

**SYNTHESIS AND IMMUNOPHARMACOLOGICAL EVALUATION OF NOVEL TLR-
7 AGONISTIC TRIAZOLE TETHERED IMIDAZOQUINOLINES**

**Ayyappa Avoni^{a,b,¶}, Sravanthi Vemireddy^{a,¶}, Shainy Sambyal^a, Syed Shafi^c, Imran
Khan^c, Halmuthur M. Sampath Kumar,^{*,a,b}**

Affiliations

*^aVaccine Immunology Laboratory, OSPC Division, CSIR-Indian Institute of Chemical
Technology, Hyderabad 500007, india*

*^bAcademy of Scientific and Innovative Research (AcSIR), Ghaziabad Uttar Pradesh 201 002,
India*

^cDepartment of Chemistry, Hamdard Univeristy, Hamdard Nagar, New Delhi, Delhi 110062

¶ Equal Contribution

**** Corresponding author:***

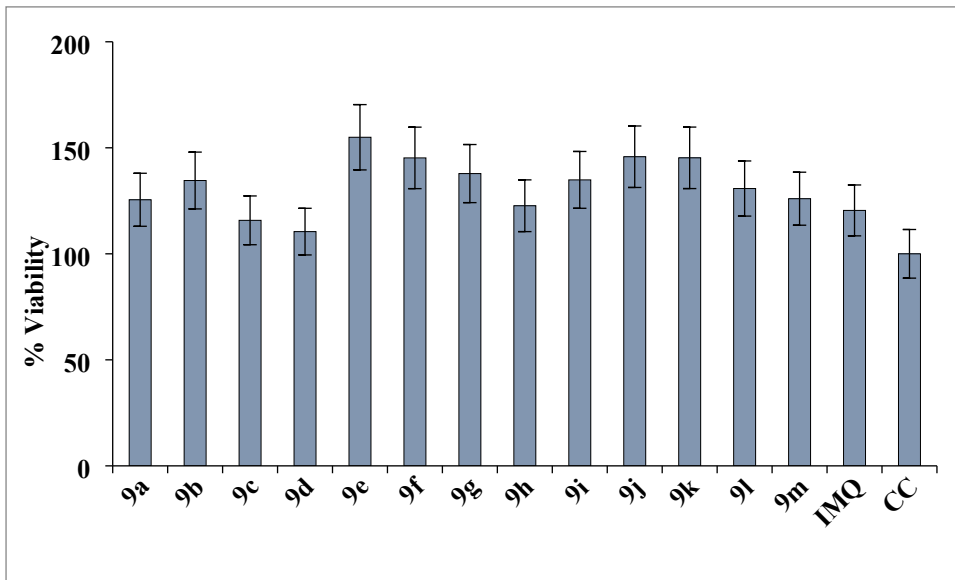
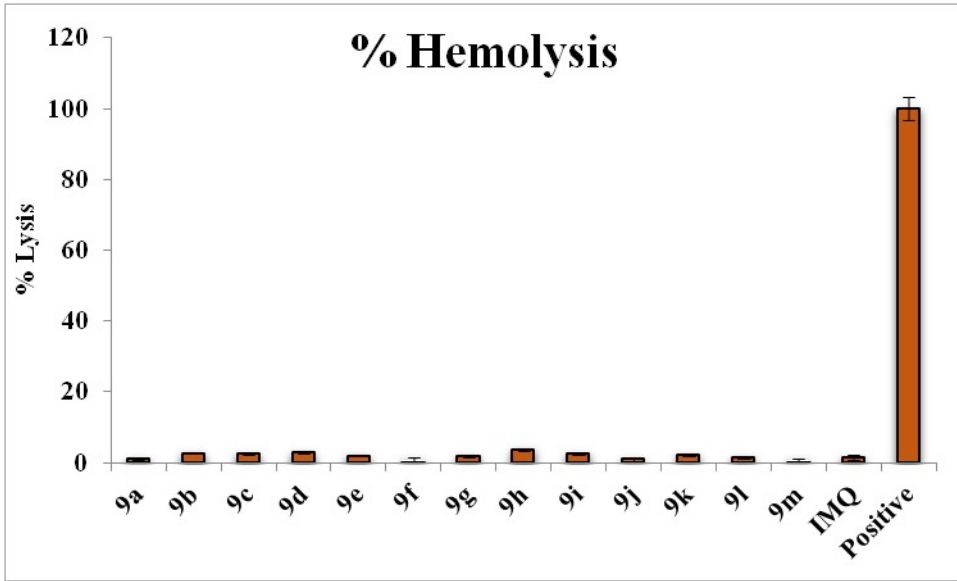
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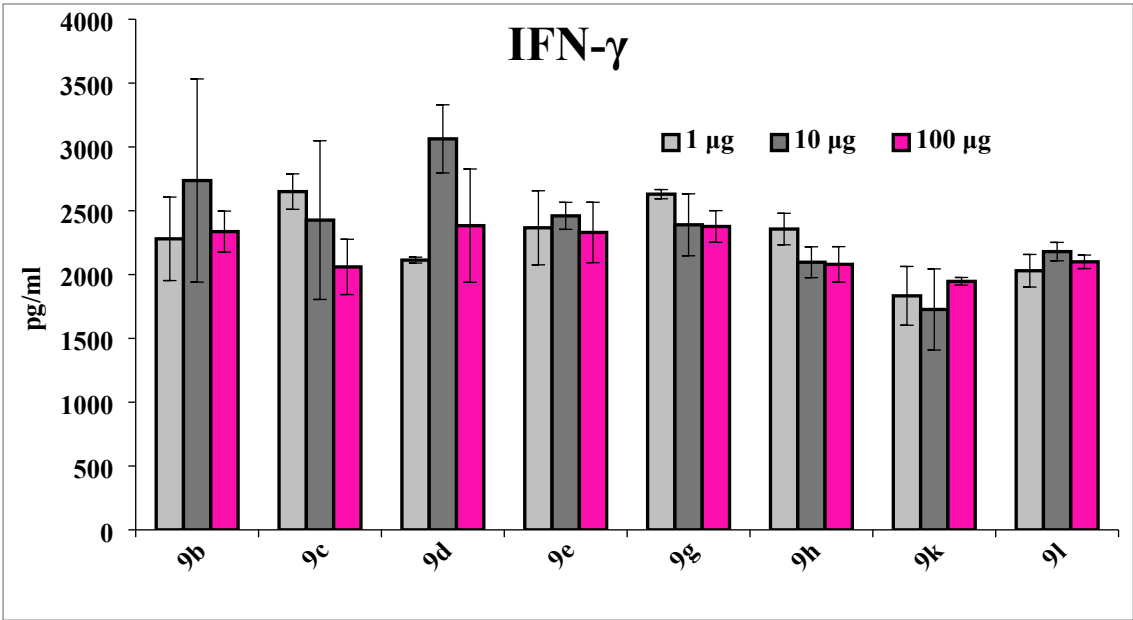
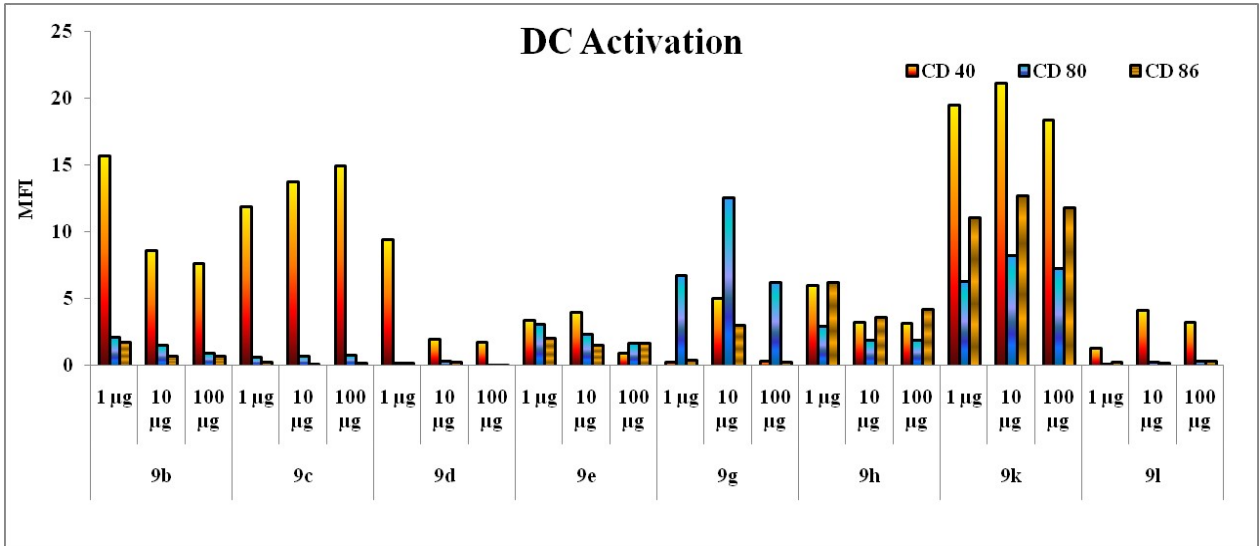
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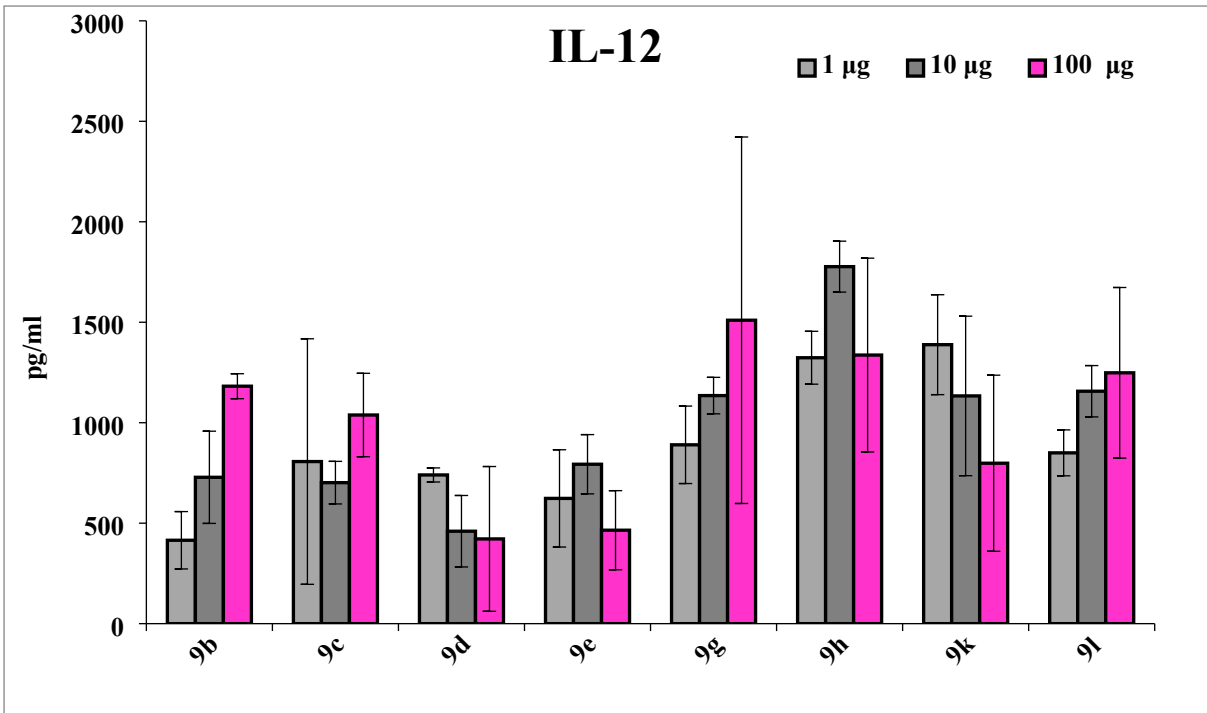
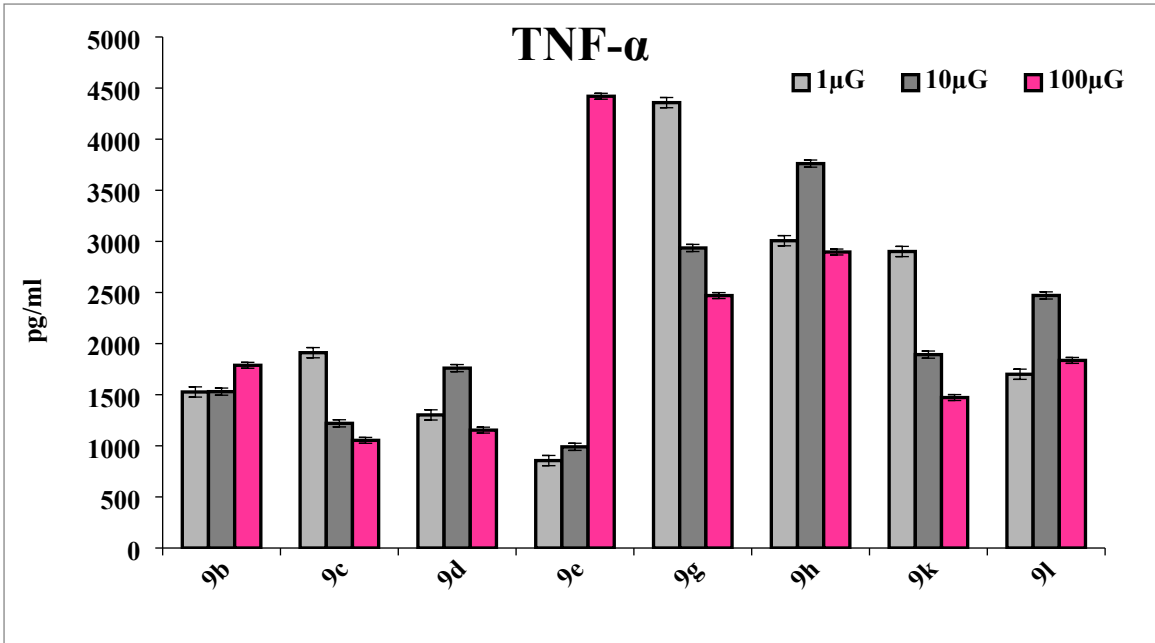
OSPC Division, CSIR-Indian Institute of Chemical Technology, Hyderabad - 500007, India

Email: sampath.iict@gov.in, Phone: +914027191824; Fax: +91-40-27160387

SUPPORTING INFORMATION







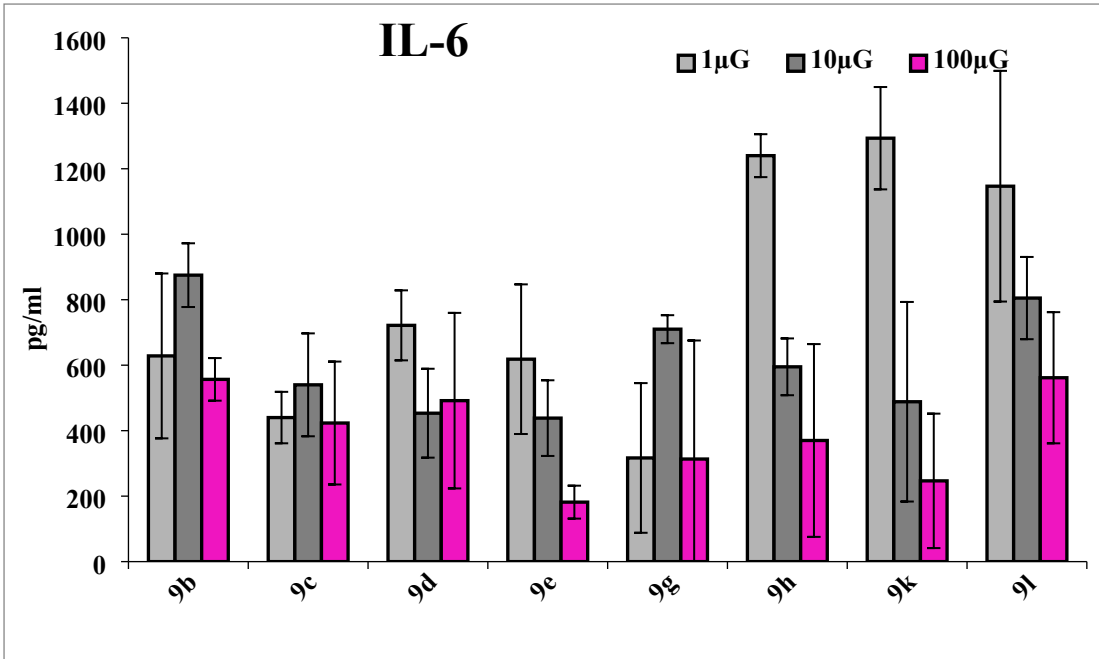


Figure S1: - Hemolysis, cytotoxicity assay, Cell surface marker and cytokine quantification from stimulated DCs.

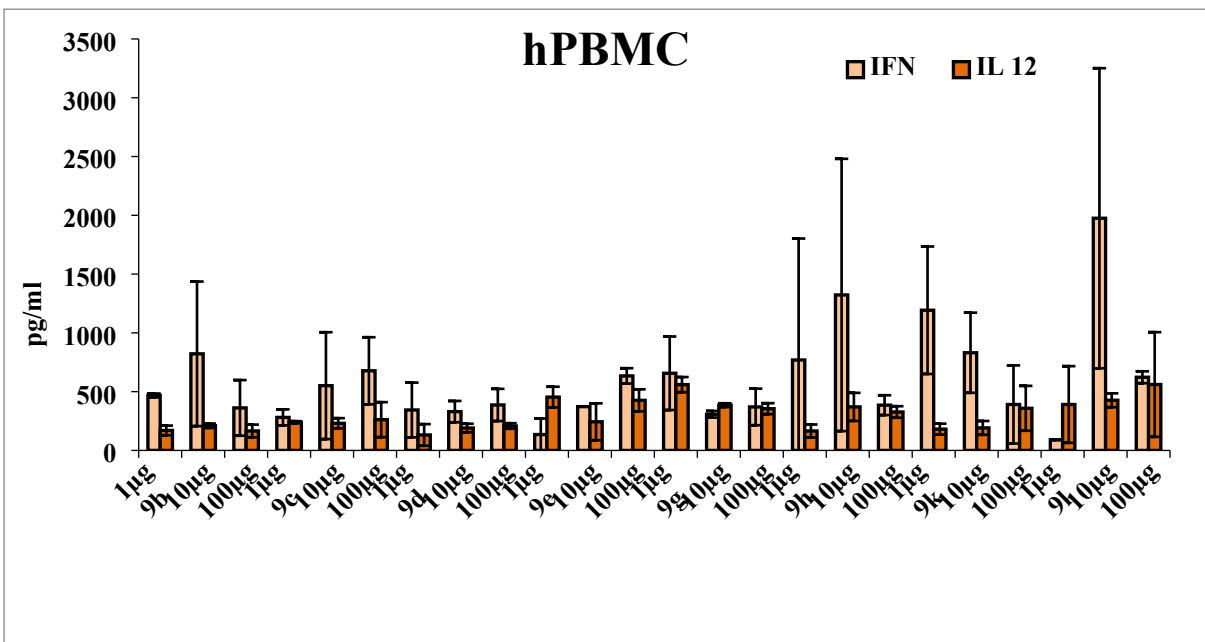
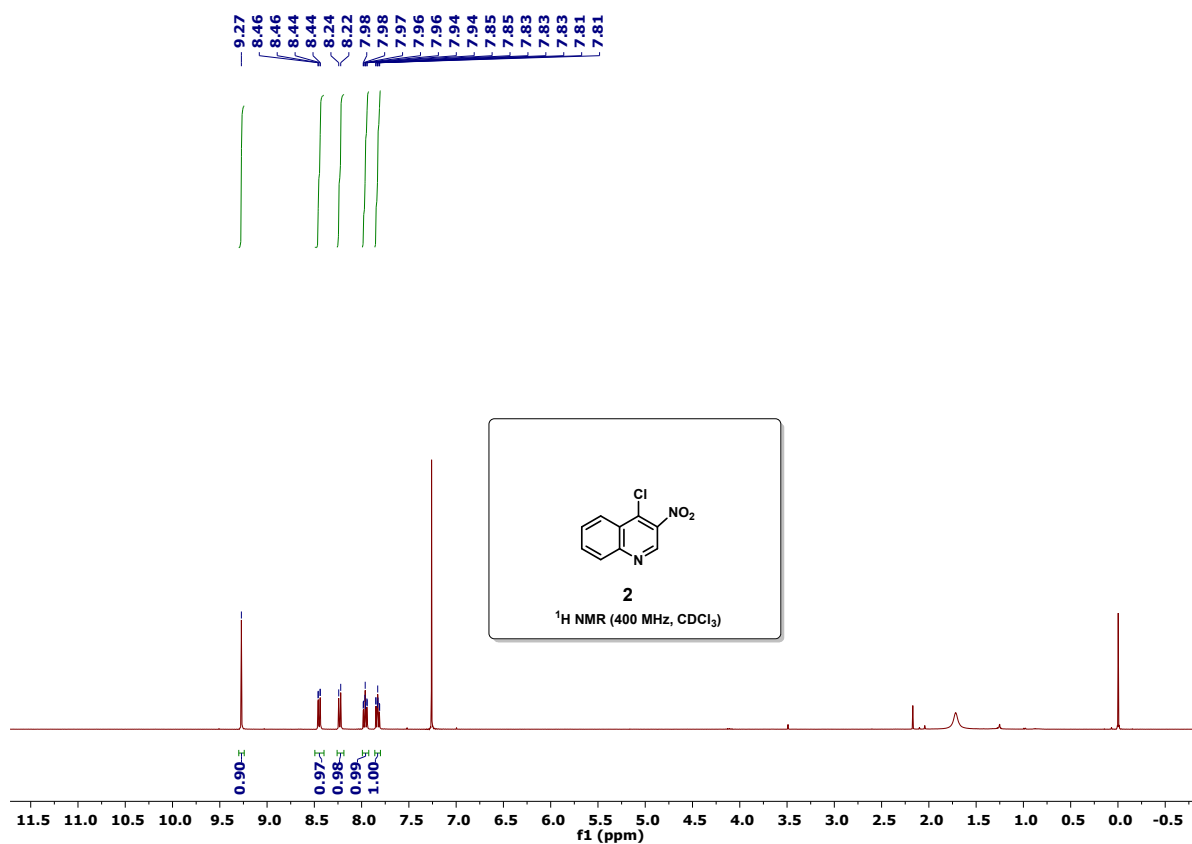


Figure S2 - IFN and IL-12 cytokine quantification from hPBMC supernatant

Spectral Copies of ^1H , ^{13}C NMR, MS (ESI) and HRMS data of Compounds:

2. 4-chloro-3-nitroquinoline



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LabSolutions

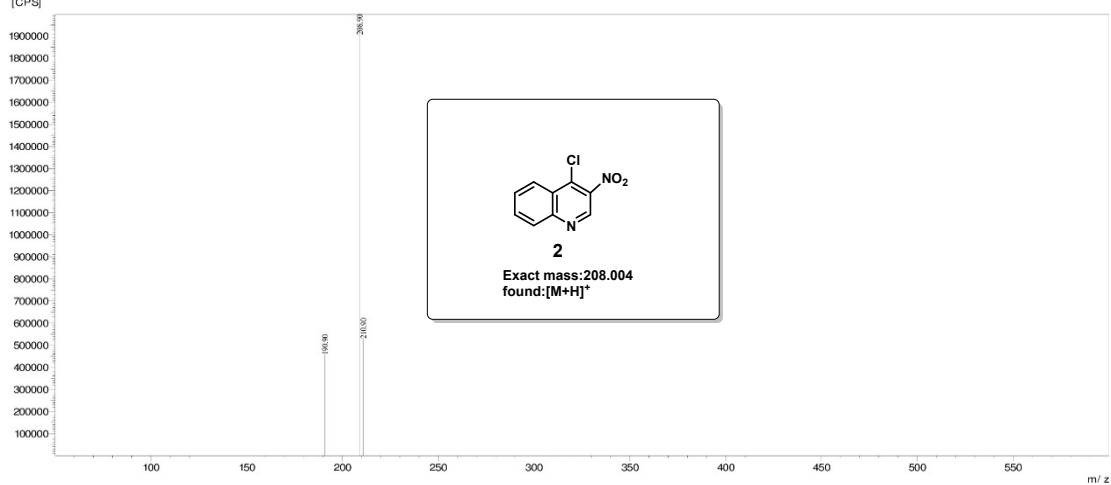
MASS REPORT

OSPC, Dr RATHOD

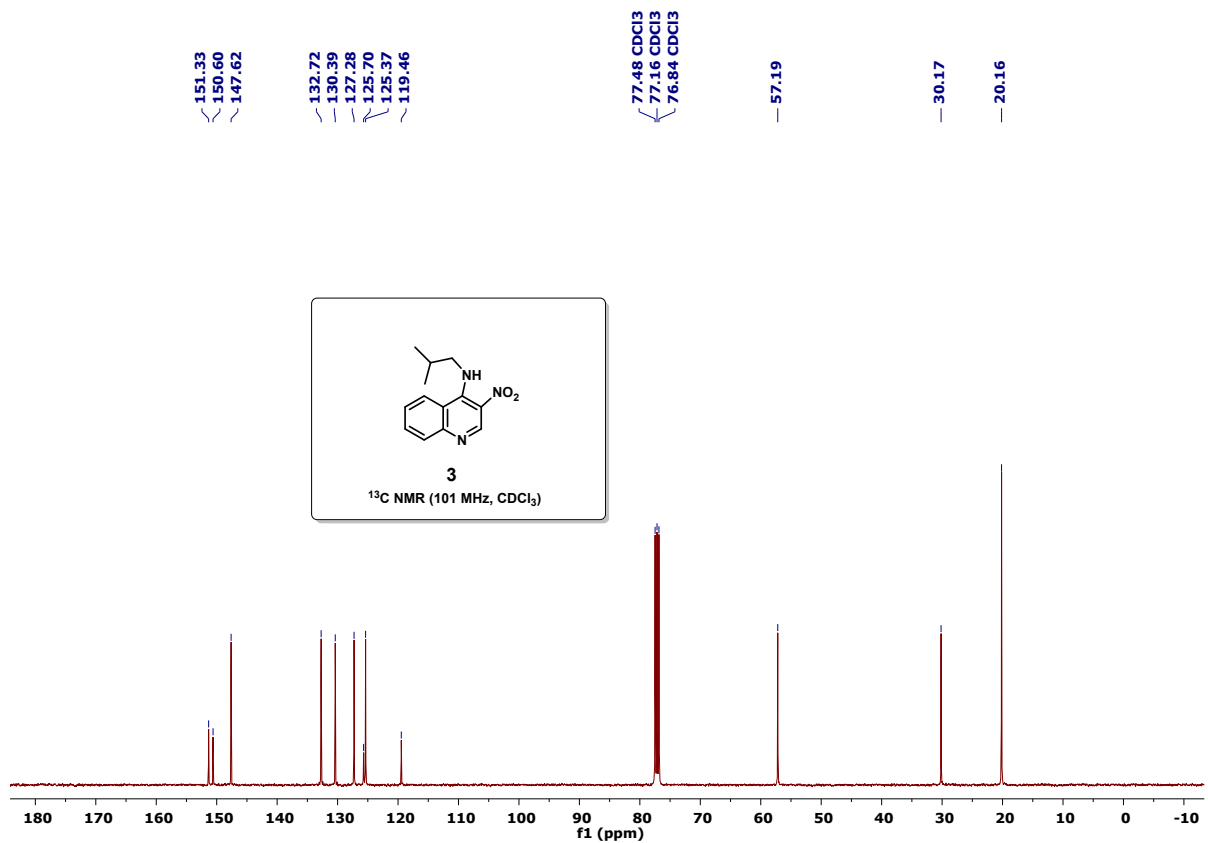
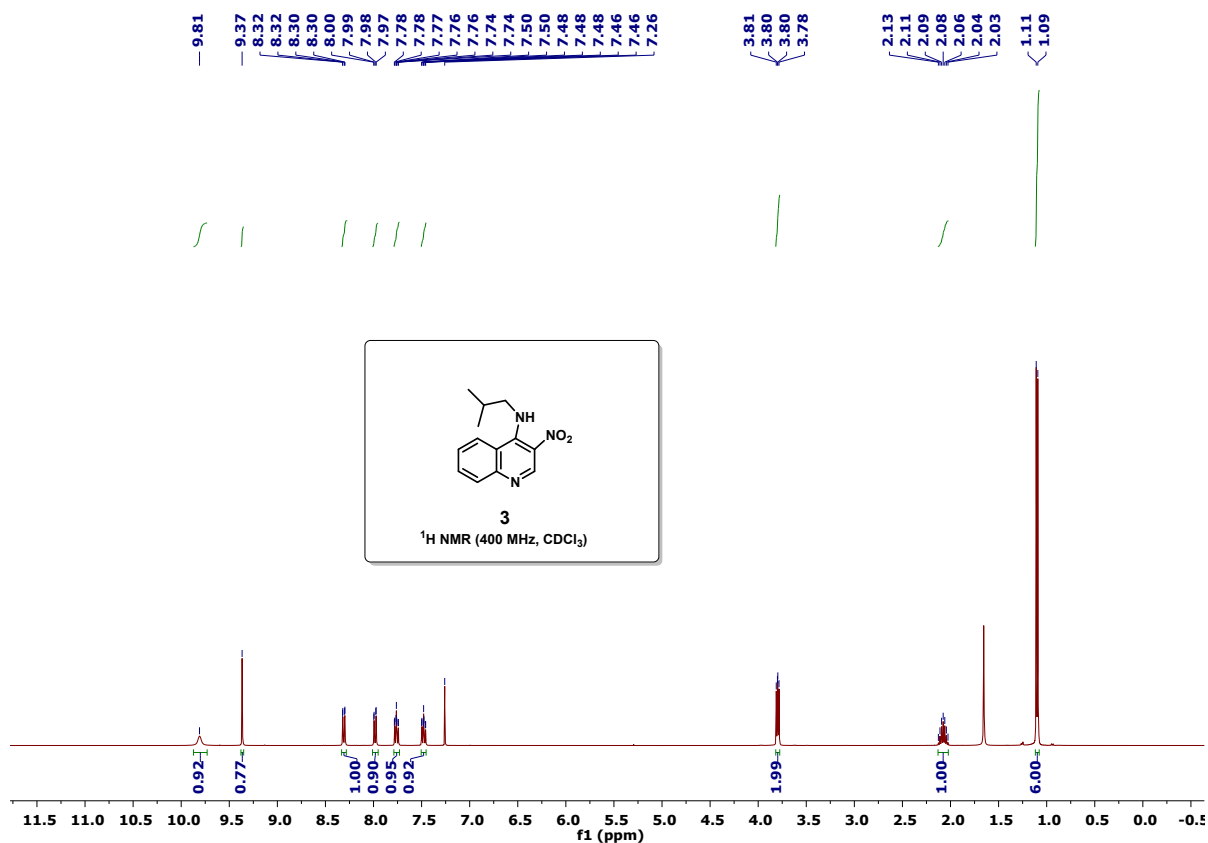
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Batch File : 23112020.lcb

MS Spectrum
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Averaged ESI Positive+
Spectrum Mode: Averaged 0.420-0.812(181-349)
[GPS]



3. 4-isobutylamino-3-nitroquinoline

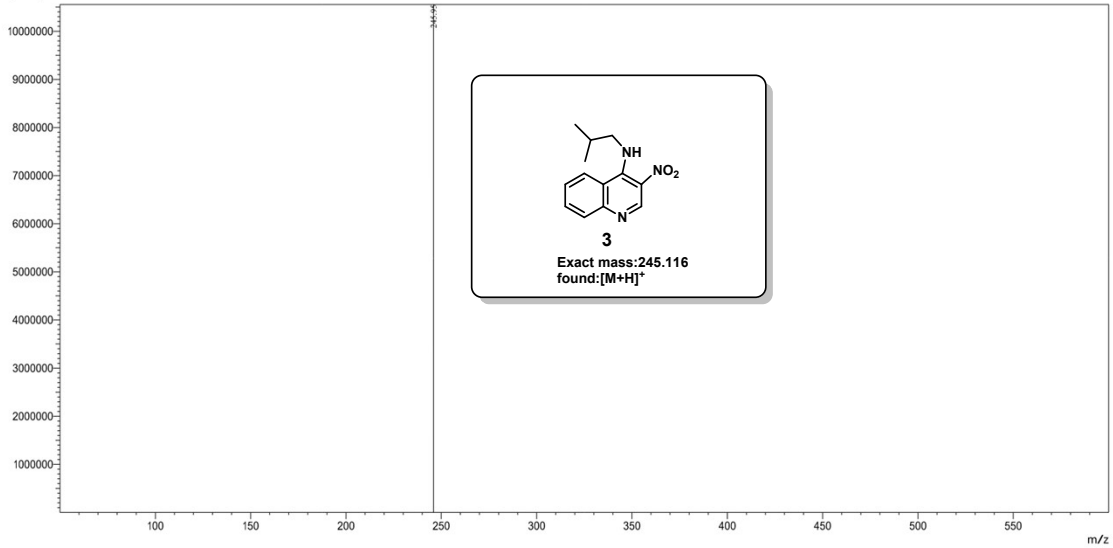


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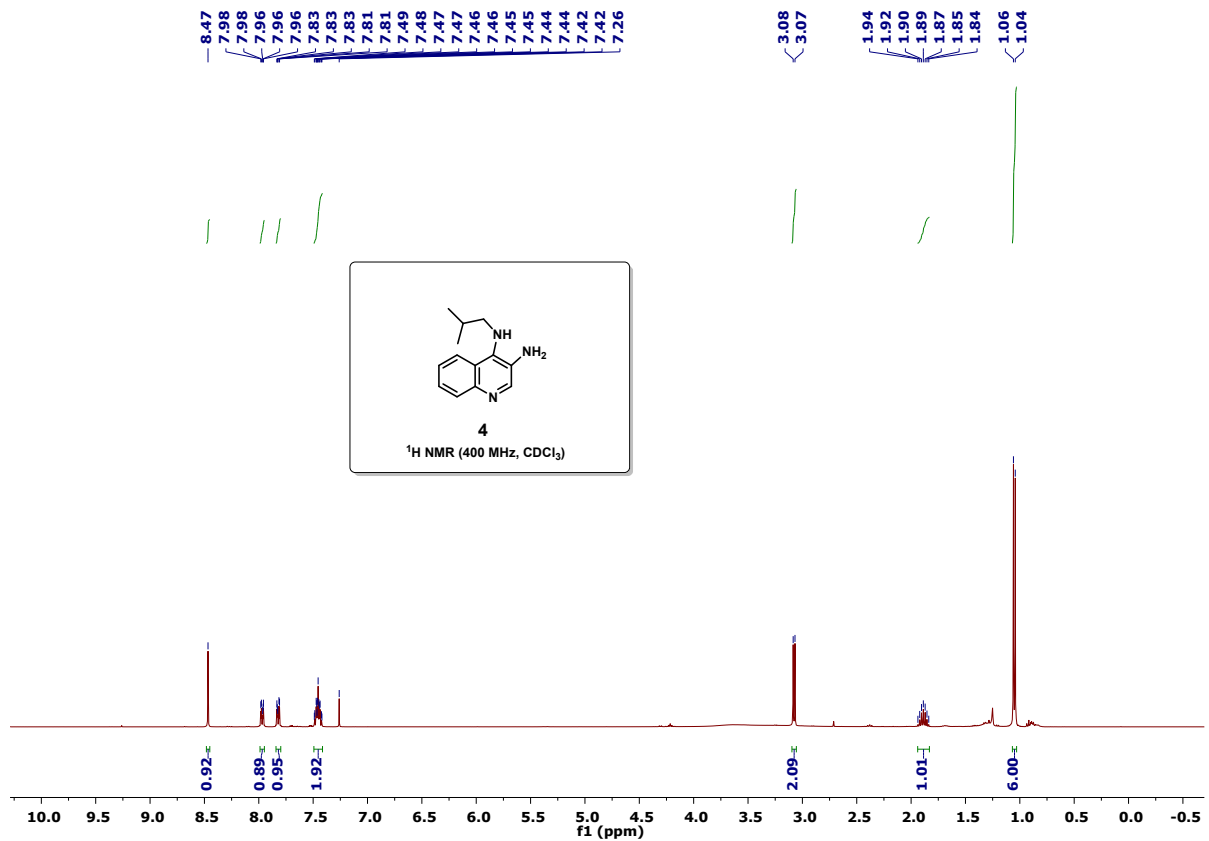
MS Spectrum
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Averaged ESI Positive+
 Spectrum Mode: Averaged 0.411-0.803(177-345)
 [CPS]



1

4.3-amino-4-isobutylaminoquinoline



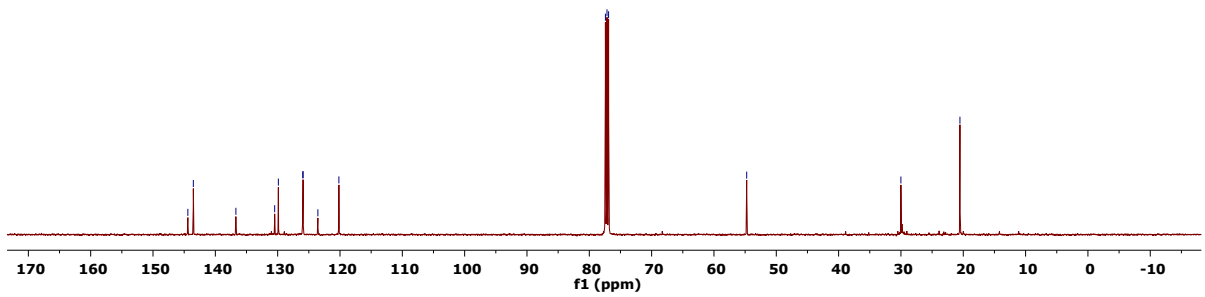
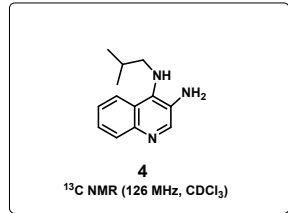
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143.53
136.70
130.48
129.89
125.97
125.92
123.55
120.18

77.41 CDCl₃
77.16 CDCl₃
76.91 CDCl₃

54.75

30.01

20.53



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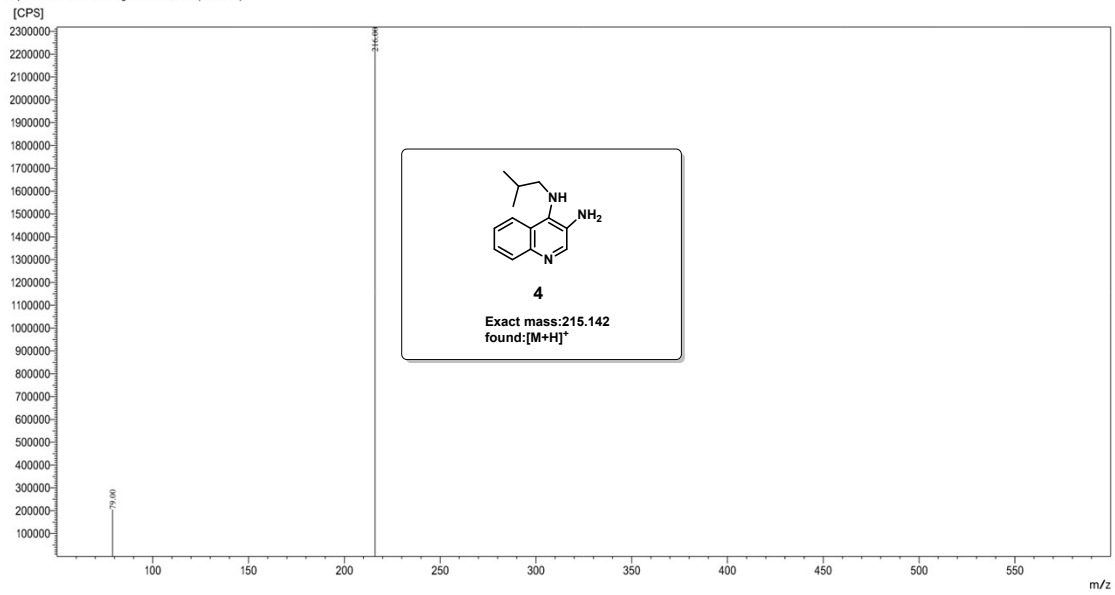
SHIMADZU
LabSolutions

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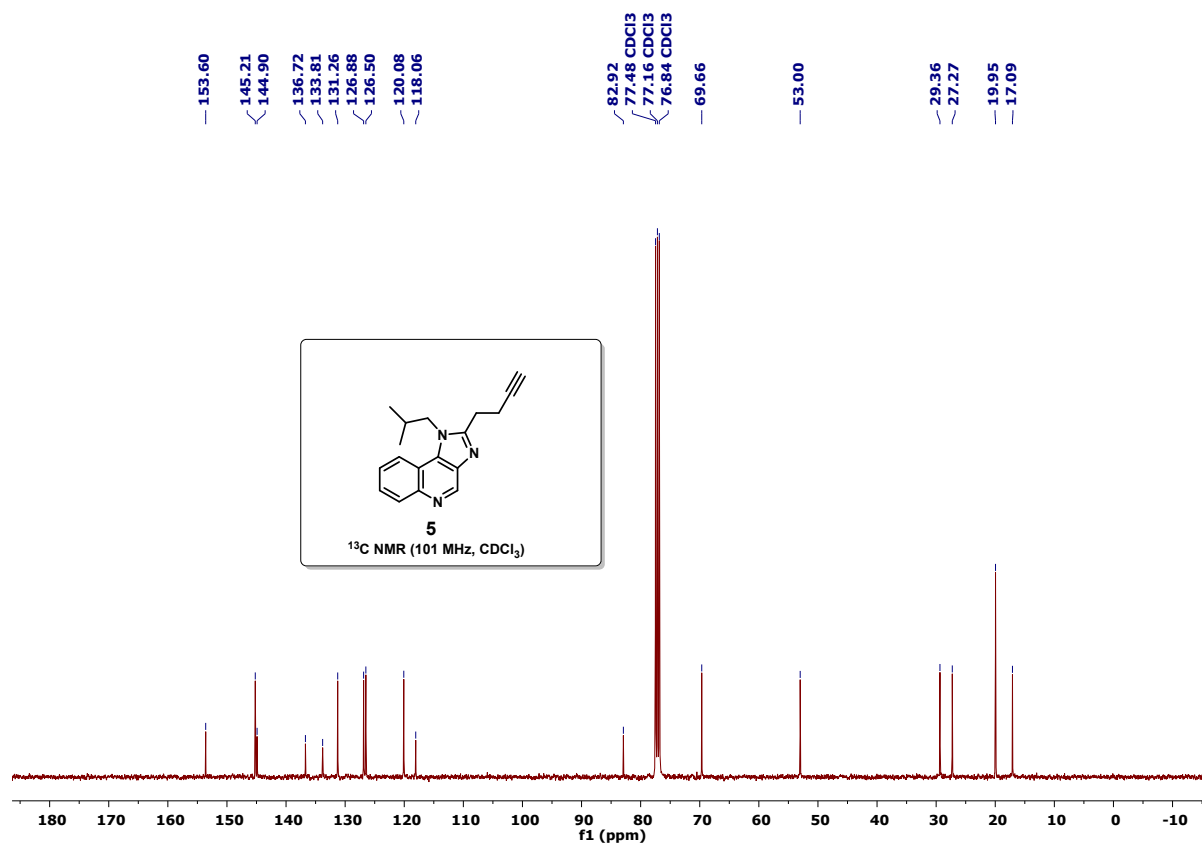
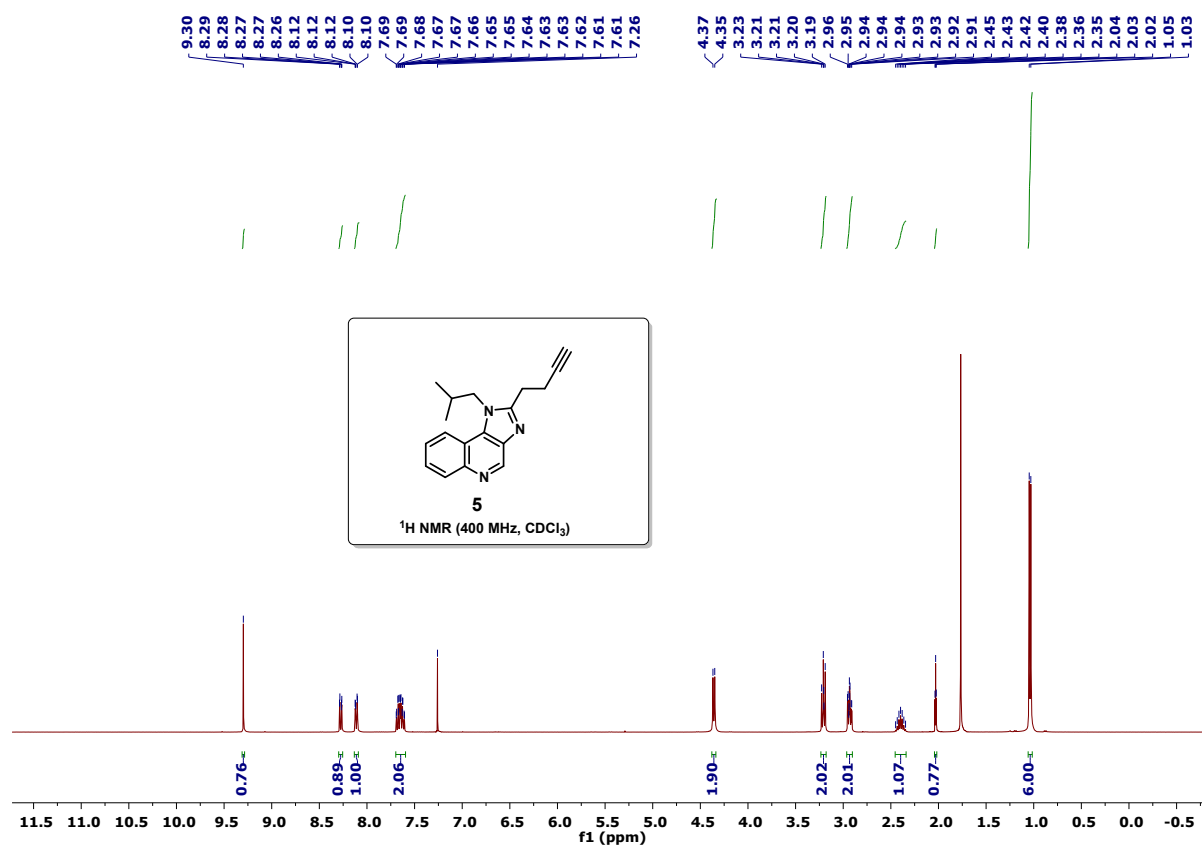
MS Spectrum
D:\DATA\NOV-2020\16112020.35.lcd

Averaged ESI Positive+
Spectrum Mode: Averaged 0.415-0.779(179-335)



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5. 2-(but-3-yn-1-yl)-1-isobutyl-1H-imidazo[4,5-c]quinoline

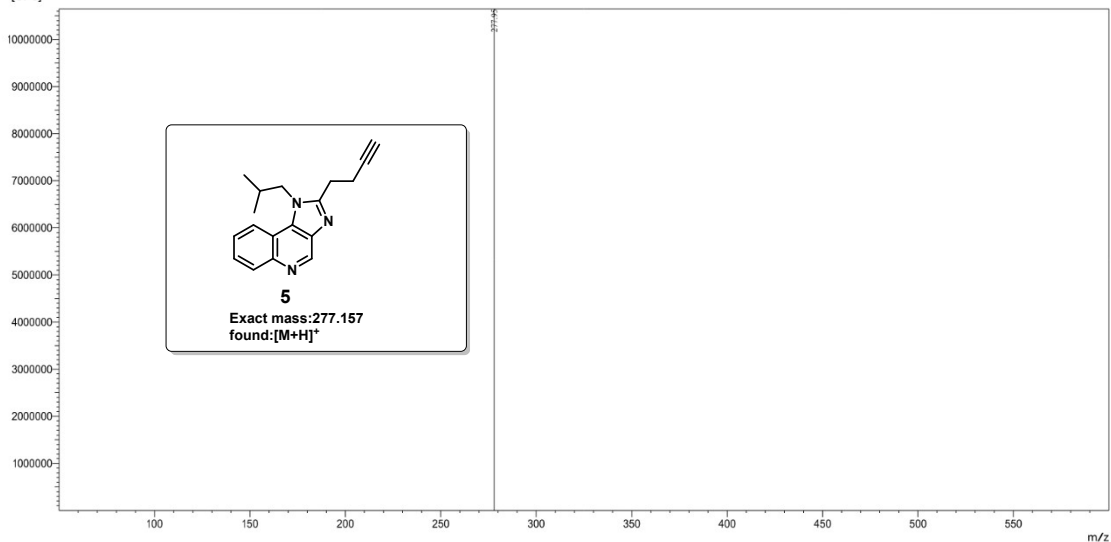


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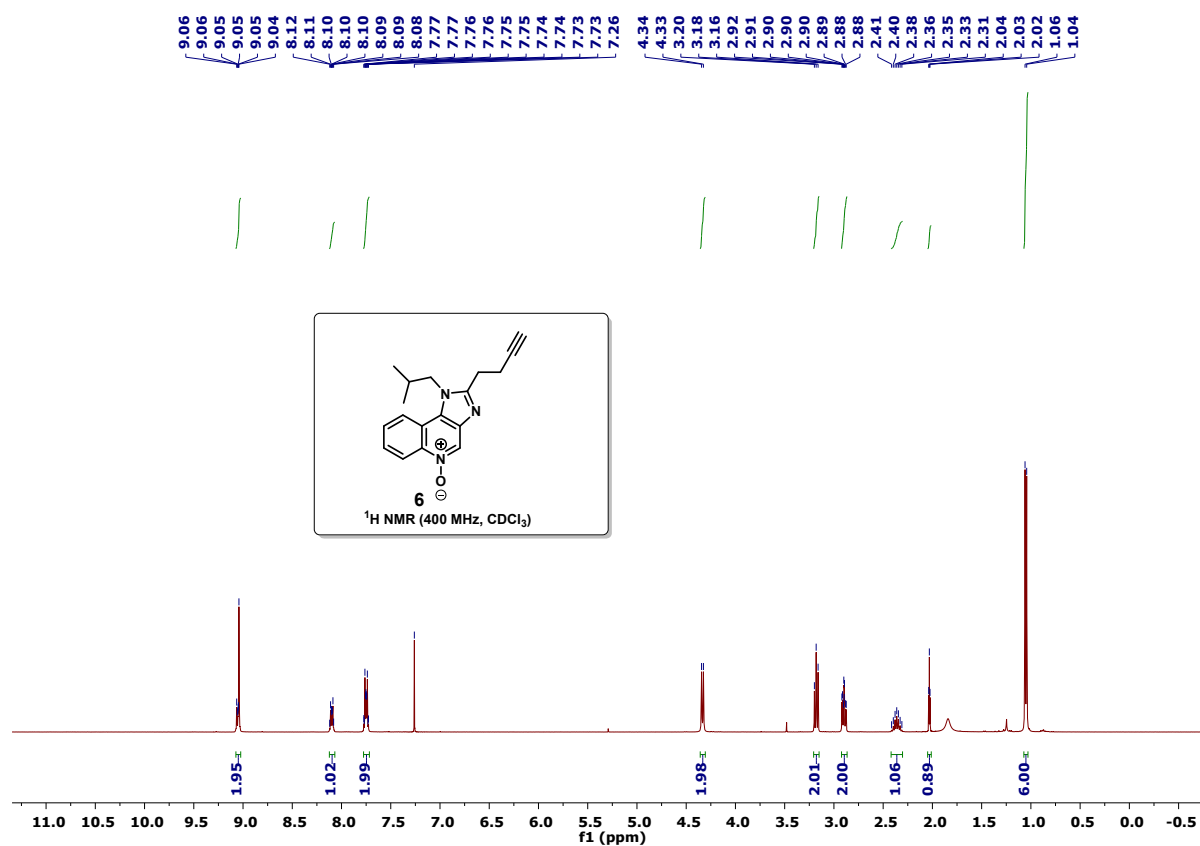
MS Spectrum
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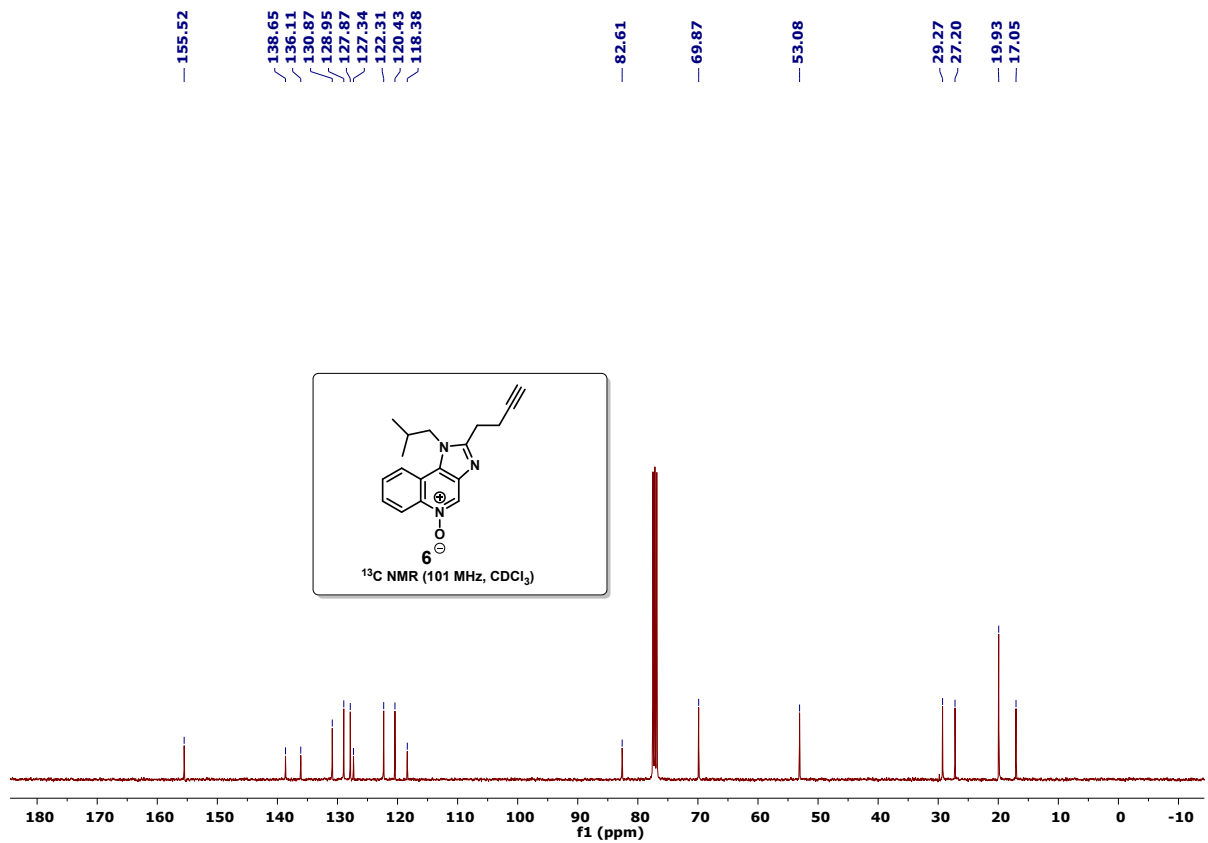
Averaged ESI Positive+
 Spectrum Mode: Averaged 0.401-0.817(173-351)
 [CPS]



1

6. 2-(but-3-yn-1-yl)-1-isobutyl-1H-imidazo[4,5-c]quinoline 5-oxide





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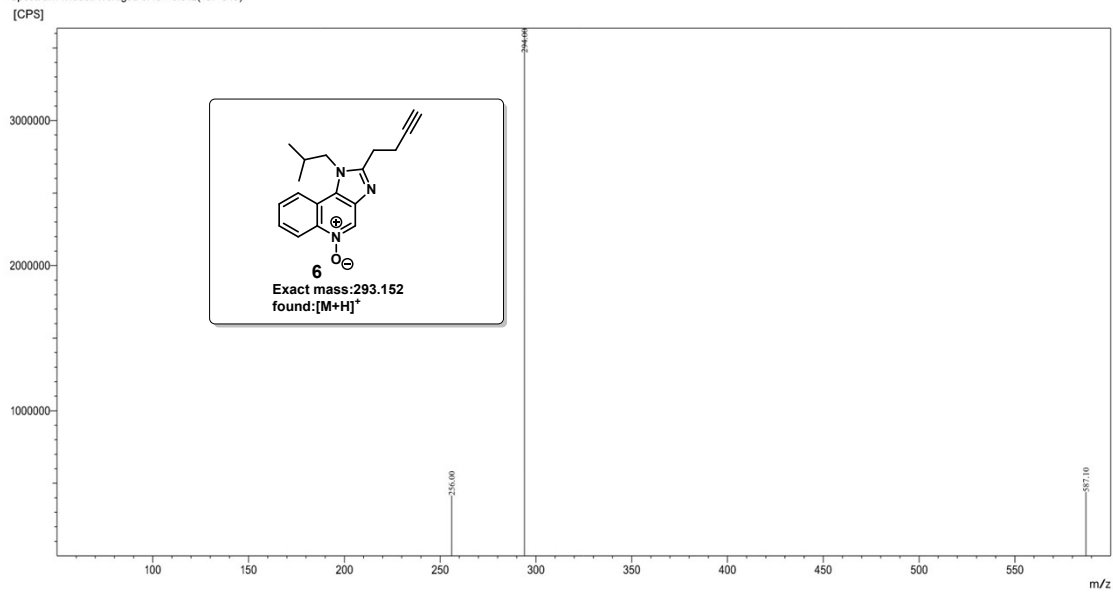
SHIMADZU
LabSolutions

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 Batch File : 18122020.1cb

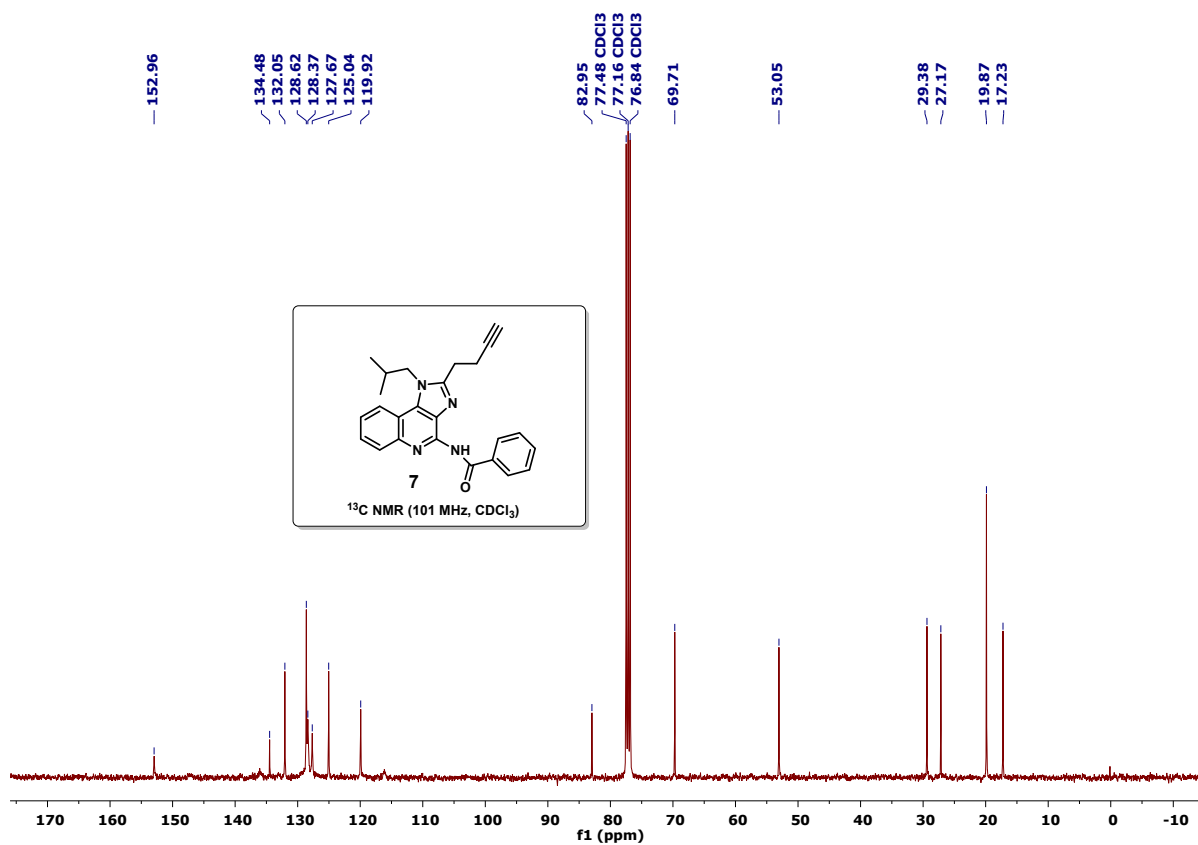
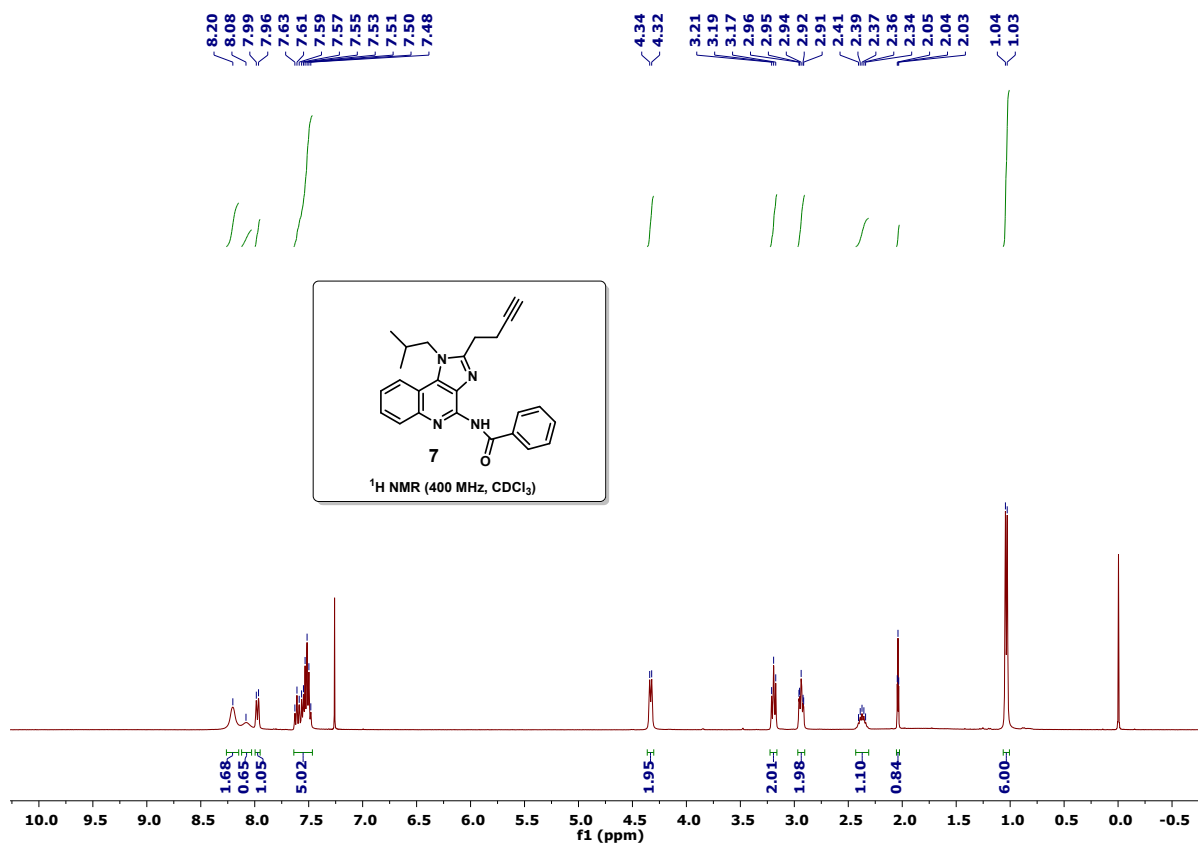
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Averaged ESI Positive+
 Spectrum Mode: Averaged 0.434-0.812(187-349)



1

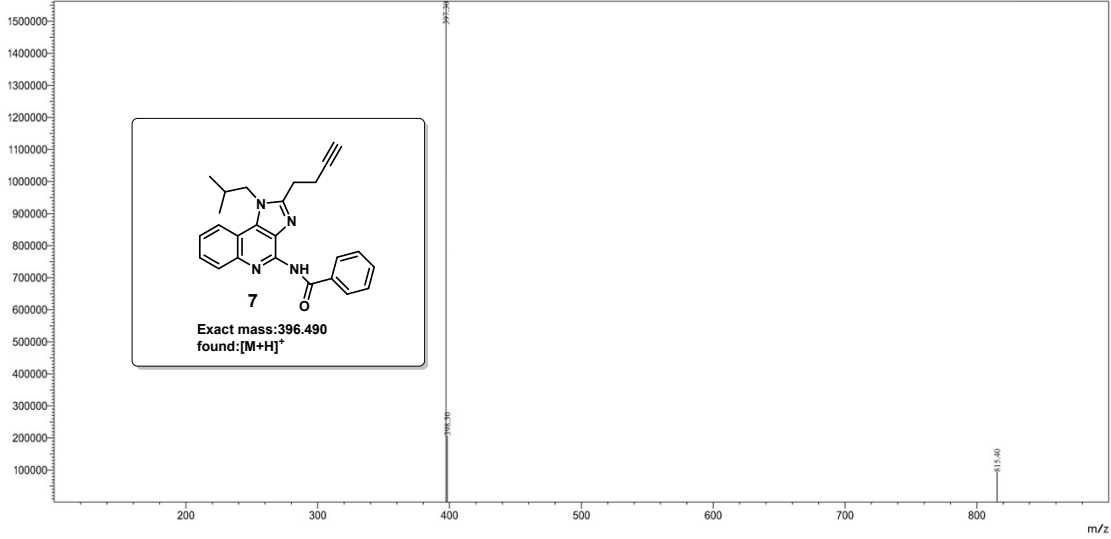
7. N-(2-(but-3-yn-1-yl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-yl)benzamide



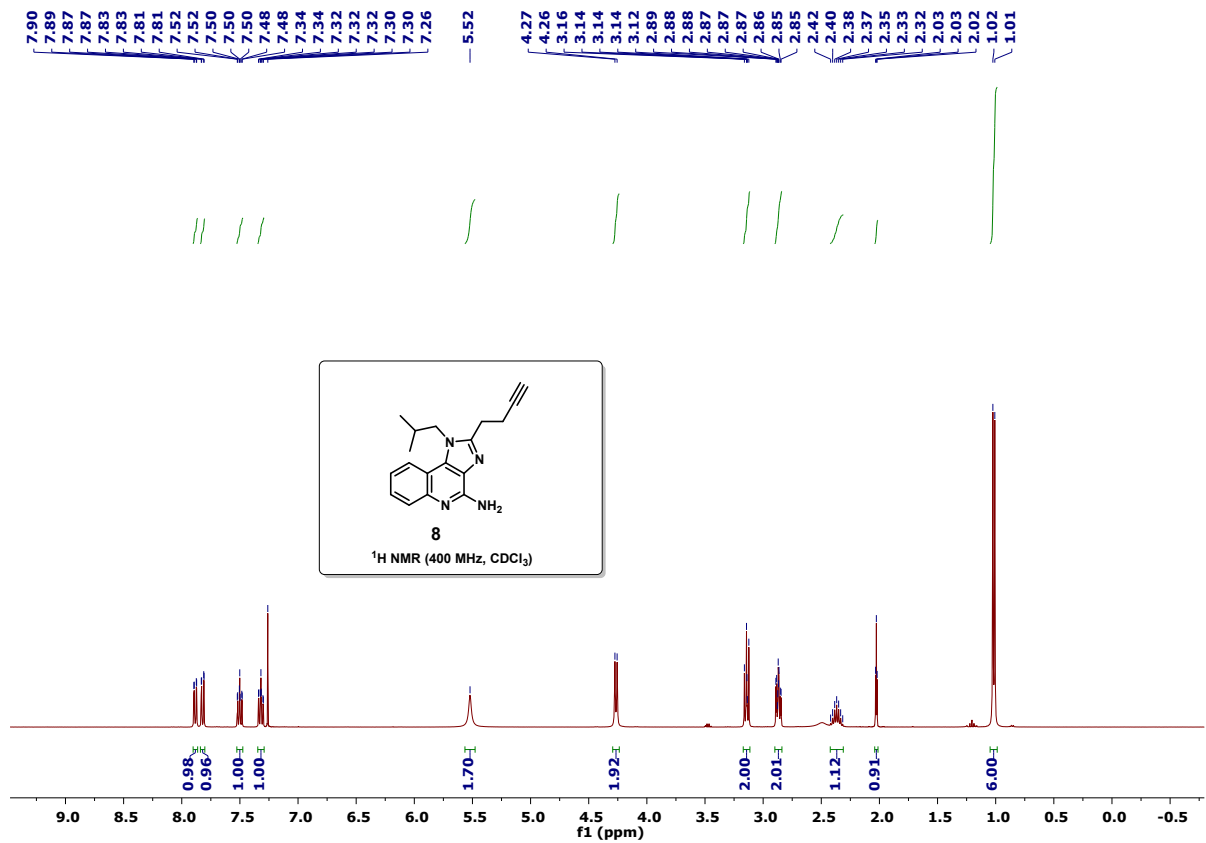
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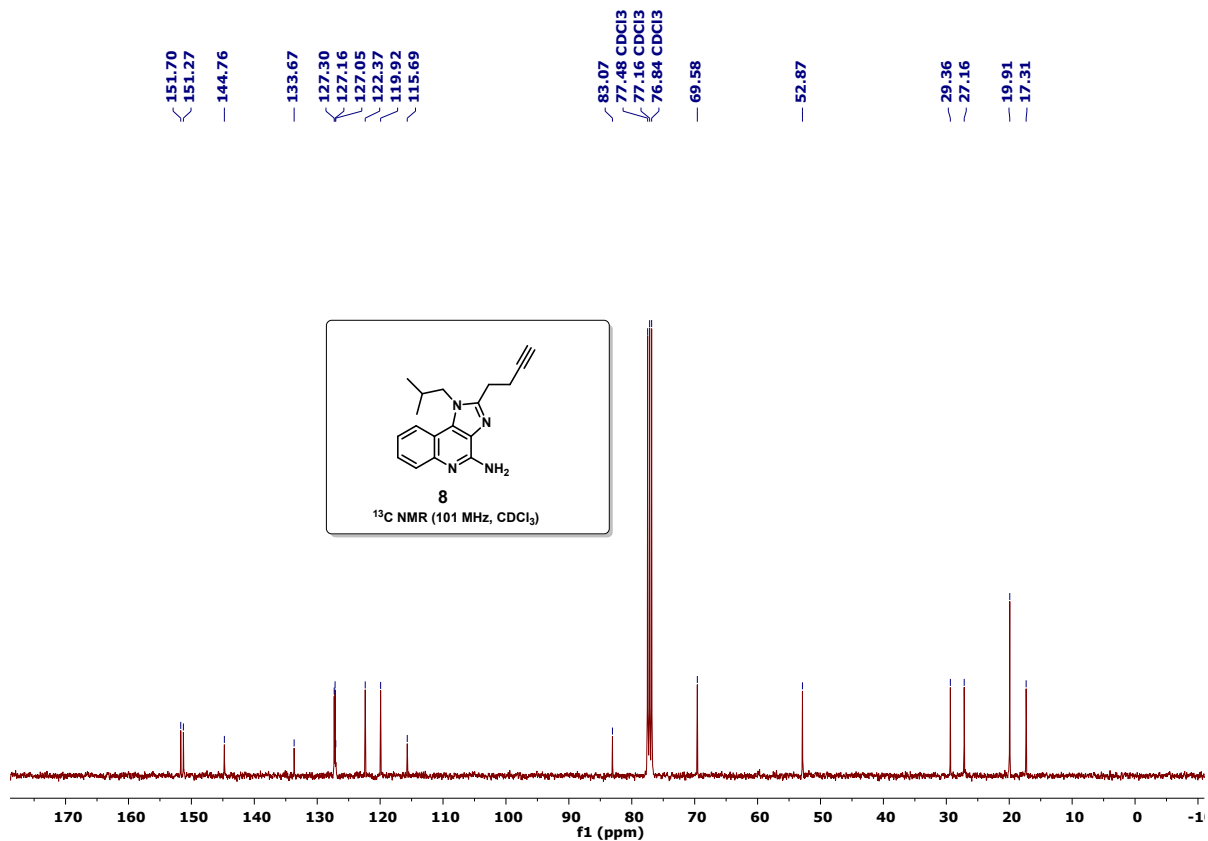
MS Spectrum
D:\DATA\JULY-2022\29072022.2.lcd

SingleESI Positive+
Spectrum Mode:Single 0.338(21)
[CPS]



8. 2-(but-3-yn-1-yl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine





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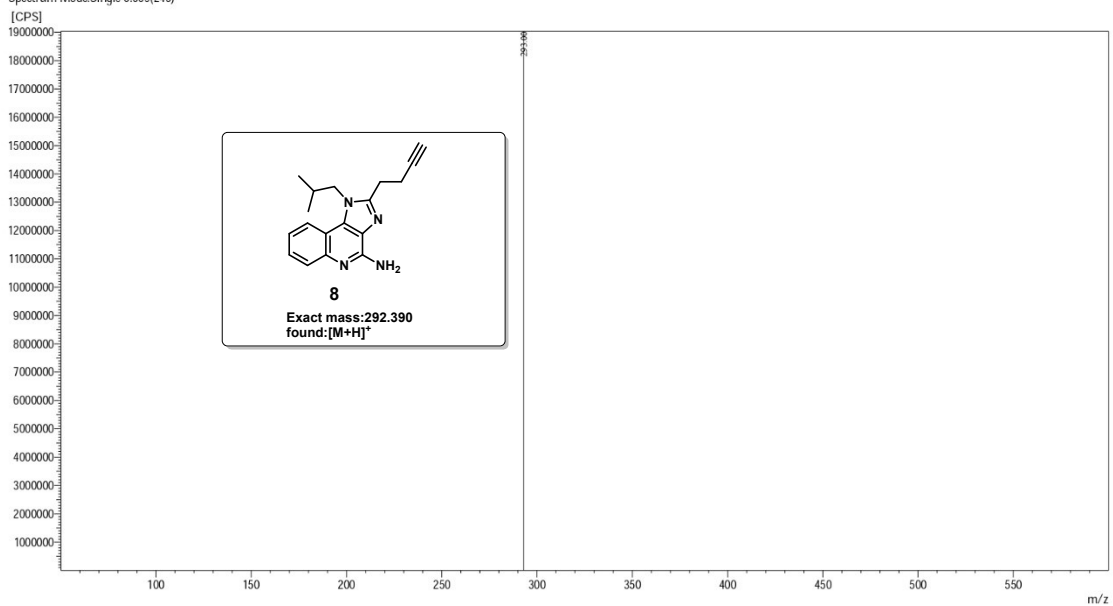
SHIMADZU
LabSolutions

MASS REPORT

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Date Acquired : 1/29/2021 1:27:19 PM
Batch File : 29012021.lcb

MS Spectrum
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Single ESI Positive+
Spectrum Mode: Single 0.569(245)



1

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Created time: 10:05:40 India Standard Time

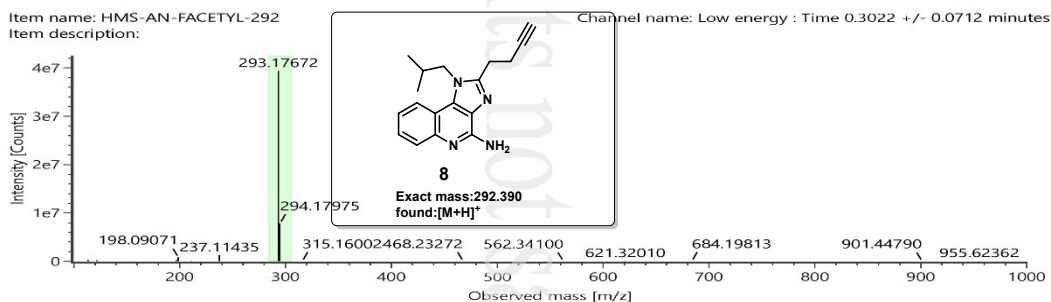
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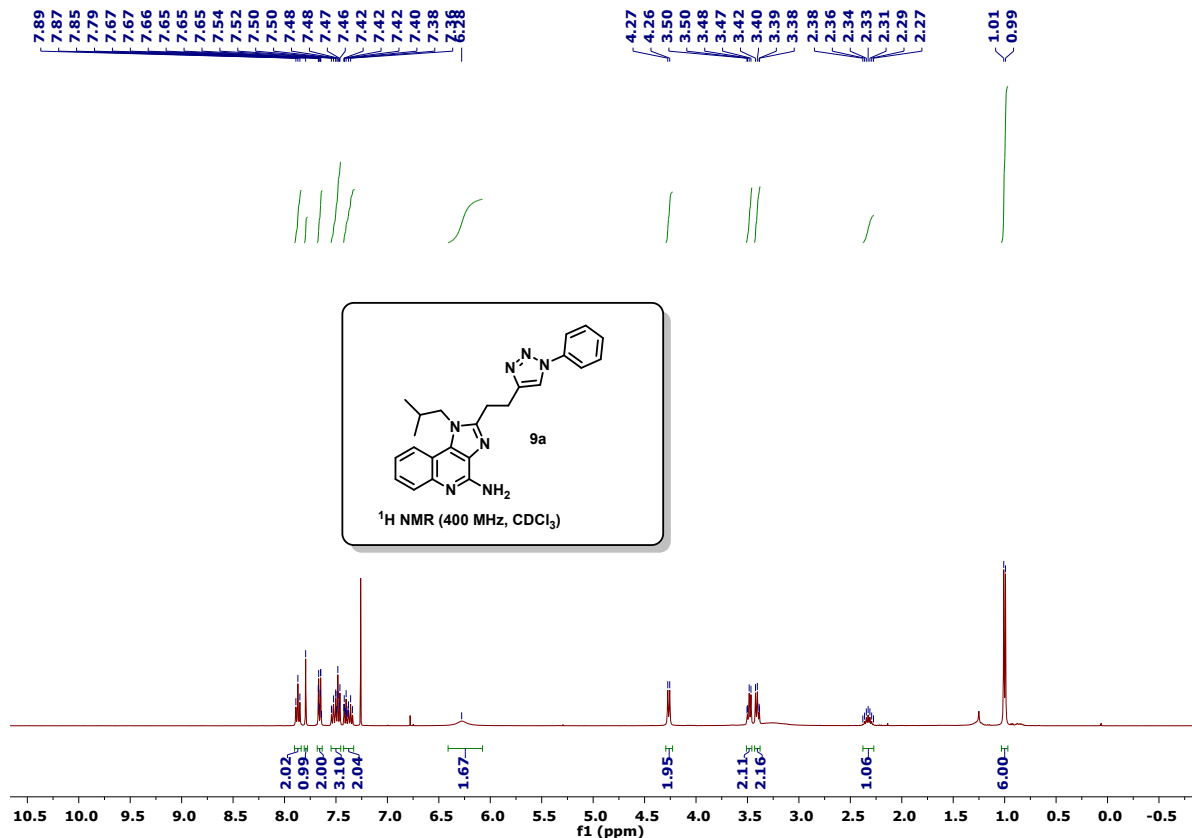
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1	C18H20N4	292.1694	292.16880	293.1767	2.2	+H

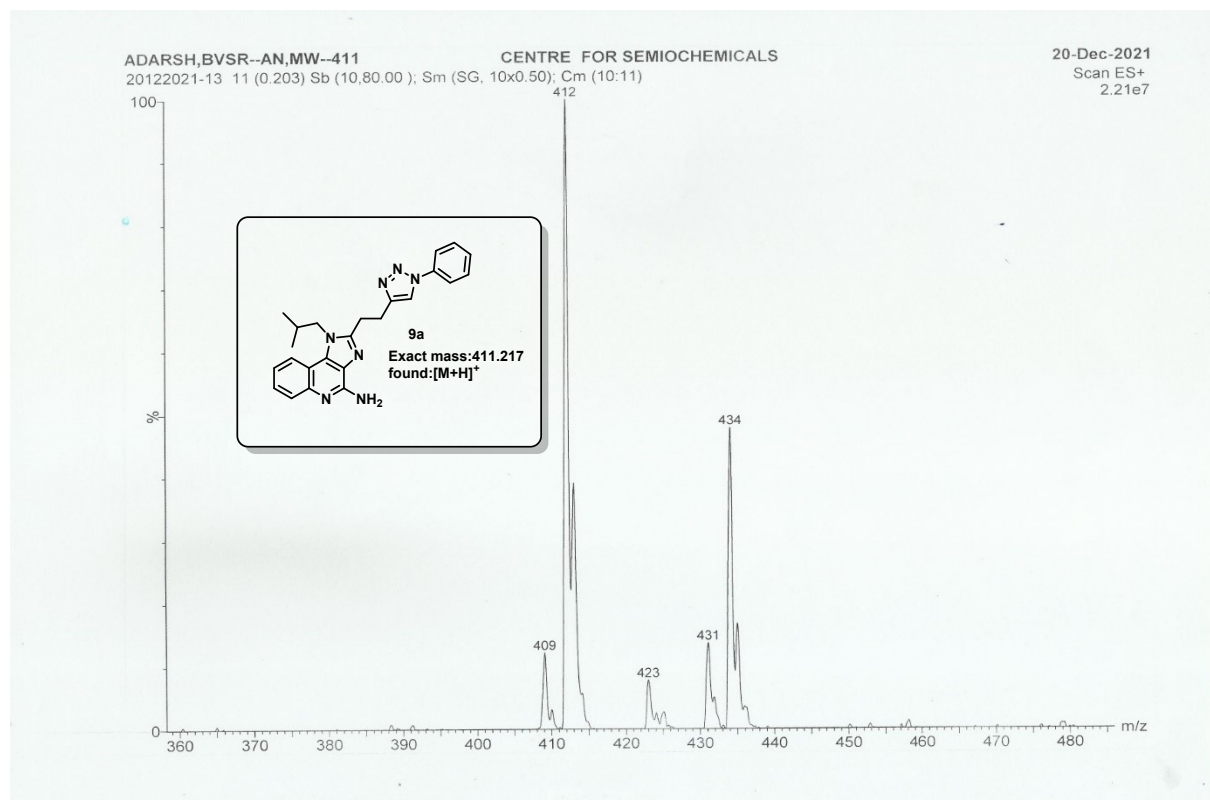
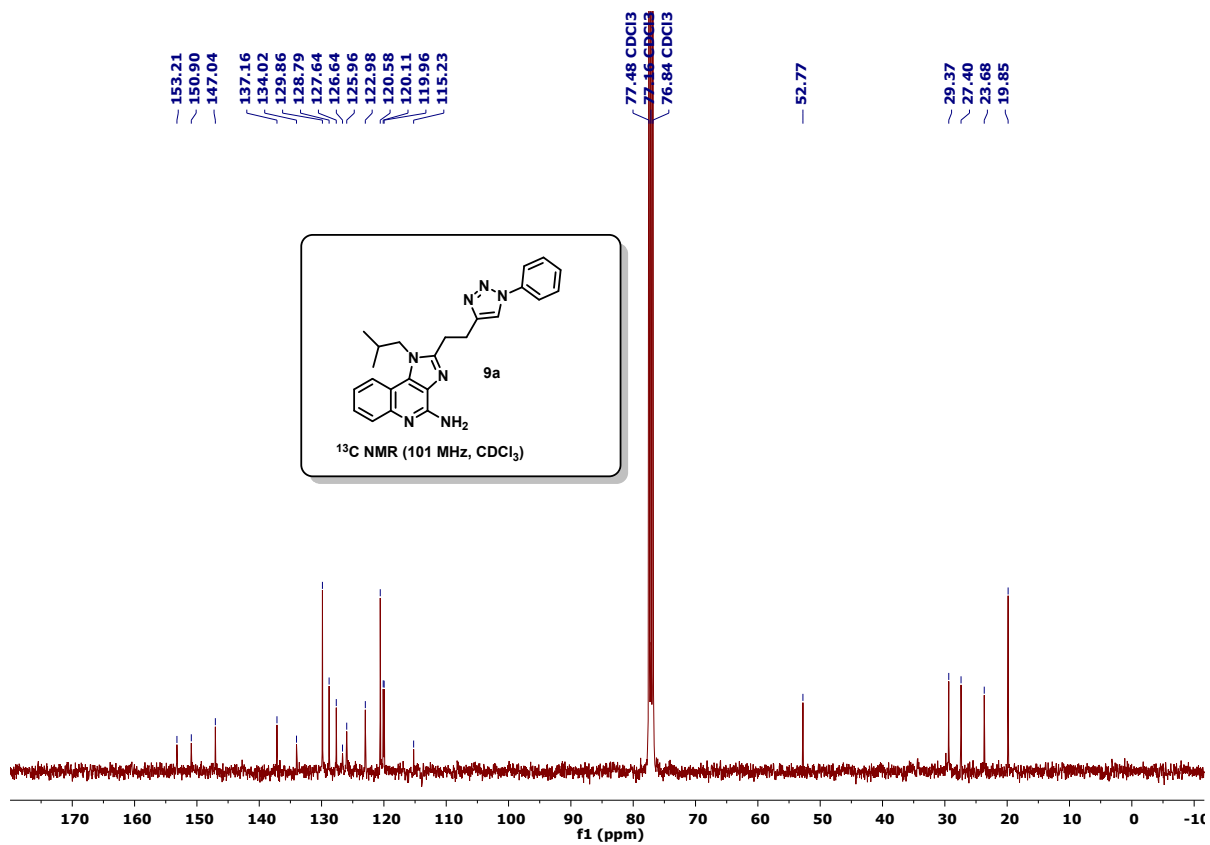
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Item name: HMS-AN-FACETYL-292
Item description:



9a. 1-isobutyl-2-(2-(1-phenyl-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine





Item name: HRMS Elemental composition Mar 07, 2022 16:47:47 India Standard Time

Created time: 17:15:49 India Standard Time

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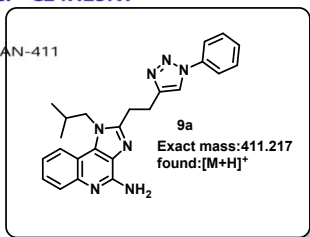
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Component name	Observed neutral mass (Da)	Neutral mass (Da)	Observed m/z	Mass error (ppm)	Adducts
C24H25N7	411.2168	411.21714	412.2240	-0.9	+H

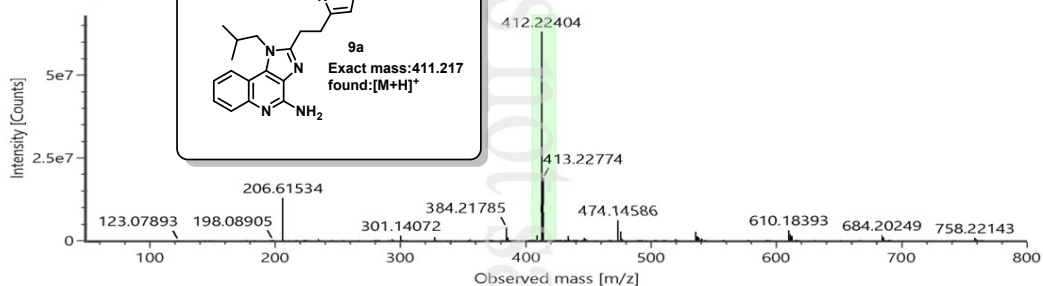
Component name: C24H25N7

Item name: HMSAN-AN-411

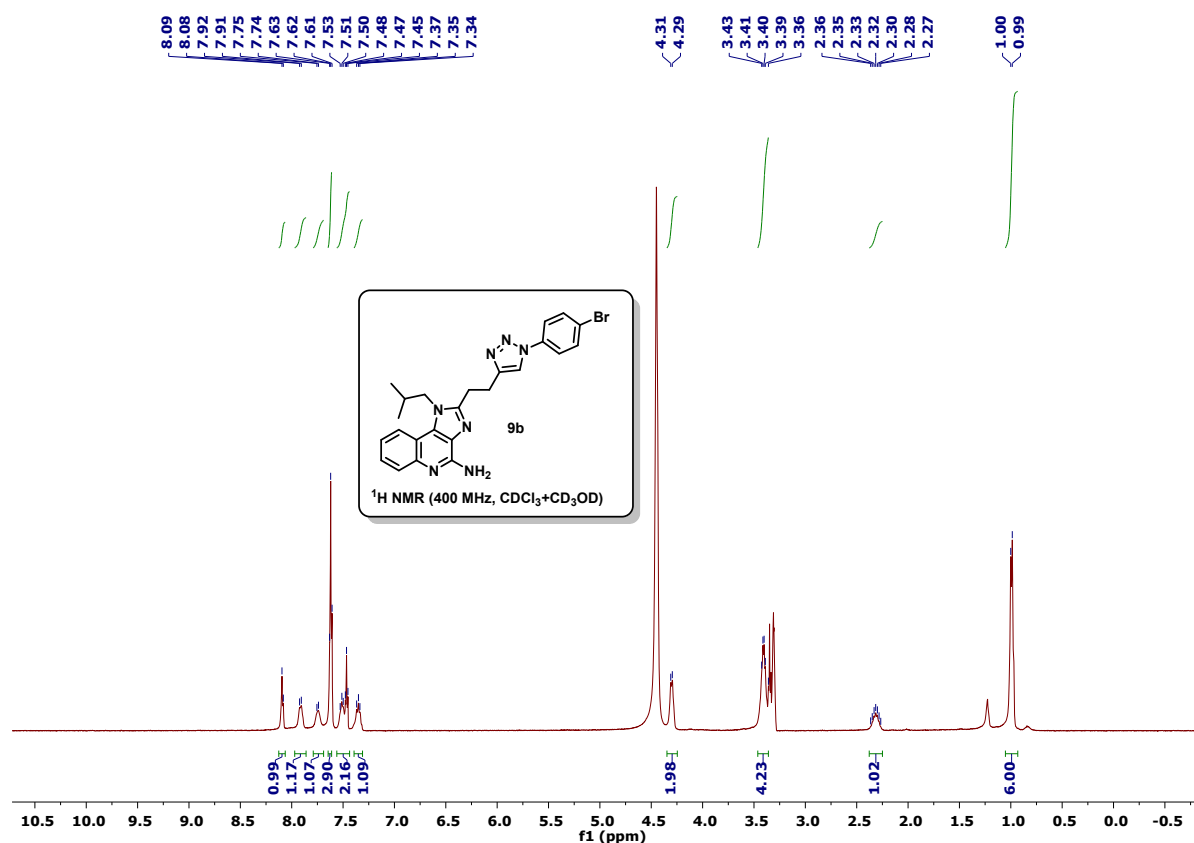
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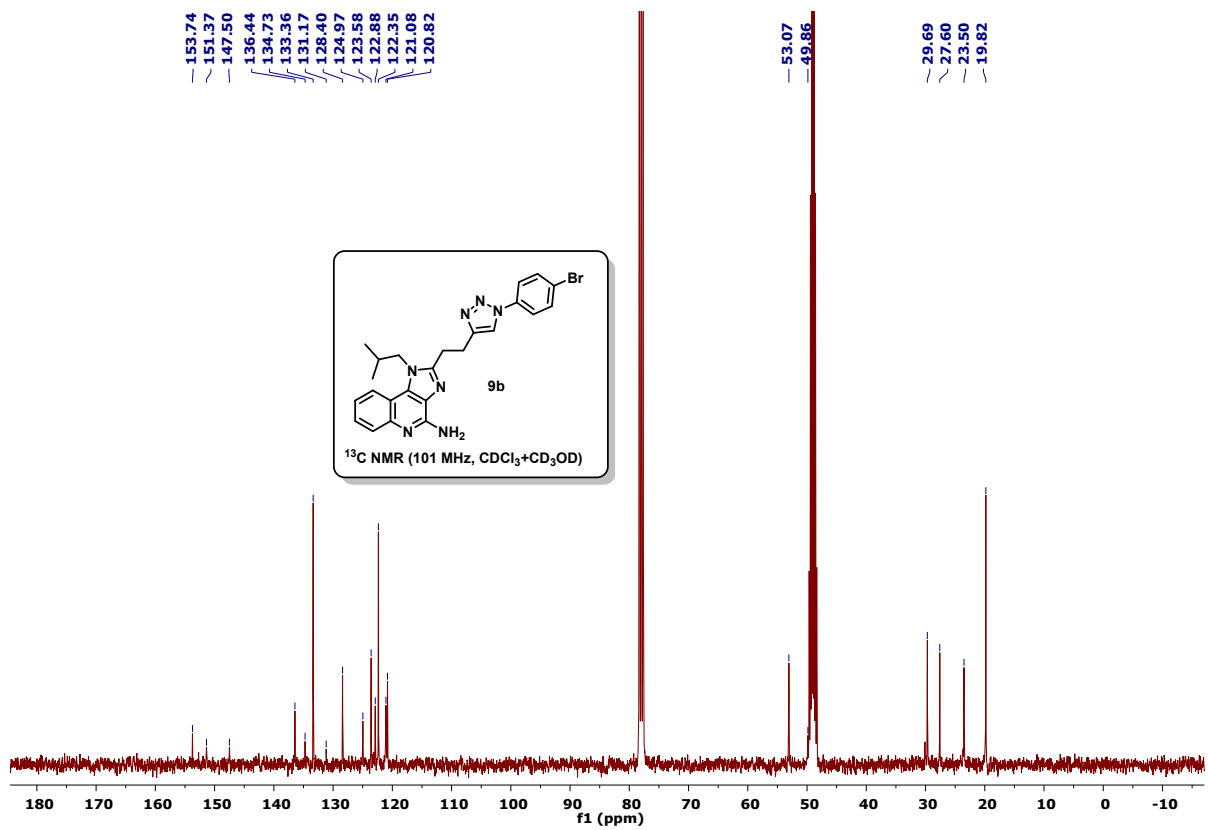


Channel name: Low energy : Time 0.3801 +/- 0.1800 minutes



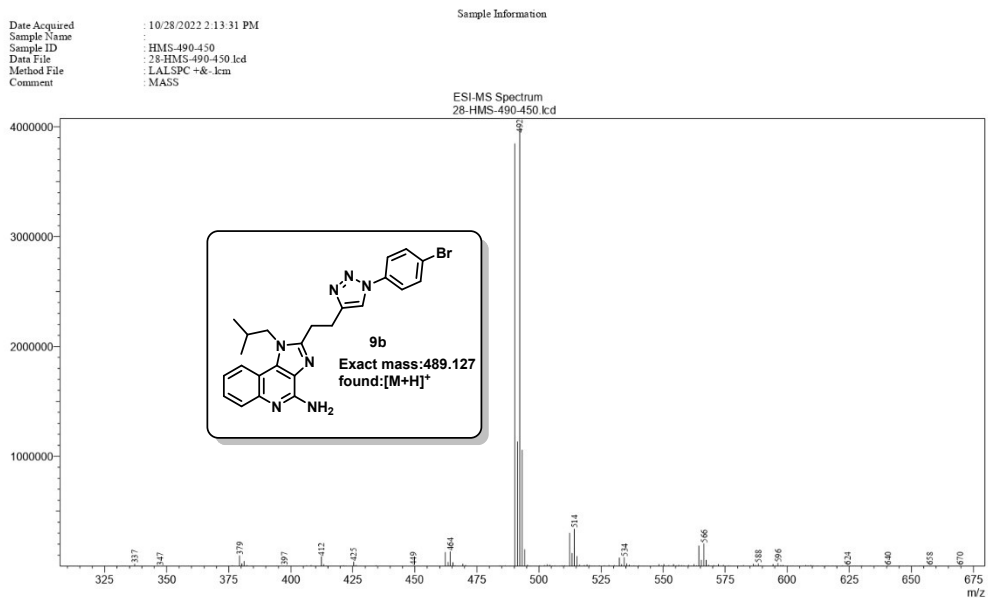
9b.2-(2-(1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine





10/28/2022 2:14:36 PM Page 1 / 1

====Department of Organic Synthesis and Process Chemistry, CSIR-IITC====



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Created time: 10:07:45 India Standard Time

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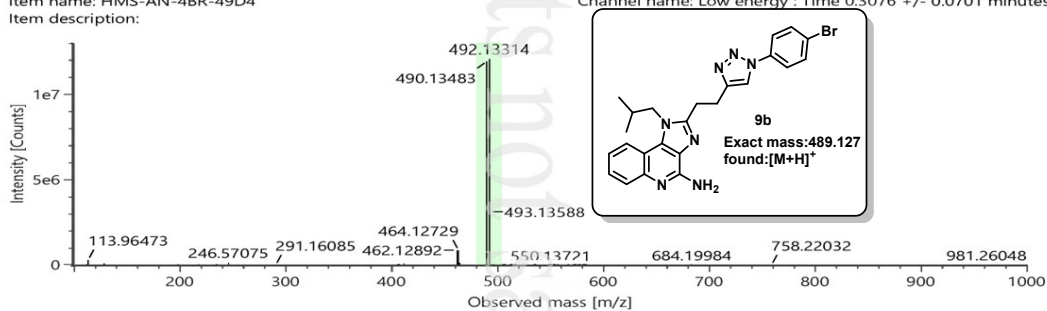
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	Component name	Observed neutral mass (Da)	Neutral mass (Da)	Observed m/z	Mass error (ppm)	Adducts
1	C24H24BrN7	489.1276	489.12766	490.1348	-0.2	+H

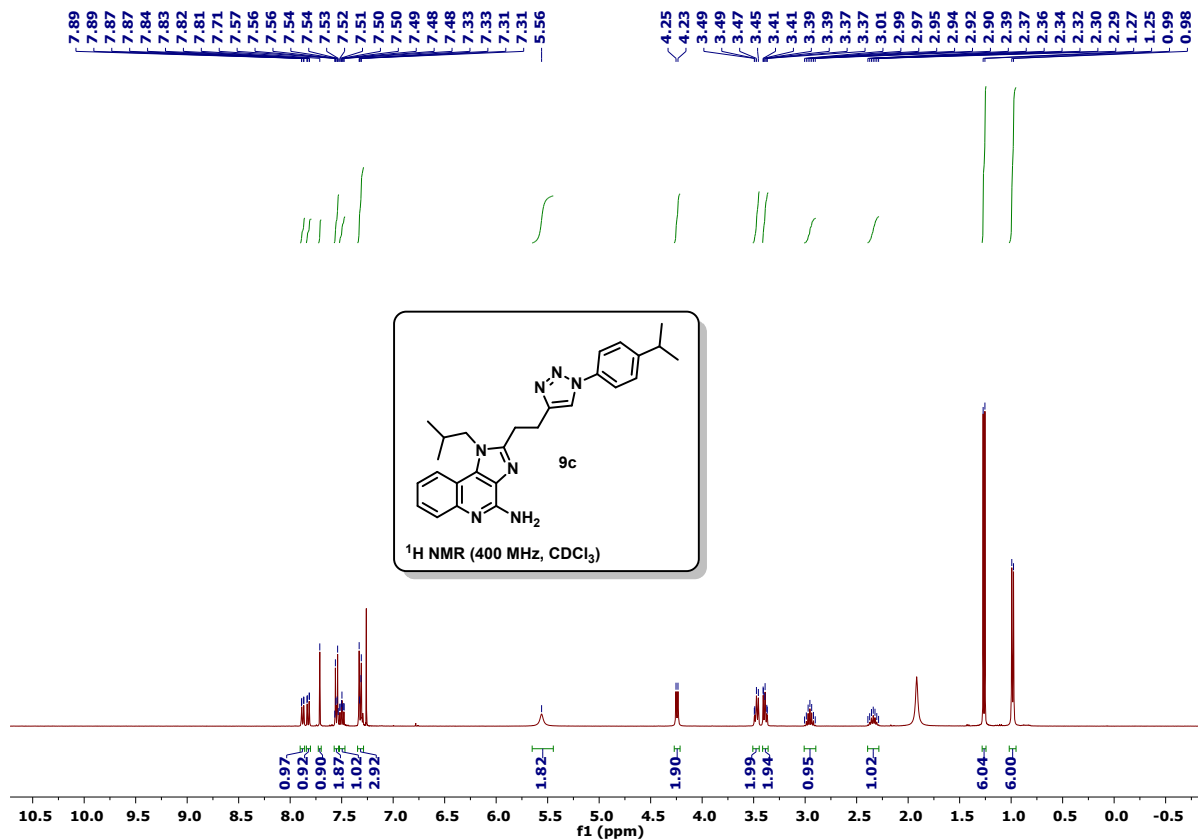
Component name: C24H24BrN7

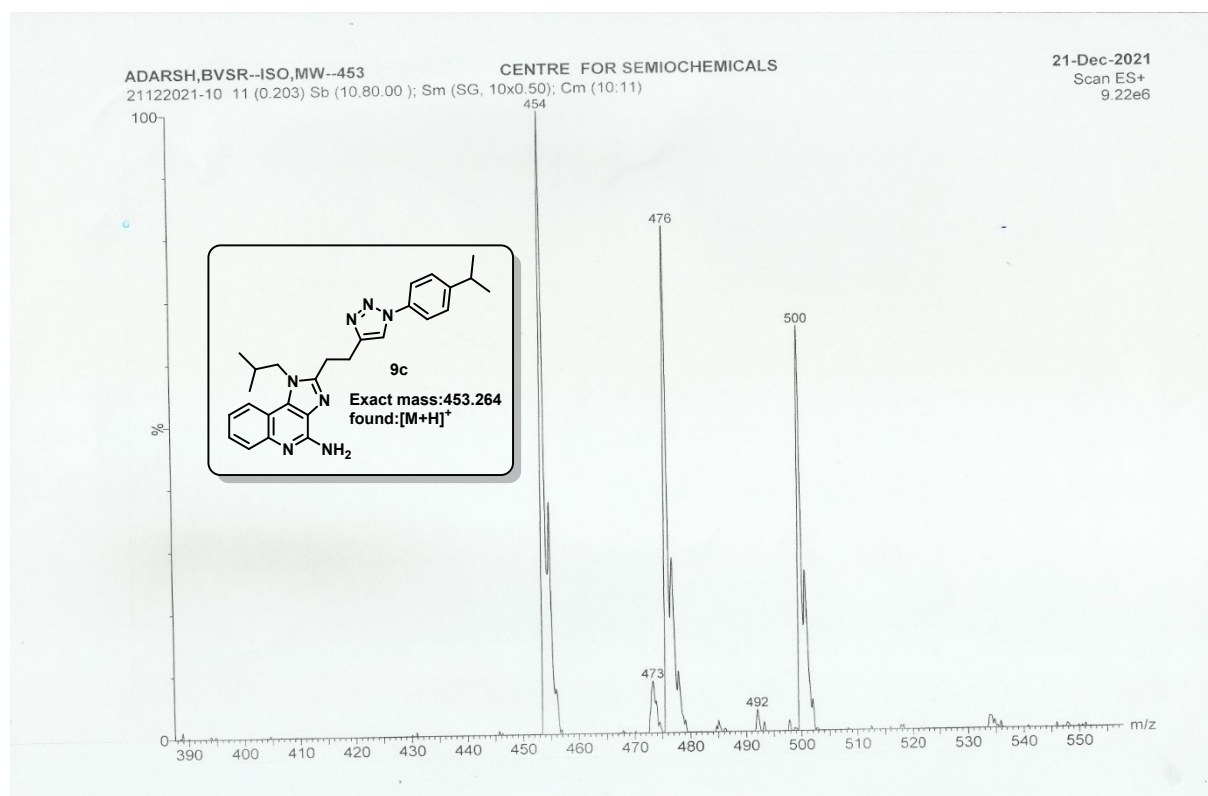
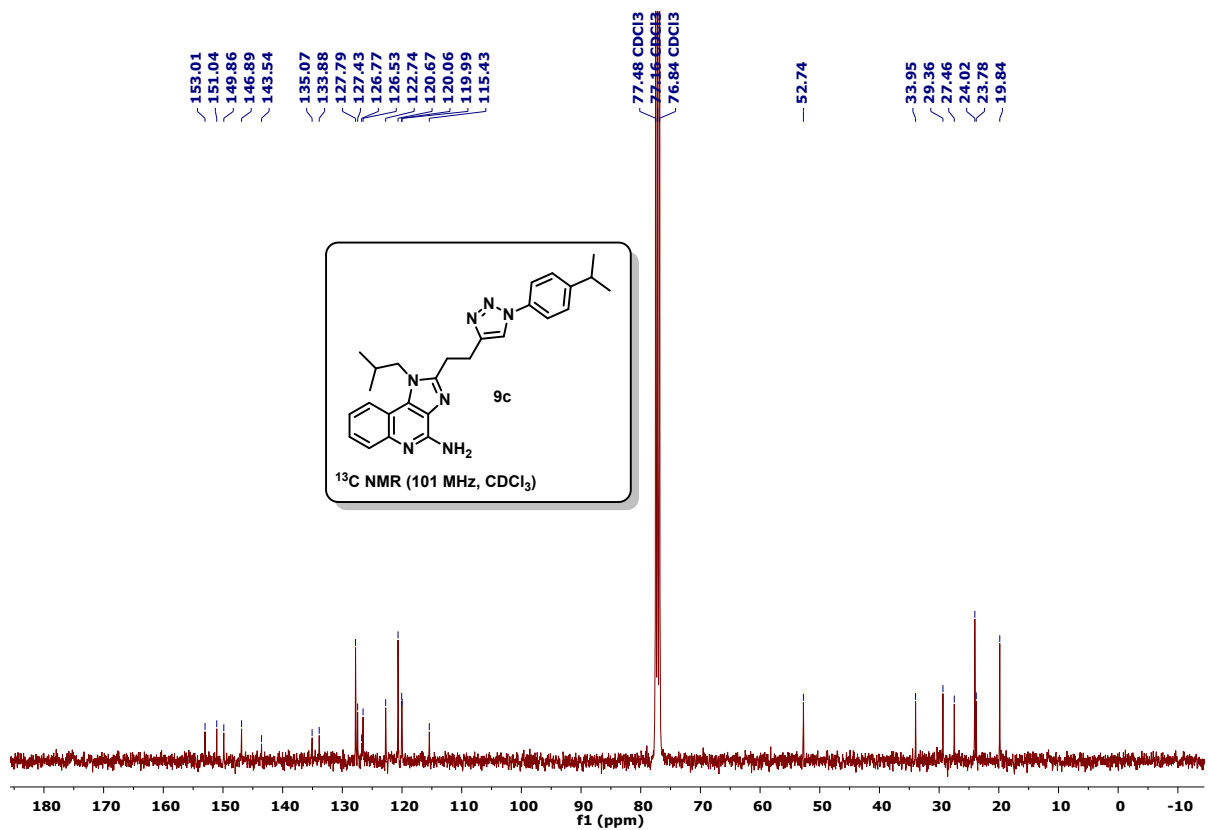
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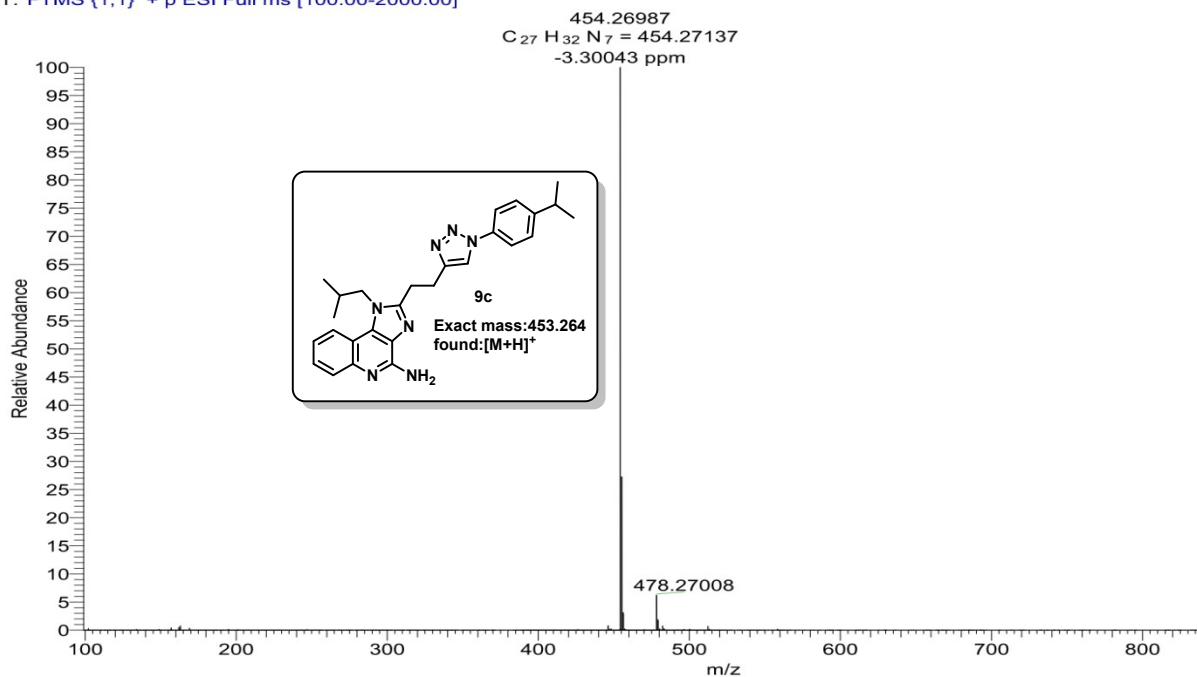


9c. 1-isobutyl-2-(2-(1-(4-isopropylphenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine

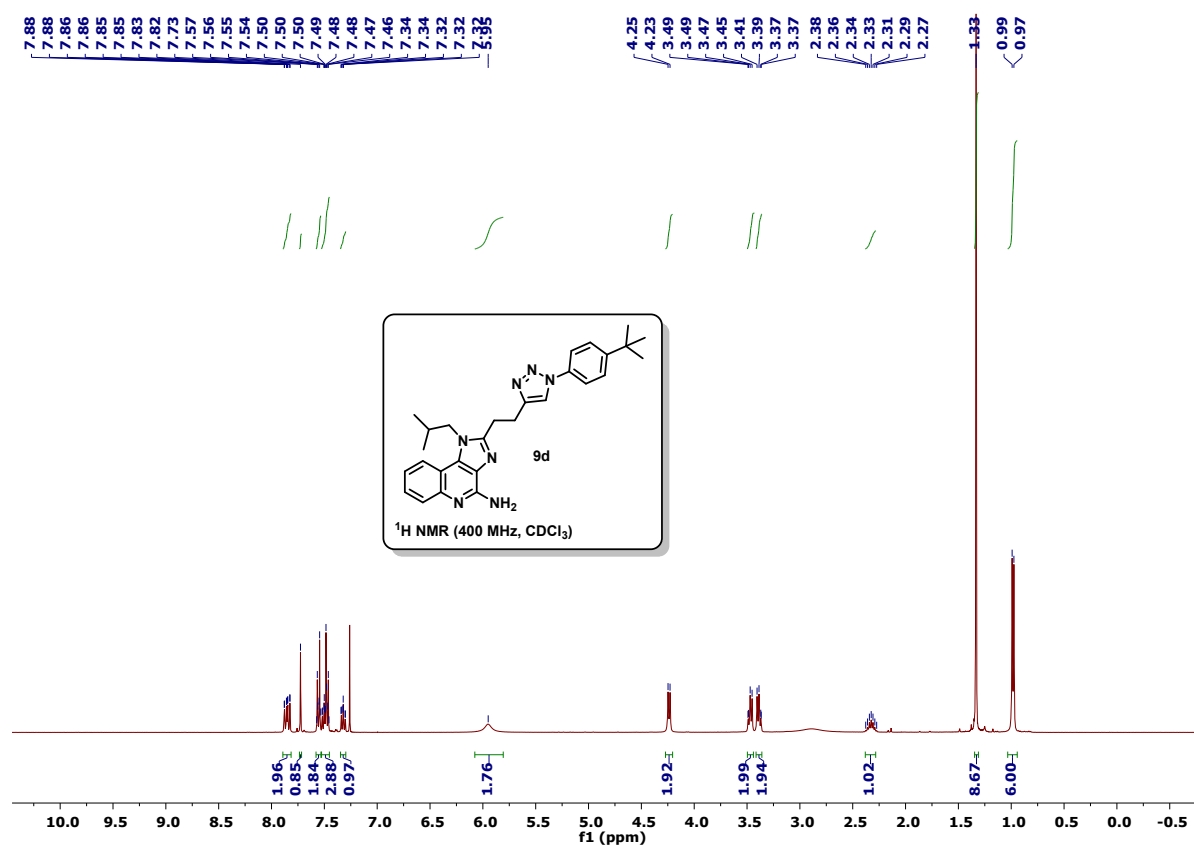




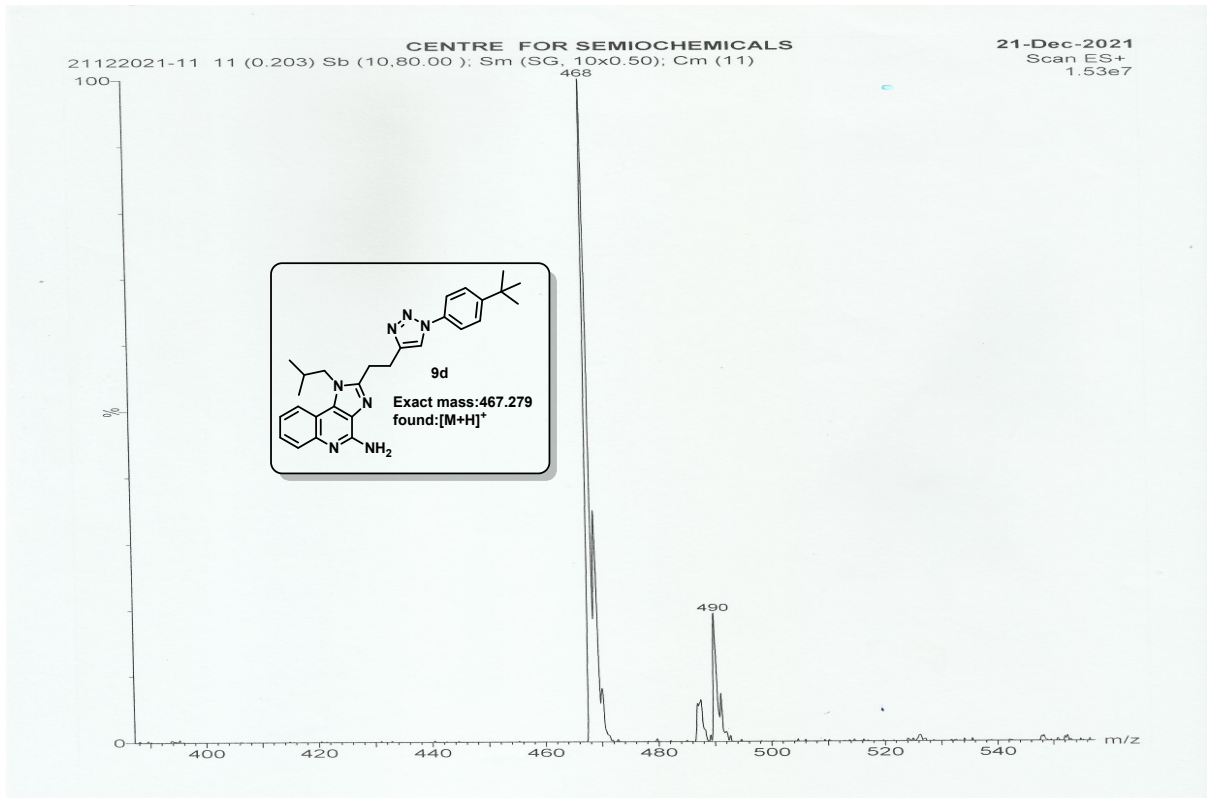
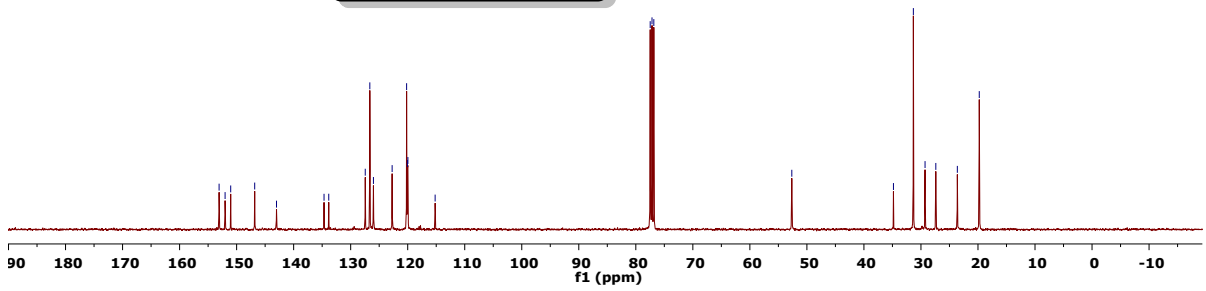
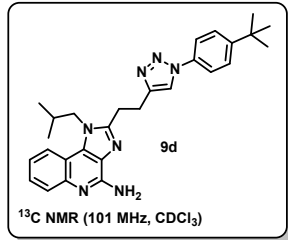
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T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]



9d. 2-(2-(1-(4-(tert-butyl)phenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine



153.07
 152.02
 151.04
 146.83
 143.01
 134.67
 133.85
 127.44
 126.64
 126.01
 122.73
 120.19
 120.06
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 115.19
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Created time: 17:20:41 India Standard Time

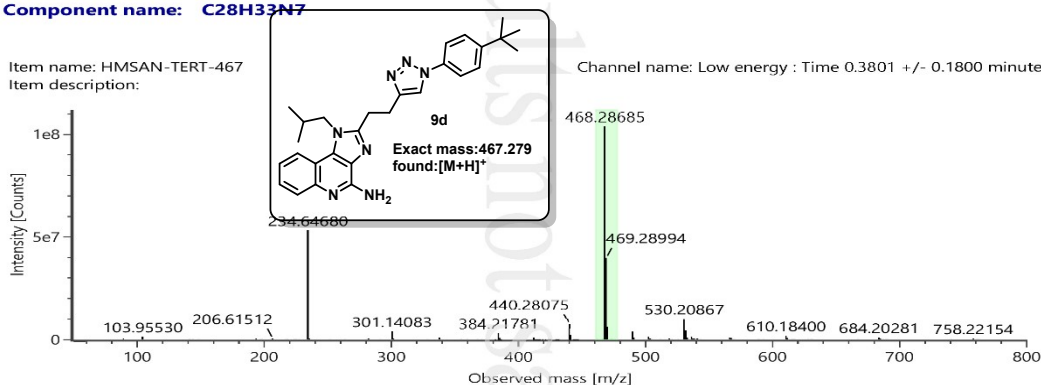
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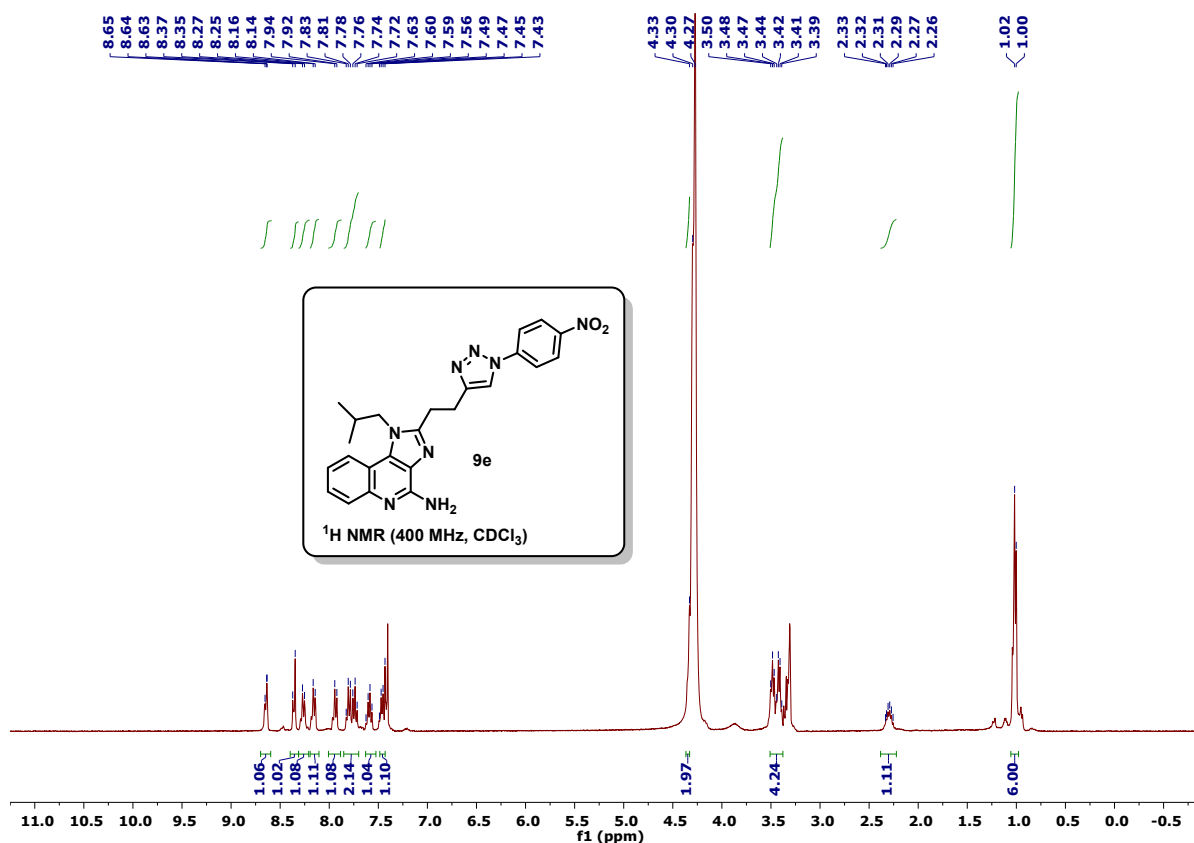
Component name	Observed neutral mass (Da)	Neutral mass (Da)	Observed m/z	Mass error (ppm)	Adducts	
1	C28H33N7	467.2796	467.27974	468.2868	-0.4	+H

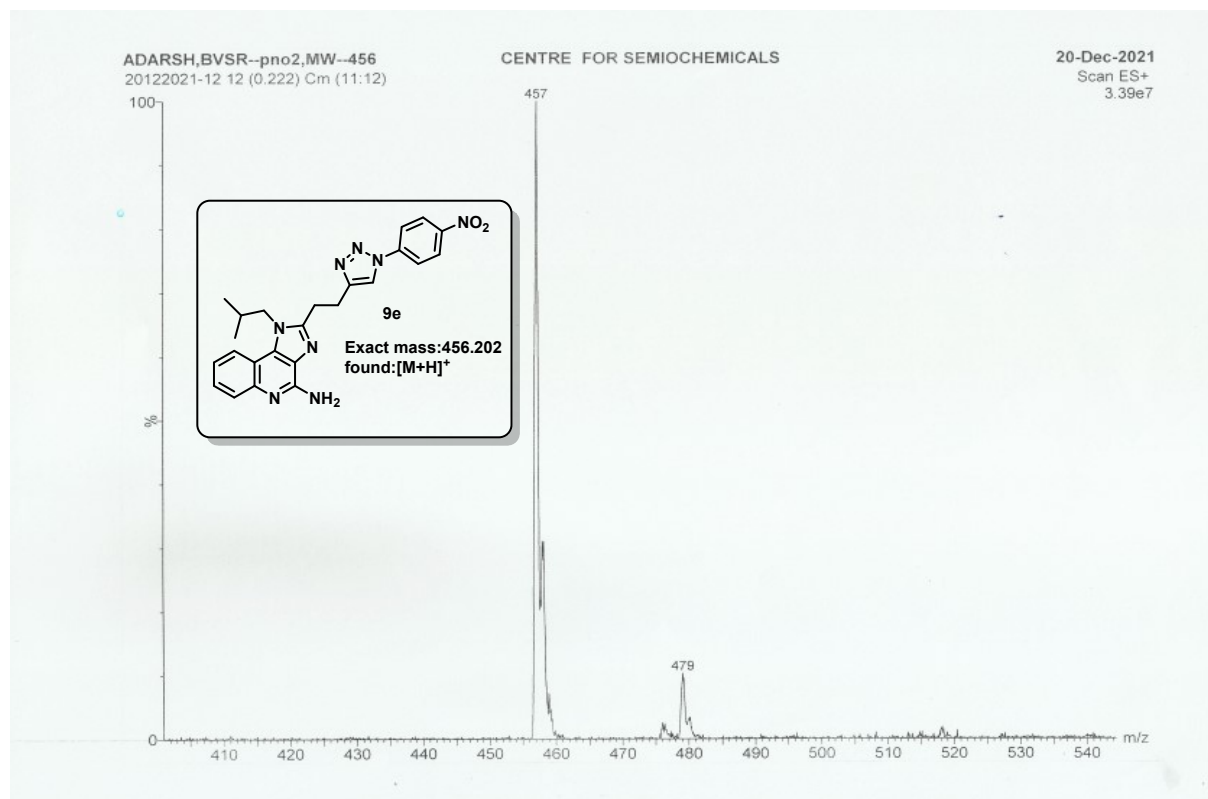
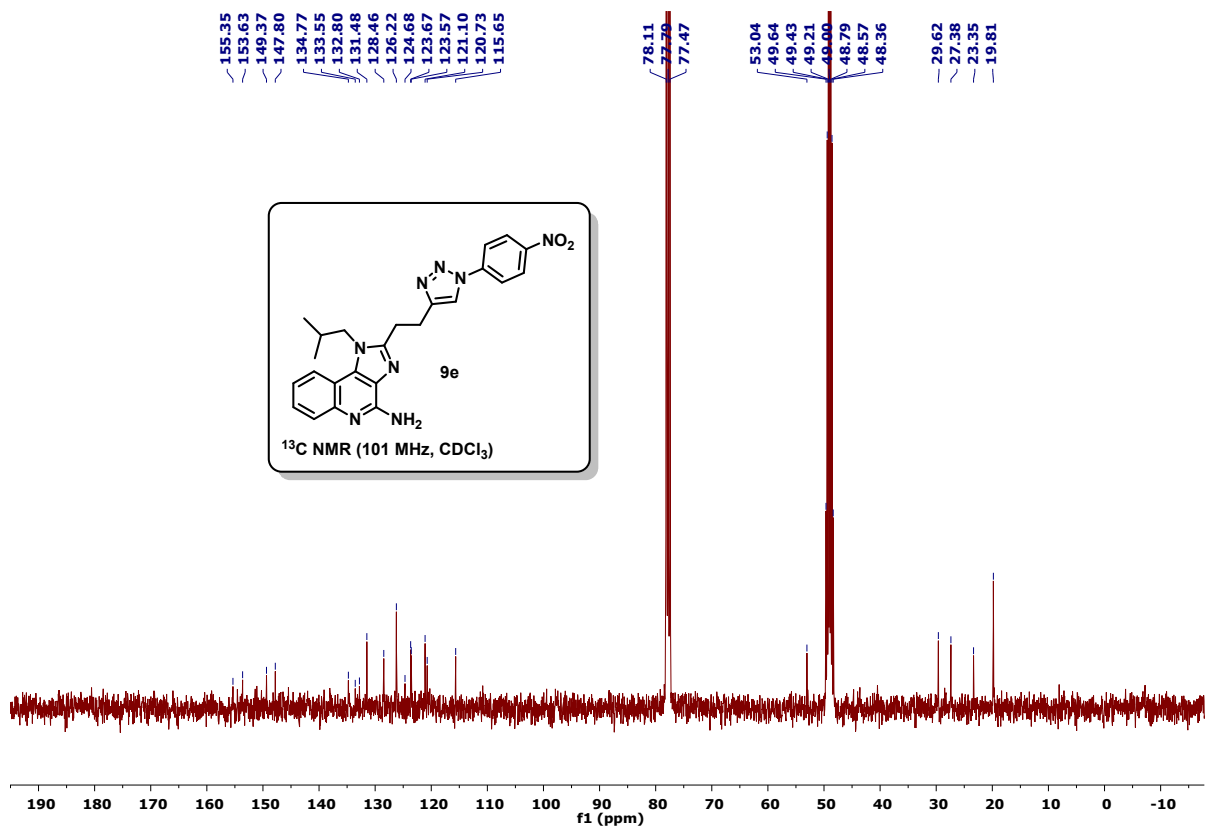
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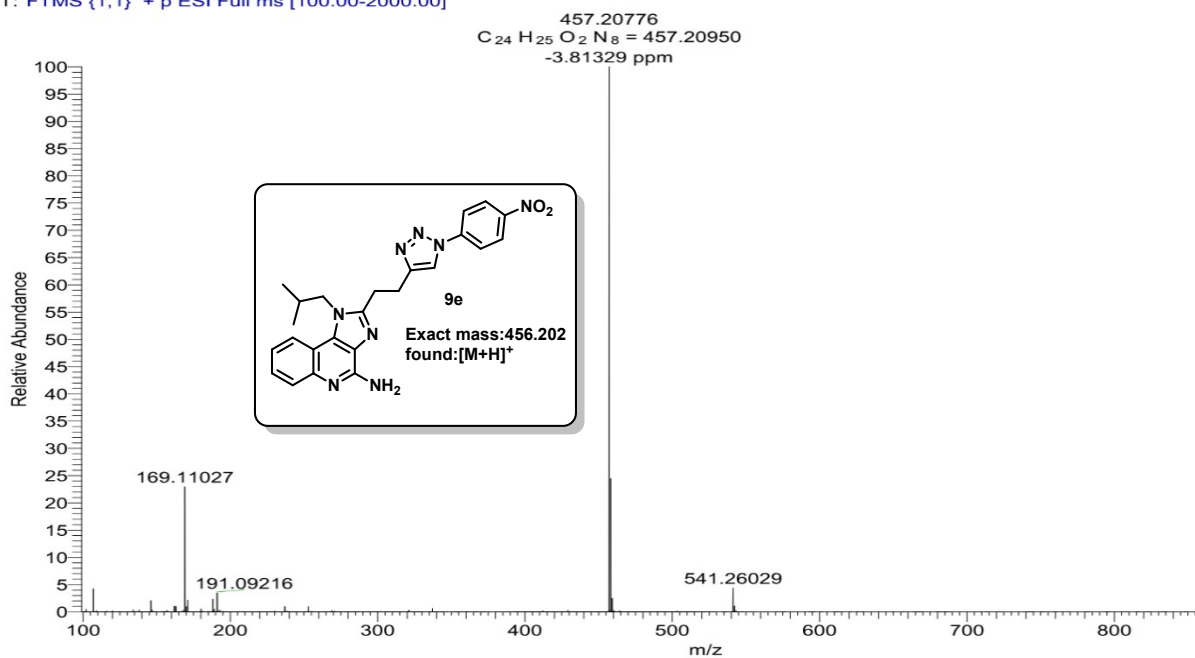


9e. 1-isobutyl-2-(2-(1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine

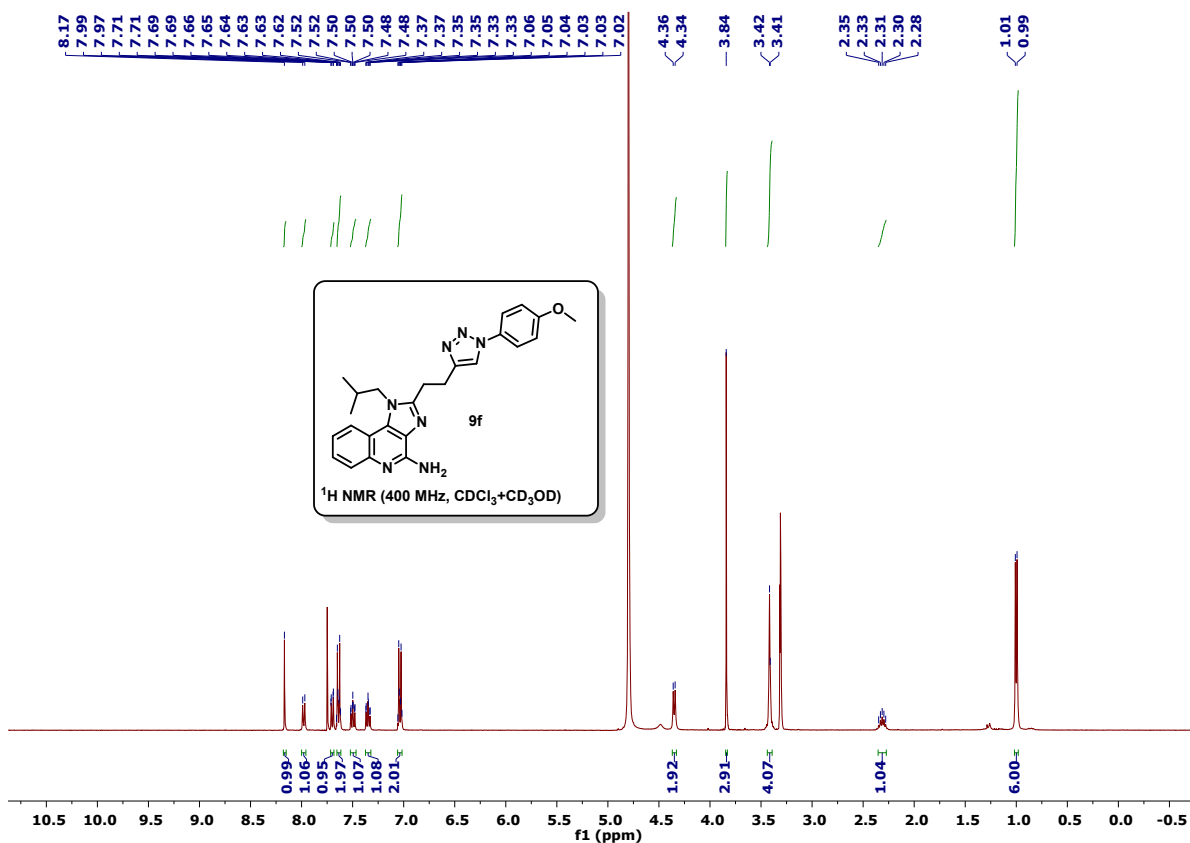


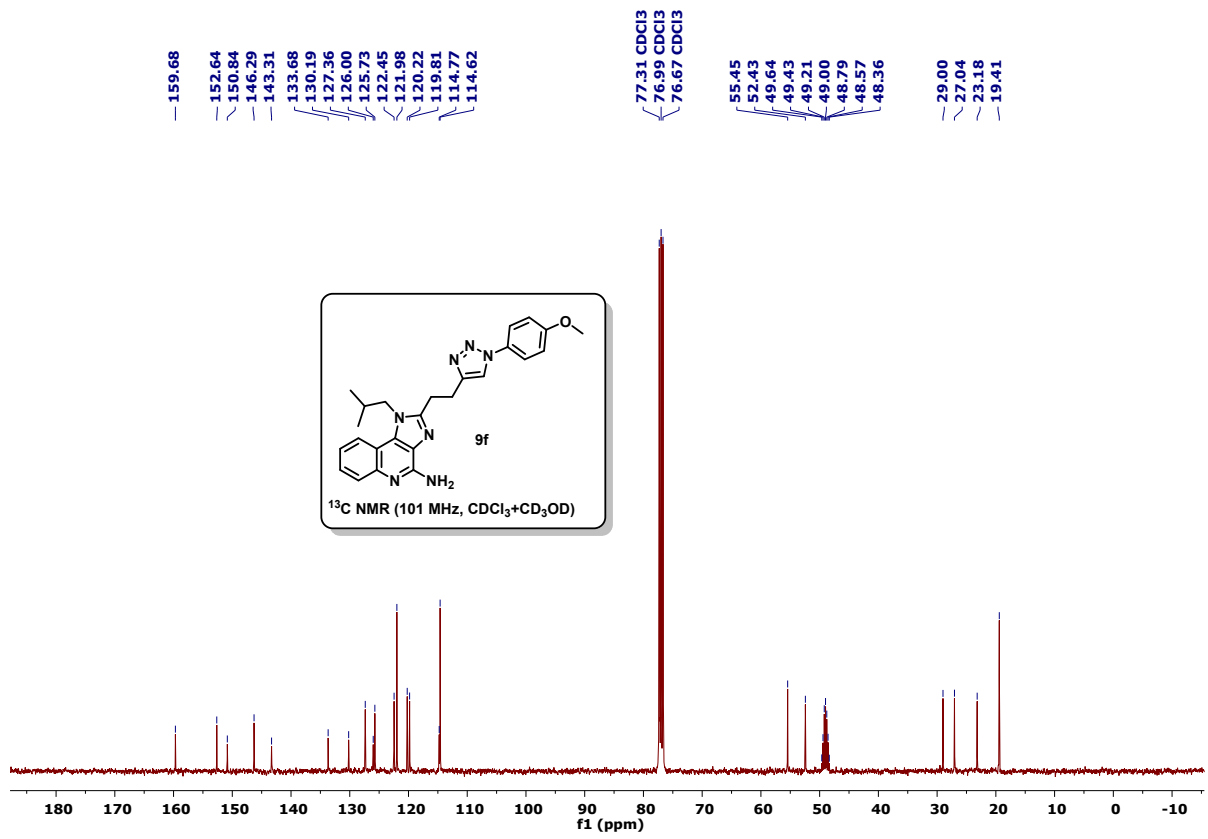


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07/26/22 16:15:45
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9f. 1-isobutyl-2-(2-(1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine





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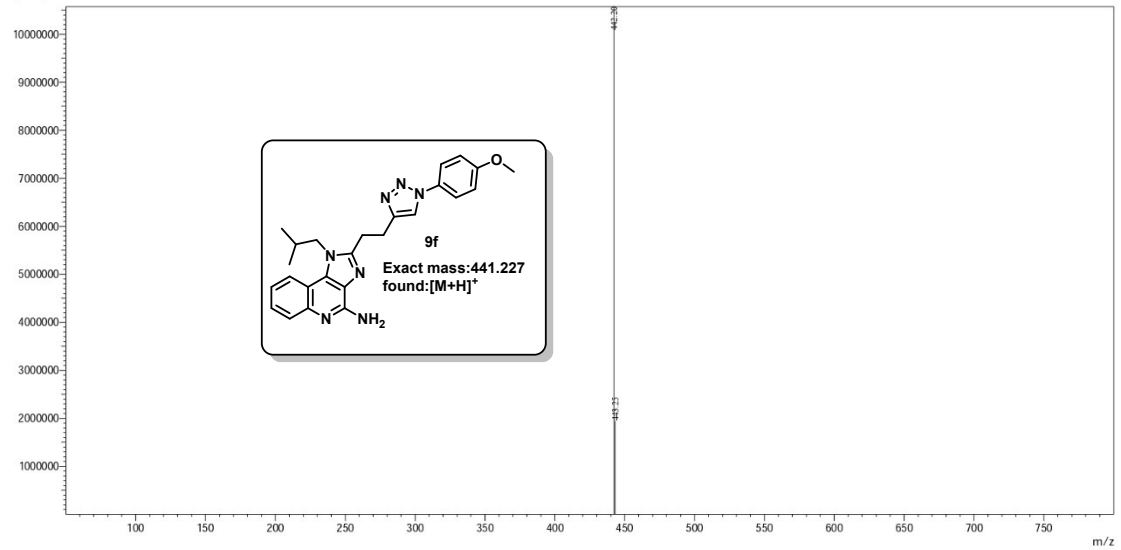


MASS REPORT

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Averaged ESI Positive+
 Spectrum Mode: Averaged 0.169-0.372(11-23)



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Created time: 14:53:24 India Standard Time

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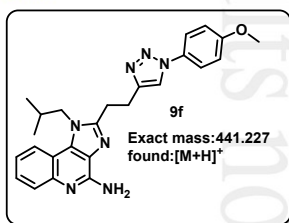
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Component name	Observed neutral mass (Da)	Neutral mass (Da)	Observed m/z	Mass error (ppm)	Adducts	
1	C25H27N7O	441.2275	441.22771	442.2347	-0.6	+H

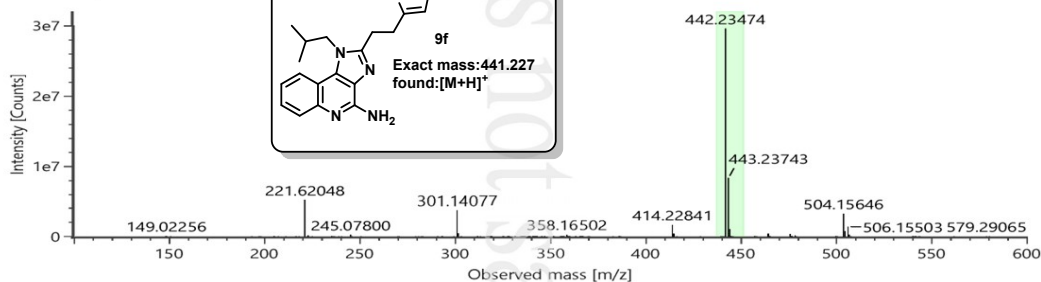
Component name: C25H27N7O

Item name: HMSAN-MA-441

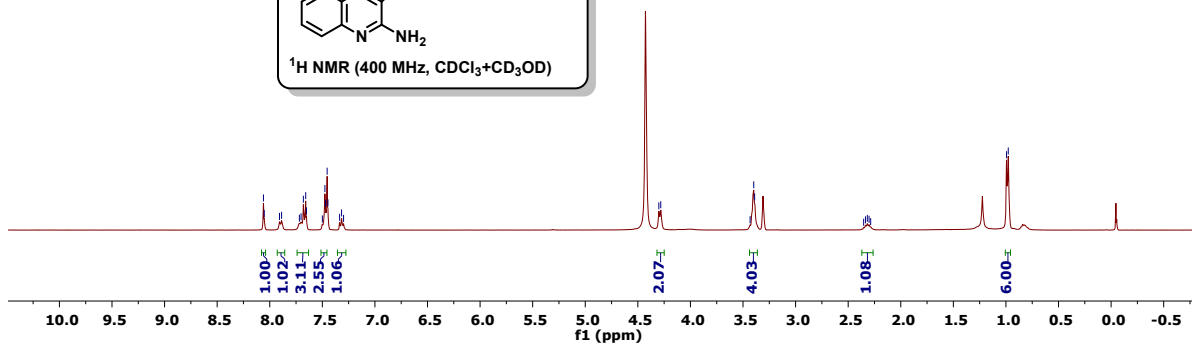
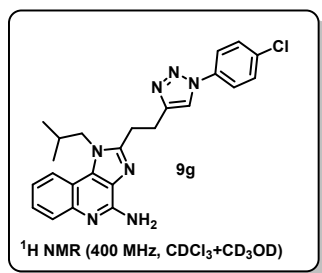
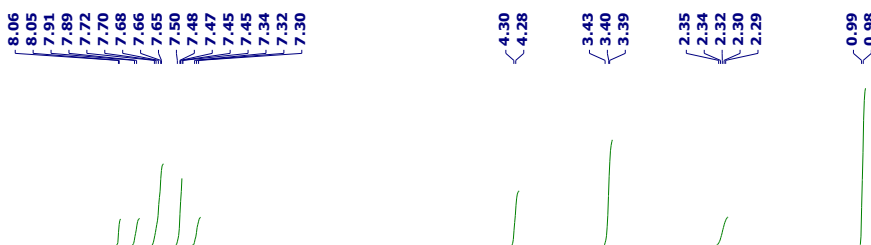
Item description:

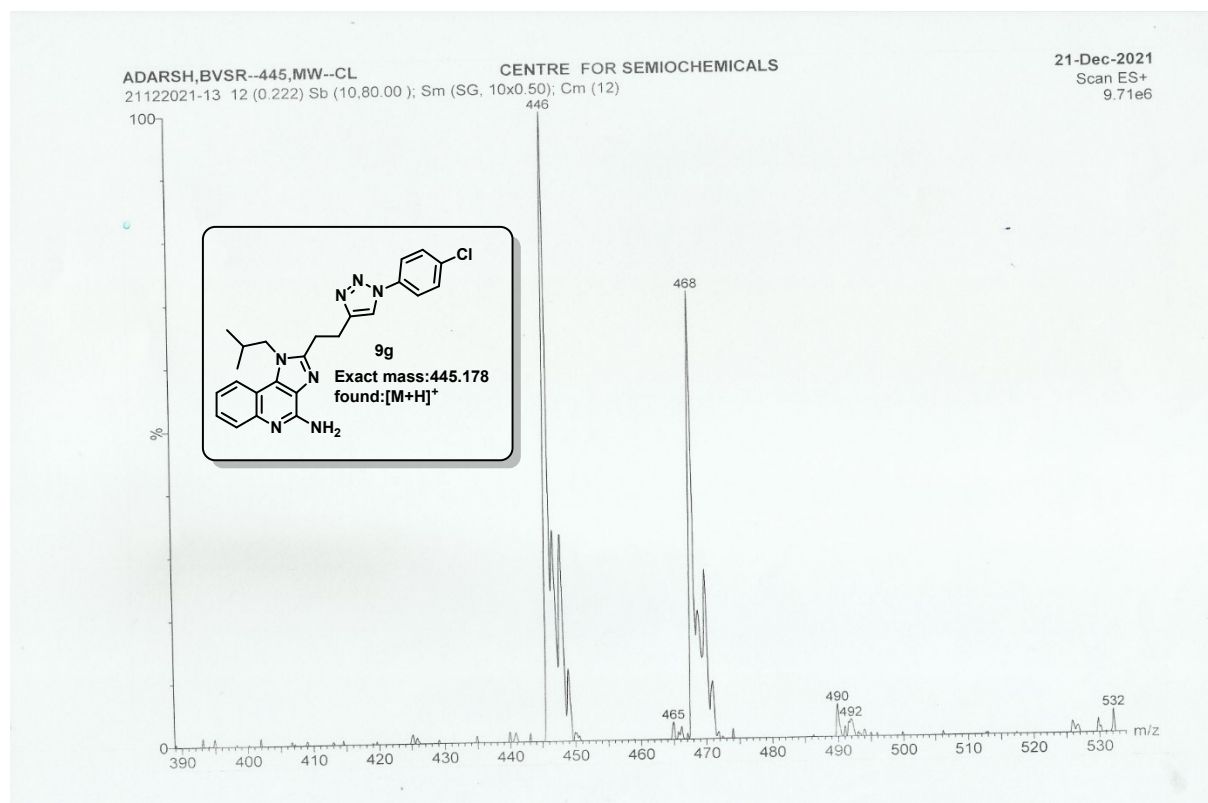
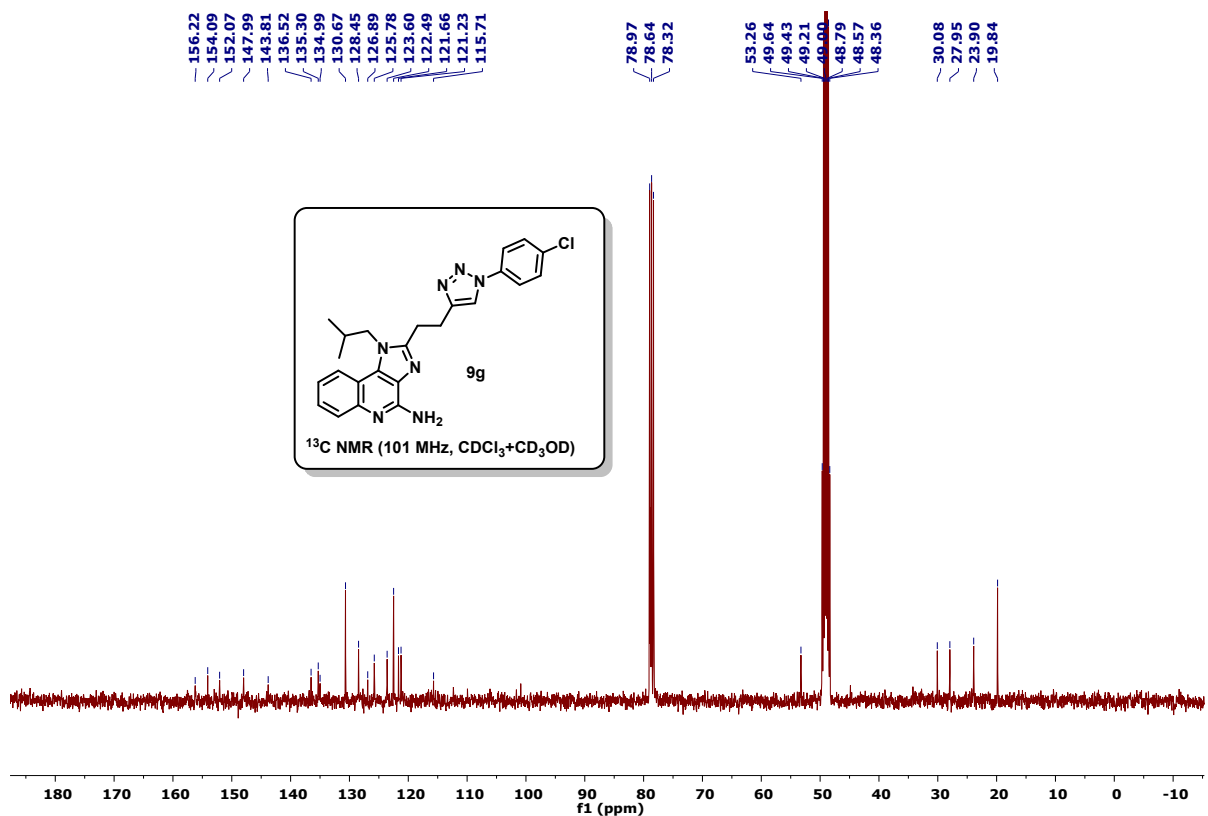


Channel name: Low energy : Time 0.3792 +/- 0.0581 minutes

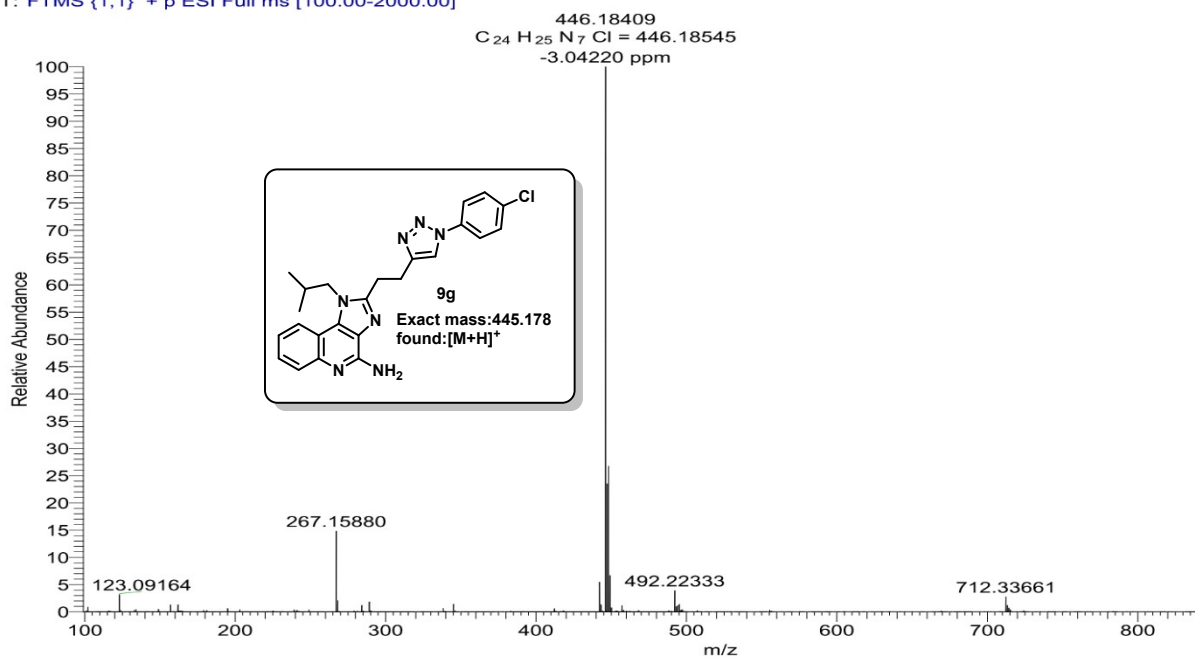


9g. 2-(2-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine

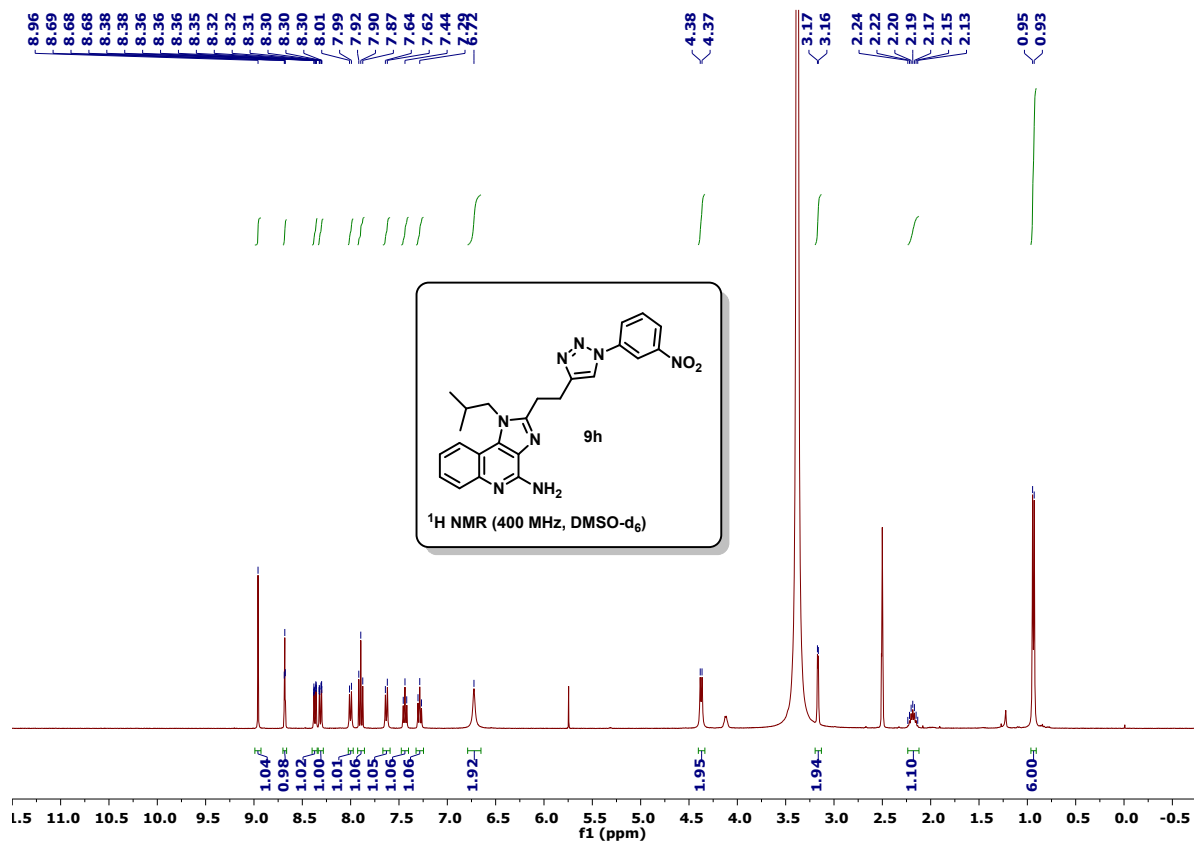


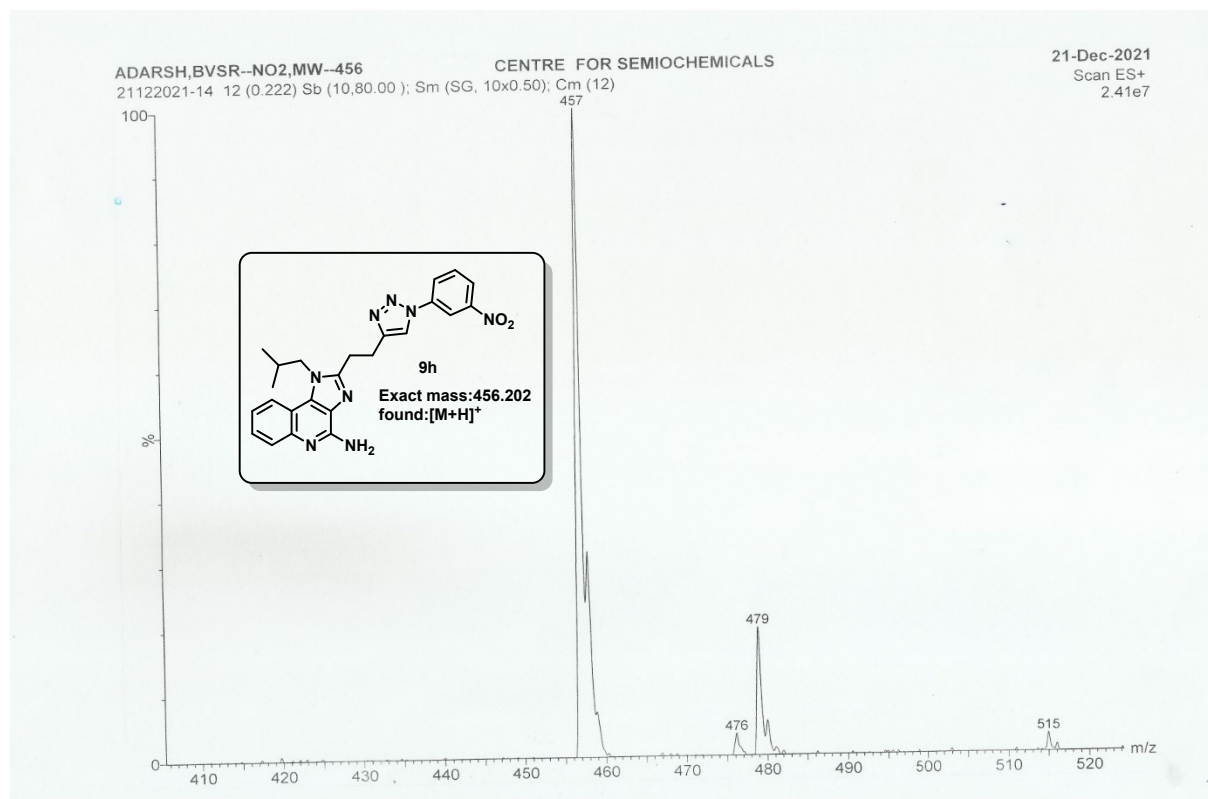
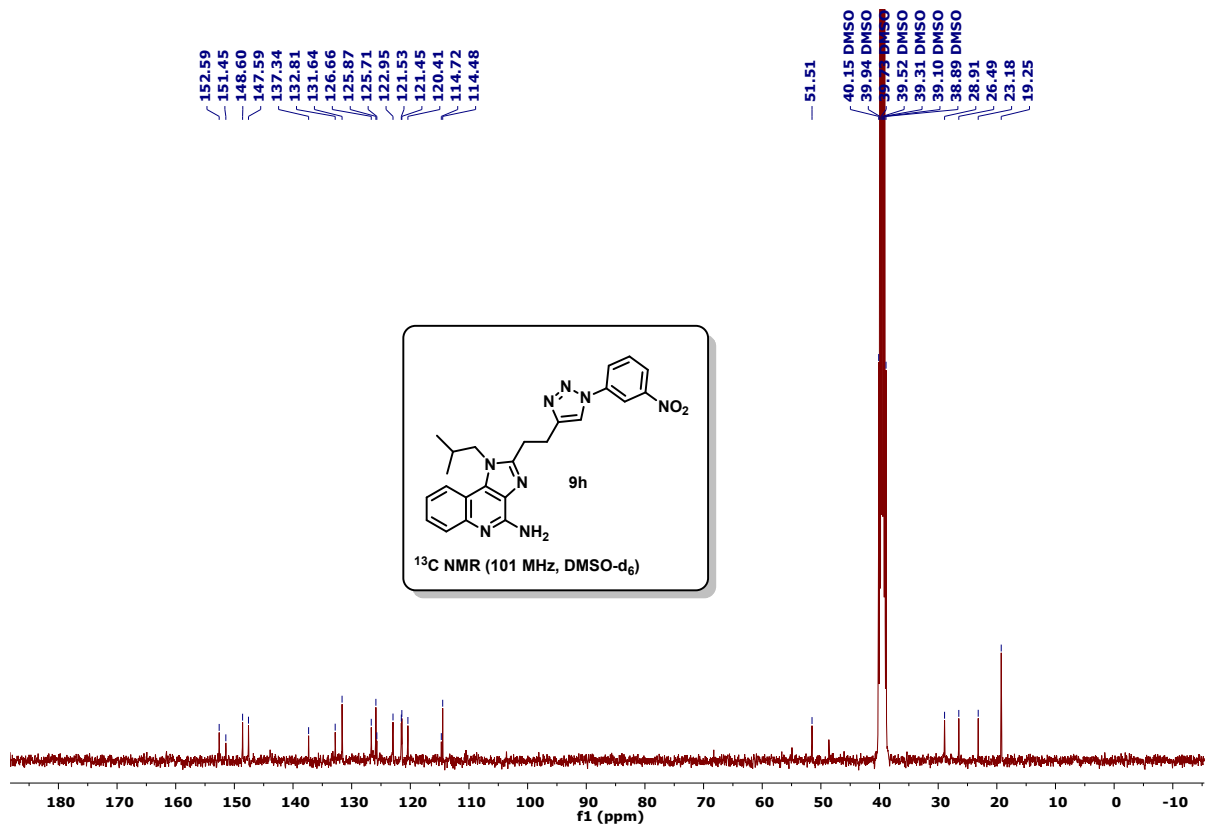


1
07/26/22 16:12:04
HMS-AN-CL #2-15 RT: 0.04-0.18 AV: 7 NL: 7.98E7
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]



9h. 1-isobutyl-2-(2-(1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine





Item name: HRMS Elemental composition Mar 08, 2022 14:13:44 India Standard Time

Created time: 14:55:19 India Standard Time

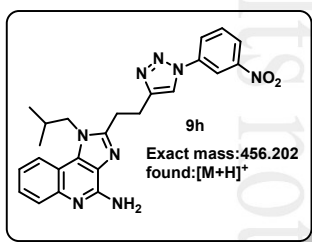
Item name: HMSAN-NO2-456

Item name: HMSAN-NO2-456, Sample position: 1:C,2, Replicate number: 1

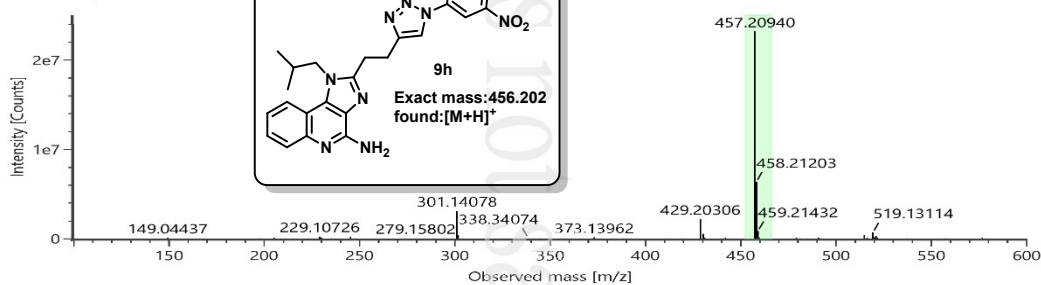
Component name	Observed neutral mass (Da)	Neutral mass (Da)	Observed m/z	Mass error (ppm)	Adducts	
1	C24H24N8O2	456.2021	456.2022	457.2094	-0.2	+H

Component name: C24H24N8O2

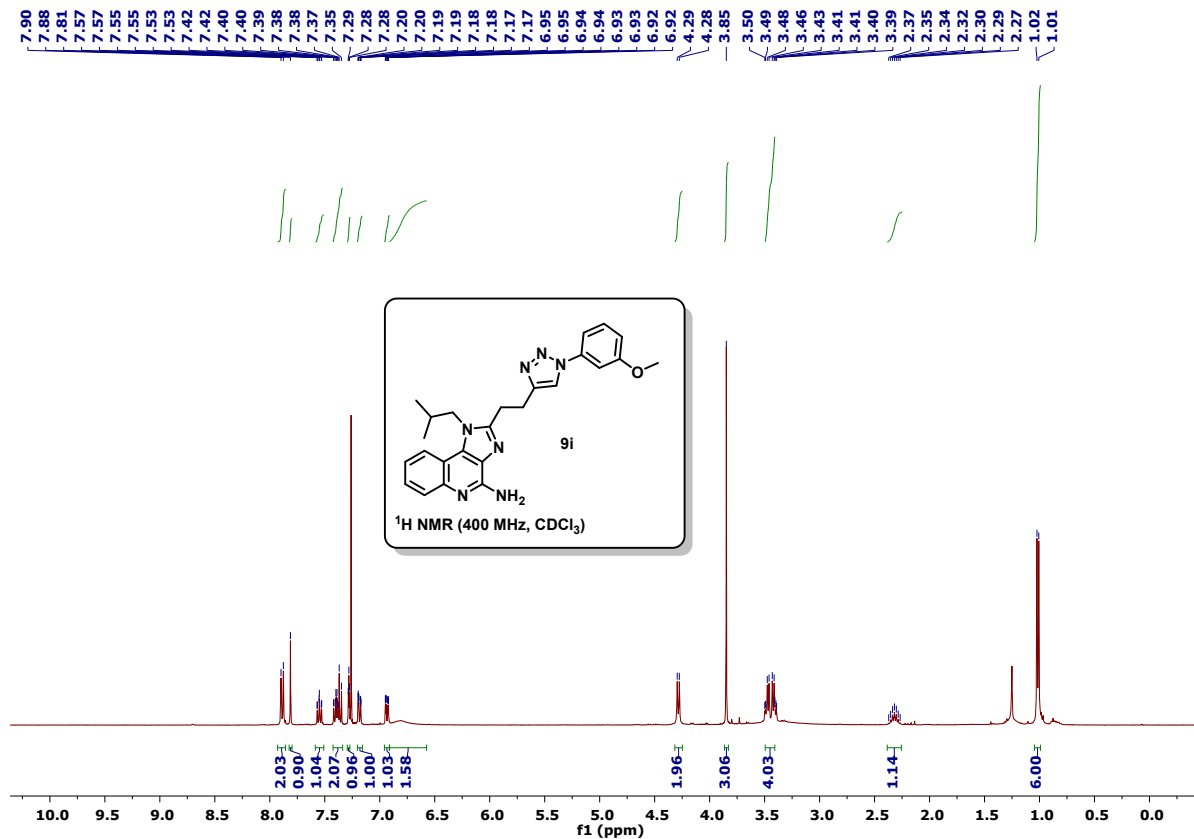
Item name: HMSAN-NO2-456
Item description:

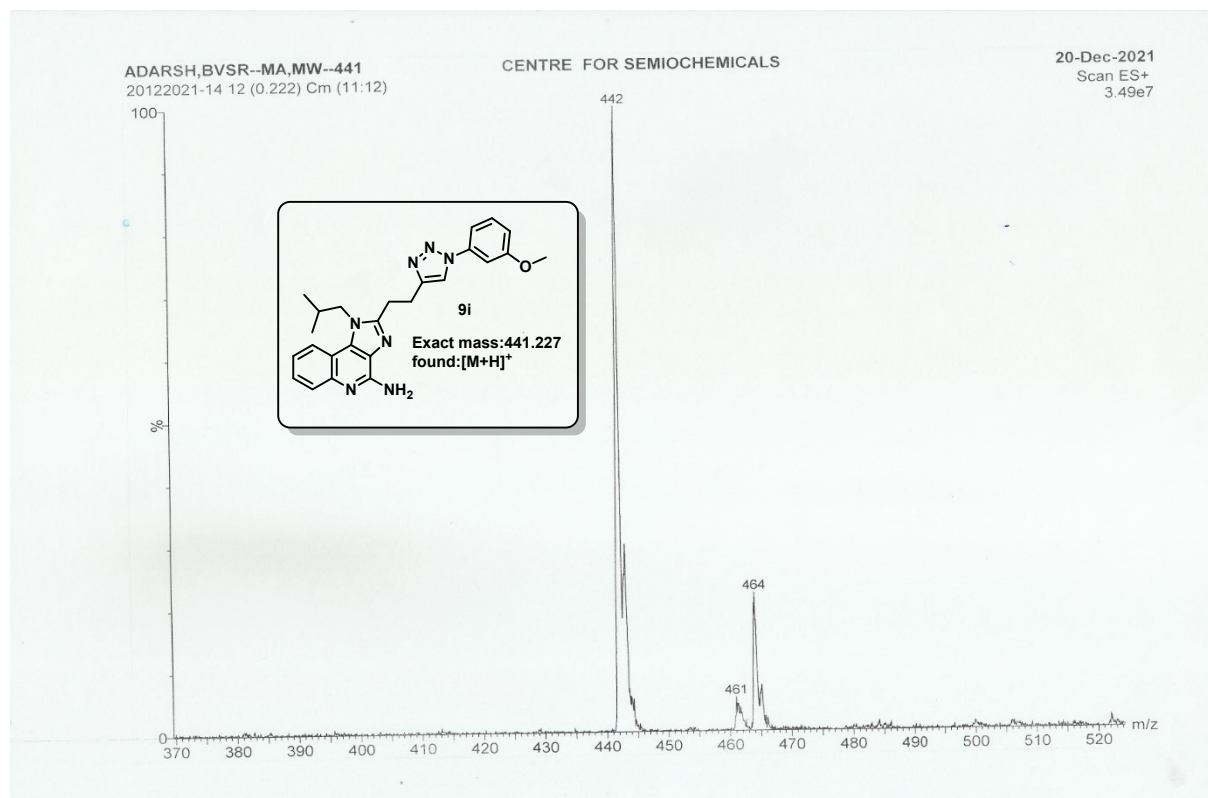
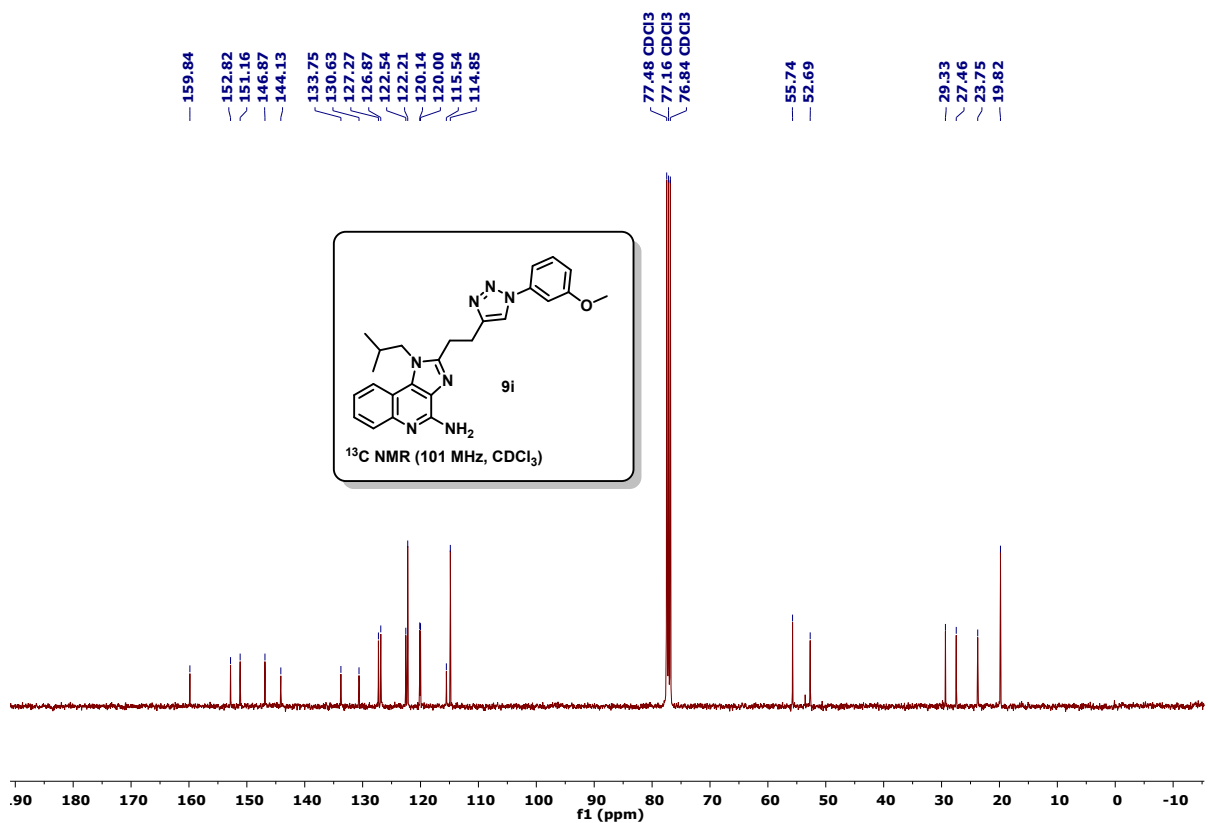


Channel name: Low energy : Time 0.3815 +/- 0.0560 minutes

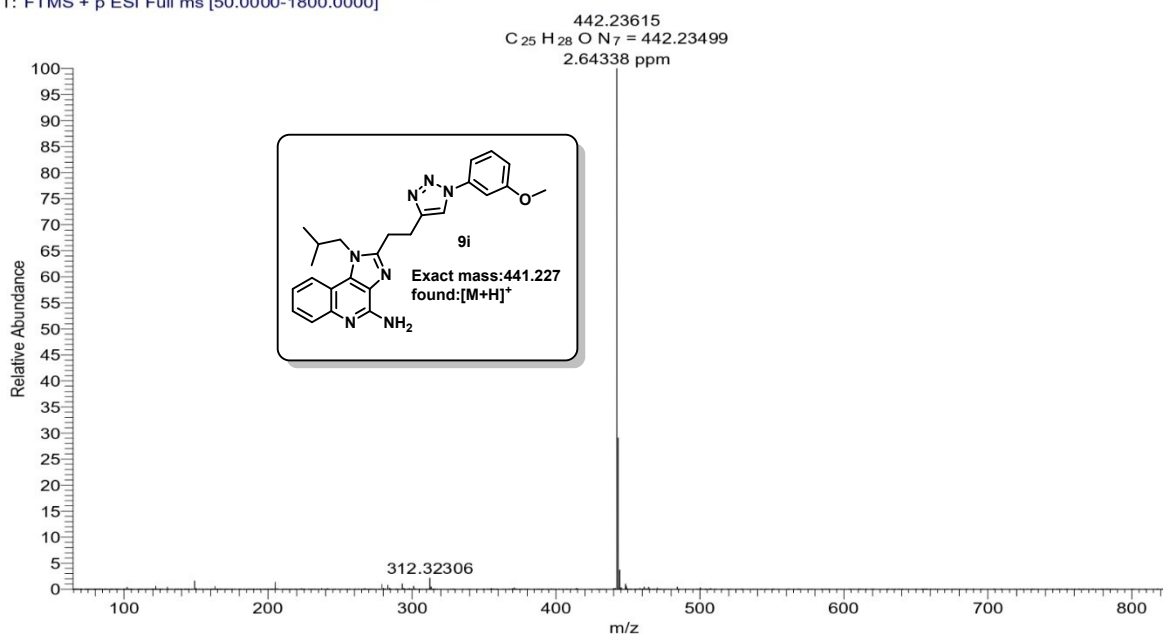


9i. 1-isobutyl-2-(2-(1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine

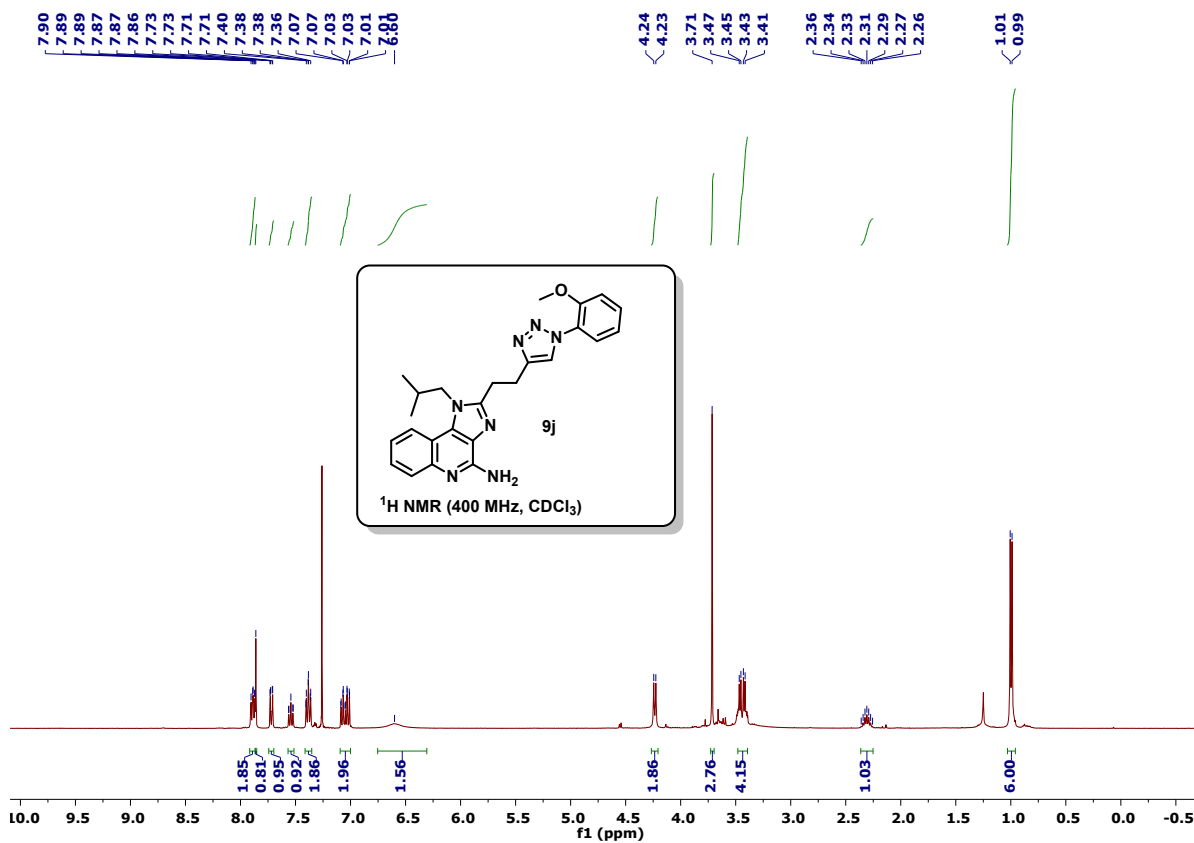


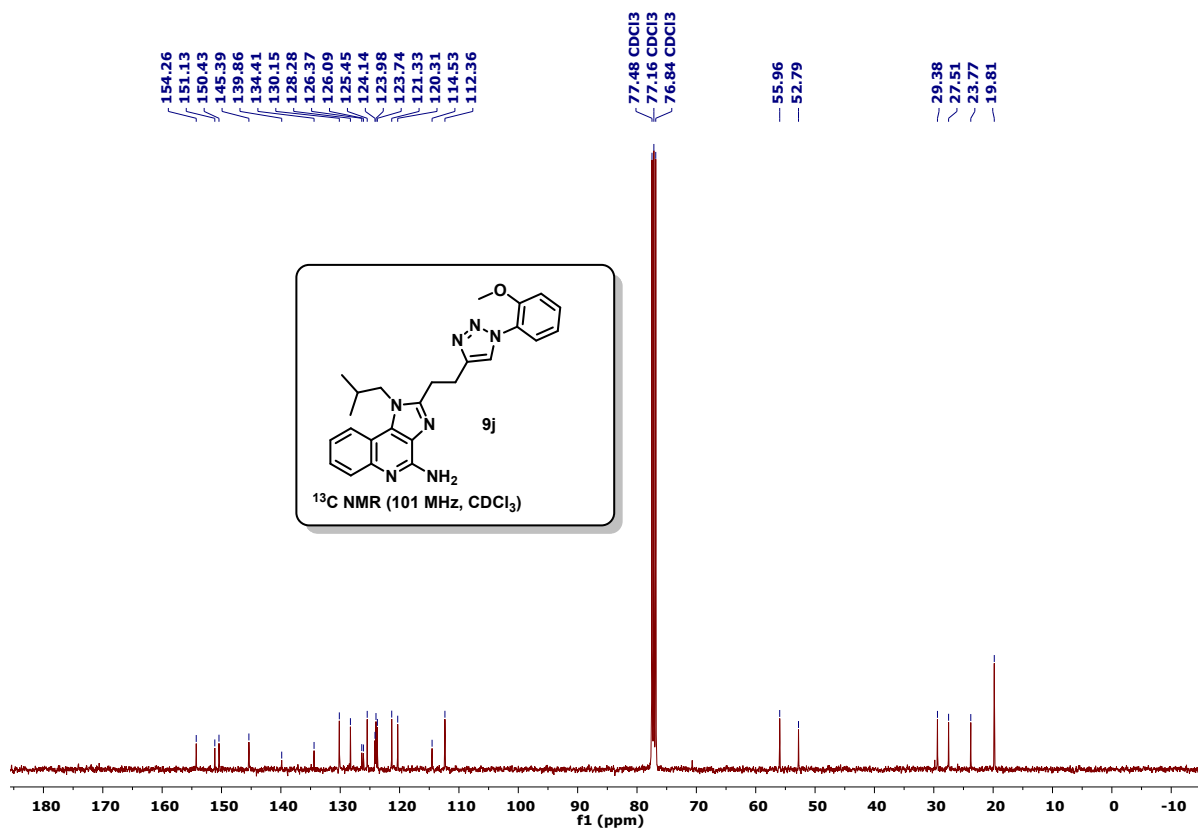


11/03/22 18:51:25

Thermo Scientific Orbitrap Exploris 120
Analysed by G SAIKRISHNAHMS-AN-M-441 #2-17 RT: 0.01-0.09 AV: 8 SB: 80 0.32-1.20 NL: 1.00E9
T: FTMS + p ESI Full ms [50.0000-1800.0000]

9j. 1-isobutyl-2-(2-(1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)ethyl)-1H-imidazo[4,5-c]quinolin-4-amine





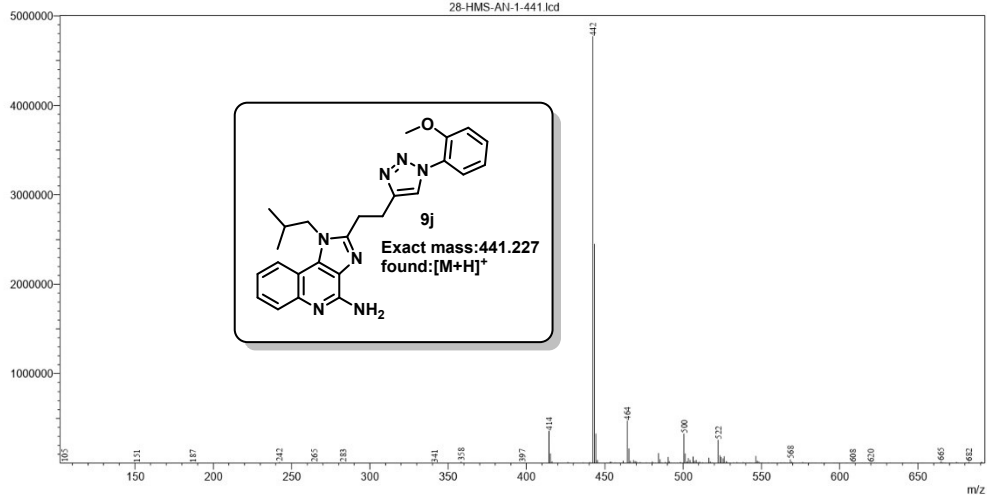
10/28/2022 2:39:07 PM Page 1 / 1

====Department of Organic Synthesis and Process Chemistry, CSIR-IICT ====

Date Acquired : 10/28/2022 2:16:17 PM
Sample Name : HMS-AN-1-441
Sample ID : HMS-AN-1-441
Data File : 28-HMS-AN-1-441.lcd
Method File : LALSPEC+&-1cm
Comment : MASS

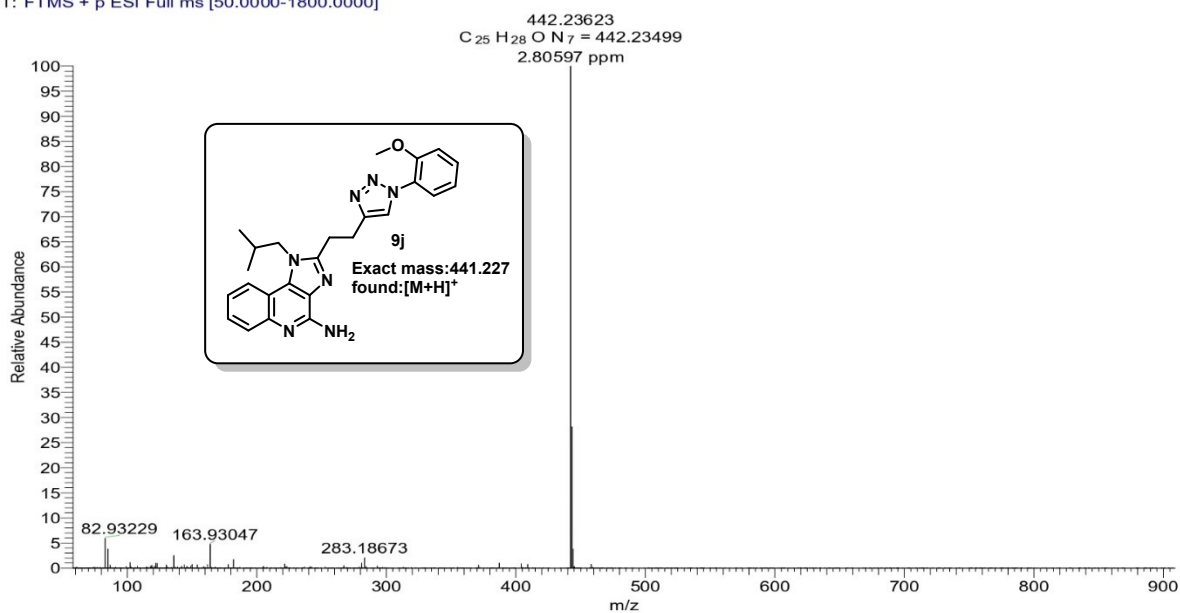
Sample Information

ESI-MS Spectrum
28-HMS-AN-1-441.lcd

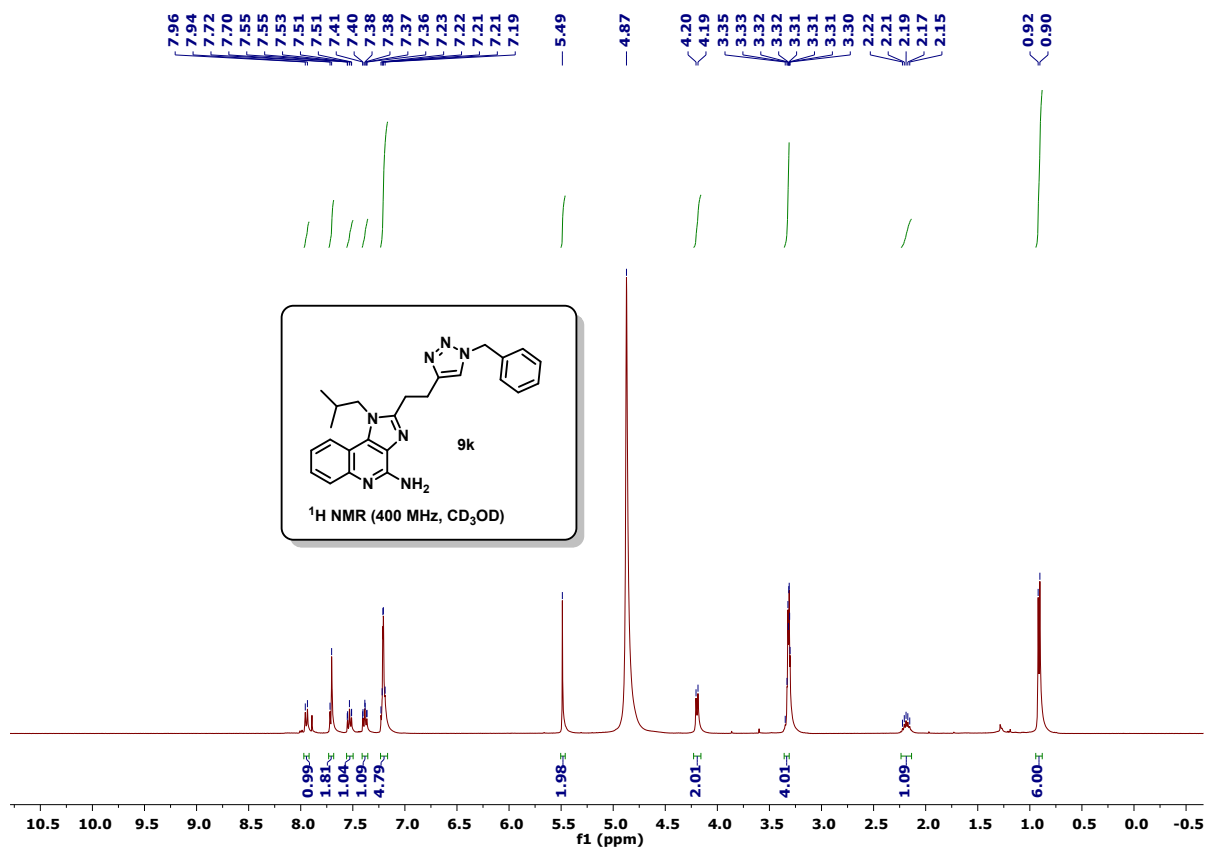


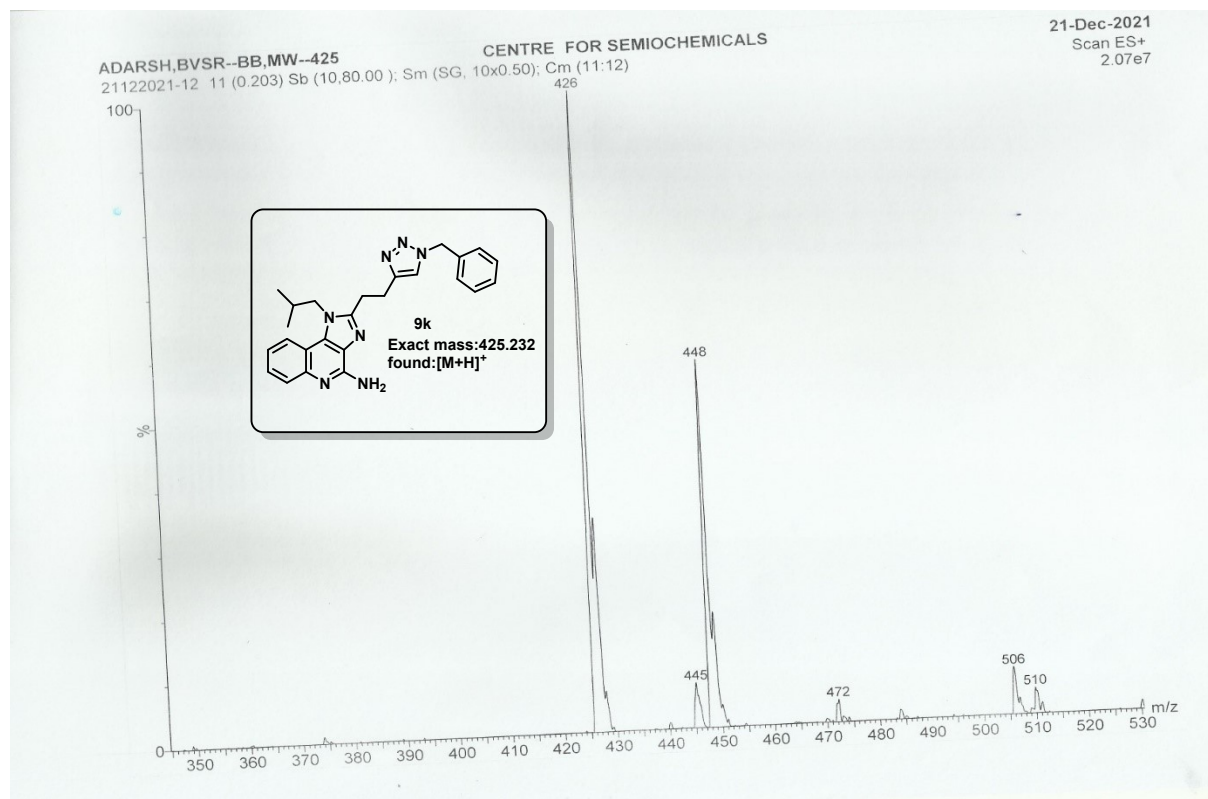
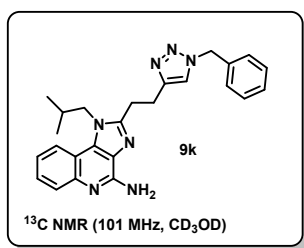
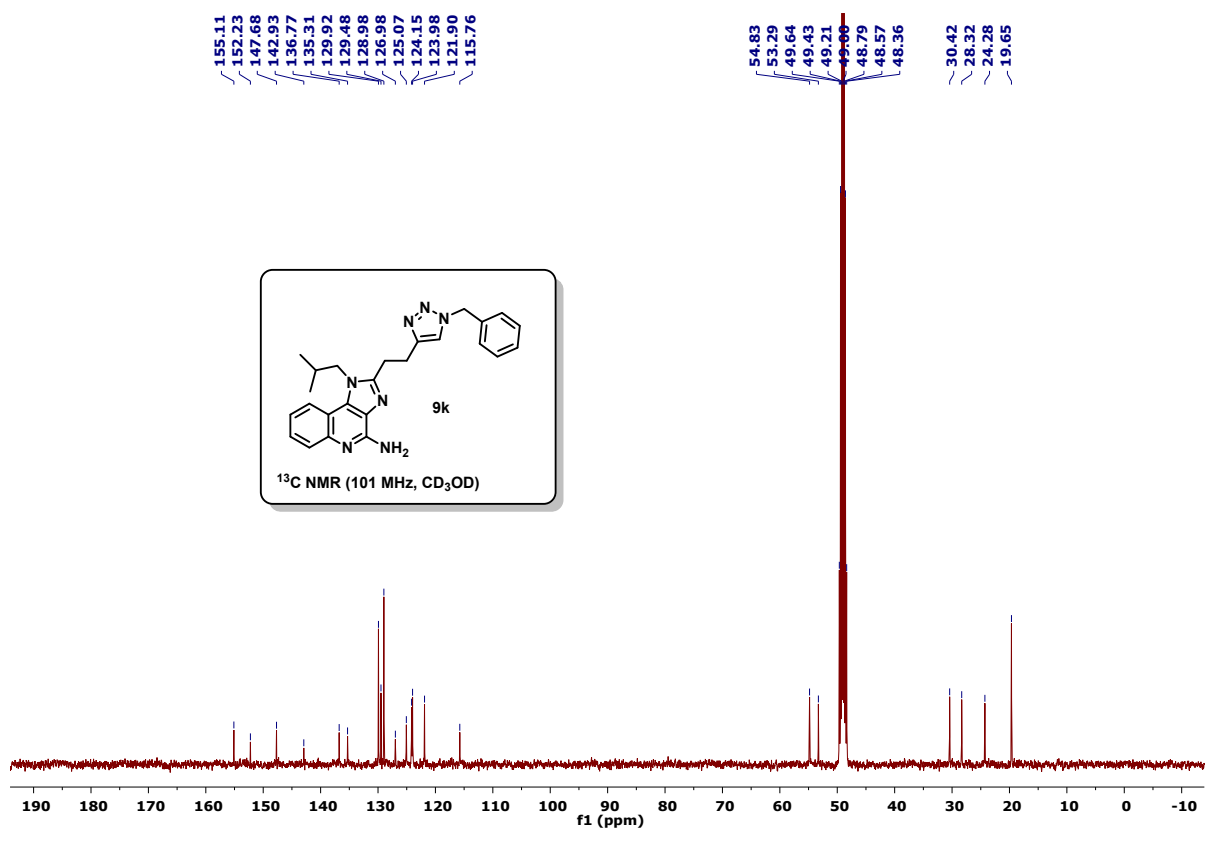
D:\Data\MASS\OCT-2022\28-HMS-AN-1-441.lcd

11/03/22 18:47:57

Thermo Scientific Orbitrap Exploris 120
Analysed by G SAIKRISHNAHMS-AN-0-441 #9-32 RT: 0.05-0.17 AV: 12 SB: 80 0.32-1.20 NL: 4.23E8
T: FTMS + p ESI Full ms [50.0000-1800.0000]

9k. 2-(2-(1-benzyl-1H-1,2,3-triazol-4-yl)ethyl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine





Item name: HRMS Elemental composition Mar 08, 2022 14:13:44 India Standard Time

Created time: 14:58:07 India Standard Time

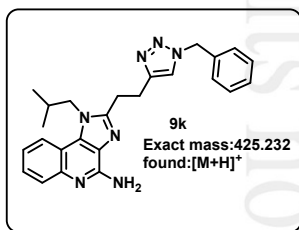
Item name: **HMSAN-BB-425**

Item name: HMSAN-BB-425, Sample position: 1:C,4, Replicate number: 1

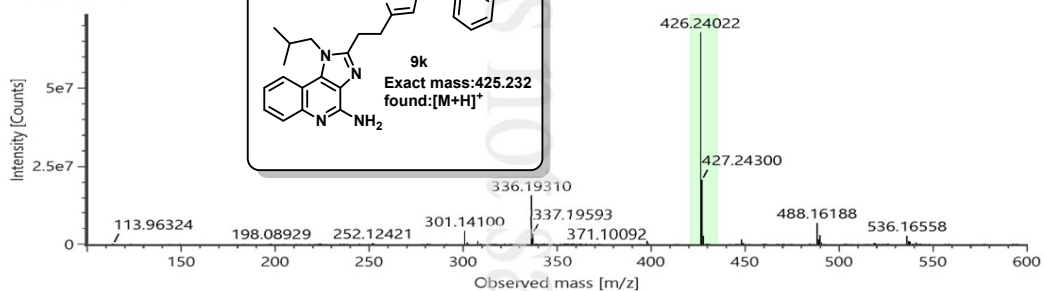
	Component name	Observed neutral mass (Da)	Neutral mass (Da)	Observed m/z	Mass error (ppm)	Adducts
1	C25H27N7	425.2329	425.23279	426.2402	0.4	+H

Component name: **C25H27N7**

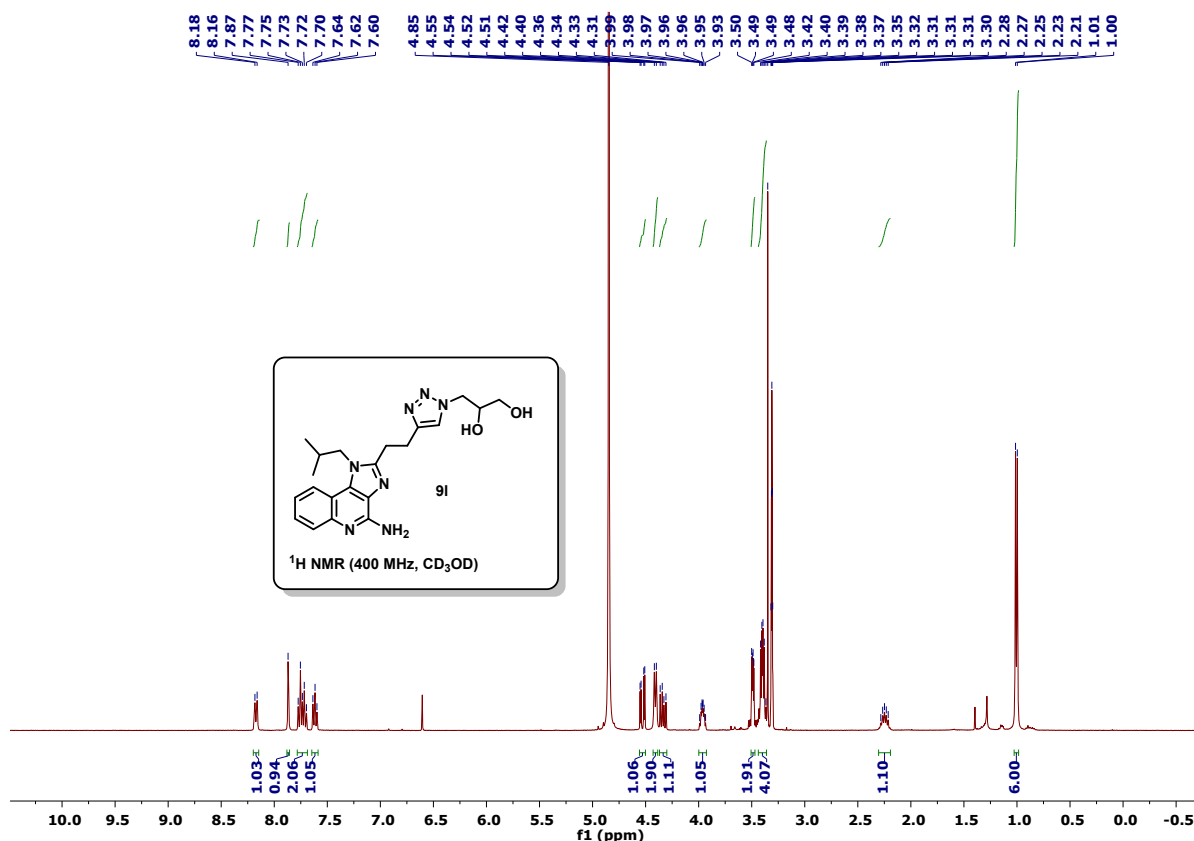
Item name: HMSAN-BB-425
Item description:

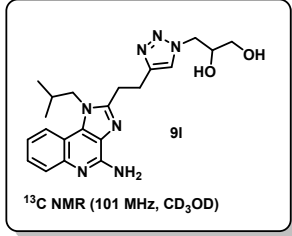
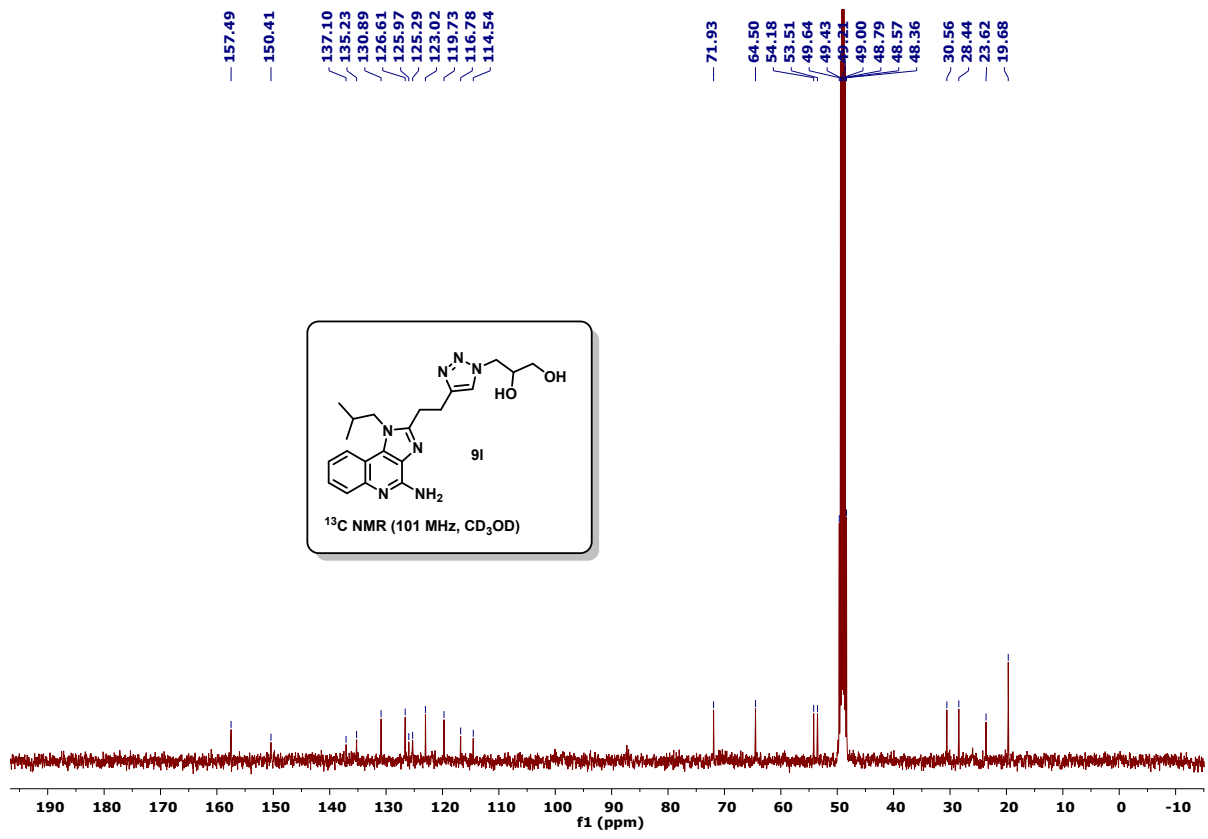


Channel name: Low energy : Time 0.3811 +/- 0.1777 minutes



9l. 3-(4-(2-(4-amino-1-isobutyl-1H-imidazo[4,5-c]quinolin-2-yl)ethyl)-1H-1,2,3-triazol-1-yl)propane-1,2-diol





SHIMADZU
 LabSolutions

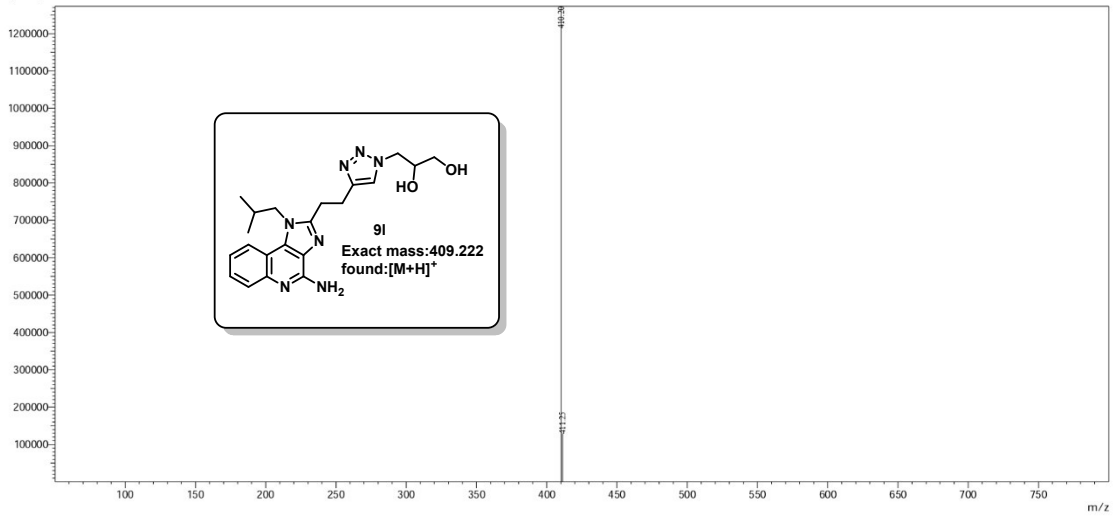
MASS REPORT

OSPC, Dr RATHOD

Sample Name : HMS-AN-20H-41.5
 Data File : 01102021.42.lcd
 Date Acquired : 10/1/2021 3:4:19 PM
 Batch File : 01102021.3.lcb

MS Spectrum
 D:\DATA\OCT-2021\01102021.42.lcd

Averaged ESI Positive+
 Spectrum Mode: Averaged 0.169-0.440(11-27)
 [CPS]



1

08/04/22 16:35:00

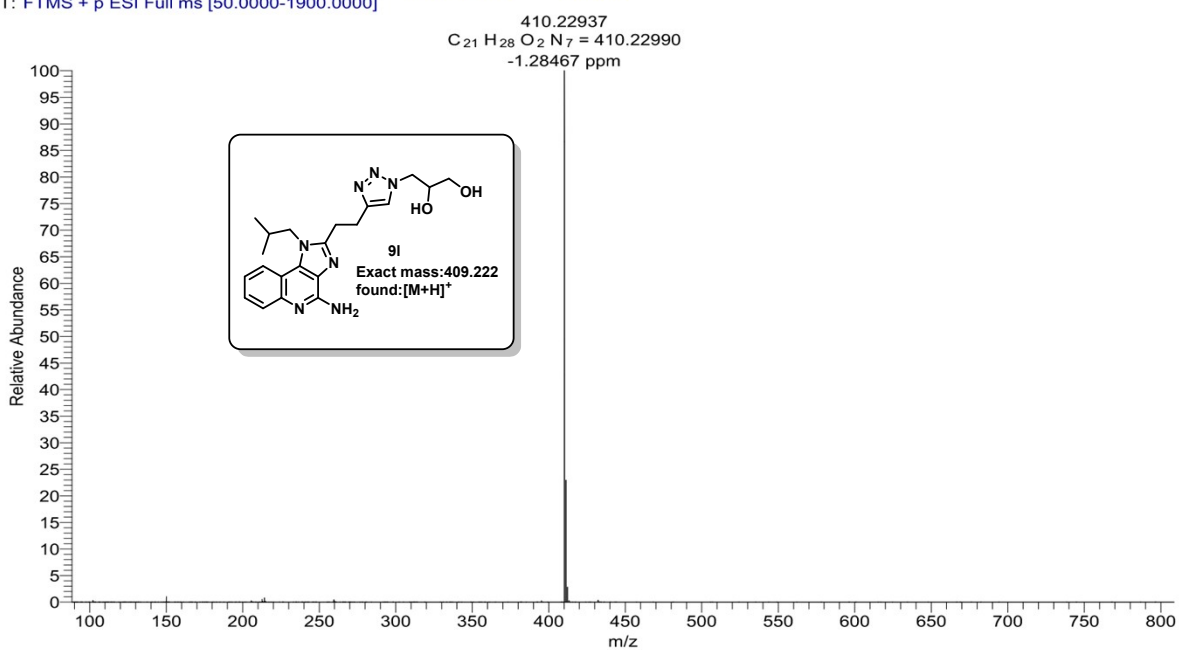
1180145675

Thermo Scientific Orbitrap Exploris 120

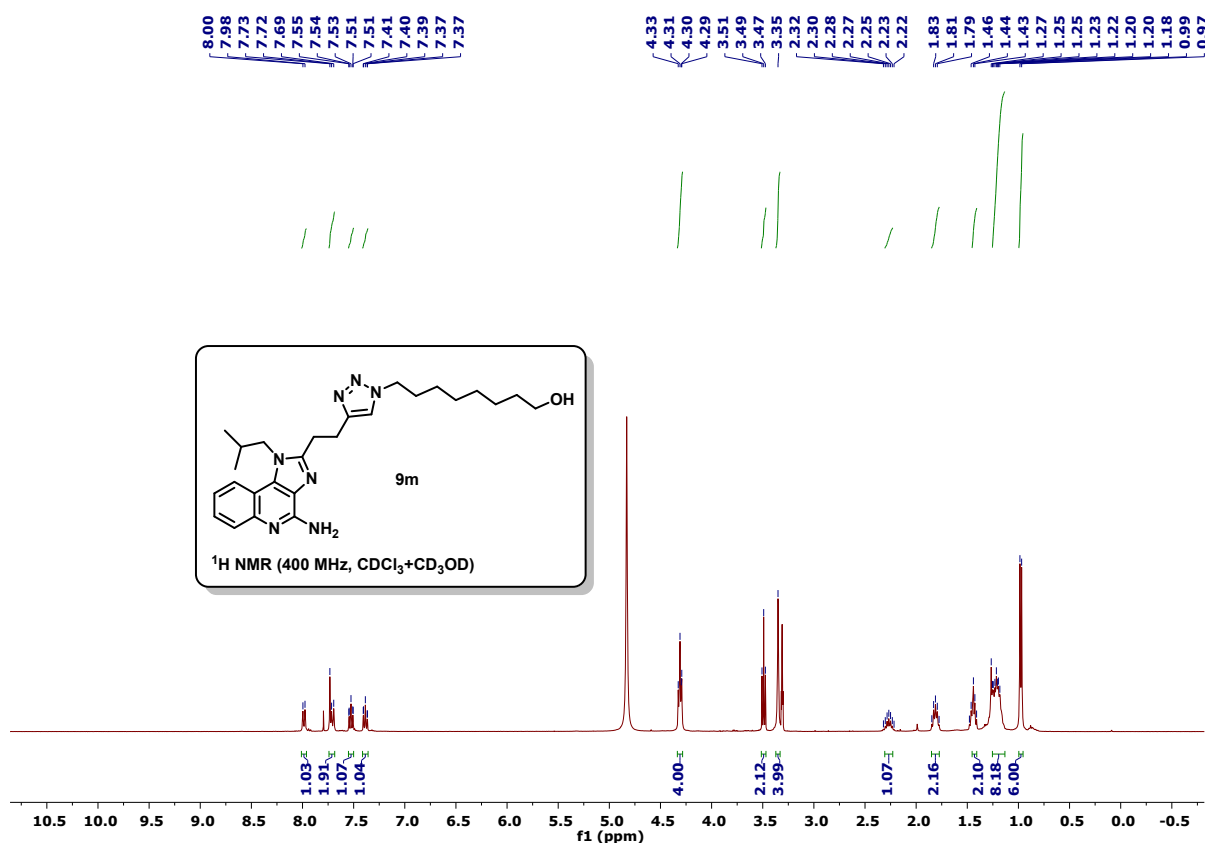
Analysed by G SAIKRISHNA

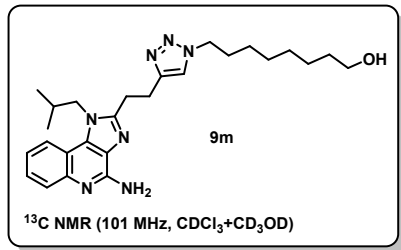
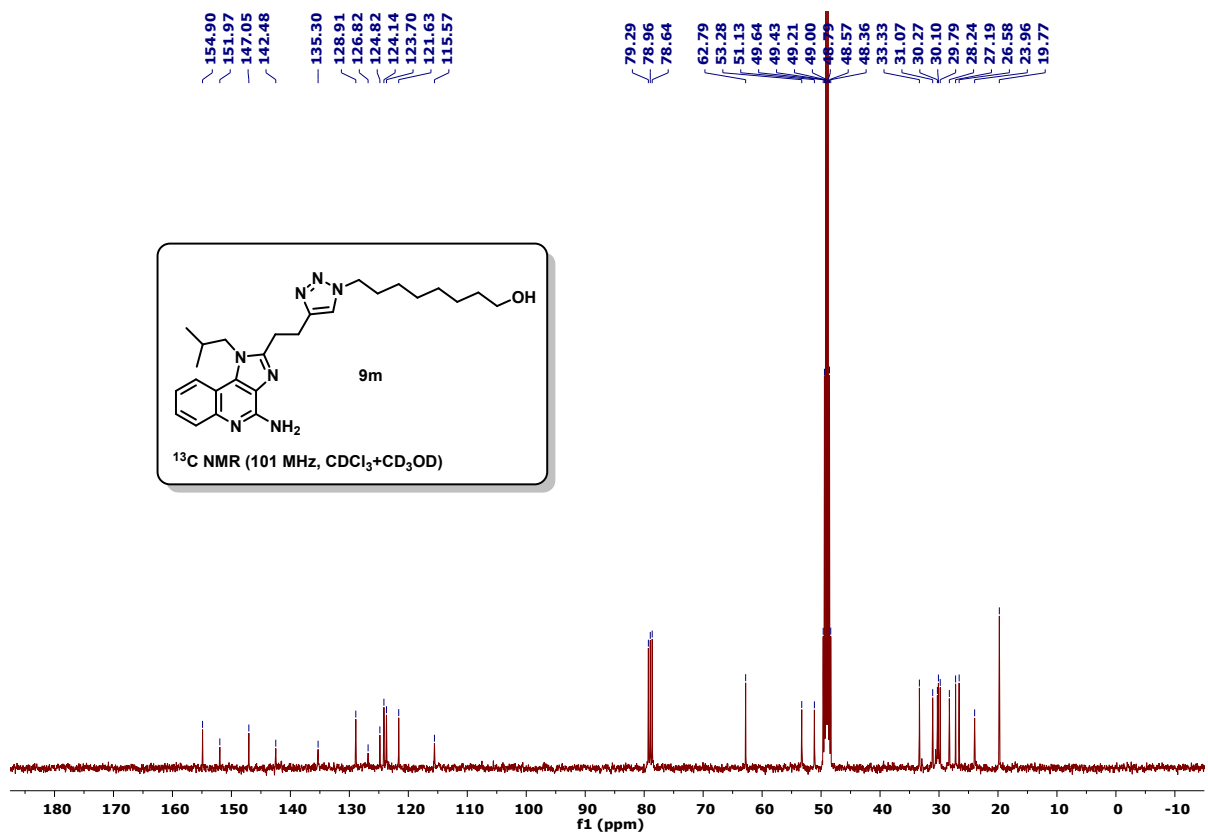
HMS-GAL2 #16-35 RT: 0.09-0.19 AV: 10 SB: 81 0.32-1.20 NL: 8.05E8

T: FTMS + p ESI Full ms [50.0000-1900.0000]



9m. 8-(4-(4-(2-(4-amino-1-isobutyl-1H-imidazo[4,5-c]quinolin-2-yl)ethyl)-1H-1,2,3-triazol-1-yl)phenyl)octan-1-ol





OSPC, Dr RATHOD

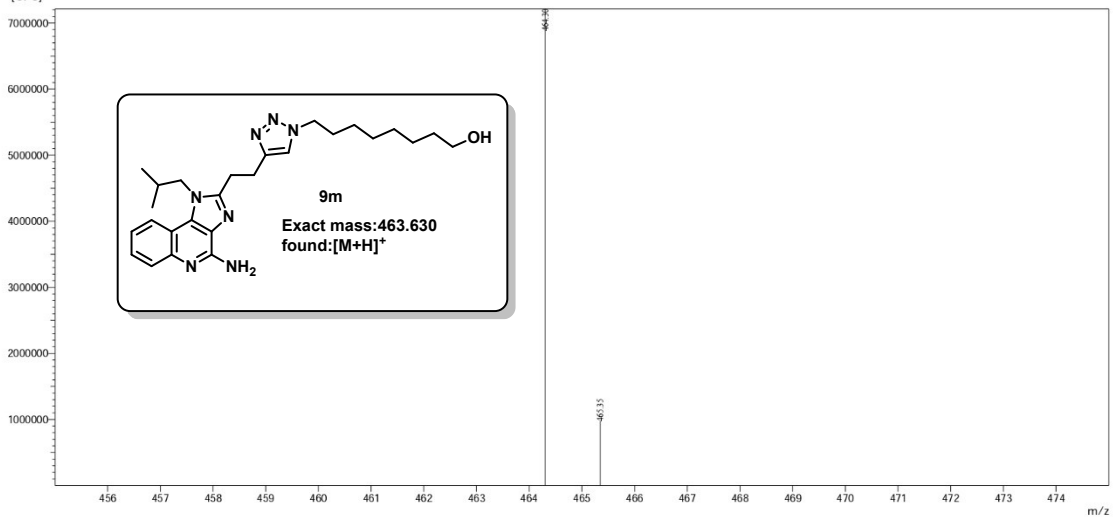
SHIMADZU
LabSolutions

MASS REPORT

Sample Name : HMS-AN-464
 Data File : 21102021.59.lcd
 Date Acquired : 10/21/2021 3:56:37 PM
 Batch File : 21102021.3.lcd

MS Spectrum
D:\DATA\OCT-2021\21102021.59.lcd

Averaged ESI Positive+
 Spectrum Mode: Averaged 0.271-0.305(17-19)
 [CPS]



1

08/04/22 16:38:10

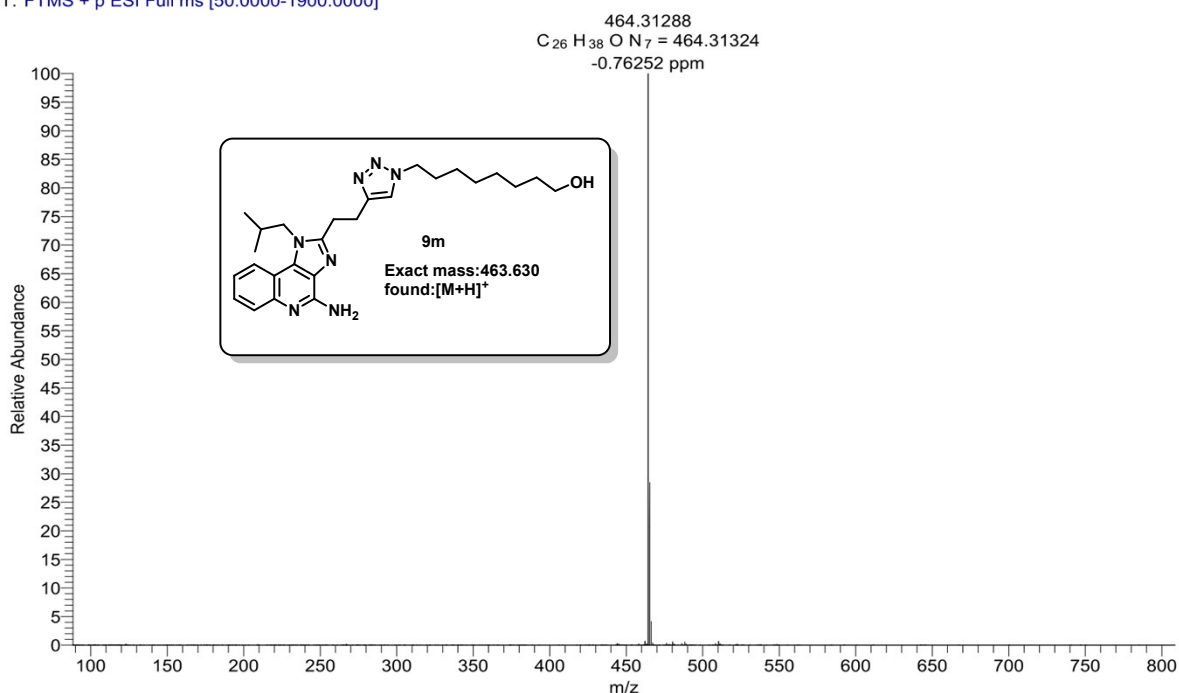
1180145676

Thermo Scientific Orbitrap Exploris 120

Analysed by G SAIKRISHNA

HMS-GAL3 #14-35 RT: 0.08-0.19 AV: 11 SB: 81 0.32-1.20 NL: 9.55E8

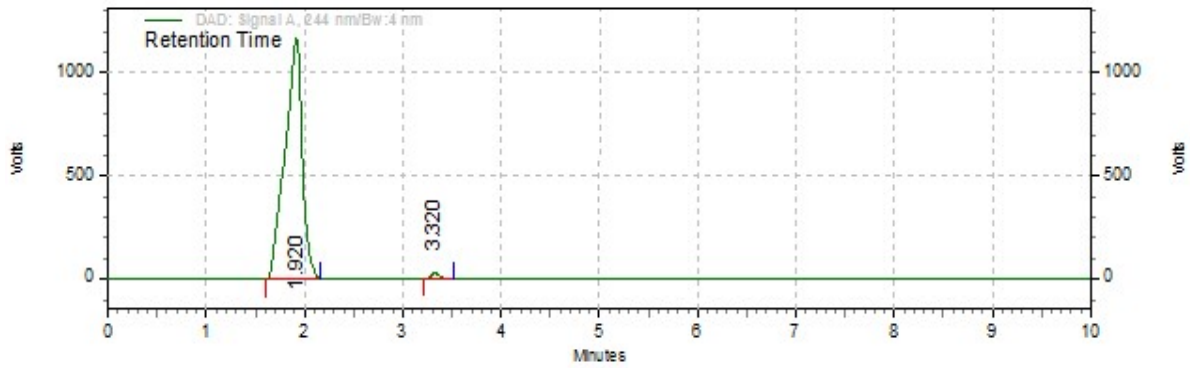
T: FTMS + p ESI Full ms [50.0000-1900.0000]



HPLC traces of compounds 9a, 9b, 9c, 9d, 9e, 9f, 9g, 9h, 9i, 9j, 9k, 9l and 9m

Analytical HPLC was carried out on an Agilent 1260 series system equipped with a DAD detector and a ZORBAX SB-C18 column (particle size: 5 μ m, dimensions: 4.6 \times 250mm), eluting with 0.1% formic acid in water (Solvent A) and CH₃CN (Solvent B), gradient 5–100% Solvent B in Solvent A over 10 min with the flow rate of 1.0 mL/min. HPLC purity was determined by UV absorption at 244 nm.

Compound 9a (Purity=97.47%)

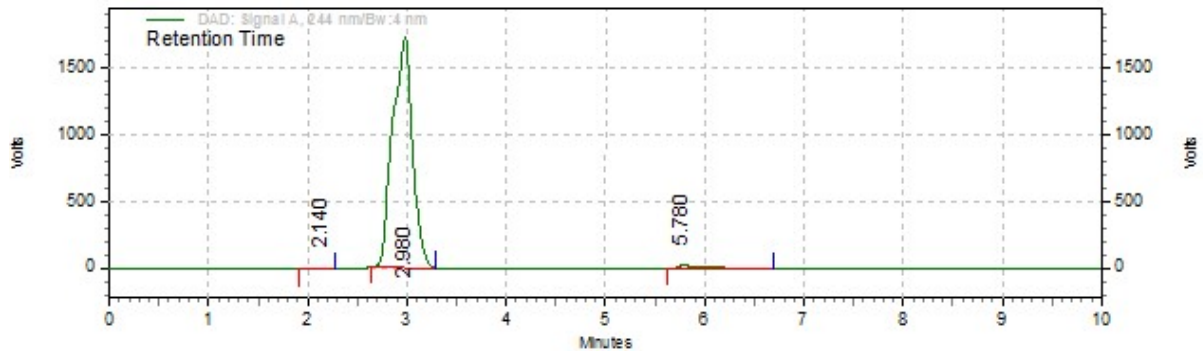


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.920	29680418	98.65	2450364	97.47
3.320	405043	1.35	63675	2.53

Totals	Area	Area %	Height	Height %
	30085461	100.00	2514039	100.00

Compound 9b (Purity=98.59%)

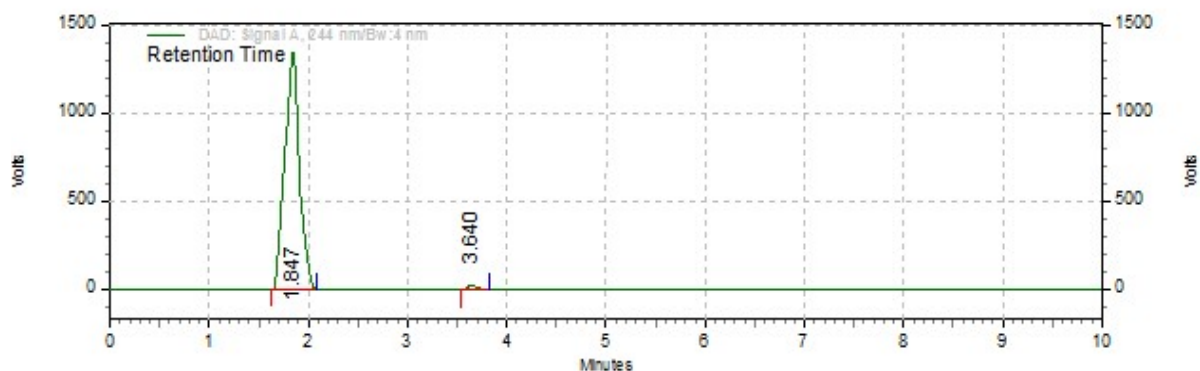


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
2.140	63191	0.12	6733	0.18
2.980	49991263	97.78	3617386	98.59
5.780	1073554	2.10	45116	1.23

Totals	Area	Area %	Height	Height %
	51128008	100.00	3669235	100.00

Compound 9c (Purity=97.79%)

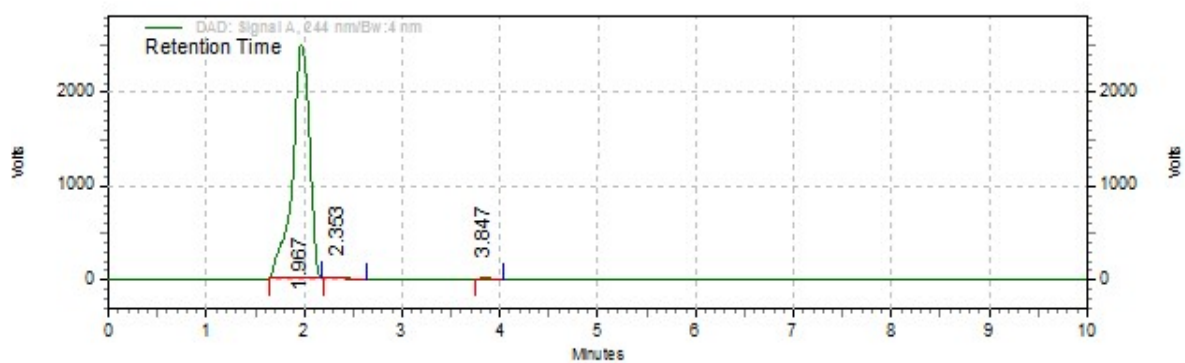


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.847	29380857	98.65	2821100	97.79
3.640	401494	1.35	63737	2.21

Totals	Area	Area %	Height	Height %
	29782351	100.00	2884837	100.00

Compound 9d (Purity=98.69%)

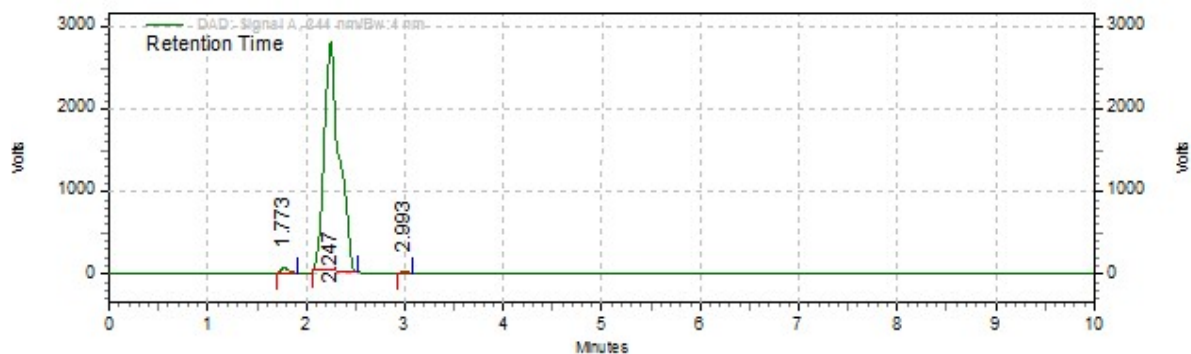


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.967	62945704	99.03	5212329	98.69
2.353	336431	0.53	30163	0.57
3.847	278073	0.44	39201	0.74

Totals	Area	Area %	Height	Height %
	63560208	100.00	5281693	100.00

Compound 9e (Purity=96.95%)

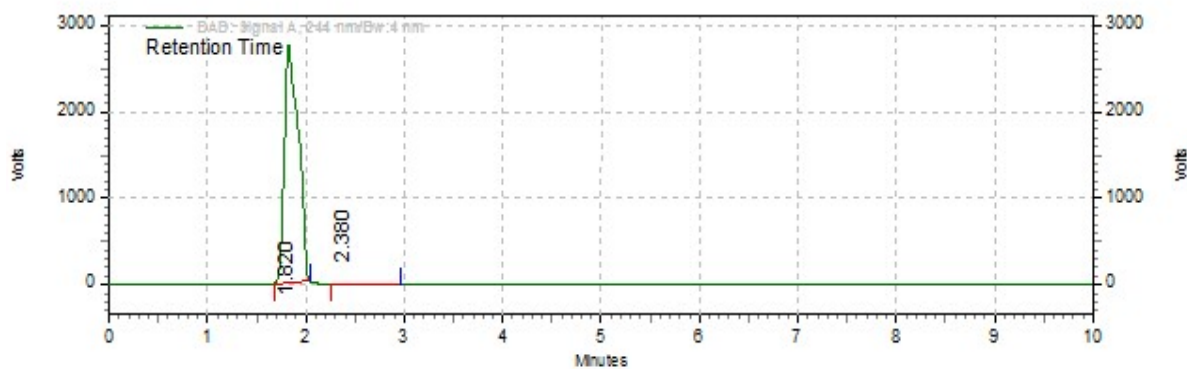


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.773	829014	1.33	145875	2.42
2.247	61400086	98.36	5842118	96.95
2.993	197736	0.32	37720	0.63

Totals	Area	Area %	Height	Height %
	62426836	100.00	6025713	100.00

Compound 9f (Purity=99.79%)

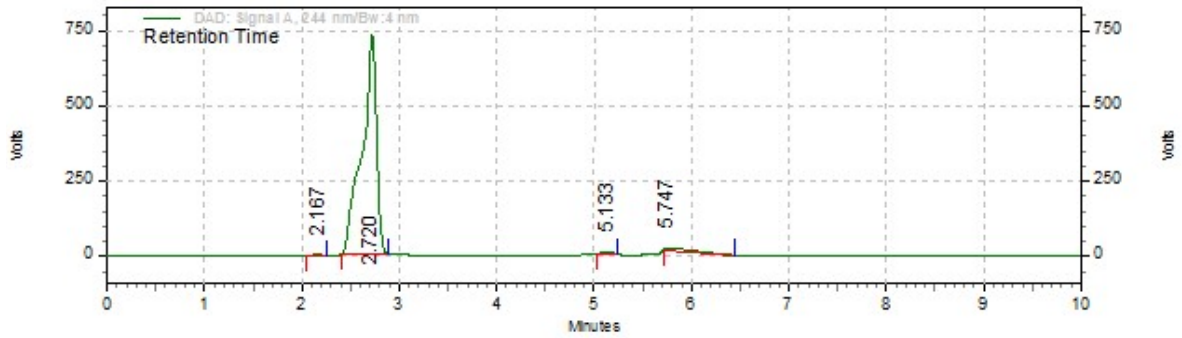


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.820	56893914	99.21	5758991	99.79
2.380	450628	0.79	12070	0.21

Totals	Area	Area %	Height	Height %
	57344542	100.00	5771061	100.00

Compound 9g (Purity=97.24%)

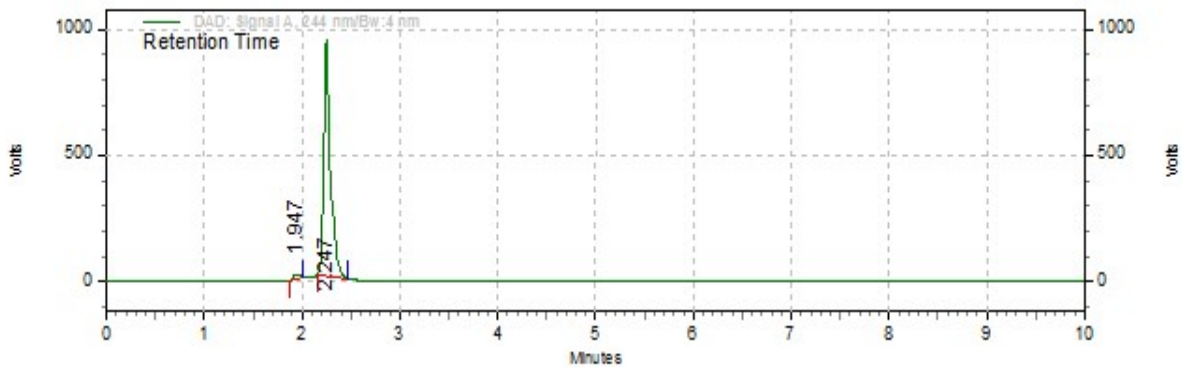


DAD: Signal A,
244 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
2.167	48811	0.30	7901	0.50
2.720	15924833	96.68	1528413	97.24
5.133	123386	0.75	17258	1.10
5.747	374332	2.27	18217	1.16

Totals	Area	Area %	Height	Height %
	16471362	100.00	1571789	100.00

Compound 9h (Purity=98.05%)

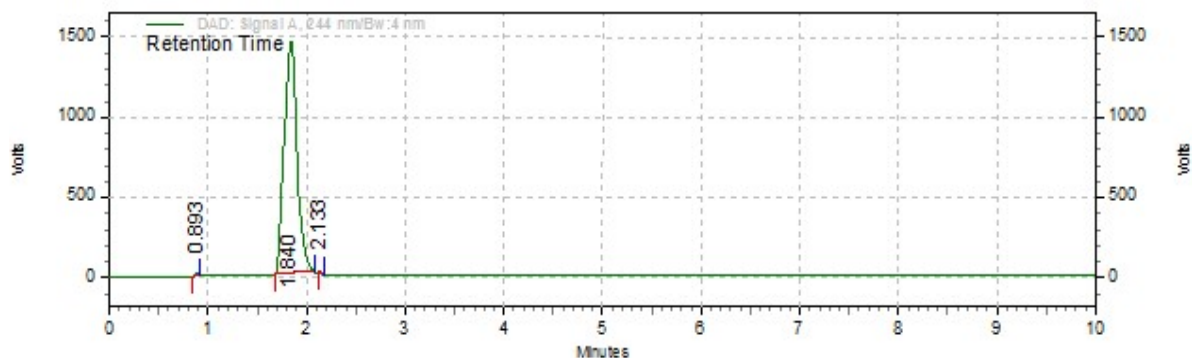


DAD: Signal A,
244 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.947	173268	1.86	39041	1.95
2.247	9164142	98.14	1965088	98.05

Totals	Area	Area %	Height	Height %
	9337410	100.00	2004129	100.00

Compound 9i (Purity=98.78%)

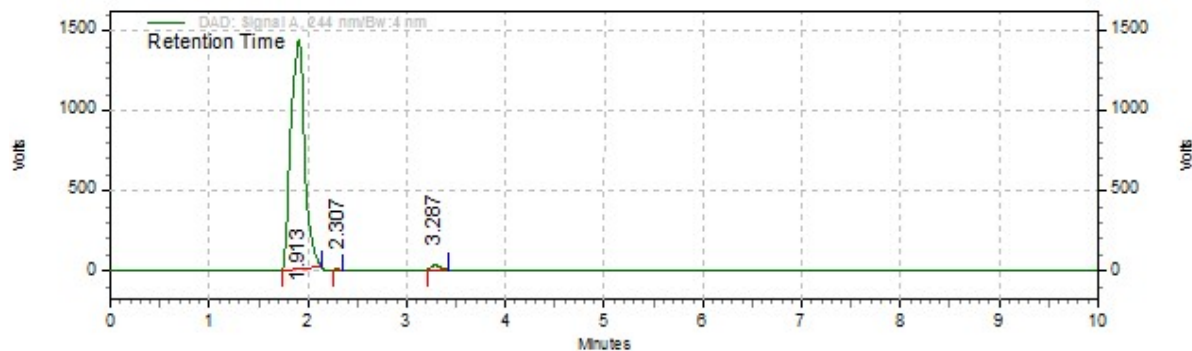


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
0.893	56598	0.20	20087	0.66
1.840	27567079	99.68	3028262	98.78
2.133	32328	0.12	17205	0.56

Totals	Area	Area %	Height	Height %
	27656005	100.00	3065554	100.00

Compound 9j (Purity=97.74%)

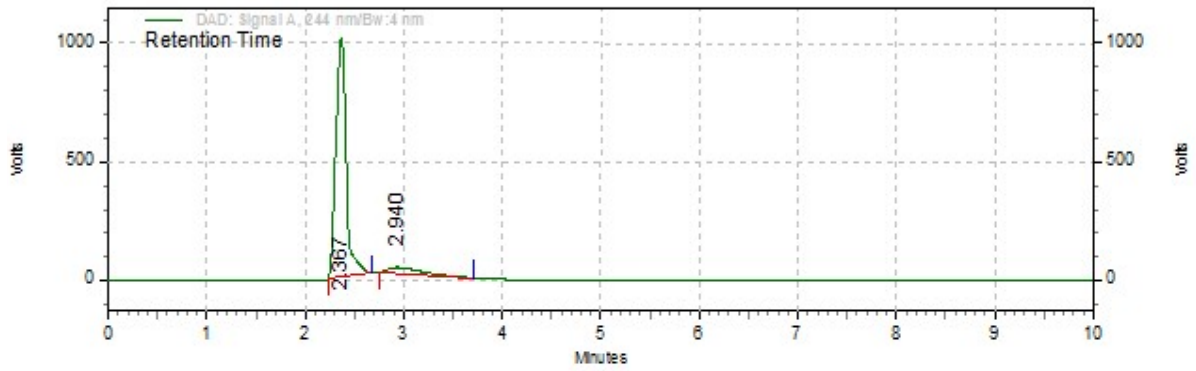


DAD: Signal A,
244 nm/Bw :4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.913	29022596	98.73	3000432	97.74
2.307	33876	0.12	12882	0.42
3.287	339292	1.15	56392	1.84

Totals	Area	Area %	Height	Height %
	29395764	100.00	3069706	100.00

Compound 9k (Purity=97.66%)



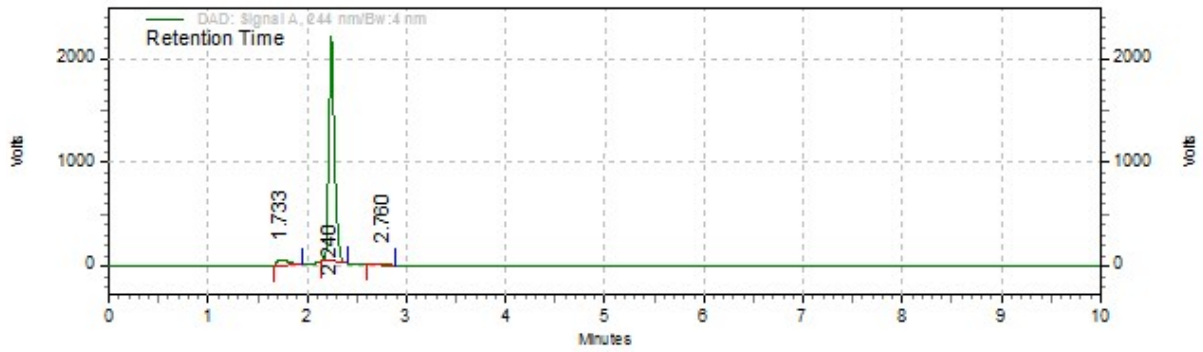
DAD: Signal A,
244 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
2.367	15236991	92.90	2111510	97.66
2.940	1164723	7.10	50575	2.34
Totals	16401714	100.00	2162085	100.00

Compound 9l (Purity=98.69%)



Compound 9m (Purity=97.58%)



DAD: Signal A,
244 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
1.733	861723	4.61	96124	2.05
2.240	17670101	94.55	4565743	97.58
2.760	156970	0.84	16973	0.36
Totals	18688794	100.00	4678840	100.00