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Structure–activity relationship studies of a novel series of anthrax lethal factor inhibitors

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ABSTRACT

We report on the identification of a novel small molecule inhibitor of anthrax lethal factor using a high-throughput screening approach. Guided by molecular docking studies, we carried out structure–activity relationship (SAR) studies and evaluated activity and selectivity of most promising compounds in in vitro enzyme inhibition assays and cellular assays. Selected compounds were further analyzed for their in vitro ADME properties, which allowed us to select two compounds for further preliminary in vivo efficacy studies. The data provided represents the basis for further pharmacology and medicinal chemistry optimizations that could result in novel anti-anthrax therapies.

1. Introduction

Anthrax lethal factor has become an attractive drug target due to its relevance to the pathogenesis of *Bacillus anthracis*, a bioterrorism agent.[1] *B. anthracis*, a rod shaped bacterium which infects humans through the respiratory system, skin, or digestive tract can be highly lethal depending upon its entry route into the human body. Although cutaneous anthrax is rarely lethal, inhalation anthrax is dangerous and usually fatal.[2] The bacteria release a toxin that kills host macrophages, consisting of three virulence factors: protective antigen (PA, 83 kDa), edema factor (EF, 89 kDa) and lethal factor (LF, 90 kDa). It has been shown that bacterial strains lacking LF are not lethal in mice.[3–6] Hence, lethal factor (LF), a Zn^{2+}-metalloprotease, plays a critical role in the cleavage of several members of the MAPKK family near their N-terminus. This cleavage prevents interaction with, and phosphorylation of, downstream MAPK, thereby inhibiting one or more signaling pathways leading to cell death in macrophages. The MAPKK family has seven members, two MEKs (MAPK/ERK (extracelluar-signal-regulated kinase) Kinase) 1 and 2 (MEK1, MEK2) and five MKKs (MKK3, MKK4, MKK5, MKK6 and MKK7). MEK1 and MEK2 specifically phosphorylate and activate the ERK1 and ERK2 MAPKs. MKK3 and MKK6 are specific for the MAP kinase $p38^{hog}$, where MKK4 and MKK7 phosphoactivate the stress-activated protein kinase JNK (c-Jun N-terminal kinase), although MKK4 can also phosphorylate $p38^{hog}$. LF cleaves MEK1, MEK2, MKK3 and MKK6 at one site while MKK4 and MKK7 are cleaved at two different sites.[7]

With the threat of lethal anthrax spores being used as a biological weapon, the current treatment includes administration of appropriate antibiotics usually prior to the occurrence of symptoms. Although antibiotics such as ciprofloxacin are effective against *B. anthracis*, high levels of secreted toxin may remain in circulation for several days which continues to damage the host even after the bacteria may have been killed.[6] Moreover, the current anthrax vaccine approved by the FDA for human use in the United States is AVA (anthrax vaccine adsorbed), which consists of a *B. anthracis* culture supernatant that has been adsorbed onto an aluminum adjuvant. By stimulating antibodies against PA, this vaccine has shown to provide protection in animal models of anthrax. However, humans treated with AVA requires six administered doses within an 18 month time period, along with annual booster immunizations, which is not an ideal dose regimen if there should be a need for rapid vaccination before or in response to bioterrorist events. Thus a combination of antibiotics and toxin inhibitors has been proposed as a rational approach for developing a more rapid response against anthrax. Since LF has been shown to act as the key virulence factor, much work has been focused on finding potent inhibitors of LF. Currently there are several potent LF inhibitors, in which some have been identified in our laboratory, however, only a few of these inhibitors are significantly effective in in vivo models. Therefore it is important that LF inhibitors not only inhibits the cleavage of MAPKK, but also are able to be bioavailable and to enter and remain active in cells since LF functions in the cytosol.

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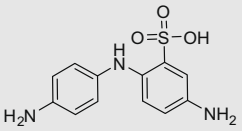
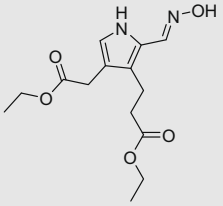
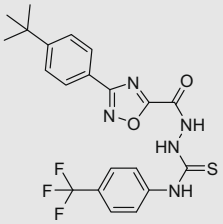
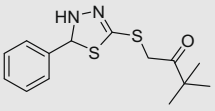
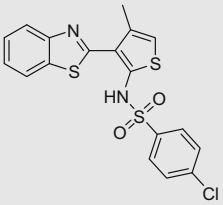
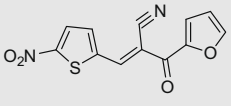
2. Results and discussion

The LF inhibitors identified thus far contain key Zn^{2+}-chelating moieties[7] such as a hydroxymate,[8–13] polyphenols/catechol,[14,15] penicillin based,[16] rhodanine,[17,18,14,6,19] aminoglycoside,[20–22] quinoline urea[23] and hydrazone.[24,25] In this study, we present the identification of a novel scaffold shown to inhibit LF in vitro and *in cell*. This scaffold was identified via screening a diverse chemical library of 16,000 drug-like molecules (HitFinder™, Maybridge, Morris Plains, NJ) utilizing high-throughput fluorescence microplate assays as previously described.[17,14,18] Kinetic studies of the most potent LF inhibitors with >50% inhibition at 20 μM, were performed and IC_{50} and K_i values were obtained by dose response measurements. A total of six compounds (Table 1) gave IC_{50} values ranging between 3 and 19 μM. Considering the ease of synthesis and guided by docking studies, we decided to focus on compound **5** (Table 1). Molecular modeling studies revealed that the docked geometry of compound **5** could fit well with the redocked molecular model of compound **7** from the previously published X-ray crystal structure by Shoop et al.[13] consisting of compound **7** in complex with LF (PDB: 1YQY) (Fig. 2). Shoop et al. demonstrated in the X-ray structure that compound **7** coordinated to the Zn^{2+} atom via the hydroxymate moiety, the thiophene group for compound **5** aligns similarly allowing us to preclude that the thiophene is coordinating the Zn^{2+} atom.

Table 1
Chemical structure and inhibitory activity of 6 hit compounds from the HTS campaign against lethal factor (LF)

#	Structure	IC_{50} (K_i) (μM)
1		3.4 (0.56)
2		4.2 (5.9)
3		4.8 (0.38)
4		12 (5.0)
5		15 (7.8)
6		19 (6.6)

Initially, we synthesized compound **5**, tested it to validate its kinetic activity and closely examined its predicted binding in the catalytic site of LF (Fig. 2). In comparing the interactions of compounds **5** and **7** in the LF catalytic site, we observed that their mode of binding was similar, in which the hydroxymate of compound **7** chelating the zinc is represented by the thiophene moiety in compound **5**, with the remaining sub-structures occupying similar subsites (Fig. 2). Based on the proposed docking geometry, we decided to perform structure–activity relationship studies in which we first explored changes amongst the sulfonylamide group to determine its relevance in binding to LF, (Table 2), with or without a methyl group at the fourth position of the thiophene ring (Tables 3 and 4). We further dissected this scaffold by exchanging the thiophene ring with an isooxazole, a benzene ring, an ethyl group or completely eliminating the thiophene group (Table 5). The synthesis of analogues which lacked the benzothiazole moiety was also carried out (Table 6) as well as the synthesis of disubstituted sulfonamides (Table 7).

The syntheses of these compounds were carried out under normal sulfonamide coupling conditions using pyridine as the solvent except for the disubstituted sulfonamides in which we used dichloromethane and triethylamine.[26] The compounds were obtained with average yields of 65–80% and purity of at least 95% for key compounds. Once synthesized and characterized, we then performed an enzymatic assay to evaluate the inhibitory activity of the resulting compounds against LF. The fluorescence peptide cleavage assay was performed in a 96 well plate (100 μL/well) as we previously reported.[19] For a number of compounds, including the six original hits, a Lineweaver–Burk analysis was also carried out to verify that the compounds are competitive against the substrate (Fig. 1).

From the data reported in Table 2 (benzothiazole-4-methylthiophene derivatives, BTMT), we first compared the lead compound **5** (Table 1) with compounds **8** and **9** and observed a loss in activity for the molecules lacking a sulfonylaryl moiety or an electron withdrawing substituent on the sulfonylaryl group. However, it seems that the position of the substituents is critical for optimal binding. For instance, if we take compounds **10** and **11**, which have a trifluoromethyl group in the *meta* or *ortho* position, respectively, compound **10** is 10 times more potent than compound **11**. A similar trend was also observed with compounds which contained a substituent in the *para* position (compounds **12**, **13**, **14**, **15**), *meta* position (compounds **32** and **33**) or disubstituted *para*/*meta* position (compounds **19**, **20**, **21** and **24**). Here the *meta* or disubstituted analogues showed improved inhibitory properties with the exception of compounds **23** and **31**. Overall, the most potent compounds of the BTMT series were compounds **21** and **10** which gave IC_{50} values of 8.9 and 9.4 μM, respectively.

As part of the evaluation of the SAR at the sulfonylamide moiety, replacement of BTMT with benzothiazole thiophene (BTT) retained enzyme potency while reducing the molecular weight of the compounds (Tables 3 and 4). Initially, we showed that a compound lacking the sulfonylaryl moiety, (compound **42**), was essentially inactive. However, reintroducing a sulfonylbenzene moiety (compound **43**) leads to an activity with an IC_{50} value of 39 μM. Comparison of the IC_{50} values of compounds **43** and **9** (Table 2) revealed that the BTT series may lead to more potent inhibitors. Analysis of the IC_{50} values with the within the BTT series, did not reveal a clear trend regarding the role of the positioning of the substituents. However, compounds that were disubstituted in the

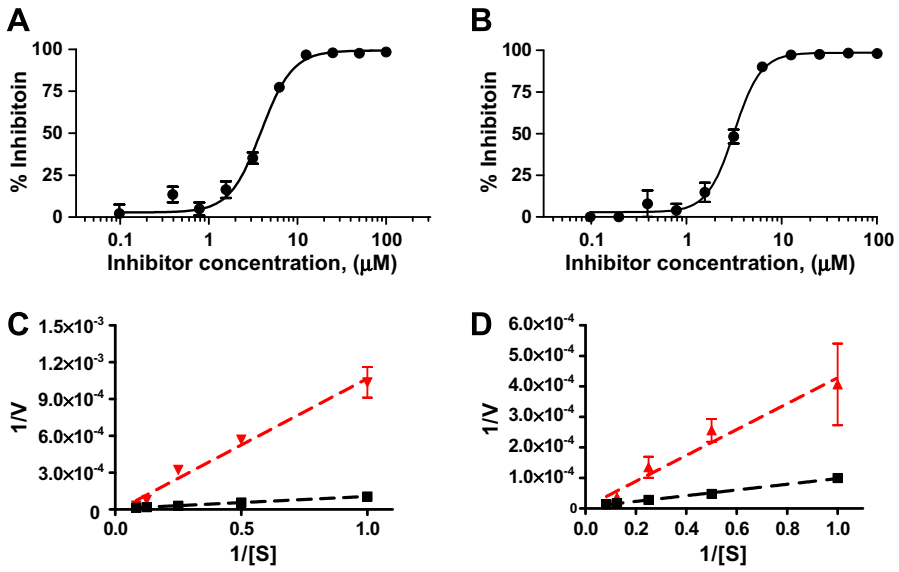


Figure 1. Kinetics of inhibition of lethal factor (LF) for compounds **70** and **71**. IC_{50} evaluation of (A) compound **70** and (B) compound **71** against LF; C) K_i evaluation of compound **70** at 10 μM; (red line; K_i = 1.01 μM); the black dashed line represents DMSO. D) K_i evaluation of compound **71** at 6 μM; (red line; K_i = 1.7 μM); the black dashed line represents DMSO.

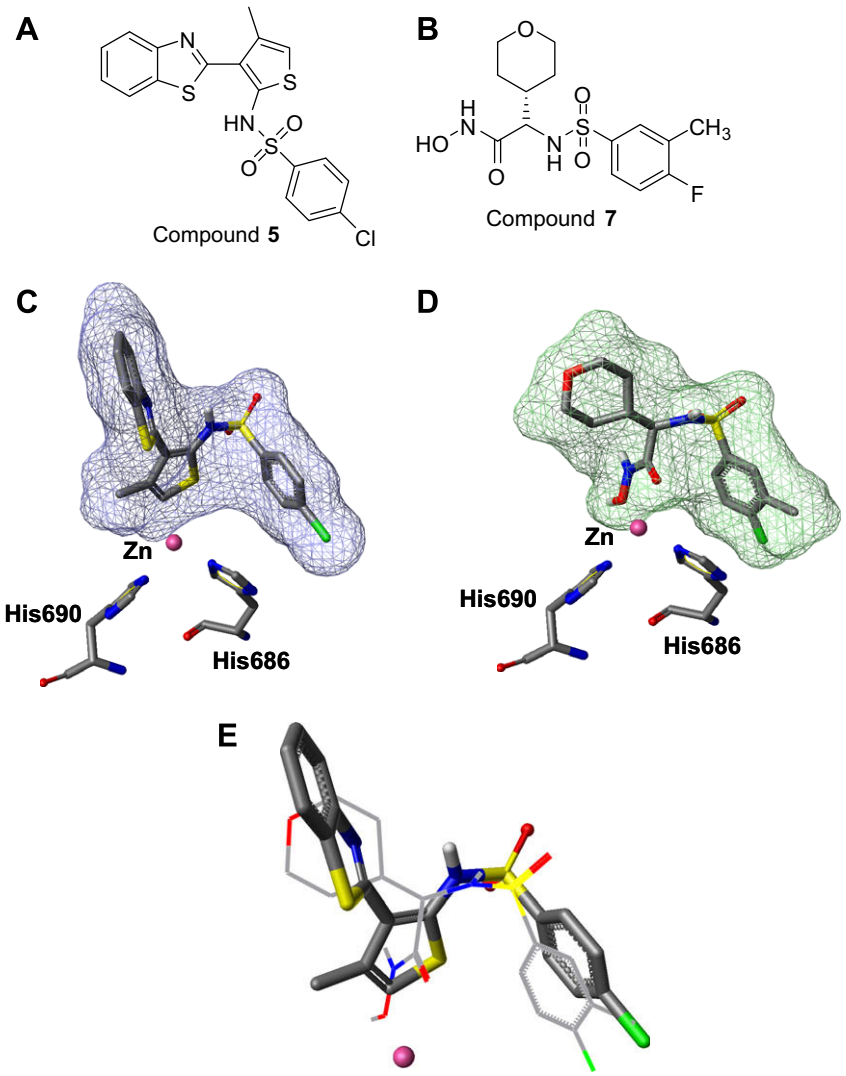
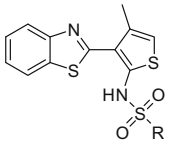
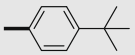
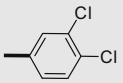
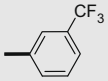
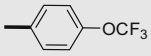
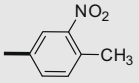
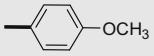
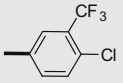
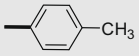
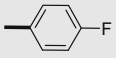
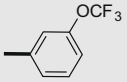
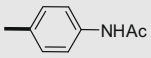
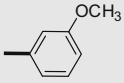
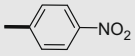
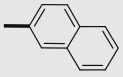
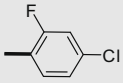
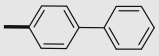
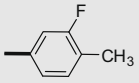
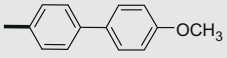
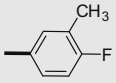
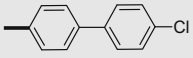
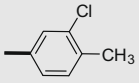
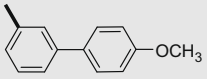
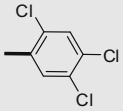
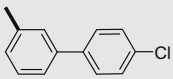
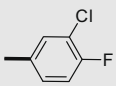
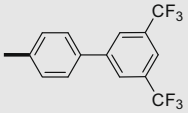
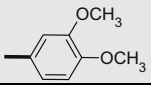
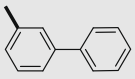


Figure 2. Molecular modeling studies; (A) Chemical structure of compound **5**. (B) Chemical structure of Compound **7**. (C) Detail of the redocked geometry of compound **7** from the X-ray coordinates in complex with LF (PDB: 1YQY).[13] Side chain residues His690 and His686 and the catalytic Zn^{2+} from LF are displayed. (D) Docked geometry of compound **5** in the catalytic site of LF. (E) Superimposition of compounds **5** (ball and stick model) and **7** (thin line model).

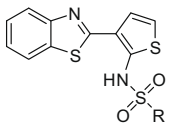
Table 2
Benzothiazole-4-methylthiophene (BTMT) derivatives and IC_{50} values against lethal factor (LF)

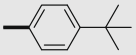
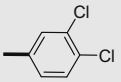
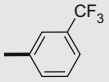
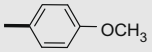
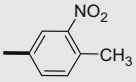
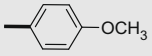
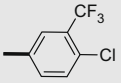
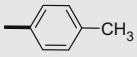
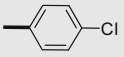
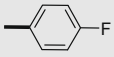
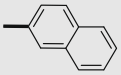
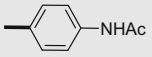
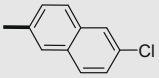
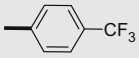
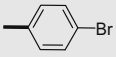
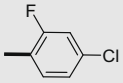
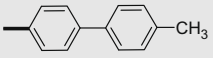
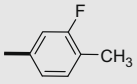
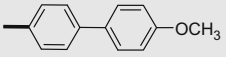
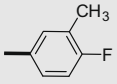
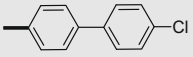
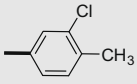
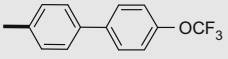
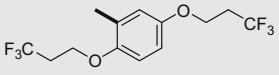
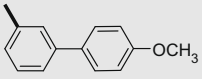
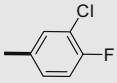
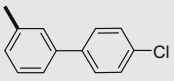
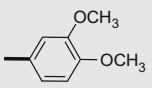
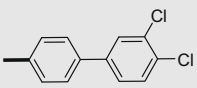
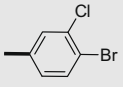
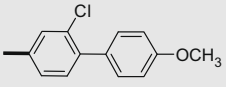


#	R	IC_{50} (μM)	#	R	IC_{50} (μM)
8	H[a]	>100	**25**		>100
9		>100	**26**		>100
10		9.4	**27**		100
11		>100	**28**		100
12		18	**29**		48
13		46	**30**		11
14		>100	**31**		>100
15		>100	**32**		11
16		14	**33**		11
17		11	**34**		12
18		19	**35**		17
19		35	**36**		21
20		17	**37**		12
21		8.9	**38**		16
22		15	**39**		22
23		>100	**40**		17
24		34	**41**		19

[a] Free amine.

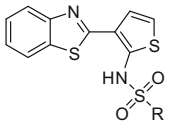
Table 3
Benzothiazole thiophene (BTT) derivatives and IC_{50} values against lethal factor (LF)

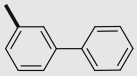
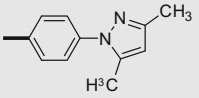
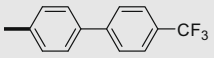
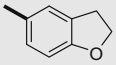
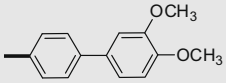
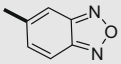
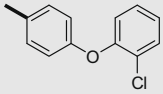
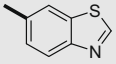
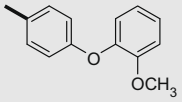
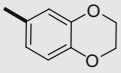
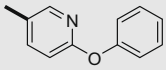
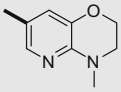
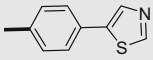
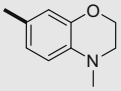
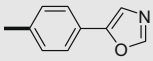
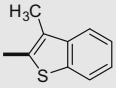
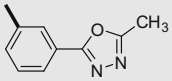
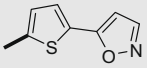
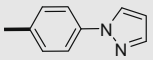
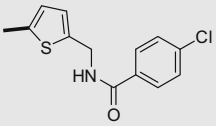
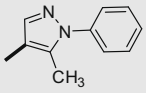
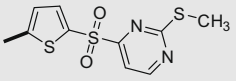
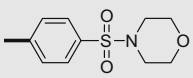
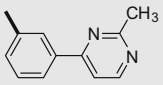


#	R	IC_{50} (μM)	#	R	IC_{50} (μM)
42	H[a]	>100	**59**		13
43		39	**60**		9
44		16	**61**		30
45		9.3	**62**		>50
46		9.6	**63**		10
47		18	**64**		10
48		13	**65**		15
49		16	**66**		8
50		24	**67**		11
51		11	**68**		8
52		18	**69**		28
53		11	**70**		3.8
54		12	**71**		3.0
55		6.6	**72**		3.9
56		39	**73**		14
57		13	**74**		18
58		19	**75**		7.2
123		4.5	**124**		3.0

[a] Free amine.

Table 4
Benzothiazole thiophene (BTT) heterocyclic derivatives and IC_{50} values against lethal factor (LF)



#	R	IC_{50} (μM)	#	R	IC_{50} (μM)
76		13	**89**		11
77		8	**90**		22
78		9	**91**		30
79		9	**92**		10
80		>50	**93**		27
81		43	**94**		27
82		12	**95**		23
83		13	**96**		10
84		25	**97**		19
85		8	**98**		14
86		>50	**99**		18
87		17	**100**		>50
88		12	**101**		19

para/*meta* position versus monosubstitution in the *meta* or *para* position appeared overall more potent (compounds **44**, **48**, **49**, **65**, **66**, **67**, **53**, **54**, **57**, **63** and **64**) with the exception of compounds **47** and **58**. This observation resulted in the design and synthesis of compound **55** which indeed has an improved IC_{50} value of 6.6 μM. On the contrary, disubstitution at the *ortho*/*meta* or *ortho*/*para* position led to compounds that lost activity dramatically (compounds **56**, **61**, **62**). Further, we synthesized compounds containing biphenylsufonyl groups that overall seem to be more potent. The most noticeable improvements were observed with compounds **70**, **71** and **72**, giving IC_{50} values of 3.8, 3.0 and 3.9 μM, respectively. Based on the SAR analysis discussed above, we subsequently synthesized the 3′,4′-disubstituted biphenyl derivatives that however led to compounds which are slightly less active (compounds **75** and **78**). Within the BTT series we also observed that disubstitution at the *meta*/*para* position, where the *meta* position had a chlorine

Table 5
Benzothiazole derivatives and IC_{50} values against lethal factor (LF)

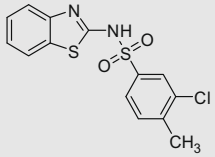
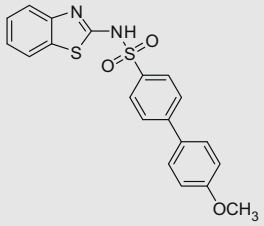
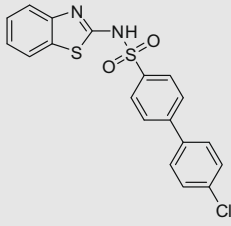
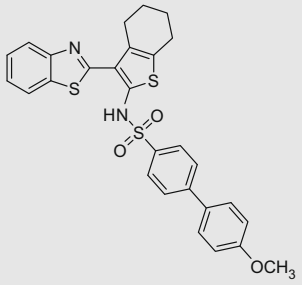
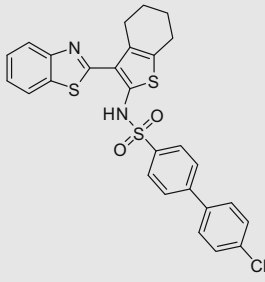
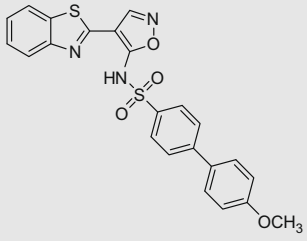
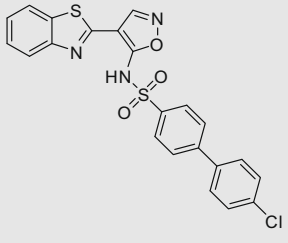
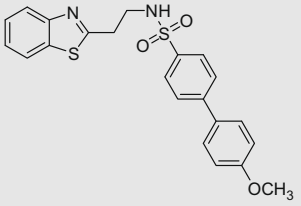
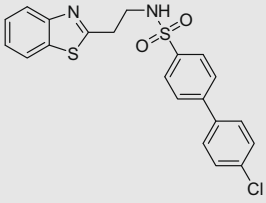
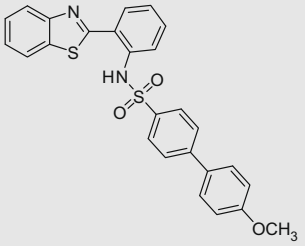
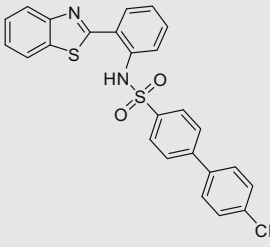
#	Structure	IC_{50} (μM)
102		28
103		>50
104		>50
105		>50
106		>50
107		>100
108		>100

Table 5 (*continued*)

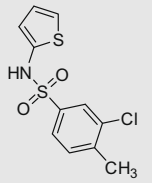
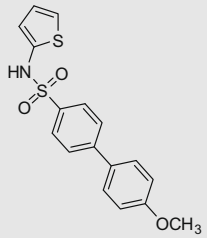
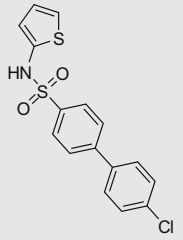
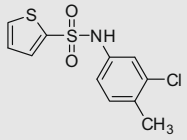
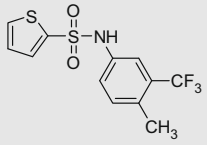
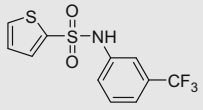
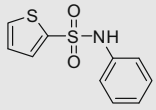
#	Structure	IC_{50} (μM)
109		>50
110		>50
111		>100
112		100

group, led to improved activity (compounds **49** vs **57**, **48** vs **55**, **65** vs **60**, **68** vs **123**). Based on these observations, we synthesized the 3-chloro-4-disubstituted biphenyl derivative **124**, in which the inhibitory activity against LF was slightly improved when compared to the related compound **70**. Moreover, the synthesis of BTT analogues with various heterocyclicsulfonyl moieties did not lead to compounds with increased affinity against LF (Table 4).

Finally, the substitution of the thiophene ring (Table 5) with various groups or its elimination resulted in compounds that lack significant inhibitory activity against LF. Similarly, compounds in which we eliminated the benzothiazole ring (Table 6), showed no inhibition at 50 μM, as well as the disubstituted sulfonylbenzene derivatives (Table 7). Overall, in comparing the BTT series with the BTMT series, we found that in most cases the binding affinity is significantly improved in the former series, with the exception of just a few compounds. Furthermore, we also found that most active compounds against LF did not inhibit (up to 100 μM) human matrix metalloproteases MMP-2 and MMP-9, the most closely related human proteases to LF.[19,14,18]

In order to assess the efficacy of the compounds in a cellular context, we measure the ability of most potent LF inhibitors to protect macrophages from LF/PA induced cell death.[19] As shown in Table 8, compound **70** gave an IC_{50} value of 506 nM in this assay, while compounds **71**, **93** and **55**, and gave IC_{50} values of 251 nM, 1.6 μM and 1 μM, respectively. Hence we identified selective and potent LF inhibitors which are effective in protecting human macrophages from anthrax toxin. In order to evaluate whether these compounds are likely to be effective in vivo, we first measured crit-

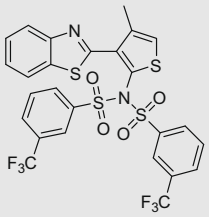
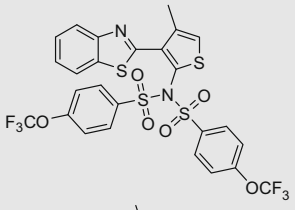
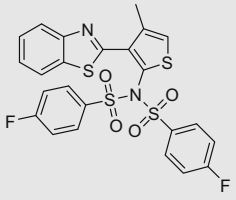
Table 6
Thiophene derivatives and IC_{50} values against lethal factor (LF)

#	Structure	IC_{50}
113		>50
114		>50
115		>50
116		>100
117		>100
118		>100
119		>100

ical in vitro parameters such as chemical stability, plasma stability, microsomal stability, solubility and cell permeability (Table 8). From these data, we concluded that except for solubility, compounds **70** and **55** were the most suited candidates for preliminary in vivo efficacy studies (Fig. 3). Preliminarily, when administered via intraperitoneal injection (daily at 25 mg/kg), compound **70** seemed to be a little more effective in protecting the mice after 8 days of post infection compared to compound **55**. While the observed protection with compound **70** is significant, further studies aimed at the determination of proper formulation of the compound (to increase solubility) and pharmacokinetics (to assess dose and regimen) must be conducted to improve the efficacy of the inhibitor.

In conclusion, we have generated and validated a novel series of LF inhibitors with low- to sub-micromolar activity. By using SAR studies, we were able to design an inhibitor against LF, which shows to be effective in macrophages and lack cytotoxicity. We were also able to show selectivity amongst MMP-2 and -9 and show that with a single daily dose of LF inhibitor we were able to observe some protection using in vivo mice models of anthrax. Thus pharmacokinetic studies of compounds **55** and **70** are underway.

Table 7
Disubstituted benzothiazole thiophene derivatives and IC_{50} values against lethal factor (LF)

	Structure	IC_{50}
120		>100
121		>100
122		>100

3. Methods and materials

3.1. Reference compounds and reagents

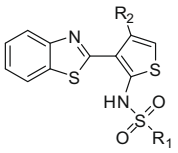
Reference compounds **8** and **42** were purchased from Ryan Scientific. All common chemicals, reagents, and buffers were purchased from Sigma–Aldrich (St. Louis, MO), Acros Organics (Geel, Belgium) or Maybridge (Morris Plains, NJ). Recombinant LF and MAPKKide® were purchased from List Biological Laboratories (Campbell, CA). The MMP-2 and MMP-9 assay kits were purchased from Anaspec, Inc. (San Jose, CA). Synthetic details of the benzothiazole derivatives are described in the methods and materials section. Characterization of each benzothiazole derivative was obtained by means of NMR spectroscopy. Elemental analysis was performed for compounds **70**, **72**, **92** and HR/MS and HPLC purity was performed for compounds **10**, **16**, **17**, **20**, **21**, **22**, **30**, **32**, **33**, **34**, **35**, **37**, **38**, **40**, **44**, **45**, **46**, **48**, **49**, **51**, **53**, **54**, **55**, **57**, **59**, **60**, **63**, **64**, **65**, **66**, **67**, **68**, **71**, **72**, **73**, **75**, **77**, **78**, **79**, **82**, **83**, **85**, **87**, **88**, **89**, **92**, **98**, **123**, **124** (Tables 9 and 10).

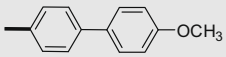
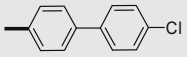
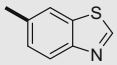
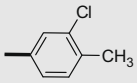
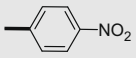
3.2. Enzymatic assays

3.2.1. MAPKKide® assay

The fluorescence peptide cleavage assay was performed in a 96 well plates (100 μL/well) in which each reaction mixture contained MAPKKide® (4 μM) and LF (50 nM) (List Biological Laboratories) in 20 mM Hepes, pH 7.4, and the screening compounds (mixture of 20; each compound at 10 μM). Kinetics of the peptide cleavage was examined for 30 min by using a fluorescent plate reader (Vic-

Table 8
In cell data along with in vitro ADME experiments for selected LF inhibitors



#	R1	R2	IC_{50} (K_i) kinetic	EC_{50}	Chem. stability (%)	Log*S* (Ug/mL)	Pampa	Metabolic stability Hept.	Plasma stability (%)
70		H	3.8 (1.0)	506 nM	84	<10[a]	Low	$t_{1/2}$ = >120 min CL_{int} = 3.6	99
71		H	3.0 (1.7)	251 nM	ND	<10[a]	Low	$t_{1/2}$ = >120 min CL_{int} = –	100[b]
92		H	10.0	1.6 μM	99.9	>100	High	$t_{1/2}$ = >120 min CL_{int} = 3.9	90
55		H	6.6	1.0 μM	99	>100	High	$t_{1/2}$ = >120 min CL_{int} = –	100
17		CH_3	11.0	ND	99	<10[a]	High	$t_{1/2}$ = >120 min CL_{int} = 0.6	100

ADME experiments conducted according to the protocols at Asinex Corporation, Moscow, Russia, http://asinex.com).[19]
ND = Not determined.
[a] This compound is insoluble at 500 μM.
[b] Results for 15 min.

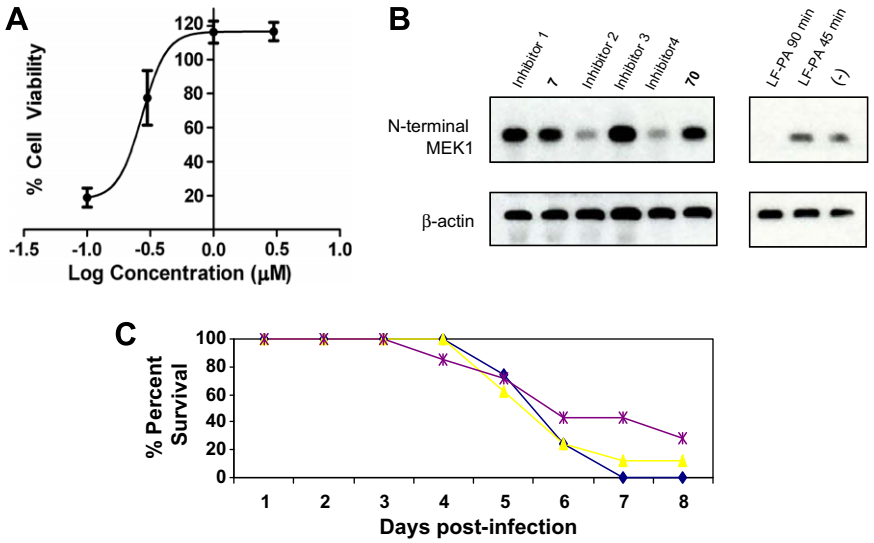


Figure 3. In cell and in vivo mice studies of the inhibition of lethal factor (LF) for compounds **70** and **55**. (A) Macrophage assay for the inhibition of LF for compound **70** (EC_{50} = 561 nM). (B) Effect of test compounds on LF cleavage of MEK1. (Left panel) HeLa cells were incubated with a mixture of LF, PA_{83}, and different concentrations of LF inhibitors (inhibitor 1 at 300 μM; compound **7**, inhibitor 2, 3, 4 at 30 μM; compound **70** at 100 μM). After 90 min, cells were lysed and protein lysates were subjected to SDS/PAGE and to immunoblotting with an anti-MEK1 antibody, specific for the N-terminus of MEK1. β-Actin was immunoblotted as loading control. Inhibitor 3 and compound **70**, along with known inhibitors 1 and compound **7**, are effective in protecting HeLa cells against LF cleavage of MEK1. Two weaker LF inhibitors 2 and 4 partially prevent the MEK1 cleavage. (Right panel) Controls included untreated cells (–) and LF-PA-only treated cells. The data show that MEK1 cleavage is complete after 90 min of LF-PA addition. (C) Compounds **70** and **55**; protection of A/J mice from anthrax. Mice given DMSO alone were used as control and all died on day 7 (blue line). The group treated with compound **70** (violet line) had the best survival ($p < 0.01$ compared to control group) while the group receiving compound **55** had slight improvement in comparison to the control ($p < 0.05$).

tor V, Perkin Elmer, Waltham, MA) at excitation and emission wavelengths of 485 and 535 nm, respectively, and IC_{50} values were obtained by dose response measurements. For selected compounds, Lineweaver–Burk analysis was also carried out to verify that the compounds are competitive against the substrate. The K_m and V_{max} values of the MAPKKide® cleavage by LF were determined at 25 °C by using the same experimental condition described above for the fluorescence screening assay but with increasing MAPKKide® concentrations (10, 5, 2.5 μM). The K_i and $K_{m(app)}$ were calculated at 5 and/or 10 μM inhibitor concentration.

Table 9
Elemental analysis of compounds **70**, **72**, **92**

#	Formula	C (%)		H (%)		N (%)		S (%)	
		Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
70	$C_{24}H_{18}N_2O_3S_3$	60.23	59.86	3.79	4.13	5.85	6.15	20.10	19.74
72	$C_{24}H_{15}F_3N_2O_3S_3$	54.12	55.03	2.84	3.46	5.26	5.21	18.06	15.99
92	$C_{18}H_{11}N_3O_2S_4$	50.33	49.23	2.58	2.49	9.78	9.83	20.10	28.97

Table 10
HRMS analysis and HPLC purity for compounds **10**, **16**, **17**, **20**, **21**, **30**, **33**–**35**, **38**, **40**, **44**, **45**, **48**, **51**, **53**, **55**, **59**, **60**, **63**, **64**, **66**–**68**, **71**, **72**, **75**, **77**, **78**, **82**, **85**, **88**, **89**, **92**, **123**, **124**

#	ESI [M−H$^+$] *m/z*		HPLC purity (%)	#	ESI [M−H$^+$] *m/z*		HPLC purity (%)
	Calcd	Found			Calcd	Found	
10	455.0164	455.0167	99.5	**60**	440.9354	440.9364	99.0
16	444.0505	444.0509	98.7	**63**	432.0141	432.0145	95.0
17	432.0141	432.0137	99.7	**64**	474.9618	474.9616	95.5
20	419.0352	419.0356	99.3	**66**	423.0290	423.0300	98.1
21	435.0057	435.0051	98.9	**67**	456.9900	456.9900	95.0
30	488.9774	488.9781	99.3	**68**	450.9239	450.9244	95.0
33	417.0396	417.0389	99.8	**71**	483.0057	483.0057	99.9
34	437.0447	437.0447	99.8	**72**	533.0270	533.0271	95.8
35	463.0603	463.0602	95.4	**75**	516.9667	516.9671	95.0
38	493.0709	493.0711	98.9	**77**	517.0320	517.0317	99.1
40	599.0351	599.0350	97.1	**78**	509.0658	509.0658	99.4
44	441.0008	441.0006	97.3	**82**	455.9963	456.9913	91.0
45	441.0008	441.0011	98.5	**85**	439.0352	439.0347	95.9
48	387.0290	387.0295	99.2	**88**	465.0508	465.0503	97.2
51	441.0008	441.0008	98.5	**89**	467.0665	467.0666	99.1
53	405.0196	405.0199	98.0	**92**	429.9807	429.9819	97.5
55	420.9900	420.9907	98.7	**123**	484.8849	484.8856	99.3
59	429.0760	429.0751	99.1	**124**	513.0163	513.0162	99.2

3.2.2. MMP-2 and -9 assay

This assay was performed as outlined in the Anaspec MMP Assay kit (Cat. No. 71151/71155). The fluorescence peptide cleavage assay was performed in a 96 well plate (50 μL/well) in which each reaction mixture contained 5-FAM/QXLTM520 (60 μL; diluted 1:100 in assay buffer) and MMP-2 or MMP-9 (10 μg/mL; pro-MMP-2 and -9 are first activated with 1 mM APMA for 20 min or 2 h, respectively) in Enzolyte™ 520 MMP-2 assay buffer and the screening compounds at 20 μM. Kinetics of the peptide cleavage was examined every 5 min for 30 min by using a fluorescent plate reader (Victor V, Perkin Elmer, Waltham, MA) at excitation and emission wavelengths of 485 and 535 nm, respectively, and % inhibition values were obtained.

3.2.3. ADME studies

In vitro ADME studies were conducted for selected compounds (Table 8) according to the protocols at Asinex Corporation (Moscow, Russia, www.asinex.com).[19]

3.2.4. Cell-based assay

Murine monocyte macrophage cells RAW 264.7 were seeded 45,000 cells per well in 96-well plates 24 h prior to treatment. Cells were seeded and maintained in Hyclone DMEM (4500 mg/L Glucose, 110 g/L Sodium Pyruvate) supplemented with 5% fetal bovine serum, 2 mM Glutamax (Invitrogen, Carlsbad, CA). Cells are replenished with fresh medium (0.1 mL/well). Cells were treated with increasing concentrations of inhibitor (from 0.3 to 30 μM) for 2 h. A hydroxamate inhibitor of the LF metalloproteinase activity, GM6001 (K_i = 5 μM) is included in the assay as a control. Anthrax protective-antigen-83 (PA83) and lethal factor (LF) are added to the final concentrations of 500 ng/mL and 35 ng/mL, respectively. After incubation for 4 h, cell viability was assessed using ATPlite Assay from Perkin Elmer (Waltham, MA). Each datum point represents triplicates of each concentration in one experiment. Viability was normalized to control cells which were treated with the vehicle, DMSO.

3.2.5. MEK1 cleavage assay

3.2.5.1. Reagents. Inhibitor 1 (GM6001, Ilomastat) purchased from United States Biological. (Swampscott, MA); Inhibitor 3 is a known LF inhibitor as described in our previously published paper.[19] Inhibitors 2 and 4 have been synthesized in our laboratories and will be described in a proceeding manuscript. The N-terminal MEK1 antibody (anti-MEK1, N-terminal) was obtained from Calbiochem (San Diego, CA) and the β-actin antibody (Monoclonal-Anti-β-actin) was purchased from Sigma. Anti-rabbit and anti-mouse-horseradish peroxidases were purchased from GE Healthcare (UK).

3.3. Cell culture/assay

HeLa (Human cervical carcinoma) cells were obtained from the American Type Culture Collection (Manassas, VA) and were cultured in Dulbecco's Modified Eagle Medium (DMEM, Cellgro) supplemented with 10% fetal bovine serum (FBS, Omega Scientific; Tarzana, CA) and 1% penicillin/streptomycin (Omega Scientific, Tarzana, CA) at 37 °C in a humidified incubator containing 5% CO_2.

Triplicate samples of approximately 2×10^4 HeLa cells were plated in individual wells of a 48-well plate and incubated for 48 h. The cells were exposed, for 90 min at 37 °C, to a pre-incubated solution of 2.5×10^{-8} M PA_{83}, 5×10^{-9} M LF and different concentrations of test compounds in serum-free DMEM medium. The analyzed inhibitors were dissolved in DMSO reaching a final DMSO concentration of 1%. Controls included untreated cells and LF-PA-only treated cells at different time points (45 and 90 min). Cells were later lysed and directly collected with reducing gel sample buffer and analyzed for MEK1 cleavage by immunoblotting as previously described.[27]

3.4. Animal procedure

Mice (eight animals per group) were infected intranasally with 4×10^5 *B. anthracis* Sterne spores. Treatment with compound **70** or **55** (25 mg/kg daily in DMSO administered via intraperitoneal injection) was started 24 h postexposure and continued for the next 8 days. Mice given DMSO alone were used as the control and all died on day 7 (blue line). The group treated with compound **70** had the best survival ($p < 0.01$ compared to control group) while the group receiving compound **55** had slight improvement in comparison to the control ($p < 0.05$).

3.5. Molecular modeling

Docking studies were performed with GOLD[28–30] and analyzed with Sybyl and Benchware 3D Explorer (Tripos, St. Louis). The X-ray coordinates of Lethal Factor PDB code 1YQY[13] and 1PWQ[10] were used to dock the compounds. For each compound, 20 solutions were generated and subsequently ranked according to Chemscore.[30] Top solutions were used to represent the docked geometry of the compounds and compared with the X-ray coordinates of compounds previously reported.

3.6. HPLC purity analysis

Reverse-phase high performance liquid chromatography (RP-HPLC) was performed on a HPLC Breeze from Waters Inc. (Milford, MA) using a Symmetry C18 (5 μM; 4.6 mm × 150 mm) reverse phase column (Waters Inc.). Mobile phase A was 0.1% trifluoroacetic acid/H_2O and mobile phase B was 0.1% trifluoroacetic acid/acetonitrile in which the flow rate was 1 mL/min for 20 min. A linear gradient program was used from 50% A and 50% B to 5% A and 95% B in 15 min followed by 5 min at 100% B. Results were analyzed using the Breeze software (Waters Inc.).

3.7. Synthetic chemistry

3.7.1. General procedure for the synthesis of monosubstituted benzothiazole sufonylamides

To a solution of the amine (0.812 mmol) in pyridine (10 mL) was added the sulfonyl chloride (1.22 mmol) dropwise and the mixture was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2 (20 mL) and extracted with 3 N HCl (3×) and the aqueous portion was extracted with CH_2Cl_2 once. The organic layer was dried with Na_2SO_4 and concentrated. Purification was done via column chromatography using ethyl acetate and hexane to give the desired compound. Note: In some cases, purification had to be done twice.

3.7.1.1. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl) benzenesulfonamide (9). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.09 (d, 1H, *J* = 8.1 Hz), 7.89 (d, 2H, *J* = 7.8 Hz), 7.57 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.45 (d, 1H, *J* = 8.4 Hz), 7.34 (dist. t, 2H, *J* = 7.8, 7.2 Hz), 6.51 (s, 1H), 2.51 (s, 3H).

3.7.1.2. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-(trifluoromethyl) benzenesulfonamide (10). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.10 (dist. t, 2H, *J* = 9.3, 2.1 Hz), 8.00 (d, 1H, *J* = 8.1 Hz), 7.89 (d, 1H, *J* = 7.8 Hz), 7.69 (d, 1H, *J* = 7.8 Hz), 7.58 (t, 1H, *J* = 8.1 Hz), 7.45 (t, 2H, *J* = 8.1 Hz), 6.58 (s, 1H), 2.51 (s, 3H).

3.7.1.3. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-2-(trifluoromethyl)benzenesulfonamide (11). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.38 (d, 1H, *J* = 9.0 Hz), 8.08 (d, 1H, *J* = 8.4 Hz), 7.89 (d, 1H, *J* = 7.8 Hz), 7.80 (d, 2H, *J* = 9.0 Hz), 7.66 (dist. t, 1H, *J* = 3.6 Hz), 7.59 (dist. t, 1H, *J* = 6.9 Hz) 7.45 (dist. t, 1H, *J* = 6.9 Hz), 6.47 (s, 1H), 2.53 (s, 3H).

3.7.1.4. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-(trifluoromethoxy)benzenesulfonamide (12). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.89 (dist. t, 3H, *J* = 9.3, 9.0 Hz), 7.58 (d, 1H, *J* = 8.1 Hz), 7.45 (t, 1H, *J* = 8.1 Hz), 7.12 (d, 2H, *J* = 8.1 Hz), 6.56 (s, 1H), 2.52 (s, 3H).

3.7.1.5. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-methoxybenzenesulfonamide (13). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 7.8 Hz), 7.90 (d, 3H, *J* = 7.8 Hz), 7.81 (d, 1H, *J* = 9.0 Hz), 7.57 (dist. t, 1H, *J* = 7.2 Hz), 6.99 (dist. t, 2H, *J* = 7.2 Hz), 6.79 (d, 1H, *J* = 9.0 Hz), 6.52 (s, 1H), 3.77 (s, 3H), 2.52 (s, 3H).

3.7.1.6. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-methylbenzenesulfonamide (14). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.09 (d, 1H, *J* = 8.1 Hz), 7.90 (d, 1H, *J* = 8.1 Hz), 7.75 (d, 2H, *J* = 8.1 Hz), 7.57 (t, 1H, *J* = 7.2 Hz), 7.43 (dist. t, 1H, *J* = 7.2 Hz), 7.13 (d,1H, *J* = 7.8 Hz), 6.50 (s, 1H), 2.51 (s, 3H), 2.32 (s, 3H).

3.7.1.7. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-fluorobenzenesulfonamide (15). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.4 Hz), 7.89 (m, 3H), 7.58 (t, 1H, *J* = 8.1 Hz), 7.47 (t, 1H, *J* = 8.1 Hz), 6.99 (t, 2H, *J* = 8.4 Hz), 6.54 (s, 1H), 2.52 (s, 3H).

3.7.1.8. *N*-(4-(*N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)sulfamoyl)phenyl)acetamide (16). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.06 (d, 1H, *J* = 8.1 Hz), 7.89 (d, 1H, *J* = 7.8 Hz), 7.80 (d, 2H, *J* = 8.7 Hz), 7.59–7.40 (m, 4H), 6.49 (s, 1H), 2.50 (s, 3H), 2.16 (s, 3H).

3.7.1.9. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-nitrobenzenesulfonamide (17). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.13 (q, 4H, *J* = 9.0 Hz), 8.07 (d, 1H, *J* = 8.1 Hz), 7.91 (d, 1H, *J* = 8.1 Hz), 7.61 (dist. t, 1H, *J* = 8.1 Hz), 7.49 (dist. t, 1H, *J* = 8.1 Hz), 6.59 (s, 1H), 2.52 (s, 3H); HRMS (ESI), *m*/*z* Calcd for $C_{18}H_{13}N_3O_4S_3$ $[M-H]^+$ 432.0141. Found 432.0137.

3.7.1.10. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-chloro-2-fluorobenzenesulfonamide (18). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.11 (d, 1H, *J* = 8.4 Hz), 7.92 (d, 1H, *J* = 7.8 Hz), 7.89 (s, 1H), 7.59 (t, 1H, *J* = 8.4 Hz), 7.46 (dist. t, 1H, *J* = 8.4 Hz), 7.20 (d, 1H, *J* = 9.6 Hz), 7.03 (d, 1H, *J* = 9.6 Hz), 6.49 (s, 1H), 2.53 (s, 3H).

3.7.1.11. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-fluoro-4-methylbenzenesulfonamide (19). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.91 (d, 1H, *J* = 7.8 Hz), 7.60–7.50 (m, 3H), 7.44 (dist. t, 1H, *J* = 7.2 Hz), 7.15 (dist. t, 1H, *J* = 7.2 Hz), 6.53 (s, 1H), 2.52 (s, 3H), 2.23 (s, 3H).

3.7.1.12. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-fluoro-3-methylbenzenesulfonamide (20). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.91 (d, 1H, *J* = 8.1 Hz), 7.69–7.62 (m, 2H), 7.60 (dist. t, 1H, *J* = 7.2 Hz), 7.44 (dist. t, 2H, *J* = 7.2 Hz) 6.92 (t, 1H, *J* = 9.0 Hz), 6.55 (s, 1H), 2.52 (s, 3H), 2.08 (s, 3H); HRMS (ESI), *m*/*z* Calcd for $C_{19}H_{15}FN_2O_2S_3$ $[M-H]^+$ 419.0352. Found 419.0356.

3.7.1.13. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-4-methylbenzenesulfonamide (21). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.4 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.83 (s, 1H), 7.63–7.54 (m, 2H), 7.43 (dist. t, 1H, *J* = 8.4 Hz), 7.16 (d, 1H, *J* = 7.8 Hz), 6.54 (s, 1H), 2.50 (s, 3H), 2.31 (s, 3H).

3.7.1.14. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-2,4,5-trichlorobenzenesulfonamide (22). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.32 (s, 1H), 8.05 (d, 1H, *J* = 8.1 Hz), 7.93 (d, 1H,

J = 8.1 Hz), 7.57 (dist. t, 1H, *J* = 7.5 Hz), 7.48 (s, 1H), 7.31 (dist. t, 1H, *J* = 6.99), 6.49 (s, 1H), 2.55 (s, 3H).

3.7.1.15. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-4-fluorobenzenesulfonamide (23). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.09 (d, 1H, *J* = 8.4 Hz), 7.93 (d, 2H, *J* = 8.1 Hz), 7.76–7.71 (m, 1H), 7.60 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.46 (dist. t, 1H, *J* = 7.8, 7.5 Hz), 7.07 (t, 1H, *J* = 8.4 Hz), 6.59 (s, 1H), 2.53 (s, 3H).

3.7.1.16. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3,4-dimethoxybenzenesulfonamide (24). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.55 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.46–7.39 (m, 2H), 7.20 (s, 1H), 6.71 (d, 1H, *J* = 8.4 Hz), 6.54 (s, 1H), 3.82 (s, 3H), 3.57 (s, 3H), 2.50 (s, 3H).

3.7.1.17. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-tert-butylbenzenesulfonamide (25). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.09 (d, 1H, *J* = 8.1 Hz), 7.89 (d, 1H, *J* = 8.1 Hz), 7.74 (d, 2H, *J* = 8.1 Hz), 7.58 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.44 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.32 (s, 2H), 6.55 (s, 1H), 2.52 (s, 3H), 1.24 (s, 9H).

3.7.1.18. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3,4-dichlorobenzenesulfonamide (26). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.94–7.91 (m, 2H), 7.63 (dist. t, 2H, *J* = 9.0, 8.1 Hz), 7.46 (dist. t, 1H, *J* = 7.8, 7.2 Hz), 7.37 (d, 1H, *J* = 9.0 Hz), 6.59 (s, 1H), 2.53 (s, 3H).

3.7.1.19. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-2-fluorobenzenesulfonamide (27). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.14 (d, 1H, *J* = 8.1 Hz), 8.00–7.11 (m, 2H), 7.62–7.52 (m, 2H), 7.46 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.18 (t, 1H, *J* = 8.1 Hz), 6.49 (s, 1H), 2.54 (s, 3H).

3.7.1.20. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-2-methylbenzenesulfonamide (28). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.06 (t, 2H, *J* = 8.7 Hz), 7.94 (d, 1H, *J* = 8.1 Hz), 7.59 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.54 (d, 1H, *J* = 8.1 Hz), 7.46 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.23 (t, 1H, *J* = 8.1 Hz), 6.44 (s, 1H), 2.76 (s, 3H), 2.54 (s, 3H).

3.7.1.21. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-nitrobenzenesulfonamide (29). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.43 (s, 1H), 8.09 (d, 1H, *J* = 8.4 Hz), 7.94–7.89 (m, 2H), 7.59 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.46 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.30 (d, 1H, *J* = 8.1 Hz), 6.60 (s, 1H), 2.56 (s, 3H), 2.52 (s, 3H).

3.7.1.22. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-chloro-3-(trifluoromethyl)benzenesulfonamide (30). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.12 (s, 1H), 8.08 (d, 1H, *J* = 7.8 Hz), 7.91 (dist. t, 1H, *J* = 7.8, 6.9 Hz), 7.60 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.48 (d, 1H, *J* = 7.5 Hz), 7.43 (d, 1H, J = 8.1 Hz), 6.61 (s, 1H), 2.53 (s, 3H).

3.7.1.23. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chlorobenzenesulfonamide (31). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.4 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.84 (s, 1H), 7.72 (d, 2H, *J* = 7.8 Hz), 7.58 (dist. t, 1H, *J* = 8.4, 8.1 Hz), 7.44 (t, 1H, *J* = 8.1 Hz), 7.42 (d, 1H, *J* = 7.8 Hz), 7.28 (d, 1H, *J* = 8.4), 6.55 (s, 1H), 2.51 (s, 3H).

3.7.1.24. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-(trifluoromethoxy)benzenesulfonamide (32). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 8.4 Hz), 7.89 (d, 1H, *J* = 8.1 Hz), 7.77 (d, 1H, *J* = 7.8 Hz), 7.71 (s, 1H), 7.58 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.44 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.36–7.22 (m, 1H), 6.57 (s, 1H), 2.51 (s, 3H).

3.7.1.25. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-methoxybenzenesulfonamide (33). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 8.1 Hz), 7.89 (d, 1H, *J* = 8.1 Hz), 7.55 (t, 1H, *J* = 8.1 Hz), 7.43 (d, 1H, *J* = 7.8 Hz), 7.34 (s, 1H), 7.22 (t, 1H, *J* = 8.1 Hz), 6.95 (d, 1H, *J* = 8.1 Hz), 6.51 (s, 1H), 3.60 (s, 3H), 2.48 (s, 3H).

3.7.1.26. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-naphthalene-2-sulfonamide (34). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.46 (s, 1H), 8.13 (d, 1H, *J* = 8.1 Hz), 7.86–7.72 (m, 5H), 7.61–7.50 (m, 3H), 7.43 (dist. t, 1H, *J* = 7.8 Hz), 7.16 (d, 1H, *J* = 7.8 Hz), 6.49 (s, 1H), 2.44 (s, 3H).

3.7.1.27. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-biphenyl-4-sulfonamide (35). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.11 (d, 1H, *J* = 8.1 Hz), 7.91–7.87 (m, 3H), 7.59 (dist. t, 1H, *J* = 7.2 Hz), 7.59 (m, 8H, *J* = 6.9 Hz), 6.55 (s, 1H), 2.51 (s, 3H).

3.7.1.28. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4′-methoxybiphenyl-4-sulfonamide (36). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.11 (d, 1H, *J* = 8.1 Hz), 7.90–7.85 (m, 3H), 7.58 (dist. t, 2H, *J* = 7.2, 6.9 Hz), 7.49–7.41 (m, 5H), 7.96 (d, 2H, *J* = 9.0 Hz), 6.54 (s, 1H), 3.86 (s, 3H).

3.7.1.29. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4′-chlorobiphenyl-4-sulfonamide (37). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.11 (d, 1H, *J* = 7.5 Hz), 7.92–7.86 (m, 3H), 7.57 (d, 1H, *J* = 8.7 Hz), 7.49–7.40 (m, 8H), 6.55 (s, 1H), 2.51 (s, 3H).

3.7.1.30. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiopen-2-yl)-4′-methoxybiphenyl-3-sulfonamide (38). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.10 (d, 1H, *J* = 7.8 Hz), 7.98 (s, 1H), 7.87 (d, 1H, *J* = 7.2 Hz), 7.76 (d, 1H, *J* = 7.8 Hz), 7.59 (d, 1H, *J* = 7.2 Hz), 7.55 (d, 1H, *J* = 7.2 Hz), 7.42 (t, 1H, *J* = 8.1 Hz), 7.35 (t, 1H, *J* = 7.8 Hz), 7.22 (d, 2H, *J* = 8.7 Hz), 6.82 (d, 1H, *J* = 8.7 Hz), 6.53 (s, 1H), 3.84 (s, 3H), 2.48 (s, 3H).

3.7.1.31. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiopen-2-yl)-4′-chlorobiphenyl-3-sulfonamide (39). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.10 (d, 1H, *J* = 8.1 Hz), 7.95 (s, 1H), 7.88 (d, 1H, *J* = 8.1 Hz), 7.80 (d, 1H, *J* = 7.8 Hz), 7.60 (d, 1H, *J* = 7.0 Hz), 7.56 (d, 1H, *J* = 7.0 Hz), 7.50–7.36 (m, 3H), 7.19 (d, 3H, *J* = 8.1 Hz), 6.57 (s, 1H), 2.50 (s, 3H).

3.7.1.32. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiopen-2-yl)-3′,5′-bis(trifluoromethyl)biphenyl-3-sulfonamide (40). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.10 (d, 1H, *J* = 8.1 Hz), 7.96–7.87 (m, 6H), 7.60 (t, 1H, *J* = 8.1 Hz), 7.52–7.42 (m, 3H), 6.59 (s, 1H), 2.51 (s, 3H).

3.7.1.33. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiopen-2-yl)-biphenyl-3-sulfonamide (41). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.10 (d, 1H, *J* = 8.1 Hz), 8.04 (s, 1H), 7.87 (d, 1H, *J* = 8.1 Hz), 7.81 (d, 1H, *J* = 7.8 Hz), 7.64 (d, 1H, *J* = 8.1 Hz), 7.55 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.45–7.35 (m, 2H), 7.30 (br s, 5H), 6.54 (s, 1H), 2.49 (s, 3H).

3.7.1.34. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)benzenesulfonamide (43). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.94 (d, 2H, *J* = 7.5 Hz), 7.85 (d, 1H, *J* = 7.8 Hz), 7.56–7.38 (m, 5H), 7.09 (d, 1H, *J* = 5.7 Hz), 6.83 (d, 2H, *J* = 5.7).

3.7.1.35. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3-(trifluoromethyl)benzenesulfonamide (44). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.18 (s, 1H), 8.06 (dist. t, 2H, *J* = 7.8 Hz), 7.87 (d, 1H, *J* = 7.8 Hz), 7.72 (d, 1H, *J* = 7.8 Hz), 7.59–7.42 (m, 4H), 7.12 (d, 1H, *J* = 5.7 Hz), 6.91 (d, 2H, *J* = 5.7 Hz).

3.7.1.36. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-2-(trifluoromethyl)benzenesulfonamide (45). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.36 (dd, 1H, *J* = 4.8, 2.7 Hz), 8.05 (d, 1H, J = 8.1 Hz), 7.85 (t, 2H, *J* = 7.5 Hz), 7.87–7.81 (m, 2H), 7.52 (t, 1H, *J* = 8.1 Hz), 7.42 (t, 2H, *J* = 8.1 Hz), 7.12 (d, 1H, *J* = 5.7 Hz), 6.81 (d, 1H, *J* = 5.7 Hz).

3.7.1.37. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-(trifluoromethoxy)benzenesulfonamide (46). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (dd, 1H, *J* = 8.7, 2.7 Hz), 7.96 (d, 2H, *J* = 8.7 Hz), 7.88 (d, 1H, *J* = 7.8 Hz), 7.57 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.42 (dist. t, 1H, *J* = 7.8, 7.5 Hz), 7.18 (d, 2H, *J* = 7.8 Hz), 7.13 (d, 1H, *J* = 5.7 Hz), 6.88 (d, 1H, *J* = 5.7 Hz).

3.7.1.38. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-methoxybenzenesulfonamide (47). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.88–7.83 (m, 3H), 7.54 (t, 1H, J = 8.1 Hz), 7.40 (t, 1H, *J* = 8.1 Hz), 7.01 (d, 1H, *J* = 5.7 Hz), 6.85–6.80 (m, 3H), 3.77 (s, 3H).

3.7.1.39. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-methylbenzenesulfonamide (48). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 7.82 (d, 2H, *J* = 8.7 Hz), 7.54 (dist. t, 1H, J = 8.7, 8.1 Hz), 7.41 (dist. t, 1H, *J* = 8.4, 7.8 Hz), 7.16 (d, 2H, *J* = 8.7 Hz), 7.09 (d, 1H, *J* = 5.7 Hz), 6.82 (d, 1H, *J* = 5.7 Hz), 2.32 (s, 3H).

3.7.1.40. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-fluorobenzenesulfonamide (49). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.96–7.91 (m, 2H), 7.88 (d, 1H, *J* = 8.1 Hz), 7.55 (t, 1H, J = 8.4 Hz), 7.42 (t, 1H, *J* = 8.4 Hz), 7.12 (d, 1H, *J* = 6.0 Hz), 7.04 (t, 2H, *J* = 8.7 Hz), 6.86 (d, 1H, *J* = 6.0 Hz).

3.7.1.41. N-(4-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)sulfamoyl)-phenyl)acetamide (50). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.99 (d, 1H, *J* = 8.1 Hz), 7.82 (d, 1H, *J* = 7.5 Hz), 7.56–7.48 (m, 3H), 7.38 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.06 (d, 1H, *J* = 5.7 Hz), 6.79 (d, 1H, *J* = 5.7 Hz), 2.13 (s, 3H).

3.7.1.42. *N*-(3-(Benzo[*d*] *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-(trifluoromethyl)benzenesulfonamide (51). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (m, 3H), 7.88 (d, 1H, *J* = 7.8 Hz), 7.64–7.53 (m, 3H), 7.43 (dist. t, 1H, *J* = 7.8, 7.2 Hz), 7.13 (d, 2H, *J* = 5.4 Hz), 6.89 (d, 1H, *J* = 5.4 Hz).

3.7.1.43. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-chloro-2-fluorobenzenesulfonamide (52). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.96–7.88 (m, 2H), 7.56 (dist. t, 1H, *J* = 8.4, 6.9 Hz), 7.44 (dist. t, 1H, *J* = 8.4, 6.9 Hz), 7.23 (d, 1H, *J* = 8.4 Hz), 7.14 (d, 1H, *J* = 5.7 Hz), 7.08 (d, 1H, *J* = 9.0 Hz), 6.83 (d, 1H, *J* = 5.7 Hz).

3.7.1.44. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-3-fluoro-4-methylbenzenesulfonamide (53). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 8.1 Hz), 7.87 (d, 1H, *J* = 8.1 Hz), 7.62–7.52 (m, 2H), 7.42 (t, 1H, *J* = 8.1 Hz), 7.20 (t, 1H, *J* = 7.5 Hz), 7.13 (d, 1H, *J* = 5.4 Hz), 6.86 (d, 1H, *J* = 5.4 Hz), 2.24 (s, 3H).

3.7.1.45. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-fluoro-3-methylbenzenesulfonamide (54). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 8.1 Hz), 7.87 (d, 1H, *J* = 8.1 Hz), 7.76 (dist. t, 1H, J = 7.8, 7.0 Hz), 7.72–7.69 (m, 1H), 7.68– 7.52 (m, 1H), 7.41 (dist. t, 1H, J = 8.1, 7.0 Hz), 7.11 (d, 1H, *J* = 5.7 Hz), 7.00–6.88 (m, 1H), 6.86 (d, 1H, *J* = 5.7 Hz), 2.13 (s, 3H).

3.7.1.46. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3-chloro-4-methylbenzenesulfonamide (55). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 8.1 Hz), 7.90 (s, 1H), 7.87 (d, 1H, *J* = 8.1 Hz), 7.67 (d, 1H, *J* = 8.1 Hz), 7.55 (t, 1H, *J* = 7.8 Hz), 7.42 (t, 1H, *J* = 7.8 Hz), 7.21 (d, 1H, *J* = 8.1 Hz), 7.13 (d, 1H, *J* = 6.0 Hz), 6.87 (d, 1H, *J* = 6.0 Hz), 2.33 (s, 3H).

3.7.1.47. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-2,5-bis(2,2,2-trifluoroethoxy)benzenesulfonamide (56). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.88 (d, 1H, *J* = 8.1 Hz), 7.66 (d, 1H, *J* = 3.0 Hz), 7.54 (dist. t, 1H, *J* = 8.4, 7.2 Hz), 7.41 (dist. t, 2H J = 8.1, 7.2 Hz), 7.15 (d, 1H, *J* = 3.0 Hz), 7.13 (d, 1H, *J* = 6.0 Hz), 6.99 (d, 1H, *J* = 8.4 Hz), 6.77 (d, 1H, *J* = 6.0 Hz), 4.46–4.33 (m, 4H).

3.7.1.48. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-3-chloro-4-fluorobenzenesulfonamide (57). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 8.7 Hz), 8.00 (d, 1H, *J* = 7.0 Hz), 7.88 (d, 1H, *J* = 8.1 Hz), 7.81–7.76 (m, 1H), 7.56 (dist. t, 1H, *J* = 8.1, 7.0 Hz), 7.42 (dist. t, 1H, *J* = 8.1, 7.0 Hz), 7.13 (d, 1H, *J* = 5.7 Hz), 7.11 (br s, 1H), 6.90 (d, 1H, *J* = 5.7 Hz).

3.7.1.49. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-3,4-dimethoxybenzenesulfonamide (58). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.03 (d, 1H, *J* = 7.8 Hz), 7.85 (d, 1H, *J* = 7.8 Hz), 7.55–7.49 (m, 2H), 7.39 (t, 1H, *J* = 8.1 Hz), 7.29 (s, 1H), 7.08 (d, 1H, *J* = 6.0 Hz), 6.85 (d, 1H, *J* = 6.0 Hz), 6.75 (d, 1H, *J* = 8.7 Hz), 3.82 (s, 3H), 3.64 (s, 3H).

3.7.1.50. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-tert-butylbenzenesulfonamide (59). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.06 (d, 1H, *J* = 8.1 Hz), 7.87 (d, 1H, *J* = 8.4 Hz), 7.82 (d, 1H, *J* = 8.4 Hz), 7.56 (dist. t, 1H, *J* = 8.4, 7.2 Hz), 7.43 (d, 1H, *J* = 7.2 Hz), 7.37 (d, 1H, *J* = 8.4 Hz), 7.13 (d, 1H, *J* = 6.0 Hz), 6.87 (d, 1H, *J* = 6.0 Hz), 1.25 (s, 9H).

3.7.1.51. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-3,4-dichlorobenzenesulfonamide (60). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.06 (d, 1H, *J* = 8.1 Hz), 8.00 (s, 1H), 7.89 (d, 1H, *J* = 8.1 Hz), 7.71 (d, 1H, *J* = 8.4 Hz), 7.57 (dist. t, 1H, *J* = 7.8, 7.5 Hz), 7.44 (dist. t, 1H, *J* = 8.1, 6.0 Hz), 7.15 (d, 1H, *J* = 5.7 Hz), 6.91 (d, 1H, *J* = 5.7 Hz).

3.7.1.52. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-3-chloro-2-fluorobenzenesulfonamide (61). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.11 (d, 1H, *J* = 8.0 Hz), 7.89 (d, 1H, *J* = 6.0 Hz), 7.57 (t, 1H, *J* = 7.0 Hz), 7.44 (t, 1H, *J* = 7.0 Hz), 7.21 (d, 1H, *J* = 8.0 Hz), 7.15 (d, 1H, *J* = 5.7 Hz), 6.84 (d, 1H, *J* = 5.7 Hz).

3.7.1.53. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-3-chloro-2-methylbenzenesulfonamide (62). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 8.1 Hz), 8.02 (d, 1H, *J* = 8.4 Hz), 7.90 (d, 1H, *J* = 8.1 Hz), 7.56 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.43 (dist. t, 1H, *J* = 7.8, 7.2 Hz), 7.24 (t, 1H, *J* = 8.1 Hz), 7.13 (d, 1H, *J* = 5.7 Hz), 6.78 (d, 1H, *J* = 5.7 Hz), 2.81 (s, 3H).

3.7.1.54. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-methyl-3-nitrobenzenesulfonamide (63). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.51 (s, 1H), 8.04 (d, 1H, *J* = 8.1 Hz), 7.97 (d, 1H, *J* = 8.1 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 7.55 (t, 1H, *J* = 7.8 Hz), 7.42 (t, 1H, *J* = 7.8 Hz), 7.33 (d, 1H, *J* = 8.1 Hz), 7.12 (d, 1H, *J* = 5.7 Hz), 6.91 (d, 1H, *J* = 5.7 Hz), 2.55 (s, 3H).

3.7.1.55. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-chloro-3-(trifluoromethyl)benzenesulfonamide (64). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.22 (s, 1H), 8.05 (d, 1H, *J* = 8.1 Hz), 7.97 (d, 1H, *J* = 8.4 Hz), 7.89 (d, 1H, *J* = 8.1 Hz), 7.58 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.51–7.42 (m, 2H), 7.15 (d, 1H, *J* = 6.0 Hz), 6.94 (d, 1H, *J* = 6.0 Hz).

3.7.1.56. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-chlorobenzenesulfonamide (65). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 7.5 Hz), 7.90–7.82 (m, 3H), 7.55 (t, 1H, *J* = 7.2 Hz), 7.44 (t, 1H

J = 7.5 Hz), 7.40–7.31 (m, 2H), 7.12 (d, 1H, *J* = 6.3 Hz), 6.86 (d, 1H, *J* = 6.3 Hz).

3.7.1.57. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-6-chloronaphthalene-2-sulfonamide (66). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.47 (s, 1H), 8.08 (d, 1H, *J* = 8.4 Hz), 7.89–7.67 (m, 5H), 7.56 (dist. t, 1H, *J* = 8.1, 7.2), 7.50–7.39 (m, 2H), 7.07 (d, 1H, *J* = 5.7 Hz), 6.82 (d, 1H, *J* = 5.7 Hz).

3.7.1.58. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-6-chloronaphthalene-2-sulfonamide (67). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.47 (s, 1H), 8.08 (d, 1H, *J* = 8.4 Hz), 7.89–7.67 (m, 5H), 7.56 (dist. t, 1H, *J* = 8.1, 7.2), 7.50–7.39 (m, 2H), 7.07 (d, 1H, *J* = 5.7 Hz), 6.82 (d, 1H, *J* = 5.7 Hz).

3.7.1.59. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-chloro-3-(trifluoromethyl)benzenesulfonamide (68). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.7 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.77 (d, 1H, *J* = 9.0 Hz), 7.56 (dist. t, 1H, *J* = 7.8, 7.5 Hz), 7.50 (d, 2H, *J* = 9.0 Hz), 7.44 (dist. t, 1H, *J* = 8.7, 8.1 Hz), 7.13 (d, 1H, *J* = 5.7 Hz), 6.88 (d, 1H, *J* = 5.7 Hz).

3.7.1.60. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4′-methylbiphenyl-4-sulfonamide (69). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 8.1 Hz), 7.95 (d, 1H, *J* = 8.1 Hz), 7.92 (d, 2H, *J* = 8.1 Hz), 7.85 (d, 2H, *J* = 8.1 Hz), 7.54 (d, 1H, *J* = 8.7 Hz), 7.50–7.20 (m, 7H), 7.10 (d, 1H, *J* = 5.7 Hz), 6.85 (d, 1H, *J* = 5.7 Hz).

3.7.1.61. *N*-(3-(Benzo[*d*]thiazol-2-yl)isoxazol-5-yl)-4′-methoxybiphenyl-4-sulfonamide (70). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.27 (s, 1H), 8.00 (d, 2H, *J* = 8.4 Hz), 7.97–7.89 (m, 2H), 7.72 (d, 2H, *J* = 7.0 Hz), 7.58 (d, 2H, *J* = 7.0 Hz), 7.49 (t, 1H, *J* = 7.0 Hz), 7.40 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 7.5 Hz), 3.90 (s, 3H).

3.7.1.62. *N*-(3-(Benzo[*d*]thiazol-2-yl)isoxazol-5-yl)-4′-chlorobiphenyl-4-sulfonamide (71). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.22 (s, 1H), 8.02 (d, 1H, *J* = 8.1 Hz), 7.94–7.86 (m, 2H), 7.71 (d, 1H, *J* = 8.4 Hz), 7.57–7.40 (m, 5H), 7.37 (d, 2H, *J* = 7.8 Hz).

3.7.1.63. *N*-(3-(Benzo[*d*]thiazol-2-yl)isoxazol-5-yl)-4′-trifluoromethoxybiphenyl-4-sulfonamide (72). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.06 (d, 2H, *J* = 7.8 Hz), 7.96 (d, 2H, *J* = 8.4 Hz), 7.85 (d, 1H, *J* = 7.8 Hz), 7.60–7.38 (m, 6H), 7.26 (d, 2H, *J* = 7.8 Hz), 7.11 (d, 1H, *J* = 6.0 Hz), 6.88 (d, 1H, *J* = 6.0 Hz).

3.7.1.64. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4′-methoxybiphenyl-3-sulfonamide (73). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (br s, 2H), 7.85 (d, 2H, *J* = 8.1 Hz), 7.80 (d, 1H, *J* = 8.1 Hz), 7.62 (d, 1H, *J* = 7.2 Hz), 7.52 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.43–7.35 (m, 3H), 7.09 (d, 1H, *J* = 6.0 Hz), 6.85 (d, 1H, *J* = 6.0 Hz), 6.80 (d, 1H, *J* = 8.7 Hz), 3.82 (s, 3H).

3.7.1.65. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4′-chlorobiphenyl-3-sulfonamide (74). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (br s, 2H), 7.84 (d, 3H, *J* = 8.1 Hz), 7.60–7.40 (m, 4H), 7.27–7.21 (m, 3H), 7.09 (d, 1H, *J* = 5.7 Hz), 6.86 (d, 1H, *J* = 5.7 Hz).

3.7.1.66. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3′,4′-dichlorobiphenyl-4-sulfonamide (75). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 7.8 Hz), 7.96 (d, 2H, *J* = 8.4 Hz), 7.86 (d, 1H, *J* = 9.0 Hz), 7.60–7.40 (m, 6H), 7.32–7.31 (m, 1H), 7.13 (d, 1H, *J* = 5.7 Hz), 6.89 (d, 1H, *J* = 5.7 Hz).

3.7.1.67. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)biphenyl-3-sulfonamide (76). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.09 (br s, 1H), 8.05 (d, 1H, *J* = 7.8 Hz), 7.84 (d, 2H, *J* = 7.8 Hz), 7.65 (d, 1H, *J* = 7.8 Hz), 7.52 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.44–7.38 (m, 3H), 7.30 (br s, 4H), 7.09 (d, 1H, *J* = 6.0 Hz), 6.85 (d, 1H, *J* = 6.0 Hz).

3.7.1.68. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4′-(trifluoromethyl)biphenyl-4-sulfonamide (77). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.98 (d, 2H, *J* = 8.1 Hz), 7.83 (d, 1H, *J* = 8.1 Hz), 7.66 (d, 2H, *J* = 8.4 Hz), 7.57–7.52 (m, 5H), 7.40 (dist. t, 3H, *J* = 7.8 Hz), 7.10 (d, 1H, *J* = 5.7 Hz), 6.86 (d, 1H, *J* = 5.7 Hz).

3.7.1.69. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3′,4′-dimethoxybiphenyl-4-sulfonamide (78). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.06 (d, 1H, *J* = 8.4 Hz), 7.94 (d, 2H, *J* = 8.4 Hz), 7.84 (d, 1H, *J* = 8.1 Hz), 7.04 (d, 1H, *J* = 8.1 Hz), 6.98 (s, 1H), 6.90 (d, 1H, *J* = 8.4 Hz), 6.84 (d, 1H, *J* = 6.0 Hz), 3.92 (s, 3H).

3.7.1.70. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-(2-chlorophenoxy)benzenesulfonamide (79). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.00 (d, 1H, *J* = 8.1 Hz), 7.88 (d, 3H, *J* = 8.7 Hz), 7.67 (d, 1H, *J* = 5.7 Hz), 7.55–7.39 (m, 2H), 7.24 (d, 2H, *J* = 7.5 Hz), 7.05–6.95 (m, 3H), 6.90 (d, 1H, *J* = 7.8 Hz), 6.79 (d, 1H, *J* = 8.7 Hz), 3.77 (s, 3H).

3.7.1.71. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-(2-methoxyphenoxy)benzenesulfonamide (80). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.00 (d, 1H, *J* = 8.1 Hz), 7.88 (d, 3H, *J* = 8.7 Hz), 7.67 (d, 1H, *J* = 5.7 Hz), 7.55–7.39 (m, 2H), 7.24 (d, 2H, *J* = 7.5 Hz), 7.05–6.95 (m, 3H), 6.90 (d, 1H, *J* = 7.8 Hz), 6.79 (d, 1H, *J* = 8.7 Hz), 3.77 (s, 3H).

3.7.1.72. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-6-phenoxypyridine-3-sulfonamide (81). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.70 (d, 1H, *J* = 2.7 Hz), 8.24 (dd, 1H, *J* = 8.7, 2.7 Hz), 7.90 (dist. t, 2H, *J* = 9.0, 8.1 Hz), 7.60–7.40 (m, 6H), 7.32–7.28 (m, 1H), 7.02 (d, 2H, *J* = 8.7 Hz), 6.83 (d, 1H, *J* = 9.0 Hz).

3.7.1.73. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4-(thiazol-5-yl)benzenesulfonamide (82). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.85 (d, 1H, *J* = 8.1 Hz), 7.65 (d, 2H, *J* = 8.4 Hz), 7.57 (dist. t, 1H, *J* = 7.2, 6.3 Hz), 7.45–7.29 (m, 2H), 7.12 (d, 1H, *J* = 6.0 Hz), 6.89 (d, 1H, *J* = 6.0 Hz), 2.64 (s, 3H).

3.7.1.74. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4-(oxazol-5-yl)benzenesulfonamide (83). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 8.1 Hz), 7.96 (d, 1H, *J* = 8.1 Hz), 7.94 (s, 1H), 7.85 (d, 1H, *J* = 8.1 Hz), 7.60 (d, 2H, *J* = 8.4 Hz), 7.56 (dist. t, 1H, *J* = 8.4, 8.1 Hz), 7.41 (t, 1H, *J* = 8.1 Hz), 7.40 (s, 1H), 7.26 (d, 1H, *J* = 2.1 Hz), 7.11 (d, 1H, *J* = 5.7 Hz), 6.86 (d, 1H, *J* = 5.7 Hz).

3.7.1.75. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide (84). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.50 (s, 1H), 8.18 (d, 1H, *J* = 8.1 Hz), 8.08 (d, 1H, J = 8.1 Hz), 8.01 (d, 1H, *J* = 7.8 Hz), 7.57–7.38 (m, 3H), 7.12 (d, 1H, *J* = 6.0 Hz), 6.90 (d, 1H, *J* = 6.0 Hz).

3.7.1.76. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4-(1H-pyrazol-1-yl)benzenesulfonamide (85). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, *J* = 8.1 Hz), 7.99 (d, 2H, *J* = 9.0 Hz), 7.89 (d, 1H, *J* = 2.0 Hz), 7.86 (d, 1H, *J* = 8.1 Hz), 7.71 (d, 3H, *J* = 9.0 Hz), 7.53 (t, 1H, *J* = 7.8 Hz), 7.41 (t, 1H, *J* = 7.8 Hz), 7.11 (d, 1H, *J* = 5.4 Hz), 6.87 (d, 1H, *J* = 5.4 Hz), 6.48 (t, 1H, *J* = 2.0 Hz).

3.7.1.77. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-5-methyl-1-phenyl-1H-pyrazole-4-sulfonamide (86). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.01 (d, 1H, *J* = 7.8 Hz), 7.92 (s, 1H), 7.90 (d, 1H, *J* = 8.1 Hz), 7.53 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.43 (d, 1H, *J* = 3.9 Hz),

7.42 (br s, 4H), 7.22 (d, 1H, *J* = 3.9 Hz), 7.19 (d, 1H, *J* = 5.7 Hz), 6.91 (d, 1H, *J* = 5.7 Hz), 2.48 (s, 3H).

3.7.1.78. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4-(morpholinosulfonyl)benzenesulfonamide (87). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 3H, *J* = 8.7 Hz), 7.89 (d, 1H, *J* = 8.4 Hz), 7.70 (d, 1H, *J* = 8.4 Hz), 7.57 (dist. t, 1H, *J* = 8.4, 7.2 Hz), 7.44 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.15 (d, 1H, *J* = 6.0 Hz), 6.94 (d, 1H, *J* = 6.0 Hz), 3.68 (t, 4H, *J* = 4.5 Hz), 2.85 (t, 4H, *J* = 4.5 Hz).

3.7.1.79. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3-(2-methylpyrimidine-4-yl)benzenesulfonamide (88). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.54 (s, 2H), 8.22 (d, 1H, *J* = 7.8 Hz), 8.07 (d, 1H, *J* = 8.1 Hz), 7.98 (d, 1H, *J* = 7.8 Hz), 7.84 (d, 1H, *J* = 8.1 Hz), 7.55–7.48 (m, 2H), 7.42 (dist. t, 1H, *J* = 7.8, 7.2 Hz), 7.25 (d, 1H, *J* = 5.4 Hz), 7.09 (d, 1H, *J* = 5.4 Hz), 6.88 (d, 1H, *J* = 5.7 Hz), 2.72 (s, 3H).

3.7.1.80. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-(3,5-dimethyl-1H-pyrazol-1-yl)benzenesulfonamide (89). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04–7.96 (m, 3H), 7.83 (d, 1H, *J* = 8.1 Hz), 7.54–7.44 (m, 3H), 7.38 (t, 1H, *J* = 8.0 Hz), 7.08 (d, 1H, *J* = 6.0 Hz), 6.82 (d, 1H, *J* = 6.0 Hz), 5.97 (s, 1H), 2.26 (s, 3H), 2.22 (s, 3H).

3.7.1.81. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3-dihydrobenzofuran-5-sulfonamide (90). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 7.8 Hz), 7.87 (d, 1H, *J* = 8.1 Hz), 7.71 (s, 1H), 7.68 (d, 1H, *J* = 8.4 Hz), 7.54 (dist. t, 1H, *J* = 8.4, 6.9 Hz), 7.41 (dist. t, 1H, *J* = 7.8, 6.9 Hz), 7.11 (d, 1H, *J* = 6.0 Hz), 6.85 (d, 1H, *J* = 6.0 Hz), 6.68 (d, 1H, *J* = 8.1 Hz).

3.7.1.82. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)benzo[c][1,2,5]-oxadiazole-5-sulfonamide (91). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.24 (d, 1H, *J* = 7.8 Hz), 8.13 (d, 1H, *J* = 7.0 Hz), 8.01 (d, 1H, *J* = 9.0 Hz), 7.83 (d, 2H, *J* = 8.1 Hz), 7.63 (dist. t, 1H, *J* = 8.1, 6.9 Hz), 7.52–7.41 (m, 3H), 7.06 (d, 1H, *J* = 5.7 Hz), 6.81 (d, 1H, *J* = 5.7 Hz).

3.7.1.83. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)benzo[d]thiazole-6-sulfonamide (92). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 9.14 (s, 1H), 8.60 (s, 1H), 8.10–8.00 (m, 3H), 7.85 (d, 1H, *J* = 8.1 Hz), 7.57 (t, 1H, *J* = 8.1 Hz), 7.42 (d, 1H, *J* = 8.1 Hz), 7.10 (d, 1H, *J* = 6.0 Hz), 6.87 (d, 1H, *J* = 6.0 Hz).

3.7.1.84. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (93). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 7.54 (t, 1H, *J* = 7.2 Hz), 7.46 (d, 1H, *J* = 2.1 Hz), 7.40 (dist. t, 1H, *J* = 8.1, 6.0 Hz), 7.11 (d, 1H, *J* = 6.0 Hz), 6.84 (d, 1H, *J* = 6.0 Hz), 6.81 (d, 1H, *J* = 8.1 Hz), 4.40–4.00 (m, 4H).

3.7.1.85. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4-methyl-3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine-7-sulfonamide (94). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.30 (d, 1H, *J* = 2.1 Hz), 8.04 (d, 1H, *J* = 8.1 Hz), 7.85 (d, 1H, *J* = 7.8 Hz), 7.53 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.39 (dist. t, 1H, *J* = 7.8, 7.2 Hz), 7.26 (d, 1H, *J* = 2.1 Hz), 7.11 (d, 1H, *J* = 6.0 Hz), 6.82 (d, 1H, *J* = 6.0 Hz), 4.11 (t, 2H, *J* = 4.5 Hz), 3.45 (t, 4H, *J* = 4.5 Hz), 3.13 (s, 3H).

3.7.1.86. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4-methyl-3,4-dihydro-2H-benzo[*b*][1,4]oxazine-7-sulfonamide (95). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.87 (d, 1H, *J* = 8.1 Hz), 7.52 (t, 1H, *J* = 8.1 Hz), 7.39 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.19 (dd, 1H, *J* = 8.4, 2.1 Hz), 7.11 (d, 1H, *J* = 6.0 Hz), 7.09 (d, 1H, *J* = 2.1 Hz), 6.84 (d, 1H, *J* = 6.0 Hz), 6.66 (d, 1H, *J* = 8.4 Hz), 4.23 (t, 2H, *J* = 4.0), 3.17 (t, 2H, *J* = 4.0).

3.7.1.87. *N*-(3-(Benzo[*d*] *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-3-methylbenzo[*b*]thiophene-2-sulfonamide (96). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.04 (d, 1H, *J* = 8.1 Hz), 7.85 (d, 1H, *J* = 8.1 Hz), 7.66–7.54 (m, 4H), 7.45–7.35 (m, 2H), 7.13 (d, 1H, *J* = 6.0 Hz), 6.89 (d, 1H, *J* = 6.0 Hz), 2.65 (s, 3H).

3.7.1.88. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-5-(isoxazol-5-yl)thiophene-2-sulfonamide (97). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.25 (s, 1H), 8.05 (d, 1H, *J* = 7.8 Hz), 7.62 (d, 1H, *J* = 3.9 Hz), 7.55 (dist. t, 1H, *J* = 7.8, 7.0 Hz), 7.41 (dist. t, 1H, *J* = 7.8, 7.0 Hz), 7.30 (d, 1H, *J* = 3.9 Hz), 7.18 (d, 1H, *J* = 5.7 Hz), 6.94 (d, 1H, *J* = 5.7 Hz), 6.40 (s, 1H).

3.7.1.89. N-((5-(*N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)sulfamoyl)thiophen-2-yl)methyl)-4-chlorobenzamide (98). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.02 (d, 1H, *J* = 7.5 Hz), 7.85 (d, 1H, *J* = 7.8 Hz), 7.78 (d, 1H, *J* = 9.0 Hz), 7.65 (d, 2H, *J* = 9.0 Hz), 7.55 (t, 2H, *J* = 8.4 Hz), 7.45–29 (m, 3H), 7.16 (d, 1H, *J* = 6.0 Hz), 6.90 (d, 1H, *J* = 6.0 Hz), 4.68 (d, 1H, *J* = 5.7 Hz).

3.7.1.90. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-5-(2-(methylthio)pyrimidin-4-yl)thiophene-2-sulfonamide (99). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.50 (d, 1H, *J* = 5.1 Hz), 8.07 (d, 1H, *J* = 7.8 Hz), 7.86 (d, 1H, *J* = 8.1 Hz), 7.64 (d, 2H, *J* = 3.9 Hz), 7.55 (t, 1H, *J* = 8.4 Hz), 7.52 (d, 1H, *J* = 3.9 Hz), 7.41 (dist. t, 1H, *J* = 7.8, 7.2 Hz), 7.18 (d, 1H, *J* = 5.7 Hz), 7.12 (d, 1H, *J* = 5.1 Hz), 6.95 (d, 1H, *J* = 5.7 Hz), 2.53 (s, 3H).

3.7.1.91. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)furan-2-sulfonamide (100). ^{1}H NMR (300 MHz, d-DMSO) δ 8.13 (br s, 1H), 7.89 (br s, 1H), 7.73 (br s, 1H), 7.30 (br s, 1H), 7.17 (d, 1H, *J* = 8.4 Hz), 7.13 (s, 1H), 6.96 (br s, 1H, *J* = 6.0 Hz), 6.76 (dist. t, 2H, *J* = 8.4, 8.1 Hz), 6.63 (br s, 1H).

3.7.1.92. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-1-(methylsulfonyl)methanesulfonamide (101). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.02 (d, 1H, *J* = 8.1 Hz), 7.84 (d, 1H, *J* = 7.8 Hz), 7.58 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.46 (dist. t, 1H, *J* = 8.1, 7.5 Hz), 7.04 (d, 1H, *J* = 6.3 Hz), 6.73 (d, 1H, *J* = 6.3 Hz), 4.63 (s, 2H), 3.41 (s, 3H).

3.7.1.93. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiopen-2-yl)-4-bromo-3-chlorobenzenesulfonamide (123). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 8.1 Hz), 7.98 (t, 1H, *J* = 1.2 Hz), 7.89 (d, 1H, *J* = 8.1 Hz), 7.60 (br s, 2H), 7.56 (dist. t, 1H, *J* = 8.7, 8.1 Hz), 7.44 (t, 1H, *J* = 8.1 Hz), 7.15 (d, 1H, *J* = 5.7 Hz), 6.10 (d, 1H, *J* = 5.7 Hz).

3.7.1.94. N-(Benzo[*d*]thiazol-2-yl)-3-chloro-4-methylbenzenesulfonamide (102). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.96 (s, 1H), 7.77 (dist. t, 2H, *J* = 7.8 Hz), 7.59 (d, 1H, *J* = 7.8 Hz), 7.44 (dist. t, 1H, *J* = 7.5 Hz), 7.35–7.30 (m, 2H), 2.43 (s, 3H).

3.7.1.95. N-(Benzo[*d*]thiazol-2-yl)-4′-methoxybiphenyl-4-sulfonamide (103). ^{1}H NMR (300 MHz, DMSO-*d*) δ 7.90 (d, 2H, *J* = 8.4 Hz), 7.83–7.79 (m, 3H), 7.67 (d, 2H, *J* = 8.7 Hz), 7.46–7.20 (m, 5H), 7.05 (d, 1H, *J* = 8.7 Hz), 3.81 (s, 3H).

3.7.1.96. *N*-(Benzo[*d*]thiazol-2-yl)-4′-chlorobiphenyl-4-sulfonamide (104). ^{1}H NMR (300 MHz, DMSO-*d*) δ 7.95–7.73 (m, 8H), 7.56 (d, 1H, *J* = 8.4 Hz), 7.40–7.30 (m, 2H), 7.23 (t, 1H, *J* = 6.6 Hz).

3.7.1.97. *N*-(3-Benzo[*d*]thiazol-2-yl)-4,5,6,7-tetrahydrobenzo[b]-thiophen-2-yl)-4′-methoxybiphenyl-4-sulfonamide (105). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.1 Hz), 7.90–7.83 (m, 3H), 7.57 (t, 2H, *J* = 8.0 Hz), 7.47–7.38 (m, 5H), 6.96 (d, 2H,

J = 6.0 Hz), 3.85 (s, 3H), 2.81 (br s, 1H), 2.72 (br s, 1H), 1.88 (br s, 4H).

3.7.1.98. *N*-(3-Benzo[*d*]thiazol-2-yl)-4,5,6,7-tetrahydrobenzo[b]-thiophen-2-yl)-4′-chlorobiphenyl-4-sulfonamide (106). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.08 (d, 1H, *J* = 8.4 Hz), 7.93–7.84 (m, 3H), 7.57 (t, 1H, *J* = 8.1 Hz), 7.50–7.44 (m, 3H), 7.40 (s, 4H), 2.83 (br s, 1H), 2.73 (br s, 1H), 1.88 (br s, 4H).

3.7.1.99. *N*-(3-(Benzo[*d*]thiazol-2-yl)isoxazol-5-yl)-4′-methoxy-biphenyl-4-sulfonamide (107). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.27 (s, 1H), 8.00 (d, 2H, *J* = 8.4 Hz), 7.97–7.89 (m, 2H), 7.72 (d, 2H, *J* = 7.0 Hz), 7.58 (d, 2H, *J* = 7.0 Hz), 7.49 (t, 1H, *J* = 7.0 Hz), 7.40 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 7.5 Hz), 3.90 (s, 3H).

3.7.1.100. *N*-(3-(Benzo[*d*]thiazol-2-yl)isoxazol-5-yl)-4′-chloro-biphenyl-4-sulfonamide (108). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.22 (s, 1H), 8.02 (d, 1H, *J* = 8.1 Hz), 7.94–7.86 (m, 2H), 7.71 (d, 1H, *J* = 8.4 Hz), 7.57–7.40 (m, 5H), 7.37 (d, 2H, *J* = 7.8 Hz).

3.7.1.101. *N*-(2-(Benzo[*d*]thiazol-2-yl)ethyll)-4′-methoxybiphenyl-4-sulfonamide (109). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.92–7.86 (m, 3H), 7.72 (d, 1H, *J* = 8.1 Hz), 7.64 (d, 2H, *J* = 8.7 Hz), 7.53 (d, 2H, *J*= 8.7 Hz), 7.50 (d, 1H, *J* = 7.8 Hz), 7.38 (t, 1H, *J* = 7.8 Hz), 7.10 (dist. t, 2H, *J* = 9.0, 7.8 Hz), 6.99 (d, 1H, *J* = 9.0 Hz), 3.86 (s, 3H), 2.79 (t, 2H, *J* = 7.0 Hz), 2.40 (t, 2H, *J* = 7.0 Hz).

3.7.1.102. *N*-(2-(Benzo[*d*]thiazol-2-yl)ethyll)-4′-chlorobiphenyl-4-sulfonamide (110). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.93 (d, 2H, *J* = 8.1 Hz), 7.87 (br s, 1H), 7.71 (d, 1H, *J* = 8.4 Hz), 7.65 (d, 2H, *J* = 8.1 Hz), 7.53–7.35 (m, 6H), 7.10 (t, 1H, *J* = 7.8 Hz), 2.82 (t, 2H, *J* = 7.0 Hz), 2.42 (t, 2H, *J* = 7.0 Hz).

3.7.1.103. *N*-(2-(Benzo[*d*]thiazol-2-yl)phenyl)-4′-methoxybiphenyl-4-sulfonamide (111). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.15 (d, 1H, *J* = 8.1 Hz), 7.86 (dist. t, 2H, J = 7.2, 6.9 Hz), 7.78 (d, 2H, *J*= 8.4 Hz), 7.72 (d, 1H, *J* = 7.8 Hz), 7.58 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.46 (dist. t, 2H, *J* = 8.4, 8.1 Hz), 7.43–7.37 (m, 4H), 7.15 (t, 1H, *J* = 7.8 Hz), 6.95 (d, 2H, J = 6.9 Hz), 3.84 (s, 3H).

3.7.1.104. *N*-(2-(Benzo[*d*]thiazol-2-yl)phenyl)-4′-chlorobiphenyl-4-sulfonamide (112). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.15 (d, 1H, *J* = 8.1 Hz), 7.87 (t, 2H, *J* = 9.0 Hz), 7.80 (d, 2H, *J*= 8.1 Hz), 7.74 (d, 1H, *J* = 8.1 Hz), 7.60 (dist. t, 1H, *J* = 7.8, 7.5 Hz), 7.47 (dist. t, 2H, *J* = 8.4, 8.1 Hz), 7.42–7.38 (m, 6H), 7.12 (dist. t, 1H, *J* = 7.8, 7.5 Hz).

3.7.1.105. 3-Chloro-4-methyl-*N*-(thiophen-2-yl)benzenesulfonamide (113). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.79 (d, 1H, J = 2.0 Hz), 7.57 (dd, 1H, *J* = 8.1, 2.0 Hz), 7.34 (d, 1H, *J* = 8.1 Hz), 7.05 (dd, 1H, *J* = 5.1, 1.2 Hz), 6.86–6.82 (m, 1H), 6.74 (dd, 1H, *J* = 5.1, 2.0 Hz), 2.45(s, 3H).

3.7.1.106. 4′-Methoxy-*N*-(thiophen-2-yl)biphenyl-4-sulfonamide (114). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.82 (d, 2H, *J* = 9.0 Hz), 7.67 (d, 2H, *J* = 8.7 Hz), 7.58 (d, 2H, *J* = 9.0 Hz), 7.04 (dist. t, 3H, *J* = 9.0, 8.7 Hz), 6.86 (dist. t, 1H, *J* = 5.7, 3.6 Hz), 6.77 (d, 1H, *J* = 3.6 Hz), 6.56 (br s, 1H), 3.89 (s, 3H).

3.7.1.107. 4′-Chloro-*N*-(thiophen-2-yl)biphenyl-4-sulfonamide (115). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.93 (d, 2H, *J* = 8.4 Hz), 7.84 (d, 2H, *J* = 8.1 Hz), 7.79 (d, 2H, *J* = 8.1 Hz), 7.74 (d, 1H, *J* = 8.7 Hz), 7.56 (d, 2H, *J* = 8.4 Hz), 7.40–7.30 (m, 2H), 7.23 (t, 1H, *J* = 6.6 Hz).

3.7.1.108. *N*-(3-Chloro-4-methylphenyl)thiophene-2-sulfonamide (116). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.61 (d, 1H, *J* = 4.2 Hz), 7.54 (d, 1H, *J* = 3.6 Hz), 7.35 (s, 1H), 7.26 (d, 2H, *J* = 3.6 Hz), 7.07 (t, 1H, *J* = 4.2 Hz), 6.91 (br s, 1H), 2.46 (s, 3H).

3.7.1.109. *N*-(4-Methyl-3-(trifluoromethyl)phenyl)thiophene-2-sulfonamide (117). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.61 (d, 1H, *J* = 4.2 Hz), 7.54 (d, 1H, *J* = 3.6 Hz), 7.35 (s, 1H), 7.26 (d, 2H, *J* = 3.6 Hz), 7.07 (t, 1H, *J* = 4.2 Hz), 6.91 (br s, 1H), 2.46 (s, 3H).

3.7.1.110. *N*-(3-Trifluoromethyl)phenyl)thiophene-2-sulfonamide (118). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.62–7.57 (m, 2H), 7.45–7.38 (m, 4H), 7.16 (br s, 1H), 7.06 (t, 1H, *J* = 3.9 Hz).

3.7.1.111. *N*-Phenylthiophene-2-sulfonamide (119). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 7.55 (t, 2H, *J* = 5.7 Hz), 7.31 (dist. t, 2H, *J* = 8.1 Hz), 7.19 (dist. t, 3H, *J* = 8.1 Hz), 7.08 (br s, 1H), 7.03 (q, 1H, *J* = 3.9, 1.2 Hz).

3.7.2. General procedure for the synthesis of disubstituted benzothiazole sufonylamides

To a solution of the amine (0.812 mmol) and the sulfonyl chloride (1.60 mmol) in dichloromethane (10 mL) was added triethylamine (29.23 mmol) dropwise and the mixture was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2 (20 mL) and extracted with 3 N HCl (3×) and the aqueous portion was extracted with CH_2Cl_2 once. The organic layer was dried with Na_2SO_4 and concentrated. Purification was done via flash column chromatography using ethyl acetate and hexane to give the desired compound.

3.7.2.1. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-3-(trifluoromethyl)-*N*-(3-(trifluoromethyl)phenylsulfonyl)benzenesulfonamide (120). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.31 (s, 2H), 8.11 (d, 2H, *J* = 7.8 Hz), 7.92 (d, 1H, *J* = 8.1 Hz), 7.75 (d, 1H, *J* = 8.1 Hz), 7.59 (d, 2H, *J* = 7.8 Hz), 7.49 (dist. t, 1H, *J* = 8.1, 7.2), 7.41 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.34 (t, 2H, *J* = 7.8 Hz), 7.25 (s, 1H), 2.46 (s, 3H).

3.7.2.2. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-(trifluoromethoxy)-*N*-(4-(trifluoromethoxy)phenylsulfonyl)benzenesulfonamide (121). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.01 (m, 5H), 7.77 (d, 1H, *J* = 7.8 Hz), 7.54 (dist. t, 1H, *J* = 8.1, 7.2 Hz), 7.42 (t, 1H, *J* = 8.1 Hz), 7.19 (s, 1H), 7.05 (d, 4H, *J* = 8.1 Hz), 2.46 (s, 3H).

3.7.2.3. *N*-(3-(Benzo[*d*]thiazol-2-yl)-4-methylthiophen-2-yl)-4-fluoro-*N*-(4-fluorophenylsulfonyl)benzenesulfonamide (122). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.03 (d, 1H, *J* = 8.1 Hz), 7.97 (dd, 4H, *J* = 9.3, 5.1 Hz), 7.80 (d, 1H, *J* = 8.1 Hz), 7.55 (t, 1H, *J* = 8.1 Hz), 7.44 (t, 1H, *J* = 8.1 Hz), 7.18 (s, 1H), 6.91 (t, 4H, *J* = 9.3 Hz), 2.41 (s, 3H).

3.7.3. General procedure for Suzuki coupling conditions[31]

A solution of bromobenzothiazole derivative (0.206 mmol), $Pd(PPh_3)_4$ (0.0206 mmol), boric acid derivative (0.247 mmol), EtOH (0.5 mL) and Na_2CO_3 (2 M, 2 mL) in toluene (10 mL) was refluxed for 18 h. The reaction mixture was filtered and concentrated. The residue was dissolved in dichloromethane and extracted with water, brine and dried with Na_2SO_4. Purification was done via flash column chromatography using ethyl acetate and hexane (1:2) to give the desired compound.

3.7.3.1. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-4′-(trifluoromethoxy)biphenyl-4-sulfonamide (72). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.05 (d, 1H, *J* = 7.8 Hz), 7.96 (d, 2H, *J* = 8.4 Hz), 7.85 (d, 1H, *J* = 7.8 Hz), 7.60–7.38 (m, 6H), 7.40 (d, 2H, *J* = 7.8 Hz), 7.11 (d, 1H, *J* = 6.0 Hz), 6.87 (d, 1H, *J* = 6.0 Hz).

3.7.3.2. *N*-(3-(Benzo[*d*]thiazol-2-yl)thiophen-2-yl)-2-chloro-4′-methoxybiphenyl-4-sulfonamide (124). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.07 (d, 1H, J = 8.1 Hz), 8.00 (s, 1H), 7.87 (d, 2H, J = 8.1 Hz), 7.77 (d, 1H, J = 8.1 Hz), 7.55 (t, 1H, J = 8.1 Hz), 7.42 (t, 1H, J = 8.1 Hz), 7.31–7.23 (m, 3H), 7.15 (d, 1H, J = 6.0 Hz), 6.96–6.90 (m, 3H), 6.81 (s, 1H), 3.86 (s, 3H).

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