

Development of Pyridine-Based Inhibitors for the Human Vaccinia-Related Kinases 1 and 2

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Supplementary Information

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Supplementary Table S1: Compound structures and ΔT_m ($^{\circ}\text{C}$) values for all 44 aminopyridine derivatives.

#	R1	R2	DSF ΔT_m ($^{\circ}\text{C}$)		#	R1	R2	DSF ΔT_m ($^{\circ}\text{C}$)	
			VRK1	VRK2				VRK1	VRK2
5			3.2 ± 0.3	2.2 ± 0.2	32			0.8 ± 0.3	1.0 ± 0.1
6			0.7 ± 0.1	1.0 ± 0.1	33			-0.1 ± 0.7	0.6 ± 0.2
7			2.2 ± 0.2	1.3 ± 0.1	34			1.2 ± 0.4	0.5 ± 0.3
8			2.3 ± 0.0	2.3 ± 0.1	35			-0.3 ± 0.5	0.5 ± 0.2
9			0.7 ± 0.2	0.7 ± 0.1	36			0.0 ± 0.1	0.4 ± 0.1
10			1.1 ± 0.3	2.3 ± 0.3	37			0.9 ± 0.2	0.4 ± 0.1
11			0.2 ± 0.3	1.2 ± 0.2	38			0.8 ± 0.0	0.4 ± 0.2
12			1.0 ± 0.1	2.0 ± 0.2	39			0.6 ± 0.4	0.3 ± 0.1
13			1.9 ± 0.1	2.6 ± 0.1	40			0.5 ± 0.0	0.3 ± 0.1
14			2.0 ± 0.2	2.3 ± 0.2	41			0.0 ± 0.1	0.2 ± 0.1
15			1.1 ± 0.2	1.7 ± 0.1	42			0.5 ± 0.1	0.2 ± 0.0
16			0.5 ± 0.4	1.7 ± 0.1	43			0.5 ± 0.1	0.1 ± 0.1
17			1.1 ± 0.1	1.1 ± 0.2	44			-0.4 ± 0.4	0.1 ± 0.1
18			2.1 ± 0.7	3.2 ± 0.0	45			0.2 ± 0.4	0.1 ± 0.0
19			2.2 ± 0.2	3.0 ± 0.0	46			0.3 ± 0.0	0.0 ± 0.0
20			-0.1 ± 0.4	0.8 ± 0.1	47			0.3 ± 0.2	0.0 ± 0.2
21			0.1 ± 0.0	0.7 ± 0.0	48			0.5 ± 0.4	-0.1 ± 0.4
22			-0.2 ± 0.0	0.3 ± 0.1	23			-0.9 ± 0.5	0.1 ± 0.2
27			1.1 ± 0.1	2.1 ± 0.1	24			-0.1 ± 0.6	0.5 ± 0.1
28			0.9 ± 0.2	1.1 ± 0.1	25			1.6 ± 0.4	-0.4 ± 0.1
29			1.4 ± 0.1	1.1 ± 0.1	26			3.5 ± 0.3	1.1 ± 0.2
30			1.4 ± 0.2	1.0 ± 0.4					
31			0.9 ± 0.2	1.0 ± 0.1					

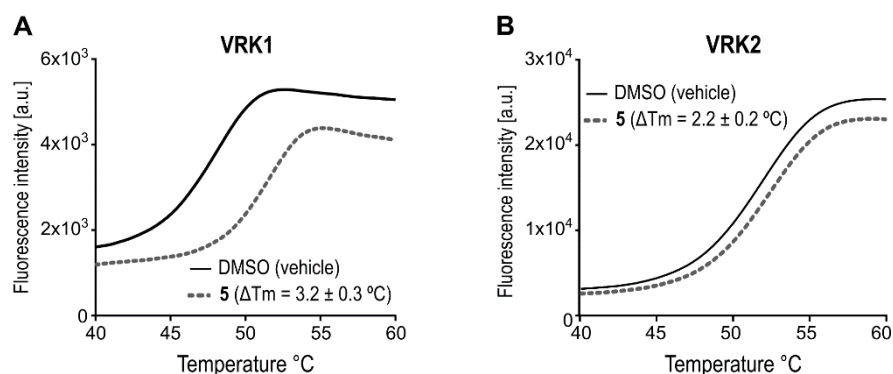
Cloning, expression, purification and crystallization

Cloning, expression and purification of VRK1-KD (residues 3-364) and VRK2-KD (residues 14-335) was reported before.^{1,2} To improve VRK1-KD crystallizability, four clusters of surface entropy reduction mutations (SER),³ were engineered into this protein: K34A/K35A/E36A; E212A/K214A/E215A; E292A/K293A/K295A and K359A/K360A. The full-length VRK1 (residues 1-396) was cloned into vector pNIC28-Bsa4 using a ligation independent cloning strategy.⁴ Our cloning strategy introduced a tobacco etch virus (TEV) protease-cleavable, N-terminal 6xHis tag. For protein production, BL21(DE3)-R3 cells, including a plasmid expressing lambda phosphatase,⁵ were cultivated in TB medium (supplemented with 50 $\mu\text{g}\cdot\text{ml}^{-1}$ kanamycin, 35 $\mu\text{g}\cdot\text{ml}^{-1}$ chloramphenicol) at 37 °C until OD600 reached ~ 3 and then cooled to 18 °C for 1 hour. Isopropyl 1-thio-D-galactopyranoside (IPTG) was added to 0.1 mM, and growth continued at 18 °C overnight. Cells were collected by centrifugation and pellets suspended in 2x lysis buffer (lysis buffer is 50 mM HEPES buffer, pH 7.5, 0.5 M NaCl, 10 mM imidazole, 0.5 mM tris(2-carboxyethyl)phosphine [TCEP], Protease Inhibitors Cocktail Set VII - Calbiochem, 1/1000 dilution) prior to flash-freezing in liquid nitrogen. After thawing, cells were lysed by sonication on ice. Proteins were purified using Ni-Sepharose resin (GE Healthcare) and eluted stepwise in binding buffer with 300 mM imidazole. Removal of hexahistidine tags was performed at 4 °C overnight using recombinant TEV protease while dialyzing against excess gel filtration buffer (25 mM HEPES, 500 mM NaCl, 0.5 mM TCEP, 5% [v/v] glycerol). Proteins were further purified by reverse affinity in Ni-Sepharose followed by gel filtration (Superdex 200 16/60, GE Healthcare). Protein in gel filtration buffer was concentrated to 14 $\text{mg}\cdot\text{ml}^{-1}$ (VRK1-KD) or 20 $\text{mg}\cdot\text{ml}^{-1}$ (VRK2-KD) using 30 kDa MWCO centrifugal concentrators (Millipore) at 4 °C. Compounds in 100% DMSO were added to protein solutions at 3-fold molar excess and incubated on ice for approximately 30 minutes. This mixture was centrifuged at 14,000 rpm for 10 minutes at 4 °C prior to setting up 150-nl volume sitting drops at three ratios of protein-inhibitor complex to reservoir solution (2:1, 1:1, or 1:2). Crystallization experiments were performed at 20 °C. Crystals were cryoprotected in reservoir solution supplemented with 20–25% glycerol before flash-freezing in liquid nitrogen for data collection. Diffraction data were collected at 100 K at the Advanced Photon Source 24ID-C (APS, Chicago), for VRK2, and at the Advanced Photon Source 24ID-E (APS, Chicago), for VRK1. The best-diffracting crystals grew under the conditions described in Table S1. Crystal optimization used Newman's buffer system.⁶

Thermal-shift assay (Differential Scanning fluorimetry – DSF)

VRK1-FL and VRK2-KD proteins were screened against an in-house collection of 44 aminopyridine compounds (Table S2). DSF measurements were made in a 384-well plate. Each well contained 20 μ L of 2 μ M kinase in 20 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol and the Applied Biosystems Protein Thermal Shift dye at the recommended dilution of 1:1000.

Compounds were solubilized in 100% DMSO to 10 mM final concentration and 100 nL was transferred using a pin transfer device (CyBio) to 10 μ M final concentration and 0.1% DMSO. Plates were sealed using optically clear films and transferred to a QuantStudio 6 qPCR instrument (Applied Biosystems). The fluorescence intensity was measured during a temperature gradient from 25 to 95 $^{\circ}$ C at a constant rate of 0.05 $^{\circ}$ C/s and protein melting temperatures were calculated based on a Boltzmann function fitting to experimental data, as implemented in the Protein Thermal Shift Software (Applied Biosystems). Protein in 0.1% DMSO was used as a reference. Compounds that caused a shift in protein melting temperature (ΔT_m) of 2 $^{\circ}$ C or higher compared to the reference were considered positive hits.



Supplementary Figure S1: DSF results for prototype compound **5**. (A, B) Shown are thermal melting curves in the presence of DMSO (vehicle) or **5** for VRK1-FL (A) and VRK2-KD (B). ΔT_m values for **5** are indicated. Values shown represent the mean (\pm standard deviation) of three independent measurements.

Enzyme inhibition assays

All enzymatic assays were performed using commercially available full-length wild-type VRK1 produced in insect cells (Carna Bioscience, Inc, Japan) and the following components from Perking Elmer Life (Shelton, USA) LANCE Ultra TR-FRET Kit: ULight–Histone 3-Thr3 peptide substrate and Eu-anti-histone 3-Thr3 detection antibody. All 384-well plates used in these assays were obtained from Corning (NY, USA).

Enzyme titration For EC₈₀ calculation, VRK1 was diluted in kinase buffer (50 mM HEPES, 5 mM MgCl₂, 1 mM EGTA, 2 mM DTT, 0.01% tween) and transferred (5 µL) to a white, flat-bottom 384-well plate containing 5 µL of a premixed solution of ATP (400 µM in kinase buffer) and ULight–Histone 3-Thr3 peptide substrate (100 nM in kinase buffer). The reaction was incubated for 30 minutes at 24 °C and stopped by addition of 5 µL EDTA (24 mM in 1x LANCE Ultra detection buffer). 5 µL of Eu-anti-histone 3-Thr3 antibody (8 nM in 1x LANCE Ultra detection buffer) was added and the reaction was incubated 1 hour at 24 °C. Fluorescence intensity was measured using a plate reader (CLARIOstar® - BMG Labtech, Ortenberg, Germany). Enzyme titration was performed twice and a representative result is shown in Supplementary Figure S2A.

K_m-ATP determination. 5 µL of a premixed solution of VRK1 (6 nM) and ULight–Histone 3-Thr3 peptide (100 nM) was aliquoted into a white, low volume, 384- round bottom well plate. The reaction was started by addition of ATP (>99% purity, Sigma Aldrich, St. Louis, MO, USA; prepared in kinase buffer) at various concentrations (ranging from 1,000 µM to 0.03 µM) and incubated for 30 minutes at 24 °C prior to quenching with 5 µL EDTA (24 mM in 1x LANCE Ultra detection buffer). 5 µL of Eu-anti-histone 3-Thr3 antibody (8 nM in 1x LANCE Ultra detection buffer) was added and the reaction was incubated 1 hour at 24 °C. Fluorescence intensity was measured using a plate reader as above. VRK1 K_m-ATP determination was performed twice and a representative result is shown in Supplementary Figure S2B.

Reaction time course. Assays were performed at the previously determined K_m-ATP. VRK1 was prepared to 6, 12 and 18 nM in kinase buffer (100 µL final volume in PCR tubes). The reaction was started by addition of 100 µL of a premixed solution containing ATP and ULight–Histone 3-Thr3 peptide in kinase buffer. The reaction was stopped by transferring 10 µL aliquots at various time points (longest reaction time: 2 hours) to a white, flat-bottom 384-well plate containing 5 µL of an EDTA solution (24mM in 1x LANCE Ultra detection buffer). 5 µL of Eu-anti-histone 3-Thr3 antibody (8 nM in 1x LANCE Ultra detection buffer) was added and the reaction was incubated 1 hour at 24 °C. Fluorescence intensity was measured using a plate reader as above. VRK1 reaction time course was performed twice and a representative result is shown in Supplementary Figure S2C.

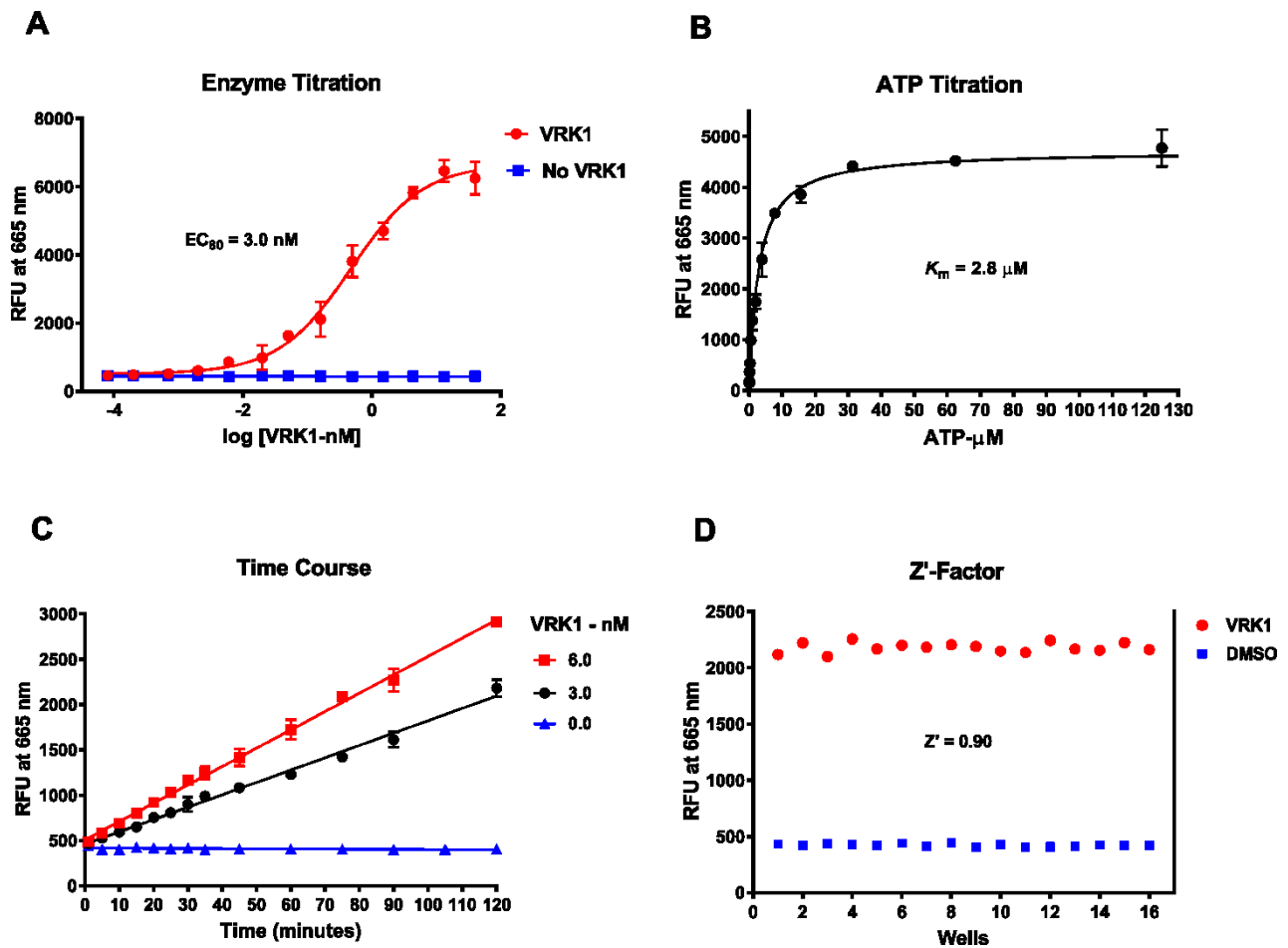
IC₅₀ determination. The inhibition of enzyme activity by pyridine derivatives was carried out at the enzyme K_m-ATP (2.8 µM) and with 6 nM enzyme. Compound stocks (in 100% DMSO) were serially diluted in 100% DMSO prior to transfer (100 nL) to a white, low volume 384-round bottom plate containing 5 µL VRK1 (at 12 nM in kinase buffer) using an automated liquid transferring system (CYBIO FeliX®, Analytik Jena, Jena, Germany). The compound-enzyme mixture was kept at 24 °C for 30 minutes. The reaction was started by the addition of 5 µL of a premixed solution containing ATP (5.6 µM) and ULight–Histone 3-Thr3 peptide (100 nM) in kinase buffer. The reaction was allowed to proceed for 75 minutes at 24 °C before quenching with 5 µL EDTA (24mM in 1x LANCE Ultra detection buffer). in detection buffer. 5 µL of Eu-anti-histone 3-Thr3 antibody (8 nM in 1x LANCE Ultra detection buffer) was added and the reaction was incubated 1 hour at 24 °C. Fluorescence intensity was measured using a plate reader as above. IC₅₀ determination

experiments were performed at least twice. Representative results are shown in Figure 2A and Supplementary Figure S3.

Z'-Factor calculation. Z'-Factor for our enzymatic assay was estimated as described before.⁷ Values for the high (16 points, DMSO only) and the low (16 points, no enzyme) ranges of the assay were recorded for every IC₅₀ experiment reported here. Enzyme reactions were performed as described above for IC₅₀ determination. A typical result is shown in Supplementary Figure S2D.

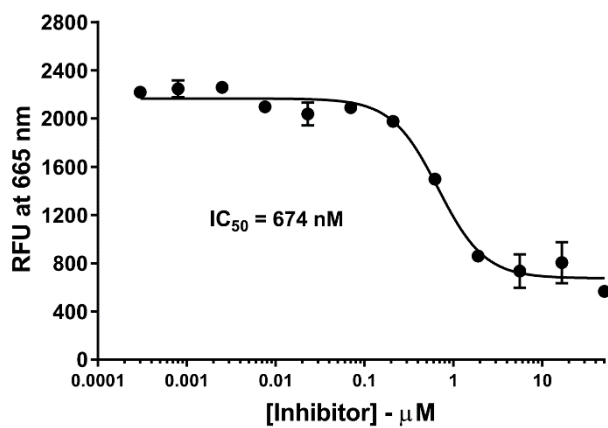
Supplementary Table S2: Enzyme Inhibition Data for VRK1-FL

Compound	IC ₅₀ (nM)	95% CI (nM)	Hill Slope
1 (BI-D1870)	33	25-44	0.7
5	260	202-332	1.4
19	674	530-860	1.6
26	150	120-188	0.8



Supplementary Figure S2: Assay development for VRK1-FL. (A) Enzyme titration curve and EC_{80} determination. (B) ATP titration curve and K_m -ATP determination. (C) Time course reaction curves. (D) Z' factor calculation. Individual data points are the mean \pm SD of three technical replicates. See Supplementary Information for further information on assay conditions.

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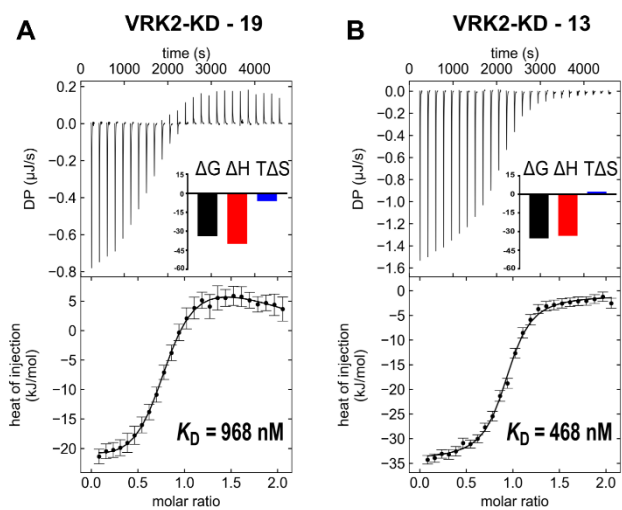
Supplementary Figure S3: IC₅₀ determination curve for compound **19** against VRK1-FL. Individual data points are the mean ± SD of three technical replicates. Estimated IC₅₀ value is shown.

Isothermal calorimetry measurements (ITC)

ITC measurements were made using a MicroCal Auto - iTC200 (Malvern, UK), set to 20 °C, 1,000 rpm stirring, 1.5 µL injections, and 180 s between each injection. For all measurements VRK1-FL and VRK2-KD were dialysed overnight against gel filtration buffer, and the dialysis buffer was used to dilute the inhibitors. VRK1-FL and VRK2-KD were titrated into a solution containing the inhibitor at one tenth of the protein concentration. The concentrations used for each measurement were: 25 µM **26** and 250 µM VRK1, and 24.7 µM **18** and 247 µM VRK2A. ITC data were analysed with NITPIC, and SEDPHAT; figures were made using GUSSE.⁸

**Supplementary Table S3: Isothermal calorimetry
measurements data**

Protein	Inhibitor	ΔG (kJ/mol)	ΔH (kJ/mol)	$T\Delta S$ (kJ/mol)	K_D (nM)
VRK1	26	-37,7	-55,6	-17,9	190
VRK2	18	-35.9	-24.9	11.0	401
VRK2	19	-33.8	-39.9	-6.2	968
VRK2	13	-35.5	-33.4	2.1	468



Supplementary Figure S4: ITC measurements of (A) **19** and (B) **13** for VRK2-KD. Values for binding energies are shown in kJ/mol.

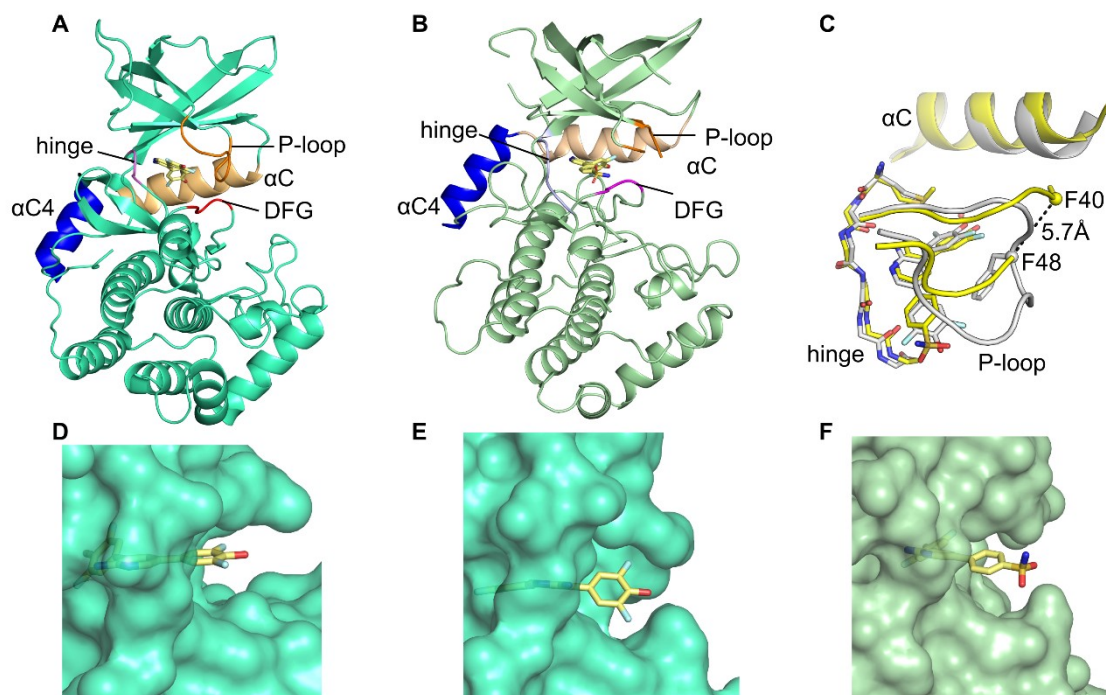
Structure solution and refinement

Diffraction data were integrated using XDS⁹ and scaled using AIMLESS from the CCP4 software suite¹⁰. Molecular replacement (MR) for VRK1-KD bound to **5** was performed with Phaser¹¹ using VRK1-KD bound to **1** (**BI-D1870**) (PDB ID 5UVF)¹². VRK2-KD bound to **1** (**BI-1870**) (PDB ID 5UU1)¹² was used as MR search model for the VRK2-KD bound to **18** dataset. Automated refinement was performed in PHENIX.¹³ Coot¹⁴ was used for manual model building and refinement. Structure validation was performed using MolProbity.¹⁵ Structure factors and coordinates have been deposited in the PDB (see Supplementary Table S4).

Supplementary Table S4- Crystallographic data

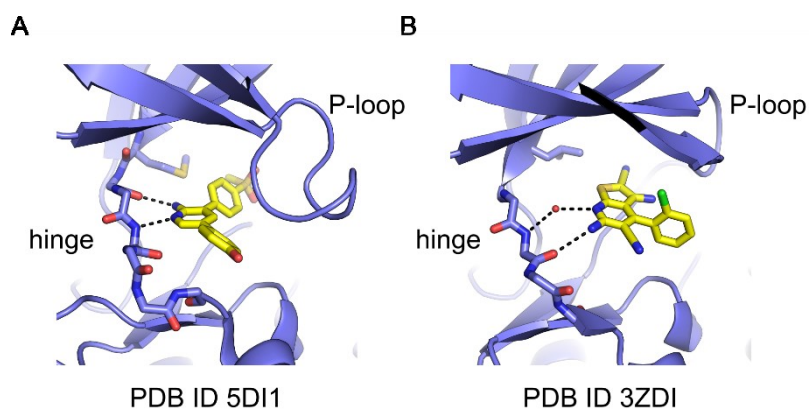
Data collection	VRK2/18	VRK1/5
X-ray source	APS 24-ID-C	APS 24-ID-E
Wavelength (Å)	0.979100	0.979180
Spatial group	P 21 21 21	P 21 21 21
Unit cell (Å)	54.53, 67.62, 171.35	92.22, 96.57, 193.06
Molecules / asymmetric unit	2	4
Resolution range (Å)	29.10-2.45	19.98-1.80
High resolution range (Å)	2.55-2.45	1.83-1.80
Unique reflections	24,095 (2,679)	159,713 (7,810)
R _{merge}	0.137 (1.773)	0.129 (2.177)
R _{p.i.m}	0.061 (0.824)	0.052 (0.882)
Mean I/σ(I)	10.8 (1.2)	13.9 (1.5)
Mean CC(1/2)	0.998 (0.707)	0.999 (0.698)
Completeness (%)	99.9 (99.9)	99.9 (100.0)
Redundancy	10.6 (10.3)	13.6 (13.6)
Refinement		
Resolution (Å)	29.10-2.45	19.98-1.80
RMS bond (Å)	0.0084	0.012
RMS angle (°)	1.994	1.497
Average B factor (Å ²)	65.06	33.22
R _{work} / R _{free} (%)	22.0 / 29.7	18.40 / 21.25
N ^o of atoms (protein/solvent/ligand)	4,466/136/52	9,904/1,173/112
Ramachandran statistics (%)		
Favored / Allowed / Outliers	98/2/0	98/2/0
PDB ID		
	6NCG	6BU6
Crystallization conditions		
	27.5% PEG3350; 200 mM Lithium Sulfate; 0.1 M Buffer system SBG, pH 6.0	22% PEG3350; 200 mM Lithium Sulfate; 0.1M Buffer system SBG, pH 7.0

Additional structural analysis



Supplementary Figure S5: (A-B) Overall structures of VRK1-KD and VRK2-KD bound to 2-aminopyridine compounds **5** and **18**, respectively. (C) Differences in the position of the conserved aromatic residue within VRK1-KD (white) and VRK2-KD (yellow) - Phe48 and Phe40, respectively. (D-E) Molecular surface representation of VRK1-KD bound to **5**. (F) Molecular surface representation of VRK1-KD bound to **18**.

Observed binding modes for 3,5-substituted 2-aminopyridines on the PDB



Supplementary Figure S6: Observed binding modes for 3,5--substituted 2-aminopyridines to human Ser/Thr kinases in the Protein Data Bank. (A) MAP4K4 bound to a 3,5-substituted 2-aminopyridine inhibitor represents the most frequently-observed binding mode for this chemical core. (B) Alternative binding mode for compounds containing a 3,5-substituted 2-aminopyridine core to human Ser/Thr kinases.

Kinase selectivity panel

Enzyme reactions for the kinase selectivity panel were performed at ProQinase (Freiburg, Germany). A radiometric protein kinase assay (33PanQinase® Activity Assay) was used to measure the kinase activity of the 48 protein kinases. All kinase assays were performed in 96-well FlashPlates™ from Perkin Elmer (Boston, MA, USA) in a 50 µL reaction volume. Assay mixture for all protein kinases contained 70 mM HEPES-NaOH pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 3 mM Na-orthovanadate, 1.2 mM DTT, 50 µg/ml PEG20000, ATP (variable amounts, corresponding to the apparent ATP-K_m of the respective kinase), [γ -³³P]-ATP (approx. 8 x 10⁵ cpm per well), protein kinase, and substrate. Final compound and DMSO concentrations in the assay were 1 µM and 1%, respectively.

The reaction cocktail was pipetted in four steps in the following order: (i) 20 µl of assay buffer, (ii) 5 µl of ATP solution (in H₂O), (iii) 5 µl of test compound (in 10 % DMSO) and (iv) 10 µl of substrate / 10 µl of enzyme solution (premixed). The protein kinase reaction cocktails were incubated at 30 °C for 60 minutes. The reaction was stopped with 50 µL of 2 % (v/v) H₃PO₄, plates were aspirated and washed two times with 200 mL 0.9 % (w/v) NaCl. Incorporation of ³³Pi (counting of “cpm”) was determined with a microplate scintillation counter (Microbeta, Wallac). All protein kinase assays were performed with a BeckmanCoulter Biomek 2000/SL robotic system.

All protein kinases provided by ProQinase were expressed in Sf9 insect cells or in *E.coli* as recombinant GST-fusion proteins or His-tagged proteins, either as full-length or enzymatically active fragments. All kinases were produced from human cDNAs and purified by either GSH-affinity chromatography or immobilized metal. Affinity tags were removed from a number of kinases during purification. The purity of the protein kinases was examined by SDS-PAGE/Coomassie staining, the identity was checked by mass spectroscopy.

For each kinase, the median value of the cpm of three wells was defined as "low control" (n=3). This value reflects unspecific binding of radioactivity to the plate in the absence of a protein kinase but in the presence of the substrate. Additionally, for each kinase the median value of the cpm of three other wells was taken as the "high control", i.e. full activity in the absence of any inhibitor (n=3). The difference between high and low control of each enzyme was taken as 100 % activity. As part of the data evaluation the low control of each kinase was subtracted from the high control value as well as from their corresponding "compounds values". The residual activity (in %) for each compound's well was calculated by using the following formula:

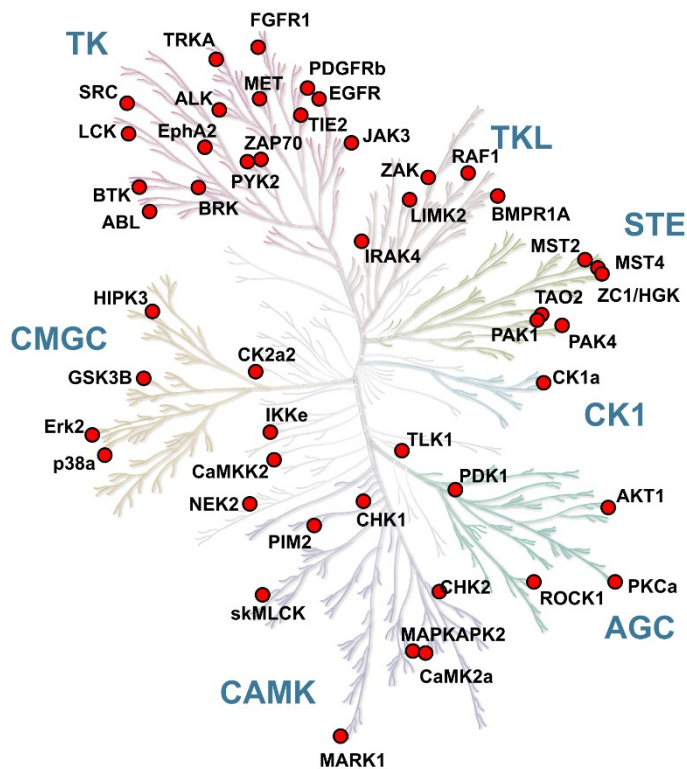
$$\text{Res. Activity (\%)} = 100 \times [(\text{signal of compounds} - \text{low control}) / (\text{high control} - \text{low control})]$$

This subset of 48 human kinases in our selectivity panel was chosen based on the criteria put forward by Bembenek, S. D. and coworkers¹⁶ and commercial availability.

Supplementary Table S5: residual activity (%) of selected kinases at 1 μ M concentration of **1**, **5** and **26**.

Kinase	1 (BI-D1870)	5	26
EPHA2	93	82	136
EGF-R	70	95	123
BRK	95	94	120
LCK	81	65	112
BMPR1A	104	59	110
IKK-epsilon	87	63	108
NEK2	86	75	107
PKC-alpha	96	35	107
MAPKAPK2	104	105	106
MST4	45	85	106
TIE2	104	93	106
ZAP70	95	103	104
HIPK3	93	78	103
BTK	86	65	102
PAK4	76	70	102
SRC	89	102	102
FGF-R1	95	75	101
TLK1	95	100	100
JAK3	45	43	99
AKT1	93	94	98
ROCK1	92	84	98
CK1-alpha1	48	77	97
TRK-A	56	54	97
PDK1	77	69	96
ABL1	81	38	95
CHK2	88	50	95
GSK3-beta	70	77	95
PDGFR-beta	83	73	95
CHK1	98	85	94
CDK2/CycA2	40	74	93
ERK2	81	81	93
CAMK2A	95	83	92
ALK	69	8	91
MARK1	90	88	90
IRAK4	57	80	85
LIMK2	53	82	104
RAF1	26	33	120
ZAK	80	59	97
TAOK2	25	8	88
MST2	45	32	86
PAK1	89	77	85
PIM2	107	65	84
PYK2	26	16	84
MET	78	35	82
p38-alpha	96	89	82
MYLK2	79	31	76
CAMKK2	68	13	47
MAP4K4	24	5	22

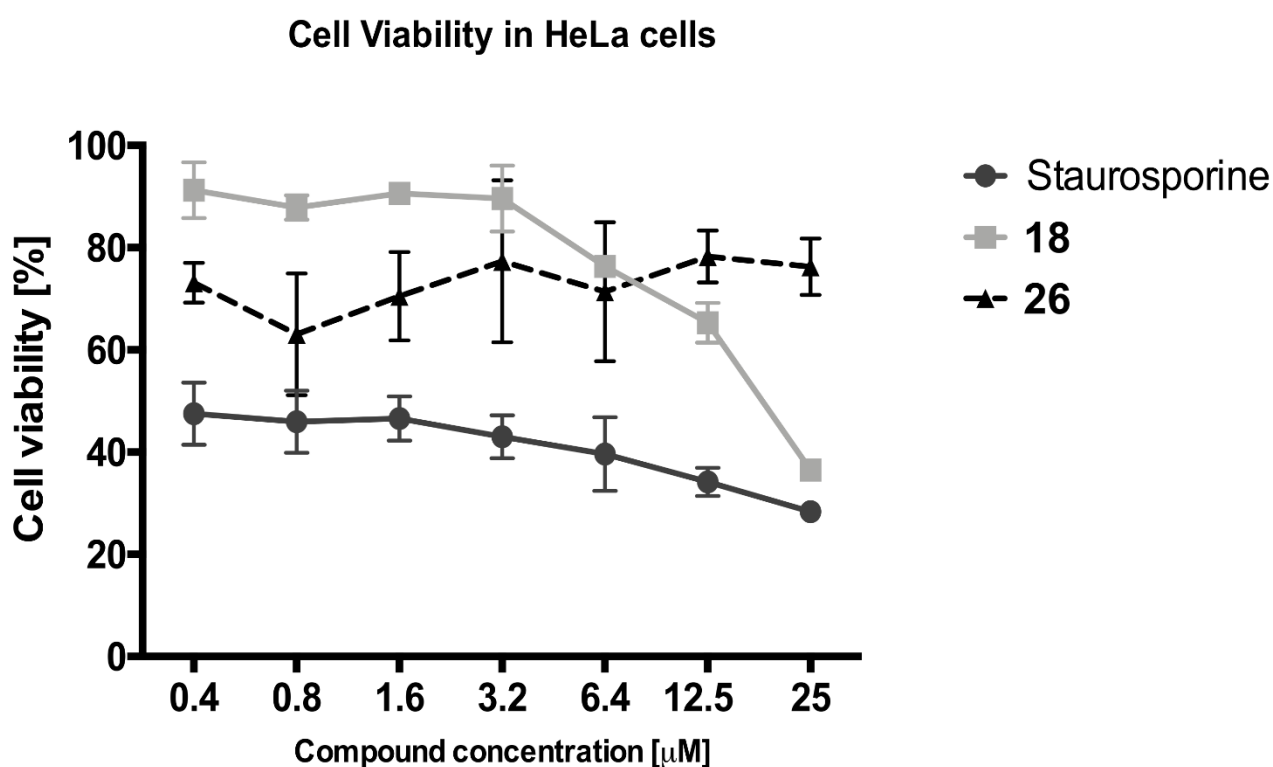
^a colors used for % remaining activity as in Figure 2



Supplementary Figure S7: Protein kinases in the selectivity panel are distributed around the human kinome. Dendrogram of the human kinome showing enzymes used for the selectivity panel (red dots). Kinase names (black font) are indicated (also on Supplementary Table S5 and Figure 2). The name of the main kinome groups is indicated by the use of a blue font. Figure made with the help of KinMap (<http://www.kinhub.org/kinmap/>).

Cell viability analysis by MTT assay

The effect of the compounds **18** and **26** on cellular proliferation was assessed by a 3-(4,5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay, using standard protocol. MTT is a water-soluble tetrazolium salt which is converted to an insoluble purple within the mitochondria. The formazan product is impermeable to the cell membranes and it accumulates in healthy cells. Its concentration can be estimated by optical density. HeLa cells were seeded in a 96-well plate and incubated during 12h with Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum in a CO₂ incubator. The medium was removed and the treatment medium was added with different concentrations of **18**, **26**, or Staurosporine (control). After 24h of treatment, 10 μ L of MTT solution (2.5 mg/mL PBS, pH 7.2) was added to each well and incubated at 37 °C for 4h in the dark. The supernatant was removed and 100 μ L of DMSO was added into each well to dissolve the formazan crystals. The absorbance was measured at 570 nm and results were expressed as the mean of three biological replicates as a percentage of control (non-treated cells as 100% viability and 5% DMSO cells as 0% viability).

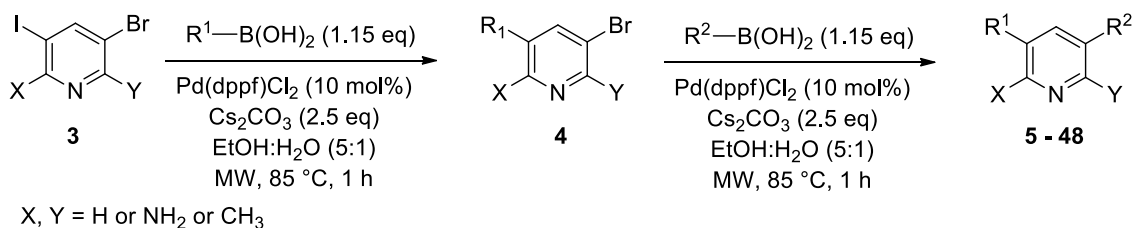


Supplementary Figure S8: Cell viability of compounds **26**, **18** and Staurosporine (used as a control). Values shown represent the mean (\pm standard deviation) of three independent measurements.

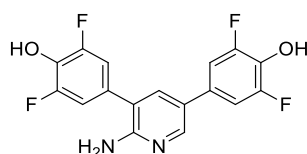
Chemistry – experimental section and analytical data

All reactions were carried out under a nitrogen atmosphere; all final compounds were characterized by ^1H NMR, ^{13}C NMR and electrospray ionization-mass spectrometry (ESI-MS). NMR spectra were recorded on a Bruker 400 MHz or 500 MHz spectrometer. All ^1H NMR experiments are reported in δ units, parts *per* million (ppm), and were measured relative to the solvent signals (DMSO) (2.50 ppm). All ^{13}C NMR spectra are reported in ppm relative to DMSO (39.52 ppm). Sample was submitted for MS analysis to control the exact mass of the compound synthesized. 1 μl of the sample (diluted for a final concentration of 0.1 $\mu\text{g}/\mu\text{l}$ in 100% acetonitrile) was analyzed by reverse phase HPLC-ESI-MS using an Acquity H-class HPLC system (Waters Corp. Milford, MA, USA) which is directly connected to the XEVO G2 Sx Q-ToF (Waters) to determine the intact mass of the small compound. The HPLC is equipped with C18 column (ACQUITY UPLC Protein BEH C18 Column, 1.7 μm , 2.1 mm X 50 mm, Waters) for small molecule separation kept at 45 $^\circ\text{C}$. The mobile phase solvent A was 0.1% Formic acid (FA) in water, and solvent B was 0.1% FA in 100% Acetonitrile (ACN). The sample were loaded at a flow rate of 0.5 $\mu\text{l}/\text{min}$, and eluted from C18 column at a flow rate of 400 $\mu\text{l}/\text{min}$ with one linear gradient step: one from 3 to 90% solvent B over 2.5 min. The column was regenerated by washing at 100% solvent B for 1.5 min and re-equilibrated at 1% solvent B for 3 min. Exact mass analysis was performed in positive ion electrospray in resolution mode. For internal calibration, the lockspray properties were the following: scan time was fixed at 0.5 seconds, with a mass window of 0.5 Da around Leu-enkephalin (556.2771 Da). The ToF-MS acquisition ranged from 50 Da to 2,000 Da with a scan time fixed at 0.5 second. The cone voltage on the ESI source was fixed at 40 V. MS Raw data was analyzed using MassLynx software developed by Waters. The purity of all final compounds was found to be >95%. Palladium catalyst, boronic acids and cesium carbonate were purchased from commercial source and used as received.

General procedure for Suzuki-Miyaura coupling

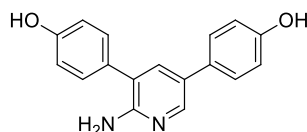


A vial equipped with a magnetic stirrer bar was charged with **3** (1 eq), Cs_2CO_3 (2.5 eq), $Pd(dppf)Cl_2$ (10 mol%), boronic acid (1.15 eq) and ethanol/water 5:1 (0.15 M). The reaction mixture was heated at 85 °C under microwave and nitrogen atmosphere for 1 hour (TLC was used to monitor the reaction). After completion, the reaction mixture was filtered through a pad of celite with ethyl acetate as the washing solvent. The product was purified by silica flash chromatography (gradient of Hexane/AcOEt) and characterized by ¹H NMR, ¹³C NMR and HRMS. We used the same procedure for both steps.



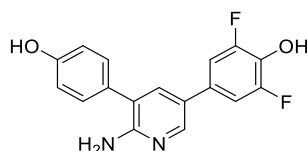
4,4'-(2-aminopyridine-3,5-diyl)bis(2,6-difluorophenol)

The compound **5** was isolated in 51% yield. ¹H NMR (500 MHz, DMSO) δ 10.27 (s, 1H), 10.13 (s, 1H), 8.27 (d, $J = 2.4$ Hz, 1H), 7.62 (d, $J = 2.4$ Hz, 1H), 7.45 – 7.35 (m, 2H), 7.25 – 7.16 (m, 2H), 5.90 (s, 2H). ¹³C NMR (126 MHz, DMSO) δ 155.94, 153.72 (d, $J = 7.7$ Hz), 153.35 (d, $J = 7.5$ Hz), 151.80 (d, $J = 7.7$ Hz), 151.42 (d, $J = 7.7$ Hz), 147.88, 144.85, 135.41, 133.00 (t, $J = 16.1$ Hz), 132.14 (t, $J = 16.3$ Hz), 128.62 (t, $J = 8.6$ Hz), 128.15 (t, $J = 8.7$ Hz), 123.09, 118.30, 112.30 (dd, $J = 15.8, 6.5$ Hz), 108.84 (dd, $J = 16.2, 6.5$ Hz). HRMS (ES): calcd for C₁₇H₁₀F₄N₂O₂ [M+H]⁺: 351.0757. Found: 351.0758.



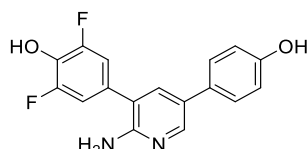
4,4'-(2-aminopyridine-3,5-diyl)diphenol

The compound **6** was isolated in 71% yield. ¹H NMR (500 MHz, DMSO) δ 9.57 (s, 1H), 9.45 (s, 1H), 8.16 (s, 1H), 7.46 (d, $J = 1.8$ Hz, 1H), 7.42 (d, $J = 8.4$ Hz, 2H), 7.33 (d, $J = 8.4$ Hz, 2H), 6.88 (d, $J = 8.4$ Hz, 2H), 6.82 (d, $J = 8.4$ Hz, 2H), 5.51 (s, 2H). ¹³C NMR (126 MHz, DMSO) δ 156.89, 156.44, 155.38, 143.56, 134.95, 129.75, 128.79, 128.60, 126.84, 125.82, 120.70, 115.79. HRMS (ES): calcd for C₁₇H₁₄N₂O₂ [M+H]⁺: 279.1128. Found: 279.1127.



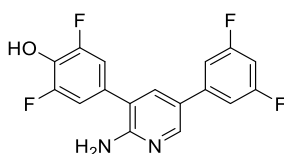
4-(6-amino-5-(4-hydroxyphenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **7** was isolated in 76% yield. ^1H NMR (500 MHz, DMSO) δ 10.09 (s, 1H), 9.56 (s, 1H), 8.22 (d, $J = 2.4$ Hz, 1H), 7.54 (d, $J = 2.3$ Hz, 1H), 7.34 (dd, $J = 14.8, 9.1$ Hz, 4H), 6.86 (d, $J = 8.5$ Hz, 2H), 5.65 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.88, 156.08, 153.70 (d, $J = 7.8$ Hz), 151.78 (d, $J = 7.8$ Hz), 143.99, 134.93, 132.05 (t, $J = 16.3$ Hz), 129.76, 128.86 (t, $J = 8.8$ Hz), 128.23, 123.25, 120.51, 115.67, 108.77 (dd, $J = 16.1, 6.3$ Hz). HRMS (ES): calcd for $\text{C}_{17}\text{H}_{12}\text{F}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 315.0945. Found: 315.0957.



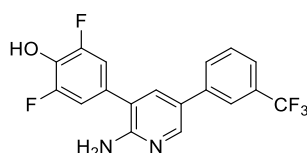
4-(2-amino-5-(4-hydroxyphenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **8** was isolated in 75% yield. ^1H NMR (500 MHz, DMSO) δ 10.26 (s, 1H), 9.40 (s, 1H), 8.17 (d, $J = 2.3$ Hz, 1H), 7.51 (d, $J = 2.3$ Hz, 1H), 7.43 (d, $J = 8.6$ Hz, 2H), 7.23 – 7.14 (m, 2H), 6.80 (d, $J = 8.6$ Hz, 2H), 5.72 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.42, 155.18, 153.33 (d, $J = 7.5$ Hz), 151.41 (d, $J = 7.7$ Hz), 144.38, 135.25, 132.91 (t, $J = 16.1$ Hz), 128.49, 126.83, 125.55, 118.37, 115.66, 112.21 (dd, $J = 15.9, 6.3$ Hz). HRMS (ES): calcd for $\text{C}_{17}\text{H}_{12}\text{F}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 315.0945. Found: 315.0957.



4-(2-amino-5-(3,5-difluorophenyl)pyridin-3-yl)-2,6-difluorophenol

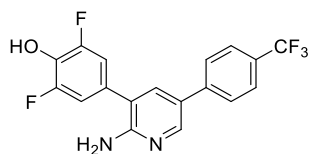
The compound **9** was isolated in 38% yield. ^1H NMR (400 MHz, DMSO) δ 10.31 (s, 1H), 8.37 (d, $J = 2.4$ Hz, 1H), 7.71 (d, $J = 2.4$ Hz, 1H), 7.47 (dd, $J = 9.4, 2.1$ Hz, 2H), 7.26 – 7.17 (m, 2H), 7.09 (tt, $J = 9.2, 2.1$ Hz, 1H), 6.07 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 163.92 (d, $J = 14.0$ Hz), 161.97 (d, $J = 14.0$ Hz), 156.74, 153.34 (d, $J = 8.0$ Hz), 151.41 (d, $J = 7.7$ Hz), 145.58, 142.61 – 140.09 (m), 135.78, 133.59 – 131.66 (m), 127.92 (d, $J = 8.7$ Hz), 122.47, 118.28, 112.32 (dd, $J = 15.9, 6.4$ Hz), 108.36 (dd, $J = 19.9, 6.1$ Hz), 101.45 (t, $J = 26.2$ Hz). HRMS (ES): calcd for $\text{C}_{17}\text{H}_{10}\text{F}_4\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$: 335.0808. Found: 335.0824.



4-(2-amino-5-(3-(trifluoromethyl)phenyl)pyridin-3-yl)-2,6-difluorophenol

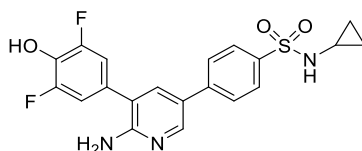
The compound **10** was isolated in 89% yield. ^1H NMR (500 MHz, DMSO) δ 10.29 (s, 1H), 8.36 (d, $J = 2.4$ Hz, 1H), 8.01 – 7.95 (m, 2H), 7.73 (d, $J = 2.4$ Hz, 1H), 7.63 (dd, $J = 8.1, 5.2$ Hz, 2H), 7.26 – 7.19 (m, 2H), 6.01 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.49, 153.35 (d, $J = 7.8$ Hz), 151.42 (d, $J = 7.7$ Hz), 145.53, 138.84, 135.95, 133.04 (t, $J = 16.2$ Hz), 130.14, 129.87 (d, $J = 5.2$ Hz), 129.54 (t, $J = 15.6$ Hz), 128.04 (t, $J = 8.7$ Hz), 127.57, 125.40, 123.44, 123.23, 122.94 (d, $J = 3.8$ Hz), 122.04

(d, $J = 3.8$ Hz), 121.06, 118.45, 112.35 (dd, $J = 15.8, 6.4$ Hz). HRMS (ES): calcd for $C_{18}H_{11}F_5N_2O$ $[M+H]^+$: 367.0870. Found: 367.0866.



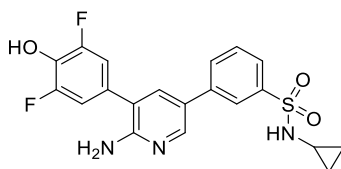
4-(2-amino-5-(4-(trifluoromethyl)phenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **11** was isolated in 62% yield. 1H NMR (500 MHz, DMSO) δ 10.30 (s, 1H), 8.37 (d, $J = 2.4$ Hz, 1H), 7.89 (d, $J = 8.2$ Hz, 2H), 7.73 (d, $J = 8.3$ Hz, 2H), 7.71 (d, $J = 2.4$ Hz, 1H), 7.25 – 7.17 (m, 2H), 6.06 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.66, 153.35 (d, $J = 7.7$ Hz), 151.43 (d, $J = 7.8$ Hz), 145.66, 141.76, 135.90, 133.07 (t, $J = 16.3$ Hz), 127.99 (t, $J = 8.6$ Hz), 126.86, 126.61, 126.09, 125.59 (dd, $J = 7.2, 3.4$ Hz), 123.33 (d, $J = 14.3$ Hz), 118.48, 112.30 (dd, $J = 15.9, 6.4$ Hz). HRMS (ES): calcd for $C_{18}H_{11}F_5N_2O$ $[M+H]^+$: 367.0870. Found: 367.0864.



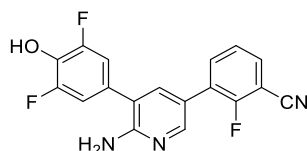
4-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-N-cyclopropylbenzenesulfonamide

The compound **12** was isolated in 61% yield. 1H NMR (500 MHz, DMSO) δ 10.29 (s, 1H), 8.40 (d, $J = 2.4$ Hz, 1H), 7.90 (dd, $J = 11.0, 5.6$ Hz, 3H), 7.81 (d, $J = 8.6$ Hz, 2H), 7.73 (d, $J = 2.4$ Hz, 1H), 7.26 – 7.18 (m, 2H), 6.08 (s, 2H), 2.12 (ddd, $J = 9.8, 6.7, 3.4$ Hz, 1H), 0.49 (td, $J = 7.0, 4.9$ Hz, 2H), 0.43 – 0.38 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.67, 153.35 (d, $J = 7.8$ Hz), 145.72, 141.56, 137.85, 135.90, 133.06 (t, $J = 16.2$ Hz), 128.17 – 127.83 (m), 127.35, 125.82, 123.17, 118.48, 112.30 (dd, $J = 15.8, 6.5$ Hz), 24.09, 5.10. HRMS (ES): calcd for $C_{20}H_{17}F_2N_3O_3S$ $[M+H]^+$: 418.1037. Found: 418.1056.



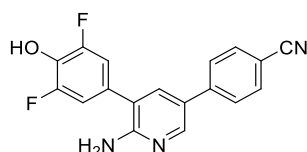
3-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-N-cyclopropylbenzenesulfonamide

The compound **13** was isolated in 66% yield. 1H NMR (500 MHz, DMSO) δ 10.29 (s, 1H), 8.33 (d, $J = 2.4$ Hz, 1H), 8.01 (t, $J = 1.6$ Hz, 1H), 7.96 (d, $J = 7.8$ Hz, 1H), 7.88 (d, $J = 2.7$ Hz, 1H), 7.72 (d, $J = 8.0$ Hz, 1H), 7.64 (dd, $J = 13.0, 5.1$ Hz, 2H), 7.25 – 7.17 (m, 2H), 6.03 (s, 2H), 2.14 – 2.08 (m, 1H), 0.48 (td, $J = 7.0, 5.0$ Hz, 2H), 0.42 – 0.38 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.56, 153.36 (d, $J = 7.6$ Hz), 151.44 (d, $J = 7.7$ Hz), 145.35, 140.92, 138.66, 135.71, 133.10 (t, $J = 16.1$ Hz), 129.70, 129.48, 127.97 (t, $J = 8.7$ Hz), 124.64, 123.30 (d, $J = 19.5$ Hz), 118.59, 112.31 (dd, $J = 15.8, 6.4$ Hz), 24.20, 5.09. HRMS (ES): calcd for $C_{20}H_{17}F_2N_3O_3S$ $[M+H]^+$: 418.1037. Found: 418.1056.



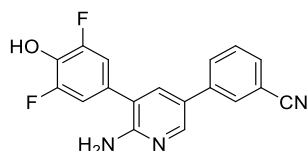
3-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-2-fluorobenzonitrile

The compound **14** was isolated in 27% yield. ^1H NMR (500 MHz, DMSO) δ 10.30 (s, 1H), 8.21 (t, J = 2.1 Hz, 1H), 7.97 (td, J = 7.9, 1.6 Hz, 1H), 7.85 (ddd, J = 7.6, 5.9, 1.6 Hz, 1H), 7.58 (s, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.23 – 7.14 (m, 2H), 6.13 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 160.42, 158.37, 156.66, 153.36 (d, J = 7.9 Hz), 151.43 (d, J = 8.3 Hz), 147.16 (d, J = 3.9 Hz), 137.53, 135.52 (d, J = 3.8 Hz), 133.34 – 132.92 (m), 132.01, 127.71 (d, J = 8.2 Hz), 126.91 (d, J = 12.1 Hz), 125.84 (d, J = 3.5 Hz), 117.87, 114.25, 112.25 (dd, J = 16.1, 6.2 Hz), 101.01. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{10}\text{F}_3\text{N}_3\text{O}$ $[\text{M}+\text{H}]^+$: 342.0854. Found: 342.0868.



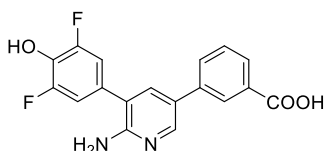
4-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)benzonitrile

The compound **15** was isolated in 63% yield. ^1H NMR (500 MHz, DMSO) δ 10.29 (s, 1H), 8.40 (d, J = 2.4 Hz, 1H), 7.89 (d, J = 8.6 Hz, 2H), 7.84 (d, J = 8.6 Hz, 2H), 7.73 (d, J = 2.4 Hz, 1H), 7.25 – 7.17 (m, 2H), 6.12 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.85, 153.34 (d, J = 7.8 Hz), 151.42 (d, J = 7.7 Hz), 145.87, 142.32, 135.86, 133.09 (t, J = 16.1 Hz), 132.70, 127.88 (t, J = 8.6 Hz), 126.08, 122.86, 119.07, 118.47, 112.31 (dd, J = 15.8, 6.5 Hz), 108.65. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_2\text{N}_3\text{O}$ $[\text{M}+\text{H}]^+$: 324.0948. Found: 324.0960.



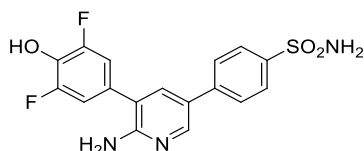
3-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)benzonitrile

The compound **16** was isolated in 64% yield. ^1H NMR (500 MHz, DMSO) δ 10.28 (s, 1H), 8.37 (d, J = 2.4 Hz, 1H), 8.19 (t, J = 1.5 Hz, 1H), 8.04 – 8.01 (m, 1H), 7.72 (ddd, J = 7.6, 4.9, 1.8 Hz, 2H), 7.60 (t, J = 7.8 Hz, 1H), 7.26 – 7.19 (m, 2H), 6.03 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.56, 153.34 (d, J = 7.7 Hz), 151.42 (d, J = 7.5 Hz), 145.50, 138.90, 135.86, 133.04 (t, J = 16.2 Hz), 130.15, 129.94 (d, J = 8.3 Hz), 129.05, 128.00 (t, J = 8.8 Hz), 122.81, 118.93, 118.41, 112.31 (dd, J = 15.9, 6.5 Hz), 112.01. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_2\text{N}_3\text{O}$ $[\text{M}+\text{H}]^+$: 324.0948. Found: 324.0960.



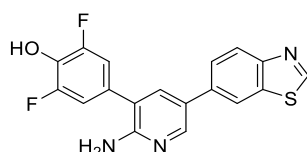
3-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)benzoic acid

The compound **17** was isolated in 25% yield. ^1H NMR (500 MHz, DMSO) δ 8.31 (d, J = 2.3 Hz, 1H), 8.14 (s, 1H), 7.87 (dd, J = 12.8, 7.8 Hz, 2H), 7.65 (d, J = 2.3 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.27 – 7.18 (m, 2H), 5.95 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.24, 153.41, 151.50 (t, J = 9.9 Hz), 145.21, 138.22 – 137.73 (m), 135.79, 129.79, 129.08, 127.33, 126.16, 124.30, 118.52, 112.29 (dd, J = 15.9, 6.6 Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{F}_2\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 343.0894. Found: 343.0906.



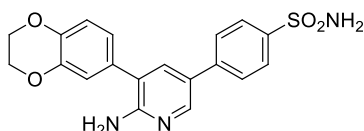
4-(6-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)benzenesulfonamide

The compound **18** was isolated in 63% yield. ¹H NMR (500 MHz, DMSO) δ 10.32 (s, 1H), 8.37 (d, *J* = 2.4 Hz, 1H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.82 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.34 (s, 2H), 7.21 (d, *J* = 7.9 Hz, 2H), 6.05 (s, 2H). ¹³C NMR (126 MHz, DMSO) δ 156.61, 153.39 (d, *J* = 7.7 Hz), 151.46 (d, *J* = 7.8 Hz), 145.64, 141.85, 140.96, 135.87, 133.16 (t, *J* = 14.6 Hz), 127.93 (t, *J* = 8.9 Hz), 126.22, 125.70, 123.39, 118.50, 112.31 (dd, *J* = 15.8, 6.5 Hz). HRMS (ES): calcd for C₁₇H₁₃F₂N₃O₃S [M+H]⁺: 378.0724. Found: 378.0739.



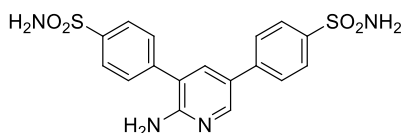
4-(2-amino-5-(benzo[d]thiazol-6-yl)pyridin-3-yl)-2,6-difluorophenol

The compound **19** was isolated in 64% yield. ¹H NMR (500 MHz, DMSO) δ 10.30 (s, 1H), 9.35 (s, 1H), 8.48 (d, *J* = 1.4 Hz, 1H), 8.39 (d, *J* = 2.4 Hz, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.85 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.74 (d, *J* = 2.3 Hz, 1H), 7.28 – 7.20 (m, 2H), 5.96 (s, 2H). ¹³C NMR (126 MHz, DMSO) δ 156.09, 155.73, 153.36 (d, *J* = 7.7 Hz), 151.82, 151.44 (d, *J* = 7.7 Hz), 145.49, 136.07, 135.27, 134.58, 133.02 (t, *J* = 16.0 Hz), 128.20 (t, *J* = 8.5 Hz), 124.40 (d, *J* = 7.7 Hz), 123.13, 119.01, 118.49, 112.29 (dd, *J* = 15.8, 6.5 Hz). HRMS (ES): calcd for C₁₈H₁₁F₂N₃OS [M+H]⁺: 356.0669. Found: 356.0658.



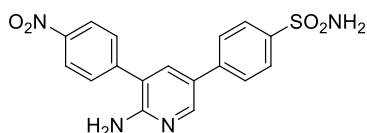
4-(6-amino-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)pyridin-3-yl)benzenesulfonamide

The compound **20** was isolated in 70% yield. ¹H NMR (500 MHz, DMSO) δ 8.35 (d, *J* = 2.4 Hz, 1H), 7.83 (q, *J* = 8.7 Hz, 4H), 7.64 (d, *J* = 2.4 Hz, 1H), 7.33 (s, 2H), 7.02 (d, *J* = 1.9 Hz, 1H), 6.99 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 5.87 (s, 2H), 4.28 (s, 4H). ¹³C NMR (126 MHz, DMSO) δ 156.65, 145.15, 143.60, 143.00, 141.80, 141.09, 135.56, 130.75, 126.24, 125.68, 123.50, 121.48, 120.04, 117.49, 117.29, 64.10. HRMS (ES): calcd for C₁₉H₁₇N₃O₄S [M+H]⁺: 384.1018. Found: 384.1028.



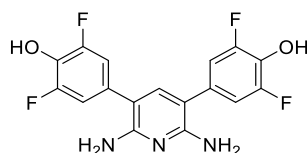
4,4'-(2-aminopyridine-3,5-diyl)dibenzenesulfonamide

The compound **21** was isolated in 52% yield. ¹H NMR (500 MHz, DMSO) δ 8.43 (d, *J* = 2.4 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 8.6 Hz, 2H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 7.0 Hz, 3H), 7.39 (s, 4H), 6.09 (s, 2H). ¹³C NMR (126 MHz, DMSO) δ 156.46, 146.22, 143.03, 141.93, 141.30, 140.87, 136.22, 129.19, 126.23 (d, *J* = 5.7 Hz), 125.74, 123.58, 119.01. HRSM (ES): calcd for C₁₇H₁₆N₄O₄S₂ [M+H]⁺: 405.0691. Found: 405.0706.



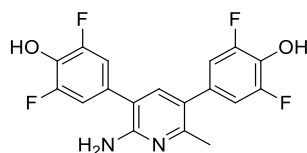
4-(6-amino-5-(4-nitrophenyl)pyridin-3-yl)benzenesulfonamide

The compound **22** was isolated in 33% yield. ^1H NMR (500 MHz, DMSO) δ 8.46 (d, J = 2.4 Hz, 1H), 8.32 (d, J = 8.8 Hz, 2H), 7.93 – 7.82 (m, 6H), 7.81 (d, J = 2.4 Hz, 1H), 7.35 (s, 2H), 6.21 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.41, 146.82, 146.52, 145.05, 141.99, 140.73, 136.23, 131.48 (d, J = 9.7 Hz), 130.15, 128.76 (d, J = 11.8 Hz), 126.25, 125.76, 123.99, 123.53, 118.24. HRMS (ES): calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$: 371.0814. Found: 371.0828.



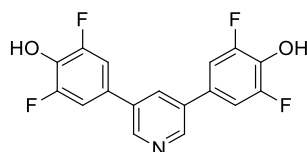
4,4'-(2,6-diaminopyridine-3,5-diyl)bis(2,6-difluorophenol)

The compound **23** was isolated in 24% yield. ^1H NMR (500 MHz, DMSO) δ 7.11 – 6.89 (m, 4H), 5.78 (d, J = 8.0 Hz, 1H), 5.55 (s, 2H), 5.17 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 158.22, 154.96, 153.28 (d, J = 7.9 Hz), 151.36 (d, J = 7.9 Hz), 139.16, 131.41 (t, J = 16.1 Hz), 130.13 (t, J = 8.8 Hz), 111.43 (dd, J = 15.6, 6.2 Hz), 106.07, 97.11. HRMS (ES): calcd for $\text{C}_{17}\text{H}_{11}\text{F}_4\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 366.0866. Found: 366.0875.



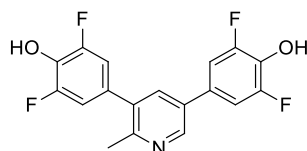
4,4'-(2-amino-6-methylpyridine-3,5-diyl)bis(2,6-difluorophenol)

The compound **24** was isolated in 55% yield. ^1H NMR (500 MHz, DMSO) δ 10.18 (s, 2H), 7.17 (s, 1H), 7.15 (d, J = 8.1 Hz, 2H), 7.07 (d, J = 9.2 Hz, 2H), 5.78 (s, 2H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 154.92, 153.33 (d, J = 7.7 Hz), 152.92 (d, J = 7.5 Hz), 152.46, 151.41 (d, J = 7.5 Hz), 151.00 (d, J = 7.8 Hz), 138.99, 132.75 (t, J = 16.1 Hz), 132.15 (t, J = 16.2 Hz), 130.47 (t, J = 8.7 Hz), 128.18 (t, J = 8.6 Hz), 123.53, 115.99, 112.57 (dd, J = 15.6, 6.2 Hz), 112.09 (dd, J = 15.8, 6.4 Hz), 22.48. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{F}_4\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 365.0913. Found: 365.0909.



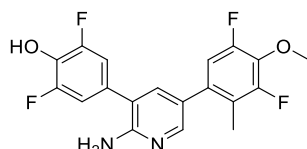
4,4'-(pyridine-3,5-diyl)bis(2,6-difluorophenol)

The compound **25** was isolated in 60% yield. ^1H NMR (500 MHz, DMSO) δ 10.47 (s, 1H), 10.34 (s, 1H), 8.86 (s, 1H), 8.11 (s, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 7.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 153.68 (d, J = 7.6 Hz), 152.84 (d, J = 5.8 Hz), 151.76 (d, J = 7.7 Hz), 150.91 (d, J = 5.9 Hz), 146.11, 135.41 (t, J = 16.2 Hz), 133.90 (t, J = 16.3 Hz), 133.37, 131.13, 127.20 (t, J = 8.7 Hz), 124.09 (dd, J = 14.1, 7.7 Hz), 116.83 (dd, J = 13.8, 4.9 Hz), 110.61 (dd, J = 16.3, 6.7 Hz). HRMS (ES): calcd for $\text{C}_{17}\text{H}_9\text{F}_4\text{NO}_2$ $[\text{M}+\text{H}]^+$: 336.0648. Found: 336.0656.



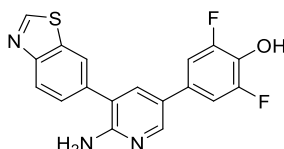
4,4'-(2-methylpyridine-3,5-diyl)bis(2,6-difluorophenol)

The compound **26** was isolated in 45% yield. $^1\text{H NMR}$ (500 MHz, DMSO) δ 10.41 (s, 2H), 8.76 (d, $J = 2.3$ Hz, 1H), 7.87 (d, $J = 2.3$ Hz, 1H), 7.57 (d, $J = 9.8$ Hz, 2H), 7.25 (d, $J = 9.2$ Hz, 2H), 2.48 (s, 3H). $^{13}\text{C NMR}$ (126 MHz, DMSO) δ 154.09, 153.70 (d, $J = 7.5$ Hz), 152.98 (d, $J = 7.7$ Hz), 151.78 (d, $J = 7.8$ Hz), 151.06 (d, $J = 7.5$ Hz), 145.35, 134.64, 134.17, 133.90 – 133.04 (m), 131.08, 129.35 (t, $J = 8.8$ Hz), 127.17 (t, $J = 8.9$ Hz), 112.94 (dd, $J = 15.9, 6.5$ Hz), 110.19 (dd, $J = 16.4, 6.5$ Hz), 22.95. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_4\text{NO}_2$ $[\text{M}+\text{H}]^+$: 350.0804. Found: 350.0794.



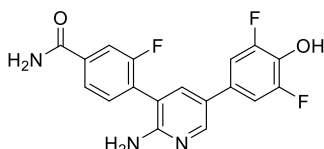
4-(2-amino-5-(3,5-difluoro-4-methoxy-2-methylphenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **27** was isolated in 63% yield. $^1\text{H NMR}$ (400 MHz, DMSO) δ 10.32 (s, 1H), 7.93 (d, $J = 2.3$ Hz, 1H), 7.33 (d, $J = 2.4$ Hz, 1H), 7.22 – 7.14 (m, 2H), 7.11 (dd, $J = 11.9, 1.9$ Hz, 1H), 5.93 (s, 2H), 3.92 (s, 3H), 2.15 (d, $J = 2.3$ Hz, 3H). $^{13}\text{C NMR}$ (126 MHz, DMSO) δ 155.87, 154.80, 153.75 (d, $J = 6.9$ Hz), 153.36 (d, $J = 7.5$ Hz), 151.81 (d, $J = 6.9$ Hz), 151.43 (d, $J = 7.7$ Hz), 147.09, 138.04, 134.76 – 134.21 (m), 133.99 (dd, $J = 8.6, 5.2$ Hz), 133.08 (d, $J = 10.5$ Hz), 127.90 (dd, $J = 9.0, 6.0$ Hz), 123.44, 119.49 (dd, $J = 14.7, 3.0$ Hz), 117.87, 112.74 (dd, $J = 19.0, 2.2$ Hz), 112.23 (dd, $J = 15.9, 6.4$ Hz), 61.78, 12.03. HRMS (ES): calcd for $\text{C}_{19}\text{H}_{14}\text{F}_4\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 379.1070. Found: 379.1077.



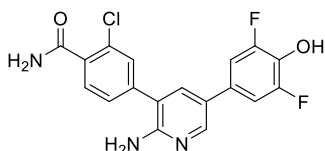
4-(6-amino-5-(benzo[d]thiazol-6-yl)pyridin-3-yl)-2,6-difluorophenol

The compound **28** was isolated in 25% yield. $^1\text{H NMR}$ (500 MHz, DMSO) δ 10.11 (s, 1H), 9.43 (s, 1H), 8.32 (d, $J = 2.5$ Hz, 2H), 8.16 (d, $J = 8.4$ Hz, 1H), 7.74 – 7.66 (m, 2H), 7.46 – 7.37 (m, 2H), 5.90 (s, 2H). $^{13}\text{C NMR}$ (101 MHz, DMSO) δ 156.93, 156.59, 154.48, 152.80, 145.45, 136.22, 135.75, 134.74, 132.60, 129.13, 127.61, 123.64, 123.17, 120.17, 109.28 (dd, $J = 15.5, 7.7$ Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_2\text{N}_3\text{OS}$ $[\text{M}+\text{H}]^+$: 356.0669. Found: 356.0658.



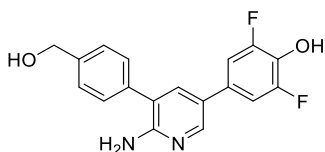
4-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-3-fluorobenzamide

The compound **29** was isolated in 62% yield. $^1\text{H NMR}$ (400 MHz, DMSO) δ 10.11 (s, 1H), 8.35 (d, $J = 2.4$ Hz, 1H), 8.12 (s, 1H), 7.85 – 7.75 (m, 2H), 7.65 (d, $J = 2.3$ Hz, 1H), 7.54 (t, $J = 7.7$ Hz, 2H), 7.42 – 7.32 (m, 2H), 5.86 (s, 2H). $^{13}\text{C NMR}$ (126 MHz, DMSO) δ 166.31, 160.31, 158.35, 156.21, 153.74 (d, $J = 7.7$ Hz), 151.82 (d, $J = 7.7$ Hz), 145.90, 136.23, 135.81 (d, $J = 6.7$ Hz), 132.71 – 131.75 (m), 131.64, 128.44 (t, $J = 8.8$ Hz), 128.04 (d, $J = 16.4$ Hz), 123.87 (d, $J = 2.6$ Hz), 122.42, 115.17, 114.98, 113.73, 108.77 (dd, $J = 16.2, 6.5$ Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{F}_3\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 360.0960. Found: 360.0962.



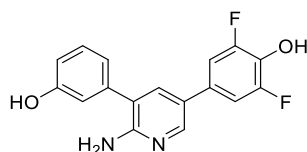
4-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-2-chlorobenzamide

The compound **30** was isolated in 61% yield. ^1H NMR (400 MHz, DMSO) δ 10.14 (s, 1H), 8.31 (d, $J = 2.4$ Hz, 1H), 7.86 (s, 1H), 7.64 (dd, $J = 5.7, 3.2$ Hz, 3H), 7.57 – 7.51 (m, 2H), 7.46 – 7.36 (m, 2H), 5.90 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.95, 155.80, 153.72 (d, $J = 7.8$ Hz), 151.80 (d, $J = 7.8$ Hz), 145.50, 140.34, 135.66 (d, $J = 11.2$ Hz), 132.23 (t, $J = 16.3$ Hz), 130.31, 129.74, 129.17, 128.50 (t, $J = 8.8$ Hz), 127.21, 123.31, 118.23, 108.93 (dd, $J = 16.2, 6.6$ Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{ClF}_2\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 376.0664. Found: 376.0664.



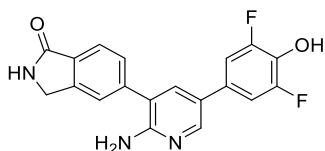
4-(6-amino-5-(4-(hydroxymethyl)phenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **31** was isolated in 18% yield. ^1H NMR (400 MHz, DMSO) δ 10.13 (s, 1H), 8.27 (d, $J = 2.4$ Hz, 1H), 7.59 (d, $J = 2.4$ Hz, 1H), 7.49 (d, $J = 8.1$ Hz, 2H), 7.42 (d, $J = 8.2$ Hz, 2H), 7.37 (d, $J = 9.9$ Hz, 2H), 5.72 (s, 2H), 5.25 (s, 1H), 4.56 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 155.94, 153.71 (d, $J = 7.7$ Hz), 151.79 (d, $J = 7.8$ Hz), 144.59, 141.82, 136.14, 135.27, 132.14 (t, $J = 16.4$ Hz), 128.72, 128.34, 126.93, 123.29, 120.25, 108.82 (dd, $J = 16.1, 6.5$ Hz), 62.64. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{14}\text{F}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 329.1102. Found: 329.1117.



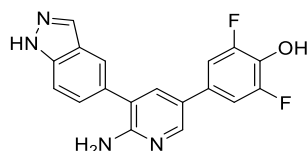
4-(6-amino-5-(3-hydroxyphenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **32** was isolated in 64% yield. ^1H NMR (400 MHz, DMSO) δ 10.11 (s, 1H), 9.56 (s, 1H), 8.26 (d, $J = 2.4$ Hz, 1H), 7.57 (d, $J = 2.4$ Hz, 1H), 7.42 – 7.32 (m, 2H), 7.27 (t, $J = 7.8$ Hz, 1H), 6.91 (dd, $J = 7.8, 4.9$ Hz, 2H), 6.78 (dd, $J = 8.0, 2.1$ Hz, 1H), 5.70 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 157.73, 155.83, 153.75 (d, $J = 7.8$ Hz), 151.83 (d, $J = 7.8$ Hz), 144.62, 139.09, 135.01, 132.21 (t, $J = 16.4$ Hz), 130.01, 128.66 (t, $J = 8.7$ Hz), 123.19, 120.45, 119.24, 115.41, 114.59, 108.81 (dd, $J = 16.1, 6.6$ Hz). HRMS (ES): calcd for $\text{C}_{17}\text{H}_{12}\text{F}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 315.0945. Found: 315.0959.



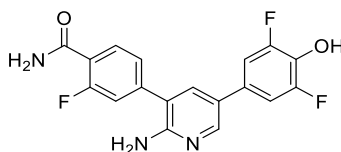
5-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)isoindolin-1-one

The compound **33** was isolated in 63% yield. ^1H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 8.58 (s, 1H), 8.32 (d, $J = 2.4$ Hz, 1H), 7.77 – 7.71 (m, 2H), 7.67 (d, $J = 2.4$ Hz, 1H), 7.62 (d, $J = 8.8$ Hz, 1H), 7.44 – 7.35 (m, 2H), 5.90 (s, 2H), 4.42 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 169.78, 155.92, 153.75 (d, $J = 7.6$ Hz), 151.84 (d, $J = 7.6$ Hz), 145.23, 144.79, 141.02, 135.60, 132.25 (t, $J = 16.2$ Hz), 131.68, 128.50 (d, $J = 13.0$ Hz), 124.08, 123.24, 119.69, 108.85 (dd, $J = 16.2, 6.6$ Hz), 45.02. HRMS (ES): calcd for $\text{C}_{19}\text{H}_{13}\text{F}_2\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 354.1049. Found: 354.1035.



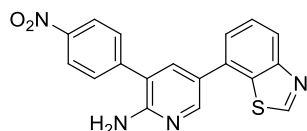
4-(6-amino-5-(1H-indazol-5-yl)pyridin-3-yl)-2,6-difluorophenol

The compound **34** was isolated in 19% yield. ^1H NMR (400 MHz, DMSO) δ 13.13 (s, 1H), 10.13 (s, 1H), 8.28 (d, $J = 2.4$ Hz, 1H), 8.12 (s, 1H), 7.90 (s, 1H), 7.67 – 7.59 (m, 2H), 7.49 (dd, $J = 8.6, 1.2$ Hz, 1H), 7.44 – 7.34 (m, 2H), 5.74 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.31, 153.73 (d, $J = 7.7$ Hz), 151.82 (d, $J = 7.8$ Hz), 144.33, 139.27, 135.54, 133.81, 132.13 (t, $J = 16.4$ Hz), 129.90, 128.79 (t, $J = 8.6$ Hz), 127.12, 123.31 (d, $J = 14.3$ Hz), 120.92, 120.60, 110.51, 108.79 (dd, $J = 16.1, 6.4$ Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{F}_2\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$: 339.1057. Found: 339.1072.



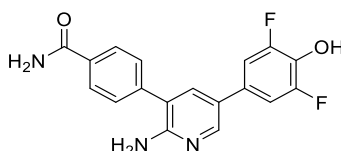
4-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-2-fluorobenzamide

The compound **35** was isolated in 61% yield. ^1H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 8.32 (d, $J = 2.4$ Hz, 1H), 7.77 (t, $J = 8.0$ Hz, 1H), 7.67 (d, $J = 2.3$ Hz, 3H), 7.50 – 7.44 (m, 2H), 7.39 (dd, $J = 15.2, 6.8$ Hz, 2H), 5.96 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 164.89, 160.72, 158.73, 155.78, 153.76 (d, $J = 7.8$ Hz), 151.84 (d, $J = 7.7$ Hz), 145.61, 142.54 (d, $J = 8.7$ Hz), 135.66, 132.29 (t, $J = 16.4$ Hz), 130.90 (d, $J = 3.0$ Hz), 128.48 (t, $J = 8.7$ Hz), 124.69, 123.27, 122.14 (d, $J = 13.8$ Hz), 118.22, 116.53, 116.34, 108.93 (dd, $J = 16.2, 6.5$ Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{F}_3\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 360.0954. Found: 360.0942.



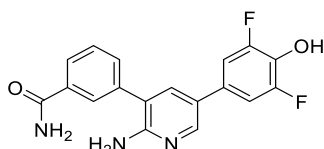
5-(benzo[d]thiazol-7-yl)-3-(4-nitrophenyl)pyridin-2-amine

The compound **36** was isolated in 26% yield. ^1H NMR (500 MHz, DMSO) δ 9.44 (s, 1H), 8.42 (d, $J = 2.4$ Hz, 1H), 8.32 (d, $J = 8.8$ Hz, 2H), 8.06 (dd, $J = 6.7, 2.5$ Hz, 1H), 7.86 (d, $J = 8.8$ Hz, 2H), 7.77 (d, $J = 2.4$ Hz, 1H), 7.63 (dd, $J = 7.1, 4.9$ Hz, 2H), 6.26 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.35, 155.97, 153.81, 147.07, 146.56, 144.86, 136.88, 133.13, 132.33, 130.05, 126.99, 124.92, 124.35, 124.08, 121.75, 118.13. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$: 349.0759. Found: 349.0769.



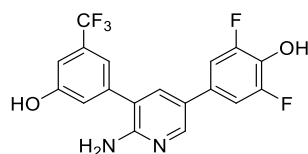
4-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)benzamide

The compound **37** was isolated in 68% yield. ^1H NMR (400 MHz, DMSO) δ 10.13 (s, 1H), 8.31 (d, $J = 2.4$ Hz, 1H), 8.04 (s, 1H), 7.99 (d, $J = 8.3$ Hz, 2H), 7.64 (dd, $J = 12.7, 5.3$ Hz, 3H), 7.44 – 7.35 (m, 3H), 5.86 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.51, 155.87, 153.74 (d, $J = 7.6$ Hz), 151.83 (d, $J = 7.7$ Hz), 145.19, 140.84, 135.52, 133.05, 132.21 (t, $J = 16.3$ Hz), 128.54, 128.09, 123.30, 119.49, 108.89 (dd, $J = 16.1, 6.6$ Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{13}\text{F}_2\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 342.1054. Found: 342.1062.



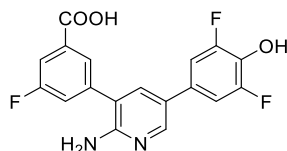
3-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)benzamide

The compound **38** was isolated in 63% yield. ^1H NMR (400 MHz, DMSO) δ 10.14 (s, 1H), 8.31 (d, J = 2.4 Hz, 1H), 8.04 (s, 1H), 7.98 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 5.3 Hz, 2H), 7.55 (t, J = 7.7 Hz, 1H), 7.44 – 7.34 (m, 3H), 5.84 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.93, 155.95, 153.77 (d, J = 7.6 Hz), 151.85 (d, J = 7.6 Hz), 145.01, 137.86, 135.63, 135.05, 132.24 (t, J = 16.5 Hz), 131.44, 128.90, 128.61 (t, J = 8.6 Hz), 127.71, 126.72, 123.25, 119.74, 108.84 (dd, J = 16.2, 6.5 Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{13}\text{F}_2\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$: 342.1054. Found: 342.1062.



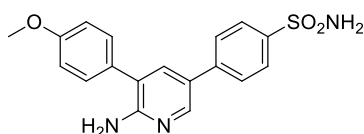
4-(6-amino-5-(3-hydroxy-5-(trifluoromethyl)phenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **39** was isolated in 60% yield. ^1H NMR (400 MHz, DMSO) δ 10.21 (s, 2H), 8.30 (d, J = 2.4 Hz, 1H), 7.64 (d, J = 2.4 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.24 (s, 1H), 7.18 (s, 1H), 7.04 (s, 1H), 5.86 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 158.20, 155.84, 153.73 (d, J = 7.6 Hz), 151.81 (d, J = 7.6 Hz), 145.28, 140.38, 135.42, 132.23 (t, J = 16.1 Hz), 131.15, 130.90, 130.65, 130.40, 128.48 (t, J = 8.7 Hz), 125.18, 123.08 (d, J = 17.8 Hz), 120.84, 119.57, 118.94, 115.88 (d, J = 3.8 Hz), 110.83 (d, J = 3.5 Hz), 108.88 (dd, J = 16.2, 6.6 Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_5\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 383.0813. Found: 383.0800.



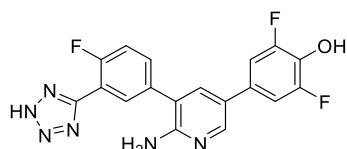
3-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-5-fluorobenzoic acid

The compound **40** was isolated in 26% yield. ^1H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.32 (d, J = 2.2 Hz, 1H), 7.90 (s, 1H), 7.68 (d, J = 2.2 Hz, 1H), 7.59 (dd, J = 26.9, 8.9 Hz, 2H), 7.46 – 7.36 (m, 2H), 5.94 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 166.85, 163.21, 161.27, 155.86, 153.73 (d, J = 8.0 Hz), 151.82 (d, J = 7.7 Hz), 145.42, 140.21 (d, J = 6.1 Hz), 135.59, 132.21 (t, J = 16.3 Hz), 128.45 (t, J = 9.0 Hz), 125.62, 123.12, 118.47, 114.66, 114.48, 108.85 (dd, J = 16.1, 6.6 Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 361.0800. Found: 361.0814.



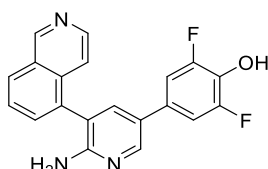
4-(6-amino-5-(4-methoxyphenyl)pyridin-3-yl)benzenesulfonamide

The compound **41** was isolated in 77% yield. ^1H NMR (500 MHz, DMSO) δ 8.36 (s, 1H), 7.84 (s, 4H), 7.66 (s, 1H), 7.47 (d, J = 7.8 Hz, 2H), 7.34 (s, 2H), 7.06 (d, J = 7.8 Hz, 2H), 5.85 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 158.73, 156.74, 145.07, 141.81, 141.13, 135.59, 131.78, 129.83, 128.93, 127.61, 126.26, 125.62 (d, J = 14.8 Hz), 123.59, 120.30, 114.41, 55.19. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 356.1069. Found: 356.1057.



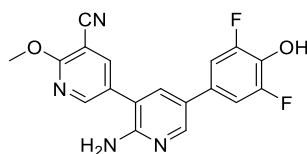
4-(6-amino-5-(4-fluoro-3-(2H-tetrazol-5-yl)phenyl)pyridin-3-yl)-2,6-difluorophenol

The compound **42** was isolated in 39% yield. ^1H NMR (400 MHz, DMSO) δ 10.11 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.05 (dd, J = 6.9, 2.1 Hz, 1H), 7.64 (d, J = 2.3 Hz, 1H), 7.42 (ddd, J = 17.1, 9.7, 6.0 Hz, 3H), 7.32 (dd, J = 10.2, 8.7 Hz, 1H), 5.78 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.07, 153.73 (d, J = 7.7 Hz), 151.81 (d, J = 7.7 Hz), 144.75, 135.28, 133.69, 132.13 (t, J = 16.4 Hz), 130.08, 129.17 (d, J = 7.9 Hz), 128.66 (t, J = 8.7 Hz), 123.19, 120.75 (d, J = 9.2 Hz), 116.77, 116.59, 108.81 (dd, J = 16.2, 6.4 Hz). HRMS (ES): calcd for $\text{C}_{18}\text{H}_{11}\text{F}_3\text{N}_6\text{O}$ $[\text{M}+\text{H}]^+$: 385.1025. Found: 385.1016.



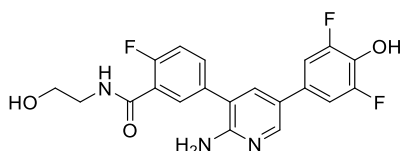
4-(6-amino-5-(isoquinolin-5-yl)pyridin-3-yl)-2,6-difluorophenol

The compound **43** was isolated in 58% yield. ^1H NMR (400 MHz, DMSO) δ 10.11 (s, 1H), 9.39 (s, 1H), 8.47 (d, J = 5.9 Hz, 1H), 8.43 (d, J = 2.5 Hz, 1H), 8.19 (dd, J = 7.6, 1.0 Hz, 1H), 7.82 – 7.73 (m, 2H), 7.69 (d, J = 2.4 Hz, 1H), 7.44 – 7.34 (m, 3H), 5.63 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 156.59, 153.75 (d, J = 7.6 Hz), 152.84, 151.83 (d, J = 7.8 Hz), 145.66, 143.25, 136.40, 134.37, 133.60, 132.36 – 131.82 (m), 128.71 (d, J = 21.4 Hz), 128.52 (d, J = 8.7 Hz), 127.83, 127.56, 122.52, 118.20, 117.24, 108.77 (dd, J = 16.2, 6.4 Hz). HRMS (ES): calcd for $\text{C}_{20}\text{H}_{13}\text{F}_2\text{N}_3\text{O}$ $[\text{M}+\text{H}]^+$: 350.1105. Found: 350.1095.



2'-amino-5'-(3,5-difluoro-4-hydroxyphenyl)-6-methoxy-[3,3'-bipyridine]-5-carbonitrile

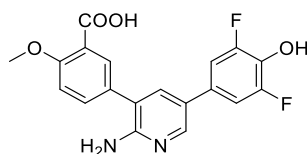
The compound **44** was isolated in 42% yield. ^1H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 8.57 (d, J = 2.4 Hz, 1H), 8.38 (d, J = 2.4 Hz, 1H), 8.33 (d, J = 2.4 Hz, 1H), 7.70 (d, J = 2.4 Hz, 1H), 7.44 – 7.35 (m, 2H), 6.06 (s, 2H), 4.05 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 162.57, 156.41, 153.75 (d, J = 7.9 Hz), 152.17 – 151.54 (m), 145.59, 144.34, 135.94, 132.27 (t, J = 14.3 Hz), 128.32 (t, J = 8.6 Hz), 127.42, 122.97, 115.35, 115.03, 108.74 (dd, J = 16.2, 6.5 Hz), 95.57, 54.58. HRMS (ES): calcd for $\text{C}_{18}\text{H}_{12}\text{F}_2\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$: 355.1007. Found: 355.1017.



5-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-2-fluoro-N-(2-hydroxyethyl)benzamide

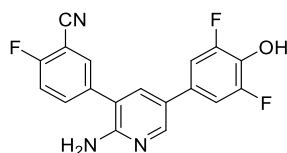
The compound **45** was isolated in 77% yield. ^1H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 8.33 (t, J = 4.9 Hz, 1H), 8.30 (d, J = 2.4 Hz, 1H), 7.72 (dd, J = 6.9, 2.3 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.43 – 7.33 (m, 3H), 5.92 (s, 2H), 4.77 (s, 1H), 3.52 (s, 2H), 3.35 (d, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 163.76, 159.61, 157.62, 155.95, 153.75 (d, J = 7.7 Hz), 151.83 (d, J = 7.6 Hz), 145.12,

135.59, 133.99, 132.71 (d, $J = 8.6$ Hz), 132.20 (t, $J = 16.5$ Hz), 130.11, 128.60 (t, $J = 8.8$ Hz), 124.41 (d, $J = 14.3$ Hz), 123.16, 118.66, 116.84, 116.66, 109.34 – 108.61 (m), 59.66, 42.09. HRMS (ES): calcd for $C_{20}H_{16}F_3N_3O_3$ $[M+H]^+$: 404.1222. Found: 404.1231.



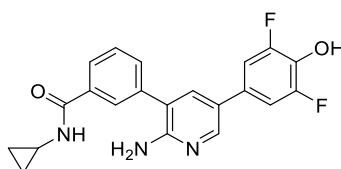
5-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-2-methoxybenzoic acid

The compound **46** was isolated in 31% yield. 1H NMR (400 MHz, DMSO) δ 8.24 (d, $J = 2.1$ Hz, 1H), 7.55 (s, 2H), 7.47 (d, $J = 7.1$ Hz, 1H), 7.36 (d, $J = 9.4$ Hz, 2H), 7.10 (d, $J = 8.7$ Hz, 1H), 5.68 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 169.07, 156.97, 156.49, 154.30 (d, $J = 7.8$ Hz), 152.38 (d, $J = 7.9$ Hz), 144.83, 135.52, 131.37, 130.22, 129.73, 129.02 – 128.69 (m), 123.82, 120.33, 113.10, 109.24 (dd, $J = 16.2, 6.1$ Hz), 56.20. HRMS (ES): calcd for $C_{19}H_{14}F_2N_2O_4$ $[M+H]^+$: 373.1000. Found: 373.0995.



5-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-2-fluorobenzonitrile

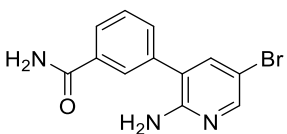
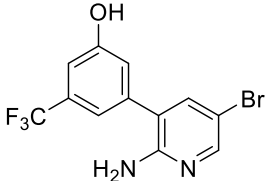
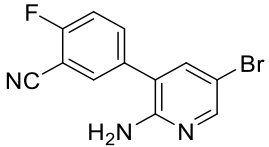
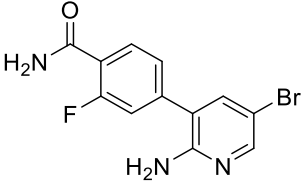
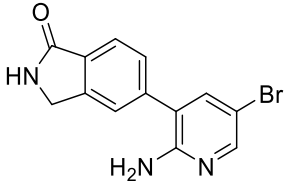
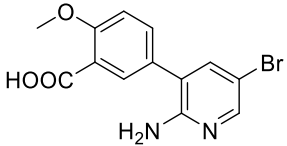
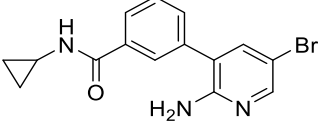
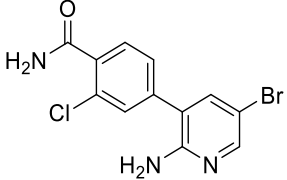
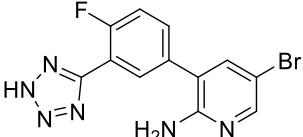
The compound **47** was isolated in 59% yield. 1H NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 8.33 (d, $J = 2.4$ Hz, 1H), 8.06 (dd, $J = 6.3, 2.3$ Hz, 1H), 7.92 (ddd, $J = 8.5, 5.3, 2.3$ Hz, 1H), 7.68 (d, $J = 2.4$ Hz, 1H), 7.61 (t, $J = 9.1$ Hz, 1H), 7.45 – 7.36 (m, 2H), 6.01 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 162.71, 160.68, 155.98, 153.75 (d, $J = 7.7$ Hz), 151.83 (d, $J = 7.8$ Hz), 145.56, 136.67 (d, $J = 8.6$ Hz), 135.82, 135.40 (d, $J = 3.4$ Hz), 134.37, 132.27 (t, $J = 16.2$ Hz), 128.37 (t, $J = 8.8$ Hz), 123.09, 117.34, 116.91 (d, $J = 19.5$ Hz), 114.11, 108.81 (dd, $J = 16.2, 6.4$ Hz), 100.63 (d, $J = 15.4$ Hz). HRMS (ES): calcd for $C_{18}H_{10}F_3N_3O$ $[M+H]^+$: 342.0849. Found: 342.0835.

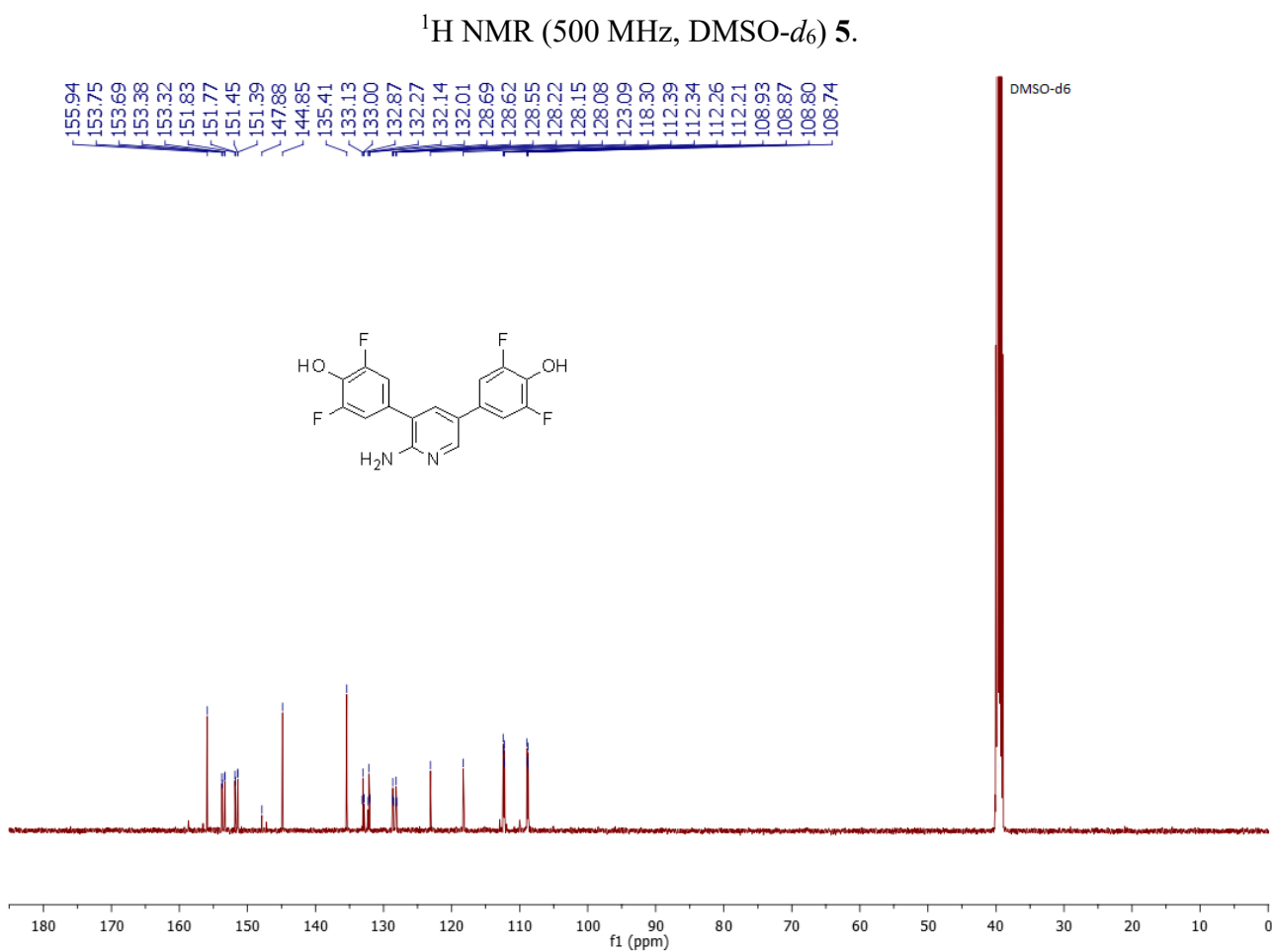
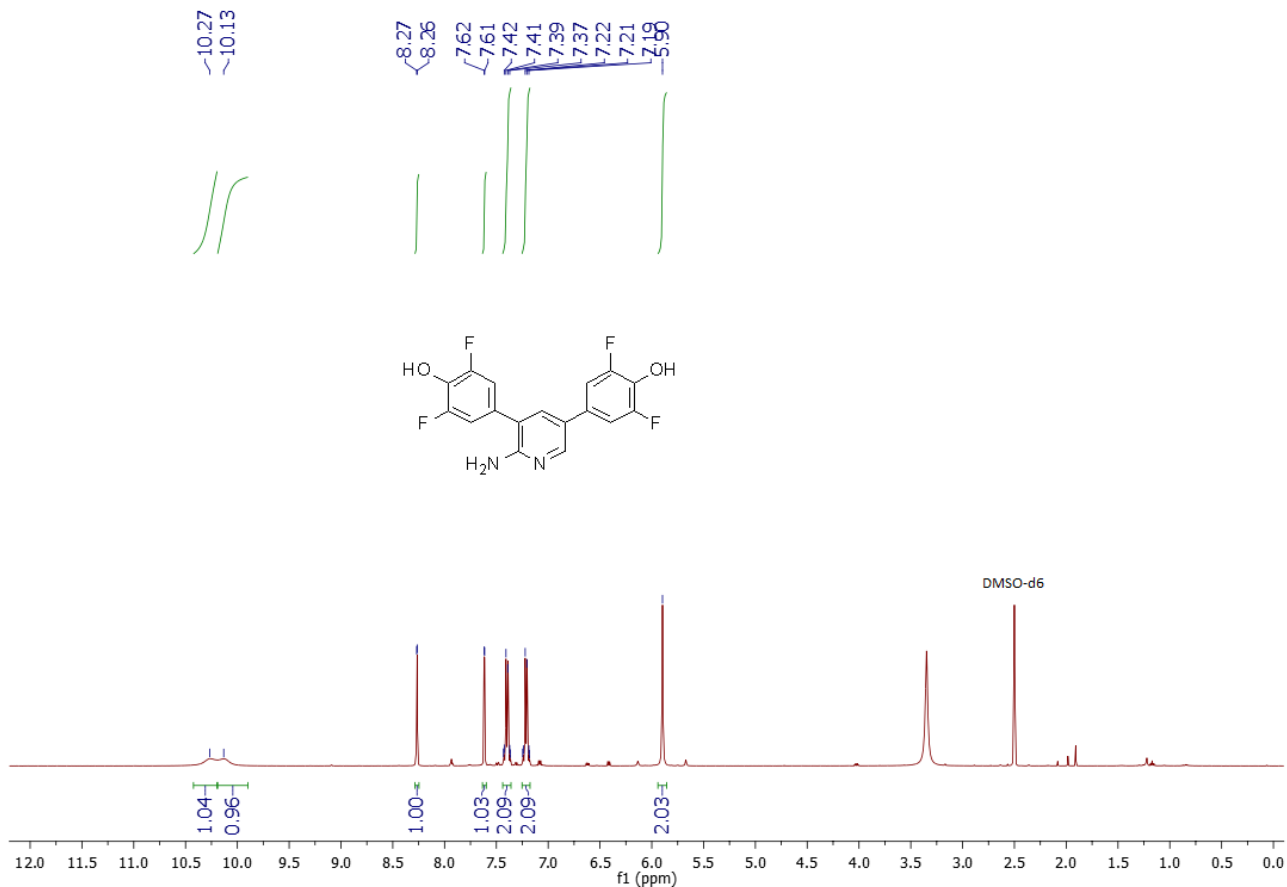


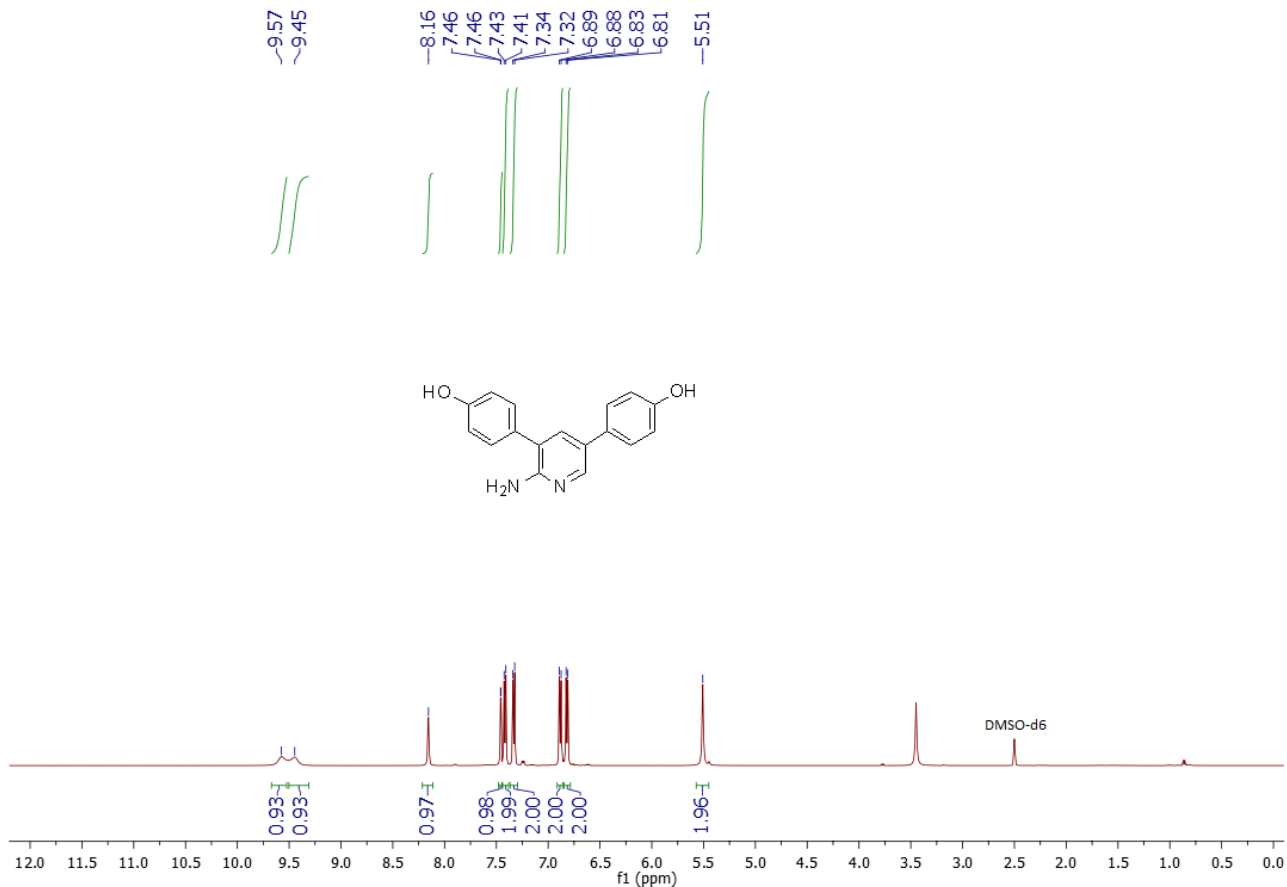
3-(2-amino-5-(3,5-difluoro-4-hydroxyphenyl)pyridin-3-yl)-N-cyclopropylbenzamide

The compound **48** was isolated in 49% yield. 1H NMR (400 MHz, DMSO) δ 10.14 (s, 1H), 8.48 (d, $J = 4.1$ Hz, 1H), 8.31 (d, $J = 2.4$ Hz, 1H), 7.91 (s, 1H), 7.82 (d, $J = 7.8$ Hz, 1H), 7.68 – 7.61 (m, 2H), 7.55 (t, $J = 7.7$ Hz, 1H), 7.43 – 7.33 (m, 2H), 5.83 (s, 2H), 2.90 – 2.81 (m, 1H), 0.70 (td, $J = 7.0, 4.8$ Hz, 2H), 0.61 – 0.52 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.39, 155.92, 153.73 (d, $J = 7.6$ Hz), 151.81 (d, $J = 7.7$ Hz), 145.02, 137.80, 135.54, 135.06, 132.16 (t, $J = 16.2$ Hz), 131.35, 128.87, 128.62 (t, $J = 8.7$ Hz), 127.29, 126.46, 123.16, 119.70, 108.82 (dd, $J = 16.1, 6.6$ Hz), 23.06, 5.76. HRMS (ES): calcd for $C_{21}H_{17}F_2N_3O_2$ $[M+H]^+$: 382.1367. Found: 382.1353.

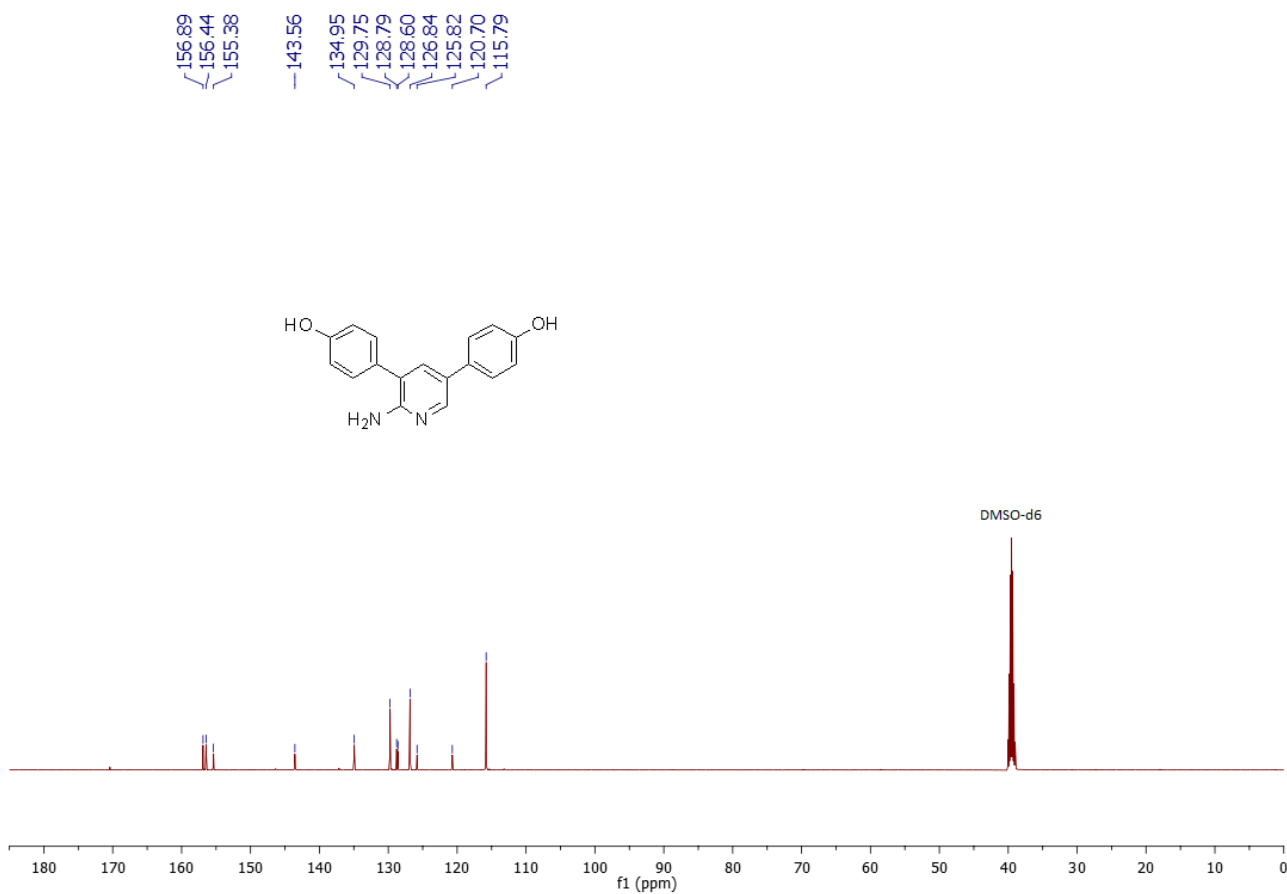
Cmpd ID	Structure	HRMS (ES)
4a		calcd for C ₁₁ H ₉ BrN ₂ O [M+H] ⁺ : 264.9977. Found: 264.9987.
4b		calcd for C ₁₂ H ₉ BrN ₂ O ₂ [M+H] ⁺ : 292.9926. Found: 292.9932.
4c		calcd for C ₁₂ H ₉ BrN ₄ O [M+H] ⁺ : 305.0038. Found: 305.0034.
4d		calcd for C ₁₄ H ₁₃ BrFN ₃ O ₂ [M+H] ⁺ : 354.0253. Found: 354.0261.
4e		calcd for C ₁₂ H ₁₀ BrN ₃ O [M+H] ⁺ : 292.0085. Found: 292.0097.
4f		calcd for C ₁₂ H ₈ BrFN ₂ O ₂ [M+H] ⁺ : 310.9831. Found: 310.9834.
4g		calcd for C ₁₂ H ₉ BrFN ₃ O [M+H] ⁺ : 309.9991. Found: 309.9989.
4h		calcd for C ₁₂ H ₁₁ BrN ₂ O [M+H] ⁺ : 279.0128. Found: 279.0117.
4i		calcd for C ₁₄ H ₁₀ BrN ₃ [M+H] ⁺ : 300.0136. Found: 300.0130.
4j		calcd for C ₁₂ H ₈ BrN ₃ O ₄ [M+H] ⁺ : 337.9776. Found: 337.9777.
4k		calcd for C ₁₂ H ₉ BrN ₄ [M+H] ⁺ : 289.0089. Found: 289.0099.

4l		calcd for C ₁₂ H ₁₀ BrN ₃ O [M+H] ⁺ : 292.0085. Found: 292.0097.
4m		calcd for C ₁₂ H ₈ BrF ₃ N ₂ O [M+H] ⁺ : 332.9850. Found: 332.9847.
4n		calcd for C ₁₂ H ₇ BrFN ₃ [M+H] ⁺ : 291.9886. Found: 291.9887.
4o		calcd for C ₁₂ H ₉ BrFN ₃ O [M+H] ⁺ : 309.9991. Found: 309.9989.
4p		calcd for C ₁₃ H ₁₀ BrN ₃ O [M+H] ⁺ : 304.0085. Found: 304.0070.
4q		calcd for C ₁₃ H ₁₁ BrN ₂ O ₃ [M+H] ⁺ : 323.0031. Found: 323.0036.
4r		calcd for C ₁₅ H ₁₄ BrN ₃ O [M+H] ⁺ : 332.0398. Found: 332.0405.
4s		calcd for C ₁₂ H ₉ BrClN ₃ O [M+H] ⁺ : 325.9690. Found: 325.9676.
4t		calcd for C ₁₂ H ₈ BrFN ₆ [M+H] ⁺ : 335.0056. Found: 335.0044.

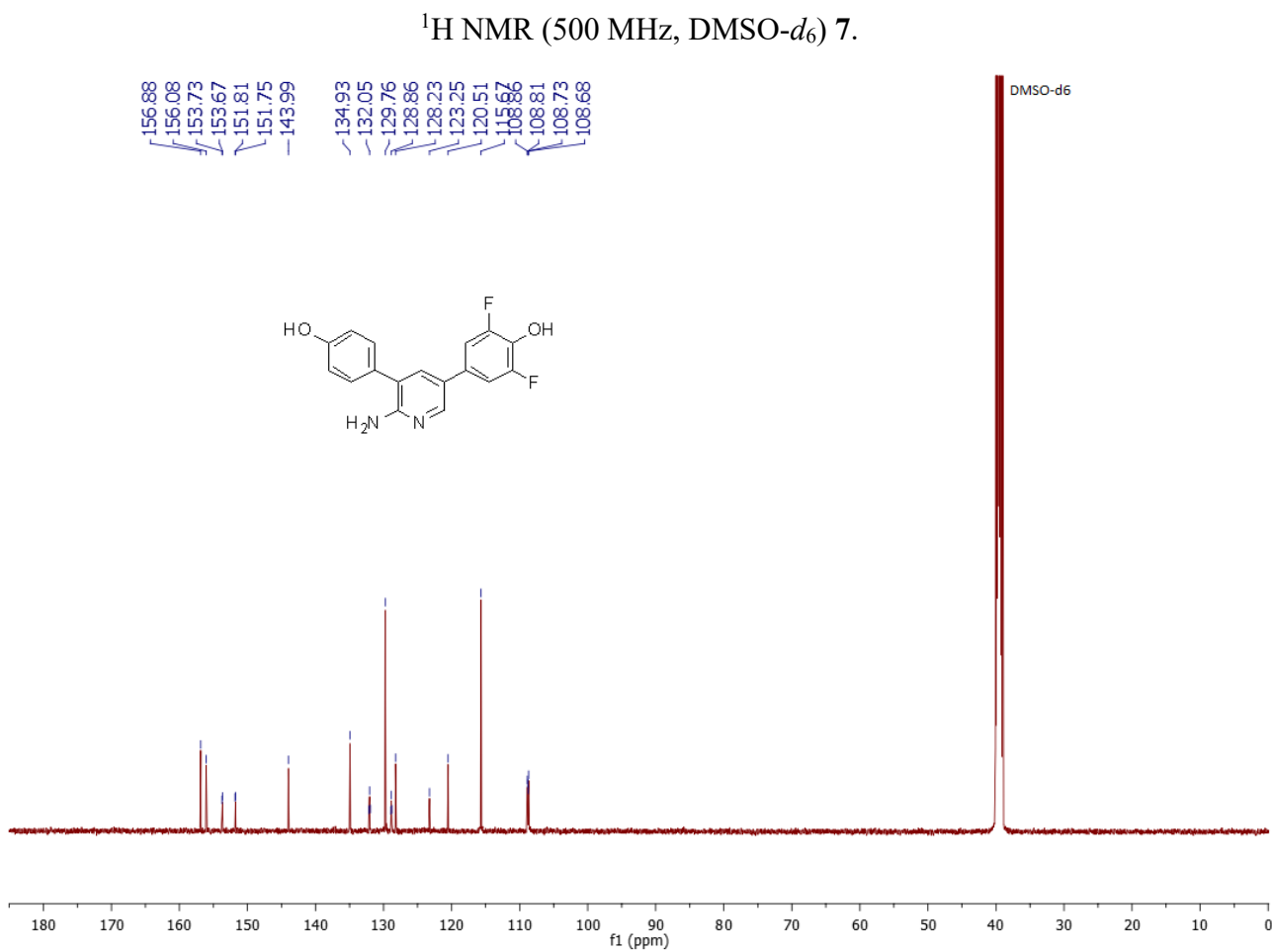
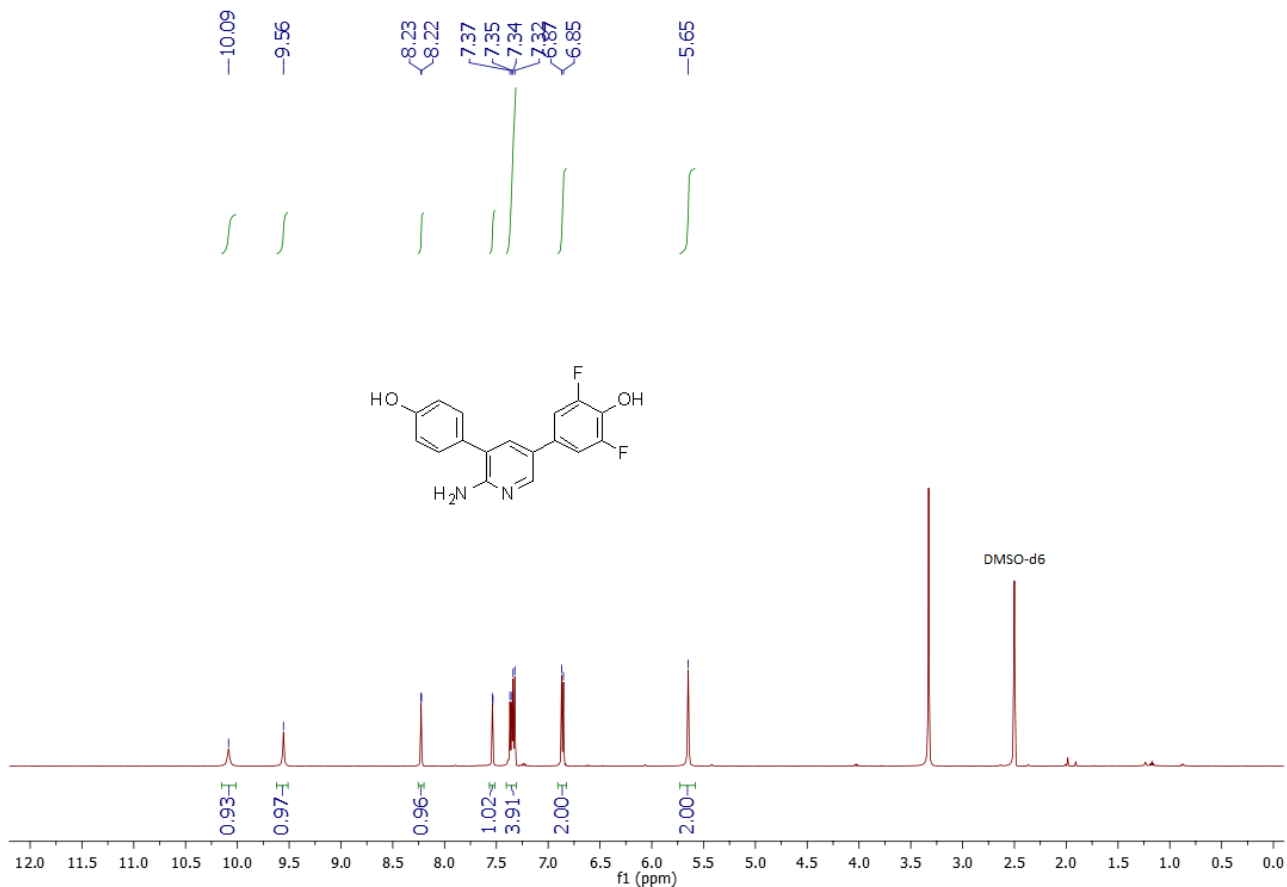


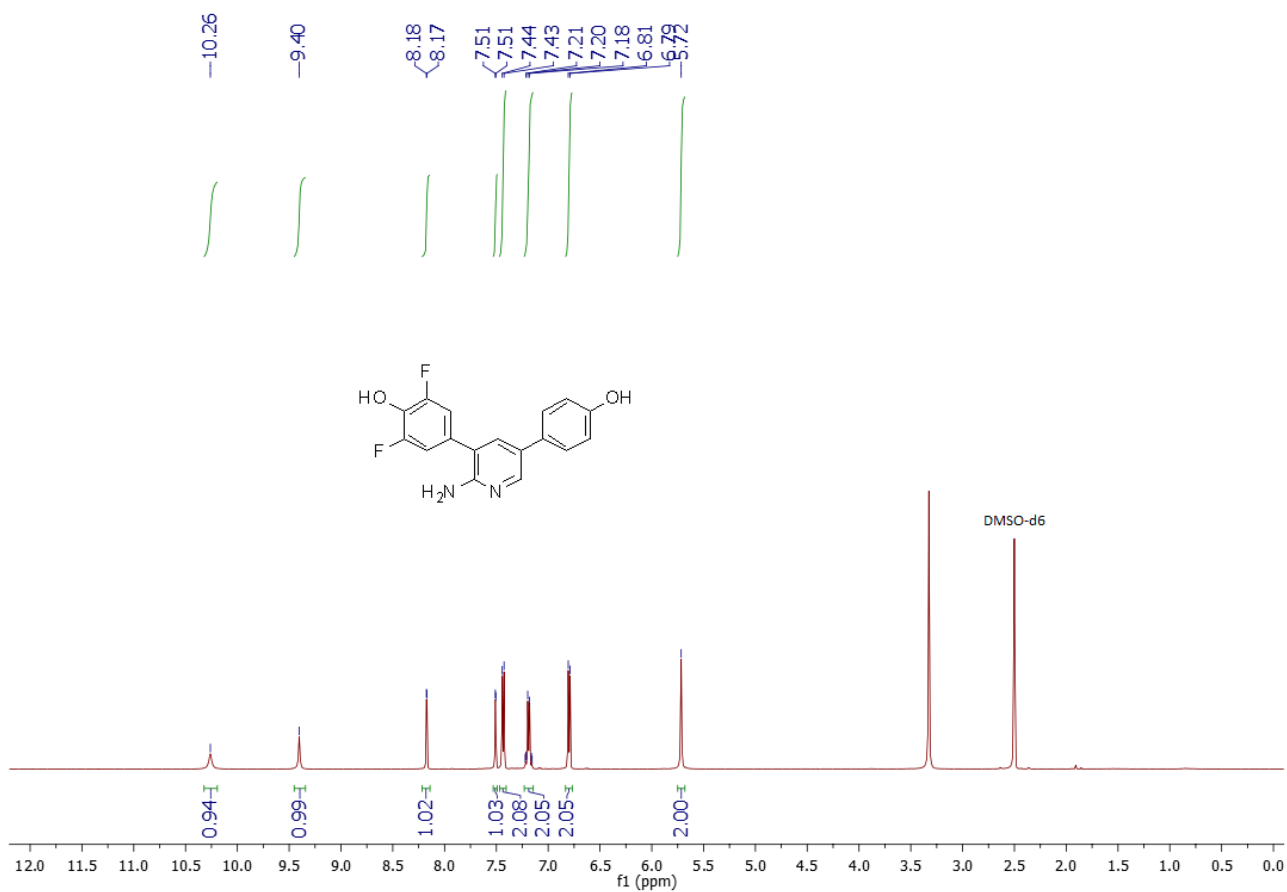


¹H NMR (500 MHz, DMSO-*d*₆) 6.

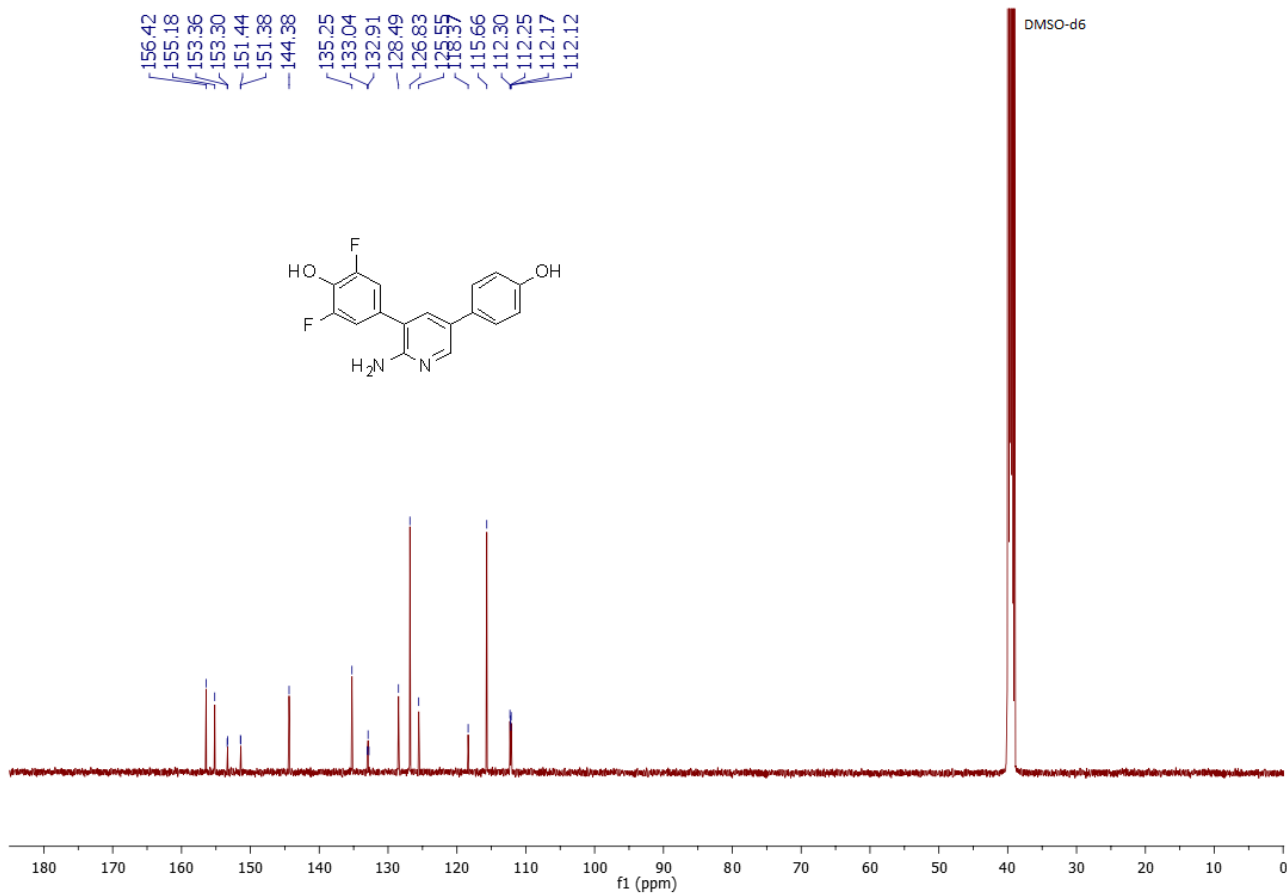


¹³C NMR (126 MHz, DMSO-*d*₆) 6.

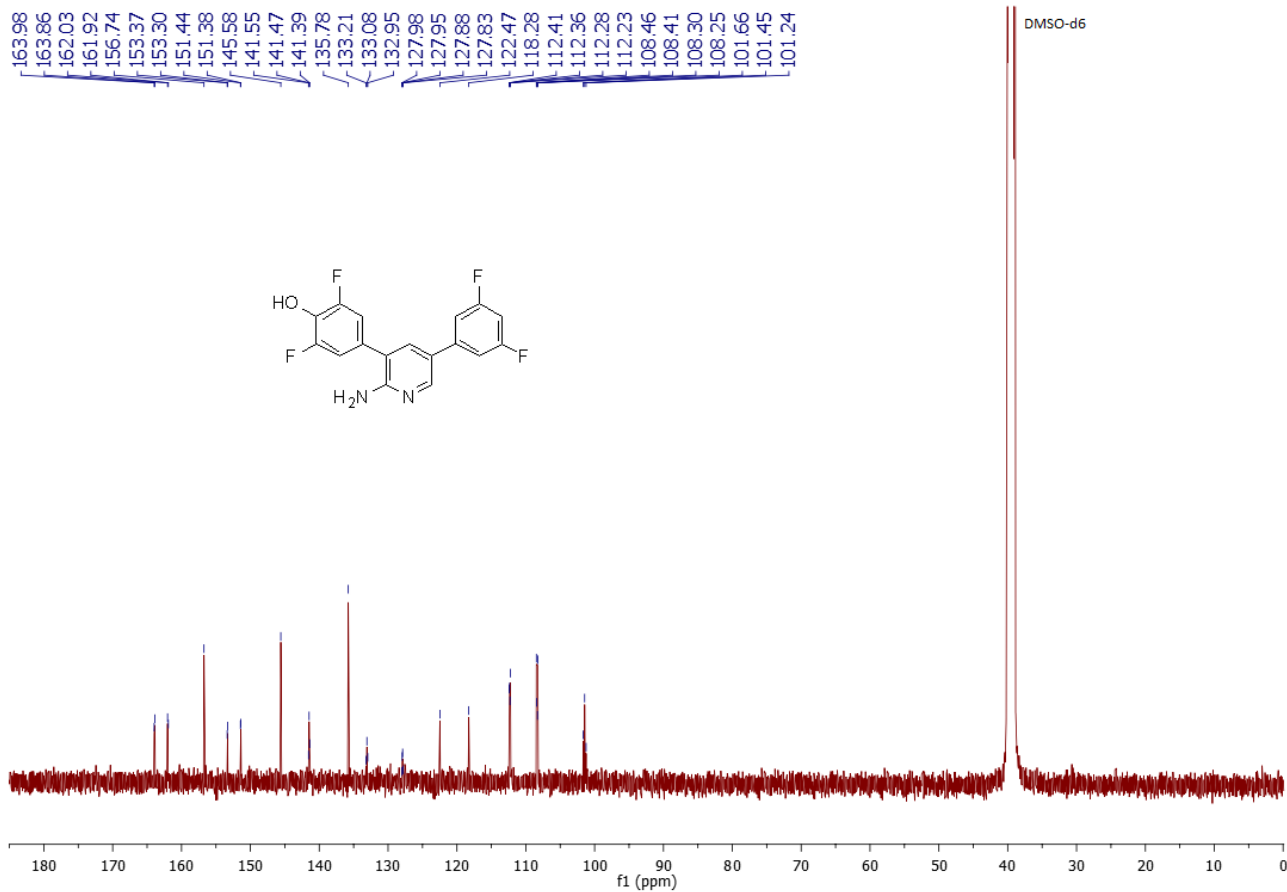
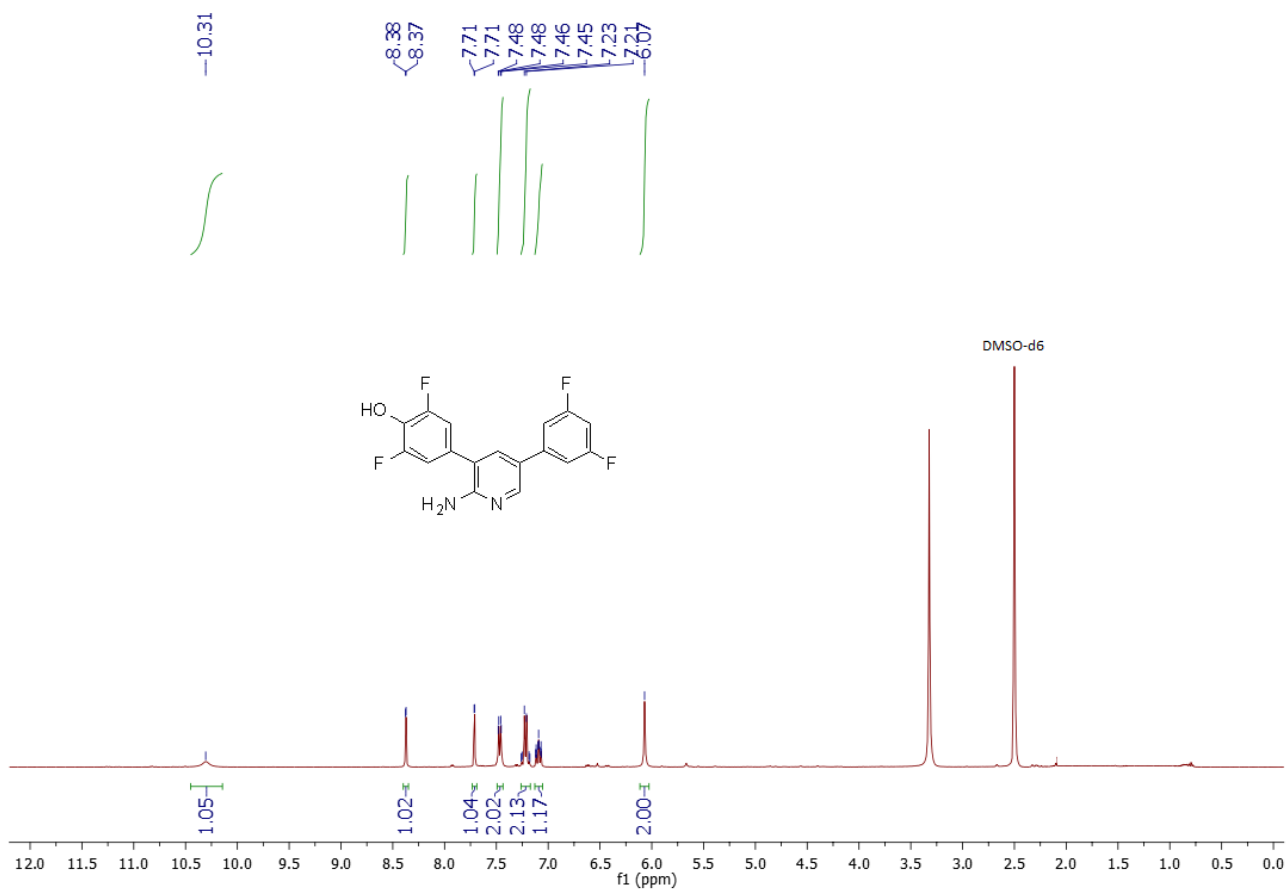


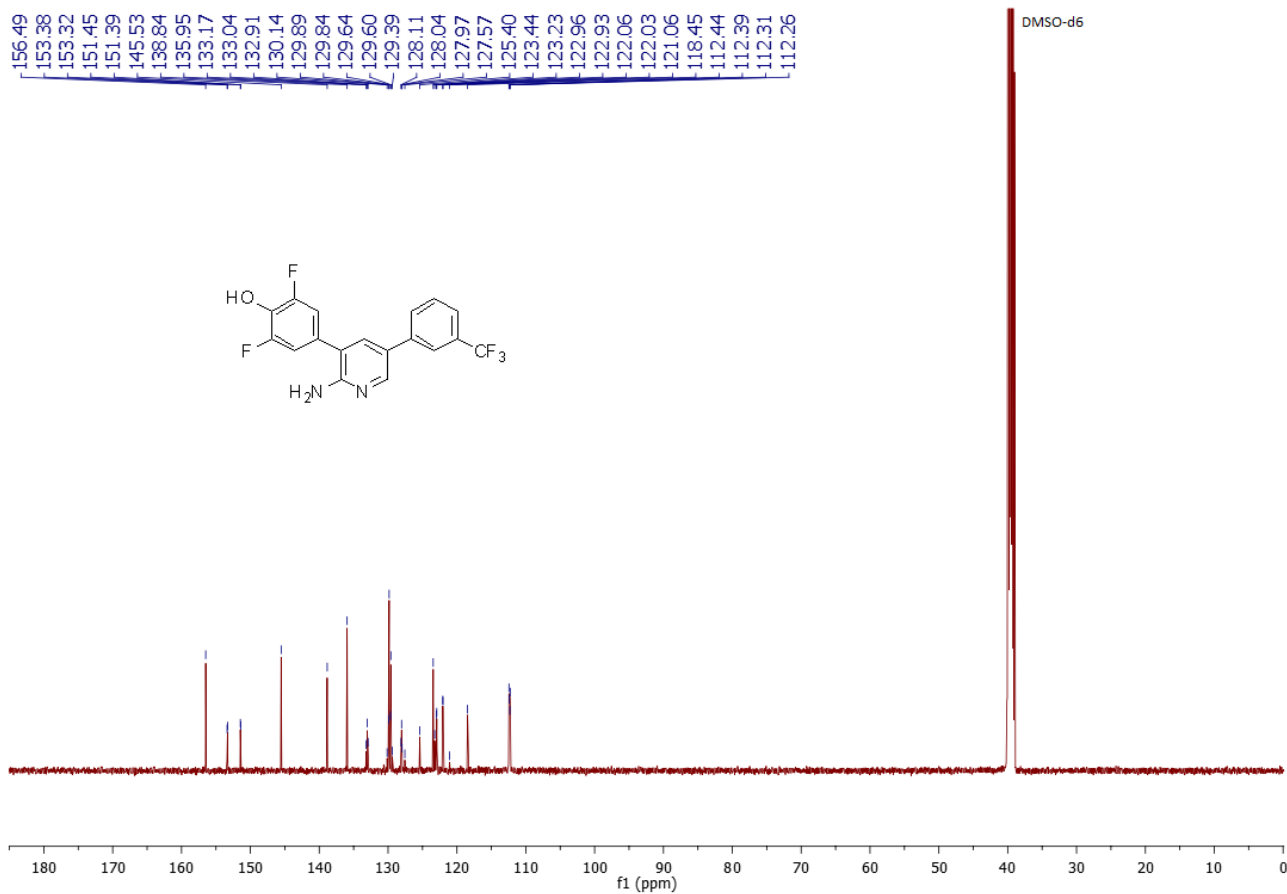
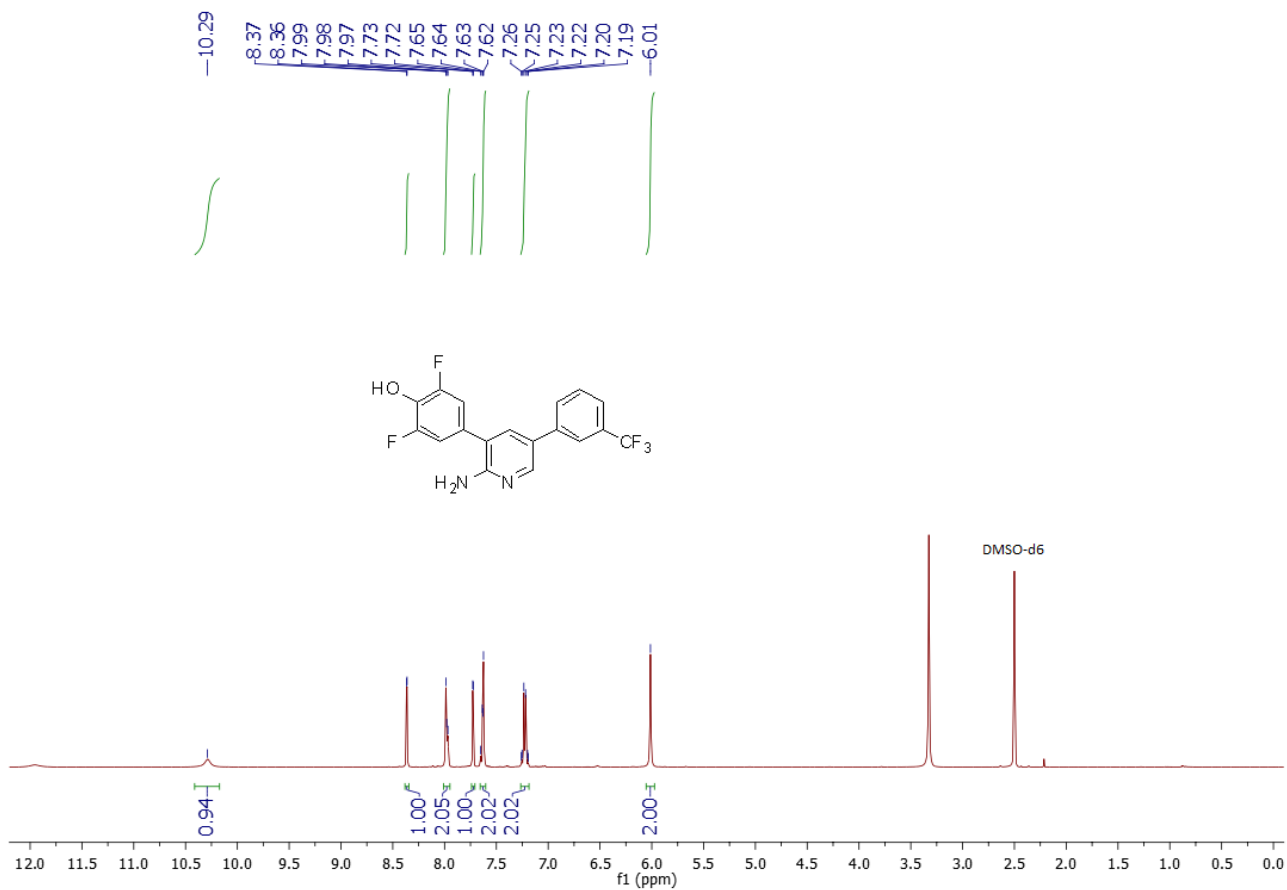


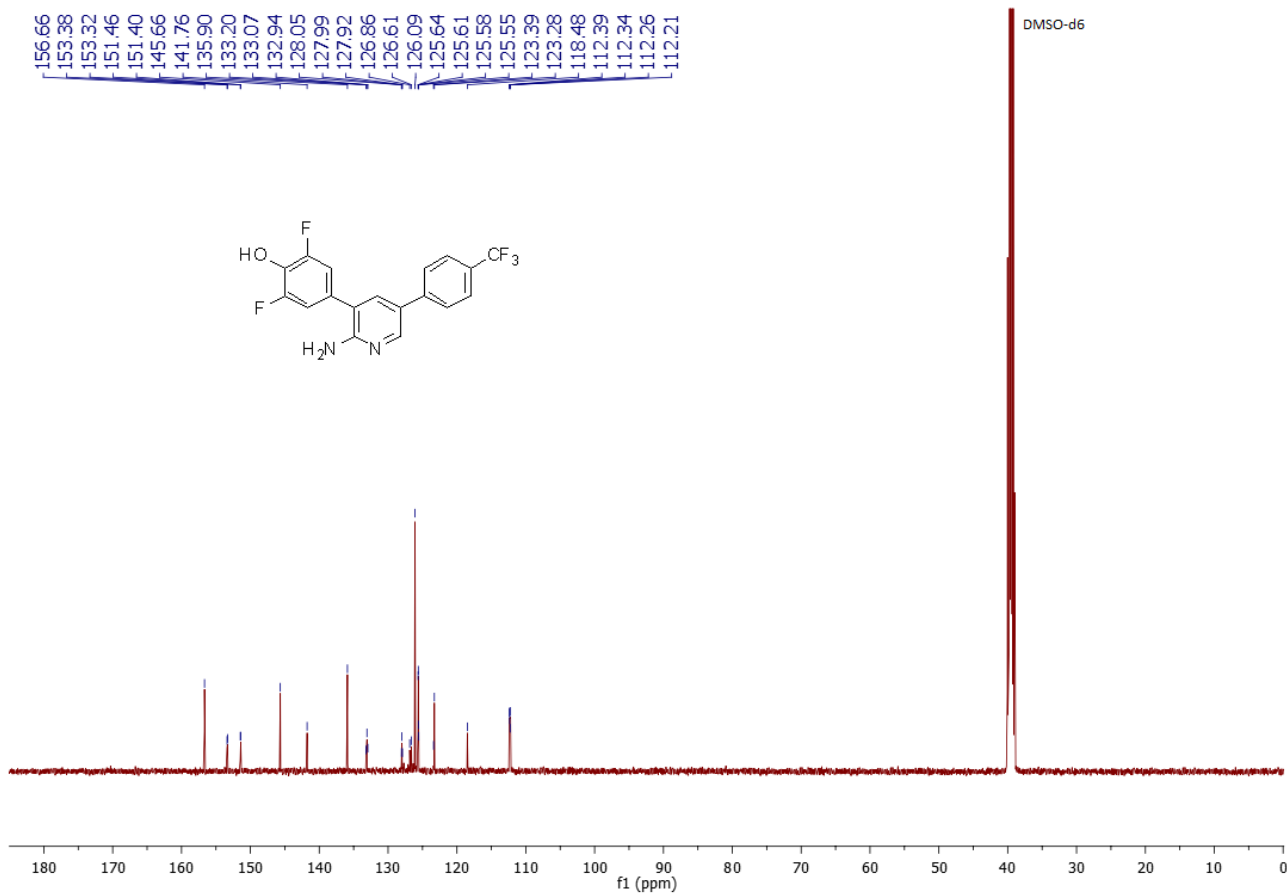
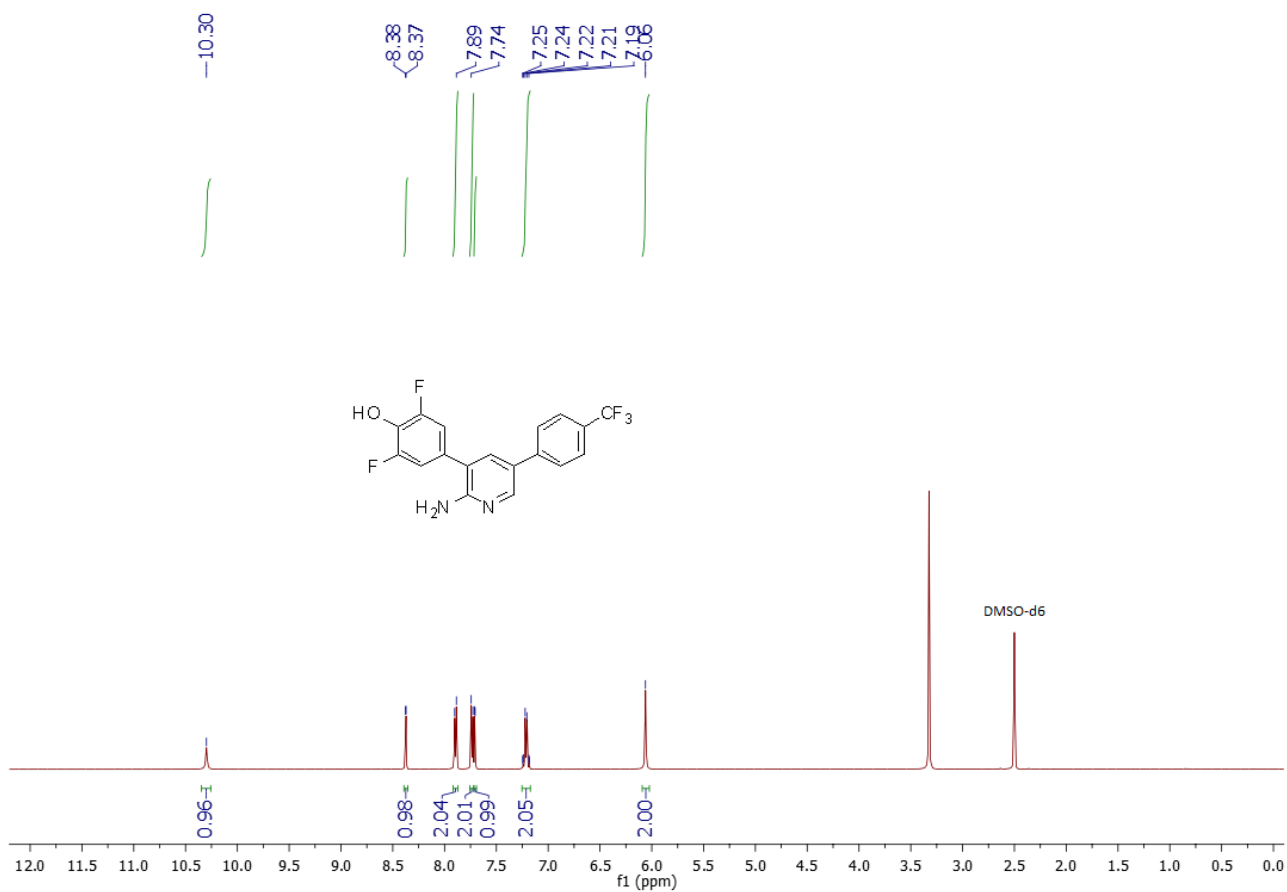
¹H NMR (500 MHz, DMSO-*d*₆) 8.

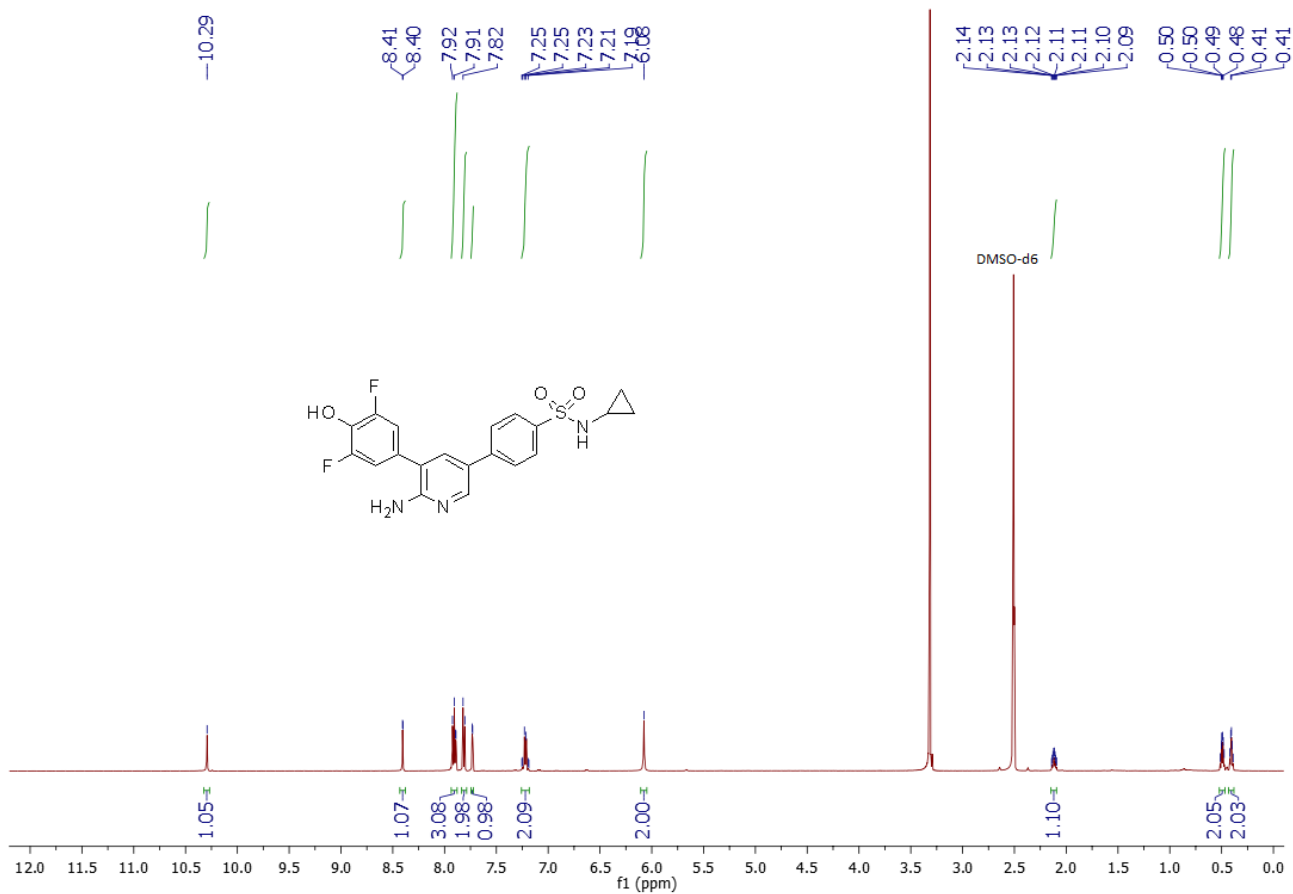


¹³C NMR (126 MHz, DMSO-*d*₆) 8.

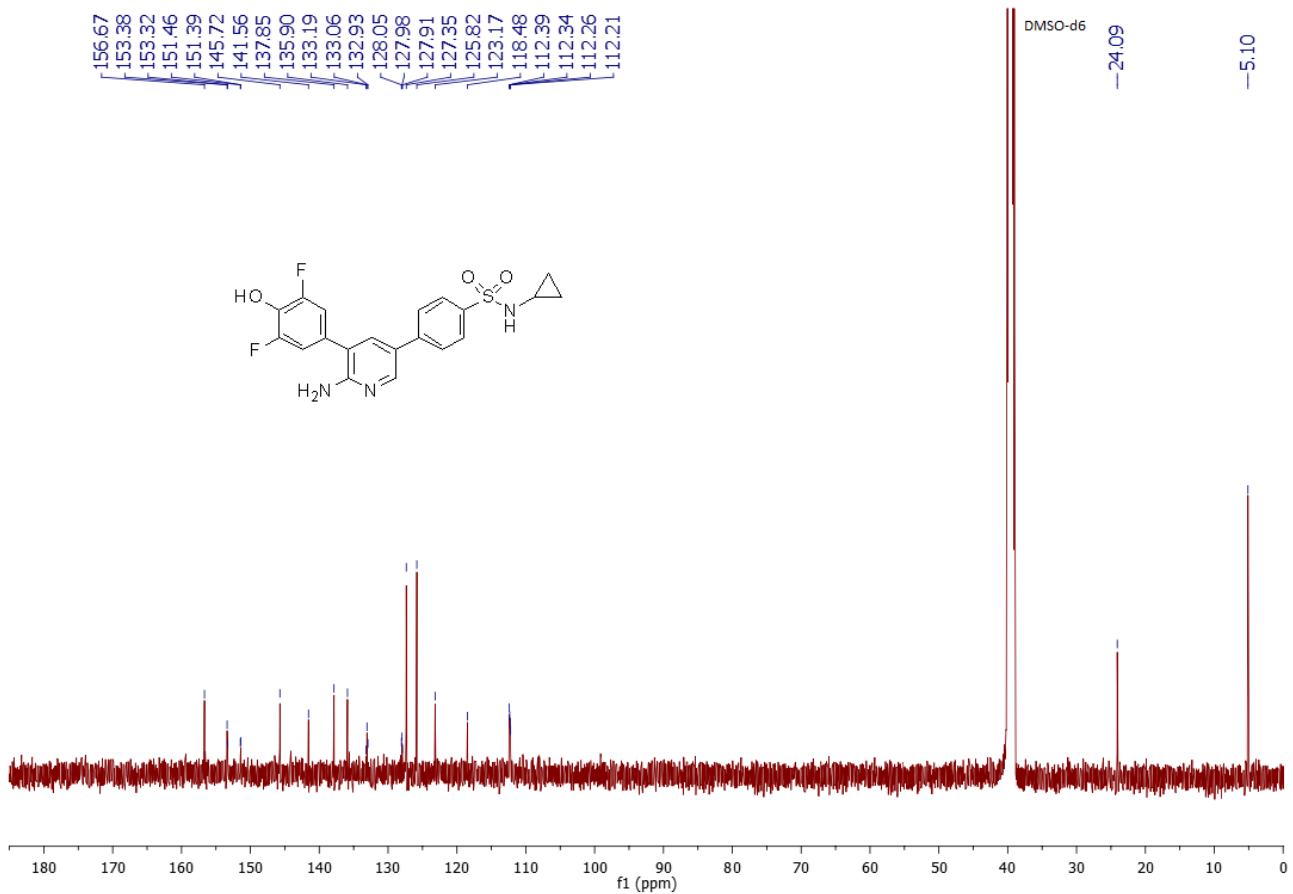




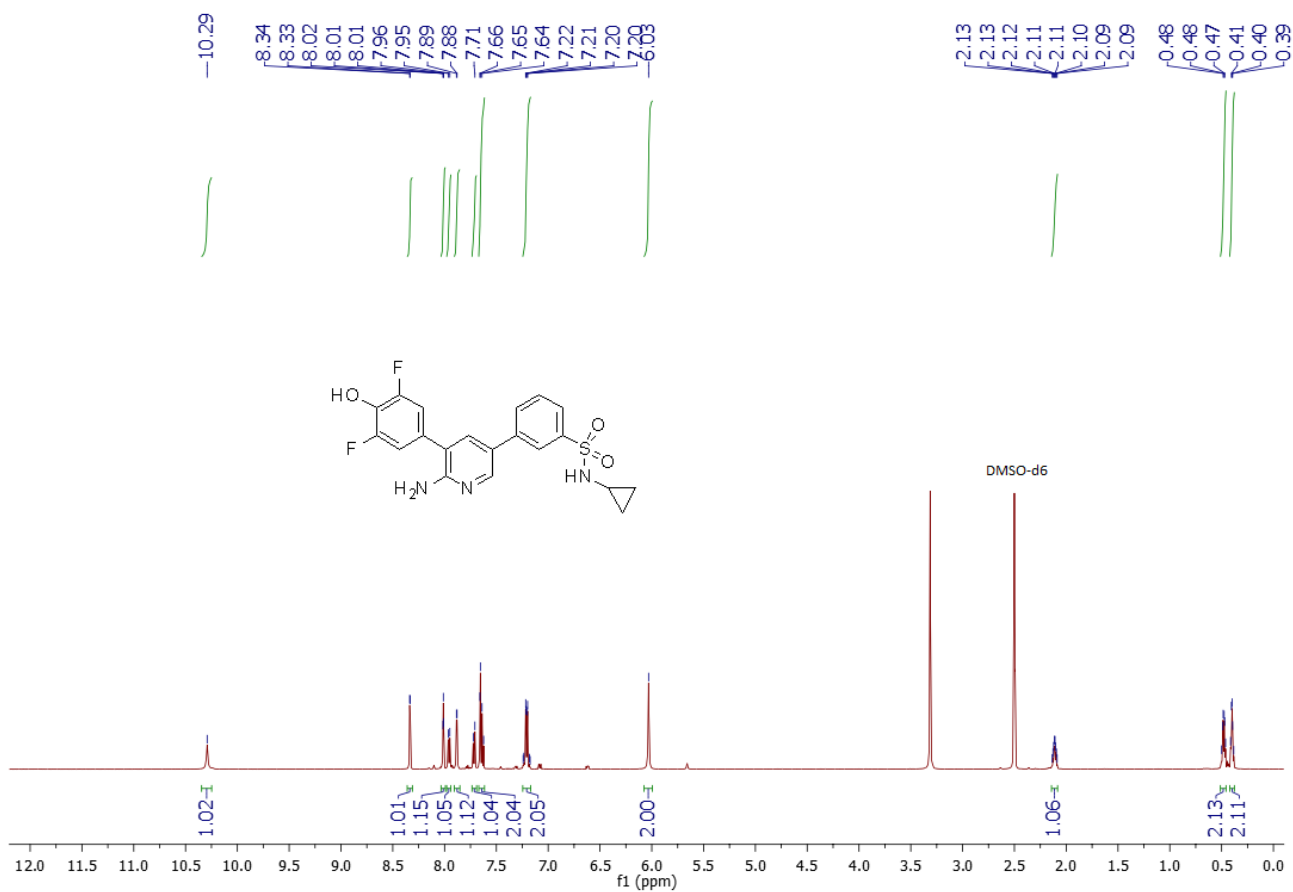




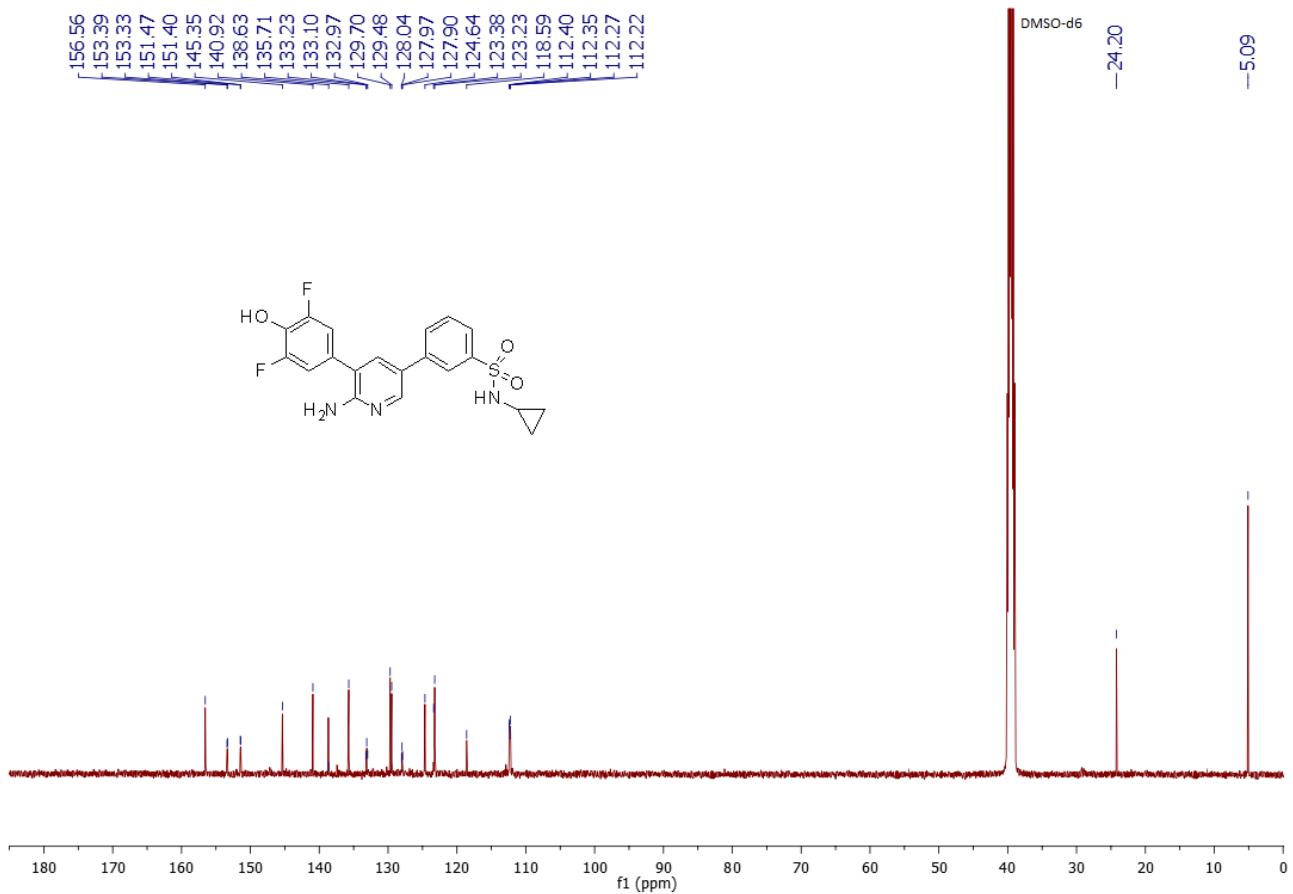
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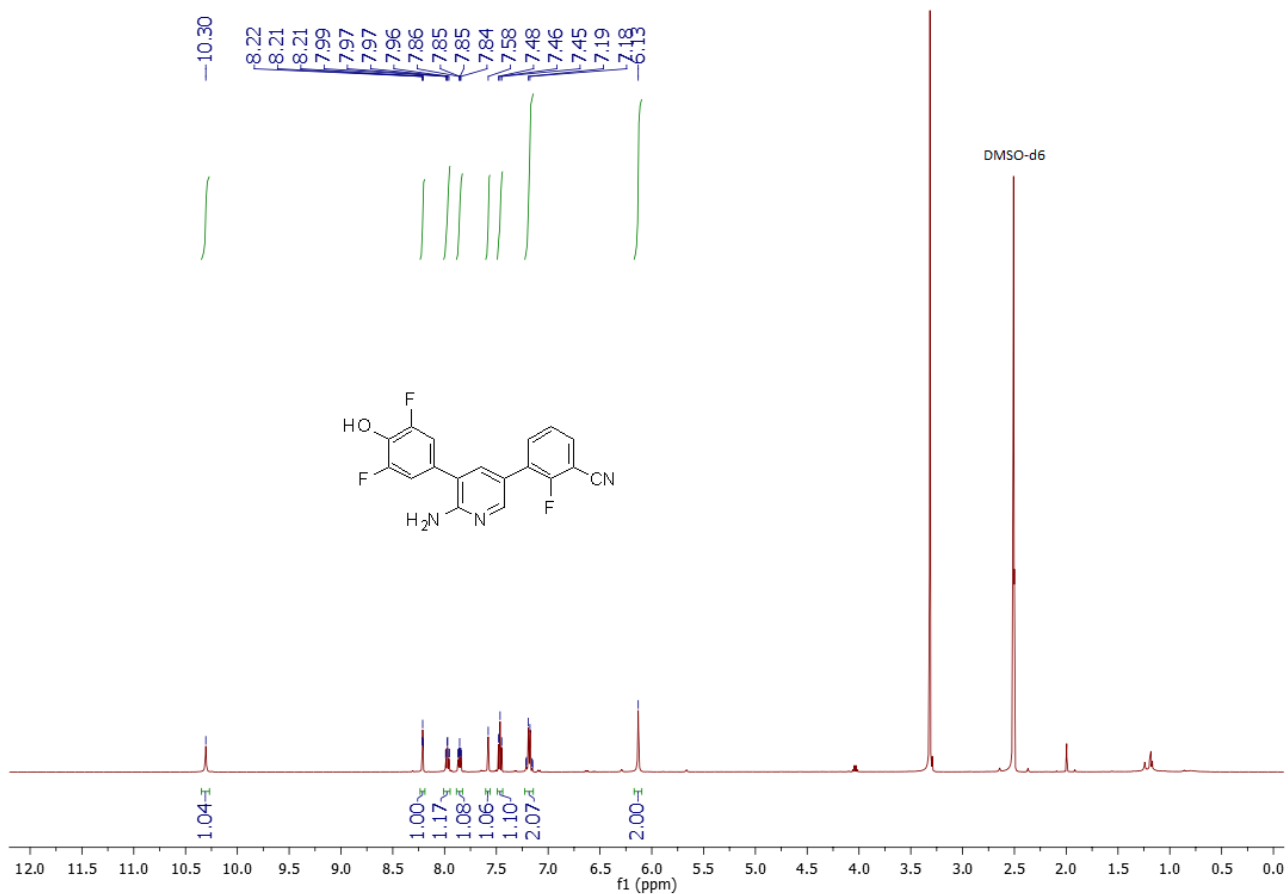
¹³C NMR (126 MHz, DMSO-*d*₆) 12.



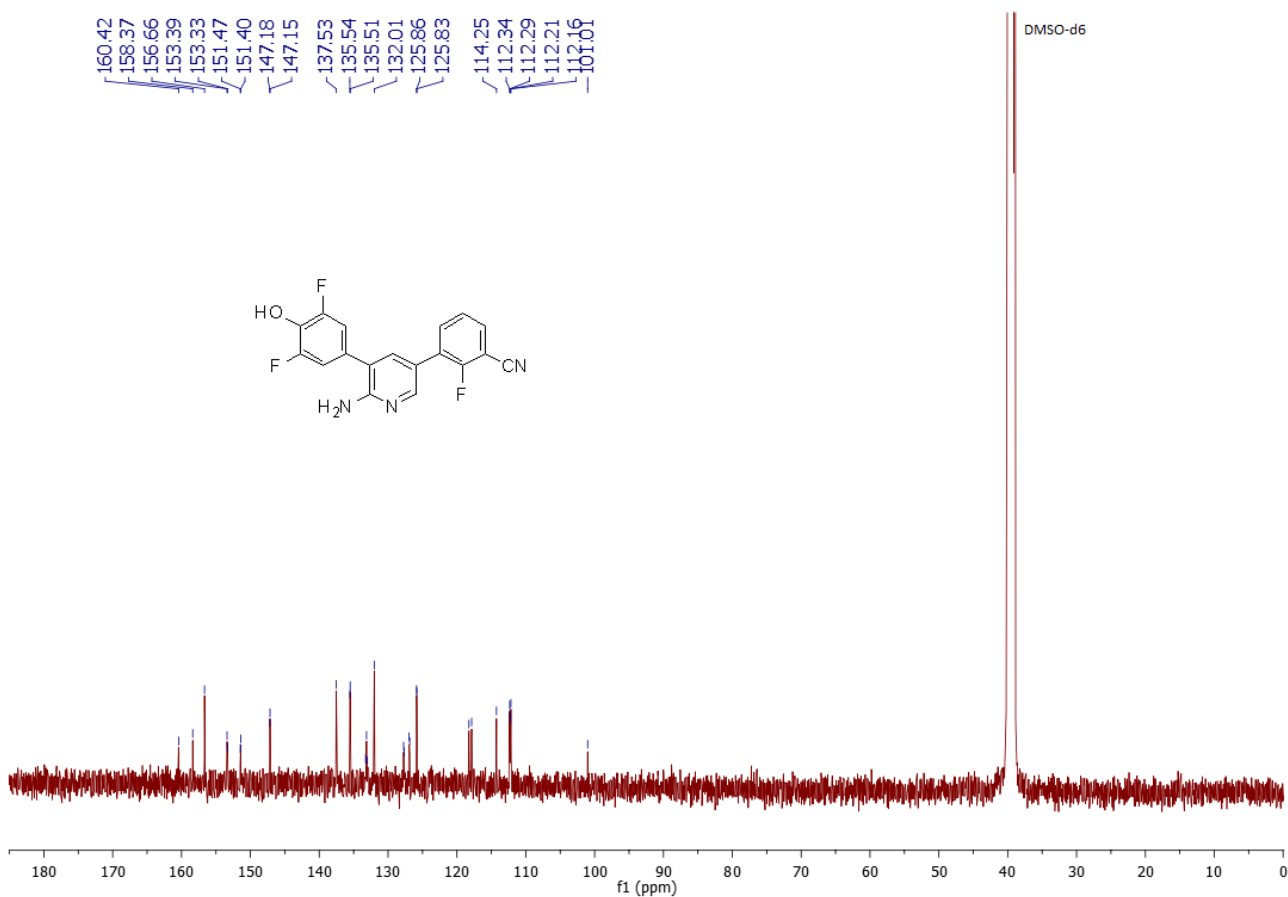
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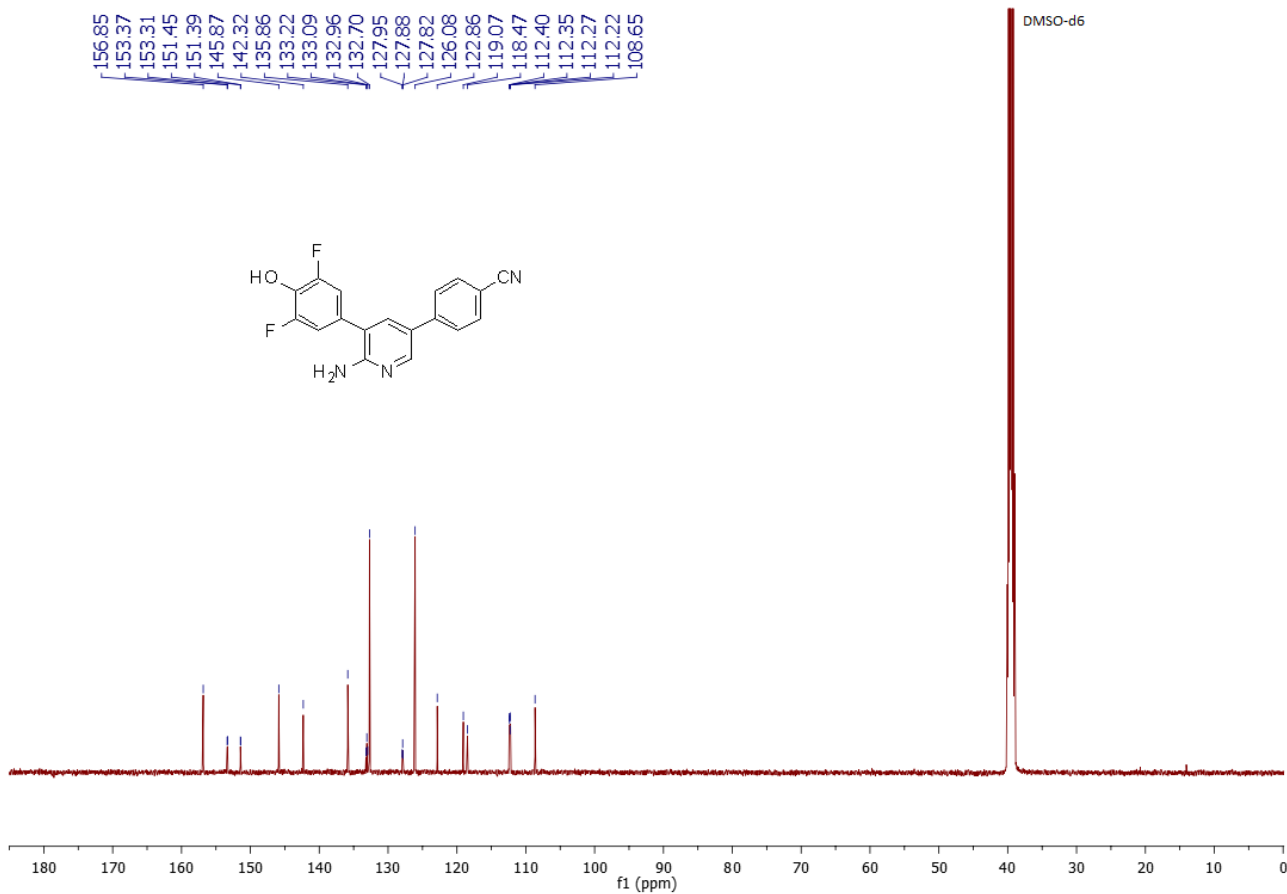
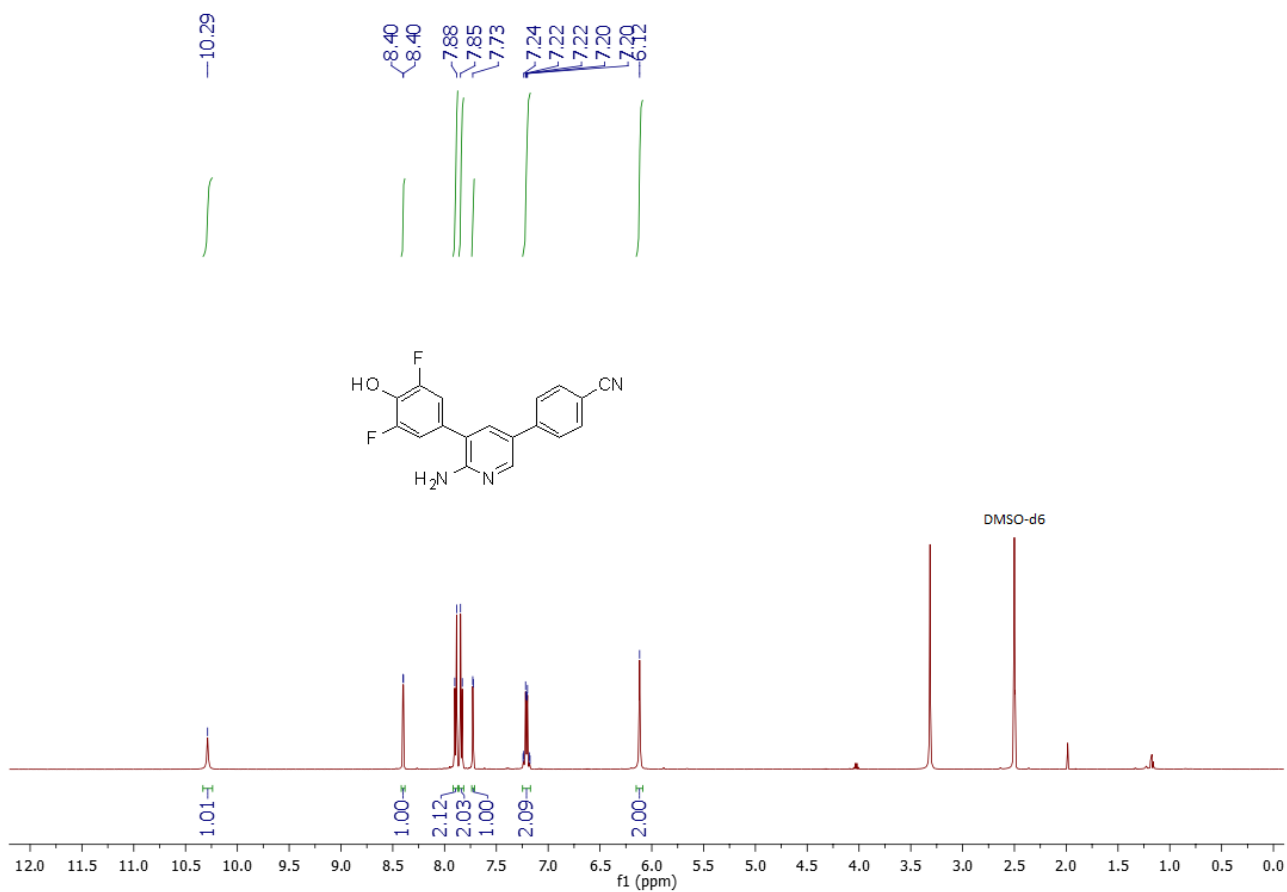
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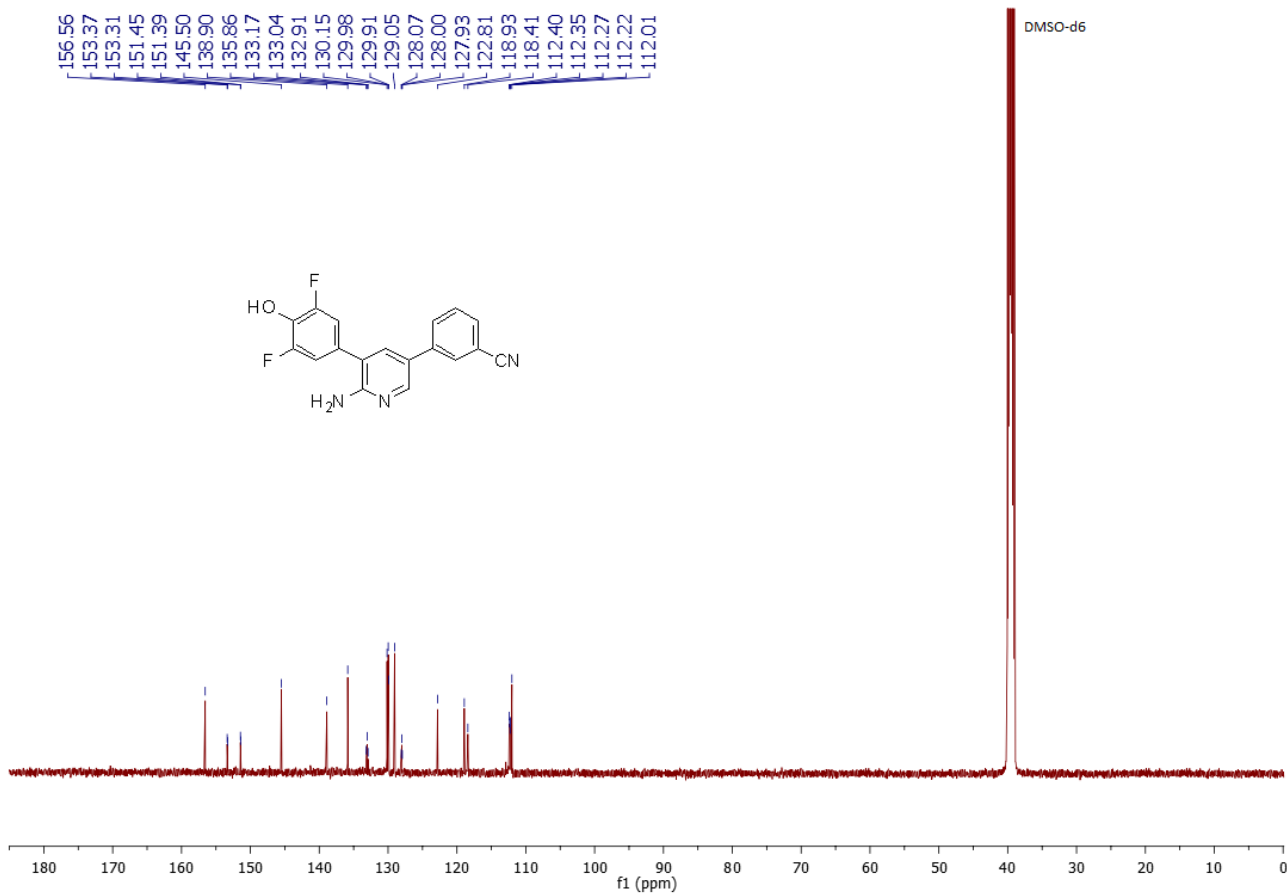
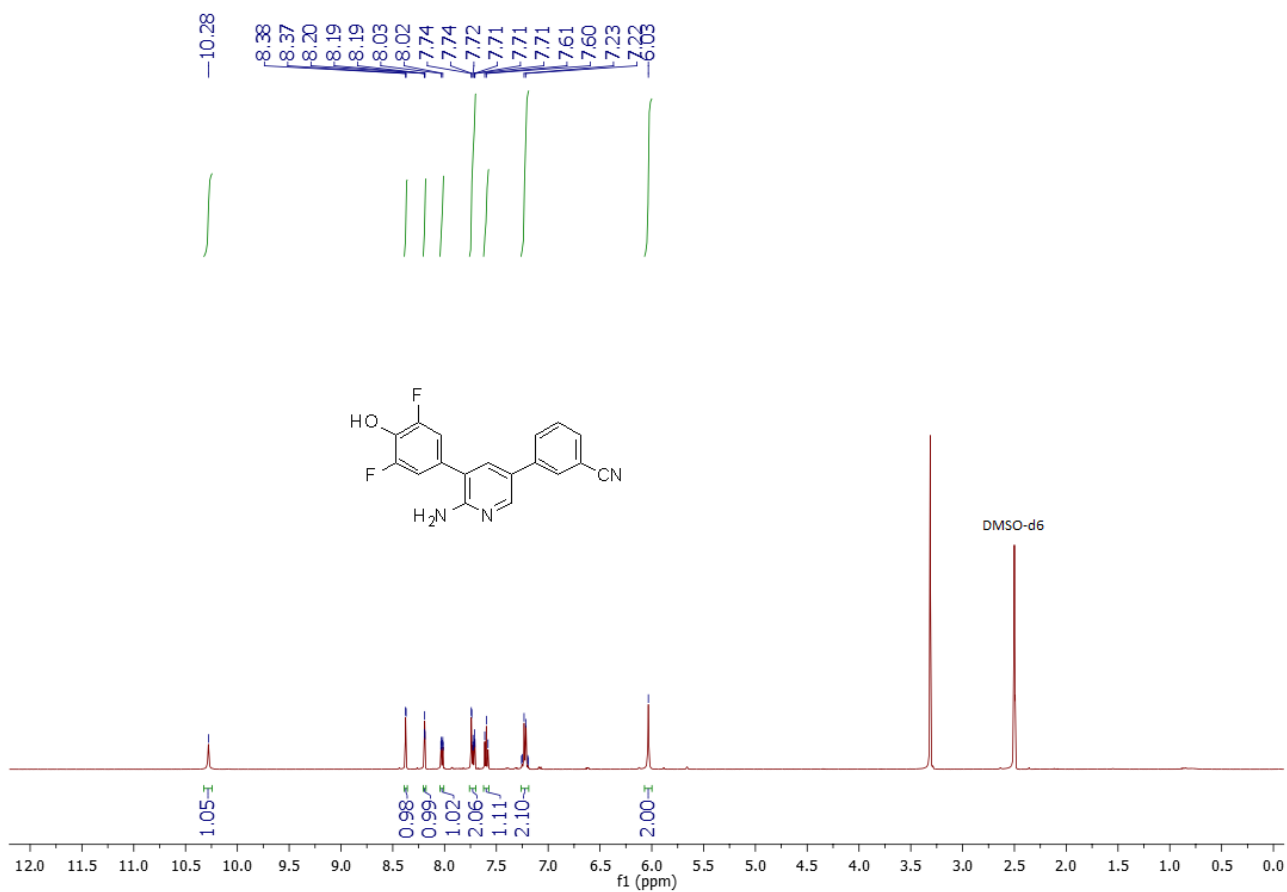


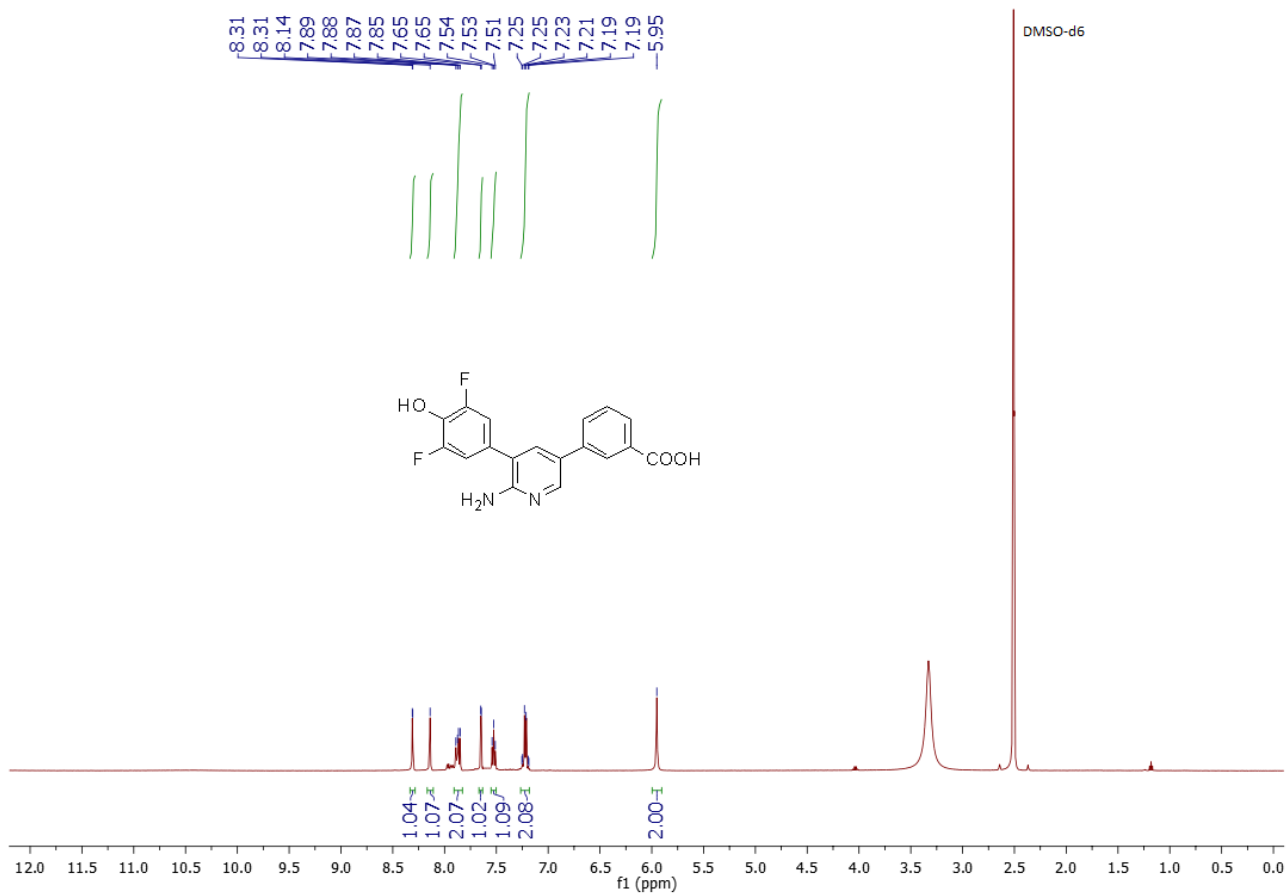
$^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$) 14.



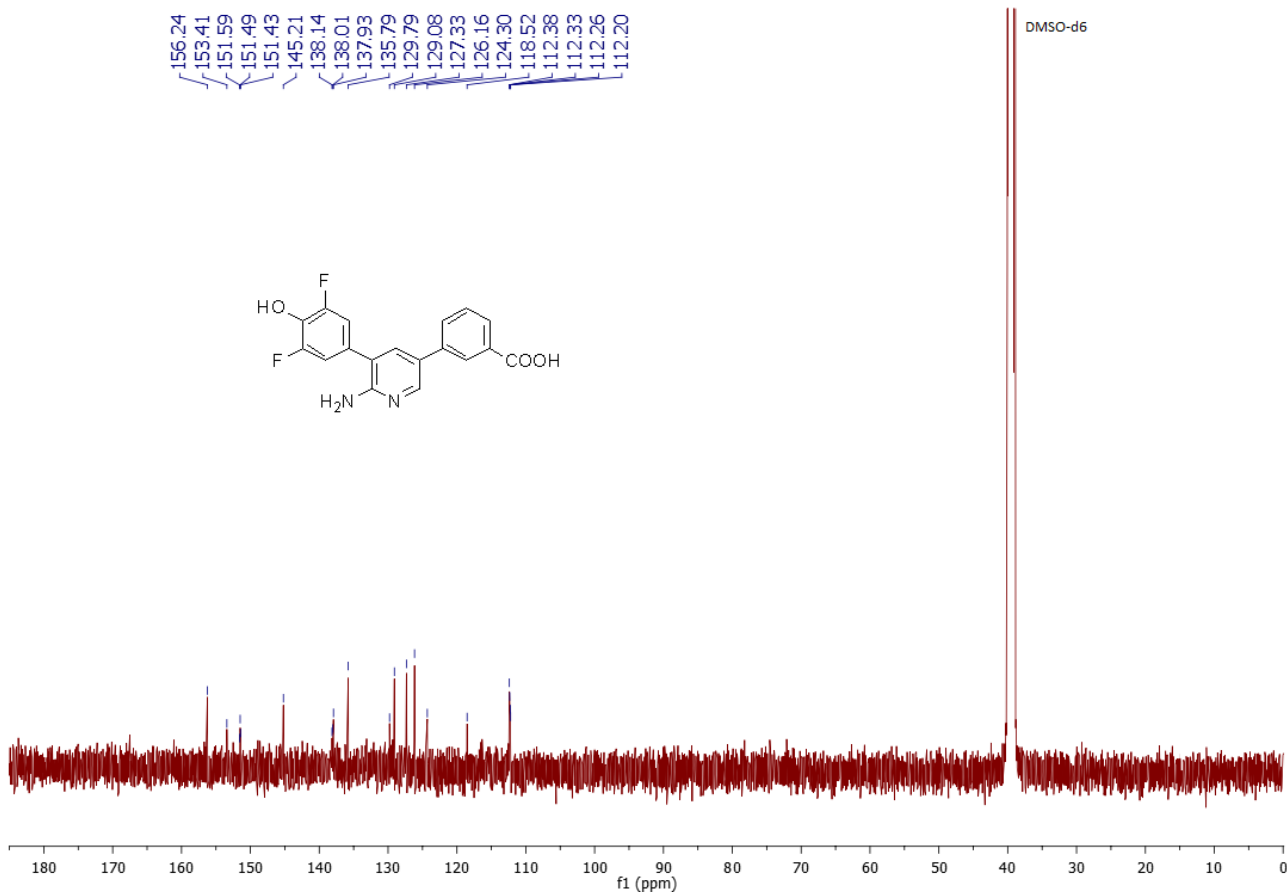
$^{13}\text{C NMR}$ (126 MHz, $\text{DMSO-}d_6$) 14.



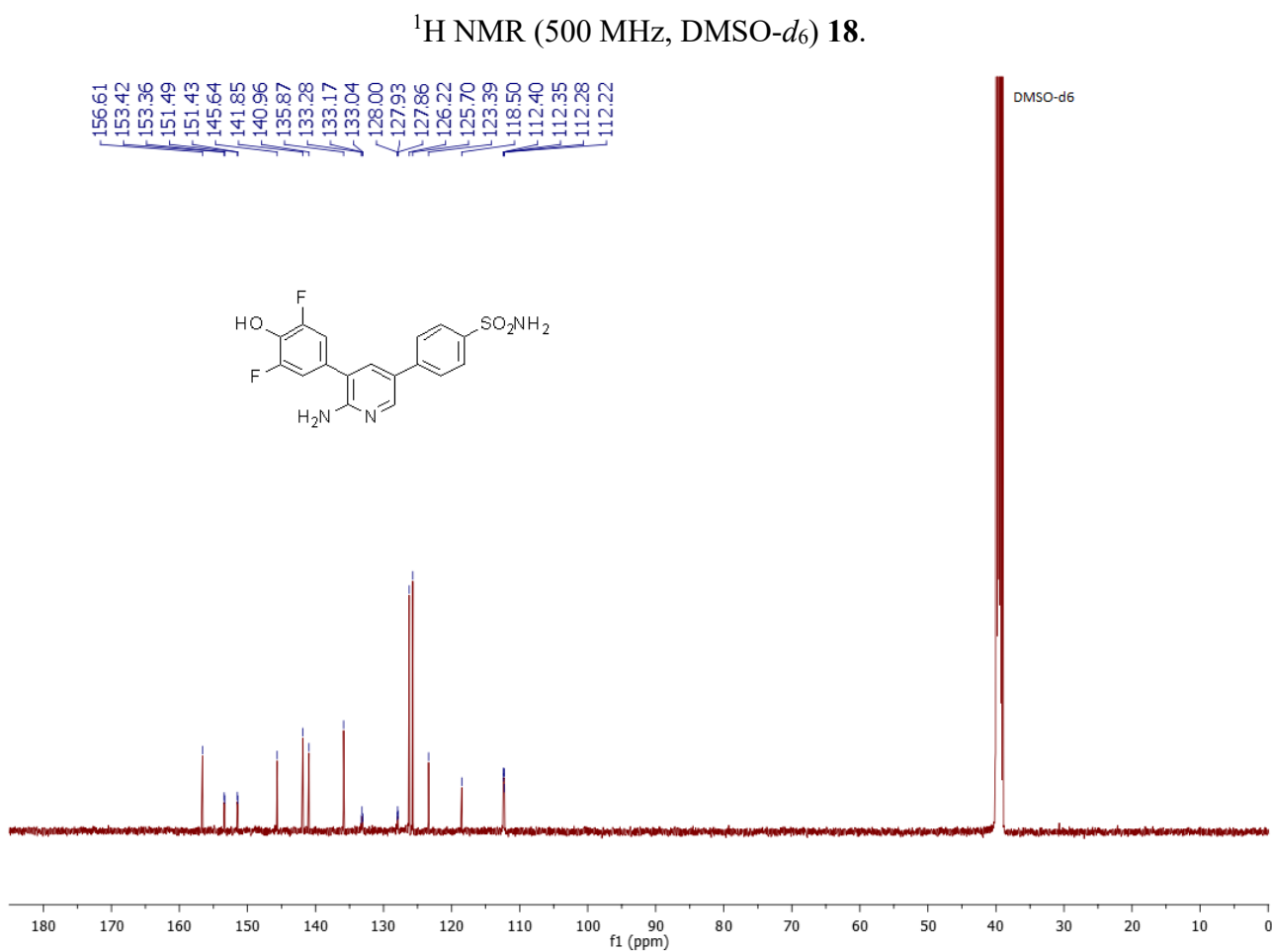
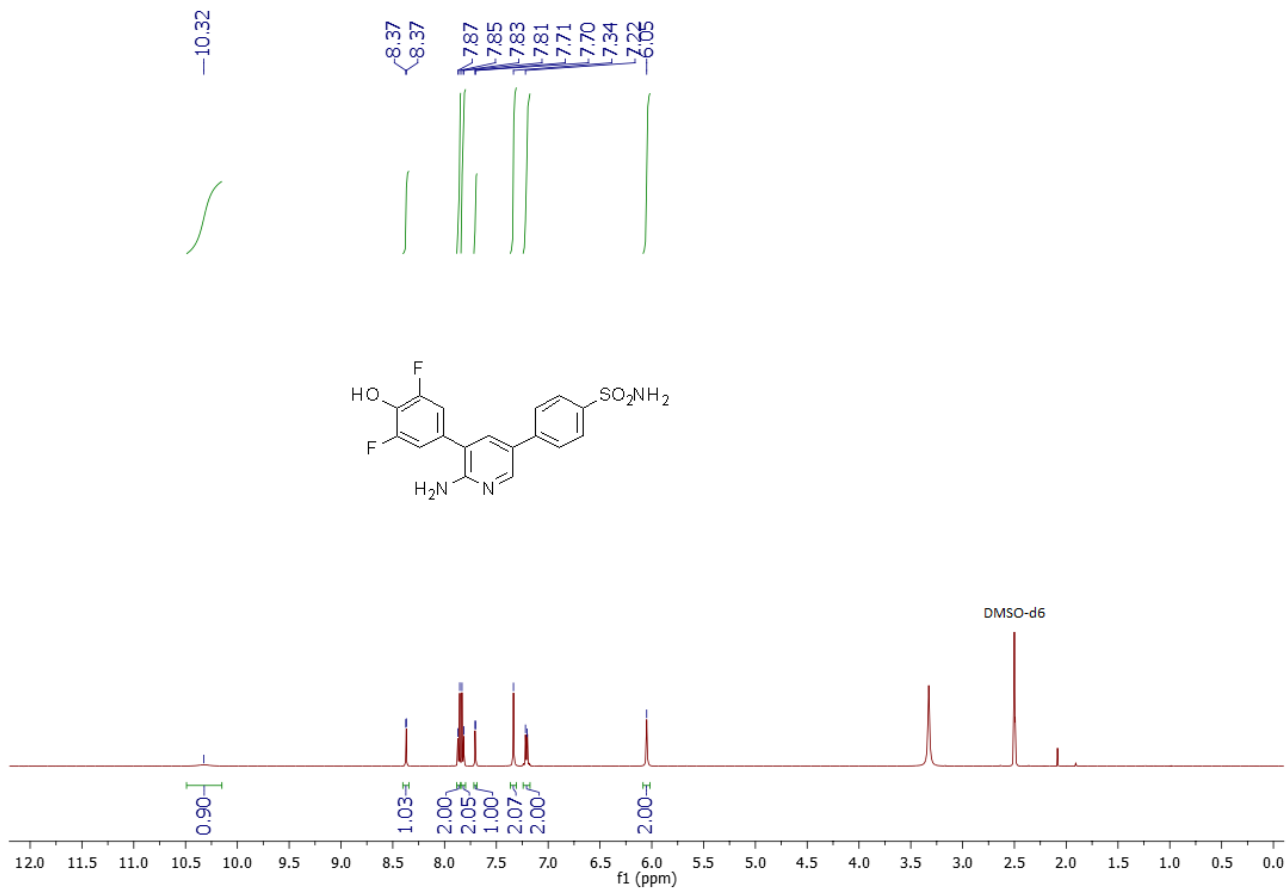


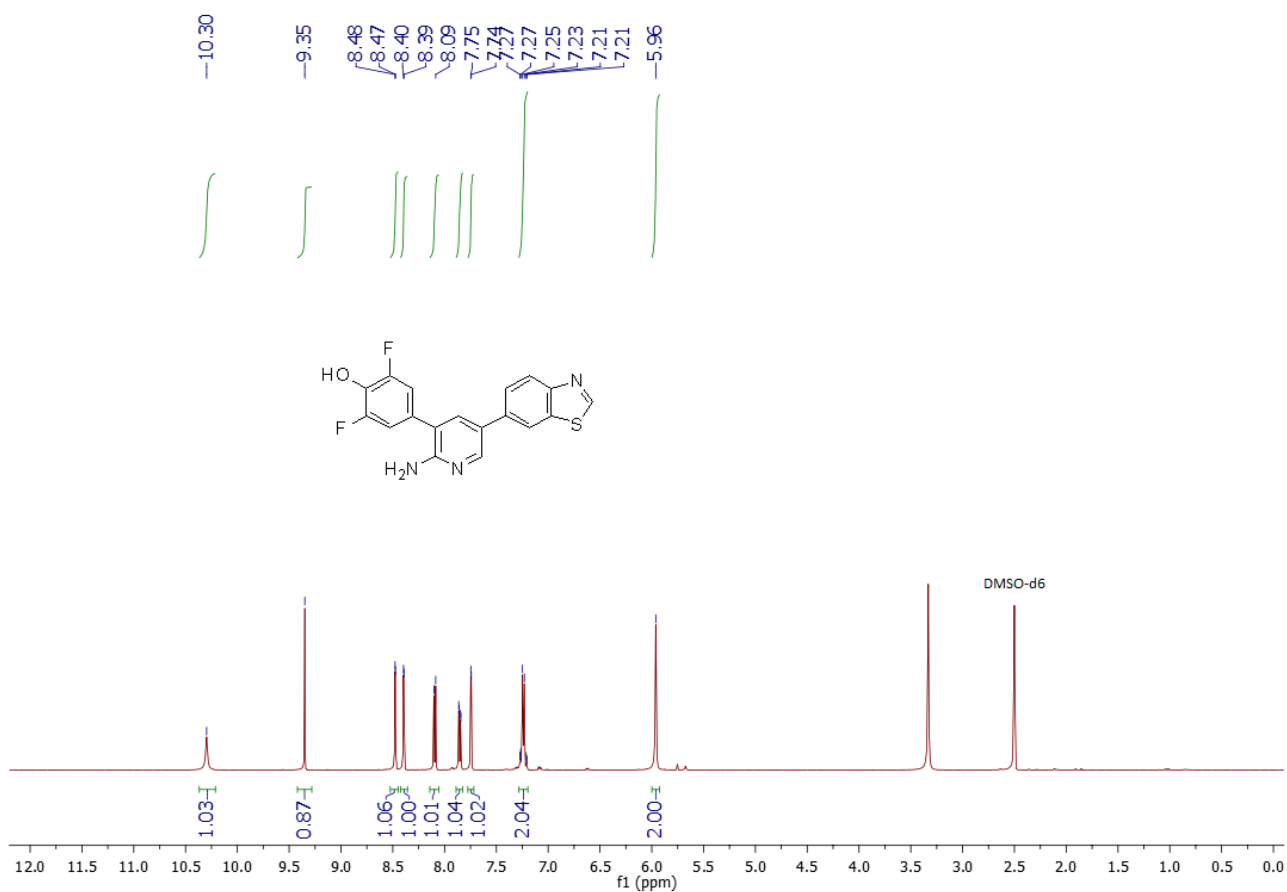


¹H NMR (500 MHz, DMSO-*d*₆) 17.

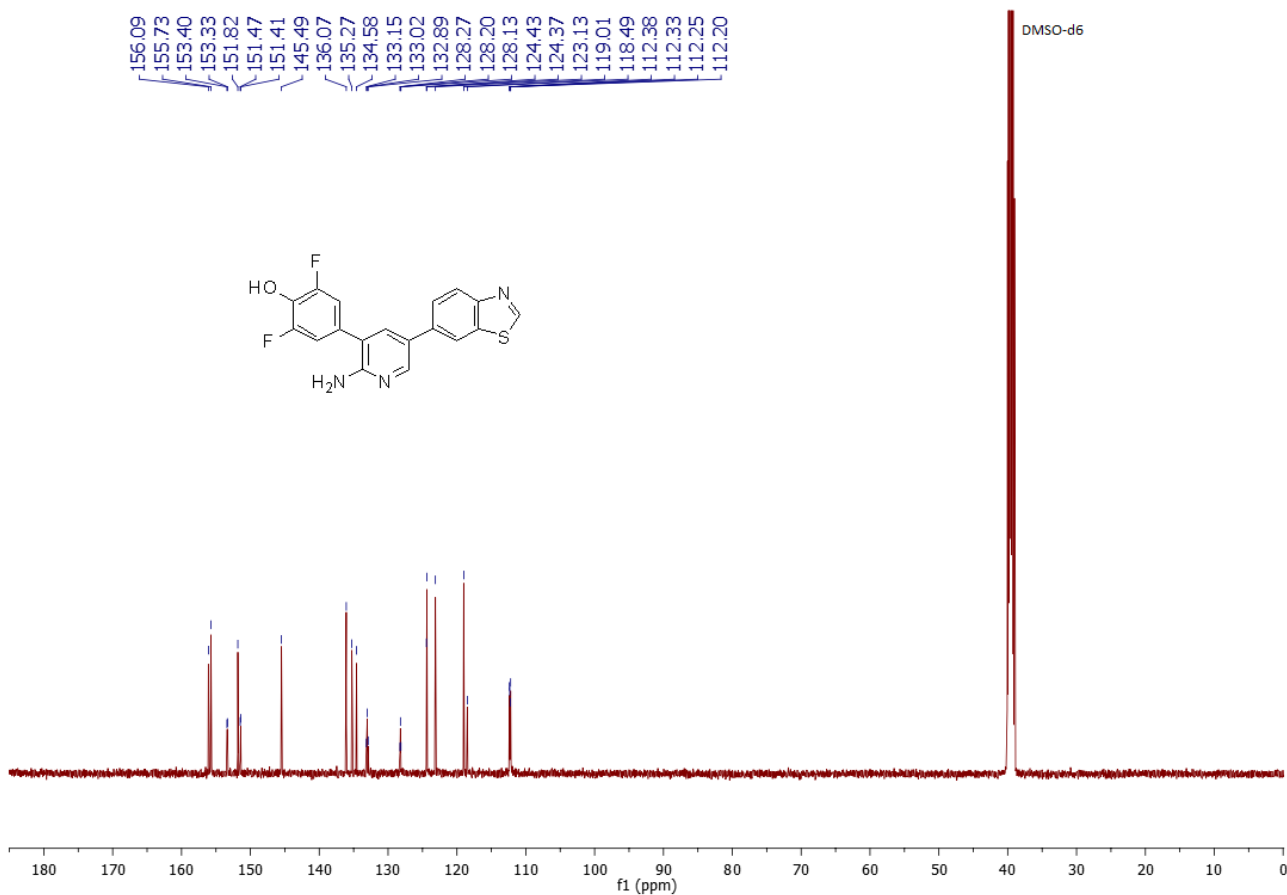


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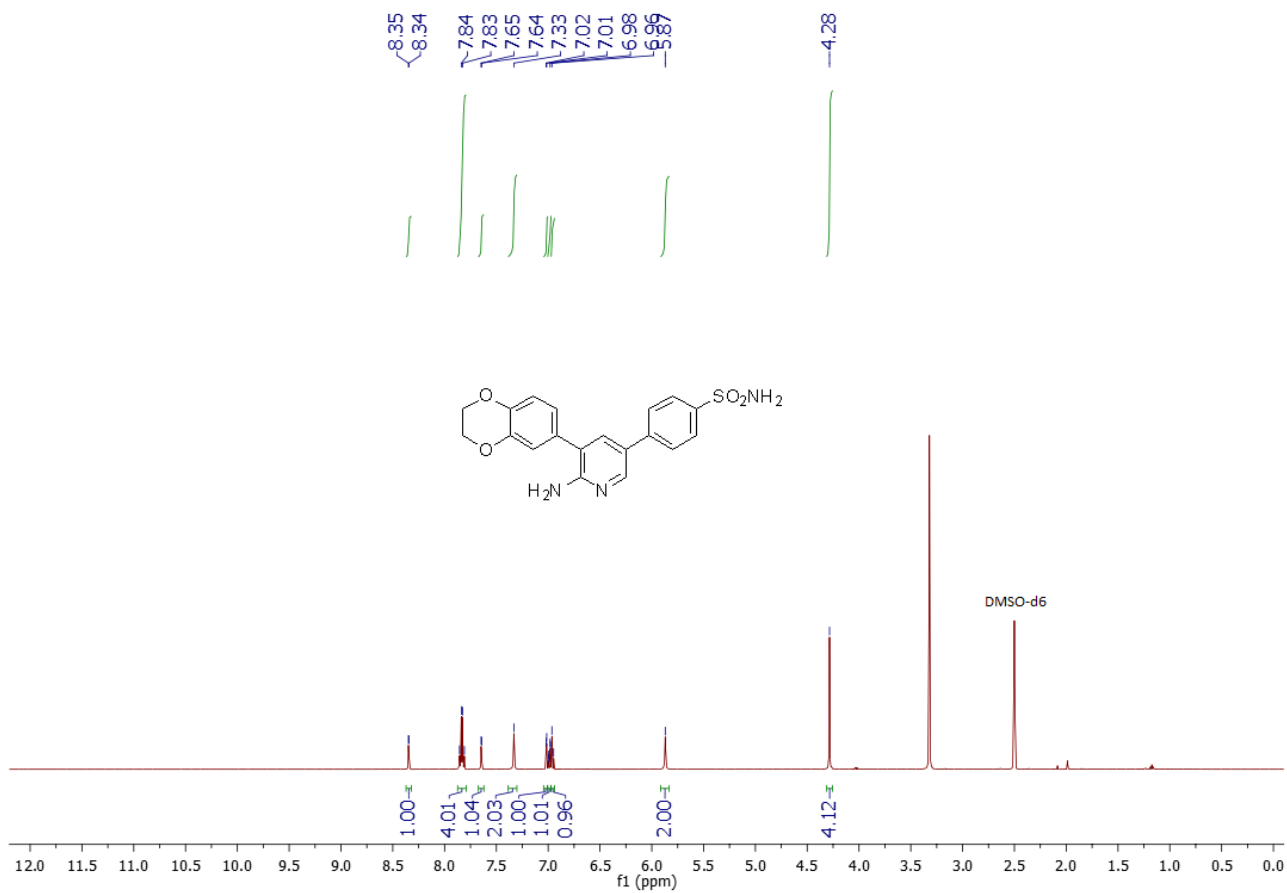




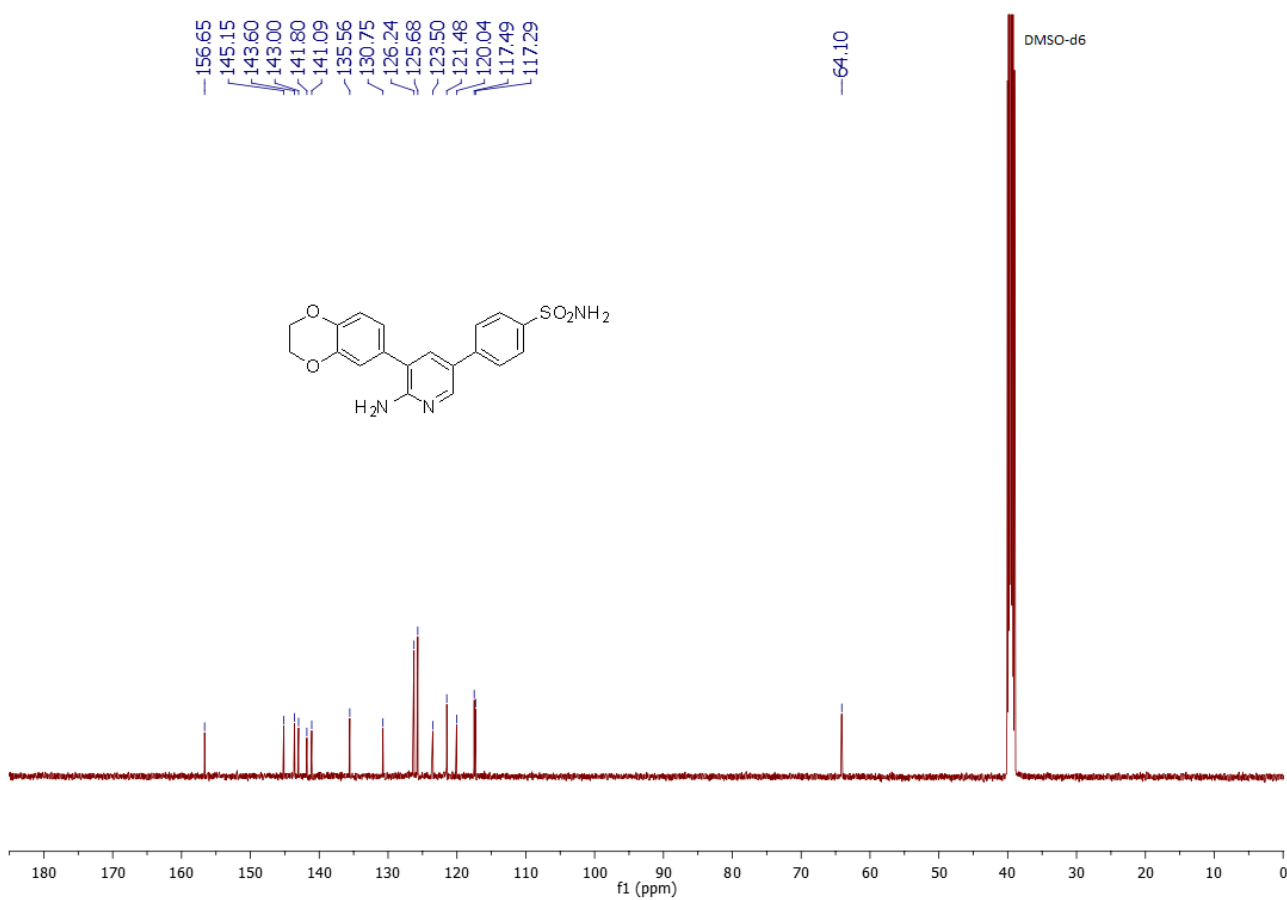
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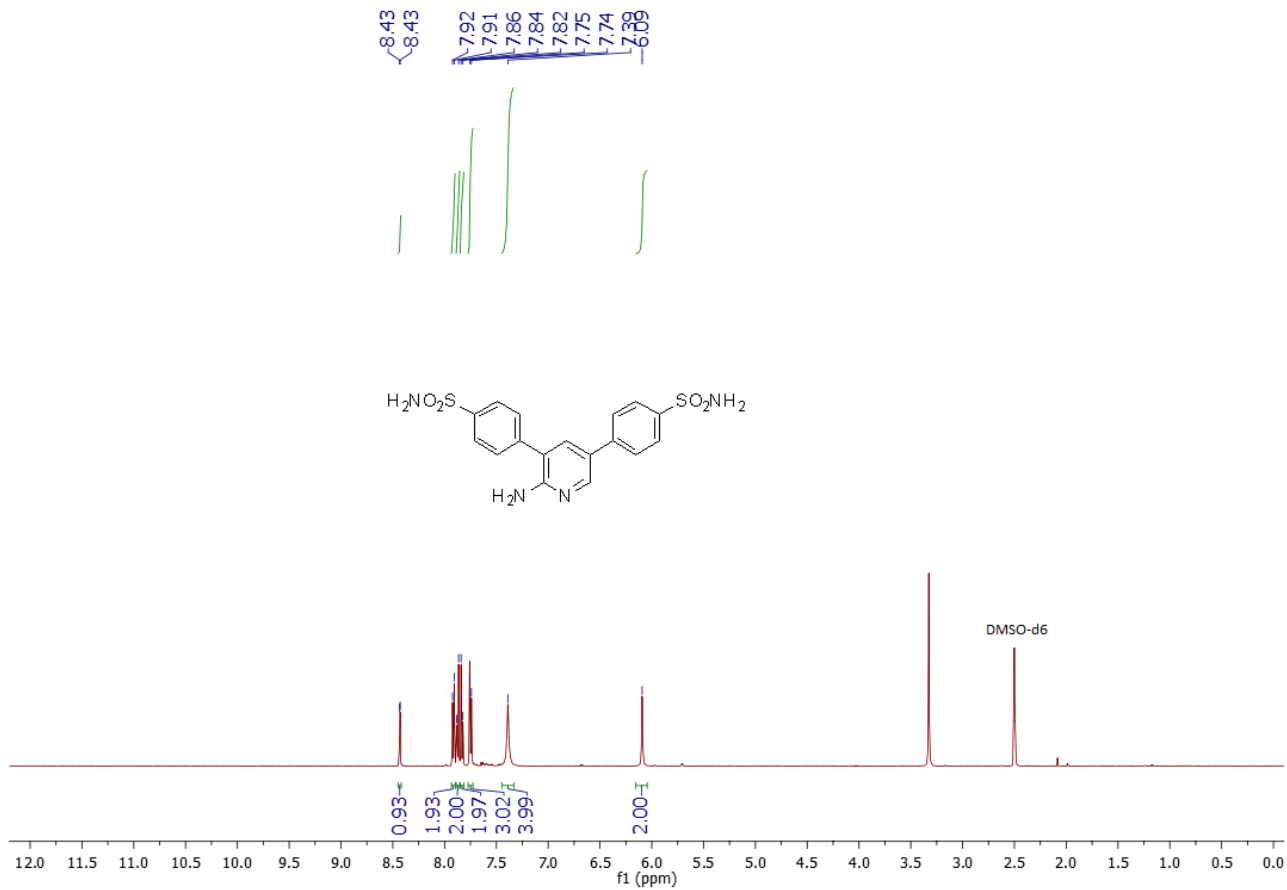
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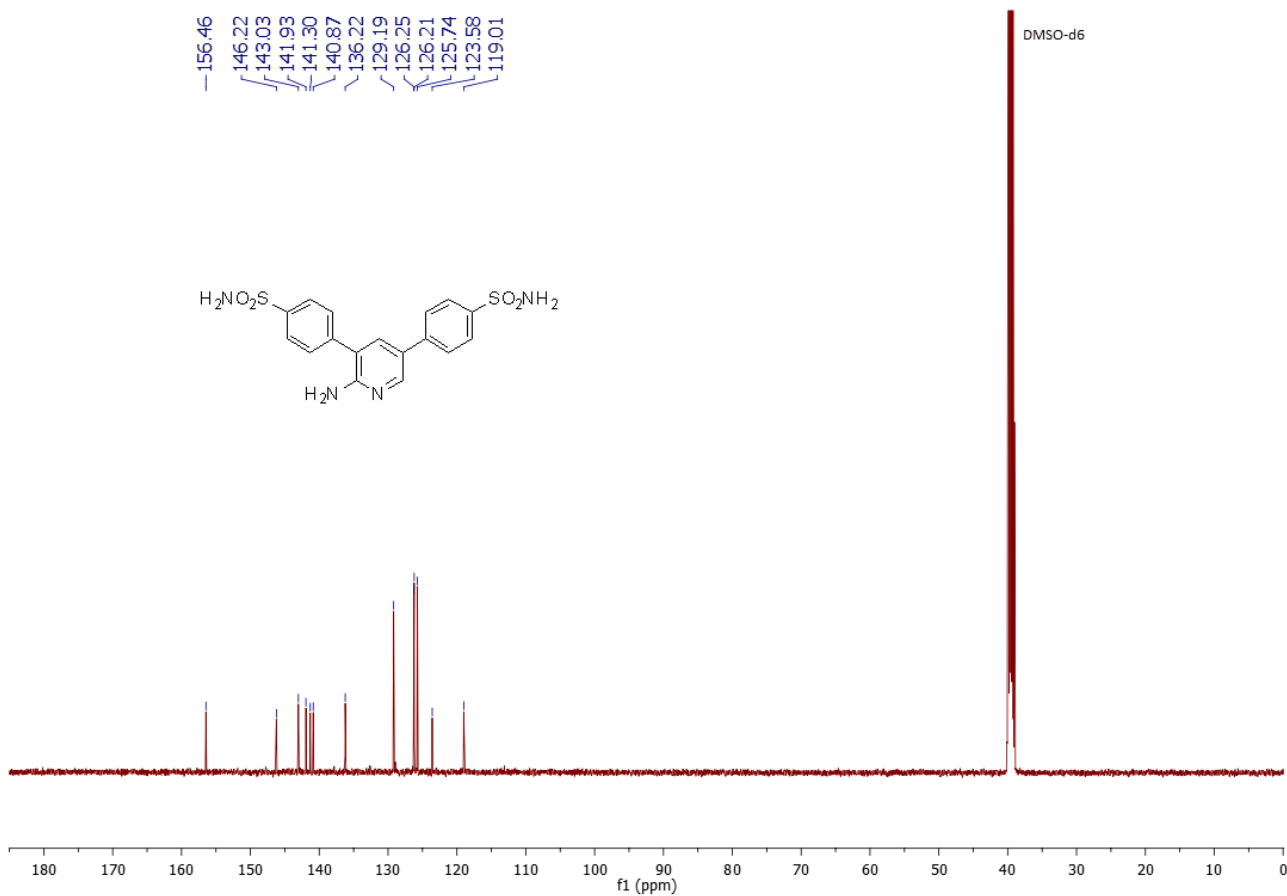
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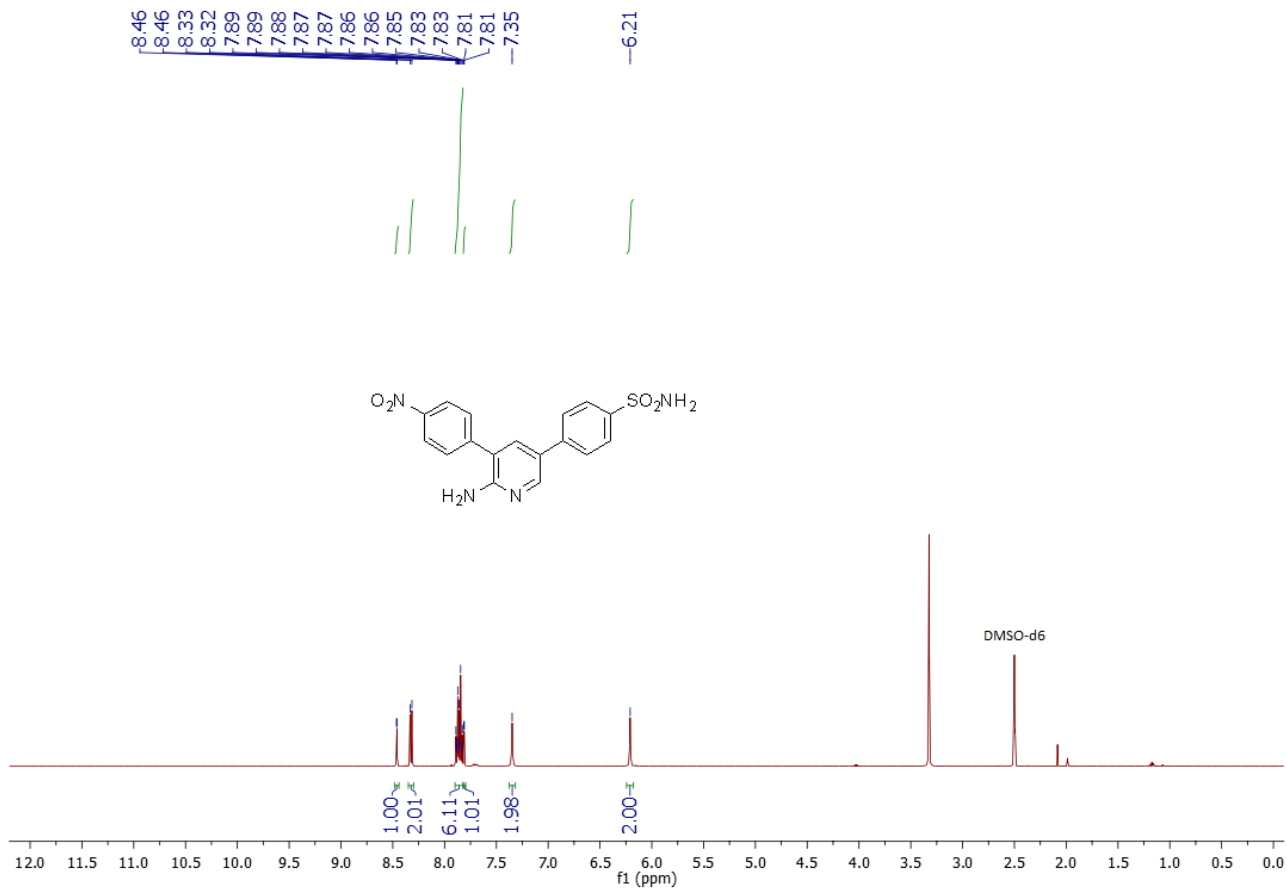
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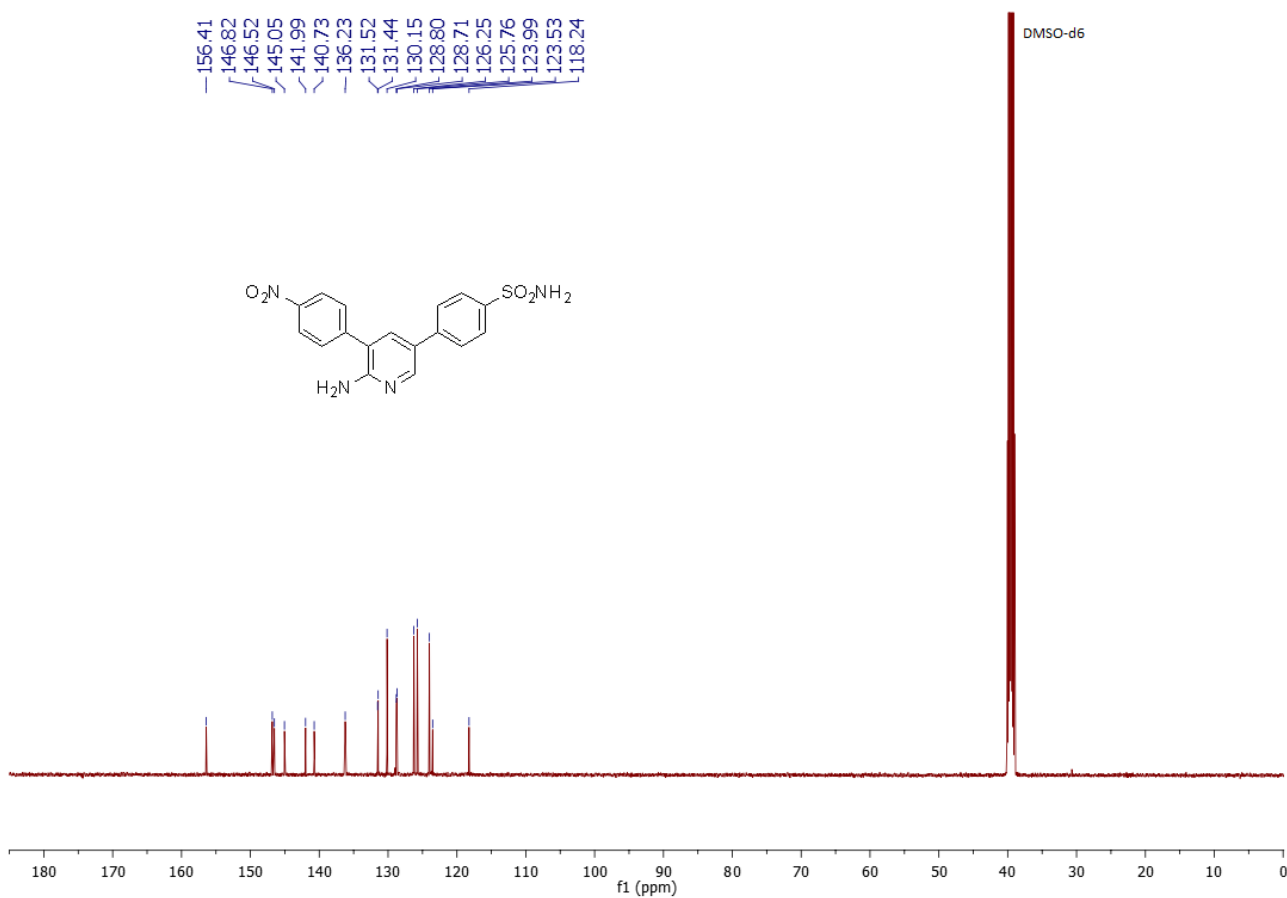
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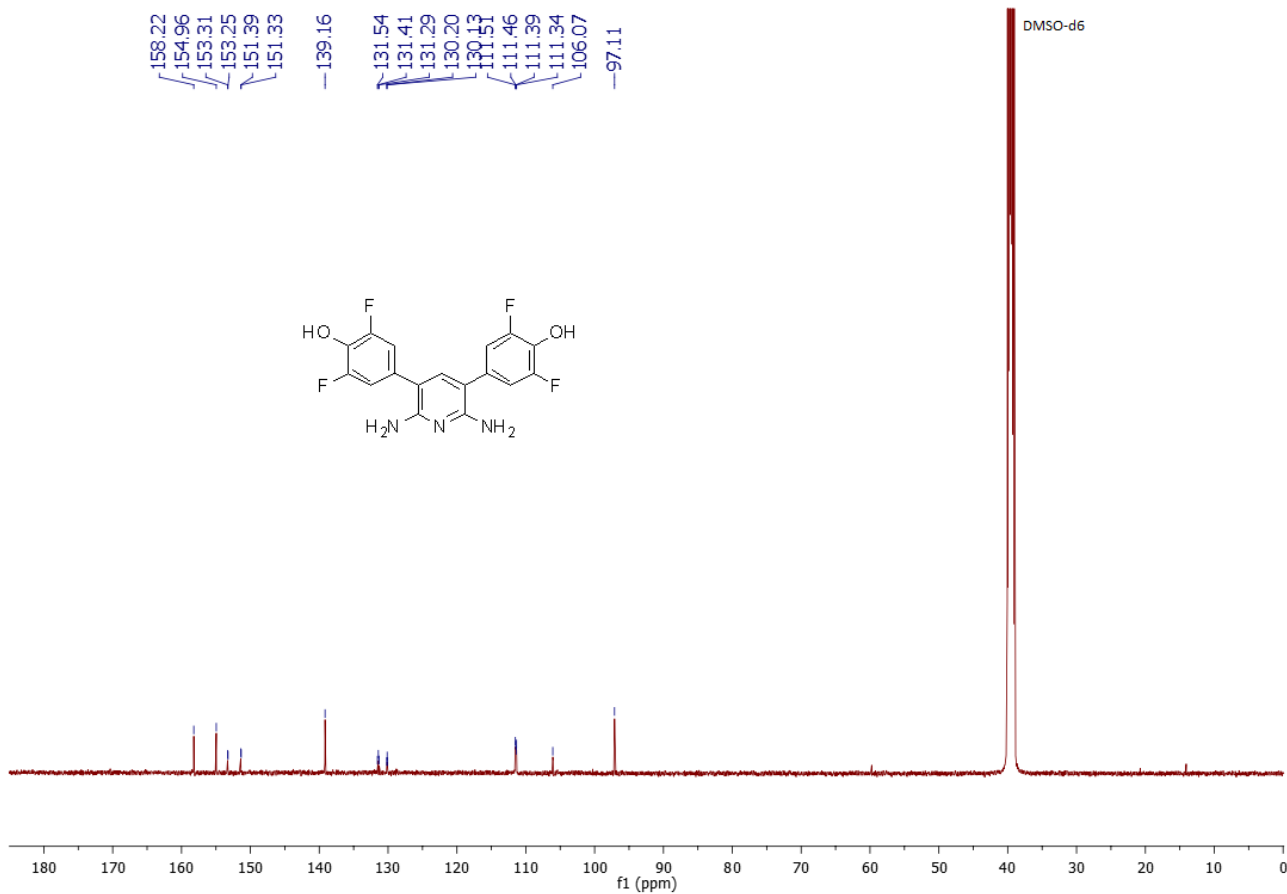
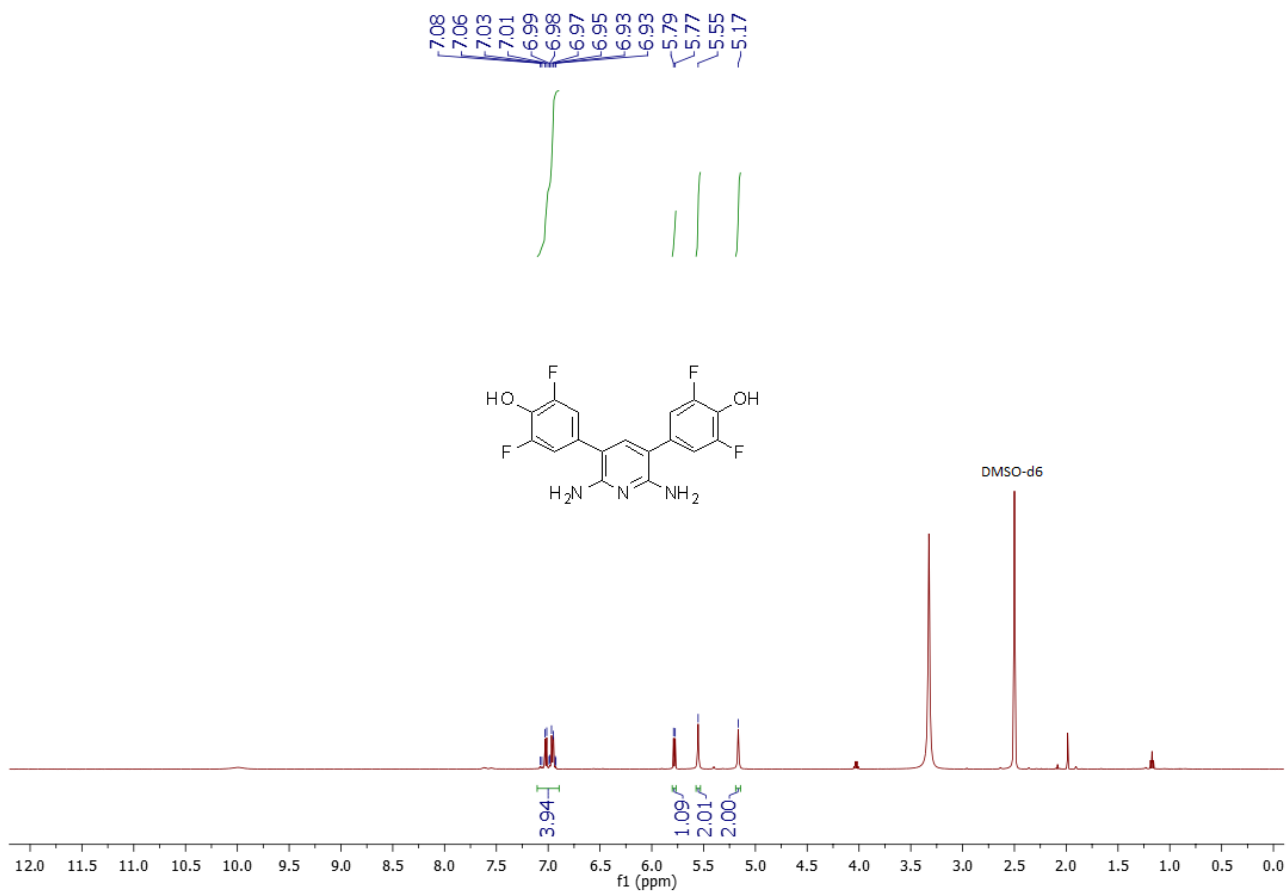
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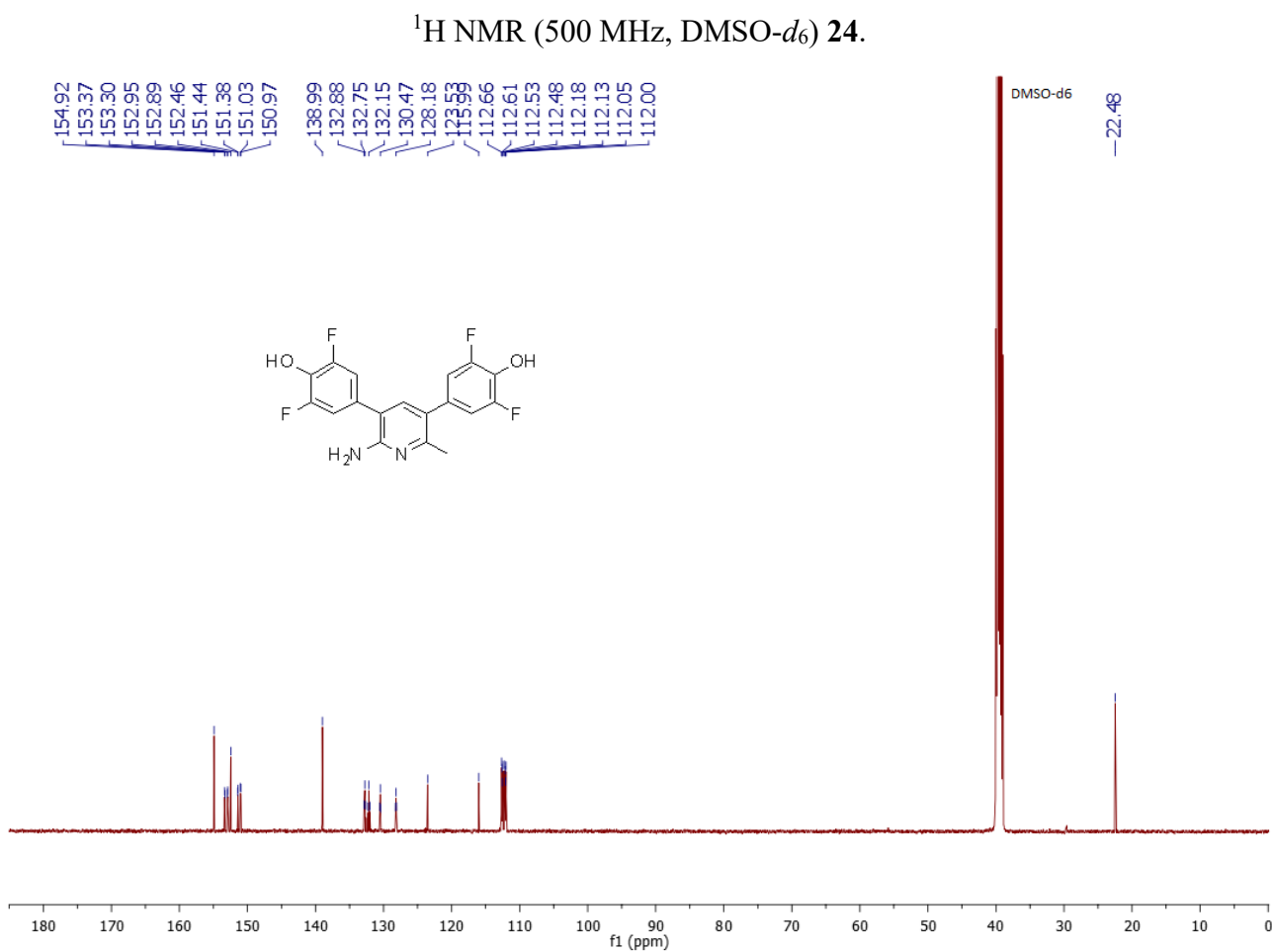
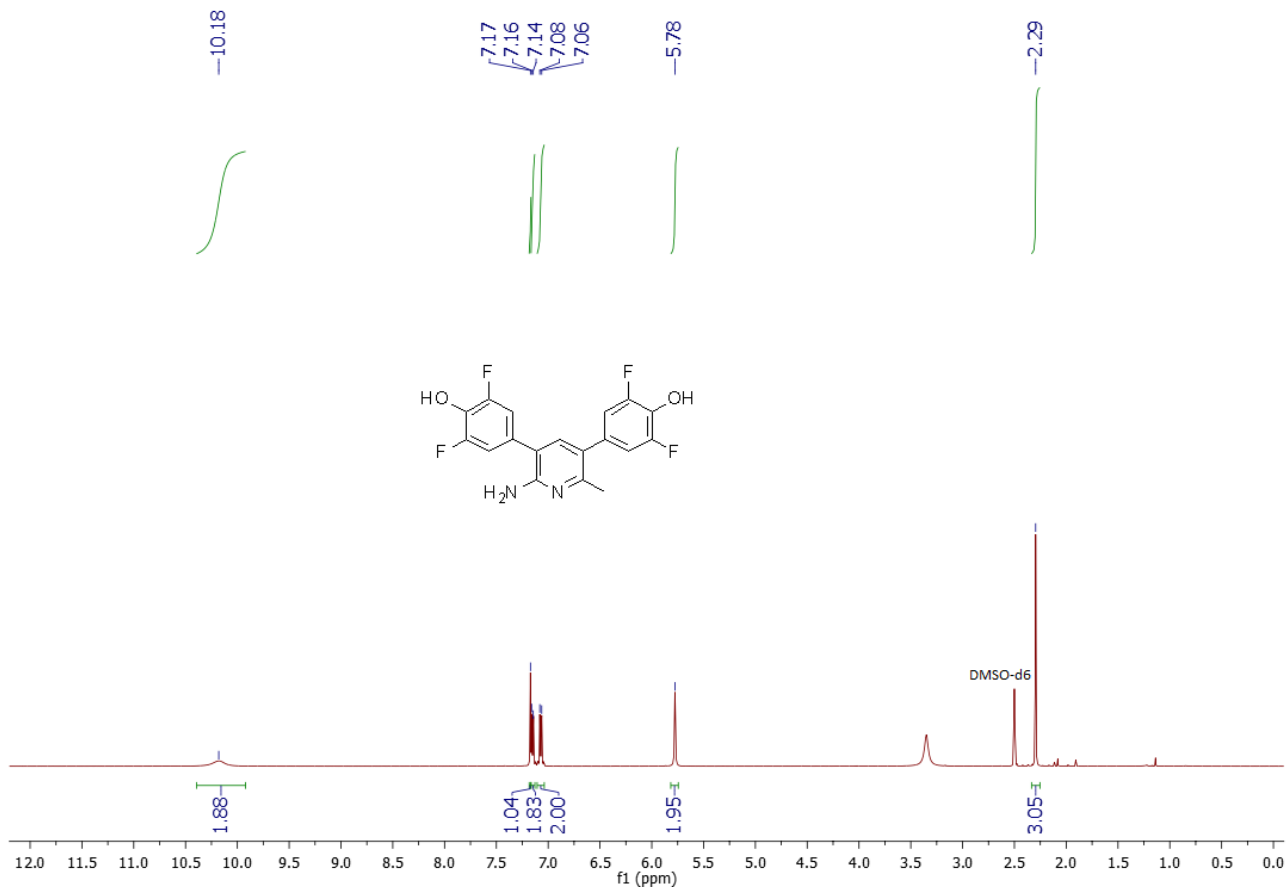


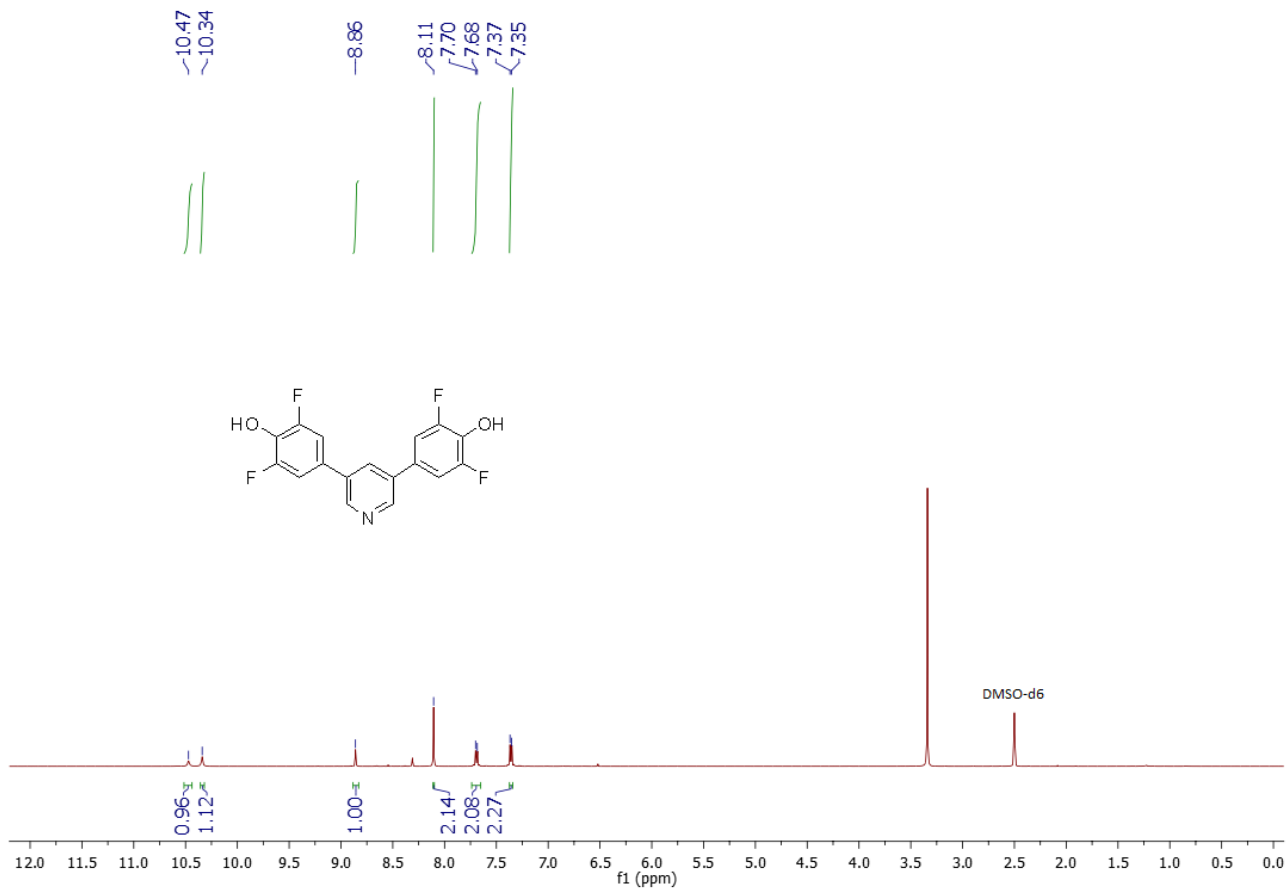
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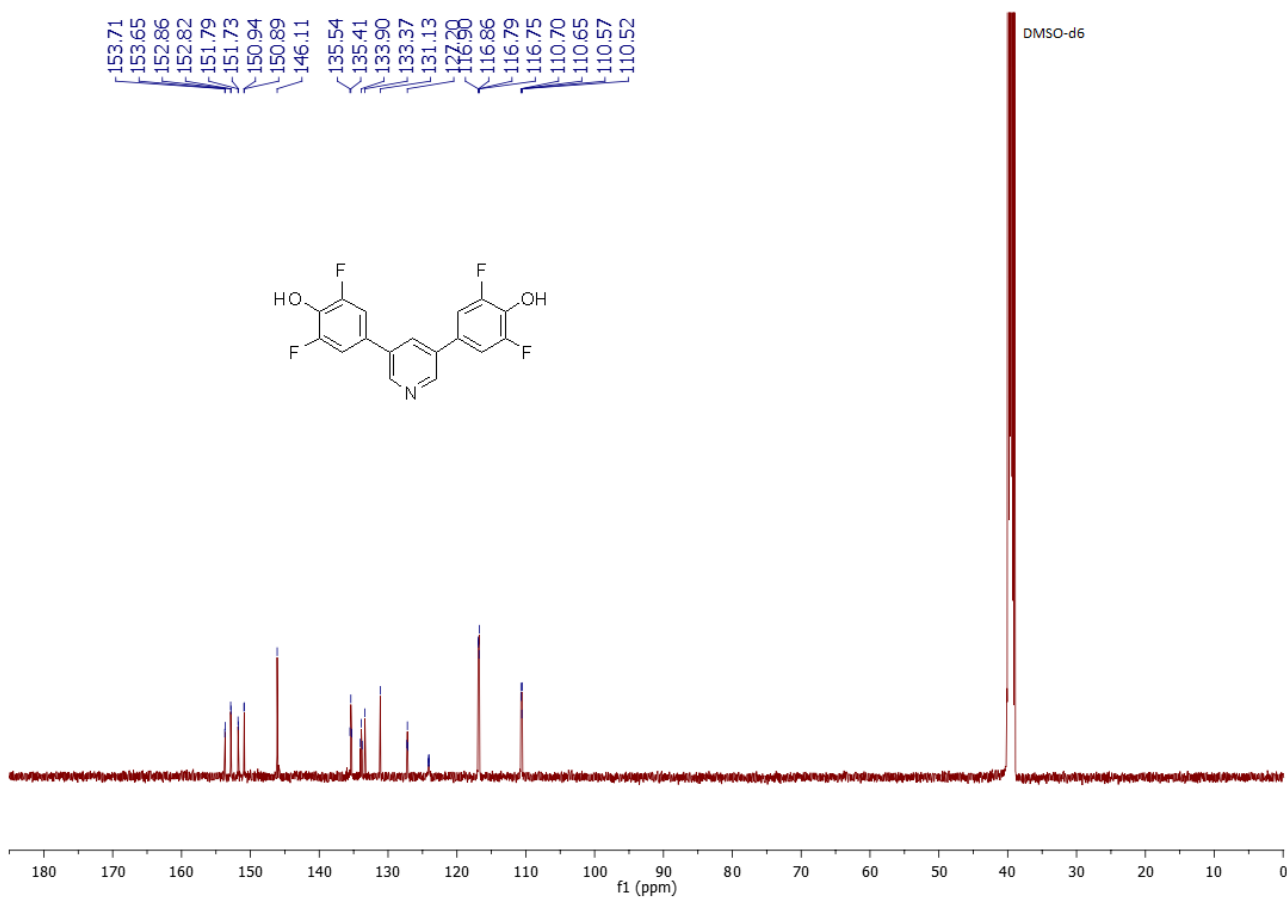
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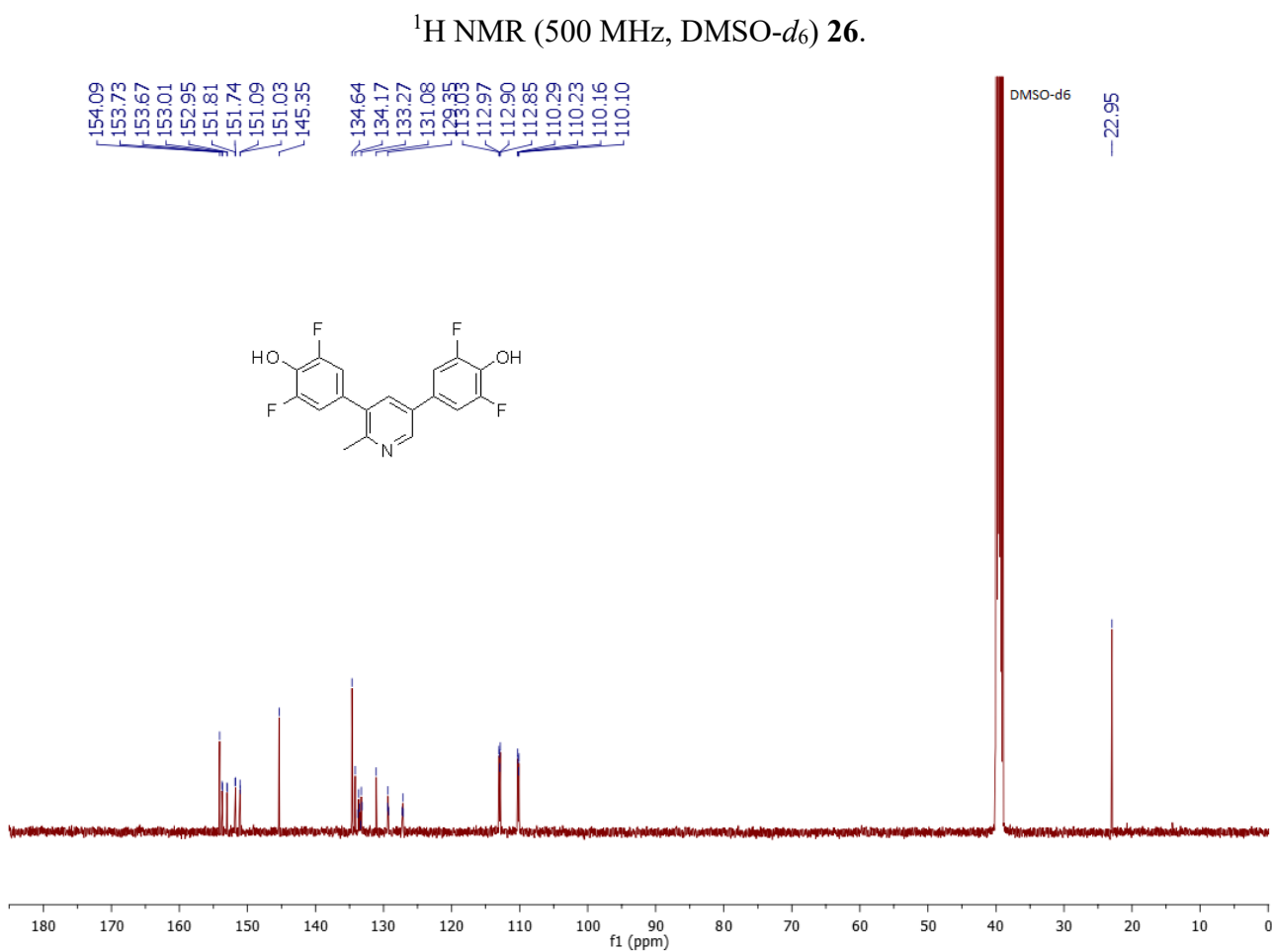
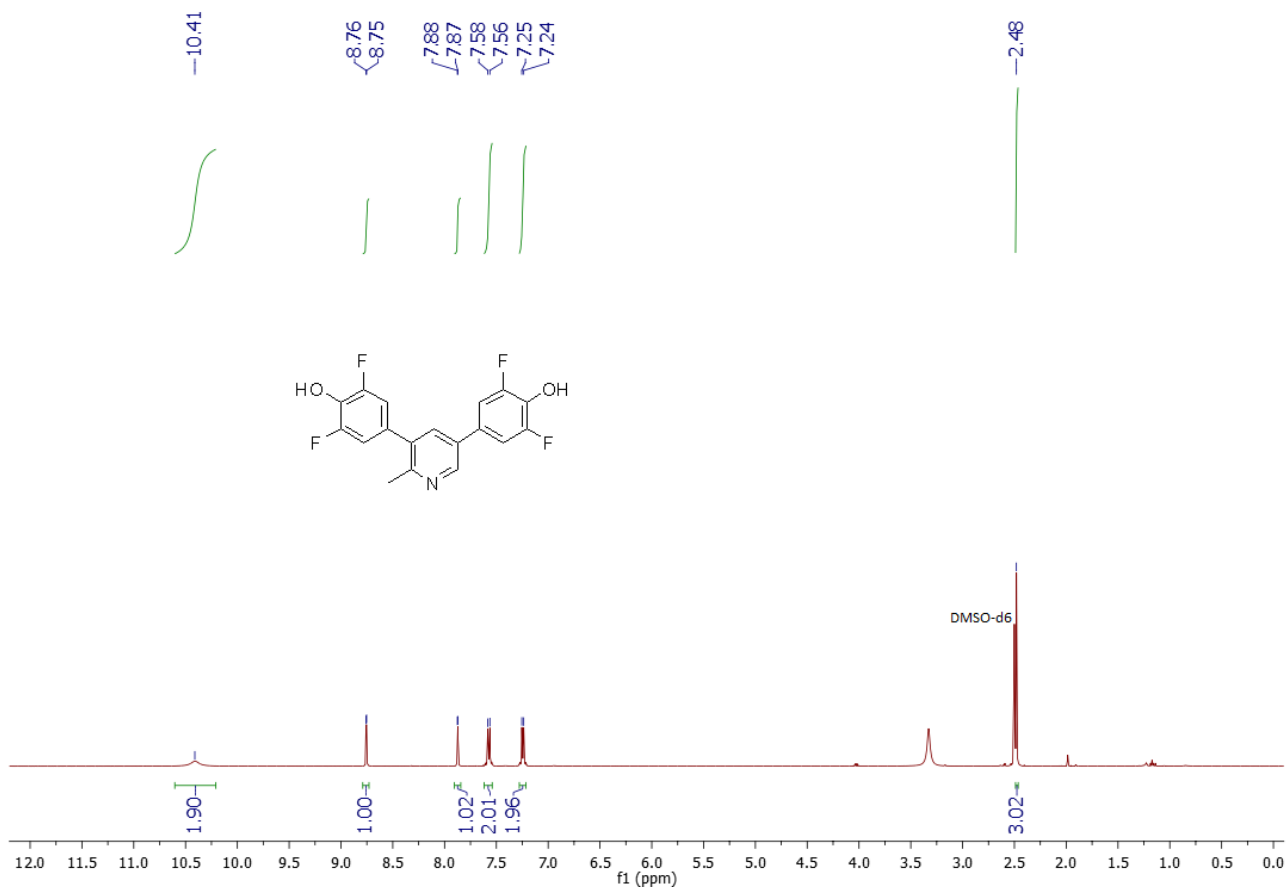


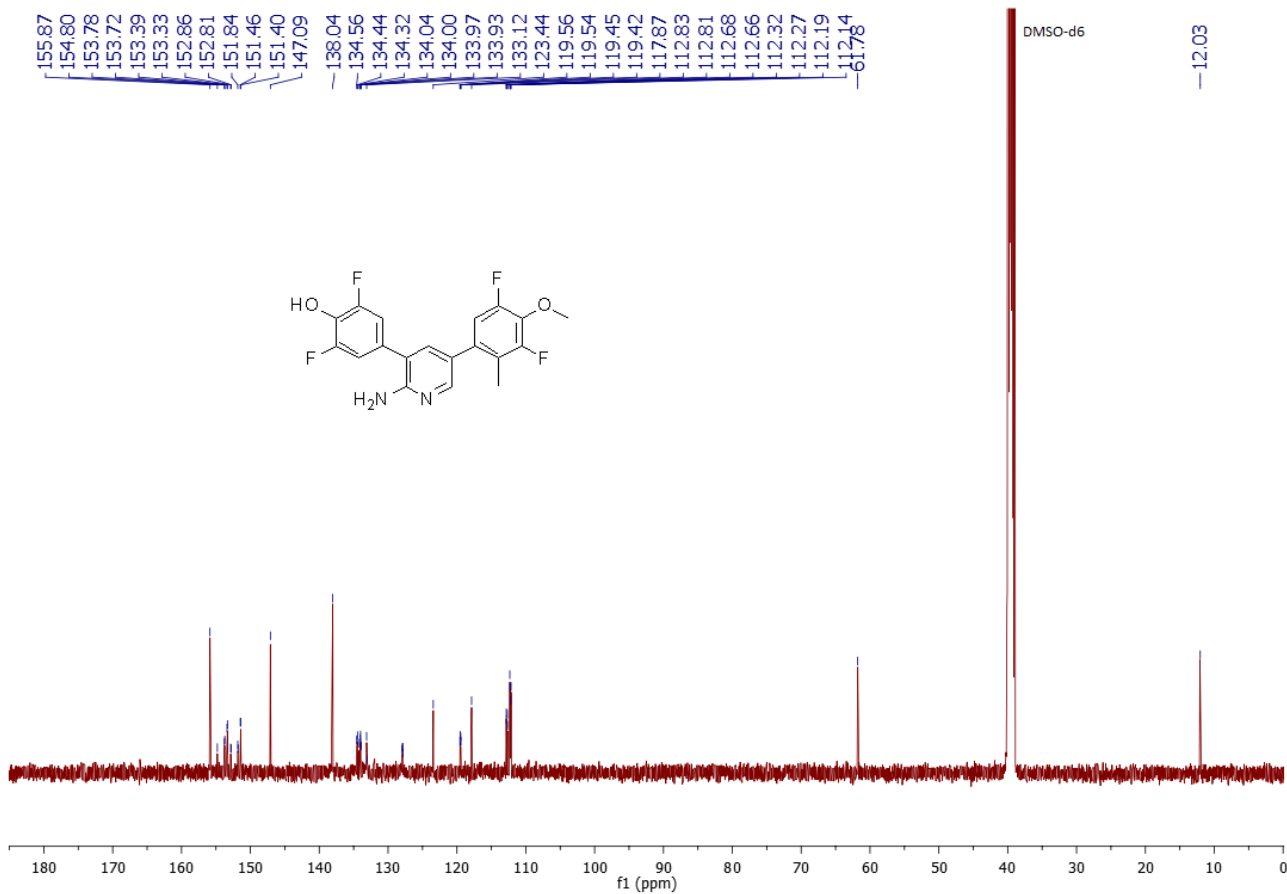
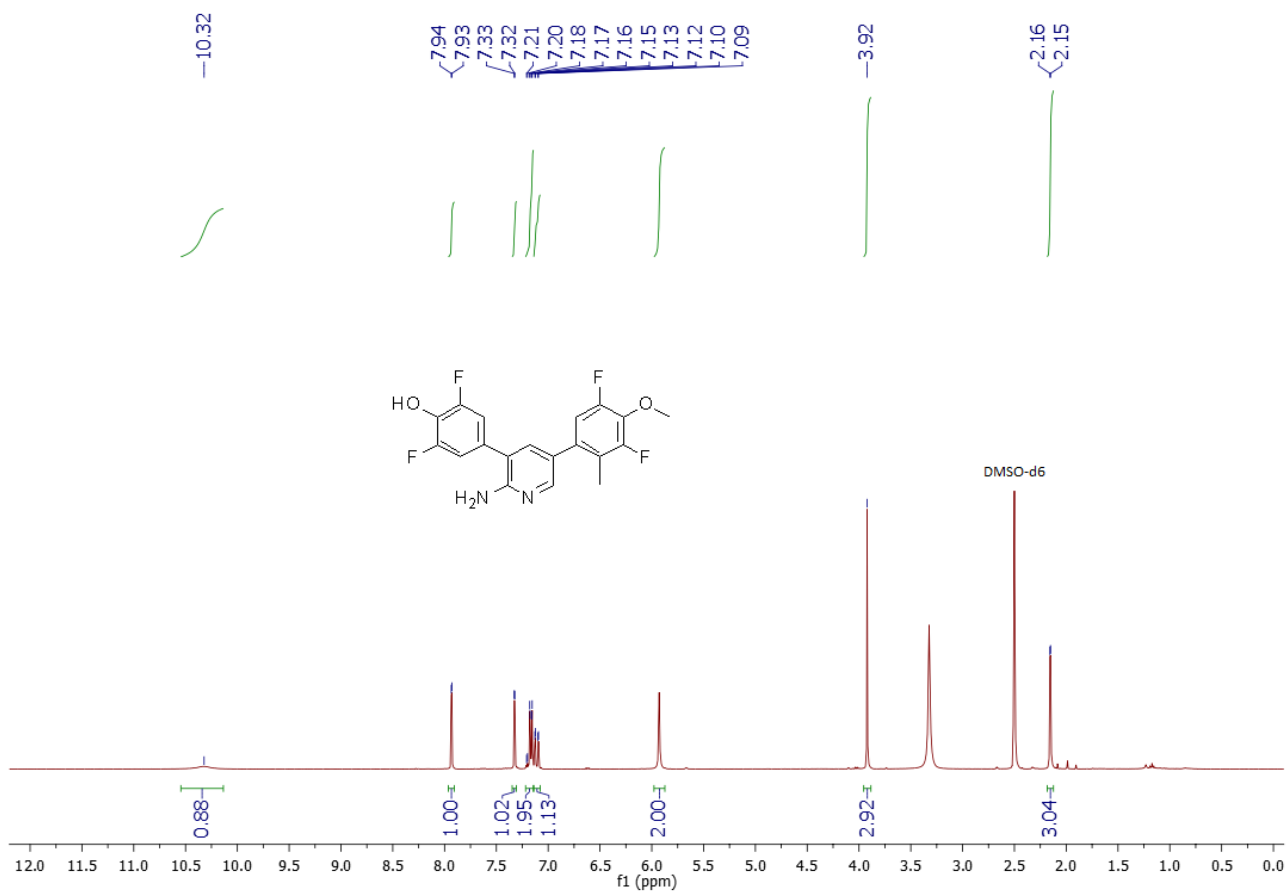


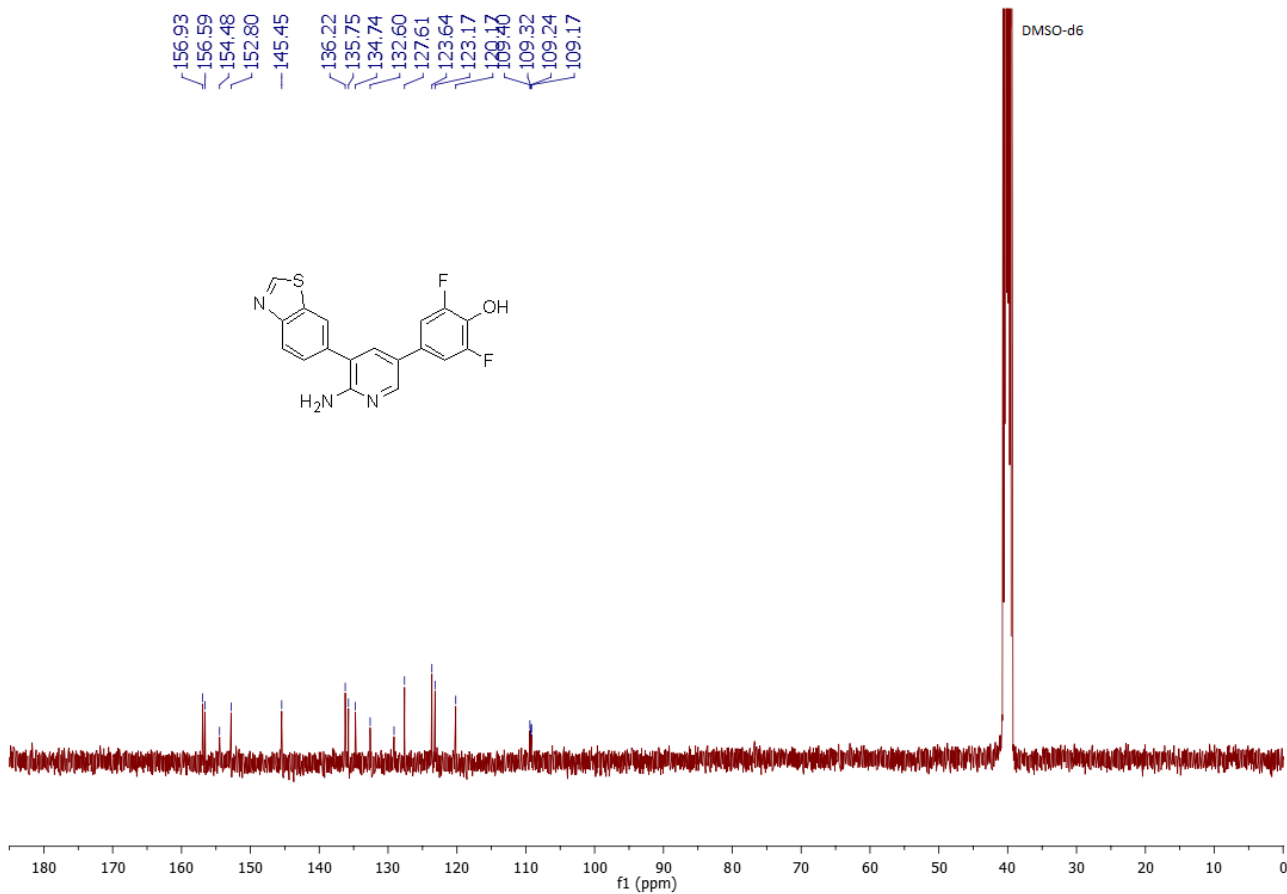
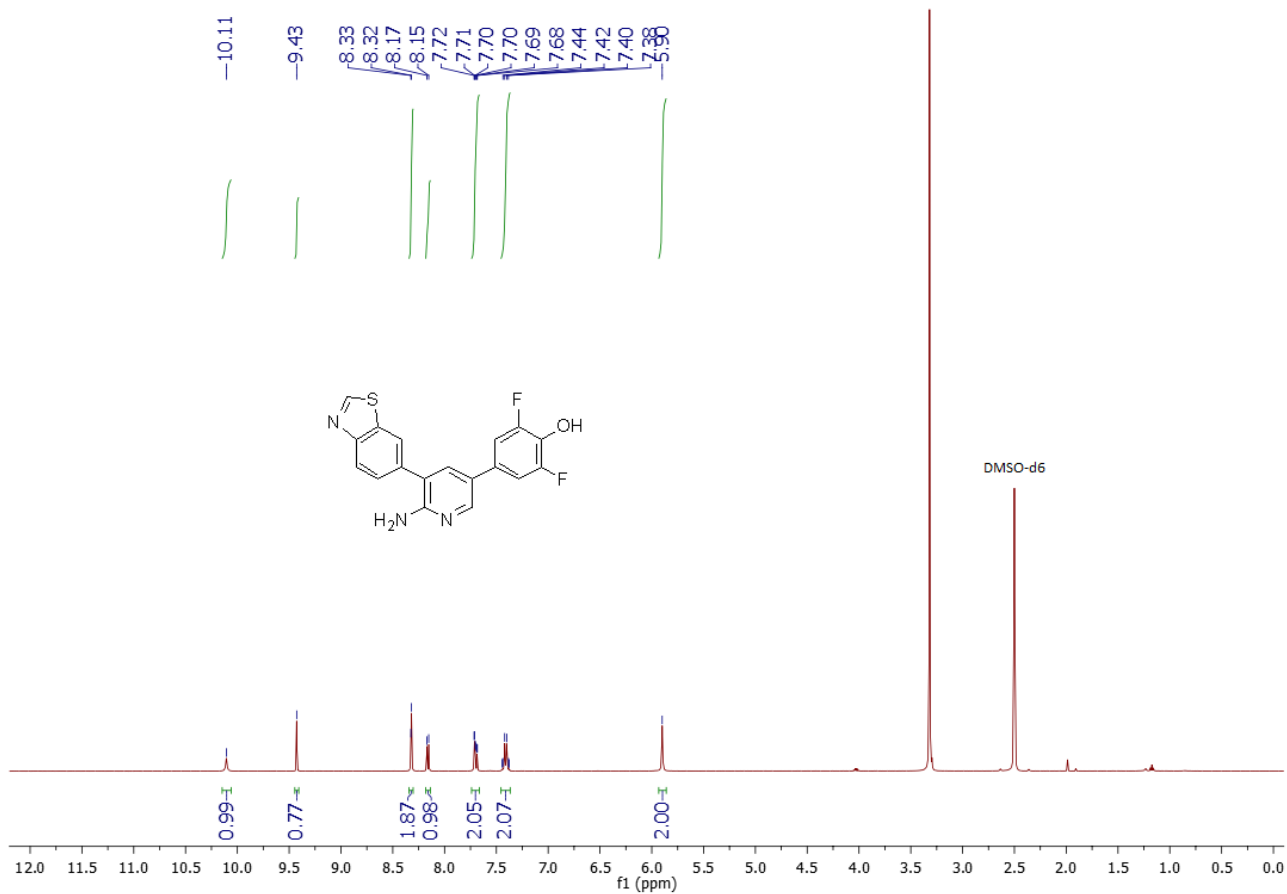
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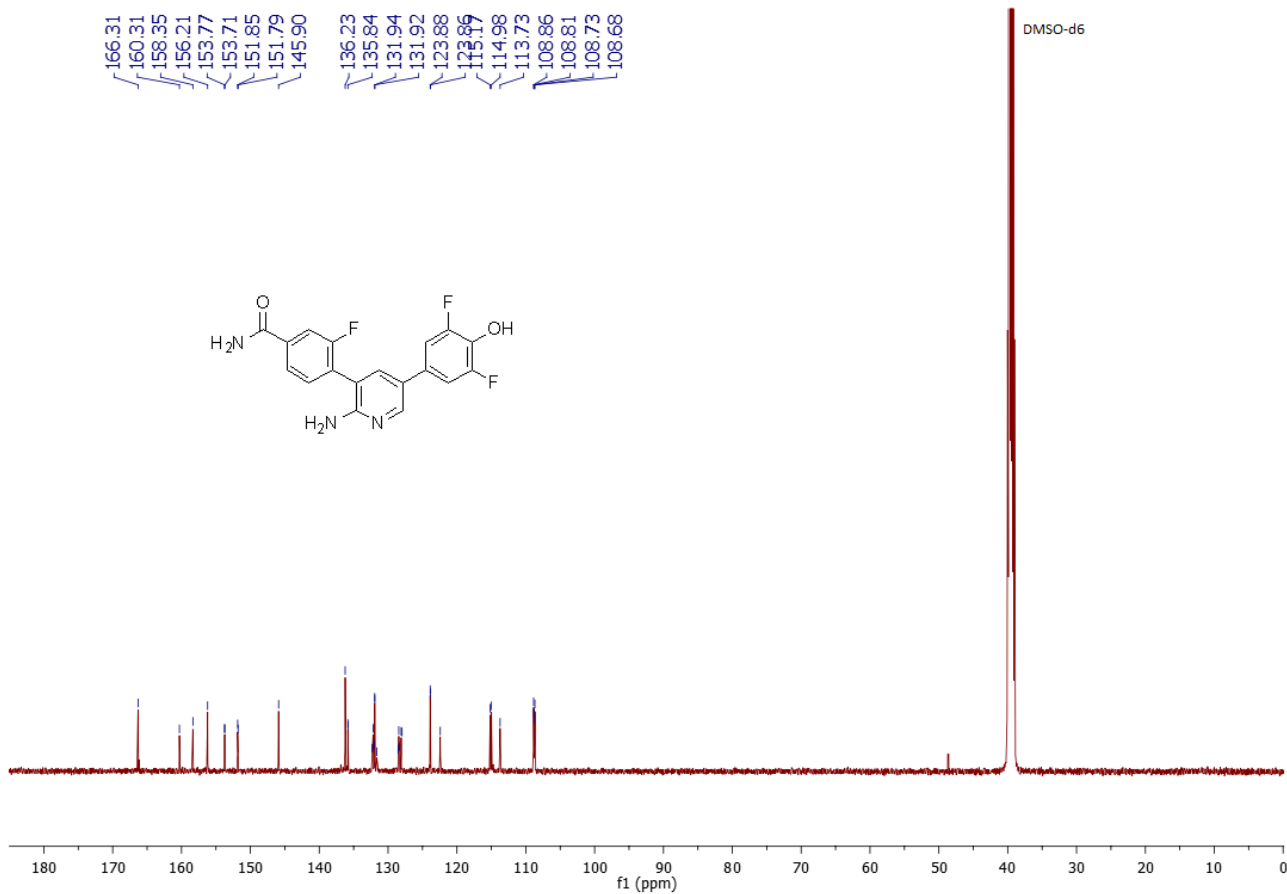
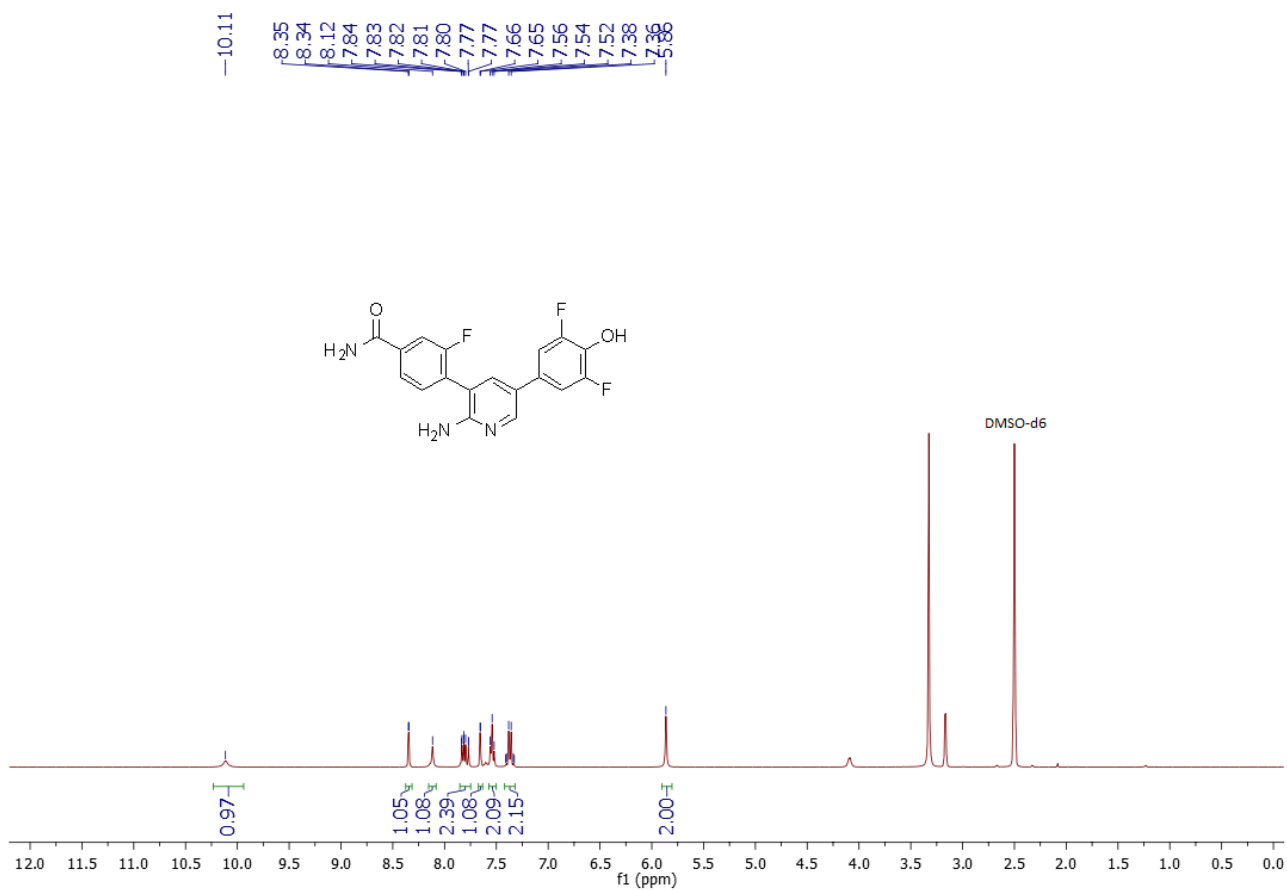


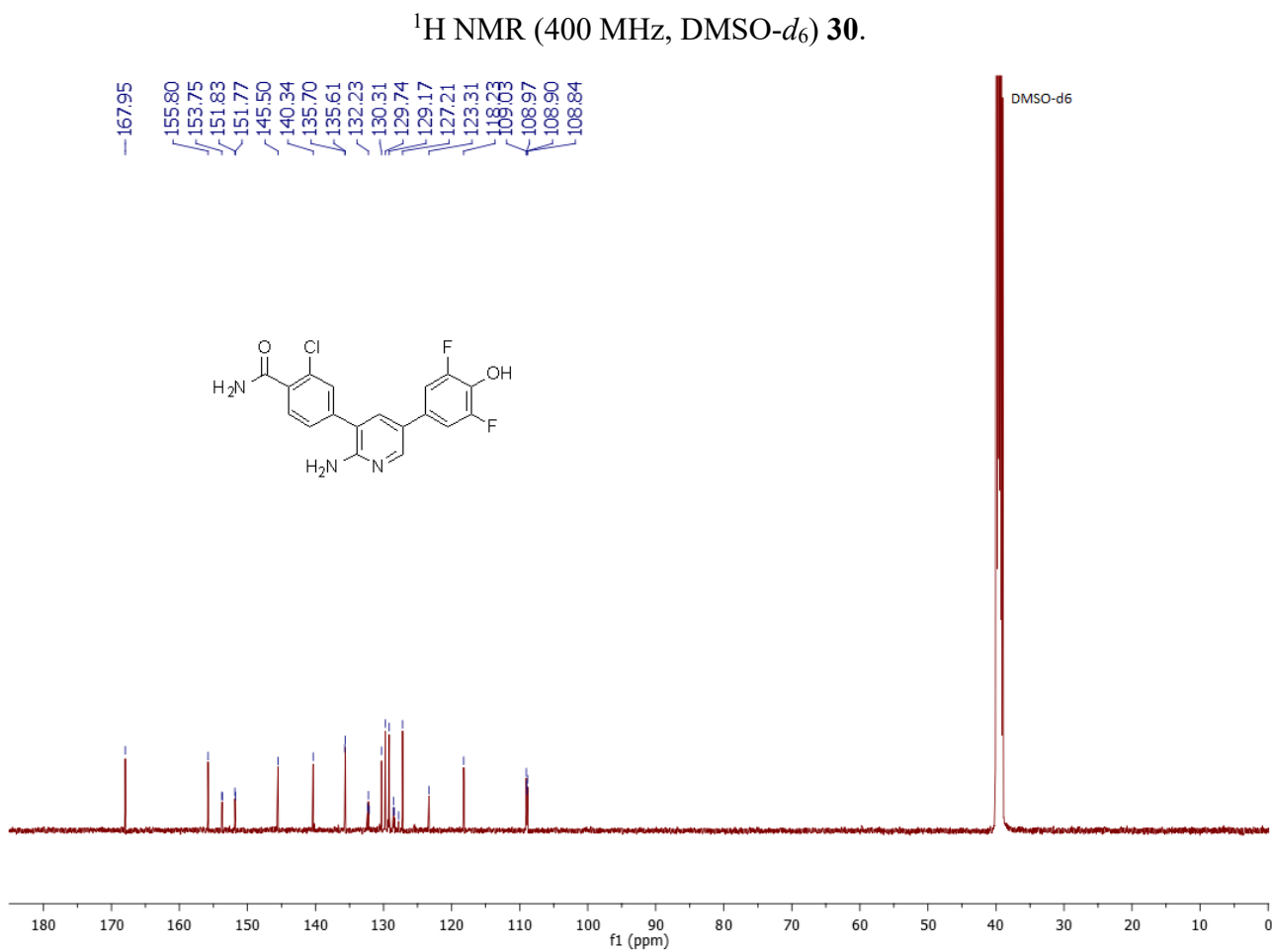
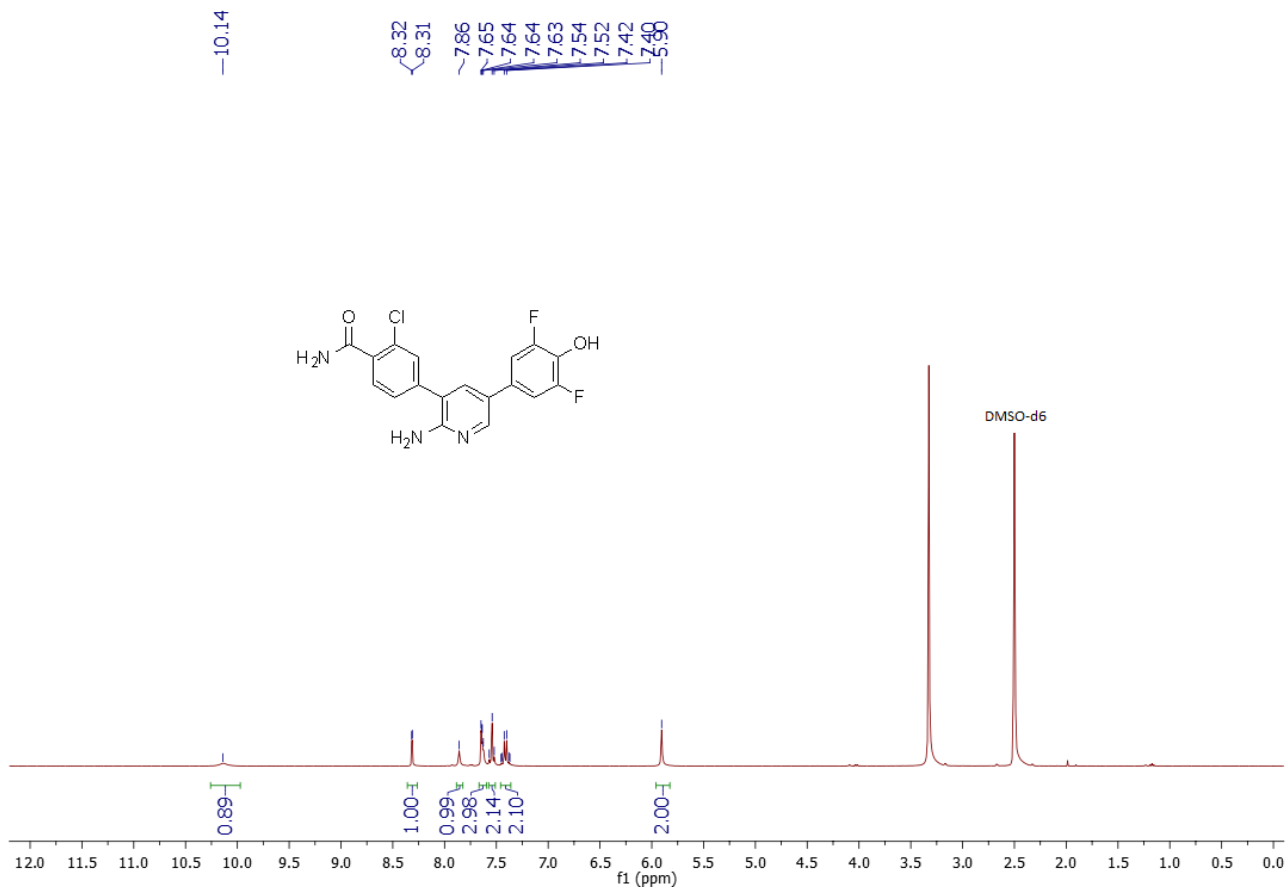
¹³C NMR (126 MHz, DMSO-*d*₆) 25.



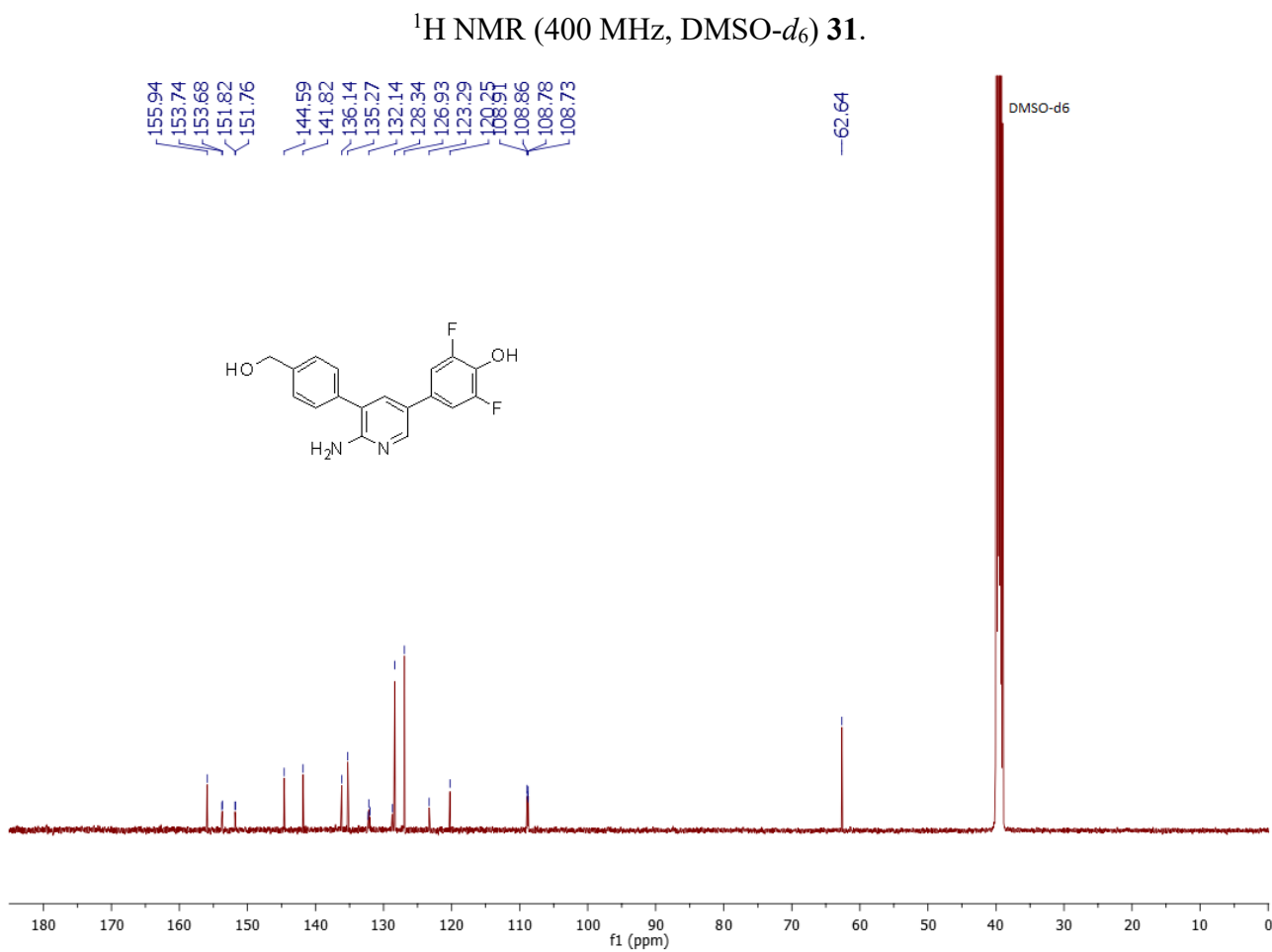
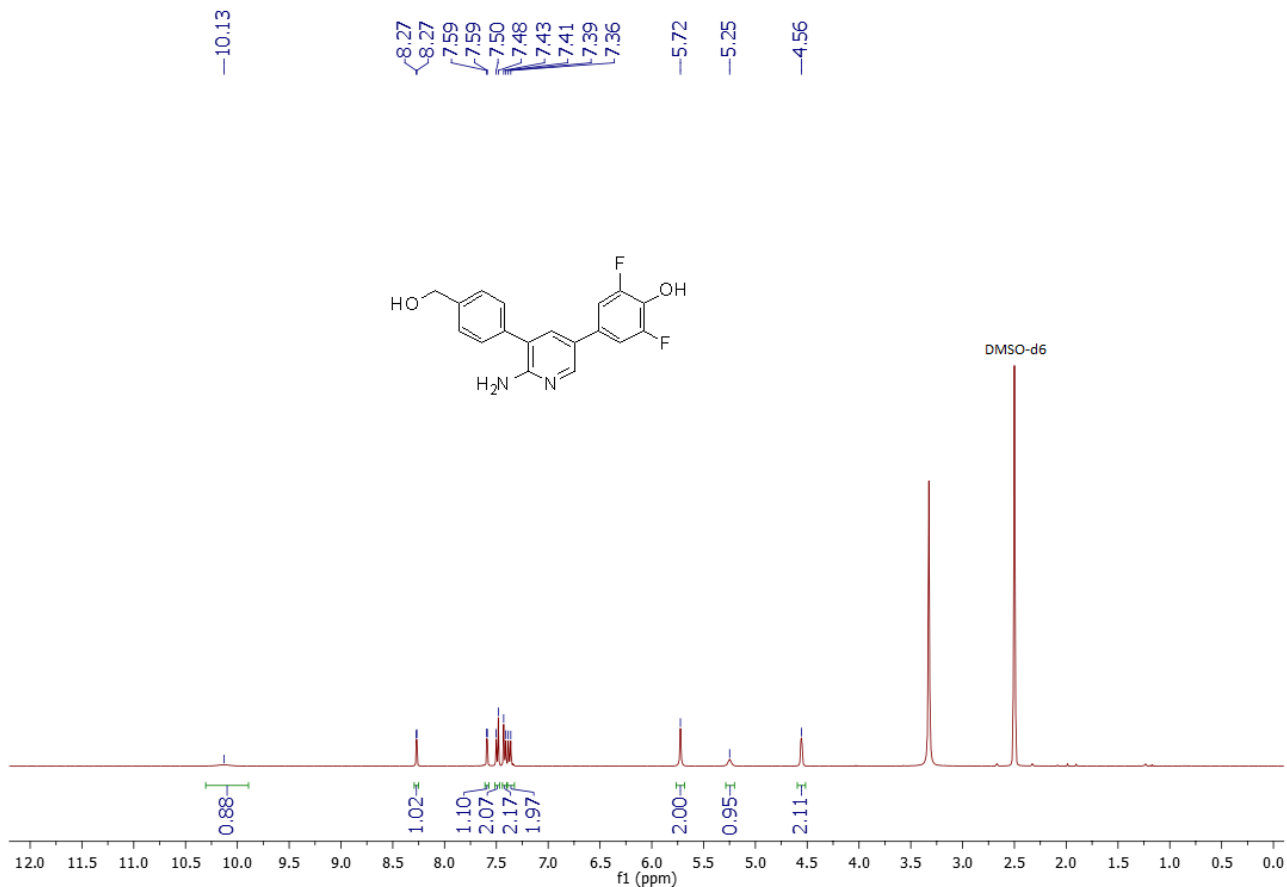


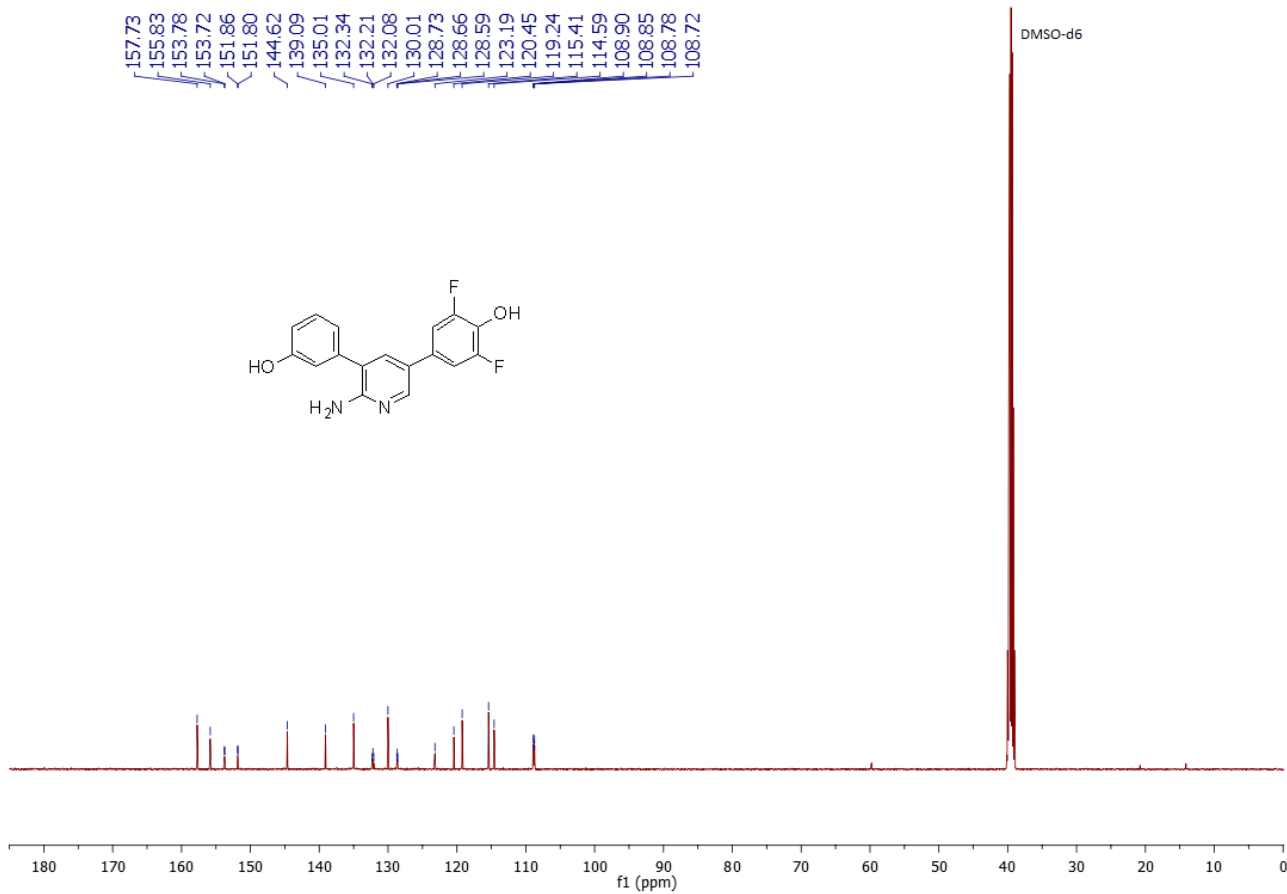
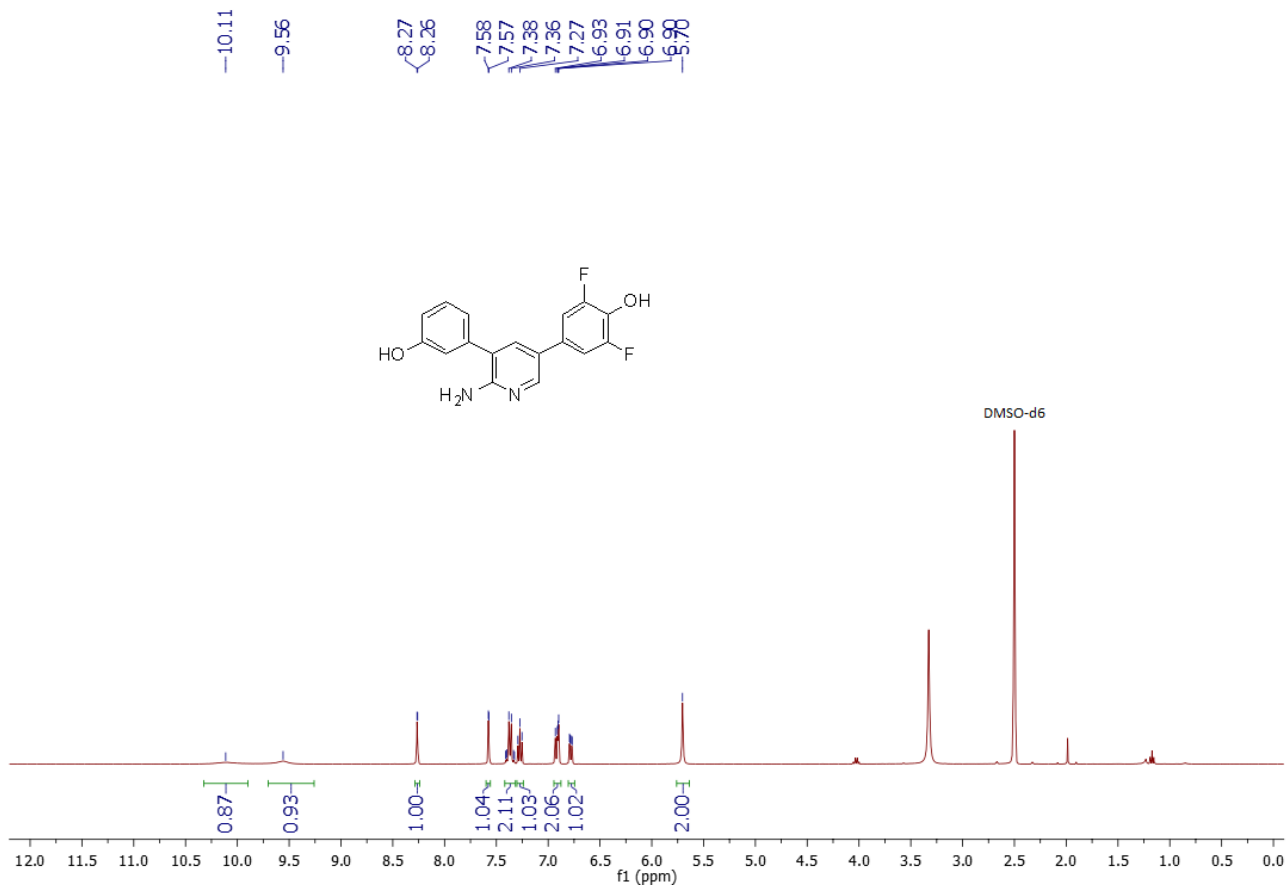


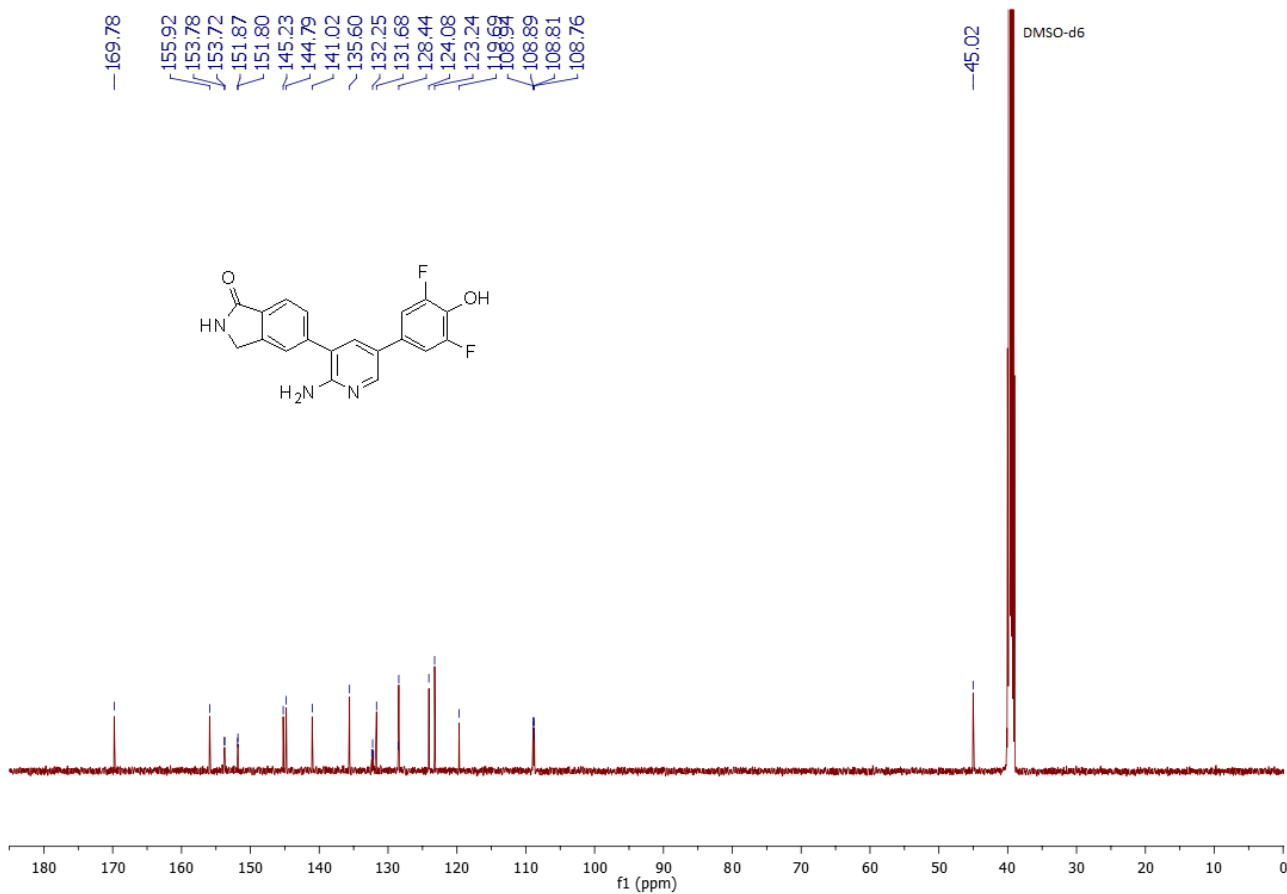
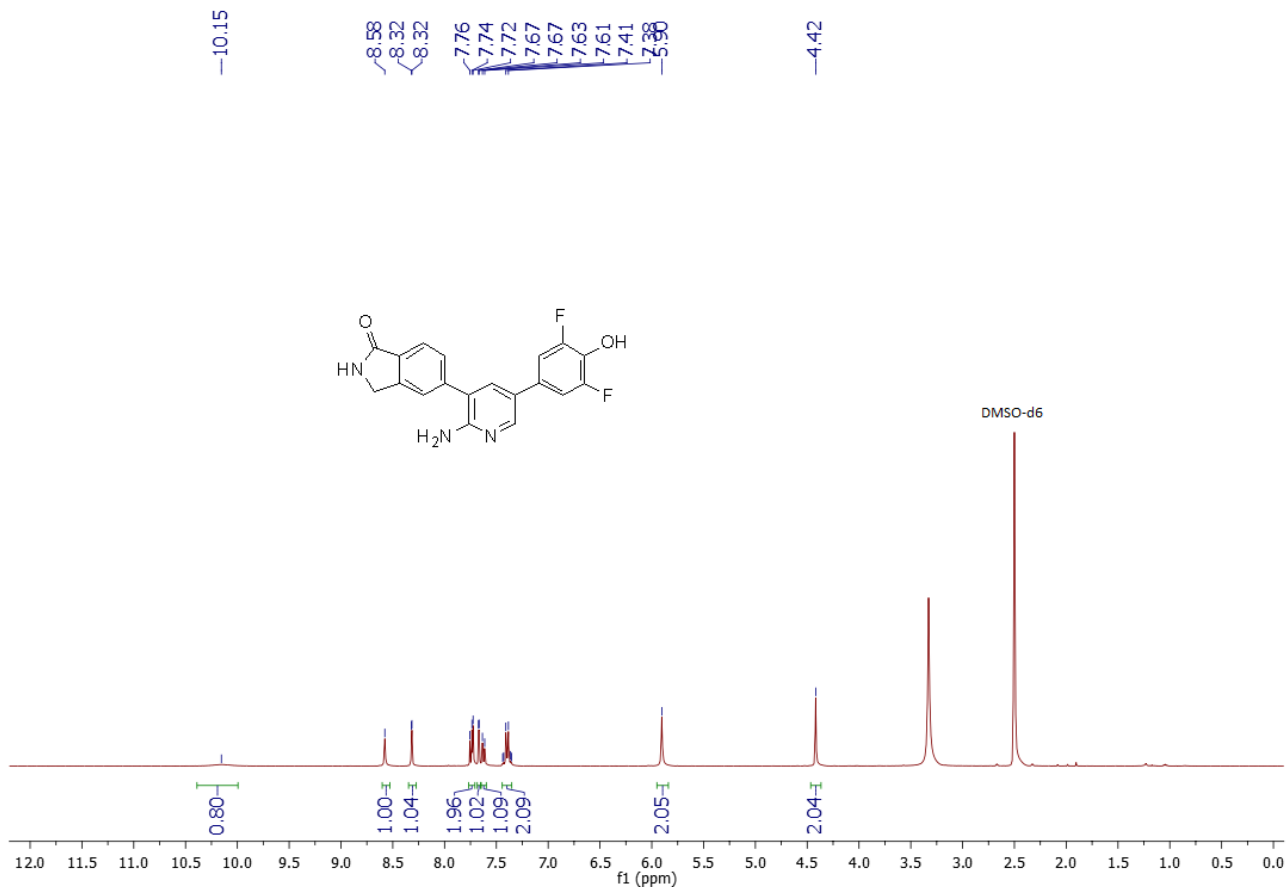




¹³C NMR (126 MHz, DMSO-*d*₆) 30.

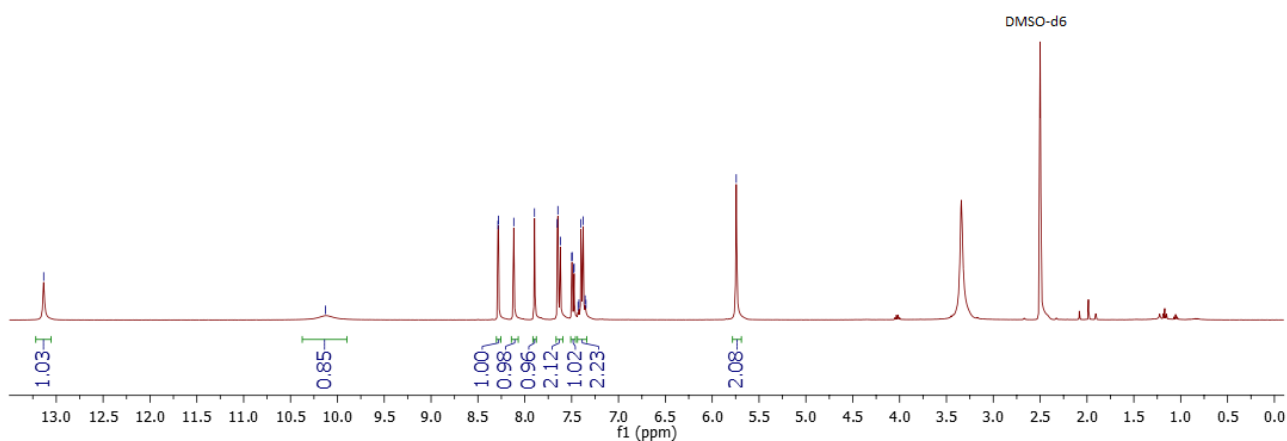
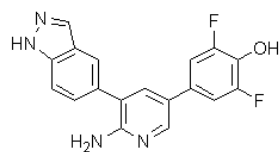






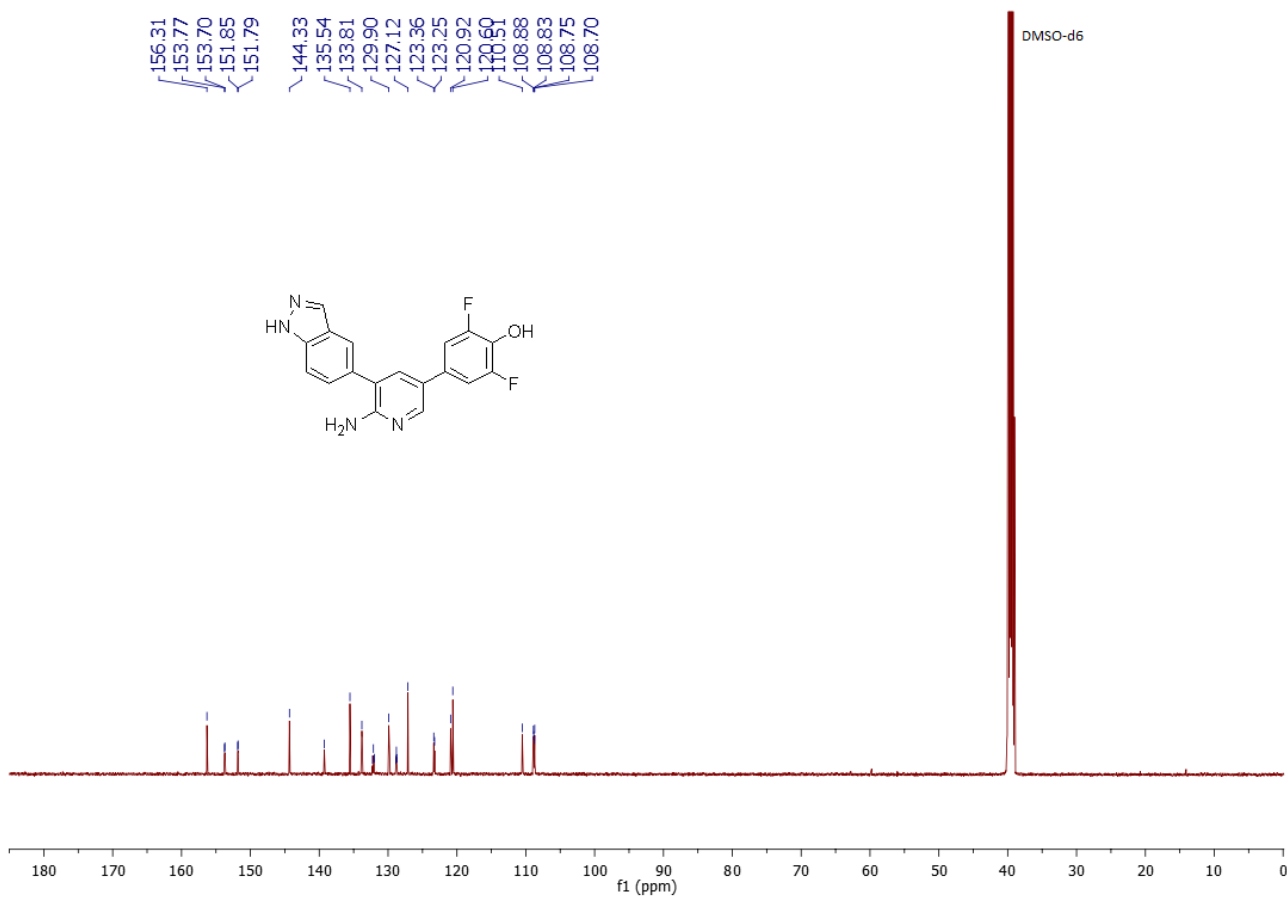
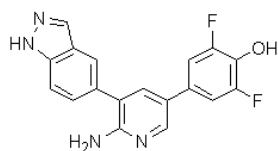
—13.13

—10.13
8.29
8.28
8.12
7.90
7.65
7.65
7.62
7.50
7.50
7.48
7.47
7.42
7.40
7.38
7.36
5.74

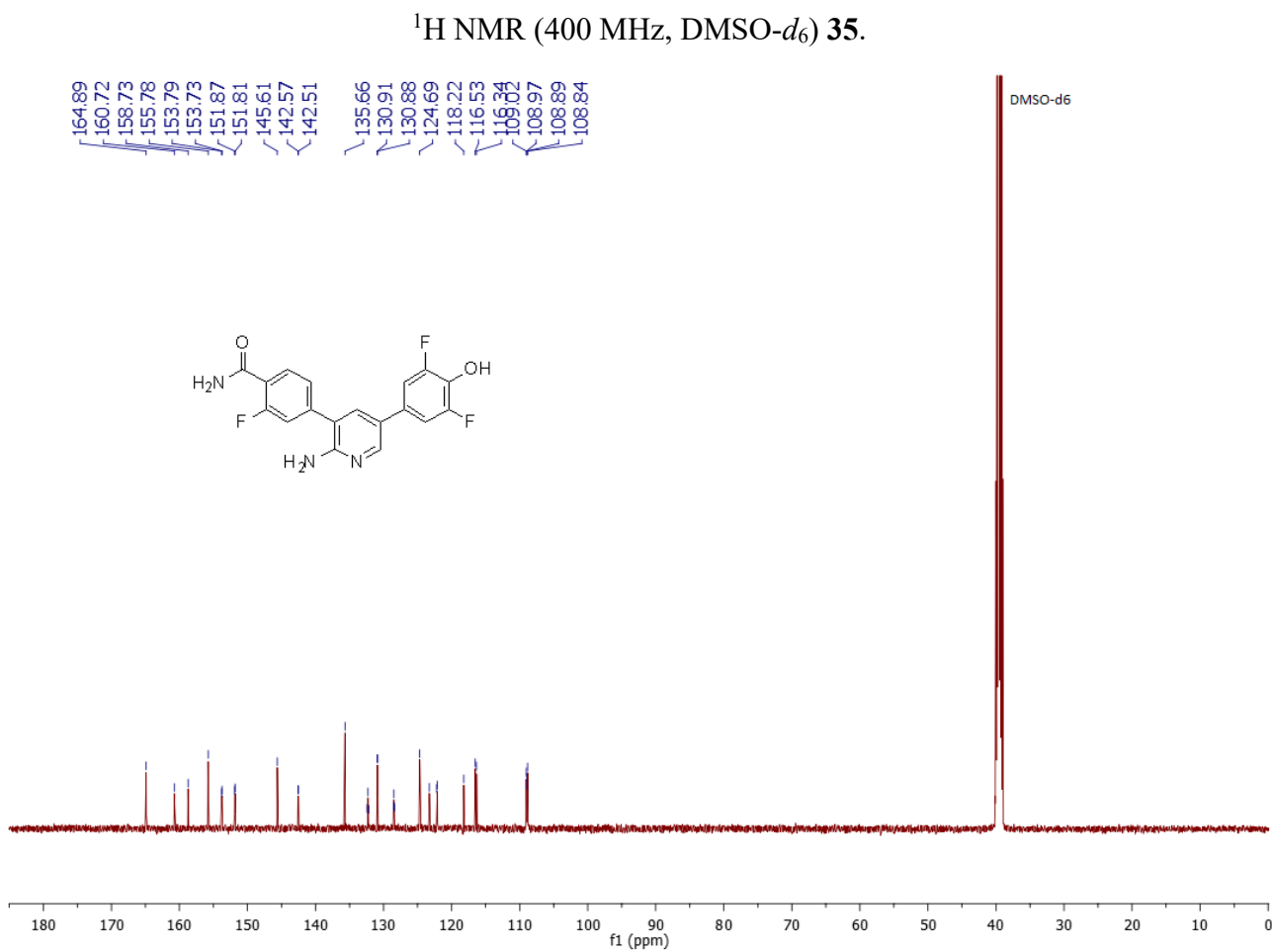
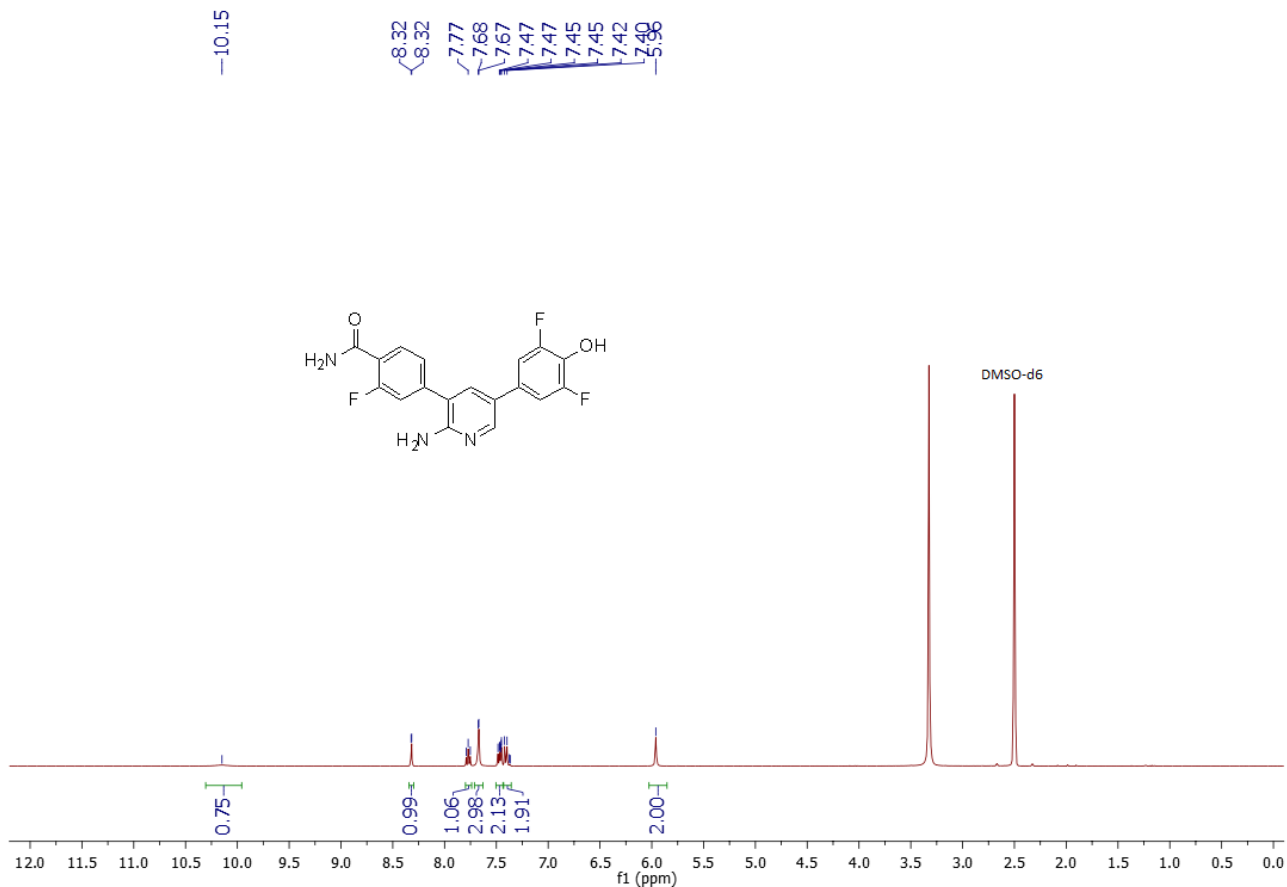


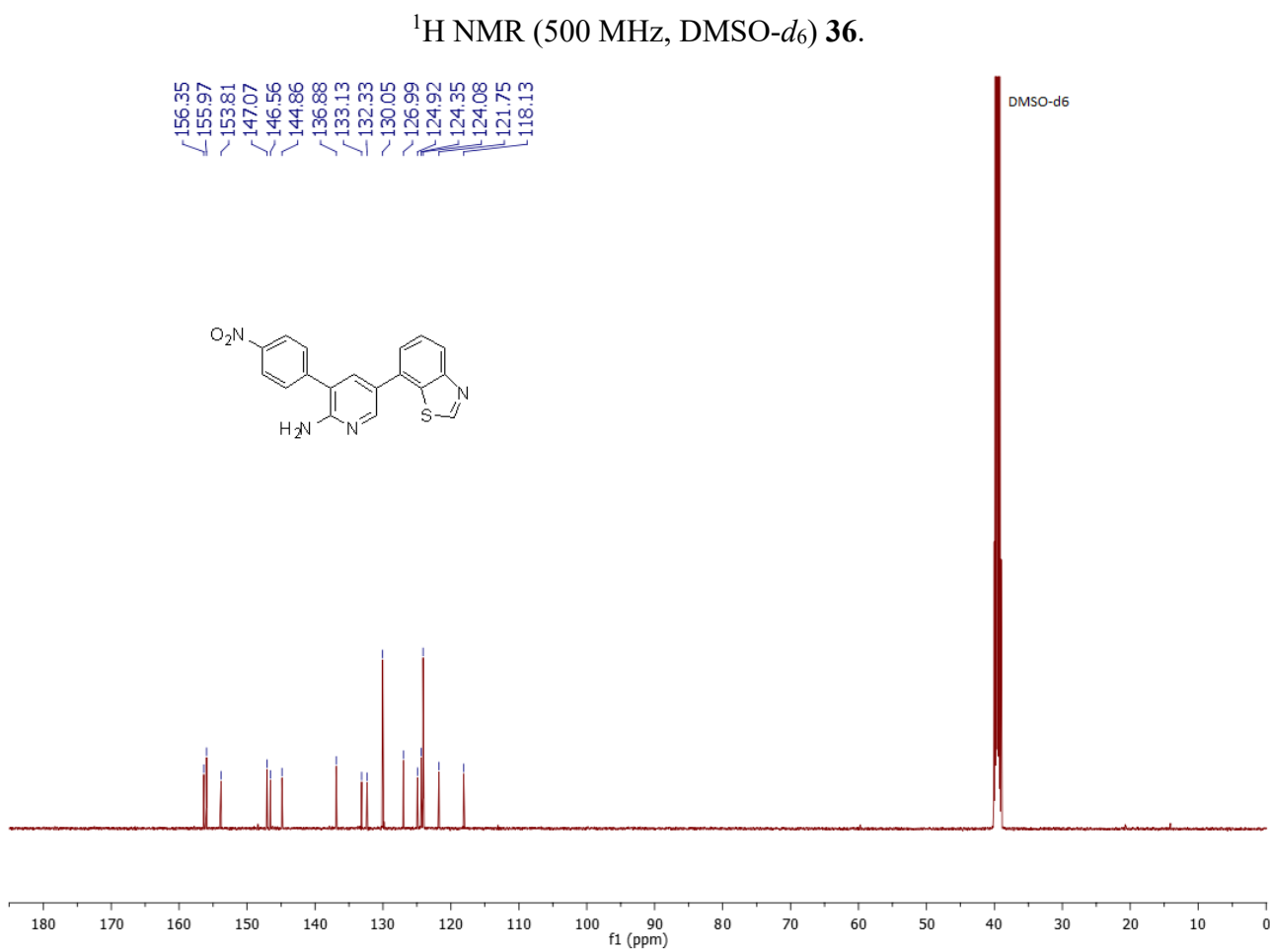
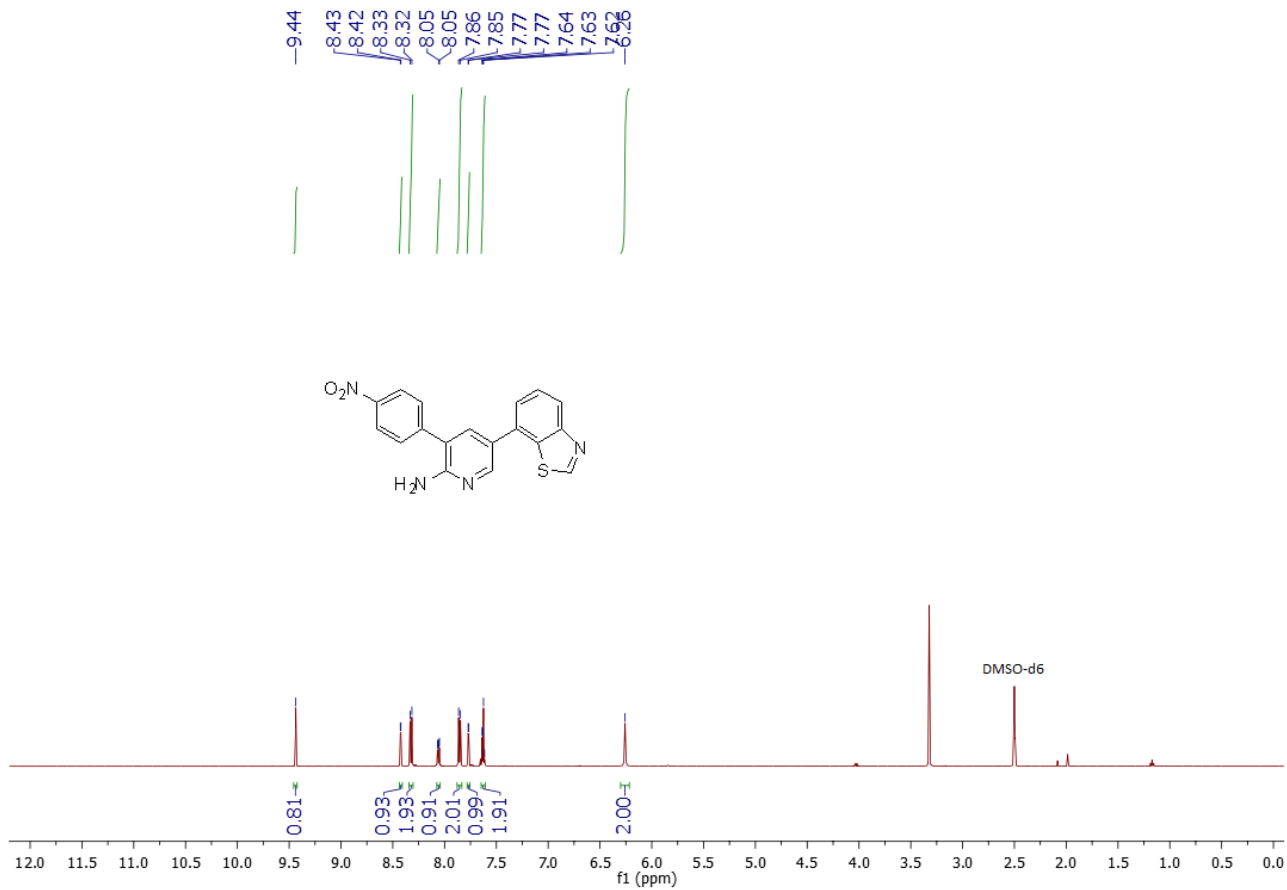
¹H NMR (400 MHz, DMSO-*d*₆) **34**.

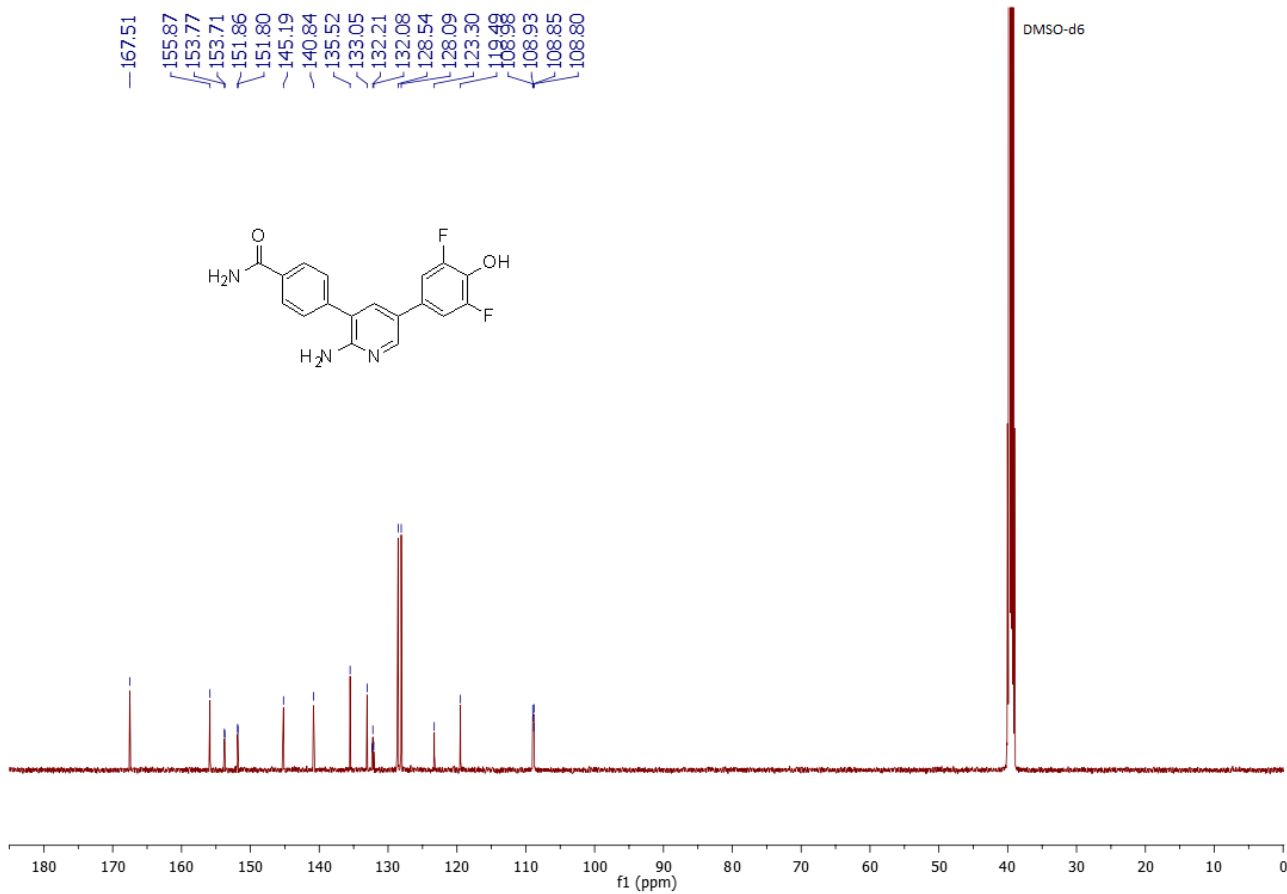
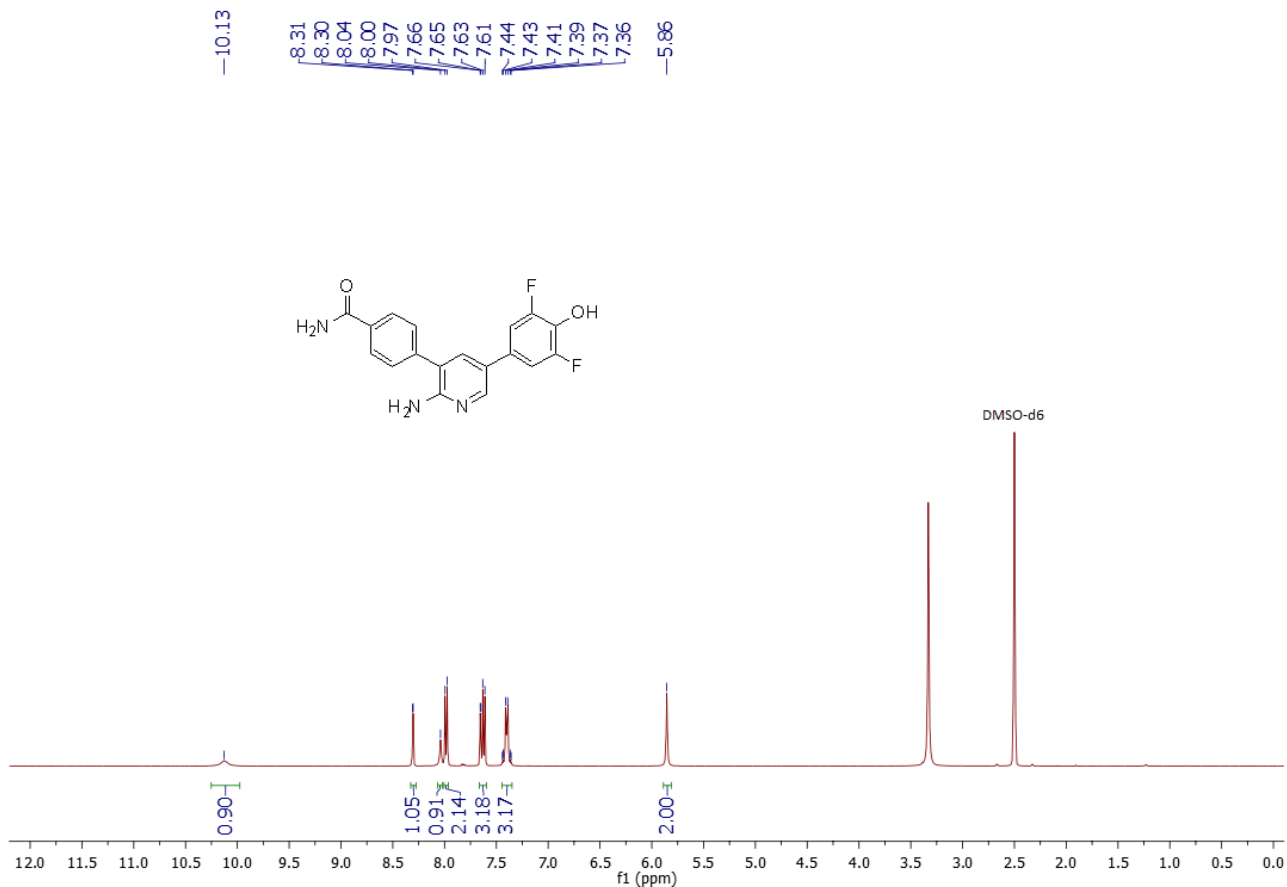
156.31
153.77
153.70
151.85
151.79
144.33
135.54
133.81
129.90
127.12
123.36
123.25
120.92
118.61
108.88
108.83
108.75
108.70

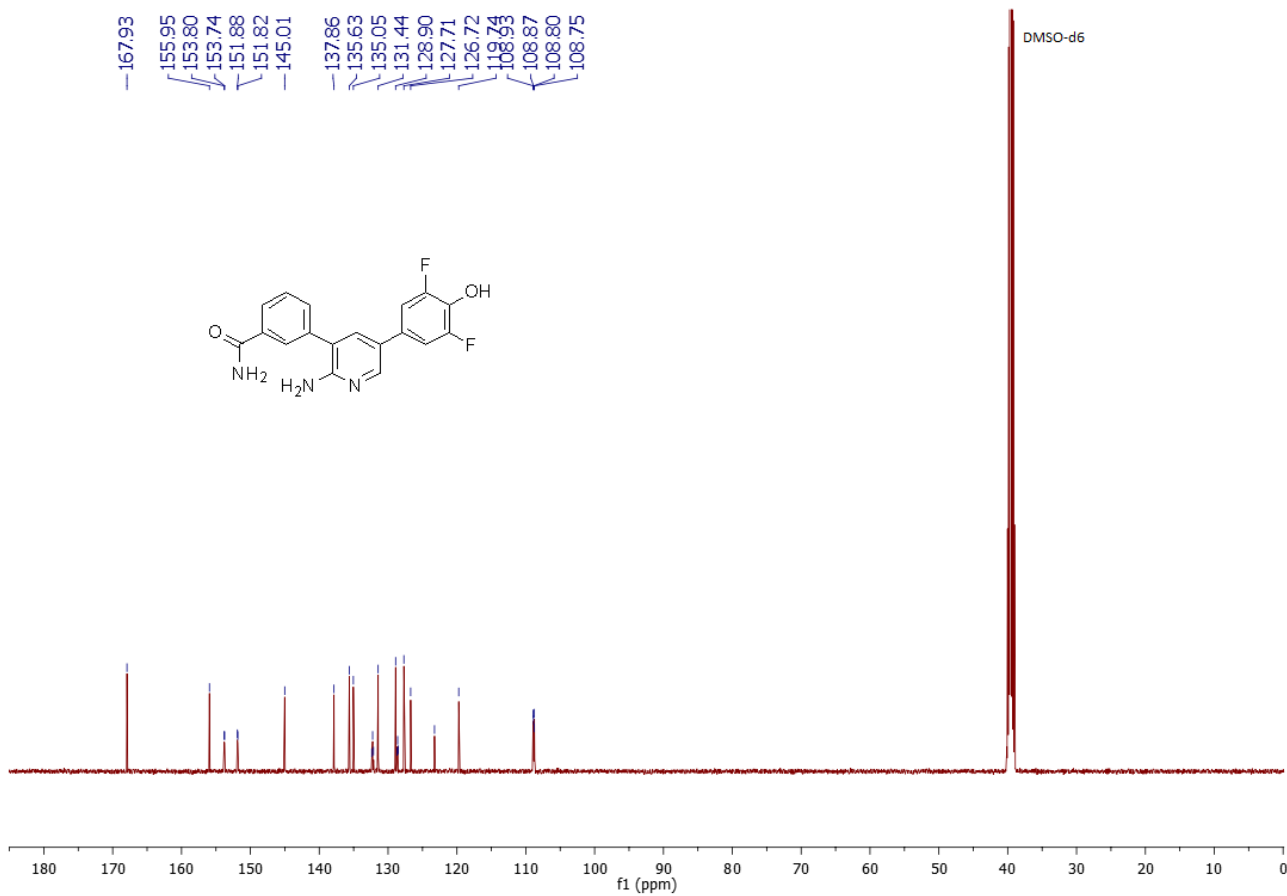
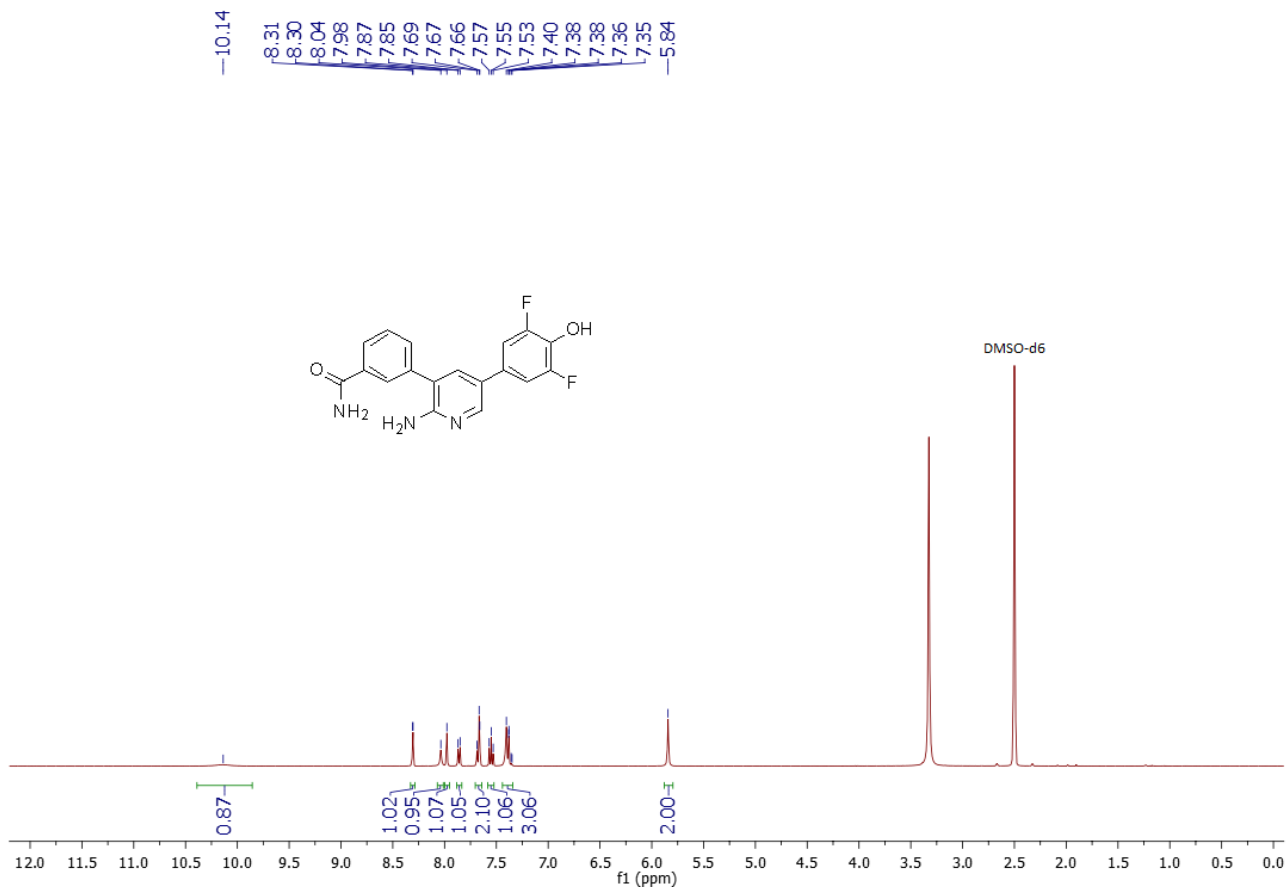


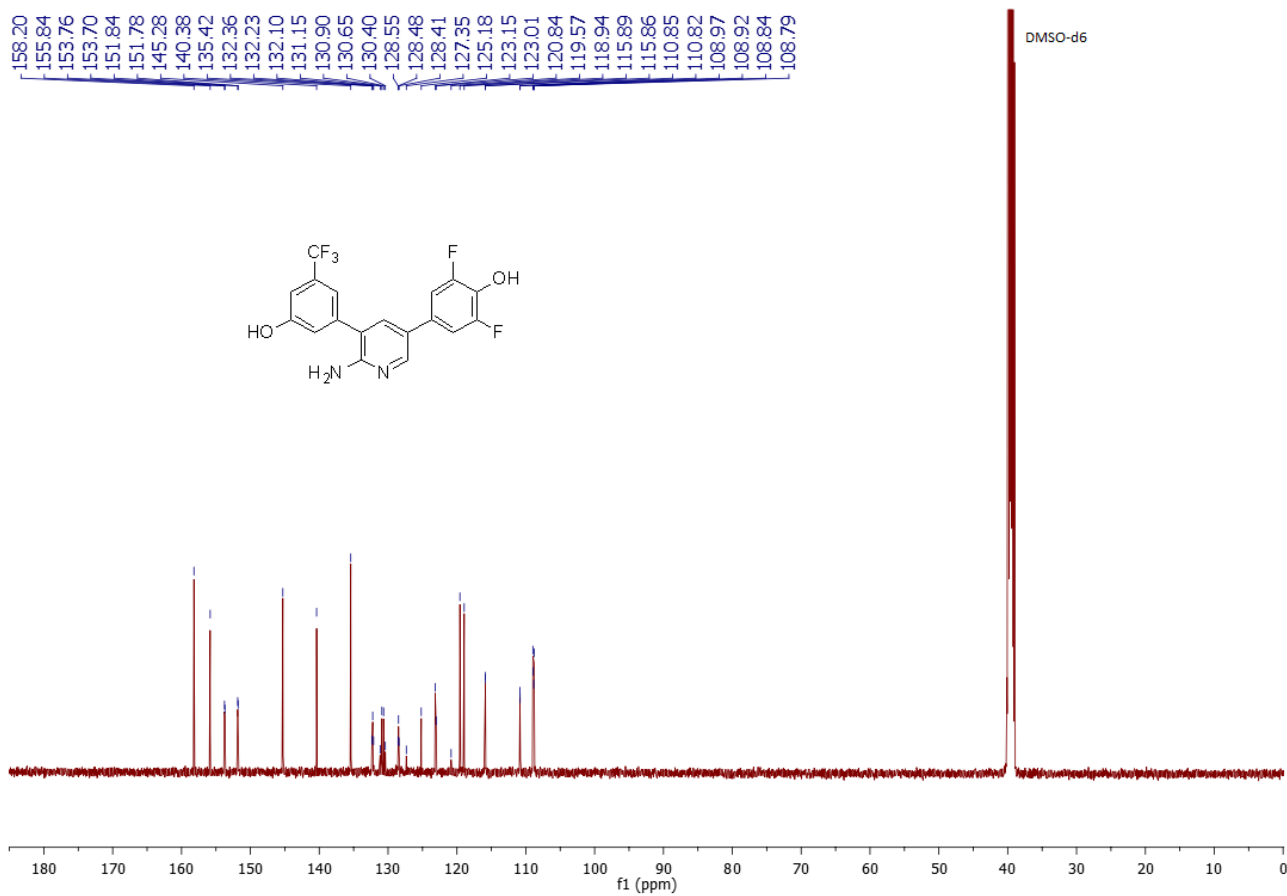
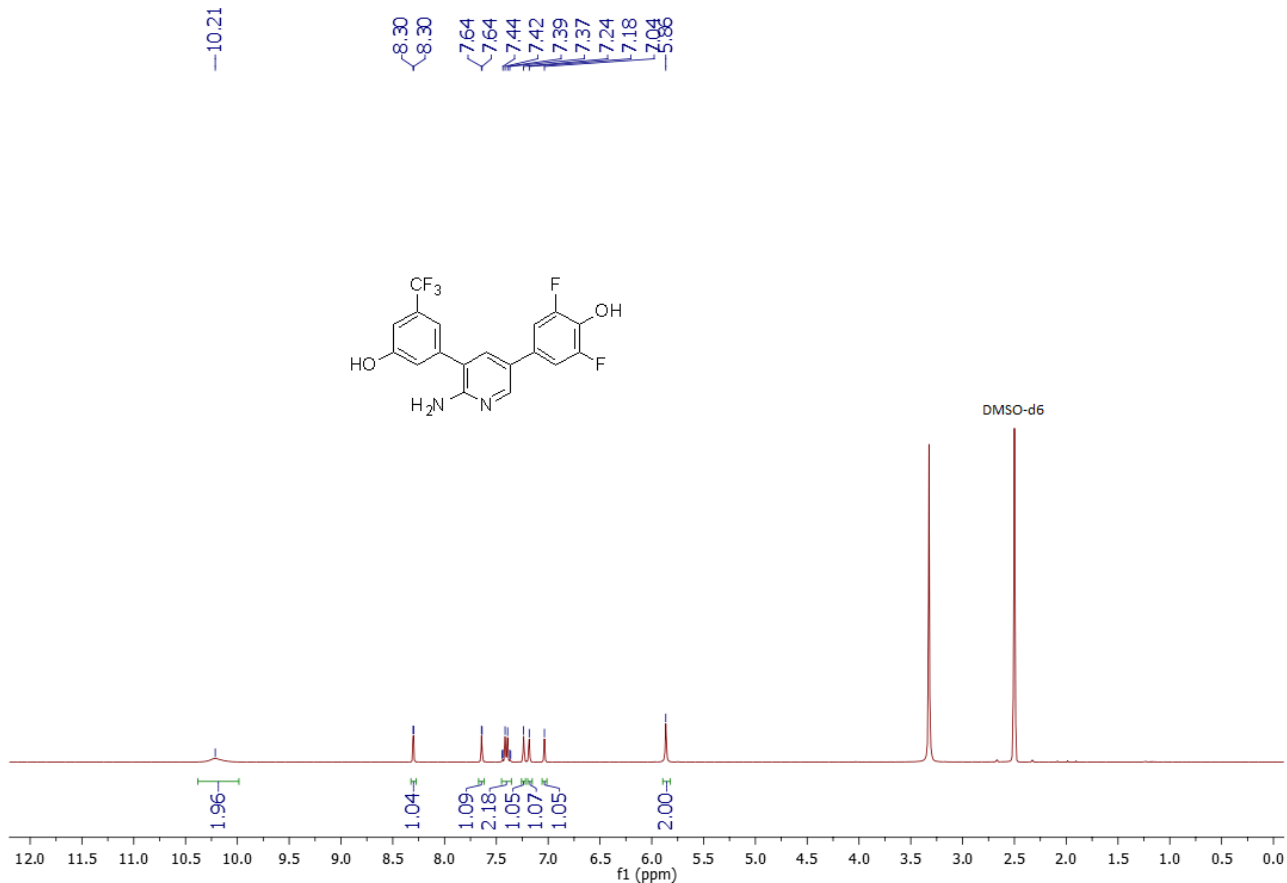
¹³C NMR (126 MHz, DMSO-*d*₆) **34**.

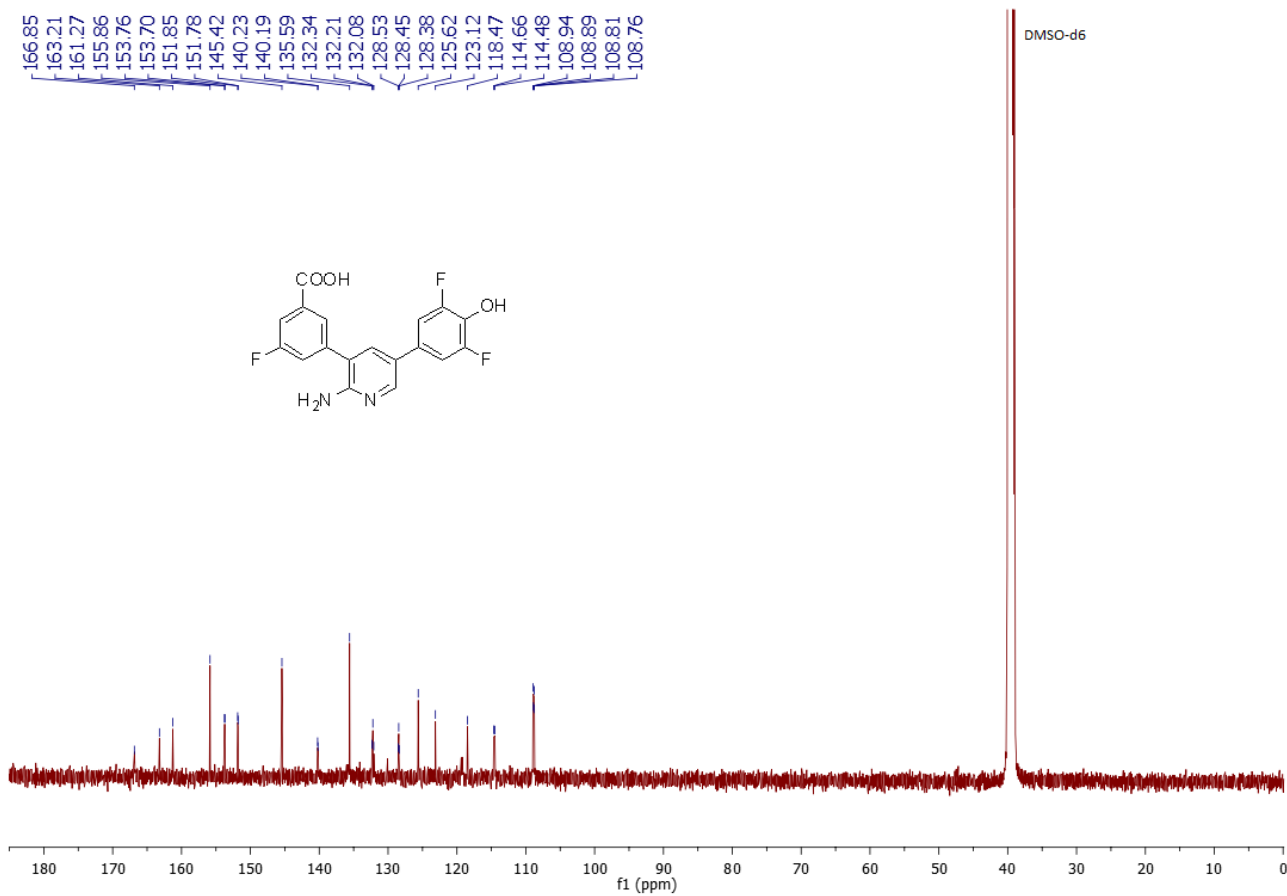
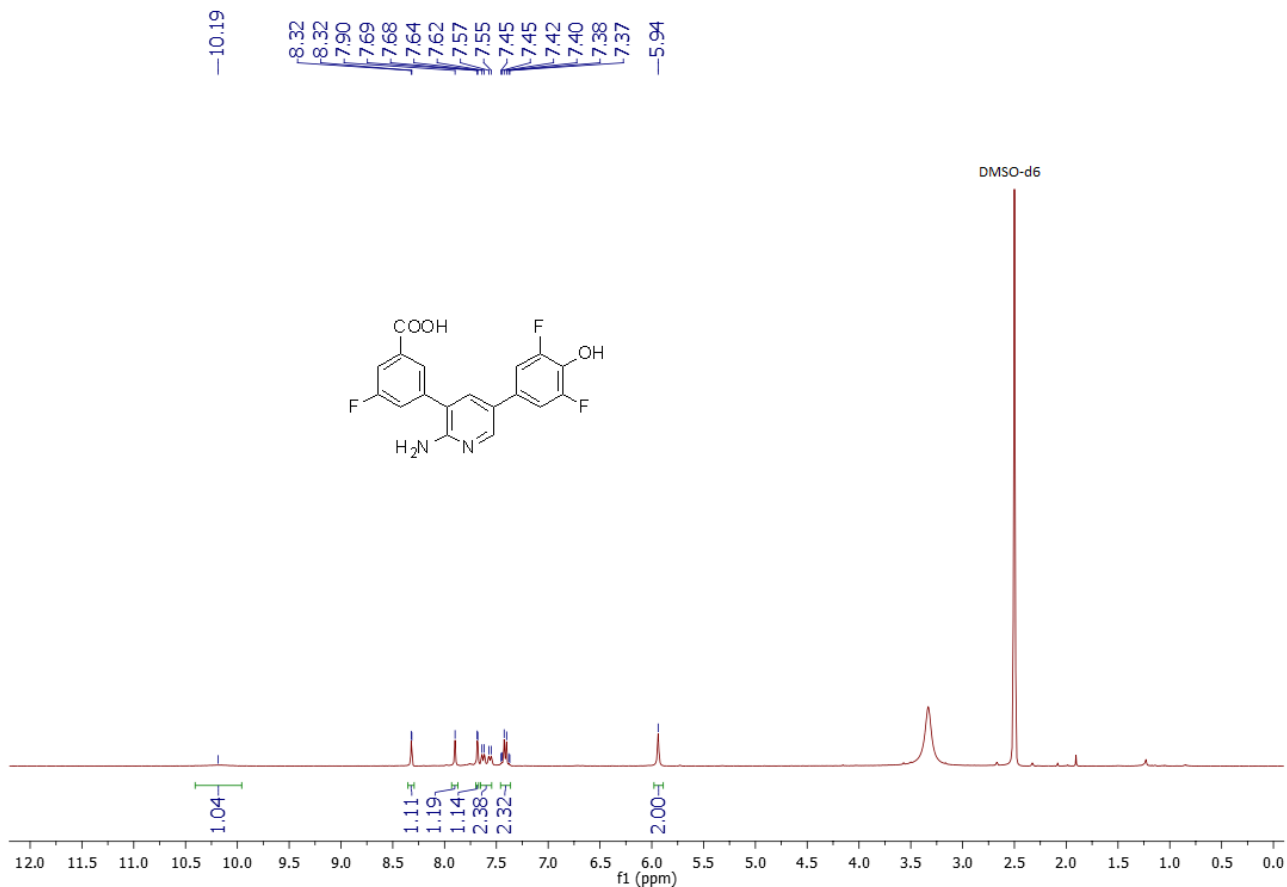


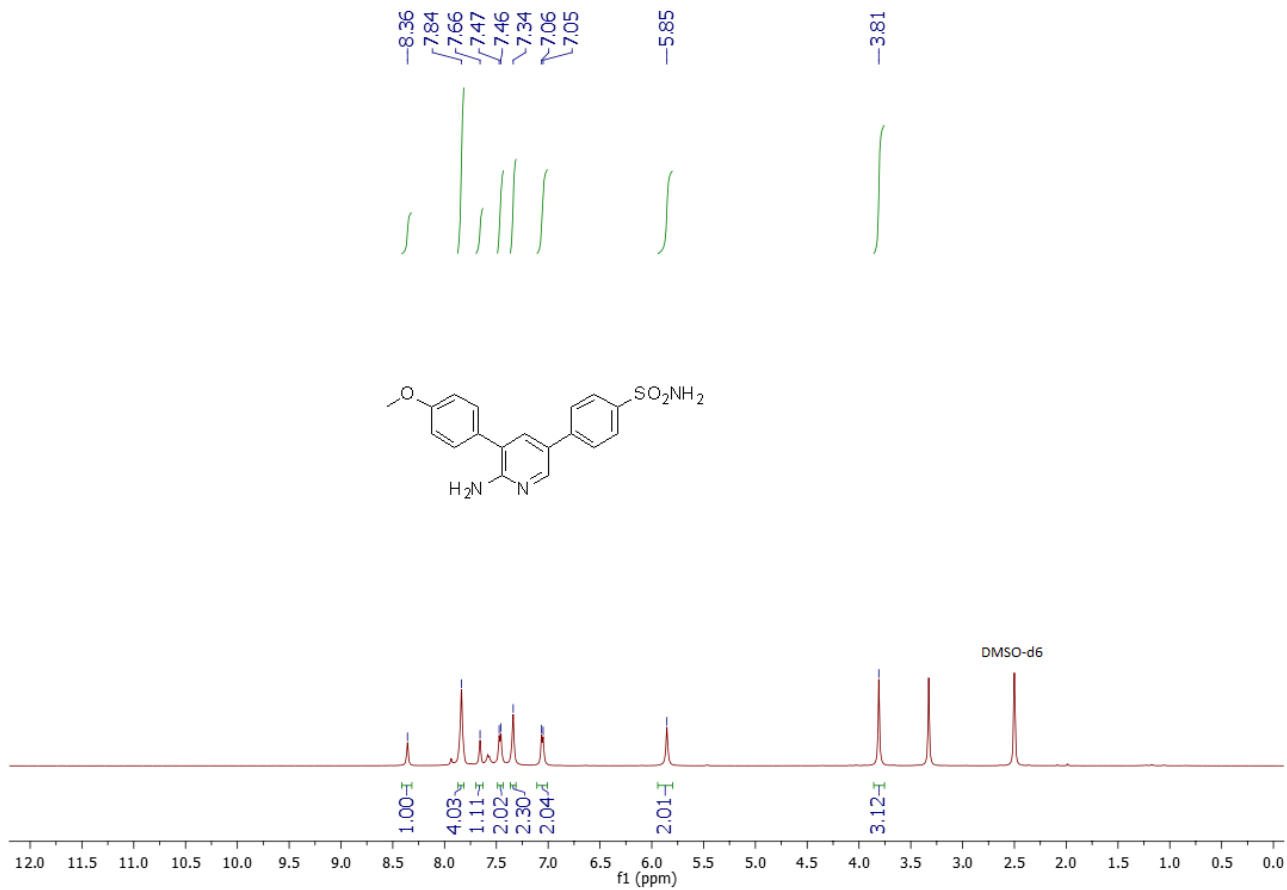




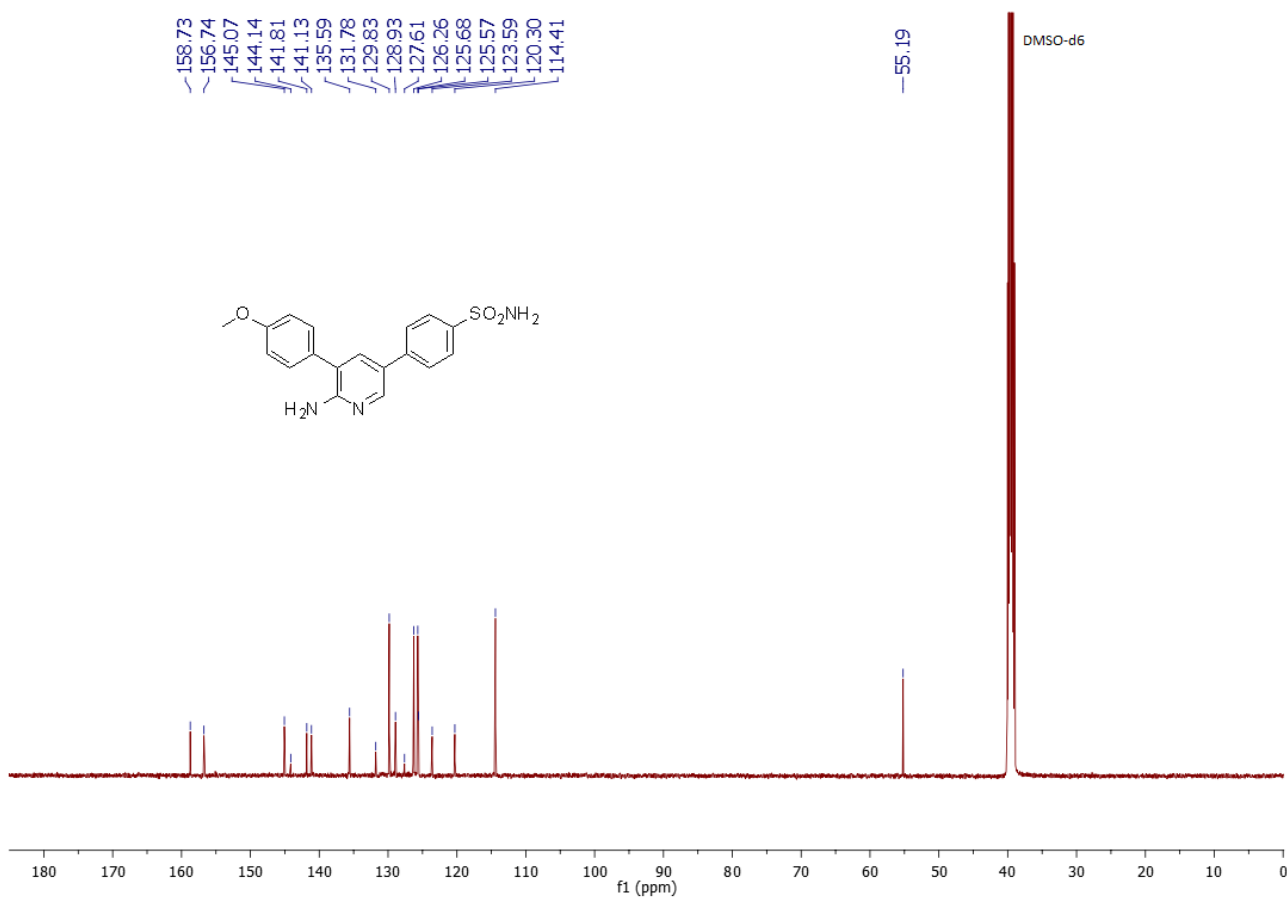




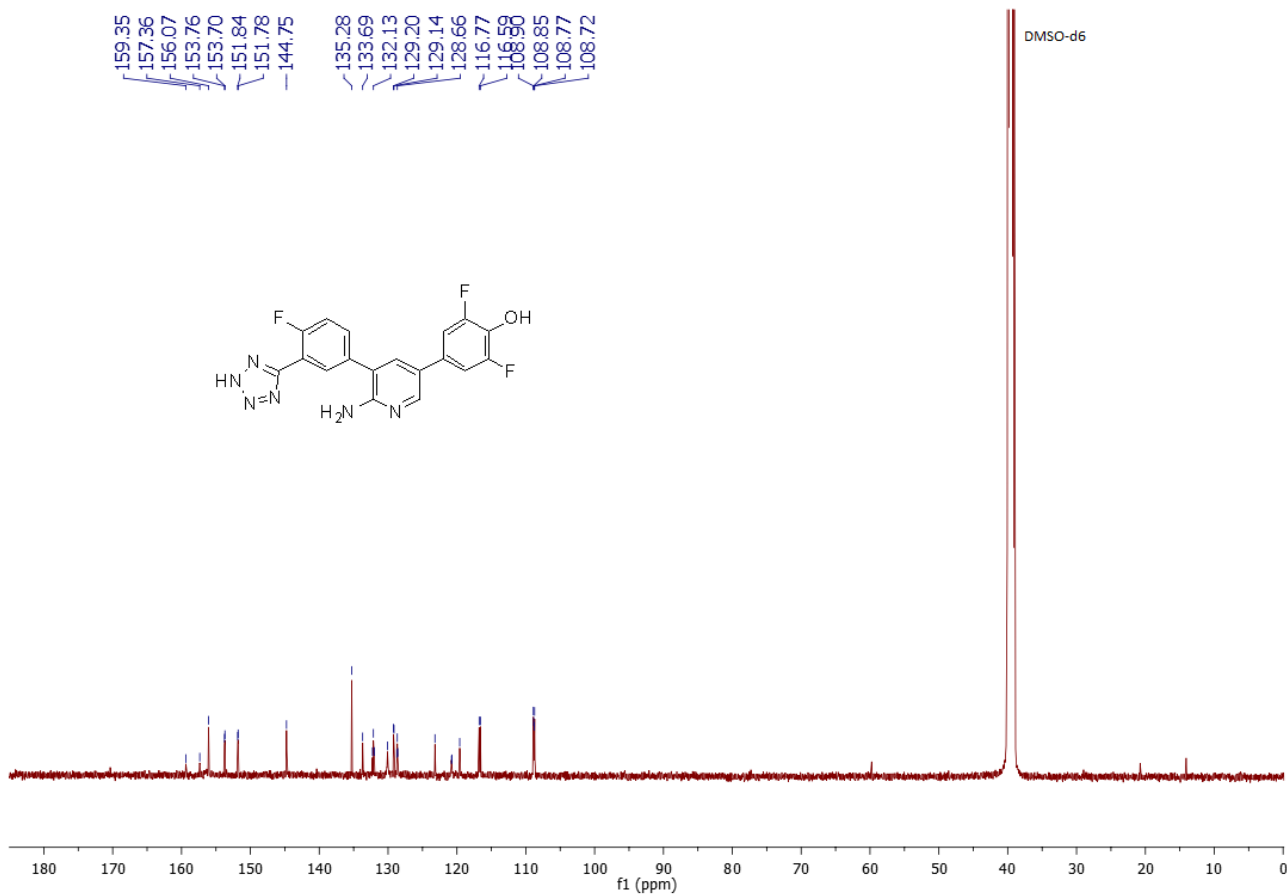
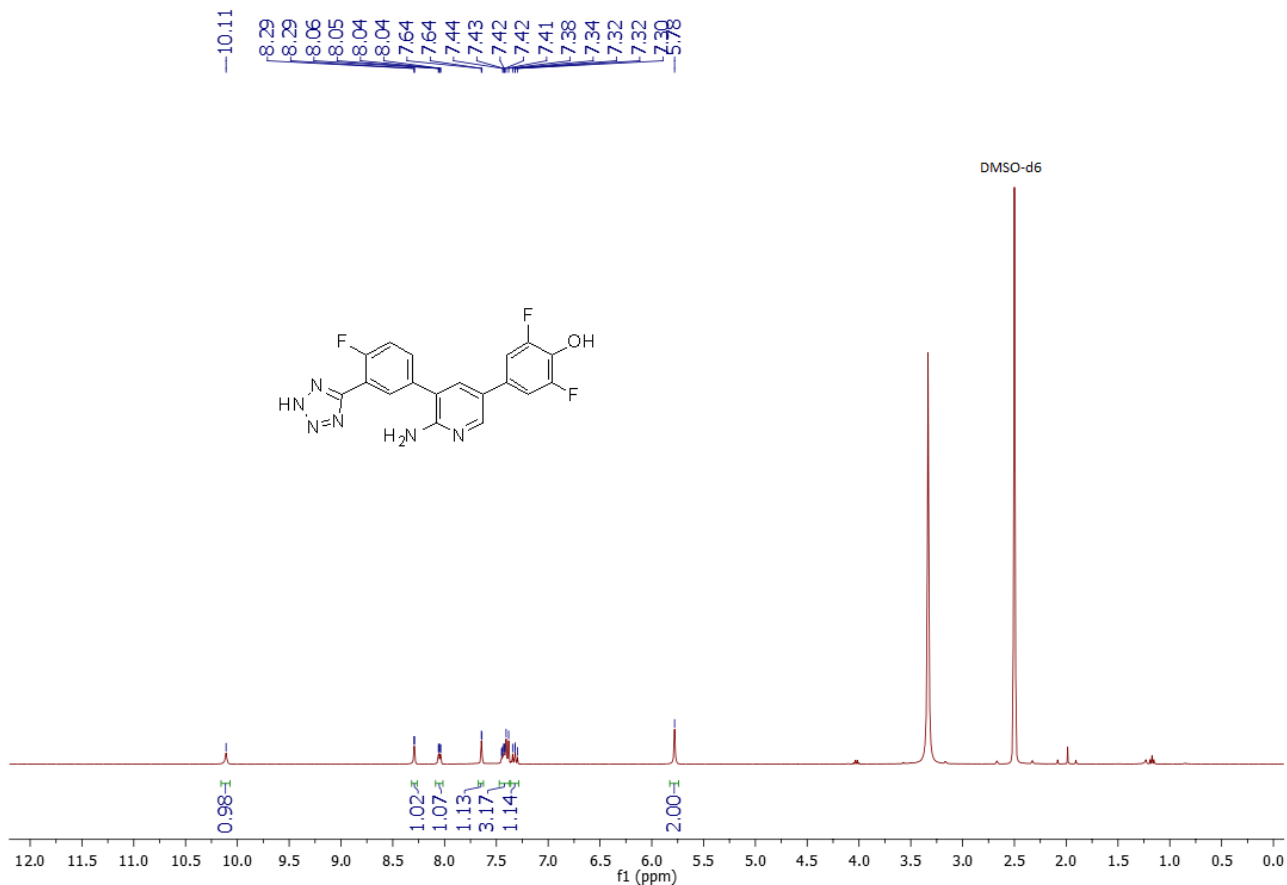


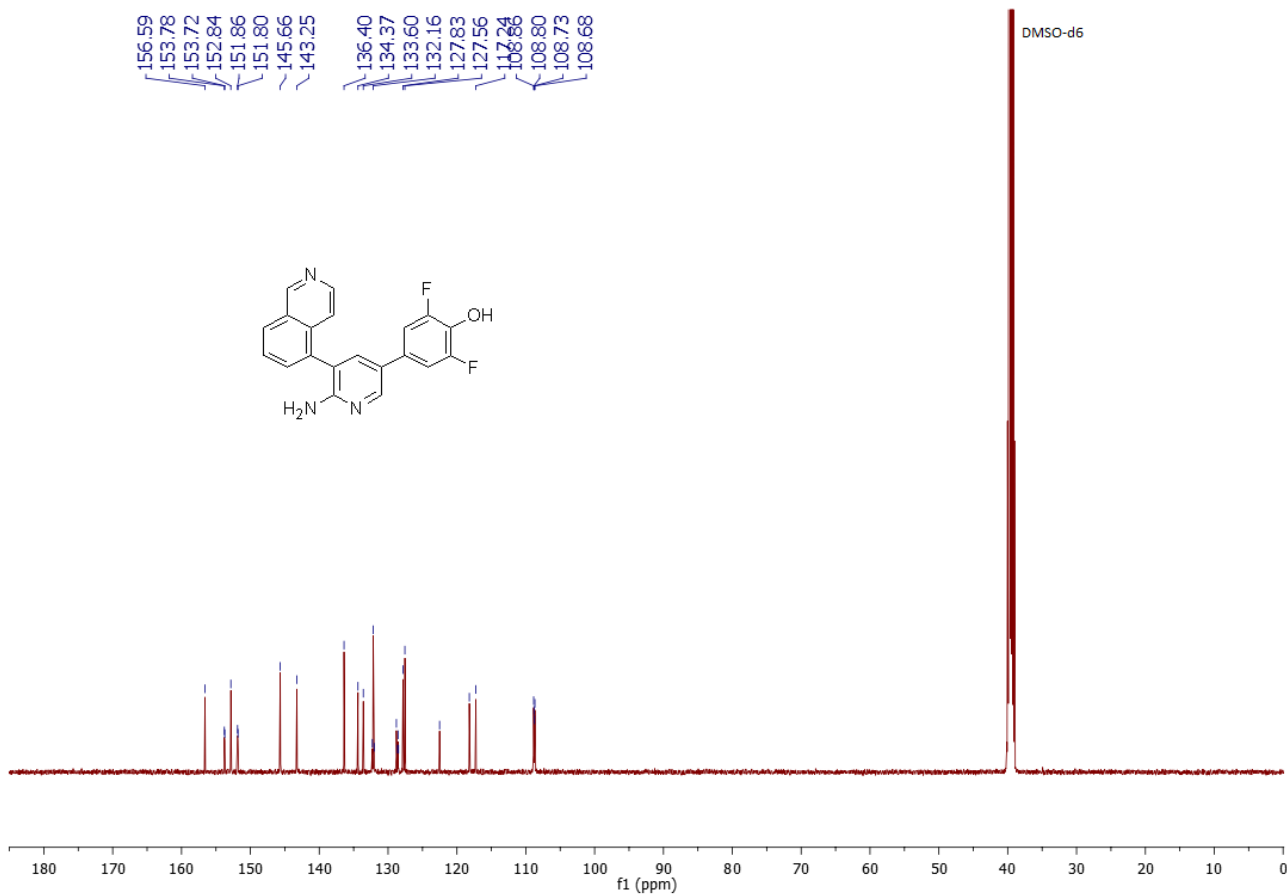
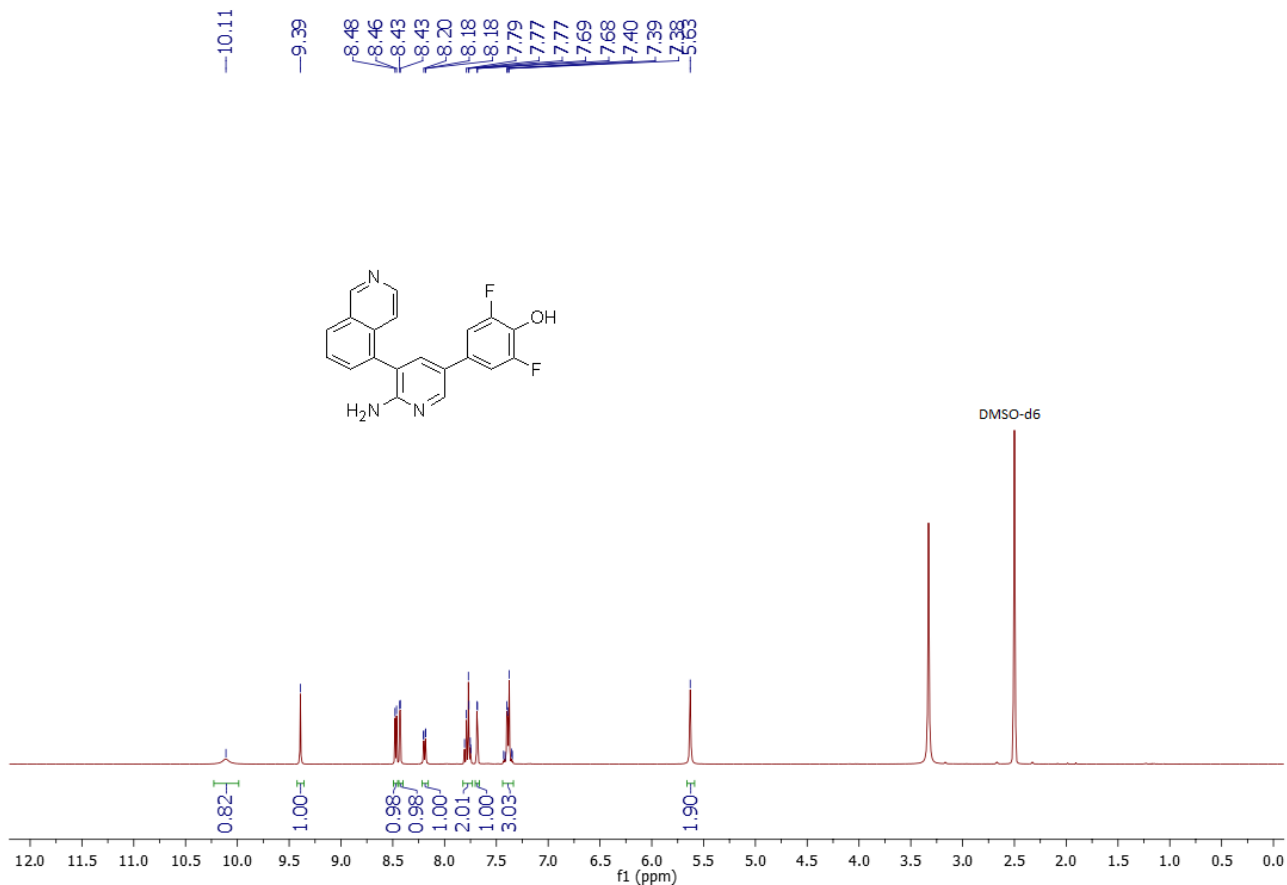


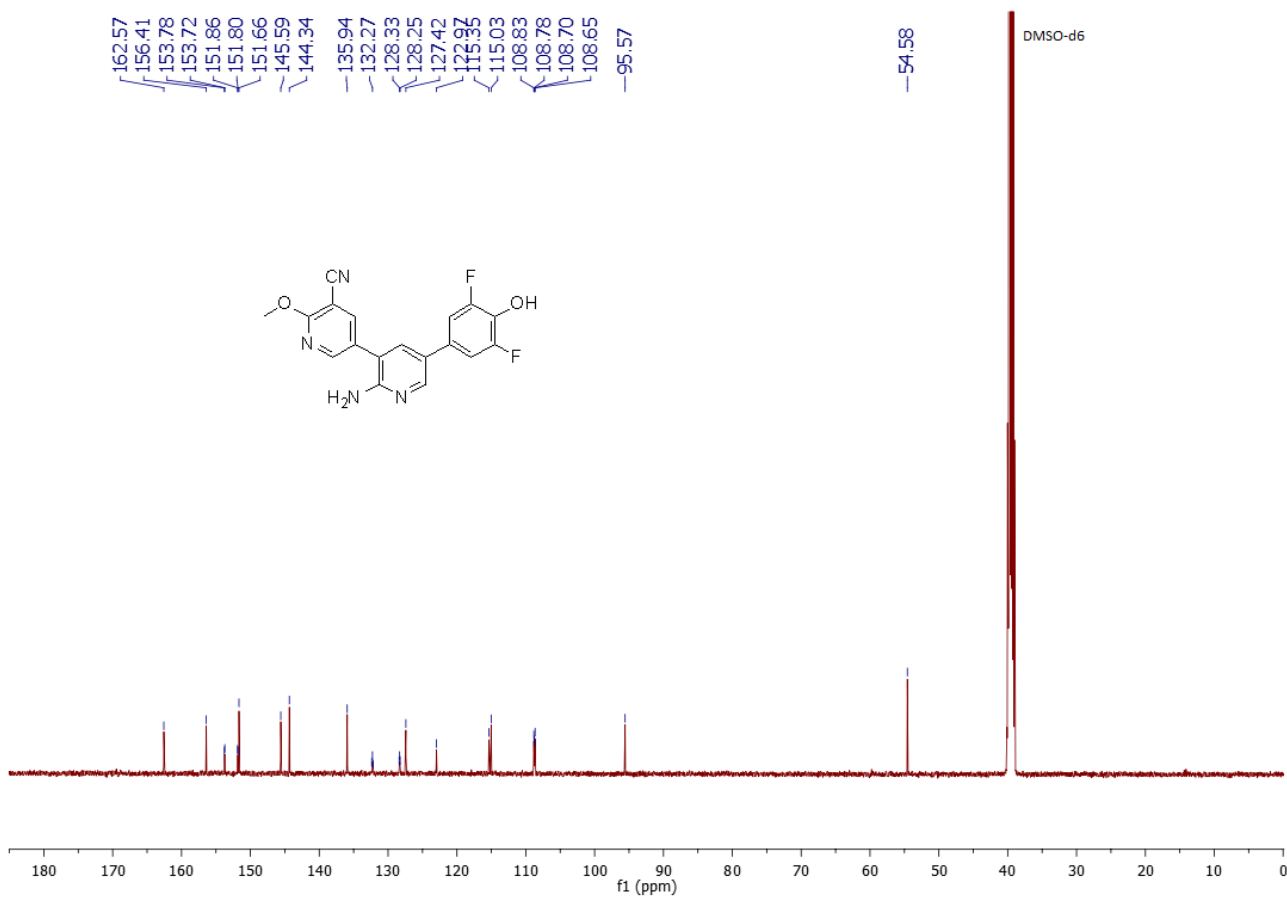
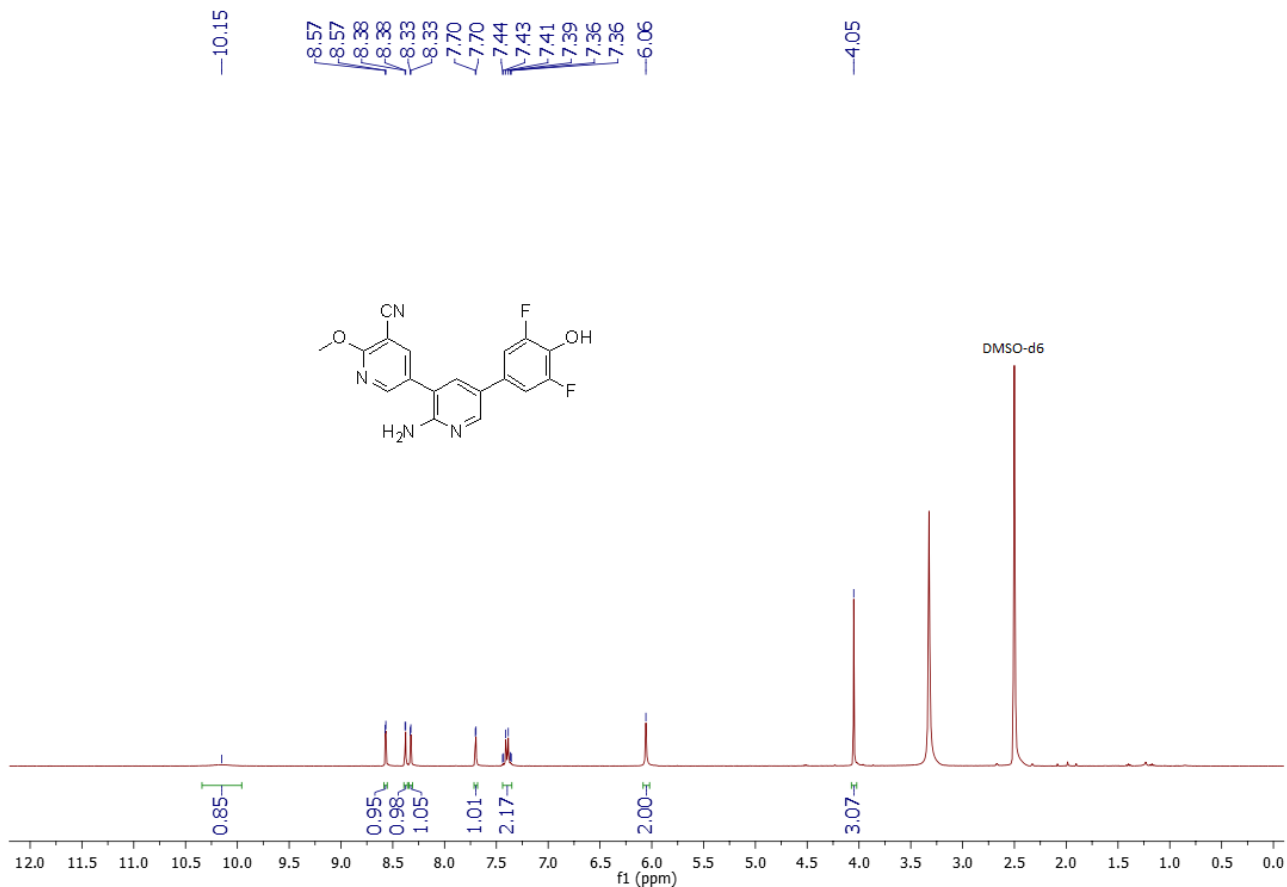
¹H NMR (500 MHz, DMSO-*d*₆) 41.

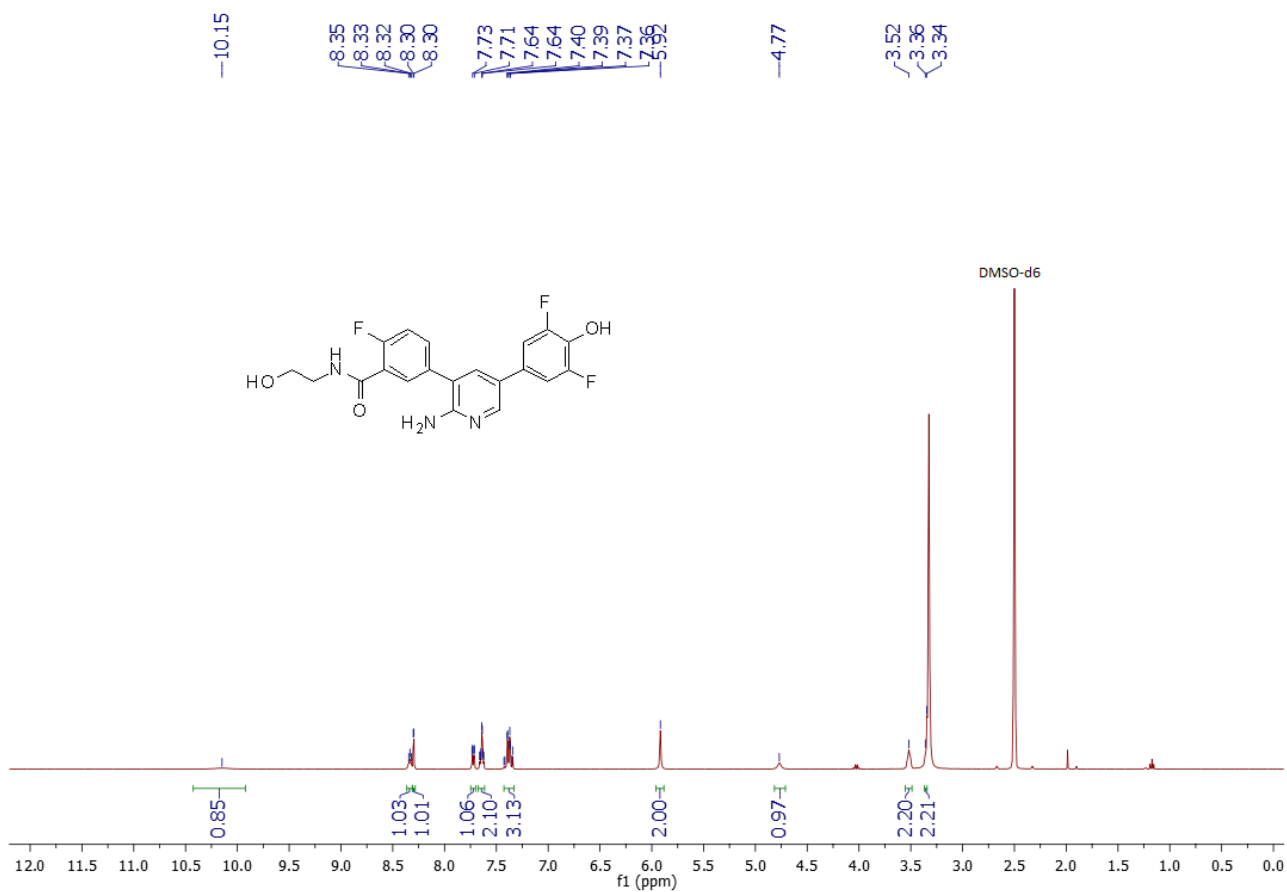


¹³C NMR (126 MHz, DMSO-*d*₆) 41.

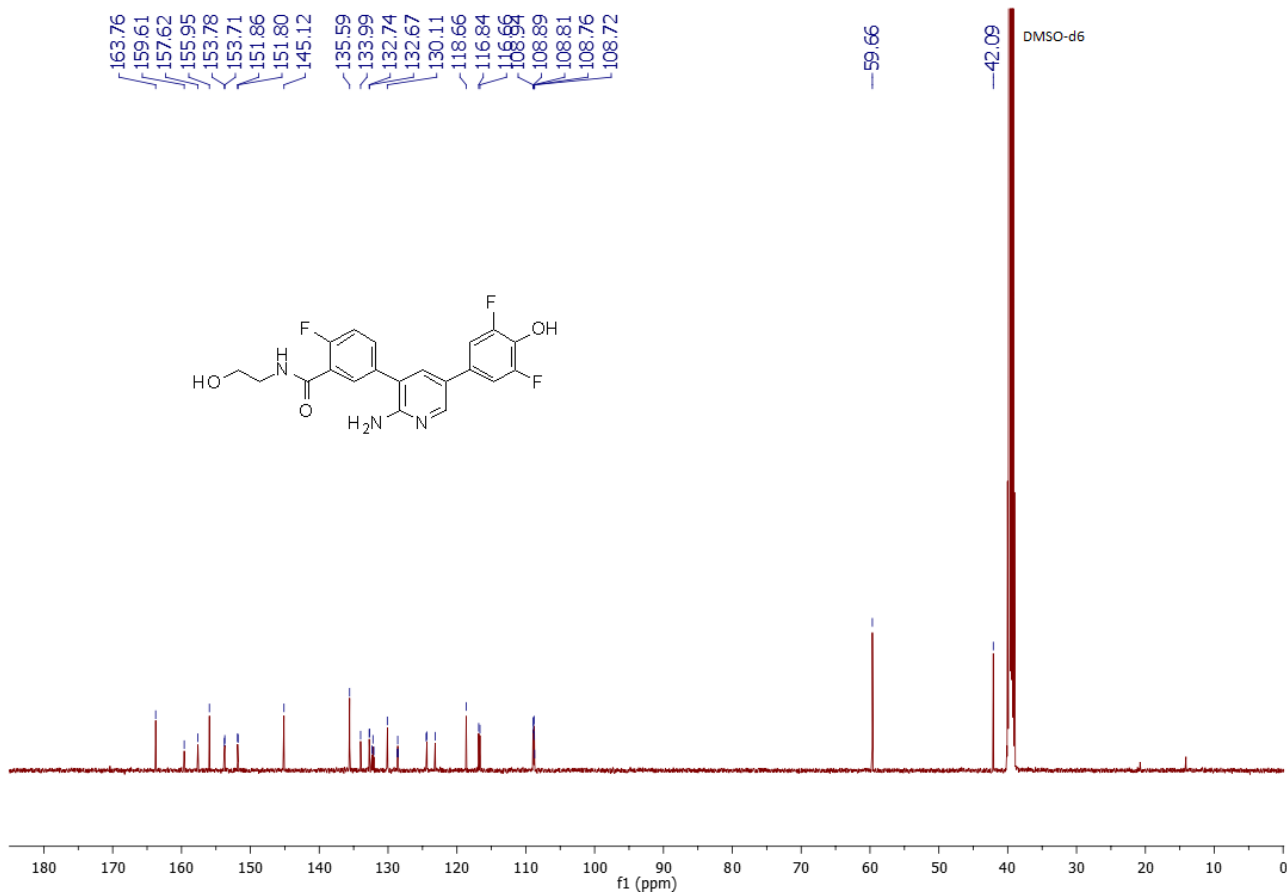




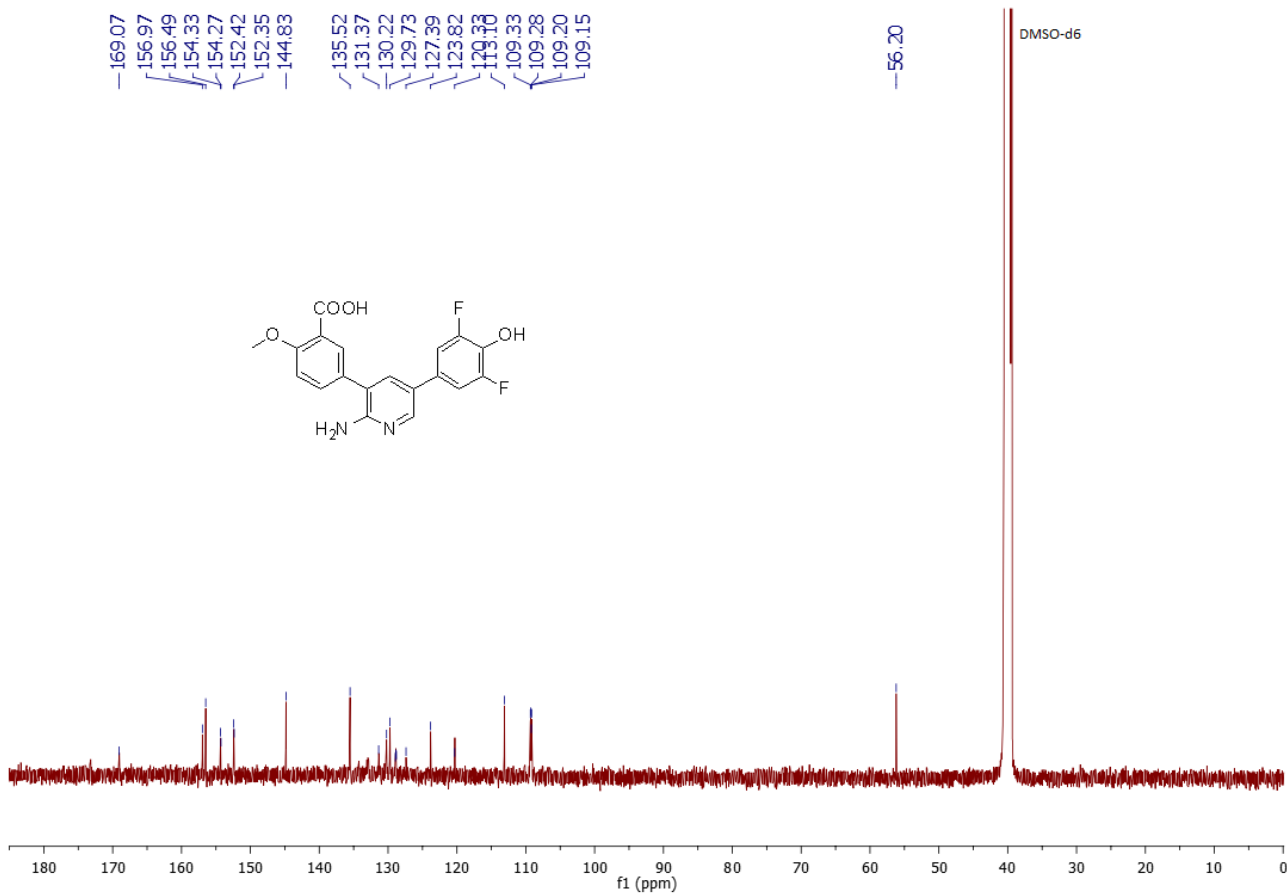
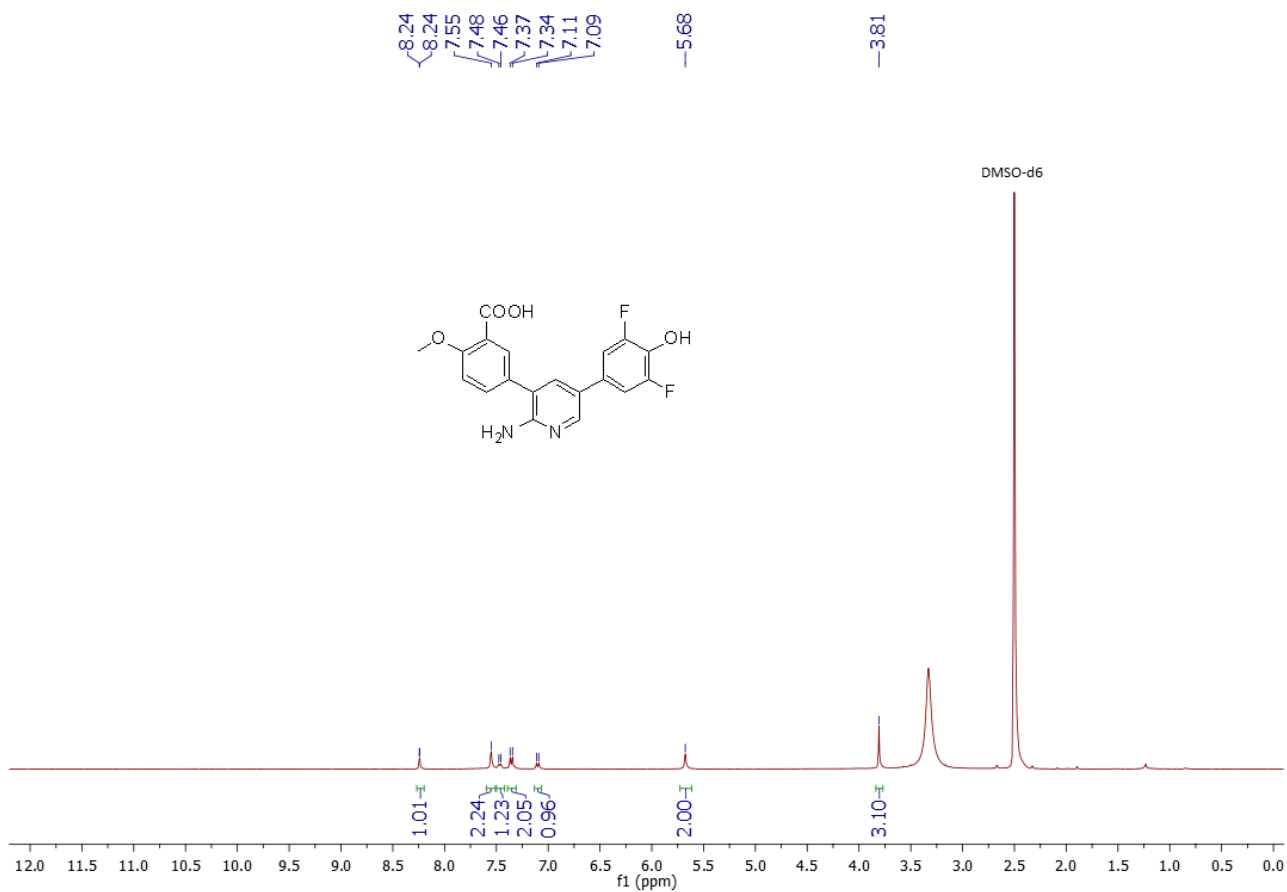


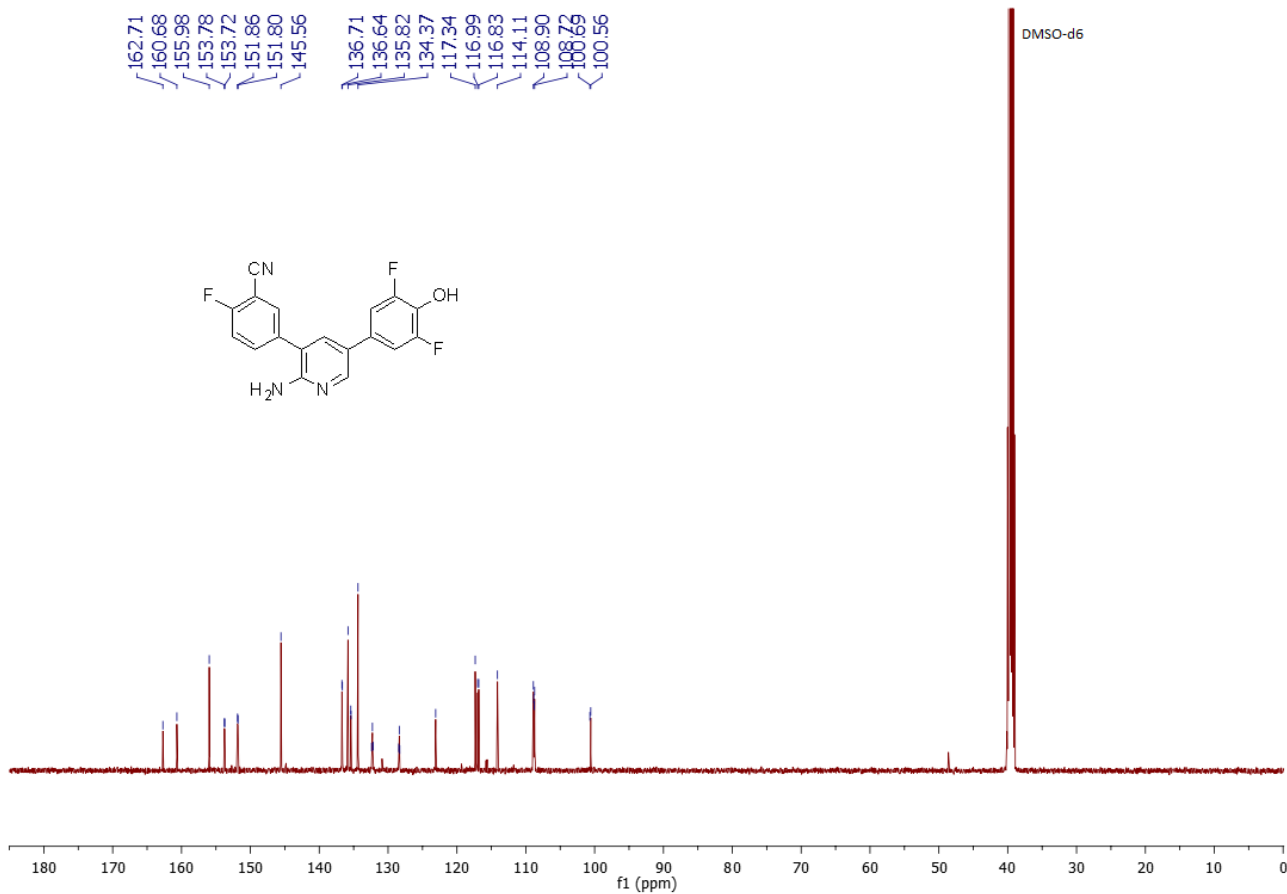
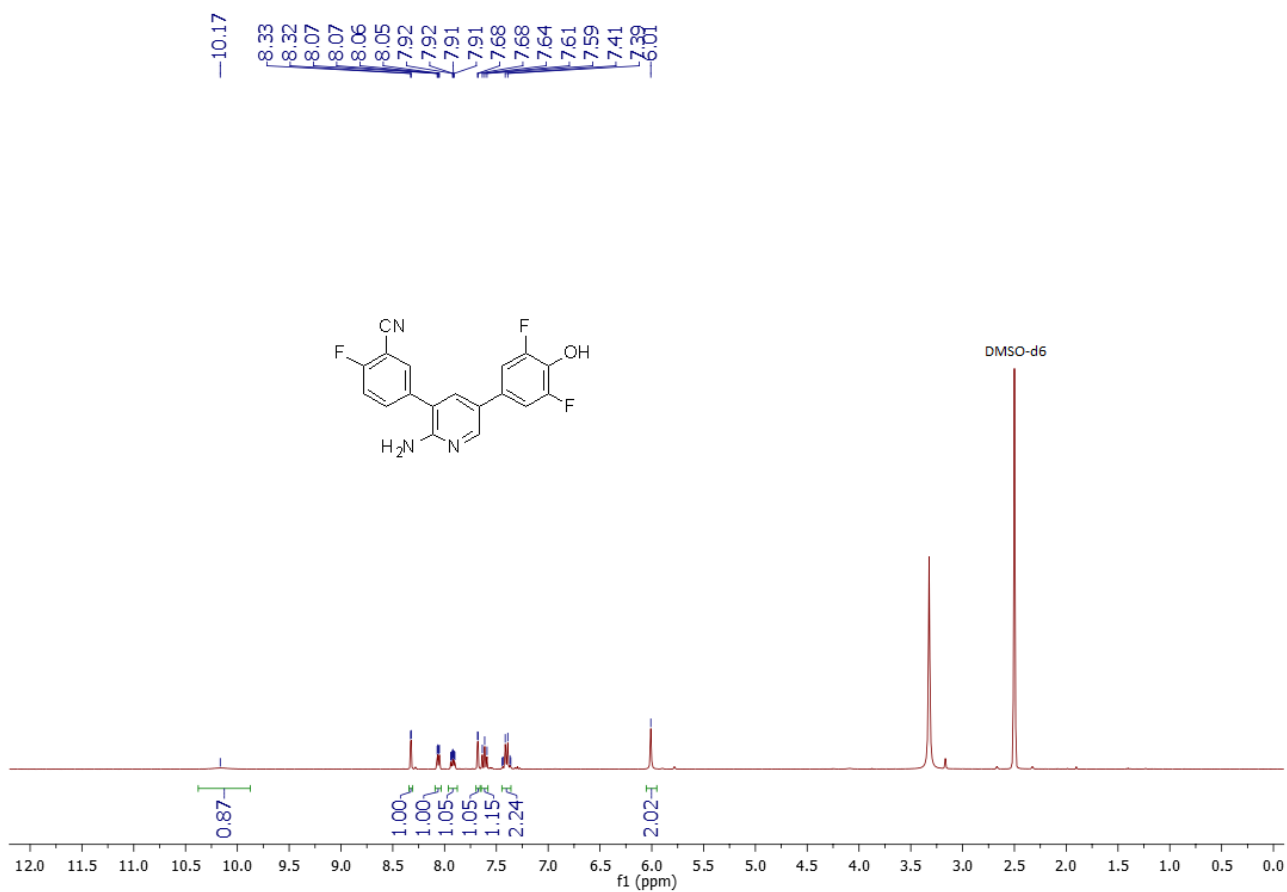


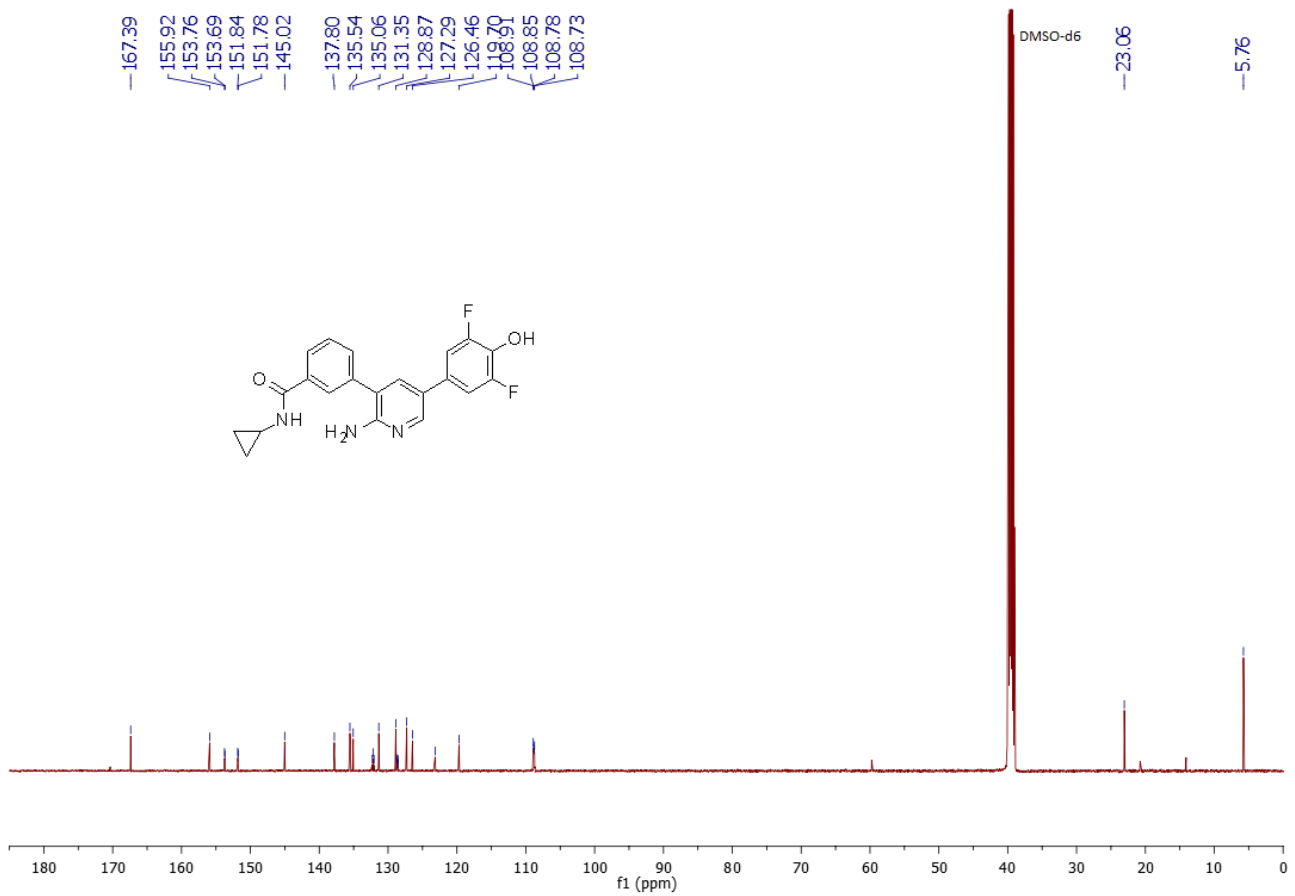
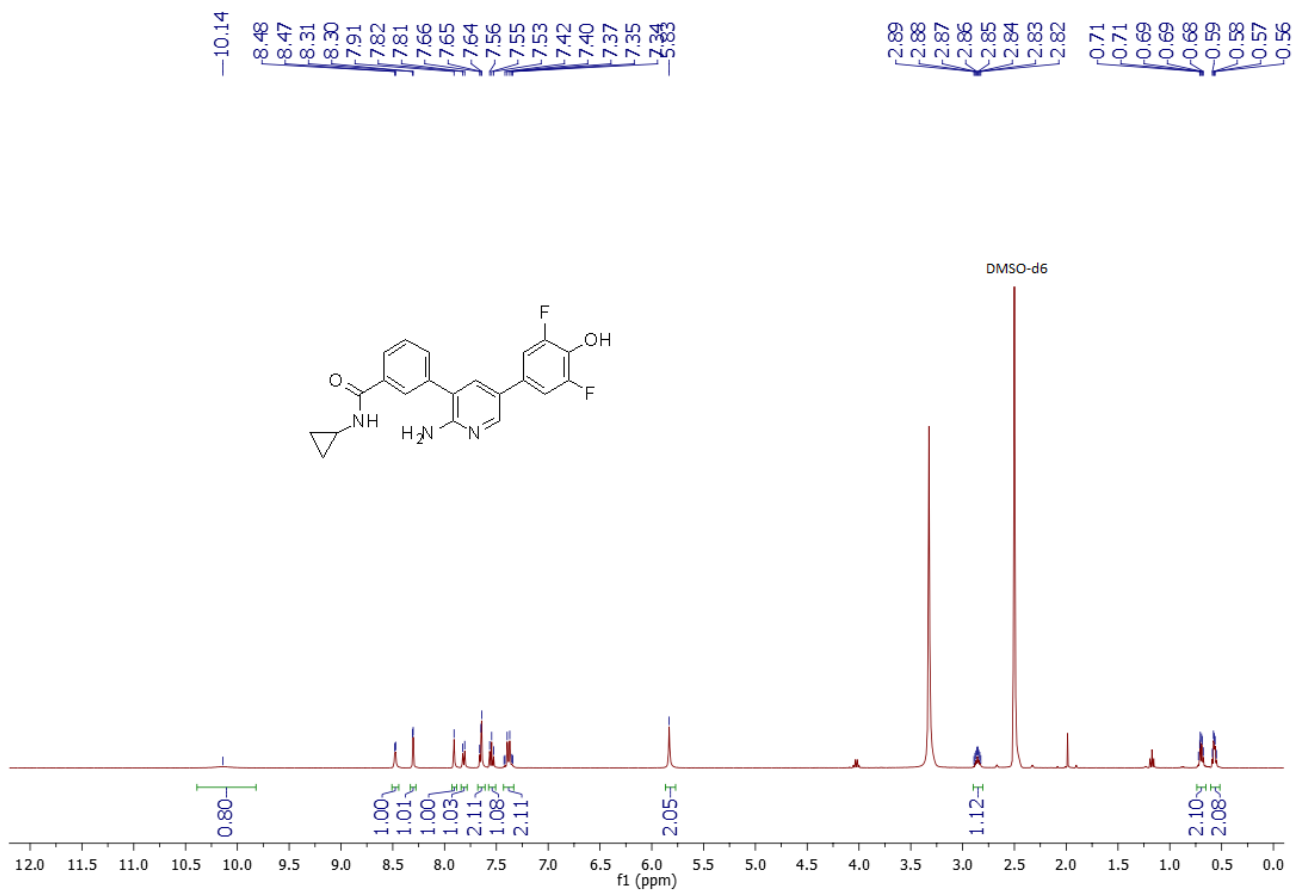
¹H NMR (400 MHz, DMSO-*d*₆) 45.



¹³C NMR (126 MHz, DMSO-*d*₆) 45.







Supplementary references

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