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Synthesis and Antimicrobial Evaluation of Nitazoxanide-Based Analogues: Identification of Selective and Broad Spectrum Activity

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A library composed of nitazoxanide-based analogues was synthesized and assayed for increased antibacterial efficacy against the pyruvate–ferredoxin oxidoreductase (PFOR) using microorganisms *Helicobacter pylori*, *Campylobacter jejuni* and *Clostridium difficile*. Derivatives were found to recapitulate and improve activity against these organisms and select analogues were tested for their ability to disrupt the PFOR enzyme directly. The library was also screened for activity against staphylococci and resulted in the identification of analogues capable of inhibiting both staphylococci and all PFOR organisms at low micromolar minimum inhibitory concentrations with low toxicity to human foreskin cells.

Introduction

The identification and optimization of drugs is exceedingly expensive and complex, and the introduction of new therapeutics to treat infectious diseases has declined significantly.[1,2] Nitazoxanide (NTZ (**1**); Figure 1) is a US Food and Drug Administration (FDA)-approved agent for treating infections caused by *Giardia lamblia* and *Cryptosporidium parvum*, but its use is limited due to poor solubility and efficacy as nearly a gram per day is required for treatment.[3–6] NTZ also exhibits broad antimicrobial action against anaerobic intestinal pathogens and also has notable activity against microbial biofilms, rotavirus, influenza, hepatitis and *Mycobacterium tuberculosis*.[7–12]

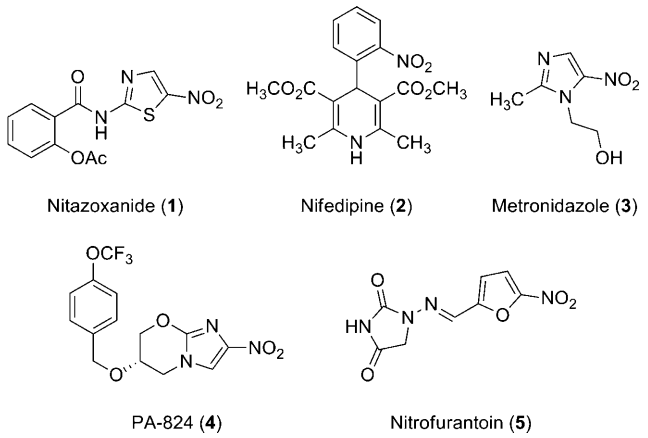

Figure 1. Representative nitro drugs.

NTZ is a unique member of the nitro-drug family (Figure 1). Unlike most nitro drugs,[3,13,14] the 5-nitro group of NTZ is metabolically stable and is not reduced as part of the mechanism of action (MoA).[15] Although nitro-group-containing drugs and analogues are seldom pursued in a drug discovery program due to mutagenic and potentially toxic side effects, the stability of NTZ and the lack of nitro reduction make it an important exception.

The broad activity of NTZ against anaerobic pathogens strongly suggests a common target. Since NTZ does not rely on metabolic reduction of the nitro group for activity, a different mechanism has been postulated to account for its efficacy. In previous mechanistic studies, we determined that the amide anion of NTZ interferes with the vitamin co-factor thiamine pyrophosphate of the essential enzyme pyruvate–ferredoxin oxidoreductase (PFOR) (Scheme 1).[15,16] PFOR is used by all strictly anaerobic bacteria, anaerobic parasites and ε-proteobacteria (Scheme 2).[15–20] Mammals and eubacteria oxidize pyruvate by the NTZ-insensitive pyruvate dehydrogenase (PDH) enzyme complex.

In these studies, NTZ was shown to completely inhibit the production of both acetyl-coenzyme A (acetyl-CoA) and CO_2 by PFOR with a K_i value of $\sim 5\times10^{-6}$ M, which is roughly two orders of magnitude lower than the K_m value for pyruvate

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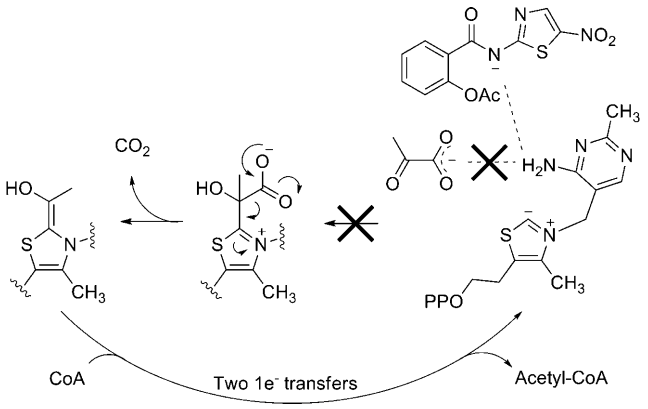
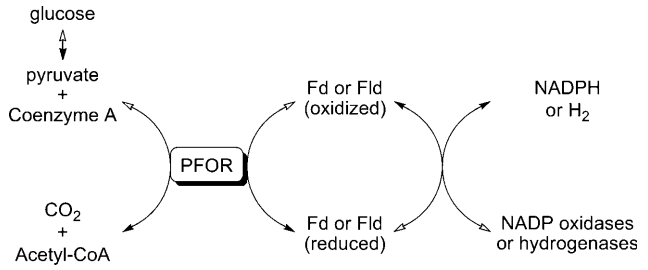


Scheme 1. Binding of pyruvate to the activated vitamin co-factor thiamine pyrophosphate (TPP). Pyruvate is then converted to acetyl-CoA. Nitazoxanide is believed to bind to and abstract a proton from the activated TPP, essentially out competing pyruvate and inhibiting the enzymatic reaction. PP: Pyrophosphate.



Scheme 2. Pyruvate:ferredoxin oxidoreductase (PFOR) enzymatic reaction. Acetyl-CoA and CO_2 are the oxidative by-products of the PFOR enzymatic reaction which requires ferredoxin (Fd) or flavodoxin (Fld) as electron acceptors. NADP oxidases or hydrogenases oxidize the reduced Fd/Fld to complete the cycle. Solid arrows indicate the forward reaction; hollow arrows indicate the reverse reaction.

($K_m = \sim 3 \times 10^{-4}$ M).[15] The anion of NTZ is the active form required for PFOR inhibition, and NTZ is not chemically modified during the enzymatic reaction. NMR analysis of the NTZ anion revealed the expected four thiazolide resonance structures involving the 5-nitro, N2′ and N3 nitrogen atoms and the amide oxygen.[15] The amide anion ($pK_a = 6.18$) or other resonance forms are postulated to interact with the N4′ of the thiamine pyrophosphate pyrimidine ring and prevent pyruvate binding (Scheme 1).

Until recently,[21] there has been little lead optimization aside from replacement of the nitro group of NTZ with halides. However, knowledge of target, as well as of the MoA, should drive lead optimization of NTZ and produce next-generation therapeutics to treat diseases that cause increased morbidity and mortality worldwide. Resistance to NTZ has not been observed clinically or induced experimentally in the laboratory, perhaps relating to drug interaction with the vitamin cofactor rather than with the enzyme directly.[22] Here, we report the use of NTZ as a tool drug for probing structure–activity relationships (SAR) to direct lead optimization against the PFOR target essential in many intestinal human pathogens.

Results and Discussion

Synthesis and biological evaluation of the 2-amino-5-nitrothiazole library against PFOR organisms

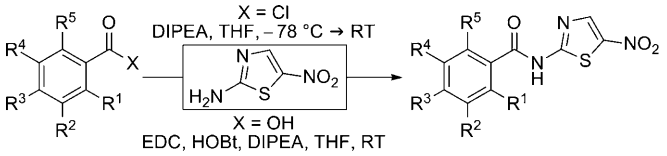
In an effort to improve the biological activity of NTZ against PFOR utilizing organisms, we envisioned the stepwise modification of benzene ring substitution patterns as well as replacement of the phenyl ring with aliphatic and heterocyclic moieties. NTZ and its derivatives have been investigated previously but tended to retain the 2-acetoxy group with only minor changes to the benzene ring.[23,24] We also sought to determine whether analogues without the 2-acetoxy group could recapitulate NTZ activity.

Helicobacter pylori and *Campylobacter jejuni* were chosen as PFOR utilizing organisms that could be quickly used to screen the NTZ library for biological activity. *H. pylori* is a microaerophilic Gram-negative bacterium that causes lifelong infections of the gastric mucosa that can lead to more severe diseases including duodenal and peptic ulcers, and gastric cancer.[25] *C. jejuni* is also a microaerophilic Gram-negative bacterium that is the most common cause of severe lower gastrointestinal infections in mammals.[26] Analogues of interest possessing increased potency against *H. pylori* and *C. jejuni* were further assessed for their activity against *Clostridium difficile*, responsible for antibiotic-associated enterocolitis in humans.[27] Secondary screens against *Escherichia coli*, *Staphylococcus aureus* (MRSA strain) and *S. epidermidis* were used to assess the activity spectrum and as a means to explore any new targets that might arise.[28] Finally, NTZ-based derivatives were tested for cytotoxic properties in a human foreskin-cell-based assay.

SAR studies began with the synthesis of benzene ring derivatives primarily bearing systematic substitutions of halides, electron-withdrawing groups and some electron-donating groups (Table 1). Analogues in this sub-library were accessed by coupling 2-amino-5-nitrothiazole (2-ANT) to the respective benzoic acid through EDC coupling or through the corresponding benzoyl chloride derivative that was either commercially available or synthesized in situ from the benzoic acid.

These analogues were then assessed for biological activity through in vitro minimum inhibitory concentration (MIC) testing against *H. pylori* and *C. jejuni*. The halide series proved useful in establishing that substitutions at any position of the ring appeared to be tolerated but yielded little effect when multiple substitutions of fluorine were investigated. Mono-substitutions of other functional groups resulted in the observation that the *para*-substituted analogues possessed more activity versus *ortho* or *meta*, with cyano analogue **21** being the exception (Table 1). Interestingly, the trifluoromethyl and methoxy analogues, which are nearly electronic opposites, possessed comparable antimicrobial potency. Similar activities for these derivatives strongly suggest that the electronic properties of the phenyl ring are not involved in the biological activity. The best library members in the series were undoubtedly the *p*-fluoro (**8**), *p*-chloro (**19**), *p*-trifluoromethyl (**24**) and *p*-methoxy (**30**) analogues, which displayed significant improvements in activity. Through the synthesis of halogen and mono-

Table 1. Synthesis and biological evaluation of halide (**6**–**19**) and mono-substituted (**20**–**31**) NTZ analogues.



NTZ halide analogue[a]	MIC [μM][b]		NTZ Mono-sub analogue[a]	MIC [μM][b]	
	H. pylori	*C. jejuni*		*H. pylori*	*C. jejuni*
Nitazoxanide (**1**)	13.0	39.1	Nitazoxanide (**1**)	13.0	39.1
R^1 = F (**6**)	0.5	5.6	R^2 = CN (**20**)	4.1	36.5
R^2 = F (**7**)	0.9	11.2	R^3 = CN (**21**)	9.1	43.8
R^3 = F (**8**)	0.9	2.8	R^1 = CF_3 (**22**)	1.6	18.9
$R^{1,3}$ = F (**9**)	0.4	7.0	R^2 = CF_3 (**23**)	3.5	4.7
$R^{2,3}$ = F (**10**)	1.8	5.3	R^3 = CF_3 (**24**)	1.6	4.7
$R^{1,5}$ = F (**11**)	0.7	14.0	R^1 = NO_2 (**25**)	1.7	27.2
$R^{1,3,5}$ = F (**12**)	4.9	9.9	R^2 = NO_2 (**26**)	1.3	27.2
R^{2-4} = F (**13**)	1.2	4.9	R^3 = NO_2 (**27**)	1.3	13.6
$R^{1,3,4}$ = F (**14**)	0.8	9.9	R^1 = OCH_3 (**28**)	1.8	17.9
R^{1-4} = F (**15**)	1.2	4.7	R^2 = OCH_3 (**29**)	1.3	7.2
R^{1-5} = F (**16**)	7.4	23.6	R^3 = OCH_3 (**30**)	1.8	4.5
R^1 = Cl (**17**)	0.3	7.8	R^2 = OCF_3 (**31**)	1.1	9.0
R^2 = Cl (**18**)	1.0	6.5	–	–	–
R^3 = Cl (**19**)	0.7	6.5	–	–	–

[a] R = H unless otherwise noted. [b] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

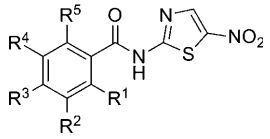
substituted derivatives, it became evident that the 2-acetoxy group was not necessary for NTZ analogue activity and that *para*-substitutions yielded improvements in activity.

Following the synthesis of the halide and mono-substituted compounds, di-substituted analogues were envisioned that would couple some of the increased activity observed in analogues reported in Table 1, as well as determine if the *ortho* oxygen could further modulate activity. Derivative **32** was accessed through methylation of *p*-trifluoromethyl salicylic acid followed by saponification of the methyl ester and EDC coupling of 2-ANT with the resulting carboxylic acid.[29] Phenol **34** was synthesized through acylation of *m*-nitro salicylic acid followed by EDC coupling of 2-ANT.[30] During the reaction, the acetyl group was cleaved and the free phenol **34** was isolated and assayed for biological activity. The remainder of the di-substituted analogues were synthesized from commercially available starting materials through coupling of the acid chloride or EDC coupling of the carboxylic acid with 2-ANT.

NTZ derivative activity did not increase with the appendage of *ortho*-oxygen atoms to the ring system in a consistent manner, and analogues **32** and **34** lost activity significantly against *C. jejuni* (Table 2). Additional di-substituted analogues bearing halogens and electron-withdrawing groups were synthesized and assayed but activity for many of these analogues also did not correlate when compared to the activity of the mono-substituted parent derivatives (Table 2). Many of the *para*-substitutions that yielded increased activity as reported in Table 1 were not further improved by additional substitutions. Activity improvements brought about by di-substitution were finally discovered when derivatives bearing both chloro and trifluoromethyl groups were appended to the phenyl ring. The general trend of the additive effects of both the chloro and trifluoromethyl groups was noteworthy for analogues **40** and **41**, and continued efforts to synthesize the fluoro derivatives may further increase biological activity.

Aliphatic analogues of NTZ were also of interest as they have been previously investigated in relation to the 2-ANT head group[31] but not in this biological context. Aliphatic derivatives were coupled to 2-ANT in a similar fashion to earlier library members via the carboxylic acid or acid chloride. In general, these derivatives possess increased activity against *H. pylori* and *C. jejuni* in comparison to NTZ (Table 3). However, most analogues lacked significant activity trends to allow any hypotheses relating to their SAR. A small

Table 2. Biological evaluation of di-substituted NTZ analogues **32**–**41**.



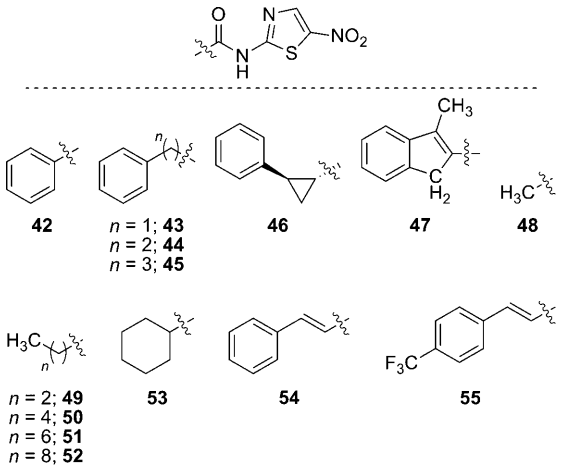
Analogue[a]	MIC [μM][b]	
	H. pylori	*C. jejuni*
Nitazoxanide (**1**)	13.0	39.1
R^1 = OCH_3; R^3 = CF_3 (**32**)	0.9	92.1
R^1 = OCH_3; R^3 = NO_2 (**33**)	0.6	9.3
R^1 = OH; R^2 = NO_2 (**34**)	19.3	103.1
R^1 = CF_3; R^3 = F (**35**)	3.4	23.9
R^1 = NO_2; R^3 = CF_3 (**36**)	8.3	88.3
R^2 = NO_2; R^3 = F (**37**)	1.6	51.2
$R^{2,4}$ = CF_3 (**38**)	5.2	41.5
R^1 = Cl; R^4 = CF_3 (**39**)	1.1	17.1
R^1 = Cl; R^2 = CF_3 (**40**)	0.9	5.7
R^2 = CF_3; R^3 = Cl (**41**)	2.8	2.8

[a] R = H unless otherwise noted. [b] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

trend is apparent correlating increased chain length to increased activity of up to six carbons in length from the amide carbonyl carbon and that further increasing length (**52** and **55**) is deleterious to activity.

With both aromatic and aliphatic NTZ derivatives synthesized and assayed for biological activity, heteroaromatic derivatives were envisioned to further explore the SAR of NTZ. Pyridine analogues were accessed through both EDC/carboxylic acid couplings and acid chloride couplings with 2-ANT. The unsubstituted pyridine derivatives (**56** and **58**) displayed low ac-

Table 3. Biological evaluation of aliphatic NTZ analogues (**42**–**55**).

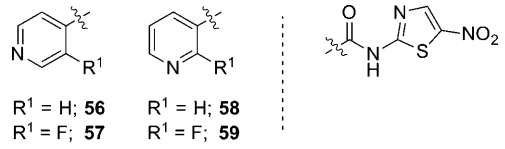


Analogue	MIC [μM][a]	
	H. pylori	*C. jejuni*
Nitazoxanide (**1**)	13.0	39.1
42	1.3	3.0
43	0.7	11.4
44	21.6	1.8
45	0.3	1.3
46	13.8	2.6
47	1.7	26.5
48	0.7	10.7
49	3.5	13.9
50	0.5	2.1
51	1.4	5.5
52	0.8	106.9
53	1.2	3.9
54	0.3	2.7
55	0.5	93.2

[a] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

tivity compared to NTZ or phenyl derivative **42** (Table 4). Two fluorine-substituted pyridine analogues were then synthesized and assayed to determine if the additive effects displayed by compounds reported in Table 1 may be applicable to heterocyclic systems. The *ortho*-fluoro-substituted pyridines (**57** and **59**) significantly improve activity compared with their unsubstituted counterparts. Sensitivity to the substitution of *ortho*-fluorine on the pyridine ring system may allow for further derivatization of pyridine-related analogues with chlorines and trifluoromethyl group substitutions (as with **40** and **41**) and is currently under investigation.

Table 4. Biological evaluation of pyridine heterocyclic NTZ analogues **56**–**59**.

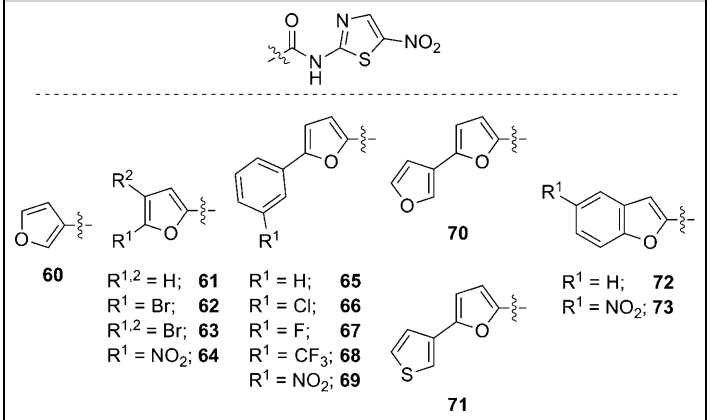


Analogue	MIC [μM][a]	
	H. pylori	*C. jejuni*
Nitazoxanide (**1**)	13.0	39.1
56	16.0	24.0
57	3.7	14.9
58	4.0	71.9
59	3.7	7.5

[a] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

Furan derivatives were investigated next and many were synthesized following the established routes. Furan compounds displayed moderate activity similar to the aromatic and substituted aromatic congeners (Table 5) as several analogues were more active than NTZ. Aryl furan 2-ANT analogues that were accessed through commercially available carboxylic acids displayed moderate to good activity against *H. pylori* and *C. jejuni* (**68** and **69**), which necessitated the synthesis of additional derivatives.

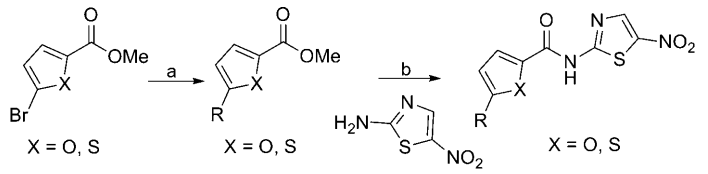
Table 5. Biological evaluation of furan and furan-related NTZ analogues **60**–**73**.



Analogue[a]	MIC [μM][b]	
	H. pylori	*C. jejuni*
Nitazoxanide (**1**)	13.0	39.1
60	0.8	12.5
61	1.0	8.4
62	1.6	12.6
63	2.8	20.2
64	21.1	7.0
65	2.4	101.5
66	1.1	22.9
67	1.5	24.0
68	1.1	8.5
69	6.9	16.7
70	1.6	13.1
71	6.2	99.6
72	0.6	110.6
73	4.5	4.5

[a] R = H unless otherwise noted. [b] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

Using the power and generality of the Suzuki–Miyaura cross coupling,[32] several *meta*-substituted furoic acid derivatives were synthesized to explore the activity around these phenyl-substituted furan analogues (Scheme 3). Analogues **65**–**67**, **70** and **71** were synthesized using the Suzuki cross coupling reaction, and the derivatives produced an SAR that displayed increased activity with the introduction of electron-withdrawing groups on the pendant phenyl ring. Analogue **68** was identi-



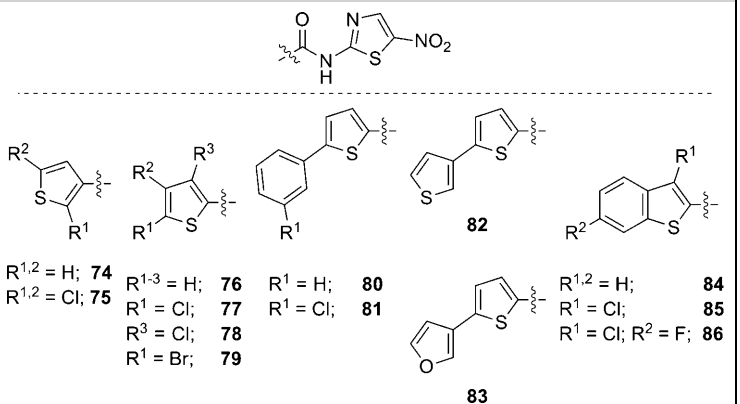
Scheme 3. Synthesis of aryl furans and aryl thiophenes. *Reagents and conditions*: a) R-$B(OH)_2$, $Pd(PPh_3)_4$, 1,4-dioxanes, Na_2CO_3, heat; b) 1. LiOH, THF/MeOH/H_2O (1:1:1); 2. EDC, HOBt, DIPEA, THF.

fied as being the best in the benzene ring series, and the differential activity of analogues **70** and **71** was noteworthy as the pendant thiophene ring (**71**) appeared to completely abolish the efficacy of the compound.

With the synthesis of a few simple pyridines and a more elaborate furan library, thiophenes were the final group to be assessed for activity. Thiophene analogues were synthesized and assayed following the established routes and several phenyl thiophene derivatives were also synthesized through Suzuki cross coupling reactions (Scheme 3).

Biological activity for the thiophene library members (Table 6) was comparable to the furan library with many of the analogues possessing potent activity against both bacteria. The Suzuki coupled analogues (**80**–**83**) display a similar trend compared to the furan derivatives and are slightly more potent in comparison (**66** versus **81**). In contrast to the furan analogues, thiophenes and benzothiophenes are more potent with increased substitution of halogens. Comparison of bi-thiophene **82** with thiophene-furan **83** is also very interesting, as the pendant furan analogue **83** is quite active compared to the former. This is in direct correlation to the furan series (**70** and **71**), with the pendant furan derivative **70** possessing the more potent activity. The activity clearly shows the pendant 3-furanyl ring (**70** and **83**) retains activity and may allow for the appendage of this heterocycle on benzene ring systems as a means to explore the SAR. Further studies including derivatization of the pendant furan ring and modifications of the attachment position are currently in progress.

Table 6. Biological evaluation of thiophene and related NTZ analogues **74**–**86**.



$R^{1,2}$ = H; **74**
$R^{1,2}$ = Cl; **75**

R^{1-3} = H; **76**
R^1 = Cl; **77**
R^3 = Cl; **78**
R^1 = Br; **79**

R^1 = H; **80**
R^1 = Cl; **81**

82

$R^{1,2}$ = H; **84**
R^1 = Cl; **85**
R^1 = Cl; R^2 = F; **86**

83

Analogue[a]	MIC [μM][b] *H. pylori*	*C. jejuni*
Nitazoxanide (**1**)	13.0	39.1
74	0.7	5.9
75	1.5	2.3
76	2.9	2.9
77	5.2	6.9
78	5.2	20.7
79	3.0	9.9
80	1.5	72.4
81	0.7	5.5
82	3.0	94.8
83	7.8	12.4
84	2.5	9.8
85	2.2	5.9
86	1.0	5.6

[a] R = H unless otherwise noted. [b] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

Evaluation of selected analogues against *C. difficile* and direct PFOR enzyme inhibition

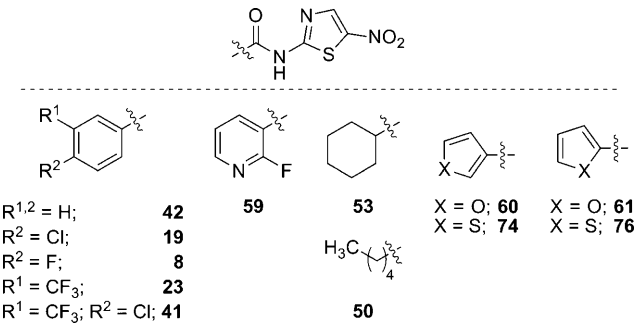
We have synthesized a library of NTZ analogues and have shown the ability of various functional groups to outperform NTZ using *H. pylori* and *C. jejuni* inhibition assays. From this library of compounds, several were chosen across the sub-libraries to be further screened for activity against the anaerobic PFOR-containing pathogen *Clostridium difficile* and in direct in vitro tests of inhibitory action against recombinant *H. pylori* PFOR purified from *E. coli* (Table 7).

C. difficile is a very common gut anaerobe that uses PFOR, however, unlike *H. pylori* and *C. jejuni*, *C. difficile* is a Gram-positive bacterium.[33,34] Many broad-spectrum antibiotics deplete natural gut floral, which enables *C. difficile* to dominate the intestinal track causing severe enterocolitis. Due to recrudescence, *C. difficile* infections are much more challenging to eradicate.[33]

Upon examining the data in Table 7 much is left to be desired for the analogues as most are less than or equal to NTZ in potency against *C. difficile*. Analogues **19** and **23** are marginally more potent than NTZ against *C. difficile* and may demonstrate the difficulties in designing new drugs to combat this bacterium. PFOR inhibition results are equally ambiguous as derivatives having efficacious effects against PFOR utilizing organisms displayed differing values in the direct enzyme inhibition assay. Activity evidenced from the PFOR enzyme assay and lack of effect in the *C. difficile* assay could be attributed to issues crossing the cell wall, slight differences in PFOR structure among these organisms or off-target effects.

Most of the library members selected and tested retained nearly equipotent inhibitory activity against the PFOR enzyme compared to NTZ but failed to significantly show increased efficacy at the enzymatic target to correlate the increase in activity against PFOR utilizing organisms. Of note are the relatively low PFOR inhibition values for analogues **23**, **41** and **61** yet the potent values for inhibition against *H. pylori*, *C. jejuni* and to some extent, *C. difficile*. PFOR

Table 7. Biological evaluation of selected analogues against *C. difficile* and direct PFOR inhibition.



$R^{1,2}$ = H; **42**
R^2 = Cl; **19**
R^2 = F; **8**
R^1 = CF_3; **23**
R^1 = CF_3; R^2 = Cl; **41**

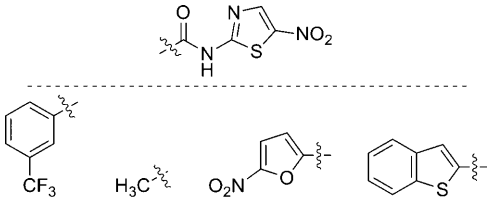
59 **53** X = O; **60** X = S; **74** X = O; **61** X = S; **76**

50

Analogue[a]	MIC [μM][b] *C. difficile*	PFOR inhibition [%][c] [Drug] = 40 μM
Nitazoxanide (**1**)	1.2	54 ± 7
42	6.0	68 ± 5[d]
19	0.8	55
8	3.3	85[d]
23	0.5	41.5 ± 5.5
41	1.4	33 ± 1
59	2.8	61 ± 13
53	5.9	54 ± 1
50	8.2	64 ± 6[d]
60	1.7	58 ± 2[d]
74	2.9	58.5 ± 3.5[d]
61	2.4	42[d]
76	1.5	56 ± 6[d]

[a] R = H unless otherwise noted. [b] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits. [c] For PFOR inhibition assays, drug concentration was fixed at 40 μM, which bench marks NTZ at ~50% inhibition. [d] Complex pattern of inhibition with two different rates.

Table 8. NTZ analogues with activity lower than 50 μM against *E. coli*.



23 **48** **64** **84**

Analogue	MIC *E. coli* [μM][a]
Nitazoxanide (**1**)	> 104.1
23	37.8
48	42.7
64	14.1
84	39.8

[a] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

results were also complicated by a complex pattern of differing rates that occurred during the PFOR assay, which indicated that some activity may be attributable to a nitroreduction mechanism. Experiments to delineate the differences in PFOR activity versus antibacterial assays and the possible role of nitro reduction in the MoA are currently being conducted.

Evaluation of library against non-PFOR utilizing organisms *E. coli, S. epidermidis* and *S. aureus*

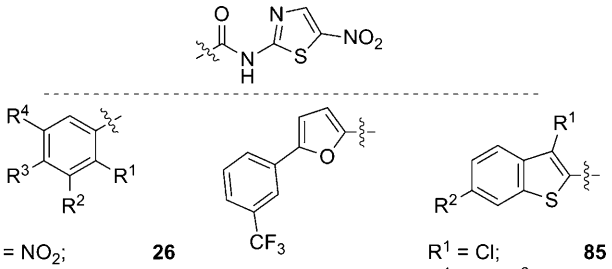
With the synthesis of an 81-membered library, we have shown that analogues based on the NTZ structure can recapitulate and improve activity against three strains of PFOR containing organisms. *E. coli* was chosen as a screening organism because NTZ has no antibacterial activity against *E. coli* (MIC > 32 μg mL^{-1}) as the putative target, PFOR, is not present. Ideally, NTZ-based analogues would also have little-to-no activity against *E. coli*. When tested, nearly the entire library had no antibacterial efficacy against *E. coli*. Only four derivatives (**23**, **48**, **64** and **84**) displayed activity lower than 50 μM indicating that the library had low off-target activity that may be related to PDH toxicity (Table 8). Low PDH toxicity was also used as a benchmark for low human toxicity as mammals also utilize PDH, and this conclusion will be further supported using human foreskin cells (see below).

Staphylococci also do not utilize PFOR for energy metabolism, and antibacterial activity would represent a new MoA and different biological target. For staphylococcal activity assessment, the entire library was screened for activity against the Gram-positive bacteria *S. aureus* methicillin-resistant strain (MRSA) and *S. epidermidis*. MRSA has become especially problematic to treat because of enhanced resistance to standard antibiotic therapies, while *S. epidermidis* has been linked to the increased rate of infection of indwelling medical devices.[35,36]

NTZ has moderate activity against MRSA and *S. epidermidis* but this action cannot be driven by the postulated PFOR MoA.[12] PDH is also not the likely target in staphylococci as the vast majority of the library was inactive at inhibiting *E. coli*. As a whole, the NTZ analogue library was relatively inactive against staphylococci with average MIC values near that of NTZ or higher (see the Supporting Information). Several derivatives displaying activity in the low micromolar range did stand out, and analogues reported in Table 9 were selected with activity ≤ 12.0 μM against *both* strains of *Staphylococcus*.

The first observation of the data strongly suggested the involvement of trifluoromethyl groups in the disruption of staphylococci versus any of the other pendant groups tested. Aromatic derivatives were also the only groups represented, with aliphatic moieties not displaying significant activity. Analogues **38** and **41** are of particular interest as they also display significant activity against PFOR utilizing organisms. Benzothiophenes **85** and **86** have moderate activity in the previous PFOR organisms and possesses potent activity against staphylococci. With the identification of several trifluoromethyl-appended NTZ analogues and benzothiophenes, in particular, derivatives capable of inhibiting bacteria across genera have been identified in this library. As staphylococci do not utilize PFOR, analogues active against both PFOR organisms and staphylococci must be acting through unique MoAs with an unknown target. SAR studies using several of the active compounds reported in Table 9 are underway to improve activity and aid in target identification.

Table 9. Biological evaluation of selected analogues against Gram-positive bacteria *S. aureus* (MRSA) and *S. epidermidis*.



$R^2 = NO_2$; **26**
$R^2 = OCF_3$; **31**
$R^{2,4} = CF_3$; **38**
$R^1 = Cl$; $R^2 = CF_3$; **40**
$R^2 = CF_3$; $R^3 = Cl$; **41**

68

$R^1 = Cl$; **85**
$R^1 = Cl$; $R^2 = F$; **86**

Analogue[a]	MIC [μM][b]	
	S. aureus (MRSA)	*S. epidermidis*
Nitazoxanide (**1**)	39.1	52.1
26	10.2	10.2
31	6.0	12.0
38	2.6	1.9
40	11.4	11.4
41	2.8	2.8
68	11.4	5.7
85	2.9	5.9
86	2.1	5.6

[a] R = H unless otherwise noted. [b] The MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits.

Evaluation of selected analogues for foreskin cell toxicity

As a final assessment of analogue activity and toxicity, a selection of the most active analogues was assayed against human foreskin cells. Table 10 summarizes the foreskin cell toxicity data and also the activity profiles of the selected NTZ derivatives. All of the NTZ analogues tested were relatively nontoxic compared to NTZ. Interestingly, all of the analogues that showed increased activity against *E. coli* were completely nontoxic to foreskin cells indicating that these analogues may, in fact, be efficacious candidates for nontoxic broad-spectrum antibiotics. Only two analogues tested, a nitro aromatic (**26**) and di-*meta* trifluoromethyl analogue (**38**), stood out as being particularly toxic. Surprisingly, the mono-trifluoromethyl analogue **23** and nitrofuran **64** were not toxic compared to compound **26** or **38.** Preliminary safety testing in mice suggests that these compounds (like NTZ) are relatively safe at 200 mg Kg^{-1} by oral administration (data not presented).[1]

Conclusions

From the synthesis and biological evaluation of NTZ-based analogues against three PFOR utilizing organisms, *E. coli* and two *Staphylococcus* strains, it is apparent that activity against these organisms can be improved beyond that of NTZ in both a broad and selective manner. Since NTZ targets PFOR, this was the logical first choice for investigation, and numerous derivatives recapitulate and outperform NTZ in terms of activity against both *H. pylori* and *C. jejuni*. We were able to determine that the 2-acetoxy group or simply an *ortho*-oxygen atom was not necessary for activity. The electronic properties of pendant benzene rings were also less important, and we postulate that steric, ionic and hydrophobic interactions play major roles in the MoA.

Recapitulating gains in activity in the halogen and mono-substituted sub-libraries with di-substituted analogues was problematic and led to nearly every derivative being less potent. Activity against PFOR organisms in the di-substituted library was discovered for trifluoromethyl/chloro combinations (**40** and **41**; Table 10), and these derivatives will be further investigated with fluorine and additional substituents. These trifluoromethyl/chloro di-substituted derivatives were also shown to be very active against both *C. difficile* and staphylococci.

Heterocyclic analogues displayed moderate-to-good activity against PFOR utilizing organisms and several had potent staphylococcal activity. Of particular note were the bi-aryl furan–furan (**70**) and thiophene–furan (**83**) analogues that displayed good activity against PFOR organisms but their pendant thiophene counterparts were completely inactive. Further studies modifying the connectivity and substitution profile of the pendant ring system of the bi-aryl groups and the substitution of furans on benzene rings are of great interest.

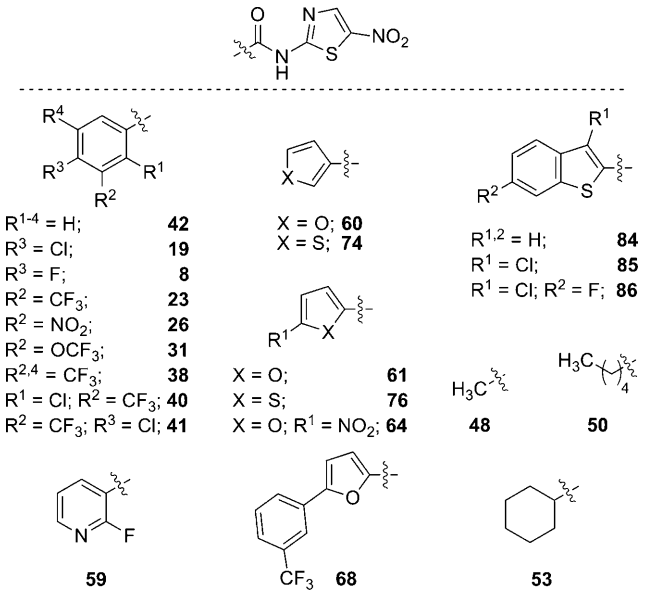
In general, *C. difficile* activity was moderate at best for the selected library members tested and may reflect the difficulty of treating this infection. As only selected analogues were tested, limited SAR can be drawn but it would be prudent to investigate halo- and trifluoromethyl-substituted furans and thiophenes for activity improvements. PFOR enzyme results for the selected derivatives did provide evidence that PFOR is being targeted and should be responsible for the activity of the NTZ-based library of analogues. However, it was clear that factors independent of PFOR inhibition may account for the activity of some analogues.

Selective activity against PFOR utilizing organisms was observed for several library members that displayed moderate-to-good activity against all PFOR organisms and in the direct enzyme assay, but were not active against staphylococci. Of particular note were the heterocyclic analogues **59**, **60**, **61**, **74** and **76** (Table 10), which represent the fluoro-substituted pyridine and unsubstituted furans and thiophenes. These analogues displayed good PFOR selectivity and moderate enzyme inhibition comparable to NTZ.

Broad-spectrum activity against PFOR, PFOR utilizing organisms and staphylococci was discovered for several trifluoromethyl-substituted benzene, aryl–furan and benzothiophene derivatives. Although compound **23** did indicate some possible activity against *E. coli*, di-substituted analogues bearing trifluoromethyl groups did not suffer from this effect (data not shown), and human foreskin toxicity data indicated that **23**, **40** and **41** are not toxic (Table 10). In particular, derivative **41** was active against all of the PFOR and staphylococci organisms yet

[1] *All experiments using animals were conducted in accordance with university and governmental guidelines on the ethical treatment of animals in scientific research.*

Table 10. Evaluation of selected analogues against human foreskin cells and a summary of biological data.



Analogue[a]	CC_{50} [μM][b] foreskin[c]	MIC [μM][b] *H. pylori*	*C. jejuni*	*C. difficile*	*S. aureus*	*S. epidermidis*
Nitazoxanide (**1**)	>52.1	13.0	39.1	1.2	39.1	52.1
42	46.3	1.3	3.0	6.0	12.0	32.1
19	56.4	0.7	6.5	0.8	2.0	26.1
8	29.9	0.9	2.8	3.3	8.4	15.0
23	>50.4	3.5	4.7	0.5	3.2	12.6
26	27.2	1.3	27.2	n.d.	10.2	10.2
31	>48.0	1.1	9.0	n.d.	6.0	12.0
38	10.4	5.2	41.5	n.d.	2.6	1.9
40	>45.5	0.9	5.7	n.d.	11.4	11.4
41	>45.5	2.8	2.8	1.4	2.8	2.8
59	>59.7	3.7	7.5	2.8	119.3	119.3
60	>66.9	0.8	12.5	1.7	50.2	33.4
74	>62.7	0.7	5.9	2.9	23.5	15.7
61	66.9	1.0	8.4	2.4	50.2	66.9
76	>62.7	2.9	2.9	1.5	15.7	31.3
64	>56.3	21.1	7.0	n.d.	112.6	28.1
68	>41.7	1.1	8.5	n.d.	11.4	5.7
84	>52.6	2.5	9.8	n.d.	6.6	13.1
85	>47.1	2.2	5.9	n.d.	2.9	5.9
86	33.5	1.0	5.6	n.d.	2.1	5.6
48	>85.5	0.7	10.7	n.d.	171.0	21.4
50	>65.8	0.5	2.1	8.2	131.5	32.9
53	>62.7	1.2	3.9	5.9	125.2	31.3

[a] R = H unless otherwise noted. [b] The CC_{50} values represent the mean of 2–3 experiments performed in triplicate, and the errors were within acceptable limits; the MIC values represent the mean of 3–6 experiments performed in triplicate, and the errors were within acceptable limits; n.d. = not determined. [c] 48 h time point.

did not display a correlating increase in the direct PFOR enzymatic inhibition assay. Amide **41**, therefore, must be acting through dual (multiple) pathways and this compound will require further investigation.

In conclusion, the exploration of NTZ-based analogues to improve activity yielded compounds capable of inhibiting across a broad spectrum and also selectively against PFOR organisms. This research has yielded much information regarding how activity can be improved but further studies and analogue derivatization are needed to continue to improve activity. Efforts are underway to access and evaluate new derivatives based on the current SAR and to begin implementation of the PFOR crystal structure in analogue evaluation and design. Coupling the results presented herein with the recent identification of head groups able to recapitulate and replace the 2-ANT[21] is expected to produce more efficacious analogues, and results are forthcoming.

Experimental Section

Biology

Determination of MIC values for H. pylori, C. jejuni *and* Staphylococci *(liquid dilution)*: *H. pylori* was grown overnight at 37 °C under microaerobic conditions in bacto brain heart infusion (BHI) medium supplemented with 4% serum. *C. jejuni* was grown in BHI medium without supplementation. Staphylococci were grown in bacto trpytic soy medium without supplementation. For the microdilution assay, bacterial cultures were diluted to a final OD_{600} value of 0.03 for *H. pylori* strain 26695, 0.01 for *C. jejuni* strain H840, and 0.01 for staphylococci. Aliquots (100 μL) were dispensed into wells of a 96-well microplate. Analogues were diluted serially starting at 32 μg mL^{-1} in DMSO, and the percent DMSO was always less than 4%. DMSO and NTZ served as controls. Plates were incubated with shaking at 37 °C in a microaerobic incubator (7% O_2 and 10% CO_2). The turbidity in the wells was read visually at 27 h or with a plate reader (Molecular Dynamics). The MIC is defined as the concentration of drug that produced no detectable bacterial growth. All experiments were performed 3–6 times in triplicate.

Determination of MIC values for C. difficile *(agar dilution)*: *C. difficile* VPI 10463 was grown anaerobically overnight in chopped meat medium (anaerobe system) from stock, and it was subcultured to a new chopped meat medium for 5 h at 37 °C. It was standardized to an optical density value of 0.1 at OD_{600}. Analogues were then diluted into the agar media at concentrations ranging from 0.125–8 μg mL^{-1}. Aliquots (10 μL) of the standardized inoculum were delivered to the surface of the agar plates. The number of viable bacteria contained in each inoculum was $\sim 7\times10^4$ and 3.5×10^4 organisms. The plates were incubated for 18 h in an anaerobic chamber and were read visually for growth or no growth. Anaerobic plates containing no compound were used as controls. All experiments were performed 3–6 times in triplicate.

Direct PFOR enzyme assay: *H. pylori* PFOR enzyme was overexpressed and purified from *E. coli* as described previously.[15] Enzymatic assays were carried out at 25 °C in 1 mL cuvettes in a modified Cary-14 spectrophotometer equipped with an OLIS data acquisition system (On Line Instrument Co.; Bogart, Georgia, USA). PFOR (EC 1.2.7.1) was assayed under anaerobic conditions with 100 mM potassium phosphate (pH 7.4), 10 mM sodium pyruvate, 5 mM benzyl viologen (BV; $\varepsilon = 9.2\ mM^{-1}cm^{-1}$ at 546 nm), 0.18 mM CoA, and 1 mM $MgCl_2$. The reaction was started by addition of enzyme, in the presence or absence of inhibitor (NTZ or derivative; 40 μM) and the reduction of redox-active BV dye was monitored at 546 nm. Inhibition of PFOR was expressed as a percentage of the control.

Determination of human foreskin cell toxicity: Human foreskin cells were plated in 96-well plates at 1.6×10^3 cells per well using medium 106 (Invitrogen). Plates were incubated overnight in a CO_2 incubator at 37 °C to allow cells to adhere to the bottom of the wells. Test compounds were serially diluted in replicate sets of plates. After 24 h, 0.02% resazurin sodium salt was added to each well of the first set of plates and placed back at 37 °C to incubate for 2 h. At that time, the plates with resazurin were read on a plate reader at OD_{570}. The second set of plates was treated in the same manner at the 48 h time point. All assays were performed in triplicate and in two independent assays. The CC_{50} value was recorded as the drug concentration that inhibited 50% of the resazurin reduction by the untreated controls. All experiments were performed 2–3 times in triplicate. DMSO concentration did not exceed 0.6%.

Chemistry

General information: All reagents were purchased from commercial suppliers and used without further purification. All reactions were run under a nitrogen or argon atmosphere unless otherwise noted. Flash chromatography was performed with 60 Å mesh standard grade silica gel (Sorbtech). ^1H and ^{13}C NMR spectra were obtained using Varian 300 MHz or 500 MHz spectrometers and recorded at 23 °C. Chemical shifts (δ) are given in parts per million (ppm) relative to the residual solvent peak: ^1H: DMSO, $\delta = 2.50$; $CDCl_3$, δ 7.27; ^{13}C: DMSO, $\delta = 39.51$ (s = singlet, br s = broad singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, ddd = doublet of doublet of doublets, m = multiplet). Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility, which is funded by the North Carolina Biotechnology Center and the NCSU Department of Chemistry.

Abbreviations: *N*,*N*-diisopropylethylamine (DIPEA), 4-dimethylaminopyridine (DMAP), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *N*-hydroxybenzotriazole (HOBt), tetrahydrofuran (THF).

Method A–acid chloride coupling: Acid chloride (~100 mg or ~0.1 mL, 1 equiv) was dissolved in THF (0.1 M) and cooled to −78 °C then 2-amino-5-nitrothiazole (1 equiv) was added in one portion. DIPEA (1.1 equiv) was added to the resulting slurry at −78 °C and the solution was held at this temperature for 10 min then allowed to warm to RT overnight. The solution was judged complete by TLC analysis (~24 h) and was diluted with EtOAc (30 mL) and washed with saturated aq $NaHCO_3$ (3×20 mL), 1 M HCl (3×20 mL) and brine (2×20 mL) then dried ($MgSO_4$), filtered and evaporated to dryness. The resulting residue was purified by gradient flash column chromatography (10–60% EtOAc/hexanes or 1–2% MeOH/CH_2Cl_2) to obtain the product.

Method B–carboxylic acid EDC coupling: Carboxylic acid (~100 mg, 1 equiv), EDC (2 equiv), HOBt (2 equiv) and DIPEA (3 equiv) were dissolved in THF (0.1 M) and stirred for 15 min. 2-Amino-5-nitrothiazole (1 equiv) was then added in one portion and the reaction was stirred at RT. Once judged complete by TLC analysis (~24 h), the resulting suspension was diluted with EtOAc (30 mL) and washed with saturated aq $NaHCO_3$ (3×20 mL), 1 M HCl (3×20 mL) and brine (2×20 mL) then dried ($MgSO_4$), filtered and evaporated to dryness. The resulting residue was purified by gradient flash column chromatography (10–60% EtOAc/hexanes or 1–2% MeOH/CH_2Cl_2) to obtain the product.

Method C–acid chloride formation: Carboxylic acid (100 mg, 1 equiv) was dissolved in CH_2Cl_2 (0.3 M) with a drop of DMF (cat.) and cooled to 0 °C then 2 M $(COCl)_2$ in CH_2Cl_2 (1.0 mL, 3 equiv) was added dropwise to the stirring solution. The slurry was allowed to warm to RT for 2 h then concentrated to dryness using hexanes to remove the excess $(COCl)_2$. The crude acid chloride obtained was used in the next step without further purification.

Method D–Suzuki coupling of furans and thiophenes: Methyl 5-bromofuran-2-carboxylate (100–150 mg, 1 equiv), $Pd(PPh_3)_4$ (5 mol%) or $PdCl_2(PPh_3)_2$ (5 mol%), 2 M Na_2CO_3 (2 equiv) and the respective boronic acid (1.3 equiv) in 1,4-dioxanes (0.1 M) was warmed to 90 °C. The solution was then held at this temperature for 5–24 h and judged complete by TLC then cooled and washed with 1 M HCl (2×20 mL), brine (2×20 mL) then dried ($MgSO_4$), filtered and evaporated to dryness. The resulting residue was then purified by flash column chromatography (5–15% EtOAc/hexanes) to obtain the product.

Method E–alkyl ester saponification: Ester (1 equiv) was dissolved in a mixture of MeOH/THF/H_2O (1 M: 1 M: 1 M) then LiOH·H_2O (3 equiv) was added. The solution was stirred for 24 h then was quenched with 1 M HCl (20 mL) and extracted with EtOAc (4× 15 mL). The combined organic layers were then washed with brine (2×20 mL) then dried ($MgSO_4$), filtered and evaporated to dryness to obtain the product.

2-Fluoro-*N*-(5-nitrothiazol-2-yl)benzamide (6): Method A yielded compound **6** (138 mg, 61%) as an orange solid: ^1H NMR (300 MHz, [D_6]DMSO): $\delta = 13.58$ (s, 1H), 8.70 (s, 1H), 7.80 (t, $J = 7.5$ Hz, 1H), 7.69 (q, $J = 7.5$ Hz, 1H), 7.48–7.30 ppm (m, 1H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 164.1$, 161.7, 159.5 (d, $J_{CF} = 253$ Hz), 142.6, 134.6 (d, $J_{CF} = 8.5$ Hz), 130.6, 124.8 (d, $J_{CF} = 3.4$ Hz), 120.9 (d, $J_{CF} = 13.0$ Hz), 116.5 ppm (d, $J_{CF} = 21.2$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_6FN_3O_3S+H]^+$: 268.0187, found 268.0193.

3-Fluoro-*N*-(5-nitrothiazol-2-yl)benzamide (7): Method A yielded compound **7** (164 mg, 73%) as a light yellow solid: ^1H NMR (300 MHz, [D_6]DMSO): $\delta = 13.69$ (s, 1H), 8.74 (s, 1H), 8.04–7.88 (m, 2H), 7.70–7.49 ppm (m, 2H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta =$ 165.3, 162.4, 161.9 (d, $J_{CF} = 244$ Hz), 142.5, 142.2, 133.1 (d, $J_{CF} =$ 7.5 Hz), 131.1 (d, $J_{CF} = 8.1$ Hz), 124.9 (d, $J_{CF} = 2.8$ Hz), 120.4 (d, $J_{CF} =$ 21.2 Hz, 1H), 115.3 ppm (d, $J_{CF} = 23.6$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_6FN_3O_3S+H]^+$: 268.0187, found 268.0195.

4-Fluoro-*N*-(5-nitrothiazol-2-yl)benzamide (8): Method A yielded compound **8** (96 mg, 43%) as a tan solid: ^1H NMR (300 MHz, [D_6]DMSO): $\delta = 13.62$ (s, 1H), 8.72 (s, 1H), 8.22 (dd, $J = 8.8$, 5.4 Hz, 2H), 7.43 ppm (t, $J = 8.9$ Hz, 2H); ^{13}C NMR (125 MHz, [D_6]DMSO): $\delta = 165.3$, 165.2 (d, $J_{CF} = 150$ Hz), 162.6, 142.6, 142.1, 131.6 (d, $J_{CF} =$ 9.5 Hz), 127.4, 115.9 ppm (d, $J_{CF} = 22.1$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_6FN_3O_3S+H]^+$: 268.0187, found 268.0196.

2,4-Difluoro-*N*-(5-nitrothiazol-2-yl)benzamide (9): Method A yielded compound **9** (158 mg, 68%) as a pale yellow solid: ^1H NMR

(500 MHz, [D$_6$]DMSO): $\delta = 13.60$ (br s, 1H), 8.70 (s, 1H), 7.91 (dd, $J = 14.9$, 8.4 Hz, 1H), 7.59–7.40 (m, 1H), 7.29 ppm (td, $J = 8.6$, 2.4 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 165.7$ (d, $J_{CF} = 12.5$ Hz), 163.7 (d, $J_{CF} = 12.2$ Hz), 162.4 (d, $J_{CF} = 198$ Hz), 161.4, 142.5, 142.2, 132.6 (d, $J_{CF} = 10.3$ Hz), 117.7 (d, $J_{CF} = 15.5$ Hz), 112.2 (d, $J_{CF} = 21.7$ Hz), 105.1 ppm (t, $J_{CF} = 26.0$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_5F_2N_3O_3S+H]^+$: 286.0092, found 286.0103.

3,4-Difluoro-*N*-(5-nitrothiazol-2-yl)benzamide (10): Method B yielded compound **10** (104 mg, 58%) as a pale yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 13.62$ (br s, 1H), 8.67 (s, 1H), 8.17 (ddd, $J = 11.2$, 7.7, 2.1 Hz, 1H), 8.14–7.95 (m, 1H), 7.64 ppm (dt, $J = 10.2$, 8.4 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 164.3$, 162.4, 152.6 (dd, $J_{CF} = 254$, 12.6 Hz), 149.2 (dd, $J_{CF} = 248$, 13.1 Hz), 142.3, 142.1, 128.2 (d, $J_{CF} = 3.8$ Hz), 126.5 (dd, $J_{CF} = 7.5$, 3.0 Hz), 118.1 ppm (dd, $J_{CF} = 18.4$, 6.1 Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_5F_2N_3O_3S+H]^+$: 286.0092, found 286.0098.

2,6-Difluoro-*N*-(5-nitrothiazol-2-yl)benzamide (11): Method A yielded compound **11** (160 mg, 70%) as a dark yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 13.97$ (s, 1H), 8.69 (s, 1H), 7.74–7.68 (m, 1H), 7.31 ppm (t, $J = 8.4$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 160.8$, 160.1, 159.0 (dd, $J_{CF} = 247$, 5.0 Hz), 142.6, 142.4, 134.2 (t, $J_{CF} = 10.2$ Hz), 112.4 (dd, $J_{CF} = 20.3$, 3.7 Hz), 111.8 ppm (t, $J_{CF} = 20.5$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_5F_2N_3O_3S+H]^+$: 286.0092, found 286.0095.

2,4,6-Trifluoro-*N*-(5-nitrothiazol-2-yl)benzamide (12): Method B with DMAP (cat.) yielded compound **12** (89 mg, 65%) as a light yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 13.97$ (br s, 1H), 8.69 (s, 1H), 7.45 ppm (t, $J = 8.9$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 163.9$ (dt, $J_{CF} = 250$, 15.7 Hz), 160.8, 159.7 (dt, $J_{CF} = 245$, 13.3 Hz), 159.2, 142.5, 142.4, 108.8 (t, $J_{CF} = 22.8$ Hz), 101.7 ppm (t, $J_{CF} = 26.7$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_4F_3N_3O_3S+H]^+$: 303.9998, found 304.0010.

3,4,5-Trifluoro-*N*-(5-nitrothiazol-2-yl)benzamide (13): Method B yielded compound **13** (68 mg, 40%) as a beige solid. After reaction completion, as judged by TLC analysis, the solution was concentrated to dryness and purified directly from the residue without dilutions or washings: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 13.78$ (br s, 1H), 8.73 (s, 1H), 8.13–8.07 ppm (m, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 163.7$, 162.4, 150.2 (dd, $J_{CF} = 249$, 6.8 Hz), 142.3, 142.2, 142.1 (dt, $J_{CF} = 255$, 15.2 Hz), 127.4, 114.0 ppm (d, $J_{CF} = 22.9$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_4F_3N_3O_3S+H]^+$: 303.9998, found 304.0007.

2,4,5-Trifluoro-*N*-(5-nitrothiazol-2-yl)benzamide (14): Method A yielded compound **14** (161 mg, 68%) as a beige solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 13.67$ (br s, 1H), 8.68 (s, 1H), 7.98 (ddd, $J = 10.3$, 9.0, 6.5 Hz, 1H), 7.79 ppm (td, $J = 10.4$, 6.5 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 162.2$, 161.5, 155.7 (dd, $J_{CF} = 254$, 9.2 Hz), 151.8 (dt, $J_{CF} = 253$, 13.8 Hz), 145.8 (dd, $J_{CF} = 246$, 9.6 Hz), 142.3, 118.9 (d, $J_{CF} = 21.2$ Hz), 117.6 (d, $J_{CF} = 15.7$ Hz), 107.4 ppm (dd, $J_{CF} = 28.3$, 22.0 Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_4F_3N_3O_3S+H]^+$: 303.9998, found 304.0007.

2,3,4,5-Tetrafluoro-*N*-(5-nitrothiazol-2-yl)benzamide (15): Method A yielded compound **15** (158 mg, 66%) as a light orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.74$ (br s, 1H), 8.73 (s, 1H), 8.14–7.75 ppm (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 161.5$, 146.0 (dd, $J_{CF} = 248$, 8.4 Hz), 145.7 (dd, $J_{CF} = 254$, 9.8 Hz), 142.3, 142.2 (dt, $J_{CF} = 256$, 13.6 Hz), 142.1, 139.1, 117.4, 112.7 ppm (d, $J_{CF} = 20.9$ Hz); HRMS (ESI): *m/z* calcd for $[C_{10}H_3F_4N_3O_3S+H]^+$: 321.9904, found 321.9911.

2,3,4,5,6-Pentafluoro-*N*-(5-nitrothiazol-2-yl)benzamide (16): Method A yielded compound **16** (95 mg, 65%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.74$ ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 160.8$, 157.3, 144.9, 143.7, 142.7, 142.2, 141.7, 138.2, 136.2 ppm; HRMS (ESI): *m/z* calcd for $[C_{10}H_2F_5N_3O_3S+H]^+$: 339.9810, found 339.9820.

2-Chloro-*N*-(5-nitrothiazol-2-yl)benzamide (17): Method A yielded compound **17** (94 mg, 42%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.74$ (s, 1H), 8.70 (s, 1H), 7.75–7.70 (m, 1H), 7.66–7.45 ppm (m, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 166.2$, 161.4, 142.6, 142.4, 132.9, 132.7, 130.4, 130.0, 129.8, 127.4 ppm; HRMS (ESI): *m/z* calcd for $[C_{10}H_6ClN_3O_3S+H]^+$: 283.9891, found 283.9900.

3-Chloro-*N*-(5-nitrothiazol-2-yl)benzamide (18): Method A yielded compound **18** (128 mg, 58%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.68$ (s, 1H), 8.17 (s, 1H), 8.05 (d, $J = 7.8$ Hz, 1H), 7.73 (d, $J = 7.0$ Hz, 1H), 7.59 ppm (t, $J = 7.9$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 166.0$, 163.8, 143.0, 142.7, 133.7, 133.5, 132.9, 130.7, 128.3, 127.3 ppm; HRMS (ESI): *m/z* calcd for $[C_{10}H_6ClN_3O_3S+H]^+$: 283.9891, found 283.9902.

4-Chloro-*N*-(5-nitrothiazol-2-yl)benzamide (19): Method A yielded compound **19** (125 mg, 57%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.67$ (s, 1H), 8.72 (s, 1H), 8.13 (d, $J = 8.5$ Hz, 2H), 7.66 ppm (d, $J = 8.7$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 165.5$, 162.5, 142.5, 142.1, 138.4, 130.5, 129.6, 128.9 ppm; HRMS (ESI): *m/z* calcd for $[C_{10}H_6ClN_3O_3S+H]^+$: 283.9891, found 283.9900.

3-Cyano-*N*-(5-nitrothiazol-2-yl)benzamide (20): Method A yielded compound **20** (145 mg, 88%) as a bright yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.68$ (s, 1H), 8.68 (s, 1H), 8.51 (s, 1H), 8.34 (d, $J = 8.0$ Hz, 1H), 8.11 (d, $J = 7.7$ Hz, 1H), 7.76 ppm (t, $J = 7.9$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 164.7$, 162.1, 141.9, 141.6, 136.0, 132.7, 132.0, 131.9, 129.7, 117.5, 111.7 ppm; HRMS (ESI): *m/z* calcd for $[C_{11}H_6N_4O_3S+H]^+$: 275.0233, found 275.0243.

4-Cyano-*N*-(5-nitrothiazol-2-yl)benzamide (21): Method C followed by Method A yielded compound **21** (134 mg, 72%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.74$ (s, 1H), 8.70 (s, 1H), 8.21 (d, $J = 8.4$ Hz, 2H), 8.03 ppm (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 165.4$, 162.3, 142.4, 142.2, 134.9, 132.7, 129.3, 118.0, 115.4 ppm; HRMS (ESI): *m/z* calcd for $[C_{11}H_6N_4O_3S+H]^+$: 275.0233, found 275.0243.

***N*-(5-Nitrothiazol-2-yl)-2-(trifluoromethyl)benzamide (22)**: Method A yielded compound **22** (55 mg, 25%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.84$ (br s, 1H), 8.71 (s, 1H), 8.07–7.71 ppm (m, 4H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 166.9$, 161.3, 132.7, 132.0 (q, $J_{CF} = 2.1$ Hz), 131.6, 129.5 (q, $J_{CF} = 265$ Hz), 129.2, 127.9 (q, $J_{CF} = 268$ Hz), 126.7 (q, $J_{CF} = 4.7$ Hz), 123.5 ppm (d, $J_{CF} = 274$ Hz); HRMS (ESI): *m/z* calcd for $[C_{11}H_6F_3N_3O_3S+H]^+$: 318.0155, found 318.0165.

***N*-(5-Nitrothiazol-2-yl)-3-(trifluoromethyl)benzamide (23)**: Method A yielded compound **23** (157 mg, 75%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 13.85$ (br s, 1H), 8.74 (s, 1H), 8.51 (s, 1H), 8.39 (d, $J = 8.2$ Hz, 1H), 8.06 (d, $J = 7.9$ Hz, 1H), 7.83 ppm (t, $J = 7.9$ Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 165.2$, 162.4, 142.5, 142.2, 132.7, 131.9, 130.1, 129.8 (q, $J_{CF} = 3.4$ Hz), 129.4 (q, $J_{CF} = 32.6$ Hz), 125.2 (q, $J_{CF} = 3.9$ Hz), 123.8 ppm (q, $J_{CF} = 273$ Hz); HRMS (ESI): *m/z* calcd for $[C_{11}H_6F_3N_3O_3S+H]^+$: 318.0155, found 318.0164.

***N*-(5-Nitrothiazol-2-yl)-4-(trifluoromethyl)benzamide (24)**: Method A yielded compound **24** (159 mg, 75%) as a light yellow solid:

^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.86 (br s, 1H), 8.74 (s, 1H), 8.30 (d, J = 8.2 Hz, 2H), 7.97 ppm (d, J = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 165.6, 162.4, 142.5, 142.2, 134.8, 132.7 (q, J_{CF} = 32.2 Hz), 129.5, 125.7 (q, J_{CF} = 3.4 Hz), 123.7 ppm (q, J_{CF} = 273 Hz); HRMS (ESI): m/z calcd for $[C_{11}H_6F_3N_3O_3S+H]^+$: 318.0155, found 318.0162.

2-Nitro-*N*-(5-nitrothiazol-2-yl)benzamide (25): Method A yielded compound **25** (83 mg, 37%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.80 (s, 1H), 8.71 (s, 1H), 8.25 (d, J = 8.2 Hz, 1H), 8.04–7.72 ppm (m, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 165.9, 161.4, 146.1, 142.5, 142.4, 134.6, 132.4, 129.8, 129.2, 124.6 ppm; HRMS (ESI): m/z calcd for $[C_{10}H_6N_4O_5S+H]^+$: 295.0132, found 295.0135.

3-Nitro-*N*-(5-nitrothiazol-2-yl)benzamide (26): Method A yielded compound **26** (131 mg, 82%) as a bright yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.92 (s, 1H), 8.94 (t, J = 1.8 Hz, 1H), 8.69 (s, 1H), 8.48 (dd, J = 8.0, 1.8 Hz, 2H), 7.85 ppm (t, J = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 164.7, 162.4, 147.8, 142.3, 134.8, 132.4, 130.5, 127.7, 123.3 ppm; HRMS (ESI): m/z calcd for $[C_{10}H_6N_4O_5S+H]^+$: 295.0132, found 295.0137.

4-Nitro-*N*-(5-nitrothiazol-2-yl)benzamide (27): Method A yielded compound **27** (133 mg, 84%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.76 (s, 1H), 8.40 (d, J = 8.5 Hz, 2H), 8.33 ppm (d, J = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 165.2, 162.4, 150.0, 142.4, 142.2, 136.5, 130.2, 123.7 ppm; HRMS (ESI): m/z calcd for $[C_{10}H_6N_4O_5S+H]^+$: 295.0132, found 295.0139.

2-Methoxy-*N*-(5-nitrothiazol-2-yl)benzamide (28): Method A with DMAP (cat.) yielded compound **28** (29 mg, 16%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.90 (s, 1H), 8.69 (s, 1H), 7.68 (dd, J = 7.6, 1.7 Hz, 1H), 7.65–7.57 (m, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 3.90 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 165.9, 161.5, 157.2, 142.7, 134.0, 130.2, 121.0, 120.6, 112.3, 56.1 ppm; HRMS (ESI): m/z calcd for $[C_{11}H_9N_3O_4S+H]^+$: 280.0387, found 280.0396.

3-Methoxy-*N*-(5-nitrothiazol-2-yl)benzamide (29): Method A yielded compound **29** (31 mg, 16%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.61 (s, 1H), 8.73 (s, 1H), 7.71 (t, J = 4.1 Hz, 2H), 7.50 (t, J = 7.8 Hz, 1H), 7.31–7.21 (m, 1H), 3.86 ppm (s, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 166.2, 162.6, 159.3, 142.7, 142.1, 132.0, 130.0, 120.9, 119.9, 113.0, 55.5 ppm; HRMS (ESI): m/z calcd for $[C_{11}H_9N_3O_4S+H]^+$: 280.0387, found 280.0395.

4-Methoxy-*N*-(5-nitrothiazol-2-yl)benzamide (30): Method A yielded compound **30** (78 mg, 38%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.37 (s, 1H), 8.70 (s, 1H), 8.14 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 8.7 Hz, 2H), 3.86 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 165.7, 163.4, 163.1, 142.8, 141.7, 130.8, 122.9, 114.1, 55.6 ppm; HRMS (ESI): m/z calcd for $[C_{11}H_9N_3O_4S+H]^+$: 280.0387, found 280.0388.

***N*-(5-Nitrothiazol-2-yl)-3-(trifluoromethoxy)benzamide (31)**: Method B yielded compound **31** (91 mg, 56%) as an orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.77 (br s, 1H), 8.74 (s, 1H), 8.20–8.14 (m, 1H), 8.12 (s, 1H), 7.78–7.67 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 165.0, 162.4, 148.4, 142.4, 142.2, 133.0, 131.0, 127.7, 125.9, 120.9, 120.0 ppm (d, J_{CF} = 256 Hz); HRMS (ESI): m/z calcd for $[C_{11}H_6F_3N_3O_4S+H]^+$: 334.0104, found 334.0115.

2-Methoxy-*N*-(5-nitrothiazol-2-yl)-4-(trifluoromethyl)benzamide (32): Method B employing known 2-methoxy-4-(trifluoromethyl)-benzoic acid[29] yielded compound **32** (61 mg, 77%) as an orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.32 (s, 1H), 8.70 (s, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.51 (s, 1H), 7.46 (d, J = 7.9 Hz, 1H), 3.96 ppm (s, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 165.3, 161.3, 157.3, 142.7, 142.2, 133.1 (q, J_{CF} = 32.0 Hz), 130.9, 125.80, 123.6 (q, J_{CF} = 273 Hz), 117.2, 109.1, 56.6 ppm; HRMS (ESI): m/z calcd for $[C_{12}H_8F_3N_3O_4S+H]^+$: 348.0260, found 348.0276.

2-Methoxy-4-nitro-*N*-(5-nitrothiazol-2-yl)benzamide (33): Method C followed by Method A yielded compound **33** (96 mg, 59%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.48 (s, 1H), 8.70 (s, 1H), 7.97–7.89 (m, 2H), 7.88–7.82 (m, 1H), 3.99 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 165.0, 161.3, 157.5, 150.4, 142.6, 131.0, 127.9, 115.4, 107.1, 56.9 ppm; HRMS (ESI): m/z calcd for $[C_{11}H_8N_4O_6S+H]^+$: 325.0237, found 325.0246.

2-Hydroxy-3-nitro-*N*-(5-nitrothiazol-2-yl)benzamide (34): Method C employing known 2-acetoxy-3-nitrobenzoic acid[30] followed by Method A yielded compound **34** (60 mg, 44%) as a yellow solid. Note: 2-O-acetyl group cleaved under the reaction conditions to afford the 2-hydroxy derivative: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.61 (s, 1H), 8.10 (dd, J = 7.7, 1.9 Hz, 1H), 7.91 (dd, J = 8.0, 1.9 Hz, 1H), 6.64 ppm (t, J = 7.8 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 167.5, 165.7, 160.8, 144.1, 140.1, 135.0, 129.8, 120.6, 112.6 ppm; HRMS (ESI): m/z calcd for $[C_{10}H_6N_4O_6S+H]^+$: 311.0081, found 311.0092.

4-Fluoro-*N*-(5-nitrothiazol-2-yl)-2-(trifluoromethyl)benzamide (35): Method A yielded compound **35** (89 mg, 40%) as a light yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.70 (s, 1H), 7.98 (dd, J = 8.6, 5.3 Hz, 1H), 7.88 (dd, J = 9.2, 2.5 Hz, 1H), 7.75 ppm (td, J = 8.4, 2.5 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 166.0, 162.9 (d, J_{CF} = 251 Hz), 161.4, 142.5, 132.4 (d, J_{CF} = 9.0 Hz), 129.0 (dd, J_{CF} = 32.8, 8.5 Hz), 128.6, 122.6 (q, J_{CF} = 272 Hz), 119.7 (d, J_{CF} = 21.4 Hz), 114.8 ppm (dd, J_{CF} = 25.9, 4.9 Hz); HRMS (ESI): m/z calcd for $[C_{11}H_5F_4N_3O_3S+H]^+$: 336.0061, found 336.0065.

2-Nitro-*N*-(5-nitrothiazol-2-yl)-4-(trifluoromethyl)benzamide (36): Method B yielded compound **36** (84 mg, 58%) as a light yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.96 (br s, 1H), 8.72 (s, 1H), 8.58 (s, 1H), 8.38 (d, J = 7.9 Hz, 1H), 8.16 ppm (d, J = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.7, 161.2, 146.6, 142.5, 142.4, 132.6, 132.0 (q, J_{CF} = 34.2 Hz), 131.4, 131.3 (d, J_{CF} = 3.4 Hz), 122.6 (d, J_{CF} = 272 Hz), 121.9 ppm (q, J_{CF} = 7.5 Hz, 1H); HRMS (ESI): m/z calcd for $[C_{11}H_5F_3N_4O_5S+H]^+$: 363.0006, found 363.0016.

4-Fluoro-3-nitro-*N*-(5-nitrothiazol-2-yl)benzamide (37): Method B yielded compound **37** (25 mg, 16%) as a pale yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.95 (d, J = 7.2 Hz, 1H), 8.76 (s, 1H), 8.60–8.38 (m, 1H), 7.90–7.78 ppm (m, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.7, 157.3 (d, J_{CF} = 268 Hz), 142.4, 136.6 (d, J_{CF} = 10.4 Hz), 128.3, 126.9, 119.3 ppm (d, J_{CF} = 21.4 Hz); HRMS (ESI): m/z calcd for $[C_{10}H_5FN_4O_5S+H]^+$: 313.0037, found 313.0044.

***N*-(5-Nitrothiazol-2-yl)-3,5-bis(trifluoromethyl)benzamide (38)**: Method B yielded compound **38** (59 mg, 40%) as a light orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.76 (s, 1H), 8.76 (s, 2H), 8.48 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 164.3, 162.6, 142.4, 133.7, 130.7 (q, J_{CF} = 33.5 Hz, 129.5 (d, J_{CF} = 3.2 Hz), 126.6 (d, J_{CF} = 3.3 Hz), 123.0 ppm (d, J_{CF} = 273 Hz); HRMS (ESI): m/z calcd for $[C_{12}H_5F_6N_3O_3S+H]^+$: 386.0029, found 386.0040.

2-Chloro-*N*-(5-nitrothiazol-2-yl)-5-(trifluoromethyl)benzamide (39): Method B yielded compound **39** (81 mg, 52%) as a light orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.85 (br s, 1H), 8.71 (s, 1H), 8.22 (d, J = 1.6 Hz, 1H), 7.97 (dd, J = 8.5, 1.8 Hz, 1H), 7.87 ppm (d, J = 8.5 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 164.8, 161.3, 142.5, 135.0, 133.7, 131.2, 129.3 (d, J_{CF} = 2.6 Hz), 127.9 (q, J_{CF} = 33.1 Hz), 127.1 (d, J_{CF} = 3.0 Hz), 123.4 (d, J_{CF} = 273 Hz),

119.4 ppm; HRMS (ESI): *m/z* calcd for $[C_{11}H_5ClF_3N_3O_3S+H]^+$: 351.9765, found 351.9767.

2-Chloro-*N*-(5-nitrothiazol-2-yl)-3-(trifluoromethyl)benzamide (40): Method B yielded compound **40** (97 mg, 62%) as a beige solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.88$ (br s, 1H), 8.72 (s, 1H), 8.13–7.97 (m, 2H), 7.73 ppm (t, $J=7.8$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=165.3$, 161.1, 142.6, 142.5, 136.0, 133.5, 130.0 (d, $J_{CF}=4.7$ Hz), 128.3, 128.2, 127.5 (q, $J_{CF}=31.0$ Hz), 122.6 ppm (q, $J_{CF}=274$ Hz); HRMS (ESI): *m/z* calcd for $[C_{11}H_5ClF_3N_3O_3S+H]^+$: 351.9765, found 351.9775.

4-Chloro-*N*-(5-nitrothiazol-2-yl)-3-(trifluoromethyl)benzamide (41): Method B yielded compound **41** (126 mg, 80%) as an orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.89$ (br s, 1H), 8.71 (d, $J=0.6$ Hz, 1H), 8.58 (d, $J=1.9$ Hz, 1H), 8.35 (dd, $J=8.4$, 2.1 Hz, 1H), 7.94 ppm (d, $J=8.4$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=$ 164.4, 162.4, 142.3, 135.7, 134.2, 132.3, 130.4, 127.9 (d, $J_{CF}=4.8$ Hz), 126.9 (q, $J_{CF}=31.6$ Hz), 122.5 ppm (q, $J_{CF}=274$ Hz); HRMS (ESI): *m/z* calcd for $[C_{11}H_5ClF_3N_3O_3S+H]^+$: 351.9765, found 351.9775.

***N*-(5-Nitrothiazol-2-yl)benzamide (42)**: Method A yielded compound **42** (90 mg, 42%) as a beige solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.61$ (s, 1H), 8.73 (s, 1H), 8.13 (d, $J=7.3$ Hz, 2H), 7.70 (t, $J=7.4$ Hz, 1H), 7.59 ppm (t, $J=7.6$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=166.4$, 162.6, 142.6, 142.1, 133.5, 130.8, 128.8, 128.6 ppm; HRMS (ESI): *m/z* calcd for $[C_{10}H_7N_3O_3S+H]^+$: 250.0281, found 250.0287.

***N*-(5-Nitrothiazol-2-yl)-2-phenylacetamide (43)**: Method B yielded compound **43** (70 mg, 36%) as a beige solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.33$ (s, 1H), 8.63 (s, 1H), 7.80–7.02 (m, 5H), 3.87 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=171.1$, 161.7, 142.7, 141.9, 134.0, 129.4, 128.5, 127.1, 41.5 ppm; HRMS (ESI): *m/z* calcd for $[C_{11}H_9N_3O_3S+H]^+$: 264.0437, found 264.0447.

***N*-(5-Nitrothiazol-2-yl)-3-phenylpropanamide (44)**: Method B yielded compound **44** (122 mg, 66%) as a pale yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=13.10$ (br s, 1H), 8.60 (s, 1H), 7.32–7.26 (m, 1H), 7.23 (d, $J=7.5$ Hz, 1H), 7.19 (t, $J=6.8$ Hz, 1H), 2.94 (t, $J=7.4$ Hz, 2H), 2.88–2.81 ppm (m, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=172.8$, 162.1, 143.1, 142.1, 140.8, 128.8, 128.7, 126.6, 37.0, 30.4 ppm; HRMS (ESI): *m/z* calcd for $[C_{12}H_{11}N_3O_3S+H]^+$: 278.0594, found 278.0604.

***N*-(5-Nitrothiazol-2-yl)-4-phenylbutanamide (45)**: Method B yielded compound **45** (133 mg, 84%) as an orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.07$ (br s, 1H), 8.60 (s, 1H), 7.35–7.05 (m, 5H), 2.64 (t, $J=7.6$ Hz, 2H), 2.56 (t, $J=7.4$ Hz, 2H), 1.94 ppm (quint., $J=7.6$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=172.8$, 161.7, 142.6, 141.6, 141.3, 128.3, 125.9, 34.4, 25.8 ppm; HRMS (ESI): *m/z* calcd for $[C_{13}H_{13}N_3O_3S+H]^+$: 292.0750, found 292.0753.

(1*R*,2*R*)-*N*-(5-Nitrothiazol-2-yl)-2-phenylcyclopropanecarboxamide (46): Method B yielded compound **46** (43 mg, 24%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=13.35$ (br s, 1H), 8.62 (s, 1H), 7.30 (t, $J=7.5$ Hz, 2H), 7.22 (t, $J=7.6$ Hz, 3H), 2.64–2.52 (m, 1H), 2.31–2.22 (m, 1H), 1.68–1.54 ppm (m, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=171.8$, 161.7, 142.8, 141.8, 139.7, 128.5, 126.6, 126.2, 27.3, 25.5, 17.1 ppm; HRMS (ESI): *m/z* calcd for $[C_{13}H_{11}N_3O_3S+H]^+$: 290.0594, found 290.0599.

3-Methyl-*N*-(5-nitrothiazol-2-yl)indene-2-carboxamide (47): Method B yielded compound **47** (25 mg, 17%) as an orange solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=12.66$ (br s, 1H), 8.61 (s, 1H), 7.66–7.55 (m, 2H), 7.48–7.39 (m, 2H), 3.98 (d, $J=2.3$ Hz, 2H), 2.57 ppm (t, $J=2.3$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=$ 162.3, 152.8, 144.3, 143.2, 142.7, 129.8, 128.3, 126.8, 124.1, 121.5, 37.8, 12.5 ppm; HRMS (ESI): *m/z* calcd for $[C_{14}H_{11}N_3O_3S+H]^+$: 302.0594, found 302.0601.

***N*-(5-Nitrothiazol-2-yl)acetamide (48)**: Method A yielded compound **48** (132 mg, 50%) as a beige solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.02$ (s, 1H), 8.56 (s, 1H), 2.21 ppm (s, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=170.3$, 161.8, 142.6, 141.7, 22.5 ppm; HRMS (ESI): *m/z* calcd for $[C_5H_5N_3O_3S+H]^+$: 188.0124, found 188.0124.

***N*-(5-Nitrothiazol-2-yl)butyramide (49)**: Method A yielded compound **49** (112 mg, 55%) as an orange solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=13.04$ (br s, 1H), 8.60 (s, 1H), 2.50 (t, $J=7.3$ Hz, 2H), 1.96–1.40 (m, 2H), 0.91 ppm (t, $J=7.4$ Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=173.0$, 161.7, 142.7, 141.6, 36.8, 17.8, 13.4 ppm; HRMS (ESI): *m/z* calcd for $[C_7H_9N_3O_3S+H]^+$: 216.0437, found 216.0439.

***N*-(5-Nitrothiazol-2-yl)hexanamide (50)**: Method A yielded compound **50** (126 mg, 71%) as a light orange solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=13.02$ (s, 1H), 8.57 (s, 1H), 2.50 (t, $J=$ 7.5 Hz, 2H), 1.67–1.44 (m, 2H), 1.44–1.07 (m, 4H), 0.85 ppm (t, $J=$ 7.0 Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=173.1$, 161.7, 142.6, 141.6, 34.9, 30.7, 24.0, 21.8, 13.8 ppm; HRMS (ESI): *m/z* calcd for $[C_9H_{13}N_3O_3S+H]^+$: 244.0750, found 244.0757.

***N*-(5-Nitrothiazol-2-yl)octanamide (51)**: Method A yielded compound **51** (61 mg, 38%) as a beige solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=13.02$ (br s, 1H), 8.59 (s, 1H), 2.50 (t, $J=7.4$ Hz, 2H), 1.75–1.43 (m, 2H), 1.31–1.19 (m, 8H), 0.84 ppm (t, $J=6.8$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=173.1$, 161.7, 142.7, 141.6, 34.9, 31.1, 28.43, 28.37, 24.3, 22.1, 13.9 ppm; HRMS (ESI): *m/z* calcd for $[C_{11}H_{17}N_3O_3S+H]^+$: 272.1063, found 272.1071.

***N*-(5-Nitrothiazol-2-yl)decanamide (52)**: Method A yielded compound **52** (53 mg, 36%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=13.02$ (br s, 1H), 8.59 (s, 1H), 2.50 (t, $J=7.4$ Hz, 2H), 1.59 (quint., $J=7.0$ Hz, 2H), 1.24 (d, $J=13.0$ Hz, 12H), 0.84 ppm (t, $J=6.8$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=173.1$, 161.7, 142.6, 141.6, 34.9, 31.3, 28.8, 28.67, 28.66, 28.4, 24.3, 22.1, 13.9 ppm; HRMS (ESI): *m/z* calcd for $[C_{13}H_{21}N_3O_3S+H]^+$: 300.1376, found 300.1383.

***N*-(5-Nitrothiazol-2-yl)cyclohexanecarboxamide (53)**: Method A yielded compound **53** (128 mg, 68%) as a beige solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=12.98$ (s, 1H), 8.57 (s, 1H), 2.58–2.46 (m, 1H), 1.83 (d, $J=12.9$ Hz, 2H), 1.80–1.64 (m, 2H), 1.62 (d, $J=11.5$ Hz, 1H), 1.38 (qd, $J=12.3$, 2.8 Hz, 2H), 1.30–1.13 ppm (m, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=175.8$, 161.9, 142.6, 141.7, 43.4, 28.5, 25.2, 24.9 ppm; HRMS (ESI): *m/z* calcd for $[C_{10}H_{13}N_3O_3S+H]^+$: 256.0750, found 256.0755.

***N*-(5-Nitrothiazol-2-yl)cinnamamide (54)**: Method B yielded compound **54** (78 mg, 42%) as a beige solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=13.31$ (s, 1H), 8.66 (s, 1H), 7.85 (d, $J=15.9$ Hz, 1H), 7.72–7.59 (m, 2H), 7.53–7.40 (m, 3H), 6.92 ppm (d, $J=15.9$ Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=164.6$, 161.9, 144.6, 142.9, 142.1, 133.9, 130.9, 129.2, 128.3, 118.1 ppm; HRMS (ESI): *m/z* calcd for $[C_{12}H_9N_3O_3S+H]^+$: 276.0437, found 276.0445.

(*E*)-*N*-(5-Nitrothiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylamide (55): Method B with DMAP (cat.) yielded compound **55** (51 mg, 32%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=$ 13.42 (s, 1H), 8.69 (s, 1H), 7.97–7.80 (m, 5H), 7.03 ppm (d, $J=$ 16.0 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=164.2$, 161.8, 142.8, 142.6, 142.2, 137.8, 129.9 (q, $J_{CF}=26.5$ Hz), 128.9, 126.0 (q, $J_{CF}=$

3.7 Hz), 124.0 (d, J_{CF} = 269 Hz), 120.9 ppm; HRMS (ESI): *m/z* calcd for $[C_{13}H_8F_3N_3O_3S+H]^+$: 344.0311, found 344.0319.

***N*-(5-Nitrothiazol-2-yl)isonicotinamide (56)**: Method A without an aqueous workup yielded compound **56** (90 mg, 56%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.85 (d, J = 4.1 Hz, 2H), 8.75 (s, 1H), 8.01 ppm (d, J = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 165.5, 162.4, 150.5, 142.5, 142.2, 138.4, 122.0 ppm; HRMS (ESI): *m/z* calcd for $[C_9H_6N_4O_3S+H]^+$: 251.0233, found 251.0239.

3-Fluoro-*N*-(5-nitrothiazol-2-yl)isonicotinamide (57): Method C followed by Method A without an aqueous workup and quenching with 2 M HCl in Et$_2$O yielded compound **57** (112 mg, 59%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.82 (s, 1H), 8.72 (s, 1H), 8.65 (d, J = 4.7 Hz, 1H), 7.82 ppm (t, J = 5.4 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.6, 161.3, 155.2 (d, J_{CF} = 262 Hz), 146.4 (d, J_{CF} = 4.8 Hz), 142.4, 139.4 (d, J_{CF} = 23.5 Hz), 127.9 (d, J_{CF} = 11.1 Hz), 123.5 ppm; HRMS (ESI): *m/z* calcd for $[C_9H_5FN_4O_3S+H]^+$: 269.0139, found 269.0142.

***N*-(5-Nitrothiazol-2-yl)nicotinamide (58)**: Method A without an aqueous workup and quenching with 2 M HCl in Et$_2$O yielded compound **58** (16 mg, 10%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.24 (s, 1H), 8.83 (d, J = 4.7 Hz, 1H), 8.74 (s, 1H), 8.45 (d, J = 8.0 Hz, 1H), 7.62 ppm (dd, J = 8.0, 4.9 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 174.5, 171.7, 162.3, 158.3, 151.5, 145.1, 136.2, 132.6 ppm; HRMS (ESI): *m/z* calcd for $[C_9H_6N_4O_3S+H]^+$: 251.0233, found 251.0238.

2-Fluoro-*N*-(5-nitrothiazol-2-yl)nicotinamide (59): Method C followed by Method A without an aqueous workup and quenching with 2 M HCl in Et$_2$O yielded compound **59** (118 mg, 62%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.77 (br s, 1H), 8.70 (s, 1H), 8.54–8.44 (m, 1H), 8.41–8.35 (m, 1H), 7.63–7.50 ppm (m, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.0 (d, J_{CF} = 5.9 Hz), 161.5, 159.4 (d, J_{CF} = 242 Hz), 151.3 (d, J_{CF} = 15.2 Hz), 142.4, 142.3, 122.3 (d, J_{CF} = 4.1 Hz), 115.9 ppm (d, J_{CF} = 28.5 Hz); HRMS (ESI): *m/z* calcd for $[C_9H_5FN_4O_3S+H]^+$: 269.0139, found 269.0143.

***N*-(5-Nitrothiazol-2-yl)furan-3-carboxamide (60)**: Method B yielded compound **60** (105 mg, 49%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.44 (br s, 1H), 8.70 (s, 1H), 8.68 (dd, J = 1.5, 0.8 Hz, 1H), 7.93–7.85 (m, 1H), 7.17–7.09 ppm (m, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.2, 161.1, 148.4, 145.1, 144.7, 142.6, 120.0, 109.1 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_5N_3O_4S+H]^+$: 240.0074, found 240.0082.

***N*-(5-Nitrothiazol-2-yl)furan-2-carboxamide (61)**: Method A yielded compound **61** (39 mg, 16%) as a beige solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.68 (s, 1H), 8.12–8.05 (m, 1H), 7.76 (d, J = 3.5 Hz, 1H), 6.78 ppm (dd, J = 3.5, 1.4 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.5, 157.0, 149.0, 145.1, 143.1, 142.5, 118.9, 113.2 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_5N_3O_4S+H]^+$: 240.0074, found 240.0077.

5-Bromo-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (62): Method B yielded compound **62** (72 mg, 44%) as a red solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.57 (br s, 1H), 8.63 (s, 1H), 7.73 (d, J = 3.7 Hz, 1H), 6.89 ppm (d, J = 3.7 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 161.9, 155.5, 146.6, 142.4, 142.1, 128.9, 120.5, 114.9 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_4BrN_3O_4S+H]^+$: 317.9179, found 317.9180.

4,5-Dibromo-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (63): Method B yielded compound **63** (53 mg, 18%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.72 (s, 1H), 7.90 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 161.9, 155.2, 146.7, 142.3, 142.1, 130.0, 121.5, 104.0 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_3Br_2N_3O_4S+H]^+$: 395.8284, found 395.8295.

5-Nitro-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (64): Method A yielded compound **64** (128 mg, 79%) as a tan solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.71 (s, 1H), 7.87 (d, J = 3.8 Hz, 1H), 7.81 ppm (d, J = 3.9 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.1, 156.3, 152.5, 145.6, 141.8, 141.5, 118.9, 113.1 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_4N_4O_6S+H]^+$: 284.9924, found 284.9928.

***N*-(5-Nitrothiazol-2-yl)-5-phenylfuran-2-carboxamide (65)**: Method D yielded the intermediate Suzuki coupling product (94 mg, 95%) as a white solid.[37] ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.86–7.76 (m, 2H), 7.50–7.34 (m, 3H), 7.28 (d, J = 3.8 Hz, 1H), 6.77 (d, J = 3.6 Hz, 1H), 3.94 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (92 mg, 100%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.84–7.75 (m, 2H), 7.52–7.43 (m, 2H), 7.44–7.36 (m, 1H), 7.32 (d, J = 3.6 Hz, 1H), 7.15 ppm (d, J = 3.6 Hz, 1H). Method B yielded compound **65** (29 mg, 24%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.79 (br s, 1H), 8.72 (s, 1H), 8.06–7.99 (m, 2H), 7.77 (d, J = 3.8 Hz, 1H), 7.55–7.47 (m, 2H), 7.46–7.40 (m, 1H), 7.29 ppm (d, J = 3.7 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.2, 157.5, 156.3, 143.9, 142.7, 142.0, 129.4, 129.0, 128.8, 125.0, 120.9, 108.6 ppm; HRMS (ESI): *m/z* calcd for $[C_{14}H_9N_3O_4S+H]^+$: 316.0387, found 316.0388.

5-(3-Chlorophenyl)-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (66): Method D yielded the intermediate Suzuki coupling product (154 mg, 88%) as a pale yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.82 (s, 1H), 7.73 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 7.44–7.40 (m, 1H), 7.39 (d, J = 3.7 Hz, 1H), 7.26 (d, J = 3.7 Hz, 1H), 3.83 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (85 mg, 89%) as a white solid.[38] ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.24 (br s, 1H), 7.87 (s, 1H), 7.77 (dd, J = 7.7, 1.1 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.50–7.42 (m, 1H), 7.33 (d, J = 3.6 Hz, 1H), 7.28 ppm (d, J = 3.6 Hz, 1H). Method B yielded compound **66** (53 mg, 45%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.70 (br s, 1H), 8.67 (s, 1H), 8.12 (t, J = 1.8 Hz, 1H), 8.00–7.92 (m, 1H), 7.71 (d, J = 3.8 Hz, 1H), 7.51 (t, J = 7.9 Hz, 1H), 7.47–7.43 (m, 1H), 7.35 ppm (d, J = 3.8 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 161.9, 156.1, 155.5, 144.2, 142.5, 142.0, 133.8, 130.8, 130.6, 128.9, 124.3, 123.4, 120.7, 109.8 ppm; HRMS (ESI): *m/z* calcd for $[C_{14}H_8ClN_3O_4S+H]^+$: 349.9997, found 350.0010.

5-(3-Fluorophenyl)-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (67): Method D yielded the intermediate Suzuki coupling product (133 mg, 83%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.70–7.62 (m, 2H), 7.54 (td, J = 8.1, 6.2 Hz, 1H), 7.45 (dd, J = 3.7, 0.4 Hz, 1H), 7.30 (d, J = 3.7 Hz, 1H), 7.26 (td, J = 8.7, 2.5 Hz, 1H), 3.85 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (93 mg, 99%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.22 (br s, 1H), 7.71–7.59 (m, 2H), 7.53 (td, J = 8.0, 6.0 Hz, 1H), 7.34 (d, J = 3.6 Hz, 1H), 7.28–7.16 ppm (m, 2H). Method B yielded compound **67** (28 mg, 29%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.71 (br s, 1H), 8.68 (s, 1H), 7.94 (d, J = 10.2 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.71 (d, J = 3.7 Hz, 1H), 7.54 (dd, J = 14.1, 7.9 Hz, 1H), 7.34 (d, J = 3.0 Hz, 1H), 7.24 ppm (td, J = 8.5, 1.8 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.8 (d, J_{CF} = 180 Hz), 161.5, 156.2, 155.8, 144.2, 142.4 (d, J_{CF} = 15.3 Hz), 142.0, 130.9, 121.0, 120.6 (d, J_{CF} = 8.6 Hz), 116.0, 115.9, 111.6 (d, J_{CF} = 24.0 Hz), 109.6 ppm (d, J_{CF} = 19.8 Hz); HRMS (ESI): *m/z* calcd for $[C_{14}H_8FN_3O_4S+H]^+$: 334.0292, found 334.0303.

***N*-(5-Nitrothiazol-2-yl)-5-(3-(trifluoromethyl)phenyl)furan-2-carboxamide (68)**: Method B with DMAP (cat.) yielded compound **68**

(79 mg, 46%) as a pale yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.82 (s, 1H), 8.72 (s, 1H), 8.35 (s, 1H), 8.32 (s, 1H), 7.82–7.67 (m, 3H), 7.50 ppm (d, J = 3.8 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.1, 156.3, 155.5, 144.5, 142.6, 142.1, 130.2, 129.7, 128.8, 125.6 (q, J_{CF} = 3.9 Hz), 124.0 (q, J_{CF} = 271 Hz), 121.3 (q, J_{CF} = 3.8 Hz), 120.8, 110.2 ppm; HRMS (ESI): *m/z* calcd for $[C_{15}H_8F_3N_3O_4S+H]^+$: 384.0260, found 384.0275.

5-(3-Nitrophenyl)-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (69): Method B yielded compound **69** (31 mg, 20%) as a bright yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.83 (br s, 1H), 8.75 (s, 1H), 8.67 (s, 1H), 8.41 (d, J = 7.8 Hz, 1H), 8.23–8.19 (m, 1H), 7.76 (t, J = 8.0 Hz, 1H), 7.72 (d, J = 3.8 Hz, 1H), 7.51 ppm (d, J = 3.7 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.1, 156.2, 154.7, 148.4, 144.7, 142.5, 142.1, 131.0, 130.6, 130.2, 123.6, 120.7, 119.1, 110.7 ppm; HRMS (ESI): *m/z* calcd for $[C_{14}H_8N_4O_6S+H]^+$: 361.0237, found 361.0241.

***N*-(5-Nitrothiazol-2-yl)-2,3′-bifuran-5-carboxamide (70)**: Method D yielded the intermediate Suzuki coupling product (133 mg, 95%) as a pale yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.22 (s, 1H), 7.84–7.77 (m, 1H), 7.36 (d, J = 3.6 Hz, 1H), 6.92–6.89 (m, 1H), 6.85 (d, J = 3.6 Hz, 1H), 3.81 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (92 mg, 99%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.08 (br s, 1H), 8.22–8.16 (m, 1H), 7.77 (t, J = 1.7 Hz, 1H), 7.27 (d, J = 3.5 Hz, 1H), 6.88 (dd, J = 1.9, 0.8 Hz, 1H), 6.80 ppm (d, J = 3.5 Hz, 1H). Method B yielded compound **70** (42 mg, 32%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.55 (s, 1H), 8.66 (s, 1H), 8.33–8.28 (m, 1H), 7.82 (d, J = 1.5 Hz, 1H), 7.73 (d, J = 3.7 Hz, 1H), 6.99 (d, J = 0.6 Hz, 1H), 6.92 ppm (d, J = 3.7 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.2, 156.1, 152.1, 144.8, 143.0, 142.6, 142.0, 141.1, 120.5, 116.5, 108.4, 108.0 ppm; HRMS (ESI): *m/z* calcd for $[C_{12}H_7N_3O_5S+H]^+$: 306.0179, found 306.0189.

***N*-(5-Nitrothiazol-2-yl)-5-(thiophen-3-yl)furan-2-carboxamide (71)**: Method D yielded the intermediate Suzuki coupling product (128 mg, 84%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.97 (dd, J = 2.9, 1.2 Hz, 1H), 7.70 (dd, J = 5.1, 2.9 Hz, 1H), 7.54 (dd, J = 5.1, 1.2 Hz, 1H), 7.40 (d, J = 3.6 Hz, 1H), 6.99 (d, J = 3.6 Hz, 1H), 3.83 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (92 mg, 99%) as a pink solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.08 (br s, 1H), 7.93 (dd, J = 2.9, 1.3 Hz, 1H), 7.69 (dd, J = 5.1, 2.9 Hz, 1H), 7.52 (dd, J = 5.1, 1.3 Hz, 1H), 7.29 (d, J = 3.6 Hz, 1H), 6.94 ppm (d, J = 3.6 Hz, 1H). Method B yielded compound **71** (23 mg, 19%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.66 (br s, 1H), 8.71 (s, 1H), 8.17 (dd, J = 2.9, 1.2 Hz, 1H), 7.74 (d, J = 3.7 Hz, 1H), 7.72 (dd, J = 5.0, 2.9 Hz, 1H), 7.67 (dd, J = 5.0, 1.2 Hz, 1H), 7.07 ppm (d, J = 3.7 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.3, 156.4, 154.5, 143.1, 142.8, 142.0, 130.7, 128.0, 125.3, 123.6, 120.8, 108.2 ppm; HRMS (ESI): *m/z* calcd for $[C_{12}H_7N_3O_4S_2+H]^+$: 321.9951, found 321.9953.

***N*-(5-Nitrothiazol-2-yl)benzofuran-2-carboxamide (72)**: Method C followed by Method A yielded compound **72** (57 mg, 32%) as a light orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.71 (s, 1H), 8.16 (s, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.61–7.49 (m, 1H), 7.39 ppm (t, J = 7.6 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.0, 157.7, 155.1, 145.9, 142.5, 142.1, 128.4, 126.7, 124.3, 123.6, 113.9, 112.2 ppm; HRMS (ESI): *m/z* calcd for $[C_{12}H_7N_3O_4S+H]^+$: 290.0230, found 290.0241.

5-Nitro-*N*-(5-nitrothiazol-2-yl)benzofuran-2-carboxamide (73): Method B yielded compound **73** (16 mg, 10%) as a light orange solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.91 (d, J = 2.4 Hz, 1H), 8.75 (s, 1H), 8.39 (dd, J = 9.2, 2.4 Hz, 1H), 8.32 (s, 1H), 8.01 ppm (d, J = 9.2 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.6, 157.5, 144.4, 142.4, 127.4, 123.3, 120.5, 114.1, 113.3, 95.8, 91.6 ppm; HRMS (ESI): *m/z* calcd for $[C_{12}H_6N_4O_6S+H]^+$: 335.0081, found 335.0088.

***N*-(5-Nitrothiazol-2-yl)thiophene-3-carboxamide (74)**: Method B yielded compound **74** (76 mg, 38%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.43 (br s, 1H), 8.75–8.68 (m, 1H), 8.65 (s, 1H), 7.75–7.72 (m, 1H), 7.72–7.67 ppm (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.4, 161.2, 142.6, 142.0, 133.7, 133.4, 127.9, 127.2 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_5N_3O_3S_2+H]^+$: 255.9845, found 255.9848.

2,5-Dichloro-*N*-(5-nitrothiazol-2-yl)thiophene-3-carboxamide (75): Method B yielded compound **75** (109 mg, 66%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.47 (br s, 1H), 8.69 (s, 1H), 7.72 ppm (d, J = 0.7 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 161.7, 159.8, 142.4, 132.9, 129.4, 126.9, 125.6 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_3Cl_2N_3O_3S_2+H]^+$: 323.9066, found 323.9074.

***N*-(5-Nitrothiazol-2-yl)thiophene-2-carboxamide (76)**: Method A yielded compound **76** (85 mg, 36%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.70 (br s, 1H), 8.72 (s, 1H), 8.32 (dd, J = 3.8, 1.1 Hz, 1H), 8.09 (dd, J = 5.0, 1.1 Hz, 1H), 7.30 ppm (dd, J = 5.0, 3.9 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.5, 160.8, 142.6, 142.0, 135.7, 135.3, 132.4, 128.9 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_5N_3O_3S_2+H]^+$: 255.9845, found 255.9846.

5-Chloro-*N*-(5-nitrothiazol-2-yl)thiophene-2-carboxamide (77): Method B yielded compound **77** (122 mg, 69%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.79 (s, 1H), 8.71 (s, 1H), 8.17 (d, J = 4.2 Hz, 1H), 7.35 ppm (d, J = 4.2 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.4, 160.0, 142.3, 142.0, 137.1, 134.9, 132.4, 129.1 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_4ClN_3O_3S_2+H]^+$: 289.9455, found 289.9465.

3-Chloro-*N*-(5-nitrothiazol-2-yl)thiophene-2-carboxamide (78): Method B yielded compound **78** (133 mg, 75%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.74 (s, 1H), 8.02 (d, J = 5.2 Hz, 1H), 7.25 ppm (d, J = 5.2 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.0, 161.9, 140.5, 139.9, 132.3, 129.9, 129.0, 128.0 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_4ClN_3O_3S_2+H]^+$: 289.9455, found 289.9467.

5-Bromo-*N*-(5-nitrothiazol-2-yl)thiophene-2-carboxamide (79): Method B yielded compound **79** (128 mg, 75%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.77 (br s, 1H), 8.71 (s, 1H), 8.11 (d, J = 4.1 Hz, 1H), 7.44 ppm (d, J = 4.1 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.3, 159.9, 142.3, 142.0, 137.5, 133.1, 132.5, 121.4 ppm; HRMS (ESI): *m/z* calcd for $[C_8H_4BrN_3O_3S_2+H]^+$: 333.8950, found 333.8959.

***N*-(5-Nitrothiazol-2-yl)-5-phenylthiophene-2-carboxamide (80)**: Method D yielded the intermediate Suzuki coupling product (92 mg, 94%) as a white solid:[39] ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.81 (d, J = 3.9 Hz, 1H), 7.78–7.69 (m, 2H), 7.62 (d, J = 4.0 Hz, 1H), 7.52–7.36 (m, 3H), 3.84 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (74 mg, 95%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.17 (br s, 1H), 7.78–7.69 (m, 3H), 7.58 (d, J = 3.9 Hz, 1H), 7.49–7.43 (m, 2H), 7.42–7.37 ppm (m, 1H). Method B yielded compound **80** (68 mg, 57%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.73 (br s, 1H), 8.72 (s, 1H), 8.32 (d, J = 4.1 Hz, 1H), 7.84–7.76 (m, 2H), 7.71 (d, J = 4.1 Hz, 1H), 7.61–7.29 ppm (m, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.6, 160.7, 151.6, 142.7, 142.0, 134.5, 133.5, 132.5, 129.4, 126.11, 125.3 ppm; HRMS (ESI): *m/z* calcd for $[C_{14}H_9N_3O_3S_2+H]^+$: 332.0158, found 332.0161.

5-(3-Chlorophenyl)-*N*-(5-nitrothiazol-2-yl)thiophene-2-carboxamide (81): Method D yielded the intermediate Suzuki coupling product (165 mg, 96%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.86 (t, J = 1.8 Hz, 1H), 7.82 (d, J = 4.0 Hz, 1H), 7.74–7.67 (m, 2H), 7.50–7.44 (m, 2H), 3.84 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (88 mg, 93%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.26 (br s, 1H), 7.83 (t, J = 1.8 Hz, 1H), 7.73 (d, J = 3.9 Hz, 1H), 7.70–7.65 (m, 2H), 7.51–7.41 ppm (m, 2H). Method B yielded compound **81** (28 mg, 33%) as a bright yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.73 (br s, 1H), 8.69 (s, 1H), 8.28 (d, J = 4.0 Hz, 1H), 7.85 (s, 1H), 7.77 (d, J = 4.0 Hz, 1H), 7.75–7.63 (m, 1H), 7.54–7.41 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 162.7, 160.8, 149.4, 142.7, 141.8, 135.6, 134.5, 134.1, 133.3, 131.2, 128.9, 126.3, 125.5, 124.8 ppm; HRMS (ESI): m/z calcd for $[C_{14}H_8ClN_3O_3S_2+H]^+$: 365.9768, found 365.9764.

***N*-(5-Nitrothiazol-2-yl)-2,3′-bithiophene-5-carboxamide (82)**: Method D yielded the intermediate Suzuki coupling product (145 mg, 95%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.99 (dd, J = 2.9, 1.3 Hz, 1H), 7.77 (d, J = 3.9 Hz, 1H), 7.70 (dd, J = 5.0, 2.9 Hz, 1H), 7.53 (dd, J = 5.0, 1.3 Hz, 1H), 7.50 (d, J = 3.9 Hz, 1H), 3.83 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (90 mg, 96%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.10 (br s, 1H), 7.95 (dd, J = 2.9, 1.3 Hz, 1H), 7.70–7.67 (m, 2H), 7.51 (dd, J = 5.0, 1.3 Hz, 1H), 7.45 ppm (d, J = 3.8 Hz, 1H). Method B yielded compound **82** (44 mg, 37%) as a yellow solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.65 (br s, 1H), 8.68 (s, 1H), 8.25 (d, J = 4.1 Hz, 1H), 8.01 (dd, J = 2.9, 1.3 Hz, 1H), 7.70 (dd, J = 5.0, 2.9 Hz, 1H), 7.55 (d, J = 4.0 Hz, 1H), 7.53 ppm (dd, J = 5.0, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.7, 160.7, 146.6, 142.7, 141.8, 133.9, 133.4, 133.3, 128.2, 126.0, 125.2, 123.3 ppm; HRMS (ESI): m/z calcd for $[C_{12}H_7N_3O_3S_3+H]^+$: 337.9722, found 337.9729.

5-(Furan-3-yl)-*N*-(5-nitrothiazol-2-yl)thiophene-2-carboxamide (83): Method D yielded the intermediate Suzuki coupling product (123 mg, 87%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.30–8.26 (m, 1H), 7.80 (t, J = 1.7 Hz, 1H), 7.76 (d, J = 3.9 Hz, 1H), 7.39 (d, J = 3.9 Hz, 1H), 6.93 (dd, J = 1.9, 0.9 Hz, 1H), 3.82 ppm (s, 3H). Method E yielded the intermediate carboxylic acid (91 mg, 98%) as a white solid: ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 13.08 (br s, 1H), 8.25 (s, 1H), 7.81–7.76 (m, 1H), 7.69–7.65 (m, 1H), 7.35 (d, J = 3.8 Hz, 1H), 6.94–6.90 ppm (m, 1H). Method B yielded compound **83** (26 mg, 21%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.66 (br s, 1H), 8.71 (s, 1H), 8.33 (s, 1H), 8.26 (d, J = 4.1 Hz, 1H), 7.82 (s, 1H), 7.47 (d, J = 4.0 Hz, 1H), 6.96 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.5, 160.6, 145.0, 143.1, 142.6, 141.9, 140.7, 133.2, 133.0, 125.3, 119.5, 109.0 ppm; HRMS (ESI): m/z calcd for $[C_{12}H_7N_3O_4S_2+H]^+$: 321.9951, found 321.9960.

***N*-(5-Nitrothiazol-2-yl)benzo[*b*]thiophene-2-carboxamide (84)**: Method A yielded compound **84** (39 mg, 25%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.97 (s, 1H), 8.75 (s, 1H), 8.67 (s, 1H), 8.12 (d, J = 7.9 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.62–7.54 (m, 1H), 7.54–7.45 ppm (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 162.3, 161.7, 142.5, 142.0, 141.4, 138.8, 135.7, 129.5, 127.6, 126.3, 125.5, 123.1 ppm; HRMS (ESI): m/z calcd for $[C_{12}H_7N_3O_3S_2+H]^+$: 306.0002, found 306.0011.

3-Chloro-*N*-(5-nitrothiazol-2-yl)benzo[*b*]thiophene-2-carboxamide (85): Method A yielded compound **85** (79 mg, 54%) as a bright yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.84 (s, 1H), 8.17 (dd, J = 6.6, 2.0 Hz, 1H), 7.98 (dd, J = 6.1, 1.9 Hz, 1H), 7.69–7.60 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.8, 163.6, 139.0, 137.7, 136.1, 128.4, 126.2, 125.9, 123.8, 123.6, 123.0 ppm; HRMS (ESI): m/z calcd for $[C_{12}H_6ClN_3O_3S_2+H]^+$: 339.9612, found 339.9616.

3-Chloro-6-fluoro-*N*-(5-nitrothiazol-2-yl)benzo[*b*]thiophene-2-carboxamide (86): Method A yielded compound **86** (69 mg, 48%) as a light yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.83 (s, 1H), 8.08 (dd, J = 9.1, 2.2 Hz, 1H), 7.97 (dd, J = 9.0, 5.1 Hz, 1H), 7.48 ppm (td, J = 9.0, 2.3 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.9, 163.5, 160.3, 139.6, 139.1, 138.4, 133.1, 129.9, 125.1, 123.5, 115.5, 109.8 ppm; HRMS (ESI): m/z calcd for $[C_{12}H_5ClFN_3O_3S_2+H]^+$: 357.9518, found 357.9529.

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