Supporting Information

Discovery of a new sulfonamide hepatitis B capsid assembly modulator

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1. Experimental Procedures for Biological Assays

Cell culture and compound treatment

HepAD38 cells were cultured with DMEM media (Welgene) supplemented with 200 unit/mL penicillin, 200 μg/mL streptomycin and 10% FBS. During maintenance and passage, the cells were cultured with 0.4 μg/mL of tetracycline. Tetracycline-free complete growth medium was changed 2 days before starting the compound treatment for induction of HBV replication. HepAD38 cells were seeded at $1*10^5$ cells/well in 48-well culture plates and treated with DMSO (0.2%, as a control) or test compounds. Each compounds were preparing 5 mM compound stock solution with DMSO. Adding 2 μL of compound stock solution in 500 μL of media (20 μM) and conducted serial dilution 3 nM to 2.2 μM. Plating 150 μL of compound solution in each well and also HepAD38 cells were seeded at $1*10^5$ cells/well. The volume of cell suspension was 150 μL of each well which were same volume of compound dilute. Final concentration ranging from 1.5 nM to 1.1 μM. And 0.4% of DMSO media were used as control. After adding cell suspension, final concentration of control wells contained 0.2% of DMSO. All procedures were conducted in duplicate.

Real-time PCR for intracellular HBV DNA

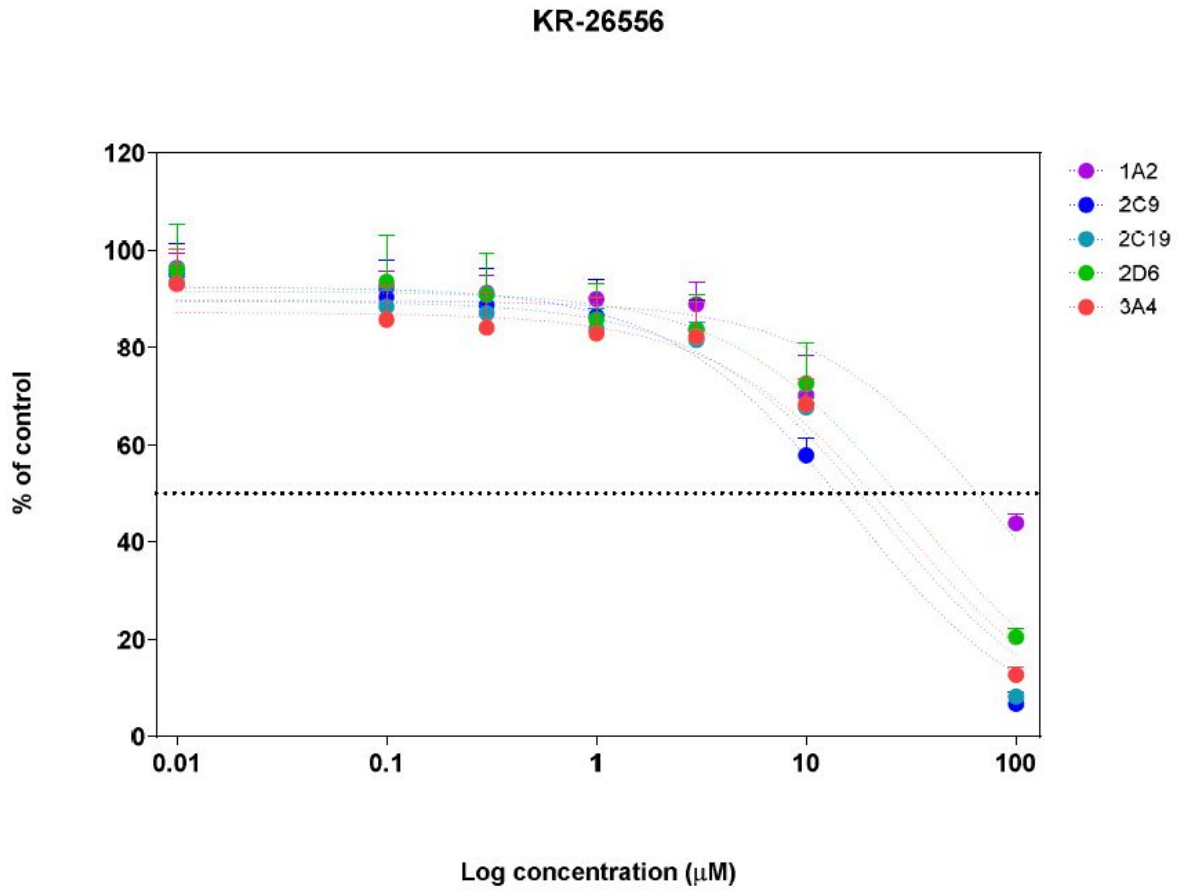
After 65 hours of treatment with compounds, the HepAD38 cells were harvested and intracellular HBV DNA was extracted by following the protocol in the DNeasy Blood & Tissue Kit (Qiagen). The primers and probes used to quantify HBV DNA were 5'-CTCGTGGTGGACTTCTCTC -3', 5'- CTGCAGGATGAAGAGGAA -3' and 5'- /56-FAM/ TGT CCT GGT /ZEN/ TAT CGC TGG ATG TGT CT /3IABkFQ/ -3'. The HBV DNA was amplified by a real-time PCR assay using the LightCycler 480 (Roche) as described this method by Lee, A. R., Lim, K.-H., Park, E.-S., Kim, D. H., Park, Y. K., Park, S., … Kim, K.-H. (2018). Multiple Functions of Cellular FLIP Are Essential for Replication of Hepatitis B Virus. *Journal of Virology*, *92*(16). All procedures were confirmed in duplicate. The variation of replicates was less than 15%.

Cell Viability Assay

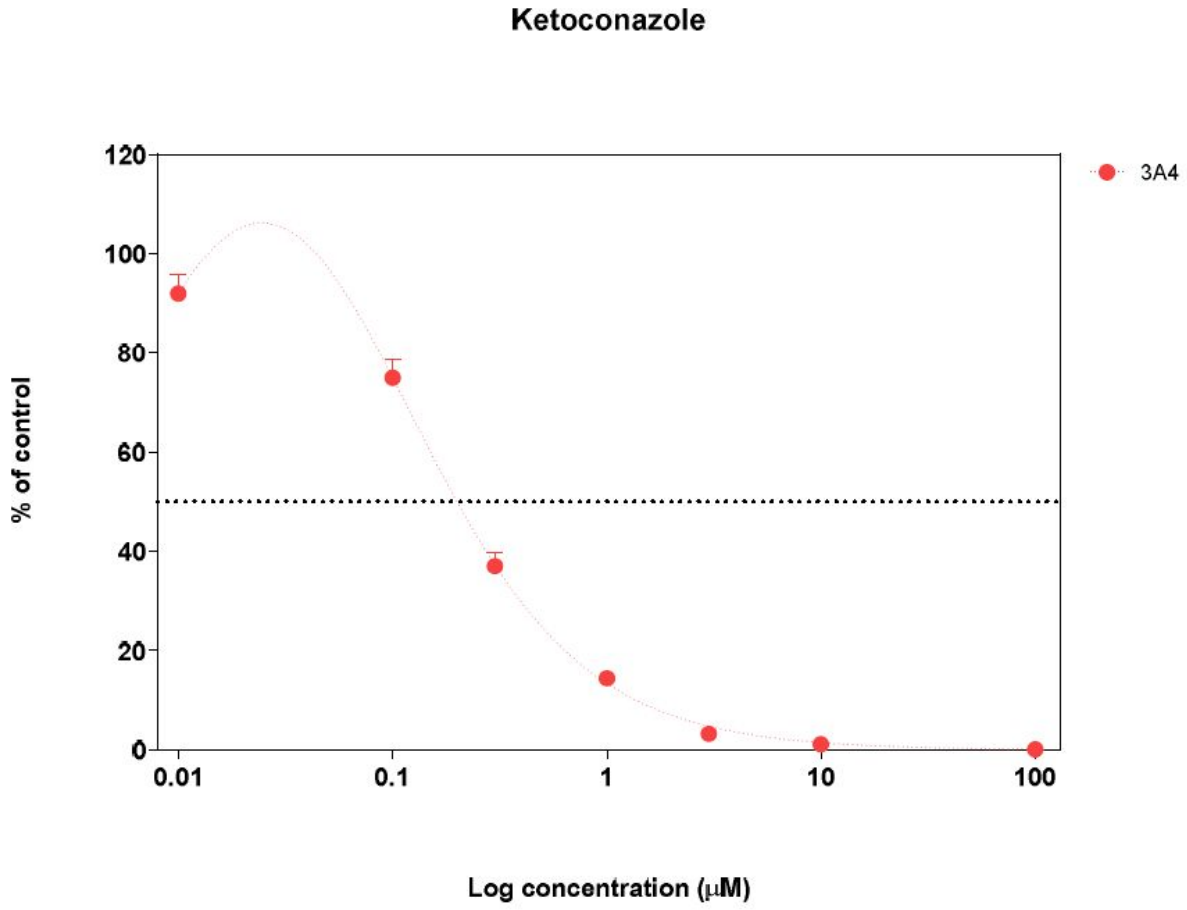
HepAD38 were cultured in Dulbecco's Modified Eagle's medium (Welgene, LM001-05) supplemented with 10% fetal bovine serum (FBS) without tetracycline for 2 days. Cells were seeded in 48-well culture plates ($1*10^5$cells per well) and were treated with 5 points (0 μM (0.8% DMSO as a control), 33 μM, 50 μM, 66 μM, 100 μM) of each compound. The cell viability was determined 65 hours after treatment by EZ-Cytox cell viability assay kit (Daeil Lab Service) according to the manufacturer's instructions. All procedures were conducted duplicate and confirmed the variation of each CC_{50} value were less than 15%. Absorbance was measured using a spectrophotometer (Spark, Tecan) at a wavelength of 450 nm.

CYP Inhibition Assay (IC_{50}, μM)

The five main cytochrome P450 isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) are investigated in Cyprotex's Cytochrome P450 Inhibition assay. Compound was diluted with Potassium Phosphate buffer and incubated at 7 concentrations from 0.01 to 100 uM with human liver microsomes with protein concentration at 10 μM α-Naphthoflavone, Sulfaphenazole, (+)-N-3-benzylnirvanol, Quinidine and Ketoconazole were used as positive control at 37 °C for 5 minutes. The mixture of test compound or positive control were pre-warmed with human liver microsomes at 37 °C for 10 min. Phenacetin, Tolubutamide, S-mephenytoin, Dextromethorphan and Sorafenib were prepared in a mixture used as substrate of CYP1A2, 2C9, 2C19, 2D6 and 3A4, respectively. After addition of 5-in-1 substrates solution and cofactor NADPH, the mixture were incubated for another 20 min. The samples were finally quenched with stop solution containing internal standard and centrifuged at 4000 rpm, at 4 °C for 15 min. Then supernatant was detected in LC-MS/MS (AB Sciex, Qtrap 4000) with HPLC (Agilent 1260) at each of the test compound concentrations.



<**Figure SI-1**. CYP assay for compound **11**>



<**Figure SI-2**. Control validation graph, Ketoconazole which is validated used system for a reference substance that inhibits CYP3A4>

Microsomal-Stability Assay

Microsomes were preincubated with a test compound for 10 min at 37 °C in 0.5 M potassium phosphate buffer (pH 7.4). Pooled liver microsomes were purchased from Corning Gentest (Tewksbury, MA, USA). The reaction mixture was composed of liver microsomes (0.5 mg protein/mL) in 100 mM PBS (pH 7.4) and the final concentration of a test compound was 1 μM. The reactions were initiated by adding NADPH solutions, test compound, 10 mM in DMSO, liver microsomal protein (Rat, Mouse, Dog(Beagle), Monkey, Mixed Gender Pooled 150-donor Human liver microsomes). After incubation times of 30 min at 37 °C, stop solution was added to terminate the reaction. Following precipitation and centrifugation, The concentrations of test compounds were determined by LC-MS/MS on an Agilent 1200 HPLC system coupled to an Agilent 6460 triple quadrupole mass spectrometer equipped with ESI source (Agilent, Santa Clara, CA, USA). The peak areas for all components were automatically integrated using Agilent 6460 Quantitative Analysis processing software. The system was validated by Buspirone which is reference compound.

Plasma Stability and Plasma Protein Binding Assay

Animal plasma (Innovative research) was added test compound stock solution (10 mM DMSO) and the mixture was spiked to prepare 5 μM of final concentration. The resulting solution was added 100 mM PBS (Thermo Fisher Scientific), after then incubated in RED plate chamber set up membrane insert. After incubation times of at 37 ^{o}C for 4 hours, stop solution was added to terminate the reaction. Following precipitation and centrifugation, the amounts of compound remaining in the samples were determined by LC-MS/ MS on an Agilent 1200 HPLC system coupled to an Agilent 6460 triple quadrupole mass spectrometer equipped with ESI source (Agilent, Santa Clara, CA, USA). The peak areas for all components were automatically integrated using Agilent 6460 Quantitative Analysis processing software. Then the result was calculated by the equation for acquiring ratio of bound compound in Plasma protein. The system was validated by reference compound.

$$\text{Free form (\%)} = (\text{Buffer chamber / Plasma chamber}) \times 100$$

$$\text{Bound form (\%)} = 100 - \text{Free form (\%)}$$

***In vivo* Rat Pharmacokinetic Study**

In vivo Pharmacokinetics of compounds was examined in male Sprague Dawley rats (7-8 weeks) via intramuscular injection and subjected to catheter surgery of the femoral vein. Then, the test compound was administered intravenously or orally. Dosing vehicles were composed of 5% DMSO and 40% PEG400 in water and dosing volume was 2 mL/kg. Blood samples were collected at different time points (n = 3 rats per time point) from the femoral vein. After centrifugation (13000 g, 3 min, 37 ºC, the plasma samples were obtained and were stored at -20 ºC until the analysis. The sample analysis was performed by LC-MS/MS as described in the above section. Pharmacokinetic and statistical analyses of plasma concentrations and statistical analysis of pharmacokinetic parameters were performed using non-compartmental analysis with Phoenix WinNonlin (v6.4; Pharsight Corp., Mountain View, CA, USA). The area under the plasma concentration-time curve from time 0 to infinity (AUC_{inf}) was calculated by the trapezoidal rule with extrapolation to time infinity. The terminal T1/2 was calculated as $\ln 2/\lambda_z$, where λ_z was the first-order rate constant associated with the terminal (log-linear) portion of the curve. Plasma clearance (CL) was calculated as dose/AUC_{inf}. The C_{max} and the time when it occurred (T_{max}) were obtained by visual inspection of the plasma concentration-time curve. The apparent volume of distribution at steady-state (Vd_{ss}) was calculated by $CL \times MRT_{inf}$ where MRT is the mean residence time extrapolated to infinity calculated as $AUMC_{inf}/AUC_{inf}$, where $AUMC_{inf}$ is the area under the first moment curve extrapolated to infinity. The bioavailability (F) was calculated as $(AUC_{non\text{-}intravenous} \times dose_{i.v.})/(AUC_{i.v} \times dose_{non\text{-}intravenous})$.

Time (h)	#1	#2	#3	Mean	S.D.
0.033	3443.6	3947.9	3854.4	3748.6	268.3
0.167	2124.6	2764.7	2555.8	2481.7	326.4
0.5	1582.8	1411.3	2005.9	1666.7	306.0
1	1321.9	1301.2	1834.3	1485.8	302.0
2	848.5	777.8	1287.4	971.2	276.1
4	487.7	282.0	564.6	444.8	146.1
6	138.3	52.0	296.9	162.4	124.2
8	83.2	28.5	144.6	85.4	58.1
24	8.9	14.1	11.8	11.6	2.6

BQL, Below the quantification limit

<**Table SI-1**. Plasma concentrations (ng/ml) after IV administration in male rats>

Time (h)	#1	#2	#3	Mean	S.D.
0.25	332.6	1069.0	1039.2	813.6	416.8
0.5	426.5	1099.0	939.2	821.6	351.4
1	362.5	675.1	719.1	585.6	194.4
2	495.5	559.0	532.3	528.9	31.9
4	671.7	318.8	319.2	436.6	203.6
6	593.3	105.4	196.7	298.4	259.4
8	432.0	56.1	107.0	198.3	203.9
24	12.3	3.7	4.4	6.8	4.8

BQL, Below the quantification limit

<**Table SI-2**. Plasma concentrations (ng/ml) after PO administration in male rats>

Subject	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC_{last} (μg·h/mL)	AUC_{∞} (μg·h/mL)	CL (L/h/kg)	Vss (L/kg)
1	NA	NA	4.69	5.84	5.90	0.85	2.73
2	NA	NA	11.27	4.81	5.04	0.99	4.05
3	NA	NA	4.06	8.25	8.32	0.60	2.06
Mean	NA	NA	6.67	6.3	6.4	0.813	2.95
SD	NA	NA	3.99	1.76	1.70	0.197	1.01

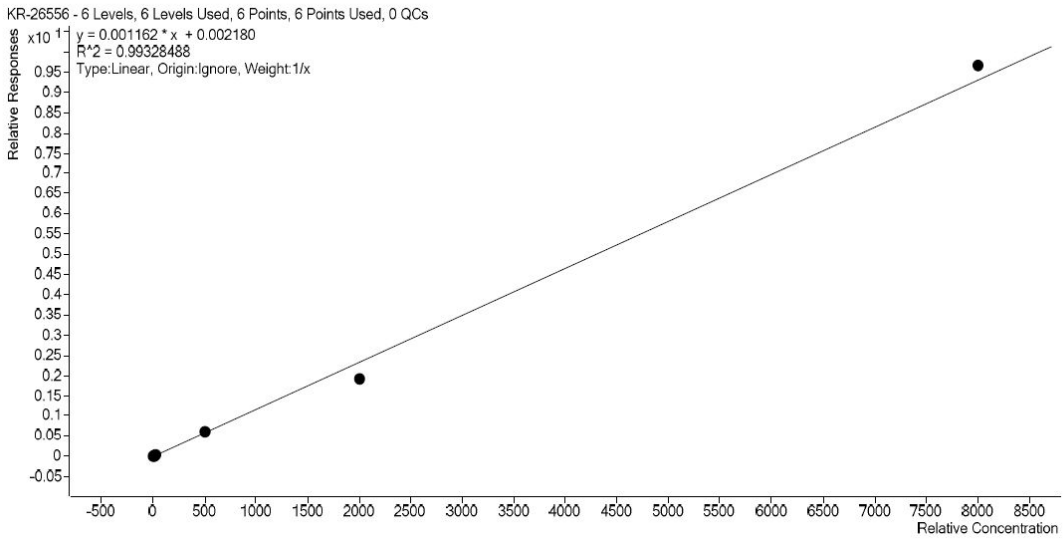
NA, not applicable; ND, not detected; NC, not calculated

<**Table SI-3**. Pharmacokinetic parameters after IV administration in male rats>

Subject	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC_{last} (μg·h/mL)	AUC_{∞} (μg·h/mL)	CL (L/h/kg)	Vss (L/kg)
1	4.00	0.67	3.18	7.77	7.83	NA	NA
2	0.50	1.10	3.87	3.41	3.43	NA	NA
3	0.25	1.04	3.28	3.98	4.00	NA	NA
Mean	1.58	0.94	3.44	5.1	5.1	NA	NA
SD	2.10	0.23	0.37	2.37	2.39	NA	NA

NA, not applicable; ND, not detected; NC, not calculated

<**Table SI-4**. Pharmacokinetic parameters after PO administration in male rats>



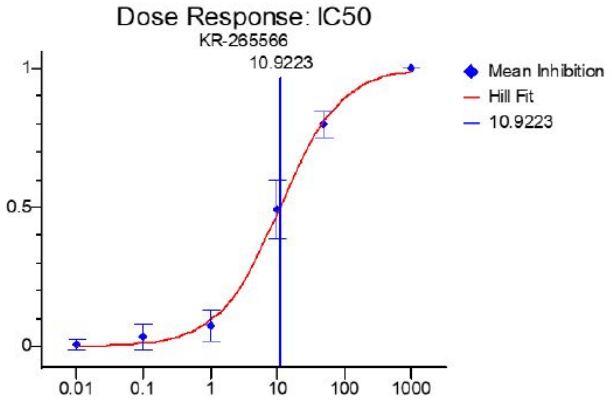
<**Figure SI-3**. The calibration of compound **11**>

hERG Patch Clamp Assay

In vitro electrophysiology manual-patch-clamp assays of selected compounds on hERG channels by using HEK293 cell line were conducted by Automated Planar Patch Clamp (PatchXpress, 7000A, in Korea Research Institute of Chemical Technology) in compliance with their Standard Operating Procedures.

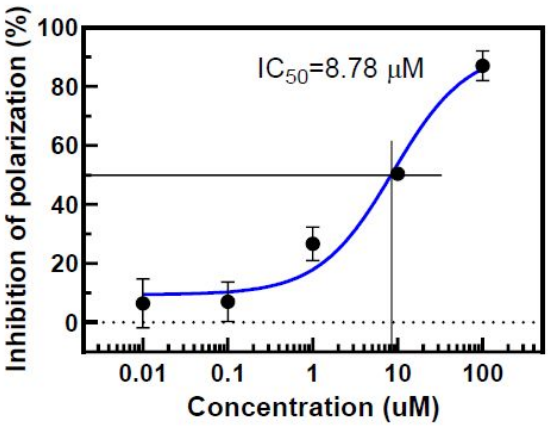
hERG Ligand Binding Assay

The Ligand binding assay is non-electrophysiological test, which is evaluated binding state between hERG protein and red fluorescent hERG channel ligand tracer through fluorescence polarization is validated cardiotoxicity of test compound. The test compound was allowed to room temperature, and test compound stock solution (10 mM DMSO solution) was treated Tracer (Astemizole, 1 mM DMSO stock, Basal signal compensation)and hERG assay buffer kit (Predictor hERG fluorescence polarization assay kit, Life technology). The result was detected by proper instrument (Infinite M 1000 Pro Microplate Reader, Tecan). The test was validated by positive control (E-4031, 3 mM DMSO stock solution).



Conc (μM)	Ave.	Std.
0.01	0.007099	0.017472
0.1	0.033615	0.045615
1	0.074847	0.058957
10	0.49145	0.105835
50	0.79675	0.045909
1000	1	0

<**Figure SI-4**. Result of hERG K^+ channel assay>



<**Figure SI-5**. Dose-dependent inhibition of polarization by compound **11**>

Treatment		IC_{50} (μM)	Conc. (μM)	% inhibition
Positive control	E-4031		10	84.2 ± 2.37
Test compounds	KR-26556	8.78	0.01	6.46 ± 8.29
			0.1	6.98 ± 6.76
			1	26.6 ± 2.71
			10	**50.4 ± 2.05**
			100	87.0 ± 5.08

<**Table SI-5**. Result of hERG ligand binding assay (mean ± SD, n = 3)>

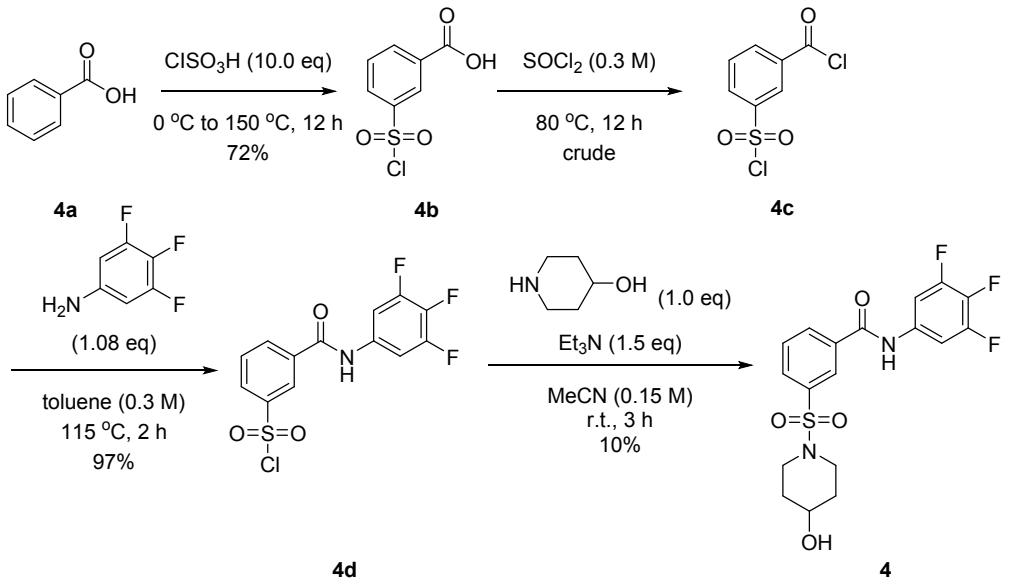
2. Chemical Synthetic Procedures

General Procedures

Unless otherwise stated, all commercially available reagents and solvents were used without further purification. DMF, CH_3CN, CH_2Cl_2, and THF were dried by using a JC Meyer solvent purification system prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. Column chromatography was performed on silica-cartridge which to use MPLC (Puchem Flash Column or Biotage ZIP KP-Sil) using proper eluent systems. ^1H NMR spectra were recorded on Bruker each 300,400,500 instrument at each 300,400,500 MHz. Chemical shifts were quoted in parts per million (ppm) referenced to the appropriate solvent peak or 0.0 ppm for tetramethylsilane. The following abbreviations were used to describe peak splitting patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, td = triplet of doublet, ddd = doublet of doublet of doublet, m = multiplet. Coupling constants, *J*, were reported in hertz unit (Hz). ^{13}C NMR spectra were recorded on Bruker 500 instrument at 126 MHz and fully decoupled by broad band proton decoupling. High resolution mass spectra (HRMS) were obtained from the Korea Research Institute of Chemical Technology by using EI ionization method. All compounds assayed were >95% pure, as determined by UPLC analysis conducted on Waters Acquity UPLC H-Class system with photodiode array (PDA) detector using a reverse-phase column with a linear H_2O/CH_3CN gradient system, 10 % to 90 % CH_3CN in H_2O.

Preparation of Key compounds

3-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (4)



3-(chlorosulfonyl)benzoic acid (4b) : In the reaction vessel are placed magnetic stirred bar and $ClSO_3H$ and cooled to 0 °C, Then Benzoic acid (610 mg, 5.00 mmol) was added portionwise. The reaction mixture was allowed to room temperature and stirred for 12 hours at 150 °C. The resulting mixture was cooled to room temperature and cautiously poured into ice water dropwise. The resulting precipitate was filtered off and washed with H_2O to obtain the crude product. (790 mg, 72%)

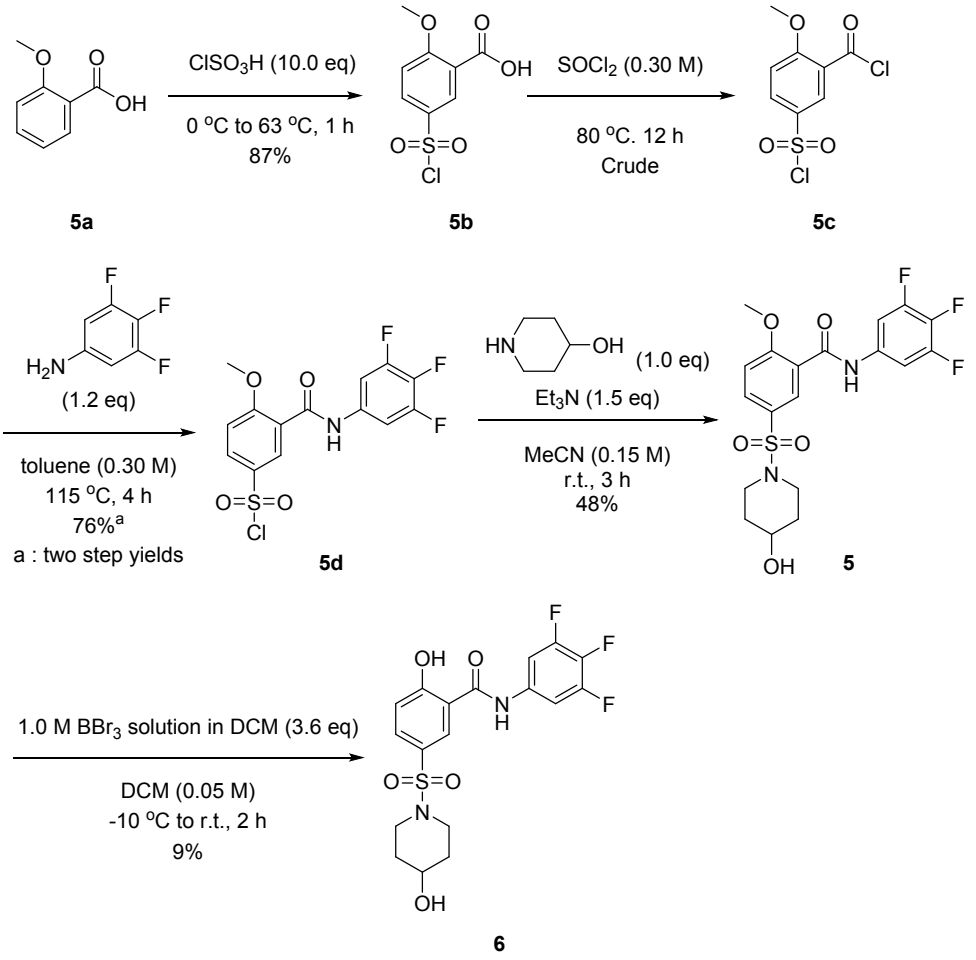
3-(chlorosulfonyl)benzoyl chloride (4c) : 3-(chlorosulfonyl)benzoic acid (780 mg, 3.53 mmol) was dissolved in $SOCl_2$ (12 mL, 0.3 M) at room temperature. The reaction mixture was stirred for 12 hours at 80 °C. The resulting mixture was concentrated *in vacuo* to obtain the crude product without further purification.

3-((3,4,5-trifluorophenyl)carbamoyl)benzenesulfonyl chloride (4d) : To a solution of 3-(chlorosulfonyl)benzoyl chloride (837 mg, 3.50 mmol) in toluene (8 mL). The reaction mixture was added slowly a suspension of 3,4,5-trifluoroaniline (570 mg, 3.80 mmol) in toluene (4 mL) and stirred for 2 hours at 115 °C. The resulting mixture was cooled to room temperature, and concentrated *in vacuo*. The residue was diluted with DCM and washed with water and brine. The combined organic layer was dried over anhydrous Na_2SO_4, evaporated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (1.20 g, 97%)

3-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (4) : To a solution of 3-((3,4,5-trifluorophenyl)carbamoyl) benzenesulfonyl chloride (1.2 g, 3.4 mmol) in MeCN (23 mL, 0.15 M) was added Piperidin-4-ol (343 mg, 3.4 mmol), Et_3N (0.7 mL, 5.1

mmol). The reaction mixture was stirred for 3 hours at room temperature. The resulting mixture was concentrated *in vacuo*. The residue was filtered off and washed with MeOH to obtain the desired product. (135.6 mg, 10%) ^{1}H NMR (300 MHz, Acetone-*d6*) δ 10.17 (s, 1H), 8.30 (d, *J* = 6.0 Hz, 2H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.73 (dd, *J* = 10.2, 6.5 Hz, 2H), 3.77 (d, *J* = 32.5 Hz, 2H), 3.29 (dd, *J* = 12.8, 5.3 Hz, 2H), 2.95 – 2.90 (m, 1H), 1.93 – 1.82 (m, 2H), 1.65 – 1.53 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.92, 136.71, 135.59, 132.61, 131.13, 130.35, 126.86, 105.35 (d, *J* = 5.7 Hz), 105.20 (d, *J* = 5.6 Hz), 64.15, 43.63, 33.32. HRMS (EI) *m*/*z* calcd for $C_{18}H_{17}F_3N_2O_4S$ $[M]^+$ 414.0861, found 414.0857.

5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-methoxy-*N*-(3,4,5-trifluorophenyl)benzamide (5) and 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-methoxy-*N*-(3,4,5-trifluorophenyl) benzamide (6)



5-(chlorosulfonyl)-2-methoxybenzoic acid (5b) : In the reaction vessel are placed magnetic stirred bar and $ClSO_3H$ (6.64 mL, 100 mmol) and cooled to 0 °C. Then 2-methoxybenzoic acid (1.52 g, 10.0 mmol) was added portionwise. The reaction mixture was stirred for 1 hour at 63

°C. The resulting solution was cooled to room temperature and poured into ice H_2O dropwise. The resulting precipitate was filtered off and washed with H_2O to obtain the desired product. (2.17 g, 87 %) ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.46 (d, J = 2.6 Hz, 1H), 8.30 (dd, J = 9.0, 2.6 Hz, 1H), 7.57 (d, J = 9.1 Hz, 1H), 4.14 (s, 3H).

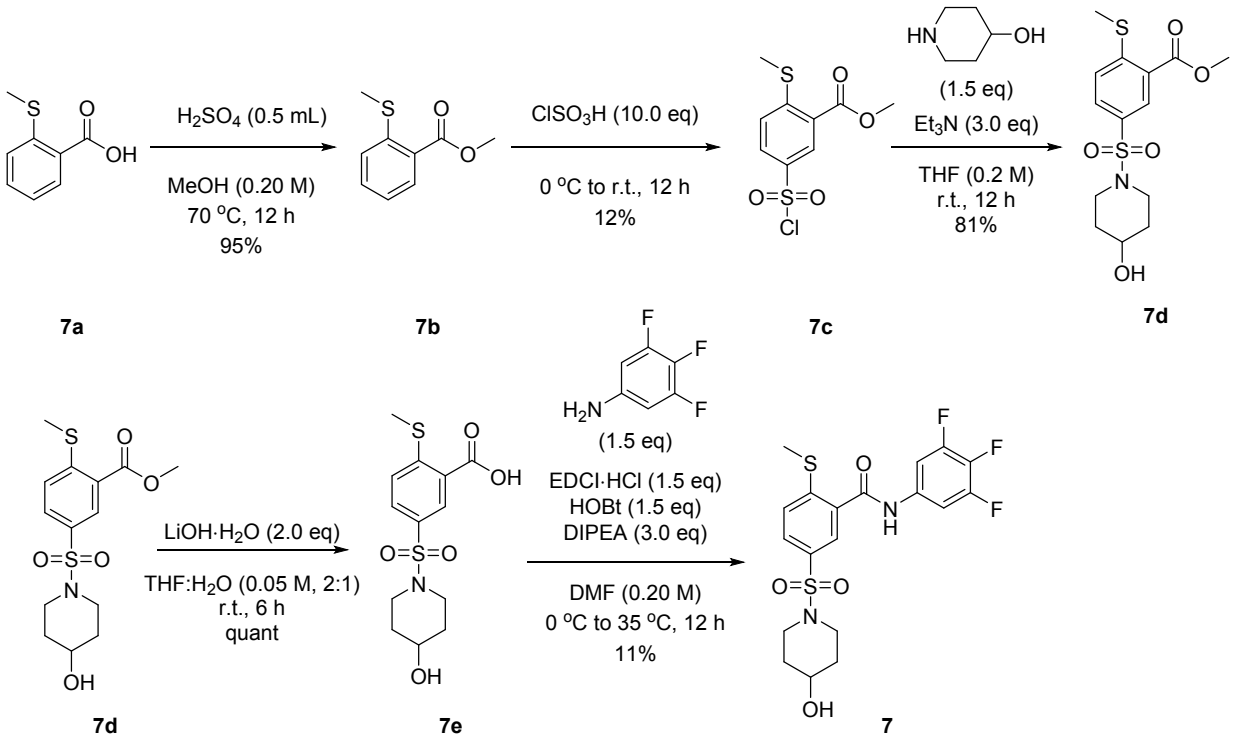
5-(chlorosulfonyl)-2-methoxybenzoyl chloride (5c) : 5-(chlorosulfonyl)-2-methoxybenzoic acid (500 mg, 2.00 mmol) was dissolved in $SOCl_2$ (8 mL, 0.25 M). The reaction mixture was stirred for 12 hours at 80 °C. The reaction mixture was cooled to room temperature, concentrated under reduced pressure. The residue was dissolved in toluene, evaporated *in vacuo* to remove remaining $SOCl_2$ several times to obtain the crude product without further purification.

4-methoxy-3-((3,4,5-trifluorophenyl)carbamoyl)benzenesulfonyl chloride (5d) : To a solution of 5-(chlorosulfonyl)-2-methoxybenzoyl chloride (538 mg) in toluene (4 mL) was added the suspension of 3,4,5-trifluoroaniline (353 mg, 2.40 mmol) in toluene (4 mL) slowly. The reaction mixture was stirred for 12 hours at 115 °C. The reaction mass was cooled to room temperature, concentrated under reduced pressure. The residue was washed with DCM, filtered off to obtain the desired product. (580 mg, 76 % two step yields)

5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-methoxy-*N*-(3,4,5-trifluorophenyl)benzamide (5) : To a solution of 4-methoxy-3-((3,4,5-trifluorophenyl)carbamoyl)benzenesulfonyl chloride (100 mg, 0.26 mmol) in MeCN (2.0 mL, 0.15 M) was added Piperidin-4-ol (27 mg, 0.26 mmol) and Et_3N (0.06 mL, 0.40 mmol). The reaction mixture was stirred for 3 hours at room temperature. The reaction mass was diluted with water and extracted with DCM. The organic layers were washed with brine and dried over Na_2SO_4, concentrated under reduce pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (55 mg, 48 %) ^{1}H NMR (300 MHz, MeOD-*d4*) δ 8.14 (d, J = 2.3 Hz, 1H), 7.92 (dd, J = 8.8 Hz, J = 2.3 Hz, 1H), 7.57 (dd, J = 9.9, 6.4 Hz, 2H), 7.38 (d, J = 8.8 Hz, 1H), 4.08 (s, 3H), 3.67 – 3.60 (m, 1H), 3.38 – 3.33 (m, 2H), 2.83 – 2.77 (m, 2H), 1.91 – 1.84 (m, 2H), 1.62 – 1.51 (m, 2H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 162.64, 162.55, 160.35, 132.87, 130.99, 129.11, 122.79, 112.80, 104.63, 104.43, 64.53, 64.41, 56.63, 43.21, 33.13 (d, J = 6.0 Hz). LC/MS $[M+H]^+$ 444.96 HRMS (EI) *m/z* calcd for $C_{19}H_{19}F_3N_2O_5S$ $[M]^+$ 444.0967, found 444.0970.

2-hydroxy-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (6) : To a solution of 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-methoxy-*N*-(3,4,5-trifluorophenyl)
benzamide (75 mg, 0.17 mmol) in DCM (3.4 mL, 0.05 M) under nitrogen atmosphere was added an excess amount of 1 M BBr_3 solution in DCM at -10 °C. The reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was quenched with MeOH and was concentrated under reduced pressure. The crude product was washed with n-hexane, and then residue was washed with diethyl ether three times. The residue was dried to afford the desired product. (46 mg, 64%) ^{1}H NMR (500 MHz, MeOD-*d4*) δ 8.36 (s, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.58 (dd, J = 9.6, 6.5 Hz, 2H), 6.99 (d, J = 8.7 Hz, 1H), 4.62 (s, 1H), 3.69 – 3.60 (m, 1H), 3.42 – 3.36 (m, 2H), 2.77 (t, J = 9.3 Hz, 2H), 1.96 – 1.85 (m, 2H), 1.65 – 1.54 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 165.67, 161.92, 151.87 – 151.61 (m), 149.93 – 149.63 (m), 132.85, 129.58, 127.17, 117.81, 117.65, 105.24 (d, J = 6.4 Hz), 105.09 (d, J = 6.1 Hz), 65.26, 43.38, 32.82. HRMS (EI) *m/z* calcd for $C_{18}H_{17}F_3N_2O_5S$ $[M]^+$ 430.0810, found 430.0970.

5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)-*N*-(3,4,5-trifluorophenyl) benzamide (7)



methyl 2-(methylthio)benzoate (7b) : To a solution of 2-(methylthio)benzoic acid (673 mg, 4.00 mmol) in MeOH (20 mL, 0.20 M) was added H_2SO_4 (0.5 mL) and stirred at 70 °C for 12 hours. The reaction mixture was cooled to room temperature, added slowly saturated $NaHCO_3$ solution until pH 7. The resulting solution was extracted with EtOAc. The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get the desired product. ^{1}H NMR (300 MHz, Acetone-d_6) δ 7.95 (dd, J = 7.8, 1.6 Hz, 1H), 7.63 – 7.49 (m, 1H), 7.47 – 7.35 (m, 1H), 7.22 (ddd, J = 8.3, 7.3, 1.2 Hz, 1H), 3.86 (s, 3H), 2.45 (s, 3H). (693 mg, 95 %)

methyl 5-(chlorosulfonyl)-2-(methylthio)benzoate (7c) : In the reaction vessel were placed magnetic stirred bar and $ClSO_3H$ (2.5 mL, 38.0 mmol) and cooled to 0 °C. Methyl 2-(methylthio)benzoate (693 mg, 3.80 mmol) was added portionwise. The reaction mixture was stirred for 12 hours at room temperature. The reaction mass was poured into ice H_2O dropwise, extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (124 mg, 12 %) ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.77 – 8.50 (m, 3H), 4.06 (s, 3H), 2.91 (s, 3H).

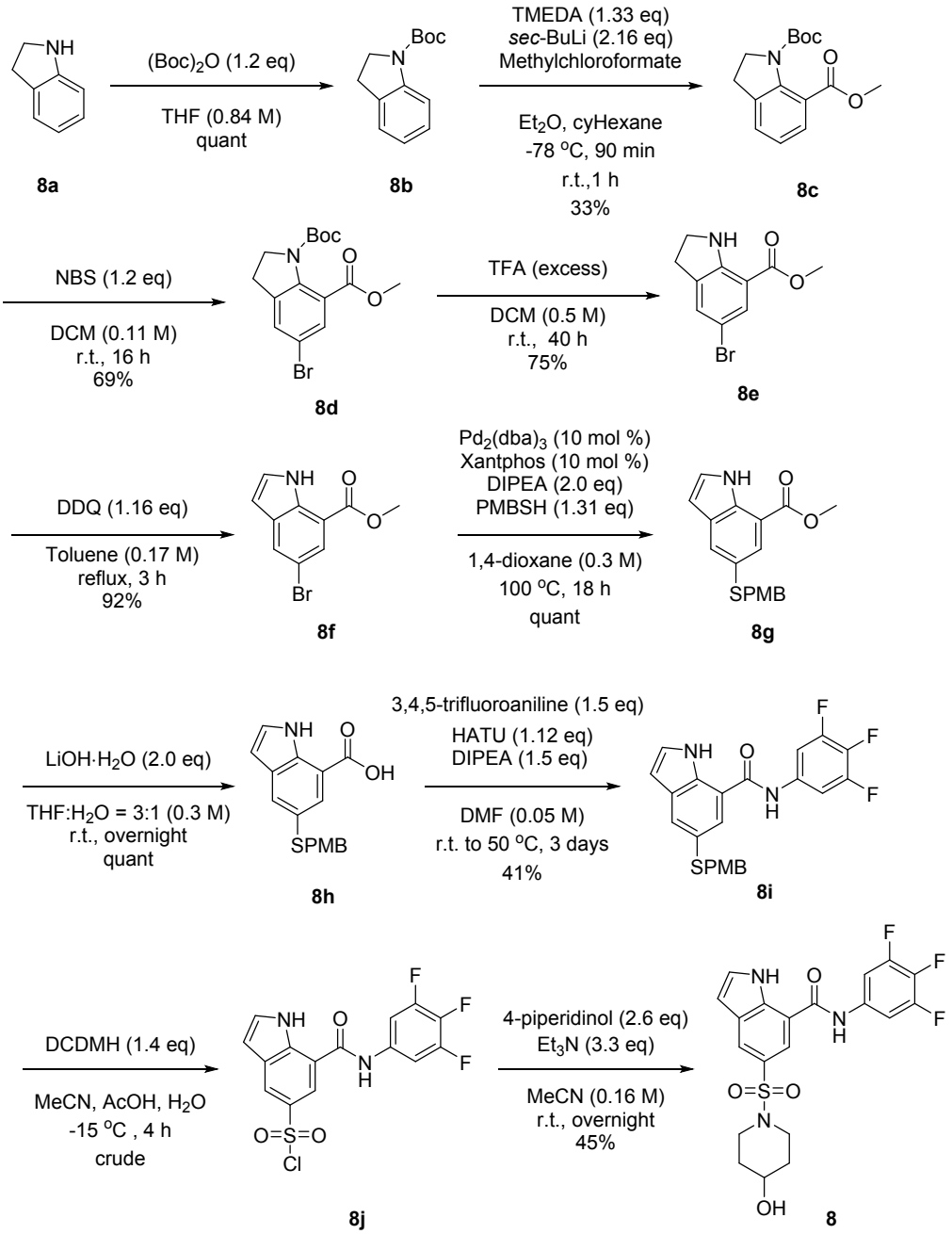
methyl 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)benzoate (7d) : To a solution

of Methyl 5-(chlorosulfonyl)-2-(methylthio)benzoate (124 mg, 0.44 mmol) in THF (2 mL, 0.20 M) was added Piperidin-4-ol (67 mg, 0.66 mmol), Et_3N (0.2 mL, 5.88 mmol). The reaction mixture was stirred at room temperature for 12 hours. The resulting solution was diluted with H_2O and extracted with $CHCl_3$. The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (123 mg, 81 %) ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.47 (d, J = 8.2 Hz, 1H), 8.40 (d, J = 1.8 Hz, 1H), 8.29 (dd, J = 8.2, 1.9 Hz, 1H), 4.01 (s, 3H), 3.86 (s, 1H), 3.73 (dd, J = 7.5, 3.8 Hz, 1H), 3.35 (ddd, J = 11.3, 7.2, 3.8 Hz, 2H), 3.03 – 2.89 (m, 3H), 2.86 (s, 3H), 1.88 (dq, J = 10.9, 3.6 Hz, 2H), 1.62 (ddd, J = 13.1, 8.2, 3.9 Hz, 2H).

5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)benzoic acid (7e) : Methyl 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)benzoate (90 mg, 0.26 mmol) was dissolved in THF:H_2O (5 mL, 0.05 M, 2:1). The reaction mixture was added $LiOH \cdot H_2O$ (22 mg, 0.52 mmol), stirred for 6 hours at room temperature. The reaction mass was acidified to pH 2 with 1 N HCl solution. The resulting precipitate was filtered off and washed with DCM and H_2O to obtain the desired product. (88 mg, quant.) ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.50 – 8.42 (m, 2H), 8.30 (dd, J = 8.2, 1.9 Hz, 1H), 3.73 (dq, J = 7.5, 3.7 Hz, 1H), 3.37 (ddd, J = 11.2, 7.2, 3.8 Hz, 2H), 3.03 – 2.91 (m, 2H), 2.84 (s, 3H), 1.89 (ddd, J = 14.1, 7.2, 3.5 Hz, 2H), 1.61 (dtd, J = 11.9, 7.8, 3.7 Hz, 2H).

5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)-*N*-(3,4,5-trifluorophenyl) benzamide (7) : To a solution of 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)benzoic acid (20 mg, 0.06 mmol) in DMF (0.6 mL, 0.10 M) was cooled to 0 °C, sequentially added EDCI·HCl (17 mg, 0.09 mmol) and HOBt (12 mg, 0.09 mmol). The reaction mixture was stirred at 0 °C for 10 minutes, then 3,4,5-trifluoroaniline (13 mg, 0.09 mmol) and DIPEA (0.05 mL, 0.18 mmol) were added. The reaction mixture was stirred at 35 °C for 12 hours. The resulting solution was diluted with H_2O, extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (12 mg, 11 %) ^{1}H NMR (300 MHz, MeOD-d_4) δ 7.85 – 7.82 (m, 2H), 7.61 (d, J = 8.2 Hz, 1H), 7.57 – 7.46 (m, 1H), 3.69 – 3.61 (m, 1H), 3.41 – 3.35 (m, 2H), 2.87 – 2.79 (m, 2H), 1.92 –1.85 (m, 2H), 1.63 – 1.52 (m, 2H). LC/MS $[M+H]^+$ 461.2

5-((4-hydroxypiperidin-1-yl)sulfonyl)-N-(3,4,5-trifluorophenyl)-1H-indole-7-carboxamide (8)



***tert*-butyl indoline-1-carboxylate (8b)** : To a solution of Indoline (5.0 g, 42.0 mmol) in THF (50.0 mL) was added di-*tert*-butylcarbonate (11.0 g, 50.0 mmol).The reaction mixture was stirred at room temperature under N_2 for 12 hours. The reaction mass was concentrated under reduced pressure. The residue was diluted with H_2O and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, evaporated *in vacuo*. The

crude mixture was purified by SiO2 column chromatography to obtain the desired product. (10.2 g, quant)

1-(*tert*-butyl) 7-methyl indoline-1,7-dicarboxylate (8c) : *ter*t-butyl indoline-1-carboxylate (2.39 g, 10.90 mmol) and TMEDA (1.69 mL, 14.50 mmol) was dissolved in dry diethyl ether (72.6 mL) at – 78 ^{o}C. The resulting solution was added *sec*-butyl lithium (1.4 M solution in cyclohexane, 15.6 mL, 21.80 mmol) dropwise over 10 minutes and the reaction left stirring for 90 minutes at this temperature. Methyl chloroformate (4.52 g, 47.85 mmol) was added to the mixture and the reaction mass was allowed to warm up to room temperature over 1 hour. The resulting solution was added H_2O carefully, separated the organic layer and washed 3 times with more water. The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (998 mg, 33%)

1-(*tert*-butyl) 7-methyl 5-bromoindoline-1,7-dicarboxylate (8d) : 1-(*tert*-butyl) 7-methyl indoline-1,7-dicarboxylate (998.0 mg, 3.599 mmol) and NBS (768.6 mg, 4.318 mmol) were dissolved in dry DCM (32.7 mL) and stirred under a nitrogen atmosphere at room temperature for 16 hours. The reaction mass was quenched sodium hydroxide solution (2 M), extracted with DCM, washed with more sodium hydroxide solution. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain the desired product. (880 mg, 69%)

methyl 5-bromoindoline-7-carboxylate (8e) : To a solution of 1-(*tert*-butyl) 7-methyl 5-bromoindoline-1,7-dicarboxylate (645.6 mg, 1.81 mmol) in DCM (3.62 mL, 0.5 M) was added TFA (0.43 mL). The reaction mixture was stirred at room temperature for 40 hours. The reaction mass was added saturated solution until pH 8, extracted with DCM. The organic layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (349 mg, 75%)

methyl 5-bromo-1H-indole-7-carboxylate (8f) : DDQ (326.5 mg, 1.44 mmol) was added portionwise to a stirred solution of methyl 5-bromoindoline-7-carboxylate (317.6 mg, 317.6 mmol) in dry toluene at room temperature under nitrogen atmosphere. The reaction mixture was stirred at reflux for 3 h. The reaction mass was cooled to room temperature, diluted with water and extracted with EtOAc. The organic layers were washed with brine, dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude was purified by SiO2 column chromatography to obtain the desired product. (289.6 mg, 92%)

methyl 5-((4-methoxybenzyl)thio)-1H-indole-7-carboxylate (8g) : A mixture of methyl 5-bromo-1H-indole-7-carboxylate (289.6 mg, 1.14 mmol), PMBSH (0.21 mL), $Pd_2(dba)_3$, (104.4 mg, 10 mol%), Xantphos (66.0 mg, 10 mol%) and DIPEA (0.40 mL, 2.28 mmol) in 1,4-dioxane (3.8 mL) was heated to 100 ^{o}C and stirred for 18 hours. The reaction mixture was cooled to room temperature, filtered through celite. The residue was diluted with water and extracted with EtOAc. The organic layers were washed with brine, dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to give the product. (375 mg, quant)

5-((4-methoxybenzyl)thio)-1H-indole-7-carboxylic acid (8h) : Methyl 5-((4-methoxybenzyl)thio)-1H-indole-7-carboxylate (375.0 mg, 1.14 mmol) was dissolved in

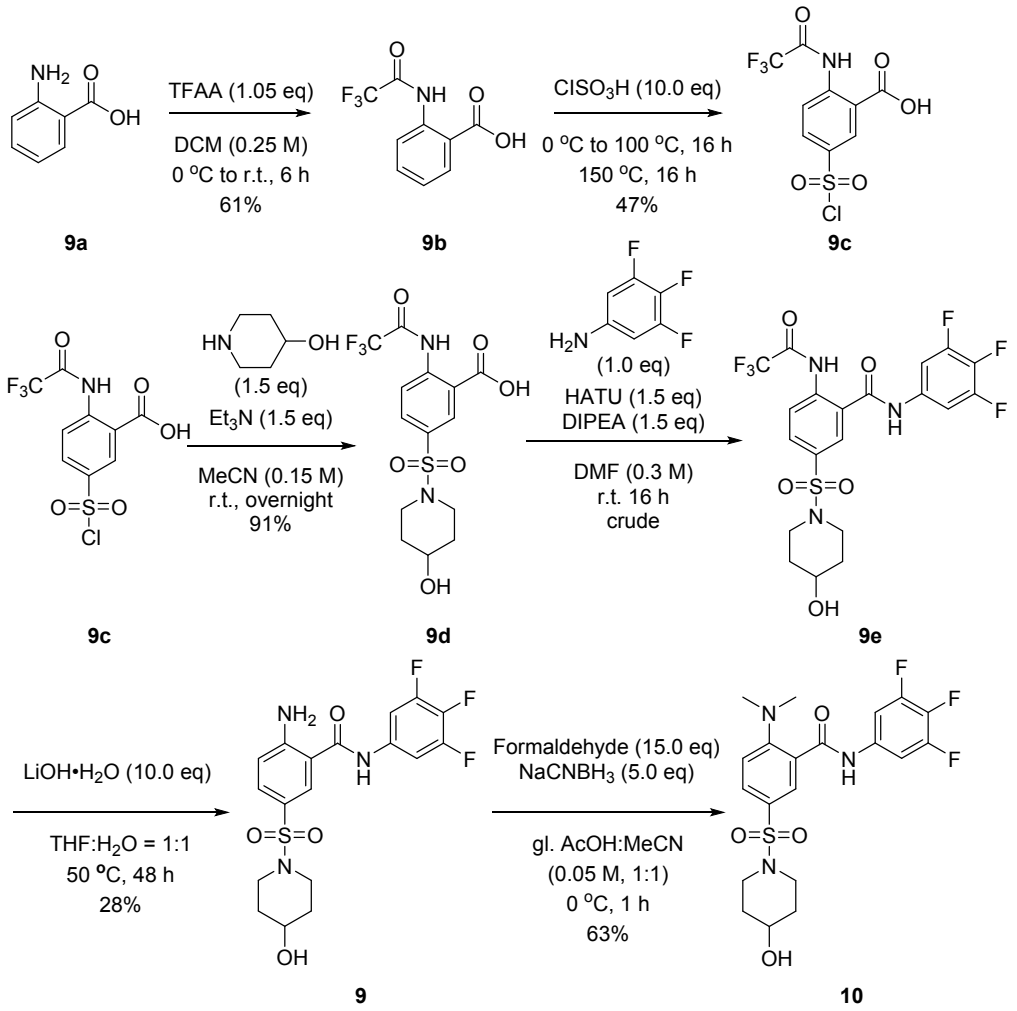
THF:H_2O (3:1, 0.3 M, 3.8 mL). The resulting solution was added $LiOH \cdot H_2O$ (95.7 mg, 2.28 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mass was acidified with 1 N HCl solution until pH 2. The resulting precipitated was filtered off to give the desired product. (357.2 mg, quant)

5-((4-methoxybenzyl)thio)-*N*-(3,4,5-trifluorophenyl)-1H-indole-7-carboxamide (8i) : To a solution of 5-((4-methoxybenzyl)thio)-1H-indole-7-carboxylic acid (250.0 mg, 0.80 mmol) in DMF (8.00 mL) was added HATU (340.7 mg, 0.90 mmol), DIPEA (0.21 mL, 1.20 mmol), 3,4,5,-trifluoroaniline (176.5 mg, 1.20 mmol). The reaction mixture was stirred at room temperature for 4 hours. After then the reaction mixture was allowed to warm at 50 ^oC and stirred for 3 days. The reaction mass was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduce pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (145.7 mg, 41%)

7-((3,4,5-trifluorophenyl)carbamoyl)-1H-indole-5-sulfonyl chloride (8j) : To a solution of 5-((4-methoxybenzyl)thio)-*N*-(3,4,5-trifluorophenyl)benzo[b]thiophene-7-carboxamide (119.2 mg, 0.270 mmol) in MeCN (6.75 mL) was added AcOH (0.084 mL), H_2O (0.17 mL), and 1,3-Dichloro-5,5-dimethylhydantoin (16.5 mg, 0.084 mmol) at -15 ^oC. The resulting solution was stirred for 4 hours. The reaction mass was diluted with water and extracted with DCM. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the crude product without further purification used directly in the next step.

5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)-1H-indole-7-carboxamide (8) : To a solution of crude 7-((3,4,5-trifluorophenyl)carbamoyl)-1H-indole-5-sulfonyl chloride in MeCN (0.16 M) was added 4-piperidinol (71 mg, 0.702 mmol), Et_3N (0.124 mL, 0.891 mmol). The resulting mixture was stirred at room temperature overnight. The reaction mass was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (54.7 mg, 45%) ^1H NMR (500 MHz, Acetonitrile-d_3) δ 10.77 (s, 1H), 9.27 (s, 1H), 8.30 (d, J = 1.5 Hz, 1H), 8.16 (d, J = 1.5 Hz, 1H), 7.63 (dd, J = 10.2, 6.4 Hz, 2H), 7.58 (s, 1H), 6.79 (dd, J = 3.3, 1.6 Hz, 1H), 3.64 – 3.50 (m, 1H), 3.39 – 3.26 (m, 2H), 2.82 – 2.75(m, 3H), 1.87 – 1.82 (m, 2H), 1.57 – 1.52 (m, 2H).^{13}C NMR (126 MHz, Acetonitrile-d_3) δ 165.46, 151.78, 149.83, 136.74, 129.53, 128.91, 126.50, 125.15, 119.25, 116.18, 105.44 (d, J = 6.2 Hz), 105.29 (d, J = 6.9 Hz), 103.33, 65.04, 43.75, 33.11. HRMS (EI) *m/z* calcd for $C_{20}H_{18}F_3N_3O_4S$ $[M]^+$ 453.0790, found 453.0964.

2-amino-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (9) and 2-(dimethylamino)-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (10)



2-(2,2,2-trifluoroacetamido)benzoic acid (9b) : To a solution of 2-aminobenzoic acid (3.0 g, 21.8 mmol) in dry DCM (87 mL) was added trifluoroacetic anhydride (3.24 mL, 23.0 mmol) dropwise at 0 °C. The resulting solution was stirred at room temperature for 6 hours. The reaction mixture was washed with saturated $NaHCO_3$ solution, the resulting precipitate was filtered off, and washed with DCM to obtain the desired product. (3.5 g, 61%) ^{1}H NMR (500 MHz, Acetone-d_6) δ 12.75 (s, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 7.9 Hz, 1H), 7.76 (t, J = 7.9 Hz, 1H), 7.38 (t, J = 7.7 Hz, 1H).

5-(chlorosulfonyl)-2-(2,2,2-trifluoroacetamido)benzoic acid (9c) : In the reaction vessel were placed magnetic stirred bar and $ClSO_3H$ (8.8 mL) and cooled at 0 °C, then 2-(2,2,2-trifluoroacetamido)benzoic acid (3.5 g, 13.2 mmol) was added portionwise. The reaction mixture was stirred at 100 °C for 16 hours, after then the reaction mixture was heated to 150

°C and stirred for 16 hours. The reaction mixture was cooled to room temperature, and put dropwise into ice water. The aqueous layer was extracted with Ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4, concentrated under reduced pressure to obtain the product. (2.04 g, 47%) ^{1}H NMR (300 MHz, Acetone-d_6) δ 9.11 (s, 1H), 8.71 (dd, J = 7.3, 2.2 Hz, 1H), 8.43 (ddd, J = 8.7, 4.3, 2.2 Hz, 1H), (dd, J = 8.7, 2.2 Hz, 1H).

5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)benzoic acid (9d) : To a solution of 5-(chlorosulfonyl)-2-(2,2,2-trifluoroacetamido)benzoic acid (500 mg, 1.51 mmol) in MeCN (10 mL, 0.15 M) was added Piperidin-4-ol (229 mg, 2.26 mmol), Et_3N (0.32 mL, 2.26 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*, The residue was diluted with EtOAc, washed with brine. The combine organic layer was dried over anhydrous $MgSO_4$, evaporated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (548 mg, 91%) ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.78 (d, J = 8.7 Hz, 1H), 8.58 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 8.7, 2.3 Hz, 1H), 3.71 (septet, J = 3.6 Hz, 1H), 3.34 – 3.27 (m, 2H), 2.90 – 2.82 (m, 2H), 1.90 – 1.82 (m, 2H), 1.63 – 1.52 (m, 2H).

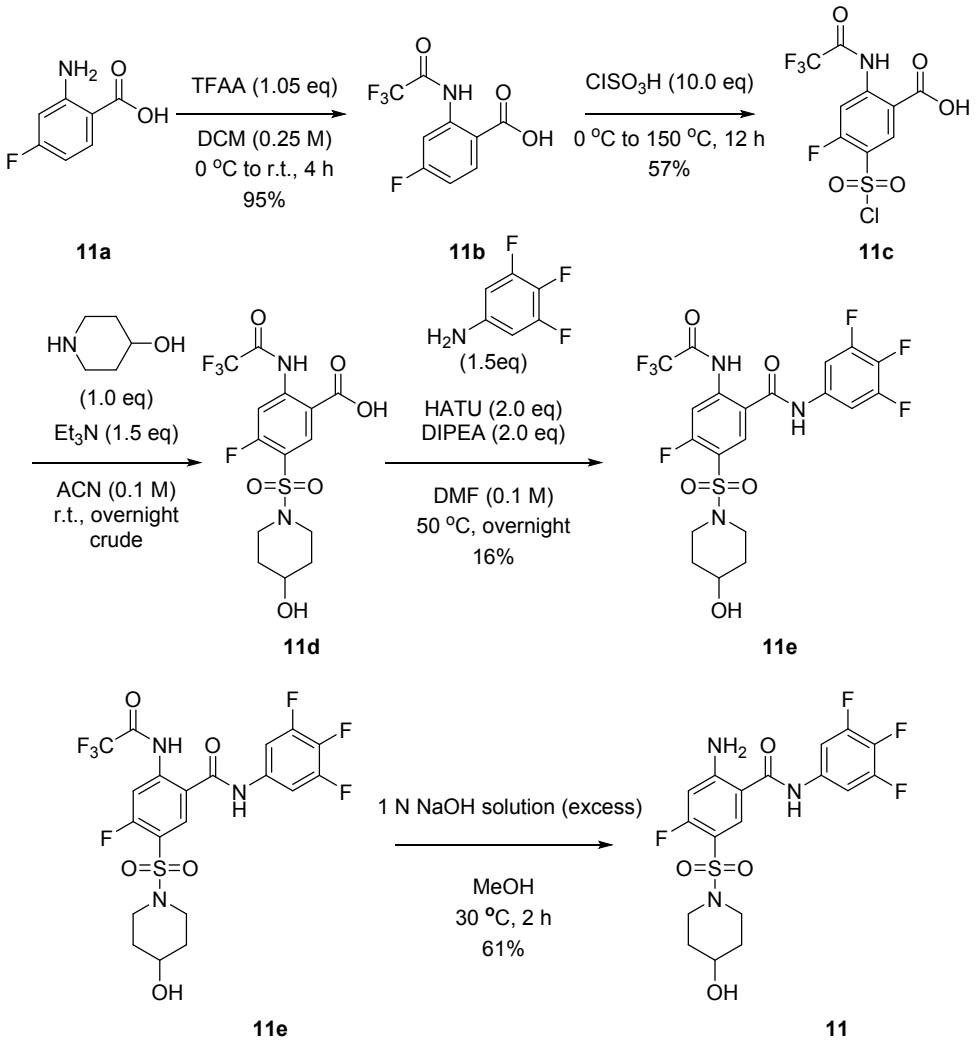
5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)-*N*-(3,4,5-trifluorophenyl)benzamide (9e) : To a solution of 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)benzoic acid (540 mg, 1.36 mmol) in DMF (4.5 mL, 0.3 M) was added HATU (775 mg, 2.04 mmol), sequentially added DIPEA (0.36 mL, 2.04 mmol). The reaction mixture was stirred at room temperature for 30 minutes. The resulting solution was added 3,4,5-trifluoroaniline (200 mg, 1.36 mmol) and stirred at room temperature for 16 hours. The reaction mixture was diluted with water, extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the crude product. (149 mg, crude)

2-amino-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (9) : To a solution of crude 5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)-*N*-(3,4,5-trifluorophenyl)benzamide (122 mg, 0.232 mmol) in THF (1 mL) was added a solution of $LiOH{\cdot}H_2O$ (97.4 mg, 2.32 mmol) in H_2O (1 mL). The reaction mixture was stirred at 50 °C for 48 hours. The resulting mixture was diluted with EtOAc, washed with brine. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (27 mg, 28%) ^{1}H NMR (300 MHz, MeOD-d_4) δ 8.00 (d, J = 2.1 Hz, 1H), 7.73 – 7.42 (m, 3H), 6.90 (d, J = 8.8 Hz, 1H), 3.74 – 3.57 (m, 1H), 3.53 – 3.37 (m, 2H), 2.83 – 2.72 (m, 2H), 1.94 –1.87 (m, 2H), 1.64–1.54 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 167.61, 153.61, 151.66, 149.71, 131.40, 129.04, 121.18, 116.30, 113.67, 104.93 (dd, J = 6.1, 1.1 Hz), 104.77 (dd, J = 6.1, 1.2 Hz), 65.43, 43.41, 32.89. HRMS (EI) *m*/*z* calcd for $C_{18}H_{18}F_3N_3O_4S$ $[M]^+$ 429.0970, found 429.0968.

2-(dimethylamino)-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (10) : 2-amino-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (44 mg, 0.10 mmol) was dissolved in gl. AcOH:MeCN (2 mL, 0.05 M, 1:1). The solution was cooled to 0 °C. After then it was added 35 % formaldehyde aqueous solution (0.15 mL, 1.54 mmol) and sequentially treated $NaBH_3CN$ (32 mg, 0.51 mmol). The reaction mixture was stirred for 3 hours at 0 °C. The reaction mass was diluted with H_2O,

extracted with EtOAc. The organic layers were washed with brine and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (29 mg, 63 %) ^{1}H NMR (300 MHz, MeOD-d_4) δ 7.83 (d, J = 2.3 Hz, 1H), 7.73 (dd, J = 8.8, 2.3 Hz, 1H), 7.60 – 7.50 (m, 2H), 7.20 (d, J = 8.8 Hz, 1H), 3.67 – 3.59 (m, 1H), 3.37 – 3.33 (m, 2H), 2.98 (s, 6H), 2.82 – 2.74 (m, 2H), 1.92 –1.83 (m, 2H), 1.63–1.52 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 167.73, 153.17, 151.84, 149.96, 130.77, 129.71, 125.46, 124.13, 116.69, 104.22 (d, J = 1.4 Hz), 104.02 (d, J = 1.3 Hz), 65.22, 43.32, 41.93, 32.81. HRMS (EI) m/z calcd for $C_{20}H_{22}F_3N_3O_4S$ $[M]^+$ 457.1283 found 457.1271.

2-amino-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (11)



4-fluoro-2-(2,2,2-trifluoroacetamido)benzoic acid (11b) : To a solution of 4-fluoroanthranilicacid (5.0 g, 32.2 mmol) in dry DCM (130 mL) was added trifluoroacetic anhydride (4.8 mL, 33.8 mmol) dropwise at 0 °C. The resulting solution was stirred at room temperature for 4 hours. The reaction mixture was added a saturated $NaHCO_3$ solution. Then the resulting precipitate was filtered off and washed with DCM to obtain the product. (7.6 g, 95%) ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.77 (s, 1H), 8.40 – 8.27 (m, 2H), 7.34 – 7.02 (m, 1H).

5-(chlorosulfonyl)-4-fluoro-2-(2,2,2-trifluoroacetamido)benzoic acid (11c) : In the reaction vessel were placed magnetic stirred bar and $ClSO_3H$ (5.2 mL) was cooled at 0 °C, Then 4-fluoro-2-(2,2,2-trifluoroacetamido)benzoic acid (2.0 g, 7.97 mmol) was added portionwise. The reaction mixture was stirred at 150 °C for 48 h. The reaction mixture was cooled to room temperature, and put dropwise into ice water. The resulting precipitate was filtered off, and washed with DCM, and removed remaining water by using toluene to obtain the product. (1.6 g, 57%) ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.76 (s, 1H), 8.40 (dd, J = 8.4, 2.6 Hz, 1H), 7.16 (td, J = 8.4, 2.6 Hz, 1H).

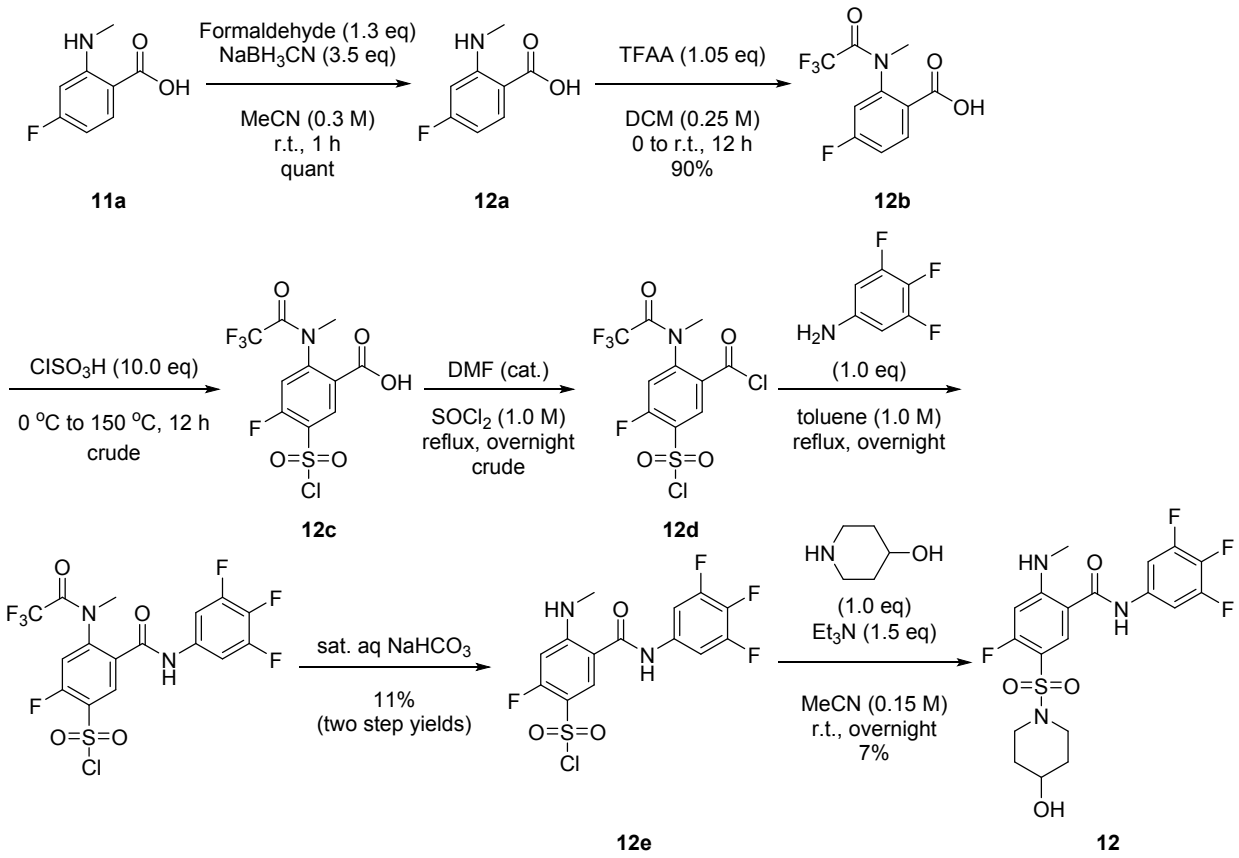
4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)benzoic acid (11d) : To a solution of 5-(chlorosulfonyl)-4-fluoro-2-(2,2,2-trifluoroacetamido)benzoic acid (1.6 g, 4.57 mmol) in MeCN (46 mL, 0.1 M) was added Piperidin-4-ol (463 mg, 4.57 mmol) and Et_3N (1.0 mL, 6.86 mmol). The reaction mixture was stirred at room temperature overnight. The resulting solution was diluted with water, extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the product. (1.33 g, crude) ^{1}H NMR (300 MHz, Acetone-d_6) δ 12.94 (s, 1H), 8.60 (d, J = 7.8 Hz, 1H), 8.54 (d, J = 12.4 Hz, 1H), 3.81 (sept, J = 4.0 Hz, 1H), 3.53 – 3.45 (m, 2H), 3.14 – 3.03 (m, 2H), 1.98 – 1.86 (m, 2H), 1.66 – 1.56 (m, 2H).

4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)-*N*-(3,4,5-trifluorophenyl)benzamide (11e) : To a solution of 4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)benzoic acid (748 mg, 1.80 mmol) in DMF (9 mL, 0.2 M) was added HATU (1.37 g, 3.61 mmol), sequentially added DIPEA (0.5 mL, 3.61 mmol). The reaction mixture was stirred at room temperature for 30 minutes. The resulting solution was added 3,4,5-trifluoroaniline (398 mg, 2.70 mmol) and stirred at room temperature overnight. The reaction mixture was diluted with water, extracted with EtOAc, dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (160 mg, 16%) ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.62 (d, J = 7.3 Hz, 1H), 7.87 (d, J = 10.4 Hz, 1H), 7.65 – 7.61 (m, 2H), 3.95 (s, 1H), 3.85 – 3.80 (m, 1H), 3.49 –3.44 (m, 2H), 3.18 –3.13 (m, 2H), 1.90 –1.86 (m, 2H), 1.63 – 1.56 (m, 2H).

2-amino-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (11) : To a solution of 4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(2,2,2-trifluoroacetamido)-*N*-(3,4,5-trifluorophenyl)benzamide (160 mg, 0.294 mmol) in MeOH (5 mL) was added a solution of 1 N NaOH solution (5 mL). The reaction mixture was stirred at room temperature for 2 hours. The resulting mixture was diluted with water, extracted with EtOAc, and washed with brine. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (80.6 mg, 61%) ^{1}H NMR (400

MHz, DMSO-d_6) δ 10.57 (s, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.62 (dd, J = 10.6, 6.5 Hz, 2H), 7.33 (s, 2H), 6.66 (d, J = 13.2 Hz, 1H), 4.71 (d, J = 4.0 Hz, 1H), 3.60 –3.56 (m, 1H), 3.29 – 3.22 (m, 2H), 2.82 (t, J = 9.8 Hz, 2H), 1.83 – 1.66 (m, 2H), 1.44 – 1.38 (m, 2H). ^{13}C NMR (126 MHz, MeOD-d_4) δ 166.96, 162.61, 160.58, 156.13 (d, J = 13.3 Hz), 134.94, 132.90 (d, J = 3.7 Hz), 110.84, 110.71, 110.54 (d, J = 1.4 Hz), 104.80 (d, J = 24.7 Hz), 102.22 (d, J = 25.3 Hz), 65.56, 42.96 (d, J = 1.9 Hz), 33.12. HRMS (EI) m/z calcd for $C_{18}H_{17}F_4N_3O_4S$ $[M]^+$ 447.0876, found 447.0866.

4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylamino)-*N*-(3,4,5-trifluorophenyl)benzamide (12)



4-fluoro-2-(methylamino)benzoic acid (12a) : To a solution of 4-fluoroanthranilic acid (10.0 g, 64.4 mmol) in MeCN (0.3 M, 214 mL) was added sodium cyanoborohydride (14.2 g, 226 mmol), formaldehyde 37% aqueous solution (6.26 mL, 100 mmol) under nitrogen atmosphere at room temperature and stirred at room temperature for 1 hour. The reaction mixture was adjusted to pH 3 by treating 1 N HCl solution. The resulting mixture was diluted with water, extracted with DCM. The combined organic layer was washed with a saturated solution of $NaHCO_3$ and brine, dried over anhydrous MgSO4 and evaporated under reduced pressure to obtain the product without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99

(dd, J = 9.3, 6.9 Hz, 3H), 7.74 (s, 2H), 6.37 (s, 2H), 6.33 (dd, J = 4.8, 2.4 Hz, 3H), 2.93 (s, 8H). LC/MS $[M+H]^+$ 170.1

4-fluoro-2-(2,2,2-trifluoro-*N*-methylacetamido)benzoic acid (12b) : To a solution of 4-fluoro-2-(methylamino)benzoic acid (1.00 g, 5.91 mmol) in dry DCM (130 mL) was added trifluoroacetic anhydride (0.86 mL, 6.21 mmol) dropwise at 0 °C. The resulting solution was stirred at room temperature for 12 hours. The reaction mixture was added to a saturated $NaHCO_3$ solution. Then the resulting precipitate was filtered off and washed with DCM to obtain the product. (1.41 g, 90%)

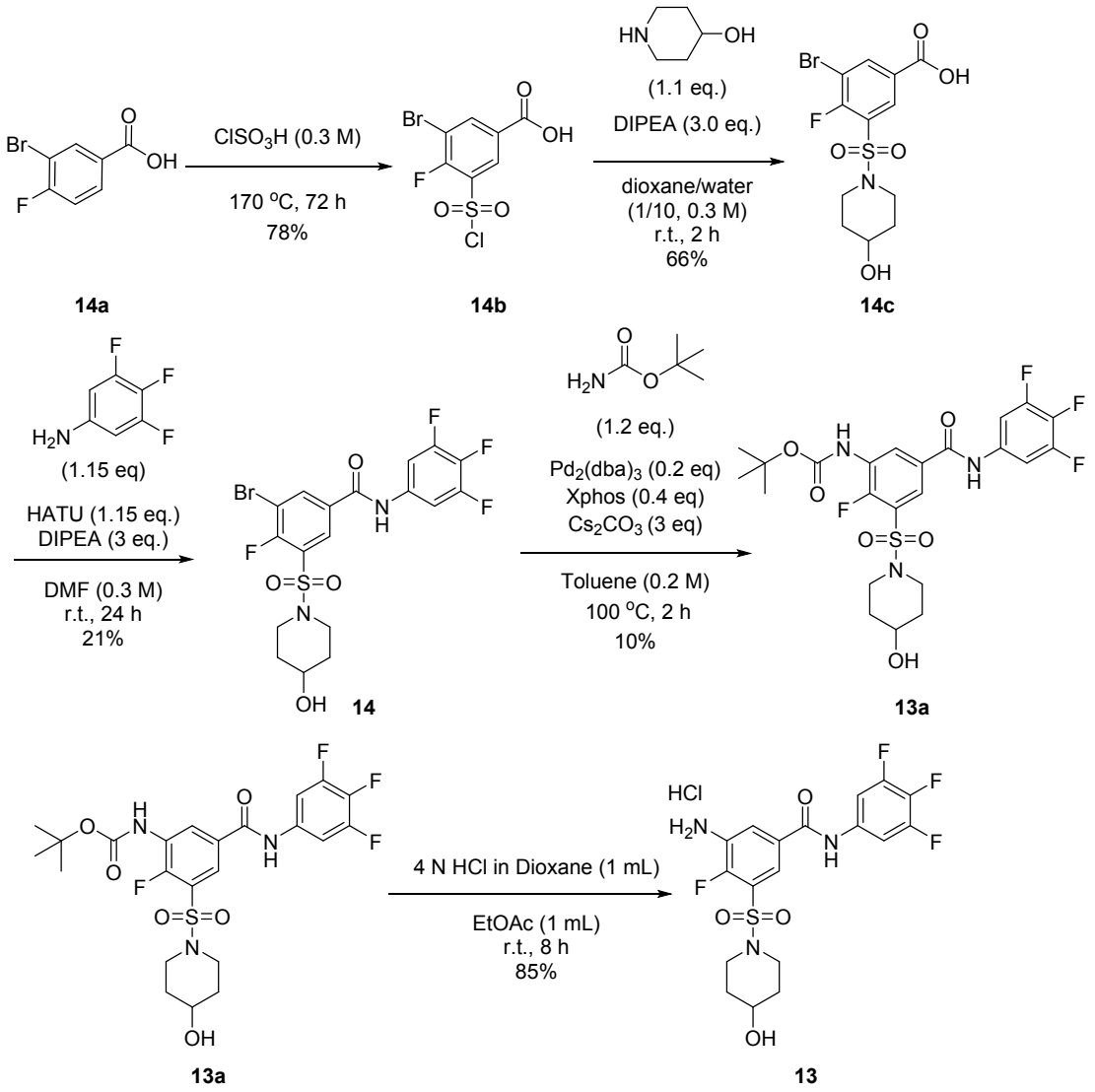
5-(chlorosulfonyl)-4-fluoro-2-(2,2,2-trifluoro-*N*-methylacetamido)benzoic acid (12c) : In the reaction vessel are placed magnetic stirred bar and $ClSO_3H$ (3.5 mL) and was cooled at 0 °C, Then 4-fluoro-2-(2,2,2-trifluoro-*N*-methylacetamido)benzoic acid (1.41 g, 5.32 mmol) was added portionwise. The reaction mixture was stirred at 150 °C for 12 hours. The reaction mixture was cooled to room temperature, and put dropwise into ice water. The resulting precipitate was filtered off, and washed with DCM. The residue was dissolved with toluene, evaporated under reduced pressure to remove remaining water to obtain the crude product (788 mg, crude)

5-(chlorosulfonyl)-4-fluoro-2-(2,2,2-trifluoro-*N*-methylacetamido)benzoyl chloride (12d) : 5-(chlorosulfonyl)-4-fluoro-2-(2,2,2-trifluoro-*N*-methylacetamido)benzoic acid (561 mg, 1.55 mmol) was dissolved in $SOCl_2$ (1.55 mL, 1.0 M). The solution was added DMF 1 drop, after then stirred and heated to 80 °C overnight. The reaction mixture was concentrated *in vacuo*. The residue was dissolved with toluene, evaporated under reduced pressure to remove remaining $SOCl_2$ to obtain the crude product for the next step directly.

2-fluoro-4-(2,2,2-trifluoro-*N*-methylacetamido)-5-((3,4,5-trifluorophenyl)carbamoyl) benzenesulfonyl chloride (12e) : To a solution of crude 5-(chlorosulfonyl)-4-fluoro-2-(2,2,2-trifluoro-*N*-methylacetamido)benzoyl chloride (1.55 mmol) in toluene (1.55 mL) was added 3,4,5-trifluoroaniline (228 mg, 1.55 mmol). The resulting solution was heated at 80 °C overnight. The reaction mixture was cooled to room temperature, evaporated under reduced pressure. The residue was diluted with saturated $NaHCO_3$ solution, stirred for 1 hour. The resulting mixture was extracted with EtOAc, washed with brine. The combined organic layer was dried over anhydrous Na_2SO_4, concentrated *in vacuo*. The crude mixture was purified by SiO2 column chromatography to obtain the product. (67.6 mg, 11% two step yields)

4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylamino)-*N*-(3,4,5-trifluorophenyl)benzamide (12) : To a solution of 2-fluoro-4-(2,2,2-trifluoro-*N*-methylacetamido)-5-((3,4,5-trifluorophenyl)carbamoyl)benzenesulfonyl chloride (65 mg, 0.16 mmol) in MeCN (1.0 mL, 0.15 M) was added Piperidin-4-ol (25 mg, 0.25 mmol), Et_3N (0.03 mL, 0.25 mmol). The reaction mixture was stirred at room temperature overnight. The resulting mixture was diluted with water, extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude mixture was purified by SiO_2 column chromatography to obtain the desired product. (5 mg, 7%) ^1H NMR (300 MHz, DMSO-d_6) δ 7.80 (d, J = 9.0 Hz, 1H), 7.54 – 7.41 (m, 2H), 6.92 (d, J = 9.1 Hz, 1H), 6.66 (s, 1H), 4.78 (s, 1H), 3.83 (s, 4H), 3.51 (s, 3H), 3.23 (s, 1H), 1.82 (s, 2H), 1.44 (d, J = 8.9 Hz, 2H).

3-bromo-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (14) and 3-amino-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (13)



3-bromo-5-(chlorosulfonyl)-4-fluorobenzoic acid (14b) : 3-Bromo-4-fluoro-benzoic acid (4 g, 18.27 mmol) was dissolved in chlorosulfonic acid (61 mL, 0.3 M) and heated at 170 °C for 72 hours. The reaction mixture was cooled to room temperature and added dropwise to an ice-water mixture. Extraction with EtOAc, drying over anhydrous $MgSO_4$, and concentration *in vacuo* to obtain the desired product (4.51 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.22 (dd, J = 6.3, 2.2 Hz, 1H), 8.11 (dd, J = 6.0, 2.3 Hz, 1H).

3-bromo-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)benzoic acid (14c) : A mixture of 3-bromo-5-(chlorosulfonyl)-4-fluorobenzoic acid (3.18 g, 10 mmol), piperidin-4-ol (1.11 g, 11 mmol), and DIPEA (5.2 mL, 30 mmol) in dioxane/water (10:1, 33 mL, 0.3 M) was stirred at

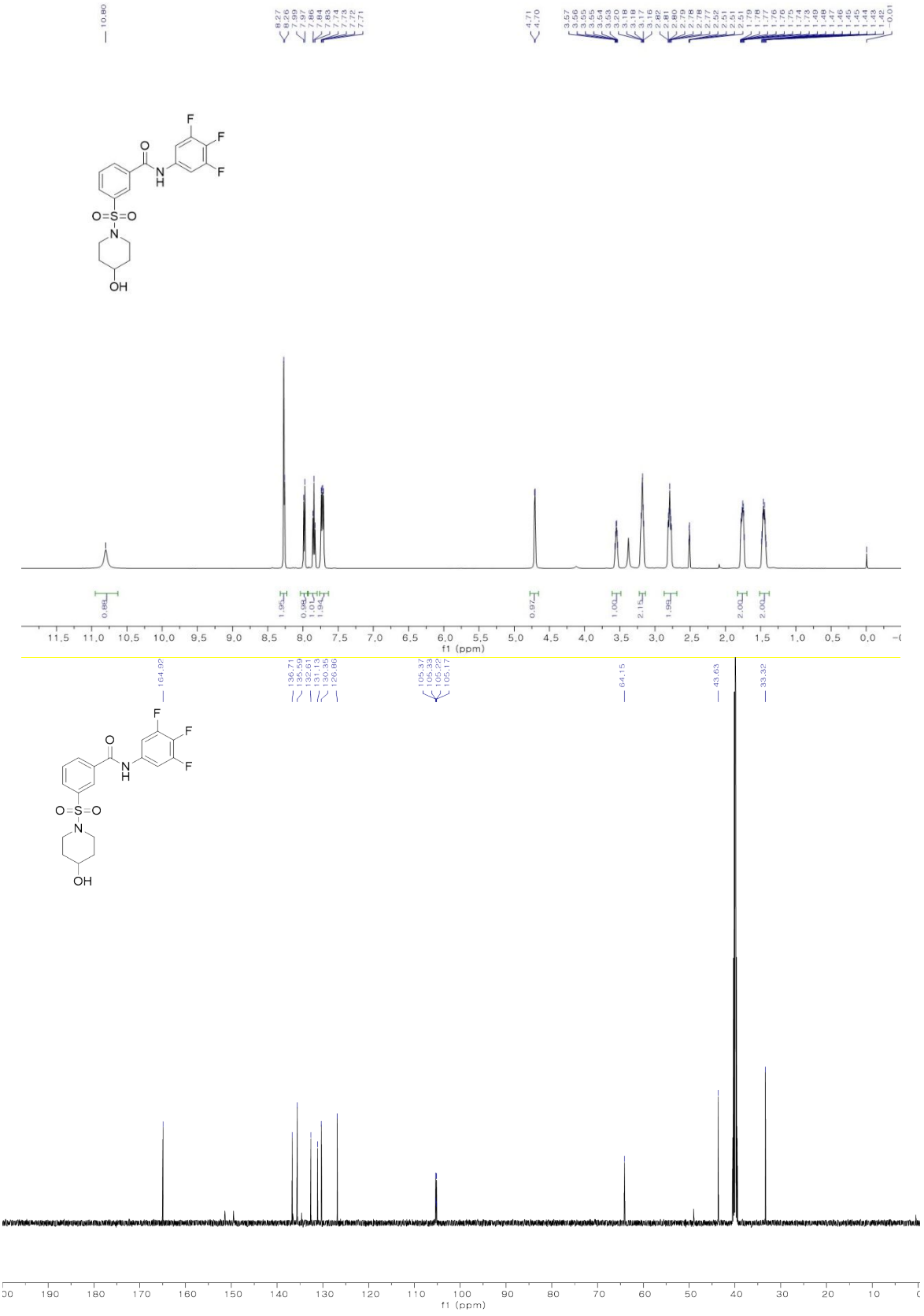
room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure. The residue was diluted with 1 N HCl solution and extracted with EtOAc three times. The combined organic layer was dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to afford the title compound (2.51 g, 66%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (dd, J = 6.1, 2.1 Hz, 1H), 8.20 (dd, J = 6.1, 2.1 Hz, 1H), 3.61 – 3.57 (m, 1H), 3.38 (dd, J = 12.3, 5.6 Hz, 2H), 2.97 (ddd, J = 12.2, 8.7, 3.3 Hz, 2H), 1.84 – 1.74 (m, 2H), 1.48 – 1.37 (m, 2H).

3-bromo-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (14) : To a solution of 3-bromo-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)benzoic acid (1.15 g, 3 mmol), 3,4,5-trifluoroaniline (508 mg, 3.45 mmol), HATU (1.13 g, 3.45 mmol), and DIPEA (1.57 mL, 9 mmol) in DMF (10 mL, 0.3 M) was stirred at room temperature for 24 hours. The reaction mixture was added with saturated $NaHCO_3$ solution, and extracted three times with EtOAc. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The crude product was purified by SiO_2 column chromatography to afford the desired product. (327 mg, 21%) ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.52 (d, J = 5.8 Hz, 1H), 8.39 (d, J = 5.7 Hz, 1H), 7.61 (dd, J = 10.0, 6.4 Hz, 2H), 3.77 (dq, J = 8.0, 4.1, 3.7 Hz, 1H), 3.57 (dt, J = 11.3, 4.8 Hz, 2H), 3.10 (t, J = 10.0 Hz, 2H), 1.93 (dd, J = 14.2, 5.7 Hz, 2H), 1.60 (qd, J = 9.1, 8.6, 4.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.80, 155.92, 138.05 (d, J = 1.3 Hz), 132.19 (d, J = 4.3 Hz), 130.28, 127.13, 126.99, 111.43, 111.25, 105.47 (d, J = 6.8 Hz), 105.32 (d, J = 5.5 Hz), 64.39, 43.42, 33.70. ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.80, 155.92, 138.05 (d, J = 1.3 Hz), 132.19 (d, J = 4.3 Hz), 130.28, 127.13, 126.99, 111.43, 111.25, 105.47 (d, J = 6.8 Hz), 105.32 (d, J = 5.5 Hz), 64.39, 43.42, 33.70. HRMS (EI) m/z calcd for $C_{18}H_{15}BrF_4N_2O_4S$ $[M]^+$ 509.9872, found 509.9883.

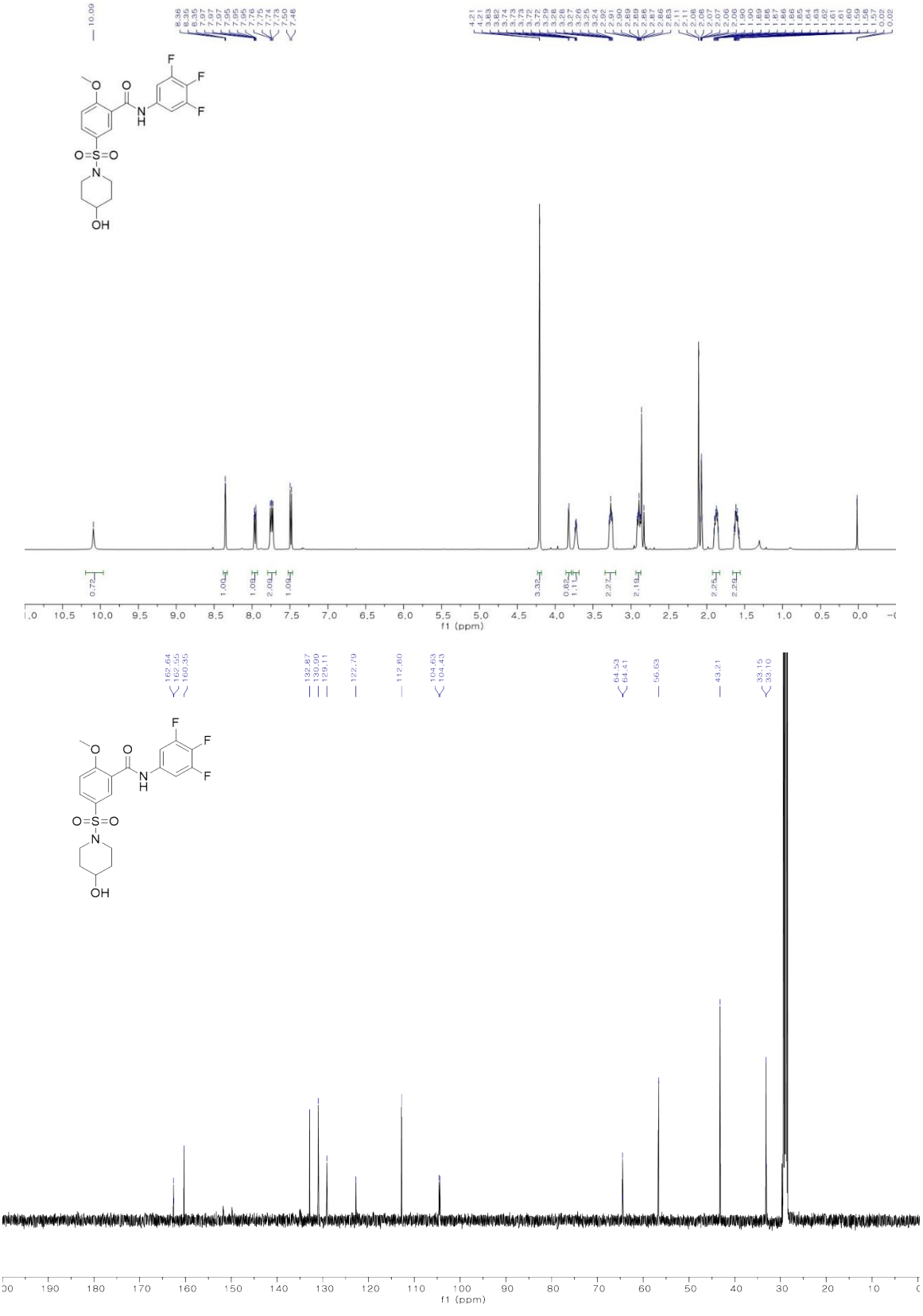
***tert*-butyl(2-fluoro-3-((4-hydroxypiperidin-1-yl)sulfonyl)-5-((3,4,5-trifluorophenyl)carbamoyl)phenyl)carbamate (13 a) :** 3-bromo-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (100 mg, 0.2 mmol), Cs_2CO_3 (196 mg, 0.6 mmol), Xphos (38 mg, 0.08 mmol), $Pd_2(dba)_3$ (37 mg, 0.04 mmol) were put in a round bottle, toluene (1 mL, 0.2 M) and *tert*-butyl carbamate (21 mg, 0.24 mmol) were added and the mixture was degassed for 5 minutes. The solution was heated at 100 ^{o}C for 2 hours. The reaction mixture was cooled to room temperature, filtered through celite, and concentrated. The reaction mixture was purified by Column Chromatography over silica gel to afford the desired product. (12 mg, 10%) ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.74 (d, J = 6.5 Hz, 1H), 8.05 (d, J = 5.0 Hz, 1H), 7.61 (dd, J = 10.0, 6.4 Hz, 2H), 3.75 (s, 1H), 3.64 – 3.49 (m, 2H), 3.05 (t, J = 10.0 Hz, 2H), 2.00 – 1.83 (m, 2H), 1.58 (d, J = 1.5 Hz, 11H).

3-amino-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide hydrogen chloride (13) : To a solution of *tert*-butyl (2-fluoro-3-((4-hydroxypiperidin-1-yl)sulfonyl)-5-((3,4,5-trifluorophenyl)carbamoyl)phenyl)carbamate (12 mg, 0.022 mmol) in EtOAc (1 mL) was added 4 N HCl solution in dioxane (1 mL) and the reaction mixture was stirred at room temperature for 8 hours. The reaction mixture was concentrated under reduced pressure, washed with cold ether, and dried to afford the title hydrochloride salt (9 mg, 85%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.67 – 7.50 (m, 4H), 3.77 – 3.73 (m, 1H), 3.59 – 3.52 (m, 2H), 3.03 (t, J = 10.7 Hz, 2H), 1.96 – 1.87 (m, 2H), 1.60 (t, J = 9.8 Hz, 2H). HRMS (EI) m/z calcd for $C_{18}H_{17}F_4N_3O_4S$ $[M]^+$ 447.0866, found 447.0876.

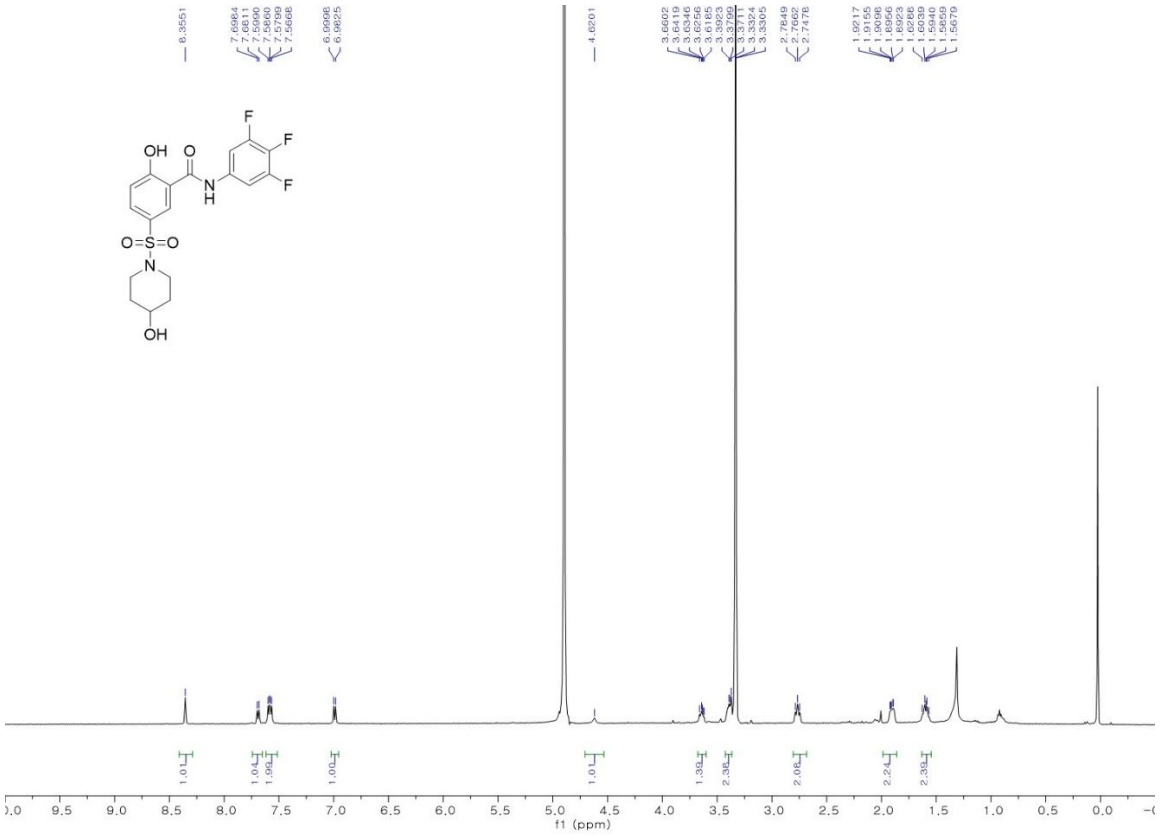
3-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide(4)

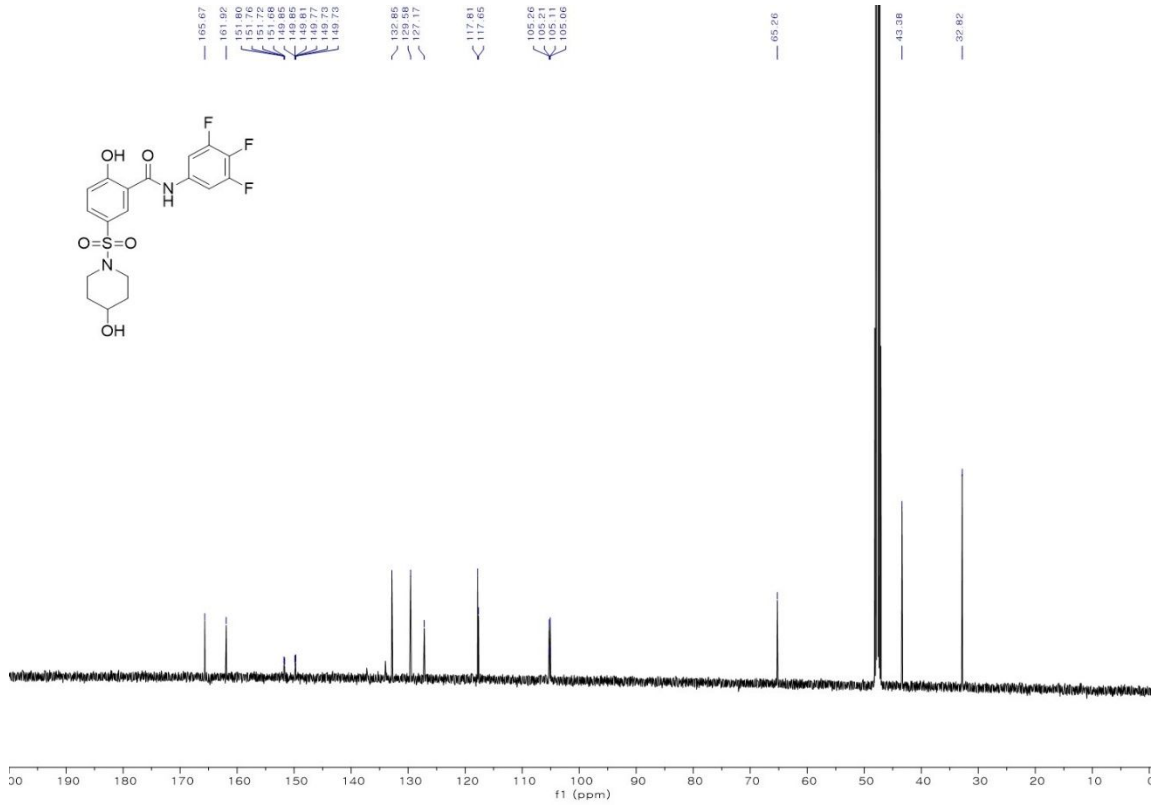


5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-methoxy-*N*-(3,4,5-trifluorophenyl)benzamide (5)

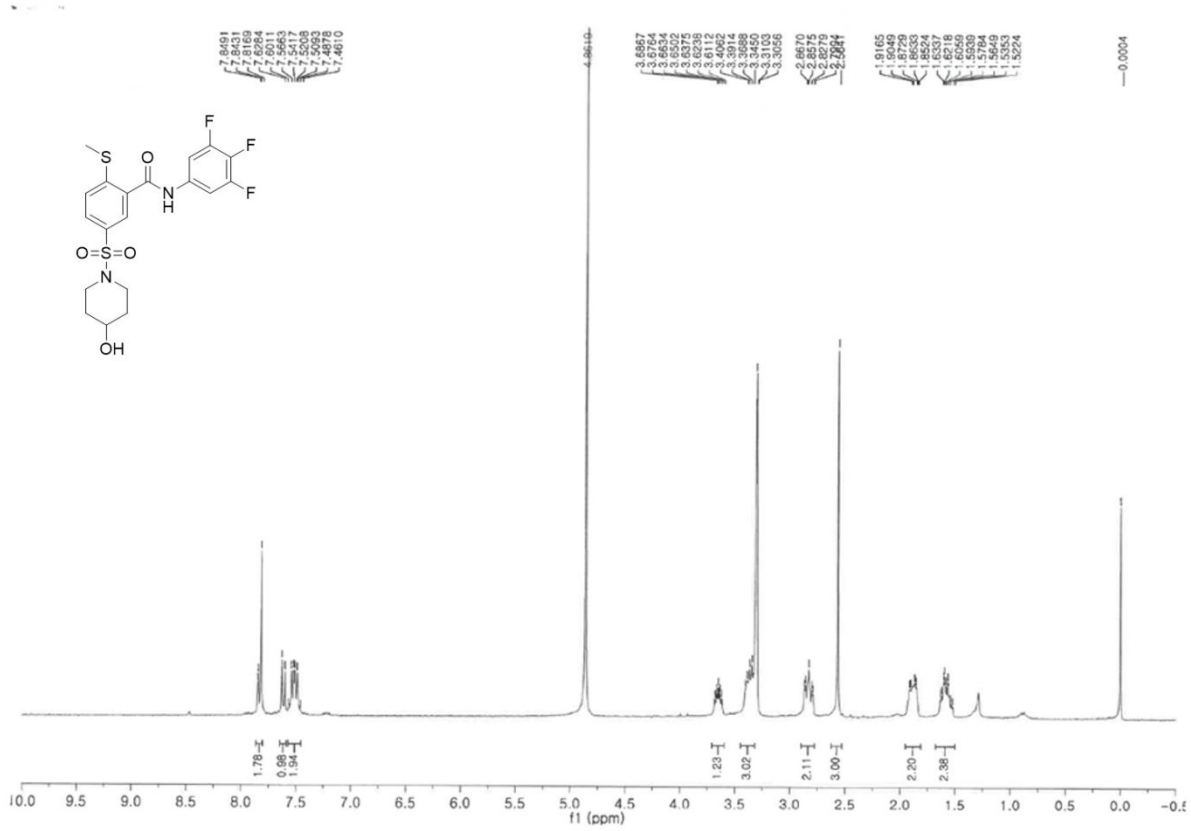


2-hydroxy-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (6)

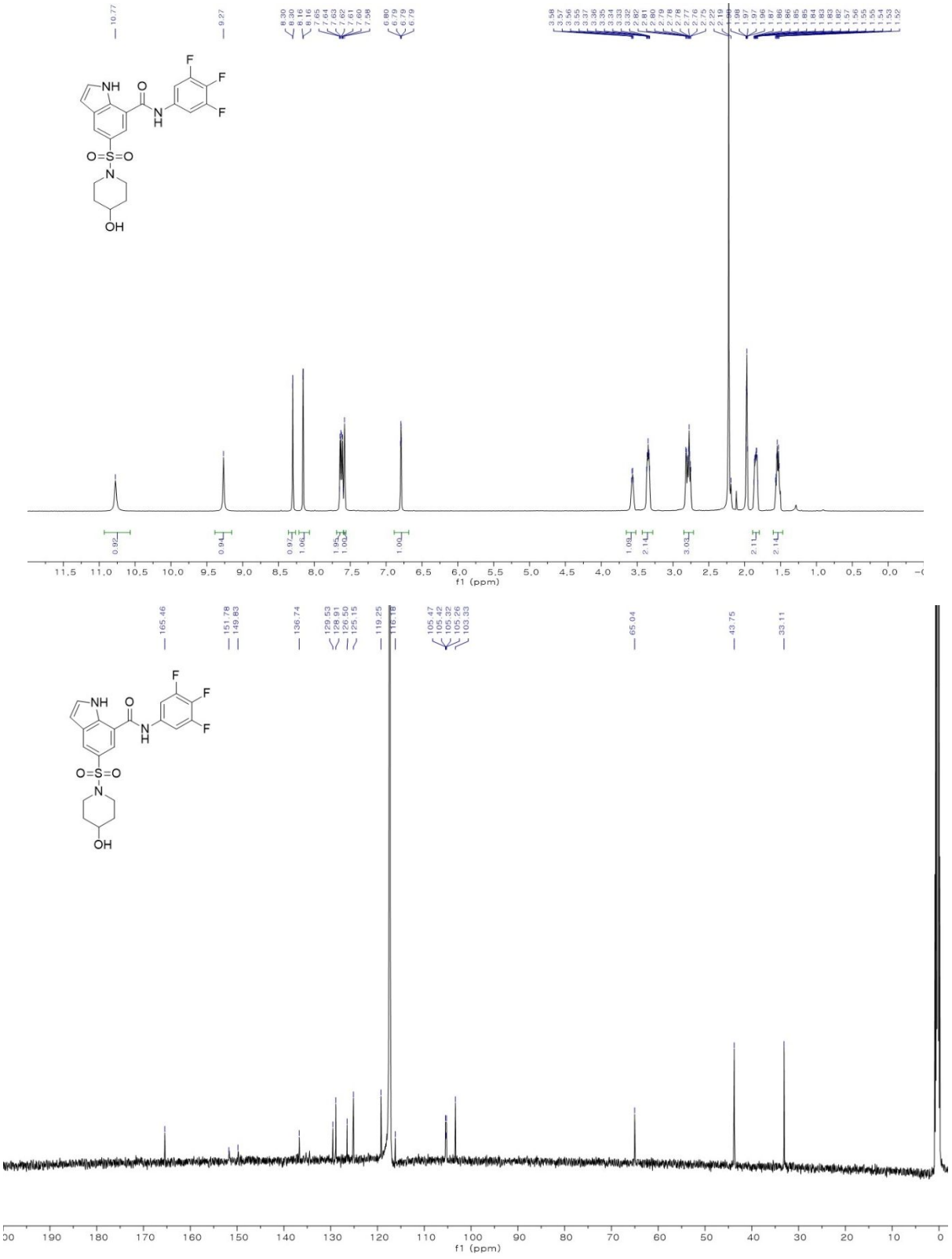




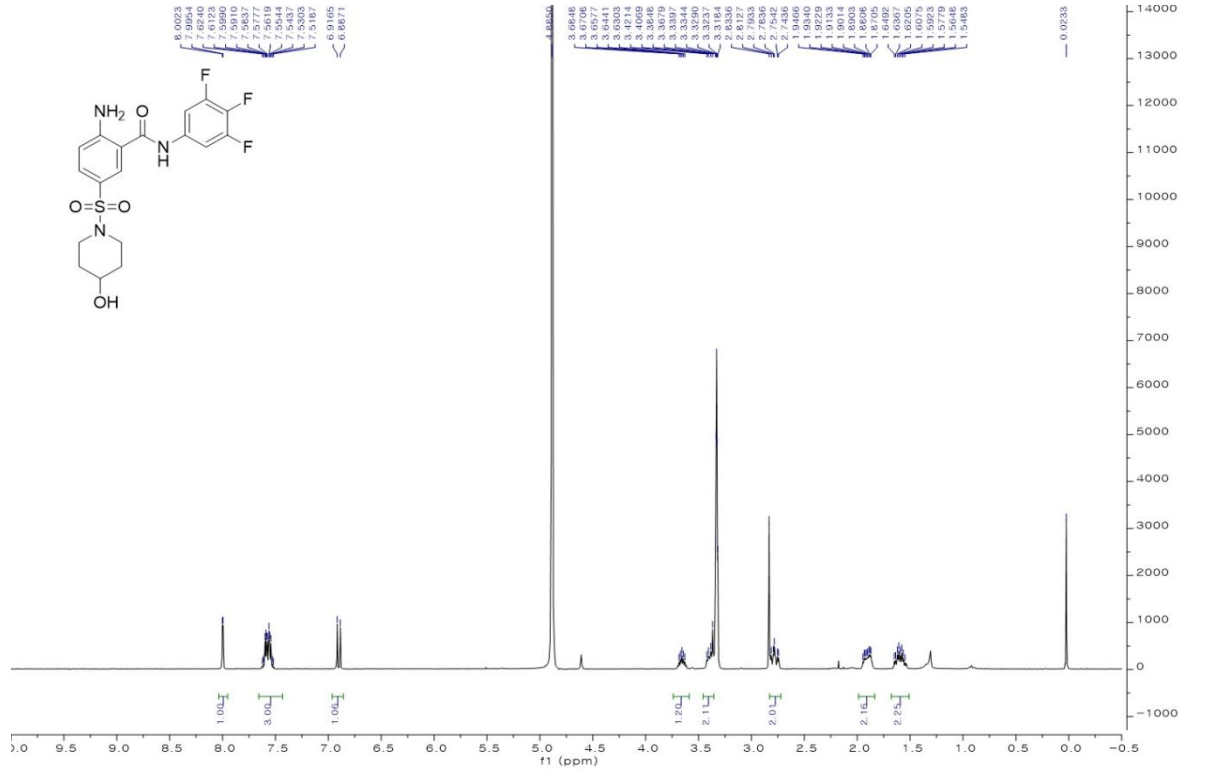
5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)-*N*-(3,4,5-trifluorophenyl) benzamide (7)

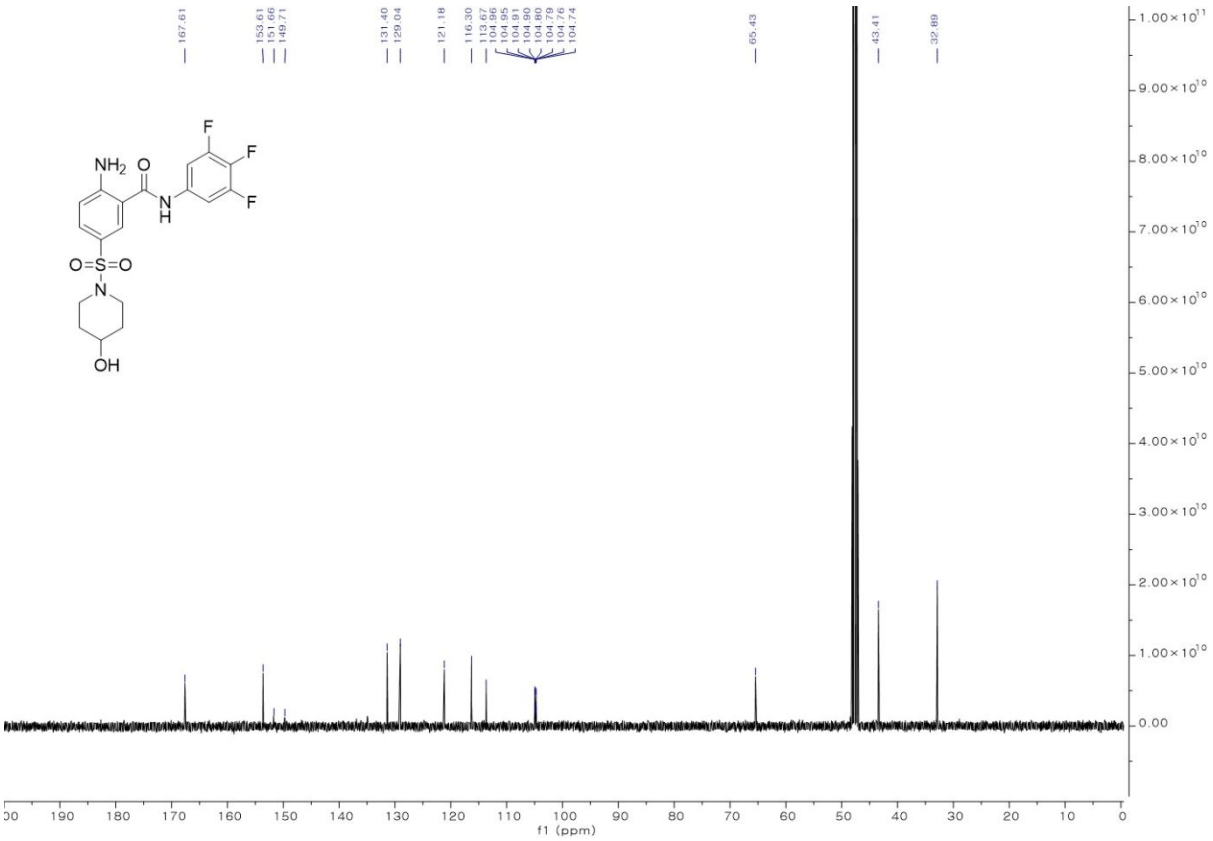


5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)-1H-indole-7-carboxamide (8)

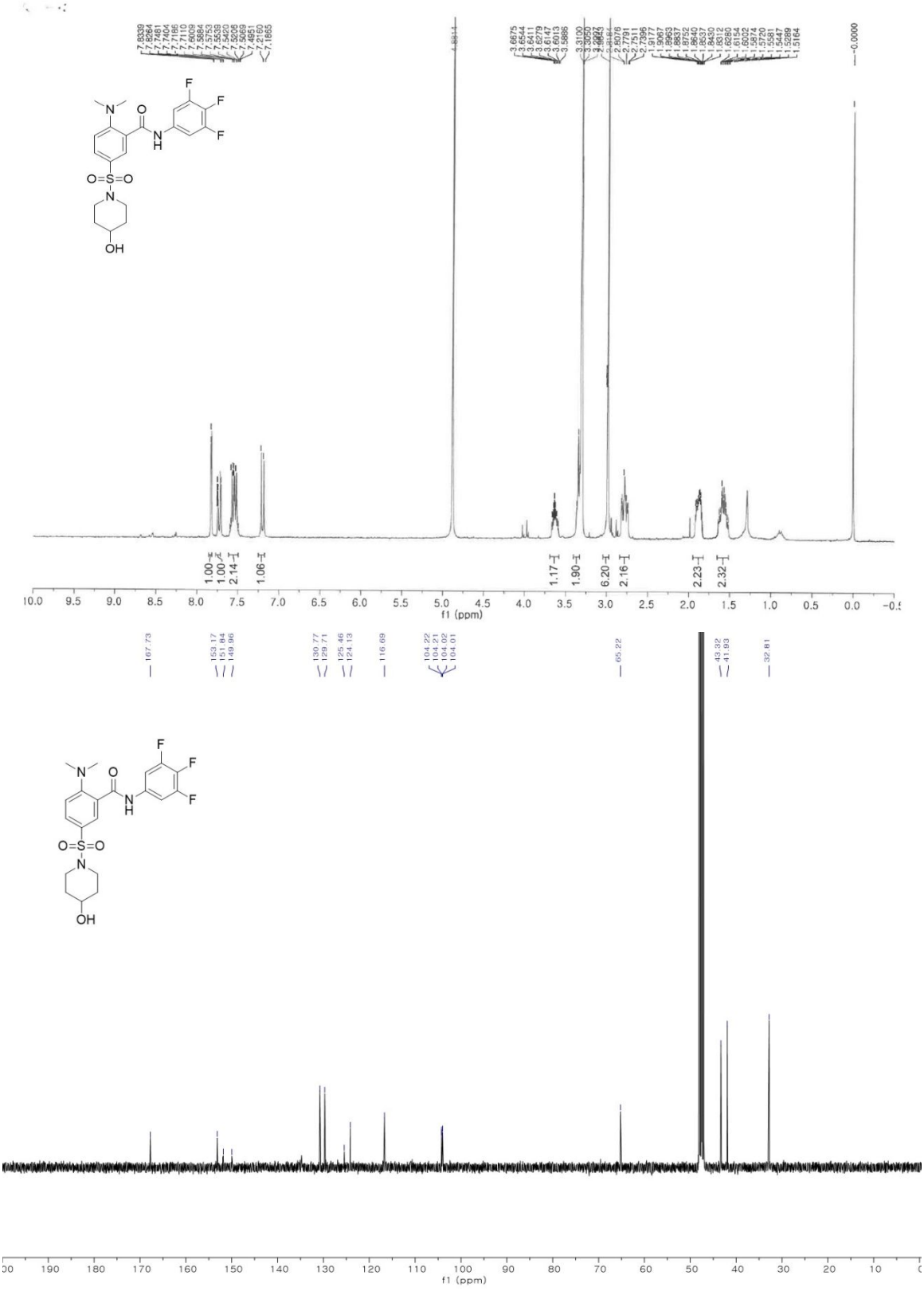


2-amino-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl)benzamide (9)

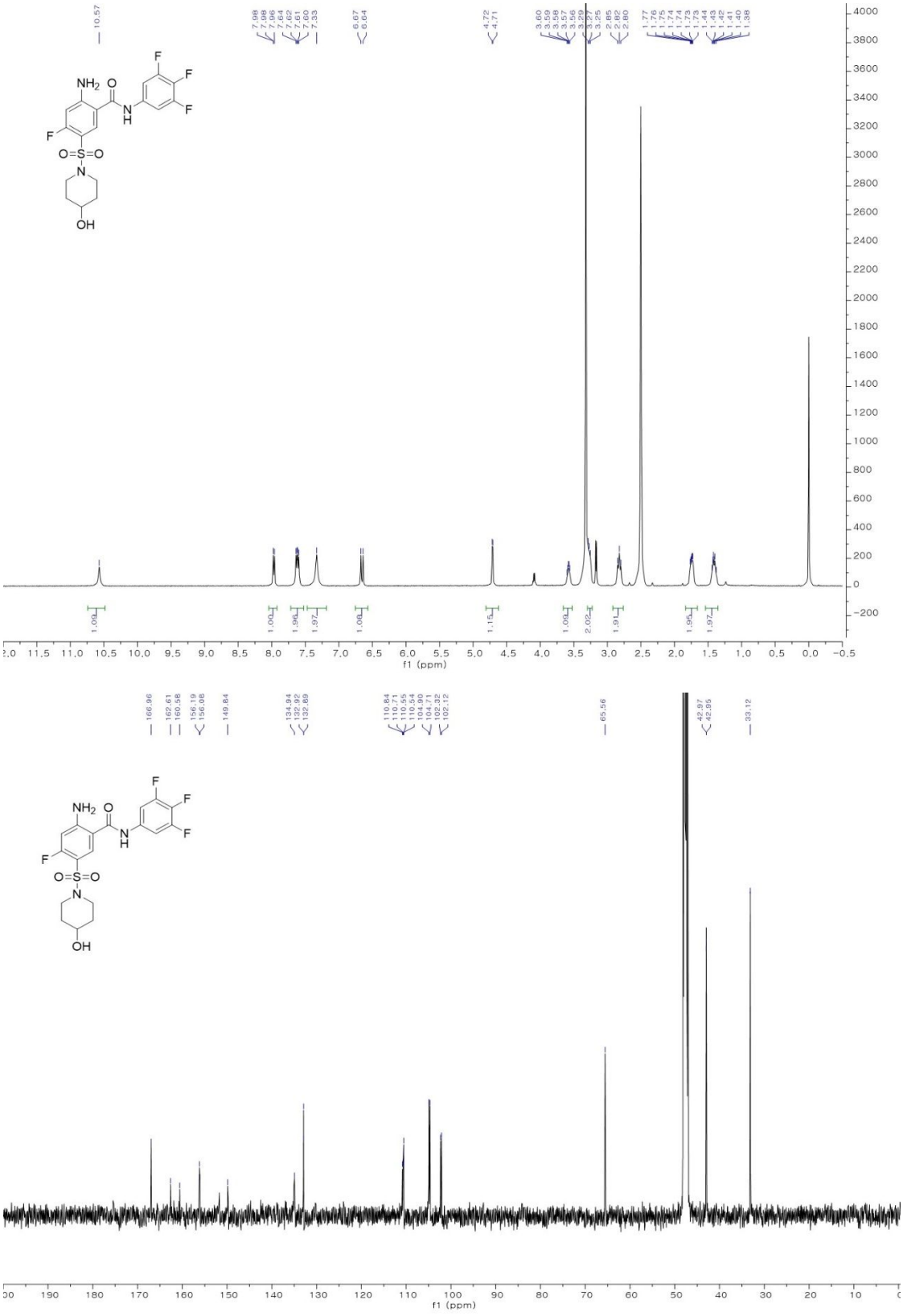




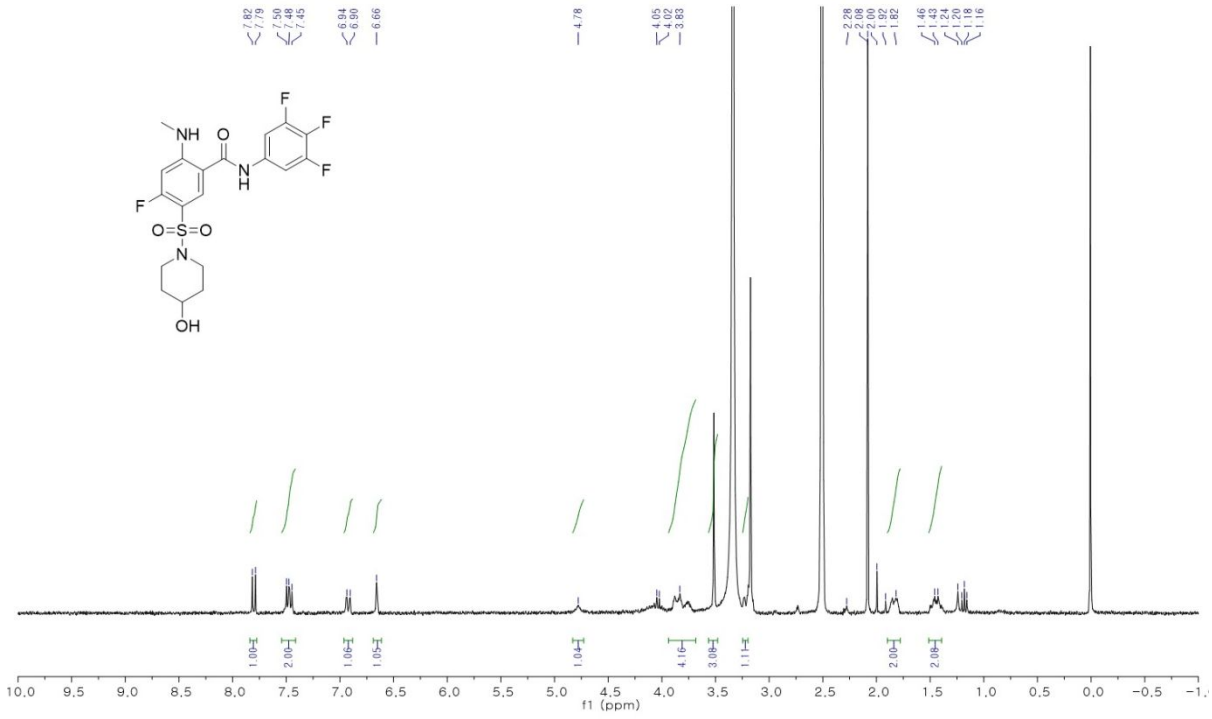
2-(dimethylamino)-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (10)



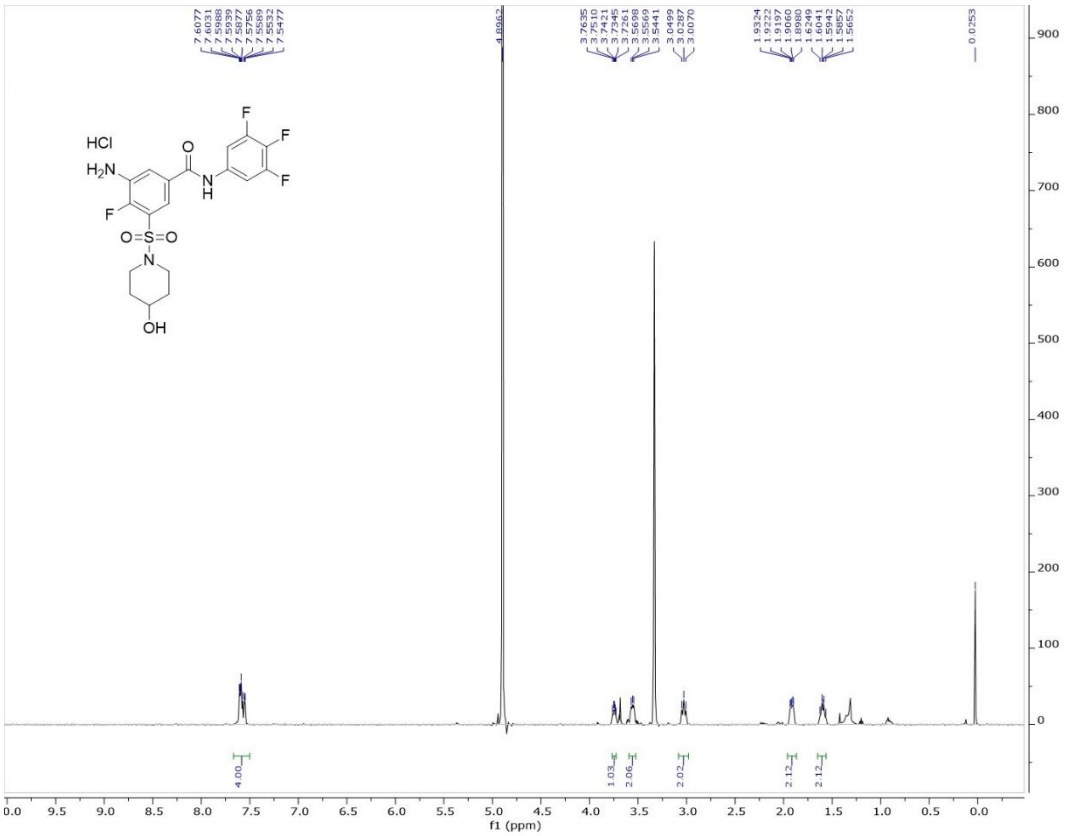
2-amino-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (11)



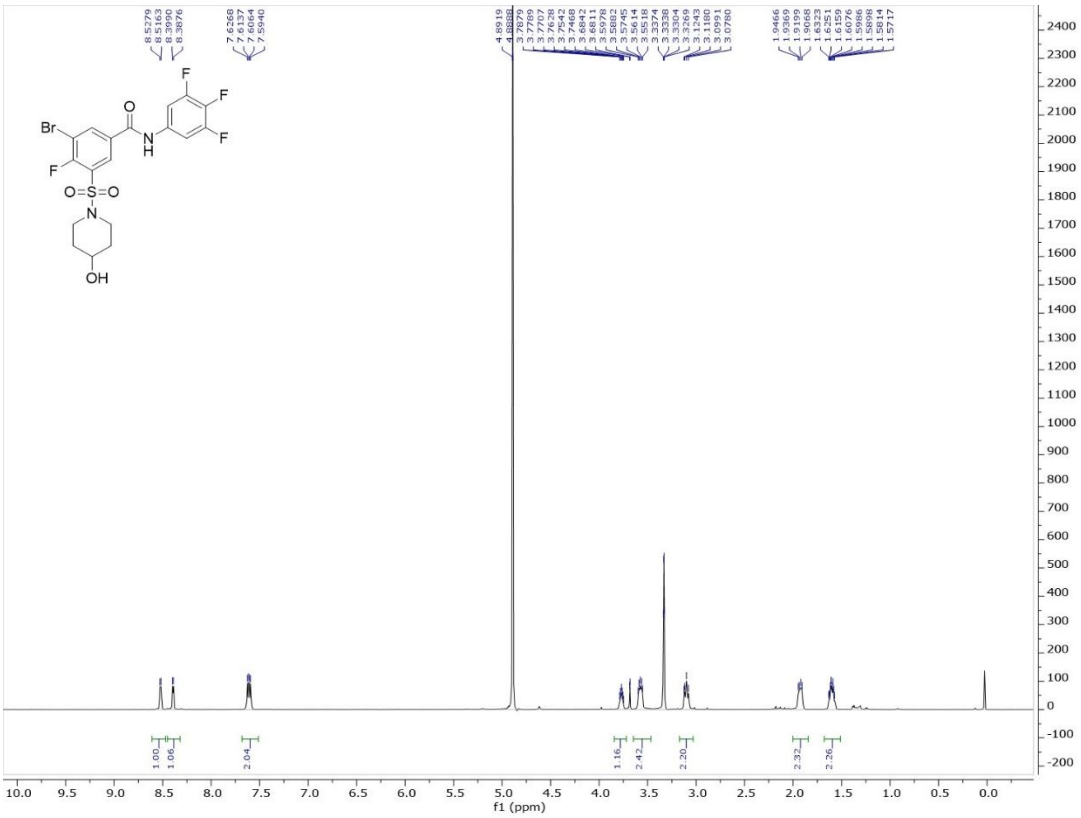
5-((4-hydroxypiperidin-1-yl)sulfonyl)-2-(methylthio)-*N*-(3,4,5-trifluorophenyl)benzamide (12)

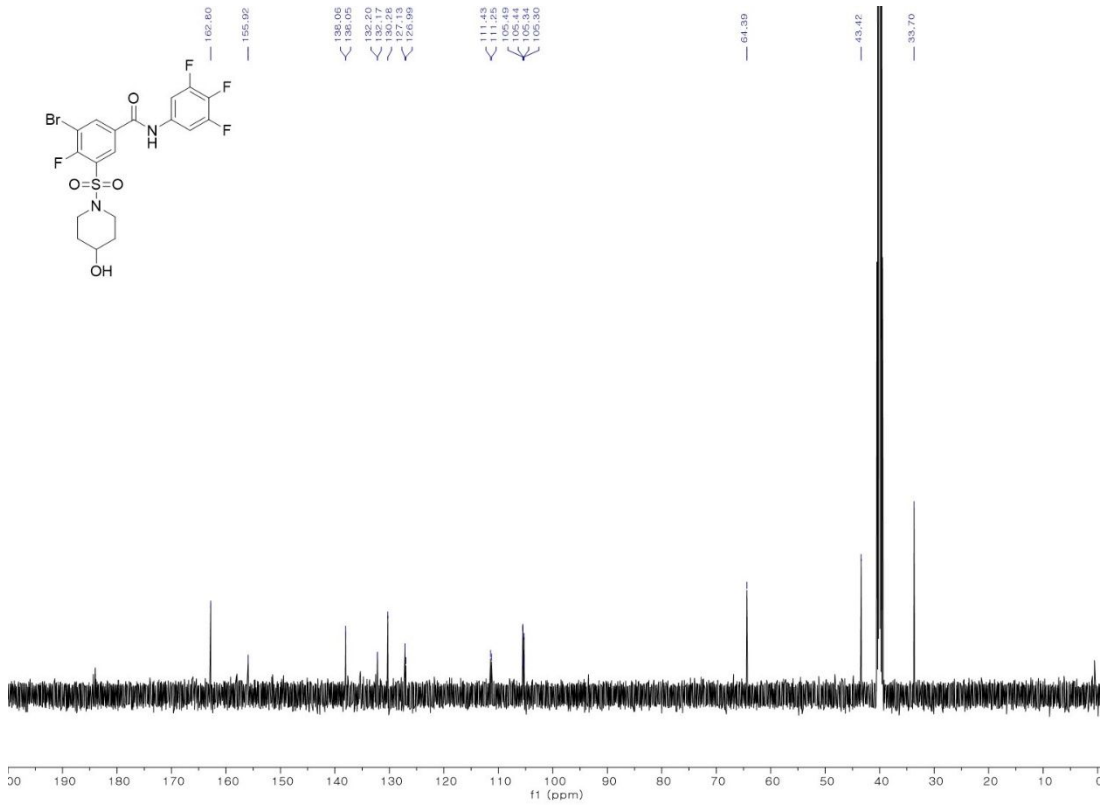


3-amino-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (13)



3-bromo-4-fluoro-5-((4-hydroxypiperidin-1-yl)sulfonyl)-*N*-(3,4,5-trifluorophenyl) benzamide (14)





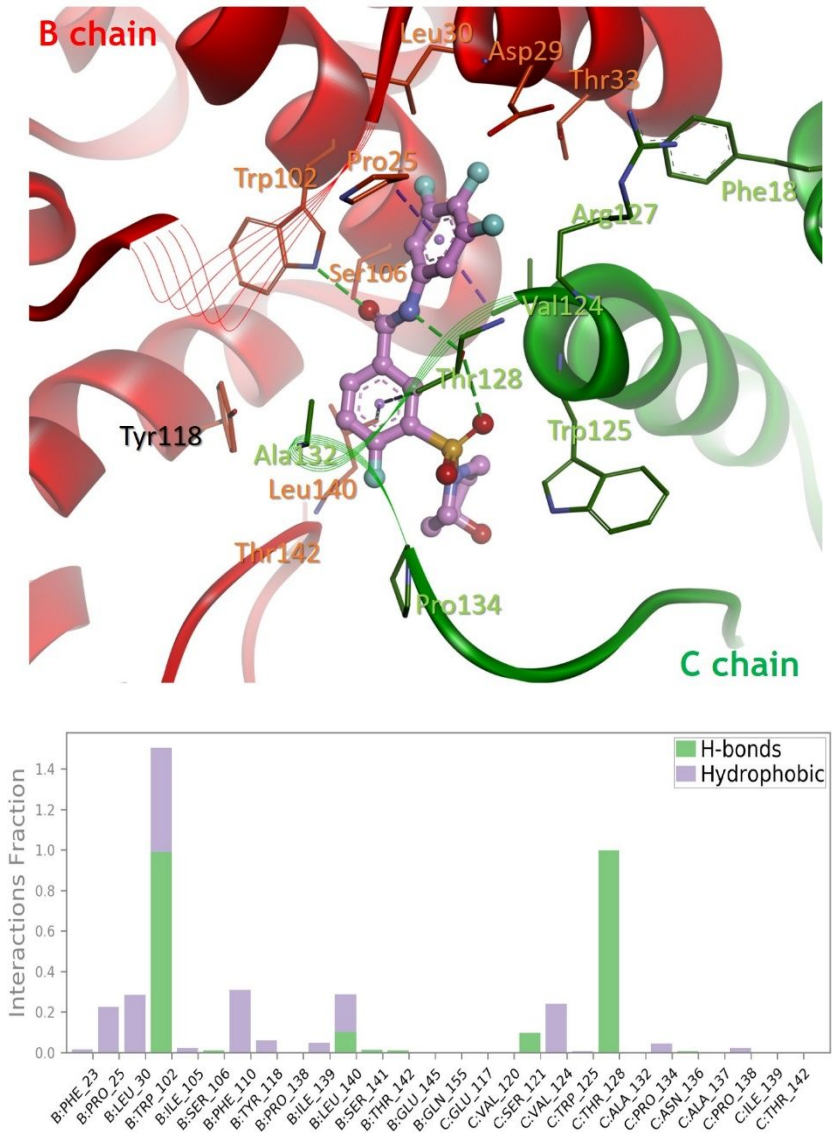
3. Molecular Dynamics Simulations

Materials and Method

Molecular docking was performed using the Schrodinger Suite 2019-3 (Schrödinger, LLC, New York, NY, USA, 2019) to predict the binding model of compound **11** with HBV core protein. The NVR-3-778 bound X-ray crystal structure of the HBV core protein (PDB cod 5T2P) was obtained from the Protein Data Bank. The protein preparation was revised using Protein Preparation Wizard in Maestro v12.1. The receptor grid box for docking was generated 25 Å × 25 Å × 25 Å size centered on complexed ligand in the binding site. The ligand was minimized using a OPLS3e force field with a dielectric constant of 80.0 in MacroModel v12.5. The ligand docking was performed using the Glide v8.4 program with Standard Precision method.

The protein-ligand docked complex models for HBV core protein bound to **11** was retrieved from the docking results, and Desmond v5.9 (Desmond Molecular Dynamics System; D. E. Shaw Research: New York, NY) and OPLS3e force field were used in the MD simulations. The System Builder was used for solvation by means of predefined TIP3P[1] water molecules in an orthorhombic box with dimensions of 10 Å x 10 Å x 10 Å, and the overall complex was neutralized by adding Cl^- counterions. The NaCl salt concentration was 0.15 mol/L. Production MD simulations 150 ns in length were carried out under periodic boundary conditions in the NPT ensemble at normal temperatures (300 K) and pressure (1.01325 bar). Recording intervals of 1.2 and 150 ps were used for energy calculation and trajectory analysis. The fractions of only hydrogen bonding and hydrophobic interactions between the protein and ligand over the course of the MD trajectory were represented by a stacked bar chart. The proposed binding model of **11** for the final frame of the MD simulations was represented using Discovery Studio 2018 (Dassault Systèmes BIOVIA, San Diego, CA, USA, 2018).

[1] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, and M.L. Klein, Comparison of simple potential functions for simulating liquid water, J. Chem. Phys. 79 (1983), pp. 926–935.



<Figure SI-6> Molecular dynamics simulation and interaction profile analysis of NVR-3-778 for HBV core protein. (A) The proposed binding model of NVR-3-778 (pink ball and stick model) for the final frame of the MD simulations. The B and C chains are displayed in the ribbon model, colored by red and green respectively. The hydrogen bonds are shown as green dashed lines and hydrophobic interactions are represented by pink dashed lines. (B) Contact histograms for the HBV core protein with NVR-3778 are represented by a colored stacked bar plot between 50 and 150 ns of the MD simulation. Only H-bonding (yellow-green colored bar) and representative hydrophobic interactions (lavender colored bar) are presented for clarity.