INTRODUCTION

Tuberculosis (TB) is an infectious disease that continues to be a global health problem despite the availability of an effective chemotherapy. According to the World Health Organization Global TB Report for 2015, there were an estimated 10.4 million new cases of TB disease, 1.4 million deaths were reported, and an additional 0.4 million deaths resulting from HIV-positive individuals. Under the directly observed treatment short course strategy, the globally recommended chemotherapy for the treatment of drug-susceptible tuberculosis involves administration of four drugs (rifampicin, isoniazid, pyrazinamide, and ethambutol) for the first 2 months followed by 4 months of rifampicin and isoniazid treatment. The major challenges facing TB treatment include this long duration of therapy, the emergence of drug resistance to current existing drugs to remove liabilities, or to improve potency for new indications, can be a more rapid route to obtaining drug candidates.

The anti-protozoan drug Nitazoxanide (NTZ, 1) may have potential for repurposing against tuberculosis. It is approved by the U.S. Food and Drug Administration (FDA) for treatment of parasitic diseases such as cryptosporidiosis, and it exhibits antibacterial activity. In bacteria such as Helicobacter pylori, 1 inhibits pyruvate-ferrodoxin oxidoreductase, but its mode of action in Caenorhabditis elegans may involve disruption of a chloride ion channel. In M. tuberculosis, it disrupts the membrane potential, interferes with the pH homeostasis machinery, and may have other modes of action. Given this polypharmacological antibacterial activity and the fact that it is an approved drug likely optimized for physicochemical and pharmacokinetic properties with a good safety profile, 1 is an attractive starting point for structure–activity relationship (SAR) studies to explore its anti-tubercular activity.

Given the encouraging anti-tubercular reports on 1, we embarked on SAR and structure–property relationship (SPR) studies to identify activity- and property-optimized analogues of 1 for characterization of mechanism of activity and progression for development into anti-tuberculosis therapeutics.
RESULTS AND DISCUSSION

We used 1 as a lead compound to conduct a systematic structure−activity relationship study of its anti-tubercular activity. Its analogues were designed to explore activity requirements for the salicylate, amide linkage, and nitrothiazole segments. A total of 56 compounds were synthesized and evaluated. The majority of the compounds were synthesized by standard amide bond coupling of two fragments.

Figure 1. General synthesis of analogues. Reagents and conditions: (a) RCOCl, Et3N, THF or RCO2H, T3P, Et3N, MeCN, rt, 16 h; (b) RNCO, Et3N, THF; (c) RSO2Cl, Et3N, DCM, rt, 16 h; (d) RCHO, Et3SiH, trifluoroacetyl (TFA), MeCN, 80°C, 4 h.

Table 1. SAR Studies of the Salicylate Region of 1

| compound | R1       | R2       | MIC (µM) | TC50 (µM) |
|----------|----------|----------|----------|-----------|
| rifampicin |          |          | 0.0040   | >50       |
| 1(NTZ)   | AcO−     | H        | 14 ± 0   | >20       |
| 2        | HO−      | H        | 20 ± 0   | >20       |
| 3        | OGlucuron| H        | >20      | >50       |
| 4        | H−       | H        | >20      | 19        |
| 5        | H2N−     | H        | >20      | 31        |
| 6        | MeHN−    | H        | >20      | 15        |
| 7        | AcHN−    | H        | >20      | ND        |
| 8        | Et(Me)2CCO2− | H      | 1.4 ± 0.070 | 6.2 |
| 9        | Et(Me)2CCOHN− | H    | 17 ± 4.9 | 4.1 |
| 10       | t-BuOOCOHN− | H    | 17 ± 4.2 | 6.6 |
| 11       | HO−      | 5-F     | >20      | ND        |
| 12       | HO−      | 5-Me    | >20      | 12        |
| 13       | HO−      | 3-Me    | >20      | 12        |
| 14       | Et(Me)2CCO2− | 4-(N-morpholinyl) | >20 | 12 |
| 15       | HO−      | 5-OH    | >20      | 13        |
| 16       | HO2C−    | H        | >20      | >50       |
| 17       | MeO−     | H        | >20      | ND        |
| 18       | 3-(Et(Me)2CCOHN−) | N/A  | 1.4 ± 0.60 | 0.9 |
| 19       | H        | N/A      | 2.4 ± 0.0071 | 20 |
| 20       | 4-OMe    | N/A      | 5.6 ± 4.2 | 11        |
| 21       | 5-OMe−   | N/A      | 5.5 ± 2.1 | 4.3       |
| 22       | 3-iPrO−  | N/A      | 16 ± 6.4 | 8.9       |
| 23       | 3-(Et(Me)2CCO2−) | N/A  | 12 ± 1.0 | 21        |
| 24       | 3-OMe    | N/A      | >20      | 37        |
| 25       | 5-(N-morpholinyl) | N/A  | >20      | 11        |

*aCompounds were tested for activity against M. tuberculosis. MIC is the minimum concentration required to inhibit the growth of M. tuberculosis in liquid culture. MICs of active compounds are the average ± standard deviation of two independent experiments. bToxic concentration (TC50) is the concentration required to inhibit growth of Vero cells by 50%. ND = not determined. Note that compounds 1 and 2 were tested at a maximum concentration of 20 µM, all other compounds were tested at a maximum concentration of 50 µM.

Chemistry. Compounds 1−30, 32−36, and 40−53, which represent direct amide analogues of 1, were prepared using standard amide bond formation protocols by reaction of an aminothiazole with a corresponding acid or activated acid derivative. The sulfonamide derivative 54 was similarly obtained from the reaction of aminothiazole with a sulfonyl chloride. Urea analogues 31 and 37−39 were prepared by a reaction between an aminothiazole and a corresponding isocyanate under standard reaction conditions. The amine
analogues S5 and S6 were prepared by reductive alkylation of an aminothiazole with an aldehyde. The synthesis is outlined in Figure 1.

**SAR Studies and Biological Activity.** We designed, synthesized, and evaluated various analogues to probe the SAR of the salicylate region of 1. Compounds were tested for activity against *M. tuberculosis* and for cytotoxicity against the Vero cell line (Tables 1 and 2). 1 itself had a modest activity against replicating *M. tuberculosis* grown aerobically, with a minimum inhibitory concentration (MIC) of 14 μM. The tizoxanide (2) and glucuronidated (3) derivatives were not active. Starting with a deconstructive approach, the removal (4) or replacement of the phenolic acetate in 1 with various amines (5, 6), a simple amide (7), methyl ether (17), or a carboxylic acid (16) resulted in analogues with no activity. Analogues incorporating more a lipophilic ester (8), amide (9, 18), or carbamate (10) showed activity, with both compounds 8 and 18 showing a 10-fold improvement in activity. The activity was, however, accompanied by an increase in cytotoxicity, resulting in a lower selectivity index. Analogues bearing various ring substitutions on the phenolic region, such as 5-fluoro (11), 5-methyl (12), 3-methyl (13), 4-(N-morpholinyl) (14), or 5-hydroxy (15), also showed no activity. A transition to 2-pyridyl analogues exemplified by 19 also resulted in improved activity and an improved separation from cytotoxicity (SI of approximately 8). Various substitutions on the 2-pyridyl unit gave analogues with modest activity, but these were also cytotoxic (20–23). The 3-methoxy (24) and 5-(N-morpholinyl) (25) analogues were inactive. Replacement of the phenol with representative aliphatic residues like methyl (26), cyclopropyl (27), cyclohexyl (28), or 2-hydroxycyclohexyl (30) resulted in loss of activity. Other heteroaromatic replacements (30–35 and 40–44) tested were also inactive. We also prepared and tested 1-naphthyl (36), cyclohexyl (38), and 2-pyridyl (39) urea analogues. All were inactive, although the phenylurea (37) had a comparable activity to the parent compound 1.

We also explored the thiazole region of 1 for SAR information (Table 3). Removal of the nitro group at C-5 eliminated all activity. Methylation of C-4 of the thiazole (46) or replacement of the nitro group with a charged methyl ester (47) resulted in inactive compounds. Similar analogues on a 2-pyridyl amide template showed some activity, but the activity was accompanied with cytotoxicity (49, 50, and 53), whereas others (48, 51, and 52) were inactive altogether.

Finally, we explored the amide linkage between the 2 aromatic residues in 1 (Table 4). A conversion to sulfonamide (54) or to benzyl type amine (55 and S6) resulted in inactive compounds.

**Pharmacokinetic Profiling of Analogues.** NTZ is rapidly metabolized in rats and is not detectable in plasma or urine.12 We wanted to determine if our analogues had improved pharmacokinetics. We evaluated four representative compounds for their pharmacokinetic properties in rats (Table 5). The volume of distribution (Vd) was low for all compounds. Plasma clearance (CL) approached rat hepatic blood flow (Qh) for two compounds, with low oral exposures (low bioavailability) suggesting that first pass metabolism was significant (Table 5). However, plasma clearance (CL) was low for 2 compounds, translating into good bioavailability.

**Analogues of 1 Have Limited Broad Spectrum Activity.** We determined the spectrum of activity for the analogues against several bacterial species. Three active and structurally diverse analogues (8, 19, and 37) were tested

### Table 2. SAR Studies of the Salicylate Region of 1

| compound | R1 | MIC (μM) | TC50 (μM) |
|----------|----|----------|-----------|
| 26 | methyl | >20 | >50 |
| 27 | cyclopropyl | >20 | >50 |
| 28 | cyclohexyl | >20 | >50 |
| 29 | 2-(OH)cyclohexyl | >20 | 37 |
| 30 | 4-pyrimidinyl | >20 | >50 |
| 31 | 1-piperidinyl | >20 | >50 |
| 32 | 2-pyrazinyl | >20 | >50 |
| 33 | 2-thiazolyl | >20 | >50 |
| 34 | 3-pyridyl | >20 | >50 |
| 35 | 4-pyridyl | >20 | >50 |
| 36 | 1-naphthylNH– | 17 ± 3.0 | >50 |
| 37 | PhNH– | 17 ± 4.2 | 7.0 |
| 38 | cyclohexylNH– | >20 | 6.1 |
| 39 | 2-pyridylNH– | >20 | 17 |
| 40 | 2-(1-methylimidazolyl) | >20 | >50 |
| 41 | 2-oxazolyl | >20 | >50 |
| 42 | 3-isoxazolyl | >20 | >50 |
| 43 | 2-(5-methylthiazolyl) | >20 | 18 |
| 44 | benzothiazolyl | >20 | 32 |

### Table 3. SAR Studies of the Thiazole Region of 1

| compound | R1 | R2 | R3 | MIC (μM) | TC50 (μM) |
|----------|----|----|----|----------|-----------|
| 45 | H | H | OAc | >20 | >50 |
| 46 | NO2 | Me | NH | >20 | 5.2 |
| 47 | COOMe | H | OAc | >20 | >50 |
| 48 | Me | H | N/A | >20 | 12 |
| 49 | CF3 | H | N/A | 5.3 ± 0.20 | 0.8 |
| 50 | NO2 | Me | N/A | 1.5 ± 0.60 | 4.2 |
| 51 | COOH | H | N/A | >20 | >50 |
| 52 | H | 2-pyridyl | N/A | >20 | 15 |
| 53 | NO2 | 2-pyridyl | N/A | 8.5 ± 2.1 | 6.4 |

Compounds were tested for activity against *M. tuberculosis*. MIC (in μM) is the minimum concentration required to inhibit the growth of *M. tuberculosis* in liquid culture. MICs of active compounds are the average ± standard deviation of two independent experiments. "Toxic concentration (TC50) in μM is the concentration required to inhibit growth of Vero cells by 50%.

**Compounds** were tested for activity against *M. tuberculosis*. MIC (in μM) is the minimum concentration required to inhibit the growth of *M. tuberculosis* in liquid culture. MICs of active compounds are the average ± standard deviation of two independent experiments except where asterisked (N = 1). "Toxic concentration (TC50) in μM is the concentration required to inhibit growth of Vero cells by 50%.

(45) eliminated all activity. Methylation of C-4 of the thiazole (46) or replacement of the nitro group with a charged methyl ester (47) resulted in inactive compounds. Similar analogues on a 2-pyridyl amide template showed some activity, but the activity was accompanied with cytotoxicity (49, 50, and 53), whereas others (48, 51, and 52) were inactive altogether.

Finally, we explored the amide linkage between the 2 aromatic residues in 1 (Table 4). A conversion to sulfonamide (54) or to benzyl type amine (55 and S6) resulted in inactive compounds.

Pharmacokinetic Profiling of Analogues. NTZ is rapidly metabolized in rats and is not detectable in plasma or urine.12 We wanted to determine if our analogues had improved pharmacokinetics. We evaluated four representative compounds for their pharmacokinetic properties in rats (Table 5). The volume of distribution (Vd) was low for all compounds. Plasma clearance (CL) approached rat hepatic blood flow (Qh) for two compounds, with low oral exposures (low bioavailability) suggesting that first pass metabolism was significant (Table 5). However, plasma clearance (CL) was low for 2 compounds, translating into good bioavailability.

Analogues of 1 Have Limited Broad Spectrum Activity. We determined the spectrum of activity for the analogues against several bacterial species. Three active and structurally diverse analogues (8, 19, and 37) were tested
against *Mycobacterium smegmatis*, two Gram-negative species (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram-positive species (*Staphylococcus aureus* and *Bacillus subtilis*), and the yeast *Saccharomyces cerevisiae* (Table 6). The Gram-positive bacteria showed some susceptibility, with *B. subtilis* being sensitive to all three compounds, whereas *M. smegmatis*, *S. cerevisiae*, and *S. aureus* were sensitive to one compound (19 or 37, respectively). The Gram-negative bacteria were not susceptible to any of the analogues.

**Table 5. PK Parameters for Selected Analogues in the Rat**

| Compound | MW (kDa) | C log P (d) | N₅₀ (Pa) | V₅₀ (L/kg) | Cl (mL/(min kg)) | PO AUC (ng h/mL) | Bioavailability (%) |
|----------|----------|-------------|----------|------------|------------------|-----------------|--------------------|
| 2        | 265.25   | 2.42        | 113.5    | 0.698 ± 0.327 | 57.5 ± 3.3       | 167 ± 10        | 5.8 ± 0.1          |
| 4        | 249.25   | 1.8         | 93.27    | 0.18 ± 0.006   | 6.1 ± 0.5        | 17500 ± 2820    | 63.5 ± 6.9         |
| 13       | 279.28   | 2.87        | 113.5    | 0.160 ± 0.012  | 8.96 ± 0.51      | 13600 ± 3890    | 72.5 ± 18.4        |
| 28       | 255.3    | 2.34        | 93.27    | 0.68 ± 0.12    | 69.8 ± 4.3       | 418 ± 96        | 17.5 ± 4.2         |

**Table 6. Spectrum of Antibacterial Activity**

| Compound | E. coli | M. smegmatis | P. aeruginosa | B. subtilis | St. aureus | Sa. cerevisiae |
|----------|---------|--------------|---------------|-------------|------------|---------------|
| 8        | >25     | >25          | >100          | 1.0         | >50        | >100          |
| 19       | >100    | 100          | >100          | 3.1         | >100       | 12.5          |
| 37       | >100    | >100         | >100          | 50          | 50         | >100          |

**Table 4. SAR Studies of the Linker Region of 1**

| Compound | Structure | MIC  | TC50 |
|----------|-----------|------|------|
| 54       | ![Structure](image1.png) | >20  | >50  |
| 55       | ![Structure](image2.png) | >20  | >50  |
| 56       | ![Structure](image3.png) | >20  | >50  |
| Rifampicin | ![Structure](image4.png) | 0.004 | >100 |

“Compounds were tested for activity against *M. tuberculosis*. MIC (in μM) is the minimum concentration required to inhibit the growth of *M. tuberculosis* in liquid culture. MICs of active compounds are the average of two independent experiments. Toxic concentration (TC₅₀, in μM) is the concentration required to inhibit growth of Vero cells by 50%. ND = not determined.

**Table 5. PK Parameters for Selected Analogues in the Rat**

“Clearance (Cl), volume of distribution (V₅₀), area-under-the-curve (PO AUC), and bioavailability (%F) are the mean ± standard deviation of three animals. Studies were conducted in accordance with ethical guidelines.

“MIC₉⁹’s were determined by the serial dilution method on a solid medium.

positive bacteria showed some susceptibility, with *B. subtilis* being sensitive to all three compounds, whereas *M. smegmatis*, *S. cerevisiae*, and *S. aureus* were sensitive to one compound (19 or 37, respectively). The Gram-negative bacteria were not susceptible to any of the analogues.

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|----------|---------|--------------|---------------|-------------|------------|---------------|
| 8        | >25     | >25          | >100          | 1.0         | >50        | >100          |
| 19       | >100    | 100          | >100          | 3.1         | >100       | 12.5          |
| 37       | >100    | >100         | >100          | 50          | 50         | >100          |

“MIC₉⁹’s were determined by the serial dilution method on a solid medium.

Bacteria showed some susceptibility, with *B. subtilis* being sensitive to all three compounds, whereas *M. smegmatis*, *S. cerevisiae*, and *S. aureus* were sensitive to one compound (19 or 37, respectively). The Gram-negative bacteria were not susceptible to any of the analogues.

**Anallogues of 1 Have Bactericidal Activity against M. tuberculosis.** Previous work had demonstrated that 1 itself had good activity against nonreplicating or persistent *M. tuberculosis*. We wanted to determine if our analogues retained bactericidal activity. Kill kinetics for compounds 19 and 37 were determined against replicating *M. tuberculosis* (Figure 2). As seen with 1, compound 37 showed bactericidal activity (at least 3 logs kill in 21 days) at concentrations equivalent to the MIC. Compound 19 was also bactericidal, but only at concentrations at or higher than 5 times the MIC. Compound 8 was also rapidly bactericidal, resulting in a >4 log kill in 7 days at 10X MIC.

Compounds 19 and 37 were also tested for the ability to kill *M. tuberculosis* under nonreplicating conditions induced by nutrient starvation. Compounds 19 and 37 demonstrated bactericidal activity under this condition as well, even at concentrations as low as 1.25X MIC, confirming that these compounds were more active against nonreplicating bacteria (Figure 3).

**CONCLUSIONS**

We conducted an SAR assessment of three segments of 1, namely, the salicylate, the nitrothiazole, and the amide linker. The salicylate and the linker regions can accommodate modifications, but the nitrothiazole region was very sensitive to change. For example, we were unable to identify a nitro-group replacement that retained activity, except in combination with a change to the 2-pyridyl amide template.
The results of our studies demonstrate that even though the series suffers from a steep selectivity barrier with some level of SAR trending with cytotoxicity, a proper manipulation of the structure could lead to promising anti-tuberculosis agents. Nitazoxanide is essentially a prodrug antiparasitic and antiviral agent that is metabolized in humans to an active metabolite, Tizoxanide. We were able to make more stable amide analogues of 1 with retained or better activity, which enabled expanded SAR exploration.

1 is active against parasites but is generally not active against aerobic bacteria. Similarly, all of its analogues tested in this study were either inactive or weakly active against bacterial species, with the exception of B. subtilis. On the other hand, the analogues tested in this study were bactericidal against M. tuberculosis under both replicating and nonreplicating conditions, which is consistent with what has previously been published. Further studies focused on understanding the mechanisms of action of this compound class in multiple organisms could shed light on why 1 and its analogues are active against parasites and M. tuberculosis but not against many other bacteria. It is worth noting that the compound class of thiazolides has been reported as active against a wide variety of helminth, protozoan parasites, anaerobic bacteria, and viruses. In the SAR for these activities, the nitro group is a prerequisite for efficient activity against extracellular but not intracellular parasites.

## MATERIALS AND METHODS

### Determination of Minimum Inhibitory Concentration

MICs were determined against M. tuberculosis H37Rv (London Pride) grown in Middlebrook 7H9 medium containing 10% v/v OADC (oleic acid, albumin, dextrose, catalase) supplement (Becton Dickinson) and 0.05% w/v Tween 80 (7H9-Tw-OADC) under aerobic conditions as previously described. Bacterial growth was measured after 5 days of incubation at 37 °C. The MIC was defined as the minimum concentration at which growth was completely inhibited, and was calculated from the inflection point of the fitted curve to the lower asymptote (zero growth).

**Cytotoxicity Assay.** The Vero cell line (ATCC CRL-1587) was grown in Dulbecco’s modified Eagle’s medium, high glucose, GlutaMAX (Invitrogen), 10% fetal bovine serum, and 1× penicillin–streptomycin solution (100 U/mL). Compounds were solubilized in dimethyl sulfoxide (DMSO) and assayed as a 10-point three-fold serial dilution. Compounds were incubated with cells for 2 days at 37 °C, 5% CO₂. CellTiter-Glo Reagent (Promega) was added, and the relative luminescent units were measured. Inhibition curves were fitted using the Levenberg–Marquardt algorithm; TC₅₀ was calculated as the compound concentration giving 50% inhibition of growth. Note that compounds 1 and 2 were tested at a maximum concentration of 20 μM, all other compounds were tested at a maximum concentration of 50 μM.

**Kill Kinetics in Replicating Conditions.** A late log phase culture of M. tuberculosis was adjusted to an OD₅₉₀ of 0.1 in 7H9-Tw-OADC, and 50 μL was used to inoculate 5 mL of 7H9-Tw-OADC containing compounds at indicated concentrations with a final DMSO concentration of 2%. Cultures were incubated standing at 37 °C, and CFUs were determined by serial dilution and plating.

**Kill Kinetics under Starvation.** Late log phase bacterial cultures were grown in 7H9-Tw-OADC, harvested, and resuspended at an OD₅₉₀ of 0.1 in PBS + 0.05% w/v tyloxapol. Cultures were incubated at 37 °C for 14 days before the compound was added at indicated concentrations (final DMSO concentration of 2%). Cultures were incubated standing at 37 °C, and CFUs were determined by serial dilution and plating.

**Spectrum.** MICs were determined using the serial dilution agar method. S. aureus RN4220 and E. coli DH5α were grown on LB agar, M. smegmatis mc²155 was grown on Middlebrook 7H10 agar plates plus 10% v/v OADC supplement, P. aeruginosa BAA 47 PA0 was cultured on Tryptic Soy Agar
plates, B. subtilis Marburg was cultured on nutrient agar plates, and S. cerevisiae Y187 was cultured on YPD agar plates. In each case, 10⁵ CFU/mL of late log phase culture was plated and incubated at 37 °C until large colonies formed on the no compound control plates. The lowest concentration of compound that had less than 1% growth was reported as the MIC₉₉.

**Compound Synthesis.** ³H and ¹³C NMR spectral data were recorded in CDCl₃ or DMSO-d₆ on a 300 or 400 MHz Bruker NMR spectrometer. Column chromatography was conducted on a Revelarish flash chromatography system. Reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. HPLC analysis was conducted on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; Phenomenex-Luna-C18, 4.8 mm × 150 mm, 5 μm, 1.0 mL/min, UV 254 nm, room temperature) with MeCN/H₂O (0.05% TFA or HCOOH buffer) gradient elution. HPLC-MS was performed on a Gilson 321 HPLC with detection performed by a Gilson 170 DAD and a Finnigan AQA mass spectrometer operating in electrospray ionization mode using a Phenomenex Gemini C18 150 × 4.6 mm column. Compound purity was determined using an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; Phenomenex-Luna-C18, 4.8 mm × 150 mm, 5 μm, 1.0 mL/min, UV 254 nm, room temperature) with MeCN/H₂O (0.05% TFA or HCOOH buffer) gradient elution. All compounds were >95% pure via LC/MS analysis. Compounds 1–3 and 47 were a generous donation from our collaborators, Lilly Research laboratories. 1–3, 26, and 27 are also commercially available. We acquired NMR and LCMS data to confirm a match to literature reports.

**2-(5-Nitrothiazol-2-ylcarbamoyl)phenyl Acetate (1).** ¹H NMR (400 MHz, DMSO-d₆): δ 13.71–13.69 (m, 1H), 8.67 (s, 1H), 7.83–7.81 (m, 1H), 7.66 (td, J = 7.8, 1.4 Hz, 1H), 7.42 (td, J = 7.6, 0.9 Hz, 1H), 7.30 (dd, J = 0.7, 8.2 Hz, 1H), 2.22 (s, 3H).

**2-Hydroxy-N-(5-nitrothiazol-2-yl)benzamide (2).** ¹H NMR (400 MHz, DMSO-d₆): δ 8.67 (s, 1H), 7.89–7.87 (m, 1H), 7.51–7.46 (m, 1H), 7.04–6.98 (m, 2H). LCMS (ESMS) calcd for C₁₅H₁₀N₄O₃:S: 306.80; found: 306.80.

**Synthesis of 2-(Amino-N-(5-nitrothiazol-2-yl)benzamide (5).** To a solution of isoanhydride (1 g, 6.13 mmol) in DMF (5 mL) in a 20 mL vial, 2-aminonitrothiazole (0.89 g, 6.13 mmol) and trimethylamine (1.86 g, 18.39 mmol, 3 equiv) were added. The vial was capped and heated to 80 °C under reflux. The reaction was monitored by LCMS analysis until completion (12 h). The reaction mixture was poured into a separatory funnel, diluted with ethyl acetate (300 mL), and washed with 1.0 N aqueous HCl (2 × 200 mL) and then with brine (200 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a brown product. This crude material was recrystallized from ethyl acetate–hexanes to afford 2-amino-N-(5-nitrothiazol-2-yl)benzamide (5) as an off-white solid (634 mg, 39% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 9.04–8.62 (brs, 2H), 7.95 (s, 1H), 7.92 (d, J = 8 Hz, 1H), 7.26 (t, J = 9.0 Hz, 1H), 6.81 (d, J = 9.0 Hz, 1H), 6.59 (t, J = 8 Hz, 1H). LCMS m/z (M + H) 265.03.

**Synthesis of 2-(Methylamino)-N-(5-nitrothiazol-2-yl)benzamide (6).** To a solution of 5-nitrothiazol-2-amine (820 mg, 6.64 mmol) and N-methylisocyanide (1 g, 5.64 mmol) in DMF (10 mL) at 0 °C, trimethylamine (1.72 g, 16.92 mmol) was added. The reaction mixture was heated to reflux and monitored by LCMS analysis until completion (12 h). The reaction mixture was poured into a separating funnel, diluted with ethyl acetate (300 mL), and washed with 1.0 N aqueous HCl (2 × 200 mL) and then with brine (200 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a brown product. This crude material was recrystallized from acetone to afford 2-(methylamino)-N-(5-nitrothiazol-2-yl)benzamide (6) as an off-white solid (543 mg, 35% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 10.41 (brs, 1H), 8.70 (s, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.45 (t, J = 9.0 Hz, 1H), 6.76 (d, J = 9.0 Hz, 1H), 6.64 (t, J = 8 Hz, 1H), 2.87 (s, 3H). LCMS m/z (M + H) 279.10.

**Synthesis of 2-Acetamido-N-(5-nitrothiazol-2-yl)benzamide (7).** A suspension of N-acetyl anthranilic acid (400 mg, 2.23 mmol), 5-nitrothiazol-2-amine (320 mg, 2.23 mmol), hydroxybenzotiazole (HOBt, 300 mg, 2.23 mmol), and triethylamine (680 mg, 6.69 mmol, 3 equiv) in DMF (25 mL) was treated with 1-[3(dimethylamino)propyl]-3-ethylcarbodiimide-HCl (EDC, 430 mg, 2.23 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. The reaction was monitored by LCMS for completion. The reaction mixture was poured into water (60 mL), extracted with ethyl acetate (2 × 100 mL), and the combined organics washed with brine (100 mL) then dried over anhydrous Na₂SO₄ to give a crude product that was purified by Gilson HPLC to give 2-acetamido-N-(5-nitrothiazol-2-yl)benzamide (7) as an off-white solid (307.4 mg, 45% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 10.21 (s, 1H), 8.69 (s, 1H), 7.62–7.75 (m, 2H), 7.57 (td, J = 7.6, 1.4 Hz, 1H), 7.26 (td, J = 7.6, 1.4 Hz, 1H), 2.04 (s, 3H). LCMS m/z (M + H) 306.80.

**Synthesis of 2-((5-Nitrothiazol-2-yl)carbamoyl)phenyl 2,2-Dimethylbutanoate (8).** Synthesis of 2-((2,2-dimethylbutanoyl)oxy)benzoic acid: To a solution of 2-hydroxybenzoic acid (1.0 g, 7.2 mmol) in pyridine (15 mL) at 0 °C under a N₂ atmosphere, 2,2-dimethylbutanoyl chloride (1.0 g, 7.9 mmol) was added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis
(EtOAc/hexane = 1:1) until completion (68 h). The reaction mixture was poured into ice cooled water (80 mL) and extracted with EtOAc (3 × 60 mL). The combined organic layer was washed with 1 N HCl solution (3 × 50 mL). It was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was washed with p-nentane to afford 2-(2-(2,2-dimethylbutanoyl)oxy)benzoic acid as a white solid (1.2 g, 71%). 1H NMR (400 MHz, CDCl3): δ 8.09 (d, J = 7.6 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 1.73 (q, J = 7.6 Hz, 2H), 1.33 (s, 6H), 0.99 (t, J = 7.6 Hz, 3H). 13C NMR (100 MHz, DMSO-d6): δ 175.745, 165.843, 148.855, 143.087, 142.570, 133.674, 133.674, 126.799, 126.434, 123.692, 126.434, 126.799, 124.706, 33.011, 24.706, 9.460. LCMS m/z (M + H) 235.10.

To a solution of 2-((2,2-dimethylbutanoyl)oxy)benzoic acid (400 mg, 1.6 mmol) and 5-nitrothiazol-2-amine (254 mg, 1.6 mmol) in DCM (20 mL) at 0 °C under a N2 atmosphere, Et3N (0.5 mL, 3.2 mmol) and 2-chloro-1-methylpyridinium iodide (408 mg, 1.6 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (2 h). The reaction mixture was poured into ice cooled water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60-120 mesh) using 1% MeOH in DCM as eluent to afford 80% that was used as such for the next step without any further purification. LCMS m/z (M + H) 364.0967; found: 364.0943 (M + 1).

Synthesis of tert-butyl (2-((5-nitrothiazol-2-yl)carbamoyl)phenyl)carbamate (10). To a solution of 2-aminobenzoic acid (0.5 g, 3.7 mmol) in DMF (10 mL) at 0 °C, Et3N (4.1 mmol) in DCM (20 mL) at 0 °C was added, and the reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (16 h). The pH of the reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (16 h). The reaction mixture was poured into ice cooled water (100 mL) and extracted with EtOAc (4 × 50 mL). The combined organic layer was washed with saturated NaHCO3 solution (2 × 25 mL), dried over anhydrous Na2SO4, and concentrated under reduced pressure. This crude material was purified by Prep-HPLC purification. This afforded tert-butyl (2-((5-nitrothiazol-2-yl)carbamoyl)phenyl)carbamate as an off-white solid (0.15 g, 18%). 1H NMR (400 MHz, DMSO-d6): δ 13.49 (brs, 1H), 9.69 (s, 1H), 8.69 (s, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 1.39 (s, 9H). LCMS m/z (M + H) 365.35.

Synthesis of 2-(2,2-dimethylbutanamido)-N-(5-nitrothiazol-2-yl)benzamide: To a solution of tert-butyl 2-((5-nitrothiazol-2-yl)carbamoyl)phenyl)carbamate (100 mg, 0.3 mmol) in DCM (5 mL) at 0 °C, 4 N HCl in 1,4-dioxan (5 mL) was added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (2 h). The reaction mixture was concentrated under reduced pressure to afford 2-amino-N-(5-nitrothiazol-2-yl)benzamide as a white solid (0.1 g, crude) that was used as such for the next step without any further purification. LCMS m/z (M + H) 265.03.

To a solution of 2-amino-N-(5-nitrothiazol-2-yl)benzamide (100 mg, 0.4 mmol) and Et3N (0.1 mL, 0.8 mmol) in ACN (5 mL) at 0 °C, 2,2-dimethylbutanoyl chloride (101 mg, 0.8 mmol) was added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (16 h). The reaction mixture was poured into ice cooled water (100 mL) and extracted with EtOAc (4 × 50 mL). The combined organic layer was washed with saturated NaHCO3 solution (2 × 25 mL), dried over anhydrous Na2SO4, and concentrated under reduced pressure. This crude material was purified by Prep-HPLC purification. This afforded 9 as an off-white solid (0.02 g, 4%). 1H NMR (400 MHz, DMSO-d6): δ 13.58 (brs, 1H), 10.89 (brs, 1H), 8.68 (s, 1H), 8.16 (m, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 2.53 (m, 1H), 1.62 (m, 2H), 1.23 (s, 6H), 0.80 (t, J = 7.6 Hz, 3H). LCMS m/z (M + H) 361.33.

Synthesis of tert-Butyl 2-((5-nitrothiazol-2-yl)carbamoyl)phenyl)carbamate (10). To a solution of 2-amino benzoic acid (0.5 g, 3.7 mmol) in THF/H2O (20 mL, 1:1), 2 N NaOH (20 mL) was added to make pH 12. To this mixture, (Boc)2O (0.9 g, 4.1 mmol) was added, and the reaction mixture was then stirred at room temperature while monitoring by TLC analysis (MeOH/DCM = 1:19) until completion (16 h). The pH of the reaction mixture was adjusted to ~5 by adding 30% aqueous citric acid, and then extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. This afforded tert-butoxycarbonyl)amino)benzoic acid as a white solid (0.7 g, 80%) that was used as such for the next step without any further purification. 1H NMR (400 MHz, CDCl3): δ 10.02 (s, 1H), 8.47 (d, J = 8.8 Hz, 1H), 8.10 (d, J = 6.8 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.04 (t, J = 7.6 Hz, 1H), 1.55 (s, 9H). LCMS m/z (M + H) 236.18.

To a solution of 5-nitrothiazol-2-amine (270 mg, 1.9 mmol) and 2-((tert-butoxycarbonyl)amino)benzoic acid (300 mg, 1.3 mmol) in DMF (10 mL) at 0 °C, EDCI-HCl (485 mg, 2.5 mmol), HOBT (432 mg, 3.2 mmol), DIPEA (0.7 mL, 3.9 mmol), and DMAP (10 mg, cat. amount) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (18 h). The reaction mixture was poured into ice cooled water (100 mL) and extracted with EtOAc (4 × 50 mL). The combined organic layer was washed with saturated NaHCO3 solution (2 × 25 mL), dried over anhydrous Na2SO4, and concentrated under reduced pressure. This crude material was purified by Prep-HPLC purification. This afforded tert-butyl (2-((5-nitrothiazol-2-yl)carbamoyl)phenyl)carbamate as an off-white solid (0.15 g, 33%). 1H NMR (400 MHz, DMSO-d6): δ 10.02 (s, 1H), 8.47 (d, J = 8.8 Hz, 1H), 8.10 (d, J = 6.8 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.04 (t, J = 7.6 Hz, 1H), 1.55 (s, 9H). LCMS m/z (M + H) 364.0967; found: 364.0943 (M + 1).
with saturated NaHCO₃ solution (2 × 25 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. This crude material was purified by Prep-HPLC purification. This afforded tert-butyl (2-((5-nitrothiazol-2-yl)carbamoyl)-phenyl)carbamate (10) as an off-white solid (0.15 g, 33%). ¹H NMR (400 MHz, DMSO-d₆): δ 13.49 (brs, 1H), 9.69 (s, 1H), 6.89 (s, 1H), 7.77 (dd, J = 7.6 Hz, 1H), 7.70 (dt, J = 8.0 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 1.39 (s, 9H). LCMS m/z (M + H) 365.35; HRMS (ESMS) calcd for C₁₉H₁₈N₄O₅S: 365.092; found: 363.0760 (M + 1).

Synthesis of 5-Fluoro-2-hydroxy-(5-nitrothiazol-2-yl)benzamide (11). A mixture of 5-fluoro-2-hydroxybenzoic acid (1.0 g, 6.4 mmol), anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 50% EtOAc in hexane as eluent to a white solid (0.43 g, 25%) that was used as such for the next step without any further purification.

To a solution of 5-nitrothiazol-2-amine (550 mg, 3.8 mmol) and 4-bromo-2-(2,2-dimethylbutanoyl)oxy)benzoic acid (1.2 g, 3.8 mmol) in DMF (12 mL) at 0 °C, TBTU (1.8 g, 5.7 mmol) and Et₃N (1.6 mL, 11.4 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc) until completion (18 h). The reaction mixture was poured into ice cooled water (100 mL) and extracted with DCM (4 × 50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. This afforded 4-bromo-2-((2,2-dimethylbutanoyl)oxy)benzoic acid as a brown oil (1.2 g, 75%) that was used as such for the next step without any further purification. LCMS m/z (M + H) 441.84.

5-Bromo-2-((5-nitrothiazol-2-yl)carbamoyl)phenyl 2,2-dimethylybutanoate (0.52 g, 1.2 mmol) was added to DMF (8 mL) in a 100 mL round bottom flask under a N₂ atmosphere. To this mixture, morpholine (0.3 mL, 2.4 mmol), Pd₃dba (0.11 g, 0.12 mmol), S-Phos (48 mg, 0.12 mmol), and Cs₂CO₃ (1.1 g, 3.6 mmol) were sequentially added. The reaction mixture was heated at 110 °C for 4 h. It was then filtered through a sintered funnel with a pad of celite and washed with EtOAc (40 mL). The filtrate was then poured into ice water (40 g) and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using 30% EtOAc–hexane as eluent to afford 5-morpholino-2-((5-nitrothiazol-2-yl)carbamoyl)phenyl 2,2-dimethylybutanoate (14) as an off-white solid (0.2 g, 35%). ¹H NMR (400 MHz, DMSO-d₆): δ 13.20 (brs, 1H), 8.85 (s, 1H), 7.76 (dt, J = 9.2 Hz, 1H), 6.92 (dd, J = 8.8 Hz, 1H), 6.66 (s, 1H), 3.72 (dd, J = 4.8 Hz, 4H), 1.64 (m, 2H), 1.25 (s, 6H), 0.86 (t, J = 7.6 Hz, 3H). LCMS m/z (M + H) 449.18; HRMS (ESMS) calcd for C₂₀H₂₄N₄O₈S: 449.1495; found: 449.1507 (M + 1).

Synthesis of 2,5-Dihydroxy-(5-nitrothiazol-2-yl)benzamide (15). To a solution of 2,5-dimethoxybenzoic acid (1.1 g, 5.5 mmol) and 5-nitrothiazol-2-amine (796 mg, 5.5 mmol) in DMF (25 mL) at 0 °C, EDCI·HCl (3.1 g, 16.5 mmol), HOBr (1.5 g, 10 mmol), and DIPEA (2.5 mL, 13.7 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (16 h). The reaction mixture was poured into ice cooled water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic...
layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 1% MeOH in DCM as eluent to afford 2,4-dimethoxy-N-(5-nitrothiazol-2-yl)benzamide as a light yellow solid (1.0 g, 62%).

1H NMR (400 MHz, DMSO-d6): δ 12.85 (s, 1H), 8.69 (s, 1H), 7.25 (s, 1H), 7.19 (d, J = 11.6 Hz, 2H), 3.86 (s, 3H), 3.77 (s, 3H). LCMS m/z (M + H) 310.08.

To a solution of 2,4-dimethoxy-N-(5-nitrothiazol-2-yl)benzamide (120 mg, 0.4 mmol) in DCM (10 mL) at 0 °C, AlCl3 (168 mg, 1.3 mmol) was added. The reaction mixture was stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (24 h). The reaction mixture was quenched with ice cooled water (30 mL) and extracted with DCM (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (150–200 mesh) using 1% MeOH in DCM as eluent to a 1% MeOH in DCM as eluent to a nitrothiazol-2-yl)benzamide (15) as a yellow solid (30 mg, 25%).

1H NMR (400 MHz, DMSO-d6): δ 13.45 (brs, 1H), 11.32 (brs, 1H), 9.31 (s, 1H), 7.31 (d, J = 2.8 Hz, 1H), 1.36 (dd, J = 8.4, 3.2 Hz, 1H), 0.91 (d, J = 8 Hz, 1H). LCMS m/z (M – H) 279.97; HRMS (ESMS) calc for C10H10N5O4S: 282.0185; found: 282.019 (M + 1).

**Synthesis of 2-((5-Nitrothiazol-2-yl)carbamoyl)benzoic Acid (16).** To a solution of isobenzofuran-1,3-dione (1.0 g, 6.7 mmol) in EtOAc (100 mL), 5-nitrothiazol-2-amine (1.0 g, 6.7 mmol) was added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (16 h). The reaction mixture was evaporated to dryness. The crude product was further purified by preparative HPLC to afford 2-((5-nitrothiazol-2-yl)carbamoyl)benzoic acid (16) as a yellow solid (156 mg, 8%).

1H NMR (400 MHz, DMSO-d6): δ 13.45 (brs, 1H), 11.32 (brs, 1H), 9.31 (s, 1H), 7.31 (d, J = 2.8 Hz, 1H), 1.36 (dd, J = 8.4, 3.2 Hz, 1H), 0.91 (d, J = 8 Hz, 1H). LCMS m/z (M + H) 279.97; HRMS (ESMS) calc for C10H10N5O4S: 282.0185; found: 282.0186 (M + 1).
Synthesis of 4-Methoxy-N-(5-nitrothiazol-2-yl)-picolinamide (20). To a solution of 4-methoxy picolinic acid (200 mg, 1.3 mmol) and 5-nitrothiazol-2-amine (188 mg, 1.3 mmol) in DCM (10 mL) at 0 °C, HOBt (265 mg, 1.9 mmol), EDCI-HCl (299 mg, 1.5 mmol), and Et3N (0.5 mL, 3.9 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc) for 16 h. The reaction mixture was poured into ice cooled water (30 mL) and extracted with DCM (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 1% MeOH in DCM as eluent to afford 4-methoxy-N-(5-nitrothiazol-2-yl)picolinamide (20) as a yellow solid (80 mg, 22%).1H NMR (400 MHz, DMSO-d6): δ 13.34 (brs, 1H), 8.71 (s, 1H), 8.61 (d, J = 5.6 Hz, 1H), 7.74 (s, 1H), 7.33 (d, J = 3.6 Hz, 1H), 3.97 (s, 3H). LCMS m/z (M + H) 279.0; HRMS (ESMS) calcd for C9H8N4O4S: 281.0345; found: 281.0343 (M + H).

Synthesis of 5-Methoxy-N-(5-nitrothiazol-2-yl)-picolinamide (21). To a solution of 5-methoxy picolinic acid (200 mg, 1.3 mmol) and 5-nitrothiazol-2-amine (189 mg, 1.3 mmol) in DCM (10 mL) at 0 °C, HOBt (193 mg, 1.4 mmol) and DCC (281 mg, 1.4 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc) for 16 h. The reaction mixture was poured into ice cooled water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 25% EtOAc in hexane as eluent to afford 5-methoxy-N-(5-nitrothiazol-2-yl)picolinamide (21) as a yellow solid (40 mg, 13%).1H NMR (400 MHz, DMSO-d6): δ 13.28 (s, 1H), 8.69 (s, 1H), 8.27 (d, J = 4.0 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.64–7.61 (dd, J = 8.8, 4.4 Hz, 1H), 3.91 (d, J = 6.4 Hz, 1H), 2.03 (m, 1H), 0.97 (d, J = 6.8 Hz, 6H).13C NMR (100 MHz, DMSO-d6): δ 165.0, 161.8, 154.7, 143.2, 142.6, 140.9, 139.2, 128.5, 122.3, 75.0, 28.0, 19.3. LCMS m/z (M + H) 323.05; HRMS (ESMS) calcd for C13H14N4O4S: 323.0814; found: 323.0815 (M + H).

Synthesis of 3-Isobutoxy-N-(5-nitrothiazol-2-yl)-pyridinyl-2,2-dimethylbutanoate (23). To a solution of 3-hydroxy picolinic acid (342 mg, 2.5 mmol) and 5-nitrothiazol-2-amine (300 mg, 2.1 mmol) in ACN (6 mL) at 0 °C, T3P (3.3 g, 10.3 mmol) and Et3N (1.7 mL, 12.4 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:1) until completion (16 h). The reaction mixture was then poured into ice cooled water (20 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 2% MeOH in DCM as eluent to afford 3-isobutoxy-N-(5-nitrothiazol-2-yl)pyridinyl-2,2-dimethylbutanoate (23) as a brown solid (318 mg, 58%). LCMS m/z (M – H) 265.07.

To a solution of 3-hydroxy-N-(5-nitrothiazol-2-yl)-picolinamide (0.1 g, 0.7 mmol) in DMF (4 mL) at 0 °C under a N2 atmosphere, 2,2-dimethylbutanoyl chloride (0.15 mL, 1.1 mmol) and K2CO3 (170 mg, 1.5 mmol) were sequentially added. The reaction mixture was then heated at 65 °C and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (4 h). The reaction mixture was poured into ice cooled water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using 25% EtOAc in hexane as eluent to afford 2-((5-nitrothiazol-2-yl)carbamoyl)pyridin-3-yl 2,2-dimethylbutanoate (23) as a light yellow solid (126 mg, 31%).1H NMR (400 MHz, DMSO-d6): δ 13.59 (brs, 1H), 8.71 (s, 1H), 8.67 (dd, J = 4.4, 1.2 Hz, 1H), 7.87 (dd, J = 8.4, 1.2 Hz, 1H), 7.83–7.80 (m, 1H), 1.69 (m, 2H), 0.90 (t, J = 7.6 Hz, 3H). LCMS m/z (M + H) 365.17; HRMS (ESMS) calcd for C13H14N4O4S: 365.092; found: 365.0913 (M + 1).

Synthesis of 3-Methoxy-N-(5-nitrothiazol-2-yl)-picolinamide (24). To a solution of 3-methoxypicolinic acid (200 mg, 1.3 mmol) and 5-nitrothiazol-2-amine (188 mg, 1.3 mmol) in DCM (10 mL) at 0 °C, HOBt (265 mg, 1.9 mmol), EDCI-HCl (296 mg, 1.5 mmol), and Et3N (0.5 mL, 3.9 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/hexane = 1:1) until completion (3 h). The reaction mixture was quenched with a solution of citric acid (5 mL). It was extracted with EtOAc (3 × 5 mL), dried over Na2SO4, and evaporated to dryness to afford 3-isobutoxypicolinic acid as a white solid (60 mg, 65%).1H NMR (400 MHz, DMSO-d6): δ 12.94 (brs, 1H), 8.12 (d, J = 4.4 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.46 (dd, J = 8.4, 4.4 Hz, 1H), 3.84 (d, J = 6.4 Hz, 2H), 2.02 (m, 1H), 0.97 (d, J = 6.4 Hz, 1H). LCMS m/z (M + H) 196.04.

To a solution of 3-isobutoxypicolinic acid (200 mg, 1.6 mmol) and 5-nitrothiazol-2-amine (237 mg, 1.6 mmol) in DCM (2.5 mL) at 0 °C, HATU (1.0 g, 2.7 mmol) and DIPEA (0.8 mL, 4.5 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 7:10) until completion (16 h). The reaction mixture was poured into ice cooled water (50 mL) and extracted with DCM (3 × 20 mL). The combined organic layer was dried over Na2SO4 and evaporated to dryness. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 30% EtOAc in hexane as eluent to afford 3-isobutoxy-N-(5-nitrothiazol-2-yl)picolinamide (22) as an off-white solid (116 mg, 40%).1H NMR (400 MHz, DMSO-d6): δ 13.28 (s, 1H), 8.69 (s, 1H), 8.27 (d, J = 4.0 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.64–7.61 (dd, J = 8.8, 4.4 Hz, 1H), 3.91 (d, J = 6.4 Hz, 1H), 2.03 (m, 1H), 0.97 (d, J = 6.8 Hz, 6H).13C NMR (100 MHz, DMSO-d6): δ 165.0, 161.8, 154.7, 143.2, 142.6, 140.9, 139.2, 128.5, 122.3, 75.0, 28.0, 19.3. LCMS m/z (M + H) 323.05; HRMS (ESMS) calcd for C13H14N4O4S: 323.0814; found: 323.0815 (M + 1).
mmol) in DCM (10 mL) at 0 °C, HOBt (265 mg, 1.9 mmol), EDCl-HCl (299 mg, 1.5 mmol), and Et3N (0.5 mL, 3.9 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (16 h). The reaction mixture was poured into ice cooled water (30 mL) and extracted with DCM (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 1% MeOH in DCM as eluent to afford 3-methoxy-N-(5-nitrothiazol-2-yl)picolinamide (24) as a yellow solid (80 mg, 22%). 1H NMR (400 MHz, DMSO-d6): δ 13.36 (s, 1H), 8.69 (s, 1H), 8.28 (d, J = 3.6 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.66 (dd, J = 8.4, 4.4 Hz, 1H), 3.90 (s, 3H). LCMS m/z (M + H) 280.99; HRMS (ESMS) calcd for C10H9N2O2S: 281.0345; found: 281.0341 (M + 1).

**Synthesis of 5-Morpholinono-N-(5-nitrothiazol-2-yl)-picolinamide (25).** To a solution of 3-bromopicolinaldehyde (2.0 g, 10.7 mmol) in MeOH (40 mL) at 0 °C, a solution of KOH (2.0 g, 36.3 mmol) in MeOH (10 mL) and a solution of I2 (4.6 g, 18.2 mmol) in MeOH (20 mL) were sequentially added, and the reaction mixture was then stirred at 0°C for 2 h while monitoring by TLC analysis (EtOAc/hexane = 3:7). The reaction mixture was quenched with 30% sodium bisulfate solution until the disappearance of brown color and extracted with DCM (3 × 100 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. This afforded methyl 5-bromopicolinate as a brown solid (1.8 g, 78%). 1H NMR (400 MHz, DMSO-d6): δ 8.80 (s, 1H), 8.04–7.97 (m, 2H), 4.01 (s, 3H). LCMS m/z (M + H) 215.92.

To a solution of methyl 5-bromopicolinate (1.8 g, 8.4 mmol) in THF/H2O (44 mL, 3:1 ratio) at 0 °C, LiOH·H2O (2.0 g, 47.4 mmol) was added. The reaction mixture was then heated to 100 °C and monitored by TLC analysis (MeOH/DCM = 1:9) until completion (0.5 h). The reaction mixture was acidified with 1 N HCl to pH 2 and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. This afforded 3-aminopicolinic acid as a white solid (1.7 g, 100%). 1H NMR (400 MHz, DMSO-d6): δ 13.44 (s, 1H), 8.84 (d, J = 1.6 Hz, 1H), 8.24 (dd, J = 8.0, 1.6 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H). LCMS m/z (M + H) 201.84.

To a solution of 3-bromopicolinic acid (1.7 g, 8.5 mmol) and 5-nitrothiazol-2-amine (1.0 g, 6.8 mmol) in DMF (17 mL), TBTU (3.3 g, 10.2 mmol) and Et3N (2.8 mL, 20.4 mmol) were sequentially added, and the reaction mixture was stirred at room temperature while monitoring by TLC analysis (MeOH/DCM = 1:9) until completion (2 h). The reaction mixture was poured into ice cooled water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using 20% MeOH in DCM as eluent to afford N-(5-nitrothiazol-2-yl)-cyclohexane-1-carboxamide (27) as a colorless liquid (41%). 1H NMR (400 MHz, DMSO-d6): δ 7.97 (d, J = 8.8 Hz, 1H), 4.76 (m, 4H), 3.41 (m, 4H). LCMS m/z (M + H) 234.68; HRMS (ESMS) calcd for C10H13N3O3S: 234.0776; found: 234.0772 (M + 1).

**Synthesis of 2-Hydroxy-N-(5-nitrothiazol-2-yl)-cyclohexane-1-carboxamide (29).** To a solution of ethyl 2-oxo cyclohexane-1-carboxylate (1.0 g, 8.5 mmol) and 5-nitrothiazol-2-amine (1.0 g, 6.8 mmol) in DMF (17 mL), TBTU (3.3 g, 10.2 mmol) and Et3N (2.8 mL, 20.4 mmol) were sequentially added and stirred for 4 h while monitoring by TLC analysis. After completion, the mixture was diluted with EtOAc (3 × 25 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using 1% MeOH in DCM as eluent to afford N-(5-nitrothiazol-2-yl)cyclohexane-1-carboxamide (28) as a brown solid (1090 mg, 43%). 1H NMR (400 MHz, DMSO-d6): δ 12.95 (s, 1H), 8.57 (s, 1H), 2.54–2.47 (m, 1H), 1.82–1.71 (m, 6H), 1.40–1.34 (m, 2H), 1.26–1.22 (m, 3H). 13C NMR (100 MHz, DMSO-d6): δ 176.357, 162.351, 143.197, 142.154, 43.831, 28.957, 25.610, 25.370. HRMS (ESMS) calcd for C10H13N3O3S: 256.0756; found: 256.0758 (M + 1).

**Synthesis of 2-Hydroxy-N-(5-nitrothiazol-2-yl)-cyclohexane-1-carboxamide (29).** To a solution of ethyl 2-oxo cyclohexane-1-carboxylate (1.0 g, 8.5 mmol) in toluene (15 mL), ethylene glycol (540 mg, 8.8 mmol) and para toluenesulfonic acid (1.6 g, 8.8 mmol) were sequentially added, and the resulting mixture was refluxed overnight. After completion, the mixture was distilled and extracted with ethyl acetate (3 × 25 mL). The combined organic layer was washed with brine solution, dried over anhydrous Na2SO4 and concentrated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel (100–200 mesh) using 20% EtOAc in hexane as eluent to afford ethyl 1,4-dioxaspiro[4.5]-decane-6-carboxylate as a colorless liquid (500 mg, 41%). LCMS m/z (M + H) 215.31.

To a solution of ethyl 1,4-dioxaspiro[4.5]-decane-6-carboxylate (400 mg, 1.8 mmol) in THF (5 mL), a solution of sodium hydroxide (110 mg, 2.8 mmol) in water (5 mL) was added, and the reaction mixture was refluxed for 12 h. After completion, the mixture was distilled and adjusted to pH 2 using 1 N HCl. The aqueous mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic layer was washed with brine solution, dried over anhydrous Na2SO4 and concentrated under reduced pressure to obtain 1,4-dioxaspiro[4.5]-decane-6-carboxylic acid as a colorless liquid (150 mg, 45%). LCMS m/z (M + H) 186.96.
To a solution of 1,4-dioxaspiro[4.5]decane-6-carboxylic acid (150 mg, 0.7 mmol) in DCM (4 mL), 5-nitrothiazol-2-amine (150 mg, 1.05 mmol), EDCI-HCl (260 mg, 1.4 mmol), HOBT (180 mg, 1.4 mmol), and Et,N (0.4 mL) were added, and the resulting mixture was stirred at room temperature overnight. After completion, the solvent was evaporated, and the resulting crude product was purified by column chromatography on silica gel (100–200 mesh) using 20% acetone in hexane as eluent to afford N-(5-nitrothiazol-2-yl)-1,4-dioxaspiro[4.5]decane-6-carboxamide as a light yellow solid (150 mg, 71%). 1H NMR (400 MHz, DMSO-d6): δ 10.27 (s, 1H), 8.30 (s, 1H), 4.09–3.98 (m, 4H), 2.84 (m, 2H), 2.05 (m, 1H), 2.00 (m, 1H), 1.88 (m, 1H), 1.74 (m, 2H), 1.47 (m, 2H), 1.37 (m, 1H). LCMS mz (M + H): 268.07.

To a solution of 1,4-dioxaspiro[4.5]decane-6-carboxylic acid (200 mg, 0.6 mmol) in acetone (5 mL), S N HCl (5 mL) was added, and the mixture was refluxed overnight. After completion of the reaction, acetone was evaporated and the aqueous mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The resulting mixture was stirred at room temperature overnight. After completion, the reaction mixture was poured into ice cooled water (40 mL) and extracted with EtOAc. Finally, it was crystallized from MeOH to a yellow solid (60 mg, 17%). 1H NMR (400 MHz, DMSO-d6): δ 12.92 (s, 1H), 8.74 (s, 1H), 8.32 (s, 1H), 2.39 (m, 2H), 2.31 (m, 2H), 1.77 (m, 4H). LCMS mz (M + H): 268.07.

To a solution of N-(5-nitrothiazol-2-yl)-1,4-dioxaspiro[4.5]decane-6-carboxamide (200 mg, 0.7 mmol) in EtOH (10 mL) was added NaBH4 (85 mg, 2.2 mmol) at 0 °C. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19). The reaction mixture was filtered and washed several times with EtOAc (3 × 50 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by preparative TLC using 5% MeOH–DCM as eluent to afford 2-hydroxy-N-(5-nitrothiazol-2-yl)cyclohexane-1-carboxamide (29) as a light yellow solid (60 mg, 30%). 1H NMR (400 MHz, DMSO-d6): δ 12.98 (brs, 1H), 8.62 (s, 1H), 4.76 (bs, 1H), 4.19 (s, 1H), 2.67 (d, J = 10.8 Hz, 1H), 1.90–1.09 (m, 8H). LCMS mz (M + H) 270: HRMS (ESMS) calcd for C10H8N3O3S: 272.0705; found: 272.0707 (M + 1).

Synthesis of N-(5-Nitrothiazol-2-yl)pyrimidine-4-carboxamide (30). 4-Methylpyrimidine (1.0 g, 10.6 mmol) in DCM (4 mL) was added to a 100 mL round bottom flask fitted with a reflux condenser. To this, KMnO4 (1.7 g) was added, then stirred at room temperature and monitored by TLC analysis (EtOAc/DCM = 1:19). The reaction mixture was then stirred at room temperature overnight and monitored by TLC analysis (EtOAc/DCM = 1:19). The reaction mixture was poured into ice cooled water (40 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 2% MeOH in DCM as eluent to afford N-(5-nitrothiazol-2-yl)pyrimidine-4-carboxamide (30) as a gray solid (421 mg, 69%). 1H NMR (400 MHz, DMSO-d6): δ 9.48 (s, 1H), 9.19 (d, J = 5.2 Hz, 1H), 8.74 (s, 1H), 8.19 (dd, J = 5.2, 1.2 Hz, 1H). LCMS mz (M + H): 252.08; HRMS (ESMS) calcd for C6H3N3O3S: 252.0219; found: 252.0065 (M − 1).

Synthesis of N-(5-Nitrothiazol-2-yl)pyridazine-2-carboxamide (31). To a solution of pyridine-1-carboxylic acid chloride (200 mg, 1.3 mmol) and 5-nitrothiazol-2-amine (197 mg, 1.3 mmol) in DCM (4 mL) at 0 °C, Et,N (1.0 mL, 6.5 mmol) was added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/DCM = 1:4) for 16 h. The reaction mixture was poured into ice cooled water (30 mL) and extracted with DCM (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 0.5% EtOAc in DCM as eluent to afford N-(5-nitrothiazol-2-yl)pyridazine-2-carboxamide (31) as a yellow solid (58 mg, 17%). 1H NMR (400 MHz, DMSO-d6): δ 11.98 (brs, 1H), 8.56 (s, 1H), 3.51 (s, 4H), 1.58 (m, 2H), 1.50 (m, 4H). LCMS mz (M + H) 257.04; HRMS (ESMS) calcd for C3H25N2O3S: 257.0708; found: 257.0706 (M + 1).

Synthesis of N-(5-Nitrothiazol-2-yl)pyrazin-2-carboxamide (32). To a solution of pyrazine-2-carboxylic acid (200 mg, 1.6 mmol) and 5-nitrothiazol-2-amine (234 mg, 1.6 mmol) in ACN (4 mL) at 0 °C were sequentially added T,P (1.5 g, 4.8 mmol) and Et,N (0.7 mL, 4.8 mmol). The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 7:10) until completion (16 h). The reaction mixture was filtered and washed several times with EtOAc. Finally, it was crystallized from MeOH to afford N-(5-nitrothiazol-2-yl)pyrazin-2-carboxamide (32) as a yellow solid (146 mg, 36%). 1H NMR (400 MHz, DMSO-d6): δ 9.62 (d, J = 1.2 Hz, 1H), 9.32 (dd, J = 2.4, 1.2 Hz, 1H), 9.11 (d, J = 2.8 Hz, 1H), 8.54 (s, 1H). LCMS mz (M − H) 249.85; HRMS (ESMS) calcd for C6H3N2O3S: 252.0219; found: 252.0204 (M + 1).

Synthesis of N-(5-Nitrothiazol-2-yl)thiazole-2-carboxamide (33). To a solution of thiazole-2-carboxylic acid (472 mg, 3.2 mmol) and 5-nitrothiazol-2-amine (350 mg, 2.7 mmol) in DCM/DMF (20 mL, 7:3 ratio) at 0 °C, HATU (1.5 g, 4.0 mmol) and DIPEA (1.6 mL, 8.1 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 7:10) until completion (16 h). The reaction mixture was filtered and washed successively with pentane, DCM, Et,O, and MeOH, which afforded N-(5-nitrothiazol-2-yl)thiazole-2-carboxamide (33) as a yellow solid (402 mg, 58%). 1H NMR (400 MHz, DMSO-d6): δ 8.55 (s, 1H), 8.01 (d, J = 2.8 Hz, 1H), 7.95 (s, 1H). LCMS mz (M + H) 257.00; HRMS (ESMS) calcd for C4H2N2O2S: 256.9803; found: 256.9779 (M + 1).

Synthesis of N-(5-Nitrothiazol-2-yl)nicotinamide (34). To a solution of nicotinic acid (200 mg, 1.6 mmol) and 5-nitrothiazol-2-amine (235 mg, 1.6 mmol) in ACN (4 mL) at 0 °C, Et,N (0.6 mL, 4.8 mmol) and T,P (3 mL, 4.8 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 7:1) until completion (12 h). The reaction mixture was poured into ice cooled water (40 mL) and extracted with
EtOAc (3 × 30 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 50% EtOAc in hexane as eluent to afford N-(5-nitrothiazol-2-yl)nicotinamide (34) as a brown solid (132 mg, 32%). 1H NMR (400 MHz, DMSO-d₆): δ 13.67 (brs, 1H), 9.23 (brs, 1H), 8.84–8.82 (m, 1H), 8.74 (s, 1H), 8.45 (d, J = 8.4 Hz, 1H), 7.64–7.61 (m, 1H). LCMS m/z (M + H) 251.07; HRMS (ESMS) calcd for C₆H₄N₂O₂S: 251.0239; found: 251.0245 (M + 1).

**Synthesis of N-(5-Nitrothiazol-2-yl)isonicotinamide (35).** To a solution of isonicotinic acid (0.5 g, 4.1 mmol) and DIPEA (1.6 mL, 9.0 mmol) were sequentially added. The reaction mixture was then stirred at room temperature while monitoring by TLC analysis (MeOH/DCM = 1:19) until completion (46 h). The reaction mixture was filtered and washed several times with DCM. It was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 40% EtOAc in hexane as eluent to afford 1-(5-nitrothiazol-2-yl)-3-phenylurea (37) as a yellow solid (254 mg, 40%). 1H NMR (400 MHz, DMSO-d₆): δ 13.81 (brs, 1H), 8.86 (s, 1H), 7.91 (s, 1H), 7.03 (s, 1H), 6.90 (s, 1H), 8.36 (d, J = 4.0 Hz, 1H), 7.86 (td, J = 8.4, 1.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.15 (m, 1H). LCMS m/z (M + H) 266.05; HRMS (ESMS) calcd for C₇H₆N₂O₂S: 266.0348; found: 266.0332 (M + 1).

**Synthesis of 1-Methyl-N-(5-nitrothiazol-2-yl)-1H-imidazole-2-carboxamide (40).** To a solution of 1-methyl-1H-imidazole-2-carboxylic acid (250 mg, 2.0 mmol) and 5-nitrothiazol-2-amine (345 mg, 2.4 mmol) in DCM/DMF (8 mL, 7:5 ratio) at 0 °C, HATU (1.0 g, 3.0 mmol) and DIPEA (1.1 mL, 6.0 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 7:10) for 16 h. The reaction mixture was filtered and washed several times with EtOAc. Finally, it was crystallized from MeOH to afford 1-(5-nitrothiazol-2-yl)-isonicotinamide (35) as a yellow solid (46 mg, 11%). 1H NMR (400 MHz, DMSO-d₆): δ 13.37 (brs, 1H), 8.66 (s, 1H), 7.91 (s, 1H), 7.03 (s, 1H), 3.98 (s, 3H). LCMS δ (M + H) 252.97. 13C NMR (100 MHz, DMSO-d₆): δ 164.473, 152.362, 143.612, 141.286, 140.171, 138.341, 129.467, 129.241, 123.970, 122.263, 119.566, 118.640. HRMS (ESMS) calcd for C₉H₇N₅O₃S: 252.0395; found: 252.0393 (M + 1).

**Synthesis of 1-Cyclohexyl-3-(5-nitrothiazol-2-yl)oxazole-2-carboxamide (41).** To a solution of oxazole-2-carboxylic acid (93 mg, 0.8 mmol) and 5-nitrothiazol-2-amine (100 mg, 0.7 mmol) in ACN (2 mL) at 0 °C, T₃P (1.5 mL, 3.4 mmol) and Et₃N (0.6 mL, 4.1 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis. The reaction mixture was poured into ice cooled water (20 mL) and extracted with DCM (3 × 30 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 40% EtOAc in hexane as eluent to afford N-(5-nitrothiazol-2-yl)-oxazole-2-carboxamide (41) as a yellow solid (34 mg, 16%). 1H NMR (400 MHz, DMSO-d₆): δ 13.56 (brs, 1H), 10.08 (s, 1H), 8.29 (s, 1H), 6.86 (s, 1H), 7.91 (s, 1H), 7.03 (s, 1H), 3.98 (s, 3H). LCMS m/z (M + H) 251.92.

**Synthesis of N-(5-Nitrothiazol-2-yl)isoxazole-3-carboxamide (42).** To a solution of isoxazole-3-carboxylic acid (200 mg, 1.8 mmol) and 5-nitrothiazol-2-amine (250 mg, 1.8 mmol) in DCM (10 mL) at 0 °C, HATU (2.0 g, 5.4 mmol) and DIPEA (1.6 mL, 9.0 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis. The reaction mixture was then stirred at room temperature and monitored by TLC analysis. After completion, the reaction was cooled, and the solid mass that appeared was filtered and washed with EtOAc and hexane to obtain 2-isocyanoatopyridine as a brown solid (326 mg, 64%). LCMS m/z (M + H) 120.8.

To a solution of 2-isocyanoatopyridine (0.3 g, 2.5 mmol) in DCM (6 mL) at 0 °C under a N₂ atmosphere, 5-nitrothiazol-2-amine (362 mg, 2.5 mmol) and Et₃N (1.7 mL, 12.5 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (46 h). The reaction mixture was filtered and washed several times with DCM. It was dried over preparative HPLC to afford 1-(5-nitrothiazol-2-yl)-3-(pyridin-2-yl)urea (39) as a yellow solid (176 mg, 27%). 1H NMR (400 MHz, DMSO-d₆): δ 12.72 (brs, 1H), 10.08 (s, 1H), 8.60 (s, 1H), 8.36 (d, J = 4.0 Hz, 1H), 7.86 (td, J = 8.4, 1.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.15 (m, 1H). LCMS m/z (M + H) 266.05; HRMS (ESMS) calcd for C₉H₉N₂O₂S: 266.0348; found: 266.0323 (M + 1).
The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using 5% EtOAc in hexane as eluent to afford N-(5-nitrothiazol-2-yl)isoxazole-3-carboxamide (42) as a yellow solid (70 mg, 16%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.24 (s, 1H), 8.73 (s, 1H), 7.23 (s, 1H). LCMS m/z (M – H) 238.99; HRMS (ESMS) calcd for C$_8$H$_6$N$_4$O$_3$S: 241.0032; not detected.

**Synthesis of 5-Methyl-N-(5-nitrothiazol-2-yl)thiazole-2-carboxamide (43).** To a solution of 5-methylthiazole-2-carboxylic acid (200 mg, 1.4 mmol) and 5-nitrothiazole-2-amine (202.8 mg, 1.4 mmol) in DCM (20 mL) at 0 °C, HATU (532 mg, 1.4 mmol) and DIPEA (315 μL, 1.8 mmol) were sequentially added. The reaction mixture was stirred at room temperature for 3 h. It was then evaporated to dryness. It was dissolved in THF (10 mL), and the reaction mixture was heated at 90 °C for 3 h. It was then evaporated to dryness. The solid was purified by column chromatography on silica gel (100–200 mesh) using 2% MeOH in DCM as eluent to a brown solid (0.45 g, 95%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.81 (brs, 1H), 8.71 (s, 2H), 2.52 (s, 3H). LCMS m/z (M + H) 263.23; HRMS (ESMS) calcd for C$_8$H$_6$N$_2$O$_3$S: 263.049; found: 263.0491 (M + 1).

**Synthesis of 5-Methyl-N-(5-nitrothiazol-2-yl)benzamide (46).** Acetic anhydride (10 mL) was added to 4-methylthiazole-2-amine (2.0 g, 17.5 mmol), which was present in a 100 mL round bottom flask fitted with a reflux condenser, and the reaction mixture was refluxed at 145 °C for 3 h. It was then evaporated to dryness using toluene as an azeotropic mixture, then filtered and dried using a high vacuum pump to afford N-(5-nitrothiazol-2-yl)benzamide (46). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.98 (s, 1H), 6.70 (s, 1H), 2.24 (s, 3H), 2.10 (s, 3H). LCMS m/z (M + H) 156.8.

To a solution of N-(4-methylthiazol-2-yl)acetamide (1.3 g, 8.3 mmol) in conc. H$_2$SO$_4$ (3.3 mL) at 0 °C, fuming HNO$_3$ (0.9 mL) was added while maintaining the inner temperature at 0 °C. The reaction mixture was then stirred at 0 °C and monitored by TLC analysis (MeOH/DCM = 3:10) until completion (2 h). The reaction mixture was poured into ice cooled water (50 g) and a solid was precipitated out. The solid was filtered and dried using a high vacuum pump to afford N-(4-methyl-5-nitrothiazol-2-yl)acetamide as a yellow solid (0.7 g, 42%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.96 (s, 1H), 2.65 (s, 3H). LCMS m/z (M + H) 199.93.

To a solution of N-(4-methyl-5-nitrothiazol-2-yl)acetamide (0.6 g, 3.0 mmol) in EtOH (6 mL) at 0 °C, conc. HCl (0.6 mL) was added, and the mixture was refluxed at 80 °C for 3 h. The reaction mixture was then evaporated to dryness. The solid was neutralized with saturated NaHCO$_3$ solution and extracted with EtOAc (3 × 30 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to afford 4-methyl-5-nitrothiazol-2-amine as a yellow solid (0.45 g, 95%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.71 (s, 2H), 2.52 (s, 3H). LCMS m/z (M + H) 159.91.

To a solution of 4-methyl-5-nitrothiazol-2-amine (100 mg, 0.7 mmol) and 2-hydroxybenzoic acid (115 mg, 0.7 mmol) in ACN (2 mL) at 0 °C, T$_3$P (1.2 mL, 2.2 mmol) and Et$_3$N (0.3 mL, 2.1 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (16 h). The reaction mixture was poured into ice cooled water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using 35% EtOAc in hexane as eluent to afford 2-hydroxy-N-(4-methyl-5-nitrothiazol-2-yl)benzamide (46) as a yellow solid (72 mg, 36%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.18 (brs, 1H), 8.72 (brs, 1H), 7.92 (dd, J = 8.0, 1.6 Hz, 1H), 7.51 (td, J = 8.0, 1.6 Hz, 1H), 7.06–6.99 (m, 2H), 2.69 (s, 3H). LCMS m/z (M + H) 279.88; HRMS (ESMS) calcd for C$_{11}$H$_8$N$_2$O$_3$: 280.0392; found: 280.0388 (M + 1).
Synthesis of N-(5-Methylthiazol-2-yl)picolinamide (48). To a solution of picolinic acid (200 mg, 1.6 mmol) and 5-methylthiazol-2-amine (184 mg, 1.6 mmol) in DCM (10 mL) at 0 °C, HATU (1.8 g, 4.8 mmol) and DIPEA (1.5 mL, 8.0 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (16 h). The reaction mixture was poured into ice cooled water (30 mL) and extracted with DCM (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 1% MeOH–DCM as eluent to afford ethyl 2-(picolinamido)thiazole-5-carboxylate as a yellow solid (385 mg, 57%). LCMS m/z (M + H) 278.29.

Ethyl 2-(picolinamido)thiazole-5-carboxylate (100 mg, 0.3 mmol) in MeOH/THF (2 mL, i:9) was added to a round bottom flask at room temperature. A solution of LiOH·H2O (45 mg, 1.1 mmol) in H2O (2 mL) was added dropwise, and the mixture was monitored by TLC analysis (MeOH/DCM = 1:9) for 16 h. The reaction mixture was evaporated to dryness and diluted with water (1.5 mL). It was then cooled to 0 °C, and the pH was adjusted to 2 using dilute HCl. A solid was precipitated out, which was filtered, washed with acetone, and dried using a high vacuum pump. Yield: 11.9 g crude as HBr salt. Mass m/z (M + H) 200.18 and (M + 2 + H) 202.21.

To a solution of 2-bromo-1-(pyridin-2-yl)ethan-1-one (5.0 g, 41.3 mmol) in AcOH (150 mL) at 0 °C, HBr in AcOH (11 mL, 185.7 mmol) and pyridinium tribromide (14.5 g, 45.4 mmol) were sequentially added. The reaction mixture was then stirred at 60 °C for 6 h and monitored by TLC analysis (EtOAc/hexane = 1:9). The reaction mixture was cooled to room temperature. EtOAc was added to the solution, and the reaction mixture was stirred at room temperature for 12 h. A white precipitate (2-bromo-1-(pyridin-2-yl)ethan-1-one) appeared, which was filtered, washed with acetone, and dried using a high vacuum pump. Yield: 12.93 g as white solid (82 mg, 59.8 mmol) in EtOH (119 mL), thiourea (4.6 g, 61.0 mmol) and HOBt (240 mg, 1.7 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 3:1). The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 3:1) until completion (16 h). The reaction mixture was poured into ice cooled water (30 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over anhydrous Na2SO4 concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 20% EtOAc in hexane as eluent to afford N-(5-(trifluoromethyl)thiazol-2-yl)nicotinamide (49) as an off-white solid (122 mg, 55%). 1H NMR (400 MHz, DMSO-d6): δ 12.93 (brs, 1H), 8.80 (s, 1H), 8.21 (s, 1H), 8.20 (s, 1H), 8.12 (t, J = 7.6 Hz, 1H), 7.75 (m, 1H). LCMS m/z (M + H) 274.06; HRMS (ESMS) calc for C9H9F2N3O: 274.0545; found: 274.0541 (M + 1).

Synthesis of N-(5-(Trifluoromethyl)thiazol-2-yl)-nicotinamide (50). To a solution of picolinic acid (100 mg, 0.8 mmol) and S-(trifluoromethyl)thiazol-2-amine (136 mg, 0.8 mmol) in ACN (2 mL) at 0 °C, T3P (1.5 mL, 2.4 mmol) and Et3N (0.3 mL, 2.4 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 3:1) until completion (3 h). The reaction mixture was poured into ice cooled water (20 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 1% MeOH in DCM as eluent to afford ethyl 2-(picolinamido)thiazole-5-carboxylate as a yellow solid (385 mg, 57%). LCMS m/z (M + H) 278.29.
stirred at 60 °C for 6 h and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (4 h). The reaction mixture was poured into ice cooled water (30 mL) and extracted with DCM (3 × 30 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 2% MeOH in DCM as eluent to afford N-(5-nitro-4-(pyridin-2-yl)thiazol-2-yl)picolinamide (53) as a yellow solid (37 mg, 12%). 1H NMR (400 MHz, DMSO-d6): δ 13.38 (brs, 1H), 8.79 (s, 1H), 8.70 (d, J = 4.0 Hz, 1H), 8.24 (d, J = 7.6 Hz, 1H), 8.13 (t, J = 7.6 Hz, 1H), 7.99 (t, J = 7.6 Hz, 1H), 7.82–7.76 (m, 2H), 7.54 (t, J = 5.6 Hz, 1H). LCMS m/z (M + H) 313.95.

To a solution of 2-methoxy-N-(5-nitrothiazol-2-yl)benzenesulfonamide (300 mg, 0.9 mmol) in DCM (15 mL) at 0 °C, AlCl3 (506 mg, 3.8 mmol) was added. The reaction mixture was stirred at room temperature and monitored by TLC analysis (MeOH/DCM = 1:19) until completion (2 h). The reaction mixture was quenched with ice cooled water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (60–120 mesh) using 1% MeOH in DCM as eluent to afford 2-methoxy-N-(5-nitrothiazol-2-yl)benzenesulfonamide as a light yellow solid (180 mg, 47%). 1H NMR (400 MHz, DMSO-d6): δ 8.35 (s, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 3.72 (s, 3H). LCMS m/z (M – H) 299.93; HRMS (ESMS) calcd for C9H7N2O3S2: 301.9905; found: 301.9912 (M + 1).

Synthesis of 2-(5-Nitrothiazol-2-yl)(methyl)phenol (55). To a solution of 2-hydroxybenzaldehyde (200 mg, 1.6 mmol) and 5-nitrothiazol-2-amine (237 mg, 1.6 mmol) in AcN (4 mL) at 0 °C, Et3SiH (1.0 mL, 6.4 mmol) and TFAA (0.5 mL, 5.0 mmol) were sequentially added. The reaction mixture was then stirred at room temperature and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (4 h). The reaction mixture was then dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 2% MeOH in DCM as eluent to afford 2-(5-Nitrothiazol-2-yl)(methyl)phenol (55) as a yellow solid (207 mg, 70%). 1H NMR (400 MHz, DMSO-d6): δ 9.96 (s, 1H), 8.26 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 7.2 Hz, 1H), 6.88 (m, 2H), 1.23 (s, 3H). LCMS m/z (M – H) 299.93; HRMS (ESMS) calcd for C9H7N2O2S2: 301.9905; found: 301.9912 (M + 1).
mL) and the organic layer was washed with saturated K₂CO₃ solution. It was then dried over Na₂SO₄ and evaporated to dryness to afford a brown solid. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 28% EtOAc in hexane as eluent to afford N-(5-nitrothiazol-2-yl)oxazole-2-carboxamide (55) as a yellow solid (81 mg, 20%). ¹H NMR (400 MHz, DMSO-d₆): δ 9.76 (s, 1H), 9.61 (brs, 1H), 8.32 (s, 1H), 7.19 (d, J = 7.2 Hz, 1H), 7.13 (t, J = 7.2 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 6.78 (t, J = 7.2 Hz, 1H), 4.49 (s, 2H). LCMS m/z (M + H) 252.06; HRMS (ESMS) calcd for C₁₀H₉N₃O₃S: 252.0443; found: 252.0441 (M + 1).

**Synthesis of 5-Nitro-N-(pyridin-2-ylmethyl)thiazol-2-amine (56).** To a solution of picolinaldehyde (200 mg, 1.9 mmol) and 5-nitrothiazol-2-amine (272 mg, 1.9 mmol) in ACN (4 mL) at 0 °C, Et₃SiH (1.1 mL, 7.6 mmol) and TFA (0.6 mL, 7.6 mmol) were sequentially added. The reaction mixture was then heated at 80 °C and monitored by TLC analysis (EtOAc/hexane = 1:1) until completion (4 h). The reaction mixture was concentrated to dryness and poured into ice cooled water (50 mL). It was extracted with EtOAc (2 × 50 mL), and the organic layer was washed with saturated K₂CO₃ solution. It was then dried over Na₂SO₄ and evaporated to dryness. The crude product was further purified by column chromatography on silica gel (100–200 mesh) using 48% EtOAc in hexane as eluent to afford 5-nitro-N-(pyridin-2-ylmethyl)thiazol-2-amine (56) as a brown solid (81 mg, 20%). ¹H NMR (400 MHz, DMSO-d₆): δ 9.87 (brs, 1H), 8.55 (d, J = 4.4 Hz, 1H), 8.31 (s, 1H), 7.80 (td, J = 7.6, 1.6 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.33 (m, 1H), 4.70 (s, 2H). LCMS m/z (M + H) 237.17; HRMS (ESMS) calcd for C₉H₈N₄O₂S: 237.0446; found: 237.0443 (M + 1).

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**ABBREVIATIONS**

MIC, minimum inhibitory concentration; TC50, half-maximum inhibitory concentration; SI, selectivity index

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