

Supporting Information

Design, Synthesis and Evaluation of New 1*H*-benzo[*d*]imidazole based PqsR Inhibitors as Adjuvant Therapy for *Pseudomonas aeruginosa* Infections

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Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or origin
PAO1-L	Wound isolate, wild type PAO1, Lausanne subline.	B. Holloway, <i>via</i> D. Haas
PAO1-L mCTX:: <i>P_{pqsA}-lux</i>	PAO1-L with chromosomal mini-CTX:: <i>P_{pqsA}-lux</i> insertion; Tc ^R	1
PAO1-N	Wound isolate, wild type PAO1, Nottingham subline	2
PA14	Burn isolate, wild type UCBPP-PA14	3, 4
PALESB58	CF isolate	5
IPCD1451	CF isolate, >95% related to PAO1, IPCD ID 1451	6, 7
IPCD1350	CF isolate, >95% related to PA14, IPCD ID 1350	6, 7
IPCD1331	CF isolate, >95% related to PAK, IPCD ID 1331	6, 7
IPCD48	CF isolate, >95% related to PA7, IPCD ID 48	6-8
PA14 mCTX:: <i>P_{pqsA}-lux</i>	PA14 with chromosomal mini-CTX:: <i>P_{pqsA}-lux</i> insertion; Tc ^R	1
<u>Plasmids</u>		
mini-CTX:: <i>P_{pqsA}-lux</i>	R6K-based mini-CTX suicide plasmid for the chromosomal insertion of a <i>P_{pqsA}-lux</i> transcriptional reporter fusion; Tc ^R	9
pMMG	pME6032Δ <i>lacI</i> constitutively expressing GFP from the <i>P_{tac}</i> promoter	10

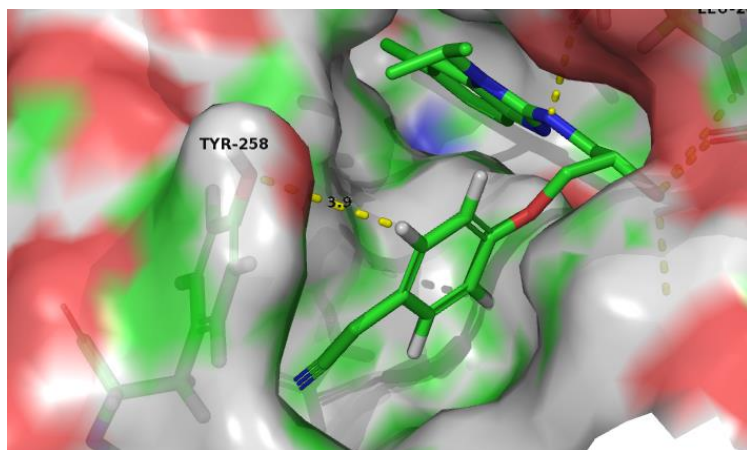


Figure S1: Schematic representation of the measurement between the *ortho*- position of the phenoxy ring of **6f** and the oxygen atom of the Tyrosine 258 (PqsR ligand binding domain).

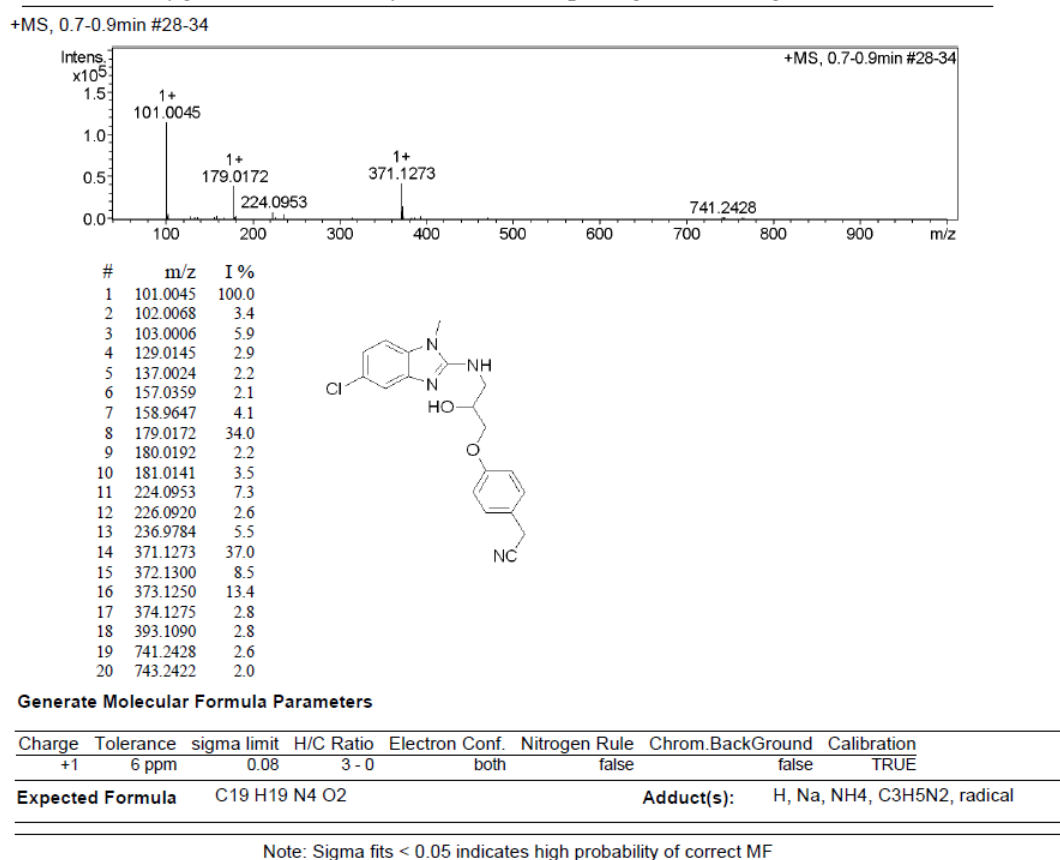
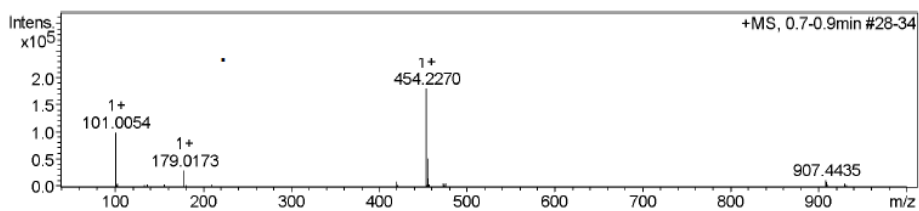
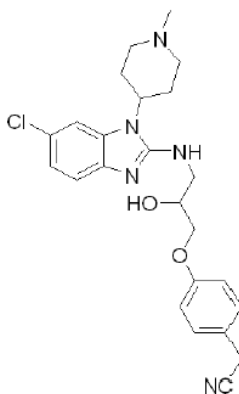


Figure S2. HRMS spectrum of **6b**.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0054	55.1
2	102.0076	1.8
3	103.0014	3.2
4	137.0025	2.0
5	157.0361	2.0
6	179.0173	16.6
7	181.0129	1.8
8	210.6240	2.1
9	420.2391	4.9
10	454.2270	100.0
11	455.2300	28.7
12	456.2274	9.2
13	457.2282	2.0
14	473.2203	3.9
15	476.2095	3.6
16	907.4435	7.1
17	908.4488	4.2
18	909.4483	2.0
19	929.4252	2.7
20	930.4261	1.6



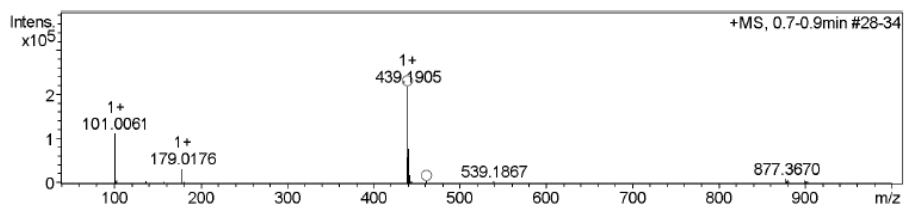
Generate Molecular Formula Parameters

Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C24 H28 Cl1 N5 O2			Adduct(s):		H, Na, NH4, C3H5N2, radical	

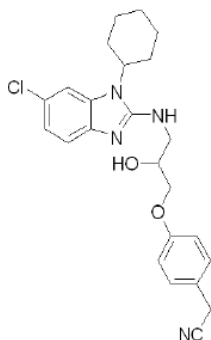
Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S3. HRMS spectrum of **6r**.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0061	51.9
2	102.0088	1.8
3	103.0023	3.0
4	137.0034	2.4
5	179.0176	14.8
6	181.0136	1.5
7	439.1905	100.0
8	440.1926	27.4
9	441.1879	35.4
10	442.1904	9.0
11	444.1688	2.2
12	461.1712	2.5
13	539.1867	2.1
14	877.3670	4.3
15	878.3705	2.5
16	879.3682	3.4
17	880.3683	1.7
18	899.3485	3.1
19	900.3522	1.8
20	901.3516	2.4



Generate Molecular Formula Parameters

Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE

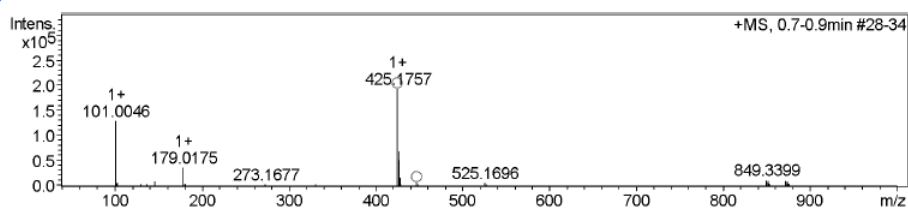
Expected Formula C₂₄ H₂₇ Cl₁ N₄ O₂ Adduct(s): H, Na, NH₄, C₃H₅N₂, radical

#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	439.1905	439.1895	2.10	0.0043	C ₂₄ H ₂₈ ClN ₄ O ₂	M+H	1.0078
1	461.1712	461.1715	0.60	0.0121	C ₂₄ H ₂₇ ClN ₄ NaO ₂	M+Na	22.9898

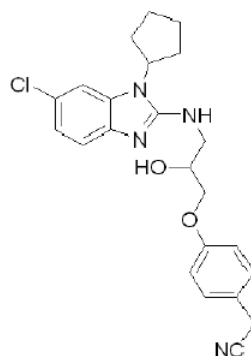
Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S4. HRMS spectrum of **6l**.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0046	66.4
2	102.0065	2.3
3	103.0007	3.7
4	146.1549	4.4
5	179.0175	18.7
6	181.0139	2.0
7	425.1757	100.0
8	426.1781	26.6
9	427.1732	35.9
10	428.1752	9.1
11	447.1562	3.5
12	525.1696	2.7
13	849.3399	6.3
14	850.3423	3.6
15	851.3395	5.1
16	852.3412	2.4
17	871.3205	5.3
18	872.3266	2.7
19	873.3179	4.2
20	874.3188	2.0



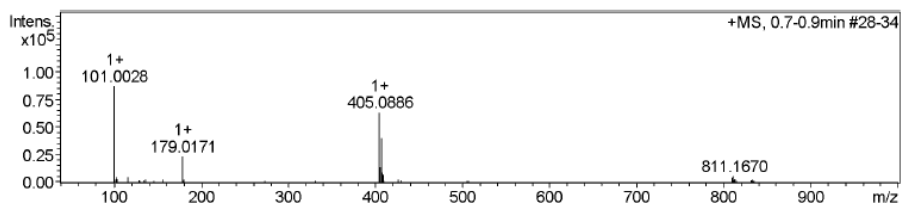
Generate Molecular Formula Parameters

Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C23 H25 Cl1 N4 O2					Adduct(s):	H, Na, NH4, C3H5N2, radical
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	425.1757	425.1739	4.40	0.0011	C23H26ClN4O2	M+H	1.0078
1	447.1562	447.1558	0.90	0.0200	C23H25ClN4NaO2	M+Na	22.9898

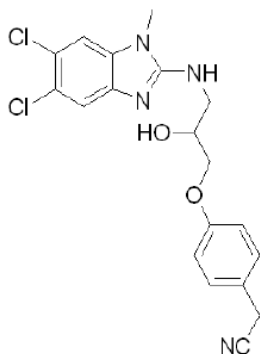
Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S5. HRMS spectrum of 6k.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0028	100.0
2	102.0054	3.3
3	102.9988	5.9
4	104.1069	4.0
5	116.9773	6.4
6	137.0016	2.9
7	157.0359	2.9
8	179.0171	27.3
9	181.0142	2.9
10	405.0886	72.6
11	406.0916	16.0
12	407.0859	46.7
13	408.0869	10.8
14	409.0846	8.4
15	427.0733	2.8
16	809.1675	5.1
17	811.1670	6.8
18	812.1694	2.9
19	813.1632	3.5
20	833.1458	3.2



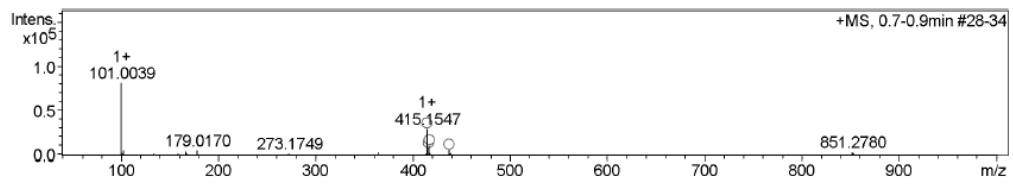
Generate Molecular Formula Paramete

Charge	Tolerance	sigma limit	H/C Ratio	Electron Contr.	Nitrogen Rule	Chrom.Background	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C21 H22 Cl2 N4 O2			Adduct(s):	H, Na, NH4, C3H5N2, radical		

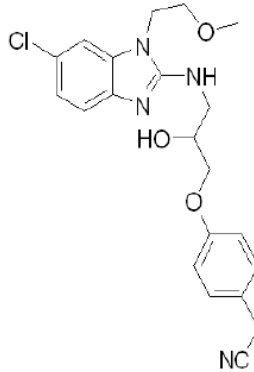
Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S6. HRMS spectrum of **6c**.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0039	100.0
2	102.0065	3.3
3	102.9999	6.0
4	134.0813	2.1
5	167.1158	5.5
6	168.9902	1.5
7	179.0170	6.5
8	273.1749	2.1
9	365.1078	3.9
10	413.1776	1.9
11	415.1547	36.4
12	416.1563	9.1
13	417.1518	12.9
14	418.1531	3.1
15	437.1360	7.3
16	438.1403	1.7
17	439.1336	2.7
18	851.2780	4.2
19	852.2757	2.2
20	853.2769	3.2



Generate Molecular Formula Parameters

No of Most Intense MS Peaks Analysed 5

Charge	Tolerance	sigma_limit	Calibration
+1	6 ppm	0.08	TRUE

Formula Min C 1 H 0 N 0 O 0 Formula Max C50 H100 N10 O10 H Na NH4 C3H5N2

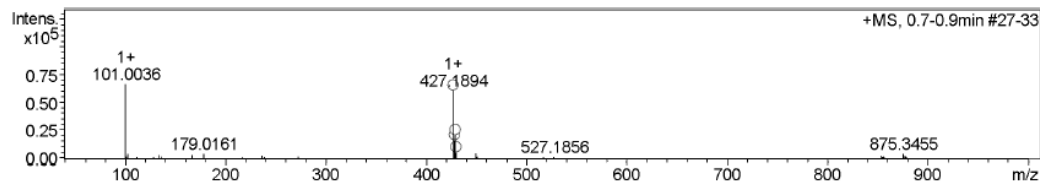
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula
2	417.1518	417.1520	0.50	0.0038	C 19 H 20 N 7 Na 1 O 3
		417.1533	3.70	0.0040	C 21 H 22 N 4 Na 1 O 4
		417.1531	3.10	0.0040	C 20 H 23 N 3 O 7
		417.1530	3.10	0.0042	C 19 H 17 N 10 O 2
		417.1517	0.20	0.0061	C 17 H 15 N 13 O 1

Note: Sigma fits < 0.05 indicates high probability of correct MF

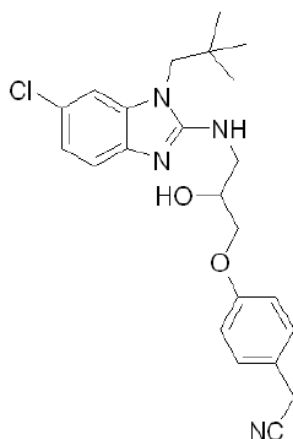
Figure S7. HRMS spectrum of **6t**.

Sample-ID	pazfs2_005	Lab	C13
Submitter	Fadi Soukarieh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_005_653078_7_01_133763.d	Acquisition Date	8/9/2023 10:43:23 AM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #27-33



#	m/z	I %
1	101.0036	100.0
2	102.0060	3.4
3	102.9999	5.8
4	134.0808	5.1
5	137.0016	2.8
6	167.1152	5.1
7	179.0161	5.9
8	237.1141	4.2
9	273.1758	2.8
10	427.1894	91.0
11	428.1919	23.9
12	429.1874	32.0
13	430.1906	8.0
14	449.1710	7.1
15	451.1675	2.7
16	853.3696	4.3
17	855.3724	3.1
18	875.3455	5.6
19	876.3515	3.0
20	877.3517	4.4



Generate Molecular Formula

tense MS Peaks Analysed 5

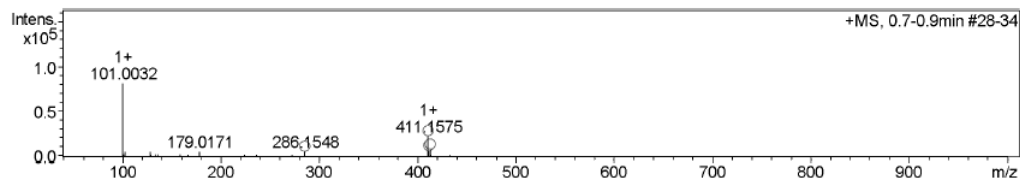
Charge	Tolerance	sigma_lim	Comparison
+1	6 ppm	0.08	TRUE

#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula
2	429.1874	429.1894	4.80	0.0029	C 22 H 27 N 3 O 6
		429.1884	2.30	0.0032	C 22 H 30 Na 1 O 7
		429.1881	1.70	0.0050	C 20 H 25 N 6 O 5
		429.1884	2.30	0.0065	C 21 H 24 N 7 Na 1 O 2
		429.1870	0.80	0.0083	C 19 H 22 N 10 Na 1 O 1
4	430.1906	430.1881	5.70	0.0167	C 11 H 25 N 11 Na 1 O 6
		430.1895	2.60	0.0168	C 13 H 27 N 8 Na 1 O 7

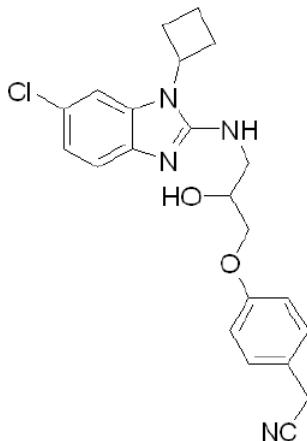
Figure S8. HRMS spectrum of **6h**.

Sample-ID	pazfs2_006	Lab	C13
Submitter	Fadi Soukarieh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_006_653079_8_01_133764.d	Acquisition Date	8/9/2023 10:45:53 AM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0032	100.0
2	102.0056	3.5
3	102.9990	6.3
4	129.0125	5.8
5	130.0110	1.8
6	134.0808	3.3
7	137.0010	2.9
8	158.9633	2.9
9	167.1170	1.8
10	179.0171	6.1
11	225.0114	1.8
12	236.9792	1.8
13	273.1758	2.1
14	286.1548	6.4
15	292.9943	1.5
16	411.1575	26.9
17	412.1616	7.1
18	413.1560	9.4
19	414.1579	2.5
20	433.1407	2.3



Generate Molecular Formula P

no. of most intense MS Peaks Analysed 5

Charge	Tolerance	sigma_limit	Calibration
+1	6 ppm	0.08	TRUE

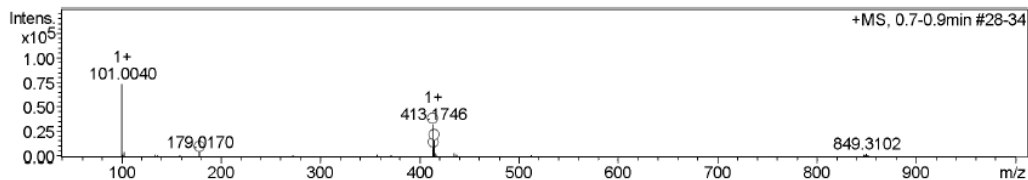
Formula Min C 1 H 0 N 0 O 0 Formula Max C50 H100 N10 O10 H Na NH4 C3H5N2

#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula
2	413.1560	413.1584	5.90	0.0088	C 22 H 22 N 4 Na 1 O 3
		413.1581	5.30	0.0114	C 20 H 17 N 10 O 1
		413.1581	5.30	0.0122	C 21 H 23 N 3 O 6
		413.1571	2.70	0.0144	C 20 H 20 N 7 Na 1 O 2
		413.1568	2.00	0.0167	C 18 H 15 N 13
4	286.1548	286.1550	0.80	0.0061	C 16 H 20 N 3 O 2
		286.1563	5.50	0.0079	C 18 H 22 O 3

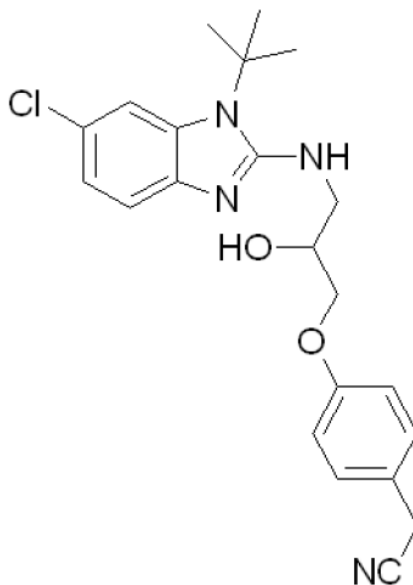
Figure S9. HRMS spectrum of **6j**.

Sample-ID	pazfs2_007	Lab	C13
Submitter	Fadi Soukarieh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_007_653080_9_01_133765.d	Acquisition Date	8/9/2023 10:48:23 AM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0040	100.0
2	102.0061	3.5
3	103.0002	5.9
4	134.0816	3.3
5	137.0021	1.8
6	179.0170	6.0
7	273.1757	1.8
8	357.1091	3.3
9	413.1746	44.6
10	414.1762	11.7
11	415.1666	22.4
12	416.1689	5.5
13	417.1542	3.4
14	435.1555	5.6
15	437.1462	3.1
16	513.1700	1.8
17	847.3205	3.0
18	849.3102	3.7
19	850.3139	1.8
20	851.3059	1.7



Generate Molecular Formula Parameter:

Charge	Tolerance	sigma_limit	C
+1	6 ppm	0.08	

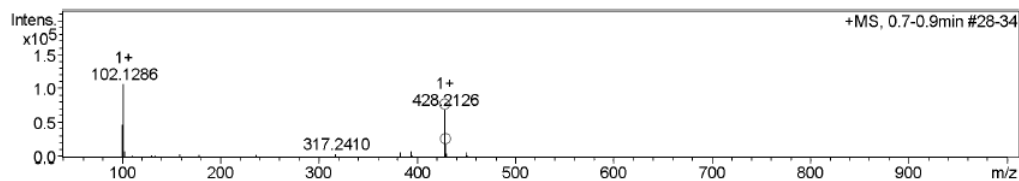
Formula Min C 1 H 0 N 0 O 0

#	meas. m/z	theo. m/z	[Err][ppm]			
1	413.1746	413.1721	6.10			
		413.1723	5.40	U.0z6r	C 25 H 26 Na 1 O 4	
		413.1734	2.80	0.0293		C 25 H 23 N 3 O 3
		413.1737	2.20	0.0323		C 26 H 22 N 4 Na 1
		413.1747	0.40	0.0352		C 27 H 25 O 4
2	415.1666	415.1642	5.90	0.0305	C 24 H 20 N 6 Na 1	
		415.1652	3.30	0.0332		C 25 H 23 N 2 O 4

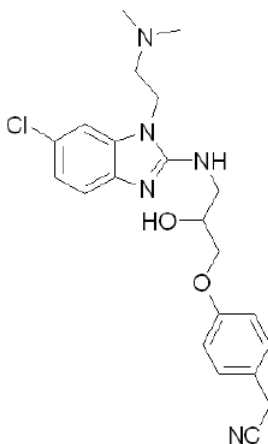
Figure S10. HRMS spectrum of **6g**.

Sample-ID	pazfs2_008	Lab	C13
Submitter	Fadi Soukariah (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_008_653081_10_01_133766.d	Acquisition Date	8/9/2023 10:50:52 AM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0041	44.2
2	102.1286	100.0
3	103.0004	2.9
4	103.1316	7.9
5	111.0932	1.7
6	130.1593	2.2
7	134.0810	1.8
8	158.9636	3.7
9	179.0170	3.0
10	236.9775	2.5
11	317.2410	4.0
12	383.1532	6.6
13	384.1596	1.5
14	394.2239	7.7
15	395.2284	1.9
16	428.2126	64.7
17	429.2150	17.0
18	430.2134	5.5
19	450.1931	5.9
20	451.1972	1.6



Generate Molecular Formula Parameters

No of Most Intense MS Peaks Analysed 5

Charge	Tolerance	sigma_limit	Calibration
+1	6 ppm	0.08	TRUE

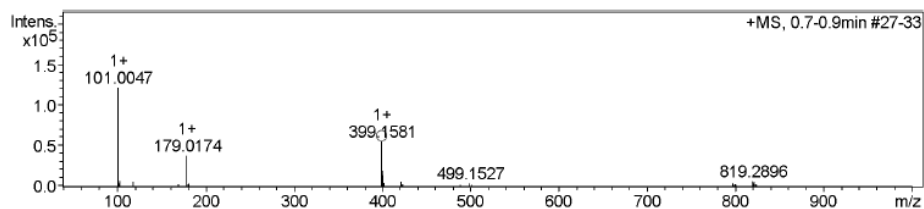
Formula Min C 1 H 0 N 0 O 0 Formula Max C50 H100 N10 O10 H Na NH4 C3H5N2

#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula
1	428.2126	428.2142	3.80	0.0348	C 19 H 29 N 6 Na 1 O 4
		428.2140	3.10	0.0372	C 17 H 24 N 12 O 2
		428.2140	3.10	0.0379	C 18 H 30 N 5 O 7
		428.2129	0.70	0.0406	C 17 H 27 N 9 Na 1 O 3
		428.2111	3.70	0.0415	C 30 H 29 Na 1 O 1
2	429.2150	429.2175	6.00	0.0110	C 28 H 28 N 3 Na 1
		429.2173	5.30	0.0150	C 27 H 29 N 2 O 3

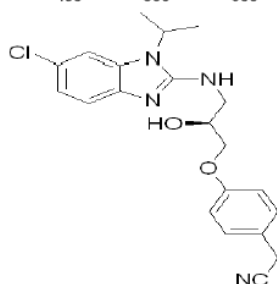
Figure S11. HRMS spectrum of **6q**.

Sample-ID	pazfs2_021	Lab	C13
Submitter	Fadi Soukariéh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_021_653086_12_01_133769.d	Acquisition Date	8/9/2023 12:21:18 PM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #27-33



#	m/z	I %
1	101.0047	100.0
2	102.0070	3.4
3	103.0011	5.8
4	118.1239	4.7
5	168.9908	2.1
6	179.0174	31.2
7	180.0189	2.0
8	181.0132	3.1
9	399.1581	45.2
10	400.1610	11.2
11	401.1542	15.9
12	402.1572	3.7
13	421.1396	4.8
14	499.1527	3.2
15	797.3108	3.3
16	799.3009	2.5
17	819.2896	5.6
18	820.2915	2.8
19	821.2826	4.4
20	822.2857	2.0



Generate Molecular Formula Parameters

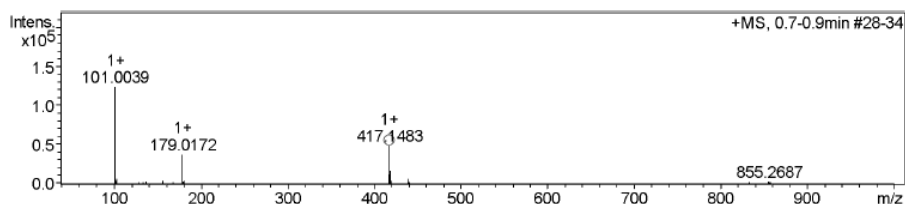
Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C21 H24 Cl1 N4 O2			Adduct(s):		H, Na, NH4, C3H5N2, radical	
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	399.1581	399.1582	0.20	0.0016	C21H24ClN4O2	M+	-0.0005

Note: Sigma fits < 0.05 indicates high probability of correct MF

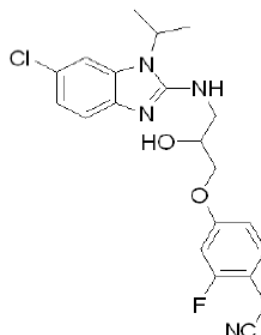
Figure S12. HRMS spectrum of **6v**.

Sample-ID	pazfs2_025	Lab	C13
Submitter	Fadi Soukariéh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_025_653087_13_01_133770.d	Acquisition Date	8/9/2023 12:23:48 PM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0039	100.0
2	102.0063	3.4
3	103.0001	5.7
4	134.0823	1.7
5	137.0021	1.9
6	157.0356	3.2
7	168.9924	1.5
8	179.0172	30.0
9	180.0192	1.9
10	181.0133	3.2
11	417.1483	39.7
12	418.1514	10.0
13	419.1459	13.9
14	420.1485	3.3
15	439.1315	5.2
16	440.1303	1.3
17	441.1285	1.8
18	833.2846	1.3
19	855.2687	2.4
20	857.2652	1.9



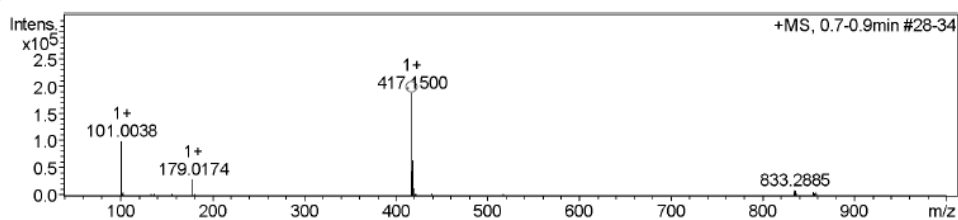
Generate Molecular Formula Parameters

Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C21 H23 Cl1 F1 N4 O2				Adduct(s):	H, Na, NH4, C3H5N2, radical	
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	417.1483	417.1488	1.10	0.0039	C21H23ClF1N4O2	M+	-0.0005

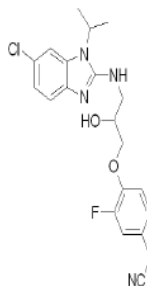
Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S13. HRMS spectrum of **60**.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0038	53.0
2	102.0062	1.8
3	102.9997	3.1
4	134.0815	1.6
5	157.0362	1.8
6	179.0174	15.8
7	181.0126	1.6
8	417.1500	100.0
9	418.1524	24.4
10	419.1474	34.4
11	420.1503	7.8
12	439.1327	2.2
13	517.1454	1.8
14	833.2885	5.6
15	834.2925	2.7
16	835.2883	4.3
17	836.2868	1.9
18	855.2703	3.9
19	856.2732	2.0
20	857.2697	2.9



Generate Molecular Formula Parameters

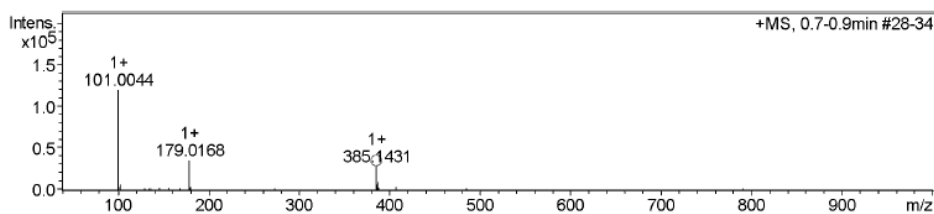
Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula		C21 H23 Cl1 F1 N4 O2			Adduct(s):		H, Na, NH4, C3H5N2, radical
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	417.1500	417.1488	2.90	0.0042	C21H23ClF1N4O2	M+	-0.0005

Note: Sigma fits < 0.05 indicates high probability of correct MF

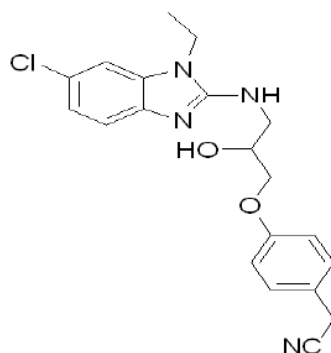
Figure S14. HRMS spectrum of **6n**.

Sample-ID	pazfs2_sen086	Lab	C13
Submitter	Fadi Soukarieh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_sen086_653089_15_01_133772.d	Acquisition Date	8/9/2023 12:28:48 PM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0044	100.0
2	102.0071	3.3
3	103.0004	5.9
4	130.1595	1.4
5	134.0815	1.5
6	137.0031	1.7
7	146.1539	2.0
8	157.0351	2.6
9	168.9927	1.7
10	179.0168	29.6
11	180.0202	1.9
12	181.0132	2.9
13	273.1687	1.2
14	385.1431	23.8
15	386.1458	5.7
16	387.1402	8.7
17	388.1407	2.0
18	407.1266	2.7
19	485.1384	1.2
20	791.2580	1.3



Generate Molecular Formula Parameters

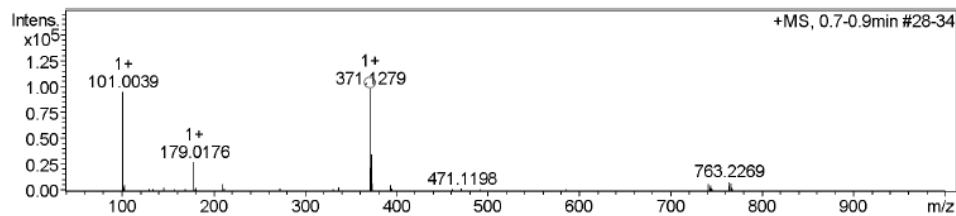
Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula		C20 H22 Cl1 N4 O2			Adduct(s):		H, Na, NH4, C3H5N2, radical
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	385.1431	385.1426	1.40	0.0073	C20H22ClN4O2	M+	-0.0005

Note: Sigma fits < 0.05 indicates high probability of correct MF

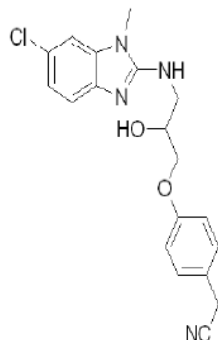
Figure S15. HRMS spectrum of **6e**.

Sample-ID	pazfs2_SEN086_Me	Lab	C13
Submitter	Fadi Soukarieh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_SEN086_Me_653090_16_01_1337	Acquisition Date	8/9/2023 12:31:19 PM
Ionisation Mode	Z3, d ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0039	96.6
2	102.0062	3.3
3	103.0003	5.9
4	146.1540	2.8
5	179.0176	28.0
6	181.0145	2.7
7	210.0794	6.4
8	337.1651	3.7
9	371.1279	100.0
10	372.1300	23.0
11	373.1256	35.3
12	374.1277	7.7
13	393.1097	6.1
14	741.2448	6.7
15	742.2481	3.1
16	743.2423	5.3
17	763.2269	9.4
18	764.2334	4.7
19	765.2245	7.2
20	766.2307	3.0



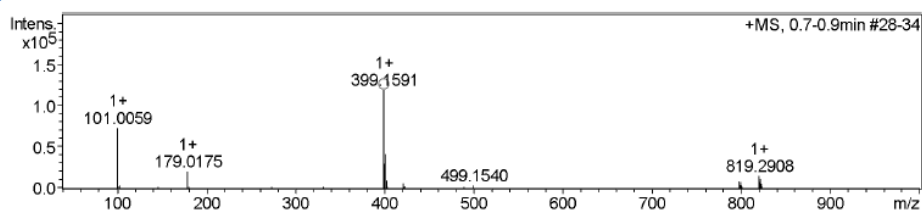
Generate Molecular Formula Parameters

Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C ₁₉ H ₂₀ Cl N ₄ O ₂				Adduct(s):	H, Na, NH ₄ , C ₃ H ₅ N ₂ , radical	
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	371.1279	371.1269	2.60	0.0041	C ₁₉ H ₂₀ ClN ₄ O ₂	M+	-0.0005

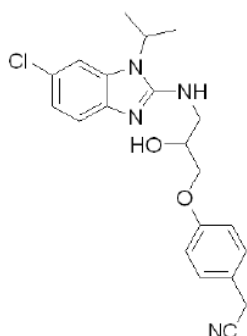
Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S16. HRMS spectrum of **6a**.

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0059	61.2
2	102.0085	2.2
3	103.0020	3.4
4	179.0175	17.3
5	399.1591	100.0
6	400.1623	25.1
7	401.1557	34.8
8	402.1583	8.6
9	421.1404	5.7
10	423.1391	2.0
11	499.1540	2.8
12	797.3091	7.8
13	798.3095	4.1
14	799.3053	6.5
15	800.3066	2.8
16	819.2908	12.9
17	820.2933	6.5
18	821.2869	10.2
19	822.2887	4.3
20	823.2854	2.6



Generate Molecular Formula Parameters

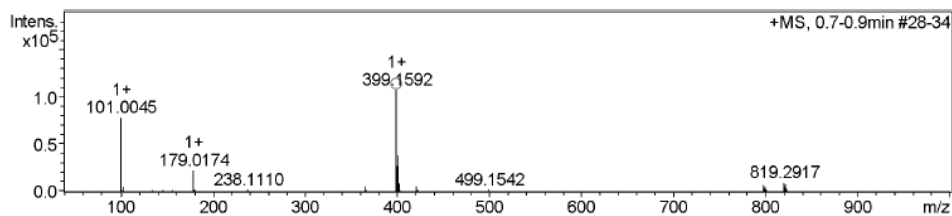
Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula	C21 H24 Cl1 N4 O2			Adduct(s):		H, Na, NH4, C3H5N2, radical	
#	meas. m/z	theo. m/z	Err [ppm]	Sigma	Formula	Adduct	Adduct Mass
1	399.1591	399.1582	2.20	0.0037	C21H24ClN4O2	M+	-0.0005

Note: Sigma fits < 0.05 indicates high probability of correct MF

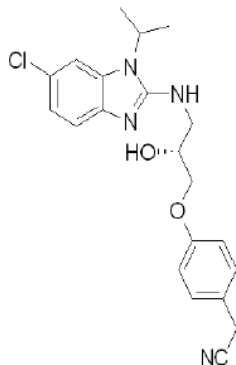
Figure S17. HRMS spectrum of **6f**.

Sample-ID	pazfs2_SEN089	Lab	C13
Submitter	Fadi Soukariéh (pazfs2)	Supervisor	Mike Stocks (pazmjs)
Analysis Name	pazfs2_SEN089_653091_17_01_133774.d	Acquisition Date	8/9/2023 12:33:49 PM
Ionisation Mode	ESI Positive	Instrument	Bruker MicroTOF

+MS, 0.7-0.9min #28-34



#	m/z	I %
1	101.0045	72.8
2	102.0069	2.5
3	103.0011	4.2
4	179.0174	20.9
5	238.1110	2.1
6	365.1964	5.2
7	399.1592	100.0
8	400.1616	24.9
9	401.1562	35.4
10	402.1592	8.2
11	421.1400	5.4
12	499.1542	2.6
13	797.3090	6.6
14	798.3108	3.4
15	799.3072	5.4
16	800.3106	2.3
17	819.2917	9.2
18	820.2941	4.8
19	821.2865	7.3
20	822.2841	3.1



Generate Molecular Formula Parameters

Charge	Tolerance	sigma limit	H/C Ratio	Electron Conf.	Nitrogen Rule	Chrom.BackGround	Calibration
+1	6 ppm	0.08	3 - 0	both	false	false	TRUE
Expected Formula		C21 H24 Cl1 N4 O2			Adduct(s):		H, Na, NH4, C3H5N2, radical
#	meas. m/z	theo. m/z	[Err][ppm]	Sigma	Formula	Adduct	Adduct Mass
1	399.1592	399.1582	2.40	0.0025	C21H24ClN4O2	M+	-0.0005

Note: Sigma fits < 0.05 indicates high probability of correct MF

Figure S18. HRMS spectrum of **6w**.

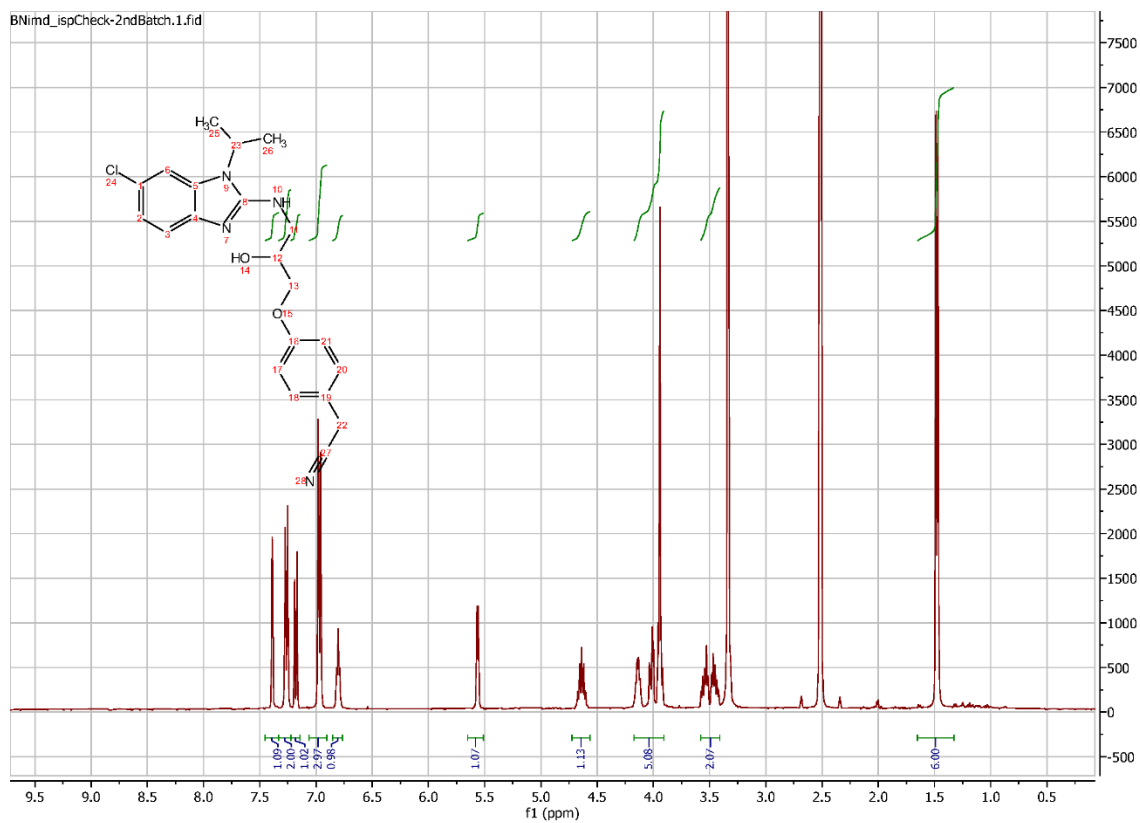


Figure S19. ¹H-NMR spectrum of **6f**.

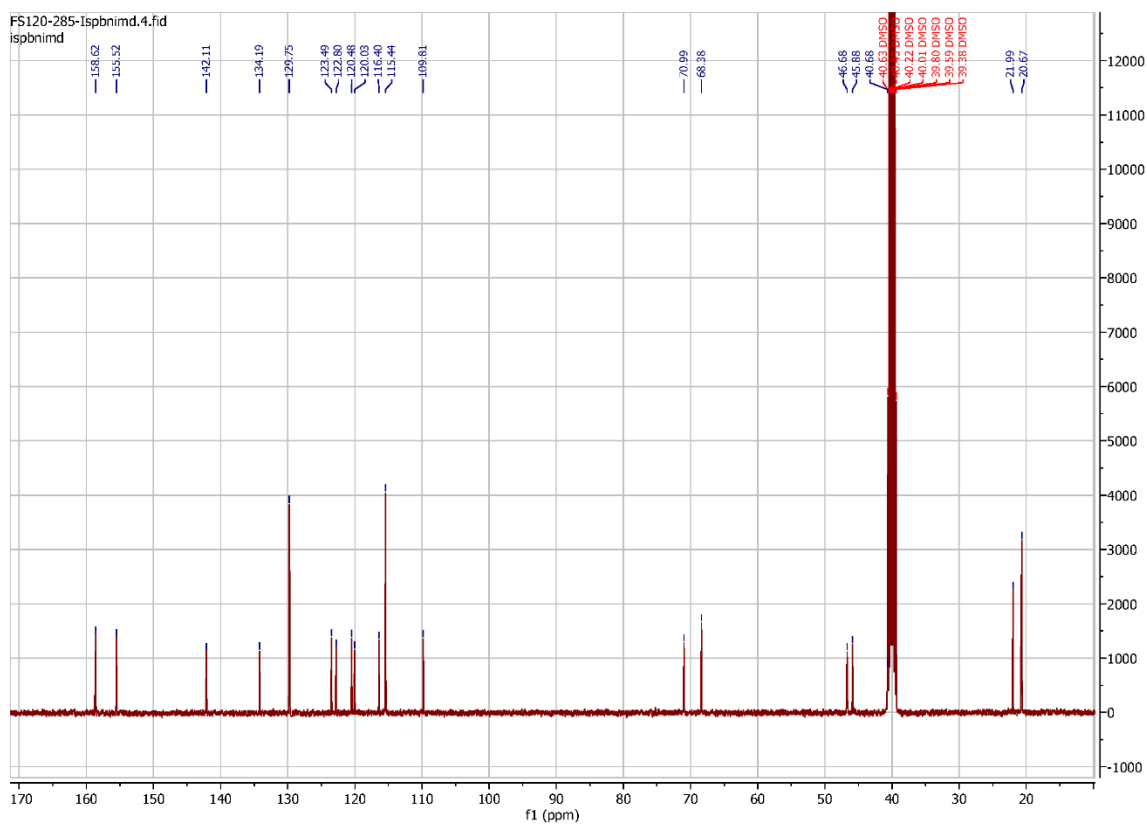


Figure S20. ¹³C-NMR spectrum of **6f**.

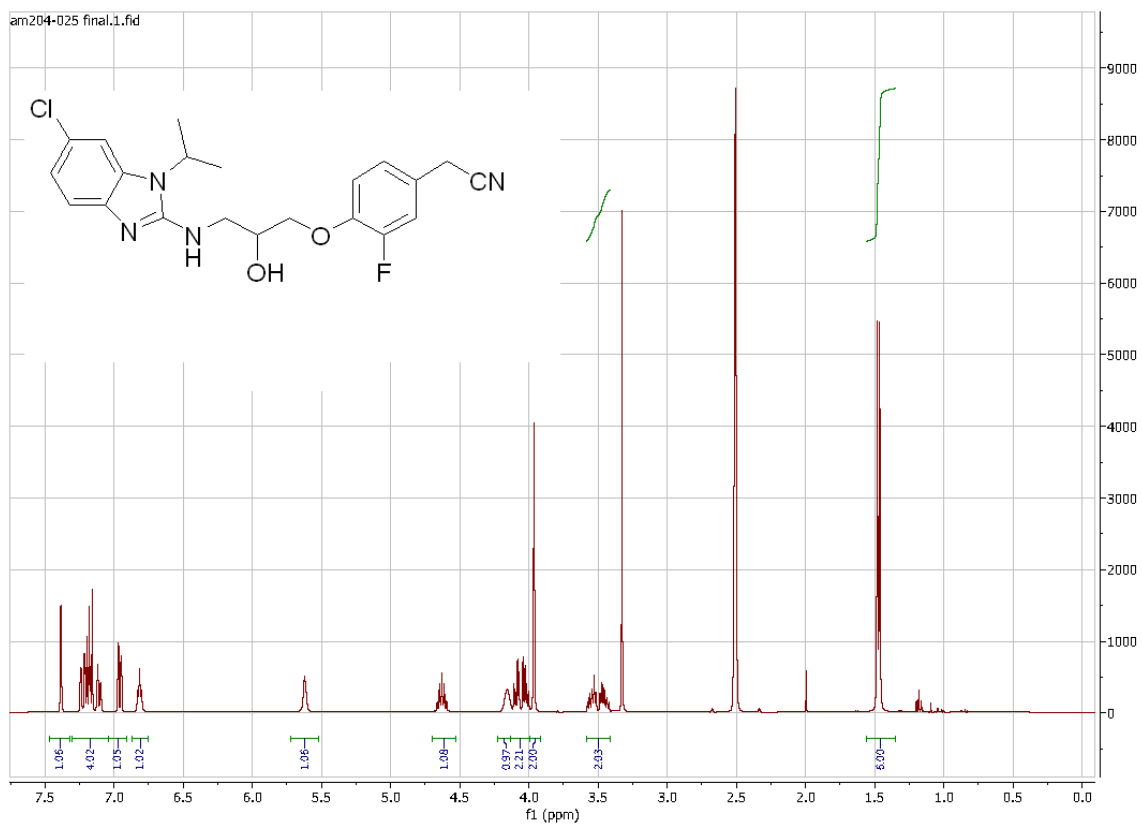


Figure S21. $^1\text{H-NMR}$ spectrum of **6n**.

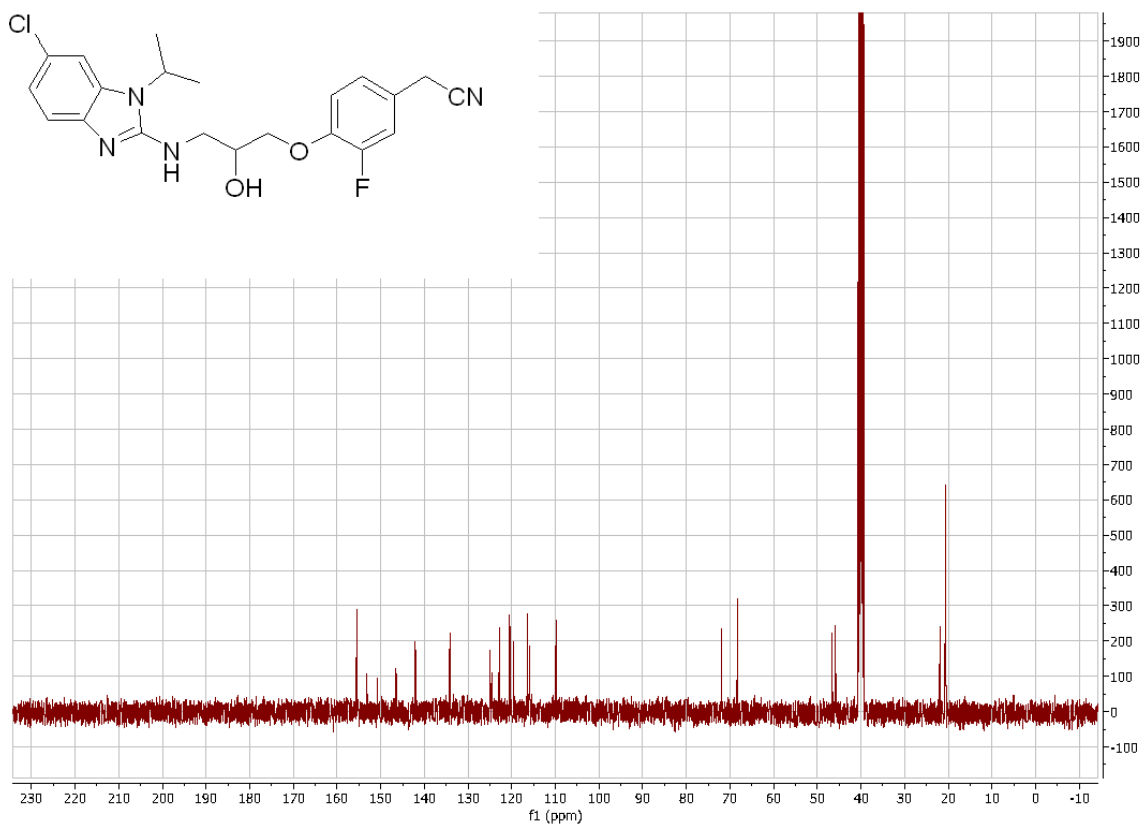


Figure S22. $^{13}\text{C-NMR}$ spectrum of **6n**.

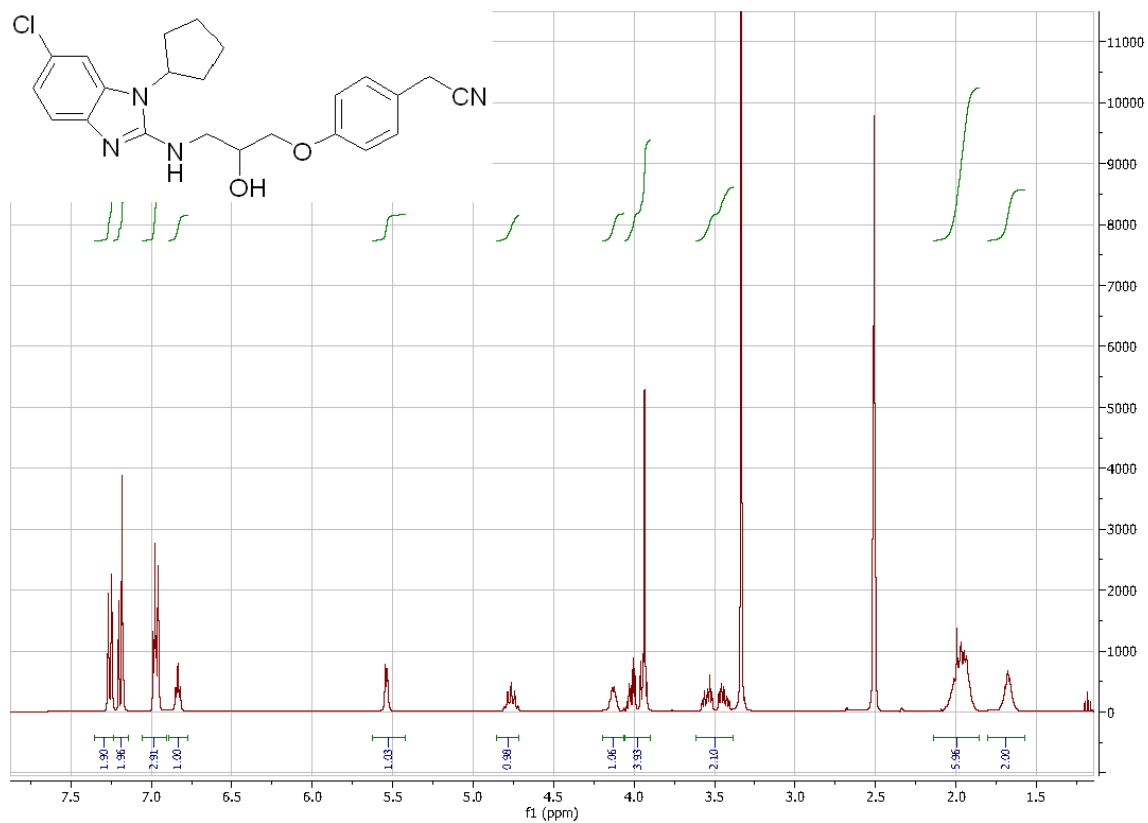


Figure S23. ¹H-NMR spectrum of **6k**.

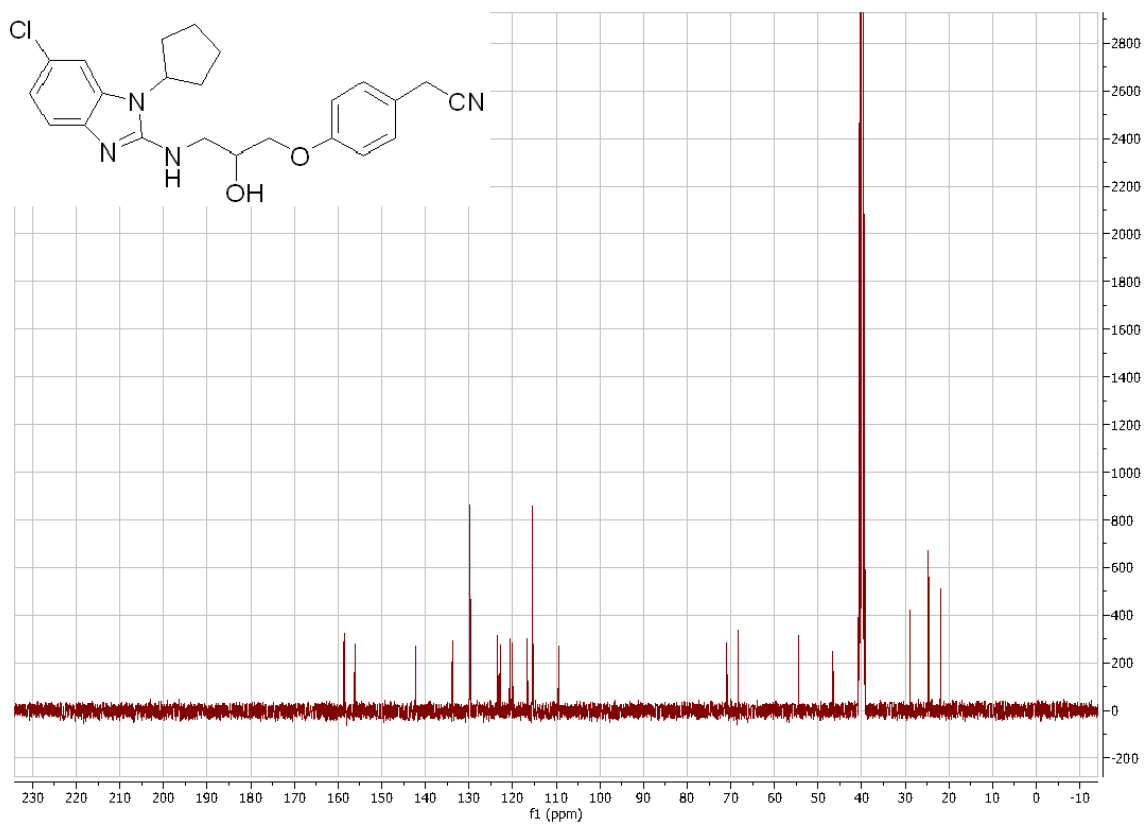


Figure S24. ¹³C-NMR spectrum of **6k**.

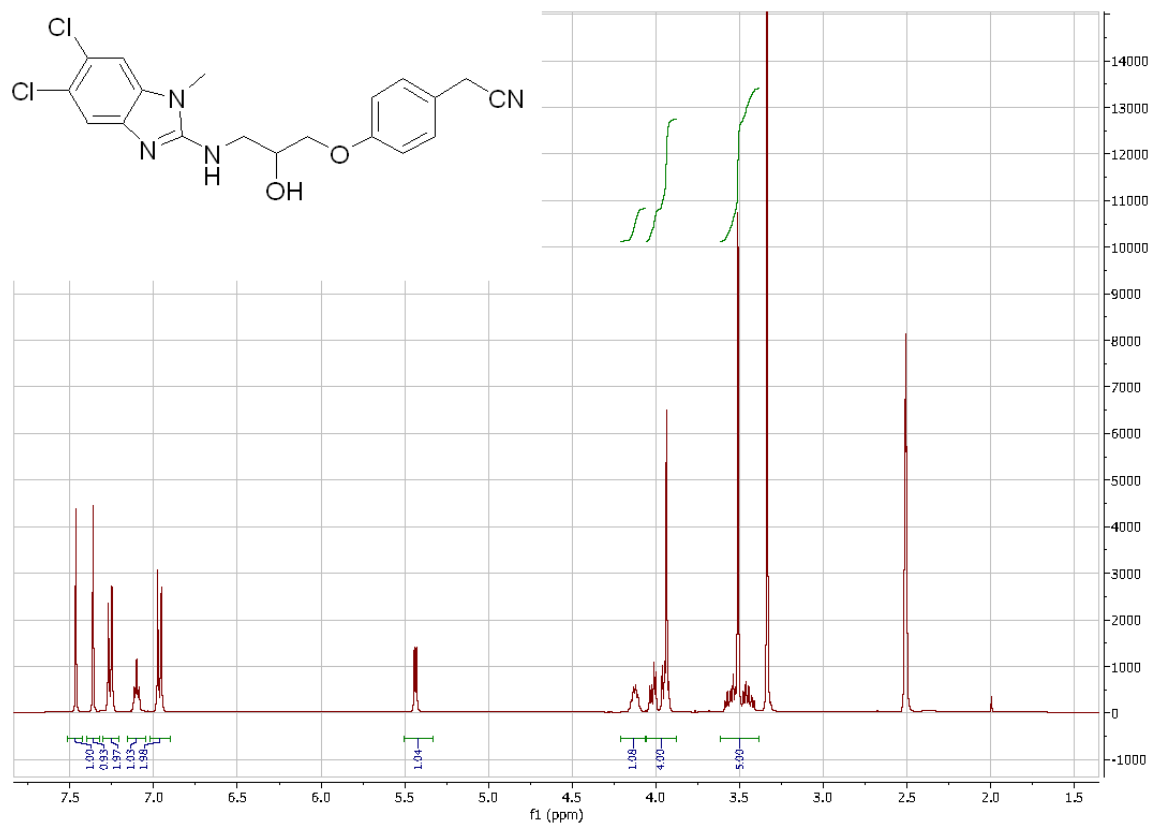


Figure S25. ¹H-NMR spectrum of **6c**.

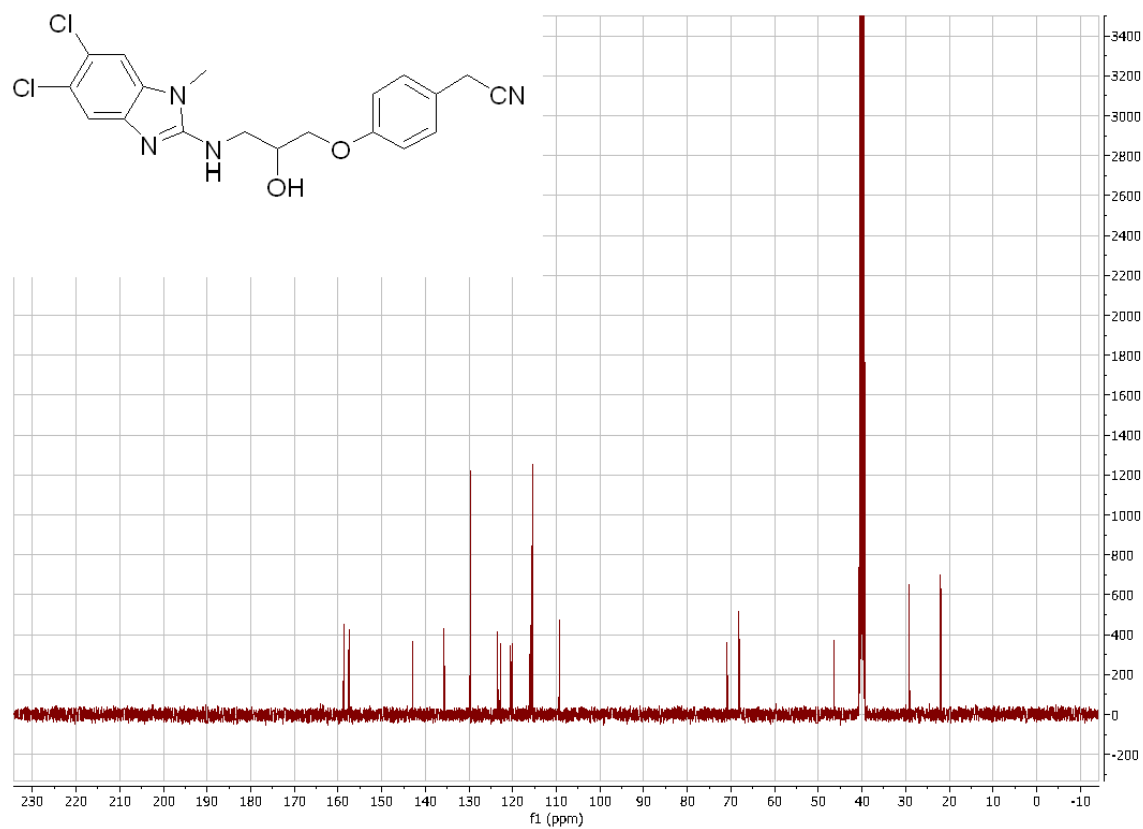


Figure S26. ¹³C-NMR spectrum of **6c**

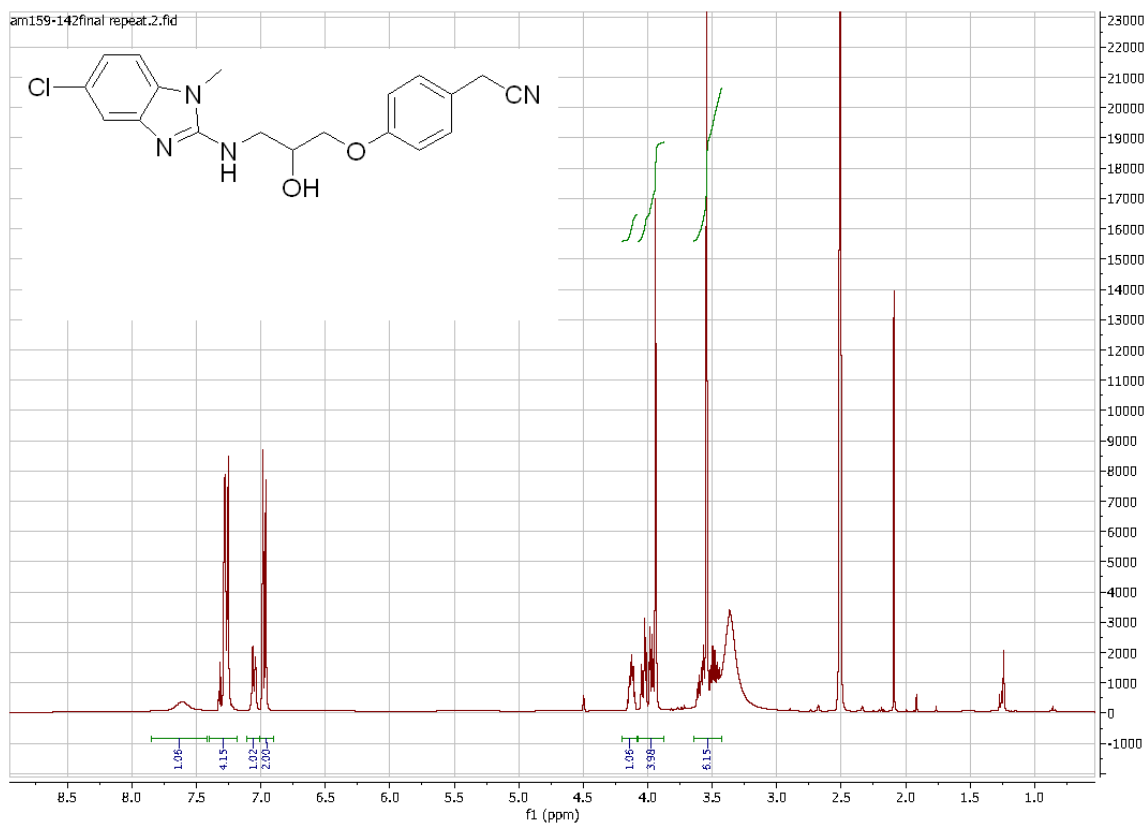


Figure S27. ¹H-NMR spectrum of **6b**.

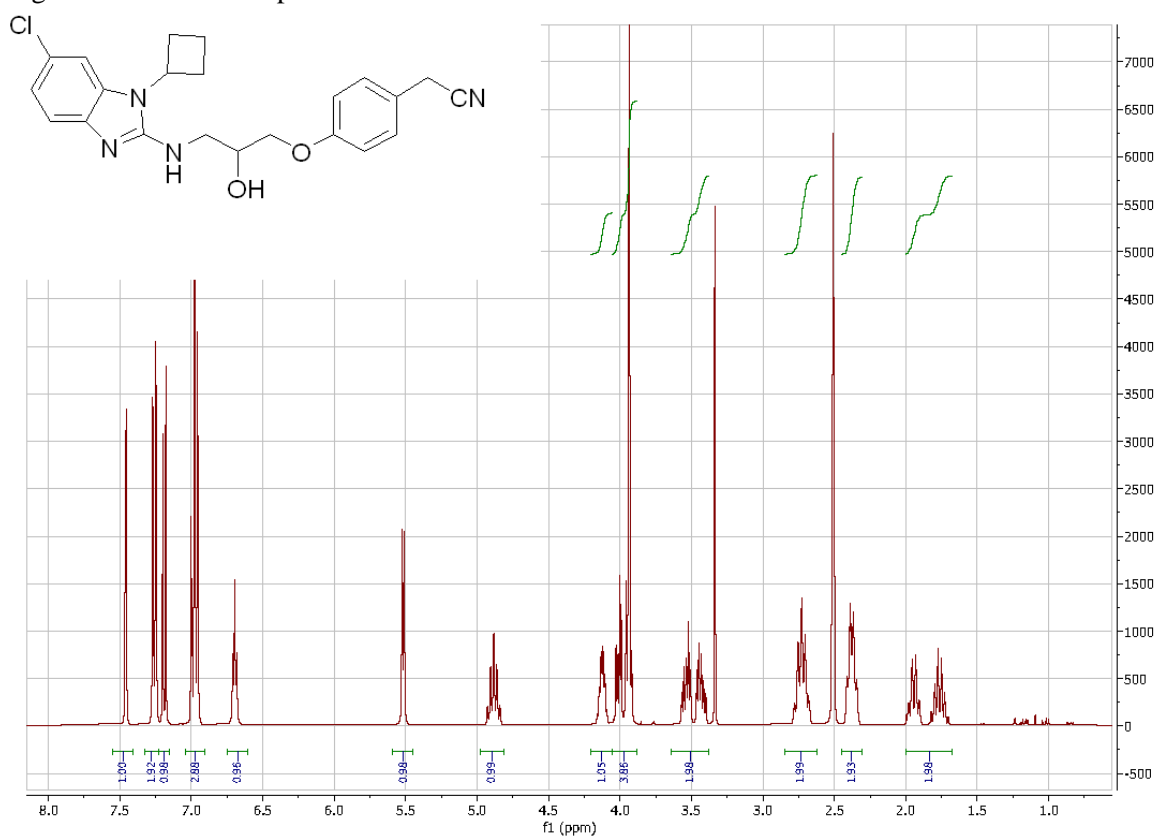


Figure S28. ¹H-NMR spectrum of **6j**

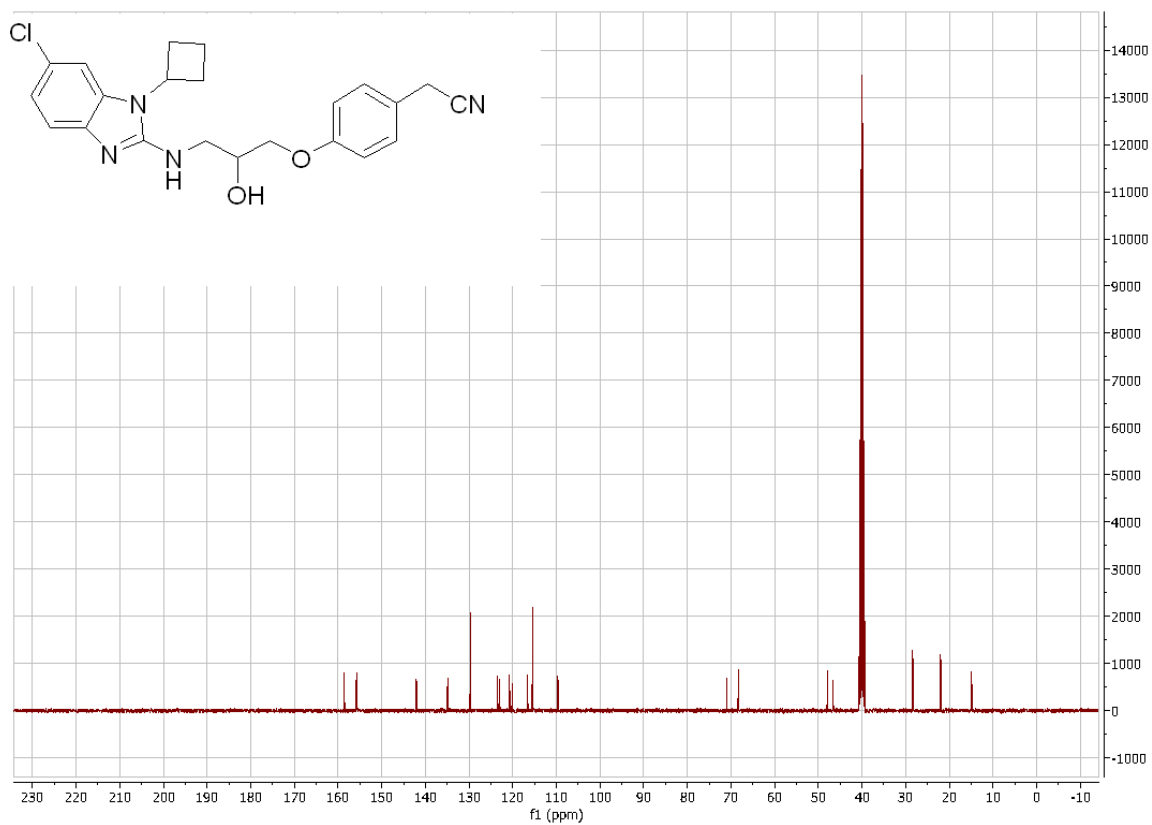


Figure S29. ^{13}C -NMR spectrum of **6j**.

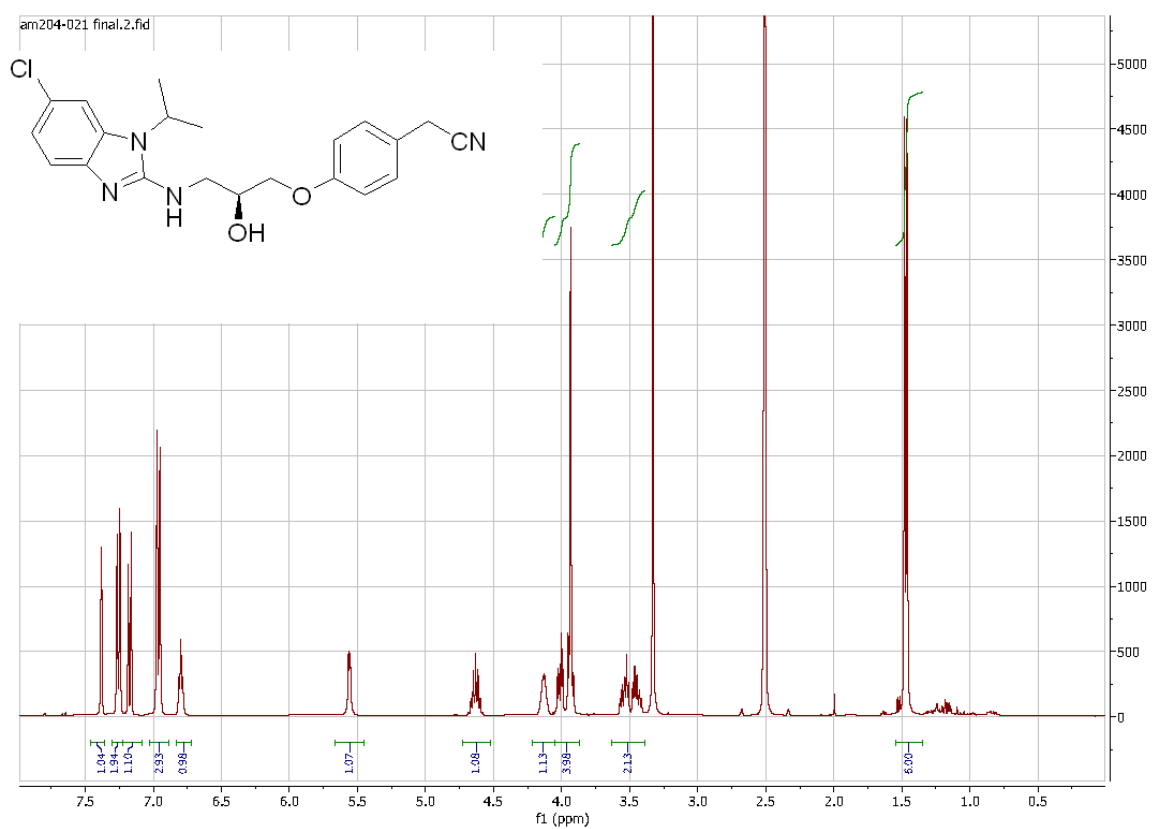


Figure S30. ^1H -NMR spectrum of **6v**.

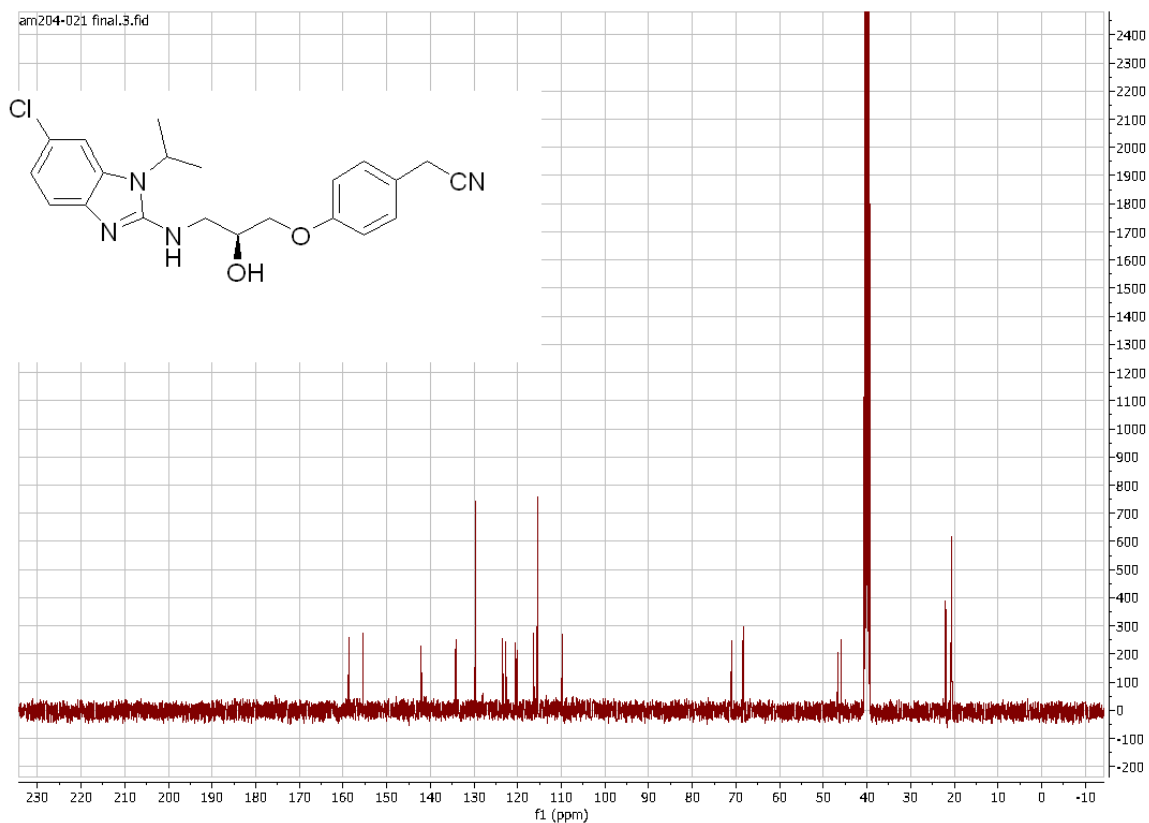


Figure S31. $^{13}\text{C-NMR}$ spectrum of **6v**.

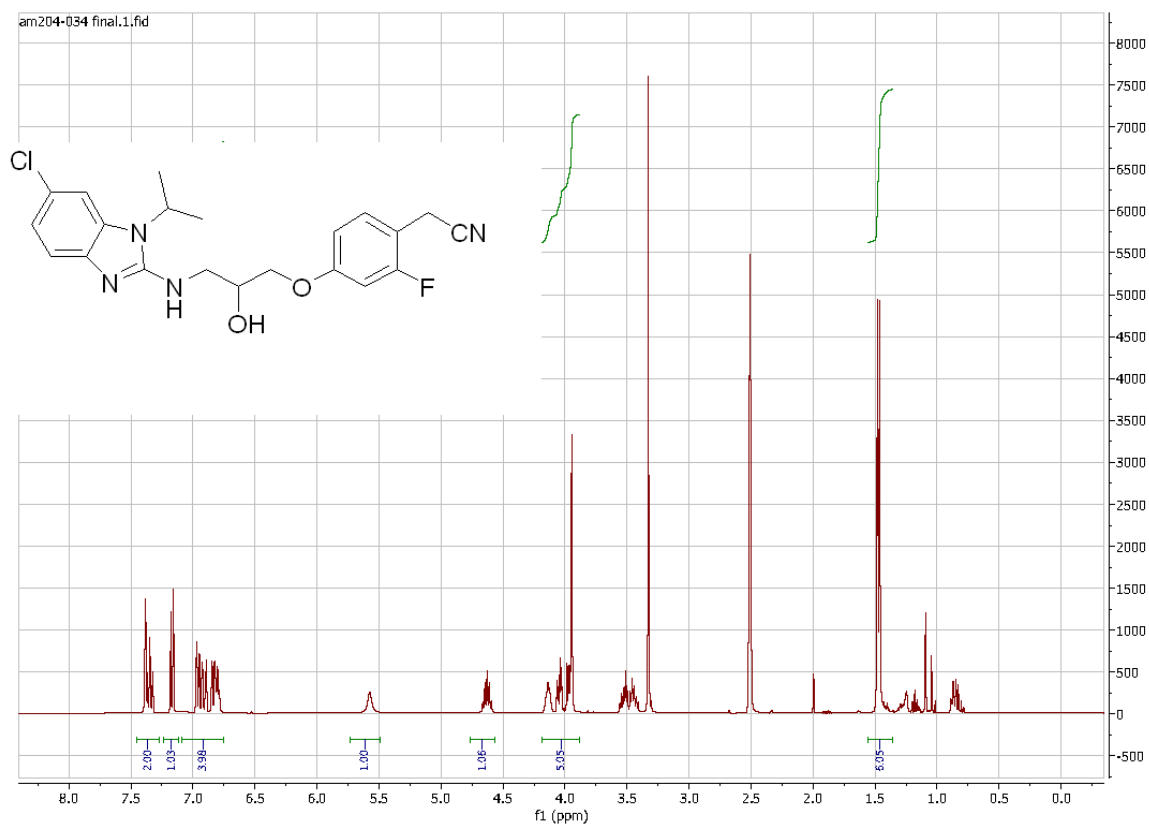


Figure S32. $^{13}\text{C-NMR}$ spectrum of **6o**.

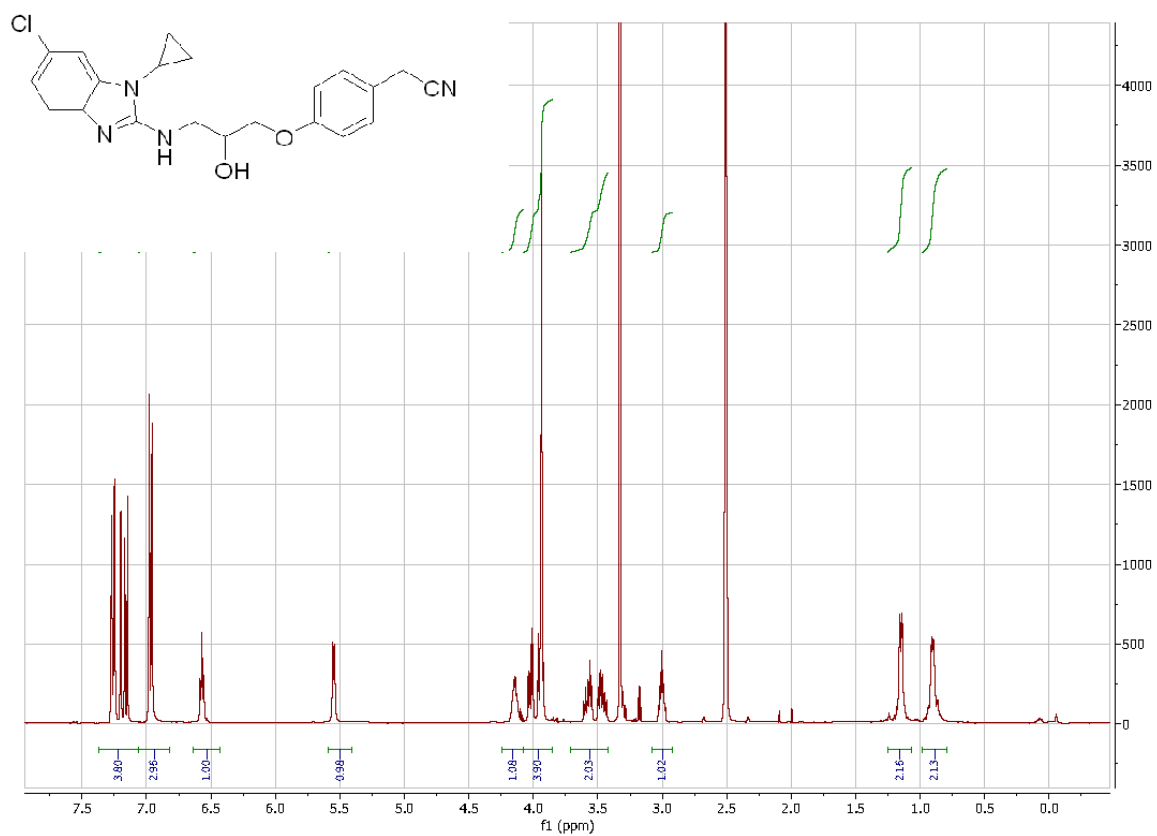


Figure S33. ¹H-NMR spectrum of **6i**.

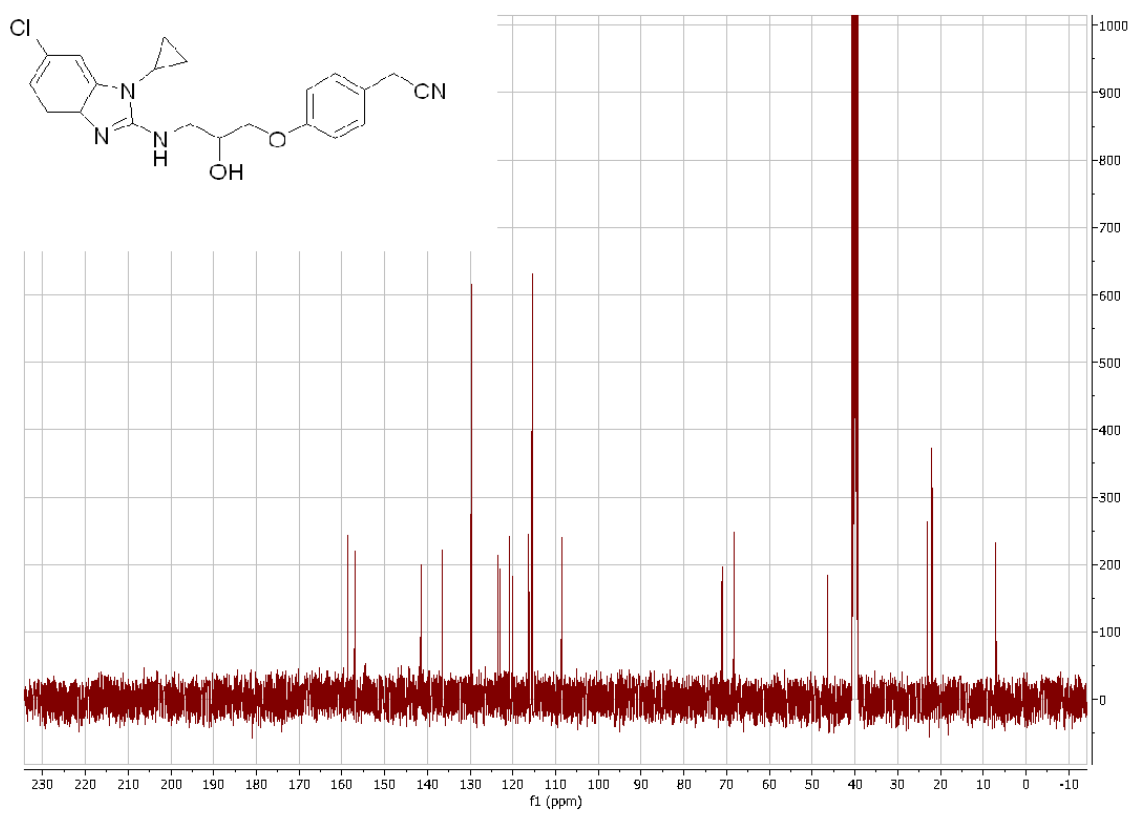


Figure S34. ¹³C-NMR spectrum of **6i**.

Printing Time: 13:35:49
Printing Date: 24 August 2023

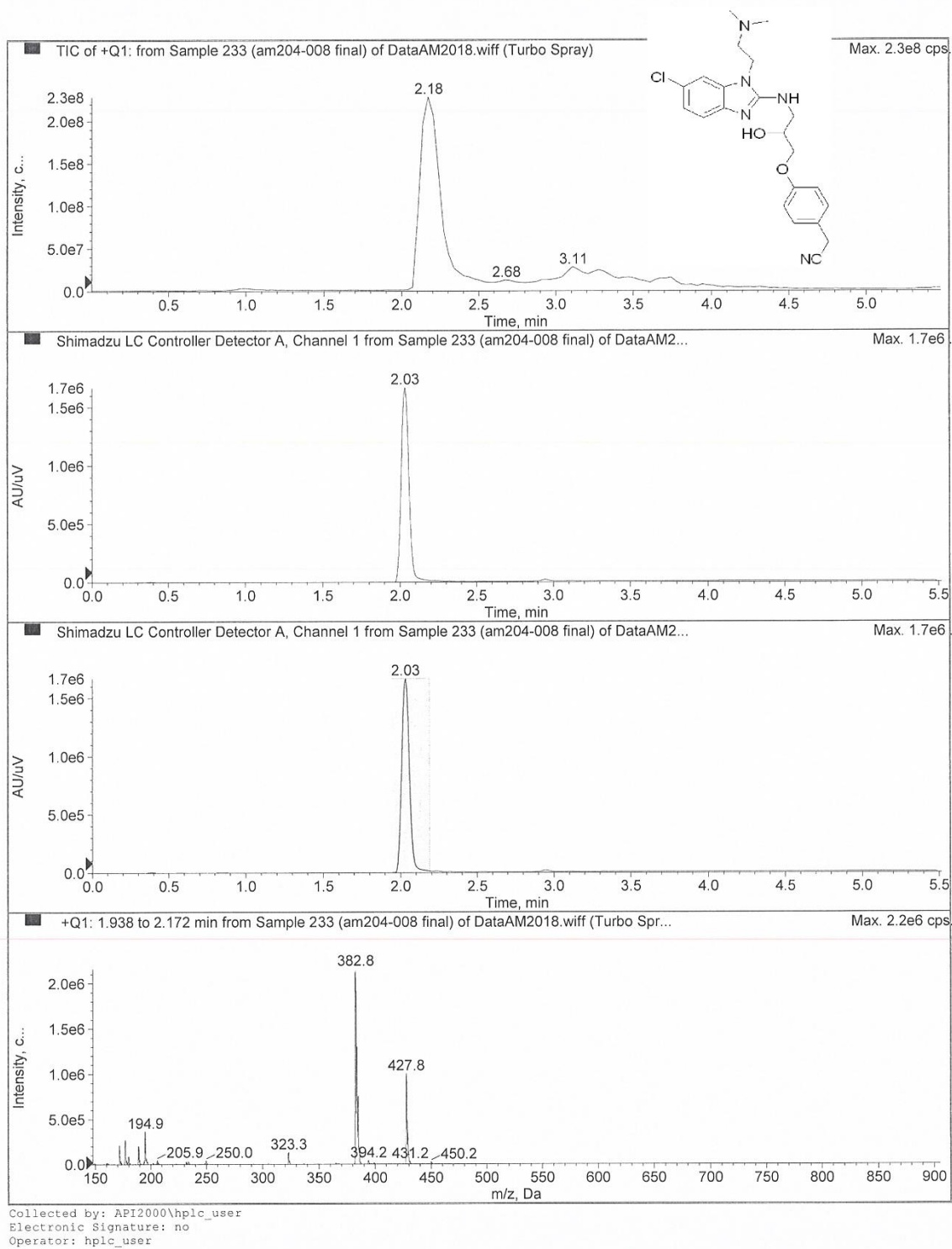
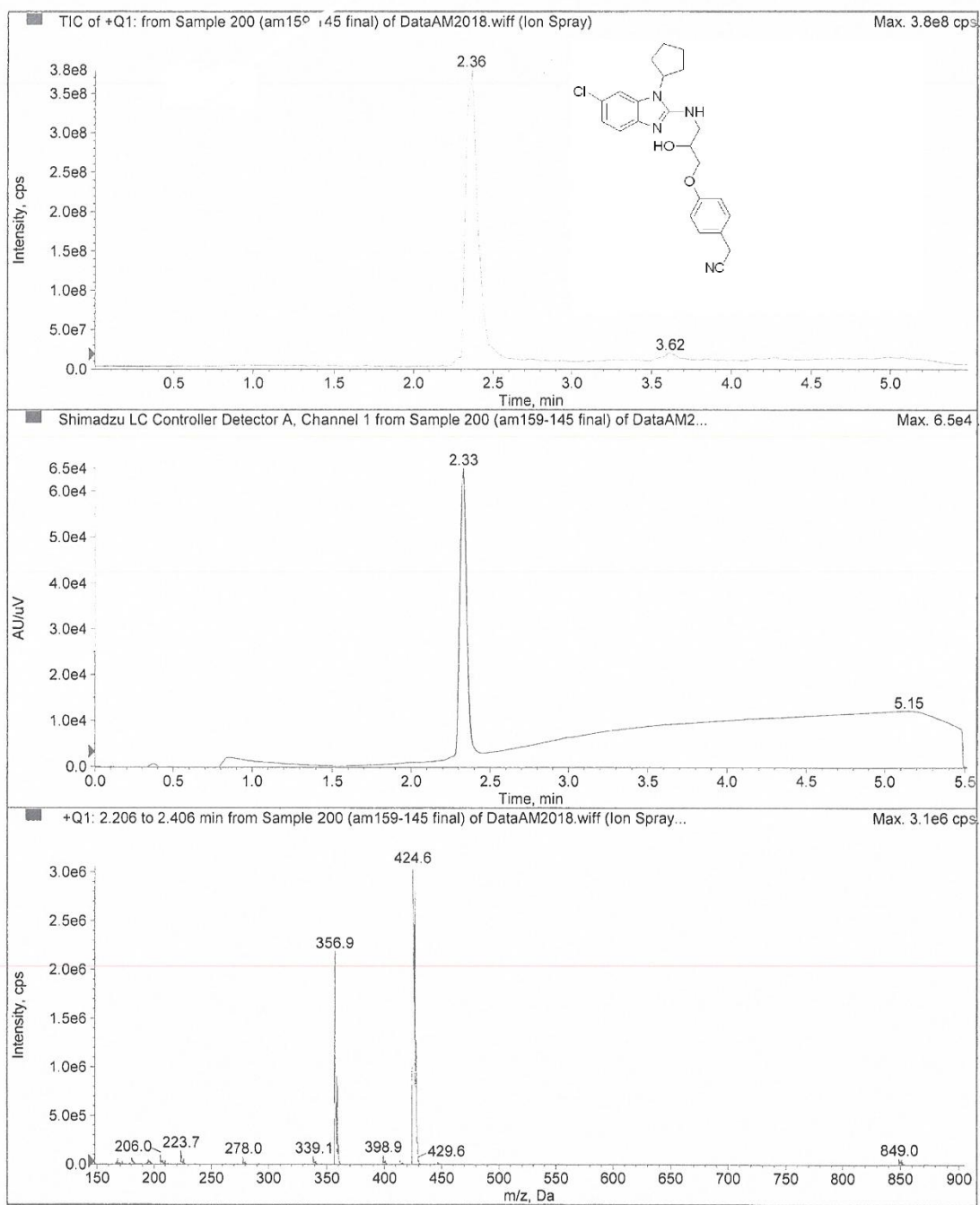


Figure S35. LCMS spectrum of **6q**.

Printing Date: 03 June 2023



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Operator: hplc_user

Figure S36. LCMS spectrum of **6k**.

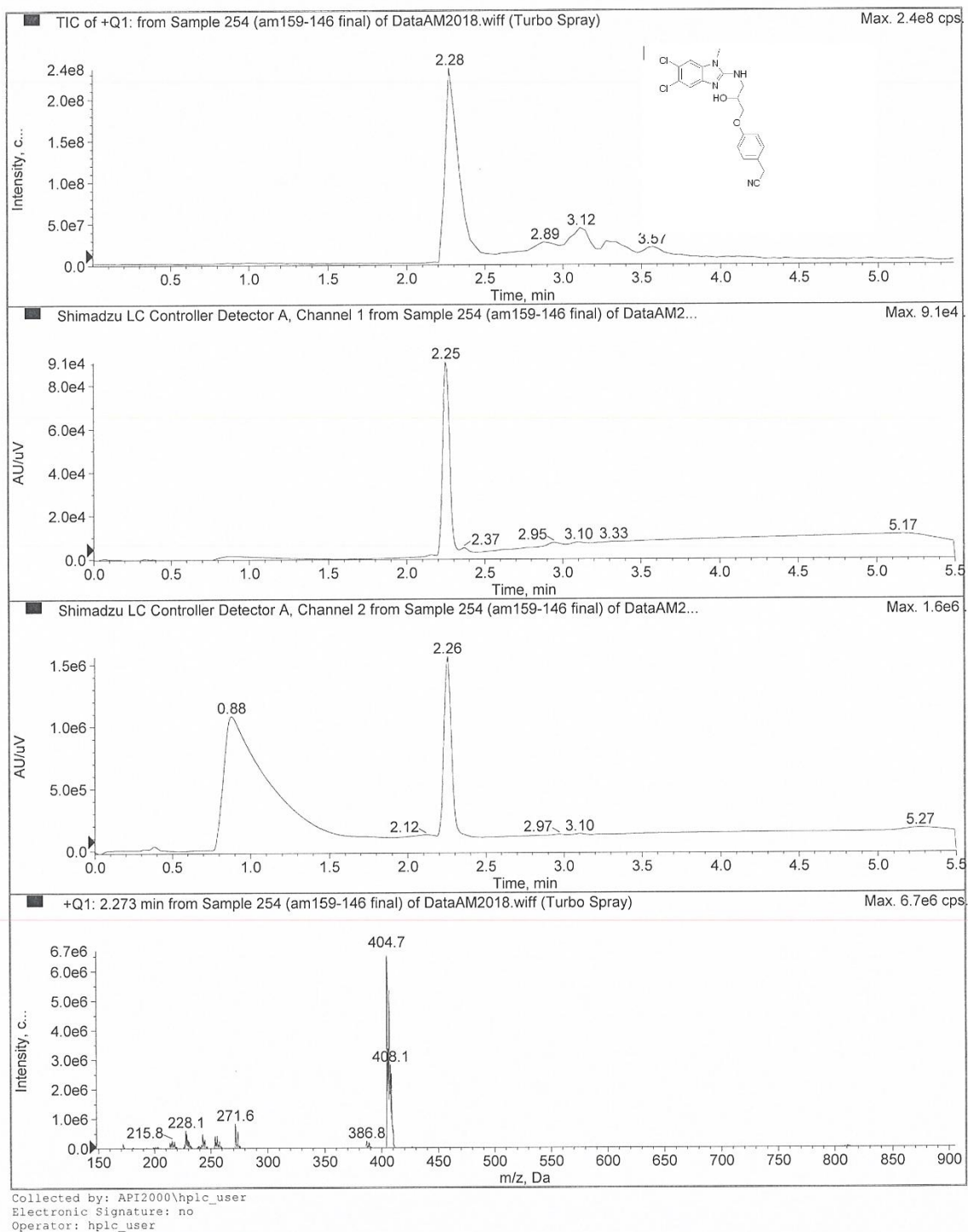


Figure S37. LCMS spectrum of 6c.

Printing Time: 13:29:55
Printing Date: 24 August 2023

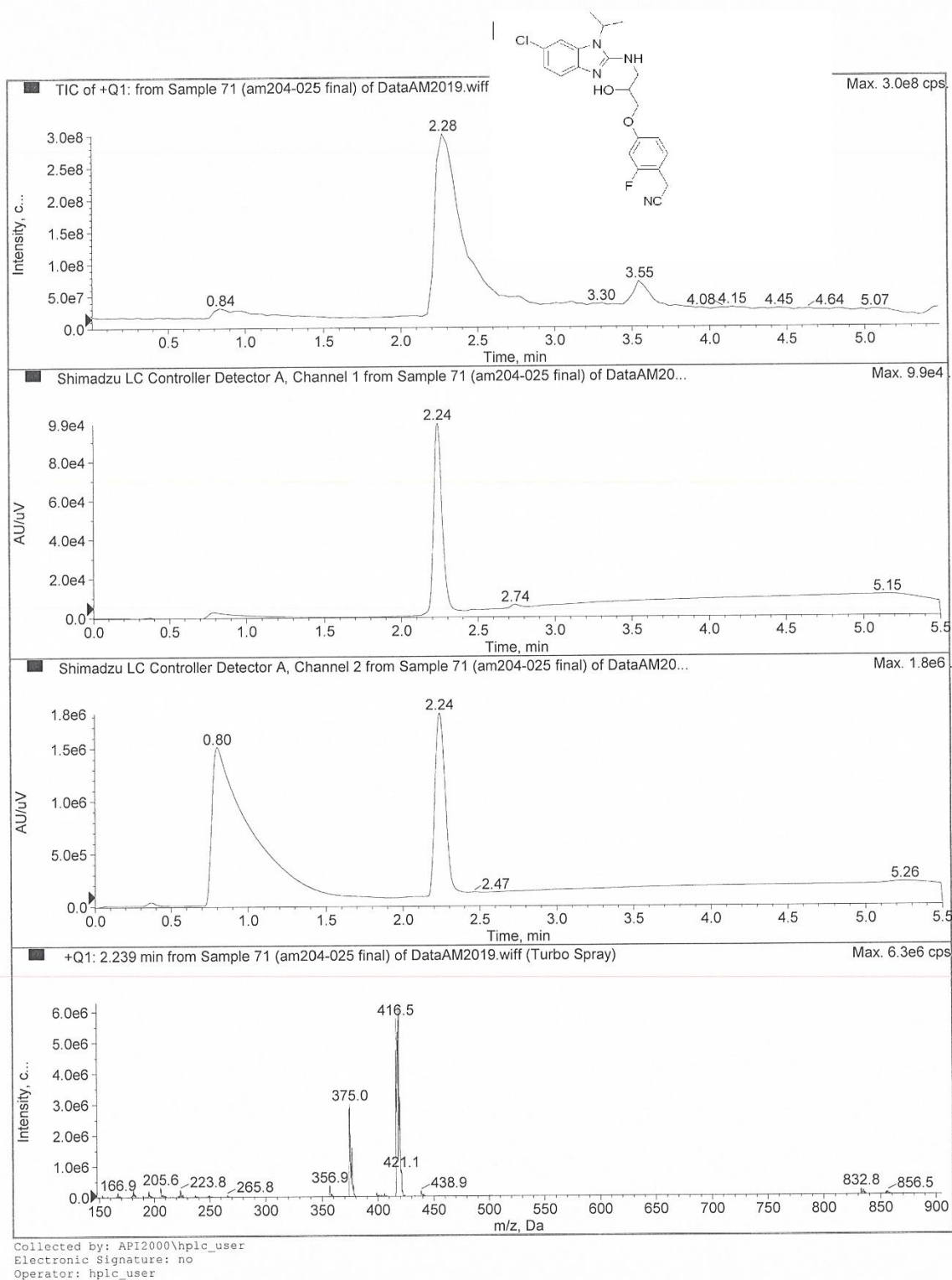
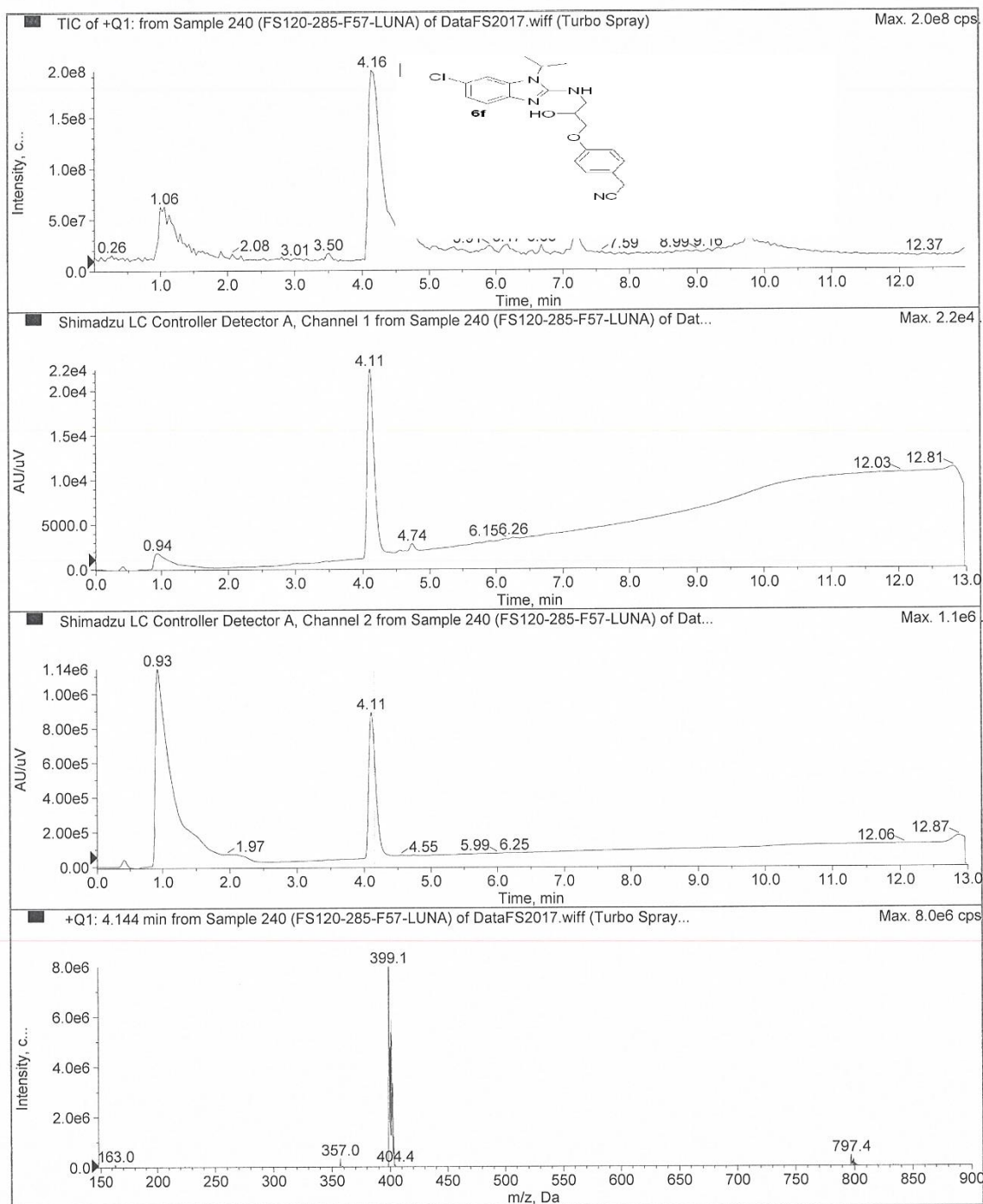


Figure S38. LCMS spectrum of 60.



Collected by: API2000\hplc_user
Electronic Signature: no
Operator: hplc_user

Figure S39. LCMS spectrum of **6f**.

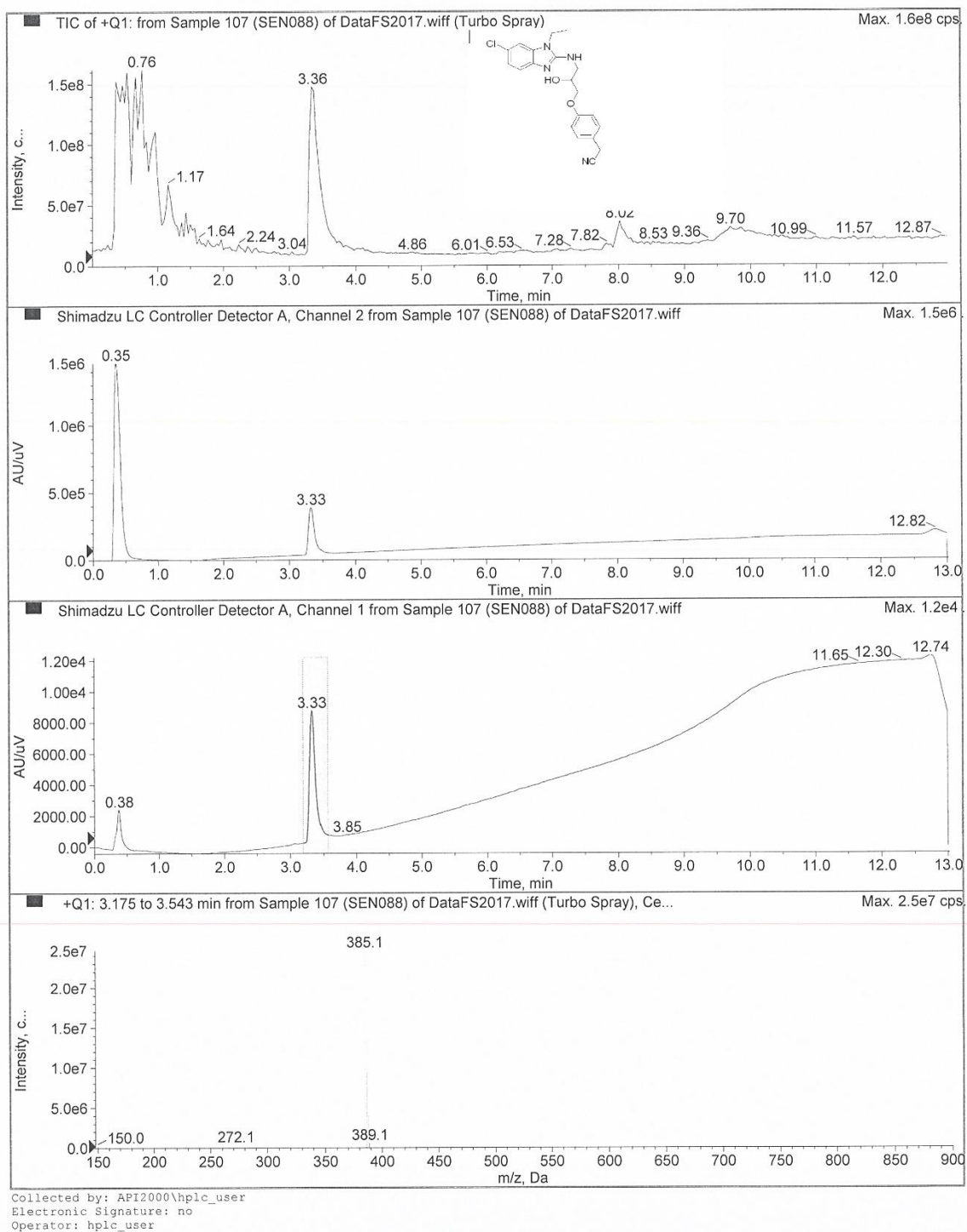


Figure S40. LCMS spectrum of **6e**.

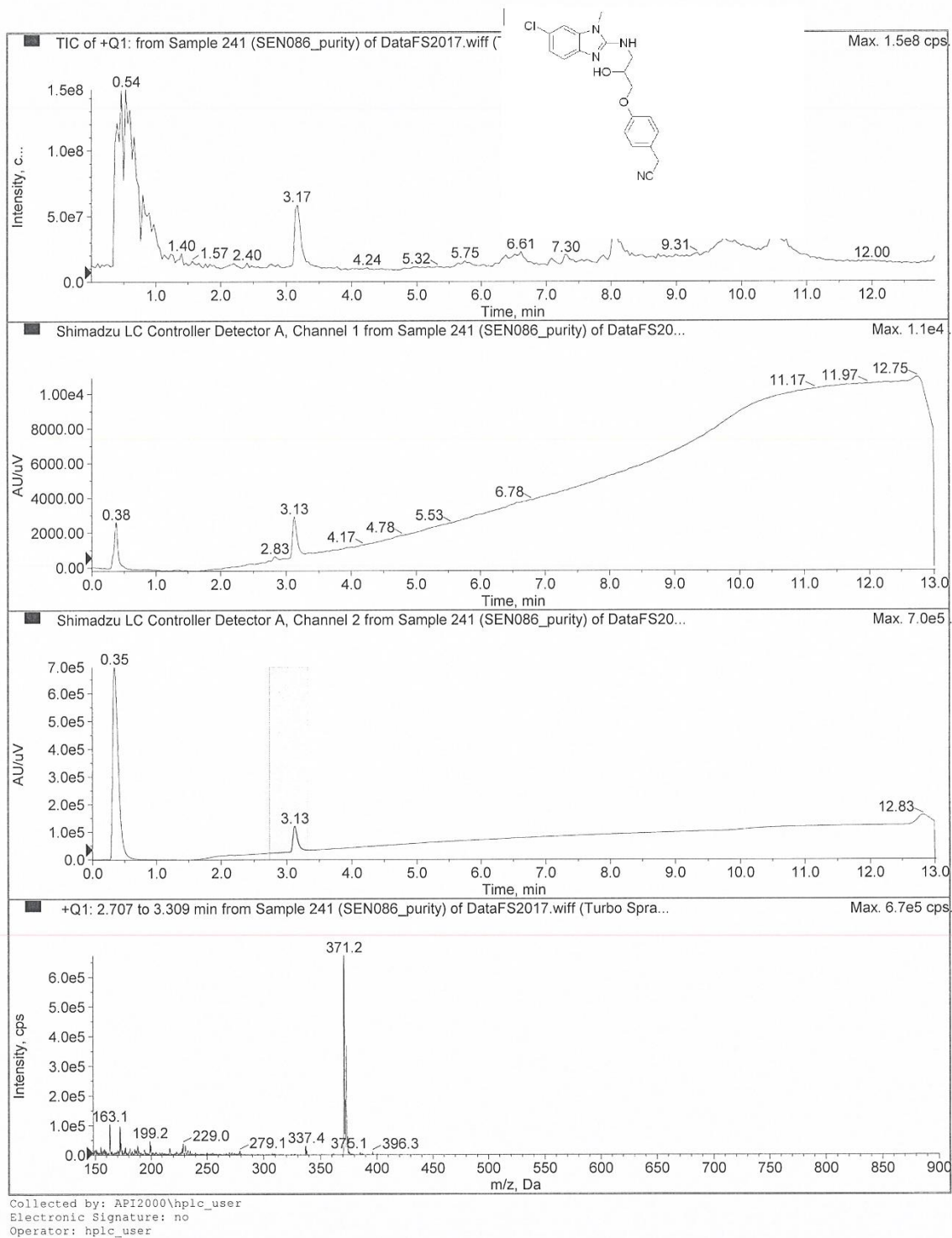


Figure S41. LCMS spectrum of **6a**.

References

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- (5) Winstanley, C.; Langille, M. G. I.; Fothergill, J. L.; Kukavica-Ibrulj, I.; Paradis-Bleau, C.; Sanschagrin, F.; Thomson, N. R.; Winsor, G. L.; Quail, M. A.; Lennard, N.; et al. Newly introduced genomic prophage islands are critical determinants of in vivo competitiveness in the Liverpool Epidemic Strain of *Pseudomonas aeruginosa*. *Genome Res* **2009**, *19* (1), 12-23. DOI: 10.1101/gr.086082.108.
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