

5-hydroxymethylcytosine features of portal venous blood predict metachronous liver metastases of colorectal cancer and reveal phosphodiesterase 4 as a therapeutic target

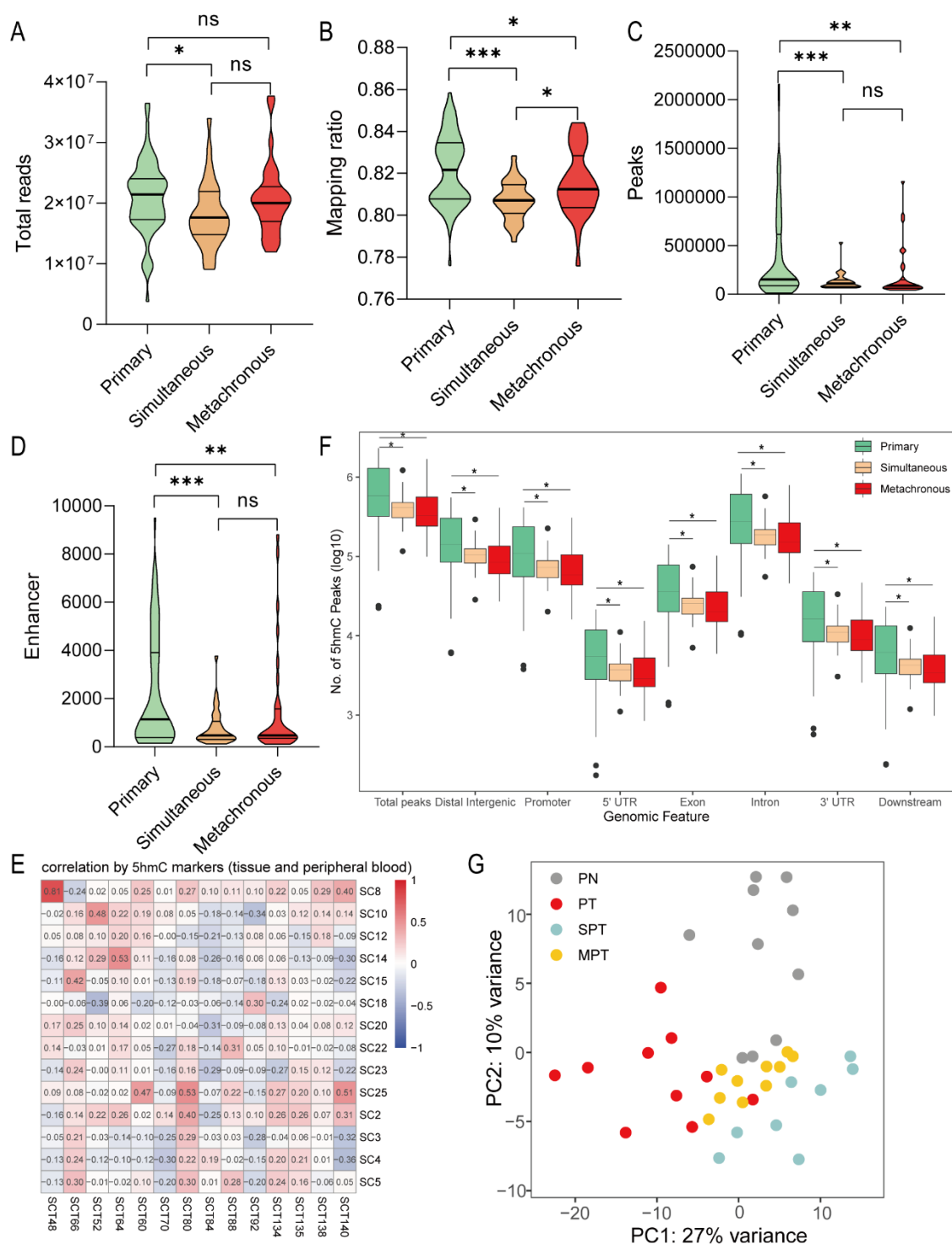


Fig S1. QC analysis for 5hmC-Seal data. (A) Total reads number for the groups. Each boxplot represents all the samples of each group. (B) Mapping ratios of the samples. Each boxplot represents all the samples of each group. (C) Total peaks number for the groups. (D) Peaks number enriched regions in enhancer for the groups. (E) Within-subject correlation is significantly higher (Wilcoxon rank-sum test $p < 0.01$) between peripheral blood cfDNA and tissue genomic DNA than that between different individuals, based on the top variable genes in cfDNA samples in terms of 5hmC modification. (F) Genome-wide 5hmC distribution in different genomic features grouped by CRC patients ($*p < 0.05$). (G) Principal component analysis (PCA) plot based on top variance hMRs in tissue gDNA samples.

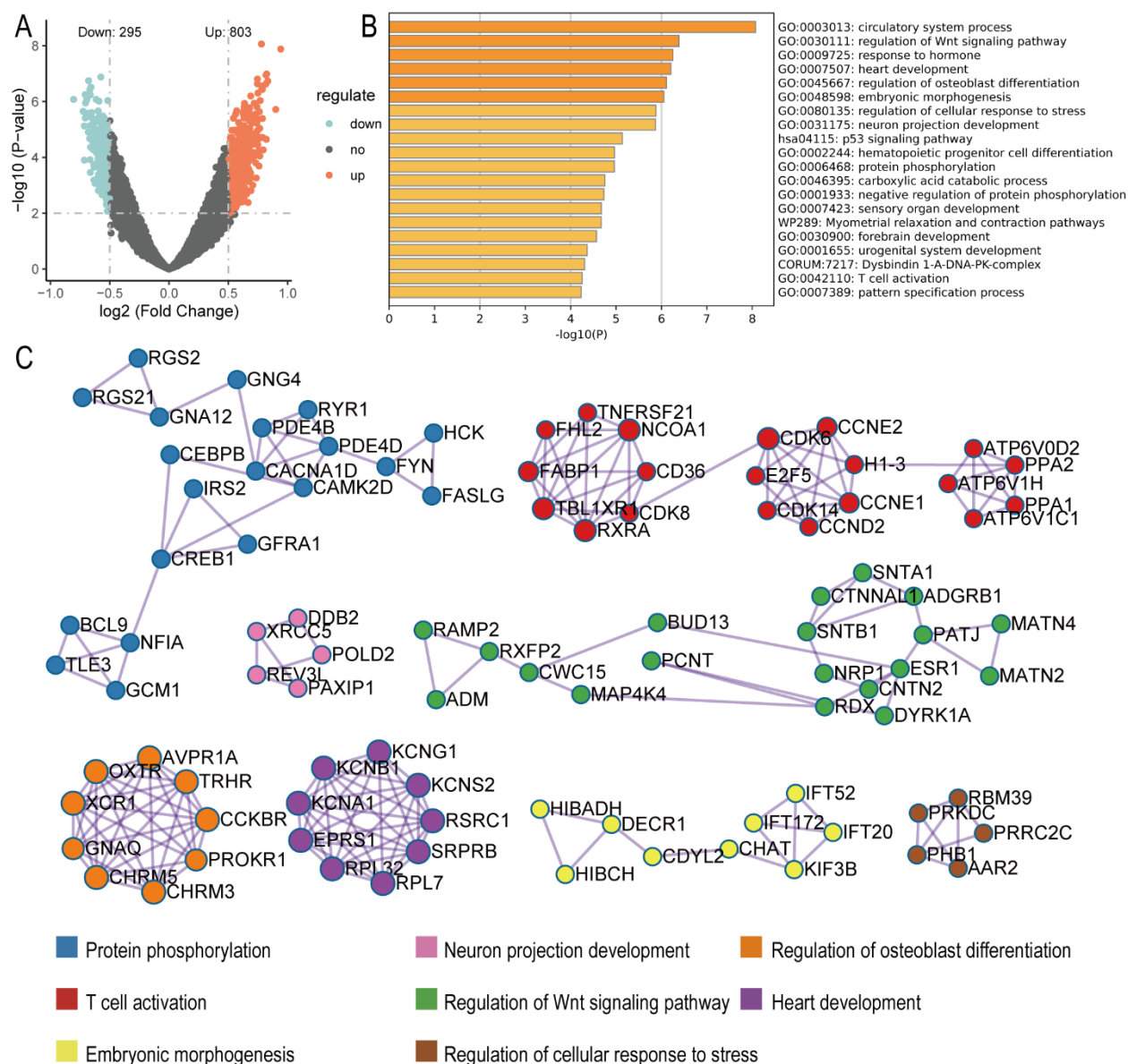


Fig S2. GO enrichment analysis and function exploration of DhMGs using Metascape. (A) Volcano plot of hMRs (SLM patients vs PC patients). Significance: ($|\log_2\text{FoldChange}| \geq 0.5$ and $p\text{-value} < 0.01$). (B) Metascape bar graph for viewing top non-redundant enrichment clusters, one per cluster, using bar graph length to represent statistical significance. (C) Ten MCODE complexes automatically identified in Metascape, colored by their identities. Their functional labels are generated based on the top-three functional enriched terms, if available visualizations of meta-analysis results based on multiple gene lists.

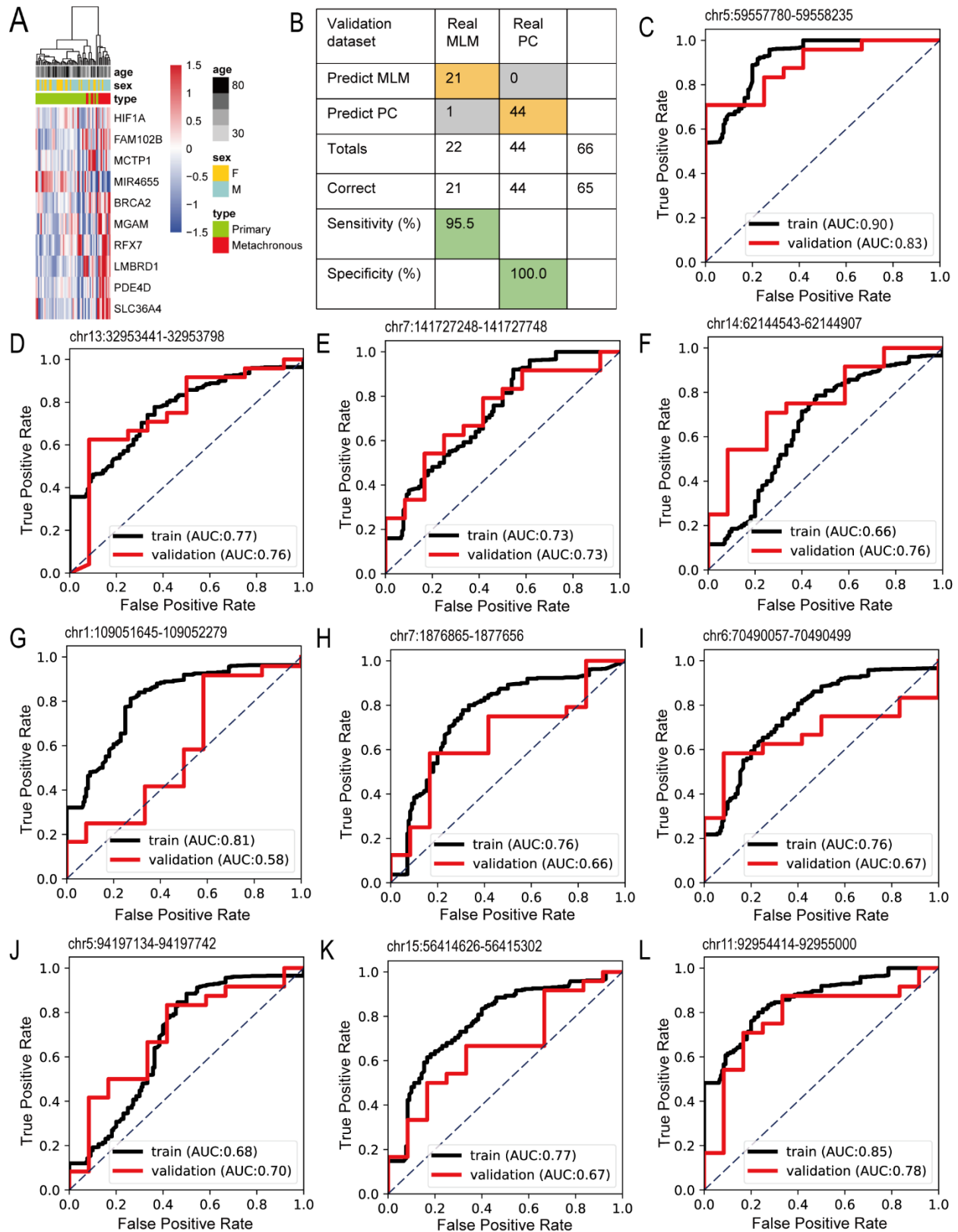


Fig S3. The individual prediction performance of each marker in the ten markers in the training and validation cohort. (A) Heatmaps of 10 5hmC markers with PC and MLM

patients in the training cohort, sex and age information labeled in the figure. (B) Confusion matrices built from the prediction model prediction in the training cohort. (C) Individual ROC curves for each marker in the training cohort and validation cohort.

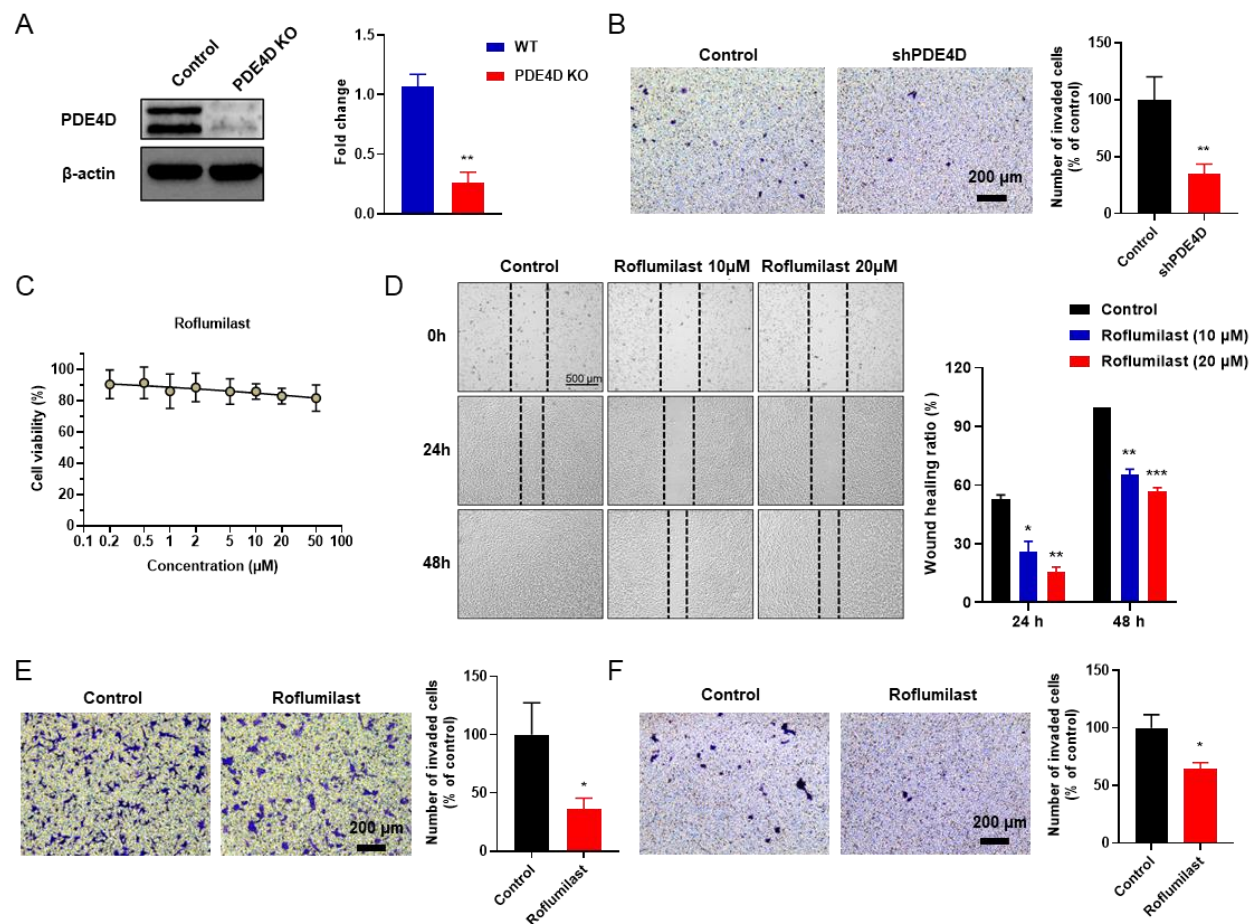


Fig. S4. PDE4D suppression inhibits migration in multiple CRC cells. (A) Western blot analysis and quantitative statistic of PDE4D expression in PDE4D K.O. cells. (B) The migration of shPDE4D SW480 cells was assessed using a transwell migration assays. Scale bar, 200 μ m. (C) Cell viability of MC38 cells treated with roflumilast. (D) Scratch assay analysis of MC38 cells treated with roflumilast. Images were acquired at 0, 24 and 48 hours. Scale bar, 500 μ m. (E to F) The migration of LoVo cells (E) and HCT116 cells (F) was assessed using a Transwell migration assays. Right, Representative images of the assay. Scale bar, 200 μ m. Data are shown as means $\pm$ SEM.

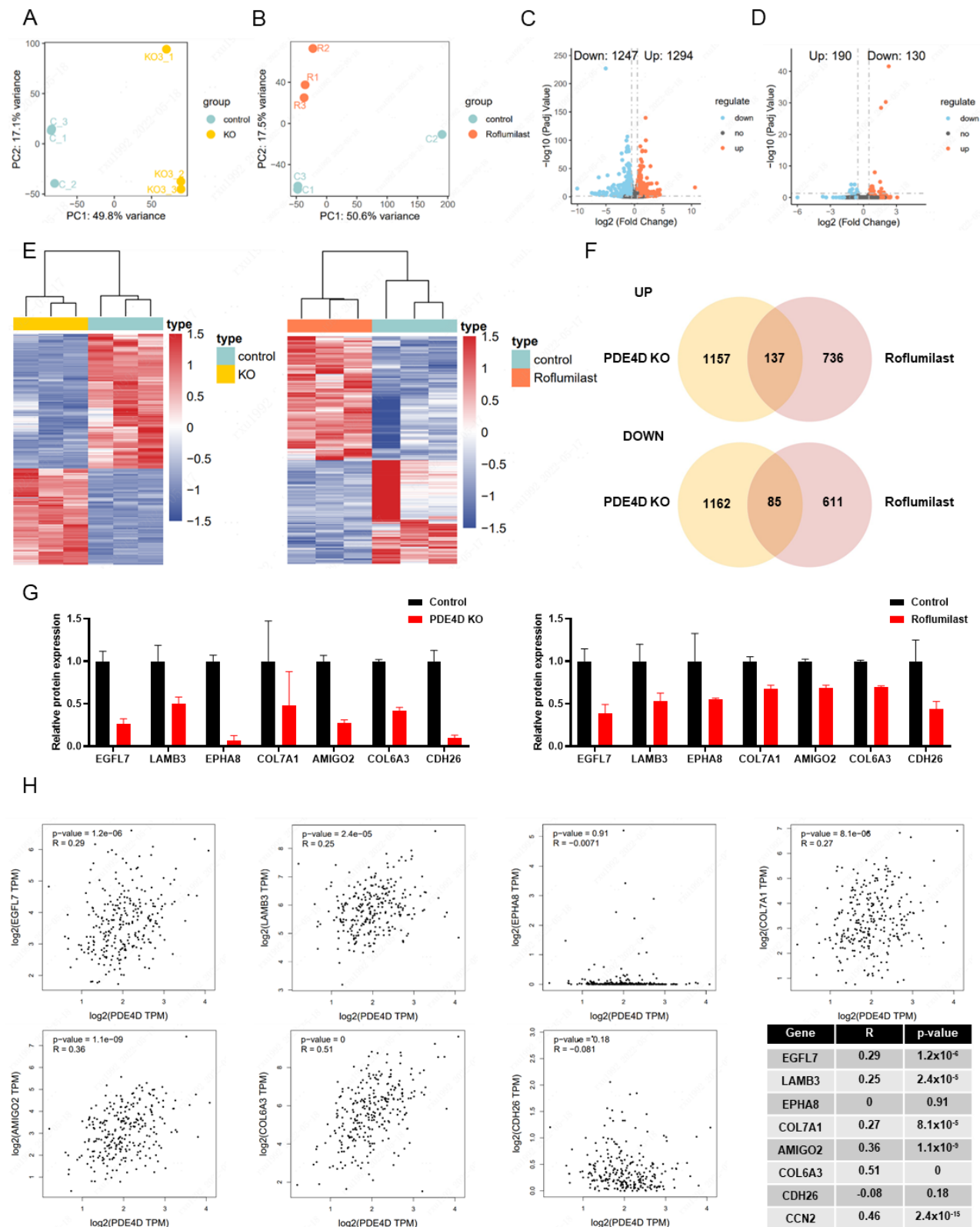


Fig. S5. Association between CCN2 and PDE4D according to RNA-seq data. (A to B) Principal component analysis (PCA) of PDE4D KO cells compared with wild type cells (A) and roflumilast treated cells compared with untreated cells (B). (C to D) Volcano plot of PDE4D KO cells (C) and roflumilast treated cells (D). Significantly alterations ($\log_2\text{FoldChange} > 0.5$, $p\text{-value} < 0.05$). (E) Gene expressions in PDE4D KO MC38 cells compared with wild type cells, as well as roflumilast treated cells compared with untreated cells analyzed by RNA-seq. (F) Venn diagram indicating overlap and specificity of upregulations and downregulations from RNA-seq data. (G) Expression of genes accumulated in cell adhesion pathway in PDE4D KO cells and roflumilast treated cells. (H) Correlation plots of the mRNA expression of PDE4D with the mRNA expressions of genes in the cell adhesion pathway, including LAMB3, EGFL7, EPHA8, COL7A1, AMIGO2, COL6A3 and CDH26 in CRC sequencing data from TCGA dataset. Data are shown as means $\pm$ SEM.

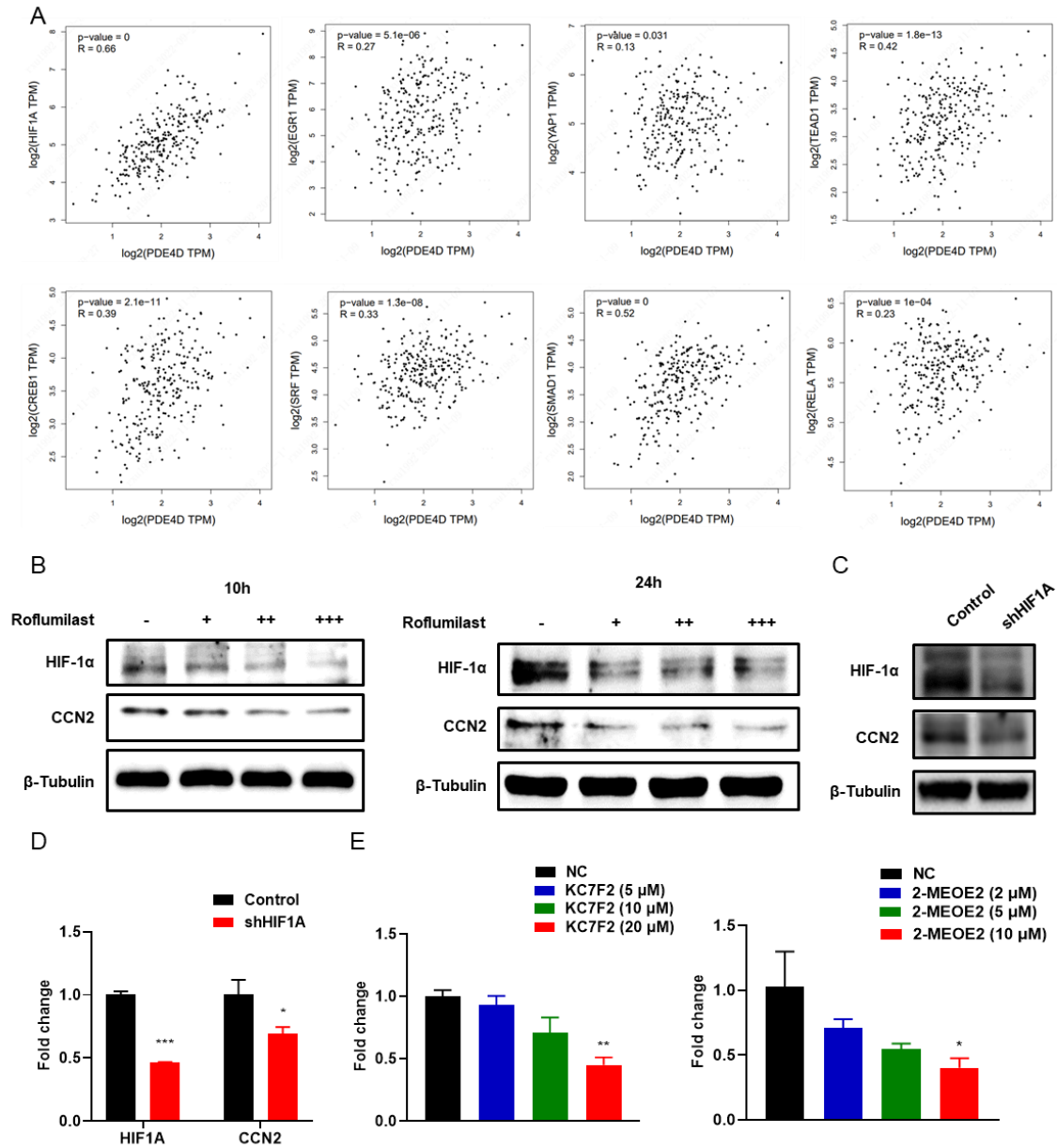


Fig. S6. PDE4 suppression inhibits HIF-1 α . (A) Correlation plots of the mRNA level of PDE4D with the mRNA level of transcription factors, including HIF1A, EGR1, YAP1, TEAD1, CREB1, SRF, SMAD1 and RELA in CRC sequencing data from TCGA dataset. (B) Western blot for HIF-1 α and CCN2 in HCT116 cells treated with roflumilast for 10h and 24h. (C) Western blot analysis of HIF-1 α and CCN2 expression in HCT116 cells treated with shHIF1A. (D) RNA expression level of HIF1A and CCN2 in HCT116 cells treated with shHIF1A. (E) RNA expression level of CCN2 in MC38 cells treated with different concentration of KC7F2 and 2-MEOE2. Data are shown as means $\pm$ SEM.

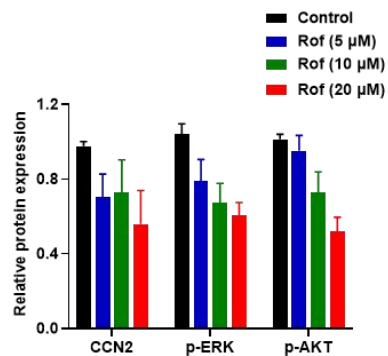
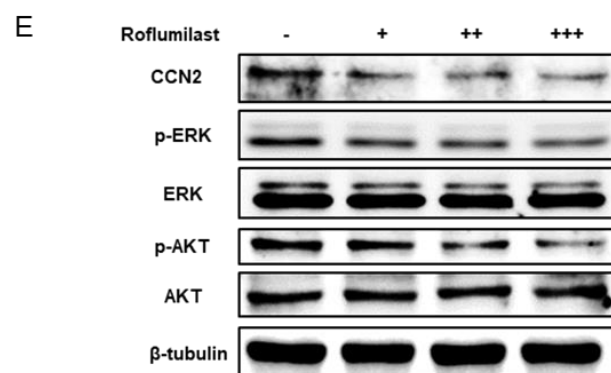
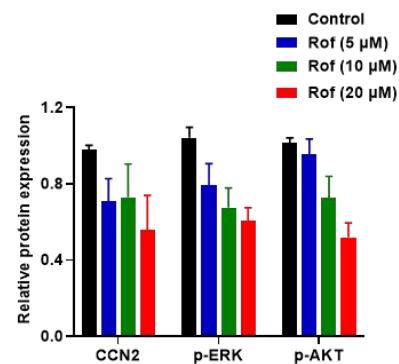
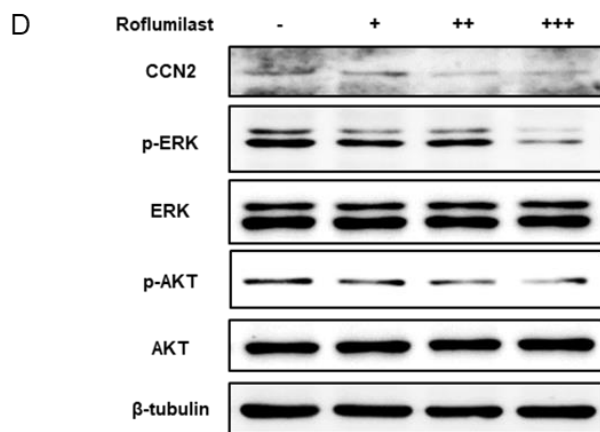
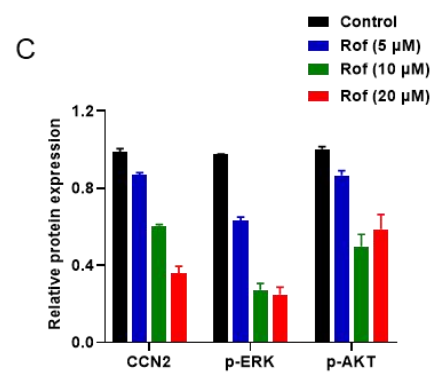
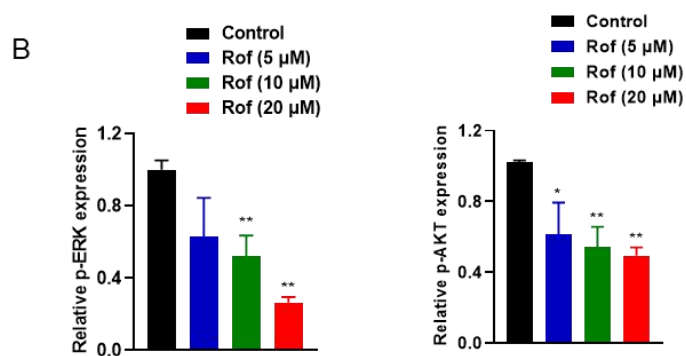
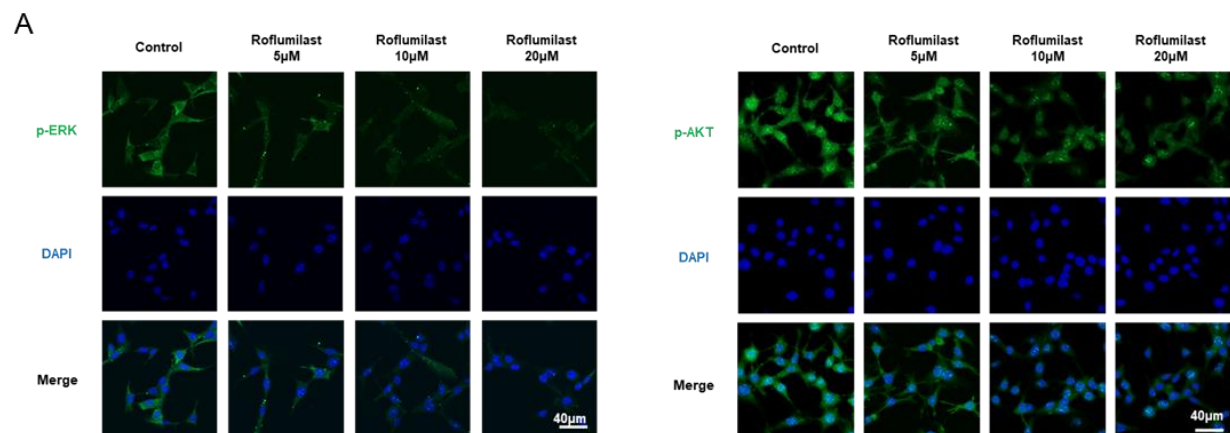


Fig. S7. Roflumilast inhibits expression of CCN2 and phosphorylation of AKT and ERK. (A) Immunofluorescence images for phospho-ERK and phospho-AKT in MC38 cells treated with roflumilast. (B) Quantitatively statistic of phospho-AKT and phospho-ERK expression. (C) Quantitatively statistic of CCN2, phospho-AKT and phospho-ERK expression in MC38 cells treated with roflumilast for 24h. (D) Western blot results for CCN2, phospho-AKT, total AKT, phospho-ERK and total ERK in MC38 cells treated with roflumilast for 48h. (E) Western blot results in HCT116 cells treated with roflumilast. Data are shown as means $\pm$ SEM.

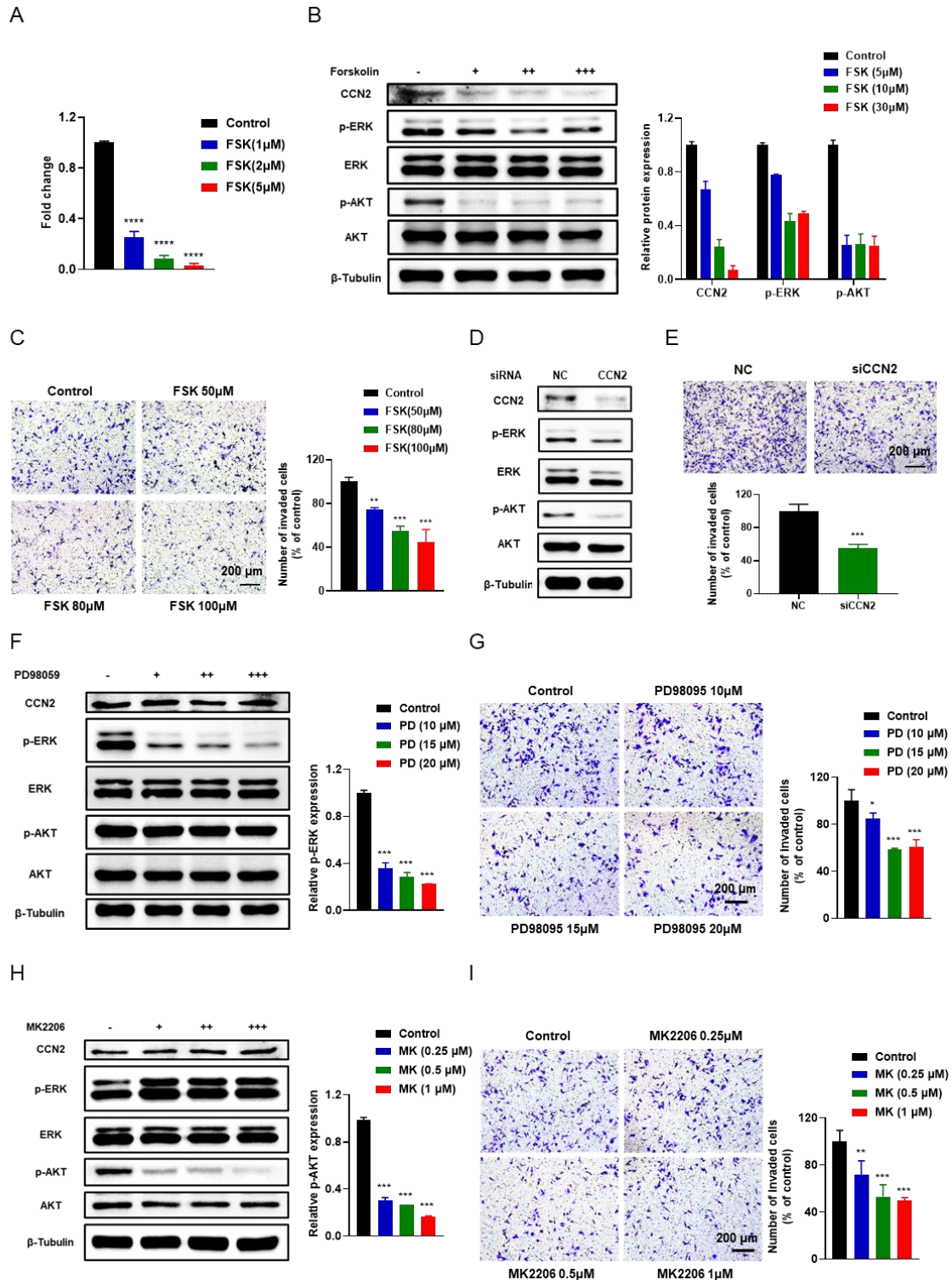


Fig. S8. CCN2 suppression inhibits metastases via p-AKT and p-ERK pathway. (A) Gene expression of CCN2 in MC38 cells treated with different concentration of FSK. (B) Western blot for CCN2, phospho-AKT, total AKT, phospho-ERK and total ERK in MC38

cells treated with FSK. (C) Transwell migration assays of MC38 cells treated with FSK. Scale bar, 200 μm . (D) CCN2 down-regulation by siRNA effect on p-AKT and p-ERK. (E) Characterization of migration ability after transfecting with CCN2-siRNA. (F) Effect of PD98059 mediated inhibiting of p-ERK after treating for 24 hours. (G) Transwell migration assays of MC38 cells treated with PD98059. (H) Effect of MK2206 mediated inhibiting of p-ERK after treating for 24 hours. (I) Effect of MK2206 mediated inhibiting of p-AKT after treating for 24 hours. Data are shown as means $\pm$ SEM.

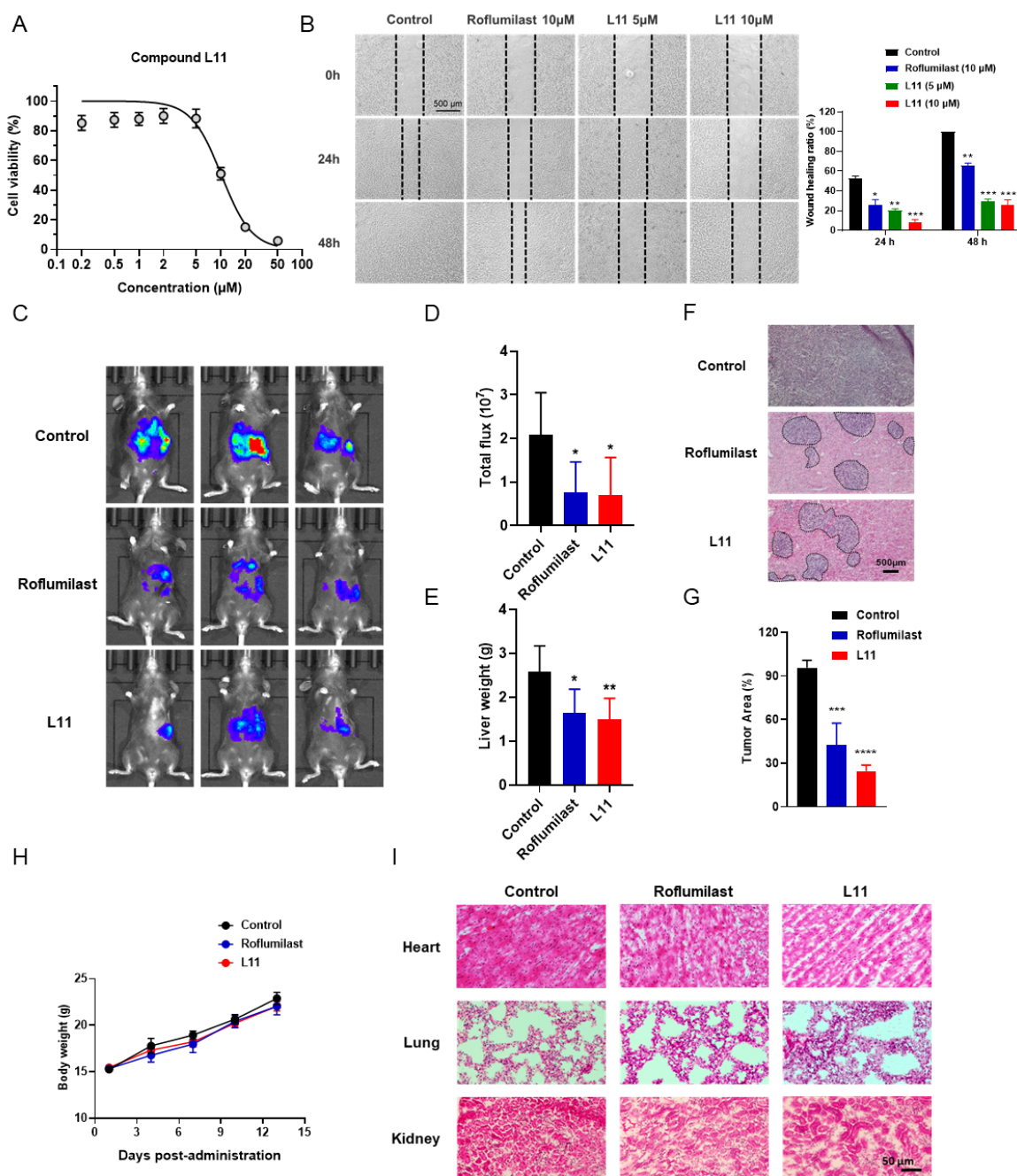


Fig. S9. Metastases inhibition and safety tests of compound L11. (A) Cell viability inhibition of MC38 cells by compound L11. (B) Scratch assay analysis of MC38 cells treated with PBS, roflumilast and compound L11. Images were acquired at 0, 24 and 48 hours. Right, quantitative analysis of scratch wound closure. Scale bar, 500 μm . (C) Fluorescence images of liver metastases mice treated with roflumilast or compound L11. (D) Quantitative analysis of fluorescence intensity of mice. (E) Statistical data of liver weight After dissecting three groups of mice. (F) Histopathological examination of liver tissue sections. (G) Quantification of liver metastases area in H&E sections. (H) Body weight curve of mice treated with PBS, roflumilast and compound L11. (I) H&E staining results of main

organs of mice, including heart, lung and kidney. Scale bar, 50 μm . Data are shown as means $\pm$ SEM.

Table S1. Coefficients for 10 DhMRs in the logistic regression model trained by the training cohort

	Coefficients	SE	Z	P> Z	97.5% CI	GeneID
Intercept	19.6651	1.971	-0.011	0.009	[-7.551, -0.785]	
chr7_141727248_141727748	1.0121	0.418	0.004	0.097	[-5.478, 7.503]	MGAM
chr7_1876865_1877656	-1.5569	0.491	-0.003	0.027	[-6.063, 2.949]	MIR4655
chr11_92954414_92955000	2.3106	0.913	0.004	0.034	[-9.736, 4.357]	SLC36A4
chr5_59557780_59558235	1.1234	0.479	0.003	0.016	[-1.683, 3.930]	PDE4D
chr5_94197134_94197742	0.4479	0.973	0.002	0.002	[-3.652, 4.548]	MCTP1
chr13_32953441_32953798	-10.4726	1.577	-0.031	0.064	[-9.952, 9.007]	BRCA2
chr6_70490057_70490499	-3.8177	0.192	-0.009	0.052	[-6.378, 8.743]	LMBRD1
chr15_56414626_56415302	-4.9716	1.273	-0.011	0.019	[-1.210, 1.267]	RFX7
chr1_109051645_109052279	2.4804	0.768	0.005	0.047	[-3.528, 8.489]	FAM102B
chr14_62144543_62144907	0.7744	0.657	0.002	0.098	[-0.2215, 1.770]	HIF1A

Abbreviation: DhMRs, Differentially 5hMc-enriched Regions. **SE**, standard errors of coefficients;

z value, Wald z-statistic value; **CI**, confidence interval.

Table S2. Inhibitory Activities of 2-arylbenzofurans towards PDE4.

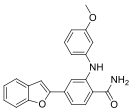
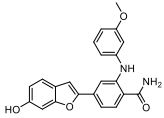
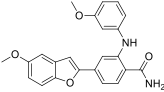
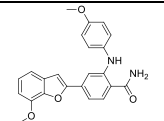
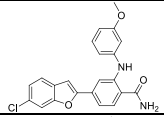
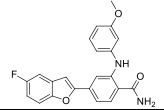
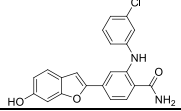
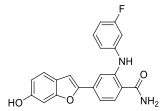
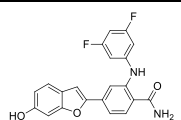
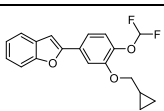
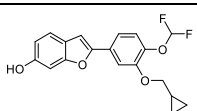
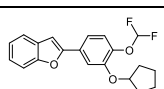
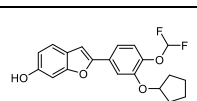
Compounds	Structure	PDE4 inhibition		IC ₅₀ (nM)
		1 μ M	100 nM	
L1		55.71%	41.94%	—
L2		68.54%	48.62%	181 $\pm$ 13
L3		24.14%	17.04%	—
L4		59.52%	28.87%	—
L5		36.91%	15.91%	—
L6		55.49%	30.29%	—
L7		77.50%	44.08%	197 $\pm$ 37.4
L8		61.62%	30.59%	—
L9		65.85%	39.13%	—
L10		37.40%	—	—
L11		83.55%	33.05%	54 $\pm$ 15.5
L12		73.33%	13.18%	910
L13		92.25%	42.73%	145.1 $\pm$ 2.3

Table S3. Statistics on diffraction data and structure refinement of PDE4D-L11.***Data collection***

Wavelength (Å)	1.54056
Temperature (K)	100
Resolution (Å)	2.10
Space group	P2 ₁ 2 ₁ 2 ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	58.36, 80.43, 164.58
<i>α</i> , <i>β</i> , <i>γ</i> (°)	90.0, 90.0, 90.0
No. reflections	45739 (4515 ^a)
Completeness (%)	99.77 (99.82 ^a)
<i>R</i> _{merge}	0.179 (0.272 ^a)
<i><I/σ(I)></i>	19.0 (4.2 ^a)
Redundancy	4.0 (5.7 ^a)

Structure refinement

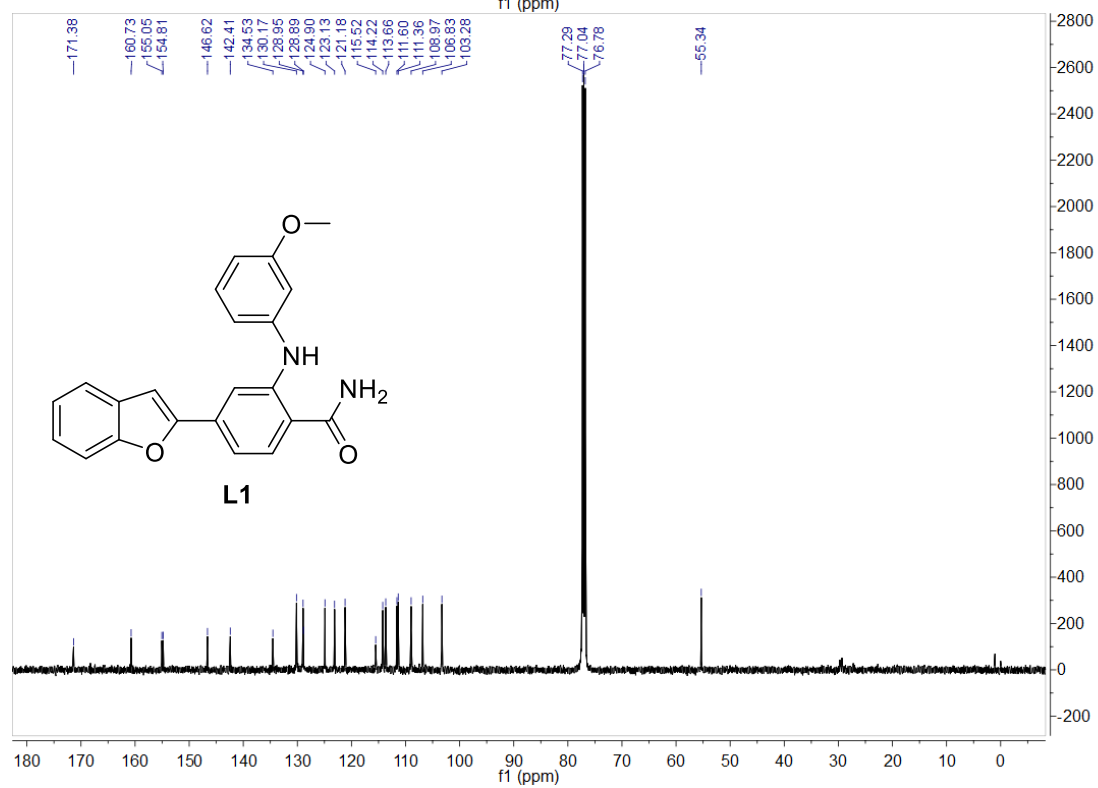
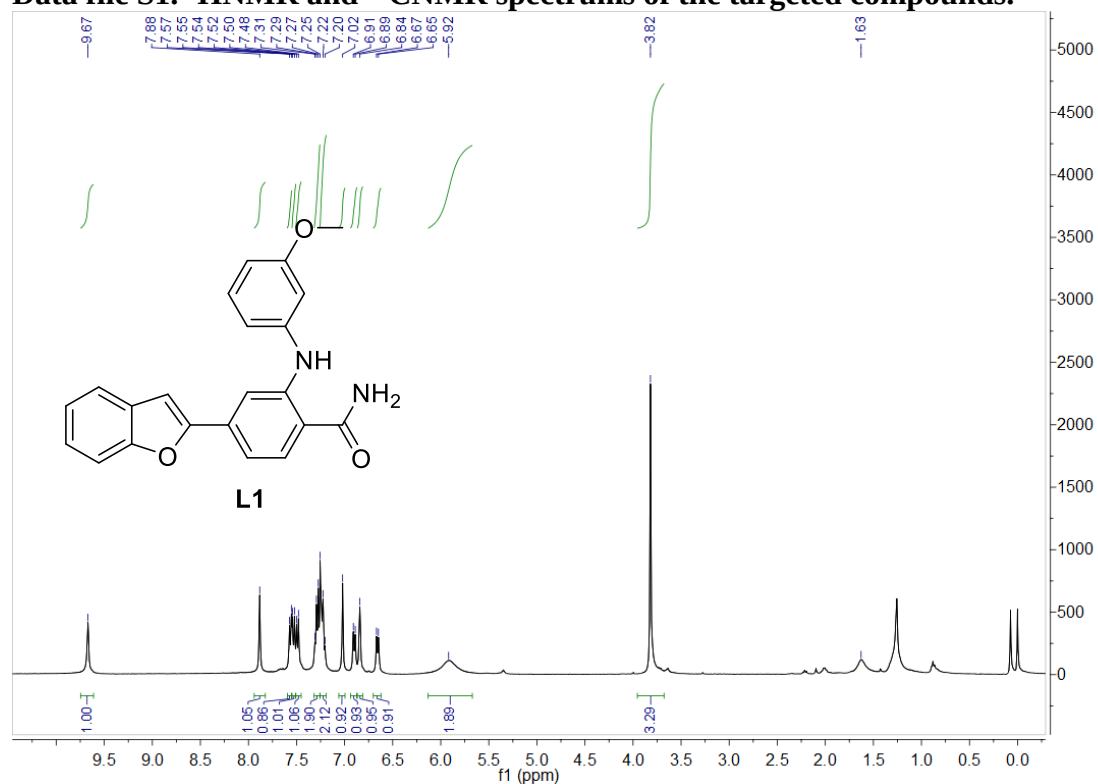
R-factor/R-free	0.227/0.278
Bond lengths	0.008 Å
Bond angles	0.912
Protein	22.58 (5208)
Inhibitor	18.32 (50)
Zn ²⁺	18.37 (2)
Mg ²⁺	12.24 (2)
Waters	25.60 (288)
Preferred	96.88%
Allowed	3.13%

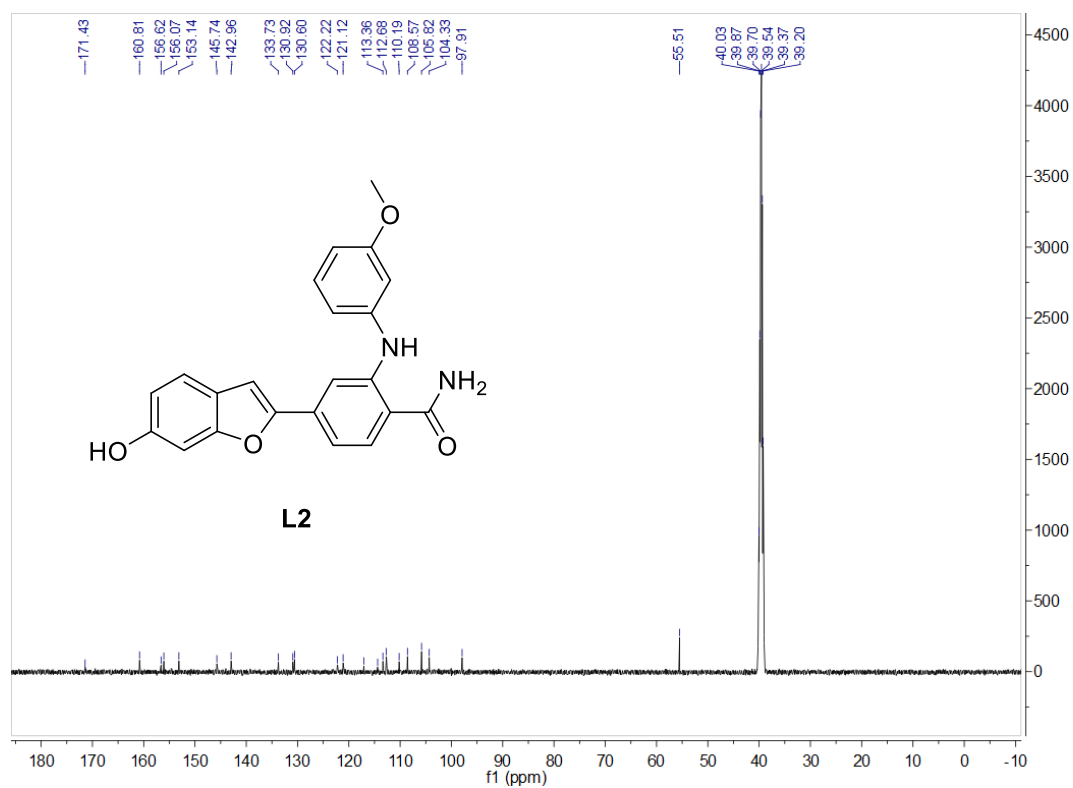
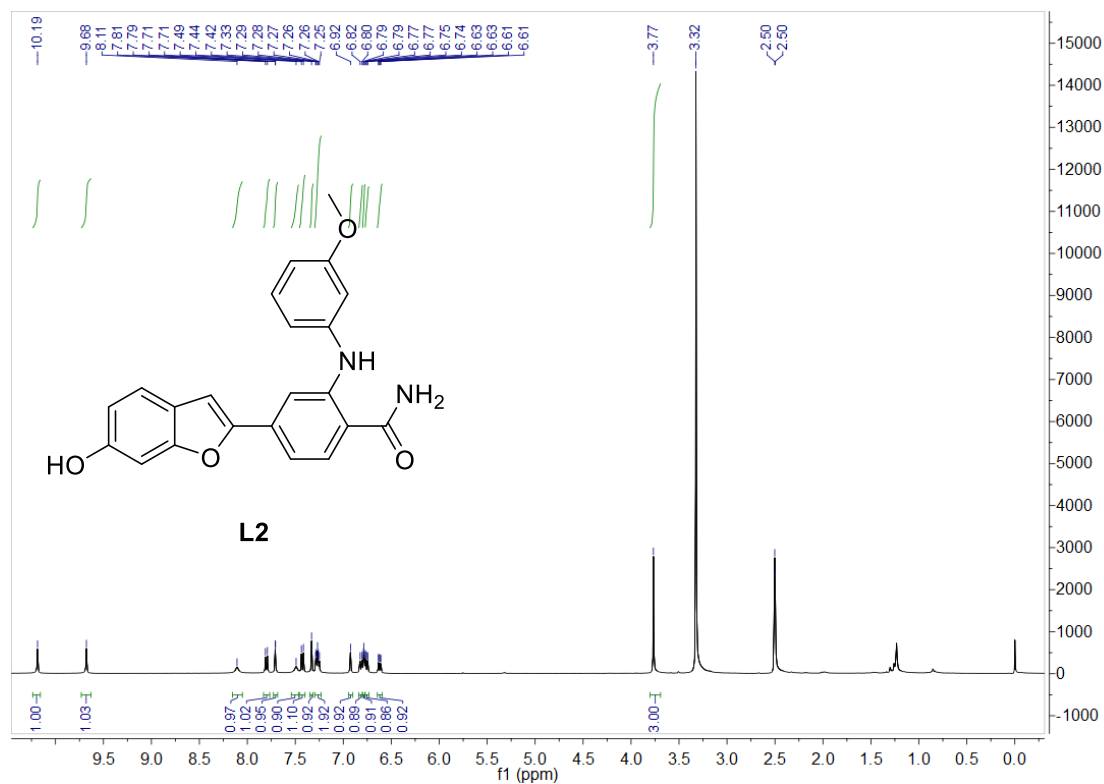
^a The highest resolution shell of 2.17 – 2.10 Å.

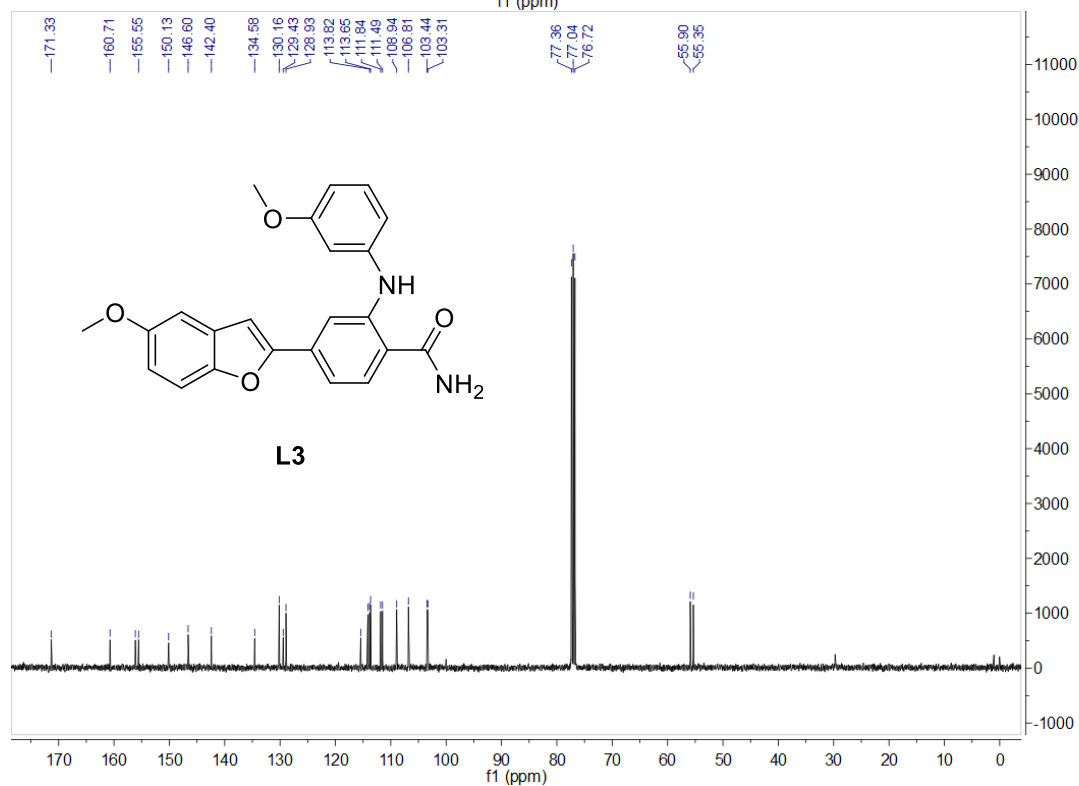
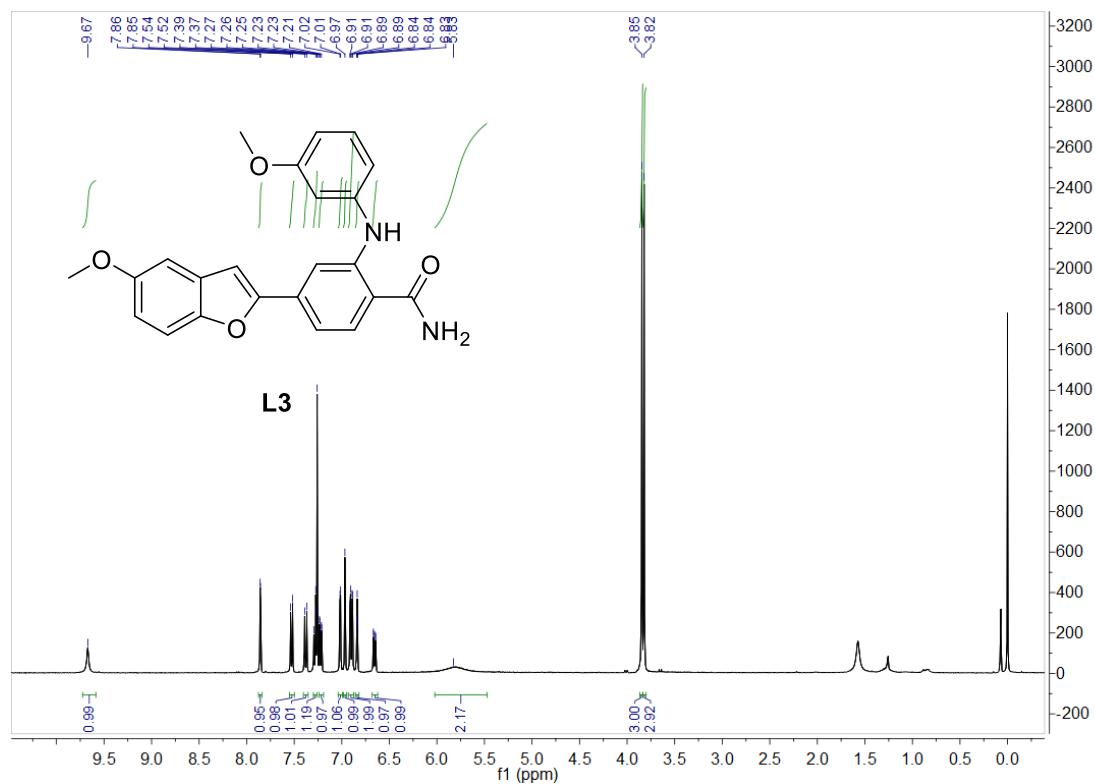
Table S4. Emetic Assays of L11 and Roflumilast on Beagle Dogs.

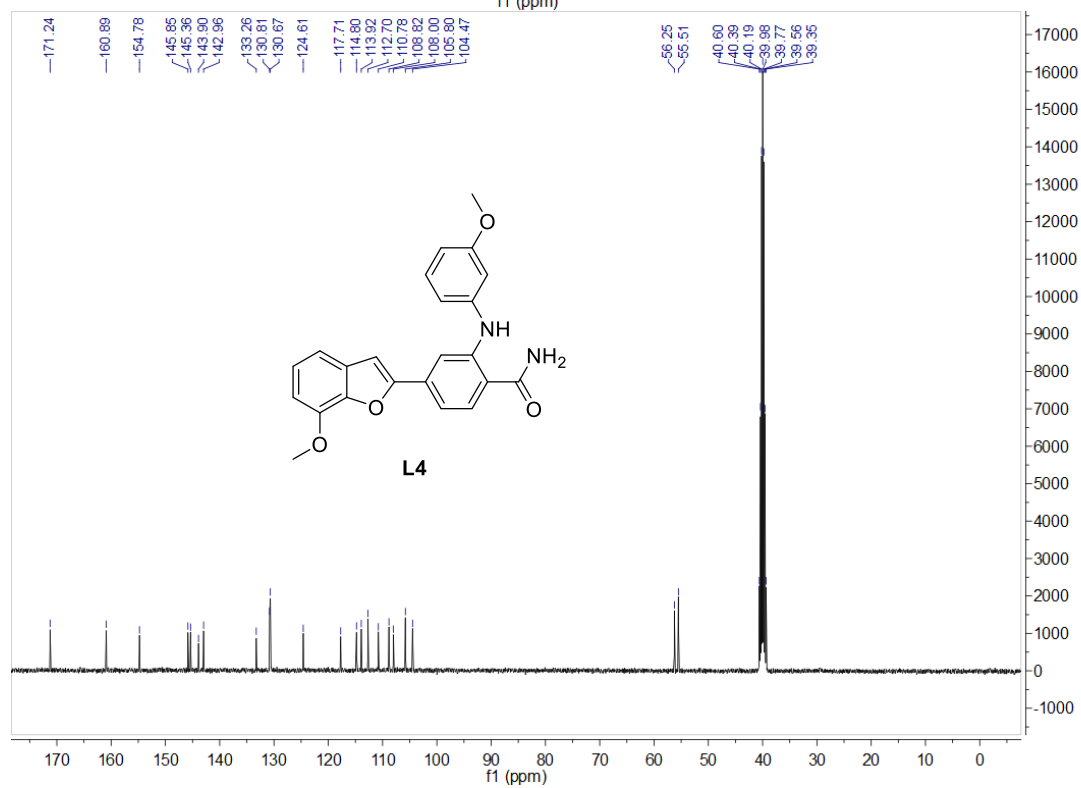
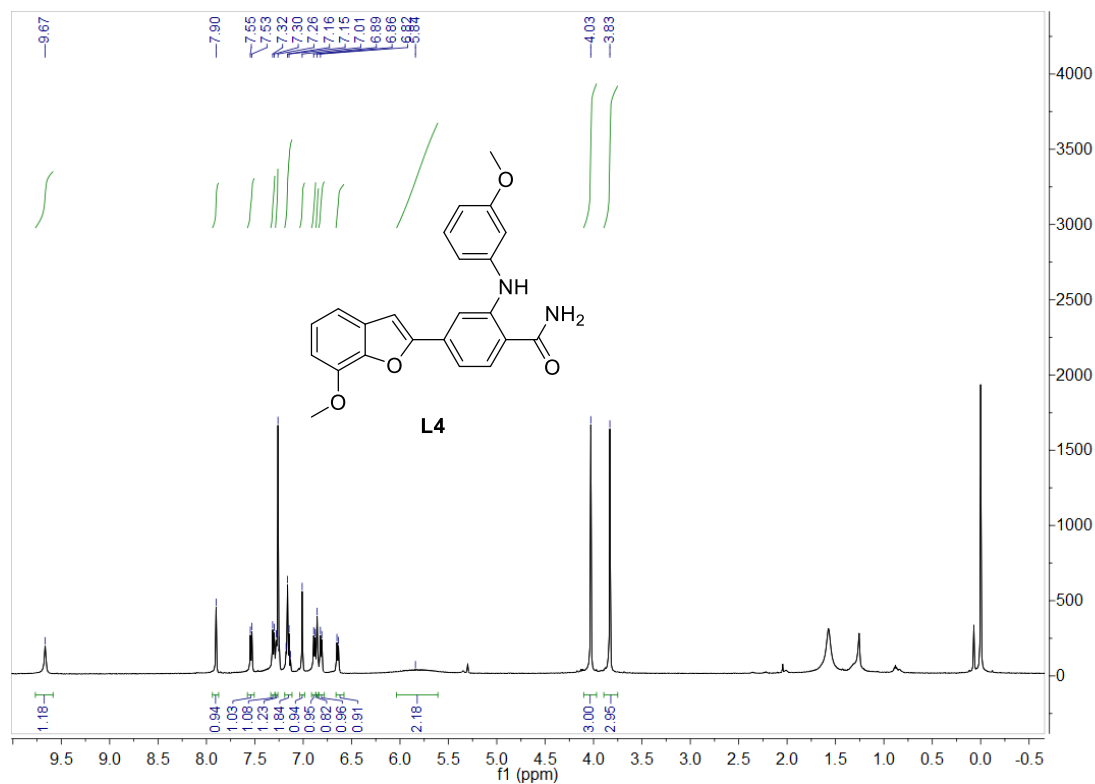
Compounds	Dose (mg/kg, po)	No. of animals with emesis/No. of test animals
L11	10	0/3
Roflumilast	1.0	3/3

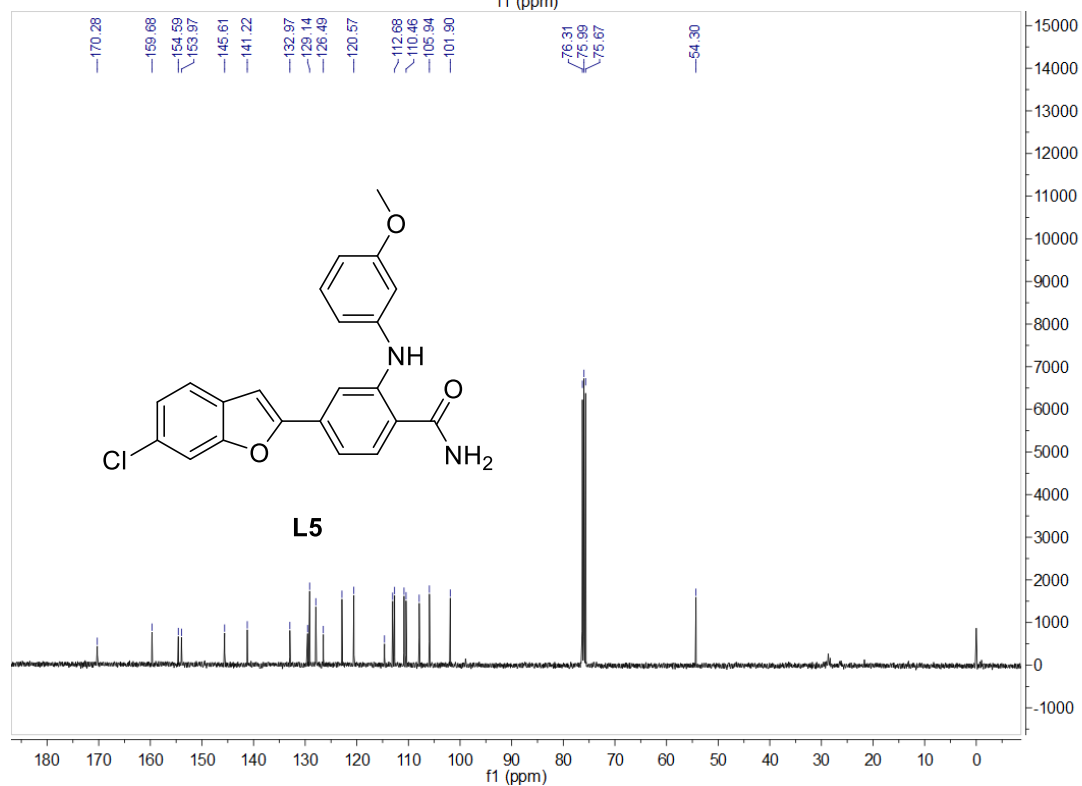
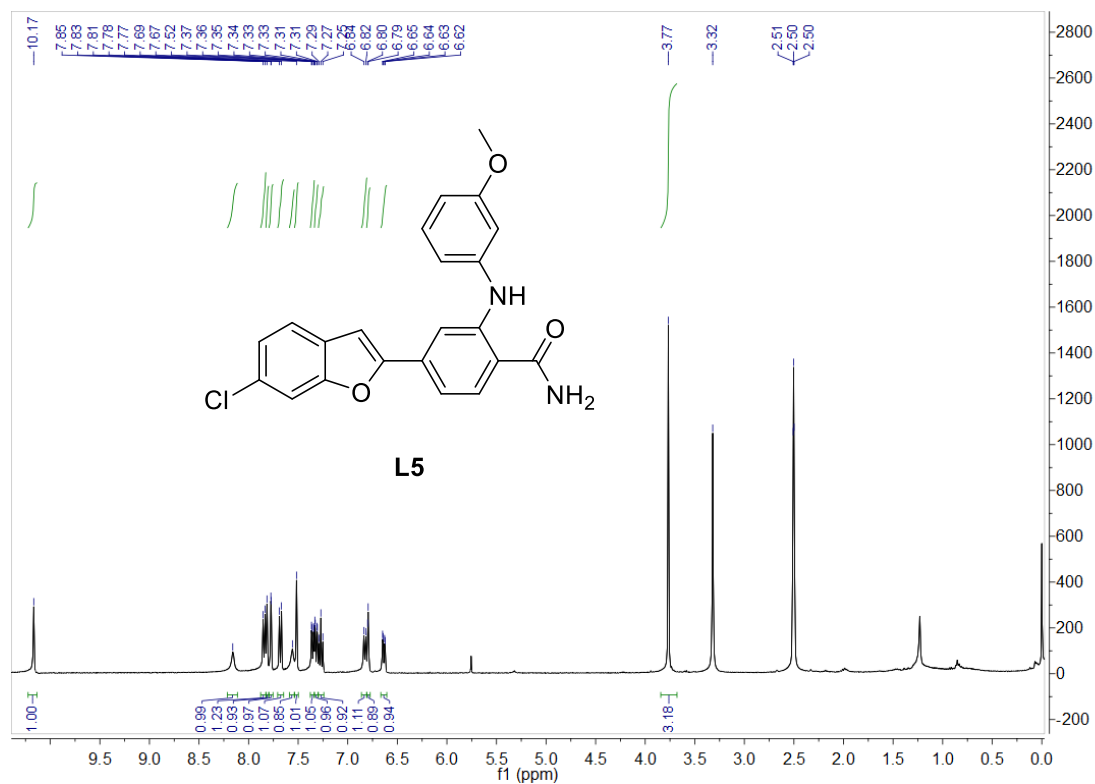
Data file S1. ^1H NMR and ^{13}C NMR spectra of the targeted compounds.

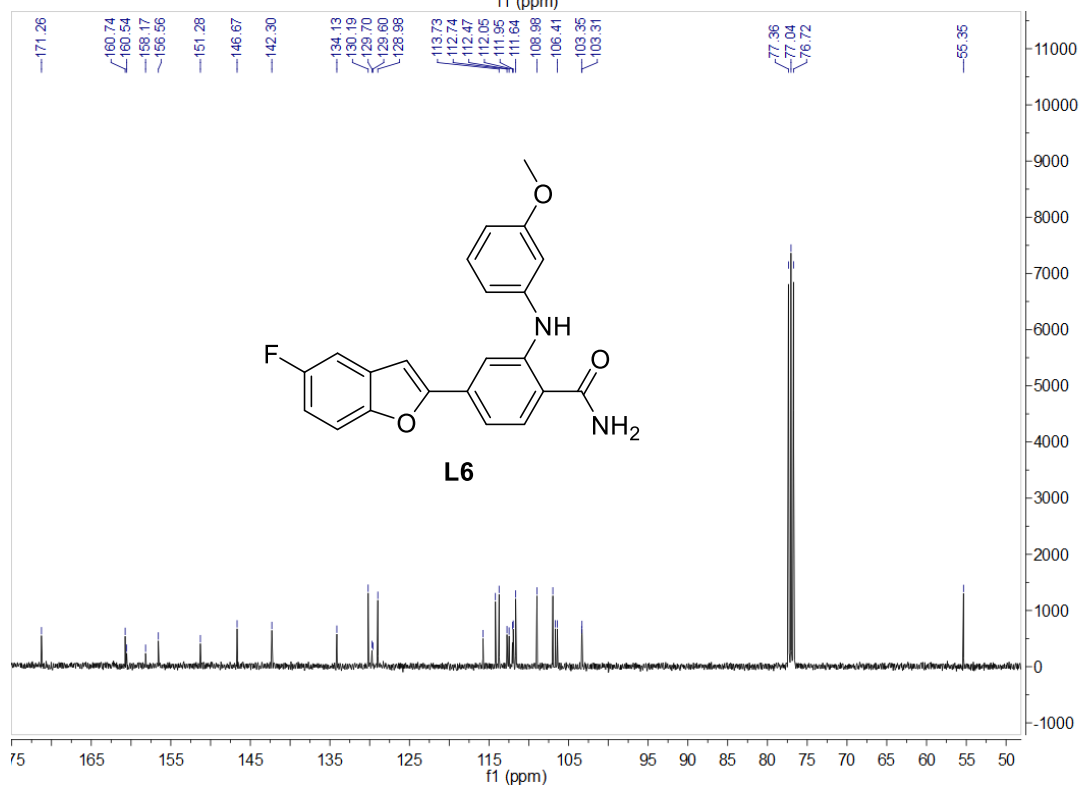
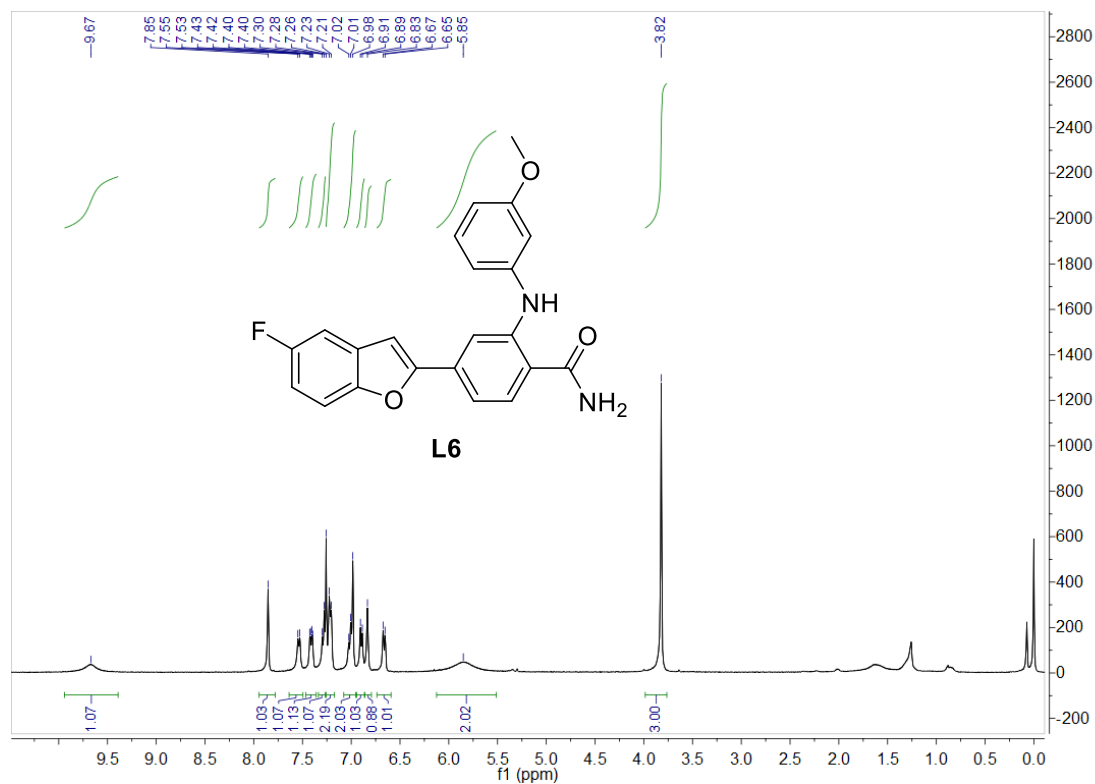


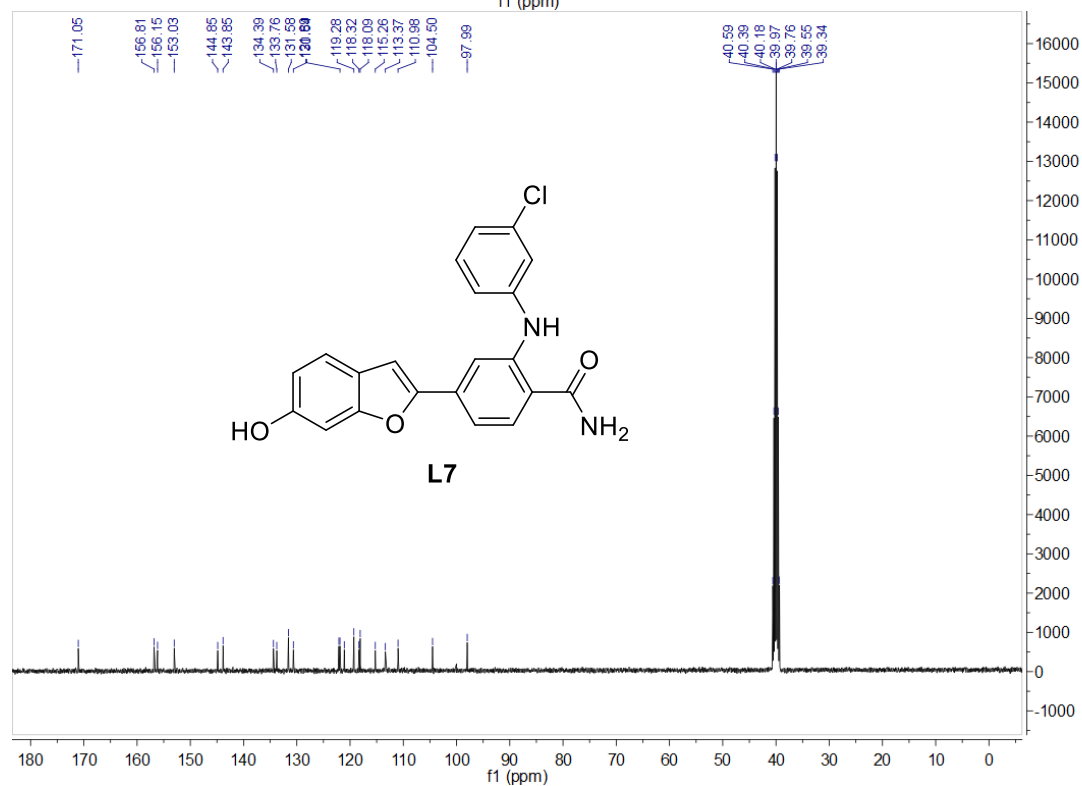
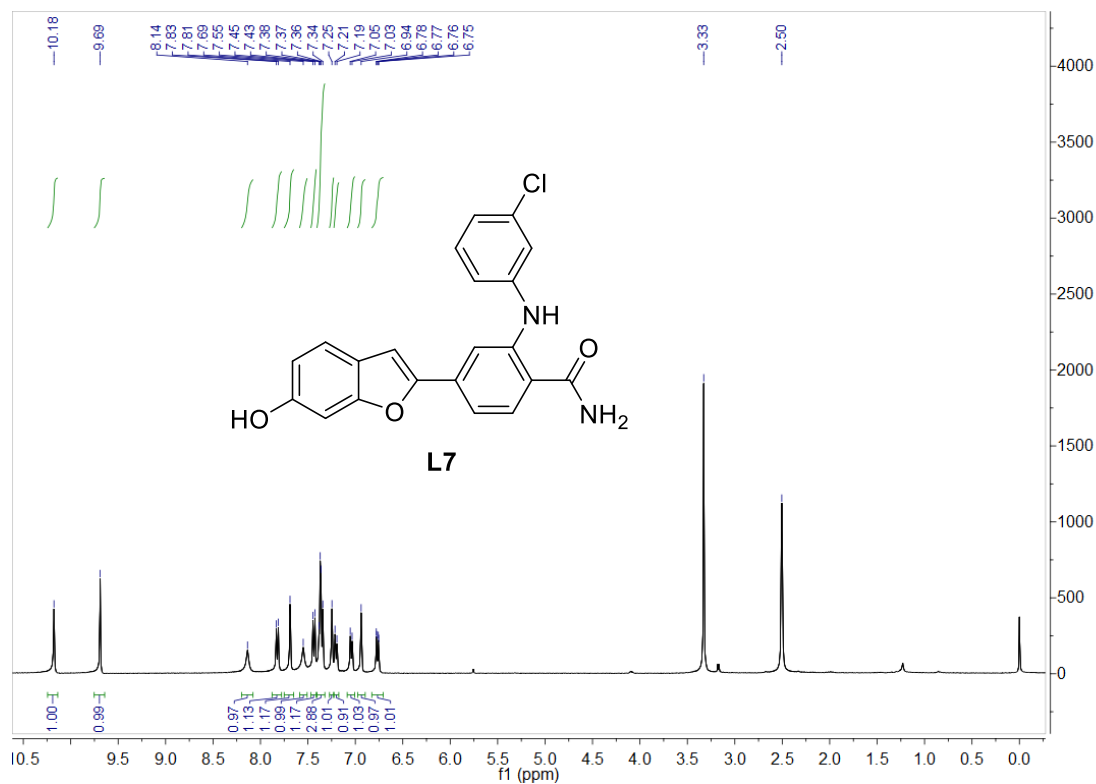


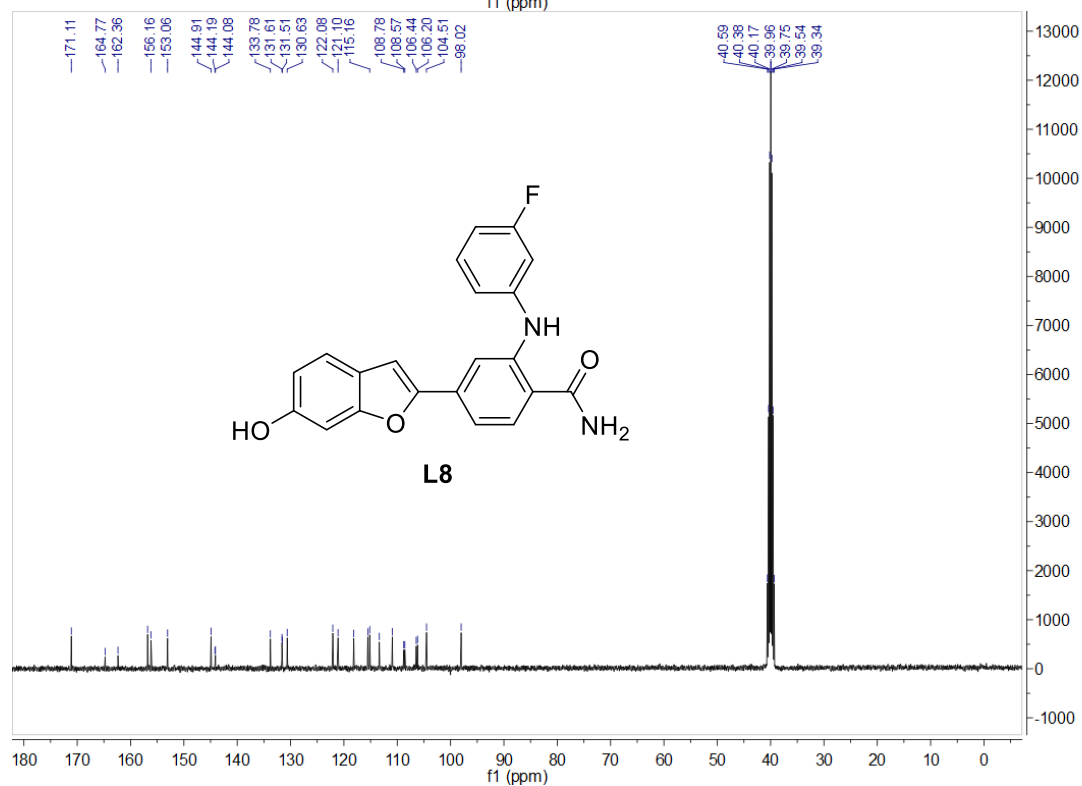
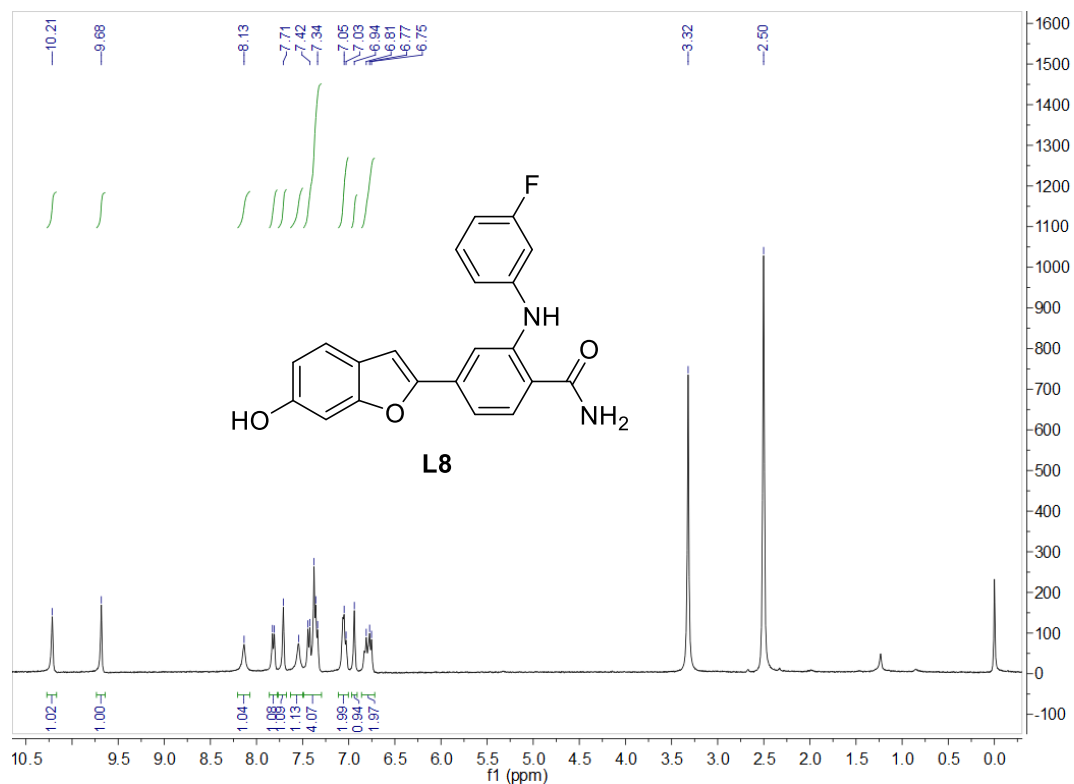


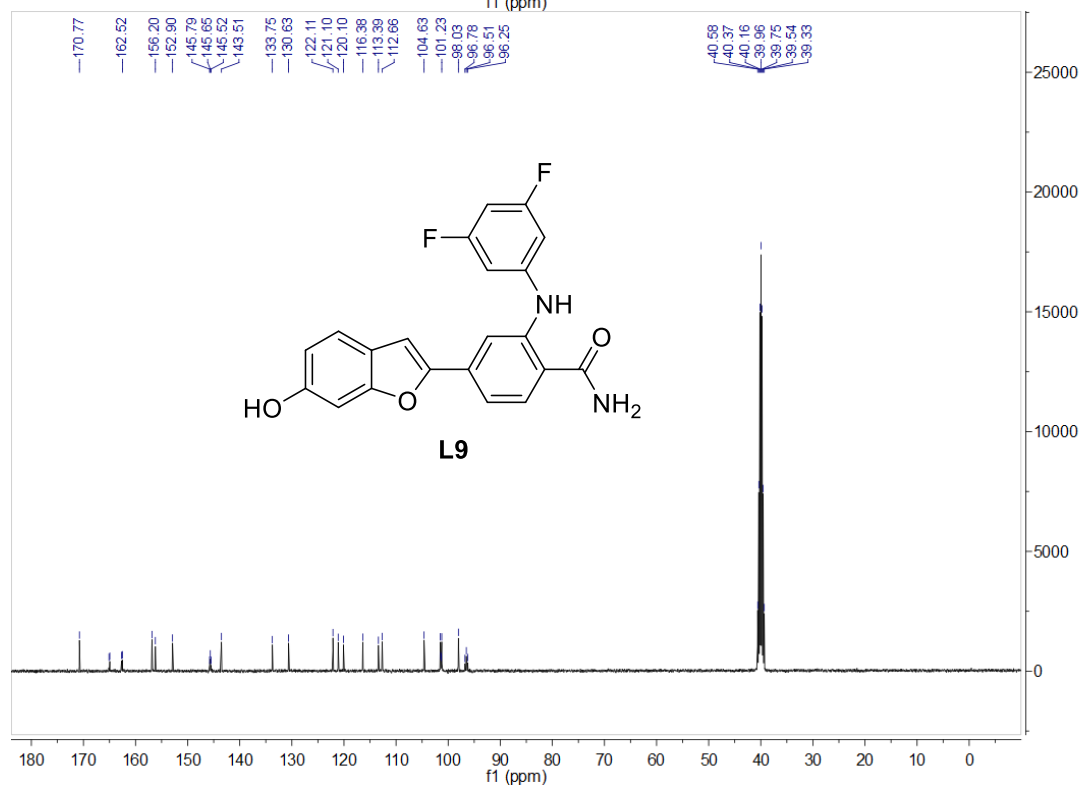
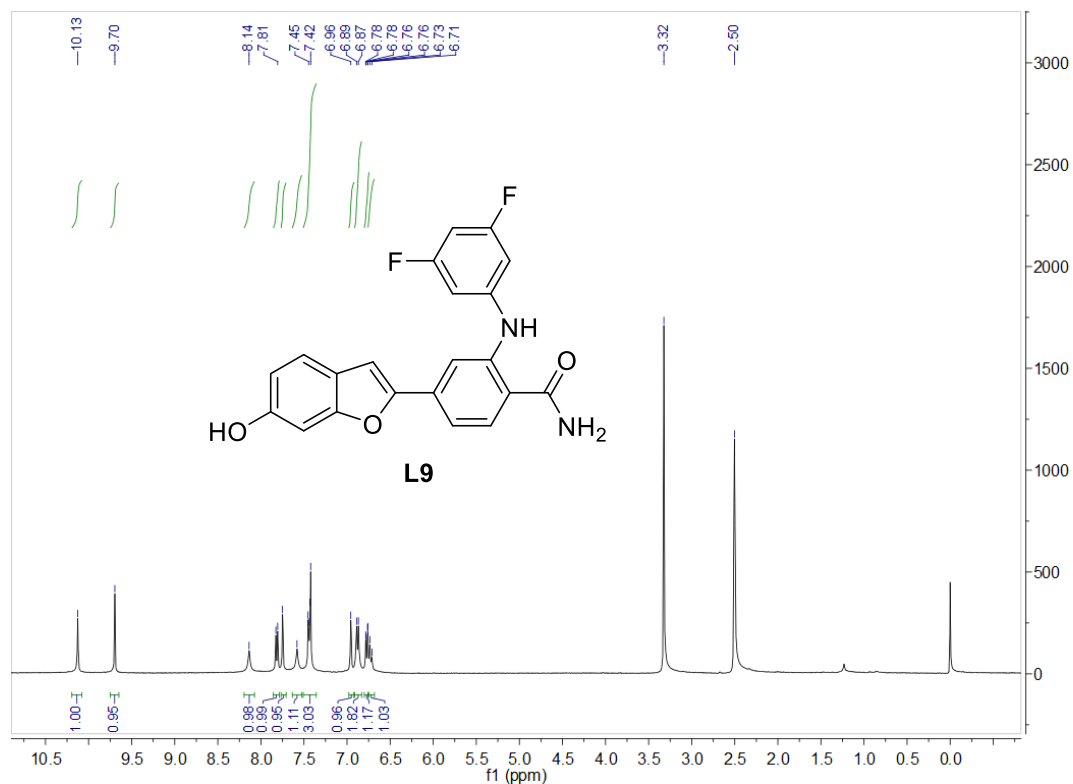


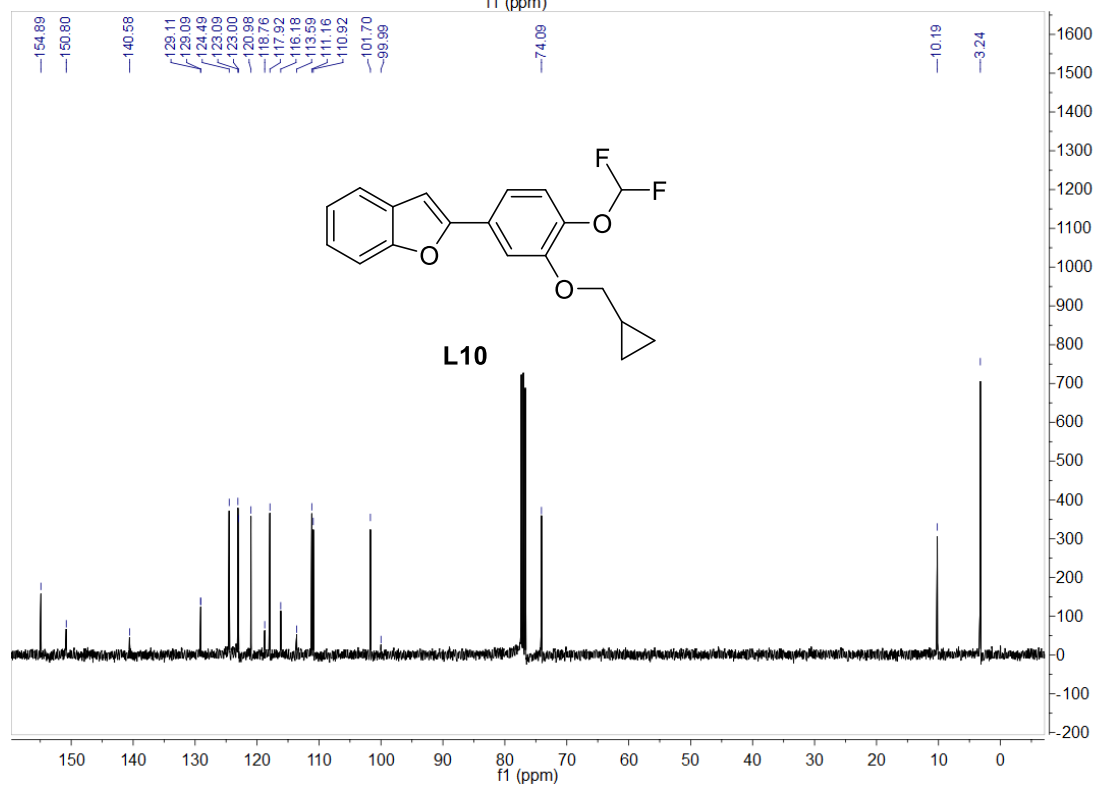
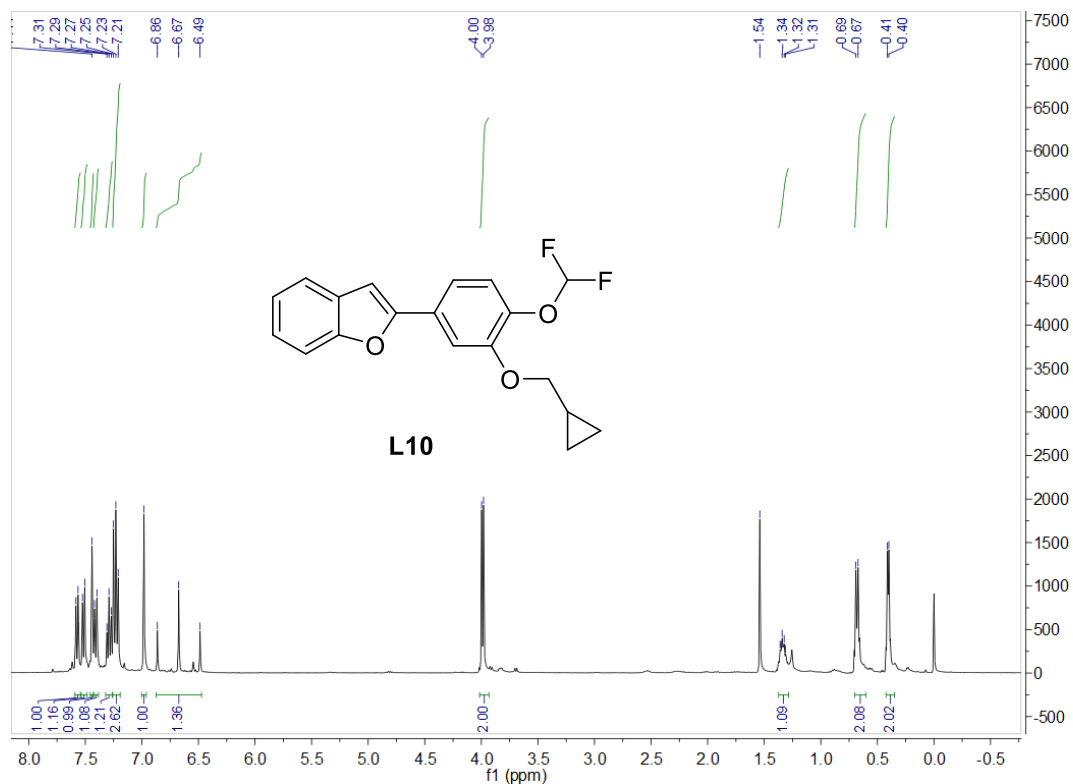


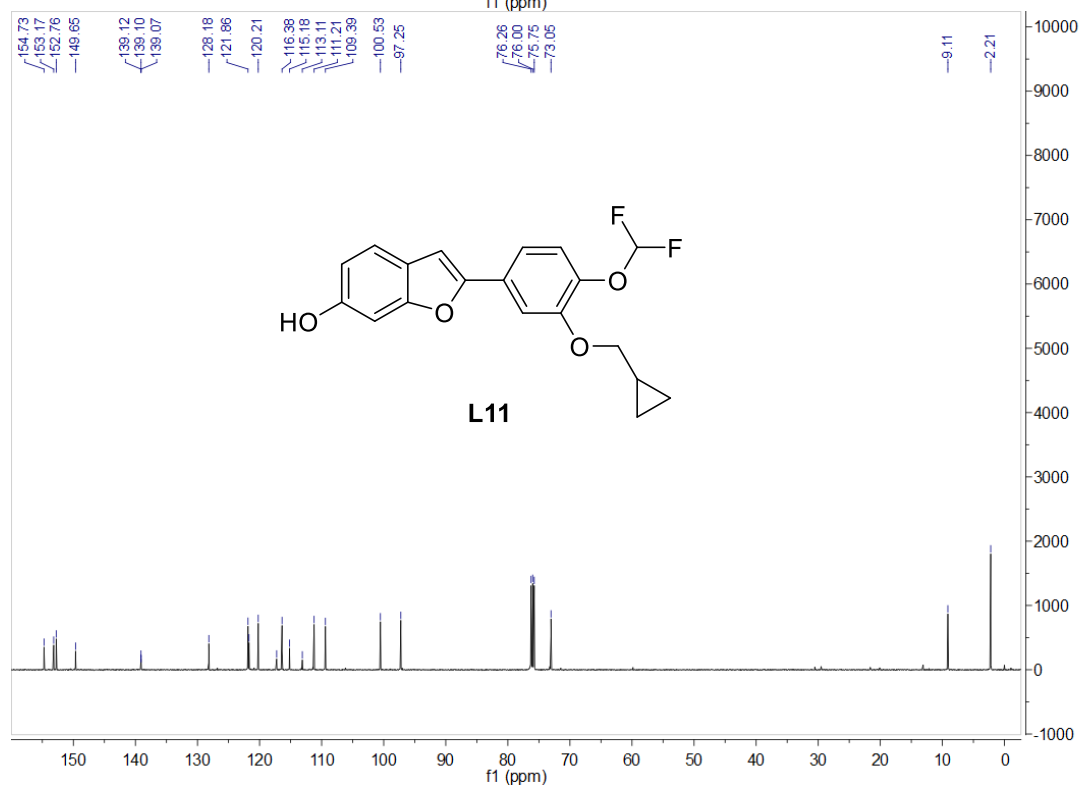
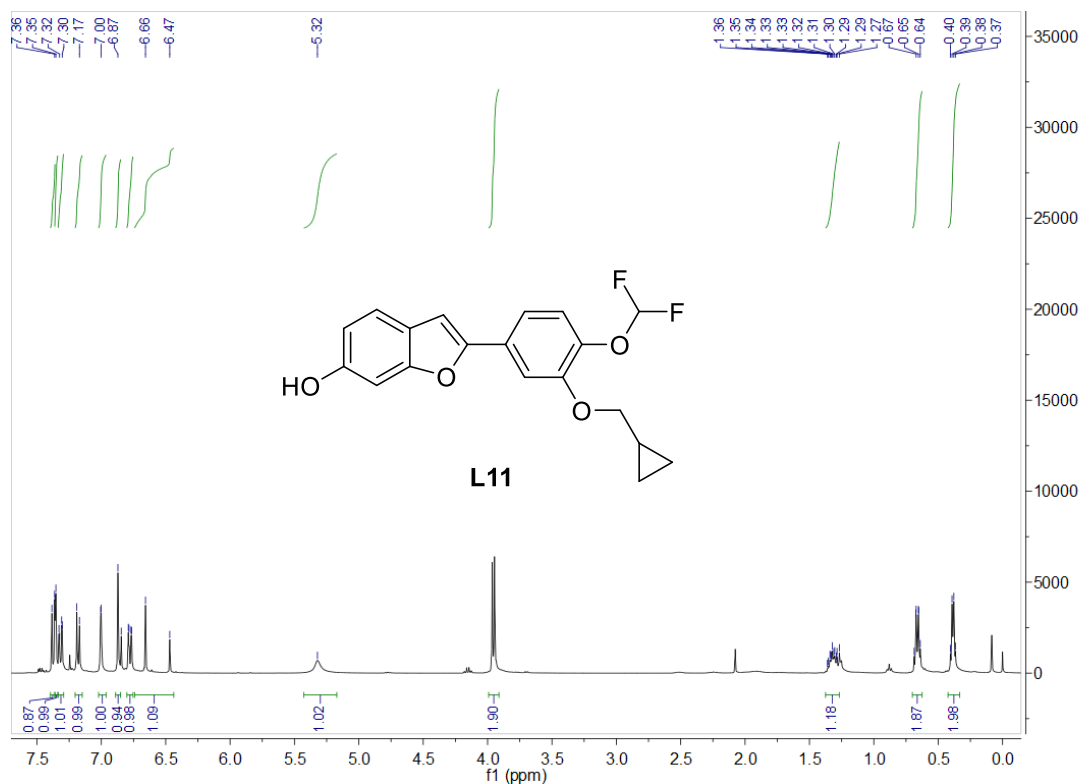


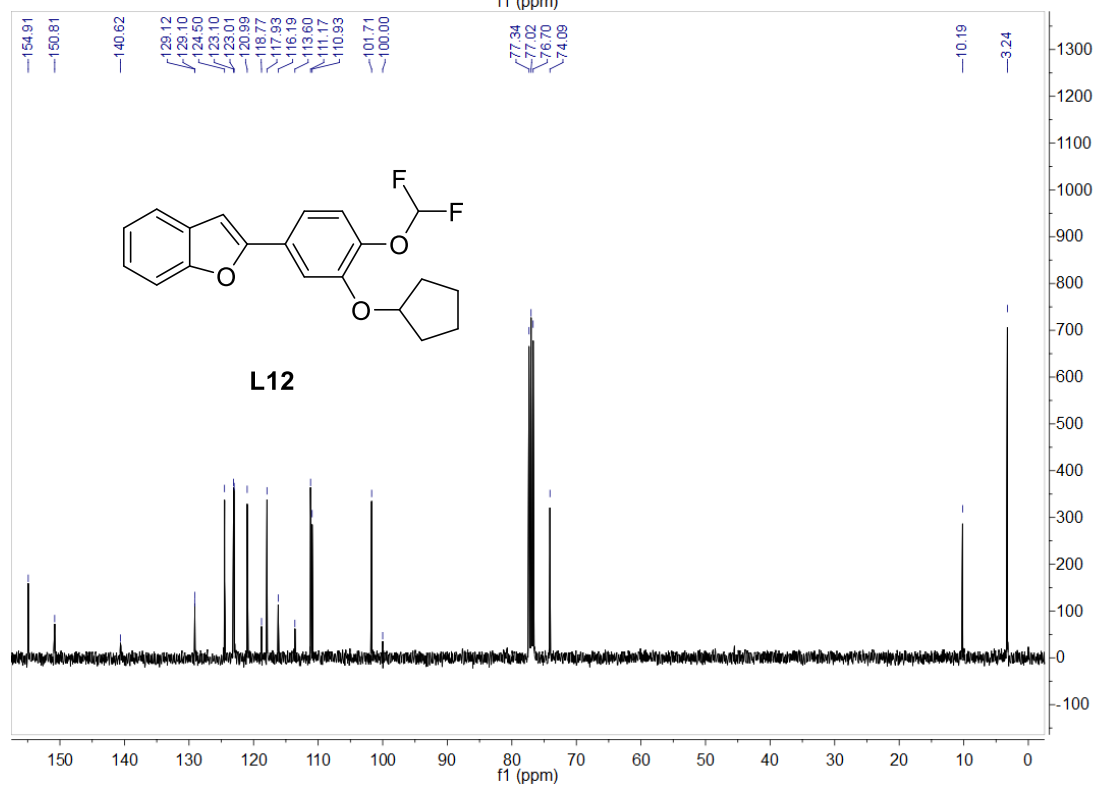
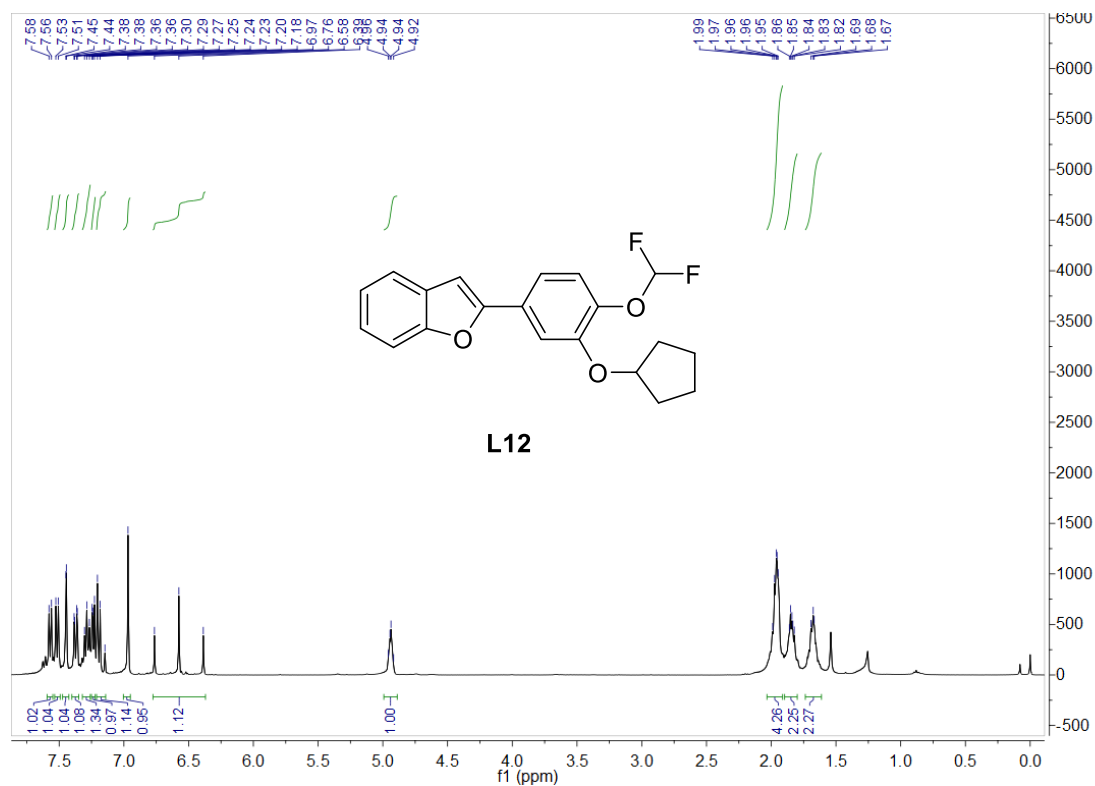


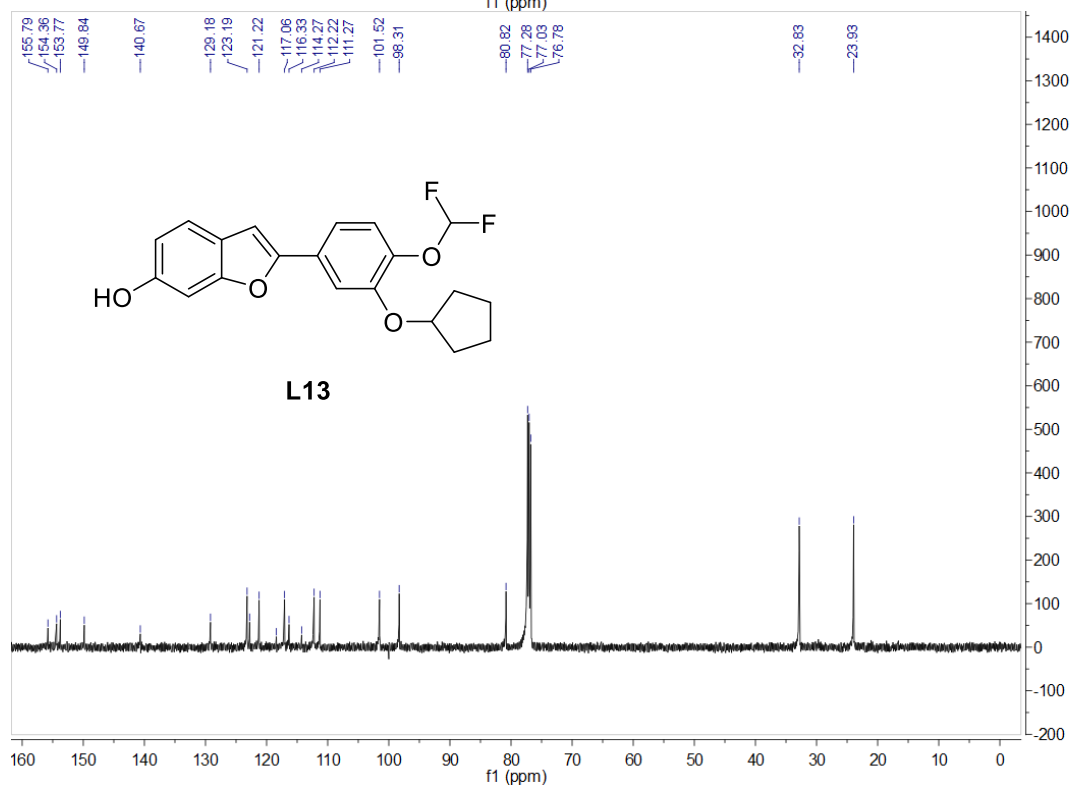
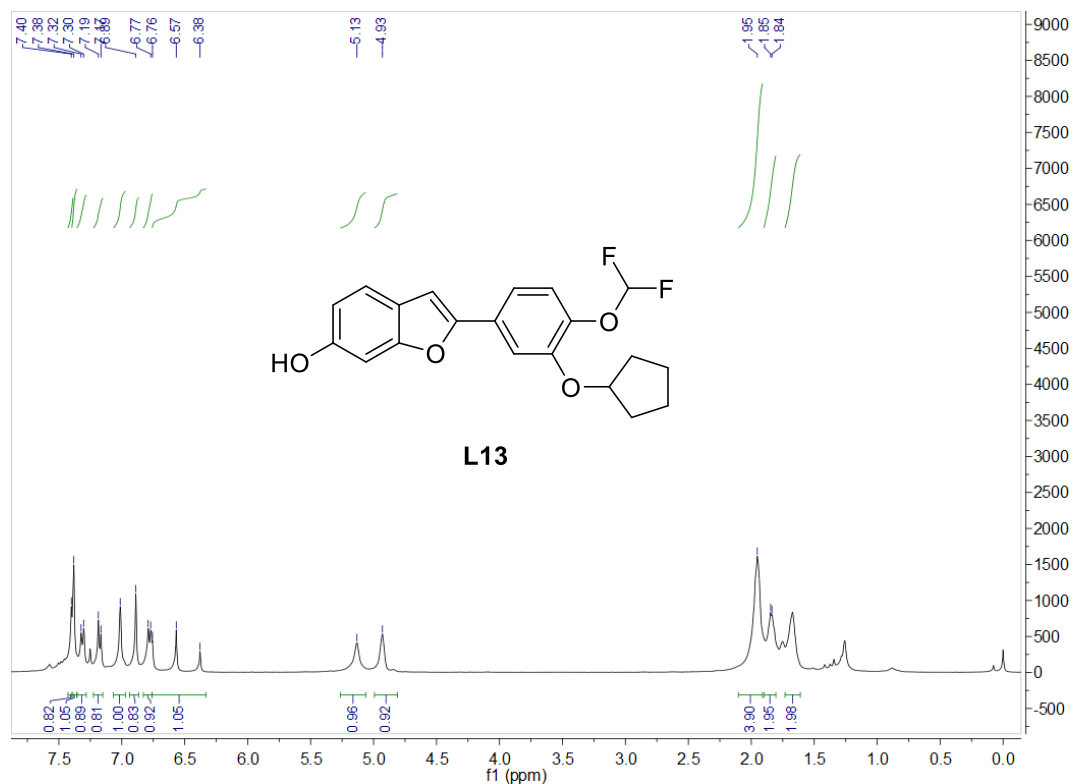












The HRMS spectra of the target compounds.

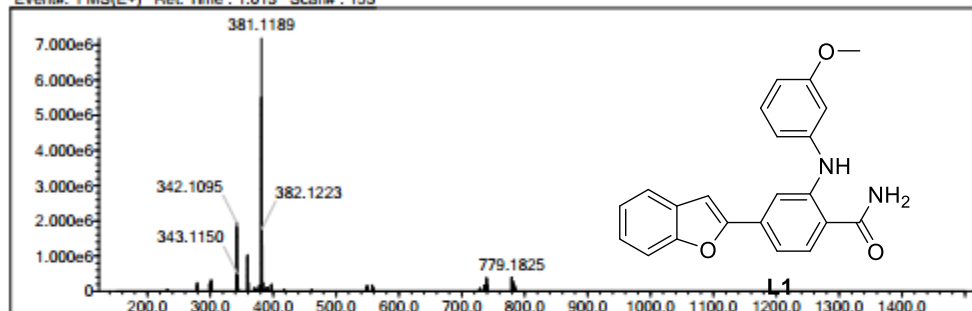
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B	3	0	0	S	2	0	0	Br	1	0	0	Na
C	4	22	22	Cl	1	0	0	Ru	2	0	0	
N	3	2	2	Fe	2	0	0	Sb	3	0	0	
O	2	3	3	Ni	2	0	0	I	3	0	0	
F	1	0	0	Cu	2	0	0	Ir	3	0	0	
Si	4	0	0	Zn	2	0	0	Pt	2	0	0	

Error Margin (mDa): 100.0
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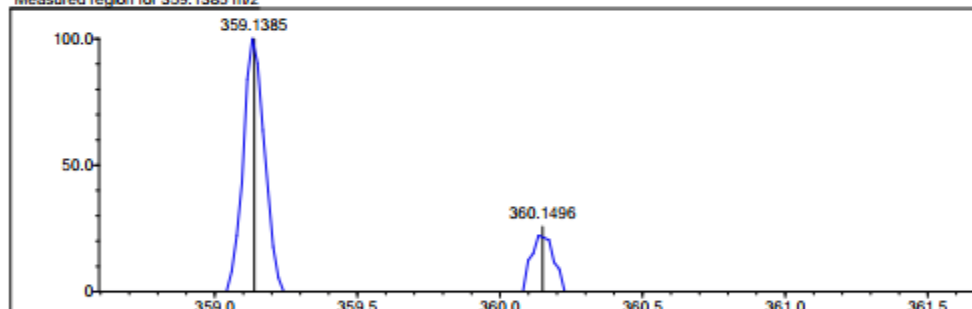
DBE Range: 10.0 - 1000.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 600

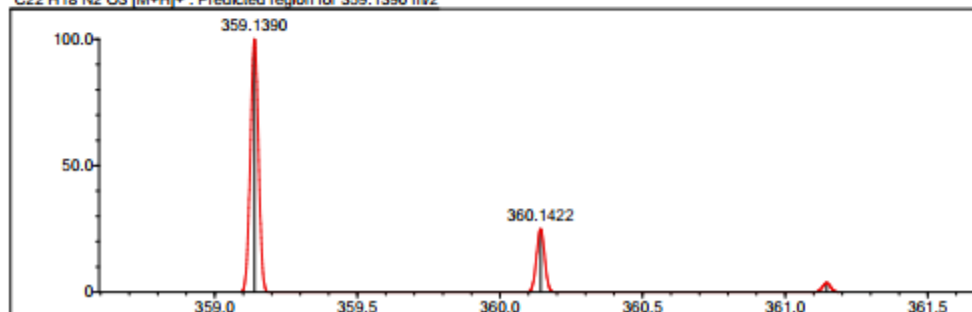
Event#: 1 MS(E+) Ret. Time : 1.013 Scan#: 153



Measured region for 359.1385 m/z



C22 H18 N2 O3 [M+H]⁺ : Predicted region for 359.1390 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	63.31	C22 H18 N2 O3	[M+H] ⁺	359.1385	359.1390	-0.5	+1.39	63.93	15.0

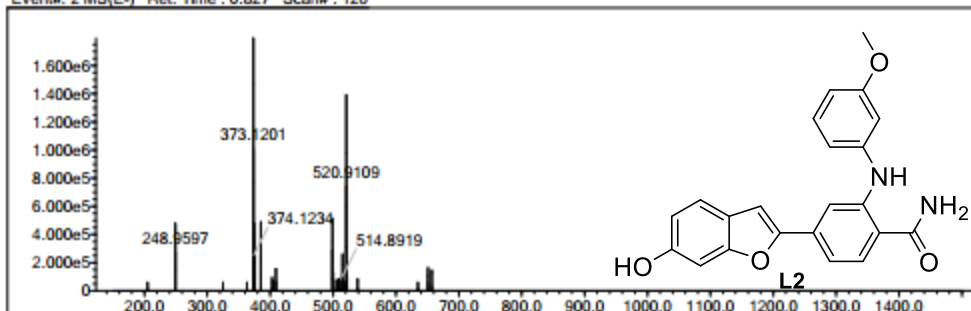
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	18	18	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	HCOO
C	4	22	22	Cl	1	0	0	Sb	3	0	0	CH3COO
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	4	4	Ni	2	0	0	Ir	3	0	0	F
F	1	0	0	Cu	2	0	0	Pt	2	0	0	Cl
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 • 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

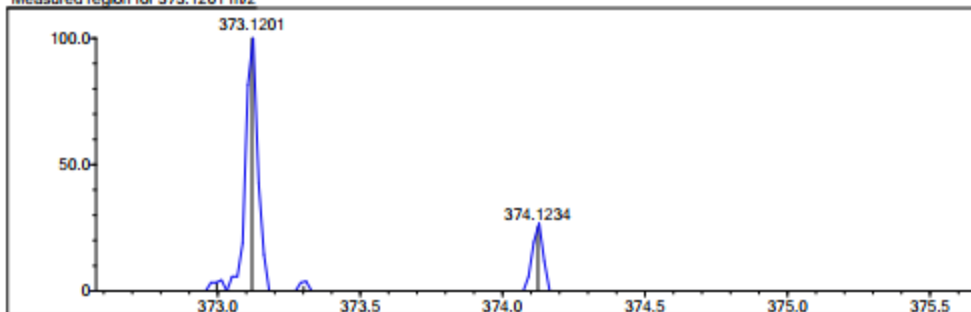
DBE Range: •100.0 • 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

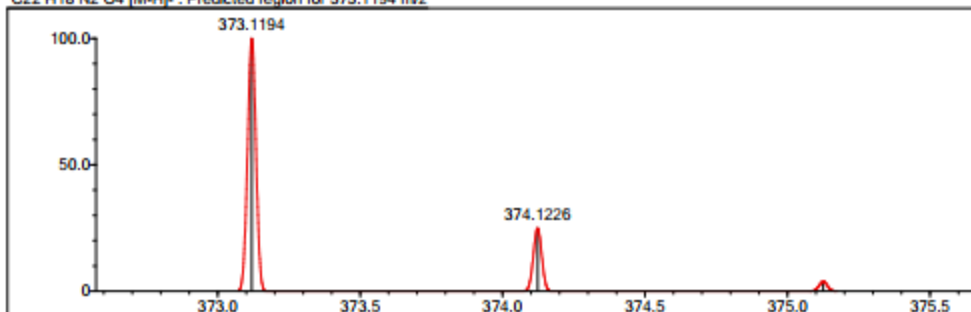
Event#: 2 MS(E-) Ret. Time : 0.827 Scan#: 126



Measured region for 373.1201 m/z



C22 H18 N2 O4 [M-H]- : Predicted region for 373.1194 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	79.81	C22 H18 N2 O4	[M-H]-	373.1201	373.1194	0.7	1.88	81.60	15.0

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	20	20	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	Na
C	4	23	23	Cl	1	0	0	Sb	3	0	0	NH4
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	4	4	Ni	2	0	0	Ir	3	0	0	
F	1	0	2	Cu	2	0	0	Pt	2	0	0	
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0

HC Ratio: 0.0 - 1000.0

Max Isotopes: all

MSn Iso RI (%): 75.00

DBE Range: -100.0 - 100.0

Apply N Rule: yes

Isotope RI (%): 1.00

MSn Logic Mode: AND

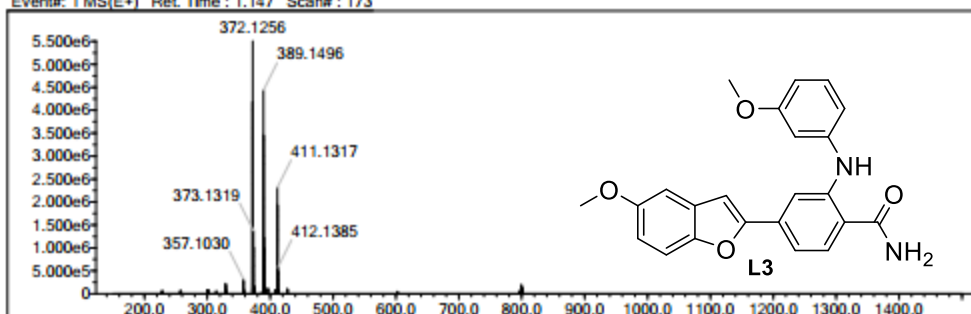
Electron Ions: both

Use MSn Info: yes

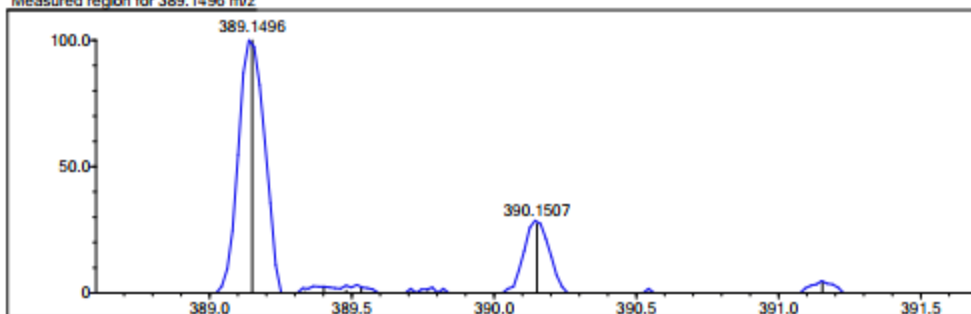
Isotope Res: 10000

Max Results: 10

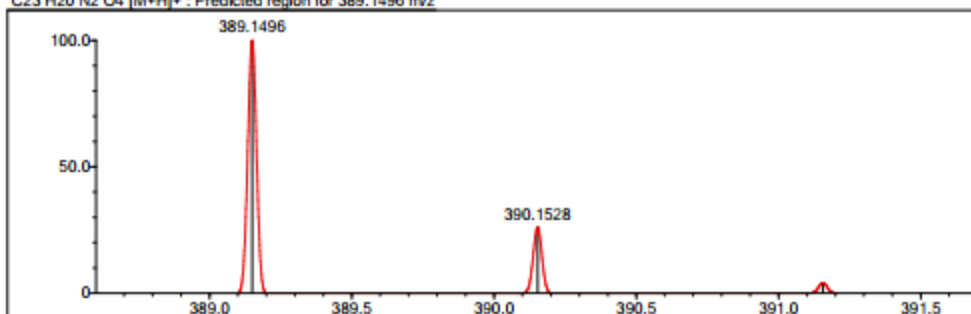
Event#: 1 MS(E+) Ret. Time : 1.147 Scan#: 173



Measured region for 389.1496 m/z



C23 H20 N2 O4 [M+H]⁺ : Predicted region for 389.1496 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	74.88	C23 H20 N2 O4	[M+H] ⁺	389.1496	389.1496	0.0	0.00	74.88	15.0

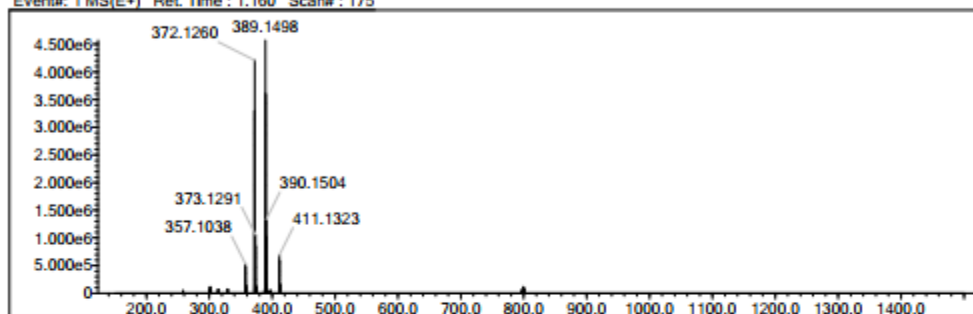
Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	20	20	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	Na
C	4	23	23	Cl	1	0	0	Sb	3	0	0	NH4
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	4	4	Ni	2	0	0	Ir	3	0	0	
F	1	0	2	Cu	2	0	0	Pt	2	0	0	
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

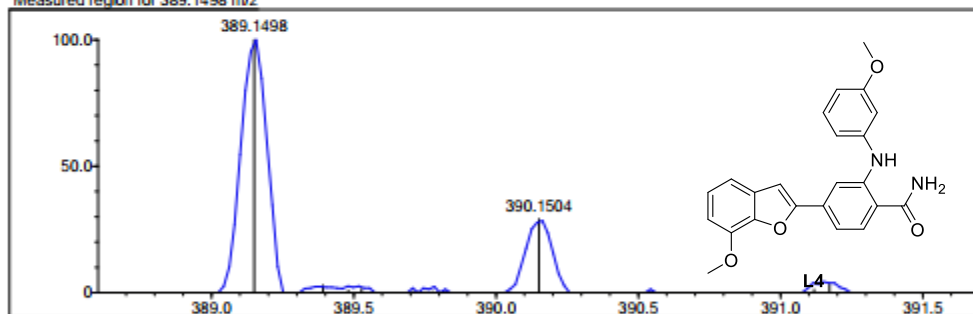
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

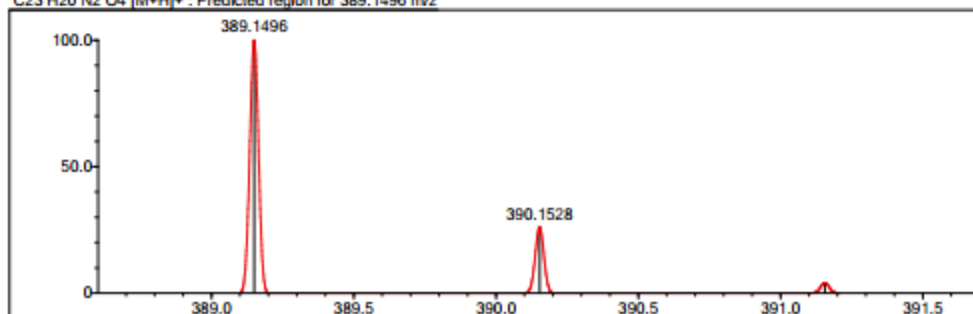
Event#: 1 MS(E+) Ret. Time : 1.160 Scan#: 175



Measured region for 389.1498 m/z



C23 H20 N2 O4 [M+H]⁺ : Predicted region for 389.1496 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	85.73	C23 H20 N2 O4	[M+H] ⁺	389.1498	389.1496	0.2	0.51	85.73	15.0

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	17	17	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	HCOO
C	4	22	22	Cl	1	1	1	Sb	3	0	0	CH3COO
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	3	3	Ni	2	0	0	Ir	3	0	0	F
F	1	0	0	Cu	2	0	0	Pt	2	0	0	Cl
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0

HC Ratio: 0.0 - 1000.0

Max Isotopes: all

MSn Iso RI (%): 75.00

DBE Range: -100.0 - 100.0

Apply N Rule: yes

Isotope RI (%): 1.00

MSn Logic Mode: AND

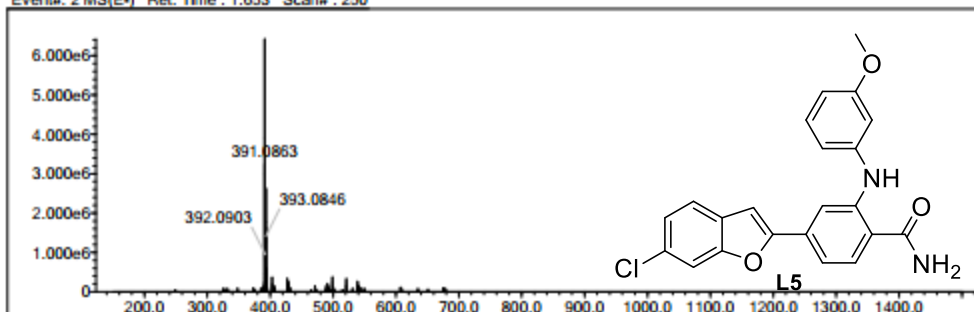
Electron Ions: both

Use MSn Info: yes

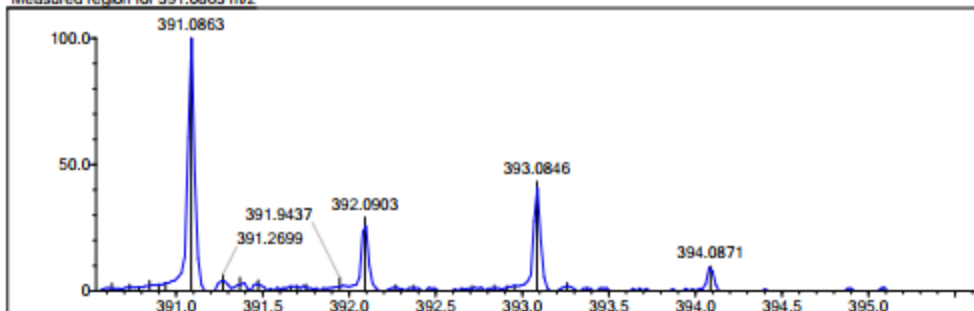
Isotope Res: 10000

Max Results: 10

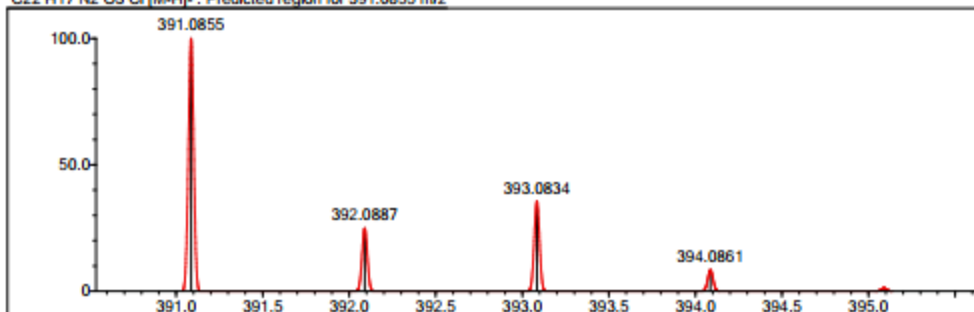
Event#: 2 MS(E-) Ret. Time : 1.653 Scan#: 250



Measured region for 391.0863 m/z



C22 H17 N2 O3 Cl [M-H]- : Predicted region for 391.0855 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	83.57	C22 H17 N2 O3 Cl	[M-H]-	391.0863	391.0855	0.8	2.05	85.82	15.0

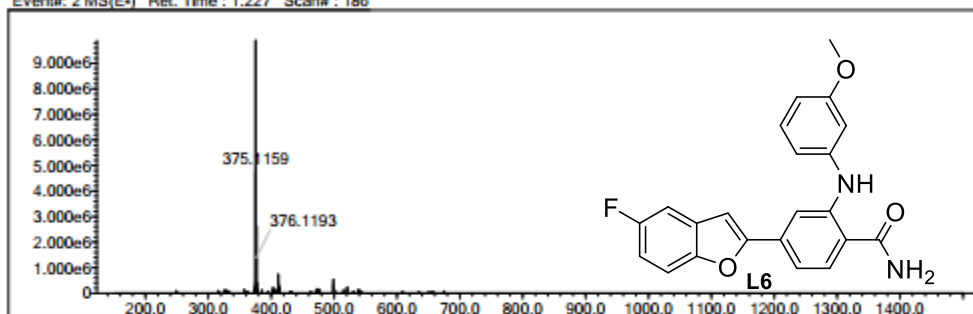
Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	17	17	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	HCOO
C	4	22	22	Cl	1	0	0	Sb	3	0	0	CH3COO
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	3	3	Ni	2	0	0	Ir	3	0	0	F
F	1	1	1	Cu	2	0	0	Pt	2	0	0	Cl
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

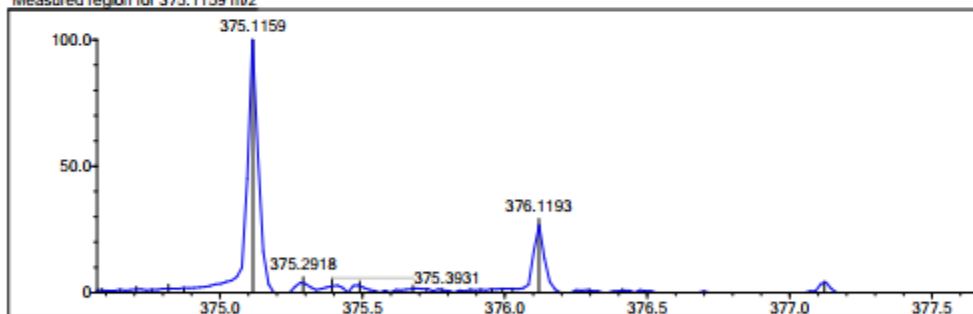
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

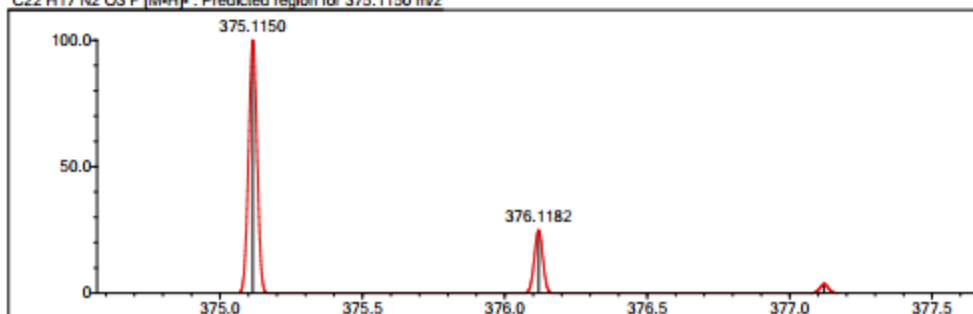
Event#: 2 MS(E-) Ret. Time : 1.227 Scan#: 186



Measured region for 375.1159 m/z



C22 H17 N2 O3 F [M-H]- : Predicted region for 375.1150 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	96.50	C22 H17 N2 O3 F	[M-H]-	375.1159	375.1150	0.9	2.40	100.00	15.0

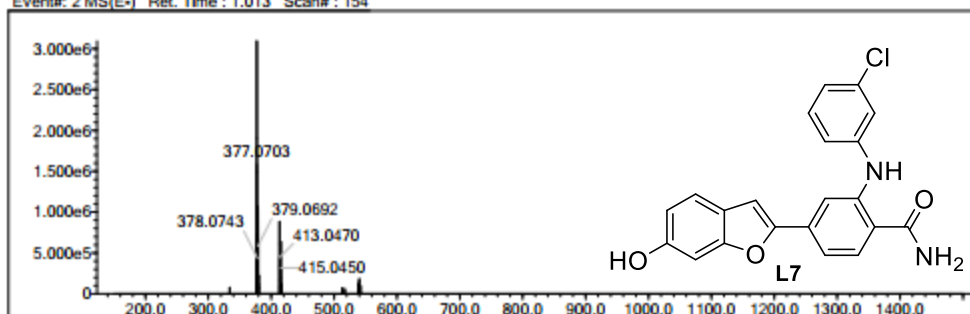
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	15	18	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	HCOO
C	4	21	21	Cl	1	1	1	Sb	3	0	0	CH3COO
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	3	4	Ni	2	0	0	Ir	3	0	0	F
F	1	0	2	Cu	2	0	0	Pt	2	0	0	Cl
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

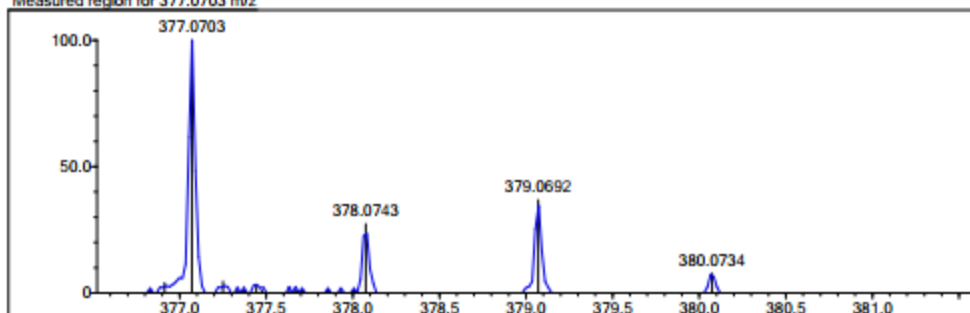
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

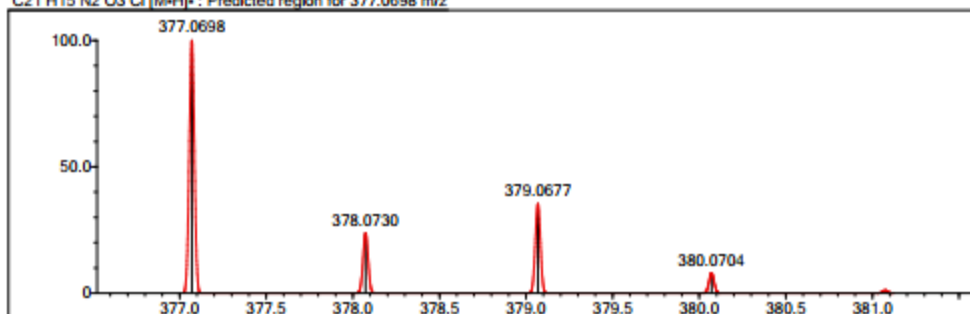
Event#: 2 MS(E-) Ret. Time : 1.013 Scan#: 154



Measured region for 377.0703 m/z



C21 H15 N2 O3 Cl [M-H]- : Predicted region for 377.0698 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isr	DBE
1	89.80	C21 H15 N2 O3 Cl	[M-H]-	377.0703	377.0698	0.5	1.33	90.55	15.0

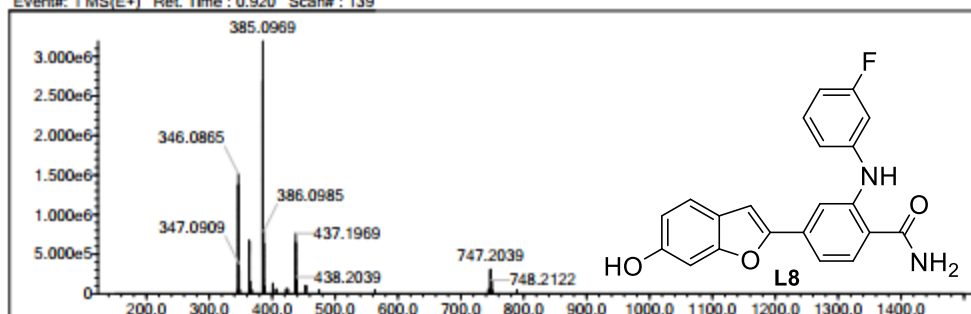
Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	15	15	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	Na
C	4	21	21	Cl	1	0	0	Sb	3	0	0	NH4
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	3	3	Ni	2	0	0	Ir	3	0	0	
F	1	1	1	Cu	2	0	0	Pt	2	0	0	
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

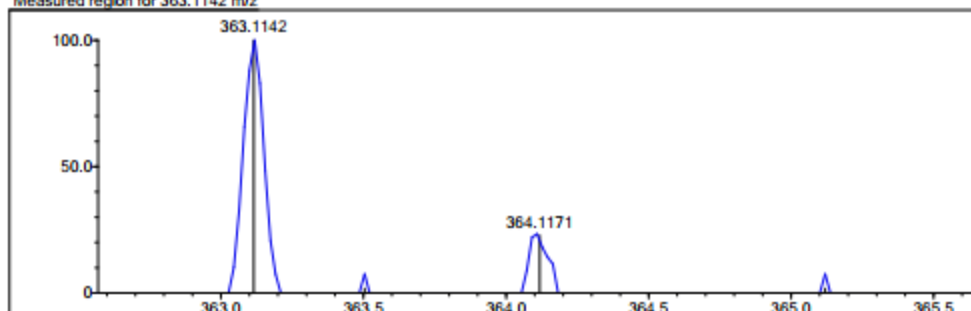
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

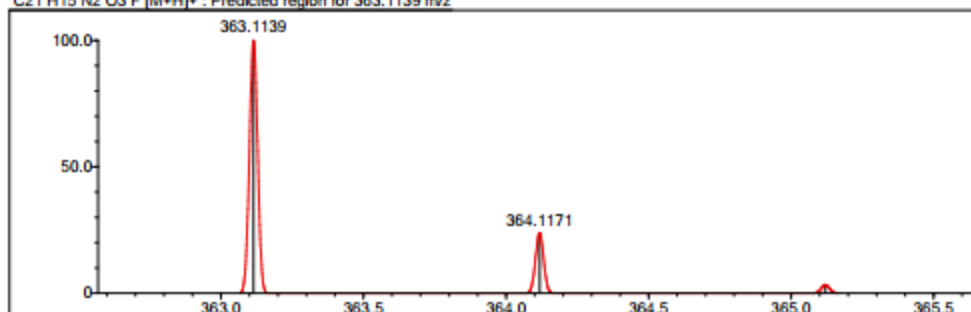
Event#: 1 MS(E+) Ret. Time : 0.920 Scan#: 139



Measured region for 363.1142 m/z



C21 H15 N2 O3 F [M+H]⁺ : Predicted region for 363.1139 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	67.33	C21 H15 N2 O3 F	[M+H] ⁺	363.1142	363.1139	0.3	0.83	67.33	15.0

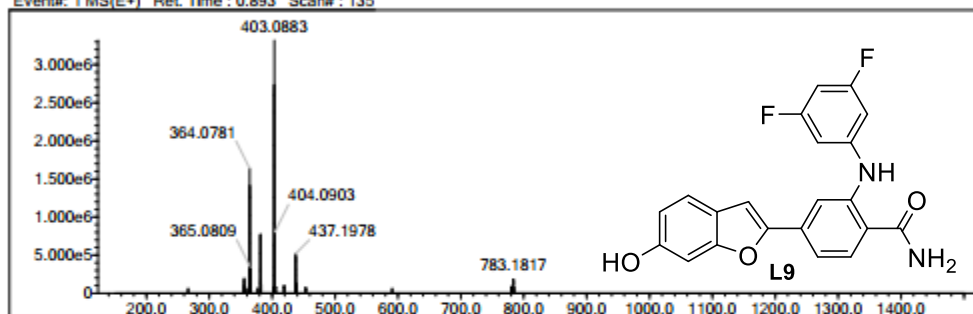
Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	14	14	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	Na
C	4	21	21	Cl	1	0	0	Sb	3	0	0	NH4
N	3	2	2	Fe	2	0	0	I	3	0	0	K
O	2	3	3	Ni	2	0	0	Ir	3	0	0	
F	1	2	2	Cu	2	0	0	Pt	2	0	0	
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 • 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

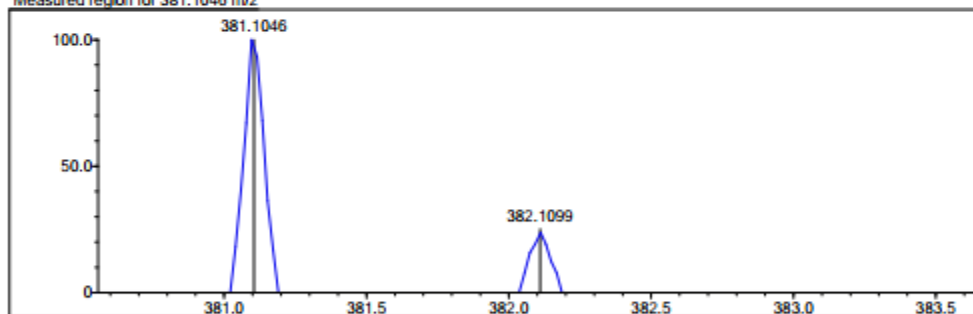
DBE Range: -100.0 • 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

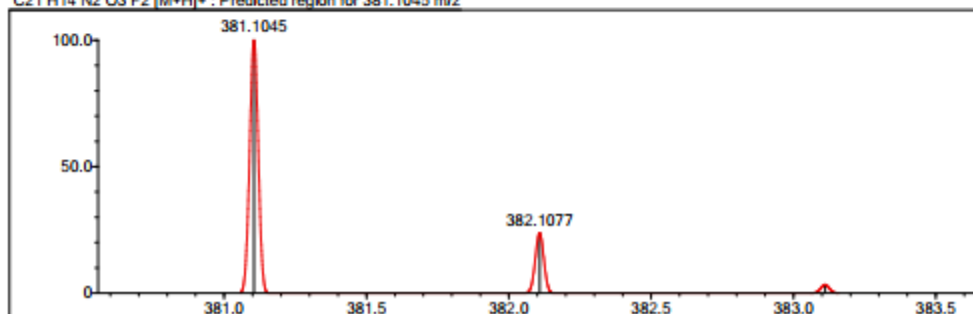
Event#: 1 MS(E+) Ret. Time : 0.893 Scan#: 135



Measured region for 381.1046 m/z



C21 H14 N2 O3 F2 [M+H]⁺ : Predicted region for 381.1045 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	81.50	C21 H14 N2 O3 F2	[M+H] ⁺	381.1046	381.1045	0.1	0.26	81.50	15.0

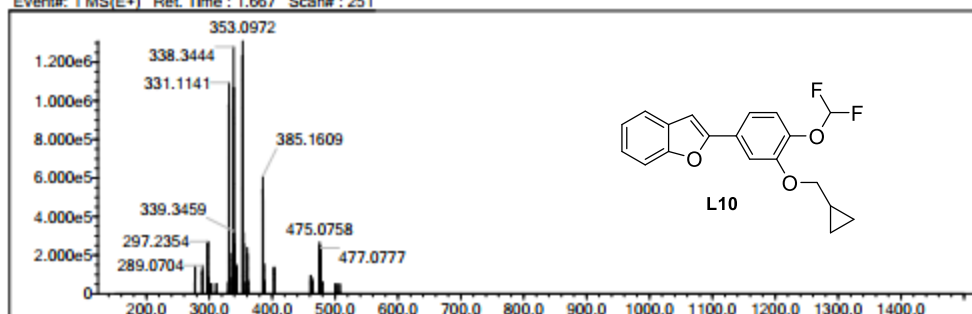
Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	16	16	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	Na
C	4	19	19	Cl	1	0	0	Sb	3	0	0	NH4
N	3	0	0	Fe	2	0	0	I	3	0	0	K
O	2	3	3	Ni	2	0	0	Ir	3	0	0	
F	1	2	2	Cu	2	0	0	Pt	2	0	0	
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

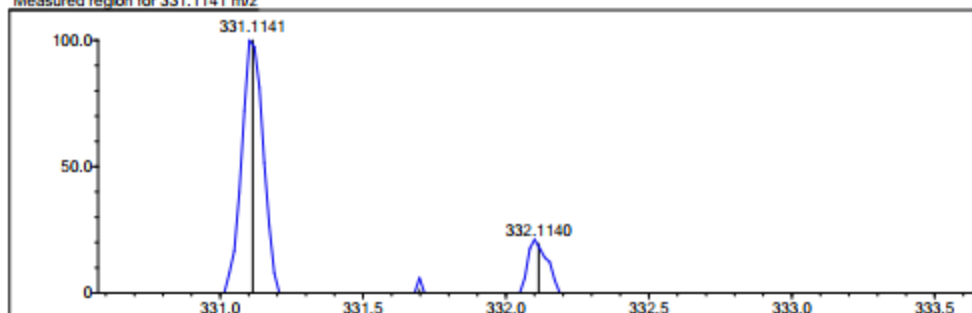
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

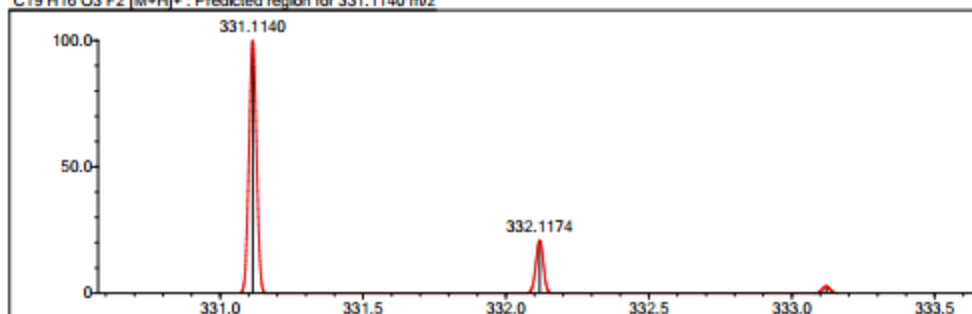
Event#: 1 MS(E+) Ret. Time : 1.667 Scan#: 251



Measured region for 331.1141 m/z



C19 H16 O3 F2 [M+H]⁺ : Predicted region for 331.1140 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	67.12	C19 H16 O3 F2	[M+H] ⁺	331.1141	331.1140	0.1	0.30	67.12	11.0

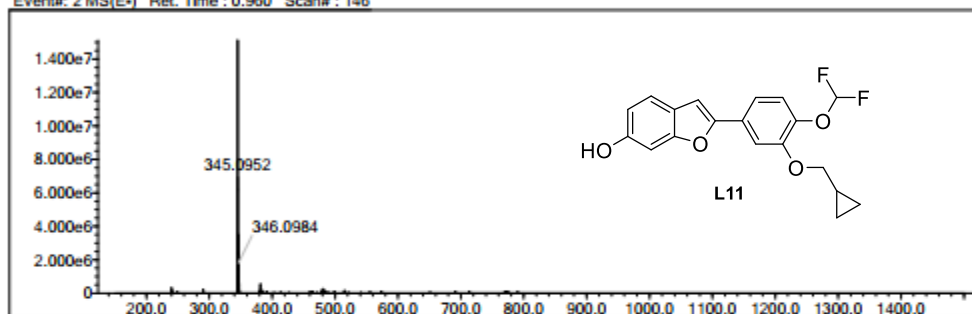
Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	16	16	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	HCOO
C	4	19	19	Cl	1	0	0	Sb	3	0	0	CH3COO
N	3	0	0	Fe	2	0	0	I	3	0	0	K
O	2	0	4	Ni	2	0	0	Ir	3	0	0	F
F	1	0	2	Cu	2	0	0	Pt	2	0	0	Cl
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

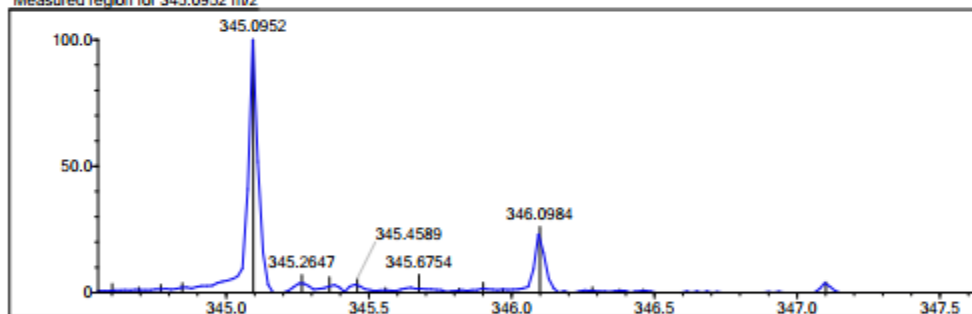
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

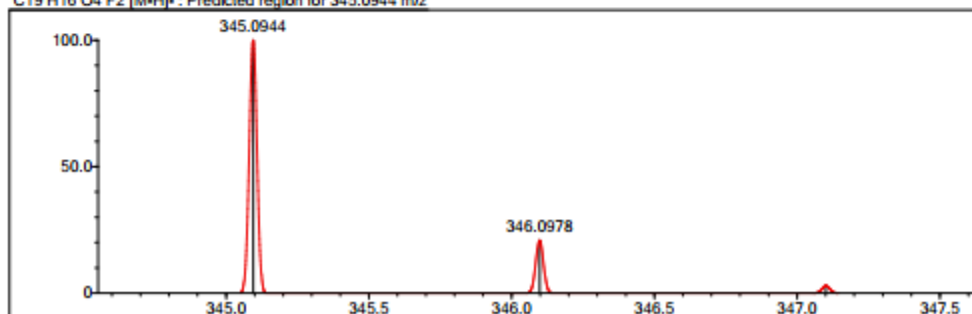
Event#: 2 MS(E-) Ret. Time : 0.960 Scan# : 146



Measured region for 345.0952 m/z



C19 H16 O4 F2 [M-H]- : Predicted region for 345.0944 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	96.18	C19 H16 O4 F2	[M-H]-	345.0952	345.0944	0.8	2.32	99.46	11.0

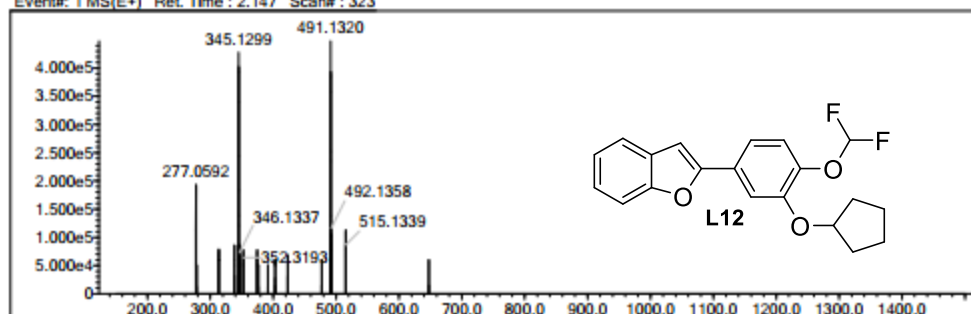
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	18	18	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	Na
C	4	20	20	Cl	1	0	0	Sb	3	0	0	NH4
N	3	0	0	Fe	2	0	0	I	3	0	0	K
O	2	3	3	Ni	2	0	0	Ir	3	0	0	
F	1	2	2	Cu	2	0	0	Pt	2	0	0	
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 - 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

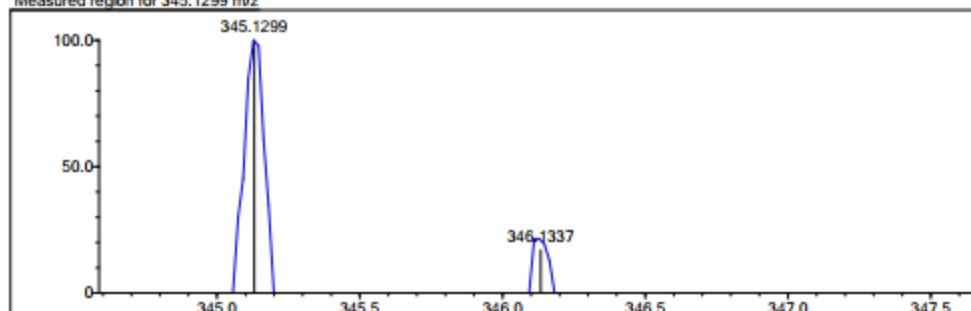
DBE Range: -100.0 - 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

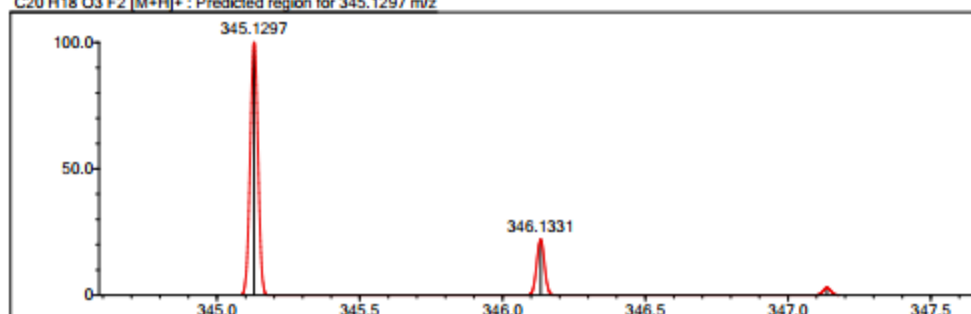
Event#: 1 MS(E+) Ret. Time: 2.147 Scan#: 323



Measured region for 345.1299 m/z



C20 H18 O3 F2 [M+H]⁺: Predicted region for 345.1297 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isr	DBE
1	84.76	C20 H18 O3 F2	[M+H] ⁺	345.1299	345.1297	0.2	0.58	84.76	11.0

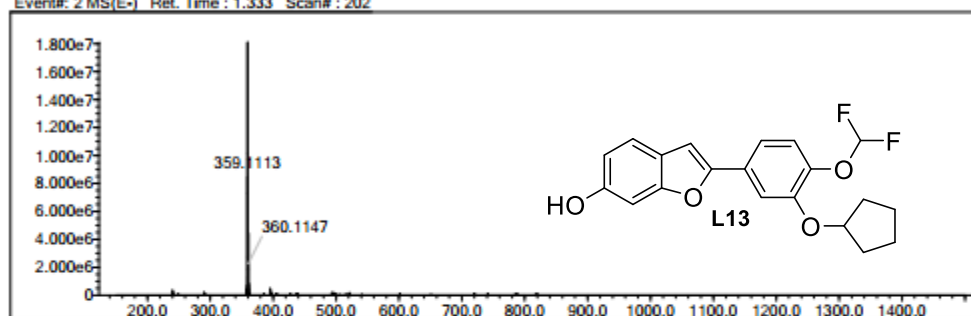
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	18	18	P	3	0	0	Br	1	0	0	H
B	3	0	0	S	2	0	0	Ru	2	0	0	HCOO
C	4	20	22	Cl	1	0	0	Sb	3	0	0	CH3COO
N	3	0	2	Fe	2	0	0	I	3	0	0	K
O	2	4	4	Ni	2	0	0	Ir	3	0	0	F
F	1	2	2	Cu	2	0	0	Pt	2	0	0	Cl
Si	4	0	0	Se	2	0	0					

Error Margin (mDa): 100.0
 HC Ratio: 0.0 • 1000.0
 Max Isotopes: all
 MSn Iso RI (%): 75.00

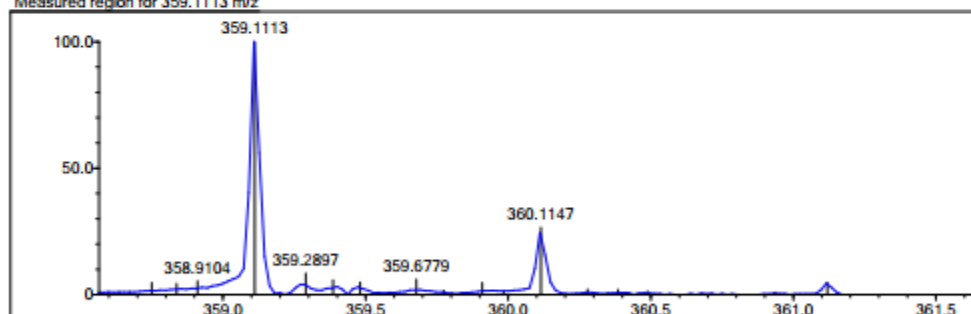
DBE Range: -100.0 • 100.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 10000
 Max Results: 10

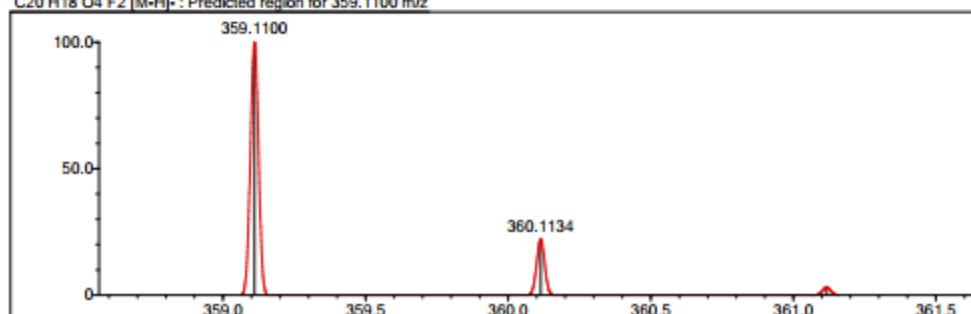
Event#: 2 MS(E-) Ret. Time : 1.333 Scan# : 202



Measured region for 359.1113 m/z



C20 H18 O4 F2 [M-H]- : Predicted region for 359.1100 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	90.00	C20 H18 O4 F2	[M-H]-	359.1113	359.1100	1.3	3.62	96.31	11.0