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Supporting Materials

New Inhibitors of Trypsin-Like Proteases. H-Bonding of an Aromatic Cyano Group with a Backbone Amide of the P_1-Binding Site Replaces Binding of a Basic Side Chain.

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General Synthetic Methods. Melting points were determined on a Thomas Hoover apparatus. ^1H NMR spectra were recorded at 300 MHz on GE QE-300 or Varian VXR-300 spectrometers. Chemical shifts were reported in parts per million relative to tetramethylsilane (δ 0.00) an internal standard for samples in CD_3OD and $CDCl_3$. Coupling constants (J values) are given in hertz (HZ) and multiplicities are assigned as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets) or dt (doublet of triplets). ^{13}C NMR spectra were obtained on a Varian VXR-300 spectrometer operating at a frequency of 75.4 MHz. ^{13}C chemical shifts are reported in ppm relative to either CD_3Cl (δ 77.0) or CD_3OD (δ 49.0) used as an internal standard. Assignments of resonances were consistent with distortionless enhancement by polarization transfer (DEPT) results. Low resolution mass spectra (LRMS) were measured on a Finnigan MAT 8230 spectrometer and high resolution mass spectra (HRMS) were measured on a VG 70-VSE spectrometer. Either chemical ionization using ammonia gas as an ion source (CI/NH_3) or fast-atom bombardment (FAB) were used.

Preparative chromatographic separation were run using E. Merck silica gel 60 (230-400 mesh) for flash columns or Sephadex LH-20 for gel filtration columns. All moisture sensitive reactions were conducted in oven-dried (125°C overnight) or flame-dried glassware under a nitrogen atmosphere. Transfers were performed by syringe or cannula. Anhydrous solvents were purchased from Aldrich in Sure-Seal bottles. Dichloromethyl boronic acid pinanediol was prepared by the method of Tsai et al., (1983).

Preparation of Inhibitors.

Cl-CH[CH_2-(m-cyanophenyl)]BO_2-$C_{10}H_{16}$ (**1**). Zinc dust (1.0 g) in 1 mL of THF was cooled to 0-5°C and a solution of *m*-cyanobenzyl bromide (1.37 g, 7.0 mmol) in 7 mL of THF was added dropwise (5 sec/drop). The reaction mixture was allowed to stir at 5°C for 2 h. A mixture consisting of LiBr (1.22 g, 14 mmol), CuCN (0.63 g, 7.0 mmol), and 6 mL of THF was placed in a 50 mL flask and cooled to -40°C; then the benzylic organozinc reagent was added by cannulation. The mixture was allowed to warm to -20°C and stir for 5 min. It was cooled to -78°C and neat dichloromethyl boronic acid pinanediol (1.47 g, 5.6 mmol) was added dropwise. The resulting mixture was stirred at -78°C for 2 h and at room temperature for 2 days. Saturated aqueous NH_4Cl (20 mL) was added to the mixture and the aqueous solution was extracted with three 20 mL portions of ether. The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give crude compound (1.8 g). It was purified by silica gel chromatography where the column was stepwise eluted with hexane (100 mL) and then 15% ether in hexane (200 mL) to give the desired product 0.53 g (27% yield). ^1H NMR (300

MHz, $CDCl_3$) δ 7.59-7.38 (m, 4H), 4.38-4.32 (m, 1H), 3.63 (dd, 1/2H, J= 16.0, 9.5 Hz), 3.48 (dd, 1/2H, J = 16.2, 8.3 Hz), 3.40-3.15 (m, 2H), 2.40-2.24 (m, 1H), 2.24-2.18 (m, 1H), 2.10-2.06 (m, 1H), 1.93-1.82 (m, 2H), 1.38 (s, 3/2H), 1.37 (s, 3/2H), 1.29 (s, 3/2H), 1.29 (s, 3/2H), 1.04 (d, 1/2H, J = 10.6Hz), 0.97 (d, 1/2H, J = 10.5 Hz), 0.84 (s, 3/2H), 0.83 (s, 3/2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 140.5, 133.6, 132.6, 130.4, 129.1, 118.5, 112.4, 86.8, 78.5, 51.0, 39.6, 38.2, 35.1, 35.0, 28.2, 26.9, 26.0, 23.8 (isomer 1); 140.6, 133.5, 132.7, 130.4, 129.1, 118.6, 112.4, 86.8, 78.6, 51.1, 39.8, 39.2, 35.1, 35.0, 28.3, 26.9, 26.1, 23.8 (isomer 2). LRMS(CI) calcd for $[M+NH_4]$ $=C_{19}H_{27}N_2O_2BCl$ 361.2, found 361.1.

$H_2NCH[CH_2C_6H_4$-m-CN$]BO_2C_{10}H_{16}$•HCl or H-boroPhe(m-CN)-$C_{10}H_{16}$•HCl (**3**). To a solution of hexamethyldisilazane (0.21 mL, 0.98 mmol) in 2 mL of THF at -78°C was added *n*-butyl lithium (1.45 M, 0.67 mL, 0.98 mmol). The solution was allowed to slowly warm to room temperature to ensure the anion generation was complete. The resulting solution was then cooled to -78°C and Cl-CH[CH_2-(*m*-cyanophenyl)]BO_2-$C_{10}H_{16}$ **1** (0.33 g, 0.98 mmol) in 2 mL of THF was added. The mixture was allowed to warm to room temperature and to stir overnight to give **2**. Solvent was evaporated and 8 mL of hexane was added to give a suspension. HCl in dioxane (4.1 N, 1.5 mL, 6.0 mmol) was added at -78°C. The mixture was slowly warmed to room temperature and stirred for 2 h. Additional hexane (6 mL) was added and crude product was isolated as a precipitant. This product was dissolved in chloroform and insoluble material was removed by filtration. The filtrate was evaporated at a reduced pressure to give an oil (~0.2 g). Final purification was achieved by chromatography

on a column of Sephedex™ LH 20 column using methanol as a solvent. H-boroPhe(*m*-CN)-$C_{10}H_{16}$•HCl was obtained as an oil (0.12 g, 34% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 7.71-7.54 (m, 4H), 4.46 (t, 1H, J = 6.9 Hz), 3.31-3.28 (m, 1H), 3.15-3.10 (m, 2H), 2.43-2.34 (m, 1H), 2.30-2.19 (m, 1H), 2.05-2.00 (t, 1H, J = 6.0 Hz), 1.94-1.80 (m, 2H), 1.41 (s, 3/2 H), 1.40 (s, 3/2H), 1.31 (s, 3/2H), 1.30 (s, 3/2H), 1.06 (d, 1/2H, J = 11.1Hz), 1.05 (d, 1/2H, J = 10.9 Hz), 0.87 (s, 3/2H), 0.86 (s, 3/2H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 139.7, 135.3, 134.1, 132.1, 131.0, 119.5, 113.7, 89.1, 80.3, 52.4, 40.7, 39.2, 36.0, 35.8, 28.8, 27.4, 27.3, 24.2 (isomer 1); 139.7, 135.3, 134.1, 132.1, 131.0, 119.5, 113.7, 89.1, 80.2, 52.2, 40.6, 39.2, 35.8, 35.7, 28.7, 27.3, 27.2, 24.2 (isomer 2). HRMS(CI) calcd for [M+H] =$C_{19}H_{26}N_2O_2B$ 325.2087, found 325.2094.

Boc-(D)Phe-Pro-boroPhe(mCN)-$C_{10}H_{16}$ (**4**). Boc-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$ was prepared by reacting Boc-(D)Phe-Pro-OH (0.43 g, 1.2 mmol), H-boroPhe(*m*CN)-$C_{10}H_{16}$•HCl **3** (0.42 g, 1.2 mmol), N-methylmorpholine (0.26 mL, 2.4 mmol), hydroxybenzotriazole•H_2O (0.36 g, 2.4 mmol), and dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) in 20 mL of dichloromethane overnight at room temperature. The reaction mixture was filtered and the filtrate was chromatographed on a 2.5 X 100 cm column of Sephedex LH-20 in methanol to yield 0.36 g of the desired product. The product was further purified by chromatography on a 2.2 X 25 cm column of Zorbax-ODS. The column was eluted isocratically with 65% acetonitrile: 35% water at a flow rate of 40 mL/min. Two major fractions were isolated, with retention times of 14.6 min and 17.3 min. Both fractions were

characterized and labeled Isomer A and Isomer B, respectively. This designation was used in subsequent experiments. Isomer A was identified as Boc-(D)Phe-Pro-(L)boroPhe(*m*CN)-$C_{10}H_{16}$ in subsequent studies. Isomer A: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.42-7.23 (m, 4H), 7.21-7.11 (m, 5H), 5.15 (bd, 1H), 4.43 (d, 1H, J = 6.8Hz), 4.35 (d, 1H, J = 7.5 Hz), 4.18 (d, 1H , J = 8.4 Hz), 3.44 (t, 1H, J = 8.2 Hz), 2.91 (d, 2H, J = 7.3 Hz), 2.86-2.75 (m, 3H), 2.49 (dd, 1H, J = 16.2, 9.3 Hz), 2.25-2.21 (m, 2H), 2.03-1.94 (m, 1H), 1.88 (t, 1H, J = 5.5Hz), 1.75-1.37 (m, 5H), 1.29 (s, 9H), 1.24 (s, 3H), 1.17 (s, 3H), 0.96 (d, 1H, J = 10.5Hz), 0.74 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.7, 171.4, 155.3, 141.5, 135.8, 134.0, 133.1, 129.7, 129.2, 128.7, 128.5, 127.1, 119.1, 112.0, 84.8, 80.0, 77.3, 58.5, 54.0, 51.5, 46.7, 40.5, 39.5, 38.7, 37.9, 36.5, 35.5, 29.5, 28.6, 28.1, 27.7, 27.0, 26.0, 23.9. HRMS(FAB) calcd for [M+H] =$C_{38}H_{50}N_4O_6B$ 669.3834, found 669.3808. Isomer B: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (bs, 1H), 7.46 (s, 1H), 7.41-7.23 (m, 3H), 7.20-7.10 (m, 5H), 5.22 (bd, 1H), 4.47 (d, 1H, J = 6.2 Hz), 4.36 (d, 1H, J = 7.3 Hz), 4.10 (d, 1H , J = 7.7 Hz), 3.41 (t, 1H, J = 8.2 Hz), 2.88 (m, 2H), 2.95-2.72 (m, 3H), 2.39 (m, 1H), 2.35-2.13 (m, 2H), 2.03-1.86 (m, 1H), 1.91 (t, 1H, J = 5.6Hz), 1.77-1.49 (m, 5H), 1.33 (s, 9H), 1.20 (s, 3H), 1.18 (s, 3H), 1.12 (d, 1H, J = 10.1 Hz), 0.75 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.4, 171.5, 155.6, 141.4, 135.6, 133.9, 133.0, 129.7, 129.2, 128.8, 128.5, 127.2, 118.9, 112.0, 84.4, 80.2, 76.9, 58.0, 54.1, 51.7, 46.4, 41.3, 39.7, 38.3, 38.0, 36.6, 35.8, 29.5, 28.7, 28.2, 27.7, 27.1, 26.0, 24.0. HRMS(FAB) calcd for [M+H] =$C_{38}H_{50}N_4O_6B$ 669.3834, found 669.3850.

H-(D)Phe-Pro-(L)boroPhe(mCN)-$C_{10}H_{16}$•HCl (**5**). Boc-(D)Phe-Pro-(D,L)boroPhe(*m*CN)-$C_{10}H_{16}$ **4** (0.21 g, 0.31 mmoles) was allowed

to react with 2 mL of 4 N HCl dioxane for 2 h at room temperature. Solvent was removed by evaporation and the residue was triturated with ether to yield 0.11 g of the desired product as a white solid. This material corresponds to H-(D)Phe-Pro-(L)boroPhe(*m*CN)-$C_{10}H_{16}$ prepared by treating chromatographically purified Boc-(D)Phe-Pro-(L)boroPhe(*m*CN)-$C_{10}H_{16}$ with HCl. ^{1}H NMR (400 MHz, CD_3OD) δ 7.68 (s, 1H), 7.62-7.42 (m, 3H), 7.39-7.26 (m, 5H), 4.47-4.41 (m, 1H), 4.40-4.37 (m, 1H), 4.16 (dd, 1H , J = 8.6, 1.8 Hz), 3.64-3.60 (m 1H), 3.35-2.84 (m, 5H), 2.59 (dd, 1H, J = 16.5, 7.1 Hz), 2.29-2.23 (m, 1H), 1.97-1.80 (m, 5H), 1.76-1.67 (m, 2H, 1.57-1.52 (m, 1H), 1.27 (s, 3H), 1.22 (s, 3H), 0.85 (d, 1H, J = 10.3 Hz), 0.82 (s, 3H). ^{13}C NMR (100.6 MHz, CD_3OD) δ 176.7, 168.9, 142.9, 135.5, 135.4, 134.4, 131.0, 130.7, 130.3, 130.1, 129.1, 120.0, 113.0, 85.1, 77.9, 59.1, 54.3, 53.1, 48.2, 44.2, 41.0, 39.1, 38.2, 37.3, 36.7, 30.2, 29.5, 27.6, 26.9, 25.2, 24.5. HRMS(FAB) calcd for [M+H] =$C_{33}H_{42}N_4O_4B$ 569.3299, found 569.3315.

H-(D)Phe-Pro-boroPhe(mCN)-OH•HCl (**8**). H-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$•HCl **5** (1.10 g, 1.8 mmol) and phenylboronic acid (1.1 g, 9.1 mmol) were allowed to stir at room temperature for 3 hr in a mixture consisting of 15 mL of water and 15 mL of ether. The phases were separated and the aqueous phase was washed with additional ether. The aqueous phase was evaporated and the residue was triturated with ether to yield the desired product, 0.83 g (1.7 mmol). HRMS(FAB) calcd for $C_{25}H_{30}BN_4O_4$ (product + ethylene glycol) [M+H]: 461.2360, found: 461.2349.

Ac-(D)Phe-Pro-(L)boroPhe(mCN)-$C_{10}H_{16}$ (**6**). H-(D)Phe-Pro-(L)boroPhe(*m*CN)-$C_{10}H_{16}$•HCl **5** (0.85 g, 1.4 mmol) was dissolved in

dioxane; acetic anhydride (0.23 g, 2.2 mmol) and saturated aqueous $NaHCO_3$ (15 mL) were added. The mixture was stirred overnight at room temperature. After removal of dioxane by evaporation, 20 mL of water were added and the product was extracted into ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to yield the product as a white solid (0.90 g). The product was purified by HPLC using the procedure described for Boc- compound except the column was eluted with 55% acetonitrile in water at a flow rate of 30 mL/min. The product, which was eluted at 7.1 min, was collected and evaporated to give 0.54 g. ^1H NMR (400 MHz, CD_3OD) δ 7.65 (s, 1H), 7.58-7.41 (m, 3H), 7.33-7.23 (m, 5H), 4.62 (d, 1H, J = 7.9 Hz), 4.49 (dd, 1H, J = 8.3, 2.7 Hz), 4.12 (dd, 1H , J = 8.8, 2.0 Hz), 3.67-3.61 (m 1H), 2.99 (d, 2H, J = 8.0 Hz), 2.89-2.81 (m, 3H), 2.67 (q, 1H, J = 8.0 Hz), 2.26-2.21 (m, 1H), 1.96-1.57 (m, 8H),1.90 (s 3H), 1.26 (s, 3H), 1.21 (s, 3H), 0.82 (d, 1H, J = 9.0 Hz), 0.81 (s, 3H). ^{13}C NMR (100.6 MHz, CD_3OD) δ 177.6, 173.4, 172.9, 143.0, 137.4, 135.4, 134.3, 130.9, 130.5, 130.3, 129.6, 128.3, 119.9, 113.0, 84.4, 77.5, 58.6, 55.0, 53.3, 48.0, 45.2, 41.1, 39.0, 38.5, 37.5, 36.8, 29.9, 29.6, 27.7, 26.8, 24.9, 24.5, 22.3. HRMS(FAB) calcd for [M+H] =$C_{35}H_{44}N_4O_5B$ 611.3405, found 611.3448.

Ac-(D)Phe-Pro-boroPhe(m-CH_2NH_2)-$C_{10}H_{16}$ (**7**). Ac-(D)Phe-Pro-boroPhe(*m*-CN)-$C_{10}H_{16}$ **6** (0.097 g) was placed in 10 mL of methanol, 10% Pd/C (30 mg) and 1N HCl (0.17 mL) were added, and the mixture was stir under H_2 at room temperature for 15 h. The solution was filtered through Celite™ and washed with 20 mL of methanol. The filtrate was concentrated under reduced pressure and the residue was triturated with ether to give pure product as white

powder (95 mg, 99% yield). HRMS(NH_3-CI) m/e calcd. for M ($C_{35}H_{47}N_4O_5B$) + H: 615.3718. Found: 615.3700. ^1H NMR (400 MHz, CD_3OD) δ 7.35-7.24 (m, 9H), 4.56-4.49 (m, 2H), 4.18 (d, 1H , J = 6.5 Hz), 4.12 (ABq, 2H, J = 13.5 Hz), 3.66-3.62 (m 1H), .34-2.80 (m, 5H), 2.61 (dd, 1H, J = 16.7, 9.2 Hz), 2.29-2.24 (m, 1H), 1.99-1.58 (m, 8H),1.77 (s 3H), 1.28 (s, 3H), 1.24 (s, 3H), 1.10 (d, 1H, J = 10.5 Hz), 0.83 (s, 3H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 176.3, 173.6, 173.1, 142.6, 137.3, 134.0, 131.2, 131.2, 130.5, 130.1, 129.7, 128.4, 127.7, 85.3, 78.0, 59.4, 55.3, 53.2, 48.1, 44.5, 44.4, 41.1, 39.1, 38.3, 37.2, 37.0, 30.0, 29.5, 27.7, 27.1, 24.7, 24.5, 22.3.