Selective dual inhibitors of the cancer-related deubiquitylating proteases USP7 and USP47

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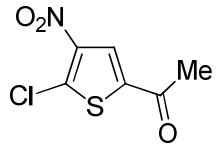
Synthetic Procedures and Compound Characterization

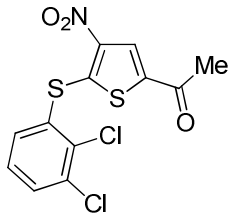
Reagents and instruments: All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry Ar. Solvents were purchased from Aldrich and Acros without further purification. Reagents and chemicals were purchased from commercial sources with purity ≥ 95% without further purification. Thin layer chromatography (TLC) was performed on EMD precoated plates (silica gel 60 F254, Art 5715, 0.25 mm) and compounds visualized by fluorescence under UV light or by staining with phosphomolybdic acid. Column chromatography was performed on EMD Silica Gel 60 (230-400 mesh) using a forced flow of 0.5–1.0 bar. ^{1}H NMR spectra were measured on a Varian Unity INOVA (300 Hz), a Bruker AVANCE-400 (400 MHz) and a Bruker AMX-500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million (ppm) and are referenced to the internal solvent signals. Coupling constants are reported in Hertz (Hz). Splitting patterns are indicated as follows: a, apparent; br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. HRMS measurements were acquired by use of Agilent Accurate-Mass TOF LC/MS mass spectrometer and a Bruker microOTOF (ESI-TOF-MS) spectrometer. cLogD values were calculated using Marvin Calculator Plugin (http://www.chemaxon.com/marvin/sketch/index.php).

Preparative HPLC: Samples were purified using a Gilson preparative HPLC system with 322 pump and UV/VIS 156. Method: Gemini C18 column 10 μm 30 × 100 mm with a linear gradient of 10-95% buffer B over 10 min with a flow rate of 20 mL/min (buffer A = 0.1% TFA in H_2O; buffer B = 0.1% TFA in CH_3CN). The eluent was monitored at 214 or 254 nm. The exact gradient used was determined by the elution behavior of the desired compound as assessed by prior analytical HPLC. Fractions containing pure target compound were identified by analytical LCMS and were combined and lyophilized.

LC/MS conditions: Agilent 6220 TOF/LC-MS System; Column: RESTEK Viva C18 (5μm 2.1 × 50 mm); Mobile phase A: H_2O/0.05% TFA; Mobile phase B: CH_3CN/0.05% TFA; Flow: 0.4 ml/min; Gradient: 0 min 10% B, 0.25 min 10% B, 0.75 min 15% B, 1.75 min 55% B, 3.25 min 90% B, 3.5 min 90% B, 3.6 min 10% B, Stop 5 min; Column temperature: 50 ^oC. All the target compounds (**1–54**) have purities of > 95% based upon LC/MS, and ^1H-NMR.

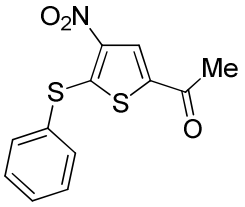


1-(5-Chloro-4-nitro-2-thienyl)ethanone (15). 2-Acetyl-5-chlorothiophene (80.3 g, 0.50 mol) was added portionwise to fuming nitric acid (500 mL) cooled in an ice-methanol bath. The reaction mixture was then stirred at ambient temperature for 30 minutes and poured into 4 liters of ice water. The solid was collected by filtration, washed with water and then dissolved in dichloromethane. After being dried over Na_2SO_4, the solution was concentrated under vacuum to give an orange solid which was recrystallized from ethanol to give a light brown product (40.9 g, 45%). ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H), 2.58 (s, 3H); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_6H_4ClNO_3S$, 204.9, found 204.9.

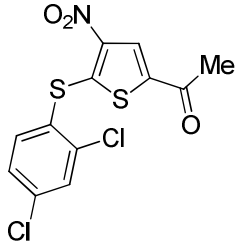


1-[(5-(2,3-Dichlorophenylsulfanyl)-4-nitro-2-thienyl]ethanone (1). 2,3-Dichlorobenzenethiol (4.3 g, 24.3 mmol) was added to a solution of sodium methoxide prepared by dissolving sodium metal (0.61 g,

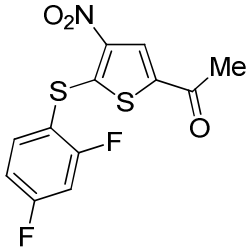
26.7 mmol) in anhydrous methanol. The mixture was stirred for 15 minutes at ambient temperature and then **15** (5 g, 24.3 mmol) added and the mixture stirred for 1 hour. Water (100 mL) was added and the solid which formed collected by filtration and dried under vacuum overnight at 40 ^{o}C. The product was recrystallized twice from acetonitrile to give a yellow solid (5.47 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (s, 1H, CH), 7.72 (m, 1H, ArH), 7.69 (m, 1H, ArH), 7.38 (t, *J* = 7.0 Hz, 1H, ArH), 2.44 (s, 3H, CH_3); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_3S_2$, 347.9323, found 347.9381.



1-(5-phenylsulfanyl-4-nitro-2-thienyl)ethanone (2). Using the method for **1**, **15** (205 mg, 1.0 mmol) and thiophenol gave **2** (151 mg, 54%) as a pale orange solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (s, 1H, CH), 7.50-7.69 (m, 5H, ArH), 2.48 (s, 3H, CH_3); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_3S_2$, 280.0102, found 280.0211.

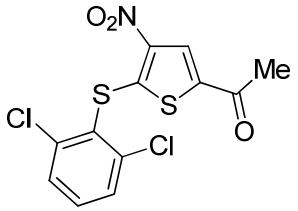


1-[5-(2,4-Dichlorophenylsulfanyl)-4-nitro-2-thienyl]ethanone (3). Using the method for **1**, **15** (166 mg, 0.8 mmol) and 2,4- dichlorobenzenethiol gave **3** (132 mg, 47.3%) as a pale orange solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09 (s, 1H, CH), 7.68 (m, 2H, ArH), 7.42 (d, 1H, ArH), 2.50 (s, 3H, CH_3); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_3S_2$, 347.9323, found 347.9450.

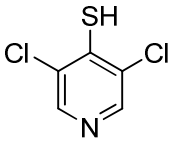


1-[5-(2,4-Difluorophenylsulfanyl)-4-nitro-2-thienyl]ethanone (4). Using the method for **1**, **15** (205 mg, 1.0 mmol) and 2,4-difluorobenzenethiol gave **4** (205 mg, 65%) as a cream colored solid. ^{1}H NMR

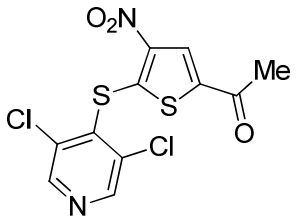
(DMSO-d_6, 400 MHz) δ 8.50 (s, 1H, CH), 7.97 (td, J = 8.5, 6.4 Hz, 1H, ArH), 7.69 (td, J = 9.5, 2.5 Hz, 1H, ArH), 7.43-7.37 (m, 1H, ArH), 2.51 (s, 3H, CH_3); HRMS (ESI) (m/z) $[M\text{-}CH_3CO]^-$ calc'd. for $C_{10}H_4F_2NO_2S_2$, 271.9652, found 271.9647.



1-[5-(2,6-Dichlorophenylsulfanyl)-4-nitro-2-thienyl]ethanone (5). Using the method of **1**, **15** (500 mg, 2.43 mmol) and 2,6-dichlorobenzenethiol gave **5** (0.44 g, 52%) as a pale orange solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.11 (s, 1H), 7.59-7.44 (m, 3H), 2.50 (s, 3H); HRMS (ESI) (m/z) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_3S_2$, 347.9323, found 347.9351.

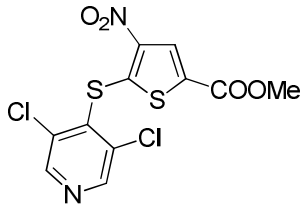


3,5-Dichloropyridine-4-thiol (17). 3,4,5-Trichloropyridine (0.47 g, 2.62 mmol) was added to a suspension of sodium hydrosulfide hydrate (0.24 g, 3.14 mmol) in 10 ml of anhydrous methanol and the mixture was stirred at 35 ^{o}C for 17 hours and then filtered. Concentration of the filtrate gave a residue which on trituration with dichloromethane gave a colorless solid (0.40 g, 76%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.88 (s, 2H); MS (ESI) (m/z) $[MH]^+$ calc'd for $C_5H_3Cl_2NS$, 179.9, found 179.9.

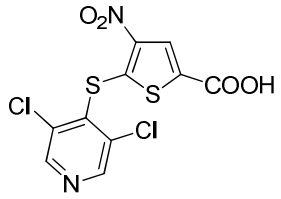


1-[5-(3,5-Dichloro-4-pyridylsulfanyl)-4-nitro-2-thienyl]ethanone (6). A suspension of **15** (100 mg, 0.55 mmol) and potassium carbonate (113 mg, 0.825 mmol) in 2 ml of toluene was stirred at ambient temperature for 18 hours and then filtered. The filtrate was diluted with 15 ml of ethyl acetate, washed twice with 2 ml of water and 2 ml of brine, and then concentrated under vacuum. The residue was purified by silica gel chromatography (hexane/EtOAc 25:1) to obtain a solid (63 mg, 37%). ^{1}H NMR

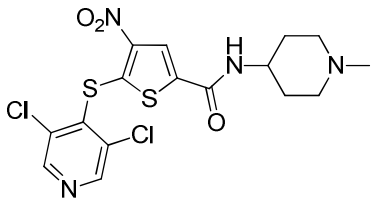
(400 MHz, CD_3OD) δ 8.85 (m, 2H), 8.36 (s, 1H), 2.52 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{11}H_7Cl_2N_2O_3S_2$, 348.9275, found 348.9268.



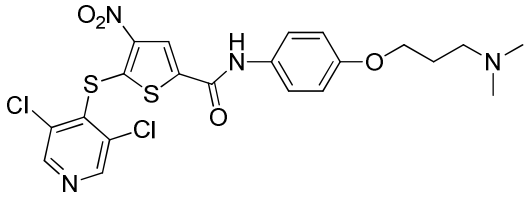
Methyl 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitrothiophene-2-carboxylate (18). A mixture of methyl 5-chloro-4-nitrothiophene-2-carboxylate **16 (**221 mg, 1.0 mmol), **17** (179 mg, 1.0 mmol) and potassium carbonate (207 mg, 1.5 mmol) in 5 ml of toluene was stirred at ambient temperature for 4 hours. The reaction mixture was then filtered, the filtrate diluted with ethyl acetate and washed twice with 2 ml of water, then twice with 2 ml of brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by silica gel chromatography (hexane/EtOAc 25:1) to give **18** (164 mg, 23%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.05 (s, 2H), 8.20 (s, 1H), 3.82 (s, 3H); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{11}H_7Cl_2N_2O_4S_2$, 364.9, found 365.0.



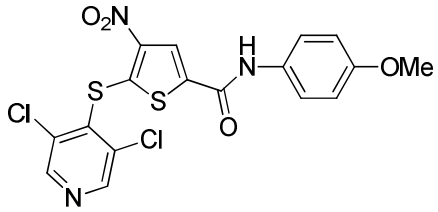
5-[(3,5-Dichloro-4-pyridyl)sulfanyl]-4-nitrothiophene-2-carboxylic acid. A mixture of **18** (275 mg, 0.75 mmol) and aqueous sodium hydroxide (1 N, 1.5 ml) in 5 ml of tetrahydrofuran was stirred at ambient temperature for 4 hours. Water (4 ml) was added, and the solution washed twice with 5 ml of ethyl ether. Aqueous HCl was added to bring the pH to 5 and the mixture extracted 5 times with 5 ml of ethyl acetate. The extract was dried over sodium sulfate and concentrated under vacuum to give a colorless solid (123 mg, 47%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.0 (s, 2H), 8.1 (s, 1H); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{10}H_5Cl_2N_2O_4S_2$, 350.9, found 350.9.



5-[(3,5-Dichloro-4-pyridyl)sulfanyl]-*N*-(1-methylpiperidin-4-yl)-4-nitrothiophene-2-carboxamide (7). O-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) (76 mg, 0.51 mmol), 1-methylpiperidine-4-amine (58 mg, 0.51 mmol), and diisopropylethylamine (20 µL, 0.12 mmol) were added to a suspension of 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitrothiophene-2-carboxylic acid (150 mg, 0.43 mmol) in 3 ml of dichloromethane. The mixture was stirred at ambient temperature for 6 hours, diluted with dichloromethane, washed with water and brine, and then dried over sodium sulfate. After being concentrated, the residue was purified by silica gel chromatography to give a solid (95 mg, 50%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.98 (m, 1H), 8.65 (m, 1H), 8.49 (m, 1H), 3.60 (m, 1H), 2.78 (m, 2H), 2.18 (s, 3H) 1.98 (m, 2H), 1.75 (m, 2H), 1.53 (m, 2H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_{17}Cl_2N_4O_3S_2$, 447.0119, found 447.0199.

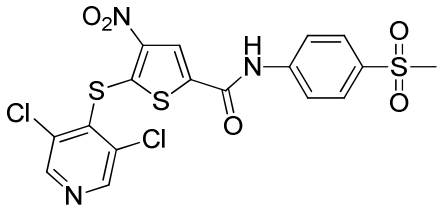


5-[(3,5-Dichloro-4-pyridyl)sulfanyl]-*N*-[4-(3-dimethylamino-propoxy)phenyl]-4-nitrothiophene-2-carboxamide (9). Chloro-*N,N,N',N'*-tetramethyluronium hexachloroantimonate (ACTU) (52 mg, 0.11 mmol) was added to a suspension of 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitrothiophene-2-carboxylic acid (35 mg, 0.1 mmol) in 1 ml of dichloromethane and the mixture stirred for 10 minutes. A solution of triethylamine (16 µL, 0.12 mmol) and *N*-(4-(3-dimethylamino-propoxy)aniline (21.6 mg, 0.12 mmol) in 0.5 ml of dichloromethane added and the mixture stirred at ambient temperature for 18 hours. Filtration through an Isolate™ PE-AX cartridge (to remove residual amines) and concentration gave a residue which was purified by reverse phase HPLC chromatography using a linear gradient of 10-95% CH_3CN over 10 minutes to give a solid (17 mg, 33%). HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{21}H_{21}Cl_2N_4O_4S_2$, 527.0381, found 527.0398.

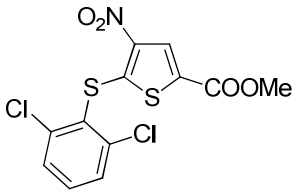


5-[(3,5-Dichloro-4-pyridyl)sulfanyl]-*N*-(4-methoxyphenyl)-4-nitrothiophene-2-carboxamide (10). Oxalyl chloride (0.93 g, 3.92 mmol) and 0.1 ml of *N,N*-dimethylformamide were added dropwise to a

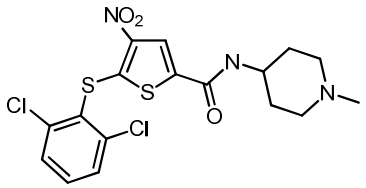
suspension of 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitrothiophene-2-carboxylic acid (0.60 g, 1.71 mmol) in 30 ml of dichloromethane and the mixture stirred at ambient temperature for 8 hours. Concentration of the mixture under vacuum gave the acid chloride as yellow oil. A 120 mg portion (0.33 mmol) of this oil was dissolved in 10 ml of dry tetrahydrofuran, chilled to 0 ^{o} C, and p-anisidine (96 mg, 0.39 mmol) and triethylamine (44 mg, 0.44 mmol) were added. The mixture was stirred at ambient temperature for 3 hours, diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography to give a solid (42 mg, 23%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.43 (s, 1H), 9.00 (s, 2H), 8.67 (s, 1H), 7.56 (m, 2H), 6.94 (m, 2H), 3.73 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{12}Cl_2N_3O_4S_2$, 455.9646, found 455.9652.



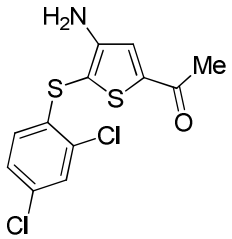
5-[(3,5-Dichloro-4-pyridyl)sulfanyl]-*N*-(4-methylsulfonyl)phenyl)-4-nitrothiophene-2-carboxamide (11). Oxalyl chloride (0.93 g, 3.92 mmol) and 0.1 ml of DMF was added dropwise to a suspension of 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitrothiophene-2-carboxylic acid (0.60 g, 1.71 mmol) in 30 ml of dichloromethane and the mixture stirred at ambient temperature for 8 hours. The mixture was concentrated under vacuum to give the acid chloride as a yellow solid. A solution of acid chloride (120 mg, 0.33 mmol) in 2 ml of dry THF was cooled to 0 ^{o}C and 4-methylsulfonylaniline (66 mg, 0.39 mmol) and triethylamine (13 mg, 0.13 mmol) added. The mixture was stirred at ambient temperature for 3 hours, then diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over sodium sulfate and concentrated to give an oil, which was purified by silica gel chromatography to give a yellow solid (60 mg, 37%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.88 (m, 1H), 9.01 (s, 2H), 8.87 (s, 1H), 7.96 (s, 4H), 3.20 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{12}Cl_2N_3O_5S_3$, 503.9316, found 503.9311.



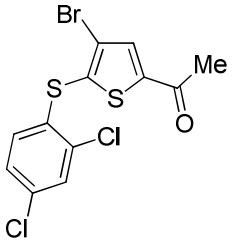
Methyl 5-[(2.6-dichlorophenyl)sulfanyl]-4-nitrothiophene-2-carboxylate. Compound **16** (5.0 g, 22.7 mmol) and 2,6-dichlorothiophenol (4.4 g, 22.7 mmol) were treated as described for **18** to give an off-white solid (5.5 g, 67%). ^{1}H NMR (DMSO-d_6, 400 MHz,) δ 8.17 (s, 1H), 7.83 (m, 2H), 7.62 (m, 1H), 3.77 (s, 3H); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_4S_2$, 363.9, found 363.9.



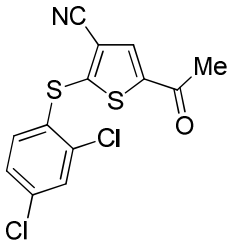
5-[(2,6-Dichlorophenyl)sulfanyl]-*N*-1-methylpiperidin-4-yl)-4-nitrothiophene-2-carboxamide (8). Methyl 5-[(2.6-dichlorophenyl)sulfanyl]-4-nitrothiophene-2-carboxylate (0.2 g, 0.95 mmol) was added to a mixture of 2 ml of concentrated sulfuric acid and 2 ml of water and stirred for 5 hours at 100 ^{o}C. The mixture was poured over 1 g of ice and stirred for 20 minutes to give a solid which was collected by filtration, washed with water and dried under vacuum at 40 ^{o}C to give a colorless solid (0.23 g, 70%). Reaction of this free acid (0.2 g, 0.57 mmol) and 1-methylpiperidine 4-amine by the procedure used to obtain **7** gave a solid (0.19 g, 75%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (m, 1H), 8.47 (m, 1H), 7.82 (m, 2H), 7.72 (m, 1H), 3.57 (m, 1H), 2.72 (m, 2H), 2.14 (s, 3H), 1.91 (m, 2H), 1.70 (m, 2H), 1.54 (m, 2H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{18}Cl_2N_3O_3S_2$, 446.0167, found 446.0130.



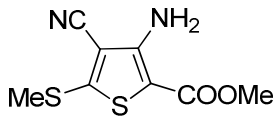
1-[4-Amino-5-(2,4-dichlorophenyl)sulfanyl-2-thienyl]ethanone (19). A mixture of 1-[5-(2,4-dichlorophenyl)sulfanyl-4-nitro-2-thienyl]ethanone (**3**) (7.0 g, 20.2 mmol), 50 ml of ethanol, 50 ml of water, ammonium chloride (4.32 g, 80.8 mmol) and iron powder (4.53 g, 80.8 mmol) was heated at 80 ^{o}C for 2 hours. The reaction mixture was filtered through celite, the filtrate concentrated under vacuum, water added to the residue, and the mixture extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated to give a solid (6.4 g, 78%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.64 (s, 1H), 7.44 (s, 1H) 7.37 (m, 1H), 6.68 (m, 1H), 2.58 (s, 3H).



1-[4-Bromo-5-(2,4-dichlorophenyl)sulfanyl-2-thienyl]ethanone (20). t-Butylnitrite (0.89 g, 8.6 mmol) was slowly added to a solution of copper (I) bromide (0.45 g, 3.14 mmol) in 25 ml of acetonitrile. The mixture was heated at 60 ^{o}C for 20 minutes, and a solution of **19** (1.0 g, 3.14 mmol) in 25 ml of acetonitrile added dropwise, and the reaction mixture heated at 60 ^{o}C for 2 hours. The reaction mixture was made basic by addition of 1*N* aqueous sodium hydroxide, and extracted with ethyl acetate. After concentration, the residue was purified by silica gel chromatography (hexane/EtOAc 50:1) to give a yellow solid (0.7 g, 58 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.83 (m, 1H), 7.47 (dd, 1H), 7.41 (m, 1H), 2.55 (s, 3H); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8BrCl_2OS_2$, 380.8, found 380.8.

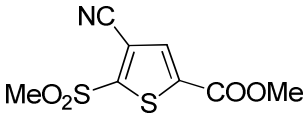


1-[4-Cyano-5-(2,4-dichlorophenyl)sulfanyl-2-thienyl]ethanone (12). Copper (I) cyanide (0.85 g, 9.4 mmol) and **20** (0.6 g, 1.6 mmol) dissolved in pyridine was irradiated (200 W) in a microwave oven at 150 ^{o}C for 30 minutes. Saturated aqueous copper (II) sulfate (20 ml) was added and the mixture extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography (hexane/EtOAc 25:1) to give **12** (0.52 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.56 (m, 1H), 7.53 (m, 1H), 7.33 (m, 1H), 2.51 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{13}H_8C_{l2}NOS_2$, 327.9424, found 327.9487.

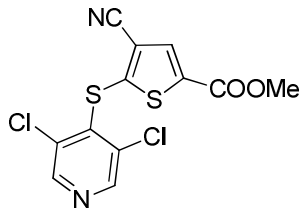


Methyl 3-amino-4-cyano-5-(methylthio)thiophene-2-carboxylate (21). 2-[Bis(methylthio)methylene] malononitrile (1.5 g, 8.8 mmol) was dissolved in 15 mL of methanol in a microwave tube. Methyl 2-mercaptoacetate (0.87 mL, 9.7 mmol) and triethylamine (1.35 mL, 9.7 mmol) were added and the

reaction mixture heated using a microwave apparatus at 130 °C for 4 minutes. The reaction mixture was cooled to room temperature and extracted four times with 50 mL of methanol. The combined extracts concentrated under vacuum to give a yellow solid (1.65 g, 82%) which was directly used for the next step without further purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.73 (s, 1H), 2.69 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_8H_8NO_4S_2$, 245.9895, found 245.9911;

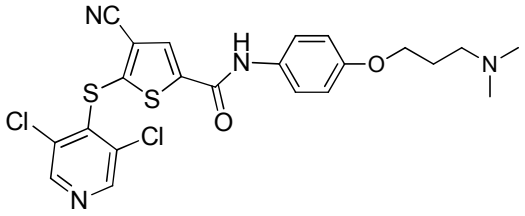


Methyl 4-cyano-5-(methylsulfonyl)thiophene-2-carboxylate (22). Isoamylnitrite (4 mL) was added dropwise to a solution of **21** (3.1 g, 13.6 mmol) in 40 mL of DMF at 65 °C while stirring. The reaction mixture was stirred at this temperature for 3 hours, after which ice water (80 mL) was added. The solid was collected by filtration, washed twice with 10 ml of water and then ethyl ether (10 mL). Drying under vacuum gave methyl 4-cyano-5-(methylthio)thiophene-2-carboxylate as a light yellow solid (2.61 g, 90%) which was used for the next step without further purification. *m*-Chloroperbenzoic acid (5.57 g, 22.6 mmol) was added over a 20 minute period while stirring at 0 °C to a solution of methyl 4-cyano-5-(methylthio)thiophene-2-carboxylate (2.4 g, 11.3 mmol) in 100 mL of CH_2Cl_2. The mixture was stirred for 30 minutes at 0 °C and then stirred overnight at ambient temperature. A saturated solution of sodium bicarbonate (50 mL) was added and the reaction was stirred for another 30 minutes. The organic layer was separated, washed with water and dried over $MgSO_4$. The residue was recrystallized to yield a light yellow solid (2.14 g, 78%) which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.84 (s, 1H), 3.91 (s, 3H), 3.58 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_8H_8NO_4S_2$, 245.9895, found 245.9911.

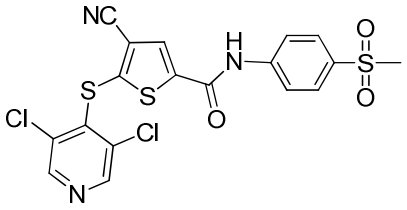


Methyl 4-Cyano-5-[(3,5-dichloropyridin-4-yl)thio]thiophene-2-carboxylate (23). Compound **22** (1.0 g, 4.0 mmol) was dissolved in 18 ml isopropyl alcohol in a microwave tube. 3,5-Dichloropyridine-4-thiol (720 mg, 4 mmol) and di-isopropylethylamine (3.3 mL, 20 mmol) were added. The reaction mixture was heated using microwave irradiation at 150 °C for 10 min. Concentration under vacuum gave a residue which was dissolved in 150 mL of ethyl acetate and the solution washed with 80 ml of

brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash silica gel chromatography (hexane/acetone 6:1) to yield a yellow solid (630 mg, 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (s, 2H), 8.19 (s, 1H), 3.81 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_7Cl_2N_2O_2S_2$, 344.9326, found 344.9395.

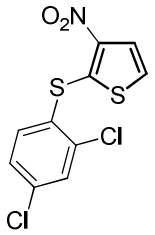


4-Cyano-5-[(3,5-dichloropyridin-4-yl)thio]-N-{4-[3-(dimethylamino)propoxy]phenyl} thiophene-2-carboxamide (13). A solution of 4-[3-(dimethylamino)propoxy]aniline (280 mg, 1.45 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of 2*N* $AlMe_3$ in toluene (2.18 ml, 4.35 mmol) while stirring at -10 ^{o}C under a nitrogen atmosphere. After the addition was completed, the reaction was allowed to warm to room temperature over a 45 minute period. A solution of **23** (0.50 g, 1.45 mmol) in CH_2Cl_2 (2 mL) was added and the reaction was heated under reflux overnight, cooled to room temperature, and hydrolyzed by slow addition of 1*N* hydrochloric acid (3.0 ml, 3.0 mmol). The mixture was stirred for 30 minutes, the organic layer separated, and the aqueous layer extracted with 3 times with 10 ml of CH_2Cl_2. The organic extracts were combined, washed with brine, dried with $MgSO_4$, and evaporated *in vacuo*. The residue was purified by preparative reverse phase HPLC using a linear gradient of 10-95% CH_3CN over 10 min with a flow rate of 20 mL/min. After lyophilization, pure product (330 mg, 45%) was obtained as a light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.83 (s, 2H), 8.26 (s, 1H), 7.58-7.55 (m, 2H), 6.97-6.93 (m, 2H), 4.04-4.01 (m, 2H), 3.23-3.20 (m, 2H), 2.77 (s, 6H), 2.11-2.06 (m, 2H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{22}H_{21}Cl_2N_4O_2S_2$, 507.0483, found 507.0583.

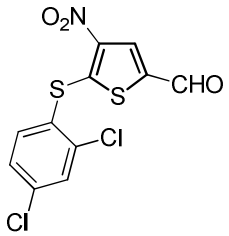


4-Cyano-5-[(3,5-dichloropyridin-4-yl)thio]-*N*-[4-(methylsulfonyl)phenyl]thiophene-2-carboxamide (14). A solution of 4-(methylsulfonyl)aniline (1.0 g, 5.8 mmol) in CH_2Cl_2 (2 mL) was added to a solution of 2*N* trimethylaluminum in toluene (4.35 mL, 8.7 mmol) while stirring at -10 ^{o}C under a nitrogen atmosphere and held at this temperature for twenty minutes. The reaction mixture was allowed

to warm to ambient temperature over a 45 minute period. A solution of **23** (1.0 g, 2.9 mmol) in CH_2Cl_2 (2 mL) was added and the reaction was heated at reflux overnight, cooled to room temperature, and hydrolyzed by slow addition of 1*N* hydrochloric acid (6.0 mL, 6.0 mmol). The mixture was stirred for 30 minutes, the organic layer was separated, and the aqueous layer was extracted 3 times 10 ml of CH_2Cl_2. The organic extracts were combined, washed with brine, dried with $MgSO_4$, and concentrated under vacuum. The residue was purified by preparative reverse phase HPLC using a linear gradient of 10-95% CH_3CN over 10 min with a flow rate of 20 mL/min. After lyophilization, pure product (447 mg, 32%) was obtained as a light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.84 (s, 2H), 8.34 (s, 1H), 7.91 (s, 4H), 3.18 (s, 3H); HRMS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{18}H_{12}Cl_2N_3O_3S_3$, 483.9418, found 483.9462.

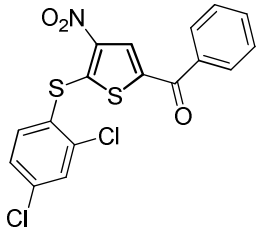


2-[(2,4-Dichlorophenyl)thio]-3-nitrothiophene (24). 2,4-Dichlorobenzenethiol (0.55 g, 3.05 mmol) was added to 0.1 M methanolic sodium methoxide solution (33.6 ml, 3.36 mmol) and the resulting solution was stirred for 15 minutes at ambient temperature. 2-chloro-3-nitrothiophene (0.5 g, 3.05 mmol) was added and the resulting mixture was stirred at ambient temperature for 2 hours. TLC analysis showed no starting material remained so the reaction was quenched by the addition of water (40 ml). The precipitate which formed was collected by filtration and dried in vacuo at 40 ºC overnight. The crude solid was crystallized from acetonitrile (20 ml) to afford **24** as a yellow solid (0.426 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (1H, d, *J* = 8.37 Hz, ArH), 7.63 (1H, d, *J* = 2.21 Hz, ArH), 7.57 (1H, d, *J* = 5.8 Hz, CH), 7.38 (1H, dd, *J* = 8.37, 2.21 Hz, ArH), 6.99 (1H, d, *J* = 5.8 Hz, CH).

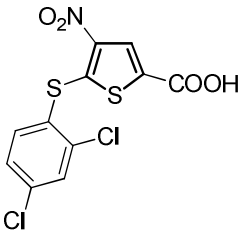


5-(2,4-Dichlorophenylsulfanyl)-4-nitrothiophene-2-carbaldehyde (25). To a suspension of potassium *tert*-butoxide (0.412 g, 3.67 mmol) in anhydrous tetrahydrofuran (20 mL), 2,4-dichlorobenzenethiol (0.6 g, 3.34 mmol) was added. The resulting mixture was stirred at ambient temperature for 15 minutes. A

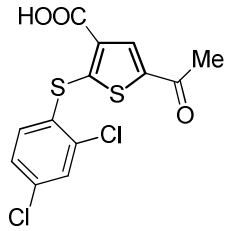
solution of 5-chloro-4-nitrothiophene-2-carbaldehyde (0.64 g, 3.34 mmol) in anhydrous tetrahydrofuran (5 ml) was added and the reaction mixture was stirred at ambient temperature for 2 hours. Then the above mixture was further heated under reflux for 2 hours. The reaction was cooled to ambient temperature and was poured into water (150 mL). The product was extracted into dichloromethane (3 x 100 mL). The organic layer was separated, washed with saturated aqueous sodium chloride solution (2 x 250 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a sticky orange solid. The crude solid was purified by silica gel chromatography (hexane/EtOAc 4:1) to afford the title product as a yellow solid (0.92 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.70 (1H, s), 8.19 (1H, s), 7.70 (1H, d, *J* = 8.37 Hz), 7.68 (1H, d, *J* = 2.21 Hz), 7.44 (1H, dd, *J* = 8.37, 2.21 Hz); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{11}H_6Cl_2NO_3S_2$, 333.9, found 333.9.



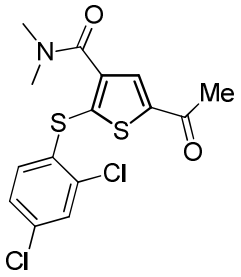
{5-[(2,4-dichlorophenyl)thio]-4-nitrothiophen-2-yl}(phenyl)methanone (26). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.11-8.10 (1H, m), 8.05-8.02 (2H, m), 7.87-7.84 (2H, m), 7.75-7.70 (2H, m), 7.61-7.57 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{10}Cl_2NO_3S_2$, 409.9, found 409.9.



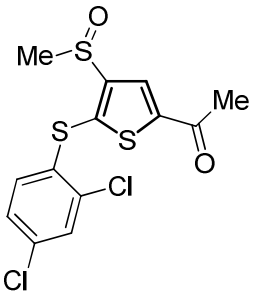
5-[(2,4-dichlorophenyl)thio]-4-nitrothiophene-2-carboxylic acid (27). ^{1}H NMR (400MHz, DMSO-*d*$_6$) δ 8.08-8.05 (2H, m), 7.99 (1H, d, *J* = 8.0 Hz), 7.71 (1H, dd, *J* = 8.0, 2.0 Hz); MS (ESI) (*m/z*) $[M-H]^-$ calc'd for $C_{11}H_4Cl_2NO_4S_2$, 347.9, found 347.9.



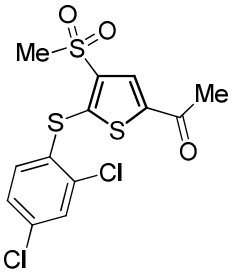
5-Acetyl-2-[(2,4-dichlorophenyl)thio]thiophene-3-carboxylic acid (28). ^{1}H NMR (400MHz, DMSO-*d*$_6$) δ 8.07 (1H, s), 7.99 (1H, d, *J* = 2.4 Hz), 7.90 (1H, d, *J* = 8.4 Hz), 7.64 (1H, dd, *J* = 8.4, 2.4 Hz). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{13}H_9Cl_2O_3S_2$, 346.9, found 346.9.



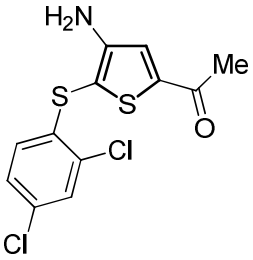
5-Acetyl-2-[(2,4-dichlorophenyl)thio]-*N,N*-dimethylthiophene-3-carboxamide (29). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, s), 7.45 (1H, d, *J* = 2.0 Hz), 7.32-7.30 (1H, m), 7.24-7.20 (1H, m), 3.05 (6H, s), 2.50 (3H, s). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{15}H_{14}Cl_2NO_2S_2$, 373.9, found 373.9.



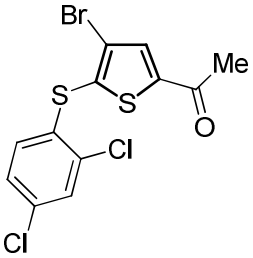
1-{5-[(2,4-Dichlorophenyl)thio]-4-(methylsulfinyl)thiophen-2-yl}ethanone (30). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.35 (1H, s), 7.86-7.85 (1H, m), 7.50 (1H, dd, *J* = 8.4, 2.0 Hz), 7.34 (1H, d, *J* = 8.4 Hz), 2.92 (3H, s), 2.58 (3H, s); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{13}H_{11}Cl_2O_2S_3$, 364.9, found 364.9.



1-{5-[(2,4-Dichlorophenyl)thio]-4-(methylsulfonyl)thiophen-2-yl}ethanone (31). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (1H, s), 7.80 (1H, d, *J* = 8.4 Hz), 7.78 (1H, d, *J* = 2.4 Hz), 7.53 (1H, dd, *J* = 8.4, 2.4 Hz), 3.30 (3H, s), 2.49 (3H, s); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{13}H_{11}Cl_2O_3S_3$, 380.9, found 380.9.

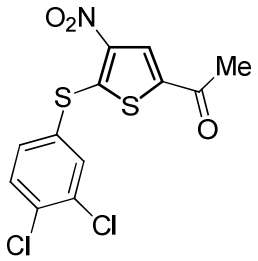


1-{4-Amino-5-[(2,4-dichlorophenyl)thio]thiophen-2-yl}ethanone (32). To a two necked 250 mL round bottom flask charged with 1-[5-(2,4-Dichlorophenylsulfanyl)-4-nitro-2-thienyl]ethanone (7.0 g, 20.2 mmol), 50 ml ethanol and 50 ml of water followed by ammonium chloride (4.32 g, 80.8 mmol) and iron powder (4.53 g, 80.8 mmol). The resulting reaction mixture was heated at 80 °C for 2 hours. The reaction mixture was then cooled to ambient temperature and was passed through a celite cartridge. The filtrate was concentrated and water was added to the residue and extracted with ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered and concentrated to afford the title product (6.4 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (1H, s), 7.44 (1H, s), 7.37 (1H, m), 6.68 (1H, m), 2.50 (3H, s). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_{10}Cl_2NOS_2$, 317.9, found 317.9.

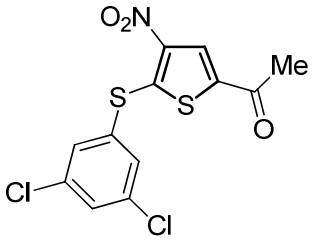


1-{4-Bromo-5-[(2,4-dichlorophenyl)thio]thiophen-2-yl}ethanone (33). To a dark solution of copper(I) bromide (0.451 g 3.14 mmol) in acetonitrile (25 mL) was added t-butyl nitrite (0.892 g, 8.6 mmol) slowly. The resulting solution was heated at 60° C for 20 min. To the above solution, was added dropwise a solution of 1-[4-amino-5-(2,4-dichlorophenyl)sulfanyl-2-thienyl]ethanone (1.0 g, 3.14

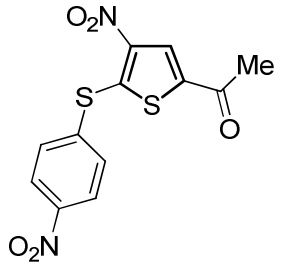
mmol) in acetonitrile (25 mL). The resulting mixture was heated to 60° C for 2 hours. The mixture was then quenched with a sodium hydroxide aqueous solution (1 M), extracted with ethyl acetate. The organic layer was separated, dried over Na_2SO_4 and concentrated. The crude solid was purified by silica gel chromatography (hexane/EtOAc 50:1) to afford the title product as a yellow solid (0.7 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (1H, s), 7.83 (1H, m), 7.47 (1H, dd), 7.11 (1H, m), 2.55 (3H, s); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8BrCl_2OS_2$, 380.8, found 380.8.



1-{5-[(3,4-Dichlorophenyl)thio]-4-nitrothiophen-2-yl}ethanone (34). Using the method for **1**, **15** (502 mg, 2.45 mmol) and 3,4-dichlorobenzenethiol gave **34** (631 mg, 75%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, s), 7.80 (1H, s), 7.70 (1H, d), 7.50 (1H, d), 2.50 (3H, s). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_3S_2$, 347.9, found 347.9.

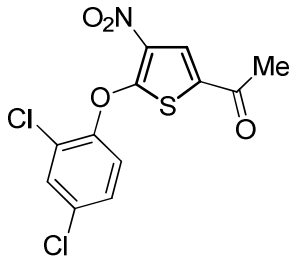


1-{5-[(3,5-Dichlorophenyl)thio]-4-nitrothiophen-2-yl}ethanone (35). Using the method for **1**, **15** (502 mg, 2.45 mmol) and 3,5-dichlorobenzenethiol gave **35** (403 mg, 48%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (1H, s), 7.57 (3H, m), 2.51 (3H, s). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_3S_2$, 347.9, found 347.9.

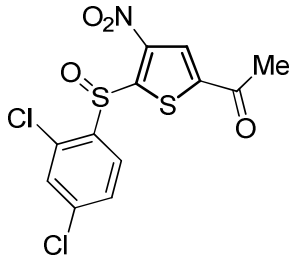


1-{4-Nitro-5-[(4-nitrophenyl)thio]thiophen-2-yl}ethanone (36). Anhydrous *N,N*-dimethylformide (5 mL) was degassed with nitrogen for 30 minutes then sodium hydride (60% dispersion in mineral oil, 0.11 g, 2.75 mmol) was added. 4-Nitrobenzenethiol (0.38 g, 2.43 mmol) was added in portions at ambient temperature and the resulting mixture was stirred at ambient temperature under nitrogen for 20

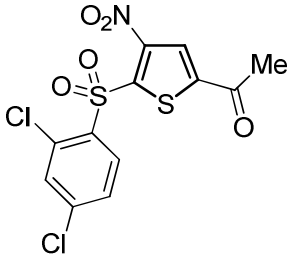
minutes. 1-(5-Chloro-4-nitro-2-thienyl)ethanone (0.5 g, 2.43 mmol) was then added and the reaction mixture was stirred at ambient temperature overnight under nitrogen. The reaction mixture was diluted with water (40 mL) and stirred for 30 minutes. The precipitated solid was collected by filtration and washed with water (2 x 4 mL). The crude solid was recrystallized from acetonitrile (12 mL) to afford the title compound as a brown solid (0.302 g, 38 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (2H, d, *J* = 8.85 Hz), 8.09 (1H, s), 7.89 (2H, d, *J* = 8.85 Hz), 2.50 (3H, s).



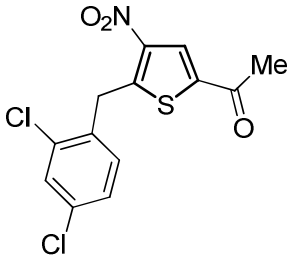
1-[5-(2,4-Dichlorophenoxy)-4-nitrothiophen-2-yl]ethanone (37). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_4S$, 331.9, found 331.9.



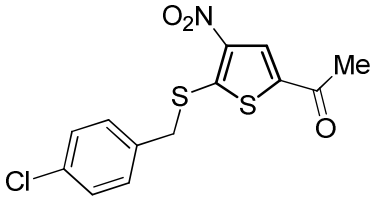
1-[5-(2,4-Dichlorophenyl)sulfinyl-4-nitro-2-thienyl]ethanone (38). To a solution of 1-[5-(2,4-dichlorophenylsulfanyl)-4-nitro-2-thienyl]ethanone (200 mg, 0.58 mmol) in dichloromethane (5 mL), *m*-chloroperoxybenzoic acid (198 mg, 1.14 mmol) was added slowly at 0 °C. The mixture was stirred at ambient temperature for 10 hours. The mixture was then diluted with dichloromethane (25 mL) and washed with aqueous sodium bicarbonate solution (3 x 10 mL), then water (2 x 10 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4, concentrated under vacuum to afford a yellow solid as the title product (0.14 g, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (1H, s), 7.96 (1H, d), 7.63 (2H, m), 2.66 (3H, s); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_8Cl_2NO_4S_2$, 363.9, found 363.9.



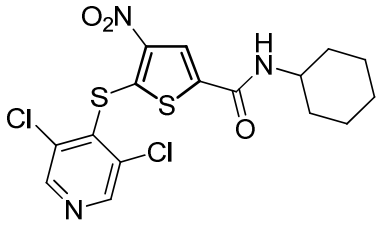
1-[5-(2,4-Dichlorophenyl)sulfonyl-4-nitro-2-thienyl]ethanone (39). To a solution of 1-[5-(2,4-dichlorophenyl)sulfinyl-4-nitro-2-thienyl]ethanone (100 mg, 0.27 mmol) in dichloromethane (2 mL), *m*-chloroperoxybenzoic acid (95 mg, 0.55 mmol) was added slowly at 0° C. The mixture was stirred at ambient temperature for 10 hours. Then the mixture was diluted with dichloromethane (10 mL) and washed with aqueous sodium bicarbonate solution (3 x 5 mL), then water (2 x 5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4, concentrated under vacuum and the residue was purified by chromatography to afford the title product (75 mg, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (1H, s), 8.32 (1H, d), 8.0 (1H, s), 7.87 (1H, m), 2.67 (3H, s).



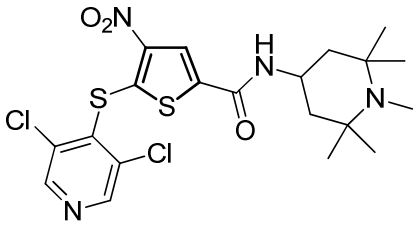
1-[5-(2,4-Dichlorobenzyl)-4-nitrothiophen-2-yl]ethanone (40). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (1H, s), 7.47 (1H, d, *J* = 2.0 Hz), 7.27 (1H, dd, *J* = 8.0, 2.0 Hz), 7.24 (1H, d, *J* = 8.0 Hz), 4.68 (s, 2H), 2.54 (s, 3H); MS (ESI) (*m/z*) $[M-H]^-$ calc'd for $C_{13}H_8Cl_2NO_3S$, 328.0, found 328.1.



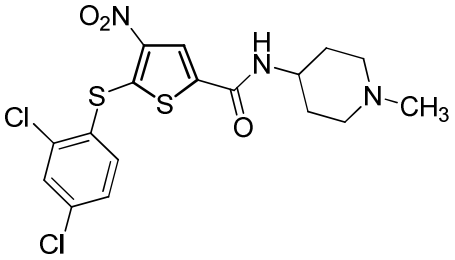
1-{5-[(4-Chlorobenzyl)thio]-4-nitrothiophen-2-yl}ethanone (41). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (1H, s), 7.57-7.54 (2H, m), 7.48-7.44 (2H, m), 4.55 (2H, s), 2.55 (3H, s); MS (ESI) (*m/z*) $[M-H]^-$ calc'd for $C_{13}H_8ClNO_3S_2$, 326.0, found 326.1.



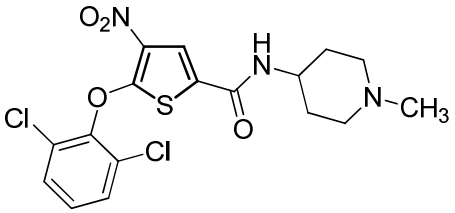
***N*-Cyclohexyl-5-[(3,5-dichloropyridin-4-yl)thio]-4-nitrothiophene-2-carboxamide (42).** Oxalyl chloride (0.934 g, 3.92 mmol) followed by *N,N*-dimethylformide (0.1 mL) was added dropwise to a suspension of 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (0.60 g, 1.71 mmol) in dichloromethane (30 mL) and the mixture was stirred at ambient temperature for 8 hours. The mixture was then allowed to be concentrated *in vacuo* to give a yellow solid. The resulting acid chloride solid was dissolved in dry tetrahydrofuran (10 mL). Cyclohexylamine (52 mg, 0.52 mmol) followed by triethylamine (0.066 g, 0.65 mmol) was added to a 2.5 mL aliquot of acid chloride solution in tetrahydrofuran at 0 ºC. The resulting mixture was stirred at ambient temperature for 3 hours. The mixture was then diluted with ethyl acetate, washed with water and brine. The organic layer was separated, dried over Na_2SO_4, filtered and the solvent was removed to afford a brown oil. The crude material was purified by silica gel chromatography (hexane/EtOAc 6:1) to afford the title product as a solid (76 mg, 34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (2H, s), 8.62 (1H, m), 8.50 (1H, s), 3.63 (1H, m), 1.71 (4H, m), 1.59 (1H, m), 1.24 (4H, m), 1.09 (1H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_{16}Cl_2N_3O_3S_2$, 431.9, found 432.0.



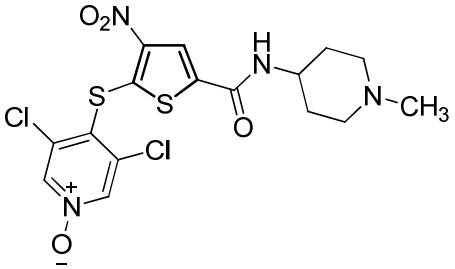
5-[(3,5-Dichloropyridin-4-yl)thio]-4-nitro-*N*-(1,2,2,6,6-pentamethylpiperidin-4-yl)thiophene-2-carboxamide (43). Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (150 mg, 0.43 mmol) and 2,2,6,6-tetramethylpiperidin-4-amine (81.0 mg, 0.51 mmol). The title compound was obtained as a solid (35.0 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.99 (2H, m), 8.81 (1H, m), 8.47 (1H, s), 7.81 (1H, m), 4.18 (1H, m), 1.96 (2H, m), 1.51 (2H, m), 1.39 (12H, m). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{20}H_{25}Cl_2N_4O_3S_2$, 503.0, found 503.0.



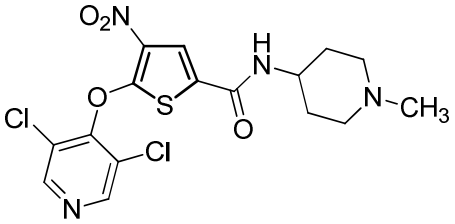
5-[(2,4-Dichlorophenyl)thio]-*N*-(1-methylpiperidin-4-yl)-4-nitrothiophene-2-carboxamide (44). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (1H, d, J = 7.6 Hz), 8.45 (1H, s), 8.04 (1H, d, J = 2.0 Hz), 7.97 (1H, d, J = 8.4 Hz), 7.69 (1H, dd, J = 8.4, 2.0 Hz), 3.65-3.55 (1H, m), 2.78-2.70 (2H, m), 2.15 (3H, s), 2.00-1.90 (2H, m), 1.75-1.68 (2H, m), 1.58-1.45 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{18}Cl_2N_3O_3S_2$, 446.0, found 446.0.



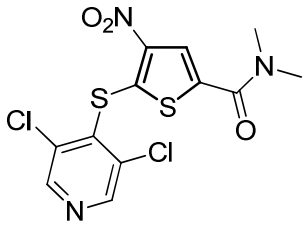
5-(2,6-Dichlorophenoxy)-*N*-(1-methylpiperidin-4-yl)-4-nitrothiophene-2-carboxamide (45). Prepared according to the procedure described for compound **7** from 5-(2,6-dichlorophenoxy)-4-nitrothiophene-2-carboxylic acid (70 mg, 0.27 mmol) and 1-methylpiperidin-4-amine (28.0 mg, 0.30 mmol). The title compound was obtained as a solid (30 mg, 26%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (1H, m), 8.39 (1H, s), 7.78 (2H, m), 7.56 (1H, m), 3.66 (1H, m), 2.85 (2H, m), 2.24 (3H, s), 1.78 (2H, m), 1.57 (2H, m), 1.23 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{18}Cl_2N_3O_4S$, 430.0, found 430.0.



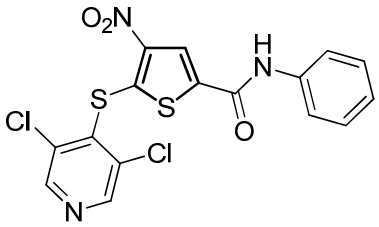
3,5-Dichloro-4-{[5-((1-methylpiperidin-4-yl)carbamoyl)-3-nitrothiophen-2-yl]thio}pyridine 1-oxide (46). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (2H, s), 8.67-8.65 (1H, m), 8.52-8.49 (1H, m), 3.65-3.55 (1H, m), 2.90-2.80 (2H, m), 2.24 (3H, s), 2.15-2.00 (1H, m), 1.77-1.74 (2H, m), 1.60-1.51 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_{17}Cl_2N_4O_4S_2$, 463.0, found 463.0.



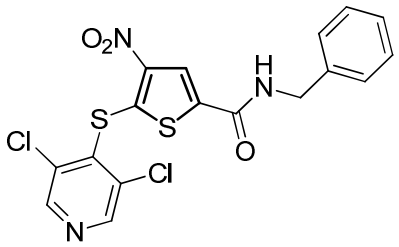
5-[(3,5-Dichloropyridin-4-yl)oxy]-*N*-(1-methylpiperidin-4-yl)-4-nitrothiophene-2-carboxamide (47). Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloropyridin-4-yl)oxy]-4-nitrothiophene-2-carboxylic acid (70 mg, 0.21 mmol) and 1-methylpiperidin-4-amine (28 mg, 0.25 mmol). The title compound was obtained as a solid (35.0 mg, 39%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (1H, m), 8.62 (2H, m), 8.57 (1H, s), 3.88 (1H, m), 3.12 (2H, m), 2.60 (2H, m), 2.50 (3H, s), 1.93 (2H, m), 1.77 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_{17}Cl_2N_4O_4S_2$, 463.0, found 463.0.



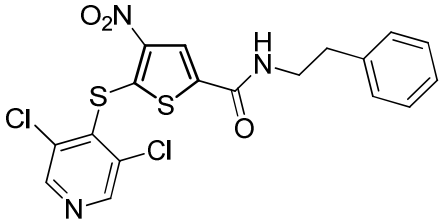
5-[(3,5-Dichloro-4-pyridyl)sulfanyl]-*N,N*-dimethyl-4-nitro-thiophene-2-carboxamide (48). Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (35 mg, 0.1 mmol) and dimethylamine (4.5 mg, 0.12 mmol). The title compound was obtained as a solid (6 mg, 16%). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{12}H_{10}Cl_2N_3O_3S_2$, 377.9, found 377.9.



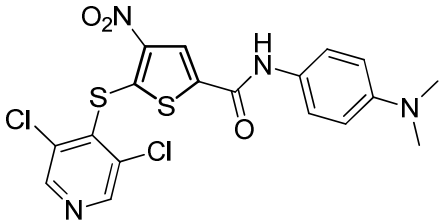
5-[(3,5-Dichloropyridin-4-yl)thio]-4-nitro-*N*-phenylthiophene-2-carboxamide (49). Prepared according to the procedure described for compound 7 from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (35 mg, 0.1 mmol) and aniline (11 mg, 0.12 mmol). The title compound was obtained as a solid (5 mg, 11%). MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_{10}Cl_2N_3O_3S_2$, 425.9, found 425.9.



***N*-Benzyl-5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxamide (50).** Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (39 mg, 0.11 mmol) and benzylamine (13 mg, 0.12 mmol). The title compound was obtained as a yellow solid (15 mg, 28%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (1H, s), 8.71 (1H, s), 8.35-8.39 (3H, m), 7.31 (2H, m), 7.27 (2H, m), 2.6 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{17}H_{12}Cl_2N_3O_3S_2$, 439.9, found 439.9.

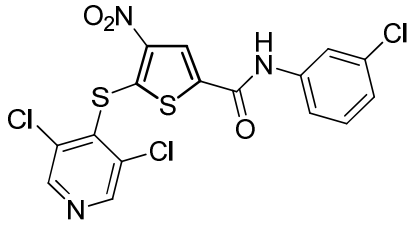


5-[(3,5-Dichloropyridin-4-yl)thio]-4-nitro-*N*-phenethylthiophene-2-carboxamide (51). Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (60 mg, 0.17 mmol) and phenethyl amine (78 mg, 0.68 mmol). The title compound was obtained as a yellow solid (70 mg, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (3H, m), 8.39 (1H, s), 7.27 (2H, m), 7.19 (3H, m), 3.42 (2H, m), 2.80 (2H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{18}H_{14}Cl_2N_3O_3S_2$, 453.9, found 453.9.

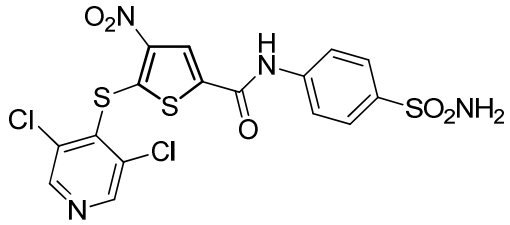


5-[(3,5-Dichloropyridin-4-yl)thio]-*N*-[4-(dimethylamino)phenyl]-4-nitrothiophene-2-carboxamide (52). Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carboxylic acid (200 mg, 0.57 mmol) and N, N'-dimethylbenzen-1,4-diamine (93 mg, 0.68 mmol). The title compound was obtained as a yellow solid (45 mg, 17%). ^{1}H

NMR (400 MHz, DMSO-d_6) δ 10.30 (1H, m), 8.99 (2H, m), 8.64 (1H, s), 7.45 (2H, m), 6.72 (2H, m), 2.86 (6H, s).



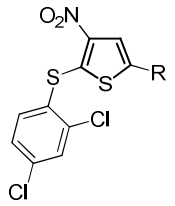
***N*-(3-Chlorophenyl)-5-[(3,5-dichloropyridin-4-yl)thio]-4-nitrothiophene-2-carboxamide (53).** Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbonyl chloride (120 mg, 0.33 mmol) and 3-chloroaniline (49 mg, 0.39 mmol). The title compound was obtained as a solid (70 mg, 47%). ^{1}H NMR (400 MHz, DMSO- d_6) δ 10.79 (1H, s), 9.01 (2H, s), 8.75 (1H, s), 7.86 (1H, s), 7.67 (1H, m), 7.39 (1H, m), 7.20 (1H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_9Cl_3N_3O_3S_2$, 459.9, found 459.9.



5-[(3,5-Dichloropyridin-4-yl)thio]-4-nitro-*N*-(4-sulfamoylphenyl)thiophene-2-carboxamide (54). Prepared according to the procedure described for compound **7** from 5-[(3,5-dichloro-4-pyridyl)sulfanyl]-4-nitro-thiophene-2-carbonyl chloride (120 mg, 0.33 mmol) and sulfanilide (84 mg, 0.39 mmol). The title compound was obtained as a solid (90 mg, 44%). ^{1}H NMR (400MHz, DMSO-d_6) δ 10.79 (1H, m), 9.01 (2H, s), 8.76 (1H, s), 7.81 (4H, s), 7.23 (2H, s), 7.40 (1H, m); MS (ESI) (*m/z*) $[MH]^+$ calc'd for $C_{16}H_{11}Cl_2N_4O_5S_3$, 504.9, found 504.9.

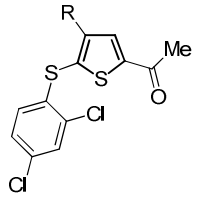
Supplemental Table 1

5-[(2,4-Dichlorophenyl)thio]-4-nitro-2-R-thiophenes



Compound	R	EC_{50} (μM)
24	-H	>31.6
25	-CHO	3.2
3	$-COCH_3$	6.9
26	$-COC_6H_5$	>31.6
27	-COOH	>31.6

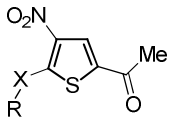
Supplemental Table 2
2-Acetyl-5-[(2,4-dichlorophenyl)thio]-R-thiophenes

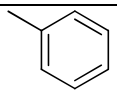
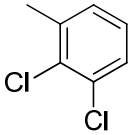
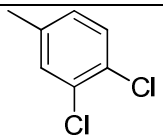
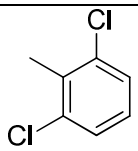
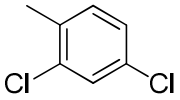
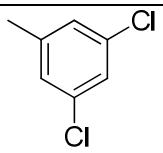
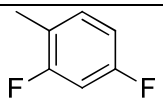
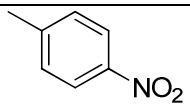
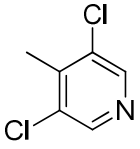


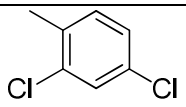
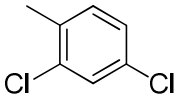
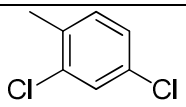
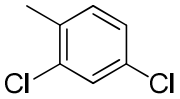
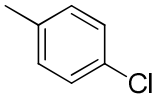
Compound	R	EC_{50} (μM)
12	-CN	19
28	-COOH	>31.6
29	$-CON(CH_3)_2$	>31.6
30	$-SOCH_3$	>31.6
31	$-SO_2CH_3$	>31.6
32	$-NH_2$	>31.6
33	-Br	>31.6

Supplemental Table 3

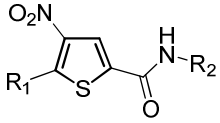
2-Acetyl-4-nitro-5-XR-thiophenes

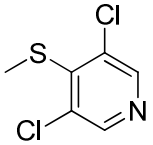
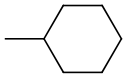
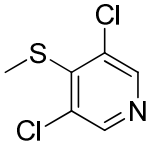
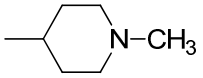
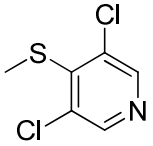
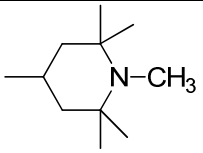
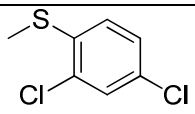
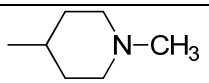


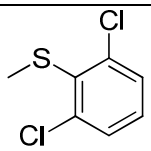
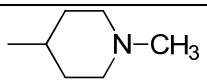
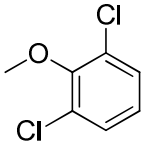
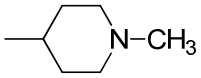
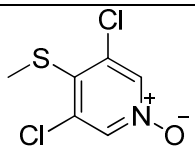
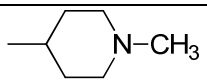
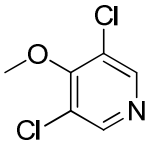
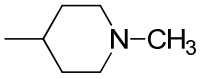
Progenra ID	R	X	EC_{50} (μM)
2		S	>31.6
1		S	4.2
34		S	3.7
5		S	2.2
3		S	6.9
35		S	6.4
4		S	8.0
36		S	7.6
6		S	2.0

37		O	3.3
38			21.2
39			12.2
40		CH_2	>31.6
41		CH_2S	>31.6

Supplemental Table 4
5-R_1-4-nitro-N-R_2-thiophene-2-carboxamides

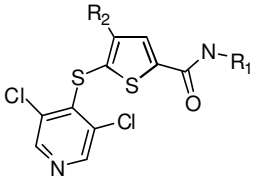


Compound	R_1	R_2	EC_{50} (μM)
42			4.0
7			1.7
43			0.91
44			5.8

8			7.8
45			>31.6
46			4.1
47			>31.6

Supplemental Table 5

5-[(3,5-Dichloropyridin-4-yl)thio]-4-R_2-N-R_1-thiophene-2-carboxamides



Compound	NR_1	R_2	EC_{50} (μM)
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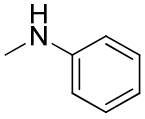
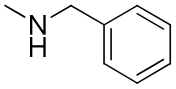
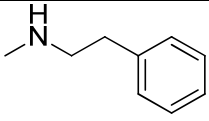
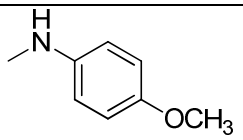
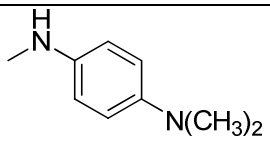
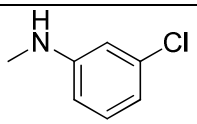
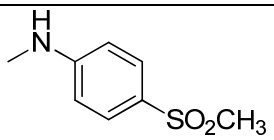
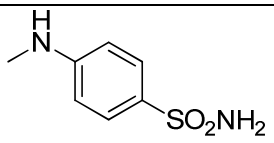
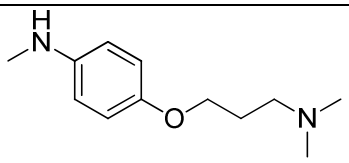
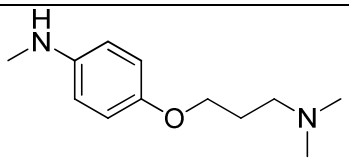
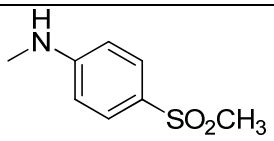
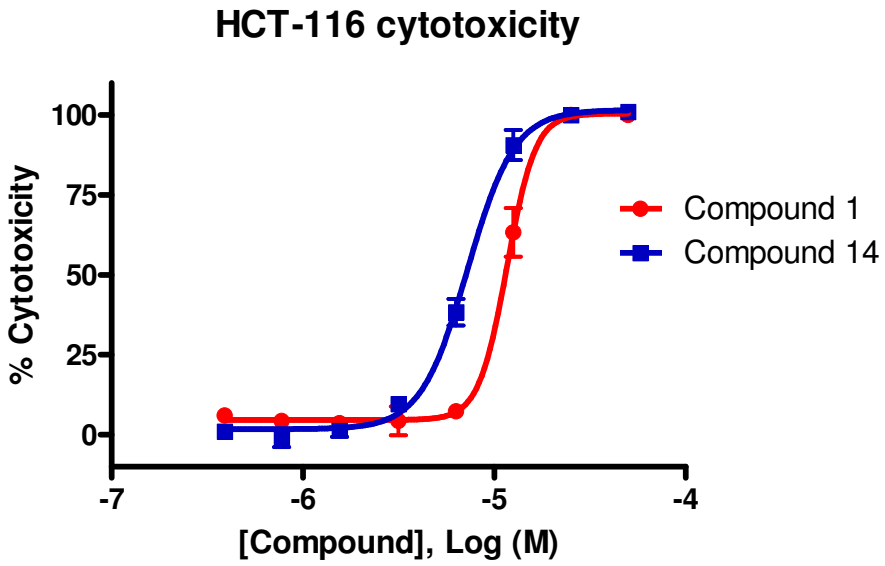
49		$-NO_2$	0.95
50		$-NO_2$	1.9
51		$-NO_2$	2.5

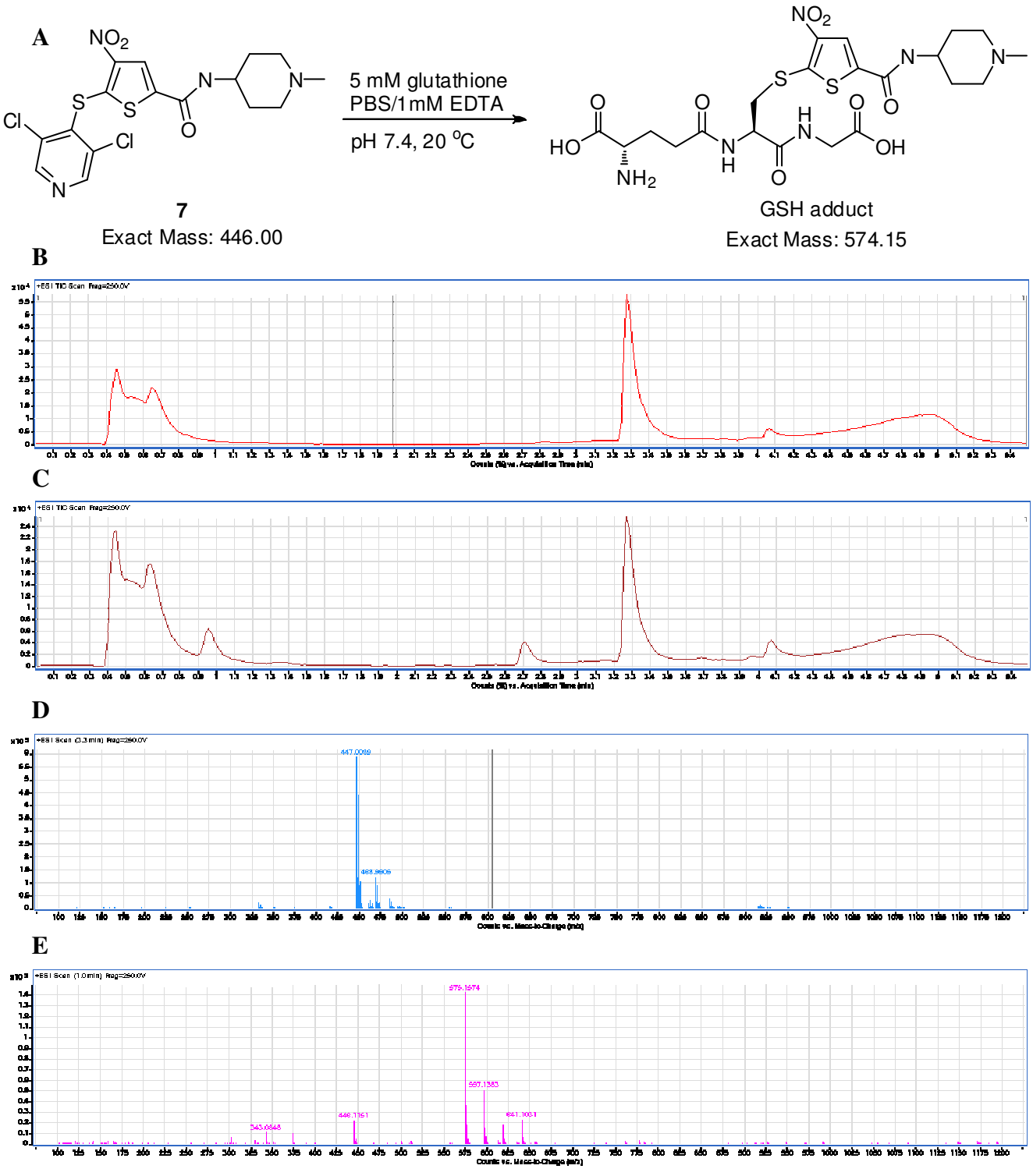
10		-NO_2	0.43
52		-NO_2	1.3
53		-NO_2	2.7
11		-NO_2	0.40
54		-NO_2	0.43
9		-NO_2	0.38
13		-CN	0.38
14		-CN	0.42

Supplemental Figure 1.



Supplemental Figure 1, HCT-116 cytotoxicity. HCT-116 cells were exposed to compound **1** or **14** for 72 hours. Compounds **1** and **14** inhibit HCT-116 growth with mean EC_{50} values of 11.4 and 7.6µM respectively. Representative curves are shown, Data shown are mean±SD of triplicate determinations.

Supplemental Figure 2



Supplemental Figure 2, GSH adduct detection with Compound **7**. A, Proposed reaction scheme; B, LC trace of the reaction mixture at 0 min (retention time of compound **7**: 3.3 min); C, LC trace of the reaction mixture at 24 min (retention time of GSH adduct: 1.0 min; retention time of compound

7: 3.3 min); D, MS trace of the peak at 3.3 min from trace B (observe 447.00, MW of compound 7: 446.00); E, MS trace of the peak at 1.0 min from trace B (observed 575.15, MW of GSH adduct: 574.15).

Supplemental Table 6. Biochemical characterization of compound **14**

Target	**EC_{50} (μM)**
USP2	>31.6
USP5	>31.6
USP7	0.42
USP8	>31.6
USP21	>31.6
USP28	>31.6
USP47	1.0
Caspase 3	>31.6
Cathepsin B	>31.6
20S CT-L	>31.6

Bioassay methods

***In vitro* enzyme activity**: The cloning, expression and purification of USP21 from BL21 (DE3) bacteria were performed using standard molecular biology techniques. USP2, USP5, USP7, USP8, USP28, USP47, Ub-PLA_2 (Ub-CHOP) and Ub-EK_L (Ub-CHOP2) were generated as described previously[1-3]. Caspase 3 and the caspase 3 substrate DEVD-Rh110 were purchased from R&D and Anaspec respectively. Deubiquitylating enzyme, cathepsin B and 20S proteasome chymotrypsin like protease activities were measured as described[1,2]. Caspase 3 activity was determined using a similar protocol. Briefly, dose ranges of compound were incubated with caspase 3 for 30 minutes before the addition of DEVD-Rh110 and reading on a fluorometric plate reader using excitation and emission maxima of 485nm and 531nm respectively. The final concentrations of caspase 3 and DEVD-Rh110 were 2nM and 100nM respectively.

GSH reactivity and plasma stability: GSH reactivity and plasma stability assessments were determined as previously detailed [4,5]. GSH adduct formation was determined by diluting compound **7** to a concentration of 133 μM in nitrogen purged PBS/1mM EDTA. The reaction was initiated by adding 20 mM reduced glutathione to the diluted compound solution. Aliquots of the reaction mixture were removed and analyzed by LC-MS. The amount of compound **7** remaining was quantified using the UV absorbance as well as the extracted ion count corresponding to the parent molecule.

Cell culture and immunoblotting: The maintenance of HCT-116 cells, cytotoxicity assessment and probing for relative expression of p53 and p21 were performed as described previously[2].

References

(1) Nicholson, B.; Leach, C. A.; Goldenberg, S. J.; Francis, D. M.; Kodrasov, M. P. et al. Characterization of ubiquitin and ubiquitin-like-protein isopeptidase activities. *Protein Sci* **2008**, *17*, 1035-1043.

(2) Altun, M.; Kramer, H. B.; Willems, L. I.; McDermott, J. L.; Leach, C. A. et al. Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. *Chem Biol* **2011**, *18*, 1401-1412.

(3) Tian, X.; Isamiddinova, N. S.; Peroutka, R. J.; Goldenberg, S. J.; Mattern, M. R. et al. Characterization of selective ubiquitin and ubiquitin-like protease inhibitors using a fluorescence-based multiplex assay format. *Assay Drug Dev Technol* **2011**, *9*, 165-173.

(4) MacFaul, P. A.; Morley, A. D.; Crawford, J. J. A simple in vitro assay for assessing the reactivity of nitrile containing compounds. *Bioorg Med Chem Lett* **2009**, *19*, 1136-1138.

(5) Di, L.; Kerns, E. H.; Hong, Y.; Chen, H. Development and application of high throughput plasma stability assay for drug discovery. *Int J Pharm* **2005**, *297*, 110-119.