Supporting information

Structure-Activity Studies of 1*H*-Imidazo[4,5-c]quinolin-4-amine Derivatives as A_3 Adenosine Receptor Positive Allosteric Modulators

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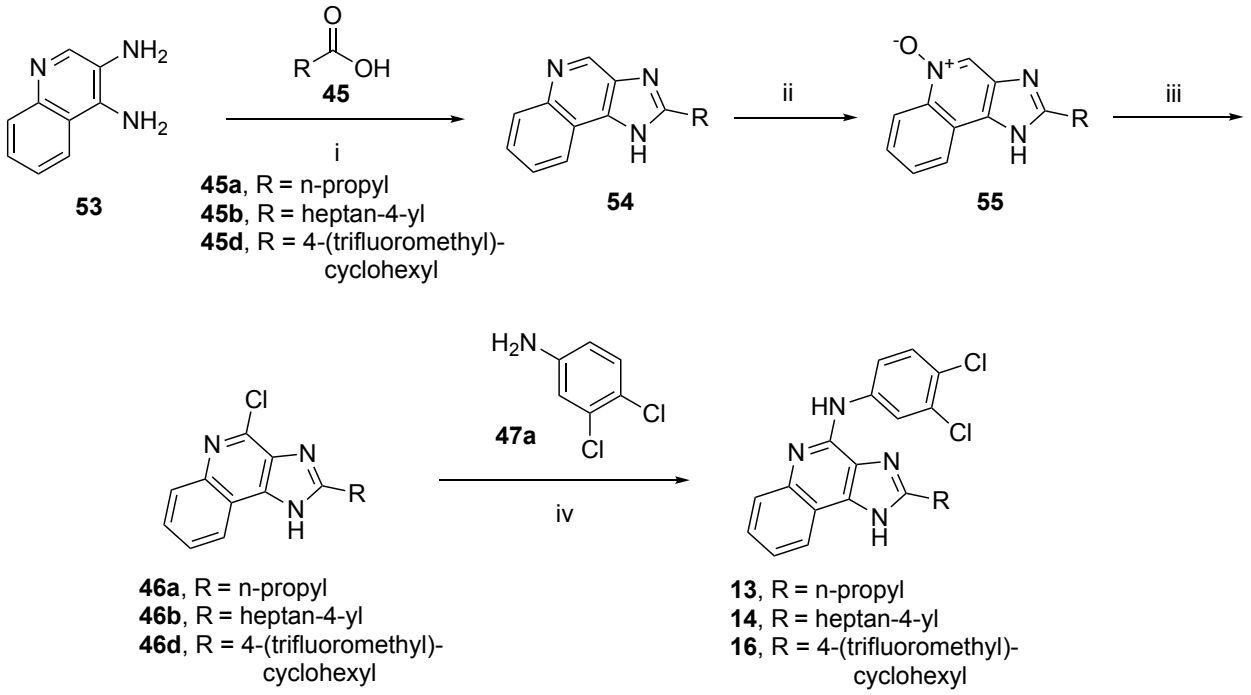
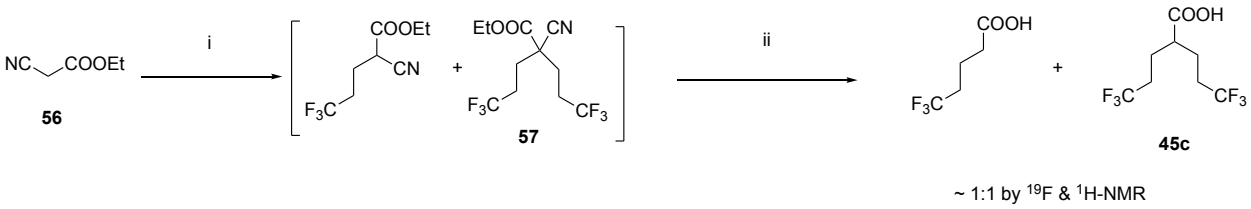
Scheme S1. Alternative route to A_3AR PAMs **13**, **14**, and **16**.



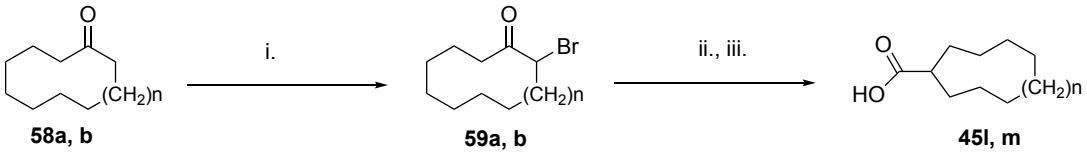
[a]Reagents and conditions: i. polyphosphoric acid, 100 °C, 8–24 h; ii. *m*-CPBA, $CHCl_3$, CH_2Cl_2, MeOH, reflux, 30 min; iii. $POCl_3$, toluene, DMF, 100 °C, 1.5 h;[1, 2] iv. General Procedure C (main text).

Scheme S2. Preparation of 5,5,5-Trifluoro-2-(3,3,3-trifluoropropyl)pentanoic Acid (**45c**).



Reagents and conditions: i. $F_3CCH_2CH_2Br$ (2.5 equiv), K_2CO_3 (2.1 equiv), DMF (sufficient to achieve a concentration of 0.4 M **57**), 60 °C, 48 h; ii. aq. NaOH (2.5 N, 50 equiv), tetrabutylammonium bromide (TBAB), 90 °C, 36 h, ~50%.

Scheme S3. Preparation of cyclononane and cyclodecane carboxylic acids (**45l–m**).

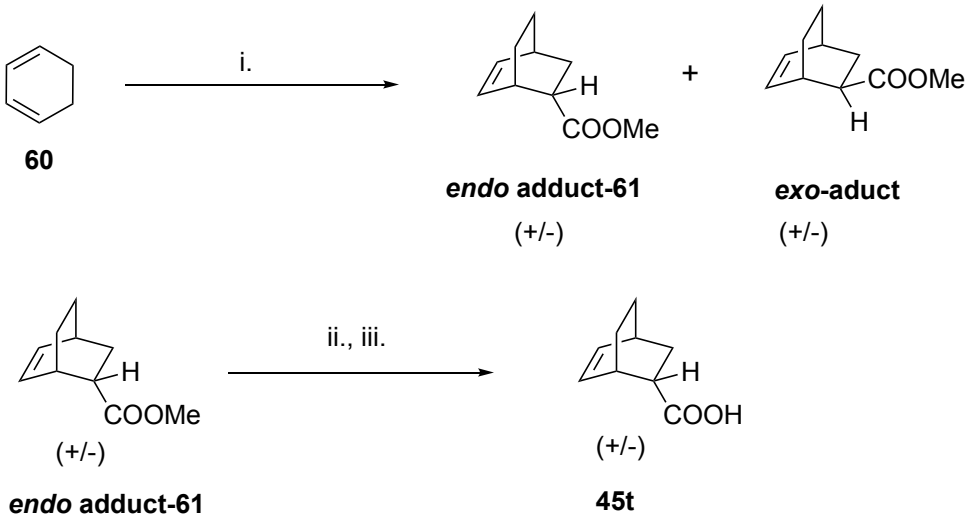


58a, b **59a, b** **45l, m**

n = 1, cyclodecanone **58a** converted to cyclononanecarboxylic acid **45l**
n = 2, cycloundecanone **58b** converted to cyclodecanecarboxylic acid **45m**

Reagents and conditions: i. NBS, PTSA, DCM, rt, 16 h; ii. $NaOCH_3$, EtO_2, rt, 20 h; iii. aqueous NaOH, reflux, 30 min, 27–29%.

Scheme S4. Preparation of (1*R*,2*R*,4*R*)- & (1*S*,2*S*,4*S*)-bicyclo[2.2.2]oct-5-ene carboxylic acid.



60

***endo* adduct-61** (+/-)

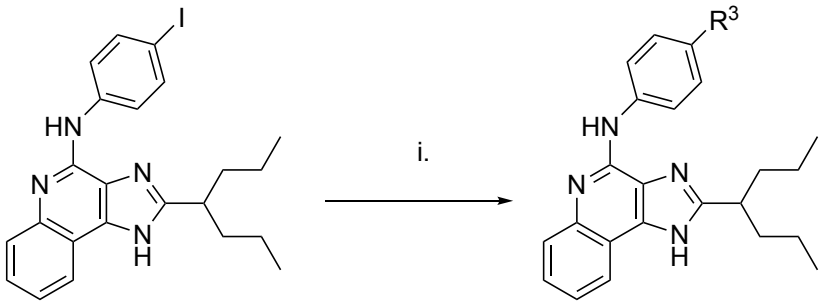
***exo*-aduct** (+/-)

***endo* adduct-61** (+/-)

45t (+/-)

Reagents and conditions: i. cyclohexa-1,3-diene, methyl acrylate, toluene, 180 °C, 22 h; ii. aqueous NaOH:MeOH, rt, 1h; iii. 1M HCl, 54%.

Scheme S5. Preparation of stannyl precursors to ^{125}I radioligand.

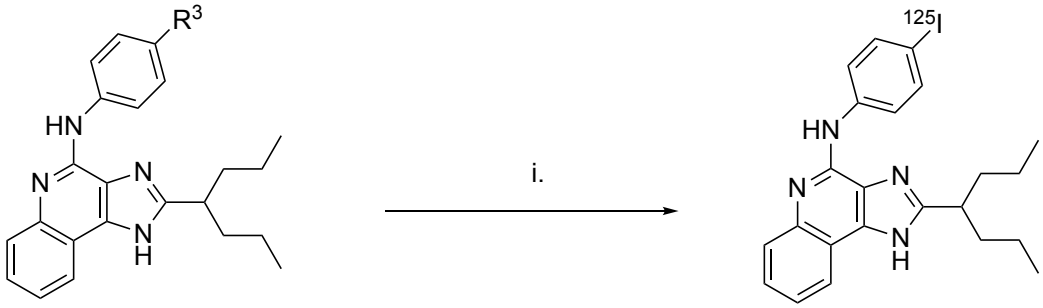


39

$R^3 = Sn(CH_3)_3$ **48**
$R^3 = Sn((CH_2)_3CH_3)_3$ **49**

Reagents and conditions: i. $PdCl_2(PPh_3)_2$, $(CH_3)_3Sn\text{-}Sn(CH_3)_3$ or $(CH_3(CH_2)_3)_3Sn\text{-}Sn((CH_2)_3CH_3)_3$ dioxane, 70 °C, 2.5 h, 10–13%.

Scheme S6. Potential radioiodination reaction to synthesize an ^{125}I radioligand.



$R^3 = Sn(CH_3)_3$ **48**
$R^3 = Sn((CH_2)_3CH_3)_3$ **49**

Reagents and conditions: i. [^{125}I]NaI, peracetic acid, rt, 10 min.

Chemical Synthesis:

All reagents and solvents were from Sigma-Aldrich (St. Louis, MO). Unless noted, ^{1}H-NMR spectra were obtained with a Bruker 400 MHz spectrometer in $CDCl_3$ (7.26 ppm), CD_3OD (HOD = 4.87 ppm), $(CD_3)_2SO$ (^{1}H=2.50 ppm), or in a mixture of $CD_3OD/CDCl_3$. The chemical shifts are expressed as ppm downfield, and coupling constants (*J*) are given in Hz. TLC analysis was carried out on glass sheets precoated with silica gel F254 (0.2 mm) from Aldrich. 3,4-Diaminoquinolines (**53**, **54** and **55**) and 2-chloroquinoline-3,4-diamines (**46a**, **b** and **d**)[3] were prepared by the literature procedures.[1, 2] The purity of final compounds (**5b**, **e** and **f**) was checked using a Hewlett−Packard 1100 HPLC equipped with an Agilent Eclipse 5 µm XDB-C18 analytical column (50 mm × 4.6 mm; Agilent Technologies Inc., Palo Alto, CA). Mobile phase: linear gradient solvent system, 10 mM TEAA (triethylammonium acetate):CH3CN from 95:5 to 0:100 in 20 min; the flow rate was 1.0 mL/min. Peaks were detected by UV absorption with a diode array detector at 230, 254, and 280 nm. All derivatives tested for biological activity showed >95 % purity in the HPLC systems. Low-resolution mass spectrometry was performed with a JEOL SX102 spectrometer with 6 kV Xe atoms following desorption from a glycerol matrix or on an Agilent LC/MS 1100 MSD, with a Waters Atlantis C18 column (Milford, MA, USA). High resolution mass spectroscopic (HRMS) measurements were performed on a proteomics optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine, unless noted. Mass accuracies were observed.

*General procedure for 2-substituted 4-chloro-1H-imidazo[4,5-c]quinolines (**46a**, **b** and **d**)[1,2]*

To a stirred solution of the appropriate 1*H*-imidazo[4,5-c]quinolin-5-oxide in a mixture of toluene (0.50 mL- 4.0 mL) and DMF (0.50 mL – 1.0 mL) at 0 °C, phosphorus oxychloride (2.6

equiv) was added and the solution stirred at rt for 10 min. The reaction mixture stirred at 110 ºC for 2-3 h. The reaction was monitored by mass spectrometry and continued until the starting material, *N*-oxide derivative (**55a**, **b** and **d)** disappeared. The reaction mixture was cooled to rt, neutralized with half saturated aqueous $NaHCO_3$ solution (10 mL) and diluted with EtOAc (25 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (2 x 15 mL). The combined organic phases were dried over Na_2SO_4 and filtered. The filtrate was concentrated by rotary evaporation to obtain the crude product, which was and purified by silica column to get the 2-substituted-4-chloro-1*H*-imidazo[4,5-c]quinolines (**46a**, **b** and **d**).

*General procedure for the synthesis of 2-substituted 1H-imidazo[4,5-c]quinoline derivatives (**54a**, **b** and **d**)*[1, 2]

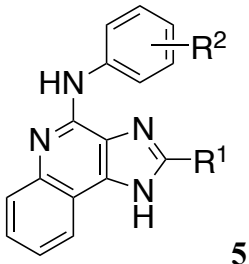
Polyphosphoric acid, 115 % H_3PO_4 basis (1.5–2.0 g) was added to 3,4-diaminoquinoline (**53**, 1.0 equiv) and the appropriate carboxylic acid (1.2–1.5 equiv). The reaction mixture was stirred at 100 °C for 8–16 h. The mixture was cooled to ~40–45 ºC and poured into a beaker with crushed ice (~30–35g). The reaction mass was slowly neutralized with con. ammonia (23%) at 0 ºC until a pH of 8–9. and diluted with EtOAc (25 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (3x 15 mL). The combined EtOAc layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated by rotary evaporation to obtain the crude, which was purified by silica column to get the 1*H*-imidazo[4,5-c]quinoline derivative.

*General procedure for 2-substituted 1H-imidazo[4,5-c]quinoline-5-oxide (**55a**, **b** and **d**)*[1, 2]

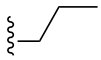
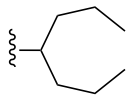
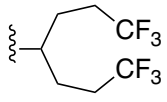
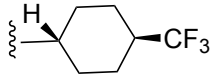
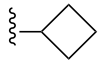
To a stirred solution of the appropriate 2-substituted-1*H*-imidazo[4,5-c]quinoline derivatives (**54a**, **b** and **d**) in a mixture of 10% MeOH in $CHCl_3$, *m*-CPBA (2.5 equiv) was added and the

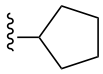
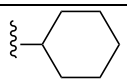
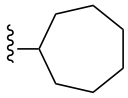
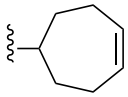
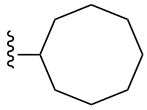
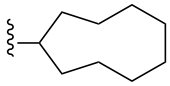
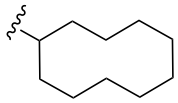
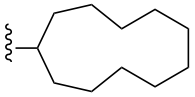
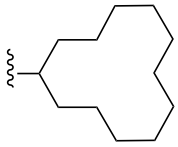
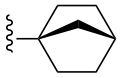
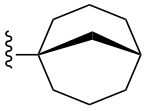
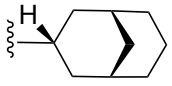
reaction mixture stirred for 30 min with gentle reflux. The reaction mixture was cooled to rt and quenched with half saturated $NaHCO_3$ (5 mL). The phases were separated, and the aqueous phase was extracted with a mixture of isopropyl alcohol and chloroform (1:2, 2 x 15 mL). The combined organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated by rotary evaporation to obtain the crude product and purified by silica column to get the 2-substituted-1*H*-imidazo[4,5-c]quinoline-5-oxide (**55a**, **b** and **d**).

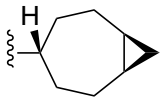
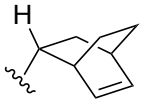
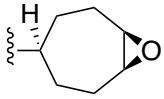
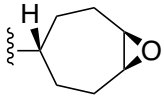
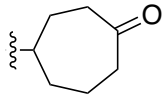
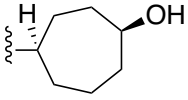
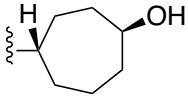
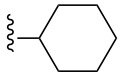
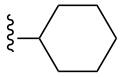
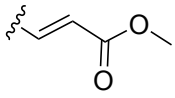
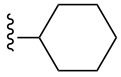
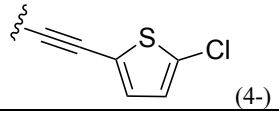
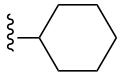
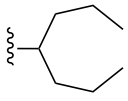
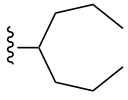
Table S1. 1*H*-Imidazo[4,5-c]quinolin-4-amine Derivatives Synthesized for Pharmacological Studies.

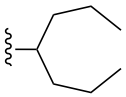


5 – **8**, **13** – **39**, **48**, **49**

Compound	**R^2**	**R^1**	**Yield[a] (%, final step)**
2-alkyl and 2-cycloalkyl derivatives			
13	3,4-Cl_2		23[b]
14	3,4-Cl_2		11[b]
15	3,4-Cl_2		50[b]
16	3,4-Cl_2		21[b]
17	3,4-Cl_2		18[b]
5	3,4-Cl_2		17[b]

6	3,4-Cl_2		23[b]
7	3,4-Cl_2		28[b]
8	3,4-Cl_2		51[b]
18	3,4-Cl_2		23[b]
19	3,4-Cl_2		27[b]
20	3,4-Cl_2		5[b]
21	3,4-Cl_2		16[b]
22	3,4-Cl_2		15[b]
23	3,4-Cl_2		25[d]
2-bicycloalkyl derivatives			
24	3,4-Cl_2		13[c]
25	3,4-Cl_2		33[c]
26	3,4-Cl_2		22[c]
27	3,4-Cl_2	 (endo)	2[c]

28	3,4-Cl_2		13[e]
29	3,4-Cl_2	 (endo)	36[d]
2-cycloalkyl derivatives with hydrophilic substitution			
30	3,4-Cl_2		6[f]
31	3,4-Cl_2		12[f]
32	3,4-Cl_2	 (±)	23[g]
33	3,4-Cl_2	 (±)	8[h]
34	3,4-Cl_2	 (±)	12[h]
2-alkyl and 2-cycloalkyl derivatives with modified 2-arylamino groups			
35	4-I		61[d]
36	4-Br		10[d]
37	 (4-)		18[i]
38	 (4-)		10[j]
39	4-I		11[d]
48	4-$Sn(CH_3)_3$		10[k]

49	4-Sn($(CH_2)_3CH_3)_3$		13[l]

a. Isolated yield.
b. Final general procedure C (viii, in Reagents and Conditions of Scheme 1) used.
c. Final general procedure D (ix, in Reagents and Conditions of Scheme 1) used.
d. Final general procedure E (x, in Reagents and Conditions of Scheme 1) used.
e. **18**, Et_2Zn, CH_2I_2 (xi, in Reagents and Conditions of Scheme 1).
f. **18**, *m*-CPBA, $CHCl_3$ (xii, in Reagents and Conditions of Scheme 1).
g. **18**, $(CH_3)_2S{\cdot}BH_3$, THF, NaOH, H_2O_2, 0 °C (xiii, in Reagents and Conditions of Scheme 1).
h. **33** and **34**, DMP, $CHCl_3$ (xiv, in Reagents and Conditions of Scheme 1).
i. **36**, $Pd(OAc)_2$, $CH_2{=}CHCOOCH_3$, Et_3N, 140 °C (xv, in Reagents and Conditions of Scheme 1).
j. **35**, $Pd(Ph_3P)_2Cl_2$, C_6H_3ClS, CuI, Et_3N, 80 °C (xvi, in Reagents and Conditions of Scheme 1).
k. **39**, $Pd(Ph_3P)_2Cl_2$, hexamethylditin, 70 °C.
l. **39**, $Pd(Ph_3P)_2Cl_2$, hexabutylditin, 70 °C.

Table S2. Effect of PAM derivatives (10 μM) on dissociation of [^{125}I]**50** (0.3 nM) using hA$_3$ARs (n=3). P-value is shown with respect to control in the absence of a PAM. Statistical significance was calculated by two-tailed Student's t-test. Data are presented as mean ± SEM. Percent radioligand remaining bound was determined 60 min after the initiation of dissociation with the competitive agonist adenosine-5′-*N*-ethylcarboxamide (**51**, 100 μM).

Compound ID	% Remaining	SEM	P-value
13	24.6	1.8	0.760
14	71.2	2.7	0.001[b]
15	45.2	5.1	0.056[b]
16	25.9	4.4	0.958[b]
17	33.3	2.4	0.271
5	36.2	3.8	0.187
6	43.4	5.2	0.075
7	54.4	3.4	0.009[b]
8	52.2	6.1	0.030[b]
18	54.1	3.4	0.001[b]
19	44.5	4.1	0.046[b]
20	37.1	4.7	0.188
21	24.6	3.1	0.786
22	24.5	4.6	0.808
23	23.9	4.5	0.735
24	45.6	3.8	0.036[b]
25	44.5	4.0	0.046[b]
12a	38	5	0.168
12b	51	3	0.013[b]
26	52.1	3.1	0.011[b]
27	46.1	4.4	0.040
28	45.7	4.9	0.048
29	42.1	5.6	0.100
30	33.2	4.3	0.350
31	39.4	3.6	0.098
32	33.1	3.7	0.328
33	33.0	3.7	0.340
34	34.9	3.0	0.210
35	57.8	4.8	0.010
36	55.8	5.2	0.015[b]
37	49.6	7.0	0.052
38	32.5	5.5	0.450
39	65.3	4.0	0.003[b]

[a] The effect of these compounds on radioligand dissociation was reported by Fisher et al.[3]

[b] Significant difference compared to control (Student T-test, P ≤0.05).

Table S3. Effect of PAM derivatives (10 μM) on the equilibrium binding of [^{125}I]**50** (0.3 nM) at the hA$_3$AR (n=3). P-value is shown with respect to control in the absence of a PAM.

Compound ID	% Change from Vehicle	SEM	P-value
13	-45.2	1.6	0.002[b]
14	70.2	9.3	0.020[b]
15	18.4	5.3	0.035[b]
16	-4.5	2.2	0.171
17	-70.9	2.3	0.006[b]
5	-23.3	3.0	0.028[b]
6	1.9	4.3	0.662
7	4.3	14.2	0.722
8	0.3	0.1	0.052
18	-11.8	1.6	0.026[b]
19	41.3	4.7	0.019[b]
20	27.8	3.2	0.007[b]
21	17.5	5.0	0.084
22	15.7	5.6	0.083
23	6.9	2.2	0.070
24	-5	1.8	0.135
25	-6.7	3.3	0.204
12a	-28.8	3.1	0.007[b]
12b	18.7	3.1	0.031[b]
26	38.3	4.6	0.012[b]
27	41	4.9	0.007[b]
28	-36.6	5.9	0.056
29	-20.5	2.5	0.032[b]
30	-21.3	3.0	0.050[b]
31	-24.1	2.8	0.040[b]
32	-19.1	2.6	0.050[b]
33	-35.8	2.1	0.023[b]
34	-31.3	3.1	0.037[b]
35	-31.1	3.5	0.04 [b]
36	-48.9	5.5	0.036[b]
37	14.6	4.0	0.011[b]
38	19.5	6.4	0.055
39	22.9	10.2	0.125

[a] The effect of these compounds on radioligand equilibrium binding was reported by Fisher et al.[3]

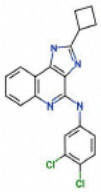
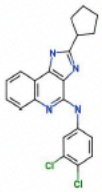
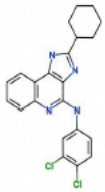
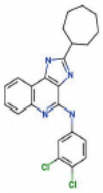
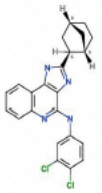
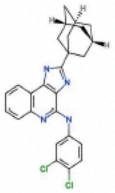
[b] Significant difference compared to control (Student T-test, P ≤0.05).

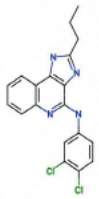
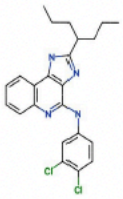
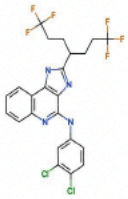
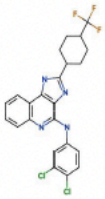
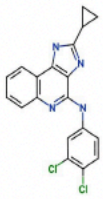
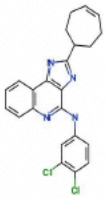
Table S4. Effect of PAM derivatives on [^{35}S]GTPγS binding induced by Cl-IB-MECA using WT hA$_3$ARs (n=3). P-value is shown with respect to control in the absence of a PAM.[a]

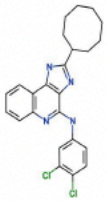
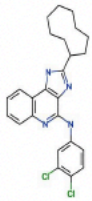
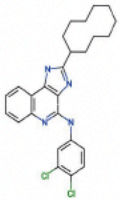
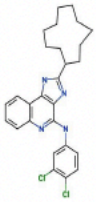
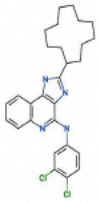
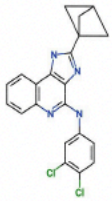
ID	DMSO			0.1 μM Compound					1.0 μM Compound					10 μM Compound				
	EC_{50} (nM)	pEC_{50}	E_{max} (%)	EC_{50} (nM)	pEC_{50}	P-value	E_{max} (%)	P-value	EC_{50} (nM)	pEC_{50}	P-value	E_{max} (%)	P-value	EC_{50} (nM)	pEC_{50}	P-value	E_{max} (%)	P-value
13	21	7.68 ± 0.08	100 ± 3	166	6.78 ± 0.16*	0.0041	132 ± 8	0.0684	852	6.24 ± 0.16*	0.0002	153 ± 12*	0.0051	305	6.52 ± 0.11*	0.0008	131 ± 6	0.0773
14	47	7.32 ± 0.16	100 ± 6	23	7.64 ± 0.16	0.5129	171 ± 9*	0.0017	17	7.77 ± 0.16	0.2041	216 ± 12*	<0.0001	25	7.60 ± 0.11	0.6711	216 ± 9*	<0.0001
15	48	7.32 ± 0.14	99 ± 5	43	7.37 ± 0.16	>0.9999	118 ± 7	0.5343	52	7.28 ± 0.20	>0.9999	159 ± 13*	0.0043	47	7.33 ± 0.13	>0.9999	185 ± 9*	0.0004
16	18	7.74 ± 0.10	99 ± 3	23	7.65 ± 0.10	>0.9999	120 ± 4*	0.0377	21	7.68 ± 0.11	>0.9999	150 ± 5*	0.0002	156	6.94 ± 0.08*	0.0012	174 ± 6*	<0.0001
17	27	7.58 ± 0.06	100 ± 2	44	7.36 ± 0.23	>0.9999	117 ± 8	>0.9999	1994	6.70 ± 0.40	0.1976	111 ± 16	>0.9999	765	6.12 ± 0.35*	0.0226	90 ± 20	>0.9999
5	27	7.57 ± 0.08	100 ± 3	48	7.32 ± 0.10	0.2748	113 ± 4	0.2896	77	7.12 ± 0.08*	0.0258	109 ± 3	0.7374	352	6.45 ± 0.11*	<0.0001	135 ± 8*	0.003
6	13	7.90 ± 0.09	100 ± 3	24	7.61 ± 0.11	0.2176	110 ± 4	0.6257	37	7.43 ± 0.11*	0.0279	142 ± 5*	0.0012	137	6.86 ± 0.08*	0.0002	201 ± 7*	<0.0001
7	15	7.82 ± 0.16	100 ± 5	20	7.70 ± 0.18	>0.9999	154 ± 9*	0.0298	23	7.64 ± 0.14	>0.9999	225 ± 10*	0.0002	73	7.14 ± 0.19	0.0649	241 ± 18*	<0.0001
8	18	7.76 ± 0.09	100 ± 3	13	7.90 ± 0.10	0.7155	120 ± 4*	0.0176	16	7.80 ± 0.05	>0.9999	175 ± 3*	<0.0001	60	7.22 ± 0.08*	0.005	218 ± 6*	<0.0001
18	36	7.44 ± 0.09	100 ± 3	24	7.61 ± 0.15	0.952	147 ± 7*	0.0107	16	7.79 ± 0.11	0.1971	241 ± 9*	<0.0001	81	7.09 ± 0.09	0.2026	287 ± 11*	<0.0001
19	38	7.42 ± 0.07	100 ± 2	30	7.52 ± 0.07	0.951	107 ± 3	0.3555	266	7.59 ± 0.05	0.3631	146 ± 2*	<0.0001	23	7.63 ± 0.08	0.1772	164 ± 4*	<0.0001
20	41	7.38 ± 0.08	100 ± 3	26	7.58 ± 0.13	0.9689	143 ± 6*	0.0459	20	7.71 ± 0.15	0.3712	241 ± 12*	<0.0001	29	7.54 ± 0.15	>0.9999	259 ± 14*	<0.0001
21	24	7.61 ± 0.08	100 ± 3	19	7.72 ± 0.15	>0.9999	109 ± 6	>0.9999	15	7.82 ± 0.14	0.9089	135 ± 7*	0.0182	16	7.80 ± 0.16	>0.9999	195 ± 10*	<0.0001
22	24	7.62 ± 0.10	100 ± 3	35	7.45 ± 0.10	>0.9999	118 ± 4	0.1214	25	7.60 ± 0.15	>0.9999	122 ± 6*	0.0527	15	7.82 ± 0.14	0.9013	166 ± 8*	<0.0001
23	30	7.52 ± 0.19	100 ± 6	14	7.86 ± 0.09	0.3327	99 ± 3	>0.9999	13	7.87 ± 0.11	0.2902	101 ± 3	>0.9999	16	7.79 ± 0.12	0.5659	116 ± 5	0.1078
24	31	7.51 ± 0.15	100 ± 5	61	7.22 ± 0.17	0.9439	141 ± 9	0.2169	40	7.39 ± 0.21	>0.9999	187 ± 14*	0.0069	236	6.63 ± 0.24*	0.0368	195 ± 22*	0.004
25	18	7.74 ± 0.15	100 ± 5	39	7.41 ± 0.16	0.3392	120 ± 6	0.3196	82	7.09 ± 0.09*	0.0257	170 ± 6*	0.0008	171	6.77 ± 0.13*	0.0026	180 ± 12*	0.0003
12a	55	7.26 ± 0.09	100 ± 3	27	7.56 ± 0.12	0.2171	103 ± 4	>0.9999	47	7.33 ± 0.11	>0.9999	154 ± 7*	0.0022	214	6.67 ± 0.09*	0.0116	258 ± 12*	<0.0001
12b	46	7.34 ± 0.09	100 ± 3	17	7.78 ± 0.19	0.275	148 ± 10	0.0729	10	8.01 ± 0.15	0.058	237 ± 12*	0.0002	30	7.52 ± 0.19	>0.9999	248 ± 19*	<0.0001
26	16	7.78 ± 0.15	97 ± 5	25	7.60 ± 0.18	>0.9999	147 ± 9	0.0799	38	7.42 ± 0.21	0.5935	219 ± 16*	0.0005	73	7.14 ± 0.17	0.103	216 ± 17*	0.0006
27	26	7.58 ± 0.15	98 ± 5	29	7.53 ± 0.17	>0.9999	141 ± 9*	0.0138	18	7.74 ± 0.12	>0.9999	187 ± 8*	0.0001	47	7.32 ± 0.10	0.6499	182 ± 8*	0.0002
28	33	7.48 ± 0.14	100 ± 5	59	7.23 ± 0.26	>0.9999	143 ± 14	0.1101	135	6.87 ± 0.16	0.1601	180 ± 14*	0.0049	116	6.94 ± 0.18	0.2343	154 ± 14*	0.0414
29	28	7.55 ± 0.15	98 ± 5	35	7.46 ± 0.18	>0.9999	129 ± 9	0.1792	131	6.88 ± 0.14*	0.0341	167 ± 12*	0.0035	400	6.40 ± 0.10*	0.0014	182 ± 13*	0.001
30	75	7.13 ± 0.18	99 ± 7	116	6.94 ± 0.21	>0.9999	131 ± 11	0.2819	99	7.01 ± 0.20	>0.9999	136 ± 11	0.1666	151	6.82 ± 0.23	0.9736	145 ± 16	0.0727
31	29	7.53 ± 0.14	98 ± 5	16	7.79 ± 0.13	0.9184	119 ± 5	0.4999	44	7.35 ± 0.22	>0.9999	173 ± 14*	0.0019	79	7.11 ± 0.16	0.3236	174 ± 12*	0.0018
32	43	7.37 ± 0.17	100 ± 6	47	7.33 ± 0.20	>0.9999	110 ± 8	>0.9999	220	0.66 ± 0.23	0.1091	160 ± 16*	0.0265	527	6.28 ± 0.20*	0.0146	151 ± 16	0.0587
33	47	7.33 ± 0.17	99 ± 6	111	6.96 ± 0.22	0.7729	132 ± 11	0.1678	85	7.07 ± 0.31	>0.9999	118 ± 14	0.7121	592	6.23 ± 0.12*	0.0218	123 ± 8	0.4338
34	30	7.53 ± 0.15	99 ± 5	92	7.03 ± 0.18	0.2238	134 ± 10	0.1852	227	6.64 ± 0.14*	0.0186	156 ± 10*	0.0215	853	6.07 ± 0.20*	0.0009	113 ± 17	>0.9999
35	59	7.23 ± 0.15	100 ± 6	60	7.22 ± 0.17	>0.9999	136 ± 8	0.1022	39	7.40 ± 0.15	>0.9999	184 ± 9*	0.001	115	6.94 ± 0.16	0.7023	206 ± 14*	0.0002
36	59	7.23 ± 0.15	102 ± 6	118	6.93 ± 0.24	0.9215	162 ± 16	0.0687	139	6.86 ± 0.20	0.6385	207 ± 17*	0.0037	329	6.48 ± 0.18	0.0796	212 ± 19*	0.0028
37	21	7.68 ± 0.18	100 ± 6	31	7.50 ± 0.15	>0.9999	133 ± 6*	0.0444	27	7.57 ± 0.19	>0.9999	164 ± 10*	0.0009	24	7.61 ± 0.14	>0.9999	190 ± 8*	<0.0001
38	39	7.41 ± 0.22	97 ± 7	61	7.21 ± 0.16	>0.9999	131 ± 8	0.0809	108	6.97 ± 0.20	0.3546	170 ± 12*	0.0012	40	7.40 ± 0.13	>0.9999	203 ± 8*	<0.0001
39	67	7.16 ± 0.15	98 ± 6	45	7.35 ± 0.13	0.9238	158 ± 8*	0.0022	45	7.34 ± 0.12	0.9803	223 ± 10*	<0.0001	41	7.39 ± 0.10	0.6941	215 ± 8*	<0.0001

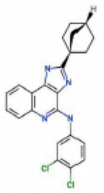
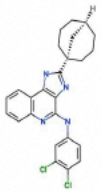
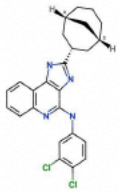
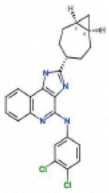
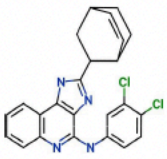
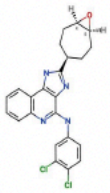
[a] Statistical significance was met when P ≤0.05 (One-way ANOVA with Bonferroni-adjusted T-test for multiple comparisons). Green highlight indicates a favorable change on agonist efficacy or potency, with respect to DMSO control for each compound, while yellow highlight indicates lower agonist affinity (higher EC_{50}).

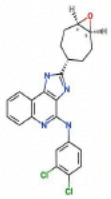
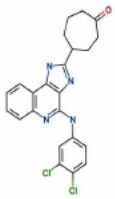
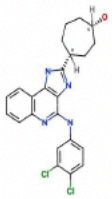
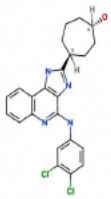
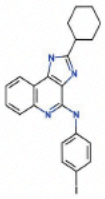
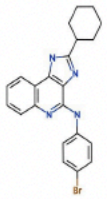
Table S5. ADMET properties calculated using the using the StarDrop software (v. 7.2), https://www.optibrium.com/stardrop-installers/.

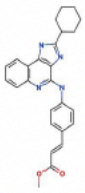
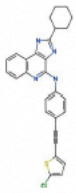
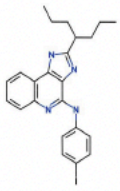
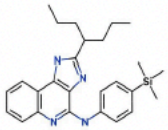
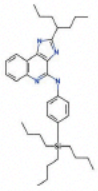
						
Compound	**5**	**6**	**7**	**8**	**12a**	**12b**
logS	-0.1472	-0.2683	-0.4178	-0.5228	-0.5111	-0.9844
logS @ pH7.4	-0.1472	-0.2683	-0.4178	-0.5228	-0.5111	-0.9844
logD	5.616	5.936	6.42	6.75	6.206	6.068
2C9 pKi	6.158	5.72	5.606	5.685	5.745	5.669
hERG pIC50	6.07	6.19	6.331	6.454	6.214	6.294
BBB log([brain]:[blood])	-0.07797	-0.06621	-0.04728	-0.03631	-0.1347	-0.187
BBB category	+	+	+	+	+	+
HIA category	+	+	+	+	+	+
P-gp category	yes	yes	yes	yes	yes	yes
2D6 affinity category	high	high	high	high	very high	very high
PPB90 category	high	high	high	high	high	high
logP	5.616	5.936	6.42	6.75	6.206	6.068
MW	383.3	397.3	411.3	425.4	423.3	463.4
HBD	2	2	2	2	2	2
HBA	4	4	4	4	4	4
TPSA	53.6	53.6	53.6	53.6	53.6	53.6
Flexibility	0.1	0.09677	0.09375	0.09091	0.08824	0.07895
Rotatable Bonds	3	3	3	3	3	3

						
Compound	**13**	**14**	**15**	**16**	**17**	**18**
logS	0.263	-0.4833	-0.4923	-0.6356	-0.01716	-0.3941
logS @ pH7.4	0.263	-0.4833	-0.4923	-0.6356	-0.01716	-0.3941
logD	5.408	6.94	6.232	6.392	5.287	6.169
2C9 pKi	6.227	5.791	5.759	5.773	6.244	6.1
hERG pIC50	5.869	6.434	6.917	6.613	5.929	6.51
BBB log([brain]:[blood])	-0.09547	0.0547	0.05113	-0.09645	-0.09293	0.07324
BBB category	+	+	+	+	+	+
HIA category	+	+	+	+	+	+
P-gp category	yes	yes	yes	yes	yes	yes
2D6 affinity category	high	high	medium	high	high	high
PPB90 category	high	high	high	high	high	high
logP	5.408	6.94	6.232	6.392	5.287	6.169
MW	371.3	427.4	535.3	479.3	369.2	423.3
HBD	2	2	2	2	2	2
HBA	4	4	4	4	4	4
TPSA	53.6	53.6	53.6	53.6	53.6	53.6
Flexibility	0.1429	0.2188	0.2368	0.1111	0.1034	0.09091
Rotatable Bonds	4	7	9	4	3	3

						
Compound	**19**	**20**	**21**	**22**	**23**	**24**
logS	-0.5902	-0.6293	-0.6825	-0.7282	-0.7668	-0.448
logS @ pH7.4	-0.5902	-0.6293	-0.6825	-0.7282	-0.7668	-0.448
logD	6.951	7.075	7.243	7.393	7.528	5.069
2C9 pKi	5.618	5.534	5.627	5.653	5.67	6.121
hERG pIC50	6.564	6.666	6.768	6.867	6.961	5.888
BBB log([brain]:[blood])	-0.03183	-0.03255	-0.03231	-0.03382	-0.03692	-0.1525
BBB category	+	+	+	+	+	+
HIA category	+	+	+	+	+	+
P-gp category	yes	yes	yes	yes	yes	yes
2D6 affinity category	high	high	high	high	high	high
PPB90 category	high	high	high	high	high	high
logP	6.951	7.075	7.243	7.393	7.528	5.069
MW	439.4	453.4	467.4	481.5	495.5	395.3
HBD	2	2	2	2	2	2
HBA	4	4	4	4	4	4
TPSA	53.6	53.6	53.6	53.6	53.6	53.6
Flexibility	0.08824	0.08571	0.08333	0.08108	0.07895	0.09375
Rotatable Bonds	3	3	3	3	3	3

						
Compound	25	26	27	28	29	30
logS	-0.6988	-0.882	-0.7376	-0.6143	-0.5777	-0.522
logS @ pH7.4	-0.6988	-0.882	-0.7376	-0.6143	-0.5777	-0.522
logD	5.593	6.074	6.789	6.535	6.303	5.861
2C9 pKi	5.727	5.636	5.682	5.703	6.209	5.698
hERG pIC50	6.225	6.458	6.423	6.375	6.468	6.121
BBB log([brain]:[blood])	-0.1496	-0.1426	-0.09721	-0.09022	0.01895	-0.2344
BBB category	+	+	+	+	+	-
HIA category	+	+	+	+	+	+
P-gp category	yes	yes	yes	yes	yes	yes
2D6 affinity category	high	high	very high	very high	very high	very high
PPB90 category	high	high	high	high	high	high
logP	5.593	6.074	6.789	6.535	6.303	5.861
MW	423.3	451.4	451.4	437.4	435.3	439.3
HBD	2	2	2	2	2	2
HBA	4	4	4	4	4	5
TPSA	53.6	53.6	53.6	53.6	53.6	66.13
Flexibility	0.08824	0.08333	0.08333	0.08571	0.08571	0.08571
Rotatable Bonds	3	3	3	3	3	3

						
Compound	**31**	**32**	**33**	**34**	**35**	**36**
logS	-0.522	-0.7006	-0.2016	-0.2016	-0.0425	0.2806
logS @ pH7.4	-0.522	-0.7006	-0.2016	-0.2016	-0.0425	0.2806
logD	5.861	5.219	5.533	5.533	5.794	5.922
2C9 pKi	5.698	5.986	5.584	5.584	5.458	5.458
hERG pIC50	6.121	6.129	6.196	6.196	6.531	6.534
BBB log([brain]:[blood])	-0.2344	-0.3541	-0.4418	-0.4418	-0.03721	-0.02661
BBB category	-	-	-	-	+	+
HIA category	+	+	+	+	+	+
P-gp category	yes	yes	yes	yes	yes	yes
2D6 affinity category	very high	high	high	high	high	high
PPB90 category	high	high	high	high	high	high
logP	5.861	5.219	5.533	5.533	5.794	5.922
MW	439.3	439.3	441.4	441.4	468.3	421.3
HBD	2	2	3	3	2	2
HBA	5	5	5	5	4	4
TPSA	66.13	70.67	73.83	73.83	53.6	53.6
Flexibility	0.08571	0.08824	0.08824	0.08824	0.09677	0.09677
Rotatable Bonds	3	3	3	3	3	3

					
Compound	**37**	**38**	**39**	**48**	**49**
logS	0.3498	-1.215	-0.1055	0.2823	-0.8281
logS @ pH7.4	0.3498	-1.215	-0.1055	0.2823	-0.8281
logD	5.123	6.705	6.262	6.527	9.4
2C9 pKi	5.397	5.81	5.606	5.607	5.485
hERG pIC50	6.35	6.761	6.634	6.61	7.772
BBB log([brain]:[blood])	-0.4501	-0.5372	0.04793	0.056	0.09369
BBB category	-	+	+	+	+
HIA category	+	+	+	+	+
P-gp category	yes	yes	yes	yes	yes
2D6 affinity category	high	high	high	high	high
PPB90 category	high	high	high	high	high
logP	5.123	6.705	6.262	6.527	9.4
MW	426.5	483	484.4	430.7	556.9
HBD	2	2	2	2	2
HBA	6	4	4	4	4
TPSA	79.9	53.6	53.6	53.6	53.6
Flexibility	0.1667	0.1282	0.2258	0.2353	0.3953
Rotatable Bonds	6	5	7	8	17

PDSP Screening

Off-target analysis of select PAM derivatives with forty-five other receptors, transporters, and channels was determined using radioligand binding assays by Psychoactive Drug Screening Program (PDSP). We thank Dr. Bryan L. Roth (Univ. North Carolina at Chapel Hill) and National Institute of Mental Health's Psychoactive Drug Screening Program (Contract # HHSN-271-2008-00025-C) for screening data.[4] Procedures: https://pdsp.unc.edu/pdspweb/content/UNC-CH%20Protocol%20Book.pdf. Unless noted in the text, no significant interactions (<50% inhibition at 10 μM) for any of the nucleosides were found at the following sites (human unless noted): $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$, $5HT_3$, $5HT_{5A}$, $5HT_6$, $5HT_7$, α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3, BZP rat brain site, D_1, D_2, D_3, D_4, D_5, $GABA_A$, H_1, H_2, H_3, H_4, M_1, M_2, M_5, δ-opioid receptor (DOR), κ-opioid receptor (KOR), μ-opioid receptor (MOR), σ_1, σ_2, DAT, NET, SERT. K_i values in μM (Table 2), or % inhibition at 10 μM (not shown), were determined.

Table S6. In vivo experimental PK determination using Wistar rats.

Dose (mg/kg, route)	**0.5 (i.v.)**	**1 (p.o.)**	**3 (p.o.)**	**10 (p.o.)**
Species of Strain	Rat and RccHan:WIST			
N mice	3	3	3	3
Feeding Condition	Fed	Fasting overnight, feed 4 h post-dosing		
Dose Volume (mg/kg b. wt.)	5			
Concentration (mg/mL)	0.1	0.2	0.6	2
Vehicles	DMSO: 20% HPBCD (10:90)	DMSO: Kolliphor EL: PBS (15:15:70)		
Blood Collection Site	Jugular vein through a catheter			
Anticoagulant	Heparin (20 IU/mL)			
Time Points (h)	0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24	0.25, 0.5, 1, 2, 4, 8, 12, 24		

Table S7. Caco-2 permeability results of compound**s 18** and **39**.

Compound Name	**Average Values**					
	Papp (10^6 cm/sec)		**Efflux Ratio**	**A to B % Recovery**	**B to A % Recovery**	**Classification**
	A to B	**B to A**				
18	0.00	0.00	NC	48.1	78.0	Low
39	0.00	0.01	NC	61.7	76.7	Low
Digoxin	0.13	9.96	74.2	82.0	84.2	Low
Propranolol	28.4	17.6	0.62	74.4	101	High
Atenolol	0.00	0.00	NC	87.8	86.1	Low

Table S8. In vivo pharmacokinetic parameters of compounds **18** and **39**.

A) compound **18**

Dose (Route)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}last}$ (h*ng/mL)	$AUC_{0\text{-}\infty}$ (h*ng/mL)	$T_{1/2}$ (h)	MRT_{last} (h)	V_d (mL/kg)	k_{el} (1/h)	F (%)	Cl (mL/h/kg)
0.5 mg/kg (i.v.)	696	0.083	1080	1120	2.38	2.64	1530	0.292	100	447
1 mg/kg (p.o.)	185	2.00	ND	ND	ND	ND	ND	ND	ND	ND
3 mg/kg (p.o.)	487	2.00	1860	1900	1.29	3.01	2930	0.539	28.7	1580
10 mg/kg (p.o.)	1780	2.00	10,200	10,300	2.60	3.98	3660	0.266	47.5	975

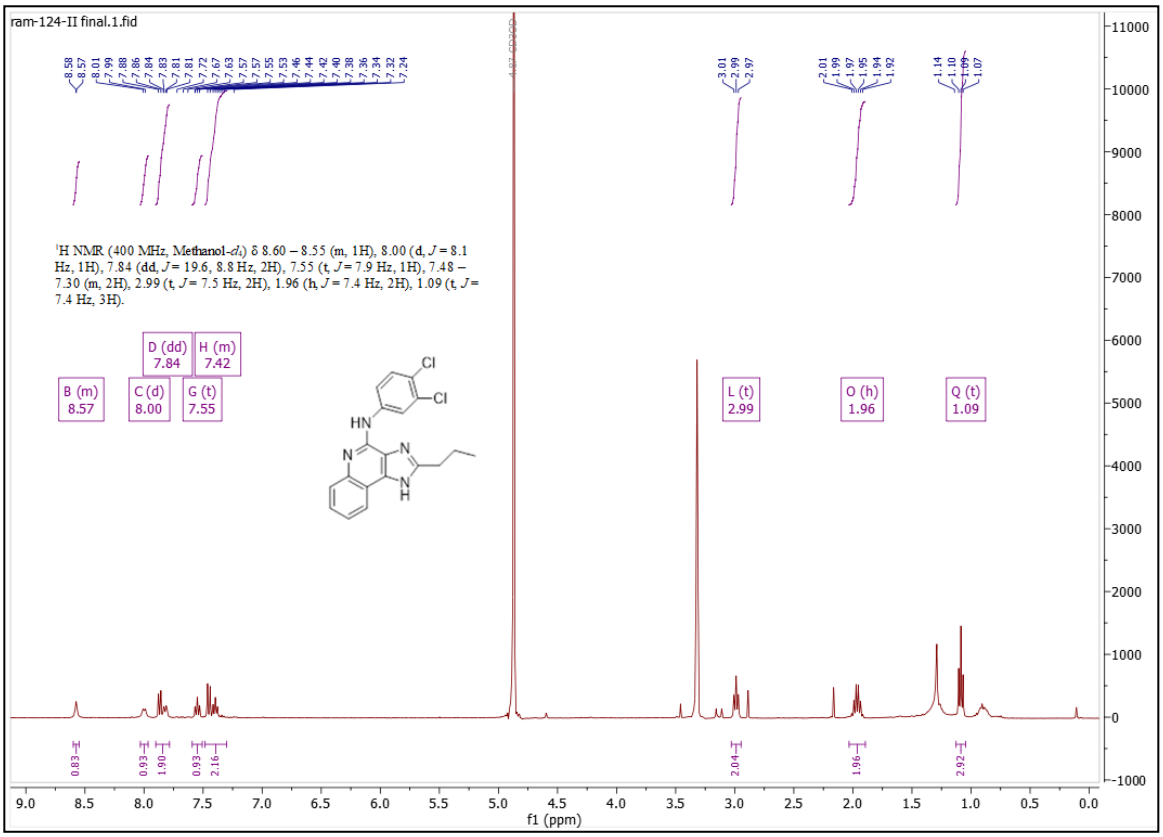
ND, not determined.

B) compound **39**

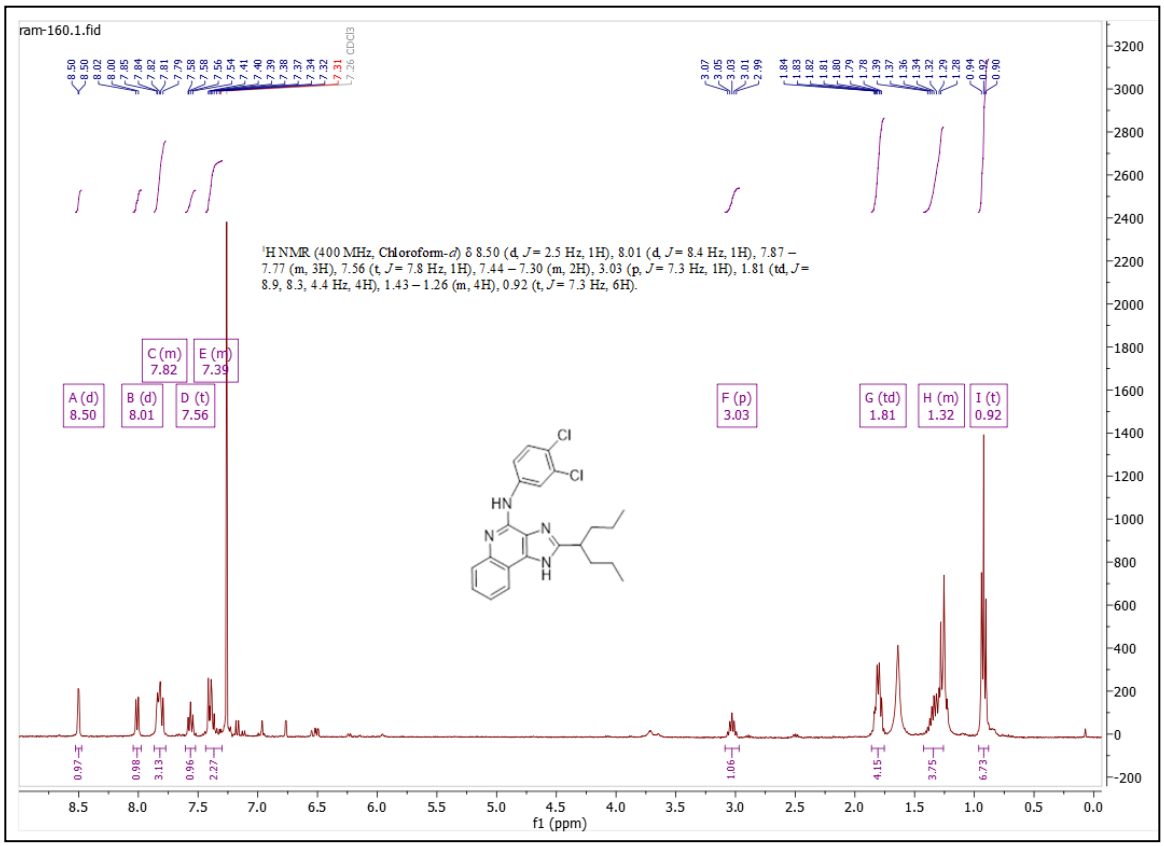
Dose (Route)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}last}$ (h*ng/mL)	$AUC_{0\text{-}\infty}$ (h*ng/mL)	$T_{1/2}$ (h)	MRT_{last} (h)	V_d (mL/kg)	k_{el} (1/h)	F (%)	Cl (mL/h/kg)
0.5 mg/kg (i.v.)	530	0.083	841	881	6.91	4.09	5660	0.100	100	568
1 mg/kg (p.o.)	142	1.000	767	778	4.71	4.41	8730	0.147	44.2	1280
3 mg/kg (p.o.)	592	2.000	3350	3380	3.44	5.62	4400	0.207	64.0	886
10 mg/kg (p.o.)	1040	4.000	10,600	10,800	3.84	7.35	5110	0.180	61.5	923

NMR Spectra

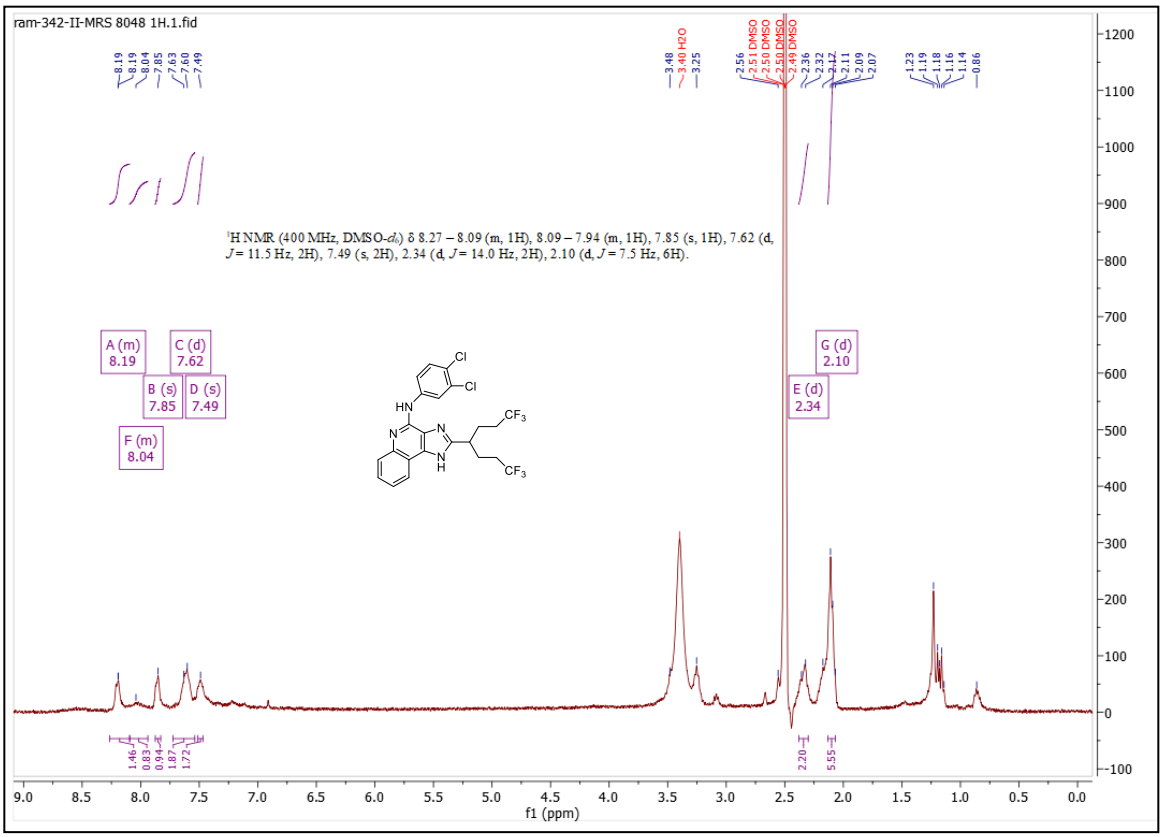
The NMR Spectra below were acquired on a Bruker ADIII-400 at 298 ºK in $CDCl_3$ plus 4 drops of MeOD.



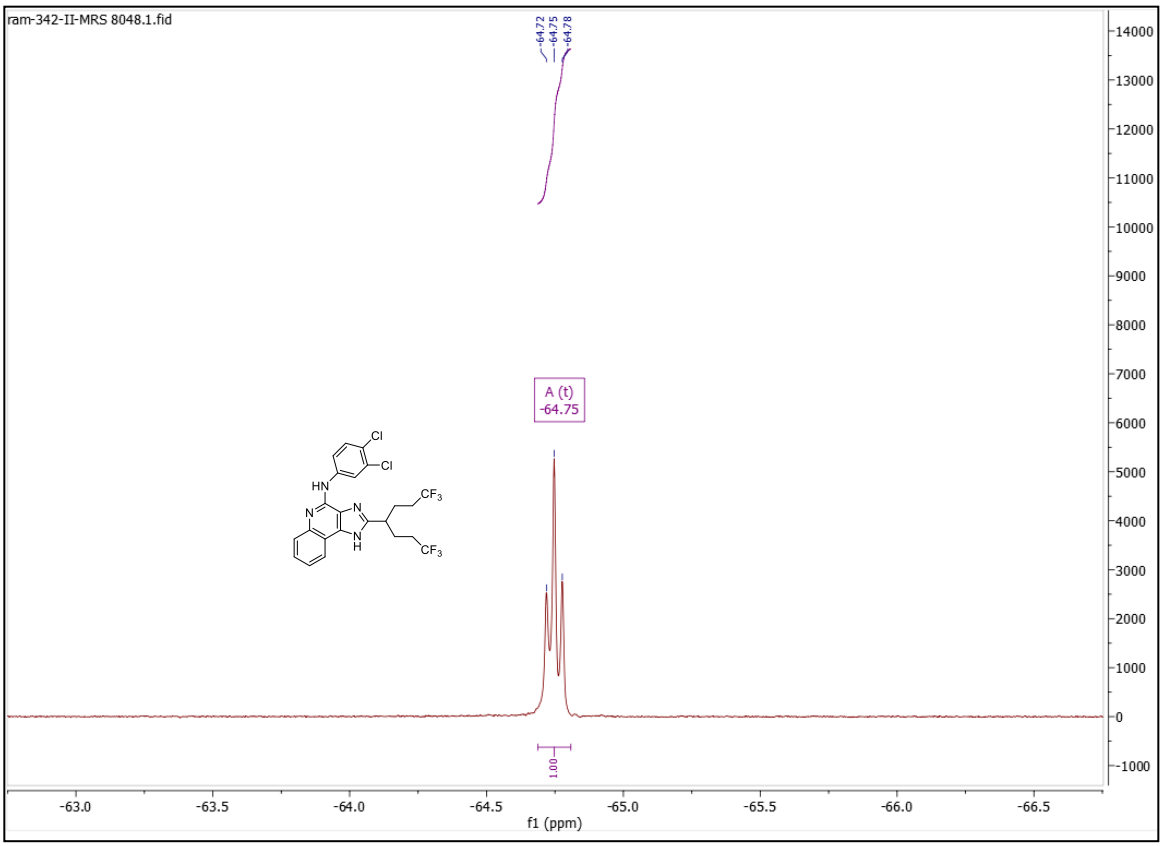
¹H NMR of 2-ethyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **13.**



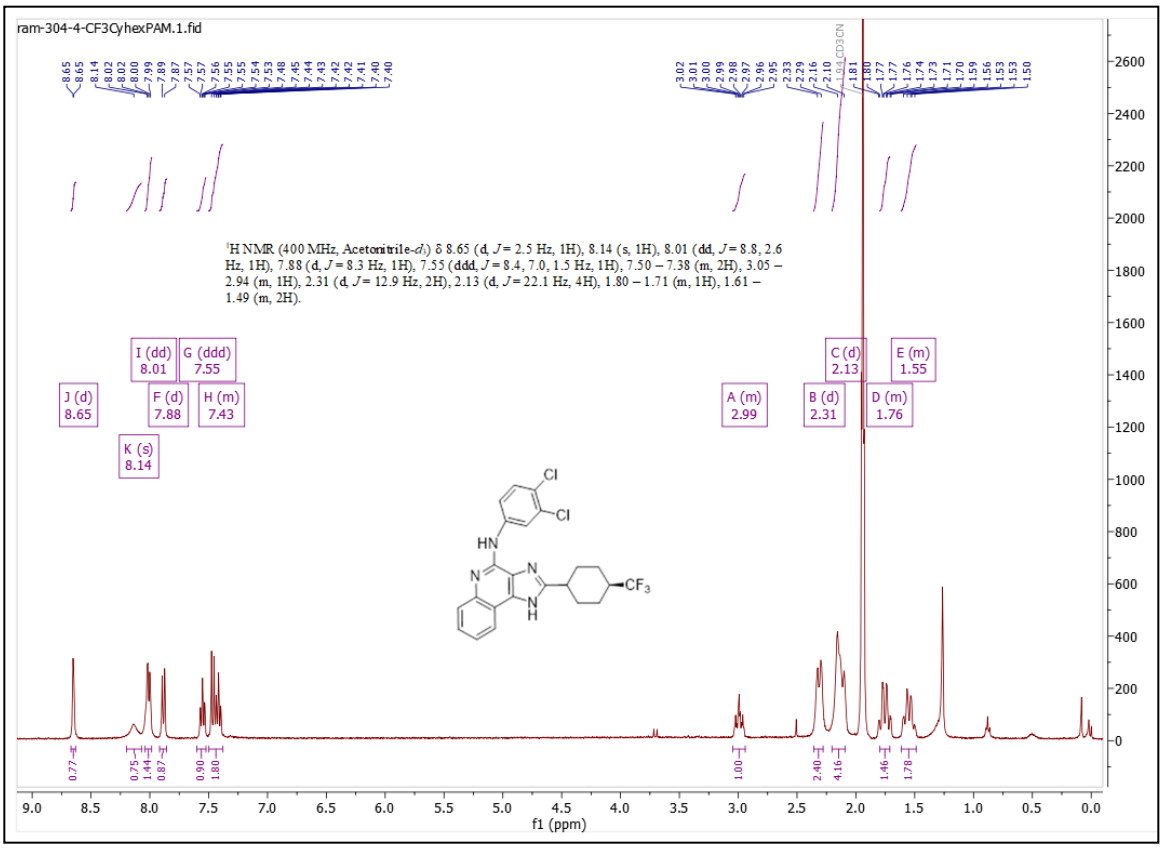
¹H NMR of 2-(heptan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **14.**



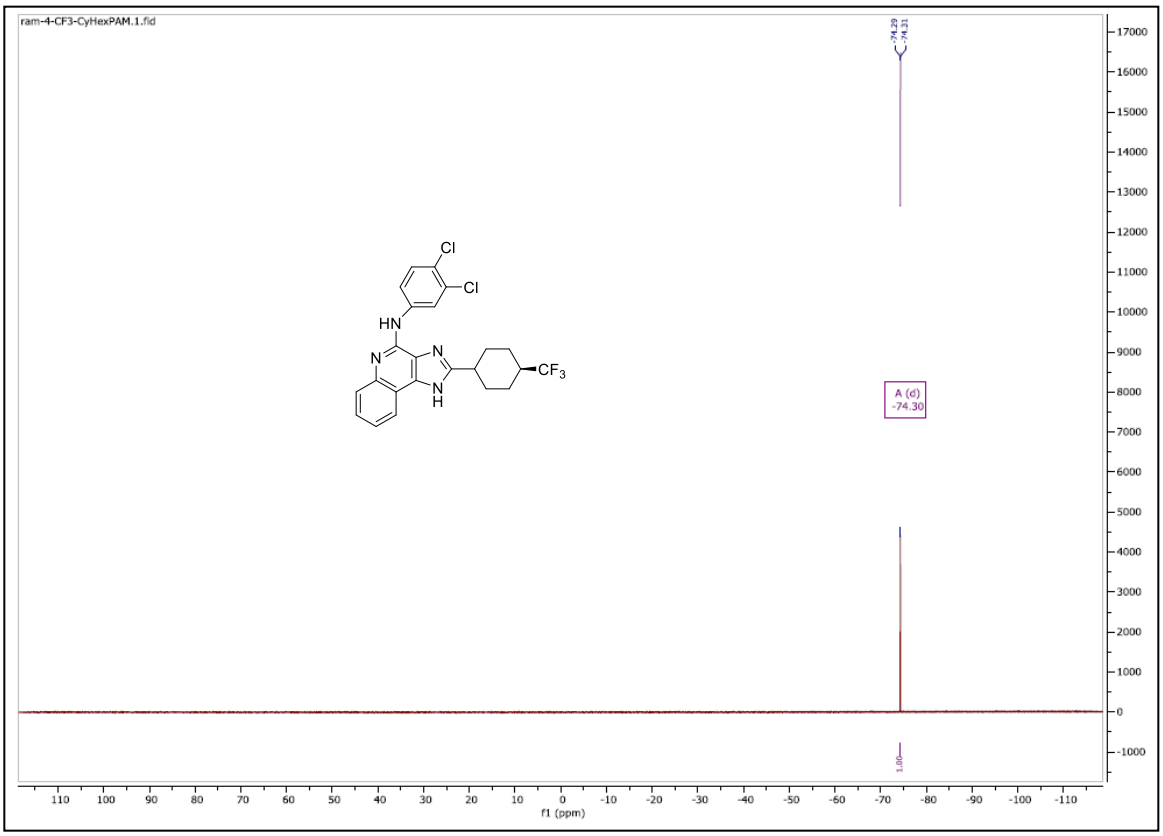
^{1}H NMR of 2-(1,1,1,7,7,7-hexafluoroheptan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **15.**



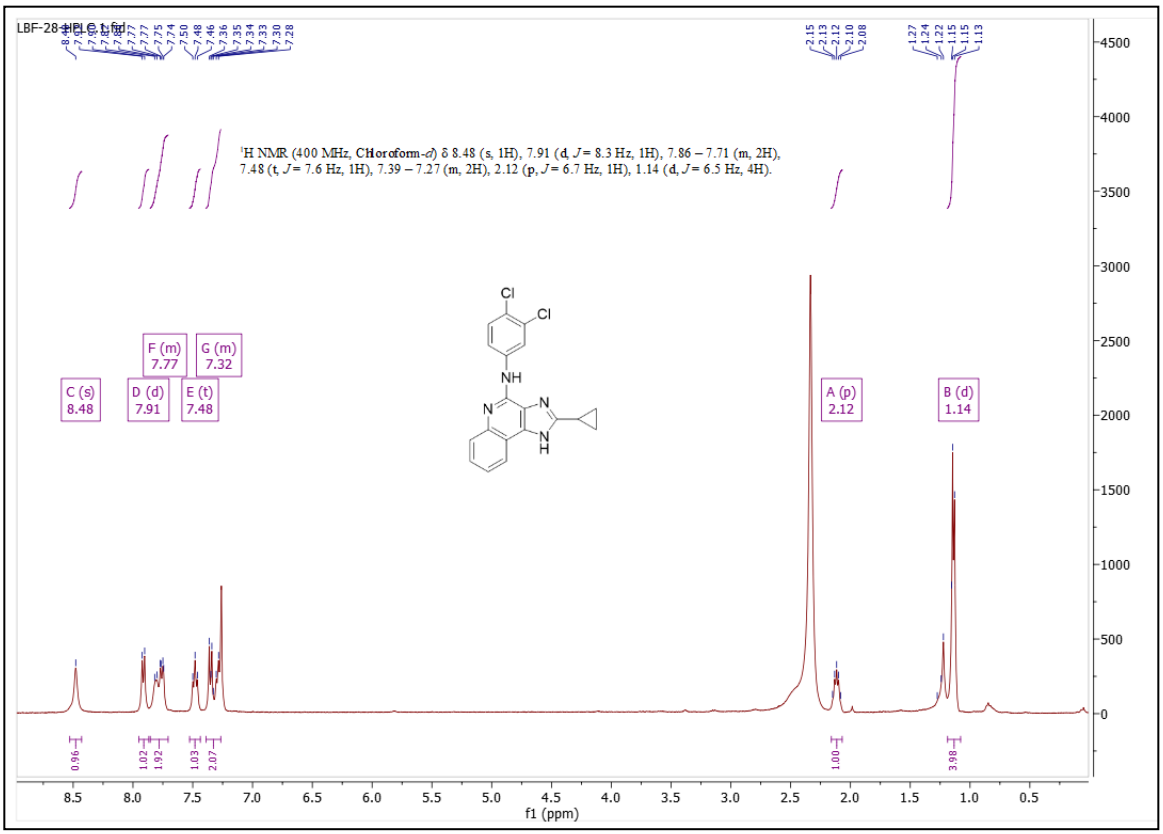
^{19}F NMR of 2-(1,1,1,7,7,7-hexafluoroheptan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **15.**



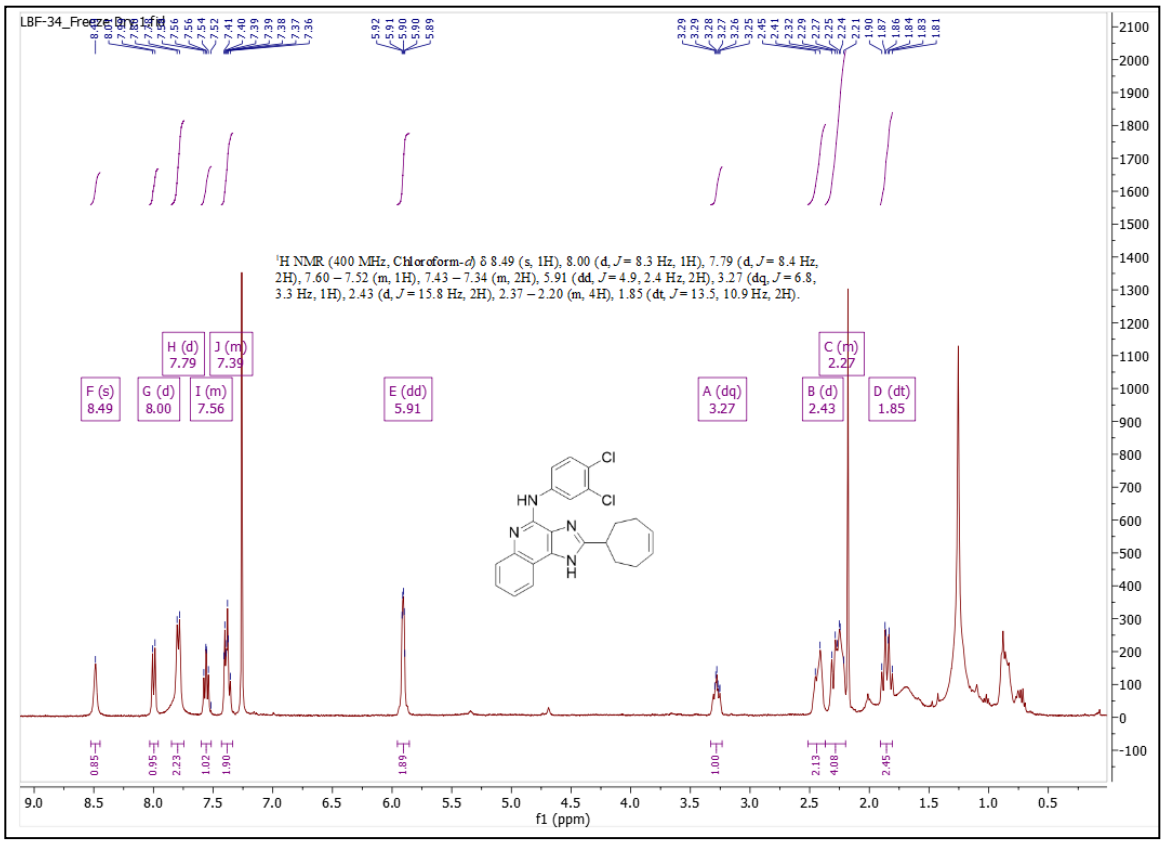
^{1}H NMR of 2-(4-(trifluoromethyl)cyclohexyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **16.**



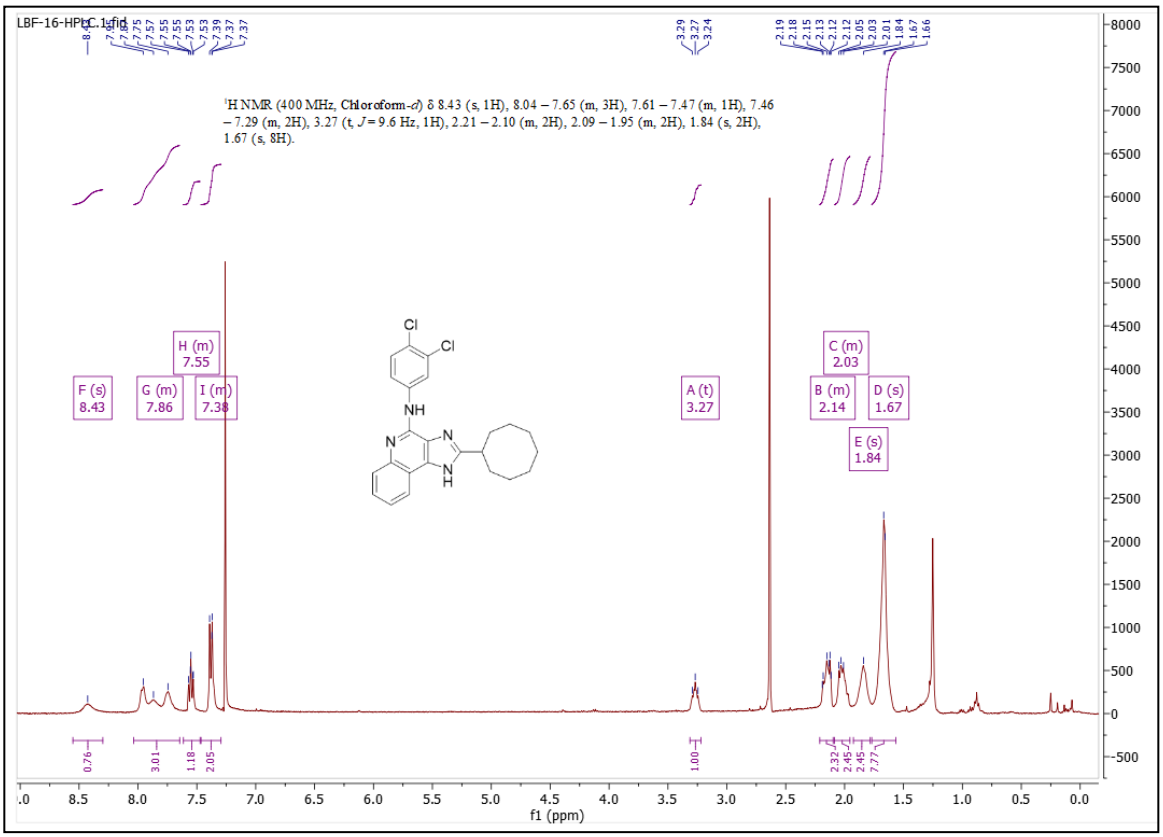
^{19}F NMR of 2-(4-(trifluoromethyl)cyclohexyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **16.**



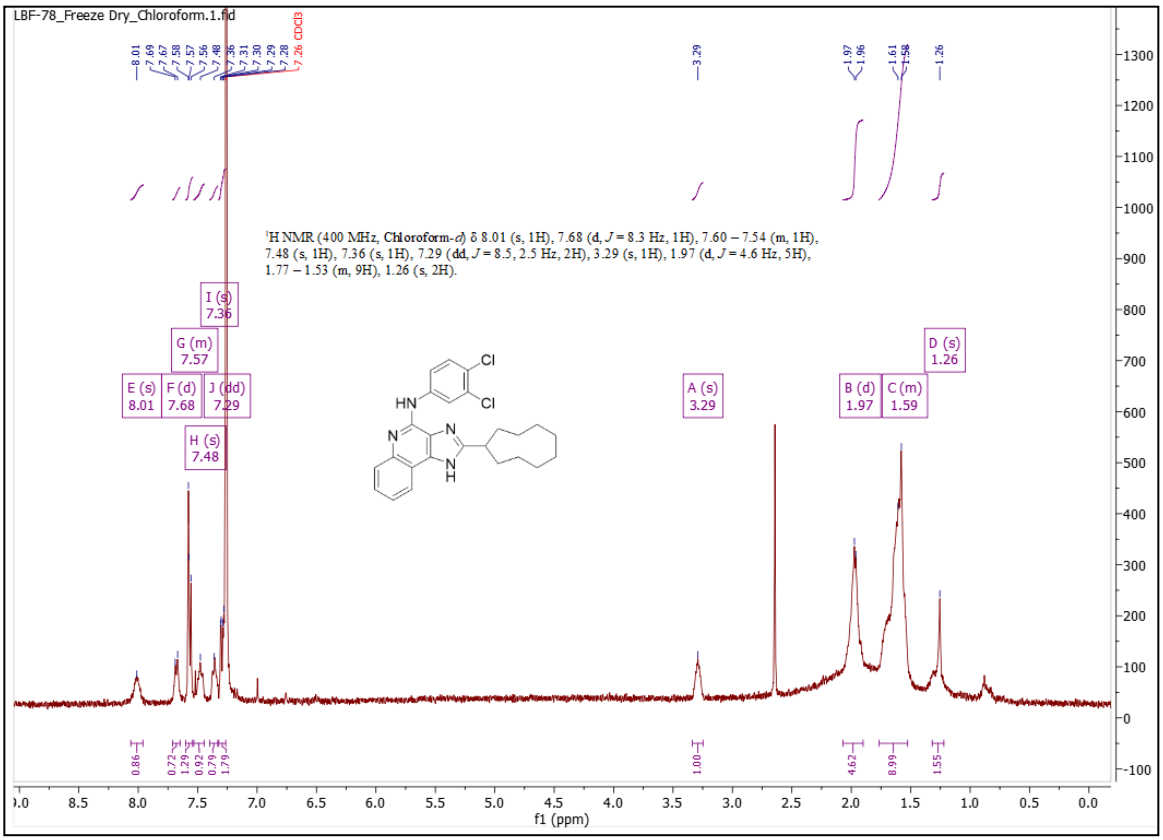
^{1}H NMR of 2-(cyclopropyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **17.**



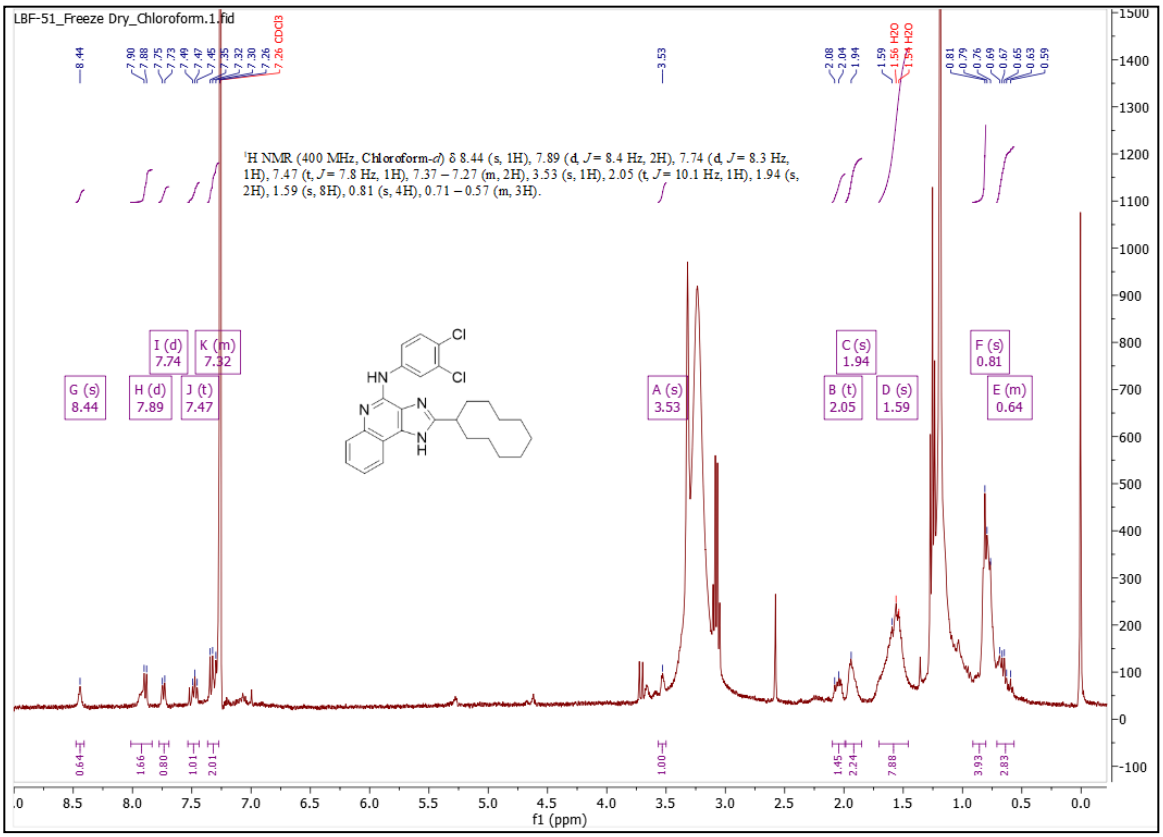
^{1}H NMR of 2-(cyclohept-4-en-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **18.**



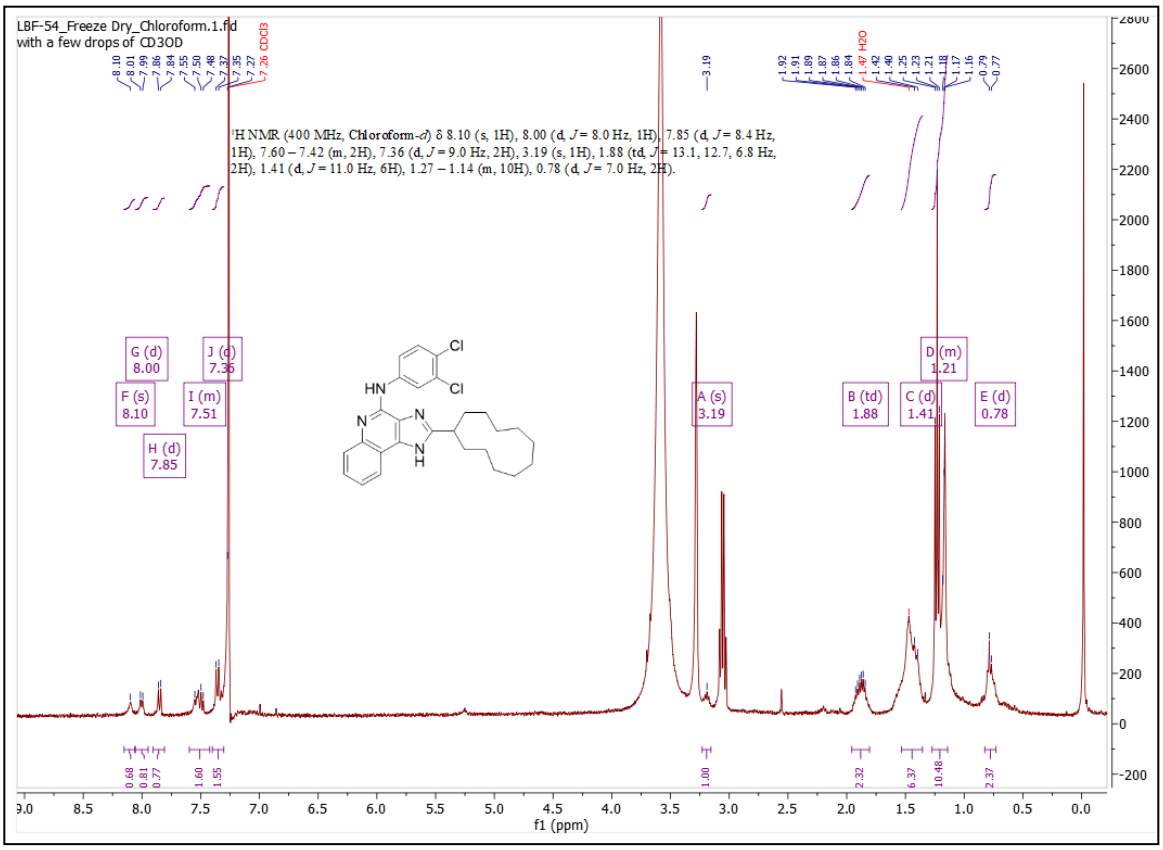
¹H NMR of 2-(cyclooctyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **19.**



^{1}H NMR of 2-(cyclononyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **20.**

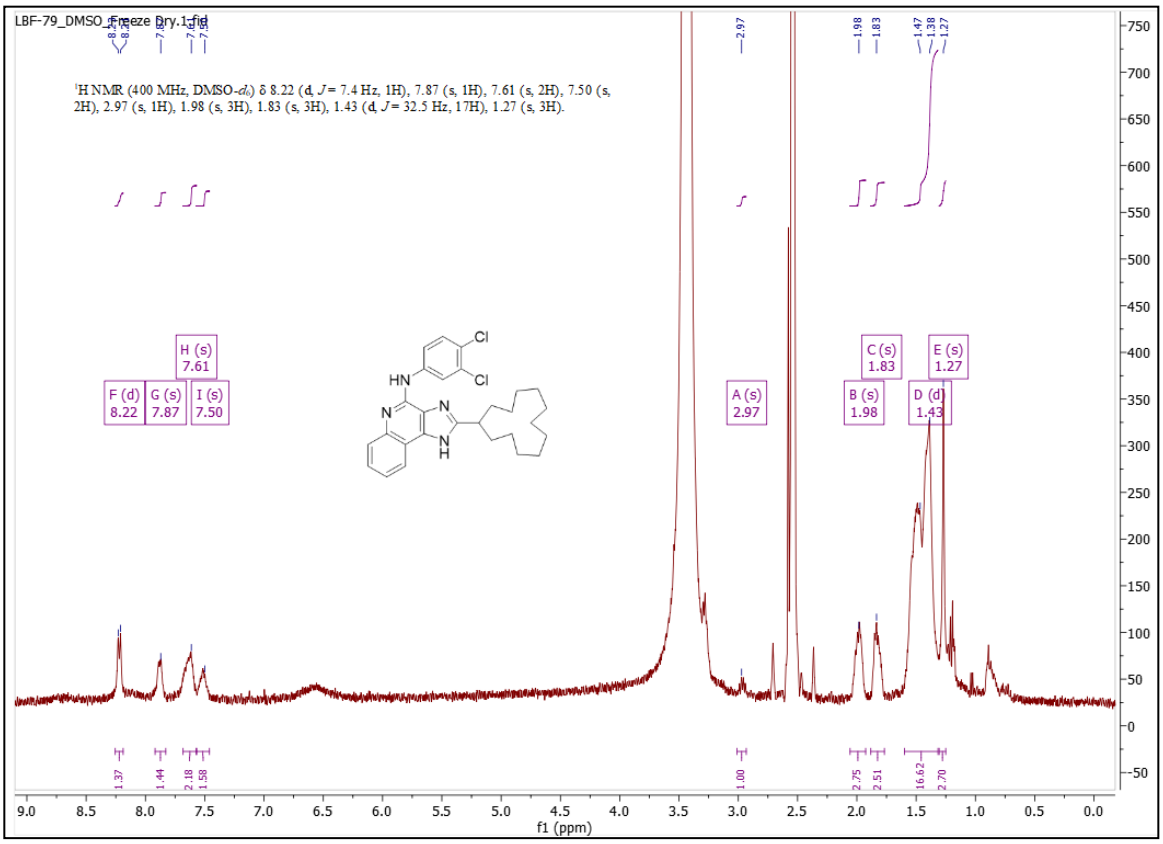


¹H NMR of 2-(cyclodecyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **21.**

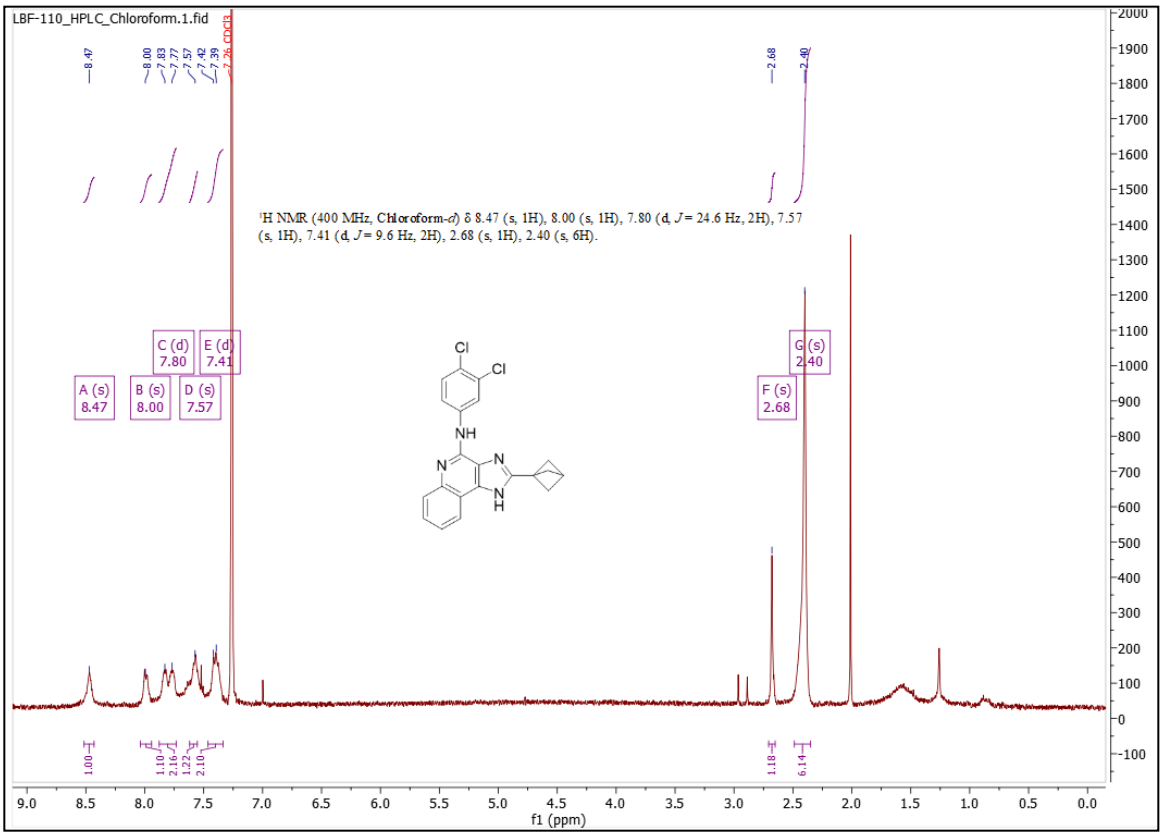


^{1}H NMR of 2-(cycloundecyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **22.**

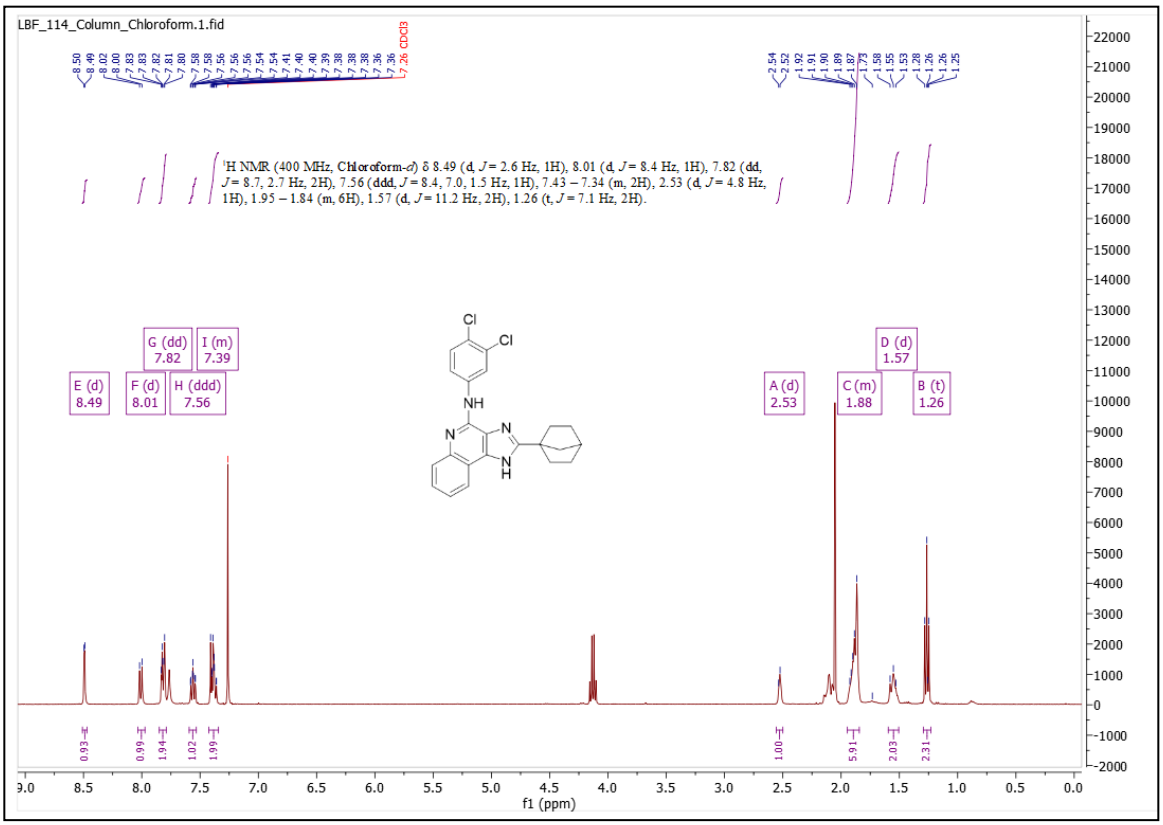
.



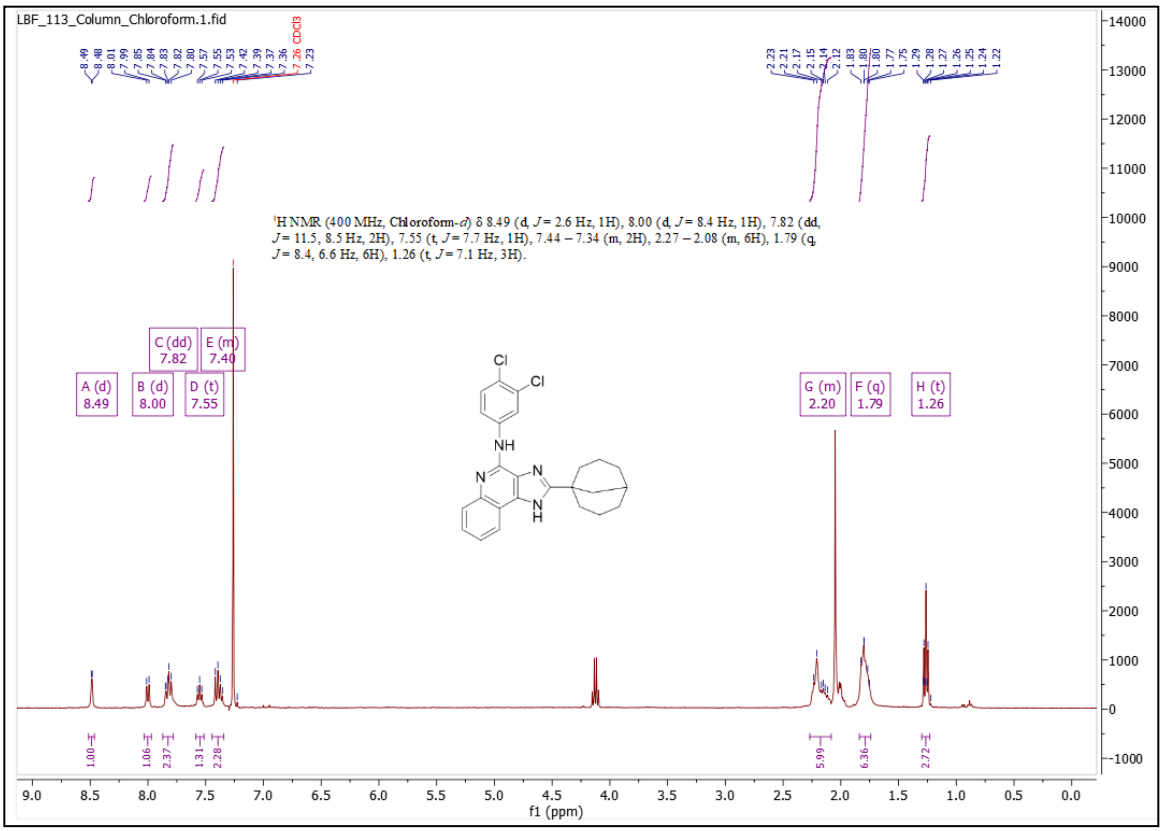
^{1}H NMR of 2-(cyclododecyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **23.**



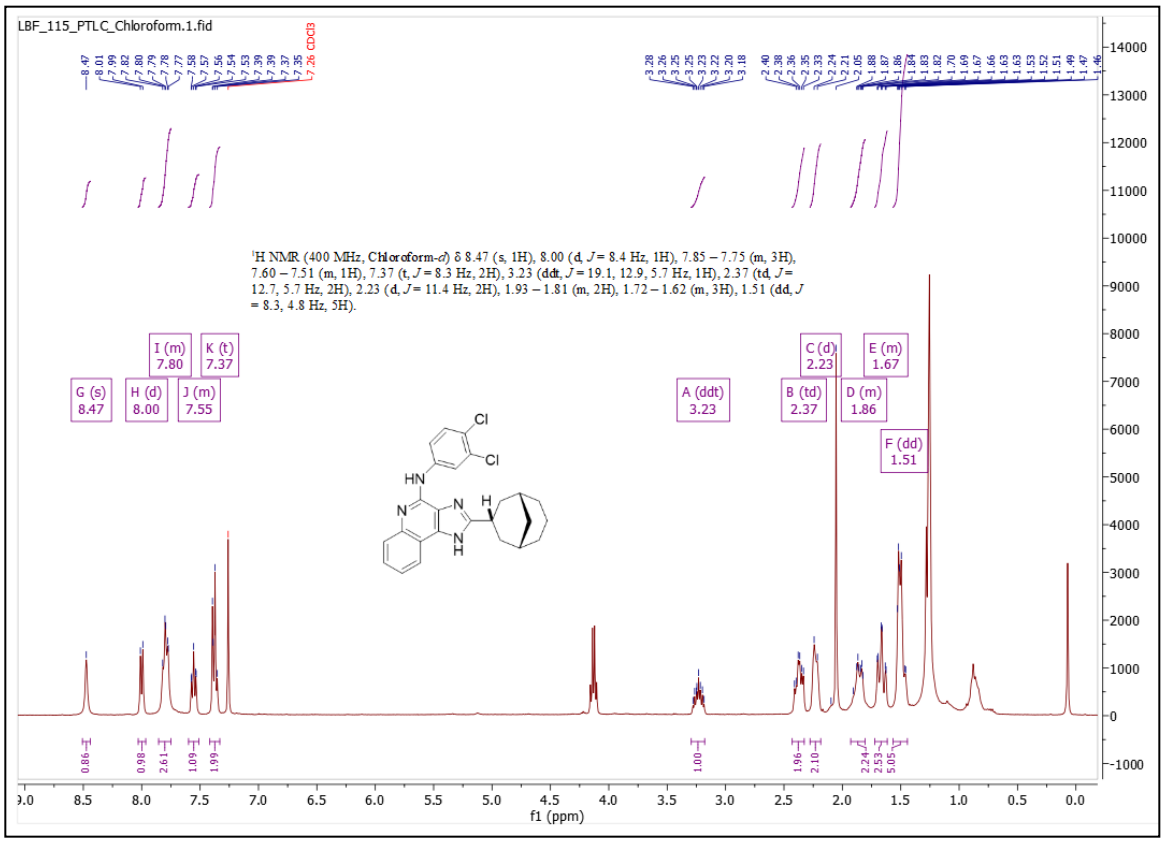
¹H NMR of 2-(bicyclo[1.1.1]heptan-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **24.**



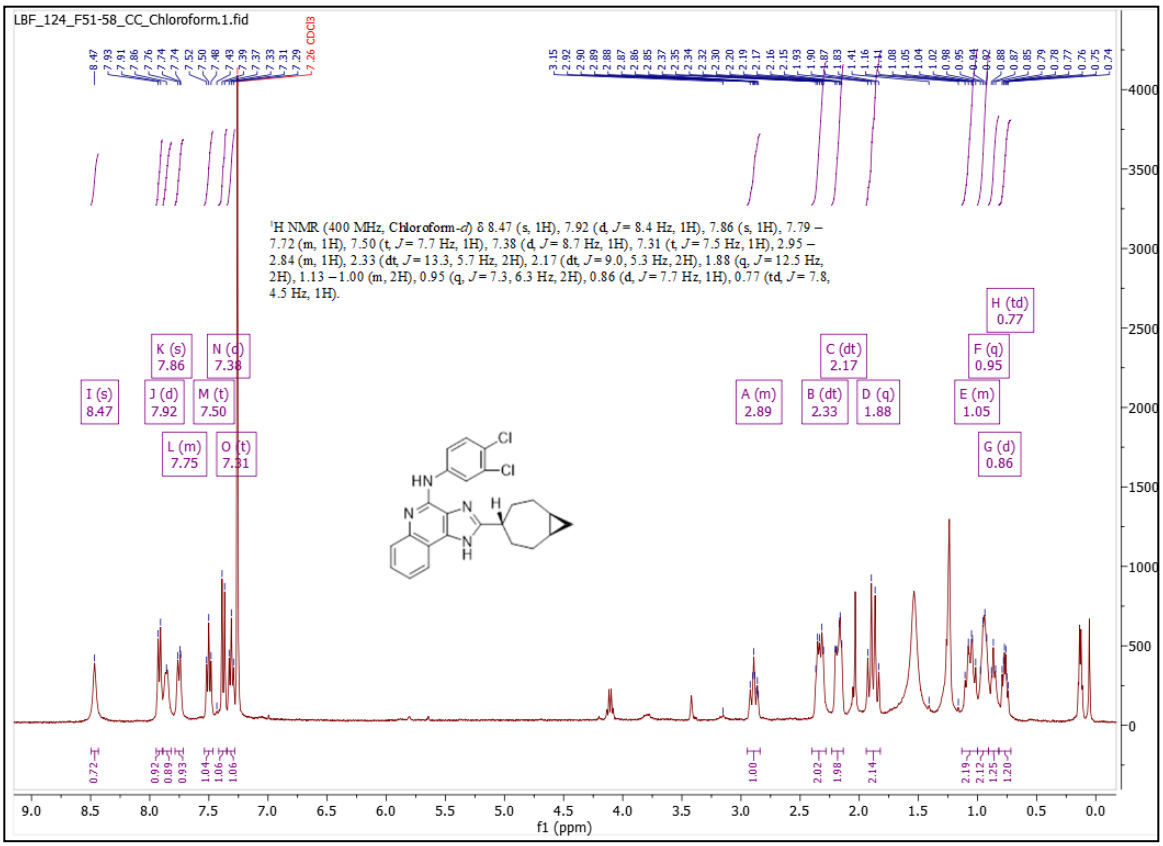
[1]H NMR of 2-(bicyclo[2.2.1]heptan-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **25.**



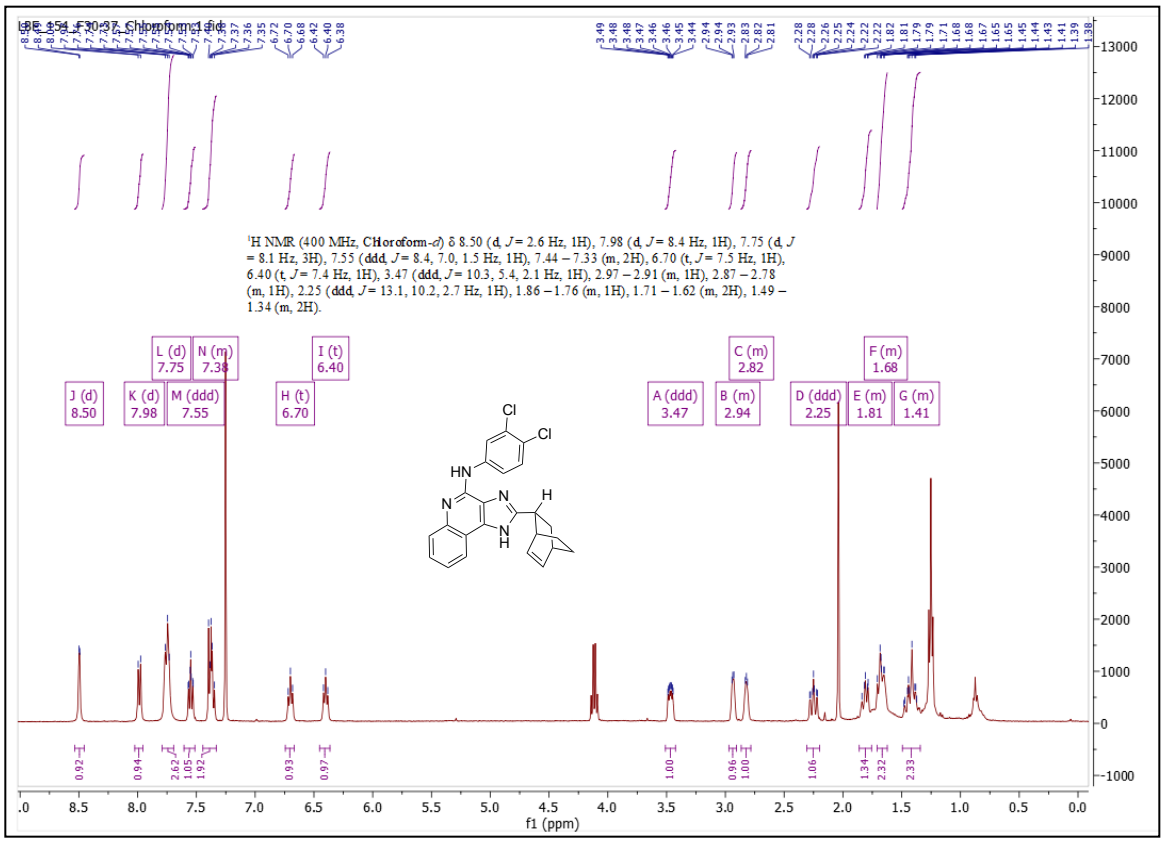
[1]H NMR of 2-(bicyclo[3.3.1]nonan-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **26.**



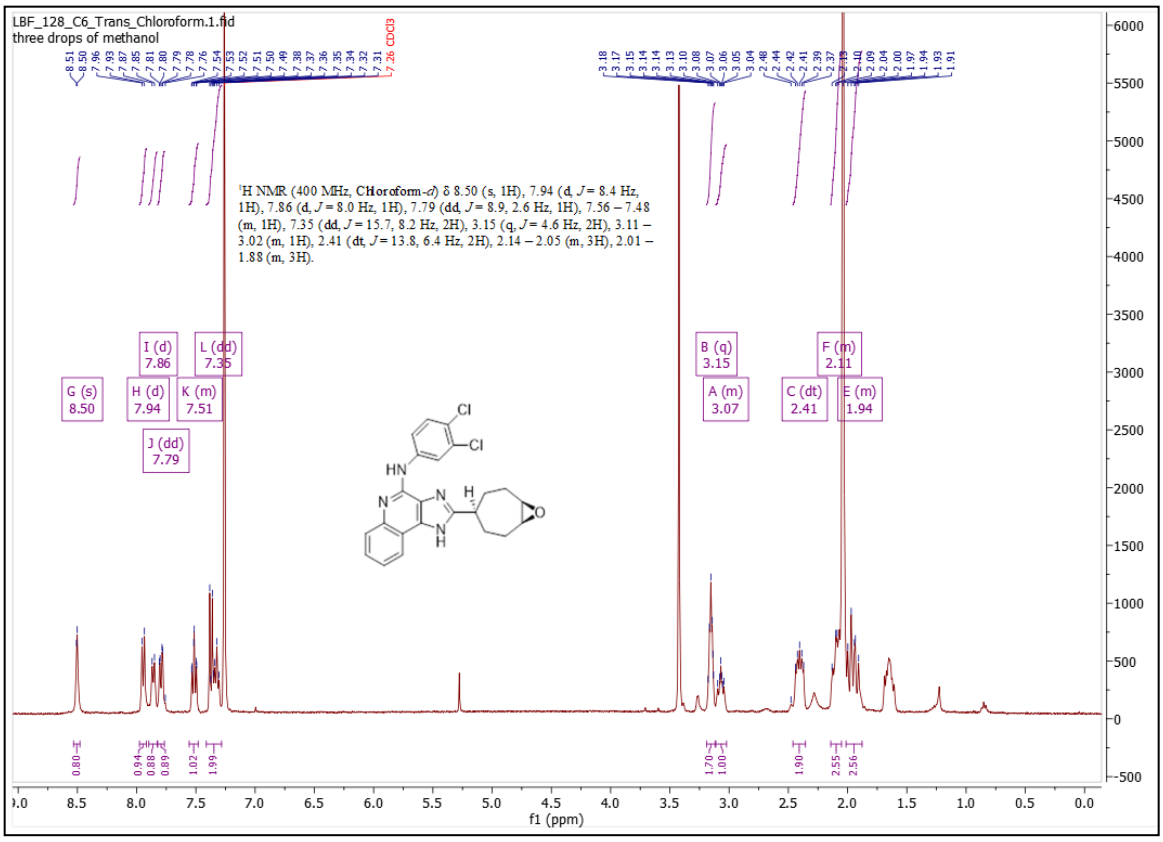
^{1}H NMR of 2-((1R,3s,5S)-bicyclo[3.3.1]nonan-3-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **27.**



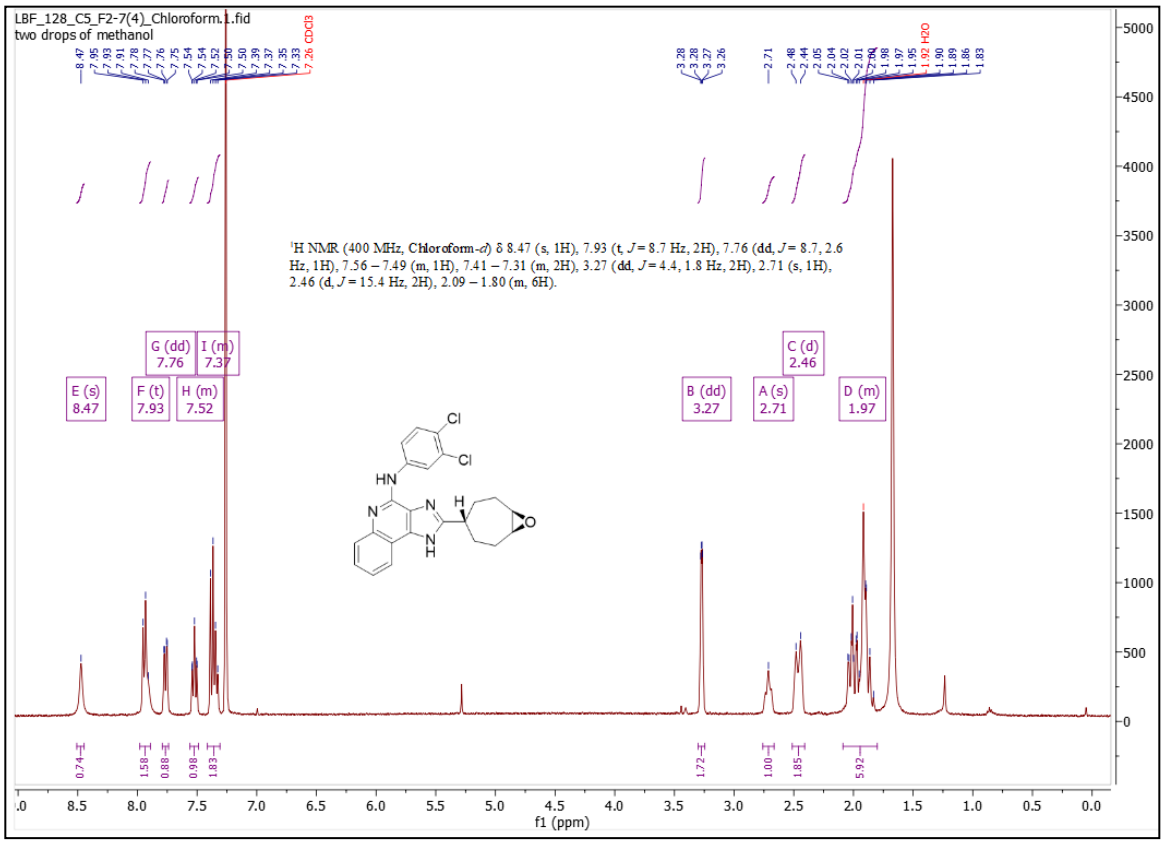
^{1}H NMR of 2-((1R,4r,7S)-bicyclo[5.1.0]octan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **28.**



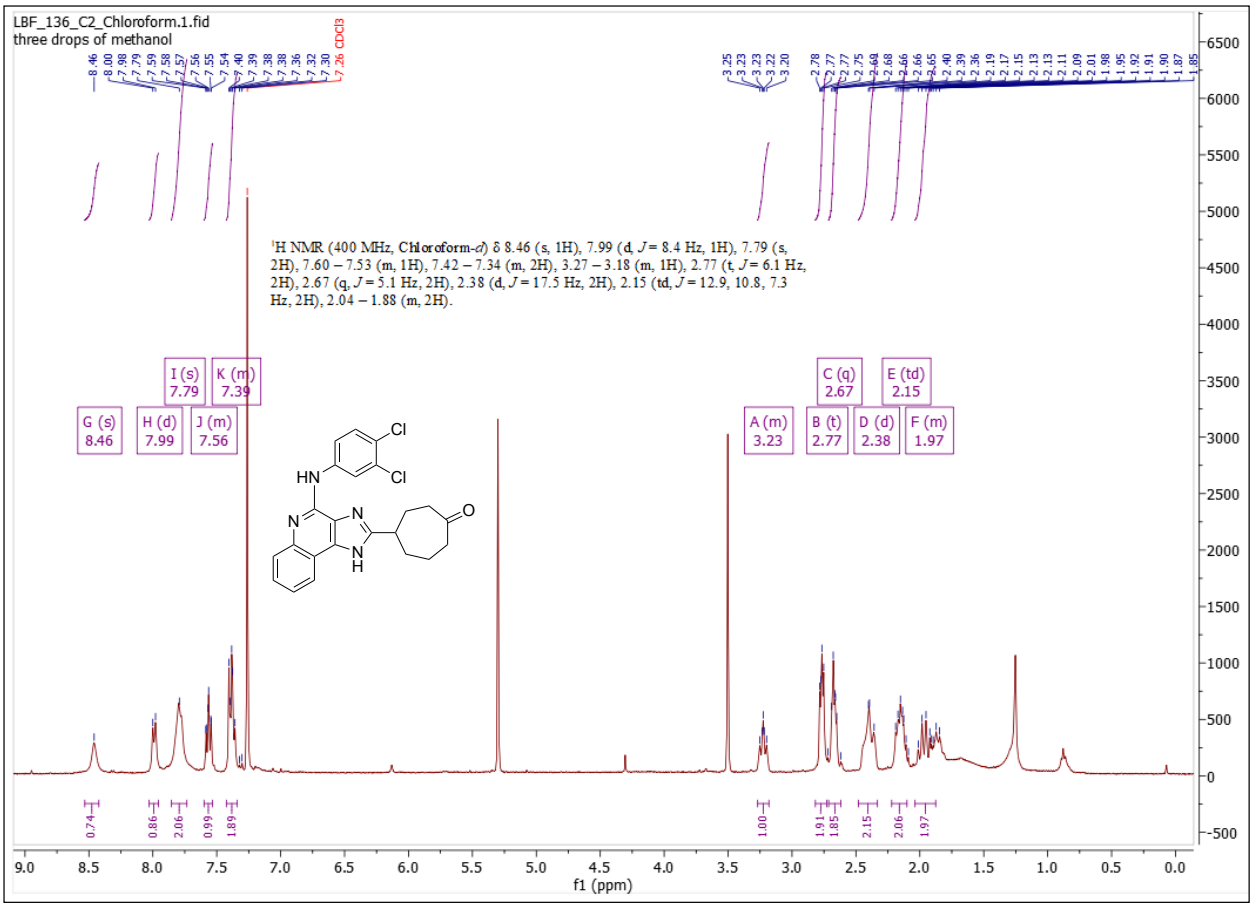
^{1}H NMR of 2-((1R,2R,4R) & (1S,2S,4S)-bicyclo[2.2.2]oct-5-en-2-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **29.**



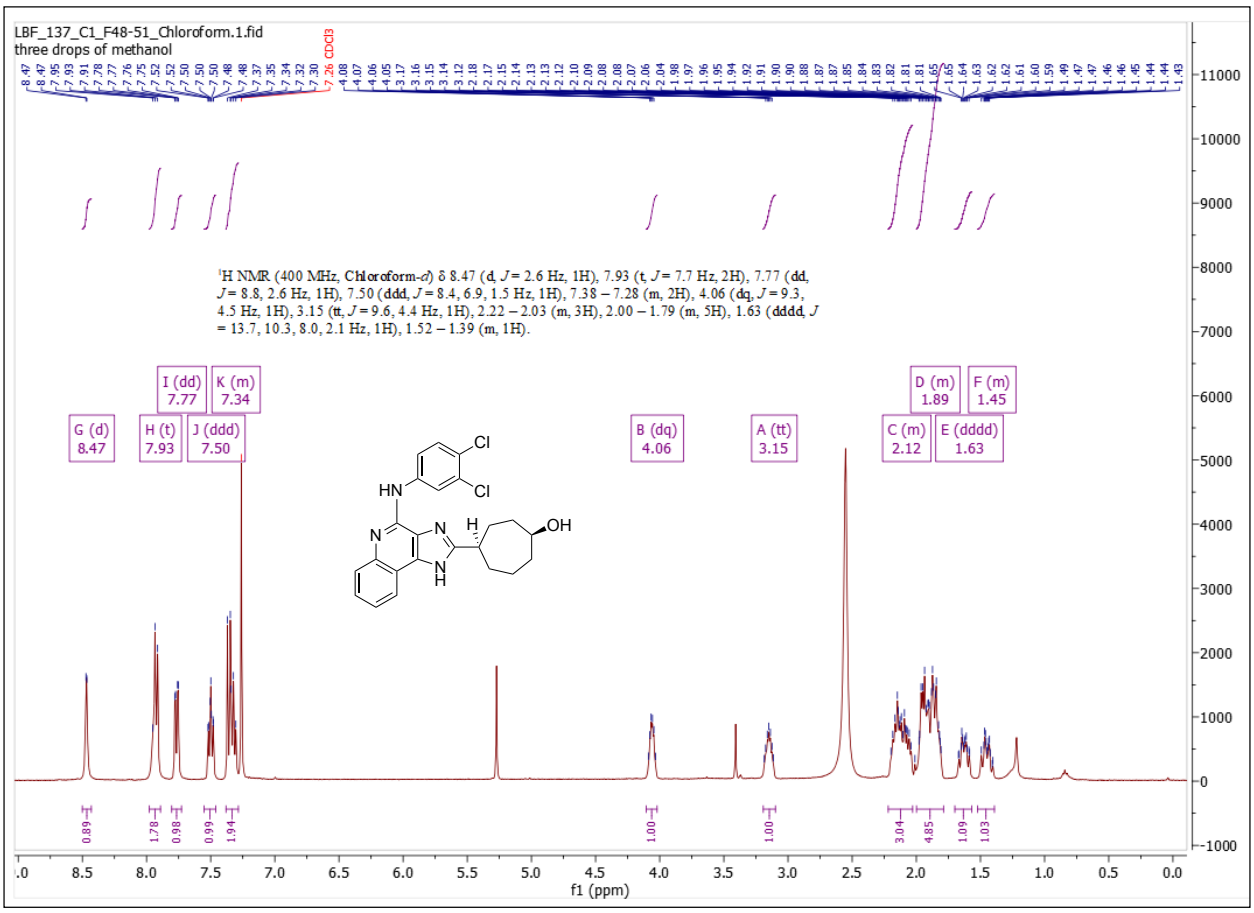
^{1}H NMR of 2-((1R,4r,7S)-8-oxabicyclo[5.1.0]octan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **30.**



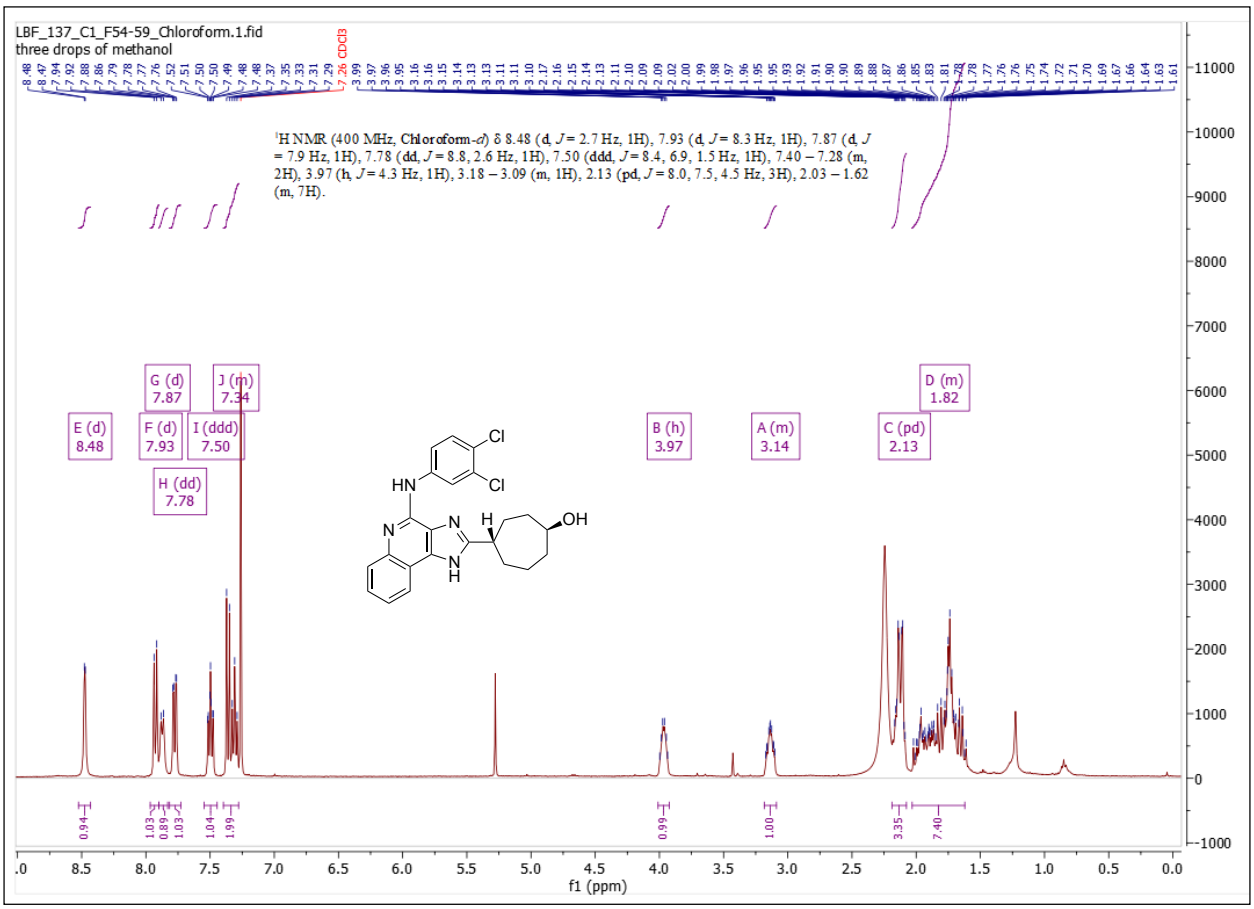
^{1}H NMR of 2-((1R,4s,7S)-8-oxabicyclo[5.1.0]octan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **31.**



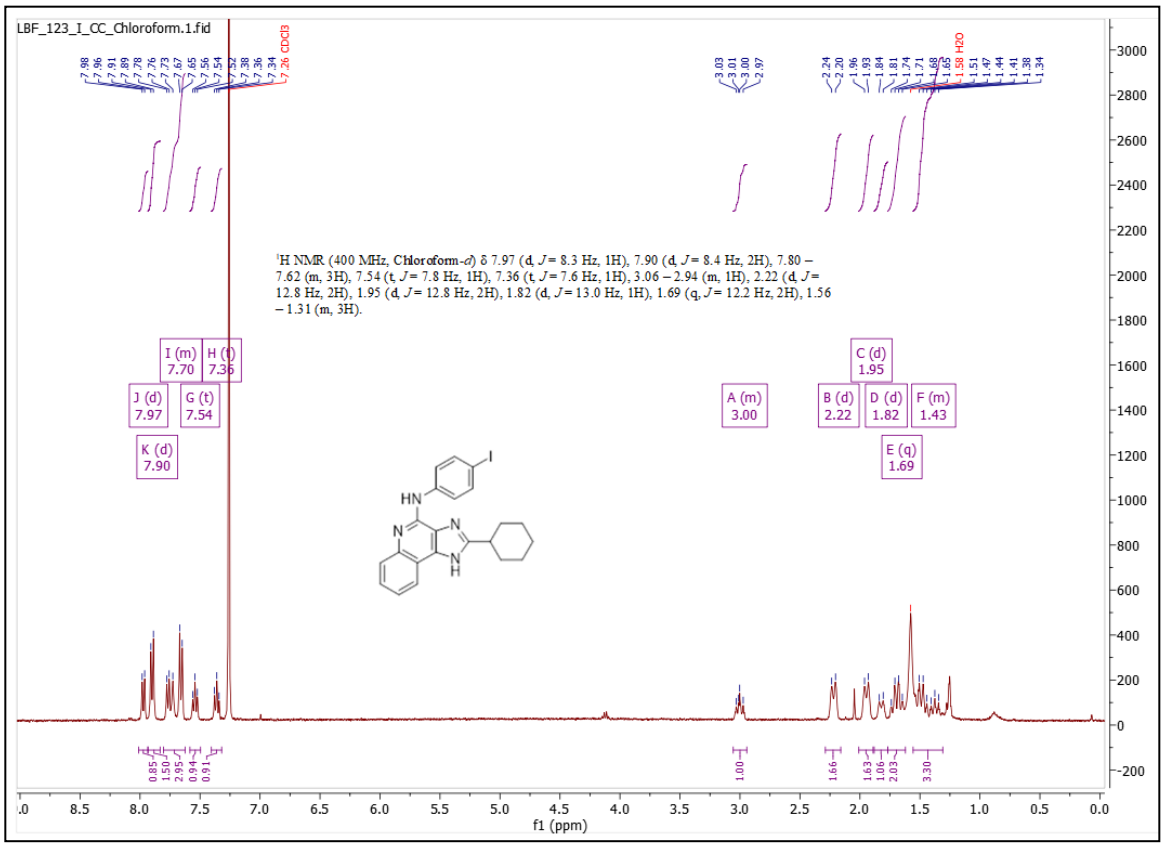
^{1}H NMR of (R)- & (S)-4-(4-((3,4-dichlorophenyl)amino)-1*H*-imidazo[4,5-c]quinolin-2-yl)cycloheptan-1-one – Compound **32.**



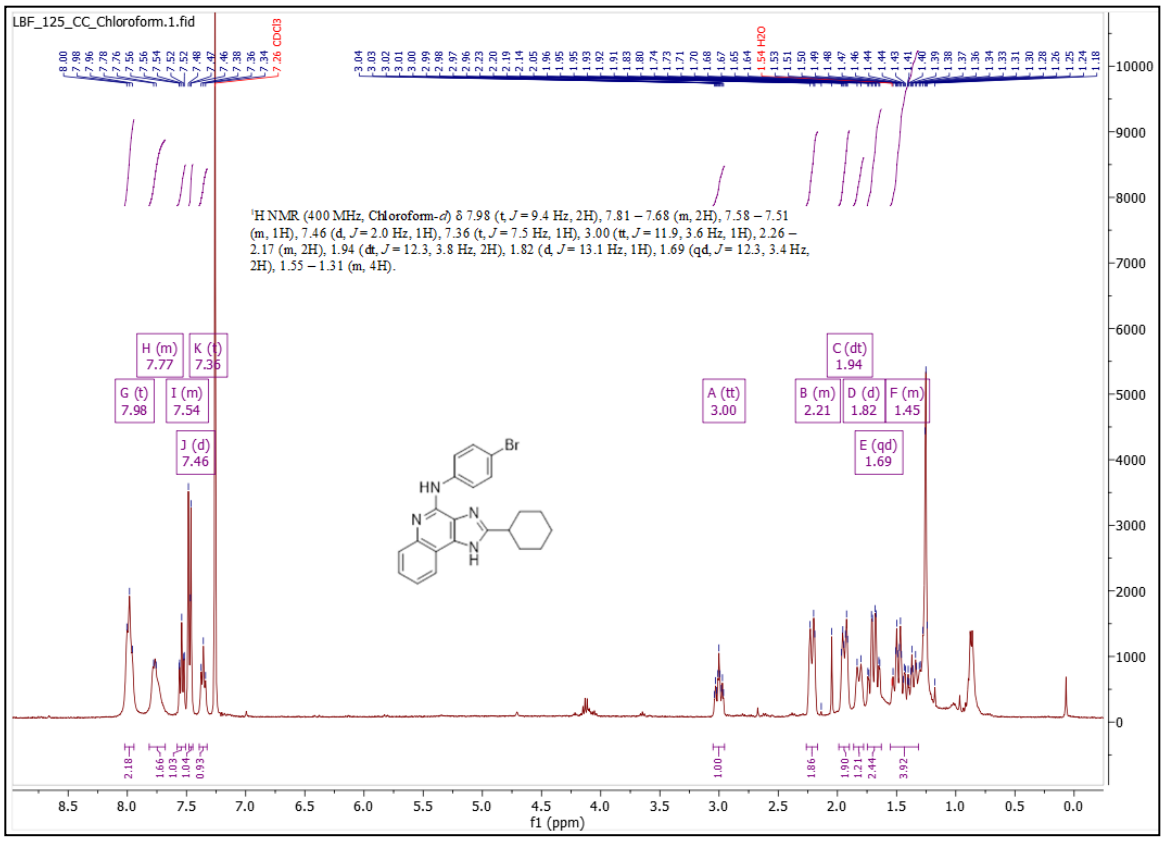
^{1}H NMR of (1R,4S)- & (1S,4R)-4-(4-((3,4-dichlorophenyl)amino)-1*H*-imidazo[4,5-c]quinolin-2-yl)cycloheptan-1-ol – Compound **33.**



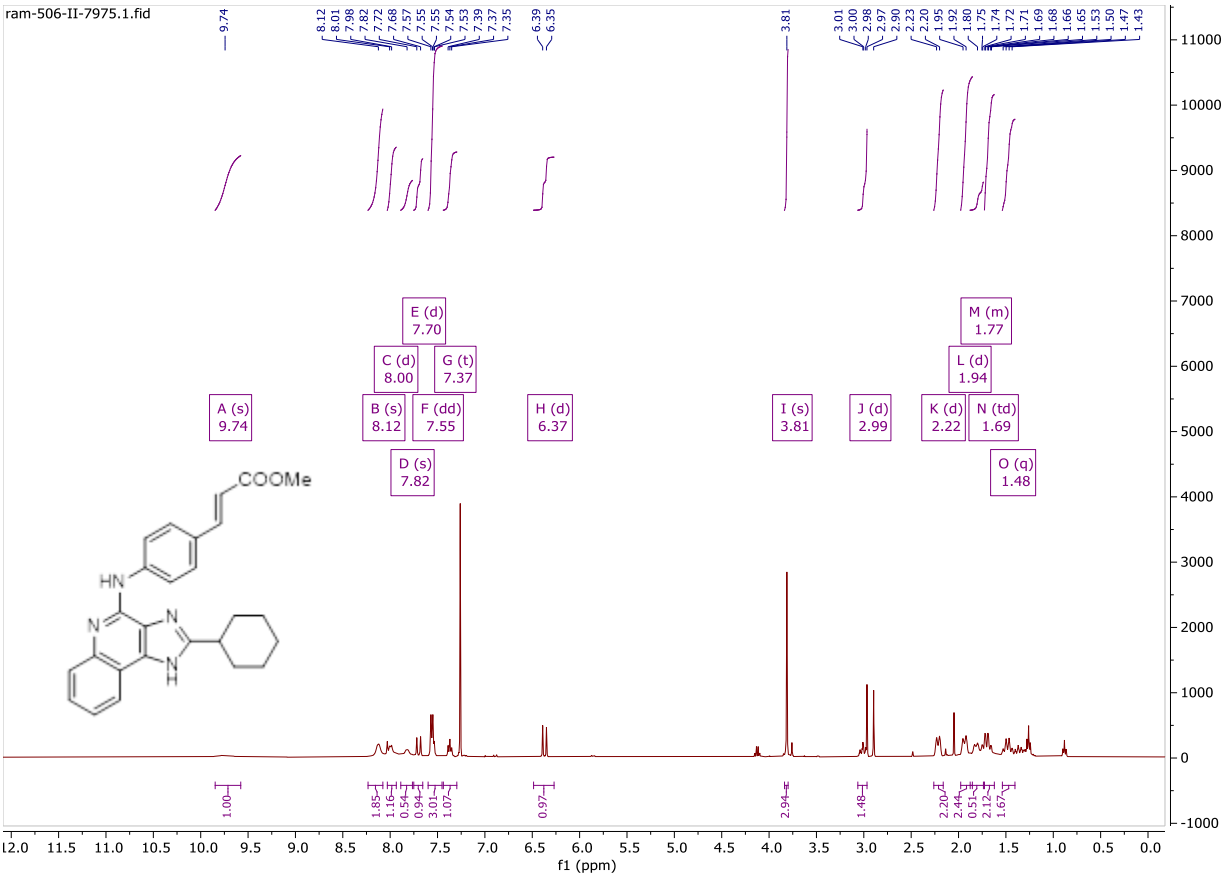
^{1}H NMR of (1R,4R)-, & (1S,4S)-4-(4-((3,4-dichlorophenyl)amino)-1*H*-imidazo[4,5-c]quinolin-2-yl)cycloheptan-1-ol – Compound **34.**



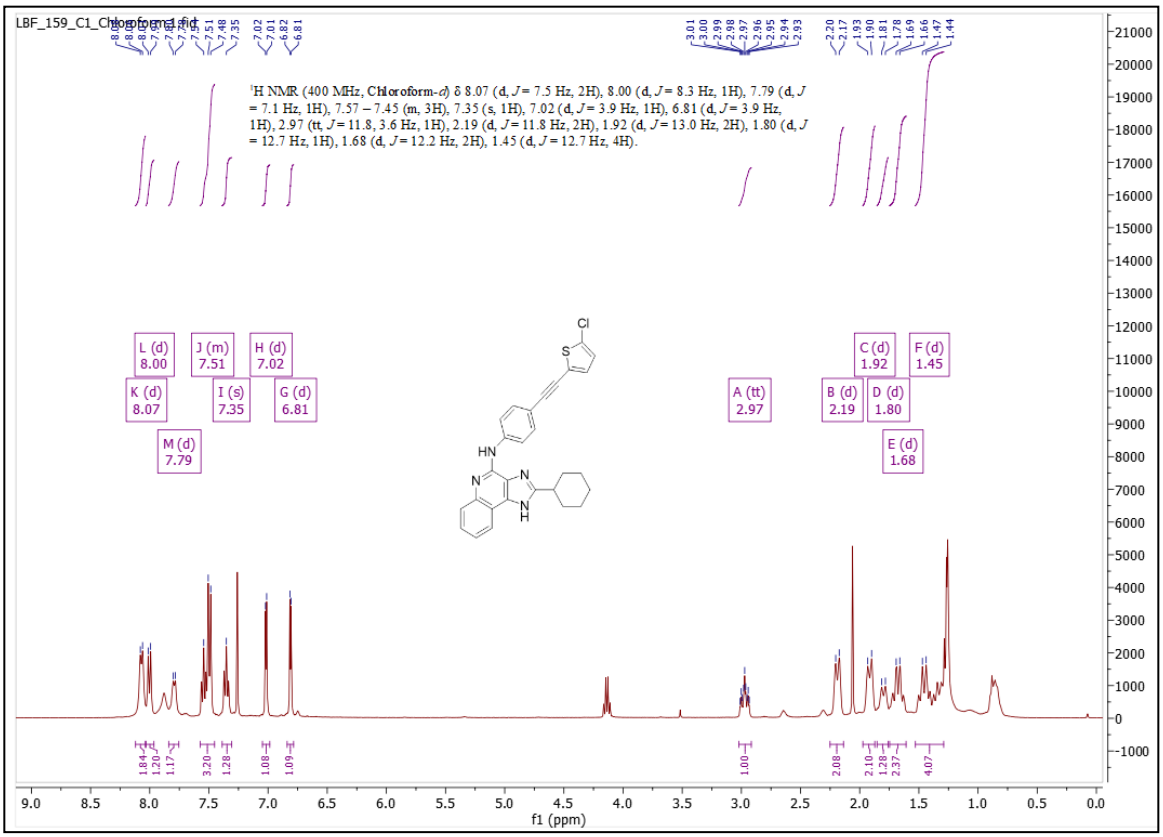
^{1}H NMR of 2-cyclohexyl-*N*-(4-iodophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **35.**



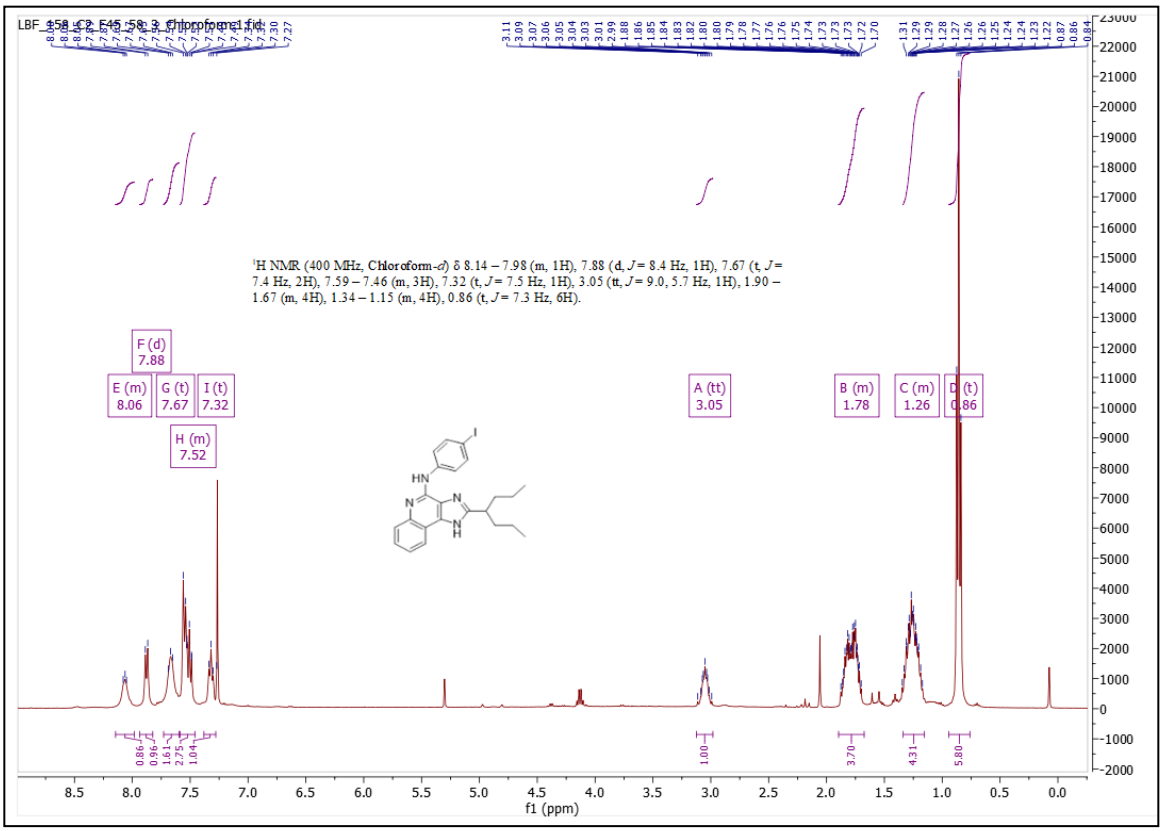
^{1}H NMR of 2-cyclohexyl-*N*-(4-bromophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **36.**



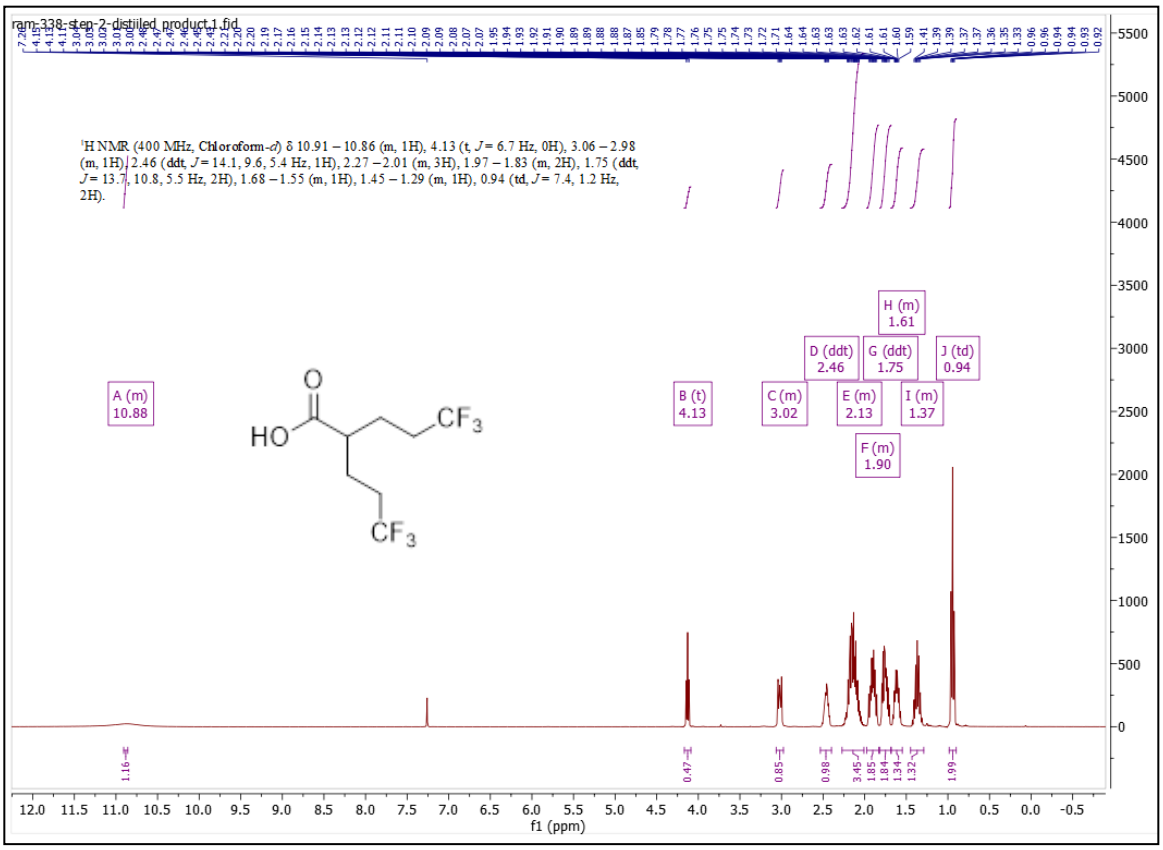
^{1}H NMR of methyl (E)-3-(4-((2-cyclohexyl-1*H*-imidazo[4,5-c]quinolin-4-yl)amino)phenyl)acrylate – Compound **37**.



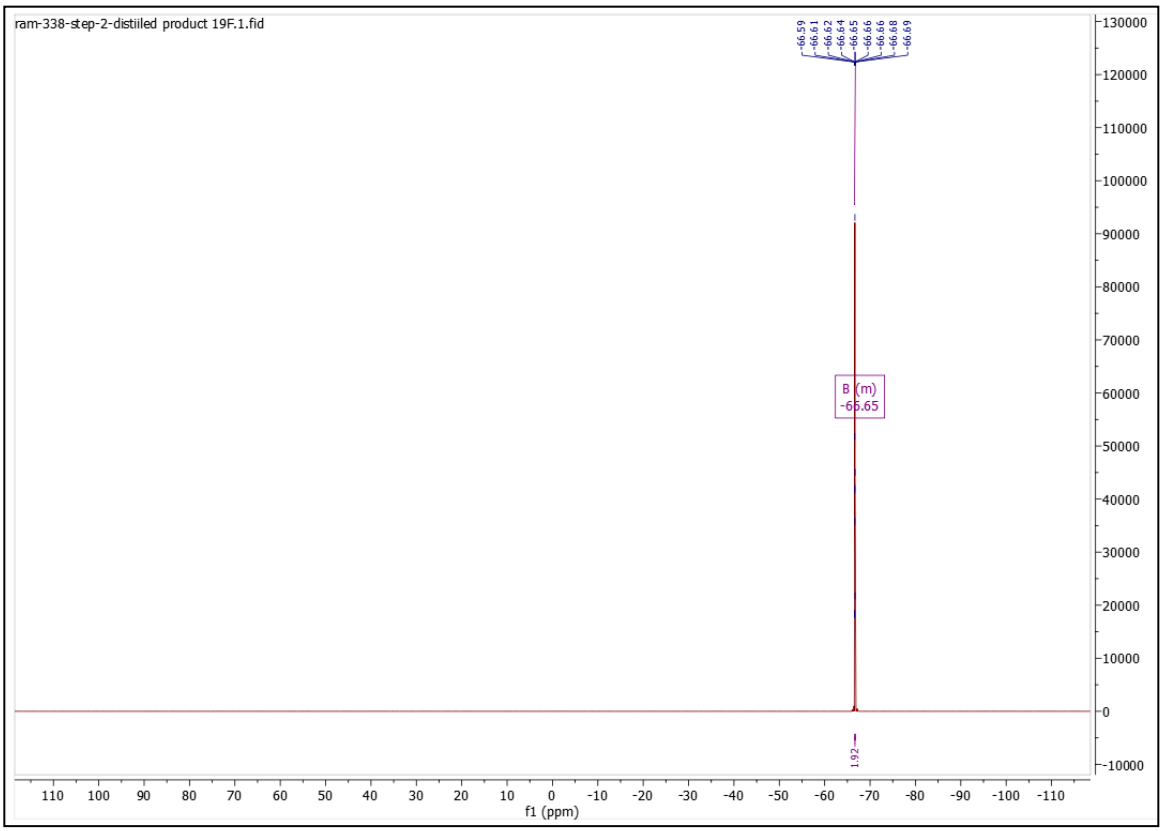
^{1}H NMR of 2-cyclohexyl-*N*-(4-((5-chlorothiophen-2-yl)ethynyl)phenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **38.**



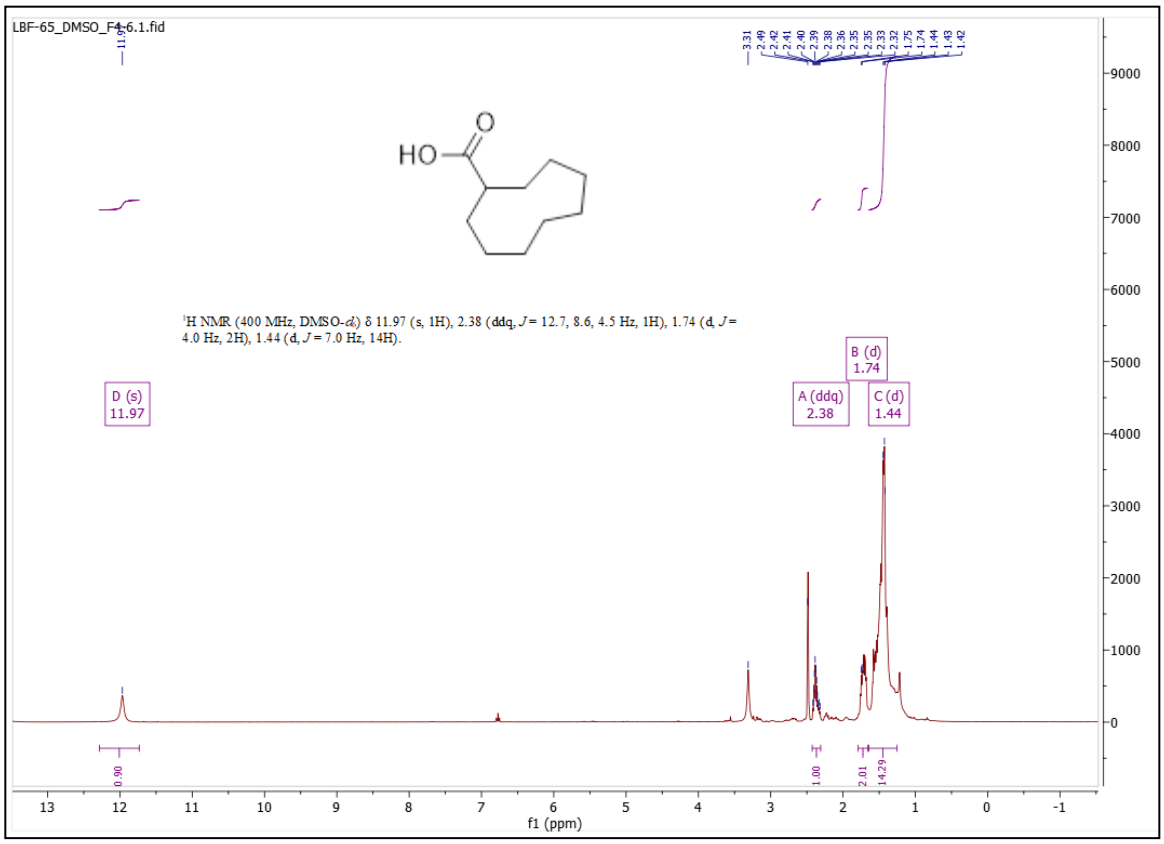
^{1}H NMR of 2-(heptan-4-yl)-*N*-(4-iodophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **39.**



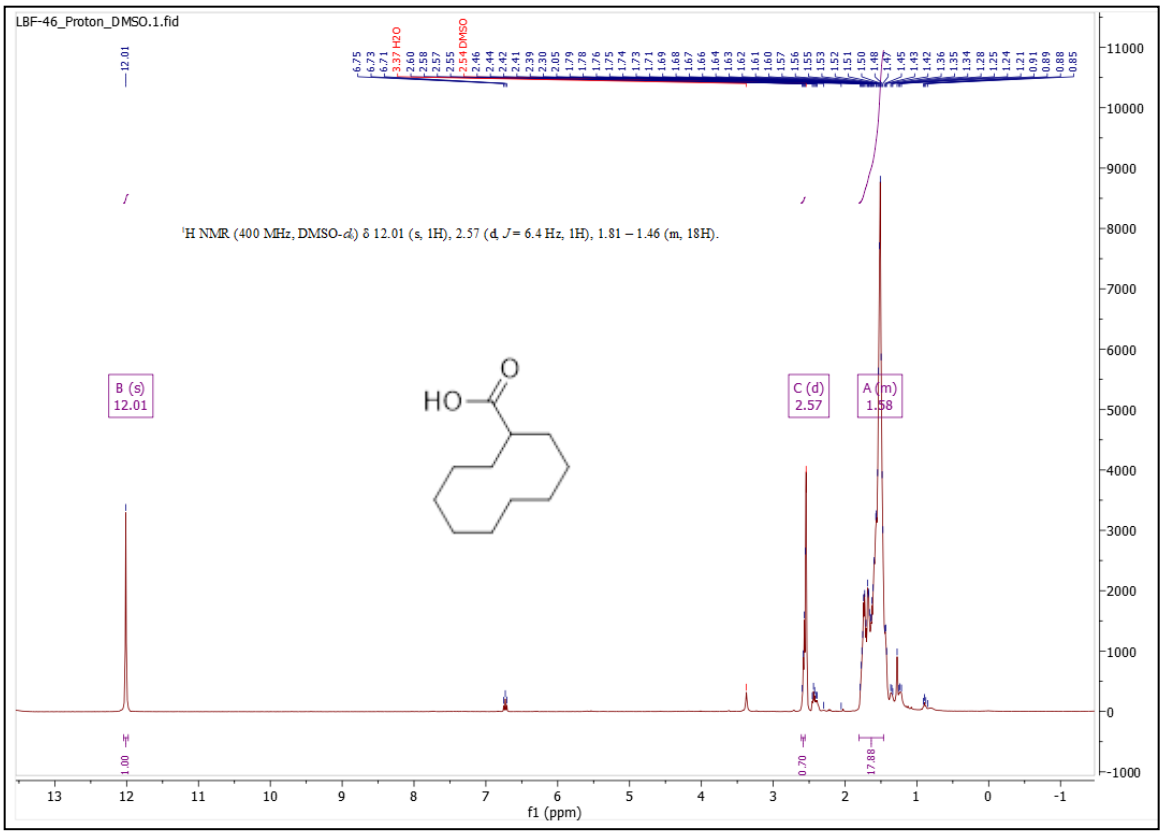
^{1}H NMR of 5,5,5-trifluoro-2-(3,3,3-trifluoropropyl)pentanoic Acid – Compound **45c.**



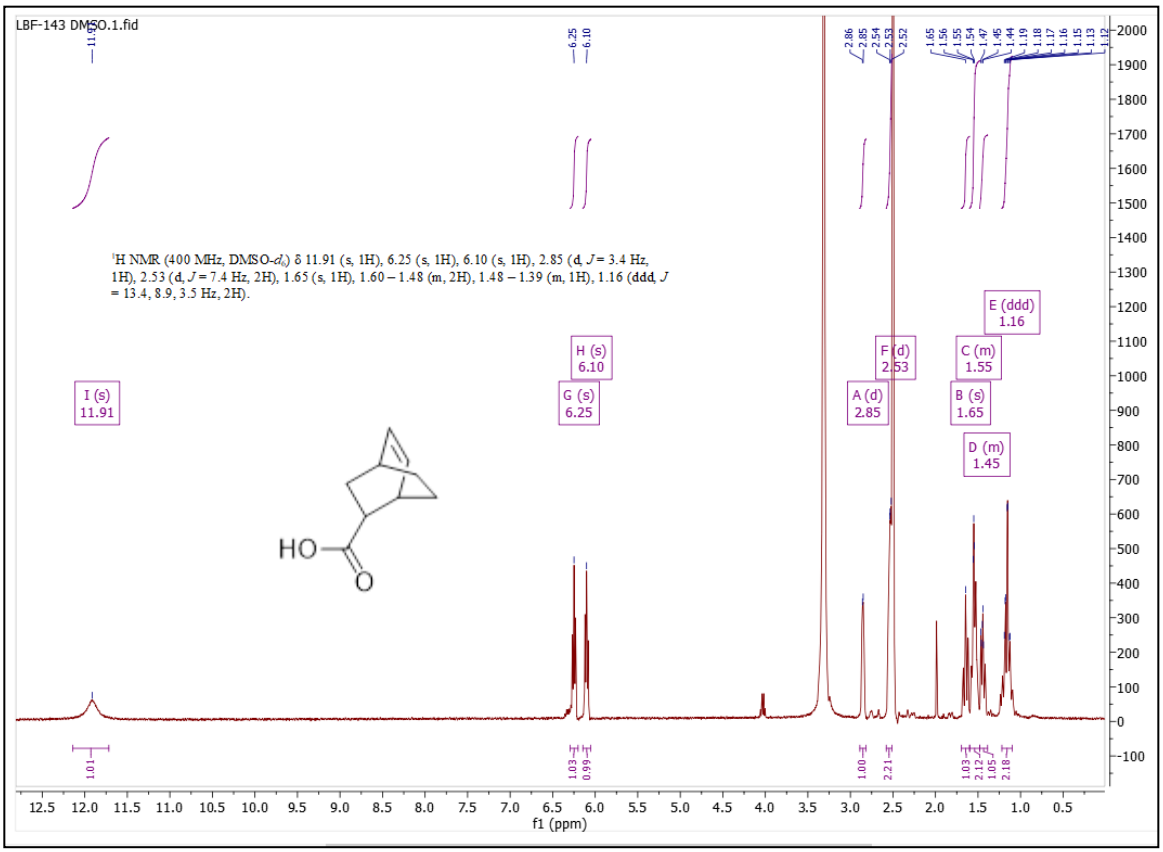
^{19}F NMR of 5,5,5-trifluoro-2-(3,3,3-trifluoropropyl)pentanoic Acid – Compound **45c.**



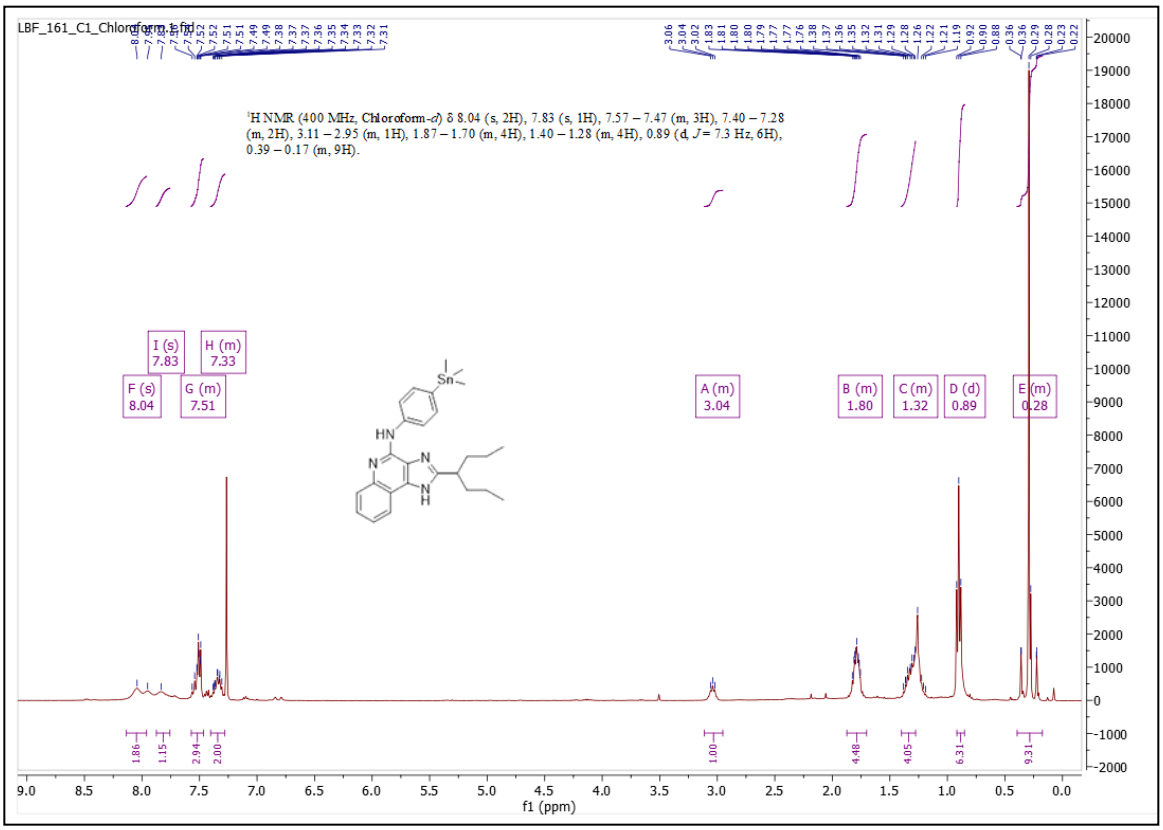
^{1}H NMR of cyclononanecarboxylic acid – Compound **45l.**



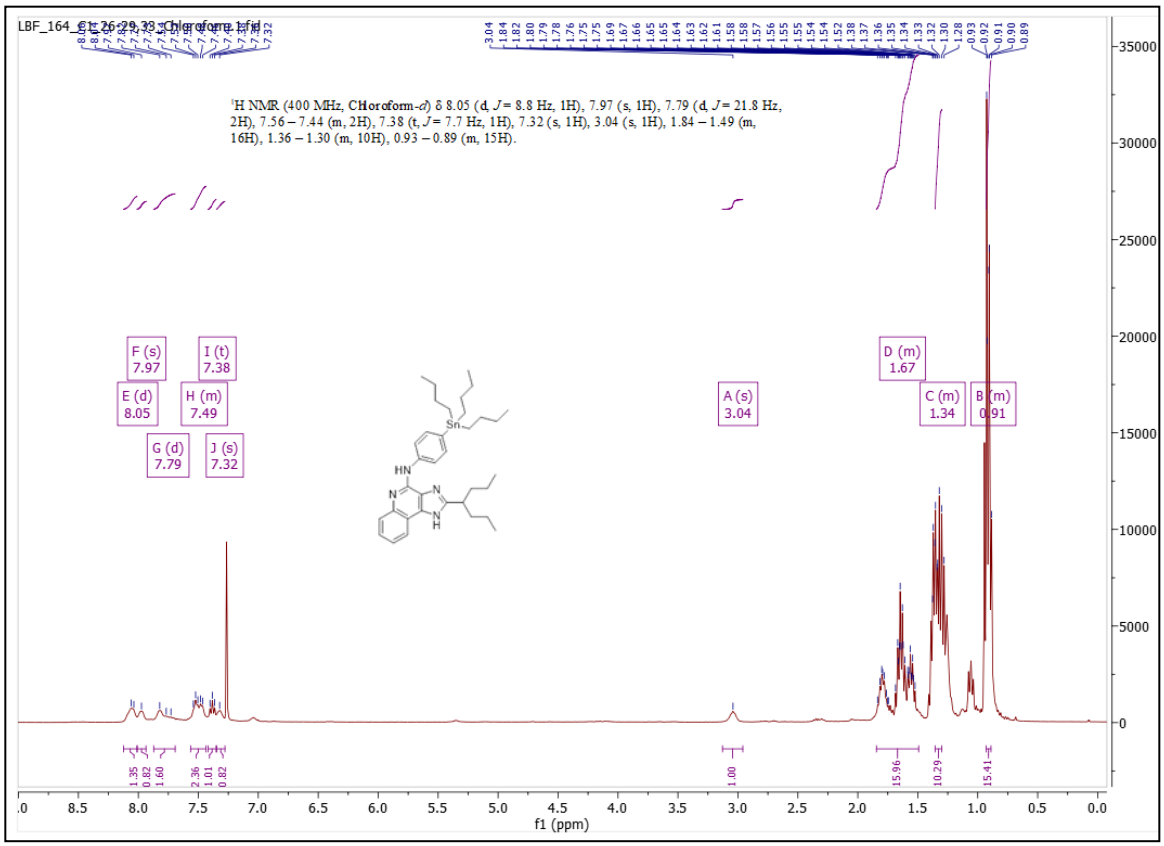
^{1}H NMR of cyclodecanecarboxylic acid – Compound **45m.**



^{1}H NMR of ((1R,2R,4R)- & (1S,2S,4S)-bicyclo[2.2.2]oct-5-ene carboxylic acid – Compound **45t.**

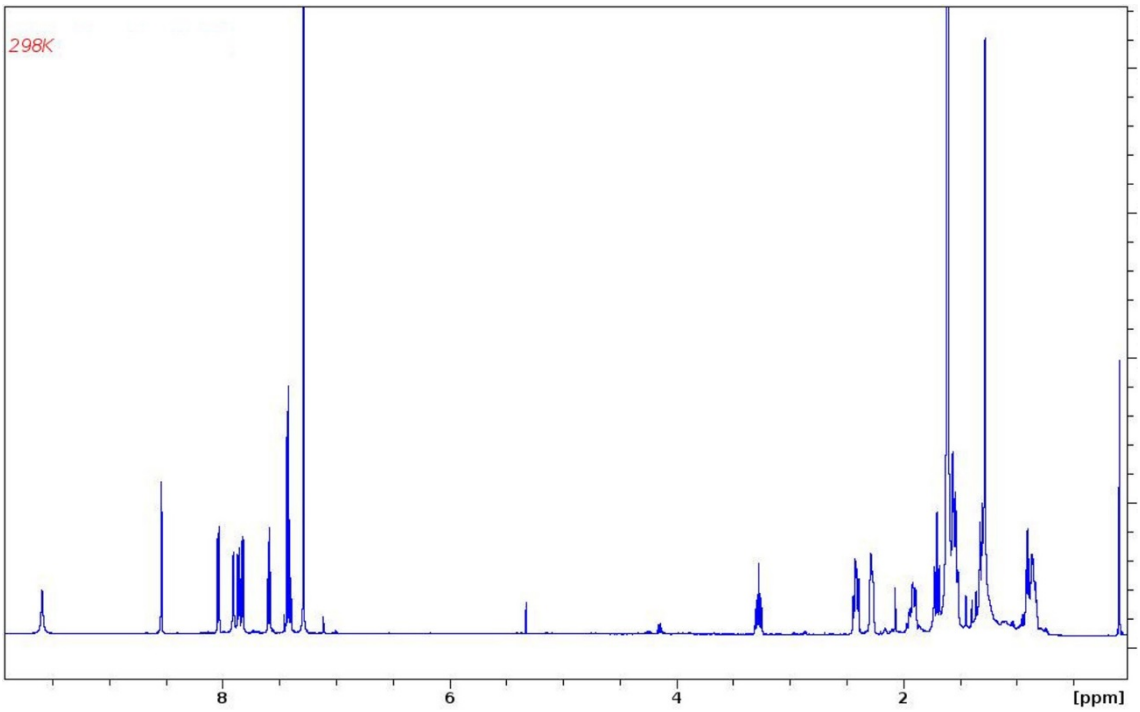


¹H NMR of 2-(heptan-4-yl)-*N*-(4-(trimethylstannyl)phenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **48.**

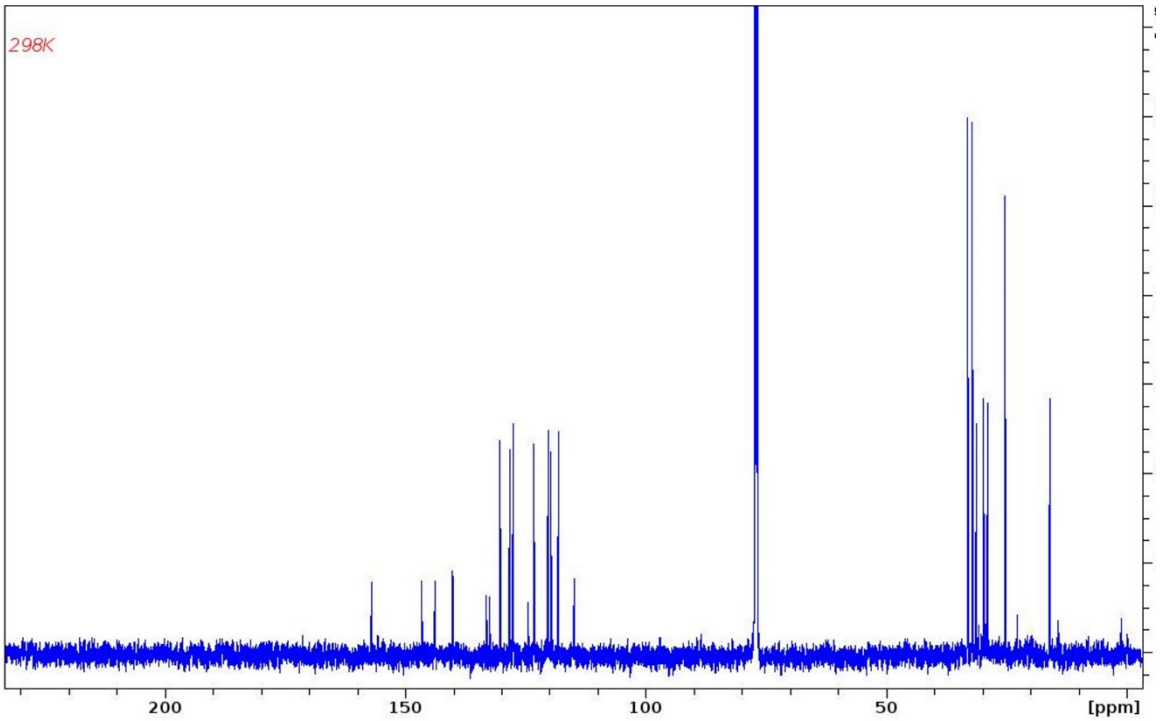


^{1}H NMR of 2-(heptan-4-yl)-*N*-(4-(tributylstannyl)phenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **49.**

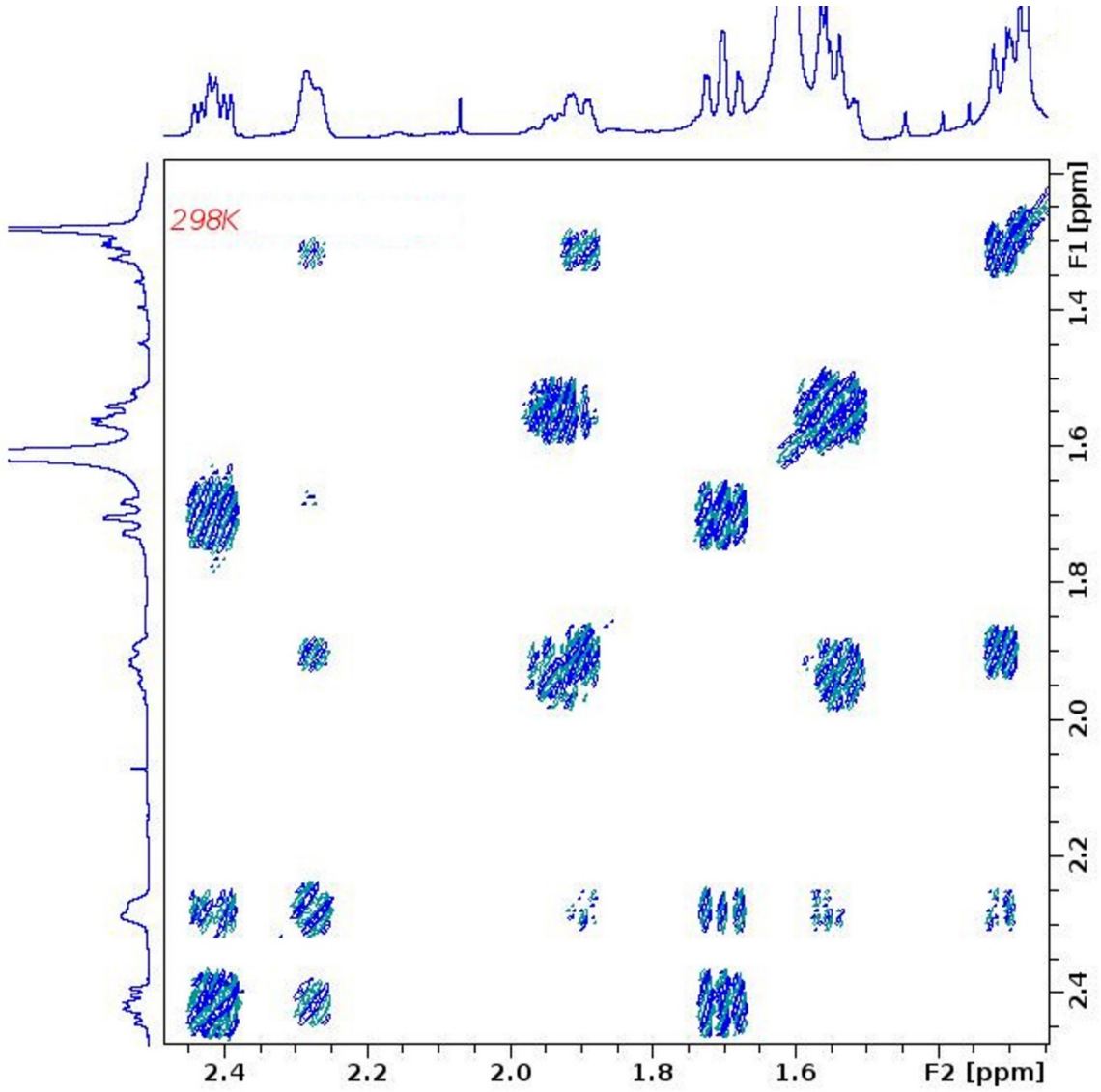
The NMR spectra below were acquired on a Bruker NEO-600 with a z-gradient, triple resonant cryoprobe *at 298˚K* in $CDCl_3$ plus 4 drops of MeOD, unless otherwise noted. They are referenced to $CDCl_3$ ^1H=7.26 ppm, ^{13}C=77.0 ppm for 275 - 298 ˚K; CD_3OD ^1H=3.31 ppm, ^{13}C=49.0 ppm for 298 K.



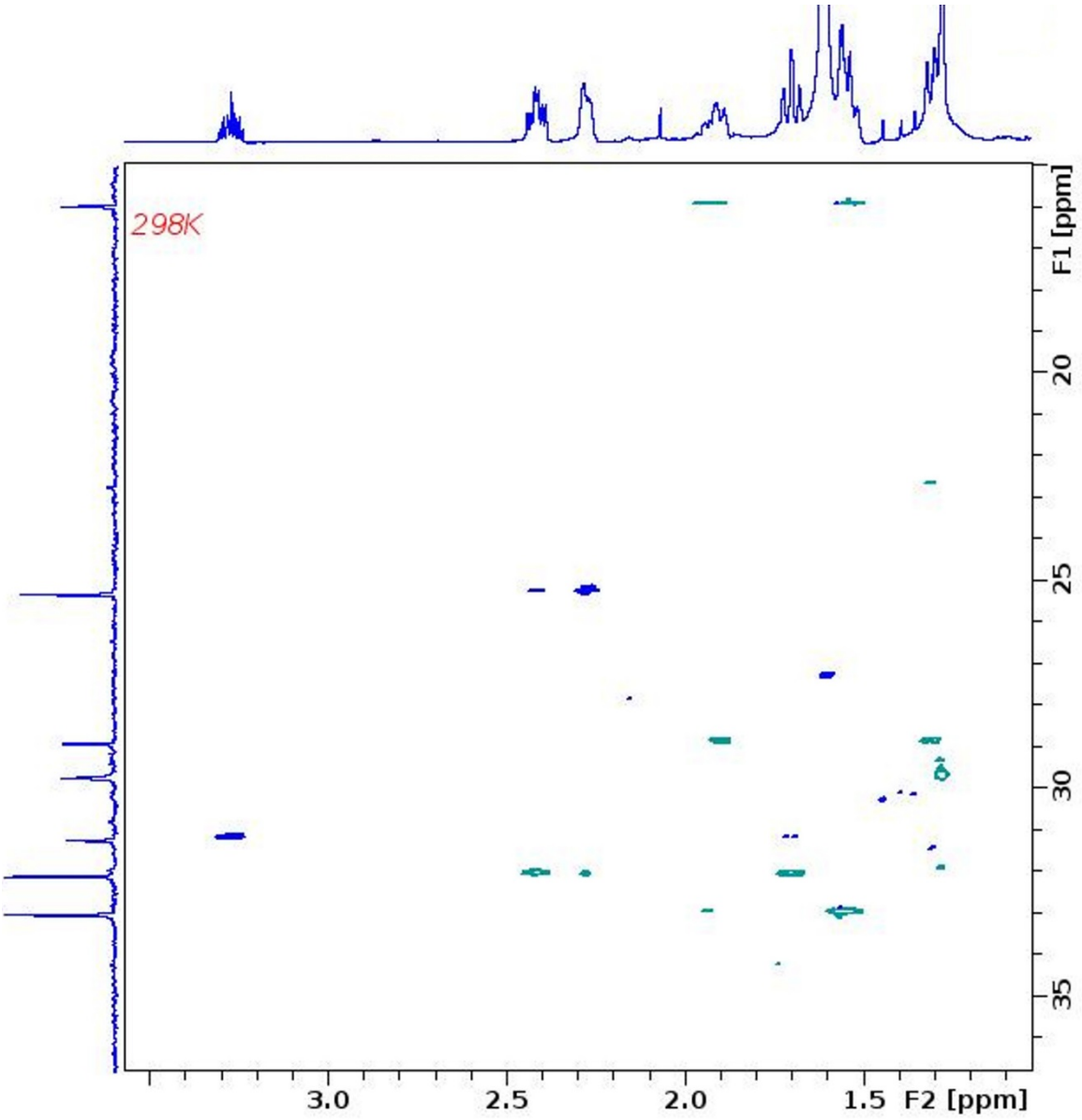
^1H spectrum of compound **27**.



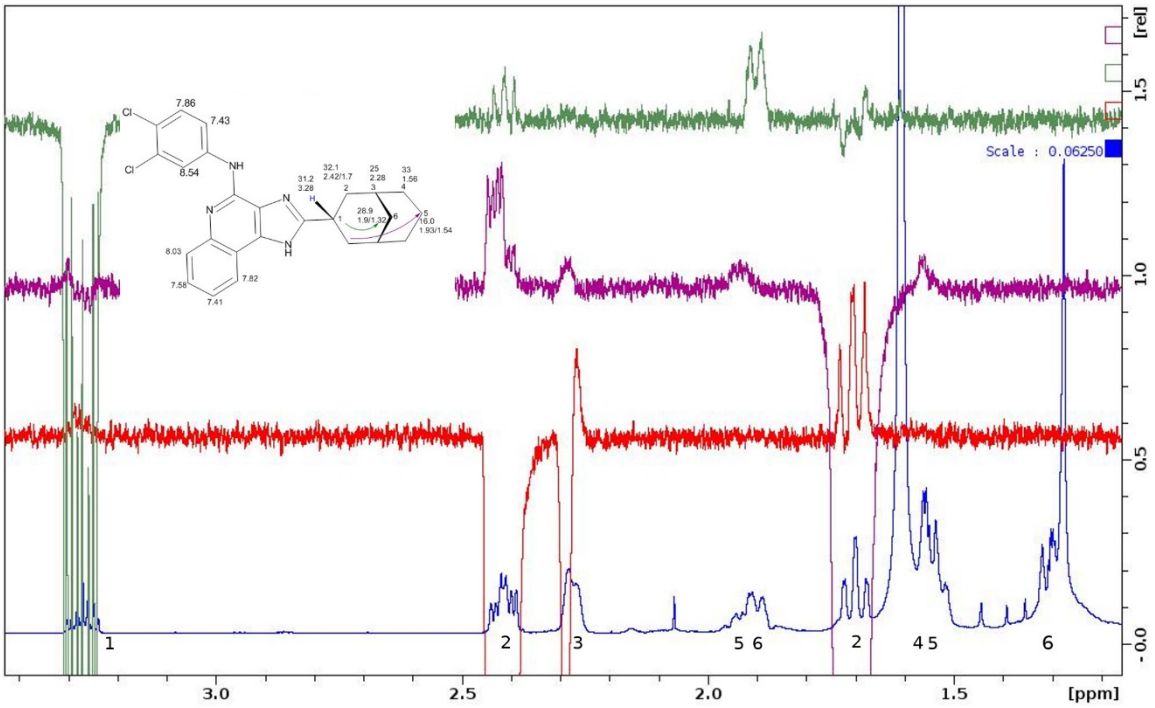
^{13}C spectrum of compound **27**.



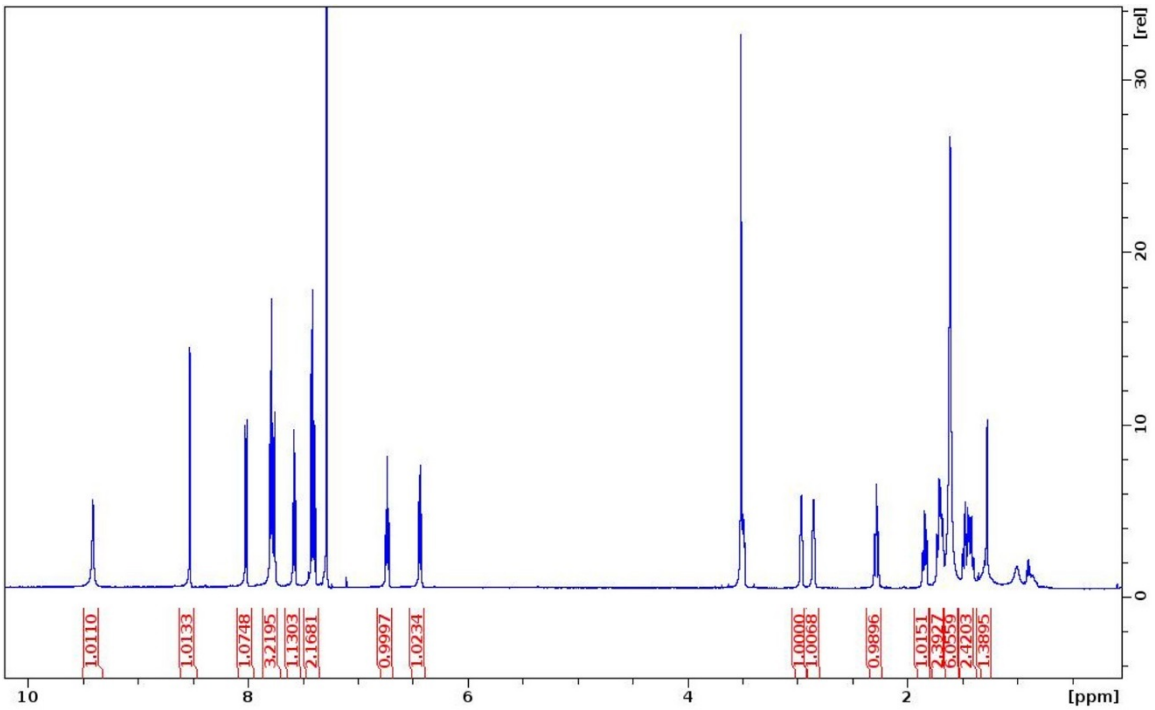
Aliphatic region of ^{1}H 3Q-COSY (mix=200ms) for compound **27**.



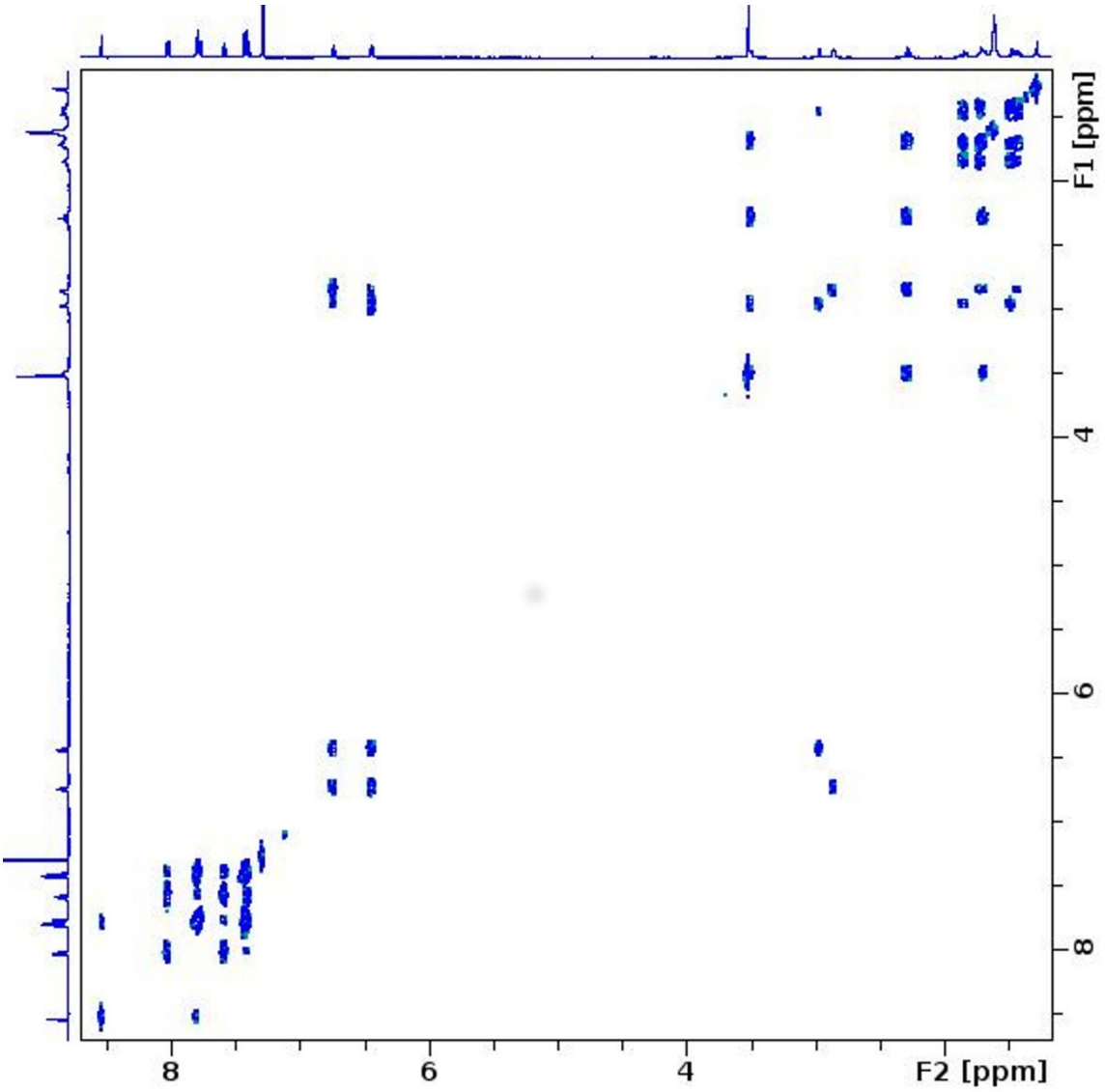
HSQC of compound **27**.



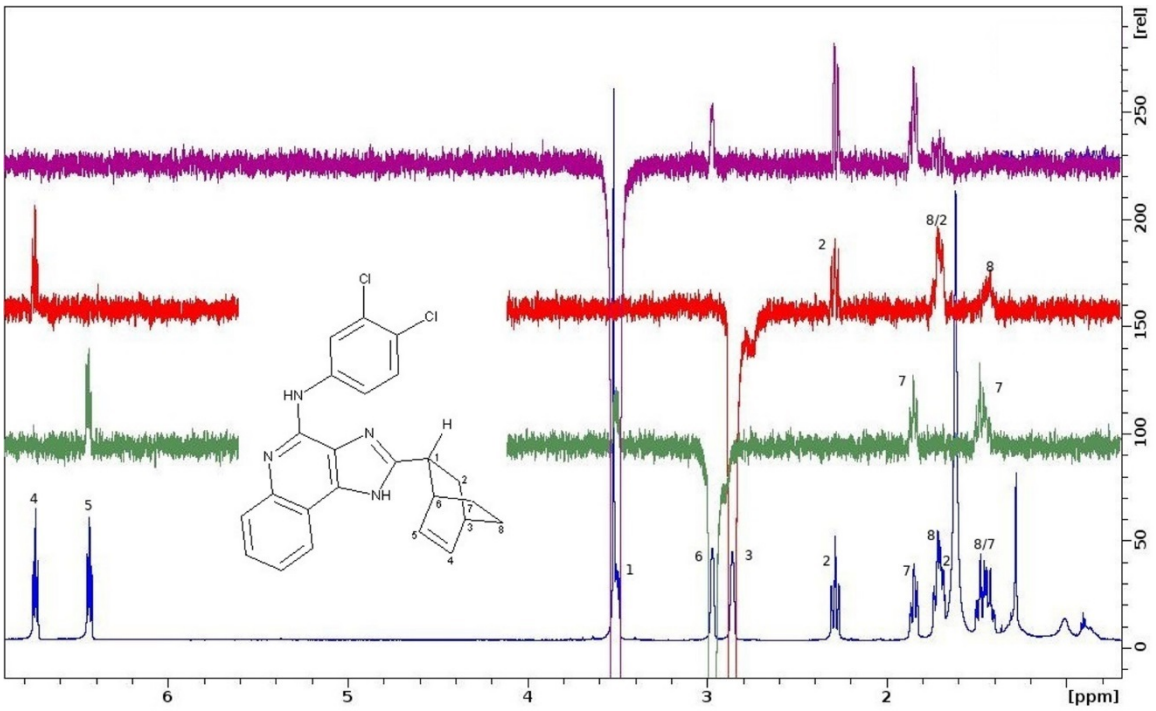
^{1}H-1D-PE-ZQS-NOE spectra (mix=200ms) for compound **27**.



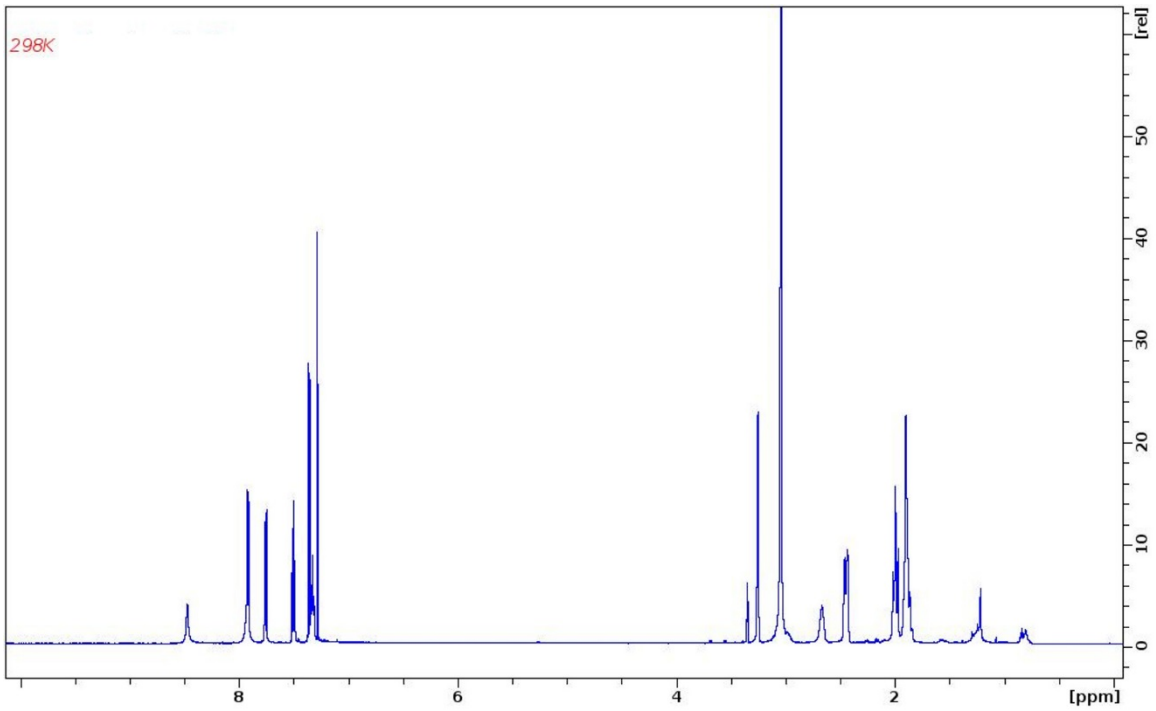
^{1}H spectrum of compound **29**.



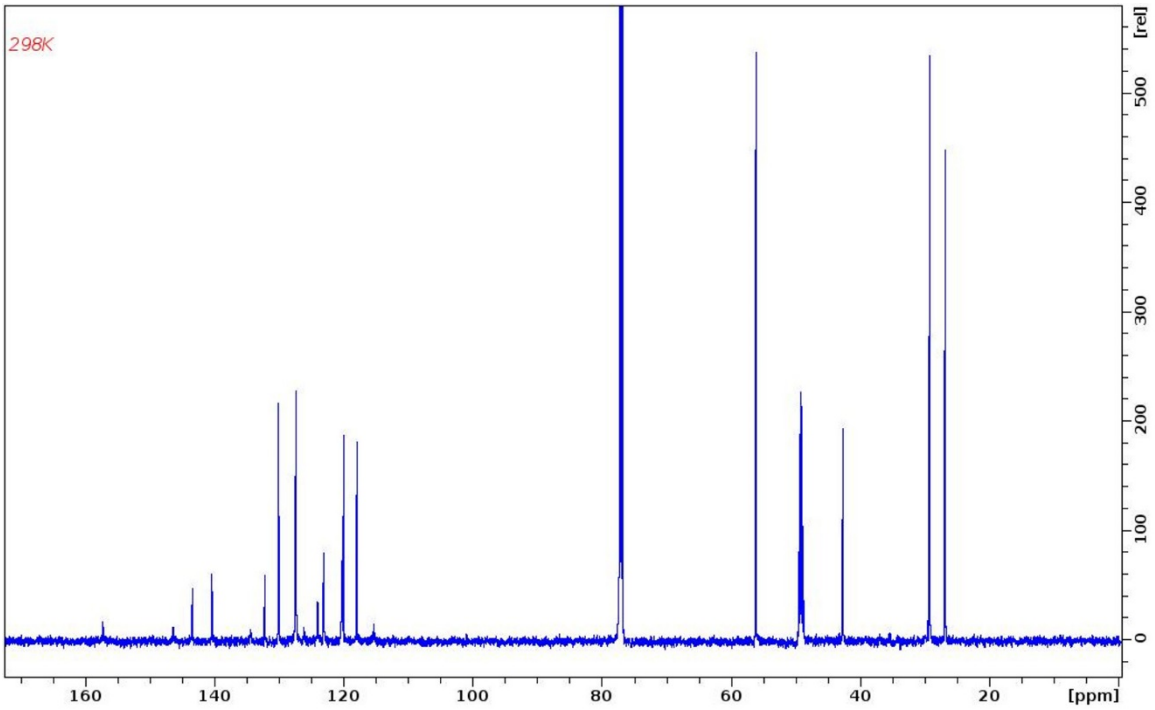
^{1}H-COSY spectrum of compound **29**.



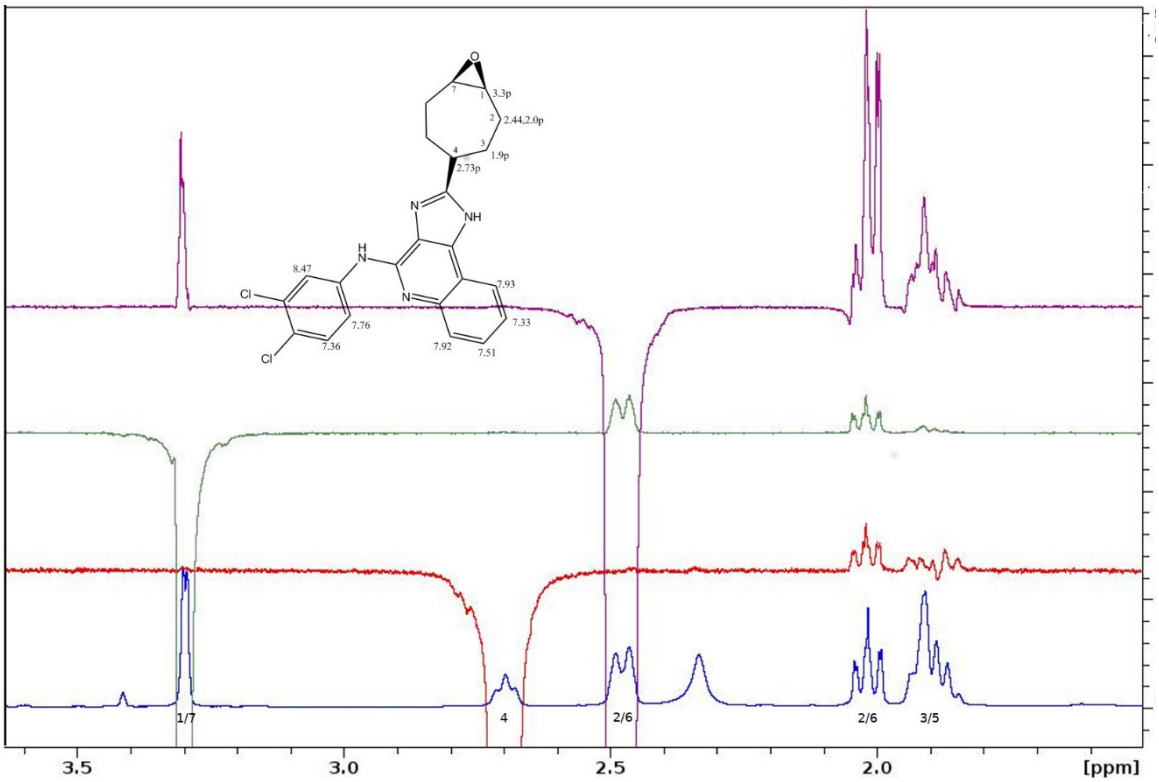
1D-NOE spectra of compound **29**. The 1/7 proximity shown in the purple trace clearly proves the *endo* conformation.



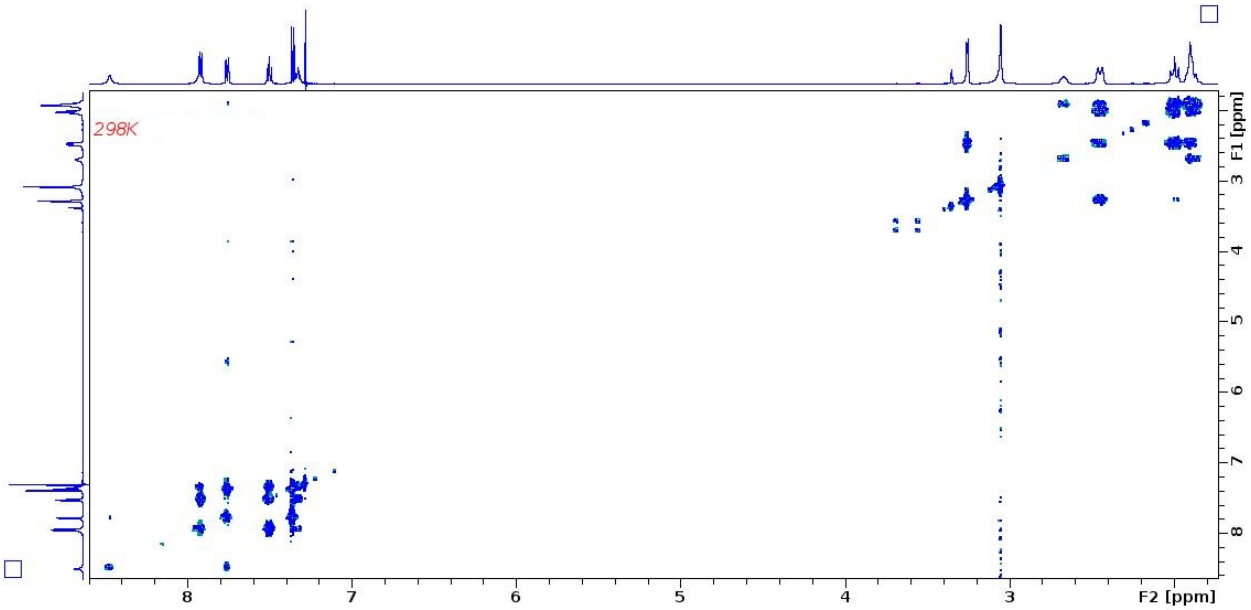
^{1}H spectrum of compound **30**.



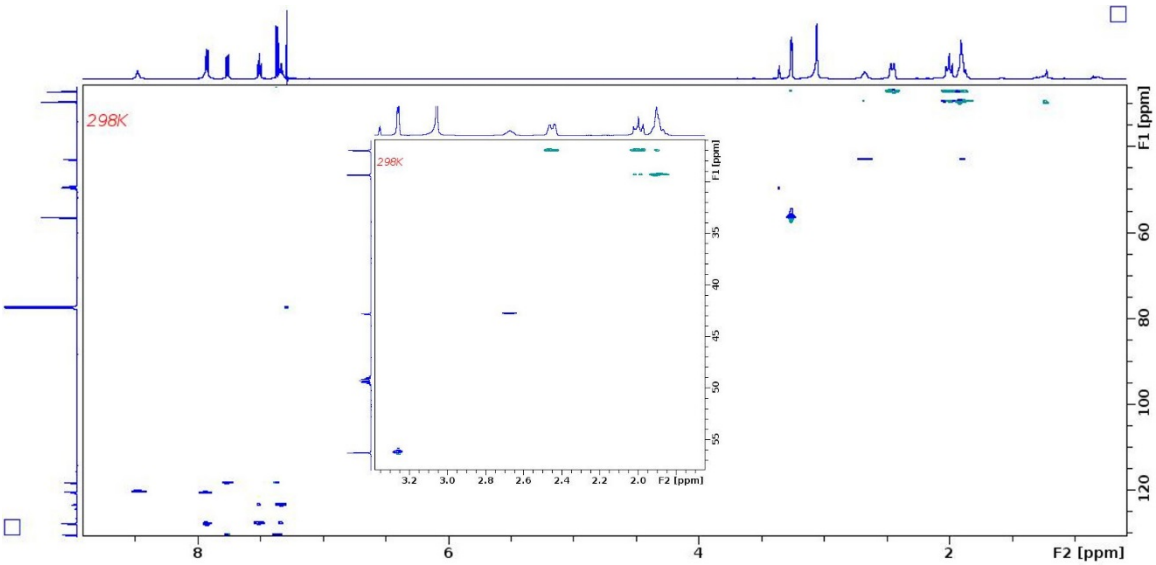
^{13}C spectrum of compound **30**.



1D-NOE spectra of compound **30** at 288˚K. The relatively equal distances of the four 2s to 1/7 (green trace) along with the 2/4 proximity (red) and shielded 3/5s are consistent with the (1R,4s,7S) isomer.

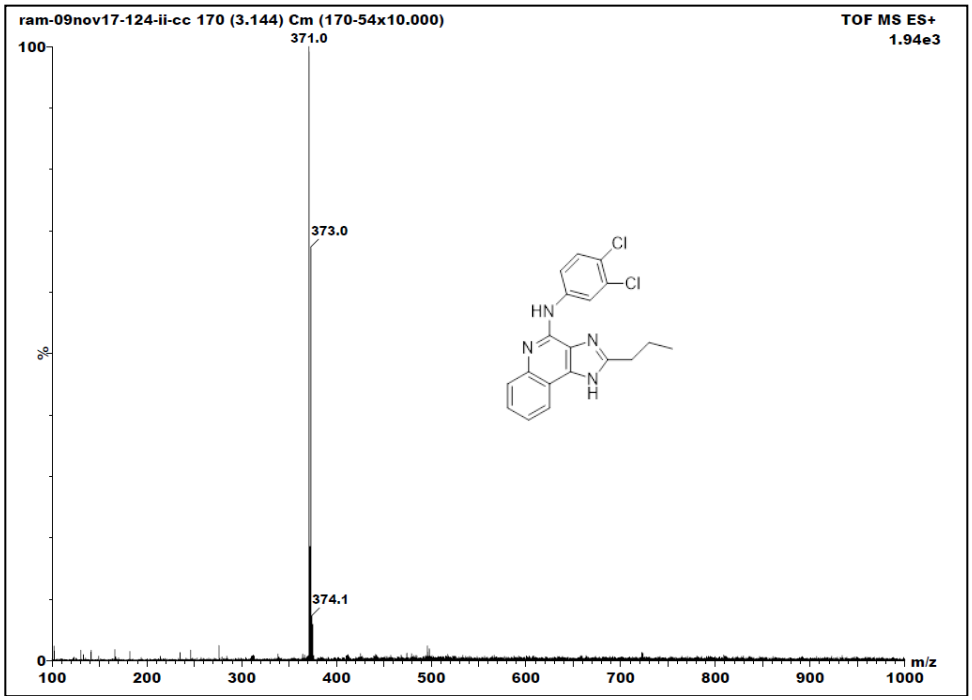


^{1}H-COSY spectrum of compound **30**.



HSQC spectrum of compound **30**.

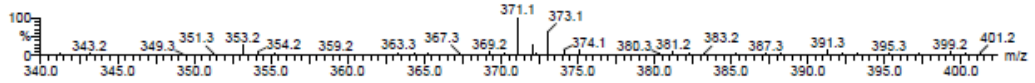
Mass Spectra



Elemental Composition Report Page 1

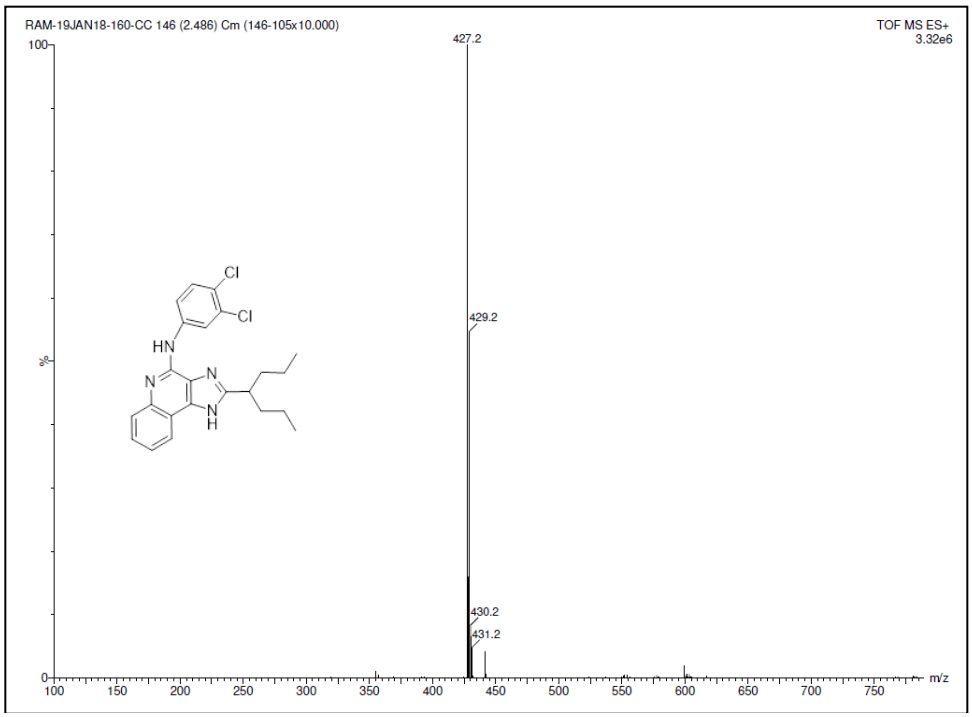
Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
42 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-20 35Cl: 2-2
09-Nov-2017
ram-09nov17-124-ii-cc 172 (3.181) Cn (Cen,3, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)
TOF MS ES+
2.55e+003



Minimum:				-2.0		
Maximum:		10.0	10.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
371.0827	371.0830	-0.3	-0.8	12.5	758.5	C19 H17 N4 35Cl2
	371.0889	-6.2	-16.7	3.5	822.8	C12 H21 N4 O5 35Cl2
	371.0736	9.1	24.5	-0.5	878.8	C8 H21 N4 O8 35Cl2

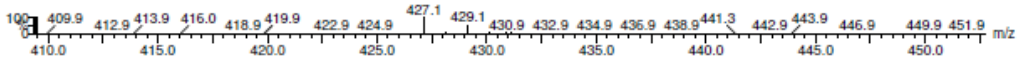
TOF MS E+ and elemental analysis of 2-propyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **13**



Elemental Composition Report **Page 1**

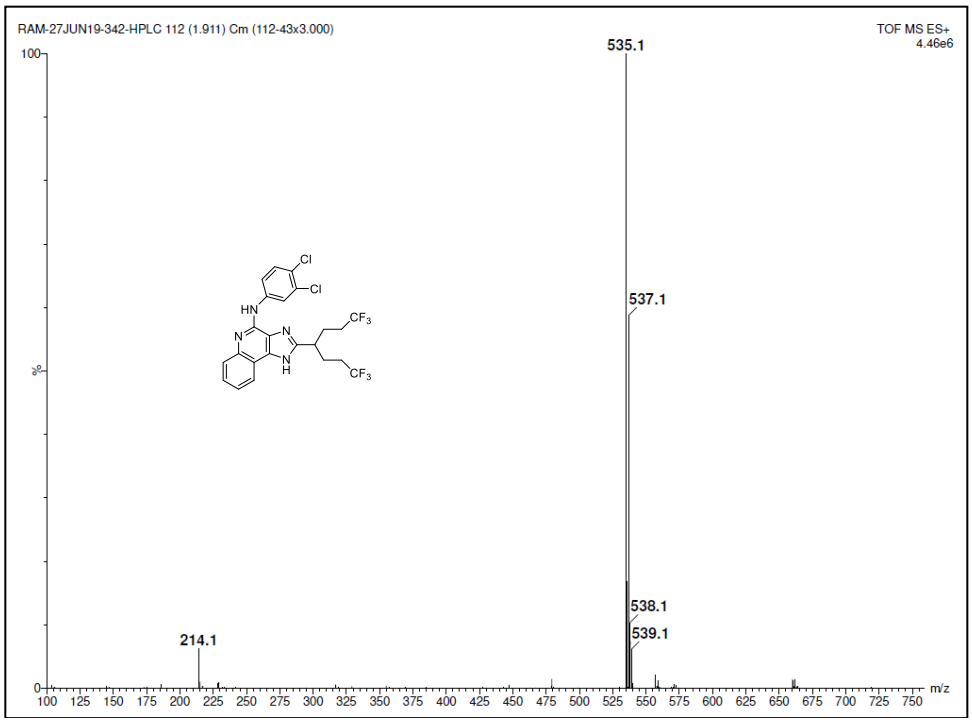
Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-60 H: 0-200 N: 4-4 O: 0-40 35Cl: 2-2
RAM-19JAN18-160-CC 148 (2.520) AM (Cen,5, 50.00, Ht,10000.0,0.00,0.70); Sm (SG, 1x2.00); Sb (15,10.00)
TOF MS ES+
2.88e+005



Minimum:				-1.5				
Maximum:		30.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
427.1462	427.1456	0.6	1.4	12.5	225.0	2.424	8.85	C23 H25 N4 35C12
	427.1515	-5.3	-12.4	3.5	224.3	1.721	17.89	C16 H29 N4 O5 35C12
	427.1362	10.0	23.4	-0.5	223.9	1.371	25.38	C12 H29 N4 O8 35C12
	427.1304	15.8	37.0	8.5	224.6	2.059	12.75	C19 H25 N4 O3 35C12
	427.1668	-20.6	-48.2	7.5	224.7	2.140	11.77	C20 H29 N4 O2 35C12
	427.1726	-26.4	-61.8	-1.5	224.0	1.454	23.36	C13 H33 N4 O7 35C12

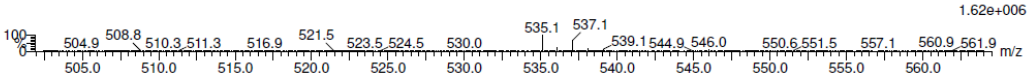
TOF MS E+ and elemental analysis of 2-(heptan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **14**



Elemental Composition Report **Page 1**

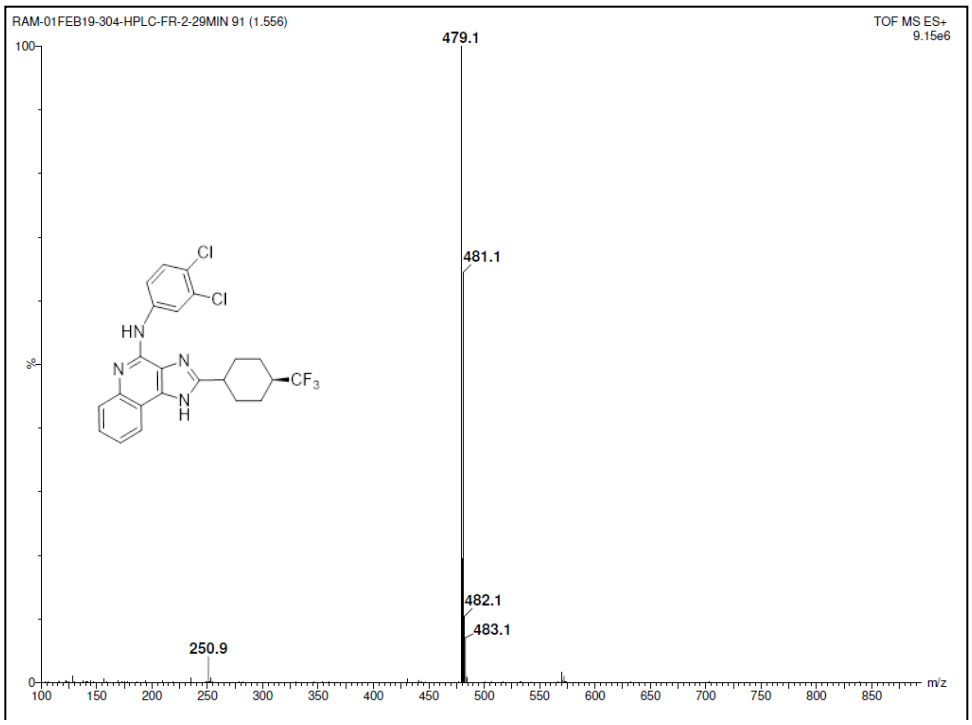
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
49 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 F: 6-6 35Cl: 2-2
RAM-27JUN19-342-HPLC 115 (1.962) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
1.62e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
535.0901	535.0891	1.0	1.9	12.5	482.5	0.758	46.85	C23 H19 N4 F6 35Cl2
	535.0950	-4.9	-9.2	3.5	482.4	0.632	53.15	C16 H23 N4 O5 F6 35Cl2

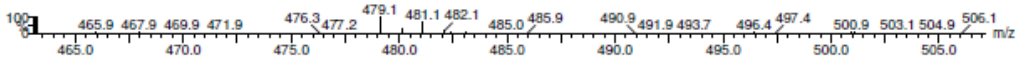
TOF MS E+ and elemental analysis of 2-(1,1,1,7,7,7-hexafluoroheptan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **15**



Elemental Composition Report **Page 1**

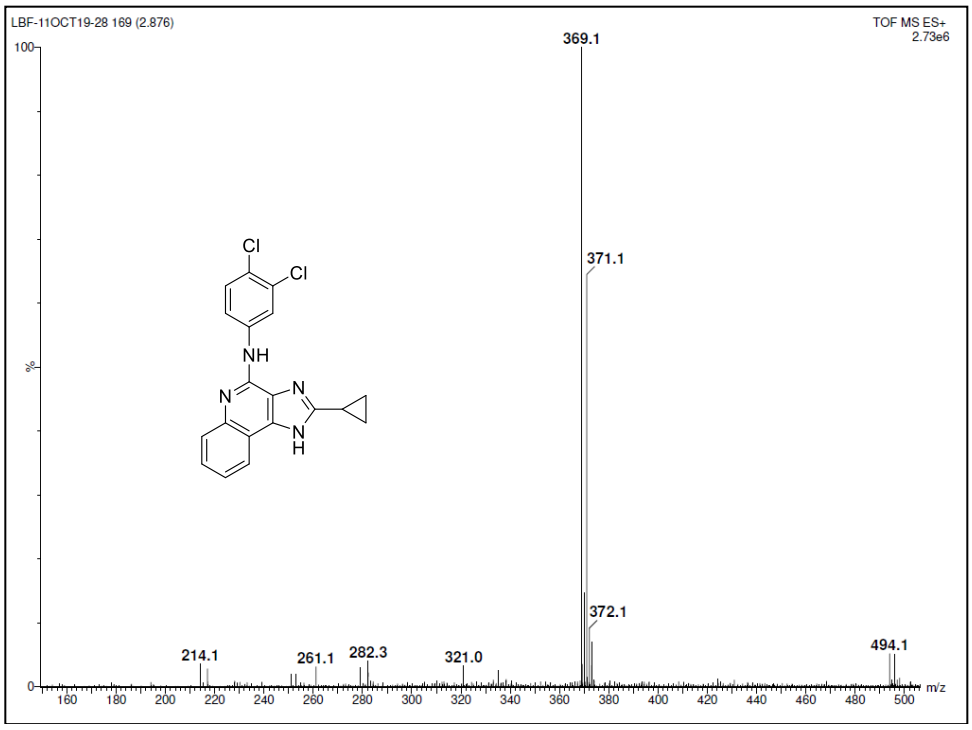
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
52 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-150 H: 0-200 N: 4-4 O: 0-60 F: 3-3 35Cl: 2-2
RAM-01FEB19-304-HPLC-FR-2-29MIN 174 (2.960) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
7.79e+005



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
479.1019	479.1017	0.2	0.4	13.5	419.9	n/a	n/a	C23 H20 N4 F3 35Cl2

TOF MS E+ and elemental analysis of 2-(4-(trifluoromethyl)cyclohexyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **16**

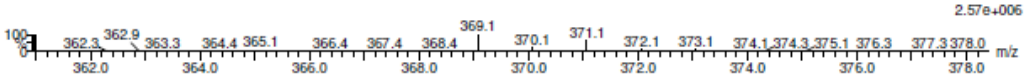


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

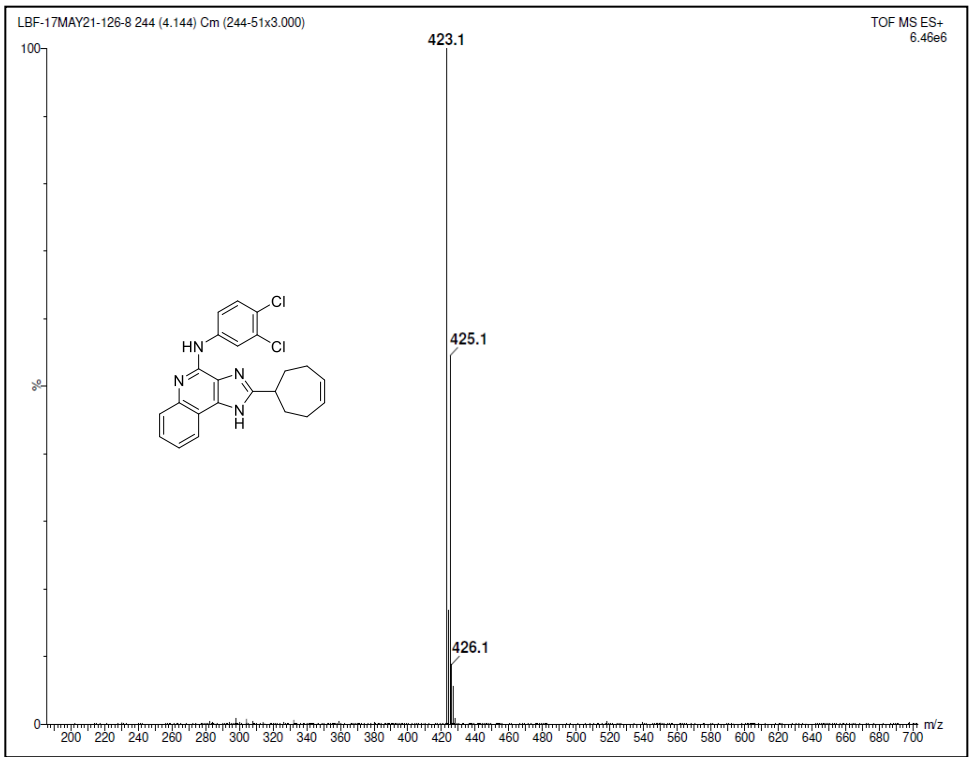
Monoisotopic Mass, Even Electron Ions
43 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 35Cl: 2-2

LBF-11OCT19-28 171 (2.909) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
2.57e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
369.0676	369.0674	0.2	0.5	13.5	624.3	n/a	n/a	C19 H15 N4 35Cl2

TOF MS E+ and elemental analysis of 2-(cyclopropyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **17**

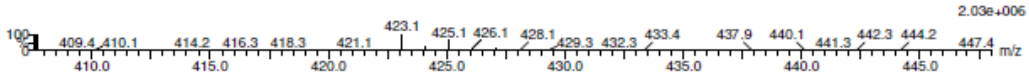


Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

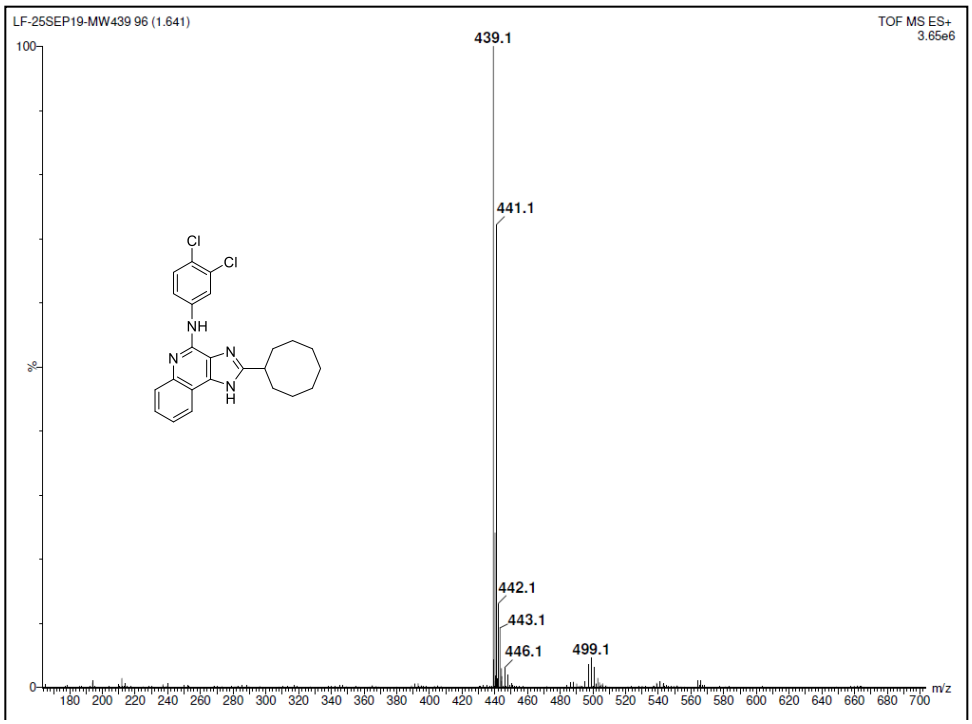
Monoisotopic Mass, Even Electron Ions
55 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2

LBF-17MAY21-126-8 248 (4.212) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
2.03e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
423.1137	423.1143	-0.6	-1.4	14.5	544.1	n/a	n/a	C23 H21 N4 35Cl2

TOF MS ES+ and elemental analysis of 2-(cyclohept-4-en-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **18**

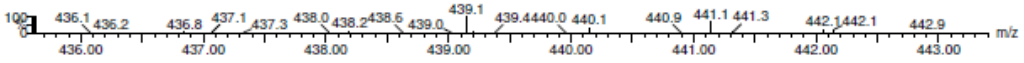


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

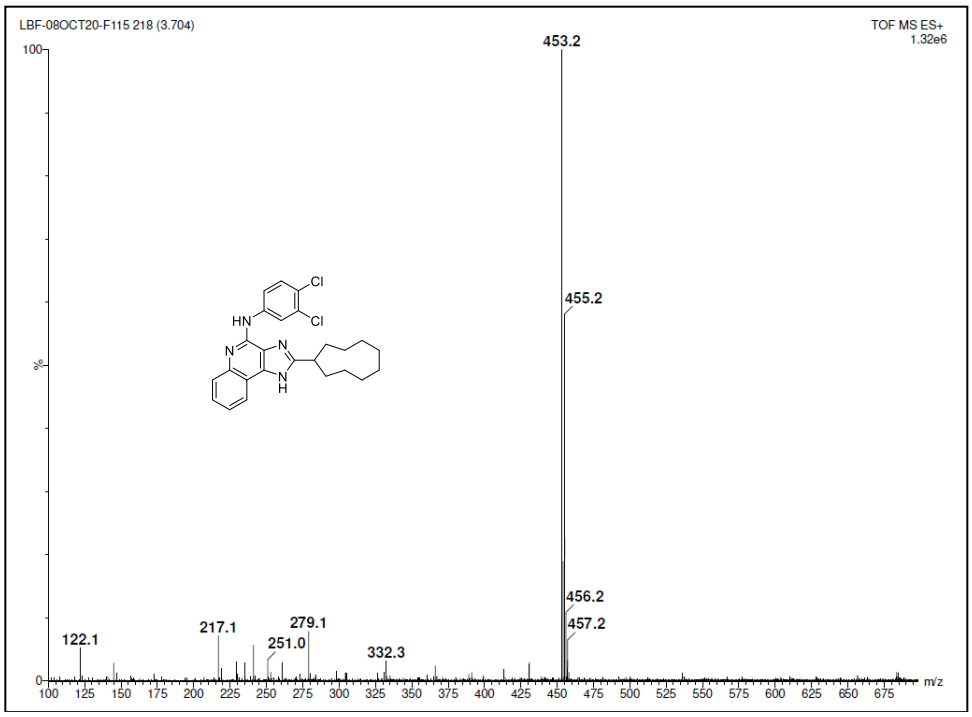
Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 35Cl: 2-2
LF-25SEP19-MW439 155 (2.639) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+

2.41e+005



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
439.1452	439.1456	-0.4	-0.9	13.5	491.0	n/a	n/a	C24 H25 N4 35Cl2

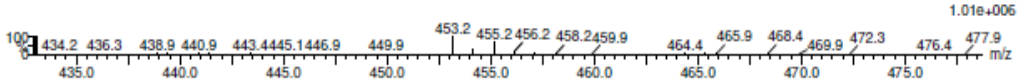
TOF MS E+ and elemental analysis of 2-(cyclooctyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **19**



Elemental Composition Report **Page 1**

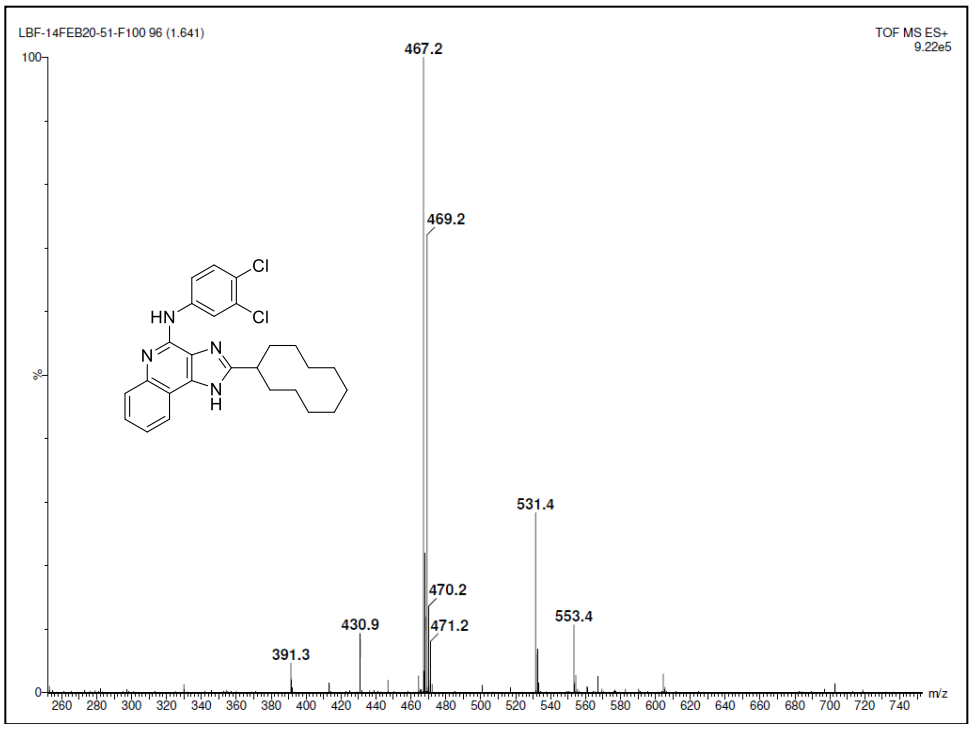
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-08OCT20-F115 222 (3.772) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
453.1616	453.1613	0.3	0.7	13.5	515.0	n/a	n/a	C25 H27 N4 35Cl2

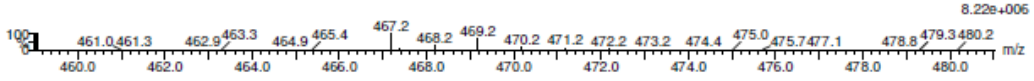
TOF MS E+ and elemental analysis of 2-(cyclononyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **20**



Elemental Composition Report **Page 1**

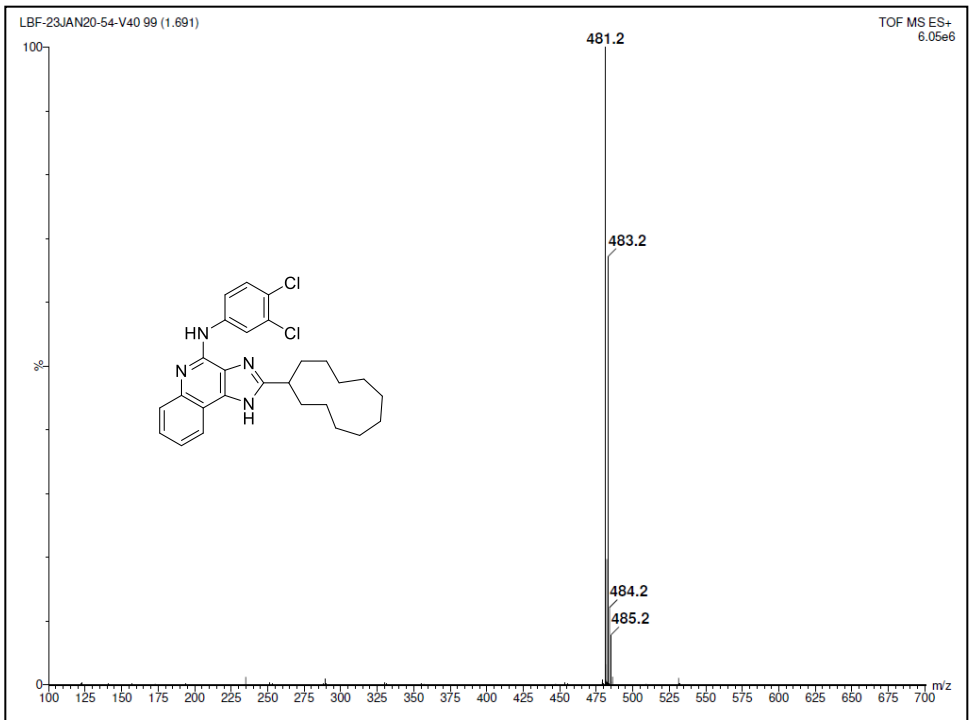
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
69 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 35Cl: 2-2
LBF-14FEB20-51-F100 99 (1.691) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
8.22e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
467.1770	467.1769	0.1	0.2	13.5	580.2	n/a	n/a	C26 H29 N4 35Cl2

TOF MS ES+ and elemental analysis of 2-(cyclodecyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **21**

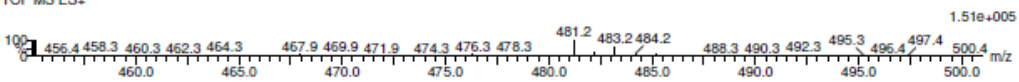


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 8.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

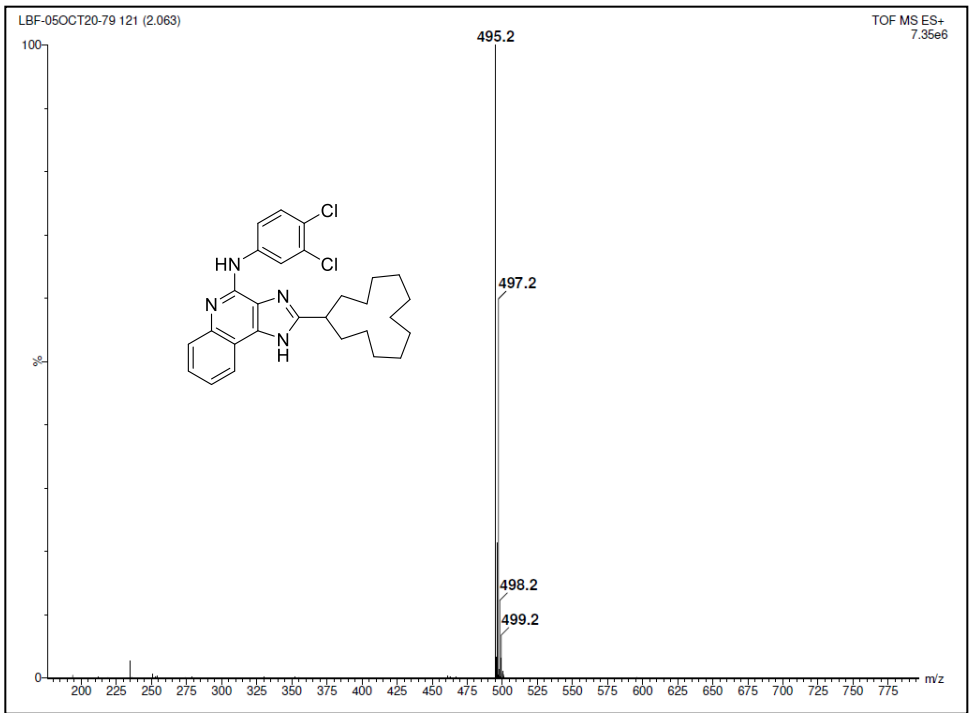
Monoisotopic Mass, Even Electron Ions
77 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 35Cl: 2-2

LBF-23JAN20-54-V40 104 (1.776) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
1.51e+005



Minimum:				-1.5				
Maximum:		8.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
481.1921	481.1926	-0.5	-1.0	13.5	414.5	0.868	41.97	C27 H31 N4 35Cl2
	481.1985	-6.4	-13.3	4.5	414.2	0.544	58.03	C20 H35 N4 O5 35Cl2

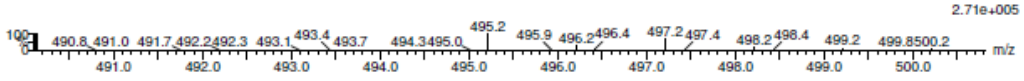
TOF MS ES+ and elemental analysis of 2-(cycloundecyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **22**



Elemental Composition Report **Page 1**

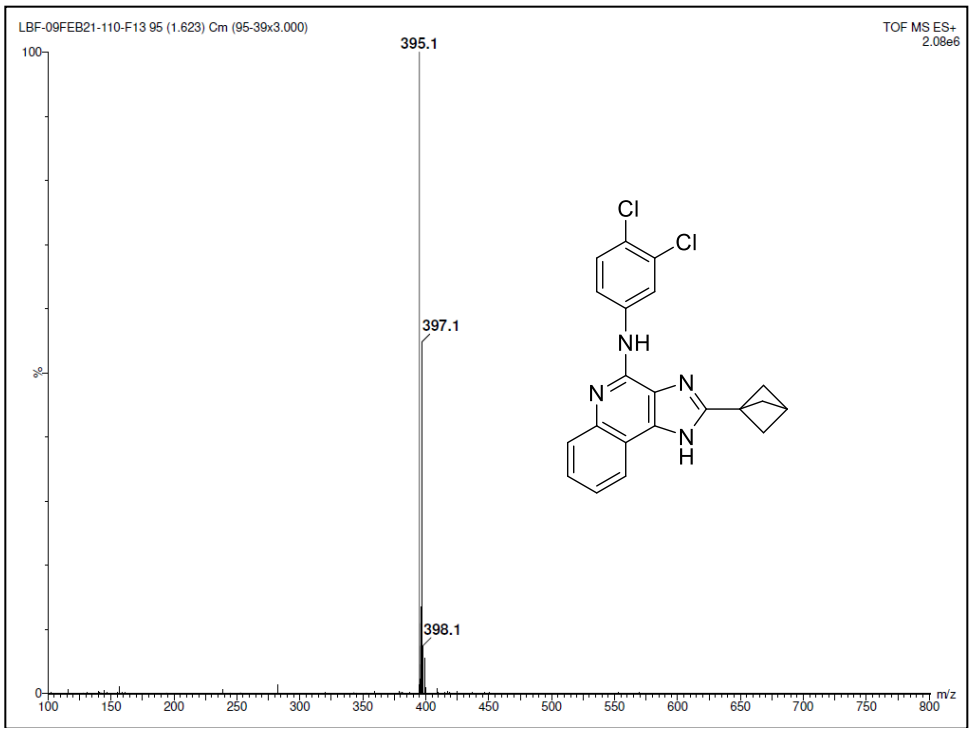
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
78 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-05OCT20-79 126 (2.148) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
495.2085	495.2082	0.3	0.6	13.5	437.1	n/a	n/a	C28 H33 N4 35Cl2

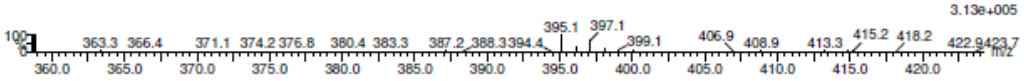
TOF MS ES+ and elemental analysis of 2-(cyclododecyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **23**



Elemental Composition Report **Page 1**

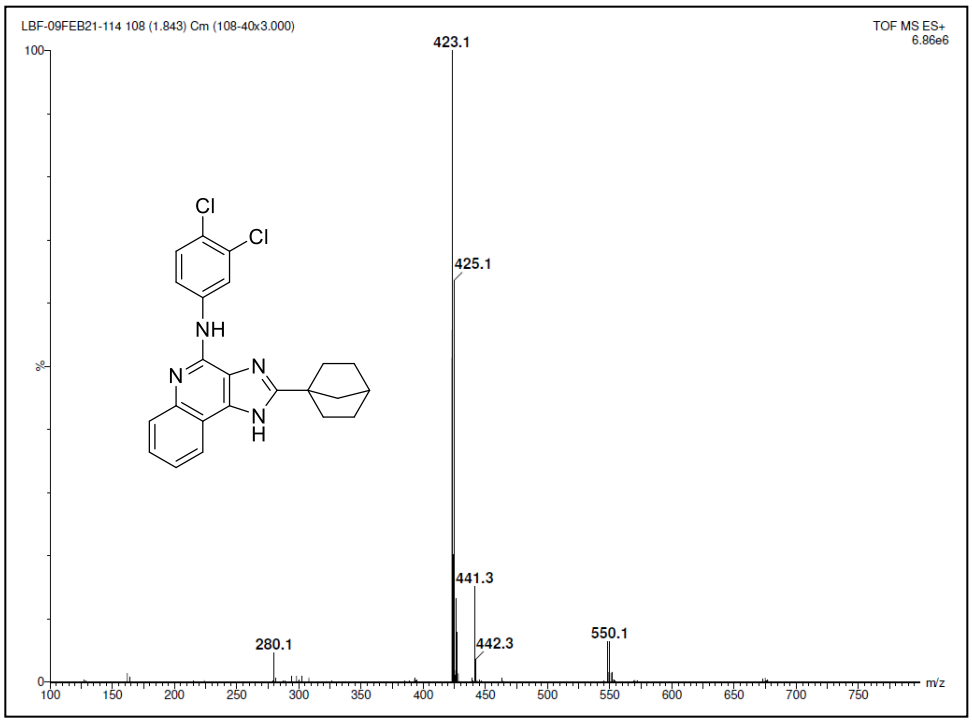
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-09FEB21-110-F13 103 (1.759) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
3.13e+005



Minimum:				-1.5				
Maximum:		5.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
395.0835	395.0830	0.5	1.3	14.5	528.2	n/a	n/a	C21 H17 N4 35C12

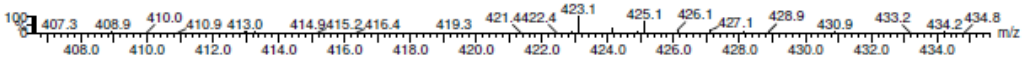
TOF MS ES+ and elemental analysis of 2-(bicyclo[1.1.1]heptan-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **24**



Elemental Composition Report **Page 1**

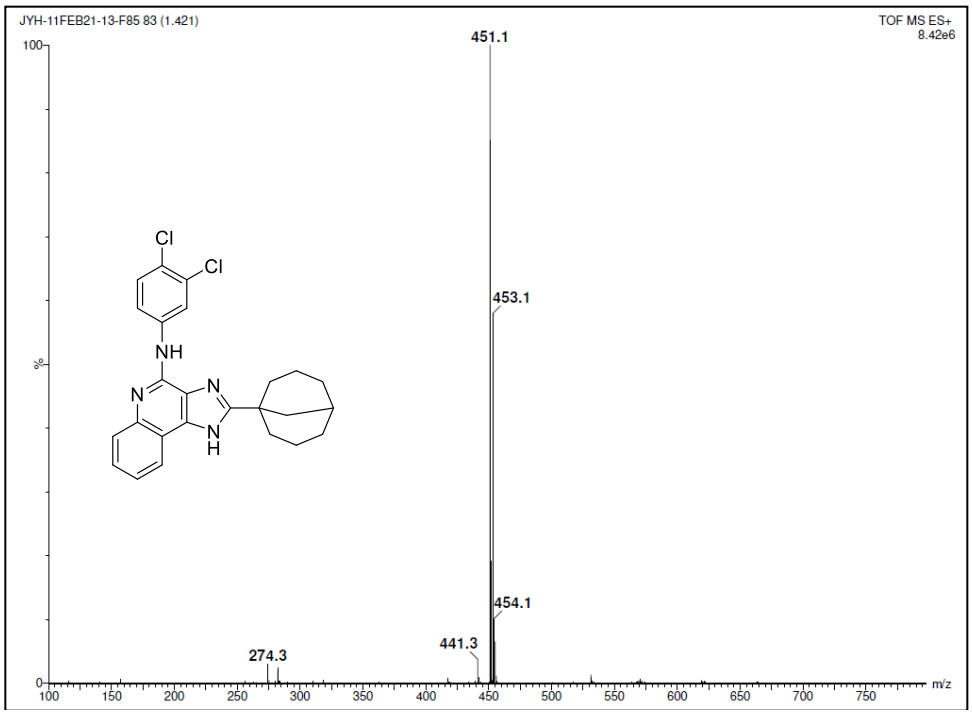
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
55 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-09FEB21-114 117 (1.996) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
7.76e+005



Minimum:				-1.5				
Maximum:		5.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
423.1140	423.1143	-0.3	-0.7	14.5	543.9	n/a	n/a	C23 H21 N4 35Cl2

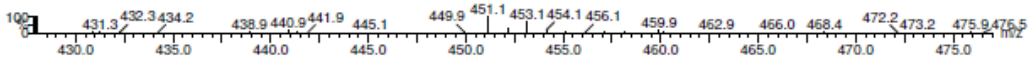
TOF MS E+ and elemental analysis of 2-(bicyclo[2.2.1]heptan-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **25**



Elemental Composition Report **Page 1**

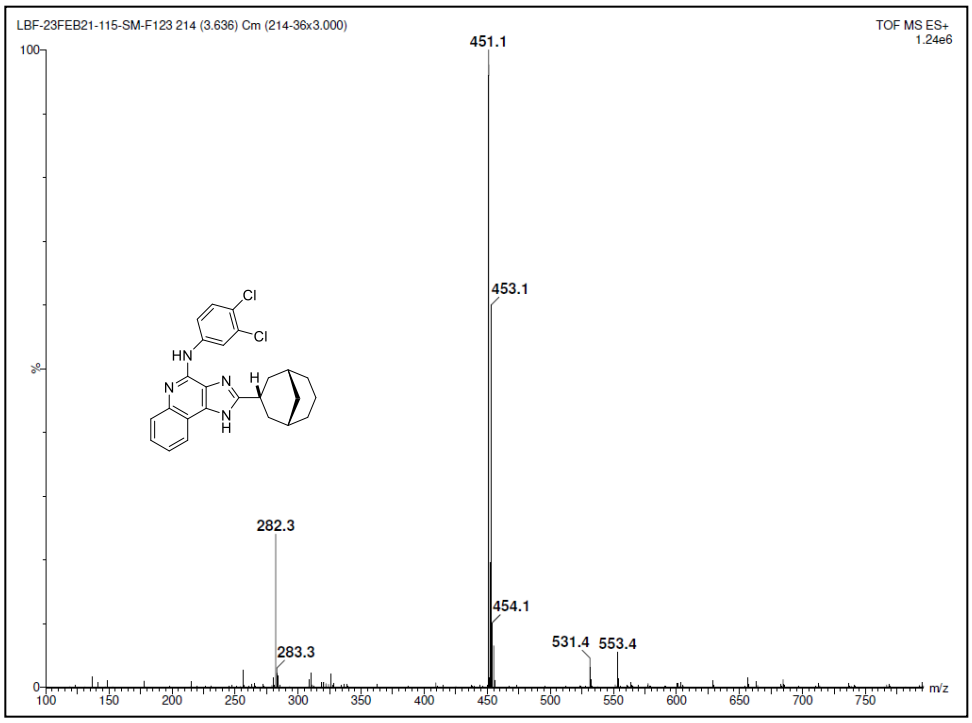
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
JYH-11FEB21-13-F85 157 (2.672) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
2.75e+005



Minimum:				-1.5				
Maximum:		5.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
451.1452	451.1456	-0.4	-0.9	14.5	428.8	n/a	n/a	C25 H25 N4 35C12

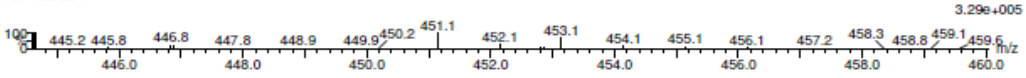
TOF MS ES+ and elemental analysis of 2-(bicyclo[3.3.1]nonan-1-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **26**



Elemental Composition Report **Page 1**

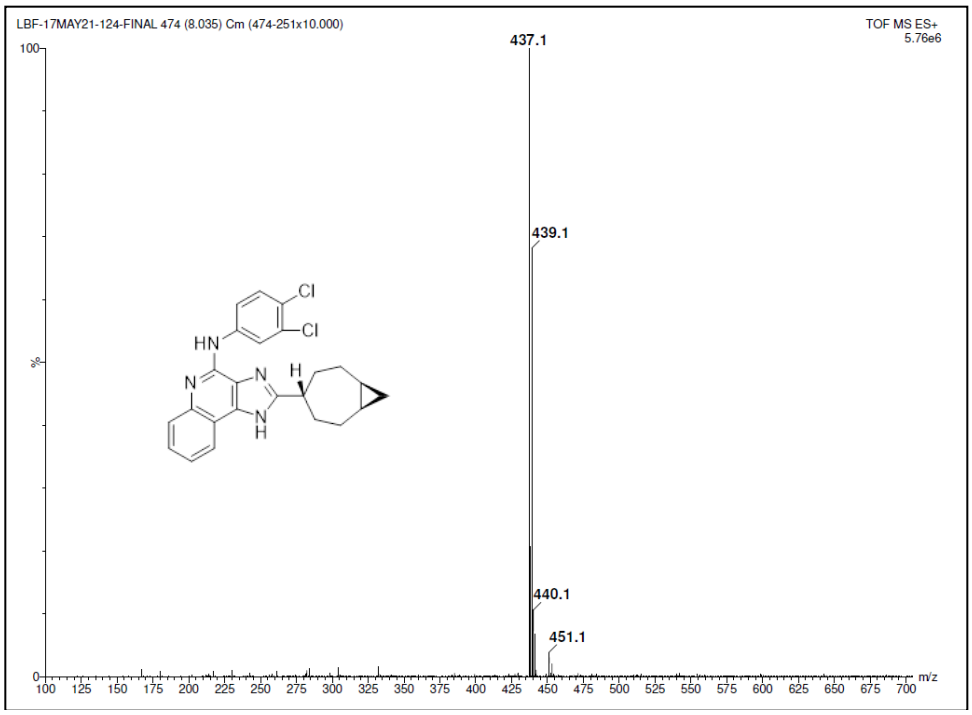
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-23FEB21-115-SM-F123 226 (3.839) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
3.29e+005



Minimum:				-1.5				
Maximum:		5.0	10.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
451.1460	451.1456	0.4	0.9	14.5	474.5	n/a	n/a	C25 H25 N4 35Cl2

TOF MS ES+ and elemental analysis of 2-((1R,3s,5S)-bicyclo[3.3.1]nonan-3-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **27**

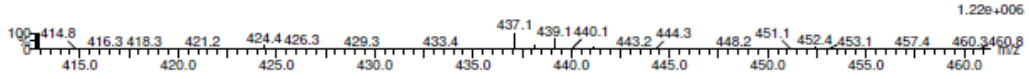


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

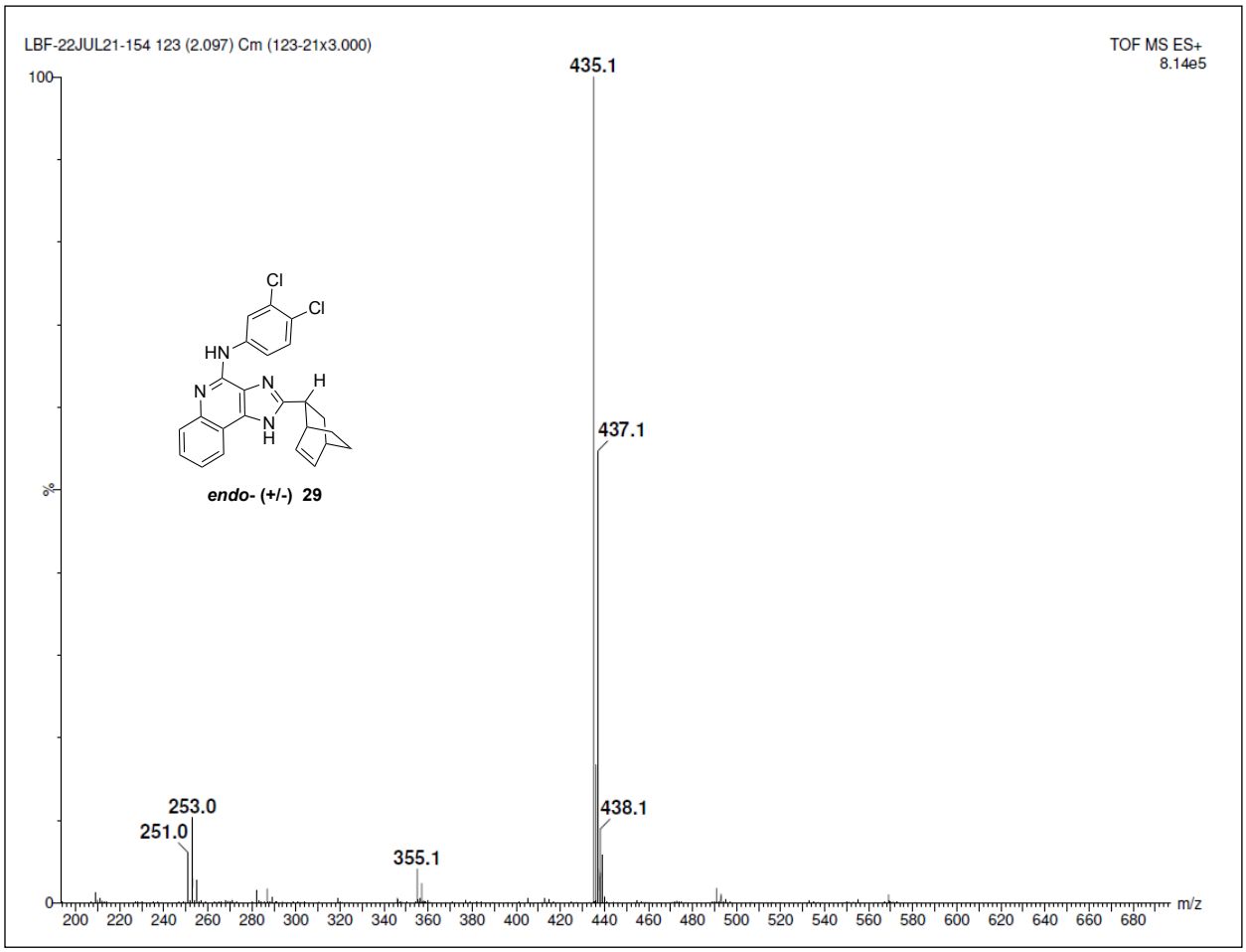
Monoisotopic Mass, Even Electron Ions
63 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2

LBF-17MAY21-124-FINAL 479 (8.119) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
1.22e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
437.1304	437.1300	0.4	0.9	14.5	476.3	n/a	n/a	C24 H23 N4 35Cl2

TOF MS ES+ and elemental analysis of 2-((1R,4r,7S)-bicyclo[5.1.0]octan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **28**

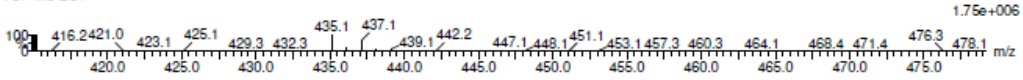


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

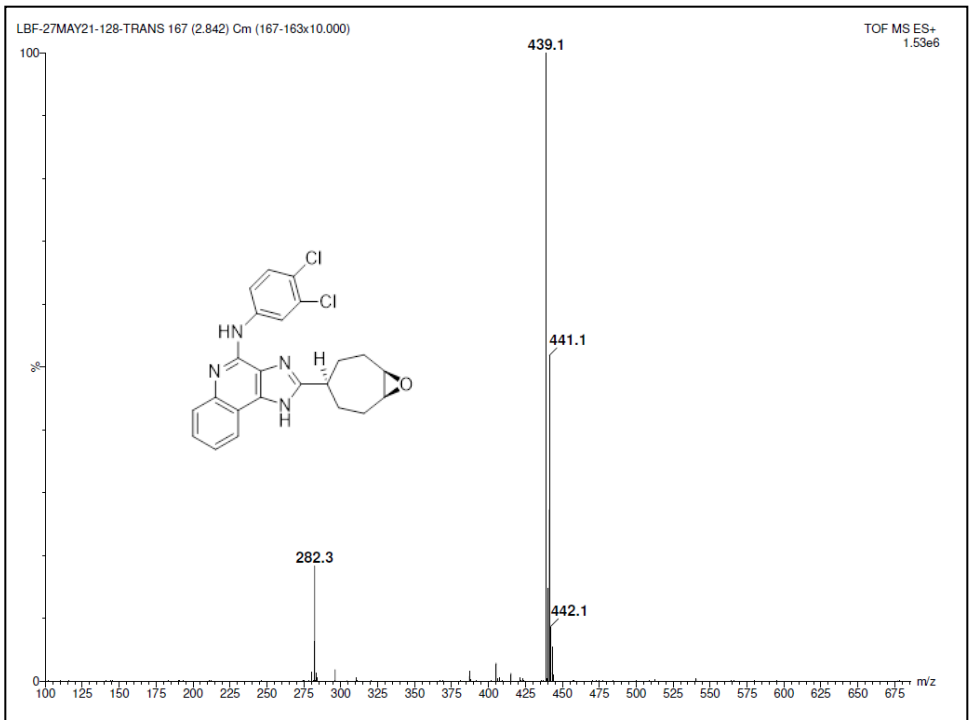
Monoisotopic Mass, Even Electron Ions
59 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-20 35Cl: 2-2

LBF-22JUL21-154 116 (1.979) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
1.75e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
435.1136	435.1143	-0.7	-1.6	15.5	509.0	n/a	n/a	C24 H21 N4 35Cl2

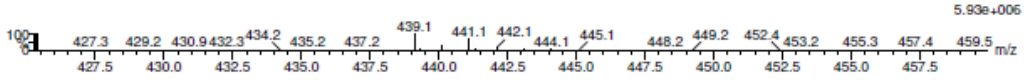
TOF MS ES+ and elemental analysis of 2-((1R,2R,4R) & (1S,2S,4S)-bicyclo[2.2.2]oct-5-en-2-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **29**



Elemental Composition Report **Page 1**

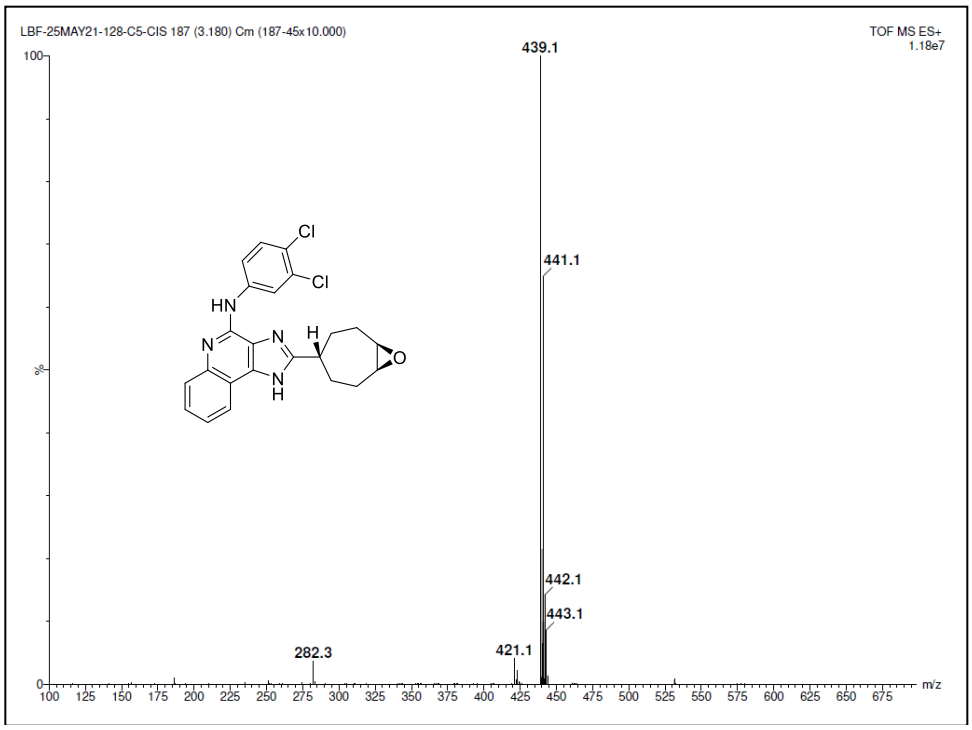
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
60 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-27MAY21-128-TRANS 166 (2.825) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
5.93e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
439.1093	439.1092	0.1	0.2	14.5	716.2	n/a	n/a	C23 H21 N4 O 35Cl2

TOF MS ES+ and elemental analysis of 2-((1R,4r,7S)-8-oxabicyclo[5.1.0]octan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **30**

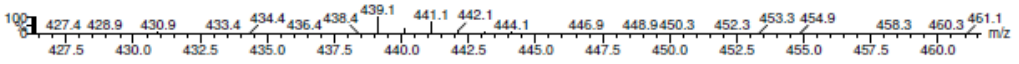


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

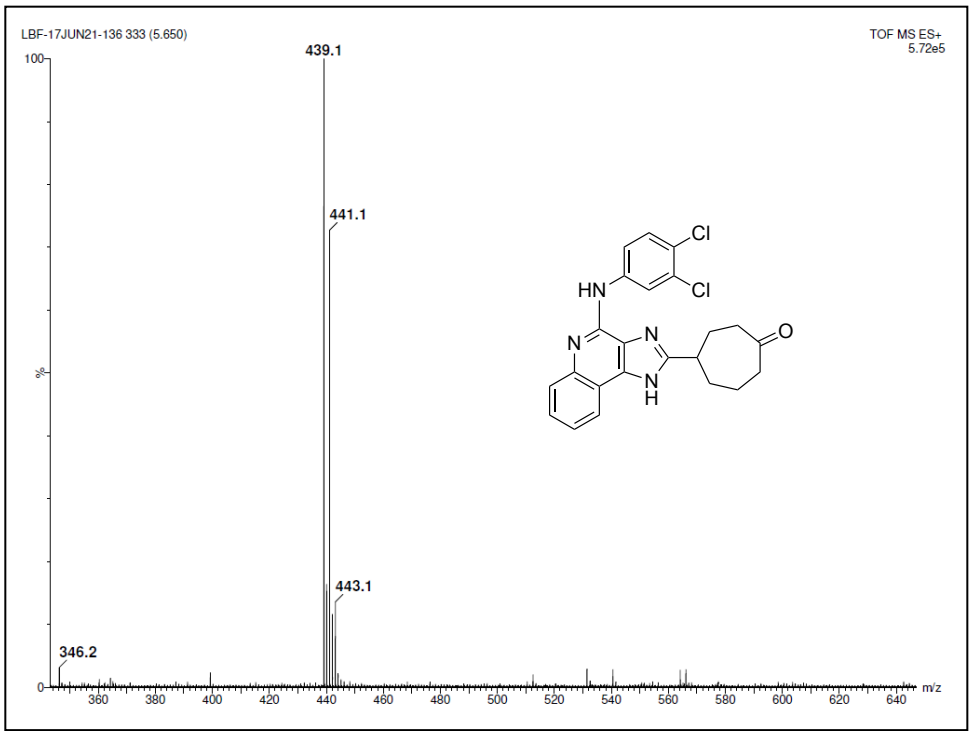
Monoisotopic Mass, Even Electron Ions
60 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-25MAY21-128-C5-CIS 191 (3.248) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+

1.59e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
439.1090	439.1092	-0.2	-0.5	14.5	481.6	n/a	n/a	C23 H21 N4 O 35Cl2

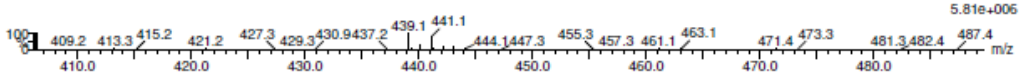
TOF MS ES+ and elemental analysis of 2-((1R,4s,7S)-8-oxabicyclo[5.1.0]octan-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **31**



Elemental Composition Report **Page 1**

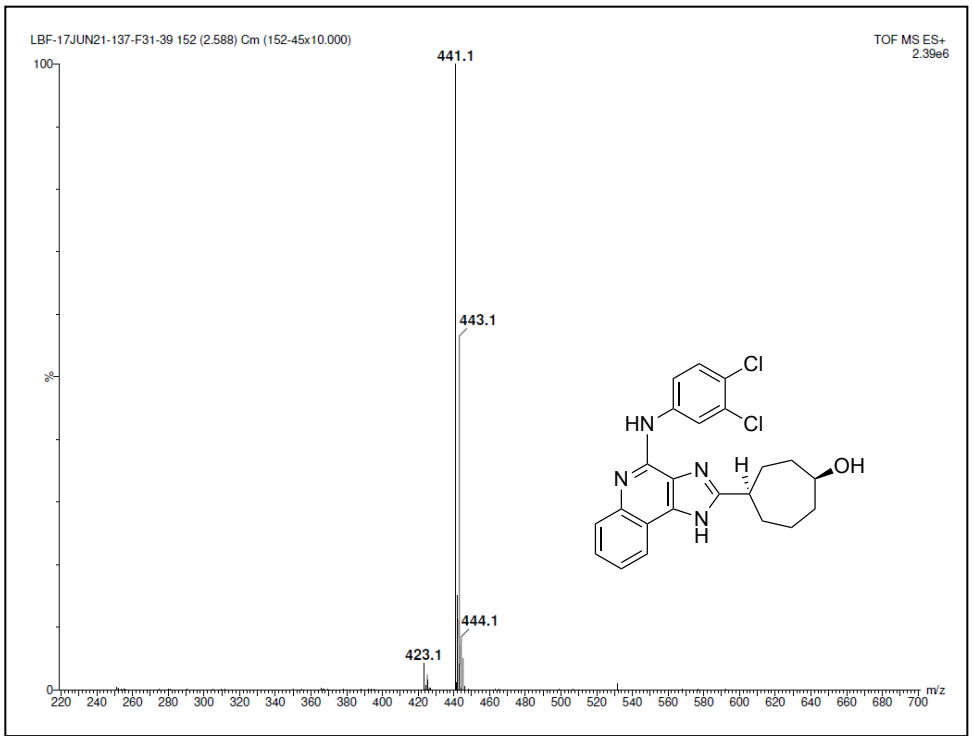
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
60 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-17JUN21-136 329 (5.582) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
5.81e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
439.1100	439.1092	0.8	1.8	14.5	626.8	n/a	n/a	C23 H21 N4 O 35Cl2

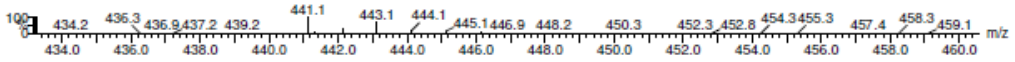
TOF MS ES+ and elemental analysis of (R)- & (S)-4-(4-((3,4-dichlorophenyl)amino)-1*H*-imidazo[4,5-c]quinolin-2-yl)cycloheptan-1-one – Compound **32**



Elemental Composition Report **Page 1**

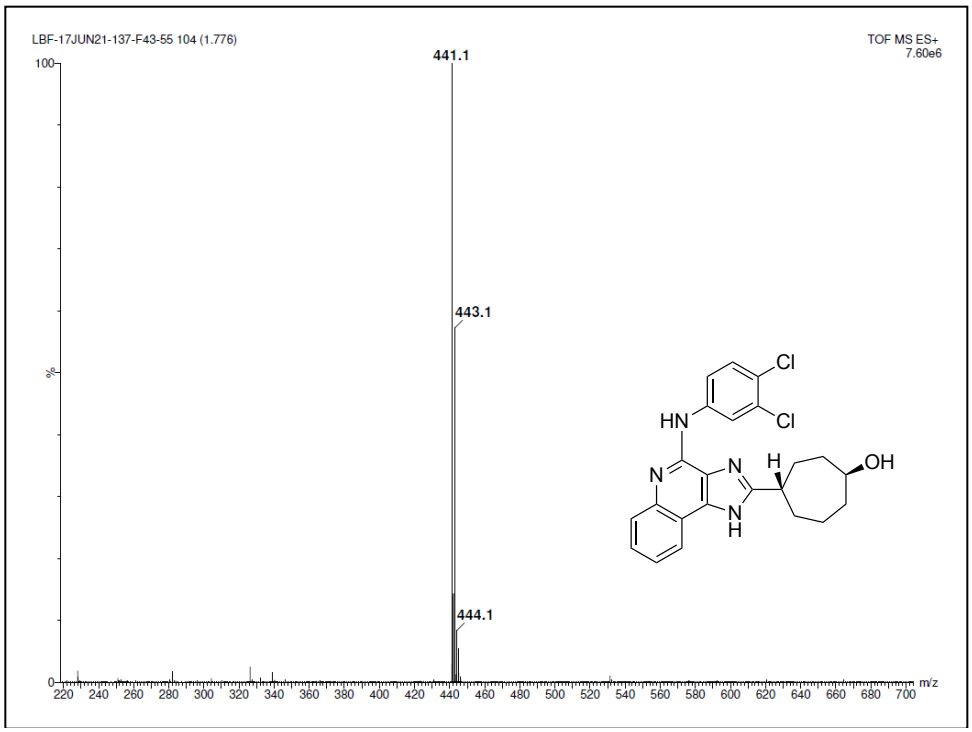
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-17JUN21-137-F31-39 152 (2.588) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
5.95e+006



	Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:					-1.5				
Maximum:			5.0	5.0	100.0				
	441.1247	441.1249	-0.2	-0.5	13.5	577.2	n/a	n/a	C23 H23 N4 O 35Cl2

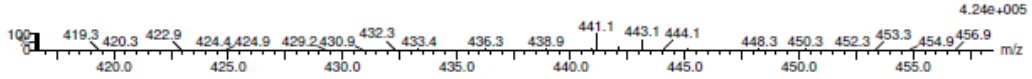
TOF MS ES+ and elemental analysis (1R,4S)- & (1S,4R)-4-(4-((3,4-dichlorophenyl)amino)-1*H*-imidazo[4,5-c]quinolin-2-yl)cycloheptan-1-ol – Compound **33**



Elemental Composition Report **Page 1**

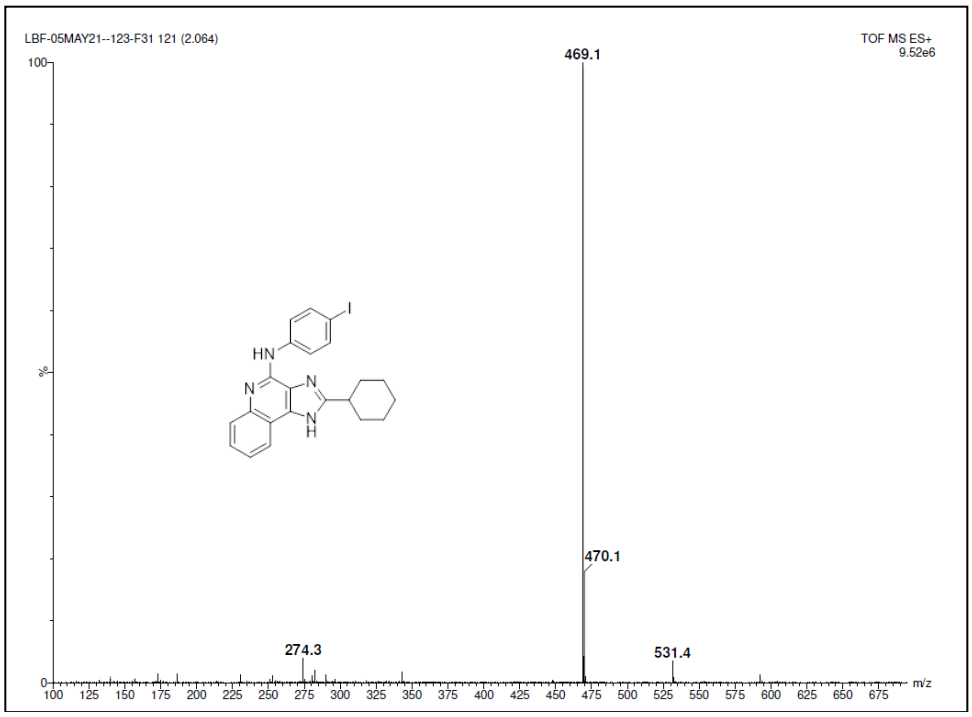
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 35Cl: 2-2
LBF-17JUN21-137-F43-55 116 (1.979) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
441.1252	441.1249	0.3	0.7	13.5	430.9	n/a	n/a	C23 H23 N4 O 35Cl2

TOF MS ES+ and elemental analysis of (1R,4R)-, & (1S,4S)-4-(4-((3,4-dichlorophenyl)amino)-1*H*-imidazo[4,5-c]quinolin-2-yl)cycloheptan-1-ol – Compound **34**

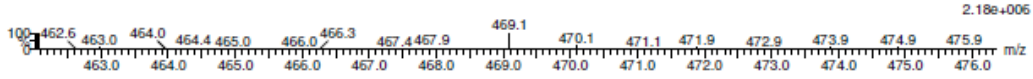


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

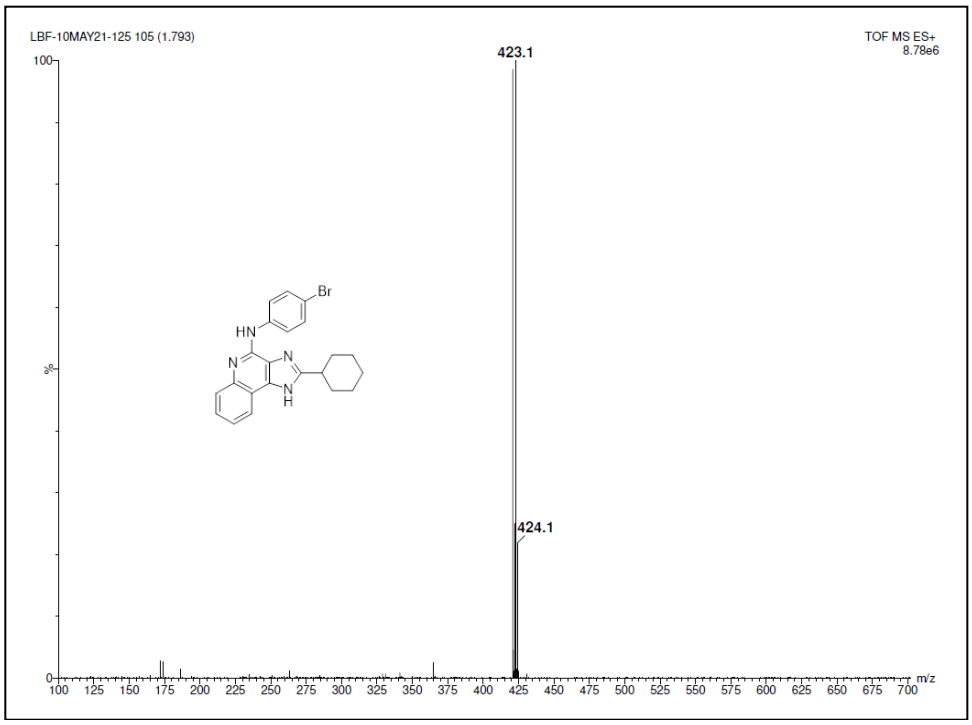
Monoisotopic Mass, Even Electron Ions
51 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 I: 1-1

LBF-05MAY21--123-F31 131 (2.233) AM2 (Ar,25000.0,0.00,0.00); ABS; Cm (131:140)
TOF MS ES+
2.18e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
469.0898	469.0889	0.9	1.9	13.5	354.4	0.434	64.76	C22 H22 N4 I
	469.0948	-5.0	-10.7	4.5	355.1	1.043	35.24	C15 H26 N4 O5 I

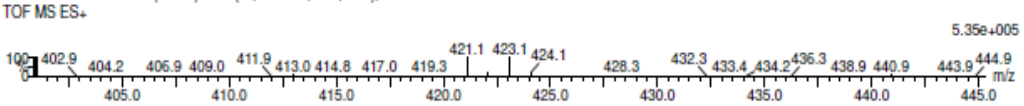
TOF MS ES+ and elemental analysis of 2-cyclohexyl-*N*-(4-iodophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **35**



Elemental Composition Report **Page 1**

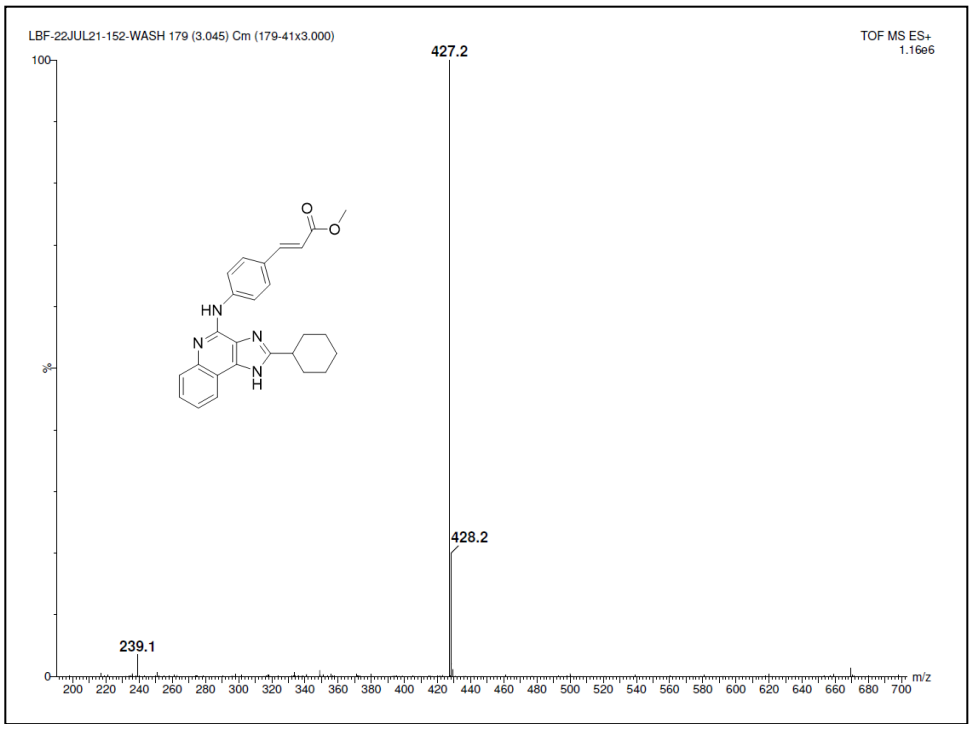
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
51 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-50 79Br: 1-1
LBF-10MAY21-125 121 (2.064) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
421.1029	421.1028	0.1	0.2	13.5	418.9	n/a	n/a	C22 H22 N4 79Br

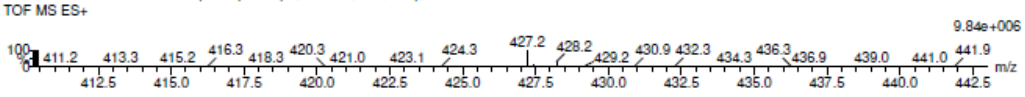
TOF MS ES+ and elemental analysis of 2-cyclohexyl-*N*-(4-bromophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **36**



Elemental Composition Report **Page 1**

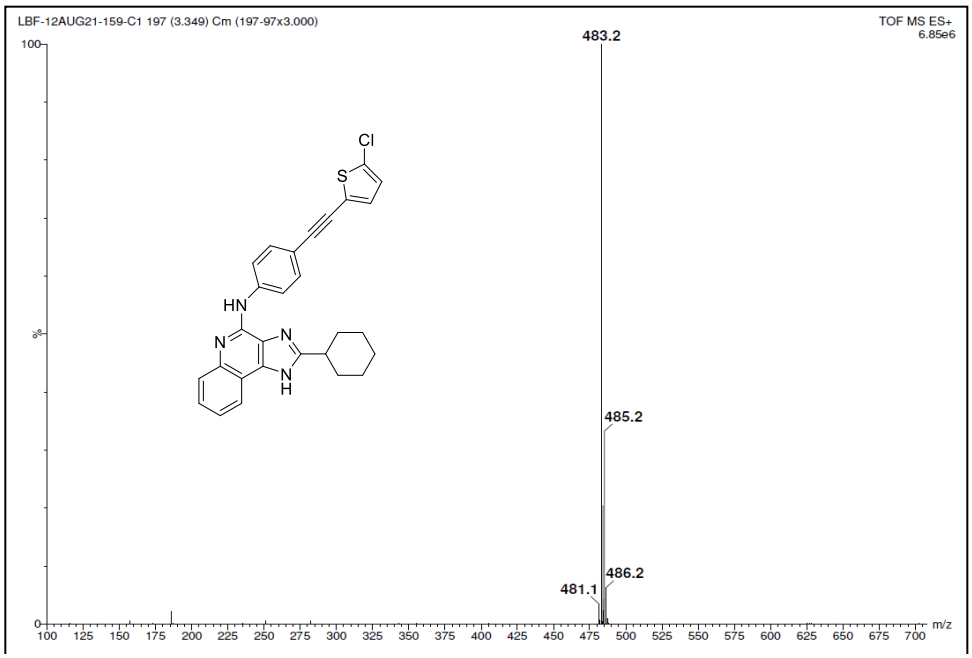
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
82 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-20
LBF-22JUL21-152-WASH 176 (2.994) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
427.2133	427.2134	-0.1	-0.2	15.5	492.6	n/a	n/a	C26 H27 N4 O2

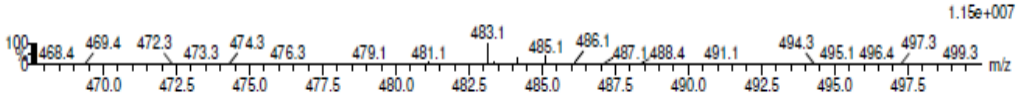
TOF MS ES+ and elemental analysis of methyl (E)- & (Z)-3-(4-((2-cyclohexyl-1H-imidazo[4,5-c]quinolin-4-yl)amino)phenyl)acrylate – Compound **37**



Elemental Composition Report **Page 1**

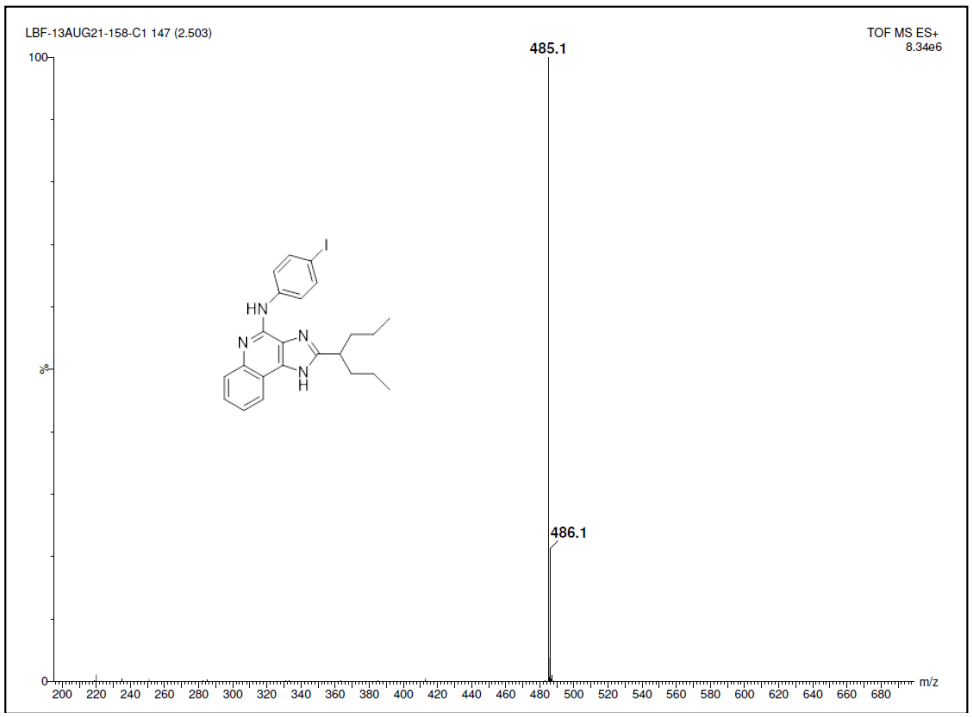
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
76 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-20 32S: 1-1 35Cl: 1-1
LBF-12AUG21-159-C1 193 (3.281) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
483.1412	483.1410	0.2	0.4	18.5	516.3	n/a	n/a	C28 H24 N4 32S 35Cl

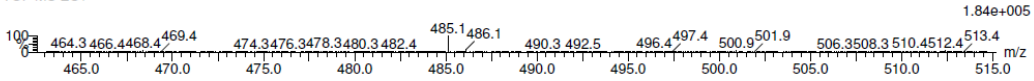
TOF MS ES+ and elemental analysis of 2-cyclohexyl-*N*-(4-((5-chlorothiophen-2-yl)ethynyl)phenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **38**



Elemental Composition Report **Page 1**

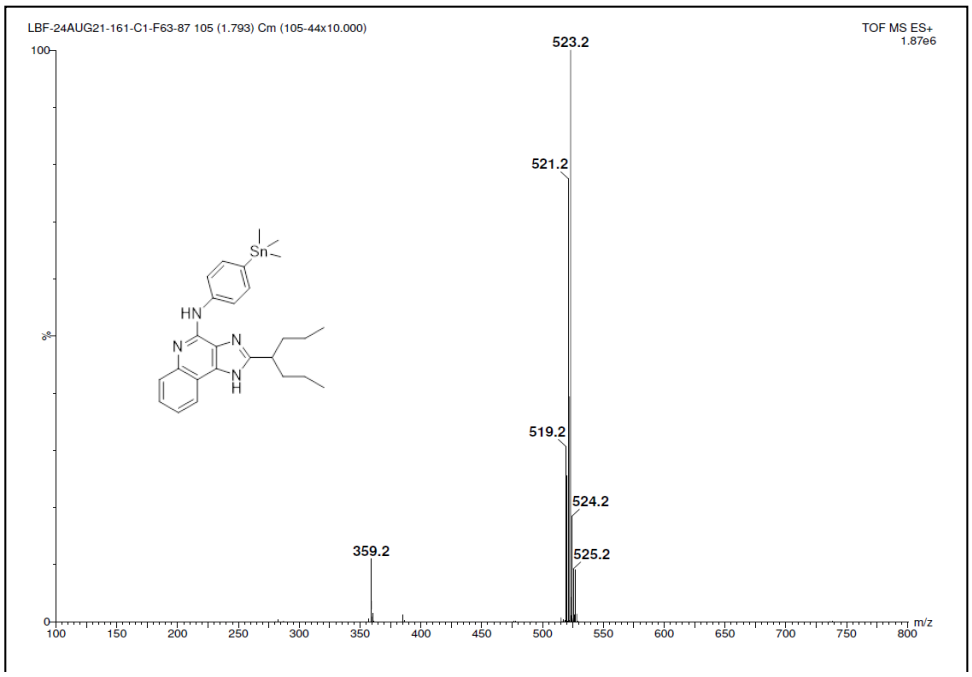
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-20 I: 1-1
LBF-13AUG21-158-C1 151 (2.571) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
485.1200	485.1202	-0.2	-0.4	12.5	329.6	n/a	n/a	C23 H26 N4 I

TOF MS ES+ and elemental analysis of 2-(heptan-4-yl)-*N*-(4-iodophenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **39**

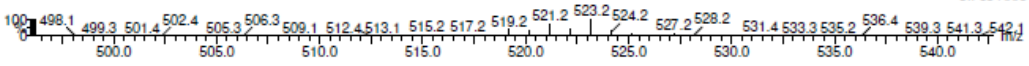


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

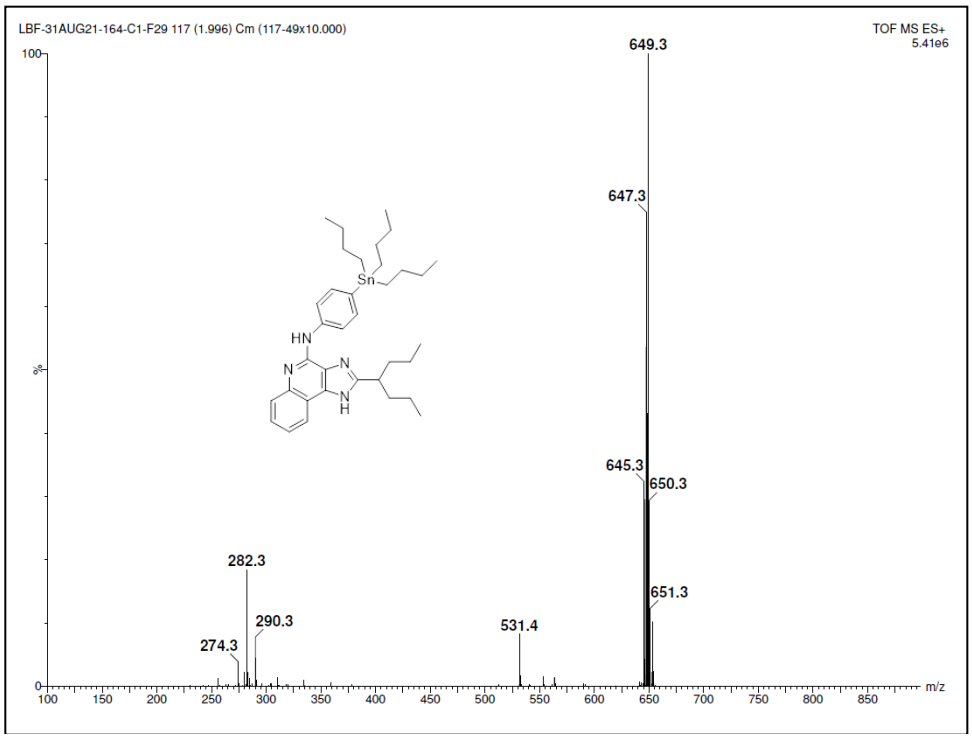
Monoisotopic Mass, Even Electron Ions
149 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-20 Na: 0-1 120Sn: 1-1

LBF-24AUG21-161-C1-F63-87 101 (1.725) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
3.76e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
523.1882	523.1884	-0.2	-0.4	12.5	498.6	1.117	32.72	C26 H35 N4 120Sn
	523.1860	2.2	4.2	9.5	498.6	1.140	31.98	C24 H36 N4 Na 120Sn
	523.1918	-3.6	-6.9	0.5	498.5	1.041	35.30	C17 H40 N4 O5 Na 120Sn

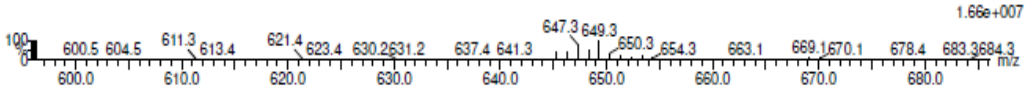
TOF MS ES+ and elemental analysis of 2-(heptan-4-yl)-*N*-(4-(trimethylstannyl)phenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **48**



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

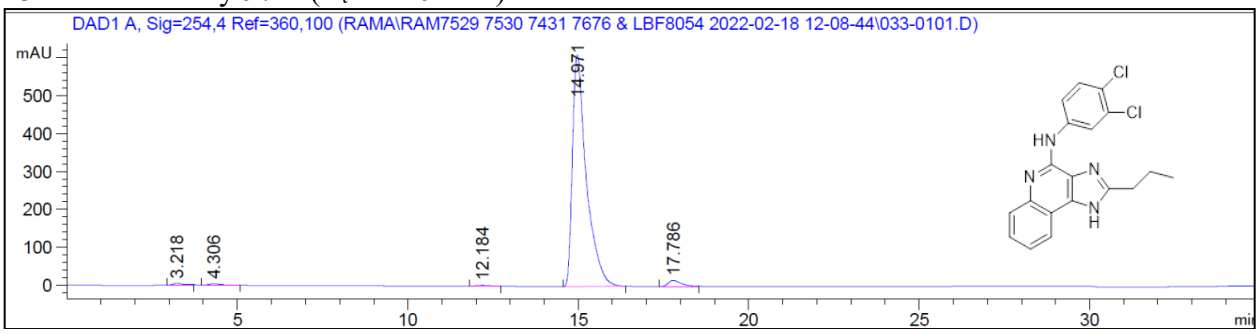
Monoisotopic Mass, Even Electron Ions
107 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-20 120Sn: 1-1
LBF-31AUG21-164-C1-F29 111 (1.894) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+
1.66e+007



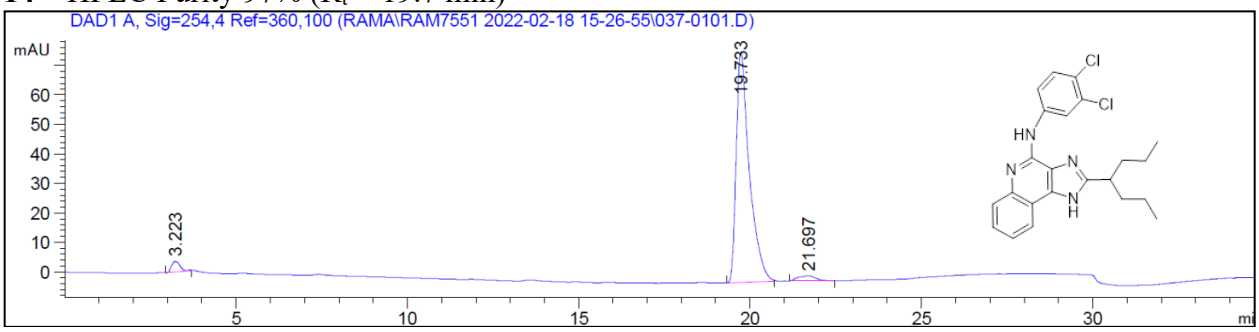
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
649.3293	649.3292	0.1	0.2	12.5	420.8	n/a	n/a	C35 H53 N4 120Sn

TOF MS ES+ and elemental analysis of 2-(heptan-4-yl)-*N*-(4-(tributylstannyl)phenyl)-1*H*-imidazo[4,5-c]quinolin-4-amine – Compound **49**

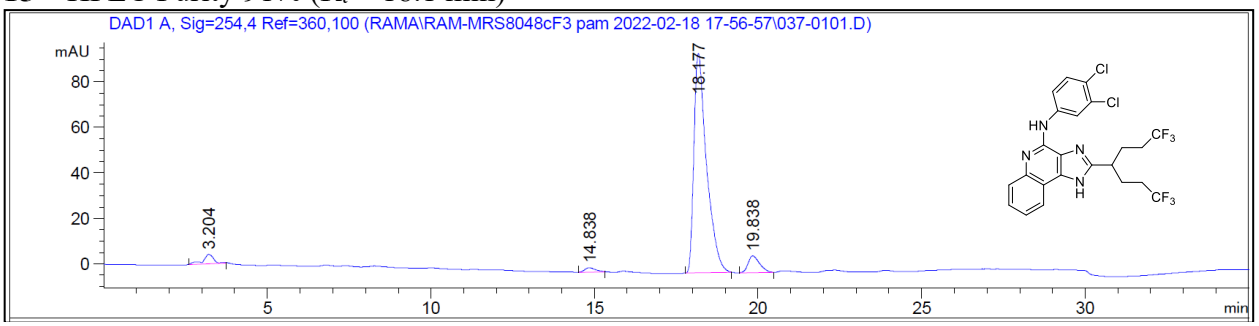
13 – HPLC Purity 97% (R_t = 14.9 min)



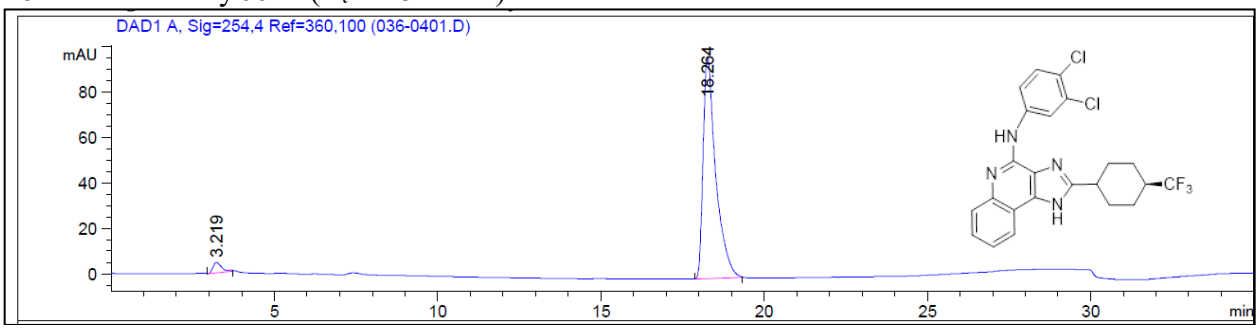
14 – HPLC Purity 97% (R_t = 19.7 min)



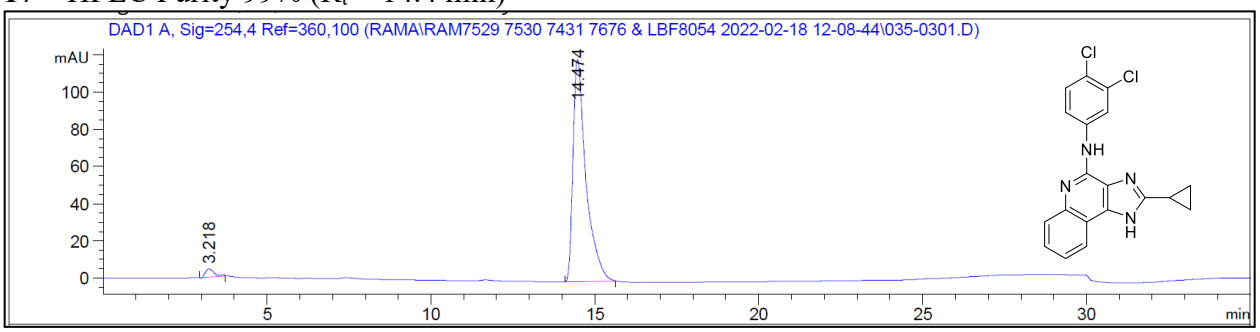
15 – HPLC Purity 91% (R_t = 18.1 min)



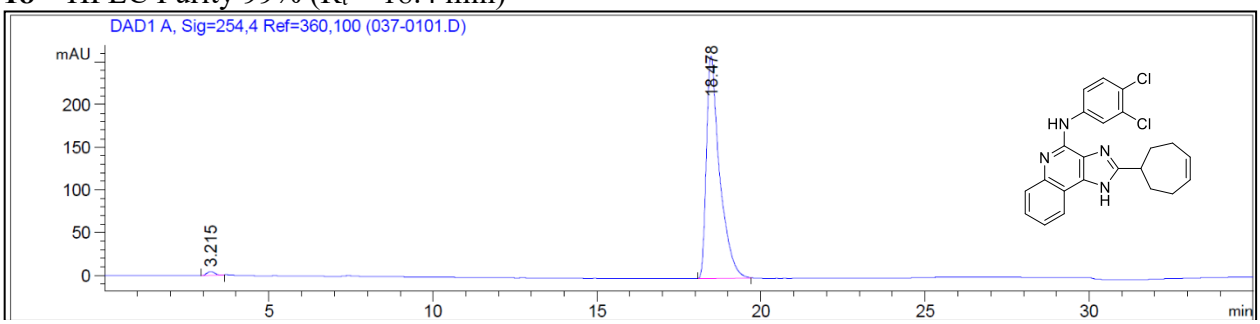
16 – HPLC Purity 99% (R_t = 18.2 min)



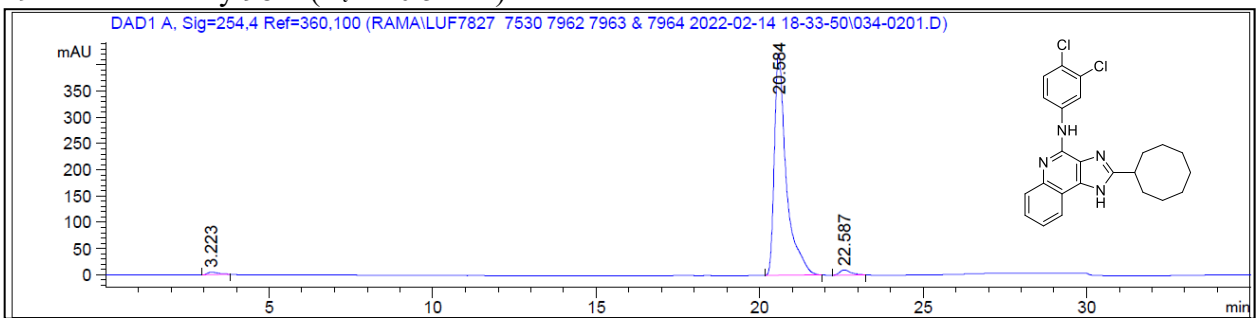
17 – HPLC Purity 99% (R_t = 14.4 min)



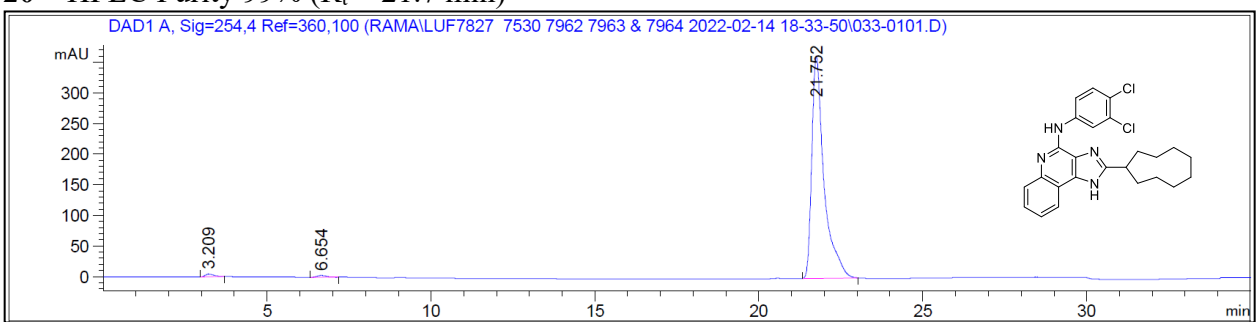
18 – HPLC Purity 99% (R_t = 18.4 min)



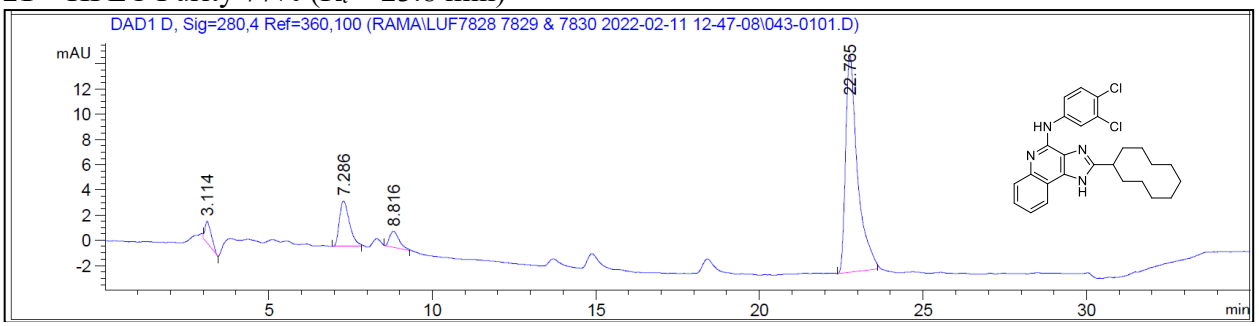
19 – HPLC Purity 98% (R_t = 20.5 min)



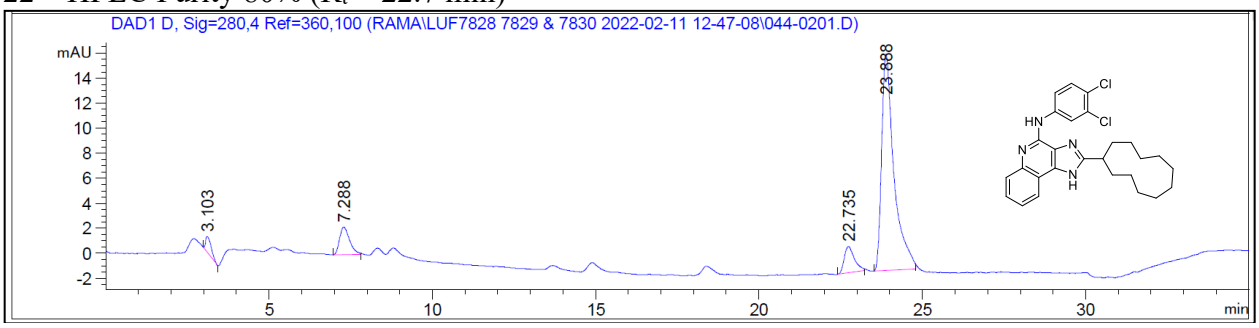
20 – HPLC Purity 99% (R_t = 21.7 min)



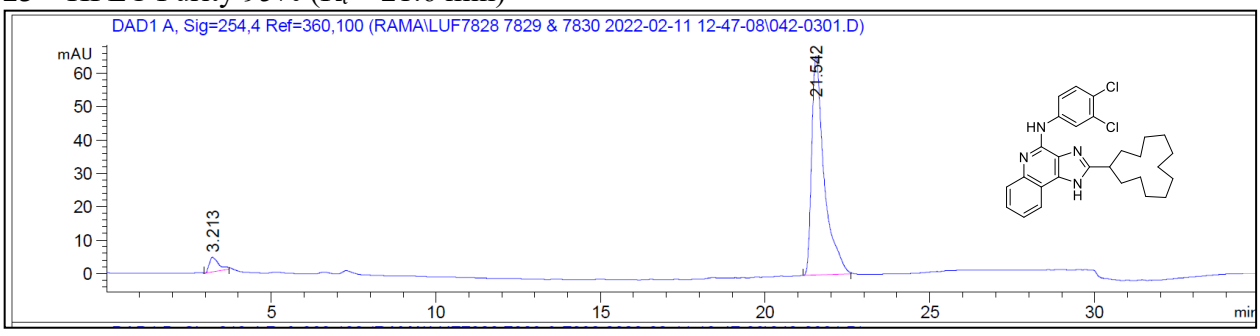
21 – HPLC Purity 77% (R_t = 23.8 min)



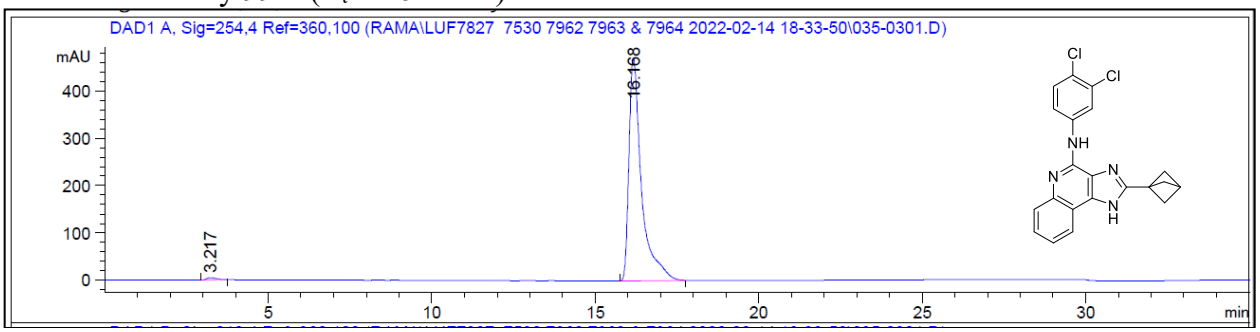
22 – HPLC Purity 80% (R_t = 22.7 min)



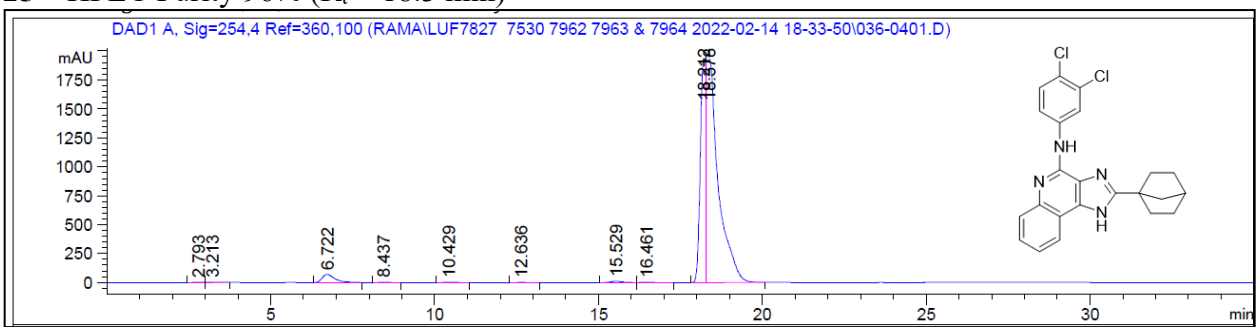
23 – HPLC Purity 95% (R_t = 21.6 min)



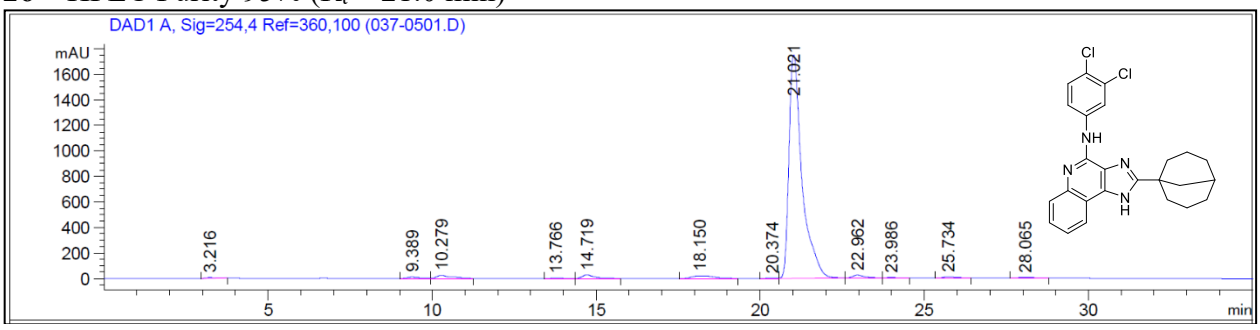
24 – HPLC Purity 99% (R_t = 16.1 min)



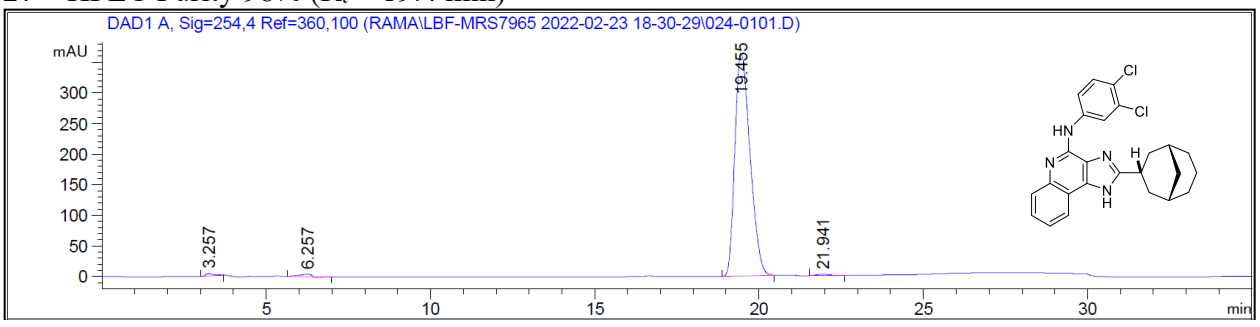
25 – HPLC Purity 96% (R_t = 18.3 min)



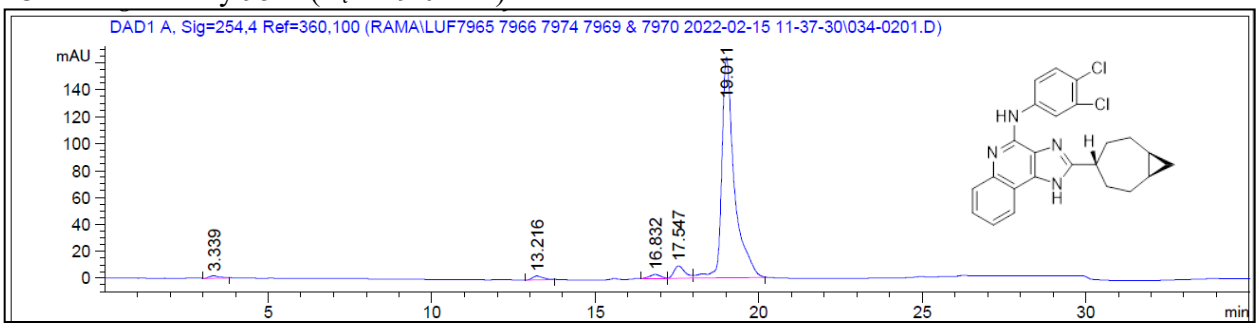
26 – HPLC Purity 95% (R_t = 21.0 min)



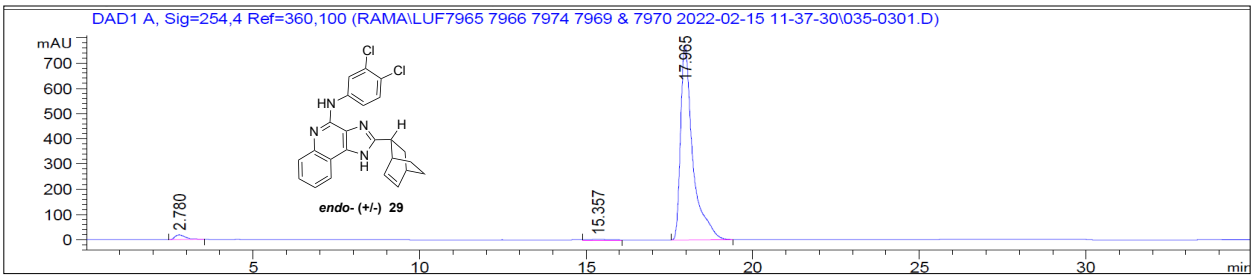
27 – HPLC Purity 98% (R_t = 19.4 min)



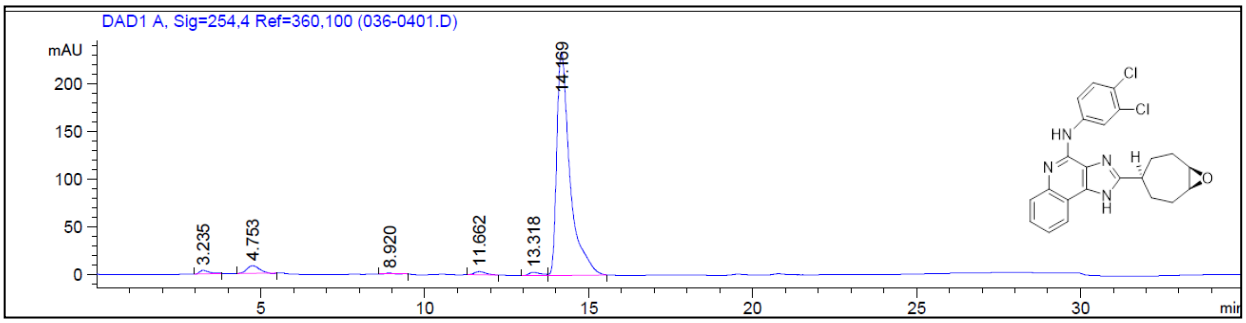
28 – HPLC Purity 95% (R_t = 19.0 min)



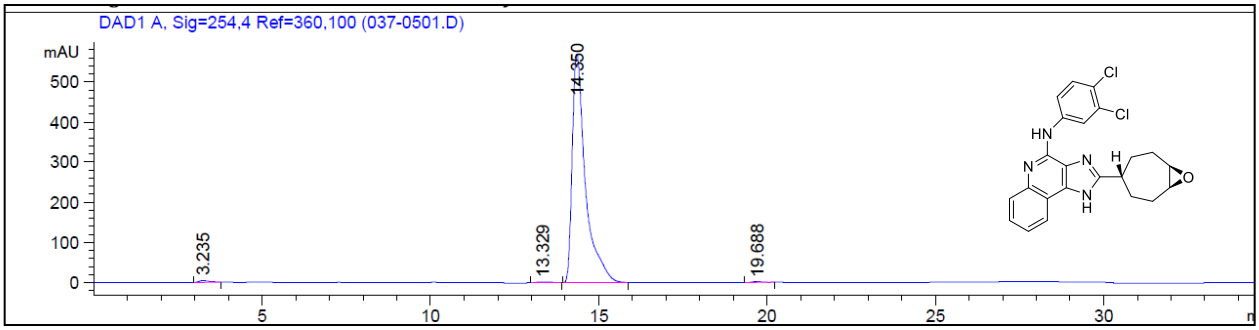
29 – HPLC Purity 99% (R_t = 17.9 min)



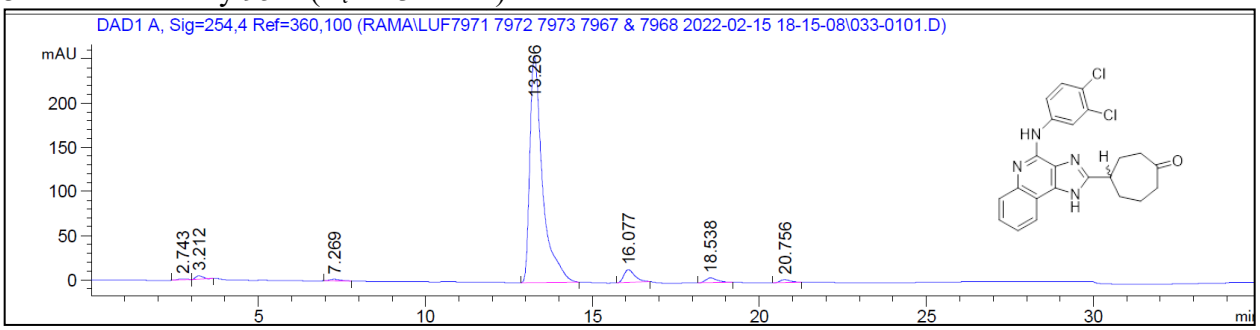
30 – HPLC Purity 96% (R_t = 14.1 min)



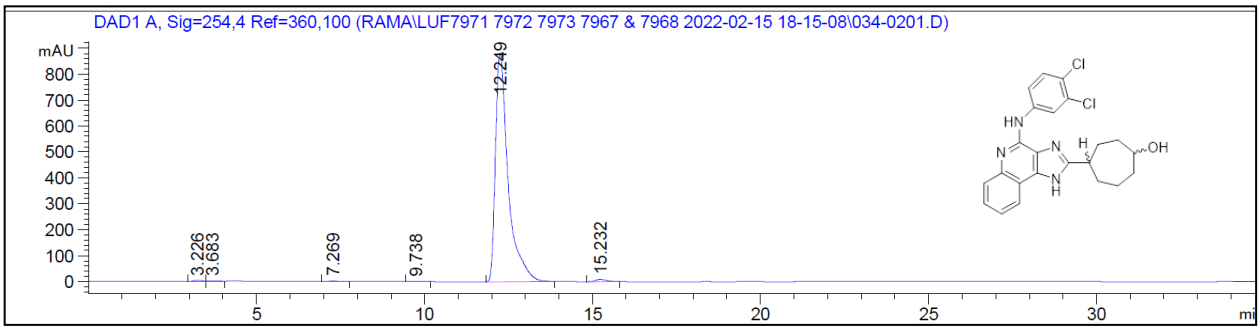
31 – HPLC Purity 99% (R_t = 14.3 min)



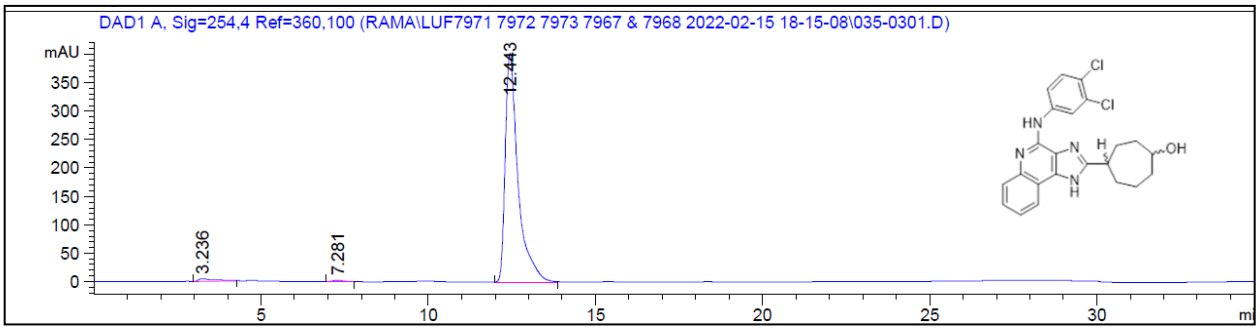
32 – HPLC Purity 95% (R_t = 13.2 min)



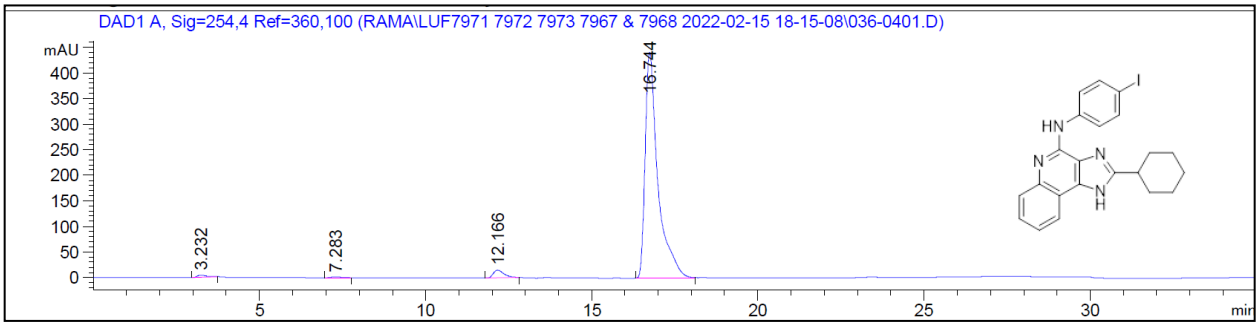
33 – HPLC Purity 95% (R_t = 12.2 min)



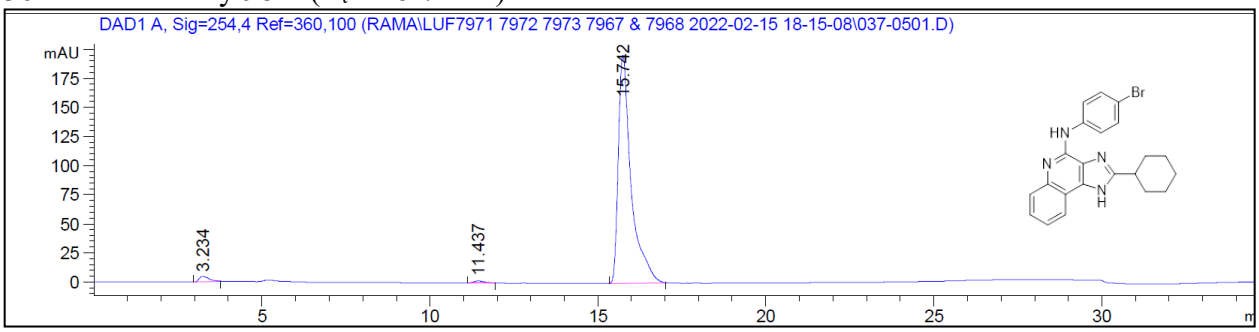
34 – HPLC Purity 98% (R_t = 12.4 min)



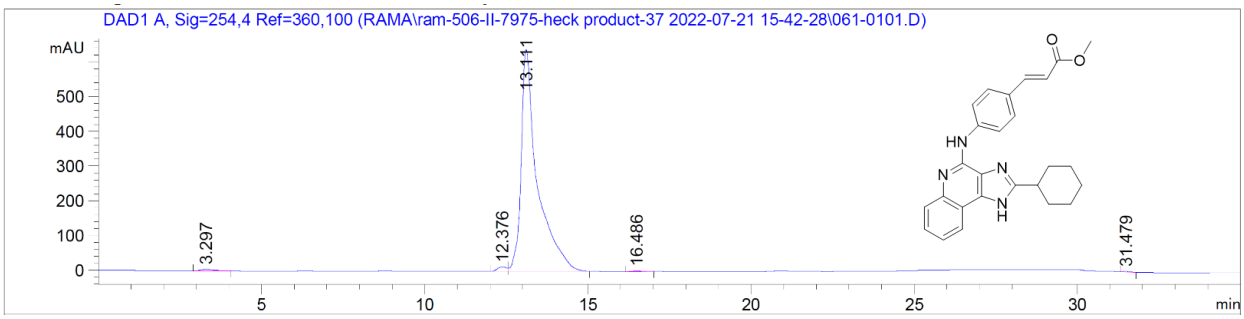
35 – HPLC Purity 96% (R_t = 16.7 min)



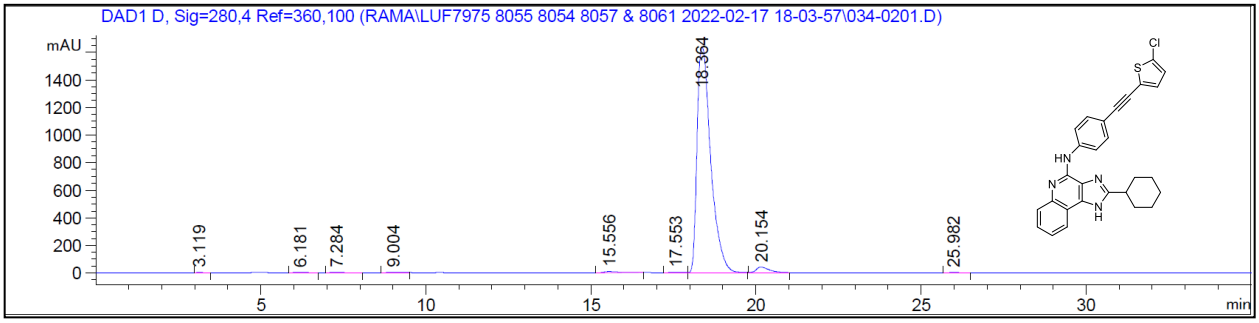
36 – HPLC Purity 98% (R_t = 15.7 min)



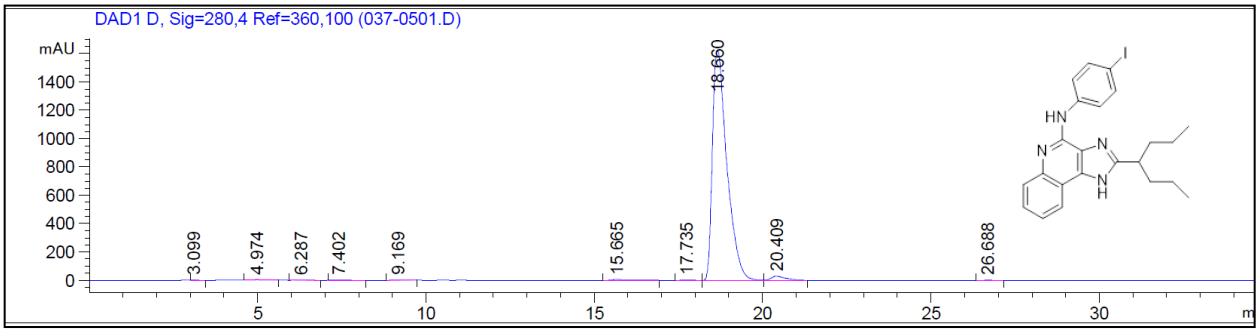
37 – HPLC Purity 98% (Rt = 13.1 min)



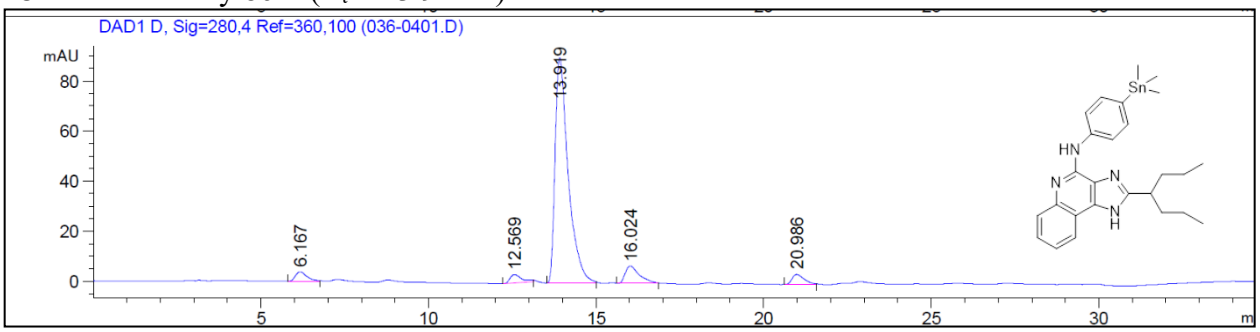
38 – HPLC Purity 96% (R_t = 16.3 min)



39 – HPLC Purity 97% (R_t = 18.6 min)



48 – HPLC Purity 86% (R_t = 13.9 min)



49 – HPLC Purity 95% (R_t = 13.9 min)

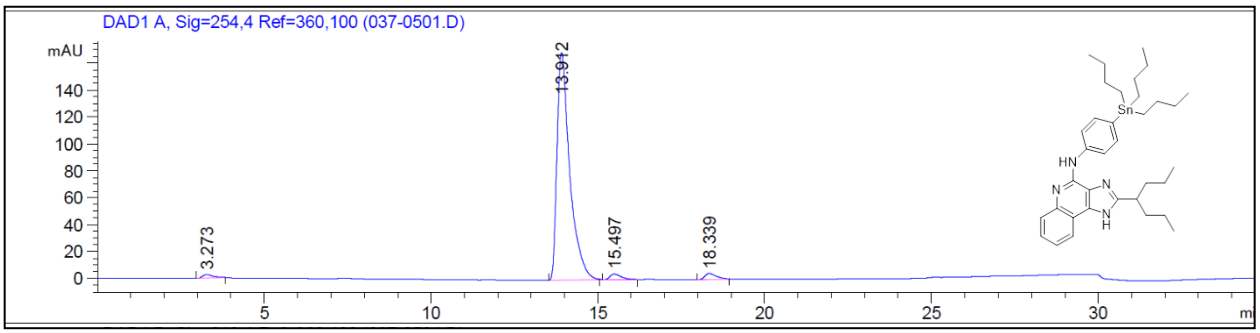
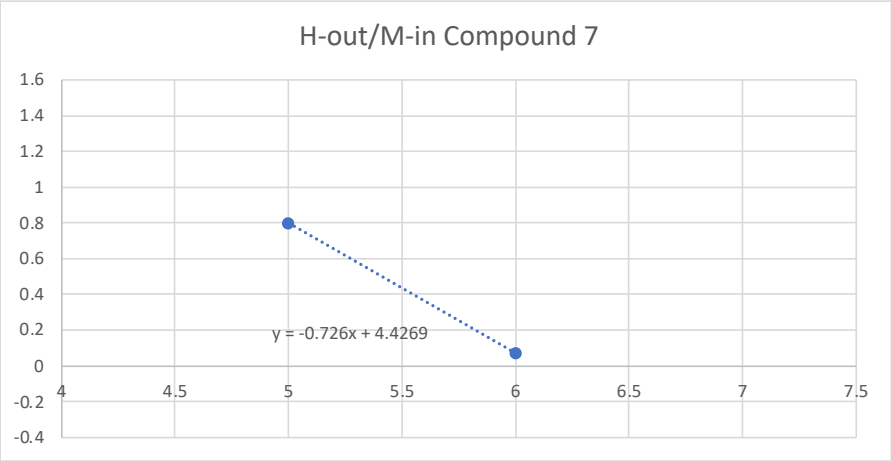
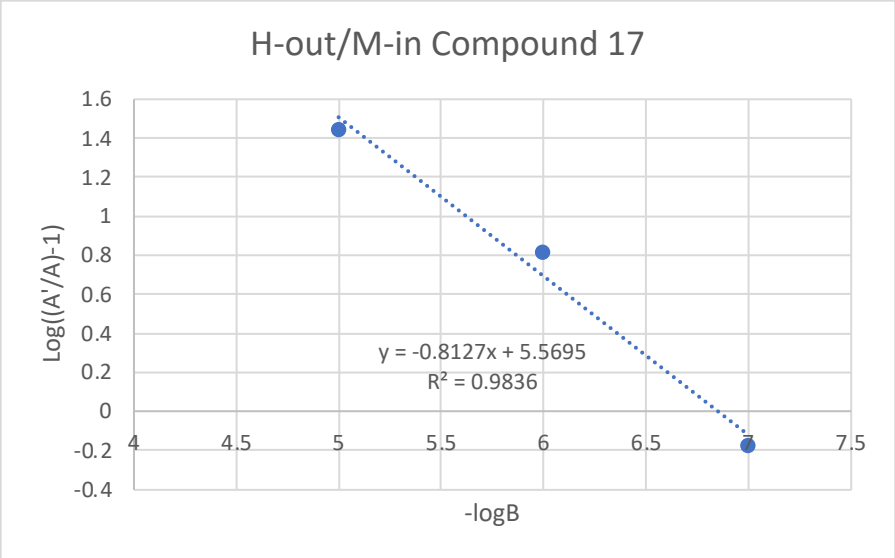


Figure S1A,B. Determination of the affinity of compounds **7** (A) and **17** (B) at the orthosteric site, using Schild analysis of shifts in the Cl-IB-MECA activation curves ([^{35}S]GTPγS binding) of the chimeric human-out/mouse-in A_3AR construct (data from Fisher et al., 2022).[3]

A) X-intercept = 6.0976, corresponds to K_B = 799 nM



B) X-intercept = 6.853, corresponds to K_B = 140 nM



Determination of the affinity of compounds **7**, **14** and **17** at the WT hAR orthosteric sites.

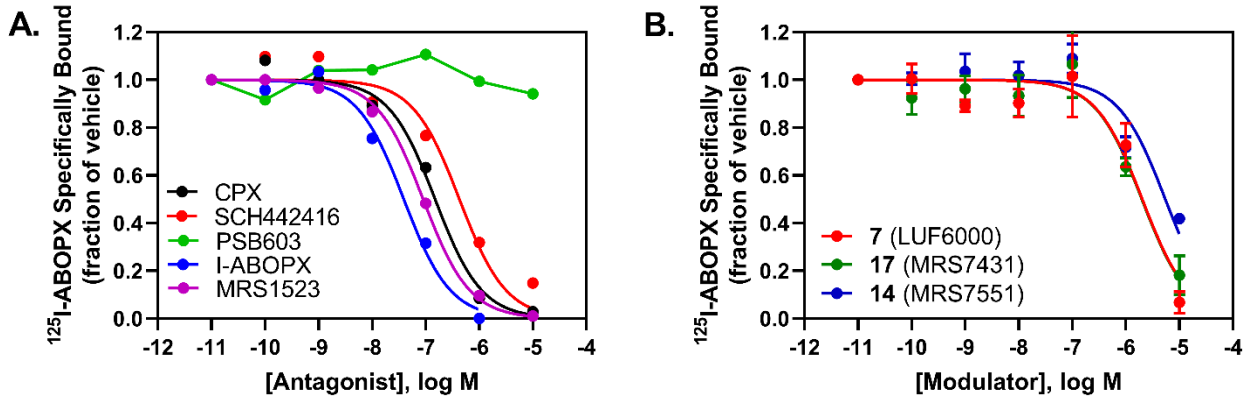


Figure S2. Effects of compounds **7**, **14** and **17** in equilibrium binding assays with the antagonist radioligand ^{125}I-ABOPX (conc. 0.4 nM, K_d 17.3±1.2 nM) and the WT hA$_3$AR. **Panel A** confirms specificity of ^{125}I-ABOPX for the hA$_3$AR based on the antagonist potency order of: I-ABOPX (38) > MRS1523 (89) > DPCPX (149) > SCH442416 (424) >> PSB603, with K_i values (nM, N=1) in parentheses. Propyl 6-ethyl-5-((ethylthio)carbonyl)-2-phenyl-4-propylnicotinate (MRS1523) is a selective A$_3$AR-selective antagonist. **Panel B** shows displacement of ^{125}I-ABOPX specific binding by compounds **7**, **14**, and **17**.

Synthesis of [^{125}I]2-(4-(3-(4-amino-3-iodobenzyl)-2,6-dioxo-1-propyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenoxy)acetic acid (^{125}I-ABOPX) was as described in Linden et al.[5,6] Binding assays were conducted with membranes (75 µg/assay tube) prepared from HEK293 cells transfected with the WT hA$_3$AR in 10 mM Tris buffer (pH 7.4) containing 10 mM Mg^{2+} in the presence of 0.4 nM ^{125}I-ABOPX and 50 nM PSB603 to block binding to A$_{2B}$ARs expressed endogenously in HEK293 cells. The K_i values of known AR antagonist ligands were consistent with binding to the A$_3$AR and in agreement with reported values (DPCPX 261 nM; I-ABOPX, 25.5 nM; and XAC, 23.4 nM).[7]

Affinity measured at non-A$_3$ hARs:
*% inhibition of specific binding by **14** at 10 µM:*
A$_1$ – [^{3}H]DPCPX (8-cyclopentyl-1,3-dipropylxanthine, 0.5 nM) 7.85±4.75%
A$_{2A}$ – [^{3}H]ZM241385 (4-[2-[7-amino-2-(2-furyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-yl-amino]ethyl]phenol, 1.0 nM) 6.67±2.96%
A$_{2B}$ – [^{3}H]DPCPX (10 nM) 0.67±3.63%

Molecular Modeling

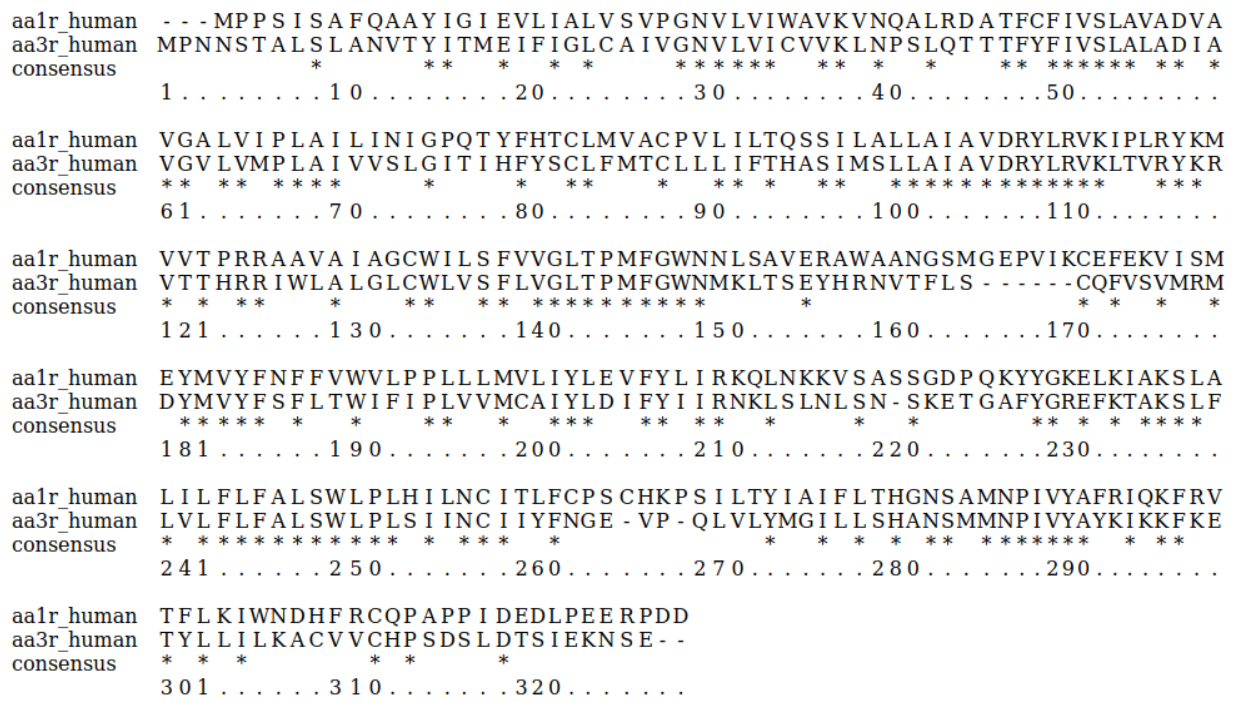


Figure S3. Sequence alignment between hA_1AR and hA_3AR according to GPCRdb (sequence identity 46%).[8] The image was generated using the software Boxshade.

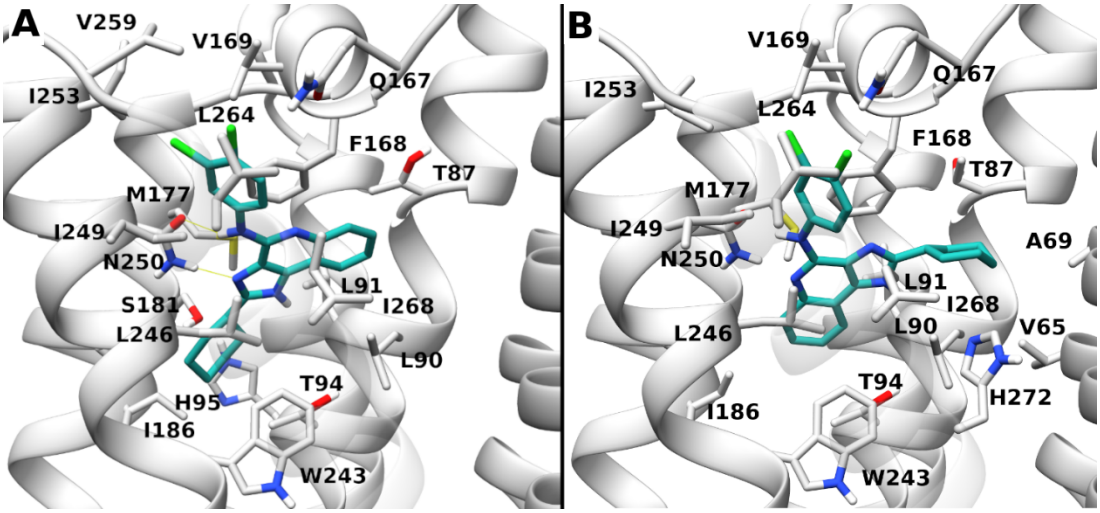


Figure S4. A) Predicted binding mode of compound **7** at the hA$_3$AR orthosteric binding site. **B)** Alternative pose of compound **7** at the hA$_3$AR orthosteric binding site (1st ranked according to Induced Fit Score).

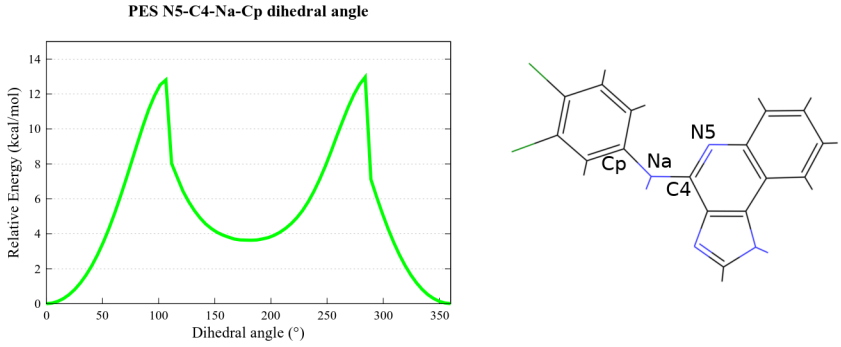


Figure S5. Potential energy surface scan of the dihedral angle defined by *N*5-C4-*N*(amino)-Cp(phenyl) atoms. The calculation was performed with the semiempirical quantum mechanical method GFNn-xTB.

References:

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