Supporting Information

Development of highly potent and selective diaminothiazole inhibitors of cyclin-dependent kinases

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Table S1: Summary of data collection and refinement for CDK2-inhibitor complexes determined during the SAR studies. Values in parentheses refer to the highest resolution shell.

			Data Collection											*Structure Refinement*								
Comp #	PDB ID	Compound PDB ID	Space group	Cell dimensions			resolution range		Unique reflections		Completeness (%)		I/σI		Rmerge [a] (%)		Mean B-factor (Å²)	rmsd [b] bonds (Å²)	rmsd angles (deg)	Rcryst [c] (%)	Rfree [d] (%)	R free reflection set size		Cross-validated coordinate error	
				a	b	c																		Luzzati plot (Å)	SigmaA (Å)
1	3QQK	X02	$P2_12_12_1$	53.8	71.99	72.33	1.85-20.00	*(1.85-1.90)*	24 442	*(1 836)*	99.3	*(98.7)*	32.8	*(4.0)*	2.6	*(20.2)*	31.2	0.008	1.3	20.1	24.1	1 084	*4.5 %*	0.24	0.11
2	3R8V	Z62	$P2_12_12_1$	53.28	71.87	72.28	1.90-20.00	*(1.90-1.97)*	21 860	*(2 099)*	96.9	*(94.8)*	53.0	*(13.3)*	6.5	*(25.0)*	24.4	0.009	1.4	19.2	22.9	1 033	*4.8 %*	0.24	0.12
3	3QTQ	X35	$P2_12_12_1$	53.4	71.39	72.08	1.80-20.00	*(1.80-1.85)*	25 820	*(1 979)*	98.5	*(97.2)*	21.9	*(6.0)*	4.2	*(25.2)*	31.5	0.010	1.4	20.1	23.3	1 033	*4.0 %*	0.24	0.09
4	3R8U	Z61	$P2_12_12_1$	53.47	72.00	72.43	2.00-20.00	*(2.00-2.10)*	19 429	*(2 575)*	99.7	*(99.8)*	19.1	*(8.8)*	7.9	*(28.5)*	24.6	0.010	1.5	20.0	23.6	1 069	*5.5 %*	0.26	0.13
5	3S00	Z60	$P2_12_12_1$	53.1	71.91	71.9	1.80-20.00	*(1.80-1.85)*	26 123	*(2 033)*	99.8	*(100)*	29.4	*(11.6)*	4.3	*(15.4)*	26.1	0.008	1.4	21.6	25.1	1 045	*4.0 %*	0.26	0.20
6	3S0O	S0Z	$P2_12_12_1$	53.21	71.83	72.15	2.00-20.00	*(2.00-2.05)*	18 378	*(1 307)*	95.3	*(97.0)*	12.8	*(4.1)*	6.6	*(25.9)*	28.4	0.009	1.4	21.3	27.1	1 103	*6.0 %*	0.31	0.24
10	3R8Z	X63	$P2_12_12_1$	53.1	71.36	71.28	1.85-20.00	*(1.85-1.90)*	23 236	*(1 770)*	97.4	*(97.9)*	22.8	*(6.4)*	3.7	*(20.3)*	29.5	0.011	1.5	20.7	25.1	1 162	*5.0 %*	0.26	0.18
16	3QTR	X36	$P2_12_12_1$	53.43	71.62	72.74	1.85-20.00	*(1.85-1.90)*	24 329	*(1 810)*	99.4	*(98.7)*	23.6	*(7.8)*	5.5	*(30.8)*	28.6	0.008	1.4	20.2	23.0	1 100	*4.5 %*	0.24	0.19
19	3RJC	0Z5	$P2_12_12_1$	53.38	71.55	71.7	1.85-20.00	*(1.85-1.90)*	23 993	*(1 825)*	99.5	*(99.7)*	22.1	*(7.2)*	4.2	*(20.2)*	33.6	0.009	1.4	20.9	25.0	1 200	*5.0 %*	0.27	0.19
26	3R9N	Z68	$P2_12_12_1$	53.38	71.62	72.74	1.75-20.00	*(1.75-1.80)*	28 636	*(2 280)*	99.4	*(98.8)*	26.5	*(12.1)*	4.9	*(17.3)*	17.5	0.008	1.4	18.9	21.0	1 100	*3.8 %*	0.21	0.13
28	3RAH	01Z	$P2_12_12_1$	53.43	71.8	72.87	1.75-20.00	*(1.75-1.80)*	28 873	*(2 297)*	99.7	*(99.9)*	24.2	*(11.3)*	7.0	*(30.0)*	16.4	0.008	1.4	19.7	22.4	1 098	*3.8 %*	0.22	0.07
30	3QTW	X38	$P2_12_12_1$	53.64	71.89	72.59	1.85-20.00	*(1.85-1.88)*	24 400	*(1 180)*	99.0	*(99.3)*	41.2	*(4.4)*	4.5	*(38.1)*	33.5	0.008	1.3	21.4	24.6	1 014	*4.4 %*	0.26	0.13
34	3QTS	X46	$P2_12_12_1$	53.41	71.62	72.66	1.90-20.00	*(1.90-1.95)*	22 288	*(1 634)*	98.6	*(99.0)*	16.9	*(8.5)*	6.9	*(23.7)*	22.4	0.010	1.5	21.4	23.1	1 093	*4.9%*	0.26	0.20
37	3RPR	25Z	$P2_12_12_1$	53.4	71.78	72.98	1.75-20.00	*(1.75-1.80)*	28 862	*(2 300)*	99.8	*(99.9)*	25.4	*(13.7)*	4.9	*(14.4)*	16.7	0.008	1.4	18.9	21.7	1 100	*3.8 %*	0.21	0.07
41	3RZB	02Z	$P2_12_12_1$	53.42	71.79	72.54	1.90-20.00	*(1.90-1.95)*	22 456	*(1 644)*	99.3	*(98.7)*	16.3	*(6.7)*	8.2	*(35.1)*	27.8	0.009	1.5	20.5	24.5	1 101	*4.6 %*	0.27	0.16
42	3QU0	X40	$P2_12_12_1$	53.37	71.66	72.41	1.95-20.00	*(1.95-2.00)*	20 747	*(1 471)*	99.5	*(99.1)*	23.1	*(3.9)*	3.8	*(16.7)*	32.5	0.011	1.4	20.0	25.1	1 100	*5.3 %*	0.28	0.12
43	3RK7	08Z	$P2_12_12_1$	53.59	71.78	72.53	1.80-20.00	*(1.80-1.90)*	26 473	*(3 891)*	99.6	*(99.6)*	19.3	*(6.1)*	5.0	*(29.5)*	31.3	0.012	1.5	20.6	22.4	1 099	*4.2 %*	0.24	0.14
44	3RK9	09Z	$P2_12_12_1$	53.5	71.7	72.59	1.85-20.00	*(1.85-1.90)*	24 357	*(1 816)*	66.9	*(99.7)*	21.9	*(6.8)*	5.2	*(33.8)*	30.8	0.008	1.3	21.1	25.2	1 097	*4.5 %*	0.26	0.14
45	3RKB	12Z	$P2_12_12_1$	53.64	71.61	72.58	2.00-20.00	*(2.00-2.05)*	19 430	*(1 365)*	99.6	*(99.7)*	15.8	*(5.6)*	7.2	*(33.7)*	32.8	0.011	1.4	20.7	24.6	1 100	*5.7 %*	0.27	0.16
48	3RK5	07Z	$P2_12_12_1$	53.35	71.83	72.42	2.00-20.00	*(2.00-2.05)*	19 336	*(1 361)*	99.7	*(99.6)*	18.9	*(6.1)*	6.4	*(34.1)*	33.6	0.010	1.4	20.6	23.8	1 103	*5.7 %*	0.26	0.17
51[e]	4GCJ	X64	$P2_12_12_1$	53.32	70.74	71.48	1.40-20.00	(1.40-1.50)	53 895	*(9 929)*	99.9	*(99.9)*	20.0	*(5.1)*	6.0	*(37.1)*	18.5	0.009	1.5	19.6	20.5	1 086	*2.1 %*	0.15	0.07
52	3QTZ	X42	$P2_12_12_1$	53.44	71.46	72.1	2.00-20.00	*(2.00-2.05)*	18 907	*(1 348)*	98.2	*(99.4)*	24.7	*(3.7)*	4.2	*(24.1)*	31.6	0.010	1.5	20.2	23.5	1 097	*5.8 %*	0.27	0.19
53	3QTU	X44	$P2_12_12_1$	53.72	71.96	72.94	1.82-20.00	*(1.82-1.85)*	25 332	*(1 186)*	97.4	*(91.1)*	54.4	*(5.9)*	5.3	*(36.4)*	38.1	0.008	1.4	19.5	24.6	997	*4.1 %*	0.27	0.18
54	3QTX	X43	$P2_12_12_1$	53.66	71.73	72.57	1.95-20.00	*(1.95-2.00)*	20 992	*(1 517)*	99.8	*(100)*	19.6	*(6.7)*	6.7	*(29.3)*	31.9	0.007	1.3	20.0	24.6	1 155	*5.5 %*	0.27	0.19
55	3RPV	26Z	$P2_12_12_1$	53.63	71.5	72.51	1.80-20.00	*(1.80-1.90)*	26 361	*(3 877)*	99.5	*(99.6)*	17.8	*(5.5)*	5.8	*(32.8)*	28.7	0.010	1.5	20.4	23.9	1 055	*4.0 %*	0.24	0.21
56	3RAL	04Z	$P2_12_12_1$	53.54	71.92	74.74	1.75-20.00	*(1.75-1.80)*	28 934	*(2 321)*	99.7	*(99.9)*	31.7	*(12.5)*	3.9	*(13.3)*	25.4	0.007	1.4	19.8	22.8	1 100	*3.8 %*	0.23	0.14
57	3RAK	03Z	$P2_12_12_1$	53.64	71.4	72.76	1.75-20.00	*(1.75-1.80)*	27 433	*(2 141)*	95.1	*(92.4)*	28.0	*(10.0)*	4.5	*(18.7)*	24.9	0.009	1.4	19.6	23.7	1 098	*4.0 %*	0.23	0.13
61	3RPY	27Z	$P2_12_12_1$	53.46	71.56	72.64	1.90-20.00	*(1.90-1.95)*	22 474	*(1 643)*	99.2	*(98.5)*	21.6	*(6.0)*	6.1	*(34.8)*	27.9	0.008	1.4	20.3	25.1	1 102	*4.9 %*	0.27	0.20
62	3SQQ	99Z	$P2_12_12_1$	53.73	71.89	72.63	1.85-20.00	*(1.85-1.90)*	24 607	*(1 861)*	99.8	*(99.8)*	23.5	*(5.8)*	5.4	*(33.5)*	26.9	0.011	1.5	20.4	23.6	1 108	*4.5 %*	0.24	0.13
66	3S1H	56Z	$P2_12_12_1$	53.65	71.75	73.24	1.75-20.00	*(1.75-1.80)*	29 112	*(2 297)*	99.8	*(99.0)*	24.2	*(7.7)*	5.0	*(24.0)*	25.6	0.009	1.5	20.9	25.0	1 107	*3.8 %*	0.25	0.23
69	3RMF	Z20	$P2_12_12_1$	53.72	71.69	72.95	1.75-20.00	*(1.75-1.80)*	28 852	*(2 253)*	99.2	*(97.6)*	26.9	*(9.9)*	4.6	*(22.5)*	26.2	0.008	1.3	20.8	23.4	1 097	*3.8 %*	0.24	0.24
82	3RNI	21Z	$P2_12_12_1$	53.54	71.85	72.1	1.95-20.00	*(1.95-2.00)*	20 830	*(1 495)*	99.9	*(99.4)*	26.1	*(6.8)*	5.0	*(29.1)*	32.1	0.009	1.4	20.9	25.5	1 104	*5.3 %*	0.27	-
87	3R9D	Z66	$P2_12_12_1$	53.47	71.69	72.94	1.95-20.00	*(1.95-2.00)*	20 940	*(1 511)*	99.7	*(99.7)*	11.2	*(4.3)*	8.3	*(34.2)*	34.0	0.009	1.4	20.8	24.6	1 089	*5.2 %*	0.27	0.16
88	3R9O	Z71	$P2_12_12_1$	53.56	71.76	72.56	1.90-20.00	*(1.90-1.95)*	22 621	*(1 658)*	99.8	*(99.6)*	20.6	*(6.2)*	5.9	*(36.1)*	32.6	0.009	1.4	20.9	24.6	1 109	*4.9 %*	0.27	0.08
90	3R9H	X67	$P2_12_12_1$	53.45	72.04	72.45	2.10-20.00	*(2.10-2.15)*	16 917	*(1 060)*	95.2	*(92.3)*	15.5	*(6.3)*	9.2	*(35.0)*	33.6	0.009	1.4	21.4	25.9	885	*5.5 %*	0.31	0.26

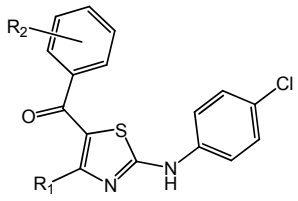
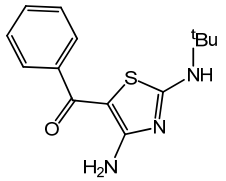
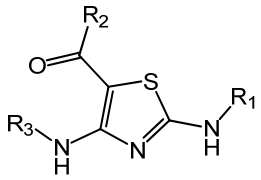
[a] Rmerge = 100 xΣh Σi | Ihi-Ih | / Σh Σi Ihi where h are unique reflection indices.

[b] rmsd = root-mean-square deviation from ideal values. which were excluded from the refinement.

[c] Rcryst = 100 xΣ | Fobs-Fmodel | / F obs where Fobs and Fmodel are observed and calculated structure factor amplitudes respectively.

[d] Rfree is Rcryst calculated for randomly chosen unique reflections. [e] The data set for compound 51 was recorded at the beamline 22-ID (SER-CAT).

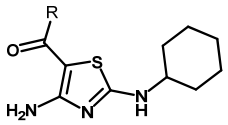
Table S2: Previously reported aminothiazole inhibitors of CDK5 and CHK1.

Compound	Modifications	PDB	Reported Inhibitory values
	R_1: -NH_2 R_2: 3-NO_2	3O0G	CDK5/p25: 2.0μM, GSK3β: 0.7μM, CDK1: 4.0μM, CK1: 124.8μM, PKA: 250.3μM, PKC: >1000μM, Src: >1000μM.
	R_1: -NH_2 R_2: 2,4-di-Cl	-	CDK5: 0.7 μM
	R_1: -NH_2 R_2: 3-CF_3	-	CDK5: 38 μM
	R_1: -NH_2 R_2: 4-Cl	-	CDK5: 66 μM
	R_1: -H R_2: 2,4-di-Cl	-	CDK5: >100 μM
	-	2WMS	CHK1: 36.9 (+/- 1.7) % at 250 μM
	R_1: 4-ClPh R_2: 3-NO_2Ph R_3: -H	3O0G	CDK5: 0.75μM
	R_1: 4-ClPh R_2: 3-FPh R_3: -H	-	CDK5: 0.7μM
	R_1: 4-ClPh R_2: 3-OMePh R_3: -H	-	CDK5: 4.0μM
	R_1: 3-Piperidyl R_2: 3-FPh R_3: -H	-	CDK5: 0.5μM
	R_1: 4-Piperidyl R_2: 3-FPh R_3: -H	-	CDK5: 6.2μM
	R_1: 3-NH_2Ph R_2: 3-FPh R_3: -H	-	CDK5: 0.031μM
	R_1: 3-NHMePh R_2: 3-FPh R_3: -H	-	CDK5: 0.044μM
	R_1: 4-NMe_2 Ph R_2: 3-FPh R_3: -H	-	CDK5: 0.068μM
	R_1: 2-Py R_2: 3-FPh R_3: -H	-	CDK5: 0.033μM
	R_1: 2-Py R_2: 2-FPh R_3: -H	-	CDK5: 0.038μM
	R_1: 2-Py R_2: 4-FPh R_3: -H	-	CDK5: 1.2μM
	R_1: 2-Py R_2: 2-OMePh R_3: -H	-	CDK5: 0.080μM
	R_1: 2-Py R_2: 3-OMePh R_3: -H	-	CDK5: 0.40μM
	R_1: 2-Py R_2: 4-OMePh R_3: -H	-	CDK5: 1.1μM
	R_1: 3-Py R_2: 2-FPh R_3: -H	-	CDK5: 0.030μM

	R_1: 3-Py R_2: 3-FPh R_3: -H	-	CDK5: 0.062μM
	R_1: 3-Py R_2: 4-FPh R_3: -H	-	CDK5: 4.1μM
	R_1: 3-Py R_2: Ph R_3: -H	-	CDK5: 0.34μM
	R_1: 3-Py R_2: 3-NO_2Ph R_3: -H	-	CDK5: 0.13μM
	R_1: 3-Py R_2: 2-OMePh R_3: -H	-	CDK5: 0.42μM
	R_1: 3-Py R_2: 3-OMePh R_3: -H	-	CDK5: 0.011μM
	R_1: 3-Py R_2: 4-OMePh R_3: -H	-	CDK5: 3.5μM
	R_1: 4-Py R_2: 2-FPh R_3: -H	-	CDK5: 0.36μM
	R_1: 4-Py R_2: 3-FPh R_3: -H	-	CDK5: 0.018μM
	R_1: 4-Py R_2: 4-FPh R_3: -H	-	CDK5: 0.40μM
	R_1: 4-Py R_2: 2-OMePh R_3: -H	-	CDK5: 0.06μM
	R_1: 4-Py R_2: 3-OMePh R_3: -H	-	CDK5: 0.20μM
	R_1: 4-Py R_2: 4-OMePh R_3: -H	-	CDK5: 0.60μM
	R_1: 3-Py R_2: 2-Py R_3: -H	-	CDK5: 0.80μM
	R_1: 3-Py R_2: 3-Py R_3: -H	-	CDK5: 0.10μM
	R_1: 3-Py R_2: 4-Py R_3: -H	-	CDK5: 1.71μM
	R_1: 3-Py R_2: 2-FPh R_3: -Me	-	CDK5: >10.0μM
	R_1: 3-(6-CF_3)Py R_2: 2-FPh R_3: -H	-	CDK5: 0.16μM

Tables S3-S6: CDK2-cyclin A2 inhibitory activities of analogues of hit compound 1. Values represent the results for duplicate or triplicate dose-response measurements with a standard deviation of ± 10 %.

Table S3



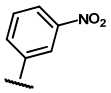
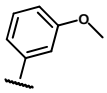
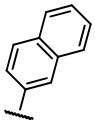
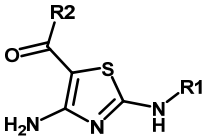
R			
Compound	**26**	**27**	**28**
IC_{50} (μM)	1.4	2.4	12
PDB ID	3R9N	-	3RAH

Table S4



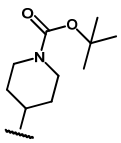
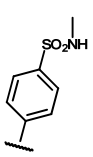
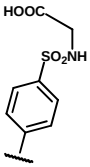
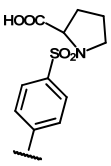
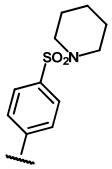
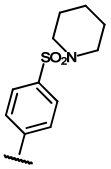
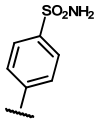
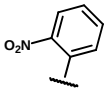
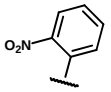
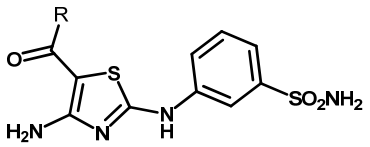
R1						
R2						
Compound	**72**	**73**	**74**	**75**	**76**	**77**
IC_{50} (μM)	3.5	17	76	89	>100	>100
PDB ID	-	-	-	-	-	-

Table S5



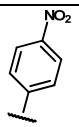
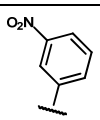
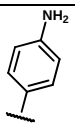
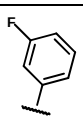
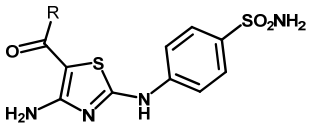
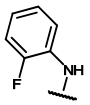
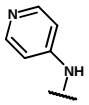
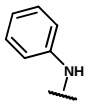
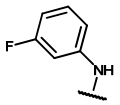
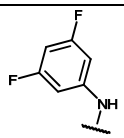
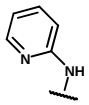
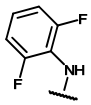
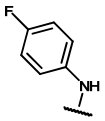
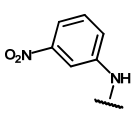
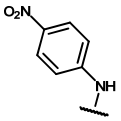
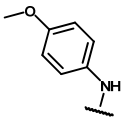
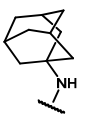
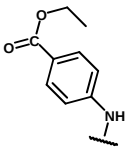
R						
Compound	**78**	**79**	**80**	**81**	**82**	**83**
IC_{50} (μM)	1.4	1.7	3.4	6.1	6.7	7.7
PDB ID	-	-	-	-	3RNI	-

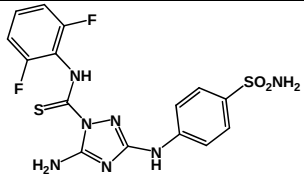
Table S6



R							
Compound	**84**	**85**	**86**	**87**	**88**	**89**	**90**
IC_{50} (μM)	21	21	28	71	>100	>100	>100
PDB ID	-	-	-	3R9D	3R9O	-	3R9H

R						
Compound	**91**	**92**	**93**	**94**	**95**	**96**
IC_{50} (μM)	>100	>100	>100	>100	>100	>100
PDB ID	-	-	-	-	-	-

CDK1/2 inhibitor III



Pan-kinase inhibitor

IC_{50} against CDK2 = < 1 nM (P^{33}-labeled assay)

PDB ligand code: DKI

CAS# 443798-55-8

Table S7: Profiling of compound 51 against a panel of 339 kinases. Residual enzymatic activity (in % of DMSO controls) was determined in single dose duplicate at a compound concentration of 0.1 μM. The ATP concentration was 10 μM. Staurosporine served as a positive control (data not shown). The experiments were performed by Reaction Biology Corp. using a P^{33}-radiolabeled assay.

KINASE	% Activity remaining	
	Data 1	Data 2
CDK5/p35	4.09	3.58
CDK5/p25	4.69	3.76
CDK2/cyclin A	5.03	4.47
CDK2/cyclin E	6.51	6.23
CDK9/cyclin T1	7.06	6.73
CDK2/Cyclin A1	9.44	6.37
CDK1/cyclin B	10.41	9.28
CDK9/cyclin K	11.66	10.44
CDK4/cyclin D1	13.12	12.05
CDK6/cyclin D1	15.23	13.25
CDK1/cyclin E	15.81	15.47
CDK4/cyclin D3	20.27	17.90
GSK3a	23.88	26.22
ERK7/MAPK15	25.02	28.13
CDK1/cyclin A	25.23	26.37
TAOK2/TAO1	33.88	33.41
GSK3b	40.06	38.25
CDK6/cyclin D3	40.30	40.46
CLK2	41.16	37.99
CLK1	44.76	47.81
BMPR2	51.26	54.04
DYRK1/DYRK1A	51.89	47.14
SRPK1	58.88	58.68
CDK3/cyclin E	60.20	57.00
MUSK	60.67	58.71
CLK3	60.78	57.40
TAOK1	61.57	58.25
SRPK2	62.20	58.63
MLK3/MAP3K11	64.33	64.70
KHS/MAP4K5	66.70	62.47
DYRK1B	67.26	62.05
IRAK4	67.47	70.97
CAMKK1	68.07	78.00
SLK/STK2	70.43	63.92
CLK4	71.94	70.83
TAOK3/JIK	75.60	79.25
IRAK1	76.84	83.51
STK33	77.55	75.29

TYK2	79.97	77.75
ZIPK/DAPK3	80.26	88.67
MST3/STK24	82.02	85.87
GCK/MAP4K2	82.48	79.62
WEE1	83.12	90.74
RIPK2	83.78	84.76
LCK2/ICK	84.09	89.26
CAMKK2	85.04	84.14
CAMK1a	85.39	93.78
WNK3	85.54	92.64
NLK	86.06	81.53
LOK/STK10	86.52	79.54
DMPK2	86.97	81.73
CK1g2	87.93	94.85
TNIK	87.96	85.82
HGK/MAP4K4	88.26	81.51
MEKK3	88.33	97.72
WNK1	88.33	100.16
BLK	88.71	96.08
CK2a2	88.99	99.23
MAPKAPK5/PRAK	89.02	87.66
ZAK/MLTK	89.30	89.47
IKKa/CHUK	89.41	88.37
PKAcg	89.89	99.54
DYRK3	90.43	92.16
NEK4	90.71	95.77
EPHA3	90.80	102.54
MLK2/MAP3K10	91.20	89.69
EPHA7	91.33	95.27
HIPK3	91.33	101.00
TXK	91.38	97.23
MYO3b	91.41	94.29
FGFR2	92.03	98.13
PKCb1	92.05	92.09
TYK1/LTK	92.08	89.97
PKG1a	92.32	99.62
NEK6	92.54	97.60
NEK3	92.64	93.01
CAMK4	92.65	90.53
HCK	92.69	94.78
Aurora C	92.98	96.60
BTK	93.09	91.79
P38d/MAPK13	93.13	96.32
ULK1	93.14	93.64
SIK1	93.18	99.80
ALK6/BMPR1B	93.20	90.14
MINK/MINK1	93.29	82.28
MLCK/MYLK	93.51	91.50

STK16	93.71	92.86
Aurora B	93.88	92.29
PIM3	93.95	97.62
DLK/MAP3K12	94.09	96.66
TGFBR2	94.11	96.16
STK38/NDR1	94.21	93.95
JNK2	94.35	103.64
HPK1/MAP4K1	94.39	96.20
OSR1/OXSR1	94.63	96.12
PLK2	94.70	95.02
TRKC	94.91	91.35
NEK9	95.00	90.43
ACK1	95.04	93.18
BRK	95.43	99.20
FGFR4	95.54	103.19
CK1g3	95.63	101.01
p70S6Kb/RPS6KB2	95.63	100.73
CDC7/DBF4	95.91	99.86
PKCb2	95.91	95.73
DYRK2	95.96	98.94
CTK/MATK	95.97	95.70
CAMK1d	96.12	96.08
ROCK2	96.16	99.23
SGK3/SGKL	96.23	94.60
LATS2	96.28	90.94
PAK4	96.34	91.12
P38b/MAPK11	96.47	93.27
AXL	96.54	98.24
RSK2	96.68	110.54
PAK6	96.77	93.34
SSTK/TSSK6	96.78	96.42
SRMS	97.01	98.02
LCK	97.14	99.02
ARAF	97.22	98.06
BMX/ETK	97.23	99.95
HIPK1	97.28	86.24
IKKe/IKBKE	97.33	94.52
YES/YES1	97.63	94.25
MLK1/MAP3K9	97.70	89.98
GRK2	97.74	98.72
CAMK2a	97.75	92.74
PHKg2	97.78	97.87
ALK5/TGFBR1	97.86	98.04
PDK1/PDPK1	97.87	101.08
EPHA6	97.87	95.07
PAK5	97.94	95.22
BRAF	98.09	93.28
CAMK2d	98.10	93.29

ERK5/MAPK7	98.12	93.52
FGFR3	98.15	96.30
CAMK1b	98.45	100.27
FMS	98.45	97.12
MEK3	98.46	97.30
ZAP70	98.74	98.54
ABL1	98.81	87.87
TIE2/TEK	98.87	102.48
FGFR1	98.91	99.10
DYRK4	98.91	105.29
GRK5	98.93	101.09
CDK7/cyclin H	98.94	94.98
DAPK2	99.10	96.70
RSK1	99.12	98.34
c-MET	99.29	104.46
IRR/INSRR	99.42	103.91
LKB1	99.46	97.85
AKT1	99.47	96.91
PYK2	99.53	103.04
CAMK1g	99.57	96.39
PKCiota	99.66	103.37
ERK2/MAPK1	99.73	100.93
ULK2	99.82	86.85
PKCnu/PRKD3	99.90	103.31
TTBK2	99.94	101.04
EPHA4	99.95	103.16
c-Src	100.00	100.73
TTBK1	100.04	101.04
PKCepsilon	100.06	104.97
NEK5	100.12	93.05
FLT4/VEGFR3	100.28	103.07
MSSK1/STK23	100.30	96.86
STK32C/YANK3	100.30	95.77
PAK2	100.32	99.84
GRK4	100.35	99.18
ROCK1	100.37	97.66
PKCa	100.46	98.03
TNK1	100.46	98.68
MAPKAPK3	100.55	99.36
PKCg	100.63	100.10
PDGFRb	100.68	97.26
MNK1	100.71	101.26
JNK3	100.71	88.76
IGF1R	100.79	97.52
NEK1	100.81	97.41
P38a/MAPK14	100.82	96.22
ALK2/ACVR1	100.96	105.73
MEK2	101.00	108.47

SIK3	101.01	101.04
FER	101.03	104.15
LRRK2	101.03	99.08
EPHB2	101.05	98.00
SIK2	101.12	94.91
CAMK2b	101.36	95.79
TLK2	101.59	103.43
JAK2	101.61	93.88
CHK2	101.62	101.20
JAK1	101.79	97.97
PKCmu/PRKD1	101.85	104.57
MARK3	101.90	96.66
MEKK1	101.94	97.37
STK39/STLK3	102.00	96.76
NEK2	102.03	100.88
EPHA5	102.03	102.82
CK1epsilon	102.05	93.94
PLK1	102.14	97.57
MKK6	102.30	93.32
FLT3	102.31	101.87
STK38L/NDR2	102.31	102.61
TBK1	102.36	91.94
RAF1	102.38	100.76
SGK2	102.53	102.54
STK22D/TSSK1	102.56	96.34
MAPKAPK2	102.58	97.77
EPHB1	102.59	100.23
TRKB	102.64	99.84
RET	102.68	101.14
PKN1/PRK1	102.70	97.14
PLK4/SAK	102.75	105.09
MRCKa/CDC42BPA	102.82	102.21
PLK3	102.88	98.18
HIPK2	102.89	100.64
PBK/TOPK	102.92	107.65
MYLK3	102.94	100.59
PIM1	103.23	99.74
CK1a1	103.23	96.53
ALK1/ACVRL1	103.29	99.65
TLK1	103.32	93.58
EGFR	103.40	105.56
FLT1/VEGFR1	103.42	99.08
MSK2/RPS6KA4	103.47	97.81
RSK4	103.59	98.39
TSSK3/STK22C	103.59	96.58
PKCtheta	103.61	98.00
DDR2	103.68	105.34
RIPK5	103.90	104.22

Haspin	104.05	100.39
MEKK2	104.06	103.42
KDR/VEGFR2	104.11	98.58
DMPK	104.14	96.89
EPHB3	104.17	101.16
GRK6	104.20	100.58
TRKA	104.21	101.68
IKKb/IKBKB	104.22	98.94
Aurora A	104.30	101.20
c-MER	104.33	99.29
CK2a	104.47	100.97
PKCeta	104.53	109.27
MST1/STK4	104.59	104.19
LYN	104.71	94.01
ERBB4/HER4	104.78	108.73
DCAMKL2	104.96	97.76
CK1g1	104.99	102.67
RSK3	105.00	96.79
ERBB2/HER2	105.01	100.55
MEK1	105.12	107.10
ABL2/ARG	105.14	102.28
FAK/PTK2	105.17	107.61
MKK4	105.24	99.62
PASK	105.29	101.37
DDR1	105.29	99.88
MARK4	105.41	97.99
c-Kit	105.41	98.15
ROS/ROS1	105.47	98.63
SYK	105.48	98.85
TEC	105.55	100.57
PKN3/PRK3	105.60	104.33
EPHB4	105.60	102.91
STK32B/YANK2	105.78	105.08
ARK5/NUAK1	105.88	94.50
PKG1b	106.08	93.49
CHK1	106.11	101.69
HIPK4	106.15	101.08
PKAcb	106.24	108.73
LYN B	106.26	100.24
EPHA1	106.37	101.77
MST2/STK3	106.39	107.10
PKCd	106.49	99.68
ALK3/BMPR1A	106.53	106.76
BRSK1	106.54	97.22
COT1/MAP3K8	106.74	101.21
GRK3	106.96	101.15
MNK2	106.98	99.06
P38g	106.99	103.95

ALK4/ACVR1B	107.14	105.36
SNARK/NUAK2	107.18	94.62
TESK1	107.19	98.71
TAK1	107.45	97.77
ASK1/MAP3K5	107.46	112.48
LIMK1	107.48	101.42
PAK1	107.51	100.25
NEK11	107.54	94.30
MELK	107.63	98.79
JNK1	107.72	99.87
PDGFRa	107.80	108.41
VRK2	107.87	92.81
MRCKb/CDC42BPB	107.92	95.86
MSK1/RPS6KA5	107.93	97.86
CAMK2g	107.96	101.45
VRK1	108.03	106.01
MARK1	108.03	97.39
AKT3	108.11	100.70
ERK1	108.22	95.47
PKN2/PRK2	108.25	91.91
MLCK2/MYLK2	108.43	101.06
NEK7	108.45	99.32
SGK1	108.48	99.73
LATS1	108.67	103.73
WNK2	108.72	103.88
TYRO3/SKY	108.76	104.64
PHKg1	108.78	103.62
EPHA8	108.84	97.64
DCAMKL1	109.10	103.45
RON/MST1R	109.17	106.49
IR	109.26	103.55
FES/FPS	109.27	99.42
ALK	109.55	98.28
JAK3	109.67	106.46
CSK	109.67	104.02
p70S6K/RPS6KB1	109.68	108.18
GRK7	109.90	103.94
EPHA2	109.93	103.60
BRSK2	110.30	106.94
DRAK1/STK17A	110.39	109.86
CK1d	110.41	101.85
PIM2	110.43	97.67
AKT2	110.44	108.98
GRK1	110.45	109.42
PKCzeta	110.63	107.12
MST4	110.72	104.46
ITK	110.76	97.20
STK25/YSK1	110.98	107.07

TSSK2	111.23	104.06
PKG2/PRKG2	111.43	110.41
RIPK3	112.50	104.71
PKA	112.74	106.50
FYN	112.76	102.96
DAPK1	113.01	118.05
ULK3	113.27	106.20
MARK2/PAR-1Ba	114.21	106.35
FGR	114.24	105.13
PAK3	114.73	103.15
PRKX	114.74	105.89
PKD2/PRKD2	114.82	109.90
FRK/PTK5	115.29	101.82

Figure S1: Evaluation of inhibitory activity on the activated CDK2-cyclin A2 complex using the DiscoveRx fluorescence-based assay. Shown are dose-response curves for representative compounds. Staurosporine served as a control. IC_{50} values and hill coefficients are indicated.

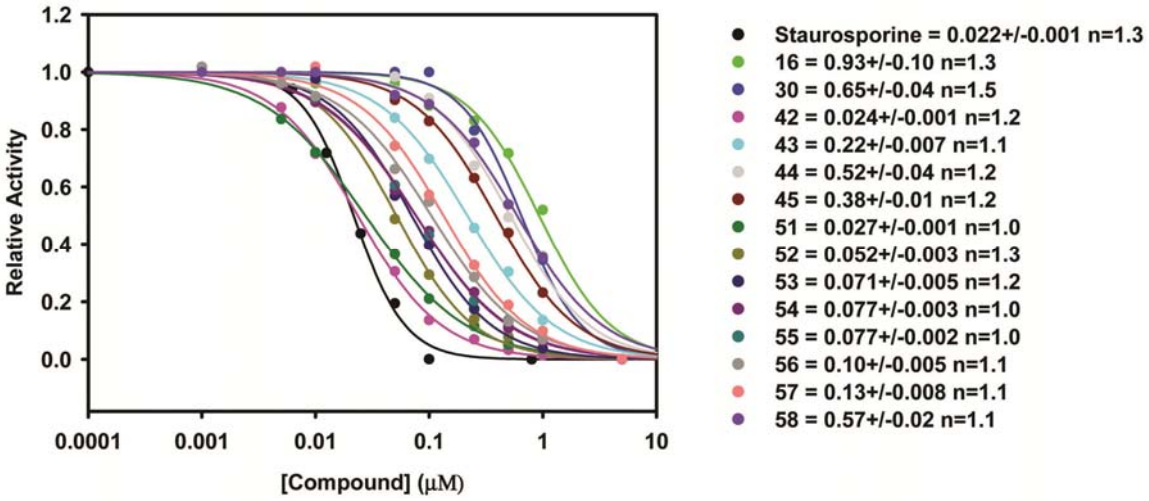


Figure S2: Inhibitory activities of compounds 42 and 52 against CDK2-cyclin A2 in comparison with staurosporine using the P^{33}-radiolabeled assay.

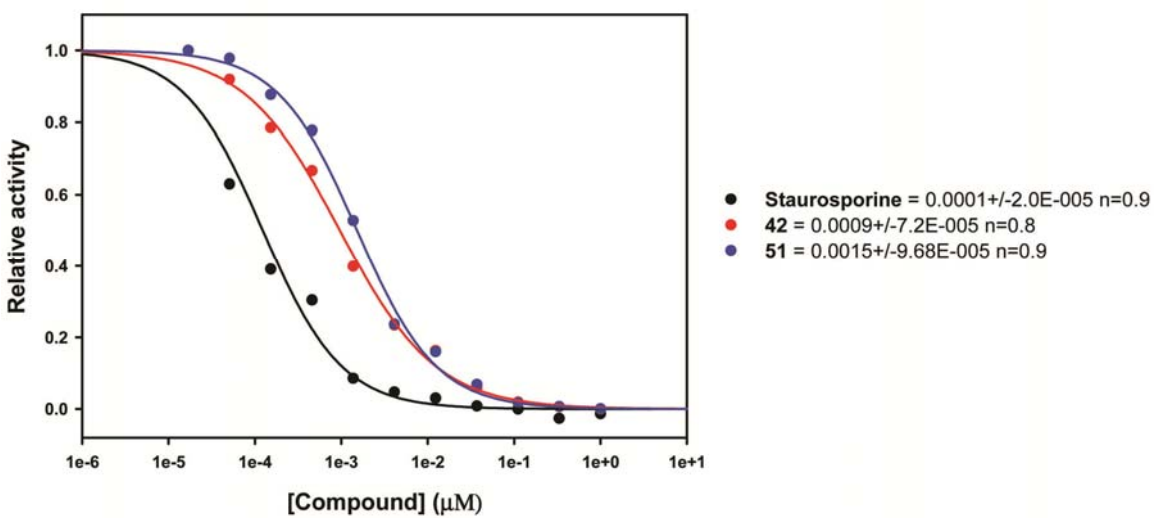


Figure S3: Broad kinase profiling (Caliper). Representative compounds were tested against 48 kinases and activities are presented as a gradient heat map from green (0% inhibition) to black (50% inhibition) to red (100% inhibition).

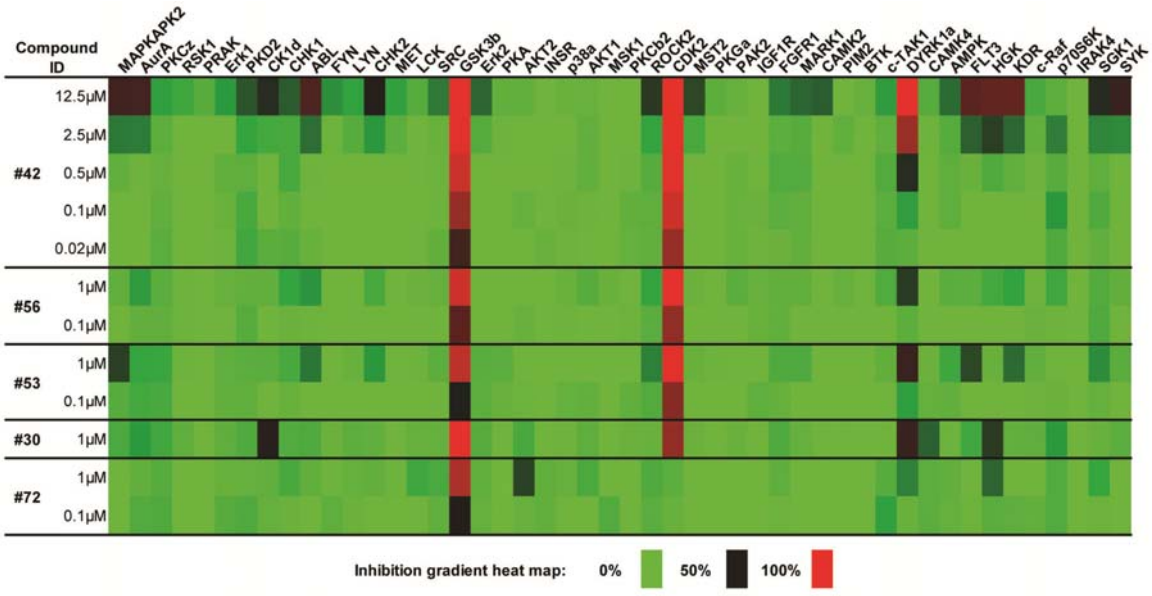


Figure S4: Inhibition of different CDK-cyclin complexes and GSK3β by compound 51. A P^{33}-radiolabeled assay was used to determine IC_{50} values with staurosporine as a control. See also Table 2 of the main text. The assays were performed by Reaction Biology Corp.

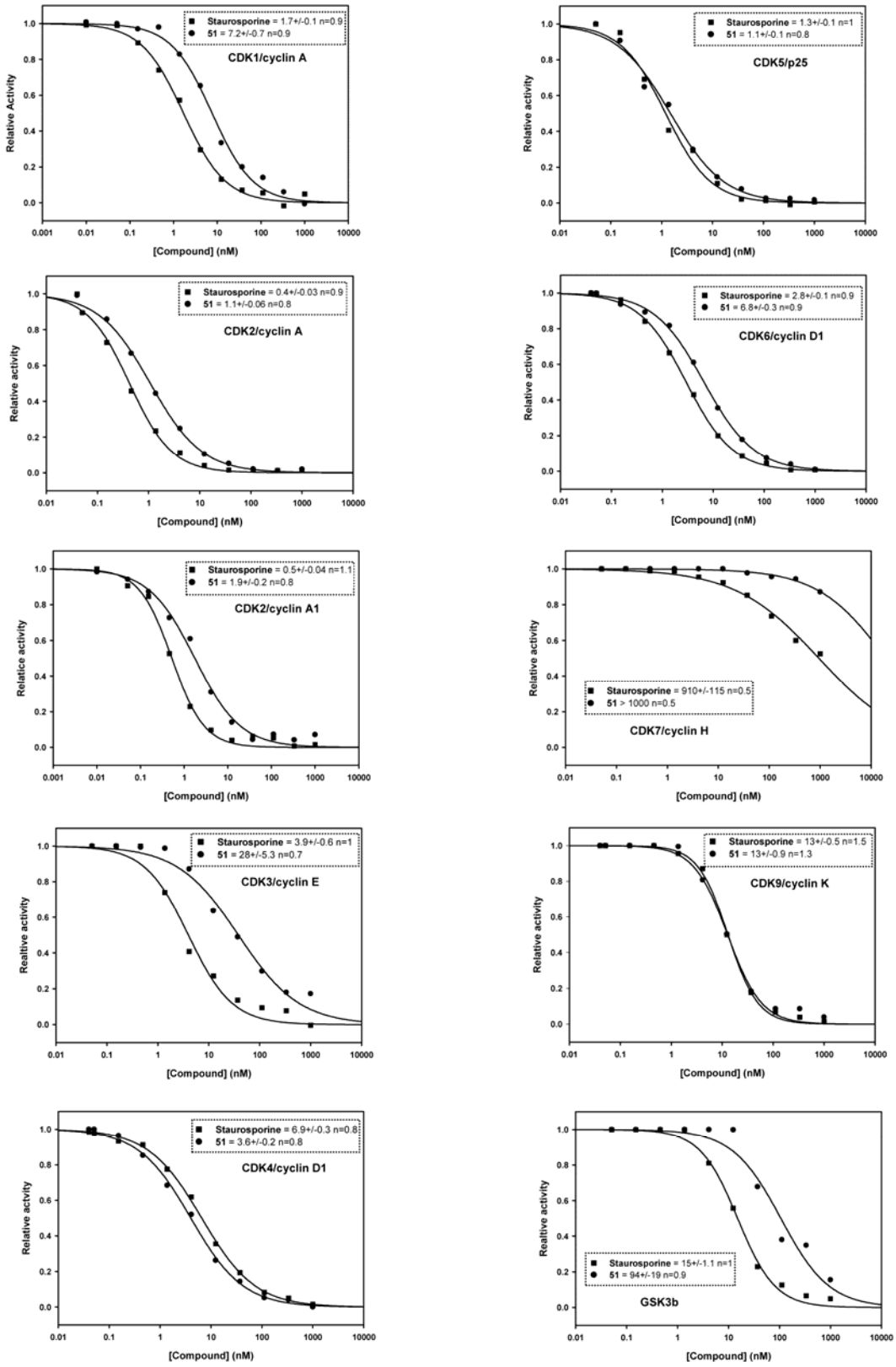


Figure S5: Crystallography of representative CDK2-inhibitor complexes. Shown are Fo-Fc electron density maps contoured at 3σ (red mesh) resulting from refinements in which the inhibitor was omitted from the model. The hinge region (residues 81-84) is shown in orange, the gatekeeper Phe80 residue in red, the DFG motif (residues 145-147) in cyan, and other residues in grey. Protein and compounds (yellow) are shown as ball and stick representations. Black and green dotted lines indicate hydrogen bonding and hydrophobic interactions, respectively.

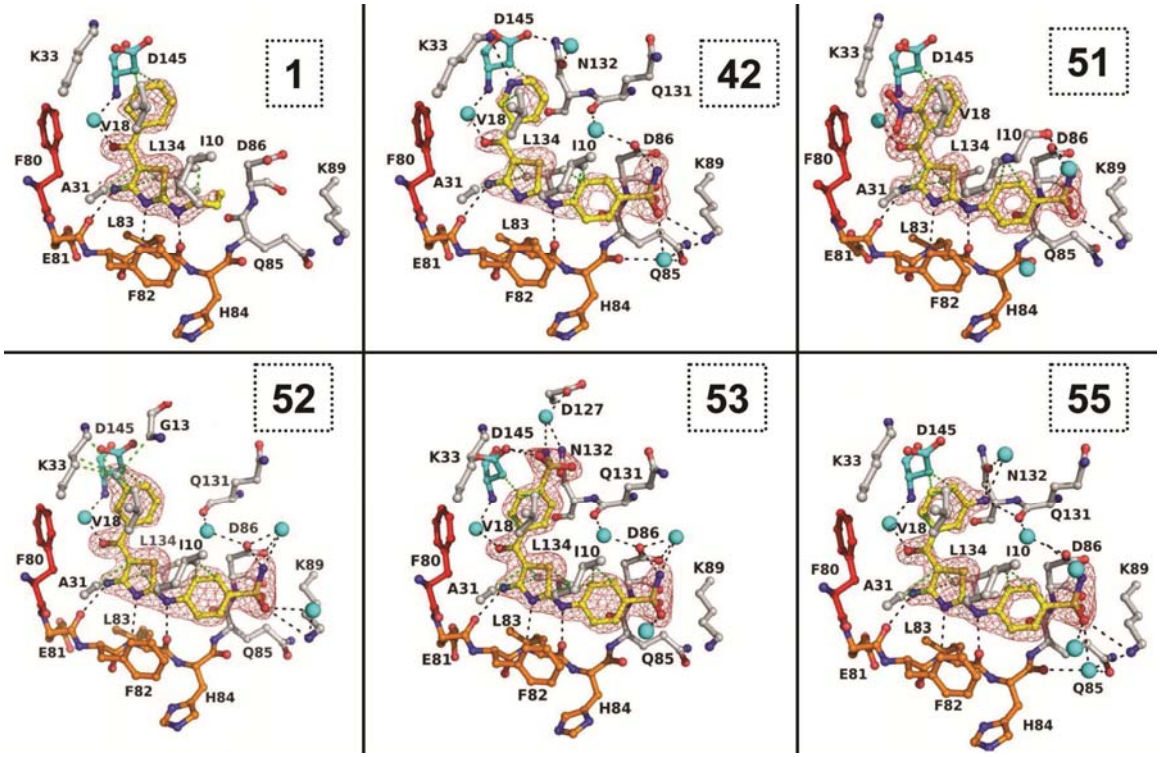
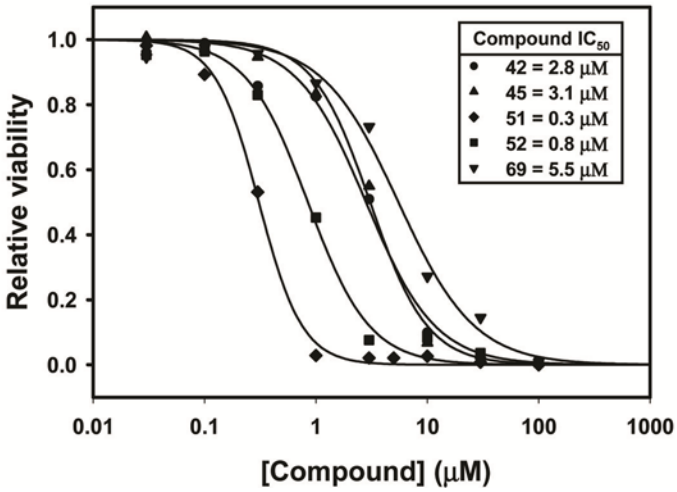
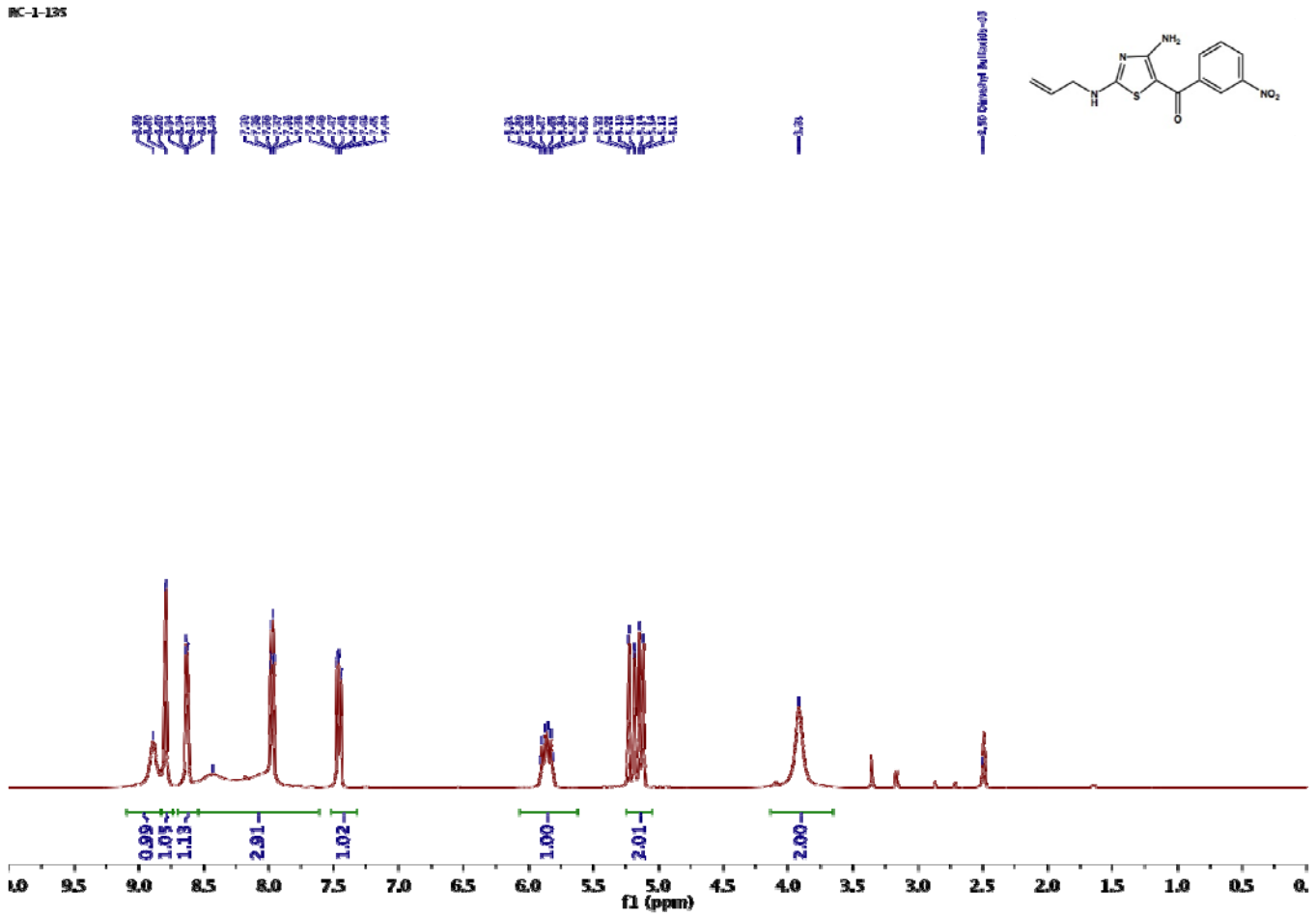


Fig S6: **Anti-proliferation activity of representative inhibitors against MDA-MB-468 breast cancer cells.** IC_{50} values were determined in triplicate.

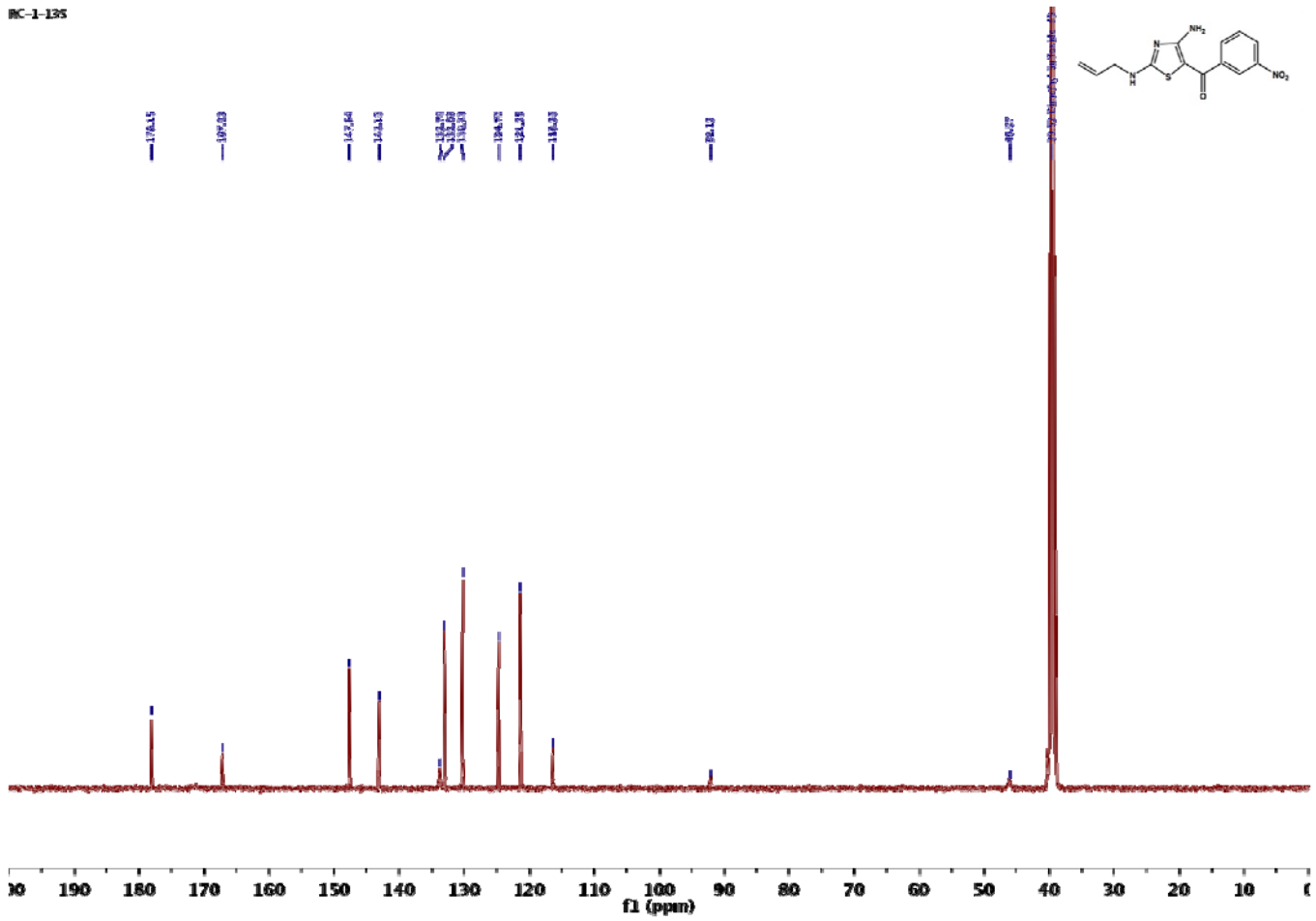


Representative compound NMR spectra

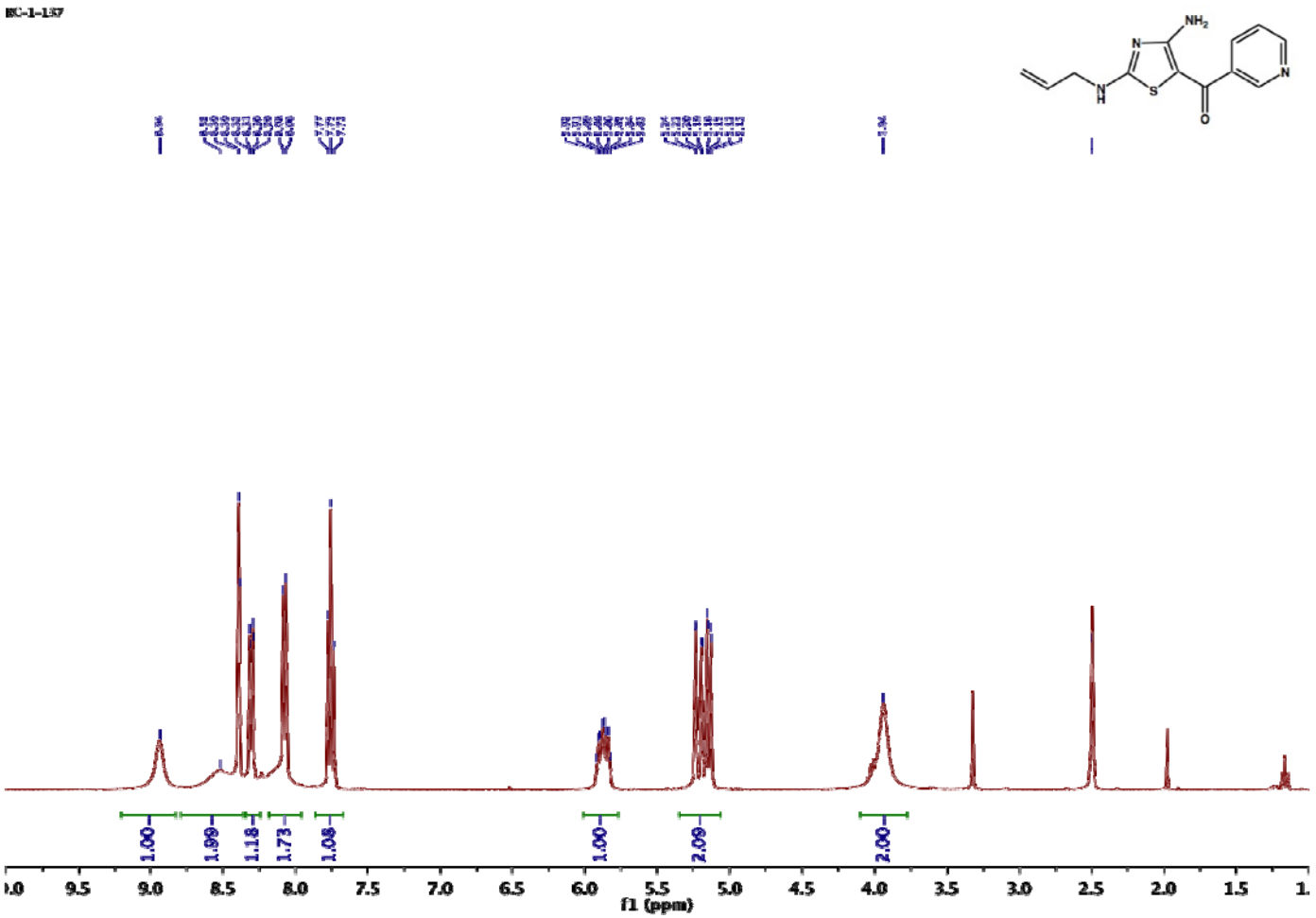
2 ^{1}H Spectrum



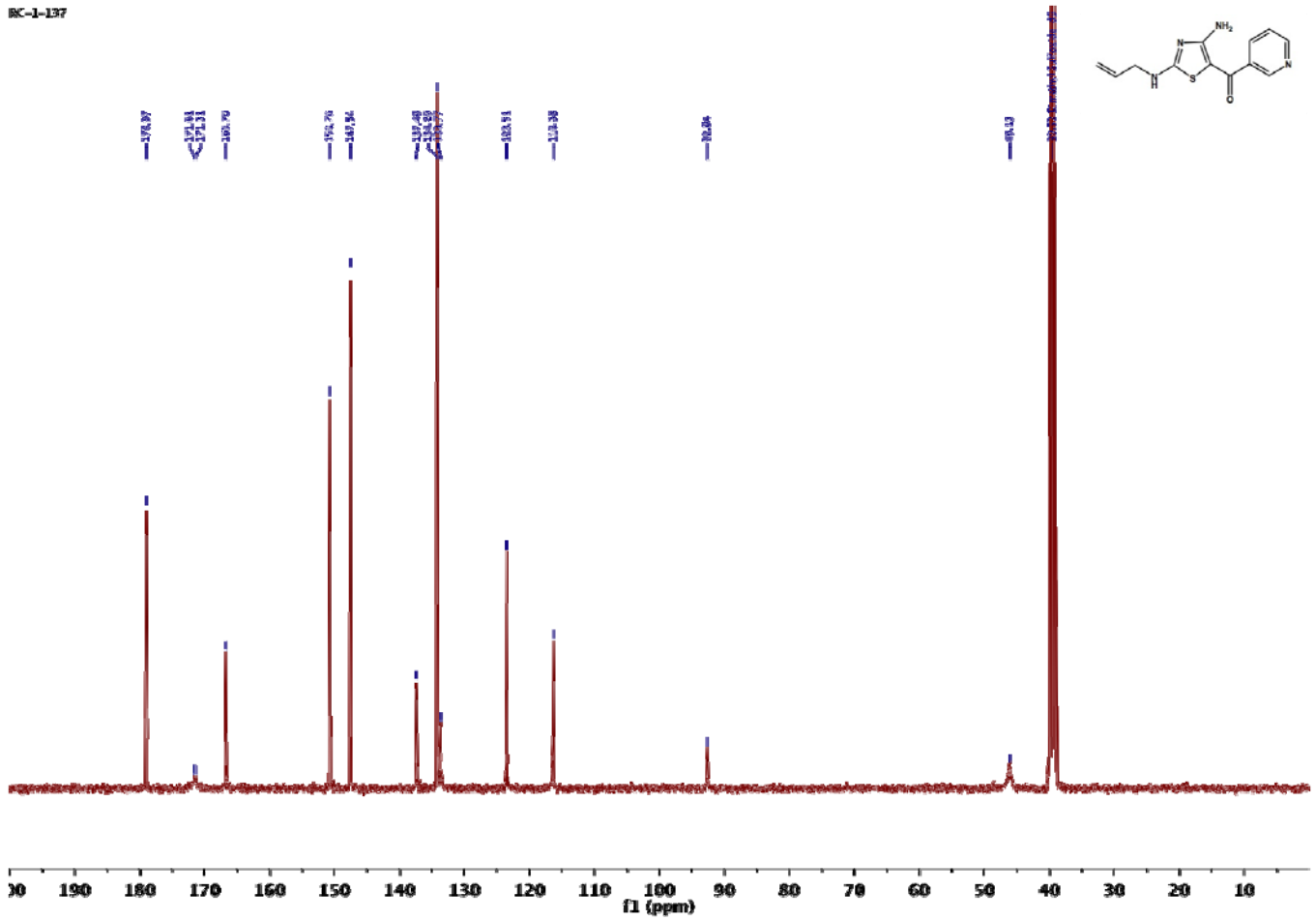
2 ^{13}C Spectrum



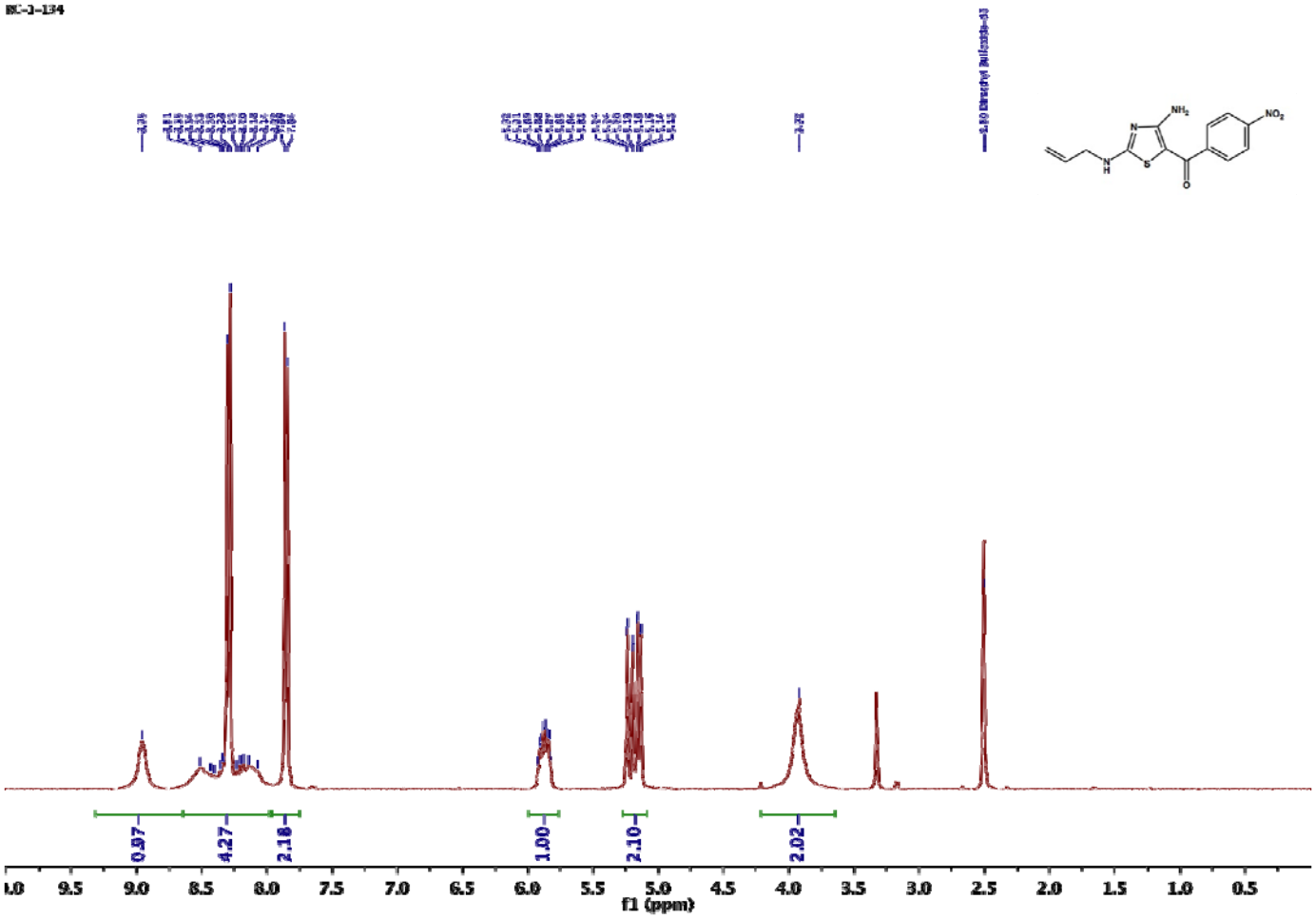
3 ^{1}H Spectrum



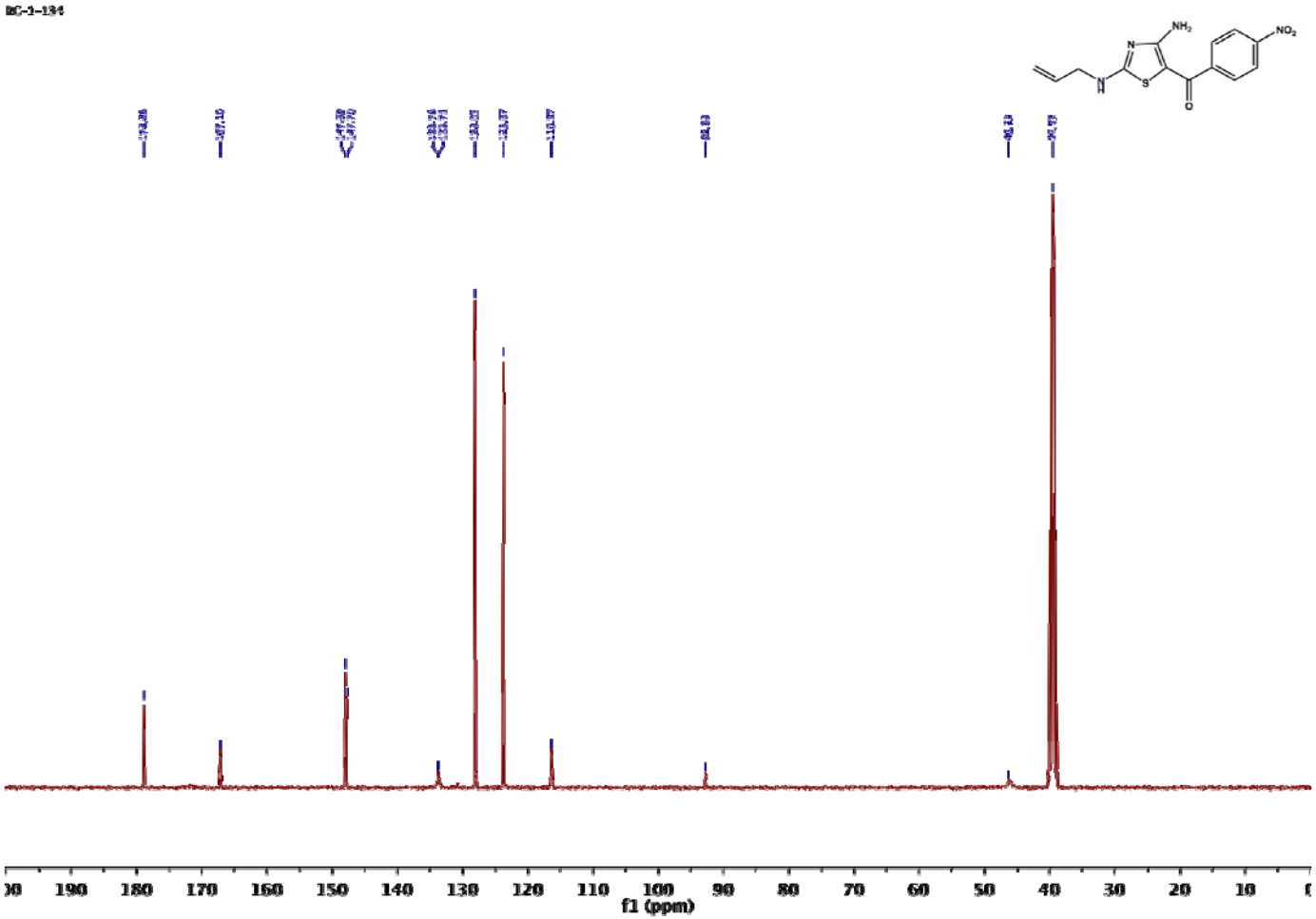
3 ^{13}C Spectrum



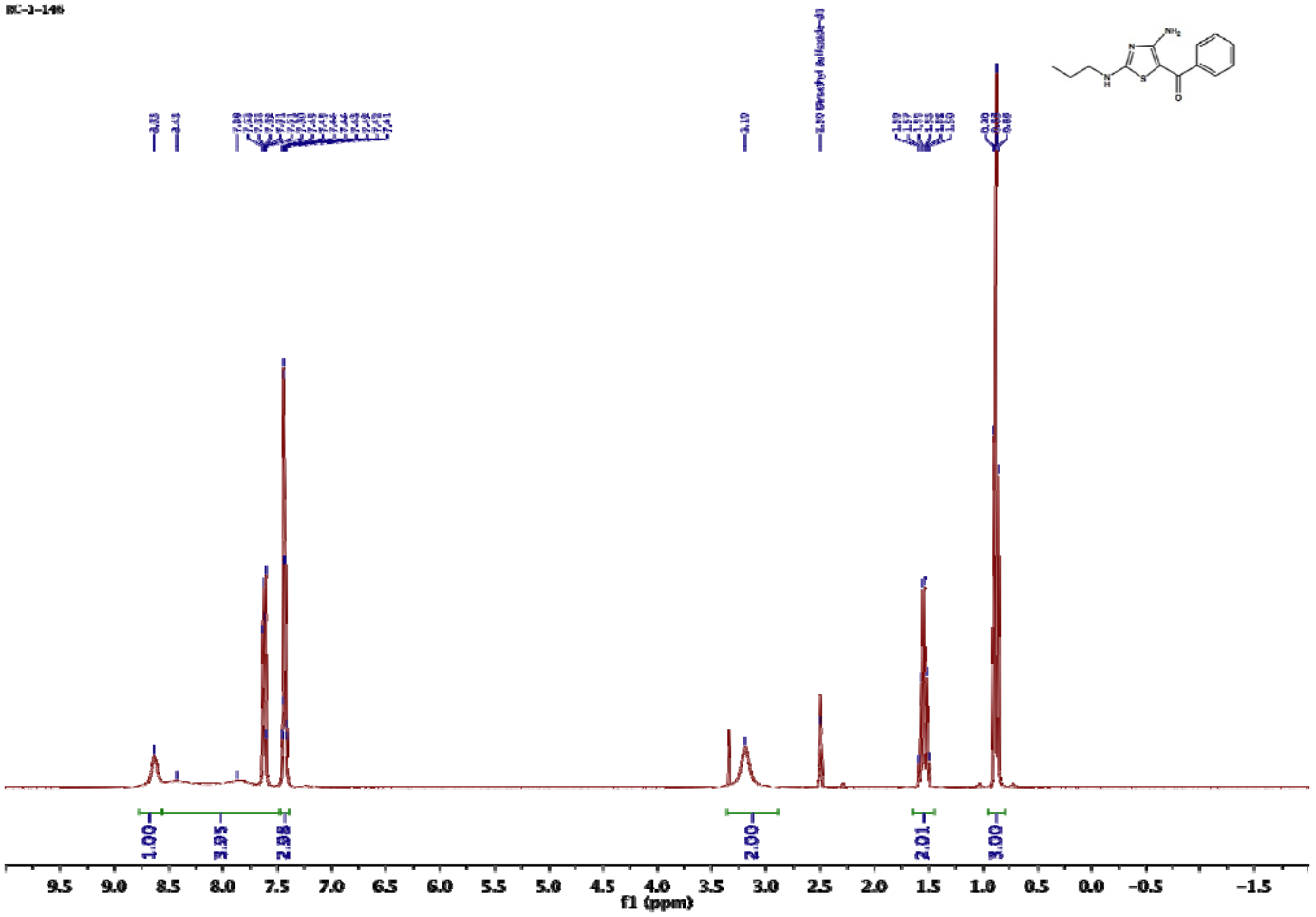
9 ^{1}H Spectrum



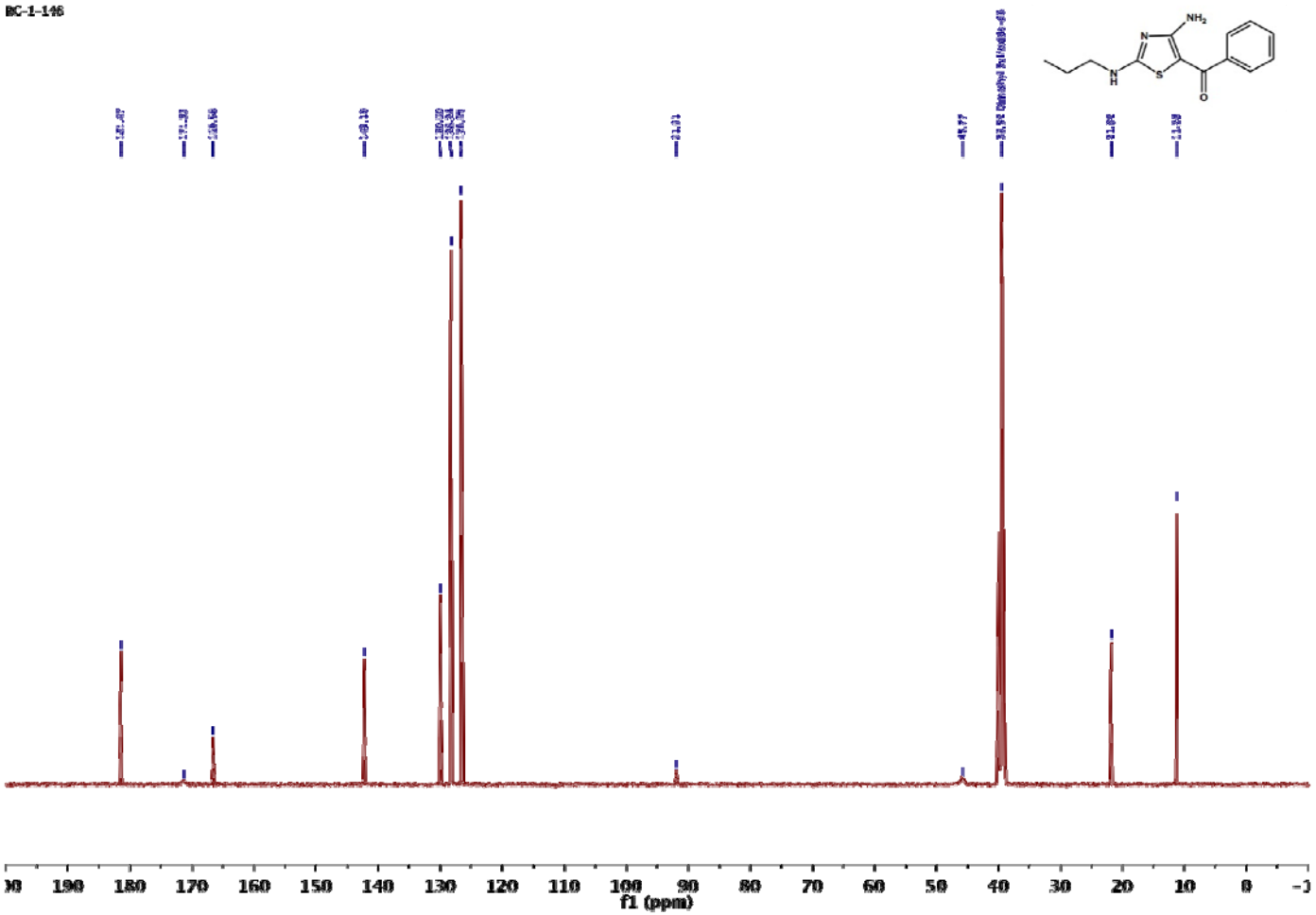
9 ^{13}C Spectrum



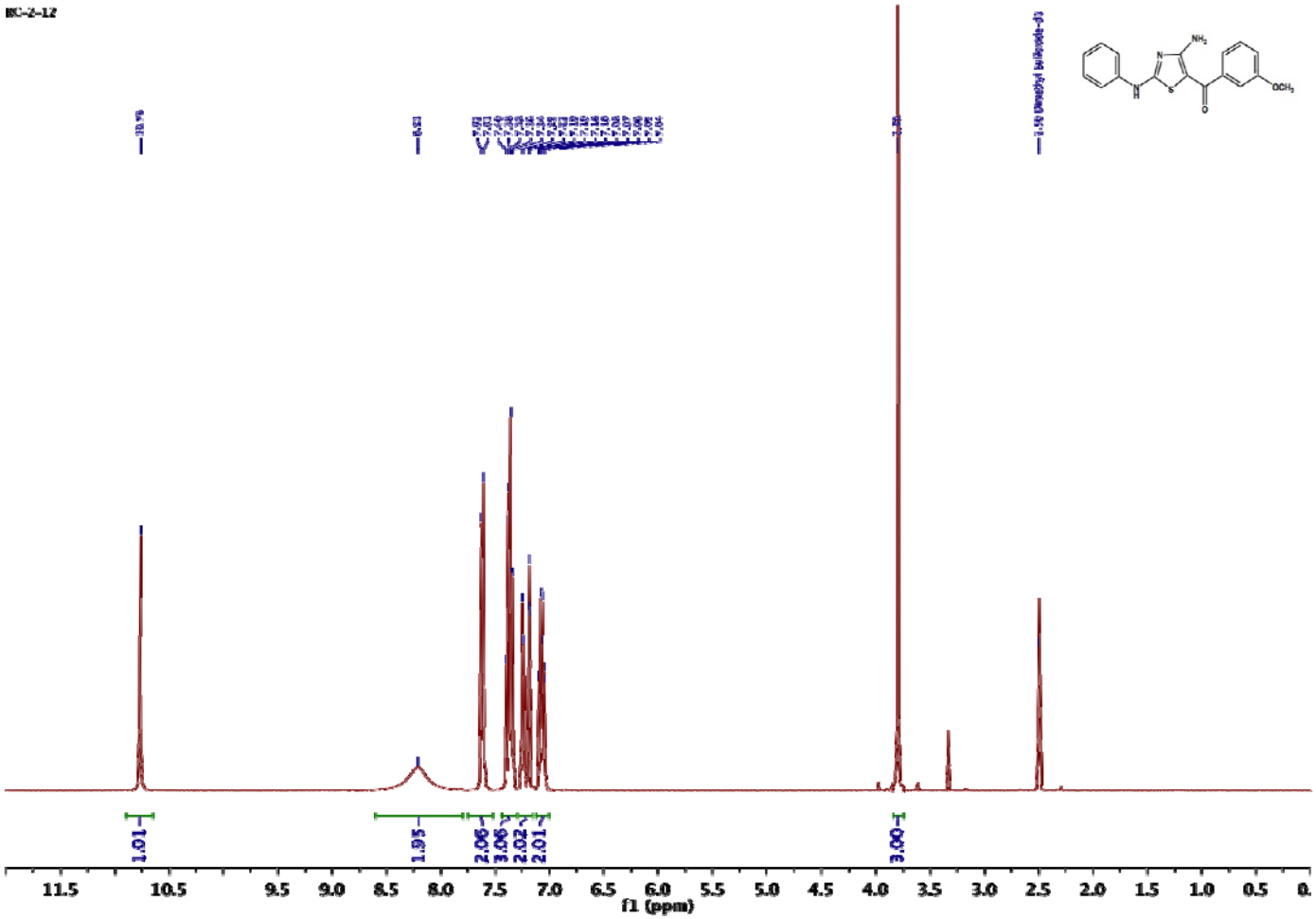
20 ^{1}H Spectrum



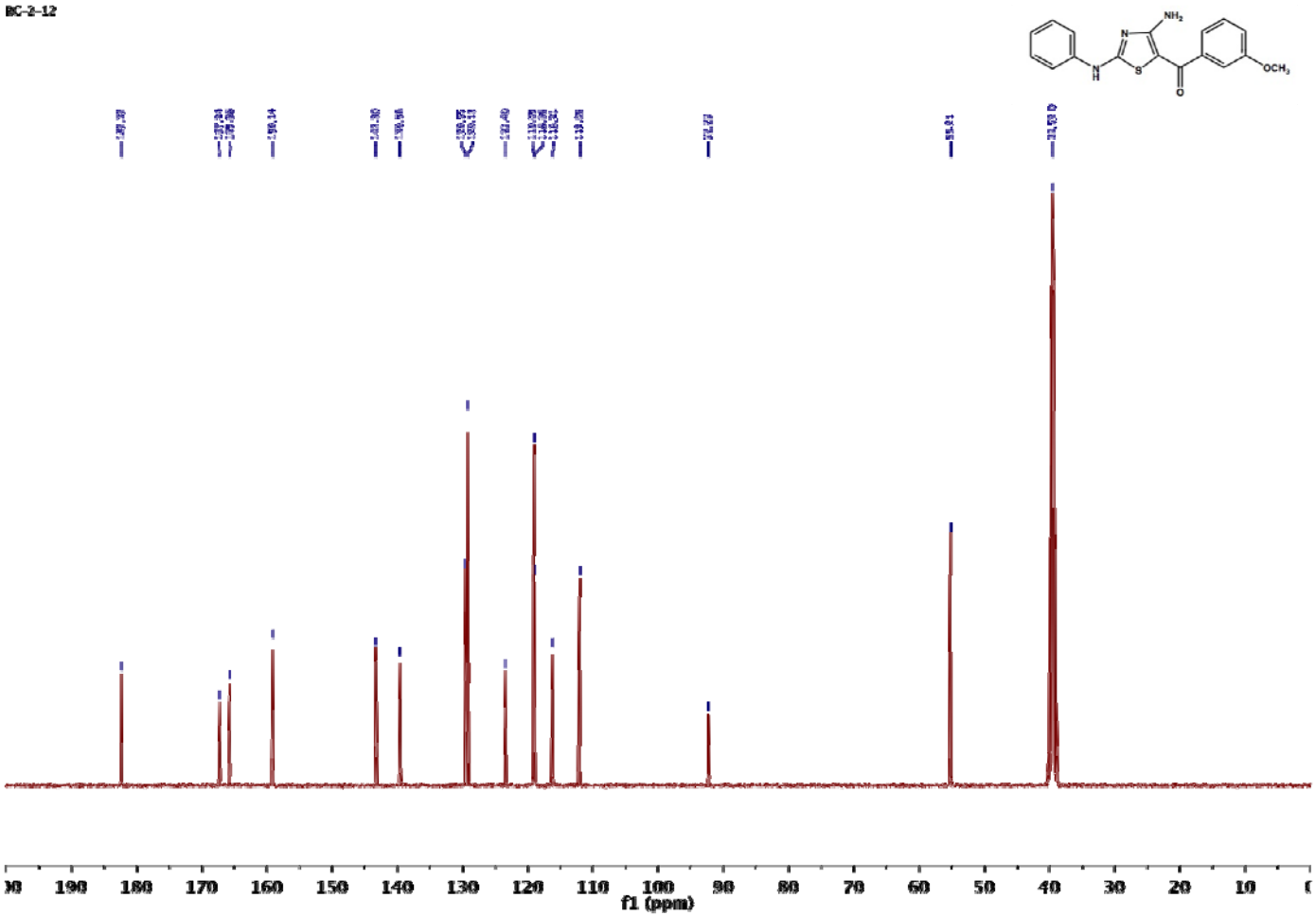
20 ^{13}C Spectrum



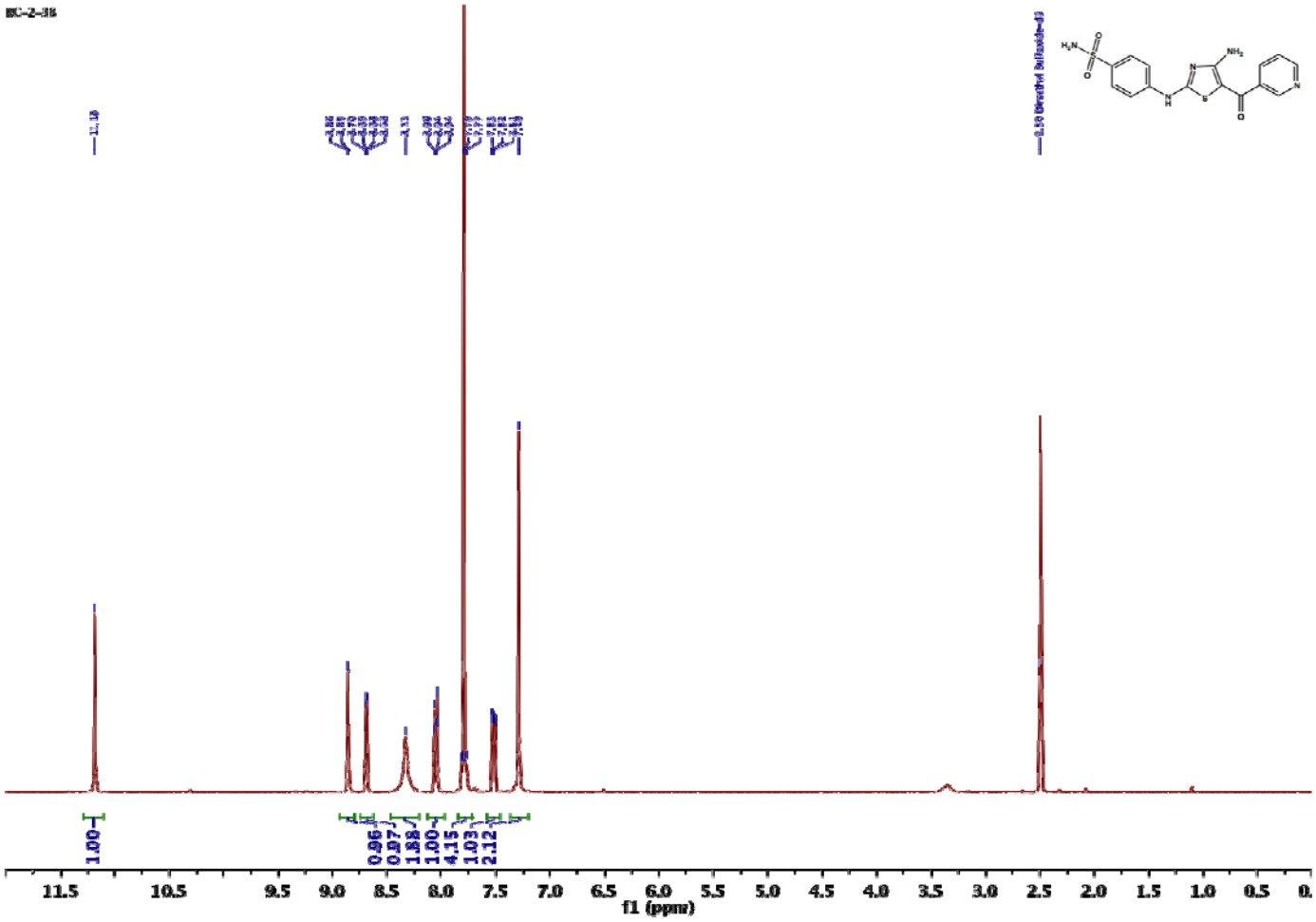
34 ^{1}H Spectrum



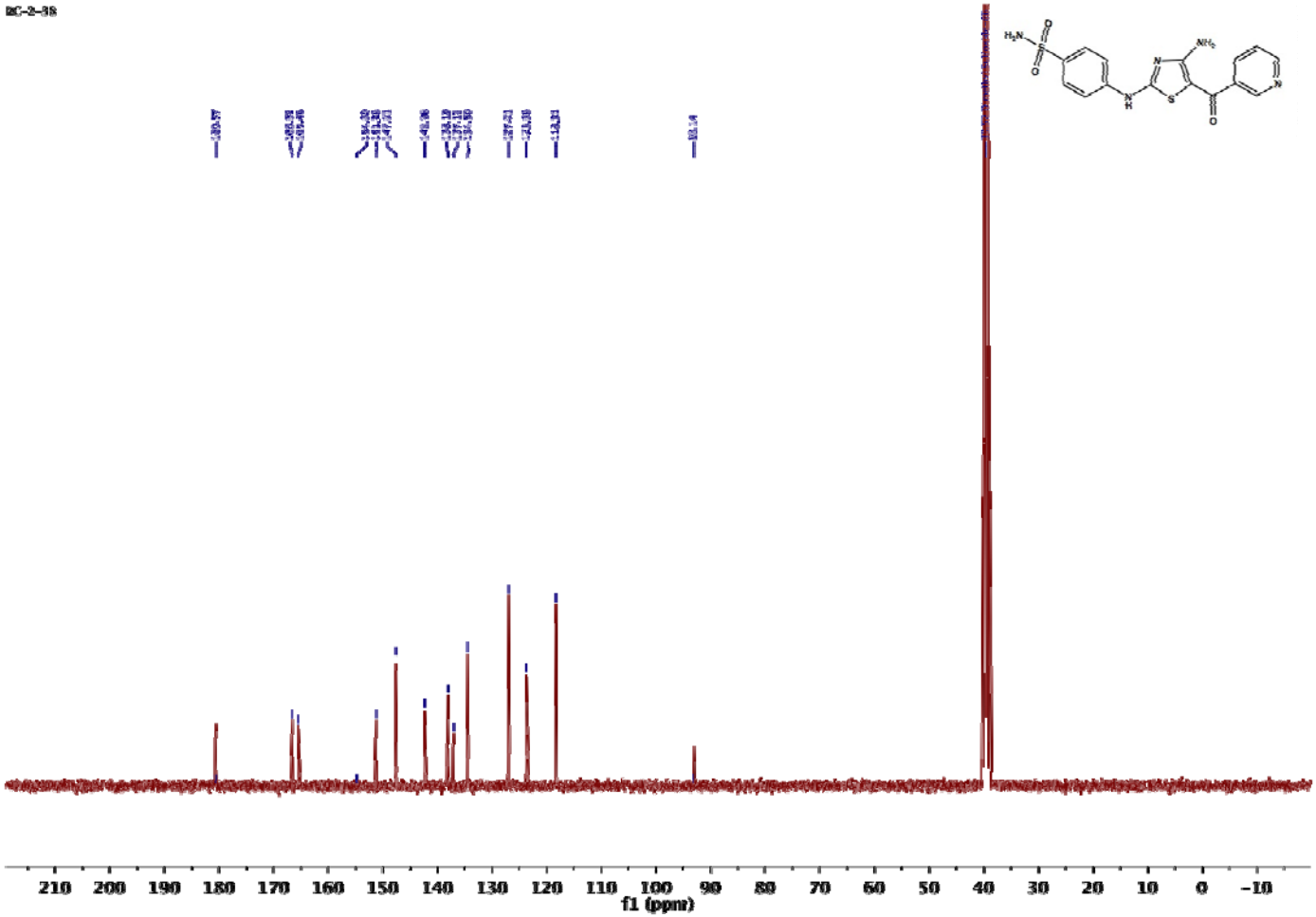
34 ^{13}C Spectrum



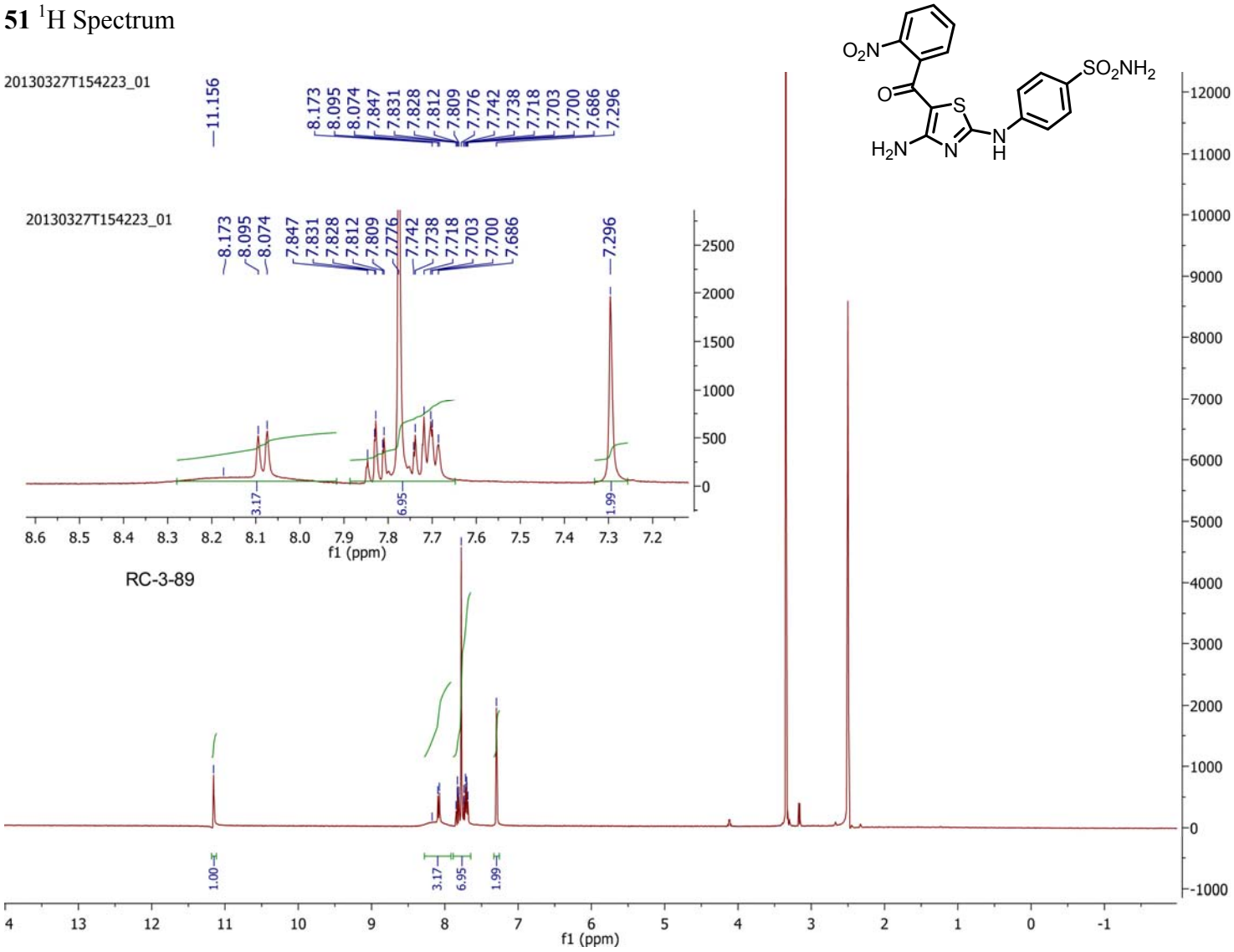
42 ^{1}H Spectrum



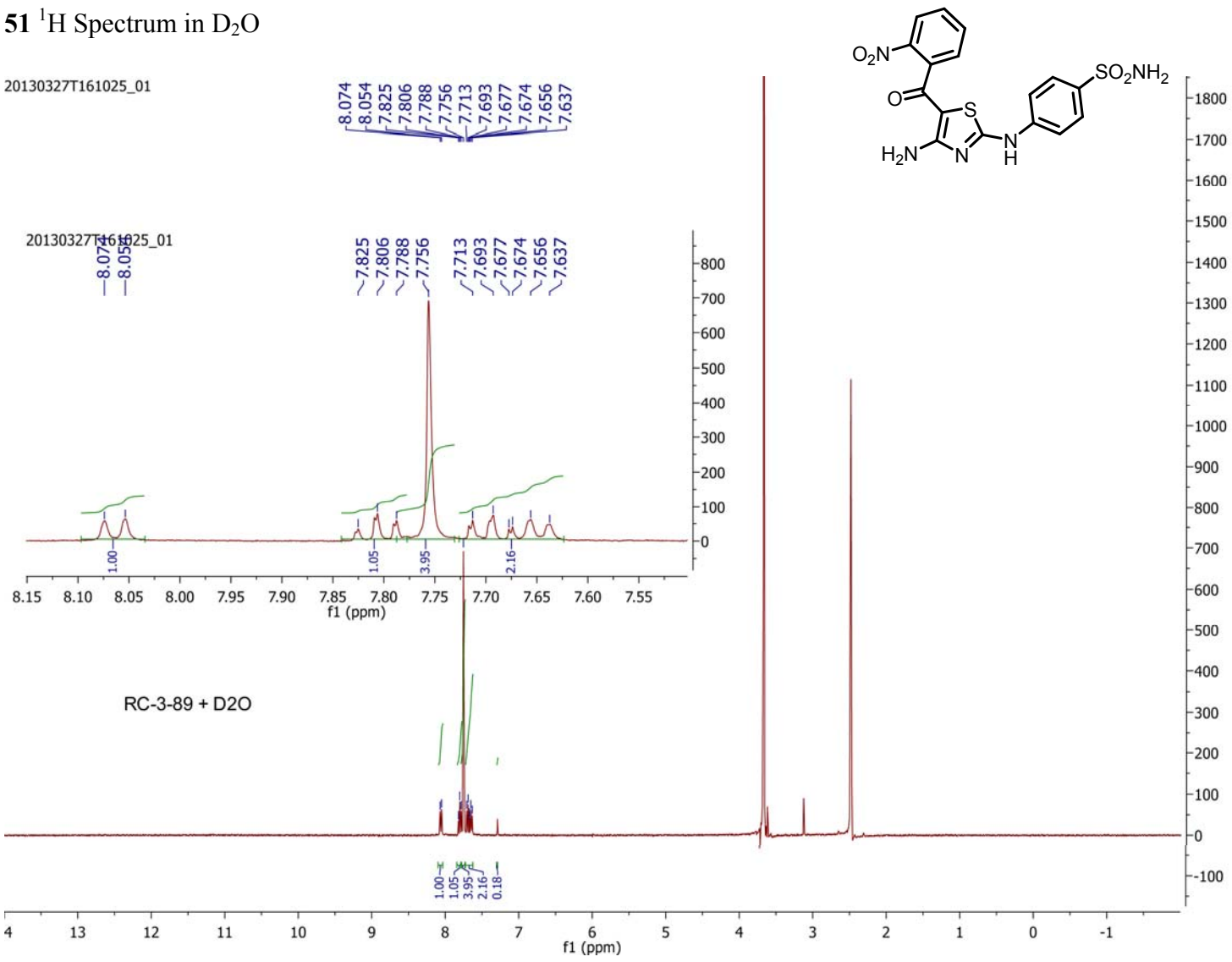
42 ^{13}C Spectrum



51 ^{1}H Spectrum



51 ^{1}H Spectrum in D_2O



51 ^{13}C Spectrum