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Article

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

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Abstract: Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate, with concomitant oxidation of NADH as the final step in the glycolytic pathway. Glycolysis plays an important role in the metabolic plasticity of cancer cells and has long been recognized as a potential therapeutic target. Thus, potent, selective inhibitors of LDH represent an attractive therapeutic approach. However, to date, pharmacological agents have failed to achieve significant target engagement *in vivo*, possibly because the protein is present in cells at very high concentrations. We report herein a lead optimization campaign focused on a pyrazole-based series of compounds, using structure-based design concepts, coupled with optimization of cellular potency, *in vitro* drug-target residence times, and *in vivo* PK properties, to identify first-in-class inhibitors that demonstrate LDH inhibition *in vivo*. The lead compounds, named **NCATS-SM1440** (**43**) and **NCATS-SM1441** (**52**) possess desirable attributes for further studying the effect of *in vivo* LDH inhibition.

INTRODUCTION

Cancer cells exhibit deregulated metabolic characteristics that are remarkably different from normal cells, demonstrating an avidity for glucose uptake and catabolism, ultimately producing lactate and generating ATP through the cytosolic, non-oxidative glycolytic pathway. Glycolysis can be the preferred NAD^+ regeneration and energy production process for cancer cells, even in the presence of oxygen, rather than the more energy-efficient mitochondrial oxidative phosphorylation. This is referred to as aerobic glycolysis, and the preference for aerobic glycolysis is also known as the Warburg effect.[1] While aerobic glycolysis is an inefficient way to generate ATP (compared with oxidative phosphorylation), the rate of ATP generation is rapid. In addition, it is hypothesized that rapidly proliferating cancer cells have adapted this approach to regenerate NAD^+ and to support the production of essential cellular building blocks such as amino acids, lipids, and nucleotides needed to support rapid cell growth.[2] Indeed, many non-cancer cells use a combination of oxidative phosphorylation and glycolysis to achieve the needed metabolic plasticity to serve their biological function. While the molecular basis of aberrant cancer metabolism and its role in cancer development and progression have yet to be fully elucidated,[3] the Warburg effect, and the enzymes in glycolysis, have long been recognized as potential targets for the selective killing of cancer cells.

Many glycolytic enzymes are overexpressed in cancer, including the lactate dehydrogenase (LDH) enzymes A (LDHA) and B (LDHB).[4,5] LDH is a tetrameric protein composed of the products of the *LDHA* (subunit M) and *LDHB* (subunit H) genes. The tetrameric combination of these gene products generates 5 LDH isoforms with different combinations of subunits depending on the cell type. The LDH5 (4M) isoform is the primary form expressed in cancer cells,[6] although other isoforms have also been reported. All LDH isoforms catalyze the last step in the glycolytic pathway converting pyruvate to lactate while

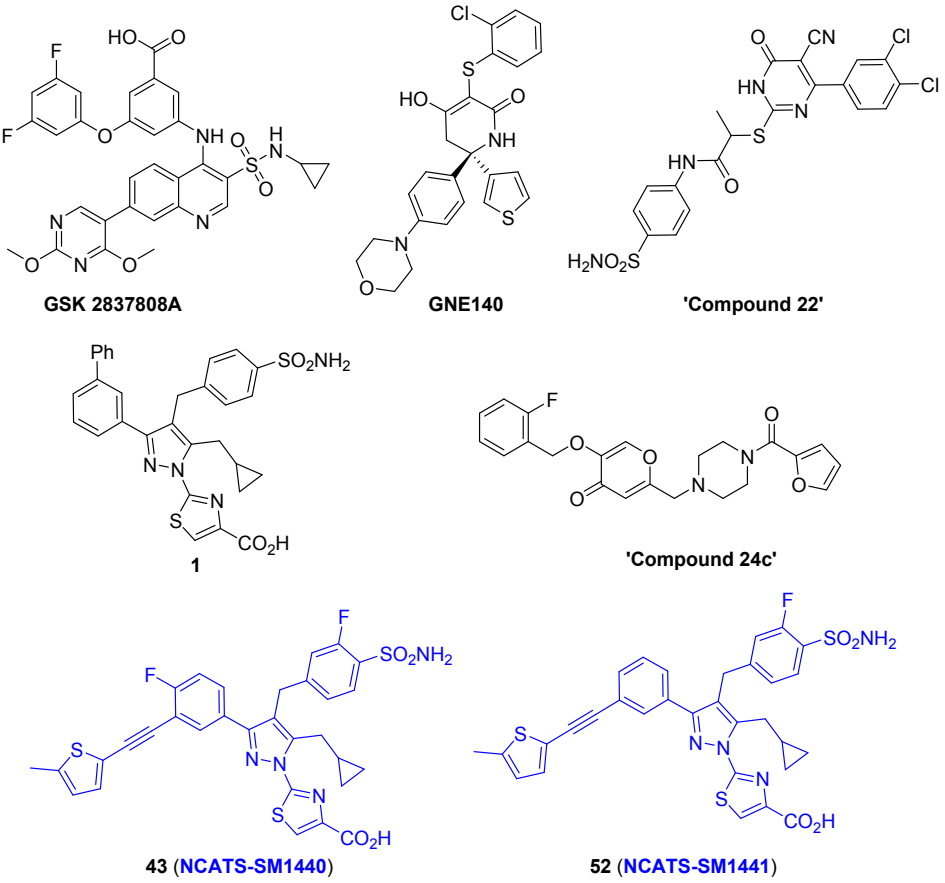
regenerating NAD^+ from NADH. The lactate produced is then secreted from cells via the monocarboxylate transporter (MCT) proteins.

Significant evidence exists to support the development of LDH inhibitors as a therapeutic option for cancer treatment. Genetic knockdown of *LDHA* has been shown to elicit cell death or delayed cell growth in cell lines from colorectal carcinoma (siRNA),[7] Burkitt lymphoma (siRNA),[8] hepatocellular carcinoma (siRNA),[9] pancreatic cancer (shRNA),[10] and mouse mammary tumor cells (shRNA).[11] For example, mouse mammary tumor cells lacking LDHA implanted as xenografts demonstrated dramatically reduced tumor growth.[11] And, in a genetically engineered mouse model (GEMM) of non-small cell lung cancer, induced knockout of mouse LDHA led to the regression of established tumors without serious systemic toxicity.[12] In contrast, LDHB knockdown has been reported not to significantly impact tumor cell survival.[7]

While the therapeutic potential of LDHA inhibition appears to be substantial, the discovery and development of LDH inhibitors has proven to be challenging. For example, because of the μM concentrations of LDH enzyme in cancer cells, an effective inhibitor will likely need to bind with exceptionally high affinity and also achieve high intracellular concentrations to enable a therapeutic level of target engagement. To date, no inhibitors of LDH which meet these criteria have been reported. The first reported LDH inhibitors came from academic groups (e.g. FX-11[13a] and NHI-2[13b]) with efforts from biotech[14] and pharmaceutical companies[15] being disclosed more recently. Molecules from GlaxoSmithKline (GSK)[16] and Genentech (GNE-140)[17] have demonstrated *in vitro* inhibition of cellular lactate production and cytotoxicity in cancer cells, but the relatively poor pharmacokinetics of these compounds limited their usefulness for testing of the therapeutic hypothesis *in vivo*. More recently, LDH inhibitors (e.g. **'Compound 24c'** in Figure 1) that show modest inhibition of

MiaPaCa-2 xenograft growth have been described; but target engagement *in vivo* was not demonstrated[18].

We recently reported the identification of a pyrazole-based hit from quantitative high-throughput screening (qHTS) and used structure-based design to develop nM inhibitors of LDH enzyme activity, exemplified by **1**.[19] Compound **1** inhibited LDH in the highly glycolytic MiaPaCa-2 (human pancreatic cancer) and A673 (human Ewing's sarcoma) cell lines, demonstrating sub-μM suppression of cellular lactate output and inhibition of cell growth. While **1** showed favorable *in vitro* ADME properties (e.g., microsomal stability, solubility), its PK profile was not suitable for *in vivo* use. Herein, we report the optimization of the pyrazole-based chemotype using binding constants (particularly off-rates or drug-target residence time) and cell-based activity to guide improvements in potency, while also optimizing pharmacokinetic properties. These efforts ultimately resulted in lead compounds **43** (**NCATS-SM1440**) and **52** (**NCATS-SM1441**) that have enabled studies of the effects of pharmacological LDH inhibition in animal models of Ewing's sarcoma[20] and human pancreatic cancers[21] as well as interrogation of a synergistic role in T cell-based immunotherapy.[22]



	Biochemical Potency	<800 nM Cellular Potency	Optimal PK profile	In vivo LDHA inhibition
GSK 2837808A	✓	X	X	X
'Compound 22'	✓	X	X	X
GNE140	✓	✓	X	X
1	✓	✓	X	X
'Compound 24c'	✓	X	**Not disclosed**	X
[a]**43 = NCATS-SM1440**	✓	✓	✓	✓
[b]**52 = NCATS-SM1441**	✓	✓	✓	✓

Figure 1. Representative previously described LDH inhibitors and comparison with new leads **43 = NCATS-SM1440** and **52 = NCATS-SM1441**. [a]Named as NCI-006 in references *20* and *21*. [b] Named as NCI-737 in reference *20.*

CHEMISTRY

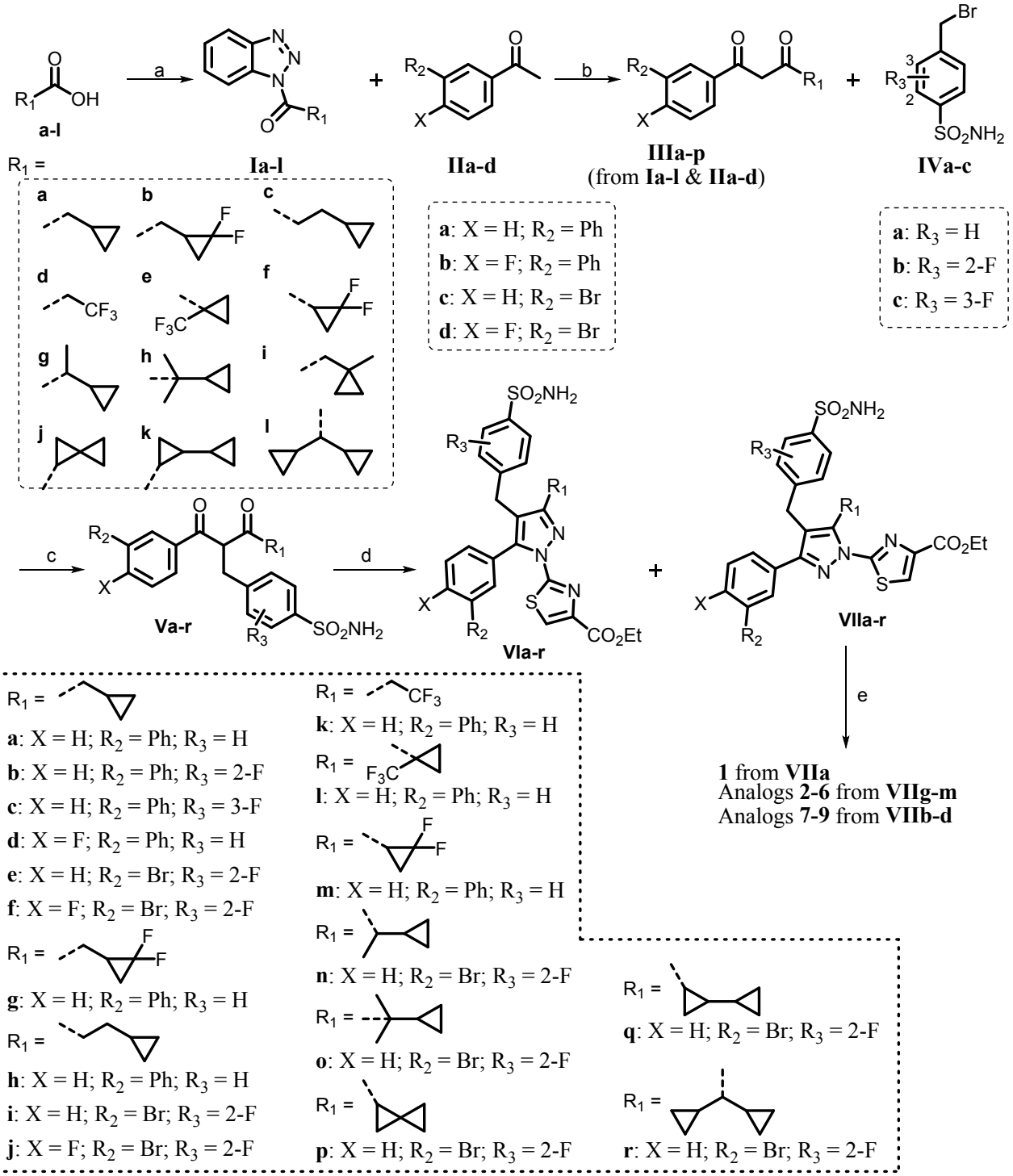
A robust linear synthetic strategy was used for the optimization of the cyclopropylmethyl and sulfonamide regions (Scheme 1 and Scheme 2). The commercially available carboxylic acids **a-l** were reacted with 1,2,3-benzotriazole in the presence of thionyl chloride to give acyl benzotriazole intermediates **Ia-l**. The reaction of these intermediates (**Ia-l**) with substituted acetophenones **IIa-d**, mediated by magnesium bromide ethyl etherate in the presence of

Hunig's base, afforded 1,3-diketone derivatives **IIIa-p** (Table S3) in good yields. Subsequently, cesium carbonate-mediated alkylation of 1,3-diketone intermediates **IIIa-p** with 4- (bromomethyl)benzenesulfonamide derivatives **IVa-c** in DMSO at room temperature generated advanced intermediates **Va-r**, as described in our previous paper[19]. Initial attempts with tosic acid-catalyzed cyclocondensation of 1,3-diketone derivatives **IIIa-p** with ethyl 2-hydrazinylthiazole-4-carboxylate hydrobromide generated a mixture of both pyrazole regioisomers. We previously reported that the 5-aryl regioisomer is inactive in LDHA assays, so formation of this regioisomer is undesired. The product ratio of the desired isomer was poor (≤10%), particularly when R_1 is electron-donating alkyl groups such as cyclopropylmethyl or cyclopropylethyl groups which were found to be optimal for activity (e.g. **Va-f, Vh-j**, and **Vn-r**). Therefore, it was neither efficient nor practical to use this cyclization protocol to synthesize the quantities of the advanced bromide containing intermediates **VIIa-f**, **VIIh-j**, and **VIIn-r** needed for late-stage optimization efforts.

The ratio of the two regioisomers produced in the reaction depends on reactivity differences dictated by the electronic environment around the two keto groups. To improve the regioisomeric ratio, we envisioned utilizing these reactivity differences by blocking the undesired reactivity of the 1-keto group via initial formation of an enamine intermediate prior to addition of the ethyl 2-hydrazinylthiazole-4-carboxylate. Extensive optimization identified improved conditions, in which heating 1,3-diketones **Va-r** in ethanol in the presence of 0.5 equivalents of both pyrrolidine and tosic acid for 1-2 h at reflux, then adding ethyl 2-hydrazinylthiazole-4-carboxylate and stirring at reflux overnight allowed us to obtain a ~ 50:50 mixture of the two regioisomers. The desired isomer was isolated as the second peak using reversed-phase (C18) chromatography, as the desired regioisomer was slightly less polar (as judged by LC/MS analysis and according to our previous reports[19]). Further reaction optimization proved elusive, and the investigation of alternative routes to obtain exclusively

the desired isomer met with limited success. Thus, for all subsequent scale-up and analog syntheses, we used the above procedure. Compound **1** and analogs **2-9** were obtained by hydrolysis of the intermediates **VIIa**, **VIIg-m**, and **VIIb-d**, respectively, using lithium hydroxide in ethanol and subsequent HPLC purification.

Scheme 1: Syntheses of intermediates **VIa-r** and analogs **1-9**[a].

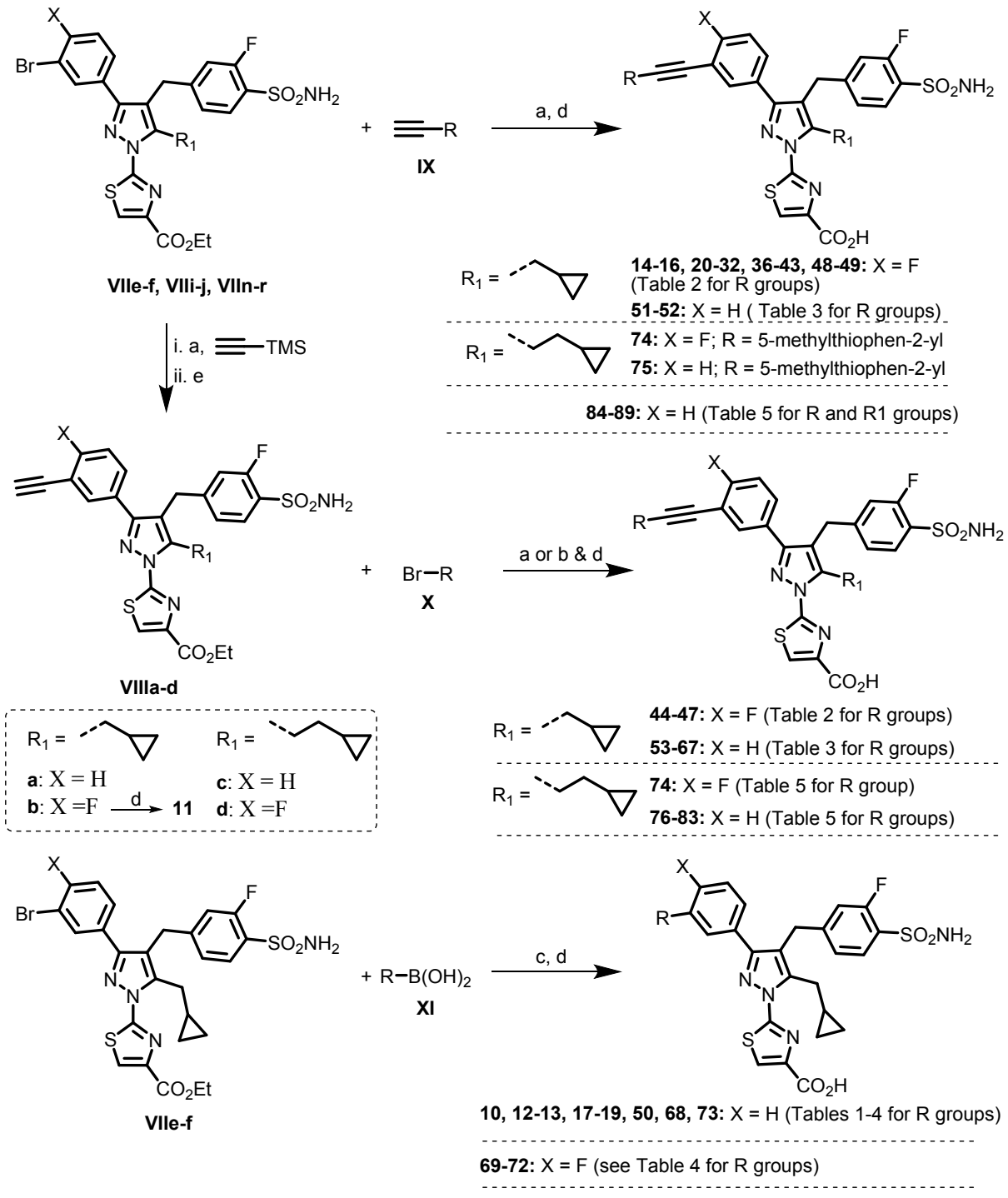


[a]Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, 4 h, 91-100% (b) $MgBr_2 \cdot OEt_2$, iPr_2NEt, CH_2Cl_2, 12 h, 60-69% (c) Cs_2CO_3, DMSO, 1 h, 55-83% (d) i. Pyrrolidine (0.5 eq), TsOH (0.5 eq), EtOH, reflux, 1-2 h ii. Ethyl 2-hydrazinylthiazole-4-carboxylate·HBr, reflux overnight (e) LiOH, THF/MeOH/H_2O, 1 h.

The synthesis of analogs **11-89** listed in Tables 2-4 was achieved utilizing either Sonogashira or Suzuki coupling of the advanced 3-bromo aryl intermediates **VIIe-f, VIIi-j**, and **VIIn-r**, followed by hydrolysis with LiOH (Scheme 2). For the Sonogashira coupling, we developed a robust room temperature, copper-free protocol catalyzed by commercially available monoligated precatalysts, [P(t-Bu)$_3$] Pd(crotyl)Cl or [DTBNpP] Pd(crotyl)Cl, in the presence of DABCO in dioxane[23]. This method allowed us to quickly generate an alkyne library and explore the SAR around the biphenyl region with access to more complex structures. Moreover, the protocol requires only a minimal work-up that could further facilitate rapid analog generation during our library synthesis and is amenable to providing large quantities of key intermediates (e.g.**VIIIa-d**) to support advanced studies on top compounds. Using this method, analogs **14-16, 20-32, 36-43, 48-49, 51-52, 74-75**, and **84-89** were synthesized directly from intermediates **VIIe-f, VIIi-j**, and **VIIn-r** with the corresponding commercially available terminal alkynes **IX** and the [P(t-Bu)$_3$] Pd(crotyl)Cl catalyst in dioxane. Syntheses of analogs **33-35** is summarized in scheme 3**.** To rapidly explore the SAR with aryl or heteroaryl R substitutions, a slightly modified 3-step protocol was used for analogs **44-47**, **53-67**, and **76-83**. The synthesis began with [P(t-Bu)$_3$]Pd(crotyl)Cl-catalyzed Sonogashira coupling of intermediates **VIIe-f** and **VIIi-j** with trimethylsilylacetylene, followed by deprotection of the TMS group with CsF, to give intermediates **VIIIa-d**. Further Sonogashira coupling of these alkyne intermediates with the corresponding aryl or heteroaryl bromides **X** using the same conditions, followed by hydrolysis with LiOH, furnished analogs **44-45, 53-61, 66-67**, and **76-79**. Analog **11** (R = H) was obtained upon hydrolysis of the intermediate

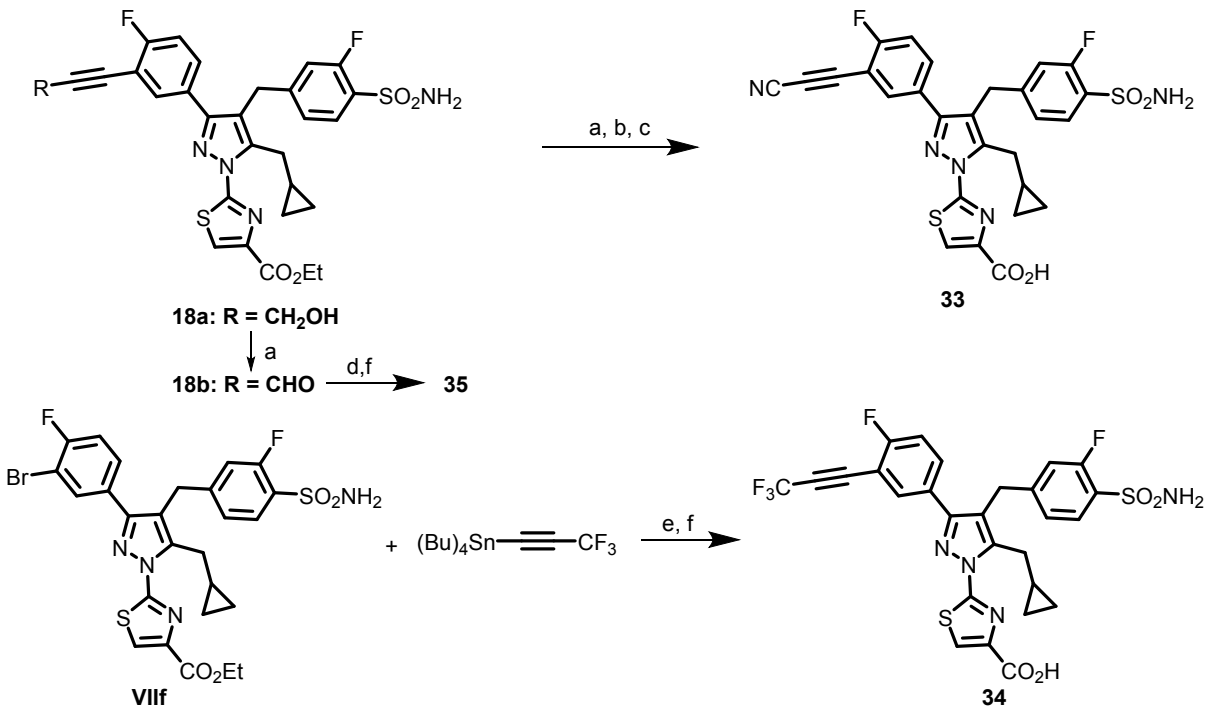
VIIIb using LiOH in a methanol-water mixture. However, the use of the [P(t-Bu)$_3$]Pd(crotyl)Cl catalyst under similar conditions failed to give the isolable product in the case of more electron-deficient heteroaryl bromides, such as 1,3-thiazolyl or 1,3-oxazolyl bromides. Fortunately, switching to a more active catalyst, [DTBNpP]Pd(crotyl)Cl, with slightly elevated reaction temperature (60 ^{o}C in dioxane) facilitated efficient product formation. Thus, Sonogashira reaction of the intermediates **VIIIa-d** with corresponding 1,3-thiazolyl or 1,3-oxazolyl bromides under those conditions in the presence of DABCO, followed by hydrolysis, furnished analogs **46-47, 62-65** and **80-82**. Due to the gaseous nature and commercially unavailability of alkyne precursors, we used SPhos precatalyst-catalyzed Suzuki coupling of the corresponding alkynyl trifluorobornates or pinacol esters, followed by hydrolysis protocol for alkyne analogs **12**-**13**, **17**-**19** and **50**. Finally, Suzuki coupling of corresponding alkenyl boronates or boronic acids with **VIIe-f**, using SPhos Pd(crotyl)Cl precatalyst in the presence of potassium phosphate base at 100 ^{o}C and subsequent hydrolysis of the ester group, provided analogs **68-73**.

Scheme 2: Syntheses of analogs **10-32** and **36**-**89**[a].



[a]Reagents and conditions: (a) [P(*t*-Bu)$_3$]Pd(crotyl)Cl, DABCO, dioxane, RT, 1-12 h (b) [DTBNpP]Pd(crotyl)Cl, DABCO, dioxane, 60 ^{o}C, 12 h (c) SPhosPd(crotyl)Cl, K_3PO_4, dioxane/H_2O, 100 ^{o}C, 0.5 h (d) LiOH, THF/MeOH/H_2O, 1 h. e) CsF, THF-EtOH, RT, 2 h

Scheme 3: Syntheses of analogs **33-35**[a].



[a]Reagents and conditions: (a) Dess-Martin periodinane, DCM, RT, 2 h (b) NaN_3, CF_3SO_3H, ACN, RT, 12 h (c) $(CH_3)_3Sn$-OH, dichloroethane, MW, 110 °C, 1 h (d) Deoxo-Fluor, DCM, RT, 12 h (e) $P(^tBu)_3.HBF_4$, $([PdCl(allyl)]_2$, dioxan, 80 °C, 4 h (f) LiOH, THF/MeOH/H_2O, 1 h.

RESULTS AND DISCUSSION

In our previous work, we described structure-guided optimization of a weakly active quantitative high-throughput screening hit into **1**, a potent, selective LDH inhibitor suitable for probing LDH function in cells[19]. Although **1** showed nanomolar inhibition of LDHA in the biochemical activity assay and was able to achieve reasonable cellular potency, additional optimization was required to identify highly potent molecules with PK properties suitable for *in vivo* studies. Here, we report a focused lead optimization effort that has identified compounds with good activity in the highly glycolytic MiaPaCa-2 and A673 cell lines and with improved pharmacokinetic properties.

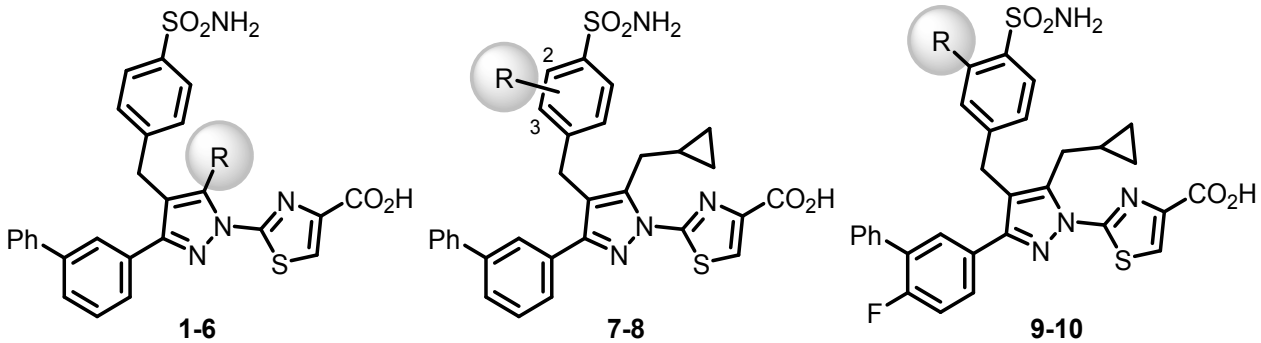
We previously showed that the thiazole carboxylic acid moiety forms a critical interaction with the active site R168 and could not be replaced by other groups. The 4-benzyl sulfonamide

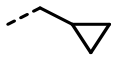
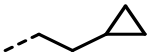
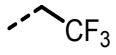
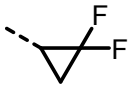
group likewise forms critical hydrogen bond interactions with Asp140, Glu191, and Ile141 that contribute significantly to the potency. Further, the cyclopropylmethyl group forms an important π stacking interaction with Tyr238 and was found to be a key substituent for conferring cellular potency. However, previous modifications of the benzyl sulfonamide or replacements for the cyclopropylmethyl group had been limited, and thus the potential to improve potency and residence time had not been fully explored. Additionally, in our previous co-crystal structure[19], the binding pose shows that the biphenyl does not fully occupy the hydrophobic pocket in this region, suggesting an opportunity to introduce other lipophilic groups in a region that appeared to be the most accommodating for further SAR optimization. Therefore, the lead optimization efforts described here have focused on these three regions of the compounds in this series.

Based on our published crystal structures of compounds in this series bound to LDHA, the SAR campaign began with more extensive investigations of the cyclopropylmethyl group. Initially, a difluoro group was introduced into the cyclopropyl ring (**2**), anticipating additional hydrogen bonding interaction with the phenol of residue Tyr238 (See Figure 2). Although analog **2** maintained biochemical potency (24 nM vs. 25 nM), it showed reduced cellular potency and cytotoxicity compared to **1** (A673 lactate = 1423 vs. 450 nM, A673 cytotox = 4642 vs. 2450 nM; MiaPaCa-2 lactate = 1721 vs. 636 nM, cytotox = 10,660 vs. 8448 nM). To extend deeper into this binding site the cyclopropylethyl (**3**) analog was made. This group resulted in reduced biochemical LDHA activity and offered only marginal improvement in cellular potency. This trend appeared to be general, as it replicated across numerous matched pairs of analogs (see **43** vs. **74**, **52** vs. **75**, **54** vs. **77**, **56** vs. **78**, **57** vs. **79**, **63** vs. **80**, **64** vs. **81**, **65** vs. **82**, and **67** vs. **83** in Tables 2-4). Replacing the cyclopropylmethyl group with other fluorinated alkyl groups (**4-6**) diminished potency in the biochemical assay and drastically reduced the cellular activity compared to **1**. Adding a branched substitution to the methylene

group (**84, 85**, and **89**) or adding substitution on the cyclopropyl ring (**86-88**) further reduced both biochemical and cellular potency, relative to **75**. The primary focus was to gain cellular potency, yet moieties other than cyclopropylmethyl or cyclopropylethyl failed to improve activity in both lactate and cytotoxicity cellular assays. Therefore, the preferred cyclopropylmethyl group was utilized in subsequent analogs. We next explored fluorine substitution at the 2- (**7**) or 3- positions (**8**) of the benzenesulfonamide ring. Analog **7**, with a 2-fluorobenzene sulfonamide substitution, showed similar biochemical potency to **1**, and slightly decreased activity in the lactate assay, but exhibited an improvement in cytotoxicity. The 3-fluoro analog, **8,** exhibited a significant decrease in activity across all assays. Initially, we were unable to rationalize these observations, as neither fluorine group appeared positioned to contribute an additional specific interaction with LDH, based on the previous crystal structure. However, crystal structures with 2-fluoro-containing analogs (**23**, **52** Figure 2) clearly show a preferred orientation of the ring, with the fluorine occupying a position pointed into the protein and away from the space occupied by the biphenyl or alkyne substitutions. Based on the previously reported correlation between the binding kinetics and the cellular potency, we subsequently found that the 2-fluoro substitution improves the off-rate of the molecule (Table 6), which might explain the enhanced cytotoxic effects in MiaPaCa-2 and A673 cellular assays. Therefore, the 2-fluoro group was retained as an optimal substitution to evaluate further modifications of the lead molecule.

Table 1. Biochemical and Cellular LDH Inhibition of **2**−**10** with Comparator **1**.



Compd No.	R	Biochemical LDHA IC_{50} (nM)[a]	A673 cells		MiaPaCa-2 cells		HEK293 Cells
			lactate inh IC_{50} (nM)[a]	Cytotox. IC_{50} (nM)[a]	lactate inh. IC_{50} (nM)[a]	Cytotox. IC_{50} (nM)[a]	CETSA IC_{50} (nM)[a]
1		24	450	2454	636	8448	-
2		25	1423	4642	1721	1066	501
3		36	509	1959	804	6195	727
4		274	9719	148[b]	19400	3168[b]	15849
5		4104	20186	>30000	12791	>30000	303
6		36	1861	24440	4760	17706	2529
7	2-F	25	613	1144	867	3376	150
8	3-F	229	4465	11471	6465	5747	-
9	H	23	835	2647	1224	8448	401
10	F	23	465	1344	585	3416	141

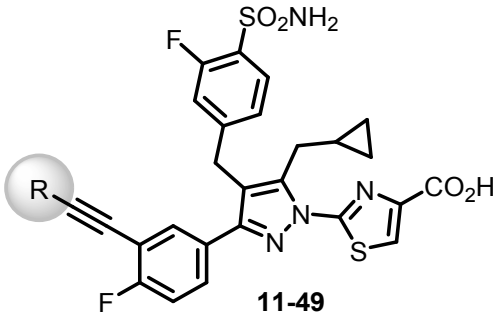
[a]IC_{50} values represent the half maximal (50%) inhibitory concentration as determined in the HTS assay using a dose response in 1536-well format. (n = 2 for lactate and n = 3 for cytotoxicity, CETSA and biochemical LDHA). [b]Efficacy < 20 % with curve class 4, therefore it should be considered as inactive.

Previous SAR[19] suggested that the 3-biphenyl moiety provided significant levels of enzyme and cellular potency. However, this functional group failed to provide the pharmacokinetic profile needed for evaluation in *in vivo* studies. An initial assessment showed no clear SAR trend regarding the presence of a 4-fluoro group (**7** and **10**, Table 1). Consequently, we employed both the 4-fluoro and 4-H for further modifications of the 3-phenyl group in this region. In addition to extensive SAR in the biphenyl series, to be published in due course, we explored alkynes and alkenes as phenyl isosteres. We rationalized that alkynes, with a linear

conformation and minimal steric bulk, could present the terminal groups to a hydrophobic region in the enzyme, and potentially provide tighter binding. In an initial SAR exploration, a simple terminal alkyne **11** (Table 2) retained biochemical activity relative to **10** (Table 1), with only modest loss in potency toward lactate output (< 2 fold) and cytotoxicity (< 3 fold) in both cell lines. We thus explored a variety of substitutions on the alkyne to better understand the SAR in this hydrophobic space in LDH (Tables 2 and 3). Initially, we explored the terminal alkyne region with numerous alkyl and cycloalkyl groups, which could occupy the hydrophobic pocket. Most changes were tolerated, with steadily improved cell-based activities noted as the size of the alkyl group increases (e.g. **16-17** and **20**). Introducing electron-withdrawing polar groups, particularly terminal -CN (**33**) and -CF_3 groups (**34**) which are strong hydrogen bond acceptors, markedly reduced cellular activity. A similar trend of lower cell potency was observed for analogs **18-19** and **35-36**, which also possess electron-withdrawing groups capable of hydrogen bond interactions. Introducing cycloalkyl groups onto the terminal alkyne region resulted in very potent compounds in the biochemical assay, but the activity in cellular assays was more variable. Groups such as cyclopropyl (**21**), cyclobutyl (**22**), and cyclopentyl (**23** & **51**) showed promising potency increases in the cellular assays. When a methylene linker was introduced (**37** vs **21** and **38** vs **23**), potency in the lactate inhibition assay increased or remained similar while less potency was observed in the cytotoxicity assays. Interestingly, when the cycloalkyl groups contained hydrogen bond acceptor or donor atoms, biochemical potency was maintained, but cellular activities significantly dropped (**24-32** and **39-41** in Table 2), consistent with our previous observations. As the SAR indicated a preference for lipophilic groups at the terminal position of the alkyne, we synthesized analog **42,** which replaced the terminal alkyl group with a 2-thienyl group. Though analog **42** was less potent in the biochemical assay (34 nM), it showed improved cellular potency (Table 2) in lactate (438 nM in A673; 307 nM in MiaPaca-2), and cytotoxicity

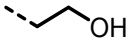
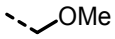
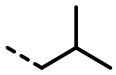
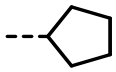
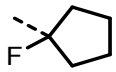
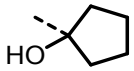
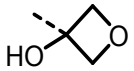
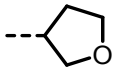
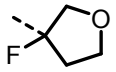
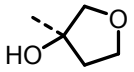
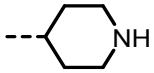
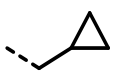
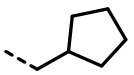
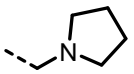
assays (265 nM in A673; 268 nM in MiaPaca-2) compared to **10**. As an unsubstituted thienyl moiety could be metabolically labile[24], we incorporated a 5-methyl thienyl group, which retained similar potency in the cellular assays (**43** and **52**, which also are named as **NCATS-SM1440** and **NCATS-SM1441** respectively). Replacing the methyl group of the thiophene with other alkyl groups (**45**, **55-57**) maintained the potency; however, bulkier substituents such as a *t*-butyl group decreased potency (**45**). Moving the 5-methyl group to the 3-position of the thiophene resulted in similar cellular potency (**53**), whereas switching to the 5-methylthien-3-yl substitution pattern (**54** and **77**) slightly decreased activity. A 3,5- (**66**) or 2,5- dimethyl substitution pattern (**67** & **83**) on the thiophene further improved cellular potency. Interestingly, introduction of halogens onto the 5-alkylthiophene significantly reduced the cellular potency (**58-60)**. Replacing the thiophene with similarly sized heterocycles was well tolerated. For instance, a 5-methylfuran (**44** and **61**) showed similar inhibition of lactate production and cytotoxicity in both cell types compared to the thiophene analogs (**43** and **52**). Introducing a methylthiazole (**46-47**, **63-65**, and **80-82**) or methyloxazole (**62**) also retained biochemical and cellular potencies. The more hydrophilic methyl imidazole (**48**) or methylpyrazole (**49**) analogs showed good biochemical potency but less favorable cellular effects in lactate and cytotoxicity assays.

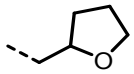
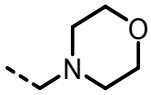
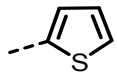
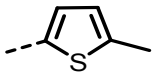
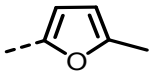
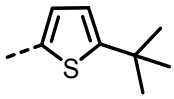
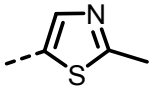
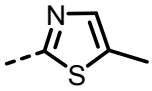
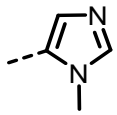
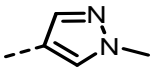
Table 2. Biochemical and Cellular LDH Inhibition of Analogs **11**−**49**.



11-49

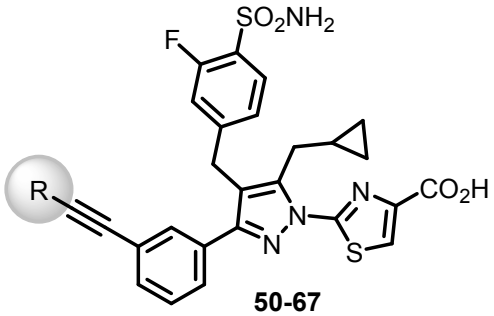
Compd No.	R	Biochemical LDHA IC_{50} (nM)[a]	A673 cells		MiaPaCa-2 cells		HEK293 Cells
			lactate inh. IC_{50} (nM)[a]	Cytotox. IC_{50} (nM)[a]	lactate inh. IC_{50} (nM)[a]	Cytotox. IC_{50} (nM)[a]	CETSA IC_{50} (nM)[a]

11	--H	9	902	4346	660	4025	253
12	$--CH_3$	11	505	332	450	322	119
13	---Et	14	487	716	470	662	112
14	---*n*-Pr	24	420	833	361	746	106
15	---*n*-Bu	50	405	773	298	845	106
16		21	284	1011	487	756	2053
17		16	141	935	137	845	75
18		3	2475	1798	1659	1327	577
19		8	769	1225	525	1092	119
20		54	284	935	487	909	89
21		9	695	132	554	323	100
22		23	378	593	428	573	133
23		22	509	100	333	314	112
24		33	450	689	454	593	95
25		7	1931	2187	1479	2178	150
26		10	1052	1177	898	1236	84
27		7	1324	2647	1047	2263	126
28		6	12241	3587	4873	2994	1499
29		7	1268	973	831	1224	89
30		1	1056	1054	867	882	201
31		5	5937	2635	3871	1738	815
32		15	12241	9052	8910	17302	2579
33	--CN	15	4010	>30000	4401	>30000	474
34	$--CF_3$	450	>30000	>30000	>30000	>30000	14005
35	$--CF_2H$	15	937	1482	741	1327	163
36		14	800	1134	822	1020	127
37		31	234	1016	259	1030	89
38		202	487	939	424	848	178
39		8	1086	973	1007	1102	267

40		9	933	1380	937	1225	178
41		107	15349	5834	18852	18061	5045
42		32	438	265	307	268	89
43 (NCATS-SM1440)		57	873	119	403	257	106
44		47	544	528	547	552	79
45		713	529	743	507	665	10602
46		33	937	569	867	488	-
47		52	1324	803	1603	909	170
48		8	1597	1542	1066	1387	299
49		9	1603	593	1141	619	189

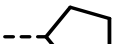
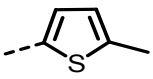
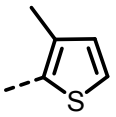
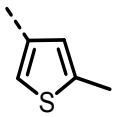
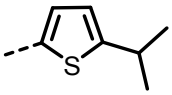
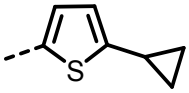
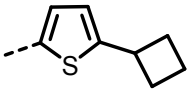
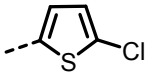
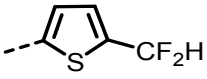
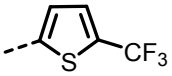
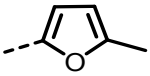
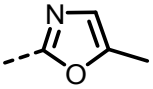
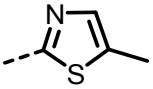
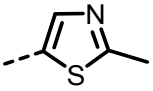
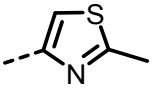
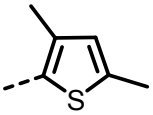
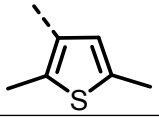
[a]IC_{50} values represent the half maximal (50%) inhibitory concentration as determined in the HTS assay using a dose response in 1536-well format. (n = 2 for lactate and n = 3 for cytotoxicity, CETSA and biochemical LDHA).

Table 3. Biochemical and Cellular LDH Inhibition of Analogs **50**−**67**.



50-67

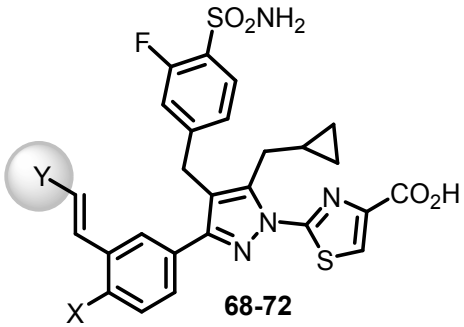
Compd	Biochemic	A673 cells	MiaPaCa-2 cells	HEK293 Cells

No.	R	al LDHA IC_{50} (nM)[a]	lactate inh. IC_{50} (nM)[a]	Cytotox IC_{50} (nM)[a]	lactate inh. IC_{50} (nM)[a]	Cytotox IC_{50} (nM)[a]	CETSA IC_{50} (nM)[a]
50		9	713	695	525	619	150
51		47	298	419	281	377	89
52 (NCATS-SM1441)		40	760	105	367	347	119
53		101	390	419	417	452	119
54		87	494	359	657	332	95
55		385	296	528	203	590	179
56		201	403	332	434	342	183
57		169	333	347	491	905	270
58		167	218	452	408	556	113
59		154	345	528	286	528	134
60		434	485	833	337	803	113
61		36	450	285	432	296	168
62		12	525	552	450	671	119
63		17	527	309	487	360	90
64		43	713	532	720	641	38
65		13	1174	347	1180	471	101
66		137	345	528	218	533	201
67		235	161	716	218	810	299

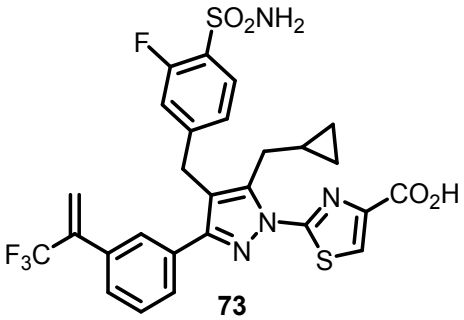
[a]IC_{50} values represent the half maximal (50%) inhibitory concentration as determined in the HTS assay using a dose response in 1536-well format. (n = 2 for lactate and n = 3 for cytotoxicity, CETSA and biochemical LDHA).

We also examined the replacement of the alkyne linker (**68-73,** Table 4) with an alkene motif. This slightly diminished the cellular potency despite low nanomolar biochemical activity. The alkene analogs **68** and **69** showed marginally inferior cellular potency compared to corresponding alkyne analogs **52** and **23**. A similar trend was also observed for other matched pairs, including for alkene analog **70** vs. alkyne **21**, **71** vs. **12**, and **72** vs. **17**.

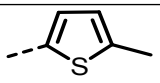
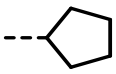
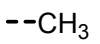
Table 4. Biochemical and Cellular LDH Inhibition of Compounds **68**−**73**.



68-72



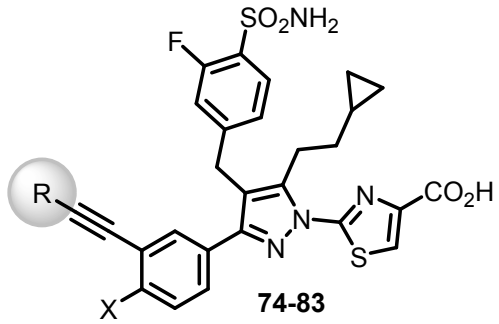
73

Compd No.	Y	X	Biochemical LDHA IC_{50} (nM)[a]	A673 cells		MiaPaCa-2 cells		HEK293 Cells
				lactate inh IC_{50} (nM)[a]	Cytotox IC_{50} (nM)[a]	lactate inh IC_{50} (nM)[a]	Cytotox IC_{50} (nM)[a]	CETSA IC_{50} (nM)[a]
68		H	52	357	452	331	471	158
69		F	74	371	488	149	454	108
70		F	15	320	833	231	837	134
71		F	11	405	614	321	528	126
72		F	59	361	2263	255	1899	112
73	-	-	39	713	16023	972	17376	398

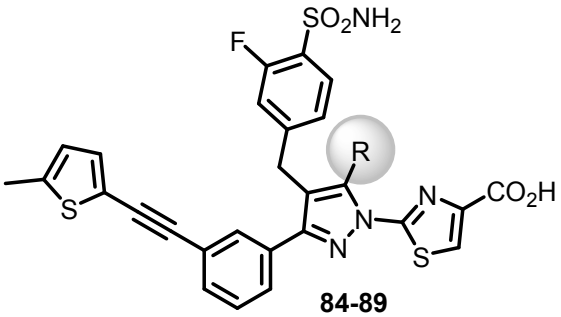
[a]IC_{50} values represent the half maximal (50%) inhibitory concentration as determined in the HTS assay using a dose response in 1536-well format. (n = 2 for lactate and n = 3 for cytotoxicity, CETSA and biochemical LDHA).

Recalling the improved cellular potency of earlier analogs with the ethylcyclopropane pyrazole substitution, we synthesized analogs **74-83** (Table 5) combining this substitution with optimal groups from the terminal alkyne region. As summarized in Table 5, analogs **74-83** showed a noteworthy improvement in cellular potency when compared to their corresponding cyclopropylmethyl analogs mentioned above.

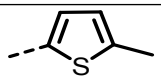
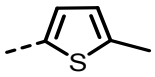
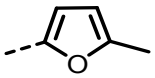
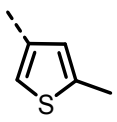
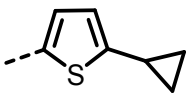
Table 5. Biochemical and Cellular LDH Inhibition of Analogs **74**−**89**.

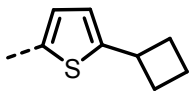
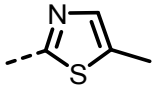
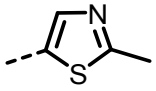
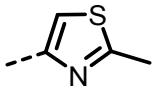
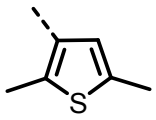
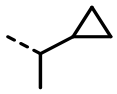
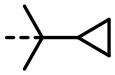
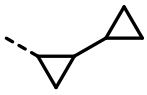
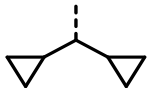


74-83



84-89

Compd No.	R	X	Biochem LDHA IC_{50} (nM)[a]	A673 cells		MiaPaCa-2 cells		HEK293 Cells
				lactate inh IC_{50} (nM)[a]	Cytotox IC_{50} (nM)[a]	lactate inh IC_{50} (nM)[a]	Cytotox IC_{50} (nM)[a]	CETSA IC_{50} (nM)[a]
74		F	202	390	218	197	226	200
75		H	117	174	187	172	171	152
76		H	52	547	137	417	137	95
77		H	160	434	197	254	223	142
78		H	306	434	320	387	265	189

79		H	636	572	571	379	496	299
80		H	33	364	180	417	173	84
81		H	24	434	218	334	218	17756
82		H	24	300	219	253	219	90
83		H	487	162	402	188	402	299
84		-	874	685	968	1027	9350	708
85		-	4540	18264	5899	5144	7427	10751
86		-	307	346	590	409	1049	251
87		-	122	485	526	459	469	175
88		-	1174	364	4686	515	5258	1027
89		-	5270	1864	26351	1219	20931	3183

[a]IC_{50} values represent the half maximal (50%) inhibitory concentration as determined in the HTS assay using a dose response in 1536-well format. (n = 2 for lactate and n = 3 for cytotoxicity, CETSA and biochemical LDHA).

In our previous report[19], we described several crystal structures with similar analogs bound to LDHA, establishing the binding mode of this series of compounds within the LDHA catalytic site, and helping to guide our lead optimization campaign toward the identification of compounds with improved potency. As part of the optimization campaign, we obtained co-crystal structures of analogs **23** and **52** bound to LDHA in the presence of cofactor NADH (Figure 2). Analogs **23** and **52** present similar binding poses as the previously reported

molecules, with the carboxylate making a clearly defined salt bridge interaction with Arg168 and a hydrogen bond with Thr247. Additionally, the cyclopropylmethyl group and cyclopropylethyl group both maintain a potential pseudo π–π interaction between the cyclopropyl group and active-site tyrosine Tyr238; this substitution was found to be vital for potent cellular activity. As demonstrated previously, the 4-benzyl sulfonamide motif makes well-defined H-bonding interactions with side chains of Asp140 and Glu191 as well as with the main chain nitrogens of Asp140, Ile141, and Glu191 to confer potent biochemical and cellular activities. Further, the cyclopentylalkyne in analog **23** and 5-methyl thienyl alkyne analog **52** presented similar binding poses compared to the biphenyl ortholog. The linear rigid confirmation of the alkynes enables the presentation of moieties that offer increased hydrophobic interactions with LDHA while offering lower lipophilicity compared with a phenyl ring. It is unclear from the crystal structure exactly how some of these variations improve cellular potency. For example, the 2-fluoro moiety on the benzenesulfonamide ring is oriented in the same way in both structures. Although this group does occupy a small sub-pocket in the enzyme, no specific interactions with the protein are evident. The changed binding kinetics that this substitution produces (*vide infra*) may instead result from subtle electronic modulation of the hydrogen bonding interactions, which ultimately provided better cellular potency. These efforts demonstrate that a multi-parameter optimization strategy that utilizes structure-based design combined with targeted improvement of residence time, can elicit significant improvements in cellular potency, at least in the context of LDHA inhibitors. All of the synthesized analogs were profiled for their biochemical activity against both LDH isozymes, LDHA and LDHB, and several compounds were tested against a related 'off-target' dehydrogenase, malate dehydrogenase (MDH). All analogs possess nearly identical potency against LDHB compared to LDHA (Table S1) and thus display a highly significant correlation coefficient (Figure S1). Recent reports allude to the need for a pan LDH inhibitor in order to

elicit a cellular phenotype[25], highlighting a possible advantage of this profile. All the analogs showed little or no biochemical activity against MDH, with over 2,500-fold selectivity for LDHA/LDHB (Table S1).

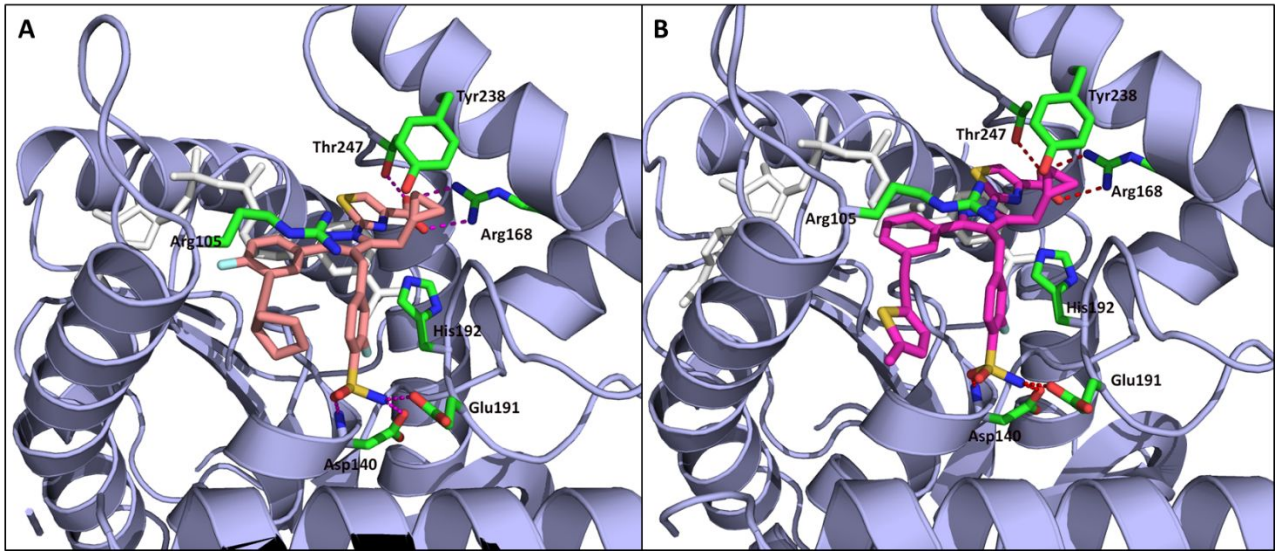


Figure 2: Crystal structure of LDHA bound to inhibitor **23** (A, pdb code 6Q0D) and **52** (B, pdb code 6Q13). LDHA is shown in ribbon (blue) and key residues in the active site are shown in green. Small molecule inhibitors are shown in sticks with salmon- and magenta-colored carbons.

While compounds inactive in the biochemical assay elicit no cellular activity, indicating the on-target nature of the observed effects, we noted only a marginal correlation between the biochemical LDHA inhibition and inhibition of cellular lactate production throughout this optimization campaign. This poor correlation may have hampered prior efforts to identify cell-active LDHA inhibitors. The unusual trend and poor correlation between biochemical and cellular potency may be due to the disparity in the concentration of LDHA in the biochemical assay (2 nM) versus the extremely high concentration of intracellular LDH (estimated to be in the range of 2−17 μM) in glycolytic tumor cells. Furthermore, the nature of the active site preferentially selects for highly lipophilic compounds with an acidic moiety such as a carboxylic acid, which can introduce additional permeability and protein binding challenges that might reduce cellular efficacy and thus influence the correlation. Though structure-guided optimization was crucial in driving the cellular potency, it was also difficult to explain the

drastically improved cytotoxic effects for certain analogs as a result of subtle structural changes during our optimization.

To address such challenges, we reasoned that a highly potent compound with a long drug-target residence time might more effectively inhibit cellular LDH activity. To better understand the reason for improved cellular activity emerging from relatively small structural changes, we complemented the structure-guided SAR with data from an SPR binding assay to analyze the top cell-active compounds, in search of compounds with longer off-rates. A summary of SPR data and the corresponding cell data is outlined in Table 6 for compounds **23**, **38**, **43**, and **52**. All analogs showed more potent binding affinities and longer off-rates (k_{off}) compared to **1**. Analysis of a larger data set across the series (including unpublished data) suggests that the compounds with long off-rates and residence times (τ) [calculated as $1/k_{off}$ (s^{-1})] offer high cellular potency in both the lactate and cytotoxicity assays. The most potent compounds exhibited off-rates that exceeded the detection limit of the assay, 10^{-6} sec^{-1}. Comparing the data presented here with our previously published results suggests that the 2-fluoro group on the sulfonamide phenyl ring and the alkyne substitution both positively contribute to longer residence times, leading to improved binding affinity and more potent cellular activity.

A good correlation was observed for the IC_{50} values for our compounds in MiaPaCa-2 and A673 cell lines, in both lactate secretion and cytotoxicity assays (Figure S2); however, the correlation between inhibition of lactate secretion and cytotoxic effect within the same cell line was poor (Figure S3). As such, a superior lactate inhibition profile did not always translate into improved cytotoxic effects for some compounds. The reason for such discrepancy is unclear but may reflect the differences in the two assay conditions. For example, in the lactate secretion assay, lactate output was measured after 30 min exposure to compound, while cytotoxicity was assessed after 48 h of incubation with compound. It is possible that the ability

of compounds to display higher cytotoxic effects depends on a longer residence time along with higher lactate inhibition.

Given the potential for the inhibitors to alter lactate production or viability due to unidentified off-target activity, we evaluated our LDHA inhibitors in a recently developed split Nano Luciferase (NLuc) high-throughput cellular thermal shift assay (SplitLuc-CETSA) to obtain concentration dependent measurements of intracellular target engagement. We have previously demonstrated the applicability and performance of our SplitLuc CETSA assay to detect LDHA target engagement, in both 384 and 1536-well plate formats, for a set of well-validated inhibitors[26]. Accordingly, we evaluated the majority of the analogs utilizing the SplitLuc-CETSA technique in HEK293T cells (Table1-5) where LDHA was appended with a *C*-terminal 15-amino acid HiBiT fusion tag. A majority of the active compounds in the lactate assay showed cellular binding and thermal stabilization of LDHA in the SplitLuc assay. In general, we observed a good correlation between CETSA IC_{50} and lactate IC_{50} (Figure S4) though we used different cell lines in the two assays. Our lead compounds **42** and **53**, along with many other compounds in the lactate assay, demonstrated stabilization of LDHA within cells. Though biochemically active compounds tended to show good CETSA activity, no clear correlation emerged between the cellular stabilization of LDHA via CETSA and the biochemical IC50. As we stated before, a similar disconnect was observed between biochemical and lactate IC_{50}. As such, biochemical LDHA screening appears to be a poor predictor of cellular effects in this series, the SplitLuc-CETSA cell-based screening platform provides a new avenue for HTS screening in a cellular context, particularly for LDHA.

Table 6. k_{off} and Residence time (τ) data correlation to cell activity for selected analogs**.**

Cpd.	K_D (nM)[a]	Residence Time τ (h)	Off rate[a] k_{off} (x10^4)	A673 cells		MiaPaCa-2 cells	
				lactate inh	cytotox	lactate inh	cytotox
				IC_{50} (nM)[a]	IC_{50} (nM)	IC_{50} (nM)[a]	IC_{50} (nM)[a]
1	0.11	0.35	8	450	2454	636	8448

23	0.0193	>277	0.01	509	100	333	314
38	0.1235	>277	0.01	487	939	424	848
43	0.0667	>277	0.01	873	119	403	257
52	0.0634	>277	0.01	760	105	367	347

[a] K_D and k_{off} were determined via SPR on a Mass-1 instrument from Sierra Sensors and residence time τ was calculated as $1/k_{off}$ (s^{-1}).

To assess the impact of the LDH inhibitors on the glycolytic pathway, the Glycolysis Stress Test (GST) was performed in A673 cells (Figure 3). Glycolytic flux is assessed by measuring the extracellular acidification rate of the media (ECAR) that results from glycolysis-dependent proton production by the cells. As shown in Figure 3, after a stable baseline is recorded, the cells were treated with compounds (**43** and **52**-Figure 3A,B) at increasing concentrations and the ECAR is measured for approximately 50 min before injection of glucose, which results in a substantial stimulation of glycolytic flux and proton production. After three further measurements, oligomycin is injected to inhibit mitochondrial oxidative phosphorylation, which results in a compensatory increase in ECAR that is then suppressed to basal levels by the hexokinase inhibitor 2-deoxyglucose. From this experiment, two glycolysis-dependent parameters can be obtained; the basal glucose-dependent ECAR (glycolysis Figure 3C) and the maximal glycolytic capacity (shown in Figure 3B). As anticipated, as LDH inhibition increases with the concentration of compounds **43** or **52**, the reserve biochemical capacity of LDH within the glycolytic pathway is exceeded, resulting in a depletion of NAD^+ and, ultimately, inhibition of the entire pathway, as evidenced by decreased ECAR.

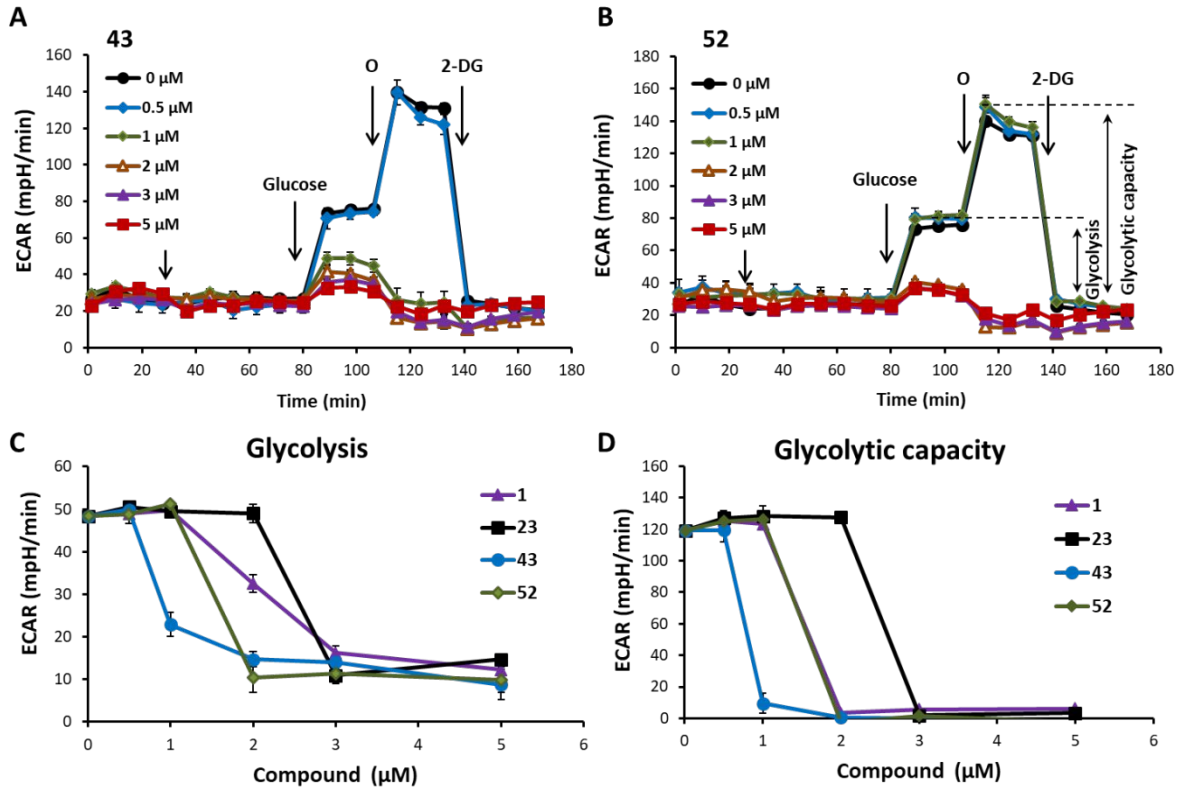


Figure 3. LDH inhibitor-dependent suppression of glycolytic flux in A673 cells. The Glycolysis Stress Test was performed in A673 cells to measure the extracellular acidification rate (ECAR) over time; cellular basal ECAR was measured, then compound **43** (panel A) or **52** (panel B) was injected at increasing concentrations. After 48 min, subsequent injections of glucose (10 mM; glycolysis), oligomycin (1 µg/mL) (O; reaching maximal glycolytic capacity), and 2-deoxyglucose (50 mM) (2-DG; inhibition of glycolysis) were made. C) Quantification of the glycolysis (ECAR after Glucose injection minus basal ECAR), and D) Quantification of Glycolytic capacity of the LDHA inhibitors, **43** and **52** in a dose-response manner in comparison with compounds **1** and **23**. Data represents the mean ± sem, n = 4-6 per group. All LDH inhibitors completely suppressed both basal and maximal glycolysis between 1-3 µM, with **43** and **52** being the most potent and **23** the least.

Compounds in the series were assessed for their Tier 1 ADME profile, using stability in rat liver microsomes (RLM), PAMPA permeability, and aqueous solubility at pH 7.4 (data shown in Table S2). Most compounds showed good aqueous kinetic solubility, presumably enabled by the free carboxylic acid on the thiazole and the benzyl sulfonamide on the 4-position of the pyrazole. However, when additional hydrophilic groups are introduced in the alkyne region (e.g. **26**, **30**, **39**, **47**-**48**, **65**, & **80** in Table S2), the solubility significantly diminished. The RLM stability varied, depending primarily on the structure of the terminal alkyne

substitutions. Analogs with *n*-alkyl groups longer than 2-carbon linkers in the terminal alkyne group (e.g., **14** & **15**; Table S2) were metabolized quickly, likely due to CYP-mediated oxidation. This metabolism was blocked with *t*-butyl (**17**), *i*-Pr (**16**), hydroxy (**18**), or cyclobutyl (**22**) replacements (Table S2). As anticipated, a similar trend was observed for analogs **20** and **21**, with *iso*-butyl and cyclopropyl groups, respectively. Importantly, all the analogs with cycloalkyl or heterocycles on the terminal alkyne exhibited high microsomal stabilities of >30 min, the highest estimable $T_{1/2}$ from a single point (15 min) measurement in our assay. PAMPA permeability was low ((1–10) × 10^{-6} cm/s)[27] in most cases, presumably due to the presence of a carboxylic acid group. Our representative compounds **43** and **52** showed low permeability in PAMPA-BBB assay (Table 7). Notably, a combination of the cyclopropylethyl group on the 5-position of the pyrazole and a methyl thiazole in the terminal alkyne (**80**-**82**; Table S2) slightly improves the permeability. A broader assessment of the in vitro ADME profile for representative compounds **43** and **52** is summarized in Table 7. In time-course studies, both representative compounds showed excellent multi-species metabolic profiles in hepatocytes ($T_{1/2}$ >150 min), cytosol ($T_{1/2}$ >120 min), plasma ($T_{1/2}$ >240 min), and liver microsomes ($T_{1/2}$ >120 min). The analogs showed very high plasma protein binding in mouse and human plasma, likely due to the presence of both carboxylic acid and sulfonamide moieties. Both compounds only showed CYP inhibition and induction at higher concentrations. Further, the compounds were not active in hERG (patch-clamp assay) and Ames profiling assays. No significant phase I and II metabolites were detected in our *in vitro* metabolic study even in the presence of GSH, which is consistent with the prolonged half-life in mouse hepatocytes. Further, both compounds showed less than two-fold PXR and AhR activation at a 10 μM.

Table 7. Summary of in vitro ADME Profiles for **43** and **52**.

Profiling Assays	**43** (**NCATS-SM1440**)	**52** (**NCATS-SM1440**)
liver microsomal $T_{1/2}$ (human, mouse & rat)	>120 min	>120 min
liver cytosolic $T_{1/2}$ (human, mouse & rat)	>120 min	>120 min
metabolic stability (hepatocytes-rat, dog, mouse)	>150 min	>150 min
Plasma Stability $T_{1/2}$	>240 min	>240 min
PAMPA permeability Pe	10.4 x 10^-6 cm/s	2.2 x 10^-6 cm/s)
PAMPA-BBB permeability Pe	16 x 10^-6 cm/s)	9.6 x 10^-6 cm/s)
aq solubility at pH 7.4	>70 µg/mL	>70 µg/mL
plasma protein binding (Fu = % fraction unbound)	0 (human), 0.7 (mouse)	0 (human), 4.4 (mouse)
CYP450 inhibition (isozyme) & IC_{50} shift for TDI determination	2C8: 80% @10 µM, 2B6: 46% @10 µM, 2C9: 45% @10 µM	2C8: 7.5 µM, 2B6: 14 µM
Metabolite ID (in vitro)	No significant Phase I or & II metabolites	No significant Phase I or & II metabolites
CYP induction	No induction at 10 µM	No induction at 10 µM
PXR and AhR Activation	<2 fold activation up to 10 µM	<2 fold activation up to 10 µM
Reactive Metabolite Formation	no GSH adducts formed	no GSH adducts formed
hERG (patch clamp)	>10 µM	>10 µM
Ames	negative	negative

Aqueous kinetic solubility (PBS buffer) and PAMPA permeability, liver microsomal & cytosolic stability studies, were conducted at NCATS. Mouse plasma stability studies were conducted at Pharmaron Inc. and involved five-time points. The microsomal stability data [mouse liver microsomes (MLM), human liver microsomes (HLM), and mouse hepatocytes] were conducted at QuintaraBio and represent the stability in the presence of NADPH and UDPGA. The parent compound was monitored at five-time points.

Encouraged by the improved cellular potency and *in vitro* ADME profiles, we evaluated top compounds for mouse PK properties following IV and PO administration (summarized in Table 8). Representative analogs (**43**-**44**, **46**-**47**, **52**, **61**, **69**, and **74**-**75**) showed improved mouse PK properties compared to **1**. IV administration at 10 mg/kg for selected alkyne analogs showed a terminal half-life of ~5 h or more, far superior to 0.85 h for **1**. Thus, both the use of the substituted alkyne as a phenyl isostere and adding 2-fluoro in the sulfonamide region seem to help improve the *in vivo* half-life. Moreover, most of these analogs, except for **46,** also showed reduced clearance. The clearance increased steadily as the steady-state volume of distribution (V_{ss}) increased, as noted for several matched pairs in the series of analogs **44**, **46**-**47**, **61**, and **69**. Despite the presence of the carboxylic acid functional group, favorable systemic exposure was achieved for several of these compounds, with plasma concentrations

in the range of cellular IC_{50} values following PO dosing at 40 or 50 mg/kg. Introducing more lipophilic groups, such as 5-methyl thienyl (**43** and **52**), 5-methyl furyl (**44** and **61**), or cyclopentyl (**69**) in the alkyne region, significantly improved the bioavailability (74% for **43**; 106% for **44**; 64% for **52**, and 83% for **69**) presumably due to higher lipophilicity leading to better permeability and absorption. Analogs **43** (AUC_{last} = 31.39 µg/mL*h & C_{max} of 5.26 µg/mL at 40 mg/kg) and **52** (AUC_{last} = 53.54 µg/mL*h & C_{max} of 8.83 µg/mL) possessed the best systemic exposure in this series. Relative to these molecules, replacing the cyclopropylmethyl group with cyclopropylethyl in the 5-position of the pyrazole and keeping 5-methyl thienyl in the alkyne region (analogs **76** and **77** with 37% & 42% respectively) significantly reduced the bioavailability and systemic exposure. A similar trend was manifested for analogs **44** and **46** that contain methyl thiazole groups at the terminal alkyne. Overall, our SAR study shows that the 5-methylthienyl group in the alkyne region is key for improved pharmacokinetic properties. Thus, analogs **43** and **52** possess suitable PK profiles for evaluation in efficacy models.

Table 8. Pharmacokinetic Profiles of Top Analogs in CD1 Mice[a].

Compd	Route	Dose	Cl (mL/min/kg)	$T_{1/2}$ (h)	[b]C_{max} (µg/mL)	AUC_{last} (µg/mL·h)	V_{ss} (L/kg)	F (%)
43	IV	10	16.1	5.2	41.79	10.57	1.79	
	PO	40		2.78	5.26	31.39		73.8
44	IV	10	75	5.1	4.77	2.32	10.3	
	PO	50		10.7	1.26	13.52		106
46	IV	10	97	5.75	5.5	1.82	13.9	
	PO	50		3.2	1.82	3.44		38
47	IV	10	128	4.9	3.32	1.36	17.9	
	PO	50		3.54	2.03	2.65		39
52	IV	10	10.4	5.3	33.82	16.53	1.7	
	PO	50		2.6	8.83	53.54		64
61	IV	10	72.3	5	4.57	2.28	12.5	
	PO	50		3.7	2.28	10.17		89
69	IV	10	74.7	5.5	6.44	2.81	13.3	
	PO	50		3.1	1.2	9.22		83
74	IV	10	74.7	6.5	35.33	6.65	2.5	
	PO	50		3.2	4.73	12.24		37
75	IV	10	18.6	5.8	58.23	9.6	1.47	

PO	50	3.7	6.85	20.03	42

[a] Values calculated from drug concentration in plasma following iv or po dosing. n = 3, 8 time points taken over 24 h. Compounds were formulated as a solution in PBS buffered saline with 1.1 equiv of NaOH (final pH 7−8). [b]C_{max} = C_0 (t = 0) for IV administration. All pharmacokinetic studies were conducted at Pharmaron, Inc.

Despite long-standing interest in drugging LDH, efforts have thus far failed to achieve sub-micromolar cellular potency while also achieving optimal PK properties. Having identified best-in-class lead compounds in terms of cellular potency and PK profile, we evaluated the top compounds in a mouse tumor model using A673 cells to demonstrate LDH inhibition *in vivo* (Figure 4). IV administration at 50 mg/kg of **43** and **52** significantly reduced LDH activity in tumors analyzed at 1 h and 6 h post-dose. These two analogs have high exposure and low clearance rates compared to analogs **47**, **69**, and **74**. In mice dosed with **52,** 80% inhibition of tumor LDH activity was observed at 6 h post-dose, compared to approximately 60 % inhibition with **43**. Whereas both **43** and **52** possess good pharmacokinetic properties, **52** has a slightly higher C_{max} and lower clearance, which correlates with its superior *in vivo* LDH inhibition profile. **56** and **74** showed moderate inhibition of LDH activity in tumors, and **14**, **47**, **69**, and **71** has less effect. **56** suppressed tumor LDH activity below 40% of control at 1 h post-dose but failed to maintain significant inhibition at 6 h, possibly due to a higher clearance. The diminished activity of analogs **47**, **69**, and **74** can be attributed to a less optimal PK profile having reduced exposure and higher clearance compared to **43** and **52** (Table 8). These data suggest an excellent correlation of *in vivo* LDH inhibition with the pharmacokinetic profile which is vital to demonstrate sustained *in vivo* LDH inhibition. It is important to note that analogs **42** and **53** are the first LDH inhibitors to demonstrate sustained target engagement *in vivo*. Of note, a clear correlation is evident between the cellular concentrations necessary to affect glycolytic flux and the concentrations achieved *in vivo* at doses that demonstrate target engagement. Consistent with these data, both compounds showed efficacy in sarcoma[20] and pancreatic[21] xenograft models at similar doses.

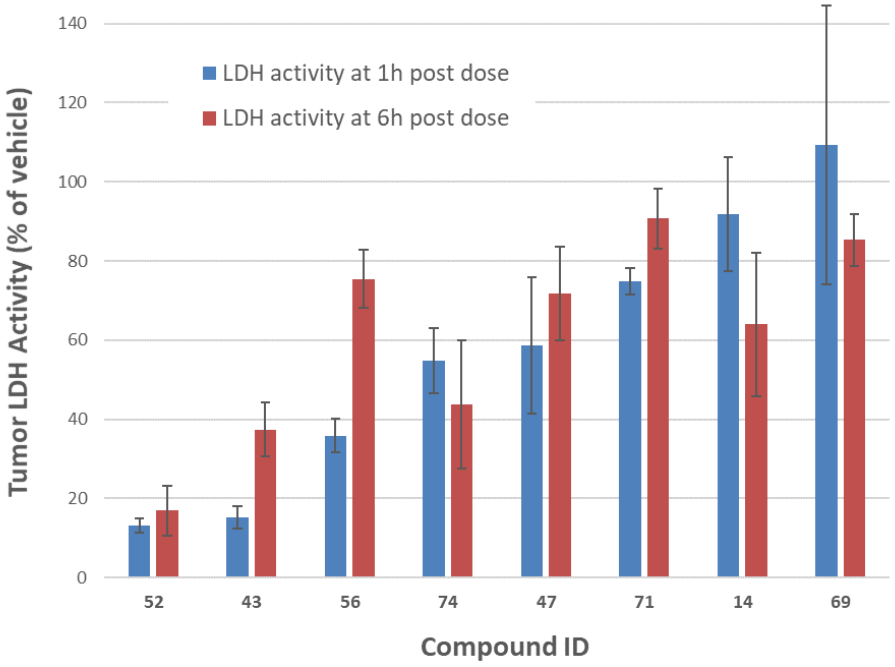


Figure 4: *In vivo* target engagement and tumor LDH activity upon administration of compounds in mice bearing A673 flank xenograft tumors. Mice received a single IV injection of LDHA inhibitors **14**, **43**, **47**, **52**, **56**, **69**, and **71 at** 50 mg/kg. At the time of sacrifice, samples of tumor and plasma were collected and flash frozen in liquid nitrogen. Compound levels in plasma and tumor were determined by LC MS/MS, and LDHA activity was measured in tumor lysates.

In order to develop an LDH inhibitor suitable for *in vivo* experiments, a diverse set of parameters were used to inform design decisions, with a focus on improving cellular potency, establishing a long residence time in the binding pocket, and identifying optimal PK properties. In our case, structure-guided optimization coupled with long residence time helped to identify lead compounds with nanomolar cellular potency. Ultimately, improvement in PK attributes allowed us to demonstrate the first notable inhibition of LDH in tumors, following dosing of mice harboring A673 xenografts. We identified critical structural features in each region of the molecule that contributed to improvements in the properties summarized in Figure 5. In short, the carboxylic acid on the thiazole ring and the 4-benzyl sulfonamide are keystone interactions critical for biochemical and cellular potency. Meanwhile, cyclopropylmethyl or cyclopropylethyl substitution on the pyrazole were crucial for cellular potency. The alkyne linker in the biphenyl region and the 2-fluoro group on the

benznesulfonamide provide a longer off-rate that further improves the cellular activity. Heterocycles, such as 5-methylthienyl, 5-methylfuryl, or thiazolyl groups distal to the alkyne, contribute to improved cellular potency, and most importantly, to improved *in vitro* ADME and *in vivo* PK properties.

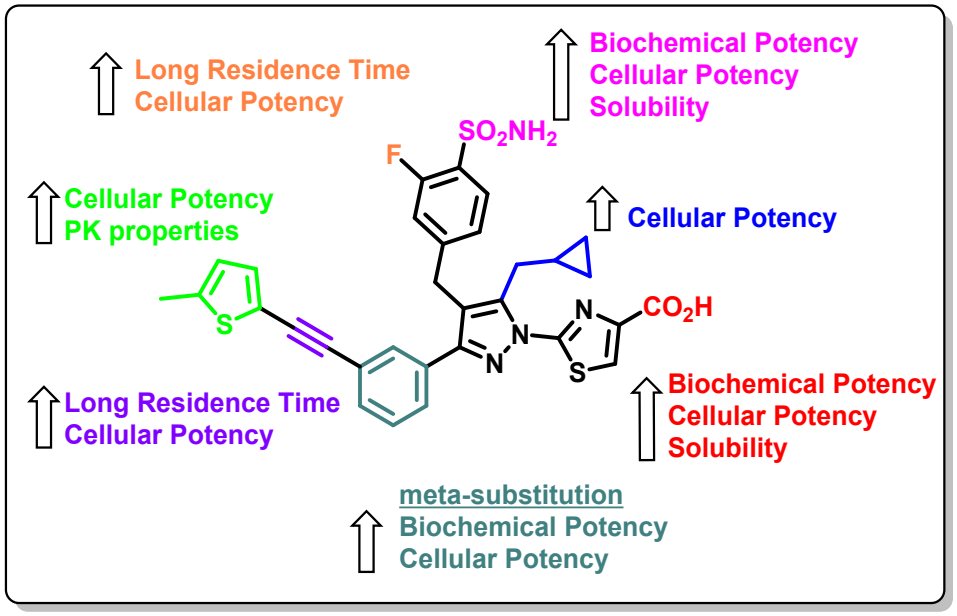


Figure 5: SAR summary and essential structural moieties contributing to the cellular potency, binding affinity, and PK properties of analog **52** (**NCATS-SM1440**) as an example.

Conclusion

Pharmaceutical companies and researchers in academia have invested significant efforts to identify small molecule inhibitors of LDH. Such attempts appear to have shown limited success in discovering potent and drug-like inhibitors that can be utilized to validate LDH as a drug target *in vivo*, a necessary step toward advancing such a molecule towards the clinic. We describe a lead optimization campaign, aided by crystallography as well as the assessment of residence time, that has resulted in LDH inhibitors with improved cellular potency, *in vitro* ADME, and *in vivo* PK properties. We report the first LDH inhibitors showing direct target engagement in human tumor xenografts. Through a systematic SAR campaign, we identified optimal structural features in each region of the molecule that contributed to slow off-rates and improved PK properties, parameters which we found to be significant determinants of

improved cellular potency and *in vivo* target engagement. We report compounds with enhanced cellular potency and properties that enable modulation of LDH activity *in vivo*, thus opening the door for studies to evaluate LDH as a pharmaceutical target in cancer models such as Ewing's sarcoma[20] or pancreatic cancers[21].

EXPERIMENTAL SECTION

Chemistry. *General Methods*. All air or moisture-sensitive reactions were performed under a positive pressure of nitrogen or argon with oven-dried glassware. Anhydrous solvents and bases such as dichloromethane, *N,N*-dimethylformamide (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). The 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. The 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 an 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).

General procedure for the synthesis of acybenzotriazole derivatives **Ia-l** *(Procedure A)*. These compounds were prepared as described previously[19]. To the 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, 1 eq) and the reaction mixture was stirred at room temperature for 0.5 h. After cooling in an ice-water bath (For larger scale, the cooling was necessary due to exothermic reaction. If the reaction mixture formed thick precipitate that was difficult to stir, then more DCM was added) the carboxylic acid (999 mmol, 1 eq) was carefully added and the mixture was stirred for 6 h. The reaction was filtered, and the filter cake was washed with DCM. To the filtrate was added bicarbonate solution slowly and the resulting mixture was stirred for 30 minutes then transferred to a separatory funnel. The organic layer was subsequently washed with bicarbonate solution and brine solution. The organic layer was dried under sodium sulfate and concentrated to provide a thick oil. The crude product was purified on a CombiFlash system using a 340 g silica column eluting with 0-20% ethyl acetate in hexanes over 10 column volumes. The first peak was collected, concentrated, and dried to get pure products **Ia-l** as oil which eventually solidifies into white solid in 91 -100% yield.

General procedure for the synthesis of 1-aryl-3-substituted propane-1,3-diones ***III*** *(Procedure B).* To a mixture of appropriate acetophenone **IIa-d** (51.0 mmol, 1 eq), magnesium

bromide diethyl etherate (32.9 g, 127 mmol, 2.5 eq) and the corresponding acybenzotriazole derivative **Ia-l** (76 mmol, 1.5 eq in CH_2Cl_2 (100 mL) was added Hunig's base (26.7 mL, 153 mmol, 3 eq) slowly (cooling is necessary for large scale) and the mixture was stirred at rt for 12 h. The reaction mixture was cooled in an ice bath and quenched with 1 M HCl. The product was extracted with CH_2Cl_2 and the organic layer was subsequently washed with brine. After drying the organic layer with $MgSO_4$, the crude product was purified on an Isco flash system using a 220 g gold column eluting with 0-30% ethyl acetate over 20 column volumes in hexanes to afford yellow oil after removing the solvent in 60-69% yield required intermediates **III**.

*General procedure for the alkylation of 1-aryl-3-substituted propane-1,3-diones **Va-r** (Procedure C)***.** 1-Aryl-3-substituted propane-1,3-dione (35.9 mmol, 1 eq) and cesium carbonate (14.05 g, 43.1 mmol, 1.2 eq) in DMSO (25 mL) was stirred at room temperature for 10 minutes. The appropriate 4-(bromomethyl)benzenesulfonamide **IVa-c** (43.1 mmol, 1.2 eq) was added in one portion and the reaction mixture was further stirred at room temperature for another 1-2 h. The resulting mixture was diluted with large excess ethyl acetate and filtered through Celite to remove any solid impurities. The filtrate was washed with saturated ammonium chloride (3X) and then with brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified directly on a flash system using a 220 g silica column eluting with 20-60% ethyl acetate in hexanes over 16 column volumes to afford pure products **Va-r** in 55-83% yield.

*General procedure for the cyclization of 1-aryl-2, 3-disubstituted propane-1,3-diones to obtain **VIa-r** and **VIIa-r** (Procedure D)*. To a mixture of the appropriate 1-aryl-2, 3-disubstituted propane-1,3-dione (2.24 mmol, 1 eq) in ethanol was added pyrrolidine (0.5 eq) and tosic acid (0.5 eq) the mixture was heated at reflux for 1 -2 h. The reaction mixture was removed from the heating block and then ethyl 2-hydrazinylthiazole-4-carboxylate hydrogen

bromide (0.600 g, 2.24 mmol, 1 eq) was added in one portion. The reaction was again heated at reflux for 12 h. The reaction mixture was concentrated, and the residue was taken up in dichloromethane and loaded to a pre-packed silica loading cartridge. The product was purified on a flash system using a 100 g silica column eluting with 20-40% ethyl acetate in hexanes to get an inseparable 1:1 mixture of regioisomers in a combined yield of 77-83%. The product mixture was then taken up in DMSO and injected onto a C18 gold column and eluted with 60-100% ACN-water (contains 0.1% TFA). The 2nd peak (the desired isomers **VIa-r**) was pooled and most of the ACN was removed under reduced pressure. The solid formed was collected by filtration, washed with water and air-dried to get pure compounds **VIa-r** in 21–36 % yield.

General procedure for Sonagashira coupling using P(t-Bu)$_3$]Pd(crotyl)Cl catalyst (Procedure E). A mixture of ethyl 2-(3-(3-bromo-4-H/fluoro phenyl)-5-(substituted)-4-(3/4-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (1 mmol), P(*t*-Bu)$_3$]Pd(crotyl)Cl precatalyst Pd-162 (5 mol %) and DABCO (2 mmol, 2 eq) in dioxan (0.5 molar concentration) was bubbled with argon for 5 minutes then the alkyne (1.5 mmol, 1.5 eq) was added and the mixture was stirred at room temperature overnight. After completion of the reaction, silica bound palladium scavenger was added and the mixture was stirred at room temperature for 1h, then diluted with ethyl acetate and filtered through a pad of celite. The filtrate was concentrated and the residue was purified on isco flash system eluting with 20-40 % ethyl acetate in hexanes over 20 column volumes to obtain pure products (NOTE: The above reaction also works with 10 mol % tri-*tert*-butylphosphonium tetrafluoroborate and 5 mol % allylpalladium chloride dimer using the same conditions).

General procedure for Sonagashira coupling using [DTBNpP]Pd(crotyl)Cl catalyst (Procedure F). A mixture of ethyl 2-(3-(3-bromo-4-H/fluoro phenyl)-5-(substituted)-4-(3/4-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (1 mmol), DTBNpP]Pd(crotyl)Cl precatalyst Pd-163, and DABCO (2 mmol, 2 eq) in dioxane (0.5 molar

concentration) was bubbled with argon for 5 minutes the alkyne (1.5 mmol, 1.5 eq) was added and the reaction mixture was stirred at 60 ^{o}C overnight. After completion of the reaction, silica bound palladium scavenger was added and the mixture was stirred at room temperature for 1h, then diluted with ethyl acetate and filtered through a pad of celite. The filtrate was concentrated and the residue was purified on isco flash system eluting with 20-100 % ethyl acetate in hexanes over 25 column volumes to obtain pure products.

A *General procedure for Suzuki coupling (Procedure G)*. To a mixture of ethyl 2-(3-(3-bromo-4-substitutedphenyl)-5-(cyclopropylmethyl)-4-(3-substituted-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carbox-ylate (0.2 mmol, 1 eq), potassium phosphate (0.4 mmol, 2 eq), SPhos Pd(crotyl)Cl precatalyst Pd-172 (2.5 mol%) and the appropriate boronic acid/pinacol ester/ trifluoroboronate in a biotage microwave vial was added dioxane (2 mL) and water (0.5 mL). The reaction mixture was bubbled with argon for few minutes then capped and stirred at 100 ^{o}C in a preheated heating block for 0.5 h. Upon completion of the reaction, the reaction mixture was cooled and stirred with a metal scavenger for 1 h. The reaction mixture was then diluted with ethyl acetate and filtered through a pad of celite. The filtrate was concentrated and purified on an isco flash system using a silica column eluting with 20-40 % ethyl acetate in hexanes to get pure products.

*General Procedure for the hydrolysis of the esters to synthesize analogs **1-89** (Procedure H)*. The corresponding ethyl ester intermediates were suspended in THF-MeOH (3/2; 5 mL) and treated with 1.5 M solution (5 eq) of aqueous lithium hydroxide. The reaction mixture was stirred for 1 h, solvent was removed by forced air and acidified with 1 M hydrochloric acid. The crude material was taken up in DMSO and purified on a preparative HPLC.

*2-(3-([1,1'-Biphenyl]-3-yl)-5-((2,2-difluorocyclopropyl)methyl)-4-(4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**2**)*. This compound was

synthesized using general procedures A, B, C, D, and H via intermediates **Ib** through **VIIg**. LC-MS Retention Time: (Method 1) = 6.071 min and (Method 2) = 3.483 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.77 – 7.67 (m, 4H), 7.63 – 7.50 (m, 2H), 7.49 – 7.41 (m, 4H), 7.39 – 7.28 (m, 5H), 4.19 (s, 2H), 3.45 - 3.35 (m, 2H), 2.27 – 2.11 (m, 1H), 1.56 – 1.30 (m, 2H); HRMS (ESI) *m/z* $(M+Na)^+$ calcd. for $C_{30}H_{24}F_2N_4NaO_4S_2$ 629.1099, found 629.1122.

*2-(3-([1,1'-Biphenyl]-3-yl)-5-(2-cyclopropylethyl)-4-(4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**3**)*. This compound was synthesized using general procedures A, B, C, D, and H via intermediates **Ic** through **VIIh**. LC-MS Retention Time: (Method 1) = 6.355 min and (Method 2) = 3.598 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 1H), 8.28 (s, 1H), 7.79 – 7.65 (m, 4H), 7.61 (dt, J = 7.7, 1.4 Hz, 1H), 7.57 – 7.48 (m, 1H), 7.52 – 7.40 (m, 4H), 7.44 – 7.33 (m, 2H), 7.38 – 7.29 (m, 4H), 4.18 (s, 2H), 3.30 – 3.22 (m, 2H), 1.45 (q, J = 7.3 Hz, 2H), 0.87 – 0.67 (m, 1H), 0.36 – 0.25 (m, 2H), 0.17 – 0.09 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.79, 152.52, 144.58, 144.09, 142.23, 140.38, 139.40, 132.38, 129.51, 129.01, 128.26, 127.73, 126.95, 126.57, 126.53, 126.00, 125.87, 125.68, 117.02, 33.32, 28.37, 24.81, 15.23, 15.20, 10.62, 4.19; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{29}N_4O_4S_2$ 585.1625, found 585.1604.

*2-(3-([1,1'-Biphenyl]-3-yl)-4-(4-sulfamoylbenzyl)-5-(2,2,2-trifluoroethyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**4**)*. This compound was synthesized using general procedures A, B, C, D, and H via intermediates **Id** through **VIIk**. LC-MS Retention Time: (Method 1) = 5.991 min and (Method 2) = 3.588 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (s, 1H), 8.33 (s, 1H), 7.74 – 7.62 (m, 5H), 7.50 (td, J = 7.6, 0.7 Hz, 1H), 7.43 (d, J = 4.3 Hz, 4H), 7.39 – 7.28 (m, 5H), 4.69 (q, J = 10.5 Hz, 2H), 4.30 (s, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{28}H_{22}F_3N_4O_4S_2$ 599.1029, found 599.1043.

*2-(3-([1,1'-Biphenyl]-3-yl)-4-(4-sulfamoylbenzyl)-5-(1-(trifluoromethyl)cyclopropyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**5**).* This compound was synthesized using general procedures A, B, C, D, and H via intermediates **Ie** through **VIII**. LC-MS Retention Time: (Method 1) = 6.031 min and (Method 2) = 3.61 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.11 (s, 1H), 8.34 (s, 1H), 7.76 – 7.70 (m, 2H), 7.69 – 7.62 (m, 3H), 7.52 – 7.45 (m, 1H), 7.45 – 7.27 (m, 9H), 4.37 (s, 2H), 1.79 – 1.09 (m, 5H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{24}F_3N_4O_4S_2$ 625.1186, found 625.1215.

*2-(3-([1,1'-Biphenyl]-3-yl)-5-(2,2-difluorocyclopropyl)-4-(4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**6**).* This compound was synthesized using general procedures A, B, C, D, and H via intermediates **If** through **VIIm**. LC-MS Retention Time: (Method 1) = 5.858 min and (Method 2) = 3.553 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 – 12.91 (m, 1H), 8.34 (s, 1H), 7.79 – 7.66 (m, 4H), 7.62 – 7.50 (m, 2H), 7.49 – 7.40 (m, 4H), 7.39 – 7.27 (m, 5H), 4.22 (s, 2H), 3.29 – 3.23 (m, 1H), 2.25 – 2.04 (m, 1H), 1.85 – 1.63 (m, 1H); HRMS (ESI) *m/z* (M+H)+ calcd. for $C_{29}H_{23}F_2N_4O_4S_2$ 593.1123, found 593.114.

*2-(3-([1,1'-Biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**7**).* This compound was synthesized using general procedures A, B, C, D, and H via intermediates **Ia** through **IVb** and **VIIb**. LC-MS Retention Time: (Method 1) = 6.133 min and (Method 2) = 3.641 min; 1H NMR (400 MHz, DMSO-d6) δ 13.16 (s, 1H), 8.30 (s, 1H), 7.73 – 7.65 (m, 3H), 7.64 – 7.58 (m, 3H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.49 – 7.41 (m, 4H), 7.40 – 7.33 (m, 1H), 7.26 – 7.07 (m, 2H), 4.20 (s, 2H), 3.19 (d, *J* = 6.9 Hz, 2H), 1.34 – 1.04 (m, 1H), 0.44 – 0.29 (m, 2H), 0.29 – 0.17 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{26}FN_4O_4S_2$ 589.1374, found 589.1363.

*2-(3-([1,1'-Biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(2-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**8**).* This compound was synthesized using general procedures A, B, C, D, and H via intermediates **Ia** through **IVc** and **VIIc**. LC-MS

Retention Time: (Method 1) = 6.234 min and (Method 2) = 3.576 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.31 (s, 1H), 7.73 – 7.64 (m, 2H), 7.62 – 7.56 (m, 2H), 7.53 (ddd, J = 7.6, 4.2, 1.2 Hz, 2H), 7.51 – 7.40 (m, 6H), 7.40 – 7.33 (m, 1H), 7.21 (t, J = 7.8 Hz, 1H), 4.14 (s, 2H), 3.20 (d, J = 6.9 Hz, 2H), 1.27 – 1.06 (m, 1H), 0.40 – 0.31 (m, 2H), 0.27 – 0.20 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{26}FN_4O_4S_2$ 589.1374, found 589.1394.

*2-(5-(Cyclopropylmethyl)-3-(6-fluoro-[1,1'-biphenyl]-3-yl)-4-(4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**9**)*. This compound was synthesized using general procedures A, B, C, D, and H via intermediates **Ia** through **IVa** and **VIId**. LC-MS Retention Time: (Method 1) = 6.297 min and (Method 2) = 3.534 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 6.68 (s, 1H), 6.11 (d, J = 8.1 Hz, 2H), 6.03 (ddd, J = 8.7, 4.7, 2.3 Hz, 1H), 5.96 (dd, J = 7.6, 2.3 Hz, 1H), 5.88 – 5.66 (m, 10H), 2.56 (s, 2H), 1.56 (d, J = 6.9 Hz, 2H), -0.37 – -0.53 (m, 1H), -1.28 (dt, J = 8.5, 2.8 Hz, 2H), -1.35 – -1.42 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{26}FN_4O_4S_2$ 589.1374, found 589.1364.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(6-fluoro-[1,1'-biphenyl]-3-yl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**10**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.78 mmol) with phenylboronic acid utlizing the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.073 min and (Method 2) = 3.54 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.30 (s, 1H), 7.72 – 7.62 (m, 2H), 7.60 (s, 2H), 7.55 (dd, J = 7.6, 2.3 Hz, 1H), 7.50 – 7.35 (m, 6H), 7.19 (dd, J = 11.3, 1.6 Hz, 1H), 7.08 (dd, J = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.24 – 3.11 (m, 2H), 1.24 – 1.10 (m, 1H), 0.45 – 0.31 (m, 2H), 0.29 – 0.17 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.77, 161.19, 151.50, 144.67, 144.47, 134.41, 129.72, 128.70, 128.67, 128.64, 128.57, 128.53, 128.29, 128.14, 126.13, 123.64, 116.95, 116.72, 116.55, 116.45, 116.23, 28.23, 28.14, 15.18, 10.32, 4.49; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}F_2N_4O_4S_2$ 607.128, found 607.129.

*2-(5-(Cyclopropylmethyl)-3-(3-ethynyl-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**11**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (3.14 mmol, 2 g) with trimethylsilylacetylene utilizing the general Sonogashira coupling procedure E. The product (3.05 mmol, 2 g) obtained was deprotected while stirring with CsF (0.51 g, 3.36 mmol, 1.1 eq) in THF/ethanol (11 mL/4 mL) at room temperature for 2 h. The reaction mixture was diluted with ethyl acetate and filtered through a celite pad. The crude product after removing the solvent was purified in an isco flash system using a silica column eluting with 20-40 % ethyl acetate in hexanes to get pure TMS deprotected product **VIIIb** in 90 % yield over 2 steps. LC-MS Retention Time: (Method 2) = 3.72 $(M+H)^+ = 583$. ^{1}H NMR (400 MHz, DMSO-d_6) 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). A portion of **VIIIb** was hydrolyzed using general procedure H to obtain analog **11**. LC-MS Retention Time: (Method 1) = 5.653 min and (Method 2) = 3.66 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.74 – 7.59 (m, 3H), 7.57 (s, 2H), 7.35 (t, *J* = 9.1 Hz, 1H), 7.14 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.04 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.55 (s, 1H), 4.16 (s, 2H), 3.16 (d, *J* = 6.9 Hz, 2H), 1.22 – 1.04 (m, 1H), 0.43 – 0.26 (m, 2H), 0.26 – 0.11 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{26}H_{21}F_2N_4O_4S_2$ 555.0967, found 555.0966.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(prop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**12**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.314 mmol, 0.2 g) with potassium trifluoro(prop-1-yn-1-yl)borate (0.069 g, 0.471 mmol) using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS

Retention Time: (Method 1) = 5.928 min and (Method 2) = 3.518 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.65 (t, J = 7.9 Hz, 1H), 7.59 (d, J = 6.6 Hz, 3H), 7.53 (ddd, J = 8.6, 5.0, 2.3 Hz, 1H), 7.30 (dd, J = 9.5, 8.6 Hz, 1H), 7.14 (dd, J = 11.4, 1.6 Hz, 1H), 7.04 (dd, J = 8.2, 1.6 Hz, 1H), 4.14 (s, 2H), 3.16 (s, 2H), 2.09 (s, 3H), 1.20 – 1.06 (m, 1H), 0.39 – 0.29 (m, 2H), 0.25 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{27}H_{23}F_2N_4O_4S_2$ 569.1123, found 569.1116.

*2-(3-(3-(But-1-yn-1-yl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**13**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 2-(1-butyn-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.294 mmol, 0.053 g) using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.118 min and (Method 2) = 3.605 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.66 (t, J = 7.9 Hz, 1H), 7.60 – 7.47 (m, 4H), 7.34 – 7.23 (m, 1H), 7.15 (dd, J = 11.3, 1.6 Hz, 1H), 7.04 (dd, J = 8.1, 1.6 Hz, 1H), 4.14 (s, 2H), 3.17 (d, J = 6.9 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H), 1.15 (s, 1H), 1.14 – 1.07 (m, 1H), 0.38 – 0.29 (m, 2H), 0.24 – 0.17 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{28}H_{25}F_2N_4O_4S_2$ 583.128, found 583.1267.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(pent-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**14**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 1-pentyne (0.294 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.246 min and (Method 2) = 3.718 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.30 (s, 1H), 7.66 (t, J = 7.9 Hz, 1H), 7.62 – 7.51 (m, 4H), 7.35 – 7.25 (m, 1H), 7.15 (dd, J = 11.3, 1.6 Hz, 1H), 7.04 (dd, J = 8.1, 1.6 Hz, 1H), 4.14 (s, 2H), 3.24 – 3.07 (m, 4H), 2.44 (t, J = 6.9 Hz, 2H), 1.56

(h, *J* = 7.2 Hz, 2H), 1.26 – 1.06 (m, 1H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.39 – 0.29 (m, 2H), 0.27 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{29}H_{27}F_2N_4O_4S_2$ 597.1436, found 597.144.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(hex-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**15**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 1-hexyne (0.294 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.511 min and (Method 2) = 3.644 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.30 (s, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.62 – 7.50 (m, 4H), 7.30 (dd, *J* = 9.4, 8.6 Hz, 1H), 7.15 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.04 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.14 (s, 2H), 3.23 – 3.10 (m, 3H), 2.46 (t, *J* = 6.9 Hz, 2H), 1.59 – 1.37 (m, 4H), 1.20 – 1.06 (m, 1H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.38 – 0.29 (m, 2H), 0.25 – 0.16 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{29}F_2N_4O_4S_2$ 611.1593, found 611.1572.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3-methylbut-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**16**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.235 mmol, 0.15 g) with 3-methyl-1-butyne (0.353 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.274 min and (Method 2) = 3.563 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.60 – 7.50 (m, 4H), 7.30 (t, *J* = 9.0 Hz, 1H), 7.16 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.14 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 2.84 (hept, *J* = 6.8 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 6H), 1.14 (ddt, *J* = 9.9, 7.5, 4.8 Hz, 1H), 0.39 – 0.28 (m, 2H), 0.27 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{29}H_{27}F_2N_4O_4S_2$ 597.1436, found 597.1424.

*2-(5-(Cyclopropylmethyl)-3-(3-(3,3-dimethylbut-1-yn-1-yl)-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**17**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.314 mmol, 0.2 g) with 2-(3,3-dimethylbut-1-yn-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.471 mmol) utlizing the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.679 min and (Method 2) = 3.754 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.66 (t, J = 7.9 Hz, 1H), 7.58 (s, 2H), 7.62 – 7.53 (m, 1H), 7.50 (dd, J = 6.9, 2.3 Hz, 1H), 7.29 (dd, J = 9.4, 8.7 Hz, 1H), 7.17 (dd, J = 11.4, 1.6 Hz, 1H), 7.05 (dd, J = 8.1, 1.6 Hz, 1H), 4.15 (s, 2H), 3.18 (d, J = 6.9 Hz, 2H), 1.29 (s, 9H), 1.20 – 1.07 (m, 1H), 0.39 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{29}F_2N_4O_4S_2$ 611.1593, found 611.1598.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(4-hydroxybut-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**18**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.235 mmol, 0.15 g) with potassium (4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)trifluoroborate (0.353 mmol) utlizing the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.211 min and (Method 2) = 3.266 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.69 – 7.60 (m, 2H), 7.58 (s, 2H), 7.54 (ddd, J = 8.7, 5.0, 2.3 Hz, 1H), 7.30 (dd, J = 9.4, 8.7 Hz, 1H), 7.14 (dd, J = 11.4, 1.6 Hz, 1H), 7.04 (dd, J = 8.2, 1.6 Hz, 1H), 4.91 (d, J = 6.0 Hz, 1H), 4.15 (s, 2H), 3.59 (q, J = 6.5 Hz, 2H), 3.17 (d, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 1.19 – 1.06 (m, 1H), 0.39 – 0.28 (m, 2H), 0.26 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{28}H_{25}F_2N_4O_4S_2$ 599.1229, found 599.123.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3-methoxyprop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**19**)*. This compound

was synthesized from coupling the advanced intermediate **VIIf** (0.235 mmol, 0.15 g) with 2-(3-methoxyprop-1-yn-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.353 mmol) utlizing the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.782 min and (Method 2) = 3.465 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.29 (s, 1H), 7.70 – 7.56 (m, 5H), 7.40 – 7.31 (m, 1H), 7.15 (dd, J = 11.4, 1.6 Hz, 1H), 7.05 (dd, J = 8.1, 1.6 Hz, 1H), 4.36 (s, 2H), 4.16 (s, 2H), 3.33 (s, 3H), 3.17 (d, J = 6.4 Hz, 2H), 1.32 – 1.01 (m, 1H), 0.39 – 0.28 (m, 2H), 0.26 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{28}H_{25}F_2N_4O_4S_2$ 599.1229, found 599.1232.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(4-methylpent-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**20**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.235 mmol, 0.15 g) with isobutylacetylene (0.353 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.536 min and (Method 2) = 3.787 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.30 (s, 1H), 7.66 (t, J = 7.9 Hz, 1H), 7.61 – 7.52 (m, 4H), 7.31 (ddd, J = 9.3, 8.0, 1.2 Hz, 1H), 7.15 (dd, J = 11.4, 1.6 Hz, 1H), 7.04 (dd, J = 8.1, 1.6 Hz, 1H), 4.14 (s, 2H), 3.16 (d, J = 6.8 Hz, 2H), 2.36 (d, J = 6.4 Hz, 2H), 1.85 (dp, J = 13.1, 6.6 Hz, 1H), 1.19 – 1.08 (m, 1H), 0.99 (d, J = 6.7 Hz, 6H), 0.38 – 0.28 (m, 2H), 0.26 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{29}F_2N_4O_4S_2$ 611.1593, found 611.1608.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(4-methylpent-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**21**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.235 mmol, 0.15 g) with ethynylcyclopropane (0.353 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.026 min and (Method 2) = 3.491 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31

(s, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.60 – 7.49 (m, 4H), 7.28 (t, *J* = 9.0 Hz, 1H), 7.15 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.04 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.14 (s, 2H), 3.16 (d, *J* = 6.6 Hz, 2H), 1.59 (tt, *J* = 8.2, 5.0 Hz, 1H), 1.14 (ddt, *J* = 14.8, 7.7, 3.7 Hz, 1H), 0.98 – 0.86 (m, 2H), 0.81 – 0.72 (m, 2H), 0.39 – 0.28 (m, 2H), 0.31 – 0.18 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.75, 161.12, 159.28, 156.76, 150.92, 147.40, 147.33, 144.58, 144.45, 132.35, 129.40, 128.89, 128.80, 128.49, 126.17, 123.66, 116.76, 116.40, 116.18, 115.96, 111.92, 111.76, 100.05, 68.25, 28.10, 15.24, 15.08, 10.30, 8.65, 4.47; HRMS (ESI) *m/z* $(M+Na)^+$ calcd. for $C_{29}H_{24}F_2N_4NaO_4S_2$ 617.1099, found 617.1126.

*2-(3-(3-(Cyclobutylethynyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**22**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.275 mmol, 0.175 g) with ethynylcyclobutane (0.412 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.392 min and (Method 2) = 3.698 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.60 – 7.51 (m, 4H), 7.30 (t, *J* = 8.9 Hz, 1H), 7.16 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.15 (s, 2H), 3.38 – 3.25 (m, 1H), 3.17 (d, *J* = 6.7 Hz, 2H), 2.38 – 2.25 (m, 2H), 2.21 – 2.06 (m, 2H), 2.05 – 1.81 (m, 2H), 1.14 (ddd, *J* = 12.7, 7.5, 5.0 Hz, 1H), 0.39 – 0.28 (m, 2H), 0.31 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+Na)^+$ calcd. for $C_{30}H_{26}F_2N_4NaO_4S_2$ 631.1256, found 631.1285.

*2-(3-(3-(Cyclopentylethynyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**23**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (3.14 mmol, 2 g) with cyclopentyl acetylene (4.39 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.319 min and (Method 2) = 3.772 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.66

(t, *J* = 7.9 Hz, 1H), 7.60 – 7.51 (m, 4H), 7.34 – 7.24 (m, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.14 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 2.89 (p, *J* = 7.2 Hz, 1H), 2.04 – 1.92 (m, 1H), 1.76 – 1.51 (m, 4H), 1.19 – 1.08 (m, 1H), 0.39 – 0.26 (m, 2H), 0.29 – 0.18 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.15, 161.75, 161.12, 159.31, 150.90, 147.33, 144.61, 144.46, 132.27, 129.56, 129.41, 128.94, 128.85, 128.52, 128.26, 126.17, 123.64, 116.70, 116.39, 116.22, 116.17, 116.00, 112.00, 72.79, 33.33, 30.08, 28.10, 24.64, 15.30, 15.23, 15.09, 10.30, 4.48; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{29}F_2N_4O_4S_2$ 623.1593, found 623.1584.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((1-fluorocyclopentyl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**24**)*. To a mixture of triethylamine trihydrofluoride (0.049 ml, 0.300 mmol) and TEA (0.021 ml, 0.150 mmol) in dichloromethane (1 mL) at -78 °C was added XtalFluor-M[28] (0.055 g, 0.225 mmol) followed by ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((1-hydroxycyclopentyl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate [prepared from the intermediate **VIIf** and 1-ethynyl-1-cyclopentanol using Sonogashira coupling procedure E] (0.1 g, 0.150 mmol). The reaction mixture was allowed to attain room temperature and stirred overnight. The reaction was quenched with aqueous sodium bicarbonate solution and extracted twice using ethyl acetate. The organic layer was concentrated and the crude product was purified in an isco flash system eluting with 10-50 % ethyl acetate in hexanes over 20 column volumes. The intermediate obtained was subsequently hydrolyed using general procedure H. LC-MS Retention Time: (Method 1) = 6.706 min and (Method 2) = 3.739 min; (sufficient amount of material was not available to run NMR); LCMS *m/z* $(M+H)^+$ calcd. for $C_{31}H_{28}F_3N_4O_4S$ 641.15, found 641.2.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((1-hydroxycyclopentyl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**25**)*. This

compound was synthesized from coupling the advanced intermediate **VIIf** (0.345 mmol, 0.22 g) with 1-ethynyl-1-cyclopentanol (0.449 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.576 min and (Method 2) = 2.651 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.62 – 7.53 (m, 2H), 7.43 (s, 2H), 7.36 – 7.27 (m, 1H), 7.15 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 5.41 (s, 1H), 4.15 (s, 2H), 3.20 – 3.13 (m, 2H), 1.97 – 1.62 (m,8H), 1.12 (tt, *J* = 11.6, 4.6 Hz, 1H), 0.38 – 0.27 (m, 2H), 0.29 – 0.17 (m, 2H); HRMS (ESI) *m/z* $(M+Na)^+$ calcd. for $C_{31}H_{28}F_2N_4NaO_5S_2$ 661.1361, found 661.1376.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((3-methyloxetan-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**26**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 3-ethynyl-3-methyloxetane (0.235 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.598 min and (Method 2) = 3.496 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H), 8.31 (s, 1H), 7.70 – 7.61 (m, 1H), 7.65 – 7.56 (m, 4H), 7.38 – 7.29 (m, 1H), 7.17 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.74 (d, *J* = 5.5 Hz, 2H), 4.45 (d, *J* = 5.6 Hz, 2H), 4.16 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 1.64 (s, 3H), 1.22 – 1.09 (m, 1H), 0.40 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{27}F_2N_4O_5S_2$ 625.1385, found 625.1388.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((3-fluorooxetan-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**27**)*. To a mixture of triethylamine trihydrofluoride (0.458 mmol, 0.075 ml) and TEA (0.229 mmol, 0.032 ml) in dichloromethane (1 mL) at -78 ^{o}C was added XtalFluor-M (0.344 mmol, 0.084 g) followed by ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((3-hydroxyoxetan-3-yl)ethynyl)phenyl)-4-

(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate [prepared from the intermediate **VIIf** and 3-ethynyloxetan-3-ol using Sonogashira coupling procedure E] (0.125 g, 0.187 mmol). The reaction mixture was allowed to attain room temperature and stirred overnight. The reaction was quenched with aqueous sodium bicarbonate solution and extracted twice using ethyl acetate. The organic layer was concentrated and the crude product was purified in an isco flash system using 12 g silica column eluting with 20-40 % ethyl acetate in hexanes over 20 column volumes. The intermediate obtained was subsequently hydrolyed using general procedure H. LC-MS Retention Time: (Method 1) = 5.767 min and (Method 2) = 3.413 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H), 8.32 (s, 1H), 7.78 (dd, *J* = 6.7, 2.3 Hz, 1H), 7.70 (ddd, *J* = 8.7, 5.1, 2.3 Hz, 1H), 7.64 (t, *J* = 7.9 Hz, 1H), 7.59 (s, 2H), 7.41 (t, *J* = 9.0 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.04 (dd, *J* = 8.1, 1.6 Hz, 1H), 5.01 – 4.94 (m, 1H), 4.94 – 4.88 (m, 2H), 4.87 (dd, *J* = 7.9, 1.1 Hz, 1H), 4.17 (s, 2H), 3.18 (t, *J* = 6.1 Hz, 2H), 1.22 – 1.07 (m, 1H), 0.40 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{29}H_{24}F_3N_4O_5S_2$ 629.1135, found 629.1147.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((3-hydroxyoxetan-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**28**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.314 mmol, 0.2 g) with 1- 3-ethynyloxetan-3-ol (0.392 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 4.877 min and (Method 2) = 3.231 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (br, 1H), 8.20 (s, 1H), 7.72 (dd, *J* = 6.9, 2.3 Hz, 1H), 7.69 – 7.50 (m, 4H), 7.41 – 7.32 (m, 1H), 7.15 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.71 (s, 1H), 4.84 – 4.72 (m, 2H), 4.65 – 4.57 (m, 2H), 4.17 (s, 2H), 3.17 (d, *J* = 6.5 Hz, 2H), 1.20 – 1.06 (m, 1H), 0.40 – 0.29 (m, 2H), 0.26 – 0.17 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{29}H_{25}F_2N_4O_6S_2$ 627.1178, found 627.1181.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((tetrahydrofuran-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**29**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 3-3-ethynyltetrahydrofuran (0.235 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.816 min and (Method 2) = 3.478 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.61 – 7.55 (m, 4H), 7.35 – 7.28 (m, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.15 (s, 2H), 3.97 (dd, *J* = 8.1, 7.3 Hz, 1H), 3.88 – 3.71 (m, 2H), 3.60 (dd, *J* = 8.1, 6.5 Hz, 1H), 3.17 (d, *J* = 6.9 Hz, 2H), 2.34 – 2.21 (m, 1H), 1.94 (ddt, *J* = 12.1, 7.8, 6.5 Hz, 1H), 1.14 (dddd, *J* = 13.1, 11.9, 5.1, 3.5 Hz, 1H), 0.40 – 0.31 (m, 2H), 0.28 – 0.16 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{27}F_2N_4O_5S_2$ 625.1385, found 625.1377.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((3-fluorotetrahydrofuran-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**30**)*. To a mixture of triethylamine trihydrofluoride (0.374 mmol, 0.061 ml) and TEA (0.187 mmol, 0.026 ml) in dichloromethane (1 mL) at -78 ^{o}C was added XtalFluor-M (0.280 mmol, 0.068 g) followed by ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((3-hydroxytetrahydrofuran-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate [prepared from the intermediate **VIIf** and 3-ethynyloxetan-3-ol using Sonogashira coupling procedure E] (0.125 g, 0.187 mmol). The reaction mixture was allowed to attain room temperature and stirred overnight. The reaction was quenched with aqueous sodium bicarbonate solution and extracted twice using ethyl acetate. The organic layer was concentrated and the crude product was purified in an isco flash system using 12 g silica column eluting with 20-40 % ethyl acetate in hexanes over 30 column volumes. The intermediate obtained was subsequently hydrolyed using general procedure H. LC-MS

Retention Time: (Method 1) = 5.587 min and (Method 2) = 3.489 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H), 8.31 (s, 1H), 7.72 – 7.62 (m, 3H), 7.59 (s, 2H), 7.39 (t, J = 9.3 Hz, 1H), 7.17 (dd, J = 11.4, 1.6 Hz, 1H), 7.04 (dd, J = 8.1, 1.6 Hz, 1H), 4.24 – 4.13 (m, 3H), 3.97 (dd, J = 8.5, 5.8 Hz, 2H), 3.87 (ddd, J = 32.4, 10.7, 0.5 Hz, 1H), 3.18 (d, J = 7.0 Hz, 2H), 2.68 – 2.52 (m, 1H), 2.49 – 2.30 (m, 1H), 1.14 (dddd, J = 13.4, 8.1, 4.9, 1.9 Hz, 1H), 0.41 – 0.31 (m, 2H), 0.27 – 0.17 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{26}F_3N_4O_5S_2$ 643.1291, found 643.1288.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((3-hydroxytetrahydrofuran-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**31**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.392 mmol, 0.25 g) with 3-ethynyltetrahydrofuran-3-ol (0.471 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 4.929 min and (Method 2) = 3.262 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.29 (s, 1H), 7.78 – 7.52 (m, 5H), 7.34 (t, J = 9.0 Hz, 1H), 7.15 (dd, J = 11.5, 1.6 Hz, 1H), 7.05 (dd, J = 8.1, 1.6 Hz, 1H), 5.94 (s, 1H), 4.16 (s, 2H), 4.00 – 3.74 (m, 4H), 3.17 (dd, J = 5.9, 1.8 Hz, 2H), 2.28 – 2.15 (m, 2H), 1.19 – 1.06 (m, 1H), 0.37 – 0.30 (m, 2H), 0.30 – 0.14 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{27}F_2N_4O_6S_2$ 641.1332, found 641.1335.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(piperidin-4-ylethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**32**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.627 mmol, 0.4 g) with tert-butyl 4-ethynylpiperidine-1-carboxylate (0.94 mmol) using the general Sonogashira coupling procedure E and subsequent Boc deprotection with TFA followed by hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 4.466 min and (Method 2) = 3.009 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.1 (br, 1H), 8.28 (s, 1H), 7.73 –6.96 (m, 8H), 5.73 (s,

1H), 4.12 (s, 2H), 3.79 (s, 2H), 3.18 (dq, *J* = 16.7, 6.8, 5.5 Hz, 4H), 3.00 (qd, *J* = 8.8, 3.4 Hz, 4H), 2.40 (d, *J* = 6.8 Hz, 1H), 1.99 (ddq, *J* = 11.1, 6.9, 4.4, 3.8 Hz, 2H), 1.74 (ddq, *J* = 13.5, 9.1, 4.3 Hz, 2H), 1.09 (t, *J* = 6.7 Hz, 1H), 1.01 – 0.86 (m, 1H), 0.35 -0.13 (m, 4H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{30}F_2N_5O_4S_2$ 638.1702, found 638.1703.

*2-(3-(3-(Cyanoethynyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**33**)*. A solution ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-(3-hydroxyprop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (0.5 g, 0.816 mmol, 1eq) in DCM (5 mL) was added Dess-MartinPeriodinane (0.519 g, 1.224 mmol, 1.5 eq) and the reaction was stirred atroom temperature for 2 h. The reaction was extracted with ethyl acetate and the organic layer was washed with 1 molar HCl and brine. The organic layer was concentrated and the crude product was purified in an isco flash system using 12 g silica column eluting with 20-100 % ethyl acetate in hexanes over 20 column volumes to obtain 260 mg of the intermediate ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-(3-oxoprop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate. LC-MS Retention Time: (Method 1) = 5.721 min; *m/z* $(M+H)^+$ for $C_{29}H_{25}F_2N_4O_5S_2$ 611.2.

The above intermediate (0.1 g, 0.164 mmol, 1 eq) and sodium azide (0.016 g, 0.246 mmol, 1.5 eq) in ACN (1 mL) was added triflic acid (0.044 ml, 0.491 mmol, 3 eq) at rt then stirred overnight. The reaction was extracted with ethyl acetate and the organic layer was washed with water, bicarbonate solution, and brine. The organic layer was concentrated and the crude product was purified in an isco flash system using 4 g silica column eluting with 20-80 % ethyl acetate in hexanes over 20 column volumes to obtain 32 mg of the intermediate ethyl 2-(3-(3-(cyanoethynyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate. LC-MS Retention Time: (Method 1) = 6.965 min; *m/z* $(M+H)^+$ for $C_{29}H_{24}F_2N_5O_4S_2$ 608.

The above intermediate ethyl 2-(3-(3-(cyanoethynyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (0.015 g, 0.025 mmol, 1 eq) and hydroxytrimethylstannane (8.93 mg, 0.049 mmol, 2 eq) in dichloroethane (1 mL) was heated in microwave at 110 °C for 1 h. The solvent was removed by forced air and the residue was taken up in 1 mL DMSO. The crude product was purified in prep HPLC. LC-MS Retention Time: (Method 1) = 5.721 min and (Method 2) = 3.41 min; (sufficient amount of material was not available to run NMR); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{27}H_{20}F_2N_5O_4S_2$ 580.0919, found 580.0935.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3,3,3-trifluoroprop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**34**)*. A mixture of **VIIf** (0.157 mmol, 0.1 g), tri(tert-butylphosphonium)tetrafluoroborate (0.016 mmol, 4.55 mg) and allylpalladium chloride dimer (7.84 μmol, 2.84 mg) in dioxane was bubbled with argon and then tributyl(3,3,3-trifluoroprop-1-yn-1-yl)stannane (0.196 mmol, 0.083 g) was added. The vial was capped and stirred at 80 °C for 4 h. LCMS showed only 15 % conversion. [NOTE: further attempt to modify the conditions did not improve the yield and the reaction did not reproduce well using same conditions.] The reaction was diluted with DCM and stirred sequentially with KF and palladium scavenger. The product obtained after filtration was directly hydrolyzed using general procedure H to obtain minute amount of the pure product. LC-MS Retention Time: (Method 1) = 5.543 min and (Method 2) = 3.484 min; (sufficient amount of material was not available to run NMR); LCMS *m/z* $(M+H)^+$ calcd. for $C_{27}H_{20}F_5N_4O_4S_2$ 623.08, found: 623.1.

*2-(5-(Cyclopropylmethyl)-3-(3-(3,3-difluoroprop-1-yn-1-yl)-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**35**)*. A solution of ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-(3-oxoprop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (0.115 mmol, 0.07g) in DCM (3

mL) was added deoxofluor (0.229 mmo, l0.042 ml) and stirred overnight at room temperature. The reaction was quenched with aqueous sodium bicarbonate solution and extracted twice using ethyl acetate. The organic layer was concentrated and the crude product was purified in an isco flash system using 6 g silica column eluting with 20-40 % ethyl acetate in hexanes over 20 column volumes. The intermediate obtained was subsequently hydrolyzed using general procedure H. LC-MS Retention Time: (Method 1) = 5.896 min and (Method 2) = 3.434 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.85 (dd, J = 6.7, 2.3 Hz, 1H), 7.74 (ddd, J = 8.7, 5.1, 2.3 Hz, 1H), 7.64 (t, J = 7.9 Hz, 1H), 7.57 (s, 2H), 7.44 (t, J = 9.1 Hz, 1H), 7.18 – 7.10 (m, 1H), 7.07 – 7.00 (m, 1H), 4.18 (s, 2H), 3.17 (d, J = 7.0 Hz, 2H), 1.21 – 1.06 (m, 1H), 0.39 – 0.28 (m, 2H), 0.27 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{27}H_{21}F_4N_4O_4S_2$ 605.0935, found 605.0949.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3-fluorobut-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**36**)*. Following the general procedure E, reaction of **VIIf** (0.275 g, 0.431 mmol) with 2-trimethylsilyloxy-3-butyne (0.604 mmol, 0.105 ml) and subsequent deprotection of the TMS group with 2 eq of K_2CO_3 in methanol provided the intermediate ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-(3-hydroxybut-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate. To a mixture of the above intermediate (0.207 mmol, 0.13 g), triethylamine trihydrofluoride (0.415 mmol, 0.068 ml) and TEA (0.207 mmol, 0.029 ml) in DCM (1 mL) was added XtalFluor-M (0.311 mmol, 0.076 g) at -78 ^{o}C and the reaction mixture was stirred overnight at room temperature. The reaction was quenched with aqueous sodium bicarbonate solution and extracted twice using ethyl acetate. The organic layer was concentrated and the crude product was purified in an isco flash system using 6 g silica column eluting with 20-40 % ethyl acetate in hexanes over 20 column volumes. The intermediate obtained was subsequently hydrolyzed using general procedure H to afford a white solid. LC-MS Retention

Time: (Method 1) = 5.811 min and (Method 2) = 3.519 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 – 13.07 (m, 1H), 8.31 (s, 1H), 7.71 – 7.62 (m, 3H), 7.58 (s, 2H), 7.38 (t, J = 8.9 Hz, 1H), 7.16 (dd, J = 11.3, 1.6 Hz, 1H), 7.04 (dd, J = 8.2, 1.6 Hz, 1H), 5.83 – 5.53 (m, 1H), 4.16 (s, 2H), 3.18 (d, J = 6.9 Hz, 2H), 1.62 (dd, J = 23.0, 6.6 Hz, 3H), 1.26 – 1.04 (m, 1H), 0.41 – 0.31 (m, 2H), 0.27 – 0.18 (m, 2H).; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{28}H_{24}F_3N_4O_4S_2$ 601.1186, found 601.1207.

*2-(5-(Cyclopropylmethyl)-3-(3-(3-cyclopropylprop-1-yn-1-yl)-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**37**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with prop-2-yn-1-ylcyclopropane (0.294 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.013 min and (Method 2) = 3.671 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.61 – 7.51 (m, 4H), 7.31 (dd, *J* = 9.4, 8.5 Hz, 1H), 7.15 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.15 (s, 2H), 3.16 (d, *J* = 6.9 Hz, 2H), 2.53 (d, *J* = 5.9 Hz, 2H), 1.13 (ddt, *J* = 9.7, 7.8, 2.9 Hz, 1H), 1.07 – 0.94 (m, 1H), 0.54 – 0.42 (m, 2H), 0.39 – 0.27 (m, 2H), 0.30 – 0.18 (m, 4H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{27}F_2N_4O_4S_2$ 609.1436, found 609.1449.

*2-(3-(3-(3-Cyclopentylprop-1-yn-1-yl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**38**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 1-3-cyclopentyl-1-propyne (0.294 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 8.045 min and (Method 2) = 3.87 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.58 (s, 2H), 7.61 – 7.51 (m, 2H), 7.30 (dd, *J* = 9.4, 8.6 Hz, 1H), 7.14 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 5.75 (s, 2H), 4.15 (s, 2H),

3.16 (d, *J* = 6.8 Hz, 2H), 2.47 (d, *J* = 6.7 Hz, 2H), 2.15 – 2.03 (m, 1H), 1.84 – 1.71 (m, 2H), 1.69 – 1.46 (m, 3H), 1.39 – 1.23 (m, 2H), 1.20 – 1.06 (m, 1H), 0.38 – 0.28 (m, 2H), 0.30 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{31}F_2N_4O_4S_2$ 637.1749, found 637.1761.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3-(pyrrolidin-1-yl)prop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**39**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 1- 1-(prop-2-yn-1-yl)pyrrolidine.HCl (0.235 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 4.49 min and (Method 2) = 2.999 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 8.32 (s, 1H), 7.76 – 7.58 (m, 5H), 7.40 (t, *J* = 9.0 Hz, 1H), 7.14 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.04 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.40 (s, 2H), 4.16 (s, 2H), 3.28 (s, 6H), 3.17 (d, *J* = 6.9 Hz, 2H), 1.96 (s, 4H), 1.27 – 1.04 (m, 1H), 0.39 – 0.28 (m, 2H), 0.27 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{30}F_2N_5O_4S_2$ 638.1702, found 638.1692.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3-(tetrahydrofuran-2-yl)prop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**40**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** (0.196 mmol, 0.125 g) with 2-(prop-2-yn-1-yl)tetrahydrofuran (0.235 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.98 min and (Method 2) = 3.412 min; (sufficient amount of material was not available to run NMR); HRMS (ESI) *m/z* $(M+Na)^+$ calcd. for $C_{31}H_{28}F_2N_4NaO_5S_2$ 661.1361, found 661.137.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3-morpholinoprop-1-yn-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**41**)*. This compound

was synthesized from coupling the advanced intermediate **VIIf** (0.627 mmol, 0.4 g) with 4-(prop-2-yn-1-yl)morpholine (1.882 mmol) using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 4.391 min and (Method 2) = 3.008 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (s, 1H), 8.18 (s, 1H), 7.65 (t, J = 7.9 Hz, 2H), 7.60 (s, 2H), 7.57 – 7.47 (m, 1H), 7.37 (t, J = 9.0 Hz, 1H), 7.13 – 7.01 (m, 2H), 3.83 (s, 2H), 3.68 -3.38 (m, 4H), 2.68 - 2.32 (m, 6H), 0.98 – 0.91 (m, 1H), 0.43 – 0.40 (m, 2H), 0.15 – 0.11 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{30}F_2N_5O_5S_2$ 654.1651, found 654.1672.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(thiophen-2-ylethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**42**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with either 4,4,5,5-tetramethyl-2-(thiophen-2-ylethynyl)-1,3,2-dioxaborolane following general Suzuki procedure G or 2-ethynylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.472 min and (Method 2) = 3.687 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.31 (s, 1H), 7.80 – 7.71 (m, 2H), 7.70 – 7.60 (m, 2H), 7.58 (s, 2H), 7.49 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.39 (dd, *J* = 9.4, 8.7 Hz, 1H), 7.21 – 7.13 (m, 2H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 7.0 Hz, 2H), 1.20 – 1.09 (m, 1H), 0.40 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.99, 161.75, 161.10, 160.26, 150.78, 144.62, 144.48, 133.54, 131.98, 130.07, 129.92, 129.55, 129.41, 128.63, 128.50, 127.97, 126.20, 123.71, 120.96, 116.89, 116.44, 116.24, 110.74, 85.57, 28.11, 15.16, 10.31, 4.47; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{23}F_2N_4O_4S_3$ 637.0844, found 637.0856.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(thiophen-2-ylethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**43**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with 2-ethynyl-5-methylthiophene

using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H (a detailed scale up procedure is provided in the supporting information). LC-MS Retention Time: (Method 1) = 6.133 min and (Method 2) = 3.694 min; ^{1}H NMR (400 MHz, DMSO-d_6) 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); 13C NMR (101 MHz, DMSO-d6) δ 161.58, 160.94, 159.18, 150.73, 147.20, 147.12, 144.47, 144.44, 143.30, 133.59, 131.80, 129.81, 129.73, 129.50, 129.35, 128.51, 128.48, 128.39, 126.25, 125.90, 123.59, 123.56, 118.47, 116.75, 116.28, 116.23, 116.07, 116.02, 110.99, 84.86, 27.99, 14.90, 10.15, 4.34; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{25}F_2N_4O_4S_3$ 651.1001, found 651.1027.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((5-methylfuran-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**44**)*. This compound was synthesized from coupling the advanced intermediate **VIIIb** with 2-bromo-5-methylfuran using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.202 min and (Method 2) = 3.593 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.23 (s, 1H), 8.30 (d, *J* = 3.2 Hz, 1H), 7.75 (dd, *J* = 6.8, 2.3 Hz, 1H), 7.70 – 7.58 (m, 4H), 7.39 (t, *J* = 9.0 Hz, 1H), 7.16 (d, *J* = 11.3 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 1H), 6.88 (d, *J* = 3.3 Hz, 1H), 6.29 – 6.23 (m, 1H), 4.17 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 2.33 (s, 3H), 1.14 (t, *J* = 7.6 Hz, 1H), 0.34 (dd, *J* = 8.0, 1.8 Hz, 2H), 0.23 (t, *J* = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.00, 161.74, 161.10, 159.28, 156.76, 154.84, 150.77, 147.29, 144.61, 144.47, 133.57, 131.70, 130.13, 128.67, 128.49, 126.22, 123.73, 118.45, 116.89, 116.48, 116.43, 116.28, 116.22, 110.55, 110.40, 107.99, 85.97, 28.11, 28.02,

15.20, 15.19, 13.51, 10.30, 4.47; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{25}F_2N_4O_5S_2$ 635.1229, found 635.124.

*2-(3-(3-((5-(Tert-butyl)thiophen-2-yl)ethynyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**45**)*. This compound was synthesized from coupling the advanced intermediate **VIIIb** with 2-bromo-5-(*tert*-butyl)thiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 7.143 min and (Method 2) = 3.959 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.32 (s, 1H), 7.73 (dd, *J* = 6.9, 2.3 Hz, 1H), 7.70 – 7.58 (m, 2H), 7.57 (s, 2H), 7.42 – 7.33 (m, 1H), 7.29 (d, *J* = 3.8 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.92 (d, *J* = 3.7 Hz, 1H), 4.17 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 1.36 (s, 9H), 1.25 – 1.07 (m, 1H), 0.40 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{34}H_{31}F_2N_4O_5S_3$ 693.147, found 693.1484.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((2-methylthiazol-5-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**46**)*. This compound was synthesized from coupling the advanced intermediate **VIIIb** with 5-bromo-2-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.093 min and (Method 2) = 3.556 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.32 (s, 1H), 8.01 (s, 1H), 7.77 (dd, *J* = 6.8, 2.3 Hz, 1H), 7.70 – 7.61 (m, 2H), 7.58 (s, 2H), 7.40 (t, *J* = 9.0 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 6.8 Hz, 2H), 2.70 (s, 3H), 1.14 (s, 1H), 0.40 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.13, 161.75, 161.09, 160.28, 159.28, 156.76, 150.71, 147.44, 147.30, 144.63, 144.48, 132.09, 129.55, 129.40, 128.67, 128.50, 126.23, 123.74, 116.91, 116.52, 116.38,

116.31, 116.23, 110.35, 87.96, 84.71, 28.10, 19.00, 15.20, 10.31, 4.48; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{24}F_2N_5O_4S_3$ 652.0953, found 652.097.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiazol-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**47**)*. This compound was synthesized from coupling the advanced intermediate **VIIIb** with 2-bromo-5-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.063 min and (Method 2) = 3.595 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.32 (s, 1H), 7.87 (dd, J = 6.8, 2.3 Hz, 1H), 7.73 – 7.62 (m, 3H), 7.58 (s, 2H), 7.43 (t, J = 9.0 Hz, 1H), 7.16 (d, J = 11.2 Hz, 1H), 7.06 (dd, J = 8.2, 1.6 Hz, 1H), 4.19 (s, 2H), 3.18 (d, J = 6.9 Hz, 2H), 2.52 (s, 3H), 1.14 (dh, J = 13.0, 6.6 Hz, 1H), 0.34 (dt, J = 8.3, 2.9 Hz, 2H), 0.23 (t, J = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.16, 161.75, 161.09, 160.63, 156.75, 150.65, 147.35, 144.62, 144.57, 144.47, 142.38, 137.77, 132.38, 131.25, 131.16, 129.54, 128.81, 128.78, 128.49, 126.25, 123.75, 116.97, 116.63, 116.44, 116.23, 109.67, 109.51, 87.66, 85.43, 28.11, 27.99, 15.31, 15.28, 15.24, 15.21, 11.66, 10.31, 4.47; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{24}F_2N_5O_4S_3$ 652.0953, found 652.097.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((1-methyl-1H-imidazol-5-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**48**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with 5-ethynyl-1-methyl-1H-imidazole using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 4.583 min and (Method 2) = 2.868 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.32 (s, 1H), 7.88 (s, 1H), 7.72 (dd, *J* = 6.8, 2.3 Hz, 1H), 7.70 – 7.62 (m, 2H), 7.59 (s, 2H), 7.45 – 7.36 (m, 2H), 7.18 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.69

(d, *J* = 0.5 Hz, 3H), 3.18 (d, *J* = 6.9 Hz, 2H), 1.22 – 1.07 (m, 1H), 0.40 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H);

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}F_2N_6O_4S_2$ 635.1344, found 635.1345.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-((1-methyl-1H-pyrazol-4-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**49**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with 4-ethynyl-1-methyl-1H-pyrazole using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.607 min and (Method 2) = 2.612 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 8.26 (s, 1H), 8.11 (d, *J* = 0.6 Hz, 1H), 7.72 (d, *J* = 0.7 Hz, 1H), 7.70 – 7.62 (m, 2H), 7.62 – 7.54 (m, 3H), 7.35 (dd, *J* = 9.4, 8.6 Hz, 1H), 7.17 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.16 (s, 2H), 3.86 (s, 3H), 3.17 (d, *J* = 6.9 Hz, 2H), 1.30 – 1.06 (m, 1H), 0.39 – 0.30 (m, 2H), 0.33 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}F_2N_6O_4S_2$ 635.1344, found 635.1345.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-(prop-1-yn-1-yl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**50**)*. This compound was synthesized from coupling the advanced intermediate **VIIe** (0.202 mmol, 0.125 g) with potassium trifluoro(prop-1-yn-1-yl)borate (0.303 mmol) using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.653 min and (Method 2) = 3.535 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.27 (s, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.59 – 7.52 (m, 3H), 7.52 – 7.45 (m, 1H), 7.42 – 7.32 (m, 2H), 7.14 (dd, *J* = 11.5, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.15 (s, 2H), 3.20 – 3.13 (m, 2H), 2.04 (s, 3H), 1.12 (dd, *J* = 12.2, 7.1 Hz, 1H), 0.38 – 0.28 (m, 2H), 0.30 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{27}H_{24}FN_4O_4S_2$ 551.1218, found 551.1208.

*2-(3-(3-(Cyclopentylethynyl)phenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**51**)*. This compound was

synthesized from coupling the advanced intermediate **VIIe** with cyclopentyl acetylene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.386 min and (Method 2) = 3.68 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.31 (s, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.58 (s, 2H), 7.55 – 7.47 (m, 2H), 7.41 – 7.32 (m, 2H), 7.16 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.15 (s, 2H), 3.17 (d, *J* = 6.8 Hz, 2H), 2.90 – 2.78 (m, 1H), 2.08 – 1.87 (m, 2H), 1.78 – 1.65 (m, 2H), 1.69 – 1.50 (m, 4H), 1.19 – 1.05 (m, 1H), 0.39 – 0.28 (m, 2H), 0.31 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{30}FN_4O_4S_2$ 605.1687, found 605.1707.

*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**)*. This compound was synthesized from coupling the advanced intermediate **VIIe** with 2-ethynyl-5-methylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time = 6.402 min $(M+H)^+$ = 632. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.32 (d, *J* = 0.9 Hz, 1H), 7.71 – 7.62 (m, 2H), 7.58 (d, *J* = 6.6 Hz, 3H), 7.52 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 3.6 Hz, 1H), 7.16 (d, *J* = 11.3 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.83 (dt, *J* = 3.5, 1.2 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.47 (d, *J* = 1.1 Hz, 3H), 1.21 – 1.09 (m, 1H), 0.35 (dt, *J* = 8.1, 2.8 Hz, 2H), 0.31 – 0.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d6) δ 161.70, 161.11, 159.24, 156.73, 151.55, 147.42, 147.34, 144.53, 144.45, 142.72, 133.19, 132.20, 131.08, 129.80, 129.50, 129.36, 129.26, 128.46, 127.59, 126.24, 126.11, 123.67, 123.64, 122.51, 119.11, 116.86, 116.36, 116.14, 91.67, 83.60, 28.10, 15.01, 10.27, 4.45. HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{26}FN_4O_4S_3$ 633.1095, found 633.1086.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((3-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**53**)*. This compound was

synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-3-methylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.488 min and (Method 2) = 3.679 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.31 (s, 1H), 7.71 – 7.62 (m, 2H), 7.65 – 7.58 (m, 1H), 7.58 – 7.51 (m, 4H), 7.46 (td, *J* = 7.7, 0.6 Hz, 1H), 7.17 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.02 (dd, *J* = 5.1, 0.5 Hz, 1H), 4.19 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.31 (s, 3H), 1.20 – 1.07 (m, 1H), 0.39 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{26}FN_4O_4S_3$ 633.1095, found 633.1099.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-3-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**54**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 3-bromo-2-methylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.516 min and (Method 2) = 3.691 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.71 – 7.61 (m, 3H), 7.58 (s, 2H), 7.58 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.50 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.44 (td, *J* = 7.6, 0.6 Hz, 1H), 7.18 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.95 (p, *J* = 1.1 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.46 (d, *J* = 1.1 Hz, 3H), 1.22 – 1.08 (m, 1H), 0.40 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{26}FN_4O_4S_3$ 633.1095, found 633.1099.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-isopropylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**55**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-isopropylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 7.071 min and (Method 2) = 3.835 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.31

(s, 1H), 7.71 – 7.62 (m, 2H), 7.62 – 7.55 (m, 1H), 7.56 (s, 2H), 7.51 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.45 (td, *J* = 7.7, 0.6 Hz, 1H), 7.25 (d, *J* = 3.6 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.88 (dd, *J* = 3.7, 1.0 Hz, 1H), 4.18 (s, 2H), 3.25 – 3.10 (m, 3H), 1.29 (d, *J* = 6.8 Hz, 6H), 1.21 – 1.09 (m, 1H), 0.40 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{33}H_{30}FN_4O_4S_3$ 661.1408, found 661.1427.

*2-(5-(Cyclopropylmethyl)-3-(3-((5-cyclopropylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**56**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-cyclopropylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.769 min and (Method 2) = 3.771 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 – 13.12 (m, 1H), 8.31 (s, 1H), 7.70 – 7.62 (m, 2H), 7.61 – 7.53 (m, 1H), 7.56 (s, 2H), 7.51 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.44 (td, *J* = 7.7, 0.6 Hz, 1H), 7.22 (d, *J* = 3.7 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.82 (dd, *J* = 3.7, 0.7 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.17 (ttd, *J* = 8.3, 4.9, 0.7 Hz, 1H), 1.20 – 1.08 (m, 1H), 1.11 – 0.98 (m, 2H), 0.79 – 0.69 (m, 2H), 0.39 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{33}H_{28}FN_4O_4S_3$ 659.1251, found 659.127.

*2-(3-(3-((5-Cyclobutylthiophen-2-yl)ethynyl)phenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**57**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-cyclobutylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 7.119 min and (Method 2) = 3.893 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.70 – 7.61 (m, 2H), 7.61 – 7.55 (m, 3H), 7.52 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.48 – 7.40 (m, 1H), 7.26 (d, *J* = 3.6 Hz, 1H), 7.16 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.88 (dd, *J*

= 3.7, 0.9 Hz, 1H), 4.18 (s, 2H), 3.79 – 3.65 (m, 1H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.45 – 2.32 (m, 2H), 2.19 – 2.04 (m, 2H), 2.08 – 1.88 (m, 1H), 1.91 – 1.78 (m, 1H), 1.21 – 1.09 (m, 1H), 0.41 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{34}H_{30}FN_4O_4S_3$ 673.1408, found 673.1391.

*2-(3-(3-((5-Chlorothiophen-2-yl)ethynyl)phenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**58**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-chlorothiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.776 min and (Method 2) = 3.844 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.31 (s, 1H), 7.74 – 7.52 (m, 6H), 7.46 (td, *J* = 7.8, 0.6 Hz, 1H), 7.34 (d, *J* = 3.9 Hz, 1H), 7.21 – 7.12 (m, 2H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.22 – 3.14 (m, 2H), 1.29 – 0.98 (m, 1H), 0.40 – 0.27 (m, 2H), 0.30 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{23}ClFN_4O_4S_3$ 653.0548, found 653.0566.

*2-(5-(Cyclopropylmethyl)-3-(3-((5-(difluoromethyl)thiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**59**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-(difluoromethyl)thiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.315 min and (Method 2) = 3.682 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.31 (s, 1H), 7.75 (td, *J* = 1.7, 0.6 Hz, 1H), 7.70 – 7.58 (m, 2H), 7.58 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.56 (s, 2H), 7.52 – 7.42 (m, 3H), 7.22 – 7.13 (m, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.19 (s, 2H), 3.19 (d, *J* = 6.9 Hz, 2H), 1.22 – 1.08 (m,1H), 0.40 – 0.27 (m, 2H), 0.30 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{24}F_3N_4O_4S_3$ 669.0906, found 669.0909.

2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-(trifluoromethyl)thiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid

(60). This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-(trifluoromethyl)thiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.897 min and (Method 2) = 3.837 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.76 (dp, *J* = 3.4, 1.2 Hz, 2H), 7.70 – 7.61 (m, 2H), 7.64 – 7.57 (m, 1H), 7.56 (s, 2H), 7.54 (dq, *J* = 3.9, 1.3 Hz, 1H), 7.49 (td, *J* = 7.8, 0.6 Hz, 1H), 7.17 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.19 (s, 2H), 3.19 (d, *J* = 6.9 Hz, 2H), 1.21 – 1.09 (m, 1H), 0.40 – 0.29 (m, 2H), 0.32 – 0.20 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{23}F_4N_4O_4S_3$ 687.0812, found 687.0807.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylfuran-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**61**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-methylfuran using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.261 min and (Method 2) = 3.591 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.32 (s, 1H), 7.70 (td, *J* = 1.7, 0.6 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.62 – 7.56 (m, 1H), 7.57 (s, 2H), 7.53 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.46 (td, *J* = 7.7, 0.6 Hz, 1H), 7.16 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.81 (dd, *J* = 3.3, 0.6 Hz, 1H), 6.23 (dq, *J* = 3.1, 1.0 Hz, 1H), 4.18 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.32 (dd, *J* = 1.0, 0.5 Hz, 3H), 1.22 – 1.07 (m, 1H), 0.39 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.76, 161.16, 154.33, 151.53, 147.46, 144.59, 144.47, 134.00, 132.31, 131.05, 129.65, 129.39, 128.49, 127.88, 126.20, 123.73, 121.98, 117.70, 116.92, 116.41, 116.20, 107.85, 92.48, 80.50, 28.12, 15.34, 15.28, 15.19, 13.50, 10.30, 4.48; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{26}FN_4O_5S_2$ 617.1323, found 617.1341.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methyloxazol-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**62**)*. This compound was

synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-methyloxazole using the general Sonogashira coupling *procedure F* and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.764 min and (Method 2) = 3.499 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.32 (s, 1H), 7.85 (td, *J* = 1.7, 0.6 Hz, 1H), 7.72 – 7.61 (m, 3H), 7.56 (s, 2H), 7.51 (td, *J* = 7.8, 0.6 Hz, 1H), 7.15 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.10 – 7.03 (m, 2H), 4.20 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 2.37 (d, *J* = 1.2 Hz, 3H), 1.20 – 1.07 (m, 1H), 0.39 – 0.29 (m, 2H), 0.27 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}FN_5O_5S_2$ 618.1276, found 618.1261.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiazol-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**63**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-5-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.875 min and (Method 2) = 3.491 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.29 (s, 1H), 7.81 (td, *J* = 1.7, 0.6 Hz, 1H), 7.70 – 7.60 (m, 4H), 7.56 (s, 2H), 7.50 (td, *J* = 7.8, 0.6 Hz, 1H), 7.19 – 7.12 (m, 1H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.20 (s, 2H), 3.28 (d, *J* = 10.6 Hz, 2H), 3.21 – 3.14 (m, 2H), 1.17 – 1.09 (m, 1H), 0.39 – 0.30 (m, 2H), 0.27 – 0.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.75, 161.14, 151.39, 147.36, 145.12, 144.61, 144.47, 142.14, 137.19, 132.41, 131.77, 130.37, 129.50, 128.78, 128.49, 126.22, 123.73, 121.04, 116.97, 116.41, 92.09, 83.00, 28.10, 15.19, 11.64, 10.31, 4.48; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}FN_5O_4S_3$ 634.1047, found 634.1056.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((2-methylthiazol-5-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**64**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 5-bromo-2-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general

procedure H. LC-MS Retention Time: (Method 1) = 5.804 min and (Method 2) = 3.468 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.31 (s, 1H), 7.96 (s, 1H), 7.72 (d, J = 1.7 Hz, 1H), 7.73 – 7.59 (m, 2H), 7.59 – 7.52 (m, 3H), 7.47 (t, J = 7.8 Hz, 1H), 7.16 (dd, J = 11.3, 1.6 Hz, 1H), 7.06 (dd, J = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.18 (d, J = 6.9 Hz, 2H), 2.69 (s, 3H), 2.07 (s, 1H), 1.20 – 1.07 (m, 1H), 0.40 – 0.27 (m, 2H), 0.30 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}FN_5O_4S_3$ 634.1047, found 634.1046.

*2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((2-methylthiazol-4-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**65**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 4-bromo-2-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.741 min and (Method 2) = 3.437 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.32 (s, 1H), 7.91 (s, 1H), 7.73 (t, J = 1.7 Hz, 1H), 7.66 (t, J = 7.9 Hz, 1H), 7.62 – 7.53 (m, 4H), 7.47 (t, J = 7.7 Hz, 1H), 7.16 (dd, J = 11.4, 1.6 Hz, 1H), 7.07 (dd, J = 8.2, 1.6 Hz, 1H), 4.19 (s, 2H), 3.18 (d, J = 6.9 Hz, 2H), 2.68 (s, 3H), 1.23 – 1.03 (m, 1H), 0.43 – 0.30 (m, 2H), 0.30 – 0.16 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{25}FN_5O_4S_3$ 634.1047, found 634.1041.

*2-(5-(Cyclopropylmethyl)-3-(3-((3,5-dimethylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**66**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 2-bromo-3,5-dimethylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.749 min and (Method 2) = 3.834 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.31 (s, 1H), 7.70 – 7.63 (m, 2H), 7.60 (dt, J = 7.6, 1.5 Hz, 1H), 7.56 (s, 2H), 7.51 (dt, J = 7.7, 1.4 Hz, 1H), 7.45 (td, J = 7.7, 0.6 Hz, 1H), 7.17 (dd, J = 11.4, 1.6 Hz, 1H), 7.07 (dd, J = 8.1, 1.6 Hz, 1H), 6.72 (dt, J = 1.4, 0.7 Hz, 1H), 4.18 (s, 2H), 3.17 (d, J = 6.9 Hz, 2H), 2.42 (d, J = 1.1 Hz, 3H), 2.23

(s, 3H), 1.19 – 1.07 (m, 1H), 0.39 – 0.31 (m, 2H), 0.26 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_4S_3$ 647.1251, found 647.1229.

*2-(5-(Cyclopropylmethyl)-3-(3-((2,5-dimethylthiophen-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**67**)*. This compound was synthesized from coupling the advanced intermediate **VIIIa** with 3-bromo-2,5-dimethylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.787 min and (Method 2) = 3.777 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.31 (s, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.58 (s, 2H), 7.51 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.45 (td, *J* = 7.6, 0.7 Hz, 1H), 7.18 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.77 (q, *J* = 1.2 Hz, 1H), 4.18 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 2.44 (d, *J* = 0.7 Hz, 3H), 2.38 (t, *J* = 0.9 Hz, 3H), 1.20 – 1.09 (m, 1H), 0.39 – 0.28 (m, 2H), 0.27 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_4S_3$ 647.1251, found 647.124.

*(E)-2-(5-(Cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-(2-(5-methylthiophen-2-yl)vinyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**68**)*. This compound was synthesized from coupling the advanced intermediate **VIIe** with (E)-4,4,5,5-tetramethyl-2-(2-(5-methylthiophen-2-yl)vinyl)-1,3,2-dioxaborolane using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.619 min and (Method 2) = 3.753 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (s, 1H), 8.30 (s, 1H), 7.70 – 7.62 (m, 2H), 7.58 – 7.51 (m, 3H), 7.43 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.23 (dd, *J* = 16.2, 0.7 Hz, 1H), 7.17 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.99 (d, *J* = 3.5 Hz, 1H), 6.83 – 6.72 (m, 2H), 4.19 (s, 2H), 3.19 (d, *J* = 6.9 Hz, 2H), 2.45 (d, *J* = 1.1 Hz, 3H), 1.21 – 1.08 (m, 1H), 0.40 – 0.29 (m, 2H), 0.32 – 0.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.79, 161.24, 152.52, 144.46, 144.42, 140.00, 139.28, 137.16, 132.14, 129.15, 128.49, 127.52, 126.57, 126.40,

126.34, 126.11, 125.90, 124.93, 123.68, 122.90, 116.83, 116.37, 116.16, 28.13, 15.31, 10.35, 4.49; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{28}FN_4O_4S_3$ 635.1251, found 635.1258.

*(E)-2-(3-(3-(2-Cyclopentylvinyl)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**69**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with (E)-(2-cyclopentylvinyl)boronic acid using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.444 min and (Method 2) = 3.792 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.30 (s, 1H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.58 (s, 2H), 7.60 – 7.54 (m, 1H), 7.49 (ddd, *J* = 8.6, 5.0, 2.3 Hz, 1H), 7.27 – 7.15 (m, 2H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.43 (dd, *J* = 16.0, 1.1 Hz, 1H), 6.10 (dd, *J* = 16.0, 8.0 Hz, 1H), 4.16 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 2.63 – 2.52 (m, 1H), 1.85 – 1.49 (m, 4H), 1.33 (ddt, *J* = 14.1, 11.9, 5.0 Hz, 2H), 1.22 – 1.07 (m, 1H), 0.39 – 0.28 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{31}F_2N_4O_4S_2$ 625.1749, found 625.1744.

*(E)-2-(5-(Cyclopropylmethyl)-3-(3-(2-cyclopropylvinyl)-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**70**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with (E)-2-(2-cyclopropylvinyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.229 min and (Method 2) = 3.694 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.59 (s, 2H), 7.53 (dd, *J* = 7.4, 2.3 Hz, 1H), 7.44 (ddd, *J* = 8.6, 5.0, 2.2 Hz, 1H), 7.25 – 7.12 (m, 2H), 7.05 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.53 (d, *J* = 15.9 Hz, 1H), 5.66 (dd, *J* = 15.9, 9.4 Hz, 1H), 4.15 (s, 2H), 3.16 (d, *J* = 6.7 Hz, 2H), 1.61 (dddd, *J* = 12.8, 9.4, 8.0, 4.7 Hz, 1H), 1.20 – 1.08 (m, 1H), 0.87 – 0.75 (m, 2H), 0.56 – 0.48 (m, 2H),

0.38 – 0.28 (m, 2H), 0.30 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{29}H_{27}F_2N_4O_4S_2$ 597.1436, found 597.1425.

*(E)-2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(prop-1-en-1-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**71**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with (E)-prop-1-en-1-ylboronic acid using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.188 min and (Method 2) = 3.502 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.30 (s, 1H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.60 (s, 2H), 7.54 (dd, *J* = 7.4, 2.3 Hz, 1H), 7.45 (ddd, *J* = 8.6, 5.0, 2.3 Hz, 1H), 7.26 – 7.14 (m, 2H), 7.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.47 (dq, *J* = 15.9, 1.7 Hz, 1H), 6.13 (dq, *J* = 16.0, 6.6 Hz, 1H), 4.14 (s, 2H), 3.18 (d, *J* = 7.0 Hz, 2H), 1.85 (dd, *J* = 6.6, 1.7 Hz, 3H), 1.22 – 1.08 (m, 1H), 0.40 – 0.29 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{27}H_{25}F_2N_4O_4S_2$ 571.128, found 571.1279.

*(E)-2-(5-(Cyclopropylmethyl)-3-(3-(3,3-dimethylbut-1-en-1-yl)-4-fluorophenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**72**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with (E)-(3,3-dimethylbut-1-en-1-yl)boronic acid using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.048 min and (Method 2) = 3.696 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.30 (d, *J* = 0.8 Hz, 1H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.58 (dd, *J* = 7.3, 2.3 Hz, 1H), 7.56 (s, 2H), 7.51 (ddd, *J* = 8.5, 5.0, 2.3 Hz, 1H), 7.28 – 7.15 (m, 2H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.36 (d, *J* = 16.4 Hz, 1H), 6.18 (d, *J* = 16.3 Hz, 1H), 4.17 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 1.24 – 1.10 (m, 1H), 1.07 (d, *J* = 1.0 Hz, 9H), 0.39 – 0.28 (m, 2H), 0.31 – 0.19 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{30}H_{31}F_2N_4O_4S_2$ 613.1749, found 613.1728.

*2-(5-(Cyclopropylmethyl)-3-(4-fluoro-3-(3,3,3-trifluoroprop-1-en-2-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**73**)*. This compound was synthesized from coupling the advanced intermediate **VIIf** with 4,4,6,6-tetramethyl-2-(3,3,3-trifluoroprop-1-en-2-yl)-1,3,2-dioxaborinane using the general Suzuki coupling procedure G and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 5.911 min and (Method 2) = 3.538 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 8.26 (s, 1H), 7.71 – 7.60 (m, 2H), 7.59 (s, 2H), 7.51 (dd, *J* = 7.2, 2.3 Hz, 1H), 7.39 (dd, *J* = 10.0, 8.6 Hz, 1H), 7.12 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.03 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.32 (dt, *J* = 2.1, 1.0 Hz, 1H), 5.97 (s, 1H), 4.14 (s, 2H), 3.17 (d, *J* = 6.9 Hz, 2H), 1.24 – 1.07 (m, 1H), 0.39 – 0.31 (m, 2H), 0.29 – 0.18 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{27}H_{22}F_5N_4O_4S_2$ 625.0997, found 625.099.

*2-(5-(2-Cyclopropylethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**74**)*. This compound was synthesized from coupling the advanced intermediate **VIIj** with 2-ethynyl-5-methylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.859 min and (Method 2) = 3.779 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 1H), 8.29 (s, 1H), 7.73 (dd, *J* = 6.9, 2.3 Hz, 1H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.61 (ddd, *J* = 8.7, 5.0, 2.3 Hz, 1H), 7.58 (s, 2H), 7.39 (dd, *J* = 9.4, 8.7 Hz, 1H), 7.29 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.19 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.85 (dt, *J* = 3.4, 1.0 Hz, 1H), 4.17 (s, 2H), 3.29 – 3.20 (m, 2H), 2.48 (d, *J* = 1.1 Hz, 3H), 1.43 (q, *J* = 7.3 Hz, 2H), 0.73 (ddt, *J* = 10.2, 7.5, 3.7 Hz, 1H), 0.36 – 0.26 (m, 2H), 0.16 – 0.08 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.75, 160.93, 160.21, 159.32, 156.81, 150.73, 147.44, 147.36, 144.69, 144.57, 143.50, 133.80, 131.92, 130.01, 129.93, 129.60, 129.46, 128.59, 126.45, 125.98, 123.71, 118.51, 116.83,

116.46, 116.24, 110.94, 88.55, 84.99, 33.27, 27.98, 24.73, 15.07, 10.55, 4.18; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{27}F_2N_4O_4S_3$ 665.1157, found 665.1174.

*2-(5-(2-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**75**)*. This compound was synthesized from coupling the advanced intermediate **VIIi** with 2-ethynyl-5-methylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.713 min and (Method 2) = 3.838 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 1H), 8.29 (s, 1H), 7.72 – 7.63 (m, 2H), 7.57 (s, 2H), 7.56 (ddt, *J* = 17.6, 7.7, 1.4 Hz, 2H), 7.46 (td, *J* = 7.7, 0.6 Hz, 1H), 7.24 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.19 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.83 (dt, *J* = 3.4, 1.1 Hz, 1H), 4.17 (s, 2H), 3.29 – 3.21 (m, 2H), 2.47 (d, *J* = 1.1 Hz, 3H), 1.43 (q, *J* = 7.3 Hz, 2H), 0.81 – 0.67 (m, 1H), 0.36 – 0.26 (m, 2H), 0.16 – 0.06 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.79, 160.96, 159.32, 156.80, 151.49, 144.72, 144.66, 142.80, 133.26, 132.28, 131.14, 129.88, 129.57, 129.43, 129.37, 128.60, 127.71, 126.31, 125.86, 123.69, 122.57, 119.12, 116.85, 116.42, 116.21, 91.71, 83.65, 33.27, 28.06, 24.74, 15.06, 10.56, 4.18; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_4S_3$ 647.1251, found 647.1272.

*2-(5-(2-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylfuran-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**76**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 2-bromo-5-methylfuran using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.449 min and (Method 2) = 3.533 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (s, 1H), 8.47 (s, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.77 (s, 3H), 7.75 – 7.62 (m, 2H), 7.37 (d, *J* = 11.2 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 1H), 6.99 (s, 1H), 6.41 (s, 1H), 4.36 (s, 2H), 2.69 (s, 2H), 1.67 – 1.56 (m, 2H), 0.93 (s, 1H), 0.50 - 0.31 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.76, 160.99, 154.34, 151.45, 147.44, 144.67, 144.58,

132.34, 131.06, 129.70, 129.44, 128.59, 127.95, 125.98, 123.73, 122.01, 117.70, 116.88, 116.43, 107.85, 92.48, 80.52, 33.26, 28.04, 24.75, 13.50, 10.56, 4.18; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_5S_2$ 631.148, found 631.1486.

*2-(5-(2-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-3-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**77**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 3-bromo-2-methylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.722 min and (Method 2) = 3.751 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.29 (s, 1H), 7.72 – 7.61 (m, 3H), 7.59 (s, 2H), 7.57 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.55 – 7.49 (m, 1H), 7.53 – 7.42 (m, 1H), 7.20 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.95 (p, *J* = 1.1 Hz, 1H), 4.17 (s, 2H), 3.29 – 3.21 (m, 2H), 2.46 (d, *J* = 1.1 Hz, 3H), 1.44 (dt, *J* = 10.3, 7.1 Hz, 2H), 0.82 – 0.67 (m, 1H), 0.36 – 0.26 (m, 2H), 0.16 – 0.08 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_4S_3$ 647.1251, found 647.1237.

*2-(5-(2-Cyclopropylethyl)-3-(3-((5-cyclopropylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**78**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 2-bromo-5-cyclopropylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 8.339 min and (Method 2) = 3.842 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.28 (s, 1H), 7.72 – 7.60 (m, 2H), 7.58 (s, 2H), 7.60 – 7.49 (m, 2H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.25 – 7.15 (m, 2H), 7.07 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.82 (dd, *J* = 3.7, 0.7 Hz, 1H), 4.17 (s, 2H), 3.29 – 3.21 (m, 2H), 2.17 (tt, *J* = 8.3, 5.0 Hz, 1H), 1.43 (q, *J* = 7.3 Hz, 2H), 1.11 – 0.98 (m, 2H), 0.81 – 0.65 (m, 3H), 0.36 – 0.25 (m, 2H), 0.16 – 0.08 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{34}H_{30}FN_4O_4S_3$ 673.1408, found 673.1392.

*2-(3-(3-((5-Cyclobutylthiophen-2-yl)ethynyl)phenyl)-5-(2-cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**79**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 2-bromo-5-cyclobutylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 7.29 min and (Method 2) = 4.251 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.28 (s, 1H), 7.72 – 7.64 (m, 2H), 7.62 – 7.54 (m, 3H), 7.53 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.46 (td, *J* = 7.7, 0.6 Hz, 1H), 7.26 (d, *J* = 3.6 Hz, 1H), 7.19 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.88 (dd, *J* = 3.7, 0.9 Hz, 1H), 4.17 (s, 2H), 3.72 (dqt, *J* = 9.0, 8.0, 1.0 Hz, 1H), 3.29 – 3.21 (m, 2H), 2.47 – 2.32 (m, 2H), 2.19 – 2.04 (m, 2H), 2.07 – 1.88 (m, 1H), 1.91 – 1.78 (m, 1H), 1.43 (q, *J* = 7.4 Hz, 2H), 0.74 (ddd, *J* = 12.6, 8.5, 5.0 Hz, 1H), 0.36 – 0.24 (m, 2H), 0.16 – 0.08 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{35}H_{32}FN_4O_4S_3$ 687.1564, found 687.1558.

*2-(5-(2-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiazol-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**80**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 2-bromo-5-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.096 min and (Method 2) = 3.581 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (s, 1H), 8.28 (s, 1H), 7.80 (td, *J* = 1.7, 0.6 Hz, 1H), 7.71 – 7.62 (m, 4H), 7.58 (s, 2H), 7.56 – 7.47 (m, 1H), 7.18 (dd, *J* = 11.4, 1.5 Hz, 1H), 7.08 (dd, *J* = 8.2, 1.6 Hz, 1H), 4.19 (s, 2H), 3.25 (s, 2H), 2.51 (s, 3H), 1.43 (q, *J* = 7.5 Hz, 2H), 0.78 – 0.69 (m, 1H), 0.36 – 0.27 (m, 2H), 0.16 – 0.08 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{27}FN_5O_4S_3$ 648.1204, found 648.1202.

*2-(5-(2-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((2-methylthiazol-5-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**81**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 5-bromo-2-methylthiazole

using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.039 min and (Method 2) = 3.566 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.28 (s, 1H), 7.96 (s, 1H), 7.74 – 7.54 (m, 7H), 7.48 (td, *J* = 7.7, 0.6 Hz, 1H), 7.19 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.24 (d, *J* = 8.2 Hz, 2H), 2.69 (s, 3H), 1.43 (q, *J* = 7.4 Hz, 2H), 0.74 (td, *J* = 7.5, 3.8 Hz, 1H), 0.36 – 0.27 (m, 2H), 0.16 – 0.08 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.55, 161.76, 160.97, 156.79, 151.40, 146.95, 144.69, 144.57, 132.33, 131.34, 130.07, 129.43, 128.58, 128.19, 125.98, 123.71, 122.00, 116.93, 116.88, 116.22, 94.67, 79.87, 33.27, 28.04, 24.74, 18.97, 10.56, 4.18; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{27}FN_5O_4S_3$ 648.1204, found 648.1191.

*2-(5-(2-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((2-methylthiazol-4-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**82**)*. This compound was synthesized from coupling the advanced intermediate **VIIIc** with 4-bromo-2-methylthiazole using the general Sonogashira coupling procedure F and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.017 min and (Method 2) = 3.538 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.30 (s, 1H), 7.92 (s, 1H), 7.73 (dt, *J* = 1.8, 1.0 Hz, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.59 (s, 2H), 7.64 – 7.54 (m, 2H), 7.53 – 7.44 (m, 1H), 7.19 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.18 (s, 2H), 3.29 – 3.21 (m, 2H), 2.68 (s, 3H), 1.43 (q, *J* = 7.3 Hz, 2H), 0.81 – 0.67 (m, 1H), 0.38 – 0.26 (m, 2H), 0.16 – 0.08 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.85, 161.76, 160.99, 151.47, 147.46, 144.68, 144.58, 132.34, 131.51, 130.19, 129.44, 129.43, 128.60, 128.05, 125.97, 124.77, 123.75, 122.17, 116.88, 116.44, 116.23, 87.46, 84.70, 33.26, 28.06, 24.76, 18.74, 10.57, 4.18; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{31}H_{27}FN_5O_4S_3$ 648.1204, found 648.1229.

*2-(5-(2-Cyclopropylethyl)-3-(3-((2,5-dimethylthiophen-3-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**83**)*. This compound

was synthesized from coupling the advanced intermediate **VIIIc** with 3-bromo-2,5-dimethylthiophene using the general Sonogashira coupling procedure E and subsequent hydrolysis using general procedure H. LC-MS Retention Time: (Method 1) = 6.982 min and (Method 2) = 3.833 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.28 (s, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.64 – 7.56 (m, 2H), 7.59 (s, 2H), 7.52 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.46 (td, *J* = 7.6, 0.7 Hz, 1H), 7.21 (dd, *J* = 11.3, 1.6 Hz, 1H), 7.09 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.77 (q, *J* = 1.1 Hz, 1H), 4.17 (s, 2H), 3.29 – 3.20 (m, 2H), 2.44 (d, *J* = 0.7 Hz, 3H), 2.38 (t, *J* = 0.9 Hz, 3H), 1.44 (q, *J* = 7.3 Hz, 2H), 0.81 – 0.67 (m, 1H), 0.36 – 0.25 (m, 2H), 0.16 – 0.08 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{33}H_{30}FN_4O_4S_3$ 661.1408, found 661.1414.

*2-(5-(1-Cyclopropylethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**84**)*. This compound was synthesized using general procedures A, B, C, D, E, and H via intermediates **If** through **VIIm**. LC-MS Retention Time: (Method 1) = 6.581 min and (Method 2) = 3.785 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (s, 1H), 8.32 (s, 1H), 7.73 – 7.63 (m, 2H), 7.59 – 7.49 (m, 4H), 7.44 (td, *J* = 7.7, 0.6 Hz, 1H), 7.23 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.17 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.83 (dt, *J* = 3.4, 1.0 Hz, 1H), 4.25 (d, *J* = 3.2 Hz, 2H), 2.47 (d, *J* = 1.1 Hz, 3H), 1.37 (d, *J* = 7.2 Hz, 3H), 0.54 - 0.46 (m, 2H), 0.29 – 0.08 (m, 4H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_4S_3$ 647.1251, found 647.1268.

*2-(5-(2-Cyclopropylpropan-2-yl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**85**)*. This compound was synthesized using general procedures A, B, C, D, E, and H via intermediates **Ig** through **VIIo**. LC-MS Retention Time: (Method 1) = 6.425 min and (Method 2) = 3.741 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27 (s, 1H), 8.58 (s, 1H), 7.74 (t, *J* = 8.0 Hz, 1H), 7.59 (s, 2H), 7.57 – 7.47 (m, 2H), 7.47 – 7.36 (m, 2H), 7.27 – 7.15 (m, 2H), 7.09 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.81 (dt, *J* = 3.4, 1.1 Hz, 1H), 4.30 (s, 2H), 2.46 (dd, *J* = 1.1, 0.4 Hz, 3H), 1.28

(tt, *J* = 8.3, 5.8 Hz, 1H), 1.19 (s, 6H), 0.29 – 0.15 (m, 4H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{33}H_{30}FN_4O_4S_3$ 661.1408, found 661.14.

*2-(4-(3-Fluoro-4-sulfamoylbenzyl)-5-((1-methylcyclopropyl)methyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**86**)*. This compound was synthesized using general procedures A, B, C, D, E, and H via intermediates **Ih** through **VIIn**. LC-MS Retention Time: (Method 1) = 6.694 min and (Method 2) = 3.751 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 8.32 (s, 1H), 7.70 (td, *J* = 1.7, 0.6 Hz, 1H), 7.64 (t, *J* = 7.9 Hz, 1H), 7.63 – 7.56 (m, 1H), 7.55 (s, 2H), 7.50 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.43 (td, *J* = 7.7, 0.6 Hz, 1H), 7.24 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.17 – 7.00 (m, 2H), 6.83 (dt, *J* = 3.4, 1.1 Hz, 1H), 4.21 (s, 2H), 3.45 (s, 2H), 2.48 (d, *J* = 1.1 Hz, 3H), 1.00 (s, 3H), 0.37 – 0.26 (m, 2H), 0.20 – 0.08 (m, 2H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{28}FN_4O_4S_3$ 647.1251, found 647.127.

*2-(4-(3-Fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-5-(spiro[2.2]pentan-1-yl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**87**)*. This compound was synthesized using general procedures A, B, C, D, E, and H via intermediates **Ii** through **VIIp**. LC-MS Retention Time: (Method 1) = 6.604 min and (Method 2) = 3.769 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H), 8.33 (d, *J* = 4.1 Hz, 1H), 7.74 – 7.38 (m, 7H), 7.24 (ddd, *J* = 3.5, 2.7, 0.5 Hz, 1H), 7.20 – 7.01 (m, 2H), 6.83 (dq, *J* = 3.4, 1.1 Hz, 1H), 4.34 – 4.12 (m, 2H), 2.77 – 2.58 (m, 1H), 2.47 (s, 3H), 1.65 – 1.08 (m, 2H), 0.77 – 0.09 (m, 3H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{32}H_{26}FN_4O_4S_3$ 645.1095, found 645.1123.

*2-(5-([1,1'-Bi(cyclopropan)]-2-yl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**88**)*. This compound was synthesized using general procedures A, B, C, D, E, and H via intermediates **Iq** through **VIIk**. LC-MS Retention Time: (Method 1) = 7.018 min and (Method 2) = 3.871 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 8.30 (s, 1H), 7.74 – 7.65 (m, 2H), 7.62

– 7.52 (m, 4H), 7.47 (td, *J* = 7.7, 0.6 Hz, 1H), 7.24 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.20 (dd, *J* = 11.4, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.83 (dt, *J* = 3.4, 1.0 Hz, 1H), 4.14 (s, 2H), 2.78 – 2.63 (m, 1H), 2.47 (d, *J* = 1.1 Hz, 3H), 1.80 (ddq, *J* = 75.6, 14.6, 7.6 Hz, 2H), 0.78 (ddt, *J* = 13.1, 8.2, 4.2 Hz, 1H), 0.55 – -0.05 (m, 4H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{33}H_{28}FN_4O_4S_3$ 659.1251, found 659.1282.

*2-(5-(Dicyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-3-(3-((5-methylthiophen-2-yl)ethynyl)phenyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid (**89**)*. This compound was synthesized using general procedures A, B, C, D, E, and H via intermediates **II** through **VIIr**. LC-MS Retention Time: (Method 1) = 6.68 min and (Method 2) = 3.741 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.11 (s, 1H), 8.32 (s, 1H), 7.72 – 7.63 (m, 2H), 7.63 – 7.54 (m, 3H), 7.51 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.43 (td, *J* = 7.7, 0.6 Hz, 1H), 7.23 (dd, *J* = 3.6, 0.5 Hz, 1H), 7.18 – 7.11 (m, 1H), 7.05 (d, *J* = 7.8 Hz, 1H), 6.83 (dt, *J* = 3.4, 1.1 Hz, 1H), 4.26 (d, *J* = 11.1 Hz, 2H), 2.47 (d, *J* = 1.1 Hz, 3H), 1.96 – 1.14 (m, 3H), 0.73 – -0.19 (m, 8H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{34}H_{30}FN_4O_4S_3$ 673.1408, found 673.1422.

Biological assays: LDH and MDH biochemical assays, cellular lactate production assay, cytotoxicity assays were performed as previously reported[19] and also provided in the supplemental documents.

Split Nano Luciferase Cellular Thermal Shift Assay (SplitLuc CETSA): The SplitLuc CETSA was performed in 1536-well plates as previously described[23]. Briefly, HEK293T cells were transiently transfected with plasmid encoding a CMV-driven LDHA open reading frame with a carboxy-terminal 86b fusion tag (GS[HiBiT]GS). Cells were transfected in a T175 flask for 24 h using $2.3x10^7$ cells, 52.5 µg plasmid DNA, and 105 µL Lipofectamine 2000. Cells were lifted, resuspended at $5x10^5$ cells /mL (DPBS with $CaCl_2$ and $MgCl_2$ plus 1 g/L glucose) and re-seeded into 1536-well cyclic olefin white plates (Aurora, cyclic olefin polymer, cat# EWB041000A) using a Multidrop Combi, at 2500 cells per well (5 µL volume).

Compounds (23 nL) were added to cells using a pin tool (Wako Automation) and incubated for 1 h at 37 °C. Plates were heated to 63.5 °C for 7.5 min using a custom machined copper heat block fitted with an internal type-T thermocouple and controlled by a Watlow temperature controller. Plates were removed from the heat block and cooled to room temperature. One µL of 6% NP40 was added per well and plates were incubated for 30 min to allow cell lysis followed by addition of 3 µL substrate containing 11S (final concentration 100 nM) and furimazine (final concentration 0.5X; from Promega 50X stock). Plates were centrifuged and analyzed for luminescence intensity using a ViewLux reader equipped with clear filters. Luminescence values were normalized to an unheated control sample.

Glycolytic stress test assay: A673 cells were cultured in Dulbecco's Modified Eagle's Medium (ATCC Catalog No. 302002) supplemented with fetal bovine serum (10%). The cells were plated into a XF96 cell culture microplate in the above medium and maintained in a 5% CO_2 incubator at 37˚C for 24h prior to the experiments. The day of the assay, compounds were diluted to the appropriate concentration in freshly prepared assay media (Seahorse basic DMEM with 2 mM Glutamine, pH 7.4 at 37 ^oC). The media in the plate with cells was then changed to assay media and maintained in a non-CO_2 incubator at 37 ˚C for 1h prior to the assay. The Seahorse XF Glycolysis Stress Test (GST) was conducted by injecting the LDH inhibitors, then, at 40 min, subsequent injections of glucose (10 mM final concentration), oligomycin (1 µg/mL final concentration), and 2-deoxyglucose (2-DG; 50 mM final concentration) as described previously.[29]

PAMPA permeability assay: The stirring double-sink PAMPA method patented by pION Inc. (Billerica, MA) was employed to determine the permeability of compounds via PAMPA passive diffusion. The PAMPA lipid membrane consisted of an artificial membrane of a proprietary lipid mixture and dodecane (Pion Inc.), optimized to predict gastrointestinal tract (GIT) passive diffusion permeability, was immobilized on a plastic matrix of a 96 well

"donor" filter plate placed above a 96 well "acceptor" plate. A pH 7.4 solution was used in both donor and acceptor wells. The test articles, stocked in 10 mM DMSO solutions, were diluted to 0.05 mM in aqueous buffer (pH 7.4), and the concentration of DMSO was 0.5% in the final solution. During the 30-min permeation period at room temperature, the test samples in the donor compartment were stirred using the Gutbox technology (Pion Inc.) to reduce the unstirred water layer. The test article concentrations in the donor and acceptor compartments were measured using an UV plate reader (Nano Quant, Infinite 200 PRO, Tecan Inc., Männedorf, Switzerland). Permeability calculations were performed using Pion Inc. software and were expressed in units of 10^{-6} cm/s.

BBB-PAMPA permeability assay: The double-sink PAMPA method was utilized for the blood-brain-barrier (BBB) permeability assay as described previously.[30] The PAMPA BBB-1 lipid (PN 110672, Pion) membrane consisted of 10% (w/v) porcine lipid brain extract dissolved in a proprietary phospholipid mixture and alkane. Like the PAMPA assay, test articles initially stocked at 10 mM DMSO solutions, were diluted to 0.05 mM in aqueous buffer (pH 7.4). Test samples were stirred using the Gutbox technology at 60 μM aqueous boundary layer setting for 1 hr at room temperature. The concentrations of compounds in the donor and acceptor compartments were measured against a reference and blank samples. The membrane permeability calculations were performed using PAMPA Evolution Software (version 3.8) from Pion Inc. and were expressed in units of 10^{-6} cm/s.

Kinetic solubility test assay: Pion's patented μSOL assay for kinetic solubility determination was used. In this assay, the classical saturation shake-flask solubility method was adapted to a 96-well microtiter plate format and a co-solvent method with *n*-propanol as the reference compound was utilized. Test compounds were prepared in 10 mM DMSO solutions (45 μL) and diluted with the co-solvent to a final drug concentration of 150 μM in the aqueous solution (pH 7.4). Samples are incubated at room temperature for 6 hrs to achieve equilibrium. The

samples were then filtered to remove any precipitate formed. The concentration of the compound in the filtrate was measured by UV absorbance. The reference drug concentration of 17 μM was used for quantitation of unknown drug concentration in filtrate. Spectroscopically pure 1-propanol was used as a cosolvent to suppress precipitation in the reference solutions. The kinetic solubility (μg/mL) was calculated with using the μSOL Evolution software.

Rat liver microsome stability assay: Single time point microsomal stability was determined in a 96-well HTS format. Sample preparation was automated using Tecan EVO 200 robot. High Resolution LC/MS (Thermo QExactive) instrument was used to measure the percentage of compound remaining after incubation using a previously described method.[31] Six standard controls were tested in each run: buspirone and propranolol (for short half-life), loperamide and diclofenac (for short to medium half-life), and carbamazepine and antipyrine (for long half-life). Briefly, the incubation consisted of 0.5 mg/mL microsomal protein, 1.0 μM drug concentration, and NADPH regeneration system (containing 0.650 mM $NADP^+$, 1.65 mM glucose 6-phosphate, 1.65 mM $MgCl_2$, and 0.2 unit/mL G6PDH) in 100 mM phosphate buffer at pH 7.4. The incubation was carried out at 37 °C for 15 min. The reaction was quenched by adding 555 μL of acetonitrile (~1:2 ratio) containing 0.28 μM albendazole (internal standard). Sample acquisition and data analysis was done using a previously described method.[29]

Mouse pharmacokinetic studies: All PK studies were conducted by Pharmaron. Male CD1 mice (sourced from Si Bei Fu Laboratory Animal Technology Co. Ltd), approximately 6-8 weeks of age and a weight of approximately 20-30 g were dosed with compounds at 10 mg/kg (IV) or 40 or 50 mg/kg (PO). The formulation (0.1 M NaOH in PBS Buffered saline, adjusted with 1N HCl to pH 7-8.5) was prepared on the day of dosing. Each dosing cohort had 3 mice and plasma was collected at 5 min, 15 min, 30 min, 1h, 2h, 4h, 8h, 12h, and 24h post dose for IV and 15 min 30 min, 1h, 2h, 4h, 8h, 12h, and 24h for PO. Approximately 0.025 mL blood

was collected via the dorsal metatarsal vein at each time point. Blood samples were then transferred into plastic micro centrifuge tubes containing Heparin-Na as anti-coagulant. Samples were then centrifuged at 4,000 g for 5 minutes at 4 ^{o}C to obtain plasma. Plasma samples were then stored in polypropylene tubes, quickly frozen and kept at -75 ^{o}C until analyzed by LC/MS/MS. The following pharmacokinetic parameters were measured $T_{1/2}$, C_0, C_{max}, T_{max}, CL, V_d, $AUC_{last,}$ and F. Animals were also monitored during the in-life phase by once daily cage side observations, any adverse clinical signs are noted as part of the PK report.

In vivo LDHA activity in tumor mouse model: Two million A673 cells were injected SQ into each female athymic nude mouse from Taconic (CrTac:NCr-Foxn1nu). Mice were divided into 4 treatment groups of 16 mice each. Each group received a single IV injection of 50 mg/kg of an LDHA inhibitor. Four mice from each group were sacrificed at 1, 3, 6, and 24 hours after dosing. Inhibitors were dissolved in standard PBS-based vehicle. At the time of sacrifice, frozen samples of tumor and plasma were collected and compound levels determined through LC MS/MS measurement against standard curves of each compound. Frozen tumor samples were also collected for LDHA activity measurements as follows. Briefly, 10 – 50 mg of frozen tumors were pulverized in liquid nitrogen followed by the addition of ten volumes of phosphate buffered saline, pH 7.4 containing 0.1% triton x-100 and incubated on ice for 1h. Samples were clarified by centrifugation and cleared tumor lysates (10-20 μg) were measured by uv-vis spectrometry for LDH activity in LDH assay buffer containing 10 mM sodium pyruvate (Sigma-Aldrich, St. Louis, MO). Reactions were initiated by the addition of 15 mM NADH (final concentration 0.3 mM) and monitored for the oxidation of NADH at 340 nM at 37°C.

Use of animal subjects

All animal studies included as part of this manuscript were performed in accordance with institutional guidelines as defined by Institutional Animal Care and Use Committee (IACUC).

Ancillary Information

Supporting Information: Additional supplemental figures, experimental procedures for representative scale up of lead compounds and key intermediates and spectroscopic data. Molecular formula strings are also available. This material is available free of charge via the internet at http://pubs.acs.org.

PDB ID Codes: 6Q0D (**23**), 6Q13 (**52**). Authors will release the atomic coordinates and experimental data upon article publication.

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Conflicts of Interest

The authors declare no competing financial interest

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Abbreviations Used: ACN, acetonitrile; CETSA, cellular thermal shift assay; LDH, lactate dehydrogenase; MeOH, methanol; MW, microwave; NCGC, NCATS Chemical Genomics Center; PAMPA, parallel artificial membrane permeability assay; PK, pharmacokinetics; qHTS, quantitative high-throughput screening; SAR, structure-activity relationships; SPR, Surface Plasmon Resonance; TBAF, Tetra-n-butylammonium fluoride TFA, trifluoroacetic acid; THF, tetrahdrofuran.

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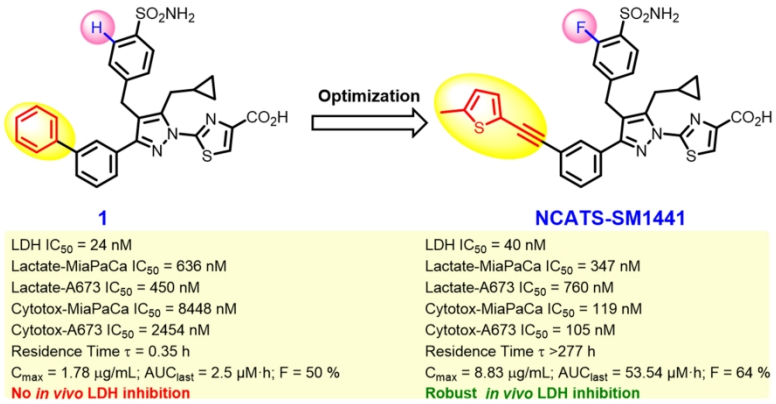

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