Supporting Information

Discovery of PIPE-791, a Potent and Brain-Penetrant Lysophosphatidic Acid Receptor 1 (LPAR1) Antagonist with Slow Tight Binding Characteristics for the Treatment of Neuroinflammatory Disorders

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Table S1. Mean Potencies, Purity, S.E.M. and Biological Replicates of Compounds

Compound	Purity @ 254 nm (%)	LPAR1 Binding K_i		LPAR1 Functional IC_{50}	
		Mean ± S.E.M (nM)	*n*	Mean ± S.E.M (nM)	*n*
13	100.0	12.6 ± 0.8	2	45.9 ± 6.3	2
14	100.0	45.0 ± 3.4	2	58.9 ± 3.9	2
15	100.0	2.1 ± 0.2	2	7.1 ± 1.7	2
18	100.0	9.1 ± 2.4	2	38.7 ± 3.5	6
19	99.9	-	-	45.3 ± 0.9	2
20	99.6	1.1 ± 0.4	2	1.7 ± 0.2	2
21	100.0	0.87 ± 0.08	2	10.0 ± 6.6	4
22	97.9	-	-	159 ± 15	2
23	99.7	-	-	1,310 ± 15	2
24	96.9	4.2 ± 0.3	2	7.6 ± 2.2	2
25	100.0	38.7 ± 12.0	2	72.9 ± 13.0	2
26	99.8	10.2 ± 1.2	2	77.3 ± 2.6	2
27	100.0	-	-	252 ± 6	2
28	100.0	-	-	899 ± 11	2
29	100.0	17.0 ± 6.6	2	49.0 ± 5.4	4
30	100.0	-	-	4,130 ± 1,015	2
31	100.0	-	-	246 ± 21	2
32	95.1	-	-	666 ± 3	2
33	98.5	-	-	154 ± 28	2
34	99.9	-	-	5,620 ± 1,515	2
35	99.7	-	-	355 ± 13	2
36	98.4	10.7 ± 3.2	2	91.5 ± 28.4	2
37	99.0	-	-	543 ± 47	2
38	95.1	2.2 ± 0.6	2	53.9 ± 10.8	2
39	96.9	2.9 ± 1.9	2	50.1 ± 5.1	2
40	99.2	1.6 ± 0.4	2	28.0 ± 6.7	2
41	100.0	-	-	45.2 ± 0.9	2
42	95.1	-	-	149 ± 83	2
43	99.9	-	-	139 ± 12	2
44	99.3	1.5 ± 0.5	2	18.4 ± 0.5	4
45	100.0	-	-	22.5 ± 8.3	2
46	100.0	-	-	9.8 ± 1.2	2
47	100.0	0.75 ± 0.08	4	9.9 ± 1.6	8
47-LM-M1	98.7	-	-	2,130 ± 490	2
47-LM-M2	100.0	-	-	233 ± 6	2
47-LM-M3	100.0	-	-	68.9 ± 7.2	2
48	100.0	-	-	9.4 ± 0.6	2
49	100.0	-	-	7.1 ± 0.6	2
50	99.7	-	-	25.9 ± 6.5	2

51	99.8	-	-	3.6 ± 0.4	2
52	99.9	-	-	1.9 ± 0.3	2

Table S2. PIPE-791 (47) Reaction Phenotyping using Recombinant CYP Enzymes

CYP	% Remaining of Unchanged **PIPE-791** (**47**) at Various Times					Half-Life (min)
	5 min	10 min	20 min	40 min	60 min	
1A2	101	98.9	103	100	98.5	>60
2B6	101	98.7	101	98.9	99.4	>60
2C8	82.0	55.6	34.6	12.1	6.0	13.2
2C9	102	101	102	98.4	99.6	>60
2C19	101	100	99.2	98.8	96.5	>60
2D6	101	103	104	101	101	>60
3A4	99.8	96.6	96.4	92.9	87.9	>60
3A5	101	101	202	97.4	97.4	>60

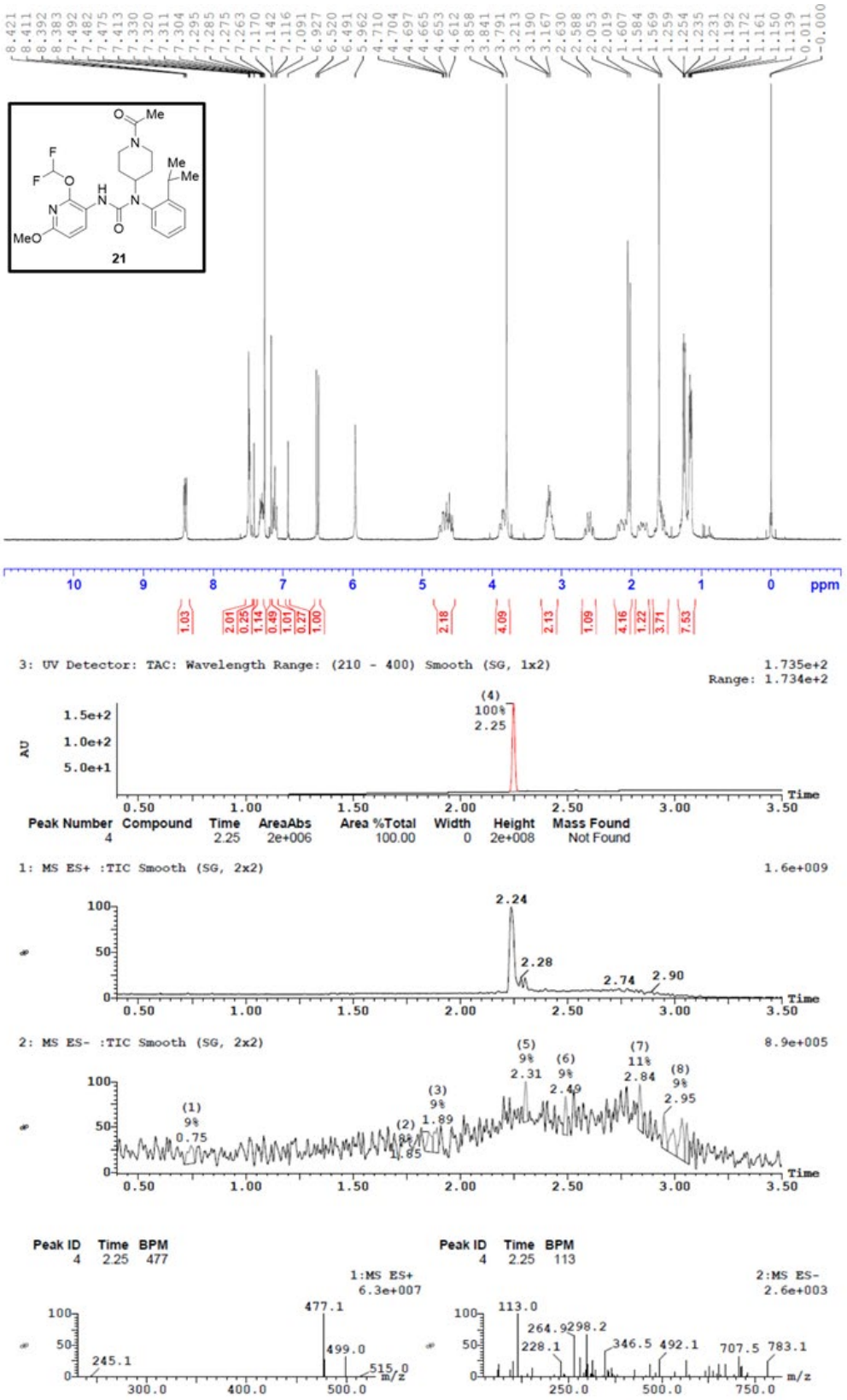
Table S3. MDR1 and BCRP Substrate Evaluation for PIPE-791 (47)

Transporter	**PIPE-791** (**47**) Concentration (μM)	Efflux Ratio		Net Flux Ratio
		Transporter-Expressing Monolayers	Control Monolayers	
MDR1	0.3	1.72	1.33	1.30
	3	1.35	1.05	1.29
	30	1.40	0.90	1.55
BCRP	0.3	1.71	1.36	1.26
	3	1.32	1.17	1.13
	30	0.97	0.98	0.99

Table S4. Transporter-Mediated Accumulation of PIPE-791 (47)

Transporter	Transporter-Mediated Accumulation Fold of **PIPE-791** (**47**) at Tested Incubation Concentrations			
	0.3 μM	1 μM	3 μM	10 μM
MATE1	1.12	0.99	1.01	1.00
MATE2-K	1.01	0.83	0.98	0.98
OAT1	0.92	0.92	0.84	0.91
OAT3	1.05	1.00	0.99	1.00
OATP1B1	1.26	1.13	1.19	1.01
OATP1B3	0.72	0.71	0.65	0.71
OCT1	1.16	0.89	1.12	1.00
OCT2	0.88	1.01	1.11	0.98
BSEP	0.75	1.37	0.86	0.88

Figure S1. ^{1}H NMR ($CDCl_3$) and LC-MS Data for Compound 21



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
477.2310	477.2313	-0.3	-0.6	10.5	C24 H31 N4 O4 F2	819.0	n/a	n/a	24	31	4	4	2

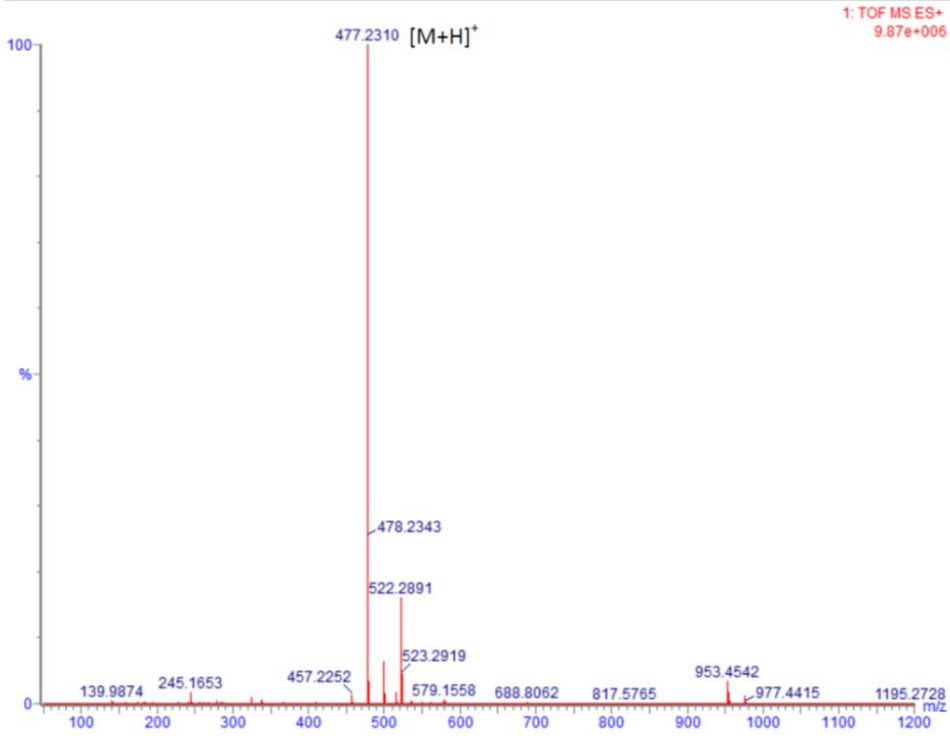
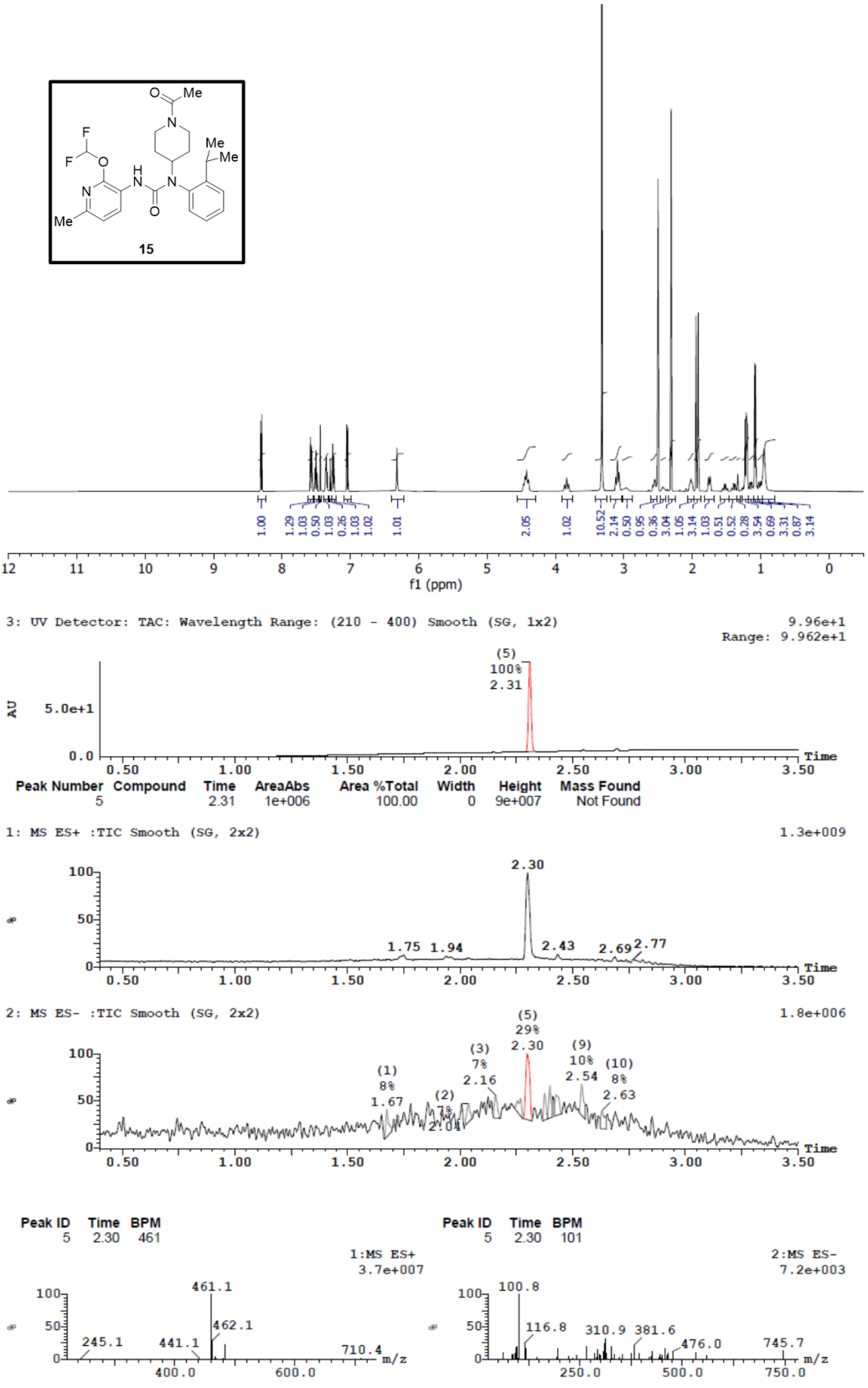


Figure S2. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 15



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
461.2362	461.2364	-0.2	-0.4	10.5	C24 H31 N4 O3 F2	834.8	n/a	n/a	24	31	4	3	2

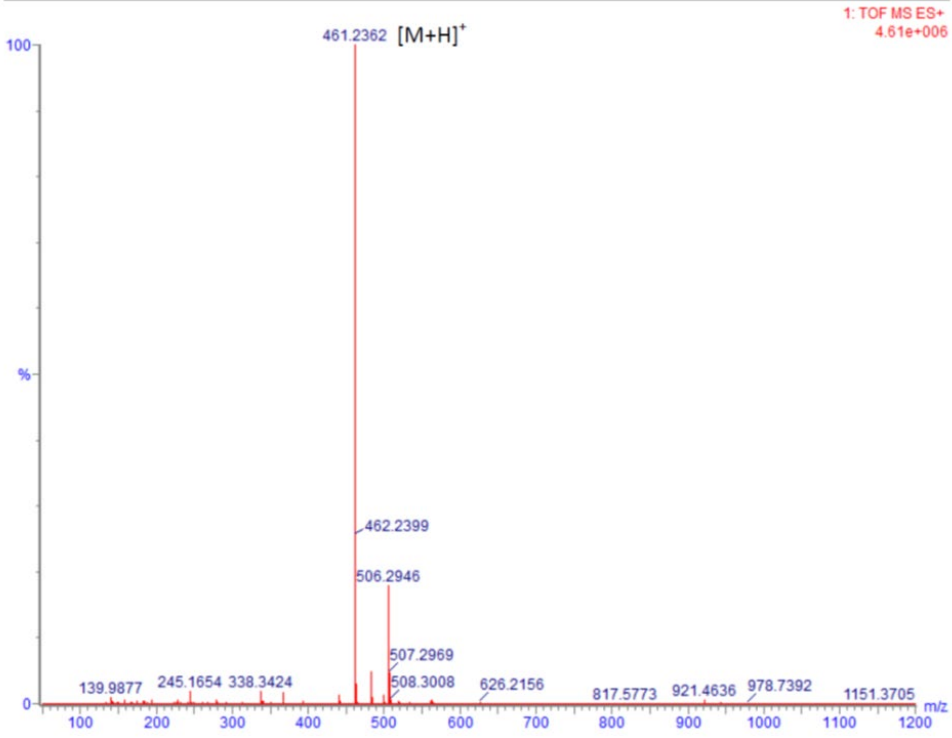
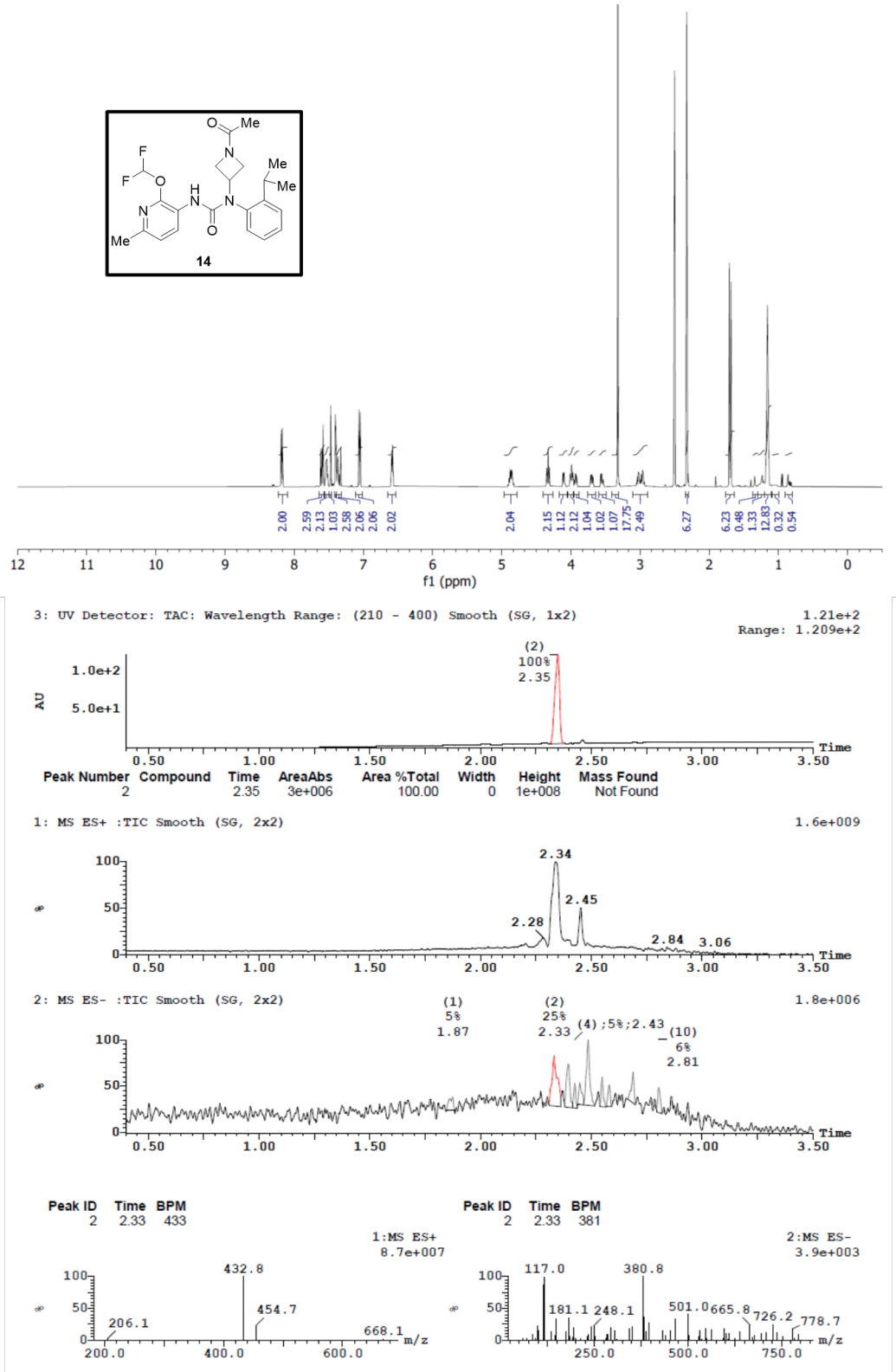


Figure S3. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 14



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
433.2056	433.2051	0.5	1.2	10.5	C22 H27 N4 O3 F2	950.3	n/a	n/a	22	27	4	3	2

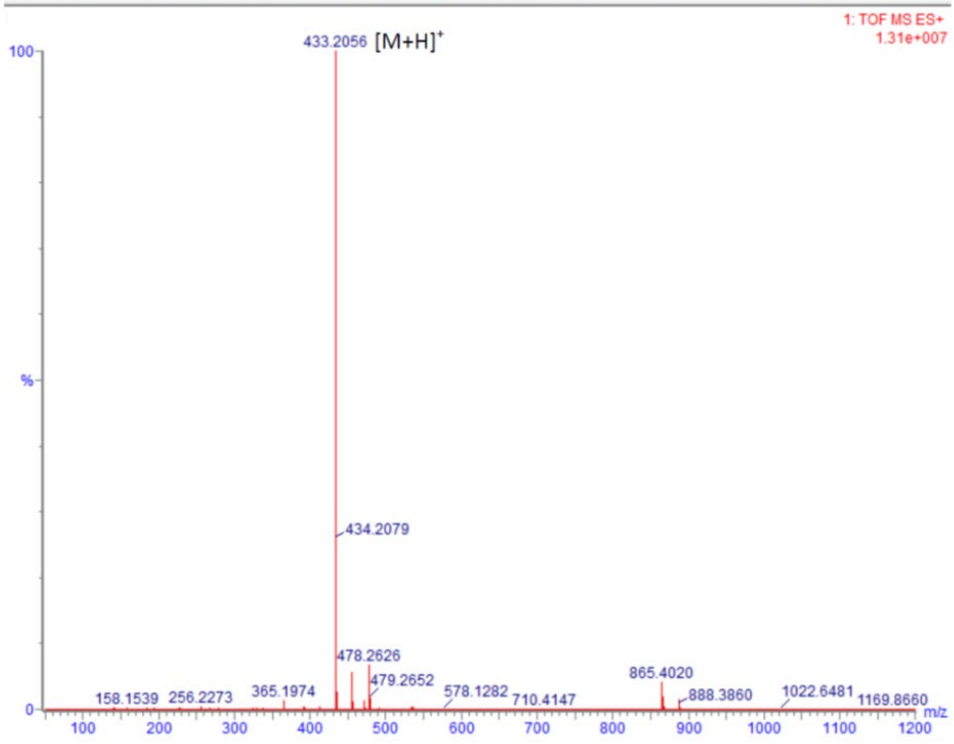
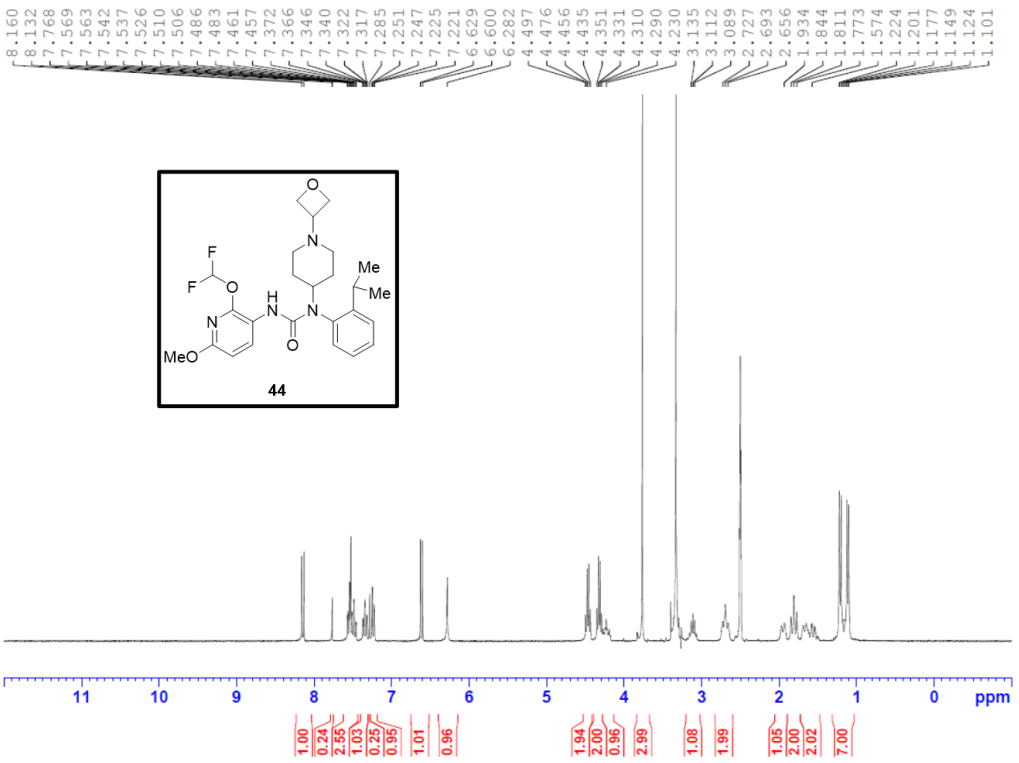
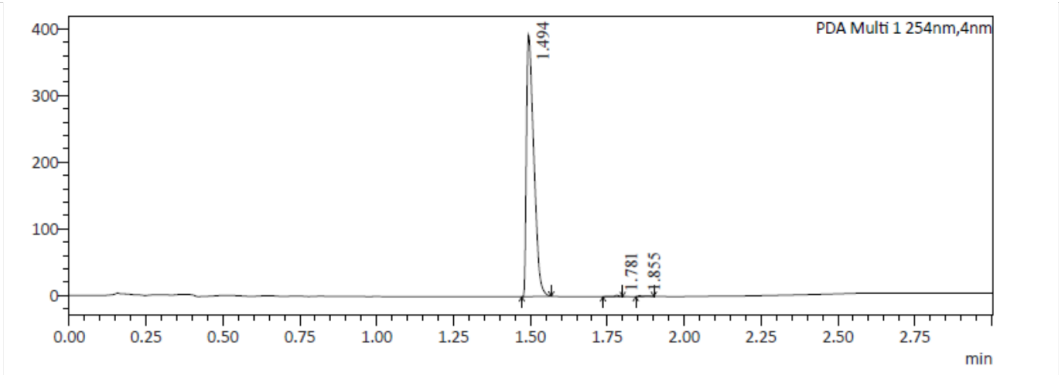


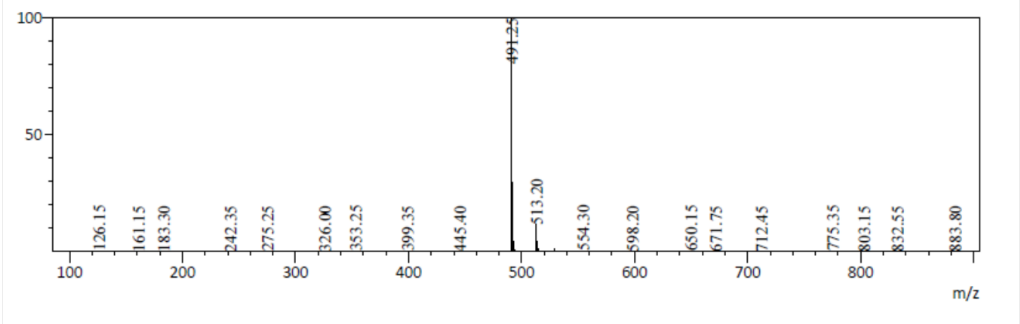
Figure S4. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 44





PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.494	393293	99.104	659827	99.272
2	1.781	2122	0.535	2523	0.380
3	1.855	1434	0.361	2314	0.348
Total		396850	100.000	664663	100.000



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
491.2469	491.2470	-0.1	-0.2	10.5	C25 H33 N4 O4 F2	780.5	n/a	n/a	25	33	4	4	2

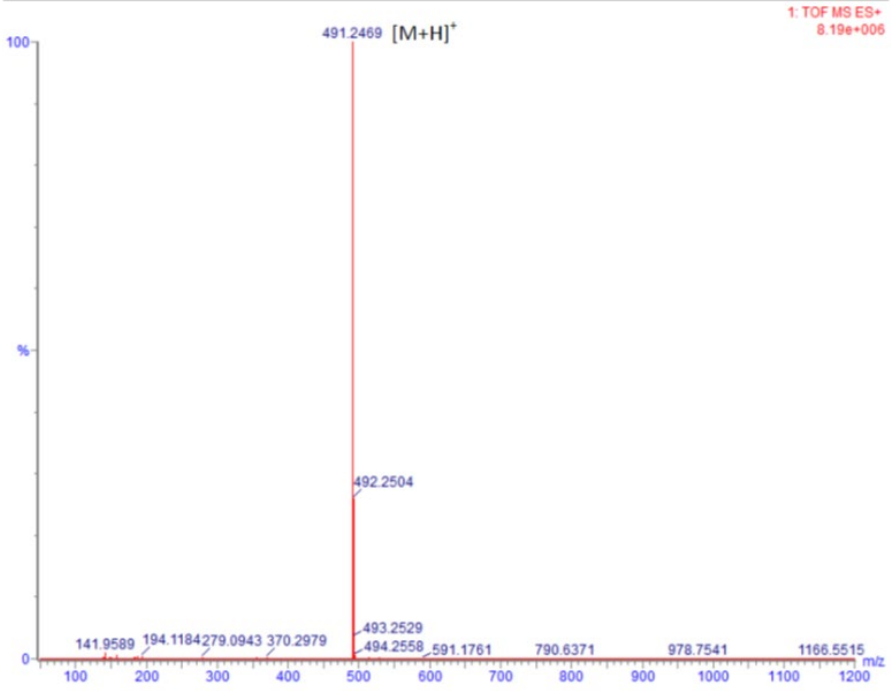
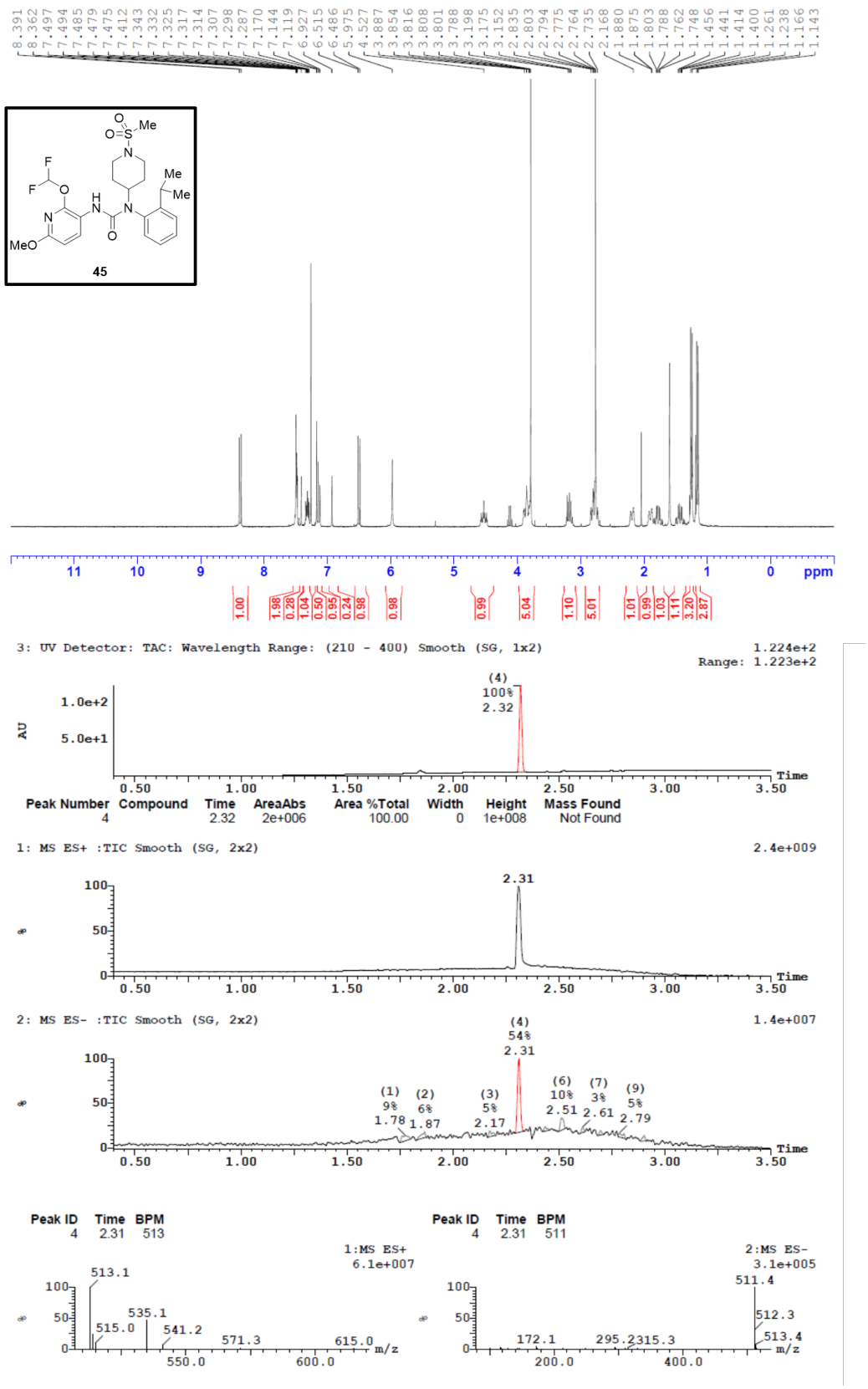


Figure S5. ^{1}H NMR ($CDCl_3$) and LC-MS Data for Compound 45



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F	S
513.1977	513.1983	-0.6	-1.2	9.5	C23 H31 N4 O5 F2 S	798.6	n/a	n/a	23	31	4	5	2	1

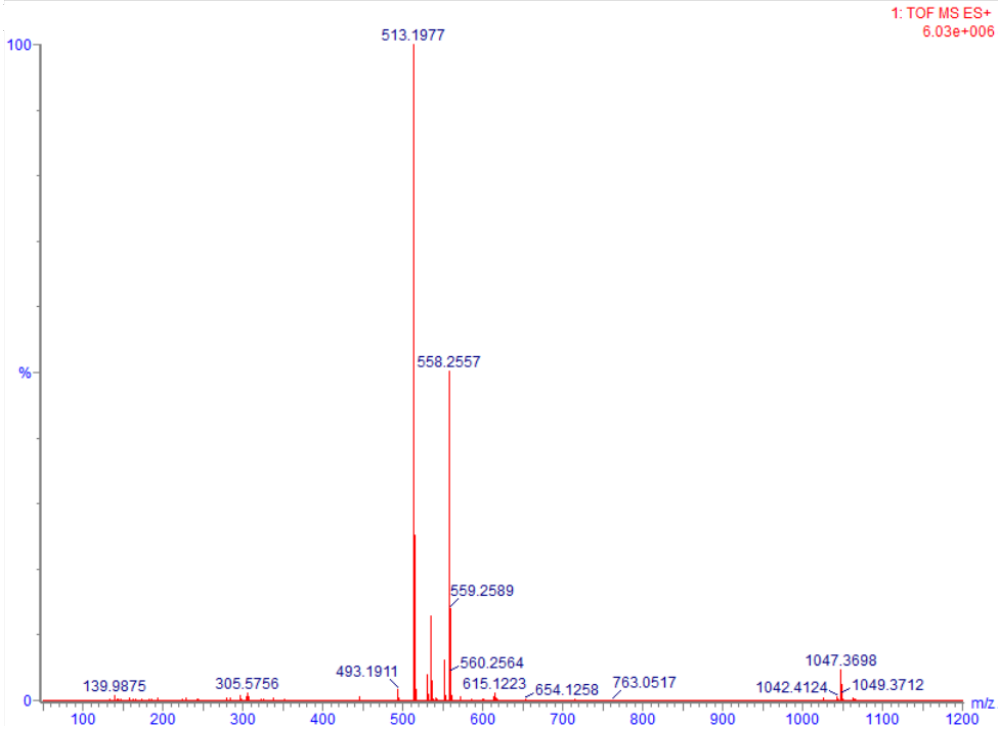
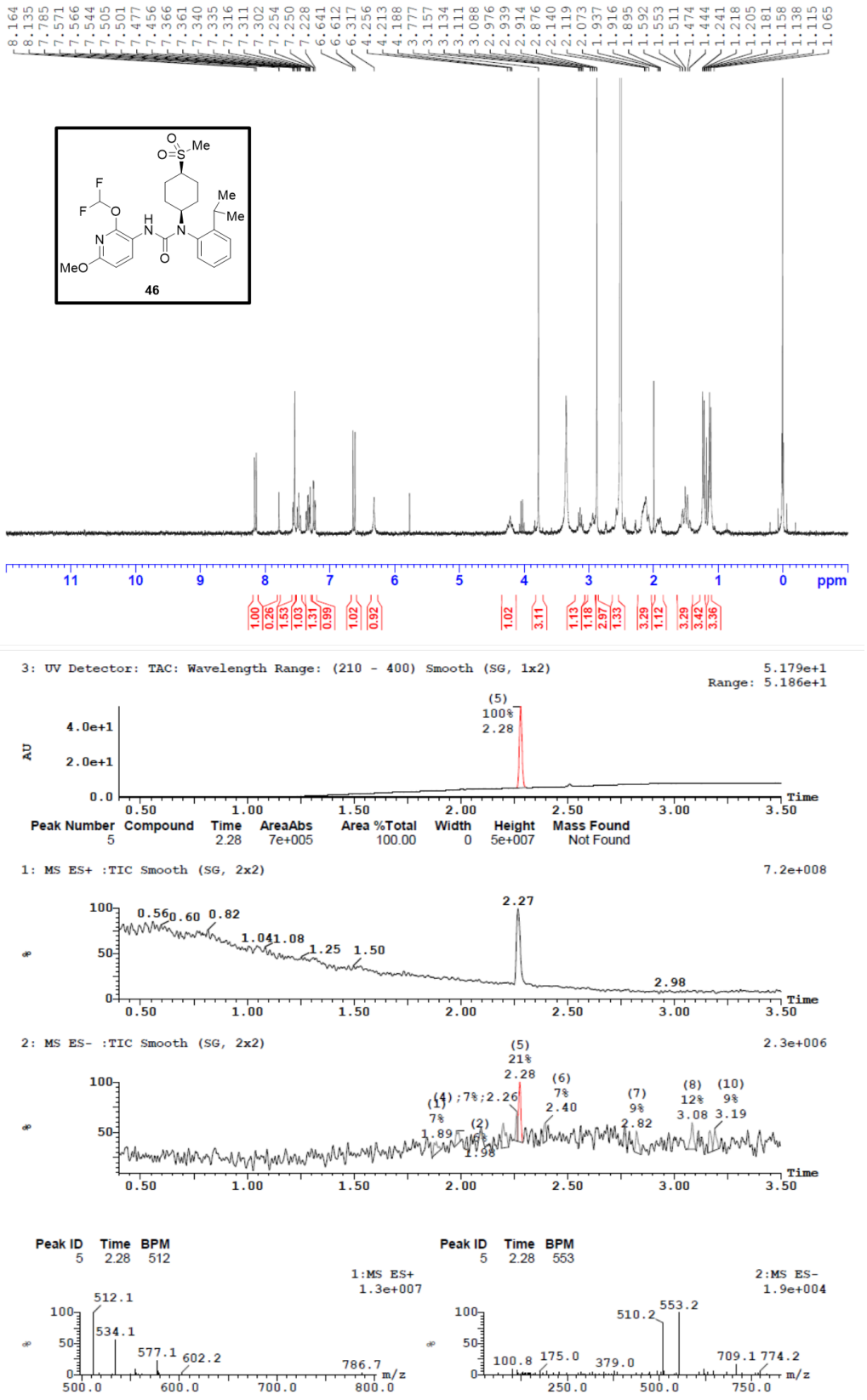


Figure S6. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 46



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F	S
512.2029	512.2031	-0.2	-0.4	9.5	C24 H32 N3 O5 F2 S	721.5	n/a	n/a	24	32	3	5	2	1

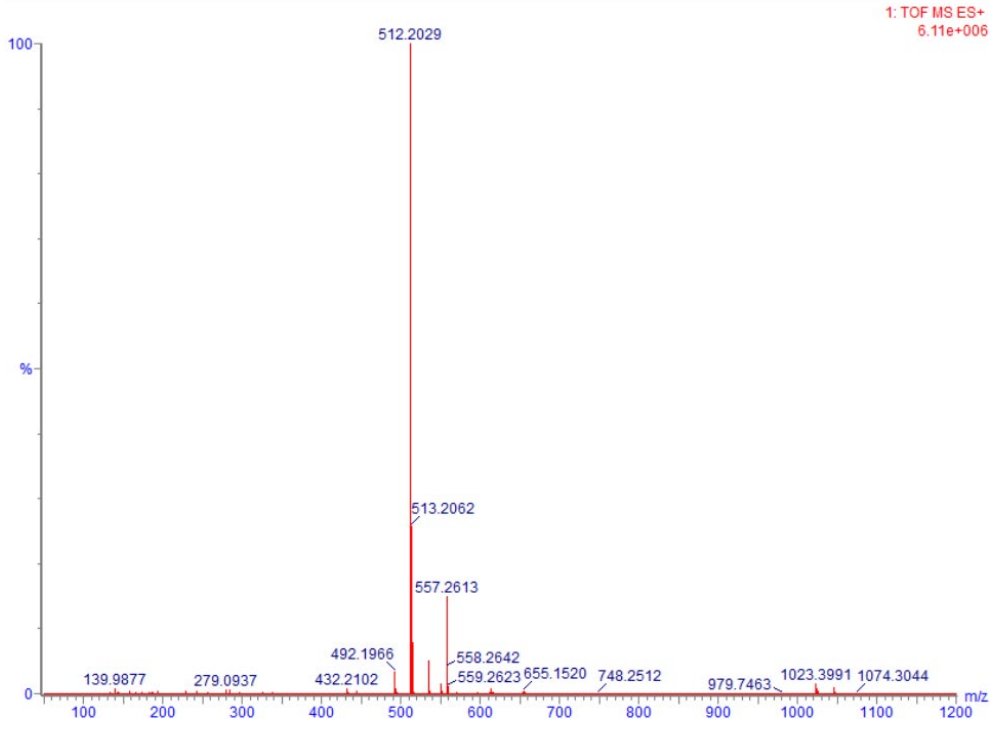
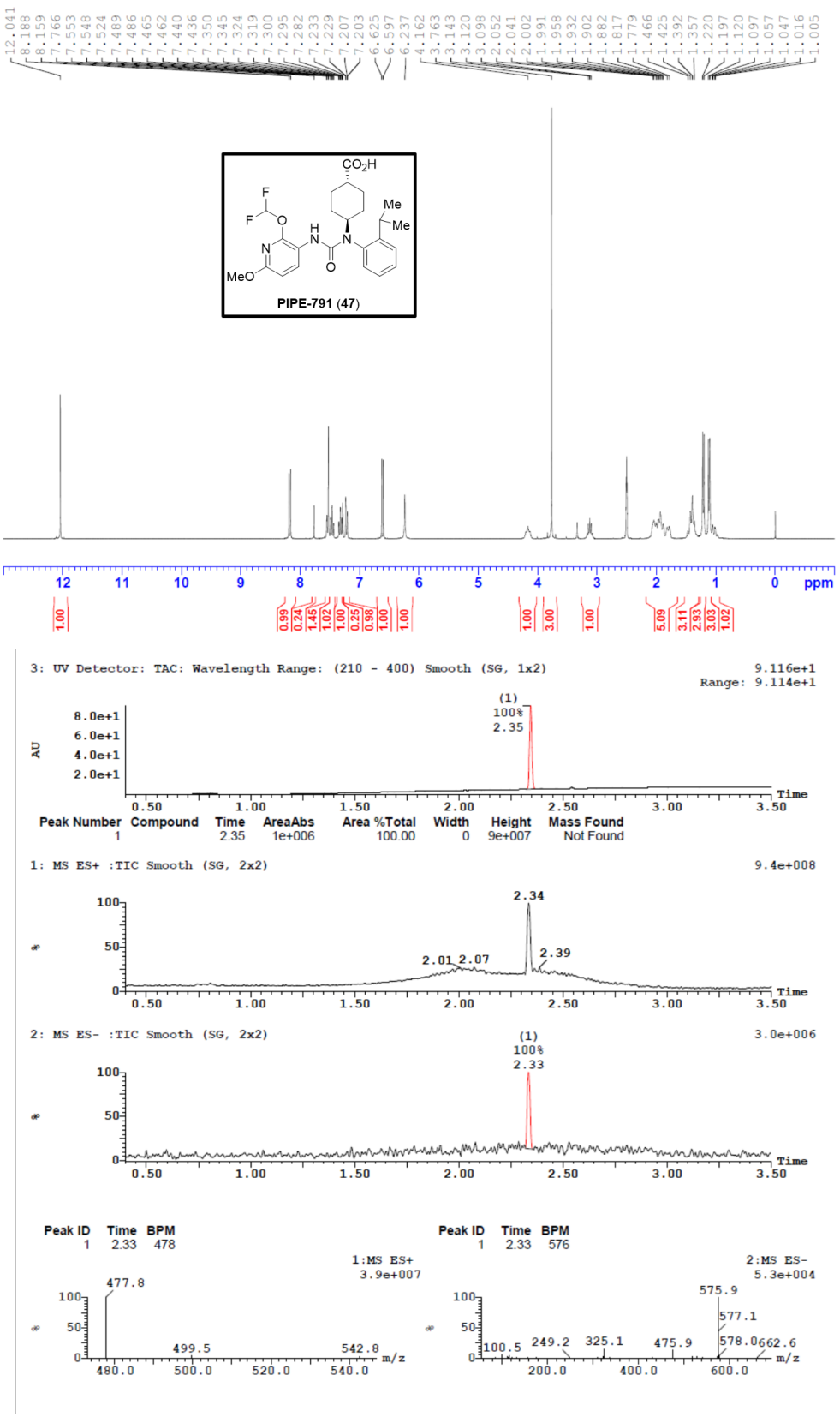


Figure S7. ^{1}H NMR (DMSO-d_6) and LC-MS Data for PIPE-791 (47)



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
478.2154	478.2154	0.0	0.0	10.5	C24 H30 N3 O5 F2	745.2	n/a	n/a	24	30	3	5	2

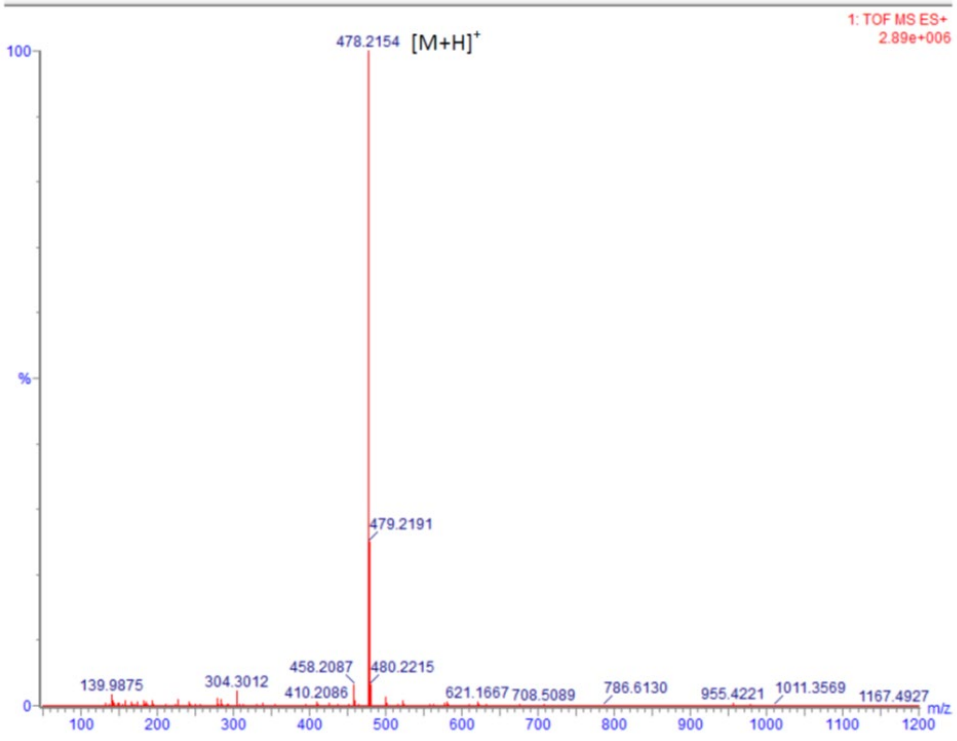


Figure S8. ^{13}C and ^{19}F NMR (DMSO-d_6) Data for PIPE-791 (47)

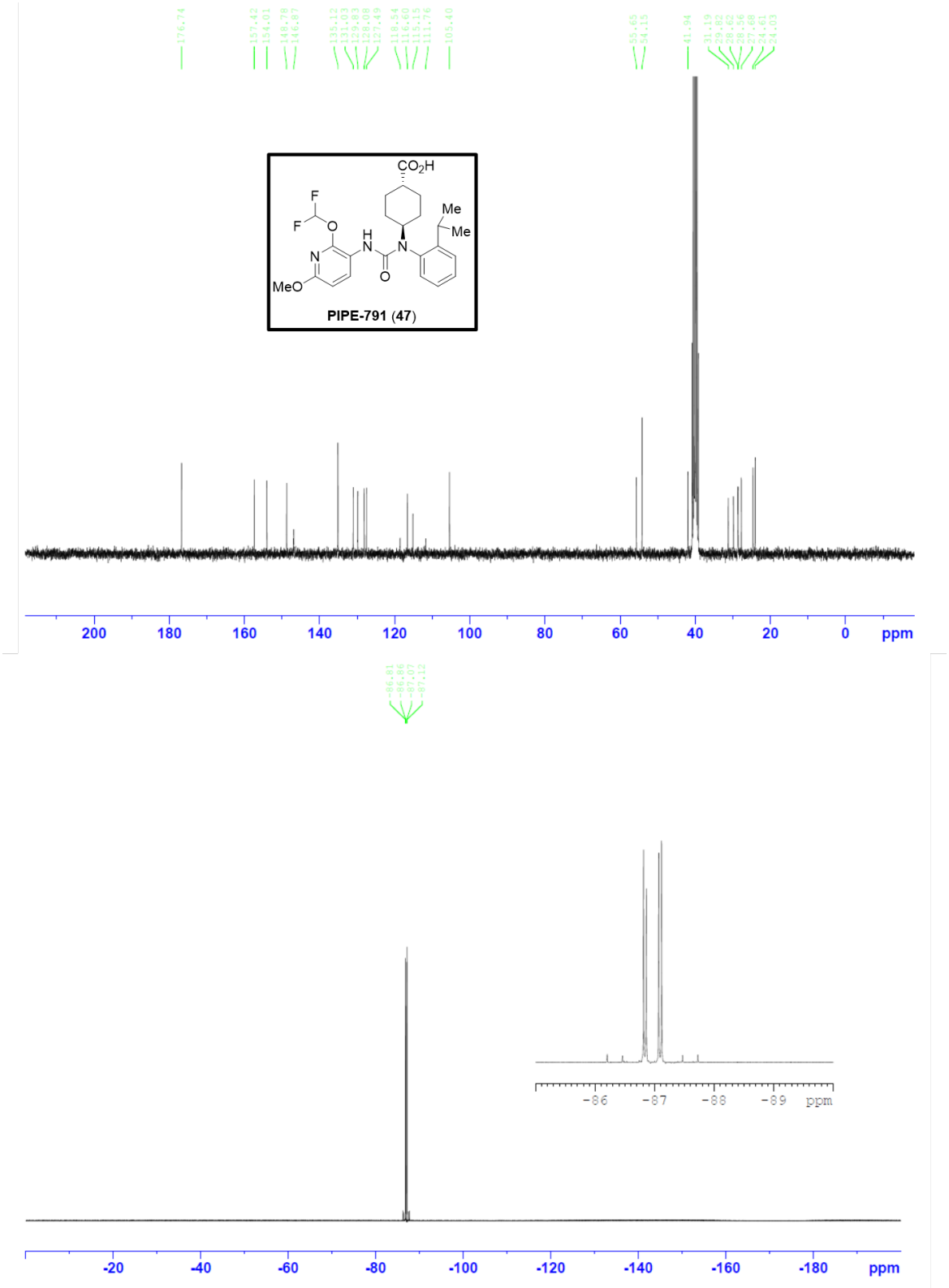
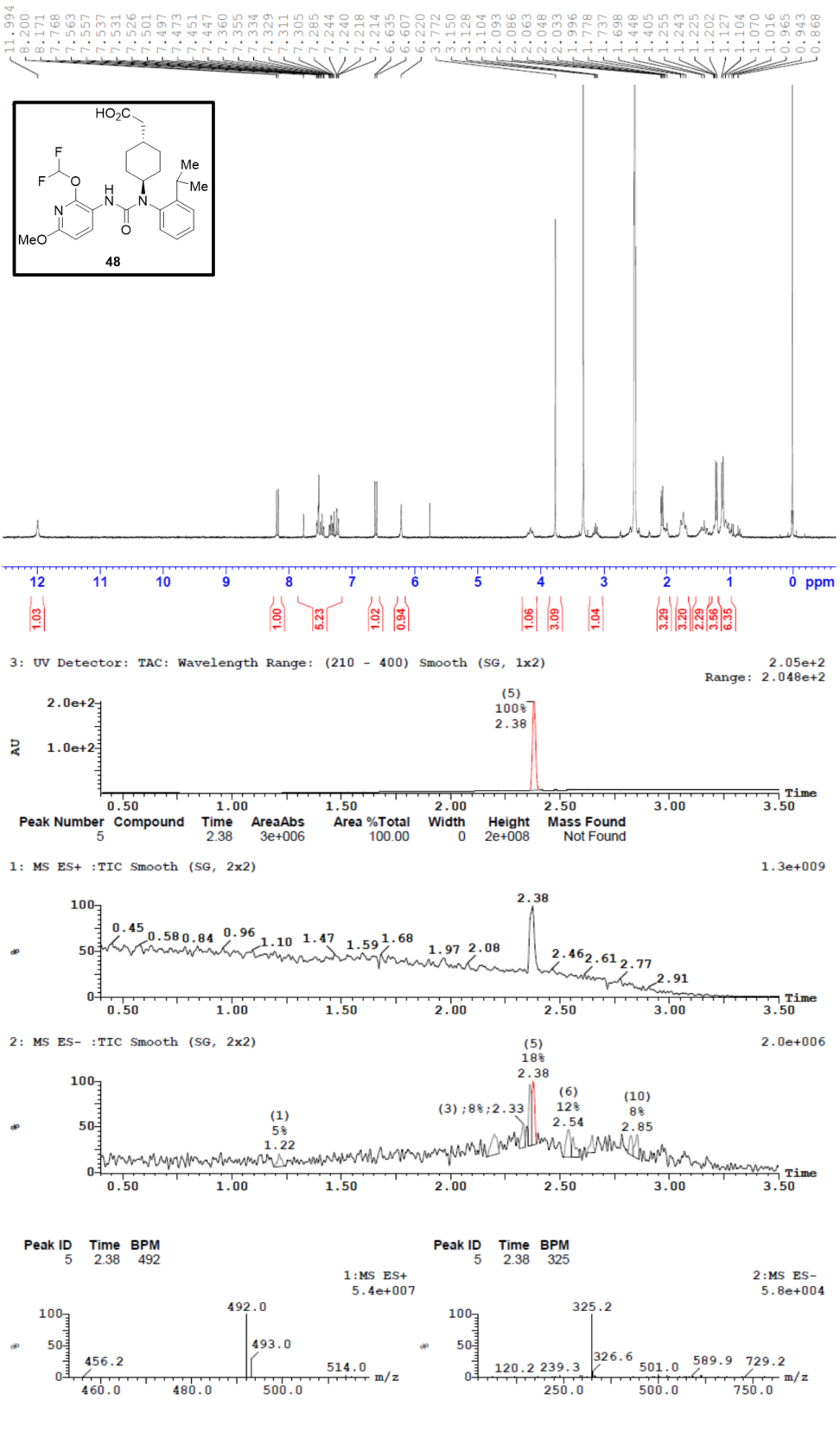


Figure S9. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 48



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
492.2310	492.2310	0.0	0.0	10.5	C25 H32 N3 O5 F2	871.0	n/a	n/a	25	32	3	5	2

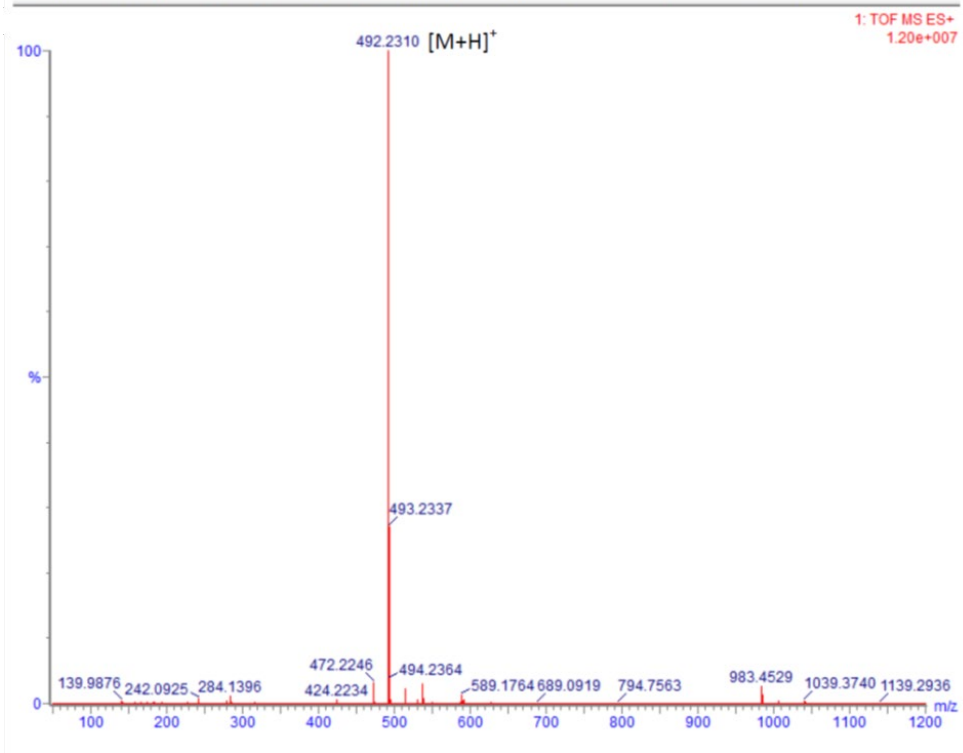
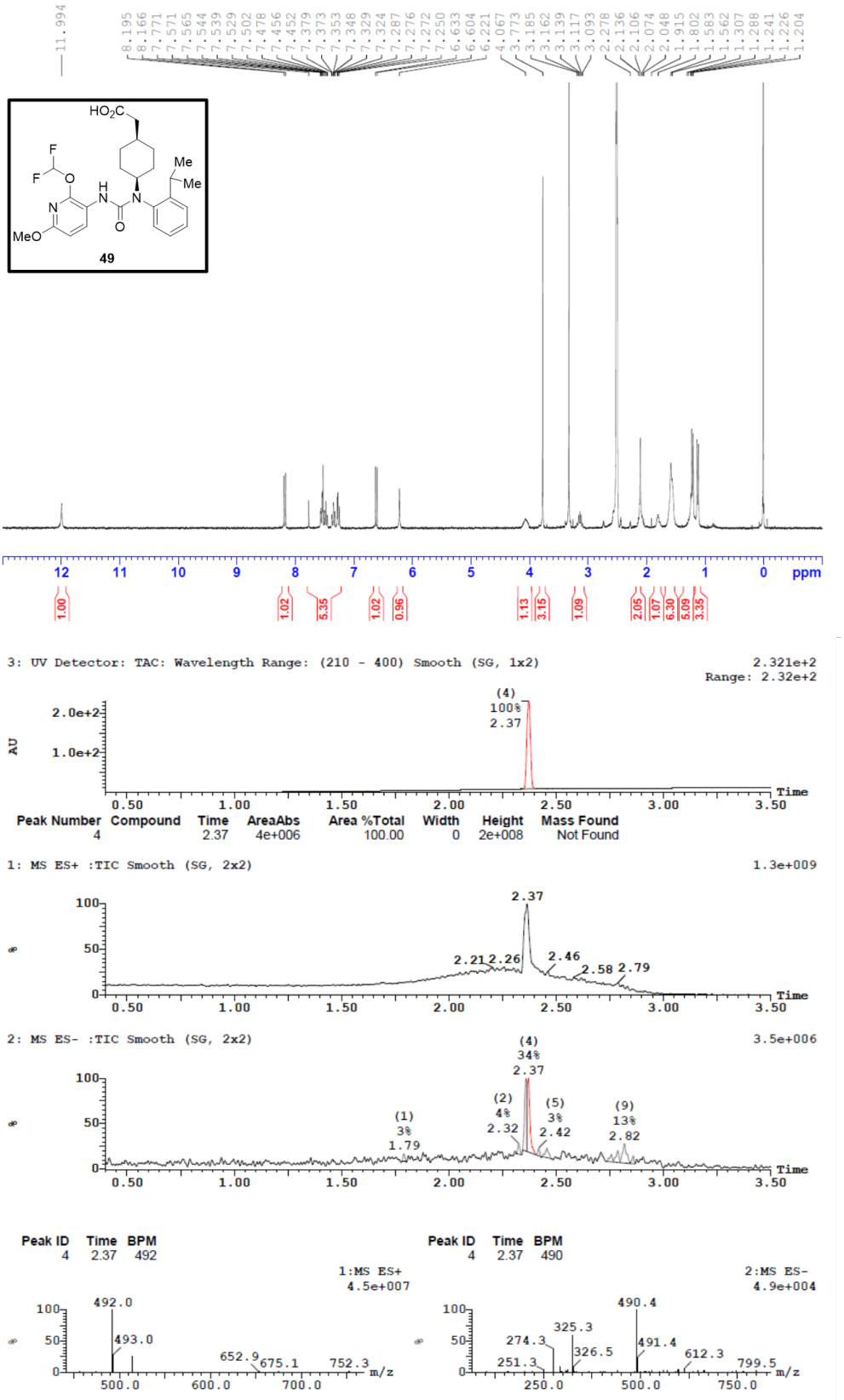


Figure S10. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 49



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
492.2304	492.2310	-0.6	-1.2	10.5	C25 H32 N3 O5 F2	834.9	n/a	n/a	25	32	3	5	2

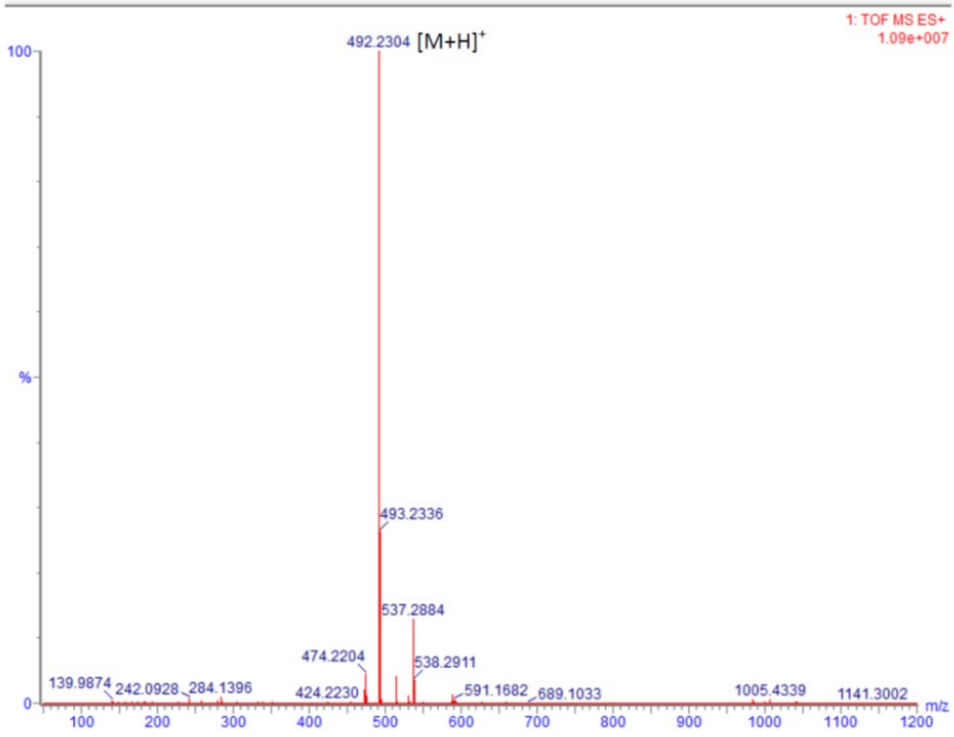
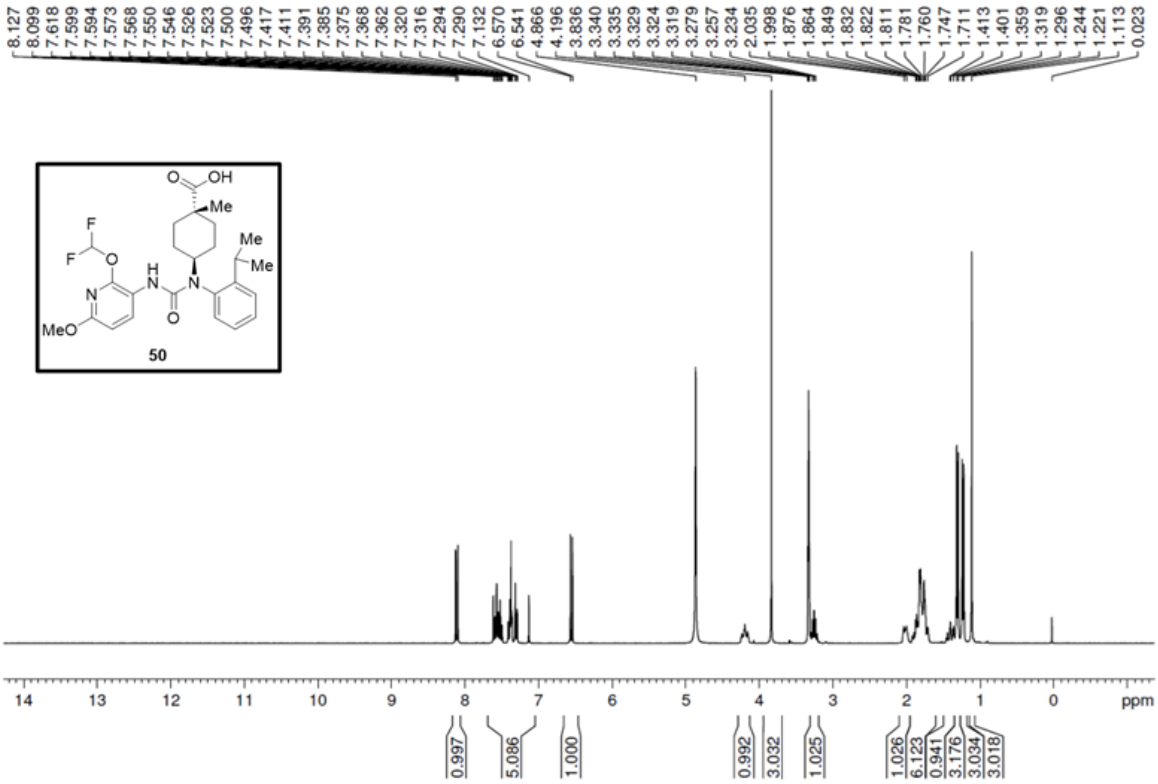
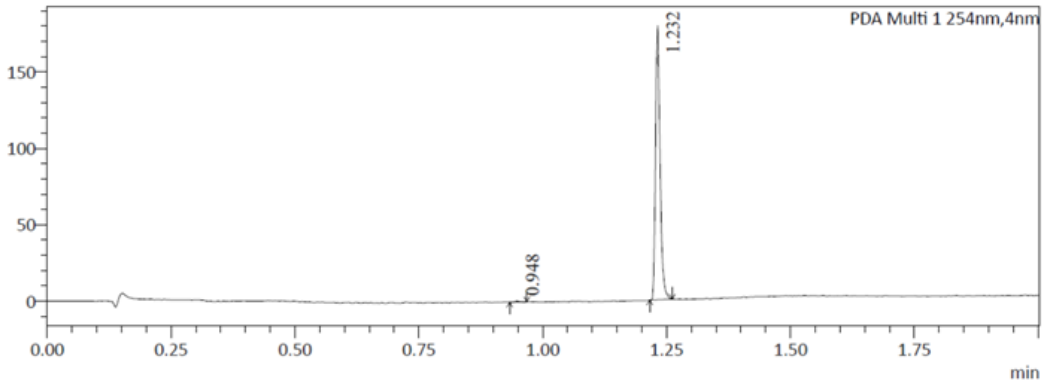


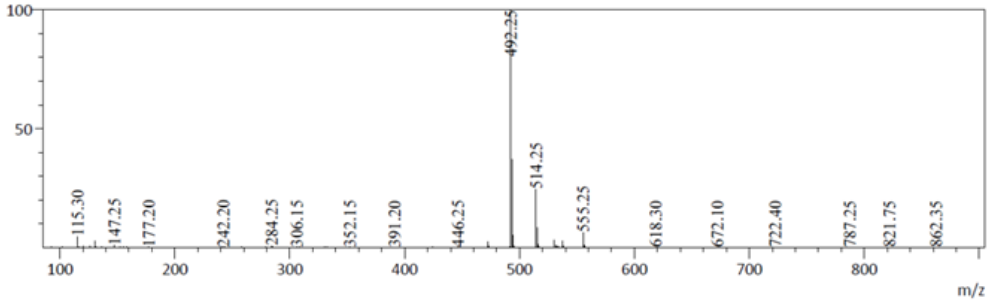
Figure S11. ^{1}H NMR (CD_3OD) and LC-MS Data for Compound 50





PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.948	695	0.385	316	0.275
2	1.232	179619	99.615	114340	99.725
Total		180314	100.000	114656	100.000



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
492.2306	492.2310	-0.4	-0.8	10.5	C25 H32 N3 O5 F2	629.2	n/a	n/a	25	32	3	5	2

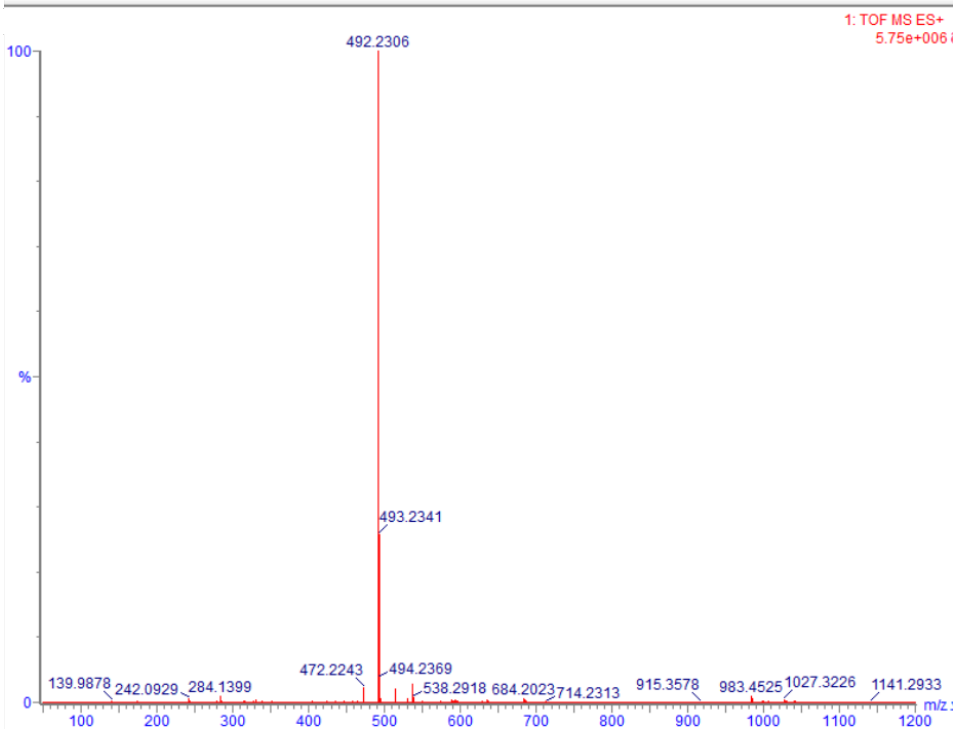
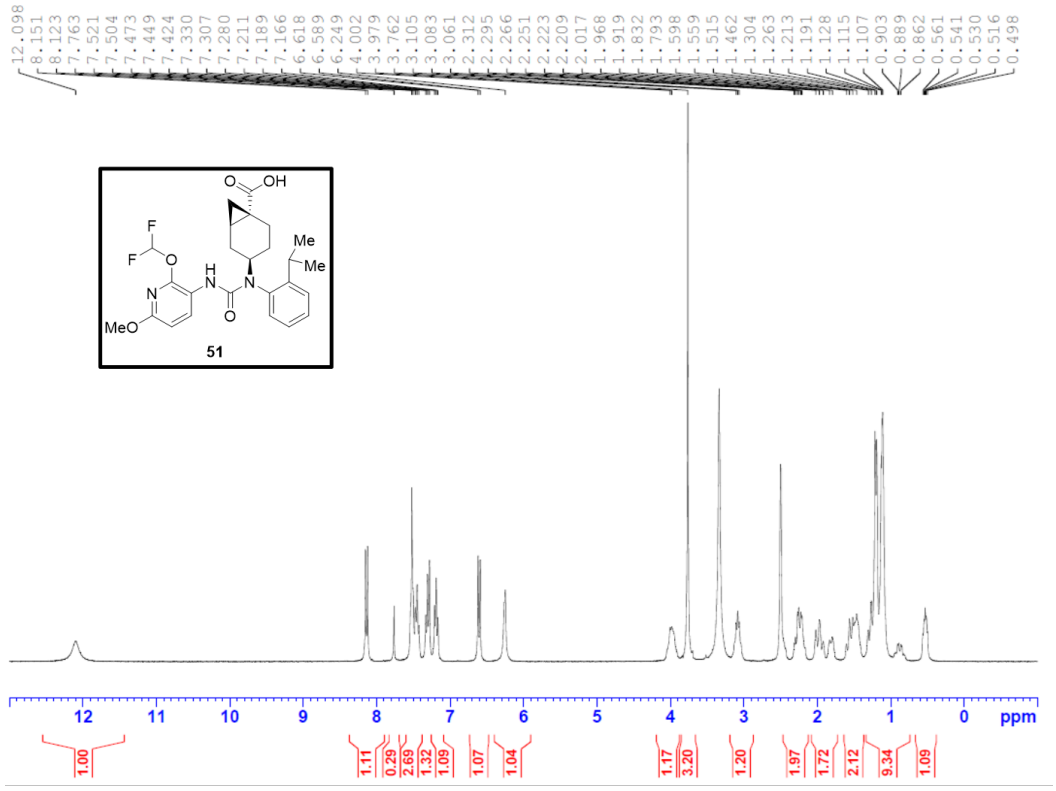
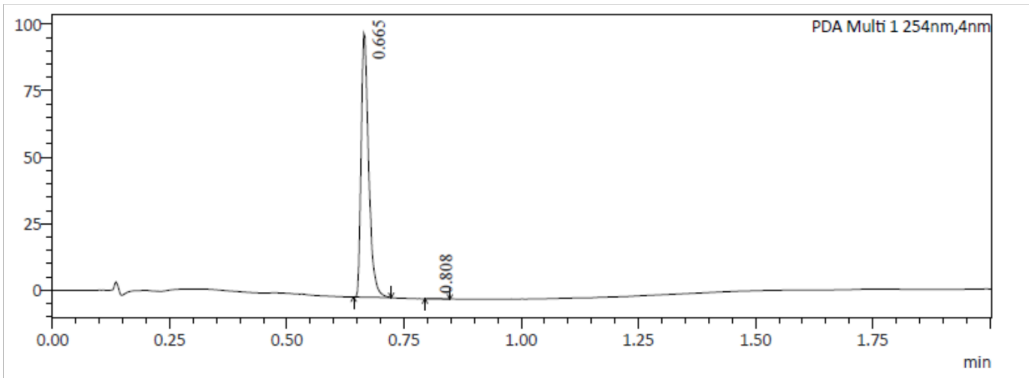


Figure S12. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 51

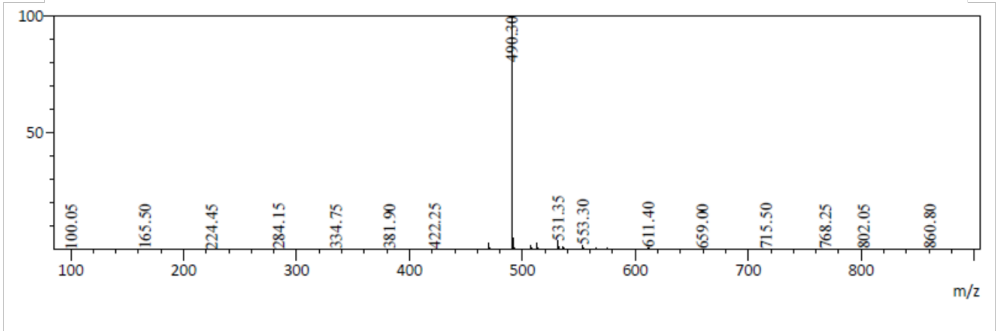




Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.665	99185	99.897	111090	99.806
2	0.808	103	0.103	215	0.194
Total		99287	100.000	111305	100.000



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
490.2157	490.2154	0.3	0.6	11.5	C25 H30 N3 O5 F2	858.9	n/a	n/a	25	30	3	5	2

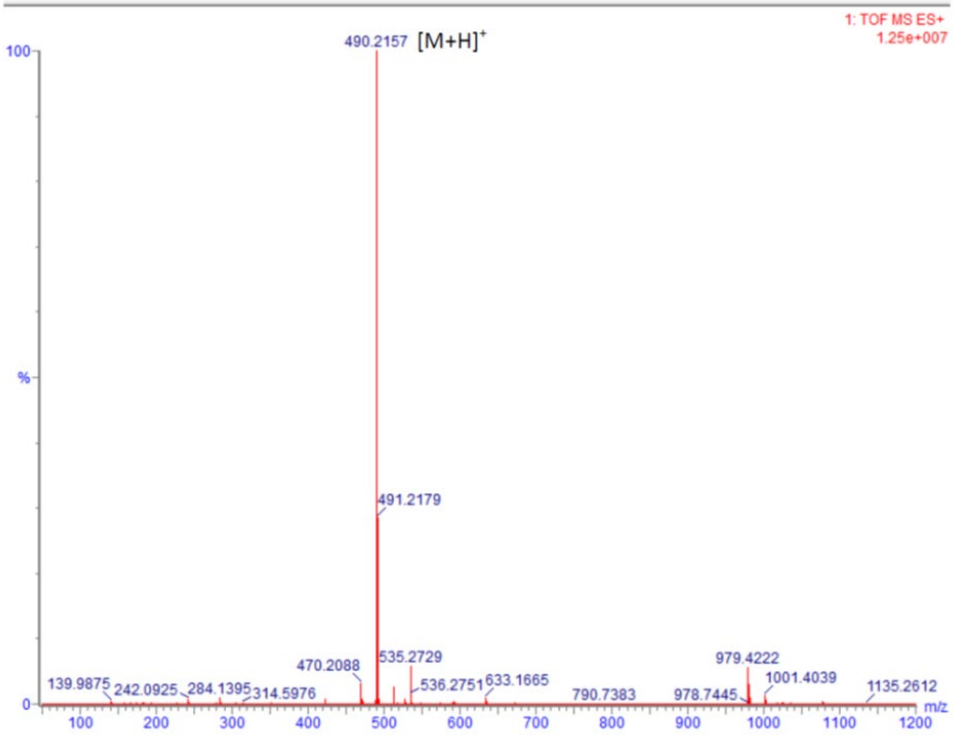
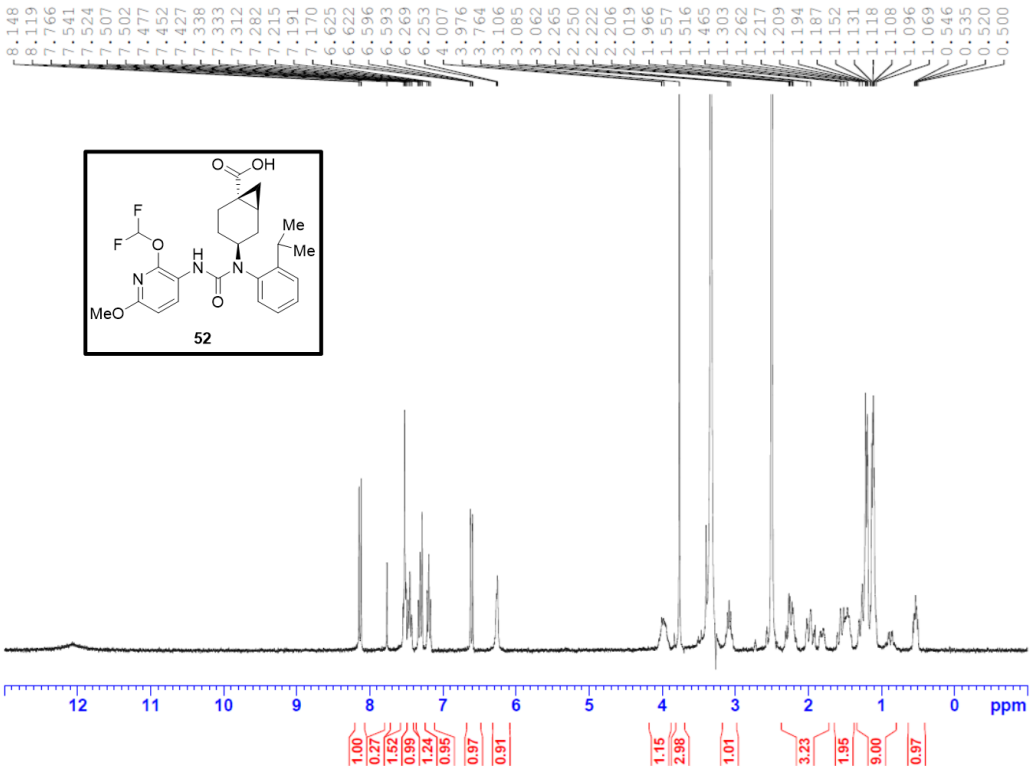
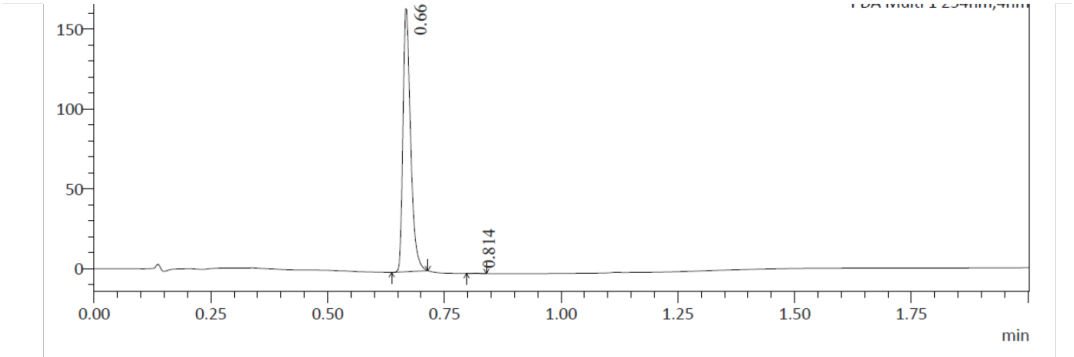


Figure S13. ^{1}H NMR (DMSO-d_6) and LC-MS Data for Compound 52

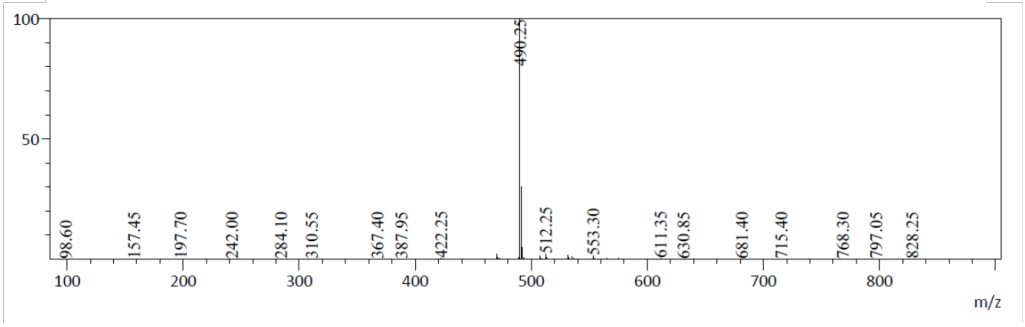




Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.668	164519	99.917	187984	99.932
2	0.814	137	0.083	129	0.068
Total		164656	100.000	188113	100.000



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	F
490.2157	490.2154	0.3	0.6	11.5	C25 H30 N3 O5 F2	824.3	n/a	n/a	25	30	3	5	2

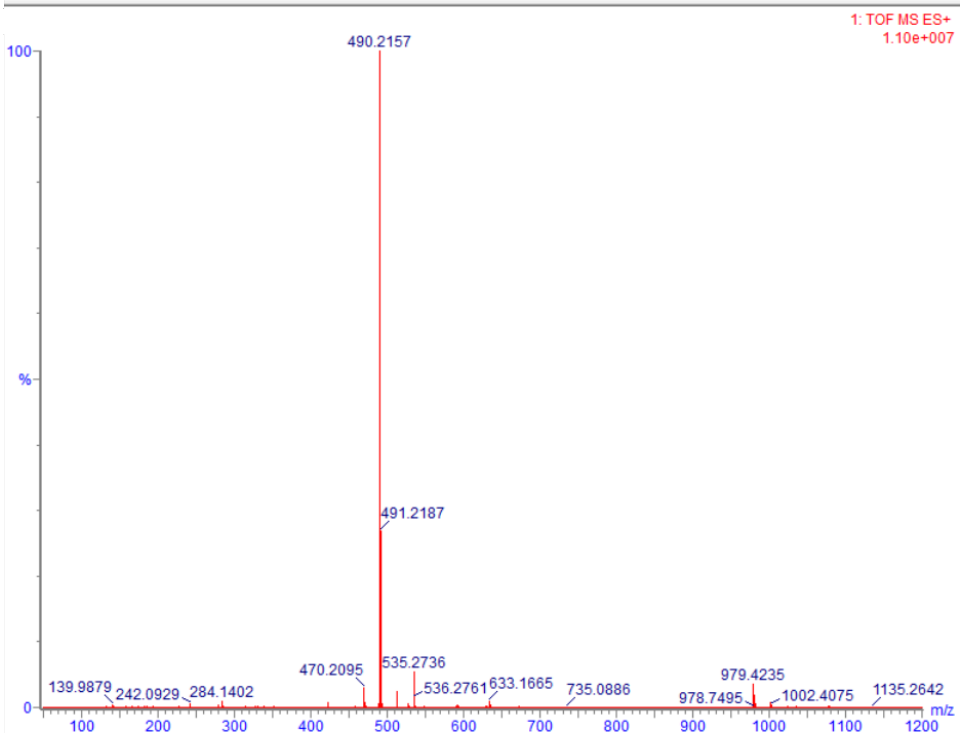
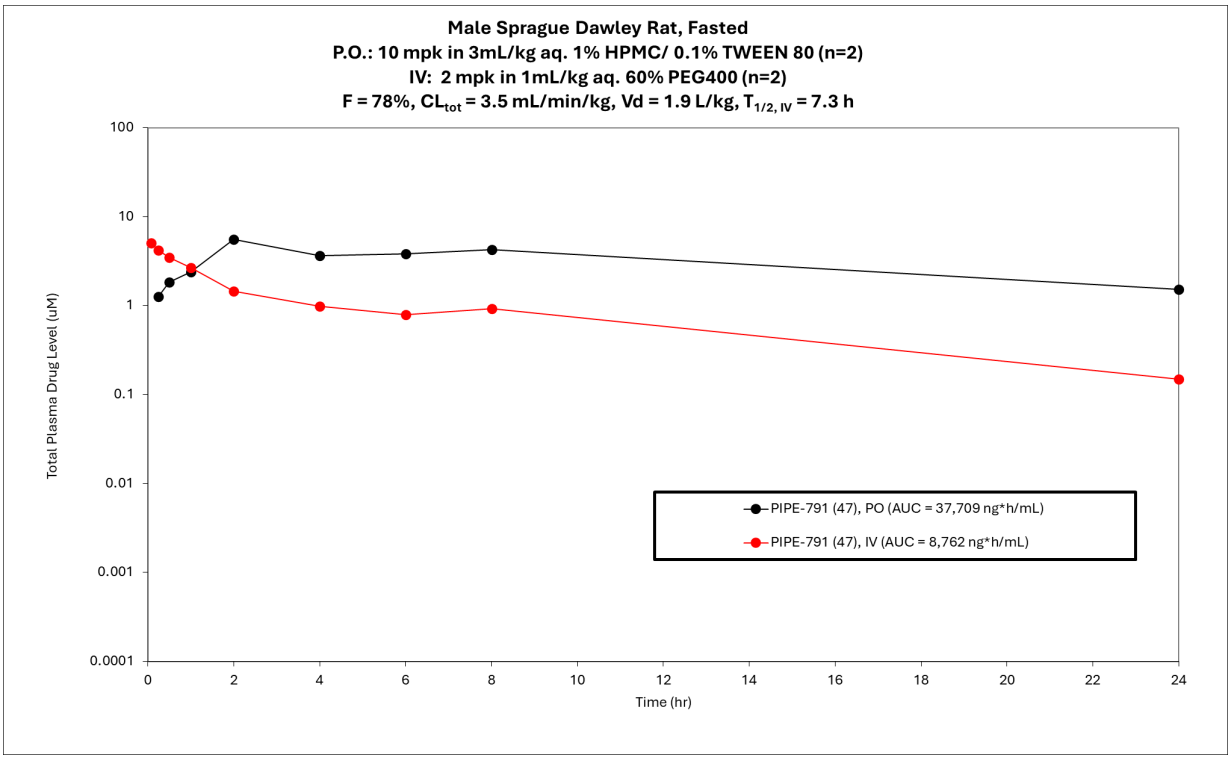


Figure S14. Sprague Dawley Rat and Beagle Dog PK Curves for PIPE-791 (47)



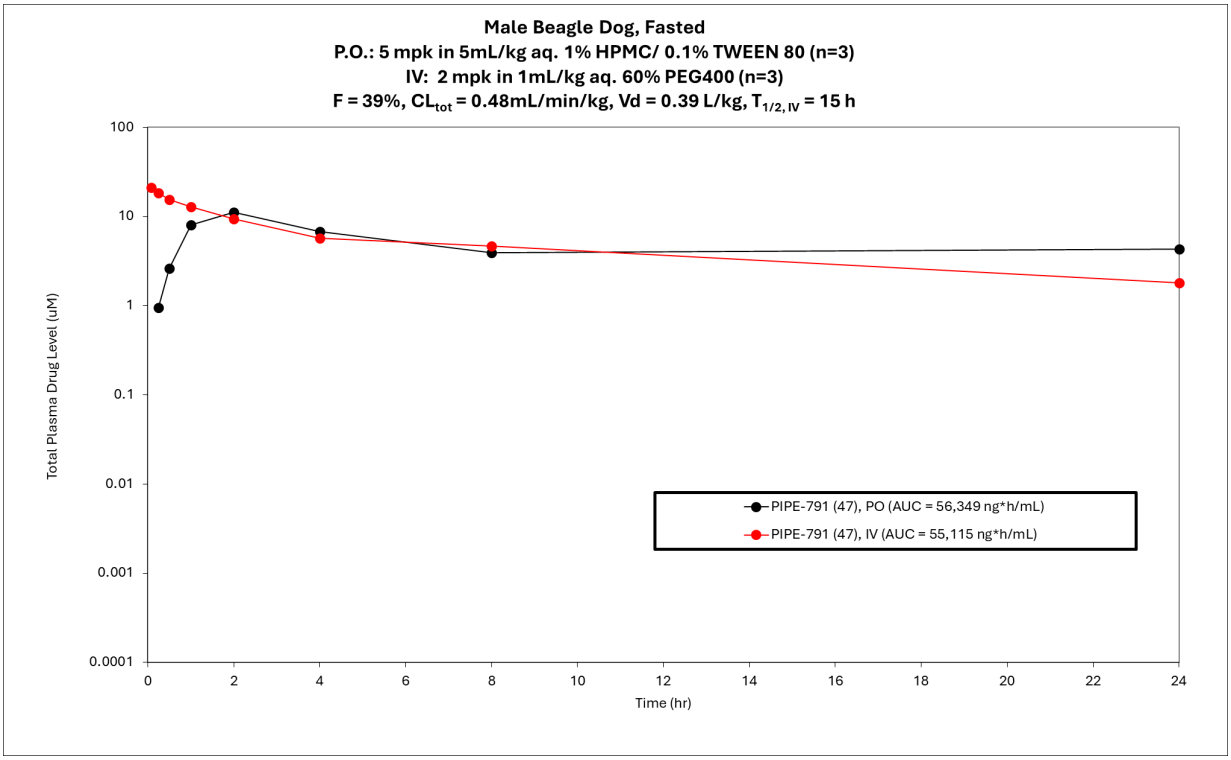
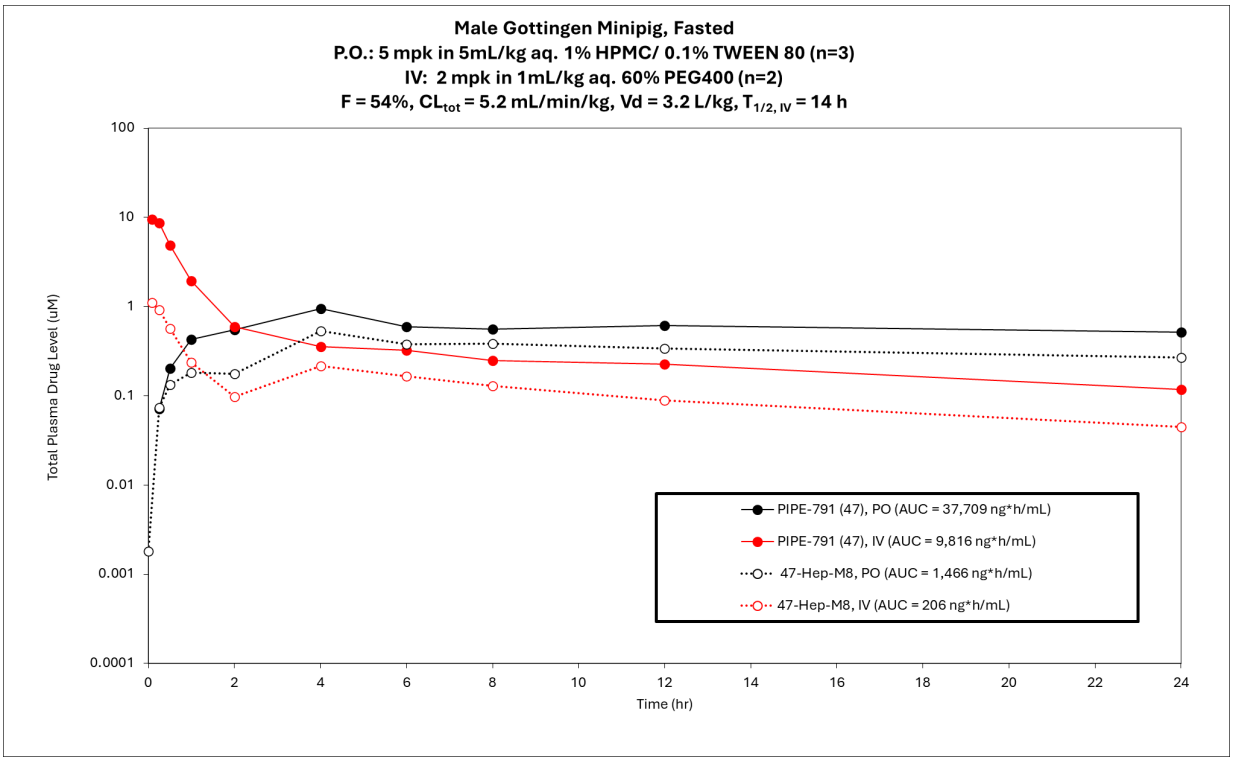


Figure S15. Göttingen Minipig and Cyno Monkey PK Curves for PIPE-791 (47)



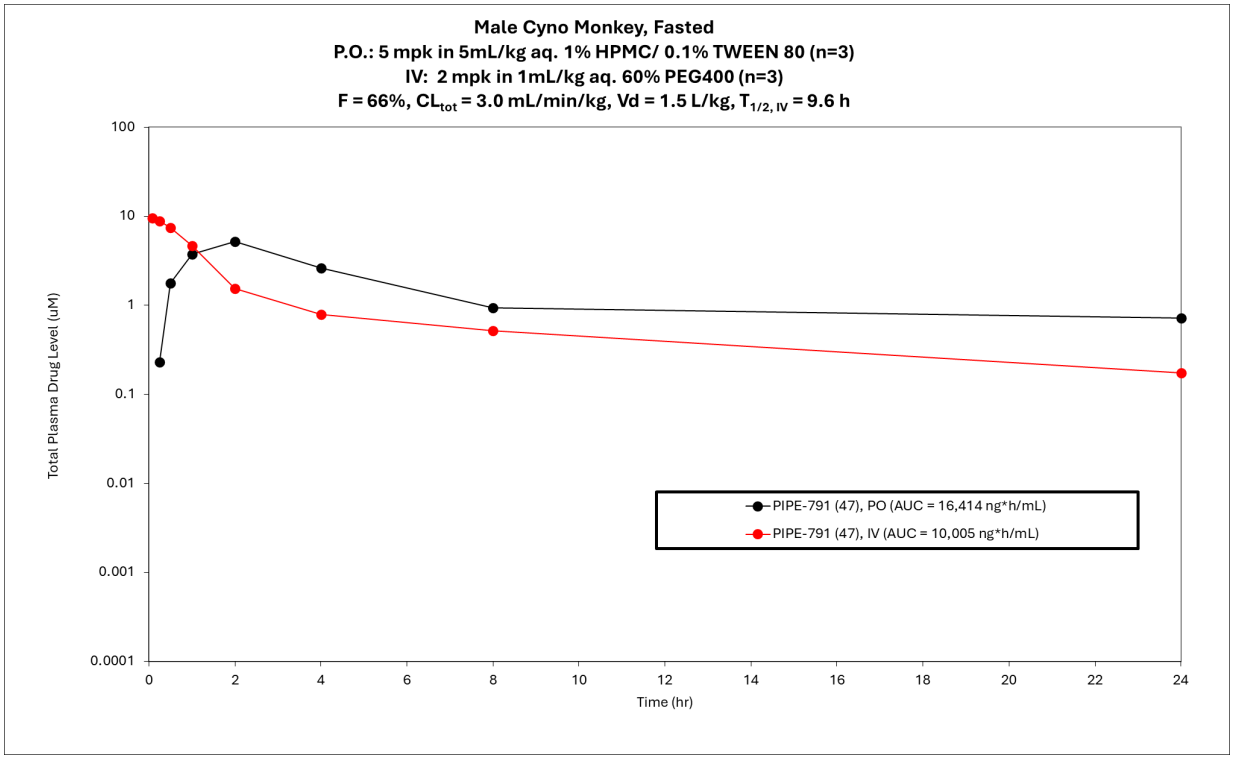


Figure S16. Eurofins SAFETYscan E/IC_{50} ELECT Data for Compound 21

% Functional Activation or Inhibition vs. Control Ligand (100%) and Vehicle (0%) at 10 µM

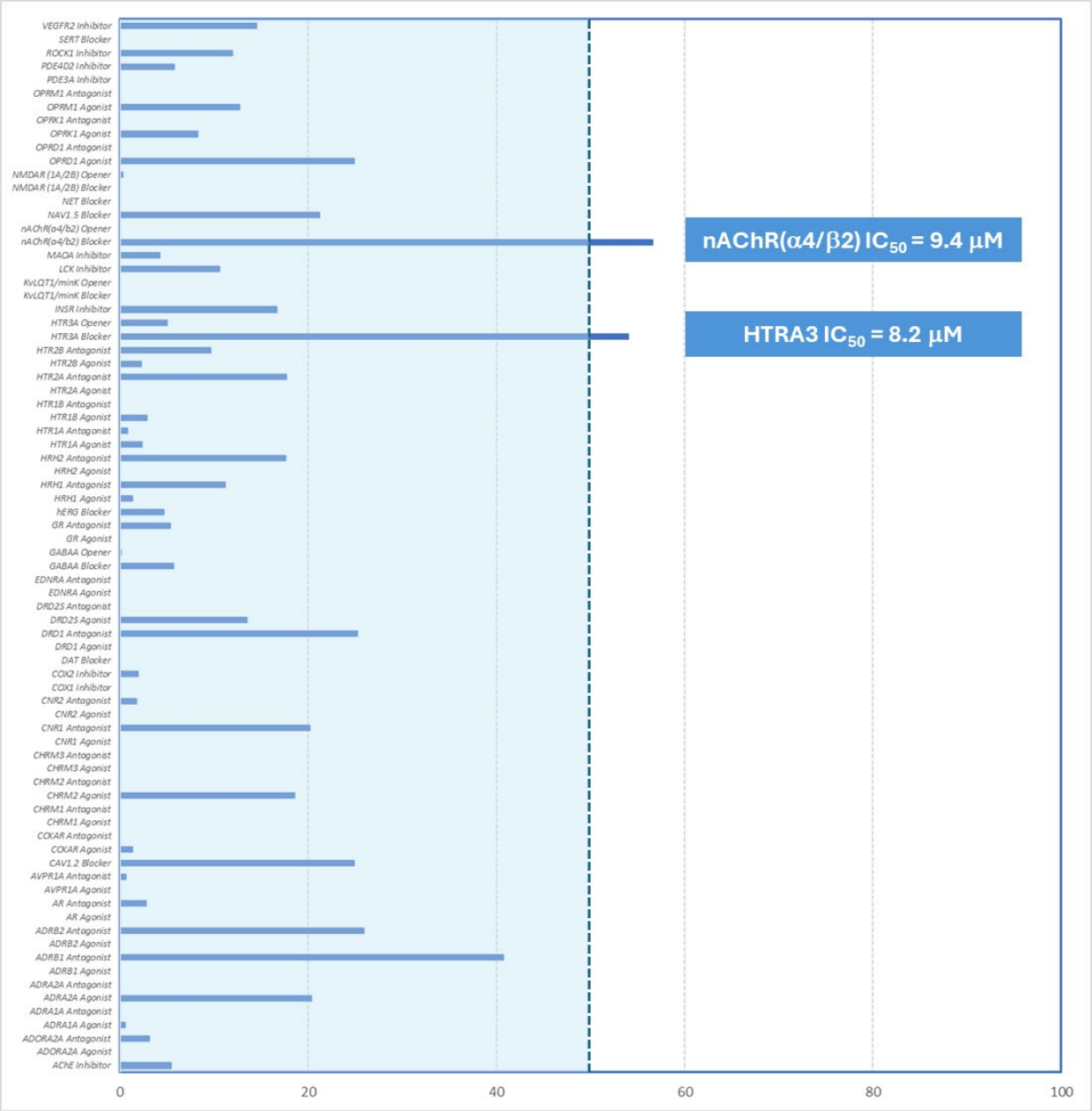


Figure S17. Eurofins SAFETYscan E/IC$_{50}$ ELECT Data for PIPE-791 (47)

% Functional Activation or Inhibition vs. Control Ligand (100%) and Vehicle (0%) @ 30 µM



Figure S18. EIC from LC-MS/MS of Compound 15 HLM and RLM Incubations

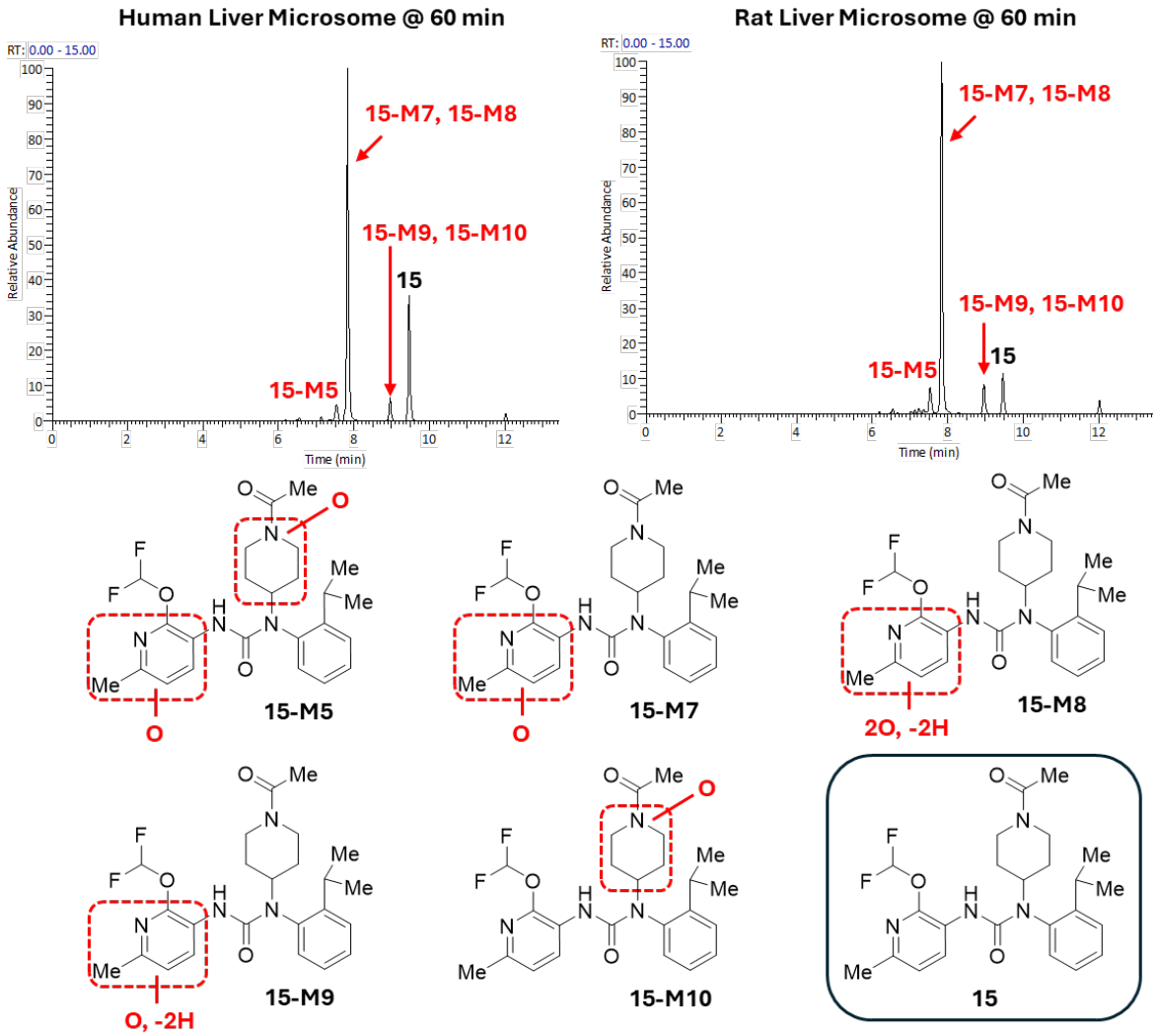


Figure S19. EIC from LC-MS/MS of PIPE-791 (47) HLM and RLM Incubations

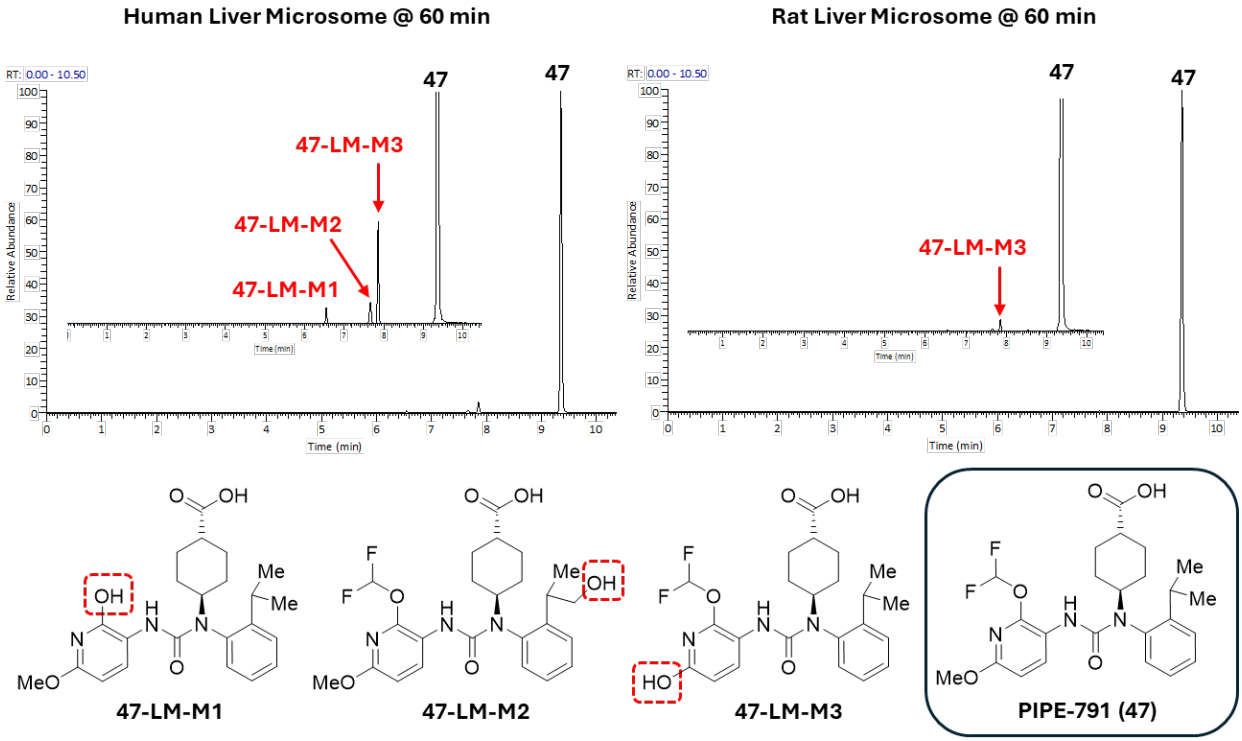
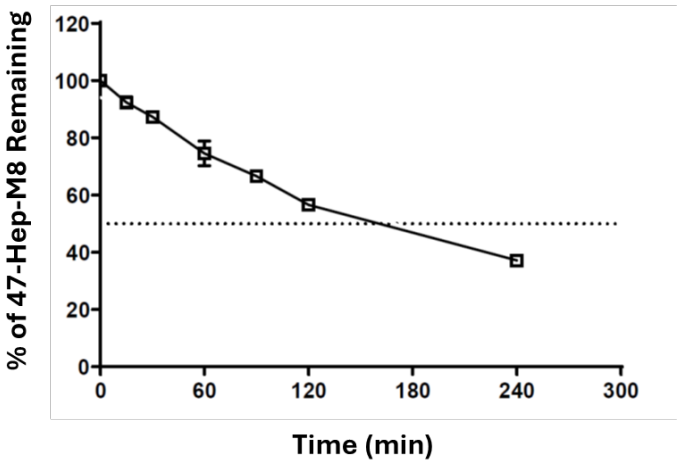


Figure S20. Stability of Acyl Glucuronide 47-Hep-M8 in Potassium Phosphate Buffer

Percent of **47-Hep-M8** Remaining with Incubtation Time in 100 mM pH 7.4 Buffer at 37°C



Chromatogram of **47-Hep-M8** and Rearranged Isomer after 240 min Incubation Time

