

# **Transformation of selective factor Xa inhibitor rivaroxaban to a dual factor Xa/thrombin inhibitor by modification of the morpholin-3-one moiety**

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## **Electronic supplementary information**

### **Contents:**

1. Synthesis
2. Biological evaluation - Enzyme assay for inhibition of serine proteases
3. Docking
4. Plasma stability

## 1. Synthesis

Chemicals obtained from Aldrich Chemical Co., Acros and Alfa Aesar were used without purification. (*S*)- *N*-(2,3-epoxypropyl)phthalimide (99% e.e.) was purchased from Sigma-Aldrich. Analytical TLC was performed on silica gel Merck 60 F254 plates (0.25 mm), using visualization with ultraviolet light and ninhydrin. Column chromatography was carried out on flash silica gel (particle size 40–240 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. Optical rotations were measured on a Perkin-Elmer 1241 MC polarimeter. The reported values for specific rotation are average values of 10 successive measurements using an integration time of 5 s. <sup>1</sup>H NMR spectra and <sup>13</sup>C NMR spectra were recorded on a Bruker AVANCE DPX300 spectrometer (<sup>1</sup>H NMR at 300.132 MHz and <sup>13</sup>C NMR at 75.475 MHz) or Bruker AVANCE III spectrometer (<sup>1</sup>H NMR at 400.130 MHz and <sup>13</sup>C NMR at 100.613 MHz) in CDCl<sub>3</sub> or DMSO-d<sub>6</sub> solution. All chemical shift values are reported in parts per million (ppm), the coupling constants (*J*) are given in hertz, and the splitting patterns are appointed as: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), td (triplet of doublets), t (triplet), dt (doublet of triplets) and m (multiplet). Mass spectra were obtained using a VG Analytical Autospec Q mass spectrometer. IR spectra were obtained on a Perkin-Elmer FTIR System Spectrum BX or Nicolet Nexus 470 FTIR spectrometers. Microanalyses were performed on a Perkin-Elmer C, H, N analyzer 240C. HPLC analyses were performed on an Agilent Technologies HP 1100 instrument with a G1365B UV-VIS detector (254 nm), a G1316A thermostat and a G1313A autosampler, using an Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm) at flow rate 1 mL/min. The eluant was a mixture of 0.1% trifluoroacetic acid in water (A) and methanol (B). Gradient: 80% A to 20% A in 15 min, to 10% A in 1 min, at 10% A for 3 min, then to 80% A in 1 min. The reported yields are yields of isolated purified products.

### **Ethyl 1-(4-nitro-phenyl)-2-oxopiperidine-4-carboxylate (1)**

Compound **2** (5.56 g, 20 mmol) was dissolved in 80 ml of dichloromethane. Benzyltriethylammonium chloride (18.24 g, 80 mmol) and potassium permanganate (12.64 g, 80 mmol) were added in two equal portions at the beginning and at the half of 7 days long refluxing of the suspension. The reaction mixture was then poured into water (150 ml) and after addition of 20% solution of sodium sulphite (300 ml) stirred for 1h at 0 °C. Obtained yellow suspension was filtered. Separated organic phase was dried over Na<sub>2</sub>SO<sub>3</sub>, filtered and concentrated under reduced pressure. The residue was purified by flash column

chromatography using EtOAc/hexane (2/1) as eluant.

Yellow crystals (2.98 g, 51%); mp 84–87 °C; IR (KBr): 3082, 2982, 2901, 1727, 1654, 1592, 1514, 1495, 1432, 1406, 1349, 1302, 1274, 1245, 1216, 1170, 1112, 1036 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 1.31 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.13–2.25 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 2.29–2.39 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 2.84 (d, 2H, *J* = 7.5 Hz, COCH<sub>2</sub>CH), 2.98–3.07 (m, 1H, COCH<sub>2</sub>CH), 3.76–3.81 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 4.23 (q, 2H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 7.49 (d, 2H, *J* = 9.1 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 8.24 (d, 2H, *J* = 9.1 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>); MS (ESI): *m/z* 293.1 (M<sup>+</sup>, 100%), 141.0 (58), 77.0 (38); HRMS (ESI) calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>5</sub> 293.1137, found 293.1146.

### General procedure for the preparation of compounds 2, 3 and 4

To a stirred solution of ethyl isonipecotate (4.62 ml, 30 mmol) or ethyl nipecotate (4.66 ml, 30 mmol) or ethyl pipecolate (9.38 ml, 60 mmol) and 1-fluoro-4-nitrobenzen (1 eq.) in dimethylsulfoxide (1M) anhydrous potassium carbonate (1.5 eq.) was added. The resulting mixture was stirred at 55 °C for 15h and after dilution with cold water extracted with dichloromethane. The organic phase was dried over Na<sub>2</sub>SO<sub>3</sub>, filtered and concentrated under reduced pressure. Compounds 3 and 4 were isolated from residue by flash column chromatography using dichloromethane or hexane/EtOAc (5/1) as eluant. Compound 2 was recrystallized from dichloromethane/hexane (1/19).

### Ethyl 1-(4-nitrophenyl)piperidine-4-carboxylate (2)

Yellow crystals (7.92 g, 95%); mp 90–93 °C (from DCM/hexane); IR (ATR): 2954, 2897, 1723, 1595, 1581, 1513, 1483, 1452, 1404, 1372, 1301, 1250, 1229, 1187, 1165, 1150, 1108, 1038, 1015 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ 1.26 (t, 3H, *J* = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.75–1.88 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>), 1.99–2.06 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.53–2.57 (m, 1H, CH<sub>2</sub>CHCH<sub>2</sub>), 3.06 (dd, 1H, *J* = 10.7 Hz, 2.7 Hz CH<sub>2</sub>NCH<sub>2</sub>), 3.10 (dd, 1H, *J* = 10.7 Hz, 2.7 Hz CH<sub>2</sub>NCH<sub>2</sub>), 3.85 (t, 1H, *J* = 3.6 Hz CH<sub>2</sub>NCH<sub>2</sub>), 3.90 (t, 1H, *J* = 3.6 Hz CH<sub>2</sub>NCH<sub>2</sub>), 4.15 (q, 2H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 6.81 (d, 2H, *J* = 9.5 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 8.09 (d, 2H, *J* = 9.5 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>); MS (ESI): *m/z* 279.1 (M<sup>+</sup>, 100%); HRMS (ESI) calc. for C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>O<sub>4</sub> 279.1345, found 279.1348.

### Ethyl 1-(4-nitrophenyl)piperidine-3-carboxylate (3)

Yellow/orange oil (7.75 g, 93%); IR (ATR): 2939, 1723, 1589, 1490, 1392, 1311, 1237, 1177, 1144, 1105, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ 1.29 (t, 3H, *J* = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.63–1.72 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.79–1.91 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>CH), 2.07–2.14 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 2.59–2.68 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 3.15 (ddd, 1H, *J* = 13.3 Hz, 10.1 Hz, 2.9 Hz,

$\text{NCH}_2\text{CH}_2$ ), 3.38 (dd, 1H,  $J = 13.4$  Hz, 9.5 Hz,  $\text{NCH}_2\text{CH}_2$ ), 3.72 (td, 1H,  $J = 13.1$  Hz, 3.7 Hz,  $\text{NCH}_2\text{CH}$ ), 3.93 (dd, 1H,  $J = 13.2$  Hz, 3.7 Hz,  $\text{NCH}_2\text{CH}$ ), 4.18 (q, 2H,  $J = 7.2$  Hz,  $\text{CH}_2\text{CH}_3$ ), 6.86 (d, 2H,  $J = 9.4$  Hz, Ar- $\text{H}^2, \text{H}^6$ ), 8.12 (d, 2H,  $J = 9.4$  Hz, Ar- $\text{H}^3, \text{H}^5$ ); MS (ESI):  $m/z$  279.1 ( $\text{M}^+$ , 100%); HRMS (ESI) calc. for  $\text{C}_{14}\text{H}_{19}\text{N}_2\text{O}_4$  279.1345, found 279.1342.

#### **Ethyl 1-(4-nitrophenyl)piperidine-2-carboxylate (4)**

Yellow/orange oil (2.37 g, 15%); IR (ATR): 2940, 2861, 1730, 1591, 1450, 1389, 1367, 1313, 1256, 1187, 1159, 1137, 1110, 1025  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300MHz)  $\delta$  1.23 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.36–1.51 (m, 1H,  $\text{CHCH}_2\text{CH}_2$ ), 1.58–1.69 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 1.73–1.81 (m, 1H,  $\text{CHCH}_2\text{CH}_2$ ), 1.85–1.98 (m, 2H,  $\text{NCH}_2\text{CH}_2$ ,  $\text{CHCH}_2$ ), 2.30–2.38 (m, 1H,  $\text{CHCH}_2$ ), 3.26–3.36 (dt, 1H,  $J = 12.5$  Hz, 3.6 Hz,  $\text{NCH}_2$ ), 3.75–3.82 (m, 1H,  $\text{NCH}_2$ ), 4.18 (q, 2H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 4.69 (dd, 1H,  $J = 5.5$  Hz, 1.7 Hz,  $\text{CHCH}_2$ ), 6.28 (d, 2H,  $J = 9.4$  Hz, Ar- $\text{H}^2, \text{H}^6$ ), 8.12 (d, 2H,  $J = 9.4$  Hz, Ar- $\text{H}^3, \text{H}^5$ ); MS (ESI):  $m/z$  279.1 ( $\text{M}^+$ , 100%); HRMS (ESI) calc. for  $\text{C}_{14}\text{H}_{19}\text{N}_2\text{O}_4$  279.1345, found 279.1346.

#### **General procedure for catalytic hydrogenation of compounds 1-4**

Mixture of starting compound (1-4) and 10% palladium on activated charcoal in absolute ethanol (0.1 M) was stirred under 1 atm  $\text{H}_2$  for 10-15h at room temperature. The progress of the reaction was monitored by TLC. After completion palladium on activated charcoal was removed by filtration. Filtrate was evaporated under reduced pressure yielding crude amine as red-brown oil. Compounds 5-8 were used without further purification.

#### **Ethyl 1-(4-aminophenyl)-2-oxopiperidine-4-carboxylate (5)**

Red-brown oil (crude product 2.51 g, 94%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300MHz)  $\delta$  1.24 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.96–2.08 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.13–2.22 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.67–2.70 (m, 2H,  $\text{COCH}_2\text{CH}$ ), 2.85–2.95 (m, 1H,  $\text{COCH}_2\text{CH}$ ), 3.52–3.56 (m, 2H,  $\text{NCH}_2\text{CH}_2$ ), 3.65 (brs, 2H,  $\text{NH}_2$ ), 4.15 (q, 2H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 6.56 (d, 2H,  $J = 8.7$  Hz, Ar- $\text{H}^2, \text{H}^6$ ), 6.90 (d, 2H,  $J = 8.7$  Hz, Ar- $\text{H}^3, \text{H}^5$ ). MS (ESI):  $m/z$  263.1 ( $\text{M}^+$ , 75%), 214.1 (45), 158.0 (68), 141.0 (100), 77.0 (68); HRMS (ESI) calc. for  $\text{C}_{14}\text{H}_{19}\text{N}_2\text{O}_3$  263.1396, found 263.1394.

#### **Ethyl 1-(4-aminophenyl)piperidine-4-carboxylate (6)**

Red-brown oil (crude product 9.25 g, 97%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.27 (t, 3H,  $J = 7.2$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.82–2.04 (m, 4H,  $\text{CH}_2\text{CHCH}_2$ ), 2.32–2.42 (m, 1H,  $\text{CH}_2\text{CHCH}_2$ ), 2.62–2.70 (m, 2H,  $\text{CH}_2\text{NCH}_2$ ), 3.40 (t, 1H,  $J = 3.7$  Hz  $\text{CH}_2\text{NCH}_2$ ), 3.44 (t, 1H,  $J = 3.7$  Hz

$\text{CH}_2\text{NCH}_2$ ), 4.15 (q, 2H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 6.63 (d, 2H,  $J = 8.8$  Hz, Ar- $\text{H}^2, \text{H}^6$ ), 6.81 (d, 2H,  $J = 8.8$  Hz, Ar- $\text{H}^3, \text{H}^5$ ),  $\text{NH}_2$  peak not seen; MS (ESI):  $m/z$  249.2 ( $\text{M}^+$ , 100%); HRMS (ESI) calc. for  $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_2$  249.1603, found 249.1599.

#### **Ethyl 1-(4-aminophenyl)piperidine-3-carboxylate (7)**

Red-brown oil (crude product 5.33 g, 90%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.28 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.55–1.88 (m, 3H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 1.97–2.04 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.65–2.75 (m, 2H,  $\text{CH}_2\text{CH}_2\text{CH}$ ,  $\text{NCH}_2\text{CH}_2$ ), 2.88 (t, 1H,  $J = 10.7$  Hz,  $\text{NCH}_2\text{CH}_2$ ), 3.29 (d, 1H,  $J = 11.9$  Hz,  $\text{NCH}_2\text{CH}$ ), 3.40 (brs, 2H,  $\text{NH}_2$ ), 3.52 (d, 1H,  $J = 11.6$  Hz,  $\text{NCH}_2\text{CH}$ ), 4.17 (q, 2H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 6.65 (d, 2H,  $J = 8.6$  Hz, Ar- $\text{H}^2, \text{H}^6$ ), 6.85 (d, 2H,  $J = 8.7$  Hz, Ar- $\text{H}^3, \text{H}^5$ ); MS (ESI)  $m/z$  249.2 ( $\text{M}^+$ , 5%), 193.1 (90), 155.0 (100), 91.1 (80); HRMS (ESI) calc. for  $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_2$  249.1603, found 249.1600.

#### **Ethyl 1-(4-aminophenyl)piperidine-2-carboxylate (8)**

Red-brown oil (crude product 1.61 g, 76%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.14 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.51–1.73 (m, 3H,  $\text{CHCH}_2\text{CH}_2$ ,  $\text{NCH}_2\text{CH}_2$ ), 1.77–1.86 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 1.91–2.11 (m, 2H,  $\text{CHCH}_2\text{CH}_2$ ), 3.04–3.43 (m, 4H,  $\text{NCH}_2$ ,  $\text{NH}_2$ ), 4.01–4.12 (m, 2H,  $\text{CH}_2\text{CH}_3$ ), 4.16 (t, 1H,  $J = 5.1$  Hz, 1.7 Hz,  $\text{CHCH}_2$ ), 6.62 (d, 2H,  $J = 8.8$  Hz, Ar- $\text{H}^2, \text{H}^6$ ), 6.83 (d, 2H,  $J = 8.8$  Hz, Ar- $\text{H}^3, \text{H}^5$ ); MS (ESI)  $m/z$  249.2 ( $\text{M}^+$ , 100%); HRMS (ESI) calc. for  $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_2$  249.1603, found 249.1597.

#### **General procedure for preparation of compounds 9-12**

Suspension of starting compound (5-8) and (*S*)-*N*-(2,3-epoxypropyl)phthalimide (1 eq.) in absolute ethanol (0.2 M) was refluxed for 15h. The crude product precipitated from clear solution at 0 °C. Compounds 9-12 were purified either by flash column chromatography using EtOAc/hexane as eluant or by recrystallization from absolute ethanol.

#### **Ethyl 1-(4-(((R)-3-(1,3-dioxisoindolin-2-yl)-2-hydroxypropyl)amino)phenyl)-2-oxopiperidine-4-carboxylate (9)**

Beige crystals (1.91 g, 43%); mp 175–177 °C (from abs. EtOH); Found: C, 64.74; H, 5.99; N, 9.18. Calc. for  $\text{C}_{25}\text{H}_{27}\text{N}_3\text{O}_6$ : C, 64.50; H, 5.85; N, 9.03%; IR (KBr): 3360, 2945, 1778, 1728, 1607, 1526, 1428, 1398, 1326, 1258, 1189, 1169, 1154, 1107, 1039  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300MHz)  $\delta$  1.31 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.63 (brs, 1H, OH), 2.07–2.17 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.21–2.30 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.74–2.78 (m, 2H,  $\text{COCH}_2\text{CH}$ ), 2.91–3.00 (m,

1H, CH<sub>2</sub>CHCH<sub>2</sub>), 3.18 (dd, 1H, *J* = 13.2 Hz, 6.5 Hz, ArNHCH<sub>2</sub>), 3.27 (dd, 1H, *J* = 13.2 Hz, 5.1 Hz, ArNHCH<sub>2</sub>), 3.61–3.65 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 3.90–3.93 (m, 2H, CON(CO)CH<sub>2</sub>CH), 4.11–4.26 (m, 4H, CH<sub>2</sub>CH<sub>3</sub>, NH, CHOH), 6.67 (d, 2H, *J* = 8.7 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.03 (d, 2H, *J* = 8.7 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), 7.75–7.78 (m, 2H, Ar-H<sup>4</sup>,H<sup>7</sup>), 7.88–7.91 (m, 2H, Ar-H<sup>5</sup>,H<sup>6</sup>); MS (ESI): *m/z* 466.2 (M<sup>+</sup>, 60), 77 (100); HRMS (ESI) calc. for C<sub>25</sub>H<sub>28</sub>N<sub>3</sub>O<sub>6</sub> 466.1978, found 466.1990.

**(R)-Ethyl 1-(4-((3-(1,3-dioxoisindolin-2-yl)-2-hydroxypropyl)amino)phenyl)piperidine-4-carboxylate (10)**

Beige crystals (2.79 g, 55%); mp 148–150 °C (from abs. EtOH); [ $\alpha$ ]<sub>D</sub><sup>20</sup> +7.3 (c 0.25 in DMSO); Found: C, 66.55; H, 6.39; N, 9.55. Calc. for C<sub>25</sub>H<sub>29</sub>N<sub>3</sub>O<sub>5</sub>: C, 66.50; H, 6.47; N, 9.31%; IR (KBr): 3324, 2944, 1772, 1715, 1516, 1466, 1429, 1395, 1308, 1253, 1172, 1090, 1030 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.28 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.83–2.05 (m, 4H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.33–2.43 (m, 1H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.65 (dd, 1H, *J* = 10.6 Hz, 2.7 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 2.69 (dd, 1H, *J* = 10.8 Hz, 2.7 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 3.15 (dd, 1H, *J* = 13.2 Hz, 6.6 Hz, ArNHCH<sub>2</sub>), 3.27 (dd, 1H, *J* = 13.2 Hz, 4.9 Hz, ArNHCH<sub>2</sub>), 3.42 (t, 1H, *J* = 3.6 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 3.46 (t, 1H, *J* = 3.6 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 3.90–3.93 (m, 2H, CON(CO)CH<sub>2</sub>CH), 4.23 (q, 3H, *J* = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>, CHOH), 6.66 (d, 2H, *J* = 8.8 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 6.86 (d, 2H, *J* = 8.7 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), 7.74–7.79 (m, 2H, Ar-H<sup>4</sup>,H<sup>7</sup>), 7.87–7.90 (m, 2H, Ar-H<sup>5</sup>,H<sup>6</sup>), *OH and NH peaks not seen*; MS (ESI): *m/z* 452.0 (M<sup>+</sup>, 70%), 406.0 (40), 261.0 (40), 248.0 (100); HRMS (ESI) calc. for C<sub>25</sub>H<sub>30</sub>N<sub>3</sub>O<sub>5</sub> 452.2185, found 452.2177.

**Ethyl 1-(4-(((R)-3-(1,3-dioxoisindolin-2-yl)-2-hydroxypropyl)amino)phenyl)piperidine-3-carboxylate (11)**

Flash-LC: EtOAc/hexane (2/1); beige crystals (4.99 g, 51%); mp 107–109 °C (from EtOAc/hexane); Found: C, 66.56; H, 6.68; N, 9.52. Calc. for C<sub>25</sub>H<sub>29</sub>N<sub>3</sub>O<sub>5</sub>: C, 66.50; H, 6.47; N, 9.31%; IR (KBr): 3504, 2933, 2361, 2342, 1706, 1520, 1398, 1301, 1168, 1104, 1035 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.29 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.55–1.88 (m, 3H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>CH), 1.99–2.06 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 2.65–2.75 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH, NCH<sub>2</sub>CH<sub>2</sub>), 2.83–2.92 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>, NCH<sub>2</sub>CH<sub>2</sub>), 3.12–3.19 (m, 1H, ArNHCH<sub>2</sub>), 3.26–3.32 (m, 2H, NCH<sub>2</sub>CH, ArNHCH<sub>2</sub>), 3.50–3.54 (m, 1H, NCH<sub>2</sub>CH), 3.91–3.93 (m, 2H, CON(CO)CH<sub>2</sub>CH), 4.18 (q, 3H, *J* = 7.3 Hz, CH<sub>2</sub>CH<sub>3</sub>, CHOH), 6.66 (d, 2H, *J* = 8.7 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 6.88 (d, 2H, *J* = 9.0 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), 7.75–7.77 (m, 2H, Ar-H<sup>4</sup>,H<sup>7</sup>), 7.88–7.90 (m, 2H, Ar-H<sup>5</sup>,H<sup>6</sup>), *NH peak not seen*; MS (ESI): *m/z* 452.2 (M<sup>+</sup>, 100%), 125.0 (100); HRMS (ESI) calc. for C<sub>25</sub>H<sub>30</sub>N<sub>3</sub>O<sub>5</sub> 452.2185, found 452.2178.

**Ethyl 1-(4-(((R)-3-(1,3-dioxoisindolin-2-yl)-2-hydroxypropyl)amino)phenyl)piperidine-2-carboxylate (12)**

Flash-LC: EtOAc/hexane (1/1); yellow-brown oil (2.19 g, 75%); IR (ATR): 3392, 2935, 2857, 1771, 1703, 1614, 1514, 14663 1428, 1392, 1300, 1242, 1177, 1156, 1098, 1031  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.14 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.51–1.73 (m, 3H,  $\text{CHCH}_2\text{CH}_2$ ,  $\text{NCH}_2\text{CH}_2$ ), 1.77–1.86 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 1.91–2.11 (m, 2H,  $\text{CHCH}_2\text{CH}_2$ ), 3.03–3.15 (m, 2H,  $\text{NCH}_2$ ,  $\text{ArNHCH}_2$ ), 3.24 (dd, 1H,  $J = 13.0$  Hz, 4.5 Hz,  $\text{ArNHCH}_2$ ), 3.33–3.41 (m, 1H,  $\text{NCH}_2$ ), 3.87–3.90 (m, 2H,  $\text{CON}(\text{CO})\text{CH}_2\text{CH}$ ), 4.00 – 4.16 (m, 4H,  $\text{CH}_2\text{CH}_3$ ,  $\text{CHCH}_2\text{CH}_2$ ,  $\text{CHOH}$ ), 4.16 (t, 1H,  $J = 5.1$  Hz, 1.7 Hz,  $\text{CHCH}_2$ ), 6.62 (d, 2H,  $J = 8.7$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 6.85 (d, 2H,  $J = 8.8$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.72–7.76 (m, 2H,  $\text{Ar-H}^4, \text{H}^7$ ), 7.85–7.87 (m, 2H,  $\text{Ar-H}^5, \text{H}^6$ ), *NH peak not seen*; MS (ESI):  $m/z$  452.2 ( $\text{M}^+$ , 100%); HRMS (ESI) calc. for  $\text{C}_{25}\text{H}_{30}\text{N}_3\text{O}_5$  452.2185, found 452.2180.

**General procedure for preparation of compounds 13-16**

*N,N'*-Carbonyldiimidazole (2 eq.) and 4-(dimethylamino)pyridine (catalytic amount) were added to a suspension of starting compound (**9-12**) in tetrahydrofuran (0.1M). The reaction mixture was stirred for 15h at 60 °C. The crude product precipitated from clear solution at 0 °C. Compounds **13-16** were purified either by flash column chromatography using dichloromethane/methanol as eluant or by recrystallization from absolute ethanol.

**Ethyl 1-(4-(((S)-5-((1,3-dioxoisindolin-2-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)-2-oxopiperidine-4-carboxylate (13)**

Beige crystals (1.41 g, 70%); mp 194–196 °C (from abs. EtOH); Found: C, 62.41; H, 5.17; N, 8.37. Calc. for  $\text{C}_{26}\text{H}_{25}\text{N}_3\text{O}_7 \times \frac{1}{2} \text{H}_2\text{O}$ : C, 62.39; H, 5.24; N, 8.40%; IR (KBr): 3448, 1752, 1709, 1648, 1518, 1479, 1416, 1341, 1304, 1222, 1195, 1137, 1093, 1030  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.30 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 2.07–2.19 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.24–2.33 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.77–2.79 (m, 2H,  $\text{COCH}_2\text{CH}$ ), 2.93–3.03 (m, 1H,  $\text{CH}_2\text{CHCH}_2$ ), 3.65–3.70 (m, 2H,  $\text{NCH}_2\text{CH}_2$ ), 3.89–4.01 (m, 2H,  $\text{ArNCH}_2\text{CH}$ ,  $\text{CON}(\text{CO})\text{CH}_2\text{CH}$ ), 4.09–4.25 (m, 4H,  $\text{CH}_2\text{CH}_3$ ,  $\text{ArNCH}_2\text{CH}$ ,  $\text{CON}(\text{CO})\text{CH}_2\text{CH}$ ), 4.93–5.02 (m, 1H,  $\text{ArNCH}_2\text{CH}$ ), 7.25 (d, 2H,  $J = 8.8$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 7.54 (d, 2H,  $J = 8.8$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.75–7.79 (m, 2H,  $\text{Ar-H}^4, \text{H}^7$ ), 7.86–7.90 (m, 2H,  $\text{Ar-H}^5, \text{H}^6$ ); MS (ESI):  $m/z$  492.2 ( $\text{M}^+$ , 45%), 141.0 (70), 77.0 (100); HRMS (ESI) calc. for  $\text{C}_{26}\text{H}_{26}\text{N}_3\text{O}_7$  492.1771, found 492.1777.

**(S)-Ethyl 1-(4-(5-((1,3-dioxoisindolin-2-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-4-carboxylate (14)**

Beige crystals (1.44 g, 49%); mp 183–188 °C (from abs. EtOH);  $[\alpha]_D^{20}$  -53.4 (c 0.25 in DMSO); Found C, 65.17; H, 5.78; N, 8.96. Calc. for  $C_{26}H_{27}N_3O_6$ : C, 65.40; H, 5.70; N, 8.80%; IR (KBr): 3462, 2953, 2811, 1742, 1710, 1516, 1403, 1315, 1223, 1192, 1138, 1088, 1047  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  1.29 (t, 3H,  $J = 7.1$  Hz,  $CH_2CH_3$ ), 1.82–2.07 (m, 4H,  $CH_2CHCH_2$ ), 2.39–2.49 (m, 1H,  $CH_2CHCH_2$ ), 2.78 (dt, 2H,  $J = 11.8$  Hz, 3.0 Hz,  $CH_2NCH_2$ ), 3.58 (t, 1H,  $J = 3.7$  Hz,  $CH_2NCH_2$ ), 3.62 (t, 1H,  $J = 3.7$  Hz,  $CH_2NCH_2$ ), 3.88 (dd, 1H,  $J = 9.1$  Hz, 5.9 Hz,  $ArNCH_2CH$ ), 3.98 (dd, 1H,  $J = 14.0$  Hz, 5.8 Hz,  $CON(CO)CH_2CH$ ), 4.07–4.21 (m, 4H,  $CH_2CH_3$ ,  $ArNCH_2CH$ ,  $CON(CO)CH_2CH$ ), 4.93–5.02 (m, 1H,  $ArNCH_2CH$ ), 6.94 (d, 2H,  $J = 9.1$  Hz,  $Ar-H^2, H^6$ ), 7.39 (d, 2H,  $J = 9.1$  Hz,  $Ar-H^3, H^5$ ), 7.76–7.79 (m, 2H,  $Ar-H^4, H^7$ ), 7.89–7.91 (m, 2H,  $Ar-H^5, H^6$ ); MS (ESI):  $m/z$  478.0 ( $M^+$ , 100%); HRMS (ESI) calc. for  $C_{26}H_{28}N_3O_6$  478.1978, found 478.1981.

**Ethyl 1-(4-((S)-5-((1,3-dioxoisindolin-2-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-3-carboxylate (15)**

Beige crystals (3.59 g, 68%); mp 153–156 °C (from abs. EtOH); Found C, 65.25; H, 5.72; N, 8.85. Calc. for  $C_{26}H_{27}N_3O_6$ : C, 65.40; H, 5.70; N, 8.80%; IR (KBr): 3473, 2939, 2812, 2361, 2343, 1780, 1616, 1526, 1467, 1310, 1234, 1030  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  1.27 (t, 3H,  $J = 7.1$  Hz,  $CH_2CH_3$ ), 1.59–1.85 (m, 3H,  $CH_2CH_2CH_2$ ,  $CH_2CH_2CH$ ), 1.98–2.04 (m, 1H,  $CH_2CH_2CH$ ), 2.61–2.70 (m, 1H,  $CH_2CH_2CH$ ), 2.75–2.83 (m, 1H,  $NCH_2CH_2$ ), 3.00 (dd, 1H,  $J = 12.2$  Hz, 9.8 Hz,  $NCH_2CH_2$ ), 3.42 (td, 1H,  $J = 12.2$  Hz, 3.2 Hz,  $NCH_2CH$ ), 3.64 (dd, 1H,  $J = 12.1$  Hz, 3.9 Hz,  $NCH_2CH$ ), 3.86 (dd, 1H,  $J = 8.9$  Hz, 6.0 Hz,  $ArNCH_2CH$ ), 3.95 (dd, 1H,  $J = 14.2$  Hz, 5.7 Hz,  $ArNCH_2CH$ ), 4.06–4.22 (m, 4H,  $CH_2CH_3$ ,  $CON(CO)CH_2CH$ ), 4.91–4.99 (m, 1H,  $ArNCH_2CH$ ), 6.93 (d, 2H,  $J = 9.2$  Hz,  $Ar-H^2, H^6$ ), 7.36 (d, 2H,  $J = 9.2$  Hz,  $Ar-H^3, H^5$ ), 7.73–7.77 (m, 2H,  $Ar-H^4, H^7$ ), 7.83–7.87 (m, 2H,  $Ar-H^5, H^6$ ); MS (ESI):  $m/z$  478.2 ( $M^+$ , 100%); HRMS (ESI) calc. for  $C_{26}H_{28}N_3O_6$  478.1978, found 478.1975.

**Ethyl 1-(4-((S)-5-((1,3-dioxoisindolin-2-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-2-carboxylate (16)**

Yellow crystals (1.34 g, 58%); mp 98–100 °C (from abs. EtOH); Calc. for  $C_{26}H_{27}N_3O_6$ : C, 65.40; H, 5.70; N, 8.80%; Found C, 65.55; H, 5.41; N, 8.84. IR (KBr): 3484, 2978, 2935, 2856, 1738, 1716, 1519, 1478, 1465, 1443, 1419, 1388, 1329, 1313, 1230, 1173, 1141, 1070, 1020, 1008  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  1.19 (t, 3H,  $J = 7.1$  Hz,  $CH_2CH_3$ ), 1.37–1.51

(m, 1H, CHCH<sub>2</sub>CH<sub>2</sub>), 1.60–1.74 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>CH<sub>2</sub>), 1.82–2.01 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>), 2.15–2.24 (m, 1H, CHCH<sub>2</sub>), 3.31–3.44 (m, 2H, NCH<sub>2</sub>), 3.87 (ddd, 1H, *J* = 9.2 Hz, 5.9 Hz, 1.1 Hz, ArNCH<sub>2</sub>CH), 3.97 (dd, 1H, *J* = 14.1 Hz, 5.8 Hz, CON(CO)CH<sub>2</sub>CH), 4.06–4.19 (m, 4H, CH<sub>2</sub>CH<sub>3</sub>, ArNCH<sub>2</sub>CH, CON(CO)CH<sub>2</sub>CH), 4.45 (dd, 1H, *J* = 5.7 Hz, 3.3 Hz, CHCH<sub>2</sub>), 4.92–5.01 (m, 1H, ArNCH<sub>2</sub>CH), 6.91 (d, 2H, *J* = 9.2 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.38 (d, 2H, *J* = 9.2 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), 7.76–7.80 (m, 2H, Ar-H<sup>4</sup>,H<sup>7</sup>), 7.89–7.91 (m, 2H, Ar-H<sup>5</sup>,H<sup>6</sup>); MS (ESI): *m/z* 478.2 (M<sup>+</sup>, 100%). HRMS (ESI) calc. for C<sub>26</sub>H<sub>28</sub>N<sub>3</sub>O<sub>6</sub> 478.1978, found 478.1973.

### General procedure for hydrazinolysis of compounds 13-16

To a solution of starting compound (**13-16**) in absolute ethanol (0.1M) hydrazine hydrate (55% solution) (3 eq.) was added. The reaction mixture was stirred for 2h at 80 °C and afterwards for 30min at 0 °C. Precipitated white crystals of phthalhydrazide were removed by filtration. Filtrate was concentrated under reduced pressure yielding crystals of crude product. Compounds **17-20** were used without further purification.

### Ethyl 1-(4-((S)-5-(aminomethyl)-2-oxooxazolidin-3-yl)phenyl)-2-oxopiperidine-4-carboxylate (**17**)

White crystals (crude product 0.99 g, 100%); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.31 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.08–2.20 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 2.25–2.34 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 2.78–2.81 (m, 2H, COCH<sub>2</sub>CH), 2.94–3.02 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>, CH<sub>2</sub>NH<sub>2</sub>), 3.12 (dd, 1H, *J* = 13.6 Hz, 4.2 Hz, CH<sub>2</sub>NH<sub>2</sub>), 3.66–3.71 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 3.87 (t, 1H, *J* = 7.7 Hz, ArNCH<sub>2</sub>CH), 4.06 (t, 1H, *J* = 8.7 Hz, ArNCH<sub>2</sub>CH), 4.23 (q, 2H, *J* = 8.7 Hz, CH<sub>2</sub>CH<sub>3</sub>), 4.64–4.72 (m, 1H, ArNCH<sub>2</sub>CH), 7.27 (d, 2H, *J* = 8.9 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.59 (d, 2H, *J* = 8.9 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), NH<sub>2</sub> peak not seen; MS (ESI): *m/z* 362.2 (M<sup>+</sup>, 100%); HRMS (ESI) calc. for C<sub>18</sub>H<sub>24</sub>N<sub>3</sub>O<sub>5</sub> 362.1716, found 362.1717.

### (S)-Ethyl 1-(4-(5-(aminomethyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-4-carboxylate (**18**)

White crystals (crude product 1.05 g, 100%); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 1.27 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.81–1.94 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.00–2.05 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.38–2.47 (m, 1H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.78 (dt, 2H, *J* = 11.7 Hz, 2.9 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 2.98 (dd, 1H, *J* = 13.5 Hz, 5.8 Hz, CHCH<sub>2</sub>NH<sub>2</sub>), 3.08 (dd, 1H, *J* = 13.6 Hz, 4.2 Hz, CHCH<sub>2</sub>NH<sub>2</sub>), 3.58 (t, 1H, *J* = 3.7 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 3.61 (t, 1H, *J* = 3.7 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 3.80 (dd, 1H, *J* = 8.9 Hz, 6.6 Hz, ArNCH<sub>2</sub>CH), 4.00 (t, 1H, *J* = 8.9 Hz, ArNCH<sub>2</sub>CH), 4.08 (q, 2H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 4.60–

4.68 (m, 1H, ArNCH<sub>2</sub>CH), 6.95 (d, 2H,  $J = 9.2$  Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.34 (d, 2H,  $J = 9.1$  Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), NH<sub>2</sub> peak not seen; MS (ESI):  $m/z$  348.2 (M<sup>+</sup>, 100%), 118.1 (60), 77.0 (30); HRMS (ESI) calc. for C<sub>18</sub>H<sub>26</sub>N<sub>3</sub>O<sub>4</sub> 348.1923, found 348.1916.

**Ethyl 1-(4-((S)-5-(aminomethyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-3-carboxylate (19)**

White crystals (crude product 1.76 g, 100%); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  1.20 (t, 3H,  $J = 7.1$  Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.56–1.75 (m, 3H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>CH), 1.88–1.92 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 2.59–2.65 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>CH), 2.74–2.89 (m, 3H, NCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>NH<sub>2</sub>), 2.96 (dd, 1H,  $J = 12.2$  Hz, 9.3 Hz, NCH<sub>2</sub>CH<sub>2</sub>), 3.36 (td, 1H,  $J = 12.0$  Hz, 3.3 Hz, NCH<sub>2</sub>CH), 3.56 (dd, 1H,  $J = 12.2$  Hz, 3.4 Hz, NCH<sub>2</sub>CH), 3.78–3.83 (m, 3H, ArNCH<sub>2</sub>CH, NH<sub>2</sub>), 4.00 (t, 1H,  $J = 8.8$  Hz, ArNCH<sub>2</sub>CH), 4.09 (q, 2H,  $J = 7.1$  Hz, CH<sub>2</sub>CH<sub>3</sub>), 4.53–4.61 (m, 1H, ArNCH<sub>2</sub>CH), 6.95 (d, 2H,  $J = 9.1$  Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.40 (d, 2H,  $J = 9.1$  Hz, Ar-H<sup>3</sup>,H<sup>5</sup>); MS (ESI):  $m/z$  348.2 (M<sup>+</sup>, 100%); HRMS (ESI) calc. for C<sub>18</sub>H<sub>26</sub>N<sub>3</sub>O<sub>4</sub> 348.1923, found 348.1918.

**Ethyl 1-(4-((S)-5-(aminomethyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-2-carboxylate (20)**

White crystals (crude product 0.98 g, 100%); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  1.11 (t, 3H,  $J = 7.1$  Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.20–1.30 (m, 1H, CHCH<sub>2</sub>CH<sub>2</sub>), 1.48–1.59 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 1.64 (td, 1H,  $J = 13.0$  Hz, 3.4 Hz, CHCH<sub>2</sub>CH<sub>2</sub>), 1.75–2.89 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>, CHCH<sub>2</sub>), 2.09 (d, 1H,  $J = 13.0$  Hz, CHCH<sub>2</sub>), 2.82 (dd, 1H,  $J = 13.6$  Hz, 5.4 Hz, CHCH<sub>2</sub>NH<sub>2</sub>), 2.88 (dd, 1H,  $J = 13.6$  Hz, 4.9 Hz, CHCH<sub>2</sub>NH<sub>2</sub>), 3.17 (dt, 1H,  $J = 11.9$  Hz, 3.3 Hz, NCH<sub>2</sub>), 3.80 (dd, 1H,  $J = 8.5$  Hz, 6.8 Hz, NCH<sub>2</sub>), 3.98–4.06 (m, 6H, ArNCH<sub>2</sub>CH, CH<sub>2</sub>CH<sub>3</sub>, NH<sub>2</sub>), 4.56–4.64 (m, 2H, ArNCH<sub>2</sub>CH, CHCH<sub>2</sub>), 6.91 (d, 2H,  $J = 9.2$  Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.36 (d, 2H,  $J = 9.2$  Hz, Ar-H<sup>3</sup>,H<sup>5</sup>); MS (ESI):  $m/z$  348.2 (M<sup>+</sup>, 100); HRMS (ESI) calc. for C<sub>18</sub>H<sub>26</sub>N<sub>3</sub>O<sub>4</sub> 348.1923, found 348.1910.

**General procedure for coupling of compounds 17-20 with 5-chlorothiophene-2-carbonyl chloride**

Solution of 5-chlorothiophene-2-carbonyl chloride (1.25 eq.) in dichloromethane (0.2M) was added dropwise to a stirred solution of starting compound (17-20) and triethylamine (2 eq.) in dichloromethane (0.05M) at 0°C. The reaction mixture was stirred for 15h at room temperature followed by removal of solvent under reduced pressure. Compounds 21-24 were purified by flash column chromatography using dichloromethane/methanol,

dichloromethane/EtOAc or EtOAc/hexane as eluant.

**Ethyl 1-(4-((S)-5-((5-chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)-2-oxopiperidine-4-carboxylate (21)**

Flash-LC: dichloromethane/methanol (19/1); white crystals (196 mg, 39%); mp 192–195 °C (from DCM/MeOH); Found: C, 54.66; H, 4.82; N, 8.30. Calc. for C<sub>23</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>5</sub>S: C, 54.60; H, 4.78; N, 8.30%; IR (KBr): 3307, 1752, 1724, 1624, 1546, 1515, 1483, 1430, 1407, 1308, 1263, 1223, 1189, 1148, 1082, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 1.22 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.92–2.04 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 2.14–2.23 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 2.55–2.57 (m, 2H, COCH<sub>2</sub>CH), 3.01–3.11 (m, 1H, CH<sub>2</sub>CHCH<sub>2</sub>), 3.48–3.72 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>, CONHCH<sub>2</sub>), 3.85 (dd, 1H, *J* = 8.8 Hz, 6.3 Hz, ArNCH<sub>2</sub>CH), 4.10–4.21 (m, 3H, CH<sub>2</sub>CH<sub>3</sub>, ArNCH<sub>2</sub>CH), 4.80–4.88 (m, 1H, ArNCH<sub>2</sub>CH), 7.19 (d, 1H, *J* = 4.0 Hz, Ar-H<sup>4</sup>), 7.27 (d, 2H, *J* = 8.9 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.53 (d, 2H, *J* = 8.9 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), 7.69 (d, 1H, *J* = 4.0 Hz, Ar-H<sup>3</sup>), 8.96 (t, 1H, *J* = 5.6 Hz, CONHCH<sub>2</sub>); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100 MHz) δ 14.07, 25.51, 34.27, 37.40, 42.20, 47.43, 49.03, 60.36, 71.28, 118.31, 126.60, 128.15, 128.43, 133.25, 136.30, 138.44, 138.63, 154.08, 160.78, 167.37, 173.20; MS (ESI): *m/z* 506.1 (M<sup>+</sup>, 100%) for <sup>35</sup>Cl, 508.1 (35) for <sup>37</sup>Cl; HRMS (ESI) calc. for C<sub>23</sub>H<sub>25</sub>ClN<sub>3</sub>O<sub>6</sub>S 506.1153, found 506.1158; HPLC: 100%, t<sub>r</sub> = 16.10 min.

**(S)-Ethyl 1-(4-(5-((5-chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-4-carboxylate (22)**

Flash-LC: dichloromethane/methanol (49/1) and dichloromethane/EtOAc (4/1); white crystals (297 mg, 58%); mp 178–181 °C (from DCM/EtOAc); [α]<sub>D</sub><sup>20</sup> = -43.7 (c 0.24 in DMSO); Found: C, 56.16; H, 5.32; N, 8.66. Calc. for C<sub>23</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>5</sub>S: C, 56.15; H, 5.33; N, 8.54%; IR (KBr): 3336, 2961, 2364, 1741, 1636, 1560, 1522, 1430, 1303, 1258, 1229, 1178, 1151, 1090, 1050 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 1.19 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.59–1.72 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>), 1.87–1.93 (m, 2H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.43–2.48 (m, 1H, CH<sub>2</sub>CHCH<sub>2</sub>), 2.73 (dt, 2H, *J* = 12.0 Hz, 2.0 Hz, CH<sub>2</sub>NCH<sub>2</sub>), 3.56–3.60 (m, 4H, CH<sub>2</sub>NCH<sub>2</sub>, CONHCH<sub>2</sub>), 3.78 (dd, 1H, *J* = 9.1 Hz, 6.3 Hz, ArNCH<sub>2</sub>CH), 4.05–4.14 (m, 3H, CH<sub>2</sub>CH<sub>3</sub>, ArNCH<sub>2</sub>CH), 4.74–4.83 (m, 1H, ArNCH<sub>2</sub>CH), 6.95 (d, 2H, *J* = 9.2 Hz, Ar-H<sup>2</sup>,H<sup>6</sup>), 7.19 (d, 1H, *J* = 4.0 Hz, Ar-H<sup>4</sup>), 7.35 (d, 2H, *J* = 9.1 Hz, Ar-H<sup>3</sup>,H<sup>5</sup>), 7.69 (d, 1H, *J* = 4.0 Hz, Ar-H<sup>3</sup>), 8.94 (t, 1H, *J* = 5.8 Hz, CONHCH<sub>2</sub>); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100 MHz) δ 14.09, 27.44, 42.26, 47.72, 48.31, 59.87, 71.10, 116.27, 119.58, 128.13, 128.40, 130.03, 133.24, 138.48, 147.61, 154.20, 160.76, 174.20, one alkyl C-atom behind solvent peak; MS (ESI): *m/z* 492.1 (M<sup>+</sup>, 100%) for <sup>35</sup>Cl,

494.1 (37) for  $^{37}\text{Cl}$ ; HRMS (ESI) calc. for  $\text{C}_{23}\text{H}_{27}\text{ClN}_3\text{O}_5\text{S}$  492.1360, found 492.1366; HPLC: 100%,  $t_r = 14.60$  min.

**Ethyl 1-(4-((S)-5-((5-chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-3-carboxylate (23)**

Flash-LC: dichloromethane/methanol (49/1); white crystals (533 mg, 54%); mp 166–168 °C (from DCM/MeOH); Found: C, 56.33; H, 5.29; N, 8.55. Calc. for  $\text{C}_{23}\text{H}_{26}\text{ClN}_3\text{O}_5\text{S}$ : C, 56.15; H, 5.33; N, 8.54%; IR (KBr): 3332, 3088, 2939, 2813, 1741, 1634, 1560, 1519, 1431, 1302, 1228, 1151, 1090, 1056, 1035  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  1.20 (t, 3H,  $J = 7.2$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.57–1.76 (m, 3H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 1.84–1.94 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.58–2.67 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.75–2.83 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.97 (dd, 1H,  $J = 12.3$  Hz, 9.3 Hz,  $\text{NCH}_2\text{CH}_2$ ), 3.37 (td, 1H,  $J = 12.1$  Hz, 3.7 Hz,  $\text{NCH}_2\text{CH}$ ), 3.54–3.61 (m, 3H,  $\text{NCH}_2\text{CH}$ ,  $\text{CONHCH}_2$ ), 3.78 (dd, 1H,  $J = 9.1$  Hz, 6.1 Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.06–4.14 (m, 3H,  $\text{CH}_2\text{CH}_3$ ,  $\text{ArNCH}_2\text{CH}$ ), 4.75–4.84 (m, 1H,  $\text{ArNCH}_2\text{CH}$ ), 6.95 (d, 2H,  $J = 9.0$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 7.19 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^4$ ), 7.36 (d, 2H,  $J = 9.0$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.69 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^3$ ), 8.95 (t, 1H,  $J = 5.8$  Hz,  $\text{CONHCH}_2$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100 MHz)  $\delta$  14.09, 23.37, 26.35, 42.25, 47.71, 49.19, 51.66, 59.95, 71.11, 116.59, 119.62, 128.13, 128.40, 130.24, 133.24, 138.48, 147.63, 154.20, 160.76, 173.07, *one alkyl C-atom behind solvent peak*; MS (ESI):  $m/z$  490.1 ( $\text{M}^+$ , 100%) for  $^{35}\text{Cl}$ , 492.1 (42) for  $^{37}\text{Cl}$ ; HRMS (ESI) calc. for  $\text{C}_{23}\text{H}_{25}\text{ClN}_3\text{O}_5\text{S}$  490.1203, found 490.1210; HPLC: 100%,  $t_r = 16.05$  min.

**Ethyl 1-(4-((S)-5-((5-chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-2-carboxylate (24)**

Flash-LC: EtOAc/hexane (2/1); white crystals (111 mg, 43%); mp 147–150 °C (from EtOAc/hexane); Found: C, 56.25; H, 5.16; N, 8.55. Calc. for  $\text{C}_{23}\text{H}_{26}\text{ClN}_3\text{O}_5\text{S}$ : C, 56.15; H, 5.33; N, 8.54%; IR (ATR): 3352, 2935, 2857, 1721, 1637, 1548, 1515, 1426, 1389, 1306, 1227, 1192, 1129, 1074, 1033  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  1.11 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.23–1.31 (m, 1H,  $\text{CHCH}_2\text{CH}_2$ ), 1.43–1.57 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 1.61–1.90 (m, 3H,  $\text{CHCH}_2\text{CH}_2$ ,  $\text{NCH}_2\text{CH}_2$ ,  $\text{CHCH}_2$ ), 2.06–2.11 (m, 1H,  $\text{CHCH}_2$ ), 3.16 (dt, 1H,  $J = 11.8$  Hz, 3.3 Hz,  $\text{NCH}_2$ ), 3.40–3.45 (m, 1H,  $\text{NCH}_2$ ), 3.58 (t, 2H,  $J = 5.6$  Hz,  $\text{CONHCH}_2$ ), 3.77 (dd, 1H,  $J = 9.1$  Hz, 6.0 Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.02 (q, 2H,  $J = 7.0$  Hz,  $\text{CH}_2\text{CH}_3$ ), 4.09 (t, 1H,  $J = 6.9$  Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.60–4.63 (m, 1H,  $\text{CHCH}_2$ ), 4.74–4.82 (m, 1H,  $\text{ArNCH}_2\text{CH}$ ), 6.90 (d, 2H,  $J = 9.3$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 7.19 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^4$ ), 7.32 (d, 2H,  $J = 9.1$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.69 (d, 2H,  $J = 4.0$  Hz,  $\text{Ar-H}^3$ ), 8.94 (t, 1H,  $J = 5.7$  Hz,  $\text{CONHCH}_2$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100

MHz)  $\delta$  14.11, 20.00, 24.84, 27.71, 42.26, 44.39, 47.72, 57.46, 59.95, 71.07, 115.25, 119.57, 128.12, 128.40, 129.55, 133.24, 138.44, 147.34, 154.22, 160.77, 172.14; MS (ESI):  $m/z$  492.1 ( $M^+$ , 100%) for  $^{35}\text{Cl}$ , 494.1 (35) for  $^{37}\text{Cl}$ ; HRMS (ESI) calc. for  $\text{C}_{23}\text{H}_{27}\text{ClN}_3\text{O}_5\text{S}$  492.1360, found 492.1337; HPLC: 96%,  $t_r$  = 17.71 min.

### General procedure for preparation of compounds 25-27

To a solution of starting compound (**21-24**) in methanol/water (1/1) (0.06M) lithium hydroxide (1.5 eq.) was added and the reaction mixture was stirred at room temperature. The progress of the reaction was monitored by TLC. After completion methanol was removed under reduced pressure. Water phase was cooled to 0°C and acidified (pH=1-2) with addition of conc. hydrochloric acid. Precipitated crystals were separated by filtration and purified by flash column chromatography using dichloromethane/methanol (9/1) as eluant.

### 1-(4-((S)-5-((5-Chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)-2-oxopiperidine-4-carboxylic acid (25)

White crystals (53 mg, 37%); mp 149–152 °C (from DCM/MeOH); Found: C, 51.68; H, 4.34; N, 8.46. Calc. for  $\text{C}_{21}\text{H}_{20}\text{ClN}_3\text{O}_6\text{S} \times \frac{1}{2} \text{H}_2\text{O}$ : C, 51.80; H, 4.35; N, 8.63%; IR (KBr): 3273, 2361, 1756, 1623, 1560, 1516, 1483, 1427, 1406, 1327, 1208, 1147  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  1.92–2.03 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.14–2.23 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.55–2.57 (m, 2H,  $\text{COCH}_2\text{CH}$ ), 2.90–3.00 (m, 1H,  $\text{CH}_2\text{CHCH}_2$ ), 3.50–3.73 (m, 4H,  $\text{NCH}_2\text{CH}_2$ ,  $\text{CONHCH}_2$ ), 3.85 (dd, 1H,  $J = 9.0$  Hz, 6.1 Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.18 (t, 1H,  $J = 9.1$  Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.79–4.88 (m, 1H,  $\text{ArNCH}_2\text{CH}$ ), 7.19 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^4$ ), 7.27 (d, 2H,  $J = 8.9$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 7.53 (d, 2H,  $J = 8.9$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.69 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^3$ ), 8.96 (t, 1H,  $J = 5.6$  Hz,  $\text{CONHCH}_2$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100 MHz)  $\delta$  25.62, 34.53, 37.57, 42.20, 47.43, 49.22, 71.26, 118.30, 126.60, 128.15, 128.43, 133.25, 136.25, 138.44, 138.72, 154.08, 160.77, 167.74, 174.98; MS (ESI):  $m/z$  478.1 ( $M^+$ , 100%) for  $^{35}\text{Cl}$ , 480.1 (37) for  $^{37}\text{Cl}$ ; HRMS (ESI) calc. for  $\text{C}_{21}\text{H}_{21}\text{ClN}_3\text{O}_6\text{S}$  478.0840, found 478.0832; HPLC: 99%,  $t_r$  = 13.93 min.

### (S)-1-(4-(5-((5-Chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-4-carboxylic acid (26)

White crystals (70 mg, 38%); mp 241–243 °C (from DCM/MeOH);  $[\alpha]_D^{20} = -47.5$  (c 0.24 in DMSO); Found: C, 54.12; H, 4.59; N, 8.96. Calc. for  $\text{C}_{21}\text{H}_{22}\text{ClN}_3\text{O}_5\text{S}$ : C, 54.37; H, 4.78; N, 9.06%; IR (ATR): 3335, 2957, 1738, 1718, 1703, 1668, 1634, 1561, 1516, 1466, 1432, 1382, 1324, 1294, 1225, 1204, 1148, 1117, 1090, 1056, 1029  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)

$\delta$  1.58–1.71 (m, 2H,  $\text{CH}_2\text{CHCH}_2$ ), 1.87–1.92 (m, 2H,  $\text{CH}_2\text{CHCH}_2$ ), 2.33–2.41 (m, 1H,  $\text{CH}_2\text{CHCH}_2$ ), 2.72 (dt, 2H,  $J = 11.8$  Hz, 2.5 Hz,  $\text{CH}_2\text{NCH}_2$ ), 3.55–3.63 (m, 4H,  $\text{CH}_2\text{NCH}_2$ ,  $\text{CONHCH}_2$ ), 3.78 (dd, 1H,  $J = 9.1$  Hz, 6.2 Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.11 (t, 1H,  $J = 9.0$  Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.74–4.83 (m, 1H,  $\text{ArNCH}_2\text{CH}$ ), 6.95 (d, 2H,  $J = 9.2$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 7.19 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^4$ ), 7.35 (d, 2H,  $J = 9.1$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.69 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^3$ ), 8.94 (t, 1H,  $J = 5.8$  Hz,  $\text{CONHCH}_2$ ), 12.20 (brs, 1H,  $\text{COOH}$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100 MHz)  $\delta$  27.55, 42.25, 47.70, 48.46, 71.09, 116.26, 119.57, 128.16, 128.41, 129.98, 133.24, 138.48, 147.71, 154.20, 160.76, 175.99, *one alkyl C-atom behind solvent peak*; MS (ESI):  $m/z$  464.1 ( $\text{M}^+$ , 100%) for  $^{35}\text{Cl}$ , 466.1 (35) for  $^{37}\text{Cl}$ ; HRMS (ESI) calc. for  $\text{C}_{21}\text{H}_{23}\text{ClN}_3\text{O}_5\text{S}$  464.1047, found 464.1068; HPLC: 99%,  $t_r = 12.13$  min.

### 1-(4-((S)-5-((5-Chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)piperidine-3-carboxylic acid (27)

White crystals (57 mg, 46%); mp 221–224 °C (from DCM/MeOH); IR (ATR): 3325, 1738, 1633, 1557, 1516, 1468, 1429, 1387, 1323, 1292, 1224, 1146, 1089, 1054, 1018  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  1.50–1.75 (m, 3H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 1.87–1.91 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.54–2.58 (m, 1H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.71–2.78 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.90 (dd, 1H,  $J = 11.9$  Hz, 9.6 Hz,  $\text{NCH}_2\text{CH}_2$ ), 3.40 (d, 2H,  $J = 11.5$  Hz,  $\text{NCH}_2\text{CH}$ ), 3.57–3.61 (m, 3H,  $\text{NCH}_2\text{CH}$ ,  $\text{CONHCH}_2$ ), 3.78 (dd, 1H,  $J = 9.0$  Hz, 6.3 Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.11 (t, 1H,  $J = 9.0$  Hz,  $\text{ArNCH}_2\text{CH}$ ), 4.75–4.83 (m, 1H,  $\text{ArNCH}_2\text{CH}$ ), 6.95 (d, 2H,  $J = 9.0$  Hz,  $\text{Ar-H}^2, \text{H}^6$ ), 7.19 (d, 1H,  $J = 4.1$  Hz,  $\text{Ar-H}^4$ ), 7.36 (d, 2H,  $J = 9.0$  Hz,  $\text{Ar-H}^3, \text{H}^5$ ), 7.69 (d, 1H,  $J = 4.0$  Hz,  $\text{Ar-H}^3$ ), 8.95 (t, 1H,  $J = 5.8$  Hz,  $\text{CONHCH}_2$ ), 12.30 (bs, 1H,  $\text{COOH}$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100 MHz)  $\delta$  23.59, 26.50, 42.25, 47.71, 49.29, 51.67, 71.10, 116.51, 119.63, 128.16, 128.41, 130.12, 133.24, 138.48, 147.72, 154.20, 160.76, 174.93; MS (ESI):  $m/z$  464.1 ( $\text{M}^+$ , 100%) for  $^{35}\text{Cl}$ , 466.1 (35) for  $^{37}\text{Cl}$ ; HRMS (ESI) calc. for  $\text{C}_{21}\text{H}_{23}\text{ClN}_3\text{O}_5\text{S}$  464.1047, found 464.1033; HPLC: 100%,  $t_r = 12.74$  min.

## 2. Biological evaluation - Enzyme assay for inhibition of serine proteases

The enzyme amidolytic method for determining inhibition was based on the spectrophotometric determination of absorbance of the product formed after amide bond cleavage of a chromogenic substrate in the presence of the enzyme.  $K_i$ , which is a quantitative measure of inhibitor potency, was determined from the kinetics of substrate hydrolysis with and without the addition of the inhibitor.<sup>1</sup> Measurements (spectrophotometer, BioTek Synergy

H4) were performed in 96-well microtiter plates with a final volume of 200  $\mu\text{L}$ . Thrombin was tested at a final concentration of 0.5 NIH E/mL ( $K_m = 2,6 \mu\text{M}$ ) with the substrate S-2238 (Chromogenix) at 20 and 40  $\mu\text{M}$  final concentration, and factor Xa at the final concentration of 1 mBAEE E/mL ( $K_m = 164 \mu\text{M}$ ) with the substrate S-2222 (Chromogenix) at 100 and 200  $\mu\text{M}$  final concentrations. Trypsin was assayed at a final concentration of 0.5 nkat/mL through the use of the substrate S-2222 (Chromogenix) at 50 and 100  $\mu\text{M}$  final concentrations. Inhibitors were dissolved in DMSO (concentration of stock solutions, 10 mmol/L) and diluted with distilled water to concentrations from 0.5 to 100  $\mu\text{M}$ . Reaction rates were measured in the presence and the absence of the inhibitor. Then 50  $\mu\text{M}$  HBSA buffer, the 50  $\mu\text{M}$  solution of each inhibitor concentration (or of HBSA buffer in case of measurement without inhibitor), and 50  $\mu\text{M}$  of enzyme solution were pipetted into the microtiter wells. The plate was incubated for 15 min at 25  $^\circ\text{C}$  and 50  $\mu\text{L}$  of chromogenic substrate then added. Absorbance at 405 nm at 25  $^\circ\text{C}$  was measured every 10 s. Measurements were carried out in triplicate with three concentrations of the inhibitor and two concentrations of the substrate. For every combination of concentrations,  $K_i$  was calculated from the change of absorbance in the initial, linear part of the curve according to the method of Cheng and Prusoff<sup>2</sup> and the final result was given as their average value. Rivaroxaban (factor Xa,  $K_i = 0.66 \pm 0.09 \text{ nM}$ ) and dabigatran (thrombin,  $K_i = 6.3 \pm 1.1 \text{ nM}$ ) were used as control.

### 3. Docking

The binding modes for the ligands to factor Xa and thrombin were studied by CDOCKER,<sup>3</sup> a docking tool based on the CHARMM force field, which is incorporated into Discovery Studio 3.0 (Accelrys Software Inc.). In CDOCKER, random ligand conformations are generated through molecular dynamics, and a variable number of translations/rotations are applied to each conformation to generate low-energy orientations of the ligand within the active site of rigid receptor. The orientations are further refined by grid-based simulated annealing (in our example 2000 heating steps, target temperature 700 K, 5000 cooling steps, target temperature 300 K), which is followed by final minimization using full potential. Final ligand poses are sorted by CHARMM energy (interaction energy plus ligand strain). The crystal structures of factor Xa (PDB entry code: 2W26, resolution 2.08  $\text{\AA}$ )<sup>4</sup> and thrombin (PDB entry code: 3RM2, resolution 1.23  $\text{\AA}$ )<sup>5</sup> were extracted from the Brookhaven Protein Database. All ligands were docked in all possible stereoisomeric forms in an active site located sphere with 7.71668  $\text{\AA}$  radius for thrombin and 8.62303  $\text{\AA}$  for factor Xa, which was generated with CreateSphere

function around subsequently removed crystal structure ligand. A total of 100 dockings for each ligand were performed and the poses with the lowest CHARMM energy were chosen for interpreting the docking results.

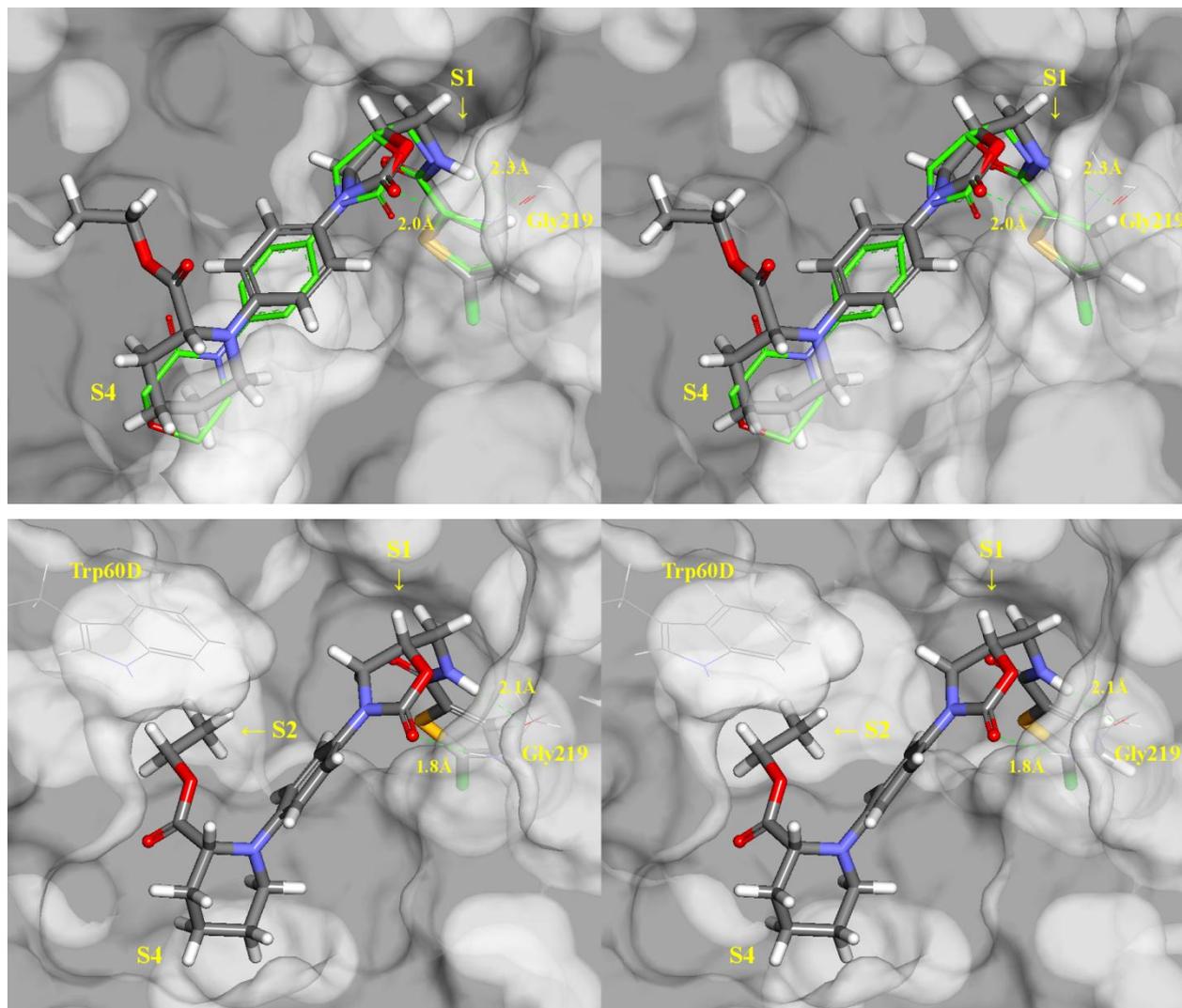


Figure: Stereograms of (2R,5'S)-**24** docked into the fXa active site<sup>4</sup> (top) and thrombin active site<sup>33</sup> (bottom). The conformation of rivaroxaban in complex with fXa (from the X-ray crystal structure) is indicated in green (top). (The figures were prepared by Discovery Studio 3.1.)

## References

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#### 4. Plasma stability study

Preliminary plasma stability study determining end-point (4 h) stability was performed for compounds **22** and **24** using a protocol described in [S. Blech, T. Ebner, E. Ludwig-Schwellinger, J. Stangier, W. Roth, The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. *Drug Metab Dispos*, **2008**, *36*, 386.

Human plasma was prepared from two male healthy volunteers. Blood was taken shortly before use and collected in heparin-containing vials as an anticoagulant. Plasma was prepared by centrifugation of blood (4000 rpm, 10 min), pooled, and used immediately. The reaction was started by addition of 10 mM solution of compound in DMSO and performed for up to 4 h at 37°C in a total volume of 2 ml. Final concentration of compound was 100 µM and DMSO concentration was 1 %.

Reaction was stopped by addition of 900 µM of acetonitrile and samples were centrifuged (18000 rpm, 10 min) and analysed using Agilent-HP 1100 HPLC system. The eluant was a mixture of 0.1% trifluoroacetic acid in water (A) and acetonitrile (B). Gradient: 90% A to 10% A in 10 min.

Compound **24** was found to be stable and no hydrolyzed compound was detected, while 5% of **22** hydrolysed. 63 percent of dabigatran etexilate (used as control) remained after 4h incubation with human plasma.