SUPPORTING INFORMATION

Novel quinolinepiperazinyl-aryltetrazoles targeting blood stage *Plasmodium falciparum*

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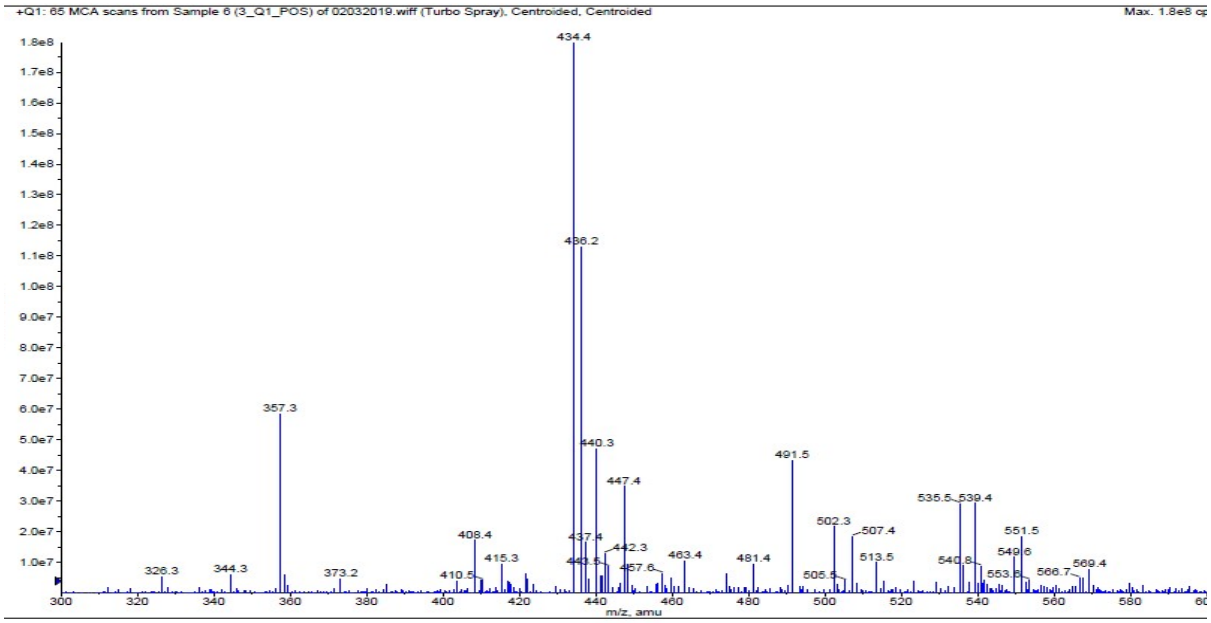
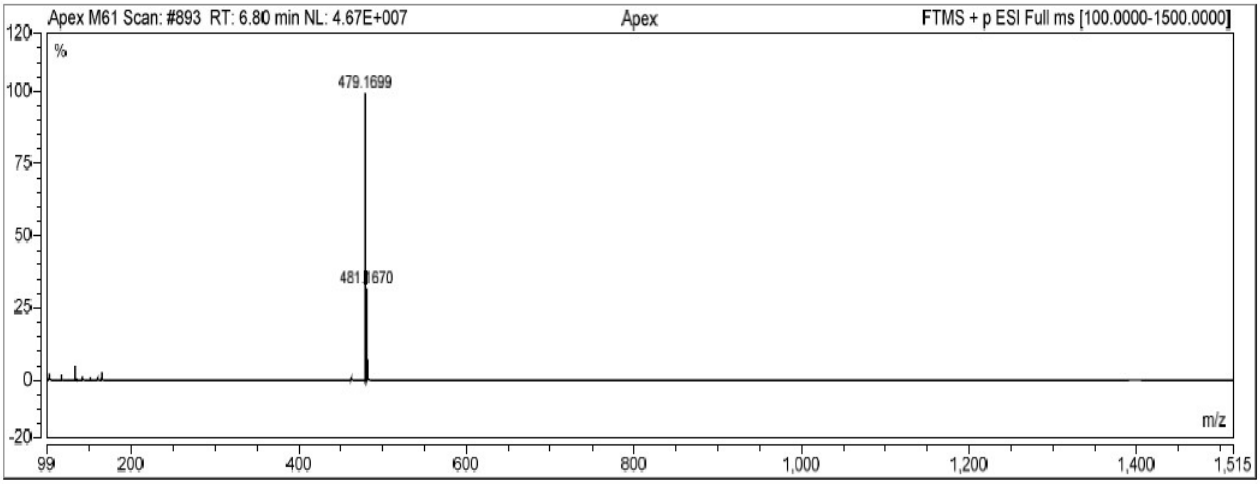
A. Characterization data of compounds

Mass spectrum of compound (**60**)

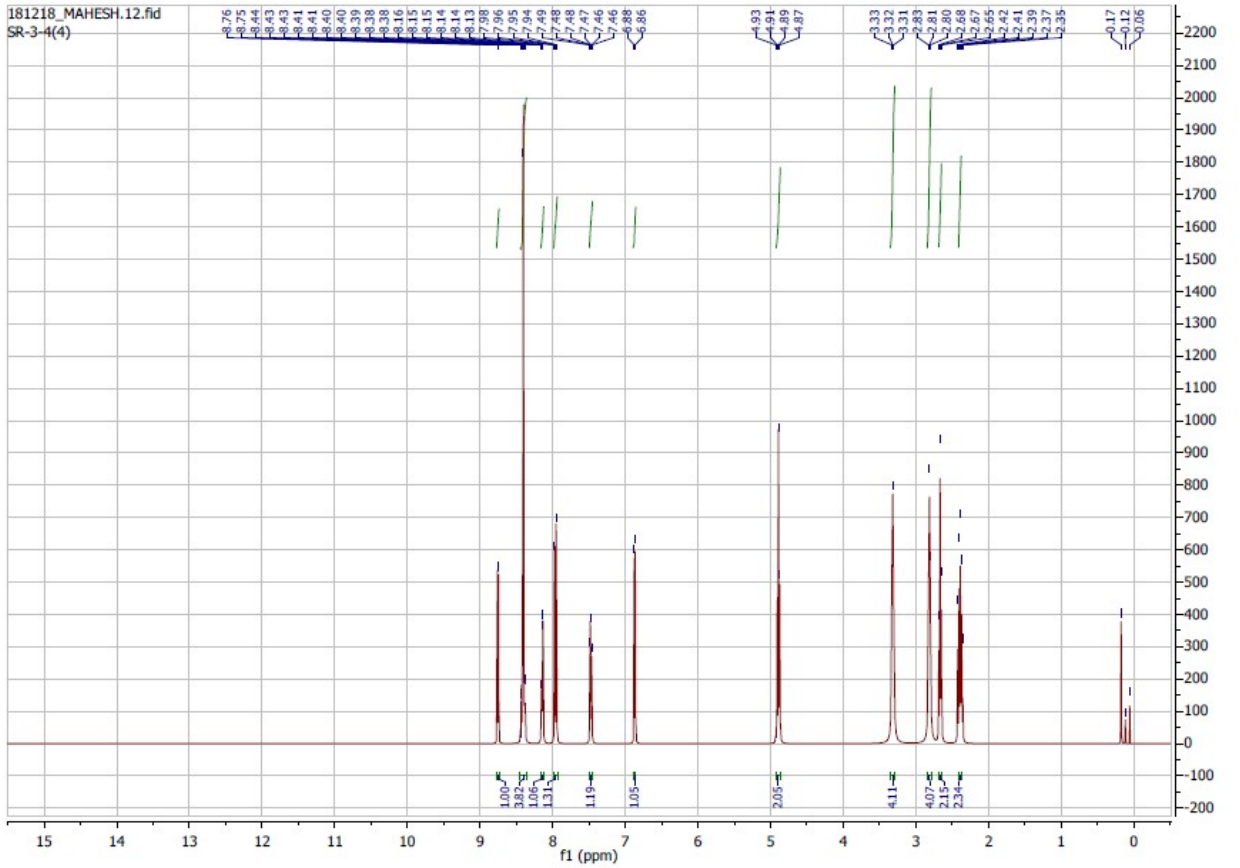


HRMS spectrum of compound (**61**):

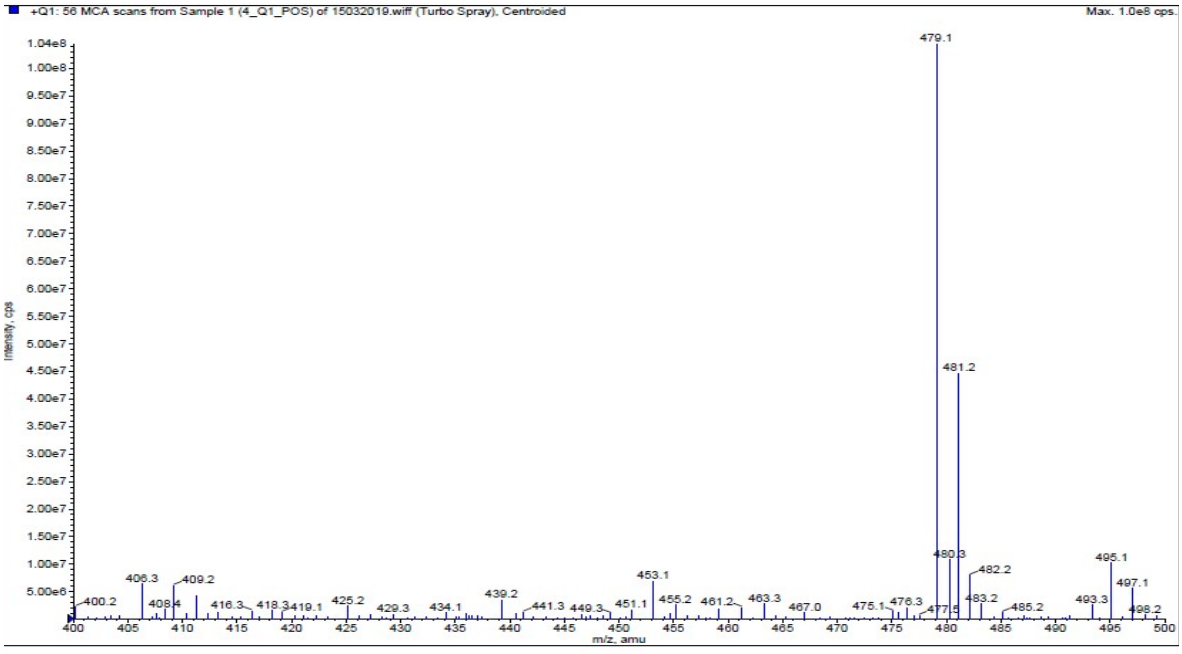
No.	Peak Name	Retention Time min	Area counts*sec	Height counts
10	M61	6.800	1206316077	46300948



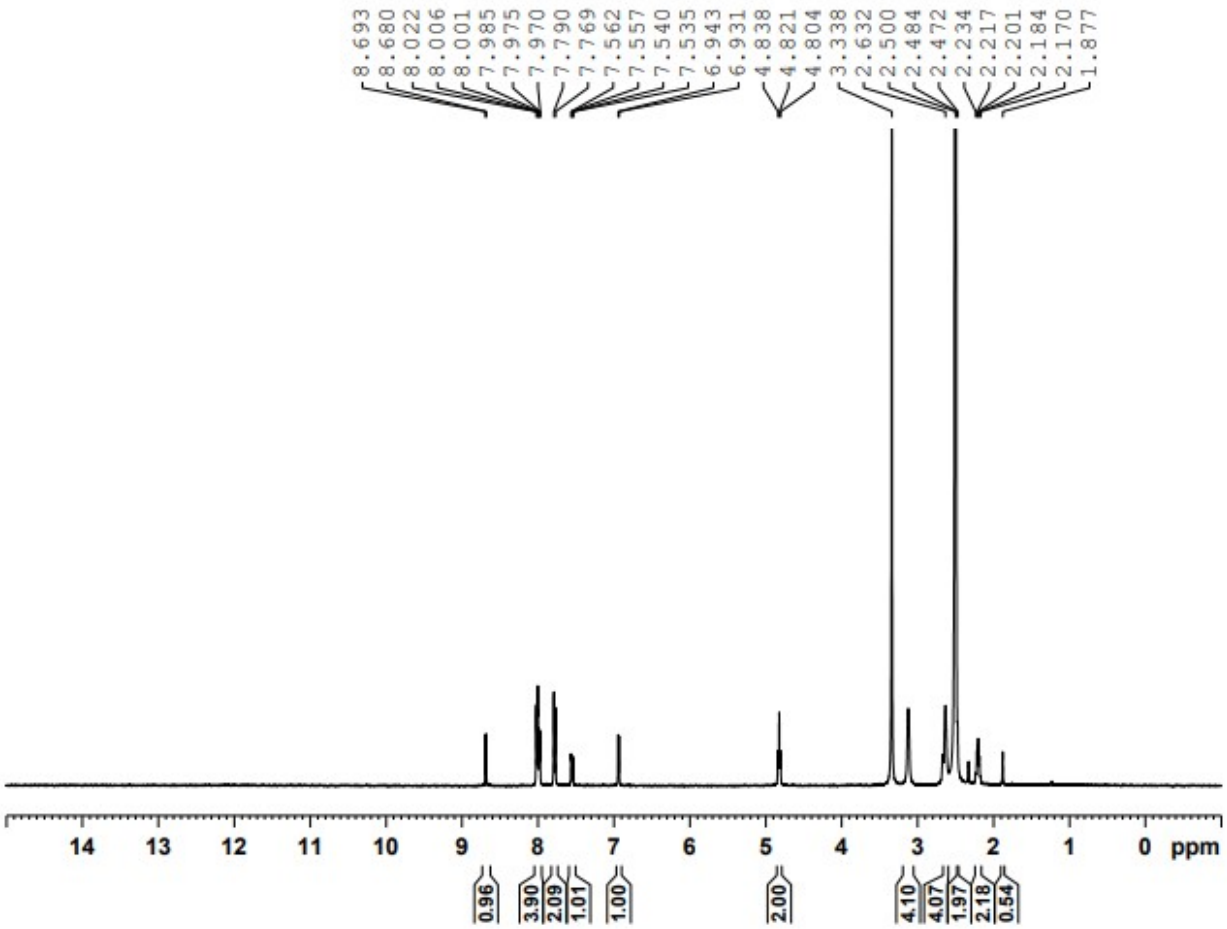
^{1}H-NMR of compound (**62**):



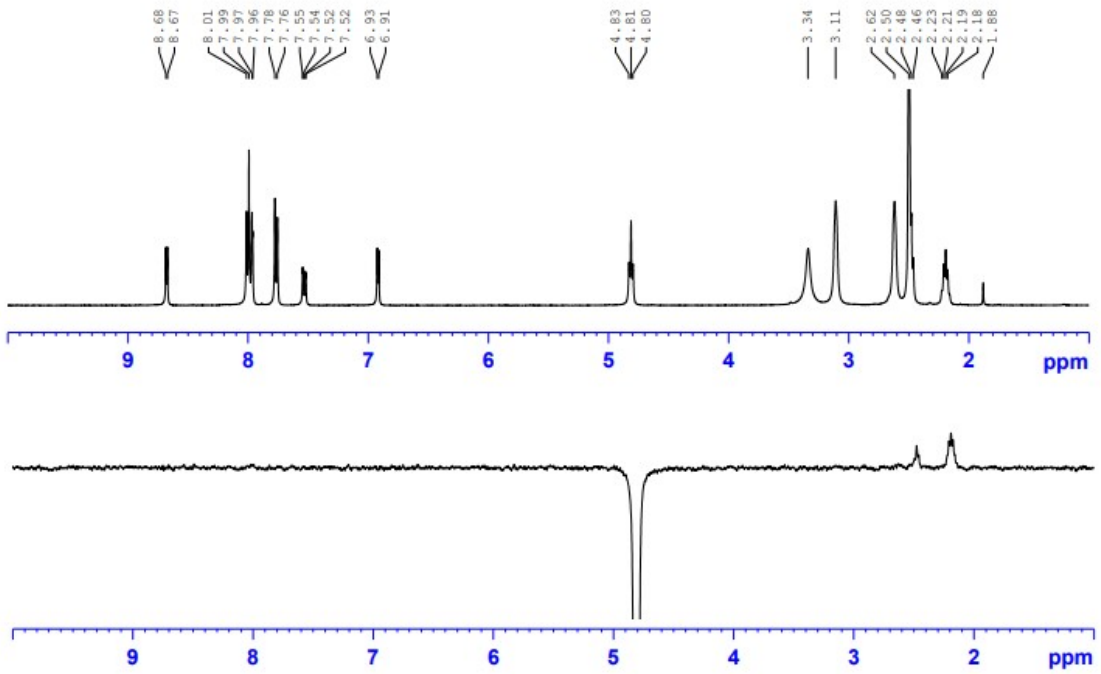
Mass spectrum of compound (**62**):



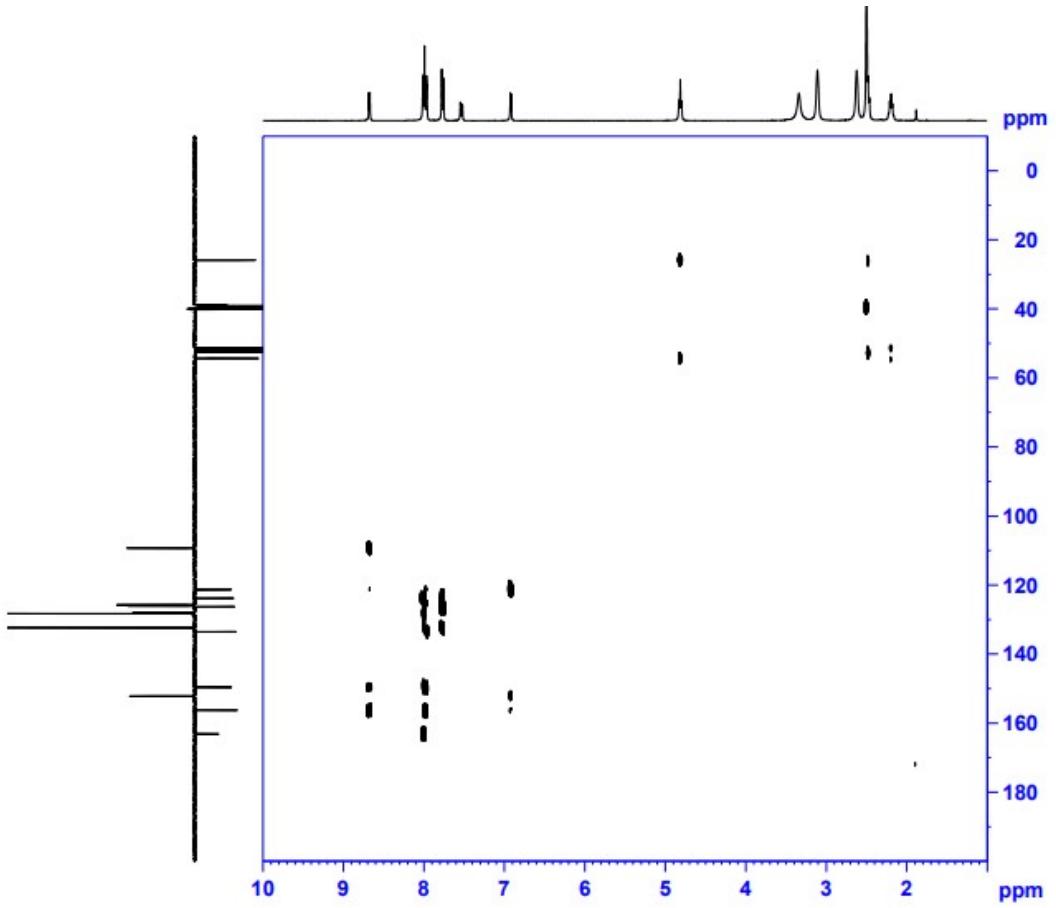
[1]H-NMR of compound (**66**):



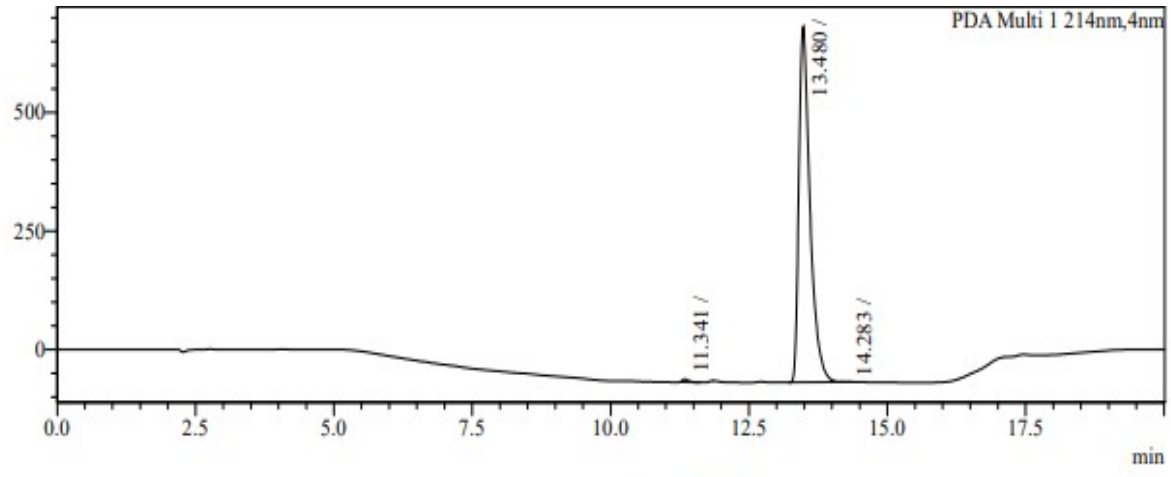
Selective Gradient NOSEY spectra of compound (**66**)**:**



HMBC spectrum of compound (**66**):



HPLC chromatogram and purity data of compound (**66**):

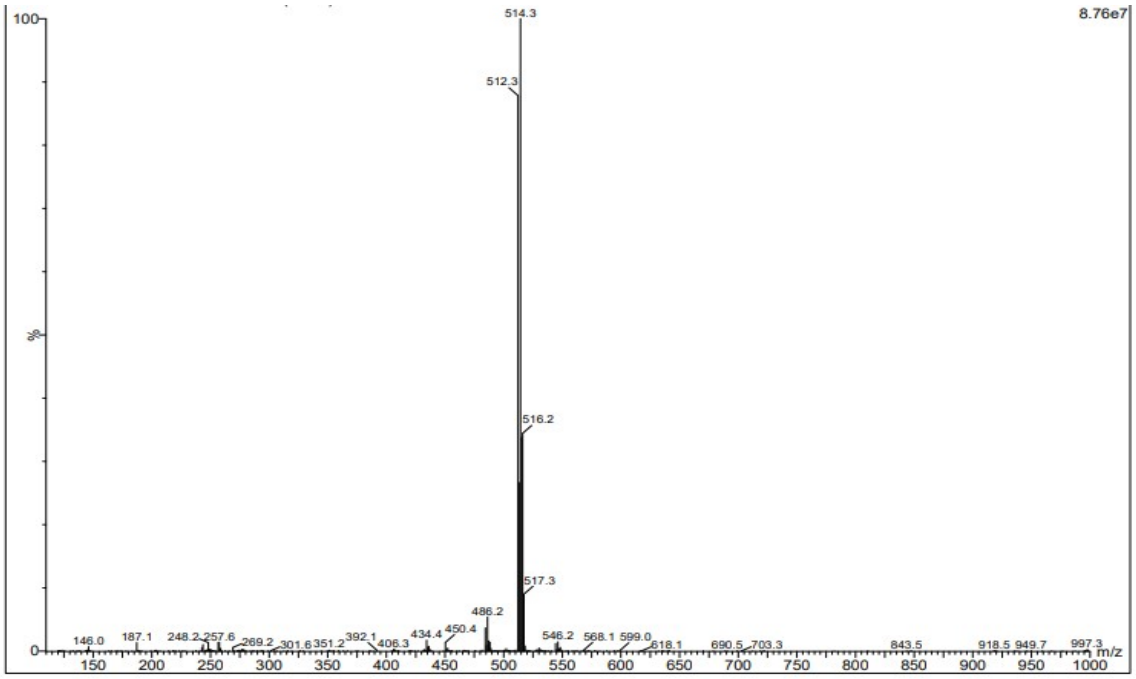


Peak Table

PDA Ch1 214nm

Peak	Ret. Time	Area	Area%
1	11.34	44864	0.42
2	13.48	10524411	99.47
3	14.28	10843	0.10
Total		10580118	100.00

UPLC-MS spectrum of compound (**66):**



HRMS spectrum of compound (**66**):

Sample Information

Item name: M1179-J04051-015-Pk2

Sample position: 1:A,5

Acquisition Method Name: HRMS

Injection volume: 0.50 µL

Analysis Information

Sample Set Created date: Nov 06, 2023 12:52:24 IST

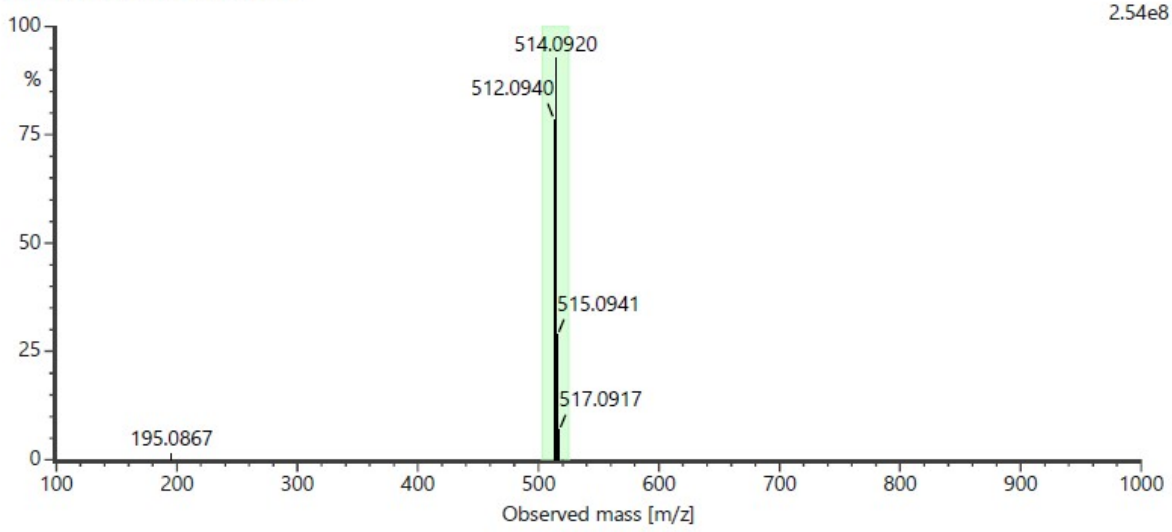
Acquisition start time: Nov 06, 2023 13:48:43 IST

ESI+ve

Item name: M1179-J04051-015-Pk2

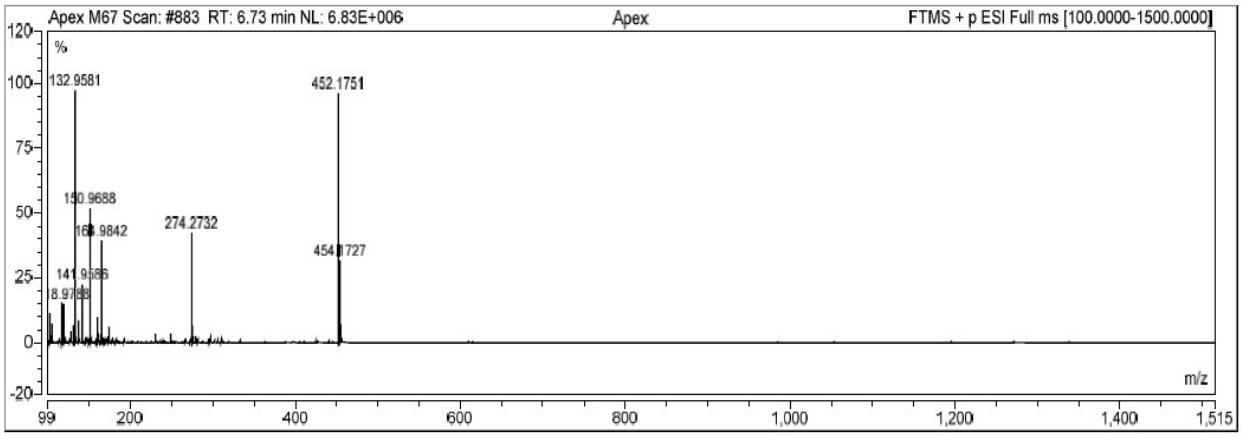
MS retention time (min): 0.1197

2.54e8

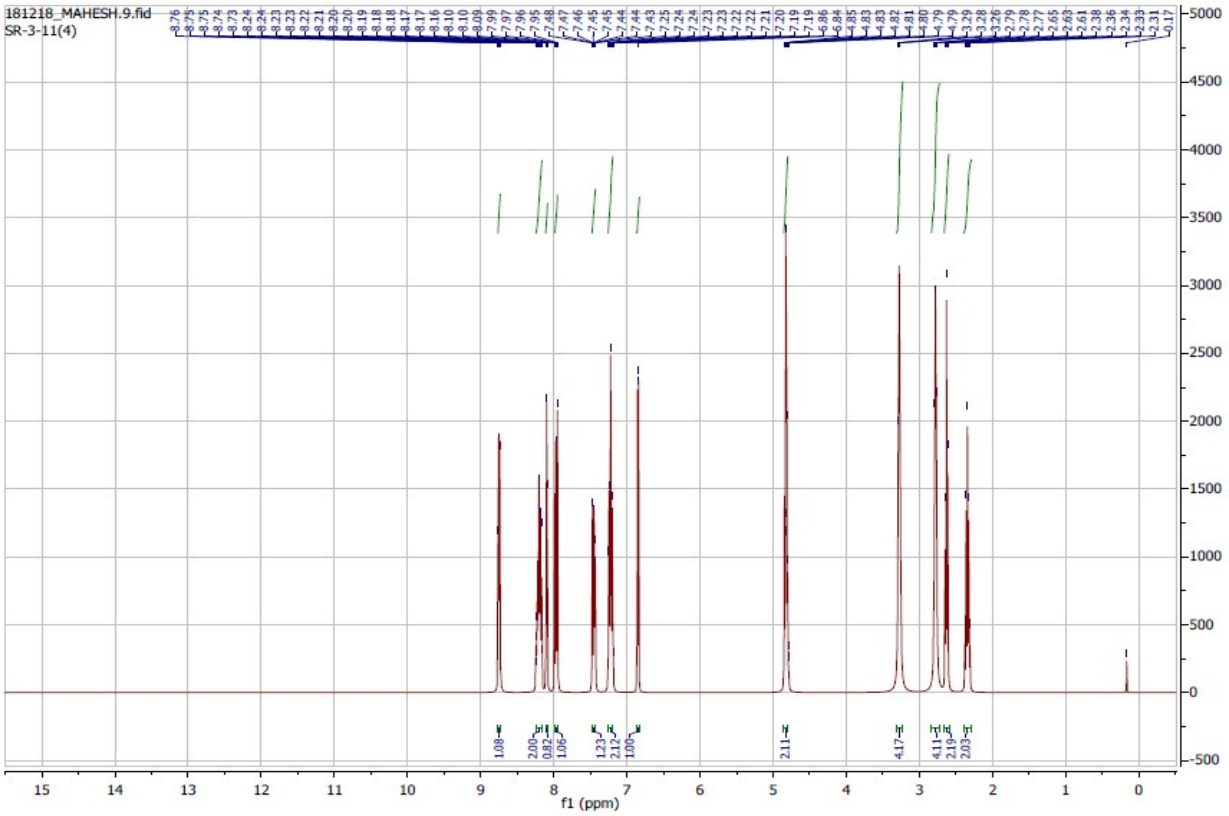


HRMS spectrum of compound (**67**):

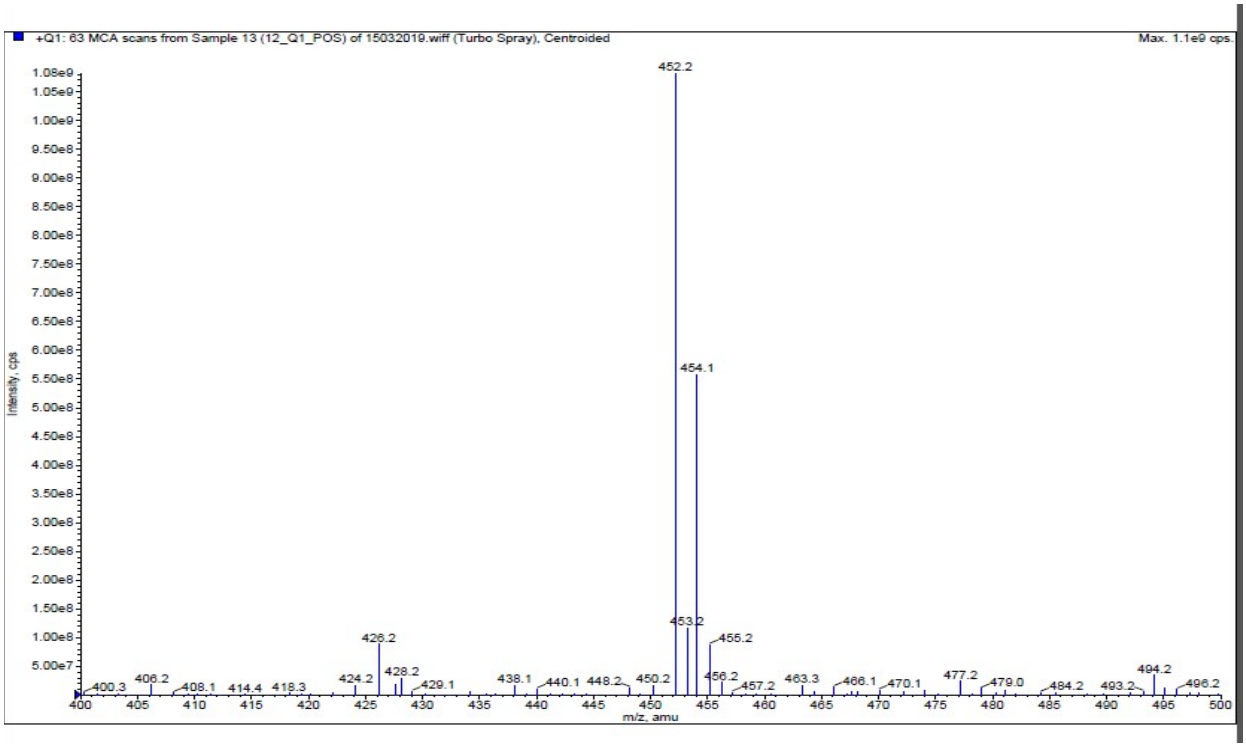
No.	Peak Name	Retention Time min	Area counts*sec	Height counts
2	M67	6.727	158589494	6593002



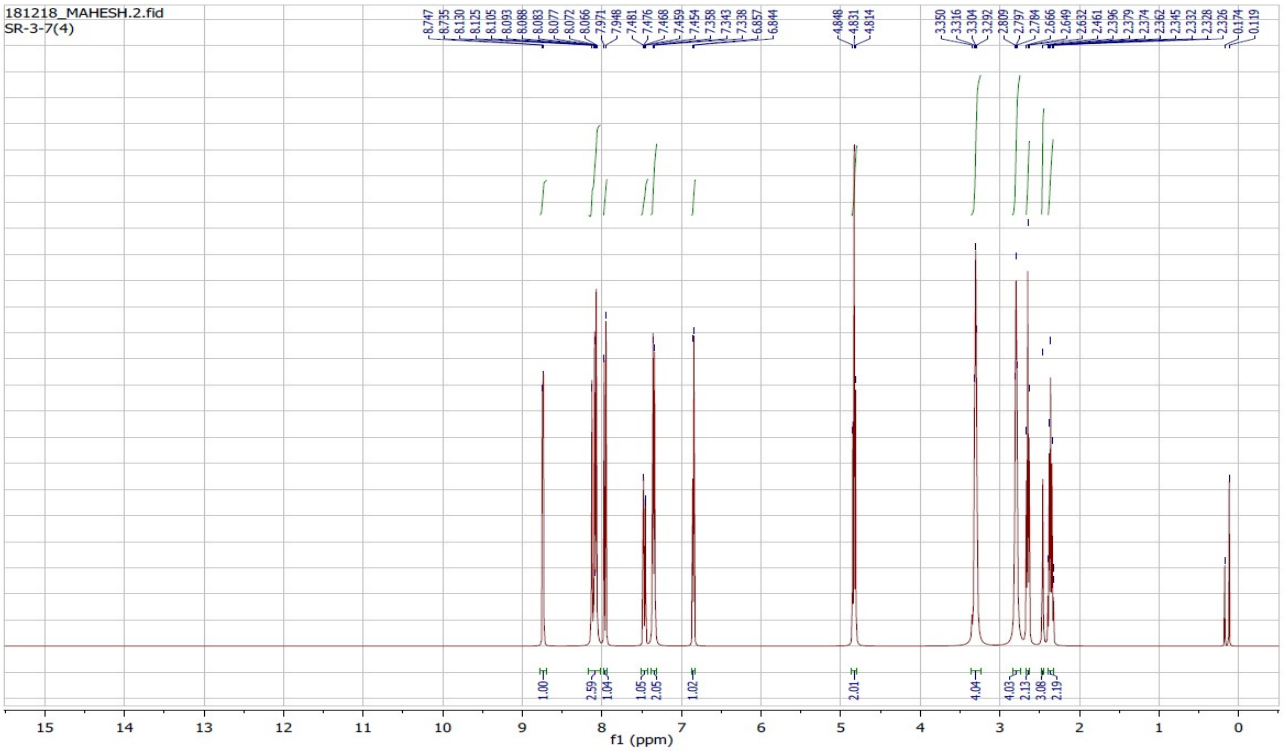
^{1}H-NMR of compound **(68)**:



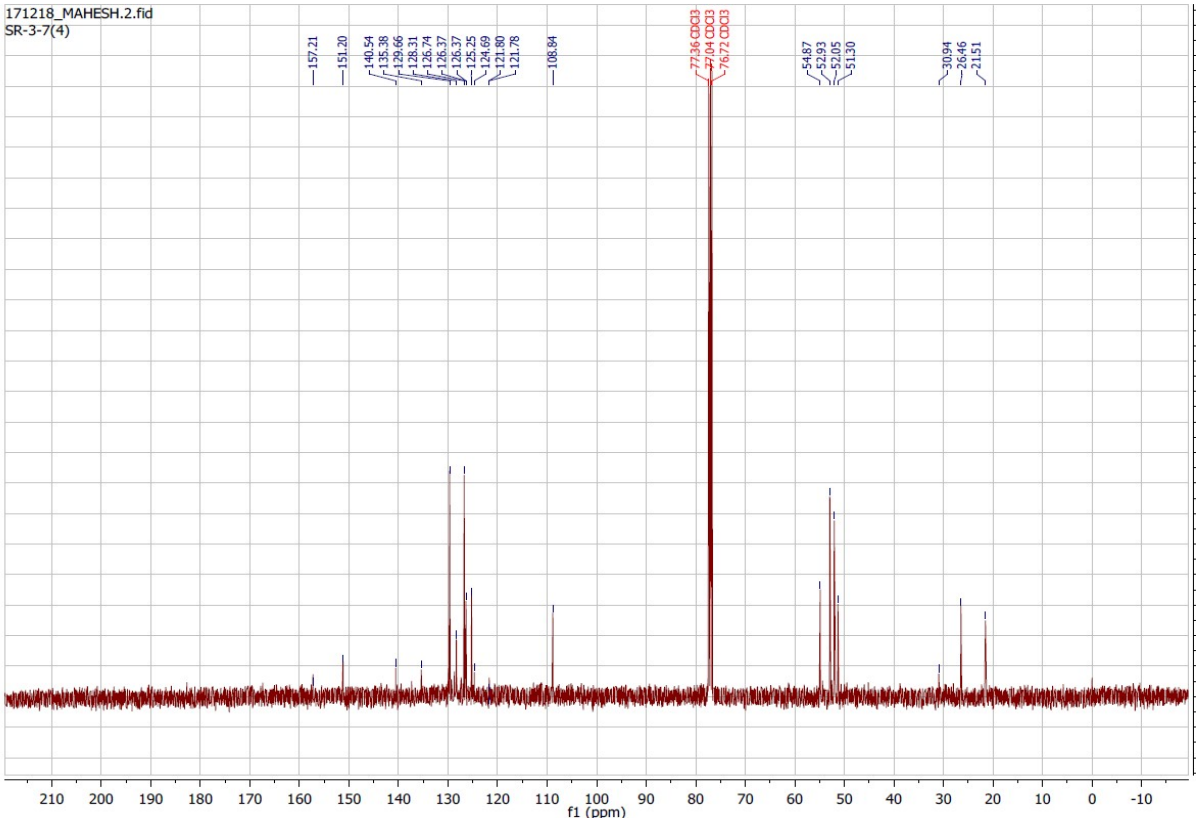
Mass spectrum of compound (**68**):



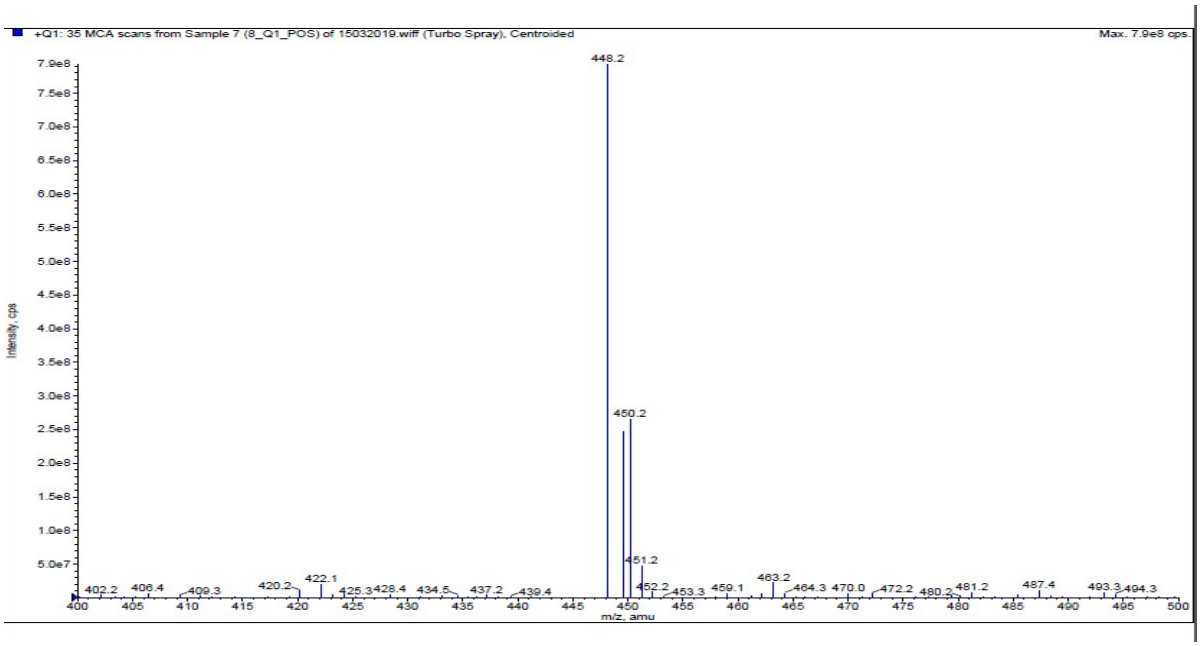
^{1}H-NMR of compound (**69**):



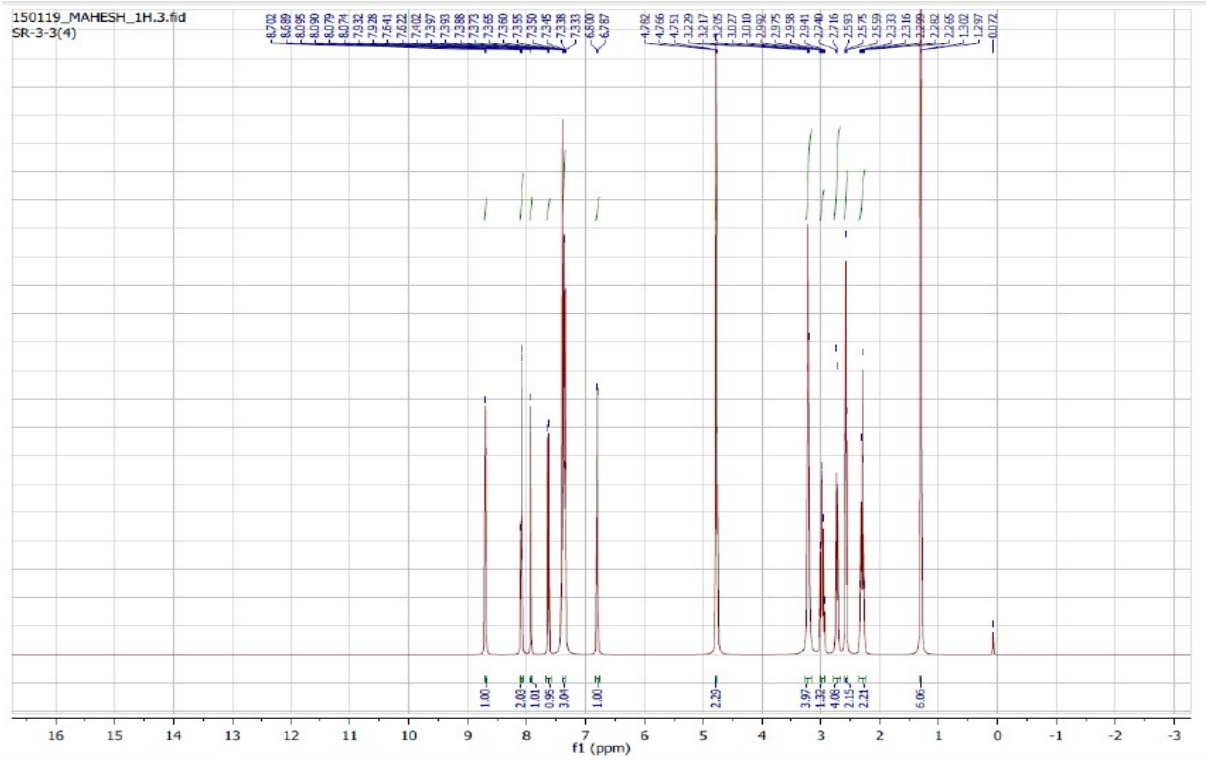
^{13}C-NMR of compound (**69**):



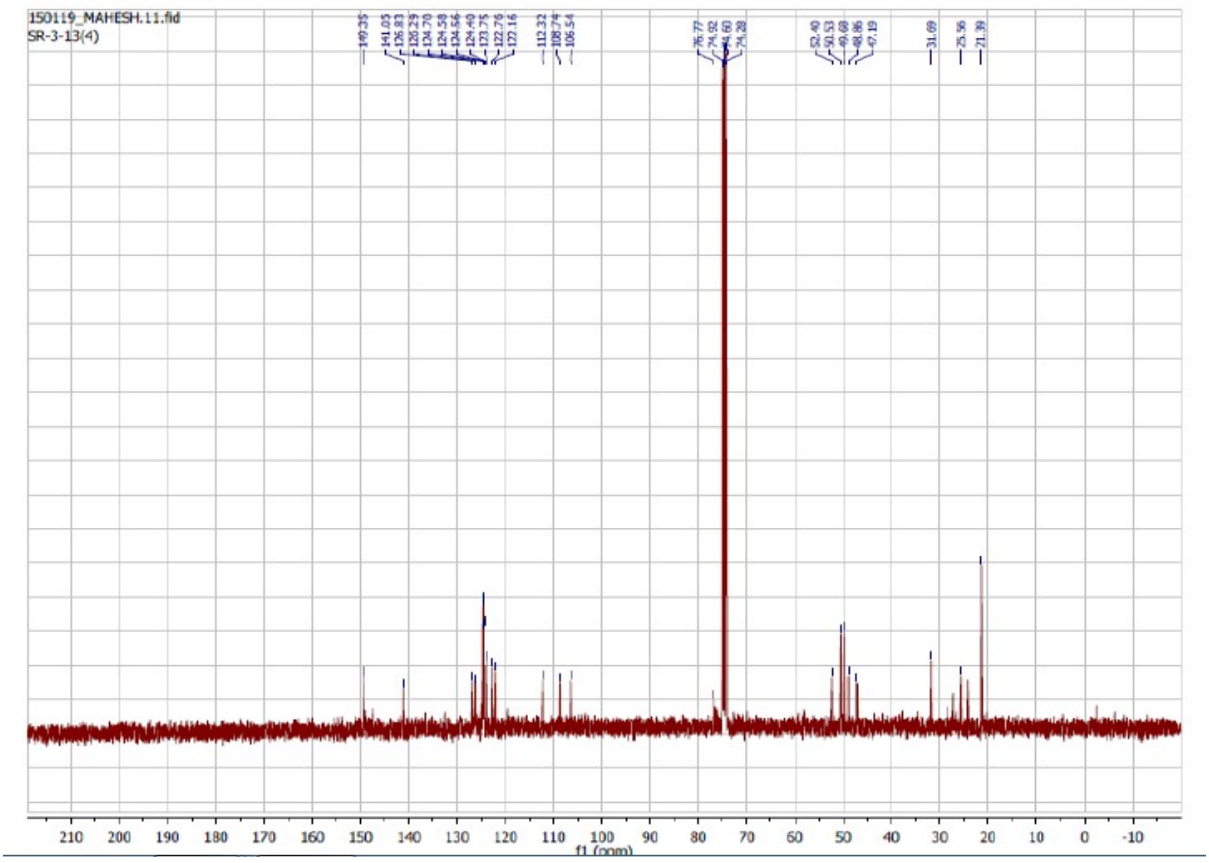
Mass spectrum of compound (**69**)**:**



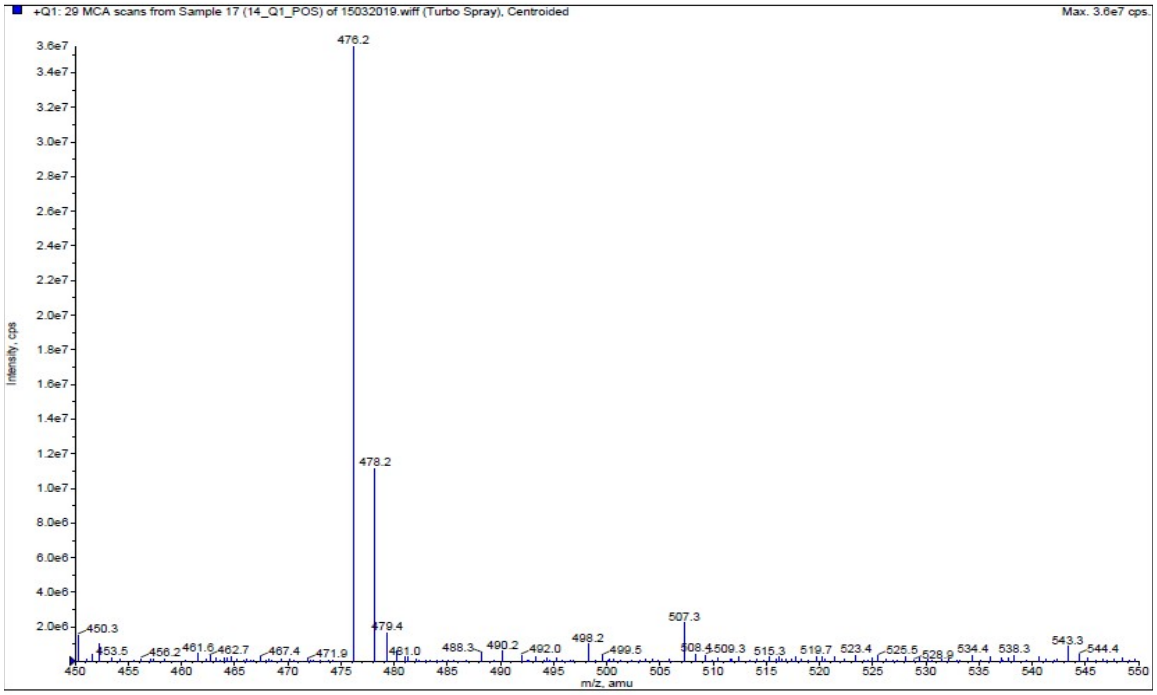
^{1}H-NMR of compound (**70**):



^{13}C-NMR of compound (**70**):

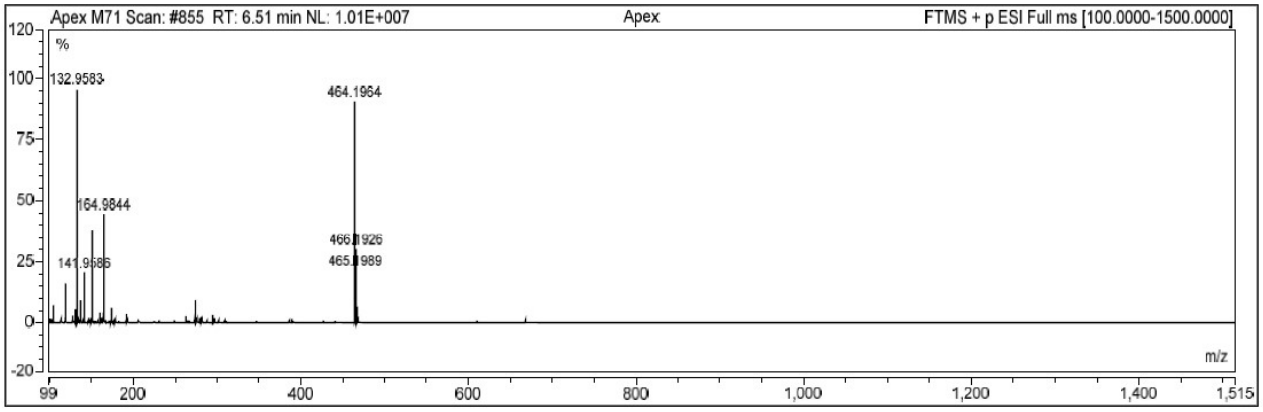


Mass spectrum of compound (**70**):

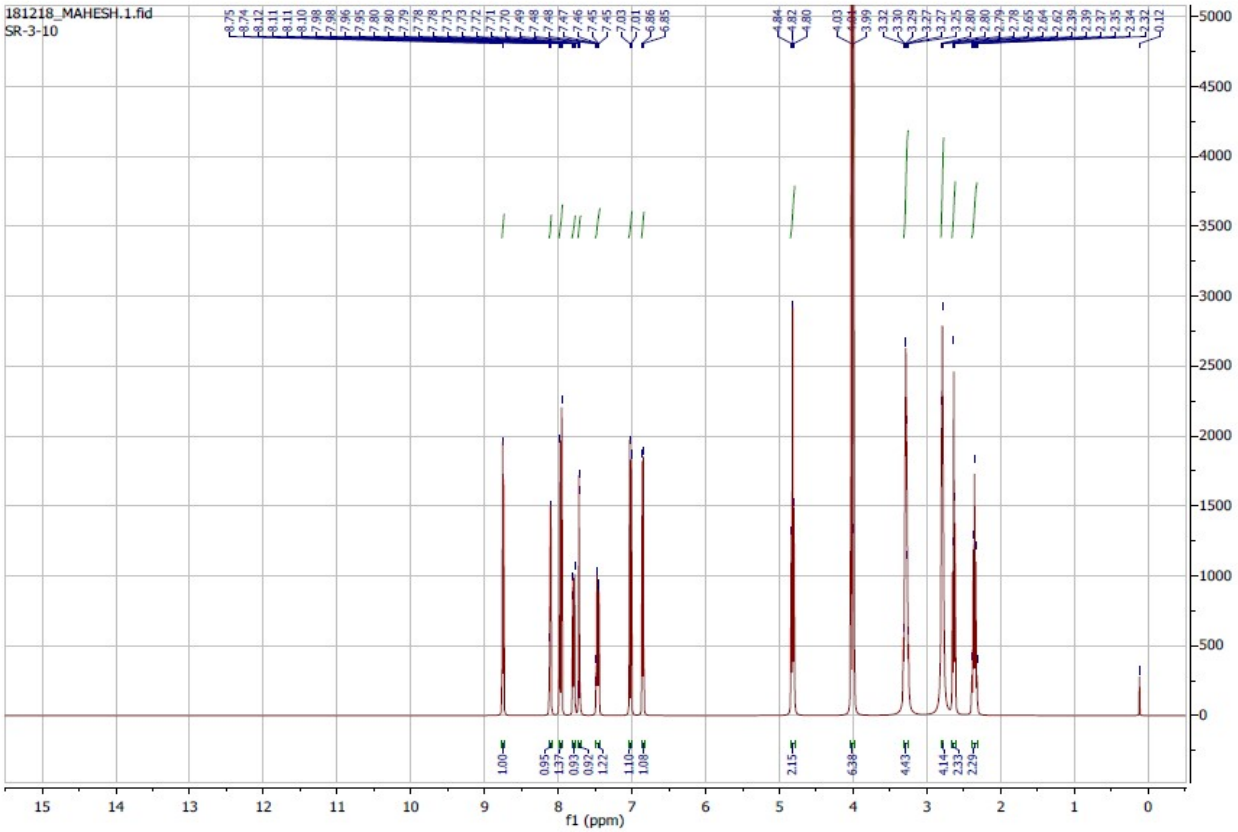


HRMS spectrum of compound (**71**):

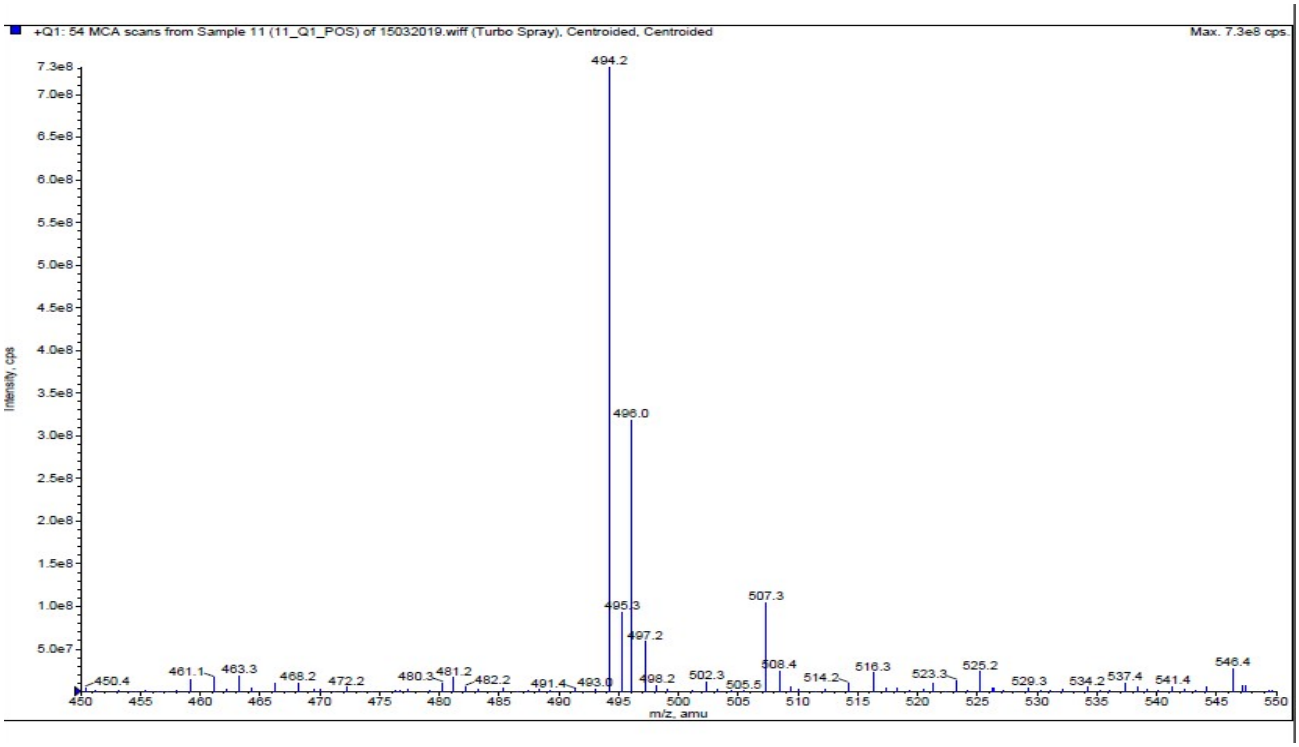
No.	Peak Name	Retention Time min	Area counts*sec	Height counts
2	M71	6.509	191546816	9149253



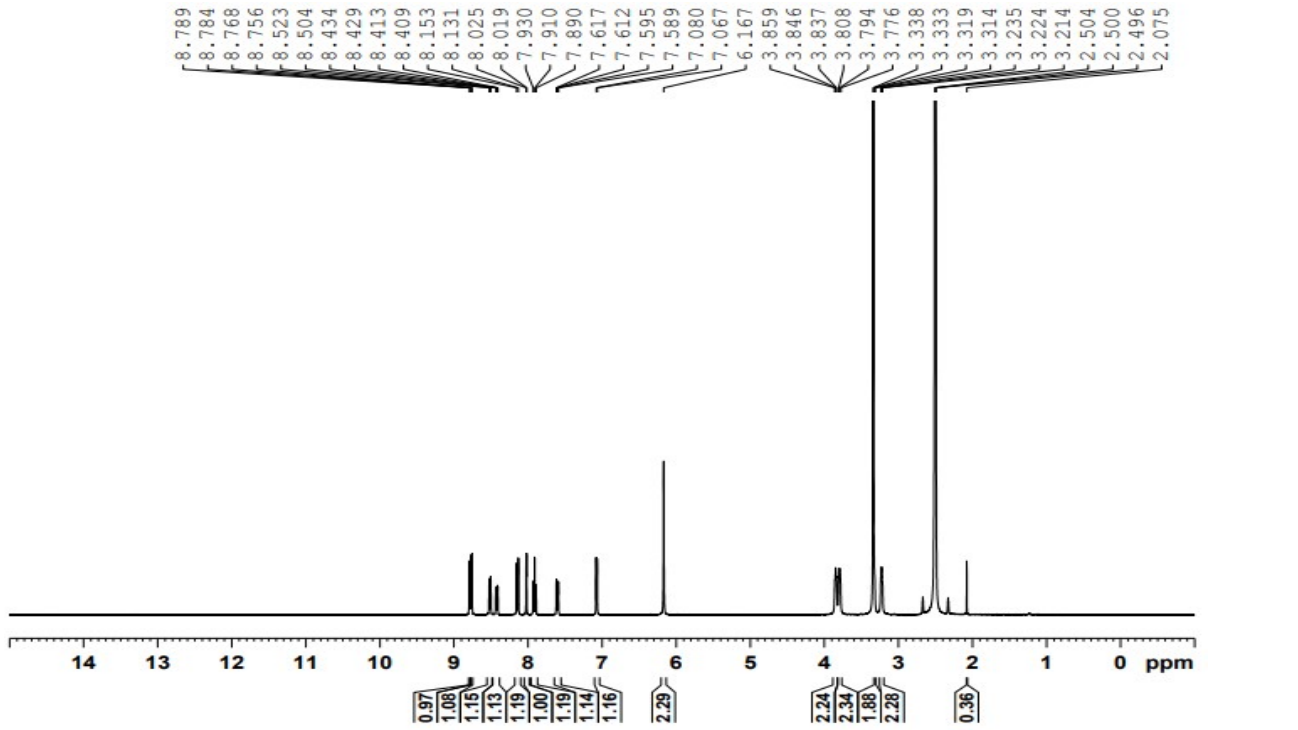
^{1}H-NMR of compound (**72)**:



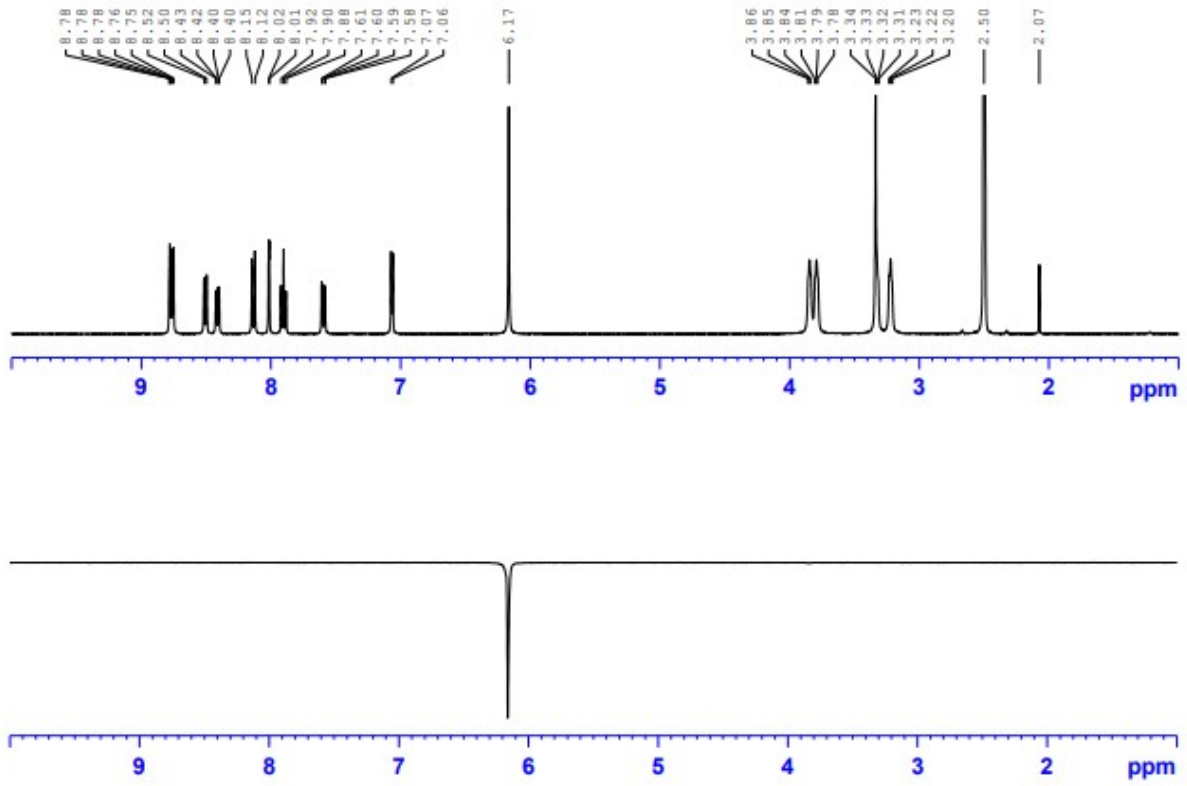
Mass spectrum of compound (**72)**:



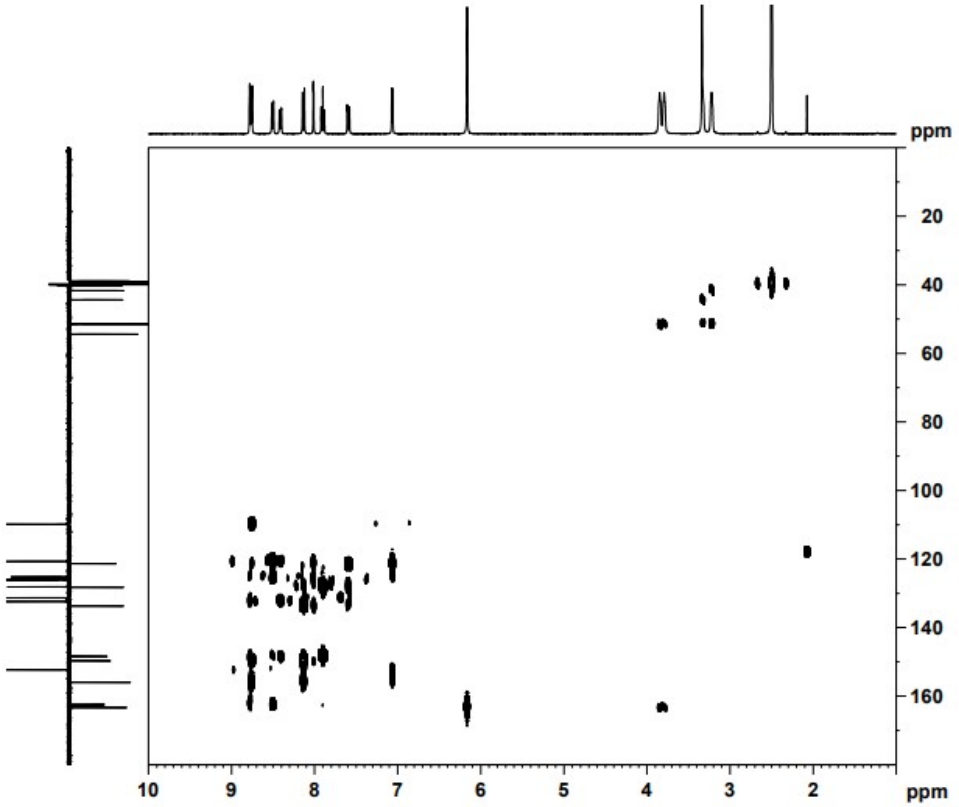
[1]H-NMR of compound (**75)**:



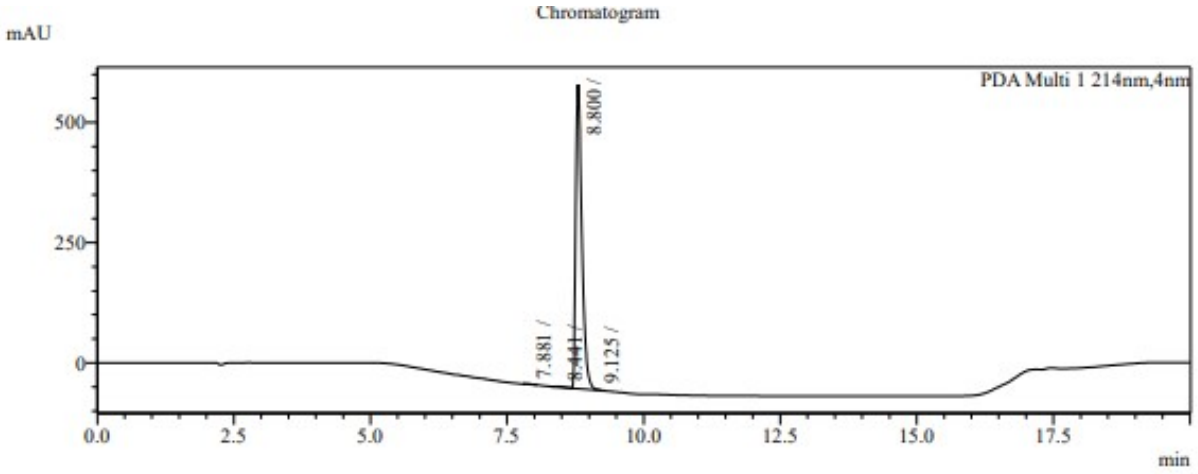
Selective Gradient NOESY spectra of compound (**75):**



HMBC spcetrum of compond (**75)**:



HPLC chromatogram and purity data of compound (**75):**

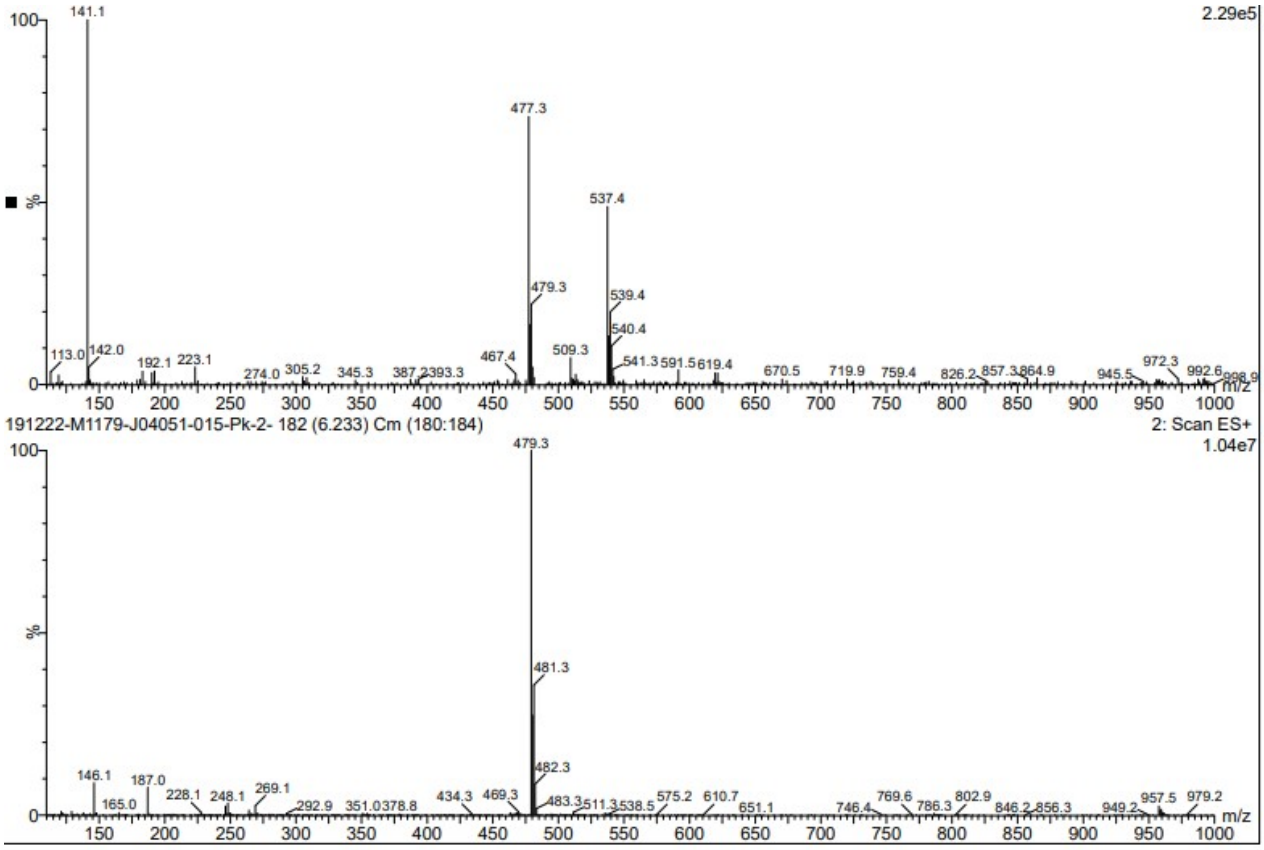


Peak Table

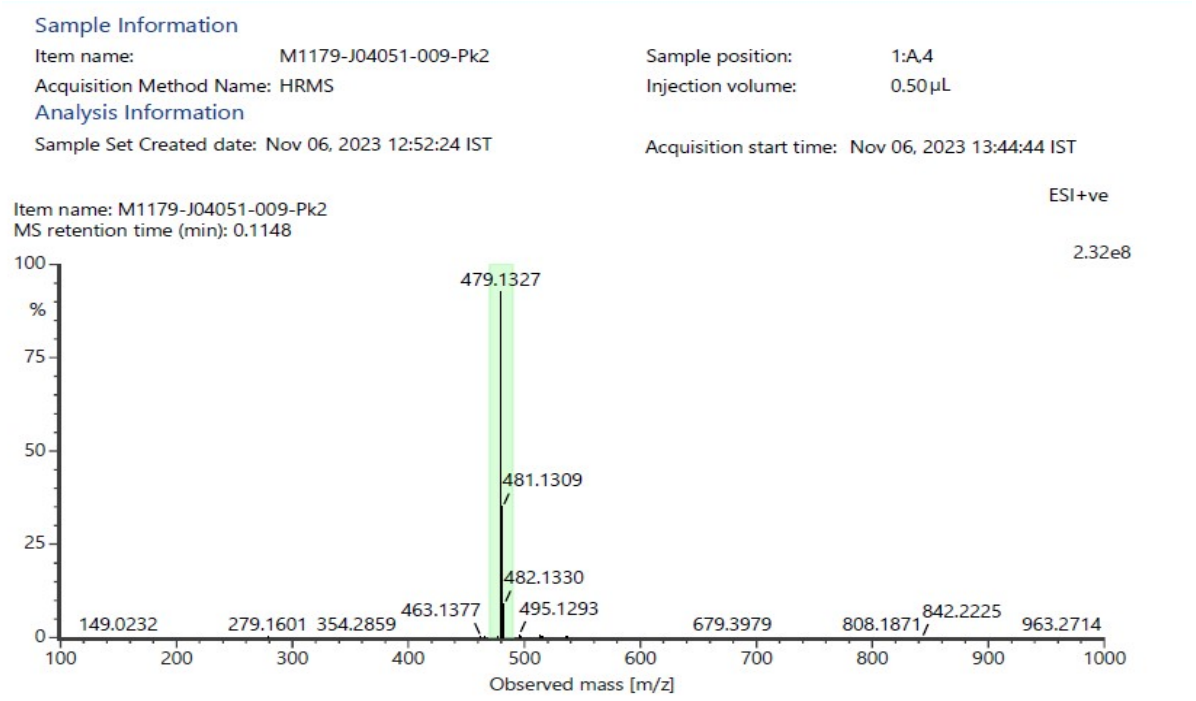
PDA Ch1 214nm

Peak	Ret. Time	Area	Area%
1	7.88	9054	0.18
2	8.44	16536	0.33
3	8.80	4969223	99.31
4	9.13	8943	0.18
Total		5003756	100.00

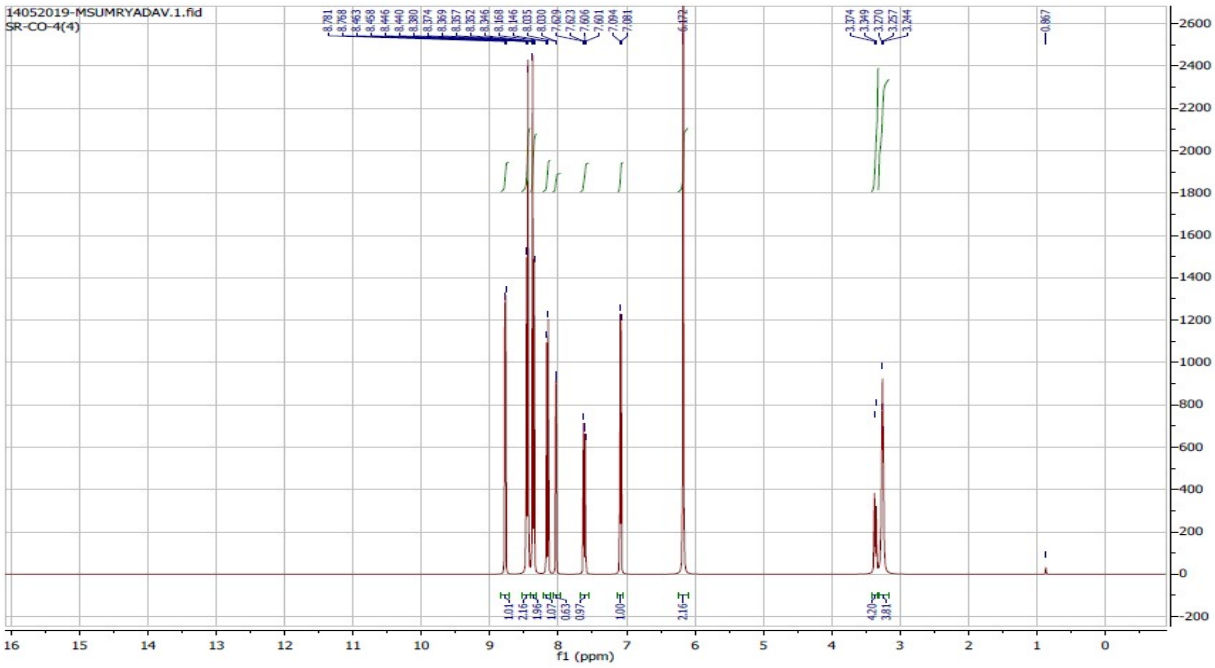
UPLC-MS spectrum of compound (**75**)**:**



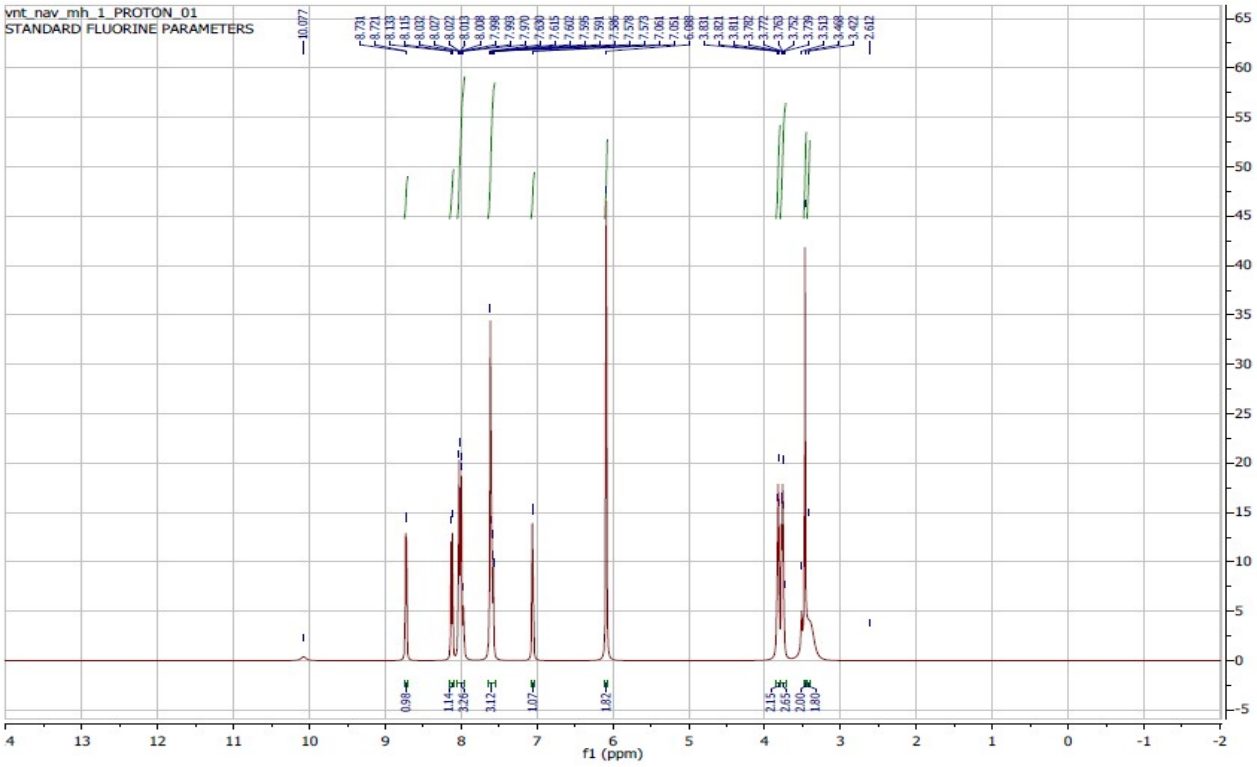
HRMS spectrum of compound (**75**):



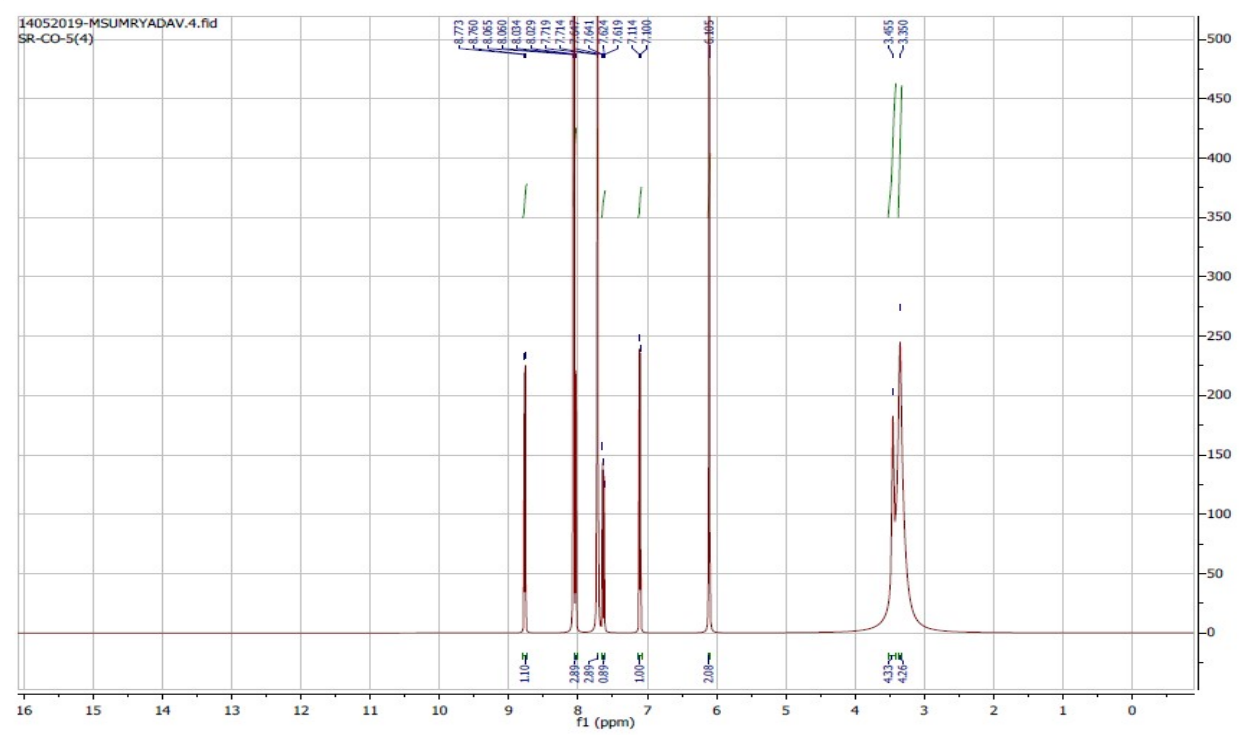
^{1}H-NMR of compound (**76**):



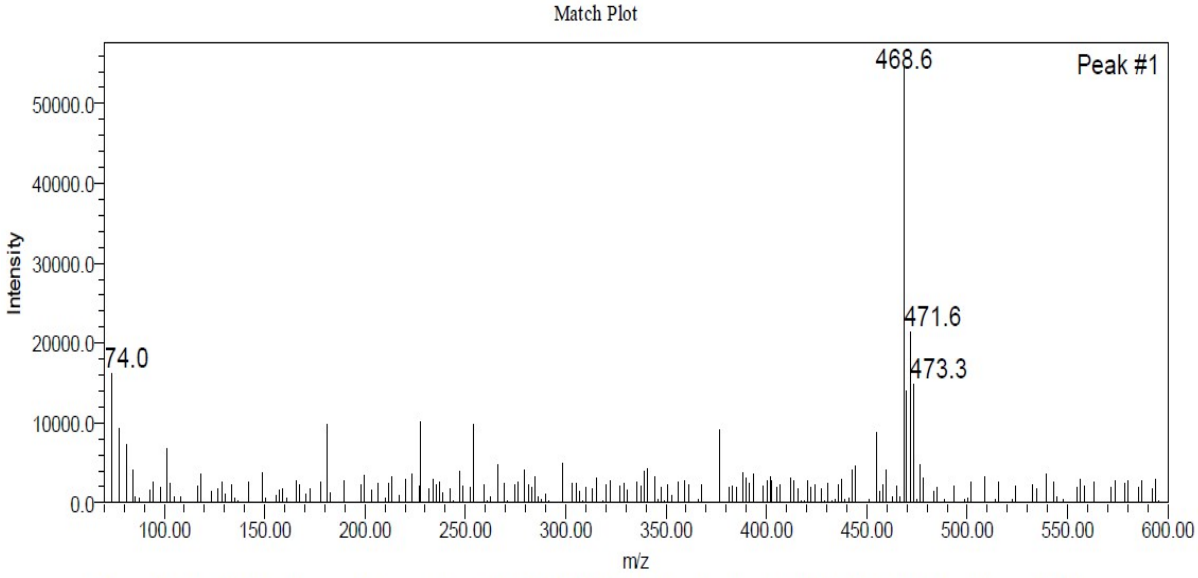
^{1}H-NMR of compound (**77**):



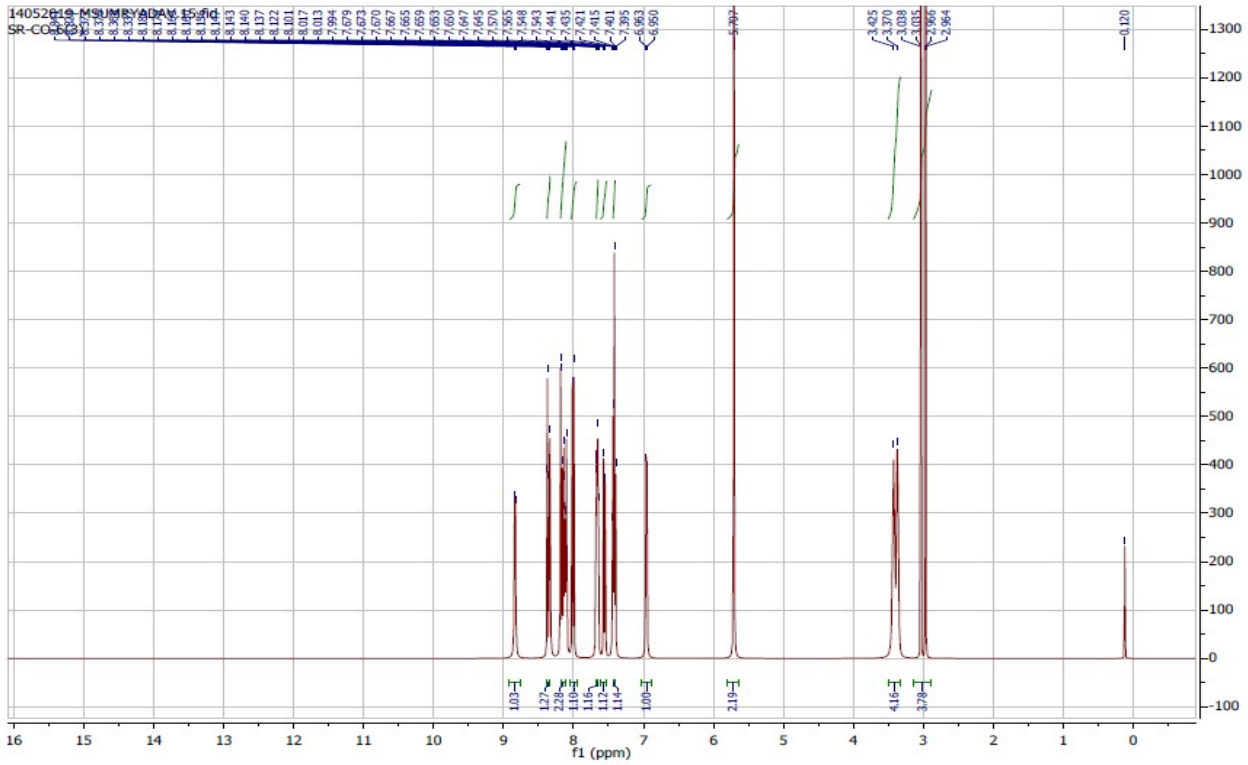
[1]H-NMR of compound (**78**):



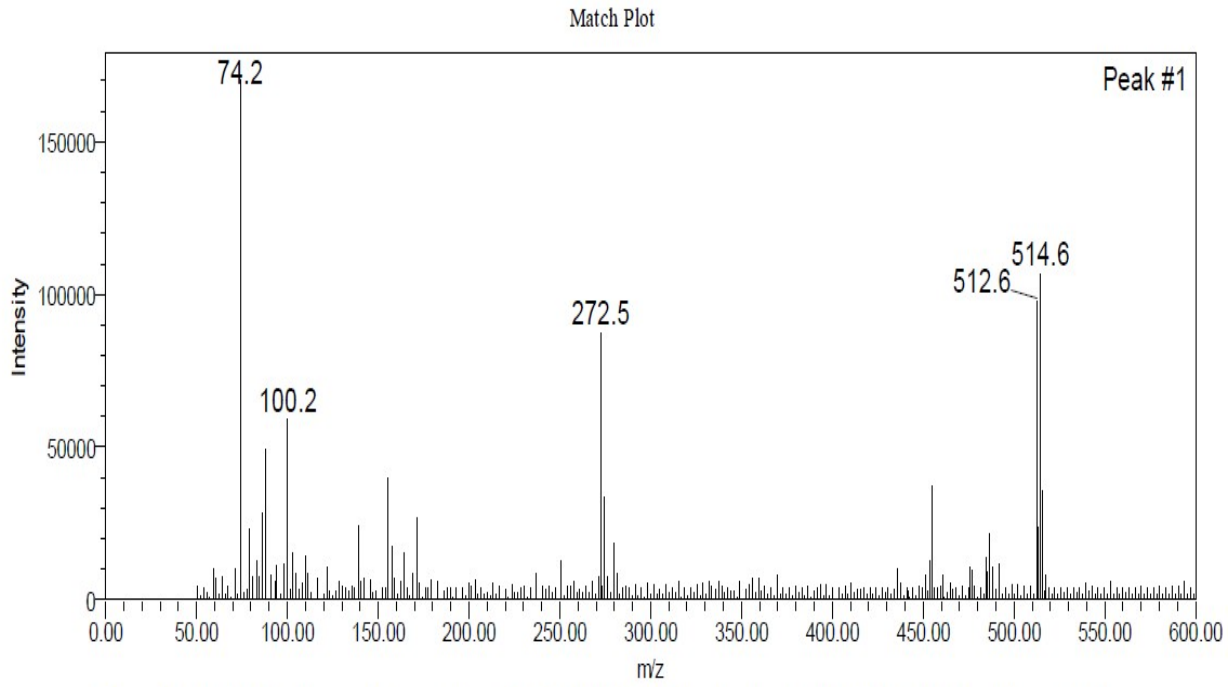
Mass spectrum of compound (**78)**:



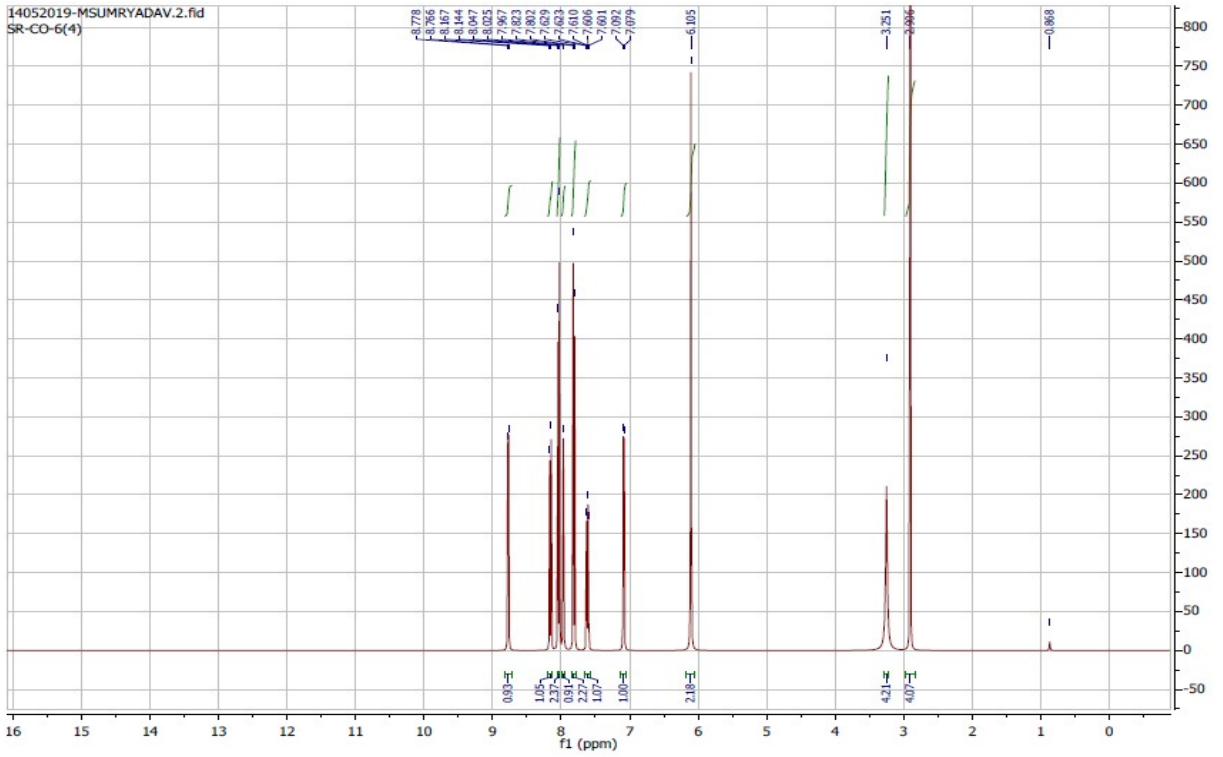
^{1}H-NMR of compound (**79)**:



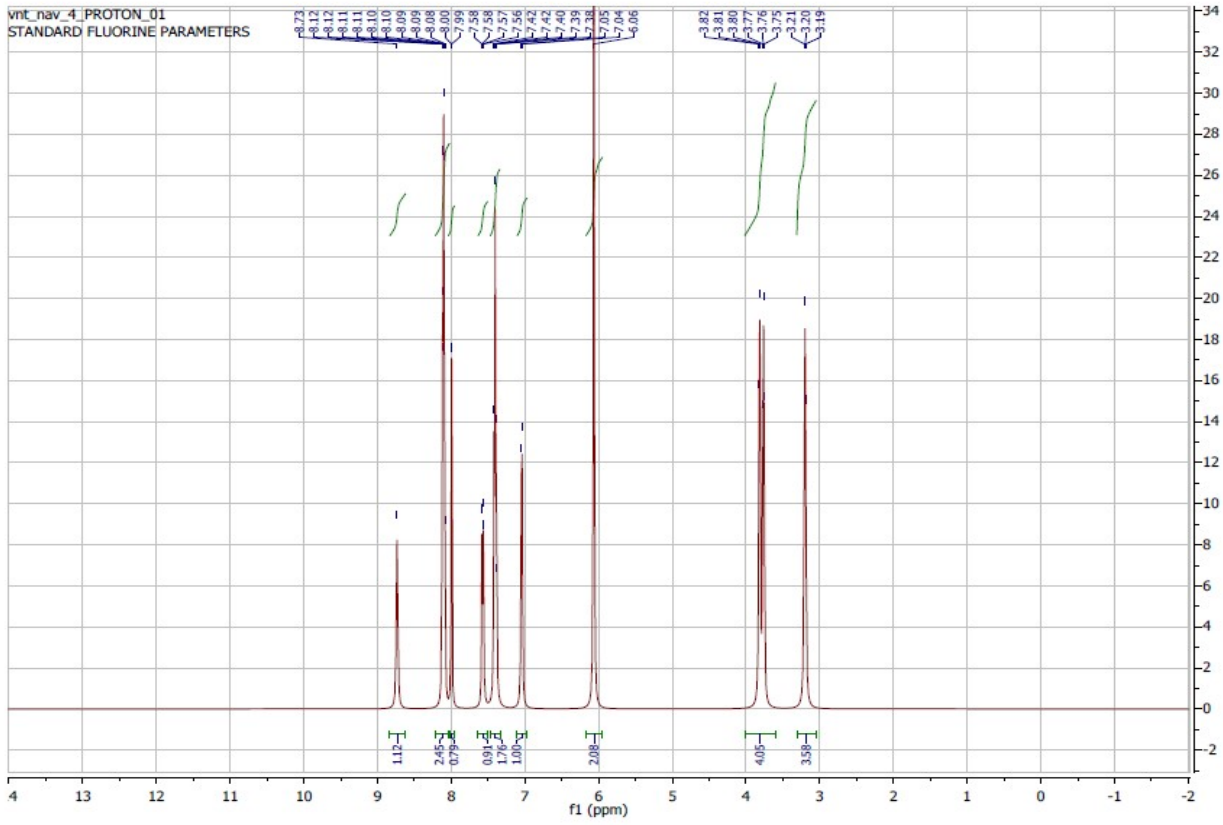
Mass Spectrum of compound (**79**):



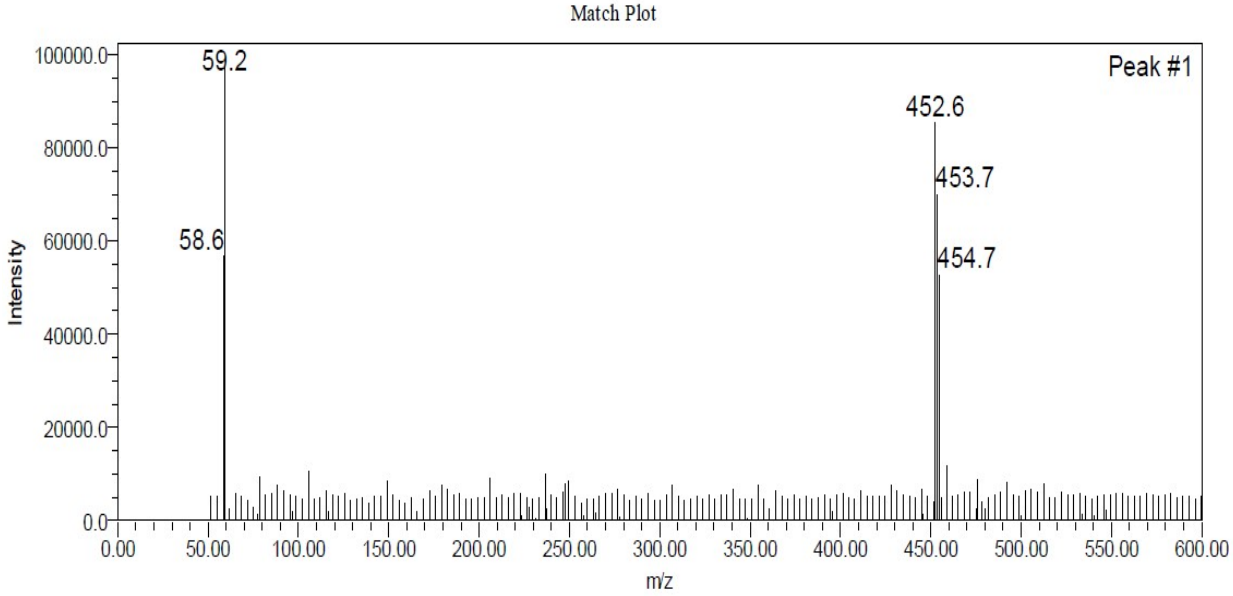
^{1}H-NMR of compound (**80**):



^{1}H-NMR of compound (**82):**

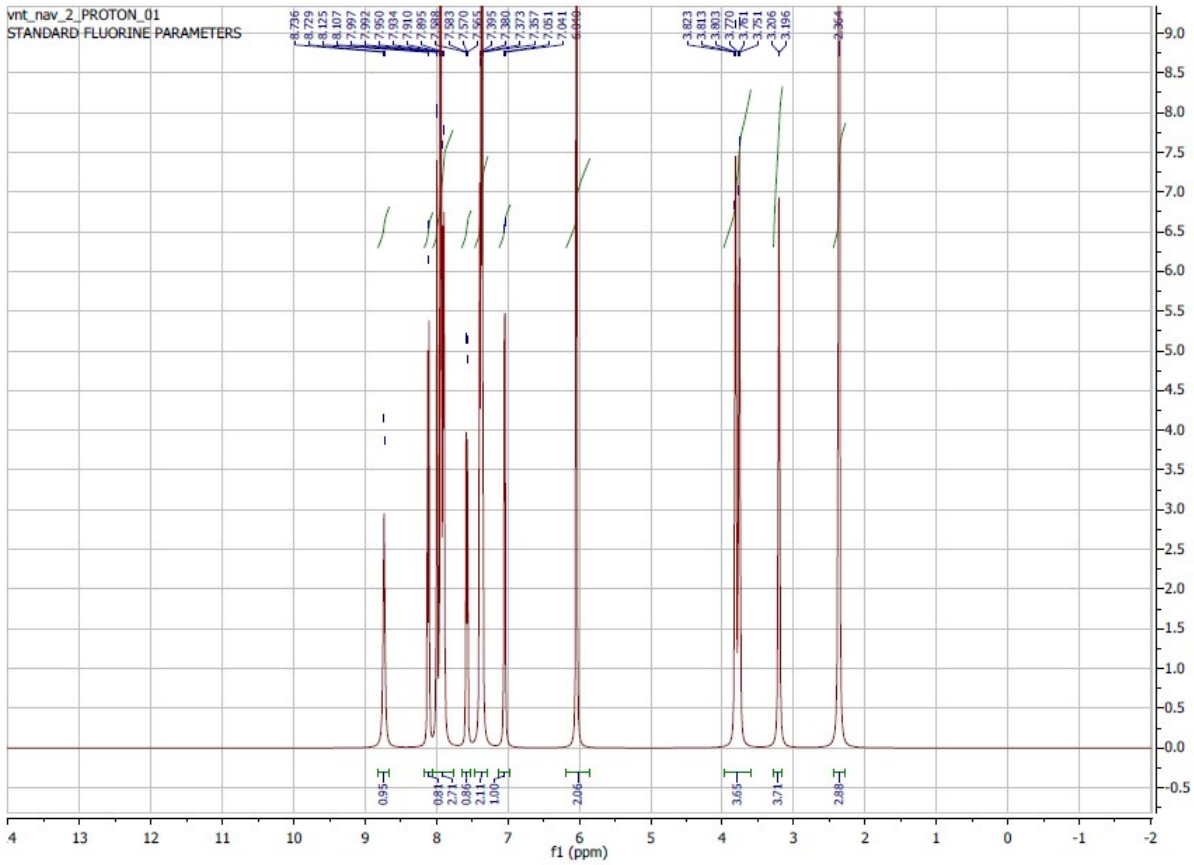


Mass Spectrum of compound (**82):**



Base Peak 59.18 Channel Description 50.00-1250.00 ES+, Centroid, CV=10 Retention Time 0.701

[1]H-NMR of compound (**83**):



HRMS spectrum of compound (**83**):

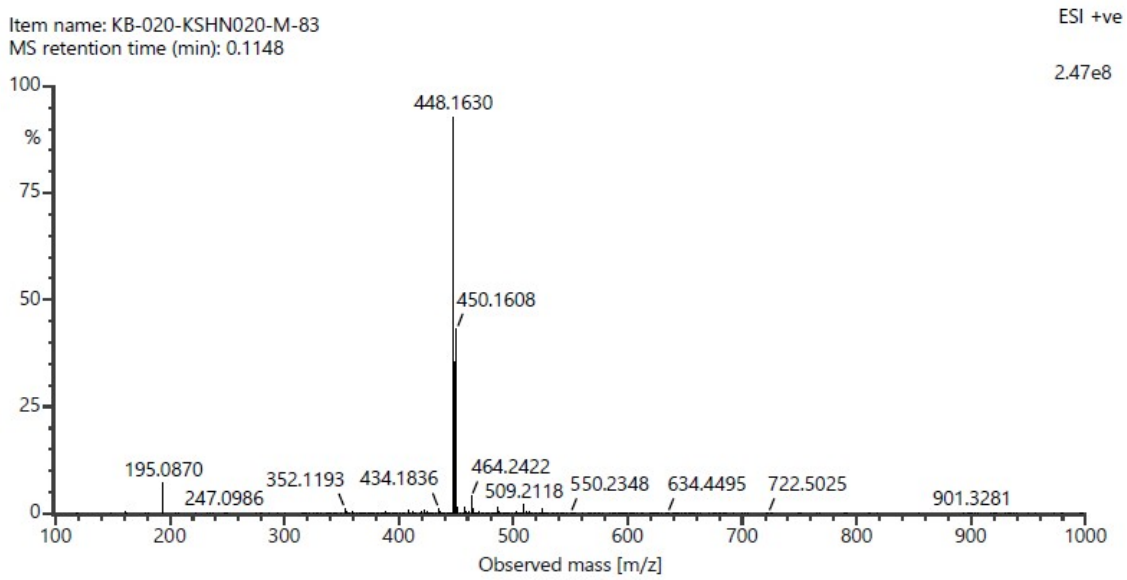
Sample Information

Item name: KB-020-KSHN020-M-83 Sample position: 1:A,6

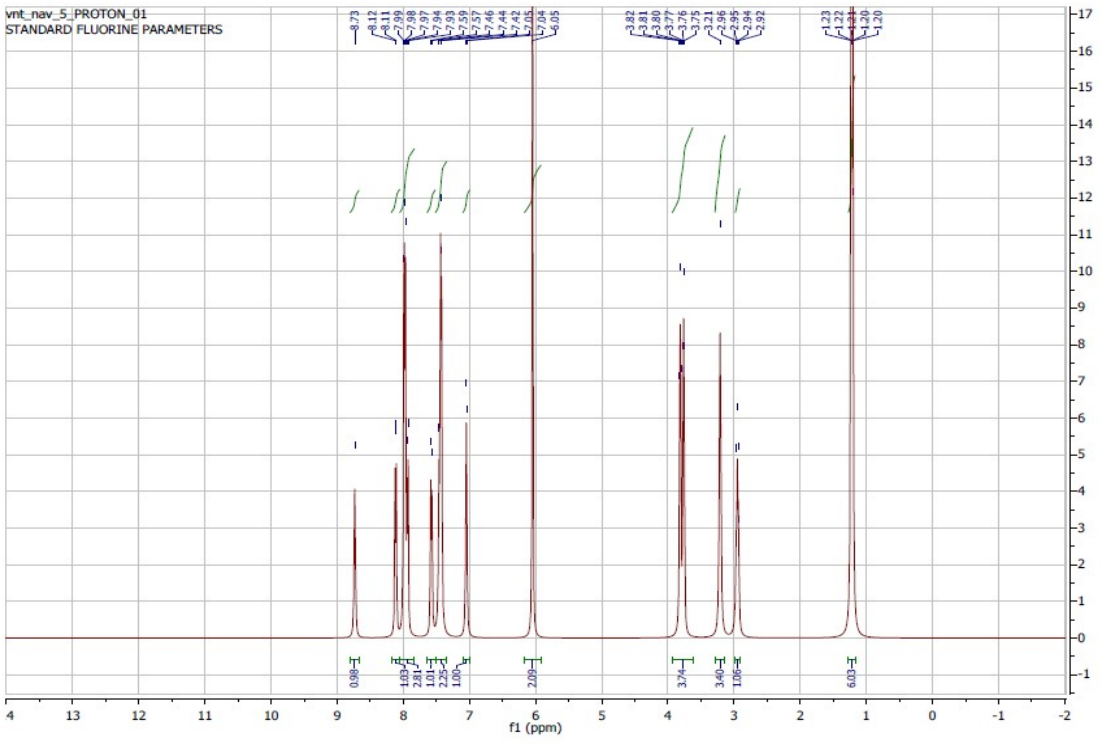
Acquisition Method Name: HRMS Injection volume: 0.50 µL

Analysis Information

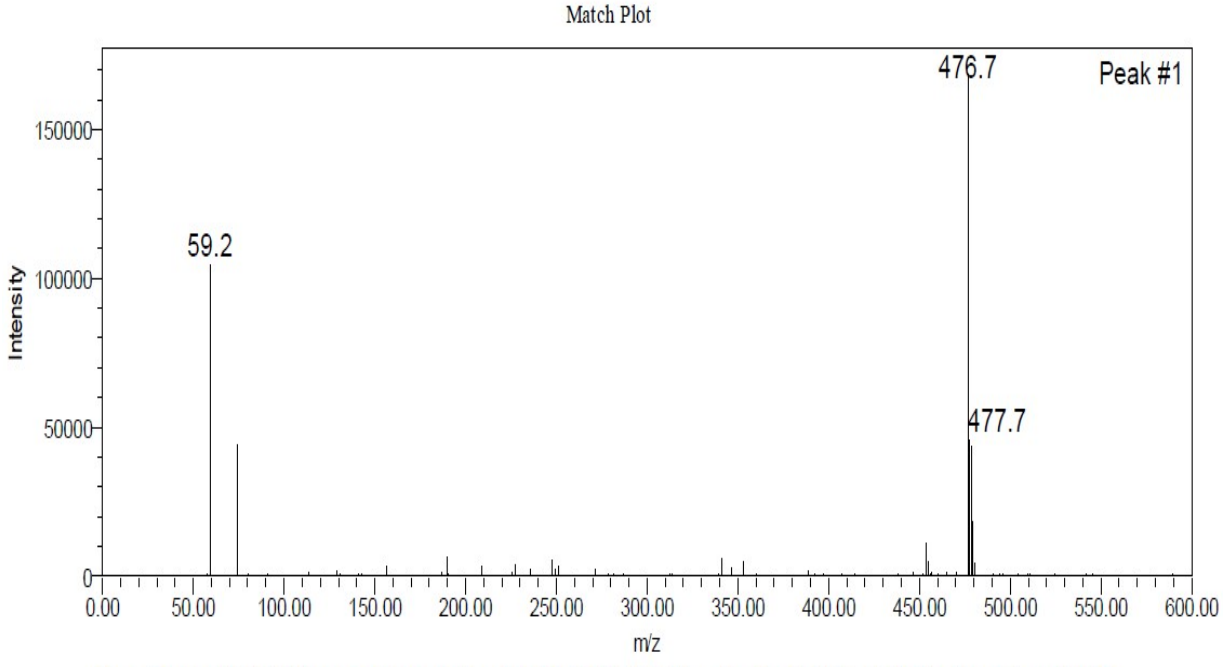
Sample Set Created date: Nov 06, 2023 12:52:24 IST Acquisition start time: Nov 06, 2023 13:52:36 IST



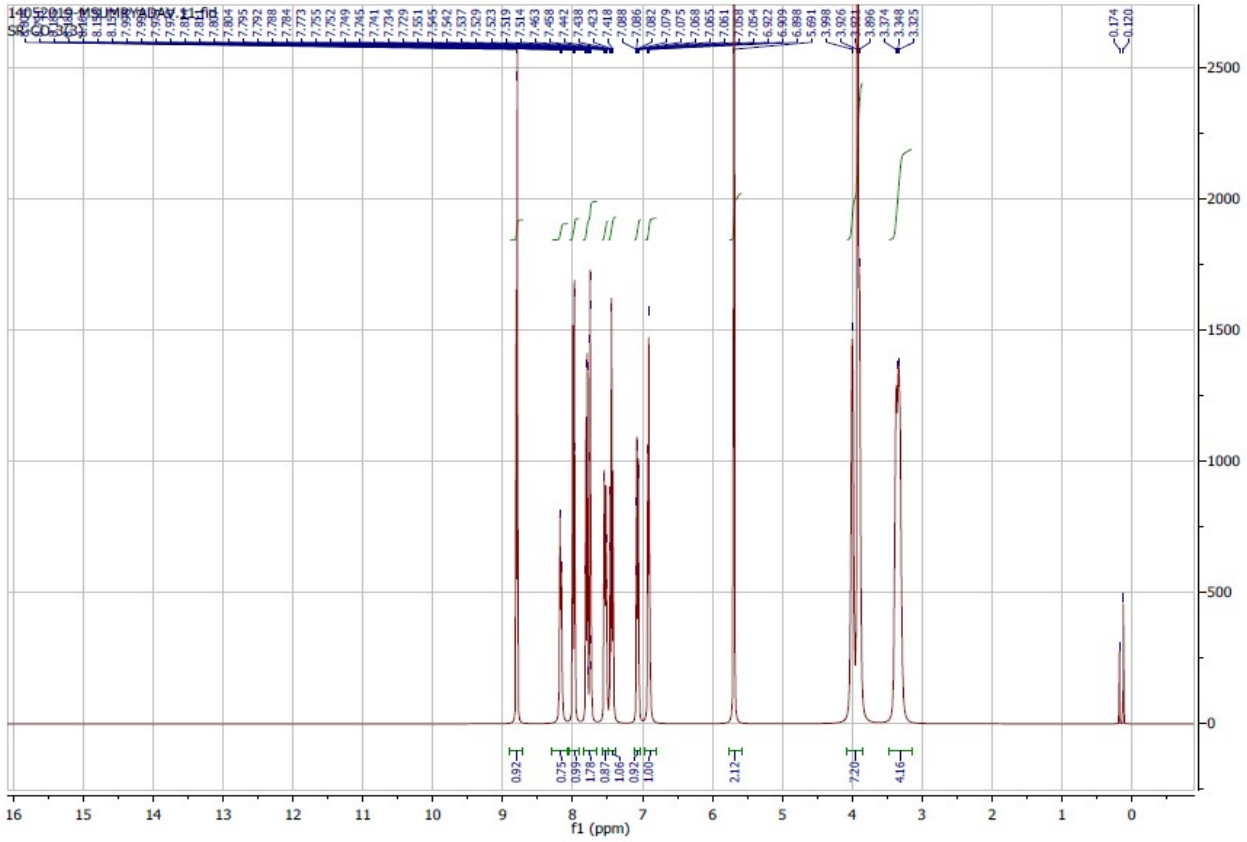
[1]H-NMR of compound (**84):**



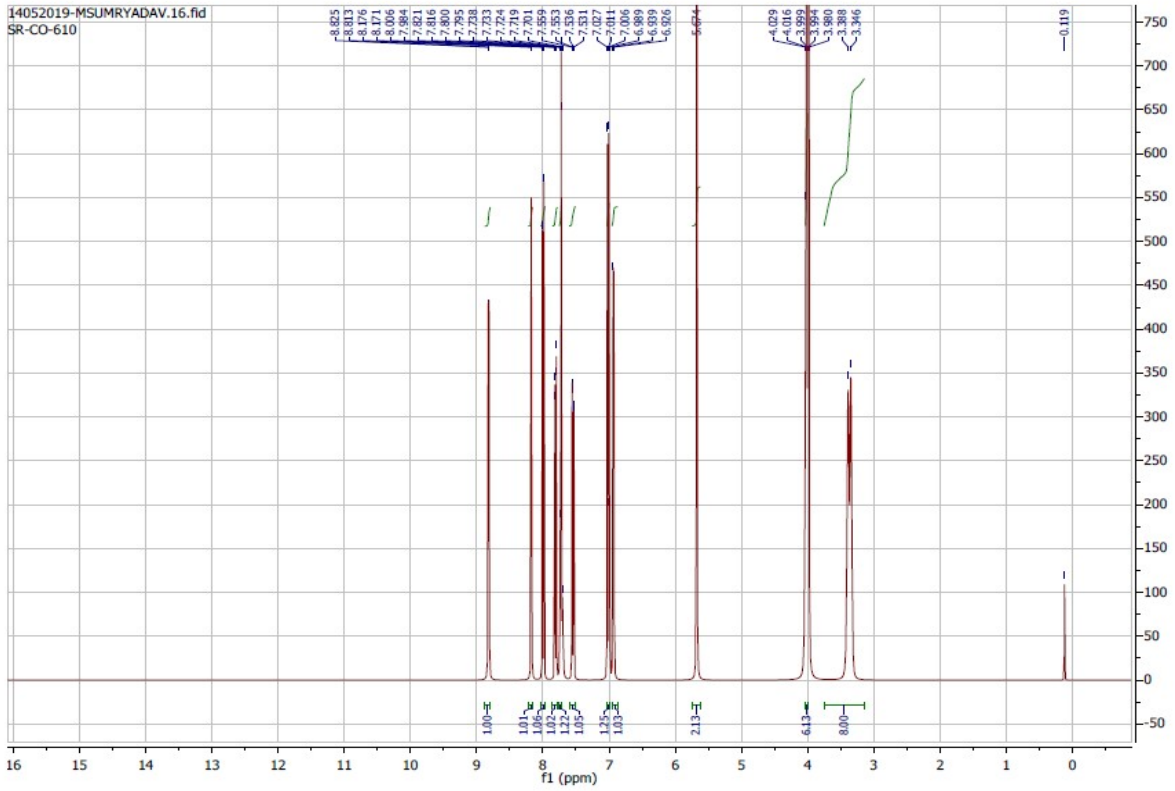
Mass Spectrum of compound (**84):**



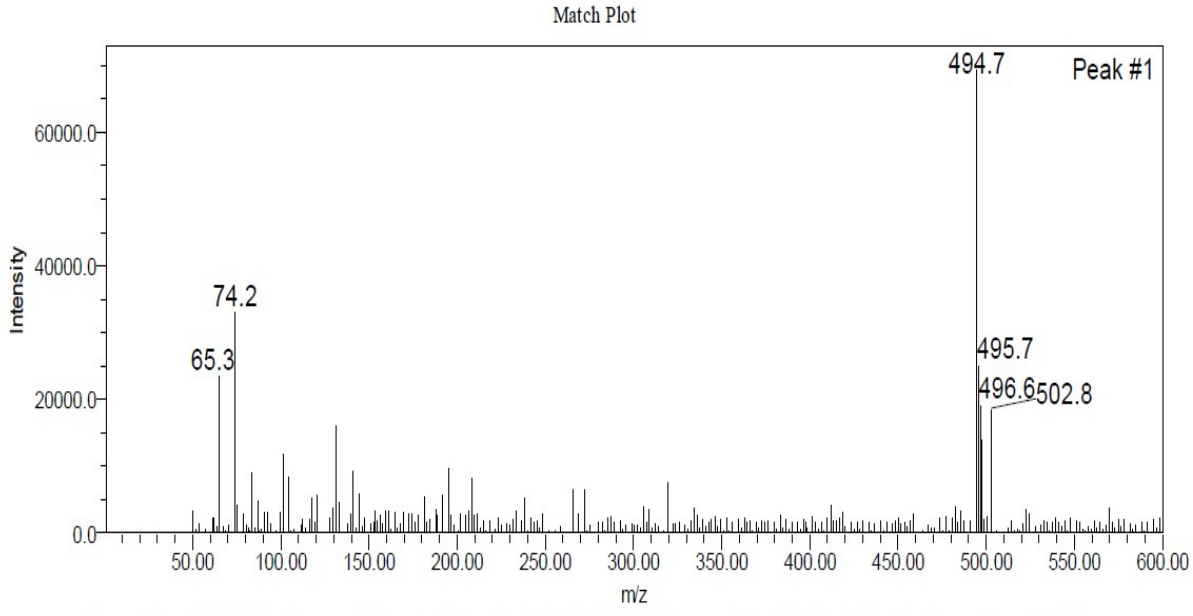
[1]H-NMR of compound (**85):**



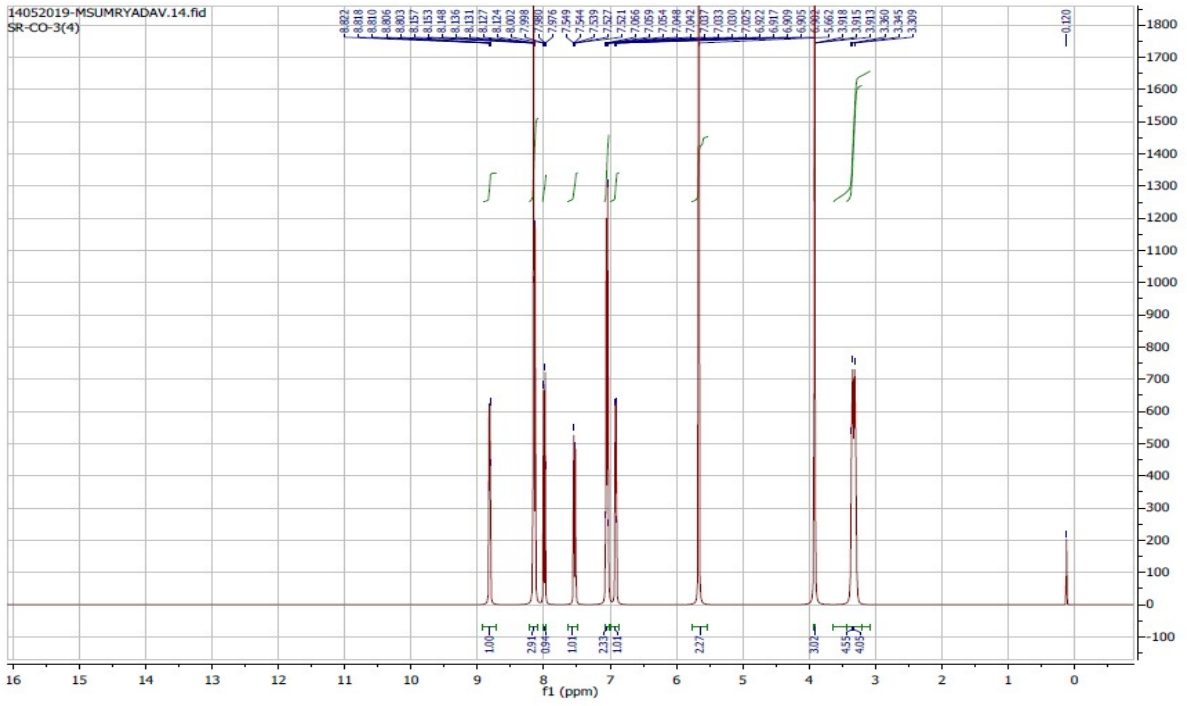
[1]H-NMR of compound (**86)**:



Mass Spectrum of compound (**86**):



^{1}H-NMR of compound (**87**):



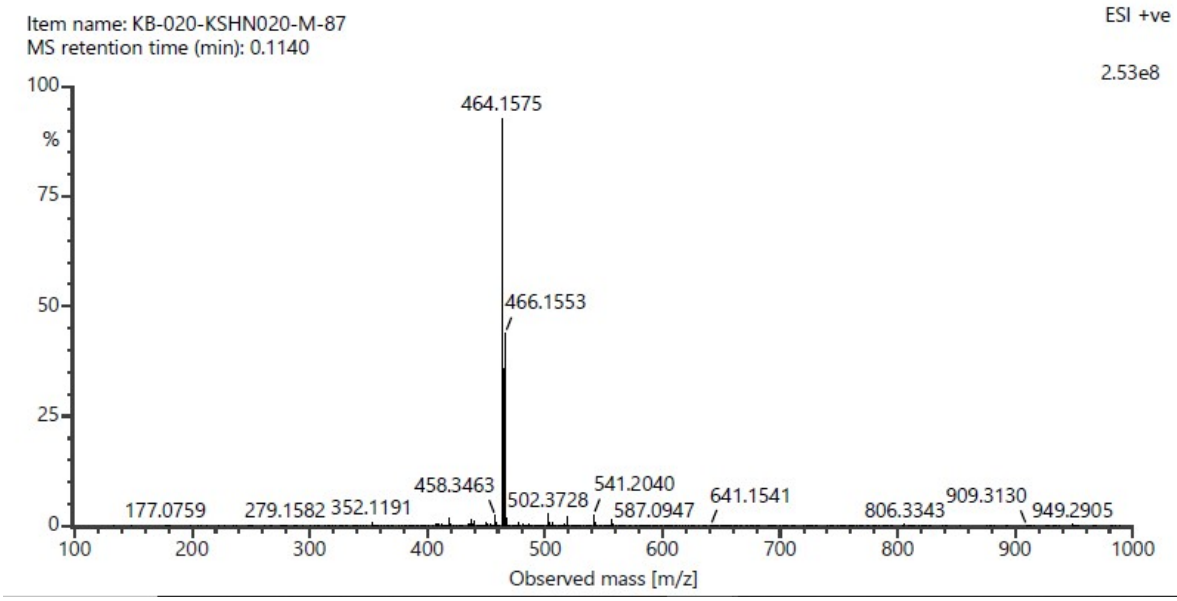
HRMS spectrum of compound (**87**):

Sample Information

Item name: KB-020-KSHN020-M-87
Sample position: 1:A,7
Acquisition Method Name: HRMS
Injection volume: 0.50 µL

Analysis Information

Sample Set Created date: Nov 06, 2023 12:52:24 IST
Acquisition start time: Nov 06, 2023 13:56:35 IST



B. Molecular dynamics simulation studies of compounds (66 and 75, in comparison to verapamil and piperquine) with wild and mutant *Pf*CRT protein

It seems (**Figure 1Sa**) that verapamil is getting diffused away from its docking site in wild *Pf*CRT whereas it is forming stable interaction with mutant *Pf*CRT. High RMSD (>3 Å) of ligand in verapamil-CRT^{WT} complex and Piperaquine in Piperaquine-CRT^{MT} complex denotes the 'ligand diffusion' away from its putative binding site. Further, with verapamil, a transition state is speculated to be formed during its interaction with CRT^{WT}, initially from 0 to 30 ns (verapamil associating with the CRT^{WT} protein) and then from 30 ns to 70 ns (verapamil diffusing away from the protein), followed by 70 ns to 100 ns (re-orienting and binding to the protein again). This is possible because verapamil is a flexible molecule (possessing many rotatable bonds) having a reactive nitrile functional group. RMSD values of <3 Å in the case of **66** and **75** with $CRT^{WT/MT}$ denote stable interactions of these two compounds with the respective proteins. This is an important observation because it indicates that even in case of mutation in the CRT, both **66** and **75** are likely to have tight binding with the mutated protein also. Further, different types of bond formations between verapamil and *Pf*CRT (**Figure 2Sa**), specifically large number of hydrophobic interactions (A79, F105, A144, L145, L148, L160, and V249) seem to be stabilizing its interaction with the mutant *Pf*CRT as compared to the wild *Pf*CRT, wherein the hydrophobic interactions with F145, L148, and L308 are quite low. On the other hand, piperaquine is making stable interactions with wild-type *Pf*CRT (ligand RMSD <2Å, **Figure 1Sb**) with ionic bond formation with Asp137, strong hydrogen bonding between L245 and nitrogen of piperaquine, and water bridges between piperaquine and Q156, D326, D329 and Q352 (**Figure 2Sb**). Piperaquine interactions with mutant *Pf*CRT highlight high fluctuations in ligand RMSD denoting movement of the ligand away from its docking site (**Figure 1Sb**). High fluctuations in ligand RMSD denote movement of the ligand away from its docking site (**Figure 1Sb**). Further, as seen in **Figure 2S**, its ionic bonding with E75, D137, D329, weak hydrogen bonding with D137, Q352 and water bridging between ligand and

protein through T76, D137 and Q352 might be the reason for its movement out of the food vacuole through *Pf*CRT. The compounds (**66** and **75**) exhibit stable interactions with *Pf*CRT and are not getting diffused away from their docking sites in both wild and mutant *Pf*CRT (**Figure 1Sc** and **1Sd**). A wide range of interactions, such as hydrogen, hydrophobic, ionic, Pi-Pi, Pi-cation interactions, and water bridges, seem to stabilize their binding with *Pf*CRT (**Figures 2Sc** and **2Sd**).

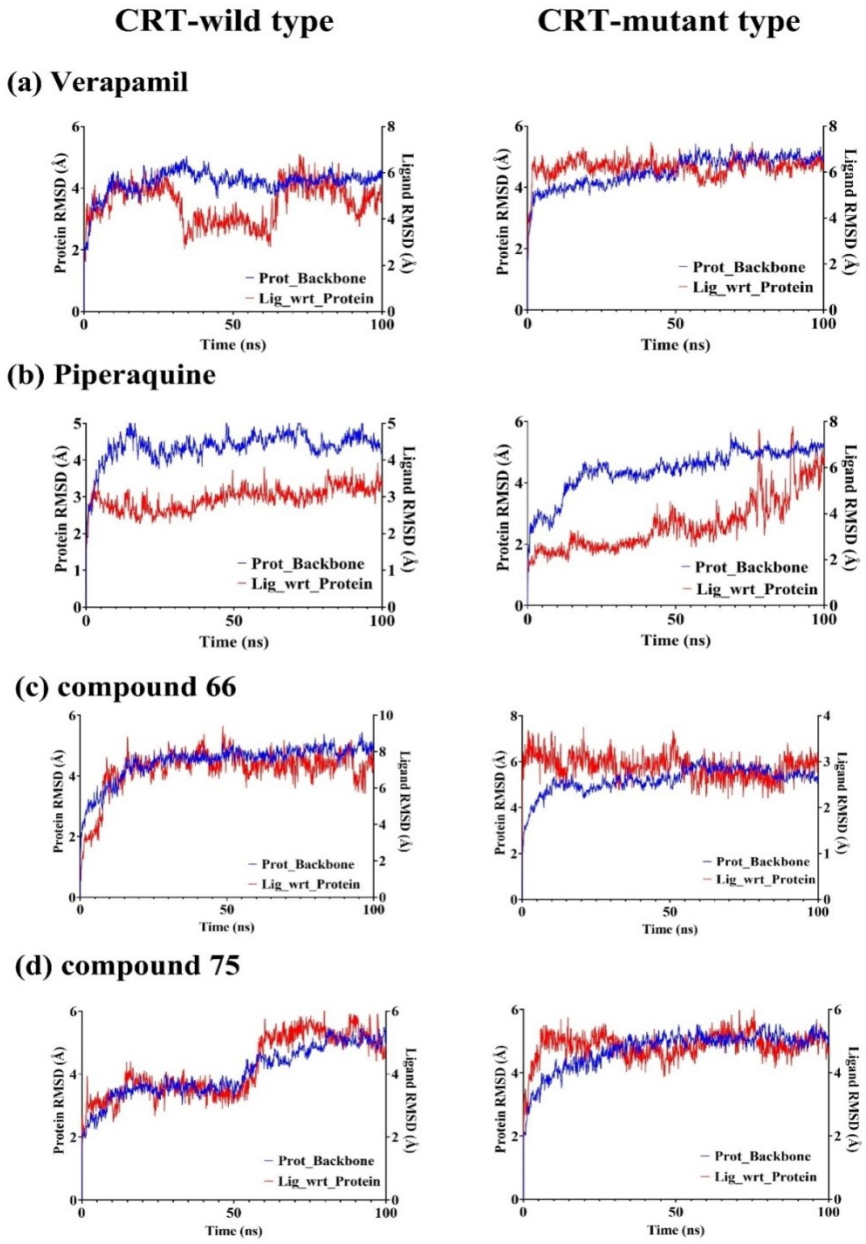


Figure 1S. RMSD plot of the ligands (**verapamil**, **piperaquine**, **66** & **75**) and the CRT proteins in association with each other during the molecular dynamics simulations. Note the high ligand RMSD denotes diffusion of the ligand away from its putative binding site.

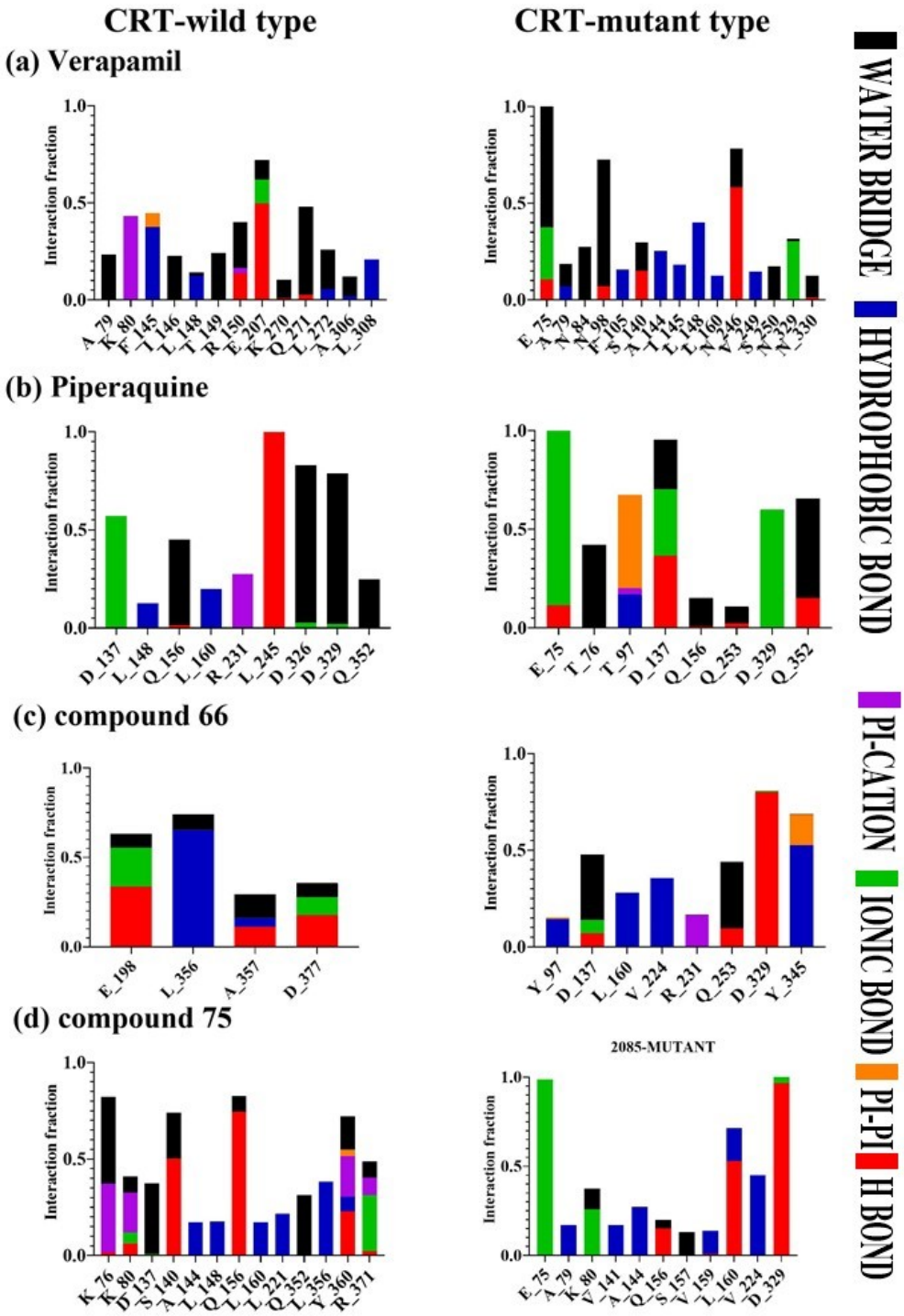


Figure 2S. Residue interaction plot of the ligands (**66** & **75**) with CRT proteins**.**