

# Supplementary data

for

## Structure-Guide Design and Optimization of Potential Druglikeness Inhibitors for TGFβRI with the Pyrrolopyrimidine Scaffold

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**Table S1** The key amino acid residues interacting with ligands in the crystal structure

PDB ID	Active data	No.of H-bonds	Hydrogen Bonds	Hydrophobic	NO.of Hydrophobic interaction
1PY5	IC <sub>50</sub> =104 nM	2	<b>HIS283, ASP351</b>	ILE211, VAL219, ALA230, VAL231, <b>LYS232</b> , LEU260, LEU278, LEU340, <b>SER280</b> , ASP281, TYR282, GLY286	12
1VJY	IC <sub>50</sub> =4 nM	3	<b>LYS232, HIS283, ASP351</b>	ILE211, VAL219, ALA230, TYR249, LEU260, <b>SER280</b> , ASP281, TYR282, , GLY286 LEU340, LEU278	11
1RW8	IC <sub>50</sub> =175 nM	1	<b>HIS283</b>	ILE211, LYS213, VAL219, ALA230, <b>LYS232</b> , TYR249, LEU260, LEU278, <b>SER280</b> , ASP281, LYS337, ASN338, LEU340, <b>ASP351</b>	14
3FAA	Ki=7.1 nM	3	<b>HIS283, ASP351, LYS232</b>	ILE211, ALA230, GLU245, TYR249, LEU260, LEU278, <b>SER280</b> , ASP281, TYR282, ASN338, LEU340	11
3GXL	IC <sub>50</sub> =25 nM	3	<b>LYS32, HIS83</b> (double H-bonds)	ILE11, GLY14, VAL19, ALA30, GLU45, TYR49, LEU78, <b>SER80</b> , ASP81, TYR82, GLY86, LYS137, LEU140, <b>ASP151</b>	14
3HMM	IC <sub>50</sub> =25 nM	2	<b>LYS32, HIS83</b>	VAL19, ALA30, GLU45, TYR49, LEU60, LEU78, VAL79, TYR82, <b>SER80</b> , ASP81, LYS137, ASN138, LEU140, <b>ASP151</b>	14

2WOU	IC <sub>50</sub> =72 nM	2	HIS283, ASP290	ILE211, VAL219, ALA230, LYS232, LEU260, LEU278, SER280, ASP281, TYR282, GLY286, LEU340, ASP351	12
2WOT	IC <sub>50</sub> =44 nM	2	HIS283, LYS232	ILE211, VAL219, ALA230, LEU260, LEU278, VAL279, SER280, ASP281, TYR282, GLU284, GLY286, ASN338, LEU340, ALA350, ASP351	15
3KCF	Ki=35 nM	2	LYS232, HIS283	ILE211, ALA230, GLU245, TYR249, LEU260, PHE262, LEU278, VAL279, SER280, ASP281, TYR282, LYS337, ASN338, LEU340, ASP351	15
2X7O	IC <sub>50</sub> =34 nM	2	ASP281, HIS283	ILE211, GLY212, VAL219, ALA230, LYS232, GLU245, LEU260, LEU278, SER280, TYR282, GLU284, HIS285, GLY286, APS290, ARG294, LEU340	16
3TZM	NR	2	HIS283, LYS232	ILE211, GLY214, ARG215, ALA230, LEU260, LEU278, SER280, ASP281, TYR282, LYS335, LYS337, ASN338, LEU340, ASP351	14
4X2J	NR	4	HIS283, TYR249, ASP351, GLU245	ILE211, VAL219, ALA230, LYS232, LEU260, SER280, ASP281, TYR282, GLY286, LEU340	10
4X2K	NR	3	HIS283, ASP281, ASP290	ILE211, VAL219, ALA230, LYS232, LEU260, SER280, ASP281, TYR282, GLY286, LEU340	10
4X2G	NR	3	ASP281, HIS283, ASP290	ILE211, VAL219, ALA230, LEU260, SER280, TYR282, SER287, LEU340, TYR603	9
4X0M	NR	1	ASP281	ILE211, VAL219, ALA230, LEU260, TYR282, HIS283, LEU340	7
4X2F	NR	3	HIS283, ASP281, ASP290	ILE211, ALA230, LEU260, TYR282, SER287, LEU340	6
5E8W	NR	3	ASP281, LYS337, HIS283	ILE211, GLY212, LYS213, GLY214, VAL219, ALA230, LYS232, LEU260, SER280, TYR282, GLY286, LEU340, ASP351	13
5E8Z	NR	3	LYS232, ASP281, HIS283	ILE211, LYS213, GLY214, ALA230, LEU260, TYR282, GLU284, GLY286, SER287, ASP290, LYS337, LEU340, ASP351	13
5FRI	IC <sub>50</sub> = 1.9 nM	2	HIS283(double H-bonds)	ILE211, VAL219, ALA230, LYS232, LEU260, SER280, ASP281, TYR282, GLU284, GKY286, LEU340, ALA350, ASP351	13
5USQ	NR	2	HIS283, ASP290	ILE211, ALA230, LYS232, LEU278, VAL279, SER280, ASP281, TYR282, GLY286, SER287, LEU340, ASP351	12
6B8Y	IC <sub>50</sub> =0.55 nM	3	HIS283, ASP351, LYS232	VAL219, ALA230, LEU260, LEU278, VAL279, SER280, ASP281, TYR282, LYS337, ASN338, LEU340,	11
5QIK	IC <sub>50</sub> =24 nM	3	LYS232, ASP351, HIS283	ILE211, LYS213, GLY214, ALA230, LEU260, SER280, ASP281, TYR282, GLU284, GLY286, LYS337, LEU340	12

5QIL	IC <sub>50</sub> =3 nM	2	HIS283, LYS232	ILE211,LYS213,GLY214,AL219,ALA230,TYR249,LEU260,LEU278,SER280,ASP281,GLY286,LEU340,ASP351	13
5QIM	IC <sub>50</sub> =6 nM	2	HIS283, LYS232	ILE211,GLY214,VAL219,ALA232,GLU245,LEU260,PHE262,LEU278,VAL279,SER280,ASP281,TYR282,GLU284,GLY286,LEU340	15
5QU0	IC <sub>50</sub> =1.6 nM	2	LYS232, HIS283	ILE211,VAL219,ALA230,LEU278,SER280,ASP281,TYR282,LEU340,ASP351	9
5QTZ	IC <sub>50</sub> =10 nM	2	HIS283, LYS232	ILE211,VAL219,ALA230,TYR249,LEU260,LEU278,SER280,ASP281,TYR282,LYS337,LEU340,ASP351	12

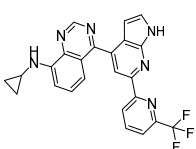
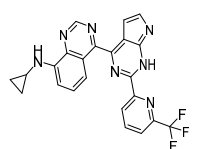
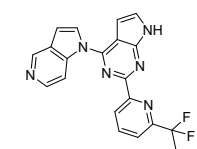
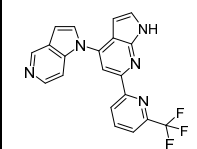
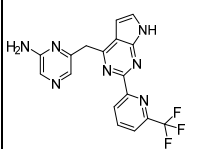
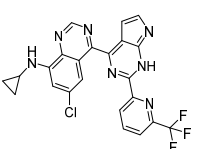
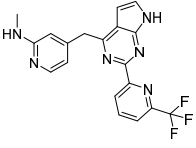
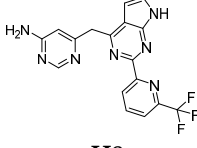
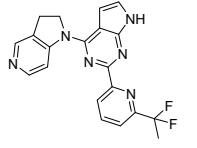
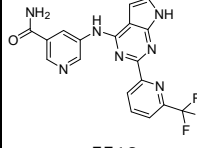
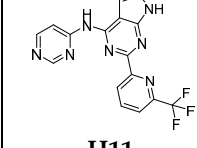
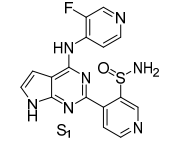
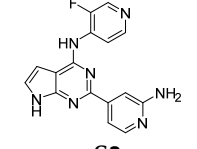
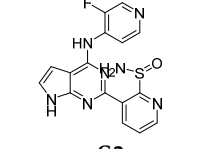
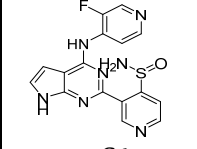
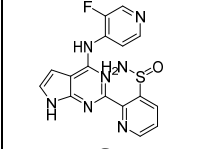
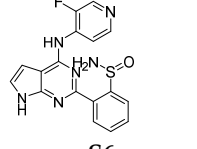
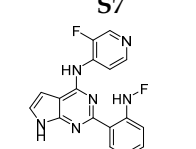
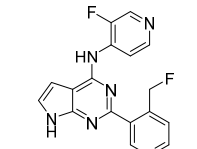
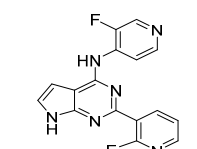
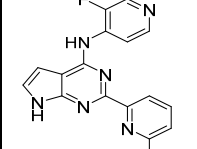
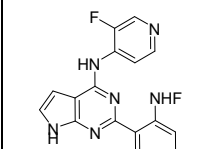
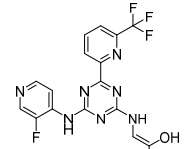
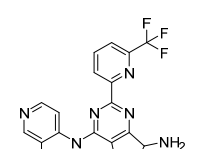
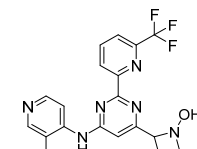
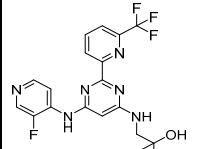
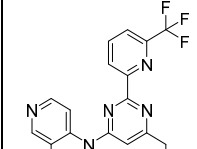
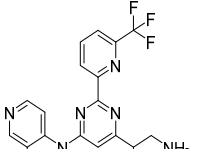
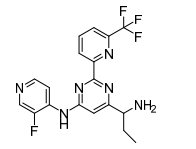
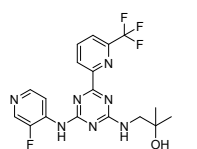
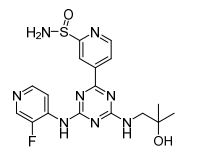
**Table S2.** The results of AUCs of scoring functions.

Score Property	Area under ROC Curve	ROC Evaluation	The ROC were calculated by DS 2020 program.
CDOCKER_ENERGY	0.539	Fall	
CDOCKER_INTERACTION_ENERGY	0.751	Fair	
PLP1	0.832	Good	
PLP2	0.828	Good	
PMF	0.921	Excellent	
PMF04	0.864	Good	
Jain	0.761	Fair	
LigScore1_Dreiding	0.745	Fair	
LigScore2_Dreiding	0.821	Good	
Ludi_1	0.871	Good	
Ludi_2	0.859	Good	
Ludi_3	0.878	Good	

**Table S3-1.** The information of the designed molecules is supported by docking to the receptor. (PDB ID:6B8Y).

Compound ID	PMF	Affinity (kcal/mol)	K <sub>i</sub> (nm)	H-Bond	Hydrophobic interaction
H1	127.36	-11.34	4.87	TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU278,TYR282,LEU340, ALA350
H2	124.63	-11.01	8.50	TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LYS232,LEU278,TYR282,LEU340,ALA350
H3	123.1	-9.27	160.34	LYS232,TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU278,LEU340,ALA350
H4	120.37	-9.47	110.61	LYS232,TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
H5	119.48	-9.84	61.27	LYS232,HIS283	ILE211,VAL219,ALA230,TYR249,LEU260,LEU278, LEU340,ALA350
H6	118.83	-9.43	122.4	TYR249,HIS283,LYS232	ILE211,VAL219,ALA230,LEU278,LEU340,ALA350
H7	115.74	-9.37	135.44	LYS232,TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,TYR282,LEU340,ALA350
H8	114.31	-9.5	108.76	LYS232,HIS283,ASP281,ASP351	ILE211,VAL219,ALA230,TYR249,LEU260,LEU278, LEU340,ALA350
H9	113.87	-9.87	61.27	LYS232, TYR249,HIS283,	ILE211,VAL219,ALA230,LEU278,LEU340,ALA350
H10	113.39	-9.39	130.95	LYS232, TYR249,HIS283, GLU284, ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
H11	112.63	-8.92	289.47	LYS232,HIS283,ASP351	ILE211,VAL219,ALA230,TYR249,LEU260,LEU278,LEU340, ALA350
S1	125.89	-9.81	64.45	LYS232,SER280,HIS283,ASN338	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
S2	124.07	-8.63	472.25	ALA230,LYS232,SER280,HIS283,ASP351,ASN338	VAL219,LEU260,LEU340,ALA350
S3	123.01	-9.87	58.24	LYS232,SER280,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
S4	122.36	-8.46	608.31	SER280,HIS283	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
S5	121.53	-9.75	71.32	LYS232, TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU340,ALA350
S6	121.22	-9.68	80.26	LYS232,SER280,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
S7	121.21	-8.28	856.56	ALA230,LYS232, TYR249, LEU278,HIS283,ASP351	ILE211,VAL219,LEU260,LEU340,ALA350
S8	120.98	-8.31	810.47	LYS232,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
S9	120.8	-8.65	456.58	LYS232, TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
S10	119.36	-8.91	293.4	LYS232,LEU278,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU340,ALA350
S11	112.25	-8.72	405.70	LYS232, HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
W1	123.06	-7.63	2.55	LYS232, TYR249,HIS283,ASP351,ASN338	ILE211,VAL219,ALA230,LEU260,LEU278,ALA350, LEU340
W2	116.8	-8.87	314.96	LYS232, TYR249,HIS283,ASP351,ASN338	ILE211,VAL219,ALA230,LEU260,LEU340
W3	116.42	-8.7	419.62	LYS232, TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU340
W4	115.12	-8.68	434.03	LYS232, TYR 249,HIS283,LYS337,ASP351	ILE211,VAL219,ALA230,LEU260,LEU340
W5	114.08	-8.71	412.61	LYS232, TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU260,LEU340
W6	113.36	-8.13	1.1	LYS232, TYR249,HIS283,ASP351,ASN338	ILE211,VAL219,ALA230,LEU260,LEU278,LEU340, ALA350
W7	112.99	-8.65	456.58	LYS232, TYR249,HIS283,ALYS337,ASN338	ILE211,VAL219,ALA230,LEU260,LEU340,ALA350
W8	112.94	-7.73	2.16	LYS232, TYR249,HIS283,ASP351,ASN338	ILE211,VAL219,ALA230,LEU278,LEU340,ALA350
S1W8	124.65	-8.74	392.23	HIS283,LYS337,ASN338,APS351	ILE211,VAL219,ALA230,LEU260,LEU340,ALA350
BMS22	113.32	-9.25	165.85	LYS232, TYR249,HIS283,ASP351	ILE211,VAL219,ALA230,LEU278,LEU340,ALA350

**Table S3-2.** The chemical structures of 30 modified molecules

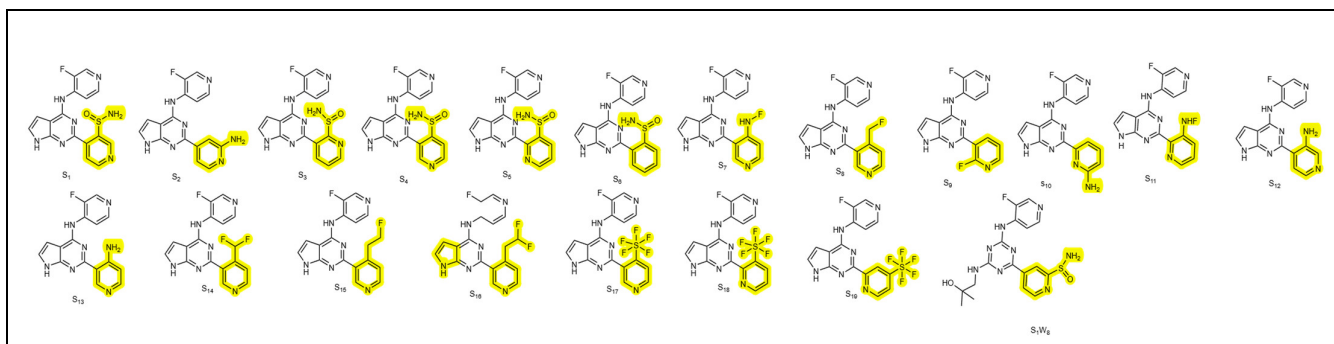
 <b>H1</b>	 <b>H2</b>	 <b>H3</b>	 <b>H4</b>	 <b>H5</b>	 <b>H6</b>
 <b>H7</b>	 <b>H8</b>	 <b>H9</b>	 <b>H10</b>	 <b>H11</b>	
 <b>S1</b>	 <b>S2</b>	 <b>S3</b>	 <b>S4</b>	 <b>S5</b>	 <b>S6</b>
 <b>S7</b>	 <b>S8</b>	 <b>S9</b>	 <b>S10</b>	 <b>S11</b>	
 <b>W1</b>	 <b>W2</b>	 <b>W3</b>	 <b>W4</b>	 <b>W5</b>	 <b>W6</b>
 <b>W7</b>	 <b>W8</b>	 <b>S1W8</b>			

**Figure S1.** The chemical structures of modified molecules from hinge region (in red), Solvent channel region (in blue), and Selective pocket region (in yellow).



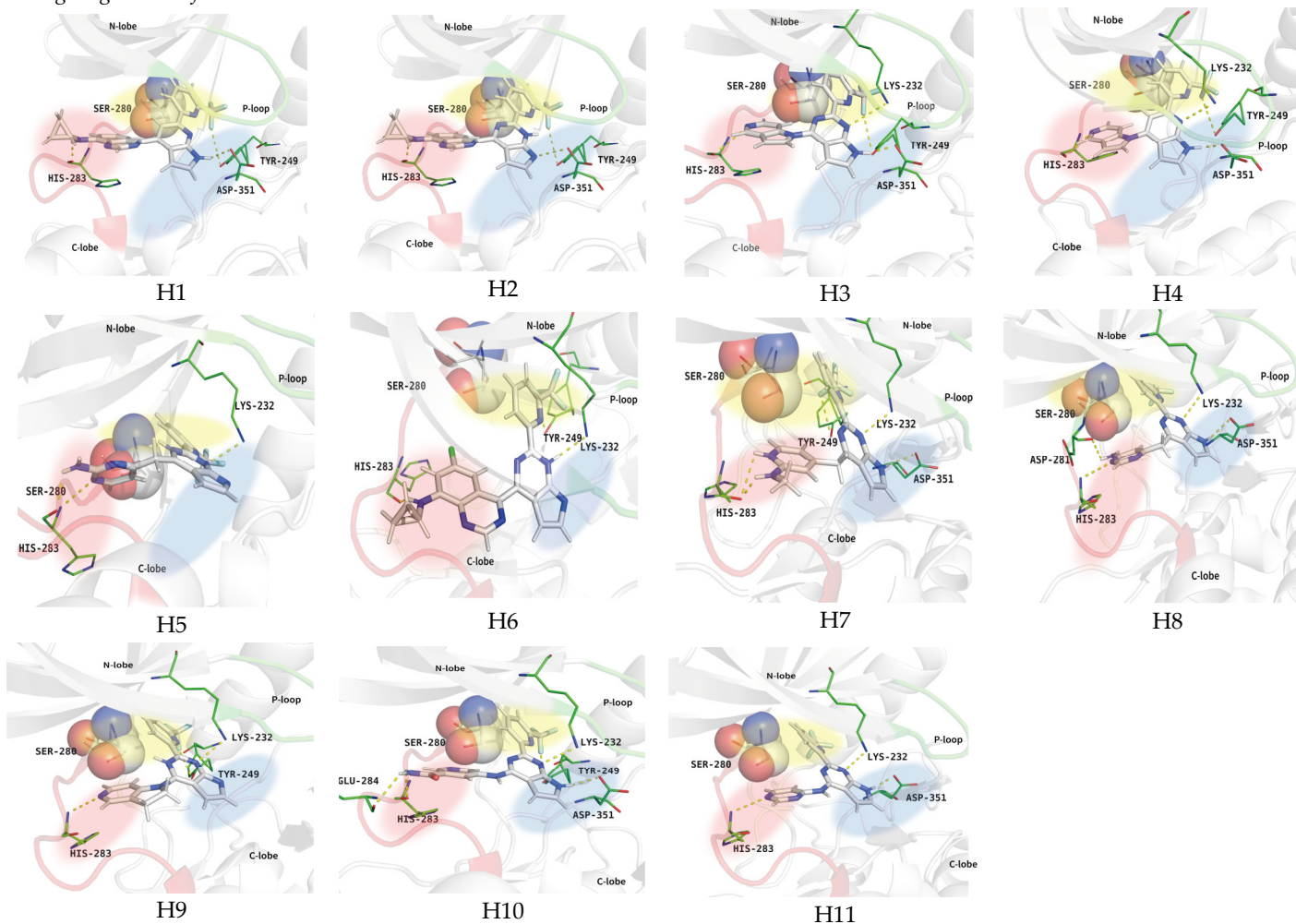




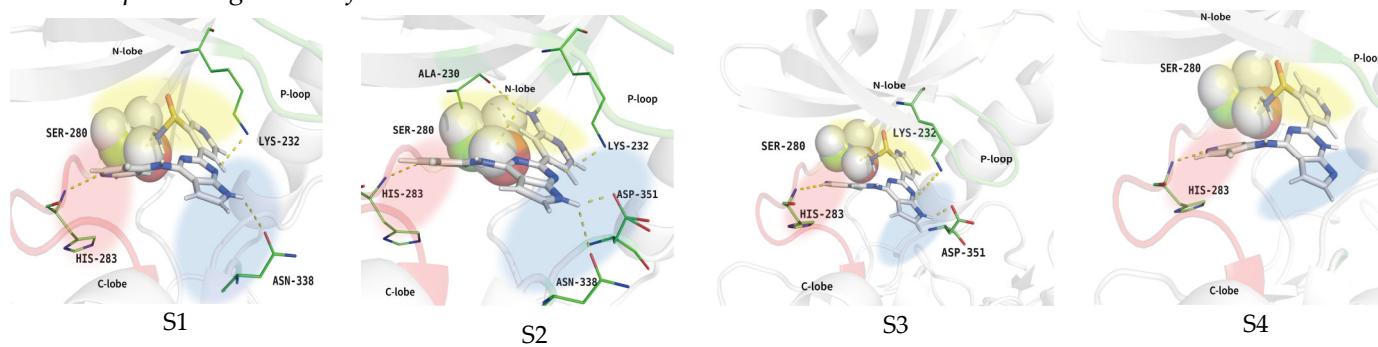


**Figure S2.3D** interaction pattern diagram between the protein and modified molecules in the crystal structure of 6B8Y.

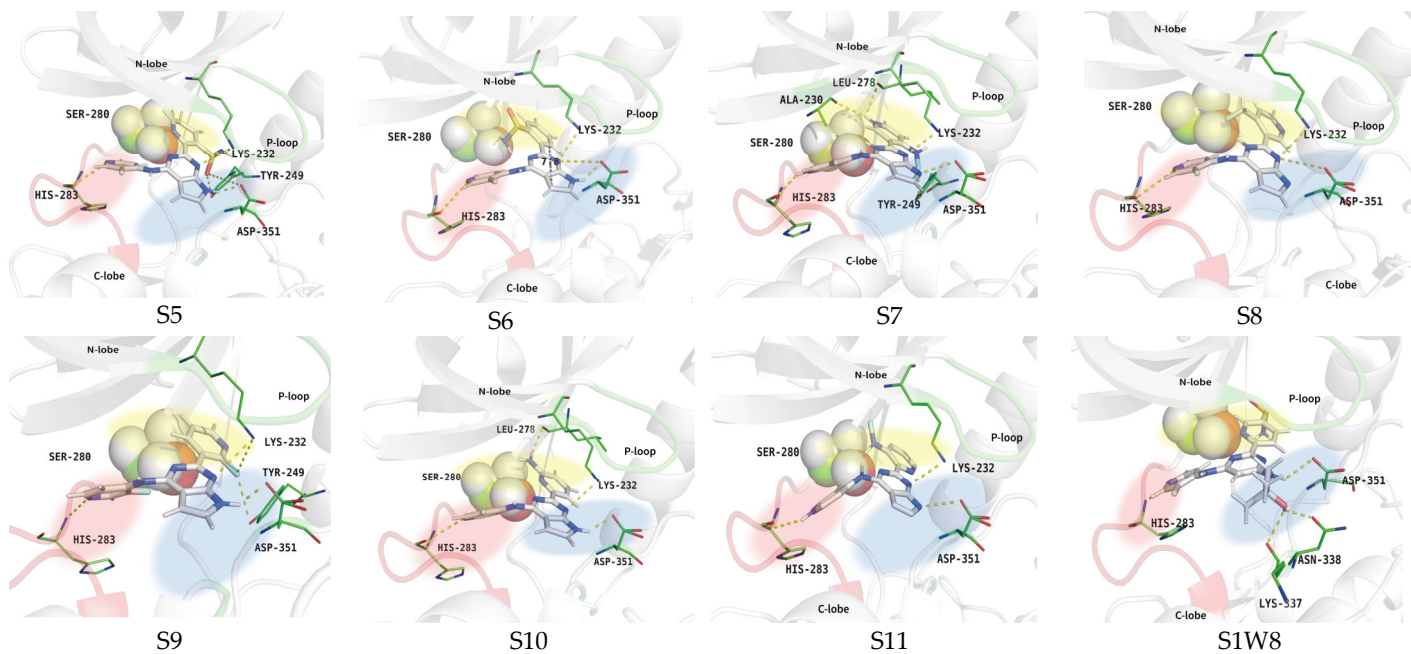
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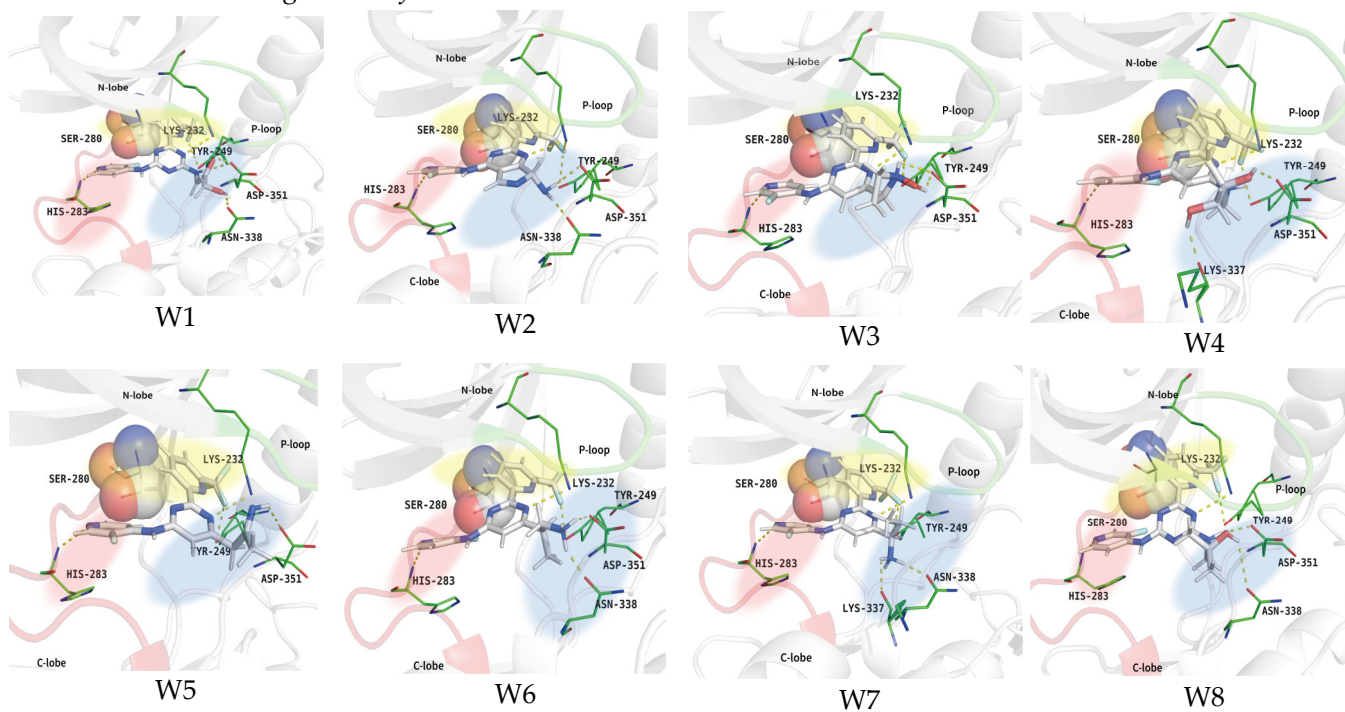
*Selective pocket region modified:*



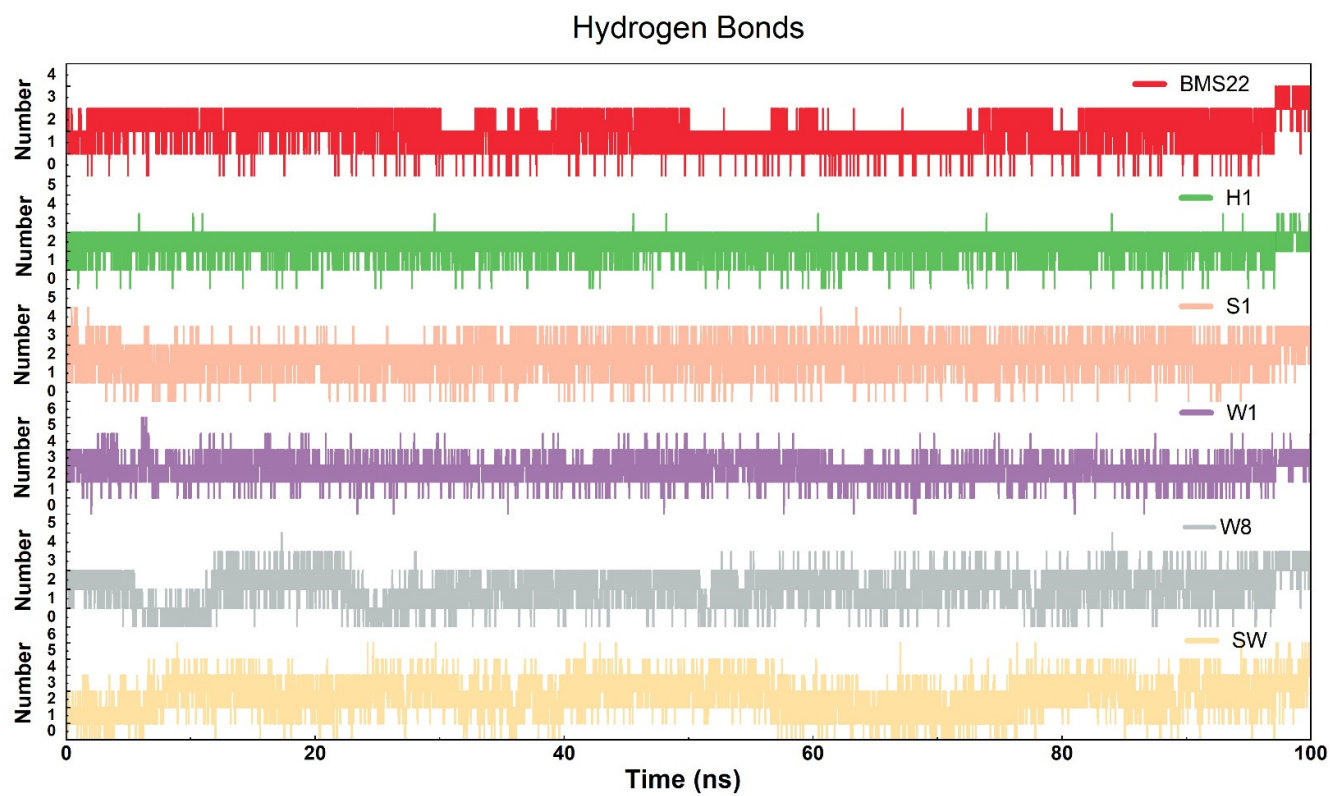




*Solvent channel region modified:*



**Figure S3.** Number of hydrogen bonds of five designed molecules and reference ligand BMS22 combined with TGF $\beta$ RI during 100 ns MD simulations.



**Table S4.** Occupancy rates of hydrogen bonds between TGFβRI and each potential inhibitor.

	acceptor	donor H	donor	frac % <sup>e</sup>
BMS22	LIG@N15	HIS_283@H	HIS_283@N <sup>a</sup>	88.38%
	ASP_351@OD1b	LIG@H30	LIG@N9	58.87%
	LIG@N3	LYS@HZ2	LYS_232@NZ	32.03%
	ASP_351@OD2b	LIG@H30	LIG@N9	22.88%
	LIG@N23	LYS_232@HZ2	LYS_232@NZ	2.81%
H1	LIG@N1	LYS_232@HZ1	LYS_232@NZ	75.70%
	ASP_351@OD1 <sup>b</sup>	LIG@H16	LIG@N5	61.22%
	HIS_283@O <sup>a</sup>	LIG@H15	LIG@N4	59.55%
	ASP_351@OD2 <sup>b</sup>	LIG@H16	LIG@N5	33.40%
	LIG@N	LYS_232@HZ1	LYS_232@NZ	1.64%
S1	LIG@N5	HIS_283@H <sup>a</sup>	HIS_283@N <sup>a</sup>	81.43%
	ASP_351@OD2 <sup>b</sup>	LIG@H7	LIG@N3	78.18%
	SER_280@OG <sup>c</sup>	LIG@H10	LIG@N6	53.47%
	ASP_281@O <sup>a</sup>	LIG@H11	LIG@N6	18.90%
	ALA_230@O	LIG@H11	LIG@N6	3.36%
W1	ASP_351@OD1 <sup>b</sup>	LIG@H42	LIG@O28	94.67%
	ASP_351@OD1 <sup>b</sup>	LIG@H41	LIG@N25	88.79%
	LIG@N21	HIS_283@H <sup>a</sup>	HIS_283@N <sup>a</sup>	80.00%
	LYS_213@O <sup>d</sup>	LIG@H43	LIG@O30	7.57%
	LIG@O28	ASN_338@HD21	ASN_338@ND2 <sup>b</sup>	3.44%
W8	LIG@N7	HIS_283@H <sup>a</sup>	HIS_283@N <sup>a</sup>	83.97%
	ASP_351@OD2 <sup>b</sup>	LIG@H16	LIG@O	49.02%
	ASP_351@OD2 <sup>b</sup>	LIG@H14	LIG@N5	26.66%
	ASP_351@OD1 <sup>b</sup>	LIG@H16	LIG@O	22.27%
	ASP_351@OD1 <sup>b</sup>	LIG@H14	LIG@N5	20.47%
	LIG@O	ASN_338@HD22	ASN_338@ND2 <sup>b</sup>	4.35%
S1W8	LIG@N4	HIS_283@H <sup>a</sup>	HIS_283@N <sup>a</sup>	85.11%
	LIG@O1	SER_280@HG1 <sup>a</sup>	SER_280@OG <sup>c</sup>	51.60%
	ASP_351@OD1 <sup>b</sup>	LIG@H17	LIG@O	38.91%
	ASP_351@OD1 <sup>b</sup>	LIG@H16	LIG@N5	36.68%
	ASP_351@OD2 <sup>b</sup>	LIG@H16	LIG@N5	18.98%
	ASP_351@OD2 <sup>b</sup>	LIG@H17	LIG@O	12.61%
	SER280@OG <sup>c</sup>	LIG@H18	LIG@N6	10.07%
	LYS_213@O <sup>d</sup>	LIG@H17	LIG@O	9.40%
	LIG@N5	ASN_338@HD22	ASN_338@ND2 <sup>b</sup>	3.39%

a. Hydrogen bond interaction with the hinge region;

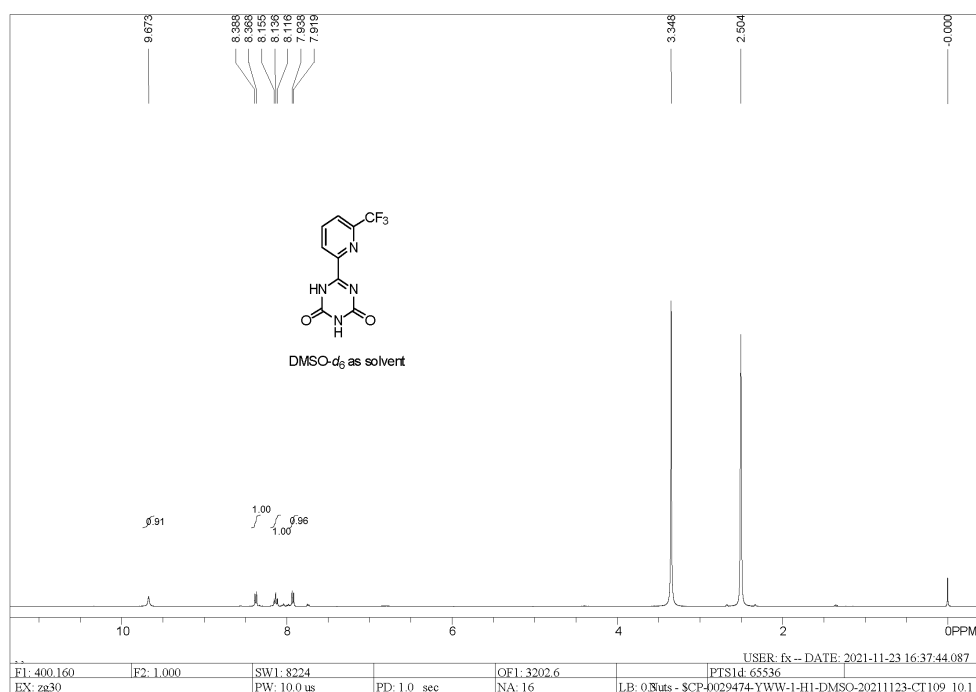
b. Hydrogen bond interaction with the solvent channel;

c. Hydrogen bond interaction with the selective hydrophobic pocket;

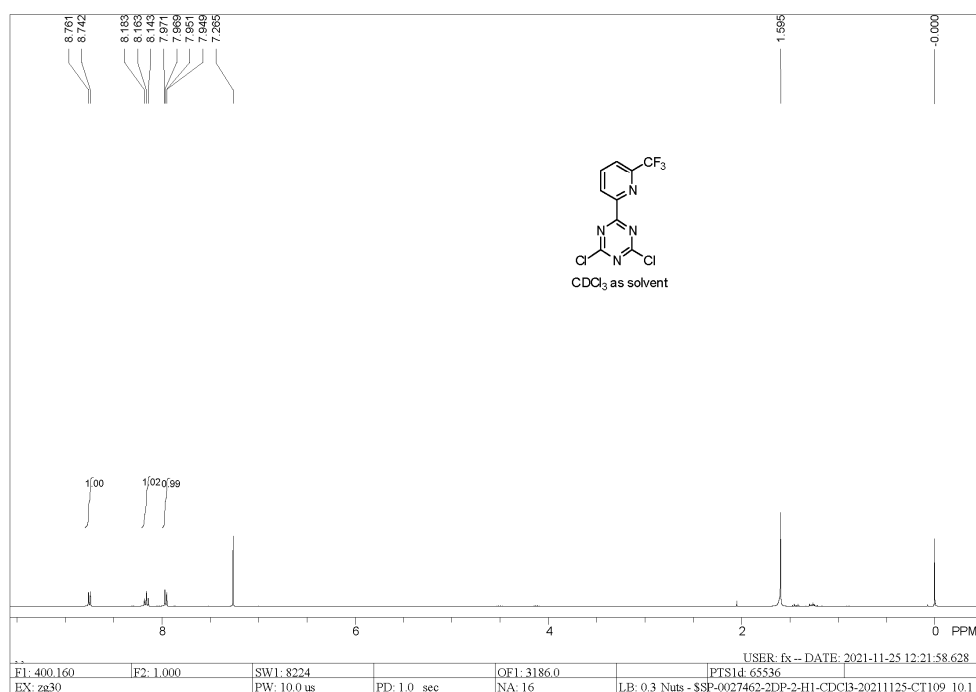
d. Hydrogen bond interaction with the P-loop region;

e. The hydrogen bonds are determined by the default criteria (acceptor... H-donor angle of &gt;120° and acceptor ... donor distance of &lt;3.5 Å) in the bonds.tcl of the VMD program

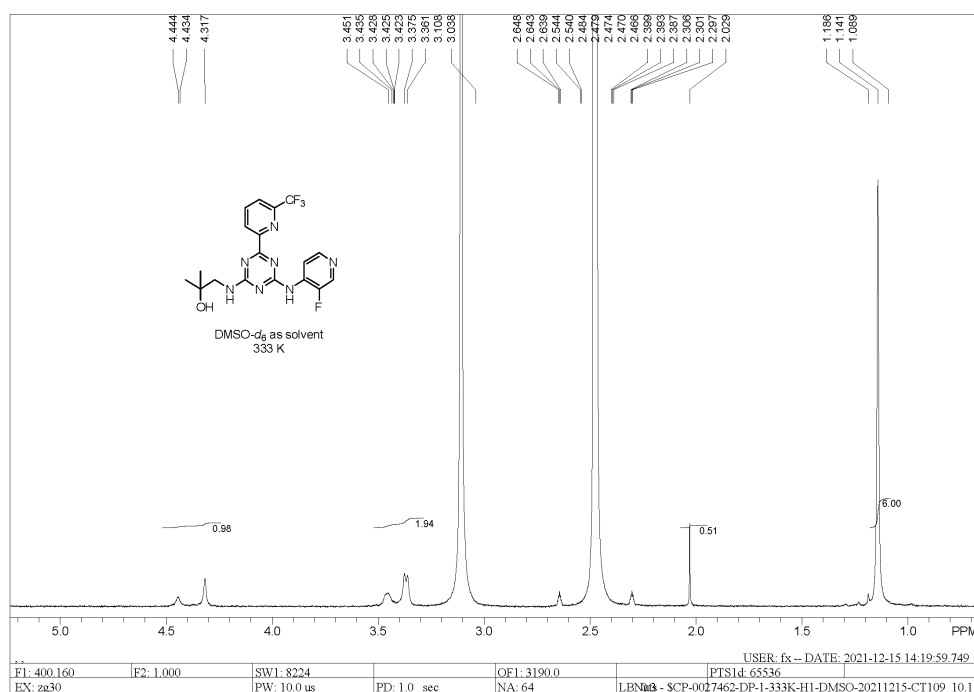
## NMR spectra of synthesized compounds



**Figure S4**  $^1\text{H}$ -NMR spectrum of 6-(6-(trifluoromethyl)pyridin-2-yl)-1,3,5-triazine-2,4(1H,3H)-dione.



**Figure S5**  $^1\text{H}$ -NMR spectrum of 2,4-dichloro-6-(6-(trifluoromethyl)pyridin-2-yl)-1,3,5-triazine.

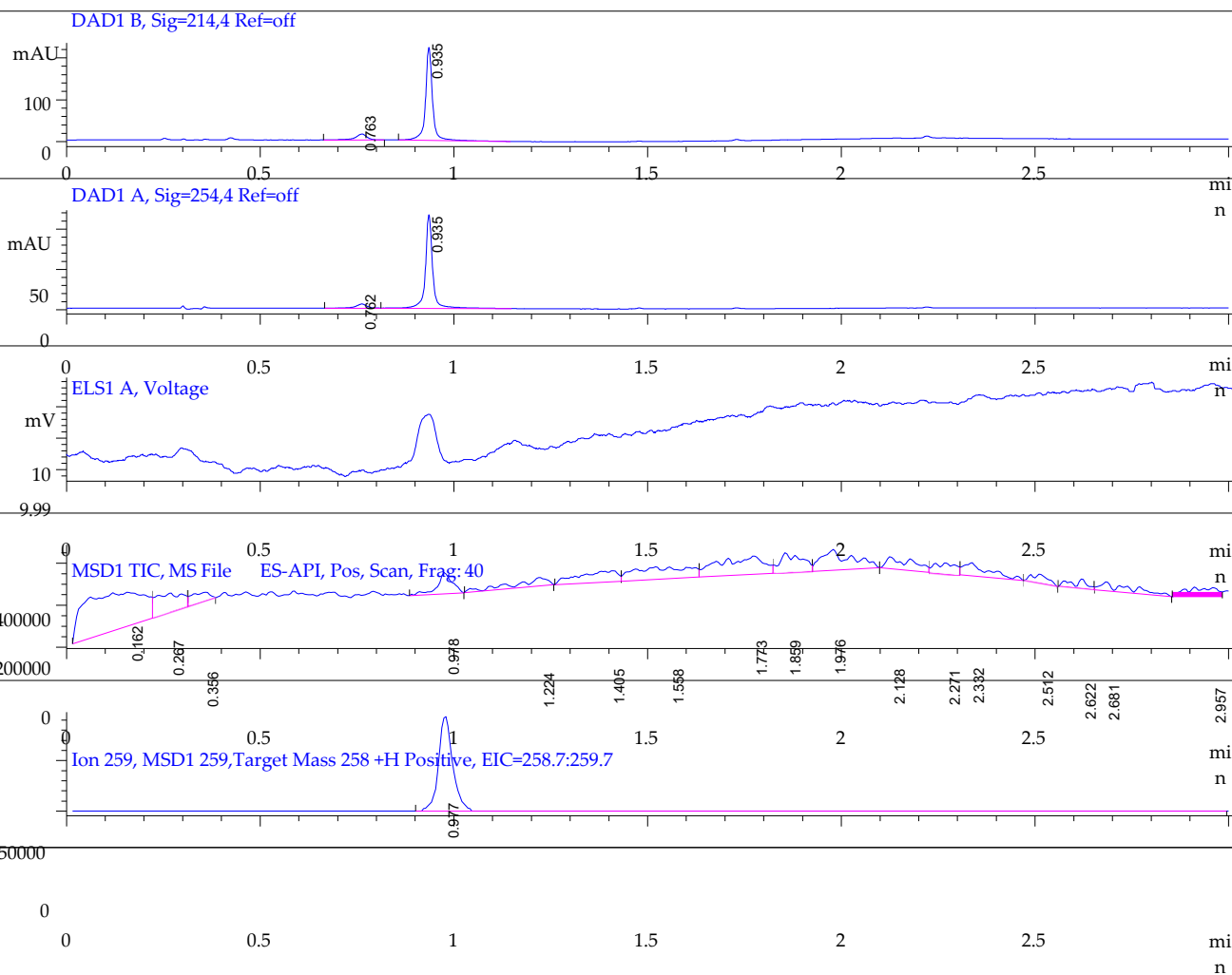


**Figure S6**  $^1\text{H}$ -NMR spectrum of 1-((4-((3-fluoropyridin-4-yl)amino)-6-(6-(trifluoromethyl)pyridin-2-yl)-1,3,5-triazin-2-yl)amino)-2-methylpropan-2-ol.



## MS report 1: 6-(6-(trifluoromethyl)pyridin-2-yl)-1,3,5-triazine-2,4(1H,3H)-dione

File ..T109\MONITOR\SP-0027462-1P1-01831-LCMS047.D Tgt Mass (EZ): 258.00  
Injection Date : 23 Nov 21 5:32 pm +0800 Seq. Line : 0  
Sample Name : SP-0027462-1P1 Location : P1-F-04  
Acq. Operator : 109monitor Inj : 1  
Spec. Reported : MS Integration Inj Volume : 0.5 ul  
Acq. Method : C:\Chem32\1\METHODS\MONITOR 3MIN-50-B-P  
Analysis Method : C:\Chem32\1\METHODS\MONITOR 3MIN-50-B-P.M  
Sample Info : Easy-Access Method: 'MONITOR 3MIN-50-B-P.M' 258.00  
Method Info : Column: Waters X Bridge C18: 50mm\*4.6 mm\*3.5 um;  
Mobile Phase: A: Water (0.01mol/L NH4HCO3) B: ACN  
Gradient : B from 5% to 95% for 1.6 min and hold 95% for 1.4 min;  
Flow rate: 2.0 ml/min;  
Column Temperature: 40 °C



## Integration Results for DAD1 B, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
0.76	0.03	29.00	13.86	9.45
0.94	0.02	277.97	221.62	90.55

## Integration Results for DAD1 A, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
0.76	0.03	10.66	5.16	6.57
0.94	0.02	151.71	116.16	93.43

## Integration Results for ELS1 A, Voltage

RetTim	Width	Area	Height	Area%
0.99	0.02	10.66	5.16	6.57
1.01	0.02	151.71	116.16	93.43

## Integration Results for MSD1 TIC, MS File

RetTime	Width	Area	Height	Area%
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LCMS047 11/23/2021 5:36:21 PM 109monitor

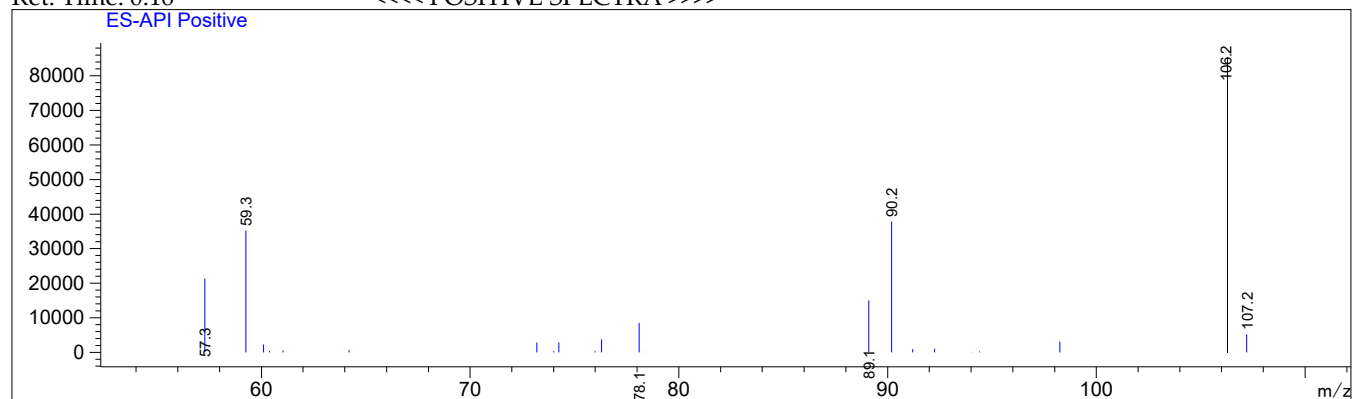
Page 1 of 7

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0.16	0.15	1894002.38	156914.33	27.63	
0.27	0.06	437014.69	89740.66	6.38	
0.36	0.05	155703.45	41483.01	2.27	
0.98	0.04	271918.50	95768.83	3.97	
1.22	0.08	221813.67	37366.83	3.24	
1.40	0.09	382647.88	53072.18	5.58	
1.56	0.12	556172.44	55576.87	8.11	
1.77	0.10	700590.00	86905.44	10.22	
1.86	0.05	365538.19	89596.53	5.33	
1.98	0.08	526599.06	92715.45	7.68	
2.13	0.06	263161.16	54866.41	3.84	
2.27	0.05	189155.39	51044.39	2.76	
2.33	0.06	277677.00	58777.80	4.05	
2.51	0.05	128105.84	43750.95	1.87	
2.62	0.04	118888.68	45586.55	1.73	
2.68	0.07	219188.27	40898.83	3.20	
2.96	0.08	146532.42	24227.98	2.14	

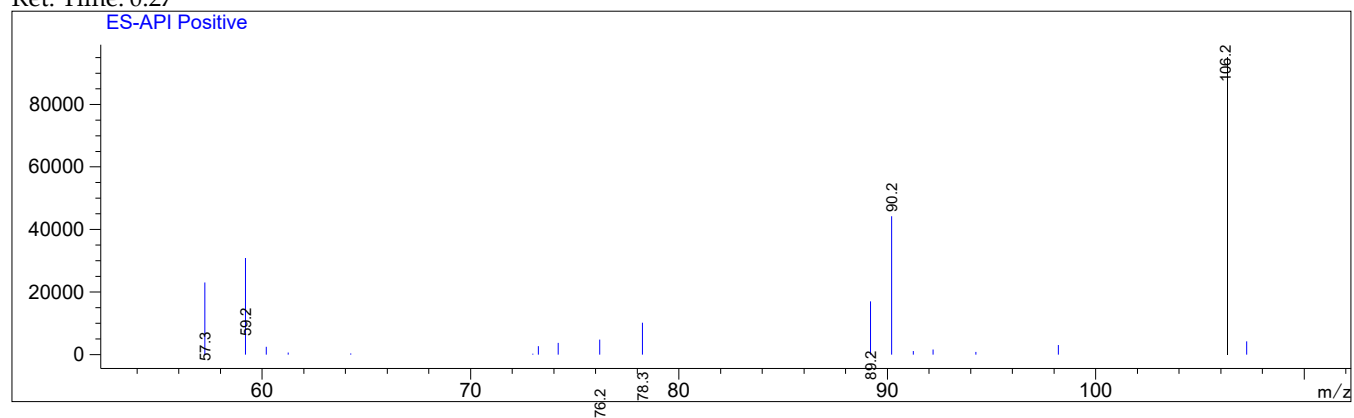


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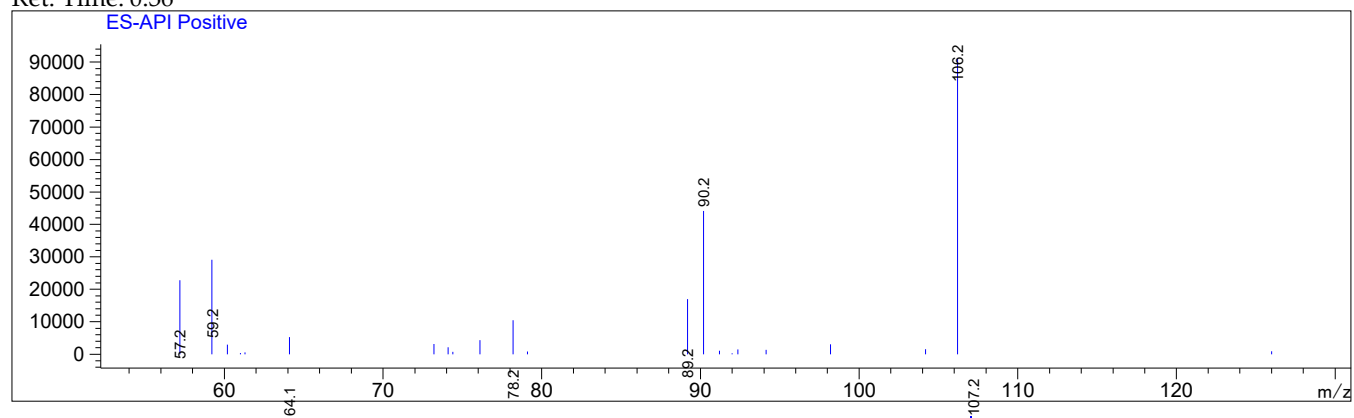
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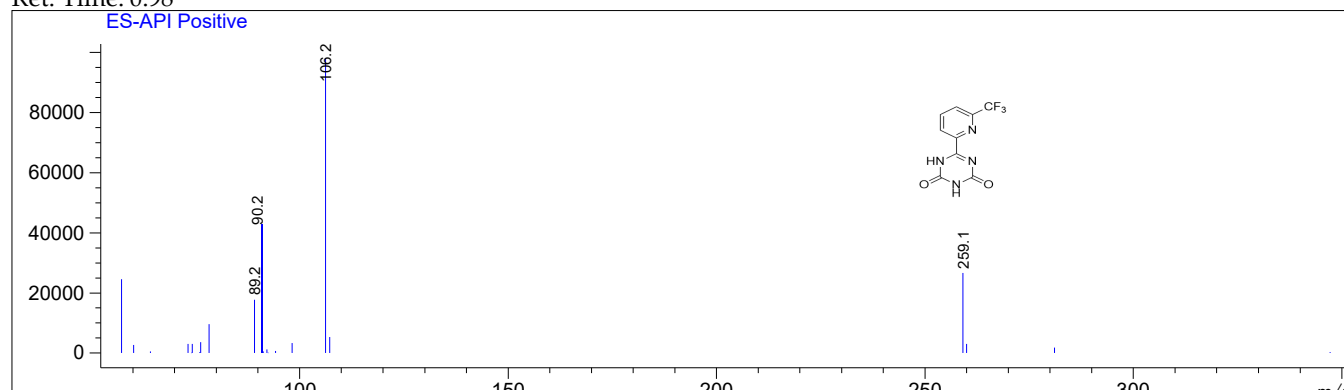
Ret. Time: 0.27



Ret. Time: 0.36



Ret. Time: 0.98





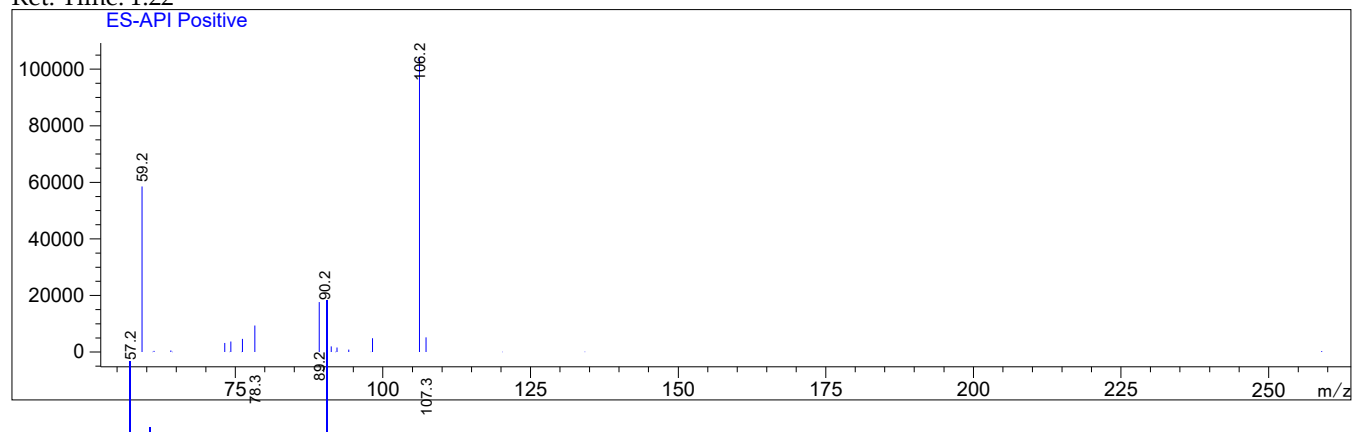
57.3

78.3

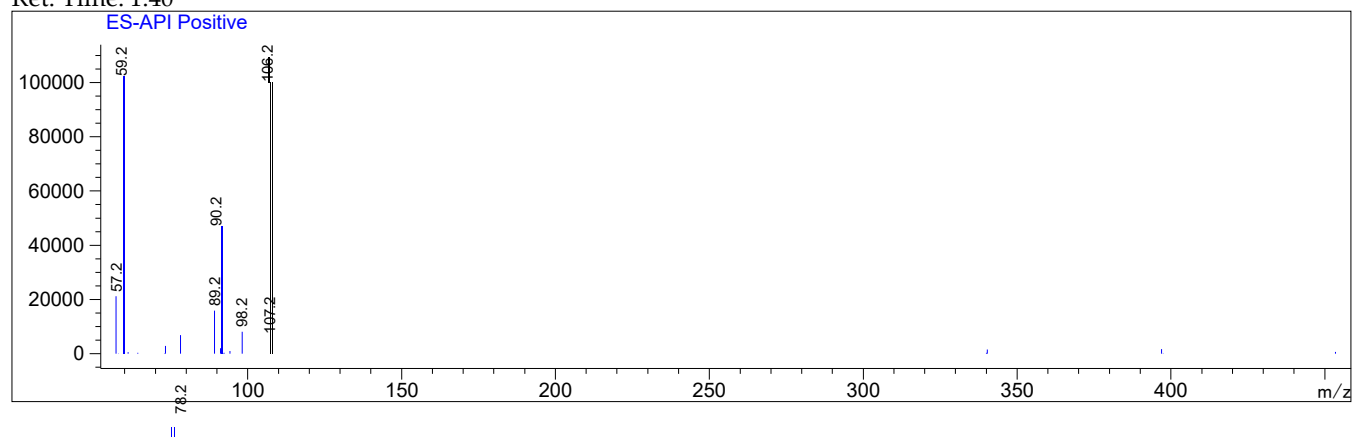
107.2

# MS Report

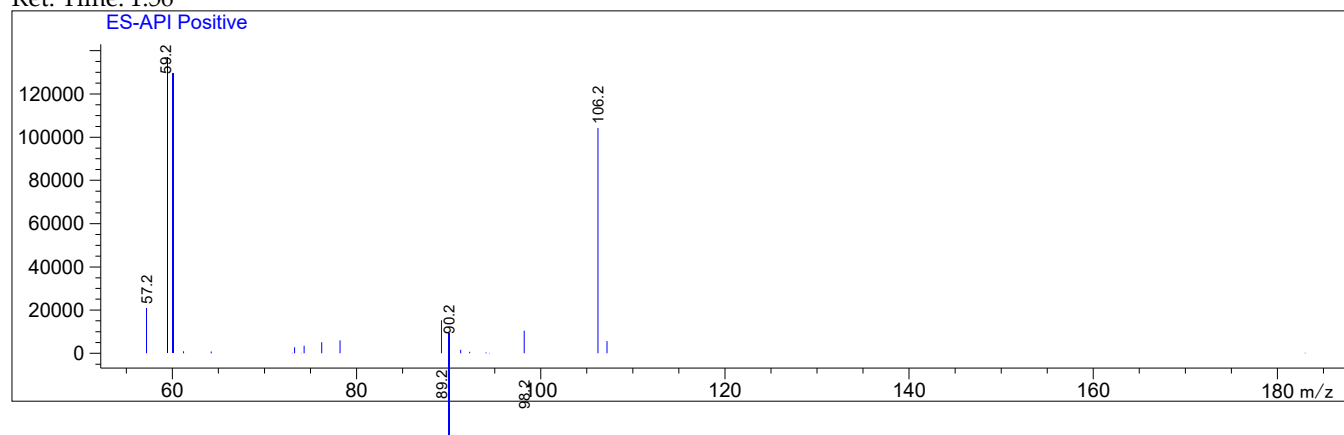
Ret. Time: 1.22



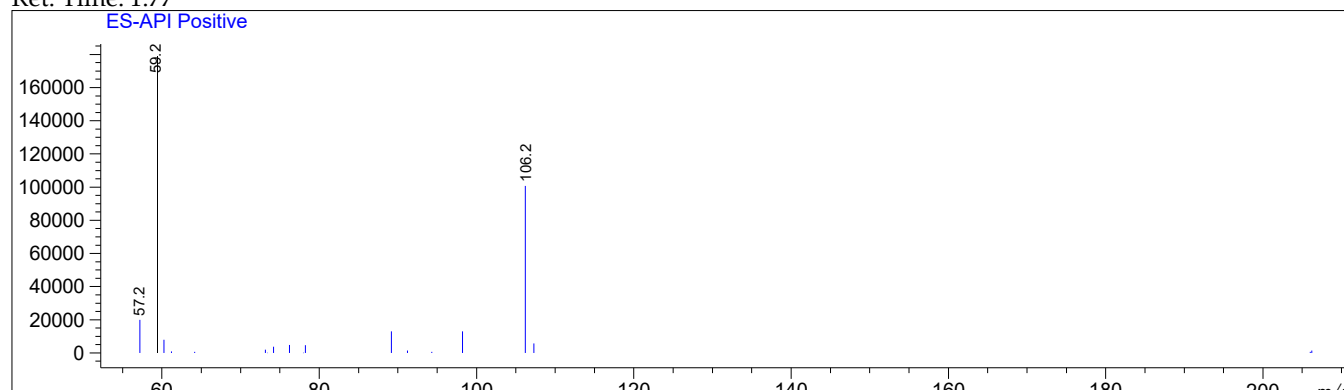
Ret. Time: 1.40



Ret. Time: 1.56



Ret. Time: 1.77

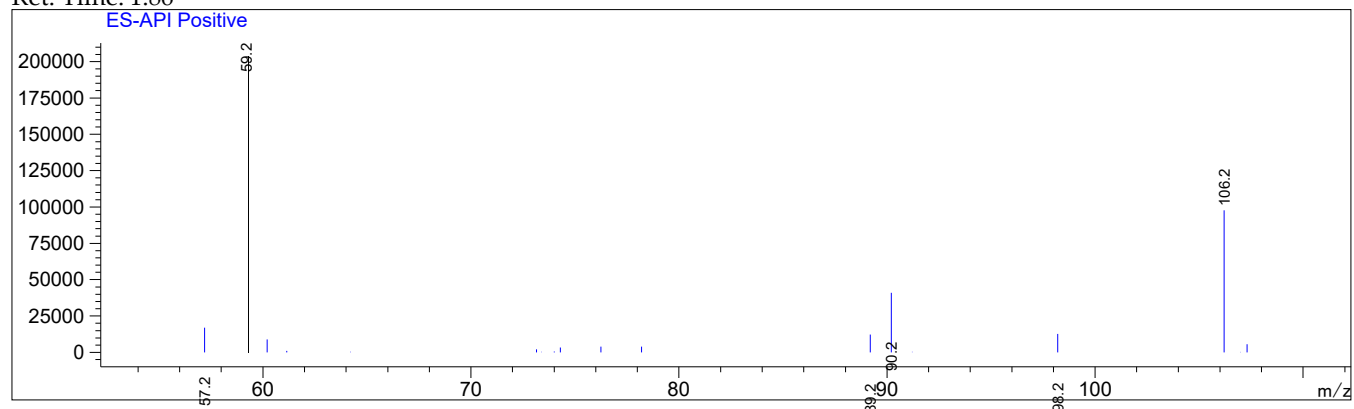


89.2 90.2

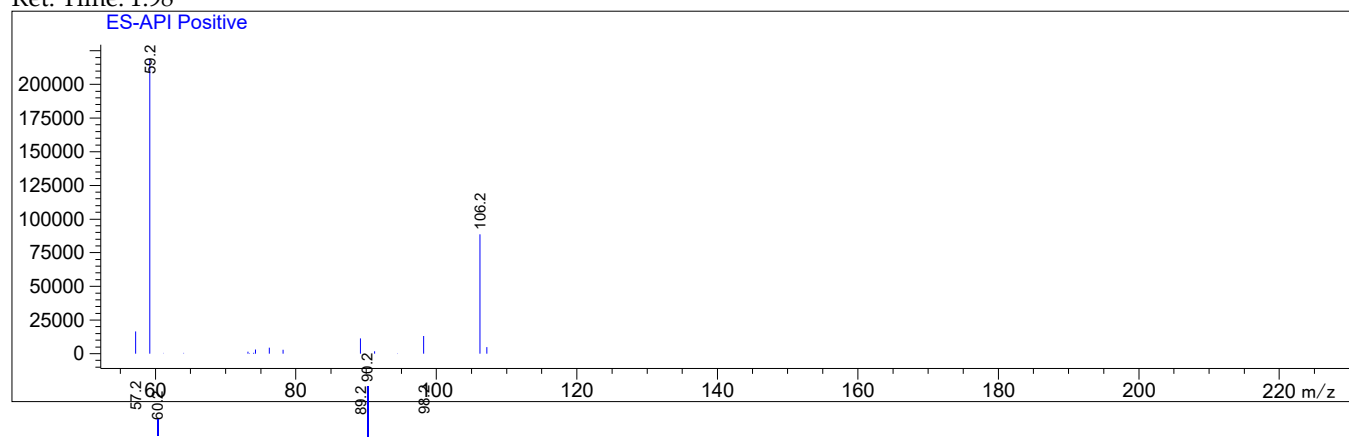
98.2

# MS Report

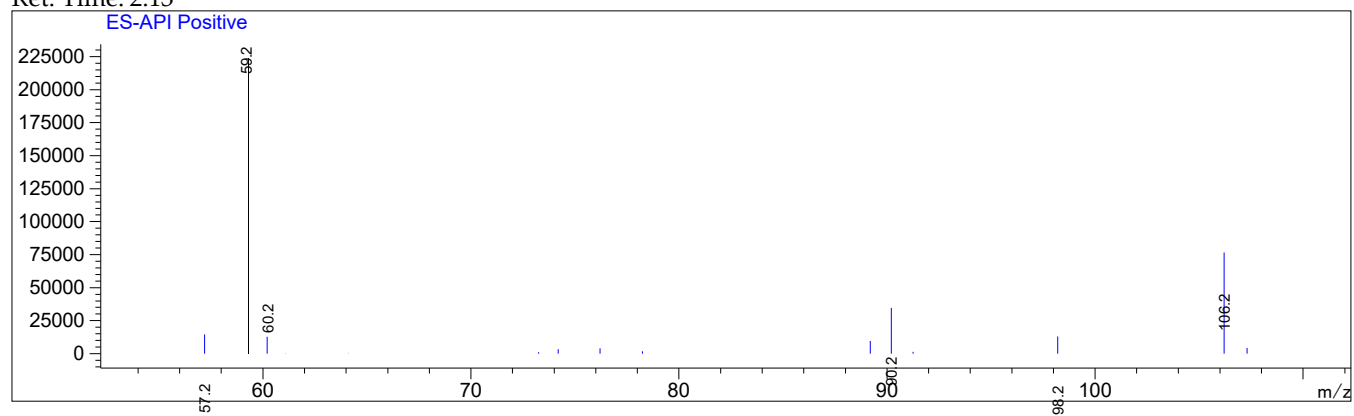
Ret. Time: 1.86



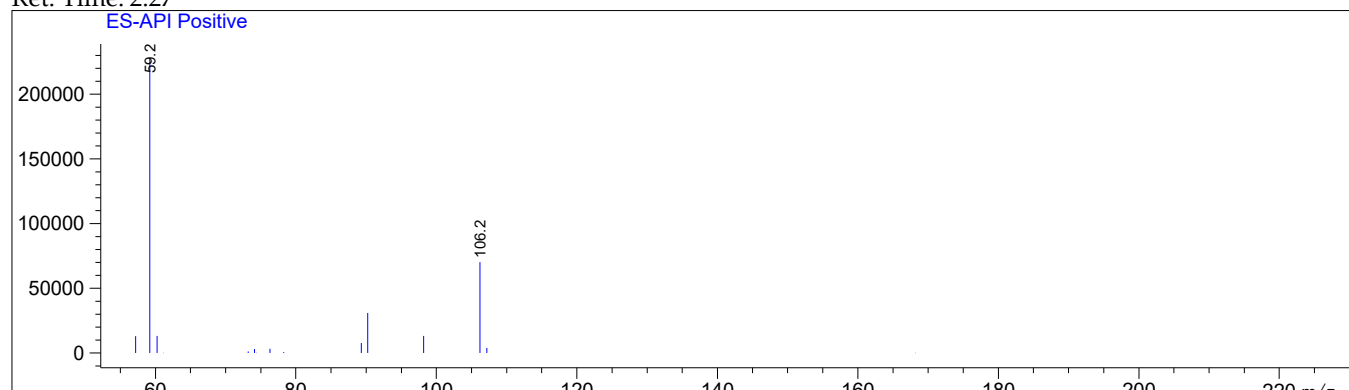
Ret. Time: 1.98



Ret. Time: 2.13



Ret. Time: 2.27



57.2  
60.3

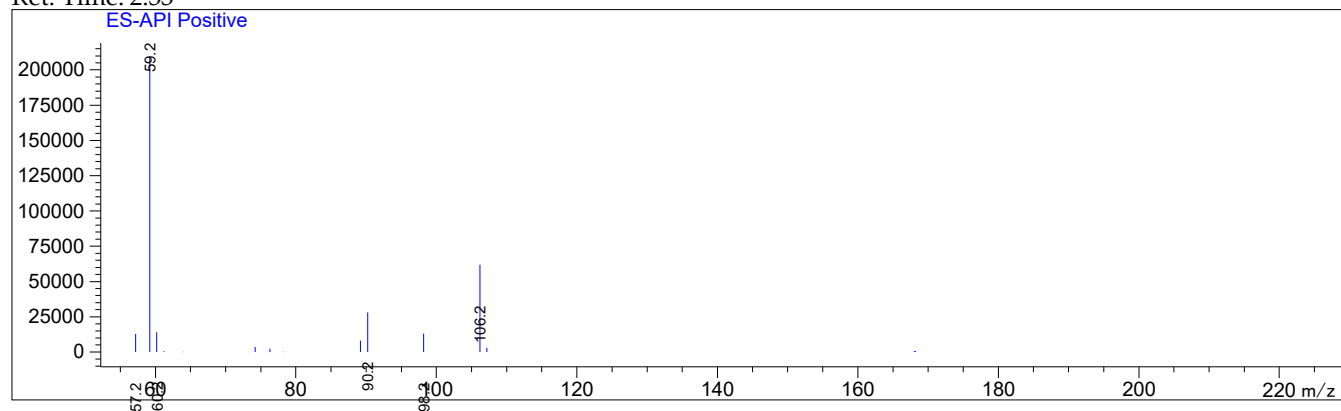
90.2

98.2

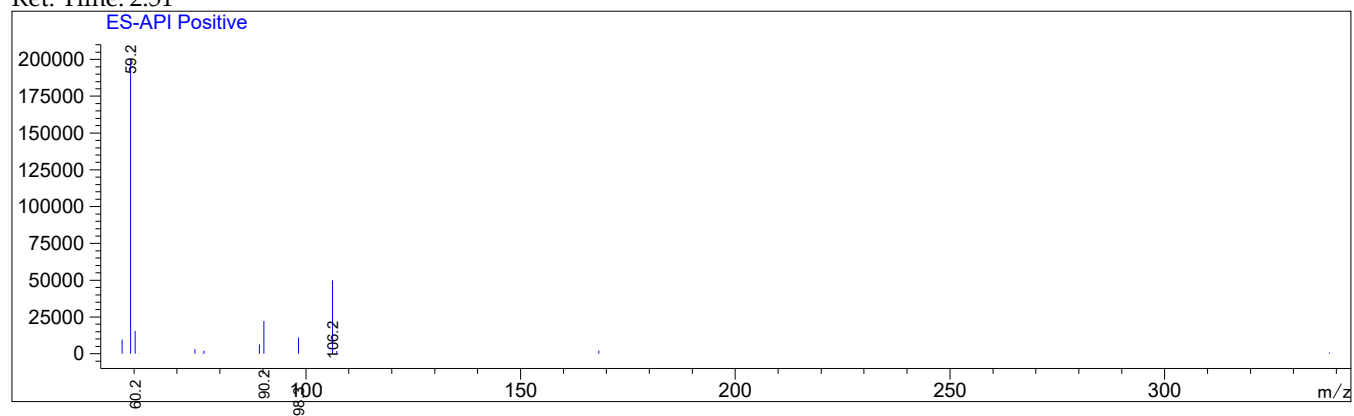


# MS Report

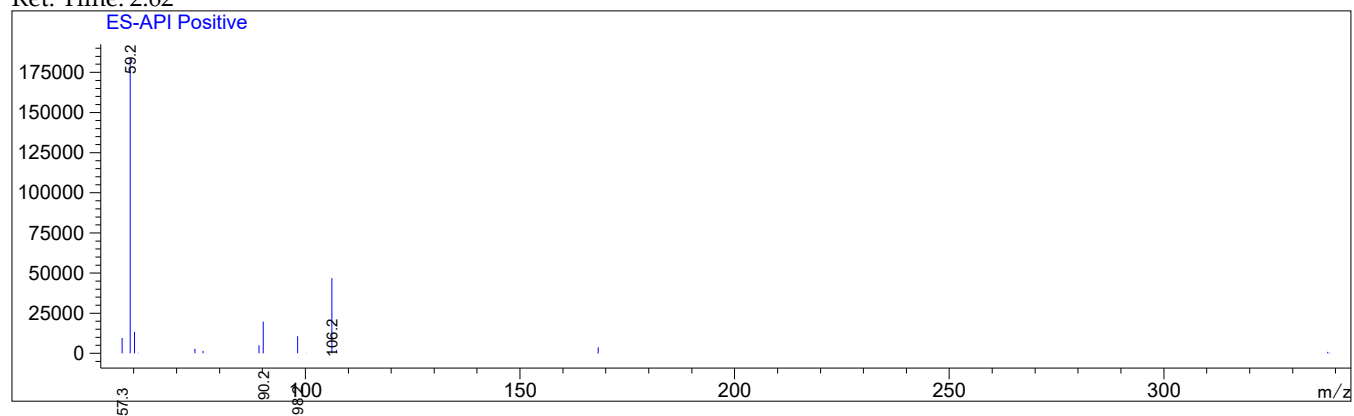
Ret. Time: 2.33



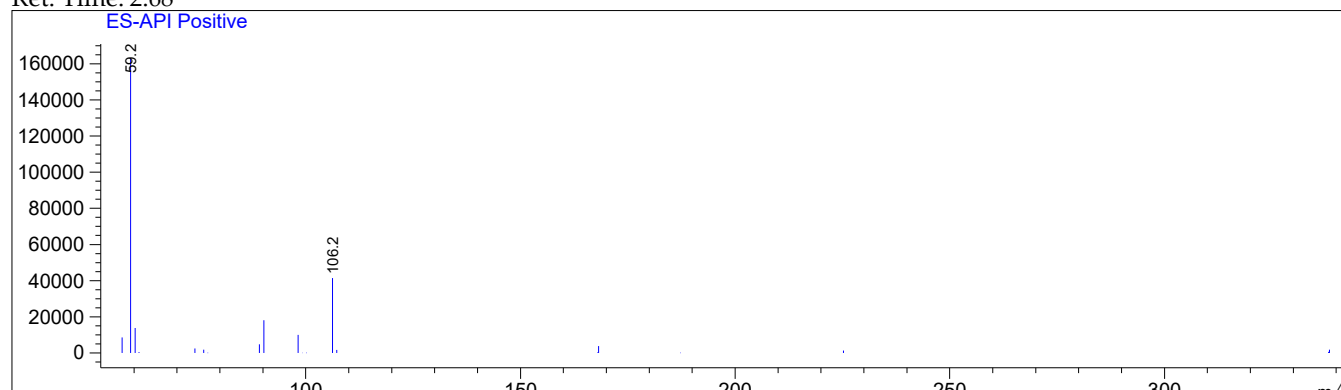
Ret. Time: 2.51



Ret. Time: 2.62



Ret. Time: 2.68



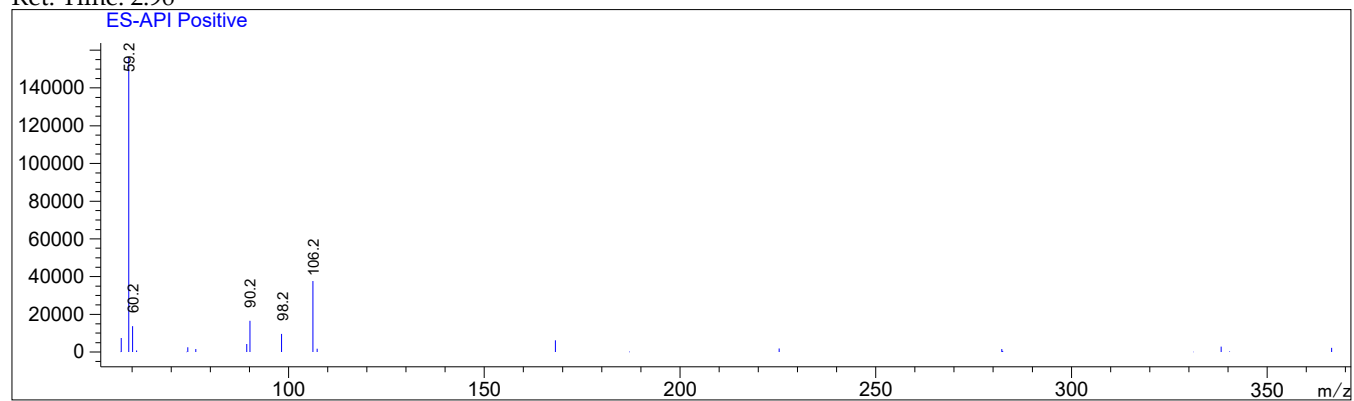
57.2

90.2

98.2

# MS Report

Ret. Time: 2.96

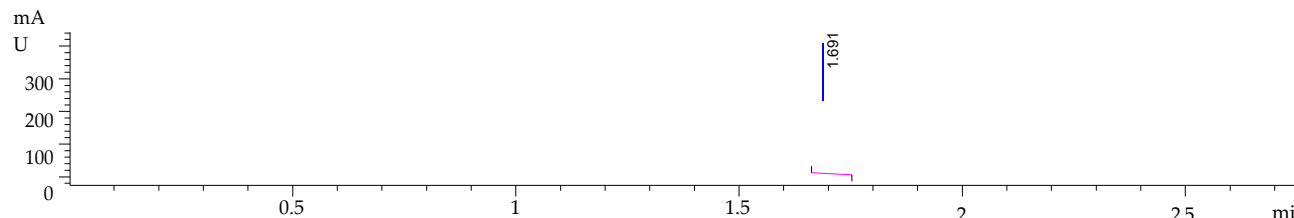




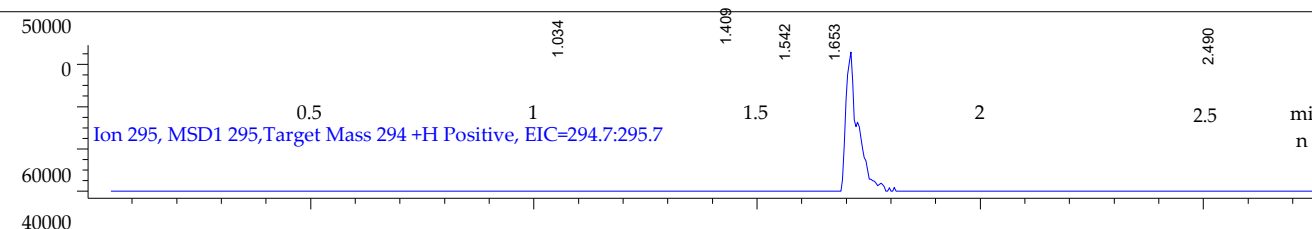
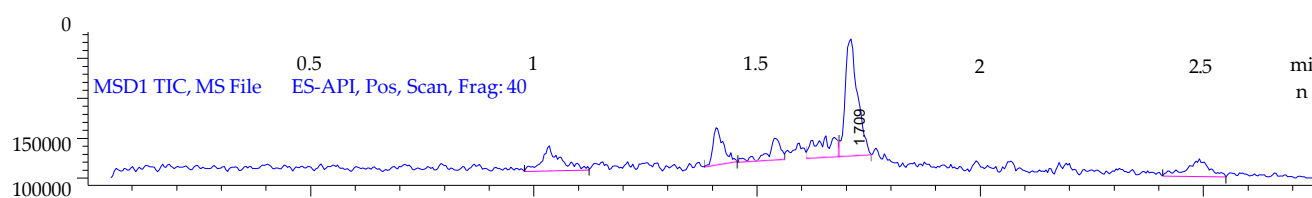
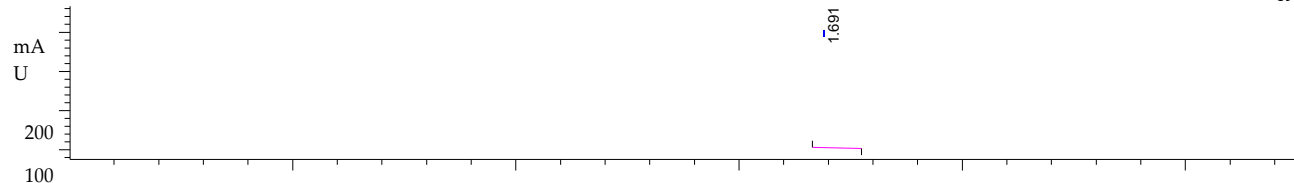
MS report 2: 2,4-dichloro-6-(6-(trifluoromethyl)pyridin-2-yl)-1,3,5-triazine

File ..09\MONITOR\SP-0027462-2P1-04381-LCMSA 046.D Tgt Mass (EZ): 294.00  
Injection Date : 25 Nov 21 11:06 am +0800 Seq. Line : 0  
Sample Name : SP-0027462-2P1 Location : P2-F-08  
Acq. Operator : 109monitor Inj : 1  
Spec. Reported : MS Integration Inj Volume : 0.5 ul  
Acq. Method : C:\Chem32\1\METHODS\MONITOR 2.75MIN-B-P  
Analysis Method : C:\Chem32\1\METHODS\MONITOR 2.75MIN-B-P.M  
Sample Info : Easy-Access Method: 'MONITOR 2.75MIN-B-P.M' 294.00  
Method Info : Column: Phenomenex Kinetex EVO C18, 50mm\*4.6 mm, 2.6 um  
Mobile Phase: A: Water (0.01mol/L NH4HCO3) B: ACN  
Gradient : 5-100% B (MeCN) in 1.75min, 100% at 2.55min, 5% at 2.65min, en  
Flow rate: 2.3 ml/min  
Column Temperature: 40 °C

DAD1 A, Sig=214,4 Ref=off



DAD1 B, Sig=254,4 Ref=off



Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.69	0.01	394.73	412.01	100.00

Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.69	0.01	331.63	343.54	100.00

Integration Results for MSD1 TIC, MS File

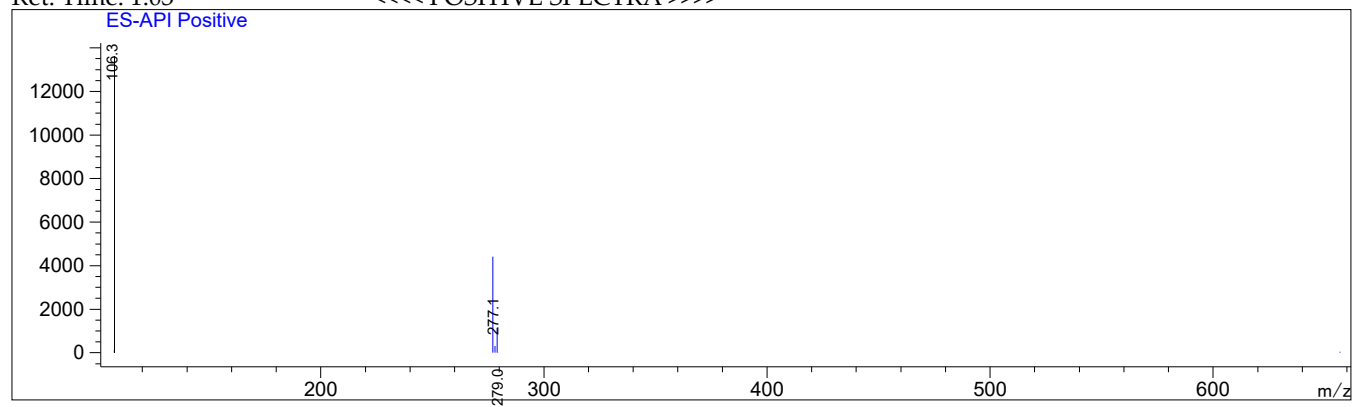
RetTim	Width	Area	Height	Area%
1.03	0.04	91138.29	31642.16	13.82
1.41	0.02	76221.73	47303.34	11.55
1.54	0.03	56649.94	26844.77	8.59



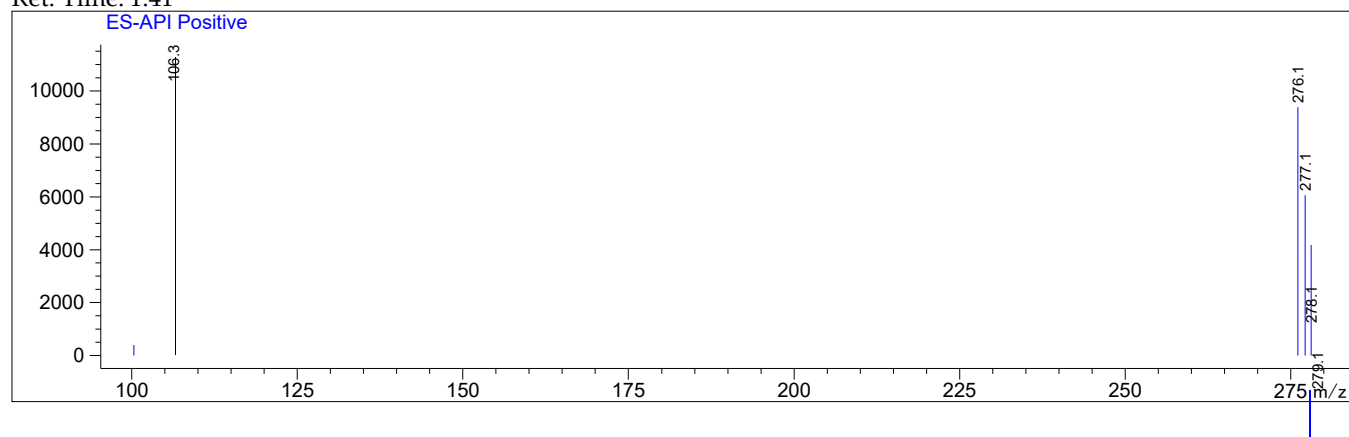
1.65	0.04	79144.08	27141.74	12.00
1.71	0.03	281485.75	147665.81	42.67
2.49	0.04	75003.82	21971.37	11.37

Ret. Time: 1.03

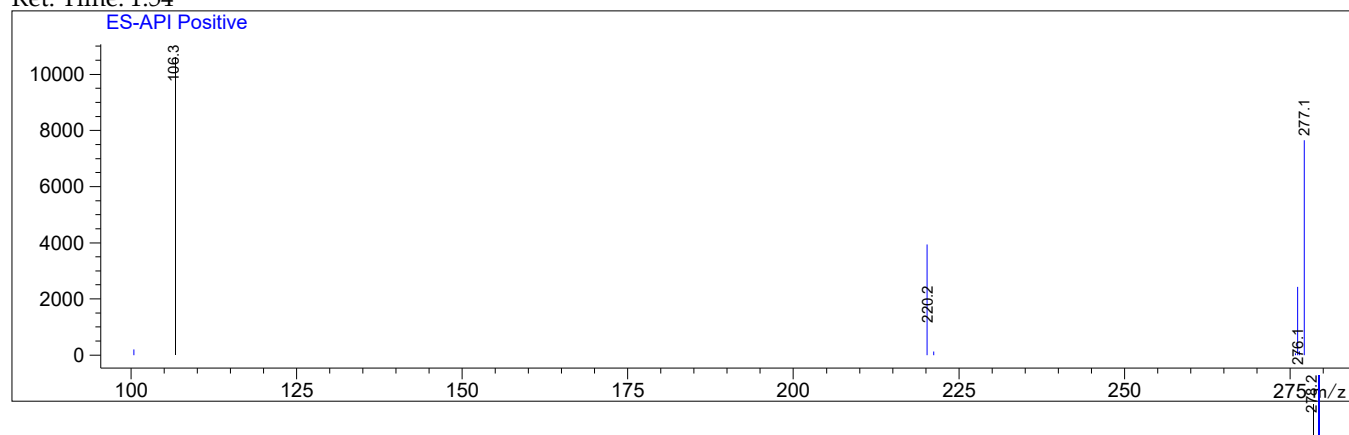
&lt;&lt;&lt;&lt; POSITIVE SPECTRA &gt;&gt;&gt;&gt;



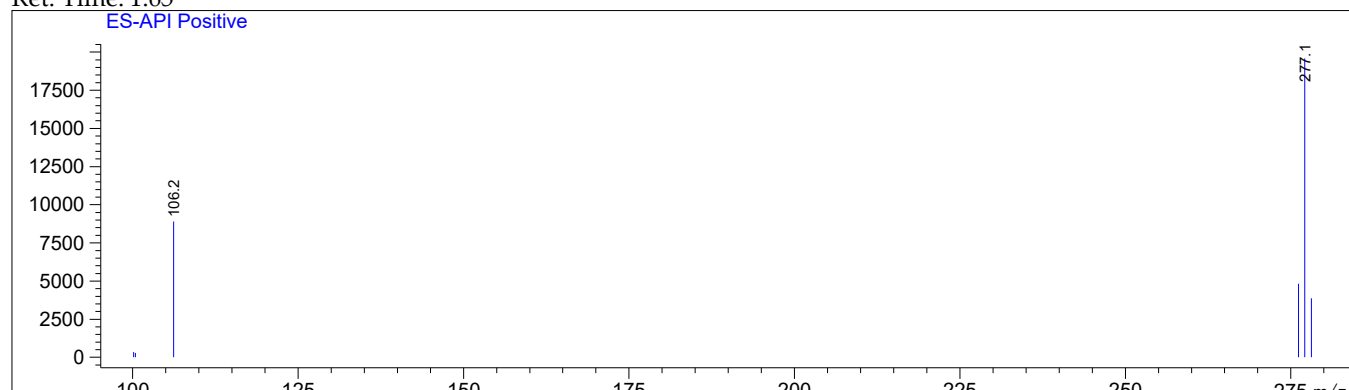
Ret. Time: 1.41



Ret. Time: 1.54



Ret. Time: 1.65

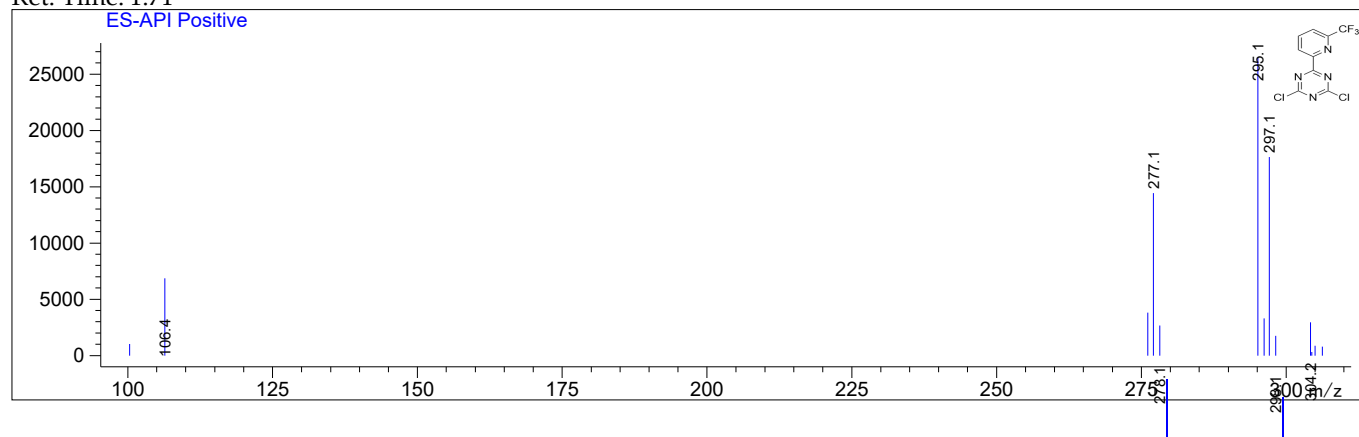


LCMSA 046 11/25/2021 11:09:25 AM 109monitor

Page 2 of 3

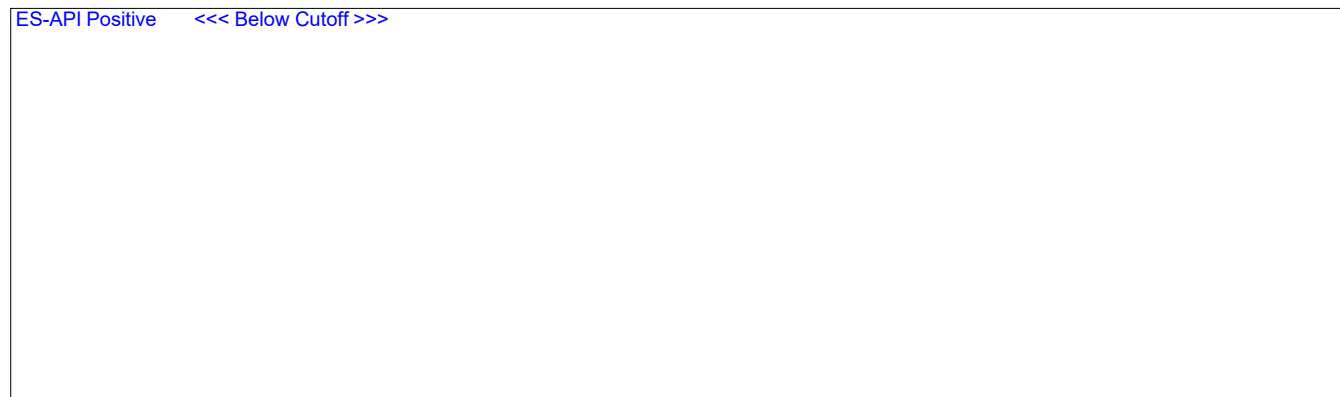
# MS Report

Ret. Time: 1.71



Ret. Time: 2.49

ES-API Positive <<< Below Cutoff >>>

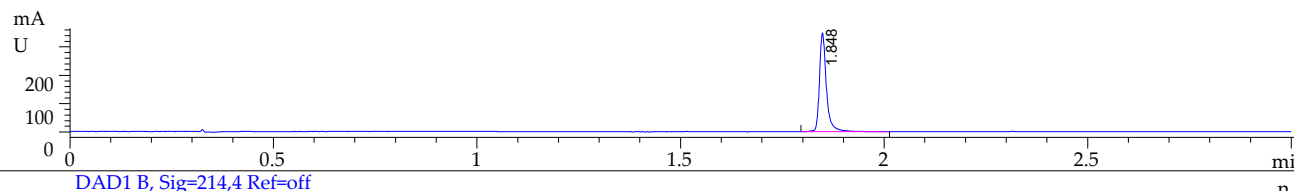




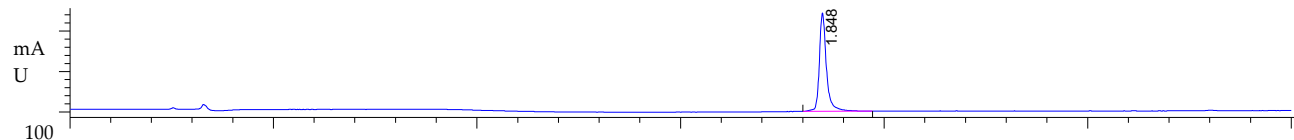
MS report 3: 1-((4-((3-fluoropyridin-4-yl)amino)-6-(6-(trifluoromethyl)pyridin-2-yl)-1,3,5- triazin-2-yl)amino)-2-methylpropan-2-ol

File .. T109\MONITOR\SP-002764-DP-1-03410-LCMS047.D Tgt Mass (EZX): 423.00  
Injection Date : 8 Dec 21 1:51 pm +0800 Seq. Line : 0  
Sample Name : SP-002764-DP-1 Location : P1-A-09  
Acq. Operator : 109monitor Inj : 1  
Spec. Reported : MS Integration Inj Volume : 3 ul  
Acq. Method : C:\Chem32\1\METHODS\MONITOR 3MIN-50-B-P  
Analysis Method : C:\CHEM32\1\METHODS\MONITOR 3MIN-B-P.M  
Sample Info : Easy-Access Method: 'MONITOR 3MIN-50-B-P.M' 423.00  
Method Info : Column: Waters X Bridge C18: 50mm\*4.6 mm\*3.5 um;  
Mobile Phase: A: Water (0.01mol/L NH4HCO3) B: ACN  
Gradient : B from 5% to 95% for 1.6 min and hold 95% for 1.4 min;  
Flow rate: 2.0 ml/min;  
Column Temperature: 40 °C

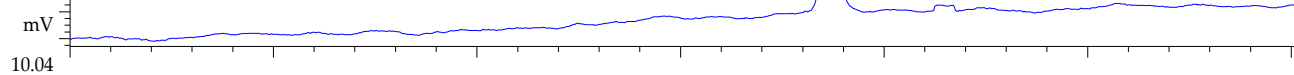
DAD1 A, Sig=254,4 Ref=off



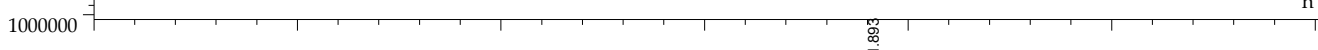
DAD1 B, Sig=214,4 Ref=off



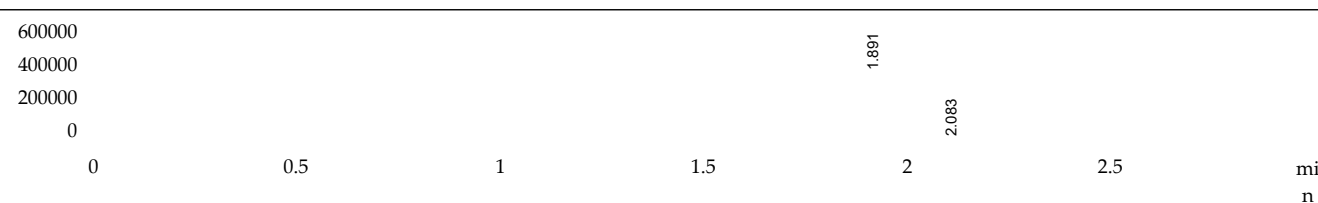
ELS1 A, Voltage



MSD1 TIC, MS File ES-API, Pos, Scan, Frag: 40



Ion 424, MSD1 424, Target Mass 423 +H Positive, EIC=423.7:424.7



Integration Results for DAD1 A, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.85	0.02	413.09	348.04	100.00

Integration Results for DAD1 B, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.85	0.02	292.27	242.33	100.00

Integration Results for ELS1 A, Voltage

RetTim	Width	Area	Height	Area%

Integration Results for MSD1 TIC, MS File

RetTime	Width	Area	Height	Area%
1.89	0.04	2232784.50	765318.81	100.00

Ret. Time: 1.89

&lt;&lt;&lt; POSITIVE SPECTRA &gt;&gt;&gt;

ES-API Positive

