

# Discovery of novel selective and orally bioavailable phosphodiesterase-1 inhibitors for the efficient treatment of idiopathic pulmonary fibrosis

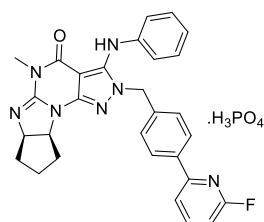
Yinuo Wu<sup>#</sup>, Yi-Jing Tian<sup>#</sup>, Mei-Ling Le<sup>#</sup>, Si-Rui Zhang, Chen Zhang, Meng-Xing Huang, Mei-Yan Jiang, Bei Zhang, and Hai-Bin Luo\*

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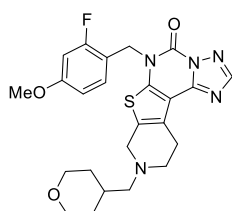
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## 1. Figure S1. Chemical structures of PDE1 inhibitors.



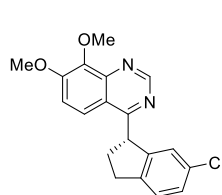
**ITI-214**

PDE1B IC<sub>50</sub>: 0.058 nM  
Selectivity over PDEs: >2759



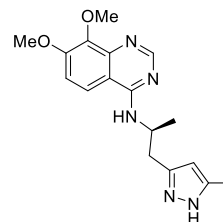
**DNS-0056**

PDE1B IC<sub>50</sub>: 26 nM  
Selectivity over PDEs: >11



**PF-04822163**

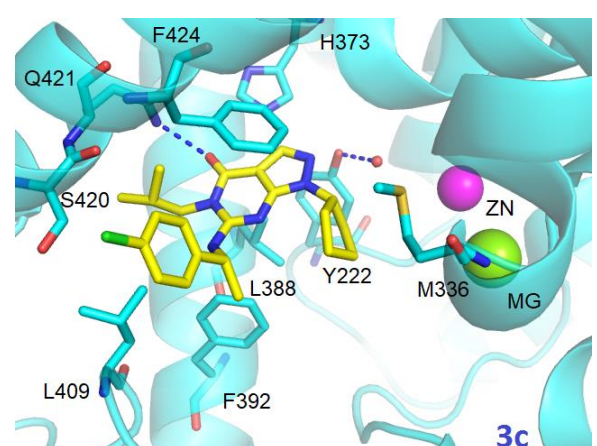
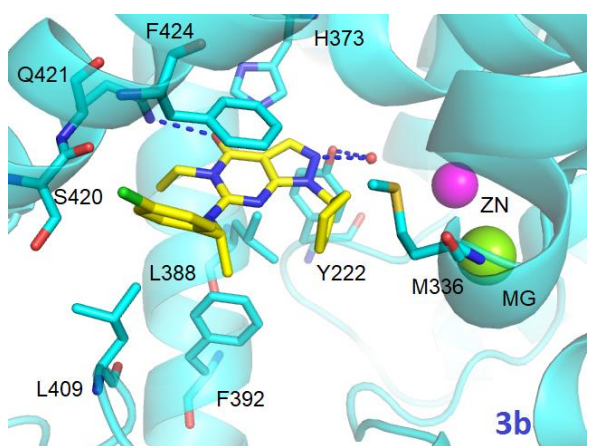
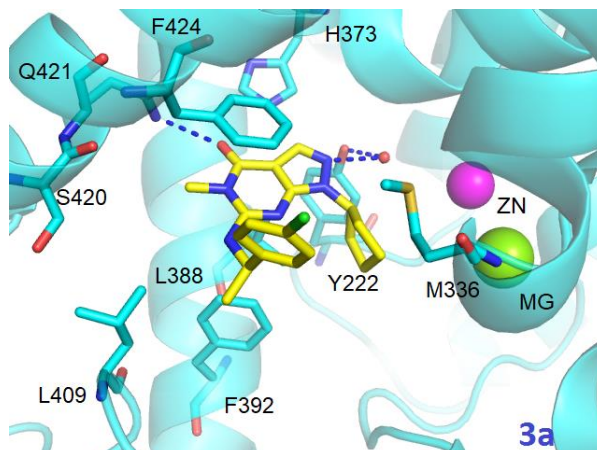
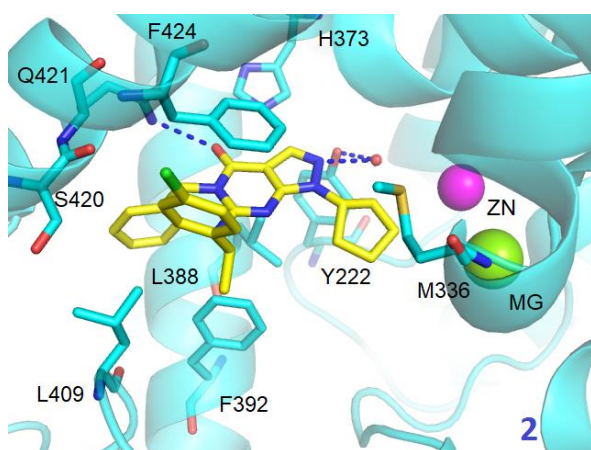
PDE1B IC<sub>50</sub>: 2.4 nM  
PDE1C IC<sub>50</sub>: 7 nM  
Selectivity over PDEs: >105

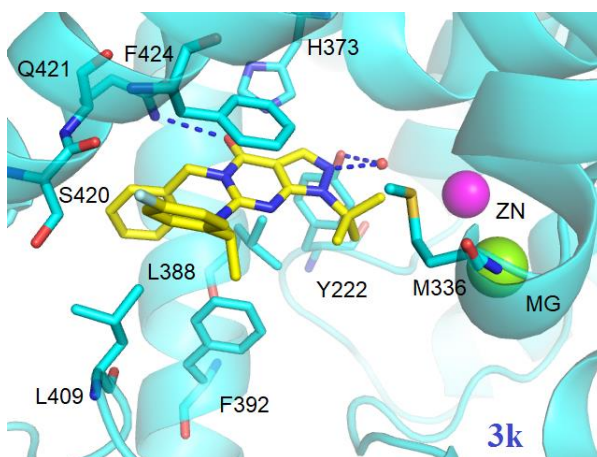
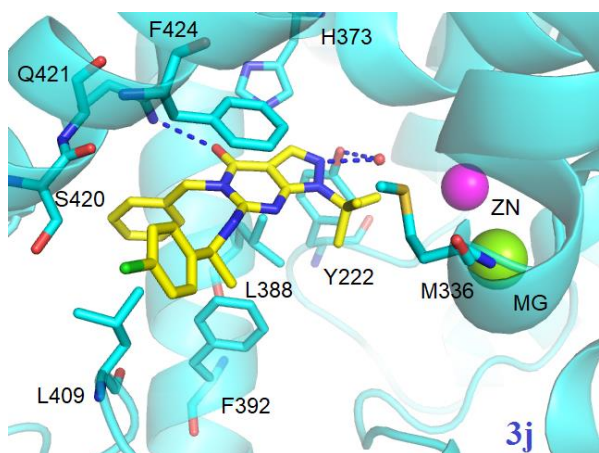
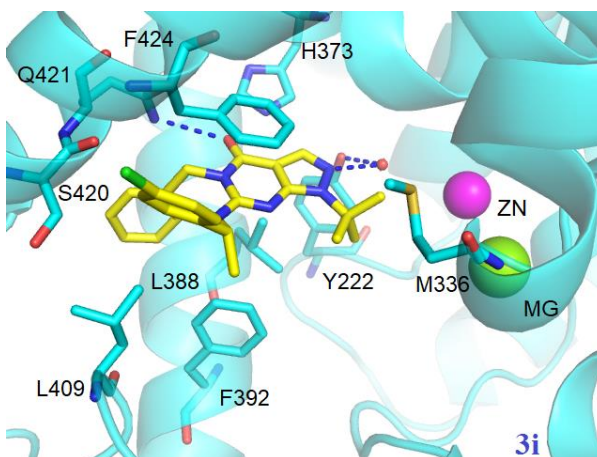
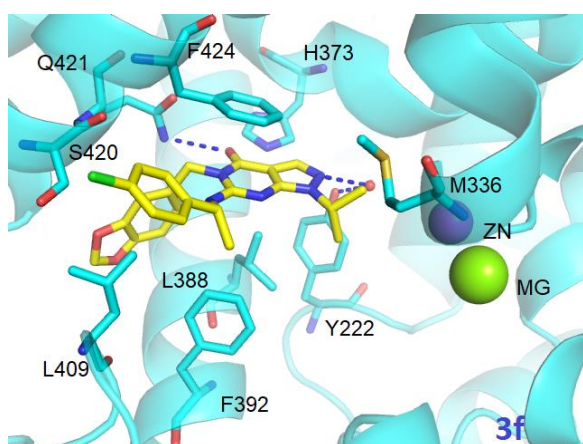
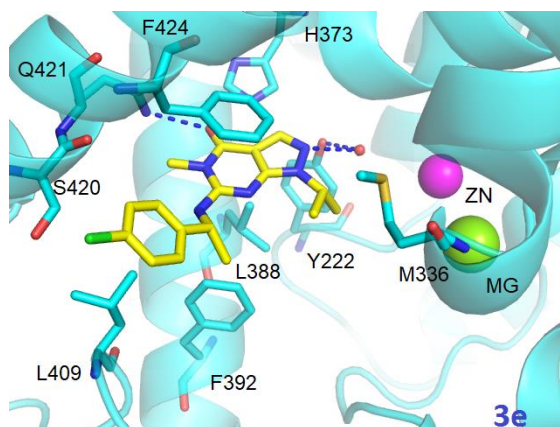
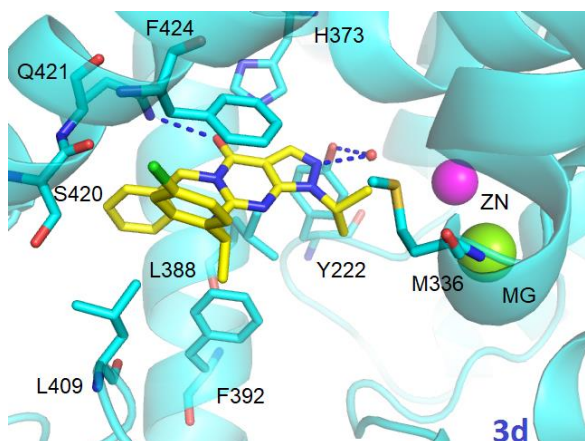


**(S)-PF-04677940**

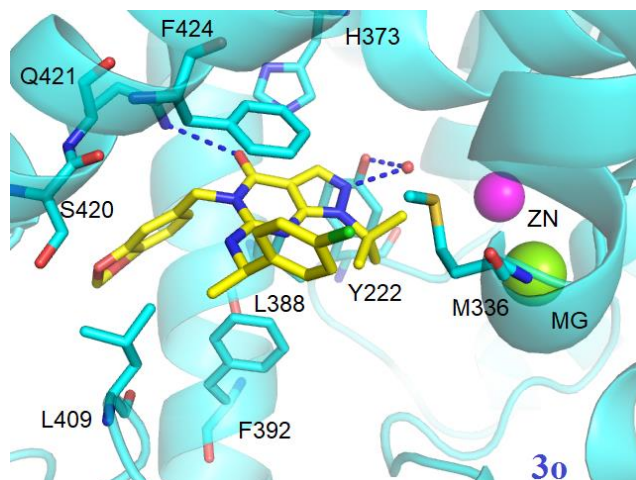
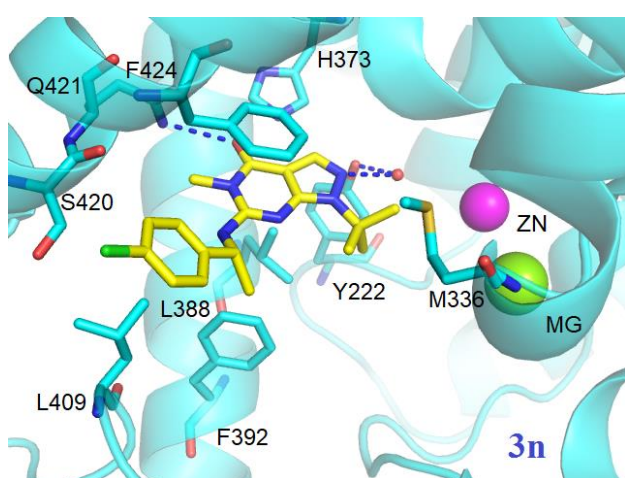
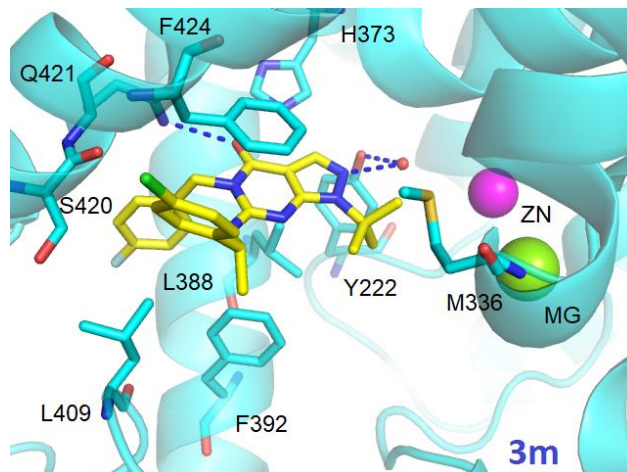
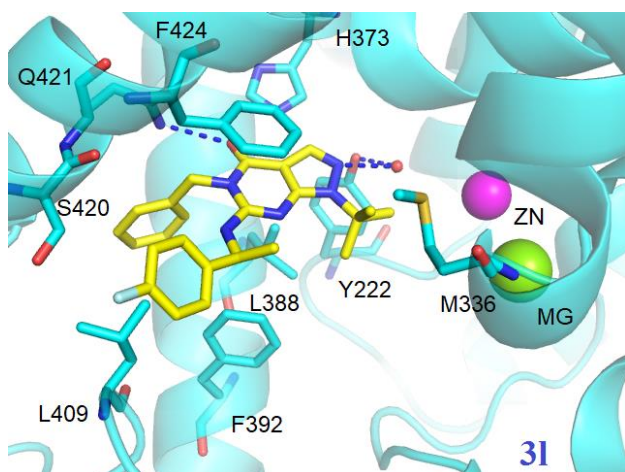
PDE1B IC<sub>50</sub>: 9 nM  
PDE1C IC<sub>50</sub>: 38 nM  
Selectivity over PDEs: >45

## 2. Figure S2: The binding modes of representative compounds









### 3. Stability of **3m** in the rat liver microsomes.

The assays were performed at the Shanghai institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China. **3m** was dissolved in 100% DMSO to prepare a 0.5mM stock solution and diluted to a final concentration of 1.5 $\mu$ M for the experiments. Ketanserin (NPGAH-CO from TCI company) was used as the positive control.

### 4. In vivo pharmacokinetics analysis of **3m**.

The pharmacokinetic properties of **3m** were analyzed by Shanghai institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China. Six male SD rats with a body weight of 230-260 g were purchased from Shanghai SIPPR-BK LAB Animal Ltd., Shanghai, China, and used for the pharmacokinetic analysis of **3m**. It was dissolved/suspended in 5% DMSO, 10% Solutol, and 85% water for intravenous administration (iv) and oral administration (po). A final dosage of 2.5 and 5.0 mg/kg rat of the formulated compounds was administered for iv and po purposes, respectively, and the blood samples were taken at various time points during a 24 h period. The concentration of the compounds in the blood was analyzed by LC-MS/MS (Shimadzu liquid chromatographic system and API4000 mass spectrometer, Applied Biosystems, Ontario, Canada).

### 5. hERG inhibition and humanCYP450 inhibition of **3m**.

The assays were performed by the Medicilon Company, Shanghai, China. hERG inhibition was performed using an automated patch clamp electrophysiology measurement in CHO-hERG cells. The CYP450 inhibition assay was performed by incubating compound **3m** with human liver microsomes and NADPH in the presence of the CYP450-isoform specific probe substrate. The IC<sub>50</sub> values of compound **3m** for five CYP isoenzymes (i.e., CYP1A2, CYP2C9, CYP2D6, CYP3A4-M

and CYP3A4-T) were determined.

## 6. Acute toxicity of compound **3m**.

The acute toxicity was tested according to similar protocols that were described in our previous study.<sup>1</sup> Thirty KM mice (22 days, 18-20 g), which were purchased from the Laboratory Animal Center of Sun Yat-Sen University (Guangzhou, China), were used to evaluate the acute toxicity of **3m**. Mice were randomly divided into three groups, and each group was given in single oral dose of 0, 1000, or 1500 mg/kg **3m** on the first day of the experiment. Mice were maintained on a 12 h light/dark cycle (light from 7:00 to 19:00) at room temperature and 60-70% relative humidity. Sterile food and water were provided according the institutional guidelines. Prior to each experiment, mice were fasted overnight and allowed free access to water. Compound **3m** was dissolved in 5% DMSO/10% Solutol/85% water solution and orally administered. Mice were observed for any abnormal behavior and mortality and weighed 4 h after **3m** was administered and then every 24h for 14 days. Animals were sacrificed on the 14th day, and tissue samples of the heart, liver, and kidney were macroscopically examined for possible damage.

## 7. Molecular Docking and Molecular Dynamics Simulations.

The X-ray crystal structure of PDE1 (PDB code: 5UP0) and **C33**-PDE9A complex (PDB ID: 4Y874) was selected for molecular modeling, and Surflex-dock embedded in the software Tripos Sybyl 2.0 was used.<sup>2,3</sup> Two metal ions crucial for the PDE's catalytic activity in the catalytic domain and water molecules coordinate these two metal ions were retained. Hydrogen atoms were added, and the ionizable residues were protonated at the neutral pH. The protomol, was generated using the

parameters by default. The parameters of `proto_thresh` and `proto_bloat` were assigned 0.5 and 0, respectively. After the protomol was the prepared, molecular docking was performed for test molecules.

After molecular docking completed, similar MD simulation procedures as previous studies were used to equilibrate the whole system. Here 8 ns MD simulations were carried out in the NPT ensemble with a constant pressure of 1 atm and a constant temperature of 300 K. The periodic boundary conditions were adopted, along with an 8 Å cutoff for long-range electrostatic interactions with the partial mesh Ewald (PME) method. The SHAKE algorithm was utilized to deal with all bonds involving hydrogen atoms, and hence the time step was set to 2 fs. An Intel Xeon E5620 CPU and an NVIDIA Tesla C2050 GPU, which are available in performing floating-point calculations, were applied to accelerate the process of MD simulations for each system. Subsequently, the 100 snapshots were isolated from the final 1.0 ns period of the MD simulation trajectories, and then used for binding-free-energy calculations by the MM-PBSA approach. For the electrostatic contribution to the solvation-free energy, the PBSA program in the Amber 16 suite was used, which could numerically solves the Poissone Boltzmann equations.

In light of the MM-PBSA method, the binding free energy ( $\Delta G_{\text{bind}}$ ) can be calculated by the following equation 1.  $G_{\text{complex}}$ ,  $G_{\text{rec}}$  and  $G_{\text{lig}}$  represent the free energies of complex, receptor and ligand, respectively.

$$\Delta G_{\text{bind}} = G_{\text{complex}} - G_{\text{rec}} - G_{\text{lig}} \quad (1)$$

Each free energy was evaluated by the sum of the MM energy  $E_{\text{MM}}$ , the solvation free energy  $G_{\text{solv}}$ , and the entropy contribution  $S$ , respectively, leading to equation 2.

$$\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - T\Delta S \quad (2)$$

$\Delta E_{\text{MM}}$  is the gas phase interaction energy and can be decomposed into  $E_{\text{MM, comp}}$ ,  $E_{\text{MM, rec}}$  and  $E_{\text{MM, lig}}$ . Solvation free energy is evaluated by the sum of the electrostatic solvation free energy and nonpolar

solvation free energy. The electrostatic solvation free energy,  $\Delta G_{PB}$ , was solved by the Poisson Boltzmann (PB) equation, while the nonpolar solvation free energy varies proportionally with the solvation accessible surface area (SASA), leading to by equation 3 and 4.

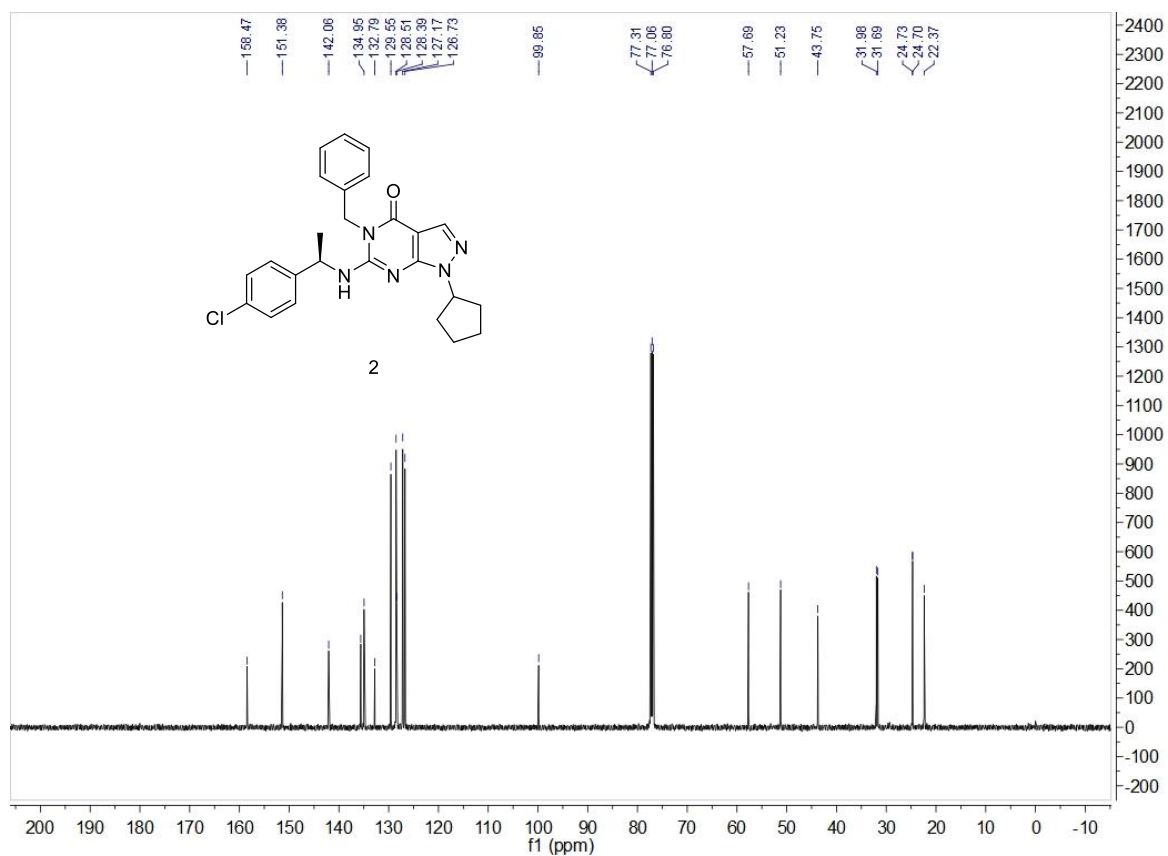
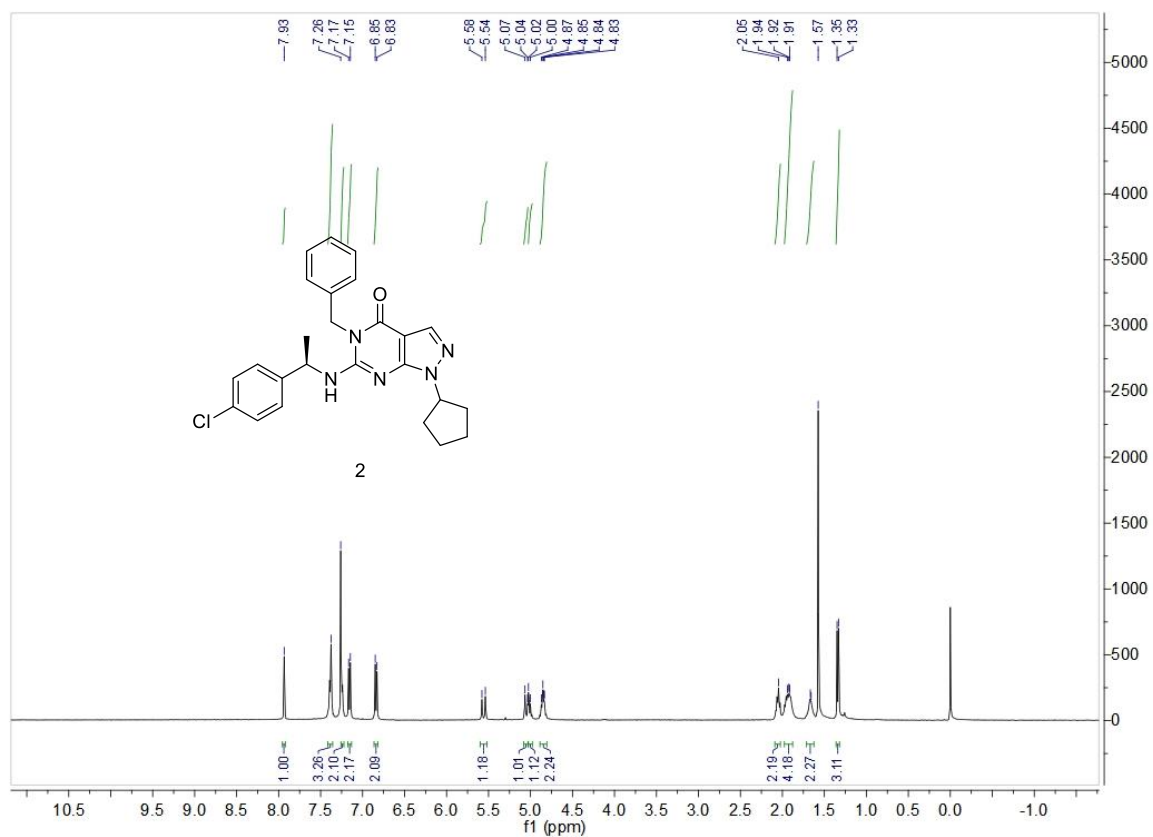
$$\Delta G_{solv} = \Delta G_{PB} + \Delta G_{np} \quad (3)$$

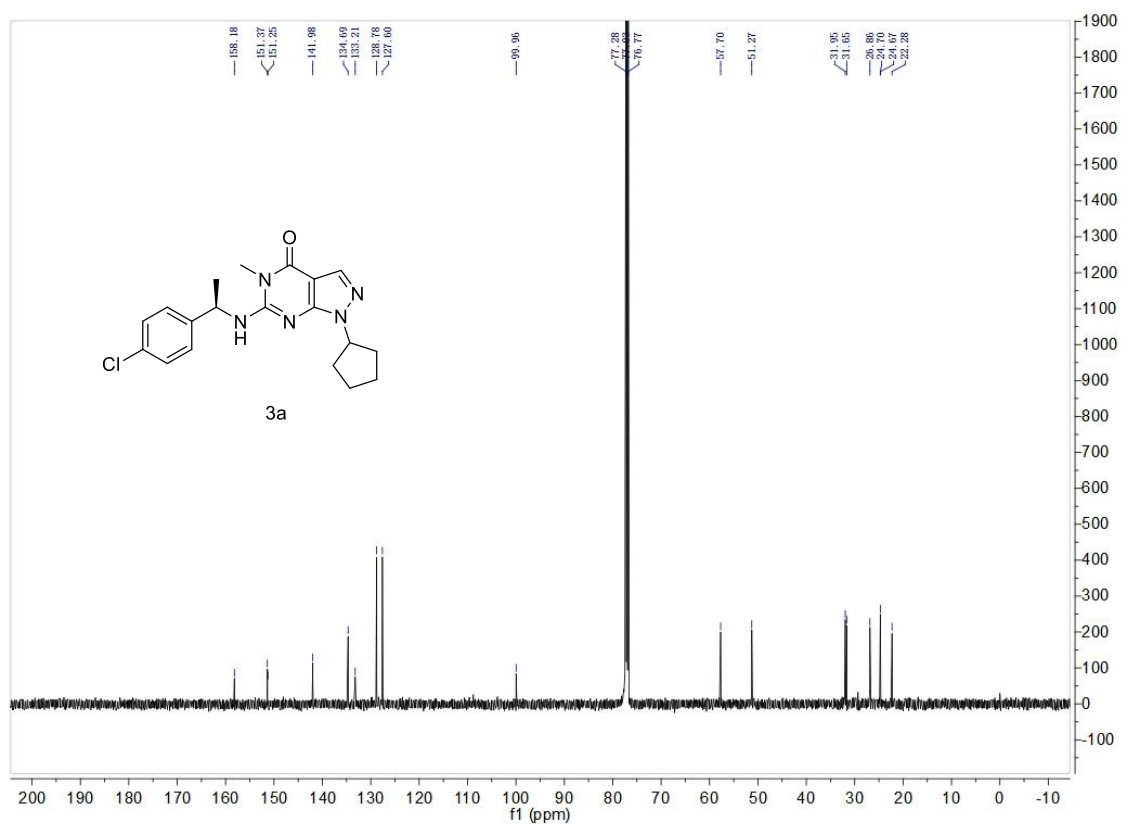
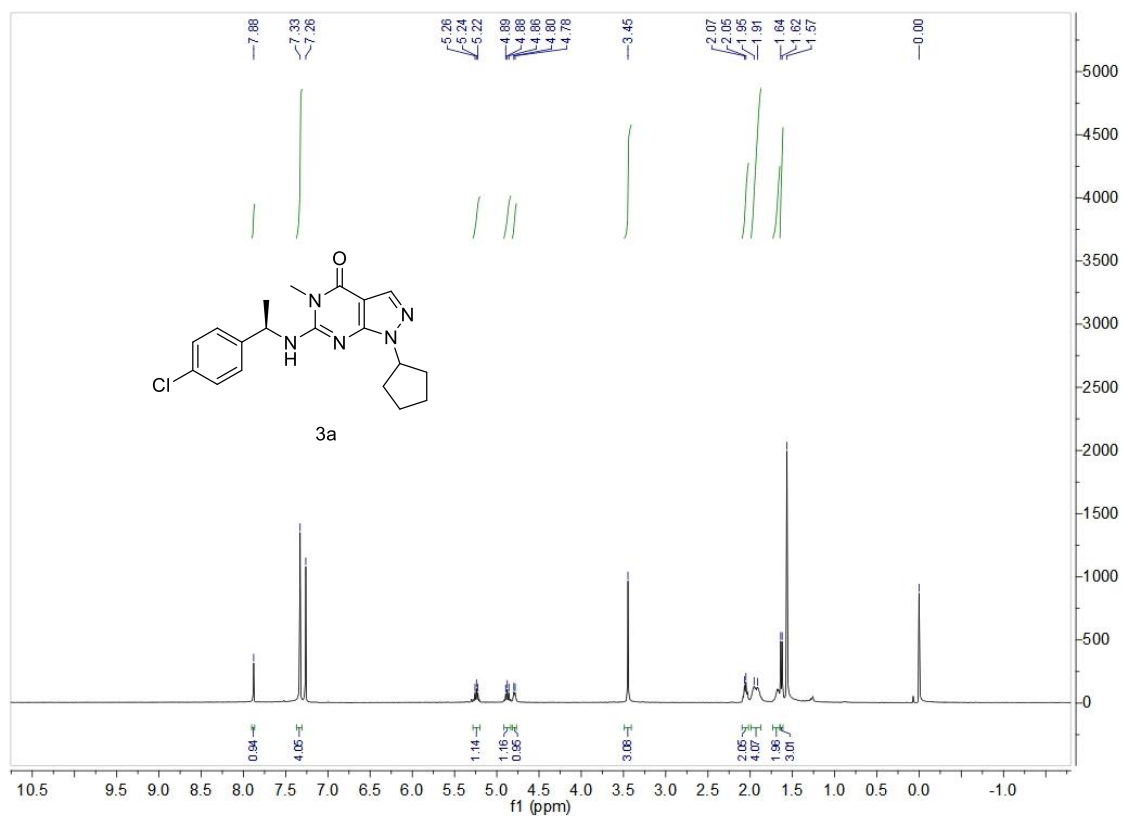
$$\Delta G_{np} = \gamma \text{ SASA} + b \quad (4)$$

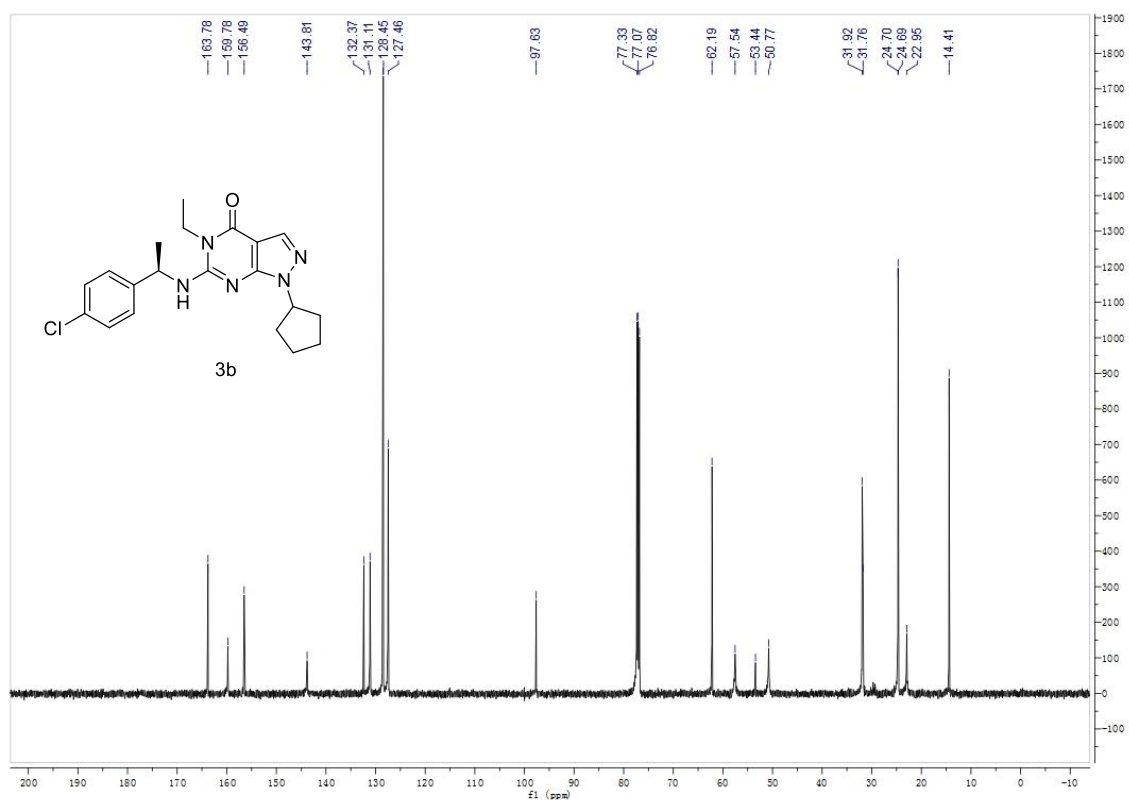
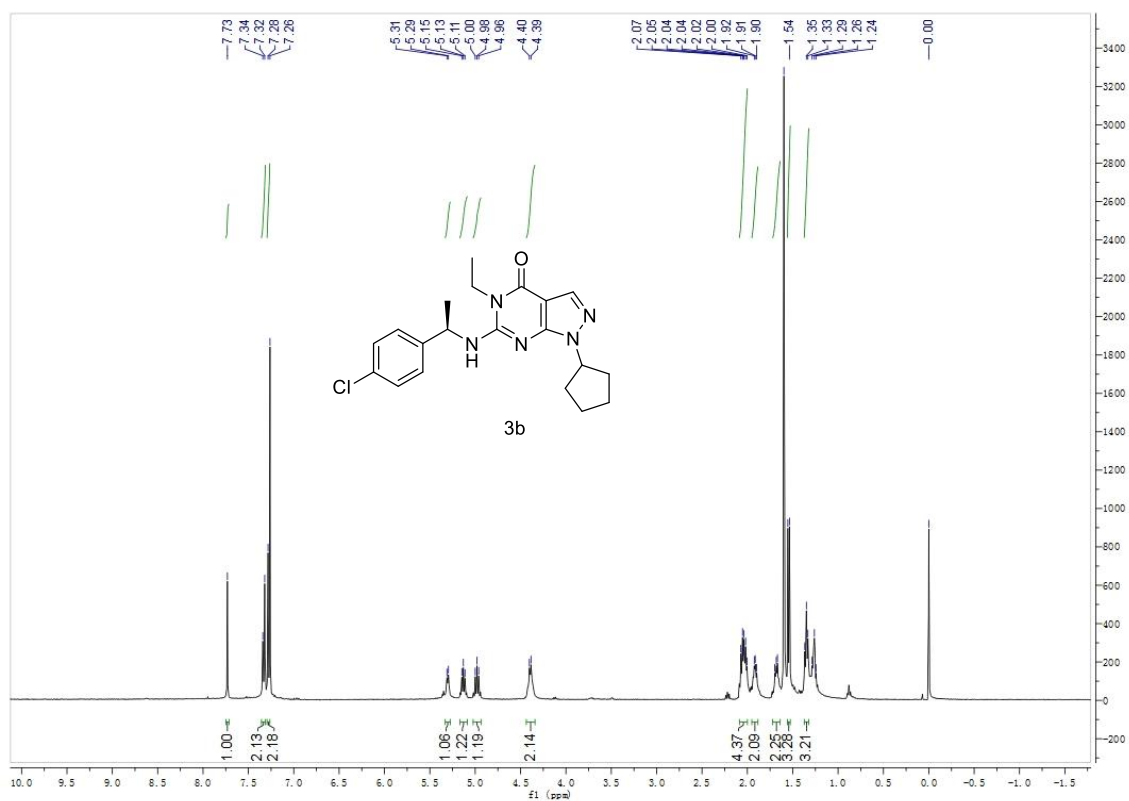
In order to get the compromise between efficiency and accuracy, entropy contribution term was omitted for  $\Delta G_{bind}$  in equation 2, since the calculations of the entropy contribution is extremely time-consuming for large protein-ligand systems. The default parameters were adopted, with  $\gamma = 0.0072$  kcal/(Å<sup>2</sup>) and  $b = 0$  kcal/mol. The charges of Mg<sup>2+</sup> and Zn<sup>2+</sup> were assigned 2.0 for PB calculations, and their bond radii were used for SA calculations.

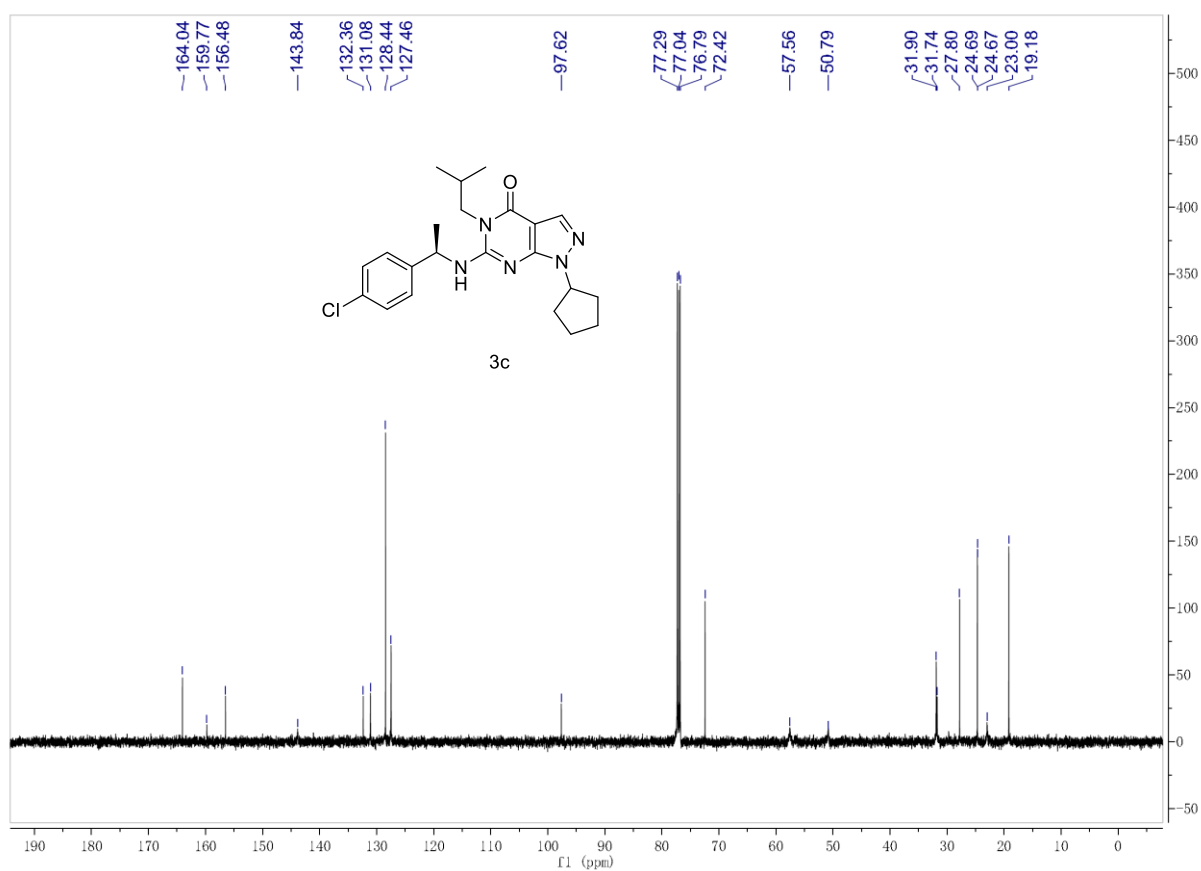
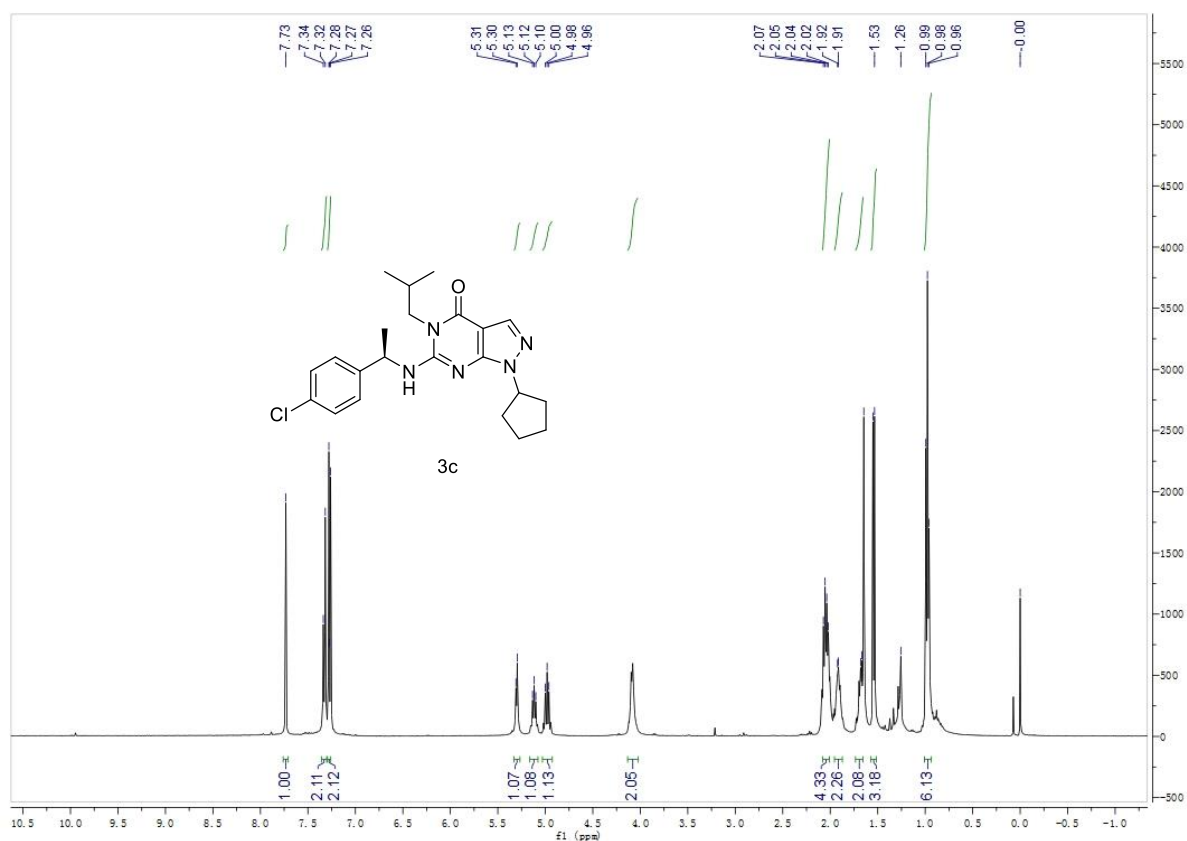


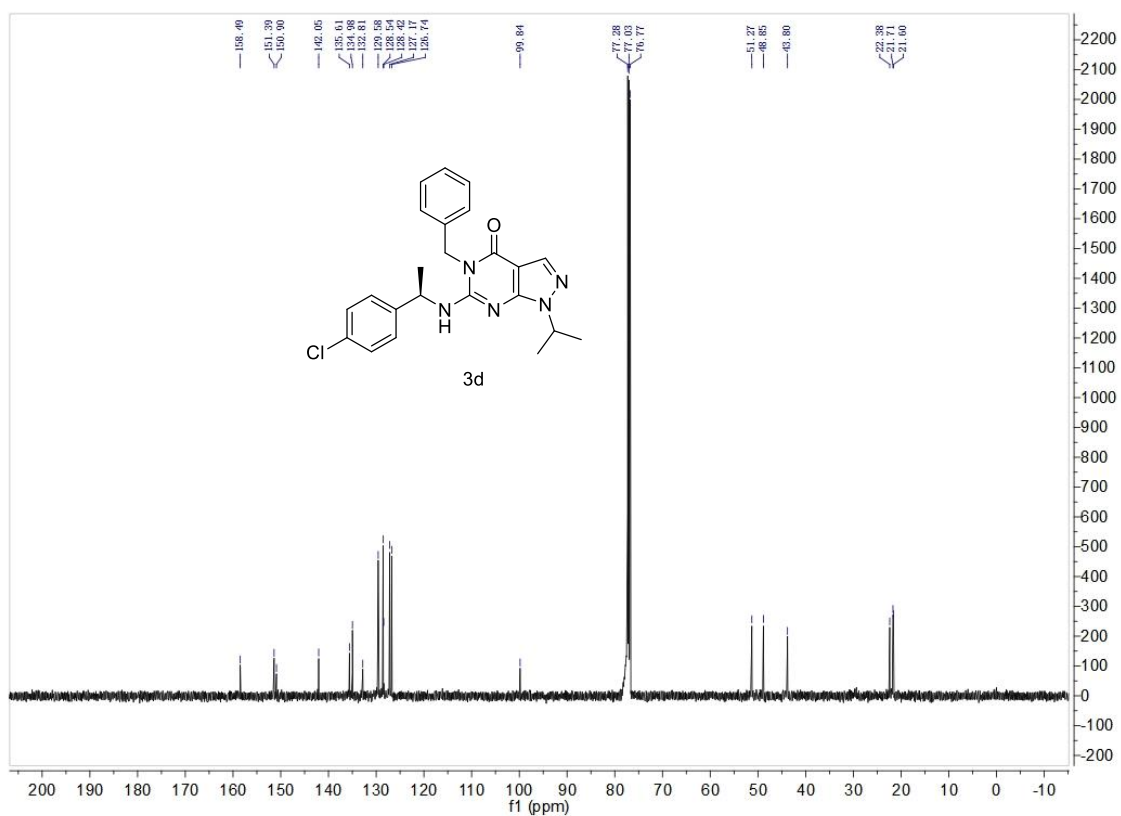
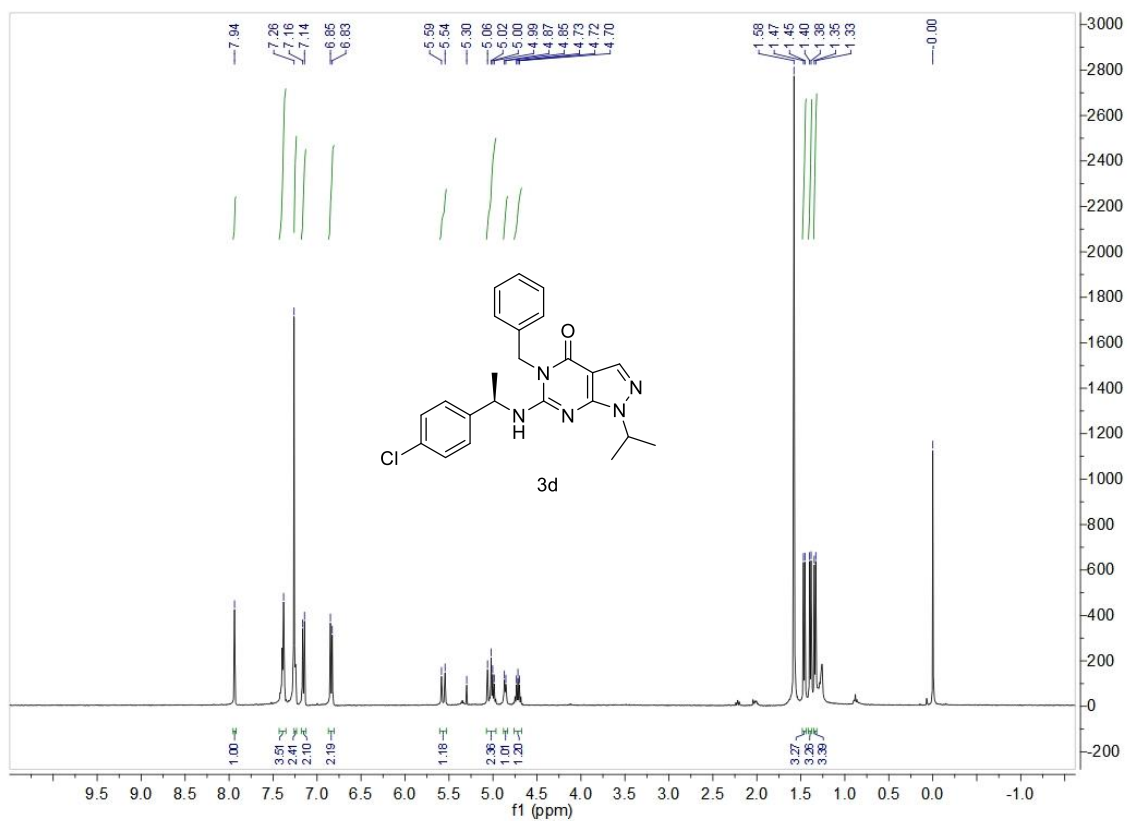
## 8. $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of the targeted compounds.

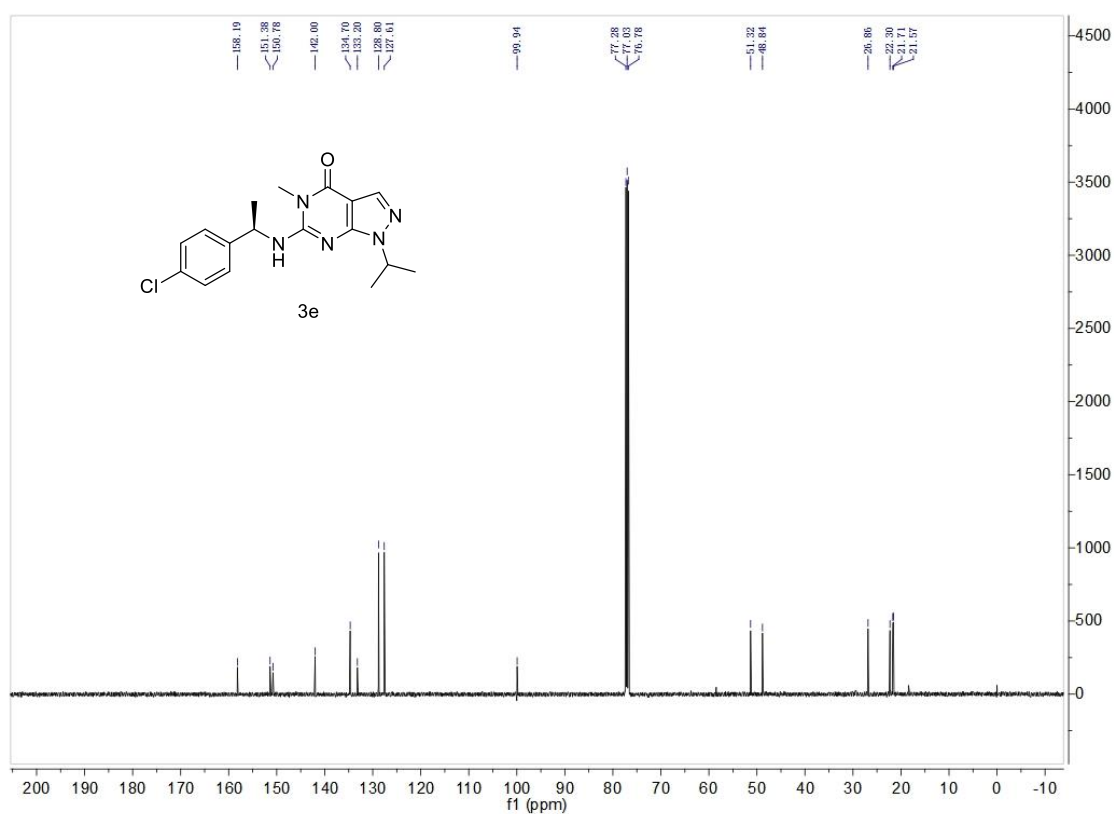
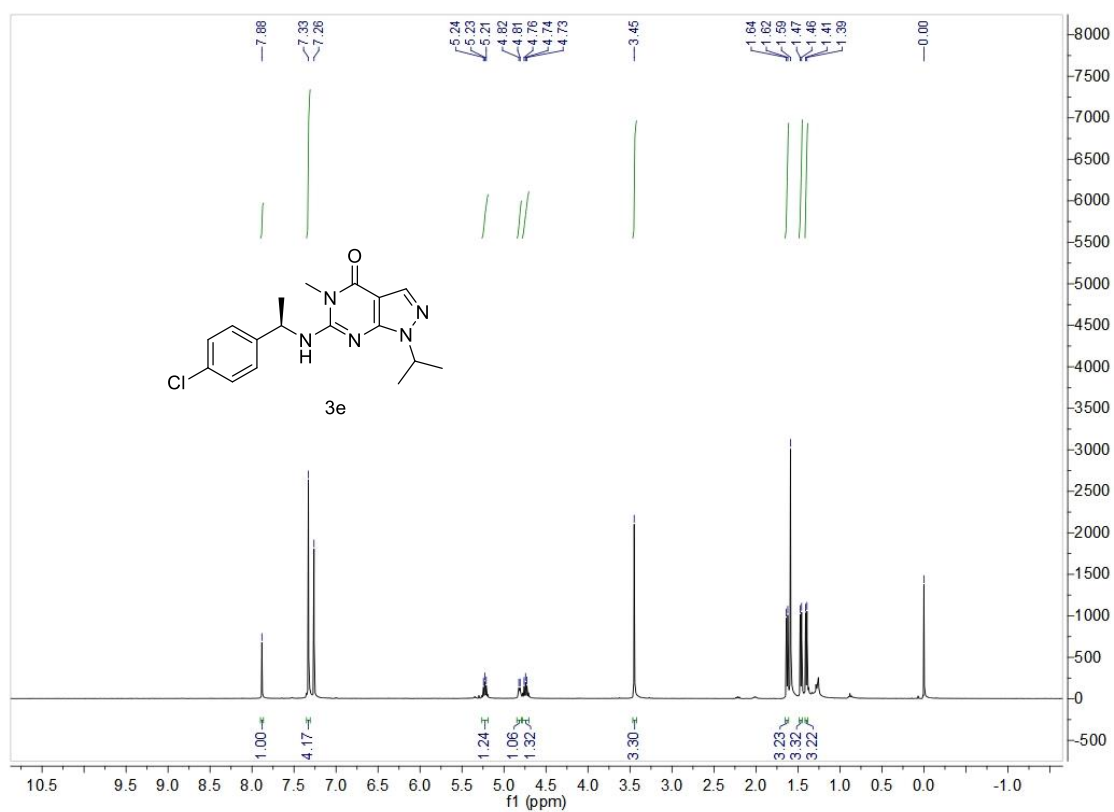




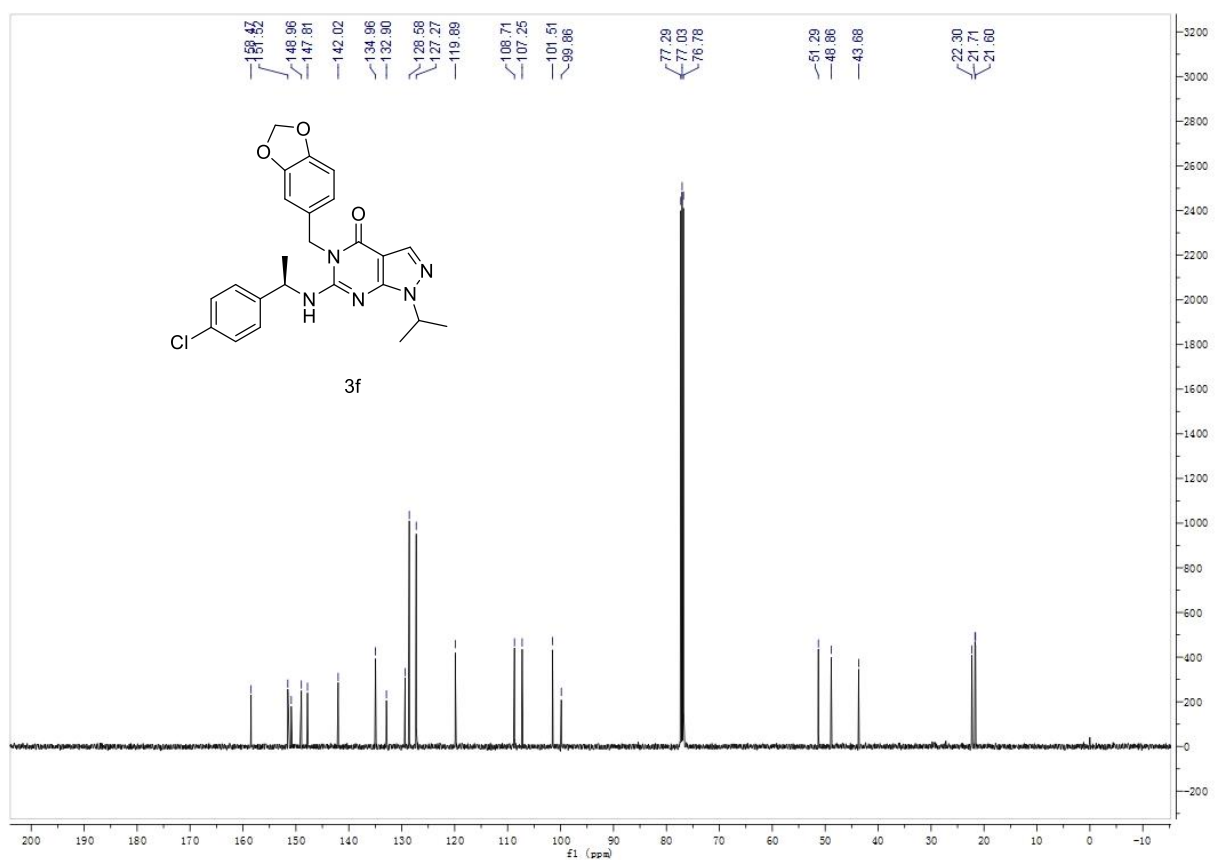
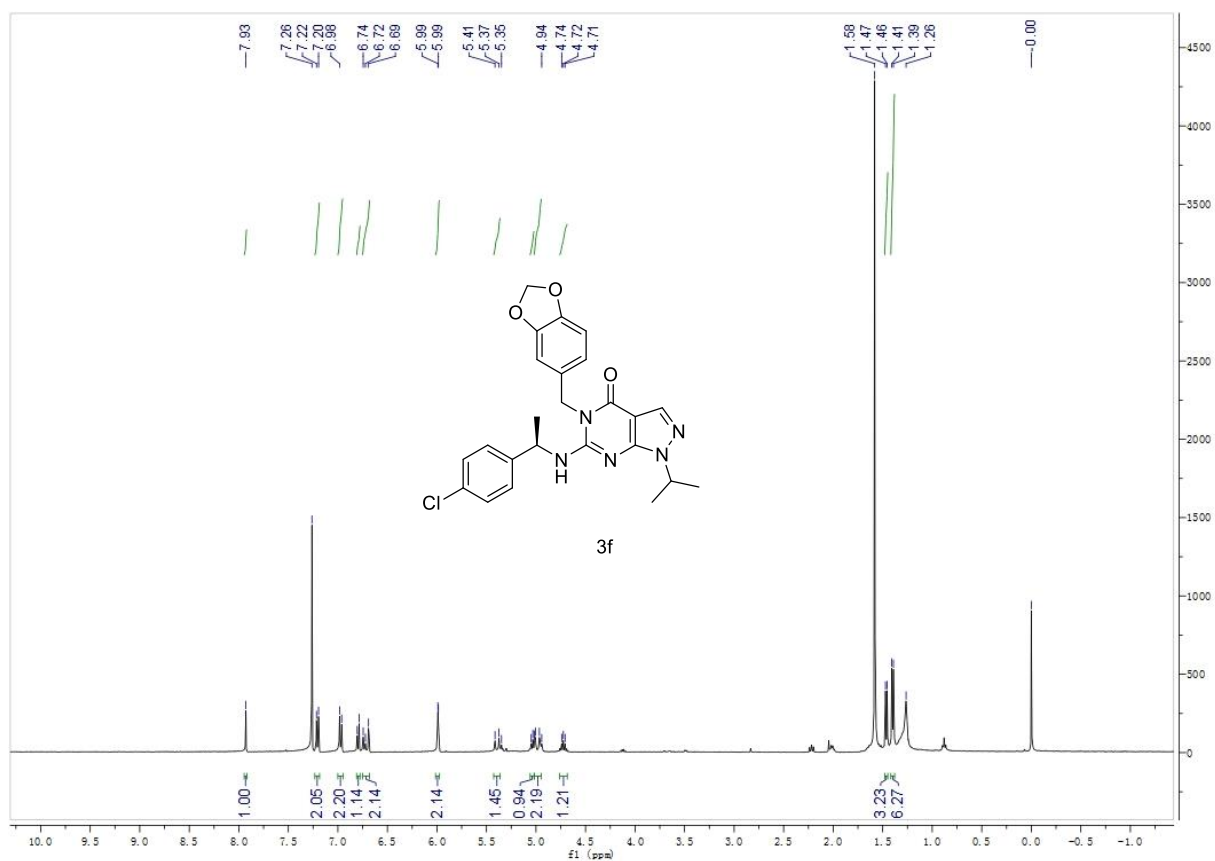


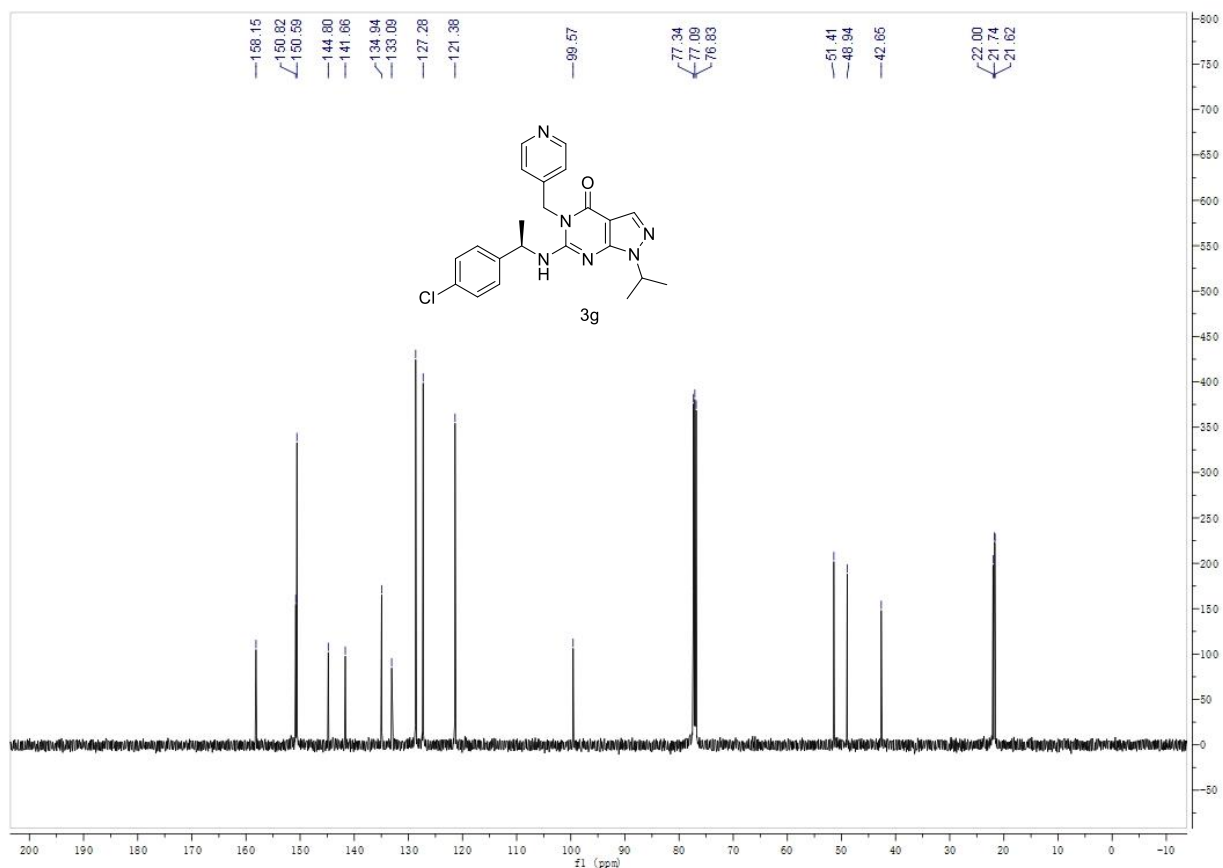
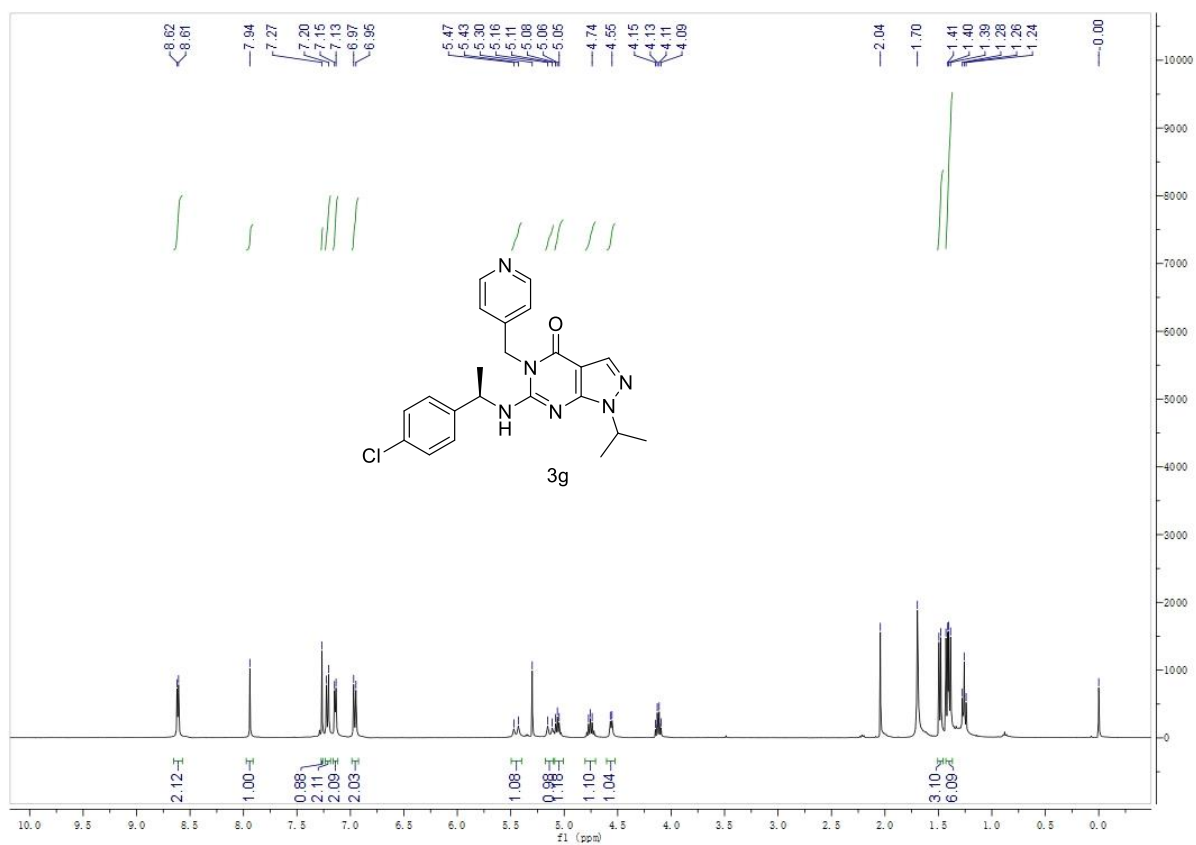


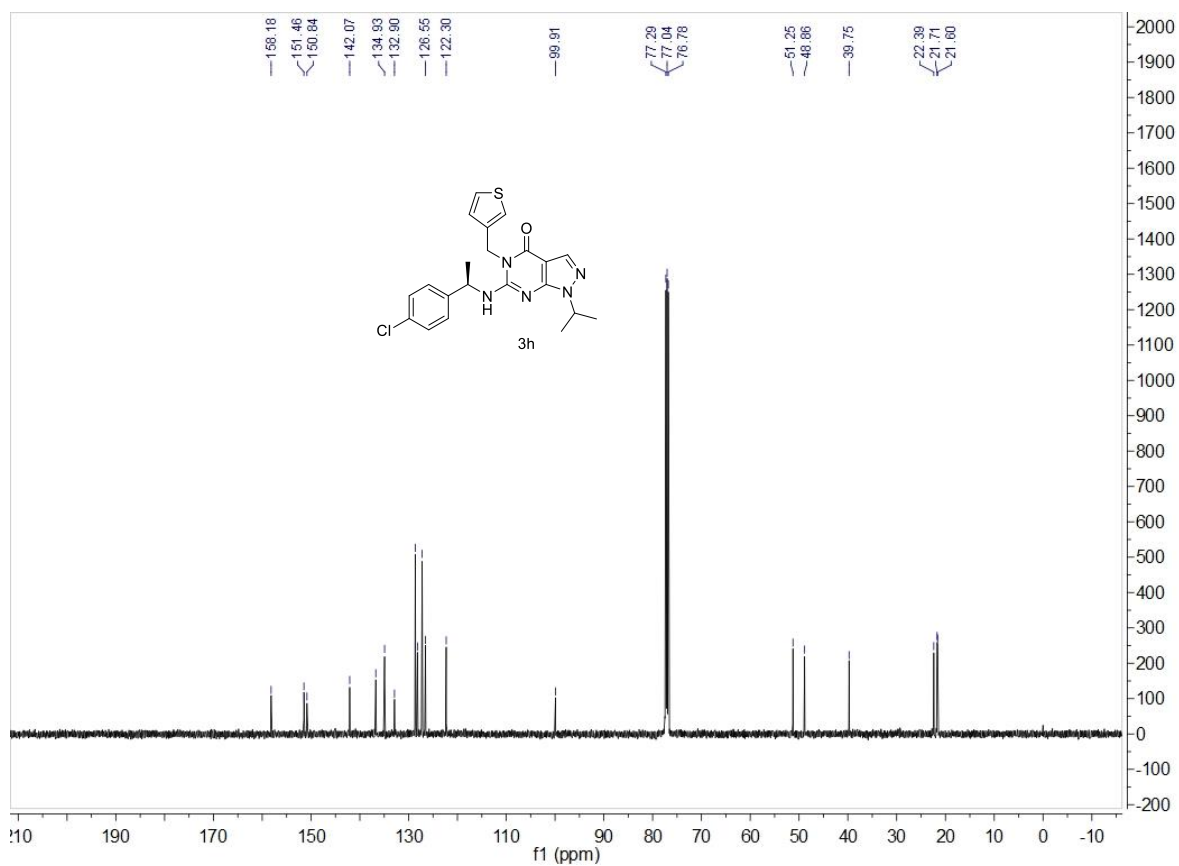
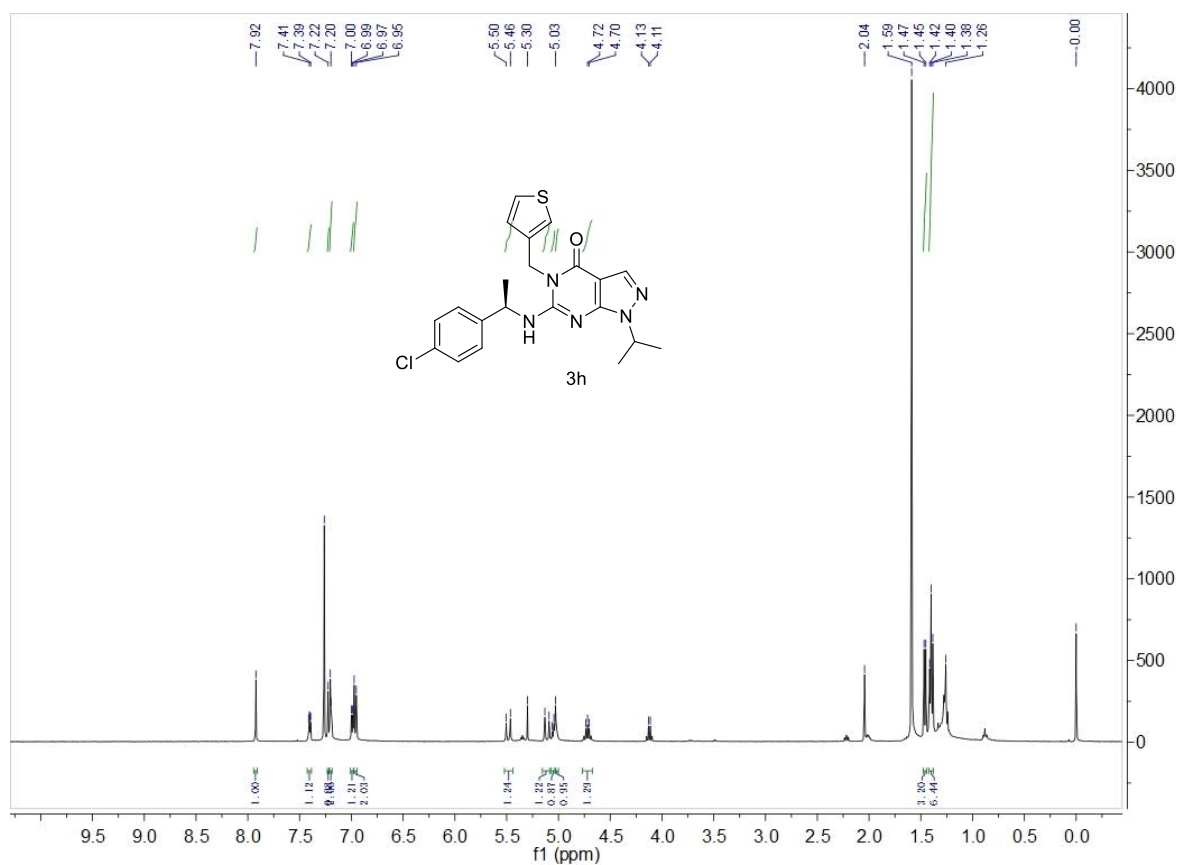


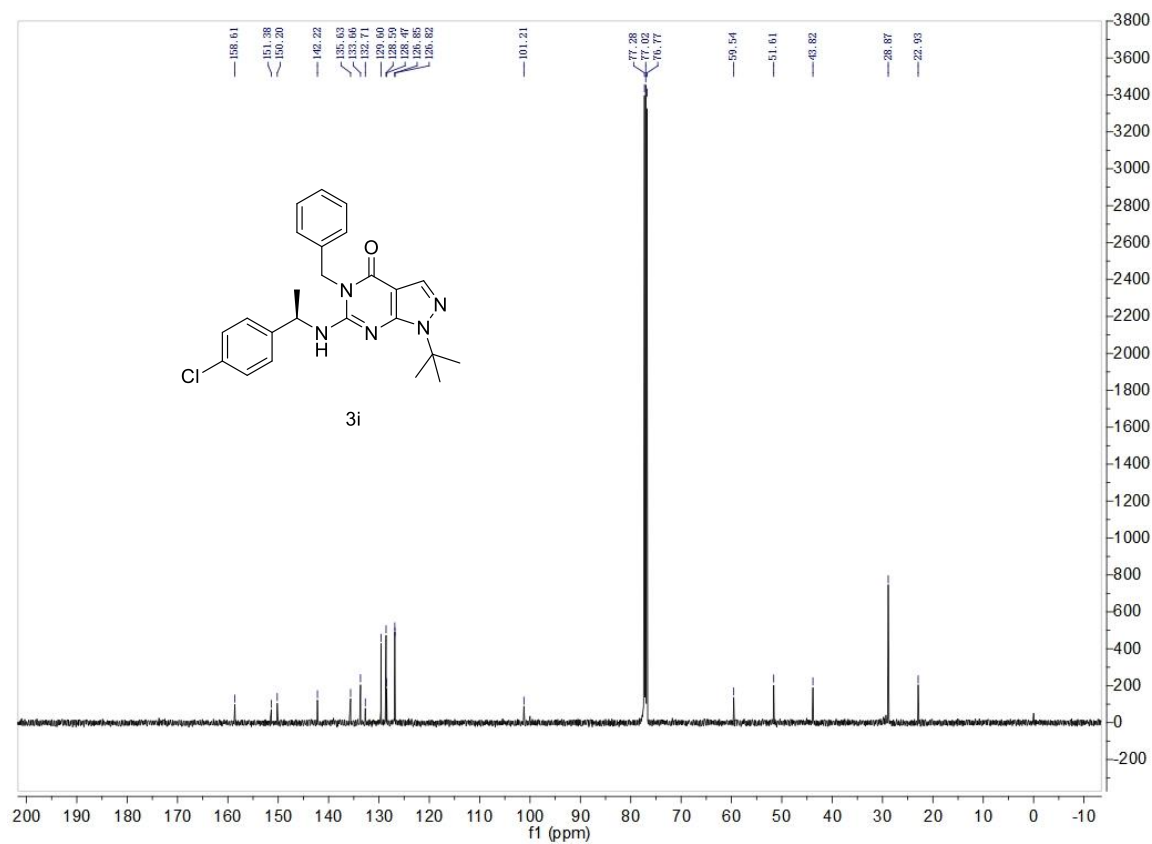
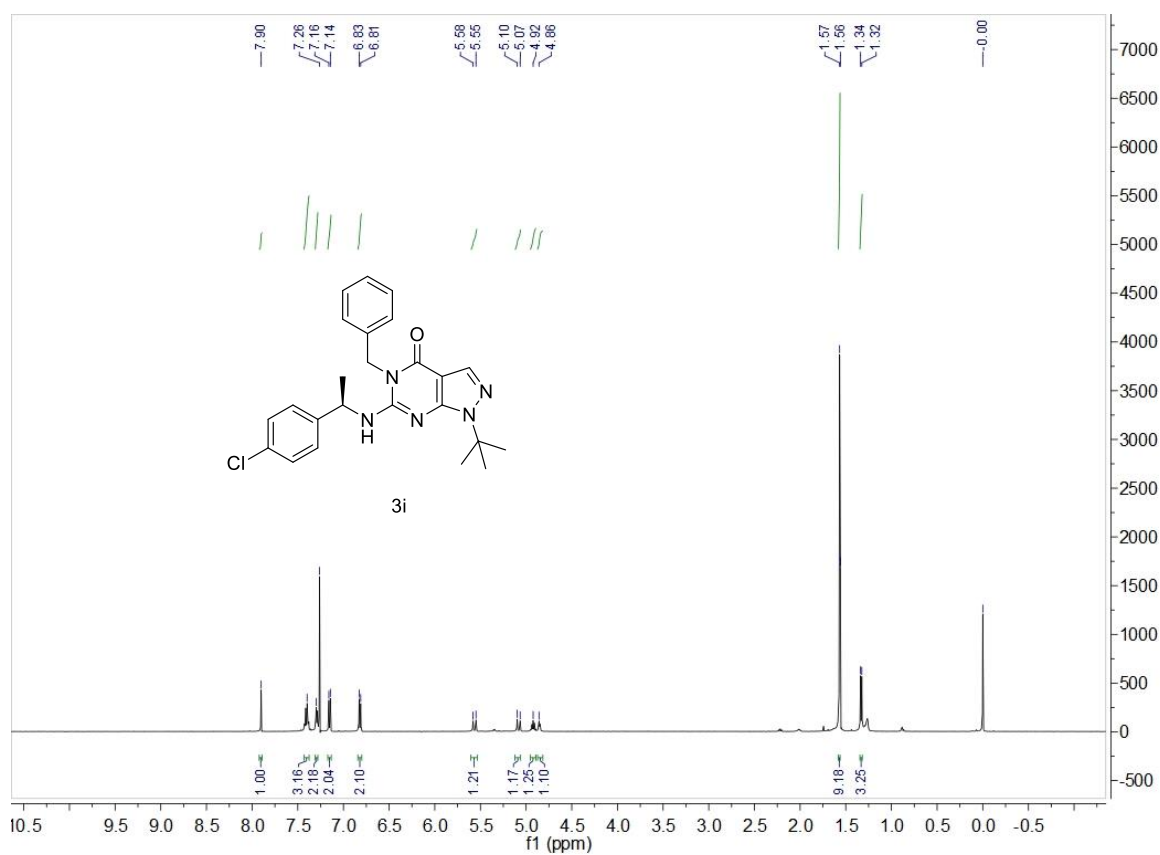


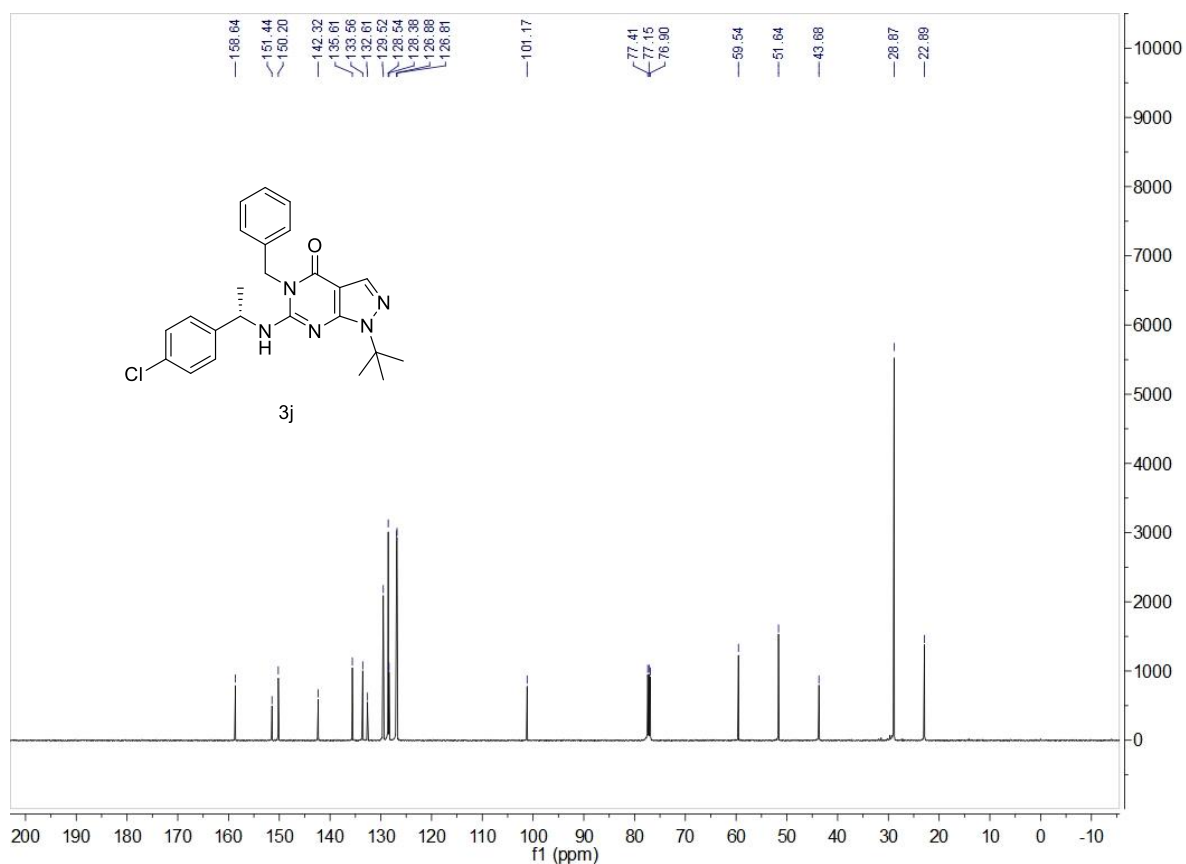
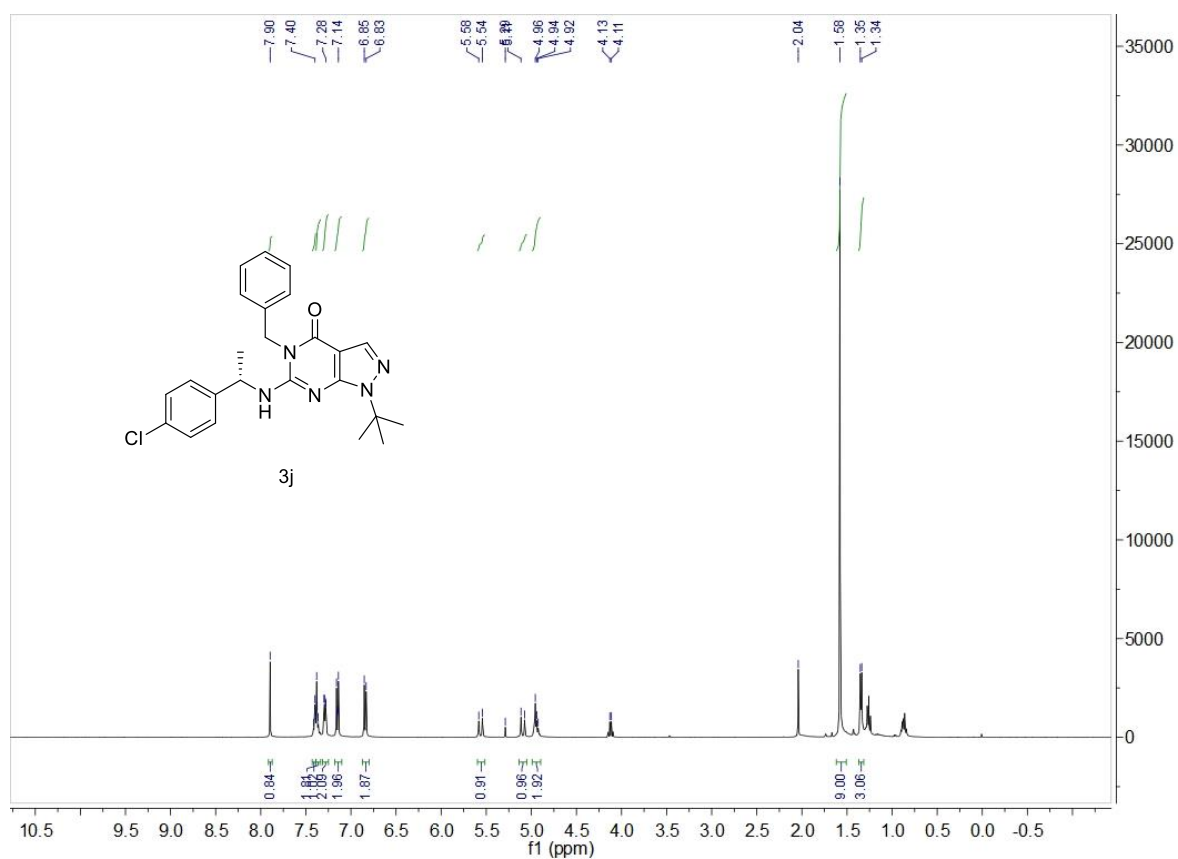


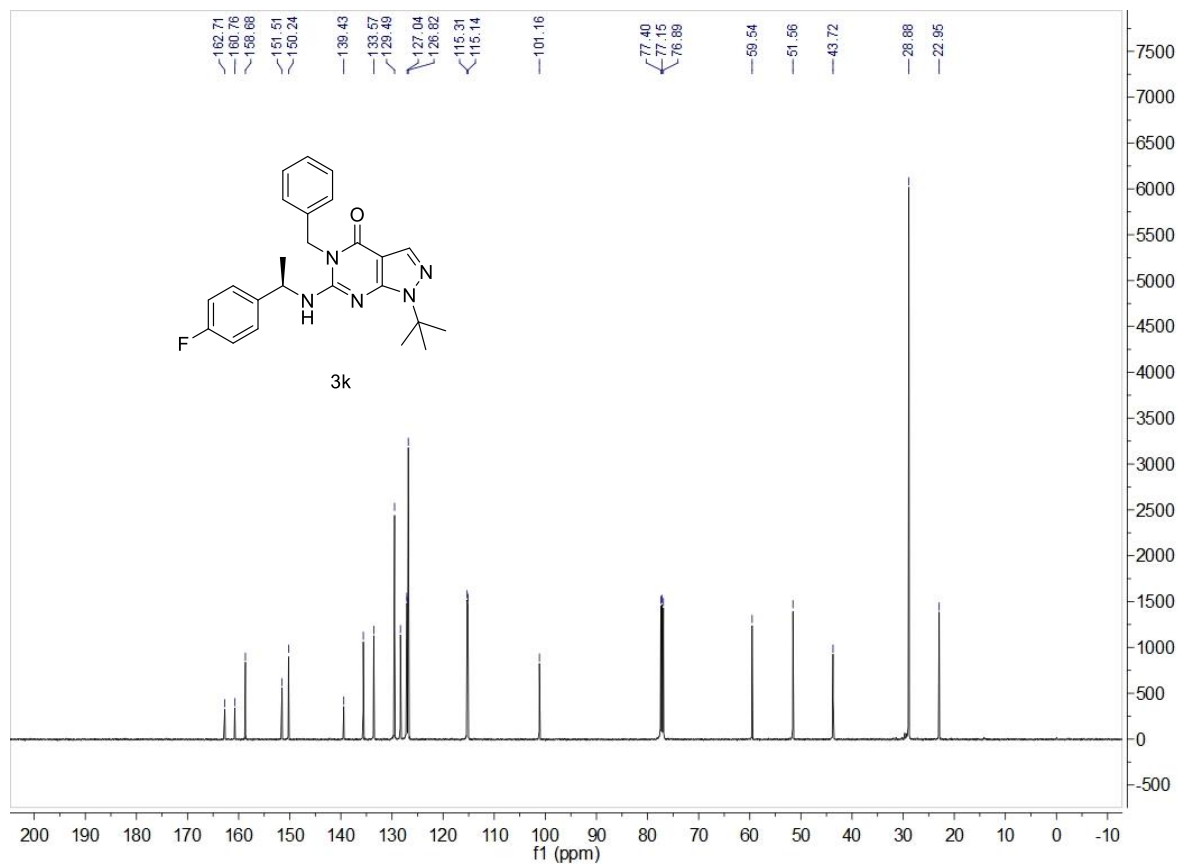
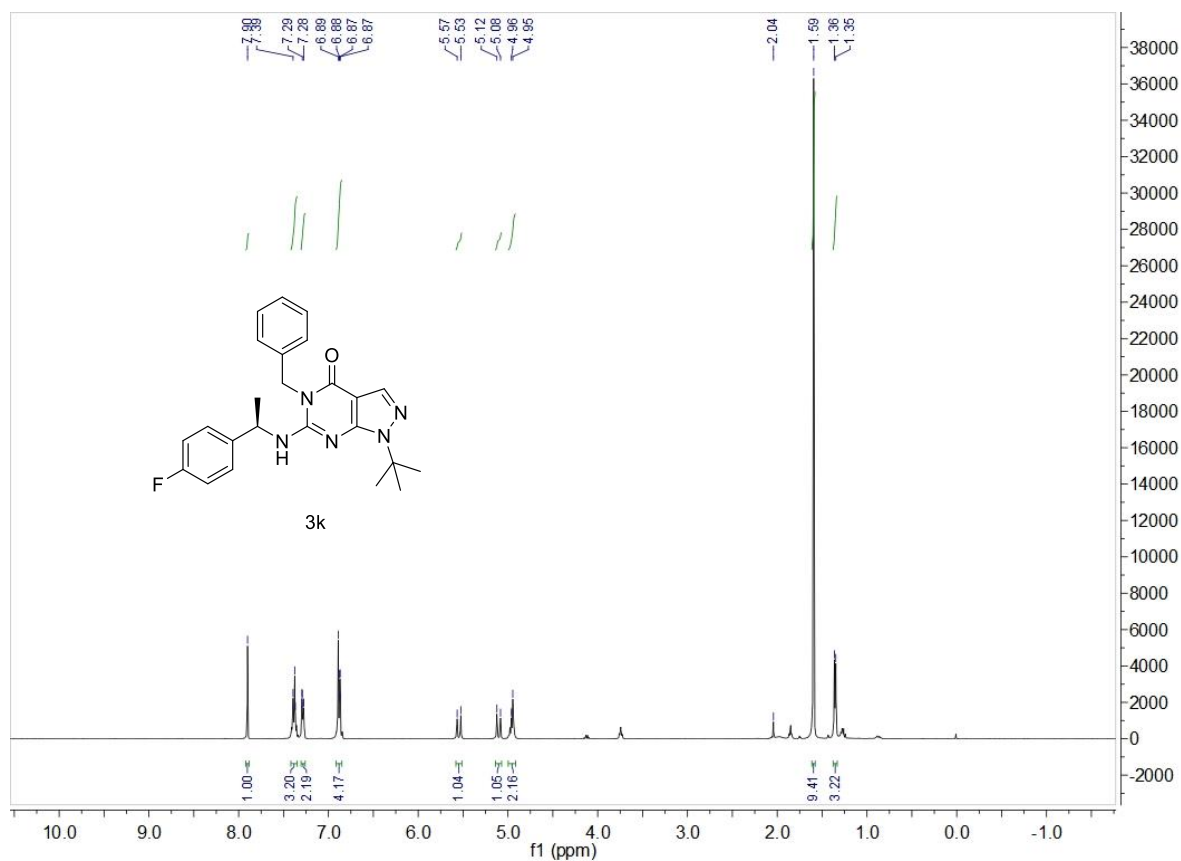




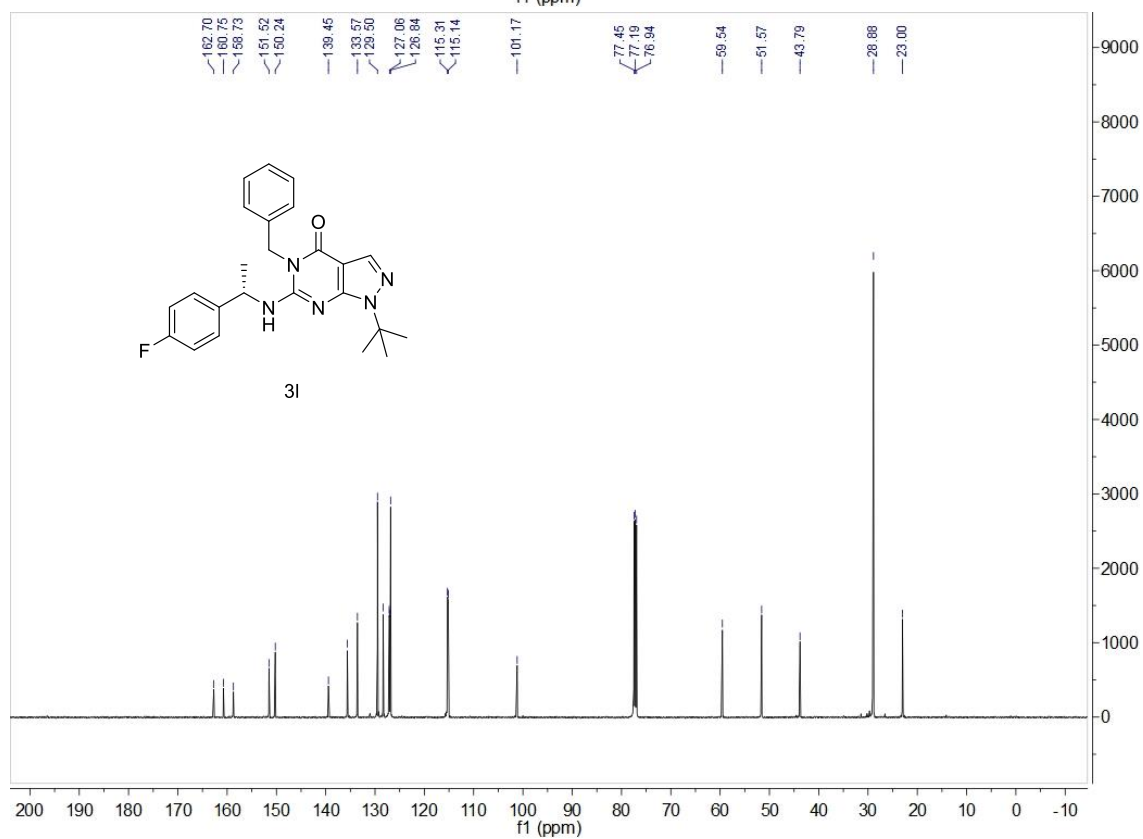
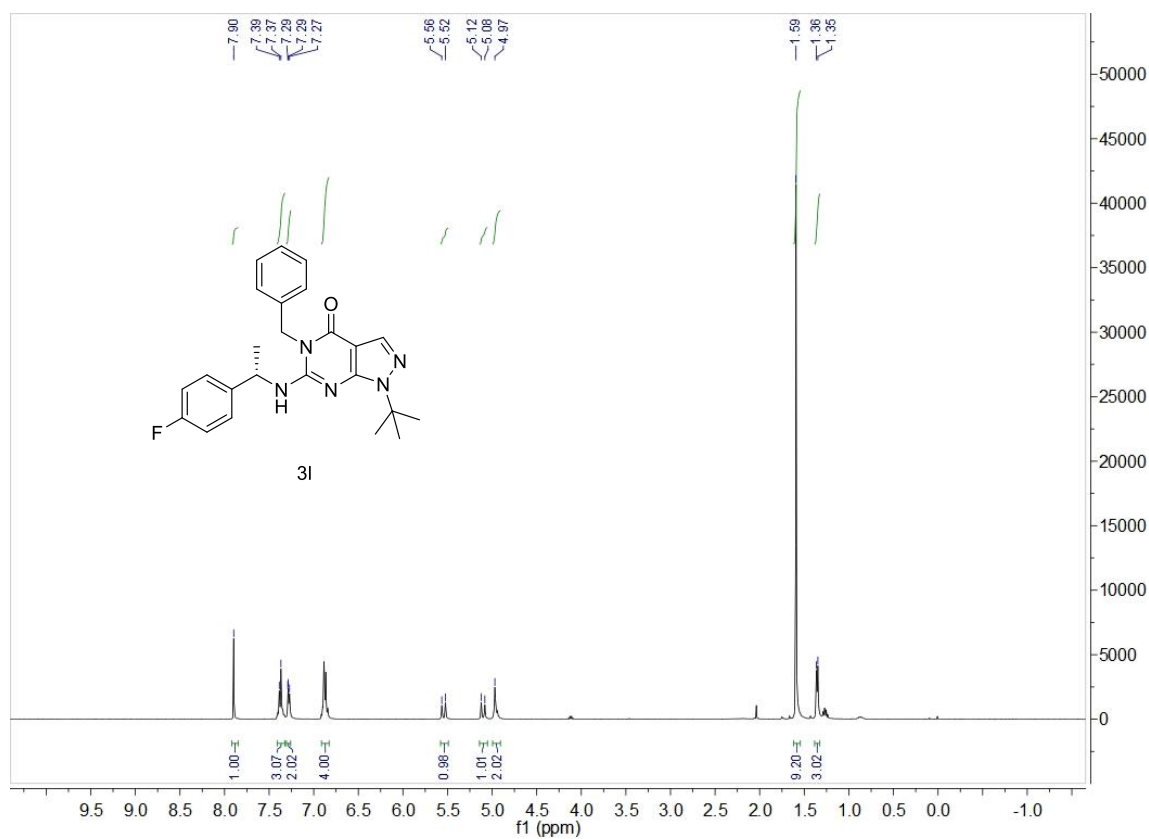


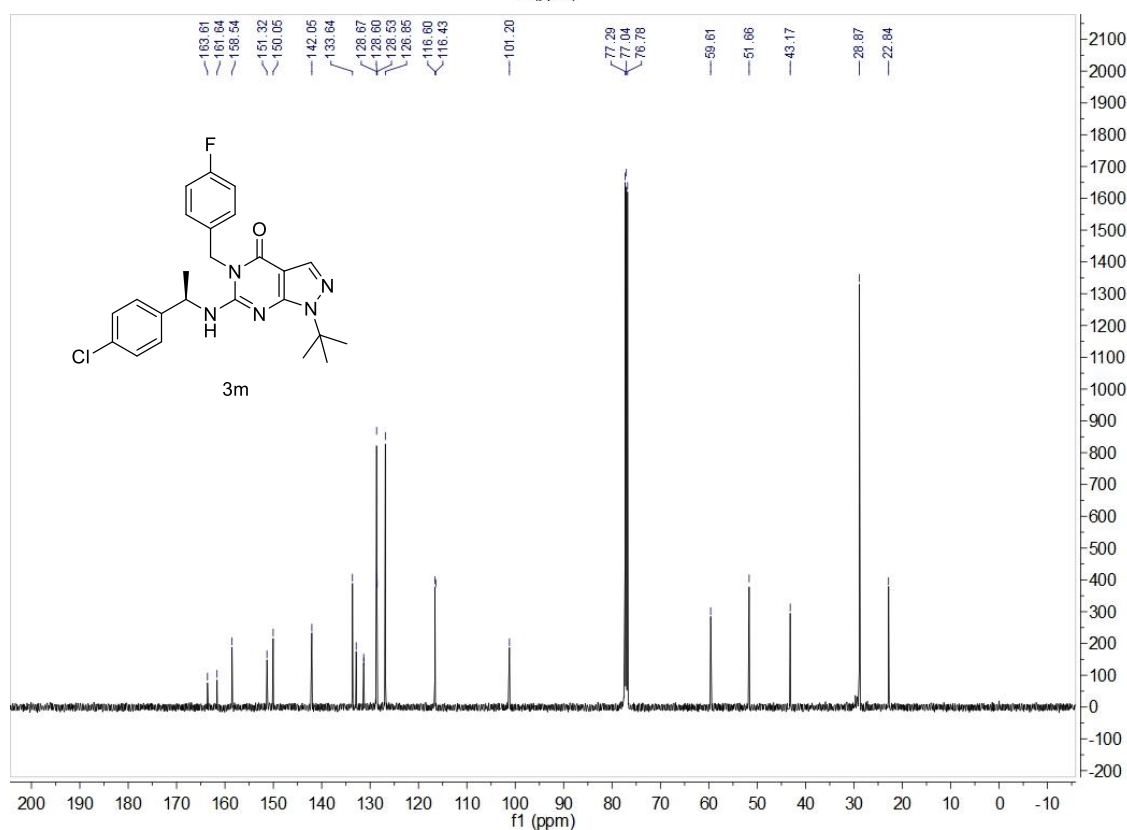
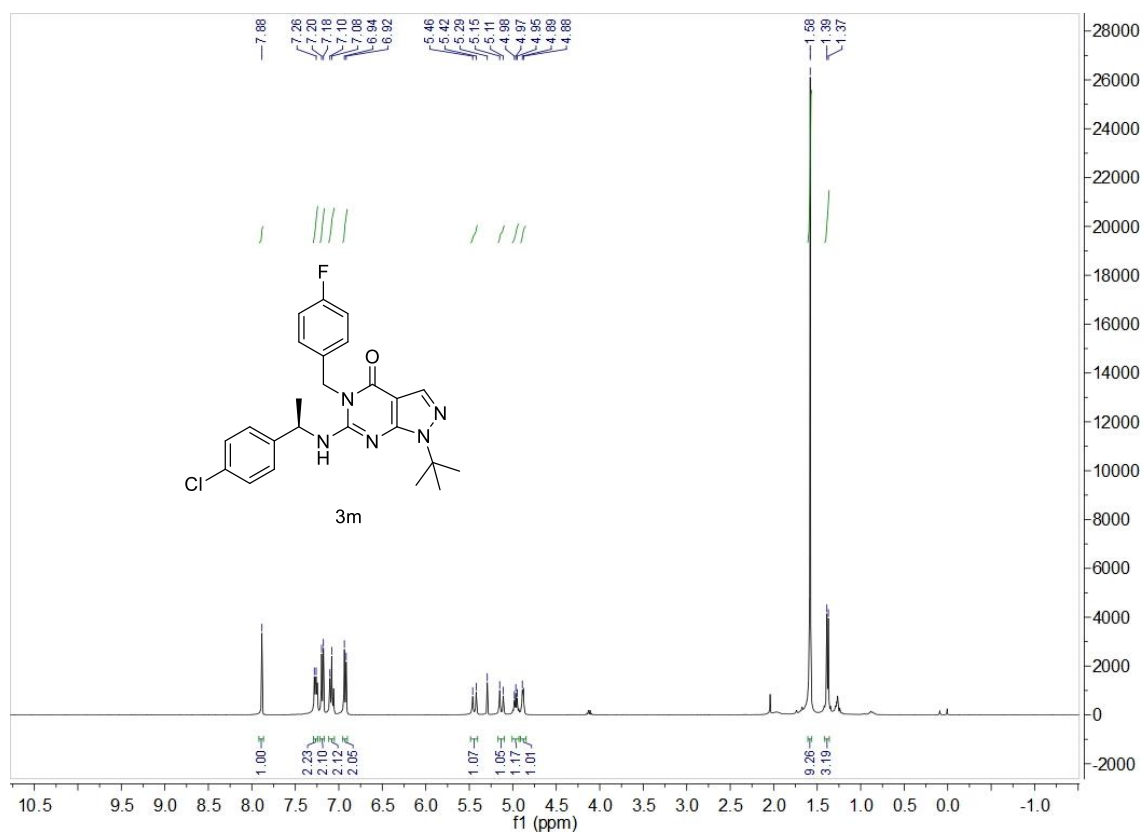


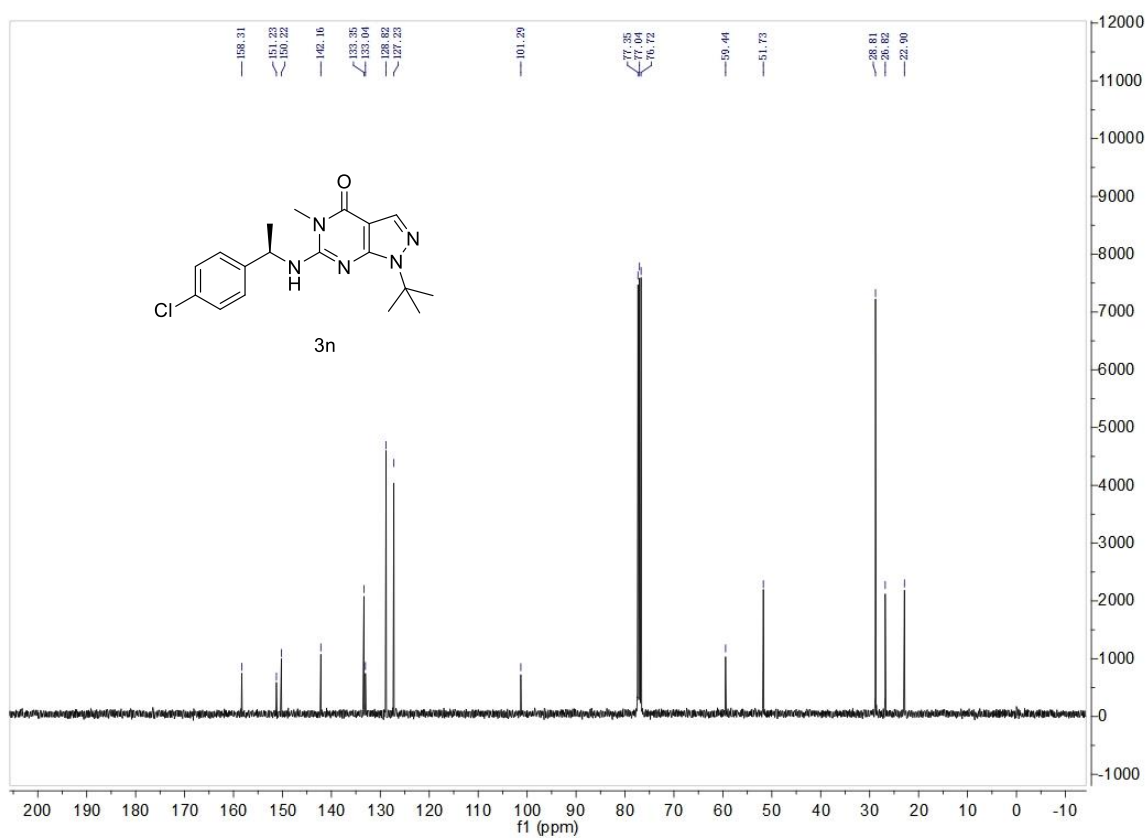
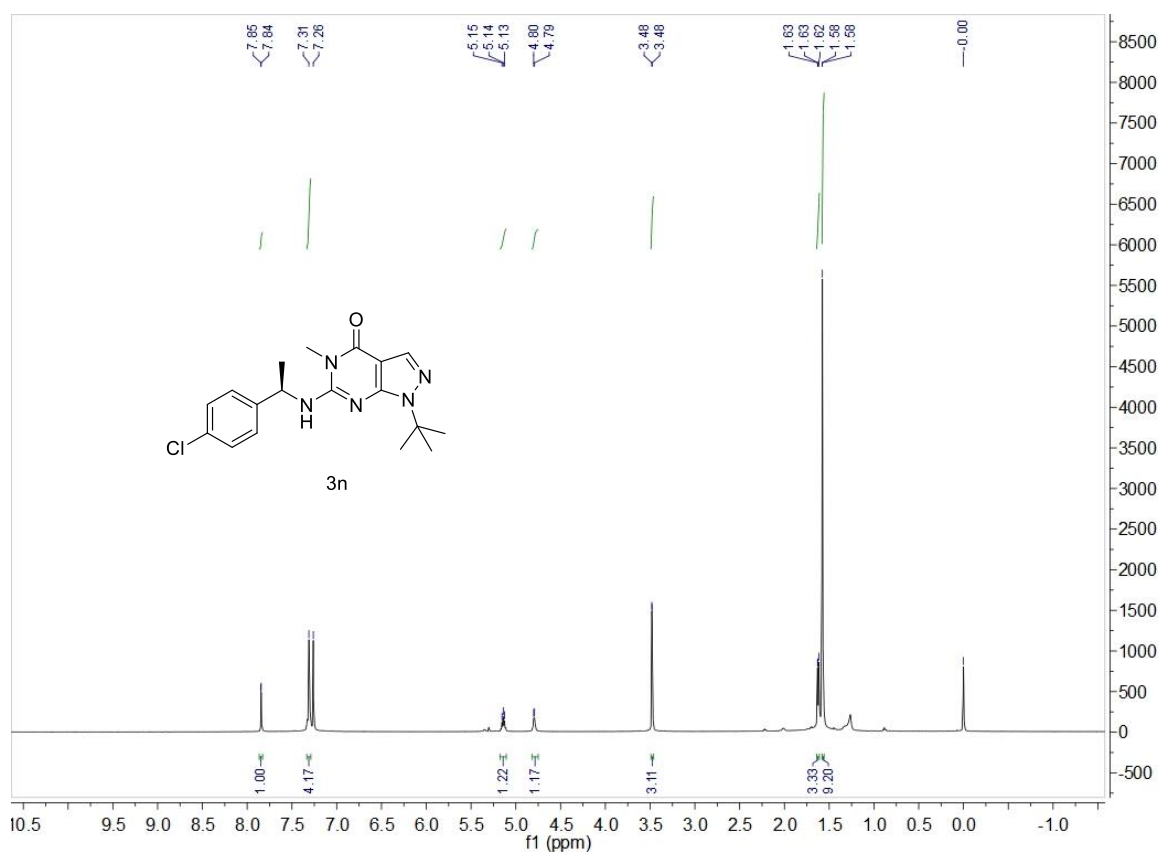


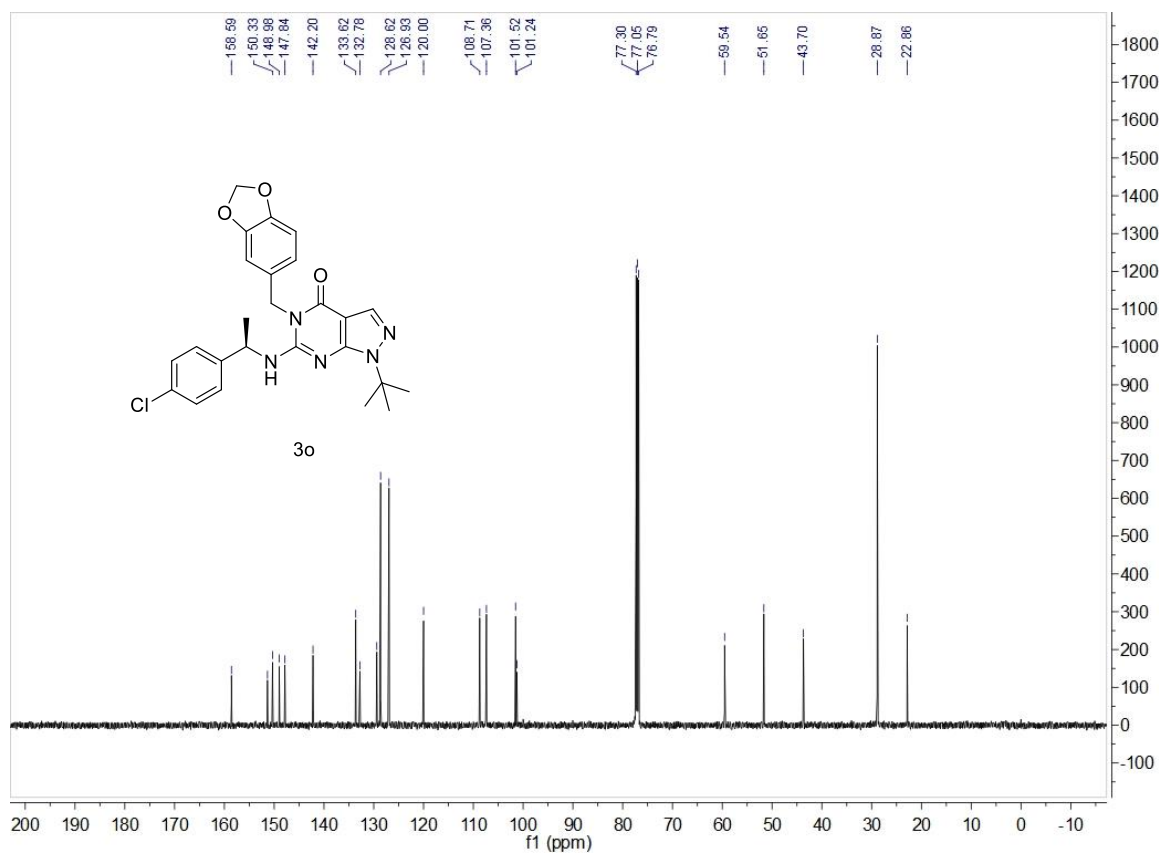
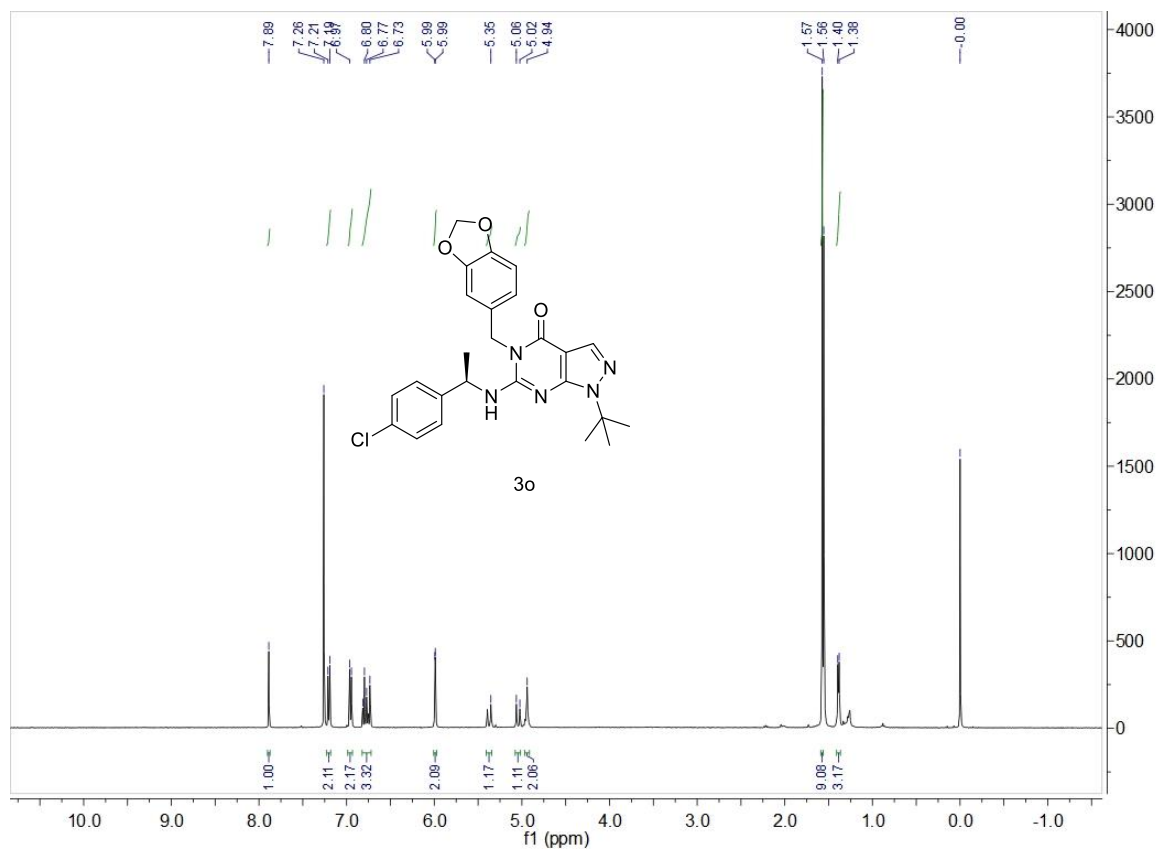


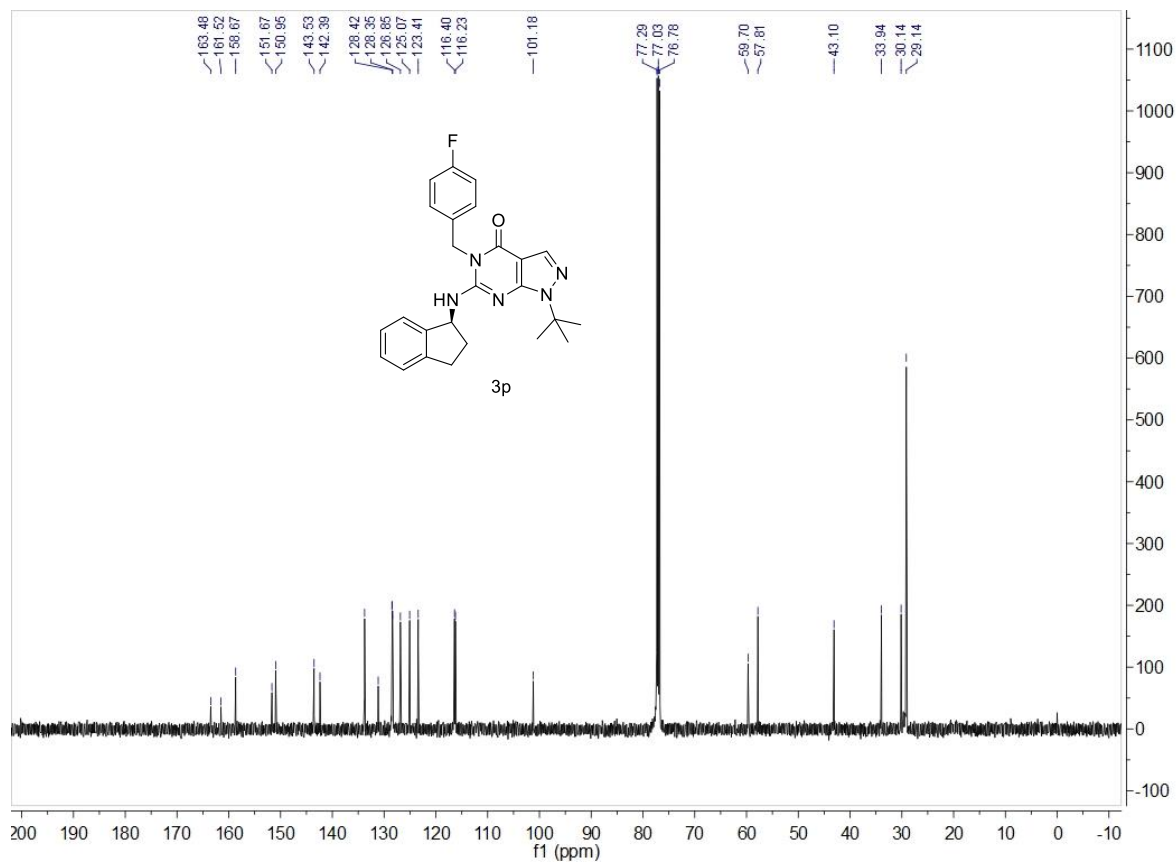
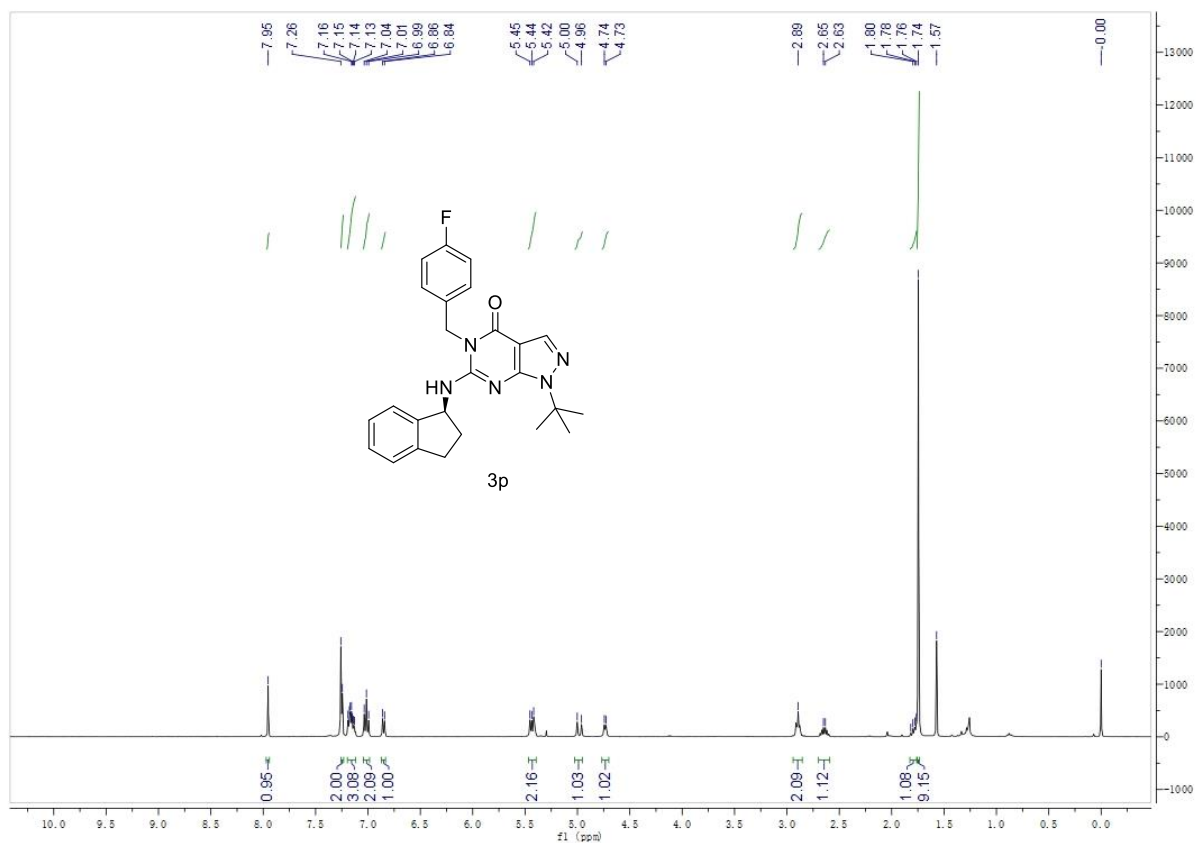


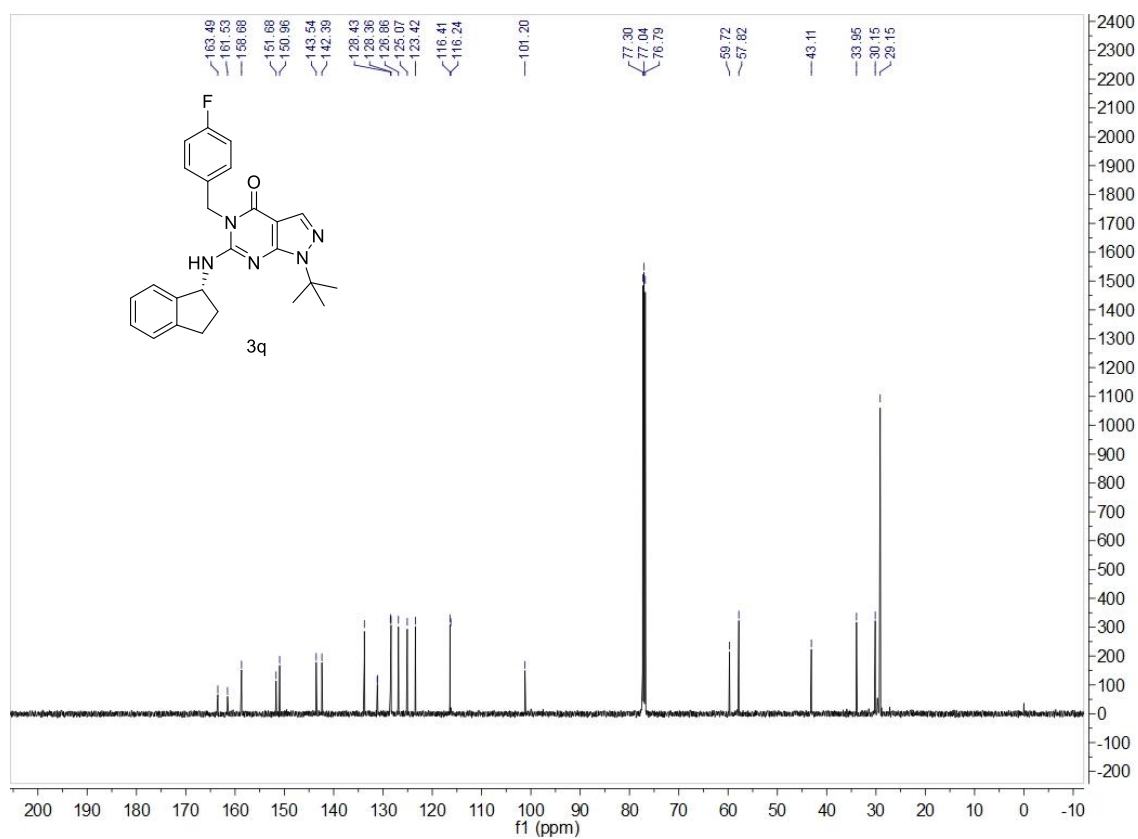
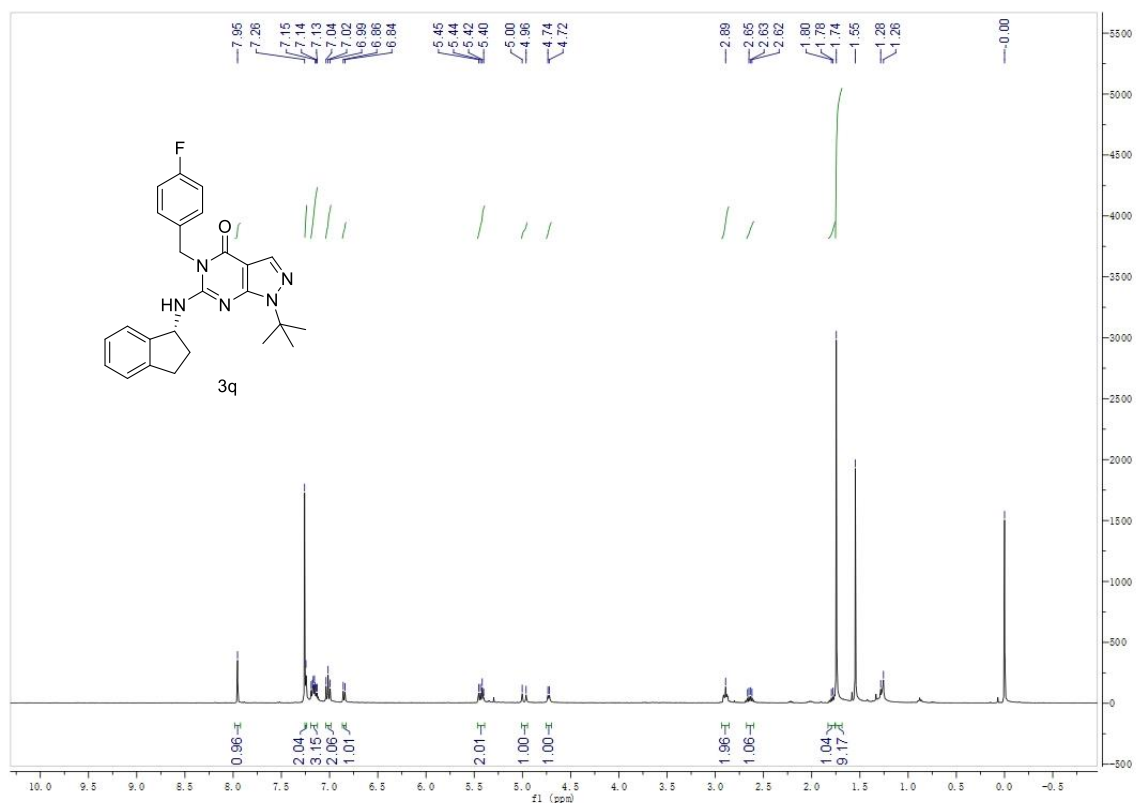




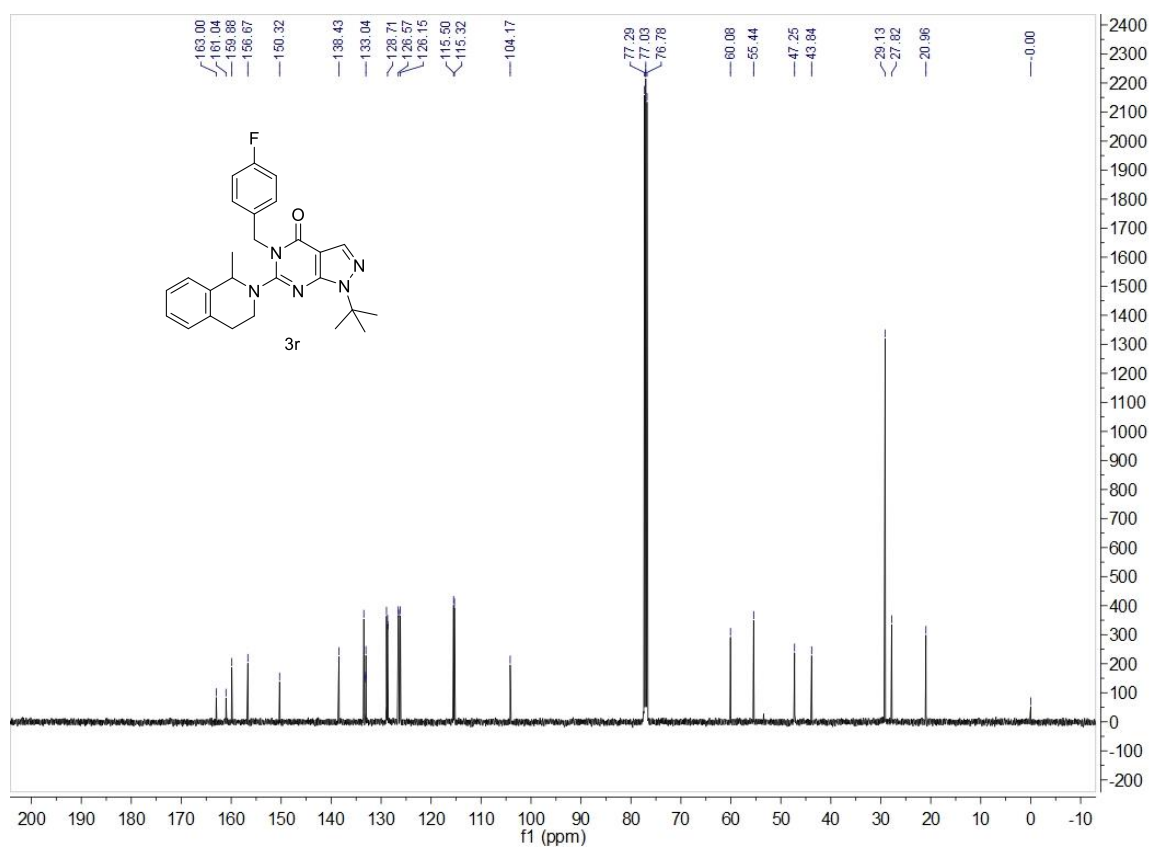
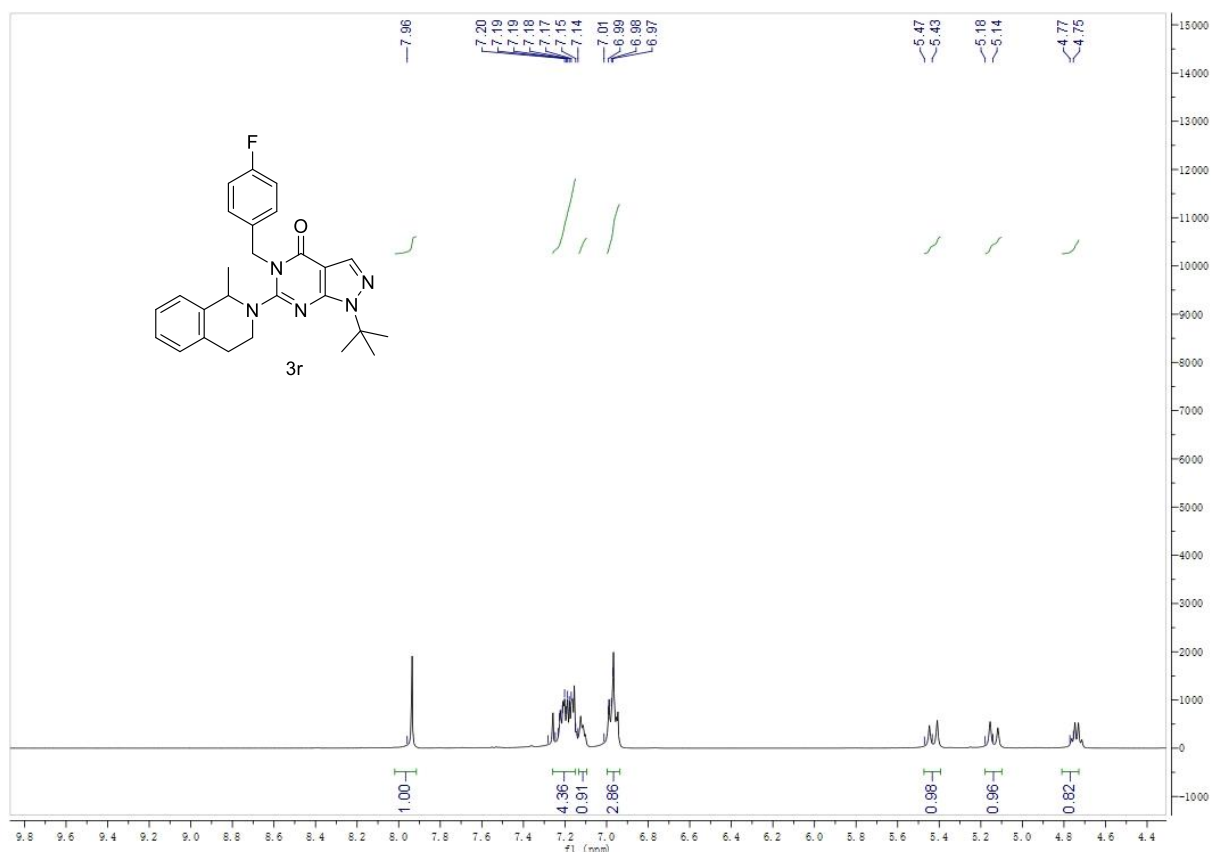












## 9. The HRMS spectrums of the target compounds.

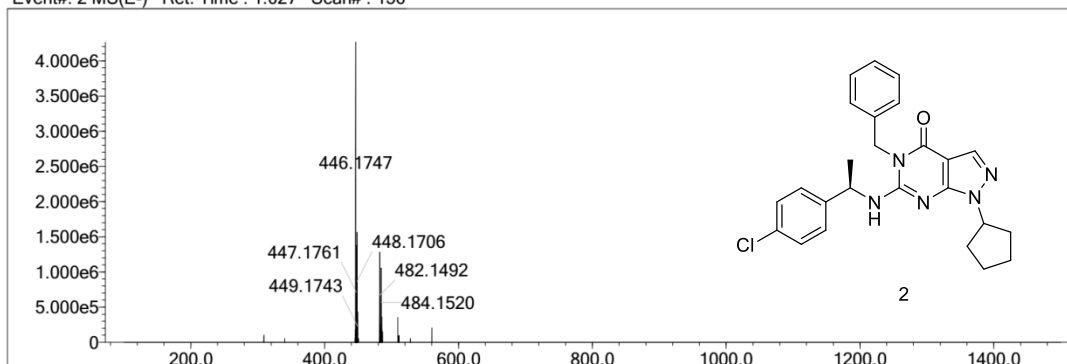
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2H	1	0	0	O	2	0	5	Cl	1	1	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

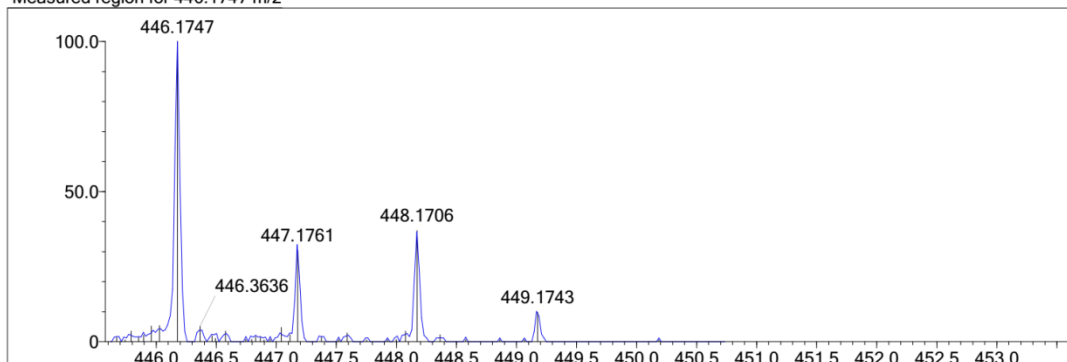
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Electron Ions: both  
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 Isotope Res: 10000  
 Max Results: 500

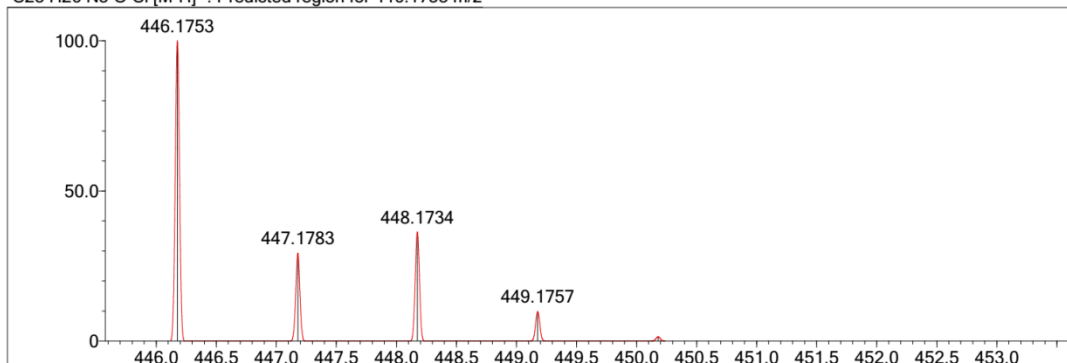
Event#: 2 MS(E-) Ret. Time : 1.027 Scan#: 156



Measured region for 446.1747 m/z



C25 H26 N5 O Cl [M-H]- : Predicted region for 446.1753 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	87.54	C25 H26 N5 O Cl	[M-H]-	446.1747	446.1753	-0.6	-1.34	88.29	15.0

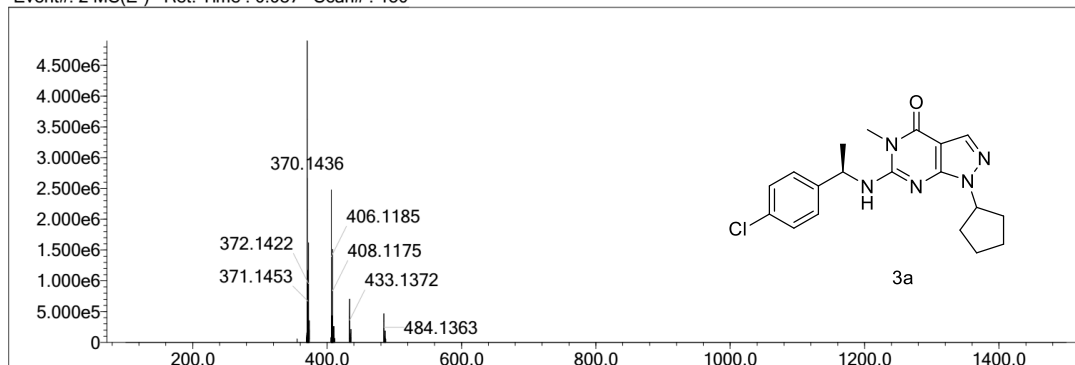
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H	1	0	30	N	3	0	10	S	2	0	0	H
2H	1	0	0	O	2	0	5	Cl	1	1	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

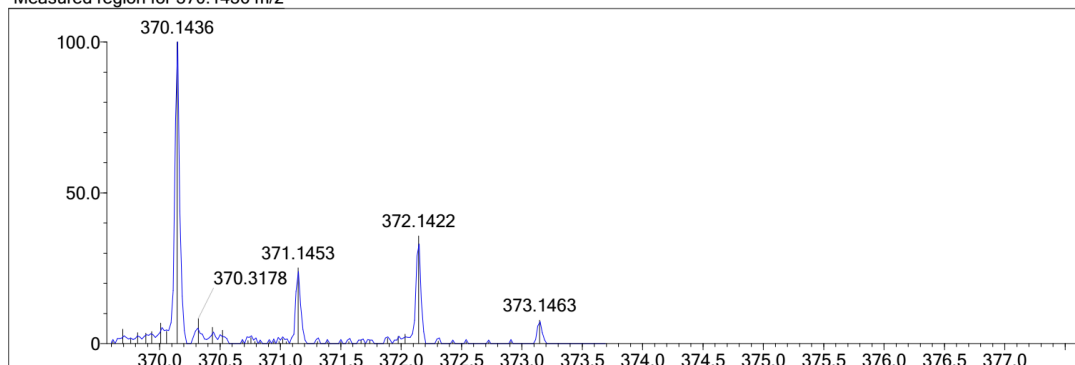
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

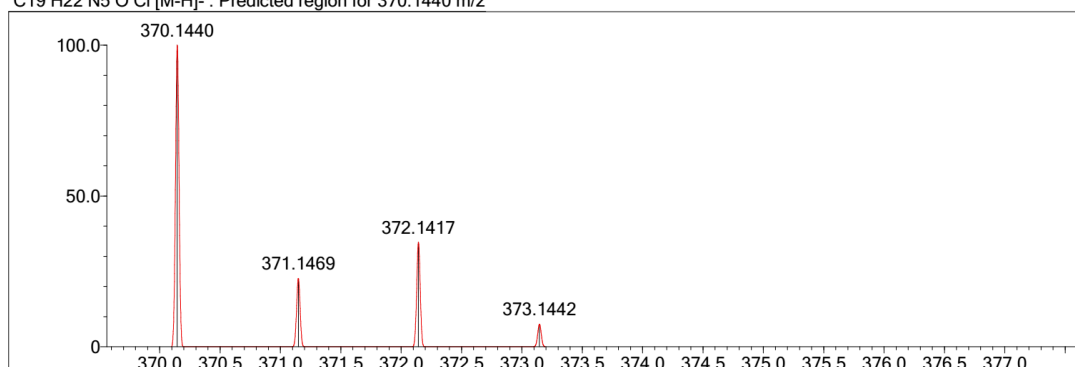
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Measured region for 370.1436 m/z



C19 H22 N5 O Cl [M-H]<sup>-</sup> : Predicted region for 370.1440 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	93.26	C19 H22 N5 O Cl	[M-H] <sup>-</sup>	370.1436	370.1440	-0.4	-1.08	93.45	11.0

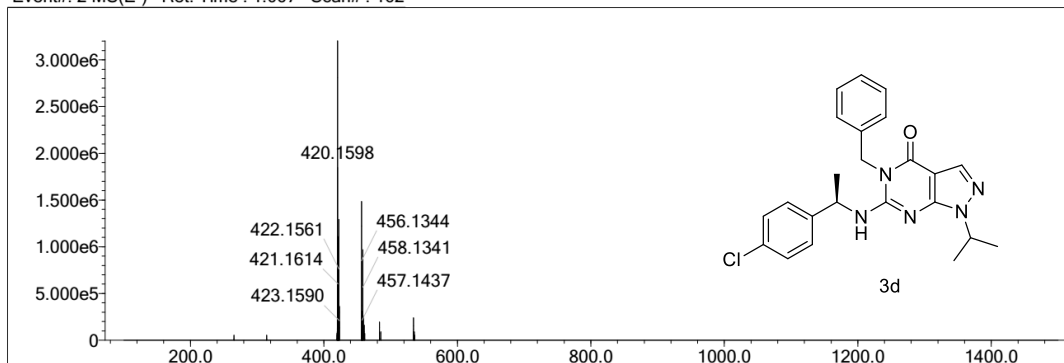
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	N	3	0	10	S	2	0	0	H
2H	1	0	0	O	2	0	5	Cl	1	1	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

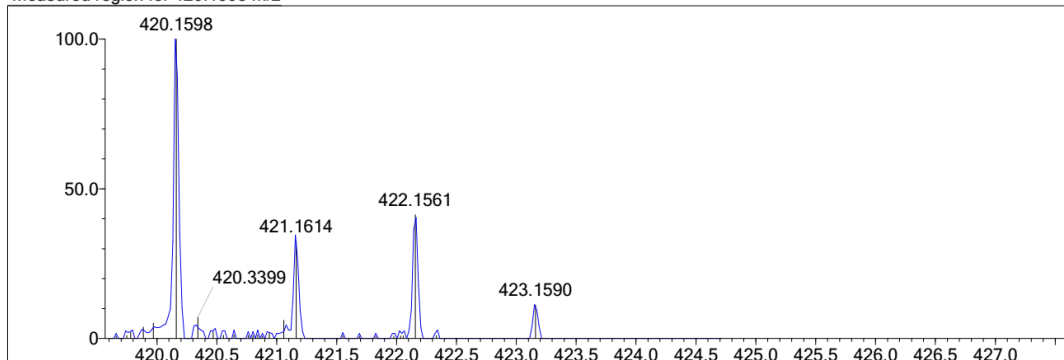
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

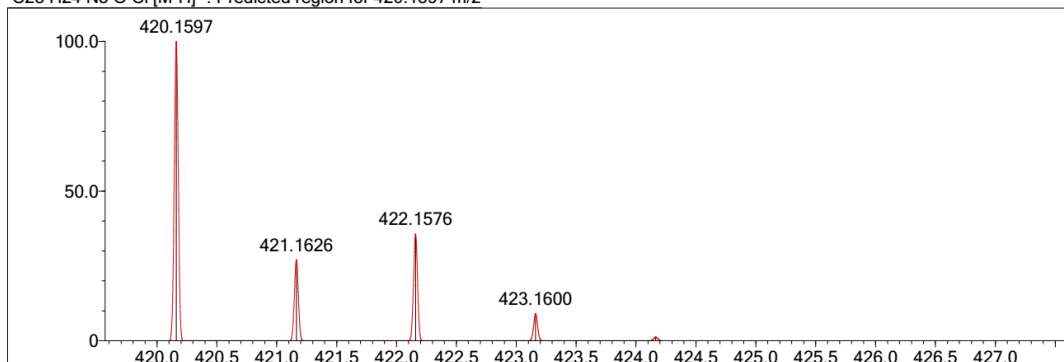
Event#: 2 MS(E-) Ret. Time : 1.067 Scan#: 162



Measured region for 420.1598 m/z



C23 H24 N5 O Cl [M-H]- : Predicted region for 420.1597 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	78.05	C23 H24 N5 O Cl	[M-H]-	420.1598	420.1597	0.1	0.24	78.05	14.0

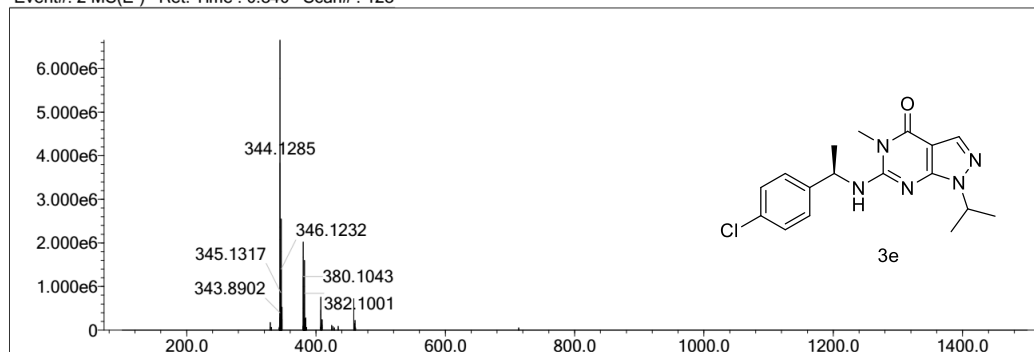
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	N	3	0	10	S	2	0	0	H
2H	1	0	0	O	2	0	5	Cl	1	1	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

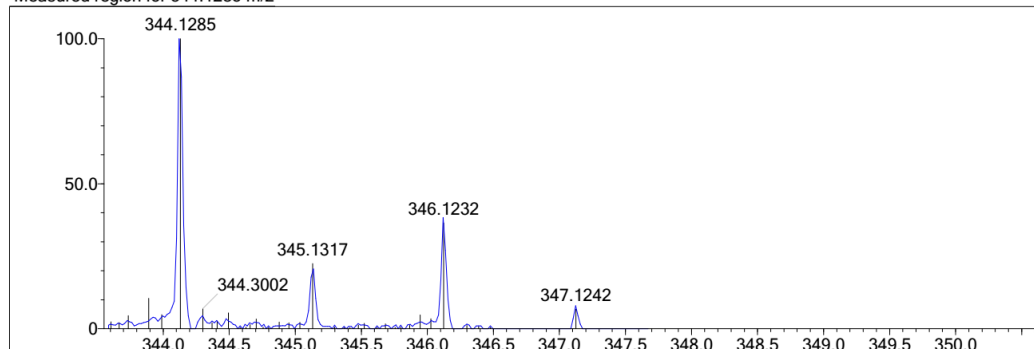
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

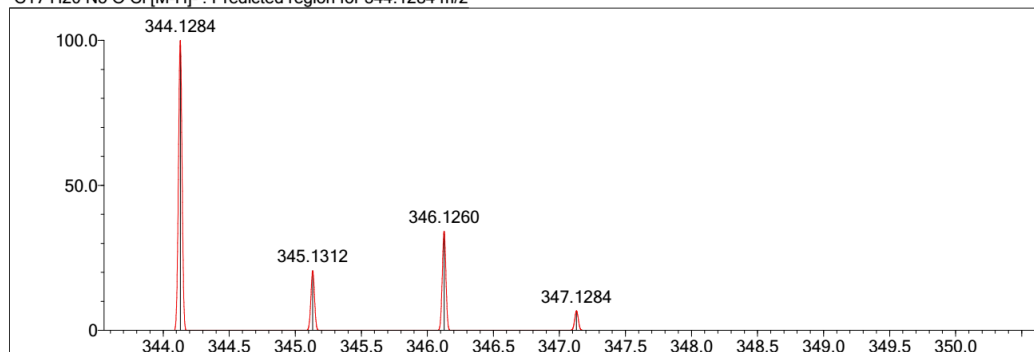
Event#: 2 MS(E-) Ret. Time : 0.840 Scan#: 128



Measured region for 344.1285 m/z



C17 H20 N5 O Cl [M-H]- : Predicted region for 344.1284 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	75.88	C17 H20 N5 O Cl	[M-H]-	344.1285	344.1284	0.1	0.29	75.88	10.0

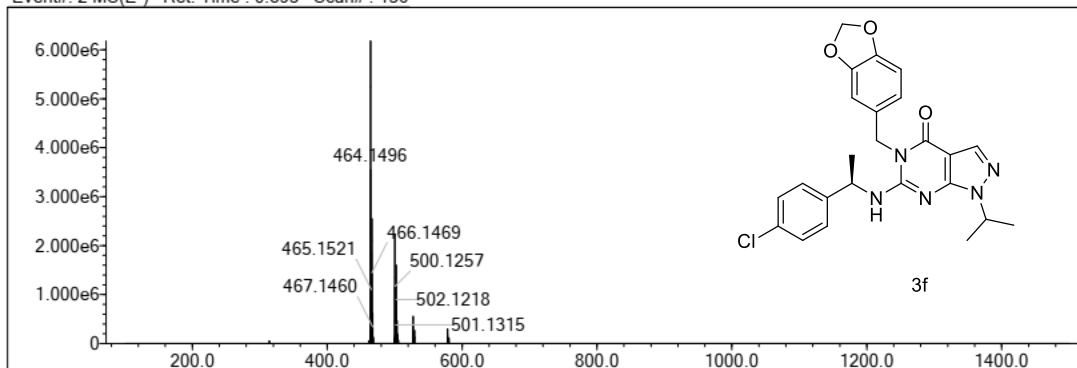
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	N	3	2	10	S	2	0	0	H
2H	1	0	0	O	2	2	4	Cl	1	0	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

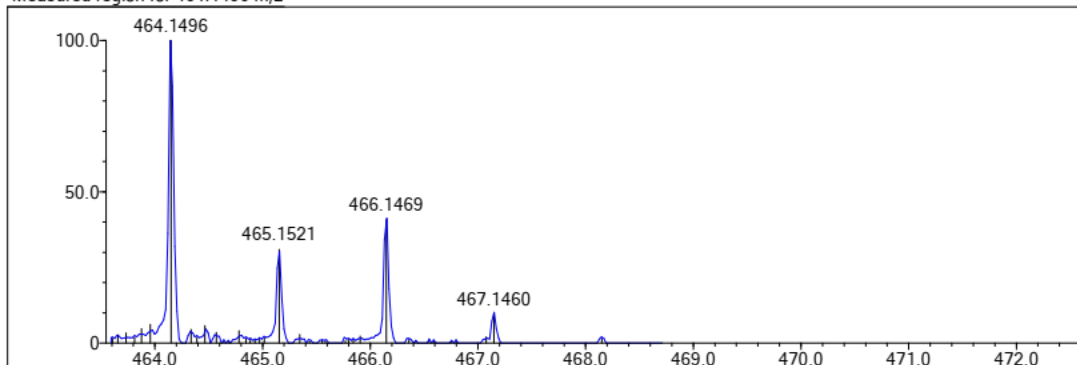
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

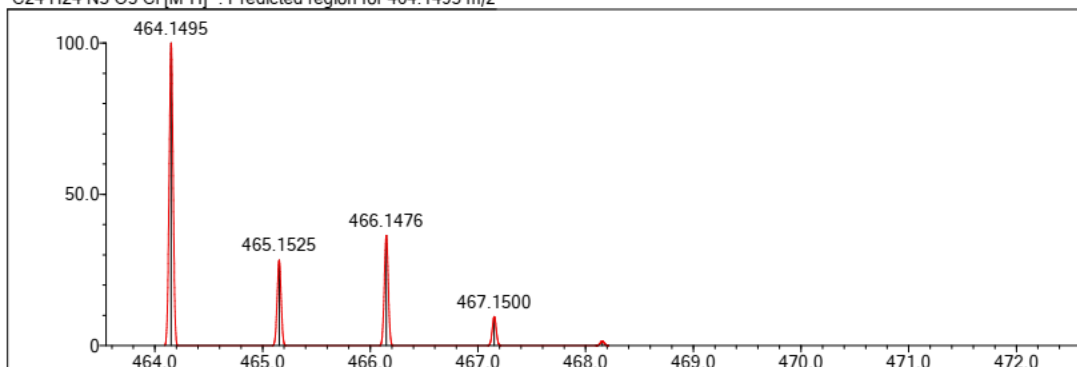
Event#: 2 MS(E-) Ret. Time : 0.893 Scan# : 136



Measured region for 464.1496 m/z



C24 H24 N5 O3 Cl [M-H]- : Predicted region for 464.1495 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	81.28	C24 H24 N5 O3 Cl	[M-H]-	464.1496	464.1495	0.1	0.22	81.28	15.0



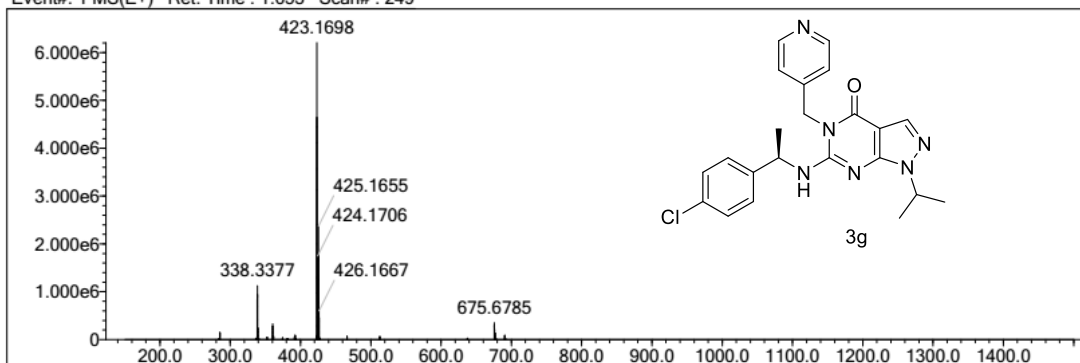
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	26	O	2	0	1	Se	2	0	0	H
2H	1	0	0	F	1	0	0	Br	1	0	0	
B	3	0	0	P	3	0	0	Pt	2	0	0	
C	4	0	25	S	2	0	0					
N	3	0	6	Cl	1	0	1					

Error Margin (ppm): 20  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

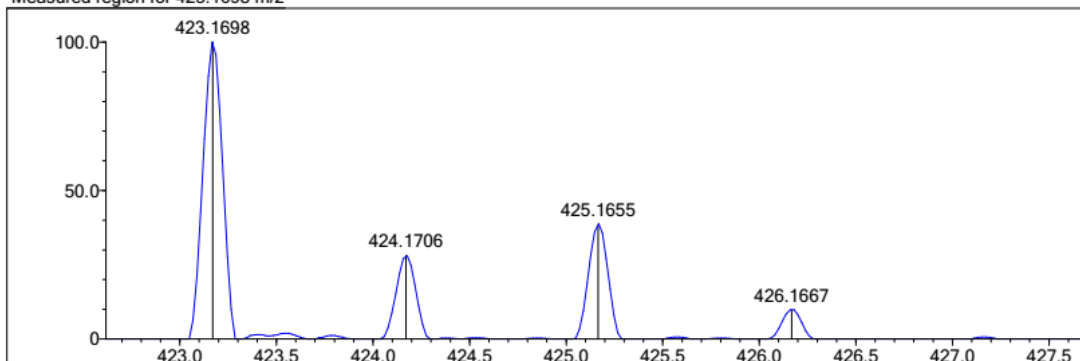
DBE Range: not fixed  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 200

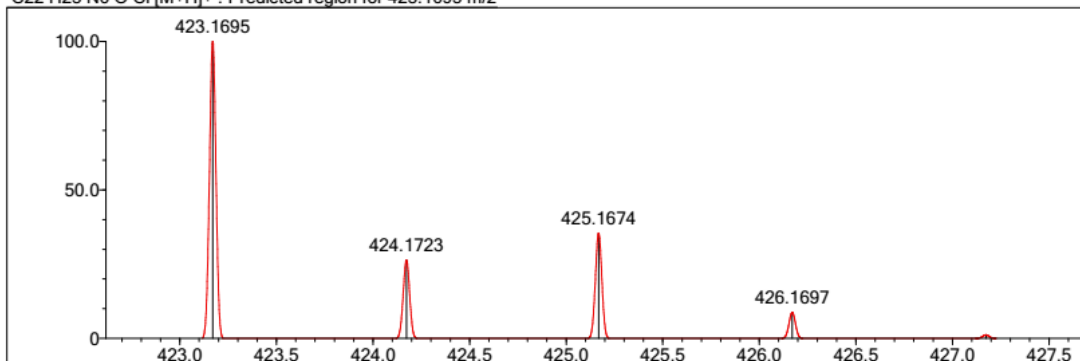
Event#: 1 MS(E+) Ret. Time : 1.653 Scan#: 249



Measured region for 423.1698 m/z



C22 H23 N6 O Cl [M+H]<sup>+</sup> : Predicted region for 423.1695 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	81.59	C22 H23 N6 O Cl	[M+H] <sup>+</sup>	423.1698	423.1695	0.3	0.71	81.59	14.0

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	25	O	2	0	1	Se	2	0	0	H
2H	1	0	0	F	1	0	0	Br	1	0	0	Na
B	3	0	0	P	3	0	0	Pt	2	0	0	
C	4	0	25	S	2	0	1					
N	3	0	6	Cl	1	0	1					

Error Margin (ppm): 20

HC Ratio: unlimited

Max Isotopes: all

MSn Iso RI (%): 75.00

DBE Range: not fixed

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

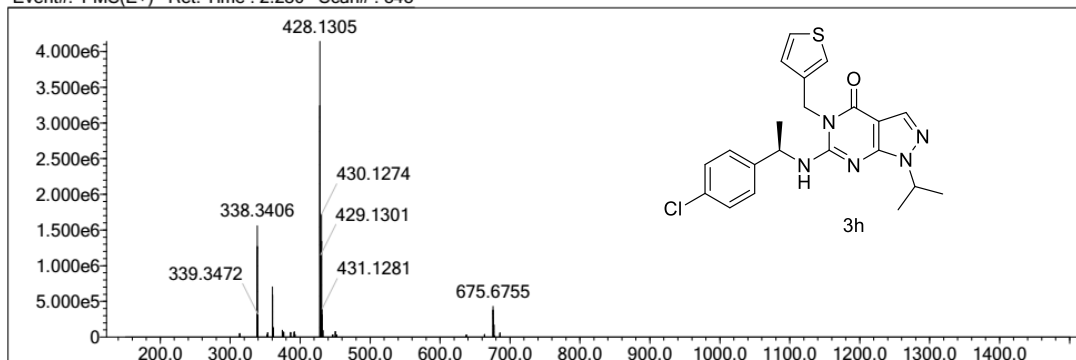
Electron Ions: both

Use MSn Info: no

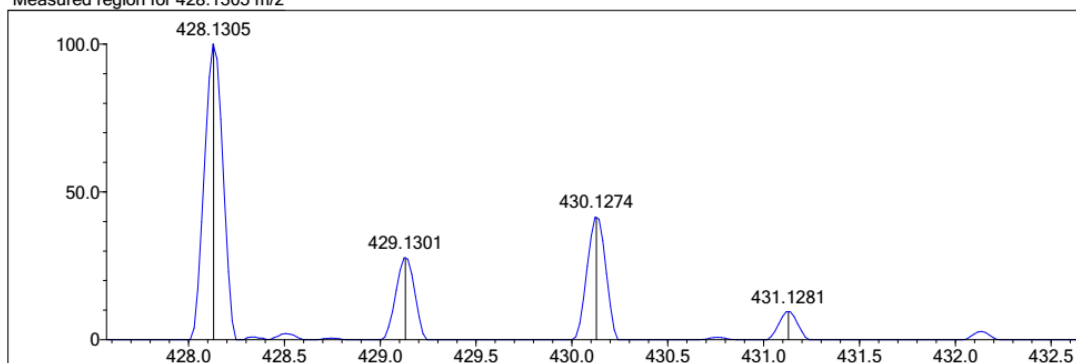
Isotope Res: 10000

Max Results: 200

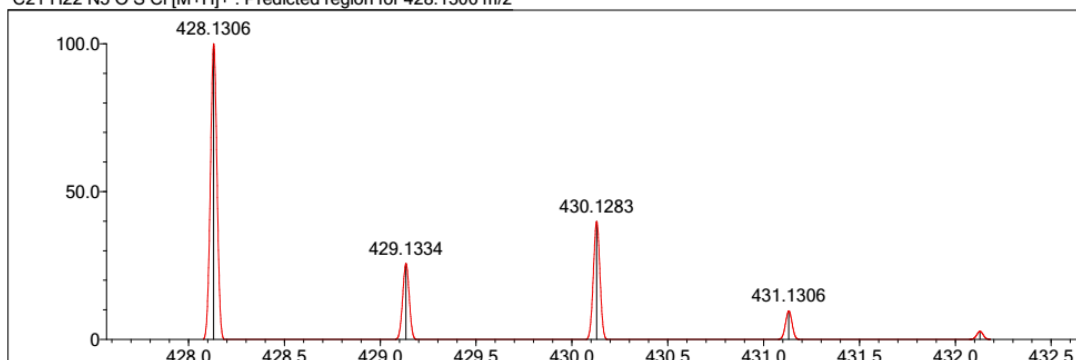
Event#: 1 MS(E+) Ret. Time : 2.280 Scan#: 343



Measured region for 428.1305 m/z



C21 H22 N5 O S Cl [M+H]<sup>+</sup> : Predicted region for 428.1306 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.69	C21 H22 N5 O S Cl	[M+H] <sup>+</sup>	428.1305	428.1306	-0.1	-0.23	90.69	13.0

Data File: \\Deep-20160624\\data1\\WuYiNuo\\LML\\LWLM1959.lcd

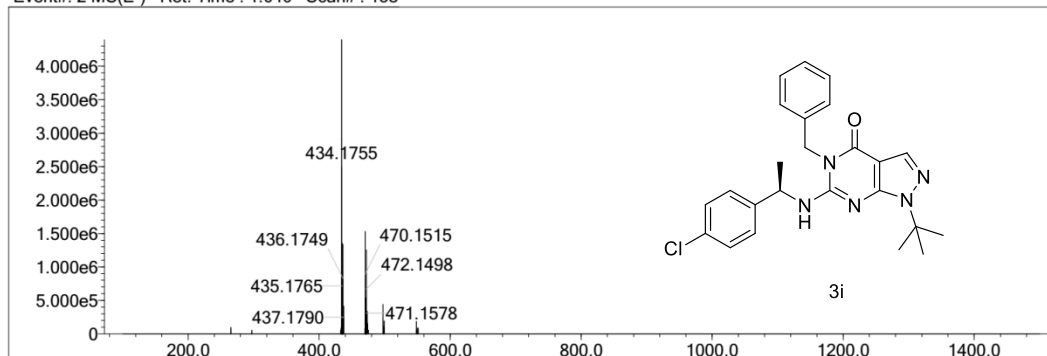
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	N	3	0	10	S	2	0	0	H
2H	1	0	0	O	2	0	5	Cl	1	1	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

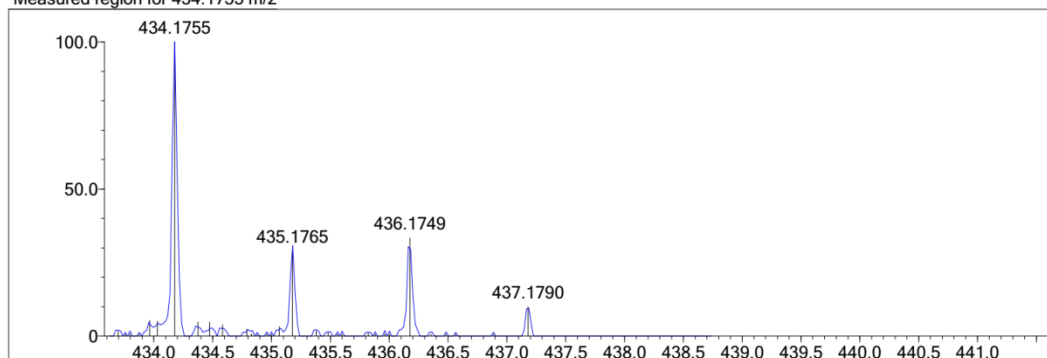
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

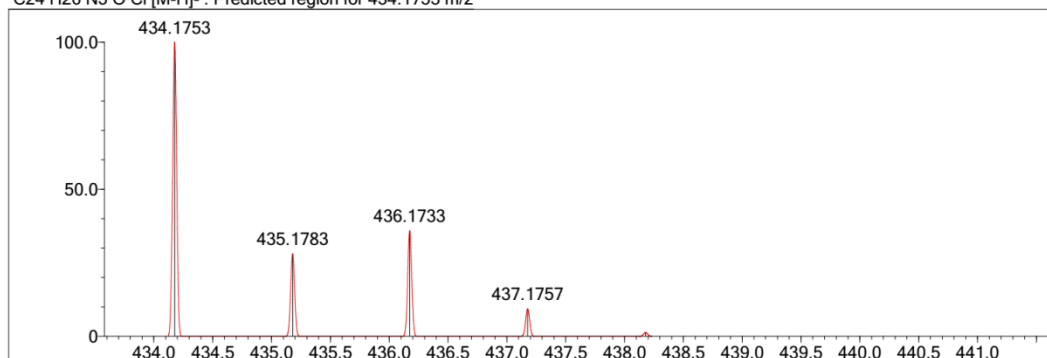
Event#: 2 MS(E-) Ret. Time : 1.040 Scan#: 158



Measured region for 434.1755 m/z



C24 H26 N5 O Cl [M-H]- : Predicted region for 434.1753 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	83.07	C24 H26 N5 O Cl	[M-H]-	434.1755	434.1753	0.2	0.46	83.07	14.0

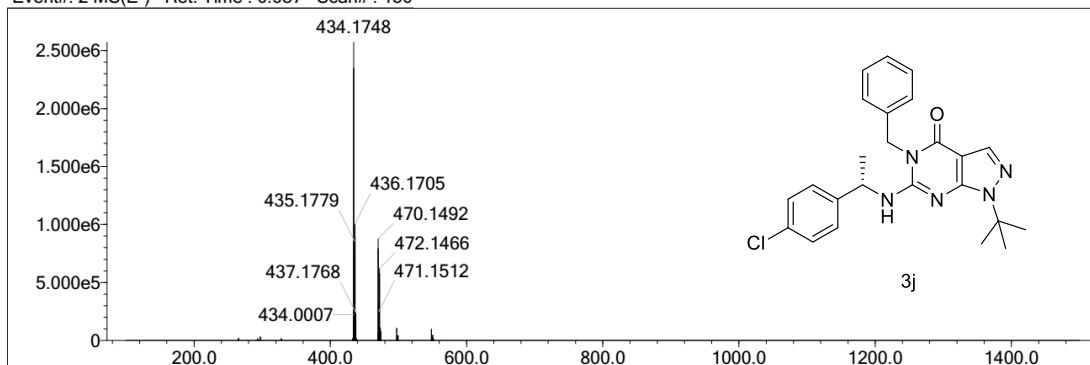
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	51	O	2	0	5	Br	1	0	0	H
2H	1	0	0	F	1	0	5	Pd	2	0	0	
B	3	0	0	P	3	0	0	I	3	0	0	
C	4	0	40	S	2	0	0					
N	3	1	10	Cl	1	0	5					

Error Margin (ppm): 20  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

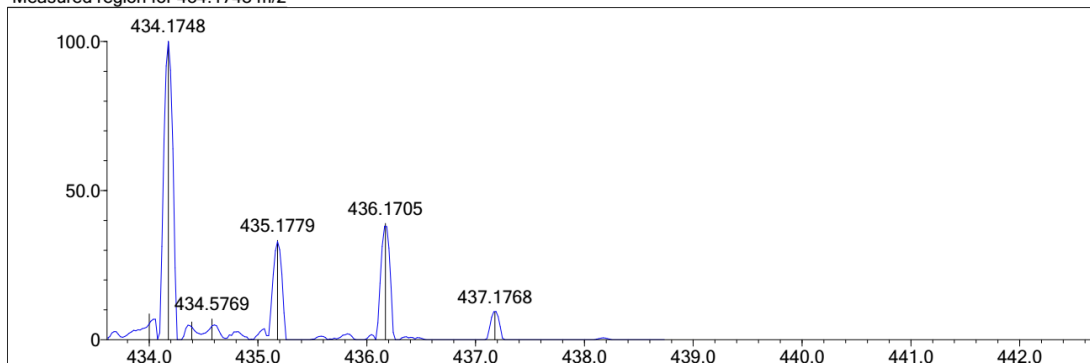
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

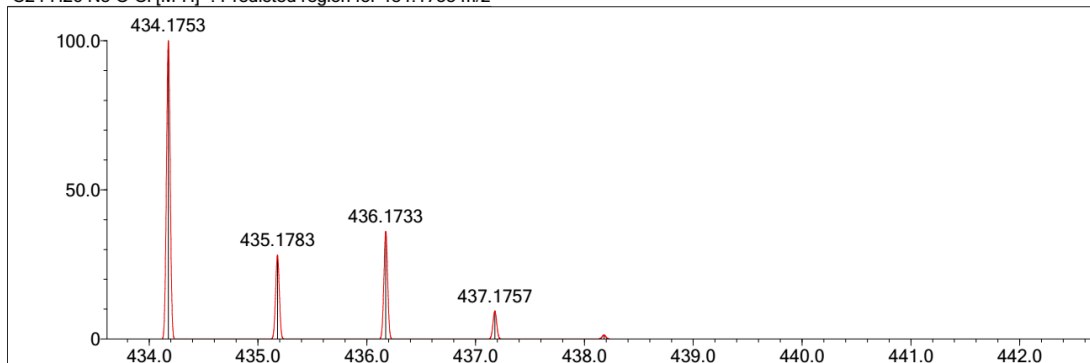
Event#: 2 MS(E-) Ret. Time : 0.987 Scan#: 150



Measured region for 434.1748 m/z



C24 H26 N5 O Cl [M-H]<sup>-</sup> : Predicted region for 434.1753 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	87.59	C24 H26 N5 O Cl	[M-H] <sup>-</sup>	434.1748	434.1753	-0.5	-1.15	87.92	14.0

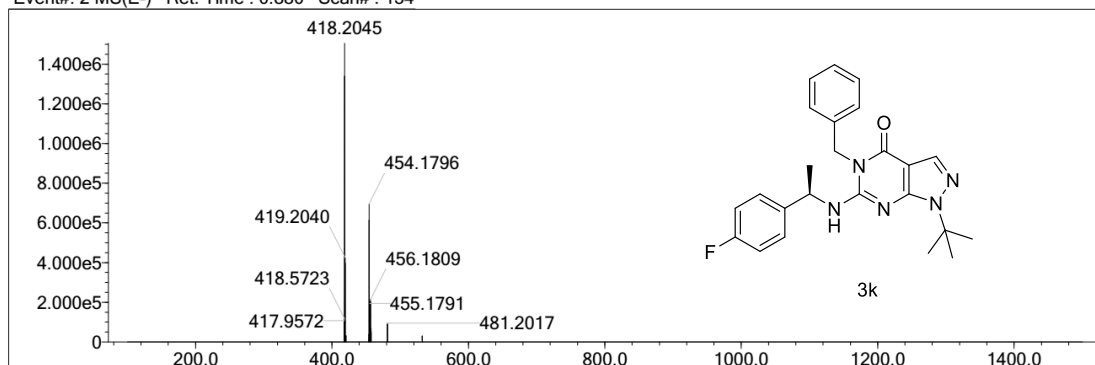
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	51	O	2	0	5	Br	1	0	0	H
2H	1	0	0	F	1	0	5	Pd	2	0	0	
B	3	0	0	P	3	0	0	I	3	0	0	
C	4	0	40	S	2	0	0					
N	3	1	10	Cl	1	0	5					

Error Margin (ppm): 20  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

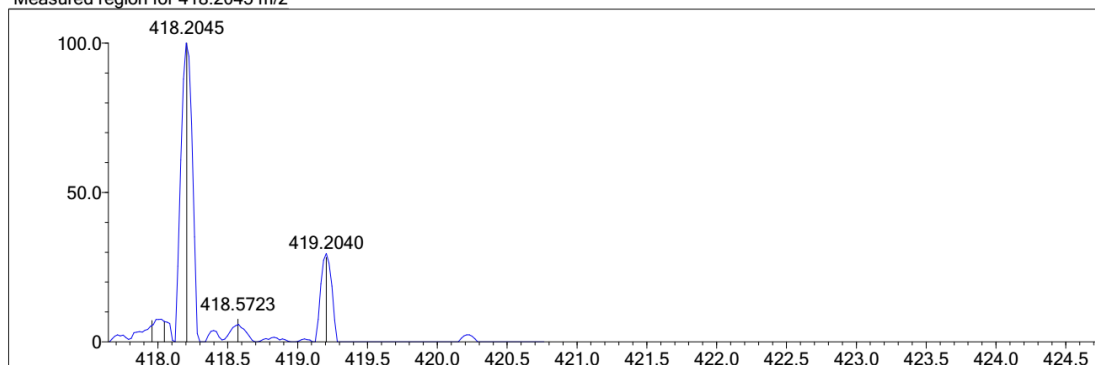
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

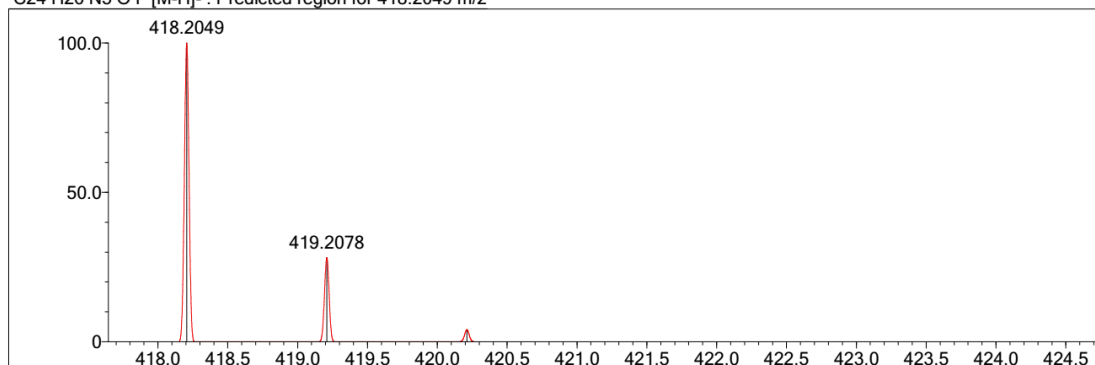
Event#: 2 MS(E-) Ret. Time : 0.880 Scan#: 134



Measured region for 418.2045 m/z



C24 H26 N5 O F [M-H]- : Predicted region for 418.2049 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	85.31	C24 H26 N5 O F	[M-H]-	418.2045	418.2049	-0.4	-0.96	85.31	14.0

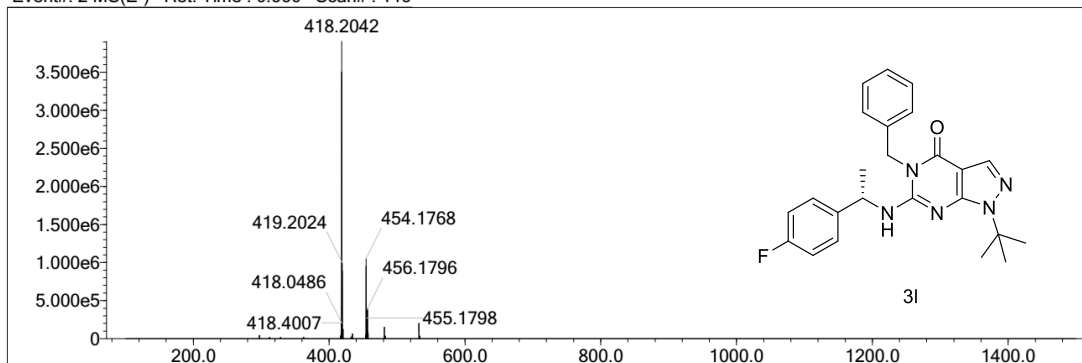
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	51	O	2	0	5	Br	1	0	0	H
2H	1	0	0	F	1	0	5	Pd	2	0	0	
B	3	0	0	P	3	0	0	I	3	0	0	
C	4	0	40	S	2	0	0					
N	3	1	10	Cl	1	0	0					

Error Margin (ppm): 20  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

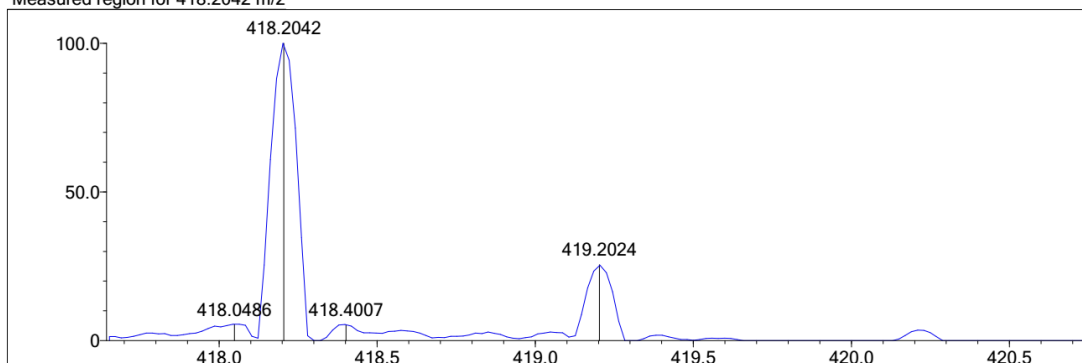
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

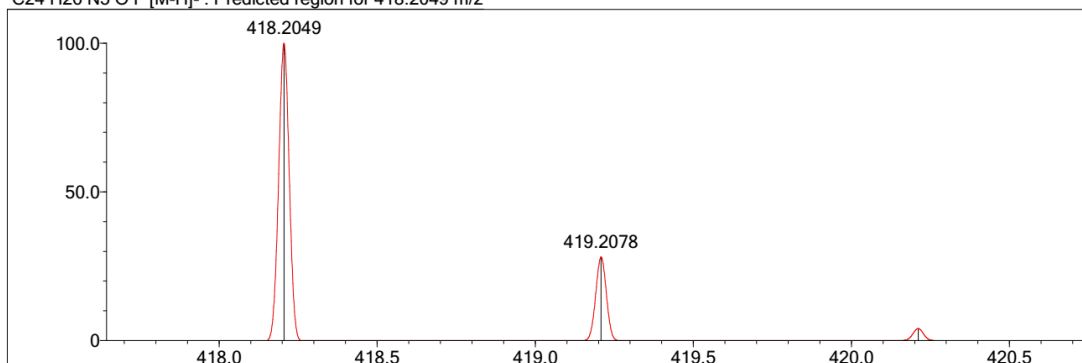
Event#: 2 MS(E-) Ret. Time : 0.960 Scan#: 146



Measured region for 418.2042 m/z



C24 H26 N5 O F [M-H]-: Predicted region for 418.2049 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	87.64	C24 H26 N5 O F	[M-H]-	418.2042	418.2049	-0.7	-1.67	89.13	14.0

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	51	O	2	0	5	Br	1	0	0	H
2H	1	0	0	F	1	0	5	Pd	2	0	0	
B	3	0	0	P	3	0	0	I	3	0	0	
C	4	0	40	S	2	0	0					
N	3	1	10	Cl	1	0	5					

Error Margin (ppm): 20

HC Ratio: unlimited

Max Isotopes: all

MSn Iso RI (%): 75.00

DBE Range: -2.0 - 200.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

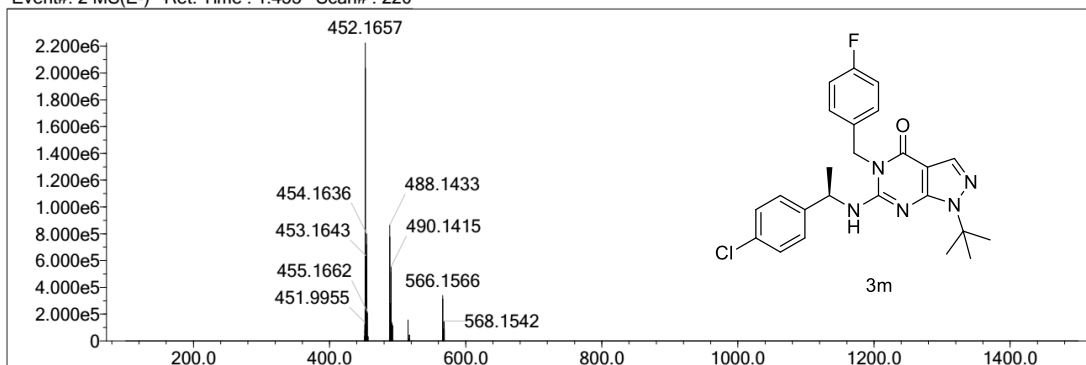
Electron Ions: both

Use MSn Info: no

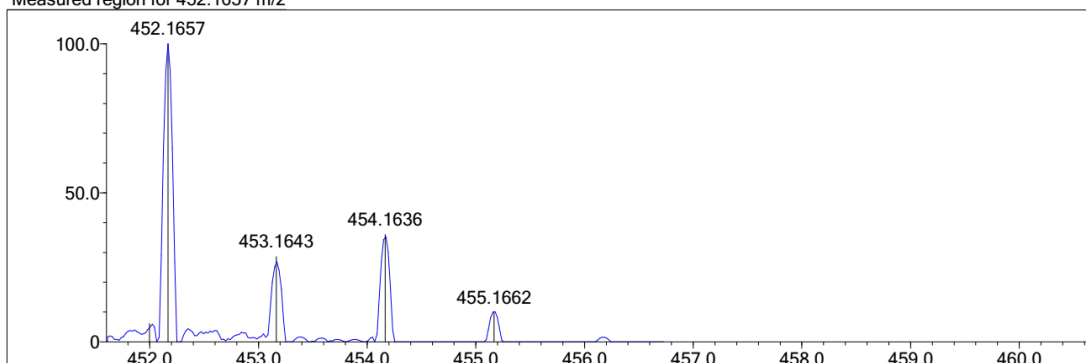
Isotope Res: 10000

Max Results: 500

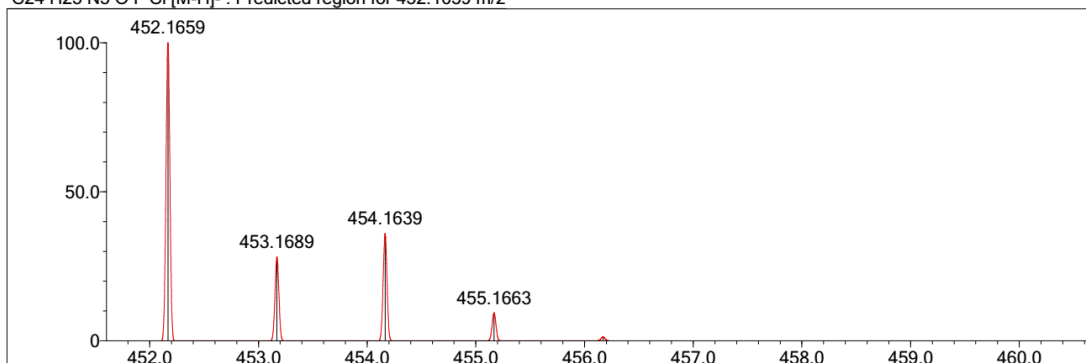
Event#: 2 MS(E-) Ret. Time : 1.453 Scan#: 220



Measured region for 452.1657 m/z



C24 H25 N5 O F Cl [M-H]- : Predicted region for 452.1659 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	99.96	C24 H25 N5 O F Cl	[M-H]-	452.1657	452.1659	-0.2	-0.44	99.96	14.0

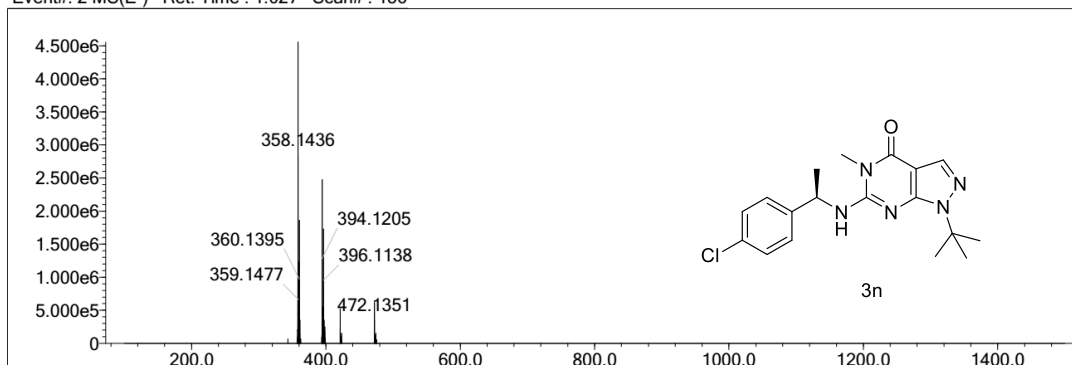
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	N	3	0	10	S	2	0	0	H
2H	1	0	0	O	2	0	5	Cl	1	1	5	HCOO
B	3	0	0	F	1	0	0	Br	1	0	0	CH3COO
C	4	0	30	P	3	0	0	Pd	2	0	0	Cl

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

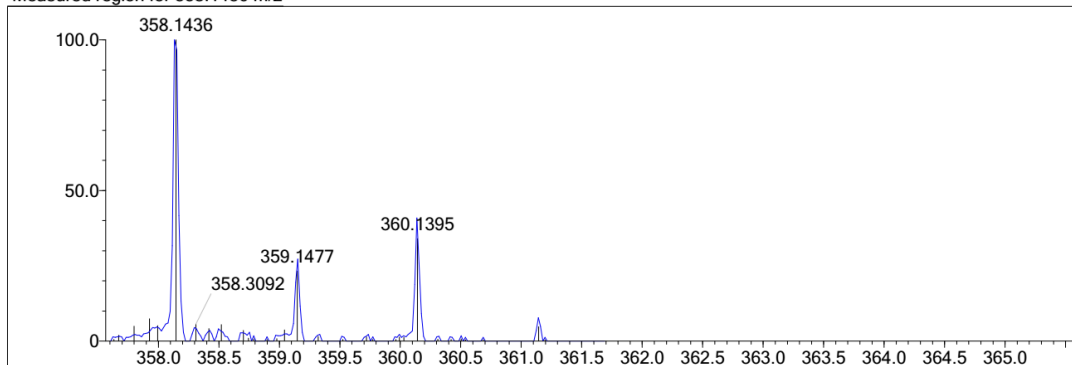
DBE Range: -2.0 - 200.0  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 500

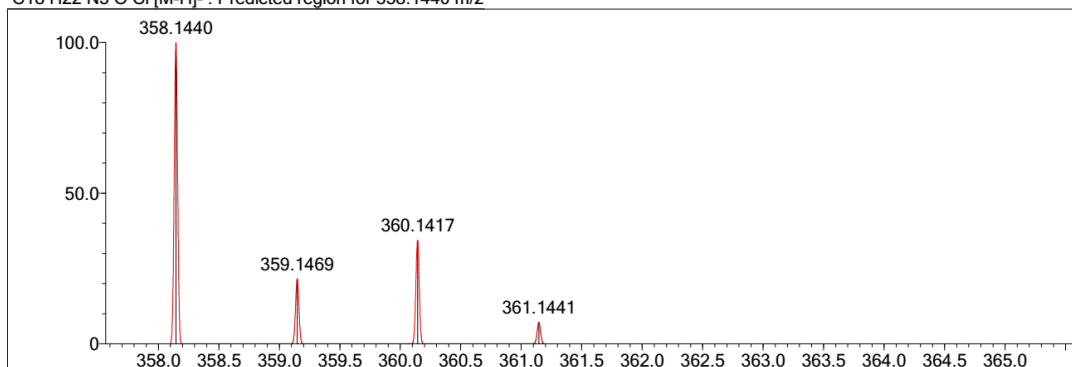
Event#: 2 MS(E-) Ret. Time : 1.027 Scan#: 156



Measured region for 358.1436 m/z



C18 H22 N5 O Cl [M-H]- : Predicted region for 358.1440 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	97.56	C18 H22 N5 O Cl	[M-H]-	358.1436	358.1440	-0.4	-1.12	97.85	10.0



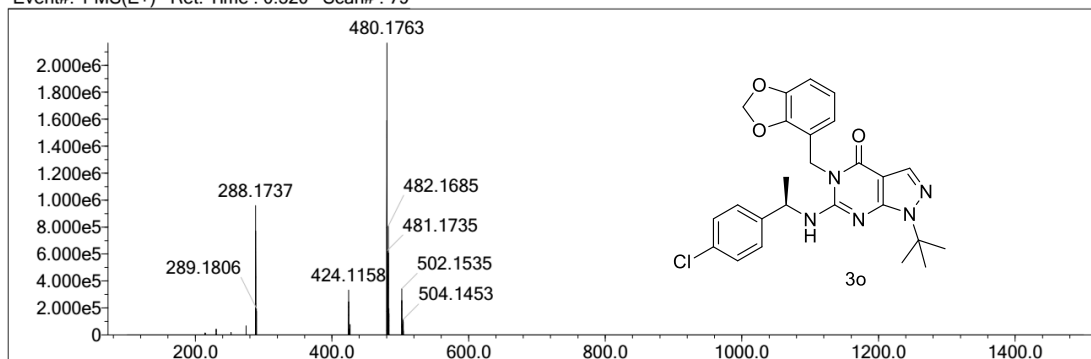
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	26	26	F	1	0	0	Se	2	0	0	H
2H	1	0	0	Si	4	0	0	Br	1	0	0	Na
B	3	0	0	P	3	0	0	Pd	2	0	0	
C	4	25	25	S	2	0	0	I	3	0	0	
N	3	5	5	Cl	1	1	1	Pt	2	0	0	
O	2	3	5	K	1	0	0					

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

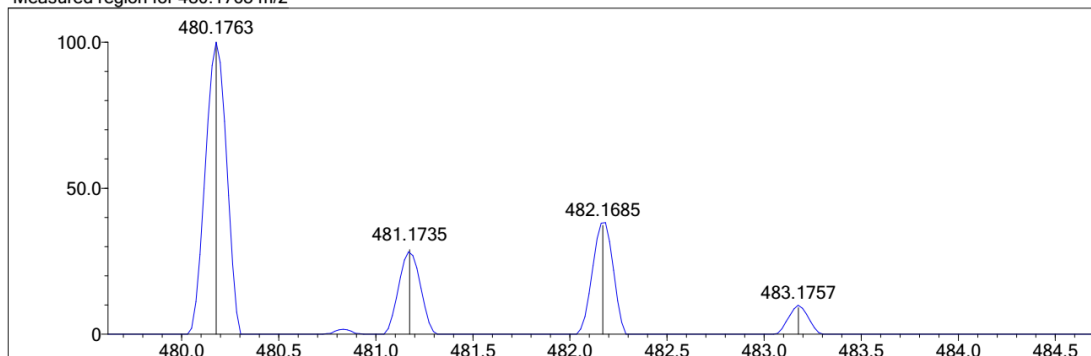
DBE Range: not fixed  
 Apply N Rule: yes  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 50

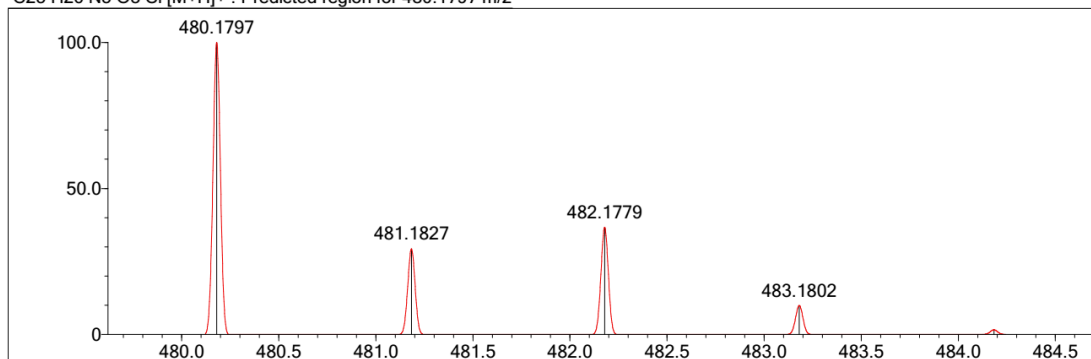
Event#: 1 MS(E+) Ret. Time : 0.520 Scan#: 79



Measured region for 480.1763 m/z



C25 H26 N5 O3 Cl [M+H]<sup>+</sup> : Predicted region for 480.1797 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	61.18	C25 H26 N5 O3 Cl	[M+H] <sup>+</sup>	480.1763	480.1797	-3.4	-7.08	88.41	15.0

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	26	O	2	0	1	Se	2	0	0	H
2H	1	0	0	F	1	0	1	Br	1	0	0	Na
B	3	0	0	P	3	0	0	Pt	2	0	0	
C	4	0	25	S	2	0	0					
N	3	0	6	Cl	1	0	0					

Error Margin (ppm): 20

HC Ratio: unlimited

Max Isotopes: all

MSn Iso RI (%): 75.00

DBE Range: not fixed

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

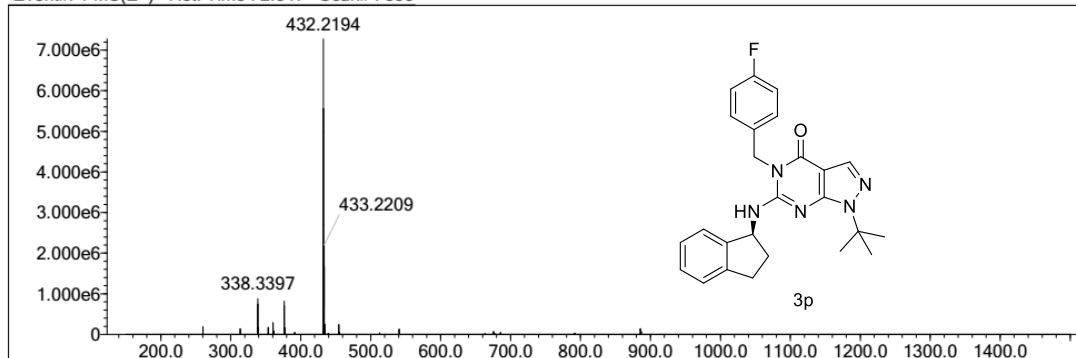
Electron Ions: both

Use MSn Info: no

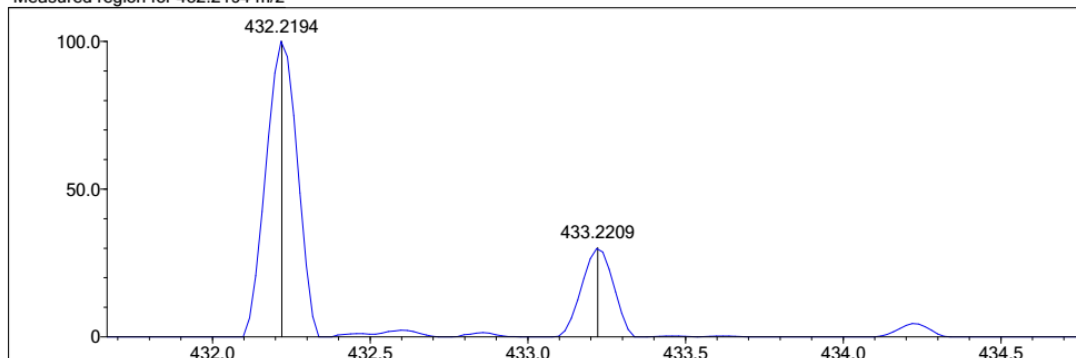
Isotope Res: 10000

Max Results: 200

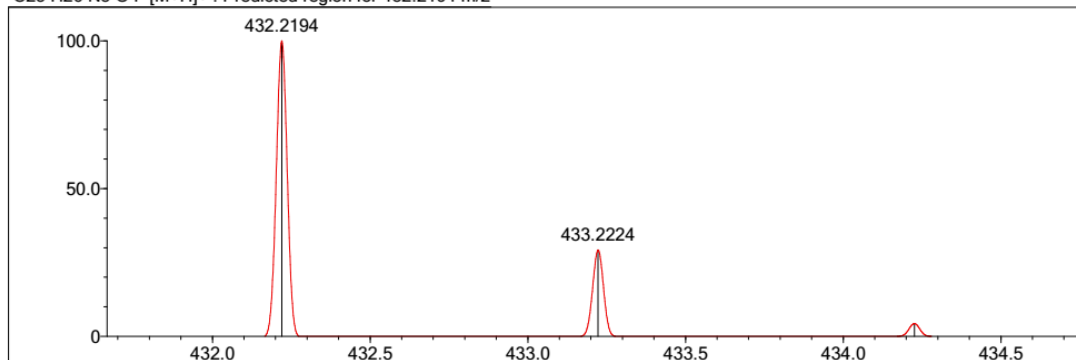
Event#: 1 MS(E+) Ret. Time : 2.347 Scan#: 353



Measured region for 432.2194 m/z



C25 H26 N5 O F [M+H]<sup>+</sup> : Predicted region for 432.2194 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	81.65	C25 H26 N5 O F	[M+H] <sup>+</sup>	432.2194	432.2194	-0.0	0.00	81.65	15.0

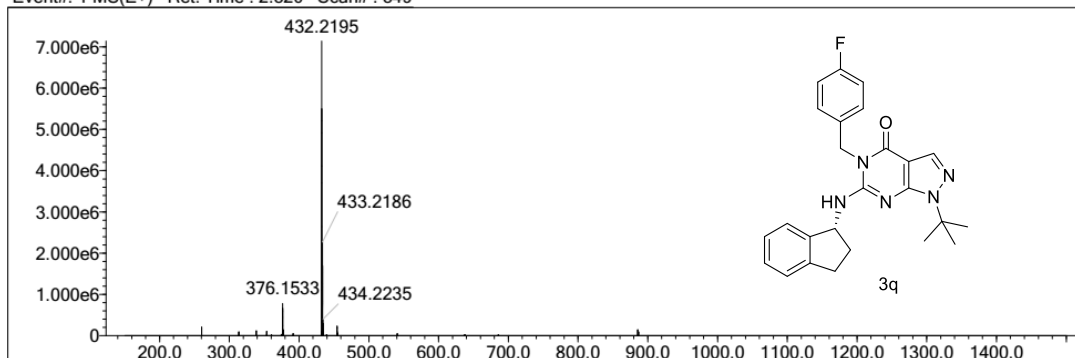
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	26	O	2	0	1	Se	2	0	0	H
2H	1	0	0	F	1	0	1	Br	1	0	0	Na
B	3	0	0	P	3	0	0	Pt	2	0	0	
C	4	0	25	S	2	0	0					
N	3	0	6	Cl	1	0	0					

Error Margin (ppm): 20  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

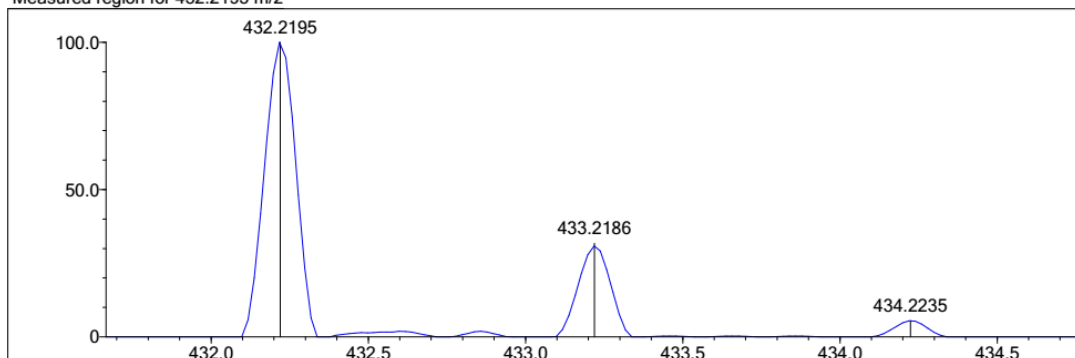
DBE Range: not fixed  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 200

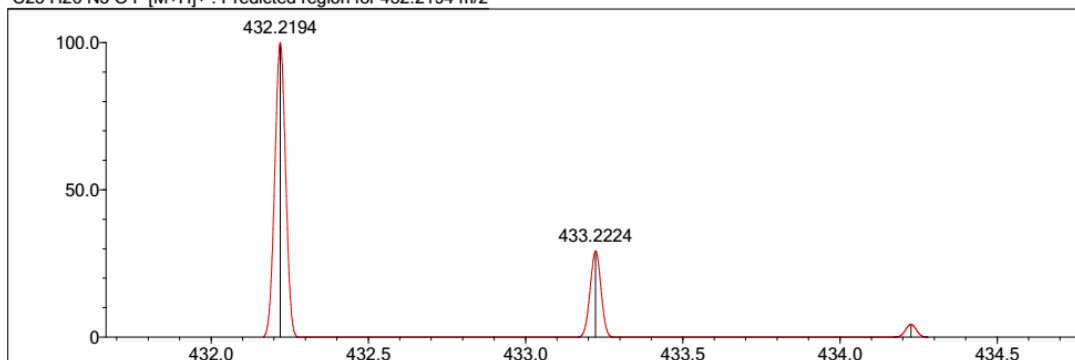
Event#: 1 MS(E+) Ret. Time : 2.320 Scan#: 349



Measured region for 432.2195 m/z



C25 H26 N5 O F [M+H]<sup>+</sup> : Predicted region for 432.2194 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	81.37	C25 H26 N5 O F	[M+H] <sup>+</sup>	432.2195	432.2194	0.1	0.23	81.37	15.0

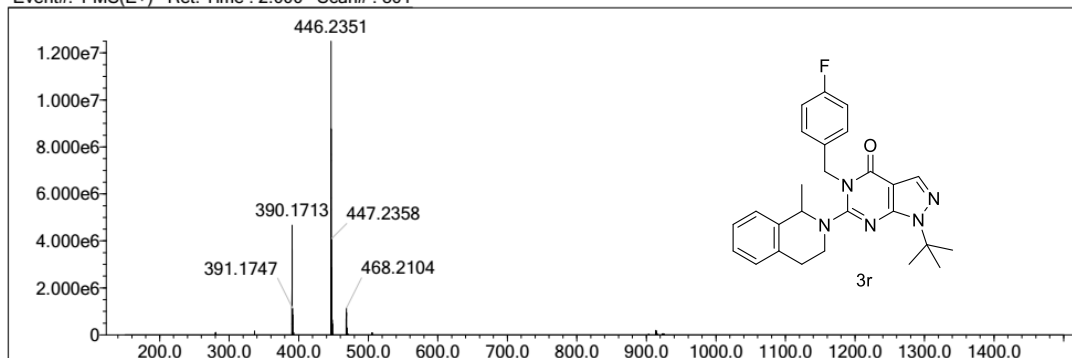
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	O	2	0	1	Se	2	0	0	H
2H	1	0	0	F	1	0	1	Br	1	0	0	Na
B	3	0	0	P	3	0	0	Pt	2	0	0	
C	4	0	26	S	2	0	0					
N	3	0	5	Cl	1	0	0					

Error Margin (ppm): 100  
 HC Ratio: unlimited  
 Max Isotopes: all  
 MSn Iso RI (%): 75.00

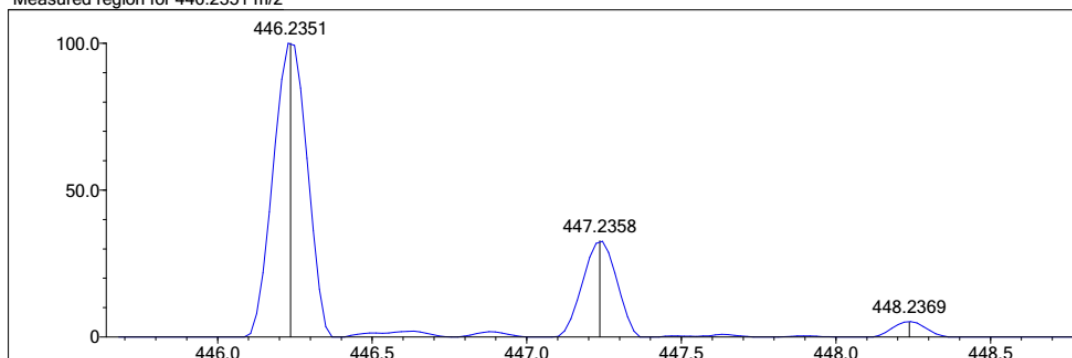
DBE Range: not fixed  
 Apply N Rule: no  
 Isotope RI (%): 1.00  
 MSn Logic Mode: AND

Electron Ions: both  
 Use MSn Info: no  
 Isotope Res: 10000  
 Max Results: 200

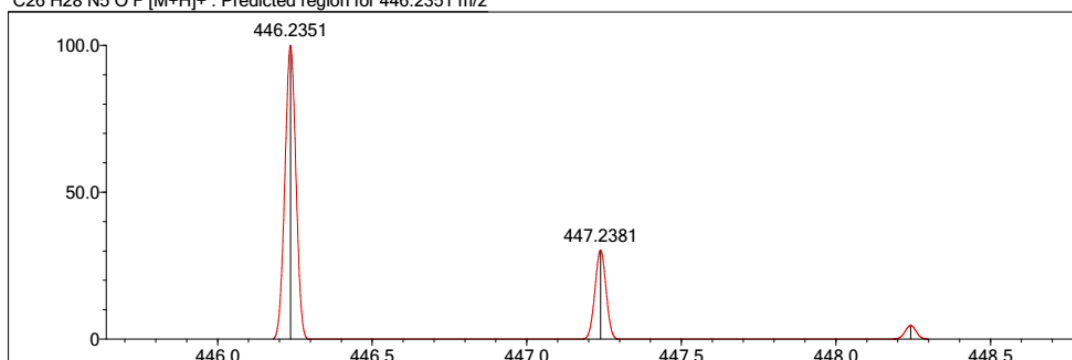
Event#: 1 MS(E+) Ret. Time : 2.000 Scan#: 301



Measured region for 446.2351 m/z



C26 H28 N5 O F [M+H]<sup>+</sup> : Predicted region for 446.2351 m/z

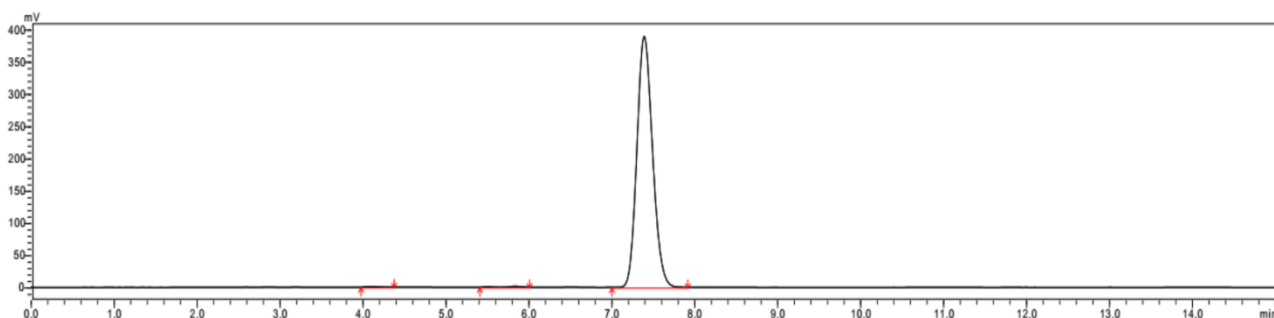


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	73.81	C26 H28 N5 O F	[M+H] <sup>+</sup>	446.2351	446.2351	0.0	0.00	73.81	15.0

## 10. The purity spectrums of the target compounds.

Compound **2**: UV detection at 254nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

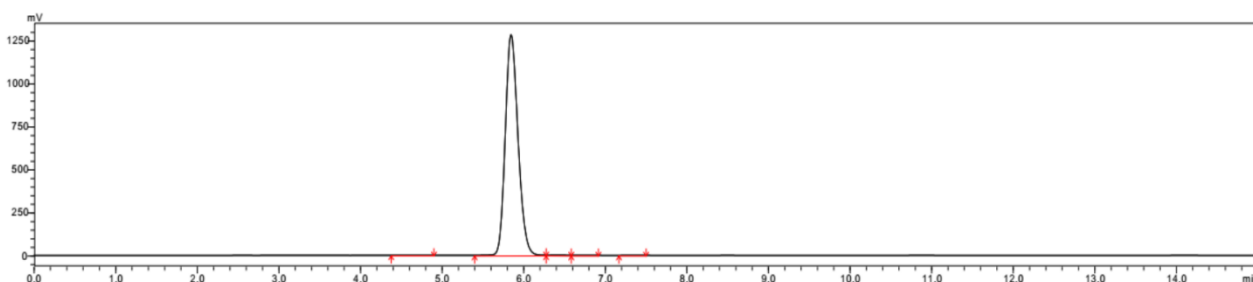
Purity: 99.3%



Peak	Retention time	Peak area	Peak height	Peak area %
1	4.093	13178	1267	0.255
2	5.844	22702	1481	0.44
3	7.396	5128863	388990	99.305
In total		5164743	391739	100

Compound **3a**: UV detection at 254nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

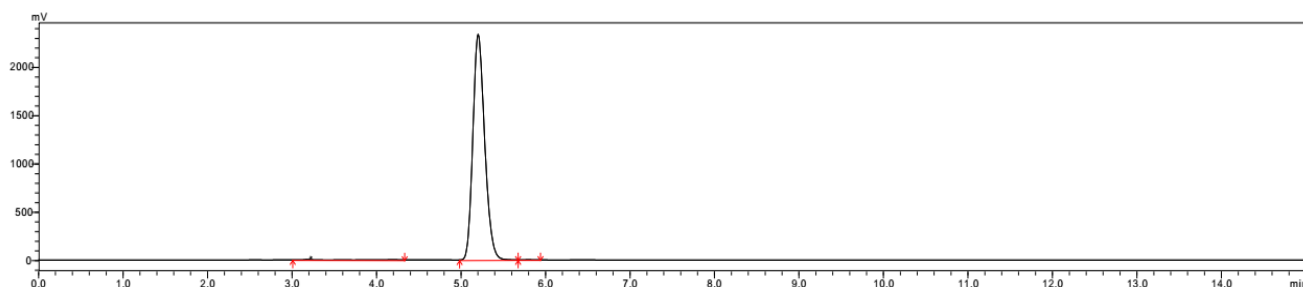
Purity: 99.6%



Peak	Retention time	Peak area	Peak height	Peak area %
1	4.544	12325	692	0.088
2	5.851	13927662	1283948	99.605
3	6.427	23056	1744	0.165
4	6.769	11668	771	0.083
5	7.366	8219	702	0.059
In total		13982930	1287858	100

Compound **3d** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

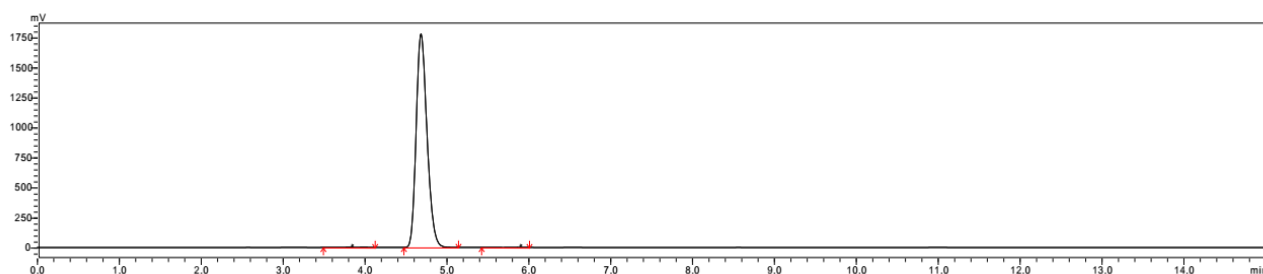
Purity: 99.7%



Peak	Retention time	Peak area	Peak height	Peak area %
1	5.2	40996	1939	0.293
2	5.667	13964068	1300242	99.707
In total		14005065	1302181	100

Compound **3e** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

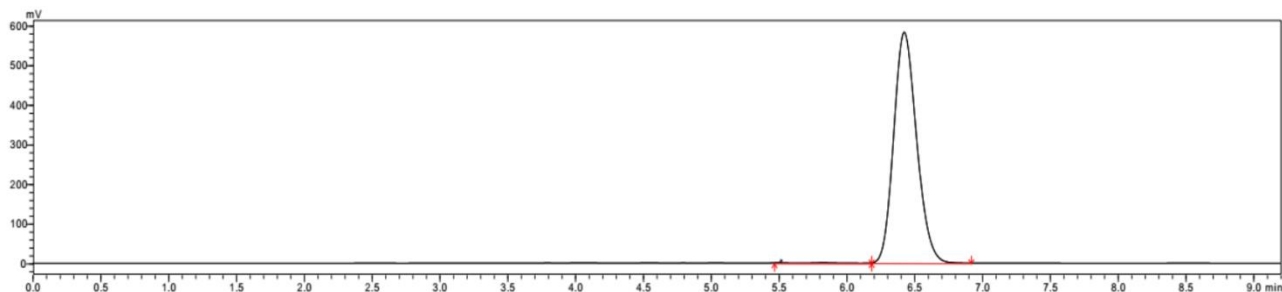
Purity: 99.7%



Peak	Retention time	Peak area	Peak height	Peak area %
1	3.813	38101	2109	0.231
2	4.689	16406837	1778960	99.677
3	5.868	15122	1360	0.092
In total		16460061	1782428	100

Compound **3g** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

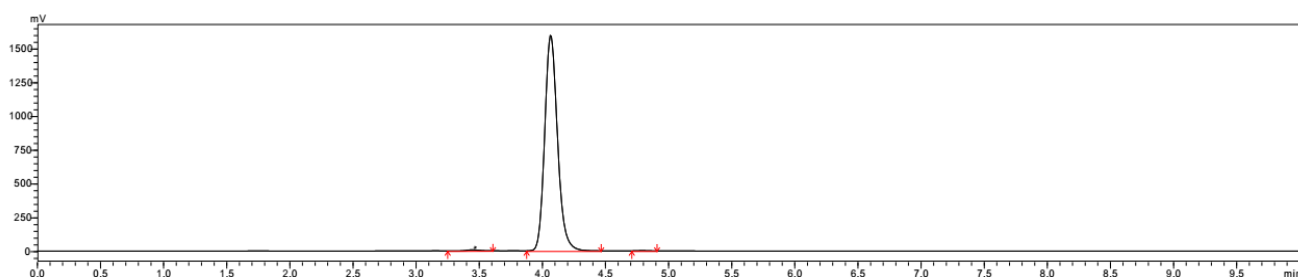
Purity: 99.8%



Peak	Retention time	Peak area	Peak height	Peak area %
1	5.495	15330	784	0.223
2	6.426	6855450	582573	99.777
In total		6870779	583357	100

Compound **3h** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

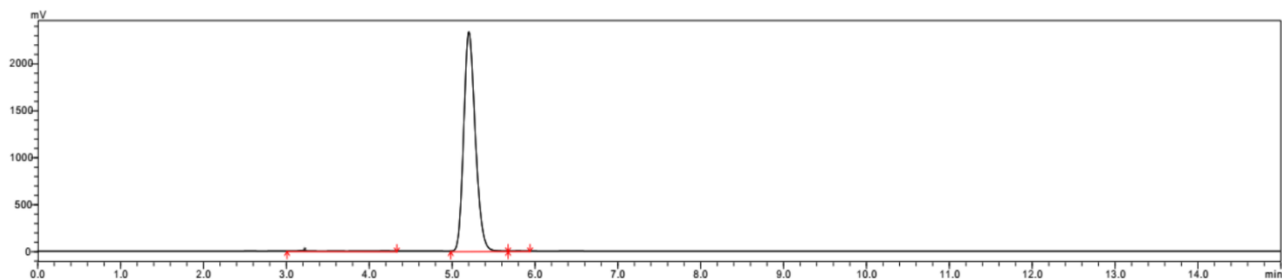
Purity: 99.4%



Peak	Retention time	Peak area	Peak height	Peak area %
1	3.445	52224	7289	0.46
2	4.07	11295035	1595970	99.403
3	4.795	15577	2264	0.137
In total		11362836	1605523	100

Compound **3k** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

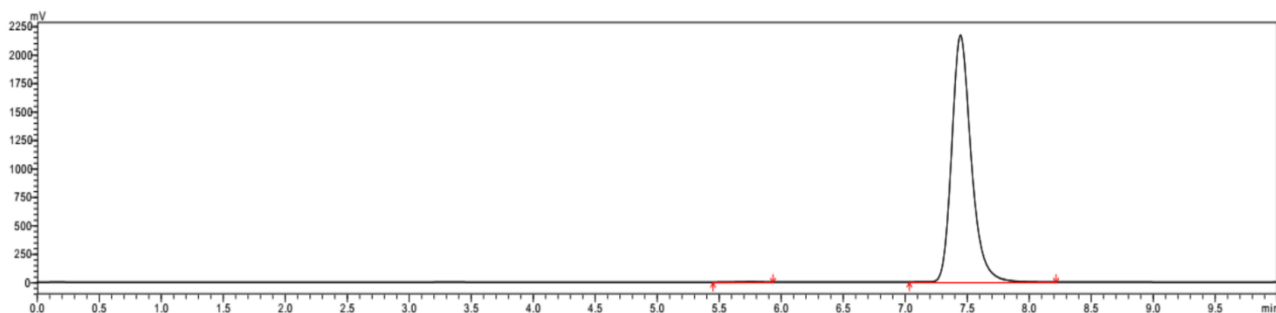
Purity: 99.7%



Peak	Retention time	Peak area	Peak height	Peak area %
1	3.19	58088	4230	0.254
2	5.209	22762820	2336323	99.671
3	5.804	17013	1723	0.074
In total		22837921	2342276	100

Compound **3l** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

Purity: 99.8%

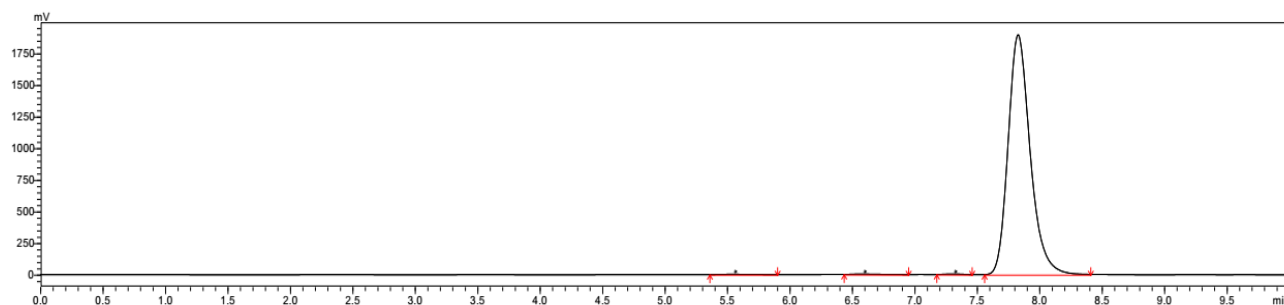


Peak	Retention time	Peak area	Peak height	Peak area %
1	5.757	48562	3721	0.205
2	7.45	23665138	2170088	99.795
In total		23713700	2173809	100



Compound **3m** : UV detection at 254 nm; elution, MeOH (100%); T = 25°C; Flow rate = 1.0 mL/min.

Purity: 99.4%



Peak	Retention time	Peak area	Peak height	Peak area %
1	5.543	57660	2837	0.245
2	6.577	53246	3599	0.227
3	7.305	35769	4075	0.152
4	7.83	23344962	1895904	99.376
In total		23491636	1906415	100

## 11. Reference

- (1) Wu, D.; Zhang, T.; Chen, Y.; Huang, Y.; Geng, H.; Yu, Y.; Zhang, C.; Lai, Z.; Wu, Y.; Guo, X.; Chen, J.; Luo, H.-B. Discovery and Optimization of Chromeno[2,3-c]pyrrol-9(2H)-ones as Novel Selective and Orally Bioavailable Phosphodiesterase 5 Inhibitors for the Treatment of Pulmonary Arterial Hypertension. *J. Med. Chem.* **2017**, *60*, 6622-6637.
- (2) Huang, M.; Shao, Y.; Hou, J.; Cui, W.; Liang, B.; Huang, Y.; Li, Z.; Wu, Y.; Zhu, X.; Liu, P.; Wan, Y.; Ke, H.; Luo, H. B., Structural Asymmetry of Phosphodiesterase-9A and a Unique Pocket for Selective Binding of a Potent Enantiomeric Inhibitor. *Mol. Pharmacol.* **2015**, *88*, 836-845.
- (3) Dyck, B.; Branstetter, B.; Gharbaoui, T.; Hudson, A. R.; Breitenbucher, J. G.; Gomez, L.; Botrous, I.; Marrone, T.; Barido, R.; Allerston, C. K.; Cedervall, E. P.; Xu, R.; Sridhar, V.; Barker, R.; Aertgeerts, K.; Schmelzer, K.; Neul, D.; Lee, D.; Massari, M. E.; Andersen, C. B.; Sebring, K.; Zhou, X.; Petroski, R.; Limberis, J.; Augustin, M.; Chun, L. E.; Edwards, T. E.; Peters, M.; Tabatabaei, A., Discovery of Selective Phosphodiesterase 1 Inhibitors with Memory Enhancing Properties. *J. Med. Chem.* **2017**, *60*, 3472-3483.