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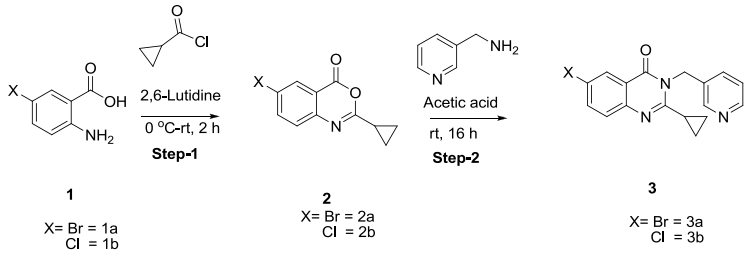
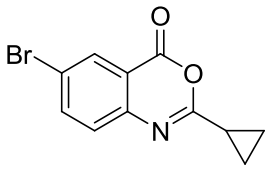
Scheme 1: Synthetic scheme for the synthesis 6-Bromo and chloro-2-cyclopropyl-3-(pyridyl-3-ylmethyl) quinazolin-4(3*H*)-ones (**3a & 3b**)

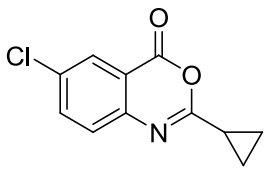


Preparation of 6-bromo-2-cyclopropyl-4H-benzo[d][1,3]oxazin-4-one (2a):



A solution of cyclopropane carbonyl chloride (1 g, 4.62mmol) was added to a stirred solution of 2-amino-5-bromobenzoic acid (1 g, 4.62 mmol) in 2, 6-Lutidine (10 V) at 0 ^{o}C for 30 min, reaction mixture was allowed to rt and maintained 2h. Reaction mixture was poured in to ice cold water and filtered the solid, washed with water. Crude compound was used for next step without any further purification. Yield (60%, 0.73 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.27 (d, J = 2.1 Hz, 1H), 7.83 (dd, J = 8.2, 2.4 Hz, 1 H), 7.37 (d, J = 9.0 Hz, 1 H), 1.97-1.90 (m, 1H), 1.32-1.26 (m, 2H), 1.15-1.08 (m, 2H). ^{13}C NMR (75 MHz $CDCl_3$): δ = 164.6, 158.3, 145.7, 139.4, 130.7, 127.7, 120.3, 118.0, 14.3, 9.6.

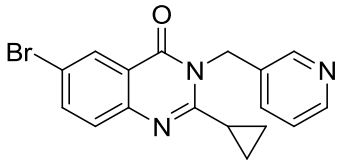
Preparation of 6-chloro-2-cyclopropyl-4H-benzo[d][1,3]oxazin-4-one (2b):



A solution of cyclopropane carbonyl chloride (1 g, 4.62mmol) was added to a stirred solution of 2-amino-5-chlorobenzoic acid (1 g, 4.62 mmol) in 2, 6-lutidine (10 V) at 0 ^{o}C for 30 min. Reaction mixture was allowed to rt and maintained for 2h. Reaction mixture was poured in to ice

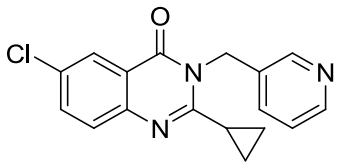
cold water and filtered the solid, washed with water. Crude compound was used for next step without any further purification. Yield (60%, 0.73 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11 (d, *J* = 2.0 Hz, 1H), 7.70 (dd, *J* = 8.4, 2.8 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 1.98-1.92 (m, 1H), 1.30-1.24 (m, 2H), 1.14 -1.11 (m, 2H). ^{13}C NMR (75 MHz $CDCl_3$): δ = 164.5, 158.4, 145.3, 136.6, 132.8, 127.6, 117.7, 14.3, 9.6.

Preparation of 6-bromo-2-cyclopropyl-3-(pyridin-3-ylmethyl) quinazolin-4(3*H*)-one (**3a**):



Pyridin-3-ylmethanamine (0.6 g, 5.63 mmol) was added to a solution of 6-bromo-2-cyclopropyl-3-(pyridin-3-ylmethyl) quinazolin-4(3*H*)-one (1g, 3.75 mmol) in acetic acid(20 V), reaction mixture was heated to reflux for 2 h. After completion of the reaction, reaction mixture was cooled to rt, the mixture was poured in to ice cold water and filter the solid. The resulting solid was applied to a silica gel column, packed in 20% EtOAc in *n*-hexane. Sequential elution with 20% EtOAc in *n*-hexane and 50% EtOAc in *n*-hexane gave 6-bromo-2-cyclopropyl-3-(pyridin-3-ylmethyl)quinazolin-4(3*H*)-one **(3a)** as a white solid. Yield (1.07 g, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.58 (s, 1 H), 8.49 (d, *J* = 4.8 Hz, 1 H), 8.20 (d, *J* = 1.6 Hz, 1 H), 7.93 (dd, *J* = 2.4, 2.4 Hz, 1 H), 7.64 (d, *J* = 8.0 Hz), 7.50 (d, *J* = 8.8 Hz, 1 H), 7.39-7.34 (m, 1 H), 5.58 (s, 2 H), 2.19-2.13 (m, 1 H), 1.10-1.06 (m, 2 H), 0.97-0.92 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.5, 158.7, 148.5, 148.2, 146.0, 137.2, 134.4, 132.2, 129.0, 128.4, 123.6, 121.3, 118.2, 44.0, 13.9, 9.4.

Preparation of 6-chloro-2-cyclopropyl-3-(pyridin-3-ylmethyl) quinazolin-4(3*H*)-one (**3b**):



Pyridin-3-ylmethanamine (0.6 g, 5.63 mmol) was added to a solution of 6-chloro-2-cyclopropyl-3-(pyridin-3-ylmethyl) quinazolin-4(3*H*)-one (1g, 3.75 mmol) in acetic acid (20 V), reaction mixture was heated to reflux for 2 h. After completion of the reaction, reaction mixture was

cooled to rt. The mixtures was poured in to ice cold water and filter the solid. The resulting solid was applied to a silica gel column packed in 20% EtOAc in n-hexane. Sequential elution with 20% EtOAc in n-hexane and 50% EtOAc in n-hexane gave 1.1 g of 6-bromo-2-cyclopropyl-3-(pyridin-3-ylmethyl)quinazolin-4(*3H*)-one as a white solid. Yield (1.07 g, 80%). ^{1}H NMR (400MHz, DMSO-d_6): δ = 8.58 (s, 1H), 8.50 (d, J = 3.6 Hz, 1 H), 8.06 (s, 1H), 7.82 (d, J =, 8.8 Hz, 1 H), 7.66 (d, J = 8.0 Hz 1 H), 7.58 (d, J = 8.8 Hz, 1 H), 7.37 (dd, J = 7.2, 4.8 Hz, 1H), 5.58 (s, 2H), 2.18-2.16 (m, 1H), 1.08-1.03 (m, 2H), 0.96-0.94 (m, 2H). ^{13}C NMR (75 MH_Z $CDCl_3$): δ = 160.5, 158.7, 148.5, 148.2, 146.0, 137.2, 134.4, 132.2, 129.0, 128.4, 123.6, 121.3, 118.2, 44.0, 13.9, 9.4.

References

[1] a) J.-F. Liu, J. Lee, A. M. Dalton, G. Bi, L. Yu, C. M. Baldino, E. McElory and M. Brown, *Tetrahedron Lett.*, 2005, 46, 1241; (b) C. Carolyn, J. D. Brackett and D. O. Bloch, *Pharmacotherapy* 2000, 20, 229; (c) P. M. Chandrika, T. Yakaiah, A. R. R. Rao, B. Narsaiah, N. C. Reddy, V. Sridhar and J. V. Rao, *Eur. J. Med. Chem*., 2008, 43, 846; (d) P. M. Chandrika, T. Yakaiah, B. Narsaiah, V. Sridhar, G. Venugopal, J. V. Rao, K. P. Kumar, U. S. N. Murthy and A. R. R. Rao, *Indian J. Chem*., 2009, 48, 840; (e) G. Rabilloud and B. Sillion, *J. Heterocyclic. Chem*., 1980, 17, 1065-1068.

Table 1 Comparison of the rate of formation of 4a in % yield with different mol% of pre-catalyst loading on 3a with $PhB(OH)_2$

	1h	2h	3h	4h	8h	16h	24h
1mol%	10	15	19	24	33	45	45
2mol%	27	36	38	42	56	66	67
5mol%	69	76	80	81	84	87	88
10mol%	93	95	96	97	97	96	96
20mol%	94	96	98	98	98	99	98
30mol%	94	94	94	95	97	96	95

Fig. 1 Comparison of the rate of formation of 4a with different mol% of pre-catalyst loading on 3a with $PhB(OH)_2$ at 80 ^oC(formation of 4a in percentages, determined by LCMS). K_3PO_4 as base and 1,4-dioxane as solvent.

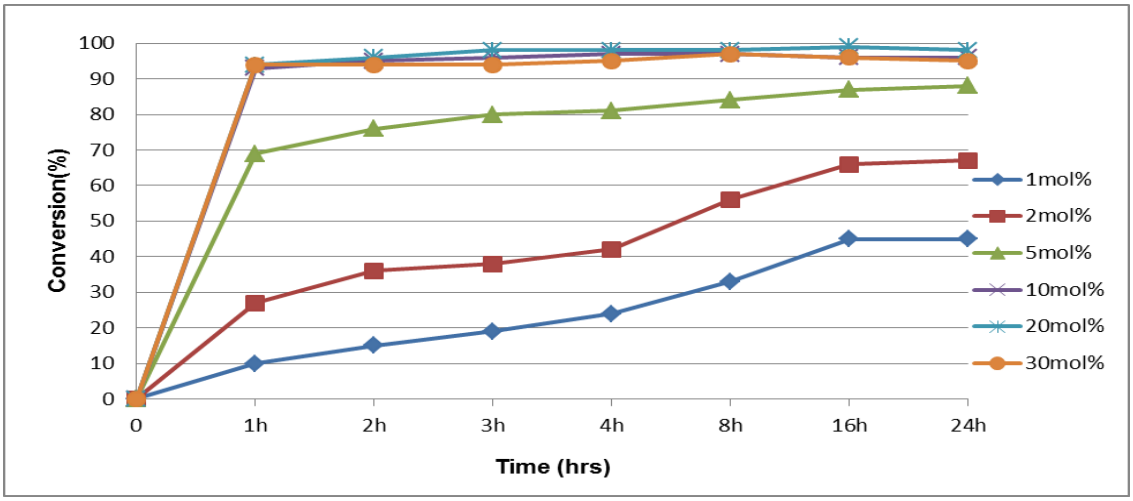


Fig. 2a Insilico studies-Sequence Alignment

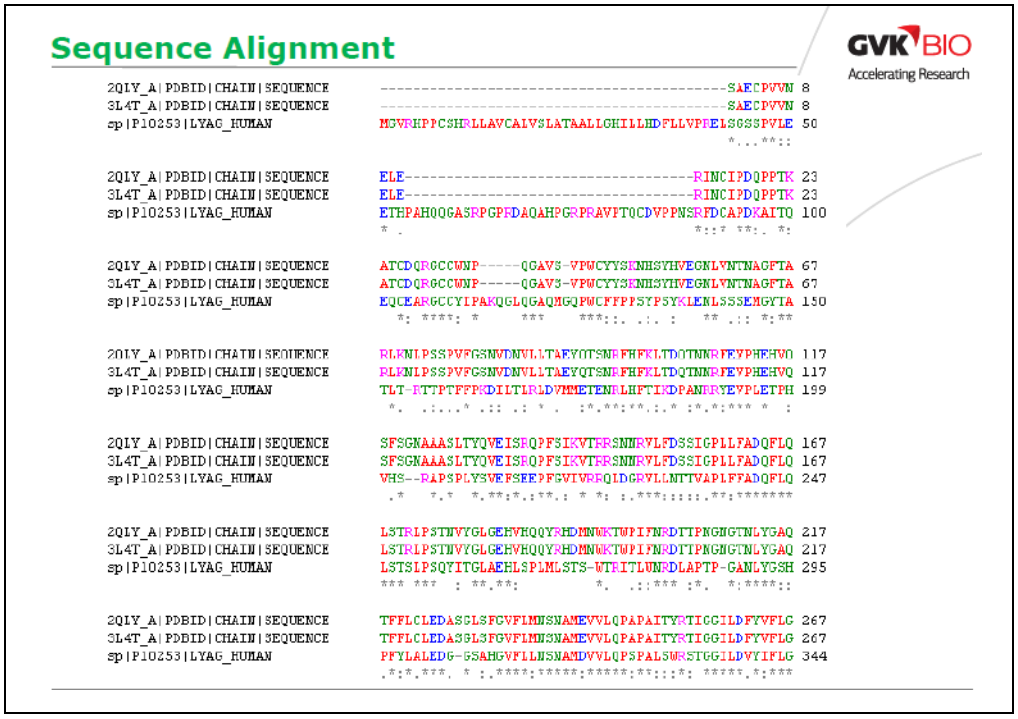


Fig. 2b Insilico studies-Sequence Alignment

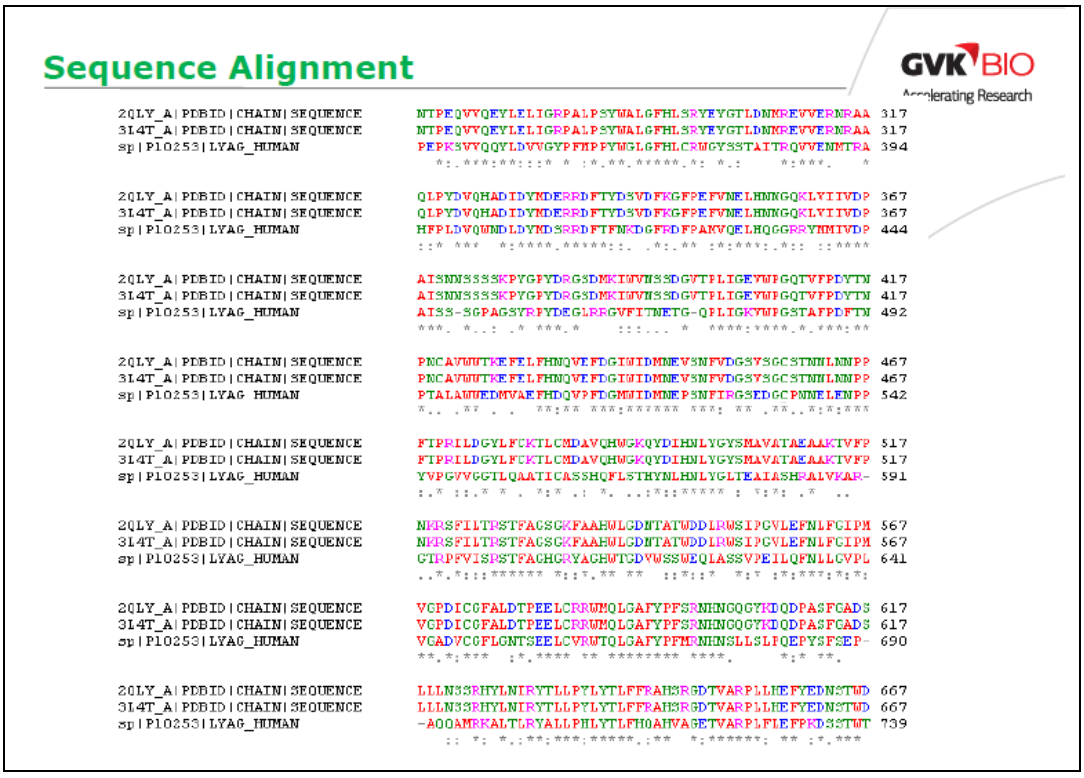


Fig. 2c Insilico studies-Sequence Alignment

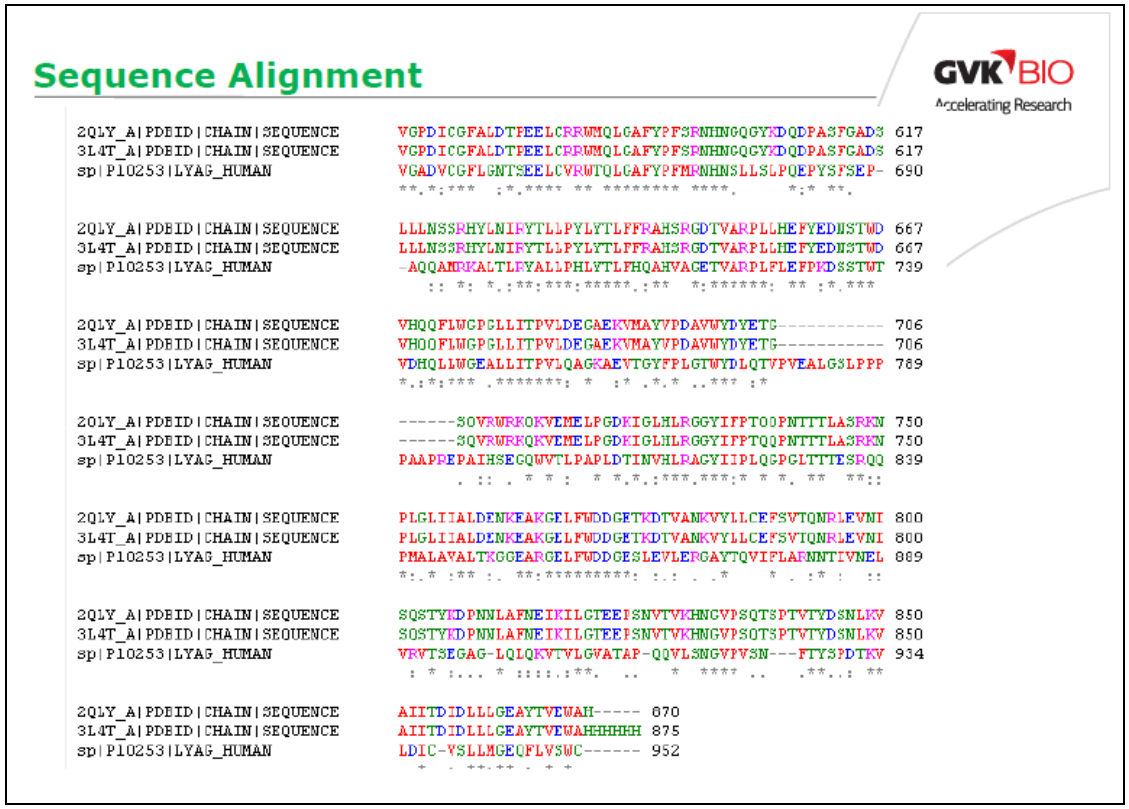


Fig 3. ELISA generated Optical Density (OD) Values

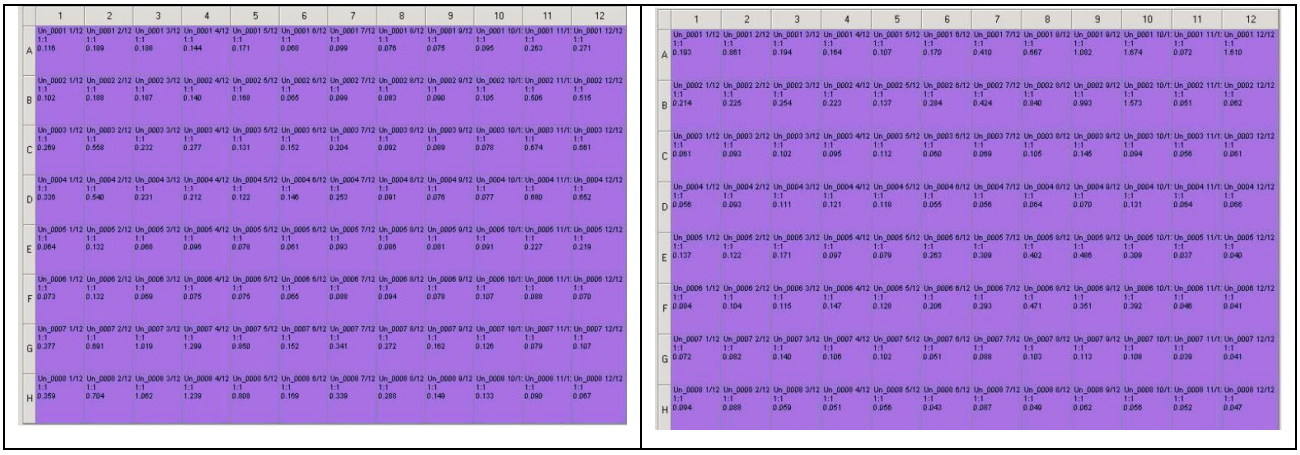


Fig 4. Screen shot of Calculations of IC50 values in Excel sheet and Screen shot of AGH 96 well pate

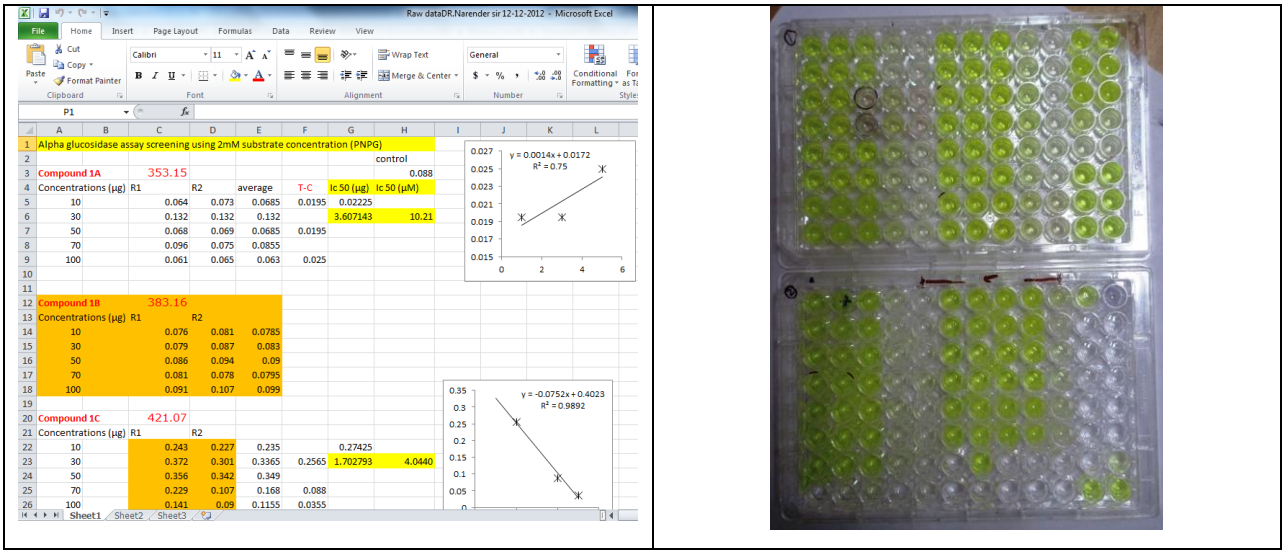
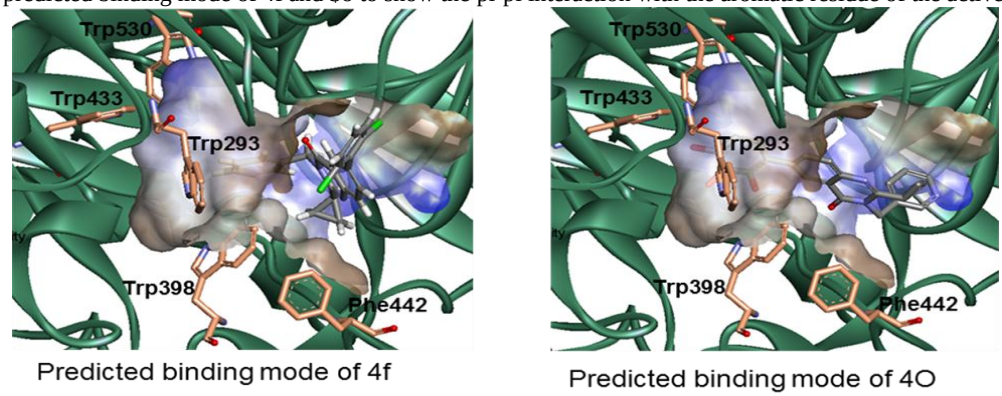


Table 2. Physicochemical properties, Lipinski properties, ADMET properties calculated for selected compounds

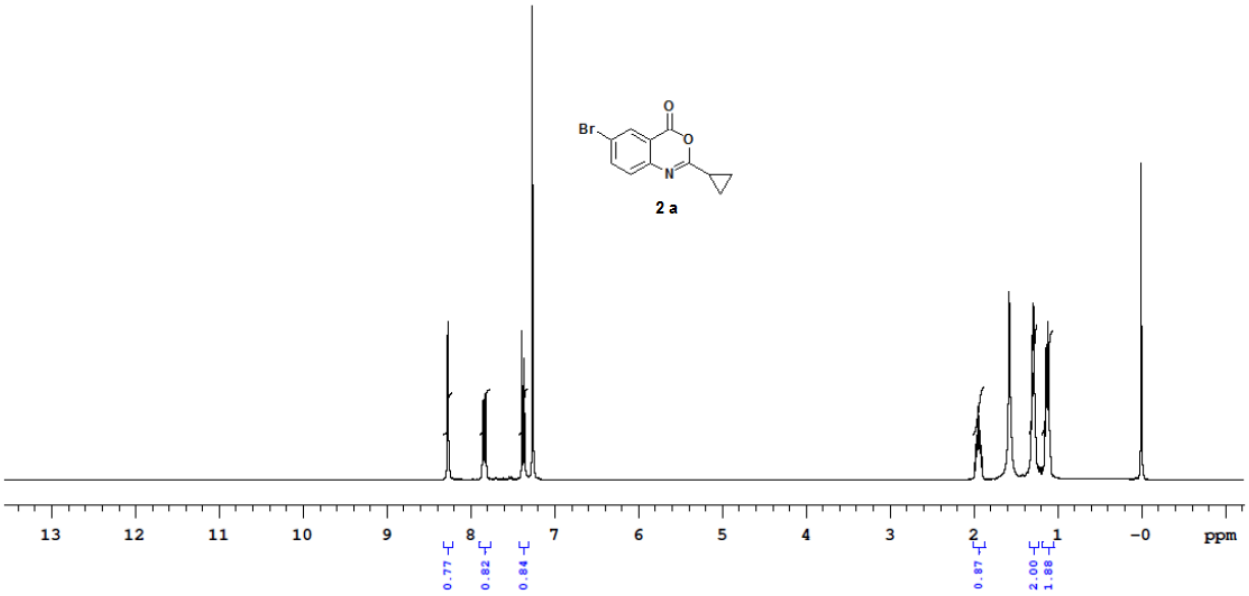
Name	PSA	HBA	NRB	NR	MW	HBD	ALogP	BBB	Abs	Sol	hERG pIC50
4q	58.45	4	4	5	354.40	0	2.59	2	0	-4.27	4.71
4u	74.24	4	4	5	371.44	1	2.62	2	0	-4.51	4.40
4x	45.56	3	5	4	333.43	0	3.88	1	0	-5.15	5.26
4w	45.56	3	4	5	317.38	0	3.05	1	0	-4.66	5.22
4y	45.56	3	3	4	291.35	0	2.71	1	0	-4.24	4.91
4s	58.70	3	4	6	393.44	0	4.34	1	0	-6.21	5.44
4r	61.35	3	4	6	392.45	1	4.04	1	0	-6.02	5.10
4t	73.80	3	4	5	359.44	0	3.41	1	0	-4.67	5.04
4a	45.56	3	4	5	353.42	0	3.74	1	0	-5.27	5.13
4p	32.67	2	4	6	402.49	0	5.80	0	0	-7.55	5.94

Abbreviations: PSA :Polar surface area; HBA :Num_H_Acceptors; NRB :Num_RotatableBonds; NR :Num_Rings;; MW :Molecular_Weight; HBD :Num_H_Donors; ALogP :ALogP;
BBB :ADMET_BBB_Level (0 high to 2 Medium penetration); Abs :ADMET_Absorption_Level (0 = Good; 1 = Moderate; 2 = Low; 3 = Very low); Sol :ADMET_Solubility (-8 low to -2 good), hERG pIC50 values should be around 5.

Fig 5. The predicted binding mode of 4f and $o to show the pi-pi interaction with the aromatic residue of the active site



The active site of the glucosidase contains hydrophobic region surrounded by Trp530, Trp433, Trp293, Trp398, Phe442 amino acids. The binding mode of these compounds 4f, 4o also reveal that the hydrophobic groups were well tightly fixed in the hydrophobic region of the glucoside active site.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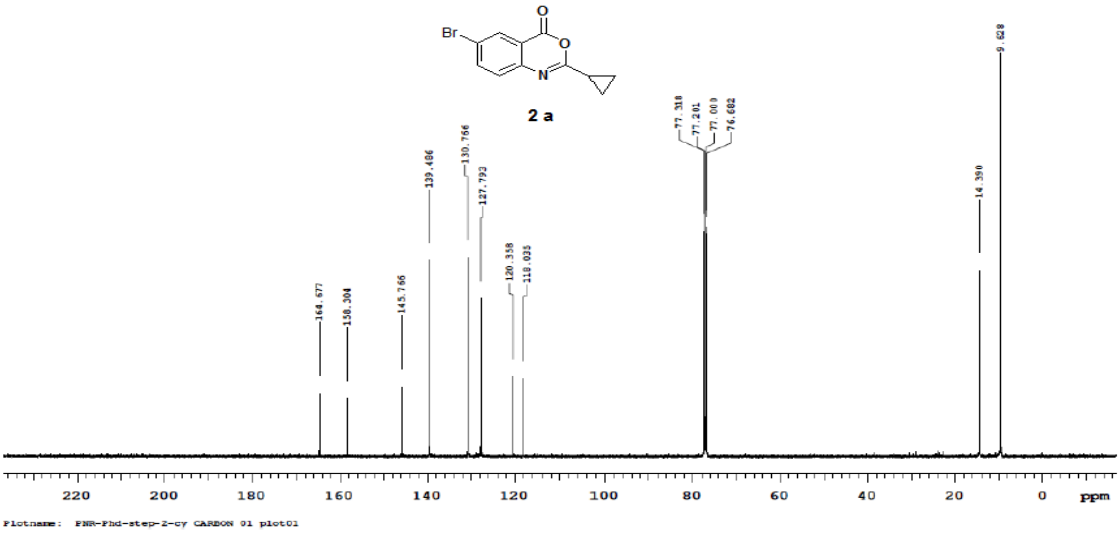
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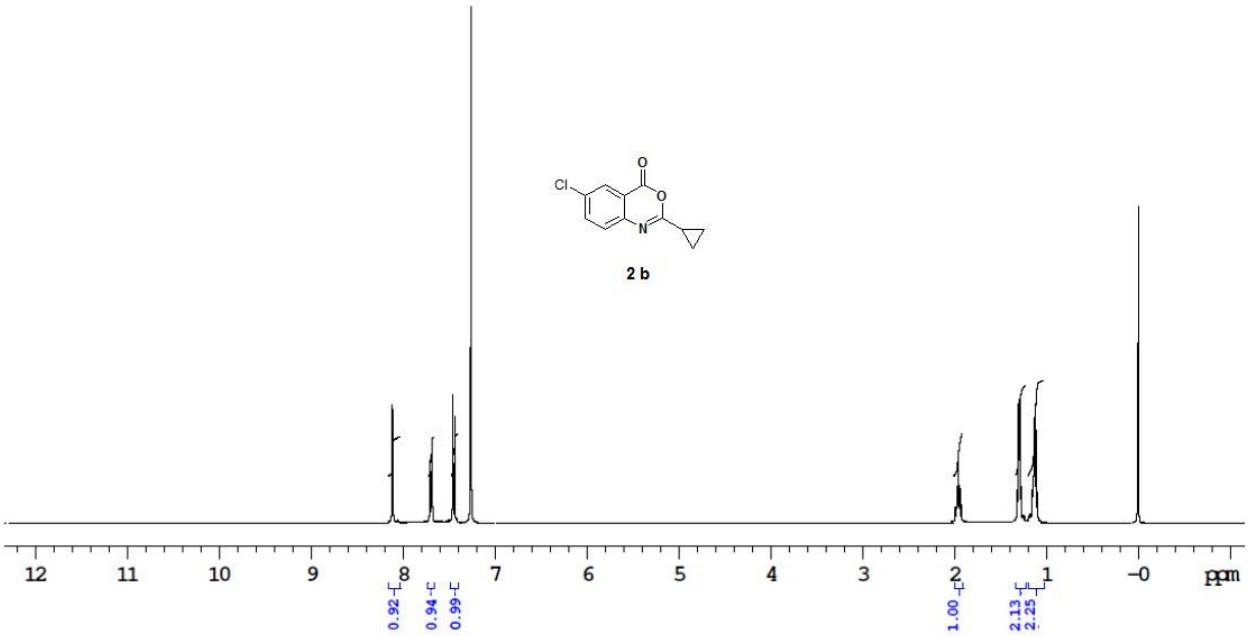

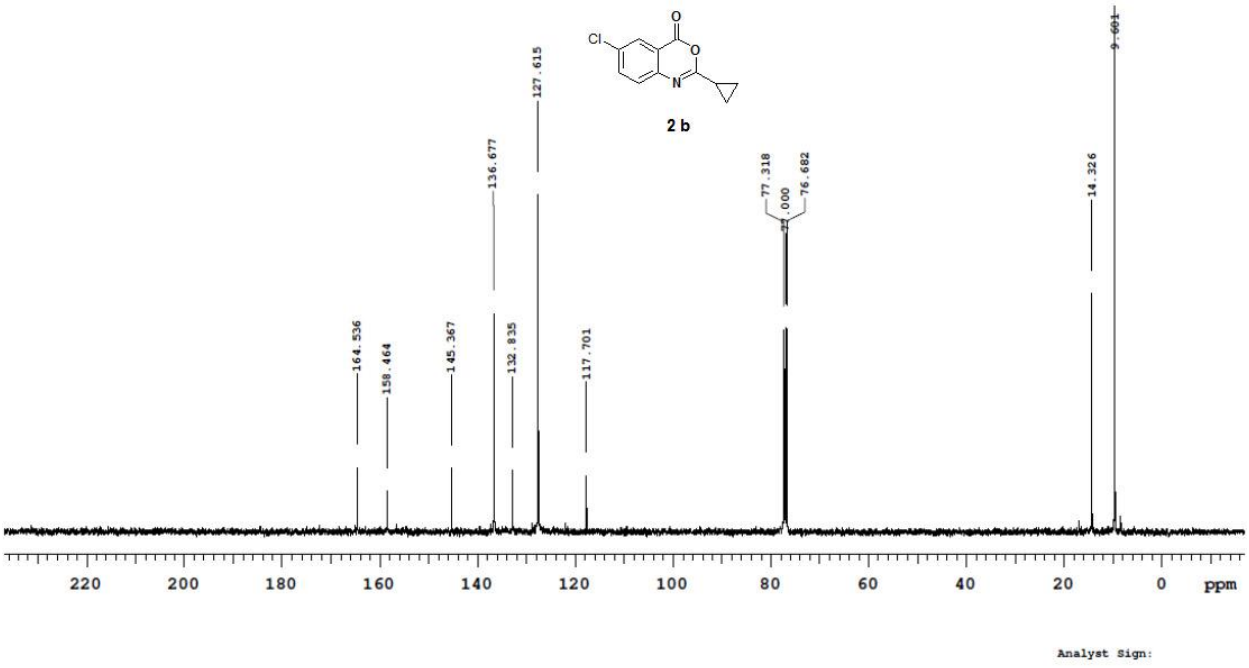

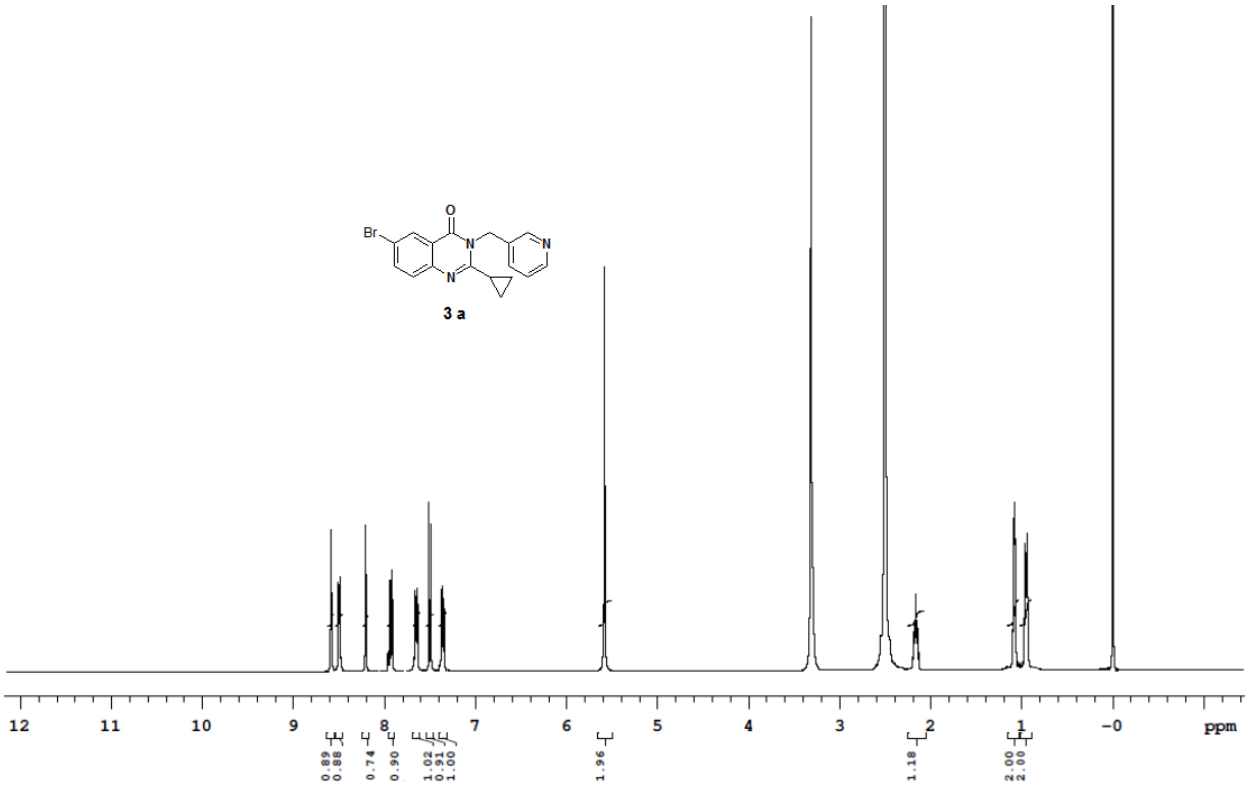

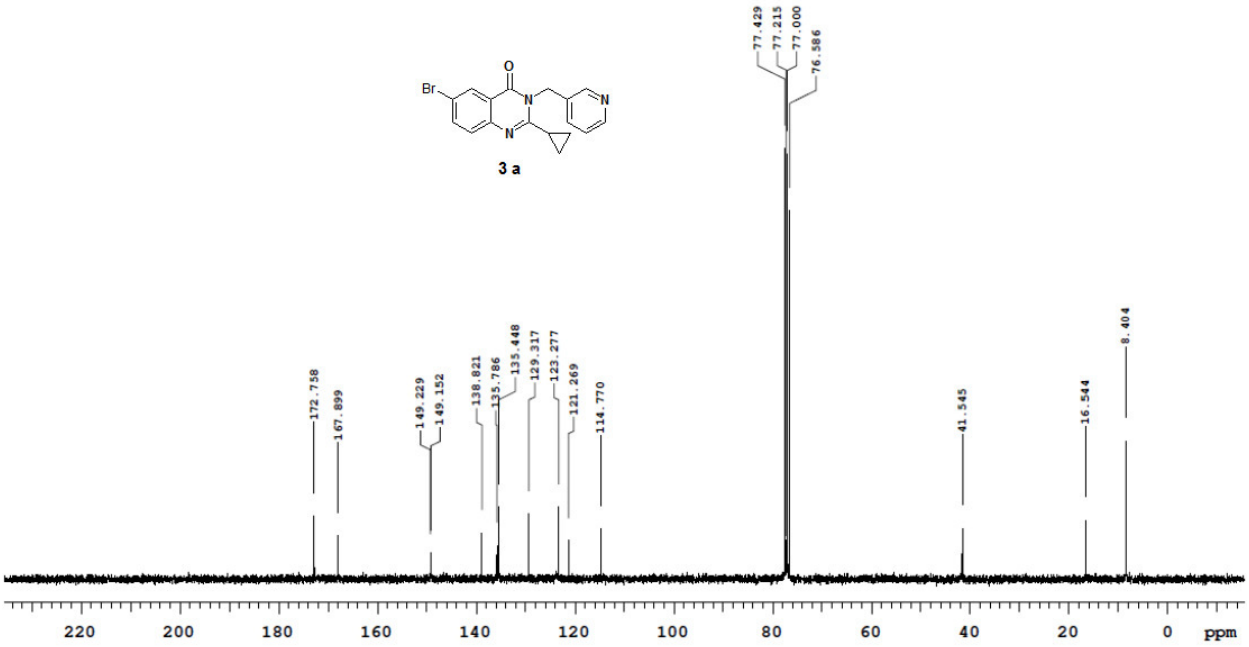

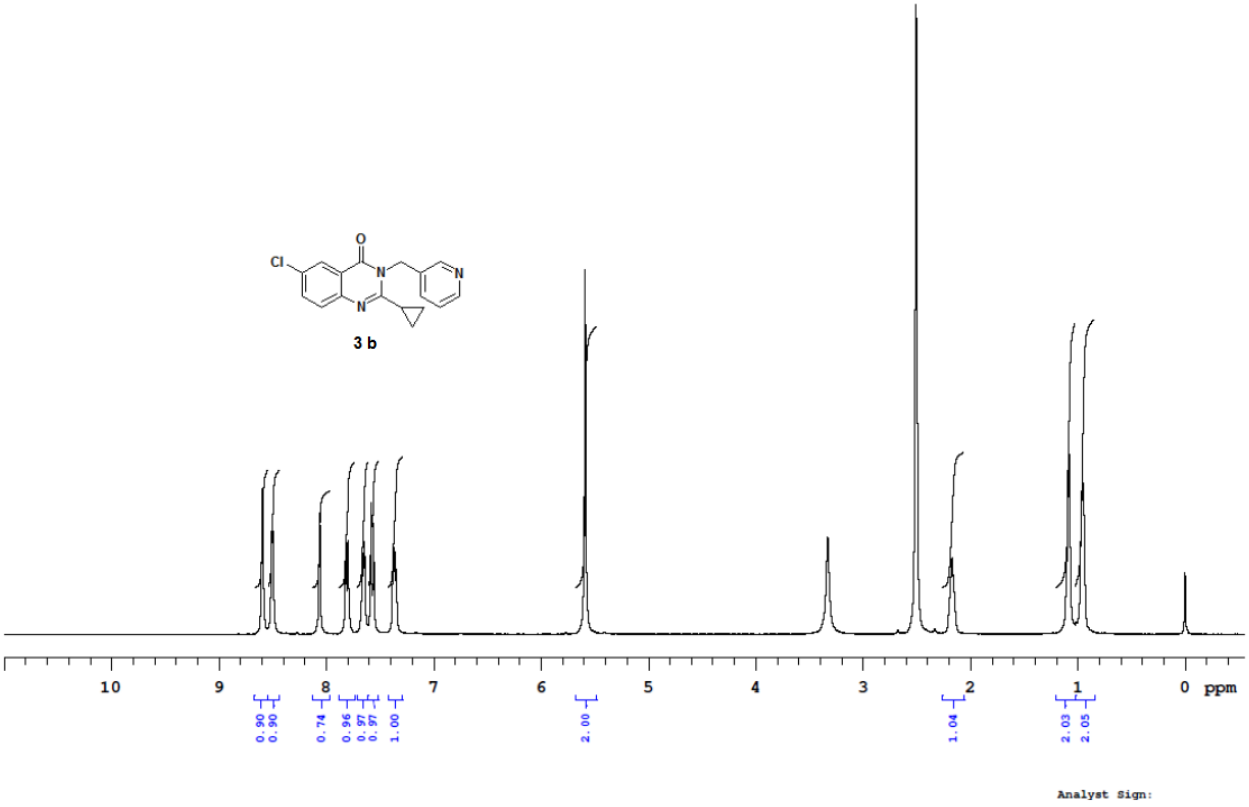

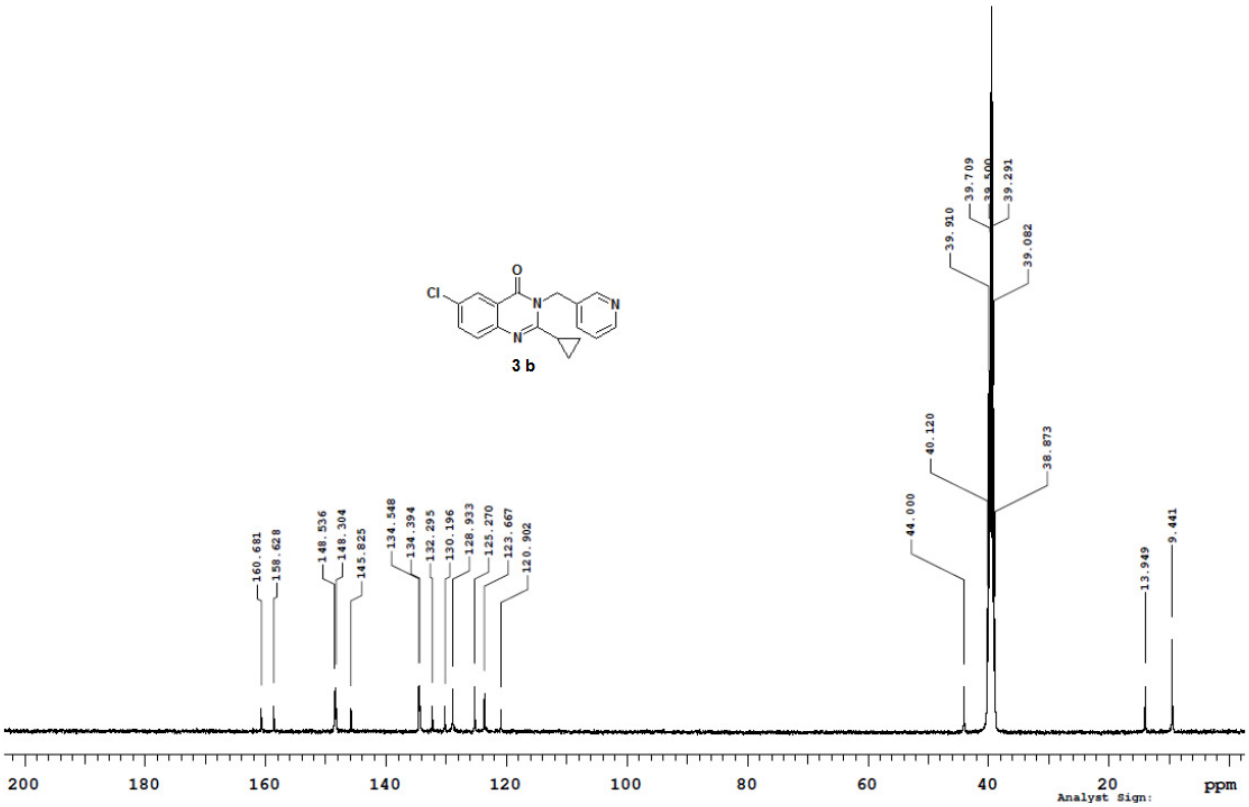

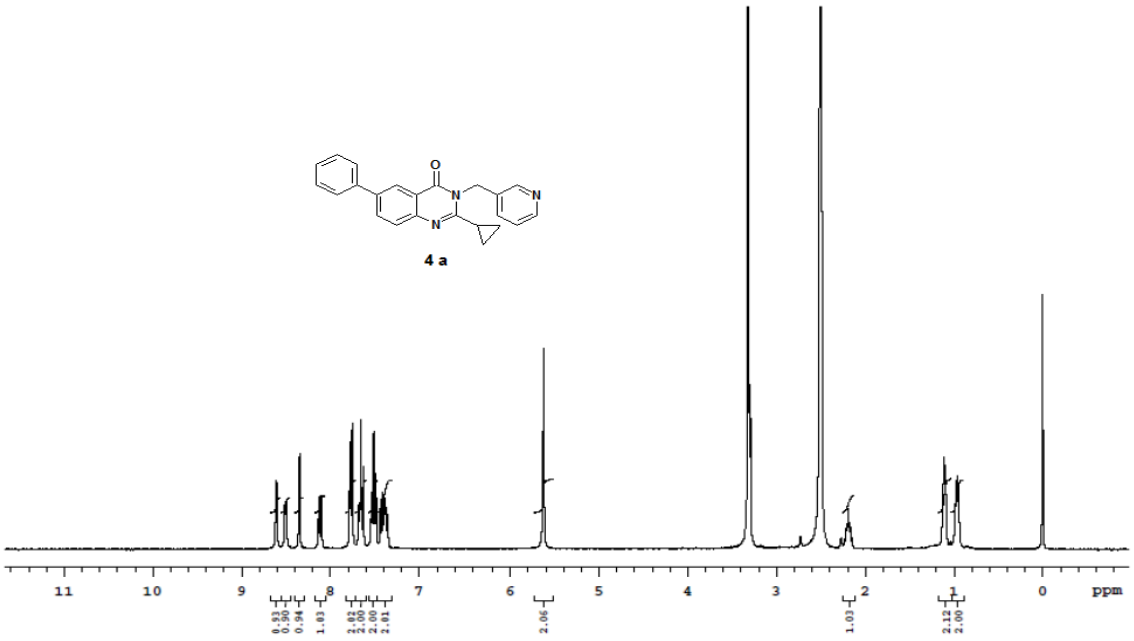
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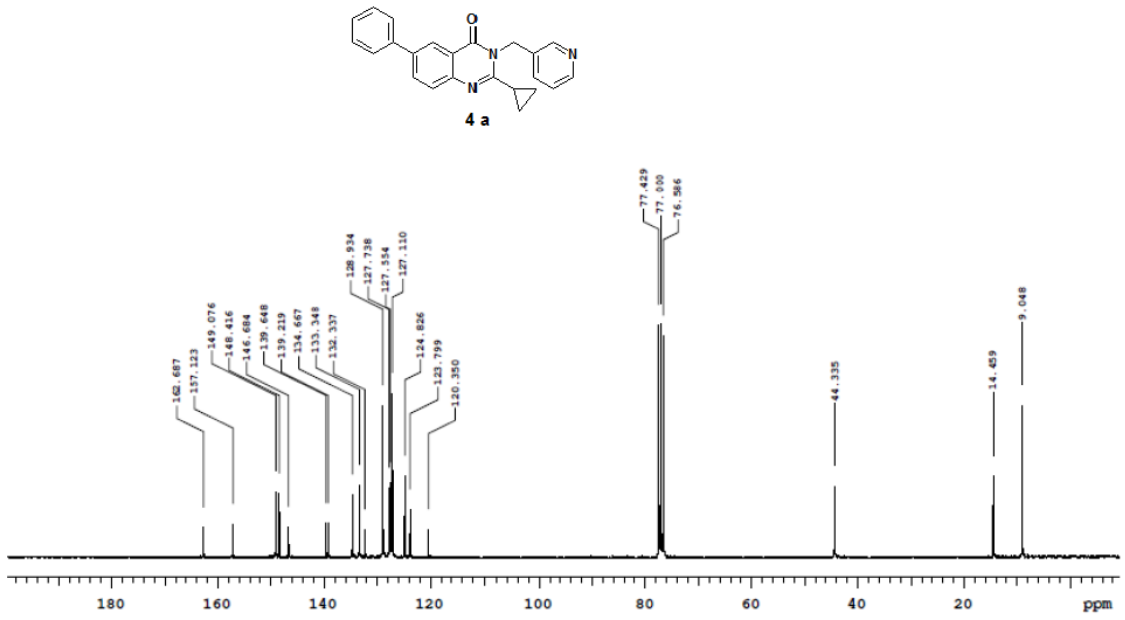
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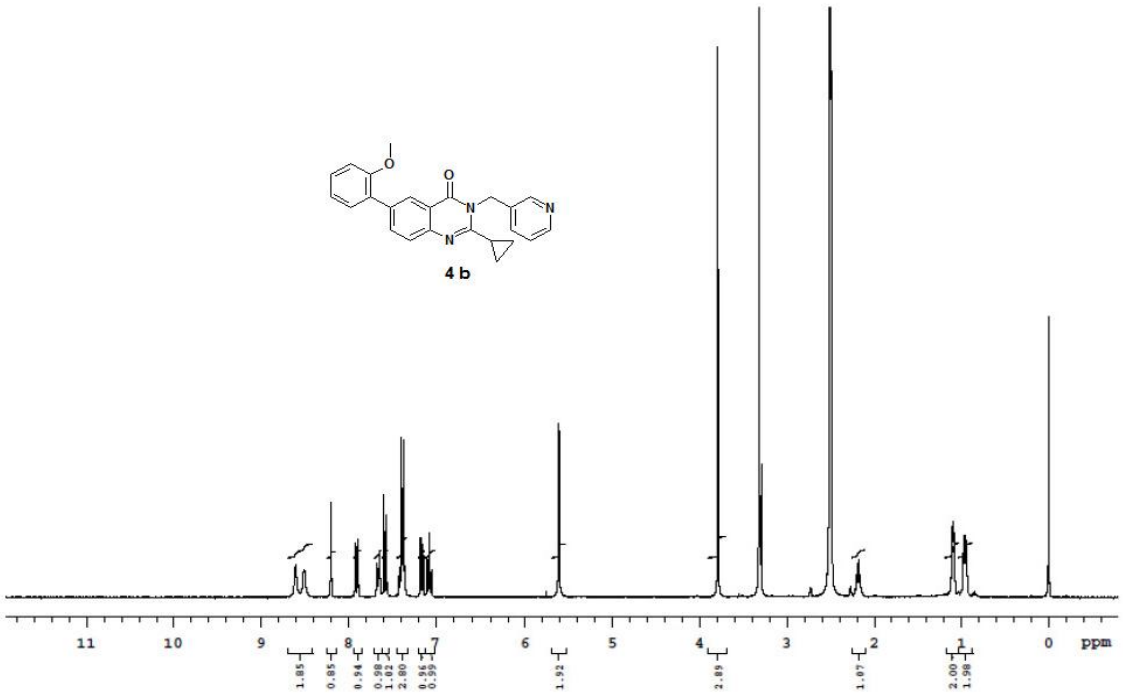

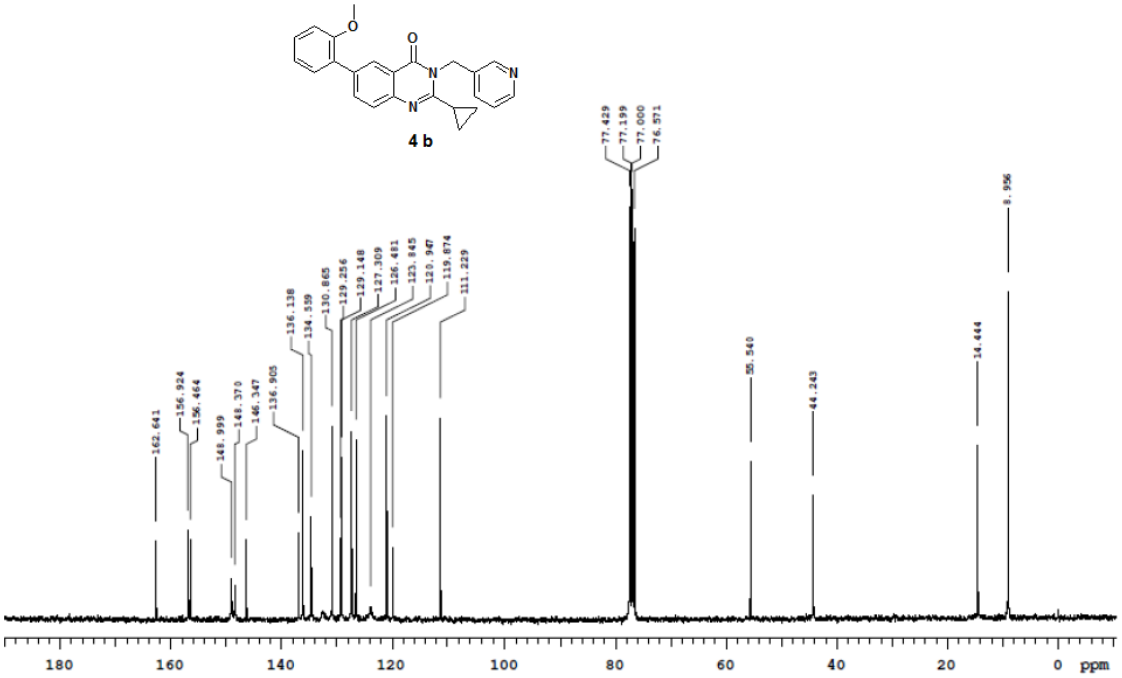

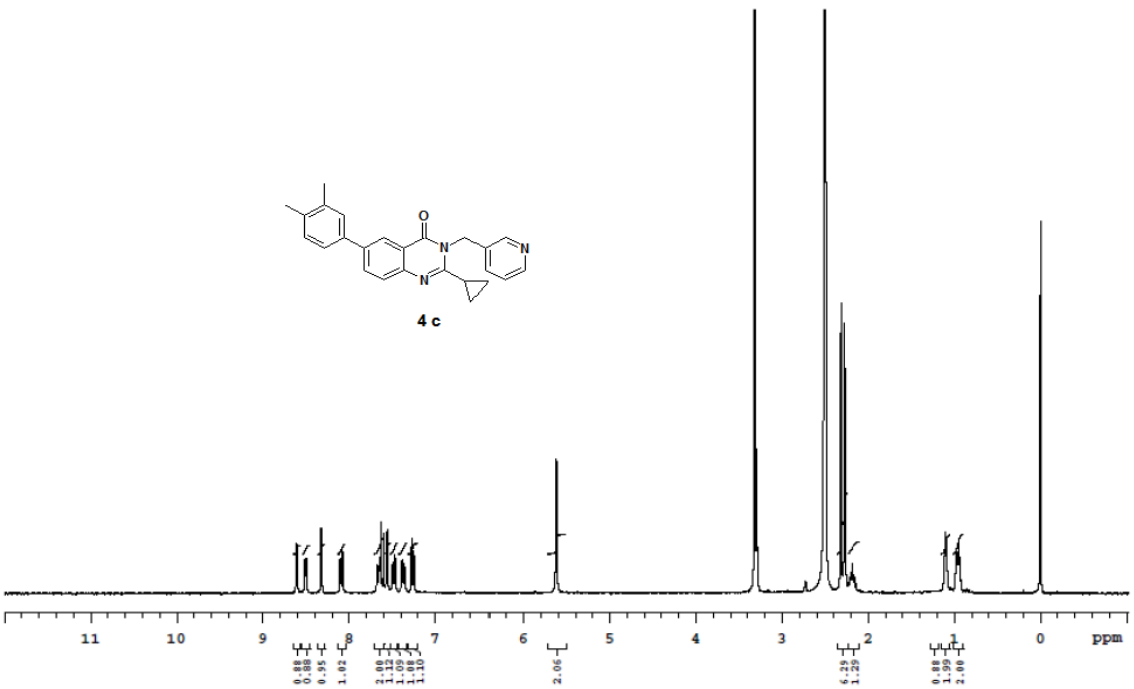

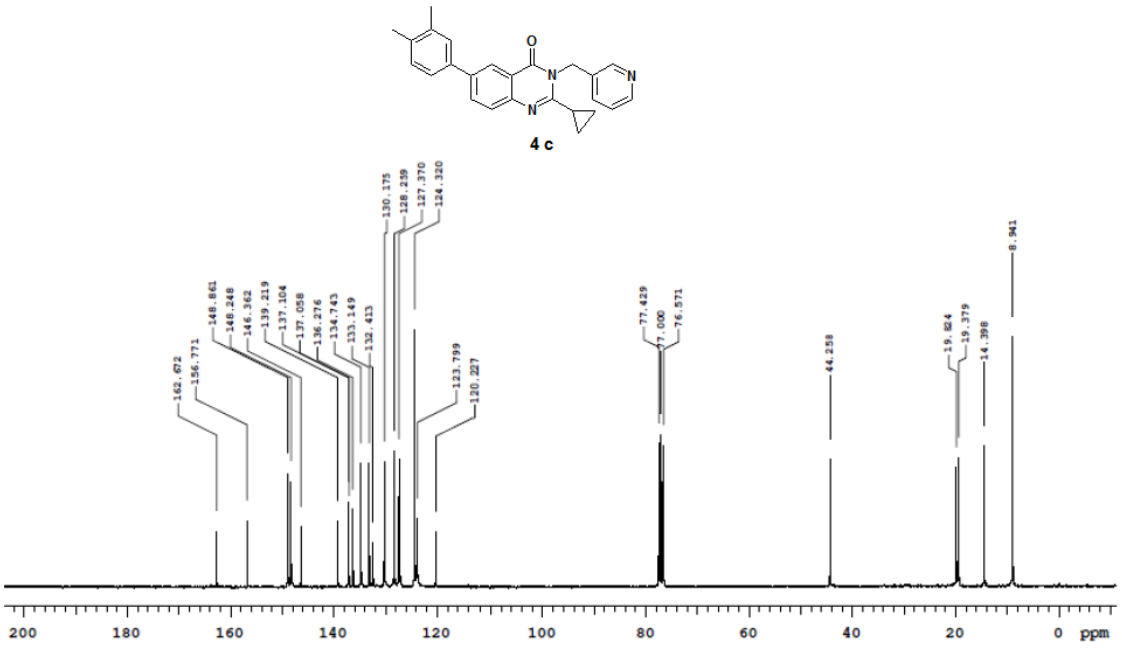

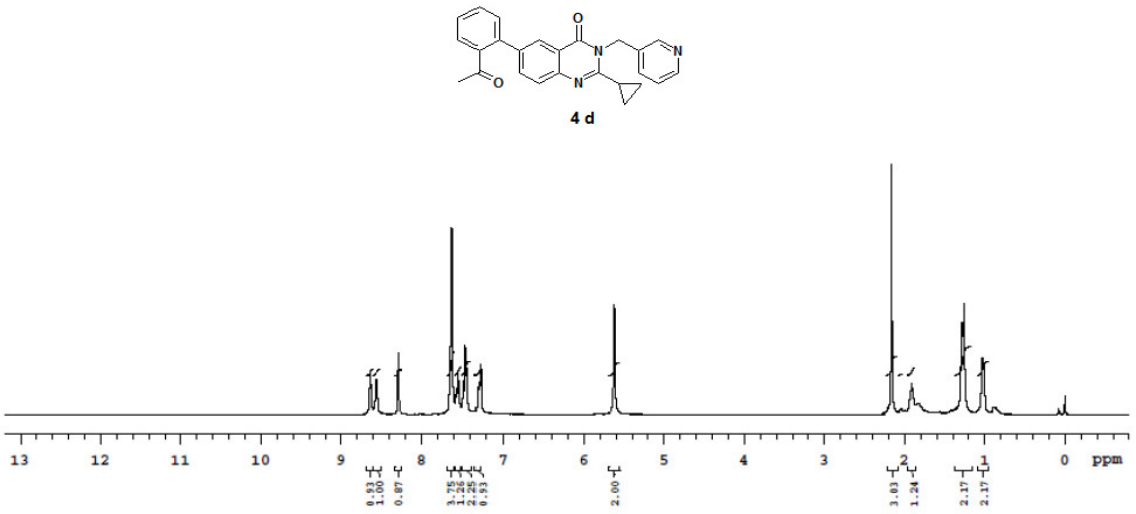

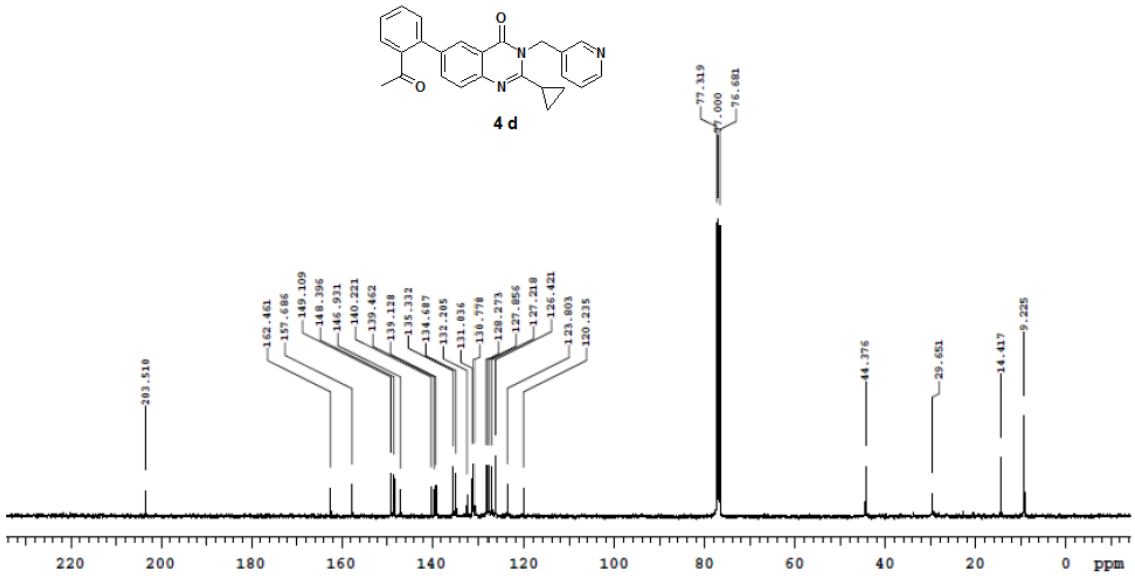

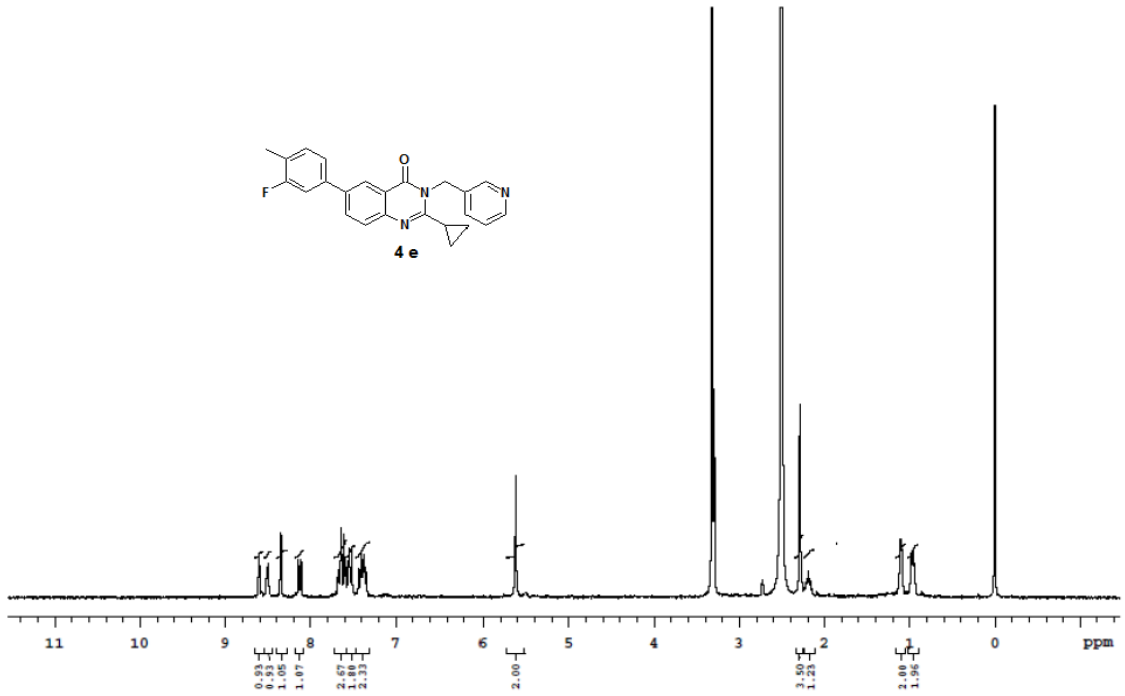

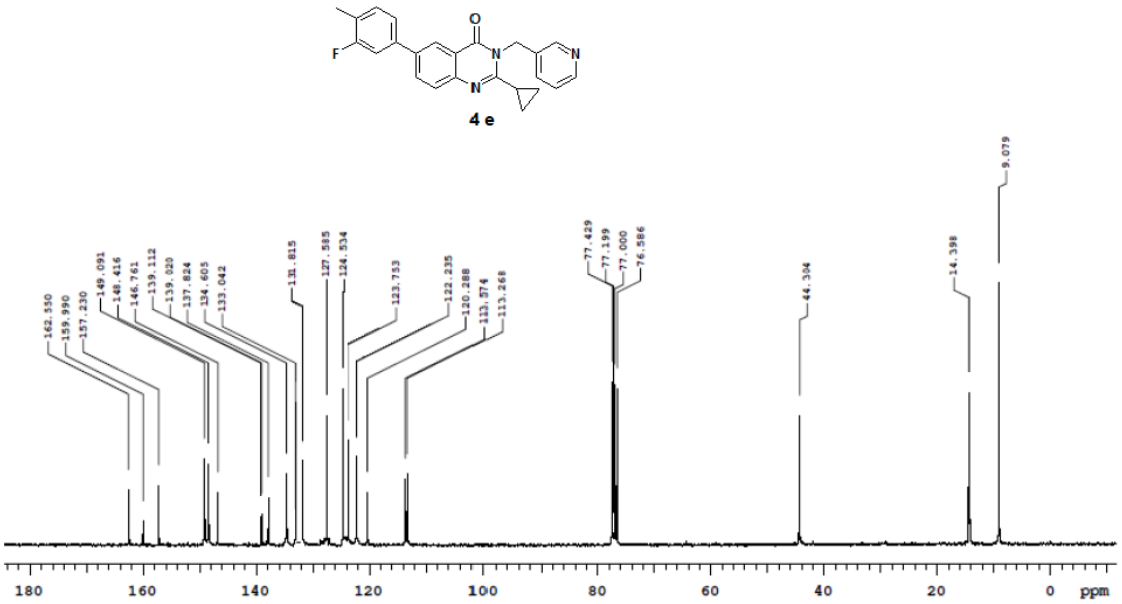

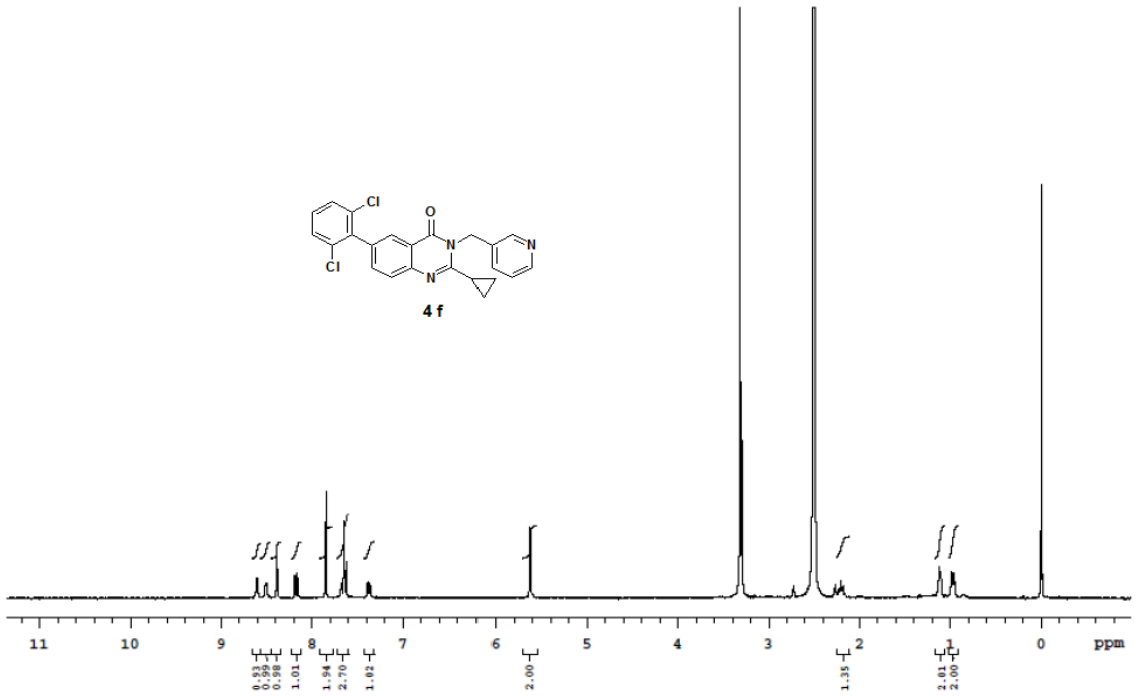

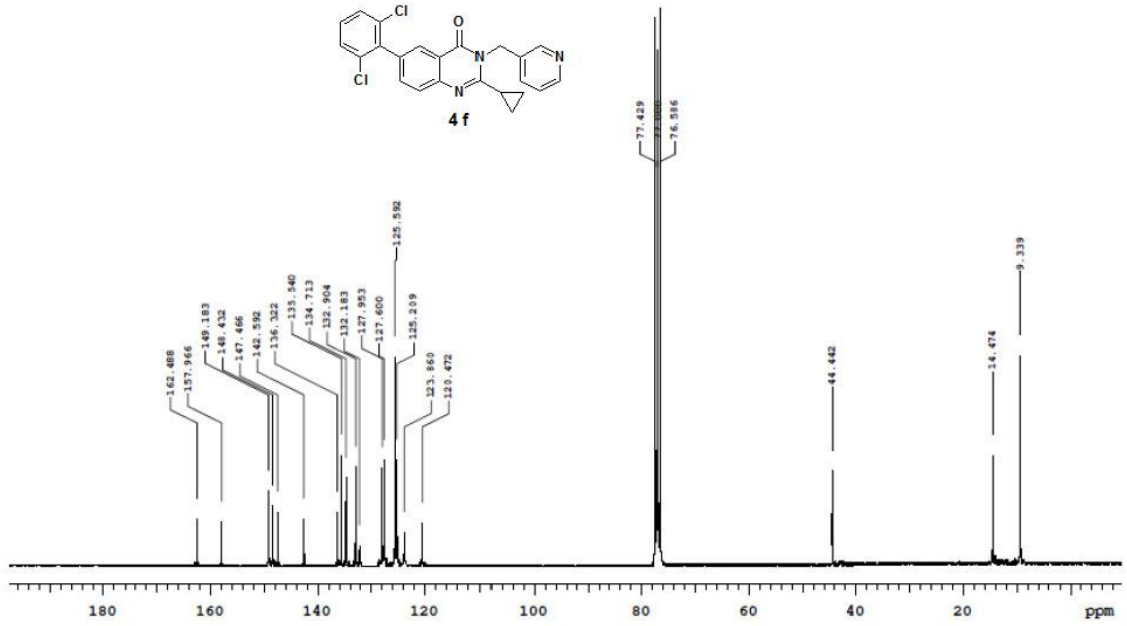

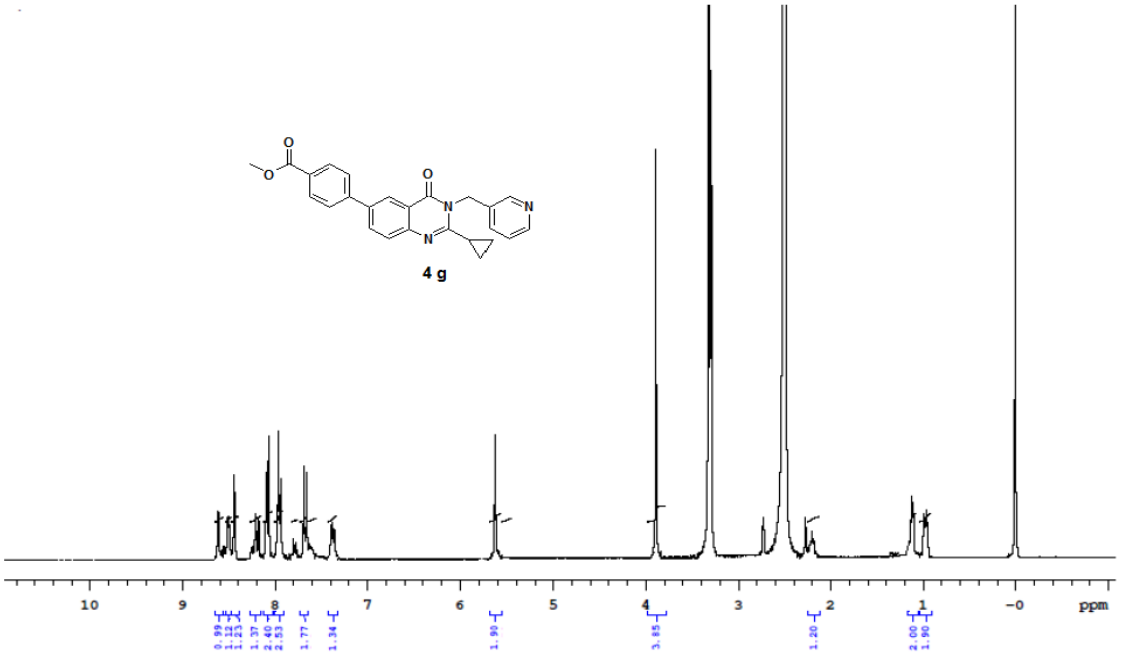

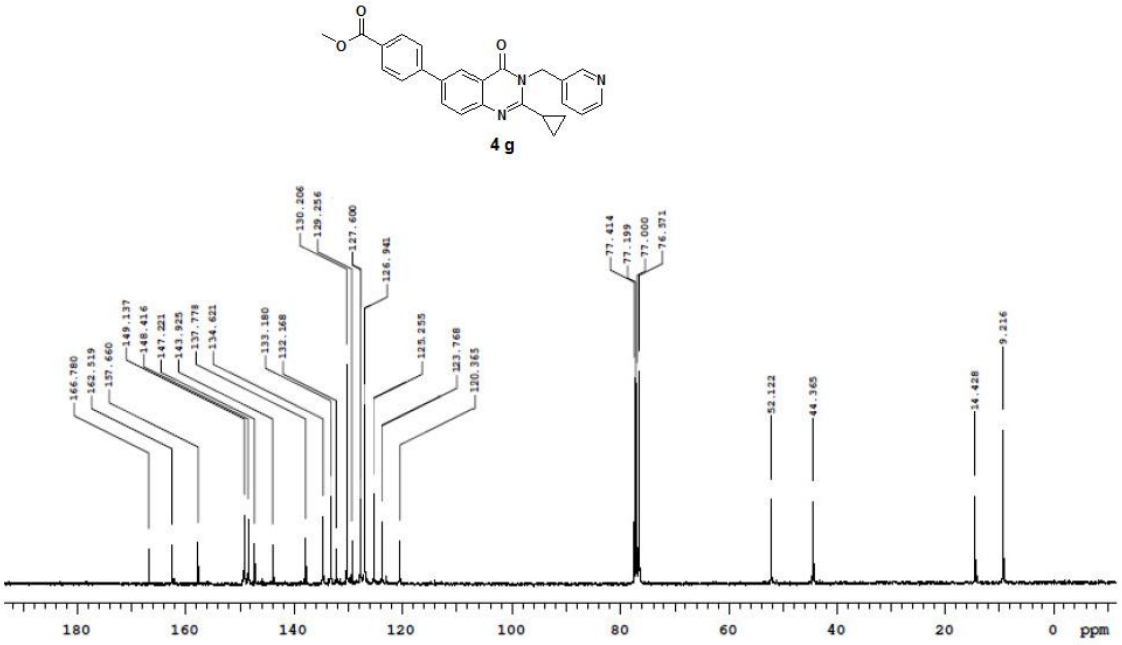

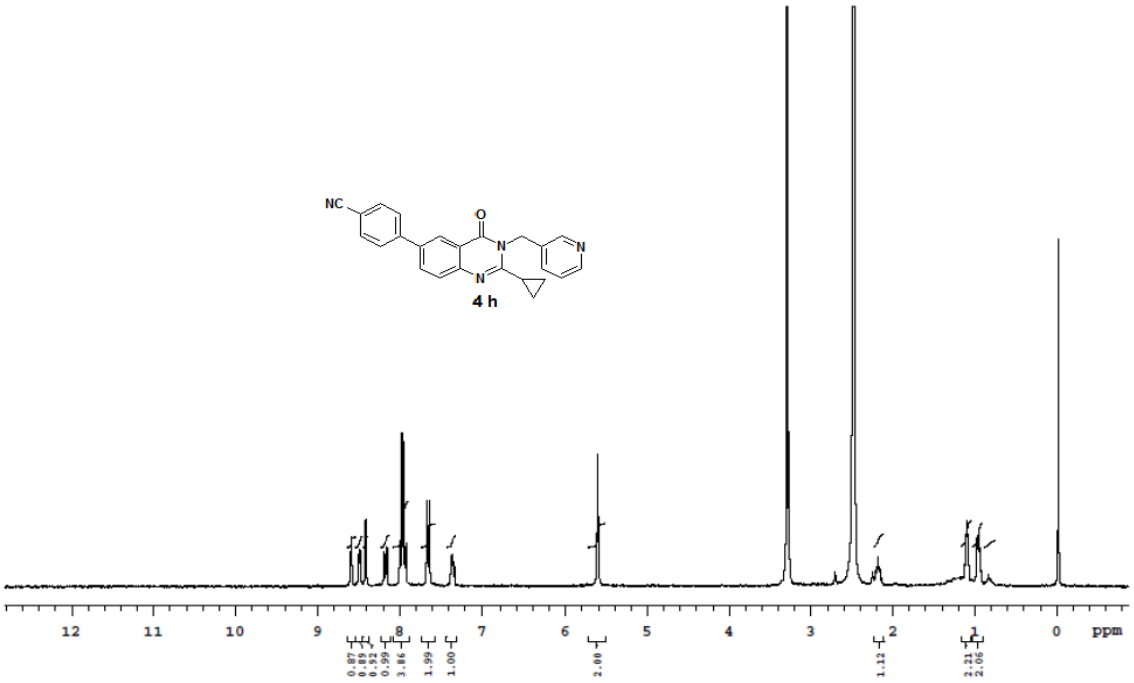

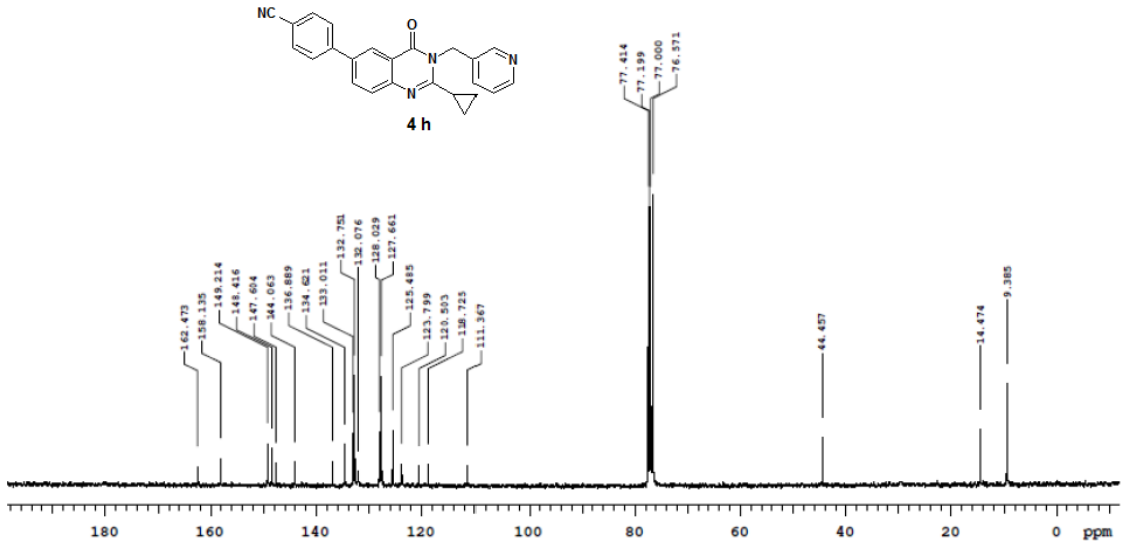

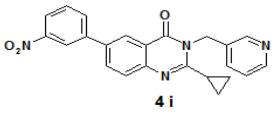

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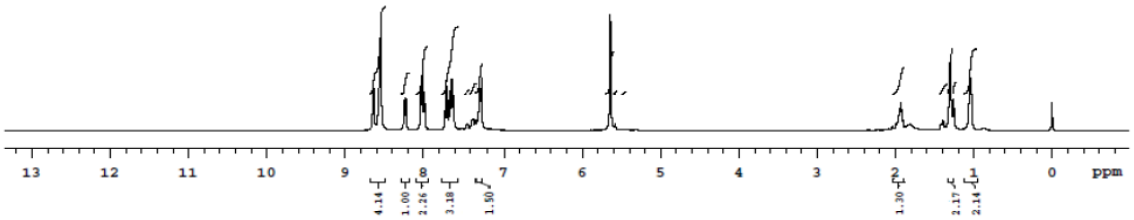

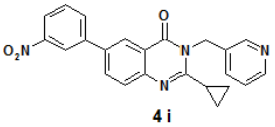

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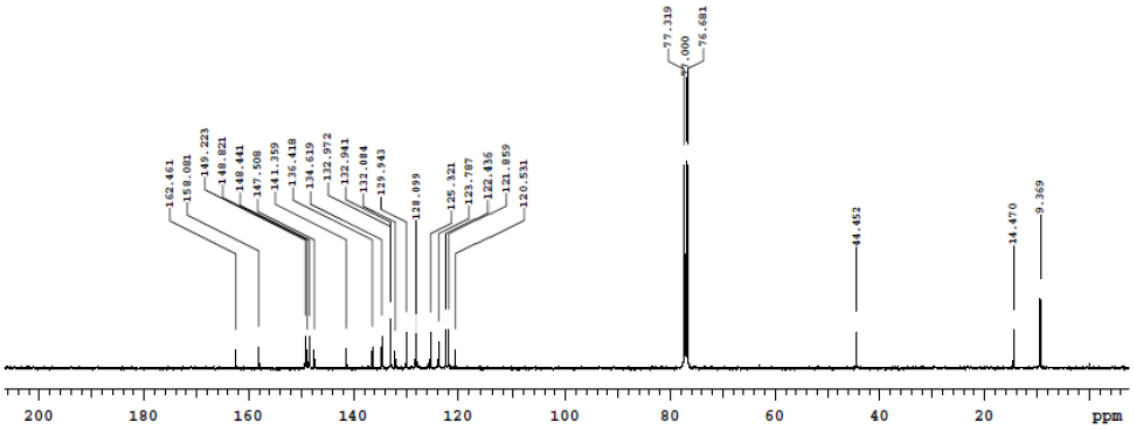

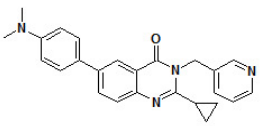

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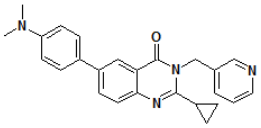

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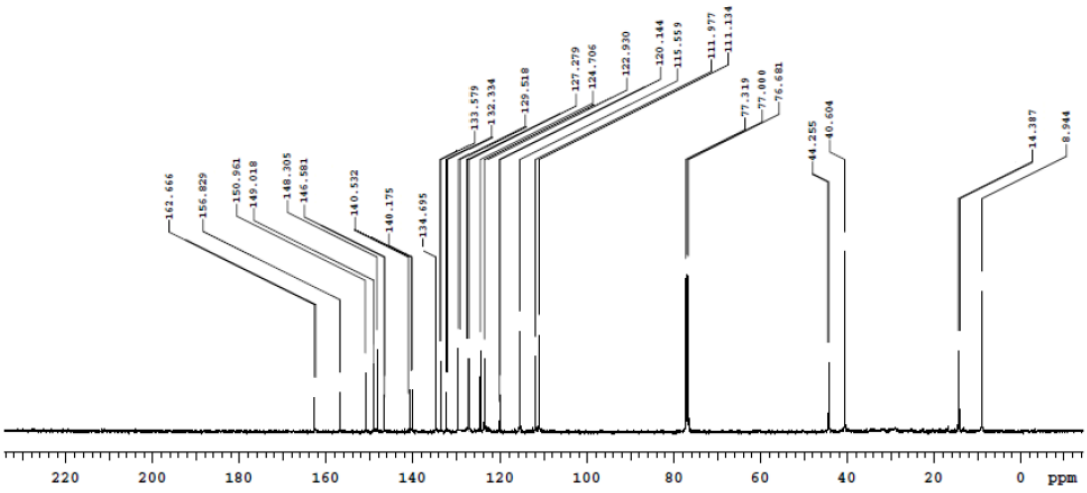

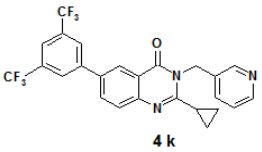

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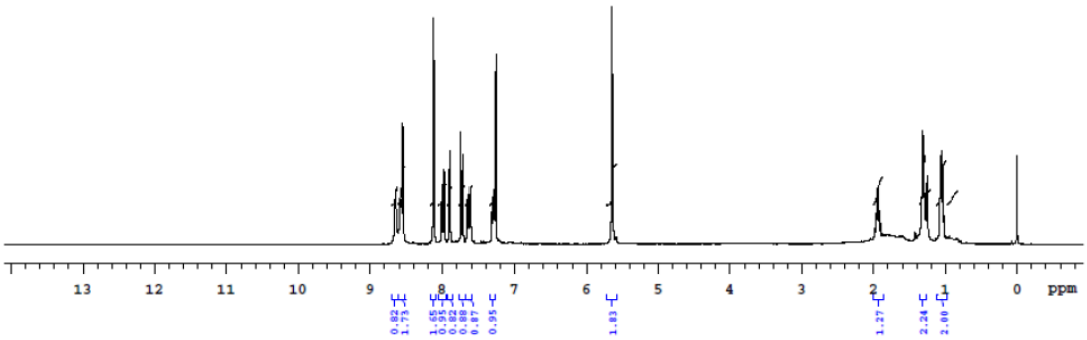

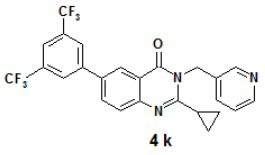

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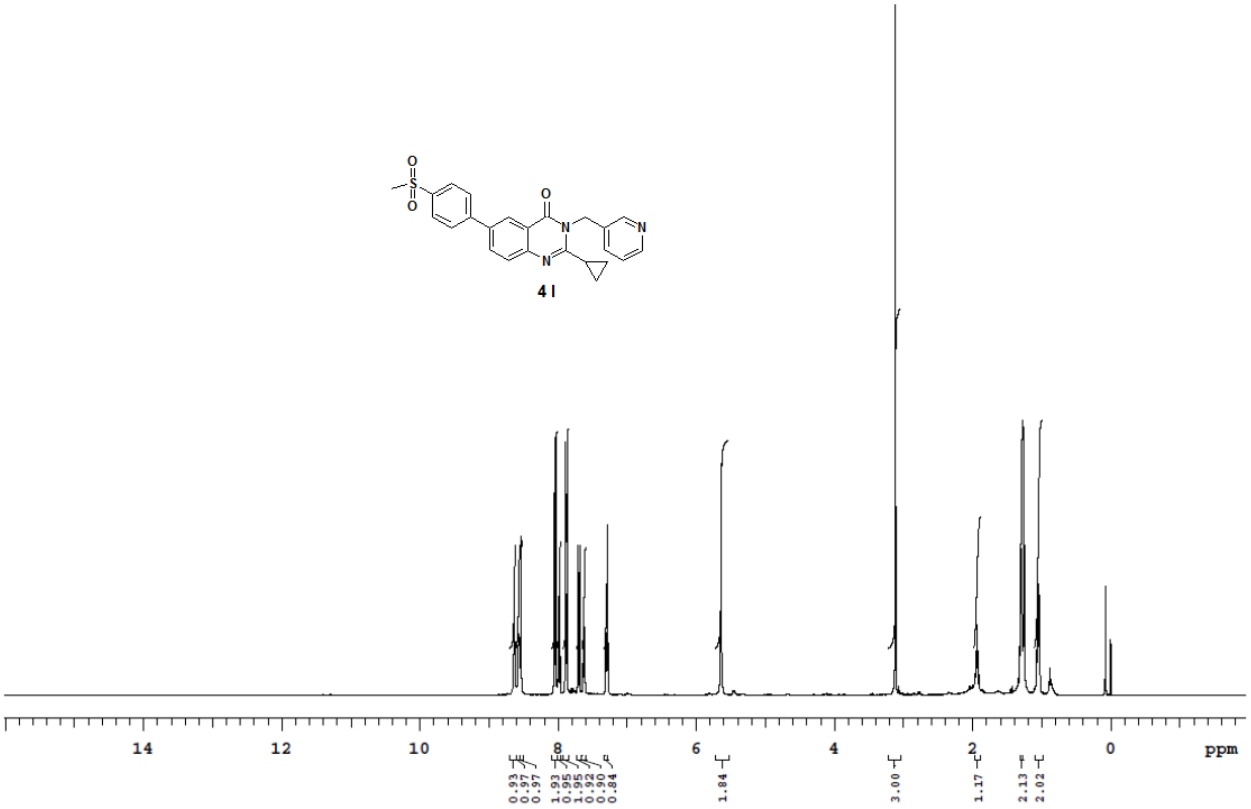

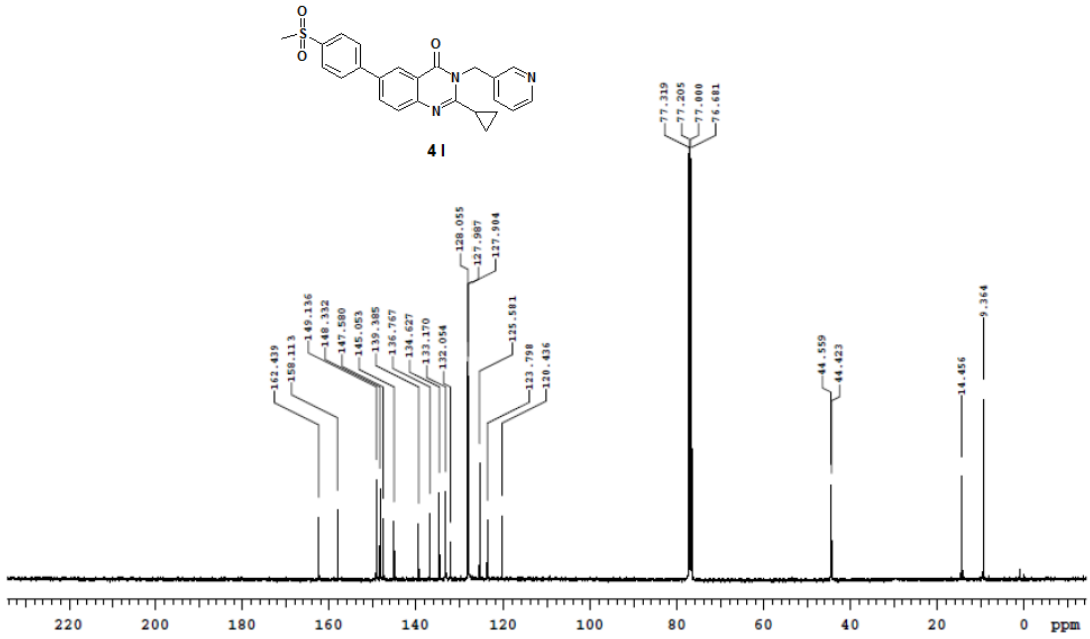

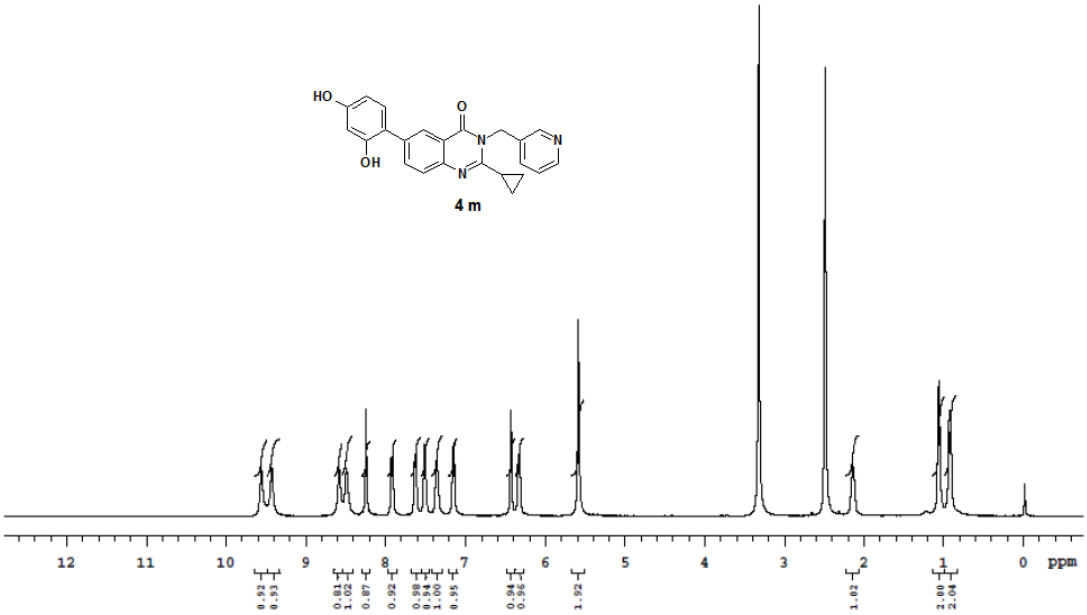

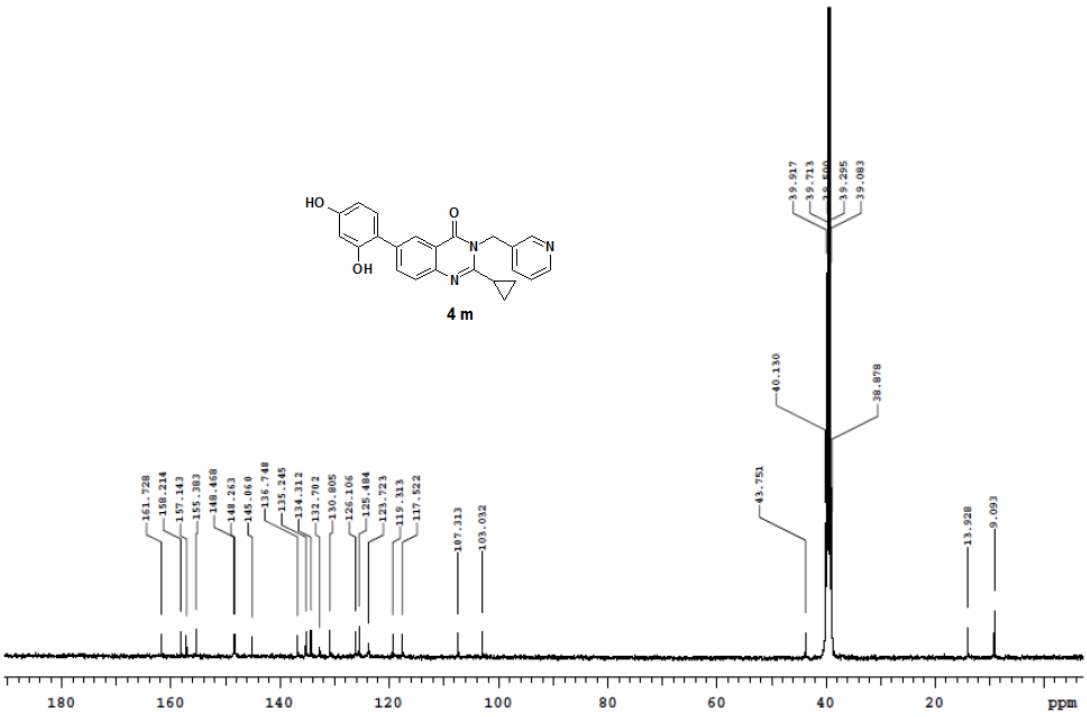

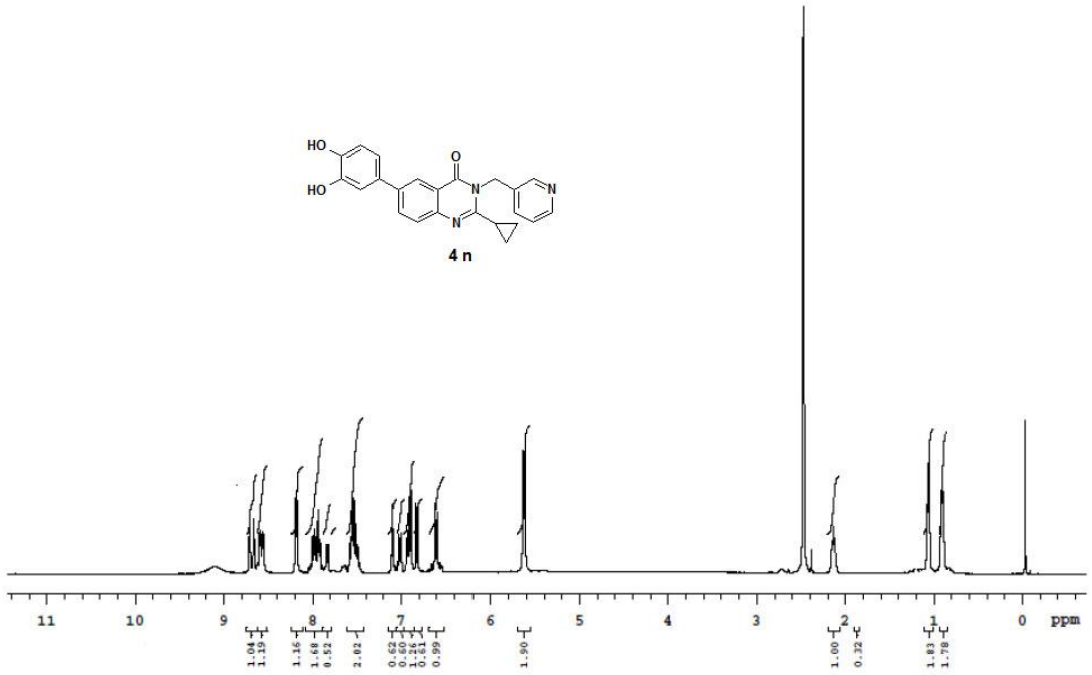

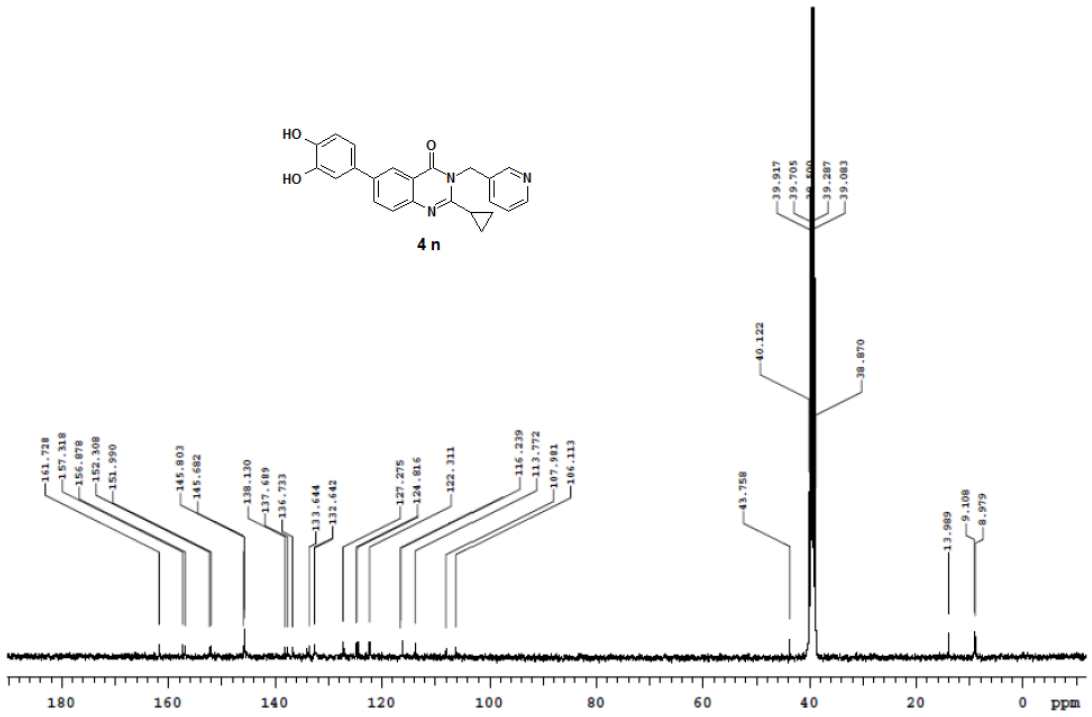

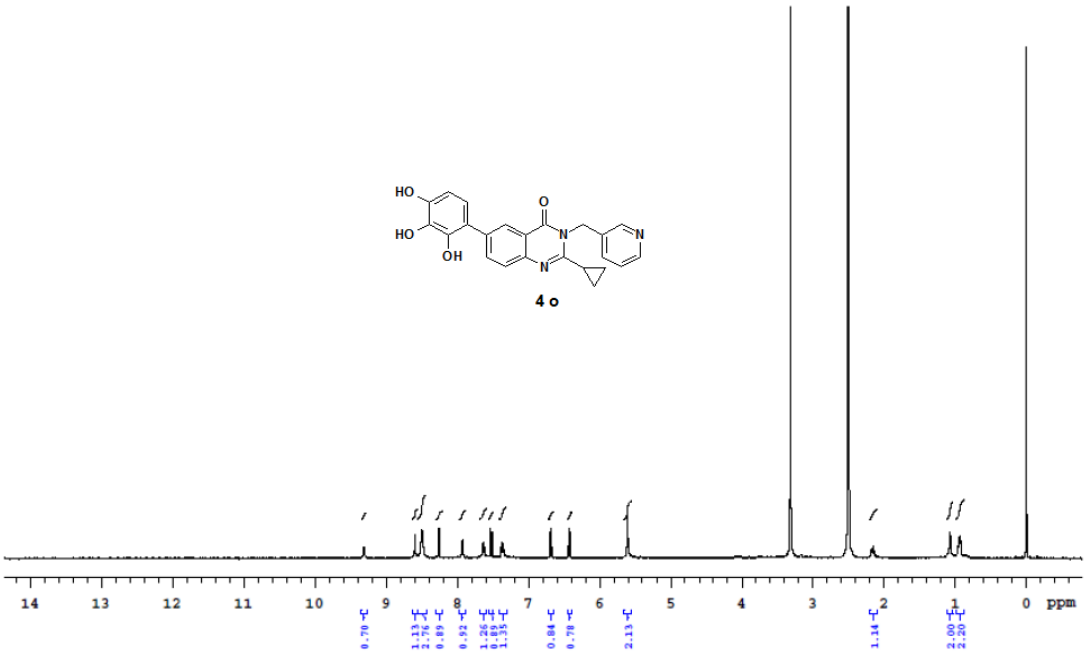

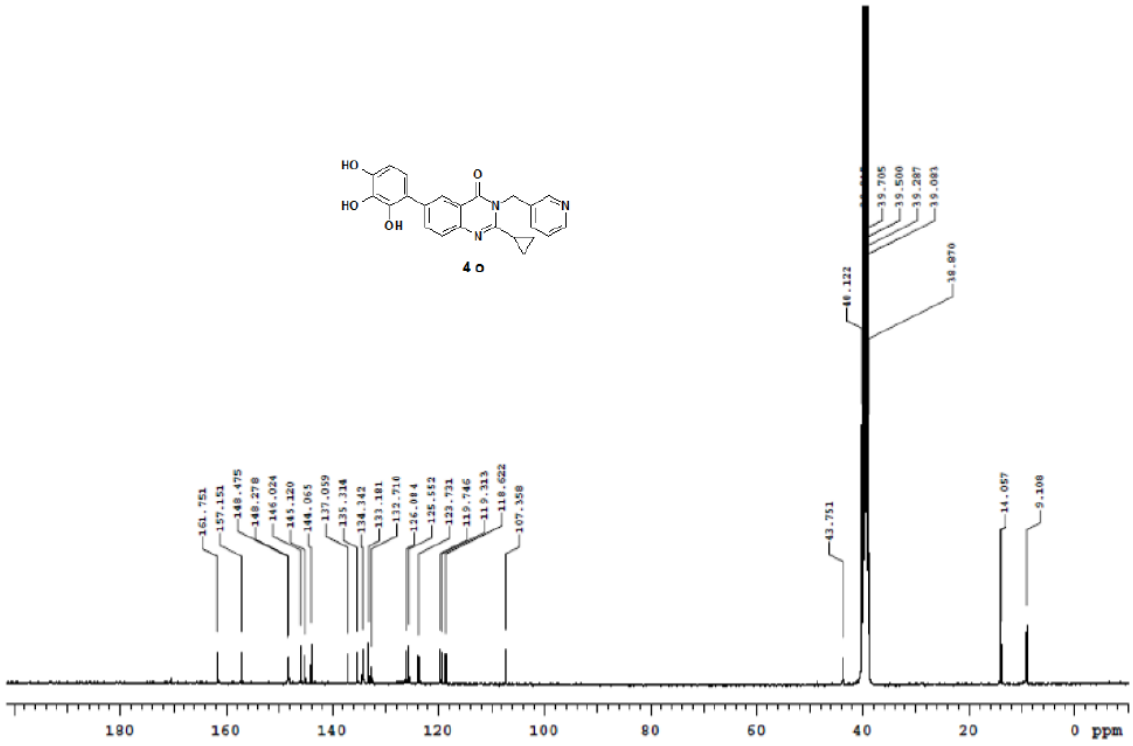

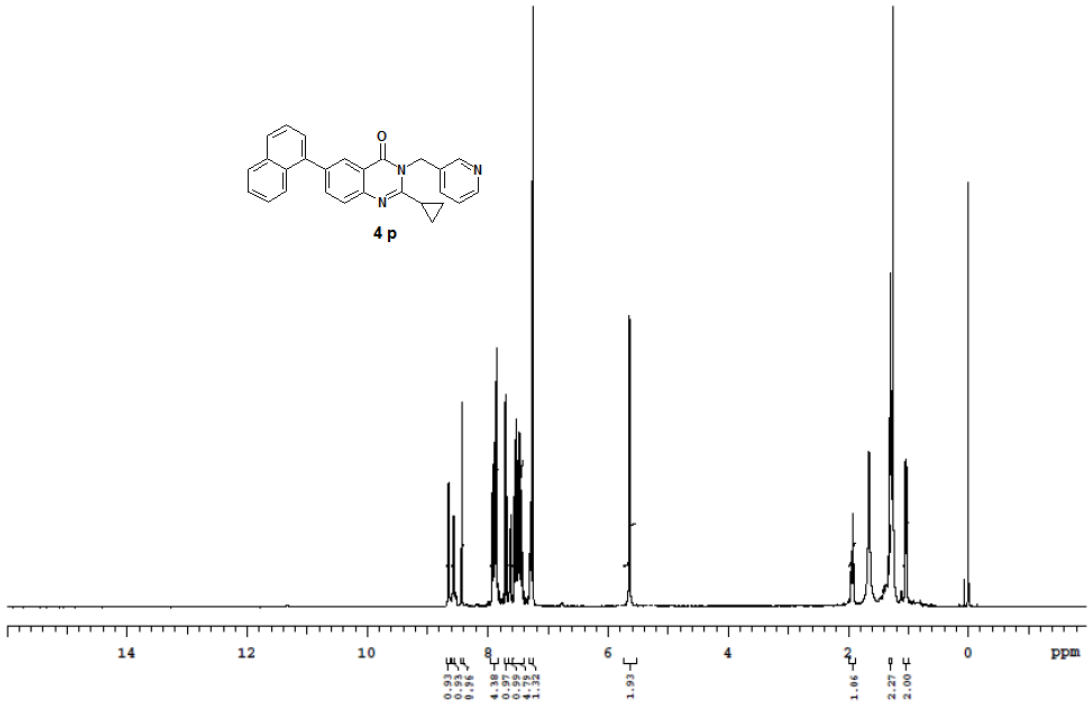

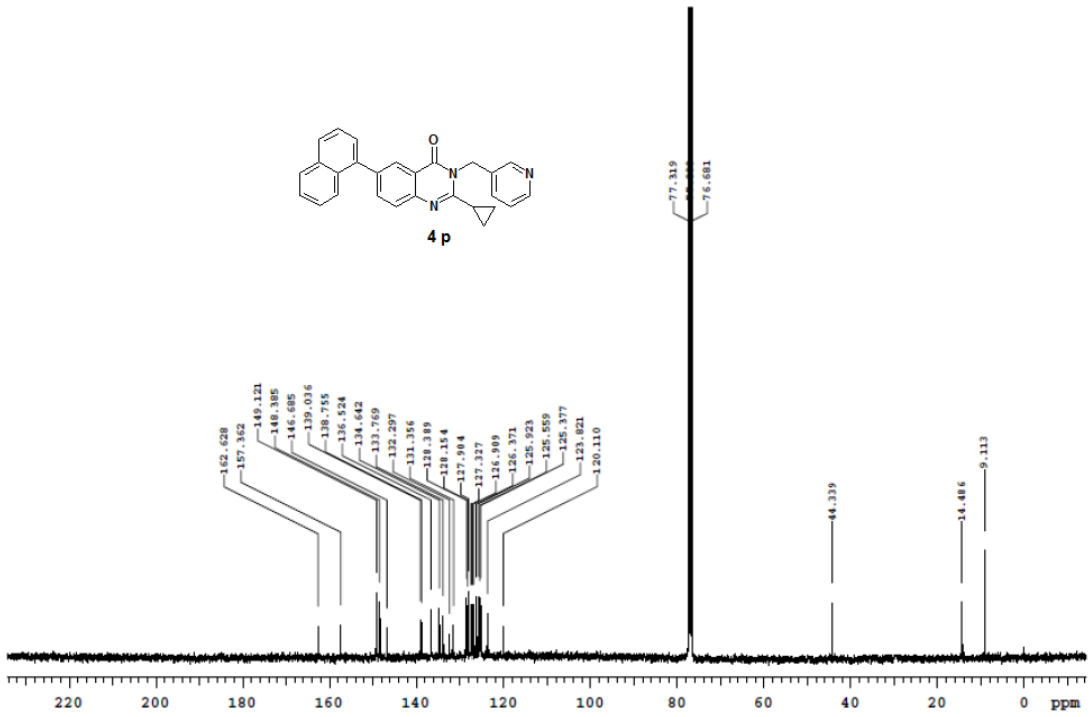

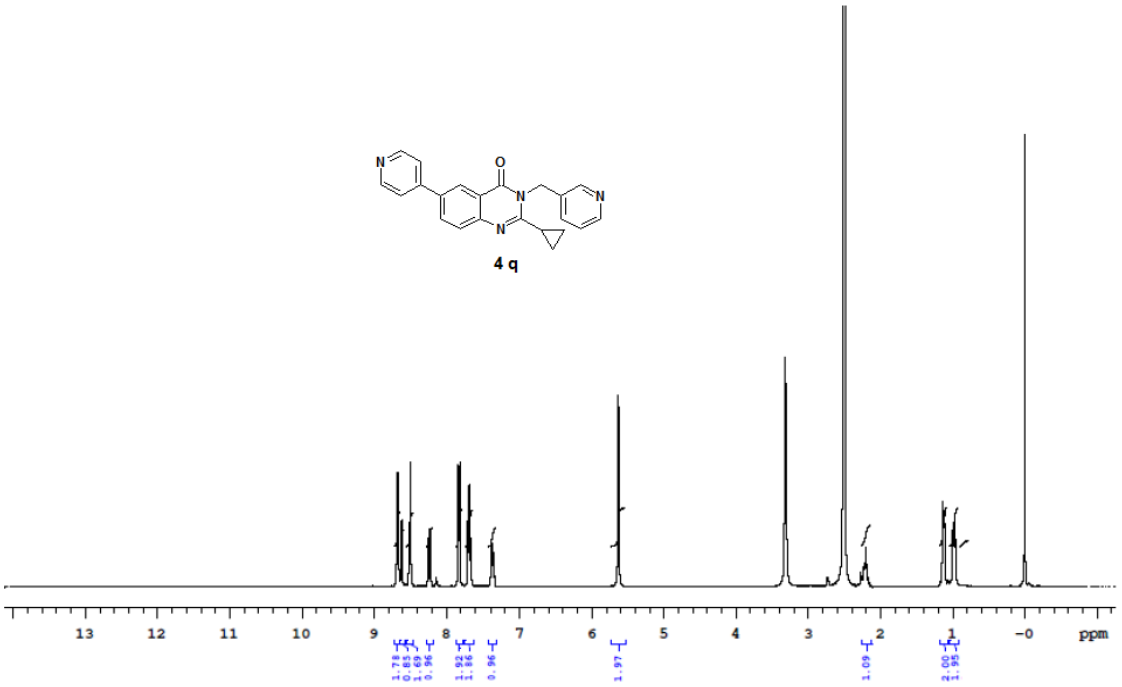

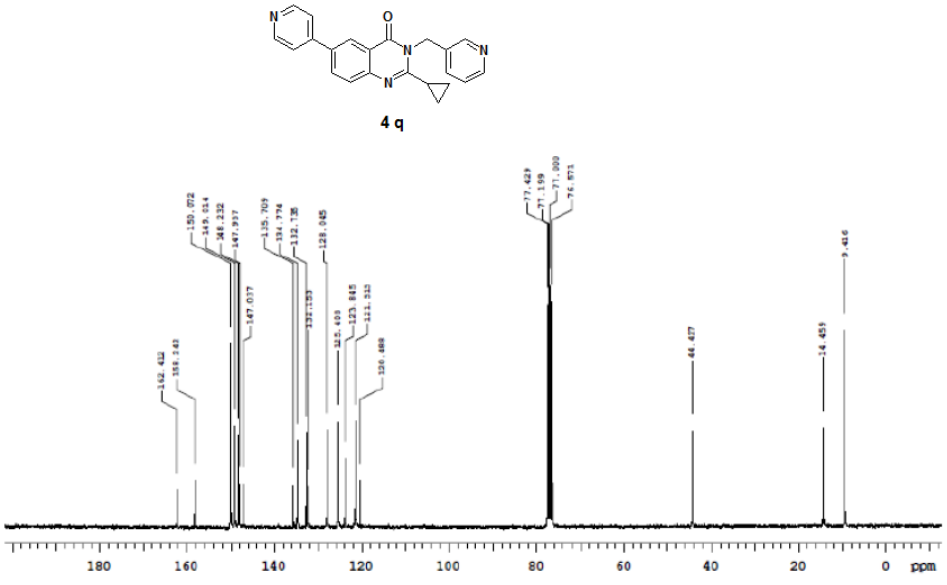

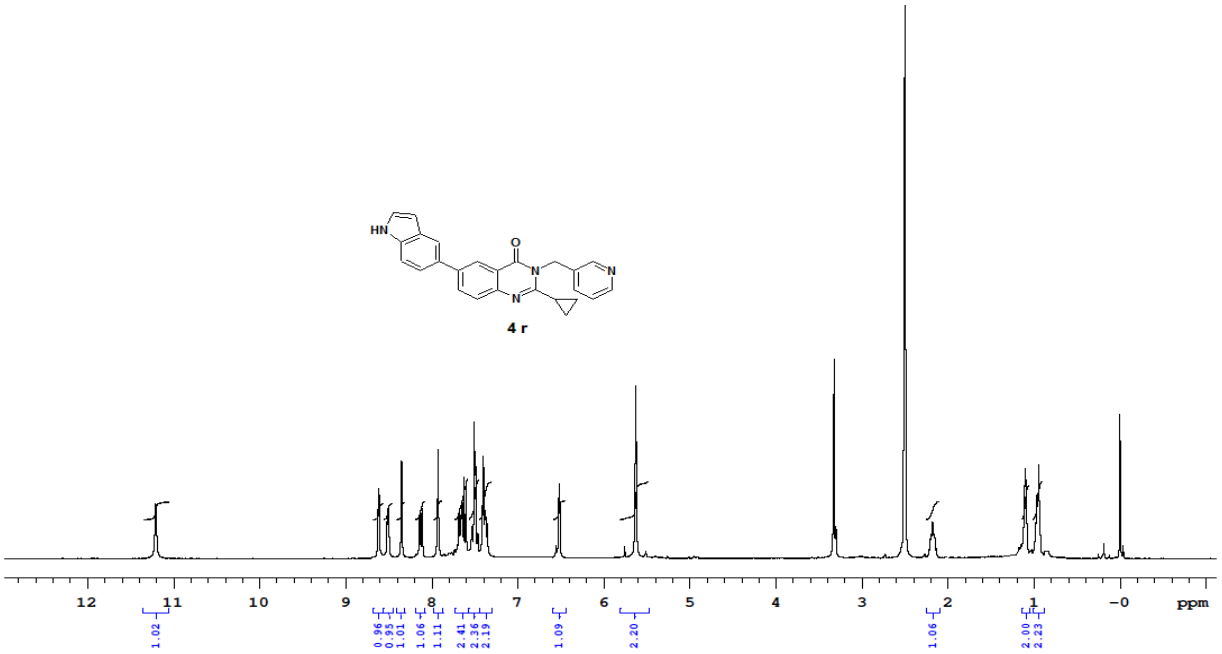

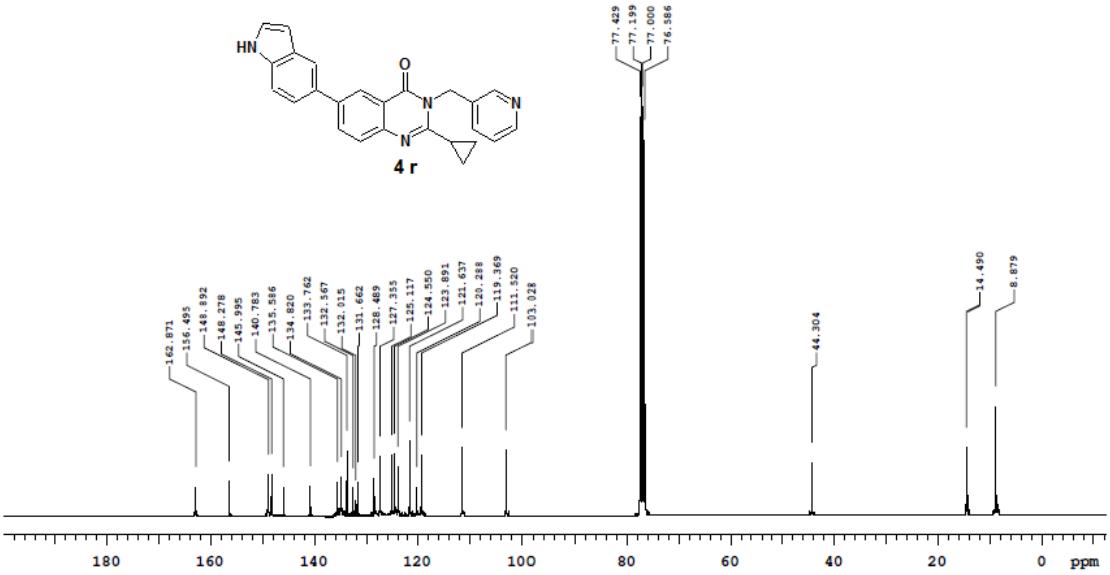

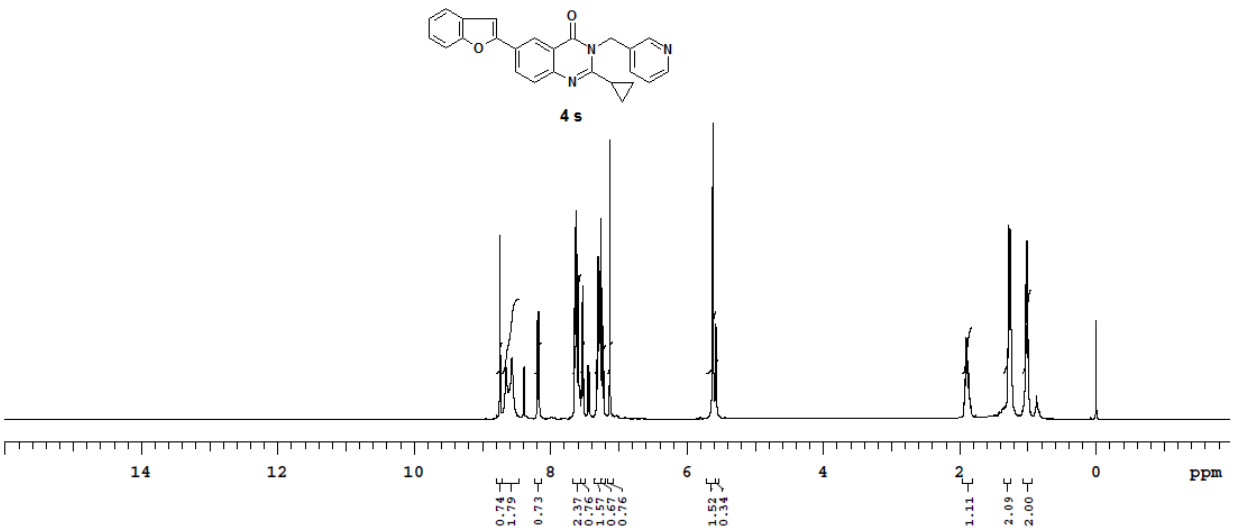

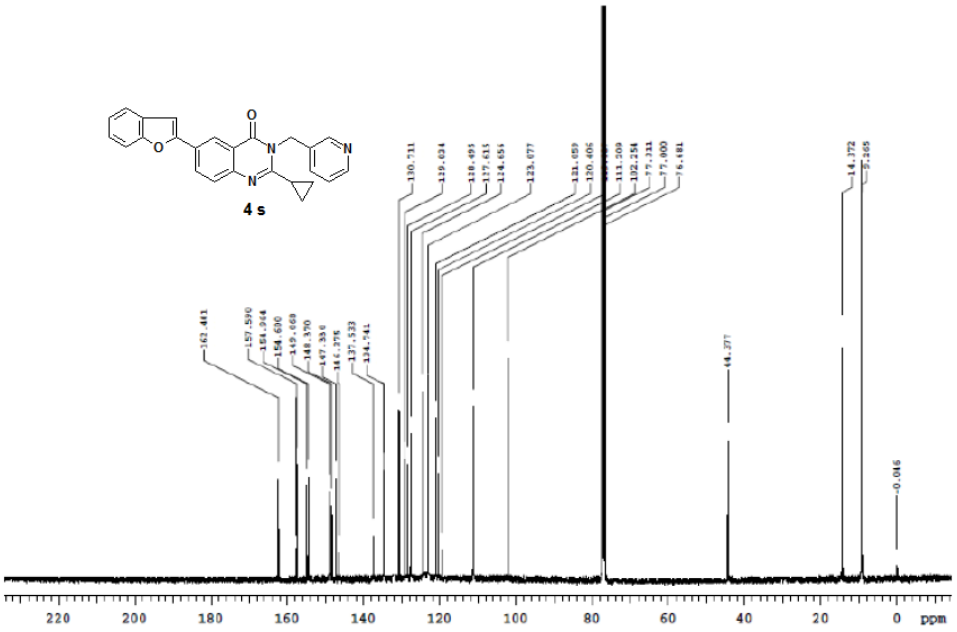

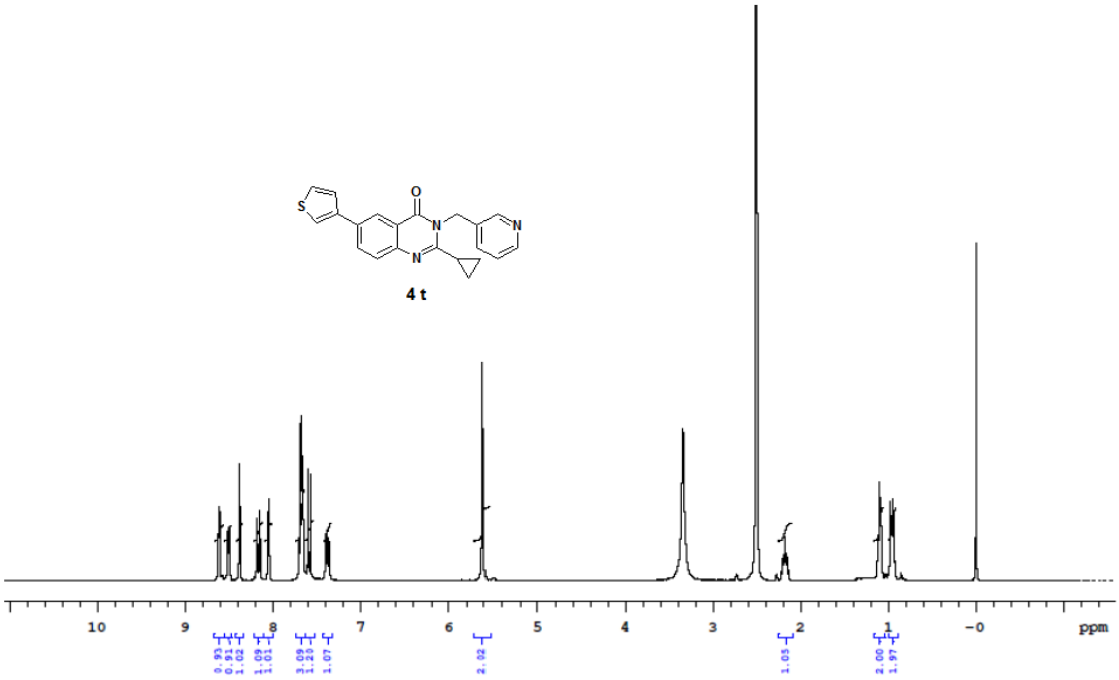

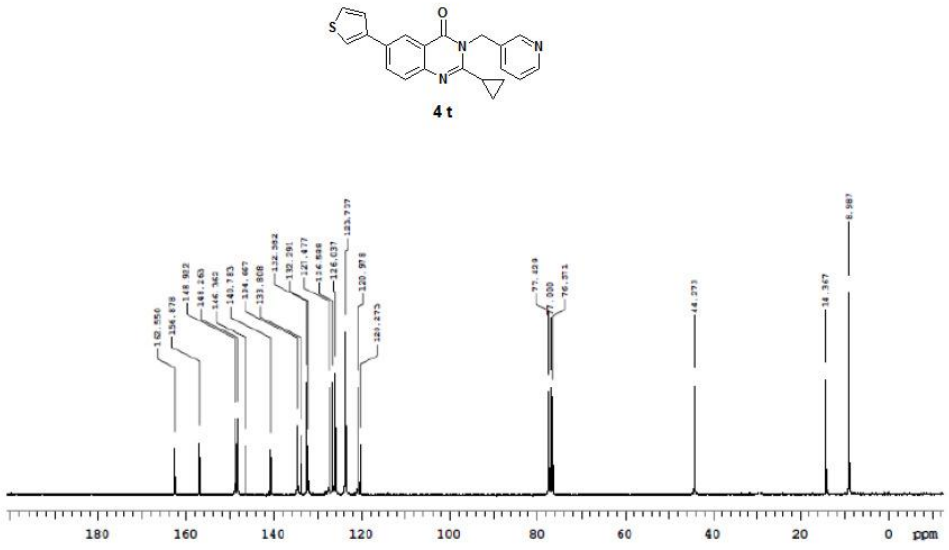

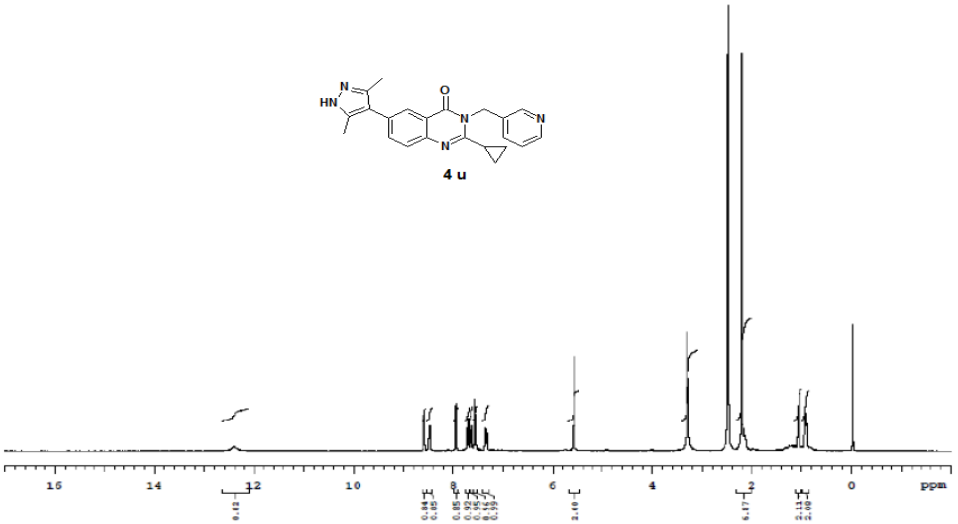

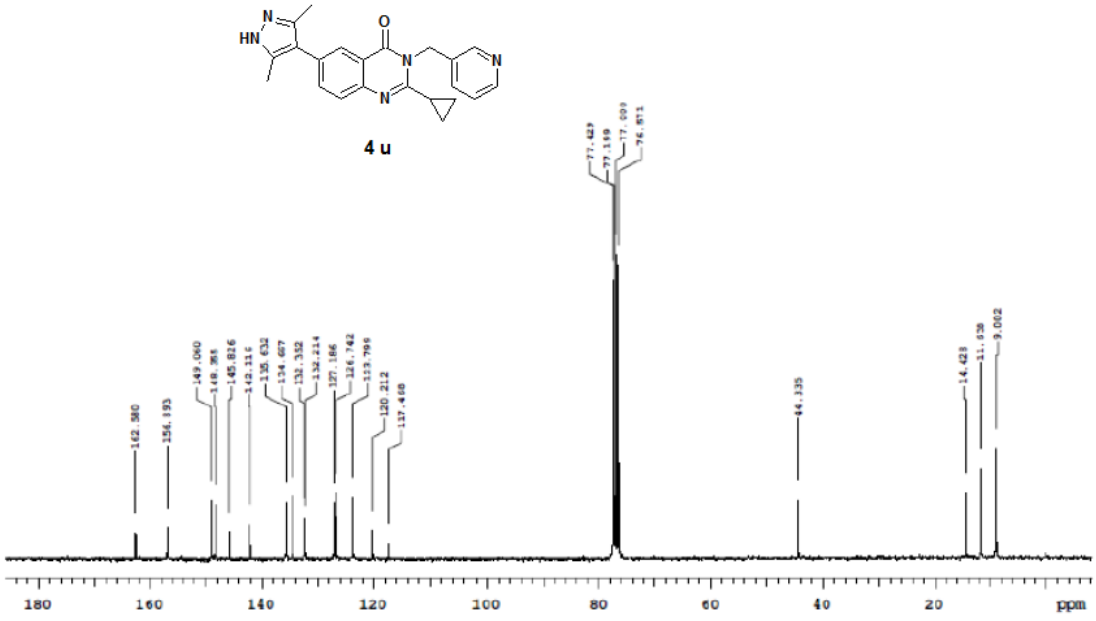

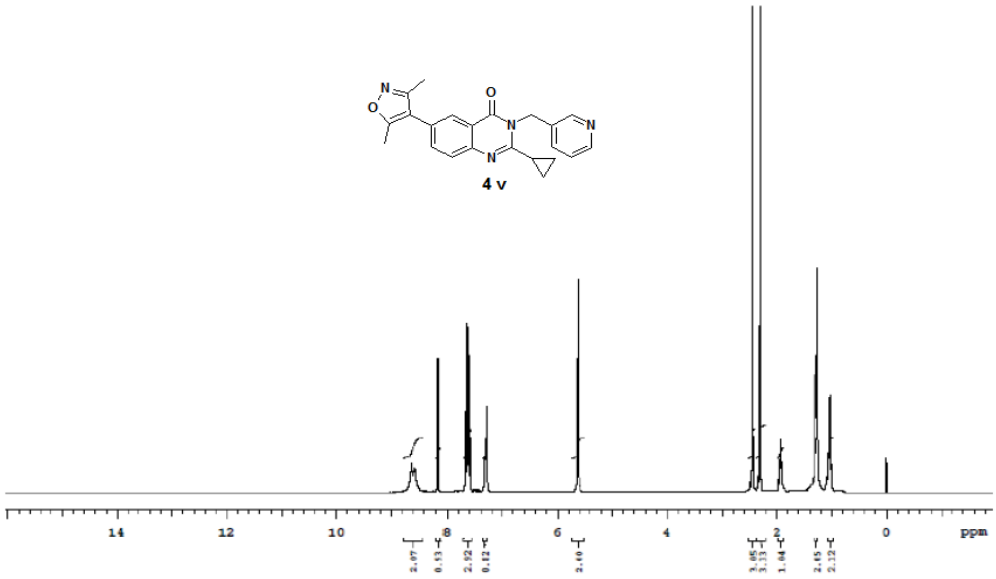

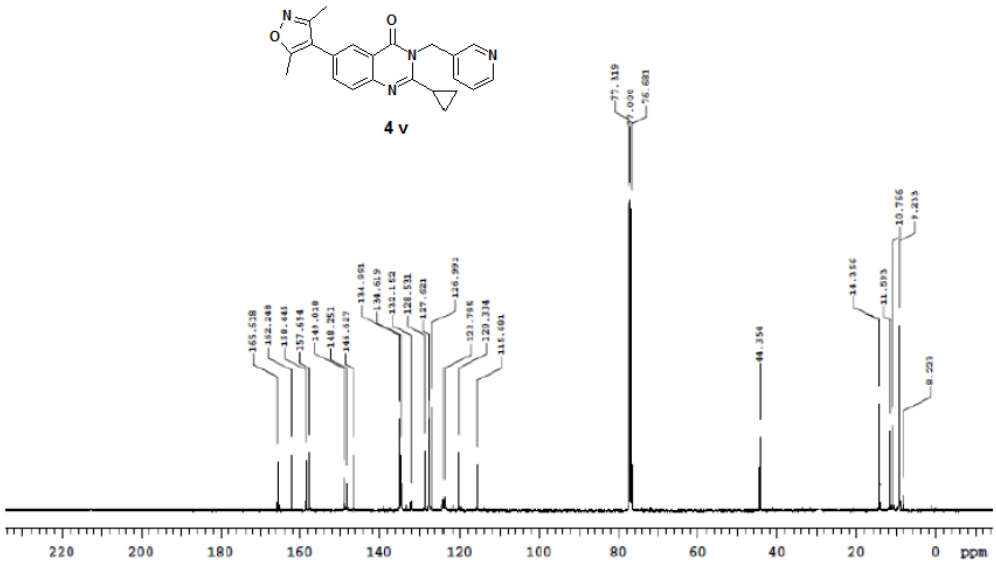

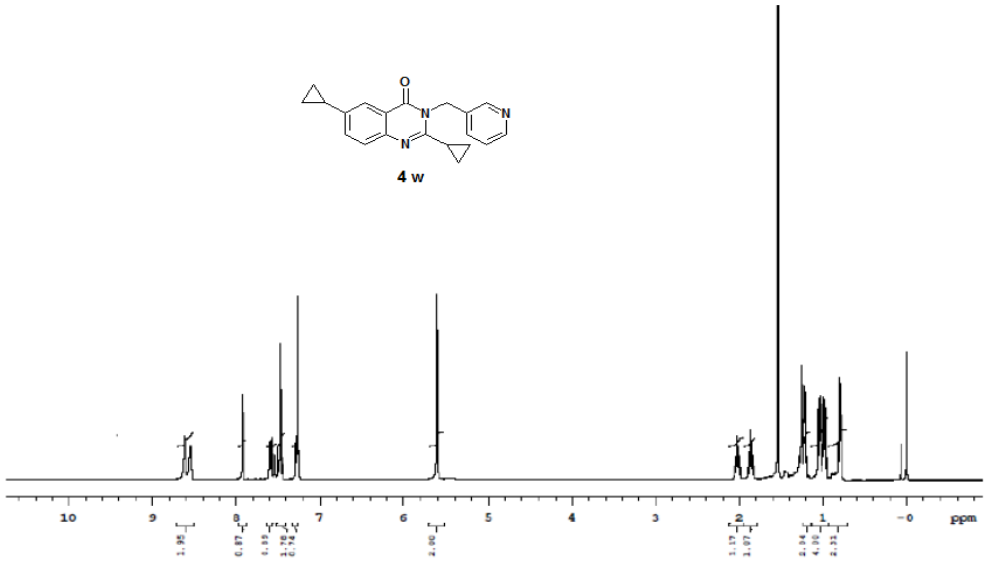

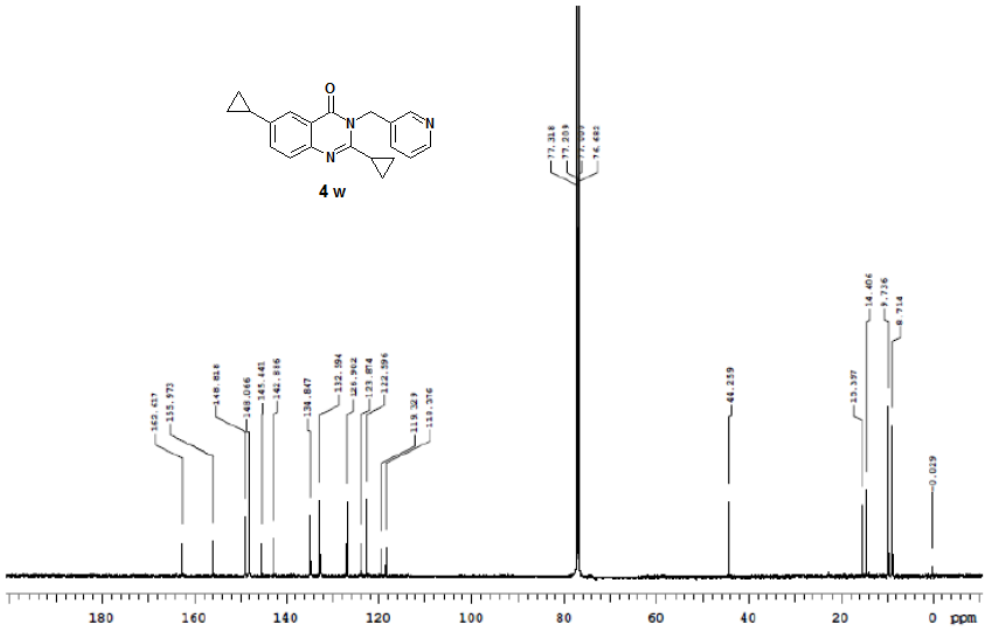

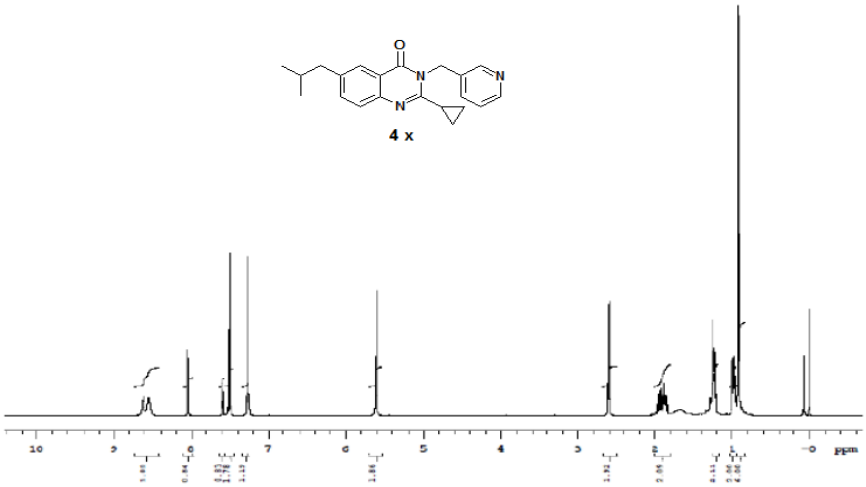

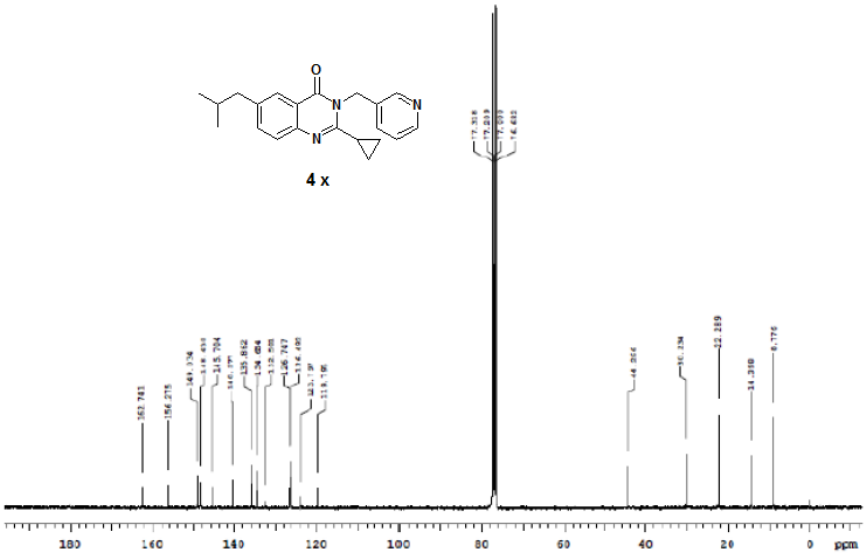

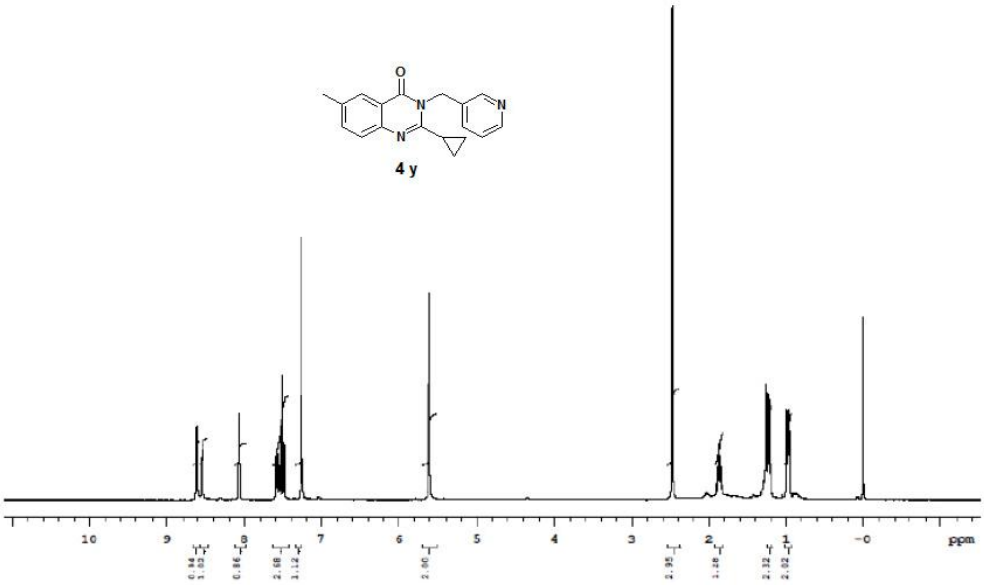

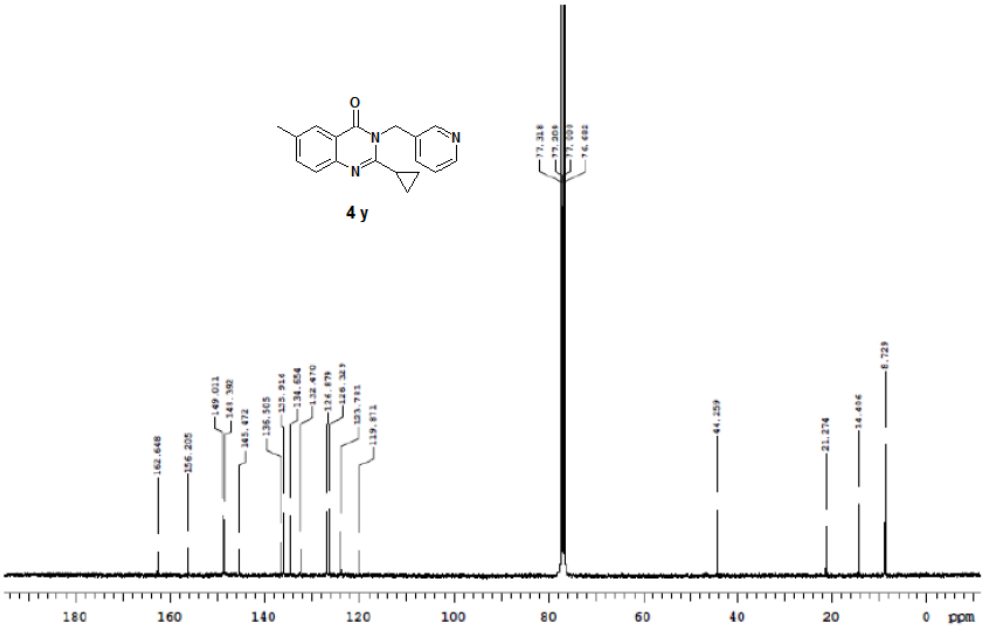

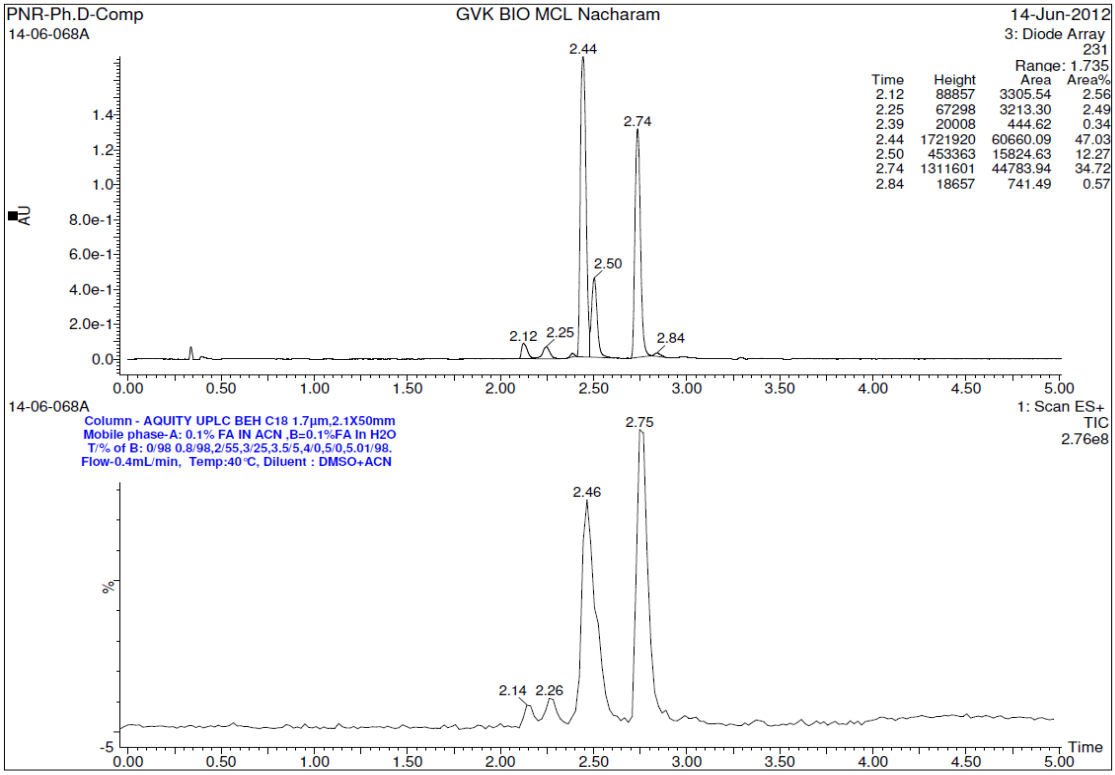
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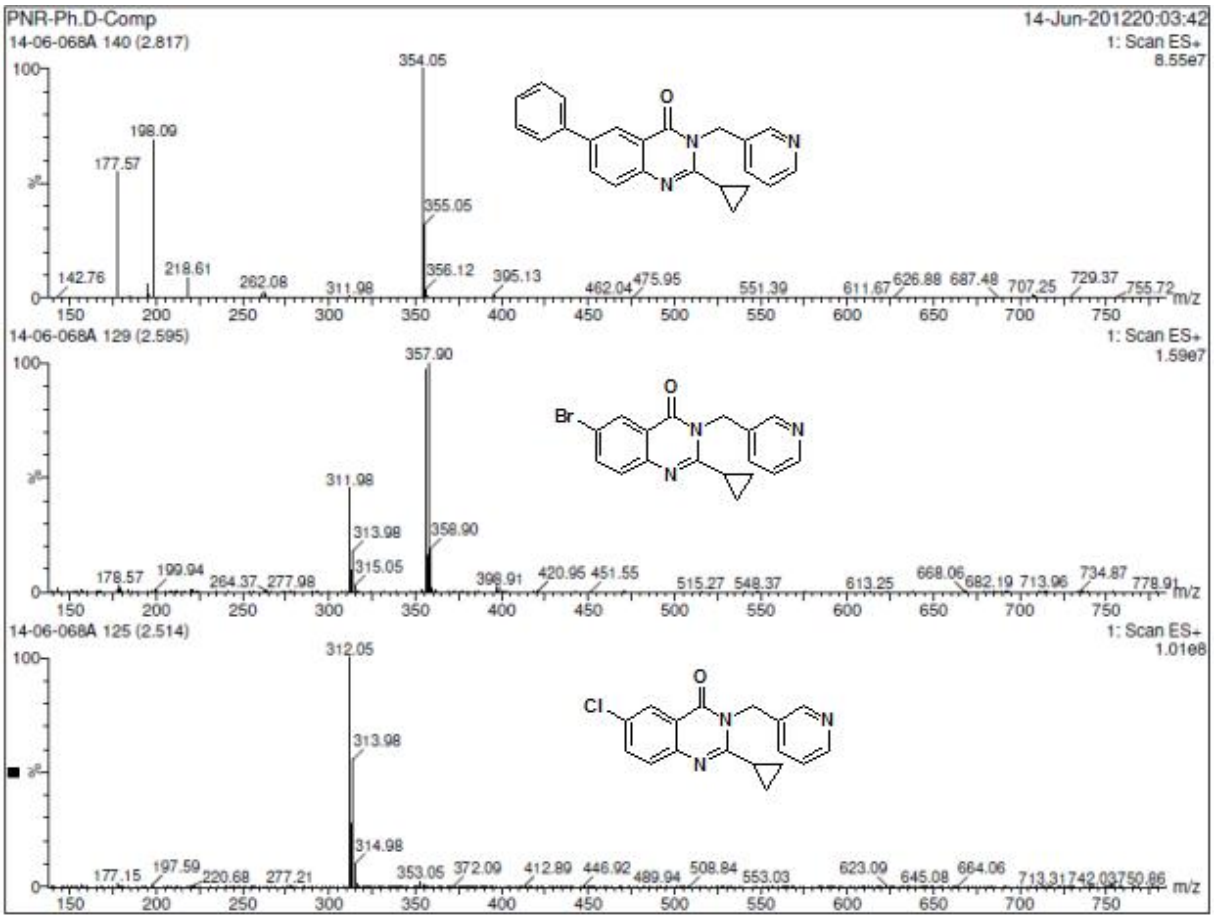
GVK BIO MCL Nacharam
14-Jun-2012
14-06-068A
3: Diode Array
231
Range: 1.735
Time Height Area Area%
2.12 88857 3305.54 2.56
2.25 67298 3213.30 2.49
2.39 20008 444.62 0.34
2.44 1721920 60660.09 47.03
2.50 453363 15824.63 12.27
2.74 1311601 44783.94 34.72
2.84 18657 741.49 0.57
AU
2.44
2.74
2.50
2.12
2.25
2.84
14-06-068A
1: Scan ES+
TIC
2.76e8
Column - AQUITY UPLC BEH C18 1.7µm,2.1X50mm
Mobile phase-A: 0.1% FA IN ACN ,B=0.1%FA In H2O
T/% of B: 0/98 0.8/98,2/55,3/25,3.5/5,4/0,5/0,5.01/98.
Flow-0.4mL/min, Temp:40°C, Diluent : DMSO+ACN
2.75
2.46
2.14
2.26
%
Time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14-Jun-201220:03:42
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355.05
142.76
218.61
262.08
311.98
356.12
395.13
462.04
475.95
551.39
611.67
626.88
687.48
707.25
729.37
755.72
m/z
14-06-068A 129 (2.595)
1: Scan ES+
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357.90
311.98
313.98
358.90
315.05
178.57
199.94
264.37
277.98
398.91
420.95
451.55
515.27
548.37
613.25
668.06
682.19
713.96
734.87
778.91
m/z
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314.98
177.15
197.59
220.68
277.21
353.05
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412.89
446.92
489.94
508.84
553.03
623.09
645.08
664.06
713.31
742.03
750.86
m/z