Supporting Information

Structural studies revealed active site distortions of human furin by a small molecule inhibitor.

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Supplementary Methods

Expression and crystallization of human furin, Inhibitor exchange.

Details about expression and purification of human furin were described previously[1, 2]. Shortly, the protease was expressed by transient transfection of human embryonic kidney cells. Three chromatography steps including immobilized metal affinity purification, immobilized inhibitor affinity chromatography, and gel permeation chromatography were applied for purification of human furin. Crystals of human furin in complex with mi-0052[1] and crystals of unliganded furin were grown as described previously[2] and subsequently used for soaking studies with the 2,5-dideoxystreptamine derived inhibitors **1**, **2** and **3** (Table 1). Orthorhombic furin crystals were soaked with inhibitors **1**, **2** and **3** essentially as in detail described for the I2-inhibitor[1], using 1 mM inhibitor in stabilizing solution. Hexagonal crystals of unliganded furin were soaked with a 5 mM inhibitor solution in 100 mM MES pH5.5, 200 mM K/NaH$_2$PO$_4$, 1 mM CaCl$_2$, 10% (v/v) DMSO, and 3.4 mM NaCl for 16 h. Soaked crystals were transferred for several seconds to 2 mM inhibitor solution in 100 mM MES pH5.5, 200 mM K/NaH2PO4, 1 mM CaCl2, 2% (v/v) DMSO, and 3.4 mM NaCl supplemented with 11% (v/v) ethylene glycol and immediately flash cooled in liquid nitrogen.

**Data collection, model building, and structure refinement**.

Diffraction data for the furin crystals in complex with **1** were collected at the BESSY-II beamline 14.1 of the Helmholtz-Zentrum Berlin (HZB)[3]. Data processing was performed with XDS[4] (v.03/2013) and programs of the CCP4-suite[5] (CCP4 v.6.3.0, CCP4 interface v.2.2.0). The structure of unliganded furin (PDB-ID: 5JXG, [2]) was adapted to the data by rigid body refinement in PHENIX[6] (v.1.9-1692). Model building was carried out in COOT[7] (v.0.6.2). PHENIX[6] (v.1.9-1692) was used for refinement. Parameter files of inhibitor **1** for refinement

were generated with the PRODRG-server[8]. Electron density annealed omit maps were calculated, omitting either the inhibitor or the catalytic residues and by performing positional refinement with simulated annealing in PHENIX. PYMOL (http://www.pymol.org) was used for molecular graphics. Residue-wise root mean square diviation (R.M.S.D.) values were calculated with SUPERPOSE as included in the CCP4-suite [5] (CCP4 v.6.3.0, CCP4 interface v.2.2.0).

Modeling of the complex of human furin with a peptide substrate.

The peptide H-ARG-ARG-VAL-ARG-ARG(↓)-SER-VAL-OH was manually docked in MAIN[9] according to the interactions observed for human furin in complex with a competitive substrate like inhibitor (PDB-ID: 5JXH, [2]). The geometry of the modeled peptide was optimized in CNS[10] (v.1.3.) The approach was essentially similar as previously described[11] but became necessary due to the small but existing differences between the mouse and the human furin structures and the different crystal forms.

Supplementary Figures
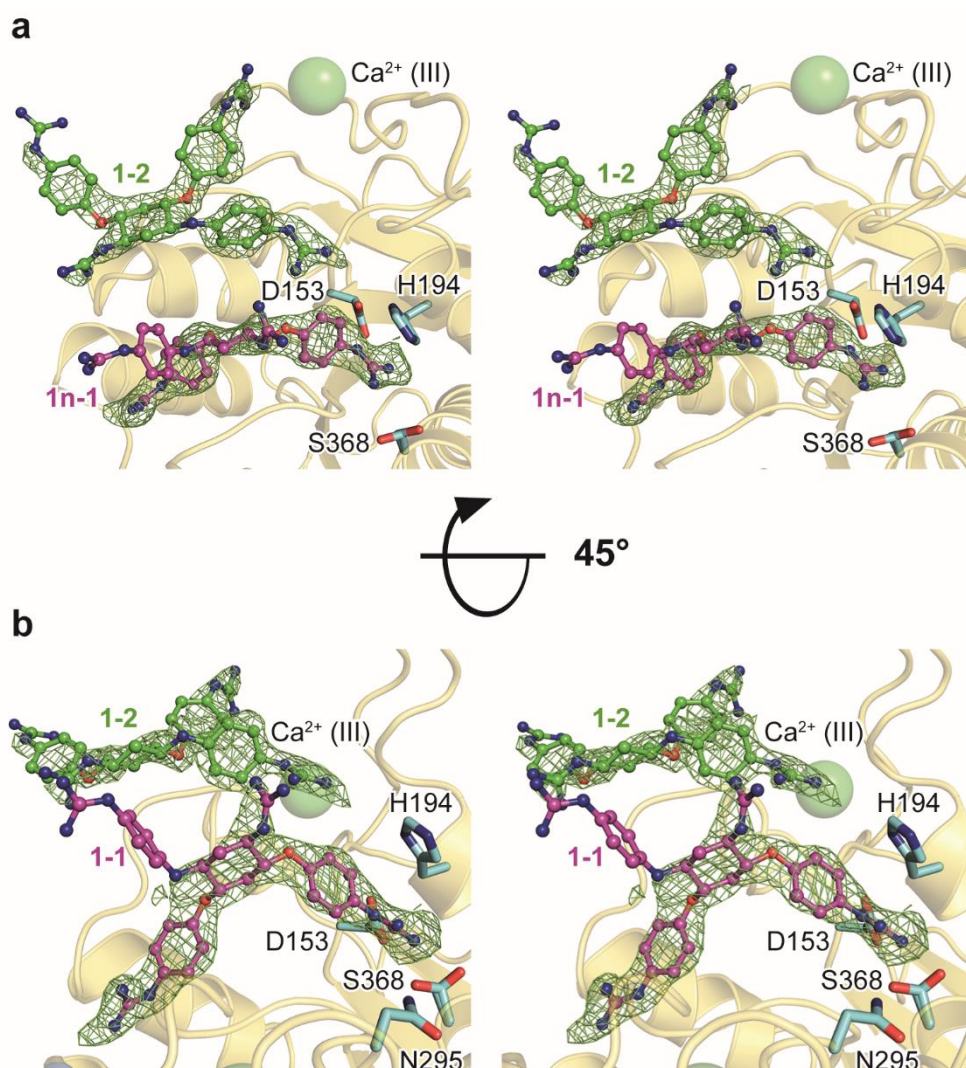
Supplementary Figure 1 Stereo representations of the furin:**1** complex. (a, b) Close view of the binding sites **1-1** and **1-2**. Human furin is shown as cartoon representation. The catalytic domain is colored in golden. Bound calcium ions are shown as green spheres. Inhibitor **1** is shown as ball and stick model with molecule **1-1** in magenta and molecule **1-2** in green. The Fo-Fc annealed omit electron density map is shown as dark green mesh contoured at 3 σ. (b) View as shown in (a) rotated by 45°.
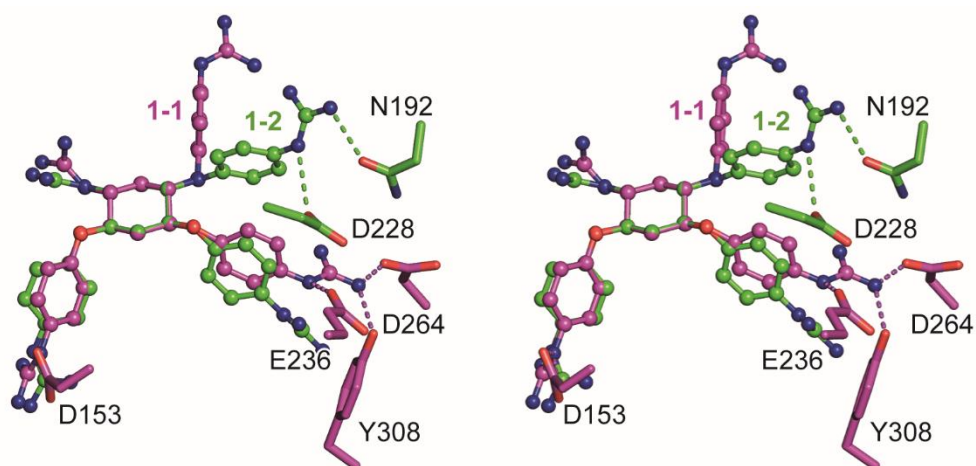
Supplementary Figure 2 Structural alignment of the molecules **1**-**1** and **1**-**2**. The molecules and interacting amino acids were aligned based on their rigid 2,5-dideoxystreptamine core structures. The inhibitor molecules **1**-**1** (magenta colored ball and stick model) and **1**-**2** (green colored ball and stick model) are given as stereo representation. Interacting residues of furin's active site cleft are shown as stick model in the color of the bound inhibitor molecule.

Supplementary References

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