Supporting Information

Enhancing the Pharmacokinetic Properties of Botulinum Neurotoxin Serotype A Protease Inhibitors Through Rational Design

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Experimental

Library Synthesis (conversion of aldehydes into acrylic hydroxamates).

Preparation of propenoate esters
To a solution of methyl diethylphosphonoacetate (2.10 g, 1.84 mL, 10 mmol) in N,N-dimethylformamide (5 mL) was added sodium bis(trimethylsilyl)amide (5 mL, 10, mmol, 2M in tetrahydrofuran). After a mild exotherm, the mixture was stirred for 10 min. The resulting solution was added to 10 vials (2 mL, each) containing a solution of one of the aldehydes (0.50 mmol, **2a-w** and **9a-f**) in N,N-dimethylformamide (0.5 mL). After shaking for 1h, hydrochloric acid (1 M, 2 mL) was added to the reaction mixture, followed by ethyl acetate/hexane (1:1, 2 mL). After shaking and allowing the layers to separate, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure to give the desired crude propenoate esters (**4a-w** and **16a-f**).

Preparation of protected hydroxamates
To a stirring solution of the propenoate ester (0.50 mmol, **4a-w** and **16a-f**) and O-(2-tetrahydropyranyl) hydroxylamine (0.50 mmol) in tetrahydrofuran (2 mL) was added sodium bis(trimethylsilyl)amide (0.50 mL, 1.0 mmol, 2M in tetrahydrofuran). After shaking for 30 min, hydrochloric acid (0.5 M, 2 mL) was added to the reaction mixture, followed by ethyl acetate (1 mL). After shaking and allowing the layers to separate, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure to give the desired crude protected hydroxamate (**5a-w** and **17a-f**).

Deprotection of hydroxamates
To a stirred solution of the protected hydroxamate (0.5 mmol, **5a-w** and **17a-f**) in methanol (1.5 mL) was added hydrogen chloride (0.25 mL, 4N in 1,4-dioxane). After stirring for 4 h, the reaction mixture was concentrated under reduced pressure. Methanol (1 mL) was added to the crude product and the mixture was again concentrated under reduced pressure. The crude product was purified by silica gel chromatography (4 g cartridge) eluting with ethyl acetate/methanol to give the desired hydroxamic acid (**6a-w** and **15a-f**) with purity $\geq$95% by HPLC.

3-(3-Chloro-benzo[b]thiophen-2-yl)-N-hydroxy-acrylamide (6a). White solid, 30% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_9ClNO_2S$ calculated 254.0037; measured 254.0038. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.15 (br s, 2H), 10.30 (br s, 1H), 8.16 (m, 1H), 7.91 (m, 1H), 7.83 (d, J = 15.6 Hz, 1H), 7.67-7.59 (m, 2H), 6.53 (d, J = 15.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.1, 136.8, 136.6, 133.6, 128.2, 128.0, 126.7, 124.0, 123.4, 122.8, 122.6.

N-Hydroxy-3-(3-methyl-benzo[b]thiophen-2-yl)-acrylamide (6b). Yellow solid, 27% yield over 3 steps. HRMS (ESI-TOF) for $C_{12}H_{12}NO_2S$ calculated 234.0583; measured 234.0592. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.22 (br s, 1H), 7.94 (m, 1H), 7.84-7.76 (m, 2H), 7.47-7.38 (m, 2H), 6.28 (d, J = 15.7 Hz, 1H), 3.57 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.8, 141.1, 138.4, 135.1, 134.3, 130.2, 127.0, 125.4, 123.4, 123.2, 120.8, 12.3.

3-(3,4-Dimethyl-thieno[2,3-b]thiophen-2-yl)-N-hydroxy-acrylamide (6c). Yellow solid, 37% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_{12}NO_2S_2$ calculated 254.0304; measured 254.0301. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (d, J = 15.6 Hz, 1H), 7.19 (s, 1H), 6.14 (d, J = 15.7 Hz, 1H), 2.50 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (135 MHz, DMSO-d_6) δ 162.5, 146.8, 136.8, 136.1, 132.9, 131.0, 129.8, 124.7, 116.7, 15.1, 12.7.

N-Hydroxy-3-(3-methyl-thiophen-2-yl)-acrylamide (6d). Off white solid, 17% yield over 3 steps. HRMS (ESI-TOF) for $C_8H_{10}NO_2S$ calculated 217.0971; measured 217.0963.

3-(5-Chloro-benzo[b]thiophen-3-yl)-N-hydroxy-acrylamide (6e). Orange solid, 29% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_9ClNO_2S$ calculated 254.0037; measured 254.0039.

N-Hydroxy-3-(1-methyl-1H-indol-2-yl)-acrylamide (6f). Yellow solid, 5% yield over 3 steps. HRMS (ESI-TOF) for $C_{12}H_{13}N_2O_2$ calculated 184.0427; measured 184.0420.

3-[5-(4-Chloro-phenyl)-furan-2-yl]-N-hydroxy-acrylamide (6g). Tan solid, 25% yield over 3 steps. HRMS (ESI-TOF) for $C_{13}H_{11}ClNO_3$ calculated 264.0422; measured 264.0424.

3-(4-Chloro-1-methyl-1H-pyrazol-3-yl)-N-hydroxy-acrylamide (6h). Off white solid, 9% yield over 3 steps. HRMS (ESI-TOF) for $C_7H_9ClN_3O_2$ calculated 202.0378; measured 202.0375.

3-(1,3-Dimethyl-1H-pyrazol-4-yl)-N-hydroxy-acrylamide (6i). Pale yellow solid, 17% yield over 3 steps. HRMS (ESI-TOF) for $C_8H_{12}N_3O_2$ calculated 182.0924; measured 182.0932.

3-(1,5-Dimethyl-1H-pyrazol-4-yl)-N-hydroxy-acrylamide (6j). White solid, 19% yield over 3 steps. HRMS (ESI-TOF) for $C_8H_{12}N_3O_2$ calculated 182.0924; measured 182.0921.

N-Hydroxy-3-(1-methyl-1H-pyrazol-4-yl)-acrylamide (6k). White solid, 27% yield over 3 steps. HRMS (ESI-TOF) for $C_7H_{10}N_3O_2$ calculated 168.0767; measured 168.0765.

3-[1-(4-Fluoro-phenyl)-1H-pyrazol-4-yl]-N-hydroxy-acrylamide (6l). White solid, 11% yield over 3 steps. HRMS (ESI-TOF) for $C_{12}H_{11}FN_3O_2$ calculated 248.083; measured 248.0838.

3-[1-(2-Fluoro-phenyl)-1H-pyrazol-4-yl]-N-hydroxy-acrylamide (6m). Off white solid, 32% yield over 3 steps. HRMS (ESI-TOF) for $C_{12}H_{11}FN_3O_2$ calculated 248.083; measured 248.0835.

N-Hydroxy-3-(4-phenyl-thiazol-2-yl)-acrylamide (6n). Yellow solid, 28% yield over 3 steps. HRMS (ESI-TOF) for $C_{12}H_{11}N_2O_2S$ calculated 247.0536; measured 247.0539.

N-Hydroxy-3-(5-pyridin-2-yl-thiophen-2-yl)-acrylamide (6o). White solid, 13% yield over 3 steps. HRMS (ESI-TOF) for $C_{12}H_{11}N_2O_2S$ calculated 247.0536; measured 247.0545.

N-Hydroxy-3-(5-methyl-3-phenyl-isoxazol-4-yl)-acrylamide (6p). White solid, 5% yield over 3 steps. HRMS (ESI-TOF) for $C_{13}H_{13}N_2O_3$ calculated 245.0921; measured 245.0926.

N-Hydroxy-3-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-acrylamide (6q). White solid, 23% yield over 3 steps. HRMS (ESI-TOF) for $C_{13}H_{14}N_3O_2$ calculated 244.108; measured 244.1079.

N-Hydroxy-3-(4-[1,2,4]triazol-1-yl-phenyl)-acrylamide (6r). White solid, 10% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_{11}N_4O_2$ calculated 231.0876; measured 231.0872.

N-Hydroxy-3-(2-piperidin-1-yl-thiazol-5-yl)-acrylamide (6s). Brown semi-solid, 16% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_{16}N_3O_2S$ calculated 254.0958; measured 254.0969.

3-(3,5-Dimethyl-isoxazol-4-yl)-N-hydroxy-acrylamide (6t). Off-white solid, 9% yield over 3 steps. HRMS (ESI-TOF) for $C_8H_{11}N_2O_3$ calculated 183.0764; measured 183.0758.

N-Hydroxy-3-(1H-indol-5-yl)-acrylamide (6u). Green solid, 10% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_{11}N_2O_2$ calculated 203.0815; measured 203.0819. ^{1}H NMR (DMSO-d_6) δ 11.25 (br s, 1H), 10.62 (br s, 1H), 8.98 (br s, 1H), 7.58-7.52 (m, 3H), 7.43 (m, 1H), 7.22 (m, 1H), 6.47-6.36 (m, 2H).

N-Hydroxy-3-(1H-indol-6-yl)-acrylamide (6v). Orange oil, 12% yield over 3 steps. HRMS (ESI-TOF) for $C_{11}H_{11}N_2O_2$ calculated 203.0815; measured 203.0823. ^{1}H NMR (DMSO-d_6) δ 11.23 (br s, 1H), 10.60 (br s, 1H, 8.94 (br s, 1H), 7.73 (br s, 1H), 7.53 (d, J = 15.7 Hz, 1H), 7.44-7.29 (m, 3H), 6.47 (m, 1H), 6.25 (d, J = 15.7 Hz, 1H).

N-Hydroxy-3-(pyridin-4-yl)-acrylamide (6w). White solid, 17% yield over 3 steps. HRMS (ESI-TOF) for $C_8H_9N_2O_2$ calculated 165.0659; measured 165.0661.

Synthesis of benzothiophen acrylic hydroxamates 15a-f

3-Chloro-6-fluoro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (8a).
To a stirring solution of 3-chloro-6-fluoro-benzo[b]thiophene-2-carboxylic acid methyl ester (**7a**, 2.0 g, 8.18 mmol) in tetrahydrofuran (25 mL) was added aqueous lithium hydroxide (16.4 mL, 1M). After one hour, the reaction mixture was poured into hydrochloric acid (20 mL, 1 M) followed by ethyl acetate (100 mL). The layers were separated and the organic layer was concentrated at reduce pressure to give the acid. To a stirring suspension of 3-chloro-6-fluoro-benzo[b]thiophene-2-carboxylic acid in N,N-dimethylformamide was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.35 g, 12.3 mmol) and hydroxybenzotriazole hydrate (1.66 g, 12.3 mmol). After 5 minutes was added (N,O)-dimethylhydroxylamine hydrochloride (1.20 g, 12.3 mmol) and triethylamine (1.7 mL, 12.3 mmol). The resulting suspension became a homogeneous solution after one hour. After 16 hours, the reaction was diluted with ethyl acetate (125 mL) and washed successively with hydrochloric acid (75 mL, 0.5 M), saturated aqueous sodium hydrogen carbonate (75 mL) and water (25 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (2.1 g, 95%). ^{1}H NMR ($CDCl_3$) δ 7.91 (m, 1H), 7.49 (m, 1H), 7.22 (m, 1H), 3.73 (s, 3H), 3.40 (s, 3H).

3-Chloro-6-fluoro-benzo[b]thiophene-2-carbaldehyde (9a). To a stirring solution of 3-chloro-6-fluoro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (**8a**, 1.0 g, 3.65 mmol) in tetrahydrofuran (10 mL) at -70 ºC under nitrogen was added a solution of lithium aluminum hydride (7.7 mL, 7.7 mmol, 1.0 M in tetrahydrofuran) dropwise over 10 minutes. After stirring for 30 minutes, the reaction was quenched by the dropwise addition of water (3 mL) and the mixture was warmed to ambient temperature. The reaction mixture was poured into ethyl acetate, the layers were separated and the organic layer was washed successively with hydrochloric acid (100 mL, 1 M) then saturated aqueous sodium chloride (100 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (780 mg, 99%). ^{1}H NMR ($CDCl_3$) δ 10.29 (s, 1H), 8.01 (m, 1H), 7.49 (m, 1H), 7.28 (m, 1H).

(2E)-3-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (16a). To a solution of methyl diethylphosphonoacetate (1.37 mL, 1.57 g, 7.4 mmol) in N,N-dimethylformamide (4 mL) was added sodium bis(trimethylsilyl)amide (3.7 mL, 7.4 mmol, 2M in tetrahydrofuran). After a mild exotherm, the mixture was stirred for 10 min. The resulting solution was added to a solution of the 3-chloro-6-fluoro-benzo[b]thiophene-2-carbaldehyde (**9a**, 780 mg, 3.7 mmol) in N,N-dimethylformamide (4 mL). After 1h, water (40 mL) was added to the reaction mixture with vigorous stirring, and the resulting suspension was filtered and dried to give the product as a white solid (1.0 g, 100%). ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 15.6 Hz, 1H), 7.79 (m, 1H), 7.44 (m, 1H), 7.18 (m, 1H), 6.33 (d, J = 15.7 Hz, 1H), 3.83 (s, 3H).

(2E)-3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide (17a). To a stirring solution of (2E)-3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (1.0 g, 3.7 mmol) and O-(2-tetrahydropyranyl) hydroxylamine (867 mg, 7.4 mmol) in tetrahydrofuran (15 mL) was added sodium bis(trimethylsilyl)amide (3.7 mL, 7.4 mmol, 2M in tetrahydrofuran). After stirring for 30 min, hydrochloric acid (0.5 N, 25 mL) was added to the reaction mixture, followed by ethyl acetate (50 mL). The layers were separated, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure. The crude product was triturated with dichloromethane (5 mL), filtered and dried to give the intermediate as a white solid (412 mg, 31%).

(2E)-3-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-N-hydroxy-(E)-acrylamide (15a). To a stirred solution of (2E)-3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide (**17a**, 400 mg) in methanol (2.5 mL) was added hydrogen chloride (5.0 mL, 4N in 1,4-dioxane). After stirring for 18h, the reaction mixture was concentrated under reduced pressure. Methanol (3 mL) was added to the crude product and the mixture was again concentrated under reduced pressure. The resulting solid was triturated with ethyl acetate (5 mL), filtered and dried to give pure product as a pale yellow solid (264 mg, 87%). HRMS (ESI-TOF) for $C_{11}H_8ClFNO_2S$ calculated 271.9943; measured 271.9938. ^{1}H NMR (DMSO-d_6) δ 10.22 (s, 1H), 8.03 (m, 1H), 7.85 (m, 1H), 7.71 (d, J = 15.7 Hz, 1H), 7.43 (m, 1H), 6.44 (d, J = 15.7 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 162.1 (J = 245 Hz), 161.9, 138.1, 137.9, 133.7, 127.7, 124.4 (J = 9 Hz), 123.5, 122.2, 115.6 (J = 25 Hz), 110.6 (J = 27 Hz).

3,6-Dichloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (8b).
To a stirring solution of 3,6-dichloro-benzo[b]thiophene-2-carboxylic acid methyl ester (**7b**, 2.0 g, 7.7 mmol) in tetrahydrofuran (25 mL) was added aqueous lithium hydroxide (15.4 mL, 1M). After one hour, the reaction mixture was poured into hydrochloric acid (20 mL, 1M) followed by ethyl acetate (100 mL). The layers were separated and the organic layer was concentrated at reduce pressure to give the acid. To a stirring suspension of 3,6-dichloro-benzo[b]thiophene-2-carboxylic acid in N,N-dimethylformamide was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.21 g, 11.6 mmol) and hydroxybenzotriazole hydrate (1.56 g, 11.6 mmol). After 5 minutes was added (N,O)-dimethylhydroxylamine hydrochloride (1.13g, 11.6 mmol) and triethylamine (1.6 mL, 11.6 mmol). The resulting suspension became a homogeneous solution after one hour. After 16 hours, the reaction was diluted with ethyl acetate (125 mL) and washed successively with hydrochloric acid (75 mL, 0.5 M), saturated aqueous sodium hydrogen carbonate (75 mL) and water (25 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (2.07 g, 93%).

3,6-Dichloro-benzo[b]thiophene-2-carbaldehyde (9b). To a stirring solution of 3,6-dichloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (**8b**, 500 mg, 1.7 mmol) in tetrahydrofuran (6 mL) at -70 ºC under nitrogen was added a solution of lithium aluminum hydride (3.6 mL, 3.7 mmol, 1.0 M in tetrahydrofuran) dropwise over 10 minutes. After stirring for 30 minutes, the reaction was quenched by the dropwise addition of water (1.5 mL) and the mixture was warmed to ambient temperature. The reaction mixture was poured into ethyl acetate, the layers were separated and the organic layer was washed successively with hydrochloric acid (50 mL, 1 M) then saturated aqueous sodium chloride (50 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (390 mg, 99%). ^{1}H NMR ($CDCl_3$) δ 10.34 (s, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.46 (dd, J = 8.5, 2.1 Hz, 1H).

(2E)-3-(3,6-Dichloro-benzo[b]thiophen-2-yl)-N-hydroxy-acrylamide (15b). **15b** was synthesized from aldehyde **9b** using procedure described for synthesis of **15a**. Yellow solid, 21% yield from aldehyde. HRMS (ESI-TOF) for $C_{11}H_8Cl_2NO_2S$ calculated 287.9647; measured 287.9658. ^{1}H NMR (DMSO-d_6) δ 10.94 (br s, 1H), 9.26 (br s, 1H), 8.25 (s, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 15.5, 1H), 7.56 (dd, J = 8.6, 2.0 Hz, 1H), 6.43 (d, J = 15.5 1H).

3-Chloro-6-methyl-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (8c). To a stirring solution of 3-chloro-6-methyl-benzo[b]thiophene-2-carboxylic acid methyl ester (**7c**, 2.0 g, 8.3 mmol) in tetrahydrofuran (25 mL) was added aqueous lithium hydroxide (16.6 mL, 1M). After one hour, the reaction mixture was poured into hydrochloric acid (20 mL, 1M) followed by ethyl acetate (100 mL). The layers were separated and the organic layer was concentrated at reduce pressure to give the acid. To a stirring suspension of 3-chloro-6-methyl-benzo[b]thiophene-2-carboxylic acid in N,N-dimethylformamide was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.39 g, 12.5 mmol) and hydroxybenzotriazole hydrate (1.68 g, 12.5 mmol). After 5 minutes was added (N,O)-dimethylhydroxylamine hydrochloride (1.21g, 12.5 mmol) and triethylamine (1.74 mL, 12.5 mmol). The resulting suspension became a homogeneous solution after one hour. After 16 hours, the reaction was diluted with ethyl acetate (125 mL) and washed successively with hydrochloric

acid (75 mL, 0.5 M), saturated aqueous sodium hydrogen carbonate (75 mL) and water (25 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (1.90 g, 86%).

3-Chloro-6-methyl-benzo[b]thiophene-2-carbaldehyde (9c). To a stirring solution of 3-chloro-6-methyl-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (**8c**, 500 mg, 1.85 mmol) in tetrahydrofuran (6 mL) at -70 ºC under nitrogen was added a solution of lithium aluminum hydride (3.9 mL, 3.9 mmol, 1.0 M in tetrahydrofuran) dropwise over 10 minutes. After stirring for 30 minutes, the reaction was quenched by the dropwise addition of water (1.5 mL) and the mixture was warmed to ambient temperature. The reaction mixture was poured into ethyl acetate, the layers were separated and the organic layer was washed successively with hydrochloric acid (50 mL, 1 M) then saturated aqueous sodium chloride (50 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (388 mg, 99%). ^{1}H NMR ($CDCl_3$) δ 10.33 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.66 (br s, 1H), 7.34 (br d, J = 8.5 Hz, 1H), 2.51 (s, 3H).

(2E)-3-(3-Chloro-6-methyl-benzo[b]thiophen-2-yl)-N-hydroxy-acrylamide (15c). 15b was synthesized from aldehyde **9c** using procedure described for synthesis of **15a**. Yellow solid, 27% yield from aldehyde. HRMS (ESI-TOF) for $C_{12}H_{10}ClNO_2S$ calculated 268.0194; measured 268.02. ^{1}H NMR (DMSO-d_6) δ 10.89 (br s, 1H), 9.21 (br s, 1H), 7.83 (s, 1H), 7.72 (d, J = 11.1 Hz, 1H), 7.68 (d, J = 3.8 Hz, 1H), 7.35 (d, J = 8.2, 1H), 6.37 (d, J = 15.5, 1H), 2.44 (s, 3H). LCMS (ESI) *m*/*z* 268.0 $(M)^+$.

3,5-Dichloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (13d). To a rapidly stirring suspension of 3-(3-chloro-phenyl)-acrylic acid **11d** (5.0 g, 27.4 mmol) and 4-dimethylaminopyridine (3.35 g, 27.4 mmol) in heptane (15 mL) at 55 ºC was dropwise added thiony chloride (10.1 mL, 140 mmol). After the addition was complete, the reaction mixture was heated to reflux (80-85 ºC) for 6 hours. Ethyl acetate (55 mL) was added to the reaction mixture and heating was continued for one hour. The hot reaction mixture was filtered and the filtrate was concentrated at reduced pressure. The resulting mixture was dissolved with dichloromethane (35 mL) followed by addition of heptane (35 mL). The resulting solution was concentrated to a volume of approximately 30 mL, cooled to 0 ºC and filtered to give 3,5-dichloro-benzo[b]thiophene-2-carbonyl chloride **12d** as a yellow solid (3.0 g, 41%). To a rapidly stirring suspension of acid chloride **12d** (1.5 g, 5.65 mmol) and (N,O)-dimethylhydroxylamine hydrochloride (661 mg, 6.78 mmol) in dichloromethane (25 mL) was added triethylamine (1.73 mL, 12.4 mmol) dropwise. After 1 hour, the resulting thick suspension was diluted with dichloromethane (100 mL) and the mixture was washed with hydrochloric acid (0.5 M, 50 mL), then satuated aqueous sodium hydrogen carbonate (25 mL). The organic layer was concentrated at reduced pressure. The crude product was purified by silica gel chromatography (40 g cartridge) eluting with ethyl acetate/hexanes (1:5) to give the desired product as a white solid in low yield (280 mg, 17%). The major product was recovered and identified as 3,7-dichloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (625 mg, 38%).

3,5-Dichloro-benzo[b]thiophene-2-carbaldehyde (9d). To a stirring solution of 3,5-Dichloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide **13d** (500 mg, 1.85 mmol) in tetrahydrofuran (6 mL) at -70 ºC under nitrogen was added a solution of lithium aluminum

hydride (3.9 mL, 3.9 mmol, 1.0 M in tetrahydrofuran) dropwise over 10 minutes. After stirring for 30 minutes, the reaction was quenched by the dropwise addition of water (1.5 mL) and the mixture was warmed to ambient temperature. The reaction mixture was poured into ethyl acetate, the layers were separated and the organic layer was washed successively with hydrochloric acid (50 mL, 1 M) then saturated aqueous sodium chloride (50 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (388 mg, 99%).

3-(3,5-Dichloro-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (16d). To a solution of methyl diethylphosphonoacetate (0.55 mL, 630 mg, 3.0 mmol) in N,N-dimethylformamide (1.5 mL) was added sodium bis(trimethylsilyl)amide (1.5 mL, 3.0 mmol, 2M in tetrahydrofuran). After a mild exotherm, the mixture was stirred for 10 min. The resulting solution was added to a solution of the 3,5-dichloro-benzo[b]thiophene-2-carbaldehyde **9d** (316 mg, 1.5 mmol) in N,N-dimethylformamide (1.5 mL). After 1h, hydrochloric acid (1N, 6 mL) was added to the reaction mixture, followed by ethyl acetate/hexane (1:1, 6 mL). After shaking and allowing the layers to separate, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure to give the product as an off-white solid (400 mg, 100%).

3-(3,5-dichloro-benzo[b]thiophen-2-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide (17d). To a stirring solution of 3-(3,5-dichloro-3-benzo[b]thiophen-2-yl)-acrylic acid methyl ester **16d** (400 mg, 1.5 mmol) and O-(2-tetrahydropyranyl) hydroxylamine (351 mg, 3.0 mmol) in tetrahydrofuran (6 mL) was added sodium bis(trimethylsilyl)amide (1.50 mL, 3.0 mmol, 2M in tetrahydrofuran). After stirring for 30 min, hydrochloric acid (0.5 N, 6 mL) was added to the reaction mixture, followed by ethyl acetate (3 mL). The layers were separated, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (8 g cartridge) eluting with ethyl acetate/hexanes (1:8) to give the product as a pale yellow solid (85 mg, 16%).

3-(3,5-Dichloro-benzo[b]thiophen-2-yl)-N-hydroxy-acrylamide (15d). To a stirred solution of 3-(3,5-dichloro-benzo[b]thiophen-2-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide **17d** (85 mg) in methanol (0.5 mL) was added hydrogen chloride (1.0 mL, 4N in 1,4-dioxane). After stirring for 3h, the reaction mixture was concentrated under reduced pressure. Methanol (1 mL) was added to the crude product and the mixture was again concentrated under reduced pressure. The resulting solid was triturated with dioxane to give pure product as a pale yellow solid (61 mg, 94%). HRMS (ESI-TOF) for $C_{11}H_8Cl_2NO_2S$ calculated 287.9647; measured 287.9649. ^{1}H NMR (DMSO-d_6) δ 10.96 (br s, 1H), 9.94 (br s, 1H), 8.15 (d, J = 8.6 Hz, 1H), 7.85 (d, J = 2.7 Hz, 1H), 7.72 (d, J = 15.4, 1H), 7.59 (dd, J = 8.5, 2.8 Hz, 1H), 6.48 (d, J = 15.4 1H). ^{13}C NMR (DMSO-d_6) δ 162.1, 143.3, 141.5, 138.4, 132.3, 130.6, 128.5, 125.1, 124.1, 123.3, 121.5.

7-Bromo-3-chloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (13e). To a rapidly stirring suspension of 3-(3-bromo-phenyl)-acrylic acid **11e** (5.0 g, 22.0 mmol) and 4-dimethylaminopyridine (3.34 g, 27.4 mmol) in heptane (15 mL) at 55 ºC was added thionyl chloride (10.1 mL, 140 mmol) dropwise. After the addition was complete, the reaction mixture was heated to reflux (80-85 ºC) for 6 hours. Ethyl acetate (55 mL) was added to the reaction mixture and heating was continued for one hour. The hot reaction mixture was filtered and the

filtrate was concentrated at reduced pressure. The resulting mixture was dissolved with dichloromethane (35 mL) followed by addition of heptane (35 mL). The resulting solution was concentrated to a volume of approximately 30 mL, cooled to 0 ºC and filtered to give 7-bromo-3-chloro-benzo[b]thiophene-2-carbonyl chloride **12e** as a yellow solid (3.5 g, 41%). To a rapidly stirring suspension of acid chloride **12e** (3.0 g, 9.7 mmol) and (N,O)-dimethylhydroxylamine hydrochloride (1.13 g, 11.6 mmol) in dichloromethane (45 mL) was added triethylamine (3.0 mL, 21.3 mmol) dropwise. After 1 hour, the resulting thick suspension was diluted with dichloromethane (150 mL) and the mixture was washed with hydrochloric acid (0.5 M, 100 mL), then satuated aqueous sodium hydrogen carbonate (50 mL). The organic layer was concentrated at reduced pressure. The crude product was triturated with methanol (25 mL) and filtered to give the product as a white solid (1.17 g, 35%).

7-Bromo-3-chloro-benzo[b]thiophene-2-carbaldehyde (9e). To a stirring solution of 7-bromo-3-chloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide **13e** (1.15 g, 3.4 mmol) in tetrahydrofuran (15 mL) at -70 ºC under nitrogen was added a solution of lithium aluminum hydride (7.2 mL, 7.2 mmol, 1.0 M in tetrahydrofuran) dropwise over 10 minutes. After stirring for 30 minutes, the reaction was quenched by the dropwise addition of water (3 mL) and the mixture was warmed to ambient temperature. The reaction mixture was poured into ethyl acetate (120 mL), the layers were separated and the organic layer was washed successively with hydrochloric acid (120 mL, 1 M) then saturated aqueous sodium chloride (60 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (930 mg, 99%).

3-(7-Bromo-3-chloro-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (16e). To a solution of methyl diethylphosphonoacetate (1.27 mL, 1.45 g, 6.8 mmol) in N,N-dimethylformamide (5 mL) was added sodium bis(trimethylsilyl)amide (3.4 mL, 6.8 mmol, 2M in tetrahydrofuran). After a mild exotherm, the mixture was stirred for 10 min. The resulting solution was added to a solution of the 7-bromo-3-chloro-benzo[b]thiophene-2-carbaldehyde **9e** (930 mg, 3.4 mmol) in N,N-dimethylformamide (5 mL). After 1h, water (60 mL) was added to the reaction mixture dropwise and the resulting suspension was filtered to give the product as a tan solid (1.0 g, 88%).

3-(7-Bromo-3-chloro-benzo[b]thiophen-2-yl)-N-hydroxy-acrylamide (15e). To a stirring solution of 3-(7-bromo-3-chloro-benzo[b]thiophen-2-yl)-acrylic acid methyl ester **16e** (275 mg, 0.83 mmol) and O-(2-tetrahydropyranyl) hydroxylamine (194 mg, 1.66 mmol) in tetrahydrofuran (4 mL) was added sodium bis(trimethylsilyl)amide (0.83 mL, 1.66 mmol, 2M in tetrahydrofuran). After stirring for 30 min, hydrochloric acid (0.5 N, 4 mL) was added to the reaction mixture, followed by ethyl acetate (4 mL). The layers were allowed to separate, the top layer was removed and the organic addition and extraction were repeated. The organic layers were combined and concentrated under reduced pressure. The crude product was dissolved in methanol (2 mL) hydrogen chloride (4.0 mL, 4N in 1,4-dioxane) was added. After stirring for 3h, the reaction mixture was concentrated under reduced pressure. Methanol (2 mL) was added to the crude product and the mixture was again concentrated under reduced pressure. The resulting solid was triturated with ethyl acetate to give pure product as an off white solid (68 mg, 25%). HRMS (ESI-TOF) for $C_{11}H_8BrClNO_2S$ calculated 331.9142; measured 331.9150. ^{1}H NMR (DMSO-d_6) δ 10.94 (br s, 1H), 9.95 (br s, 1H), 7.87 (dd, J = 7.9, 2.4 Hz, 1H), 7.81 (dd, J = 7.8, 2.2 1H), 7.73 (d, J = 15.4 1H), 7.52 (app t, J = 7.9 Hz, 1H), 6.56 (d, J = 15.4 1H). ^{13}C NMR (DMSO-d_6) δ 161.7, 138.0, 138.7, 134.7, 130.7, 128.5, 127.3, 124.5, 123.3, 122.1, 116.1.

6-Chloro-3-methyl-benzo[b]thiophene-2-carbaldehyde (9f). To a stirring solution of 2-bromo-5-chloro-3-methyl-benzo[b]thiophene (**14**, 500 mg, 1.91 mmol) in tetrahydrofuran (10 mL) at -70 ºC under nitrogen was added a solution of n-butyllithium (0.84 mL, 2.1 mmol, 2.5 M in hexane). After 30 minutes, N,N-dimethylformamide (0.44 mL, 420 mg, 5.73 mmol) was added and the reaction mixture was allowed to warm to ambient temperature. After one hour at ambient temperature, the reaction mixture was poured into water (25 mL) and the resulting precipitate was filtered and rinsed with water (5 mL). The solid was collected and dried to give the product as a white solid (335 mg, 83%).

(2E)-3-(5-Chloro-3-methyl-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (16f). To a solution of methyl diethylphosphonoacetate (0.55 mL, 630 mg, 3.0 mmol) in N,N-dimethylformamide (1.5 mL) was added sodium bis(trimethylsilyl)amide (1.5 mL, 3.0 mmol, 2M in tetrahydrofuran). After a mild exotherm, the mixture was stirred for 10 min. The resulting solution was added to a solution of the 6-chloro-3-methyl-benzo[b]thiophene-2-carbaldehyde (**9f**, 316 mg, 1.5 mmol) in N,N-dimethylformamide (1.5 mL). After 1h, hydrochloric acid (1N, 6 mL) was added to the reaction mixture, followed by ethyl acetate/hexane (1:1, 6 mL). After shaking and allowing the layers to separate, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure to give the product as an off-white solid (400 mg, 100%).

3-(5-Chloro-3-methyl-benzo[b]thiophen-2-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide (17f).
To a stirring solution of (2E)-3-(5-chloro-3-methyl-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (**16f**, 400 mg, 1.5 mmol) and O-(2-tetrahydropyranyl) hydroxylamine (351 mg, 3.0 mmol) in tetrahydrofuran (6 mL) was added sodium bis(trimethylsilyl)amide (1.50 mL, 3.0 mmol, 2M in tetrahydrofuran). After stirring for 30 min, hydrochloric acid (0.5 N, 6 mL) was added to the reaction mixture, followed by ethyl acetate (3 mL). The layers were separated, the top layer was removed and the organic addition and extraction was repeated. The organic layers were combined and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (8 g cartridge) eluting with ethyl acetate/hexanes (1:8) to give the product as a pale yellow solid (85 mg, 16%).

(2E)-3-(5-Chloro-3-methyl-benzo[b]thiophen-2-yl)-N-hydroxy-acrylamide (15f).
To a stirred solution of (2E)-3-(5-chloro-3-methyl-benzo[b]thiophen-2-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide (**17f**, 85 mg) in methanol (0.5 mL) was added hydrogen chloride (1.0 mL, 4M in 1,4-dioxane). After stirring for 3h, the reaction mixture was concentrated under reduced pressure. Methanol (1 mL) was added to the crude product and the mixture was again concentrated under reduced pressure. The resulting solid was triturated with dioxane to give pure product as a pale yellow solid (61 mg, 94%). HRMS (ESI-TOF) for $C_{12}H_{11}ClNO_2S$ calculated 268.0194; measured 268.0185. ^{1}H NMR (DMSO-d_6) δ 7.97 (d, J = 8.5 Hz, 1H), 7.89 (d, J = 2.9 Hz, 1H), 7.77 (d, J = 15.7 Hz, 1H), 7.45 (dd, J = 8.5, 2.8 Hz, 1H), 6.31 (d, J = 15.6 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 162.6, 142.5, 136.8, 136.4, 134.5, 130.6, 129.9, 126.9, 124.9, 122.9, 121.7, 12.3.

Synthesis of derivative 18 (with saturated hydroxamate linker)

3-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-propionic acid methyl ester (17). To a stirring suspension of platinum (IV) oxide (50 mg) in ethyl acetate (3 mL) was added (2E)-3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-acrylic acid methyl ester (**16a**, 250 mg, 0.93 mmol) in ethyl acetate (2 mL). The reaction mixture was stirred under an atmosphere of hydrogen for 18 hours. The reaction mixture was filtered through Celite, rinsed with ethyl acetate and the combined filtrates were concentrated at reduced pressure. The crude product was purified by silica gel chromatography (8 g cartridge) eluting with ethyl acetate/hexanes (1:9 to 3:7) to give the product as a colorless oil (160 mg, 63%). ^{1}H NMR ($CDCl_3$) δ 7.68 (m, 1H), 7.42 (m, 1H), 7.18 (m, 1H), 3.73 (s, 3H), 3.25 (app t, 7.5 Hz, 2H), 2.72 (app t, 7.5 Hz, 2H).

3-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-N-hydroxy-propionamide (18). To a stirring solution of 3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-propionic acid methyl ester (**17**, 160 mg, 0.59 mmol) in methanol (1 mL) was added aqueous lithium hydroxide (1.17 mL, 1M). After one hour, the reaction mixture was poured into hydrochloric acid (20 mL, 1M) followed by ethyl acetate (3 x 30 mL). The layers were separated and the combined organic layer was concentrated at reduce pressure to give the acid. To a stirring suspension of 3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-propionic acid in N,N-dimethylformamide was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (168 mg, 0.88 mmol) and hydroxybenzotriazole hydrate (119 mg, 0.88 mmol). After 5 minutes was added O-(2-tetrahydropyranyl) hydroxylamine (103 mg, 0.88 mmol) and the resulting suspension became a homogeneous solution after one hour. After 2 hours, the reaction was diluted with ethyl acetate (30 mL) and washed successively with hydrochloric acid (20 mL, 0.5 M), saturated aqueous sodium hydrogen carbonate (20 mL) and water (10 mL). The organic layer was concentrated at reduced pressure to give the product as a white solid (145 mg, 81%). To a stirring solution of 3-(3-chloro-6-fluoro-benzo[b]thiophen-2-yl)-N-(tetrahydro-pyran-2-yloxy)-propionamide (145 mg) in methanol (1 mL) was added hydrogen chloride (2.0 mL, 4N in 1,4-dioxane). After stirring for 3 hours, the reaction mixture was concentrated under reduced pressure. Methanol (3 mL) was added to the crude product and the mixture was again concentrated under reduced pressure. The crude product was purified by silica gel chromatography (4 g cartridge) eluting with ethyl acetate/hexanes (2:8 to 9:1) to give the product as a white solid (63 mg, 57%). HRMS (ESI-TOF) for $C_{11}H_{10}ClFNO_2S$ calculated 274.0099; measured 274.0112. ^{1}H NMR (DMSO-d_6) δ 10.44 (br s, 1H), 8.79 (br s, 1H), 7.92 (m, 1H), 7.72 (m, 1H), 7.35 (m, 1H), 3.15 (app t, 7.6 Hz, 2H), 3.38 (app t, 7.6 Hz, 2H).

Synthesis of benzothiophene methyl acrylic derivatives

1-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-ethanone (10a). To a stirring solution of 3-chloro-6-fluoro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (**8a**, 273 mg, 1.0 mmol) in tetrahydrofuran (5 mL) at 3 ºC under nitrogen was added a solution of methylmagnesium bromide (0.67 mL, 2.0 mmol, 3.0 M in diethyl ether) dropwise over 10 minutes, keeping the temperature >10 ºC. After stirring for 30 minutes, the reaction was poured into saturated aqueous ammonium chloride (30 mL) and ethyl acetate (30 mL) was added, the layers were separated and the organic layer was concentrated at reduced pressure to give the

product as a white solid (220 mg, 96%). ^{1}H NMR ($CDCl_3$) δ 7.93 (m, 1H), 7.52 (m, 1H), 7.24 (m, 1H), 2.80 (s, 3H).

1-(3,6-Dichloro-benzo[b]thiophen-2-yl)-ethanone (10b). To a stirring solution of 3,6-dichloro-benzo[b]thiophene-2-carboxylic acid methoxy-methyl-amide (**8b**, 500 mg, 1.7 mmol) in tetrahydrofuran (9 mL) at 3 ºC under nitrogen was added a solution of methylmagnesium bromide (1.1 mL, 3.4 mmol, 3.0 M in diethyl ether) dropwise over 10 minutes, keeping the temperature >10 ºC. After stirring for 30 minutes, the reaction was poured into saturated aqueous ammonium chloride (30 mL) and ethyl acetate (30 mL) was added, the layers were separated and the organic layer was concentrated at reduced pressure to give the product as a white solid (410 mg, 98%). ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 2.0 Hz, 1H), 7.45 (dd, J = 8.5, 2.1 Hz, 1H).

(2E)-3-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-but-2-enoic acid hydroxyamide (20a-(*E*)) and (2Z)-3-(3-Chloro-6-fluoro-benzo[b]thiophen-2-yl)-but-2-enoic acid hydroxyamide (20a-(*Z*)). 20a-(*Z/E*) were synthesized from ketone **10a** using procedure described for synthesis of **15a**. The isomers were separated by silica gel chromatography (4 g cartridge) eluting with ethyl acetate/hexanes (2:8 to 3:7). (*E*) isomer: yellow solid, 23% overall yield from ketone. HRMS (ESI-TOF) for $C_{12}H_{10}ClFNO_2S$ calculated 286.0099; measured 286.0111. ^{1}H NMR (DMSO-d_6) δ 10.85 (s, 1H), 9.02 (br s, 1H), 8.00 (dd, J = 9.2, 2.3 Hz, 1H), 7.82 (dd, J = 8.9, 5.2 Hz, 1H), 7.45-7.38 (m, 1H), 6.23 (s, 1H), 2.82 (s, 3H). LCMS (ESI) *m/z* 285.9 $(M)^+$. (*Z*) isomer: brown oil, 8.3% overall yield from ketone. HRMS (ESI-TOF) for $C_{12}H_{10}ClFNO_2S$ calculated 286.0099; measured 286.0094. ^{1}H NMR (DMSO-d_6) δ 10.20 (br s, 1H), 7.98 (dd, J = 9.2, 2.3 Hz, 1H), 7.76 (dd, J = 8.9, 5.2 Hz, 1H), 7.42-7.35 (m, 1H), 6.10 (s, 1H), 2.10 (s, 3H).

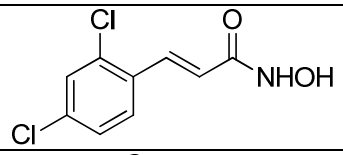
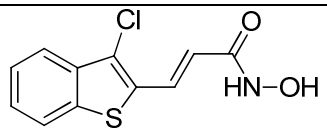
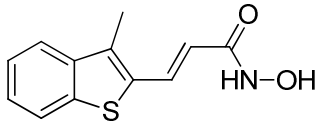
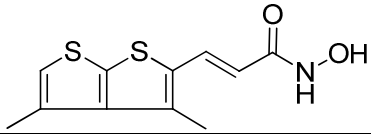
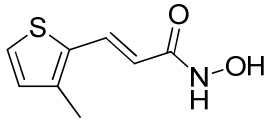
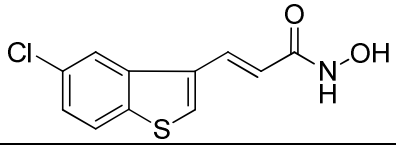
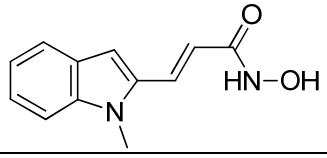
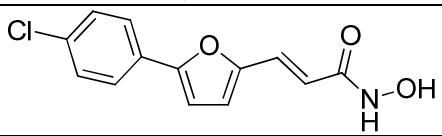
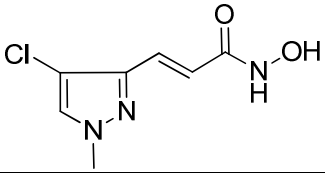
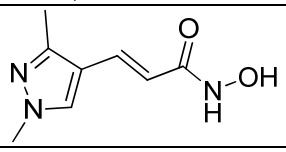
(2E)-3-(3,6-Dichloro-benzo[b]thiophen-2-yl)-but-2-enoic acid hydroxyamide (20b-(*E*)) and (2Z)-3-(3,6-Dichloro-benzo[b]thiophen-2-yl)-but-2-enoic acid hydroxyamide (20b-(*Z*)). 20b-(*Z/E*) were synthesized from ketone **10b** using procedure described for synthesis of **15a**. The isomers were separated by silica gel chromatography (4 g cartridge) eluting with ethyl acetate/hexanes (2:8 to 3:7). (*E*) isomer: yellow solid, 27% overall yield from ketone. HRMS (ESI-TOF) for $C_{12}H_{10}Cl_2NO_2S$ calculated 301.9804; measured 301.9811. ^{1}H NMR (DMSO-d_6) δ 10.86 (br s, 1H), 8.24 (s, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.56 (dd, J = 8.7, 1.8 Hz, 1H), 6.25 (s, 1H), 2.57 (s, 3H). LCMS (ESI) *m/z* 301.84 $(M)^+$. (*Z*) isomer: brown oil, 11% overall yield from ketone. HRMS (ESI-TOF) for $C_{12}H_{10}Cl_2NO_2S$ calculated 301.9804; measured 301.9807. ^{1}H NMR (DMSO-d_6) δ 10.25 (br s, 1H), 8.20 (s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.7 Hz, 1H), 6.13 (s, 1H), 2.13 (s, 3H).

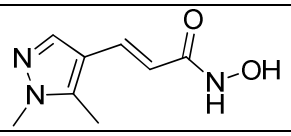
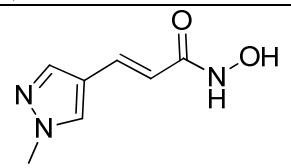
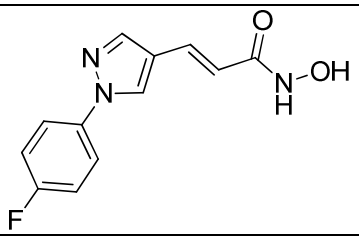
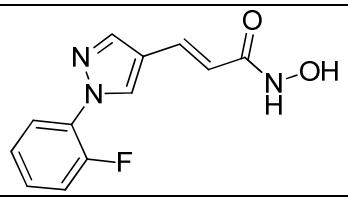
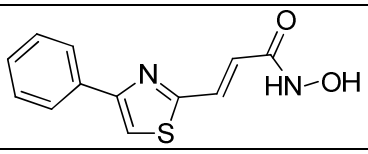
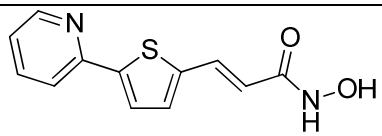
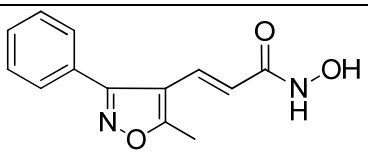
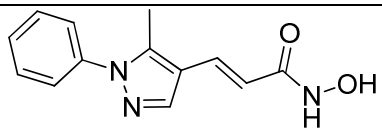
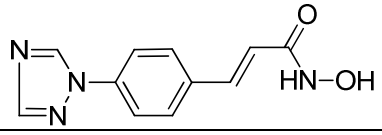
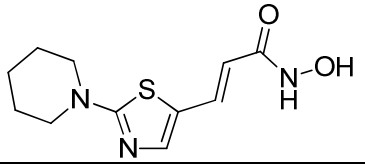
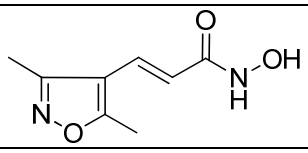
Plasma Protein Binding. Plasma protein binding is calculated following incubation of a 2.5 μM solution of analyte in plasma.[1] Nanosep centrifugal devices (molecular weight cut off 10,000, Pall Life Sciences, Ann Arbor, MI) were used to separate free and bound compound. The percent of free analyte was calculated by LC/MS/MS determination of the percent of the concentration of analyte in the filtrate relative to the total concentration. This percentage is then subtracted from 100 to determine the protein bound fraction.

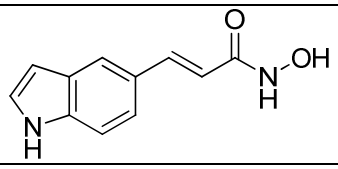
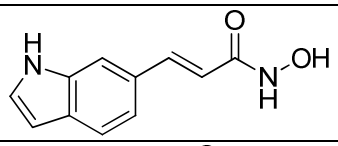
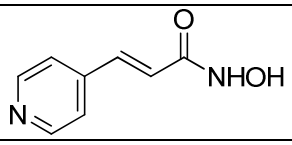
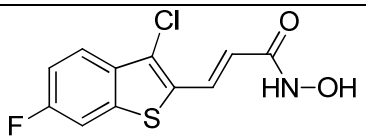
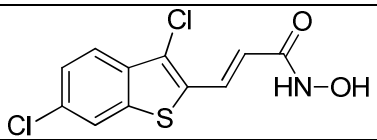
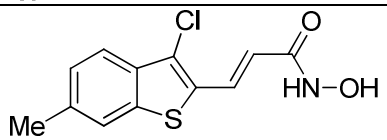
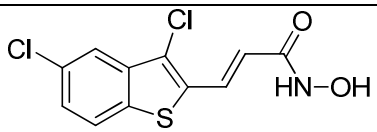
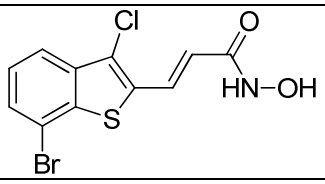
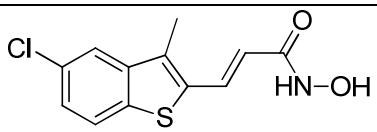
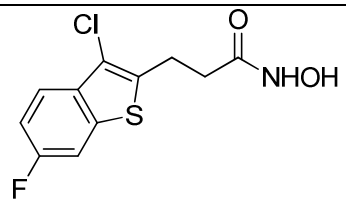
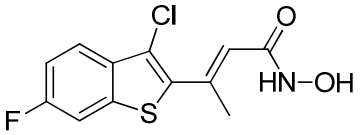
Aqueous Solubility. Aqueous solubility was determined using a miniaturized shake flask method at pH 6.8 after a 24 hour incubation at room temperature on a shaker set to 600 rpm.[2]

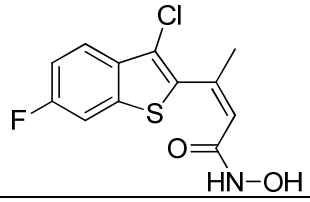
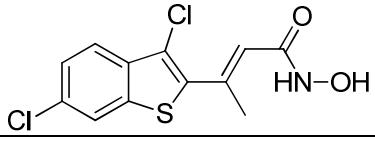
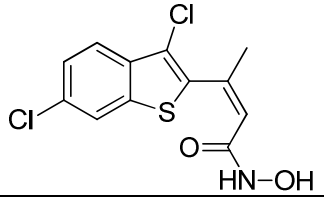
Whatman (Clifton, NJ) Mini-UniPrep syringeless filter were used to filter the resulting solution. Aqueous solubility was determined on the filtrate by LC/MS/MS determination of the concentration of compound in the filtrate.

Supporting Table S1. Structures of all tested hydroxamates and inhibitory data

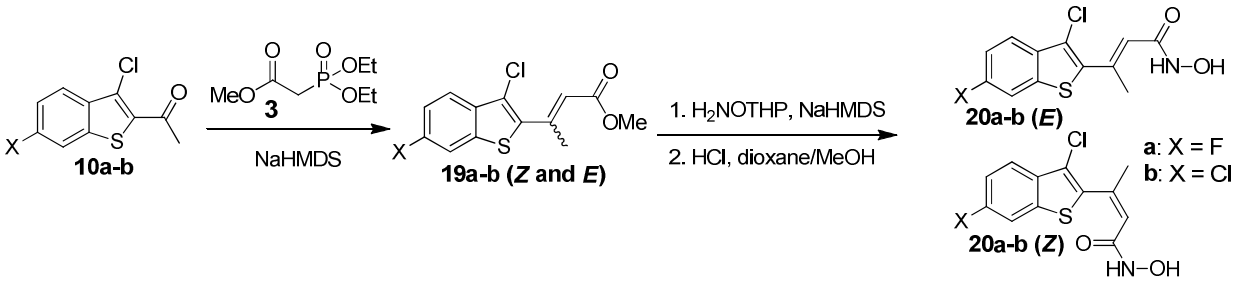
Compound	Structure	IC_{50} (μM)[a]
1		0.67 ± 0.036
6a		0.34 ± 0.023
6b		0.63 ± 0.035
6c		5.9 ± 0.69
6d		23.5
6e		27.6
6f		10.9
6g		100
6h		>100
6i		>100

6j		>100
6k		>100
6l		>100
6m		>100
6n		>100
6o		>100
6p		53
6q		>100
6r		>100
6s		>100
6t		>100

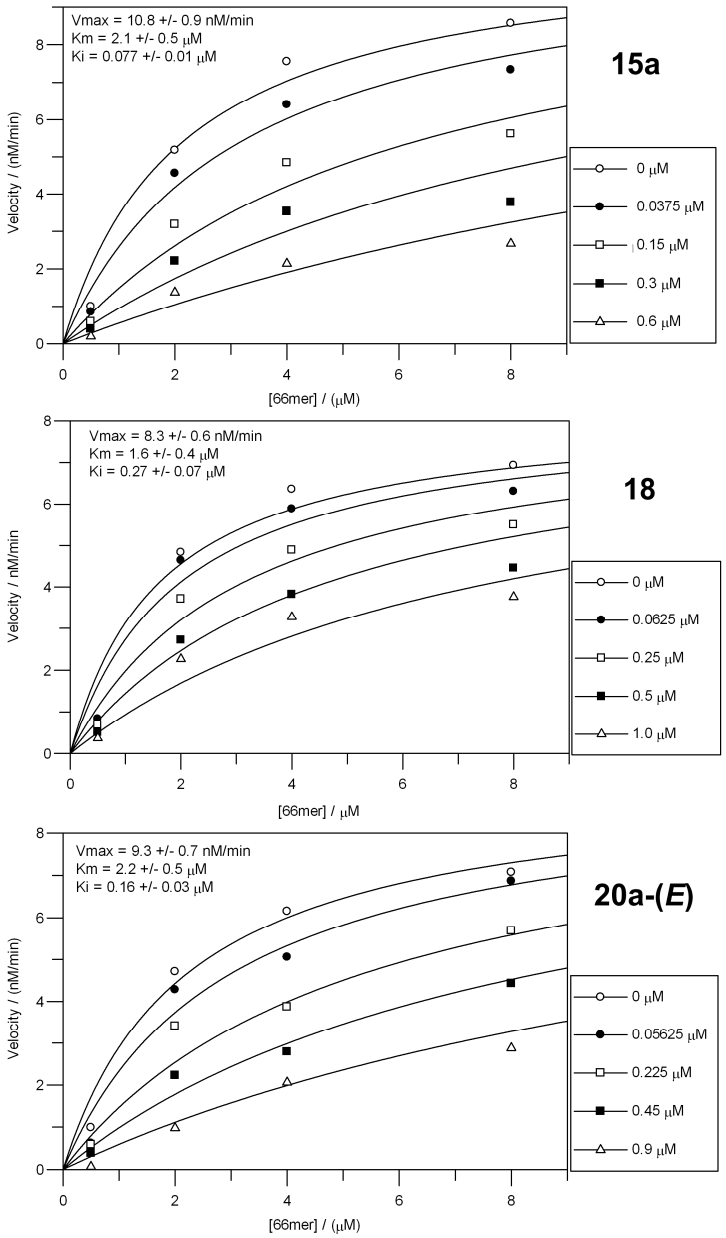
6u		81.1
6v		>100
6w		>100
15a		0.15 ± 0.004
15b		0.26 ± 0.019
15c		1.94 ± 0.12
15d		0.35 ± 0.074
15e		26.3
15f		0.21 ± 0.014
18		0.53 ± 0.029
20a-(E)		0.43 ± 0.025

20a-(Z)		6.22
20b-(E)		0.41 ± 0.023
20b-(Z)		4.78

(a) IC_{50} values determined in SNAPtide assay. IC_{50} is calculated from at least three experiments if standard error of mean is given.



Supporting Scheme S1. Synthesis of acrylic hydroxamates with methyl substituent on double bond



Supporting Figure S1. Kinetic data for inhibitors **15a**, **18** and **20a-(*E*)** in SNAP25[141-206] cleavage assay

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1. Lee, K.-J., Mower, R., Hollenbeck, T., Castelo, J., Johnson, N., Gordon, P., Sinko, P., Holme, K., and Lee, Y.-H. Modulation of Nonspecific Binding in Ultrafiltration Protein Binding Studies. *Pharm. Res.* **2003**, *20*, 1015-1021.

2. Zhou, L., Yang, L., Tilton, S., and Wang, J. Development of a High Throughput Equilibrium Solubility Assay Using Miniaturized Shake Flask Method in Early Drug Discovery. *J. Pharm. Sci.* **2007**, *96*, 3052-3062.