Identification of an Orally Efficacious Matrix Metalloprotease 12 Inhibitor for Potential Treatment of Asthma

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I. Supporting Info: Biological Assays

a. Determination of IC_{50} against human MMP-12.

The assays for human MMP-12 and MMP-13 activity were performed by incubating 20 μM of the fluorogenic peptide substrate MCA-Pro-Leu-Gly-Leu-Dpa(DNP)-Ala-Arg (Anaspec, San Jose, CA) with 0.5 nM recombinant human MMP-12 or MMP-13 catalytic domain along with various concentrations of compound in 50 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM $CaCl_2$, 0.005 % Brij-35 and 10% DMSO. The rate of increase in fluorescent signal was measured on a Safire plate reader (Tecan, Männedorf, Switzerland) exciting at a wavelength of 325 nm and measuring at an emission wavelength of 395 nm. The enzymes were expressed in *E. coli*, refolded from insoluble inclusion bodies, and purified.

b. Determination of IC_{50} against mouse MMP-12.

The assay to measure potency in mouse MMP-12 catalytic domain was identical in format to the assay used for the human MMP enzymes with the exception that 3 nM mouse MMP-12 was required to obtain similar catalytic rates. Cleavage of 20 μM of the MCA-Pro-Leu-Gly-Leu-Dpa (DNP)-Ala-Arg peptide was measured over time, monitoring λ_{ex}: 325 nm and λ_{em}: 395 (Tecan Safire 2, Tecan, Männedorf, Switzerland).

c. Determination of IC_{50} against sheep MMP-12.

The assay to measure inhibitor potency in sheep MMP-12 catalytic domain was identical in format to the assay used for the human MMP enzymes. The concentration of sheep MMP-12 in the assay was 0.5 nM. Cleavage of 20 μM of the MCA-Pro-Leu-Gly-Leu-Dpa (DNP)-Ala-Arg peptide was measured over time, monitoring λ_{ex}: 325 nm and λ_{em}: 395 nm.

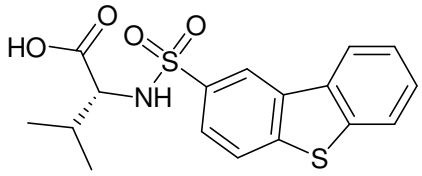
d. Evaluation of the efficacy of a MMP-12 specific inhibitor in a sheep asthma model.

Compounds were dosed either intravenously or per oral route twice daily, the day before challenge, and then, the following day (day of *Ascaris suum* challenge) 1 hour

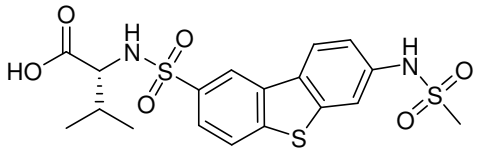
prior to challenge and 8 hours post challenge. Increases in airway resistance were measured throughout the day to capture both the early phase asthmatic response (EAR) and late phase asthmatic response (LAR). Airway hyper responsiveness (AHR) to aerosolized carbachol was measured the following day (24 hours post challenge). Following AHR measurements, lungs were lavaged and total cell counts were quantified in the BAL fluid.

II. Supporting Info: Chemistry

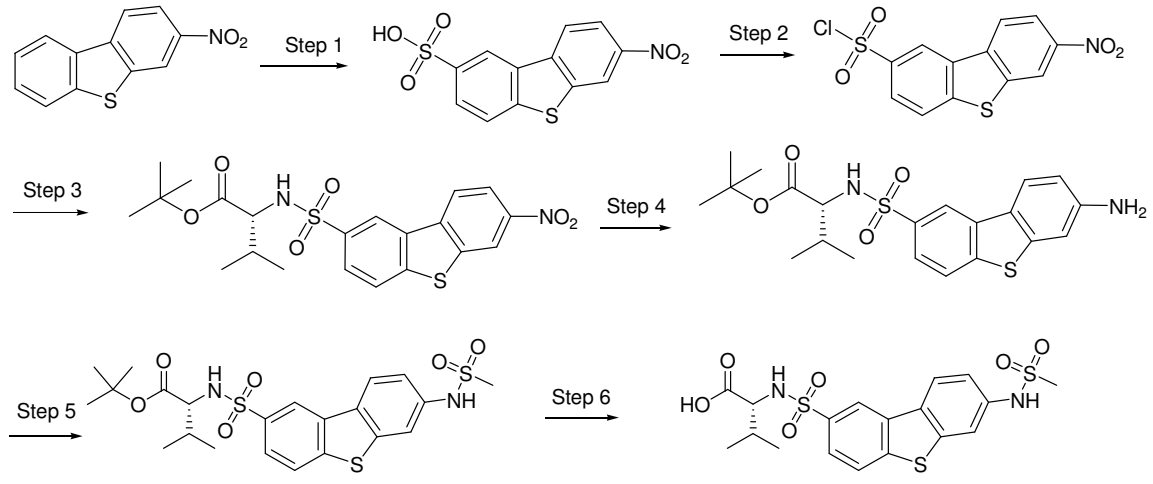
All reagents and solvents were of commercial quality and used without further purification. Column chromatography was performed using Merck silica gel 60 (230-400 mesh). Proton nuclear magnetic spectroscopy ^{1}H NMR spectra (300MHz or 400 MHz) were obtained on a Bruker 300/400 spectrometers. Chemical shifts are reported in parts per million relative to Me_4Si as internal standard. Conventional abbreviations used for signal shape are: s. singlet; d. doublet (apparent), t. triplet (apparent), m. multiplet; br. broad. Low-resolution mass spectra (MS) were obtained using a Micromass Platform Electrospray Ionization Quadrapole mass spectrometer. High resolution exact mass measurements (HRMS) were performed on a Bruker ApexIII 7T FT/ICR/MS. All intermediates were characterized by ^{1}H NMR. All new final SAR compounds were determined to be consistent with proposed structure by ^{1}H NMR, MS, HRMS and were greater than 95% pure in two solvent systems (HPLC Method 1: H_2O-CH_3CN and HPLC Method 2: H_2O-MeOH) as determined using an Agilent 1100 HPLC instrument on a C18 column.



(*R*)-2-(Dibenzo[b,d]thiophene-2-sulfonamido)-3-methylbutanoic acid (**3a**). The title compound was prepared as a white solid following a known procedure[1] for the preparation of its enatiomer **3b**. ^{1}H NMR (400 MHz, MeOD) δ ppm 1.10 (d, *J* = 6.82 Hz, 3H), 1.19 (d, *J* = 6.82 Hz, 3H), 2.16 - 2.34 (m, 1H), 3.83 (d, *J* = 5.31 Hz, 1H), 7.10 (dd, *J* = 8.59, 2.02 Hz, 1H), 7.33 (d, *J* = 2.02 Hz, 1H), 8.02 (dd, *J* = 8.34, 1.77 Hz, 1H), 8.20 (d, *J* = 8.59 Hz, 1H), 8.30 (d, *J* = 8.34 Hz, 1H), 8.45 (d, *J* = 1.01 Hz, 1H).



(*R*)-3-Methyl-2-(7-(methylsulfonamido)dibenzo[*b,d*]thiophene-2-sulfonamido)butanoic acid (**4a**). The title compound **4a** was prepared following the synthetic scheme and procedures described below.



[1] O'Brien, P. M.; Picard, J. A.; Sliskovic, D. R.; White, A. D. Method of inhibiting matrix metalloproteinase. US patent 6,906,092 B2. **2005**.

Step 1: 7-Nitrodibenzo[*b,d*]thiophene-2-sulfonic acid. To a round-bottom flask containing 3-nitrodibenzo[*b,d*]thiophene (1.0 g, 4.37 mmol) in 10 mL of trifluoroacetic acid was slowly added chlorosulfonic acid (0.5 mL) at ambient temperature. The resulting suspension was stirred for 2 hours. The reaction mixture was filtered and 1.31 g of 7-nitrodibenzo[*b,d*]thiophene-2-sulfonic acid was collected from the filtrate as a pale yellow solid in 97% yield.

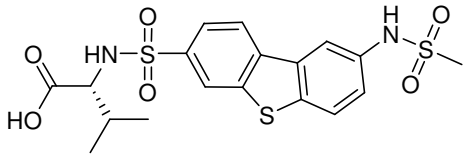
Step 2: 7-Nitrodibenzo[*b,d*]thiophene-2-sulfonyl chloride. 7-Nitrodibenzo[*b,d*]thiophene-2-sulfonic acid (1.0 g, 3.2 mmol) obtained from Step 1 was mixed with 20 mL of thionyl chloride ($SOCl_2$) followed by slow addition of a few drops of dimethylformamide (DMF). The mixture was stirred at 80 °C for 24 hours. The excess $SOCl_2$ was removed under reduced pressure. The crude product was triturated with ice water and filtered. 7-Nitrodibenzo[*b,d*]thiophene-2-sulfonyl chloride (0.95 g) was collected from the filtrate as an off-white solid in 90% yield.

Step 3: (*R*)-*tert*-Butyl 3-methyl-2-(7-nitrodibenzo[*b,d*]thiophene-2-sulfonamido) butanoate. To a slurry of 7-nitrodibenzo[*b,d*]thiophene-2-sulfonyl chloride (450 mg, 1.37 mmol) from Step 2 and (*R*)-*tert*-butyl-2-amino-3-methylbutanoate hydrochloride (318 mg, 1.52 mmol) in DCM (CH_2Cl_2, 8 mL) was slowly added *N,N*-diisopropylethylamine (520 mg, 4 mmol) at 0 °C. The resulting mixture was stirred for 4 hours and allowed to warm to ambient temperature. The reaction mixture was purified by column chromatography to provide 510 mg of the (*R*)-valine sulfonamide as a white solid in 80% yield.

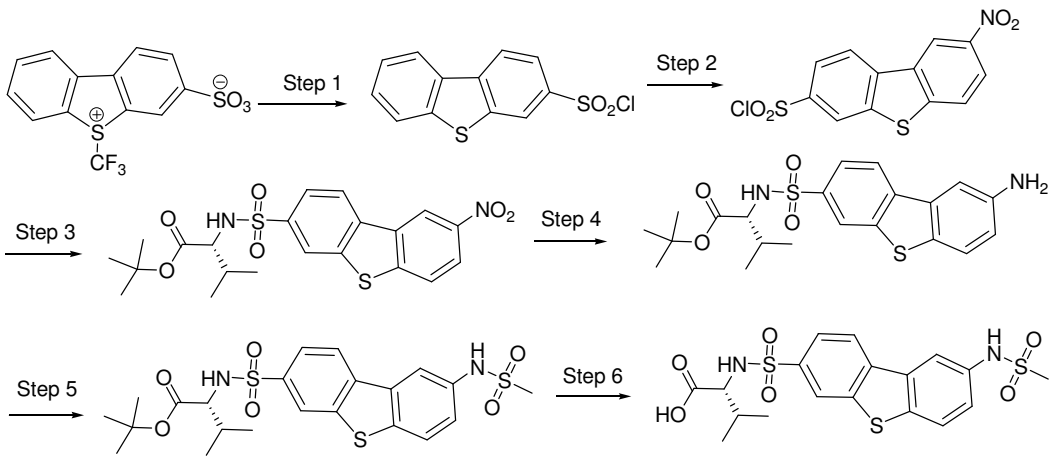
Step 4: (*R*)-*tert*-butyl 2-(7-Aminodibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoate. The nitro sulfonamide obtained in Step 3 (500 mg) was mixed with 20 mL of MeOH and 100 mg of 10% palladium on carbon (Pd/C). The reaction was carried out in a Parr shaker at ambient temperature under hydrogen (50 psi) overnight. The reaction mixture was filtered through Celite® and concentrated to provide (*R*)-*tert*-butyl 2-(7-aminodibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoate (430 mg) as an off-white solid in 92% yield.

Step 5: (*R*)-*tert*-Butyl 3-methyl-2-(7-(methylsulfonamido)dibenzo[*b,d*]thiophene-2-sulfonamido)butanoate. A solution of the aryl amine (109 mg, 0.25 mmol) from Step 4 and dimethylaminopyridine (DMAP, 37 mg, 0.3 mmol) in DCM (3 mL) was treated with methyl chloroformate (28 mg, 30 mmol). The mixture was stirred at ambient temperature overnight and purified by silica gel column chromatography to provide 129 mg of (*R*)-*tert*-butyl 3-methyl-2-(7-(methylsulfonamido) dibenzo[*b,d*]thiophene-2-sulfonamido)butanoate as a white solid in quantitative yield.

Step 6: (*R*)-3-Methyl-2-(7-(methylsulfonamido)dibenzo[*b,d*]thiophene-2-sulfonamido) butanoic acid (**4a**). The sulfonamide t-butyl ester (121 mg, 0.24 mmol) from Step 5 was dissolved in 2 mL of TFA/CH_2Cl_2 (1:1), and the solution was stirred at ambient temperature for 3 hours. The solvents were removed under reduced pressure and the residue was triturated in acetonitrile/water followed by a freeze-dry process. (*R*)-3-methyl-2-(7-(methylsulfonamido)dibenzo[*b,d*]thiophene-2-sulfonamido)butanoic acid (97 mg) was obtained as a white solid in 90% yield. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.80 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H), 1.86 - 2.04 (m, 1H), 3.10 (s, 3H), 3.64 (dd, *J* = 5.8, 9.6 Hz, 1H), 7.39 (dd, *J* = 1.9, 8.7 Hz, 1H), 7.83 (dd, *J* = 1.8, 8.6 Hz, 1H), 7.87 (d, *J* = 2.0 Hz, 1H), 8.11 (d, *J* = 9.9 Hz, 1H), 8.19 (d, *J* = 8.6 Hz, 1H), 8.41 (d, *J* = 8.6 Hz, 1H), 8.61 (d, *J* = 1.5 Hz, 1H), 10.17 (s, 1H), 12.54 (s, 1H). HRMS: calcd for $C_{18}H_{20}N_2O_6S_3$+H^+, 457.05562; found (ESI-FTMS, $[M+H]^+$), 457.0548.



(*R*)-3-Methyl-2-(8-(methylsulfonamido)dibenzo[*b,d*]thiophene-3-sulfonamido)butanoic acid (**5a**). The title compound **5a** was prepared following the synthetic scheme and procedures described below.



Step 1: Dibenzo[*b,d*]thiophene-3-sulfonyl chloride. 5-(trifluoromethyl)-5*H*-dibenzo[*b,d*]thiophenium-3-sulfonate (3.0 g, 9.0 mmol) was mixed with 30 mL of thionyl chloride ($SOCl_2$) followed by slow addition of a few drops of dimethylformamide (DMF). The mixture was stirred at 80 °C for 24 hours. The excess $SOCl_2$ was removed under reduced pressure. The crude product was triturated with ice water and filtered. Dibenzo[*b,d*]thiophene-3-sulfonyl chloride (2.35 g) was collected from the filtrate as an off-white solid in 92% yield.

Step 2: 8-Nitrodibenzo[*b,d*]thiophene-3-sulfonyl chloride. Dibenzo[*b,d*]thiophene-3-sulfonyl chloride (2.0 g, 7.1 mmol) from Step 1 was mixed with trifluoroacetic acid (20 mL) and cooled with ice/water bath. Fuming nitric acid (>90%, 0.29 mL) was added dropwise over 5 min. The cooling bath was removed and the mixture was stirred at ambient temperature for 2 hours. The reaction mixture was filtered and 8-nitrodibenzo[*b,d*]thiophene-3-sulfonyl chloride (1.1 g) was collected from the filtrate as an off-white solid in 47% yield.

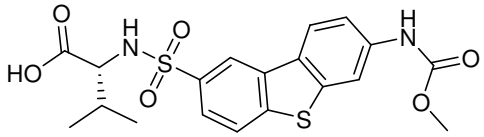
Step 3: (*R*)-*tert*-Butyl 3-methyl-2-(8-nitrodibenzo[*b,d*]thiophene-3-sulfonamido)butanoate. A mixture of 8-nitrodibenzo[*b,d*]thiophene-3-sulfonyl chloride (450 mg, 1.37 mmol) from Step 2 and (*R*)-*tert*-butyl 2-amino-3-methylbutanoate hydrochloride (318 mg, 1.52 mmol) in DCM (8 mL) at 0 °C underwent slow addition of *N,N*-diisopropylethylamine (520 mg, 4 mmol). The resulting mixture was stirred for 4 hours and allowed to warm to ambient temperature. The reaction mixture was purified

by column chromatography to provide 460 mg of the (*R*)-*tert*-butyl 3-methyl-2-(8-nitrodibenzo[*b,d*]thiophene-3-sulfonamido)butanoate as a white solid in 72% yield.

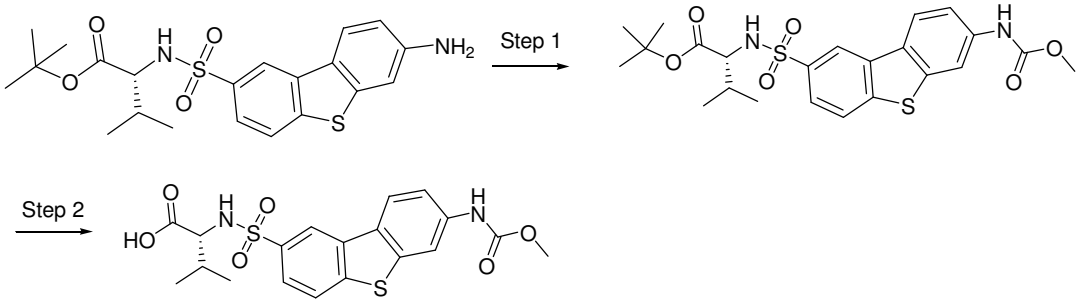
Step 4: (*R*)-*tert*-Butyl 2-(8-aminodibenzo[*b,d*]thiophene-3-sulfonamido)-3-methylbutanoate. The valine sulfonamide from Step 3 (450 mg) was mixed with 40 mL of MeOH and 100 mg of 10% palladium on carbon (Pd/C). The reaction was carried out in a Parr shaker (H_2, 50 psi) at ambient temperature overnight. The reaction mixture was filtered through Celite® and concentrated to provide (*R*)-*tert*-butyl 2-(8-aminodibenzo[*b,d*]thiophene-3-sulfonamido)-3-methylbutanoate (320 mg) as an off-white solid in 88% yield.

Step 5: (*R*)-*tert*-Butyl 3-methyl-2-(8-(methylsulfonamido)dibenzo[*b,d*]thiophene-3-sulfonamido)butanoate. The aryl amine (109 mg, 0.25 mmol) from Step 4 and dimethylaminopyridine (DMAP, 37 mg, 0.3 mmol) were dissolved in 3 mL of CH_2Cl_2, followed by the addition of methyl chloroformate (32 mg, 28 mmol). The mixture was stirred at ambient temperature overnight and purified by silica gel column chromatography to provide 65 mg of (*R*)-*tert*-butyl 3-methyl-2-(8-(methylsulfonamido)dibenzo[*b,d*]thiophene-3-sulfonamido)butanoate as a white solid in 51% yield.

Step 6: (*R*)-3-Methyl-2-(8-(methylsulfonamido)dibenzo[*b,d*]thiophene-3-sulfonamido)butanoic acid (**5a**). The sulfonamide t-butyl ester (65 mg, 0.13 mmol) from Step 5 was dissolved in 2 mL of TFA/CH_2Cl_2 (1:1), and the solution was stirred at ambient temperature for 3 hours. The solvents were removed under reduced pressure and the residue was triturated in acetonitrile/water followed by a freeze-dry process. (*R*)-3-methyl-2-(8-(methylsulfonamido)dibenzo[*b,d*]thiophene-3-sulfonamido)butanoic acid (97 mg) was obtained as a white solid in 86% yield. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.79 (d, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 6.82 Hz, 3H), 1.87 - 2.02 (m, 1H), 3.07 (s, 3H), 3.61 (dd, *J* = 5.9, 9.5 Hz, 1H), 7.46 (dd, *J* = 2.0, 8.7 Hz, 1H), 7.88 (dd, *J* = 1.5, 8.3 Hz, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 8.16 (d, *J* = 9.4 Hz, 1H), 8.19 (d, *J* = 2.0 Hz, 1H), 8.43 (d, *J* = 8.6 Hz, 1H), 8.46 (d, *J* = 1.8 Hz, 1H), 9.96 (s, 1H), 12.53 (s, 1H). HRMS: calcd for $C_{18}H_{20}N_2O_6S_3$+H^+, 457.05562; found (ESI-FTMS, $[M+H]^+$), 457.0546.



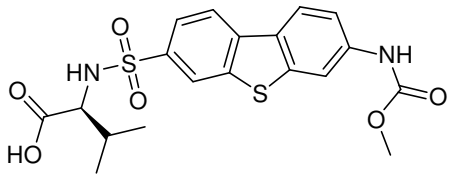
(*R*)-2-(7-(Methoxycarbonylamino)dibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoic acid (**6a**). The title compound **6a** was prepared following the scheme and synthetic procedures described below.



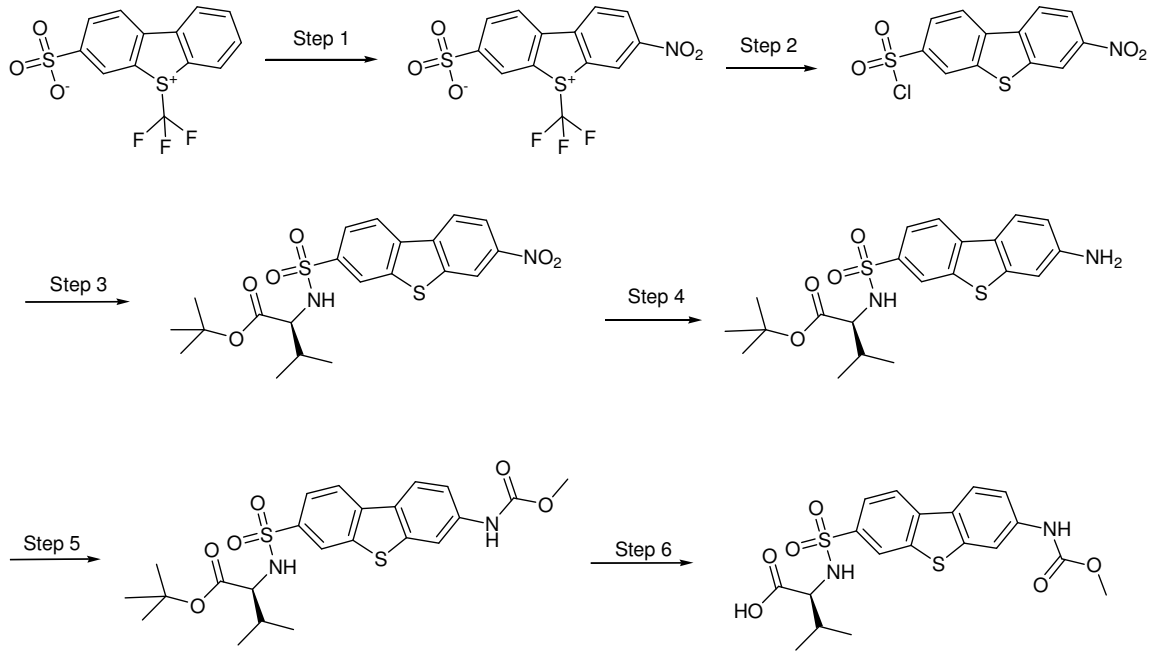
Step 1: (*R*)-*tert*-Butyl 2-(7-(methoxycarbonylamino)dibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoate. A solution of (*R*)-*tert*-butyl 2-(7-aminodibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoate (product of Step 4 in preparation of **4b**, 109 mg, 0.25 mmol) and dimethylaminopyridine (DMAP, 37 mg, 0.3 mmol) in DCM (2 mL) was treated with methyl chloroformate (28 mg, 0.30 mmol). The mixture was stirred at ambient temperature overnight and purified by silica gel column chromatography to provide (*R*)-*tert*-butyl 2-(7-(methoxycarbonylamino) dibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoate as a white solid in 94% yield.

Step 2: (*R*)-2-(7-(Methoxycarbonylamino)dibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoic acid (**6a**). The sulfonamide *t*-butyl ester (116 mg, 0.24 mmol) from Step 1 was dissolved in 2 mL of TFA/CH_2Cl_2 (1:1), and the solution was stirred at ambient temperature for 3 hours. The solvents were removed under reduced pressure and the residue was triturated in acetonitrile/water followed by a freeze-dry process. (*R*)-2-(7-(methoxycarbonylamino)dibenzo[*b,d*]thiophene-2-sulfonamido)-3-methylbutanoic acid was obtained as a white solid (91 mg). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.81 (d, *J* = 6.8

Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H), 1.88 - 2.03 (m, 1H), 3.64 (dd, *J* = 5.9, 9.5 Hz, 1H), 3.72 (s, 3H), 7.56 (dd, *J* = 1.9, 8.7 Hz, 1H), 7.81 (dd, *J* = 1.8, 8.6 Hz, 1H), 8.10 (d, *J* = 9.6 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 8.22 (d, *J* = 1.8 z, 1H), 8.35 (d, *J* = 8.6 Hz, 1H), 8.57 (d, *J* = 1.5 Hz, 1H), 10.07 (s, 1H), 12.54 (s, 1H). HRMS: calcd for $C_{19}H_{20}N_2O_6S_2+H^+$, 437.08355; found (ESI-FTMS, $[M+H]^+$), 437.0833.



(*S*)-2-(7-(Methoxycarbonylamino)dibenzo[*b,d*]thiophene-3-sulfonamido)-3-methylbutanoic acid (**9b**). The title compound was prepared following the synthetic scheme and procedures described below.



Step 1: 3-Nitro-5-trifluoromethyl-dibenzothiophenium-7-sulfonate. S-(trifluoromethyl)dibenzothiophenium-3-sulfonate (5.0 g) was added in portions to a mixture of 30 % oleum (3.3 mL) and 90% HNO_3 (1.7 ml). The mixture was stirred at ambient temperature overnight and then slowly dropped into diethyl ether (250 mL) at 0 °C. The precipitated solid was recovered by filtration and dried under vacuum providing

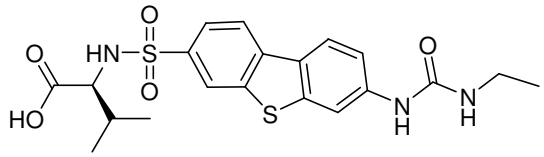
3-nitro-5-trifluoromethyl-dibenzothiophenium-7-sulfonate (5.37 g, 95 % yield) as a light brown solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.64 (d, *J* = 1.9 Hz, 1H), 9.05 (d, *J* = 0.9 Hz, 1H), 8.85 (dd, *J* = 8.7, 2.0 Hz, 1H), 8.75 (d, *J* = 8.8 Hz, 1H), 8.65 (d, *J* = 7.9 Hz, 1H), 8.26 (dd, *J* = 8.0, 1.4 Hz, 1H).

Step 2: 7-Nitro-dibenzothiophene-3-sulfonyl chloride. The 3-nitro-5-trifluoromethyl-dibenzothiophenium-7-sulfonate (5 g) was dissolved in thionyl chloride (35 mL), a few drops of DMF were added, and the mixture was heated at 80 °C for 24 hours. The excess of thionyl chloride was removed under reduced pressure and the residue triturated twice with DCM to provide a quantitative yield of the desired product that was used for the next step without further purification. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.09 (d, *J* = 2.0 Hz, 1H), 8.61 (d, *J* = 8.5 Hz, 1H), 8.49 (dd, *J* = 8.5, 0.6 Hz, 1H), 8.34 (dd, *J* = 1.5, 0.6 Hz, 1H), 8.33 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.83 (dd, *J* = 8.2, 1.5 Hz, 1H).

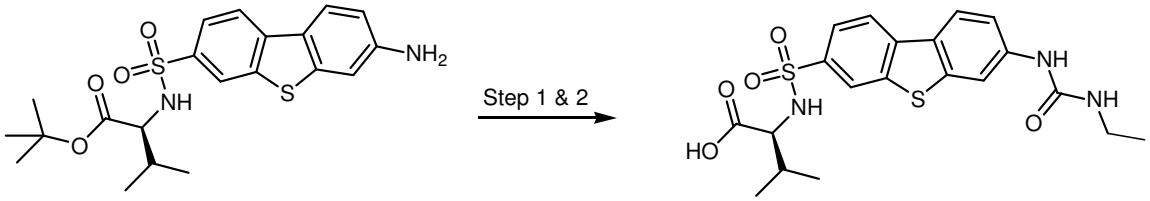
Step 3: (*S*)-3-Methyl-2-(7-nitro-dibenzothiophene-3-sulfonylamino)-butyric acid *tert*-butyl ester. The title compound was prepared as a white solid following the procedures described for the preparation of **27** (Step 2). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 0.89 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 1.15 (s, 9H), 2.00 (m, 1H), 3.63 (d, *J* = 6.3 Hz, 1H), 7.81 (s, 1H), 7.98 (dd, *J* = 8.5, 1.9 Hz, 1H), 8.35 (dd, *J* = 8.5, 1.9 Hz, 1H), 8.56 (dd, *J* = 1.6, 0.6 Hz, 1H), 8.66 (d, *J* = 8.2 Hz, 1H), 8.67 (d, *J* = 8.5 Hz, 1H), 9.05 (d, *J* = 2.2 Hz, 1H). ESI-POS $[M-H]^+$ 465.1.

Step 4: (*S*)-2-(7-Amino-dibenzothiophene-3-sulfonylamino)-3-methyl-butyric acid *tert*-butyl ester. The nitro compound from Step 3 was dissolved in MeOH and shaken overnight under an atmosphere of hydrogen (40 psi) in the presence of 10% Pd/C (10% w/w). The mixture was filtered through Celite® and the solvent removed under reduced pressure to provide a quantitative yield of the desired product. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 0.82 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 1.07 (s, 9H), 1.92 (m, 1H), 3.50 (dd, *J* = 9.7, 6.3 Hz, 1H), 5.71 (s, 2H), 6.79 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.05 (d, *J* = 1.9 Hz, 1H), 7.72 (dd, *J* = 8.5, 1.9 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 8.15 (d, *J* = 8.2 Hz, 1H), 8.21 (d, *J* = 1.9 Hz, 1H). ESI-POS $[M-H]^+$ 435.1.

Step 5: (*S*)-2-(7-Methoxycarbonylamino-dibenzothiophene-3-sulfonylamino)-3-methyl-butyric acid (**9b**). Following procedures for the preparation of MMP408 and using methyl chloroformate for the *N*-derivatization step, compound **9b** was prepared as a white solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 0.80 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 1.95 (m, 1H), 3.61 (m, 1H), 3.72 (s, 3H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.91 (dd, *J* = 8.5, 1.6 Hz, 1H), 8.10 (m, 1H), 8.42 (d, *J* = 8.8 Hz, 1H), 8.50 (d, *J* = 8.5 Hz, 1H), 8.52 (d, *J* = 1.3 Hz, 1H), 9.46 (s, 1H), 12.45 (s, 1H). ESI-POS $[M-H]^+$ 437.0.



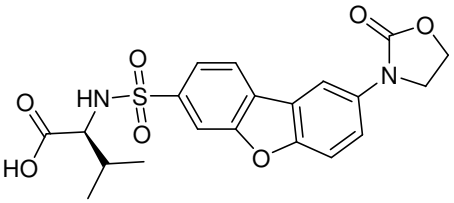
(*S*)-2-(7-(3-Ethylureido)dibenzo[*b,d*]thiophene-3-sulfonamido)-3-methylbutanoic acid (**20**). The title compound was prepared following the scheme and synthetic procedures described below.



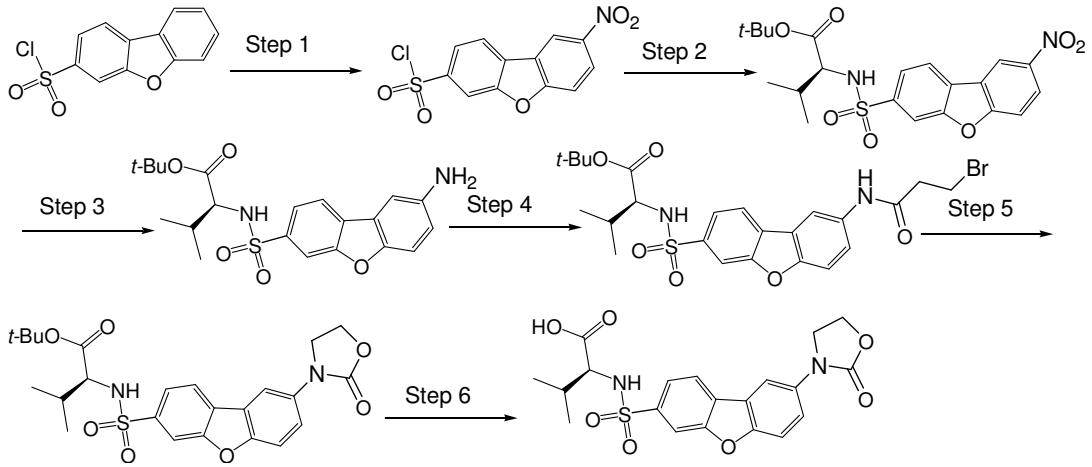
Step 1: (*S*)-*tert*-Butyl 2-(7-(3-ethylureido)dibenzo[*b,d*]thiophene-3-sulfonamido)-3-methylbutanoate. A solution of the amine (250 mg, 0.57 mmol) from Step 4 (see procedures for the preparation of **9b**) in DCM (5 mL) was treated with ethylisocyanate (1.2 eq), and the mixture was stirred for 24 hours at ambient temperature. The volatiles were evaporated and the crude was purified using silica gel chromatography.

Step 2: (*S*)-2-(7-(3-Ethylureido)dibenzo[*b,d*]thiophene-3-sulfonamido)-3-methylbutanoic acid. The product from Step 1 was dissolved in 30 % TFA in DCM (2 mL) and the solution was stirred at ambient temperature for 3 hours. The solvents were removed under reduced pressure and the residue was triturated with acetonitrile/water followed by a freeze-dry process. (*S*)-2-[7-(3-ethyl-ureido)-dibenzothiophene-3-sulfonylamino]-3-

methyl-butyric acid was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 0.81 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H), 1.09 (t, *J* = 5.6 Hz, 3H), 1.95 (m, 1H), 3.15 (dq, *J* = 7.0, 5.6 Hz, 2H), 3.58 (m, 1H), 6.27 (t, *J* = 5.5 Hz, 1H), 7.45 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.81 (dd, *J* = 8.5, 1.8 Hz, 1H), 8.00 (d, *J* = 9.0 Hz, 1H), 8.23 (d, *J* = 1.8 Hz, 1H), 8.26 (d, *J* = 8.3 Hz, 1H), 8.34 (d, *J* = 8.3 Hz, 1H), 8.36 (d, *J* = 1.8 Hz, 1H), 8.82 (s, 1H), 12.51 (s, 1H). ESI-POS $[M-H]^+$ 450.1.



(*S*)-3-Methyl-2-(8-(2-oxooxazolidin-3-yl)dibenzo[*b,d*]furan-3-sulfonamido)butanoic acid (**27**, MMP145). The title compound was prepared following the synthetic scheme and procedures described below.



Step 1: 8-Nitro-dibenzofuran-3-sulfonyl chloride. Dibenzo[*b,d*]furan-3-sulfonyl chloride (10.64 g, 40 mmol) was dissolved in DCM (60 mL) and with stirred for 30 minutes at ambient temperature. After the addition of trifluoroacetic acid (100 mL) to the solution, HNO_3 (10.6g, 168 mmol) was added dropwise. The mixture was stirred at ambient temperature for 6 hours, and concentrated under reduced pressure. TFA (60 mL) was

added and the mixture was filtered. The filter cake was washed with cold water to provide the product (10.11 g) as a yellow solid (yield 78%).

Step 2: (*S*)-3-Methyl-2-(8-nitro-dibenzofuran-3-sulfonylamino)-butyric acid *tert*-butyl ester. In a round bottomed flask (*L*)-valine *t*-butyl ester (HCl salt, 6.80 g, 32.4 mmol) and diisopropylethylamine (11.3 mL) were mixed in DCM (250 mL). The product obtained in Step 1 (10.11 g, 32.4 mmol) was then added slowly to the above solution maintained at 0 °C. The ice bath was removed, and the reaction was allowed to warm to ambient temperature over 2 hours. Water (200 mL) was added to the reaction flask, and DCM was removed under reduced pressure. The suspension was filtered, and the filter cake was washed with water and dried to give the product (13.80 g, yield 94%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.8 Hz, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 1.13 (s, 9H), 2.09 (td, *J* = 6.8, 4.6 Hz, 1H), 3.74 (dd, *J* = 9.9, 4.6 Hz, 1H), 5.22 (d, *J* = 9.9 Hz, 1H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.95 (dd, *J* = 8.1, 1.26 Hz, 1H), 8.08 - 8.22 (m, 2H), 8.50 (dd, *J* = 9.2, 2.2 Hz, 1H), 8.94 (d, *J* = 2.5 Hz, 1H).

Step 3: (*S*)-2-(8-Amino-dibenzofuran-3-sulfonylamino)-3-methyl-butyric acid *tert*-butyl ester. The product obtained in Step 2 (13.80 g) was dissolved in methanol (150 mL) followed by addition of 10% Pd/C (0.6 g, 50% water). The reaction mixture was placed in a Parr shaker under an atmosphere of hydrogen (50 psi) for 6 hours. The suspension was filtered through Celite®, and the solvent removed under reduced pressure to afford the product (13.72 g, yield 98%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H), 1.10 (s, 9H), 2.05 (td, *J* = 6.8, 4.55 Hz, 1H), 3.69 (dd, *J* = 9.9, 4.55 Hz, 1H), 5.18 (d, *J* = 9.9 Hz, 1H), 6.91 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.23 (d, *J* = 2.5 Hz, 1H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.78 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.99 (d, *J* = 1.0 Hz, 1H).

Step 4: (*S*)-2-[8-(3-Bromo-propionylamino)-dibenzofuran-3-sulfonylamino]-3-methyl-butyric acid *tert*-butyl ester. A solution of (*S*)-2-(8-amino-dibenzofuran-3-sulfonylamino)-3-methyl-butyric acid *tert*-butyl ester (7.80 g, 21 mmol) in DCM (50 mL) was cooled to 0 ^{o}C and treated with pyridine (2.0 equiv.) followed by a slow addition of bromoethyl chloroformate (1.2 equiv.). The reaction mixture was warmed from 0 ^{o}C to ambient temperature over 2 hours, then was diluted with water and 1 N HCl. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (40 mL x 3).

The combined organic layers were washed with 0.5 N HCl (30 mL x 2), brine (30 mL x 2), and dried over sodium sulfate. Evaporation of the solvent under reduced pressure provided the product (10.08 g, yield 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H), 1.10 (s, 9H), 2.07 (d, *J* = 4.6 Hz, 1H), 3.62 (t, *J* = 5.8 Hz, 2H), 3.71 (dd, *J* = 9.9, 4.6 Hz, 1H), 4.54 (t, *J* = 5.9 Hz, 2H), 5.19 (d, *J* = 9.9 Hz, 1H), 6.86 (s, 1H) 7.37 (dd, *J* = 8.9, 2.27 Hz, 1H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.83 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.96 - 8.08 (m, 2H), 8.23 (s, 1H); MS: calcd for $C_{24}H_{29}BrN_2O_7S$ ($M+H^+$) 570.47, found (ESI-FTMS, $[M-H]^+$) 568.09.

Step 5: (*S*)-3-Methyl-2-[8-(2-oxo-oxazolidin-3-yl)-dibenzofuran-3-sulfonylamino]-butyric acid *tert*-butyl ester. A solution of the crude material obtained in Step 4 in DMF (50 mL) was treated with cesium carbonate (2.0 equiv.) and the mixture was stirred at 60 ^{o}C for 4 hours. The mixture was diluted with water and filtered to give (*S*)-3-methyl-2-[8-(2-oxo-oxazolidin-3-yl)-dibenzofuran-3-sulfonylamino]-butyric acid *tert*-butyl ester (8.02 g, yield 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 1.10 (s, 9H), 2.07 (td, *J* = 6.9, 4.67 Hz, 1H), 3.72 (dd, *J* = 10.0, 4.42 Hz, 1H), 4.19 (dd, *J* = 9.0, 7.2 Hz, 2H), 4.57 (dd, *J* = 8.7, 7.2 Hz, 2H), 5.20 (d, *J* = 9.9 Hz, 1H), 7.54 - 7.71 (m, 2H), 7.84 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.05 (dd, *J* = 4.8, 3.3 Hz, 2H), 8.25 (d, *J* = 1.5 Hz, 1H); MS: calcd for $C_{24}H_{28}N_2O_7S$ (M^+) 488.55, found (ESI-FTMS, $[M-H]^+$) 487.50.

Step 6: (*S*)-3-Methyl-2-[8-(2-oxo-oxazolidin-3-yl)-dibenzofuran-3-sulfonylamino]-butyric acid. A solution of the crude product (7.25 g) obtained in Step 5 in 20% trifluoroacetic acid in DCM (50 mL) was stirred at ambient temperature for 2 hours, whereupon the solvent was removed under reduced pressure. HPLC purification gave (*S*)-3-methyl-2-[8-(2-oxo-oxazolidin-3-yl)-dibenzofuran-3-sulfonylamino]butyric acid **27** (5.06 g, yield 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (d, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 1.88 - 1.98 (m, 1H), 3.61 (dd, *J* = 9.6, 6.1 Hz, 1H), 4.12 - 4.26 (m, 2H), 4.51 (dd, *J* =9.1, 6.8 Hz, 2H), 7.76 - 7.86 (m, 2H), 7.93 (dd, *J* = 9.1, 2.5 Hz, 1H), 8.06 (d, *J* = 1.3 Hz, 1H), 8.18 (d, *J* = 9.6 Hz, 1H), 8.30 - 8.39 (m, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 17.8, 19.0, 30.4, 45.5, 61.3, 61.5, 110.3, 111.6, 112.1, 120.3, 121.5, 121.7, 122.7, 126.9, 134.9, 140.2, 153.0, 155.0, 155.2, 172.0; HRMS: calcd for $C_{20}H_{20}N_2O_7S$

($M+H^+$) 433.10640, found (ESI-FTMS, $[M+H]^+$) 433.10635; CHN: calcd for $C_{20}H_{20}N_2O_7S$ C: 55.55%, H: 4.66%, N: 6.48%, found C: 55.53%, H: 4.67%, N: 6.75%.