Supporting Information

Discovery of potent and specific dihydroisoxazole inhibitors of human transglutaminase 2

Cornelius Klöck†, Zachary Herrera†, Megan Albertelli§, and Chaitan Khosla*,†,‡

Departments of †Chemistry, ‡Chemical Engineering and §Comparative Medicine

Stanford University, Stanford CA 94305
1. Supplemental Figures

Figure S1: Standard curves for LC-MS analysis of inhibitor concentrations in plasma (A) and consistency of internal standard peak area across samples (B)

#27: very low ion counts for all masses, disregarded in analysis
#33 blank

#1: very low ion counts for all masses, disregarded in analysis
#39 blank
2. Supplemental Tables

| #   | R =     | X= | k_{app}/K_i [mM min^{-1}] |
|-----|---------|----|---------------------------|
|     |         |    | TG1 | TG2 |
| S1a | 3-OH (cis) | Cl | 0.6 | < 0.5 |
| S1b | 3-OH (trans) | Cl | 5.7 | 2.0 |
| S1c | 4-OH (cis) | Cl | 1.7 | 0.6 |
| S1d | 4-OH (trans) | Cl | 2.1 | 3.6 |
| 4e  | 4-OH (trans) | Br | 4.8 | 12.5 |

Table S1. Potency and specificity of inhibitors containing hydroxy-substituted prolines
| #   | R               | \( \frac{k_{\text{ass}}}{K_i} \) [mM\(^{-1}\) min\(^{-1}\)] | TG1 | TG2 |
|-----|-----------------|-------------------------------------------------|-----|-----|
|     |                 |                                                 |     |     |
| S2a | (pyrazyl)       | 1.5                                             |     |     |
| S2b | (quinoxalyl)    | 2.7                                             |     |     |
| S2c | n = 0           | 0.78                                            | poor|     |
| S2d | n = 1           | poor                                            | poor| poor|
| S2e | n = 2           | poor                                            | poor| poor|

Table S2. Potency and specificity of inhibitors with N-terminal amides
3. Supplemental Synthetic Methods

(2S,3R)-quinolin-3-ylmethyl 2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl-3-hydroxyprrolidine-1-carboxylate (S1a).

The synthesis followed the general procedure in steps 1 and 3 but deviated in the deprotection of the Boc group in step 2. Here, the intermediate was dissolved in 4M HCl in dioxane and the mixture was stirred for 30 minutes before cold ether was added, furnishing a viscous oil formed on the walls of the vial. The liquid was decanted off and the viscous oil was rinsed with more ether and then taken up in little methanol and triturated with ether, furnishing the deprotected product as its HCl salt in the form of a white solid. Inadvertently, under these conditions, bromide was replaced for chloride in the DHI moiety. The remainder of the synthesis followed the standard conditions and the title compound was obtained as a white solid that precipitated in high purity from a solution in ethyl acetate. \(^{1}\)H NMR (500 MHz, DMSO-\(d_6\), mixture of rotational isomers) \(\delta\) 8.92 & 8.83 (2 d, \(J = 2.2\) Hz, 1H), 8.34 & 8.24 (2 d, \(J = 1.2\) Hz, 1H), 8.16 – 7.93 (m, 3H), 7.81 – 7.73 (m, 1H), 7.63 (ddd, \(J = 8.1, 6.9, 1.2\) Hz, 1H), 5.38 – 5.12 (m, 3H), 4.85 – 4.77 & 4.72 – 4.66 (2 m, 1H), 4.44 – 4.32 (m, 1H), 4.29 & 4.18 (2 d, \(J = 7.1\) Hz, 1H), 3.61 – 3.31 (m, 3H, partly obscured by residual water), 3.24 – 3.06 (m, 3H), 2.00 – 1.90 (m, 1H), 1.90 – 1.82 (m, 1H). \(^{13}\)C NMR (126 MHz, DMSO-\(d_6\), mixture of rotational isomers) \(\delta\) 169.81 (d, \(J = 35.2\) Hz), 153.90 (d, \(J = 10.3\) Hz), 150.45 (d, \(J = 39.5\) Hz), 149.47 (d, \(J = 21.9\) Hz), 147.10 (d, \(J = 11.6\) Hz), 134.32 (d, \(J = 85.5\) Hz), 130.04, 129.63 (d, \(J = 16.3\) Hz), 128.76, 128.07 (d, \(J = 16.6\) Hz), 127.28 (d, \(J = 3.6\) Hz), 126.94 (d, \(J = 3.9\) Hz), 81.14 (d, \(J = 9.4\) Hz), 70.56 (d, \(J = 108.7\) Hz), 63.86 (d, \(J = 3.0\) Hz), 63.60 (d, \(J = 83.9\) Hz), 44.32 (d, \(J = 41.1\) Hz), 40.48 (d, \(J = 22.3\) Hz), 40.10, 31.84 (d, \(J = 89.6\) Hz). HRMS (ESI-QTOF) m/z: calculated for C\(_{20}\)H\(_{22}\)ClN\(_4\)O\(_5\)\(^+\) [M+H]\(^+\) 433.12732; found 433.12779.

(2S,3S)-quinolin-3-ylmethyl 2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl-3-hydroxyprrolidine-1-carboxylate (S1b).

Compound S1b was prepared by the procedure described for S1a, furnishing the title compound as a white solid. \(^{1}\)H NMR (500 MHz, DMSO-\(d_6\), mixture of rotational isomers) \(\delta\) 8.94 & 8.84 (2
d, J = 2.2 Hz, 1H), 8.44 & 8.35 (2 t, J = 6.0 Hz, 1H), 8.36 & 8.25 (2 d, J = 1.4 Hz, 1H), 8.07 – 7.94 (m, 2H), 7.77 (dddd, J = 8.4, 7.0, 5.6, 1.5 Hz, 1H), 7.63 (dddd, J = 8.1, 6.9, 3.1, 1.2 Hz, 1H), 5.38 & 5.36 (2 d, J = 4.0 Hz, 1H), 5.34 – 5.19 (m, 2H), 4.81 & 4.71 (2 ddt, J = 10.6, 7.5, 5.2 Hz, 1H), 4.17 – 4.04 (m, 2H), 3.64 – 3.16 (m, 5H, partly obscured by residual water), 2.98 (ddd, 
J = 24.9, 17.5, 7.3 Hz, 1H), 1.97 – 1.83 (m, 1H), 1.82 – 1.71 (m, 1H). 13C NMR (126 MHz, DMSO-d6, mixture of rotational isomers) δ 170.64 (d, J = 24.7 Hz), 154.10 (d, J = 46.8 Hz), 150.50 (d, J = 33.7 Hz), 149.03 (d, J = 24.4 Hz), 147.13 (d, J = 10.2 Hz), 134.44 (d, J = 64.8 Hz), 130.02 (d, J = 6.1 Hz), 129.66 (d, J = 11.8 Hz), 128.77, 128.09 (d, J = 13.8 Hz), 127.28 (d, J = 5.0 Hz), 126.96 (d, J = 2.8 Hz), 80.79 (d, J = 12.8 Hz), 74.01 (d, J = 129.8 Hz), 69.17 (d, J = 79.5 Hz), 63.99 (d, J = 22.3 Hz), 44.92 (d, J = 62.1 Hz), 41.30 (d, J = 24.8 Hz), 40.42, 31.99 (d, J = 102.6 Hz). HRMS (ESI-QTOF) m/z: calculated for C20H22ClN4O5+ [M+H]+ 433.12732; found 433.12771.

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-hydroxypyrrolidine-1-carboxylate (S1c).

Compound S1c was prepared by the procedure described for S1a, furnishing the title compound as a white solid after purification by preparative TLC. 1H NMR (500 MHz, DMSO-d6, mixture of rotational isomers) δ 8.93 & 8.85 (2 d, J = 2.1 Hz, 1H), 8.41 – 8.33 (m, 1H), 8.30 – 8.21 (m, 1H), 8.06 – 7.93 (m, 2H), 7.77 (dddd, J = 8.4, 6.7, 5.1, 1.5 Hz, 1H), 7.63 (dddd, J = 8.1, 6.9, 2.8, 1.2 Hz, 1H), 5.36 – 5.19 (m, 2H), 5.17 & 5.14 (2 d, J = 5.5 Hz, 1H), 4.80 & 4.66 (2 ddt, J = 10.6, 7.7, 5.4 Hz, 1H) 4.30 – 4.15 (m, 2H), 3.63 & 3.58 (2 dd, J = 10.7, 5.3 Hz, 1H), 3.44 – 3.13 (m, 4H, partly obscured by residual water), 3.01 & 2.92 (2 dd, J = 17.5, 7.6 Hz, 1H), 2.38 – 2.25 (m, 1H), 1.80 – 1.71 (m, 1H). 13C NMR (126 MHz, DMSO-d6, mixture of rotational isomers) δ 173.04 (d, J = 34.7 Hz), 154.00 (d, J = 51.6 Hz), 150.54 (d, J = 18.9 Hz), 149.15 (d, J = 32.4 Hz), 147.12 (d, J = 8.1 Hz), 134.51 (d, J = 45.0 Hz), 129.94 (d, J = 3.8 Hz), 129.67 (d, J = 10.6 Hz), 128.75, 128.09 (d, J = 13.8 Hz), 127.27 (d, J = 5.4 Hz), 126.95 (d, J = 2.7 Hz), 80.89 (d, J = 17.9 Hz), 68.35 (d, J = 108.7 Hz), 64.08 (d, J = 6.7 Hz), 59.01 (d, J = 81.7 Hz), 54.76 (d, J = 40.9 Hz), 41.16 (d, J = 35.5 Hz), 40.41, 38.07. HRMS (ESI-QTOF) m/z: calculated for C20H22ClN4O5+ [M+H]+ 433.12732; found 433.12772.
(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl-4-hydroxypryrrolidine-1-carboxylate (S1d).

Compound S1d was prepared by the procedure described for S1a, furnishing the title compound as a white solid. $^1$H NMR (500 MHz, DMSO-$d_6$, mixture of rotational isomers) $\delta$ 8.92 & 8.84 (2 d, $J = 2.2$ Hz, 1H), 8.41 & 8.33 (2 t, $J = 6.0$ Hz, 1H), 8.33 & 8.25 (2 d, $J = 1.5$ Hz, 1H), 8.03 (ddd, $J = 8.5$, 3.9, 1.1 Hz, 1H), 8.00 & 7.96 (2 dd, $J = 8.3$, 1.4 Hz, 1H), 7.81 – 7.73 (m, 1H), 7.68 – 7.59 (m, 1H), 5.34 – 5.18 (m, 2H), 5.10 (d, $J = 3.5$ Hz, 1H), 4.81 & 4.65 (2 ddt, $J = 10.4$, 7.5, 5.0 Hz, 1H), 4.39 – 4.22 (m, 2H), 3.60 – 3.09 (m, 5H), 2.99 & 2.89 (dd, $J = 17.5$, 7.4 Hz, 1H), 2.16 – 2.02 (m, 1H), 1.91 – 1.77 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$, mixture of rotational isomers) $\delta$ 172.79 (d, $J = 45.5$ Hz), 154.01 (d, $J = 29.2$ Hz), 150.51 (d, $J = 22.7$ Hz), 148.98 (d, $J = 36.9$ Hz), 147.12 (d, $J = 9.5$ Hz), 134.44 (d, $J = 58.0$ Hz), 129.99 (d, $J = 6.8$ Hz), 129.66 (d, $J = 14.0$ Hz), 128.77, 128.07 (d, $J = 14.2$ Hz), 127.27 (d, $J = 4.8$ Hz), 126.96 (d, $J = 4.8$ Hz), 80.98 (d, $J = 23.8$ Hz), 68.22 (d, $J = 85.7$ Hz), 63.99, 58.78 (d, $J = 79.7$ Hz), 55.24 (d, $J = 63.8$ Hz), 41.22 (d, $J = 39.1$ Hz), 40.34, 38.86. HRMS (ESI-QTOF) m/z: calculated for C$_{20}$H$_{22}$ClN$_4$O$_5^+$ [M+H]$^+$ 433.12732; found 433.12700.

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(pyrazine-2-carbonyl)pyrrolidine-2-carboxamide (S2a).

Pyrazine-2-carboxylic acid (67.4 mg, 0.543 mmol), EDCI (120 mg, 0.625 mmol) and HOBt hydrate (73.4 mg, 0.543 mmol) were dissolved in 1 mL DMF and stirred for 10 minutes before 4-methylmorpholine (110 mg, 1.09 mmol) and (S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)pyrrolidine-2-carboxamide (150 mg, 0.543 mmol) in 1 mL DMF were added. The mixture was stirred at room temperature for 2 hours before it was diluted with 20 mL water and extracted with 50 mL ethyl acetate. The organic layer was washed with brine (3 * 30 mL), dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by preparative TLC (10*20 cm plate, 1 mm thickness, developed with 80% ethyl acetate / pentane), furnishing (S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(pyrazine-2-carbonyl)pyrrolidine-2-carboxamide (27 mg, 0.071 mmol, 13.0 % yield) as a yellowish solid. $^1$H NMR (500 MHz, DMSO-$d_6$, mixture of rotational isomers) $\delta$ 8.94 (d, $J = 10.1$ Hz, 1H), 8.77 & 8.71 (2 m, 1H), 8.71 & 8.56 (2 m, 1H), 8.28 & 8.21 (2 t, $J = 6.1$ Hz, 1H), 4.85 & 4.53 – 4.42 (dd / m, $J =$
8.6, 3.5 Hz, 1H), 4.80 – 4.68 & 4.53 – 4.42 (2 m, 1H), 3.78 (t, J = 6.7 Hz, 1H), 3.65 (q, J = 7.1, 6.2 Hz, 1H), 3.51 – 3.17 (m, 2H, partly obscured by residual water), 3.10 (t, J = 5.8 Hz, 1H), 3.05 & 2.86 (2 dd, J = 17.6, 7.5 Hz, 1H), 2.31 – 2.12 (m, 1H), 1.85 (m, 3H). 13C NMR (126 MHz, Chloroform-d, mixture of rotational isomers) δ 172.72 (d, J = 136.8 Hz), 165.01 (d, J = 80.2 Hz), 147.98 (d, J = 87.5 Hz), 146.51 (d, J = 60.5 Hz), 146.12 (d, J = 16.3 Hz), 142.19 (d, J = 58.4 Hz), 137.89 (d, J = 36.6 Hz), 80.44 (d, J = 18.1 Hz), 62.44 (d, J = 161.2 Hz), 49.30 (d, J = 213.1 Hz), 43.88 (d, J = 15.6 Hz), 41.73 (d, J = 58.3 Hz), 30.46 (d, J = 459.4 Hz), 23.91 (d, J = 469.7 Hz). HRMS (ESI-QTOF) m/z: calculated for C14H17BrN5O3+[M+H]+ 382.05093; found 382.04936.

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(quinoxaline-2-carbonyl)pyrrolidine-2-carboxamide (S2b).

Compound S2b was prepared from (S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)pyrrolidine-2-carboxamide and quinoxaline-2-carboxylic acid by the same procedure as compound S2a, and obtained as a reddish solid (38 mg, 0.088 mmol, 15.3 % yield) after preparative TLC. 1H NMR (500 MHz, DMSO-d6, mixture of rotational isomers) δ 9.23 & 9.20 (2 s, 1H), 8.34 & 8.29 (2 t, J = 6.1 Hz, 1H), 8.19 – 8.05 (m, 2H), 8.00 – 7.91 (m, 2H), 5.11 & 4.55 (2 dd, J = 8.5, 3.7 Hz, 1H), 4.76 & 4.37 (2 ddt, J = 10.4, 7.5, 4.9 Hz, 1H), 3.95 (t, J = 6.7 Hz, 1H), 3.78 – 3.66 (m, 1H), 3.46 & 3.13 (2 ddd, J = 14.0, 6.4, 5.1 Hz, 1H), 3.24 & 2.96 (2 dt, J = 14.2, 5.1 Hz, 1H), 3.07 & 2.71 (2 dd, J = 17.6, 7.3 Hz, 1H), 3.39 & 3.02 (2 dd, J = 17.6, 10.8 Hz, 1H), 2.35 – 2.16 (m, 1H), 2.00 – 1.79 (m, 3H). 13C NMR (126 MHz, Chloroform-d, mixture of rotational isomers) δ 172.91 (d, J = 180.4 Hz), 165.14 (d, J = 79.1 Hz), 146.55 (d, J = 120.6 Hz), 145.69 (d, J = 25.2 Hz), 142.67 (d, J = 6.3 Hz), 139.89 (d, J = 63.5 Hz), 137.75 (d, J = 66.4 Hz), 131.73 (d, J = 22.7 Hz), 130.84 (d, J = 18.1 Hz), 129.81 (d, J = 29.6 Hz), 129.43 (d, J = 5.1 Hz), 80.40 (d, J = 36.4 Hz), 62.81 (d, J = 231.6 Hz), 49.52 (d, J = 200.9 Hz), 43.84, 41.77 (d, J = 56.7 Hz), 30.60 (d, J = 483.5 Hz), 23.96 (d, J = 463.9 Hz). HRMS (ESI-QTOF) m/z: calculated for C18H19BrN5O3+[M+H]+ 432.06658; found 432.06196.

(S)-1-benzoyl-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)pyrrolidine-2-carboxamide (S2c).
Compound S2c was prepared analogously to compound S2a, except that the amine was used as its TFA salt and an additional equivalent of 4-methylmorpholine added, furnishing the product as a clear oil (35.4 mg, 0.093 mmol, 24.2 % yield) after preparative TLC. $^1$H NMR (500 MHz, DMSO-$d_6$, mixture of rotational isomers) $\delta$ 8.21 & 8.13 (2 t, $J = 6.1$ Hz, 1H), 7.56 – 7.24 (m, 5H), 4.74 & 4.51 (ddt, $J = 11.2$, 7.6, 5.1 Hz, 1H), 4.42 & 4.21 (2 dd, $J = 8.5$, 5.2 Hz, 1H), 3.65 – 3.49 (m, 1H), 3.46 – 3.10 (m, 4H, partly obscured by residual water), 3.05 & 2.89 (2 dd, $J = 17.6$, 7.4 Hz, 1H), 2.25 – 2.09 (m, 1H), 1.93 – 1.72 (m, 3H). HRMS (ESI-QTOF) m/z: calculated for C$_{16}$H$_{19}$BrN$_3$O$_3$ $^+$ [M+H]$^+$ 380.06043; found 380.06009.

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(2-phenylacetyl)pyrrolidine-2-carboxamide (S2d).

Compound S2d was prepared by the procedure of compound S2c, furnishing the product as a clear oil (33 mg, 0.084 mmol, 21.8 % yield) after preparative TLC. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.38 – 7.31 (m, 2H), 7.31 – 7.22 (m, 3H, partly obscured by solvent), 7.11 (t, $J = 5.8$ Hz, 1H), 4.75 (ddt, $J = 9.9$, 8.0, 4.8 Hz, 1H), 4.56 (dd, $J = 8.2$, 2.6 Hz, 1H), 3.71 (s, 2H), 3.64 – 3.40 (m, 4H), 3.18 (dd, $J = 17.4$, 10.6 Hz, 1H), 2.97 (dd, $J = 17.4$, 8.0 Hz, 1H), 2.31 (ddd, $J = 12.3$, 6.2, 3.1 Hz, 1H), 2.11 – 1.84 (m, 3H). HRMS (ESI-QTOF) m/z: calculated for C$_{17}$H$_{21}$BrN$_3$O$_3$ $^+$ [M+H]$^+$ 394.07608; found 394.07578.

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(3-phenylpropanoyl)pyrrolidine-2-carboxamide (S2e).

Compound S2e was prepared by the procedure of compound S2c, furnishing the product as a white solid (46.5 mg, 0.114 mmol, 29.6 % yield) after preparative TLC. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.37 – 7.16 (m, 5H, partly obscured by solvent), 7.05 (t, $J = 5.1$ Hz, 1H), 4.78 (ddt, $J = 12.4$, 8.9, 4.5 Hz, 1H), 4.52 (dd, $J = 8.1$, 2.4 Hz, 1H), 3.58 (ddd, $J = 14.4$, 6.8, 4.9 Hz, 1H), 3.52 – 3.39 (m, 2H), 3.32 (td, $J = 9.4$, 6.7 Hz, 1H), 3.21 (dd, $J = 17.5$, 10.6 Hz, 1H), 3.07 – 2.93 (m, 3H), 2.63 (td, $J = 7.6$, 3.5 Hz, 2H), 2.28 (ddd, $J = 12.2$, 6.4, 3.3 Hz, 1H), 2.10 – 1.82 (m, 3H). HRMS (ESI-QTOF) m/z: calculated for C$_{18}$H$_{23}$BrN$_3$O$_3$ $^+$ [M+H]$^+$ 408.09173; found 408.09159.

quinolin-3-ylmethyl 1H-imidazole-1-carboxylate (S3a).
Building block S3a was prepared according to our previously published procedure.¹

3-fluorobenzyl 1H-imidazole-1-carboxylate (S3b).
3-fluorobenzaldehyde (0.855 ml, 8.06 mmol) was diluted with 30 mL absolute ethanol, stirred on an ice bath and lithium borohydride (2M in THF) (1.209 ml, 2.42 mmol) was added dropwise until TLC showed full conversion of the starting material. The reaction was quenched by addition of a few drops of water, followed by dropwise addition of 1 M hydrochloric acid until evolution of gas ceased and the pH of the mixture reached a neutral level as judged by a pH paper. Ethanol was removed under reduced pressure and the resulting slurry was diluted with more water (ca. 30 mL) and extracted with ethyl acetate (75 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and the volatiles were removed under reduced pressure, furnishing the alcohol in nearly quantitative yield as a clear oil, which was diluted with 5 mL acetonitrile. Separately, 1,1'-carbonyldiimidazole (1.960 g, 12.09 mmol) was suspended in 10 mL dry acetonitrile, added to the alcohol and the mixture stirred for 30 minutes. The mixture was placed in a freezer overnight and a white solid could be filtered off next day. To remove residual imidazole from this solid, it was dissolved in equal parts ethyl acetate and water and the organic layer was washed with sodium bicarbonate twice and brine and eventually dried over sodium sulfate. The volatiles were removed, furnishing 3-fluorobenzyl 1H-imidazole-1-carboxylate (256 mg, 1.163 mmol, 14.4 % yield over two steps) as a white solid in unsatisfactory overall yield but good purity.¹H NMR (500 MHz, Chloroform-d) δ 8.15 – 8.03 (m, 1H), 7.37 (t, J = 1.5 Hz, 1H), 7.30 (td, J = 8.0, 5.8 Hz, 1H), 7.15 (dt, J = 7.7, 1.2 Hz, 1H), 7.08 (dt, J = 9.3, 2.1 Hz, 1H), 7.03 – 6.97 (m, 2H), 5.33 (s, 2H). ¹³C NMR (126 MHz, Chloroform-d), 162.68 (d, J = 247.2 Hz), 136.27 (d, J = 7.5 Hz), 130.65, 130.41 (d, J = 8.4 Hz), 123.99 (d, J = 3.1 Hz), 117.02, 115.93 (d, J = 21.0 Hz), 115.32 (d, J = 22.1 Hz), 68.68 (d, J = 1.5 Hz).

2,3-dimethoxybenzyl 1H-imidazole-1-carboxylate (S3c).
(2,3-dimethoxyphenyl)methanol (250 mg, 1.49 mmol) and 1,1'-carbonyldiimidazole (362 mg, 2.23 mmol) were dissolved in 5 mL dry acetonitrile each and combined and stirred for one hour. Instead of crystallizing the product, acetonitrile was evaporated from the reaction mixture and the residue diluted in 75 mL ethyl acetate and washed with sodium bicarbonate solution (2* 50 mL) and brine (50 mL), furnishing 2,3-dimethoxybenzyl 1H-imidazole-1-carboxylate (383 mg,
1.46 mmol, 98 % yield) as a clear oil that slowly solidified in the cold. \(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 8.13 (t, \(J = 1.0\) Hz, 1H), 7.41 (dd, \(J = 1.7, 1.2\) Hz, 1H), 7.11 – 7.02 (m, 2H), 7.01 – 6.94 (m, 2H), 5.46 (s, 2H), 3.88 (s, 3H), 3.87 (s, 3H). \(^13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 152.82, 148.70, 147.95, 137.19, 130.67, 127.83, 124.29, 122.08, 117.24, 113.68, 65.41, 61.23, 55.89.

3-(benzyloxy)benzyl 1\(H\)-imidazol-1-carboxylate (S3d).

Compound S3d was prepared from 3-(benzyloxy)benzaldehyde (500 mg, 2.36 mmol) using the procedure described for compound S3b. Instead of crystallizing the product in the second step, the crude reaction mixture was evaporated, extracted and washed as described for compound S3c, affording 3-(benzyloxy)benzyl 1\(H\)-imidazol-1-carboxylate (708 mg, 2.30 mmol, 97 % yield) as a colorless oil that slowly solidified in the freezer. \(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 8.14 (t, \(J = 1.0\) Hz, 1H), 7.45 – 7.30 (m, 7H), 7.08 – 6.99 (m, 4H), 5.38 (s, 2H), 5.09 (s, 2H). \(^13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 159.10, 148.63, 137.20, 136.65, 135.52, 130.75, 130.05, 128.69, 128.15, 127.51, 121.07, 117.22, 115.46, 115.16, 70.10, 69.68.

pyridin-2-ylmethyl 1\(H\)-imidazol-1-carboxylate (S3e).

1,1’-Carbonyldiimidazole (2.229 g, 13.75 mmol) was dissolved in 20 mL dry acetonitrile at room temperature and pyridin-2-ylmethanol (0.884 ml, 9.16 mmol) was added neat to the vigorously stirred solution. The reaction was allowed to proceed for 30 minutes at which point the volume of acetonitrile was reduced on the rotary evaporator. The resulting oily slurry was taken up in 75 mL ethyl acetate and 75 mL water and partitioned. The organic layer was washed with sodium bicarbonate solution (2 * 50 mL) and brine (1 * 50 mL) and eventually dried over anhydrous sodium sulfate. The volatiles were removed under reduced pressure, furnishing pyridin-2-ylmethyl 1\(H\)-imidazol-1-carboxylate (1.297 g, 6.38 mmol, 69.7 % yield) as a yellow mobile oil that slowly solidified on standing. The crude product was analyzed by 1H NMR in CDCl\(_3\) and deemed sufficiently pure.\(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 8.63 (ddd, \(J = 4.9, 1.8, 0.9\) Hz, 1H), 8.20 (t, \(J = 1.0\) Hz, 1H), 7.76 (td, \(J = 7.7, 1.8\) Hz, 1H), 7.49 (t, \(J = 1.4\) Hz, 1H), 7.44 (d, \(J = 7.9\) Hz, 1H), 7.30 (ddd, \(J = 7.6, 4.8, 1.1\) Hz, 1H), 7.08 (dd, \(J = 1.7, 0.9\) Hz, 1H), 5.53 (s, 2H). \(^13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 153.65, 149.64, 148.37, 137.04, 136.87, 130.57, 123.43, 122.05, 117.07, 69.69.
pyridin-3-ylmethyl 1H-imidazole-1-carboxylate (S3f).

The title compound (0.978 g, 4.81 mmol, 52.5 % yield) was prepared according to the procedure outlined in for compound S3e. ¹H NMR (500 MHz, Chloroform-d) δ 8.74 (s, 1H), 8.66 (d, J = 4.8 Hz, 1H), 8.16 (s, 1H), 7.82 (d, J = 8.6 Hz, 1H), 7.44 (s, 1H), 7.38 (t, J = 6.5 Hz, 1H), 7.07 (s, 1H), 5.46 (s, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 150.37, 149.92, 148.29, 136.94, 136.41, 130.65, 129.59, 123.58, 116.98, 67.07.

pyridin-4-ylmethyl 1H-imidazole-1-carboxylate (S3g).

The title compound (0.434 g, 2.14 mmol, 23.3 % yield) was prepared according to the procedure outlined in for compound S3e, except that both reagents were individually dissolved in 10 mL acetonitrile each and then combined. ¹H NMR (500 MHz, Chloroform-d) δ 8.69 – 8.62 (m, 2H), 8.16 (t, J = 1.0 Hz, 1H), 7.44 (t, J = 1.4 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.08 (d, J = 0.9 Hz, 1H), 5.41 (s, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 149.98, 147.98, 142.51, 136.77, 130.58, 121.68, 116.80, 77.16, 67.19.

(S)-1-(naphthalen-2-yl)ethyl 1H-imidazole-1-carboxylate (S3h).

Commercial (S)-1-(naphthalen-2-yl)ethanol (500 mg, 2.90 mmol) was dissolved in 5 mL anhydrous acetonitrile and separately, di(1H-imidazol-1-yl)methanone (942 mg, 5.81 mmol) was suspended in 5 mL acetonitrile. The solutions were combined and after about 30 minutes, large quantities of a white fluffy solid precipitated. The solid was filtered and washed with little acetonitrile, furnishing (S)-1-(naphthalen-2-yl)ethyl 1H-imidazole-1-carboxylate that contained about 30 mol% residual imidazole as judged by NMR (537 mg, approximately 63% yield when corrected for imidazole content), but which was used in the inhibitor synthesis without further purification. ¹H NMR (500 MHz, Chloroform-d) δ 8.18 (t, J = 1.0 Hz, 1H), 7.92 – 7.81 (m, 4H), 7.55 – 7.48 (m, 3H), 7.45 (t, J = 1.4 Hz, 1H), 7.07 (dd, J = 1.7, 0.9 Hz, 1H), 6.24 (q, J = 6.6 Hz, 1H), 1.83 (d, J = 6.6 Hz, 3H).

(R)-1-(naphthalen-2-yl)ethyl 1H-imidazole-1-carboxylate (S3i).

The title compound was prepared by the same procedure as compound S3h, furnishing 628 mg of a fluffy white solid with 30 mol% residual imidazole (by NMR, approximately 74% yield
when corrected for imidazole content), that was used directly without further purification.\(^1\)\(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 8.18 (t, \(J = 1.0\) Hz, 1H), 7.92 – 7.82 (m, 4H), 7.56 – 7.48 (m, 3H), 7.45 (t, \(J = 1.5\) Hz, 1H), 7.07 (dd, \(J = 1.7, 0.9\) Hz, 1H), 6.24 (q, \(J = 6.6\) Hz, 1H), 1.82 (d, \(J = 6.6\) Hz, 3H).

**4-nitrophenyl (quinolin-3-ylmethyl) carbonate (S4a).**

Building block S4a was prepared according to our previously published procedure.\(^2\)

**4-nitrophenyl (quinolin-4-ylmethyl) carbonate (S4b).**

Adapting our previously published procedure,\(^2\) quinoline-4-carbaldehyde (3 g, 19.09 mmol) was dissolved in absolute ethanol (40 mL), cooled in a dry-ice acetone bath and lithium borohydride (4.77 ml, 9.54 mmol) added dropwise. Once TLC showed consumption of the starting material, the cooling bath was removed and 1 M aqueous HCl carefully added. After the reaction had reached room temperature and additional HCl would not yield more gas evolution, the mixture was neutralized with aqueous sodium bicarbonate and then concentrated under reduced pressure. The residue was extracted with ethyl acetate (3x) and the combined organic fractions were dried over sodium sulfate and evaporated, yielding the alcohol as an orange oil that solidified on standing. The alcohol was taken up in anhydrous DCM (30 mL), N-methylmorpholine (4.20 ml, 38.2 mmol) was added and the solution cooled in an ice-bath. Separately, 4-nitrophenyl chloroformate (5.77 g, 28.6 mmol) was dissolved in DCM (15 mL) and added dropwise to the cooled solution of the alcohol. After addition had been completed, the mixture was allowed to warm to room temperature and stirred overnight. Aqueous sodium bicarbonate and ethyl acetate were then added to the reaction mixture and partitioned. The organic layer was washed with sodium bicarbonate solution (3x) and brine. The organic phase was dried over sodium sulfate and evaporated, furnishing a red crude product that solidified on standing that and that was purified by silica gel chromatography (20% to 50% ethyl acetate in pentane, affording 4-nitrophenyl (quinolin-4-ylmethyl) carbonate (3.1 g, 9.56 mmol, 50.1 % yield over two steps) as an off-white solid. \(^1\)H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 8.97 (d, \(J = 4.4\) Hz, 1H), 8.35 – 8.26 (m, 2H), 8.24 – 8.13 (m, 1H), 8.01 (dt, \(J = 8.4, 0.9\) Hz, 1H), 7.80 (ddd, \(J = 8.4, 6.8, 1.4\) Hz, 1H), 7.67 (ddd, \(J = 8.3, 6.9, 1.3\) Hz, 1H), 7.55 (d, \(J = 4.4\) Hz, 1H), 7.41 (d, \(J = 9.2\) Hz, 2H), 5.80 (d, \(J = 0.8\) Hz, 2H).
4-nitrophenyl (quinoxalin-2-ylmethyl) carbonate (S4c).

Following the procedure of Robinson and Taylor, benzene-1,2-diamine (1.5 g, 13.9 mmol) and 1,3-dihydroxypropan-2-one (1.25 g, 13.9 mmol) were dissolved in DMSO (15 mL) and the solution heated to 80 °C. Then, 10 mol% triethylamine (0.193 ml, 1.39 mmol) was added, followed by 2 mol% palladium(II) acetate (0.062 g, 0.277 mmol). The mixture was stirred and heated while open to air. After 3 hours, the solution was diluted with water (100 mL) and extracted with ethyl acetate (150 mL). The organic layer was washed four times with a buffer of phosphoric acid and monobasic sodium phosphate (pH = 2), followed by aqueous sodium bicarbonate. Quinoxalin-2-ylmethanol (0.67 g, 4.18 mmol, 30.2 % yield) was isolated by silica gel chromatography. An aliquot of the alcohol pooled from two preparations (1.0 g, 6.2 mmol) was then reacted with nitrophenyl chloroformate (1.9 g, 9.4 mmol) for three hours as described for compound S4b and in the literature. The solid orange-red crude product was taken up in little ethyl acetate. A white solid that poorly dissolved was filtered off. The remainder was applied to a silica gel column and eluted with gradient of ethyl acetate in pentane (20% to 50%). Suitable fractions were pooled to yield an orange solid. Both the initially filtered white solid as well as the orange solid from the silica gel column were identified as the title compound (1.4 g, 4.3 mmol, 69 % combined yield). 1H NMR (400 MHz, Chloroform-d) δ 9.02 (s, 1H), 8.36 – 8.25 (m, 2H), 8.22 – 8.09 (m, 2H), 7.87 – 7.76 (m, 2H), 7.51 – 7.39 (m, 2H), 5.64 (s, 2H).

(S)-2-(5-amino-2-(((benzyloxy)carbonyl)amino)-5-oxopentanamido)acetic acid (ZQS)

(S)-5-amino-2-(((benzyloxy)carbonyl)amino)-5-oxopentanoic acid (17 g, 60.7 mmol), (S)-methyl 2-amino-3-hydroxypropanoate, HCl (9.44 g, 60.7 mmol), EDCI (13.37 g, 69.8 mmol) and HOBt (8.20 g, 60.7 mmol) were dissolved in 70 mL and N-methylmorpholine (13.34 ml, 121 mmol) was added. The mixture was stirred for 30 minutes by which time a large amount of solid had formed. The mixture was poured into 400 mL of water and vigorously stirred for another 30 minutes. The solid was filtered, washed with little water and the crude ester then hydrolyzed by the method of Sondheimer. To this end, the solid was dissolved in 250 mL of 80% aqueous ethanol at room temperature and 1 M NaOH was added in small aliquots and the conversion was monitored by TLC (15% EtOH in EtOAc) and staining with KMnO₄. Once TLC showed full conversion, an equimolar amount of HCl was added and the solution evaporated under reduced
pressure. The resulting residue was taken up in little acidic water and stirred for 30 minutes. A bright white solid was then collected by filtration, washed with little water and dried to furnish (S)-2-((S)-5-amino-2-(((benzyloxy)carbonyl)amino)-5-oxopentanamido)-3-hydroxypropanoic acid (2.346 g, 6.39 mmol, 10.53 % yield) in high purity but modest yield. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.04 (d, \(J = 7.8\) Hz, 1H), 7.45 (d, \(J = 8.4\) Hz, 1H), 7.40 – 7.28 (m, 5H), 7.25 (br s, 1H), 6.77 (br s, 1H), 5.02 (s, 2H), 4.25 (dt, \(J = 7.8, 4.4\) Hz, 1H), 4.07 (td, \(J = 8.8, 4.8\) Hz, 1H), 3.73 (dd, \(J = 10.9, 4.9\) Hz, 1H), 3.61 (dd, \(J = 10.9, 4.1\) Hz, 1H), 2.21 – 2.05 (m, 2H), 1.91 (ddt, \(J = 14.7, 9.4, 5.7\) Hz, 1H), 1.68 (dt, \(J = 13.6, 9.4, 6.1\) Hz, 1H). HRMS (ESI-QTOF) m/z: calculated for C\(_{16}\)H\(_{20}\)N\(_3\)O\(_7\) \([M-H]^-\) 366.13067; found 366.13065.
4. Supplemental Compound Characterization

a. Compound 2:

(S)-quinolin-3-ylmethyl 3-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-3,4-dihydro-1H-pyrido[3,4-b]indole-2(9H)-carboxylate

LC-MS of compound 2 (Abs<sub>280</sub> and TIC traces, peak spectrum and peak table)
| m/z     | z | Abund  | % Abundance |
|---------|---|--------|-------------|
| 562.10843 | 1 | 1389391.6 | 97.44       |
| 563.11097 | 1 | 400436.9   | 28.08       |
| 564.10694 | 1 | 1425941.1  | 100         |
| 565.10908 | 1 | 399124.3   | 27.99       |
NMR of compound 2 (1H spectrum)
b. Compound 3a:

(S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)azetidine-1-carboxylate

LC-MS of compound 3a (Abs280 & TIC traces, peak spectrum & peak table)
NMR of compound 3a (\(^1\)H and \(^{13}\)C spectra)
c. Compound 3b:

(S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoylelpiperidine-1-carboxylate

LC-MS of compound 3b (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 3b (1H spectrum)
d. Compound 3c:

(R)-quinolin-3-ylmethyl 3-(((3S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate, TFA salt

LC-MS of compound 3c (Abs_{280} and TIC traces, peak spectrum and peak table)
NMR of compound 3c (1H and 13C spectra)
e. Compound 3d:

(S)-quinolin-3-ylmethyl 2-(2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)amino)-2-oxoethyl)pyrrolidine-1-carboxylate

LC-MS of compound 3d (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 3d (¹H and ¹³C spectra)
f. Compound 4a:

(S)-quinolin-3-ylmethyl 2-((((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-2-methylpyrrolidine-1-carboxylate

LC-MS of compound 4a (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 4a (¹H spectrum)
g. Compound 4b:

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-fluoro-pyrrolidine-1-carboxylate

LC-MS of compound 4b (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 4b (1H and 13C spectra)
h. Compound 4d:

(2S,4R)-quinolin-3-ylmethyl 4-amino-2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate (bis-TFA salt)

LC-MS of compound 4d (Abs280 trace)

(eluted in the void volume before MS acquisition commenced at 2 min)
NMR of compound 4d (1H and 13C spectra)
i. Compound 4e:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-hydroxy-pyrrolidine-1-carboxylate

LC-MS of compound 4e (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 4e (¹H and ¹³C spectra)
j. Compound 5a:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-methoxy-
pyrrolidine-1-carboxylate

LC-MS of compound 5a (Abs250 and TIC traces, peak spectrum and peak table)
NMR of compound 5a (¹H spectrum)
k. Compound 5c:

(2S,4R)-quinolin-3-ylmethyl 4-(benzyl oxy)-2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 5c (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 5c (1H and 13C spectra)
1. Compound 6:

(2S,4R)-quinolin-3-ylmethyl 4-benzyl-2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-carbamoyl)pyrrolidine-1-carboxylate, TFA salt

LC-MS of compound 6 (Abs$_{280}$ and TIC traces, peak spectrum and peak table)

| m/z   | z | Abund      | % Abundance |
|-------|---|------------|-------------|
| 551.12961 | 1 | 1966800.3  | 97.79       |
| 552.13145 | 1 | 612375.3   | 30.45       |
| 553.12816 | 1 | 2011163.3  | 100         |
| 554.12974 | 1 | 615605     | 30.61       |
NMR of compound 6 (1H and 13C spectra)
m. Compound 7a:

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-phenyl-pyrrolidine-1-carboxylate

LC-MS of compound 7a (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 7a (¹H and ¹³C spectra)
n. Compound 7b:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-phenylpyrrolidine-1-carboxylate

LC-MS of compound 7b (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 7b (1H spectrum)
o. Compound 7c:

\[
(2S,4S)-\text{quinolin-3-ylmethyl} \quad 2-\text{(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl}-4-(2-hydroxyphenyl)\text{pyrrolidine-1-carboxylate, TFA salt}
\]

LC-MS of compound 7c (Abs\text{250} and TIC traces, peak spectrum and peak table)

| m/z  | z  | Abund  | % Abundance |
|------|----|--------|-------------|
| 553.1075 | 1 | 721806.3 | 96.77 |
| 554.11052 | 1 | 192258.4 | 25.78 |
| 555.10594 | 1 | 745905.4 | 100 |
| 556.10876 | 1 | 192047 | 25.75 |
NMR of compound 7c (1H spectrum)
p. Compound 7d:

\[
(2S,4S)\text{-quinolin-3-ylmethyl 2-}((\text{(S)-3-bromo-4,5-dihydroisoxazol-5-yl})\text{methyl})\text{carbamoyl})\text{-4-(3-hydroxyphenyl)pyrrolidine-1-carboxylate, TFA salt}
\]

LC-MS of compound 7d (Abs\text{280 and TIC traces, peak spectrum and peak table})
NMR of compound 7d (1H spectrum)
q. Compound 7e:

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(4-hydroxyphenyl)pyrrolidine-1-carboxylate

LC-MS of compound 7e (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 7e (1H and 13C spectra)
r. Compound 7f:

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(3-chlorophenyl)pyrrolidine-1-carboxylate, TFA salt

LC-MS of compound 7f (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 7f (1H spectrum)
s. Compound 7g:

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(4-chlorophenyl)pyrrolidine-1-carboxylate, TFA salt

LC-MS of compound 7g (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 7g (1H spectrum)
t. Compound 7h:

(2S,4S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(5-fluoro-1H-indol-3-yl)pyrrolidine-1-carboxylate, TFA salt

LC-MS of compound 7h (Abs\textsubscript{280} and TIC traces, peak spectrum and peak table)

| m/z   | Intensity | % Abundance |
|-------|-----------|-------------|
| 121.05087 | 51790.4  | 10.2        |
| 594.11403 | 491232.5 | 96.78       |
| 595.11702 | 142349.8 | 28.04       |
| 596.1124  | 50579.8  | 100         |
| 597.11541 | 143279.2 | 28.23       |
NMR of compound 7h (1H spectrum)
u. Compound 8:

(S)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-phenyl-2,5-dihydro-1H-pyrrole-1-carboxylate, TFA salt

LC-MS of compound 8 (Abs$_{280}$ and TIC traces, peak spectrum and peak table)

| m/z   | z | Abund  | % Abundance |
|-------|---|--------|-------------|
| 535.09716 | 1 | 991554 | 98.28       |
| 536.1001  | 1 | 261184.4 | 25.89      |
| 537.09557 | 1 | 100864.6 | 100        |
| 538.09819 | 1 | 259109.1 | 25.68       |
NMR of compound 8 (1H spectrum)
v. Compound 9a:

\[
(2S,4R)\text{-quinolin-3-ylmethyl} \quad 4\text{-benzamido-2-(((S)-3-bromo-4,5\text{-dihydroisoxazol-5-yl)methyl})-carbamoyl)pyrrolidine-1-carboxylate}
\]

LC-MS of compound 9a (Abs$_{280}$ and TIC traces, peak spectrum and peak table)

| m/z  | z | Abund  | % Abundance |
|------|---|--------|-------------|
| 580.11897 | 1 | 1436267.9 | 97.27       |
| 581.12152 | 1 | 407883.9  | 27.62       |
| 582.11748 | 1 | 1476576.6 | 100         |
| 583.11957 | 1 | 407799.1  | 27.62       |
NMR of compound 9a (¹H spectrum)
w. Compound 9b:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(2-hydroxybenzamido)pyrrolidine-1-carboxylate

LC-MS of compound 9b (Abs280 and TIC traces, peak spectrum and peak table)

| m/z   | Charge | Abundance | % Abundance |
|-------|--------|-----------|-------------|
| 596.11434 | 1      | 1742863.8 | 97.27       |
| 597.1163  | 1      | 524844.1  | 29.29       |
| 598.11285  | 1      | 1791747.5 | 100         |
| 599.11448  | 1      | 527373.4  | 29.43       |
NMR of compound 9b (1H spectrum)
x. Compound 9c:

(2S,4R)-quinolin-3-ylmethyl 2-((((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(3-hydroxybenzamido)pyrrolidine-1-carboxylate

LC-MS of compound 9c (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 9c (1H spectrum)
y. Compound 9d:

(2S,4R)-quinolin-3-ylmethyl 2-((((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(4-hydroxybenzamido)pyrrolidine-1-carboxylate

LC-MS of compound 9d (Abs 280 and TIC traces, peak spectrum and peak table)
NMR of compound 9d ('H spectrum)
z. Compound 9e:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(nicotinamido)pyrrolidine-1-carboxylate

LC-MS of compound 9e (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 9e (¹H and ¹³C spectra)
aa. Compound 9f:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-(pyrazine-2-carboxamido)pyrrolidine-1-carboxylate

LC-MS of compound 9f (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 9f ('H spectrum)
bb. Compound 9g:

1-(((3R,5S)-5-((((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-1-((quinolin-3-ylmethoxy)carbonyl)pyrroloidin-3-yl)carbamoyl)cyclobutanecarboxylic acid, TFA salt

LC-MS of compound 9g (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 9g (1H spectrum)
cc. Compound 10a:

(S)-tert-butyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10a (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 10a (1H and 13C spectra)
dd. Compound 10a’:

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)pyrrolidine-2-carboxamide, TFA

NMR of compound 10a’ (1H spectrum)
ee. Compound 10b:

(S)-prop-2-yn-1-yl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoylpyrrolidine-1-carboxylate

LC-MS of compound 10b (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 10b (1H and 13C spectra)
Compound 10c:

(S)-benzyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10c (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 10c (¹H and ¹³C spectra)
gg. Compound 10d:

(S)-3-fluorobenzyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10d (Abs$_{280}$ and TIC traces, peak spectrum and peak table)

| $m/z$ | $z$ | Abund | % Abundance |
|------|-----|-------|-------------|
| 428.06224 | 1  | 1177742.4 | 99.72 |
| 429.0653 | 1  | 226483.2  | 19.18 |
| 430.0603 | 1  | 1181063.3 | 100 |
| 431.06328 | 1  | 224957.1  | 19.05 |

| $m/z$ | $z$ | Abund | % Abundance |
|------|-----|-------|-------------|
| 450.04442 | 1  | 758671.6  | 64.24 |
| 451.0469 | 1  | 142775.5  | 12.09 |
| 452.04268 | 1  | 748074.4  | 63.34 |
| 453.04501 | 1  | 139372.2  | 11.8 |
NMR of compound 10d (¹H spectrum)
hh. Compound 10f:

(S)-2,3-dimethoxybenzyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10f (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 10f (¹H and ¹³C spectra)
ii. Compound 10g:

(S)-3-(benzyloxy)benzyl 2-((((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10g (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 10g (1H and 13C spectra)
jj. Compound 10h:

(S)-pyridin-2-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10h (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 10h (1H spectrum)
Compound 10i:

(S)-pyridin-3-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

NMR of compound 10i (1H spectrum)
II. Compound 10j:

\[(S)\text{-pyridin-4-ylmethyl}\quad 2\text{-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl}pyrroldidine-1\text{-carboxylate}\]

NMR of compound 10j (\(^1\)H spectrum)
Compound 10k:

(S)-quinolin-4-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoylpyrrolidine-1-carboxylate

LC-MS of compound 10k (Abs$_{280}$ and TIC traces, peak spectrum and peak table)

| m/z     | z | Abund  | % Abundance |
|---------|---|--------|-------------|
| 461.08155 | 1 | 1035916.2 | 98.97       |
| 462.08449 | 1 | 210135   | 20.08       |
| 463.07884 | 1 | 1046745.3 | 100         |
| 464.08248 | 1 | 208493.3 | 19.92       |
NMR of compound 10k (1H and 13C spectra)
nn. Compound 10l:

(S)-quinoxalin-2-ylmethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoylpyrrolidine-1-carboxylate

LC-MS of compound 10l (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 10l (1H and 13C spectra)
Compound 10m:

(S)-(S)-1-(naphthalen-2-yl)ethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoylpyrrolidine-1-carboxylate

LC-MS of compound 10m (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound 10m (\textsuperscript{1}H spectrum)
pp. Compound 10n:

(S)-(R)-1-(naphthalen-2-yl)ethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10n (Abs\textsubscript{280} and TIC traces, peak spectrum and peak table)
NMR of compound 10n (1H spectrum)
Compound 10o:

(S)-(R)-1-(quinolin-3-yl)ethyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate

LC-MS of compound 10o (Abs₂₅₀ and TIC traces, peak spectrum and peak table)
NMR of compound 10o (1H spectrum)
rr. Compound 10p:

(S)-(1H-benzo[d]imidazol-2-yl)methyl 2-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-pyrrolidine-1-carboxylate

LC-MS of compound 10p (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound 10p (¹H and ¹³C spectra)
ss. Compound 10q:

\[(S)-(1\text{-methyl-1H-benzo}[d]\text{imidazol-2-yl})\text{methyl} 2-(((S)-3\text{-bromo-4,5-dihydroisoxazol-5-yl})\text{methyl})\text{-carbamoyl} \text{pyrrolidine-1-carboxylate}\]

LC-MS of compound 10q (Abs \text{280} and TIC traces, peak spectrum and peak table)
NMR of compound 10q (1H spectrum)
tt. Compound S1a:

(2S,3R)-quinolin-3-ylmethyl 2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-3-hydroxy-3-pyrrolidine-1-carboxylate

LC-MS of compound S1a (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound S1a (1H and 13C spectra)
uu. Compound S1b:

\[
(2S,3S)\text{-quinolin-3-ylmethyl} \quad 2\text{-}(((S)\text{-3-chloro-4,5-dihydroisoxazol-5-yl)methyl}\text{carbamoyl})\text{-3-hydroxy}\
\text{pyrrolidine-1-carboxylate}
\]

LC-MS of compound S1b (Abs\text{280} and TIC traces, peak spectrum and peak table)
NMR of compound S1b (\(^1\)H and \(^1\)C spectra)
**vv. Compound S1c:**

\[(2S,4S)-\text{quinolin-3-ylmethyl } \rightarrow \text{2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl}-4-\text{hydroxypyrrolidine-1-carboxylate} \]

LC-MS of compound S1c (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound S1c (1H and 13C spectra)
Compound S1d:

(2S,4R)-quinolin-3-ylmethyl 2-(((S)-3-chloro-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)-4-hydroxypyrrolidine-1-carboxylate

LC-MS of compound S1d (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound S1d ($^1$H and $^{13}$C spectra)
xx. Compound S2a:

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(pyrazine-2-carbonyl)pyrrolidine-2-carboxamide

LC-MS of compound S2a (Abs280 and TIC traces, peak spectrum and peak table)
NMR of compound S2a (¹H and ¹³C spectra)
yy. Compound S2b:

(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(quinoxaline-2-carbonyl)pyrrolidine-2-carboxamide

LC-MS of compound S2b (Abs\textsubscript{280} and TIC traces, peak spectrum and peak table)

| m/z  | z  | Abund | % Abundance |
|------|----|-------|-------------|
| 121.05087 | 1  | 64081.9 | 20.48       |
| 430.0504   | 1  | 102158.7 | 32.65      |
| 431.05605  | 1  | 46627    | 14.9        |
| 432.06196  | 1  | 312894.4 | 100         |
| 433.06239  | 1  | 82687.1  | 26.43       |

| m/z  | z  | Abund | % Abundance |
|------|----|-------|-------------|
| 434.06378 | 1  | 242607.4 | 77.54      |
| 435.06674 | 1  | 45825.8  | 14.65      |
| 454.0478  | 1  | 115637.1 | 36.96      |
| 456.04601 | 1  | 115239   | 36.83      |
| 487.10394 | 1  | 51887    | 16.52      |
NMR of compound S2b (1H and 13C spectra)
**zz. Compound S2c:**

(S)-1-benzoyl-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)pyrrolidine-2-carboxamide

LC-MS of compound S2c (Abs254 and TIC traces, peak spectrum and peak table)
NMR of compound S2c (¹H spectrum)
(S)-N-(((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(2-phenylacetyl)pyrrolidine-2-carboxamide

LC-MS of compound S2d (Abs$_{280}$ and TIC traces, peak spectrum and peak table)
NMR of compound S2d (¹H spectrum)
Compound S2e:

(S)-N-((S)-3-bromo-4,5-dihydroisoxazol-5-yl)methyl)-1-(3-phenylpropanoyl)pyrrolidine-2-carboxamide

LC-MS of compound S2e (Abs$_{280}$ and TIC traces, peak spectrum and peak table)

| $m/z$ | z | Abund | % Abundance |
|-------|---|-------|-------------|
| 121.05087 | 1 | 80473.8 | 13.54 |
| 230.1177 | 1 | 66867.2 | 11.25 |
| 408.09159 | 1 | 594431.6 | 100 |
| 409.09485 | 1 | 107076.1 | 18.01 |

| $m/z$ | z | Abund | % Abundance |
|-------|---|-------|-------------|
| 410.0897 | 1 | 592215.3 | 99.63 |
| 411.0929 | 1 | 106286.1 | 17.88 |
| 430.07342 | 1 | 315909.8 | 53.14 |
| 432.07162 | 1 | 316465.6 | 53.24 |
NMR of compound S2e (1H spectrum)
(S)-2-(5-amino-2-(((benzyloxy)carbonyl)amino)-5-oxopentanamido)acetic acid

NMR of ZQS (1H spectrum)
5. References

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