Supporting information

Pyrazole-Based Lactate Dehydrogenase (LDH) Inhibitors with Optimized Cell Activity and Pharmacokinetic Properties

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Table of Contents

Table S1. Ligand Efficiency (LE), Biochemical LDHB and MDH Inhibition of Compounds **2-89**[a].

Compd No.	LDHB IC_{50} ± SD (μM)[a]	LE[b]	MDH IC_{50}± SD (μM)	Compd No.	LDHB IC_{50} ± SD (μM)[a]	LE[b]	MDH IC_{50} ± SD (μM)
2	0.008 ± 0.001	0.02	ND	**46**	0.019 ± 0.001	0.01	34.499
3	0.007 ± 0.001	0.05	ND	**47**	0.024 ± 0	0.02	21.768
4	0.01 ± 0.001	0.11	ND	**48**	0.005 ± 0.002	0.02	30.747
5	0.09 ± 0.011	0.14	ND	**49**	0.006 ± 0.001	0.03	38.709
6	1.373 ± 0.158	0.04	ND	**50**	0.007 ± 0.001	0.02	21.768
7	0.006 ± 0	0.03	ND	**51**	0.021 ± 0.006	0.07	27.404
8	0.007 ± 0.001	0.09	ND	**52**	0.043 ± 0.017	0.06	36.604 ± 2.219
9	0.055 ± 0.006	0.01	ND	**53**	0.041 ± 0.009	0.05	34.499
10	0.005 ± 0.001	0.04	ND	**54**	0.047 ± 0.003	0.04	38.709
11	0.007 ± 0.002	0.01	15.41	**55**	0.16 ± 0.02	0.07	24.424
12	0.007 ± 0	0.04	34.499	**56**	0.097 ± 0.011	0.05	30.748
13	0.01 ± 0	0.04	27.404	**57**	0.306 ± 0	0.02	ND
14	0.015 ± 0.001	0.05	27.404	**58**	0.078 ± 0.015	0.07	34.499
15	0.024 ± 0.003	0.05	30.748	**59**	0.075 ± 0.013	0.05	34.499
16	0.013 ± 0.001	0.07	24.424	**60**	0.173 ± 0.02	0.03	21.768
17	0.01 ± 0.002	0.1	21.768	**61**	0.018 ± 0.003	0.04	34.499
18	0.005 ± 0.001	0.05	38.709	**62**	0.01 ± 0.002	0.03	34.499
19	0.007 ± 0.002	0.02	27.404	**63**	0.01 ± 0.002	0.03	18.212
20	0.026 ± 0.002	0.07	27.404	**64**	0.001 ± 0.001	0.02	34.499
21	0.003 ± 0	0.02	ND	**65**	0.012 ± 0.003	0.01	28.864
22	0.018 ± 0.001	0.05	27.404	**66**	0.074 ± 0.018	0.06	34.499
23	0.006 ± 0.001	0.04	ND	**67**	0.101 ± 0.013	0.1	30.748
24	0.023 ± 0.002	0.04	30.748	**68**	0.025 ± 0.003	0.15	34.499
25	0.006 ± 0.001	0.04	34.499	**69**	0.04 ± 0.003	0.13	24.424
26	0.006 ± 0.001	0.01	30.748	**70**	0.009 ± 0.002	0.21	34.499
27	0.009 ± 0.003	0.02	30.748	**71**	0.006 ± 0.001	0.23	27.404
28	0.007 ± 0.001	0.11	34.499	**72**	0.024 ± 0.007	0.15	19.4
29	0.007 ± 0.002	0.02	34.499	**73**	0.014 ± 0.001	0.13	30.748
30	0.006 ± 0.001	0.01	34.499	**74**	0.081 ± 0.018	0.08	34.499
31	0.004 ± 0	0.08	48.732	**75**	0.084 ± 0.014	0.11	38.709
32	0.005 ± 0.001	0.12	ND	**76**	0.043 ± 0.017	0.15	36.338
33	0.012 ± 0	0.07	34.499	**77**	0.062 ± 0.012	0.1	34.499
34	0.789 ± 0.253	0.14	30.748	**78**	0.173 ± 0.02	0.06	30.748
35	0.013 ± 0.001	0.01	30.748	**79**	0.331 ± 0.022	0.03	30.748
36	0.009 ± 0.001	0.02	27.404	**80**	0.02 ± 0.006	0.16	32.386
37	0.017 ± 0	0.08	30.748	**81**	0.017 ± 0.007	0.18	32.386
38	0.111 ± 0.06	0.04	30.748	**82**	0.012 ± 0.001	0.18	34.499
39	0.007 ± 0.001	0.01	ND	**83**	0.209 ± 0.014	0.04	34.499
40	0.006 ± 0.002	0.01	30.748	**84**	0.31 ± 0.058	0.01	34.499
41	0.042 ± 0.017	0.15	ND	**85**	0.804 ± 0.102	0.08	34.499
42	0.024 ± 0.004	0.04	34.5	**86**	0.091 ± 0.016	0.06	34.499
43	0.033 ± 0.008	0.07	30.004 ± 3.54	**87**	0.086 ± 0	0.11	30.748
44	0.025 ± 0.006	0.03	34.499	**88**	0.547 ± 0.063	0.01	30.748
45	0.401 ± 0.027	0.03	24.424	**89**	0.941 ± 0.16	0.09	34.499

[a]IC_{50} values represent the half maximal (50%) inhibitory concentration as determined in the HTS assay (n = 3 for LDHB and n =1 for MDH) using a dose response in 1536-well format. [b]Ligand efficiency (LE) was calculated using the following equation: 1.37* (pIC_{50}/HA). HA = Heavy atoms (non-hydrogen). pIC_{50} = -logIC_{50} of LDHA activity.

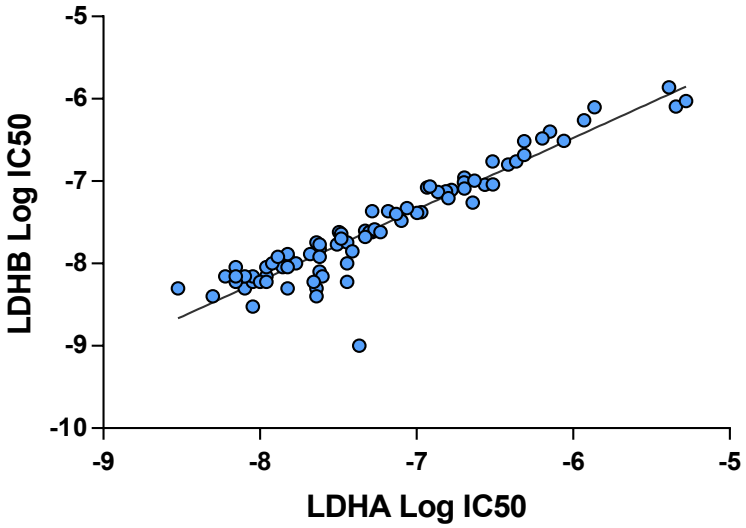
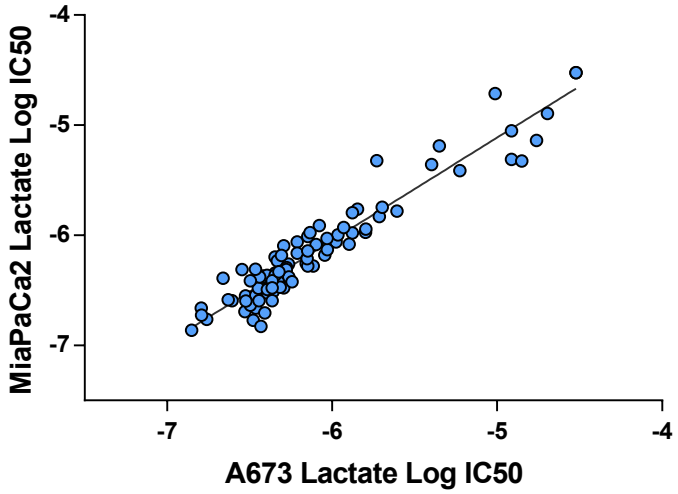


Figure S1: Correlation between LDHA and LDHB biochemical activities.

A



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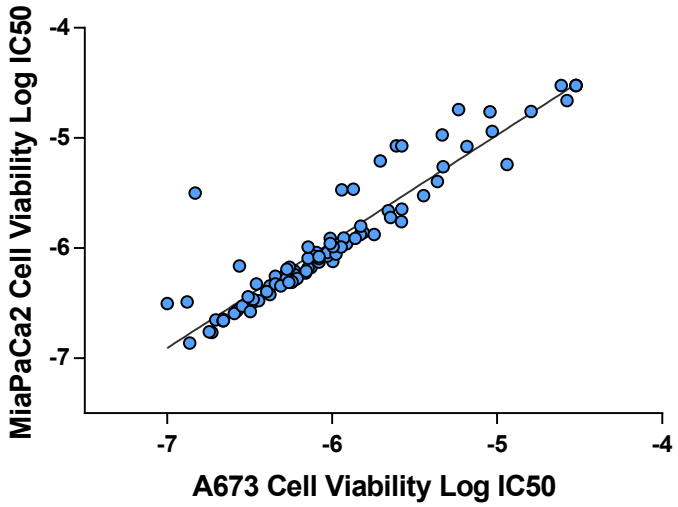
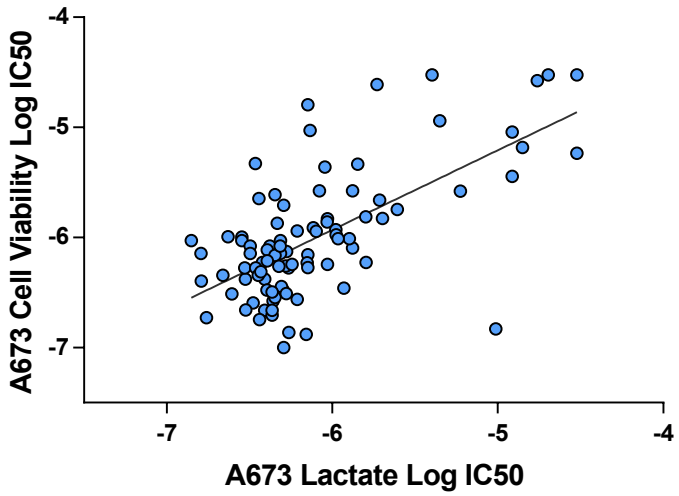


Figure S2: A) Correlation of A673 Lactate vs MiaPaCa2 Lactate Data. B) A673 Cytotoxicity vs MiaPaCa2 Cytotoxicity.



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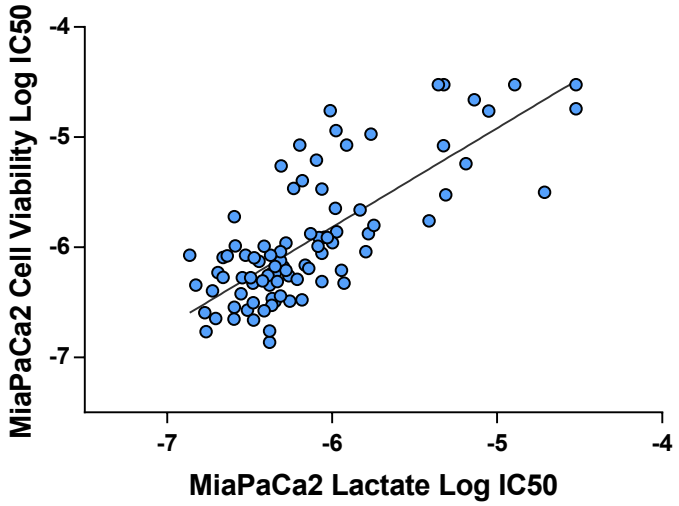


Figure S3: A) Correlation of A673 Lactate vs A673 Cytotoxic effects. B) MiaPaCa2 Lactate vs MiaPaCa2 Cytotoxicity.

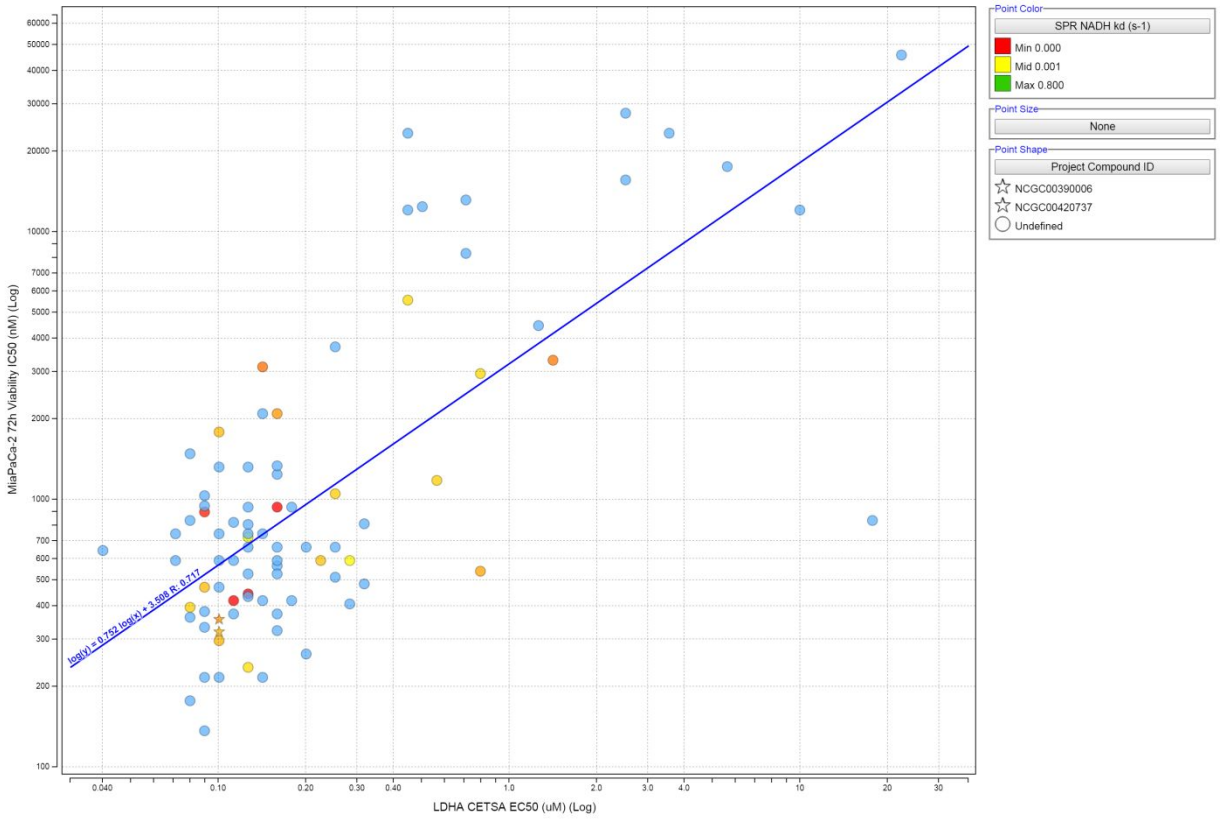


Figure S4: A) Correlation of MiaPaCa-2 72h Viability vs LDHA CETSA binding activity in HEK293 cells.

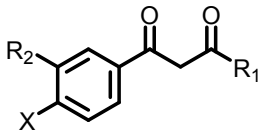
Table S2. *In vitro* ADME Profiles of compounds **2-89**[a]

Compd	[a]Metabolic Stability [m]	[b]Permeability [1e-6 cm/sec]	[c]Solubility [µg/ml]	Compd	[a]Metabolic Stability [m]	[b]Permeability [1e-6 cm/sec]	[c]Solubility [µg/ml]
2	>30	<1	69.5	**46**	>30	<1	76.9
3	>30	18.4	59.6	**47**	>30	10.7	3
4	>30	1.3	73.4	**48**	>30	<1	<1
5	>30	6.1	59.7	**49**	>30	ND	72.7
6	>30	2.9	71.7	**50**	>30	1.4	66.7
7	>30	3.1	56.7	**51**	>30	ND	70
8	>30	2.8	57.7	**52**	>30	1.6	78.6
9	>30	7	57.4	**53**	>30	ND	78.4
10	>30	1.5	62.4	**54**	>30	1.7	>93
11	>30	<1	>82	**55**	>30	1.9	77.2
12	>30	<1	64.6	**56**	>30	1.1	>97
13	>30	5.9	66.7	**57**	>30	1.4	62.4
14	15	9.6	71.2	**58**	>30	1.2	70.8
15	ND	4.1	>90	**59**	>30	<1	73.6
16	6.2	3.0	>88	**60**	>30	1.3	>101
17	>30	8	71.7	**61**	>30	7.8	>91
18	>30	4.8	75.0	**62**	>30	ND	71.6

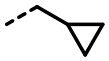
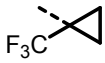
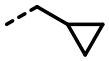
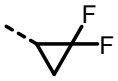
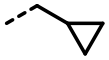
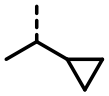
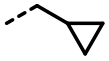
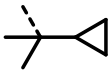
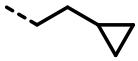
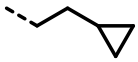
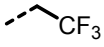
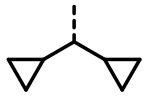
19	>30	2.2	68	**63**	>30	6.43	<1
20	29.1	8.1	>90	**64**	>30	<1	>94
21	13.9	8	66. 3	**65**	>30	17	>94
22	4.3	6.6	76.1	**66**	>30	<1	71.7
23	>30	5.3	71.9	**67**	>30	1.7	79.6
24	ND	10.2	58.7	**68**	>30	1.7	>94
25	ND	4	>94	**69**	ND	16.7	73. 3
26	>30	<1	13.2	**70**	>30	ND	62.9
27	>30	2.1	>93	**71**	>30	6	>84
28	>30	1.9	>93	**72**	>30	9.7	72.9
29	>30	3.5	>92	**73**	27.7	0.9	56.8
30	>30	2.2	18.9	**74**	ND	6.1	77.4
31	>30	2.1	>95	**75**	>30	3.9	>95
32	>30	4.3	28.9	**76**	>30	18.2	>93
33	>30	19.3	66.5	**77**	>30	1.3	77.7
34	ND	ND	ND	**78**	>30	<1	77.7
35	ND	2.7	66.3	**79**	>30	<1	69.7
36	>30	<1	67.5	**80**	>30	131.2	<1
37	>30	19.1	62.3	**81**	>30	13.5	>96
38	>30	<1.6	75.6	**82**	>30	201.2	>96
39	>30	16.9	3.7	**83**	>30	4.73	>98
40	>30	3.8	70.8	**84**	>30	ND	ND
41	>30	<1.4	>97	**85**	>30	<1	>98
42	>30	1.02	73.4	**86**	>30	7.5	77.3
43	>30	2.2	71.6	**87**	>30	<1	71.8
44	>30	1.3	73.8	**88**	>30	216.1	82
45	>30	0.7	83	**89**	>30	49.6	84.4

[a]The microsomal stability data [rat liver microsomes (RLM)], [b]PAMPA permeability, and [c]Aqueous kinetic solubility (PBS buffer) were conducted at NCATS.

Table S3. Structures of intermediates **IIIa-p** for scheme 1 in the manuscript



IIIa-p

Compd No.	X	R_1	R_2	Compd No.	X	R_1	R_2
IIIa	H		Ph	**IIIi**	H		Ph
IIIb	F		Ph	**IIIj**	H		Ph
IIIc	H		Br	**IIIk**	H		Br
IIId	F		Br	**IIIl**	H		Br
IIIe	H		Ph	**IIIm**	H		Br
IIIf	H		Br	**IIIn**	H		Br
IIIg	F		Br	**IIIo**	H		Br
IIIh	H		Ph	**IIIp**	H		Br

PDB CODES: 6Q0D (**23**); 6Q13 (**52**)**.**

Table S4. Data collection and refinement statistics for the 2 LDHA crystal structures.

Parameters	**hLDHA + 52 + NADH**	**hLDHA + 23 + NADH**
PDB Code	6Q13	6Q0D
Data collection		
X-ray source	APS 21-ID-G	APS 21-ID-F
Date of data collection	November 5, 2016	November 4, 2015
Co-crystallization conditions	2 mM ligand; 1mM NADH, harvested 3 days after setup	4 mM ligand; 4 mM NADH, harvested 3 day after setup

Space group	$P2_12_12$	$C2$
Cell dimensions (*a*, *b*, *c*, α, β, γ):	134.80, 94.74, 121.56, 90, 90, 90	212.04, 128.07, 104,14, 90.0, 19.35, 90.0
Resolution limit (Å)	2.00 (2.05-2.00)	2.05 (2.10-2.05)
Total unique reflections	104,626	150,548
Completeness (%)	99.1 (99.9)	99.1 (99.5)
Multiplicity	5.84 (5.85)	3.2 (3.2)
<I>/σ(<I>)	19.02 (3.08)	12.91 (2.4)
R_{merge}	0.073 (0.573)	0.064 (0.494)
Refinement		
Total reflections in refinement	104,552 (7,313)	150,548
Reflections in R_{free}	1,916 (144)	2014
R_{factor}/ R_{free}	0.169/0.209	0.184/0.224
Molprobity score	1.09	1.37
Number of atoms		
Protein	10,154	15,548
Ligand	348	588
Waters/others	981/56	754
RMS deviations		
Bond lengths (Å)	0.007	0.007
Bond angles (˚)	0.927	0.900

Protein Purification for Crystallization.

The crystallography construct of hLDHA corresponds to UniProt accession number P00338 residues 2-332 with an N-terminal $(His)_6$-Smt fusion tag. After lysis of the cell paste, lysate was applied to a HisTrap Nickel chelating column. The $(His)_6$ tag binds to the resin and eluted at about 200 mM imidazole. The combined fractions containing the target protein were digested overnight with ULP-protease and applied to a second Ni column (HisTrap FF). Cleaved hLDHA flows through while uncleaved His-tagged protein, liberated HisSmt tag, and ULP-protease are retained on the column. Final polishing was performed by size exclusion chromatography on S-100 in a buffer of 25 mM HEPES pH 7.2, 150 mM NaCl. The retention time on SEC was consistent with a tetramer of LDHA protein subunits. Purified protein was concentrated to 20 mg/ml and frozen at -80˚C for subsequent crystallization experiments. Typical yields were > 5 mg of protein per liter of *E. coli* culture.

Crystallization.

Crystallization was carried out at 16 ˚C using the sitting drop vapor diffusion method. Crystals with NCGC00420737 were obtained by co-crystallizing hLDHA at 20 mg/mL with 2 mM ligand and 1 mM NADH against a reservoir solution containing 10% (w/v) PEG 8000 and 100 mM imidazole (pH 8.0). Crystals with **23** were grown from hLDHA with 4 mM ligand and NADH set against a reservoir solution containing 5% PEG 1000, 100 mM sodium phosphate/citrate (pH 4.2) and 40% (v/v) reagent alcohol. In each case, crystals appeared spontaneously in a few days and were stable for several weeks.

Crystal Structure Solution and Refinement.

Diffraction data were collected from various X-ray sources as outlined in (Supplemental Table S3). All diffraction data were processed using XDS and scaled using XSCALE.[1] Structures were solved by molecular replacement using the program PHASER[2] with a published structure of hLDHA as a search model. Models were refined using alternating cycles of manual rebuilding in Coot[3] and automated refinement in *PHENIX*[4].

SPR Methods. Surface Plasmon Resonance (SPR) binding studies were performed by BioSensor Tools LLC (Salt Lake City, UT) using Biacore T100 SPR systems (GE Healthcare Life Sciences). His-tagged hLDHA was amine coupled to a CM5 sensor chip to provide three different surface densities that ranged between 2000 and 250 RU. During coupling NADH was included with

hLDHA to protect the NADH binding site. Flow cell 2 was activated and blocked as a control surface. The running buffer contained HBS-p and 1% DMSO. Data were collected at 25 ^{o}C.

Surfaces were tested for consistent, functional LDHA by measuring NADH binding up to 0.25 mM using a two-fold dilution series replicated four times. Running buffer included HBS-p with 1% DMSO at 25 ^{o}C. NADH bound in a concentration-dependent manner and binding responses correlated with surface capacity in that higher density surfaces produced higher RU. Stoichiometry of binding was 0.6 to 0.7 based on expected Rmax. The average K_D for NADH on the different surfaces from different experiments ranged from 6.3 to 12 μM.

Compounds were tested routinely in a 3-fold dilution series for binding to the different density LDHA surfaces using HBS-p, 1% DMSO with 0.5 mM NADH. To examine effects of cofactor binding, compounds were tested in HBS-p, 1% DMSO without added NADH. Global fits were determined for the data on each different density surface using a1:1 interaction model including a step for mass transport.

Biological Assays

LDH biochemical assays: Briefly, 3 μL of recombinant human lactate dehydrogenase 5 (LDHA, #A38558H, Meridian Life Science, Inc., Memphis, TN) in LDH assay buffer (200 mM Tris HCl pH 7.4, 100 μM EDTA and 0.01% Tween-20) was added to a black solid-bottom 1536-well assay plate (Greiner Bio-One) using a BioRAPTR FRD dispenser (Beckman Coulter, Brea, CA). A 1536-well pintool dispenser (Wako Automation, San Diego, CA) was used to transfer 23 nL of DMSO-solubilized compound (both library and vehicle controls) to each 1536-well assay plate. Following compound transfer, 1 μL of substrate solution containing NADH and sodium pyruvate (Sigma-Aldrich, St. Louis, MO) in LDH assay buffer was dispensed via BioRAPTR FRD to initiate the reaction. Final concentrations in the 4 μL reaction volume were 2 nM LDHA enzyme, 0.06 mM NADH and 0.2 mM sodium pyruvate. Following a 5 minute incubation period at room temperature, 1 μL of detection reagent (*Clostridium kluyveri* diaphorase (Sigma-Aldrich) and resazurin sodium salt (Sigma-Aldrich) in LDH assay buffer) was added to a total volume of 5 μL. Final concentrations of detection reagents were 0.133 mg/mL diaphorase and 37 μM resazurin. Plates were immediately transferred to a ViewLux microplate imager (PerkinElmer, Waltham, MA), and any resulting resorufin fluorescence was measured (ex540, em590 nm) at 0 and 20

minutes. Fluorescence was normalized using enzyme-free and DMSO-treated control wells on each plate. Human lactate dehydrogenase 1 (LDHB, #A38155H, Meridian Life Science, Inc., Memphis, TN) was assayed as described above for LDHA.

MDH biochemical assay: Briefly, 3 µL of MDH solution (containing 13.33 IU/mL malate dehydrogenase from porcine heart, 0.2 mM NAD, 0.067mg/mL diaphorase and 0.067 mM resazurin in MDH assay buffer (50 mM Tris pH 8.0, 5 mM $MgCl_2$, 0.01% Brij 3)) was added to a black solid bottom 1536-well assay plate (Greiner Bio-One) using a BioRAPTR FRD dispenser (Beckman Coulter, Brea, CA). A 1536-well pintool dispenser outfitted with 20 nL pins (Wako Automation, San Diego, CA) was used to transfer 23 nL of DMSO-solubilized compound (Cherrypick plates) to each 1536-well assay plate. Following compound transfer, plates were incubated in room temperature for 10 min. 1 µL of substrate solution containing malic acid (160uM) was dispensed via BioRAPTR FRD to initiate the reaction. Plates were immediately transferred to a ViewLux microplate imager (PerkinElmer, Waltham, MA), and any resulting resorufin fluorescence was measured (ex540, em590 nm) at 0 and 5 min. Well fluorescence was normalized using enzyme-free and DMSO-treated control wells on each plate, and changes in fluorescence (ΔRFU) were calculated using the difference in fluorescent signal for each well at 5 versus 0 minutes.

Cell Lines: The MiaPaCa-2 human pancreatic carcinoma and A673 human Ewing's sarcoma cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured in DMEM (Invitrogen 11965118) culture medium supplemented with 10% fetal bovine serum and 100 units/mL Penicillin, 100 µg/mL Streptomycin and maintained in a 37 °C, 5% CO_2/95% humidified air incubator.

Cellular Lactate production assay: A673 and MiaPaCa-2 cells were cultured as described above and plated in 1536-well black clear bottom tissue culture plates using a Multidrop Combi peristaltic dispenser (ThermoFisher, Waltham, MA) at a density of 500 cells/well in 4 µL of non-supplemented DMEM (Invitrogen 31053036) culture medium. A 1536-well pintool dispenser outfitted with 20 nL pins (Wako Automation, San Diego, CA) was used to transfer 23 nL of compound in DMSO to the 1536-well assay plates. After 2 hr incubation at 37 °C, 2 µL of reconstituted Lactate Reaction Mix (BioVision K607-100) was dispensed into each well using a

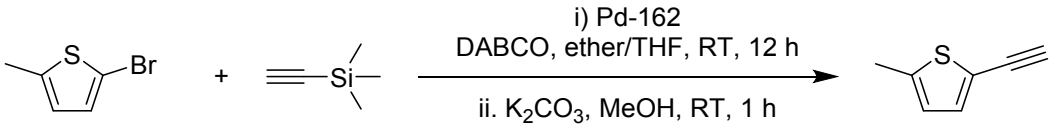
BioRAPTR FRD dispenser (Beckman Coulter, Brea, CA). Plates were incubated at room temperature for 30 minutes, transferred to a ViewLux microplate imager (PerkinElmer, Waltham, MA) and the fluorescence (Ex/Em 525/598 nm) and absorbance (573 nm) were measured accordingly. The Biovision assay kit uses a lactate oxidase-coupled biochemical reaction to measure lactate concentrations. All compounds were tested in a cell-free counter-assay with the Biovision assay kit to ensure compounds were not cross-inhibiting the reporter assay.

Cytotoxicity assay: A673 and MiaPaCa-2 cells were cultured as described above, and plated in 1536-well white solid tissue culture plates using a Multidrop Combi peristaltic dispenser (ThermoFisher, Waltham, MA) at a density of 500 cells/well in 5 μL of DMEM (Invitrogen 11965118) culture medium supplemented with 10 % fetal bovine serum and 100 units/mL Penicillin, 100 μg/mL Streptomycin. A 1536-well pintool dispenser outfitted with 20 nL pins (Wako Automation, San Diego, CA) was used to transfer 23 nL of compound in DMSO to the 1536-well assay plates. After 48 hr incubation at 37 °C, 2.5 μL of CellTiter-Glo (Promega) was dispensed into each well using a BioRAPTR FRD dispenser (Beckman Coulter, Brea, CA). Plates were incubated at room temperature for 10 minutes, transferred to a ViewLux microplate imager (PerkinElmer, Waltham, MA) and the ATP-coupled luminescence was measured using a 1 second exposure.

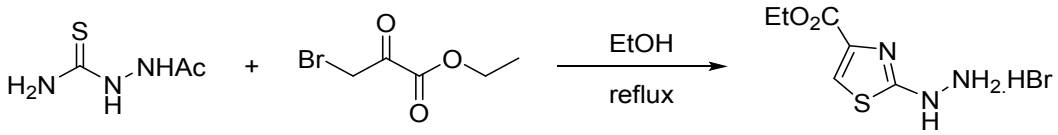
General Methods for Chemistry. All air or moisture sensitive reactions were performed under positive pressure of nitrogen or argon with oven-dried glassware. Anhydrous solvents and bases such as dichloromethane, *N,N*-dimethylforamide (DMF), acetonitrile, ethanol, DMSO, dioxan DABCO were purchased from Sigma-Aldrich. Palladium catalysts were purchased from Johnson Matthey and used as such. Preparative purification was performed on a Waters semi-preparative HPLC system using a Phenomenex Luna C18 column (5 micron, 30 x 75 mm) at a flow rate of 45 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Fraction collection was triggered by UV detection (220 nm). Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). Method 1: A 7 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8 minute run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 micron, 3 x 75 mm) was used at a temperature of 50 °C. Method

2: A 3 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5 minute run time at a flow rate of 1 mL/min. A Phenomenex Gemini Phenyl column (3 micron, 3 x 100 mm) was used at a temperature of 50 °C. Purity determination was performed using an Agilent Diode Array Detector for both Method 1 and Method 2. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometers. Chemical shifts are reported in ppm with undeuterated solvent (DMSO-d_6 at 2.49 ppm) as internal standard for DMSO-d_6 solutions. All of the analogs tested in the biological assays have purity greater than 95%, based on both analytical methods. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02).

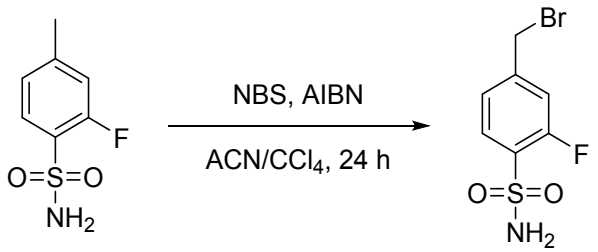
Representative scale-up experimental procedures for key intermediates and lead compounds.



Synthesis of 2-ethynyl-5-methylthiophene: To a mixture of 2-bromo-5-methylthiophene (32.2 ml, 282 mmol, 1 eq) in ether (200 ml)/THF (40 mL) was added DABCO (63.4 g, 565 mmol, 2 eq) and [P(*t*-Bu)$_3$] Pd(crotyl)Cl (https://matthey.com/products-and-services/pharmaceutical-and-medical/catalysts/pd-162, cat # Pd-162) (2.82 g, 7.06 mmol, 5 mol %) then bubbled with argon for 5 minutes ,followed by addition of ethyne, 1-trimethylsilyl- (59.9 ml, 424 mmol, 1.5 eq) and was stirred overnight. The reaction was diluted with pentane and filtered through pad of silica. The filtrate was carefully concentrated at low bath temp and the residue was taken in methanol and stirred with K_2CO_3 (39.0 g, 282 mmol, 1 eq) for 1 h. The reaction was diluted with ether/pentane and filtered through plug of silica. The filtrate was concentrated and purified in isco normal phase eluting with pet ether. The peak was collected and concentrated to obtain relatively pure product. The product was further purified using distillation at 60 ^{o}C @25 bar pressure (bath temp 100 ^{o}C). The pure compound is colorless which slowly turns into yellow then brown upon exposure to air or light. Stored in the fridge under argon. LC-MS Retention Time: (Method 2) = 3.469 min.

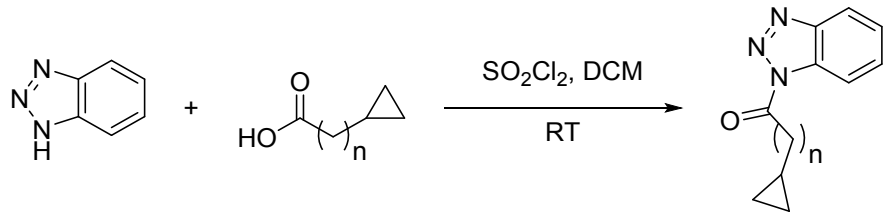


Synthesis of ethyl 2-hydrazineylthiazole-4-carboxylate hydrobromide: To a suspension of 2-acetylhydrazinecarbothioamide (15 g, 113 mmol) in ethanol (200 mL) was added ethyl bromopyruvate (15.71 ml, 113 mmol) and the mixture was stirred at rt for 30 minutes until the solution becomes clear, then refluxed for 12 h. (The acetyl group falls off in situ to form the product). The reaction was concentrated and agitated with 20 mL of MeOH and 300 mL of ether. The yellow precipitate was collected by filtration and washed with ether and dried to obtain a yellow solid as HBr salt (23.2 g; 77 %). LC-MS Retention Time: (Method 2) = 2.60 min $(M+H)^+$ = 188. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.72 (s, 1H), 7.52 (s, 1H), 4.93 (s, 2H), 4.19 (qd, J = 7.1, 0.7 Hz, 2H), 1.25 (td, J = 7.1, 0.7 Hz, 3H).



IVb

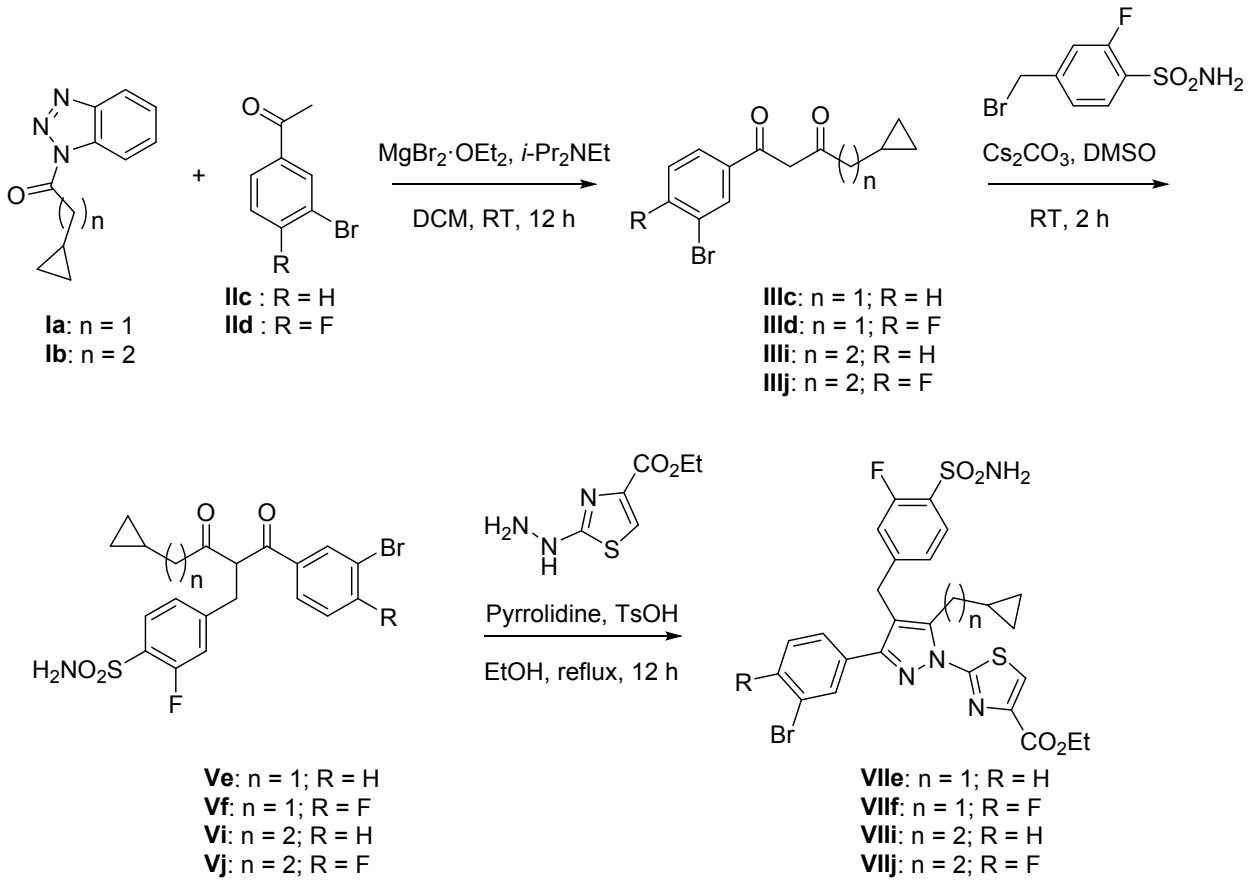
Synthesis of 4-(bromomethyl)-2-fluorobenzenesulfonamide (**IVb**): A mixture of 2-fluoro-4-methylbenzenesulfonamide (25 g, 132 mmol, 1 eq), NBS (30.6 g, 172 mmol, 1.3 eq) and AIBN (2.170 g, 13.21 mmol, 10 mol % added in portions for every 6 hrs. to push the reaction further) in CCl_4 (100 mL)/ ACN (200 mL) was refluxed for 24 h. The solvent was evaporated then the residue was suspended in ethyl acetate and filtered. The filtrate was subsequently washed with sodium thiosulfate solution, saturated sodium bicarbonate and brine solutions the organic layer was dried with sodium sulfate. The crude material was mixed with silica and dry loaded to a loading cartridge. The compound was purified on an isco flash system using a 220 g silica column (in 2 batches) eluting with 5-100 % ethyl acetate in hexanes over 16 column volumes. (Note: slight impurity is still present). LC-MS Retention Time: (Method 2) = 2.788 min $(M+H)^+$ = 270. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.78 (t, J = 7.9 Hz, 1H), 7.69 (s, 2H), 7.58 – 7.41 (m, 2H), 4.74 (s, 2H).



Ia: n = 1
Ib: n = 2

Synthesis of intermediates Ia-b (representative procedure for 1-(1H-benzo[d][1,2,3]triazol-1-yl)-2-cyclopropylethan-1-one Ia): To a solution of 1H-benzo[d][1,2,3]triazole (476 g, 3995 mmol, 4 eq) in DCM (600 mL) was added thionyl chloride (72.9 ml, 999 mmol, 1eq) and the mixture was stirred at rt for 0.5 h then 2-cyclopropylacetic acid (93 ml, 999 mmol, 1 eq) was carefully added upon cooling in an ice water bath (for larger scale cooling necessary due to exothermic reaction, if the reaction mixture solidifies add more DCM) and stirred for 6 h. The reaction was filtered, and the filter cake was washed with DCM. The filtrate was added bicarbonate solution slowly and stirred for 30 minutes then transferred to a separatory funnel. The organic layer washed with bicarbonate solution then with brine. Concentrated and purified on Isco flash silica system using 340 g silica column eluting with 0-20 % ethyl acetate in hexanes over 10 column volumes (done in 6 batches). The first peak was collected and dried to get oil, which solidifies eventually (Yield 92 %) to give *1-(1H-benzo[d][1,2,3]triazol-1-yl)-2-cyclopropylethan-1-one* **Ia**. LC-MS Retention Time: (Method 2) = 3.51 min $(M+H)^+ = 202$.

1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-cyclopropylpropan-1-one **Ib**. This compound was synthesized following the same procedure used for **Ia** starting from 3-cyclopropylpropanoic acid (131 mmol, 15g) to obtain white solid in 93 % yield. LC-MS Retention Time: (Method 2) = 3.6 min $(M+H)^+ = 216$.



Synthesis of intermediates IIIc-d and **IIIi-j** (Representative procedure for **IIIc-d**): 3'-Bromoacetophenone **IIc** or 3-bromo-4-fluoroacetophenone **IId** (754 mmol, 1eq) 1-(1H-benzo[d][1,2,3]triazol-1-yl)-2-cyclopropylethan-1-one **Ia** (167 g, 829 mmol, 1.2 eq) was charged with 1000 mL DCM then magnesium bromide diethyl etherate (487 g, 1884 mmol, 2.5 eq) was added in one portion in a 4 necked flask set up with overhead stirrer. The reaction was cooled in an ice bath then Hunig's base (395 ml, 2261 mmol, 3 eq) as added drop wise over 15 minutes through a dropping funnel. The reaction was stirred overnight. The reaction was placed in an ice bath then added ice cubes slowly while stirring vigorously (*Caution*: *exothermic reaction*). The addition of ice continued until no more exothermic reaction occurs, after which added 1 molar HCl dropwise under ice cooling followed further acidification with 6 molar HCl. The reaction mixture was then extracted with DCM, and the organic layer was washed with brine. The DCM layer was dried with magnesium sulfate and concentrated. The crude product after removing the solvent was purified on a flash system using 340 g Biotage columns eluting with gradient elution (0-30 % ethyl acetate in hexanes over 20 column volumes (in 8 batches) to get yellow liquid as a first peak.

1-(3-Bromophenyl)-4-cyclopropylbutane-1,3-dione ***(IIIc)***. Yield = 62 %, LCMS Retention time: (Method 2) = 3.84 min. (usually as in keto enol form another peak around 3.44 min) $(M+H)^+$ = 281.

1-(3-Bromo-4-fluorophenyl)-4-cyclopropylbutane-1,3-dione ***(IIId)***. Yield = 74 %, LC-MS Retention Time (Method 2) = 3.85 min. (usually as in keto enol form another peak around 3.46 min) $(M+H)^+$ = 301.

1-(3-Bromophenyl)-5-cyclopropylpentane-1,3-dione ***(IIIi)***. This compound was synthesized following the above general procedure from 3'-bromoacetophenone **IIc** (106 mmol, 21.02 g) and 1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-cyclopropylpropan-1-one **Ib** (116 mmol, 25 g). Yield = 64 %, LC-MS Retention Time (Method 2) = 3.98 min. $(M+H)^+$ = 295.

1-(3-bromophenyl)-5-cyclopropylpentane-1,3-dione ***(IIIj)***. This compound was synthesized following the above general procedure from 3'-bromoacetophenone **IId** (17.97 mmol, 3.9 g) and 1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-cyclopropylpropan-1-one **Ib** (19.77 mmol, 4.25 g). Yield = 64 %, LC-MS Retention Time (Method 2) = 4.06 min. $(M+H)^+$ = 313.

Synthesis of intermediates Ve-f and **Vi-j** (Representative procedure for **Ve-f**): To a mixture of 1-(3-bromophenyl)-4-cyclopropylbutane-1, 3-dione **IIIc** or 1-(3-bromo-4-fluorophenyl)-4-cyclopropylbutane-1,3-dione **IIId** (329 mmol) in DMSO (300 mL) was added Cs_2CO_3 (129 g, 395 mmol) and the reaction mixture was stirred at RT for 10 minutes. To the above mixture was added 4-(bromomethyl)-2-fluorobenzenesulfonamide **IVb** (88 g, 329 mmol) portion wise upon cooling in ice water bath, then the mixture was allowed to stir at room temperature for 1 h. The reaction was diluted with ethyl acetate and filtered to remove the solids. The filtrate was quenched with 100 mL of 1 molar HCl and extracted with ethyl acetate. The aqueous layer was again extracted twice with ethyl acetate then the combined organic layer was washed with saturated ammonium chloride solution 3 times. The combined organic layer was dried with magnesium sulfate and concentrated. The crude material was taken in DCM and purified in Isco flash system using 330 g gold columns (distributed into 5 columns).

4-(2-(3-Bromobenzoyl)-4-cyclopropyl-3-oxobutyl)-2-fluorobenzenesulfonamide ***(Ve)***. Yield = 79 %, LCMS Retention time: (Method 2) = 3.388 min. $(M+Na)^+$ = 490.

4-(2-(3-Bromo-4-fluorobenzoyl)-4-cyclopropyl-3-oxobutyl)-2-fluorobenzenesulfonamide ***(Vf)***. Yield = 75 %, LCMS Retention time: (Method 2) = 3.36 min. $(M+H)^+$ = 488.

4-(2-(3-bromobenzoyl)-5-cyclopropyl-3-oxopentyl)-2-fluorobenzenesulfonamide ***(Vi)***. This compound was synthesized following the above general procedure from 1-(3-bromophenyl)-5-cyclopropylpentane-1,3-dione **IIIi** (66.1 mmol, 19.5 g) and 1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-cyclopropylpropan-1-one **Ib** (116 mmol, 17.71 g). Yield = 78 %, LC-MS Retention Time (Method 2) = 3.53 min. $(M+H)^+$ = 482.

4-(2-(3-Bromo-4-fluorobenzoyl)-5-cyclopropyl-3-oxopentyl)-2-fluorobenzenesulfonamide ***(Vj)***. This compound was synthesized following the above general procedure from 4-(2-(3-bromo-4-fluorobenzoyl)-5-cyclopropyl-3-oxopentyl)-2-fluorobenzenesulfonamide **IIIj** (11.5 mmol, 3.6 g) and 4-(bromomethyl)-2-fluorobenzenesulfonamide **IVb** (10.92 mmol, 2.93 g). Yield = 65 %, LC-MS Retention Time (Method 2) = 3.57 min. $(M+H)^+$ = 501.

Synthesis of intermediates VIIe-f and **VIIi-j** (Representative procedure for **VIIe-f**): To a mixture of **Ve** or **Vf** (53.4 mmol) and tosic acid (5.08 g, 26.7 mmol, 0.5 eq) in ethanol (100 mL) was added pyrrolidine (2.207 ml, 26.7 mmol, 0.5 eq) and the mixture was refluxed for 1 h. The reaction was cooled and ethyl 2-hydrazinylthiazole-4-carboxylate and HBr (22.36 g, 64.1 mmol) was added and the mixture was heated at reflux overnight. The reaction was concentrated and the residue was taken in DCM and immediately loaded to a silica loading cartridge. The compound was purified on an Isco flash system using 330 g gold column eluting with 20-40 % ethyl acetate in hexanes over 20 column volumes. The pure product, containing a mixture of two regioisomers, were further separated on a reverse phase flash system using a 415 g gold column eluting with 60-100 % ACN (0.1% TFA) in water (0.1 % TFA) over 20 column volumes. The 2nd peak was pooled, and concentrated and the residue was stirred with a clear solution of bicarbonate. The white precipitate formed was collected by filtration. The filter cake was thoroughly washed with water and air-dried and finally in a vacuum desiccator under P_2O_5 to get pure white solids.

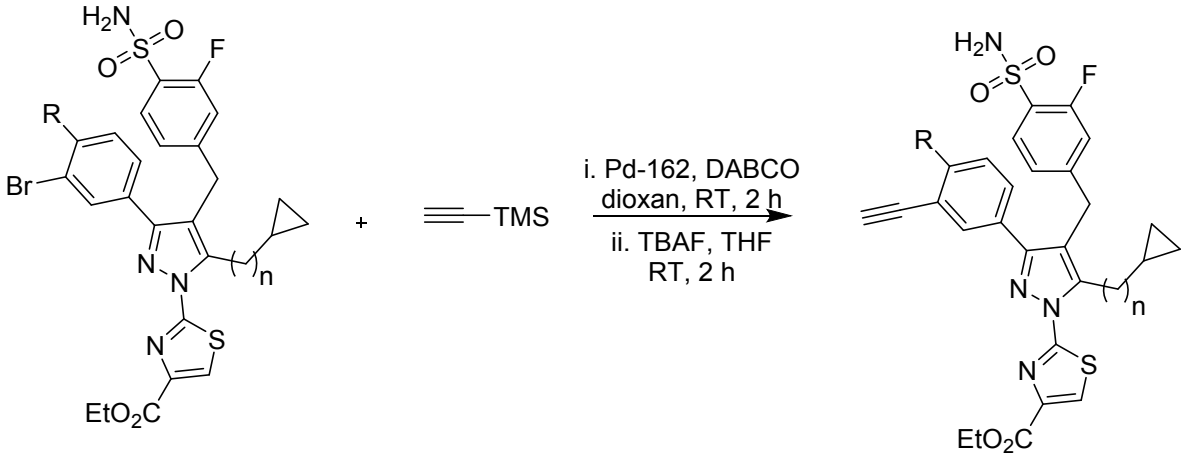
Ethyl 2-(3-(3-bromophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate ***(VIIe)***: Yield = 33 %, LCMS Retention time: (Method 1) = 6.62 min. $(M+H)^+$ = 621. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.38 (s, 1H), 7.72 (t, *J* = 1.8 Hz, 1H), 7.66 (dd, *J* = 8.5, 7.4 Hz, 1H), 7.62 – 7.54 (m, 4H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.19 – 7.13 (m, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.32 (q, *J* = 7.1 Hz, 2H), 4.17 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.21 – 1.08 (m, 1H), 0.40 – 0.32 (m, 2H), 0.29 – 0.20 (m, 2H).

Ethyl 2-(3-(3-bromo-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate ***(VIIf)***: Yield = 34 %, LCMS Retention time: (Method 1) =

6.924 min. $(M+H)^+$ = 639. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.38 (s, 1H), 7.83 (dd, *J* = 6.7, 2.2 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.62 – 7.56 (m, 3H), 7.42 (t, *J* = 8.7 Hz, 1H), 7.15 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.32 (q, *J* = 7.1 Hz, 2H), 4.17 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.22 – 1.08 (m, 1H), 0.41 – 0.31 (m, 2H), 0.29 – 0.22 (m, 2H).

Ethyl 2-(3-(3-bromophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate ***(VIIi)***: This compound was synthesized following the above general procedure from 4-(2-(3-bromobenzoyl)-5-cyclopropyl-3-oxopentyl)-2-fluorobenzenesulfonamide **Vi** (46 mmol, 22.2 g). Yield = 45 %, LCMS Retention time: (Method 2) = 3.912 min. $(M+H)^+$ = 635.

Ethyl 2-(3-(3-bromophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate ***(VIIj)***: This compound was synthesized following the above general procedure from 4-(2-(3-bromo-4-fluorobenzoyl)-5-cyclopropyl-3-oxopentyl)-2-fluorobenzenesulfonamide **Vj** (7.45 mmol, 3.73 g). Yield = 41 %, LCMS Retention time: (Method 2) = 3.928 min. $(M+H)^+$ = 653.



Ve: n = 1; R = H
Vf: n = 1; R = F
Vi: n = 2; R = H

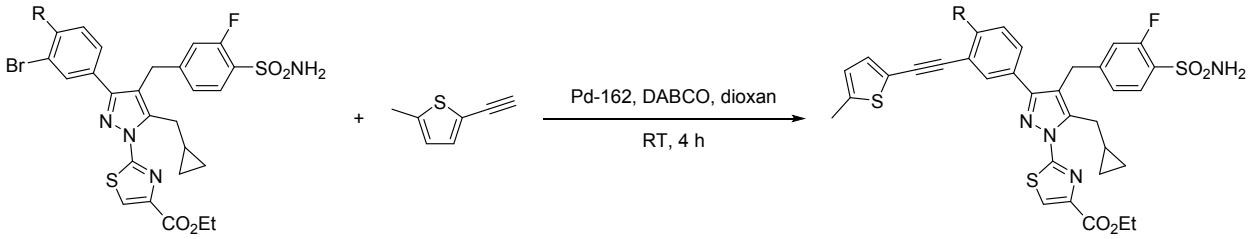
VIIIa: n = 1; R = H
VIIIb: n = 1; R = F
VIIIc: n = 2; R = H

Representative procedure for the synthesis intermediates VIIIa-c: A mixture of ethyl 2-(3-(3-bromophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate **Ve** (10.7 g, 17.27 mmol), [P(t-Bu)3] Pd(crotyl)Cl (cat # Pd-162) (0.345 g, 0.864 mmol) and DABCO (3.87 g, 34.5 mmol) in dioxane was bubbled with argon then ethynyltrimethylsilane (4.79 ml, 34.5 mmol) was added and the mixture stirred at rt for 2 h. The

reaction was diluted with ethyl acetate then filtered through celite. The filtrate was concentrated and loaded directly to a 330 g gold column eluting with 10-40 % ethyl acetate in hexanes over 20 column volumes. The product fraction obtained after evaporating the solvent was treated with 1 molar TBAF in THF (17.3 mL, 17.27 mmol) and stirred at rt for 2 h. After completion of the reaction, excess THF was removed under diminished pressure. The crude product was extracted with ethyl acetate and subsequently washed with water and brine. The crude product was purified on a flash system eluting with 20-50 % ethyl acetate in hexanes over 22 column volumes using a 220 g gold column to yield 8.06 g (83%) of *ethyl 2-(5-(cyclopropylmethyl)-3-(3-ethynylphenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate* ***(VIIIa)***. LCMS Retention time: (Method 2) = 3.68 min. $(M+H)^+$ = 565.

Ethyl 2-(5-(cyclopropylmethyl)-3-(3-ethynyl-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate ***(VIIIb)***. LC-MS Retention Time: (Method 2) = 3.72 $(M+H)^+$ = 583. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.38 (d, *J* = 1.2 Hz, 1H), 7.75 – 7.60 (m, 3H), 7.57 (s, 2H), 7.39 – 7.31 (m, 1H), 7.14 (dd, *J* = 11.3, 1.7 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.55 (s, 1H), 4.32 (qd, *J* = 7.1, 1.1 Hz, 2H), 4.16 (s, 2H), 3.16 (d, *J* = 6.9 Hz, 2H), 1.32 (td, *J* = 7.1, 1.1 Hz, 2H), 1.23 – 1.08 (m, 1H), 0.39 – 0.31 (m, 2H), 0.25 (dt, *J* = 5.1, 1.4 Hz, 2H).

Ethyl 2-(5-(2-cyclopropylethyl)-3-(3-ethynylphenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate ***(VIIIc)***. LCMS Retention time: (Method 2) = 3.763 min. $(M+H)^+$ = 579.



VIIe: R = H
VIIe: R = F

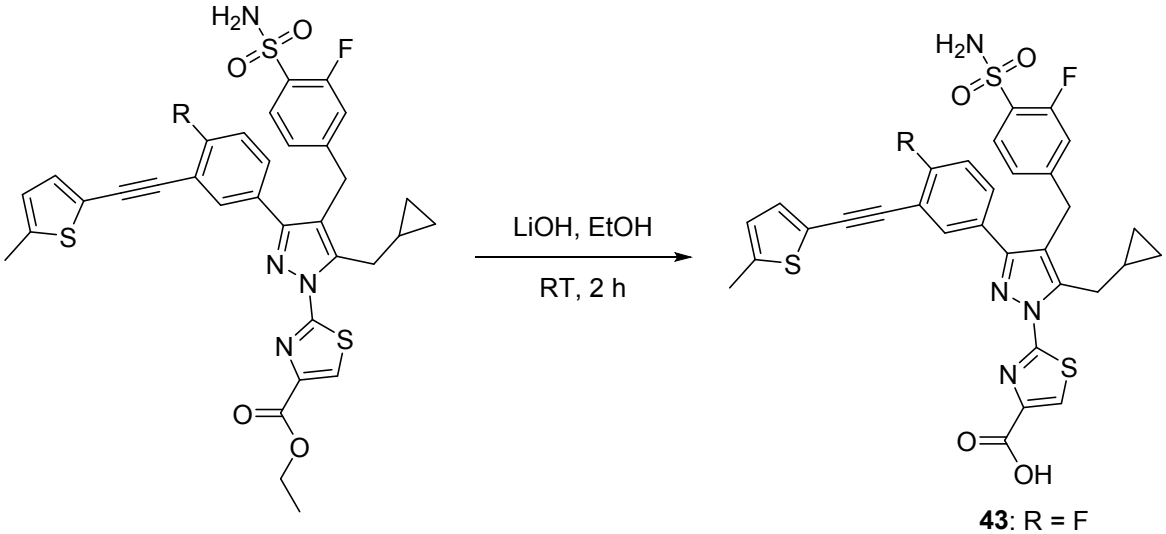
R = H
R = F

A mixture of **VIIe** or **VIIf** (14.12 mmol), 2-ethynyl-5-methylthiophene (2.242 g, 18.35 mmol), and DABCO (3.17 g, 28.2 mmol) in dioxan (30 mL) was bubbled with argon for 10 minutes, then [P(t-Bu)$_3$] Pd(crotyl)Cl (Pd-162) (0.141 g, 0.353 mmol) was added and the mixture stirred at rt for 4 -12 h. After completion of the reaction, Pd scavenger silia DMT was added and the mixture was stirred for 2 h at RT. The reaction mixture was diluted with ethyl acetate and filtered through a plug of silica. The filtrate was concentrated and purified on an Isco flash system using a 330 g gold

column eluting with 15-40 % ethyl acetate in hexanes over 20 CV. The product had some yellow color (pure by LC) which is further purified in reverse phase flash system using a 415 g gold column eluting with 60-100 % ACN (0.1 % TFA) in water (0.1 % TFA) over 25 column (elutes with around 80 % ACN). The fractions were pooled and concentrated then neutralized with bicarbonate solution. The precipitate was collected by filtration and washed with water then air dried followed by vacuum drying under P_2O_5 to get white solids.

Ethyl 2-(5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate: Yield = 83 %. LC-MS Retention Time: (Method 1) = 7.338 min $(M+H)^+$ = 661.

Ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate: Yield = 90 %. LC-MS Retention Time: (Method 1) = 7.53 min $(M+H)^+$ = 679. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.38 (s, 1H), 7.74 (dd, *J* = 6.9, 2.3 Hz, 1H), 7.70 – 7.59 (m, 2H), 7.57 (s, 2H), 7.37 (t, *J* = 9.1 Hz, 1H), 7.29 (dd, *J* = 3.6, 0.6 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.90 – 6.82 (m, 1H), 4.32 (q, *J* = 7.1 Hz, 2H), 4.18 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.22 – 1.09 (m, 1H), 0.43 – 0.33 (m, 2H), 0.29 – 0.22 (m, 2H).



43: R = F
52: R = H

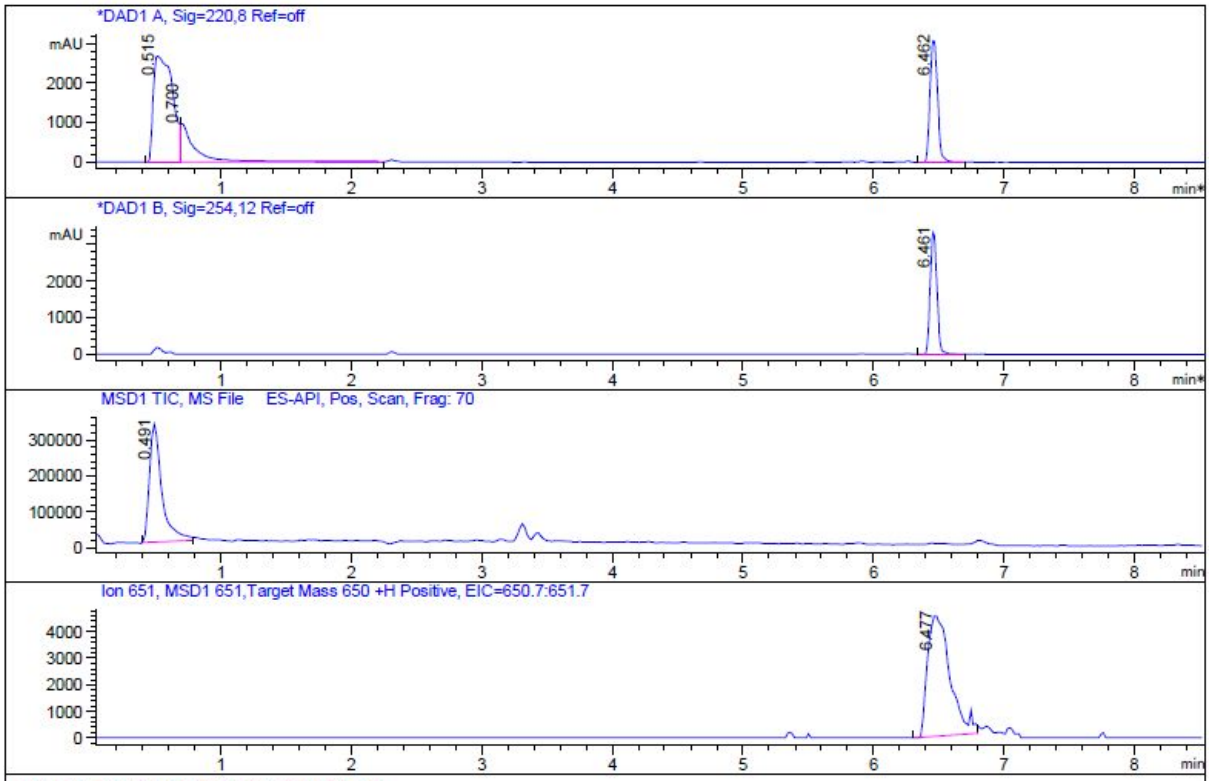
*Synthesis of 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**43**).* To a mixture containing ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (8.65

g, 12.74 mmol) and EtOH (150 mL) was added LiOH (42.5 ml, 63.7 mmol, 5 eq) and the mixture was stirred at RT for 2 h. After completion of the reaction, most of the solvent was removed and the residue was diluted with water (50 mL). The reaction mixture was acidified with 1 molar HCl. The precipitate formed was stirred at RT for 1 h then collected by filtration. The filter cake was thoroughly washed with cold water. The milky precipitate was further suspended in hot water and stirred for 30 minutes and filtered again. The precipitate was washed with hot water and with cold ethanol to get the white solid which was further dried under air overnight and finally in a vacuum oven overnight at 80 ^{o}C. Yield 7.3 g as white solid (88 %). LC-MS Retention Time = 6.133 min $(M+H)^+$ = 651. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.14 (s, 1H), 8.29 (s, 1H), 7.71 (dd, *J* = 6.9, 2.3 Hz, 1H), 7.66 – 7.54 (m, 4H), 7.35 (dd, *J* = 9.4, 8.7 Hz, 1H), 7.26 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.14 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.03 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.83 (dq, *J* = 3.6, 1.0 Hz, 1H), 4.15 (s, 2H), 3.15 (d, *J* = 6.9 Hz, 2H), 2.47 – 2.45 (m, 3H), 1.20 – 1.06 (m, 1H), 0.38 – 0.29 (m, 2H), 0.24 – 0.15 (m, 2H).

*Synthesis of 2-(5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**52**):* A mixture containing ethyl 2-(5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)-phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (10.9 g, 16.50 mmol) in ethanol (150 mL) was treated with 1.5 molar LiOH (55.0 ml, 82 mmol, 5 eq) and the reaction was stirred at RT for 2 h. After removing most of the solvent, the reaction was diluted with 100 mL of water and acidified with 1 molar HCl. The precipitate formed was collected by filtration and washed with water. The wet precipitate was suspended in hot water and sonicated for 2 h followed by stirring for another 1 h (alternatively the precipitate can be stirred overnight). The precipitate formed was collected by filtration, washed with water and a mixture of cold water/ethanol (1/1) and air dried. Finally, the compound was dried under high vacuum under P_2O_5 overnight to get pure product as white solid 10.2 g (98 %). LC-MS Retention Time = 6.402 min $(M+H)^+$ = 632. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.18 (s, 1H), 8.32 (s, 1H), 7.74 – 7.62 (m, 2H), 7.56 (s, 0H), 7.52 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.48 – 7.40 (m, 1H), 7.24 (d, *J* = 3.5 Hz, 1H), 7.17 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.83 (dq, *J* = 3.4, 1.1 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.47 (d, *J* = 1.1 Hz, 3H), 1.23 – 1.09 (m, 1H), 0.39 – 0.31 (m, 2H), 0.27 – 0.20 (m, 2H).

Figure S4: LCMS for **43**

```
File ..ANTUKALLUG\02-18\200218-GRB061-0151-13477.D Tgt Mass (EZX): 650.00
Injection Date    : 20 Feb 18  12:04 pm -0500          Seq. Line : 0
Sample Name       : GRB061-015                    Location  : P1-D-03
Acq. Operator     : Ganesha Rai                          Inj : 1
Spec. Reported    : UV Integration                 Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD.M
Sample Info : Easy-Access Method: 'FINAL_GRAD'  650.00
Method Info : Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Agilent Eclipse XDB-C18 3 micron 3 x 75mm
```



Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.52	0.14	27394.09	2676.13	56.91	179
0.70	0.14	8205.07	967.65	17.05	179
6.46	0.07	12533.18	3103.14	26.04	651

Integration Results for DAD1 B, Sig=254,12 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
6.46	0.06	11868.17	3325.29	100.00	651

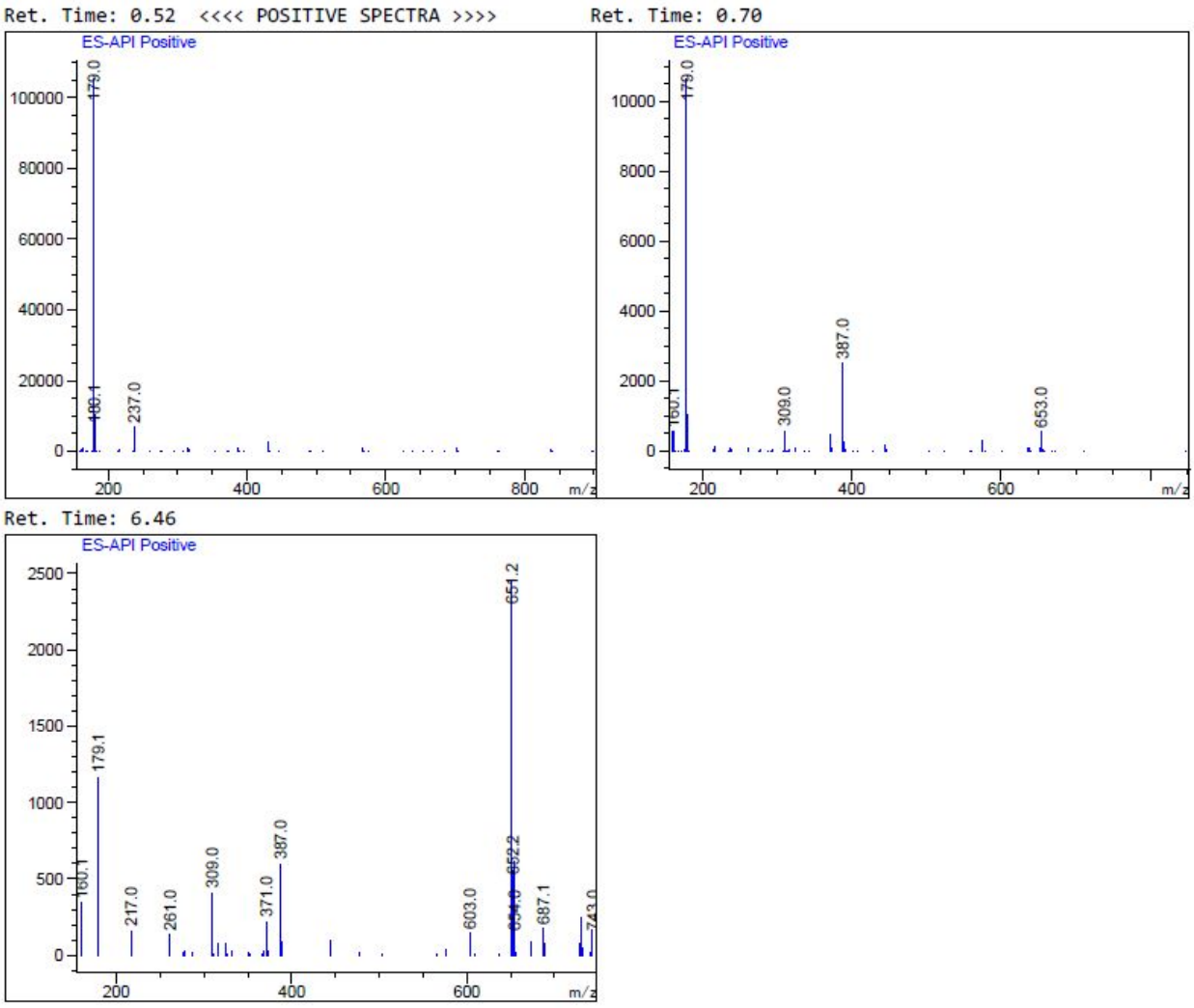
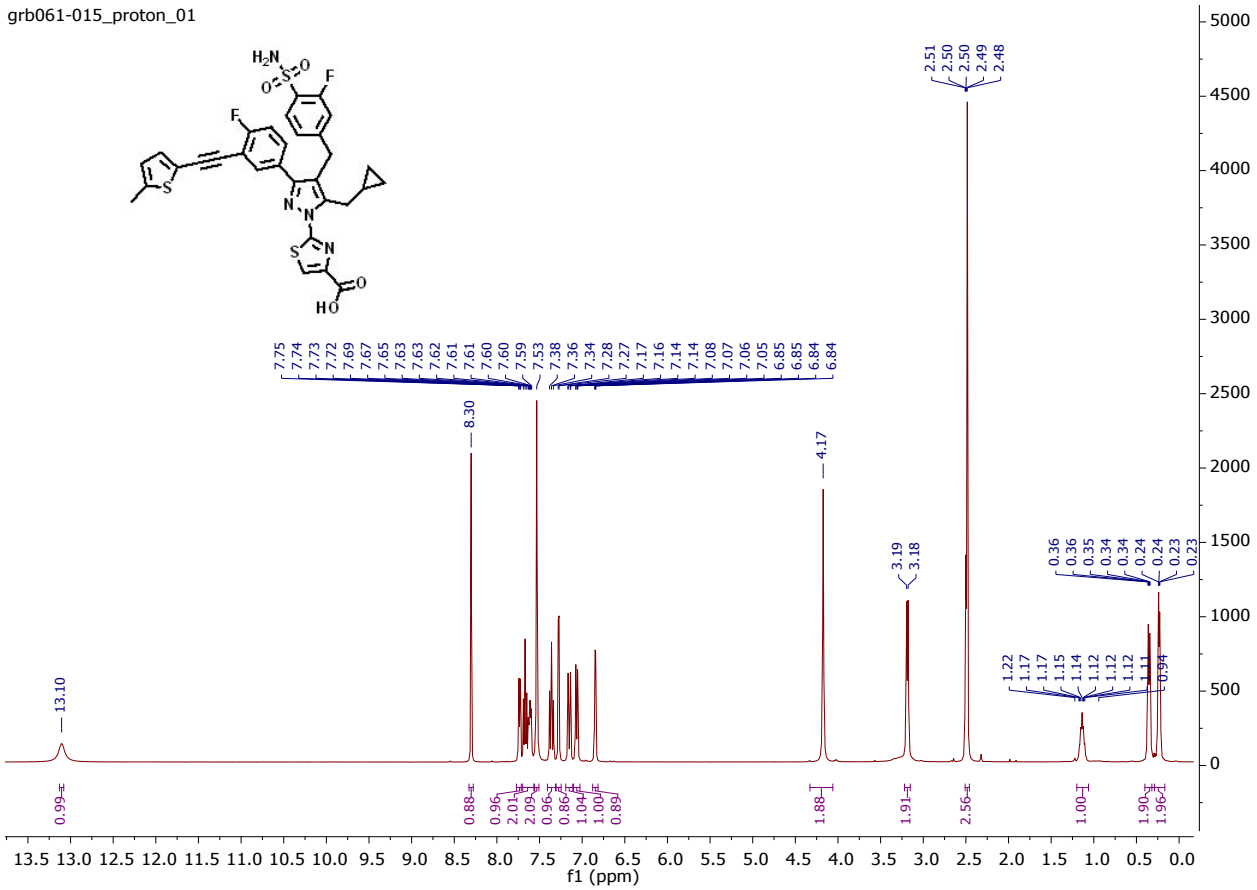


Figure S5: Proton NMR and Fluorine NMR for **43**


grb061-015_proton_01
f1 (ppm)

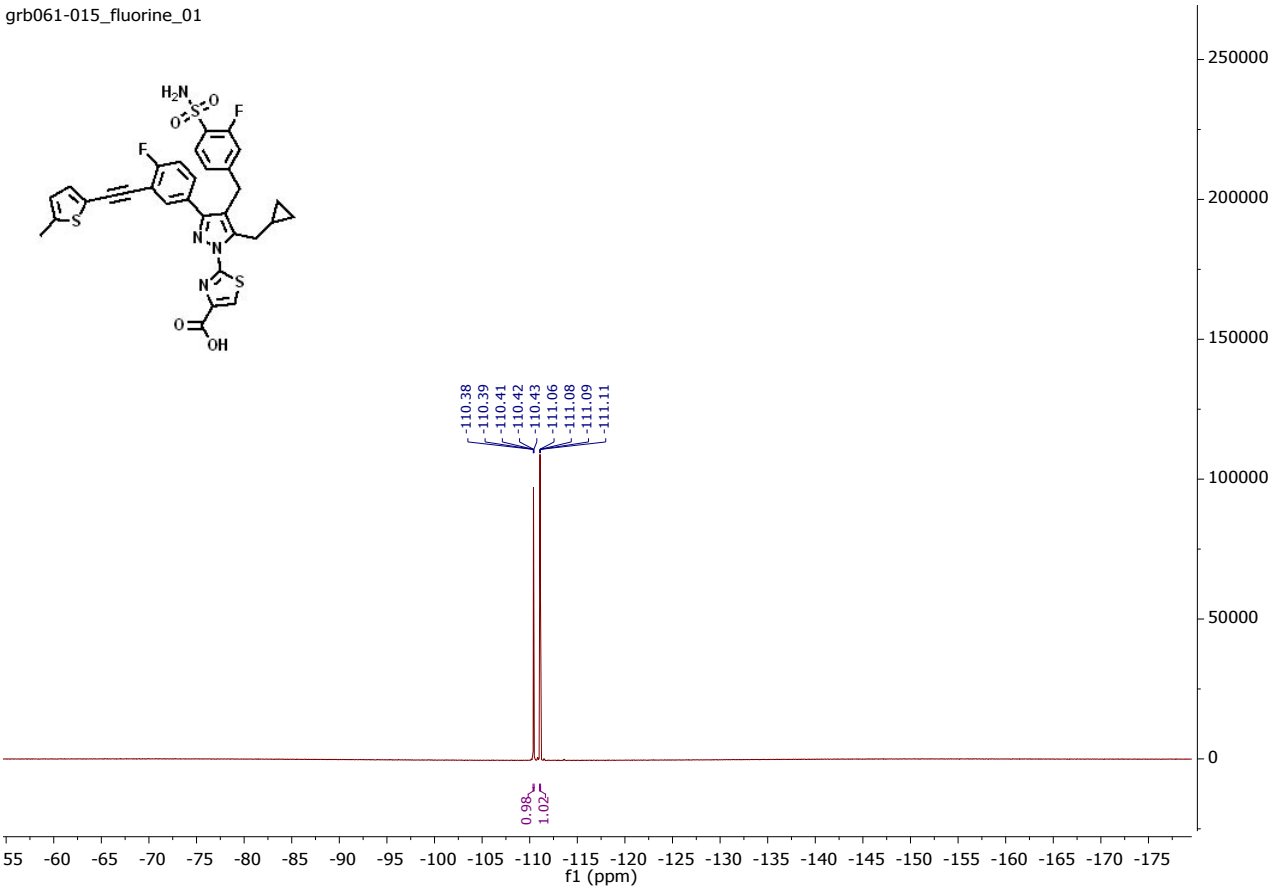


Figure S6: ^{13}C NMR spectra for **43**

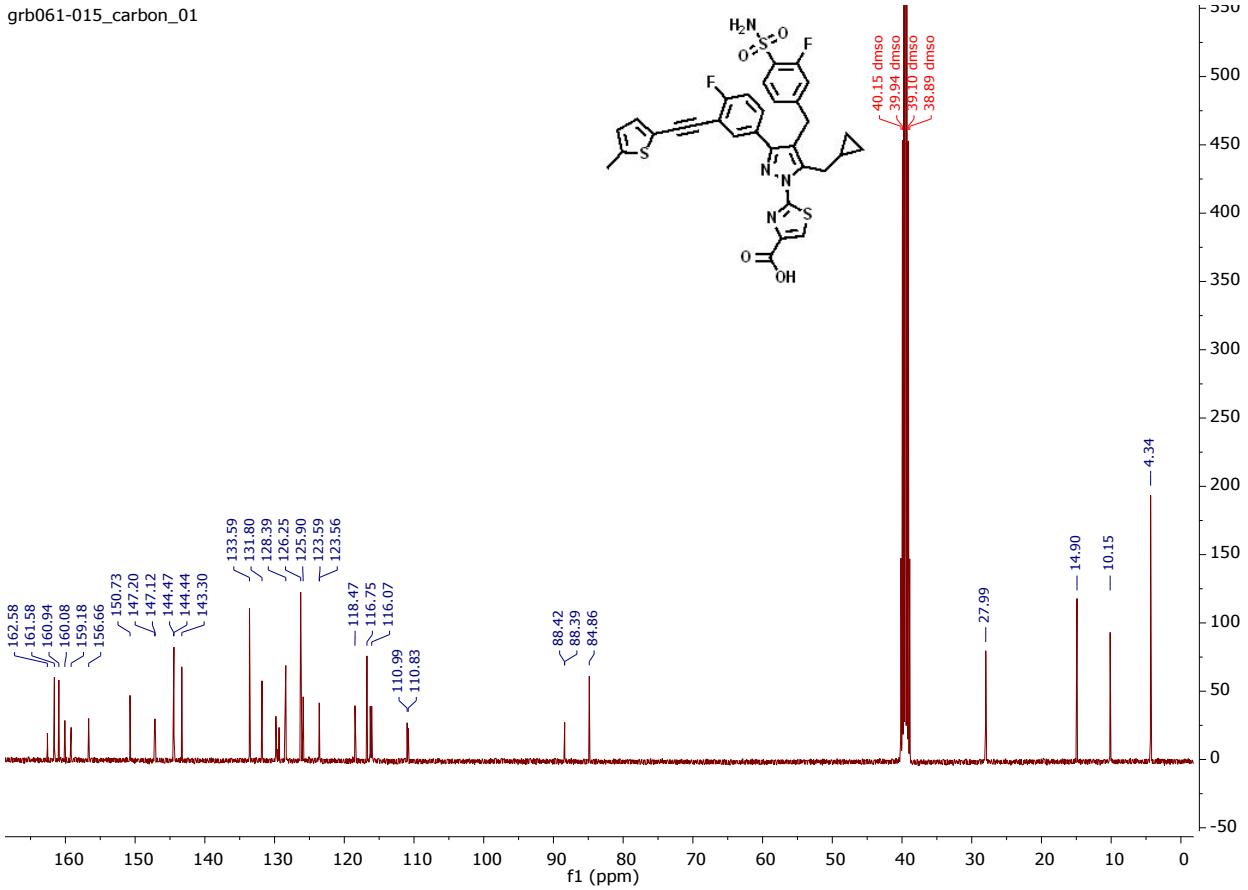

grb061-015_carbon_01
162.58
161.58
160.94
160.08
159.18
156.66
150.73
147.20
147.12
144.47
144.44
143.30
133.59
131.80
128.39
126.25
125.90
123.59
123.56
118.47
116.75
116.07
110.99
110.83
88.42
88.39
84.86
40.15 dmso
39.94 dmso
39.10 dmso
38.89 dmso
27.99
14.90
10.15
4.34
f1 (ppm)

Figure S7: LCMS for for **52**

```
File ..ANTUKALLUG\02-18\200218-GRB061-0141-46812.D Tgt Mass (EZX): 632.00
Injection Date    : 20 Feb 18   2:32 pm -0500          Seq. Line : 0
Sample Name       : grb061-014                  Location  : P1-F-02
Acq. Operator     : Ganesha Rai                           Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD.M
Sample Info : Easy-Access Method: 'FINAL_GRAD'  632.00
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
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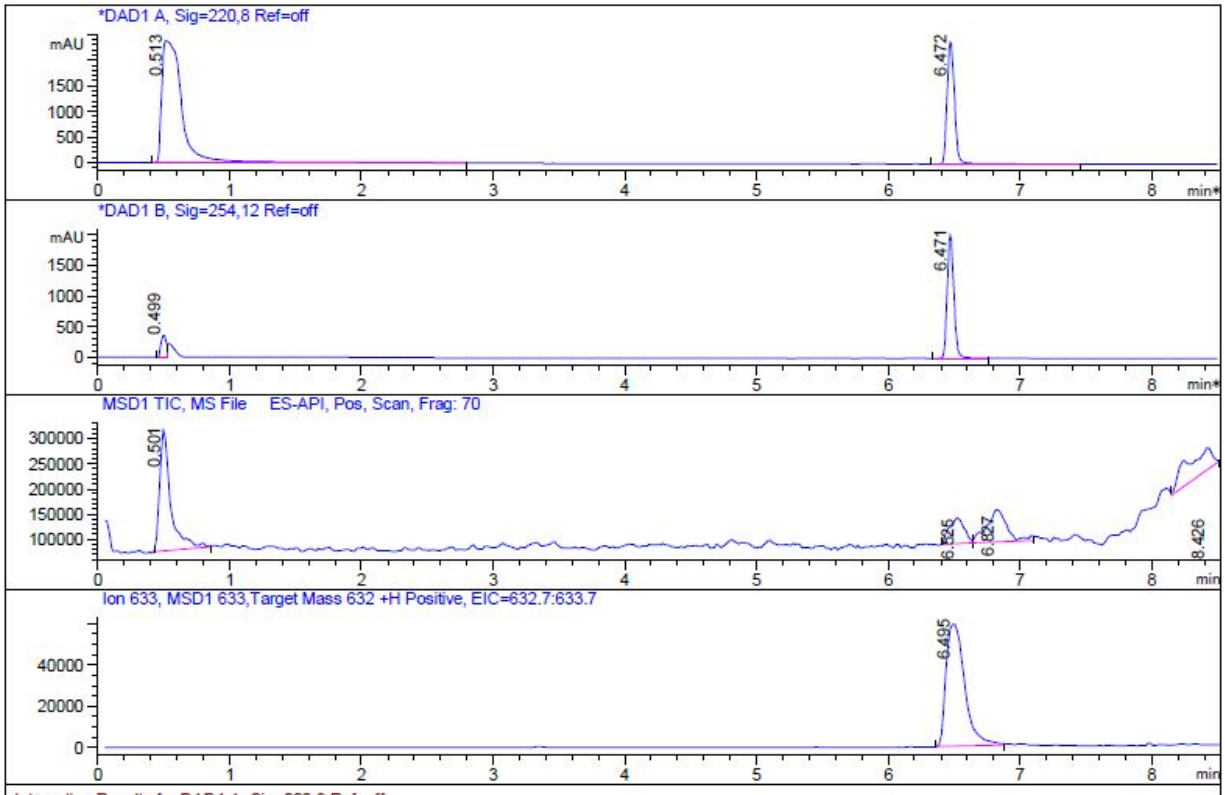


Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.51	0.14	25955.47	2397.40	73.15	179
6.47	0.06	9529.08	2393.91	26.85	179

Integration Results for DAD1 B, Sig=254,12 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.50	0.05	1034.19	358.91	12.54	179
6.47	0.06	7212.60	2022.89	87.46	633

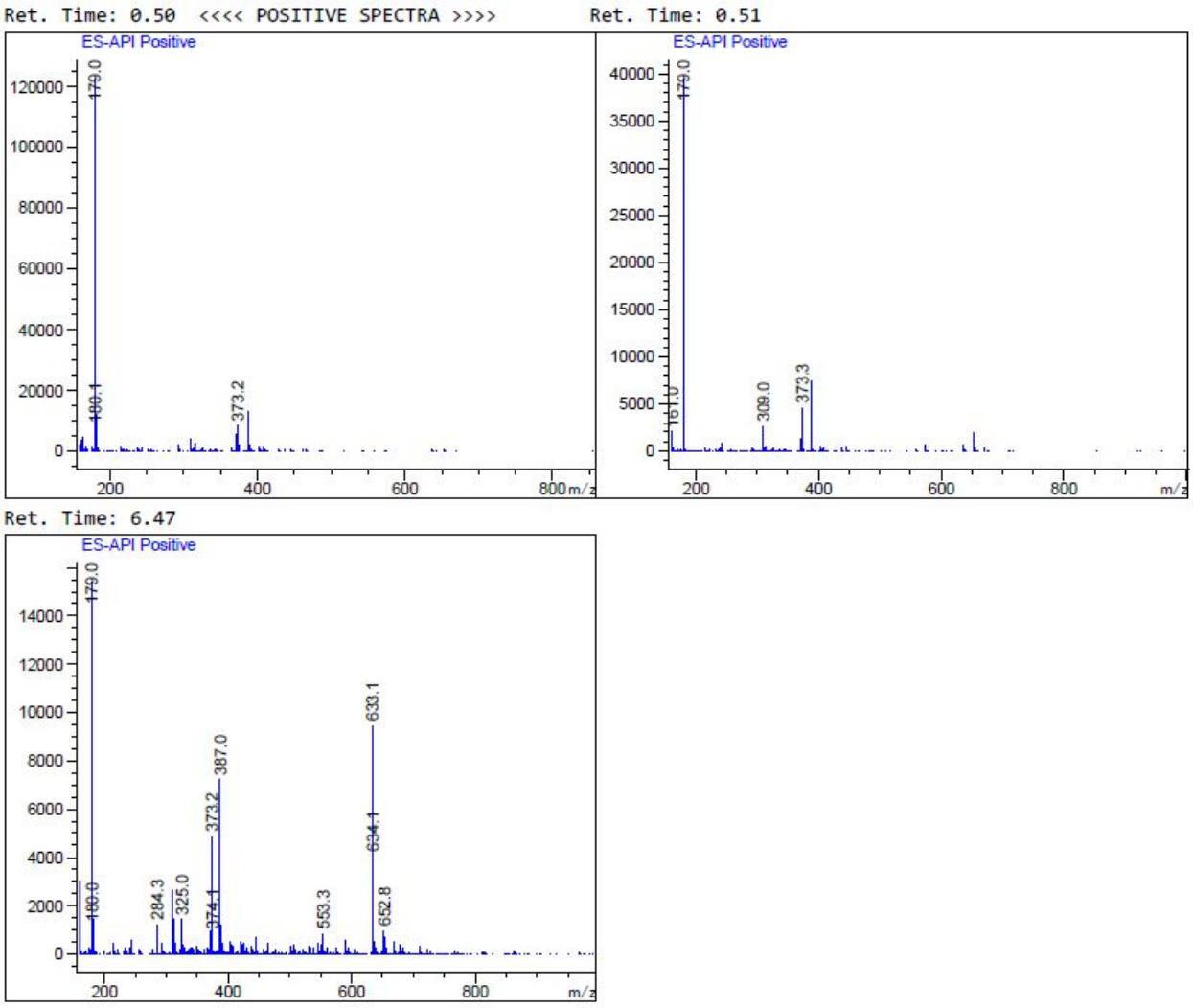
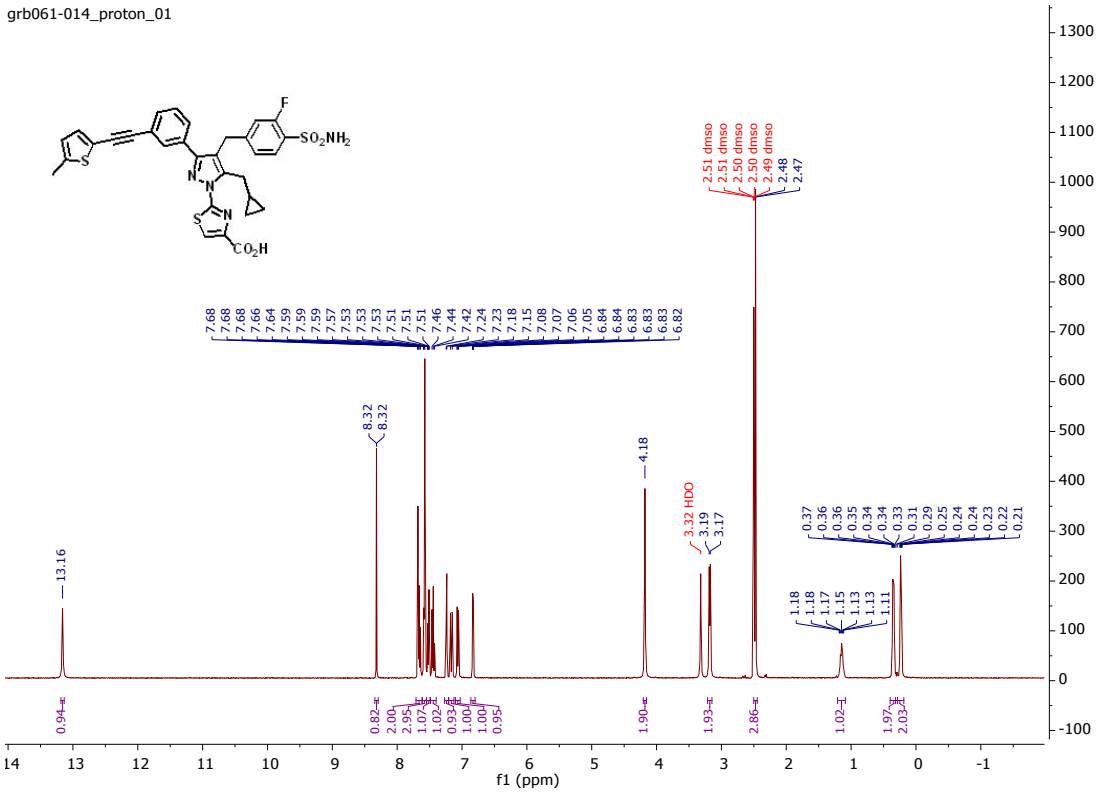


Figure S8: Proton NMR and Fluorine NMR for **52**


grb061-014_proton_01
F
SO2NH2
S
N
N
S
N
CO2H
13.16
8.32
8.32
4.18
3.32 HDO
3.19
3.17
2.51 dmso
2.51 dmso
2.50 dmso
2.50 dmso
2.49 dmso
2.48
2.47
f1 (ppm)

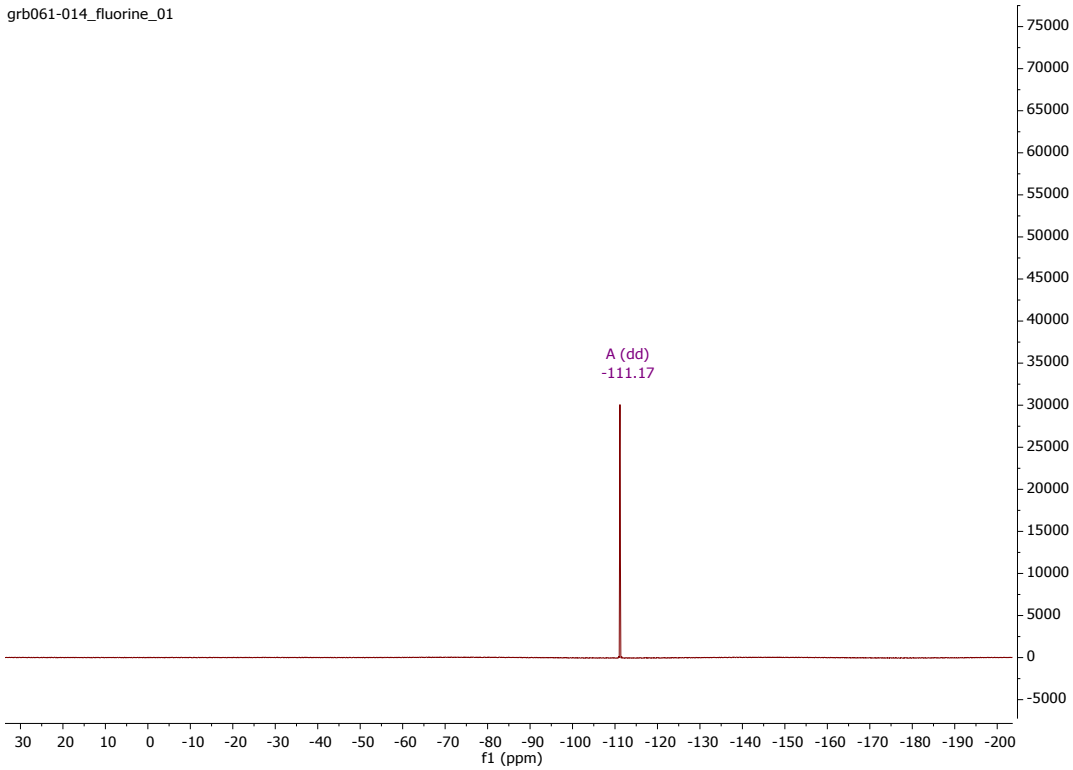
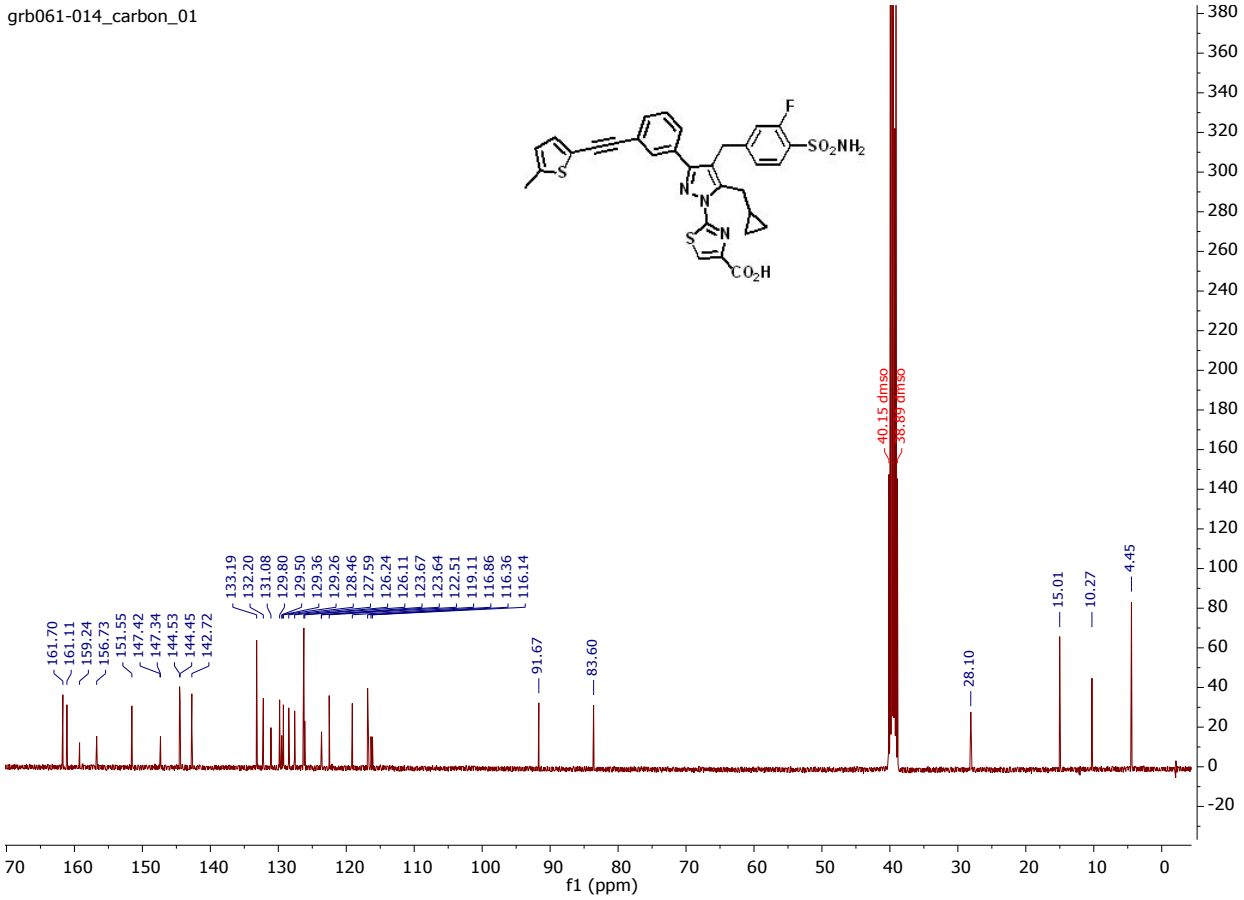

grb061-014_fluorine_01
A (dd)
-111.17
f1 (ppm)

Figure S9: ^{13}C NMR spectra for **52**



Figures S10: Originial LCMS, proton, and carbon NMR for key compounds

```
File ..MSCHEM\12-14\161214-GRB045-014_PK21-18138.D Tgt Mass (EZX): 584.71
Injection Date    : 16 Dec 14    9:01 am -0500          Seq. Line : 0
Sample Name       : GRB045-014_pk2               Location  : P2-A-12
Acq. Operator     : M. S. Chemist                          Inj : 1
Spec. Reported    : UV Integration                 Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NP.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NP.M
Sample Info : Easy-Access Method: 'SUBMISSION'  584.71
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
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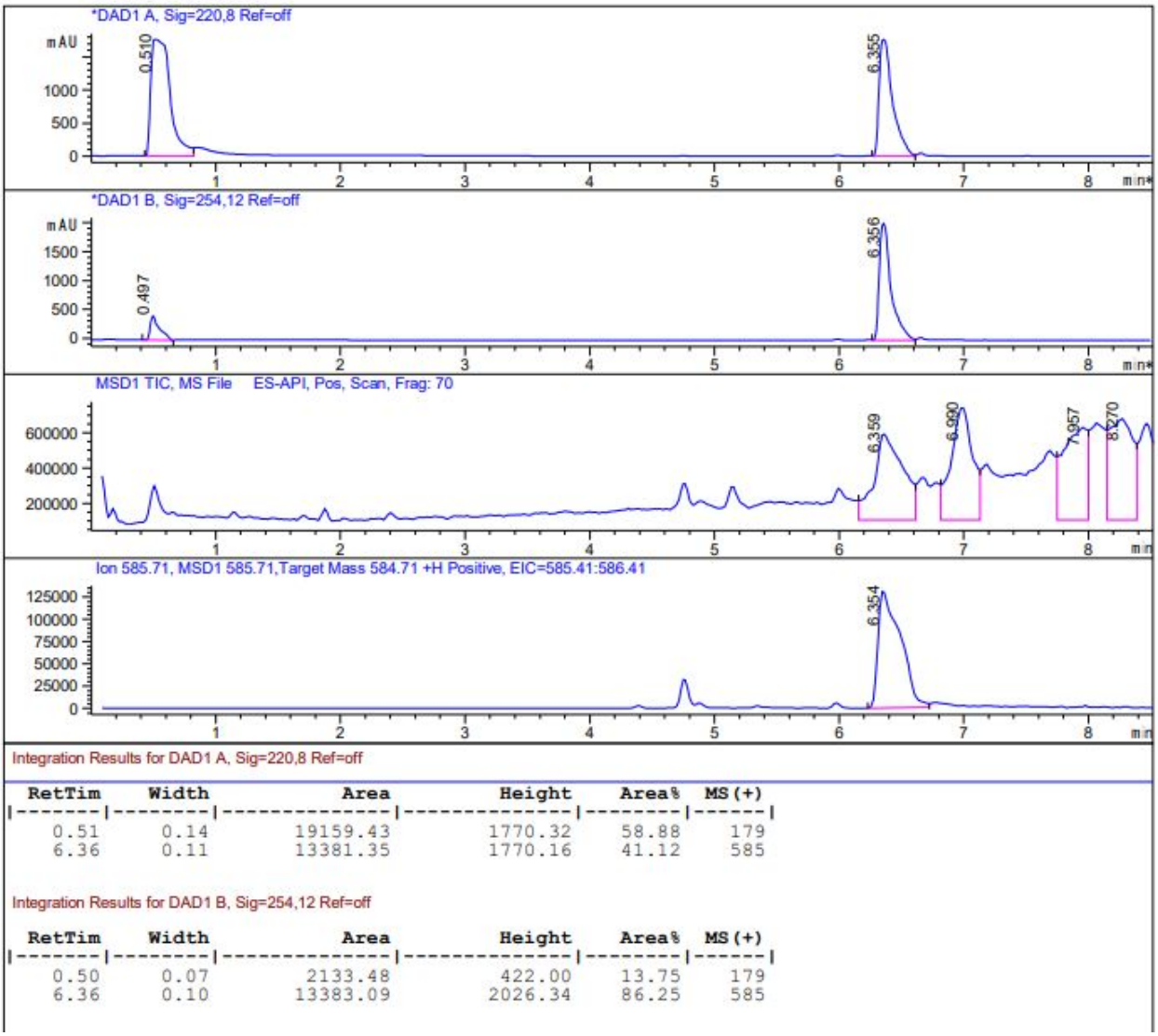
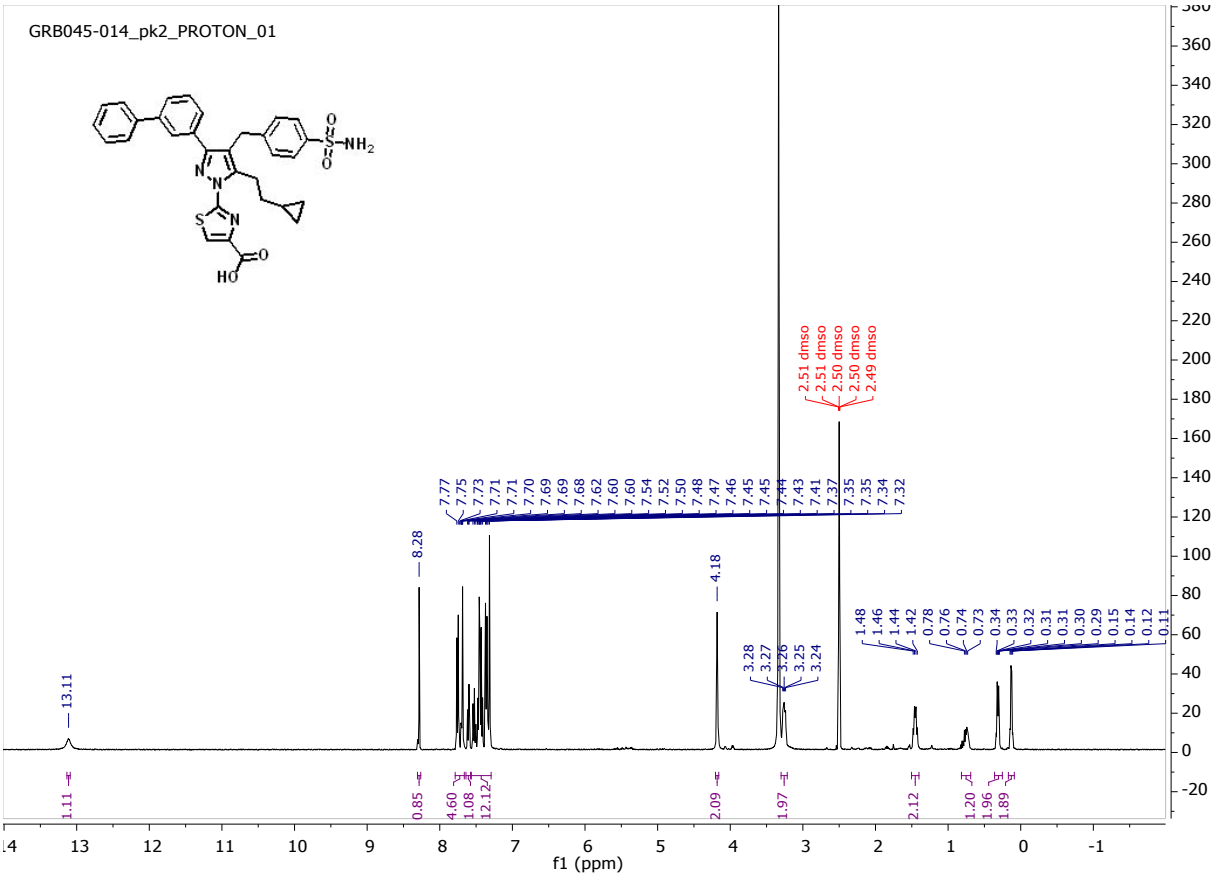


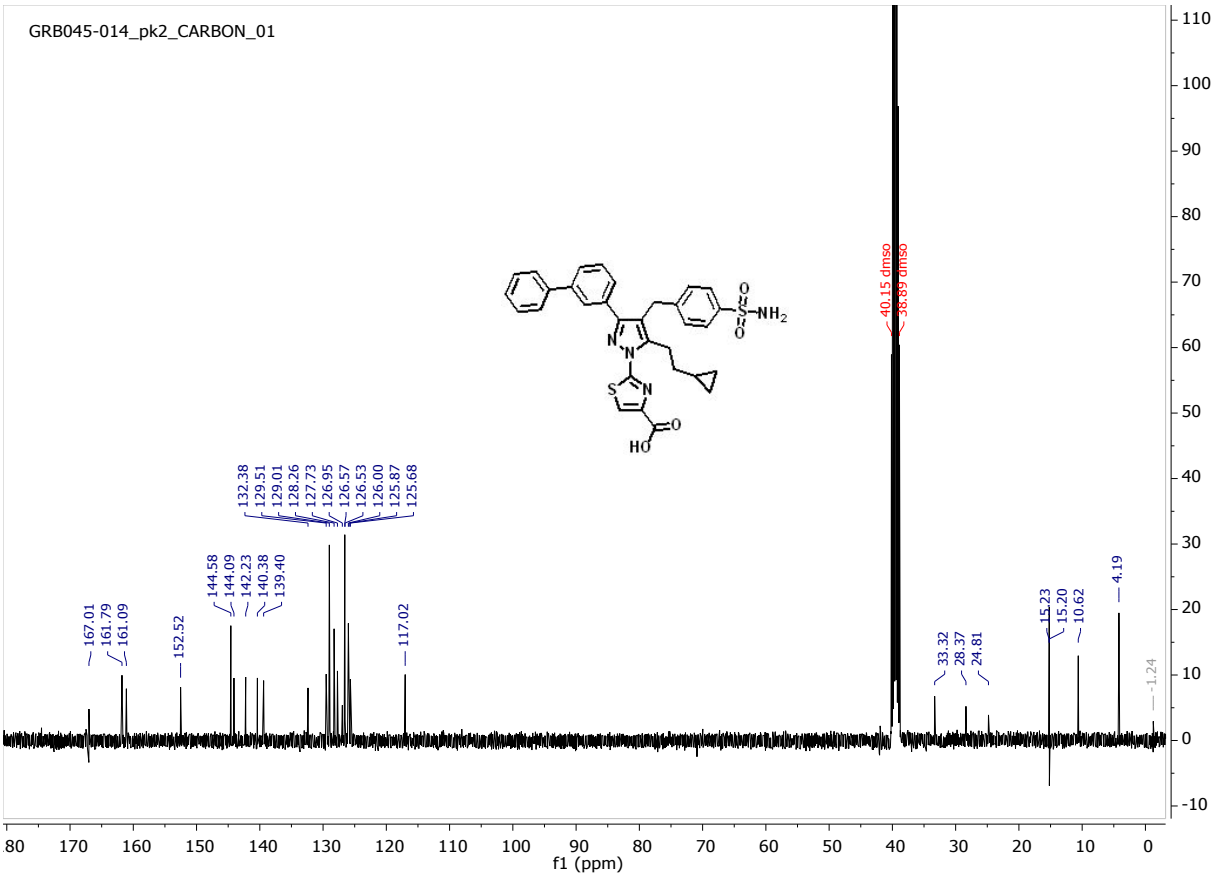
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.51	0.14	19159.43	1770.32	58.88	179
6.36	0.11	13381.35	1770.16	41.12	585

Integration Results for DAD1 B, Sig=254,12 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.50	0.07	2133.48	422.00	13.75	179
6.36	0.10	13383.09	2026.34	86.25	585


GRB045-014_pk2_PROTON_01
13.11
8.28
7.77
7.75
7.73
7.71
7.71
7.70
7.69
7.69
7.68
7.62
7.60
7.60
7.54
7.52
7.50
7.48
7.47
7.46
7.45
7.45
7.44
7.43
7.41
7.37
7.35
7.35
7.34
7.32
4.18
3.28
3.27
3.26
3.25
3.24
2.51 dmso
2.51 dmso
2.50 dmso
2.50 dmso
2.49 dmso
1.48
1.46
1.44
1.42
0.78
0.76
0.74
0.73
0.34
0.33
0.32
0.31
0.31
0.30
0.29
0.15
0.14
0.12
0.11
1.11
0.85
4.60
1.08
1.21
2.09
1.97
2.12
1.20
1.96
1.89
f1 (ppm)



LCMS, proton and carbon NMR for compound 10

```
File ..RAW\MSCHEM\05-15\050515-GRB048-0681-22157.D Tgt Mass (EZX): 606.66
Injection Date    :  5 May 15   3:19 pm -0500          Seq. Line : 0
Sample Name       : GRB048-068                    Location  : P2-A-08
Acq. Operator     : M. S. Chemist                           Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : Easy-Access Method: 'SUBMISSION'  606.66
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
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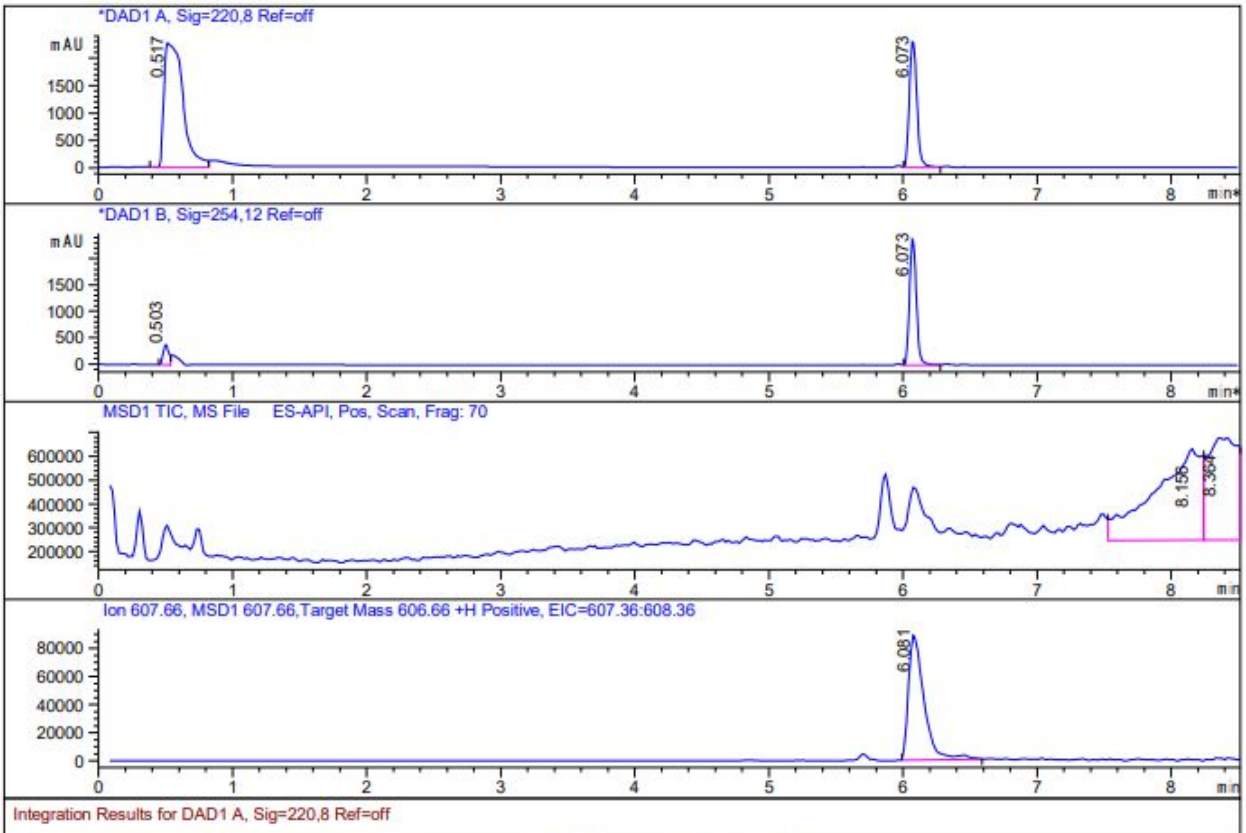
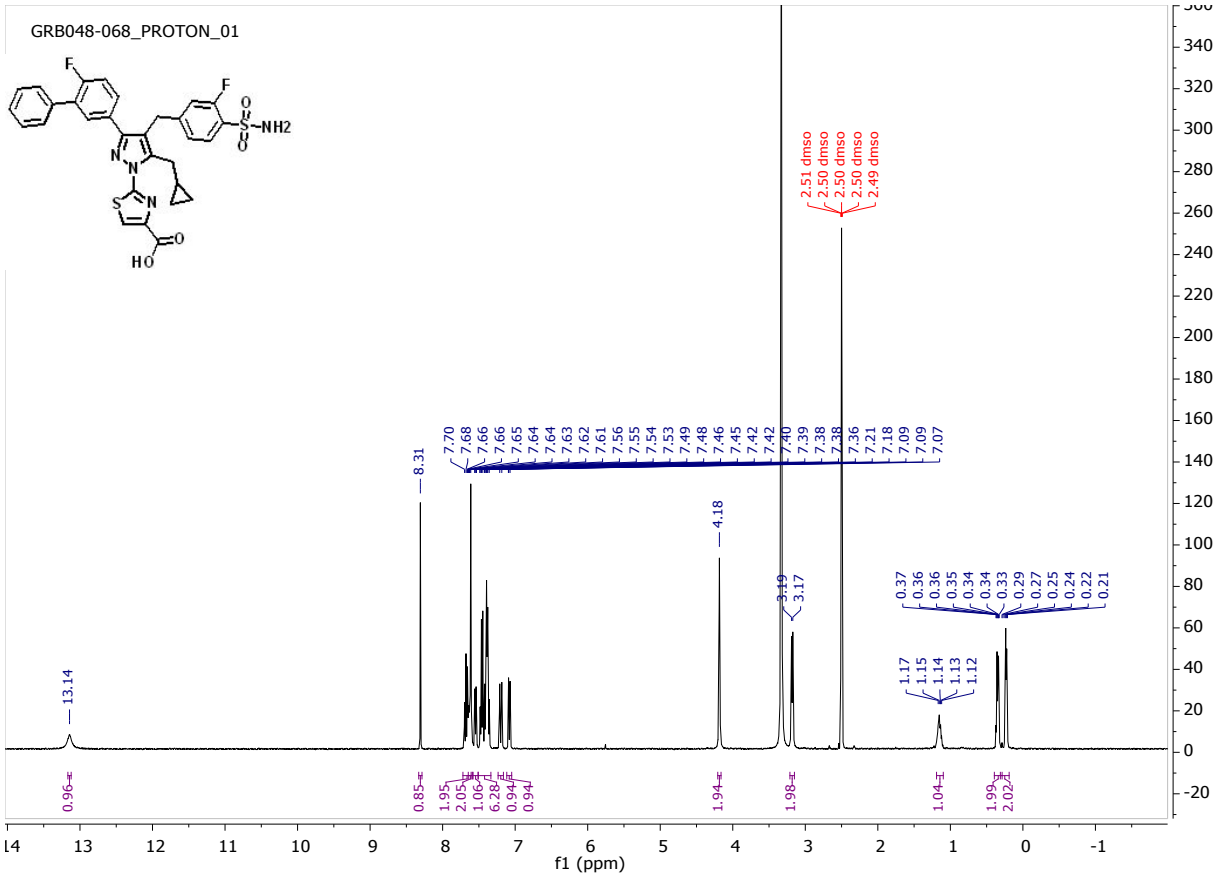


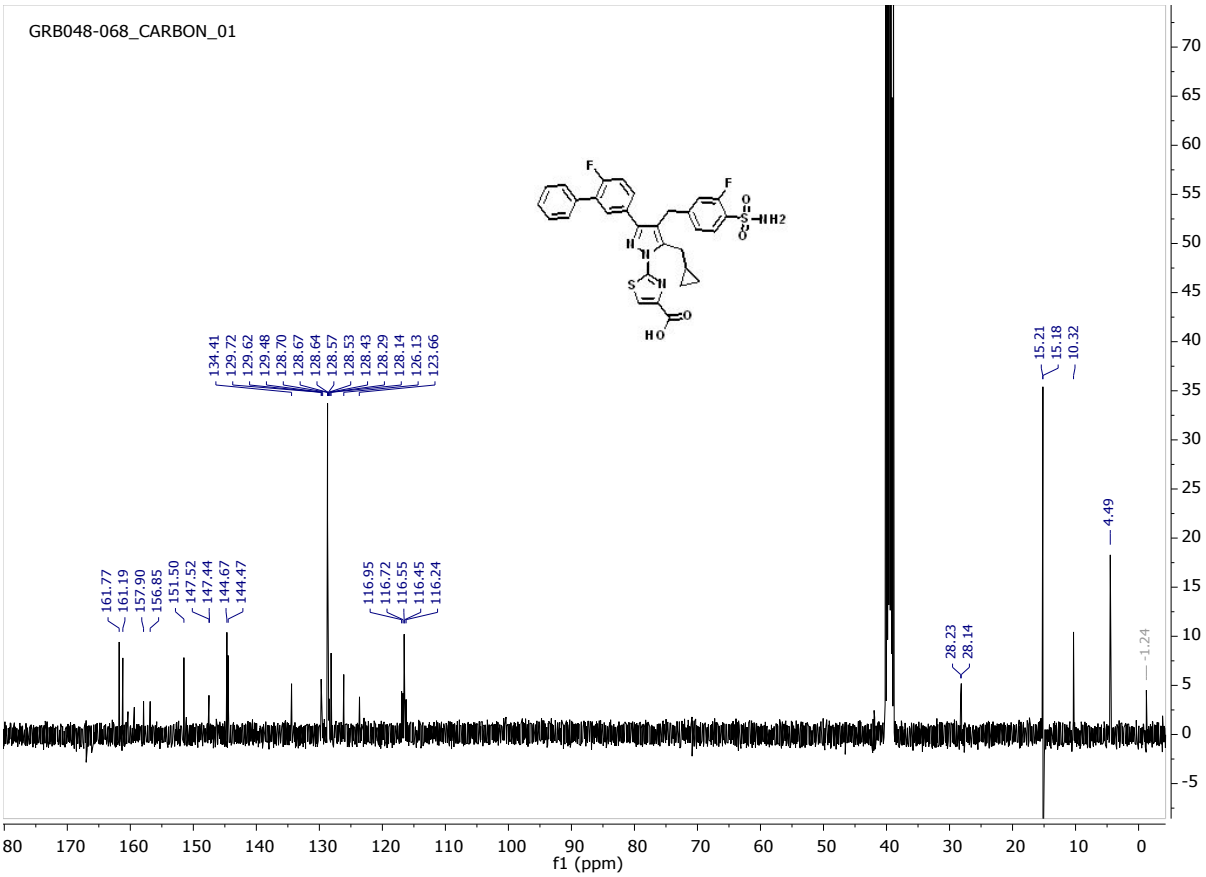
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.52	0.13	22419.12	2267.75	71.11	179
6.07	0.06	9108.02	2303.65	28.89	607

Integration Results for DAD1 B, Sig=254,12 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.50	0.05	1138.32	384.21	11.67	179
6.07	0.06	8620.12	2397.59	88.33	607


GRB048-068_PROTON_01
F
F
O
S
NH2
O
N
N
S
N
O
HO
2.51 dmso
2.50 dmso
2.50 dmso
2.50 dmso
2.49 dmso
8.31
7.70
7.68
7.66
7.66
7.65
7.64
7.64
7.63
7.62
7.61
7.56
7.55
7.54
7.53
7.49
7.48
7.46
7.45
7.42
7.42
7.40
7.39
7.38
7.38
7.36
7.21
7.18
7.09
7.09
7.07
4.18
3.19
3.17
13.14
0.37
0.36
0.36
0.35
0.34
0.34
0.33
0.29
0.27
0.25
0.24
0.22
0.21
1.17
1.15
1.14
1.13
1.12
0.96
0.85
1.95
2.05
1.06
6.28
0.94
0.94
1.94
1.98
1.04
1.99
2.02
f1 (ppm)



LCMS, proton and carbon NMR for compound 21

```
File ..RAW\MSCHEM\10-15\281015-GRB052-0041-27864.D Tgt Mass (EZX): 594.65
Injection Date    : 28 Oct 15   1:46 pm -0500          Seq. Line : 0
Sample Name       : GRB052-004                    Location  : P1-A-08
Acq. Operator     : M. S. Chemist                           Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : Easy-Access Method: 'SUBMISSION'  594.65
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
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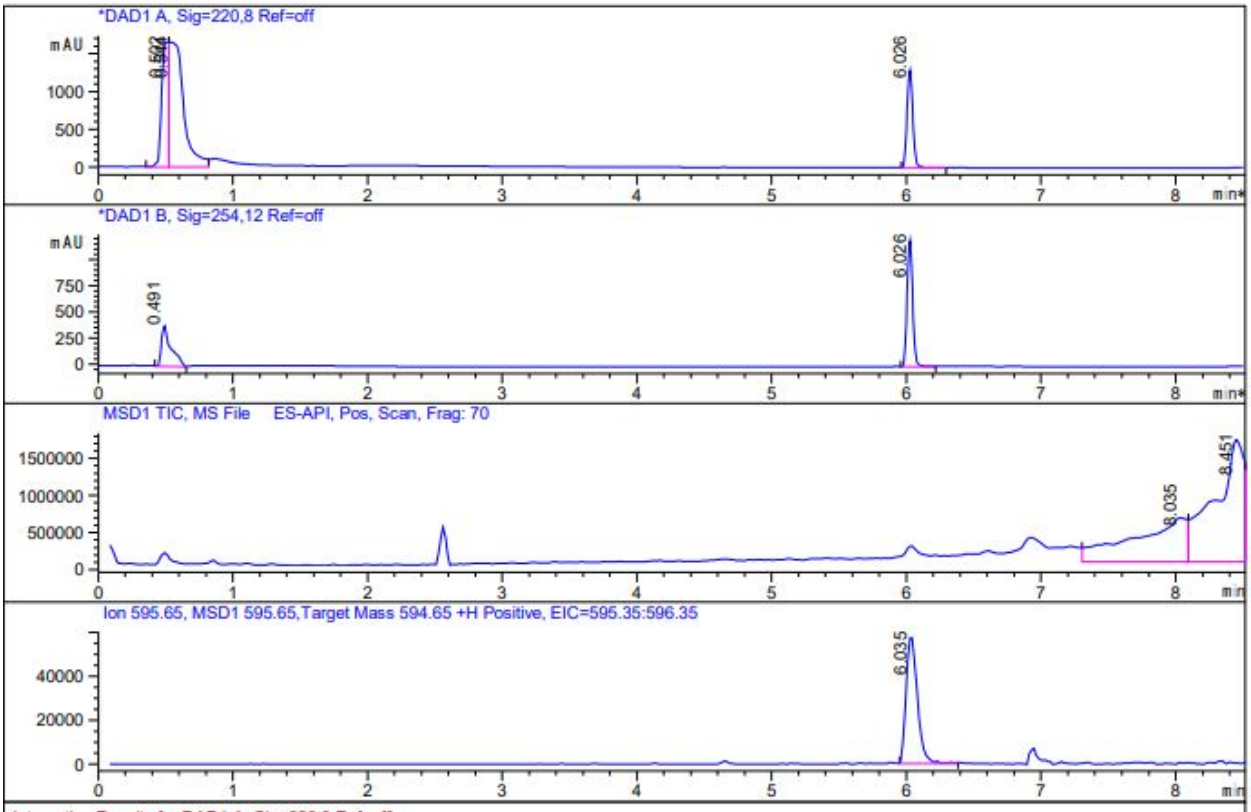
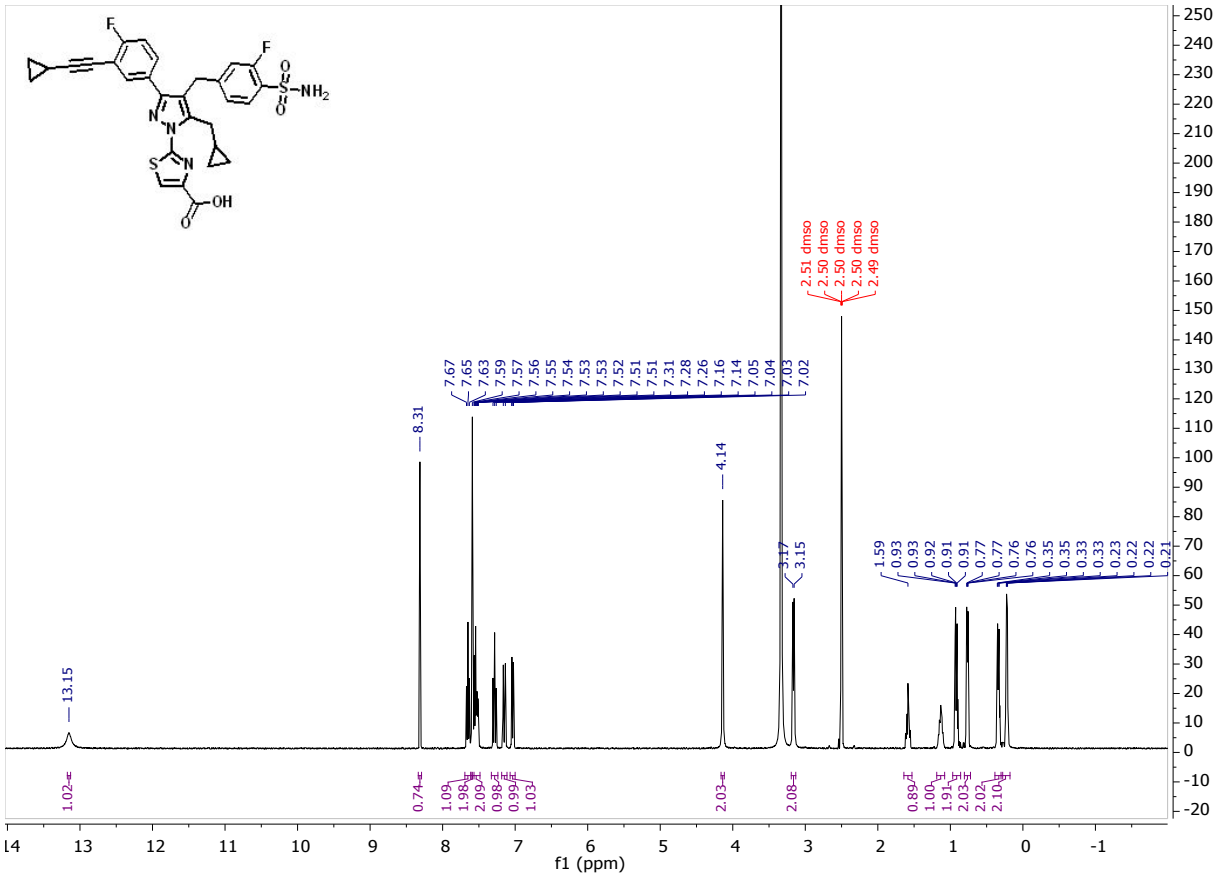


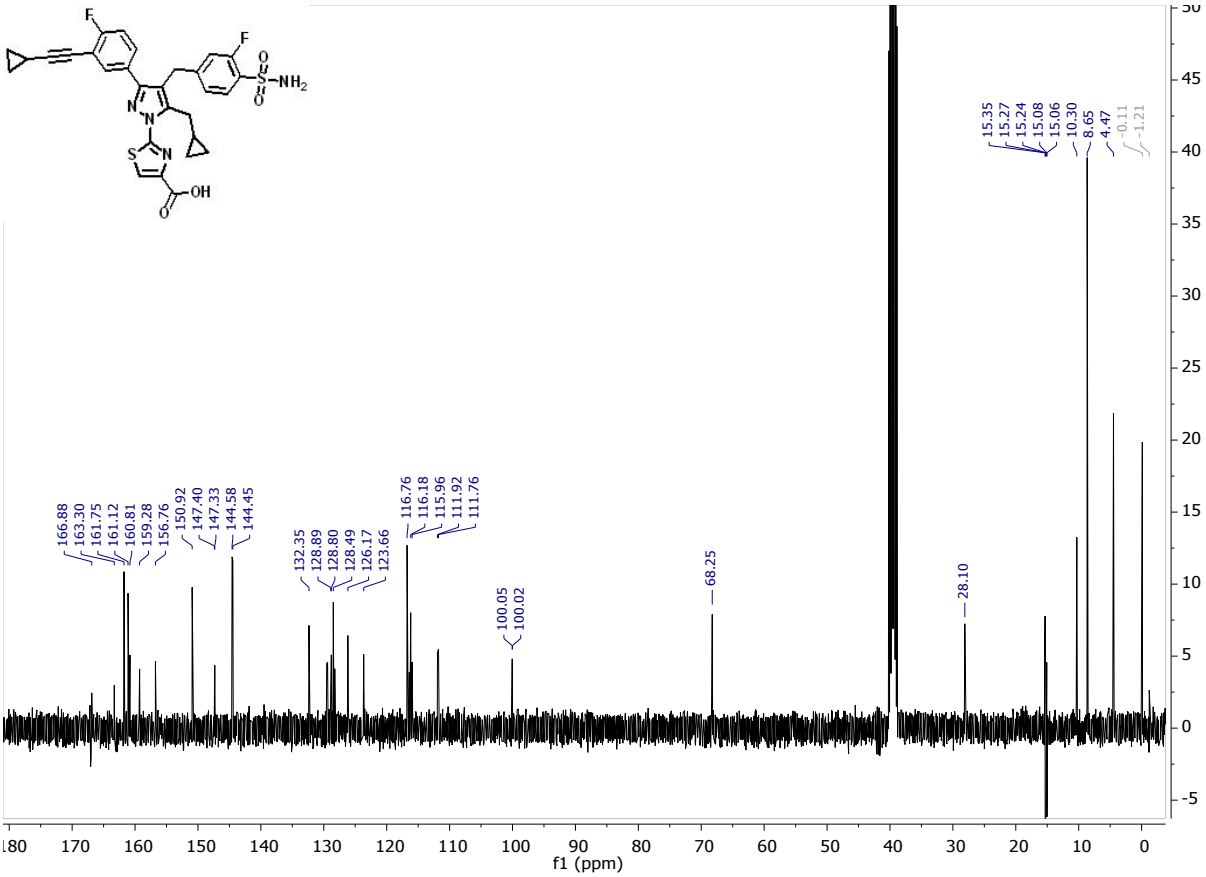
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.50	0.05	5711.87	1658.70	25.98	179
0.54	0.09	12344.24	1651.44	56.14	179
6.03	0.05	3931.44	1295.61	17.88	595

Integration Results for DAD1 B, Sig=254,12 Ref=off

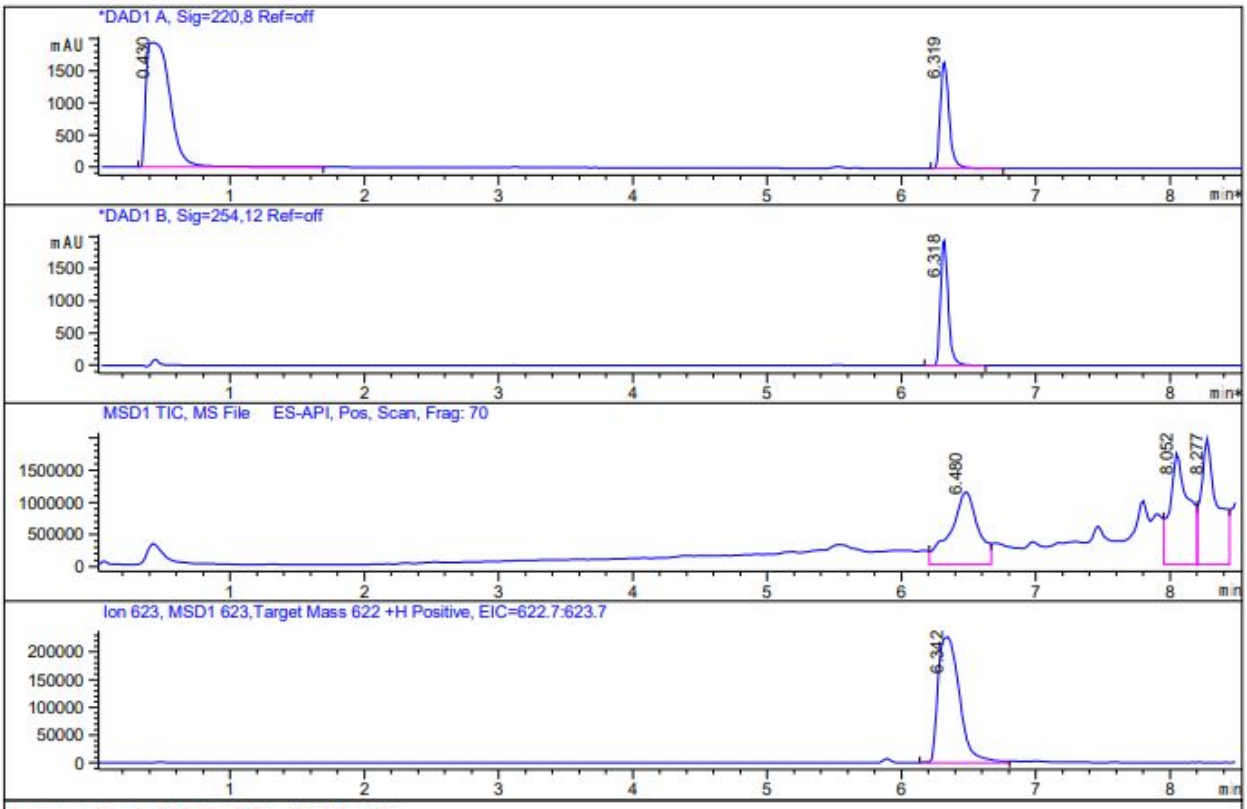
RetTim	Width	Area	Height	Area%	MS(+)
0.49	0.07	1909.57	385.11	34.87	179
6.03	0.05	3567.01	1209.70	65.13	595


F
F
O
S
O
NH2
N
N
S
N
OH
O
13.15
8.31
4.14
3.17
3.15
2.51 dmso
2.50 dmso
2.50 dmso
2.50 dmso
2.49 dmso
7.67
7.65
7.63
7.59
7.57
7.56
7.55
7.54
7.53
7.53
7.52
7.51
7.51
7.31
7.28
7.26
7.16
7.14
7.05
7.04
7.03
7.02
1.59
0.93
0.93
0.92
0.91
0.91
0.77
0.77
0.76
0.76
0.35
0.35
0.33
0.33
0.23
0.22
0.22
0.21
1.02
0.74
1.09
1.98
2.09
0.98
0.99
1.03
2.03
2.08
0.89
1.00
1.91
2.03
2.02
2.10
f1 (ppm)



LCMS, proton and carbon NMR for compound 23

```
File ..ANTUKALLUG\10-15\141015-GRB051-0931-15214.D Tgt Mass (EZX): 622.00
Injection Date    : 14 Oct 15   8:41 am -0500          Seq. Line : 0
Sample Name       : GRB051-093                    Location  : Vial 31
Acq. Operator     : Ganesha Rai                          Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : PURE IN VIAL  000001LTQ  Easy-Access Method: 'SUBMISSION'  622.00
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
```

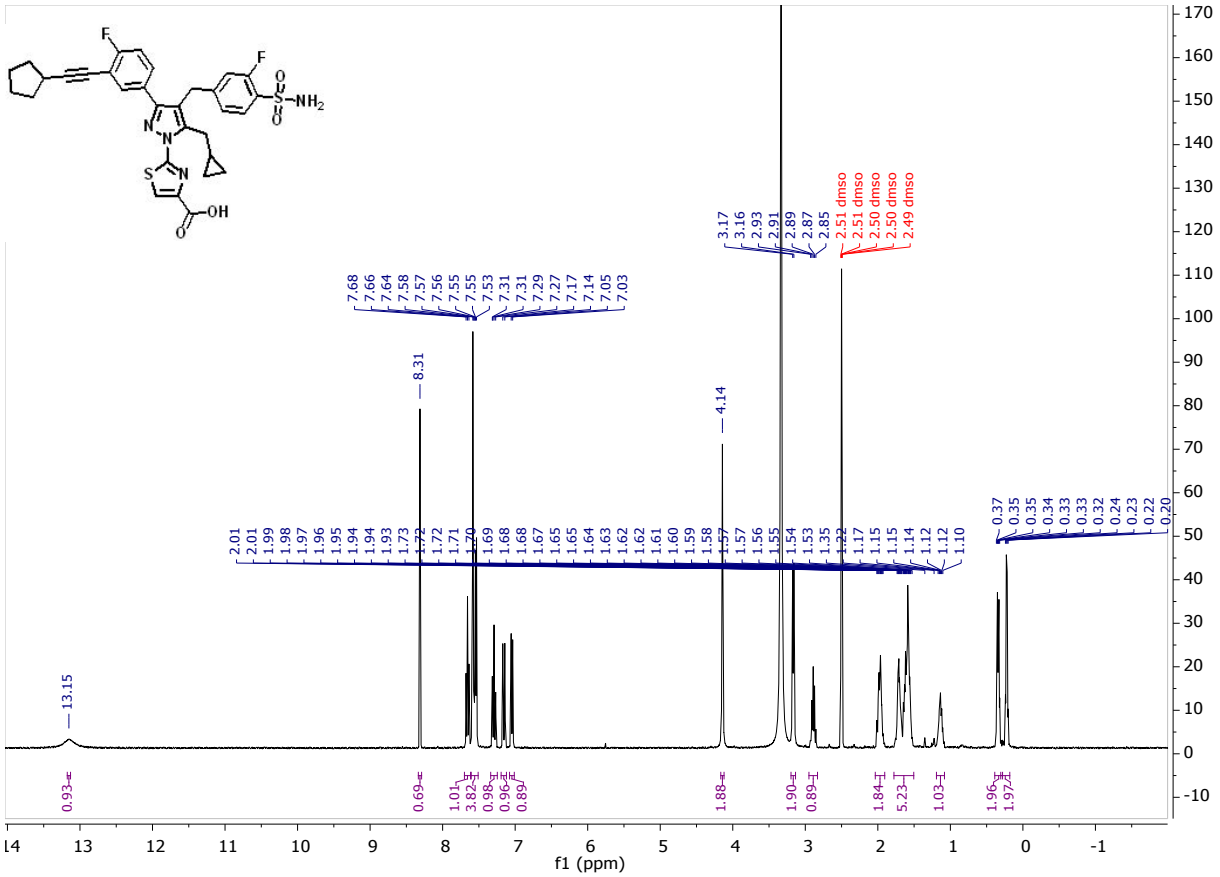


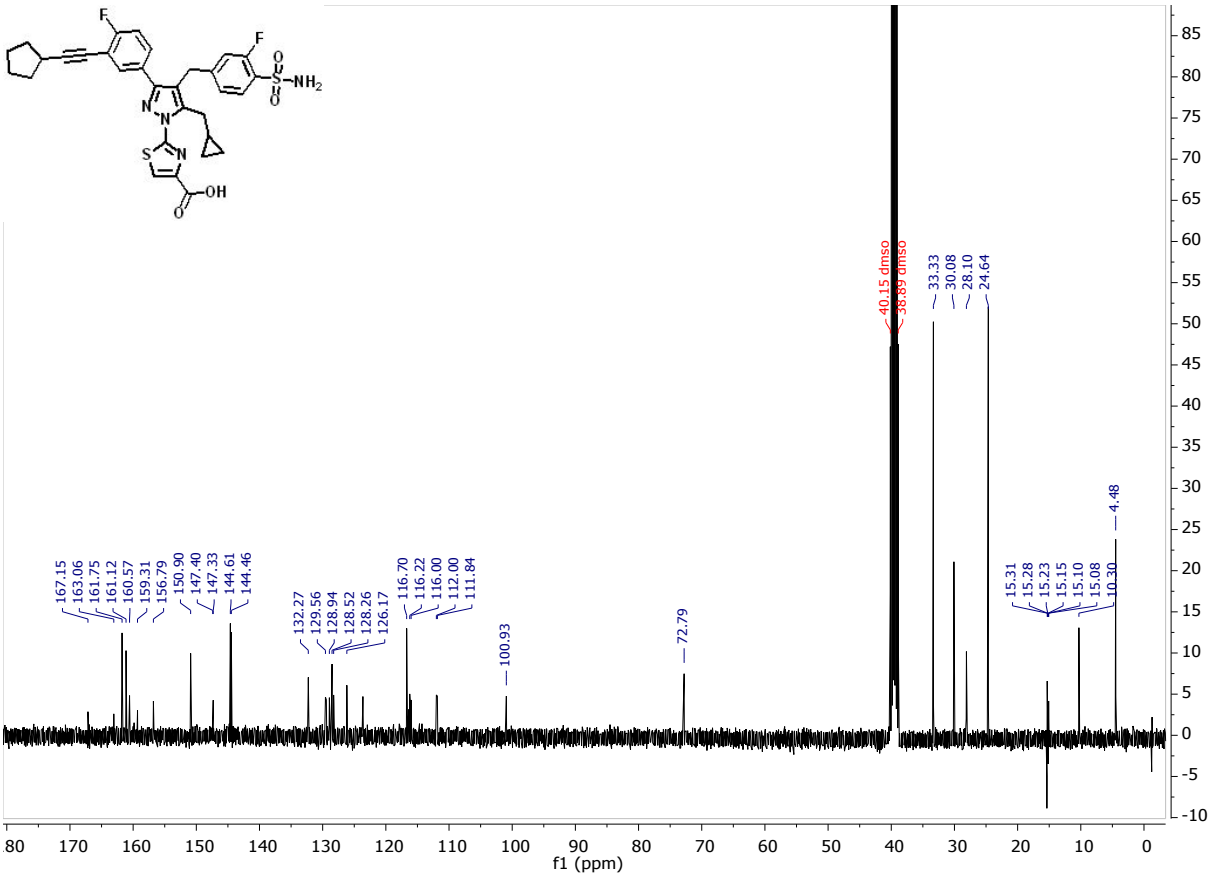
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.43	0.20	23781.88	1941.75	75.73	179
6.32	0.07	7620.09	1650.08	24.27	623

Integration Results for DAD1 B, Sig=254,12 Ref=off

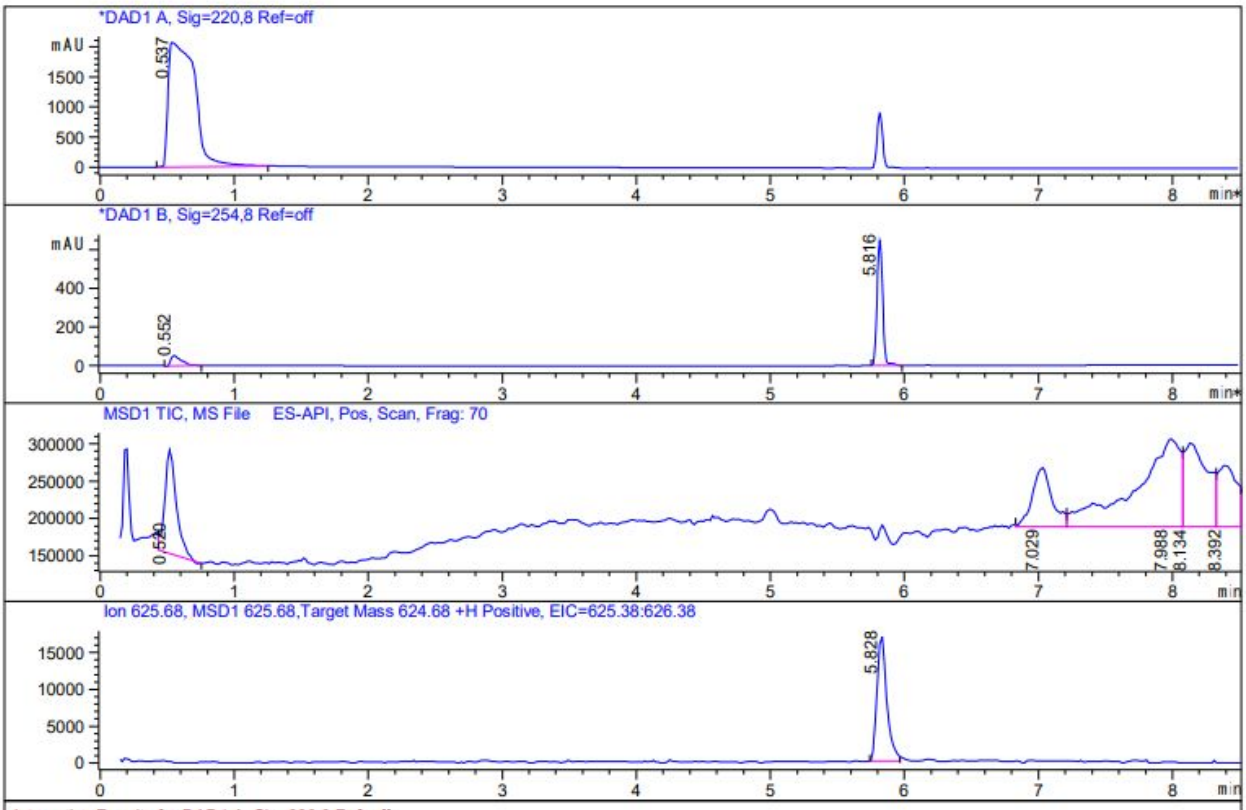
RetTim	Width	Area	Height	Area%	MS(+)
6.32	0.06	7795.78	1947.98	100.00	623


F
F
O
S
NH2
O
N
N
S
N
OH
O
7.68
7.66
7.64
7.58
7.57
7.56
7.55
7.55
7.53
7.31
7.31
7.29
7.27
7.17
7.14
7.05
7.03
3.17
3.16
2.93
2.91
2.89
2.87
2.85
2.51 dmso
2.51 dmso
2.50 dmso
2.50 dmso
2.49 dmso
8.31
4.14
13.15
f1 (ppm)



LCMS, proton and carbon NMR for compound 29

```
File ..RAW\MSCHEM\09-15\160915-GRB051-0321-19969.D Tgt Mass (EZX): 624.68
Injection Date    : 16 Sep 15  12:35 pm -0500          Seq. Line : 0
Sample Name       : GRB051-032                    Location  : P1-F-04
Acq. Operator     : M. S. Chemist                           Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : Easy-Access Method: 'SUBMISSION'  624.68
Method Info : Long Gradient 4% to 100% ACN over 7 minutes (0.05%TFA)
              Luna  C18 3.0 x 75 mm
```

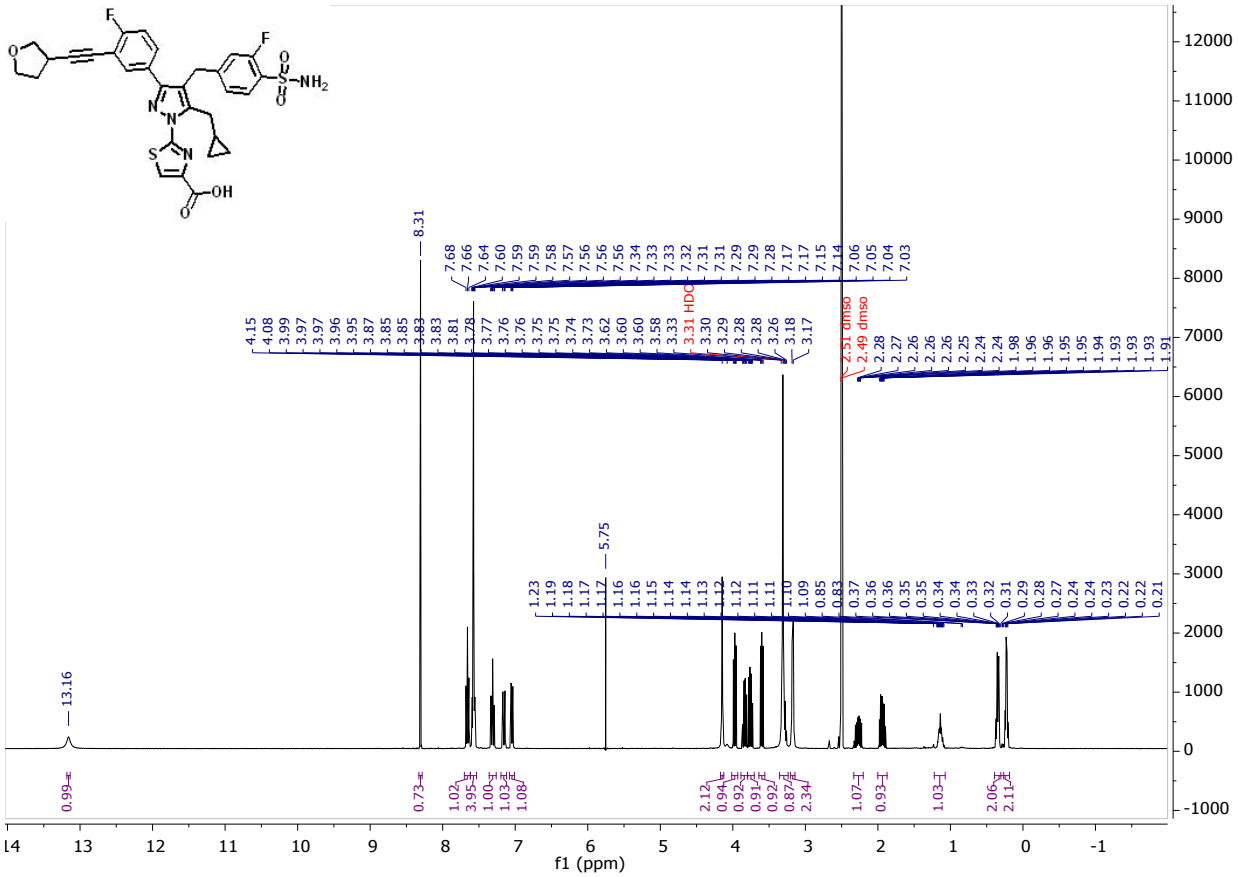


Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.54	0.18	28509.21	2071.35	100.00	225

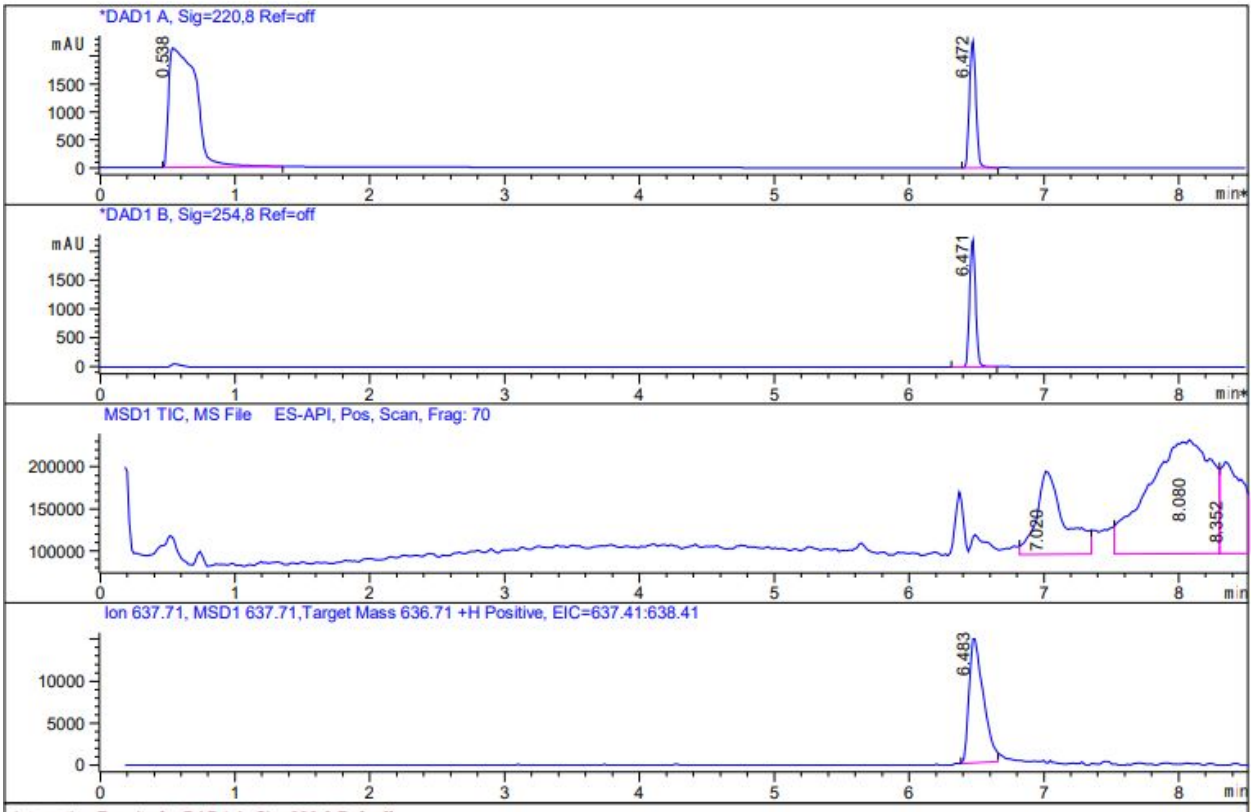
Integration Results for DAD1 B, Sig=254,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.55	0.08	298.22	52.82	13.48	179
5.82	0.05	1914.09	651.66	86.52	625



LCMS, proton and carbon NMR for compound 42

```
File ..RAW\MSCHEM\08-15\180815-GRB050-0581-19488.D Tgt Mass (EZX): 636.71
Injection Date    : 18 Aug 15  11:44 am -0500          Seq. Line : 0
Sample Name       : GRB050-058                    Location  : P2-B-01
Acq. Operator     : M. S. Chemist                           Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NP.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NP.M
Sample Info : Easy-Access Method: 'SUBMISSION'  636.71
Method Info : Long Gradient 4% to 100% ACN over 7 minutes (0.05%TFA)
              Luna  C18 3.0 x 75 mm
```

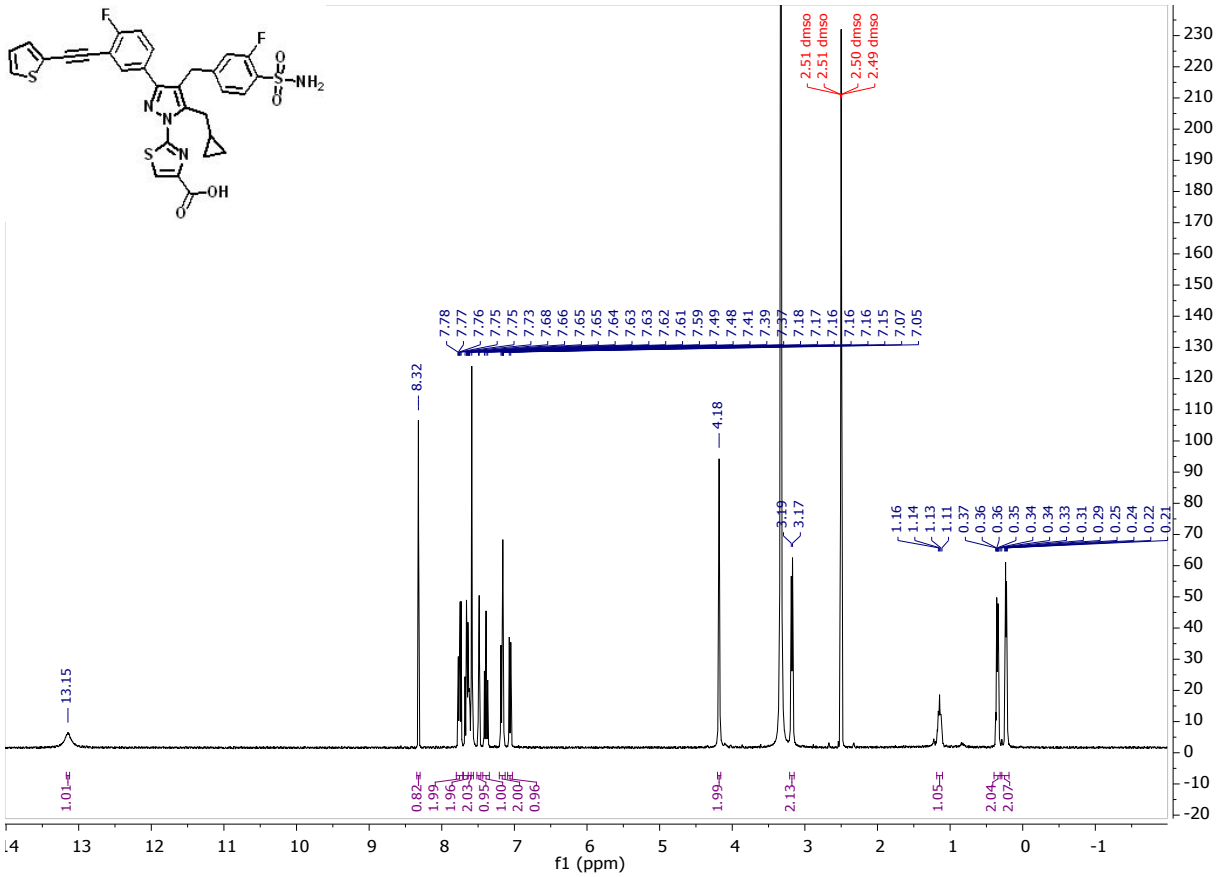


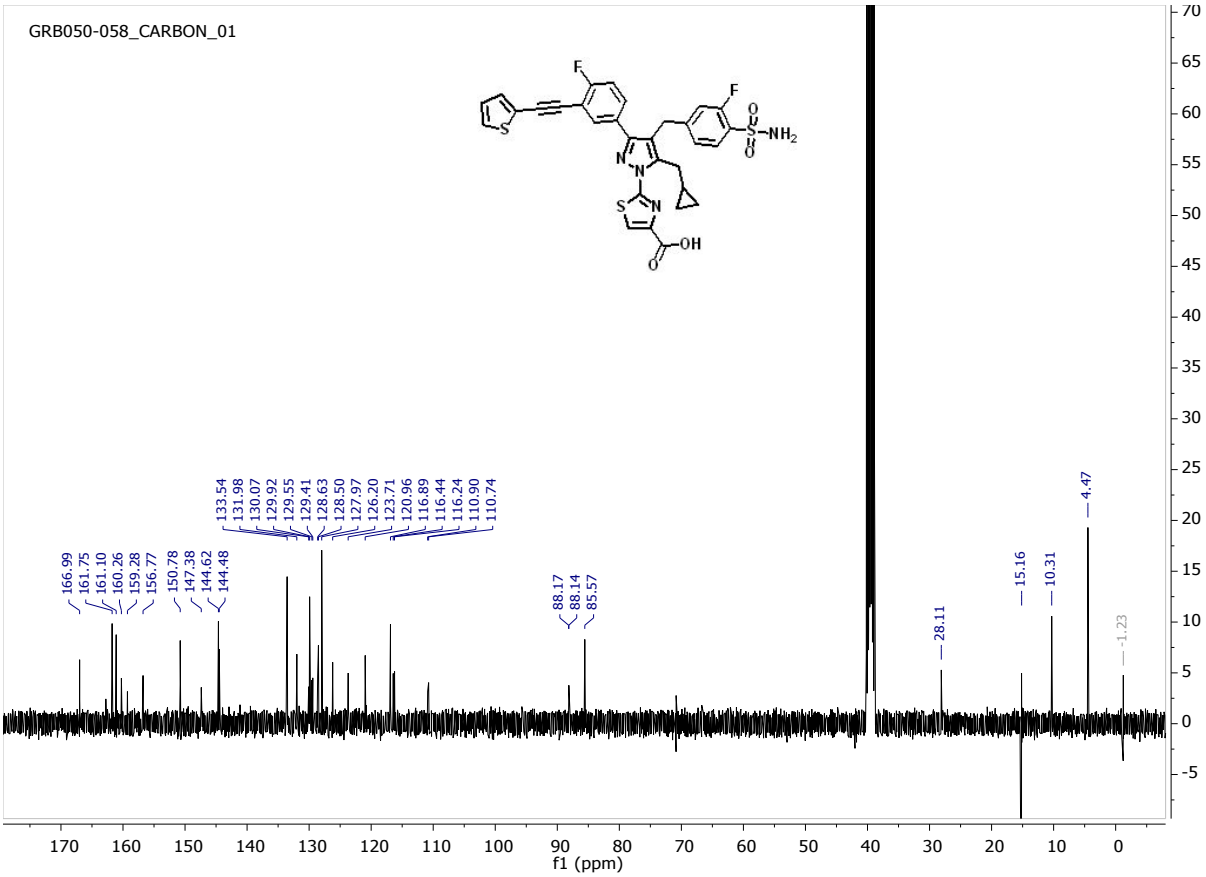
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.54	0.18	29650.13	2129.36	79.45	179
6.47	0.05	7667.35	2263.50	20.55	637

Integration Results for DAD1 B, Sig=254,8 Ref=off

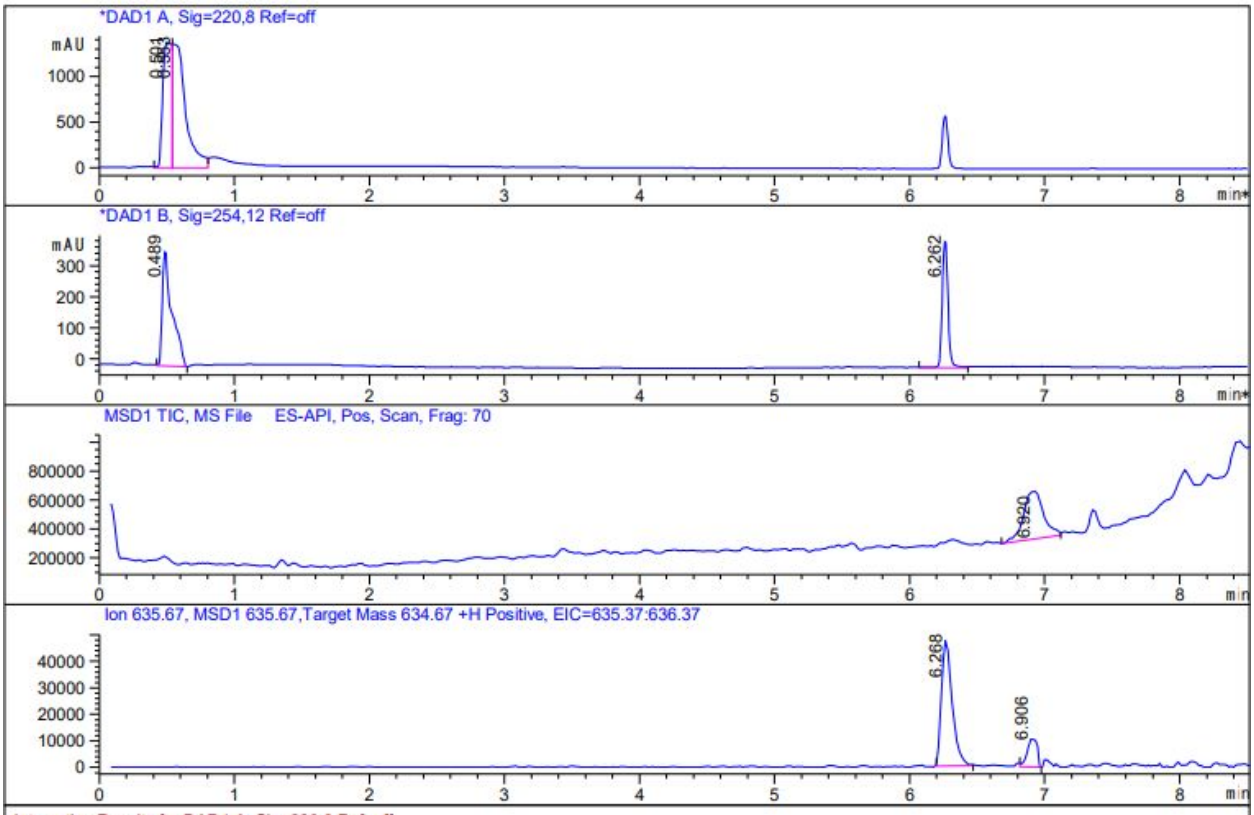
RetTim	Width	Area	Height	Area%	MS(+)
6.47	0.05	6814.04	2192.51	100.00	637


F
F
O
S
NH2
O
N
N
S
N
OH
O
2.51 dmso
2.51 dmso
2.50 dmso
2.49 dmso
7.78
7.77
7.76
7.75
7.75
7.73
7.68
7.66
7.65
7.65
7.64
7.63
7.63
7.62
7.61
7.59
7.49
7.48
7.41
7.39
7.37
7.18
7.17
7.16
7.16
7.16
7.15
7.07
7.05
8.32
4.18
3.19
3.17
1.16
1.14
1.13
1.11
0.37
0.36
0.36
0.35
0.34
0.34
0.33
0.31
0.29
0.25
0.24
0.22
0.21
13.15
1.01
0.82
1.99
1.96
2.03
0.95
1.00
2.00
0.96
1.99
2.13
1.05
2.04
2.07
f1 (ppm)



LCMS, proton and carbon NMR for compound 44

```
File ..RAW\MSCHEM\12-15\161215-GRB053-0301-29121.D Tgt Mass (EZX): 634.67
Injection Date    : 16 Dec 15   3:41 pm -0500          Seq. Line : 0
Sample Name       : GRB053-030                    Location  : P1-A-06
Acq. Operator     : M. S. Chemist                           Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : Easy-Access Method: 'SUBMISSION'  634.67
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
```

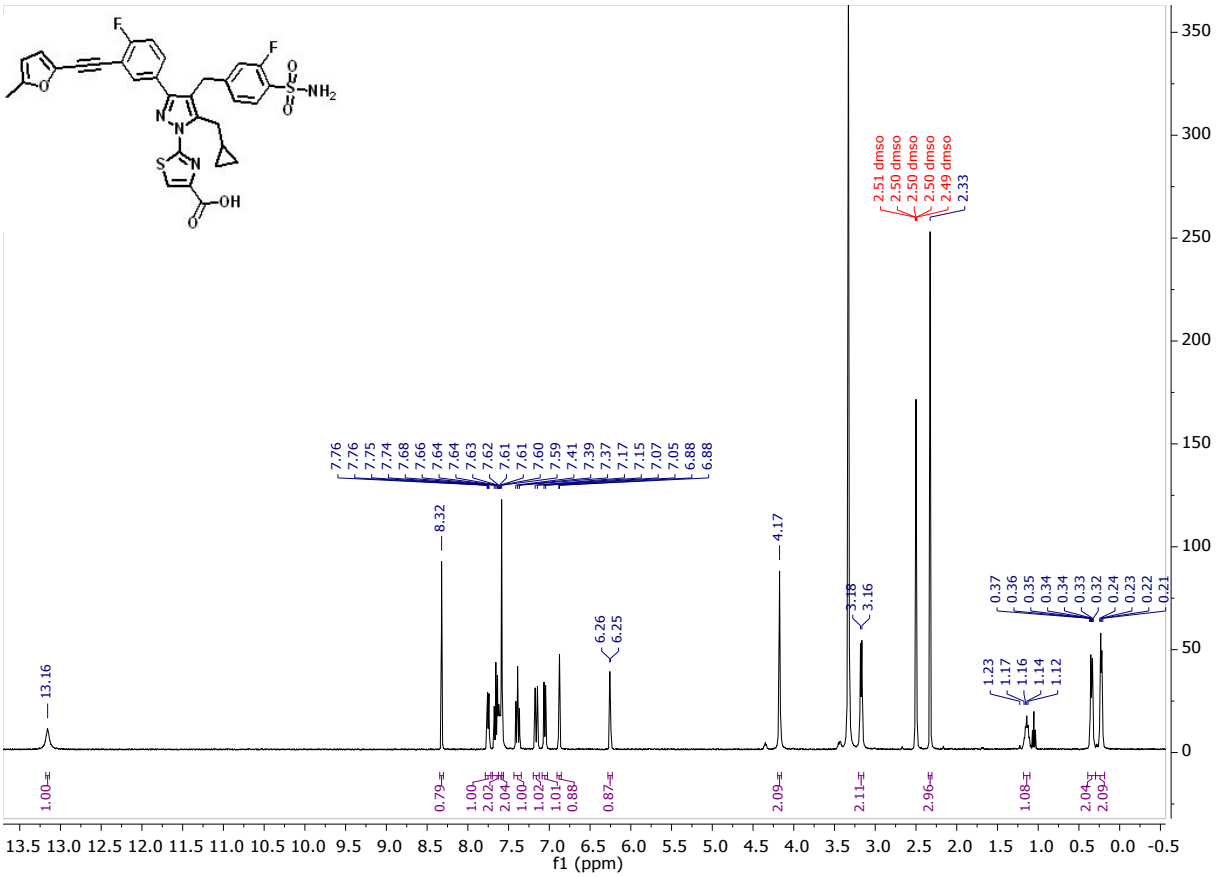


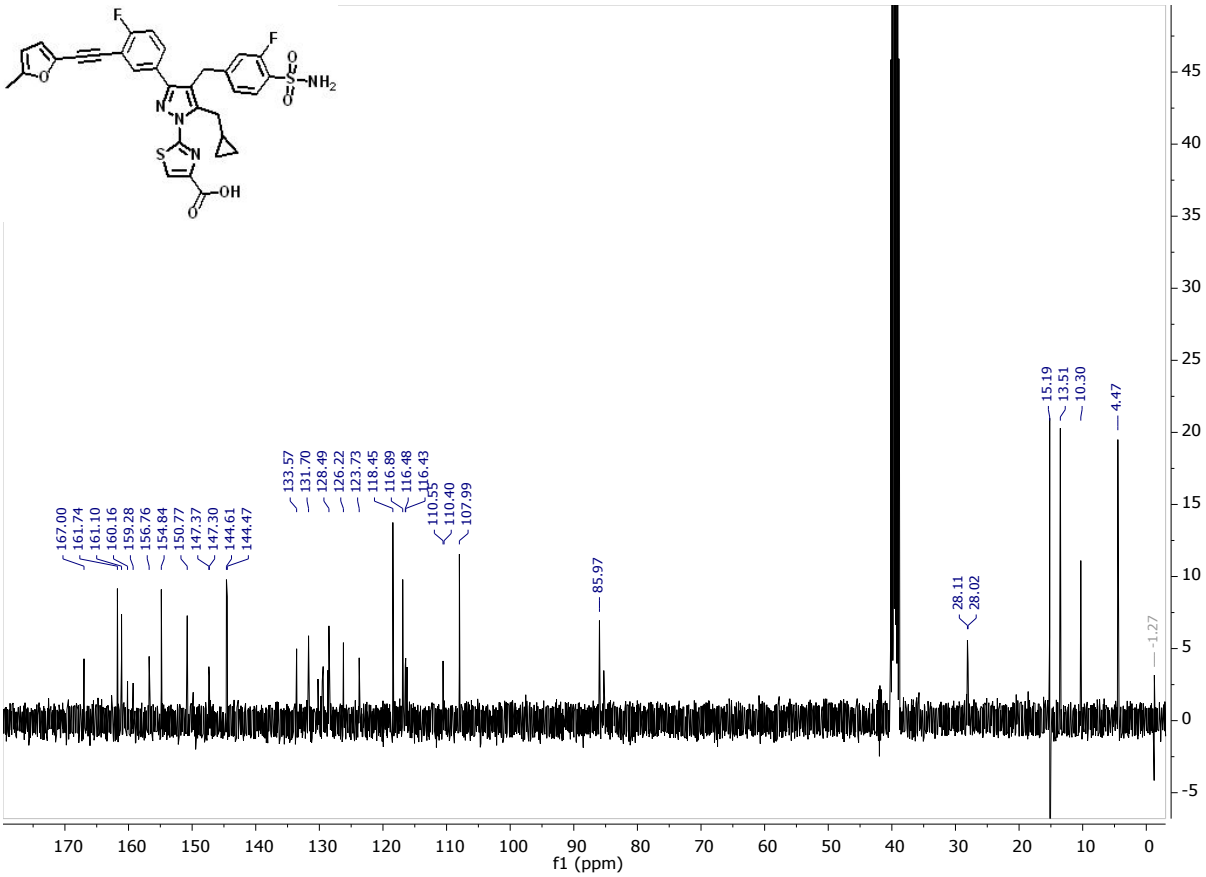
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.50	0.07	6303.15	1373.20	41.28	179
0.55	0.11	8967.60	1348.43	58.72	179

Integration Results for DAD1 B, Sig=254,12 Ref=off

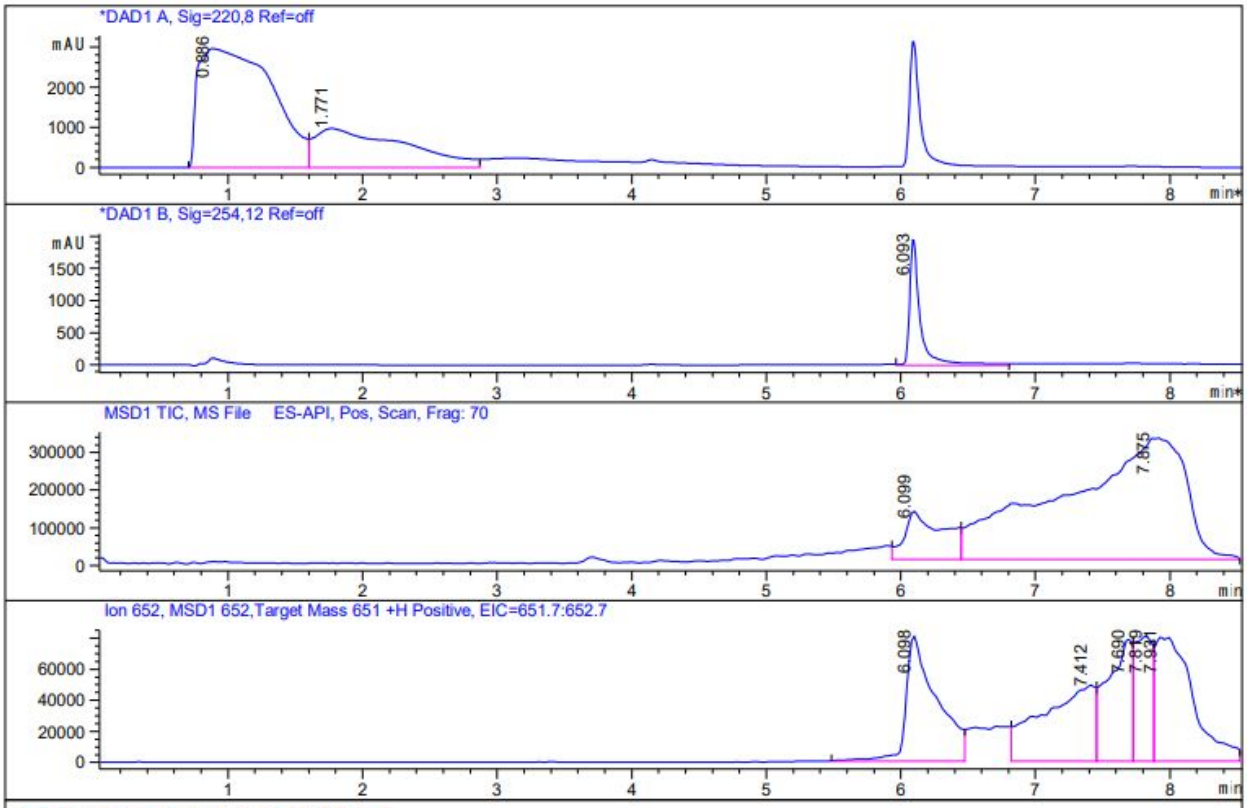
RetTim	Width	Area	Height	Area%	MS(+)
0.49	0.07	1848.79	369.43	59.54	179
6.26	0.05	1256.17	410.94	40.46	635


F
F
O
S
NH2
O
N
N
S
N
OH
O
13.16
8.32
7.76
7.76
7.75
7.74
7.68
7.66
7.64
7.64
7.63
7.62
7.61
7.61
7.60
7.59
7.41
7.39
7.37
7.17
7.15
7.07
7.05
6.88
6.88
6.26
6.25
4.17
3.18
3.16
2.51 dmso
2.50 dmso
2.50 dmso
2.50 dmso
2.49 dmso
2.33
1.23
1.17
1.16
1.14
1.12
0.37
0.36
0.35
0.34
0.34
0.33
0.32
0.24
0.23
0.22
0.21
1.00
0.79
1.00
2.02
2.04
1.00
1.02
1.01
0.88
0.87
2.09
2.11
2.96
1.08
2.04
2.09
f1 (ppm)



LCMS, proton and carbon NMR for compound 46

```
File ..ANTUKALLUG\07-16\270716-GRB056-0722-00436.D Tgt Mass (EZX): 651.00
Injection Date    : 27 Jul 16    9:14 am -0500          Seq. Line : 0
Sample Name       : GRB056-072                    Location  : P1-E-06
Acq. Operator     : Ganesha Rai                             Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : 000002UXA  Easy-Access Method: 'SUBMISSION'  651.00
Method Info : Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Agilent Eclipse XDB-C18 3 micron 3 x 75mm
```

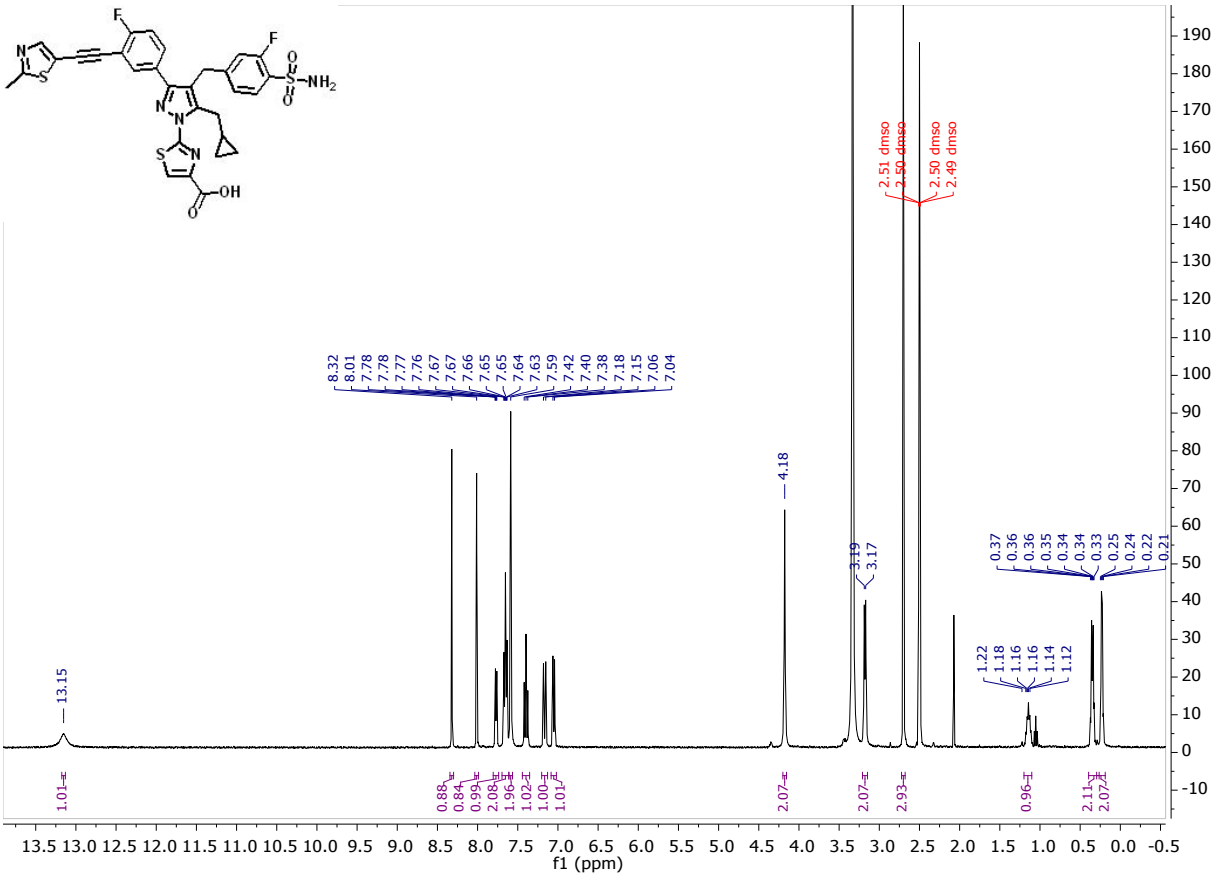


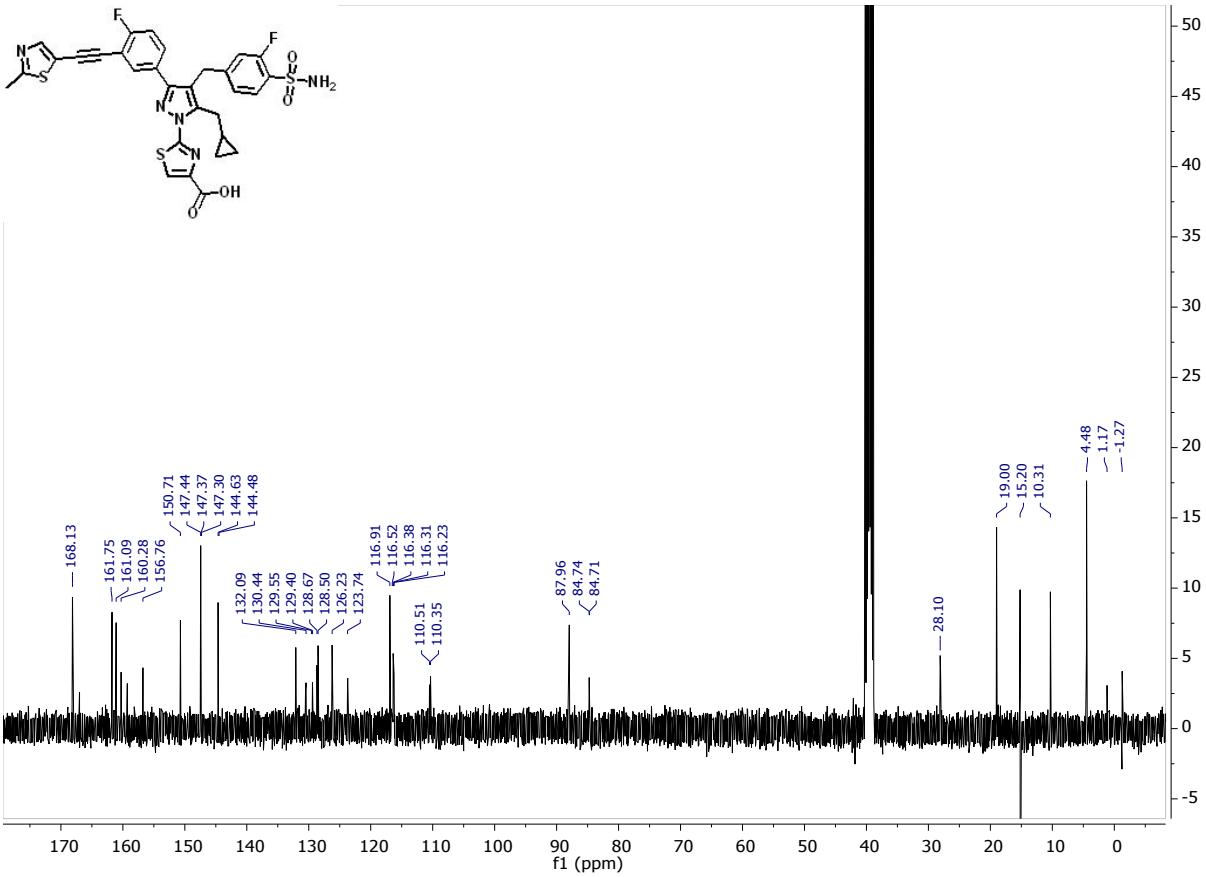
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.89	0.48	113278.52	2963.00	71.29	169
1.77	0.61	45623.04	976.04	28.71	ND

Integration Results for DAD1 B, Sig=254,12 Ref=off

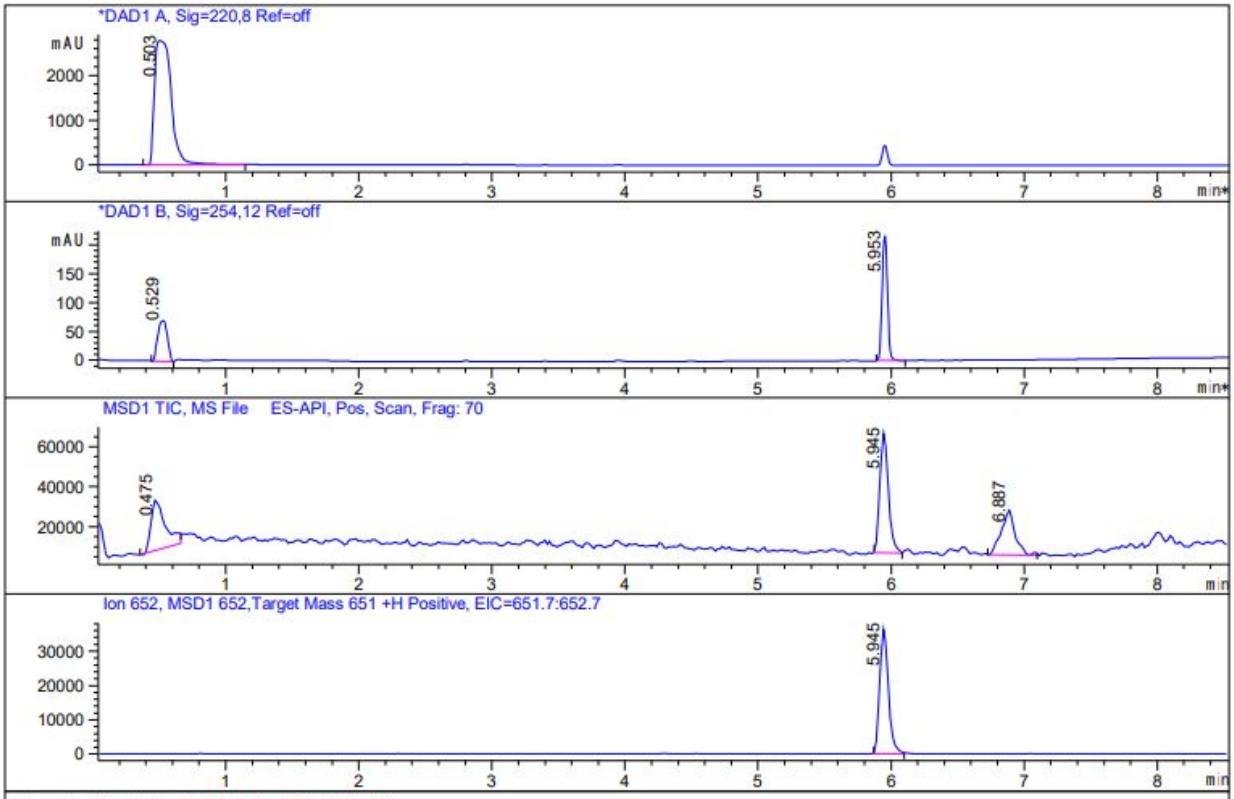
RetTim	Width	Area	Height	Area%	MS(+)
6.09	0.08	10660.35	1957.69	100.00	652


F
F
O
S
NH2
O
N
S
N
N
S
N
OH
O
8.32
8.01
7.78
7.78
7.77
7.76
7.67
7.67
7.66
7.65
7.65
7.64
7.63
7.59
7.42
7.40
7.38
7.18
7.15
7.06
7.04
13.15
4.18
3.19
3.17
2.51 dmso
2.50 dmso
2.50 dmso
2.49 dmso
1.22
1.18
1.16
1.16
1.14
1.12
0.37
0.36
0.36
0.35
0.34
0.34
0.33
0.25
0.24
0.22
0.21
1.01
0.88
0.84
0.99
2.08
1.96
1.02
1.00
1.01
2.07
2.07
2.93
0.96
2.11
2.07
f1 (ppm)



LCMS, proton and carbon NMR for compound 47

```
File ..ANTUKALLUG\07-16\250716-GRB056-0731-00345.D Tgt Mass (EZX): 651.00
Injection Date    : 25 Jul 16  11:30 am -0500          Seq. Line : 0
Sample Name       : GRB056-073                      Location  : P1-A-07
Acq. Operator     : Ganesha Rai                              Inj : 1
Spec. Reported    : UV Integration                  Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD.M
Sample Info : Easy-Access Method: 'FINAL_GRAD'  651.00
Method Info : Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Agilent Eclipse XDB-C18 3 micron 3 x 75mm
```

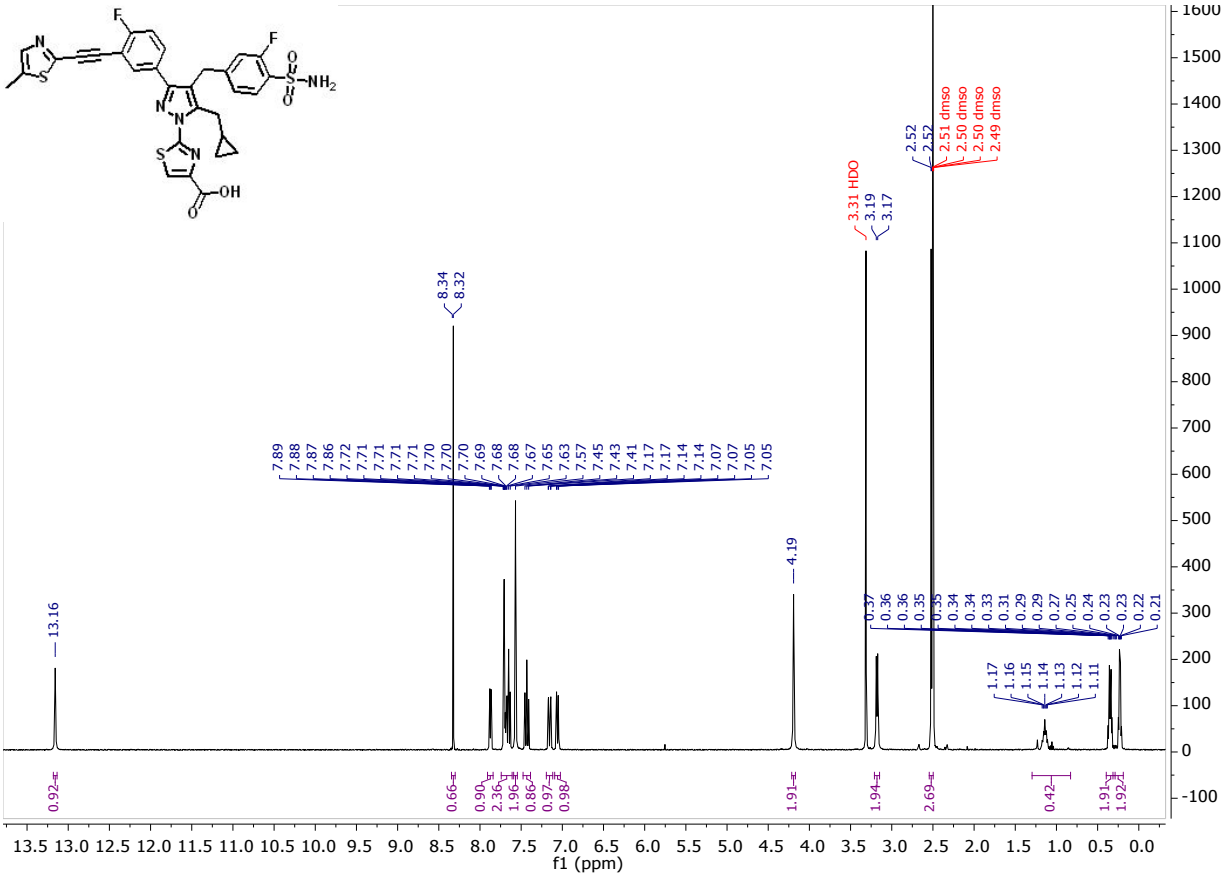


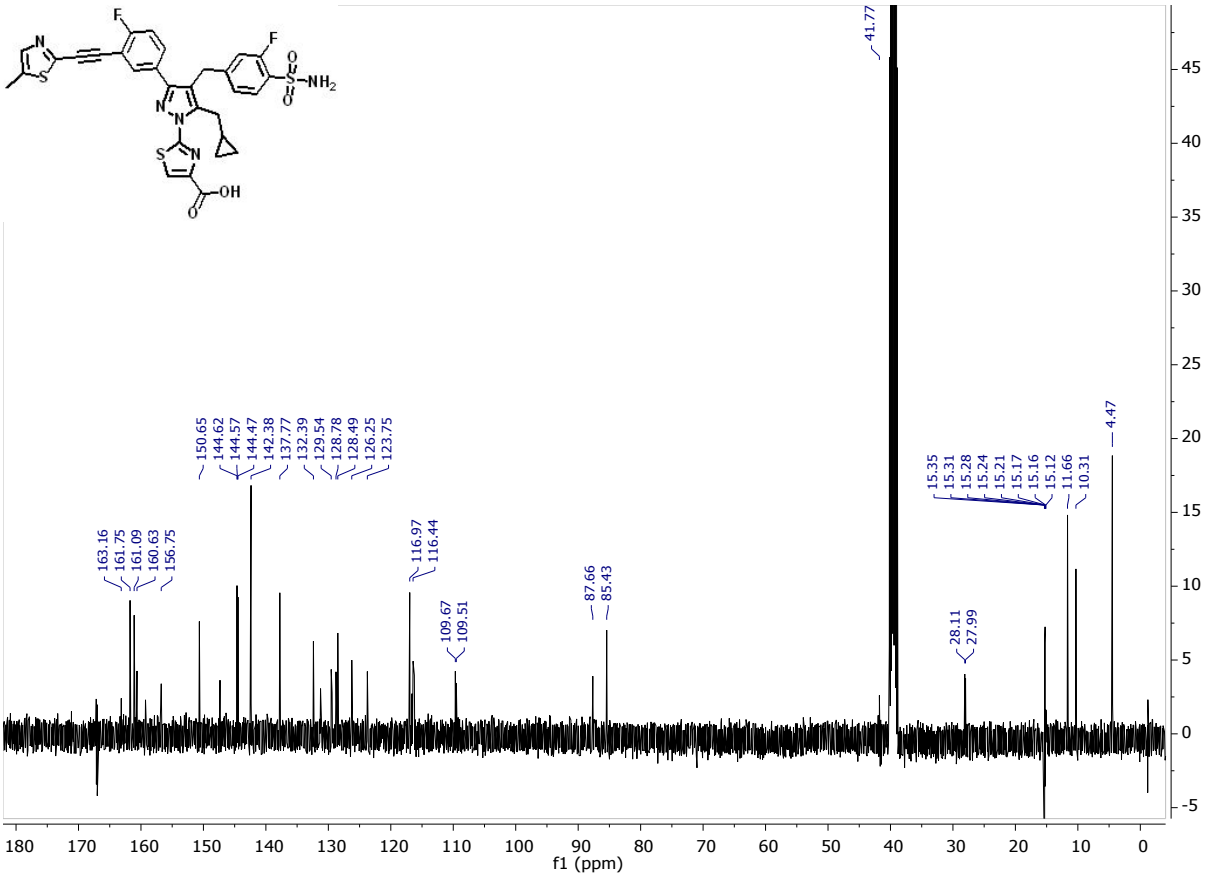
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.50	0.14	24343.61	2774.34	100.00	179

Integration Results for DAD1 B, Sig=254,12 Ref=off

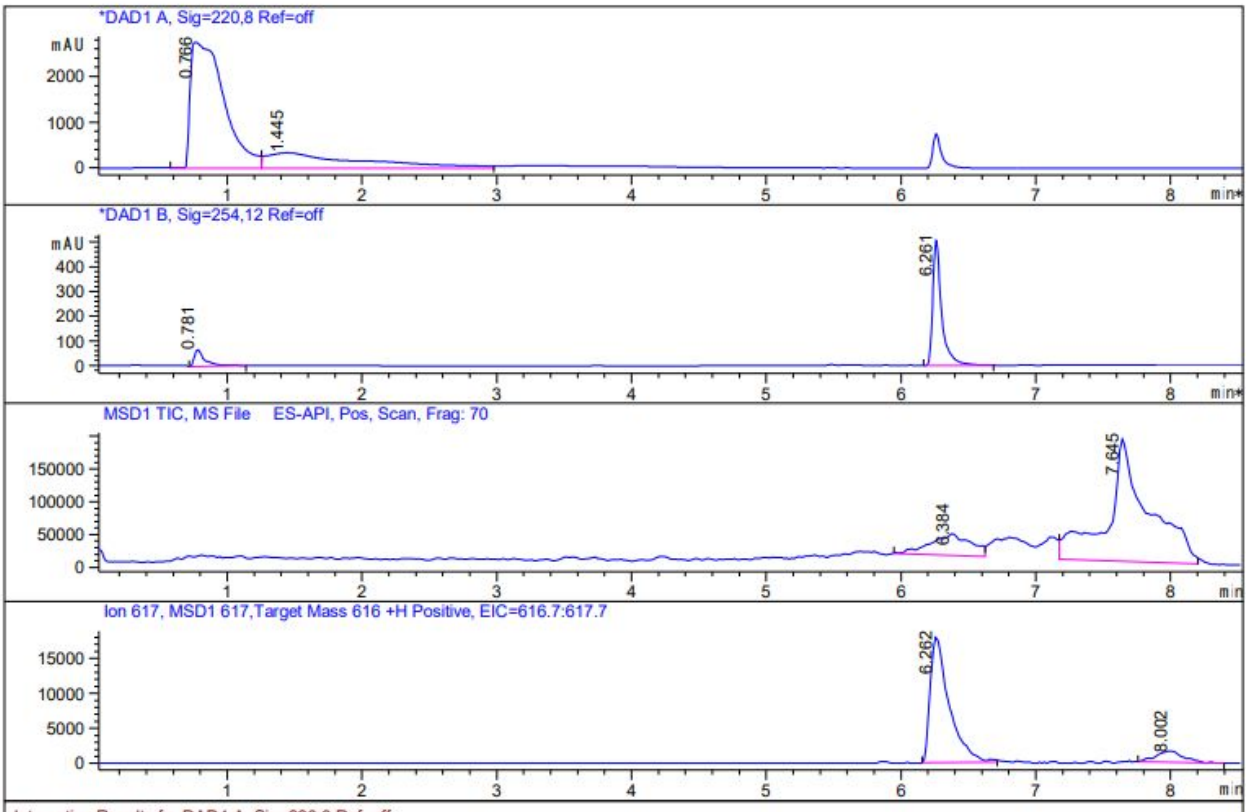
RetTim	Width	Area	Height	Area%	MS(+)
0.53	0.09	353.86	70.19	36.75	179
5.95	0.05	608.98	215.27	63.25	652


F
F
N
S
N
N
S
N
O
S
NH2
O
OH
O
13.16
8.34
8.32
7.89
7.88
7.87
7.86
7.72
7.71
7.71
7.71
7.70
7.70
7.70
7.69
7.68
7.68
7.67
7.65
7.63
7.57
7.45
7.43
7.41
7.17
7.17
7.14
7.14
7.07
7.07
7.05
7.05
4.19
3.31 HDO
3.19
3.17
2.52
2.52
2.51 dmso
2.50 dmso
2.50 dmso
2.49 dmso
0.37
0.36
0.36
0.35
0.35
0.34
0.34
0.33
0.31
0.29
0.29
0.27
0.25
0.24
0.23
0.23
0.22
0.21
1.17
1.16
1.15
1.14
1.13
1.12
1.11
0.92
0.66
0.90
2.36
1.96
0.86
0.97
0.98
1.91
1.94
2.69
0.42
1.91
1.92
f1 (ppm)



LCMS, proton and carbon NMR for compound 61

```
File ..ANTUKALLUG\07-16\250716-GRB056-0702-00341.D Tgt Mass (EZX): 616.00
Injection Date    : 25 Jul 16  10:49 am -0500          Seq. Line : 0
Sample Name       : GRB056-070                      Location  : P1-A-03
Acq. Operator     : Ganesha Rai                              Inj : 1
Spec. Reported    : UV Integration                  Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : 000002V28  Easy-Access Method: 'SUBMISSION'  616.00
Method Info : Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Agilent Eclipse XDB-C18 3 micron 3 x 75mm
```

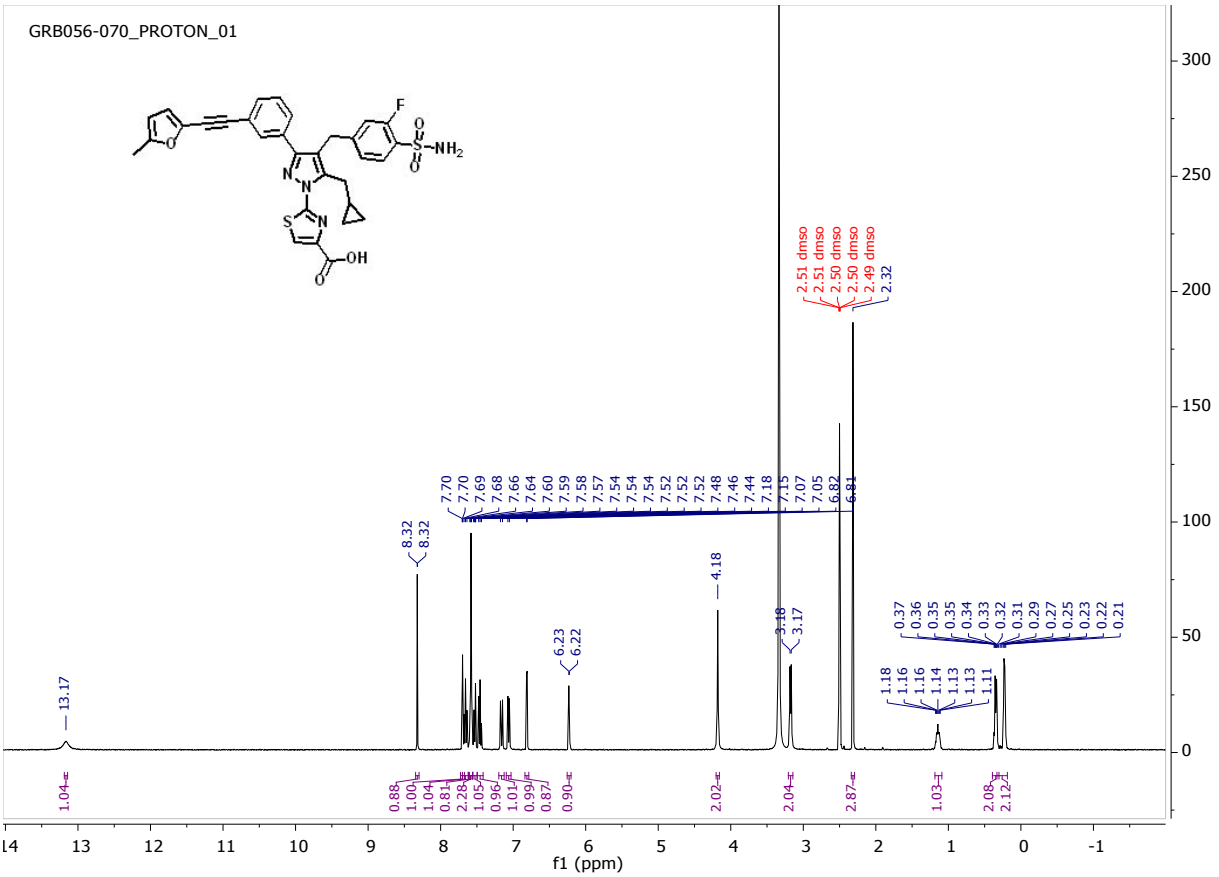


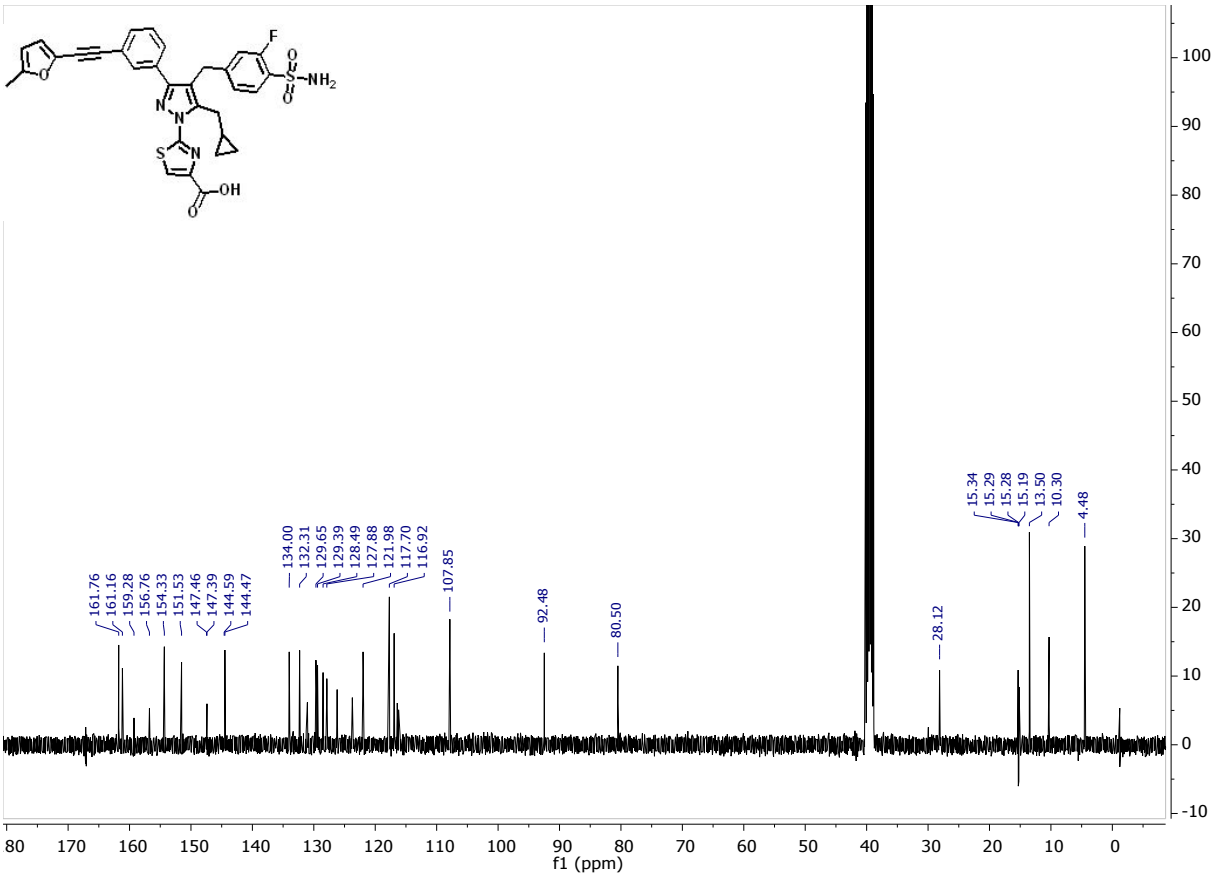
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.77	0.23	47873.43	2749.31	74.56	179
1.44	0.64	16336.11	334.58	25.44	179

Integration Results for DAD1 B, Sig=254,12 Ref=off

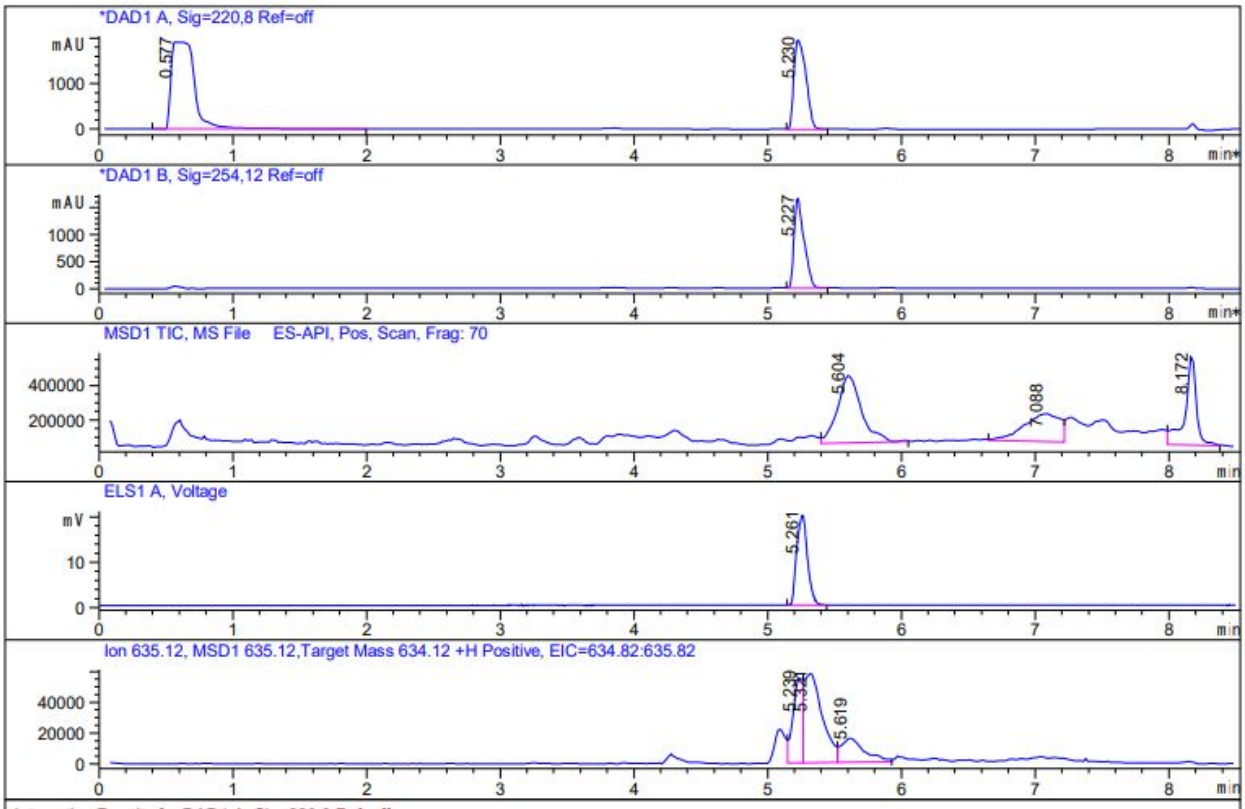
RetTim	Width	Area	Height	Area%	MS(+)
0.78	0.08	390.10	68.15	14.35	179
6.26	0.07	2328.81	508.47	85.65	617


GRB056-070_PROTON_01
f1 (ppm)



LCMS, proton and carbon NMR for compound 68

```
File ..TKINZ\03-16\300316-NCGC00481000-011-15534.D Tgt Mass (EZX): 634.12
Injection Date    : 30-Mar-16, 21:12:19                Seq. Line : 0
Sample Name       : NCGC00481000-01                 Location  : P2-H-05
Acq. Operator     : Zina Itkin                           Inj : 0
Spec. Reported    : UV Integration                  Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD.M
Sample Info : 0202680346  Easy-Access Method: 'FINAL_GRAD'  634.12
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
```

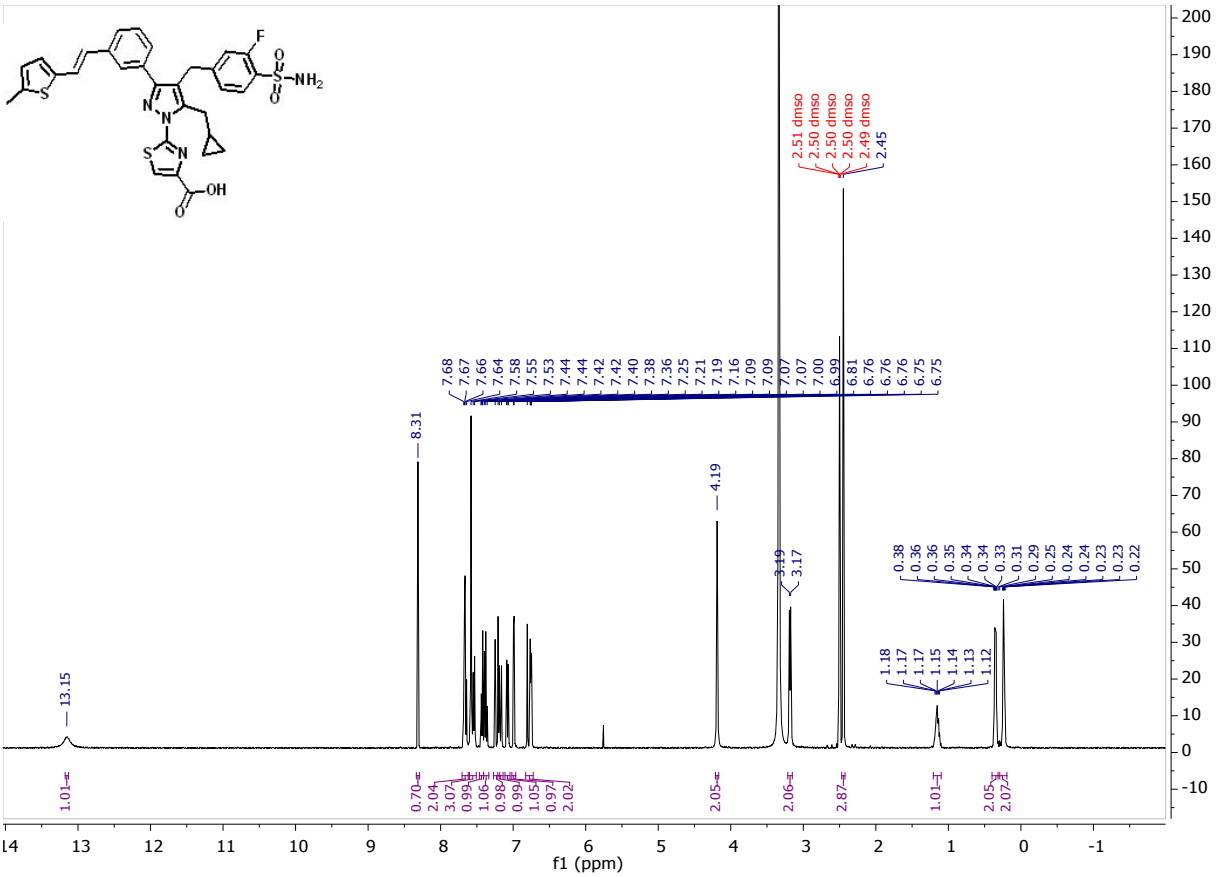


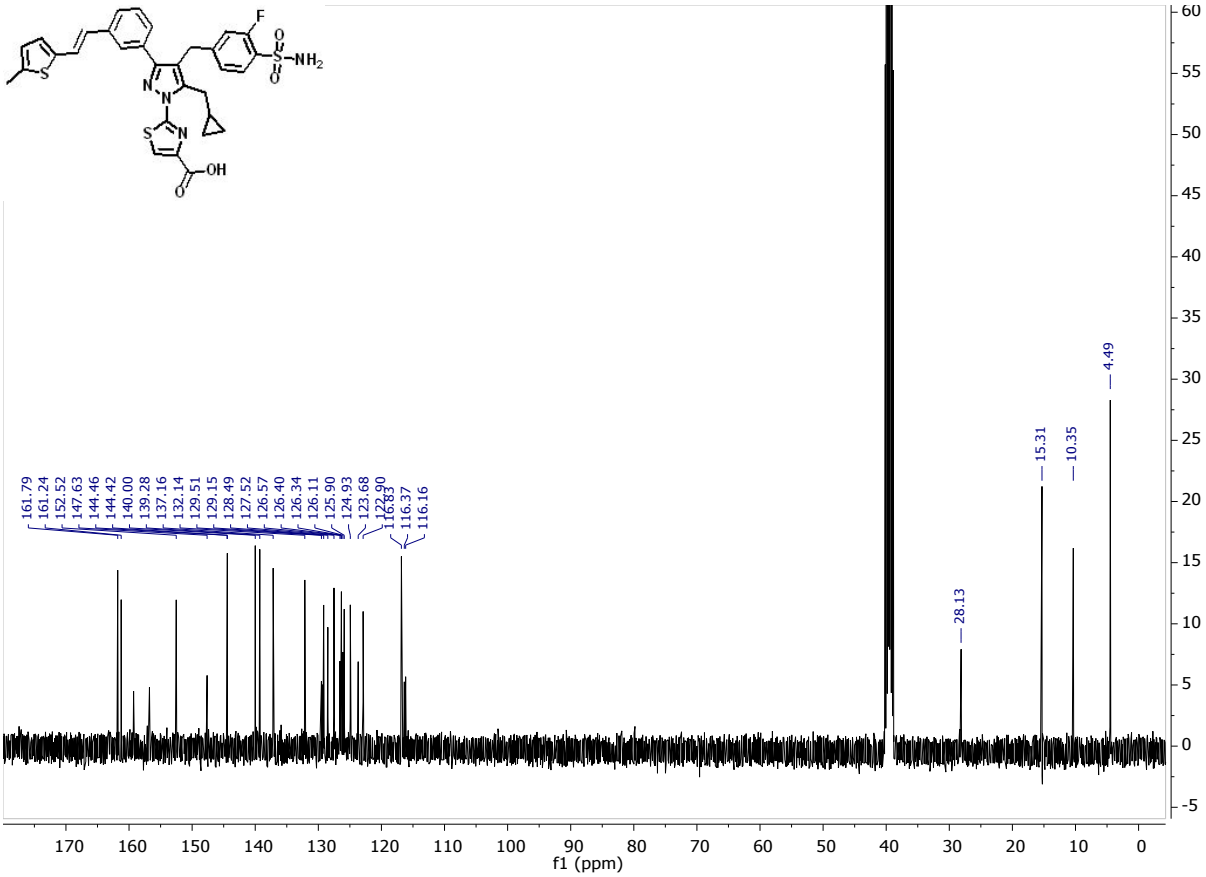
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.58	0.16	23189.96	1914.55	65.27	179
5.23	0.11	12340.28	1956.11	34.73	635

Integration Results for DAD1 B, Sig=254,12 Ref=off

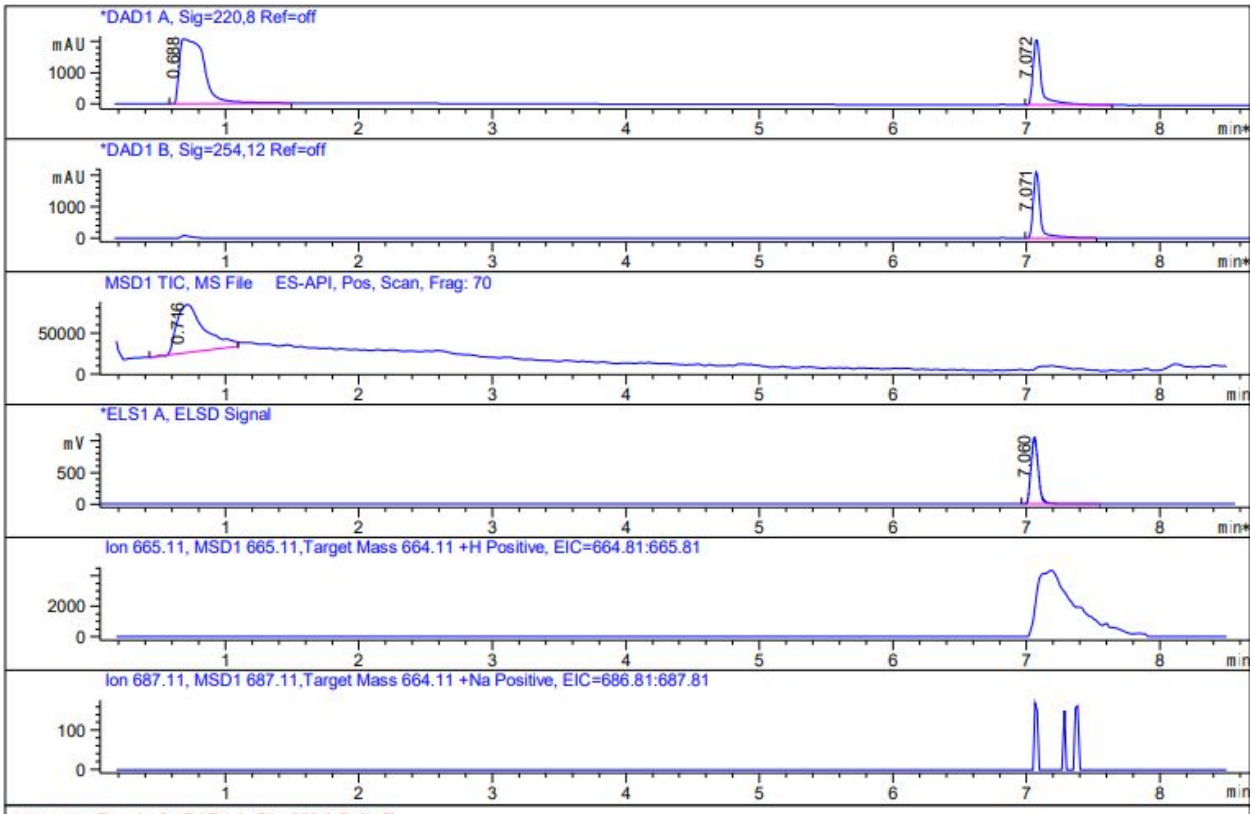
RetTim	Width	Area	Height	Area%	MS(+)
5.23	0.07	8680.45	1661.99	100.00	635


F
O
S
NH2
O
N
N
S
N
OH
O
13.15
8.31
4.19
3.19
3.17
2.51 dmso
2.50 dmso
2.50 dmso
2.50 dmso
2.49 dmso
2.45
7.68
7.67
7.66
7.64
7.58
7.55
7.53
7.44
7.44
7.42
7.42
7.40
7.38
7.36
7.25
7.21
7.19
7.16
7.09
7.09
7.07
7.07
7.00
6.99
6.81
6.76
6.76
6.76
6.75
6.75
0.38
0.36
0.36
0.35
0.34
0.34
0.33
0.31
0.29
0.25
0.24
0.24
0.23
0.23
0.22
1.18
1.17
1.17
1.15
1.14
1.13
1.12
1.01
0.70
2.04
3.07
0.99
1.06
0.98
0.99
1.05
0.97
2.02
2.05
2.06
2.87
1.01
2.05
2.07
f1 (ppm)



LCMS, proton and carbon NMR for compound 74

```
File ..CHEM\09-17\250917-NCGC00478989-0211-43049.D Tgt Mass (EZX): 664.11
Injection Date    : 25-Sep-17, 12:56:56                Seq. Line : 0
Sample Name       : NCGC00478989-02                 Location  : P1-D-01
Acq. Operator     : Paul Shinn                             Inj : 0
Spec. Reported    : UV Integration                  Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : S000002W7  Easy-Access Method: 'FINAL_GRAD_NO_PRINT'  664.11
Method Info : Long Gradient 4% to 100% ACN over 7 minutes (0.05%TFA)
              Luna  C18 3.0 x 75 mm
```

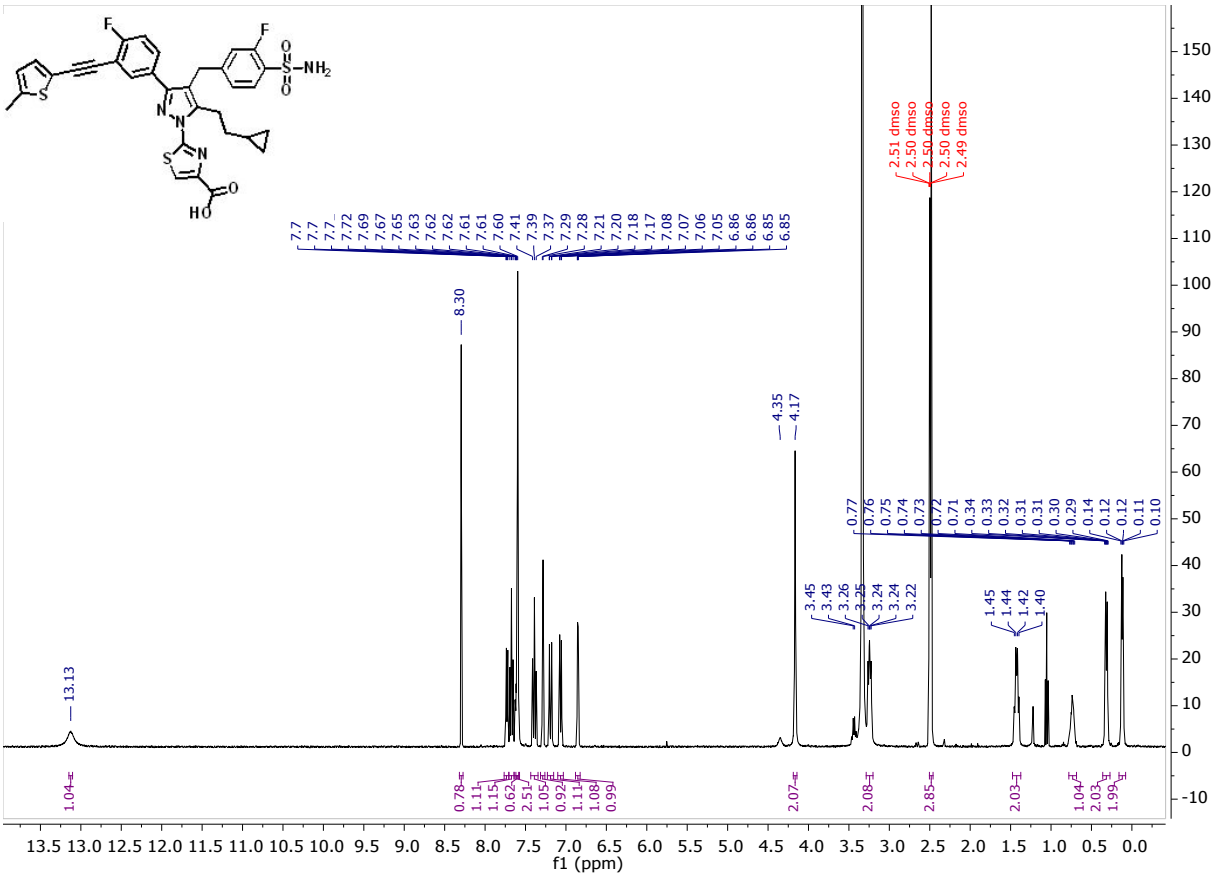


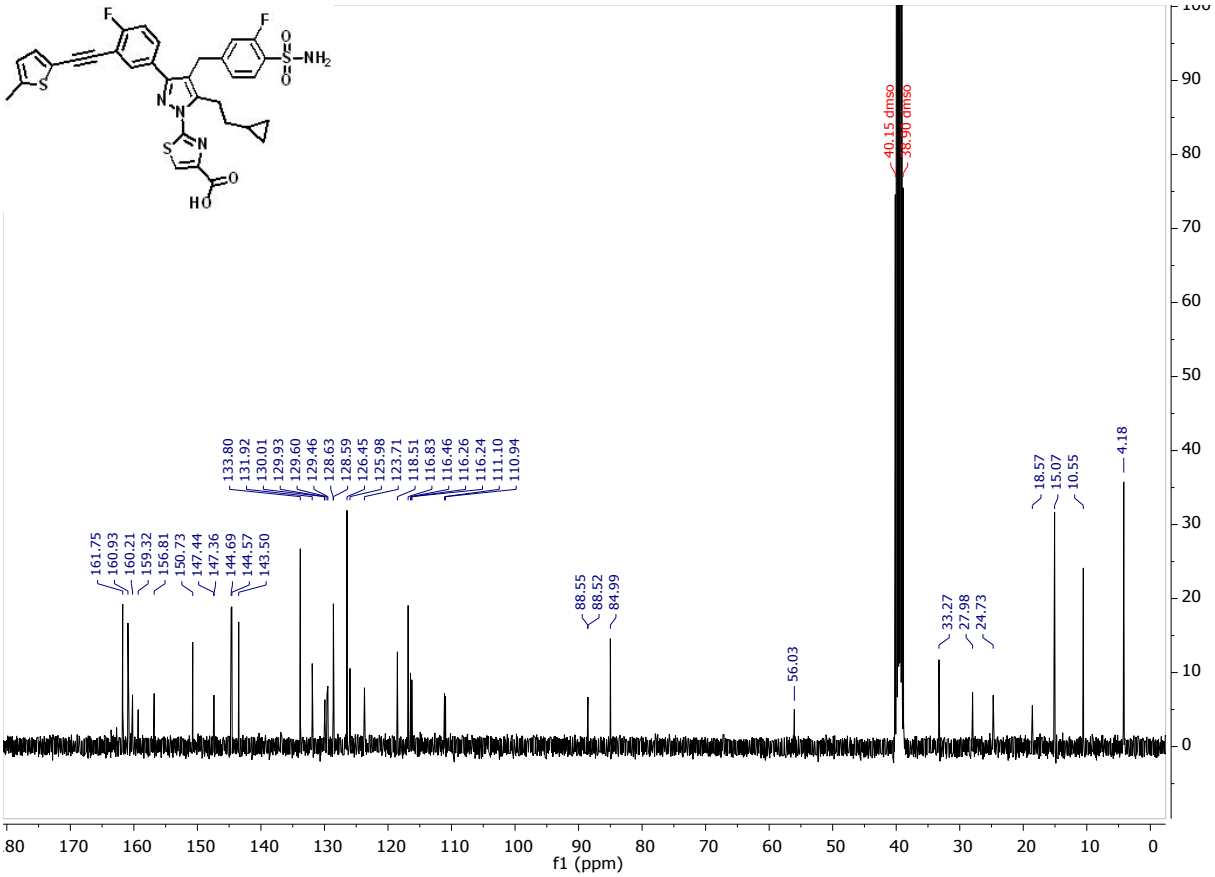
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.69	0.17	26821.06	2060.80	74.37	179
7.07	0.07	9244.50	2089.18	25.63	665

Integration Results for DAD1 B, Sig=254,12 Ref=off

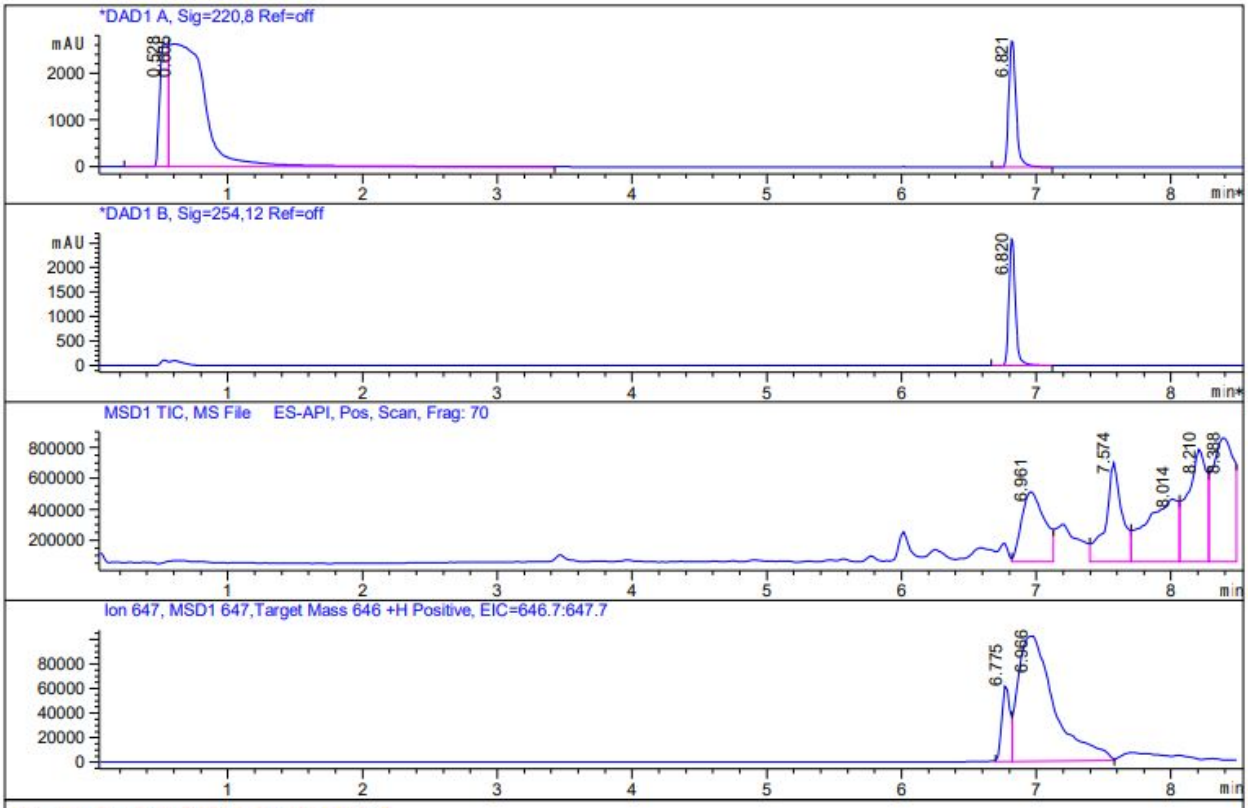
RetTim	Width	Area	Height	Area%	MS(+)
7.07	0.06	7832.47	2111.10	100.00	665


F
F
O
S
NH2
O
S
N
N
S
N
O
HO
7.7
7.7
7.7
7.72
7.69
7.67
7.65
7.63
7.62
7.62
7.61
7.61
7.60
7.41
7.39
7.37
7.29
7.28
7.21
7.20
7.18
7.17
7.08
7.07
7.06
7.05
6.86
6.86
6.85
6.85
8.30
4.35
4.17
2.51 dmso
2.50 dmso
2.50 dmso
2.50 dmso
2.49 dmso
0.77
0.76
0.75
0.74
0.73
0.72
0.71
0.34
0.33
0.32
0.31
0.31
0.30
0.29
0.14
0.12
0.12
0.11
0.10
3.45
3.43
3.26
3.25
3.24
3.24
3.22
1.45
1.44
1.42
1.40
13.13
1.04
0.78
1.11
1.15
0.62
2.51
1.05
0.92
1.11
1.08
0.99
2.07
2.08
2.85
2.03
1.04
2.03
1.99
f1 (ppm)



LCMS, proton and carbon NMR for compound 75

```
File ..ANTUKALLUG\04-16\200416-GRB055-0852-22013.D Tgt Mass (EZX): 646.00
Injection Date    : 20 Apr 16   4:04 pm -0500          Seq. Line : 0
Sample Name       : GRB055-085                    Location  : Vial 34
Acq. Operator     : Ganesha Rai                         Inj : 1
Spec. Reported    : UV Integration                Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD_NO_PRINT.M
Sample Info : S000002XH  Easy-Access Method: 'SUBMISSION'  646.00
Method Info : Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Luna C18 3 micron 3 x 75mm
```

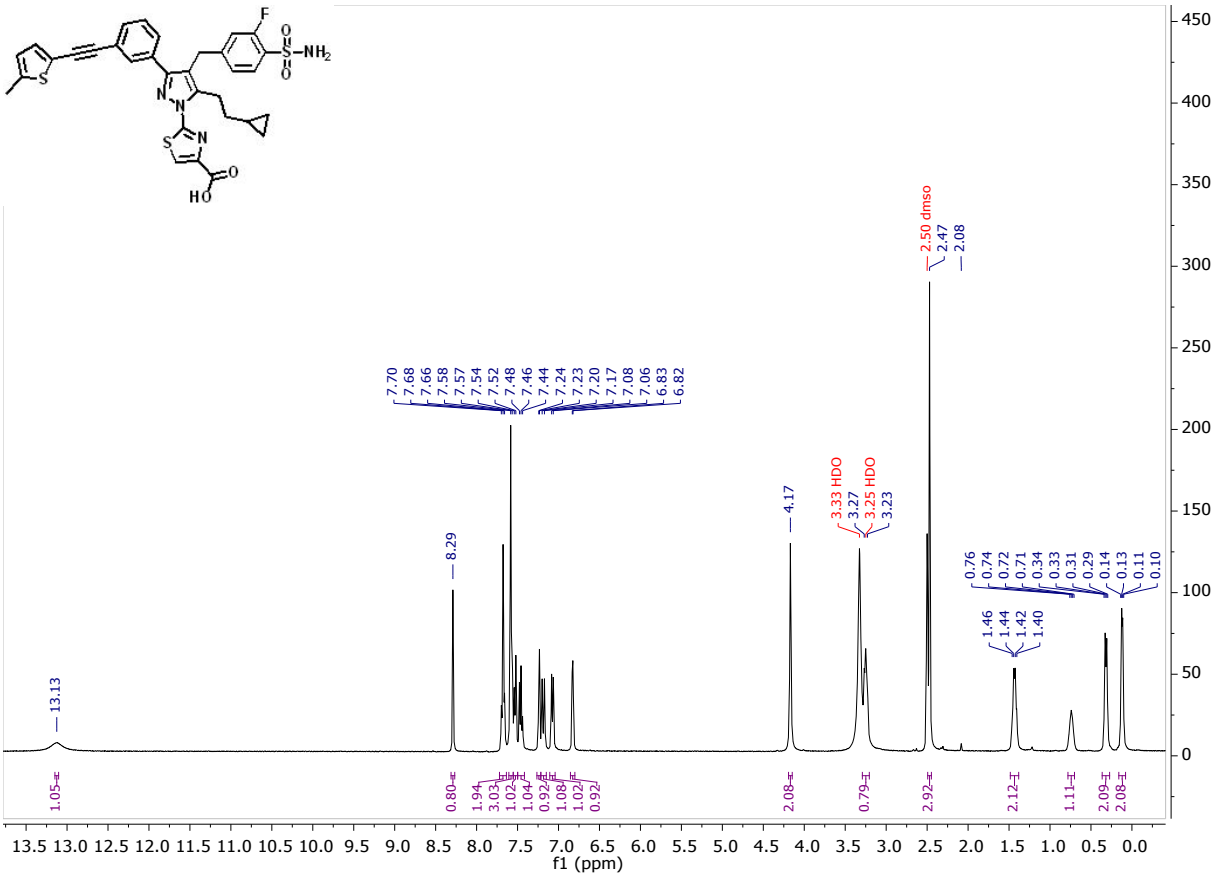


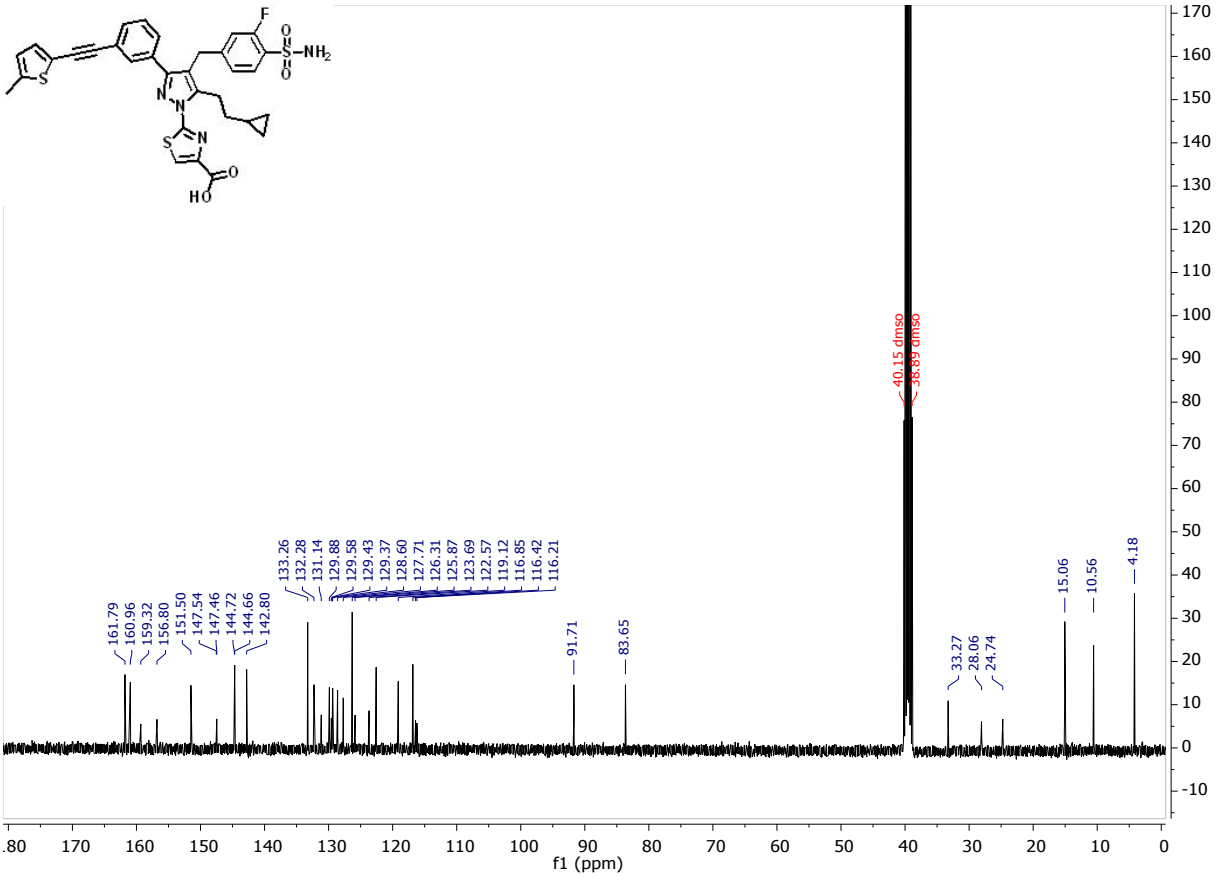
Integration Results for DAD1 A, Sig=220,8 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
0.53	0.06	11062.23	2636.11	15.79	249
0.60	0.23	48792.48	2625.21	69.66	249
6.82	0.06	10188.77	2717.80	14.55	647

Integration Results for DAD1 B, Sig=254,12 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
6.82	0.05	8565.91	2584.16	100.00	647


F
O
S
NH2
O
N
N
S
N
O
HO
13.13
8.29
7.70
7.68
7.66
7.58
7.57
7.54
7.52
7.48
7.46
7.44
7.24
7.23
7.20
7.17
7.08
7.06
6.83
6.82
4.17
3.33 HDO
3.27
3.25 HDO
3.23
2.50 dmso
2.47
2.08
1.46
1.44
1.42
1.40
0.76
0.74
0.72
0.71
0.34
0.33
0.31
0.29
0.14
0.13
0.11
0.10
1.05
0.80
1.94
3.03
1.02
1.04
0.92
1.08
1.02
0.92
2.08
0.79
2.92
2.12
1.11
2.09
2.08
f1 (ppm)



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