Supporting Information

2,8-Disubstituted-1,6-naphthyridines and 4,6-disubstituted-isoquinolines with potent, selective affinity for CDK8/19

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Table S1. Mouse pharmacokinetic data for compounds **7**, **8** and **17**

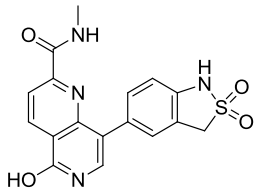
Compd	Cl (L/hr/kg)	Vd (L/hr)	F (%)	Caco-2	
				Papp $_{A\ to\ B}$	ER
7	11.6	2.7	16	38.3	1.02
8	Not measurable*	Not measurable	Not measurable	5.94	7.26
17	23	5.8	~8	ND	ND

*Clearance was too high to be quantified

Table S2. Aldehyde oxidase metabolism data

Series	Compd	% of parent compd remaining after 45 min	Clint AO (mL/min/kg)
Naphthyridine	**7**	-	598
Naphthyridine	**8**	20	-
Naphthyridine	**13**	0	-
Naphthyridine	**19**	-	418
Naphthyridine	**25**	0	367
Naphthyridine	**26**	-	>1000
Isoquinoline	**27**	0	-
Isoquinoline	**29**	1	-
Isoquinoline	**30**	0	-
Isoquinoline	**32**	3	-

Figure S1. Comparison of ^{1}H NMR of the major metabolite isolated from Met-ID of **37** in human hepatocytes with ^{1}H NMR of the synthesised sample



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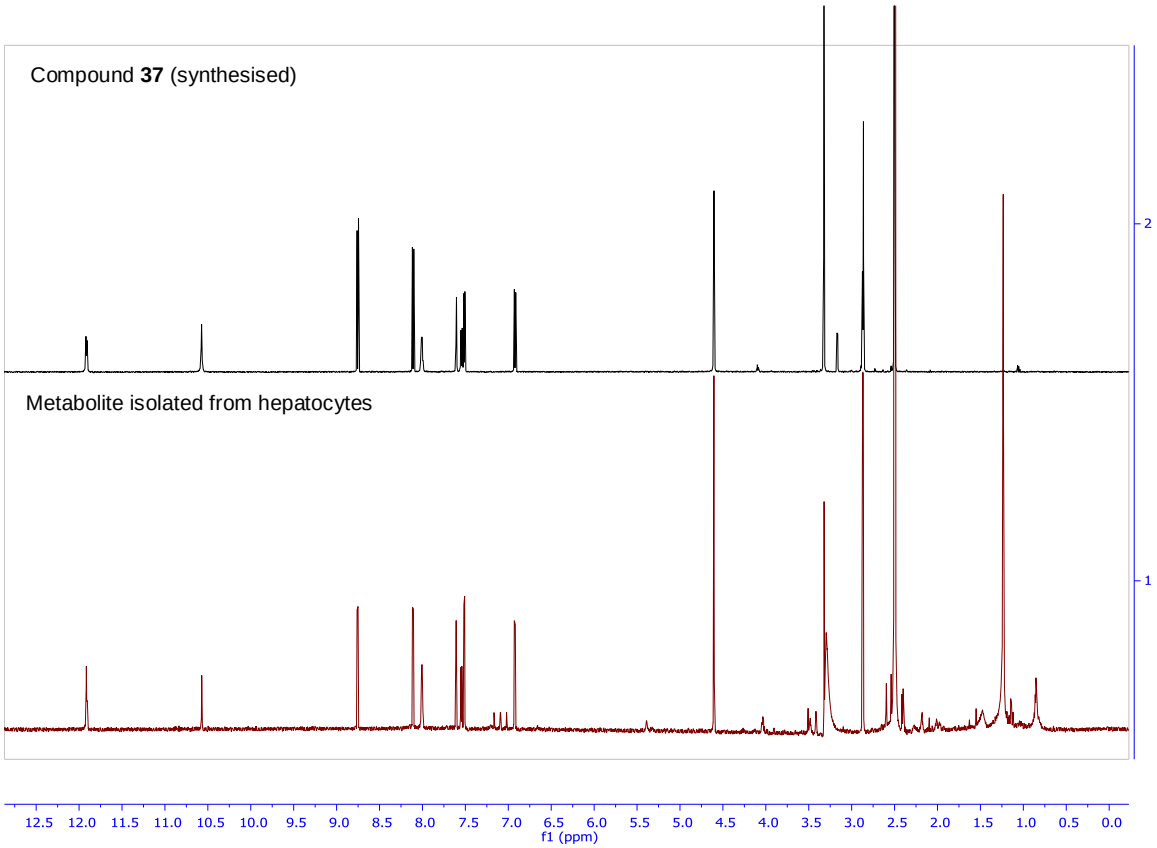


Table S3. Introduction of pendant polar functionality to abrogate AO-mediated metabolism

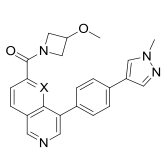
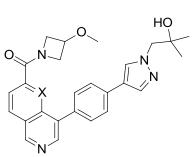
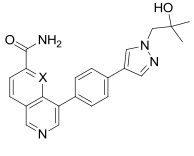
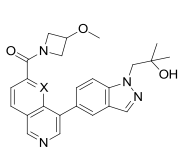
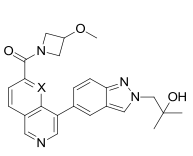
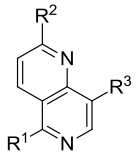
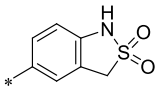
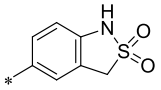
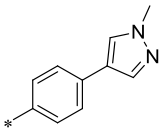
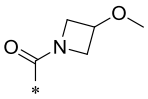
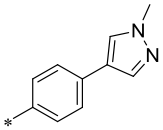
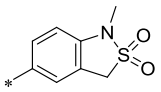
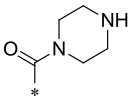
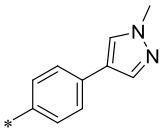
						
X = N	Compound	**13**	**38**		**39**	**40**
	AO (% parent compd remaining)	0	0	-	0	0
	CDK8 IC_{50} (nM)	5.2±4.7	6.7±0.21	-	72±23	8.5±1.5
	7dF3 IC_{50} (nM)	2.5±0.8	2.8±0.7	-	225±76	17.9±5.5
X = C	Compound	**29**	**41**	**42**	**43**	**44**
	AO (% parent compd remaining)	1	0	7	2	0
	CDK8 IC_{50} (nM)	11±5.7	17±2.1	3.0±0.71	275±35.4	32±13
	7dF3 IC_{50} (nM)	16±8.3	13.2±15.2	0.6±0.2	921±148	92.7±41.1

Table S4. C-5 substituted naphthyridines



Compd	R^1	R^2	R^3	CDK8 IC_{50} (nM)	7dF3 IC_{50} (nM)	AO*
37	OH			225±64	9605±2434	100%
45	OMe			3450±778	>30000	ND
46	Me			220±28	273±43	9
47	CHF_2			2300±424	2430±661	ND
48	NH_2			7.2±1.3	9.3±2.3	15
49	NH_2			100±14	559±469	<4.2

*Clint expressed in mL/min/kg except for compound **37** where % of parent compound remaining after 45 min is given. ND = Not determined

Table S5. Comparison of AO-mediated metabolism in the AO-enzyme assay with the Litmus Test.[10]

compd	Series	AO substrate (Enzyme assay)	AO substrate (Litmus Test)
26	Naphthyridine	Yes	Yes
7	Naphthyridine	Yes	Yes
48	Aminonaphthyridine	No	No
13	Naphthyridine	Yes	Yes
51	Aminonaphthyridine	No	No
28	Isoquinoline	Yes	Yes
64	Aminoisoquinoline	No	No
29	Isoquinoline	Yes	Yes
59	Aminoisoquinoline	No	No
31	Isoquinoline	Yes	Yes
63	Aminoisoquinoline	No	No
34	Isoquinoline	Yes	Yes
65	Aminoisoquinoline	No	No

Enzyme assay: "Yes" if Clint >500 mL/min/kg or the % of parent compound remaining after 45 min incubation is <40%. Litmus test: "Yes" if conversion of the parent compound into the M+50 product is greater than 70% based on TIC chromatogram. "No" if conversion into the M+50 product is less than 10%.

Table S6. Activity of compounds **51** and **59** tested at 1 µM across a panel of 264 and 307 kinases respectively (Millipore Panel: data expressed as percentage effect versus each kinase tested at its respective Km for ATP). FLT4 IC_{50} was determined for compound **51** (6.3 µM).

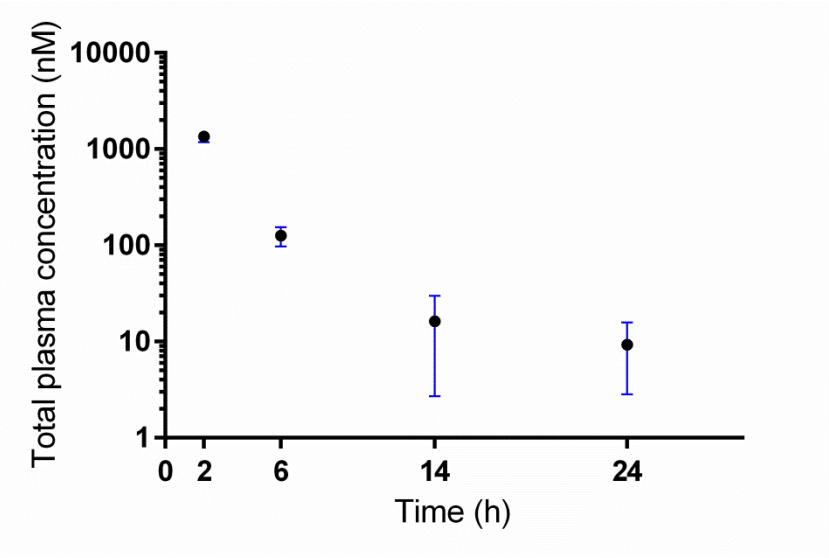
	51	59		51	59
ABL	20	-10	**EPHA4**	2	1
ABL(T315I)		5	**EPHA3**	10	10
ALK	-20	-30	**EPHA5**	-1	3
ALK4	9	8	**EPHA7**	-4	1
ARG	11	-7	**EPHA8**	-9	-12
ARK5	2	-14	**EPHB1**	13	-44
ASK1	0	-10	**EPHB2**	7	24
AURKA	-3	-21	**EPHB3**	2	-10
AXL	27	-12	**EPHB4**	8	6
BLK	0	-26	**ERBB4**	-8	-34
BMX	-3	1	**FER**	-18	3
BRK	10	5	**FES**	-3	-7
BRSK1	6	2	**FGFR1**	10	11
BRSK2	1	5	**FGFR2**	-3	-6
CAMKI	-10	-5	**FGFR3**	7	-4
CAMKIV	-17	23	**FGFR4**	3	14
CDK1/CYCLINB	1	-5	**FGR**	19	7
CDK2/CYCLINA	5	-5	**FLT1**	-9	-15
CDK2/CYCLINE	-1	1	**FLT3**	0	16
CDK3/CYCLINE	4	-5	**FLT3(D835Y)**		-10
CDK5/P25	3	11	**FLT4**	-54	-62
CDK5/P35	8	-2	**FMS**	-37	-14
CDK6/CYCLIND3	-10	-14	**FYN**	1	8
CDK7/CYCLINH/MAT1	20	-4	**GSK3ALPHA**	-17	-29
CDK9/CYCLINT1	8	-14	**GSK3BETA**	1	-9
CHK1	-8	-9	**HCK**	-6	-3
CHK2	-1	-10	**HIPK1**	5	0
CK1(Y)		4	**HIPK2**	-8	16
CK1DELTA	1	-10	**HIPK3**	6	4
CK2	5	8	**IGF-1R**	19	9
CK2ALPHA2	0	13	**IKKALPHA**	-17	28
CSK	21	7	**IKKBETA**	-14	8
DAPK1	0	-5	**IR**	3	-15
DAPK2	5	12	**IRR**	-2	-5
DDR2	6	24	**IRAK1**	8	14
DMPK	7	-11	**IRAK4**	-9	1
DRAK1	1	-18	**ITK**	-6	2
DYRK2	-6	-9	**JAK2**	-2	3
EEF-2K	2	8	**JAK3**	2	14
EGFR	-45	14	**JNK1ALPHA1**	-4	-11
EGFR(L858R)		-15	**JNK2ALPHA2**	-6	2
EGFR(L861Q)		-9	**JNK3**	-33	-8
EPHA1	7	4	**KDR**	0	-5
EPHA2	-12	7	**CKIT**	1	-3

	51	59		51	59
CKIT(D816V)		-3	**PDK1**	-8	-10
LCK	2	-18	**PI3K BETA**	-3	-2
LIMK1	0	5	**PI3K DELTA**	-10	-10
LKB1	-5	-10	**PI3K GAMMA**	-18	-7
LOK	3	1	**PIM-1**	-10	-13
LYN	-1	0	**PIM-2**	16	14
MAPK1	-13	-20	**PKA**	-1	20
MAPK2	6	-4	**PKBALPHA**	-11	4
MAPKAP-K2	6	-9	**PKBBETA**	13	-24
MAPKAP-K3	-15	-7	**PKBGAMMA**	-4	-6
MARK1	-5	-14	**PKCALPHA**	-10	-2
MEK1	-18	-9	**PKCBETAI**	-1	15
MET	22	-19	**PKCBII**	-4	-3
MINK	-11	-13	**PKCDELTA**	4	0
MKK4		3	**PKCEPSILON**	14	8
MKK6	-4	-14	**PKCGAMMA**	-1	9
MKK7BETA	9	-23	**PKCETA**	5	-4
MLCK	0	-20	**PKCIOTA**	-16	2
MLK1	-3	-30	**PKCMU**	-3	10
MNK2	-7	-4	**PRKCQ**	5	-12
MRCKALPHA	2	0	**PKCZETA**	4	-13
MRCKBETA	-4	13	**PKG1ALPHA**	0	17
MSK1	-2	0	**PKG1BETA**	1	1
MSK2	7	-12	**PKD2**	-1	-5
MSSK1	7	13	**PLK3**	-9	5
MST1	9	-10	**PRAK**	15	-17
MST2	-5	-14	**PRK2**	-3	-11
MST3	7	-34	**PRKX**	43	6
MUSK	3	-18	**PTK5**	-8	-4
NEK2	-5	-9	**PYK2**	-4	-1
NEK3	-14	-2	**CRAF**	-20	0
NEK6	-2	-34	**RET**	-2	6
NEK7	-6	-26	**RIPK2**	-16	-5
NEK11	3	-22	**ROCK-I**	32	2
NLK	-3	-5	**ROCK-II**	-2	-12
P70S6K	-25	29	**RON**	15	-11
PAK2	-17	-18	**ROS**	14	-4
PAK4	4	10	**RSE**	17	-6
PAK5	-23	-7	**RSK1**	13	-14
PAK6	-12	25	**RSK2**	6	0
PAR-1BALPHA	-9	-4	**RSK3**	4	4
PASK	-8	-4	**SAPK2A**	-4	-9
PDGFRALPHA	-3	11	**SAPK2A(T106M)**		1
PDGFRBETA	2	17	**SAPK2B**	0	43

	51	59		51	59
SAPK3	-11	-11	**CKIT(V654A)**		6
SAPK4	2	-13	**FGFR1(V561M)**		-6
SGK	14	14	**VRK2**	4	-3
SGK2	-46	38	**PDGFRALPHA(V561D)**		-9
SGK3	-13	9	**TAO2**	-5	-7
SIK	-3	-5	**CHK2(R145W)**		-6
SNK	8	3	**CHK2(I157T)**		-9
SRC	7	-6	**FGFR2(N549H)**		-14
SRPK1	3	-24	**GCK**	-26	1
SRPK2	0	-2	**HASPIN**	-7	-12
STK33	-15	1	**PIM-3**	-9	9
SYK	-10	-11	**TAO3**	-16	-6
TAK1	-9	-13	**TLK2**	2	-5
TBK1	-23	17	**SRC(1-530)**		-3
TIE2	-6	-18	**SRC(T341M)**		-18
TRKA	-23	-25	**TAO1**	-1	9
TRKB	-23	0	**ABL (H396P)**		5
TSSK1	5	-14	**ABL (Q252H)**		9
TSSK2	15	-26	**GRK7**	3	8
WNK2	18	-3	**ACK1**	0	-10
WNK3	-9	4	**ULK3**	2	8
YES	-22	-18	**ABL (M351T)**		2
ZAP-70	-3	-6	**ABL(Y253F)**		-6
ZIPK	25	-5	**BTK(R28H)**		-12
CK1GAMMA1	-8	-8	**CLK2**	-18	-3
CK1GAMMA2	-24	-9	**RET (V804L)**		-9
CK1GAMMA3	-18	19	**RET(V804M)**		7
DCAMKL2	0	0	**TIE2(R849W)**		-12
GRK5	6	22	**TIE2(Y897S)**		-6
GRK6	4	8	**TXK**	-13	-7
PHKGAMMA2	10	2	**ULK2**	4	8
CAMKIIBETA	-10	5	**MTOR**	-12	-5
CAMKIIGAMMA	5	2	**PLK1**	1	-6
CAMKIIDELTA	-10	8	**MTOR/FKBP12**	-4	4
CLK3	2	-19	**PI3 KINASE (P110B/P85B)**		-1
CKIT(D816H)		-1	**MELK**	-10	-8
CKIT(V560G)		-21	**TEC ACTIVATED**	-18	13
EGFR(T790M)		-2	**IGF-1R, ACTIVATED**	-18	-10
EGFR(T790M,L858R)		-2	**IR, ACTIVATED**	-15	-2
FAK	3	-18	**PI3 KINASE (P85A)**	-12	0
MER	-3	-4	**PI3 KINASE (E542K/P85A)**		-3
PDGFRALPHA(D842V)		1	**PI3 KINASE (E542K/P85A)**		-2
RSK4	9	13	**PI3 KINASE (H1047R/P85A)**		-2
CAMKIDELTA	-3	5	**PI3 KINASE (P65A)**		-4

	51	59
PEK	-6	1
cMET (D1246H)		1
cMET (D1246N)		3
cMET (M1268T)		5
cMET (Y1248D)		19
cMET (Y1248H)		6
FMS(Y969C)		-31
HCK	-3	0
cMET (Y1248C)		-19
PIP4K2A	-5	1
AURKB	0	14
AURKC	6	-10
LCK	-1	4
TGFBR1	-5	-4
PRKAA1	-1	-3
PRKAA2	-1	2

Figure S2. Plasma concentrations of **51** after a single oral dose (5 mg/kg) in the SW620 human tumor xenograft PK/PD study (mouse ppb = 96.2 %).



Methods:

Aldehyde oxidase (AO) assay: Compounds were tested according to either or both of the following formats: A) Test compound (1 µM) was incubated in presence of human cytosolic proteins (0.5 mg/ml); percentage parent compound remaining after 45 minutes was determined. Carbazeran and zaleplon were used as positive and negative controls respectively. Percent remaining >90% indicates negligible degradation; < 90 and > 60% indicates potential degradation; < 60% parent compound remaining suggest that cytoslic clearance may represent a relevant route of elimination. B) Test compound (1 µM) was incubated in presence of human cytosolic proteins (0.5 mg/ml) with and without a selective inhibitor of AO, hydralazine (10 µM) and percentage test compound remaining was determined at time points between 0 and 90 minutes to allow calculation of intrinsic clearance (Clint). Carbazeran and zaleplon were used as positive and negative controls respectively.

Human tumour xenograft PK/PD study

Cell Culture and Animal Model: The human colorectal adenocarcinoma cell line SW620 (ATCC, #CCL-227) was cultured and expanded at 37°C in 5% CO_2 without antibiotics using Dulbecco modified Eagle medium F12 supplemented with 10% fetal calf serum and 2 mM L-Glutamine (Invitrogen).

Animal procedures were performed in accordance with the German Laws for Animal Welfare and were approved by the local governmental authority (Hessian State Agency for Veterinary Services and Consumer Protection). Animals were acclimatised for a week prior to use and general health status was monitored during the experiments.

Tumors were established *in vivo* by subcutaneous injection of 5 million cells in 100 µl PBS into the right flanks of 6- to 8-week-old male Hd2 Rag mice [C;129P2-H2^{d}-TgH(II2rg)^{tm1Bm}-TgH(Rag2)^{tm1Alt}N4] (bred at Taconic). Tumors were allowed to grow until a volume range of 300-500mm³ was achieved: calculated from digital calliper measurements (volume = L x W x W/2; L longer diameter and W shorter diameter). Mice with appropriate tumor volumes were stratified into control and treatment groups (n=6 / group). Single administrations of vehicle or **51** (formulated in 0.5% Methocel / 0.25% Tween20 / water) were given by oral gavage and tumors and plasma were collected at the respective time points after dosing.

Tumor xenograft processing and Luminex assay: Tumors were excised and samples of 80 – 120 mg weight were transferred to Precellys tubes (Peqlab, #91-PCS-CK28), immediately snap-frozen in liquid nitrogen and stored at -80 °C until use. 500 µl lysis buffer (Extraction Buffer I from ProteoExtract Subcellular Proteome Extraction Kit (S-PEK, Merck Millipore, #539790) freshly supplemented with Phosphatase Inhibitor Cocktail II (Merck Millipore, #524625, dilution 1:100), Protease Inhibitor Cocktail Set III (Merck Millipore, #539134, dilution 1:1000), Benzonase (Novagen, #70664, dilution 1:10000), beta-glycerolphosphate (Merck Millipore, #35675, dilution 1:50) were added and samples were ground using a Precellys 24 (PEQLAB) at 6500 rpm for 2x 10 sec. Homogenates were transferred into a fresh tube and spun at 13000 rpm at 4 C for 20 minutes. Supernatants were collected, aliquoted and snap-frozen in liquid nitrogen. Tumor lysates were diluted 1:5 in lysis buffer and the concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, #23227).

Level of phospho-STAT1^{SER727} and total STAT1 were quantified in a Luminex-based bead assay. Briefly, 100 µg of total protein from each sample (three technical replicates) were incubated overnight with microspheres coupled with total STAT1 antibodies (mouse anti STAT1, R&D, #MAB14901). After three washes, detection antibodies for total (rabbit anti STAT1, Santa Cruz, #sc-346, dilution 1:500) or phospho-STAT1 (rabbit anti phospho-STAT1^{SER727}, Cell Signaling, #8826, dilution 1:250), were added for 1 hour. Samples were washed three times and incubated with phycoerythrin-labelled secondary antibody (donkey anti rabbit-PE, Dianova #711-116-152, dilution 1:800) for one hour. Levels of phospho-STAT1^{SER727} and total STAT1 were measured on a Luminex 200 machine according to the manufacturer's instructions. Level of phospho-STAT1^{SER727} were normalized against levels of total STAT1 in each sample and calculated as percent of vehicle controls.

Pharmacokinetic analysis: Plasma samples were measured by LC-MS/MS on a Sciex API4000 following separation on a Chromolith FastGradient RP-18e column, 50-3mm (Merck KGaA, Darmstadt, Germany) with conditions of 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B). The column was equilibrated and the gradient program was started with initial conditions of 100% A and 0% B, held over 0.6 min, a fast linear gradient over 0.9 min to 10% A and 90% B, held over 1.5 min (all at 0.8 ml/min flow rate), followed by a rapid back-equilibration at 100% A and 0% B over 0.5 min, at 2.0 ml/min flow rate.

Detection was achieved in a positive electrospray ionization mode by multiple reaction monitoring for compound **51** and the internal standard analogue (8-bromo-4-[3-(4-methyl-pyrazol-1-yl)-azetidin-1-yl]-quinazoline) (415.12>357.02 at 40eV for compound **51**, and 343.85>262.00 at 50eV for the internal standard). Samples were quantified by external standard calibration in blank plasma (7 point calibration ranging from 2.00 to 5000 ng/mL).

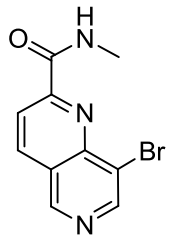
Synthetic Procedures

Commercially available starting materials, reagents and dry solvents were used as supplied. Column chromatography was performed on a Biotage SP1 purification system using Thomson or Biotage Flash silica cartridges or on a Companion purification system using Interchim silica cartridges. Ion exchange chromatography was performed using acidic Isolute Flash SCX-II columns. Preparative HPLC was conducted according the following method: injections of the sample were made onto a Phenomenex Gemini column (10 μm, 250 x 21.2 mm, C18, Phenomenex, Torrance, USA). Chromatographic separation at room temperature was carried out using Gilson GX-281 Liquid Handler system combined with a Gilson 322 HPLC pump (Gilson, Middleton, USA) over a 15 minute gradient elution from 10:90 to 100:0 MeCN:water (both modified with 0.1% formic acid) at a flow rate of 20 mL/min. ^{1}H NMR spectra were recorded on a Bruker Avance-500. Samples were prepared as solutions in a deuterated solvent and referenced to the appropriate internal non-deuterated solvent peak. ^{13}C NMR spectra were recorded at 126 MHz using an internal deuterium lock. The following internal references were used: $CDCl_3$ (δ_C 77.2), CD_3OD (δ_C 49.0) and DMSO-d_6 (δ_C 39.5). LC/MS and HRMS analysis was performed on an Agilent 1200 series HPLC and diode array detector coupled to a 6210 time of flight mass spectrometer with dual multimode APCI/ESI source. Analytical separation was carried out according to the following Methods: Method A, analytical separation was carried out at 30°C on a Merck Chromolith Flash column (RP-18e, 25 x 2 mm) using a flow rate of 0.75 mL/min in a 4 minute gradient elution with detection at 254 nm. The mobile phase was a mixture of methanol (solvent A) and water (solvent B), both containing formic acid at 0.1%. Gradient elution was as follows: 5:95 (A/B) to 100:0 (A/B) over 2.5 min, 100:0 (A/B) for 1 min, and then reversion back to 5:95 (A/B) over 0.1 min, finally 5:95 (A/B) for 0.4 min.; Method B, analytical separation was carried out at 30°C on a Merck Purospher STAR column (RP-18e, 30 x 4 mm) using a flow rate of 1.5 mL/min in a 4 minute gradient elution with detection at 254 nm. The mobile phase was a mixture of methanol (solvent A) and water (solvent B), both containing formic acid at 0.1%. Gradient elution was as follows: 10:90 (A/B) to 90:10 (A/B) over 2.5 min, 90:10 (A/B) for 1 min, and then reversion back to 10:90 (A/B) over 0.3 min, finally 10:90 (A/B) for 0.2 min.; Method C, analytical separation was carried out at 40°C on a Merck Purospher STAR column (RP-18e, 30 x 4 mm) using a flow rate of 3 mL/min in a 2 minute gradient elution with detection at 254 nm. The mobile phase was a mixture of methanol (solvent A) and water (solvent B), both containing formic acid at 0.1%. Gradient elution was as follows: 10:90 (A/B) to 90:10 (A/B)

over 1.25 min, 90:10 (A/B) for 0.5 min, and then reversion back to 10:90 (A/B) over 0.15 min, finally 10:90 (A/B) for 0.1 min; Method D, analytical separation was carried out on a Chromolith Performance column (RP-18e, 100 x 3 mm) using a flow rate of 2.0 mL/min in a 4.8 min gradient elution with detection at 220 nm. The mobile phase was a mixture of water (solvent A) and acetonitrile (solvent B) both containing 0.1% TFA. Gradient elution was as follows: 99:1 (A/B) over 0.2 min, the 99:1 to 0:100 (A/B) over 3.6 min, 0:100 (A/B) for 0.4 min, and then reversion back to 99:1 (A/B) over 0.1 min and finally 99:1 (A/B) for 0.5 min. The following references masses were used for HRMS analysis: caffeine $[M+H]^+$ 195.087652; (hexakis(1*H*,1*H*,3*H*-tetrafluoropentoxy)phosphazene $[M+H]^+$ 922.009798) and hexakis(2,2-difluoroethoxy)phosphazene $[M+H]^+$ 622.02896 or reserpine $[M+H]^+$ 609.280657. All compounds submitted for biological testing were determined to be > 95% pure by Methods A, B, C or D.

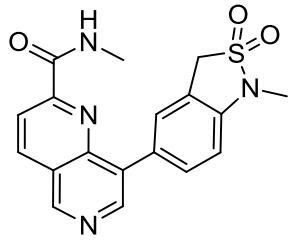
Preparation of compound 7 (Table 1)

8-Bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide



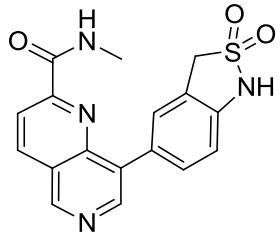
To 8-bromo-1,6-naphthyridine-2-carboxylic acid (commercially available, 250 mg, 0.988 mmol) in DMF (8.2 mL) was added HATU (451 mg, 1.19 mmol), methylamine in methanol (2 M, 1.48 mL, 2.96 mmol) and DIPEA (690 µL, 3.95 mmol). The reaction mixture was stirred at rt for 1 h 30 min and concentrated. The crude product was purified by Biotage column chromatography (DCM/EtOH 99:1 to 95:5). The solid obtained was diluted in DCM and washed with water. The organic layer was dried over MgSO4, filtered and concentrated *in vacuo* to give 8-bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide as a cream solid (245 mg, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H), 9.03 (s, 1H), 8.51 (d, *J* = 8.4 Hz, 1H), 8.48 (d, *J* = 8.4 Hz, 1H), 8.28 (bs, 1H), 3.14 (d, *J* = 5.1 Hz, 3H); LC–MS (method A, ESI, m/z) t_R = 1.19 min, 266/268 $(M+H)^+$.

N-methyl-8-(1-methyl-2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-1,6-naphthyridine-2-carboxamide (**7**)



8-Bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide (40 mg, 0.15 mmol), 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-dihydrobenzo[*c*]isothiazole 2,2-dioxide[11] (51 mg, 0.17 mmol) and $Pd(dppf)Cl_2.CH_2Cl_2$ (6 mg, 7 µmol) were loaded in a microwave vial and then acetonitrile (2.6 mL) and sodium carbonate in water (421 µL, 0.210 mmol) were added. The reaction mixture was heated at 120 °C for 1 h under microwave irradiation and the solvents were evaporated. The crude material was purified by Biotage column chromatography (DCM/EtOH 99:1 to 92:8) and filtered on a SCX-2 column to give *N*-methyl-8-(1-methyl-2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-1,6-naphthyridine-2-carboxamide **7** (30 mg, 54% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.80 (s, 1H), 8.54 (d, *J* = 8.5 Hz, 1H), 8.49 (d, *J* = 8.5 Hz, 1H), 7.85 – 7.78 (m, 1H), 7.74 – 7.70 (m, 1H), 7.61 – 7.59 (m, 1H), 6.93 (d, *J* = 8.2 Hz, 1H), 4.47 (s, 2H), 3.26 (s, 3H), 3.05 (d, *J* = 5.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.3, 153.6, 152.5, 147.1, 146.5, 142.0, 138.0, 133.0, 132.3, 129.3, 127.5, 124.3, 120.6, 117.8, 109.1, 51.0, 26.7, 26.6; LC–MS (method A, ESI, m/z) t_R = 2.39 min, 369 $(M+H)^+$; ESI-HRMS calcd for $C_{18}H_{17}N_4O_3S$ $(M+H)^+$ 369.1016, found 369.1010.

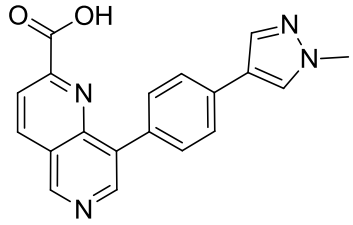
Preparation of compound 8 (Table 1)



8-Bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide (53 mg, 0.20 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-dihydrobenzo[*c*]isothiazole 2,2-dioxide[11] (65 mg, 0.22 mmol) and Pd(dppf)Cl_2.CH_2Cl_2 (8 mg, 10 µmol) were loaded in a microwave vial and then acetonitrile (3.4 mL) and sodium carbonate in water (558 µL, 0.279 mmol) were added. The reaction mixture was heated at 120 °C for 1 h under microwave irradiation and the solvents were evaporated. The crude material was purified by Biotage column chromatography (DCM/EtOH 98:2 to 93:7) and filtered on a SCX-2 column to give 8-(2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-*N*-methyl-1,6-naphthyridine-2-carboxamide **8** (50 mg, 71% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.75 (s, 1H), 9.46 (s, 1H), 8.84 – 8.82 (m, 2H), 8.30 (d, *J* = 8.4 Hz, 1H), 8.20 (q, *J* = 4.9 Hz, 1H), 7.82 – 7.80 (m, 1H), 7.76 – 7.72 (m, 1H), 7.01 (d, *J* = 8.2 Hz, 1H), 4.67 (s, 2H), 2.90 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO- d6) δ 164.2, 154.0, 152.5, 146.6, 145.7, 140.0, 138.7, 132.2, 131.4, 128.1, 128.0, 123.8, 120.3, 120.1, 111.2, 52.0, 26.4; LC–MS (method A, ESI, m/z) t_R = 2.17 min, 355 $(M+H)^+$; ESI-HRMS calcd for $C_{17}H_{15}N_4O_3S$ $(M+H)^+$ 355.0859, found 355.0854.

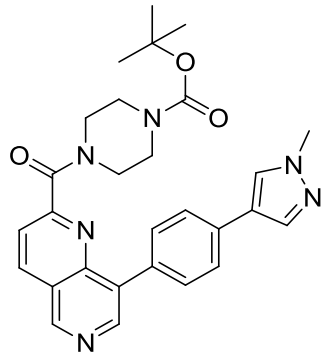
Preparation of compound 17 (Table 2)

8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carboxylic acid



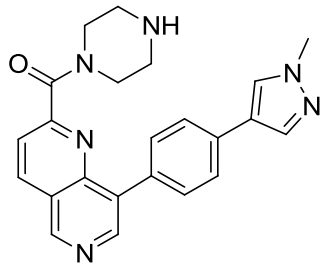
8-Bromo-1,6-naphthyridine-2-carboxylic acid (commercially available, 300 mg, 1.19 mmol), 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole[9] (337 mg, 1.19 mmol), Pd(dppf)Cl_2. CH_2Cl_2 (43 mg, 0.059 mmol) were loaded in a microwave vial. 0.5 M aqueous sodium carbonate solution (3.3 mL, 1.7 mmol) and acetonitrile (10 mL) were added. The reaction mixture was heated at 120 °C for 1 h under microwave irradiation and the solvents were evaporated. The crude material was purified by Biotage column chromatography (DCM/MEOH 90:10 to 50:50 containing 0.3% acetic acid) to give 8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carboxylic acid as impure grey solid which was used directly in the next step (600 mg, 60% pure, 92% corrected yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.75 (s, 1H), 8.54 (d, *J* = 8.4 Hz, 1H), 8.23 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 0.9 Hz, 1H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.2 Hz, 2H), 3.90 (s, 3H); LC–MS (method B, ESI, m/z) t_R = 2.41 min, 331 $(M+H)^+$;

tert-Butyl 4-(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate



To a mixture of 8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carboxylic acid (60% pure, 100 mg, 0.182 mmol) in DMF (5 mL) was added *tert*-butyl piperazine-1-carboxylate (118 mg, 0.636 mmol), triethylamine (0.302 mL, 2.18 mmol) and HATU (242 mg, 0.636 mmol). The reaction mixture was stirred at rt for 1 h and then diluted with water and EtOAc. The layers were separated; the organic layer was washed with water. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 94:4) to give *tert*-butyl 4-(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate (66 mg, 73% yield) as a pale yellow oil which was used directly in the next step. LC–MS (method B, ESI, m/z) t_R = 2.84 min, 499 $(M+H)^+$.

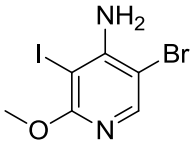
(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)(piperazin-1-yl)methanone (**17**)



To a solution of *tert*-butyl 4-(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate (66 mg, 0.13 mmol) in DCM (3.5 mL) was added trifluoroacetic acid (0.483 mL, 2.65 mmol) at 0 °C and the mixture was stirred at rt for 2 h. The solvent was evaporated and the residue was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 85:15), further purified by ion exchange (SCX2-cartridge, loading with DCM/MeOH 9:1, elution with DCM/1N NH_3 in MeOH 9:1) and by prep. HPLC to give (8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)(piperazin-1-yl)methanone **17** (22 mg, 76% yield) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 9.38 (s, 1H), 8.80 (s, 1H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.19 (s, 1H), 7.94 (s, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.67 (s, 4H), 3.89 (s, 3H), 3.65 – 3.54 (m, 2H), 3.49 – 3.45 (m, 2H), 2.82 – 2.75 (m, 2H), 2.74 – 2.69 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d6) δ 165.9, 158.0, 152.3, 146.6, 145.8, 137.9, 136.2, 132.7, 132.7, 132.3, 131.1, 128.0, 124.6, 122.8, 122.2, 121.6, 47.7, 46.0, 45.3, 42.7, 38.7; LC–MS (method B, ESI, m/z) t_R = 1.87 min, 399 $(M+H)^+$; ESI-HRMS calcd for $C_{23}H_{23}N_6O$ $(M+H)^+$ 399.1928, found 399.1917.

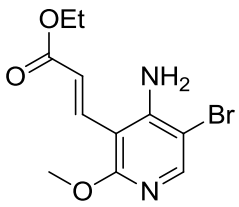
Preparation of compound 37 (Figure S1)

5-Bromo-3-iodo-2-methoxypyridin-4-amine.



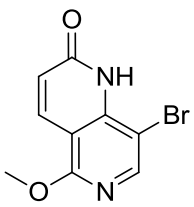
To 3-iodo-2-methoxypyridin-4-amine (commercially available, 1.00 g, 4.00 mmol) in solution in acetonitrile (40 mL) were added *N*-bromosuccinimide (783 mg, 4.40 mmol) and acetic acid (1 mL). The reaction mixture was stirred at rt for 3 h and concentrated. Water and EtOAc were added to the residue. The layers were separated, the aqueous layer was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude was purified by Biotage column chromatography (cyclohexane/EtOAc 100:0 to 95:5) to give 5-bromo-3-iodo-2-methoxypyridin-4-amine (1.25 g, 86% pure, 82% corrected yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (s, 1H), 5.13 (bs, 2H), 3.94 (s, 3H); LC – MS (method C, ESI, m/z) t_R = 1.38 min, 329/331 $(M+H)^+$.

Ethyl (*E*)-3-(4-amino-5-bromo-2-methoxypyridin-3-yl)acrylate



5-bromo-3-iodo-2-methoxypyridin-4-amine (1.21 g, 3.68 mmol), tri-o-tolylphosphine (0.090 g, 0.29 mmol) and palladium(II)acetate (0.033 g, 0.15 mmol) were introduced in a vial and then DMF (10 mL), triethylamine (0.718 mL, 5.15 mmol) and ethyl acrylate (0.598 mL, 5.52 mmol) were added. The reaction mixture was stirred at 100 °C for 15 h. Water and DCM were added and the layers were separated. The organic layer was washed with water and then the aqueous layers were extracted twice with DCM. The organic layer was dried over $MgSO_4$ and concentrated. The crude was purified by Biotage column chromatography (DCM, 100%) to give ethyl (*E*)-3-(4-amino-5-bromo-2-methoxypyridin-3-yl)acrylate as a colourless solid (684 mg, 62% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.70 (d, *J* = 16.1 Hz, 1H), 6.59 (d, *J* = 16.1 Hz, 1H), 5.04 (bs, 2H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.95 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H); LC – MS (method C, ESI, m/z) t_R = 1.46 min, 301/303 $(M+H)^+$.

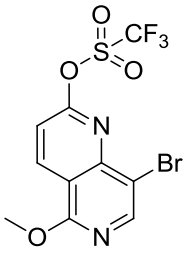
8-Bromo-5-methoxy-1,6-naphthyridin-2(1*H*)-one



To a solution of (*E*)-ethyl 3-(4-amino-5-bromo-2-methoxypyridin-3-yl)acrylate (684 mg, 2.27 mmol) in ethanol (9.5 mL) was added sodium methanethiolate (164 mg, 2.34 mmol) and the mixture was stirred at rt for 1.5 h. Water and DCM were added to the reaction mixture. The layers were separated and the aqueous layer was extracted three times with DCM. The organic layers were combined, dried over $MgSO_4$ and concentrated. The crude was purified by Biotage

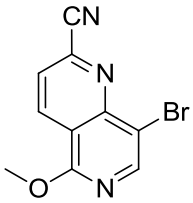
column chromatography (DCM/EtOAc 100:0 to 80:20) to give 8-bromo-5-methoxy-1,6-naphthyridin-2(1*H*)-one as a colourless solid (510 mg, 88% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (bs, 1H), 8.24 (s, 1H), 8.02 (d, *J* = 9.6 Hz, 1H), 6.64 (d, *J* = 9.6 Hz, 1H), 4.07 (s, 3H); LC – MS (method C, ESI, m/z) t_R = 1.22 min, 255/257 $(M+H)^+$.

8-Bromo-5-methoxy-1,6-naphthyridin-2-yl trifluoromethanesulfonate



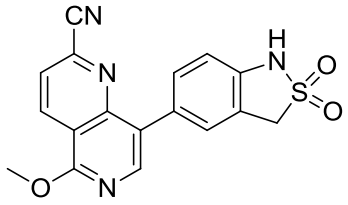
To a solution of 8-bromo-5-methoxy-1,6-naphthyridin-2(1*H*)-one (50 mg, 0.20 mmol) in DCM (1.2 mL) were added triethylamine (55 µL, 0.39 mmol) and trifluoromethanesulfonic anhydride (40 µL, 0.24 mmol). After 30 min, saturated aqueous $NaHCO_3$ solution and DCM were added, the layers were separated and the aqueous layer was extracted three times with DCM. The organic layer were dried over $MgSO_4$, filtered and concentrated. The crude product was used without any purification in the next step. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.75 (d, *J* = 8.7 Hz, 1H), 8.52 (s, 1H), 7.35 (d, *J* = 8.7 Hz, 1H), 4.17 (s, 3H); LC – MS (method C, ESI, m/z) t_R = 1.69 min, 387/389 $(M+H)^+$.

8-Bromo-5-methoxy-1,6-naphthyridine-2-carbonitrile



8-Bromo-5-methoxy-1,6-naphthyridin-2-yl trifluoromethanesulfonate (215 mg, 0.556 mmol), $Pd(PPh_3)_4$ (64 mg, 0.056 mmol) and zinc cyanide (72 mg, 0.61 mmol) were loaded in a microwave vial and then DMF (3.7 mL) was added. The reaction mixture was heated at 60 °C for 2.5 h in an oil bath. The reaction mixture was concentrated and purified by Biotage column chromatography (cyclohexane/DCM 50:50 to 0:100) to give 8-bromo-5-methoxy-1,6-naphthyridine-2-carbonitrile (99 mg, 67% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, *J* = 8.3 Hz, 1H), 8.55 (s, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 4.18 (s, 3H); LC – MS (method C, ESI, m/z) t_R = 1.57 min, 264/266 $(M+H)^+$.

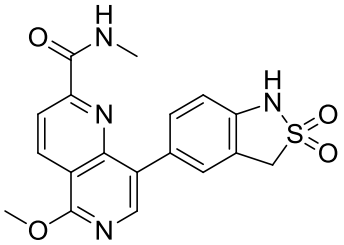
8-(2,2-Dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-methoxy-1,6-naphthyridine-2-carbonitrile



8-Bromo-5-methoxy-1,6-naphthyridine-2-carbonitrile (47 mg, 0.18 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-dihydrobenzo[*c*]isothiazole 2,2-dioxide[11] (58 mg, 0.20 mmol) and $Pd(dppf)Cl_2.CH_2Cl_2$ (7 mg, 9 µmol) were loaded in a microwave vial and then degassed acetonitrile (3 mL) and 0.5

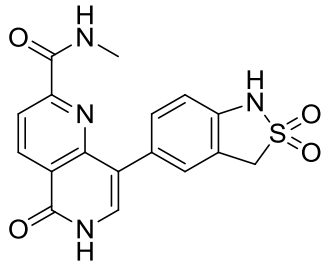
M sodium carbonate in water (500 µL, 0.249 mmol) were added. The reaction was heated at 120 ºC for 60 min under microwave irradiation. The crude was purified by Biotage column chromatography (DCM/EtOH 100:0 to 98:2) to give 8-(2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-methoxy-1,6-naphthyridine-2-carbonitrile as a yellow solid (100 mg, 70% yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.68 (s, 1H), 8.86 (d, *J* = 8.4 Hz, 1H), 8.38 (s, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.56 (bs, 1H), 7.54-7.51 (m, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 4.63 (s, 2H), 4.15 (s, 3H); LC – MS (method C, ESI, m/z) t_R = 1.31 min, 353 $(M+H)^+$.

8-(2,2-Dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-methoxy-*N*-methyl-1,6-naphthyridine-2-carboxamide (**45**)



To 8-(2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-methoxy-1,6-naphthyridine-2-carbonitrile (95 mg, 0.27 mmol) in ethanol (2.10 mL) was added 2 M sodium hydroxide (2.10 mL, 4.18 mmol). The reaction mixture was stirred at rt for 1 h and at 40 °C for 2.5 h. 1 M hydrogen chloride in dioxane (4.2 mL, 4.18 mmol) was added to the reaction mixture and the solution was concentrated. The crude was solubilized in DMF (2.25 mL) and HATU (123 mg, 0.324 mmol) was added. The reaction mixture was stirred for 15 min before the addition of 2 M methylamine in THF (410 µL, 0.821 mmol). The mixture was stirred at rt for 15 min before the addition of DIPEA (206 µL, 1.18 mmol). The resulting solution was then stirred at rt for 1.5 h. The reaction mixture was concentrated and the crude material purified by Biotage column chromatography (DCM/EtOH 99:1 to 96:4). The product obtained was then solubilised in DCM and washed with water to give 8-(2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-methoxy-*N*-methyl-1,6-naphthyridine-2-carboxamide **45** (24 mg, contaminated by 3% of tetramethylurea, 23% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (d, *J* = 8.5 Hz, 1H), 8.41 (d, *J* = 8.5 Hz, 1H), 8.25 (s, 1H), 7.85 (bd, *J* = 5.1 Hz, 1H), 7.59 (ddt, *J* = 8.2, 1.7, 0.7 Hz, 1H), 7.52 (s, 1H), 7.32 (bs, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 4.51 (s, 2H), 4.21 (s, 3H), 3.05 (d, *J* = 5.1 Hz, 3H); LC – MS (method B, ESI, m/z) t_R = 2.59 min, 385 $(M+H)^+$.

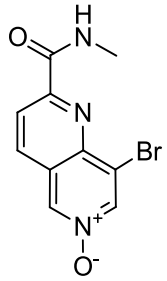
8-(2,2-Dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-hydroxy-*N*-methyl-1,6-naphthyridine-2-carboxamide (**37**)



To 8-(2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-methoxy-*N*-methyl-1,6-naphthy-ridine-2-carboxamide (14 mg, 0.036 mmol) in ethanol (0.6 mL) was added pyridine hydrochloride salt (68 mg, 0.59 mmol). The reaction mixture was heated at 150 °C for 40 min. Sat. $NaHCO_3$ solution and DCM were added. The mixture was concentrated and the resulting residue was solubilised in water and acidified to pH 2. The solution was concentrated and the residue was washed with DCM/EtOH, EtOH and acetone. The filtrates were combined (35 mg) and purified by Biotage column chromatography (DCM/EtOH 98:2 to 92:8) to give 8-(2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-5-hydroxy-*N*-methyl-1,6-naphthyridine-2-carboxamide **37** (8 mg, 59% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 11.91 (d, *J* = 6.1 Hz, 1H), 10.57 (s, 1H), 8.75 (d, *J* = 8.2 Hz, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 8.01 (q, *J* = 4.9 Hz, 1H), 7.61 (d, *J* = 1.9 Hz, 1H), 7.54 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.51 (d, *J* = 6.0 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 4.60 (s, 2H), 2.87 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 164.0, 161.1, 153.2, 150.9, 139.2, 137.9, 132.4, 130.2, 128.3, 126.9, 123.1, 120.0, 119.2, 117.3, 111.2, 52.0, 26.3; LC – MS (method B, ESI, m/z) t_R = 1.93 min, 371 $(M+H)^+$. ESI-HRMS calcd for $C_{17}H_{15}N_4O_4S$ $(M+H)^+$ 371.0809, found 371.0803.

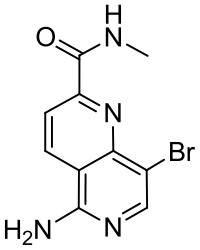
Preparation of compound 48 (Table 4)

8-Bromo-2-(methylcarbamoyl)-1,6-naphthyridine 6-oxide



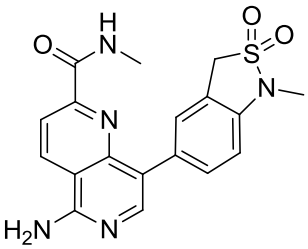
In a 12 mL screw-capped vessel 8-bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide (171 mg, 0.646 mmol) was dissolved in DCM (4 mL) and treated with 3-chloroperoxybenzoic acid (191 mg, 0.776 mmol) at rt. The orange solution was stirred overnight at rt. Additional 3-chloroperoxybenzoic acid (191 mg, 0.776 mmol) was added and the reaction mixture was stirred for an additional 3 h at rt. The reaction mixture was treated with 1 N NaOH solution and DCM and the layers separated. The organic layer was washed with water, dried over $Na_2S_2O_4$, filtered and evaporated to dryness to give 57 mg (90% pure, 28% yield) of 8-bromo-2-(methylcarbamoyl)-1,6-naphthyridine 6-oxide as pale beige solid which was used directly in the next step. LC – MS (method D, ESI, m/z) t_R = 1.69 min, 282/284 $(M+H)^+$.

5-Amino-8-bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide



In a 12 mL screw-capped vessel 8-bromo-2-(methylcarbamoyl)-1,6-naphthyridine 6-oxide (90% pure, 59 mg, 0.19 mmol) was suspended in pyridine (3 mL). Toluene-4-sulfonyl chloride (43 mg, 0.23 mmol) was added and the reaction mixture was stirred for 30 min at rt. Ethanolamine (282 µL, 4.72 mmol) was added. The reaction mixture turned dark red and was stirred for an additional 30 min. The red mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water, dried over $Na_2S_2O_4$, filtered and evaporated to dryness to give 49 mg (85% pure, 79% yield) of 5-amino-8-bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide as a brown solid which was used directly in the next step. LC – MS (method D, ESI, m/z) t_R = 1.66 min, 281/283 $(M+H)^+$.

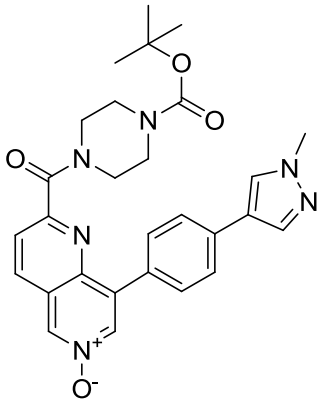
5-Amino-*N*-methyl-8-(1-methyl-2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-1,6-naphthyridine-2-carboxamide (**48**)



To a solution of 5-amino-8-bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide (85% pure, 49 mg, 0.15 mmol) in *N*,*N*-dimethylformamide (2.5 mL) were added 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3-dihydrobenzo[*c*]isothiazole 2,2-dioxide[11] (55 mg, 0.18 mmol), 0.5 M aqueous sodium carbonate solution (0.59 mL, 0.30 mmol) and Pd(dppf)Cl_2. CH_2Cl_2 (6 mg, 0.01 mmol). The vial was flushed with nitrogen twice and heated at 120°C for 1 h under microwave irradiation. The reaction mixture was treated with water and solid sodium chloride. The resulting brown precipitate was filtered off, washed with water and purified by flash chromatography (Companion, DCM/methanol gradient). This impure solid obtained was suspended in acetonitrile, filtered, washed with acetonitrile to give 5-amino-*N*-methyl-8-(1-methyl-2,2-dioxido-1,3-dihydrobenzo[*c*]isothiazol-5-yl)-1,6-naphthyridine-2-carboxamide **48** (17 mg, 29% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.88 (d, *J* = 8.5 Hz, 1H), 8.14 (s, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 8.07 – 8.02 (m, 1H), 7.70 (s, 1H), 7.68 – 7.63 (m, 1H), 7.36 (s, 2H), 7.04 (d, *J* = 8.2 Hz, 1H), 4.74 (s, 2H), 3.10 (s, 3H), 2.88 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 164.3, 157.6, 152.6, 148.0, 146.4, 140.3, 135.1, 130.3, 130.1, 127.0, 121.2, 118.3, 117.8, 112.8, 109.3, 50.4, 26.3, 26.2; LC – MS (method B, ESI, m/z) t_R = 1.72 min, 384 $(M+H)^+$; ESI-HRMS calcd for $C_{18}H_{18}N_5O_3S$ $(M+H)^+$ 384.1125, found 384.1118.

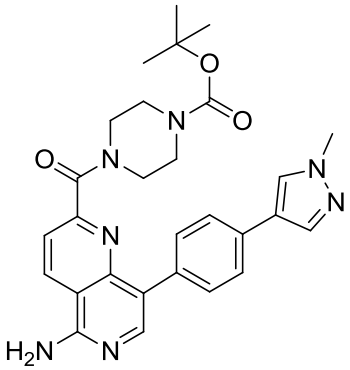
Preparation of compound 49 (Table 4)

2-(4-(*tert*-Butoxycarbonyl)piperazine-1-carbonyl)-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine 6-oxide



To a mixture of *tert*-butyl 4-(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate (171 mg, 0.343 mmol) in DCM (3 mL) was added 3-chloroperoxybenzoic acid (93 mg, 0.38 mmol) and the mixture was stirred at rt for 1 h. The mixture was diluted with 1N NaOH and DCM and the layers were separated. The aqueous layer was extracted with DCM twice. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 80:20) to give 2-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine 6-oxide (125 mg, 71% yield) as a yellow solid. ^{1}H-NMR (500 MHz, CD_3OD) δ 9.10 (d, *J* = 2.1 Hz, 1H), 8.56 (d, *J* = 8.6 Hz, 1H), 8.53 (d, *J* = 2.1 Hz, 1H), 8.10 (s, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.94 (d, *J* = 0.9 Hz, 1H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 3.96 (s, 3H), 3.78 - 3.72 (m, 2H), 3.62 (s, 2H), 3.51 (s, 2H), 3.27 (s, 2H), 1.40 (s, 9H); LC – MS (method C, ESI, mz) t_R = 1.29 min, 515 $(M+H)^+$.

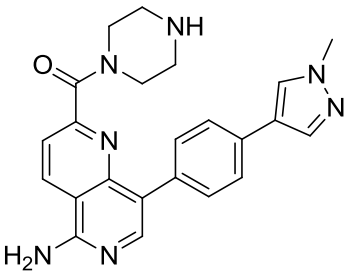
tert-Butyl 4-(5-amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate



To a suspension of 2-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine 6-oxide (120 mg, 0.233 mmol) in pyridine (5.5 mL, 47 mmol) was added 4-toluenesulfonyl chloride (53 mg, 0.28 mmol) and the reaction mixture was stirred at rt for 40 min. Ethanolamine (353 µL, 5.83 mmol) was then added and the reaction mixture was stirred at rt for 45 min. The mixture was then diluted with water and EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 90:10) to give a yellow solid (32 mg) which was combined with the precipitate formed in the aqueous layer (53 mg, isolated by filtration)

to give *tert*-butyl 4-(5-amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate (85 mg, 71% yield) as a yellow solid. ^{1}H-NMR (500 MHz, DMSO-d6) δ 8.81 (d, *J* = 8.6 Hz, 1H), 8.15 (s, 1H), 8.13 (s, 1H), 7.87 (d, *J* = 0.8 Hz, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.28 (bs, 2H), 3.87 (s, 3H), 3.65 - 3.58 (m, 2H), 3.54 - 3.47 (m, 2H), 3.45 - 3.37 (m, 2H), 3.29 - 3.20 (m, 2H), 1.37 (s, 9H); LC – MS (method C, ESI, m/z) t_R = 1.18 min, 514 $(M+H)^+$.

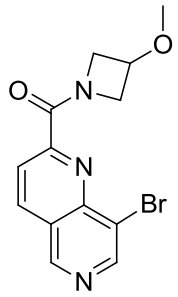
(5-Amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)(piperazin-1-yl)methanone (**49**)



To a solution of *tert*-butyl 4-(5-amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine-2-carbonyl)piperazine-1-carboxylate (85 mg, 0.17 mmol) in DCM (4.5 mL) was added trifluoroacetic acid (0.483 mL, 3.31 mmol) at 0 °C and the mixture was stirred at rt for 1 h. Trifluoroacetic acid was removed by azeotropic distillation with toluene and the residue was purified by Biotage column chromatography (DCM/25% aq. NH_4OH in MeOH (9/1), 100:0 to 50:50) and further purified by ion exchange (SCX2 cartridge, loading with DCM/MeOH 9/1, elution with DCM/1N NH_3 in MeOH 9:1) to give a yellow residue. This residue was triturated with hot EtOAc and was filtered to give 40 mg of material, which was further purified by prep. HPLC to give (5-amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)(piperazin-1-yl)methanone **49** (38 mg, 56% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.78 (d, *J* = 8.5 Hz, 1H), 8.16 (s, 1H), 8.11 (s, 1H), 7.88 (s, 1H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.25 (s, 2H), 3.87 (s, 3H), 3.57 – 3.53 (m, 2H), 3.42 – 3.37 (m, 2H), 2.75 – 2.70 (m, 2H), 2.66 – 2.61 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d6) δ 166.2, 157.5, 156.9, 148.1, 146.3, 136.0, 134.5, 134.3, 130.7, 130.4, 127.7, 124.4, 122.0, 121.8, 119.7, 111.4, 47.9, 46.0, 45.4, 42.7, 38.7; LC – MS (method C, ESI, m/z) t_R = 0.69 min, 414 $(M+H)^+$; ESI-HRMS calcd for $C_{23}H_{24}N_7O$ $(M+H)^+$ 414.2037, found 414.2030.

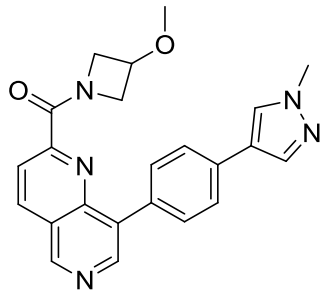
Preparation of compound 51 (Table 5)

(8-Bromo-1,6-naphthyridin-2-yl)(3-methoxyazetidin-1-yl)methanone



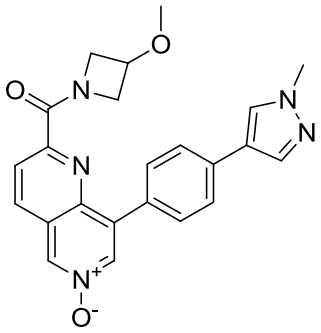
To a mixture of 8-bromo-1,6-naphthyridine-2-carboxylic acid (commercially available, 300 mg, 1.19 mmol) in DMF (12 mL) was added 3-methoxy-azetidine HCl salt (513 mg, 4.15 mmol), triethylamine (1.97 mL, 14.2 mmol) and HATU (1.58 g, 4.15 mmol) and the reaction mixture was stirred at rt for 1 h. Water and EtOAc were added and the layers separated. The organic layer was washed with water. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuum. The resulting brown oil was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 96:4) to give (8-bromo-1,6-naphthyridin-2-yl)(3-methoxyazetidin-1-yl)methanone (340 mg, quant. yield) as a brown sticky solid. ^{1}H-NMR (500 MHz, DMSO-d6) δ 9.48 (s, 1H), 9.10 (s, 1H), 8.82 (d, *J* = 8.5 Hz, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 5.08 - 5.02 (m, 1H), 4.71 - 4.67 (m, 1H), 4.45 - 4.28 (m, 2H), 4.00 - 3.94 (m, 1H), 3.27 (s, 3H). LC – MS (method C, ESI, m/z) t_R = 1.36 min, 322/324 $(M+H)^+$.

(3-Methoxyazetidin-1-yl)(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)methanone



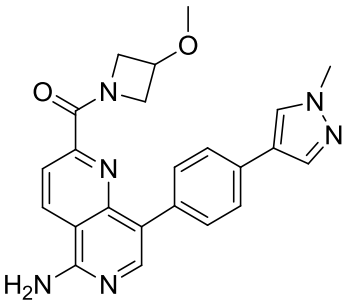
(8-Bromo-1,6-naphthyridin-2-yl)(3-methoxyazetidin-1-yl)methanone (280 mg, 0.869 mmol), 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole[9] (321 mg, 1.13 mmol) and Pd(dppf)Cl_2.CH_2Cl_2 (36 mg, 0.043 mmol) were loaded in a microwave vial and then acetonitrile (11 mL) and 0.5 M sodium carbonate in water (2.40 mL, 1.21 mmol) were added. The reaction was heated at 120 ºC for 60 min and concentrated. The crude product was purified by Biotage column chromatography (DCM/EtOH 99:1 to 80:20) to give (3-methoxyazetidin-1-yl)(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)methanone (300 mg, 86% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.83 (s, 1H), 8.46 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 8.5 Hz, 1H), 7.82 (d, *J* = 0.8 Hz, 1H), 7.70 (d, *J* = 0.8 Hz, 1H), 7.66 - 7.58 (m, 4H), 4.50 (ddd, *J* = 11.9, 6.0, 1.6 Hz, 1H), 4.39 - 4.25 (m, 2H), 4.14 - 4.02 (m, 2H), 3.98 (s, 3H), 3.17 (s, 3H); LC – MS (method B, ESI, m/z) t_R = 2.62 min, 400 $(M+H)^+$.

2-(3-Methoxyazetidine-1-carbonyl)-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine 6-oxide



To a mixture of (3-methoxyazetidin-1-yl)(8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)methanone (20 mg, 0.050 mmol) in DCM (0.5 mL) was added 3-chloroperoxybenzoic acid (15 mg, 0.060 mmol) and the reaction mixture was stirred at rt for 1.5 h. 1 N NaOH and DCM were added and the layers were separated. The aqueous layer was extracted with DCM twice. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuum. The residue was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 91:9) to give 2-(3-methoxyazetidine-1-carbonyl)-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine 6-oxide (14 mg, 67%) as a yellow solid which was used directly in the next step. LC – MS (method C, ESI, m/z) t_R = 1.15 min, 416 $(M+H)^+$.

(5-Amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)(3-methoxyazetidin-1-yl)methanone (**51**)

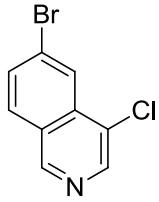


To a suspension of 2-(3-methoxyazetidine-1-carbonyl)-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridine 6-oxide (14 mg, 0.034 mmol) in pyridine (792 µL, 6.74 mmol) was added 4-toluenesulfonyl chloride (7.7 mg, 0.040 mmol) and the mixture was stirred at rt for 30 min. Ethanolamine (51 µl, 0.84 mmol) was then added and the reaction mixture was stirred at rt for 45 min. The mixture was then diluted with water and EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by Biotage column chromatography (DCM/EtOH, 100:0 to 80:20) and further purified by ion exchange (SCX2 cartridge, loading with DCM/MeOH 9/1, elution with DCM/1N NH_3 in MeOH 9/1) and by prep. HPLC to give (5-amino-8-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-1,6-naphthyridin-2-yl)(3-methoxyazetidin-1-yl)methanone **51** (6.5 mg, 47% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.84 (d, *J* = 8.7 Hz, 1H), 8.16 (s, 1H), 8.09 (s, 1H), 8.03 (d, *J* = 8.7 Hz, 1H), 7.88 (d, *J* = 0.8 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.36 (bs, 2H), 4.44 – 4.38 (m, 1H), 4.25 – 4.19 (m, 1H), 4.17 – 4.07 (m, 2H), 3.88 (s, 3H), 3.84 – 3.78 (m, 1H), 3.08 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 163.8, 157.5, 153.8, 148.7, 145.6, 135.9, 134.6, 134.4, 131.0, 130.8, 127.6,

124.3, 122.7, 121.8, 119.2, 112.1, 69.3, 61.3, 55.5, 55.1, 38.7; LC – MS (method C, ESI, m/z) t_R = 1.06 min, 415 $(M+H)^+$. ESI-HRMS calcd for $C_{23}H_{23}N_6O_2$ $(M+H)^+$ 415.1877, found 415.1872

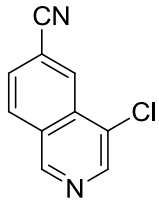
Preparation of compound 59 (Table 5)

6-Bromo-4-chloroisoquinoline



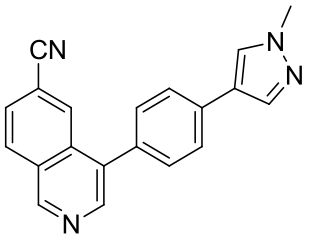
6-Bromoisoquinoline (commercially available, 2.0 g, 9.6 mmol) in solution in sulfuryl chloride (5.0 mL, 62 mmol) was heated at 60 °C for 5 min. Another 5 mL of sulfuryl chloride were added and the reaction mixture was heated for 25 min. Sat. $NaCO_3$ aq. solution was added to the reaction mixture and then EtOAc was added. The layers were separated. The aqueous layer was extracted three times with EtOAc and the organic layers were combined and dried over $MgSO_4$. The crude product was purified by Biotage column chromatography (DCM/EtOH 99.9:0.01) to give 6-bromo-4-chloroisoquinoline (1.30 g, 56% yield) as a cream solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.62 (s, 1H), 8.43 – 8.38 (m, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.79 (dd, *J* = 8.7, 1.8 Hz, 1H); LC – MS (method C, ESI, m/z) t_R = 1.58 min, 242/244/246 $(M+H)^+$.

4-Chloroisoquinoline-6-carbonitrile



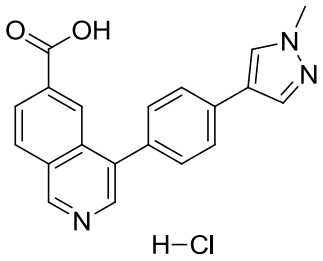
Two reactions were set up as follow: 6-bromo-4-chloroisoquinoline (500 mg, 2.06 mmol), zinc cyanide (266 mg, 2.27 mmol) and $Pd(PPh_3)_4$ (238 mg, 0.206 mmol) were loaded in a microwave vial and then DMF (14 mL) was added. The reaction mixture was heated for 1 h 30 min at 60 °C. The crude was concentrated and purified by Biotage column chromatography (DCM/EtOH 99.9/0.1 to 97.5/2.5) to give 4-chloroisoquinoline-6-carbonitrile (245 mg, 31% yield) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.74 (s, 1H), 8.62 (dt, *J* = 1.6, 0.8 Hz, 1H), 8.15 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.86 (dd, *J* = 8.4, 1.6 Hz, 1H); LC – MS (method C, ESI, m/z) t_R = 1.30 min, 189/191 $(M+H)^+$.

4-(4-(1-Methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline-6-carbonitrile



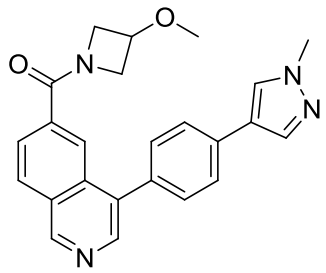
4-Chloroisoquinoline-6-carbonitrile (121 mg, 0.642 mmol), 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole[9] (219 mg, 0.770 mmol) and $Pd(dppf)Cl_2.CH_2Cl_2$ (26 mg, 0.032 mmol) were loaded in a microwave vial and then acetonitrile (11 mL) and 0.5 M sodium carbonate in water (1.80 mL, 0.898 mmol) were added. The reaction was heated at 150 ºC for 60 min. and concentrated. The crude product was purified by Biotage column chromatography (DCM/EtOH 99.9:0.1 to 98:2) to give 4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline-6-carbonitrile (142 mg, 71% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.68 (s, 1H), 8.39 (d, *J* = 1.5 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 0.8 Hz, 1H), 7.81 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.74 (s, 1H), 7.71 – 7.67 (m, 2H), 7.53 – 7.47 (m, 2H), 4.02 (s, 3H); LC – MS (method C, ESI, m/z) t_R = 2.86 min, 311 $(M+H)^+$.

4-(4-(1-Methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline-6-carboxylic acid hydrochloride



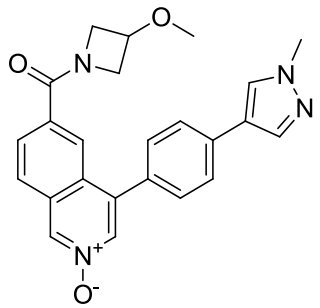
To 4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline-6-carbonitrile (100 mg, 0.322 mmol) in ethanol (2.50 mL) was added 2 M sodium hydroxide (2.50 mL, 5.00 mmol). The reaction was heated at 100 °C for 1 h 30 min. Hydrogen chloride (5.00 mL, 5.00 mmol) was added to the reaction mixture and the solution was concentrated. Isopropanol was added to the residue, the salts were filtered and the filtrate was concentrated. 4 N HCl in dioxane was added to the residue obtained. The solution was evaporated to obtain 4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline-6-carboxylic acid hydrochloride (100 mg, 85 % yield) as a yellow solid which was used directly in the next step. ^{1}H NMR (500 MHz, DMSO-d6) δ 9.70 (s, 1H), 8.68 (s, 1H), 8.60 (s, 1H), 8.51 (d, *J* = 8.5 Hz, 1H), 8.32 – 8.27 (m, 2H), 8.01 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 3.91 (s, 3H); LC – MS (method B, ESI, m/z) t_R = 2.58 min, 330 $(M+H)^+$.

(3-Methoxy-azetidin-1-yl)-{4-[4-(1-methyl-1*H*-pyrazol-4-yl)-phenyl]-isoquinolin-6-yl}-methanone



To a suspension of 4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline-6-carboxylic acid (60 mg, 0.18 mmol) in DMF (1.5 mL) was added HATU (83 mg, 0.22 mmol). The reaction mixture was stirred for 10 min before the addition of 3-methoxyazetidine hydrochloride (68 mg, 0.55 mmol). The mixture was stirred for 35 min before the addition of DIPEA (159 µL, 0.911 mmol). The resulting solution was then stirred at rt for 21 h. Additional HATU (83 mg, 0.22 mmol), 3-methoxyazetidine hydrochloride (68 mg, 0.55 mmol) and DIPEA (200 µL) were added. The reaction mixture was stirred at rt for another 3 h and concentrated. The crude product was purified by Biotage column chromatography (DCM/EtOH 99:1 to 80:20). The isolated product was then solubilised in DCM and washed with water. The organic layers were dried over $MgSO_4$ and concentrated to give (3-methoxy-azetidin-1-yl)-{4-[4-(1-methyl-1*H*-pyrazol-4-yl)-phenyl]-isoquinolin-6-yl}-methanone as a white solid (40 mg, 55% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (s, 1H), 8.58 (s, 1H), 8.17 (s, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 7.87 (dd, *J*= 8.4, 1.4 Hz, 1H), 7.86 (s, 1H), 7.72 (s, 1H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 4.40-4.30 (m, 2H), 4.26-4.21 (m, 1H), 4.12-4.06 (m, 2H), 4.00 (s, 3H), 3.29 (s, 3H); LC – MS (method B, ESI, m/z) t_R = 2.44 min, 399 $(M+H)^+$; ESI-HRMS calcd for $C_{24}H_{23}N_4O_2$ 399.1816 $(M+H)^+$, 399.1808.

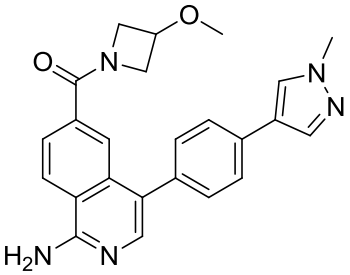
6-(3-Methoxyazetidine-1-carbonyl)-4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline 2-oxide



To a solution of (3-methoxyazetidin-1-yl)(4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl) isoquinolin-6-yl)methanone (30 mg, 0.075 mmol) in DCM (753 µL) was added 3-chloroperoxybenzoic acid (13 mg, 0.075 mmol) and the reaction mixture was stirred at rt for 1h. Additional 4 mg of 3-chloroperoxybenzoic acid were added. After 30 min, the conversion was not complete and another 2 mg of 3-chloroperoxybenzoic acid were added and the reaction mixture was stirred for another 30 min at rt. 1N NaOH and DCM were added to the reaction mixture and the layers were separated. The aqueous layers were extracted with DCM and the organics layers were dried over $MgSO_4$ and concentrated. The crude product was used in the next step without any purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.18 (d, *J* = 1.5 Hz, 1H), 8.07 (s,

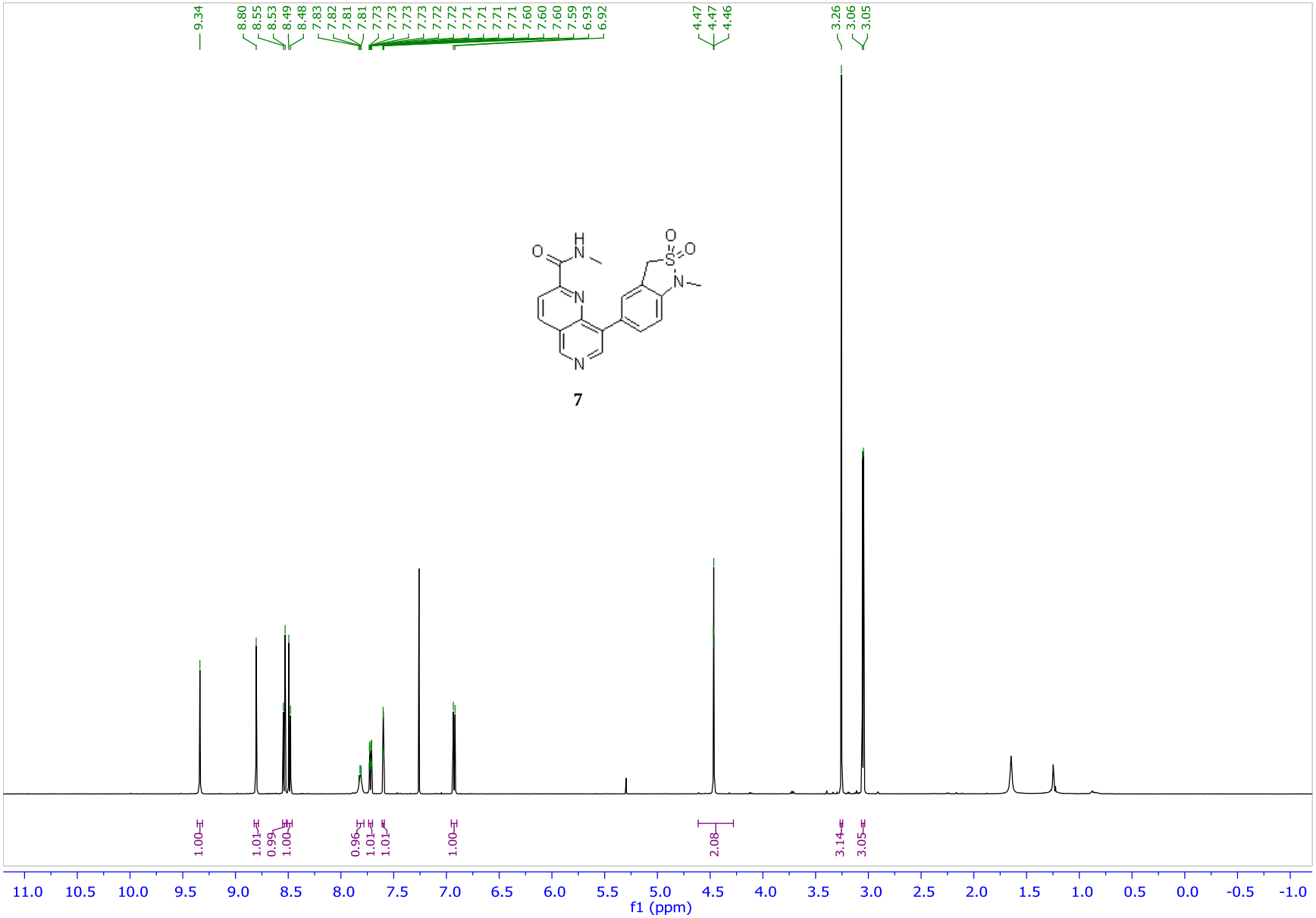
1H), 7.89-7.80 (m, 2H,), 7.86 (s, 1H), 7.73(s, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 4.40-4.30 (m, 2H), 4.27-4.21 (m, 1H), 4.12-4.04 (m, 2H), 4.00 (s, 3H), 3.30 (s, 3H); LC – MS (method C, ESI, m/z) Rt = 1.15 min – 415 $(M+H)^+$.

(1-Amino-4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinolin-6-yl)(3-methoxyazetidin-1-yl)methanone (**59**)



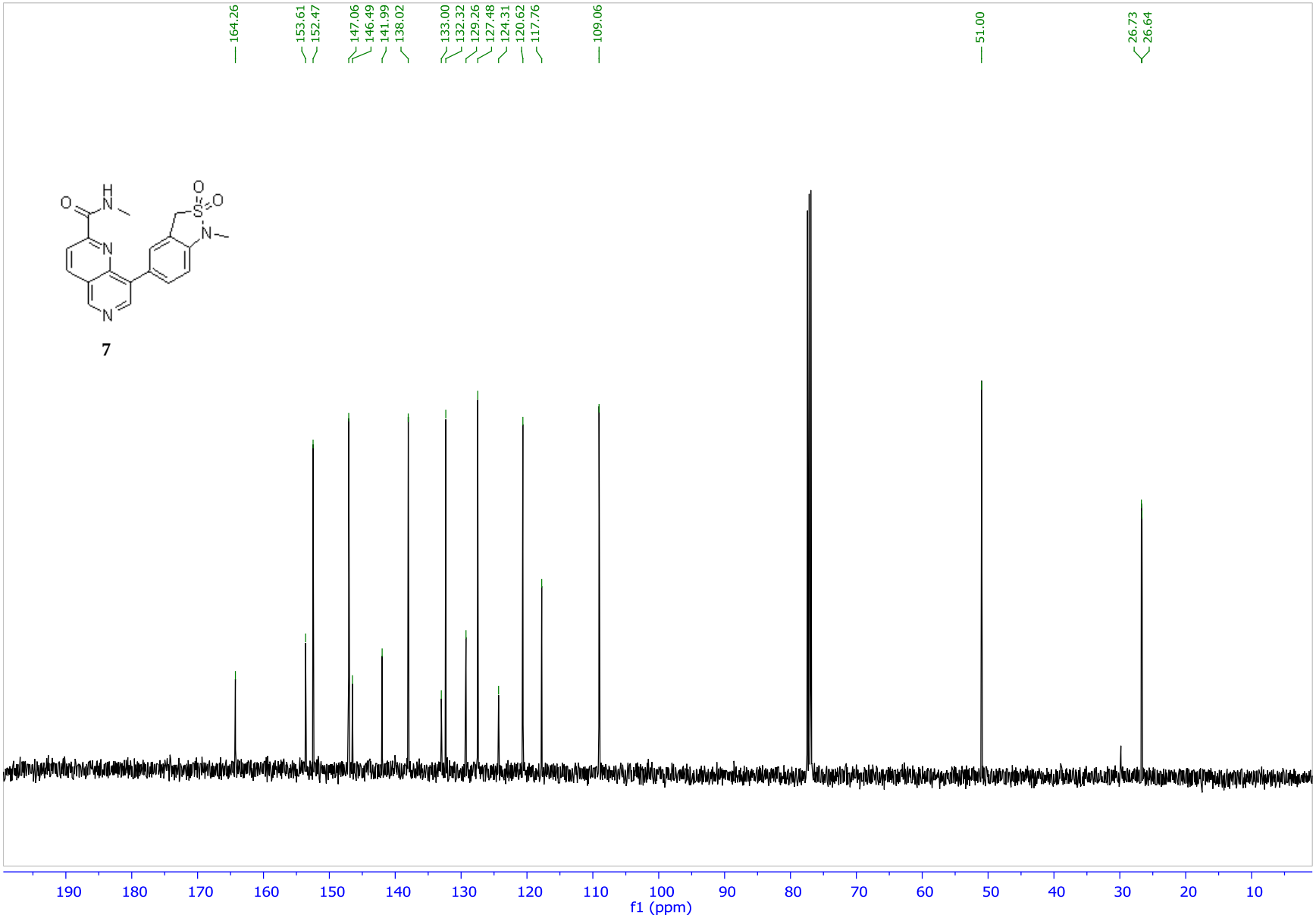
To a suspension of 6-(3-methoxyazetidine-1-carbonyl)-4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinoline 2-oxide (26 mg, 0.063 mmol) in pyridine (1.50 mL) was added 4-toluenesulfonlyl chloride (14 mg, 0.075 mmol) and the reaction mixture was stirred at rt for 1 h 15 min. Ethanolamine (95 µL, 1.6 mmol) was added and the reaction mixture was stirred at rt for 1 h. The mixture was diluted with water and EtOAc. The layers were separated and the aqueous layers were extracted three times with EtOAc. The organic layers were combined, dried over $MgSO_4$ and concentrated. The crude was purified by Biotage column chromatography (DCM/EtOH 96:4 to 82:18) to give (1-amino-4-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)isoquinolin-6-yl)(3-methoxyazetidin-1-yl)methanone **59** as a yellow solid (20 mg, 77% yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.34 (d, *J* = 8.5 Hz, 1H), 8.20 (s, 1H), 7.93 (s, 2H), 7.84 (s, 1H), 7.71 – 7.67 (m, 3H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.07 (s, 2H), 4.43 – 4.36 (m, 1H), 4.26 – 4.18 (m, 2H), 4.10 – 4.03 (m, 1H), 3.89 (s, 3H), 3.87 – 3.79 (m, 1H), 3.20 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 168.7, 156.9, 142.3, 136.1, 135.0, 134.5, 134.2, 131.4, 130.3, 127.9, 125.2, 124.8, 124.0, 123.8, 122.2, 121.6, 117.4, 69.0, 59.8, 55.6, 55.4, 38.7; LC – MS (method B, ESI, m/z) t_R = 2.03 min, 414 $(M+H)^+$; ESI-HRMS: calcd for $C_{24}H_{24}N_5O$ $(M+H)^+$ 414.1925, found 414.1904

^{1}H NMR ($CDCl_3$, 500 MHz)

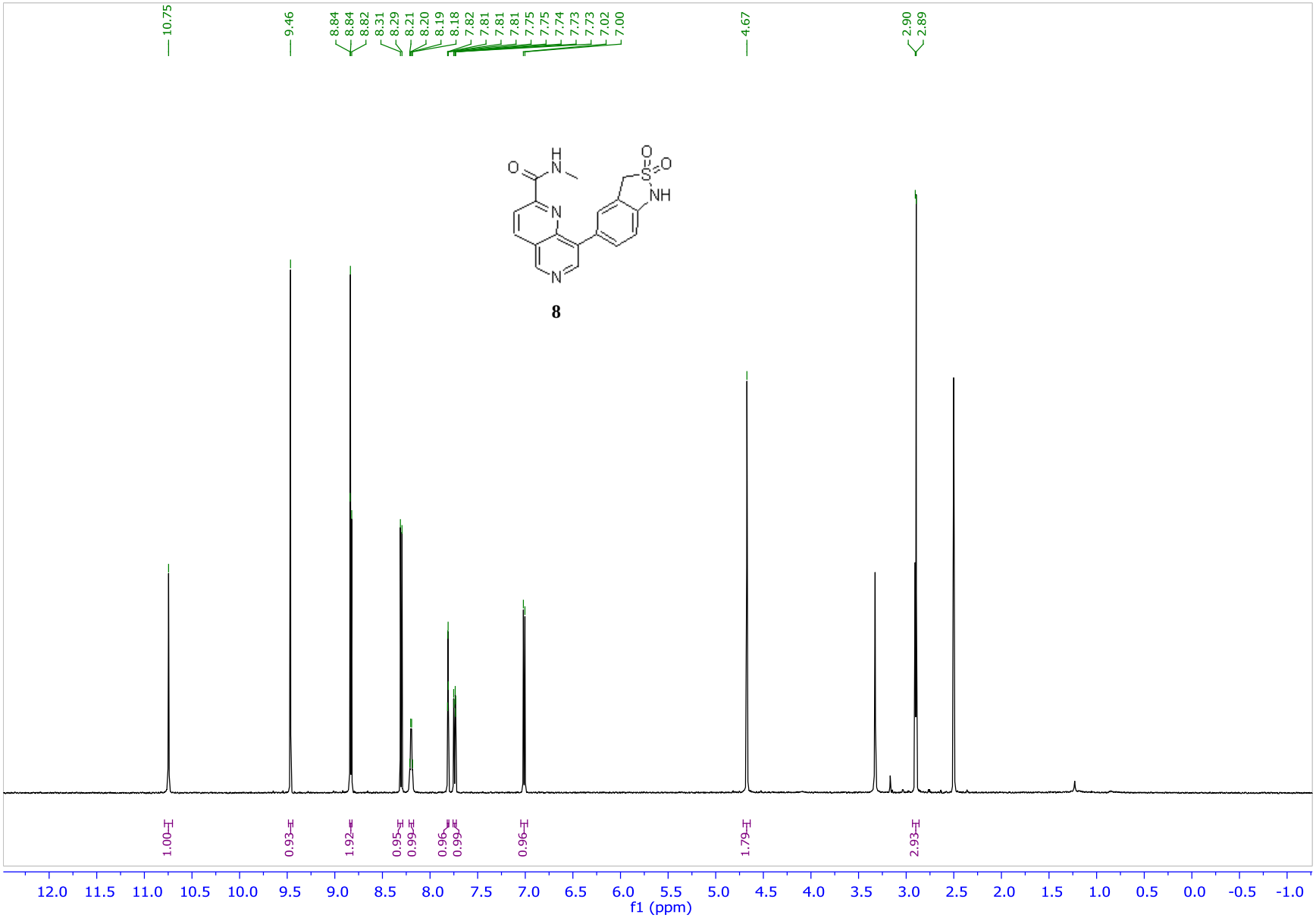


7

^{13}C NMR ($CDCl_3$, 500 MHz)

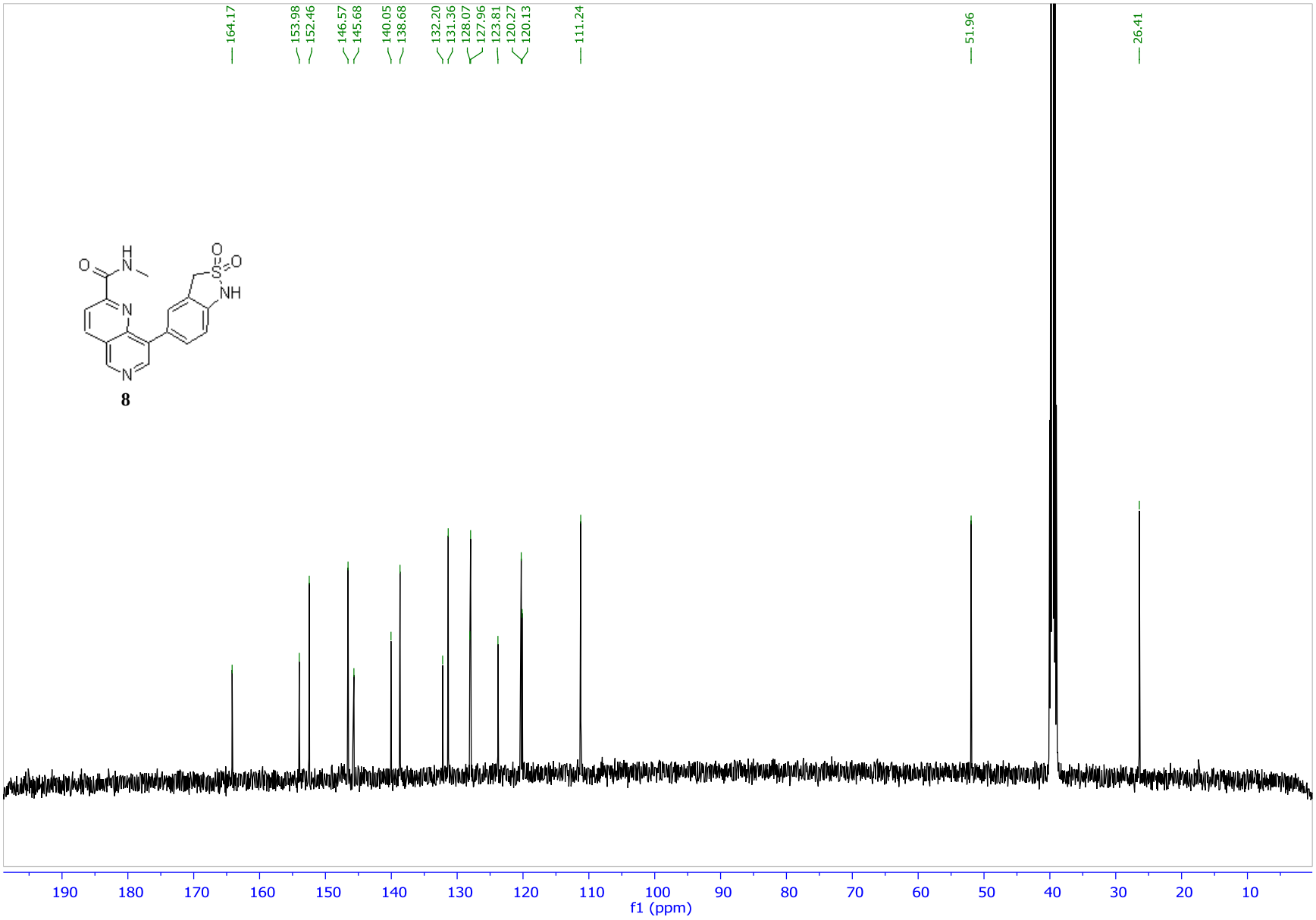


^{1}H NMR (DMSO-d6, 500 MHz)

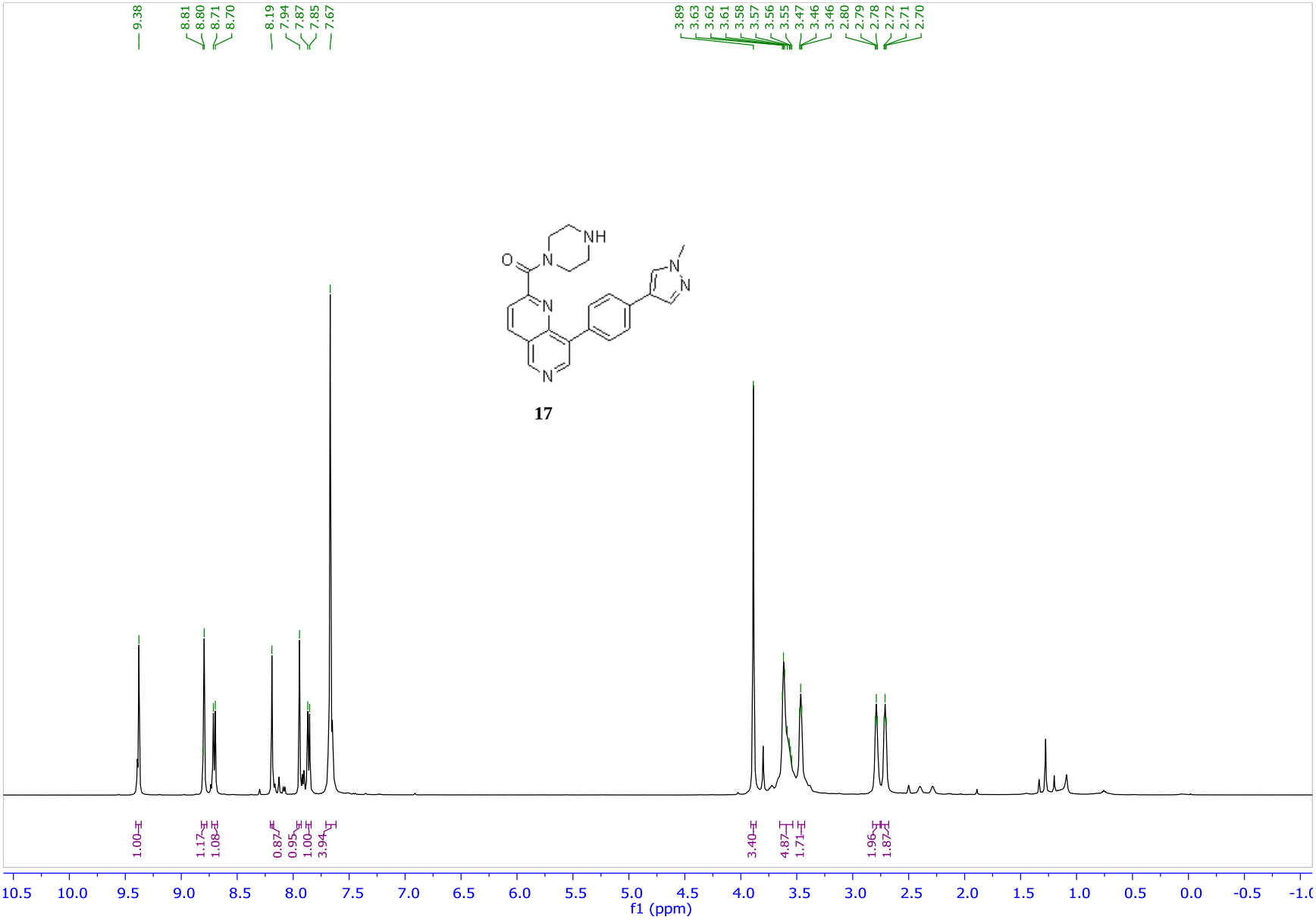


8

^{1}H NMR (DMSO-d6, 500 MHz)

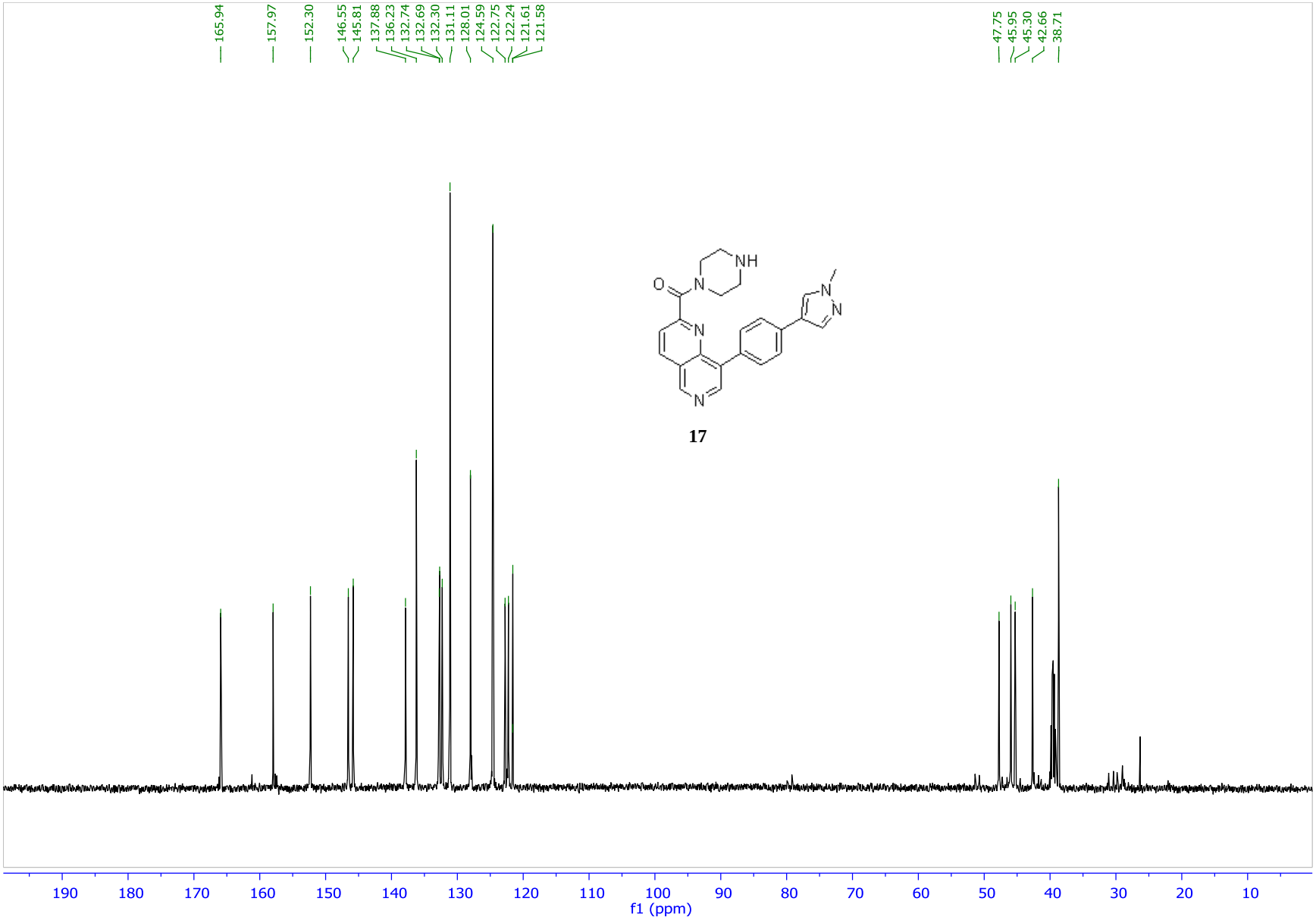


^{1}H NMR (DMSO-d6, 500 MHz)



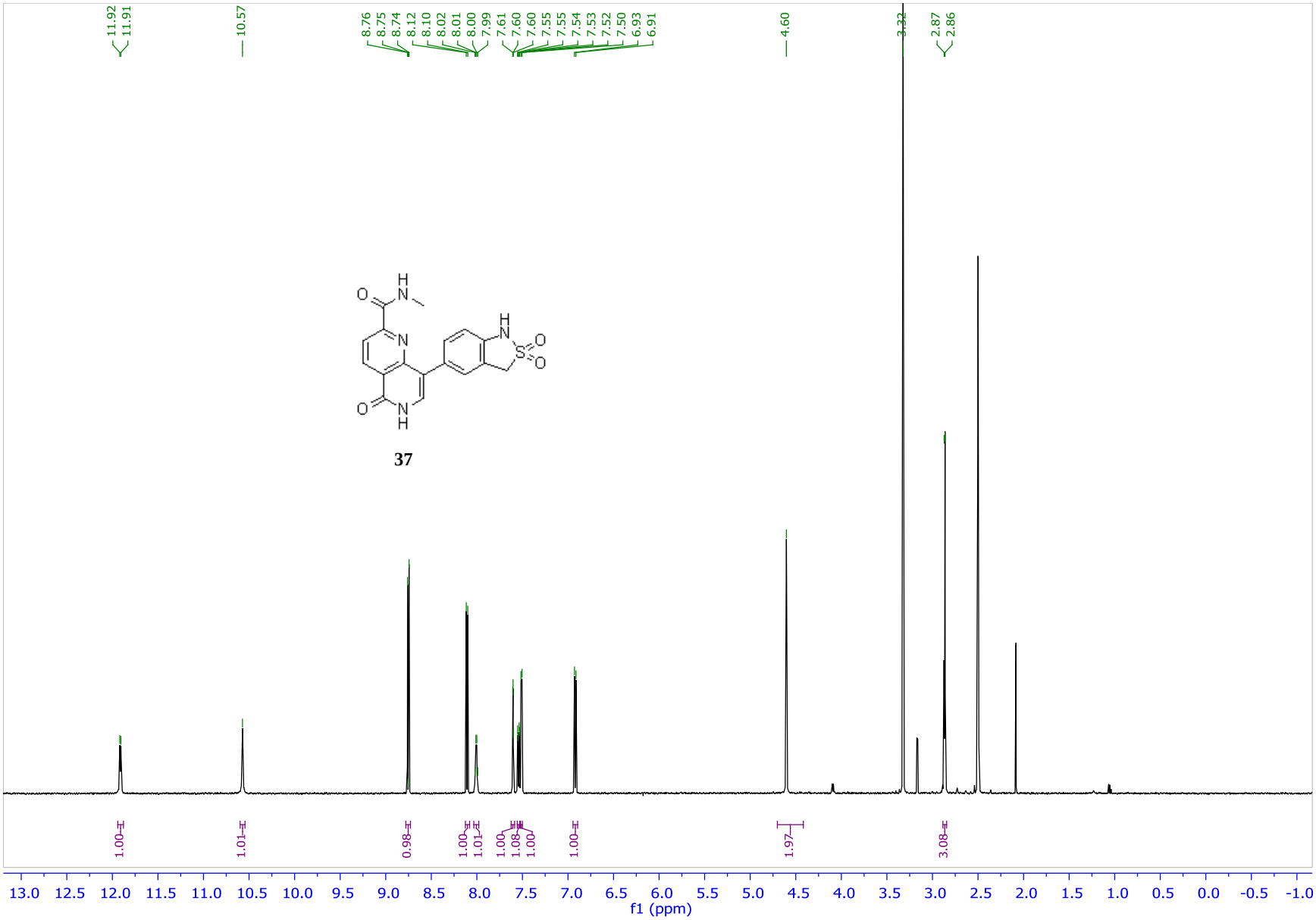
17

^{13}C NMR (DMSO-d6, 500 MHz)



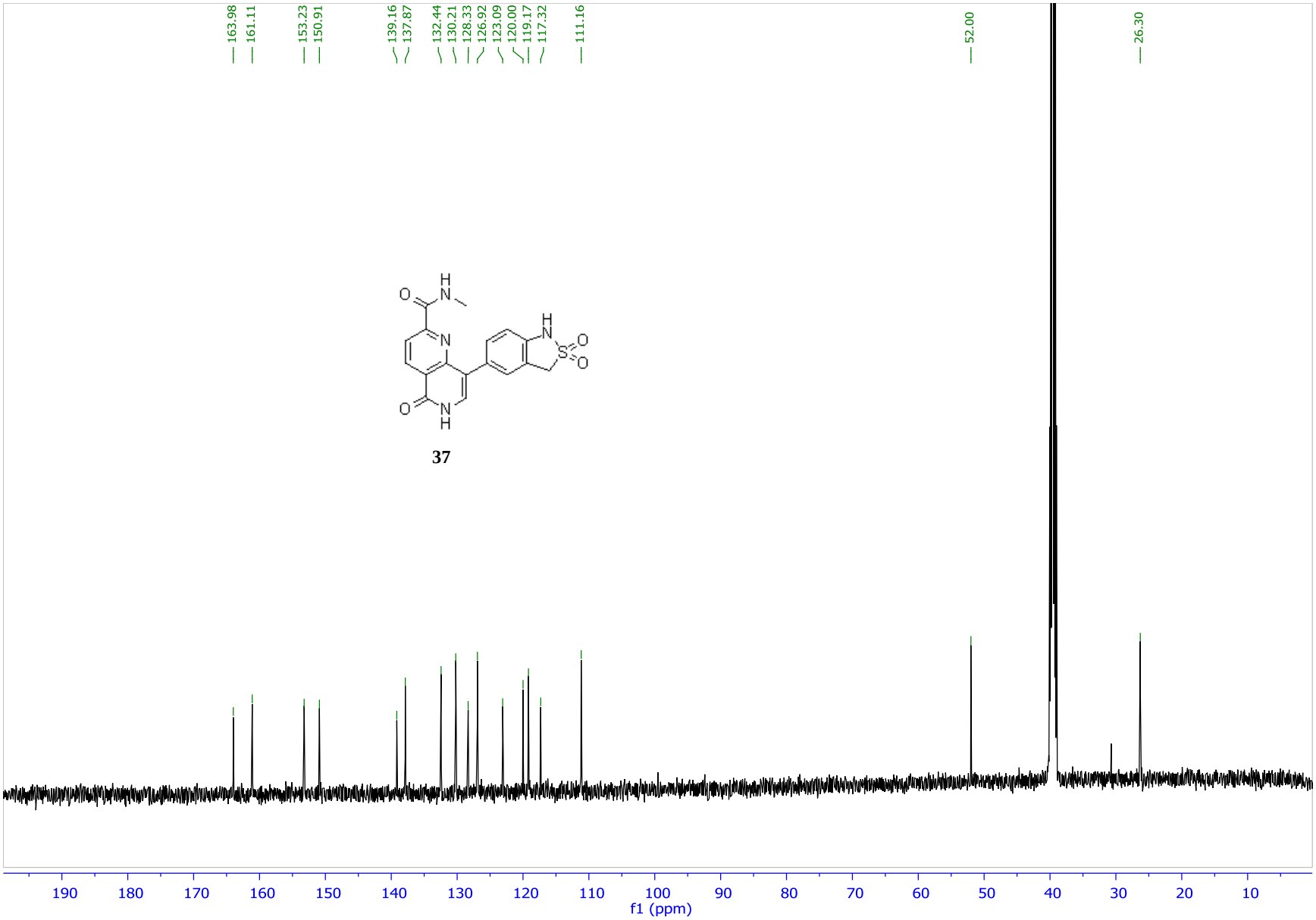
17

^{1}H NMR (DMSO-d6, 500 MHz)



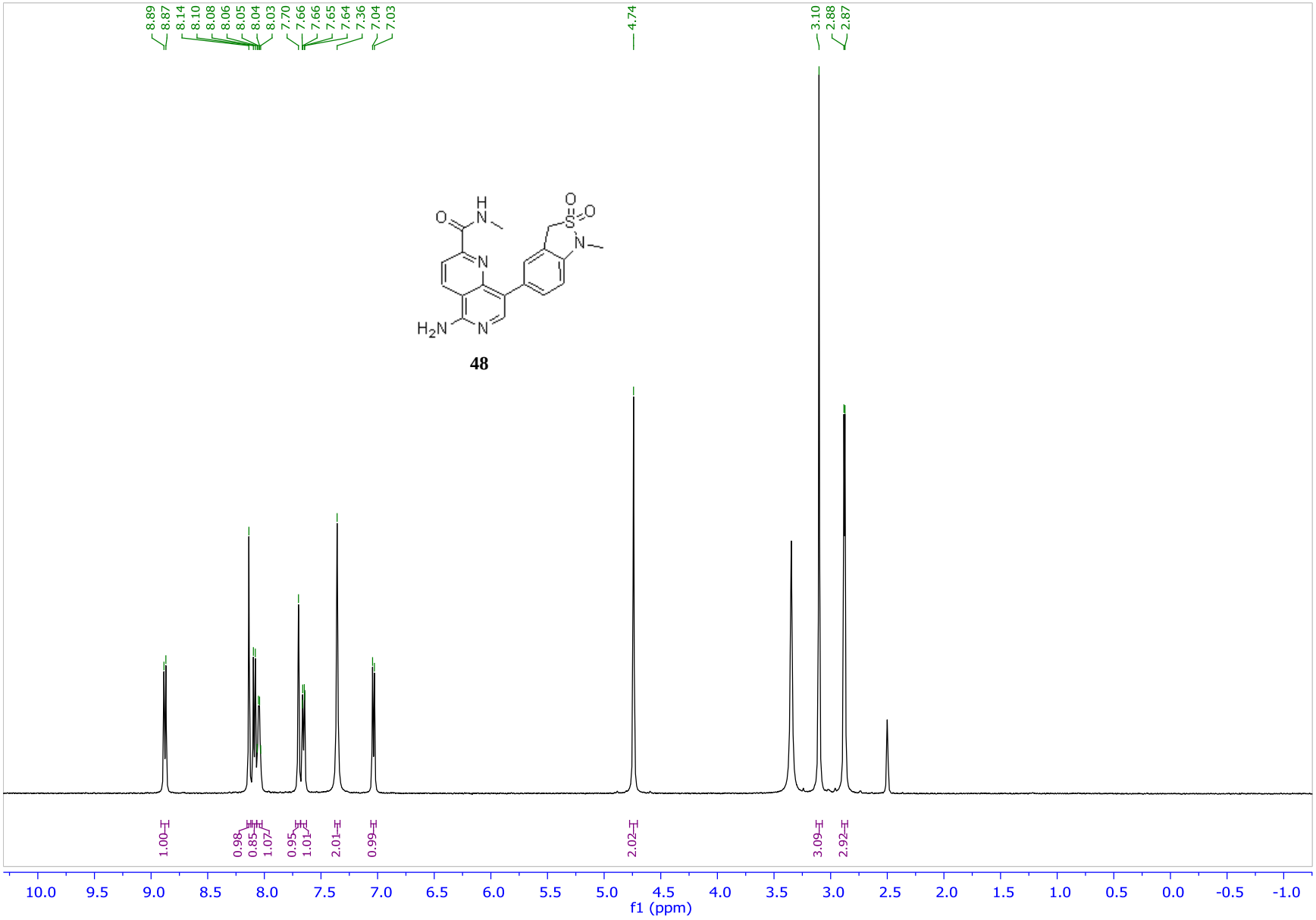
37

^{13}C NMR (DMSO-d6, 500 MHz)



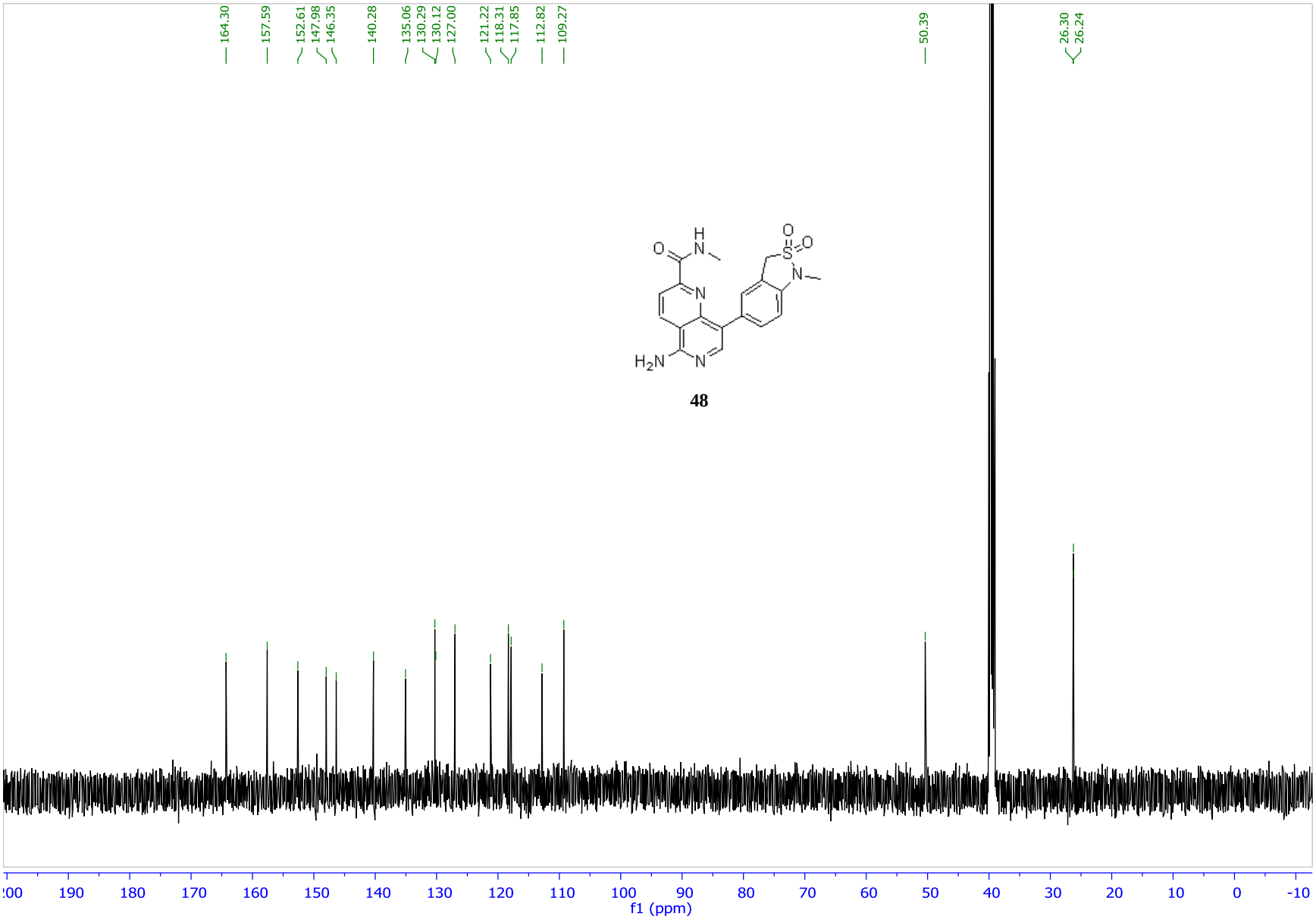
37

^{1}H NMR (DMSO-d6, 500 MHz)



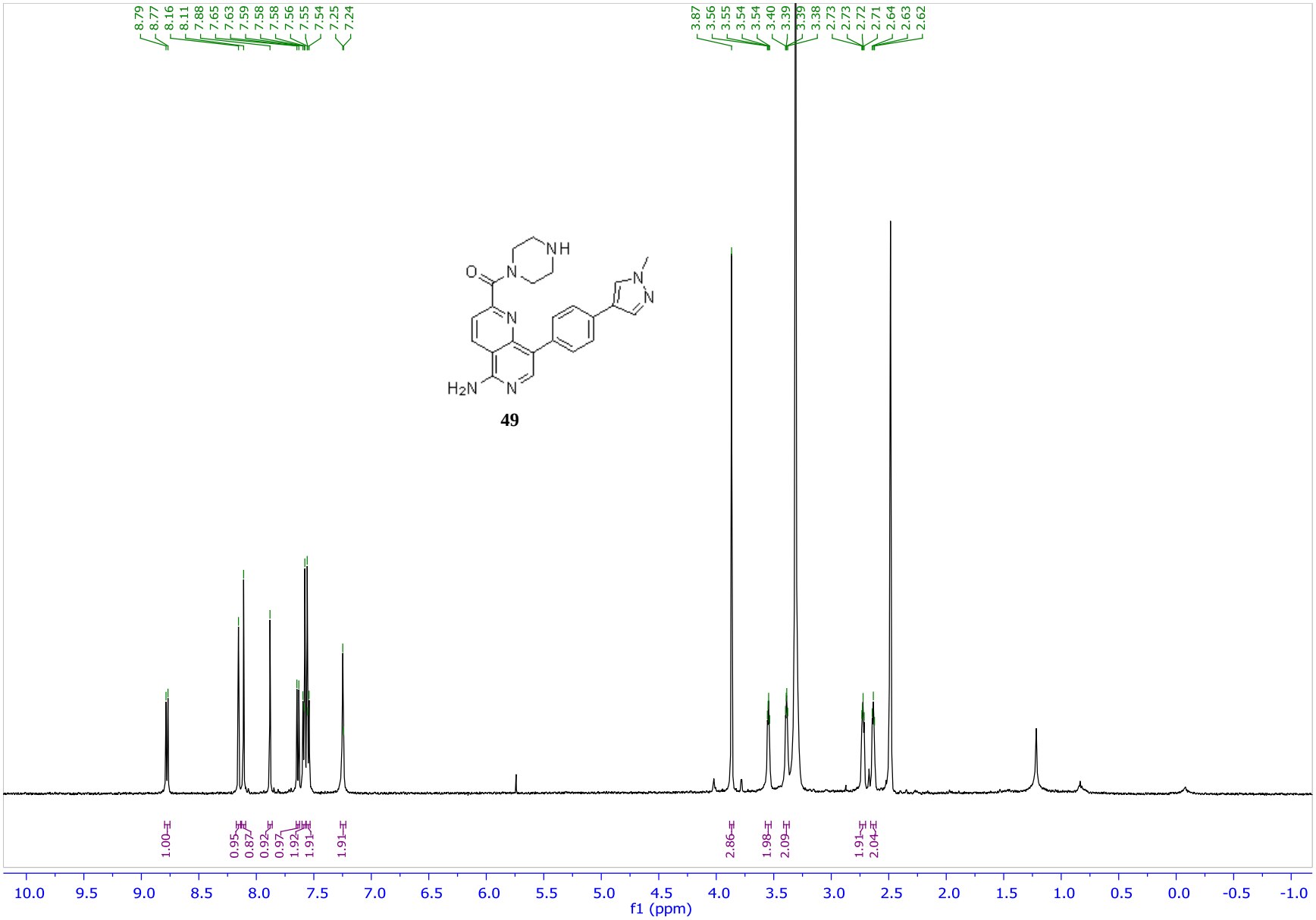
48

^{13}C NMR (DMSO-d6, 500 MHz)



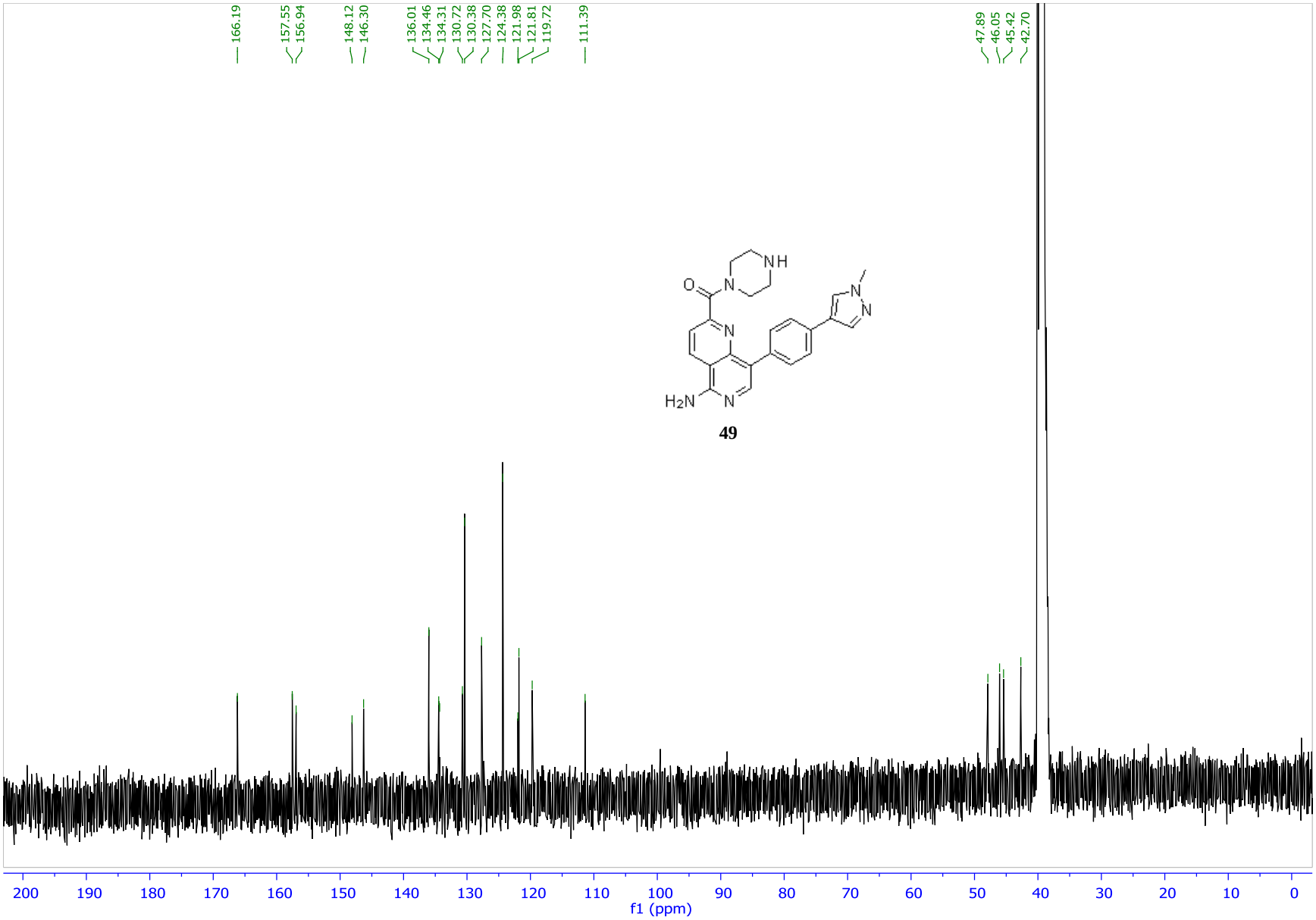
48

^{1}H NMR (DMSO-d6, 500 MHz)



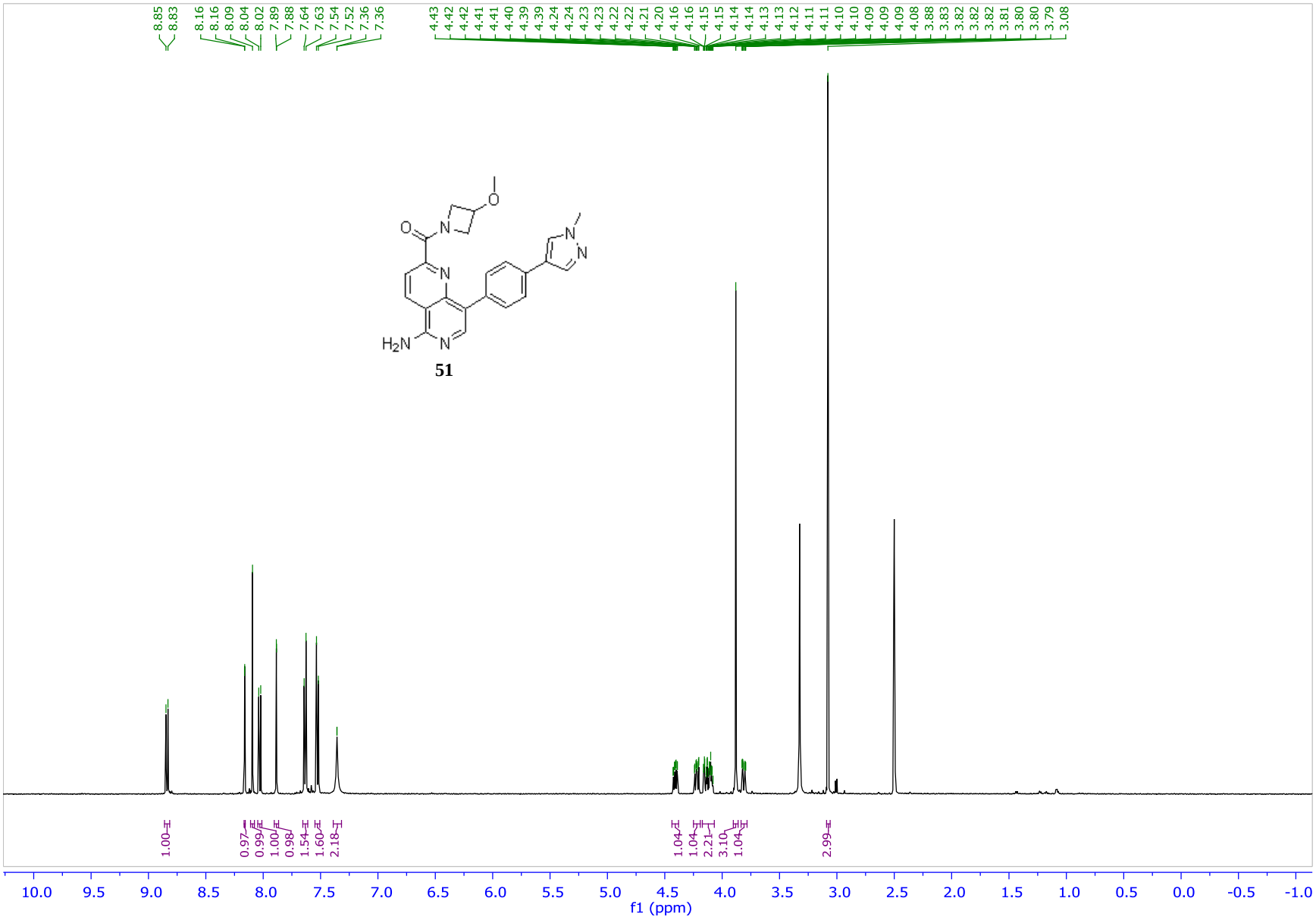
49

^{13}C NMR (DMSO-d6, 500 MHz)

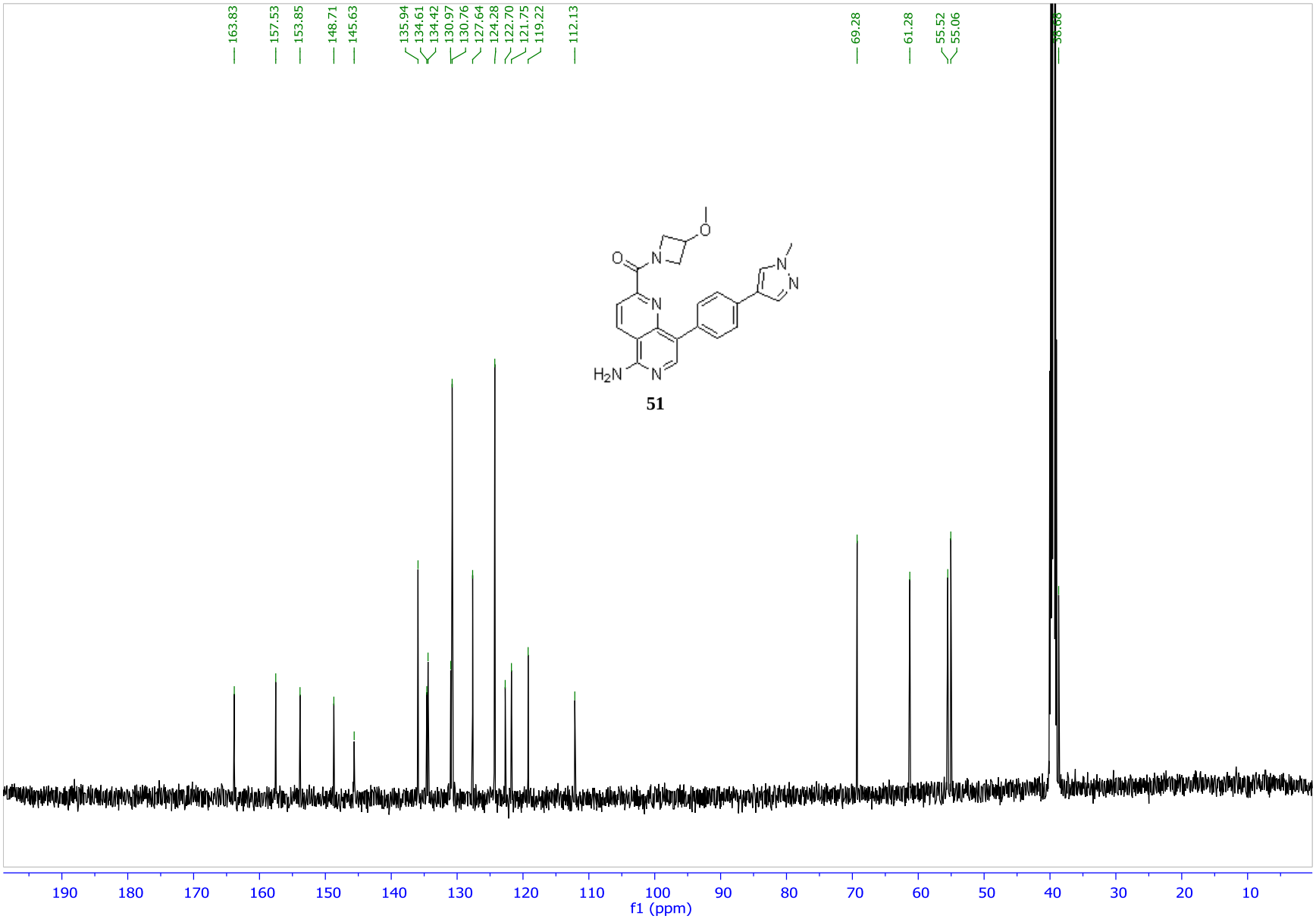


49

^{1}H NMR (DMSO-d6, 500 MHz)

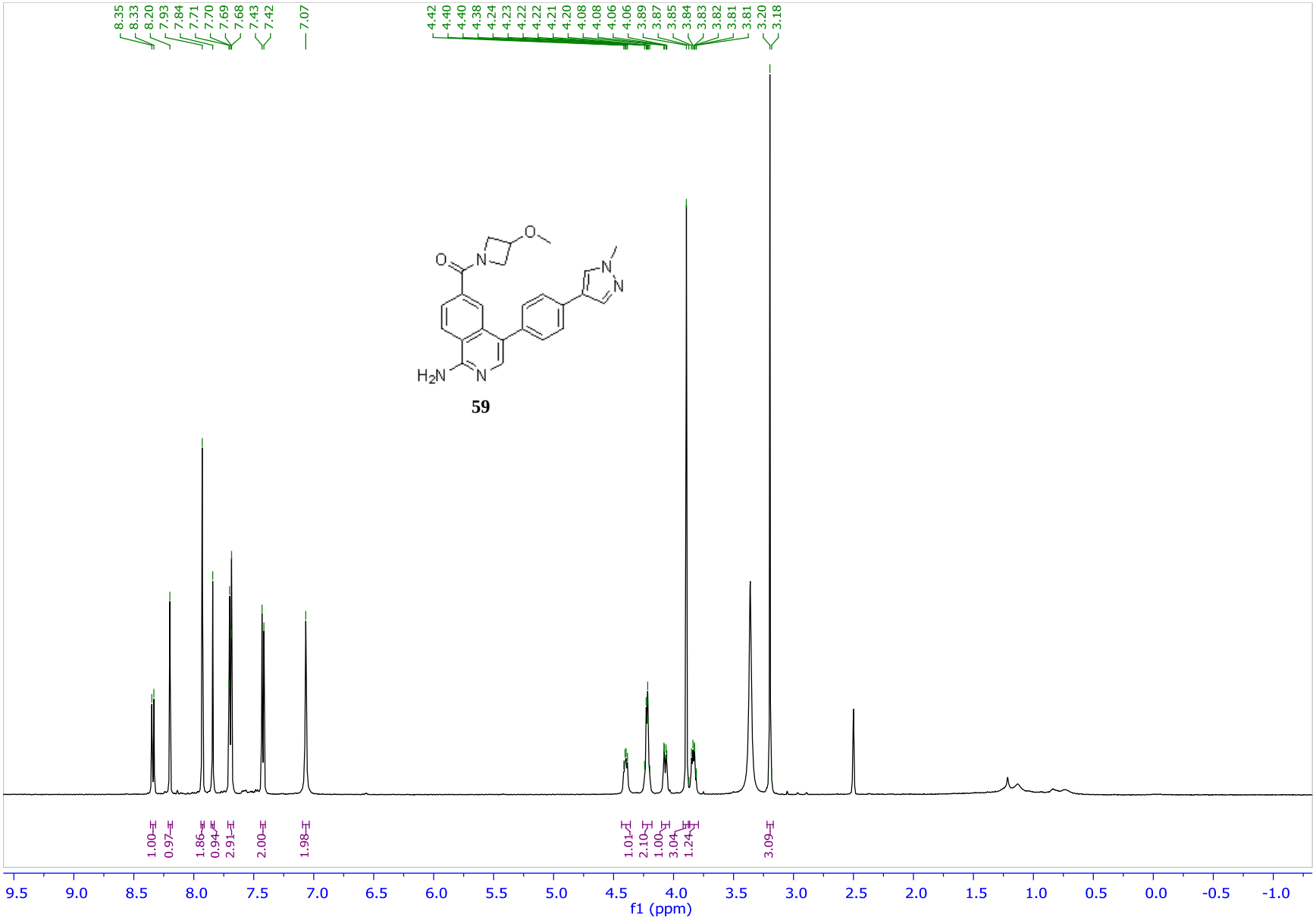


^{13}C NMR (DMSO-d6, 500 MHz)

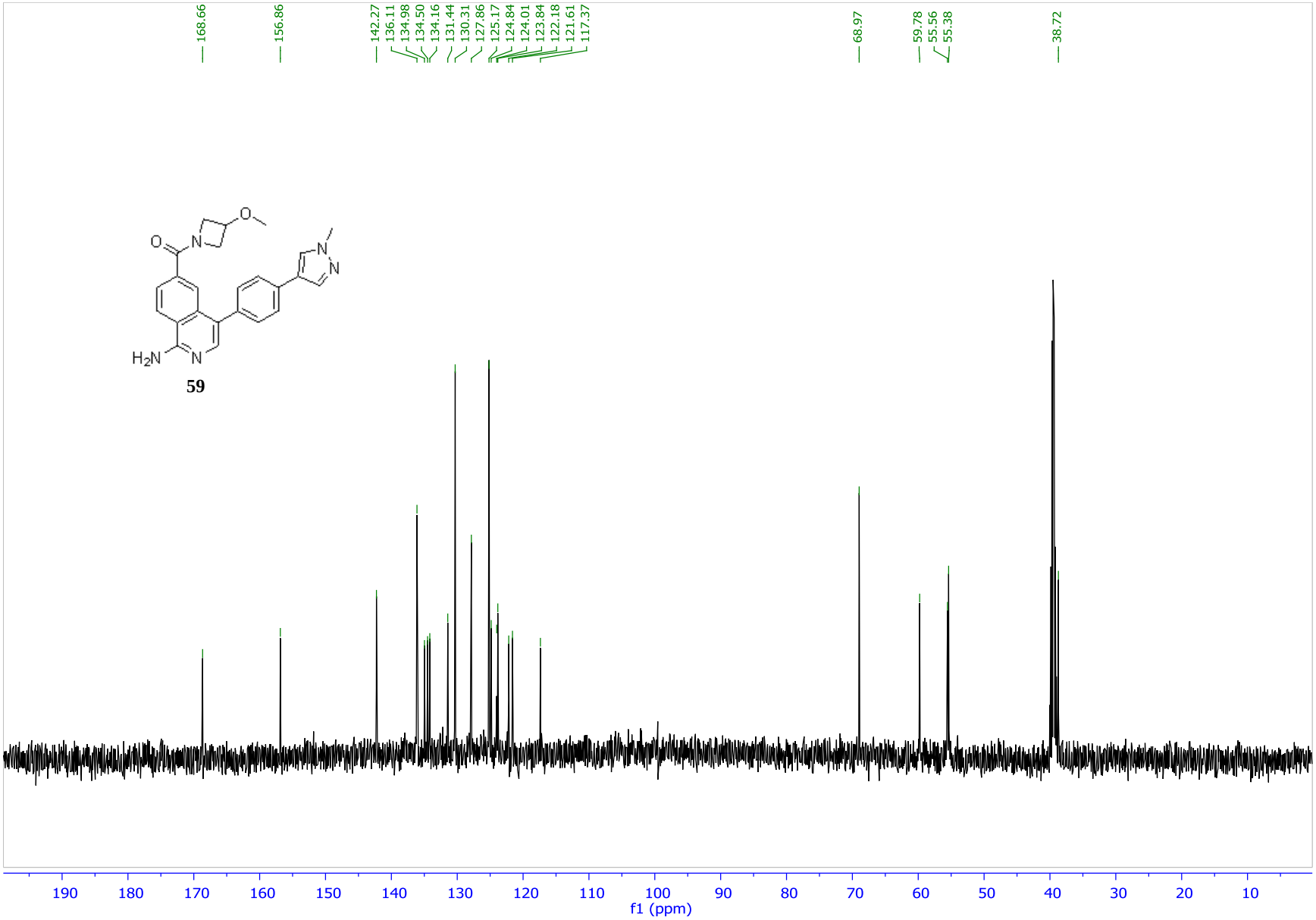


51

^{1}H NMR (DMSO-d6, 500 MHz)



^{13}C NMR (DMSO-d6, 500 MHz)



Crystallographic data and refinement parameters for protein crystal structure of CDK8/cyclin C bound to compound 7

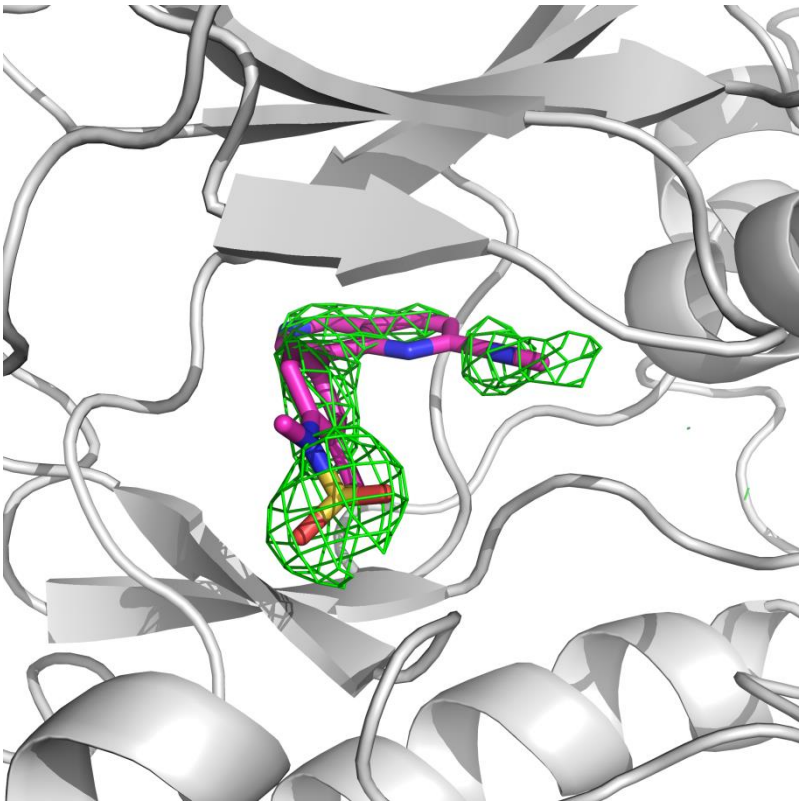
Data-collection details	Compound 7
Space group	$P2_12_12_1$
Unit-Cell parameters [Å, °]	a=70.75, b= 71.28, c= 171.95; α = 90.0 β = 90.0 γ = 90.0
Resolution [Å] [a]	2.60 (2.85-2.60)
Data collection wavelength [Å]	1.00000
Number of measurements [a]	108731 (24845)
Number of unique reflections [a]	26767 (6346)
Completeness[%] [a]	97.2 (97.2)
R_{merge} [%][a]	8.4 (50.3)
Mean(I)/sd [a]	17.74 (3.33)
Refinement	
Resolution [Å]	85.98-2.60
R_{work}/R_{free}	0.237 / 0.274[c]
Rmsd bond length [Å]	0.010
Rmsd bond angle [°]	1.22
Average B-factor [Å^2]	
Protein	42.8
Ligand	45.0
Water	33.5
Formic acid	47.0
All atoms	42.7
Number of atoms	
Protein atoms	5064
Ligand	26
Water	49
Formic acid	6
All atoms	5145
Ramachandran	
Core [%]	93.8
Allowed [%]	6.2
Generously allowed [%]	0.0
Dissallowed [%]	0.0

[a]Values for the last resolution shell shown in brackets;

[b]Using randomly selected 3.3% of data

[c]Using randomly selected 3.5% of data

Fo-Fc Omit map at 2.0 σ cut-off for protein crystal structure of CDK8/cyclin C bound to compound 7



Fo-Fc Omit map at 2.5 σ cut-off for protein crystal structure of CDK8/cyclin C bound to compound 7