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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.

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 Zn²⁺-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 (extracellular-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

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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.

Results and Discussion

The LF inhibitors identified thus far contain key Zn^{2+} -chelating moieties⁷ such as a hydroxymate^{8–13}, polyphenols/catechol^{14,15}, penicillin based¹⁶, rhodanine^{17,18,14,6,19}, aminoglycoside^{20–22}, quinoline urea²³, 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 μ M, 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 μ M. 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) (Figure 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.

Initially, we synthesized compound **5**, tested it to validate its kinetic activity and closely examined its predicted binding in the catalytic site of LF (Figure 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 (Figure 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 (Table 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 μ L/well) as we previously reported¹⁹. For a number of compounds, including the 6 original hits, a Lineweaver-Burk analysis was also carried out to verify that the compounds are competitive against the substrate (Figure 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 ten 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 IC₅₀ values of 8.9 and 9.4 μ M, 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 (Table 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 IC₅₀ value of 39 μ M. Comparison of the IC₅₀ values of compounds **43** and **9** (Table 2) revealed that the BTT series may lead to more potent inhibitors. Analysis of the IC₅₀ values 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 *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 IC₅₀ value of 6.6 μ M. 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 biphenylsulfonyl groups that overall seem to be more potent. The most noticeable improvements were observed with compounds **70**, **71**, and **72**, giving IC₅₀ values of 3.8, 3.0 and 3.9 μ M, 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 group, led to improved activity (compounds **49** versus **57**, **48** versus **55**, **65** versus **60**, **68** versus **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 μ M, 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 μ M) 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 IC₅₀ value of 506 nM in this assay, while compounds **71**, **93**, and **55**, and gave IC₅₀ values of 251 nM, 1.6 μ M and 1 μ M, 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 critical *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 (Figure 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.

Methods and Materials

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 experimental 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** (Table 9 and 10).

Enzymatic Assays

MAPKKide[®] Assay: The fluorescence peptide cleavage assay was performed in a 96 well plates (100 μ L/well) in which each reaction mixture contained MAPKKide[®] (4 μ M) 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 μ M). Kinetics of the peptide cleavage was examined 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 IC₅₀ 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 μ M). The K_i and $K_{m(app)}$ were calculated at 5 and/or 10 μ M inhibitor concentration.

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 μ L/well) in which each reaction mixture contained 5-FAM/QXLTM520 (60 μ L; diluted 1:100 in assay buffer) and MMP-2 or MMP-9 (10 μ g/mL; pro-MMP-2 and -9 are first activated with 1mM APMA for 20 min or 2 hrs respectively) in Enzolyte[™] 520 MMP-2 assay buffer and the screening compounds at 20 μ M. Kinetics of the peptide cleavage was examined every five minutes 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.

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

Cell-Based Assay—Murine monocyte macrophage cells RAW 264.7 were seeded 45,000 cells per well in 96-well plates 24 hours 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, 2mM Glutamax (Invitrogen, Carlsbad, CA). Cells are replenished with fresh medium (0.1ml/well). Cells were treated with increasing concentrations of inhibitor (from 0.3 to 30 μ M) for 2 hours. A hydroxamate inhibitor of the LF metalloproteinase activity, GM6001 (K_i = 5 μ M) is included in the assay as a control. Anthrax protective-antigen-83 (PA83) and lethal factor (LF) are added to the final concentrations of 500ng/ml and 35ng/ml, respectively. After incubation for 4 hours, cell viability was assessed using ATPlite Assay from PerkinElmer (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.

MEK1 Cleavage Assay

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).

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% CO₂.

Triplicate samples of approximately 2×10^4 HeLa cells were plated in individual wells of a 48-well plate and incubated for 48h. The cells were exposed, for 90 minutes at 37 °C, to a pre-incubated solution of 2.5×10^{-8} M PA₈₃, 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 minutes). Cells were later lysed and directly collected with reducing gel sample buffer and analyzed for MEK1 cleavage by immunoblotting as previously described.²⁷

Animal Procedure—Mice (eight animals per group) were infected intranasally with 4×10^5 *Bacillus 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 ($p < 0.05$).

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 1YQY¹³ and 1PWQ¹⁰ 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.

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 μ M; 4.6 mm $\times$ 150 mm) reverse phase column (Waters Inc.). Mobile phase A was 0.1% trifluoroacetic acid/H₂O 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.).

Synthetic Chemistry

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 CH₂Cl₂ (20 mL) and extracted with 3N HCl (3X) and the aqueous portion was extracted with CH₂Cl₂ once. The organic layer was dried with Na₂SO₄ 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

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl) benzenesulfonamide (**9**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-(trifluoromethyl) benzenesulfonamide (**10**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-2-(trifluoromethyl)benzenesulfonamide (**11**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-(trifluoromethoxy)benzenesulfonamide (**12**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-methoxybenzenesulfonamide (**13**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-methylbenzenesulfonamide (**14**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-fluorobenzenesulfonamide (**15**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(4-(N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)sulfamoyl)phenyl)acetamide (**16**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-nitrobenzenesulfonamide (**17**).

¹H NMR (300 MHz, d-CDCl₃) 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 C₁₈H₁₃N₃O₄S₃ [M - H]⁺ 432.0141 Found 432.0137.

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-chloro-2-fluorobenzenesulfonamide (**18**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-fluoro-4-methylbenzenesulfonamide (**19**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-fluoro-3-methylbenzenesulfonamide (**20**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.08 (d, 1H, $J = 8.1\text{ Hz}$), 7.91 (d, 1H, $J = 8.1\text{ Hz}$), 7.69-7.62 (m, 2H), 7.60 (dist. t, 1H, $J = 7.2\text{ Hz}$), 7.44 (dist. t, 2H, $J = 7.2\text{ Hz}$), 6.92 (t, 1H, $J = 9.0\text{ Hz}$), 6.55 (s, 1H), 2.52 (s, 3H), 2.08 (s, 3H); HRMS (ESI), m/z Calcd. For $\text{C}_{19}\text{H}_{15}\text{FN}_2\text{O}_2\text{S}_3$ $[\text{M} - \text{H}]^+$ 419.0352 Found 419.0356.

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-4-methylbenzenesulfonamide (**21**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.08 (d, 1H, $J = 8.4\text{ Hz}$), 7.90 (d, 1H, $J = 7.8\text{ Hz}$), 7.83 (s, 1H), 7.63-7.54 (m, 2H), 7.43 (dist. t, 1H, $J = 8.4\text{ Hz}$), 7.16 (d, 1H, $J = 7.8\text{ Hz}$), 6.54 (s, 1H), 2.50 (s, 3H), 2.31 (s, 3H);

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-2,4,5-trichlorobenzenesulfonamide (**22**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.32 (s, 1H), 8.05 (d, 1H, $J = 8.1\text{ Hz}$), 7.93 (d, 1H, $J = 8.1\text{ Hz}$), 7.57 (dist. t, 1H, $J = 7.5\text{ Hz}$), 7.48 (s, 1H), 7.31 (dist. t, 1H, $J = 6.99$), 6.49 (s, 1H), 2.55 (s, 3H).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-4-fluorobenzenesulfonamide (**23**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.09 (d, 1H, $J = 8.4\text{ Hz}$), 7.93 (d, 2H, $J = 8.1\text{ Hz}$), 7.76-7.71 (m, 1H), 7.60 (dist. t, 1H, $J = 8.1, 7.5\text{ Hz}$), 7.46 (dist. t, 1H, $J = 7.8, 7.5\text{ Hz}$), 7.07 (t, 1H, $J = 8.4\text{ Hz}$), 6.59 (s, 1H), 2.53 (s, 3H).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3,4-dimethoxybenzenesulfonamide (**24**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.08 (d, 1H, $J = 8.1\text{ Hz}$), 7.90 (d, 1H, $J = 7.8\text{ Hz}$), 7.55 (dist. t, 1H, $J = 8.1, 7.2\text{ Hz}$), 7.46-7.39 (m, 2H), 7.20 (s, 1H), 6.71 (d, 1H, $J = 8.4\text{ Hz}$), 6.54 (s, 1H), 3.82 (s, 3H), 3.57 (s, 3H), 2.50 (s, 3H).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-tert-butylbenzenesulfonamide (**25**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.09 (d, 1H, $J = 8.1\text{ Hz}$), 7.89 (d, 1H, $J = 8.1\text{ Hz}$), 7.74 (d, 2H, $J = 8.1\text{ Hz}$), 7.58 (dist. t, 1H, $J = 8.1, 7.2\text{ Hz}$), 7.44 (dist. t, 1H, $J = 8.1, 7.2\text{ Hz}$), 7.32 (s, 2H), 6.55 (s, 1H), 2.52 (s, 3H), 1.24 (s, 9H).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3,4-dichlorobenzenesulfonamide (**26**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.08 (d, 1H, $J = 8.1\text{ Hz}$), 7.94-7.91 (m, 2H), 7.63 (dist. t, 2H, $J = 9.0, 8.1\text{ Hz}$), 7.46 (dist. t, 1H, $J = 7.8, 7.2\text{ Hz}$), 7.37 (d, 1H, $J = 9.0\text{ Hz}$), 6.59 (s, 1H), 2.53 (s, 3H).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-2-fluorobenzenesulfonamide (**27**).

^1H NMR (300 MHz, d-CDCl_3) δ 8.14 (d, 1H, $J = 8.1\text{ Hz}$), 8.00-7.11 (m, 2H), 7.62-7.52 (m, 2H), 7.46 (dist. t, 1H, $J = 8.1, 7.2\text{ Hz}$), 7.18 (t, 1H, $J = 8.1\text{ Hz}$), 6.49 (s, 1H), 2.54 (s, 3H).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chloro-2-methylbenzenesulfonamide (**28**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-nitrobenzenesulfonamide (**29**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-chloro-3-(trifluoromethyl)benzenesulfonamide (**30**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-chlorobenzenesulfonamide (**31**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-(trifluoromethoxy)benzenesulfonamide (**32**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-methoxybenzenesulfonamide (**33**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)naphthalene-2-sulfonamide (**34**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)biphenyl-4-sulfonamide (**35**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4'-methoxybiphenyl-4-sulfonamide (**36**).

^1H NMR (300 MHz, d-CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4'-chlorobiphenyl-4-sulfonamide (**37**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiopen-2-yl)-4'-methoxybiphenyl-3-sulfonamide (**38**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiopen-2-yl)-4'-chlorobiphenyl-3-sulfonamide (**39**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiopen-2-yl)-3',5'-bis(trifluoromethyl)biphenyl-3-sulfonamide (**40**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiopen-2-yl)biphenyl-3-sulfonamide (**41**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)benzenesulfonamide (**43**).

^1H NMR (300 MHz, d- CDCl_3) 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, $J = 5.7$).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3-(trifluoromethyl)benzenesulfonamide (**44**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-2-(trifluoromethyl)benzenesulfonamide (**45**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-(trifluoromethoxy)benzenesulfonamide (**46**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-methoxybenzenesulfonamide (**47**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-methylbenzenesulfonamide (**48**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-fluorobenzenesulfonamide (**49**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(4-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)sulfamoyl)phenyl)acetamide (**50**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-(trifluoromethyl)benzenesulfonamide (**51**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-chloro-2-fluorobenzenesulfonamide (**52**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3-fluoro-4-methylbenzenesulfonamide (**53**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-fluoro-3-methylbenzenesulfonamide (**54**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3-chloro-4-methylbenzenesulfonamide (**55**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-2,5-bis(2,2,2-trifluoroethoxy)benzenesulfonamide (**56**).

^1H NMR (300 MHz, d- CDCl_3) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3-chloro-4-fluorobenzenesulfonamide (**57**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3,4-dimethoxybenzenesulfonamide (**58**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-tert-butylbenzenesulfonamide (**59**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3,4-dichlorobenzenesulfonamide (**60**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3-chloro-2-fluorobenzenesulfonamide (**61**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-3-chloro-2-methylbenzenesulfonamide (**62**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-methyl-3-nitrobenzenesulfonamide (**63**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-chloro-3-(trifluoromethyl)benzenesulfonamide (**64**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-chlorobenzenesulfonamide (**65**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-6-chloronaphthalene-2-sulfonamide (**66**).

¹H NMR (300 MHz, d-CDCl₃) δ8.47 (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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-6-chloronaphthalene-2-sulfonamide (**67**).

¹H NMR (300 MHz, d-CDCl₃) δ8.47 (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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-chloro-3-(trifluoromethyl)benzenesulfonamide (**68**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4'-methylbiphenyl-4-sulfonamide (**69**).

¹H NMR (300 MHz, d-CDCl₃) δ8.07 (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).

N-(3-(benzo[d]thiazol-2-yl)isoxazol-5-yl)-4'-methoxybiphenyl-4-sulfonamide (**70**).

¹H NMR (300 MHz, d-CDCl₃) δ8.27 (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).

N-(3-(benzo[d]thiazol-2-yl)isoxazol-5-yl)-4'-chlorobiphenyl-4-sulfonamide (**71**).

¹H NMR (300 MHz, d-CDCl₃) δ8.22 (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).

N-(3-(benzo[d]thiazol-2-yl)isoxazol-5-yl)-4'-trifluoromethoxybiphenyl-4-sulfonamide (**72**).

¹H NMR (300 MHz, d-CDCl₃) δ8.06 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4'-methoxybiphenyl-3-sulfonamide (**73**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4'-chlorobiphenyl-3-sulfonamide (**74**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-3',4'-dichlorobiphenyl-4-sulfonamide (**75**).

¹H NMR (300 MHz, d-CDCl₃) δ8.07 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)biphenyl-3-sulfonamide (**76**).

¹H NMR (300 MHz, d-CDCl₃) δ8.09 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4'-(trifluoromethyl)biphenyl-4-sulfonamide (**77**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-3',4'-dimethoxybiphenyl-4-sulfonamide (**78**).

¹H NMR (300 MHz, d-CDCl₃) δ8.06 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(2-chlorophenoxy)benzenesulfonamide (**79**).

¹H NMR (300 MHz, d-CDCl₃) δ8.00 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(2-methoxyphenoxy)benzenesulfonamide (**80**).

¹H NMR (300 MHz, d-CDCl₃) δ8.00 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-6-phenoxy pyridine-3-sulfonamide (**81**).

¹H NMR (300 MHz, d-CDCl₃) δ8.70 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(thiazol-5-yl)benzenesulfonamide (**82**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(oxazol-5-yl)benzenesulfonamide (**83**).

¹H NMR (300 MHz, d-CDCl₃) δ8.07 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide (**84**).

¹H NMR (300 MHz, d-CDCl₃) δ8.50 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(1H-pyrazol-1-yl)benzenesulfonamide (**85**).

¹H NMR (300 MHz, d-CDCl₃) δ8.07 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-5-methyl-1-phenyl-1H-pyrazole-4-sulfonamide (**86**).

¹H NMR (300 MHz, d-CDCl₃) δ8.01 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(morpholinosulfonyl)benzenesulfonamide (**87**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-3-(2-methylpyrimidine-4-yl)benzenesulfonamide (**88**).

¹H NMR (300 MHz, d-CDCl₃) δ8.54 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-(3,5-dimethyl-1H-pyrazol-1-yl)benzenesulfonamide (**89**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 – 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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-3-dihydrobenzofuran-5-sulfonamide (**90**).

¹H NMR (300 MHz, d-CDCl₃) δ8.05 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)benzo[c][1,2,5]oxadiazole-5-sulfonamide (**91**).

¹H NMR (300 MHz, d-CDCl₃) δ8.24 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)benzo[d]thiazole-6-sulfonamide (**92**).

¹H NMR (300 MHz, d-CDCl₃) δ9.14 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**93**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

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**).

¹H NMR (300 MHz, d-CDCl₃) δ8.30 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazine-7-sulfonamide (**95**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-3-methylbenzo[b]thiophene-2-sulfonamide (**96**).

¹H NMR (300 MHz, d-CDCl₃) δ8.04 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-5-(isoxazol-5-yl)thiophene-2-sulfonamide (**97**).

¹H NMR (300 MHz, d-CDCl₃) δ8.25 (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).

N-((5-(N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)sulfamoyl)thiophen-2-yl)methyl)-4-chlorobenzamide (**98**).

¹H NMR (300 MHz, d-CDCl₃) δ8.02 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-5-(2-(methylthio)pyrimidin-4-yl)thiophene-2-sulfonamide (**99**).

¹H NMR (300 MHz, d-CDCl₃) δ8.50 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)furan-2-sulfonamide (**100**).

¹H NMR (300 MHz, d-DMSO) δ8.13 (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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-1-(methylsulfonyl)methanesulfonamide (**101**).

¹H NMR (300 MHz, d-CDCl₃) δ8.02 (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).

N-(3-(benzo[d]thiazol-2-yl)thiopen-2-yl)-4-bromo-3-chlorobenzenesulfonamide (**123**).

¹H NMR (300 MHz, d-CDCl₃) δ8.05 (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).

N-(benzo[d]thiazol-2-yl)-3-chloro-4-methylbenzenesulfonamide (**102**).

¹H NMR (300 MHz, d-CDCl₃) δ7.96 (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).

N-(benzo[d]thiazol-2-yl)-4'-methoxybiphenyl-4-sulfonamide (**103**).

¹H NMR (300 MHz, d-DMSO) δ7.90 (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).

N-(benzo[d]thiazol-2-yl)-4'-chlorobiphenyl-4-sulfonamide (**104**).

¹H NMR (300 MHz, d-DMSO) δ7.95-7.73 (m, 8H), 7.56 (d, 1H, *J* = 8.4 Hz), 7.40-7.30 (m, 2H), 7.23 (t, 1H, *J* = 6.6 Hz).

N-(3-benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-4'-methoxybiphenyl-4-sulfonamide (**105**).

¹H NMR (300 MHz, d-CDCl₃) δ8.08 (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).

N-(3-benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-4'-chlorobiphenyl-4-sulfonamide (**106**).

¹H NMR (300 MHz, d-CDCl₃) δ8.08 (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).

N-(3-(benzo[d]thiazol-2-yl)isoxazol-5-yl)-4'-methoxybiphenyl-4-sulfonamide (**107**).

¹H NMR (300 MHz, d-CDCl₃) δ8.27 (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).

N-(3-(benzo[d]thiazol-2-yl)isoxazol-5-yl)-4'-chlorobiphenyl-4-sulfonamide (**108**).

¹H NMR (300 MHz, d-CDCl₃) δ8.22 (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).

N-(2-(benzo[d]thiazol-2-yl)ethyl)-4'-methoxybiphenyl-4-sulfonamide (**109**).

¹H NMR (300 MHz, d-CDCl₃) δ7.92 - 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).

N-(2-(benzo[d]thiazol-2-yl)ethyl)-4'-chlorobiphenyl-4-sulfonamide (**110**).

¹H NMR (300 MHz, d-CDCl₃) δ7.93 (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).

N-(2-(benzo[d]thiazol-2-yl)phenyl)-4'-methoxybiphenyl-4-sulfonamide (**111**).

¹H NMR (300 MHz, d-CDCl₃) δ8.15 (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).

N-(2-(benzo[d]thiazol-2-yl)phenyl)-4'-chlorobiphenyl-4-sulfonamide (**112**).

¹H NMR (300 MHz, d-CDCl₃) δ8.15 (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-chloro-4-methyl-N-(thiophen-2-yl)benzenesulfonamide (**113**).

¹H NMR (300 MHz, d-CDCl₃) δ7.79 (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).

4'-methoxy-N-(thiophen-2-yl)biphenyl-4-sulfonamide (**114**).

¹H NMR (300 MHz, d-CDCl₃) δ7.82 (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).

4'-chloro-N-(thiophen-2-yl)biphenyl-4-sulfonamide (**115**).

¹H NMR (300 MHz, d-CDCl₃) δ7.93 (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).

N-(3-chloro-4-methylphenyl)thiophene-2-sulfonamide (**116**).

¹H NMR (300 MHz, d-CDCl₃) δ7.61 (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).

N-(4-methyl-3-(trifluoromethyl)phenyl)thiophene-2-sulfonamide (**117**).

¹H NMR (300 MHz, d-CDCl₃) δ7.61 (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).

N-(3-trifluoromethylphenyl)thiophene-2-sulfonamide (**118**).

¹H NMR (300 MHz, d-CDCl₃) δ7.62–7.57 (m, 2H), 7.45–7.38 (m, 4H), 7.16 (br s, 1H), 7.06 (t, 1H, *J* = 3.9 Hz).

N-phenylthiophene-2-sulfonamide (**119**).

¹H NMR (300 MHz, d-CDCl₃) δ7.55 (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).

General procedure for the synthesis of disubstituted benzothiazole sulfonylamides: 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 CH₂Cl₂ (20 mL) and extracted with 3N HCl (3X) and the aqueous portion was extracted with CH₂Cl₂ once. The organic layer was dried with Na₂SO₄ and concentrated. Purification was done via flash column chromatography using ethyl acetate and hexane to give the desired compound.

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-3-(trifluoromethyl)-N-(3-(trifluoromethyl)phenylsulfonyl)benzenesulfonamide (**120**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-(trifluoromethoxy)-N-(4-(trifluoromethoxy)phenylsulfonyl)benzenesulfonamide (**121**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)-4-methylthiophen-2-yl)-4-fluoro-N-(4-fluorophenylsulfonyl)benzenesulfonamide (**122**).

¹H NMR (300 MHz, d-CDCl₃) 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).

General Procedure for Suzuki Coupling Conditions³¹: A solution of bromobenzothiazole derivative (0.206 mmol), Pd(PPh₃)₄ (0.0206 mmol), boric acid derivative (0.247 mmol), EtOH (0.5 mL) and Na₂CO₃ (2 M, 2 mL) in toluene (10 mL) was refluxed for 18 hrs. The reaction mixture was filtered and concentrated. The residue was dissolved in dichloromethane and extracted with water, brine and dried with Na₂SO₄. Purification was done via flash column chromatography using ethyl acetate and hexane (1:2) to give the desired compound.

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-4'-(trifluoromethoxy)biphenyl-4-sulfonamide (**72**).

¹H NMR (300 MHz, d-CDCl₃) 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).

N-(3-(benzo[d]thiazol-2-yl)thiophen-2-yl)-2-chloro-4'-methoxybiphenyl-4-sulfonamide (**124**).

¹H NMR (300 MHz, d-CDCl₃) 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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Figure 1.

Kinetics of inhibition of lethal factor (LF) for compounds **70** and **71**. IC₅₀ 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 Zn²⁺ 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).

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** ($\text{EC}_{50} = 561 \text{ nM}$) (B) Effect of test compounds on LF cleavage of MEK1. (Left panel) HeLa cells were incubated with a mixture of LF, PA₈₃, 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 minutes, 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 minutes 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 ($p < 0.05$).

Table 1

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

#	Structure	IC ₅₀ (Ki)
1		3.4 (0.56) μM
2		4.2 (5.9) μM
3		4.8 (0.38) μM
4		12 (5.0) μM

#	Structure	IC ₅₀ (Ki)
5	<chem>Cc1cc(C=Nc2ccc(Cl)cc2)sc1-c3nc4ccccc4n3</chem>	15 (7.8) μ M
6	<chem>O=[N+]([O-])c1ccsc1/C=C/C(=O)C2=CC=CC=O2</chem>	19 (6.6) μ M

benzothiazole-4-methylthiophene (BTMT) derivatives and IC₅₀ values against lethal factor (LF).

Table 2

IC ₅₀ μM	#	R	
	>100	25
	>100	26

IC ₅₀ μM	
	R		9.4
	IC ₅₀ μM	
	R		27

IC ₅₀ μM	IC ₅₀ μM	IC ₅₀ μM	#	R
100			28

R		IC ₅₀ μM	#	IC ₅₀ μM
48	29	18
R

R		IC ₅₀ μM	#
<p>Chemical structure of a 4-chloro-2-(trifluoromethyl)phenyl group.</p>	11	46	30

	IC ₅₀ μM	>100
	R	#	31

IC ₅₀ μM	R	IC ₅₀ μM	#
11	<p>Chemical structure of a 4-(4-(trifluoromethyl)phenyl)phenyl group. It consists of a central benzene ring with a trifluoromethyl group (-CF₃) at the para position and a phenyl ring at the other para position.</p>	>100	32
	<p>Chemical structure of a 4-(4-fluorophenyl)phenyl group. It consists of a central benzene ring with a fluorine atom (-F) at the para position and a phenyl ring at the other para position.</p>

IC ₅₀ μM		R		#	
11		<p>Chemical structure of 4-(4-ethoxyphenyl)-2-methyl-2H-benzothienopyran-6-yl sulfonamide. The R group is a 4-ethoxyphenyl group.</p>		33	
14		<p>Chemical structure of 4-(4-acetamidophenyl)-2-methyl-2H-benzothienopyran-6-yl sulfonamide. The R group is a 4-acetamidophenyl group.</p>		34	
11		<p>Chemical structure of 4-(4-nitrophenyl)-2-methyl-2H-benzothienopyran-6-yl sulfonamide. The R group is a 4-nitrophenyl group.</p>		12

R		IC ₅₀ μM	#
<p>Chemical structure of R group 17: 4-(4-chlorophenyl)-4'-methylbiphenyl.</p>	<p>Chemical structure of R group 19: 4-(4-chlorophenyl)-4'-methylbiphenyl.</p>	17	35
		19	
<p>Chemical structure of R group 21: 4-(4-chlorophenyl)-4'-methylbiphenyl.</p>	<p>Chemical structure of R group 35: 4-(4-chlorophenyl)-4'-methylbiphenyl.</p>	21	36
		35

IC ₅₀ μM		12	
R		<p>Chemical structure of R group 12: 4-(4-bromophenyl)phenyl.</p>	
#		37	
IC ₅₀ μM		17	
R		<p>Chemical structure of R group 17: 4-(4-(trifluoromethyl)phenyl)phenyl.</p>

	IC ₅₀ μM	#
	R	R
	16	38

IC ₅₀ μM		22	
R		<p>Chemical structure of the R group: 4-chloro-2-methylphenyl.</p>	
#		39	
IC ₅₀ μM		15	
R		<p>Chemical structure of the R group: 1,3,5-trideuterio-4-methylphenyl.</p>

IC ₅₀ μM		17	
R		<p>Chemical structure of R group 17: 4-(4-methylphenyl)-2,6-difluorophenyl.</p>	
#		40	
IC ₅₀ μM		>100	
R		<p>Chemical structure of R group: 3-chloro-4-fluorophenyl.</p>

R		IC ₅₀ μM	#
<p>Chemical structure of a biphenyl group with a methyl substituent. The biphenyl system is shown with a methyl group attached to one of the rings.</p>		34	41
		19	

Table 3 Benzothiazole thiophene (BTT) derivatives and IC₅₀ values against lethal factor (LF).

IC ₅₀ μM		#		
		R			
	13		59	>100	
9			60	39

IC ₅₀ μM		R	
30		<p>Chemical structure of a 2-fluoro-3-chlorophenyl group. It consists of a benzene ring with a fluorine atom at the 2-position and a chlorine atom at the 3-position.</p>	
	61		
16		<p>Chemical structure of a 4-(trifluoromethyl)phenyl group. It consists of a benzene ring with a trifluoromethyl group (-CF₃) attached to its 4-position.</p>

IC ₅₀ μM	R	IC ₅₀ μM	#
>50	<p>Chemical structure of a 1,3-bis(4-chlorophenyl)propane derivative. It consists of a central propane chain with a 4-chlorophenyl group attached to each end.</p>	9.3	62
	<p>Chemical structure of a 1,3-bis(4-fluorophenyl)propane derivative. It consists of a central propane chain with a 4-fluorophenyl group attached to each end.</p>

	IC₅₀ μM	#
	R
	63
	R
	9.6
	IC₅₀ μM	10

IC ₅₀ μM	R	IC ₅₀ μM	#
10	<p>Chemical structure of a benzene ring with a trifluoromethyl group (-CF₃) and a chlorine atom (-Cl) at the para position.</p>	18	64
	<p>Chemical structure of a benzene ring with a trifluoromethyl group (-CF₃) and a chlorine atom (-Cl) at the para position.</p>

	R	IC ₅₀ μM	#		15
			13	65
	R	IC ₅₀ μM	#		8
			16	99

IC₅₀ μM	R		#	IC₅₀ μM	
	11			67	24
	8			68	11

<div>IC₅₀ μM</div>	<div>R</div>	<div>IC₅₀ μM</div>	<div>#</div>	<div>R</div>
28		18	69
3.8		11	70

R		IC ₅₀ μM	#
<p>Chemical structure of a biphenyl derivative. It consists of two benzene rings connected by a single bond. One ring has a chlorine atom (Cl) at the para position, and the other ring has a methyl group (CH₃) at the para position.</p>		12	71
R		3.0

R		IC ₅₀ μM	#
		6.6	72
		3.9	
		39	73
		13	74
		14	
		18

R		IC ₅₀ μM	#
7.2	75
19	124
3.0	

Table 4

thiophene (BTT) heterocyclic derivatives and IC₅₀ values against lethal factor (LF).

IC ₅₀ μM	11
IC ₅₀ μM	13
IC ₅₀ μM	89

R	IC ₅₀ μM	#	IC ₅₀ μM
22	90	8

IC ₅₀ μM	10
R	<chem>Cc1ccc2sc(cc12)-</chem>
IC ₅₀ μM	#
9	92
R	<chem>c1ccc(cc1)Cc2ccccc2</chem>

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IC ₅₀ μM	R	IC ₅₀ μM	#
27		>50	93

<p>IC₅₀ μM</p>	<p>27</p>
<p>IC₅₀ μM</p>	<p>43</p>
<p>R</p>	<p>#</p>	<p>94</p>
<p>R</p>	<p>IC₅₀ μM</p>	<p>27</p>

IC ₅₀ μM	23
IC ₅₀ μM	12
#	95
R

IC ₅₀ μM	R	IC ₅₀ μM	#
10		13	96

R		IC ₅₀ μM	#	IC ₅₀ μM	R	IC ₅₀ μM
25	97		19	

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R

IC₅₀
μM

14

R

IC₅₀
μM

8

#

98

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IC ₅₀ μM	R	IC ₅₀ μM	#
>50		17	100

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R		IC ₅₀ μM	#	IC ₅₀ μM
12	101
19		

Table 5
Benzothiazole derivatives and IC₅₀ values against lethal factor (LF).

#	Structure	IC ₅₀ μ M
102	<chem>CC1=CC=C(C=C1)SC2=CC=CC=C2C(=O)C(C)SC3=CC=C(C=C3)C</chem>	28

#	Structure	IC ₅₀ μ M
103	<chem>CCOC(=O)c1ccc(cc1)-c2cc(ccc2)S(=O)(=O)Nc3cc4ccccc4s3</chem>	>50

#	Structure	IC ₅₀ μ M
104	<chem>Clc1ccc(cc1)-c2ccc(cc2)C(=O)Oc3c[nH]c4ccccc34</chem>	>50

#	Structure	IC ₅₀ μ M
105		>50
106		>50

#	Structure	IC ₅₀ μM
107		>100
108		>100
109		>50
110		>50

#	Structure	IC ₅₀ μ M
111	<chem>CC1=CC=C(C=C1)-C2=CC=CC=C2S(=O)(=O)NC3=CC=C(C=C3)C4=NC5=CC=CC=C5S4</chem>	>100
112	<chem>ClC1=CC=C(C=C1)-C2=CC=CC=C2S(=O)(=O)NC3=CC=C(C=C3)C4=NC5=CC=CC=C5S4</chem>	100 μ M

Table 6Thiophene derivatives and IC₅₀ values against lethal factor (LF).

#	Structure	IC ₅₀
113	<chem>CC1=CC=C(S(=O)(=O)C2=CC=C(Cl)C=C2Cl)S1</chem>	>50 μM

#	Structure	IC ₅₀
114	<chem>CCOC(=O)c1ccc(cc1)-c2ccc(cc2)C(=O)CCc3cc(C)c(C(=O)O)c3</chem>	>50 μM

#	Structure	IC ₅₀
115	<chem>Clc1ccc(cc1)-c2ccc(cc2)S(=O)(=O)CC3=CC=CC=C3</chem>	>50 μM
116	<chem>Clc1cc(Cl)ccc1NS(=O)(=O)c2ccsc2</chem>	>100 μM

#	Structure	IC ₅₀
117	<chem>FC(F)(F)c1cc(ccc1NS(=O)(=O)c2ccccc2)C(F)(F)F</chem>	>100 μM
118	<chem>FC(F)(F)c1cc(ccc1NS(=O)(=O)c2ccccc2)C(F)(F)F</chem>	>100 μM

#	Structure	IC ₅₀
119	<chem>O=C1C(=C(C=C1)C2=CC=CC=C2N2C(=O)C=CC2=O)C3=CC=CC=C3</chem>	>100 μM

Table 7Disubstituted benzothiazole thiophene derivatives and IC₅₀ values against lethal factor (LF).

#	Structure	IC ₅₀
120		>100 μM

#	Structure	IC ₅₀
121		>100 μM
122		>100 μM

In cell data along with *in vitro* ADME experiments for selected LF inhibitors. ADME experiments conducted according to the protocols at Asinex Corporation, Moscow, Russia, <http://asinex.com>).¹⁹

<div> </div>									
#	R1	R2	IC ₅₀ (Ki) Kinetic	EC ₅₀	Chem. Stability	LogS Ug/mL	Pampa	Metabolic stability Hept.	Plasma Stability
70		H	3.8(1.0) μM	506 nM	84%	<10 [*]	Low	t _{1/2} = >120min CL _{int} = 3.6	99%
71		H	3.0(1.7) μM	251 nM	ND	<10 [*]	Low	t _{1/2} = >120min CL _{int} = –	100% ^{**}
92		H	10.0 μM	1.6 μM	99.9%	>100	High	t _{1/2} = >120min CL _{int} = 3.9	90%
55		H	6.6 μM	1.0 μM	99%	>100	High	t _{1/2} = >120min CL _{int} = –	100%
17		CH ₃	11.0 μM	ND	99%	<10 [*]	High	t _{1/2} = >120min CL _{int} = 0.6	100%

ND = Not Determined

^{*} This compound is insoluble at 500 μM

^{**} Results for 15 min.

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

#	Formula	C%		H%		N%		S%	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
70	C ₂₄ H ₁₈ N ₂ O ₃ S ₃	60.23	59.86	3.79	4.13	5.85	6.15	20.10	19.74
72	C ₂₄ H ₁₃ F ₃ N ₂ O ₃ S ₃	54.12	55.03	2.84	3.46	5.26	5.21	18.06	15.99
92	C ₁₈ H ₁₁ N ₃ O ₂ S 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**, **34**, **35**, **38**, **40**, **44**, **45**, **48**, **51**, **53**, **55**, **59**, **60**, **63**, **64**, **66**, **67**, **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
	Calc.	Found			Calc.	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 %