Warren et al. PS Hoffman corresponding author.

Supplemental material

S1. Chemical Synthesis

General Synthetic Materials and Methods. All nonaqueous reactions were carried out in oven or flame-dried glassware under an argon or nitrogen atmosphere with dry solvents and magnetic stirring, unless otherwise stated. The argon and nitrogen were dried by passing through a tube of Drierite. Anhydrous diethyl ether (Et_2O), chloroform ($CHCl_3$), Dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), and tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were purchased from Aldrich or VMR Chemicals and used as received. THF was dried over activated molecular sieves (4 Å) prior to use. All other reagents were purchased from Acros chemicals and Aldrich chemicals. Except as indicated otherwise, reactions were monitored by thin layer chromatography (TLC) using 0.25 mm Whatman precoated silica gel plates. Flash chromatography was performed with the indicated solvents and Dynamic Adsorbents silica gel (particle size 0.023 – 0.040 mm). Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Varian UnityInova 500/51 or Varian UnityInova 300/54 at 300K unless otherwise noted. Chemical shifts are reported in ppm (δ) values relative to the solvent as follows: $CDCl_3$ (δ 7.24 for proton and δ 77.0 for carbon NMR), DMSO-d_6 (δ 2.50 for proton and δ 39.5 for carbon NMR). All high-resolution mass spectrometry was carried out by the Mass Spectrometry Laboratory in the School of Chemical Sciences at the University of Illinois Urbana-Champagne (Urbana, IL).

Other abbreviations: 1,1'-bis(diphenylphosphino)ferrocene (dppf), 4-dimethylaminopyridine (DMAP), 9-borabicyclo[3.3.1]nonane (9-BBN), acetic acid (AcOH), N,N-diisopropylethylamine (DIEA), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 1-

hydroxybenzotriazole hydrate (HOBt), tert-butanol (tBuOH), triethylamine (TEA), trifluoroacetic acid (TFA).

General Procedure A: Williamson Ether Synthesis. To a solution of a phenol (1.0 eq.), sodium iodide (1.1 eq.), alkyl bromide (1.5 eq.) in DMF (0.3 M) at rt was added finely ground anhydrous potassium carbonate (2.0 eq.), then heated 60 ºC, and then let react for 12 h. The reaction was quenched with sat. $NaHCO_3$ (100x the volume of DMF) and extracted into EtOAc (100x the volume of DMF). The organic layer was washed 3x with neat water (100x the volume of DMF), dried with Na_2SO_4, evaporated to dryness, and immediately purified by flash chromatography.

General Procedure B: Saponification. A methyl ester (1.0 eq.) was dissolved in a mixture of MeOH:THF:H_2O (1:1:1, 0.3 M) then LiOH•H_2O (3.0 eq.) was added. The solution was stirred for 12 hours at 60 ºC then cooled to rt and quenched with 1M HCl (100x the volume of the reaction) and extracted 3x into EtOAc (100x the volume of reaction). The organic layer was dried with Na_2SO_4 and evaporated to a solid. No further purification was necessary.

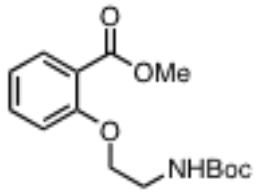
General Procedure C: Amide Coupling. An aryl carboxylic acid (1.0 eq.), EDC (1.1 eq.), HOBT (1.1 eq.), TEA (4.0 eq.) and DMAP (0.1 eq.) were dissolved in THF (0.1 M) and stirred for 15 mins. An aromatic amine (1.5 eq.) was then added in one portion and the reaction was stirred at 55 °C. Once judged complete by TLC analysis (~24h), the resulting suspension was diluted with EtOAc (100x the volume of reaction) and washed with 1M HCl (100x the volume of reaction), sat. $NaHCO_3$ (100x the volume of reaction) and then dried with Na_2SO_4 and evaporation to dryness. The resulting residue was purified by flash column chromatography.

General Procedure D: N-Boc Deprotection. To a solution of a Boc protected amine in CH_2Cl_2 (0.1 M) was added trifluoroacetic acid (equal volume to CH_2Cl_2) at rt and let stir for 2 h,

before being evaporated to dryness. The reaction residue was then treated with 6 N HCl (2 eq.) and then dry ether to induce crystallization of the amine•HCl salt, and the suspension evaporated to dryness. The salt was then dissolved in neat water, frozen, and the water removed via sublimation to yield the deprotected product.

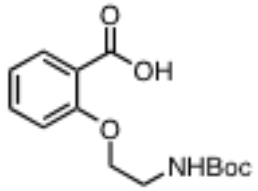
The synthesis of **VPC16a1011**, **VPC161183**, **VPC16b1094**, and **VPC161195** has previously been described (1).

Methyl 2-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzoate (1).



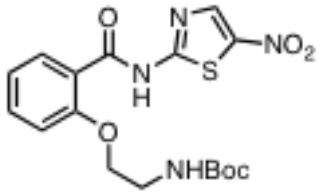
General procedure A was used to couple methyl 2-hydroxybenzoate (2.0 mL, 15.4 mmol) and *tert*-butyl (2-bromoethyl)carbamate. Purified by flash chromatography (10 – 50 % ethyl acetate / hexanes) to yield 4.54 g of the title compound. 99%. Clear and colorless oil. ^{1}HNMR (300 MHz, DMSO) δ 7.64 (dd, J = 7.6, 1.1 Hz, 1H), 7.51 (td, J = 7.5, 0.9 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.01 (dd, J = 14.6, 7.1 Hz, 1H), 6.83 (t, J = 5.3 Hz, 1H), 4.03 (t, J = 5.9 Hz, 2H), 3.79 (s, J = 0.7 Hz, 3H), 3.31 (dd, J = 6.7, 5.5 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, DMSO) δ 166.37, 157.35, 155.57, 133.44, 130.66, 120.61, 120.47, 114.04, 77.82, 67.40, 51.81, 38.98, 28.18.

2-(2-((tert-Butoxycarbonyl)amino)ethoxy)benzoic acid (2).



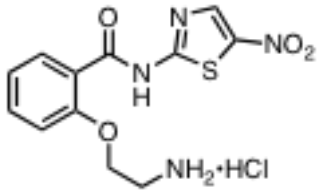
General procedure B was used to hydrolyze methyl ester **1** (4.22 g, 14.29 mmol) to yield 3.81 g of the title compound. 95%. White foam. ^{1}H NMR (300 MHz, DMSO) δ 12.53 (s, 1H), 7.64 (dd, J = 7.6, 1.6 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.85 (t, J = 5.4 Hz, 1H), 4.04 (t, J = 6.0 Hz, 2H), 3.30 (dd, J = 7.4, 5.9 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (75 MHz, DMSO) δ 167.30, 157.28, 155.65, 133.13, 130.80, 121.71, 120.58, 114.14, 77.92, 67.58, 39.31, 28.23.

tert-Butyl (2-(2-((5-nitrothiazol-2-yl)carbamoyl)phenoxy)ethyl)carbamate (3).



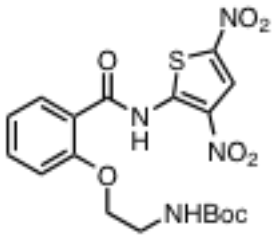
General procedure C was used to couple carboxylic acid **2** (1.50 g, 5.33 mmol) to 2-Amino-5-nitrothiazole to yield 827 mg of the title compound. 38%. Tan solid. ^{1}H NMR (300 MHz, DMSO) δ 12.75 (s, 1H), 8.69 (s, 1H), 7.71 (dd, J = 7.6, 1.7 Hz, 1H), 7.59 (t, J = 7.9 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 7.05 (t, J = 5.4 Hz, 1H), 4.15 (t, J = 5.7 Hz, 2H), 3.34 (d, J = 5.9, 5.1 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR (75 MHz, DMSO) δ 165.66, 161.55, 156.46, 155.70, 142.81, 141.94, 134.16, 130.60, 121.01, 120.87, 113.17, 77.90, 67.58, 39.23, 28.16.

2-(2-Aminoethoxy)-N-(5-nitrothiazol-2-yl)benzamide hydrochloride (VPC161219).



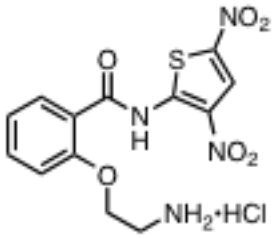
General procedure D was used to deprotect N-Boc **3** (1.34 g, 3.23 mmol) to yield 1.12 g of the title compound. 100%. Tan solid. ^{1}H NMR (300 MHz, DMSO) δ 12.94 (s, 1H), 8.71 (s, 1H), 8.24 (s, 3H), 7.69 (d, J = 7.6 Hz, 1H), 7.62 (t, J = 7.2 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 4.35 (t, J = 4.8 Hz, 2H), 3.24 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 165.79, 161.78, 155.66, 142.79, 141.88, 134.06, 130.75, 121.57, 121.27, 113.16, 65.16, 38.19.

tert-Butyl (2-(2-((3,5-dinitrothiophen-2-yl)carbamoyl)phenoxy)ethyl)carbamate (4).



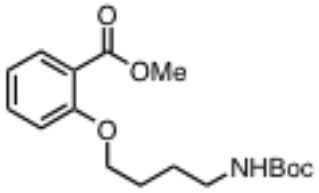
General procedure C was used to couple carboxylic acid **2** (969 mg, 3.45 mmol) to 3,5-dinitrothiophen-2-amine to yield 1.055 g of the title compound. 68%. Yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 13.04 (s, J = 49.8 Hz, 1H), 8.58 (s, 1H), 8.11 (d, J = 7.8 Hz, 1H), 7.72 (dd, J = 11.3, 4.3 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.06 (t, J = 5.6 Hz, 1H), 4.43 (t, J = 4.8 Hz, 2H), 3.44 (dd, J = 5.3, 4.8 Hz, 2H), 1.25 (s, J = 23.1 Hz, 9H). ^{13}C NMR (126 MHz, DMSO) δ 163.54, 157.32, 155.69, 146.62, 138.96, 136.29, 132.31, 130.05, 123.03, 121.81, 117.28, 113.93, 77.78, 69.10, 38.58, 28.01.

2-(2-Aminoethoxy)-N-(3,5-dinitrothiophen-2-yl)benzamide hydrochloride (VPC16b2031).



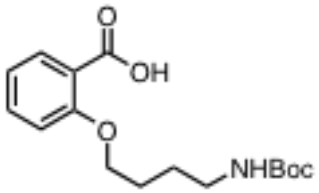
General procedure D was used to deprotect N-Boc **4** (750 mg, 1.67 mmol) to yield 578 mg of the title compound. 90%. Yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 12.77 (s, 1H), 8.55 (s, 1H), 8.46 (s, 3H), 8.12 (dd, J = 7.8, 1.5 Hz, 1H), 7.75 (t, J = 7.7 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.25 (t, J = 7.5 Hz, 1H), 4.65 (t, J = 4.8 Hz, 2H), 3.49 – 3.40 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 163.47, 156.60, 146.86, 139.08, 136.43, 132.49, 129.93, 122.92, 122.17, 117.28, 113.67, 66.45, 37.57.

Methyl 2-(4-((tert-butoxycarbonyl)amino)butoxy)benzoate (5).



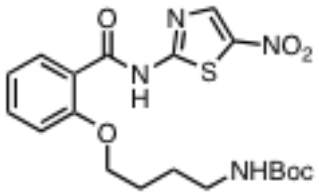
General procedure A was used to couple methyl 2-hydroxybenzoate (800 µL, 6.17 mmol) and tert-butyl (4-bromobutyl)carbamate. Purified by flash chromatography (10 – 50 % ethyl acetate / hexanes) to yield 1.24 g of the title compound. 62%. Clear amber oil. ^{1}H NMR (300 MHz, DMSO) δ 7.62 (d, J = 6.4 Hz, 1H), 7.50 (t, J = 7.2 Hz, 1H), 7.12 (d, J = 8.3 Hz, 1H), 6.99 (t, J = 7.4 Hz, 1H), 6.91 – 6.78 (m, 1H), 4.02 (t, J = 6.0 Hz, 2H), 3.78 (s, 3H), 2.97 (dd, J = 6.3, 6.2 Hz, 2H), 1.78 – 1.62 (m, 2H), 1.62 – 1.45 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (75 MHz, DMSO) δ 157.56, 155.61, 133.40, 130.61, 120.28, 119.99, 113.48, 77.33, 67.92, 51.73, 39.50, 28.24, 26.10, 26.05.

2-(4-((tert-Butoxycarbonyl)amino)butoxy)benzoic acid (6).



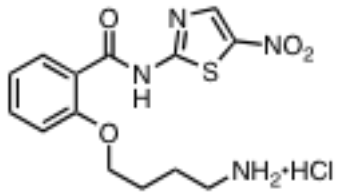
General procedure B was used to hydrolyze methyl ester **5** (1.20 g, 3.71 mmol) to yield 1.128 g of the title compound. 98%. Amber wax. ^{1}H NMR (300 MHz, DMSO) δ 12.49 (s, 1H), 7.60 (d, J = 6.1 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 6.97 (t, J = 7.5 Hz, 1H), 6.84 (t, J = 5.4 Hz, 1H), 4.01 (t, J = 6.3 Hz, 2H), 2.96 (dd, J = 6.4, 6.2 Hz, 2H), 1.68 (dt, J = 6.4 Hz, 2H), 1.53 (dt, J = 7.0 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (75 MHz, DMSO) δ 167.44, 157.36, 155.63, 132.86, 130.53, 121.66, 119.96, 113.41, 77.36, 67.94, 39.50, 28.27, 26.02.

tert-butyl (4-(2-((5-nitrothiazol-2-yl)carbamoyl)phenoxy)butyl)carbamate (7).



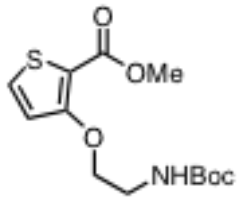
General procedure C was used to couple carboxylic acid **6** (1.10 g, 3.56 mmol) to 2-Amino-5-nitrothiazole to yield 567 mg of the title compound. 37%. Beige solid. ^{1}H NMR (500 MHz, DMSO) δ 12.70 (s, 1H), 8.66 (s, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.59 (t, J = 7.9 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.81 – 6.72 (m, 1H), 4.14 (t, J = 6.3 Hz, 2H), 2.95 (dd, J = 6.3, 6.2 Hz, 2H), 1.83 – 1.70 (m, 2H), 1.53 (dt, J = 7.0, 6.7 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 165.52, 161.33, 156.81, 155.57, 142.76, 142.01, 134.19, 130.37, 120.70, 113.22, 77.36, 68.37, 39.51, 28.22, 25.89.

2-(4-aminobutoxy)-N-(5-nitrothiazol-2-yl)benzamide (VPC162096).



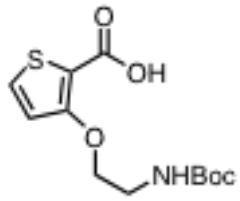
General procedure D was used to deprotect N-Boc **7** (500 mg, 1.15 mmol) to yield 427 mg of the title compound. 100%. Beige solid. ^{1}H NMR (500 MHz, DMSO) δ 12.78 (s, 1H), 8.67 (s, 1H), 7.80 (s, 3H), 7.71 (dd, J = 7.6, 1.0 Hz, 1H), 7.65 – 7.55 (m, 1H), 7.21 (t, J = 22.6 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 4.16 (t, J = 6.2 Hz, 2H), 2.91 – 2.80 (m, 2H), 1.91 – 1.78 (m, 2H), 1.70 (dt, J = 7.4, 7.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 165.46, 161.24, 156.56, 142.71, 141.87, 134.05, 130.23, 120.72, 120.65, 113.18, 67.85, 38.19, 25.38, 23.40.

Methyl 3-(2-((tert-butoxycarbonyl)amino)ethoxy)thiophene-2-carboxylate (8).



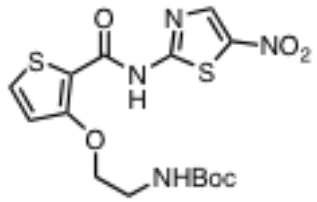
General procedure A was used to couple methyl 3-hydroxythiophene-2-carboxylate (1.0 g, 6.32 mmol) and *tert*-butyl (2-bromoethyl)carbamate. Purified by flash chromatography (10 – 50 % ethyl acetate / hexanes) to yield 1.89 g of the title compound. 99%. Thick amber oil. ^{1}H NMR (500 MHz, DMSO) δ 7.81 (d, J = 5.6 Hz, 1H), 7.11 (d, J = 5.5 Hz, 1H), 6.91 (t, J = 5.5 Hz, 1H), 4.12 (t, J = 6.0 Hz, 2H), 3.72 (s, 3H), 3.27 (q, J = 5.9 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 161.32, 160.87, 155.63, 132.03, 118.22, 108.73, 77.88, 70.00, 51.38, 39.40, 28.19.

3-(2-((tert-butoxycarbonyl)amino)ethoxy)thiophene-2-carboxylic acid (9).



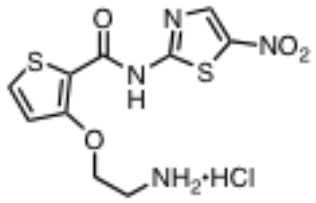
General procedure B was used to hydrolyze methyl ester **8** (1.84 g, 6.11 mmol) to yield 1.43 g of the title compound. 82%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 12.43 (s, 1H), 7.74 (d, J = 5.5 Hz, 1H), 7.08 (d, J = 5.5 Hz, 1H), 6.91 (t, J = 5.5 Hz, 1H), 4.10 (t, J = 6.1 Hz, 2H), 3.25 (q, J = 6.0 Hz, 2H), 1.37 (s, 9H). 13C NMR (126 MHz, DMSO) δ 162.42, 160.29, 155.68, 131.30, 118.42, 110.48, 77.93, 70.04, 39.45, 28.24.

tert-Butyl (2-((2-((5-nitrothiazol-2-yl)carbamoyl)thiophen-3-yl)oxy)ethyl)carbamate (10).



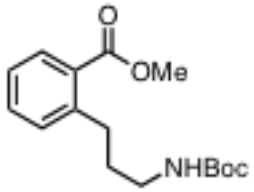
General procedure C was used to couple carboxylic acid **9** (100 mg, 0.35 mmol) to 2-Amino-5-nitrothiazole to yield 87 mg of the title compound. 60%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 11.30 (s, 1H), 8.67 (s, 1H), 8.06 (d, J = 5.5 Hz, 1H), 7.32 – 7.20 (m, 2H), 4.34 (t, J = 5.1 Hz, 2H), 3.39 (dd, J = 10.5, 5.2 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 160.89, 159.43, 159.08, 155.82, 142.54, 134.92, 117.67, 78.00, 71.87, 39.33, 28.11.

3-(2-aminoethoxy)-N-(5-nitrothiazol-2-yl)thiophene-2-carboxamide hydrochloride (VPC162125).



General procedure D was used to deprotect N-Boc **10** (25 mg, 0.060 mmol) to yield 21 mg of the title compound. 100%. Pale yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 11.62 (s, 1H), 8.70 (s, 1H), 8.51 (s, 3H), 8.20 – 7.93 (m, 1H), 7.39 – 7.15 (m, 1H), 4.61 – 4.45 (m, 2H), 2.69 – 2.21 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 160.87, 159.32, 157.78, 142.37, 141.94, 134.83, 117.50, 111.79, 68.71, 38.16.

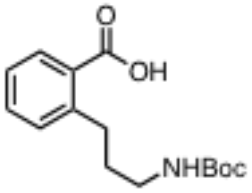
Methyl 2-(3-((tert-butoxycarbonyl)amino)propyl)benzoate (11).



tert-Butyl allylcarbamate (113 mg, 0.72 mmol) and THF (1.5 mL) were charged into a flame-dried round bottom flask followed by the dropwise addition of 0.5M 9-BBN in THF (1.92 mL). After 2 h, 2M Cs_2CO_3 (0.72 mL), methyl 2-iodobenzoate (0.07 mL, 0.48 mmol) and Pd(dppf)Cl_2 (27.7 mg, 5 mol%) were added to the flask and held at room temperature. Once judged complete by TLC (~24 h), the crude mixture was diluted with EtOAc (30 mL) and washed with sat. NH_4Cl (2 x 20 mL) and brine (2 x 20 mL) then dried ($MgSO_4$) followed by filtration and evaporation to dryness. The resulting residue was purified by gradient flash column chromatography (5-30% EtOAc/hexanes) to yield 130 mg of methyl 2-(3-(*tert*-butoxycarbonylamino)propyl)benzoate. 93%. Amber oil. ^{1}H NMR (500 MHz, DMSO) δ 7.76 (dd, J = 7.8, 1.1 Hz, 1H), 7.49 (td, J = 7.5, 1.4 Hz, 1H), 7.39 – 7.26 (m, 2H), 6.85 (t, J = 5.3 Hz,

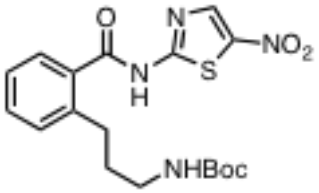
1H), 3.82 (s, 3H), 2.93 (dd, J = 6.7, 6.2 Hz, 2H), 2.87 – 2.81 (m, 2H), 1.62 (dt, J = 7.4, 7.4 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 167.52, 155.57, 142.96, 132.03, 130.91, 130.07, 129.41, 126.11, 77.38, 51.97, 39.77, 31.61, 30.99, 28.27.

2-(3-((tert-Butoxycarbonyl)amino)propyl)benzoic acid (12).



General procedure B was used to hydrolyze methyl ester **11** (130 mg, 0.45 mmol) to yield 120 mg of the title compound. 96%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 12.82 (s, 1H), 7.77 (dd, J = 7.7, 1.1 Hz, 1H), 7.45 (td, J = 7.5, 1.3 Hz, 1H), 7.35 – 7.23 (m, 2H), 6.81 (t, J = 5.3 Hz, 1H), 2.98 – 2.84 (m, 4H), 1.64 (dt, J = 7.4, 7.4 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 168.83, 155.55, 142.88, 131.58, 130.78, 130.47, 130.21, 125.93, 77.38, 39.83, 31.53, 30.88, 28.27.

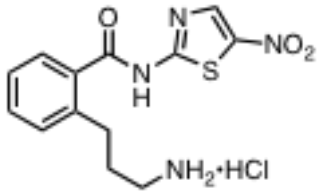
tert-Butyl (3-(2-((5-nitrothiazol-2-yl)carbamoyl)phenyl)propyl)carbamate (13).



General procedure C was used to couple carboxylic acid **12** (95 mg, 0.34 mmol) to 2-Amino-5-nitrothiazole to yield 58 mg of the title compound. 42%. Light yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 13.54 (s, 1H), 8.69 (s, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.41 – 7.33 (m, 2H), 6.81 (t, J = 5.5 Hz, 1H), 2.91 (dd, J = 6.5, 6.3 Hz, 2H), 2.79 – 2.68 (m,

2H), 1.68 – 1.57 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 168.72, 161.99, 155.53, 142.62, 142.03, 141.15, 132.26, 131.45, 130.16, 128.45, 125.88, 77.34, 39.67, 31.46, 30.00, 28.20.

2-(3-Aminopropyl)-N-(5-nitrothiazol-2-yl)benzamide hydrochloride (VPC162134 / Amixicile).



General procedure D was used to deprotect N-Boc **13** (310 mg, 0.76 mmol) to yield 257 mg of the title compound. 98%. Pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.60 (s, 1H), 8.71 (s, 1H), 8.05 (bs, 3H), 7.65 (d, J = 7.7 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.47 – 7.35 (m, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.79 – 2.71 (m, 2H), 1.87 (quint., J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.6, 162.1, 142.7, 142.0, 140.3, 132.2, 131.7, 130.2, 128.8, 126.3, 38.4, 29.6, 28.9; HRMS (ESI) calcd for $[C_{13}H_{14}N_4O_3S + H]^+$ 307.0859, found 307.0855.

REFERENCES

1. **Ballard, T. E., X. Wang, I. Olekhnovich, T. Koerner, C. Seymour, J. Salamoun, M. Warthan, P. S. Hoffman, T. L. Macdonald**. 2011. Synthesis and Antimicrobial Evaluation of Nitazoxanide-Based Analogues: Identification of Selective and Broad Spectrum Activity. ChemMedChem. **6**:362-377.

S2. Clinical score matrix.

Clinical Scoring System for Mice Infected with *Clostridium difficile*

Category	Scores*				
	0	**1**	**2**	**3**	**4**
Weight loss	None	<10%	10-15%	15-20%	>20%
Activity	Normal	Alert/slow moving	Lethargic/shaky	Inactive unless prodded	Not moving
Posture	Normal	Back slanted	Hunched	Hunched/nose down	
Coat	Normal	Piloerection	Rough skin	Very ruffled/puff/ Ungroomed	
Diarrhea	Normal	Soft stool/discolored (yellowish)	Wet stained tail/ mucous +/- blood	Liquid/no stool (ileus)	
Eyes/Nose	Normal	Squinted ½ closed	Squinted/discharge	Closed/discharge	

**Clinical Score=sum of all parameter scores. Total possible score=20. Normal=0; Found dead=20. Mice with score of ≥ 14 are euthanized.*