Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Mayank, [a,b*] Ashutosh Singh,[c] Kumar Udit Saumya,[c] Mayank Joshi,[d] Navneet Kaur,[e*] Neha Gargc,[c,f*], Narinder Singh[a*]

[a]Department of Chemistry, Indian Institute of Technology Ropar, Punjab 140001, India,
nsingh@iitrpr.ac.in
[b]University College of Pharmacy and Guru Kashi University, Talwandi Sabo
mayank6103@gmail.com
[c] School of Basic Sciences, Indian Institute of Technology Mandi, Himachal Pradesh 175005, India,
[d]M. M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana, 133207, India.
[e]Department of Chemistry, Punjab University, Chandigarh, 160014, India.
navneetkaur@pu.ac.in
[f]Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh-221005, India.
nehagarg@bhu.ac.in

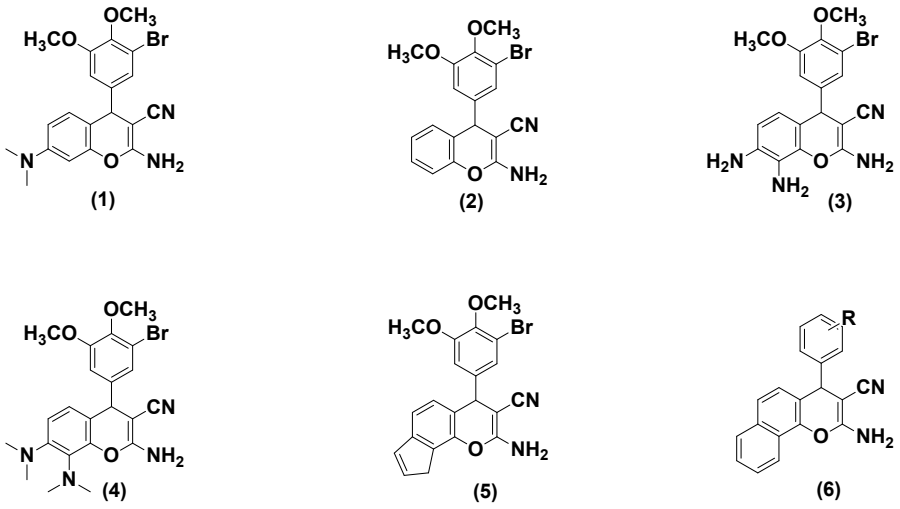


Figure SF 1.(1) Structure of MX-58151 **(1)**; **(2-5)** other compounds of the MX-58151 series reported to binding colchicine binding site of α/β tubulin isoforms and **(6)**Basic scaffold of2-amino-4-phenyl-4H-benzo[h]chromene-3-carbonitrilederivatives used in our studies.

Synthesis of chromene derivatives

The reaction scheme for the synthesis of chromene derivatives is as represented in figure SF1. Many methods for the synthesis of chromene derivatives are available in literature.[1-3] Herein, for the synthesis of chromene derivatives, one equivalent of each α-Naphthol, malononitrile, and aldehyde derivatives was reflexed in methanol for 6 hrs. Few drops of triethylamine were used as a catalyst and after completion of the reaction, the products were purified by recrystallizing using methanol as a solvent. The structural features of the synthesized compounds are represented in figure SF2 below. The characterization of the synthesized compounds was done by ^{1}H NMRspectroscopic and mass spectrometric technique. The characterization data of the compounds were provided in figure SF3-SF14 below. Other methods for the synthesis of chromene derivatives are also reported

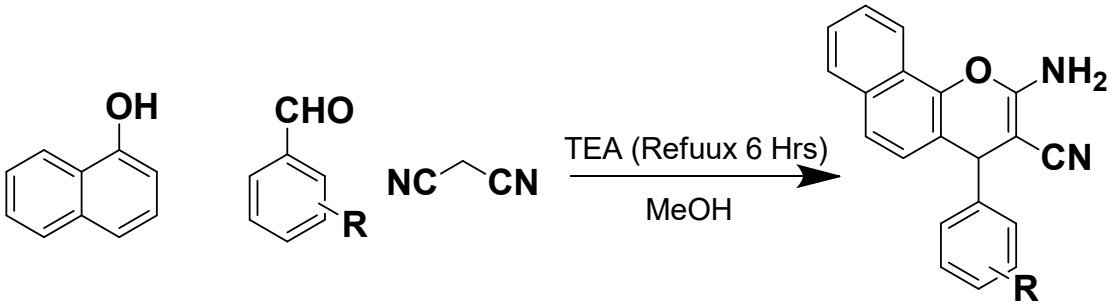


Figure SF2. Structure of chromene derivatives used in the study

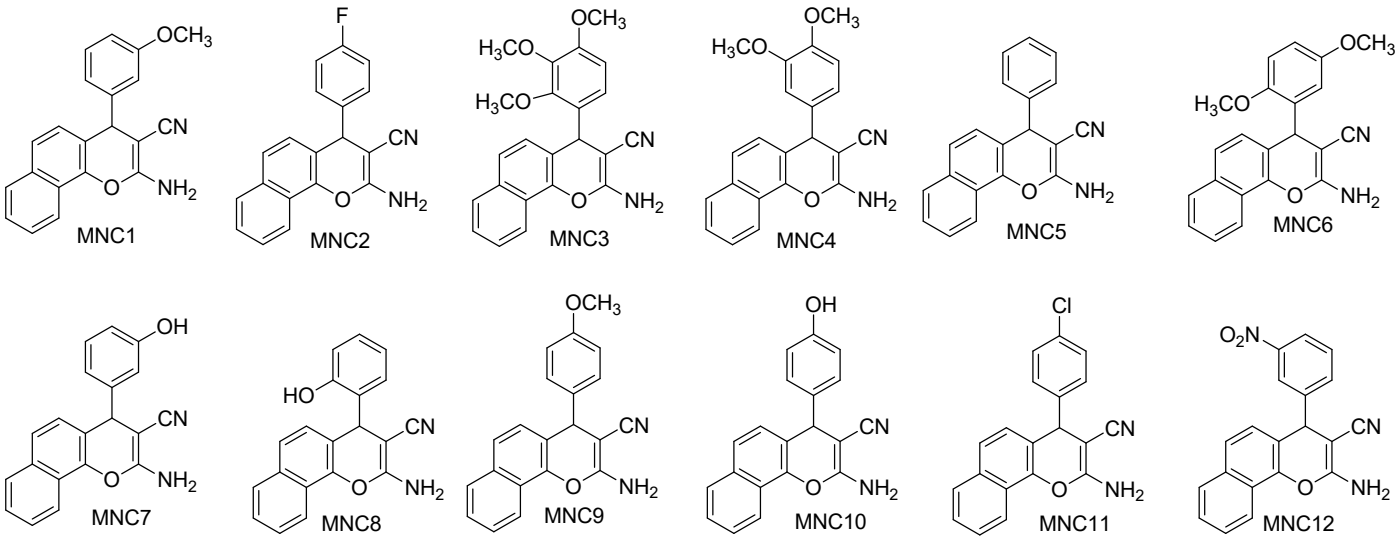
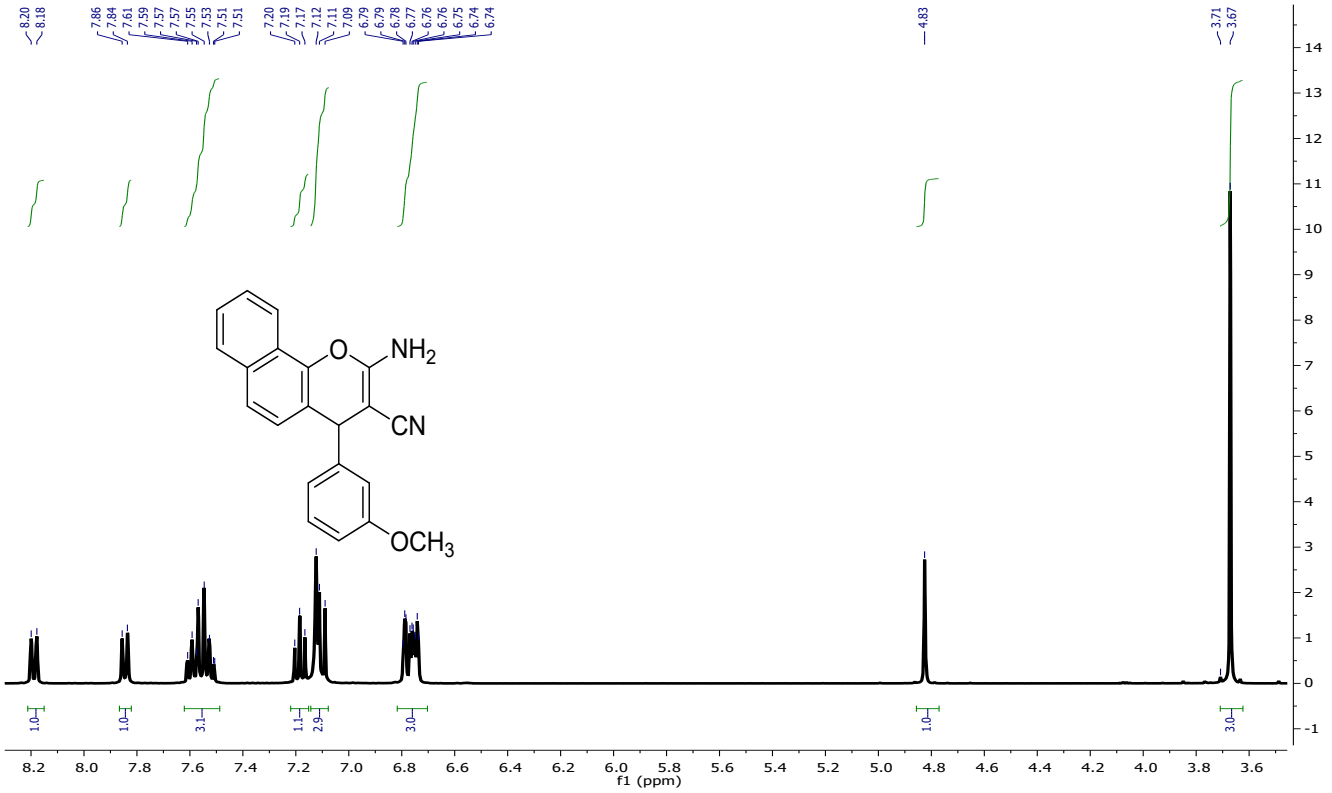
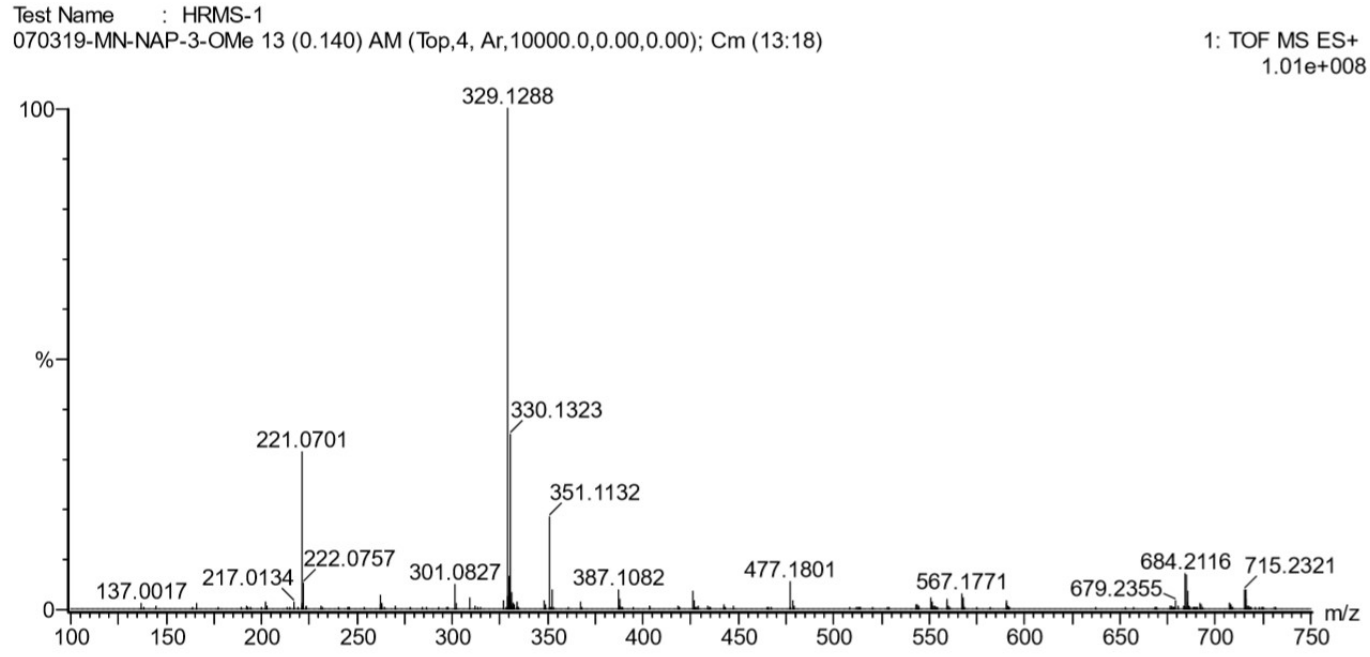


Figure SF3. Structure of chromene derivatives used in the study





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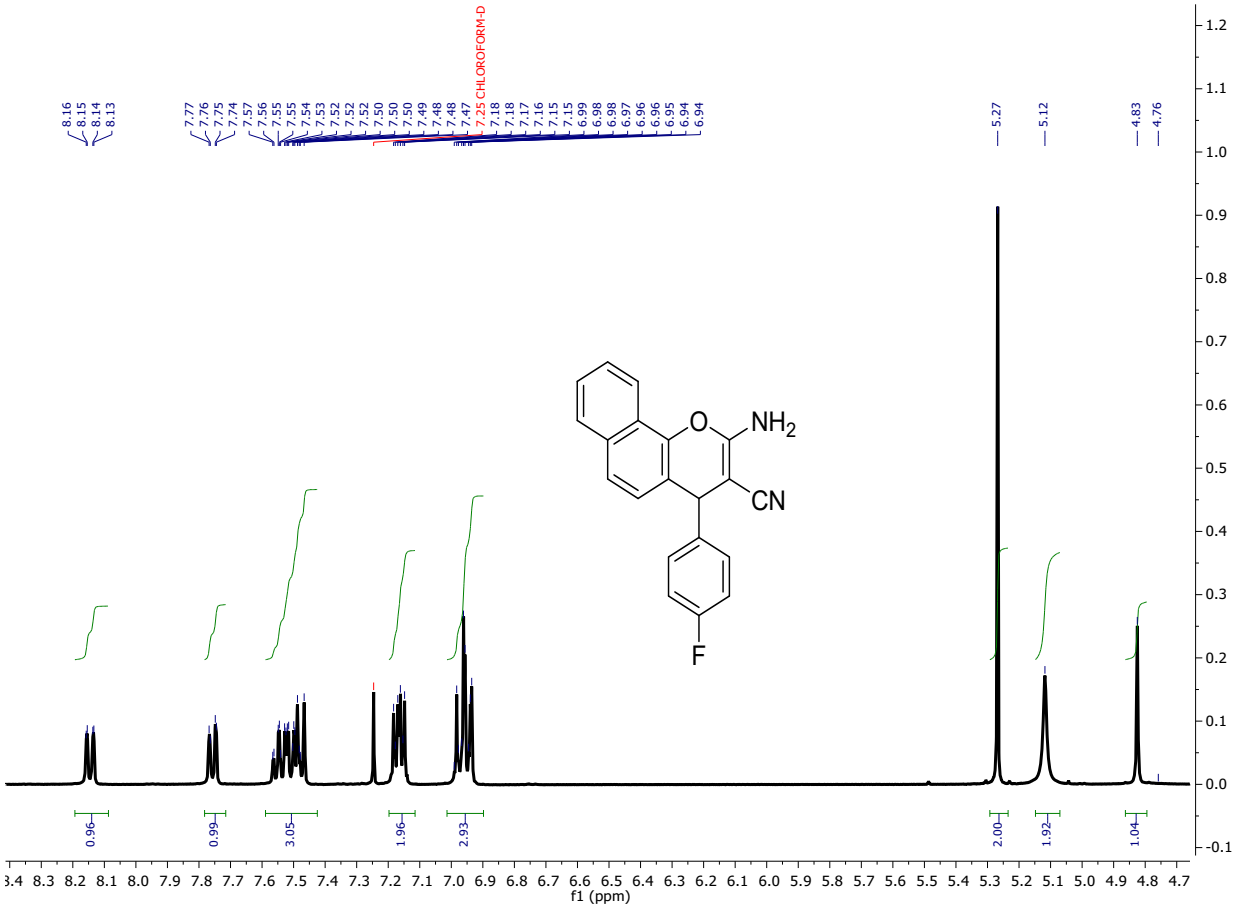
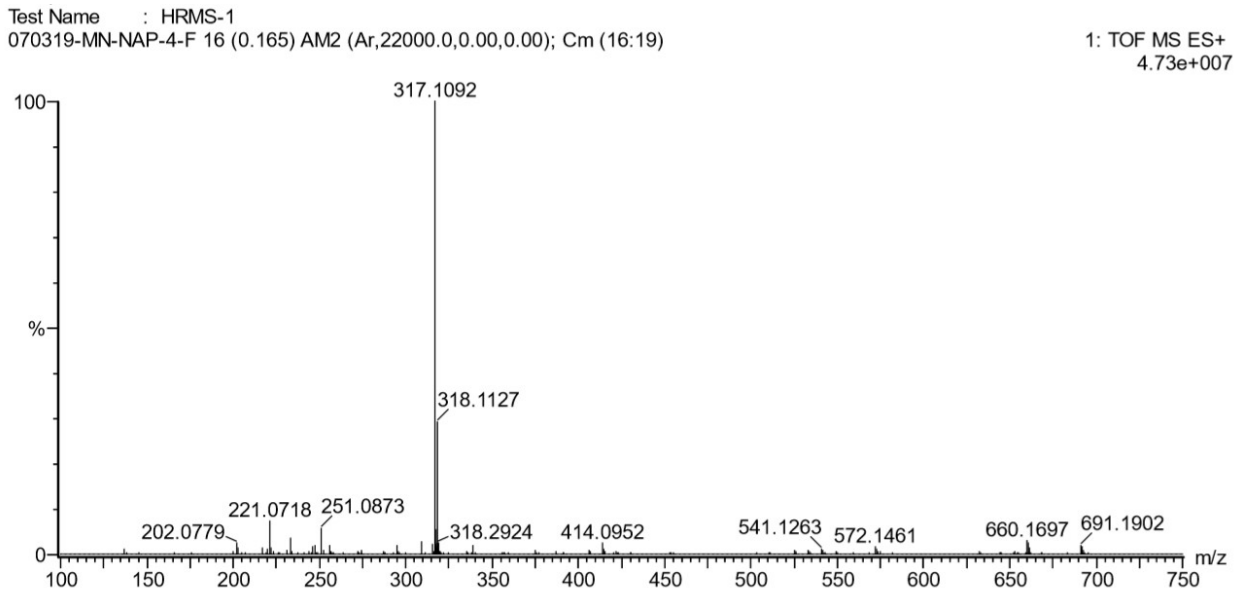
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
329.1288	329.1290	-0.2	-0.6	14.5	1105.1	n/a	n/a	C21 H17 N2 O2

Figure SF4.^{1}H NMR and HRMS of MNC-1 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{21}H_{16}N_2O_2^+$ 329.12; found 329.1288)





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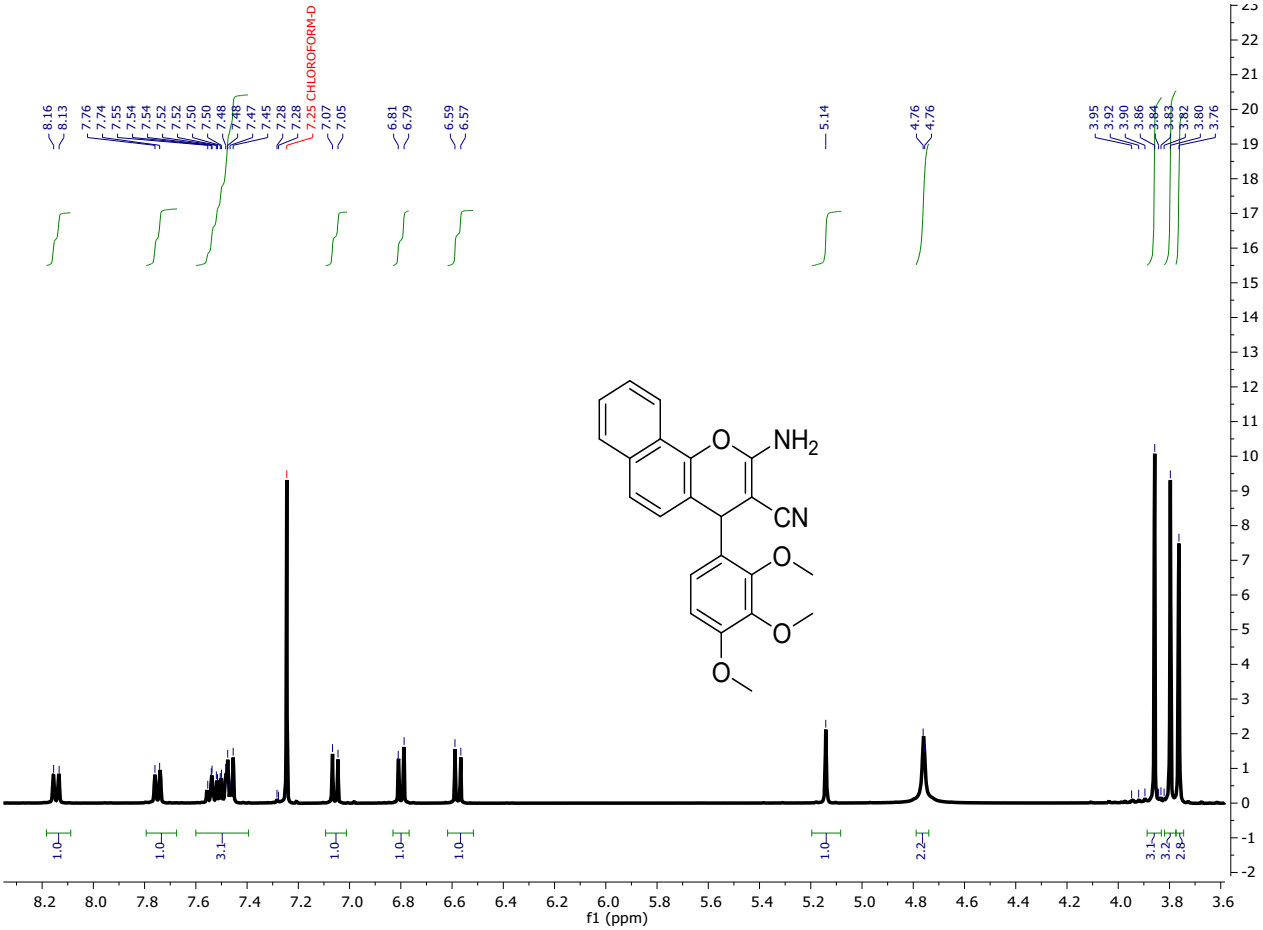
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
317.1092	317.1090	0.2	0.6	14.5	847.2	n/a	n/a	C20 H14 N2 O F

Figure SF5.^{1}H NMR and HRMS of MNC-2 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{13}FN_2O^+$ 317.10; found 317.1092)



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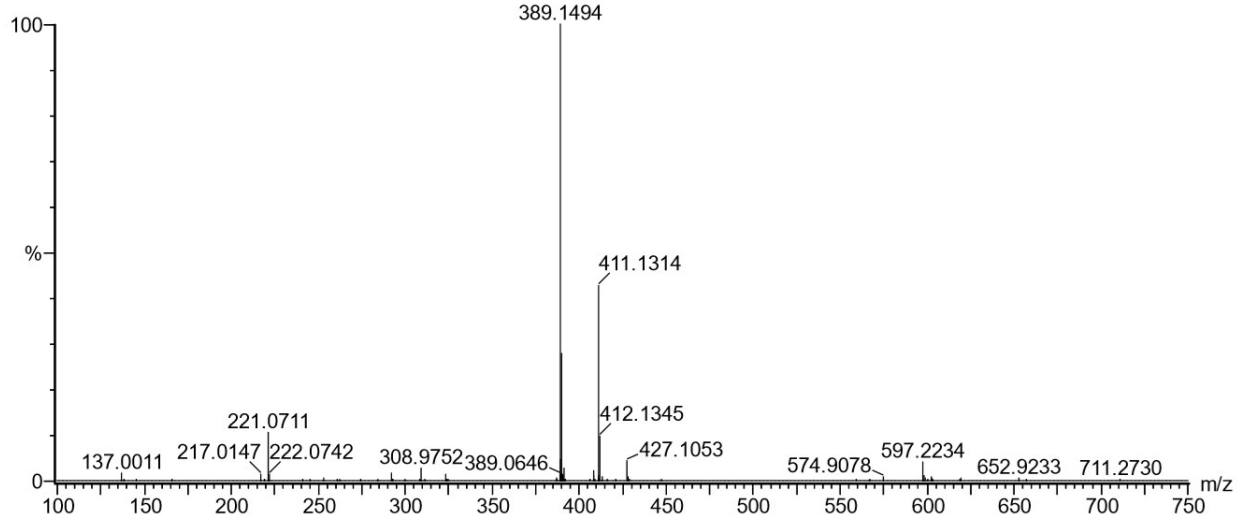
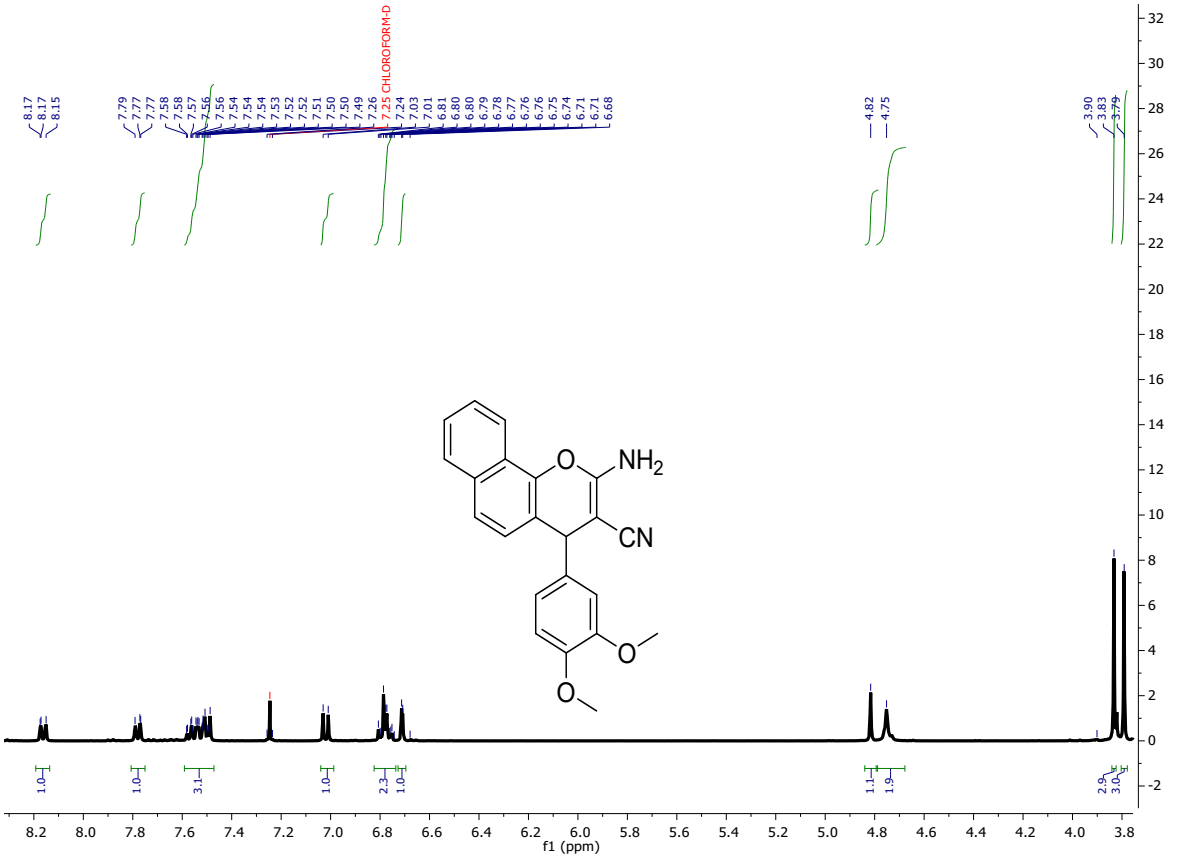


Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
389.1494	389.1501	-0.7	-1.8	14.5	656.9	n/a	n/a	C23 H21 N2 O4

Figure SF6.^{1}H NMR and HRMS of MNC-3 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{23}H_{20}N_2O_4^+$ 389.14; found 389.1494)



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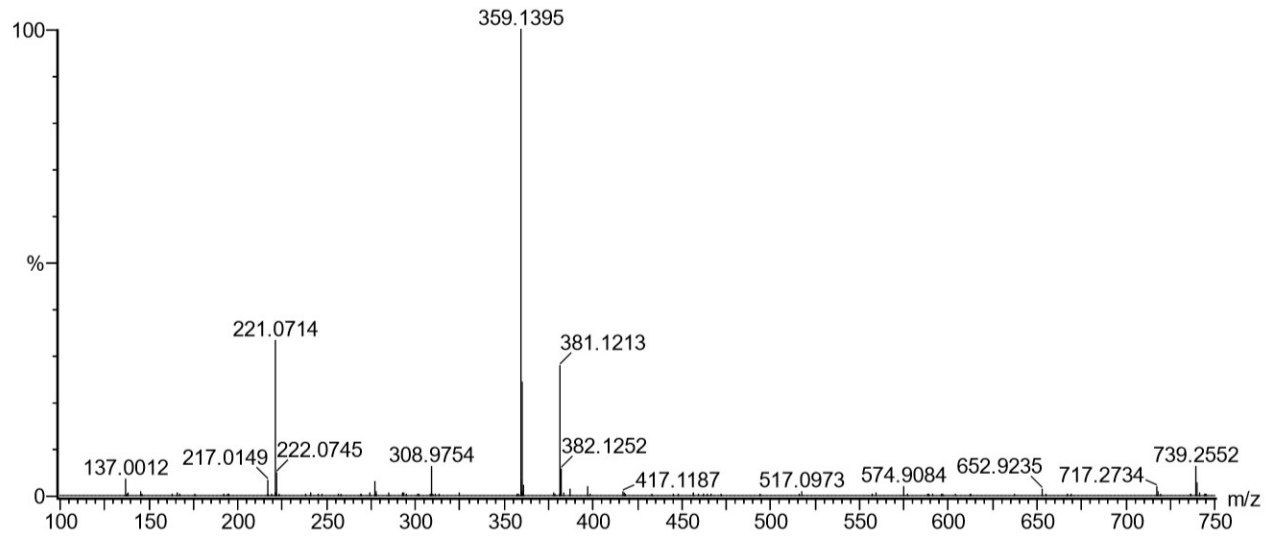
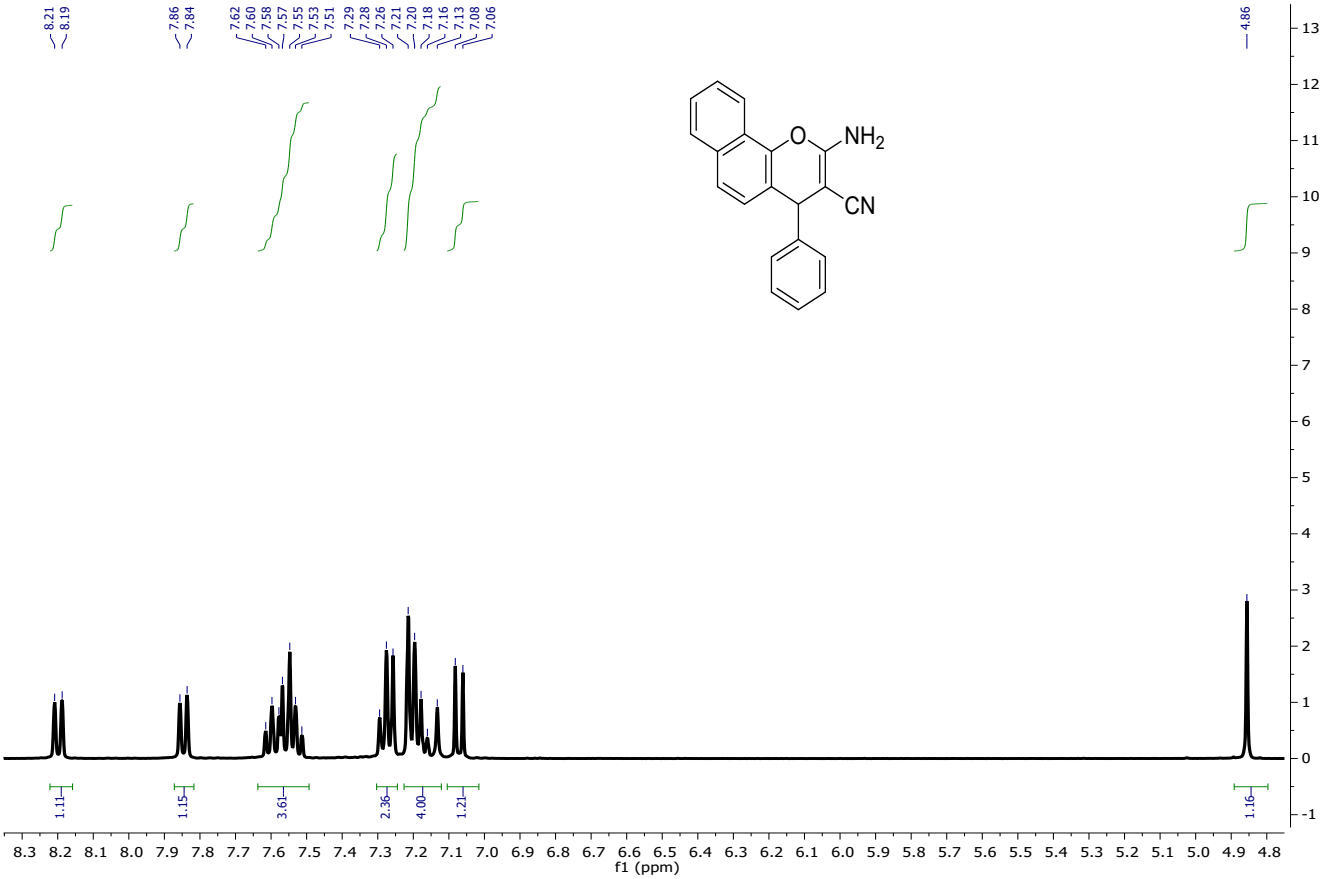
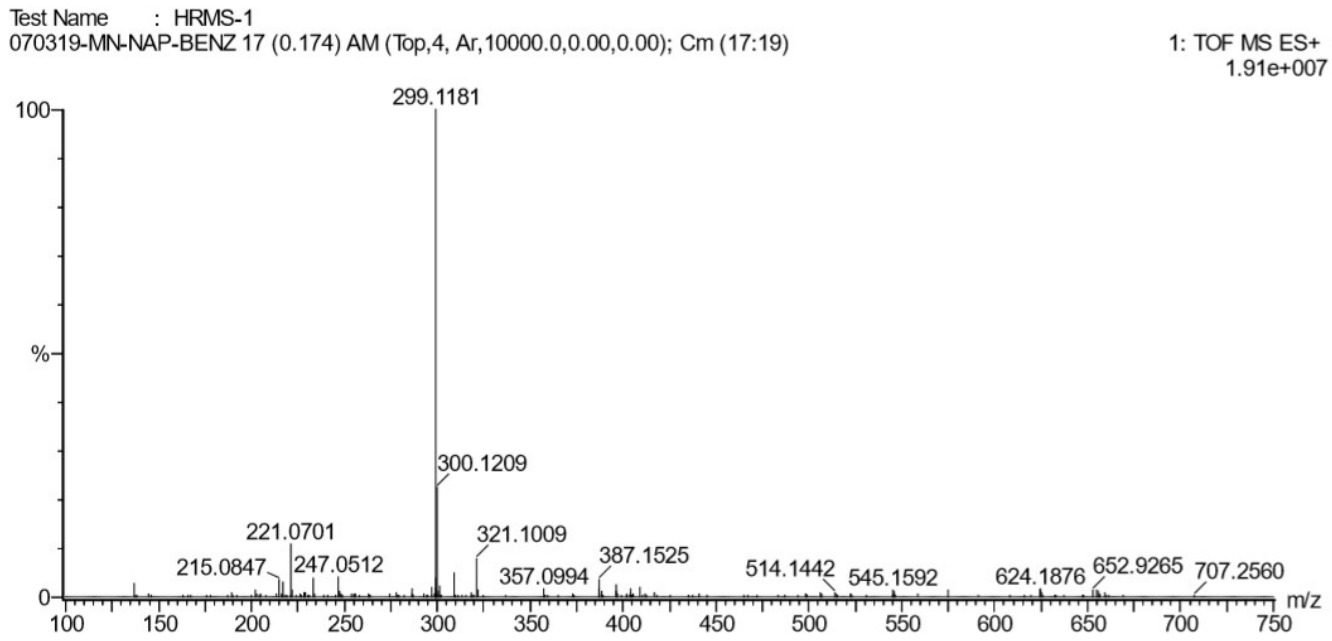


Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
359.1395	359.1396	-0.1	-0.3	14.5	674.0	n/a	n/a	C22 H19 N2 O3

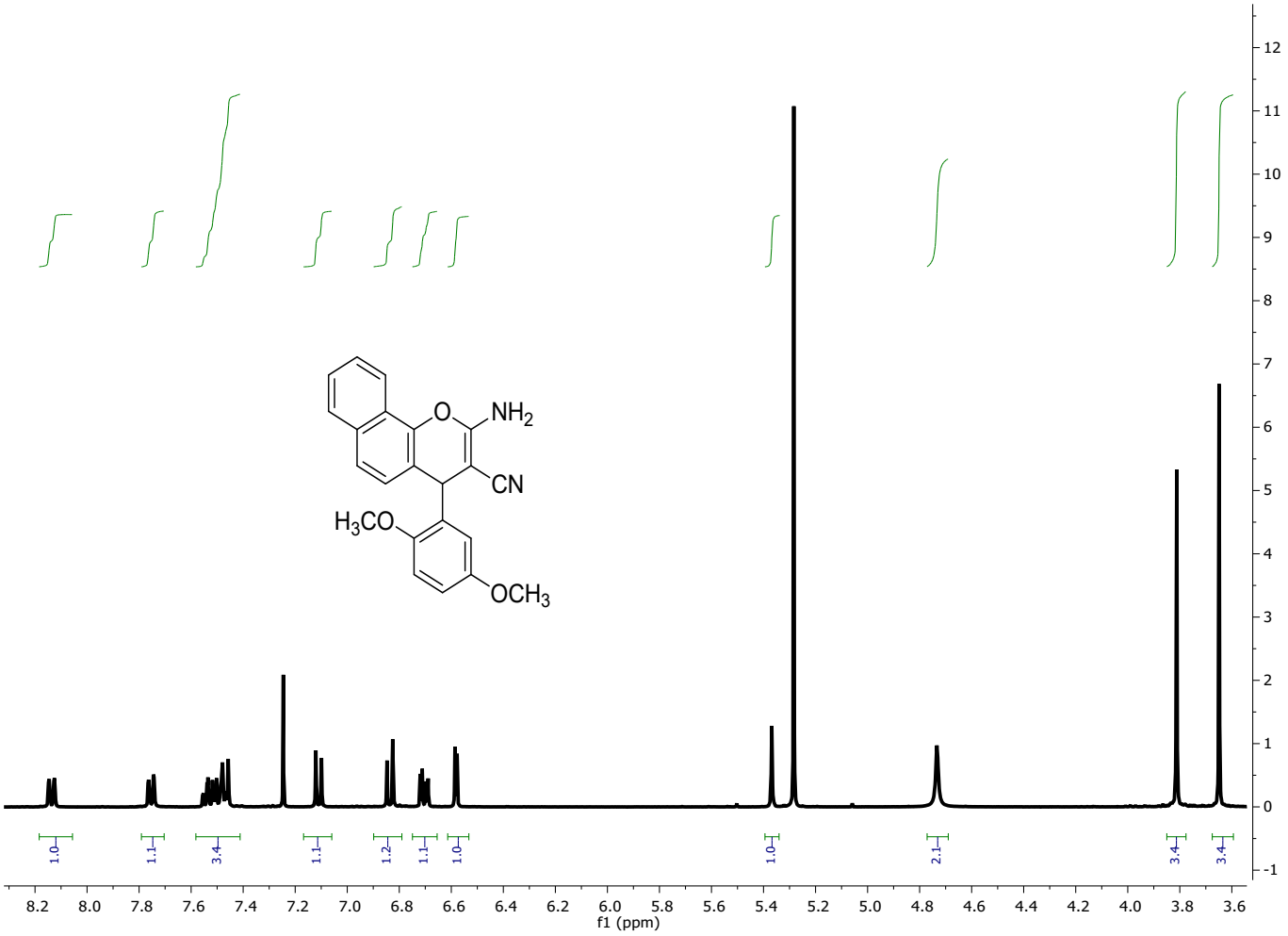
Figure SF7.^{1}H NMR and HRMS of MNC-4 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{22}H_{18}N_2O_2^+$ 359.13; found 359.1395)

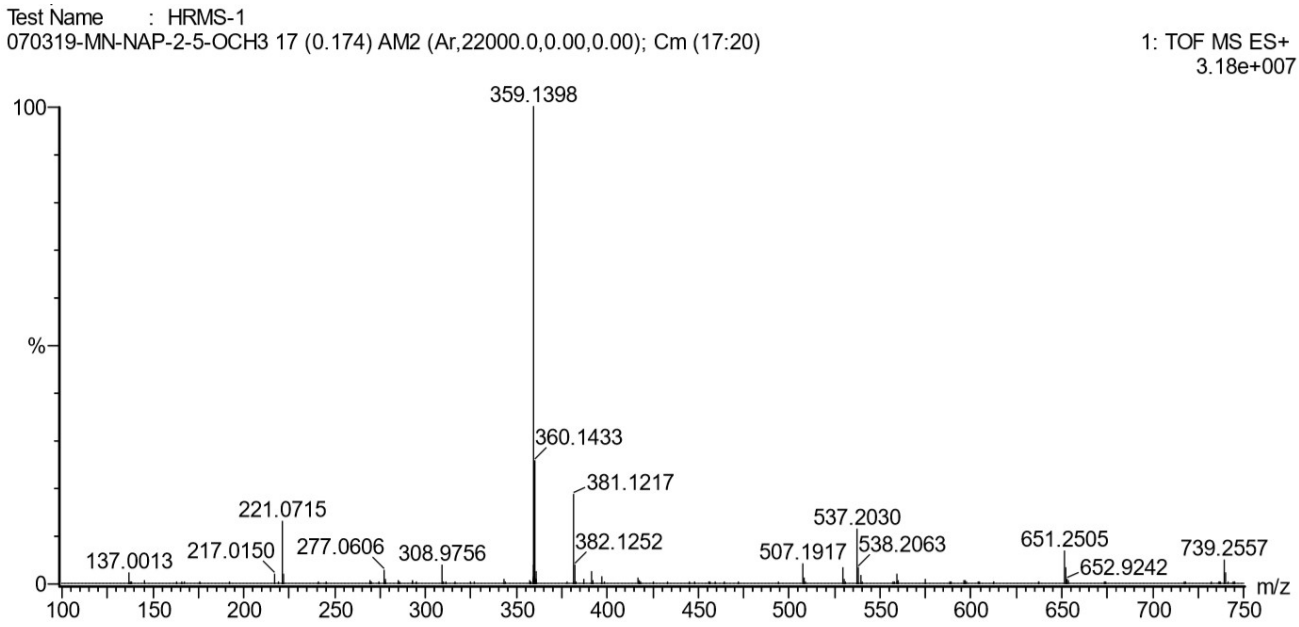




Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
299.1181	299.1184	-0.3	-1.0	14.5	753.6	n/a	n/a	C20 H15 N2 O

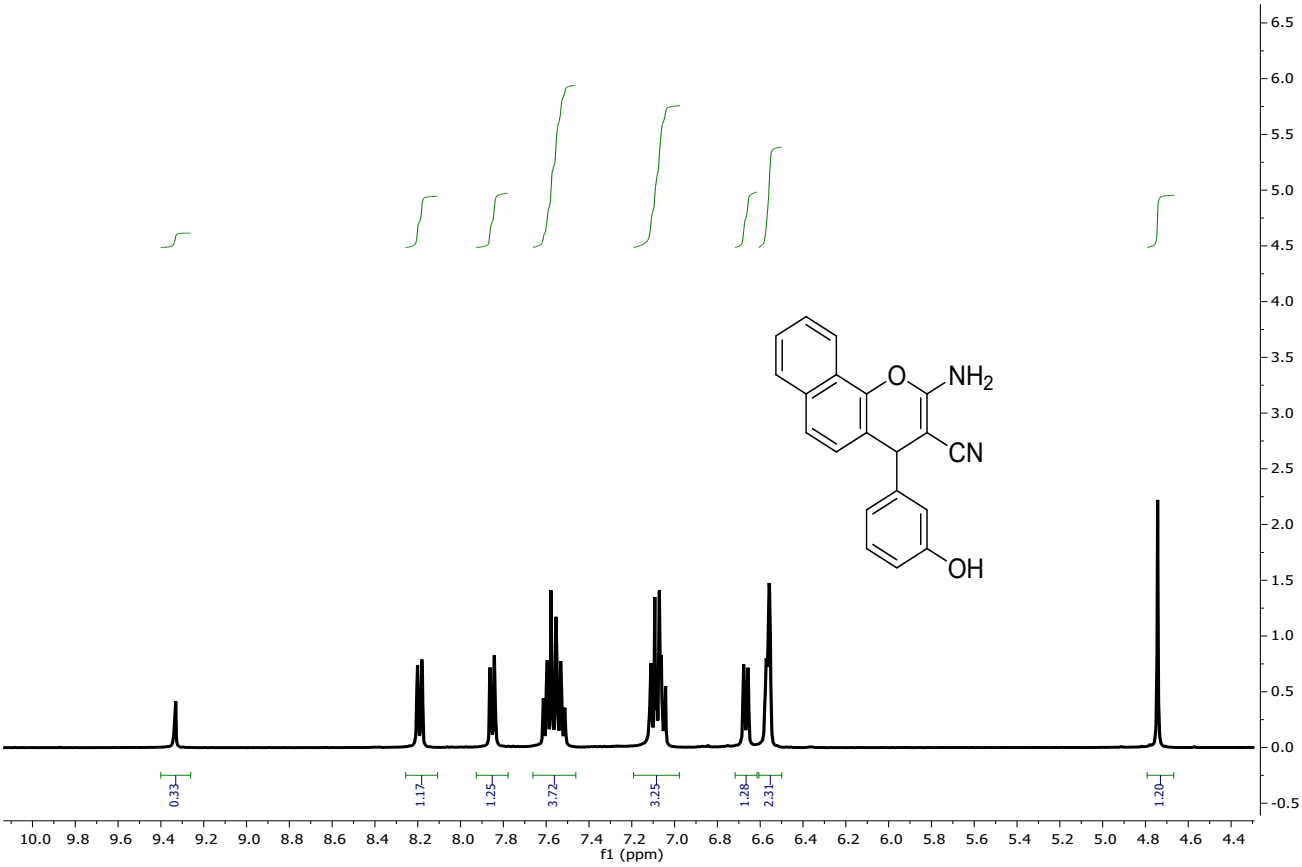
Figure SF8.^{1}H NMR and HRMS of MNC-5 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{14}N_2O^+$ 299.11; found 299.1181)

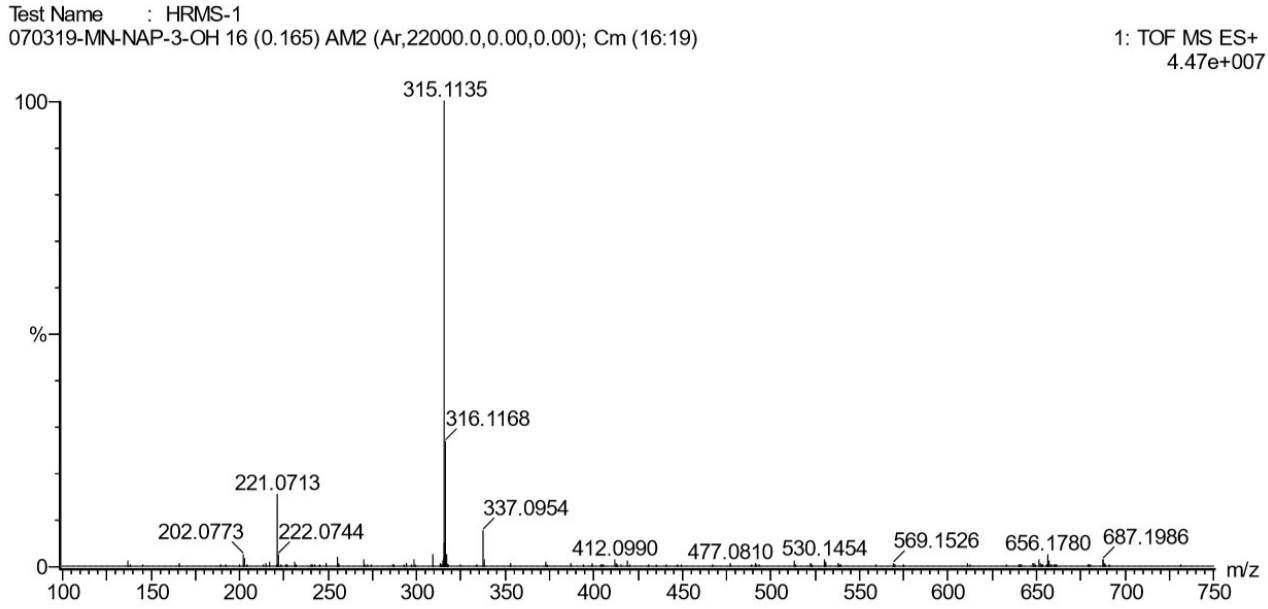




Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
359.1398	359.1396	0.2	0.6	14.5	735.2	n/a	n/a	C22 H19 N2 O3

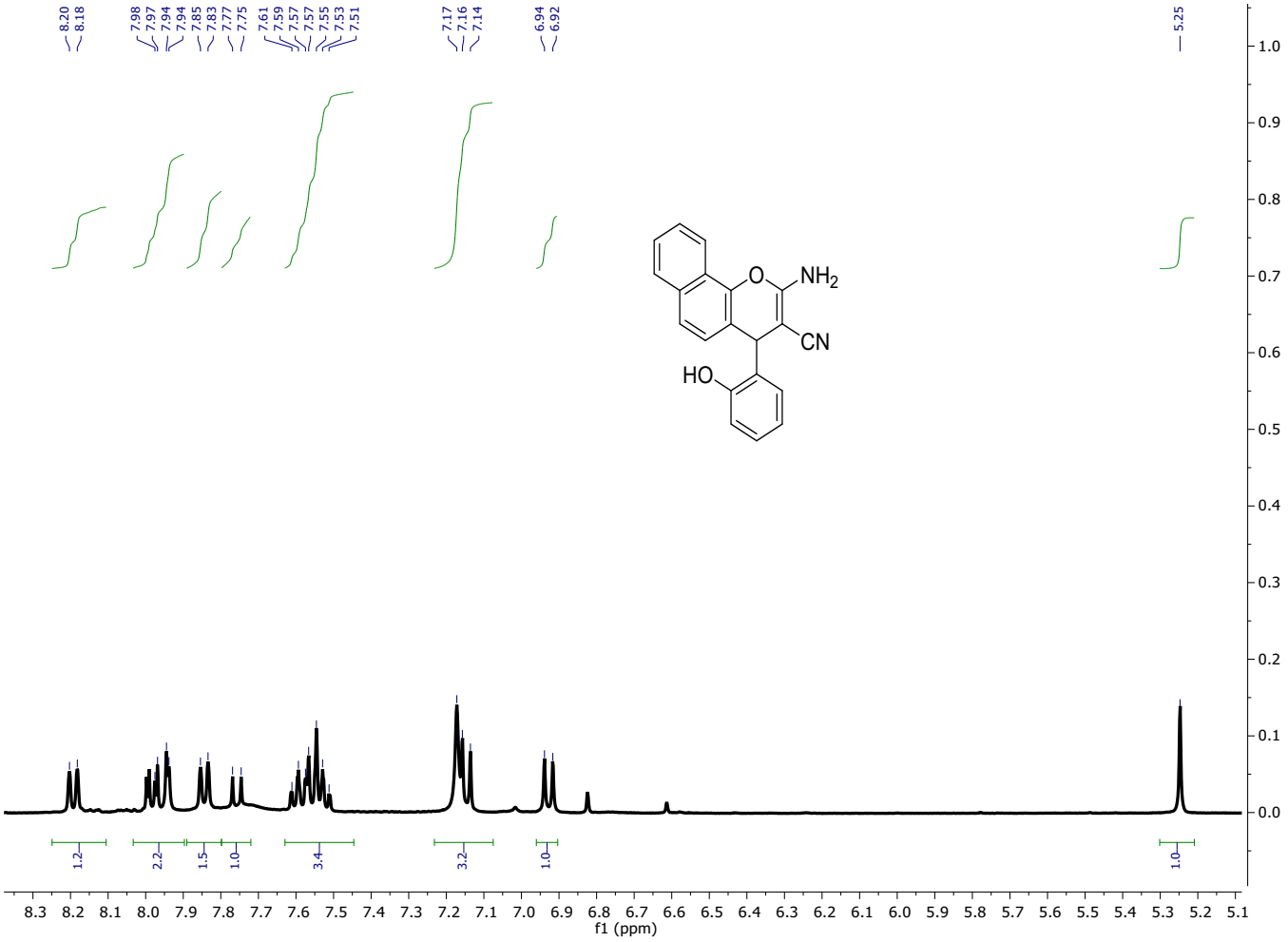
Figure SF9.^{1}H NMR and HRMS of MNC-6 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{22}H_{18}N_2O_3^+$ 359.13; found 359.1398)

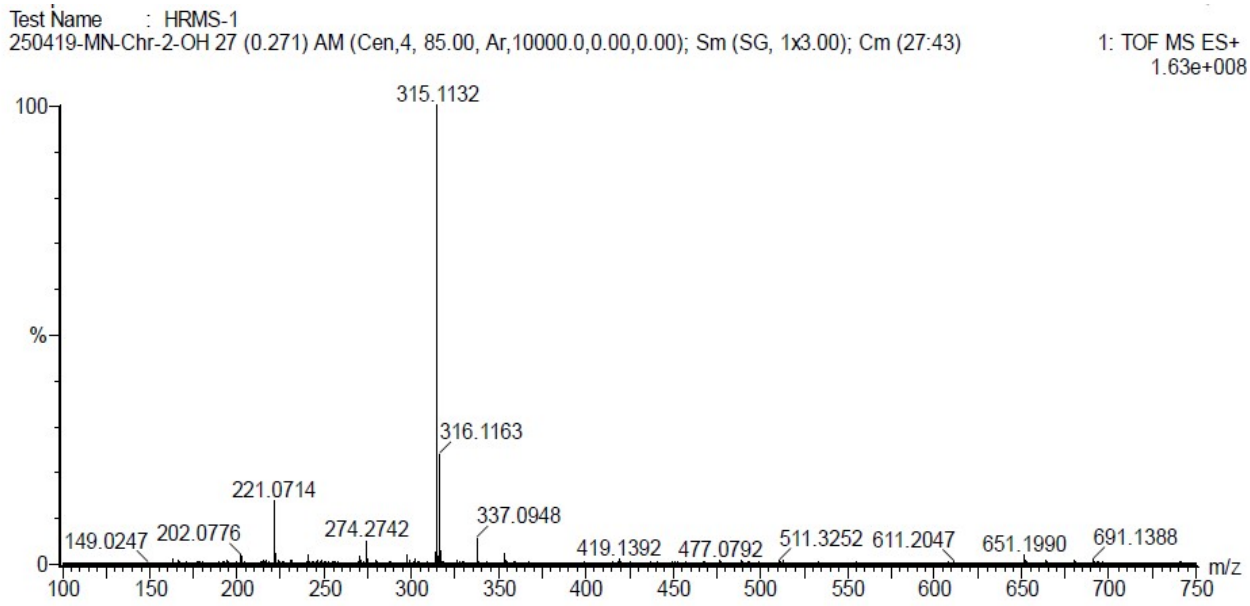




Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
315.1135	315.1134	0.1	0.3	14.5	783.1	n/a	n/a	C20 H15 N2 O2

Figure SF10.^{1}H NMR and HRMS of MNC-7 used in the study (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{14}N_2O_2^+$ 315.11; found 315.1135)

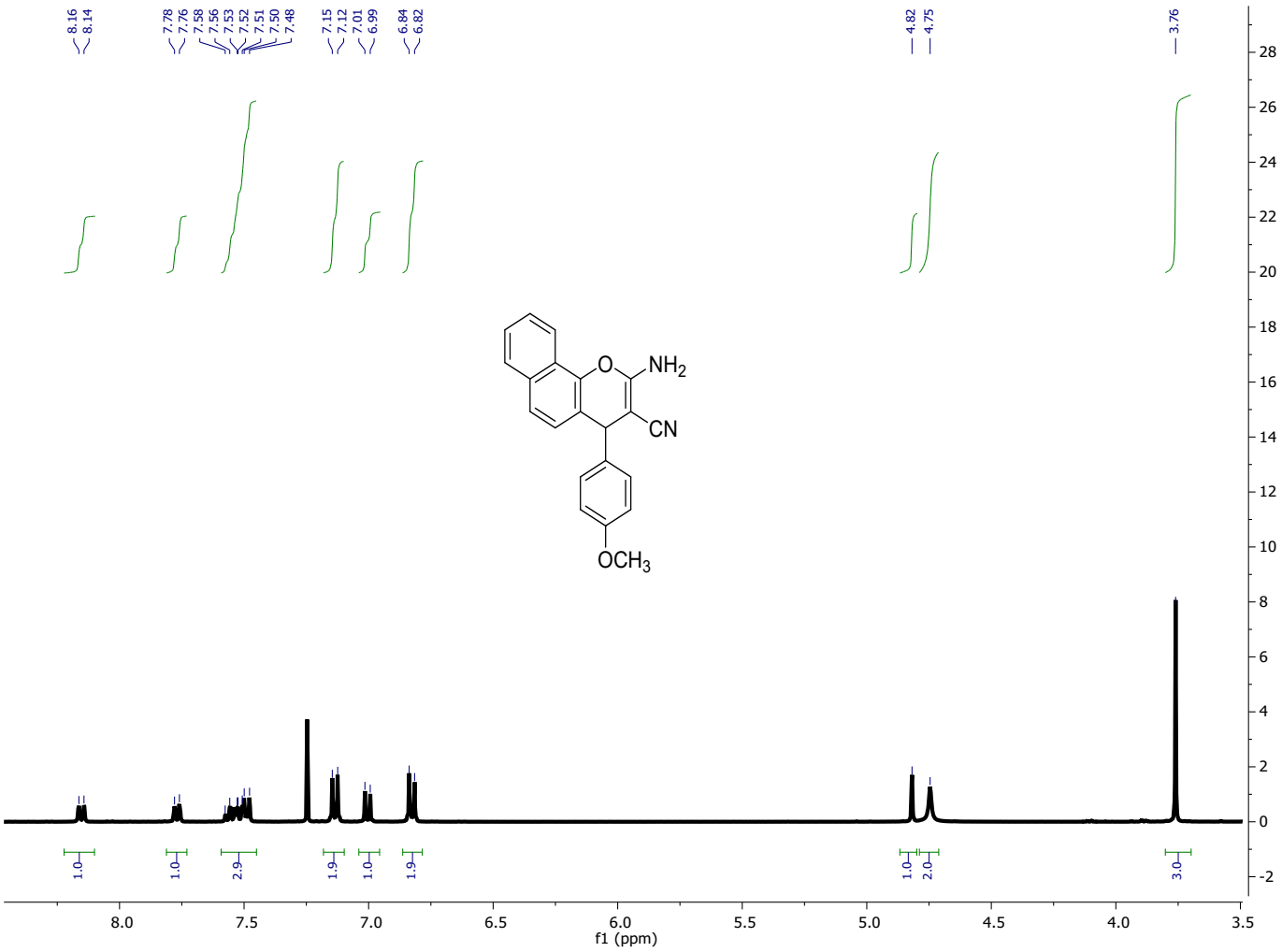


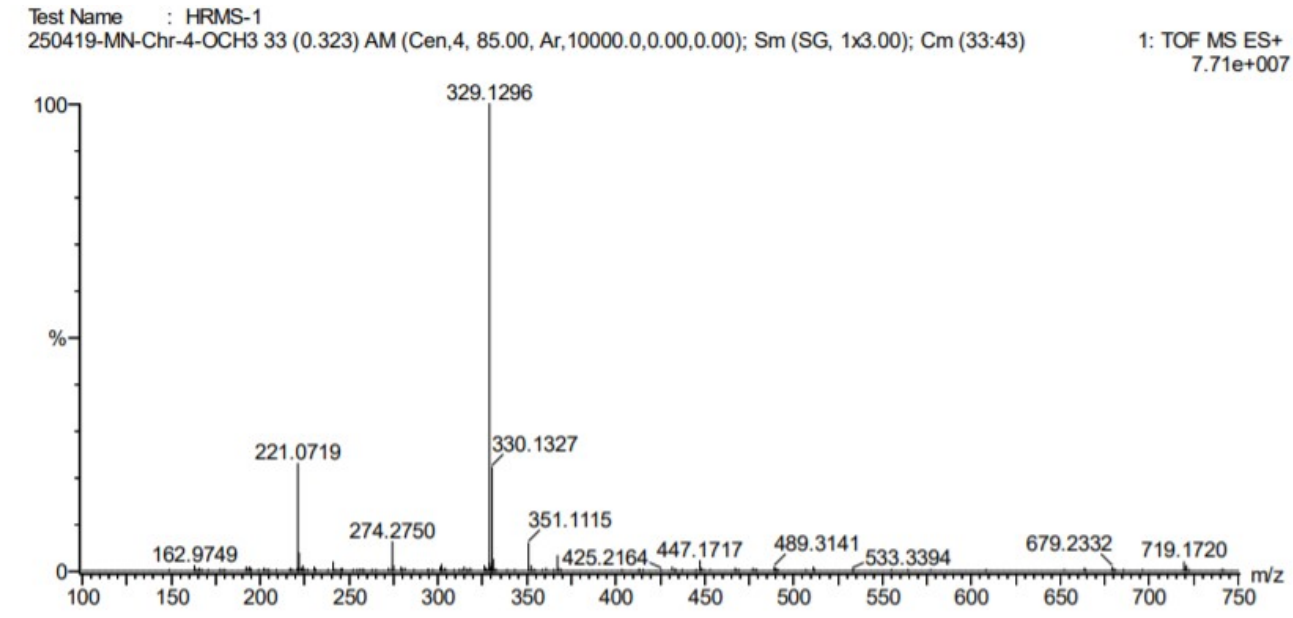


Minimum: -1.5
Maximum: 5.0 5.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
315.1132	315.1134	-0.2	-0.6	14.5	984.4	n/a	n/a	C20 H15 N2 O2

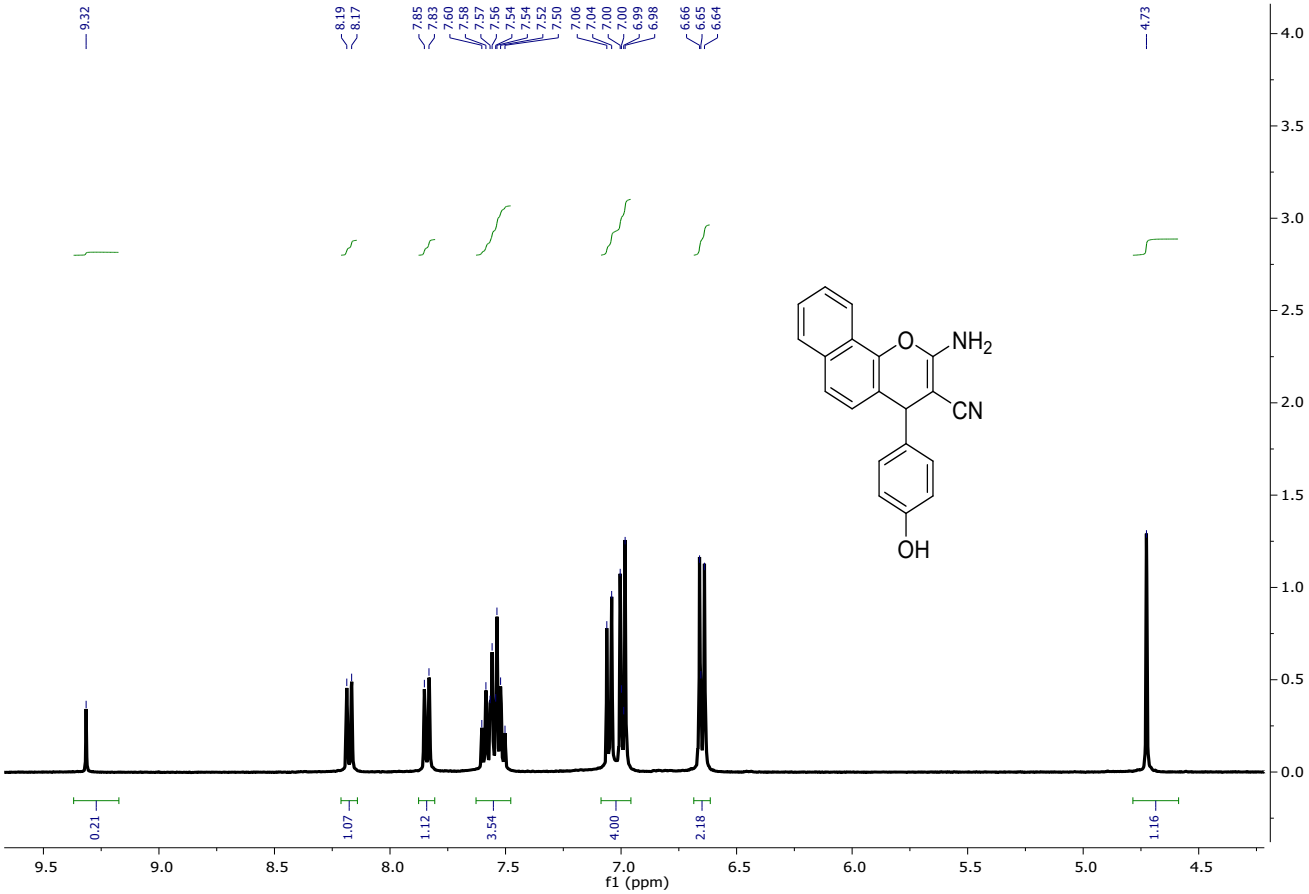
Figure SF11.^{1}H NMR of MNC-8 used in the study (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{14}N_2O_2^+$ 315.34; found 315.1132)

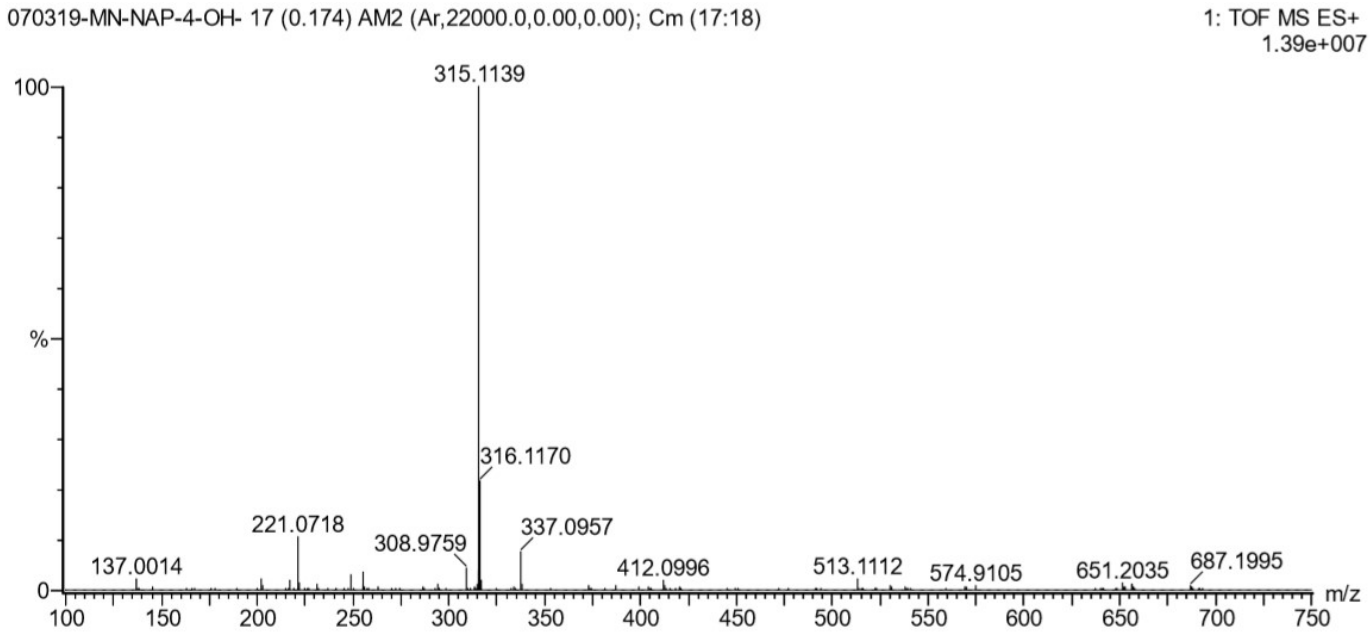




Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
329.1296	329.1290	0.6	1.8	14.5	956.4	n/a	n/a	C21 H17 N2 O2

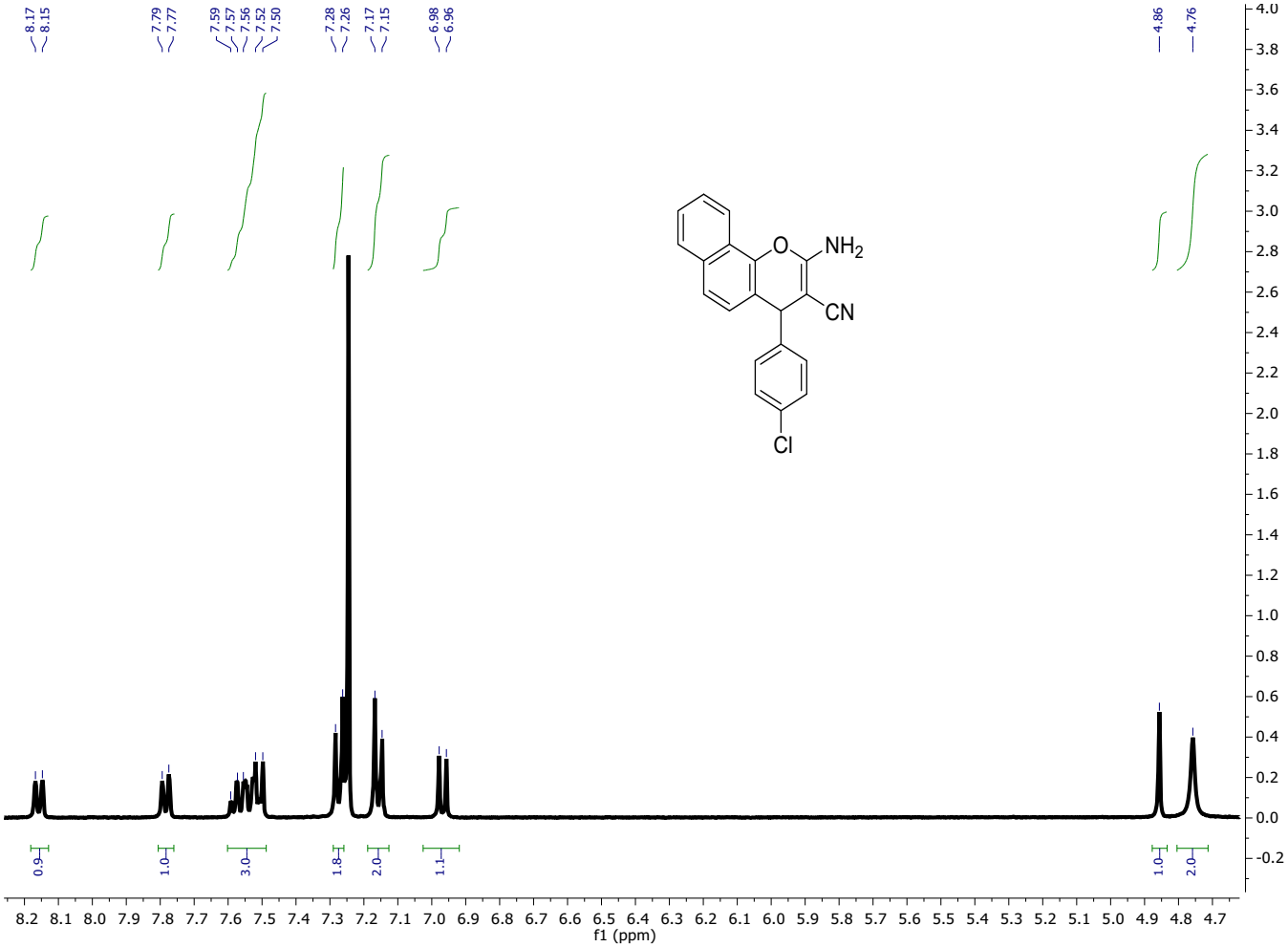
Figure SF12.^{1}H NMR of MNC-9 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{21}H_{16}N_2O_2^+$ 339.120; found 329.1296)

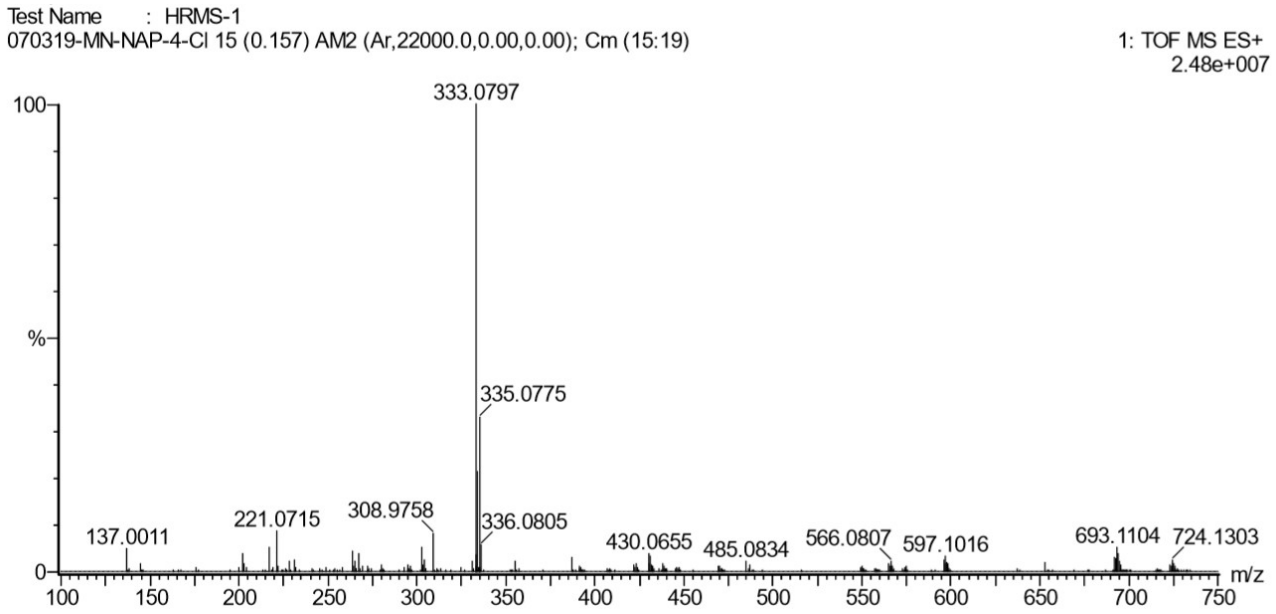




Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
315.1139	315.1134	0.5	1.6	14.5	715.4	n/a	n/a	C20 H15 N2 O2

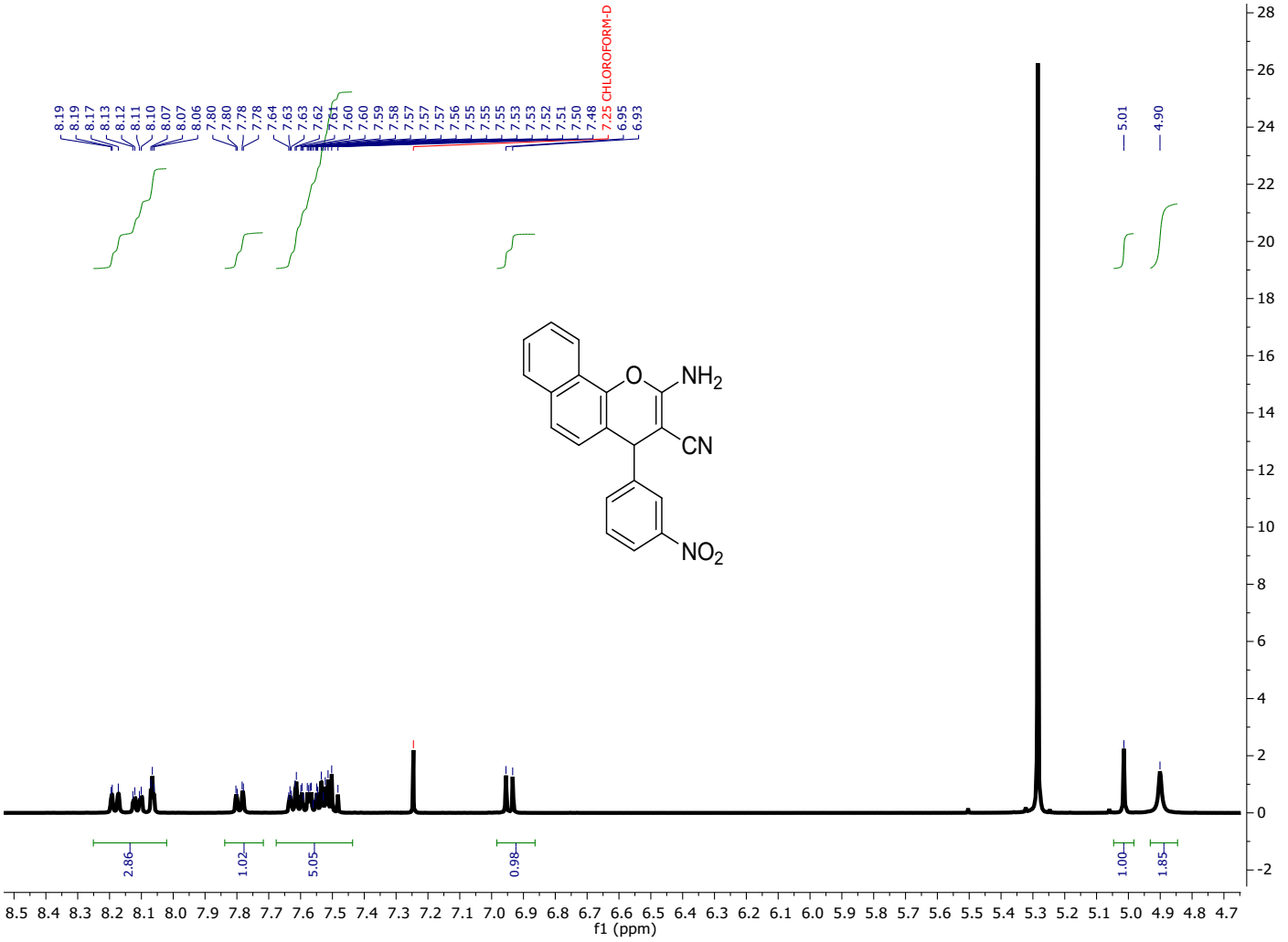
Figure SF13.[1]H NMR and HRMS of MNC-10 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{14}N_2O_2^+$ 315.110; found 315.1139)





Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
333.0797	333.0795	0.2	0.6	14.5	706.3	n/a	n/a	C20 H14 N2 O Cl

Figure SF14.^{1}H NMR and HRMSof MNC-11 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{13}ClN_2O^+$ 333.070; found 333.0797)



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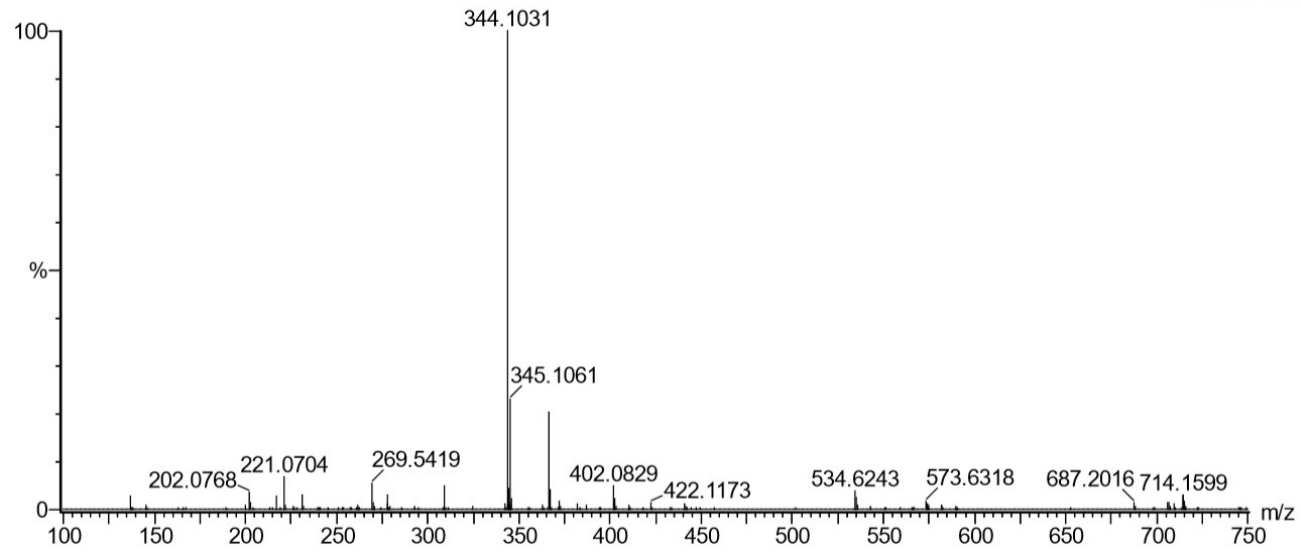
3.06e+007



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
344.1031	344.1035	-0.4	-1.2	15.5	790.5	n/a	n/a	C20 H14 N3 O3

Figure SF15.^{1}H NMR of MNC-12 used in the study, (For HRMS m/z: $[M+H]^+$ calcd for $C_{20}H_{13}N_3O_3^+$ 344.100; found 344.104)

Crystal growth and structural analysis

The crystals of the compound MNC-10 were grown by slow evaporation technique at ambient temperature. SCXRD experiments of MNC-1 single crystal was performed on a Bruker AXS KAPPA APEX-II CCD diffractometer (Monochromatic Mo Kα radiation). Unit cell determination, data collection was done at 296.0 K and data reduction were made using the Bruker APEX-III package. The crystal structures were solved using Olex[2] package equipped with XT[3] and were further refined using XL. Crystal diagram was created using Mercury software. The details of the single-crystal X-ray diffraction data collection, structure solution and refinement are given in Table 1.

Table ST1: Crystallographic table of MNC-1

Data/Salt	**MNC-1**
Empirical formula	$C_{20}H_{14}N_2O_2$
CCDC number	2236979
Formula weight	314.33
Crystal system	Monoclinic
Space group	$P2_1/c$
a(Å)	13.4145(5)
b(Å)	10.6112(4)
c(Å)	11.6042(3)
α(°)	90
β(°)	109.3990(10)
γ(°)	90
V(Å^3)	1558.01(9)
Z	4
ρ_{calc}(g/cm^{-3})	1.340
Temperature (K)	296.0(2)
μ/ mm^{-1}	0.088
$2\theta_{min,\ max}$ ()	5.01 to 56.694
F (000)	656.0
$h_{min,max}$; $k_{min,max}$; $l_{min,max}$	-17, 17; -14, 14; -15, 15
Total no. of reflections	40495
R_{int}	0.0494
No. of unique reflections	3883
R_1 [I>2σ(I)]	0.0472
wR2 (all data)	0.1232
GooF on F^2	1.051
$\Delta\rho_{max,min}$/eÅ^{-3}	0.14/-0.23

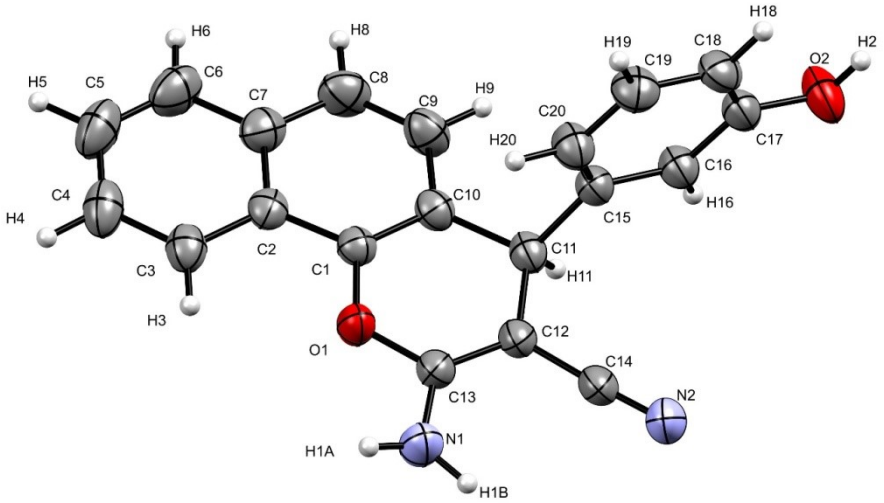


Figure SF16: Asymmetric unit of MNC-1 (Thermal ellipsoid probability 50%)

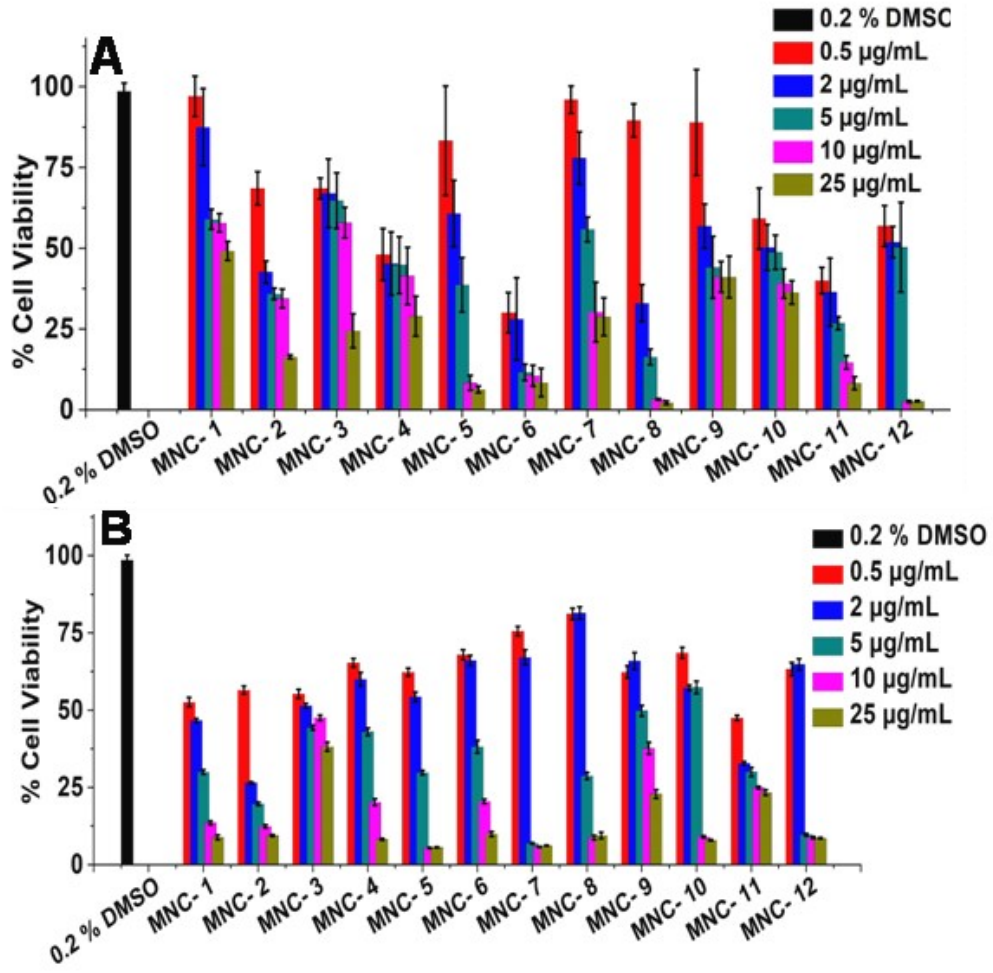


Figure SF17. Anticancer activity of chromene derivatives as obtained for HeLa (**A)** and A549 (B) cancer cell lines.

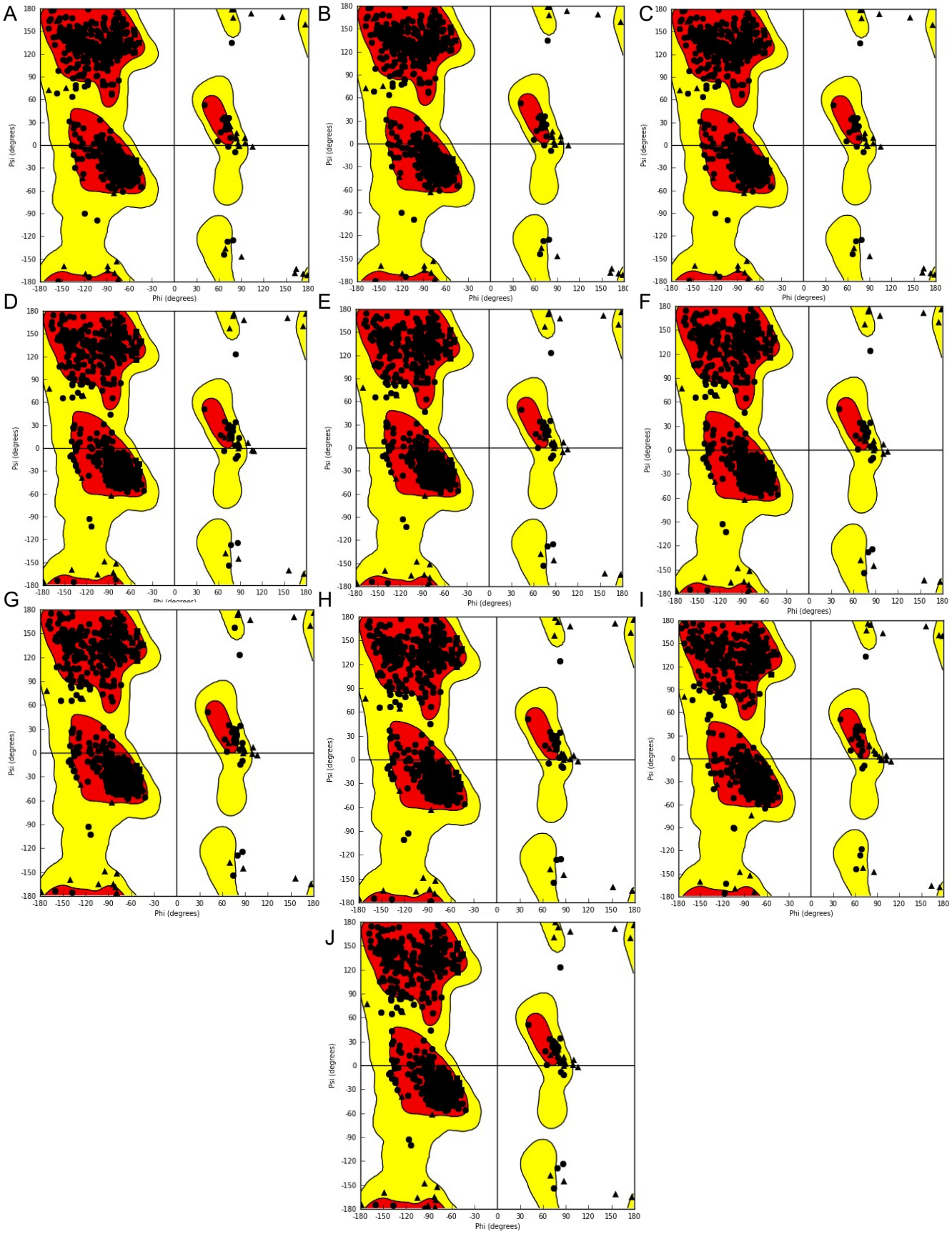


Figure SF18. A-J, Ramachandran plots for microtubule isoform BI, BIIa, BIIb, BIII, BIVa, BIVb, BV, BVI, BVIII, and BVIII respectively.

Table ST2: Ramachandran plot analysis revealing the percentage of total amino acid residues present in favorable, allowed, and outlier regions in the case of all the ten isoforms.

S. No	Isoform	Residues Percentage		
		Favorable region	Allowed Region	Outliers region
1	BI	97.8%	1.8%	0.3%
2	BIIa	97.7%	2.0%	0.3%
3	BIIb	97.7%	2.0%	0.3%
4	BIII	96.4%	3.1%	0.5%
5	BIVa	96.4%	3.1%	0.5%
6	BIVb	96.7%	2.9%	0.5%
7	BV	96.5%	3.0%	0.5%
8	BVI	96.3%	3.3%	0.3%
9	BVII	97.0%	2.8%	0.2%
10	BVIII	96.7%	3.0%	0.3%

Table ST3: Binding position of all the studied molecules in the case of tubulin isoforms.

CODE	R/S Configuration	S/D* Binding pattern compounds against each isoform									
		B1	BIIA	BIIb	BIII	BIVa	BIVb	BV	BVI	BVII	BVIII
MNC-1	R	S	S	D	S	S	S	S	S	S	S
	S	D	S	D	S	S	D	S	D	D	D
MNC-2	R	S	D	S	S	S	S	S	S	D	D
	S	S	S	D	D	S	S	S	S	S	S
MNC-3	R	D	D	S	D	S	D	S	S	S	S
	S	S	S	D	D	S	S	D	S	S	D
MNC-4	R	D	D	S	D	S	S	D	S	D	S
	S	D	D	S	S	S	S	S	S	S	S
MNC-5	R	D	S	S	S	S	D	S	S	S	S
	S	S	S	S	S	S	S	S	S	S	S
MNC-6	R	S	S	S	S	S	S	D	S	S	S
	S	D	D	S	S	S	S	D	S	S	S
MNC-7	R	S	S	S	S	S	S	S	S	S	S
	S	S	S	D	S	S	S	D	S	S	S
MNC-8	R	S	S	D	D	S	S	S	S	S	S
	S	D	S	D	D	D	D	D	D	S	D
MNC-9	R	D	D	S	D	S	S	S	S	S	S
	S	D	D	S	S	S	S	S	S	S	S
MNC-10	R	S	S	S	S	S	S	S	D	S	S
	S	S	S	S	D	S	S	D	D	S	D
MNC-11	R	D	D	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	D	S	S	S
MNC-12	R	D	D	D	S	S	D	S	S	D	S
	S	S	D	D	S	S	D	D	D	S	D

***S =** Binding of compounds at the same site where colchicine binds or some degree of overlapping, **D** = Binding of compound at the site different from colchicine binding site.

Table ST4: Maximum side-chain RMSF fluctuating residues of MNC-1 bounded microtubule isoforms as obtained from 50 ns of MD simulation studies.

β1		β 2b		β 2b		β 3		β 4a	
Residue Index	RMSF	Residue Index	RMSF	Residue Index	RMSF	Residue Index	RMSF	Residue Index	RMSF
873	5.11	718	9.171	440	8.384	440	5.896	440	14.855
440	4.999	440	6.292	439	6.022	283	5.522	438	12.973
40	4.836	722	6.048	283	5.01	717	5.059	439	12.648
282	4.227	283	5.935	285	4.883	40	4.88	721	11.97
283	4.037	720	5.818	438	4.515	873	4.264	163	11.947
81	3.828	723	5.66	873	4.474	723	4.257	718	11.704
163	3.697	719	5.445	40	4.431	718	4.247	661	11.35
285	3.456	724	5.43	870	4.274	285	4.244	345	11.243
42	3.44	721	5.123	718	4.266	724	4.102	720	11.158
84	3.435	717	4.733	284	4.126	282	3.996	660	10.996
β4b		β5		β6		β7		β8	
Residue Index	RMSF	Residue Index	RMSF	Residue Index	RMSF	Residue Index	RMSF	Residue Index	RMSF
42	7.197	440	6.835	440	15.136	873	8.595	722	7.385
40	6.444	283	6.127	438	14.738	872	6.488	723	7.305
43	6.195	40	4.813	439	14.079	283	5.982	721	6.538
283	6.023	439	4.747	349	13.993	440	5.913	724	6.431
440	5.946	41	4.681	724	13.836	722	5.719	1	5.488
41	5.304	42	4.625	721	13.654	718	5.115	725	4.367
44	5.302	285	4.545	720	13.547	723	5.075	748	4.333
46	5.099	438	4.528	725	13.153	720	4.834	718	4.068
724	4.61	39	4.428	437	13.066	439	4.625	2	3.906
38	4.423	539	4.286	660	12.709	721	4.469	720	3.787

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