SUPPORTING INFORMATION

Discovery of Highly Potent 2-Sulfonyl-Pyrimidinyl Derivatives for Apoptosis Inhibition and Ischemia Treatment

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Materials

All chemical reagents were used as supplied by Sigma-Aldrich, J&K and Alfa Aesar Chemicals. DCM, DMF, acetonitrile were distilled from calcium hydride; THF was distilled from sodium/benzophenone ketyl prior to use. CellTiter-Glo® Luminescent Cell Viability Assay kit (G7570,G7573) was bought from Promega. DCFH-DA (D399), TMRM (T668), Mitotracker® Red CMXRos (M7512) were bought from Thermo Fisher Scientifc. zVAD (FMK001) was bought from R&D System. DMEM Medium, Fetal Bovine Serum (12483-020) and Penicillin-Streptomycin (15140-122) were bought from Life Technology. Doxycycline (D9891) was bought from Sigma-Aldrich. G418 (345819) was bought from Calbiochem. Cytochrome c antibody(556432) was bought from BD Pharmingen. Bim antibody (2819) was bought from Cell Signaling. Sprague Dawley rats were ordered from Charles River. Nylon suture and syringe were ordered from RWD Life Science.

^{1}HNMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperature with stated solvants. ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (^{1}H, δ 7.26; ^{13}C, δ77.00). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constants. High-resolution mass spectra were obtained using Agilent Techmologies 6540 UHD Accurate-Mass Q-TOF LC/MS. Mass spectra was obtained by HPLC/MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA)Detector). The system was equipped with a Waters C_{18} 5μm SunFire separation column(150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade acetonitrile (solvent B) with a flow rate of 0.3 mL/min. LC-MSMS data was obtained using Thermo FINNIGAN TSQ Quantum DISCOVERY MAX. The system was equipped with a Kinetex-C18 110A column (3 mm * 30 mm ID, 2.6 um, phenomenex).

Experimental Section

High throughput screening. A chemical library containing 200000 compounds (UTSW library) was screened according to the following procedure: U2OS_Bim cells (a cell line in which the BH3-only protein Bim can be inducibly expressed by the addition of Doxycycline to the growth medium)[1] were plated in 384-well plates with 30μl medium at a density of 500 cells per well. 16 hrs after plating, test compounds were transferred from stock plates to the assay plates with the cultured cells. Positive control (20 μM zVAD) and negative control (DMSO) were added to every plate. 1 hr after compound treatment, 0.1ug/mL Doxycyclin (DOX) was added to induce the expression of the Bim protein. After 24 hrs, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit (Promega, G7570) according to the manufacturer's instructions.

Luminescence was recorded with a PerkinElmer EnSpire Multimode Plate Reader. Compounds that could rescue cell viability to a level above 50% were selected; these compounds were then screened a second time for assurance.

Immunostaining of cytochrome c. U2OS_Bim cells were plated in Lab-Tek eight-chambered slides (Thermo Scientific). 24 hrs later, cells were treated with 20uM hit compound for one hour. Cell were washed by PBS for 10 min and fixed with 2% PFA for 30 min at room temperature. Following three additional washes in PBS, cells were incubated in PBS containing 0.1% Triton X-100 for 10 min. Cytochrome c antibody (diluted in 5% BSA in PBST) was incubated with the cells at 4 °C overnight. Cells were then washed three times with PBST and incubated with secondary antibody at room temperature for 1 h. Following three additional washes in PBS, the slides were covered and sealed and then examined with a Zeiss LSM 510 confocal microscope.

Apoptosis inhibition assay. U2OS_Bim or U2OS_tBid cells were plated at a density of 3000 cells per well in 96-well plates. 24 hrs later, cells were treated with indicated compounds for one hour. The cells were then treated with 0.1 μg/mL DOX to trigger apoptosis. 24 hours after the addition of DOX, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit. Cell survival rate was calculated. Data are represented as means ± standard deviation of duplicates.

Testing the binding reversibility of the 2-sulfonyl-pyrimidinyl derivatives compounds. U2OS_Bim cells were plated at a density of 3000 cells per well in 96-well plates. 24 hrs later, duplicate sets of cells were treated with experimental compounds for indicated time. One subset of these cells was treated with 0.1 μg/mL DOX to trigger apoptosis. The remaining subset of cells was washed free of the experimental compound at different time point using warmed medium, 3 times, before being treated with 0.1 μg/mL DOX. 24 hours after the addition of DOX, cell viability was monitored by measuring the ATP levels using a CellTiter-Glo kit. Cell survival rates were calculated. Data are represented as means ± standard deviation of duplicates.

Compounds Stability Assays. 100nM of Compounds **33** or **40** were incubated with 2mM GSH in PBS buffer (pH = 7.4) at 37 °C. The reaction was monitored at various time points using LC-MS/MS, and the amount of the remaining compound was analyzed by Lcquan 2.5 Software.

TMRM staining. U2OS_Bim cells were plated at a density of 3000 cells per well in 96-well optical plates. 24 hrs later, cells were treated with experimental compounds for one hour. The cells were then treated with 0.1 μg/mL DOX to trigger apoptosis. 4 hrs later, 50nM TMRM was added to each well and incubated for 30min. Cells were washed 3 times with warmed PBS buffer and examined with a Zeiss LSM 510 confocal microscope.

ROS measurement. U2OS_Bim cells were plated at a density of 10000 cells per well in 96-well optical plate. 24 hrs later, cells were treated with experimental compounds for 2 hrs. The cells were then treated with 0.1 μg/mL DOX to trigger apoptosis. 4 hrs later, cells were washed 3 times with PBS and then incubated in PBS with 2 μM DCFH-DA for 30 min at 37°C. Cells were then

washed twice with PBS and fluorescence was detected with a PerkinElmer EnSpire Multimode Plate Reader (λ_{ex}= 485 nm and λ_{em}= 525 nm). As a positive control, following the DCFH-DA incubation and PBS washing, 50 μM of H_2O_2 was added to untreated cells and followed by fluorescence analysis. Immediately after fluorescence detection, cell viability was determined by measuring the ATP levels using a Cell Titer-Glo kit. Mean ROS levels were recorded as ROS fluorescence/cell viability and ROS increases as compared with the negative control group were calculated.

Detection of Bim expression level. U2OS_Bim cells were plated at a density of 50000 cells per well in 6-well plates. 24 hrs later, cells were treated with indicated compounds for 2hrs and then celles were washed and treated with 0.1 μg/mL DOX to trigger apoptosis. 18 hours after the addition of DOX, cells were collected and lysed with 300μL lysis buffer (50 mM Tris (pH 8.0), 137mMNaCl,1mMEDTA,1%TritonX-100,10%glycerol,1× mixture protease inhibitor) per sample. 10μL of cell lysate was used for Western blot of Bim and actin.

Transient focal cerebral ischemia. Animal procedures were approved by NIBS local ethic committee. Transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) in rats as described previously, with slight modifications.[2] Briefly, male Sprague Dawley rats (weight 280-320 g) were anesthetized with 2% isoflurane. The left common, external, and internal carotid arteries (CCA, ECA, and ICA) were exposed. A 4-0 monofilament nylon suture with a silicon-coated tip was introduced through an incision of the ECA into the ICA to occlude the origin of the MCA for 1 h. Compound solutions were injected into the left striatum after the suture insertion, at the following coordinates in reference to the bregma: AP, 0.4; ML, 3.0; DV, -5.0 mm (from the dura). Solution was injected at a rate of 1 μL/minute using a Hamilton syringe. The syringe was left in place for 5 min before being slowly retracted. Animals were sacrificed 24 h after the MCAO. Brains were removed and sliced into sections of 2 mm thickness. Infarct size was examined via staining with 1.5 % 2, 3, 5-triphenyltetrazolium chloride.

Supporting Tables, Figures and Discussions

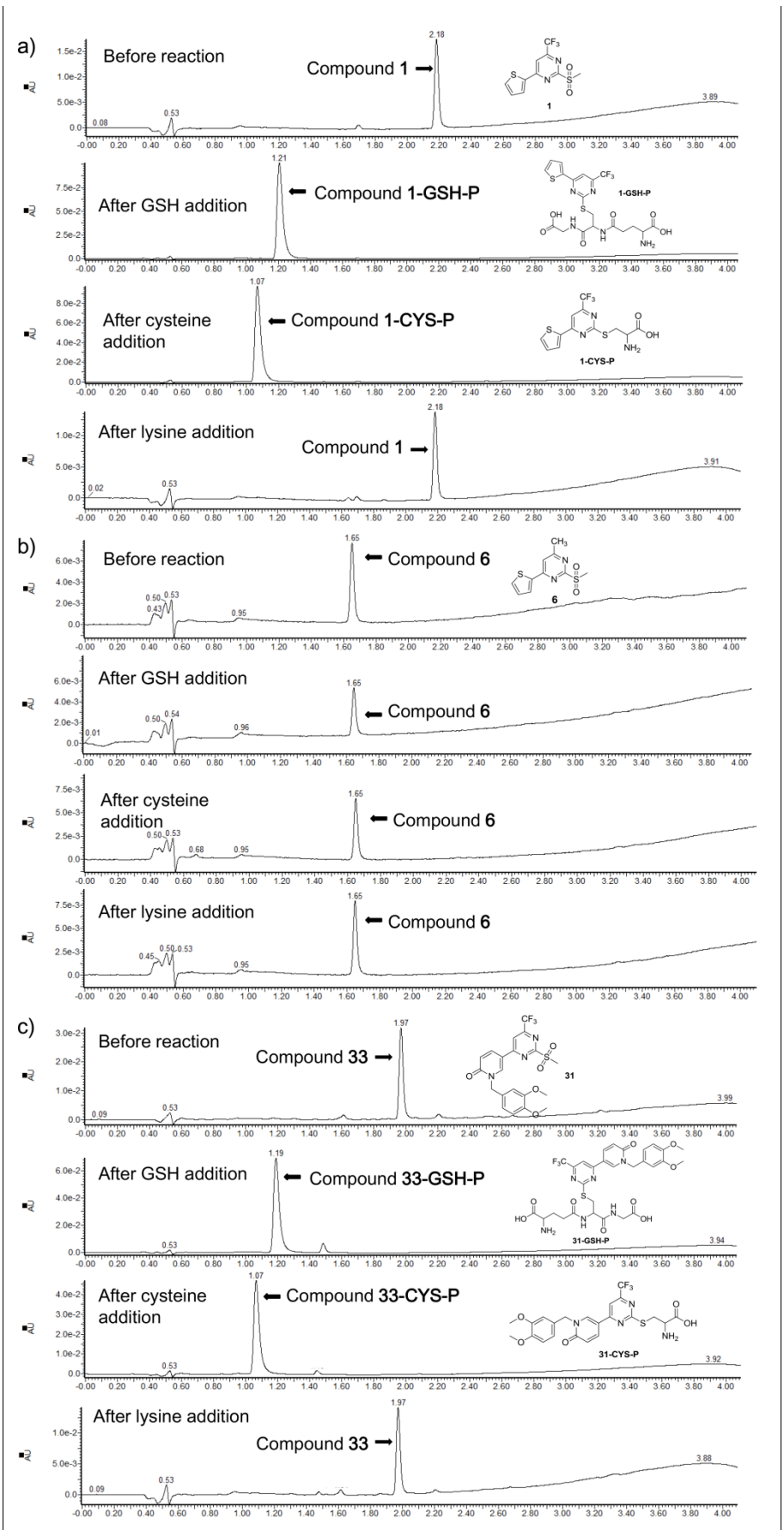
Table S1. Apoptosis inhibition activity on U2OS_tBid cell line

Compound No.	EC_{50}(nM)	Compound No.	EC_{50}(nM)
20	1130±24	**26**	109±21
21	809±188	**27**	122±28
22	136±11	**28**	158±12
23	186±78	**33**	32±5.9
24	4382±994	**42**	0.23±0.04
25	535±68	**(TC9-305)**	

Table S2. Comparison of EC_{50} between wash and no-wash assay

No.	EC_{50} [a] (no wash)	EC_{50} [a] (wash)	No.	EC_{50} [a] (no wash)	EC_{50} [a] (wash)
hit	4010±686	3895±305	**23**	66±4.2	45±16
1	749±111	661±232	**25**	57±1.7	62±9.9
3	1135±242	1268±385	**27**	69±9.7	66±21.3
10	2201±238	5058±1582	**28**	94±9.6	168±16.3
20	1157±66	1069±74	**33**	33±0.87	7±1.08
21	1143±235	997±425	**42**	0.42±0.05	0.53±0.03
22	257±27	305±42	**ZVAD**	69217±10560	No activity

[a]: cellular apoptosis inhibition activity.


a)
Before reaction
Compound 1
After GSH addition
Compound 1-GSH-P
After cysteine addition
Compound 1-CYS-P
After lysine addition
Compound 1
b)
Before reaction
Compound 6
After GSH addition
Compound 6
After cysteine addition
Compound 6
After lysine addition
Compound 6
c)
Before reaction
Compound 33
After GSH addition
Compound 33-GSH-P
After cysteine addition
Compound 33-CYS-P
After lysine addition
Compound 33

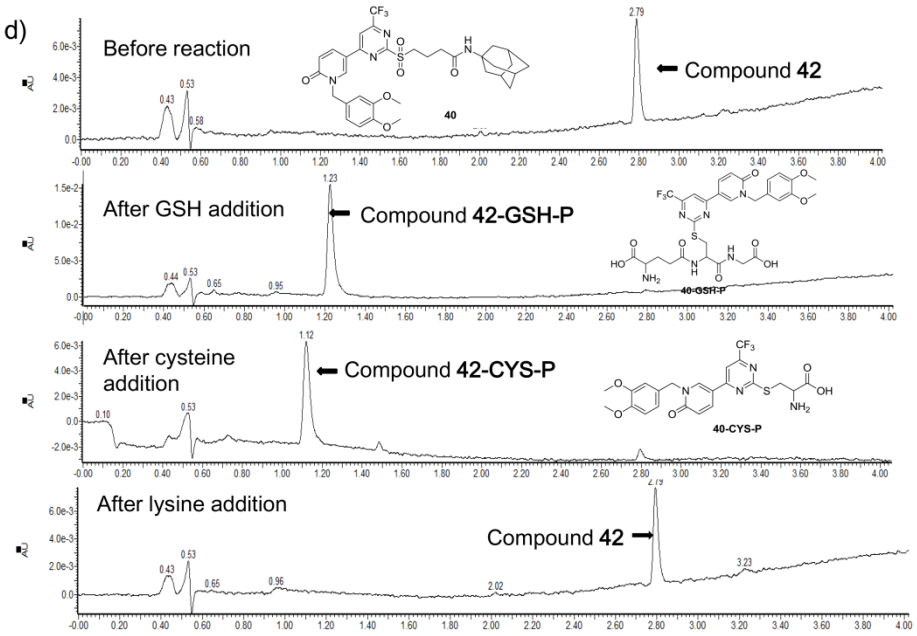


Figure S1. Model reaction between cysteine/lysine/GSH and compound **1**, **6**, **33** and **42** detected by UPLC. Compound **1**/**6**/**33**/**42**: 20μM; cysteine/lysine/GSH: 100μM; reaction time: 2 hr.

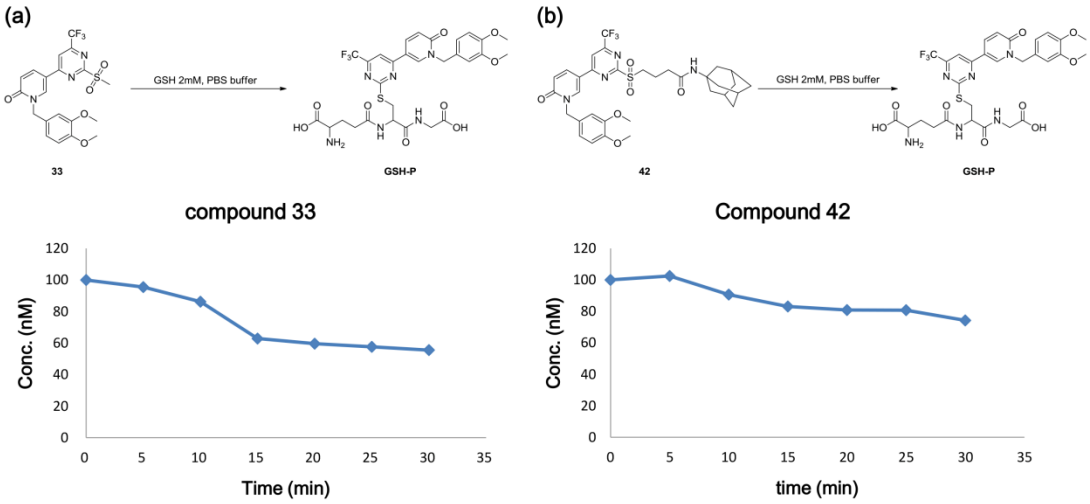
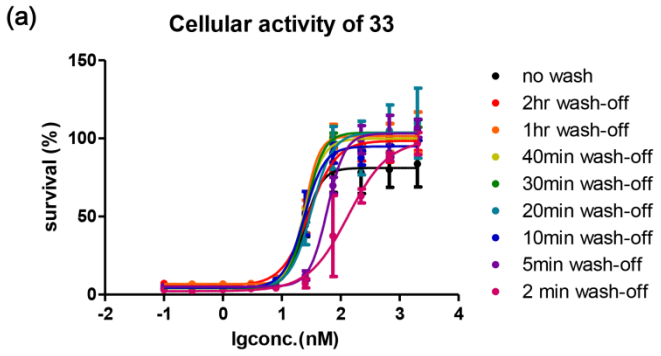
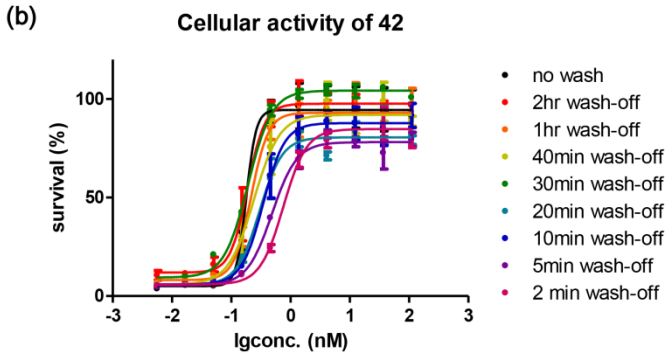


Figure S2. Reactivity of compounds **33** (100nM) and **42** (100nM) with GSH (2mM) in PBS buffer monitored by LC-MS/MS.

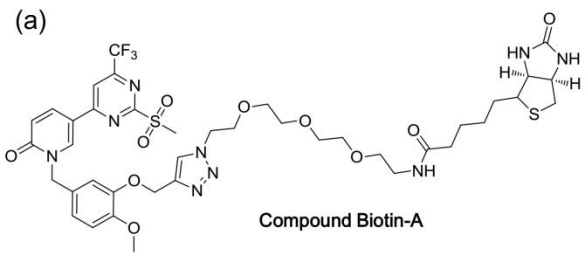


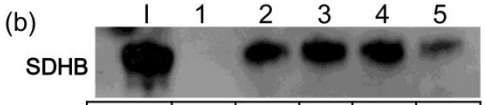
condition	EC50 (nM)
No wash	24.22
2hr wash-off	29.16
1hr wash-off	24.46
40min wash-off	24.01
30min wash-off	25.82
20min wash-off	32.99
10min wash-off	23.99
5min wash-off	59.09
2min wash-off	134.6



condition	EC50 (nM)
No wash	0.1787
2hr wash-off	0.1879
1hr wash-off	0.2165
40min wash-off	0.2411
30min wash-off	0.1922
20min wash-off	0.2997
10min wash-off	0.3452
5min wash-off	0.4869
2min wash-off	0.7566

Figure S3. Cellular activity of compounds **33** (a) and **42** (b) under different wash-off conditions. Left: activity curve of compounds under different wash-off conditions; right: EC_{50} values under different wash-off conditions.





No.	1	2	3	4	5
probe	(-)	(+)			
time	2 hr	2 hr	1 hr	30 min	10 min

Figure S4. Affinity pulldown using probe molecule compound Biotin-A at different time point. (a), structure of compound Biotin-A; (b), western blot of SDHB after pulldown. I: input (level of SDHB in cell lysate).

Discussion about the reactivity of this series of compounds with GSH and target protein SDHB:

(i), the reactivity of our compounds with GSH under physical concentrations (2mM GSH in PBS buffer) was detected. The reaction process was monitored by MS/MS. Compound **33** (original numbered as **31**, EC_{50}=25nM) and compound **42** (original numbered as **40**, EC_{50}=0.42nM) were detected as representatives of our compounds. Considering the EC_{50} values of **33** and **42**, the concentration of 100nM for these compounds was used in the model reactions.

(ii), cellular wash assay was applied to monitor the *in vivo* irreversible binding ability of our compounds with the target. Cells were incubated with compounds **33** or **42** for different periods of time varying between 2min and 2hr and then free compounds were washed away. Results showed that EC_{50} values at incubation time longer than 30min remained stable, indicating the target

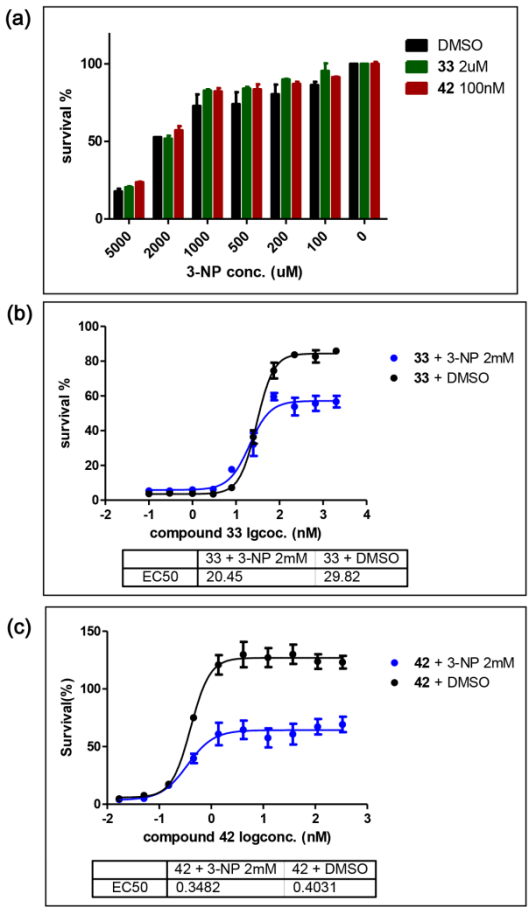
binding process have completed at this time point. Also, compounds activity at incubation time shorter than 30 min showed a gradual increase along the extension of incubation time. The cellular activity of compounds at 2-10 min incubation suggested that compound-target binding have already progressed to a large extent.

(iii), affinity pulldown experiment using the active biotinylated compound (compound Biotin-A) was carried out to detect the covalent binding with the target SDHB at different time point. This compound was used in our previous Mol Cell paper in target identification.[3] Pulldown experiment was carried out as previously described.[3] Cell lysate was incubated with Biotin-A coated streptavidin agarose beads or free beads for indicated period of time. Western blot of SDHB in the eluted fraction showed that obvious covalent binding could be observed at 10min. Besides, compound incubation at 30min to 2hr yielded the same level of target binding, indicating that covalent binding with SDHB completed with 30 minutes. GSH also exist in cell lysate, but its existence didn't affect the covalent binding of compound Biotin-A with SDHB.

(iv), from results in (i) to (iii), we concluded that the covalent reaction between our compounds and SDHB (both *in vivo* and in cell lysate) was faster and more prone to happen, when compared with their reactions with GSH. We believe that both kinds of covalent reaction happen inside cells when our compounds are added, but the reaction with GSH was kind of an unspecific reaction.

Table S3. Physicochemical properties (clogP, TPSA, Rotatable Bonds, MW) of the compounds

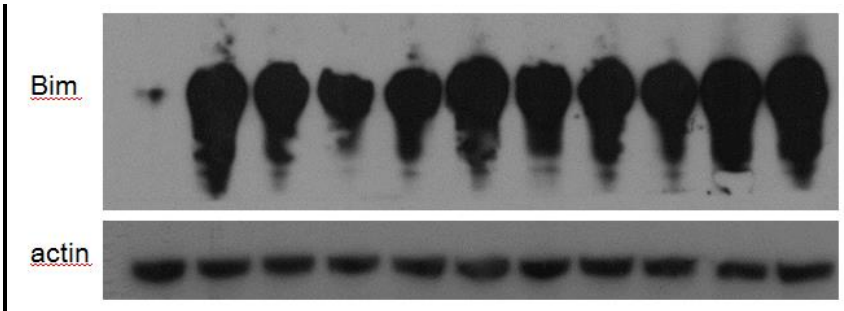
No.	M.W	Rotatable Bonds	logP(o/w)	TPSA	No.	M.W	Rotatable Bonds	logP(o/w)	TPSA
1	308.30	3	1.47876	59.92	**22**	443.83	5	2.3357601	80.23
2	322.33	4	1.99176	59.92	**23**	443.83	5	2.2987599	80.23
3	394.39	8	1.67676	86.22	**24**	425.38	5	1.43576	100.46
4	398.42	6	3.3547599	59.92	**25**	439.41	6	1.69976	89.46
5	460.49	6	5.2267599	59.92	**26**	453.43	7	2.04076	89.46
6	254.33	2	0.84200001	59.92	**27**	467.46	8	2.6547599	89.46
7	281.31	3	0.046	84.64	**28**	439.41	6	1.66276	89.46
8	322.43	3	2.0339999	59.92	**29**	433.4	6	2.22176	80.23
9	323.39	3	0.528	80.23	**30**	433.4	6	2.1847601	80.23
10	302.27	3	1.94876	59.92	**31**	434.39	6	1.40376	104.02
11	316.3	3	2.24476	59.92	**32**	434.39	6	1.36676	104.02
12	316.3	6	4.6295199	119.84	**33**	469.43	7	1.4055001	98.69
13	316.3	3	2.2467599	59.92	**34**	525.54	11	3.2444999	98.69
14	360.31	5	1.88776	86.22	**35**	553.59	13	4.1285	98.69
15	360.31	5	1.92676	86.22	**36**	513.49	10	1.3235	107.92
16	360.31	5	1.88976	86.22	**37**	527.51	11	1.7654999	107.92
17	394.37	5	3.5958	69.15	**38**	568.57	12	1.2595	119
18	373.35	5	1.4818	80.23	**39**	594.6	12	1.7915	119
19	319.26	3	-0.27824	89.02	**40**	608.63	12	2.2335	119
20	409.38	5	1.70676	80.23	**41**	540.51	11	0.70649999	141.78
21	443.83	5	2.2967601	80.23	**42**	674.73	13	3.7065001	127.79



	33 + 3-NP 2mM	33 + DMSO
EC50	20.45	29.82

	42 + 3-NP 2mM	42 + DMSO
EC50	0.3482	0.4031

Figure S5. Mitochondrial Complex II inhibitor 3-nitropropionate (3-NP) and Compound **33**/**42** are mutually non-competitive with each other. (a), compound **33** (2μM) and **42** (100nM) did not block the cell death caused by 3-NP (2mM and 5mM); (b) 3-NP (2mM) did not affect the EC_{50} values of **33** and **42** on apoptosis inhibition.

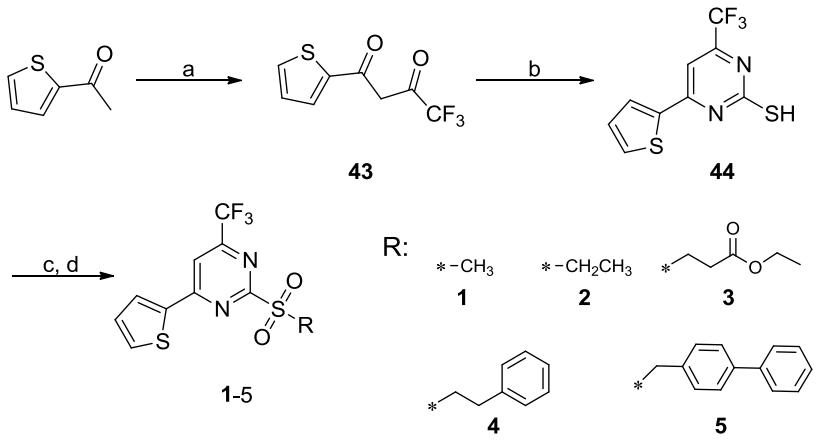


DOX	(-)	(+)									
cmpd	DMSO	zVAD	**23**	**26**	**27**	**28**	**33**	**38**	**39**	**40**	**42**
Conc. (nM)	---	40000	1000	1000	1000	1000	200	1000	200	10	10
EC50 (nM)	-----	69000	66	110	69	94	25	164	27	1.4	0.42

Figure S6. Bim overexpression is not affected by compounds treatment. Western blot of Bim and actin was detected at 18 hrs after compounds treatment and apoptosis induction. Cells treated with DMSO and without apoptosis induction were used as negative control; cells treated with zVAD and with apoptosis induction were used as positive control.

Synthetic Procedures

Scheme 1. Generalized synthesis route for compounds **1-5**



Reagents and conditions: (a) CF_3COOEt, NaOMe, MeOH, 0℃ to 80 ℃, overnight. (b) $(NH_2)_2CS$, AcOH, EtOH, 95℃, microwave, 2hrs. (c) RX, K_2CO_3, DMF, rt, 3hrs.(X=Cl or Br) (d) mCPBA, DCM, rt, 3hrs.

4,4,4-trifluoro-1-(thiophen-2-yl)butane-1,3-dione (43) 1-(thiophen-2-yl) ethanone (20g, 16mmol) was added to a solution of NaOMe (10.3g, 19mmol) in MeOH at 0℃ dropwise, and the mixture was stirred at room temperature for 1hr. Then the mixture was cooled to 0℃, and ethyl 2,2,2-trifluoroacetate(27g, 19mmol) was added in portions, and the whole reaction mixture was stirred and refluxed at 80°C overnight. After the organic solvent was evaporated in vacuo, the risidue was dissolved in H_2O(200mL), acified by HCl(120mL, 1N), and extracted by EtOAc(200mL) 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (PE/EA= 20/1), to give 4,4,4-trifluoro-1-(thiophen-2-yl)butane-1,3-dione (**43**) as a light red solid in 32% yield. ^{1}H NMR (400 MHz, $CDCl_3$): 7.84(dd, J=1.2, 4.0Hz, 1H), 7.76(dd, J=1.2, 4.8Hz, 1H), 7.21(dd, J=4.0, 4.8Hz, 1H), 6.45(s, 1H).

4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine-2-thiol (44) AcOH (0.8mL) was added to a solution of **43** (1g, 4.5mmol) and thiourea(1.7g, 22.5mmol) in MeOH (4mL) in a 25 mL microwave tube under N_2 atmosphere. The reaction mixture was microwaved at 95°C for 2hrs. The reaction mixture was filtered. Solvents were removed in vacuo from the filtrate, then the residue was extracted 3 times with EtOAc and H_2O. The organic layer was combined, washed by brine, dried over Na_2SO_4, then the solvent was evaporated in vacuo. The solid residue was further purified by silica gel column chromatograpy(PE/EA=1/1) to give 4-(thiophen-2-yl)-6-(trifluoromethyl) pyrimidine-2-thiol as an orange solid (**44**) in 50% yield. ^{1}H NMR ($CDCl_3$)： δ 7.86(dd, J=1.2, 4.0Hz, 1H), 7.63(dd, J=1.2, 5.2Hz, 1H), 7.51(s, 1H), 7.20(dd,

J=4.0, 5.2Hz, 1H).

2-(methylsulfonyl)-4-(thiophen-2-yl)-6-(trifluoromethyl) pyrimidine (1) 1.1 eq of K_2CO_3(35mg, 0.25mmol) was added to a solution of **44** (60mg, 023mmol) and CH_3I (36mg, 0.25mmol) in DMF (1mL), and the reaction mixture was stirred at room temperature for 2 hrs. The reaction mixture was then extracted by EtOAc/H_2O (15mL/15mL) 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography (PE/EA=1/1) to give 2-(methylthio)-4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine as a white solid.

mCPBA(85mg, 0.49mmol) was added to a solution of 2-(methylthio)-4-(thiophen-2-yl)-6-(trifluoromethyl) pyrimidine (54mg, 0.196mmol) in DCM, and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by satured $NaHCO_3$/DCM (10mL/10mL) 3 times. The organic layer was combined, washed by brine, dried over Na_2SO_4 and further purified silica gel column chromatography (PE/EA= 1/1) to give **1** in 28% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78(s, 1H), 8.53(dd, J=1.2, 4.0Hz, 1H), 8.11(dd, J=1.2, 4.8Hz, 1H), 7.38(dd, J=4.0, 4.8Hz, 1H), 3.48(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.93, 163.51, 156.74, 139.16, 134.63, 131.67, 129.61, 121.35, 118.61, 113.23, 39.14. HRMS (ESI): $[M+H]^+$ calculated for $[C_{10}H_8F_3N_2O_2S_2^+]$, 308.9974, found 308.9974. UPLC purity 95%.

2-(ethylsulfonyl)-4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine (2) The titled compound was prepared in 15% yield as a light yellow solid from **44** (60mg, 023mmol) and iodoethane (27mg, 0.25mmol) according to the procedure for **1**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78(s, 1H), 8.52(dd, J=1.2, 4.0Hz, 1H), 8.11(dd, J=1.2, 4.8Hz, 1H), 7.38(dd, J=4.0, 4.8Hz, 1H), 3.67(q, J=7.2Hz, 2H), 1.34(t, J=7.2Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.33, 163.32, 157.24, 139.17, 134.54, 131.64, 129.59, 124.68, 121.34, 118.60, 115.85, 113.13, 45.81, 7.02. HRMS (ESI): $[M+H]^+$ calculated for $[C_{11}H_{10}F_3N_2O_2S_2^+]$, 323.0130, found 323.0130. UPLC purity 95%.

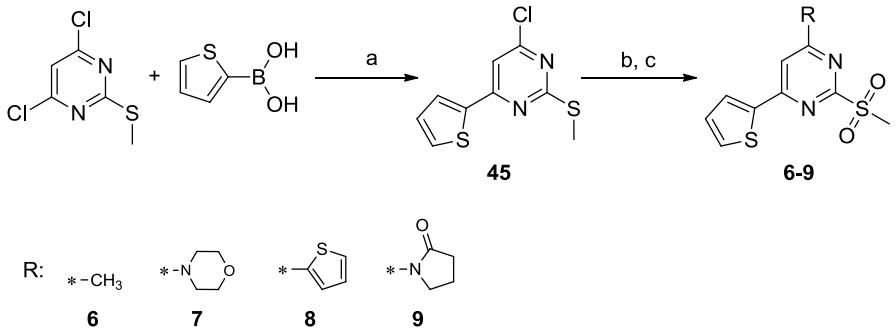
Ethyl 3-((4-(thiophen-2-yl)-6-(trifluoromethyl) pyrimidin-2-yl) sulfonyl) propanoate (3) The titled compound was prepared in 36% yield as a white solid from **44** and ethyl 3-chloropropanoate according to the procedure for **1**. ^{1}H NMR(400Hz, CDCl3) δ 8.03 (dd, *J*=1.2, 4.0 Hz, 1H), 7.94 (s, 1H), 7.75 (dd, *J*=1.2, 4.8 Hz, 1H), 7.26 (dd, *J*=4.0, 4.8 Hz, 1H), 4.17 (q, *J*=7.2 Hz, 2H), 3.95 (t, *J*=7.6 Hz, 2H), 3.01 (t, *J*=7.6 Hz, 2H), 1.27 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 170.15, 166.39, 163.54, 157.39 157.02, 139.16, 134.73, 134.67, 131.71, 129.60, 113.29, 61.65. HRMS (ESI): $[M+H]^+$ calculated for $[C_{14}H_{14}F_3N_2O_4S_2^+]$, 395.0346, found. 395.0343. UPLC purity 95%.

2-(([1,1'-biphenyl]-4-ylmethyl)sulfonyl)-4-(thiophen-2-yl)-6-(trifluoromethyl) pyrimidine (4) The titled compound was prepared in 11% yield as a light yellow soild from **44** (60mg, 0.23mmol) and 4-(chloromethyl)-1,1'-biphenyl (50mg, 0.25mmol) according to the the procedure for **1**. ^{1}H NMR (400 MHz, DMSO-d_6): δ8.79 (s, 1H), 8.54 (dd, J=1.2, 4.0Hz, 1H), 8.13 (dd, J=1.2, 5.2Hz, 1H), 7.64-7.68 (m, 4H), 7.48-7.52 (m, 2H), 7.43-7.48 (m, 2H), 7.36-7.40 (m, 2H) ^{13}C NMR (100

MHz, $CDCl_3$): 166.25, 163.51, 157.09, 156.72, 142.05, 140.36, 139.22, 134.59, 132.02, 131.63, 129.58, 128.95, 127.78, 127.67, 127.24, 125.54, 121.48, 118.70, 113.14, 113.12, 105.11, 57.18. HRMS (ESI): $[M+H]^+$ calculated for $[C_{15}H_{15}F_3N_3O_3S_2^+]$ 461.0600, found 461.0595. UPLC purity 95%.

2-(phenethylsulfonyl)-4-(thiophen-2-yl)-6-(trifluoromethyl) pyrimidine (5) The titled compound was prepared in 23% yield as a white solid from **44** (158mg, 0.61mmol) and (2-bromoethyl)benzene(39mg, 0.21mmol) according to the procedure for **1**. ^1H NMR(400Hz, $CDCl_3$) δ7.98(dd, J=0.8, 4.0Hz, 1H), 7.84(s, 1H), 7.74(dd, J=0.8, 4.8Hz, 1H), 7.24(dd, J=4.0, 4.8Hz, 1H), 7.12-7.21(m, 5H), 3.87-3.92(m, 2H), 3.21-3.27(m, 2H). HRMS (ESI): $[M+H]^+$ calculated for $[C_{17}H_{14}F_3N_2O_2S_2^+]$, 399.0443, found 399.0446 . UPLC purity 95%.

Scheme 2. Generalized synthesis route for compounds **6-9**



Reagents and conditions: (a) $Pd(PPh_3)_4$, $Na2CO_3$, DME/H_2O=2/1, 80℃, 6 hrs. (b), for **6**, CH_3MgCl, $Fe(acac)_3$, THF/NMP=5/1, 0℃, 3hr; for **7**, morpholine, K_2CO_3, DMF, 50℃,microwave, 2hrs; for **8**, thiophen-2-ylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O=2/1, 150℃,microwave, 2hrs; for **9**, pyrrolidin-2-one, binap, $Pd_2(dba)_3$, K_3PO_4, dioxane, 90℃, 6 hrs. (c), mCPBA, DCM, rt, 3hrs.

4-chloro-2-(methylthio)-6-(thiophen-2-yl)pyrimidine (45) To a solution of 4,6-dichloro-2-(methylthio)pyrimidine(400mg, 2.06mmol) and thiophen-2-ylboronic acid(300mg,2.26mmol) in DME/H_2O(4mL/2mL) was added $Pd(PPh_3)_4$ (69mg, 0.06mmol) and Na_2CO_3 (546mg, 4.12mmol) in portions under N_2 atmosphere. The reaction mixture was heated and refluxed for 2hrs. The reaction mixture was cooled to room temperature, and filtered over celite. Solvent was evaoporated in vacuo, and the residue was extracted by $EtOAc/H_2O$(30mL/30mL) 3 times. The organic layer was combined, washed by brine, dried over Na_2SO_4, and further purified by silica gel column chromatography (PE/EA=20/1) to give 4-chloro-2-(methylthio)-6-(thiophen-2-yl)pyrimidine (40) as a white solid in 27% yield. ^1H NMR(400Hz, CDCl3) δ 7.76(dd, J=0.8, 4.0Hz, 1H), 7.56(dd, J=0.8, 4.8Hz, 1H), 7.72(s, 1H),

7.16(dd, J=4.0, 4.8Hz, 1H), 2.62(s, 3H).

4-methyl-2-(methylsulfonyl)-6-(thiophen-2-yl)pyrimidine (6) To a solution of **45** (70mg, 0.29mmol) in THF/NMP(2mL/0.4mL) was added $Fe(acac)_3$(6mg, 0.01mmol) followed by CH_3MgCl(30mg, 0.35mmol) dropwise at -20^oC under N_2 atmosphere. The reaction mixture was stirred at -20^oC to room temperature overnight. The reaction mixture was filtered, and solvents were removed from the filtrate in vacuo. The residue was extracted by EtOAc/H_2O 3 times. The organic layer was washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=10/1) to give 4-methyl-2-(methylthio)-6-(thiophen-2-yl) pyrimidine as a light yellow oil in 20% yield.

mCPBA(24mg, 0.14mmol) was added to a solution of 4-methyl-2-(methylthio)-6-(thiophen-2-yl) pyrimidine (12mg, 0.05mmol) in DCM and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by DCM and satured $NaHCO_3$ solution 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=1/1) to give 4-methyl-2-(methylsulfonyl) -6-(thiophen-2-yl) pyrimidine as a white solid in 40% yield. HRMS (ESI): $[M+H]^+$ calculated for $[C_{10}H_{11}N_2O_2S_2^+]$, 255.0256, found 255.0269. UPLC purity 95%.

4-(2-(methylsulfonyl)-6-(thiophen-2-yl)pyrimidin-4-yl)morpholine (7) morpholine (15mg, 0.17mmol) and K_2CO_3 (24mg, 0.17mmol) under N_2 atmosphere was added to a solution of **45** (30mg, 0.12mmol) in DMF in portions, and the reaction mixture was microwaved at 150^oC for 2hrs. The reaction mixture was extracted by EtOAc/H_2O 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=20/1) to give 4-(2-(methylthio)-6-(thiophen-2-yl)pyrimidin-4-yl)morpholine as a white solid in 41% yield.

mCPBA(36mg, 0.21mmol) was added to a solution of 4-(2-(methylthio)-6-(thiophen-2-yl)pyrimidin-4-yl)morpholine (13mg, 0.05mmol) in DCM and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by DCM and satured $NaHCO_3$ solution 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=1/1) to give 4-(2-(methylsulfonyl)-6-(thiophen-2-yl)pyrimidin-4-yl)morpholine as a white solid in 30% yield. ^1H NMR(400Hz, CDCl3) δ 7.76(dd, J=1.2, 4.0Hz, 1H), 7,51(dd, J=1.2, 5.2Hz, 1H), 7.14(dd, J=4.0, 5.2Hz, 1H), 6.78(s, 1H), 3.83~3.73(m, 8H), 3.35(s, 3H). UPLC purity 95%.

2-(methylsulfonyl)-4,6-di(thiophen-2-yl)pyrimidine (8) To a solution of **45** (300mg, 1.54mmol) and thiophen-2-ylboronic acid (800mg, 6.19mmol) in DME/H_2O(4mL/2mL) was added $Pd(PPh_3)_4$ (56mg, 0.05mmol) followed by Na_2CO_3 (655mg, 6.19mmol) under N_2 atmosphere. The reaction mixture was microwaved at 150^oC for 2hrs. The reaction mixture was cooled to room temperature, and filtered over celite. Solvent was evaoporated in vacuo, and the residue was extracted by EtOAc and H_2O 3 times. The organic layer was combined, washed by brine, dried over Na_2SO_4,

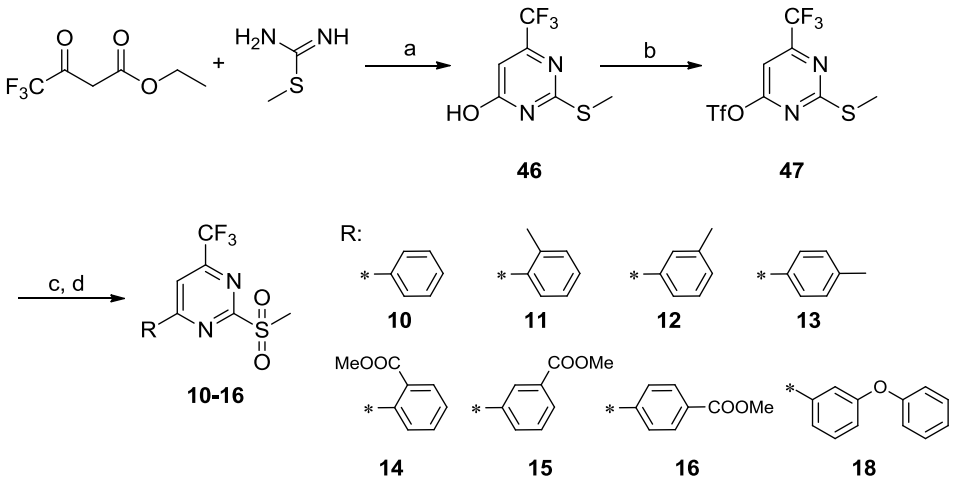
and further purified by silica gel column chromatography (PE/EA=100/1) to give 110 mg of 2-(methylthio)-4,6-di(thiophen-2-yl)pyrimidine as a white solid in 30% yield.

mCPBA(32mg, 0.19mmol) was added to a solution of 2-(methylthio)-4,6-di(thiophen-2-yl)pyrimidine (27mg, 0.09mmol) in DCM and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by DCM and satured $NaHCO_3$ solution 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=1/1) to give 2-(methylsulfonyl)-4,6-di(thiophen-2-yl)pyrimidine as a white solid in 20% yield. ^1H NMR(400Hz, $CDCl_3$) δ7,97 (dd, J=1.0, 3.8Hz, 2H), 7.83 (s, 1H), 7.64(dd, J=1.0, 5.0Hz, 2H), 7.21(dd, J=3.8, 5.0Hz, 2H), 3.46(s, 3H). HRMS (ESI): $[M+H]^+$ calculated for $[C_{13}H_{11}N_2O_2S_3^+]$, 322.9977, found 322.9974. UPLC purity 95%.

1-(2-(methylsulfonyl)-6-(thiophen-2-yl)pyrimidin-4-yl)pyrrolidin-2-one (9) To a solution of **45** (30mg, 0.12mmol) and pyrrolidin-2-one(21mg, 0.24mmol) in dry dioxane was added binap(8mg, 0.01mmol), $Pd_2(dba)_3$(6mg, 0.006mmol) and K_3PO_4(65mg, 0.30mmol) under N_2 atmoshpere. The reaction was refluxed at 160^{o}C for 8hrs. The reaction mixture was cooled to room temperature, filtered over celite. Solvents were removed from the filtrate in vacuo, then the residue was extracted by EtOAc/H_2O 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=4/1) to give 1-(2-(methylthio)-6-(thiophen-2-yl)pyrimidin-4-yl)pyrrolidin-2-one as a yellow oil in 33% yield.

mCPBA(20mg, 0.12mmol) was added to a solution of 1-(2-(methylthio)-6-(thiophen-2-yl)pyrimidin-4-yl)pyrrolidin-2-one (12mg, 0.04mmol) in DCM and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by DCM and satured $NaHCO_3$ solution 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=1/1) to give 1-(2-(methylsulfonyl)-6-(thiophen-2-yl) pyrimidin-4-yl) pyrrolidin-2-one as a white solid in 15% yiled. ^1H NMR(400Hz, $CDCl_3$) δ 8.33(s,1H), 7.91(d, J=4.0Hz, 1H), 7.58(d, J=4.8Hz, 1H), 7.17-7.20(m, 1H), 4.20(t, J=7.2Hz, 2H), 3.40(s, 3H), 2.73(t, J=8.0Hz, 2H), 2.16-2.24(m, 2H). HRMS (ESI): $[M+H]^+$ calculated for $[C_{13}H_{14}N_3O_3S_2^+]$, 324.0471, found 324.0469. UPLC purity 95%.

Scheme 3. Generalized synthesis route for compounds **10-16 and 18**



Reagents and conditions: (a) NaOH, EtOH/H_2O, microwave, 90℃, 2hrs. (b) $(CF_3SO_2)_2O$, DIEA, DCM, overnight. (c) $RB(OH)_2$, $Pd(dppf)Cl_2$, Na_2CO_3, dioxane/H_2O=2/1, 80℃, 6 hrs. (d) mCPBA, DCM, rt, 3hrs.

2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-ol (46) To a solution of ethyl 4,4,4-trifluoro-3-oxobutanoate(2g, 10.9mmol) and methyl carbamimidothioate(4g, 21.3mmol) in EtOH(10mL) was added 10N NaOH solution (2mL) dropwise under N_2 atmosphere. The reaction mixture was microwaved at 90°C for 2hrs. The reaction mixture was cooled to room temperature, then solvents were evaporated in vacuo. The residue was dissolved in H_2O and acidified to PH=2.0 with 1N HCl, then extracted by DCM 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further purified by recrystalization with DCM/PE to give 1.8g of 2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-ol as a white solid (**46**) in 78% yield. ^{1}H NMR (400 MHz, DMSO): δ 6.59(s, 1H), 2.51(s, 3H).

2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl trifluoro methane sulfonate (47) To a solution of **46** (100mg, 0.48mmol) and DIEA (184mg, 1.4mmol) in DCM (2ml) was added drop wise trifluoromethane sulfonic anhydride and (201mg, 0.71mmol) at 0℃and stirred at RT over night. The mixture was extracted with H_2O/DCM (50mL/50ml) 3 times, washed with brine (50mlx3) and dried with Na_2SO_4, and then concentrated in vacuo and purified by chromatography (PE/EA=10/1) to give the desired product as a light yellow oil, 2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl trifluoromethanesulfonate in 55% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.05 (s, 1H), 2.61(s, 3H).

2-(methylsulfonyl)-4-phenyl-6-(trifluoromethyl)pyrimidine (10) To a solution of **47** (50mg, 0.146mmol) and phenylboronic acid (18mg, 0.146mmol) in dioxane (2mL) was added $PdCl_2$(dppf) (10mg, 0.01mmol) followed by Na_2CO_3 (2N, 1mL) under N_2 atmosphere. The reaction mixture was refluxed at 90°C for 5hrs. The reaction mixture was cooled to room temperature and filtered over celite. Solvents were removed from the filtrate in vacuo, and the residue was extracted by

DCM/H_2O(20mL/20mL) 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further purified by silica gel column chromatography(PE/EA)=30/1 to give 2-(methylthio)-4-phenyl-6-(trifluoromethyl) pyrimidine as a light yellow solid in 76% yield.

mCPBA(48mg, 0.28mmol) was added to a solution of 2-(methylthio)-4-phenyl-6-(trifluoromethyl) pyrimidine (30mg, 0.11mmol) in DCM and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by DCM and satured $NaHCO_3$ solution 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=1/1) to give 42-(methylsulfonyl)-4-phenyl-6-(trifluoromethyl) pyrimidine as a light yellow solid in 36% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.26-8.24 (m, 2H), 8.20 (s, 1H), 7.57-7.67 (m, 3H), 3.480 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.26, 167.09, 157.22, 157.35, 133.79, 133.66, 131.07, 129.73, 128.98, 128.19, 121.47, 118.73, 113.77, 114.74, 39.25. HRMS (ESI): $[M+H]^+$ calculated for $[C_{12}H_{10}F_3N_2O_2S^+]$, 303.0409, found 303.0409. UPLC purity 95%.

2-(methylsulfonyl)-4-(o-tolyl)-6-(trifluoromethyl)pyrimidine (11) The titled compound (was prepared in a total yield of 4% as a light yellow soild from **47** (50mg, 0.146mmol) and o-tolylboronic acid (20mg, 0.146mmol) according to the procedure for **10**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.86(s, 1H), 7.58-7.61(m, 1H), 7.46-7.61(m, 1H), 7.37-7.41(m, 2H), 3.46(s, 3H), 2.56(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.24, 166.28, 157.07, 156.70, 137.48, 134.57, 132.08, 131.75, 130.37, 126.80, 121.26, 118.69, 118.52, 39.08, 20.83. HRMS (ESI): calculated for $[C_{13}H_{12}F_3N_2O_2S^+]$, 317.0566, found 317.0565. UPLC purity 95%.

2-(methylsulfonyl)-4-(m-tolyl)-6-(trifluoromethyl)pyrimidine (12) The titled compound was prepared in a yield of 4% as a light yellow soild from **47** (50mg, 0.146mmol) and m-tolylboronic acid (20mg, 0.146mmol) according to the procedure for **10**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.18(s, 1H), 8.06(s, 1H), 8.00-8.03(m, 1H), 7.46-7.68(m, 2H), 3.48(s, 3H), 2.49(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.48，167.04，157.56，157.19，139.74，134.64，133.64，129.59，128.71，125.37，121.50，118.76，114.81，114.78，39.26，21.62. HRMS (ESI): calculated for $[C_{13}H_{12}F_3N_2O_2S^+]$, 317.0566, found 317.0565. UPLC purity 95%.

2-(methylsulfonyl)-4-(p-tolyl)-6-(trifluoromethyl)pyrimidine (14) The titled compound was prepared in a yield of 4% as a light yellow soild from **47** (50mg, 0.146mmol) and p-tolylboronic acid (20mg, 0.146mmol) according to the procedure for **10**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15(d, J=8.4Hz, 2H), 8.14(s, 1H), 7.38(d, J=8.4Hz, 2H), 3.47(s, 3H), 2.48(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.96, 166.85, 157.31, 156.94, 144.81, 130.76, 130.30, 127.98, 114.13, 39.06, 21.7. HRMS (ESI): calculated for $[C_{13}H_{12}F_3N_2O_2S^+]$, 317.0566, found 317.0566. UPLC purity 95%.

Methyl 2-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl) benzoate (14) The titled compound was prepared in a yield of 25% as a light yellow soild from **47** (50mg, 0.146mmol) and 2-(methoxycarbonyl)phenylboronic acid (26mg, 0.146mmol) according to the procedure for **10**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 7.96-8.00(m, 1H), 7.97(s, 1H), 7.63-7.73(m, 3H), 3.81(s, 3H),

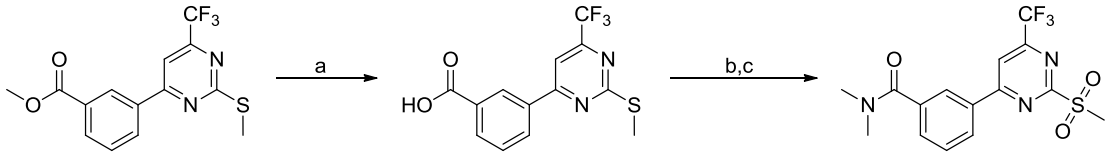
3.42(s, 3H). LC-MS (ESI) *m/z*: calcd for [$C_{14}H_{12}F_3N_2O_4S^+$], 361.0, found 361.4. UPLC purity 95%.

Methyl 3-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl) benzoate (15) The titled compound was prepared in a yield of 26% as a light yellow soild from **47** (50mg, 0.146mmol) and 3-methoxycarbonyl)phenylboronic acid (26mg, 0.146mmol) according to the procedure for **10**. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.17(s, 1H), 7.76-7.79(m, 2H), 7.50(t, J=8.4Hz, 1H), 7.18(dd, J=3.4, 8.4Hz, 1H), 3.93(s, 3H), 3.48(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.83, 170.89, 167.37, 136.70, 132.01, 131.11, 131.07, 130.61, 130.18, 116.55, 116.52, 52.63, 40.30. HRMS (ESI): calculated for [$C_{14}H_{12}F_3N_2O_4S^+$], 361.0464, found 361.0464. UPLC purity 95%.

Methyl 4-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl) benzoate (16) The titled compound was prepared in a yield of 13% as a light yellow soild from **47** (50mg, 0.146mmol) and 4-methoxycarbonyl-phenylboronic acid (26mg, 0.146mmol) according to the procedure for **10**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31-8.34(m, 2H), 8.23-8.26(m, 2H), 8.25(s, 1H), 3.99(s, 3H), 3.49(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 168.23, 167.24, 166.04, 137.39, 134.54, 131.95, 130.75, 128.19, 121.18, 115.33, 52.56, 39.03. HRMS (ESI): calculated for [$C_{14}H_{12}F_3N_2O_4S^+$], 361.0464, found 361.0464. UPLC purity 95%.

2-(methylsulfonyl)-4-(3-phenoxyphenyl)-6-(trifluoromethyl)pyrimidine (18) The titled compound was prepared in a yield of 21% as a light yellow soild from **47** (50mg, 0.146mmol) and (3-phenoxyphenyl)boronic acid (41 mg, 0.14 mmol) according to the procedure for **10**. ^{1}H NMR:(400Mz, CDCl3): δ 8.13 (s, 1H), 7.95 (dd, *J*=0.8, 7.6 Hz, 1H), 7.87 (s, 1H), 7.54 (t, *J*=8.0 Hz, 1H), 7.39 (t, *J*=8.0 Hz, 2H), 7.18 (dt, *J*=0.8, 7.6 Hz, 2H), 7.06 (d, *J*=8.0 Hz, 2H), 3.44 (s, 3H). LC-MS (ESI) *m/z* calculated for [$C_{18}H_{14}F_3N_2O_3S^+$]: 395.4, found 395.3. UPLC purity 95%.

Scheme 4. Generalized synthesis route for compounds **17**



15 **48** **17**

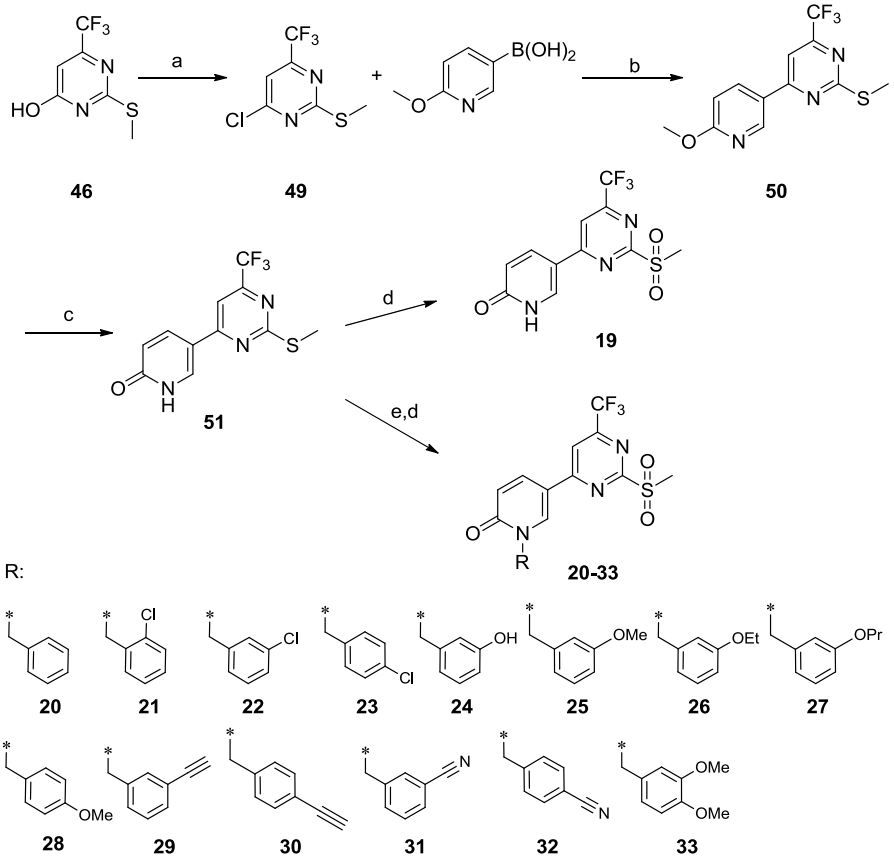
Reagents and conditions: (a) 4N NaOH, THF, RT, 4hrs. (b) dimethylamine, DIPEA, HATU, RT, 16hrs. (c) mCPBA, DCM, rt, 3hrs.

3-(2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl)benzoic acid (48) 4N NaOH(aq, 1mL)was added to a solution of 15 (100mg, 0.305mmol) in THF (8mL) dropwise. The reaction mixture was then stirred at room temperature for 4hrs. Solvents were evaporated in vacuo, and the residue was extracted by EtOAC and H_2O 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4, concentrated and further purified by silica gel column chromatography to give 95mg of **48** as a white solid.

N,N-dimethyl-3-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)benzamide (17) To a solution of **48** (95mg, 0. 3mmol) in DMF (1mL) was added dimethylamine (1eq), DIPEA(2.5eq), HATU(1.2eq) and the reaction mixture was stirred at room temperature overnight. Solvents were evaporated in vacuo, and the residue was extracted by EtOAC and H_2O 3 times. The organic layer was combined, washed with brine, dried over Na2SO4, concentrated and further purified by silica gel column chromatography to give 100 mg of N,N-dimethyl-3-(2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl)benzamide (89%) as a white solid.

mCPBA(24mg, 0.14mmol) was added to a solution of N,N-dimethyl-3-(2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl)benzamide (20mg, 0.06mmol) in DCM and the reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was extracted by DCM and satured $NaHCO_3$ solution 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further pufrified by silica gel column chromatography(PE/EA=1/1) to give **17** as a light yellow solid in 25% yield. ^{1}H NMR (400 MHz, DMSO): δ 8.97(s, 1H), 8.49-8.53(m, 1H), 8.46(s, 1H), 7.70-7.72(m, 2H), 3.56(s, 3H), 3.04(s, 3H), 2.94(s, 3H). LC-MS (ESI) *m/z*: calcd for [$C_{15}H_{15}F_3N_3O_3S^+$], 374.0, found 374.5. UPLC purity 95%.

Scheme 5. Generalized synthesis route for compounds **19-33**



Reagents and conditions: (a) $POCl_3$, 120℃, 3hrs. (b) ($Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O=2/1, 90℃, 6hrs. (c) HBr(aq)/EtOH=10/3 120℃, 3 hrs. (d) Oxone, MeOH/H_2O, rt, 3hrs. (e), NaH, RX, DMF, 0℃ to rt, 3hr.

4-chloro-2-(methylthio)-6-(trifluoromethyl)pyrimidine (49) A solution of **46** (1.4g, 6.67mmol) in $POCl_3$(15mL) was refluxed at 120^{o}C for 3hrs. The reaction mixture was cooled to room temperature, and $POCl_3$ was removed in vacuo. The residue was extracted by DCM and icy H_2O 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and concentrated to give 4-chloro-2-(methylthio)-6-(trifluoromethyl)pyrimidine as a light yellow oil in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$): 7.27(s, 1H), 2.60(s, 3H).

4-(6-methoxypyridin-3-yl)-2-(methylthio)-6-(trifluoromethyl) pyrimidine (50) To a solution of **49** (1g, 4.4mmol) and (6-methoxypyridin-3-yl)boronic acid(0.8g, 5.3mmol) in dioxane/H_2O(10mL/5mL) was added $Pd(PPh_3)_4$ (250mg, 0.22mmol) followed by Na_2CO_3 (930mg, 8.7mmol) under N_2 atmosphere. The reaction mixture was refluxed at 90^{o}C for 5hrs. The reaction mixture was cooled to room temperature and filtered over celite. Solvents were removed from the filtrate in vacuo, and the residue was extracted by DCM and H_2O 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further purified by silica gel column chromatography(PE/EA)=30/1 to give 4-(6-methoxypyridin-3-yl)-2-(methylthio)-6-(trifluoromethyl) pyrimidine as a colorless oil in 70% yield. ^{1}H NMR (400 MHz, CDCl3-d_6): δ 8.94 (d, *J*=2.4 Hz, 1H), 8. 32 (dd, *J*=2.4, 8.8 Hz, 1H), 7.56 (s, 1H), 6.87 (d, *J*=8.8 Hz, 1H), 4.62 (s, 3H), 2.65 (s, 3H).

5-(2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl) pyridin-2(1H)-one (51) To a solution of **50** (917mg, 3.05mmol) in EtOH(10mL) was added 3mL HBr(aq) dropwise. The reaction mixture was refluxed at 120^{o}C for 3 hrs. The reaction mixture was cooled to room temperature, and alkalized to pH=6.0 with statured Na_2CO_3 solution. EtOH was then removed from the mixtureby evaporation in vacuo, then the mixture was filtered. The solid part was dried to give 5-(2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one as a white solid in 98% yield. ^{1}H NMR (400 MHz, DMSO): δ 8.61 (d, *J*=2.8 Hz, 1H), 8. 33 (dd, *J*=2.8, 9.6 Hz, 1H), 8.13 (s, 1H), 6.49 (d, *J*=9.6 Hz, 1H), 2.60 (s, 3H).

5-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (19) An aqueous solution of Oxone (5eq) was added dropwise to a solution of **51** (100mg, 0.348mmol) in MeOH. The reaction mixture was stirred at room temperature for 8hrs. Solvents were evaporated from the reaction mixture, then the residue was extracted by EtOAc/H_2O 3 times. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further purified by silica gel column chromatography(PE/EA=6/1) to give 5-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (**19**) in a yield of 63%

as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 12.58(s, 1H), 8.80(d, J=2.8Hz, 1H), 8.73(s, 1H), 8.41(dd, J=2.8, 9.6Hz, 1H), 6.53(d, J=9.6Hz, 1H), 3.52(s, 3H). ^{13}C NMR (100 MHz, DMSO): 165.76, 165.44, 162.45, 155.18, 154.83, 140.68, 138.27, 121.72, 120.21, 118.99, 114.13, 114.10, 112.53, 39.01. HRMS (ESI): calculated for [$C_{11}H_9F_3N_3O_3S^+$], 320.0311, found 320.0311. UPLC purity 95%.

1-Benzyl-5-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (20) To a solution of **51** (100 mg, 0.32 mmol) in anhydrous DMF (10 mL) was added NaH (8.4 mg, 0.35 mmol) at 0^{o}C and then stirred for 30 min. Then to the mixture was added benzyl bromide (66 mg, 0.38 mmol) and stirred at RT for 5h. Then the reaction mixture was poured into water and extracted with EA (3×20 mL) and the organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (200~300 mesh silica gel, eluted with PE/EtOAc = 10:1) to give the product as a white solid in 57% yield.

To a solution of 1-benzyl-5-(2-(methylthio)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (10 mg, 0.03 mmol) in DCM (2 mL) was added m-CPBA (10 mg, 0.06 mmol) at RT. Then the mixture was stirred at RT for 3 h. The solvent was removed and extracted with DCM (3×15ml). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by Pre-TLC (EtOAc/PE= 1:2) to obtain **20** as a white solid in 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, *J*=2.8 Hz, 1H), 7.99 (dd, *J*=2.8, 9.6 Hz, 1H), 7.82 (s, 1H), 7.41-7.34 (m, 5H), 6.76 (d, *J*=9.6 Hz, 1H), 5.27 (s, 2H), 3.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.89, 165.57, 162.09, 157.41, 157.04, 141.69, 136.06, 135.11, 131.04, 129.29, 128.96, 128.74, 128.35, 121.49, 113.28, 112.35, 112.32, 53.44, 39.08. HRMS (ESI): calculated for [$C_{18}H_{15}F_3N_3O_3S^+$], 410.0781, found 410.0782. UPLC purity 95%.

1-(2-chlorobenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl) pyridin-2(1H)-one (21) The titled compound was prepared as a white solid in 18% yield from **51** (20mg, 0.07mmol) and 1-(bromomethyl)-2-chlorobenzene (16mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, $CDCl_3$): δ 8.67 (d, *J*=2.8 Hz, 1H), 8.00 (dd, *J*=2.8, 9.6 Hz, 1H), 7.86 (s, 1H), 7.33-7.30 (m, 3H), 7.25-7.22 (m, 1H), 6.76 (d, *J*=9.6 Hz, 1H), 5.24 (s, 2H), 3.42 (s, 3H). Mass(m/z): 444.5 $[M+H]^+$. HRMS (ESI): calculated for [$C_{18}H_{14}ClF_3N_3O_3S^+$], 444.0391, found 444.0397. UPLC purity 95%.

1-(3-chlorobenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (22) The titled compound was prepared as a white solid in 18% yield from **51** (20mg, 0.07mmol) and 1-(bromomethyl)-3-chlorobenzene (16mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, $CDCl_3$): δ 8.72 (d, *J*=2.8 Hz, 1H), 8.01 (dd, *J*=2.8, 9.6 Hz, 1H), 7.81 (s, 1H), 7.45-7.42 (m, 1H), 7.34-7.28 (m, 3H), 6.75 (d, *J*=9.6 Hz, 1H), 5.37 (s, 2H), 3.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.98, 165.47, 162.07, 157.63, 157.26, 141.97, 141.93, 136.15, 133.86, 132.52, 130.74, 130.23, 127.71, 121.53, 113.26, 112.22, 51.13, 39.07. HRMS (ESI): calculated for [$C_{18}H_{14}ClF_3N_3O_3S^+$], 444.0391, found 444.0399. UPLC purity

95%.

1-(4-chlorobenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (23) The titled compound was prepared as a white solid in 21% yield from **51** (20mg, 0.07mmol) and 1-(bromomethyl)-4-chlorobenzene (16mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, $CDCl_3$): δ 8.69 (d, *J*=2.4 Hz, 1H), 8.02 (dd, *J*=2.4, 9.6 Hz, 1H) , 7.86 (s, 1H), 7.34-7.27 (m, 4H), 6.76 (d, *J*=9.6 Hz, 1H), 5.24 (s, 2H), 3.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.55, 161.95, 141.65, 141.62, 136.09, 134.82, 133.67, 132.27, 129.73, 129.71, 129.47, 121.69, 113.39, 112.34, 112.31, 112.28, 53.09, 39.12, 31.09, 29.35. HRMS (ESI): calculated for [$C_{18}H_{14}ClF_3N_3O_3S^+$], 444.0391, found 444.0393. UPLC purity 95%.

1-(3-hydroxybenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (24) The titled compound was prepared as a white solid in 18% yield from **51** (20mg, 0.07mmol) and (3-(bromomethyl)phenoxy)(tert-butyl)dimethylsilane (24mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR(400Hz, $CDCl_3$) δ 8.66(d, J=2.4Hz, 1H), 7.97(dd, J=2.4, 9.6Hz, 1H), 7.83(s, 1H), 7.24(d, J=8.0Hz, 1H), 6.92(d, J=9.6Hz, 1H), 6.84-6.86(m, 1H), 6.80-6.83(m, 1H), 6.74(d, J=9.6Hz, 1H), 5.20(s, 2H), 3.41(s, 3H). ^{13}C NMR (100 MHz, DMSO): 165.82, 165.21, 161.27, 157.57, 143.81, 137.90, 137.58, 129.68, 119.90, 117.98, 114.64, 114.21, 114.17, 114.15, 114.12, 113.03, 52.32, 39.02. HRMS (ESI): calculated for [$C_{18}H_{15}F_3N_3O_4S^+$], 426.0730, found 426.0736. UPLC purity 95%.

1-(3-methoxybenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (25) The titled compound was prepared as a white solid in 14% yield from **51** (20mg, 0.07mmol) and 1-(bromomethyl)-3-methoxybenzene (16mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, $CDCl_3$): δ 8.64 (d, *J*=2.8 Hz, 1H), 8.01 (dd, *J*=2.8, 9.6 Hz, 1H), 7.82 (s, 1H), 7.31-7.26 (m, 1H), 6.92-6.86 (m, 3H), 6.75 (d, *J*=9.6 Hz, 1H), 5.23 (s, 2H), 3.80 (s, 3H) , 3.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.90, 165.55, 162.07, 160.28, 157.46, 157.09, 141.70, 141.64, 136.58, 136.01, 130.46, 130.38, 121.52, 120.41, 114.08, 113.25, 112.28, 55.53, 55.43, 53.22, 39.10. HRMS (ESI): calculated for [$C_{19}H_{17}F_3N_3O_4S^+$], 440.0886, found 440.0892. UPLC purity 95%.

1-(3-ethoxybenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (26) The titled compound was prepared as a white solid in 7% yield from **51** (20mg, 0.07mmol) and 1-(bromomethyl)-3-ethoxybenzene (17mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR(400Hz, $CDCl_3$) δ 8.62(d, J=2.4Hz, 1H), 7.99(dd, J=2.4, 9.6Hz, 1H), 7.81(s, 1H), 7.28(dd, J=7.6, 8.8Hz, 1H), 6.84-6.91(m, 3H), 6.75(d, J=9.6Hz, 1H), 5.23(s, 2H), 4.02(q, J=6.8Hz, 2H), 3.39(s, 3H), 1.40(t, J=6.8Hz, 3H). UPLC purity 95%.

5-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)-1-(3-propoxybenzyl)pyridin-2(1H)-one (27) The titled compound was prepared as a white solid in 8% yield from **51** (20mg, 0.07mmol) and 1-(bromomethyl)-3-propoxybenzene (18mg, 0.08mmol) according to the

procedure for **20**. ^{1}H NMR(400Hz, CDCl3) δ 8. 62(dd, J=2.4Hz, 1H), 8.00(dd, J=2.4, 9.6Hz, 1H), 7.81(s, 1H), 7.15-7.30(m, 1H), 6.85-6.91(m, 3H), 6.76(d, J=9.6Hz, 1H), 5.23(s, 2H), 3.91(t, J=6.4Hz, 2H), 3.39(s, 3H), 1.74-1.84(m, 2H), 1.22(t, J=7.6Hz, 3H). HRMS (ESI): calculated for [$C_{21}H_{21}F_3N_3O_4S^+$], 468.1199, found 468.1205. UPLC purity 95%.

1-(4-Methoxybenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (28) The titled compound was prepared in a yield of 11% as a white solid from **51** (30 mg, 0.10 mmol) and 1-(chloromethyl)-4-methoxybenzene (22 mg, 0.11 mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, $CDCl_3$): δ 8.63 (d, *J*=2.0 Hz, 1H), 7.97 (dd, *J*=2.0, 9.6 Hz, 1H), 7.81 (s, 1H), 7.32 (d, *J*=8.4 Hz, 2H), 6.90 (d, *J*=8.4 Hz, 2H), 6.73 (d, *J*=9.6 Hz, 1H), 5.19 (s, 2H), 3.79 (s, 3H), 3.39 (s, 3H). Mass(m/z): 440.2 $[M+H]^+$. ^{13}C NMR (100 MHz, $CDCl_3$): 166.94, 165.61, 165.14, 162.14, 160.06, 157.08, 141.47, 135.85, 130.16, 130.12, 127.04, 121.49, 118.62, 114.71, 113.12, 112.14, 55.54, 53.06, 39.07. HRMS (ESI): calculated for [$C_{19}H_{17}F_3N_3O_4S^+$], 440.0886, found 440.0892. UPLC purity 95%.

1-(3-ethynylbenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl) pyridin-2(1H)-one (29): The titled compound was prepared in a yield of 19% as a yellow solid from **51** (60mg, 0.2mmol) and 1-(bromomethyl)-3-ethynylbenzene (38mg, 0.19mmol) according to the procedure for **20**. ^{1}H NMR(400Hz, CDCl3) δ 8.01(dd, J=2.8, 9.6Hz, 1H), 7.87(s, 1H), 7.71(dd, J=3.2, 6Hz, 1H), 7.52(dd, J=3.6, 5.6 Hz, 1H), 7.56(s, 1H), 7.34~7.32(m, 2H), 6.75(d, J=9.6Hz, 1H), 5,34(s, 2H), 3.40(s, 3H), 3.08(s, 1H). HRMS (ESI): calculated for [$C_{20}H_{15}F_3N_3O_3S^+$], 434.0781, found 434.0794. UPLC purity 95%.

1-(4-ethynylbenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl) pyridin-2(1H)-one (30) The titled compound was prepared in a yield of 64% as a yellow solid from **51** (130mg, 0.45mmol) and 1-(bromomethyl)-4-ethynylbenzene (80mg, 0.41mmol) according to the procedure for **20**. ^{1}H NMR(400Hz, $CDCl_3$) δ 8.68(d, J=2.4Hz, 1H), 8.00(dd, J=2.4, 9.6Hz, 1H), 7.88(s, 1H), 7.45(d, J=8.0Hz, 2H), 7.29(d, J=8.0Hz, 2H), 6.72(d, J=9.6Hz, 1H), 5.23(s, 2H), 3.39(s, 3H), 3.08(s, 1H). HRMS (ESI): calculated for [$C_{20}H_{15}F_3N_3O_3S^+$], 434.0781, found 434.0794. UPLC purity 95%.

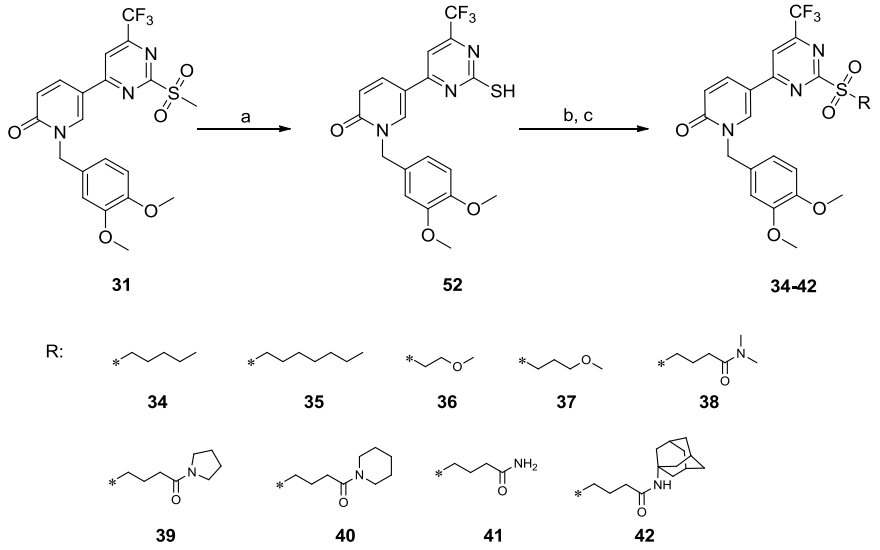
3-((5-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)-2-oxopyridin-1(2H)-yl)methyl)benzonitrile (31) The titled compound was prepared as a white solid in 13% yield from **51** (20mg, 0.07mmol) and 3-(bromomethyl)benzonitrile (16mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, DMSO): δ 9.30 (d, J=2.4Hz, 1H), 8.69 (s, 1H), 8.46 (dd, J=2.4, 9.6Hz, 1H), 7.84 (s, 1H), 7.79 (d, J=8.4Hz, 1H), 7.70 (d, J=8.0Hz, 1H), 7.59 (t, J=8.0Hz, 1H), 6.65 (d, J=9.6Hz, 1H), 5.30 (s, 2H), 3.55 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ165.84, 165.26, 161.25, 155.23, 154.87, 144.02, 138.00, 137.85, 132.67, 131.57, 131.28, 129.88, 119.91, 118.59, 114.26, 114.23, 113.29, 111.51, 52.27, 39.02. HRMS (ESI): calculated for [$C_{19}H_{14}F_3N_4O_3S^+$], 435.0733, found 435.0765. UPLC purity 95%.

4-((5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)-2- oxopyridin- 1(2H)-yl)methyl)

benzonitrile (32) The titled compound was prepared as a white solid in 16% yield from **51** (20mg, 0.07mmol) and 4-(bromomethyl)benzonitrile (16mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, DMSO): δ 9.31 (d, J=2.4Hz, 1H), 8.69 (s, 1H), 8.47 (dd, J=2.4, 9.6Hz, 1H), 7.84 (d, J=8.0Hz, 2H), 7.51 (d, J=8.0Hz, 2H), 6.66 (d, J=9.6Hz, 1H), 5.34 (s, 2H), 3.55 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ165.84, 165.21, 161.22, 155.28, 154.92, 144.03, 142.05, 137.89, 132.56, 128.34, 121.70, 119.93, 118.97, 118.65, 114.24, 114.21, 113.29, 110.44, 52.55, 39.03. HRMS (ESI): calculated for [$C_{19}H_{14}F_3N_4O_3S^+$], 435.0733, found 435.0761. UPLC purity 95%.

1-(3,4-dimethoxybenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl) pyrimidin-4-yl)pyridin-2(1H)-one (33) The titled compound was prepared as a white solid in 16% yield from **51** (20mg, 0.07mmol) and 4-(bromomethyl)-1,2-dimethoxybenzene (19mg, 0.08mmol) according to the procedure for **20**. ^{1}H NMR:(400Mz, $CDCl_3$): δ 8.68 (d, *J*=2.4 Hz, 1H), 7.98 (dd, *J*=2.4, 9.6 Hz, 1H) , 7.82 (s, 1H), 6.94 (s, 1H), 6.92 (d, *J*=8.0 Hz, 1H), 6.84 (d, *J*=8.0 Hz, 1H), 6.73 (d, *J*=9.6 Hz, 1H), 5.18 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.84, 165.58, 162.15, 157.37, 149.59, 141.60, 141.55, 135.94, 127.66, 121.37, 121.13, 113.21, 112.28, 112.25, 111.85, 111.47, 56.19, 56.10, 56.01, 53.12, 39.04, 39.02. HRMS (ESI): calculated for [$C_{20}H_{19}F_3N_3O_5S^+$], 470.0992, found 470.0986. UPLC purity 95%.

Scheme6. Generalized synthesis route for compounds **34-42**



Reagents and conditions: (a), NaSH, DMF, 0℃ to rt, 3hr. (b), RX, K_2CO_3, DMF, 0℃ to rt, 3hr. (c), Oxone, MeOH/H_2O, rt, 3hrs.

1-(3,4-dimethoxybenzyl)-5-(2-mercapto-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (52) NaHS(28mg, 0.38mmol) was added to a solution of 1-(3,4-dimethoxybenzyl)-5-(2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-

one (**33**) (60mg, 0.13mmol) in DMF(2mL) under N_2 atmosphere. The reaction mixture was stirred at room temperature for 2hrs. The reaction mixture was acidified to PH=5 by 1N HCl, then extracted by DCM/H_2O. The organic layer was combined, washed with brine, dried over Na_2SO_4 and further purified by silica gel column chromatography(PE/EA=1/1) to give 50mg of 1-(3,4-dimethoxybenzyl)-5-(2-mercapto-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one as a yellow solid (98%).

1-(3,4-dimethoxybenzyl)-5-(2-(pentylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (**34**) The titled compound was prepared in a yield of 77% (21mg, 0.04mmol) as a white soild from **52** (22 mg, 0.052 mmol) and 1-bromopentane (12mg, 0.078mmol) according to the procedure for **1.** ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.69 (d, J=2.8Hz, 1H), 7,97 (dd, J=2.4, 9.6Hz, 1H), 7.81 (s, 1H), 6.95-6,91(m, 2H), 6.83(d, 1H, J=8.4Hz), 6.74(d,1H,J9.6Hz), 5.19(s, 2H), 3.87(s, 3H), 3.86(s, 3H), 3.58-3.54(m, 2H), 1.94-1.96(m, 2H), 1.50-1.32(m, 4H), 0.91(t, 3H, J=7.2Hz). HRMS (ESI): calculated for [$C_{24}H_{27}F_3N_3O_5S^+$], 526.1618, found 526.1619. UPLC purity 95%.

1-(3,4-dimethoxybenzyl)-5-(2-(heptylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (**35**) The titled compound was prepared in a yield of 46% (13mg, 0.023mmol) as a white soild from **52** (21 mg, 0.05 mmol) and 1-bromo-3-methoxypropane (11mg, 0.075mmol) according to the procedure for **1.** ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.69 (d, J = 2.4 Hz, 1H), 7.98 (dd, J = 2.8, 9.6 Hz, 1H),7.79 (s, 1H), 6.94-6.90 (m, 2H), 6.84 (dm J = 8.4 Hz, 1H), 6.75 (d, J = 9.6 Hz, 1H), 5.19 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.56 (t, J = 8.0 Hz, 2H), 1.90-1.84 (m, 2H), 1.34-1.24 (m, 6H), 0.86 (t, J = 6.8 Hz, 3H). HRMS (ESI): calculated for [$C_{26}H_{31}F_3N_3O_5S^+$], 554.1931, found 554.1963. UPLC purity 95%.

1-(3,4-dimethoxybenzyl)-5-(2-((2-methoxyethyl)sulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl) pyridin-2(1H)-one (**36**) The titled compound was prepared in a yield of 68% (21mg, 0.041mmol) as a white soild from **52** (24 mg, 0.06 mmol) and1-bromo-2-methoxyethane (12mg, 0.09mmol) according to the procedure for **1.** ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 2.8 Hz, 1H), 8.01 (dd, J = 2.8, 9.6 Hz, 1H), 7.81 (s, 1H), 6.95-6.92 (m, 2H), 6.85 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 9.6 Hz, 1H), 5.19 (s, 2H), 3.59-3.85 (m, 8H), 3.80 (t, J = 5.6 Hz, 2H), 3.16 (s, 3H). HRMS (ESI): calculated for [$C_{22}H_{23}F_3N_3O_6S^+$], 514.1254, found 514.1262. UPLC purity 95%.

1-(3,4-dimethoxybenzyl)-5-(2-((3-methoxypropyl)sulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (**37**) The titled compound was prepared in a yield of 80% (21mg, 0.04mmol) as a white soild from **52** (21 mg, 0.05 mmol) and 1-bromo-3-methoxypropane (11mg, 0.075mmol) according to the procedure for **1.** ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.70 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 2.4, 9.6 Hz, 1H), 7.81 (s, 1H), 6.96-6.91 (m, 2H), 6.85 (d, J = 8.4 Hz, 1H), 6.74 (d, J = 9.6 Hz, 1H), 5.19 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.69 (t, J = 7.6 Hz, 2H), 3.52 (t, J = 5.6 Hz, 2H), 3.30 (s, 3H), 2.21-2.14 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ166.57, 165.49, 162.14, 157.88, 157.51, 157.21, 156.76, 149.61, 149.53, 141.58, 135.96, 127.86, 121.44, 121.05, 113.29, 112.13, 111.81,

111.50, 70.17, 58.69, 56.16, 56.07, 53.15, 48.54, 32.06, 29.79, 23.00, 14.25. HRMS (ESI): calculated for [$C_{23}H_{25}F_3N_4O_6S^+$], 528.1411, found 528.1415. UPLC purity 95%.

4-((4-(1-(3,4-dimethoxybenzyl)-6-oxo-1,6-dihydropyridin-3-yl)-6-(trifluoromethyl)pyrimidin-2-yl)sulfonyl)-N,N-dimethylbutanamide (**38**) The titled compound was prepared in a yield of 15% (3mg, 0.0053mmol) as a white solid from **52** (15 mg, 0.035mmol) and 4-bromo-N,N-dimethylbutanamide (21mg, 0.11mmol) according to the procedure for **1.** ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.96 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 2.8, 9.6 Hz, 1H), 7.62 (s, 1H), 7.02 (d, J = 2.0 Hz, 1H), 6.99 (dd, J = 2.0, 8.0 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.78 (d, J = 9.6 Hz, 1H), 5.28 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.75 (t, J = 7.2 Hz, 2H), 3.03 (s, 3H), 2.93 (s, 3H), 2.62 (t, J = 6.8 Hz, 2H), 2.30-2.24 (m, 2H). HRMS (ESI): calculated for [$C_{25}H_{28}F_3N_4O_6S^+$], 569.1676, found 569.1699. UPLC purity 95%.

1-(3,4-dimethoxybenzyl)-5-(2-((4-oxo-4-(pyrrolidin-1-yl)butyl)sulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (**39**) The titled compound was prepared in a yield of 28% (9mg, 0.015mmol) as a colorless oil from **52** (23mg, 0.054mmol) and 4-bromo-1-(pyrrolidin-1-yl)butan-1-one (36mg, 0.16mmol) according to the procedure for **1**. ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.07 (d, J = 2.8 Hz, 1H), 7.96 (dd, J = 2.8, 9.6 Hz, 1H), 7.82 (s, 1H), 7.04 (d, J = 2.0 Hz, 1H), 7.01 (dd, J = 2.0, 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 9.6 Hz, 1H), 5.29 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81-3.77 (m, 2H), 3.40 (t, J = 6.8 Hz, 4H), 2.54 (t, J = 6.4 Hz, 2H), 2.29-2.22 (m, 2H), 1.96 (q, J = 6.4 Hz, 2H), 1.87-1.82 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ169.76, 166.46, 165.45, 162.34, 158.44, 157.96, 157.59, 157.27, 149.61, 149.37, 142.51, 135.92, 128.39, 121.38, 121.30, 113.28, 112.11, 111.55, 56.22, 56.17, 53.09, 50.83, 46.77, 46.07, 32.78, 29.93, 26.27, 24.59, 18.52. HRMS (ESI): calculated for [$C_{27}H_{30}F_3N_4O_6S^+$], 595.1833, found 595.1853. UPLC purity 95%.

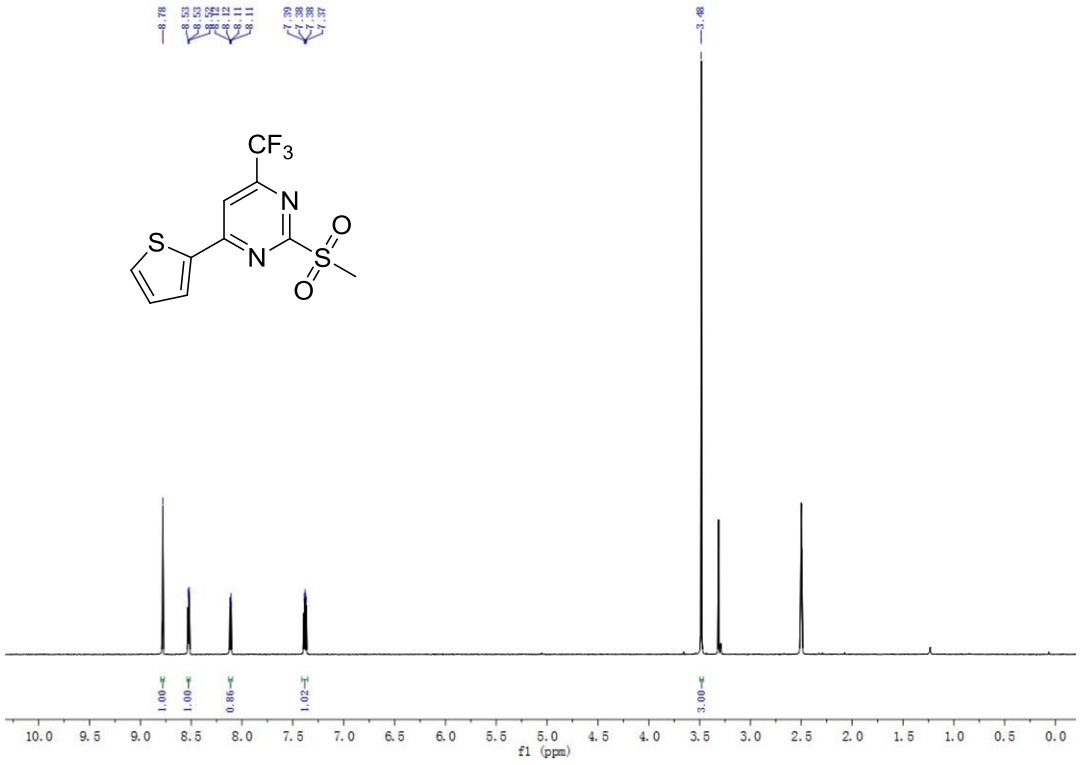
1-(3,4-dimethoxybenzyl)-5-(2-((4-oxo-4-(piperidin-1-yl)butyl)sulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl)pyridin-2(1H)-one (**40**) The titled compound was prepared in a yield of 75% (27mg, 0.044mmol) as a white solid from **52** (25mg, 0.059mmol) and 4-bromo-1-(piperidin-1-yl)butan-1-one (41mg, 0.18mmol) according to the procedure for **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, J = 2.8 Hz, 1H), 7.98 (dd, J = 2.8, 9.6 Hz, 1H), 7.83 (s, 1H), 7.01 (d, J = 2.0 Hz, 1H), 6.97 (dd, J = 2.0, 8.0 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 9.6 Hz, 1H), 5.25 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.77-3.73 (m, 2H), 3.48 (m, 2H), 3.37 (m, 2H), 2.58 (t, J = 6.8 Hz, 2H), 2.28-2.21 (m, 2H), 1.65-1.59 (m, 2H), 1.54 (m, 2H), 1.47 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ169.28, 166.45, 165.34, 162.17, 157.82, 157.49, 157.08, 149.44, 149.32, 142.24, 135.95, 132.47, 131.03, 128.99, 128.15, 121.27, 121.09, 113.21, 112.13, 111.95, 111.45, 71.94, 56.11, 56.05, 53.05, 50.82, 31.32, 24.53, 19.23, 18.70. HRMS (ESI): calculated for [$C_{28}H_{32}F_3N_4O_6S^+$], 609.1989, found 609.2019. UPLC purity 95%.

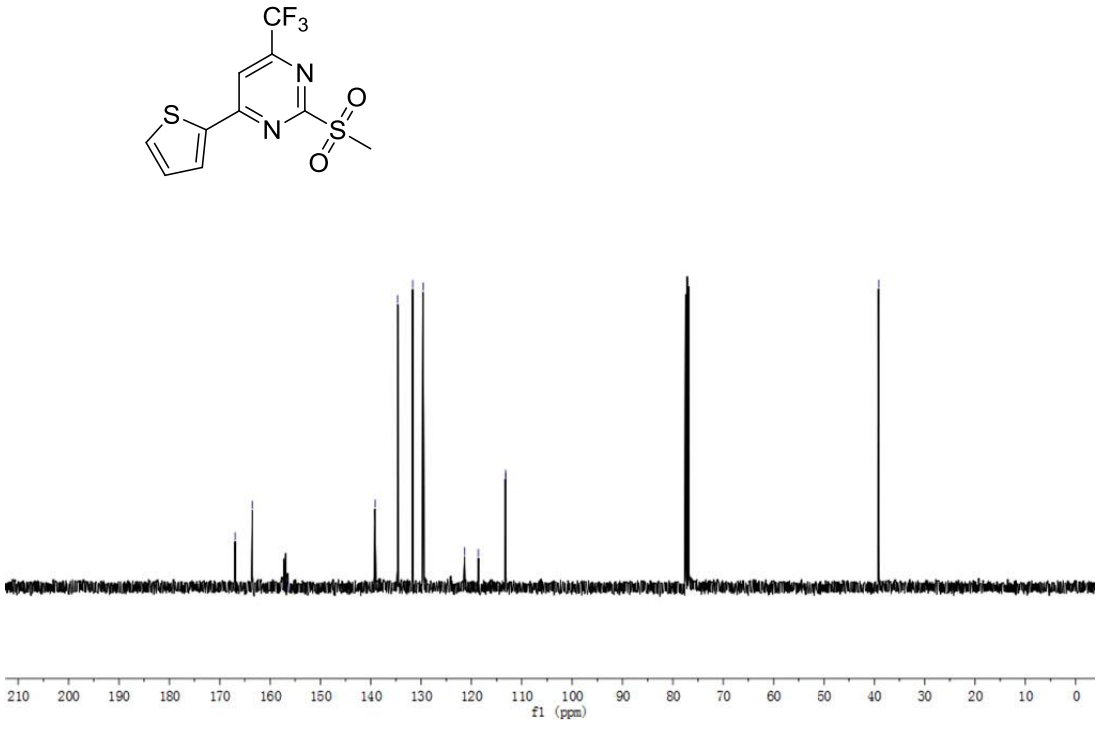
4-((4-(1-(3,4-dimethoxybenzyl)-6-oxo-1,6-dihydropyridin-3-yl)-6-(trifluoromethyl)pyrimidin-2-yl)sulfonyl)butanamide (**41**) The titled compound (9mg, 35% yield) was prepared as a white

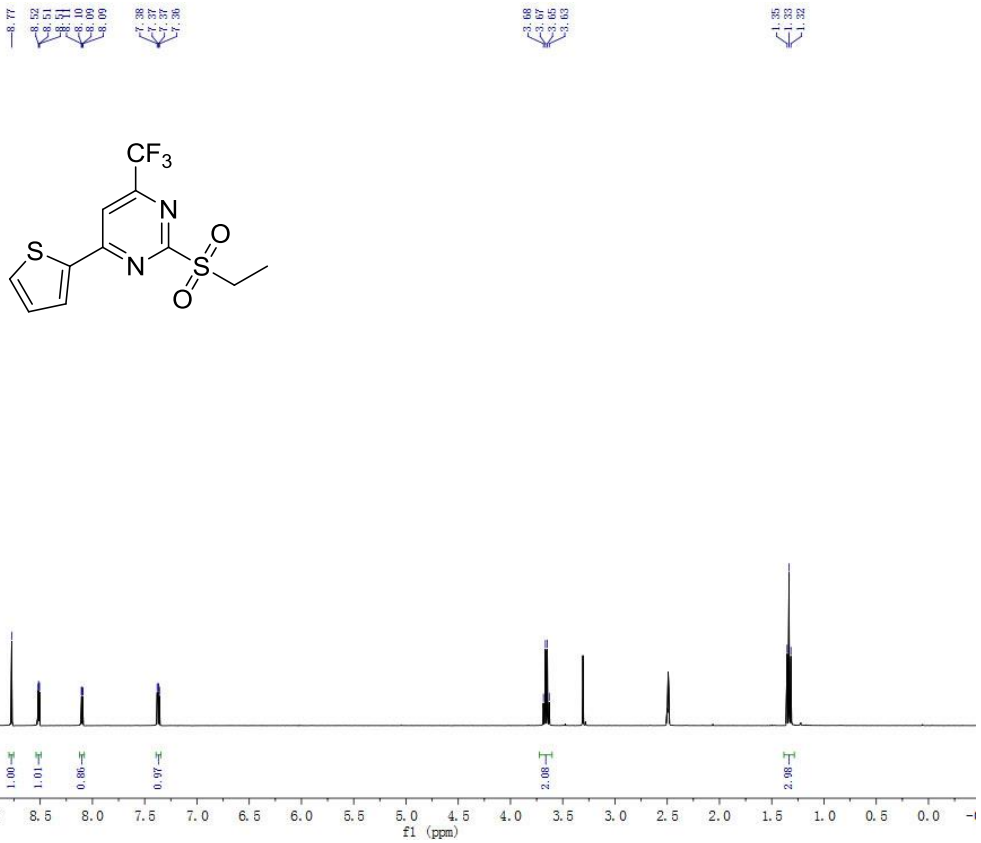
solid from **52** (20mg, 0.047mmol) and4-bromobutanamide (24mg, 0.14mmol) according to the procedure for **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, J = 2.4 Hz, 1H), 7.97 (dd, J = 2.8, 10.0 Hz, 1H), 7.82 (s, 1H), 6.98 (m, 1H), 6.96-6.94 (m, 1H), 6.86 (d, J = 8.4 Hz, 1H), 6.74 (d, J = 10.0 Hz, 1H), 5.63 (br, 1H), 5.54 (br, 1H), 5.23 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.70 (t, J = 7.2 Hz, 2H), 2.50 (t, J = 6.4 Hz, 2H), 2.29-2.21 (m, 2H). HRMS (ESI): calculated for [$C_{23}H_{24}F_3N_4O_6S^+$], 541.1363, found 541.1368. UPLC purity 95%.

N-((3s,5s,7s)-adamantan-1-yl)-4-((4-(1-(3,4-dimethoxybenzyl)-6-oxo-1,2,3,6-tetrahydropyridin-3-yl)-6-(trifluoromethyl)pyrimidin-2-yl)sulfonyl)butanamide (**42,** also named as **TC9-305**) The titled compound (9mg, 16% yield) was prepared as a white solid from **52** (40mg, 0.094mmol) and N-((1S,3s)-adamantan-1-yl)-4-bromobutanamide (30mg, 0.1mmol) according to the procedure for **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85(d, 1H, J=2.4Hz), 7.96(dd, 1H, J=2.4, 9.6Hz), 7.81(s, 1H), 6.99(d,1H, J=2.0Hz), 6.70(dd, 1H, J=2.0, 4.0Hz), 6.84(d, 1H, J=8.0Hz), 6.72(d, 1H, J=9.6Hz), 5.26(s, 2H), 3.87(s, 3H), 3.86(s, 3H), 3.67(t, 2H, J=7.2Hz), 2.36(t, 2H, J=6.8Hz), 2.26-2.22(m, 2H), 2.04-2.01 (m, 3H), 1.96-1.95(m, 6H), 1.27-1.24(m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.89, 166.32, 165.48, 162.06, 157.76, 157.41, 149.46, 141.88, 135.81, 130.02, 127.87, 121.46, 121.22, 113.16, 112.08, 111.99, 111.36, 56.15, 56.08, 53.15, 52.32, 50.99, 41.79, 36.39, 35.15, 29.51, 27.35, 25.67, 22.83, 18.83. HRMS (ESI): calculated for [$C_{33}H_{38}F_3N_4O_6S^+$], 675.2459, found 675.2479. UPLC purity 95%.

^{1}H and ^{13}C NMR spectra



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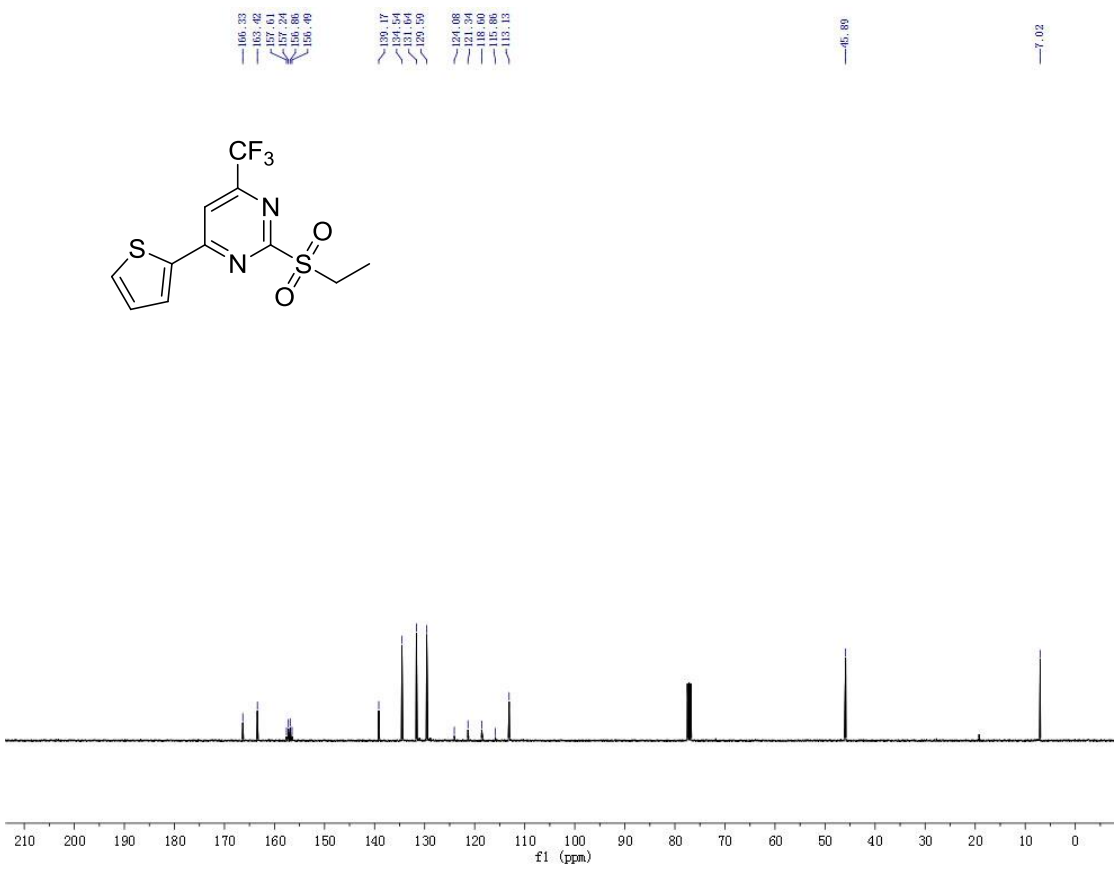
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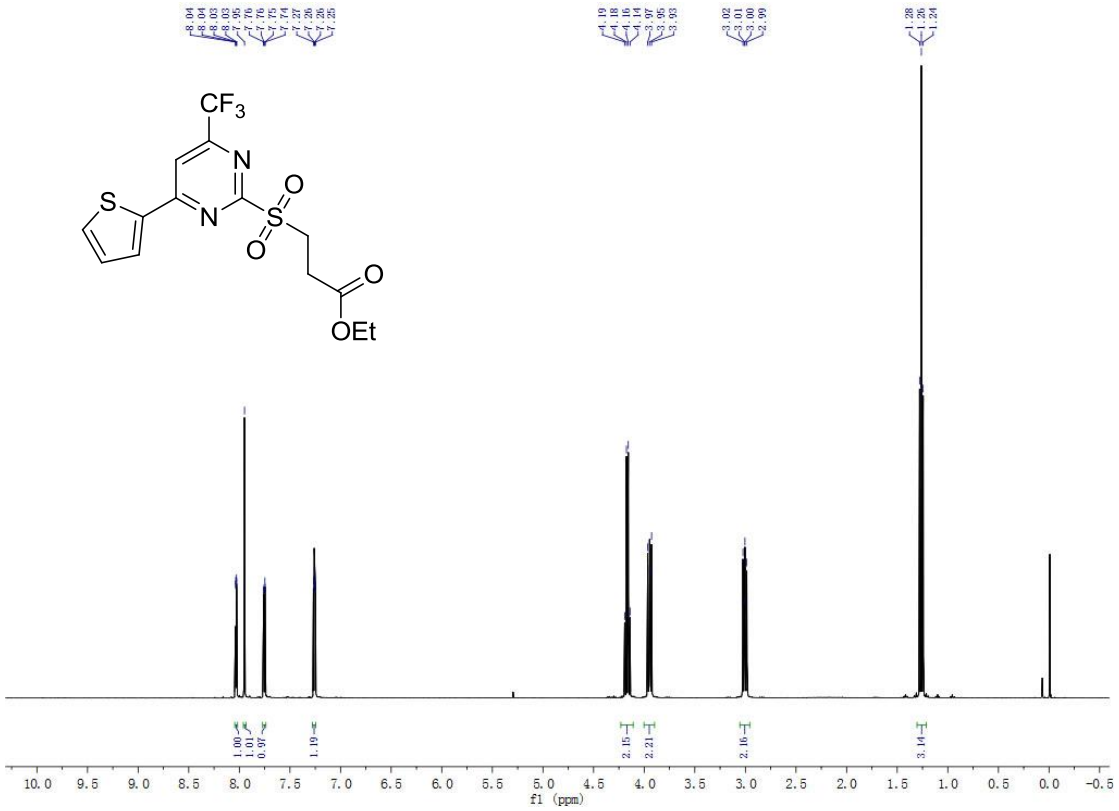
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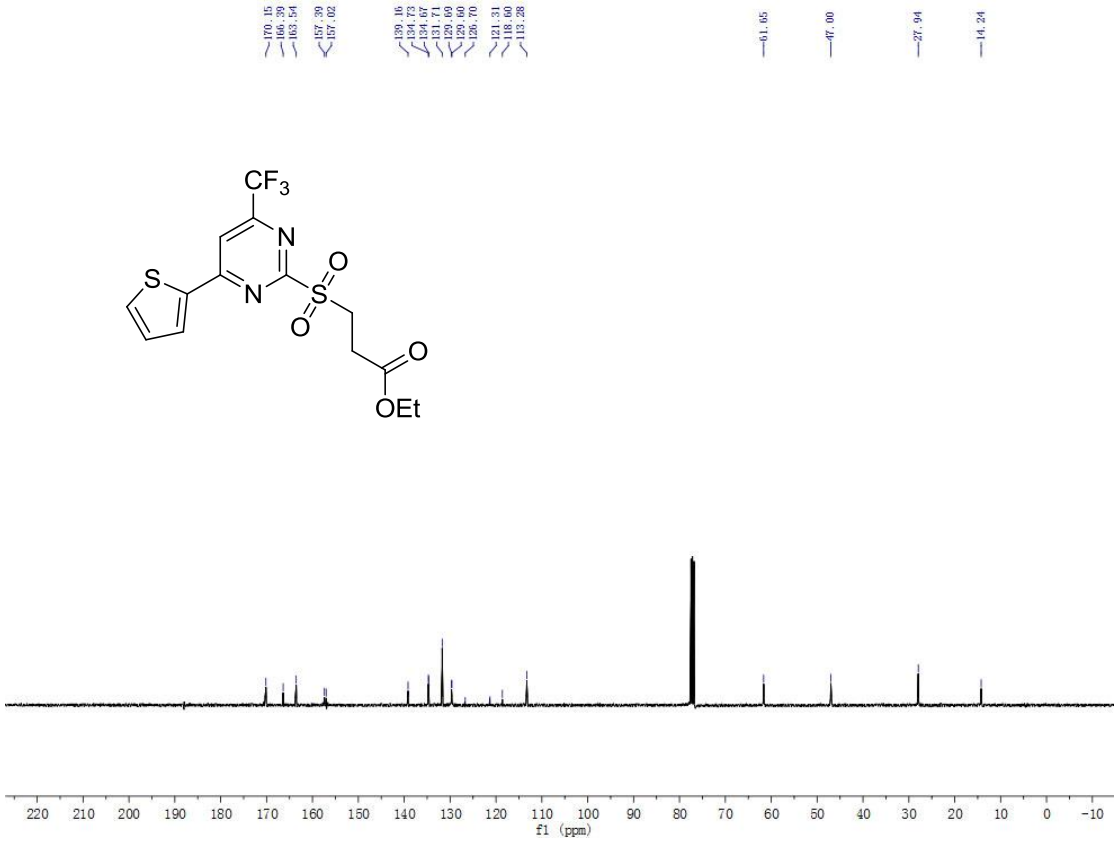
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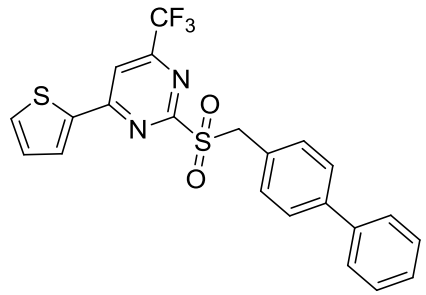
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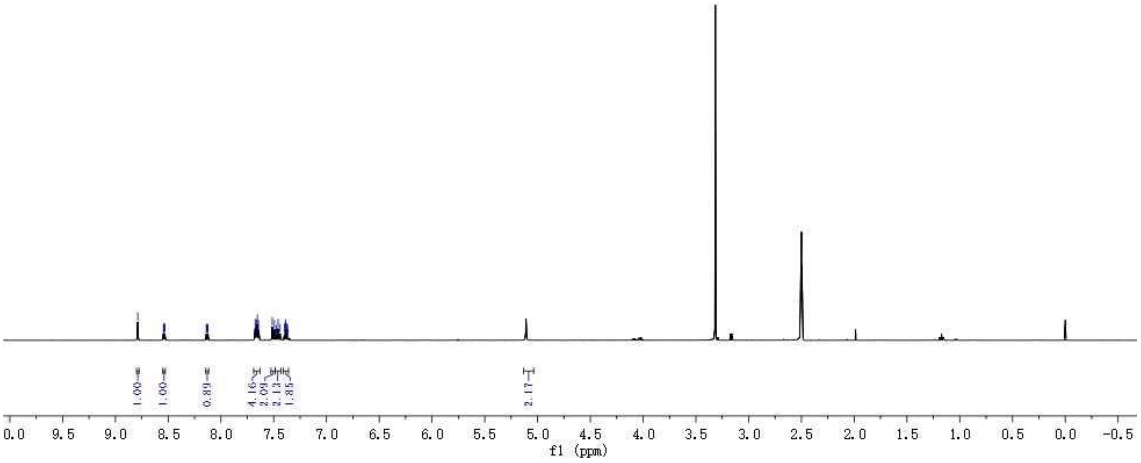
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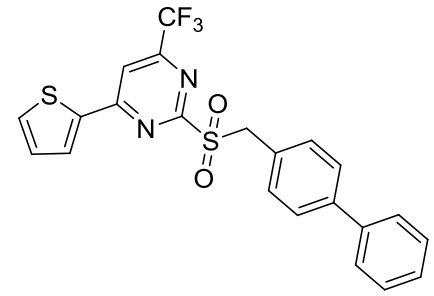
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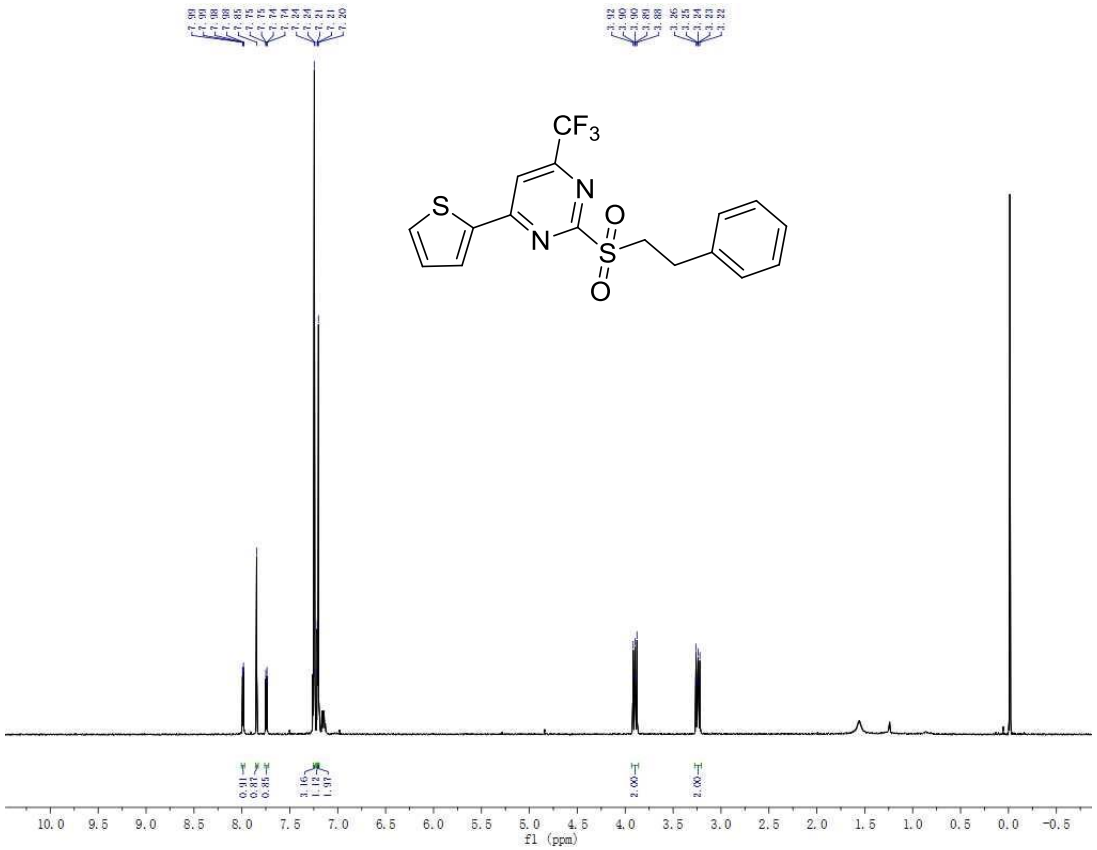
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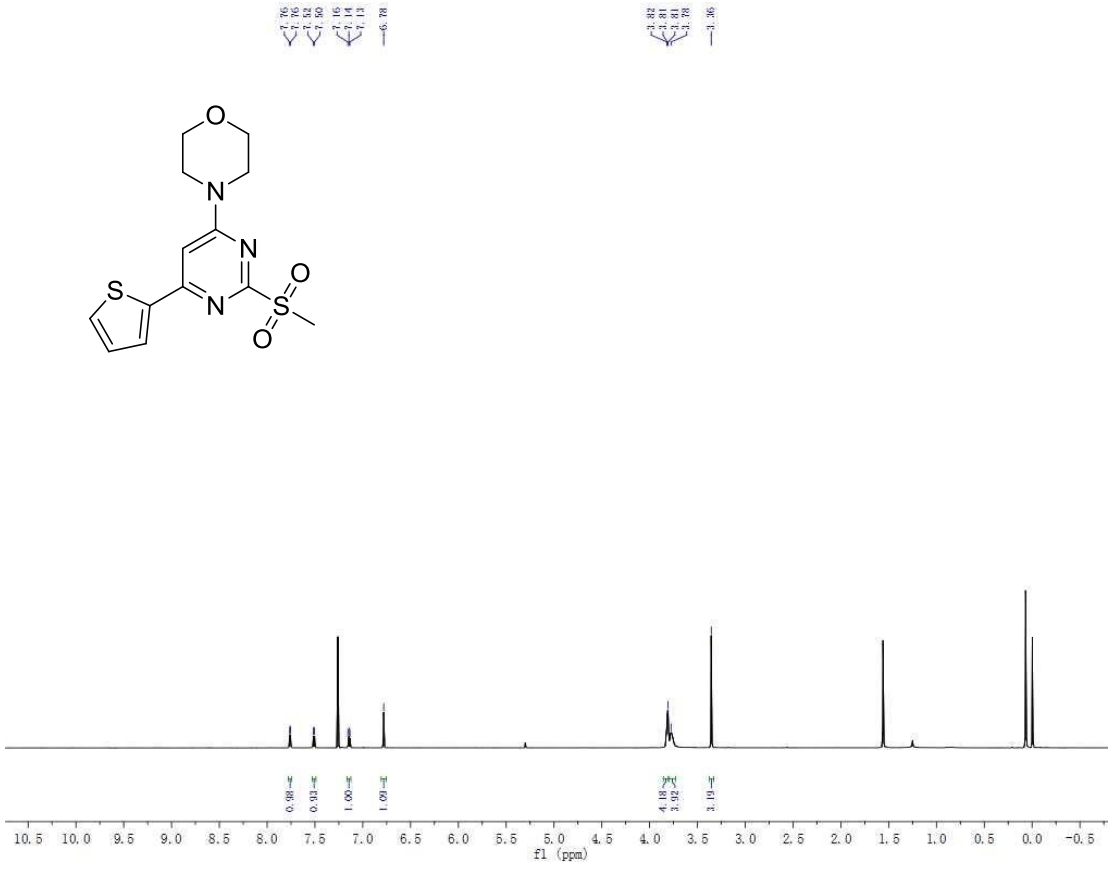
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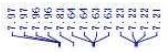

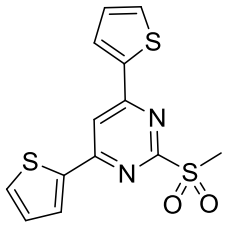

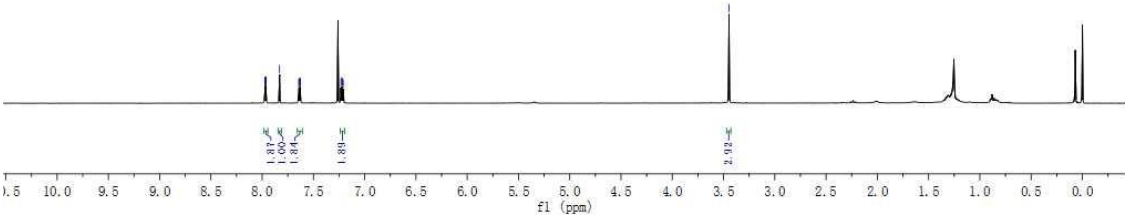
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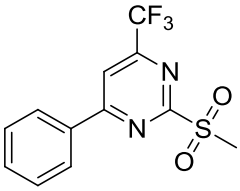

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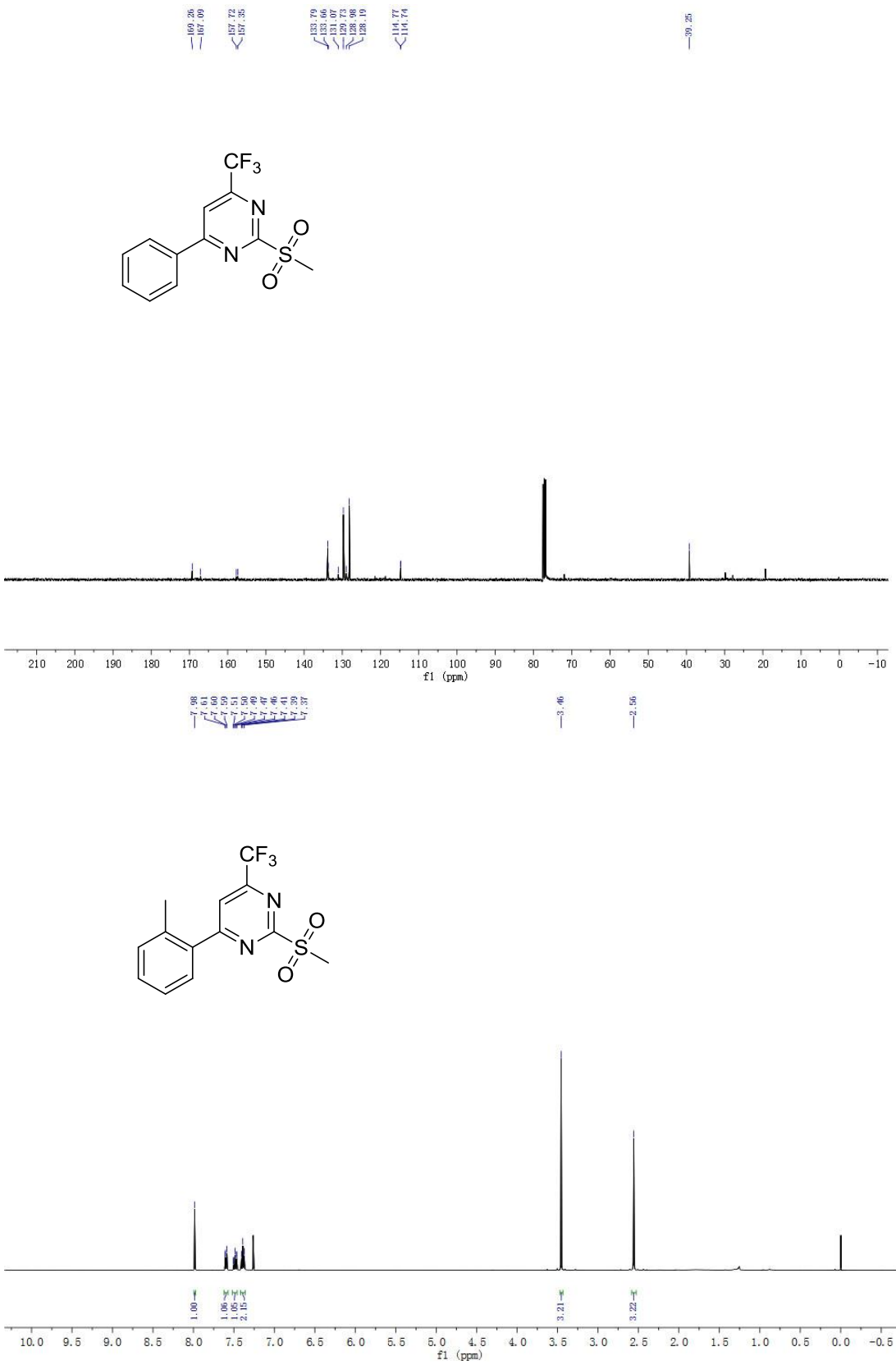
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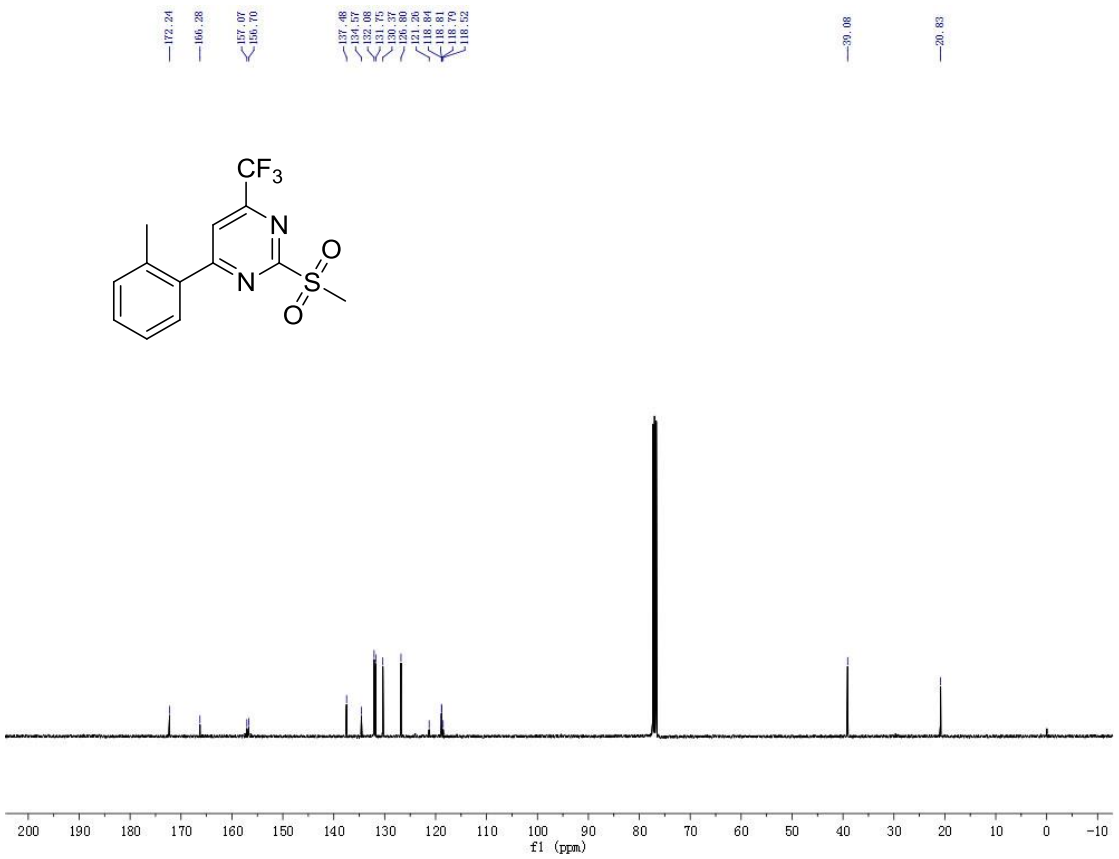
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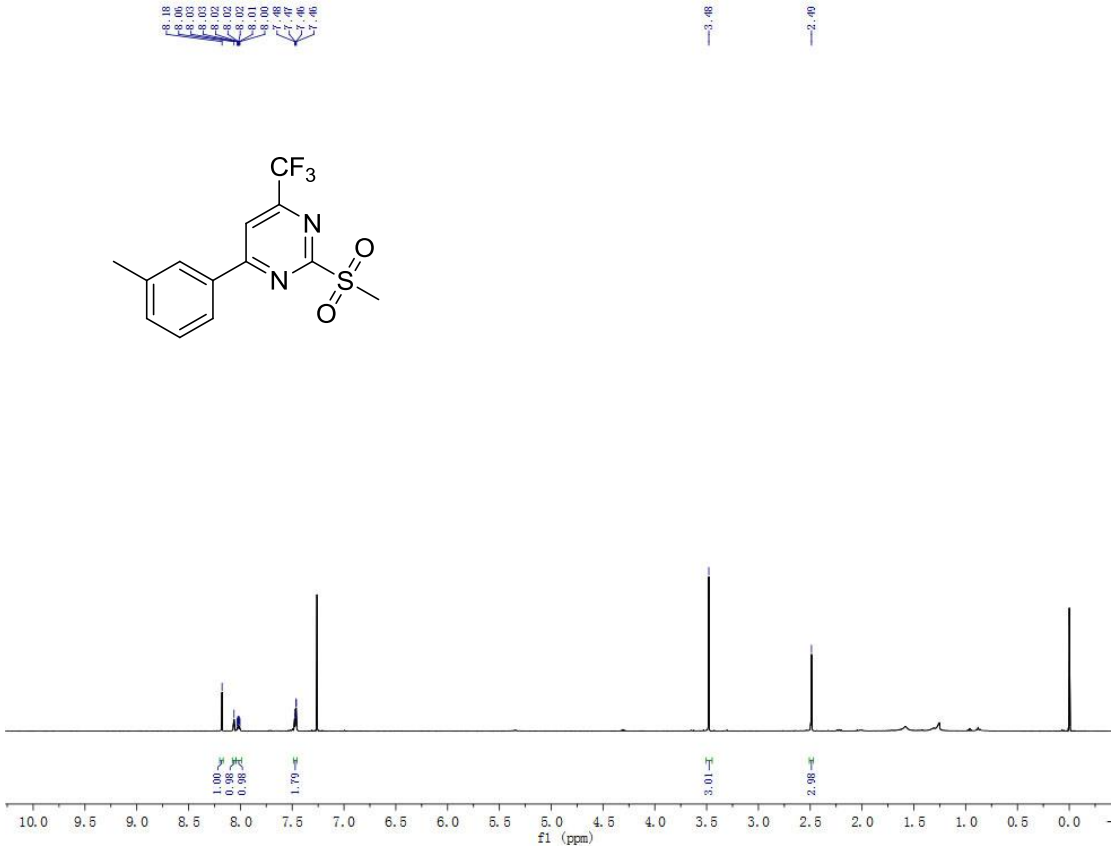
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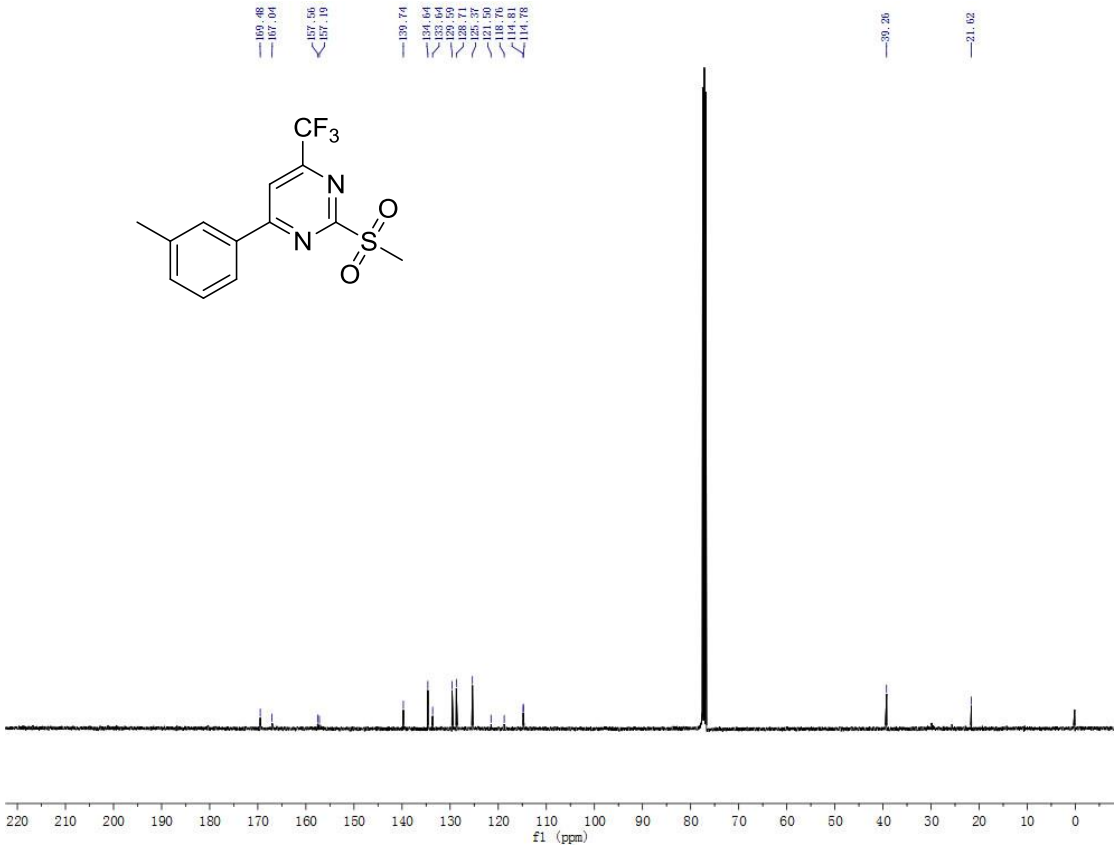
0.98
1.79
3.01
2.98
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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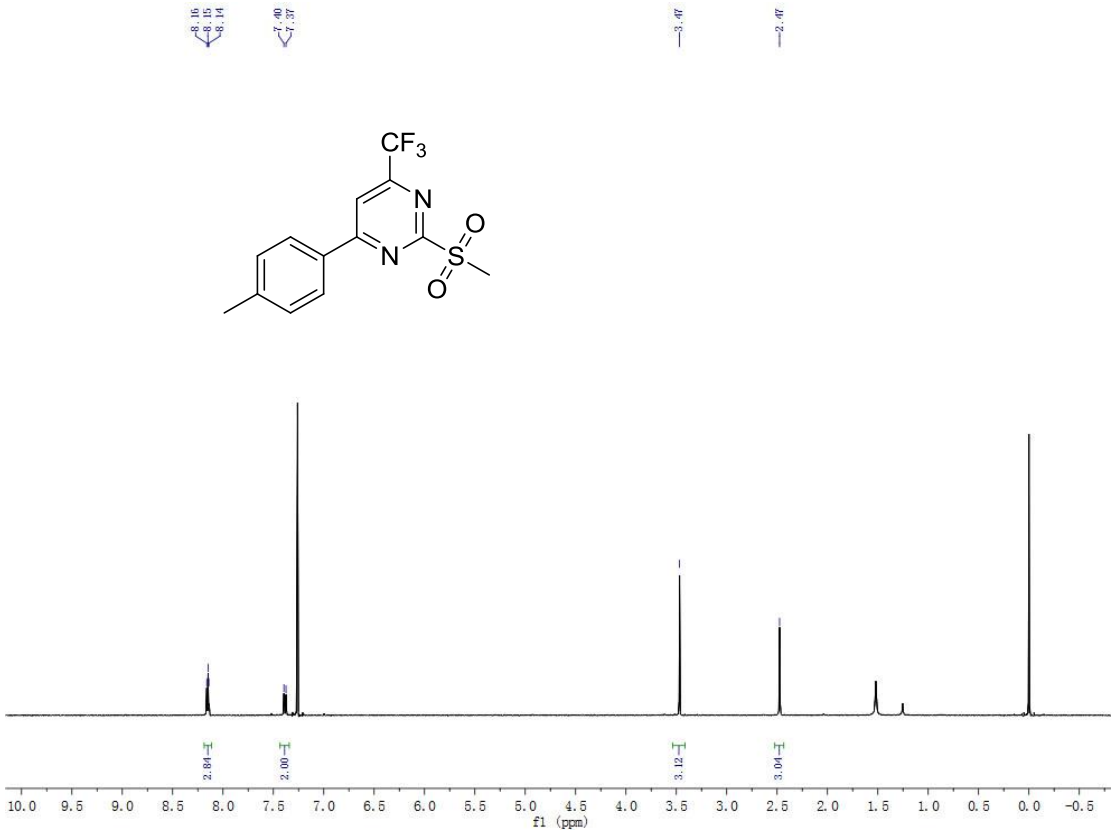
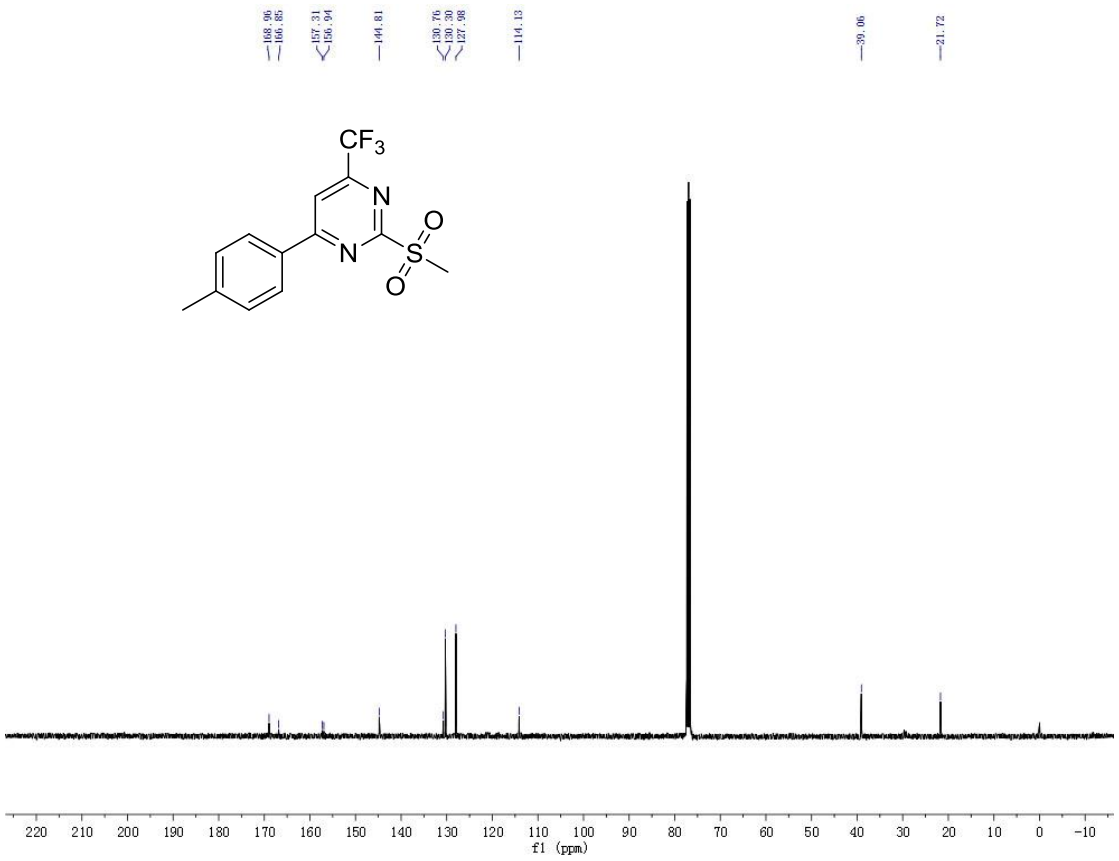
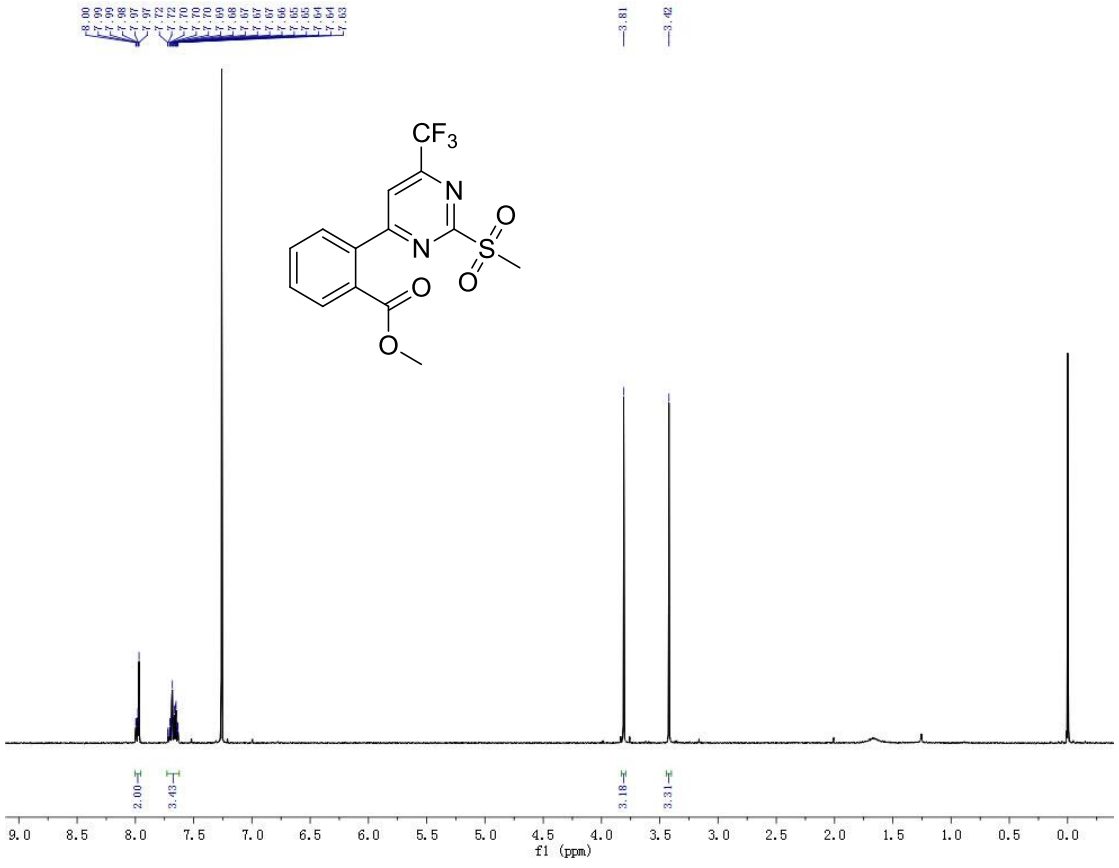
167.04
157.56
157.19
139.74
134.64
133.64
129.59
128.71
125.57
121.50
118.76
114.81
114.78
39.26
21.62
CF3
N
N
O
S
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


8.16
8.15
8.14
7.40
7.37
3.47
2.47
CF3
N
N
O
S
O
2.84
2.00
3.12
3.04
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


168.96
166.85
157.31
156.94
144.81
130.76
130.30
127.98
114.13
39.06
21.72
CF3
N
N
O
S
O
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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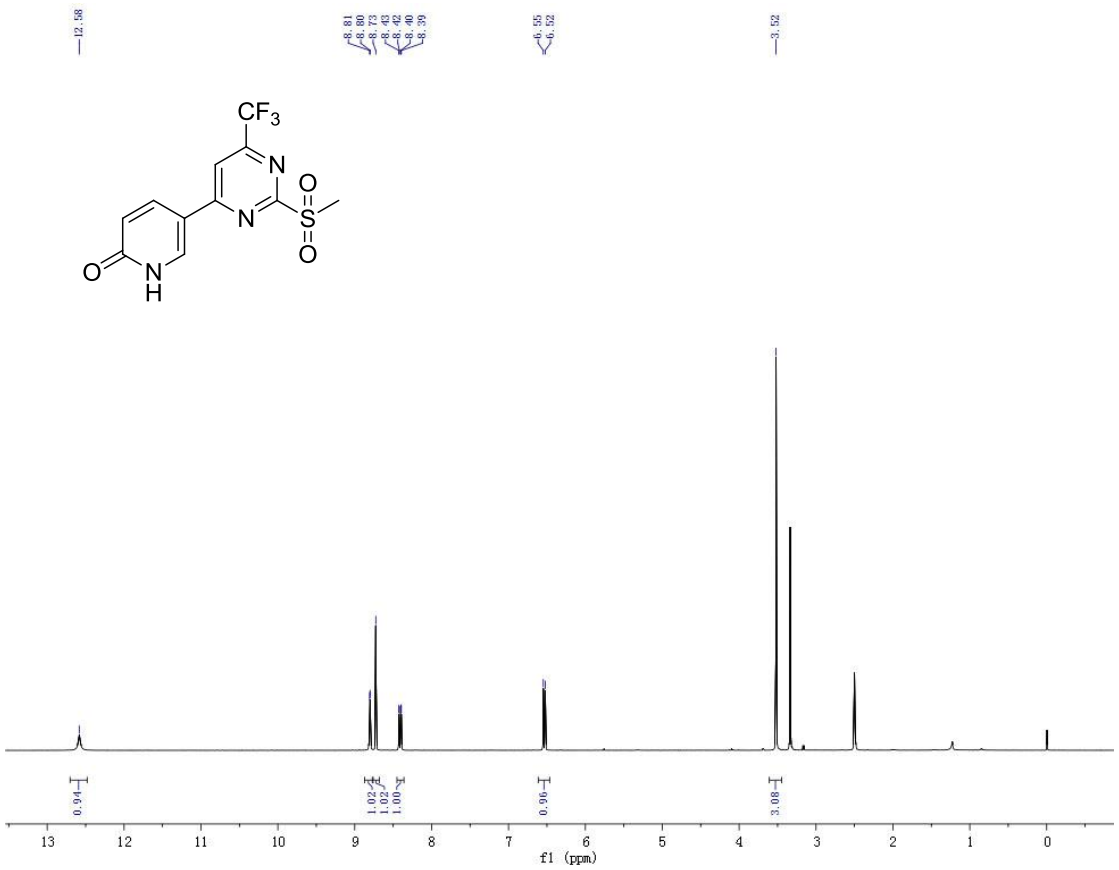
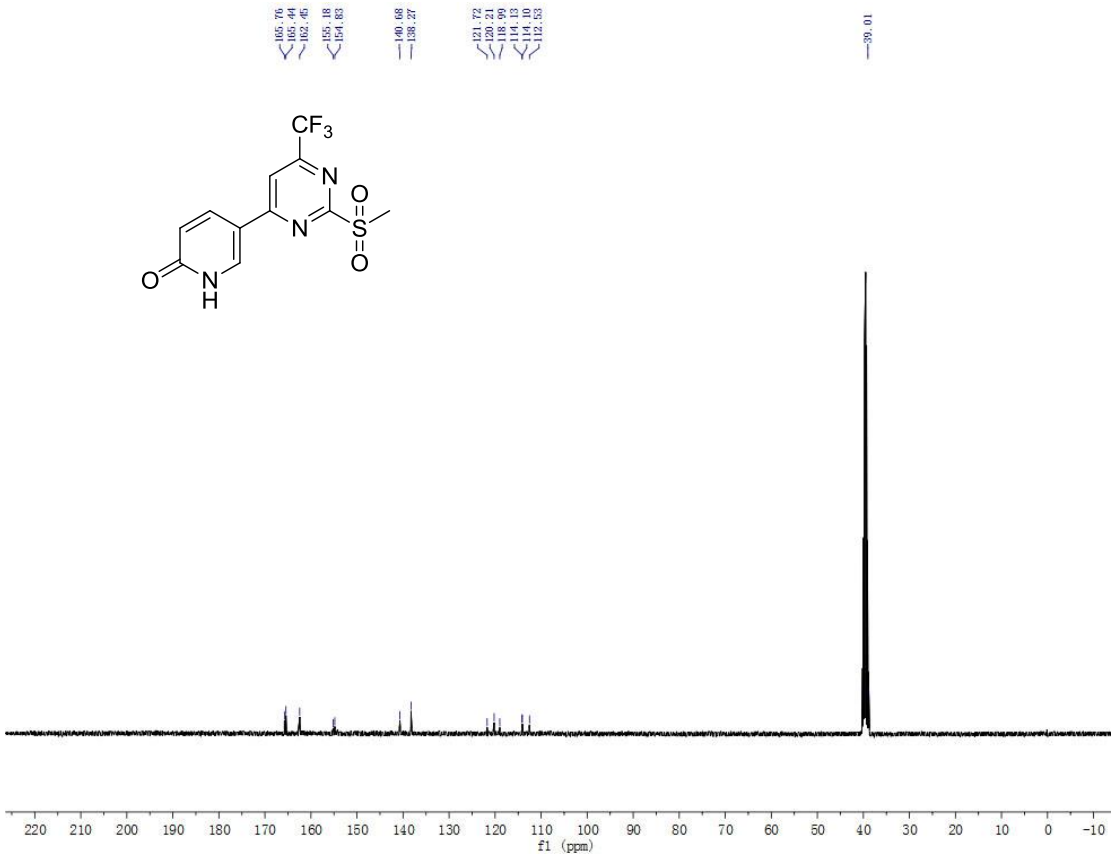
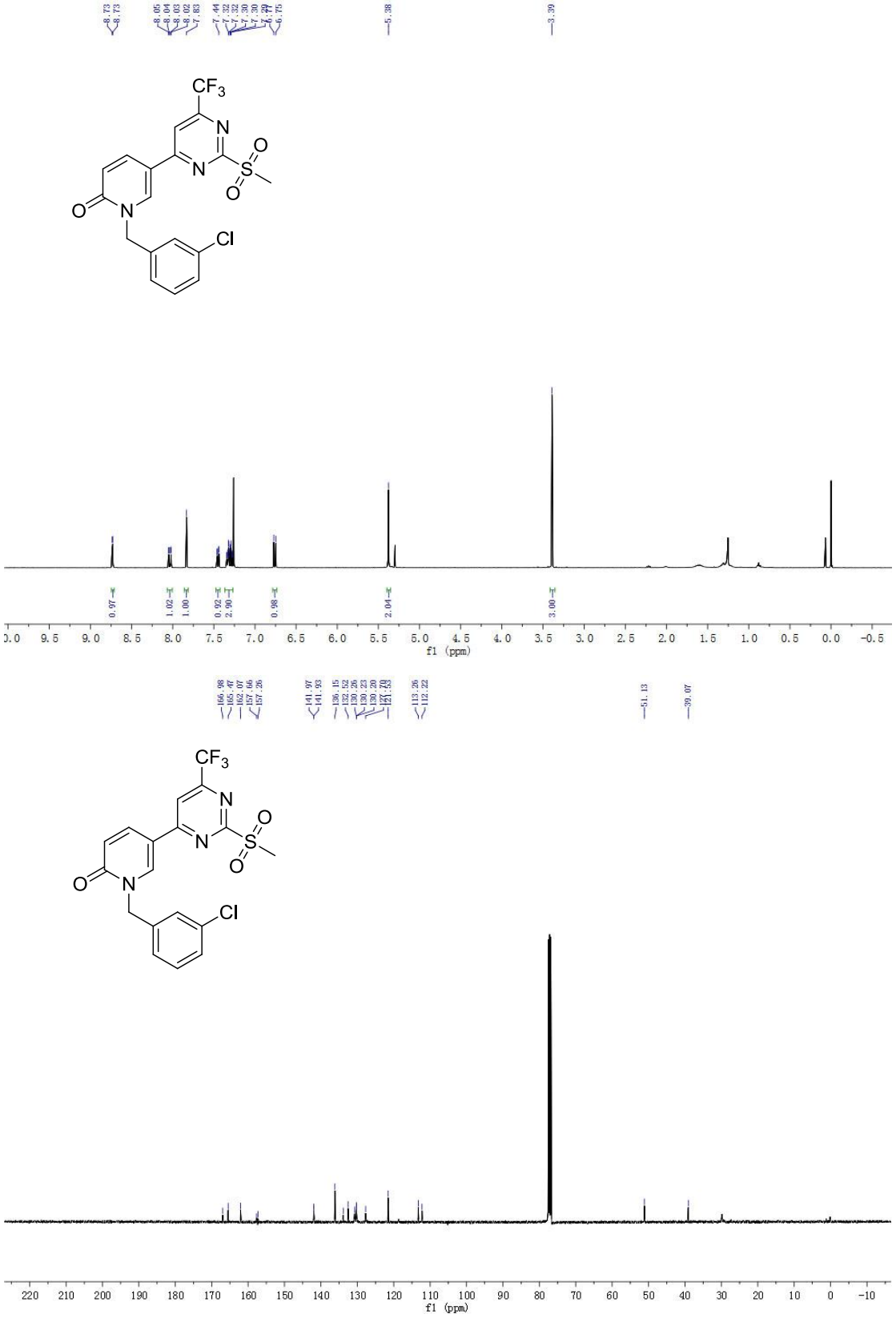
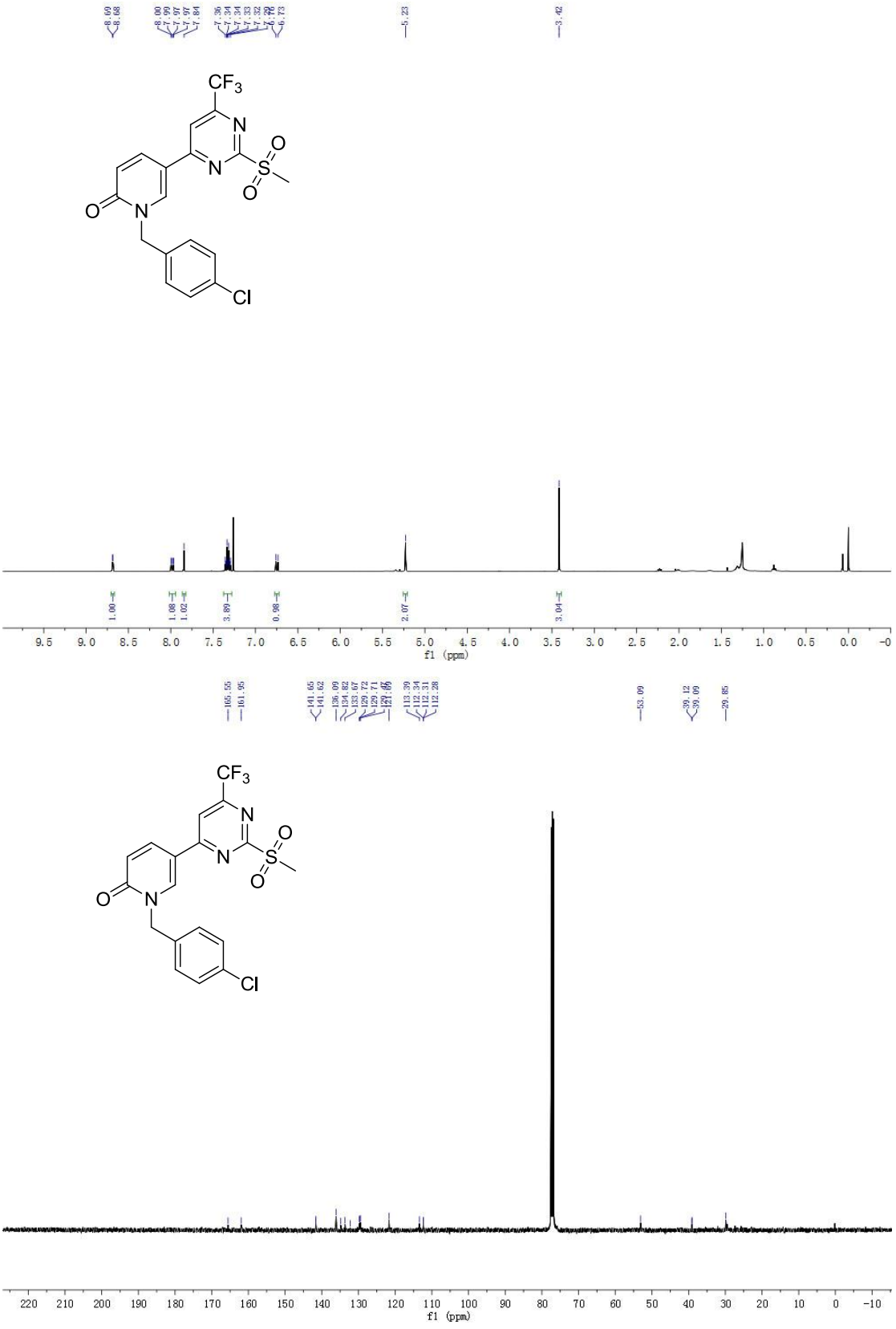
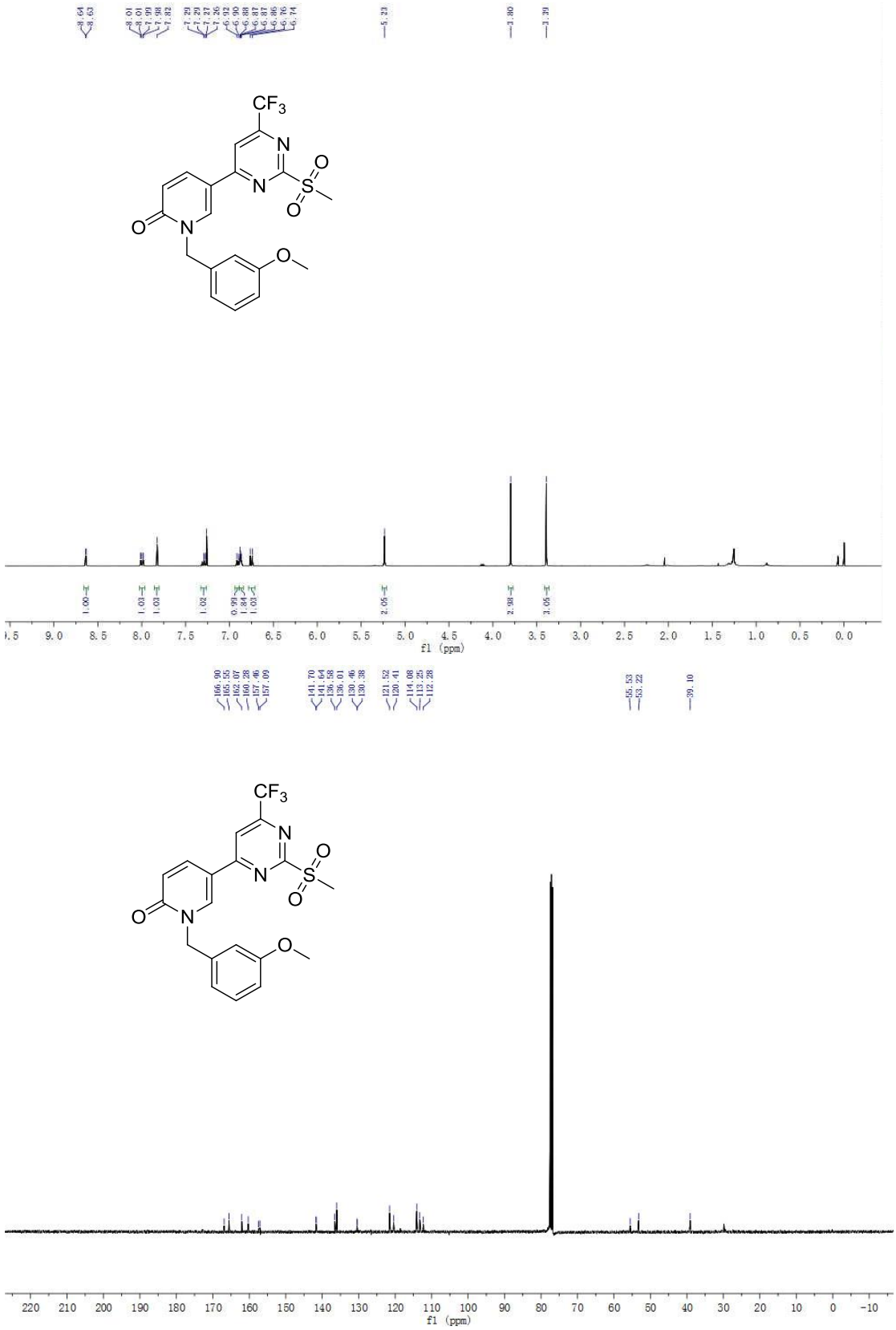
7.99
7.99
7.98
7.97
7.97
7.72
7.70
7.70
7.70
7.69
7.68
7.67
7.67
7.66
7.65
7.65
7.64
7.64
7.63
3.81
3.42
CF3
N
N
O
S
O
O
O
O
2.00
3.43
3.18
3.31
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


12.58
8.81
8.80
8.73
8.43
8.42
8.40
8.39
6.55
6.52
3.52
CF3
N
O
S
O
N
O
H
0.94
1.02
1.02
1.00
0.96
3.08
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

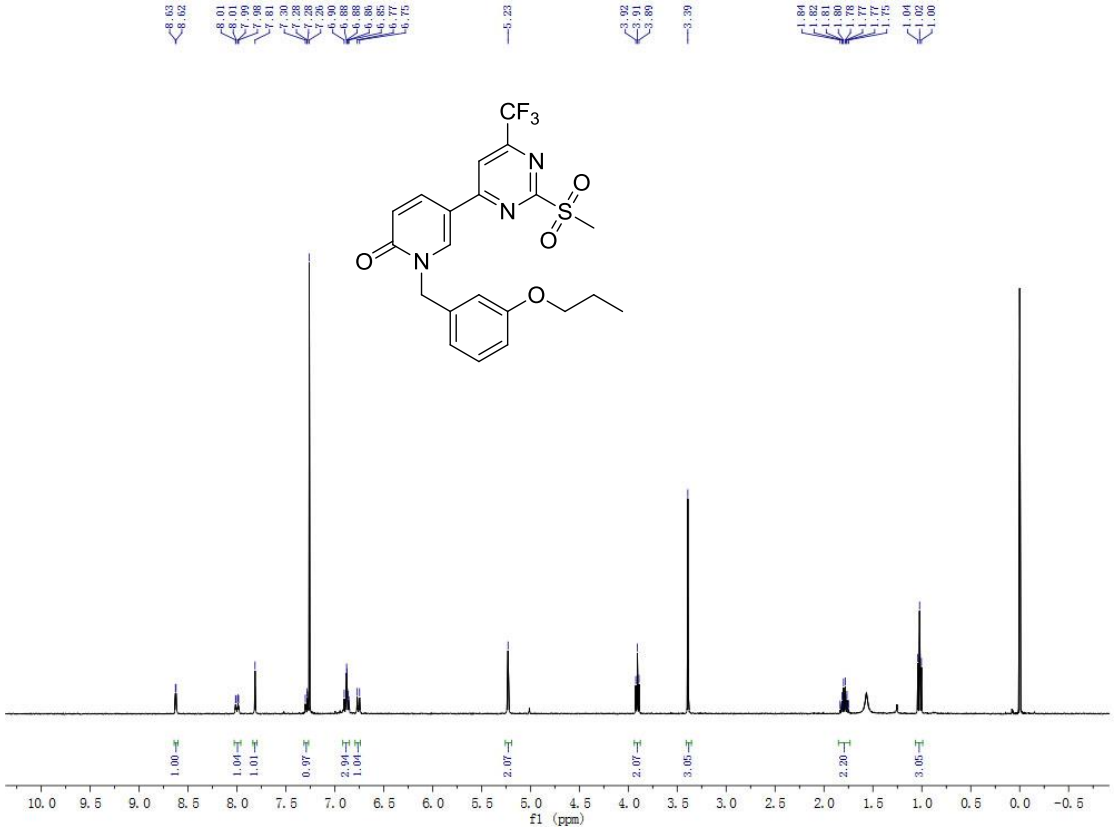

165.76
165.44
162.45
155.18
154.63
140.68
138.27
121.72
120.31
118.89
114.13
114.10
112.53
39.01
CF3
N
O
S
O
N
O
H
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

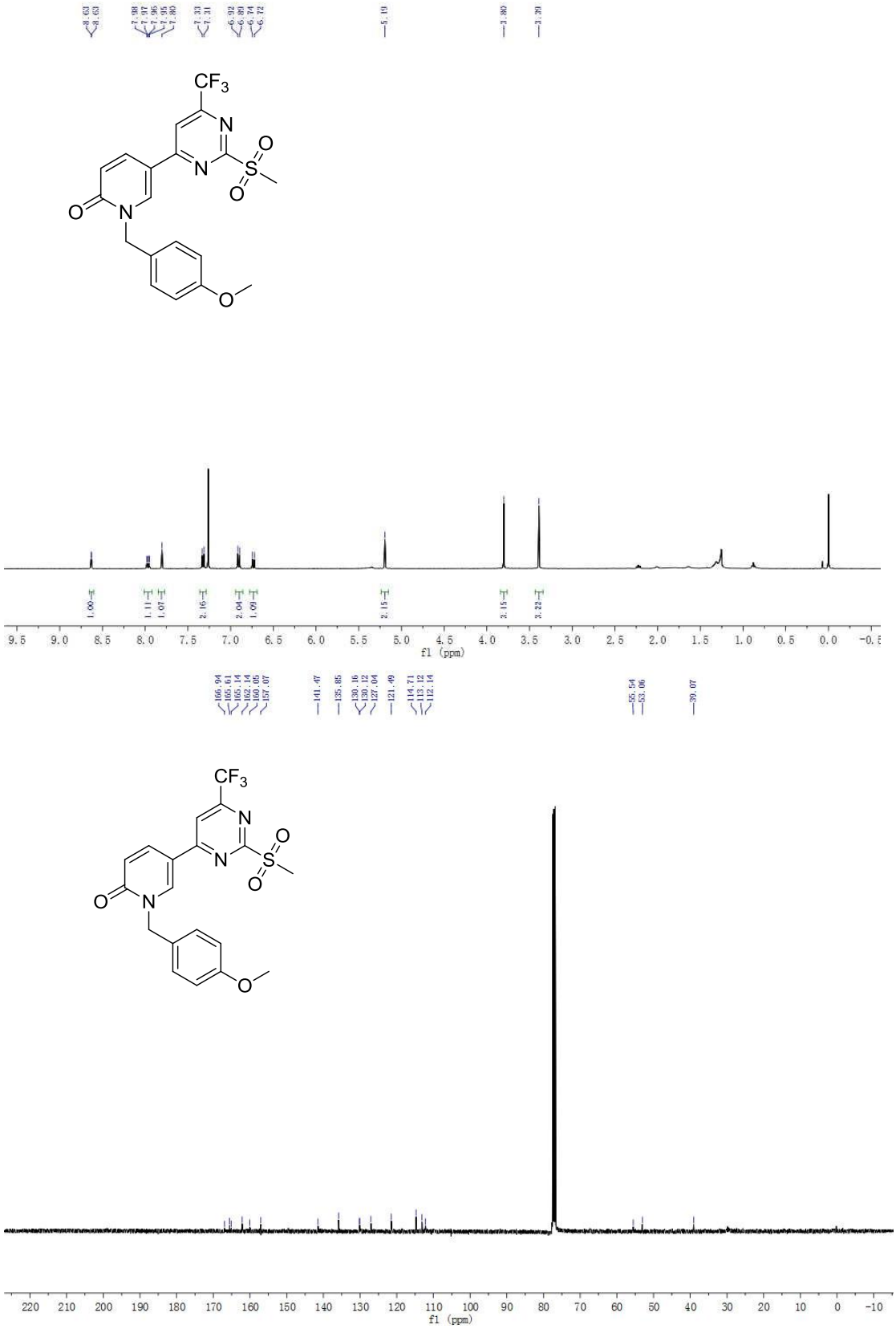

8.73
8.73
8.05
8.04
8.03
8.02
7.83
7.44
7.32
7.32
7.30
7.30
6.75
5.38
3.39
CF3
N
N
O
S
O
O
N
Cl
0.97
1.02
1.00
0.92
2.90
0.98
2.04
3.00
f1 (ppm)
166.98
165.47
162.07
157.66
157.26
141.97
141.93
136.15
132.52
130.26
130.23
130.20
113.26
112.22
51.13
39.07
CF3
N
N
O
S
O
O
N
Cl
f1 (ppm)

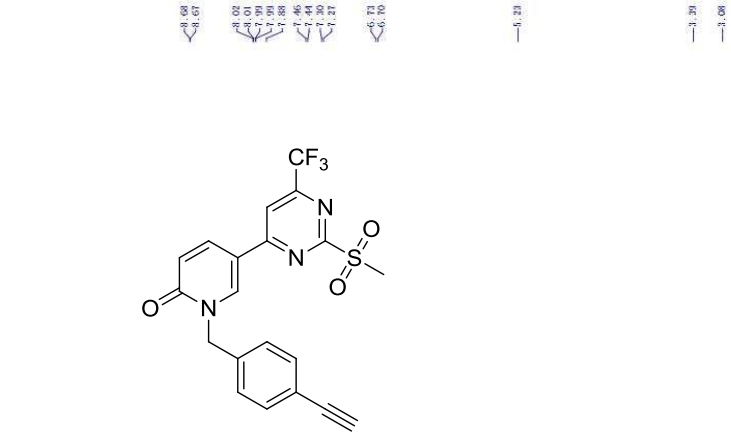

CF3
N
N
S
O
O
O
N
Cl
1.00
1.08
1.02
3.89
0.98
2.07
3.04
f1 (ppm)
f1 (ppm)

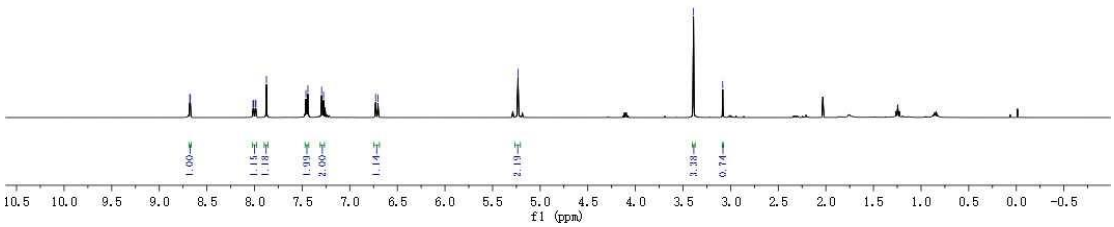

CF3
N
N
O
S
O
O
N
O
f1 (ppm)

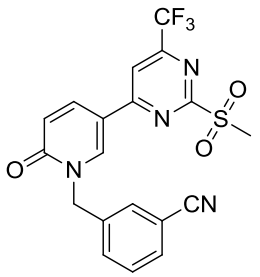

CF3
N
N
O
S
O
O
N
O
f1 (ppm)

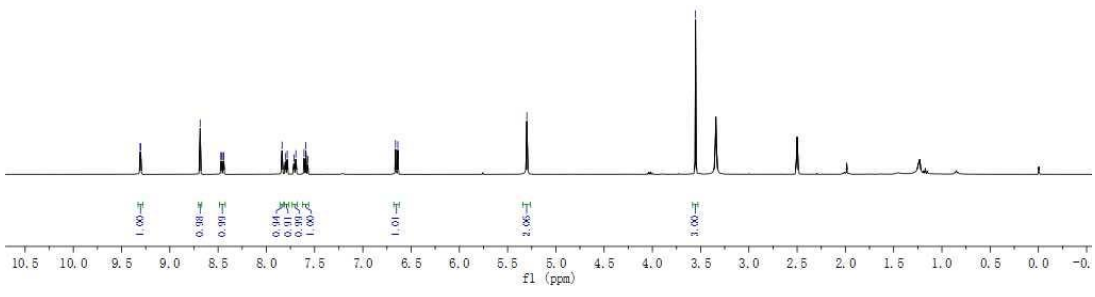

CF3
N
N
O
S
O
O
N
O
f1 (ppm)

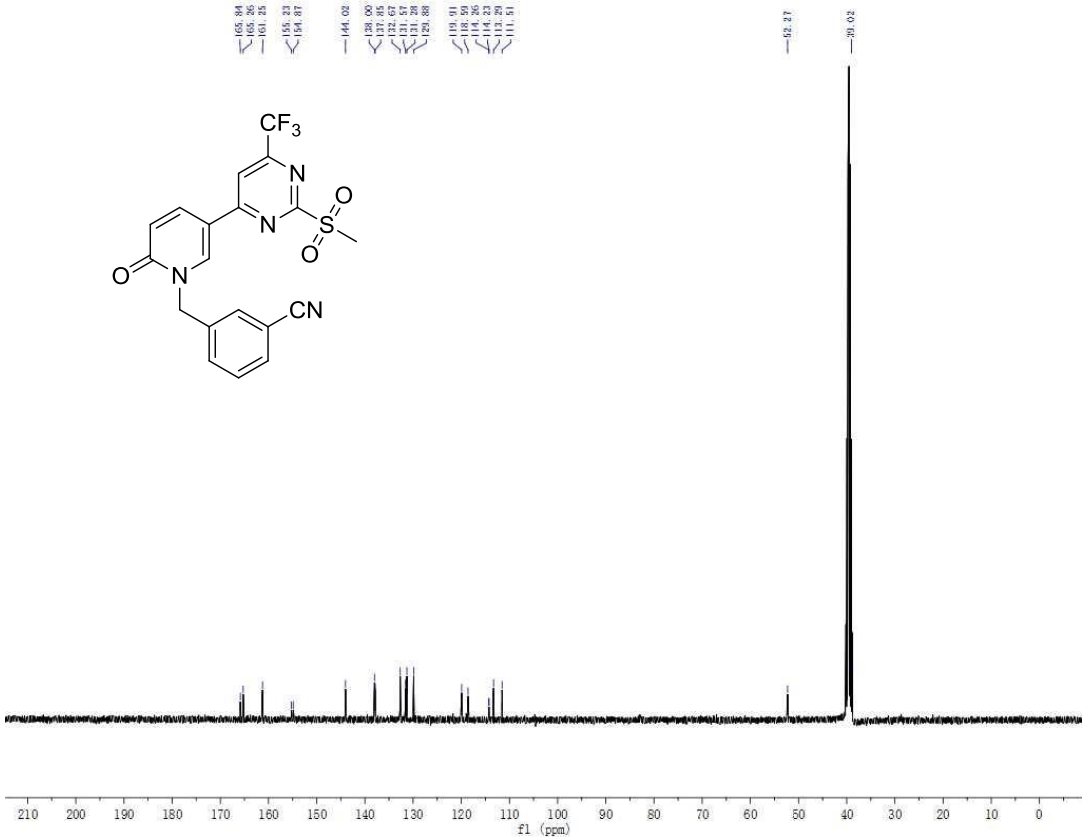

8.63
8.63
7.98
7.97
7.96
7.95
7.80
7.33
7.31
6.92
6.89
6.74
6.72
5.19
3.80
3.39
CF3
N
N
O
S
O
O
N
O
O
1.00
1.11
1.07
2.16
2.04
1.09
2.15
3.15
3.22
f1 (ppm)
166.94
165.61
165.14
162.14
160.05
157.07
141.47
135.85
130.16
130.12
127.04
121.49
114.71
113.12
112.14
55.54
53.06
39.07
CF3
N
N
O
S
O
O
N
O
O
f1 (ppm)

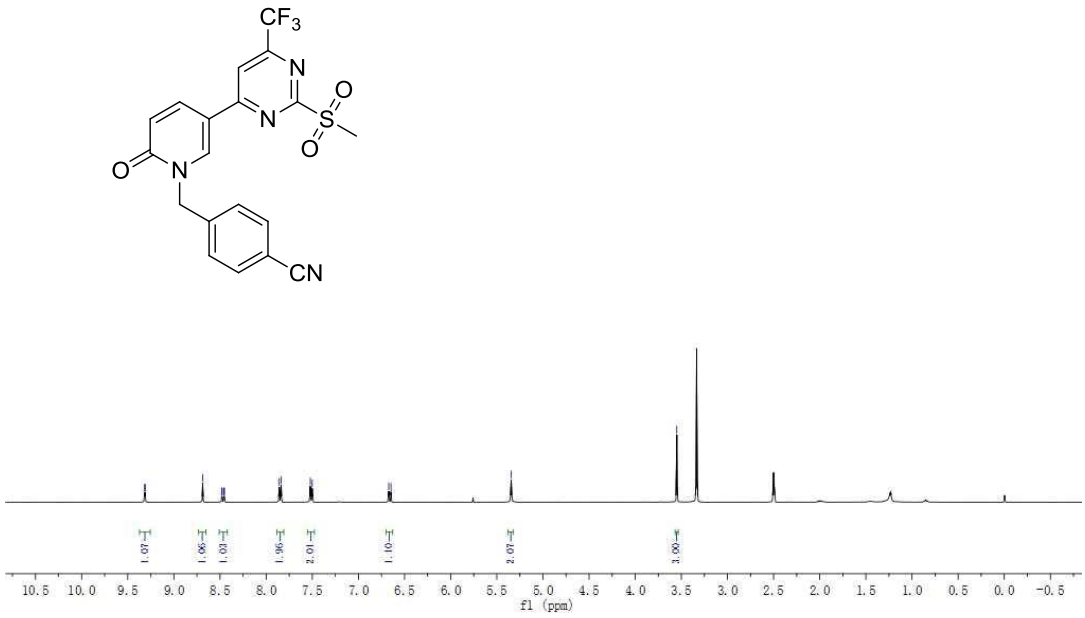


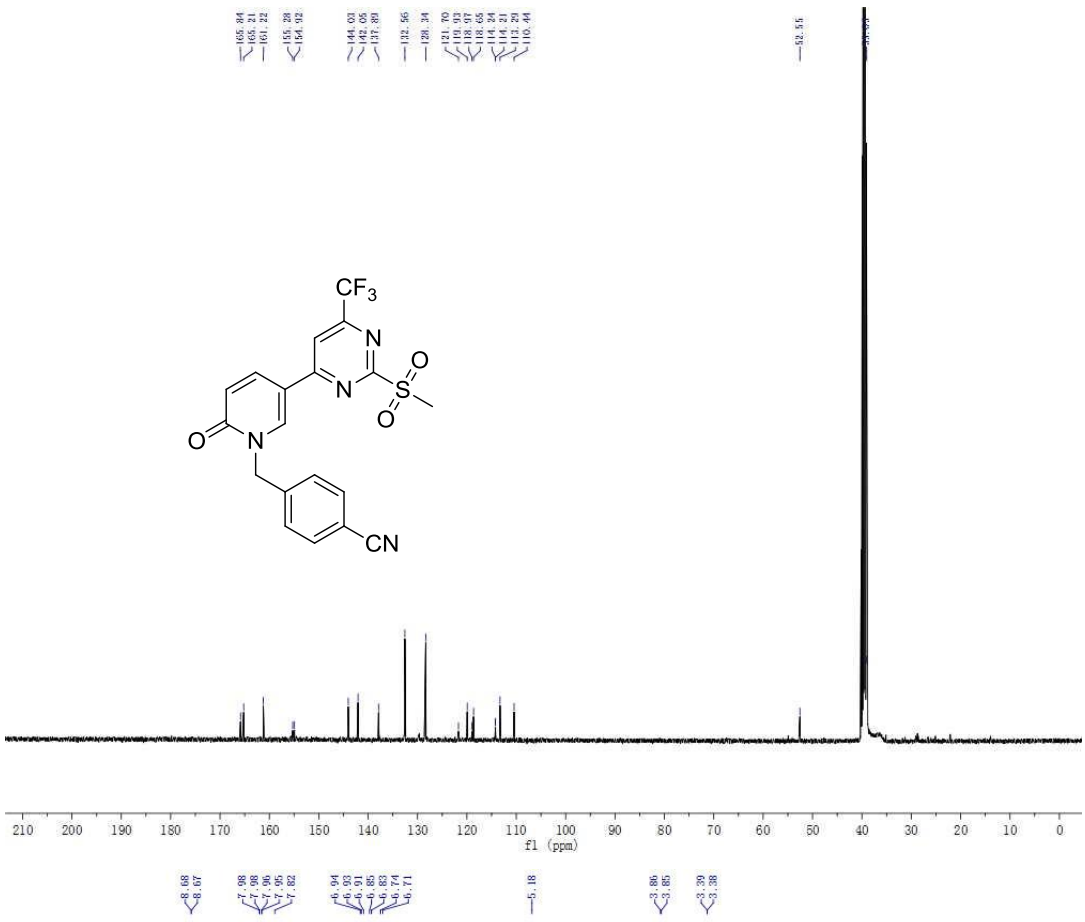


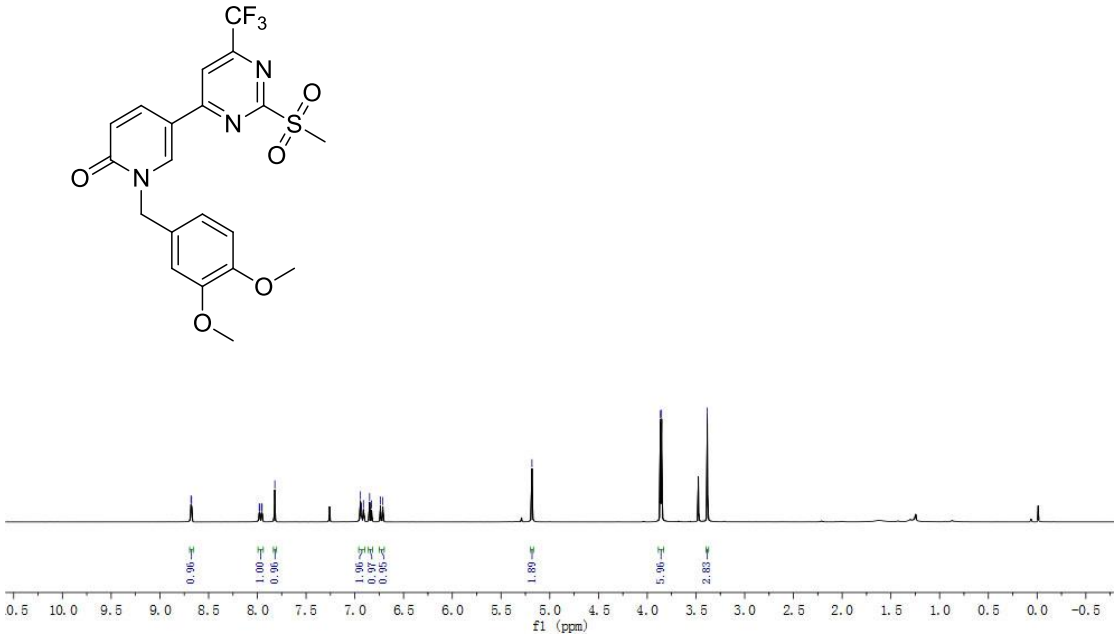


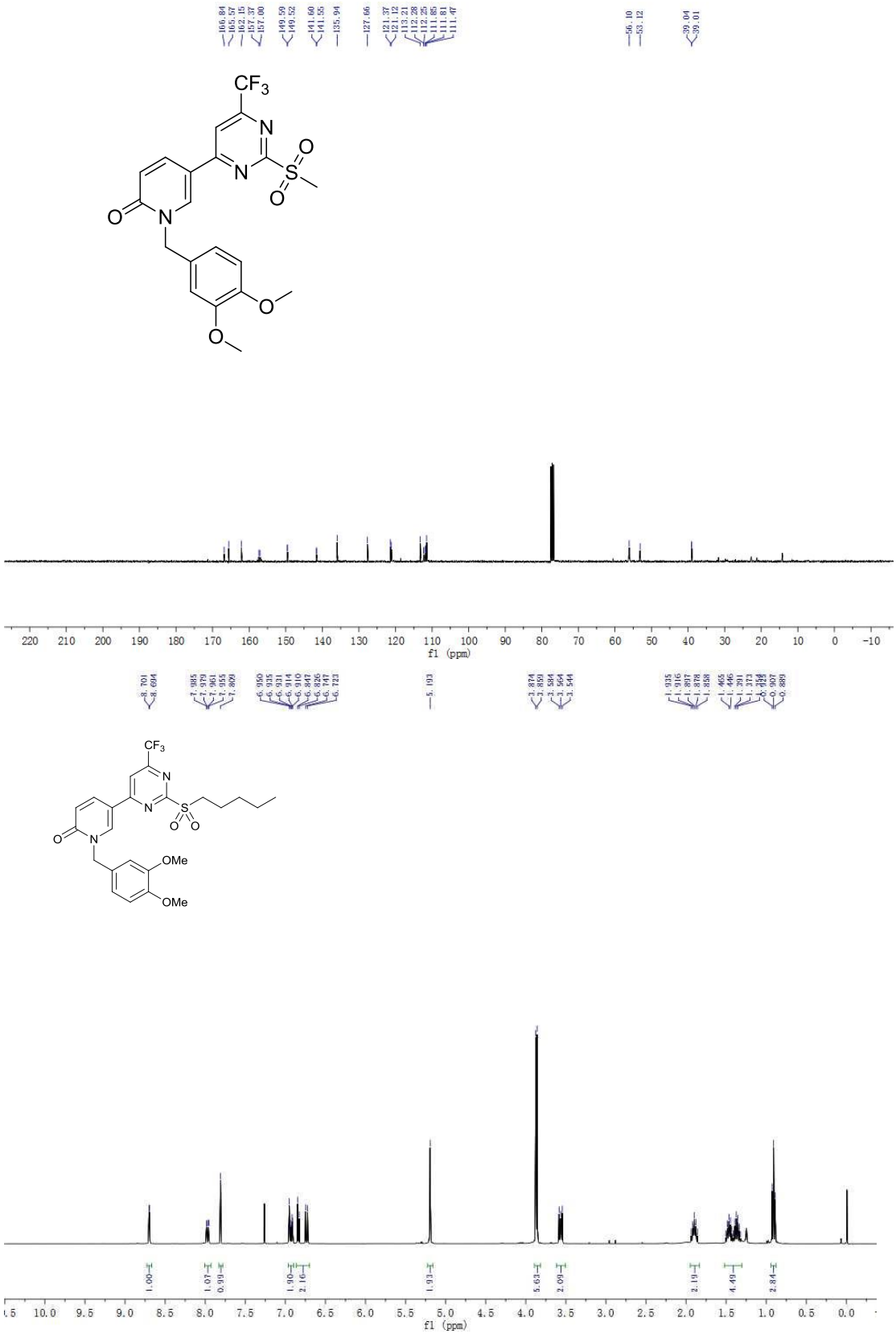


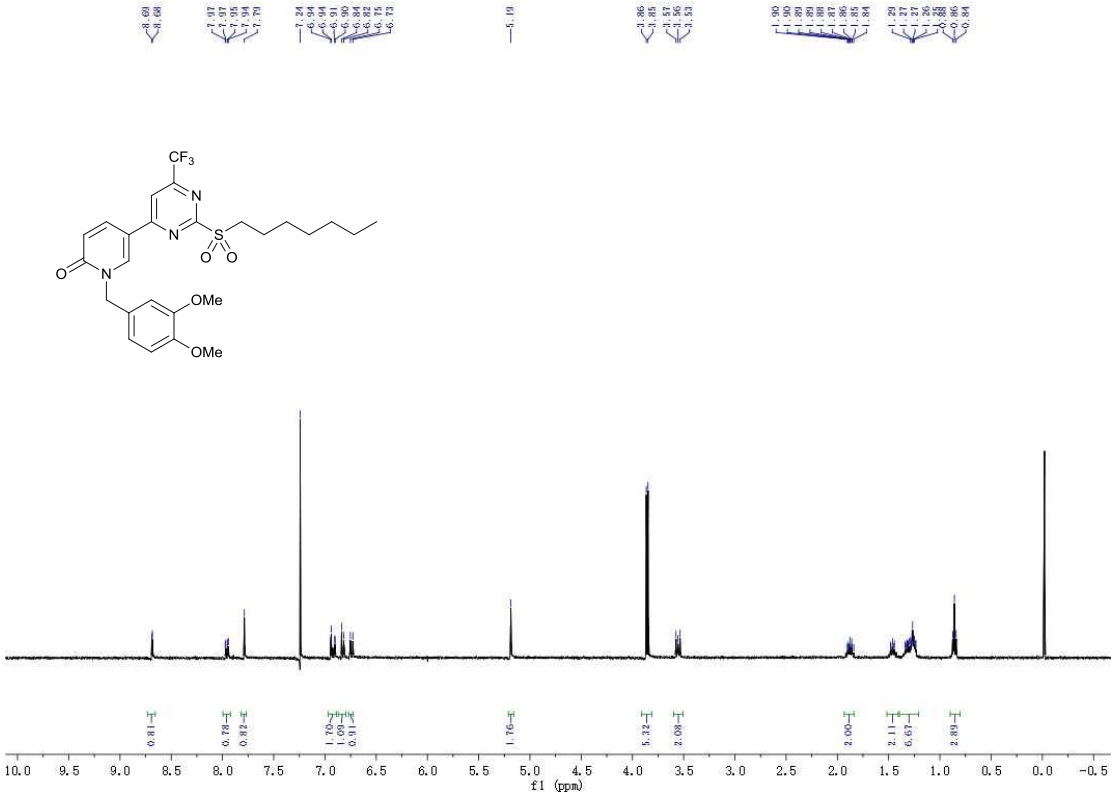

CF3
N
N
O
S
O
O
N
CN
f1 (ppm)

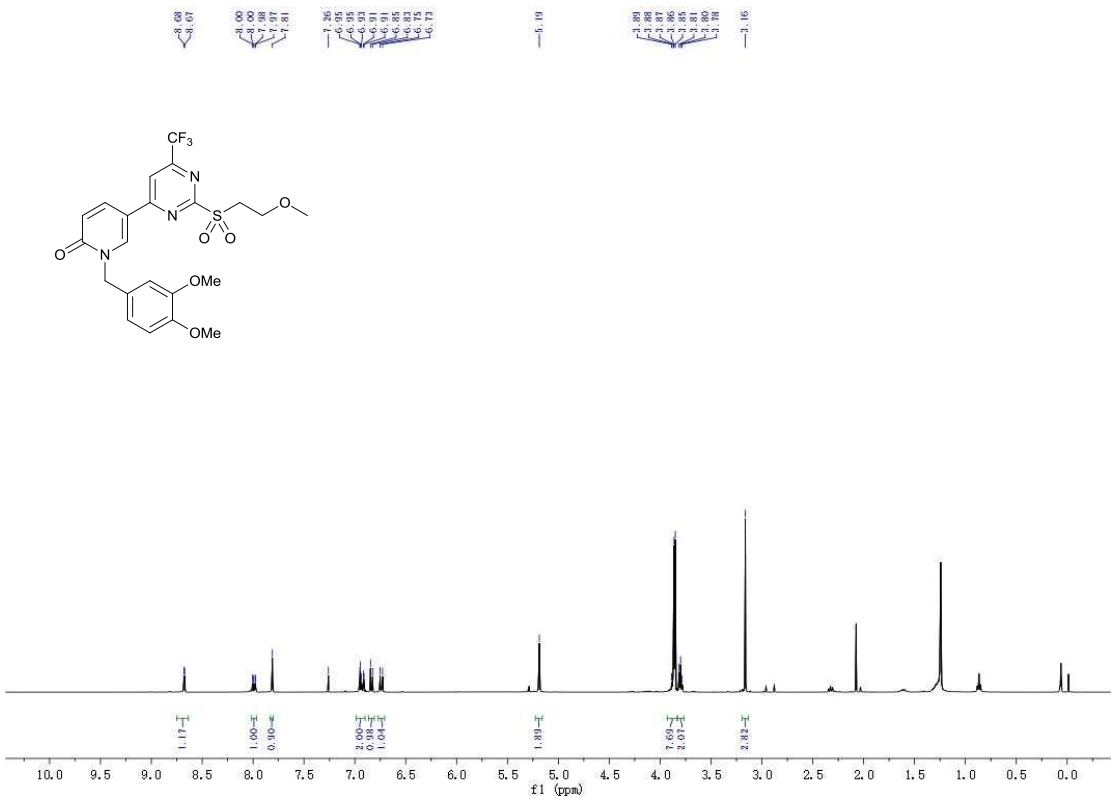

CF3
N
N
O
S
O
O
N
CN
f1 (ppm)

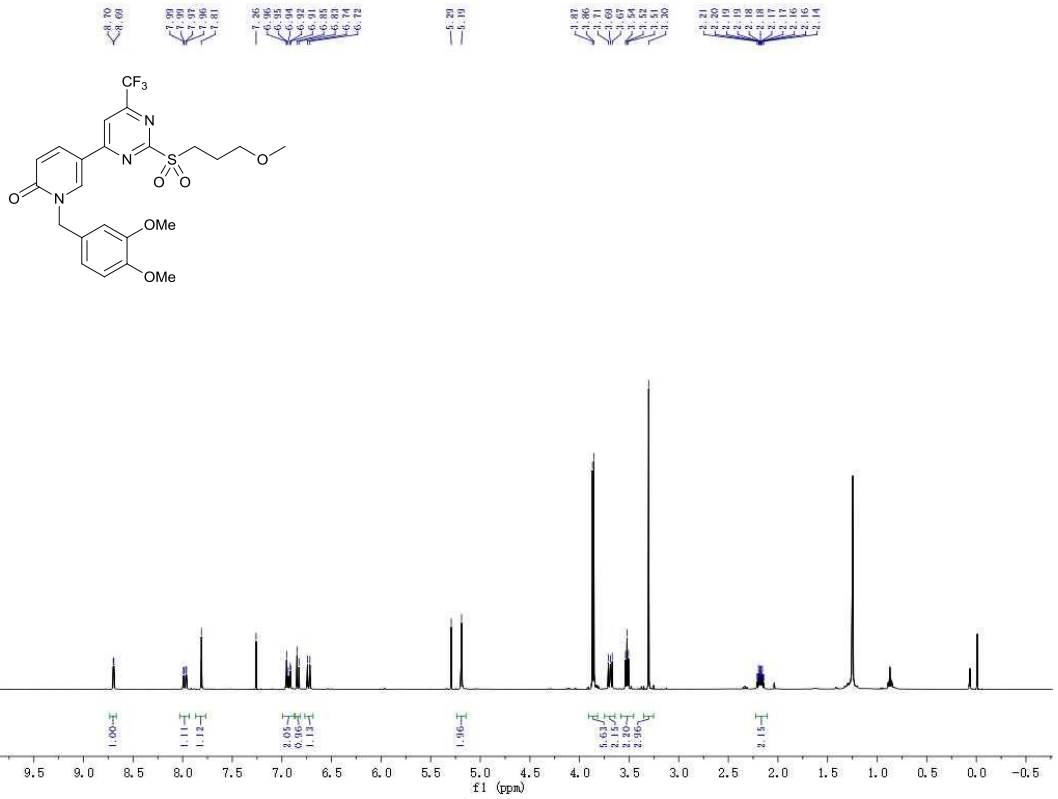

CF3
N
N
O
S
O
O
N
CN
f1 (ppm)

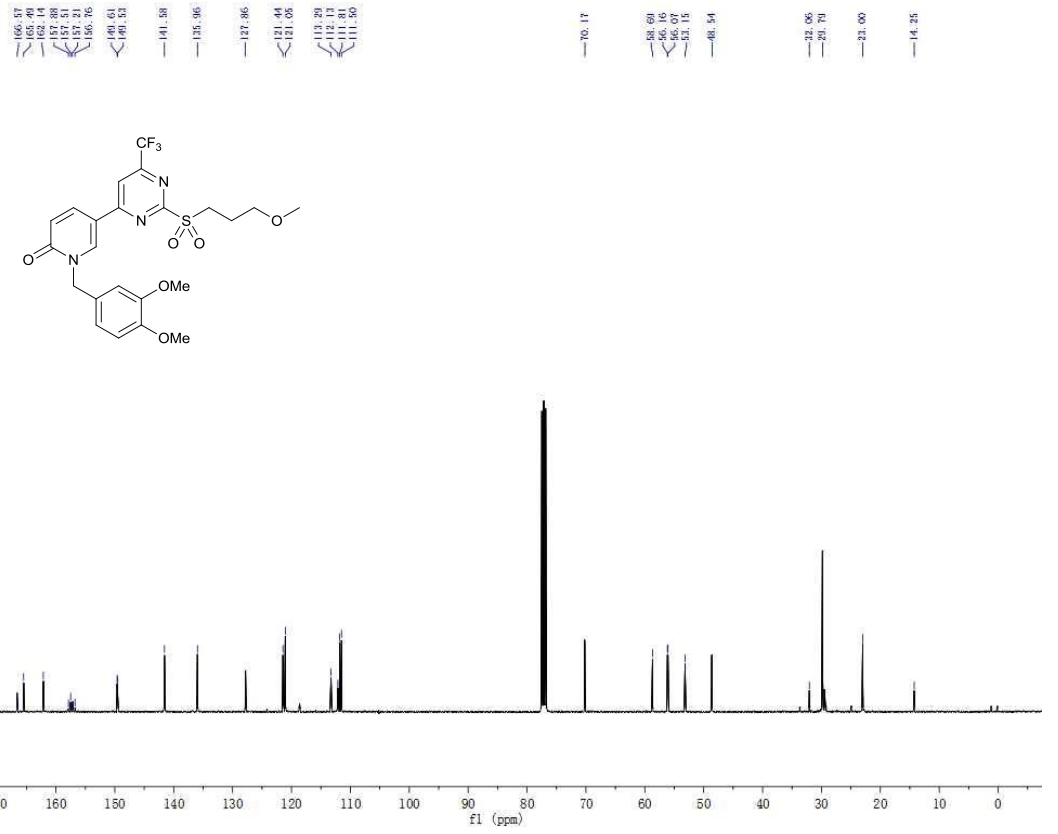

CF3
N
N
O
S
O
O
N
O
O
f1 (ppm)

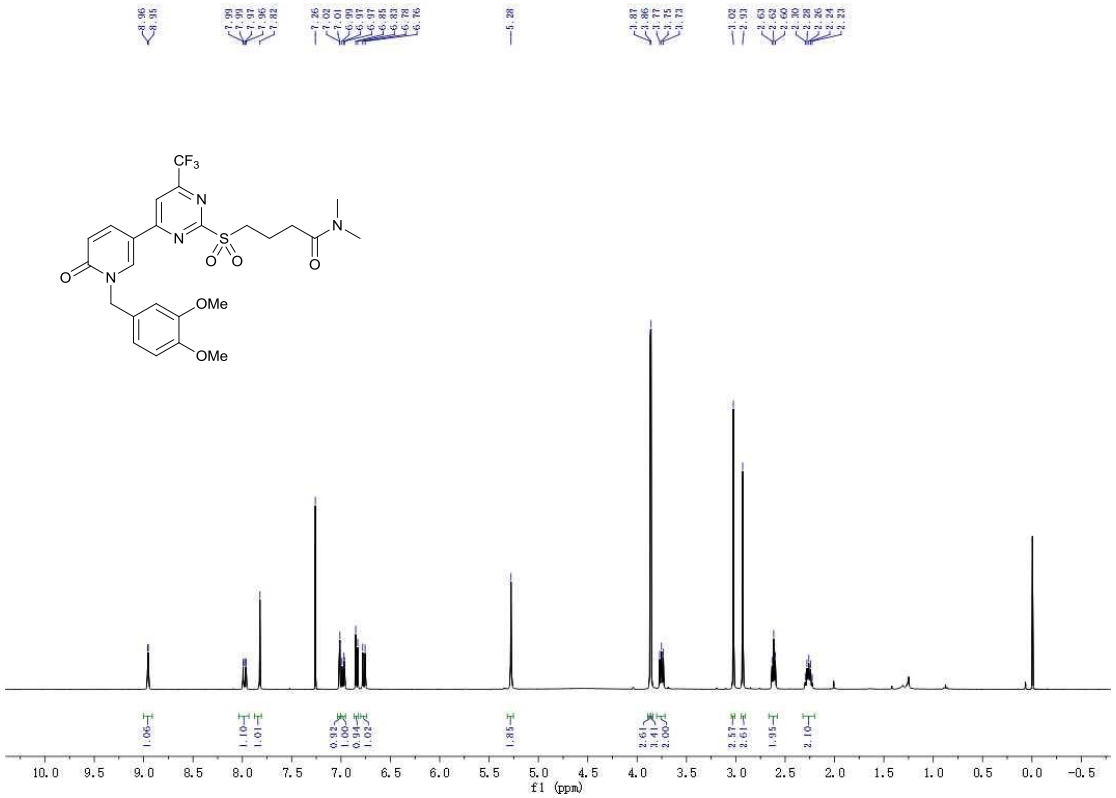

166.84
165.57
162.15
157.37
157.00
149.59
149.52
141.60
141.55
135.94
127.66
121.37
121.12
113.21
112.28
112.25
111.85
111.81
111.47
56.10
53.12
39.04
39.01
CF3
N
N
O
S
O
O
N
O
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
8.701
8.694
7.985
7.979
7.966
7.955
7.809
6.950
6.935
6.931
6.914
6.910
6.847
6.826
6.797
6.723
5.193
3.874
3.859
3.564
3.544
1.935
1.916
1.897
1.878
1.858
1.465
1.446
1.391
1.373
0.907
0.889
CF3
N
N
S
O
O
O
N
OMe
OMe
1.00
1.07
0.99
1.90
2.16
1.93
5.63
2.09
2.19
4.49
2.84
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

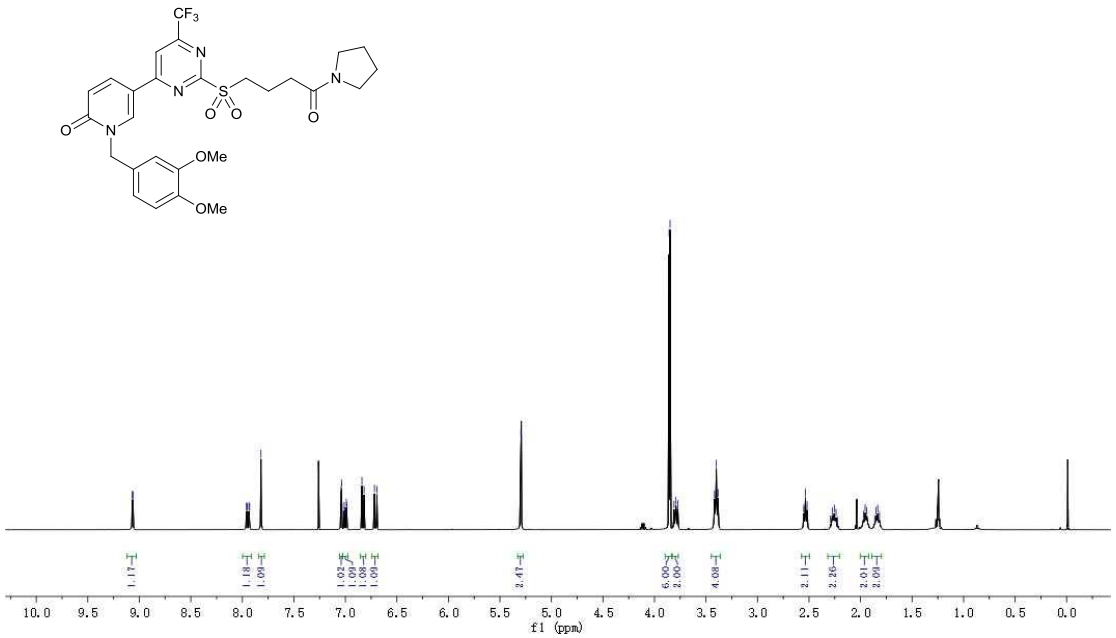


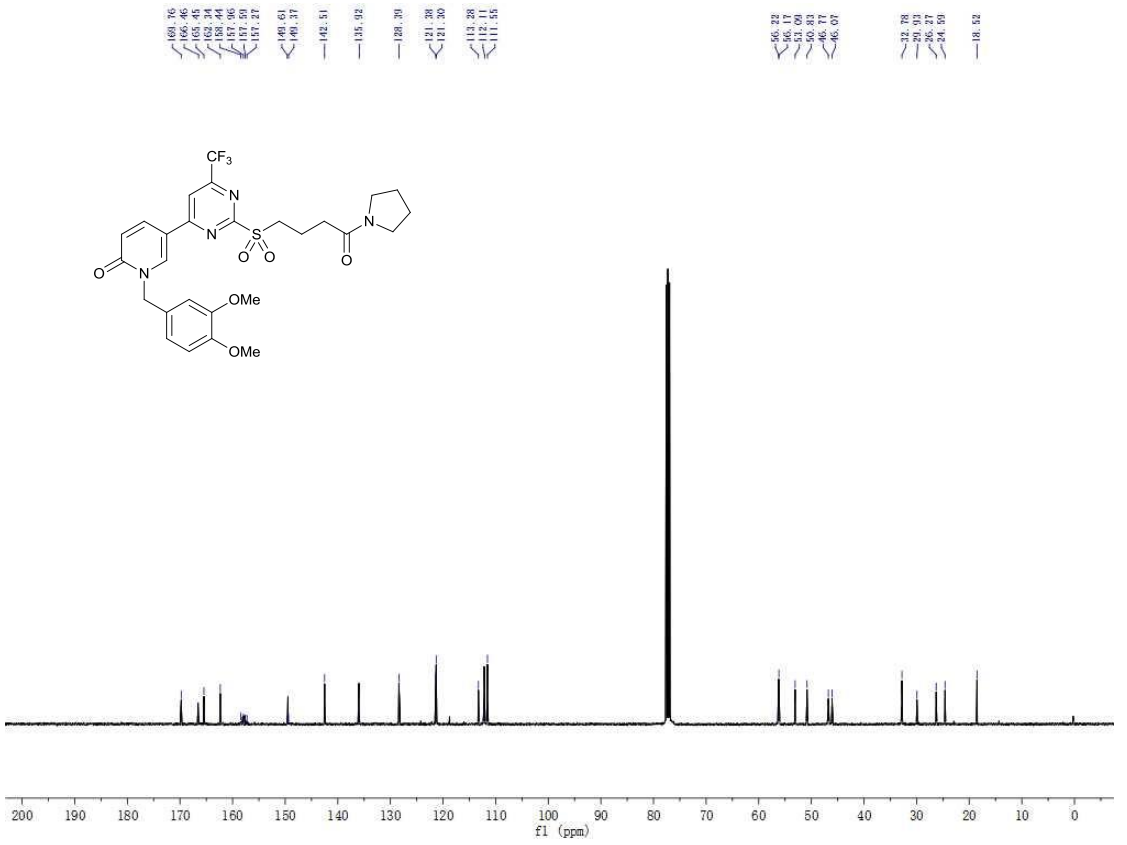


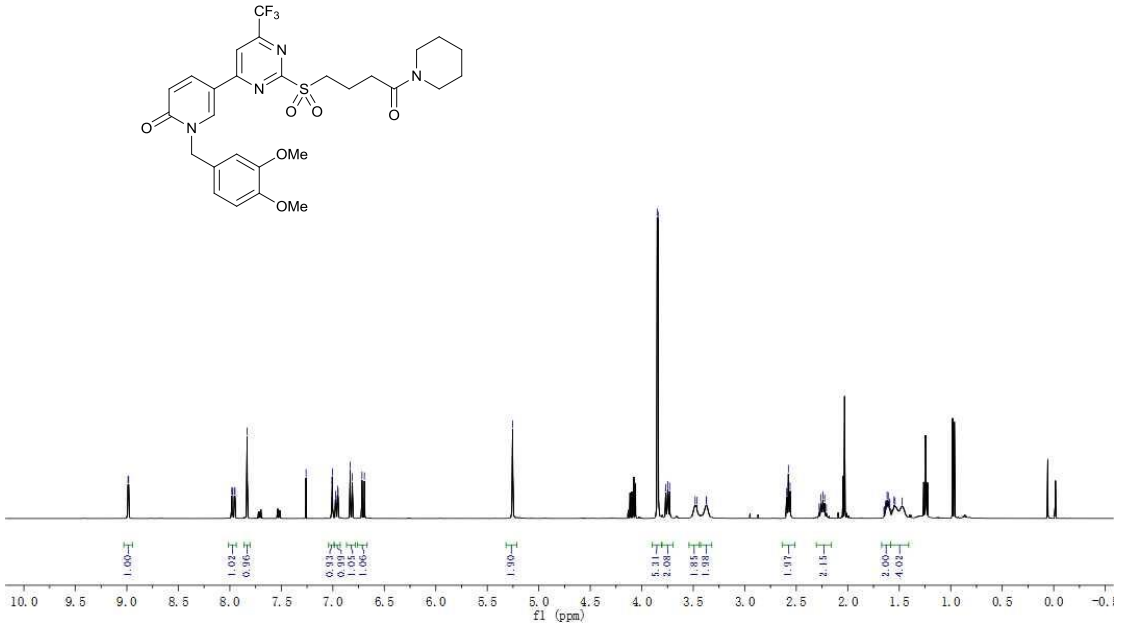


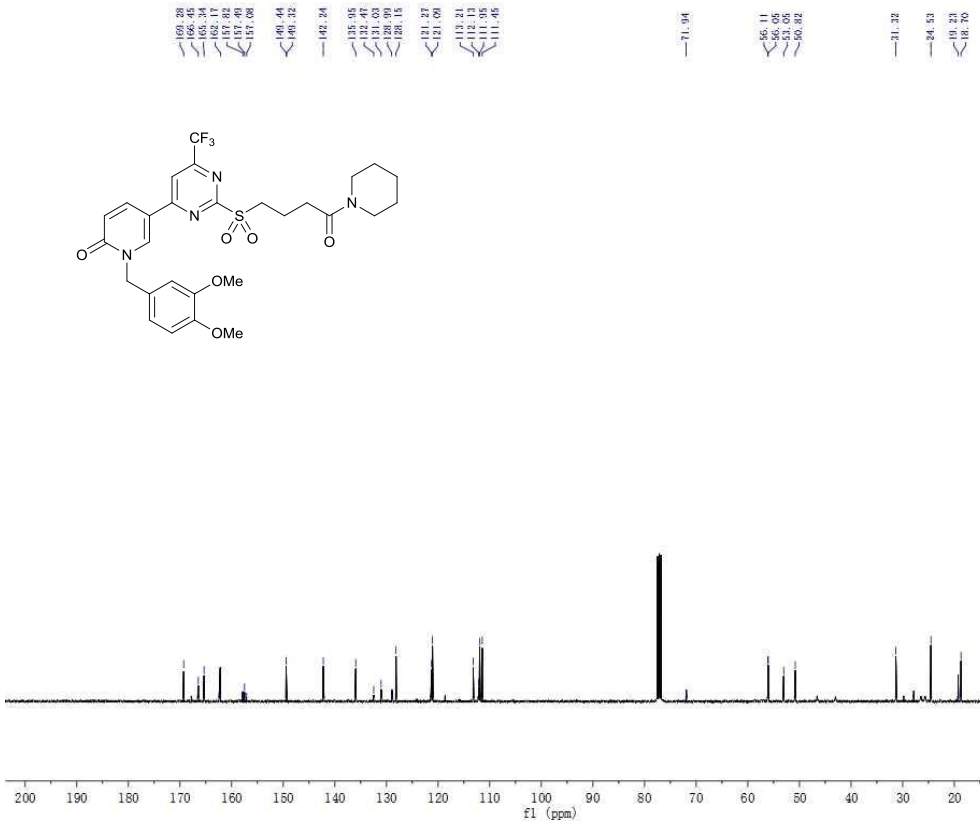



CF3
N
N
S
O
O
N
O
O
N
OMe
OMe
f1 (ppm)

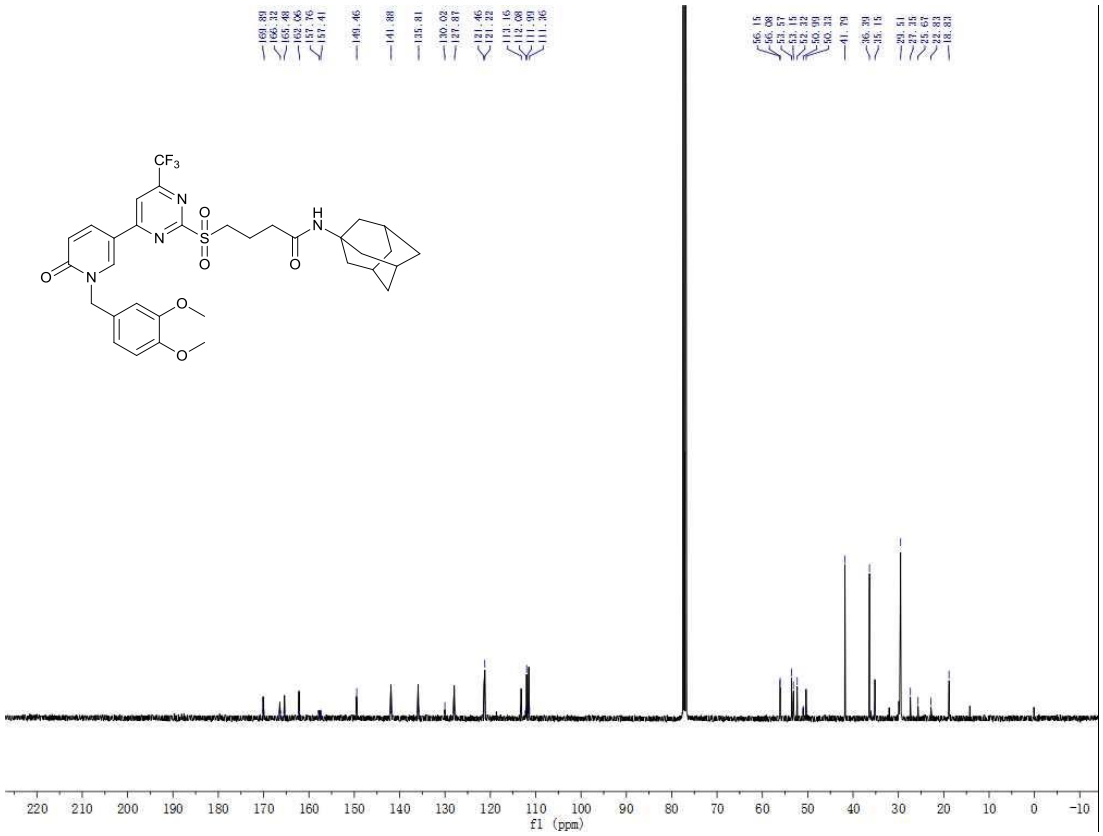

CF3
N
N
S
O
O
N
O
O
N
OMe
OMe
f1 (ppm)

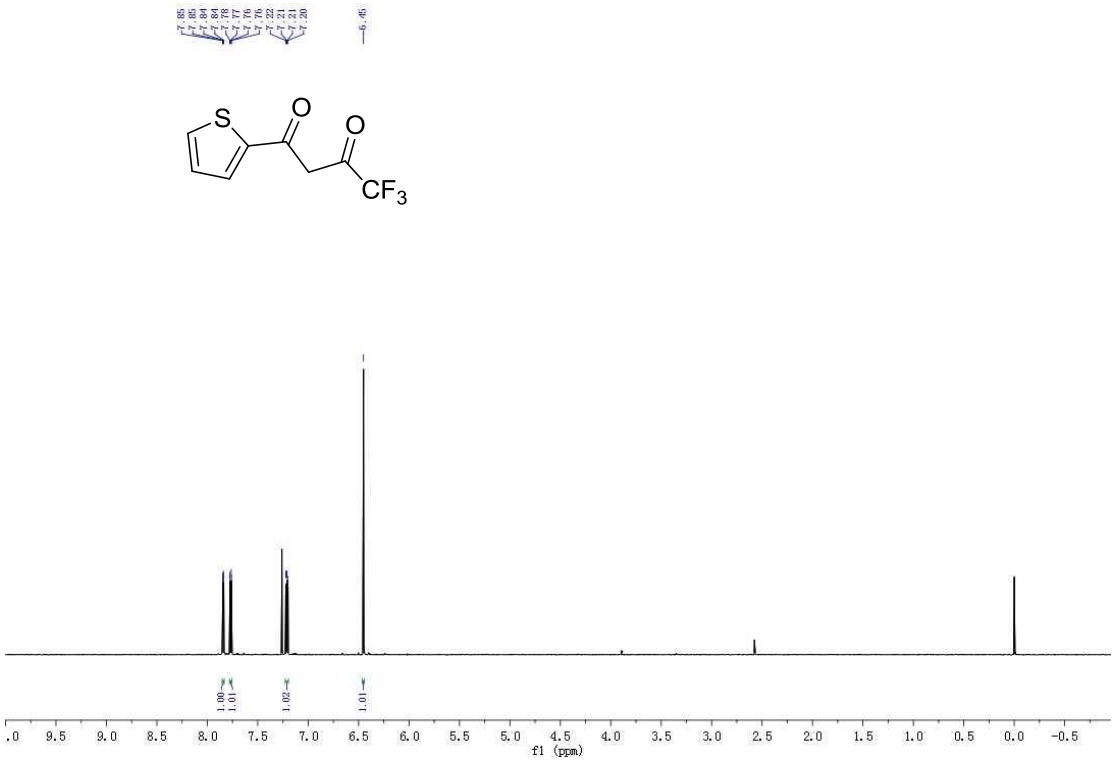

CF3
N
N
S
O
O
N
O
O
N
OMe
OMe
f1 (ppm)

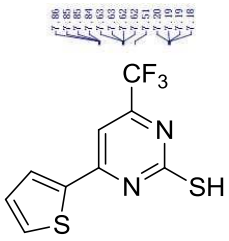

CF3
N
N
S
O
O
N
O
O
N
OMe
OMe
f1 (ppm)

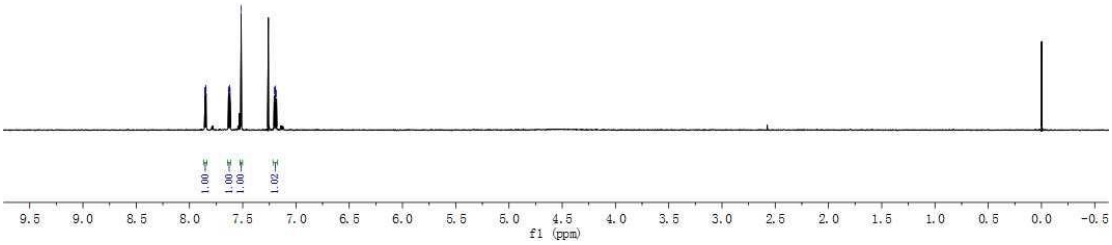

169.28
166.45
165.34
162.17
157.82
157.49
157.08
149.44
149.32
142.24
135.95
132.47
131.03
128.99
128.15
121.27
121.09
113.21
112.13
111.95
111.45
71.94
56.11
56.05
53.05
50.82
31.32
24.53
19.23
18.70
CF3
N
N
S
O
O
O
N
O
N
OMe
OMe
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

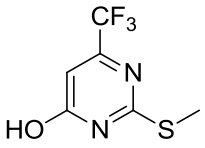

8.86
8.85
7.97
7.97
7.95
7.94
7.81
6.99
6.98
6.96
6.86
6.83
6.74
6.71
5.26
3.87
3.86
3.69
3.65
2.39
2.37
2.35
2.24
2.23
2.21
2.03
1.97
1.25
CF3
N
N
S
O
O
O
H
N
O
N
O
O
1.00
1.05
0.96
1.11
1.11
1.12
1.02
2.66
2.07
2.02
2.00
2.32
2.62
3.29
5.96
6.68
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


CF3
N
N
O
S
O
O
H
N
O
N
O
O
f1 (ppm)

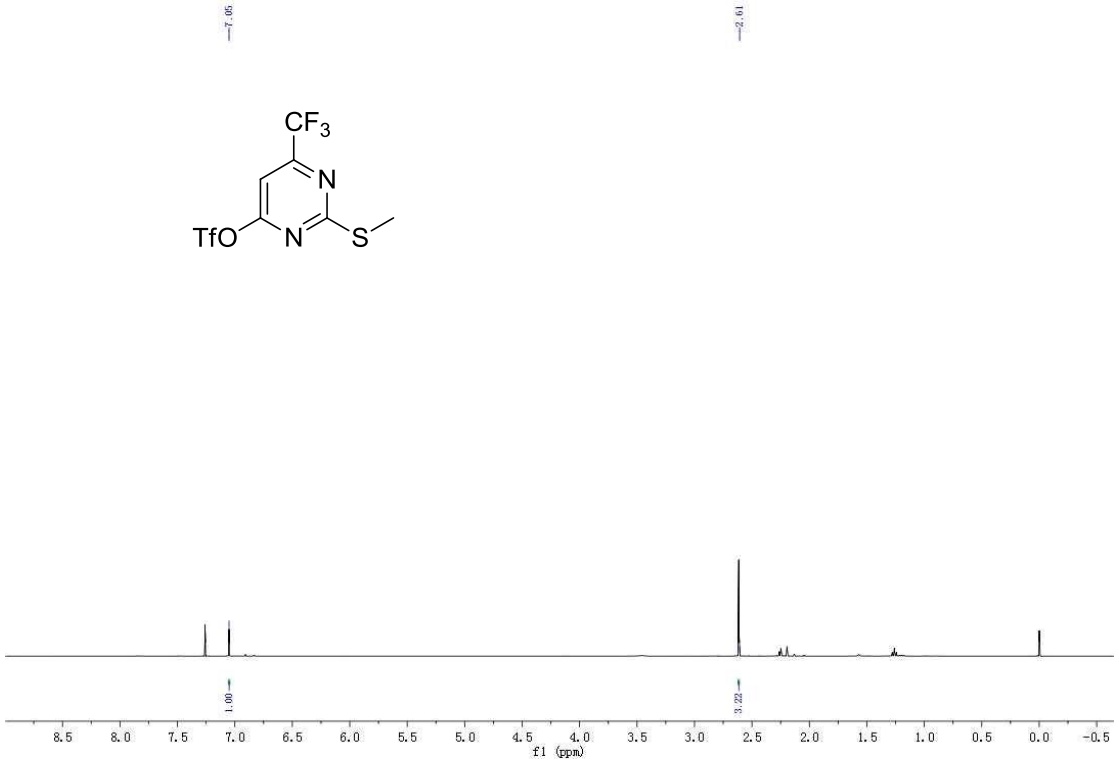

S
O
O
CF3
f1 (ppm)

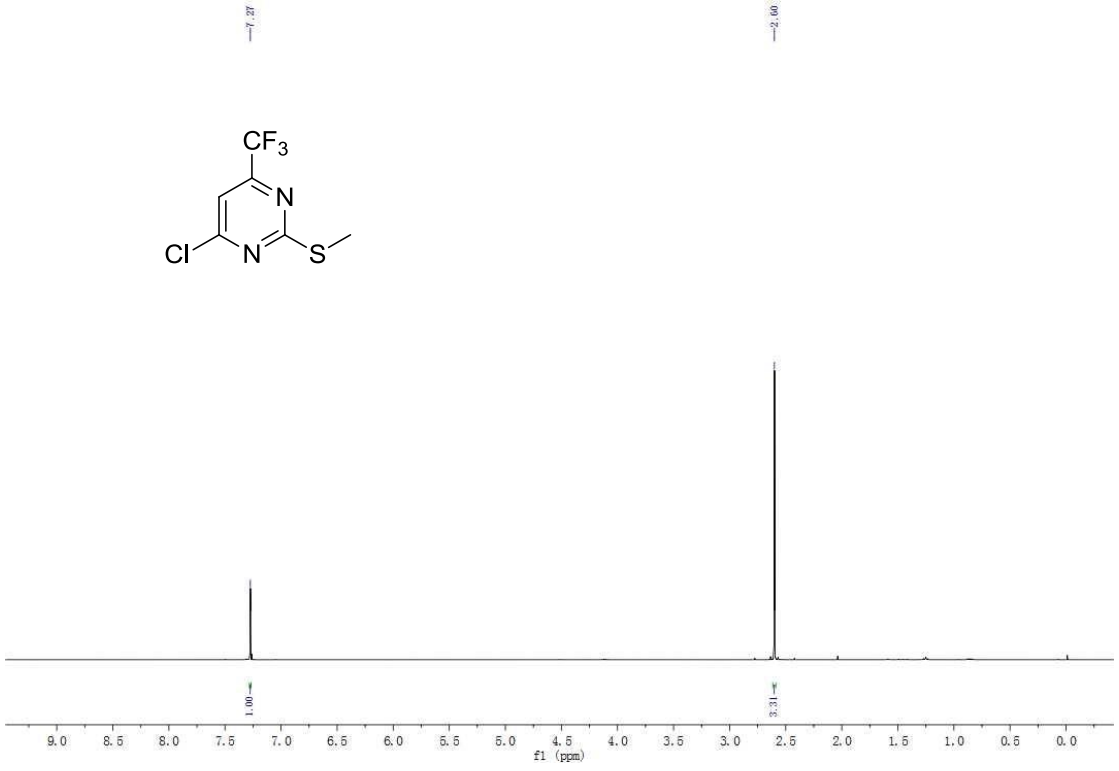

CF3
N
N
SH
S

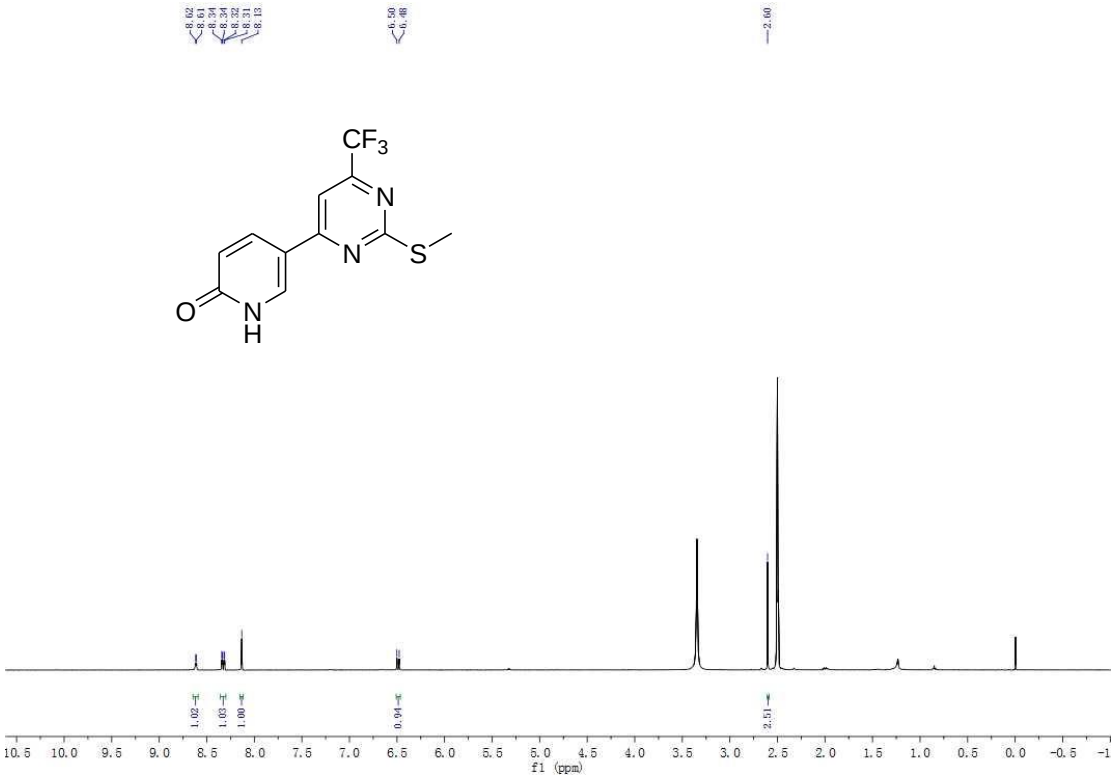

1.00
1.00
1.00
1.02
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


CF3
N
HO
N
S

6.50
2.51
1.00
4.38
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)


7.05
2.61
CF3
N
TfO
N
S
1.00
3.22
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


7.27
2.60
CF3
N
Cl
N
S
1.00
3.31
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


8.62
8.61
8.34
8.33
8.32
8.31
8.13
6.50
6.48
2.60
CF3
N
N
S
O
N
H
1.02
1.03
1.00
0.94
2.51
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
f1 (ppm)

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