Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Synthesis, Activity and Structure-Activity Relationship of Noroviral Protease Inhibitors

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Experimental Section

All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). All compounds were characterized by ^{1}H spectrum on a Varian (Palo Alto, CA) 400-MR spectrometer. The purities were determined by a Shimadzu Prominence HPLC using a Zorbax C18 column (4.6 x 250 mm; methanol:water = 70:30; flow rate = 1 mL/min; monitored at 254 and 280 nm). The purities of all compounds were found to be >95%.

General synthetic method A (for synthesizing compound **1** with an aldehyde functionality): To a solution of Z-Glu-OMe (2.95 g, 10 mmol) in DMF (50 mL) were added Et_3N (7 mL, 50 mmol) and HOBT (2.03 g, 15 mmol) at 0 ^{0}C followed, after 10 min, by adding $NH(CH_3)_2$·HCl (2.45 g, 30 mmol) and EDCI (2.88 g, 15 mmol). The reaction mixture was stirred overnight at room temperature before quenched with water (50 mL). The product was extracted with EtOAc (3 x 20 mL) and the combined organic phases washed successively with 1 M HCl (10 mL), 1 M NaOH (10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified with column chromatography (silica gel, EtOAc/Hexanes 1:1) to give α-amino-Z-protected **17** (X^1=X^2=Me, 2.91g, 90%) as a colorless oil.

The product thus obtained (1.61 g, 5 mmol) was dissolved in MeOH (20 mL) containing 5% Pd/C (300 mg). The reaction was vigorously stirred under H_2 atmosphere (1 atm) for 5 h. The reaction mixture was filtered through Celite, wahed and the filtrate was concentrated under reduced pressure to give **17** (921 mg, 98%) as a colorless oil, which could be used in the next step without further purification.

To a solution of **17** (921 mg, 5 mmol) and Z-Phe-OH (1.64 g, 5.5 mmol) in DMF (30 mL) were added Et_3N (2.1 mL, 15 mmol) and HOBT (743 mg, 5.5 mmol) at 0 ^{0}C. After stirring for 45 min, EDC (1.44 g, 7.5 mmol) was added at 0 $^\circ$C and the reaction mixture allowed to stir overnight at room temperature. The reaction was quenched with water (30 mL) and extracted with EtOAc (3 x 15 mL). The combined organic phases were successively washed with 1 M HCl (10 mL), 1 M NaOH (10 mL)

and brine (10 mL), dried and concentrated. The remaining residue was purified with column chromatography (silica gel, EtOAc/Hexanes 1:1 then EtOAc) to give α-amino-Z-protected **20** (2.08 g, 88%) as a colorless oil.

Hydrogenation of the product (1.41 g, 3 mmol) with 5% Pd/C was performed using the above method to give **20** (925 mg, 92%) as a colorless oil. The coupling reaction between **20** (536 mg, 1.6 mmol) and Z-Leu-OH (636 mg, 2.4 mmol) was done using the EDC/HOBT method as described above to give **21** as a colorless oil (784 mg, 84%).

Compound **21** (285 mg, 0.49 mmol) was dissolved in a mixture of THF/MeOH (v/v, 4:1, 5 mL). $NaBH_4$ (36.2 mg, 0.98 mmol) was added slowly at 0 ^{0}C followed by the addition of LiCl (42 mg, 0.98 mmol). The reaction mixture was stirred overnight at room temperature and concentrated under reduced pressure. The resulting residue was subjected to column chromatography (silica gel, EtOAc then 5% MeOH in EtOAc) to give the corresponding alcohol, which was dissolved in dichloromethane (6 mL). Dess–Martin periodinane (382 mg, 0.93 mmol) was added at $^\circ$C and after stirring for 4h, the reaction was quenched by adding saturated $Na_2S_2O_3$ solution (5 mL), $NaHCO_3$ (5 mL) and diethyl ether (50 mL) and stirred for 30 min. The organic layer was separated, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography (silica gel, EtOAc then 2% MeOH in EtOAc) to give **1** as a white solid (221 mg, 87%).

General synthetic method B (for synthesizing compound **11** with an α,β-unsaturated ester): To a solution of triethyl phosphonoacetate (44 µL, 0.22 mmol) in THF (5 mL) was added NaH (9.7 mg, 0.24 mmol, 60% in oil) at 0 ºC. After 1 h, compound **1** (110 mg, 0.2 mmol) was added and the reaction mixture was stirred overnight at room temperature. The reaction was quenched with saturated NH_4Cl (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over $NaSO_4$, filtered and concentrated under reduced pressure. The residue was purified with column chromatography (silica gel, EtOAc) to give compound **11** (98 mg, 78%) as a white solid.

General synthetic method C (for synthesizing compound **10** with a methyl ketone functionality): To a solution of **1** (121 mg, 0.23 mmol) in THF (4 mL) at -78 °C was added methyl magnesium bromide (0.39 mL, 3 M solution in THF, 1.15 mmol) slowly. After stirring for 1h, the reaction was gradually warmed to room temperature over 1 h and then quenched by adding saturated NH_4Cl (10 mL). The product was extracted with EtOAc (3 x 10 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified with column chromatography (silica gel, EtOAc then 5% MeOH in MeOH) to give the corresponding alcohol (82 mg, 66%) as a colorless oil, which was dissolved in dichloromethane (5 mL). Dess-Martin periodinane (129 mg, 0.30 mmol) was added at 0°C and after stirring for 4h, the reaction was quenched by adding saturated $Na_2S_2O_3$ solution (5 mL), $NaHCO_3$ (5 mL) and diethyl ether (30 mL) and stirred for 30 min. The organic layer was separated, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography (silica gel, EtOAc then 2% MeOH in EtOAc) to give **10** as a white solid (66 mg, 82%).

Compound characterization

Compound 1: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.38 (d, *J* = 7.2 Hz, 1H), 7.60 (br, 1 H), 7.39-7.17 (m, 10 H), 6.62 (br, 1 H), 5.18-4.99 (m, 3 H), 4.73-4.66 (m, 1 H), 4.21-4.03 (m, 1 H), 3.18-3.01 (m, 2 H), 2.97 (s, 3 H), 2.91 (s, 3 H), 2.42-2.24 (m, 1 H), 2.22-1.83 (m, 3 H), 1.64-1.35 (m, 3 H), 0.92 (d, *J* = 4.4 Hz, 3 H), 0.87 (d, *J* = 4.4 Hz, 3 H).

Compound 2: ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.35 (s, 1 H), 7.89-7.83 (m, 1 H), 7.43-7.21 (m, 5 H), 4.99 (s, 2 H), 4.39-4.18 (m, 1 H), 4.11-3.98 (m, 2 H), 2.95 (s, 3 H), 2.75 (s, 3 H), 2.31-2.27 (m, 1 H), 2.21-2.16 (m, 1 H), 1.87-1.79 (m, 1 H), 1.62-1.38 (m, 4 H), 1.17 (d, *J* = 6.0 Hz, 3 H), 0.87 (d, *J* = 6.8 Hz, 3 H), 0.81 (d, *J* = 6.8 Hz, 3 H).

Compound 3: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.47 (d, *J* = 8.0 Hz, 1H) 7.41-7.15 (m, 10 H), 6.83 (br, 1 H), 5.41 (m, 1 H), 5.13-4.93 (m, 2 H), 4.71-4.59 (m, 1 H), 4.42-4.27 (m, 1 H), 4.19-4.05 (m, 1 H), 3.68-

3.62 (m, 1 H), 3.34-3.29 (m, 1 H), 3.20-2.95 (m, 2 H), 1.92 (s, 3 H), 1.61-1.37 (m, 3 H), 0.95 (d, J = 4.4 Hz, 3 H), 0.88 (d, J = 4.4 Hz, 3 H).

Compound 4: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.37 (s, 1 H), 8.02 (d, J = 3.2 Hz,1 H), 7.91 (d, J = 3.2 Hz,1 H), 7.51 (s, 1 H), 7.34-7.26 (m, 5 H), 6.78 (s,1 H), 4.99 (s, 2 H), 4.26-4.18 (m, 2 H), 4.11-4.07 (m, 1 H), 2.85 (s, 3 H), 2.76 (s, 3 H), 2.84-2.75 (m, 2 H), 2.31-2.26 (m, 2 H), 2.25-1.99 (m, 1 H), 1.82-1.77 (m, 1 H), 1.61-1.42 (m, 3 H), 0.87 (d, J = 4.2 Hz, 3 H), 0.81 (d, J = 4.2 Hz, 3 H).

Compound 5: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.53 (s, 1H), 7.78 (br, 1 H), 7.37-7.20 (m, 5 H), 7.03 (m, 1 H), 6.79 (br, 1 H), 5.07 (s, 2 H), 4.56-4.21 (m, 2 H), 4.12-4.07 (m, 1 H), 2.97 (s, 3 H), 2.92 (s, 3 H), 2.42-1.95 (m, 8 H), 1.62-1.55 (m, 3 H), 0.94 (d, J = 6.0 Hz, 3 H), 0.90 (d, J = 6.0 Hz, 3 H).

Compound 6: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.56 (s, 1 H), 7.92 (br, 1 H), 7.61 (br, 1 H), 7.43-7.28 (m, 5 H), 5.58 (t, J = 6.4 Hz, 1 H), 5.18-5.07 (m, 2 H), 4.61-4.34 (m, 3 H), 3.82-3.64 (m, 2 H), 3.01 (s, 3 H), 2.97 (s, 3 H), 3.01-2.84 (m, 2 H), 2.57-1.94 (m, 9 H), 1.04 (d, J = 6.4 Hz, 3 H), 0.94 (d, J = 6.4 Hz, 3 H).

Compound 7: ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.22 (s, 1 H), 7.94 (br, 1 H), 7.41-7.12 (m, 10 H), 6.83 (br, 1 H), 6.09 (dd, J = 3.2, 11.2 Hz, 1 H), 4.97 (s, 2 H), 4.52-4.21 (m, 3 H), 3.62 (br, 1 H), 3.28-3.17 (m, 2 H), 3.02-2.97 (m, 1 H), 2.86 (s, 3 H), 2.76 (s, 3 H), 2.42-1.99 (m, 4 H), 1.82-1.79 (m, 1 H), 1.59-1.51 (m, 1H).

Compound 8: ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.36 (s, 1 H), 8.26 (J = 6.8 Hz, 1 H), 7.46-7.21 (m, 5 H), 4.98 (s, 2 H), 4.75-4.62 (m, 1 H), 4.40-4.18 (m, 2 H), 3.61-3.42 (m, 2 H), 2.89 (s, 3 H), 2.76 (s, 3 H), 2.35-2.07 (m, 2 H), 2.01-1.27 (m, 9 H), 0.85 (d, J = 6.4 Hz, 3 H), 0.83 (d, J = 6.4 Hz, 3 H).

Compound 9: ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.43 (s, 1 H), 7.29-7.19 (m, 5 H), 5.82-5.73 (m, 1 H), 4.42-4.18 (m, 2 H), 3.08-3.05 (m, 2 H), 2.96 (s, 3 H), 2.90 (s, 3 H), 2.43-2.39 (m, 1 H), 2.29-2.19 (m, 1 H), 1.95 (s, 3 H), 2.05-1.56 (5 H), 0.85 (d, J = 6.4 Hz, 3 H), 0.83 (d, J = 6.4 Hz, 3 H).

Compound 10: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.18 (m, 10 H), 6.58 (d, J = 4.0 Hz,1 H), 5.10-5.05 (m, 2 H), 4.71-4.65 (m, 1 H), 4.47-4.38 (m, 1 H), 4.17-4.08 (m, 1 H), 3.11-3.07 (m, 2 H), 2.93 (s, 3 H),

2.90 (s, 3 H), 2.39-2.14 (m, 2 H), 2.14 (s, 3 H), 1.93-1.41 (m, 5 H), 0.91 (d, *J* = 6.0 Hz, 3 H), 0.88 (d, *J* = 6.0 Hz, 3 H).

Compound 11: ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.99-7.93 (m, 1 H), 7.38-7.14 (m, 10 H), 5.57 (d, *J* = 16.0 Hz, 1 H), 5.02-4.92 (m, 2 H), 4.52-4.38 (m, 2 H), 4.10 (q, *J* = 7.2 Hz, 2 H), 4.00-3.94 (m, 1 H), 2.95-2.89 (m, 2 H), 2.86 (s, 3 H), 2.76 (s, 3 H), 2.35-2.17 (m, 2 H), 1.81-1.64 (m, 1 H), 1.61-1.42 (m, 2 H), 1.41-1.23 (m, 2 H), 1.20 (t, *J* = 7.2 Hz, 3 H), 0.80 (d, *J* = 6.8 Hz, 3 H), 0.75 (d, *J* = 6.8 Hz, 3 H).

Compound 12: ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.02-7.96 (m, 2 H), 7.41-7.13 (m, 10 H), 6.71-6.65 (m, 1 H), 5.64 (d, *J* = 16.0 Hz, 1 H), 5.05-4.96 (m, 2 H), 4.51-4.44 (m, 1 H), 4.39-4.33 (m, 1 H), 4.09 (q, *J* = 7.2 Hz, 2 H), 3.98-3.92 (m, 1 H), 2.93 (dd, *J* = 8.0, 3.2 Hz, 1 H), 2.84 (dd, *J* = 8.0, 3.2 Hz, 1 H), 2.37-2.21 (m, 1 H), 2.05 (t, *J* = 8.0, Hz, 1 H), 1.77-1.58 (m, 2 H), 1.51-1.27 (m, 3 H), 1.18 (t, *J* = 7.2 Hz, 3 H), 0.81 (d, *J* = 6.0 Hz, 3 H), 0.78 (d, *J* = 6.0 Hz, 3 H).

Compound 13: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.16 (m, 10 H), 6.95 (d, *J* = 4.0 Hz, 1 H), 6.32 (m, 1 H), 5.79 (d, *J* = 16.0 Hz, 1 H), 5.21-4.97 (m, 3 H), 4.81 (m, 1 H), 4.61-4.57 (m, 1 H), 4.11 (q, *J* = 7.2 Hz, 2 H), 3.98 (br, 1 H), 3.65-3.37 (m, 2 H), 3.21-2.94 (m, 2 H), 1.97 (s, 3 H), 1.63-1.27 (m, 3 H), 1.24 (t, *J* = 7.2 Hz, 3 H), 0.93 (d, *J* = 4.0 Hz, 3 H), 0.87 (d, *J* = 4.0 Hz, 3 H).

Compound 14: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (s, 1H), 7.97 (br, 1 H), 7.35-7.16 (m, 10 H), 6.60 (br, 1 H), 5.21 (br, 1 H), 5.19-4.98 (m, 2 H), 4.65-4.47 (m, 2 H), 4.21-4.05 (m, 2 H), 3.34 (s, 3 H), 3.18-3.03 (m, 2 H), 2.52-2.31 (m, 2 H), 2.21-1.82 (m, 3 H), 1.67-1.43 (m, 2 H), 1.43-1.39 (m, 1 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.87 (d, *J* = 6.4 Hz, 3 H).

Compound 15: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.37 (s, 1H), 7.41-7.13 (m, 10 H), 6.61 (br, 1 H), 6.34 (br, 1 H), 5.17-4.97 (m, 2 H), 4.79-4.58 (m, 1 H), 4.18-3.99 (m, 2 H), 3.12-2.98 (m, 2 H), 2.78-2.63 (m, 1 H), 2.57-2.23 (m, 2 H), 2.05-1.98 (m, 1 H), 1.63-1.37 (m, 4 H), 0.94 (d, *J* = 6.4 Hz, 3 H), 0.88 (d, *J* = 6.4 Hz, 3 H), 0.76-0.74 (m, 2 H), 0.52-0.50 (m, 2 H).

Compound 16: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (s, 1H), 7.91 (d, *J* = 3.2 Hz, 1 H), 7.36-7.17 (m, 10 H), 6.01 (d, *J* = 3.2 Hz,1 H), 5.09 (s, 2 H), 5.09-5.05 (m, 1 H), 4.78-4.75 (m, 1 H), 4.20-4.15 (m, 1 H), 3.38 (t, *J* = 6.8 Hz, 2 H), 3.33 (t, *J* = 6.8 Hz, 2 H), 3.14-3.10 (m, 2 H), 2.42-2.31 (m, 1 H), 2.21-2.19 (m, 1 H), 1.97-1.93 (m, 2 H), 1.86-1.83 (m, 2 H), 1.63-1.57 (m, 5 H), 0.92 (d, *J* = 6.4 Hz, 3 H), 0.89 (d, *J* = 6.4 Hz, 3 H).

Enzyme inhibition. Enzyme activities of recombinant His_6-tagged NV/HOV protease, purified using Ni-NTA column, in the presence of tri-peptide substrate-based inhibitors were analyzed by a FRET assay using a fluorescently-labeled peptide substrate (EDANS-EPDFHLQGPEDLAK-Dabcyl) as described previously.[1] Briefly, 0.5 μM final concentration of protease (NV or HOV) was incubated with increasing concentrations of the inhibitor (0, 0.05, 0.2, 1, 5, 20, 100 and 150 μM) in a buffer consisting of 50 mM NaH_2PO_4 (pH 8), 50 mM NaCl, and 5 mM TCEP for 45 min at room temperature, and florescence measurements were carried out immediately after adding 30 μM (final concentration) of the peptide substrate, over 30 min at 1.0 min intervals at 37 °C. The final volume of the assay mixture in each well is 100 μL. The fluorescence was measured at excitation/emission wavelengths of 360 and 460 nm and the initial velocities calculated. Subsequently, the inhibition constants (K_i) for each inhibitor were calculated using Morrison tight-binding modeling (Prism 5.0, GraphPad). All reactions were performed in a 96 well format.

Homology modeling. The 3D structure for HOV protease was predicted using Phyre web server (Ref. 20), using the crystal structure of NVPro (PDB ID: 2FYQ) as the template. Structure analyses were performed by superimposing the two structures using Maestro (version 9.1, Schrödinger Inc.).

References:

1. C. E. Zeitler, M. K. Estes and B. V. Venkataram Prasad, *J. Virol*. 2006, **80**, 5050.