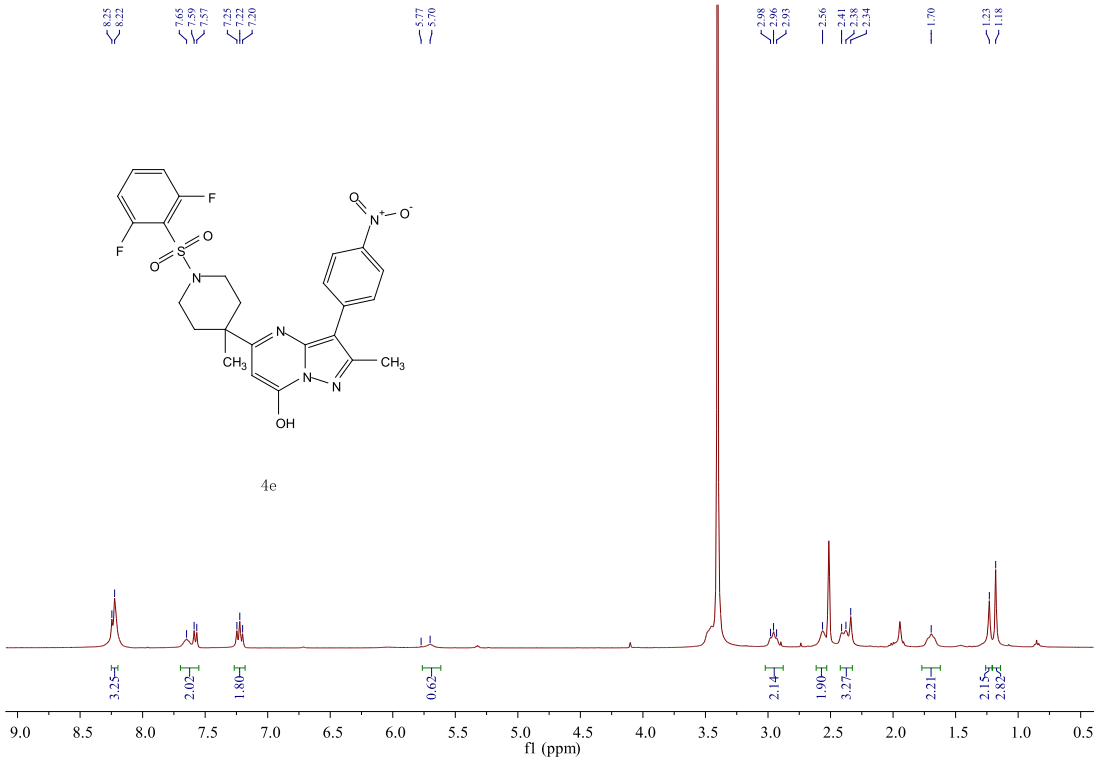
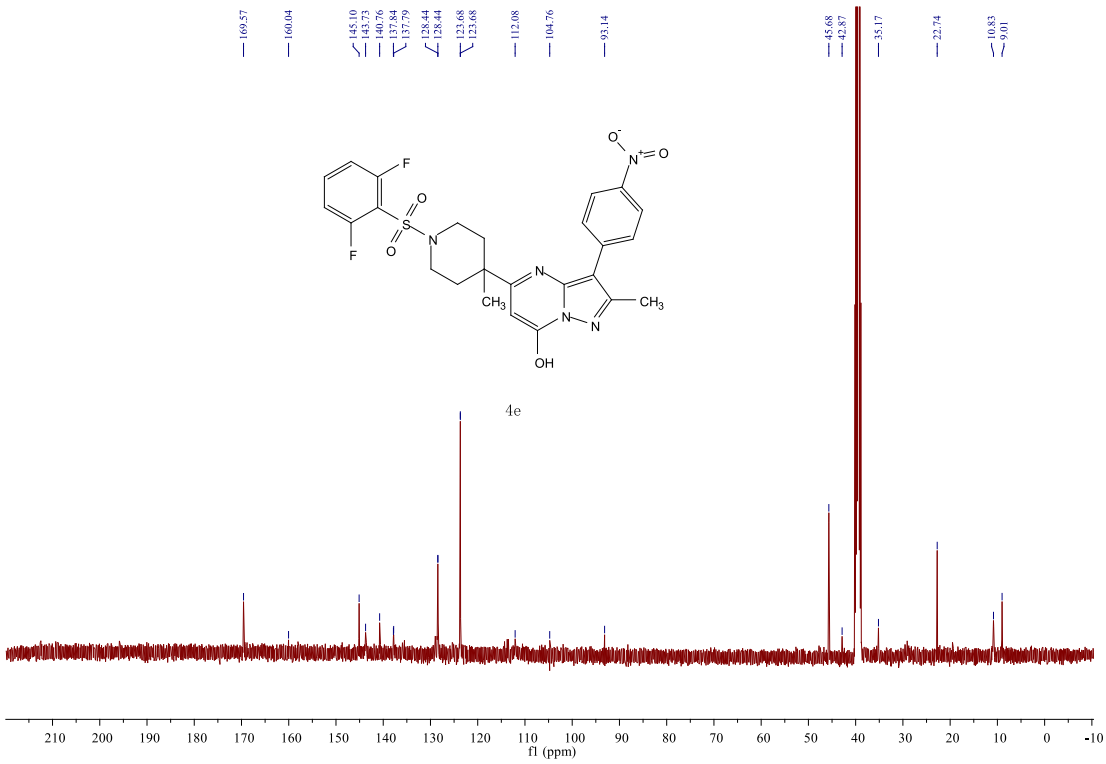
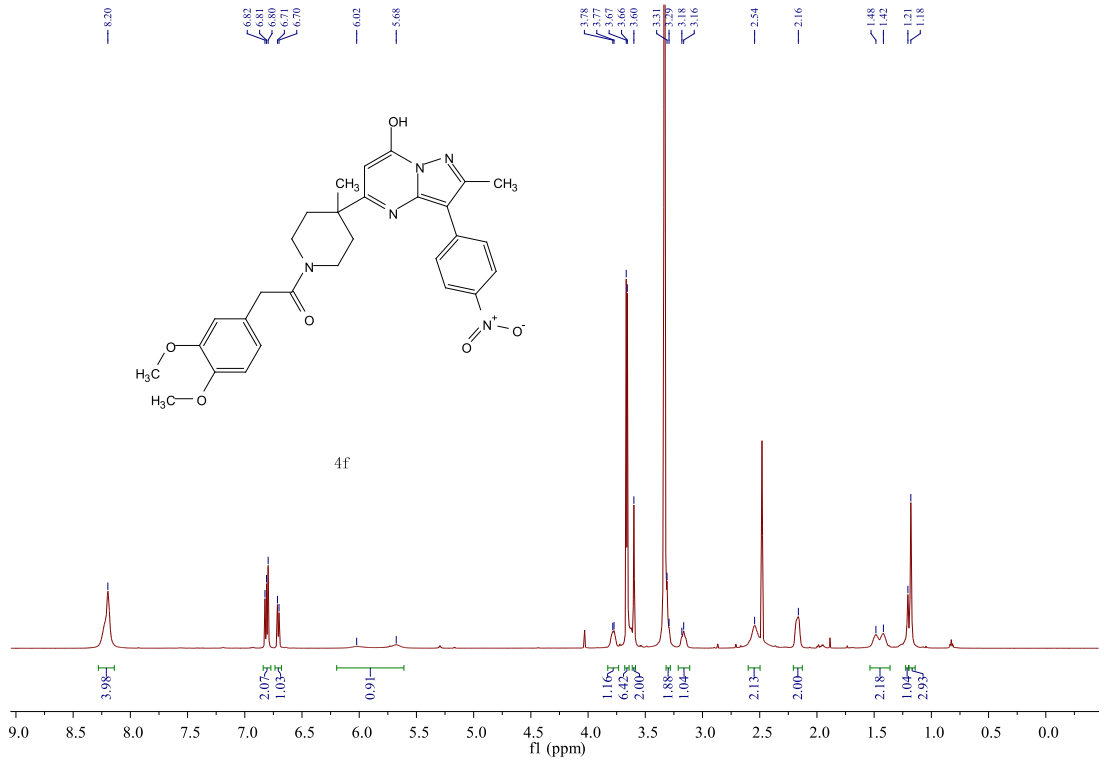

7.42
7.20
5.87
5.34
4.65
3.71
2.30
2.10
1.80
1.77
1.40
1.27
F
O
N
N
N
N
OH
CH3
H3C
H3C
CH3
CH3
4d
2.02
2.00
0.43
0.43
1.12
3.22
2.90
1.93
1.94
3.01
9.27
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

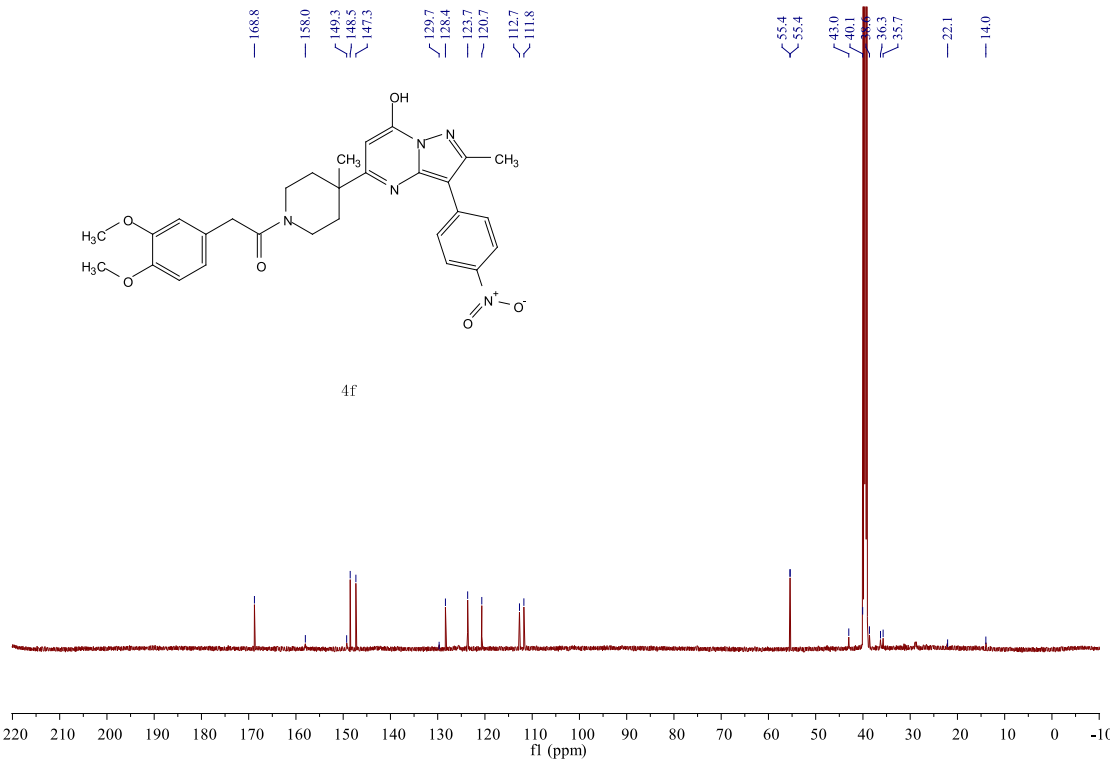
^{1}H NMR and ^{13}C NMR for 4e



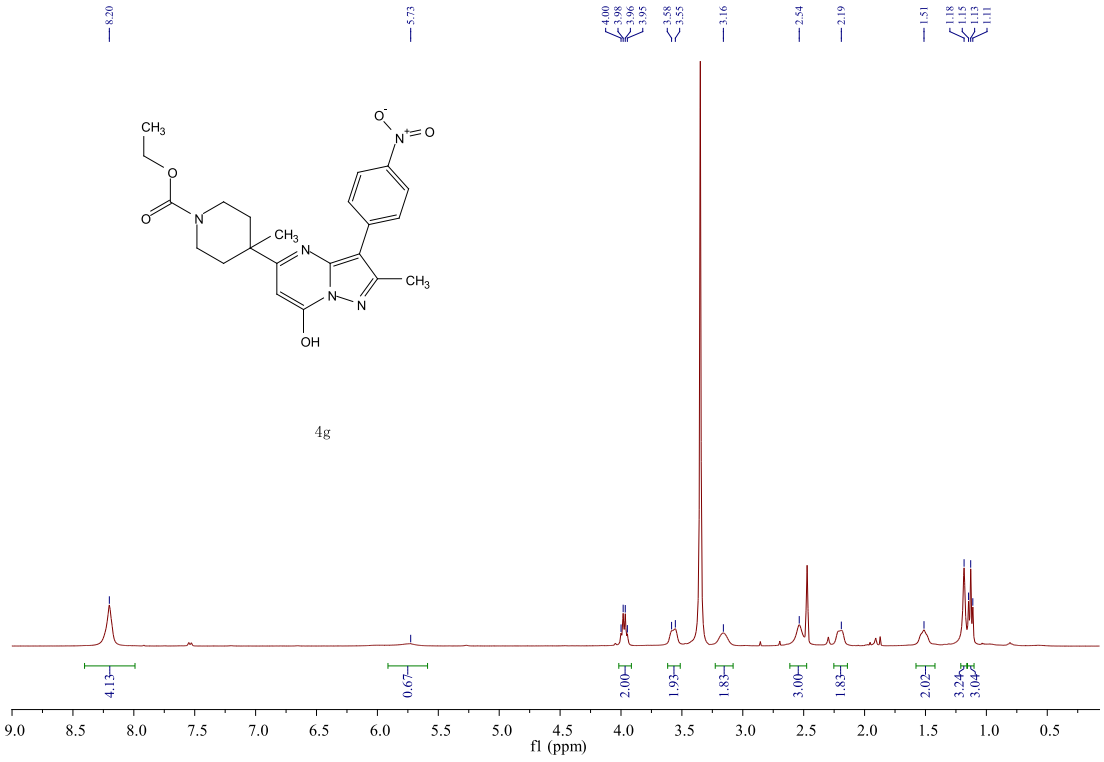


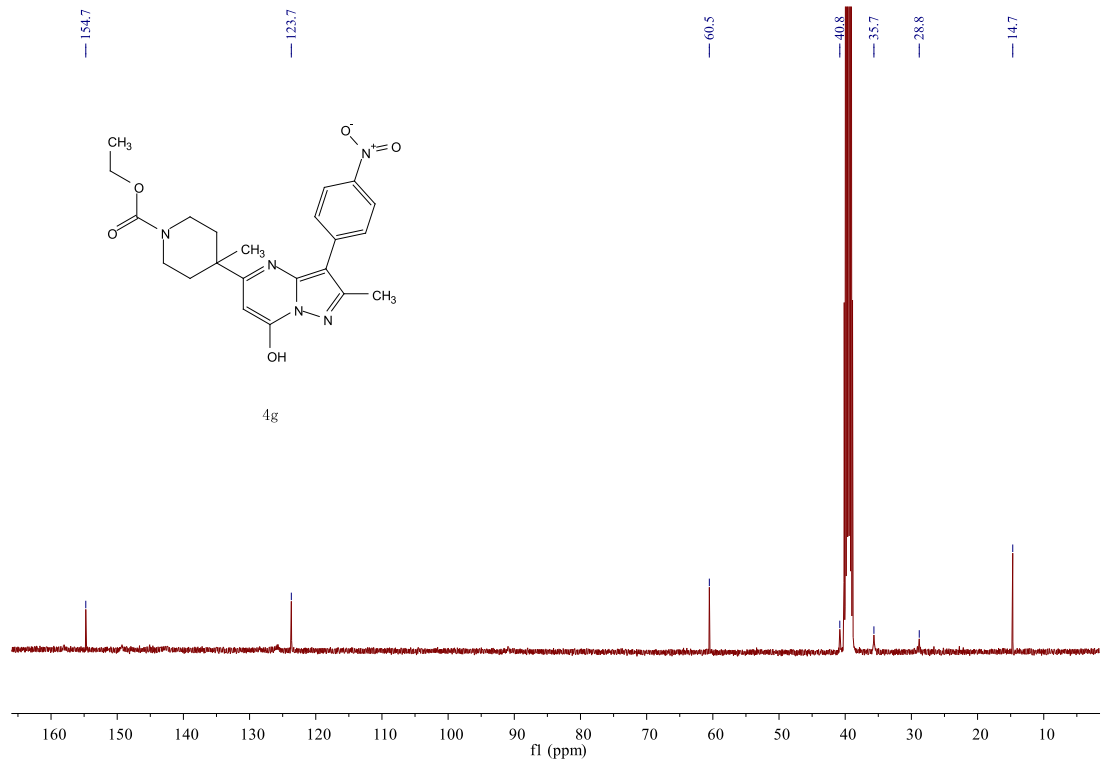
^{1}H NMR and ^{13}C NMR for 4f



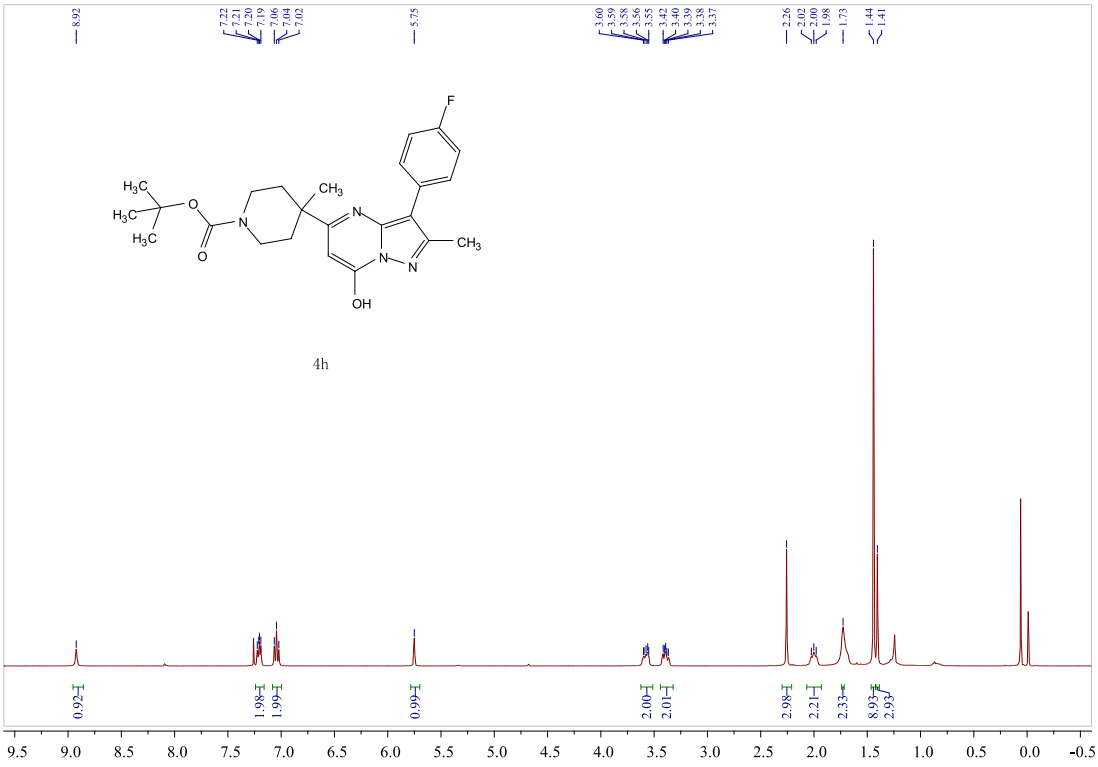


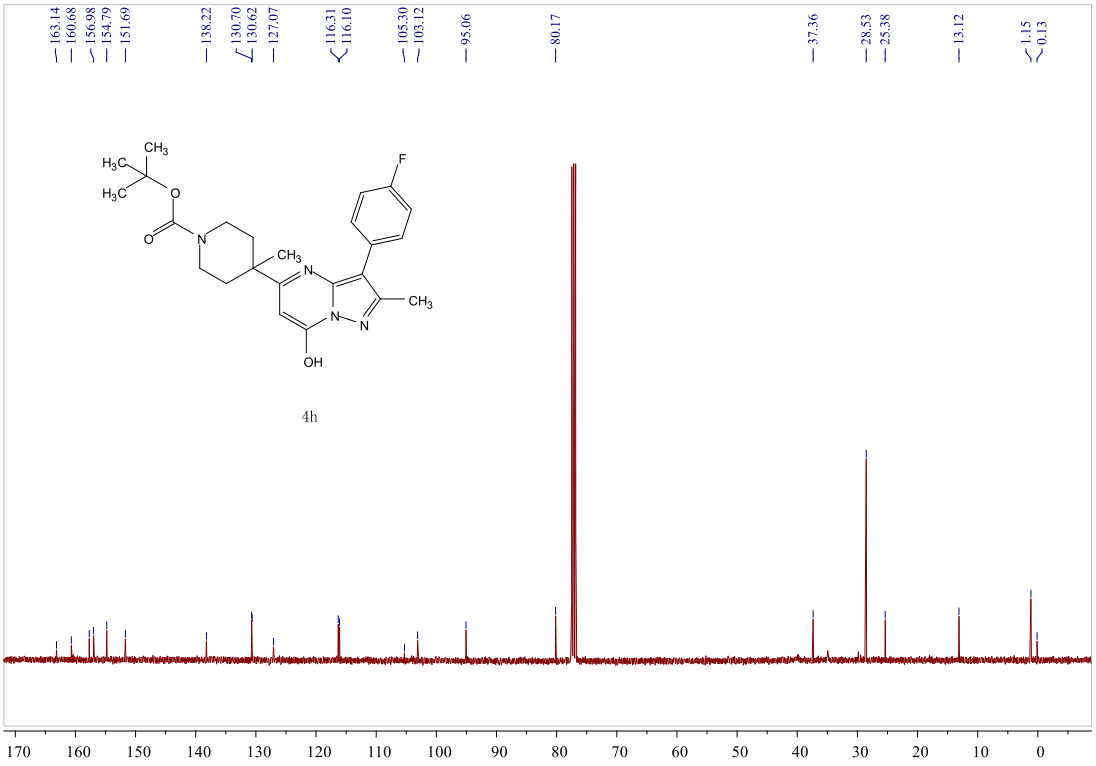
^{1}H NMR and ^{13}C NMR for 4g





^{1}H NMR and ^{13}C NMR for 4h





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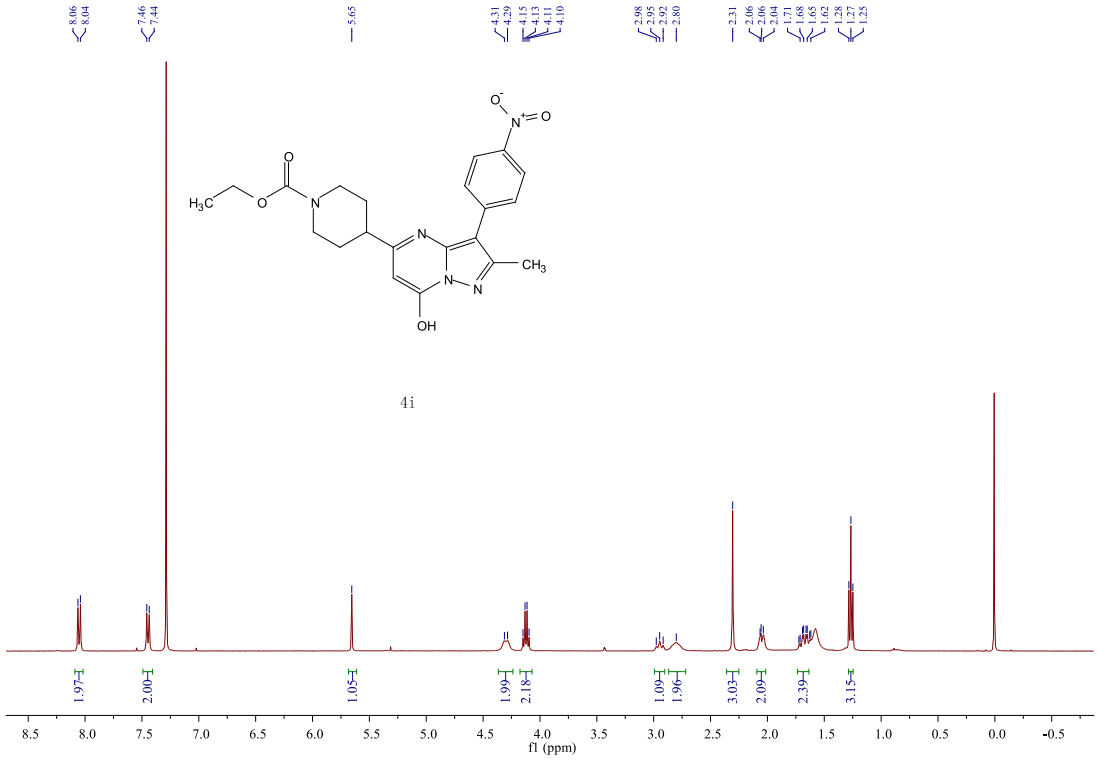
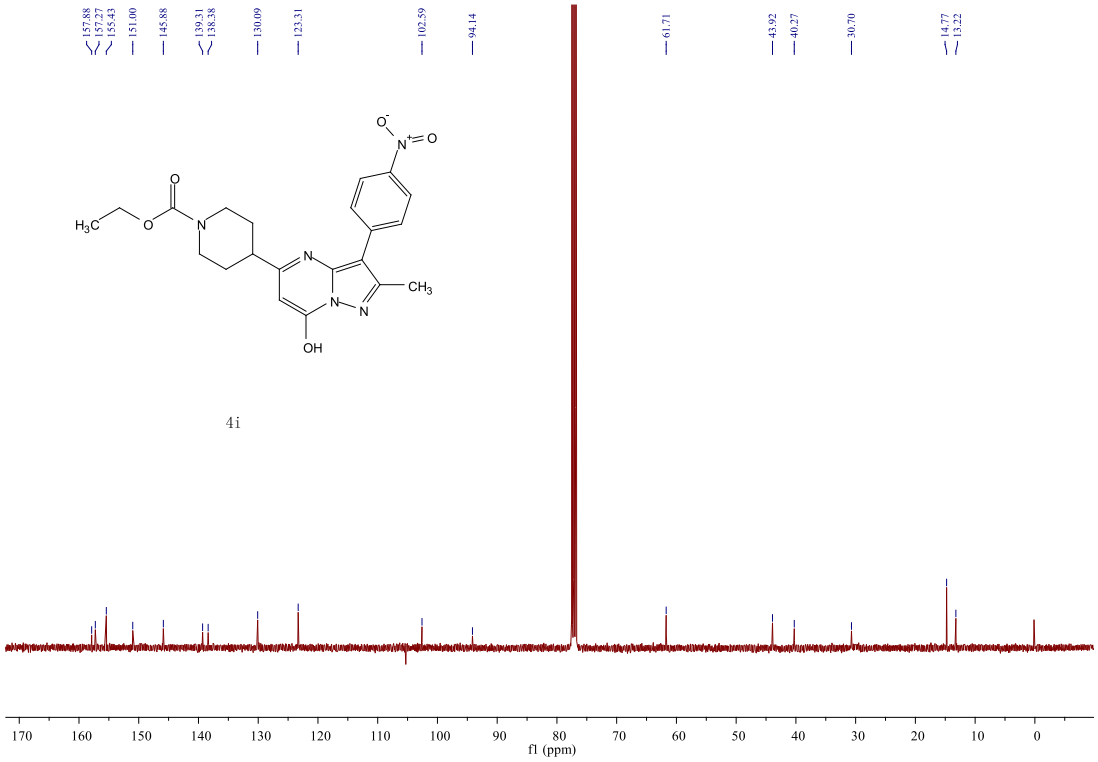
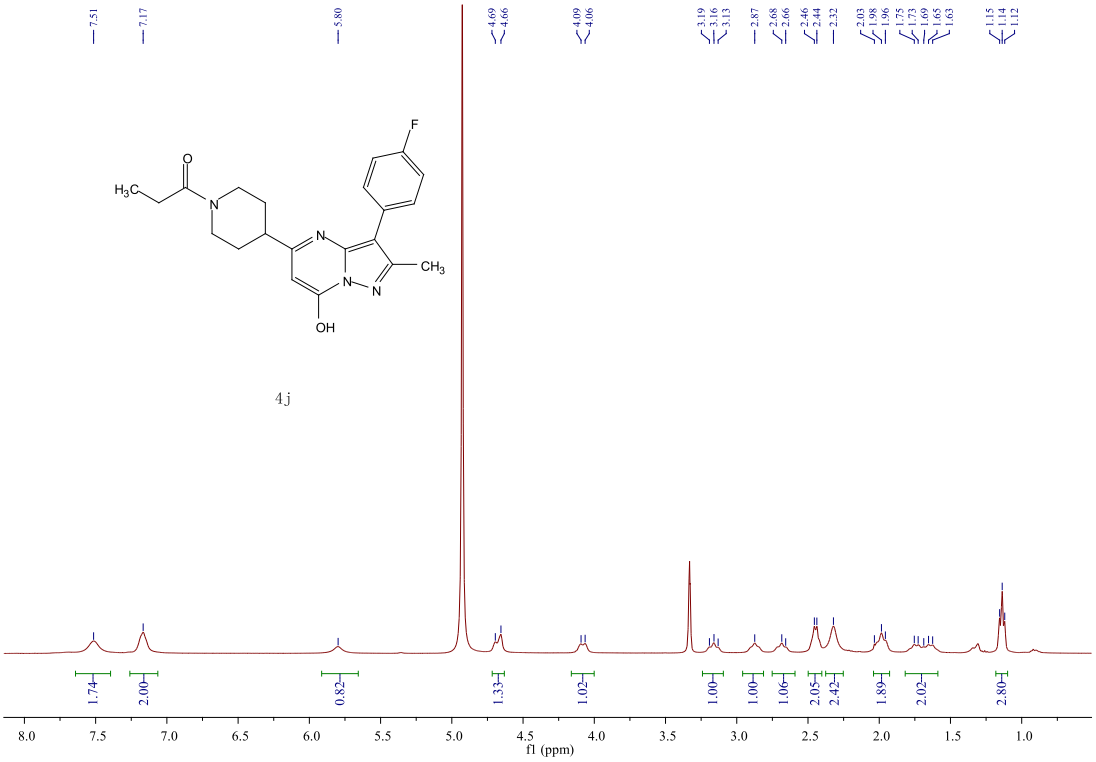
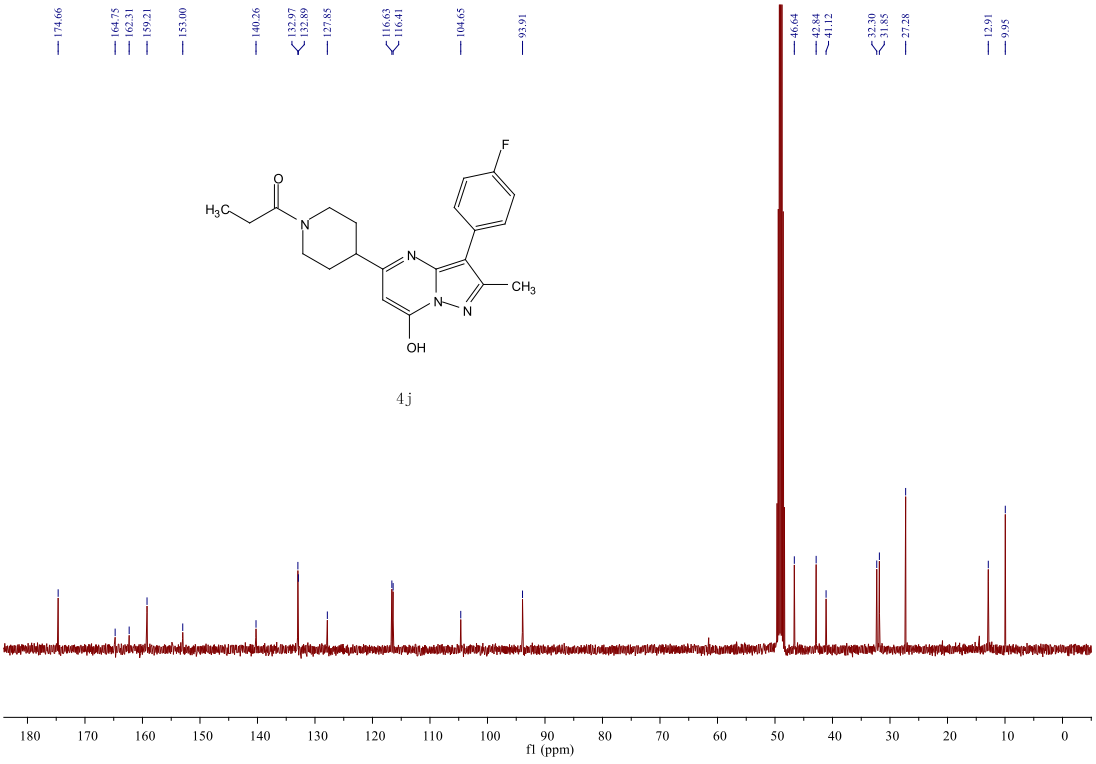


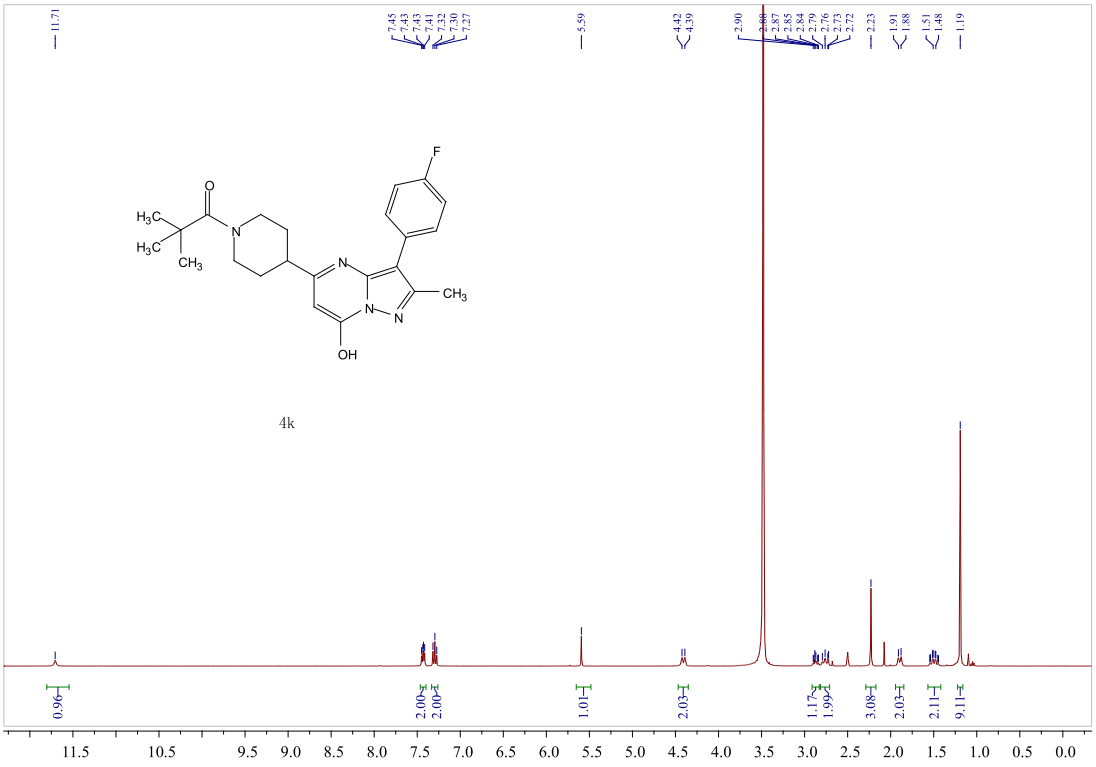


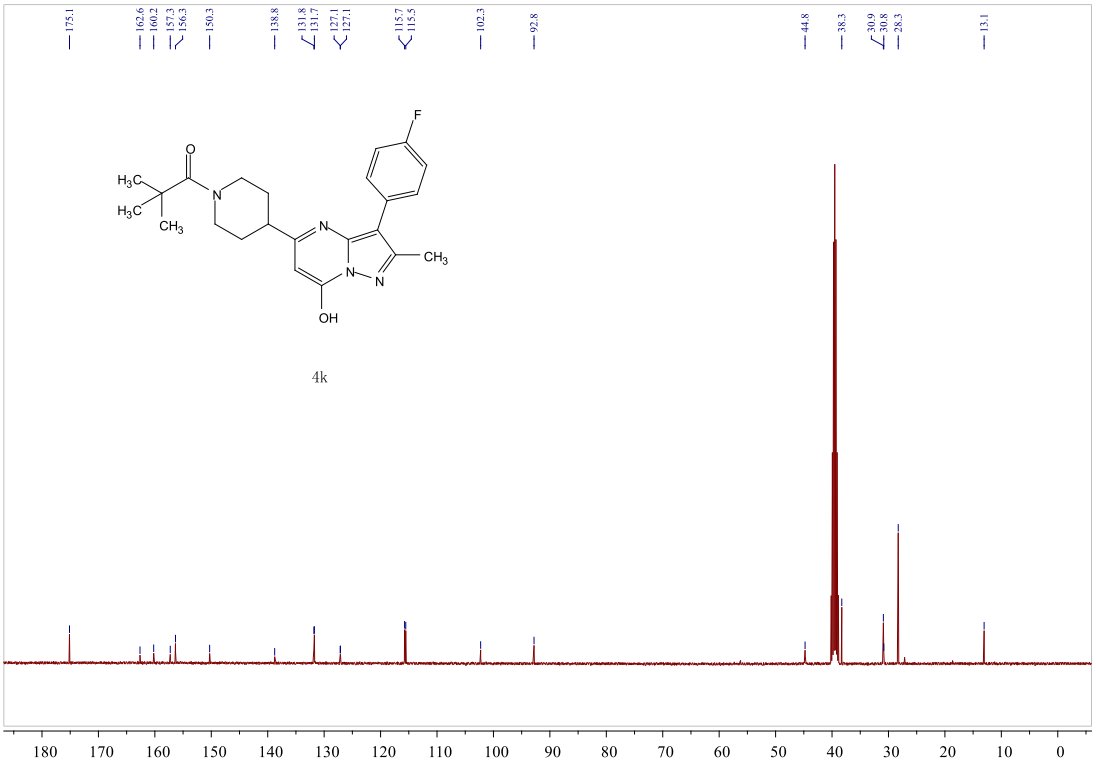
^{1}H NMR and ^{13}C NMR for 4j





^{1}H NMR and ^{13}C NMR for 4k





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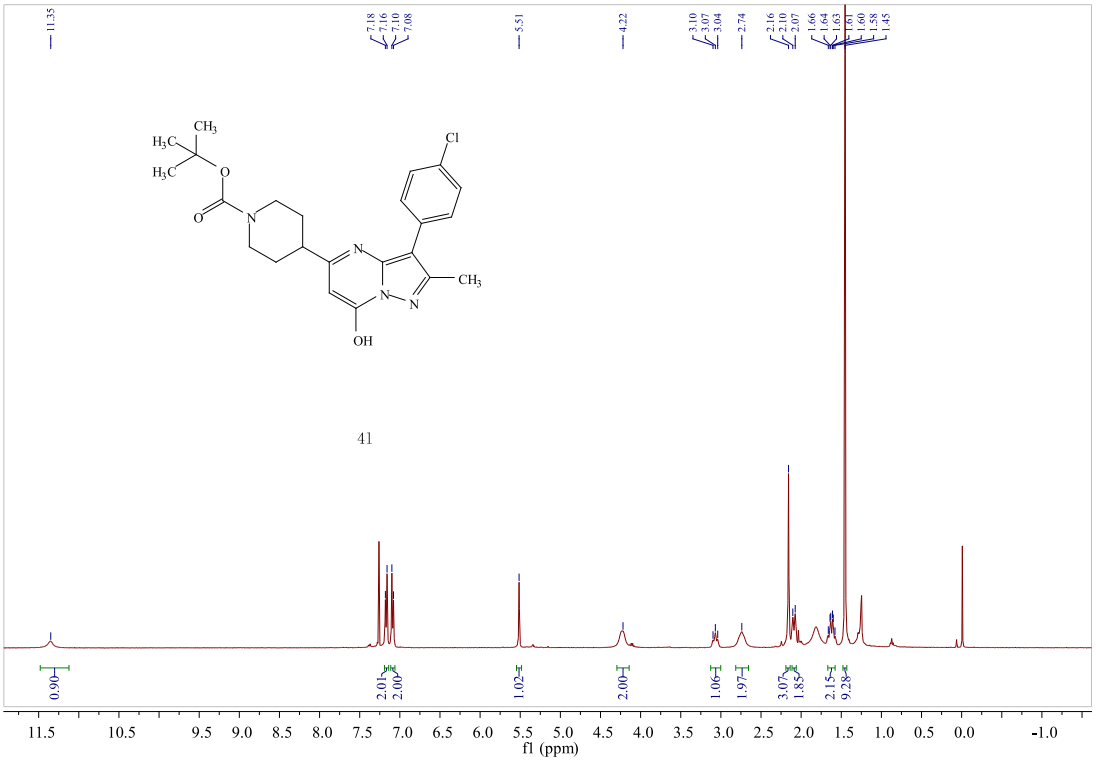

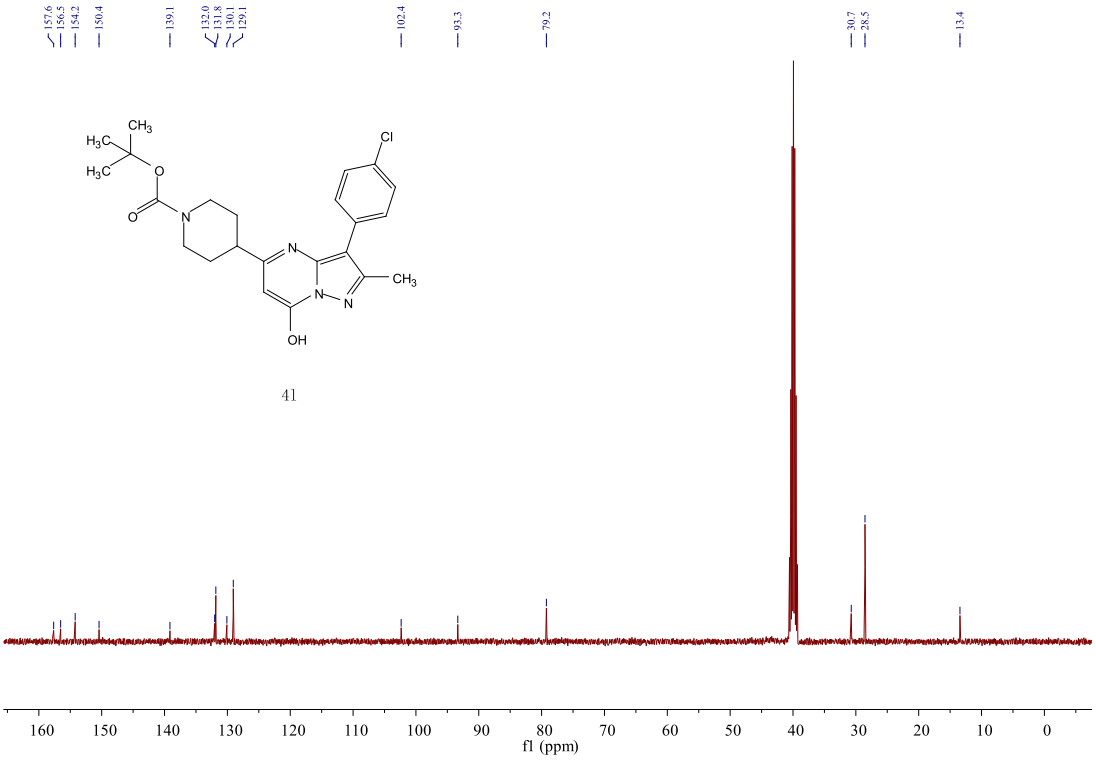

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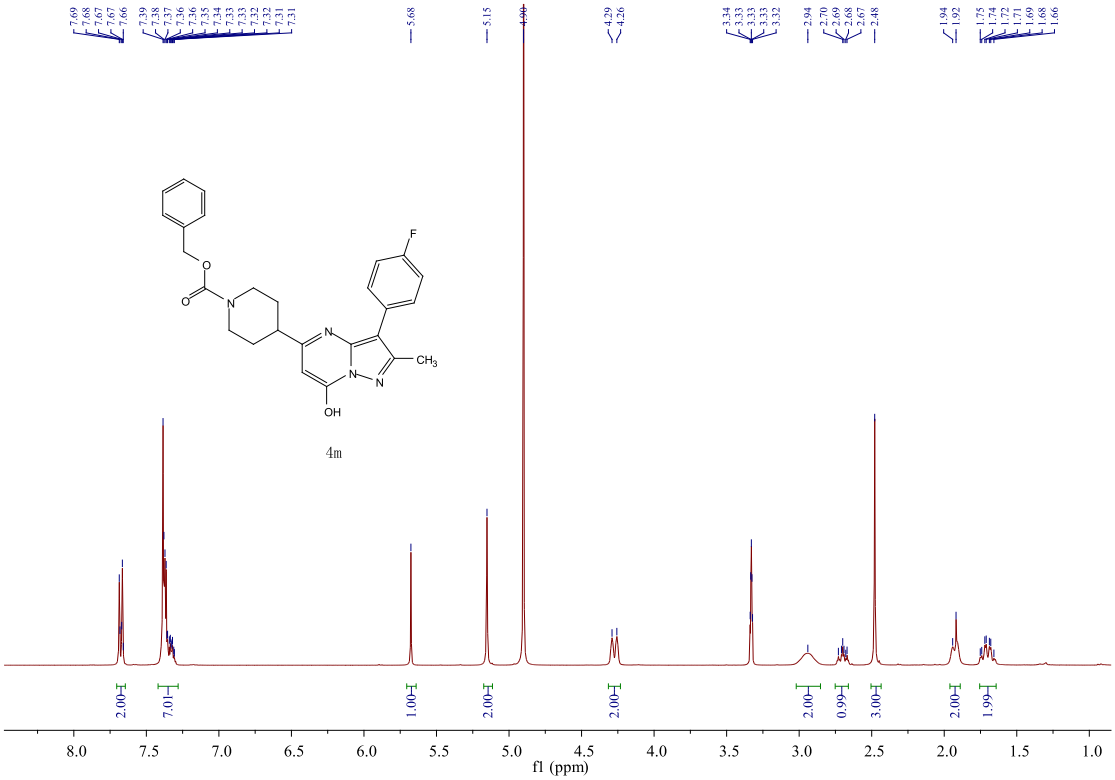

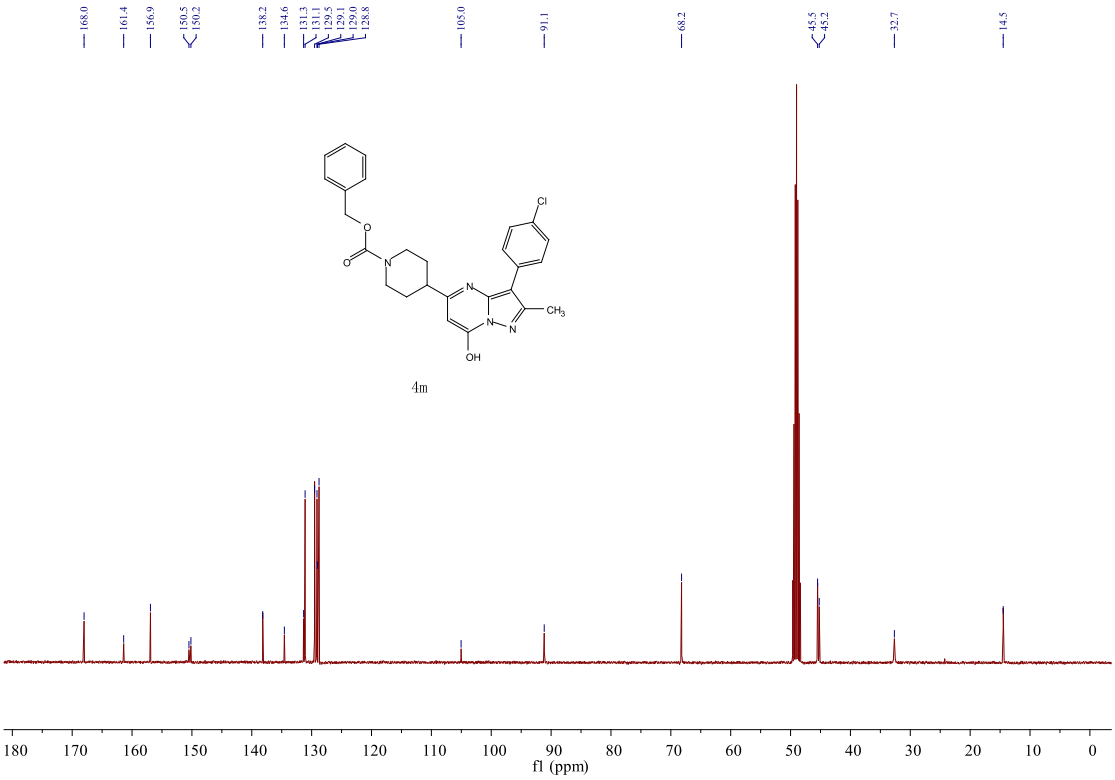

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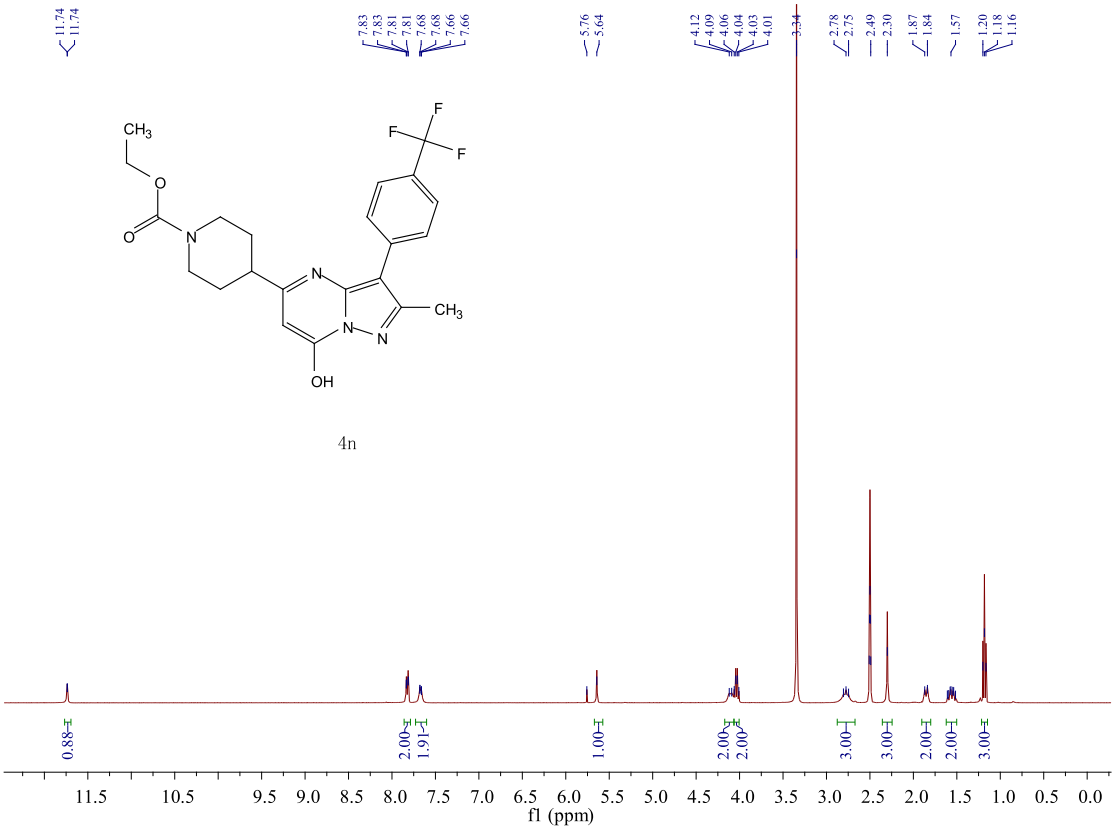

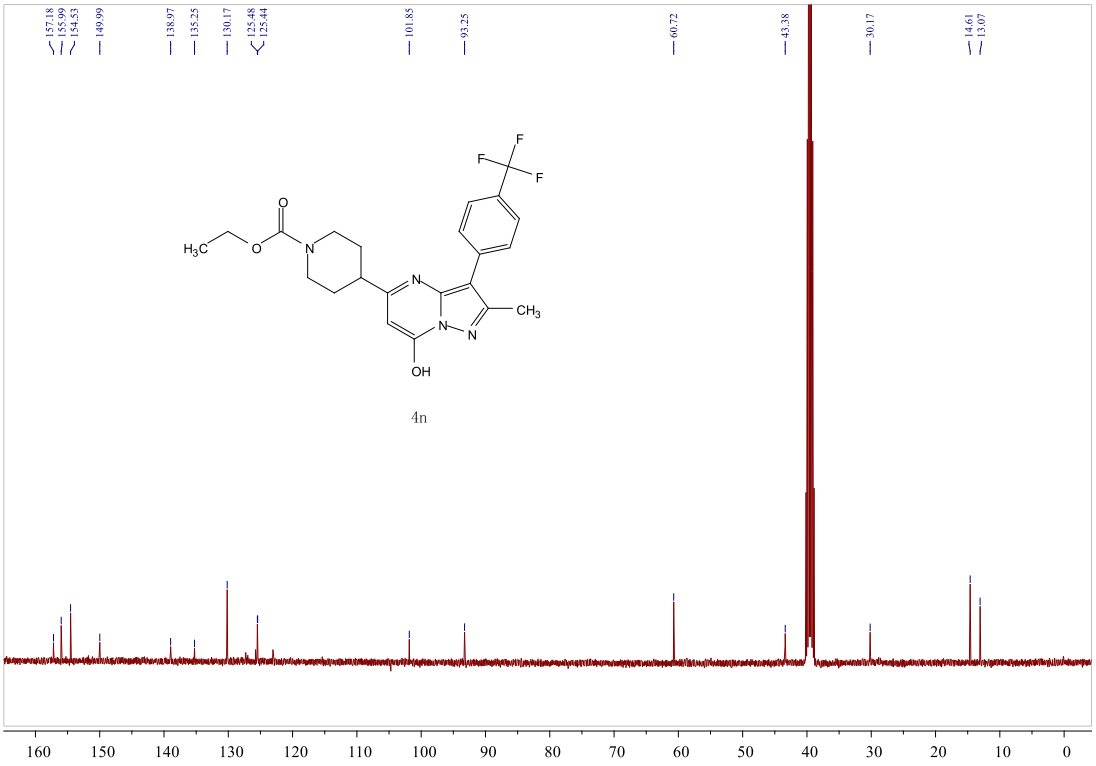

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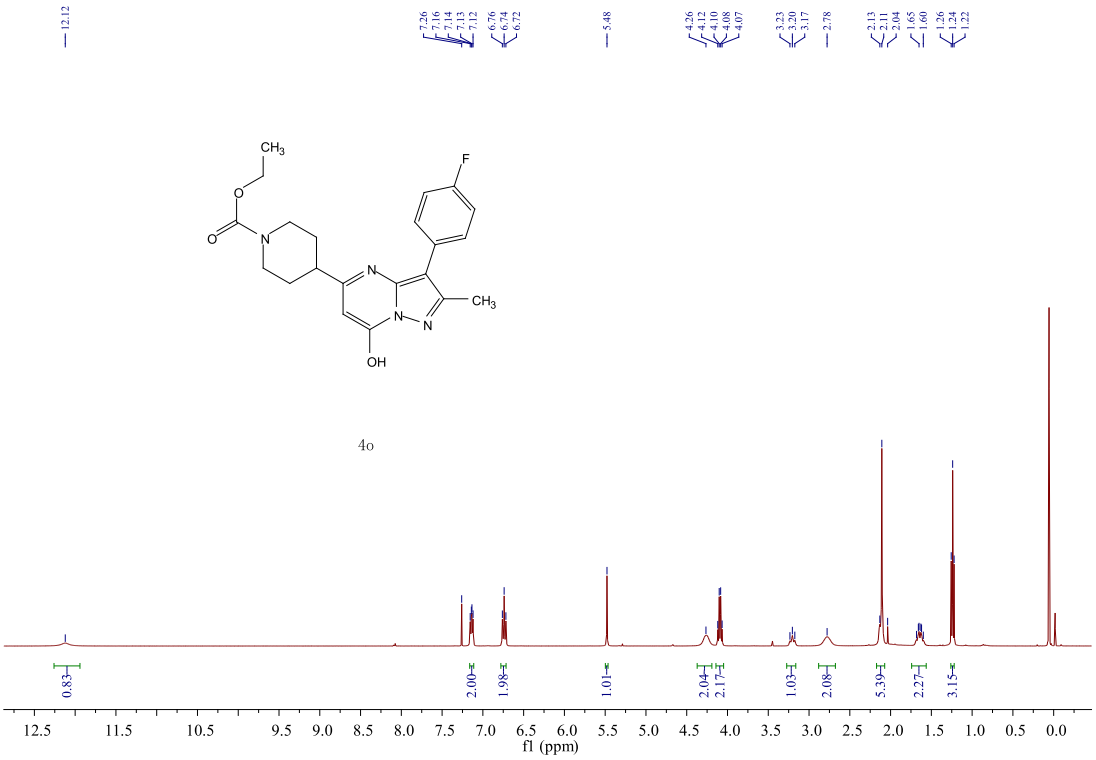

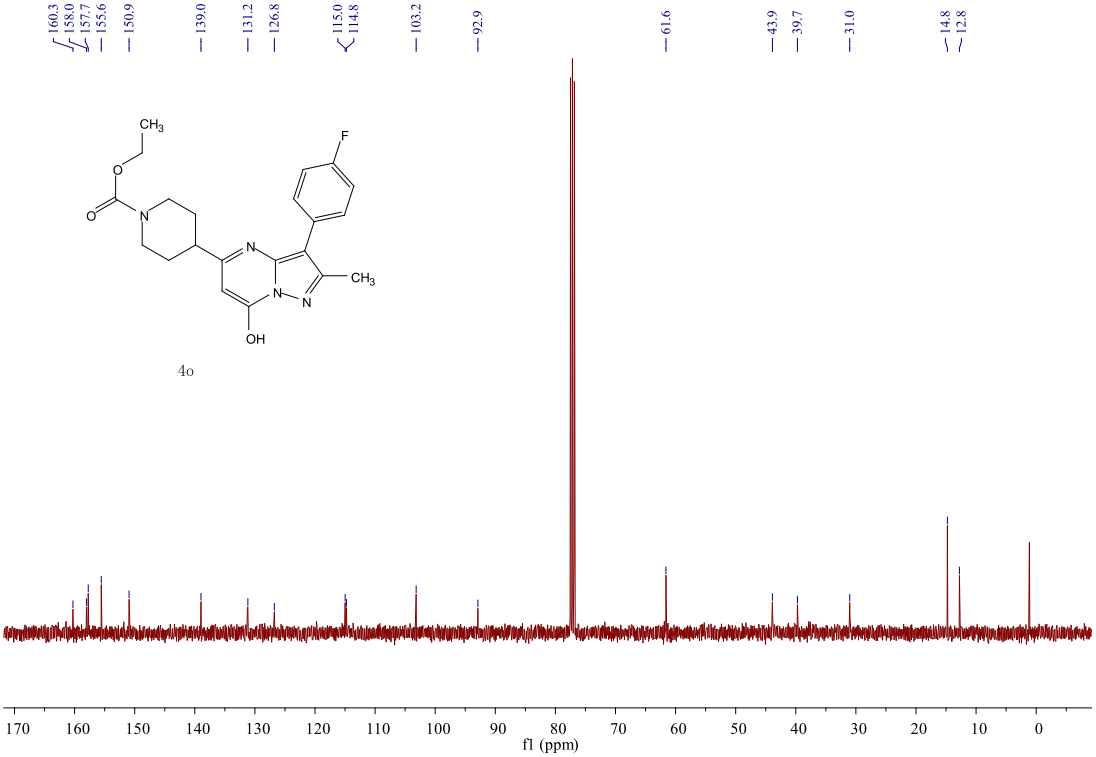

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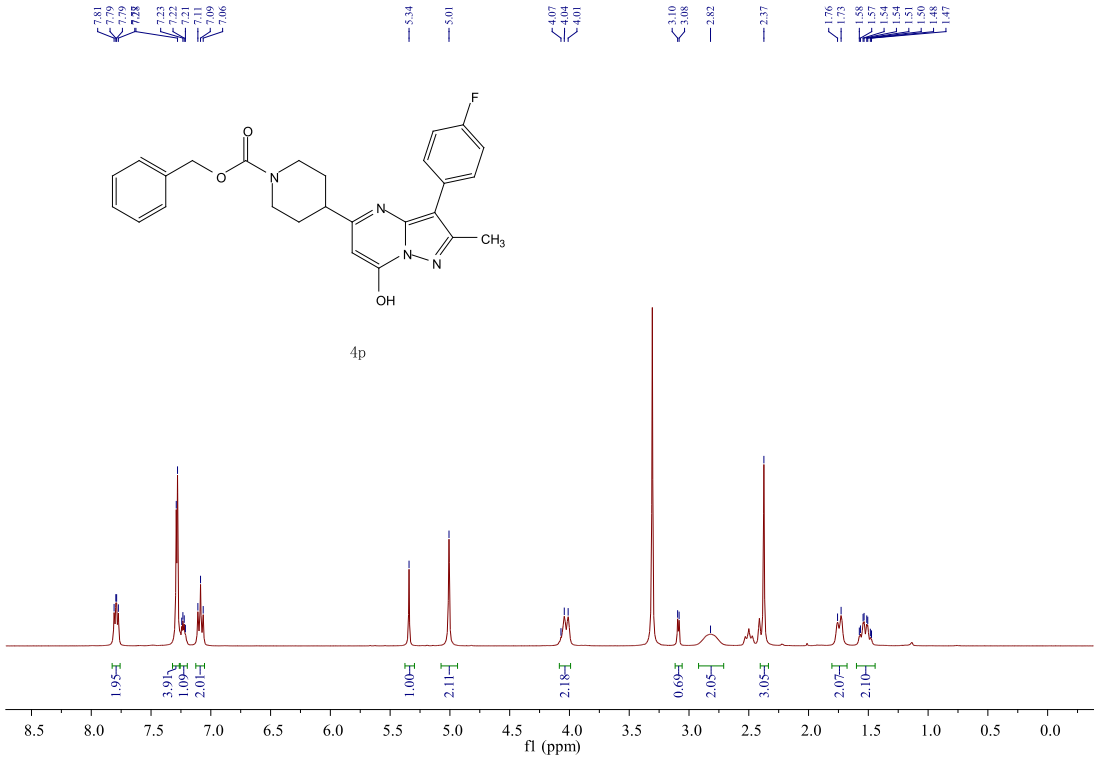
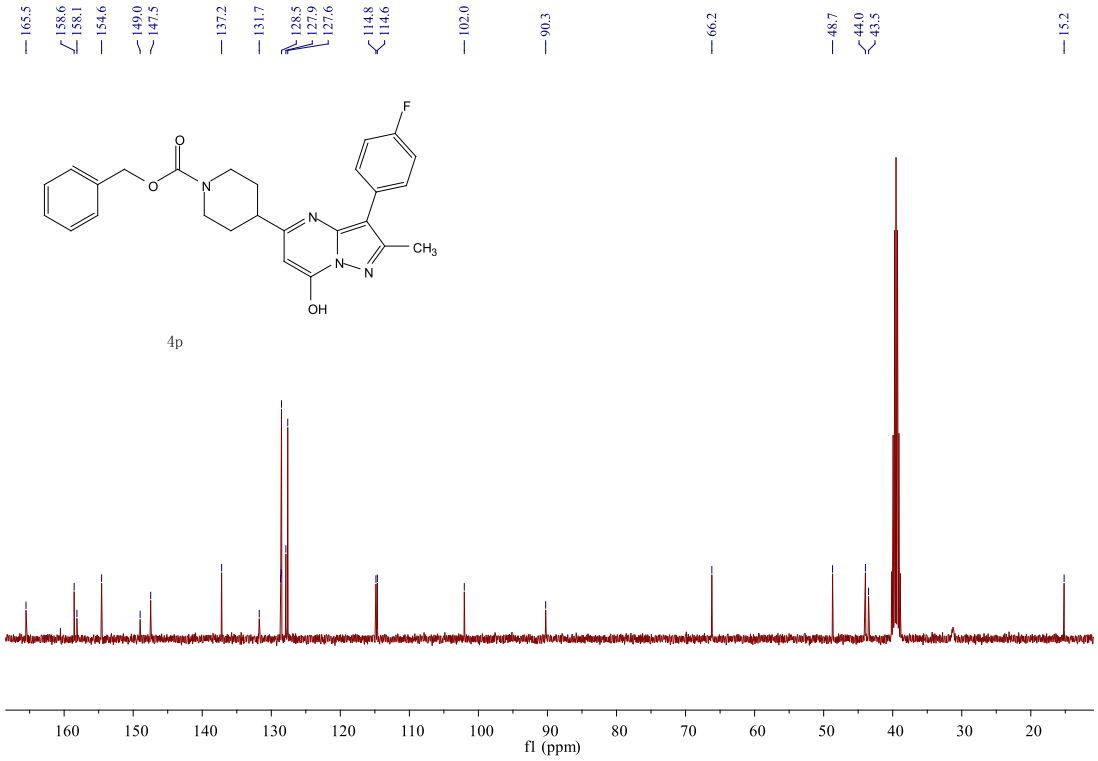




PART 3. Reversed Phase HPLC results of 4a-4p

All the reversed phase HPLC were conducted on DIONEX Ultimate 3000 and all the results were obtained with the UV detection at 254 nm. Compounds **4d**, **4e**, **4m** were taken with Method A (Table 1) while the others were taken with Method B (Table 2). The results are listed in Table 3.

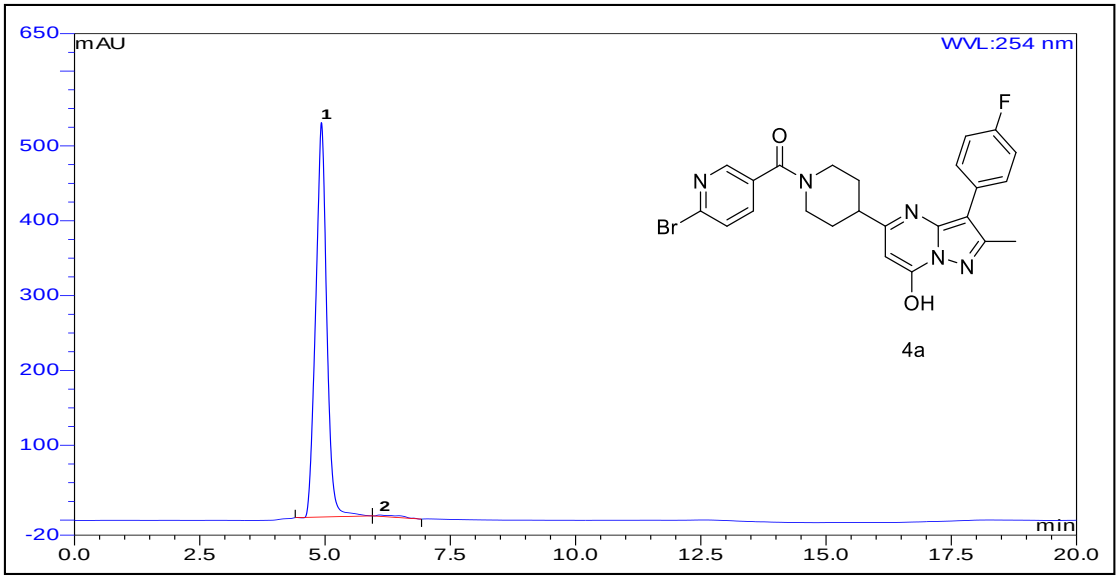
Table 1. Reversed Phase HPLC Method A

Time(min)	(%)(H_2O+0.1% Et_3N)	(%)MeOH
0	30	70
5	30	70
7	45	55
9	45	55
11	30	70
20	30	70

Table 2. Reversed Phase HPLC Method B

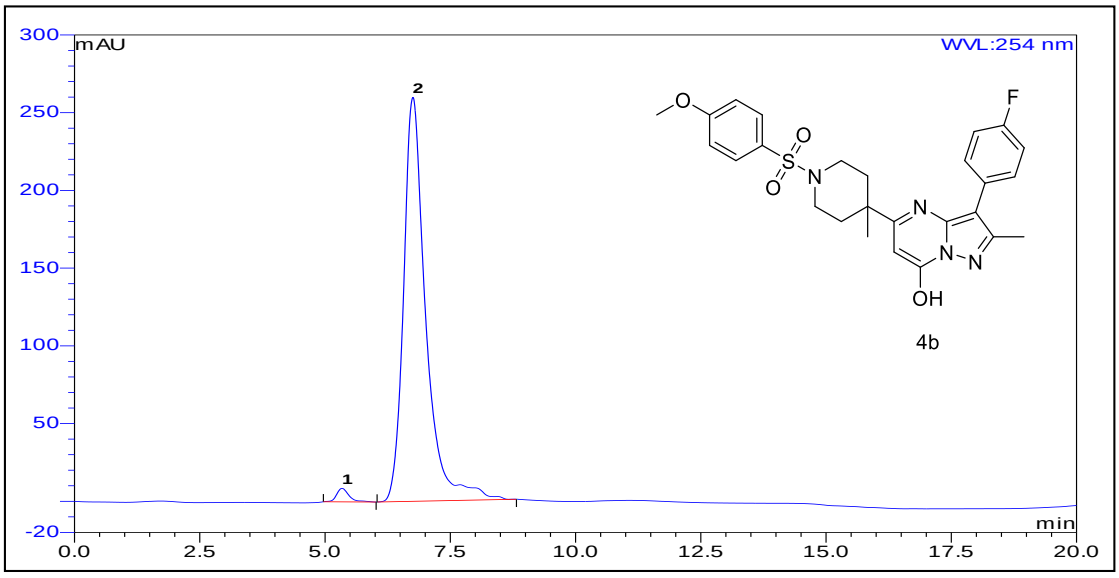
Time(min)	(%)(H_2O+0.1% Et_3N)	(%)MeOH
0	20	80
5	20	80
7	45	55
9	45	55
11	20	80
20	20	80

Reversed Phase for **4a** (Method A)



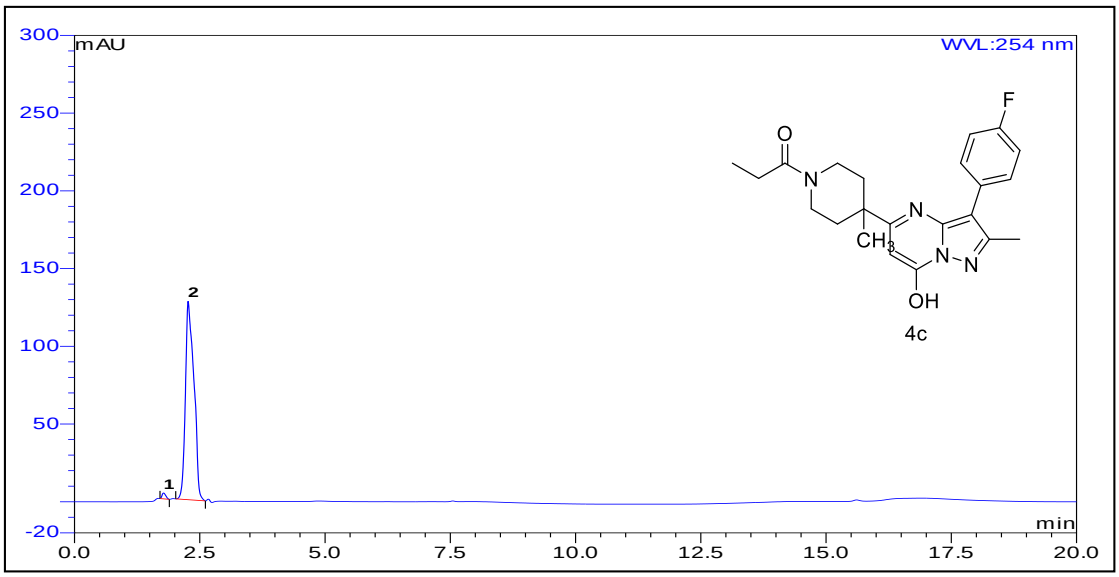
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	4.93	526.95	134.64	99.08
2	6.09	1.76	1.26	0.92
Total		628.71	135.90	100.00

Reversed Phase for **4b** (Method A)



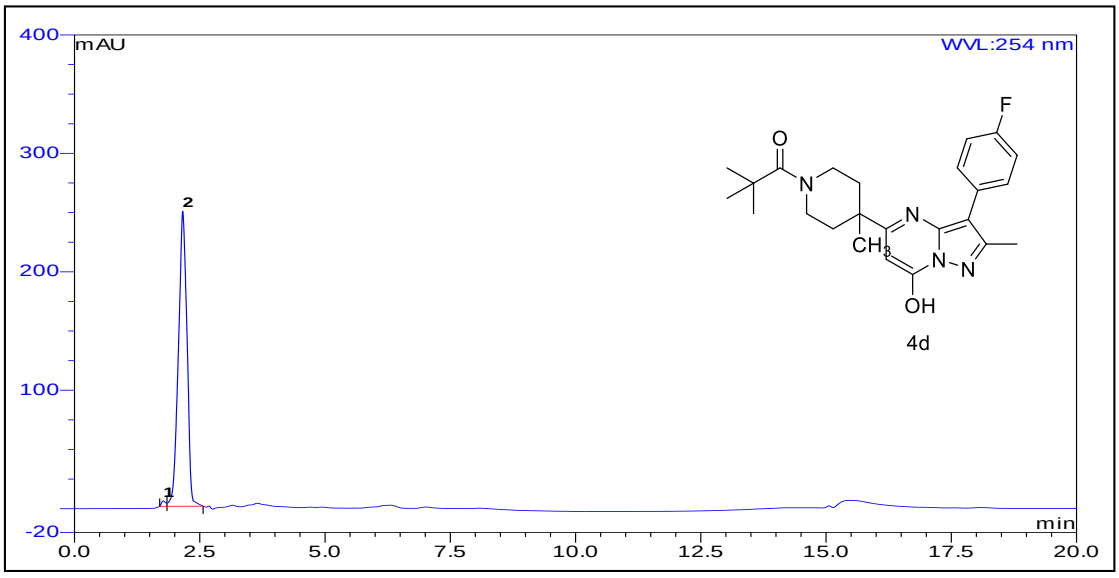
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	5.34	8.69	2.51	1.85
2	6.75	259.86	133.30	98.51
Total		268.54	135.81	100.00

Reversed Phase for **4c** (Method A)



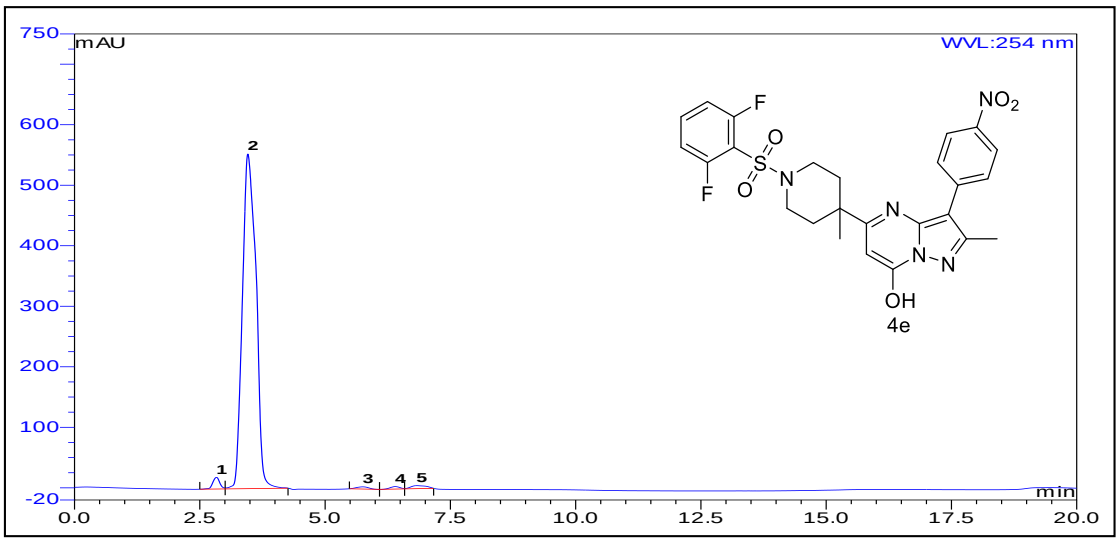
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	1.78	3.81	0.33	1.29
2	2.27	127.56	25.10	98.71
Total		131.37	25.43	100.00

Reversed Phase for **4d** (Method B)



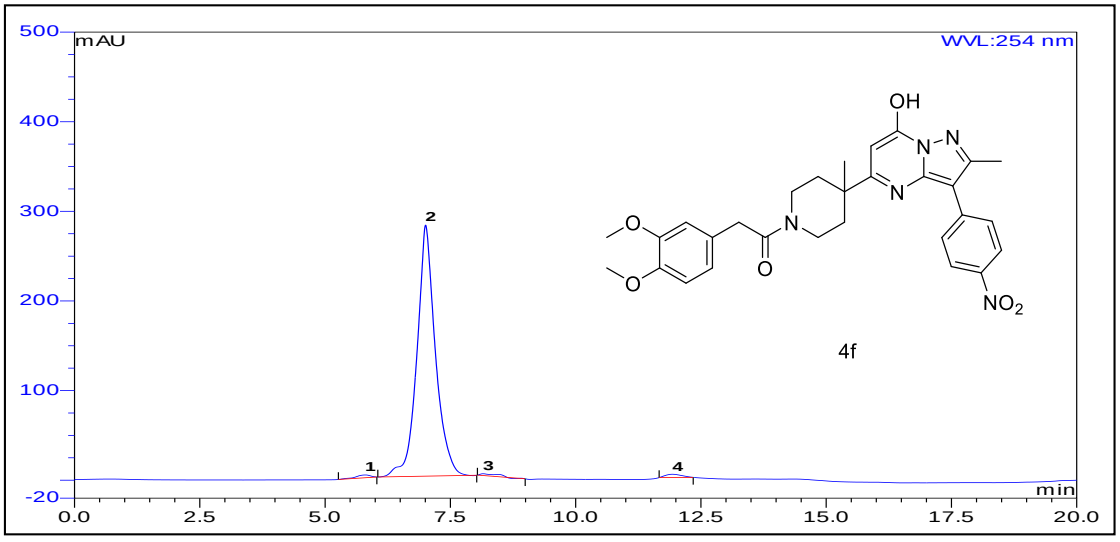
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	1.77	4.54	0.44	0.89
2	2.16	249.09	49.37	99.11
Total		253.63	49.81	100.00

Reversed Phase for **4e**(Method B)



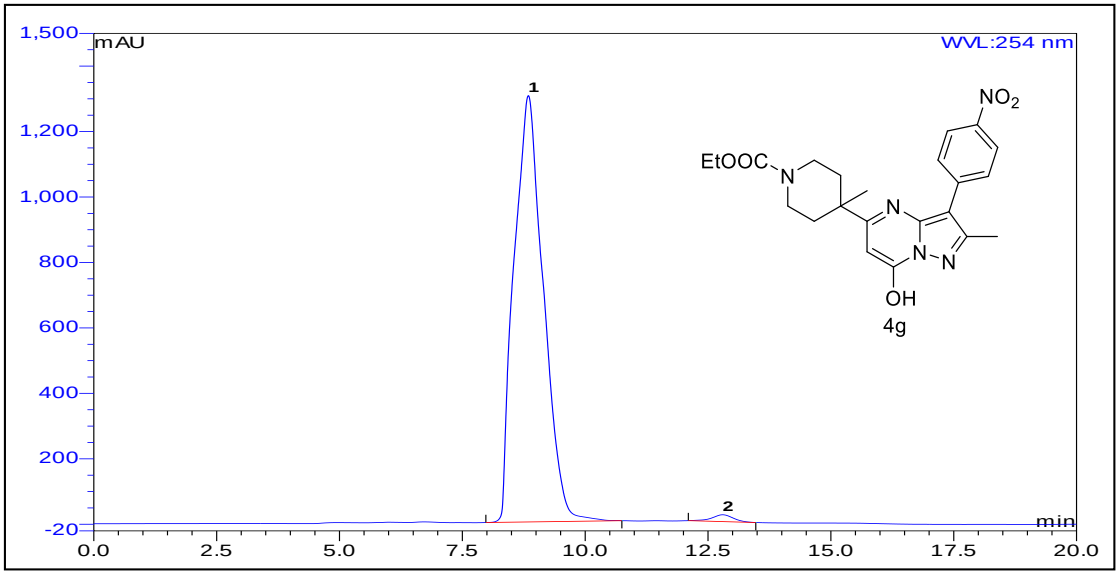
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	2.83	19.23	3.13	1.73
2	3.46	552.86	174.37	96.21
3	5.75	3.63	1.01	0.56
4	6.40	4.13	0.92	0.51
5	6.83	4.98	1.81	1.00
Total		584.84	181.25	100.00

Reversed Phase for **4f** (Method A)



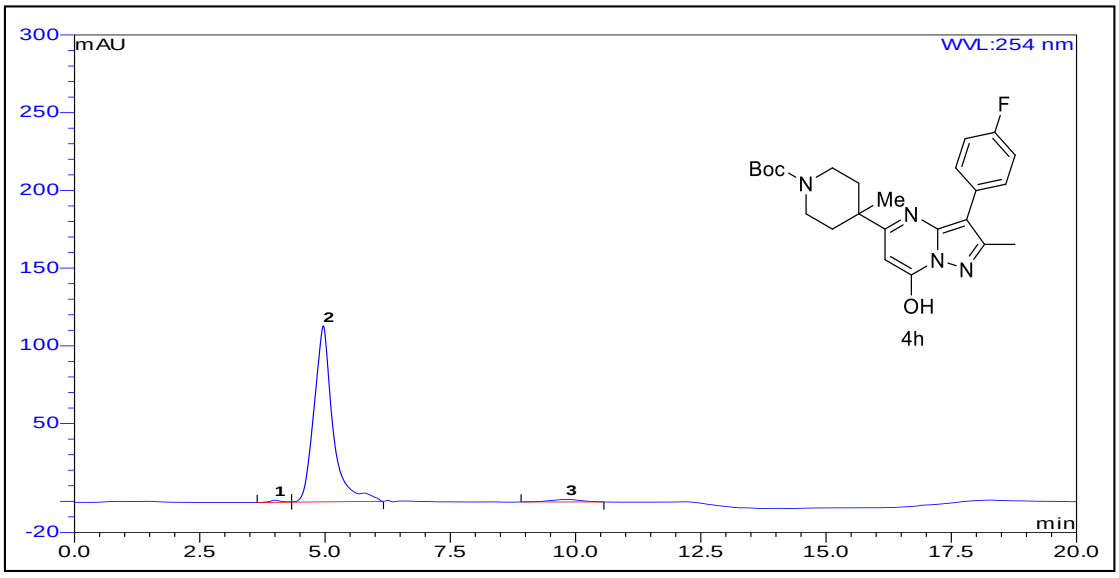
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	5.80	3.11	1.08	0.88
2	7.01	280.16	119.40	97.24
3	8.15	1.99	0.94	0.76
4	11.93	3.62	1.37	1.11
Total		288.88	122.79	100.00

Reversed Phase for **4g** (Method A)



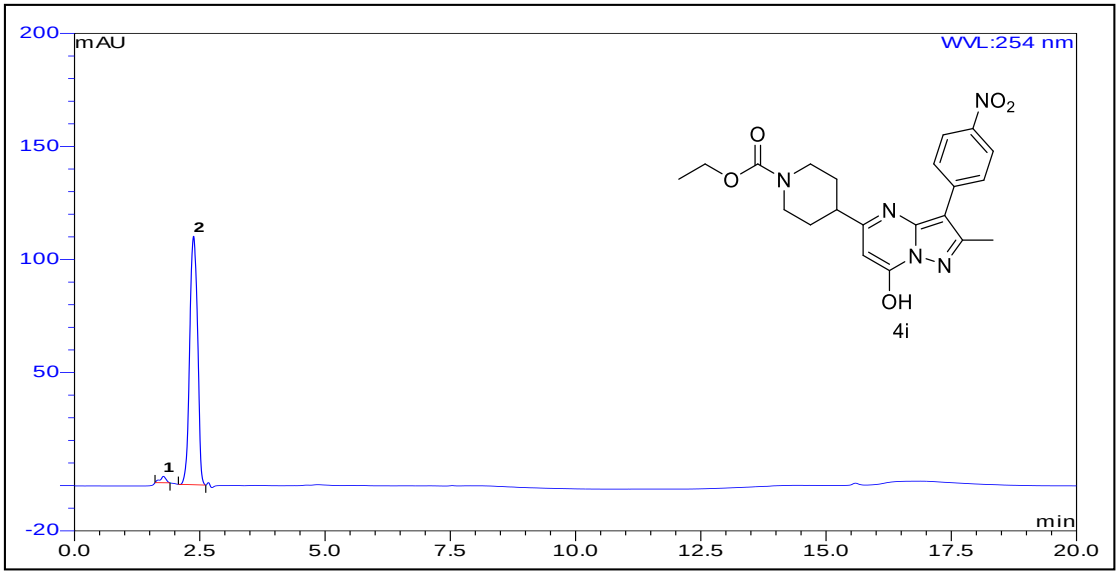
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	8.85	1302.57	901.50	98.85
2	12.80	20.78	10.48	1.15
Total		1323.35	911.98	100.00

Reversed Phase for **4h** (Method A)



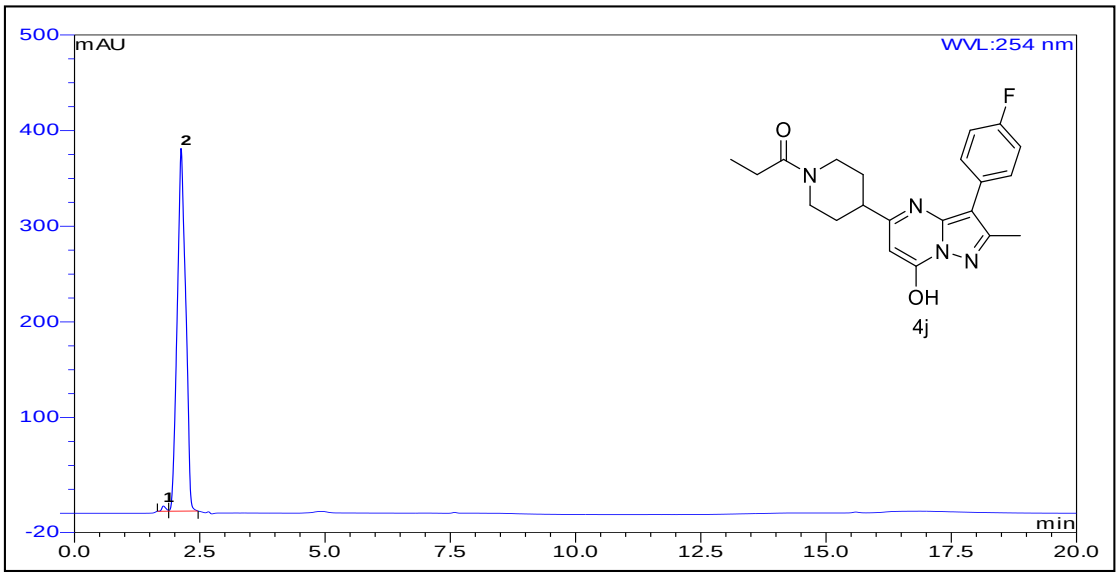
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	4.00	1.38	0.36	0.71
2	4.97	113.30	49.06	97.02
3	9.81	1.62	1.15	2.27
Total		116.30	50.57	100.00

Reversed Phase for **4i** (Method A)



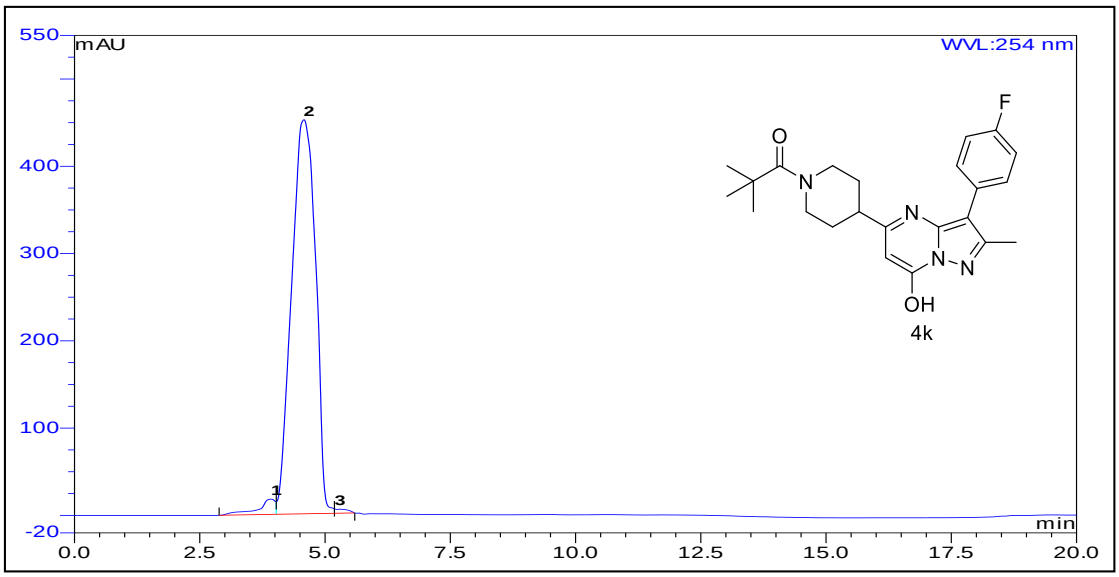
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	1.77	2.85	0.40	1.95
2	2.38	109.89	20.01	98.05
Total		112.74	20.41	100.00

Reversed Phase for **4j** (Method A)



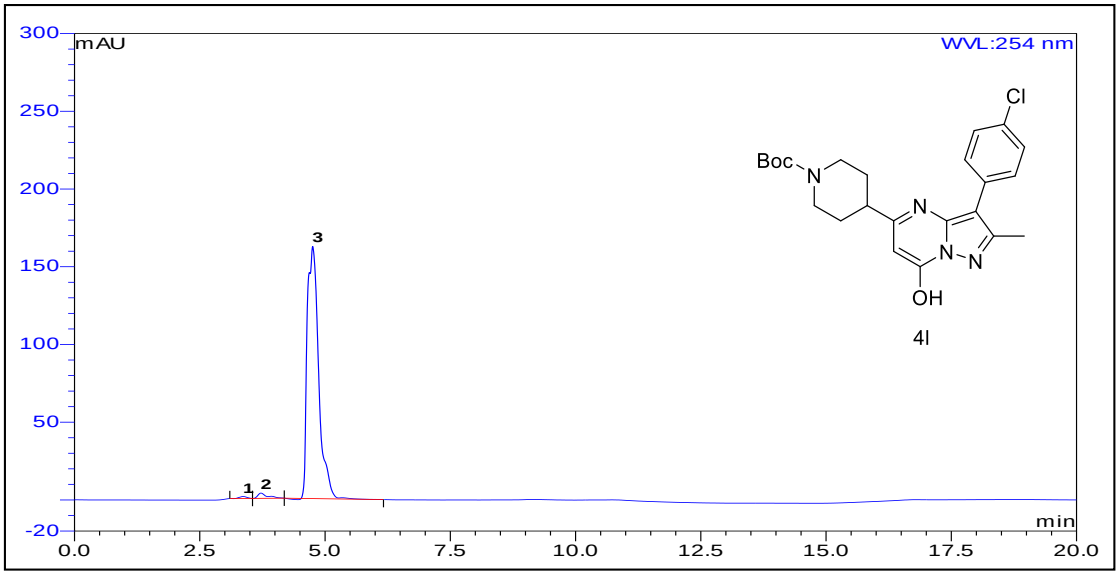
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	1.77	5.44	0.51	0.69
2	2.13	379.08	74.09	99.31
Total		384.51	74.60	100.00

Reversed Phase for **4k** (Method A)



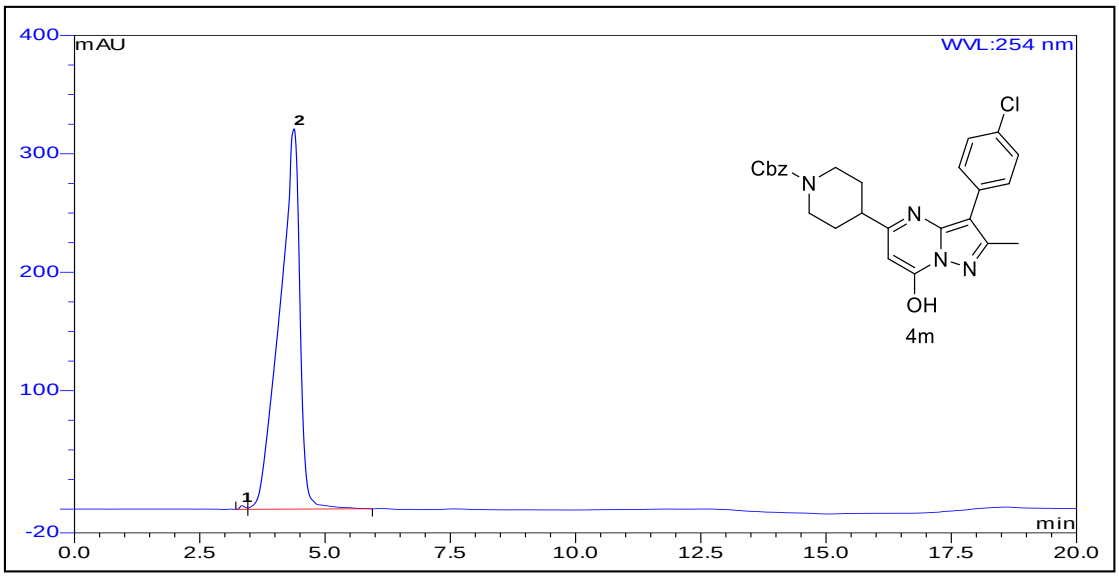
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	3.93	17.44	7.10	2.74
2	4.58	451.57	250.31	96.72
3	5.19	5.02	1.40	0.54
Total		116.30	50.57	100.00

Reversed Phase for **4l** (Method A)



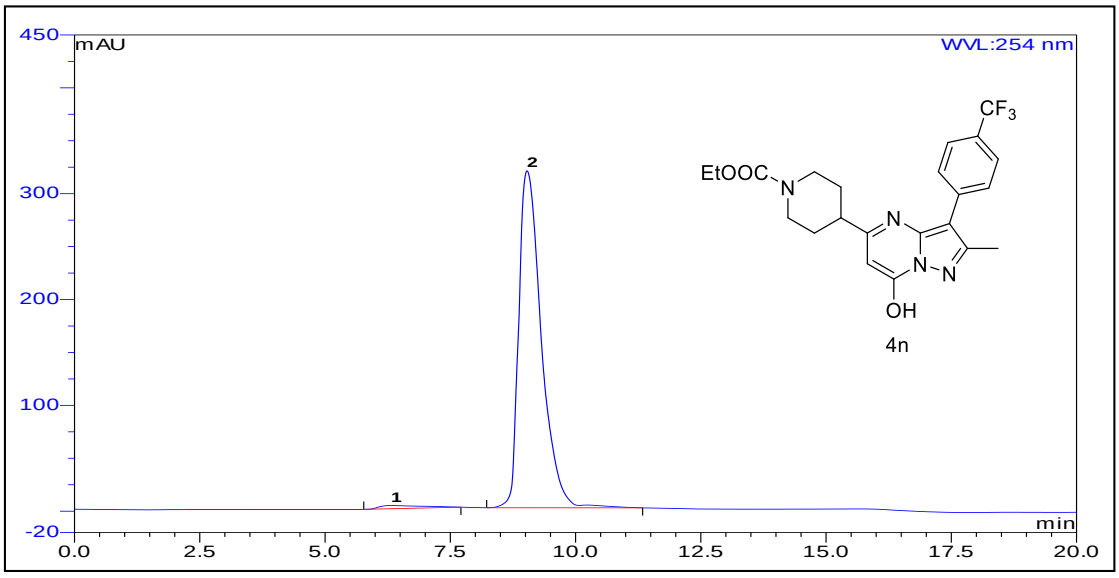
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	3.37	1.28	0.21	0.48
2	3.72	3.36	0.75	1.70
3	4.75	162.21	43.14	97.82
Total		166.85	44.10	100.00

Reversed Phase for **4m** (Method B)



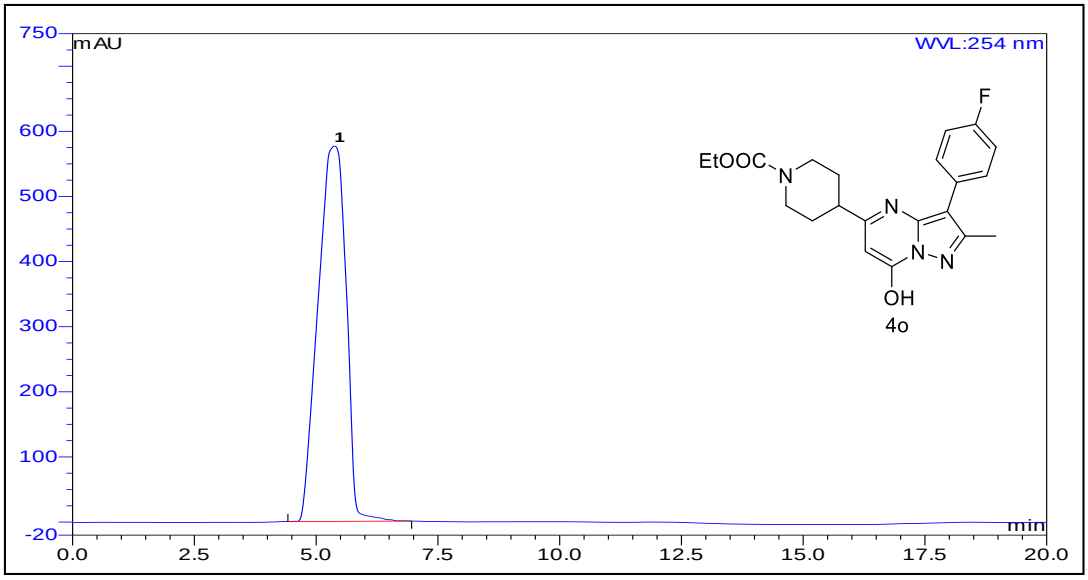
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	3.34	3.03	0.37	0.24
2	4.39	321.04	151.14	99.76
Total		324.07	151.51	100.00

Reversed Phase for **4n** (Method A)



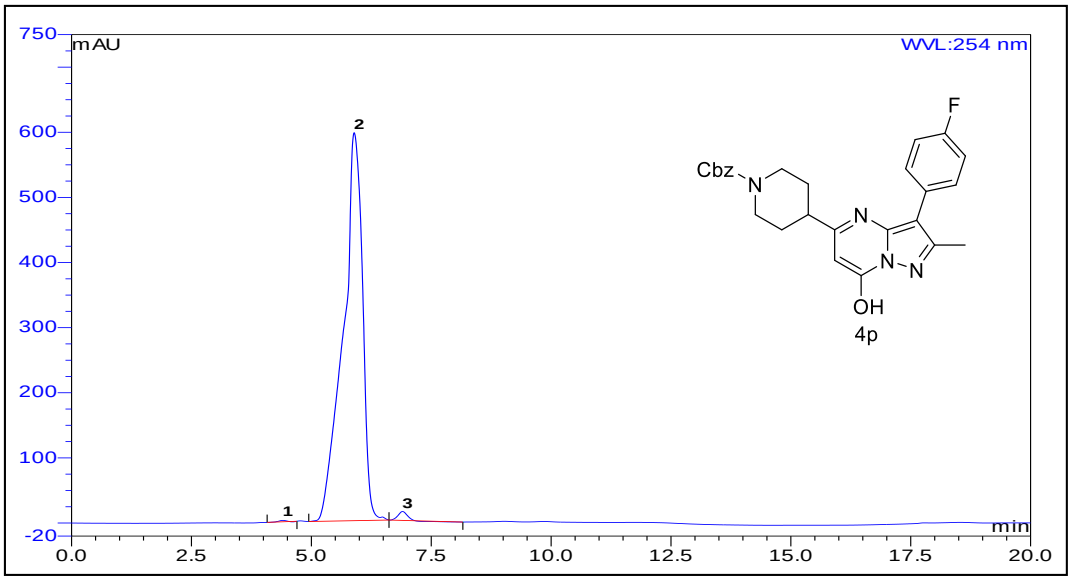
No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	6.32	3.00	2.94	1.73
2	9.03	318.38	166.63	98.27
Total		321.38	169.57	100.00

Reversed Phase for **4o** (Method A)



No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	5.38	576.25	384.75	100
Total		576.25	384.75	100.00

Reversed Phase for **4p** (Method A)



No.	Ret. Time min	Height mAU	Area mAU*min	Rel.Area %
1	4.41	2.12	0.33	0.12
2	5.89	595.74	279.66	98.94
3	6.90	13.68	2.67	0.94
Total		166.85	282.66	100.00