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Limiting the Number of Potential Binding Modes by Introducing Symmetry into Ligands: Structure-Based Design of Inhibitors for Trypsin-Like Serine Proteases

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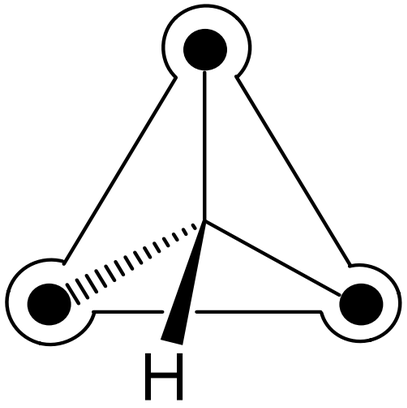
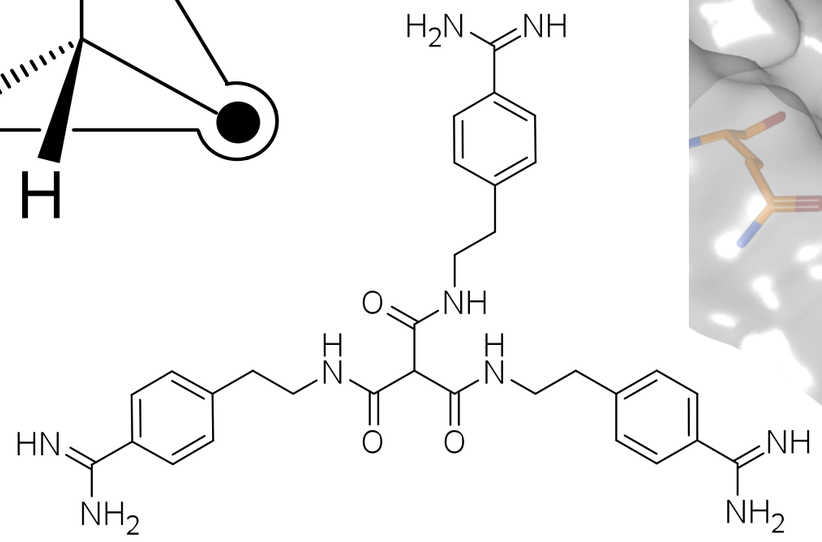
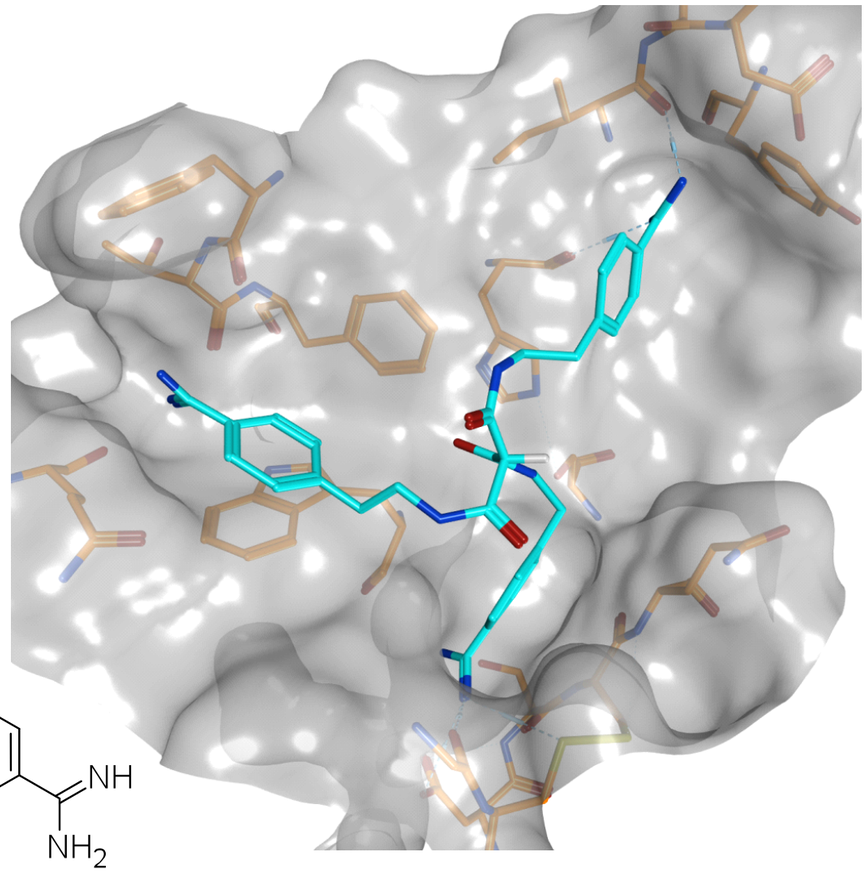
Limiting the Number of Potential Binding Modes by Introducing Symmetry into Ligands: Structure-Based Design of Inhibitors for Trypsin-Like Serine Proteases







Abstract: In the absence of X-ray data, the exploration of compound binding modes continues to be a challenging task. For structure-based design, specific features of active sites in different targets play a major role in rationalizing ligand binding characteristics. For example, dibasic compounds have been reported as potent inhibitors of various trypsin-like serine proteases, the active sites of which contain several binding pockets that can be targeted by cationic moieties. This results in several possible orientations within the active site, complicating the binding mode prediction of such compounds by docking tools. Therefore, we introduced symmetry in bi- and tribasic compounds to reduce conformational space in docking calculations and to simplify binding mode selection by limiting the number of possible pocket occupations. Asymmetric bisbenzamidines were used as starting points for a multistage and structure-guided optimization. A series of 24 final compounds with either two or three benzamidine substructures was ultimately synthesized and evaluated as inhibitors of five serine proteases, leading to potent symmetric inhibitors for the pharmaceutical drug targets matriptase, matriptase-2, thrombin and factor Xa. This study underlines the relevance of ligand symmetry for chemical biology.

Keywords: benzamidines; chirality; peptidomimetics; serine proteases; symmetry

Introduction

Serine proteases of interest:

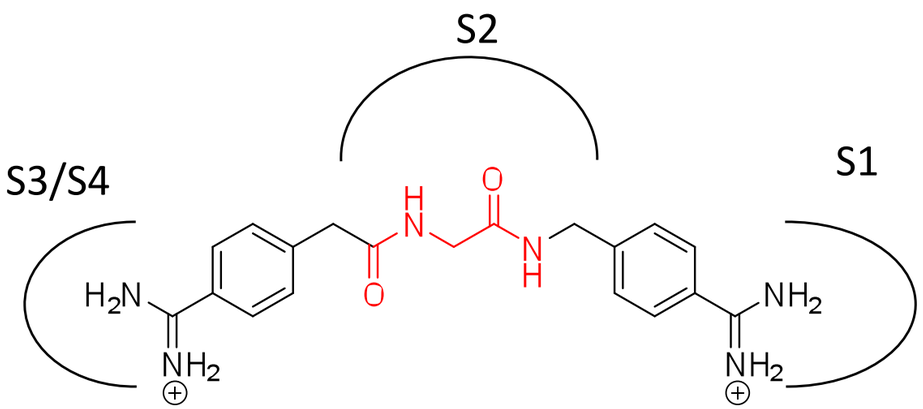
- Matriptase: implicated with tumorigenesis
- Matriptase 2: critical function in human iron homeostasis
- Thrombin and Factor Xa: coagulation proteases and drug targets for the treatment of thromboembolic complications

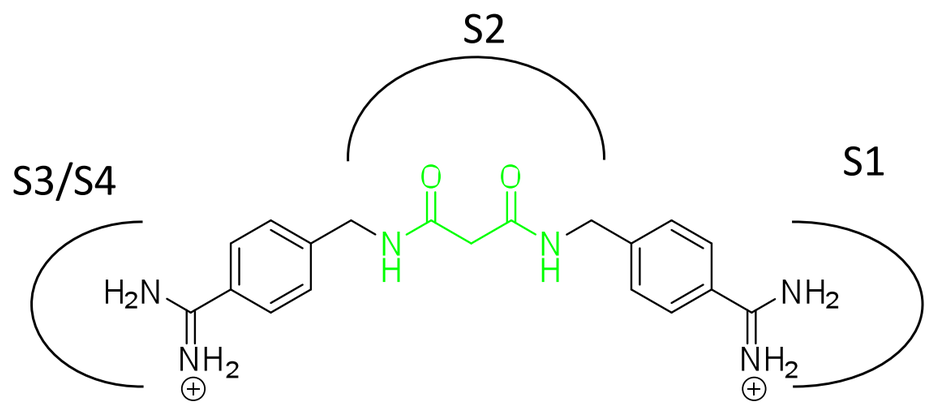
Common target features:

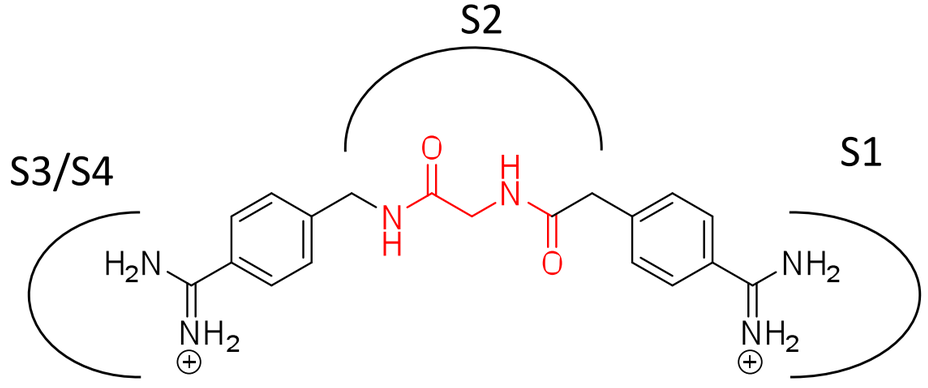
- Can be inhibited by arginine mimetics like benzamidines
- In particular dibasic compounds (i.e. bisbenzamidines) were reported as potent inhibitors

Compound design

- Asymmetric vs. symmetric inhibitors





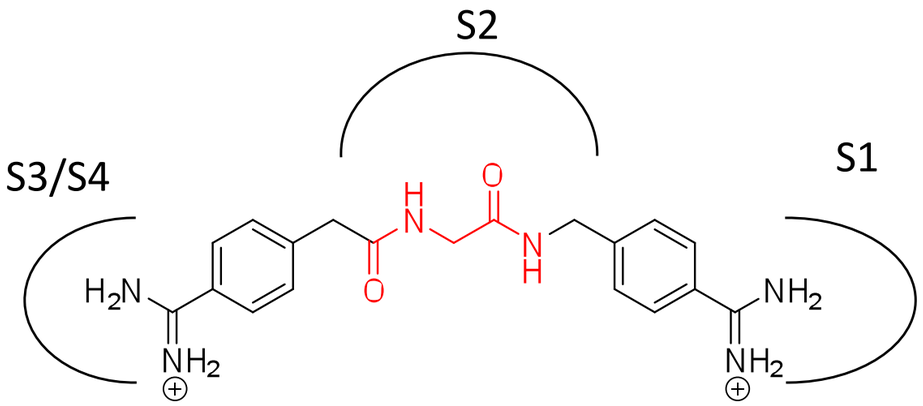


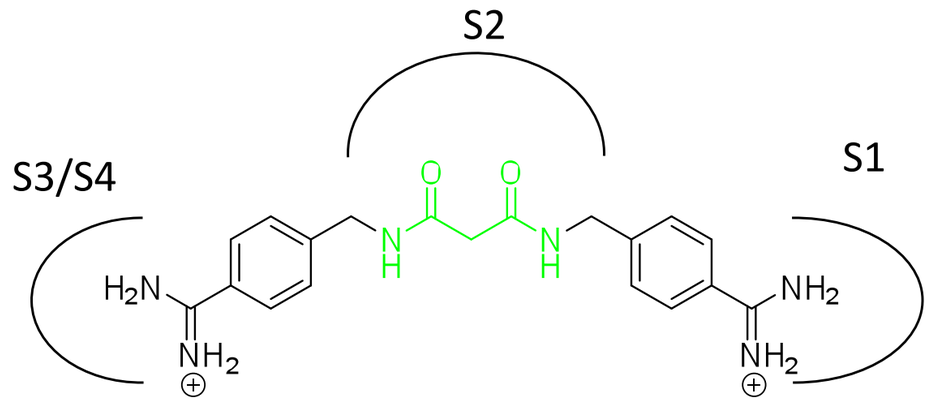
Symmetric inhibitors reduce the number of possible pocket occupations of benzamidine residues within the active site of selected serine proteases

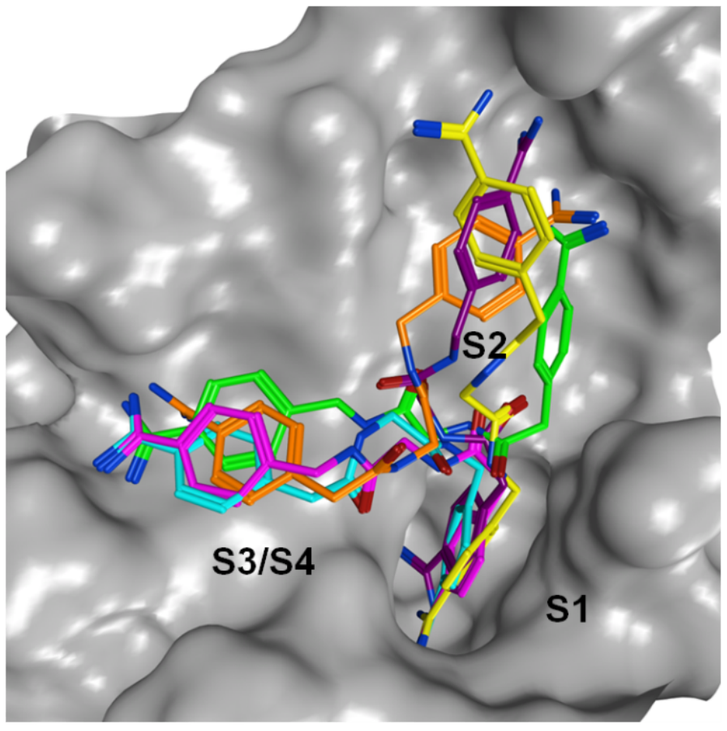
The introduction of symmetry facilitates structure-based compound design

Compound design

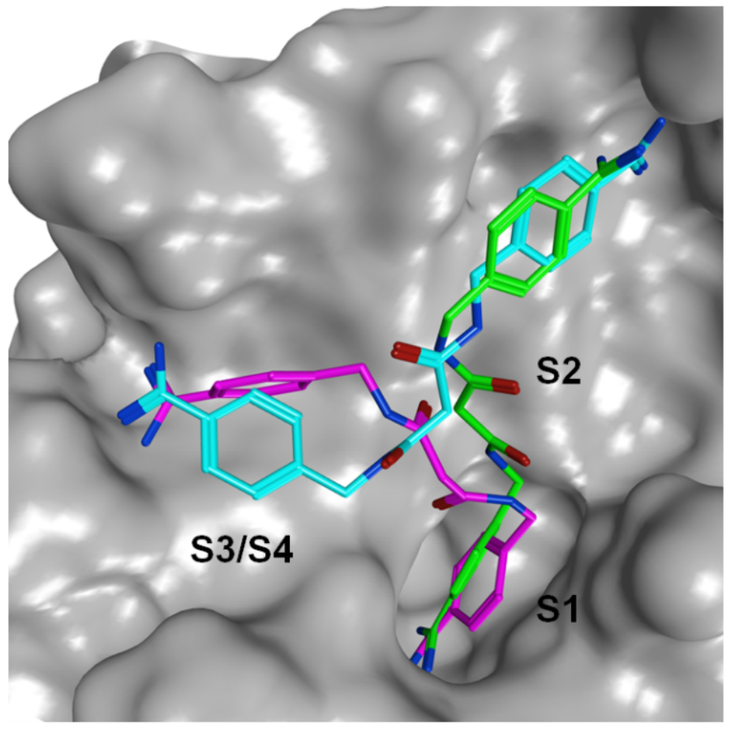
- Asymmetric vs. symmetric inhibitors



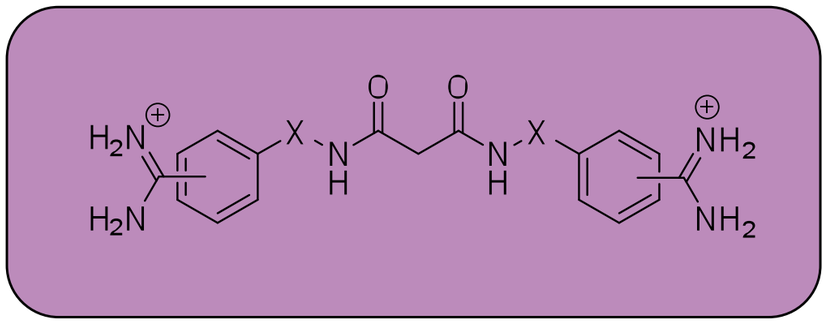




Even more possible pocket occupations of benzamidine residues (S1, S2, and S3/4) were observed in docking studies on matriptase and matriptase-2

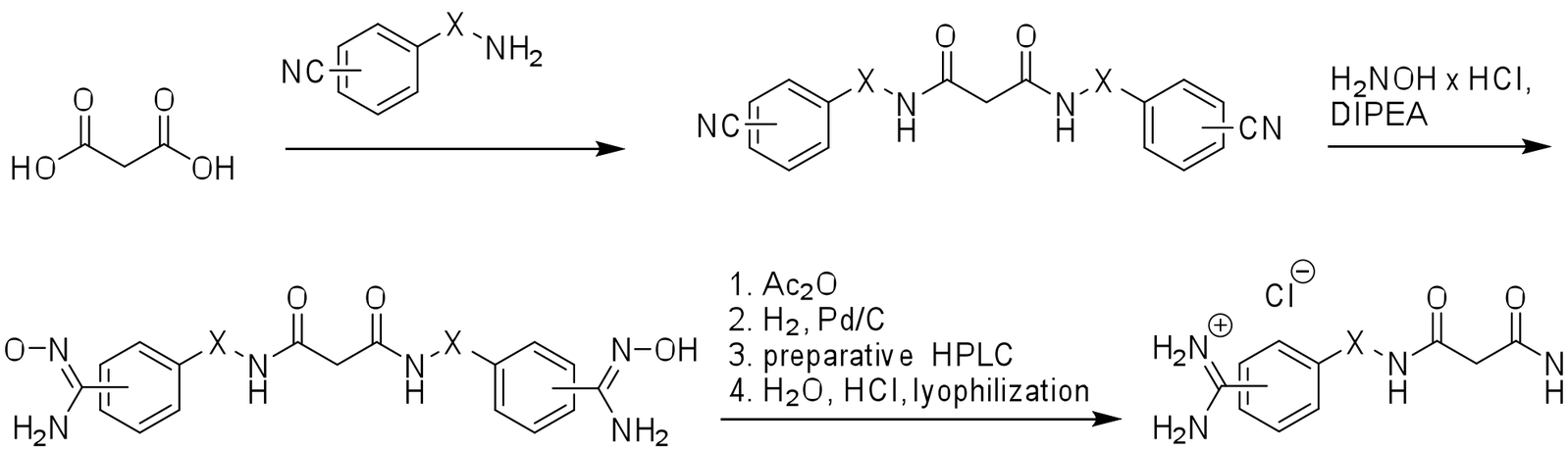


Synthesis



Variations:

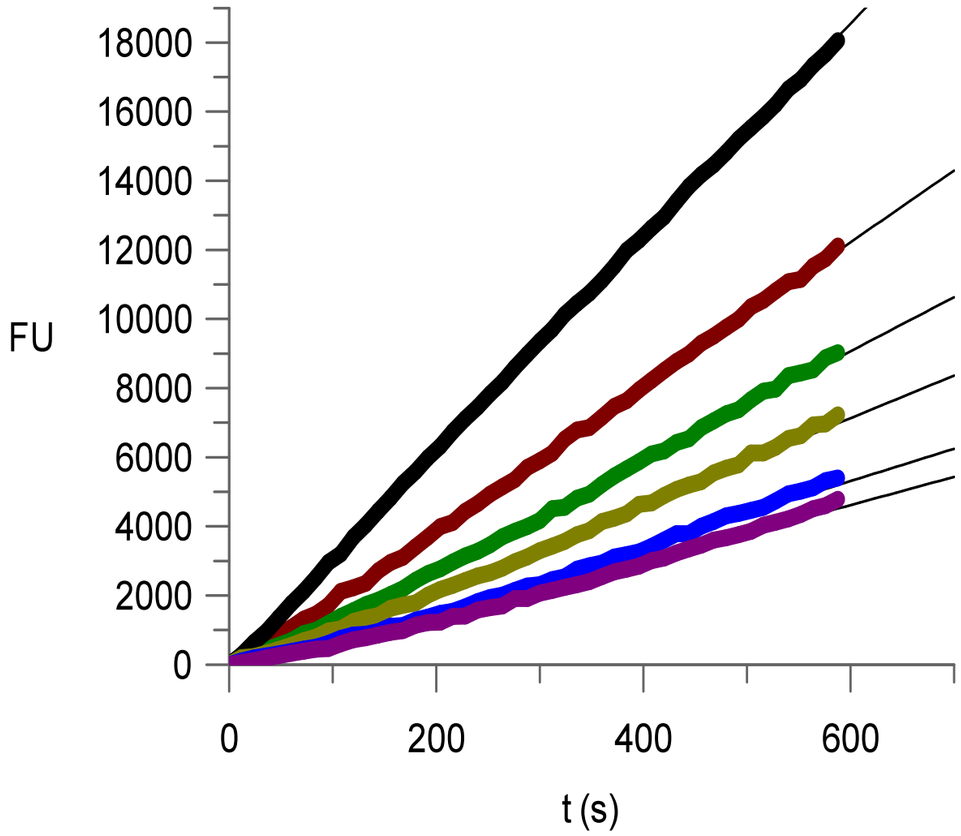
- Linker length (X=1-3 atoms)
- Linker type
- Aromatic substitution (*para* or *meta*)



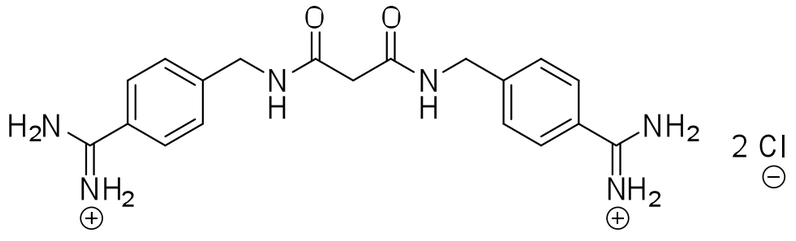
Characterization of all compounds:

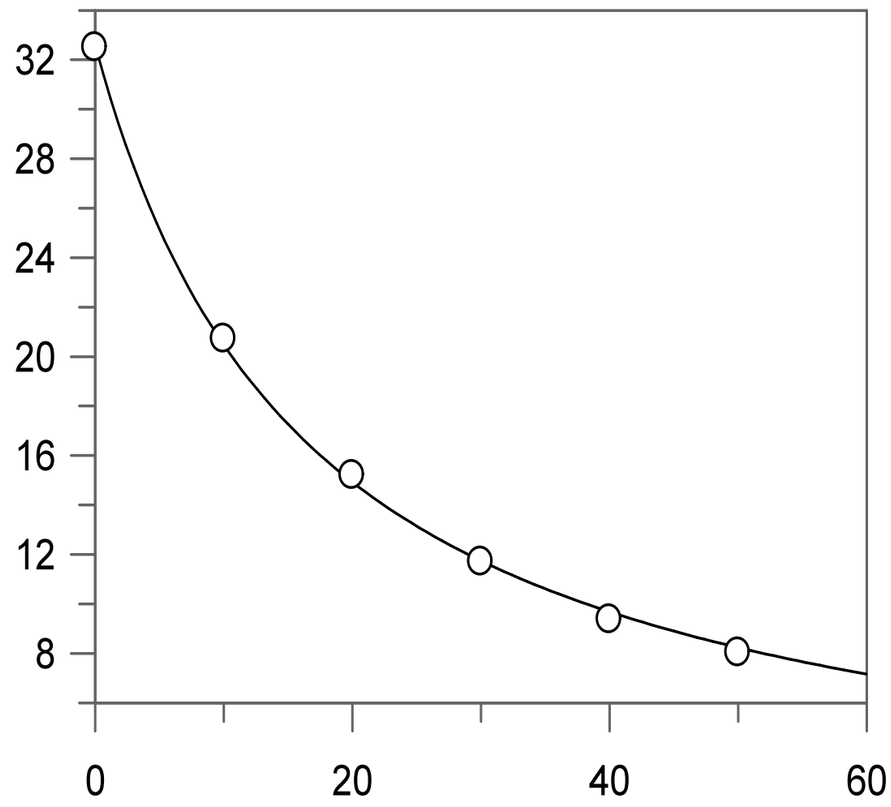
- ^{13}C and ^{1}H NMR; HRMS; LC/MS

Biochemical evaluation



Chemical Formula: $C_{19}H_{24}Cl_2N_6O_2$
Molecular Weight: 439,34

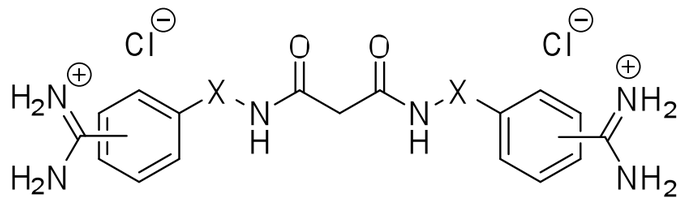




Parameter	Value	Std. Error
v0	32,5988	0,2607
IC50	16,8993	0,3723

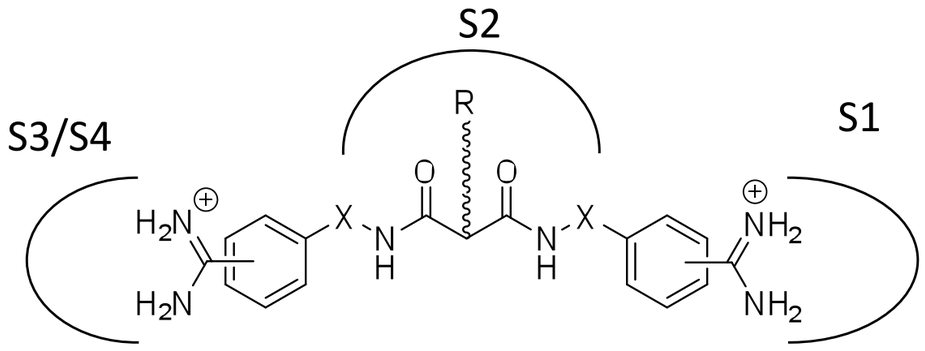
Matriptase-2: K_i = 8,01 μM

Inhibitory activities



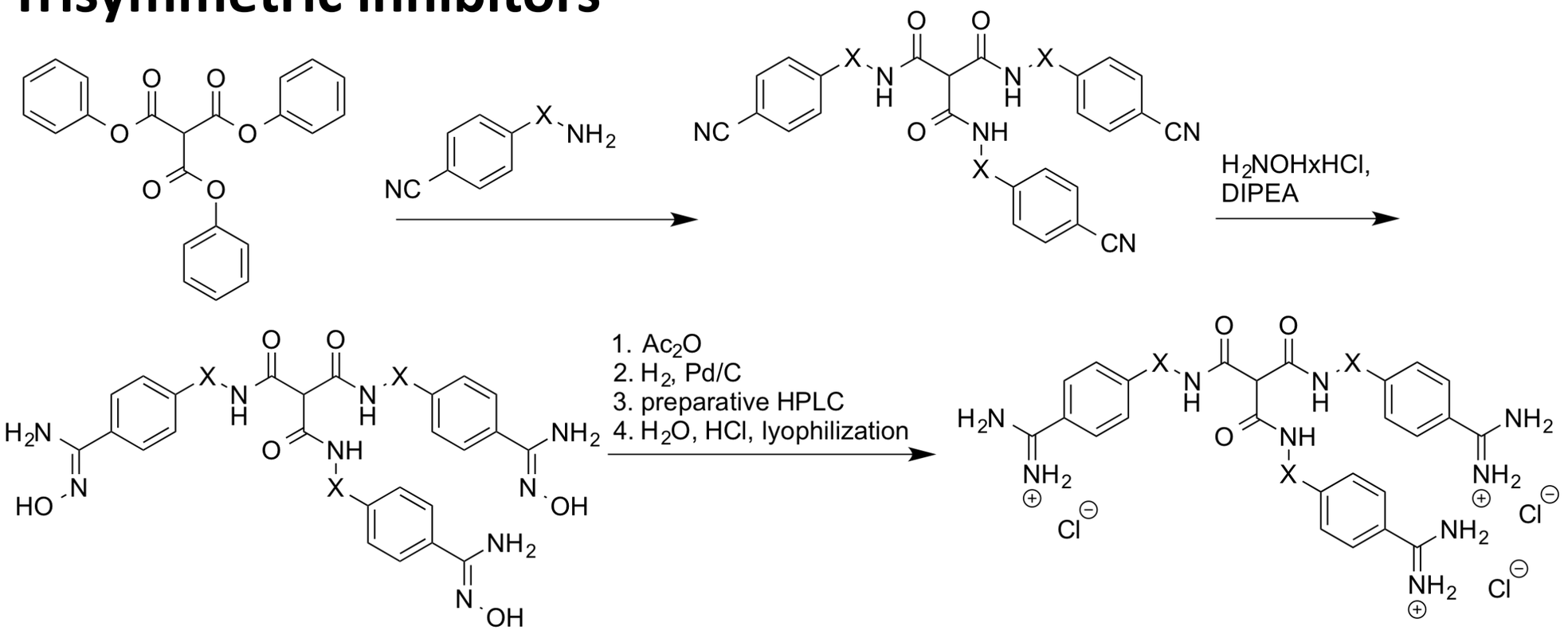
Arom. subst.	Linker (X)	Human Matriptase K_i (μM)	Human Matriptase-2 K_i (μM)	Human Thrombin K_i (μM)	Bovine Factor Xa K_i (μM)
para	CH_2	6.62 ± 0.30	8.01 ± 0.26	14.6 ± 0.3	8.74 ± 0.42
meta	CH_2	6.74 ± 0.25	45.3 ± 1.4	41.3 ± 1.0	5.26 ± 0.39
para	CH_2CH_2	4.23 ± 0.15	8.24 ± 0.25	35.2 ± 0.7	15.3 ± 0.5
meta	CH_2CH_2	10.0 ± 0.6	39.4 ± 1.2	9.04 ± 0.64	5.98 ± 0.35
para	OCH_2CH_2	24.6 ± 1.2	9.09 ± 0.67	17.1 ± 1.3	21.3 ± 1.7
meta	OCH_2CH_2	3.39 ± 0.19	12.3 ± 1.0	2.50 ± 0.11	0.751 ± 0.029

Compound optimization



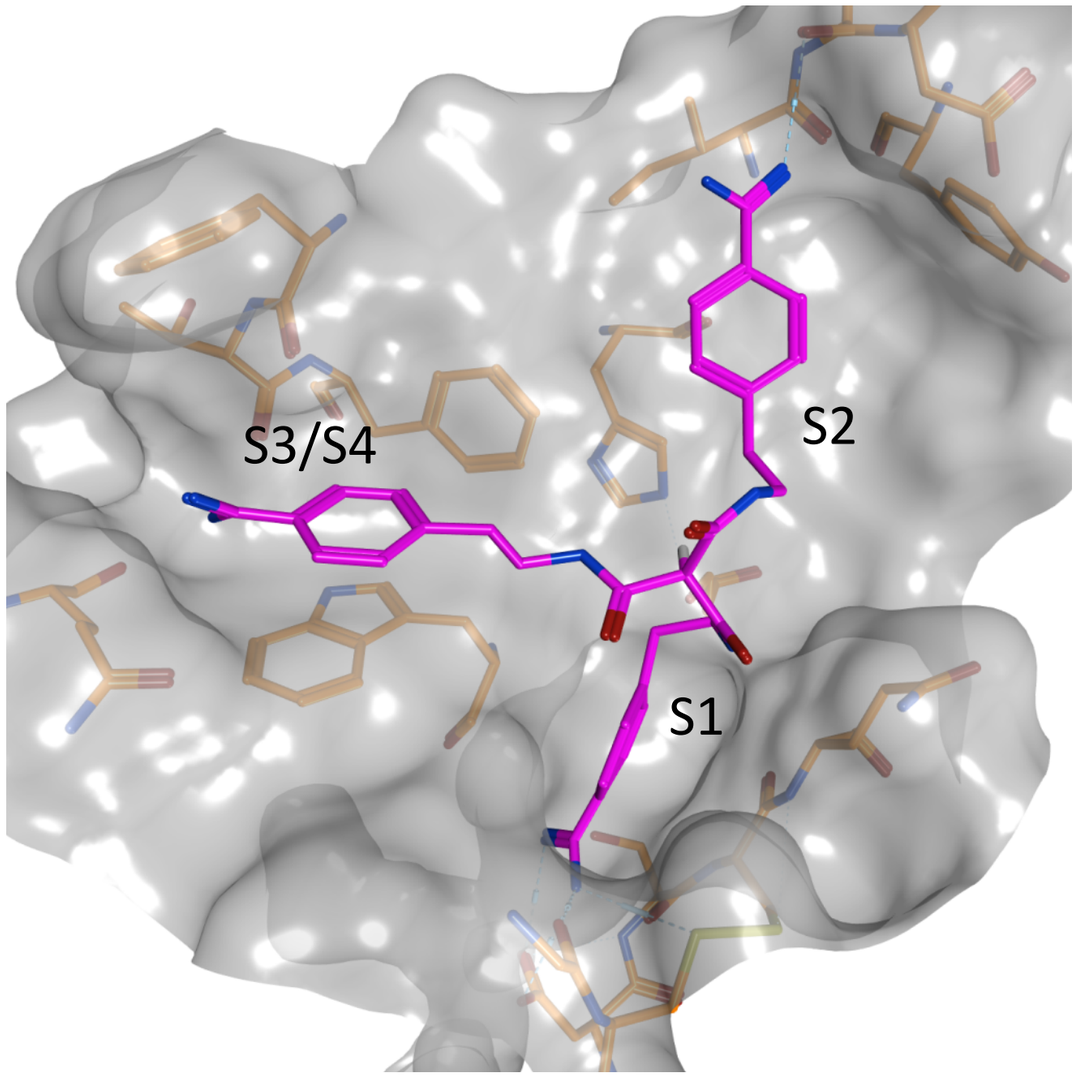
- Virtual evaluation of different branching residues (R) by molecular docking
- Synthesis of candidate compounds and biochemical evaluation
- Preferred branching residues in virtual screening:
  - Basic branching residues for Matriptase and Matriptase 2
  - Lipophilic moieties for Thrombin and Factor Xa

Trisymmetric inhibitors

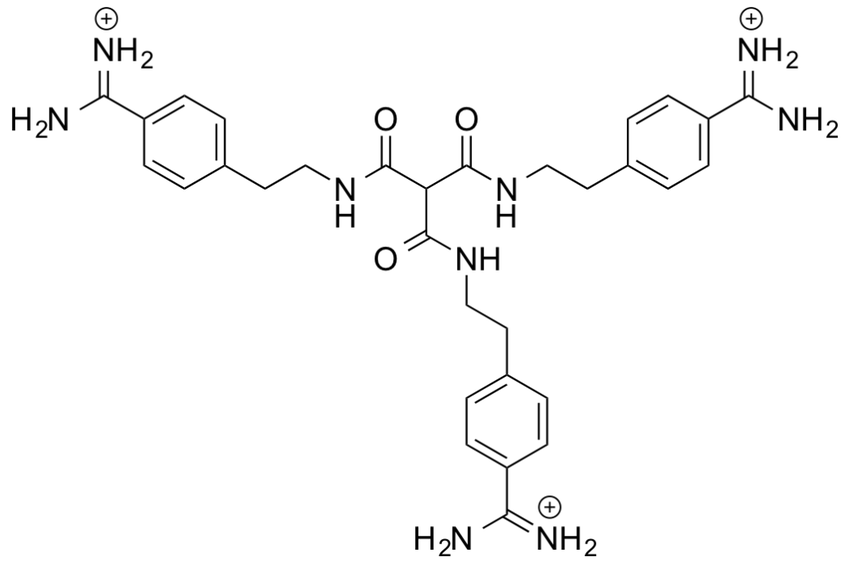


Linker (X)	Human Matriptase K_i (μM)	Human Matriptase-2 K_i (μM)	Human Thrombin K_i (μM)	Bovine Factor Xa K_i (μM)
CH_2	3.11 ± 0.16	3.86 ± 0.27	18.8 ± 0.8	5.24 ± 0.34
CH_2CH_2	0.393 ± 0.004	1.46 ± 0.10	10.3 ± 0.2	14.7 ± 0.5
OCH_2CH_2	4.01 ± 0.11	4.59 ± 0.28	18.9 ± 1.7	15.7 ± 0.8

Trisymmetric inhibitors

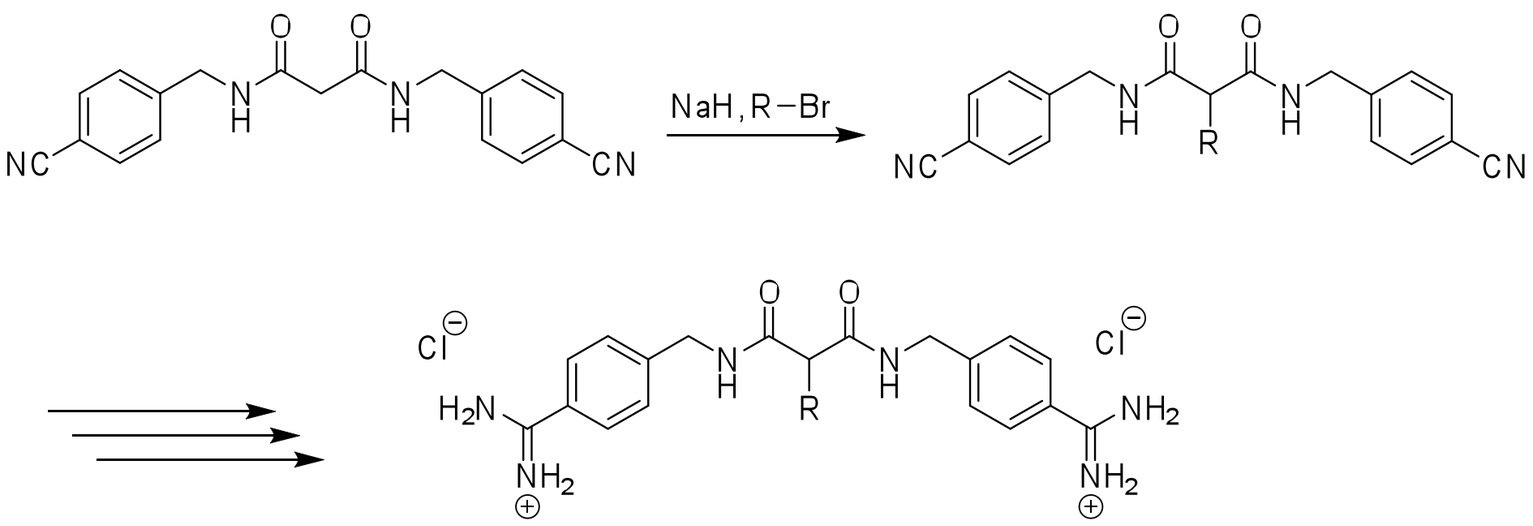


Putative binding mode of a tribasic and trisymmetric inhibitor in the active site of Matriptase

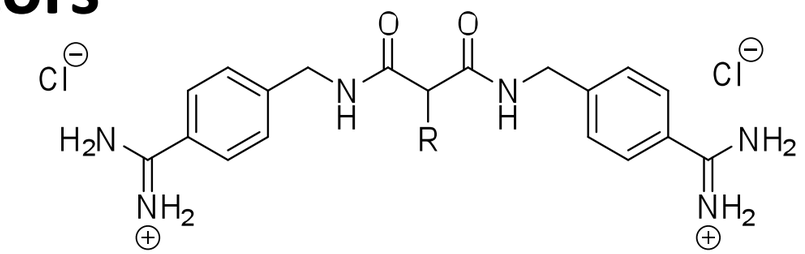


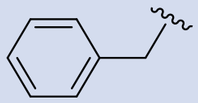
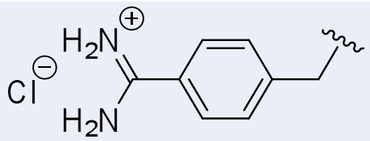
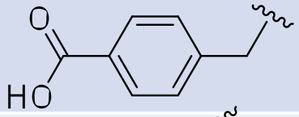
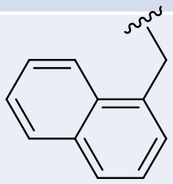
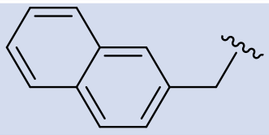
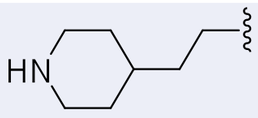
Matriptase: K_i = 0.393 µM

Branched inhibitors



Branched inhibitors

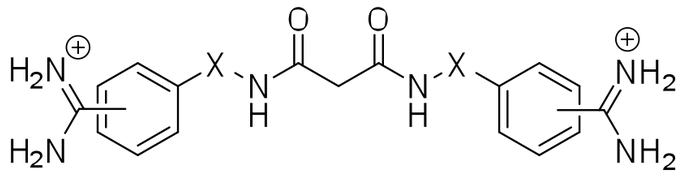


Branching residue	Human Matriptase K_i (μM)	Human Matriptase-2 K_i (μM)	Human Thrombin K_i (μM)	Bovine Factor Xa K_i (μM)
	12.3 ± 0.2	15.1 ± 0.3	21.6 ± 1.4	10.1 ± 0.6
	6.79 ± 0.20	4.98 ± 0.37	38.3 ± 1.6	33.4 ± 2.2
	287 ± 17	134 ± 3	210 ± 16	81.7 ± 5.9
	9.92 ± 0.27	5.73 ± 0.20	9.78 ± 0.58	1.46 ± 0.13
	17.0 ± 1.4	15.6 ± 0.9	14.0 ± 0.7	13.8 ± 0.4
	1.32 ± 0.05	3.71 ± 0.40	57.8 ± 2.7	33.7 ± 3.1

Compound optimization pathway

Matriptase:

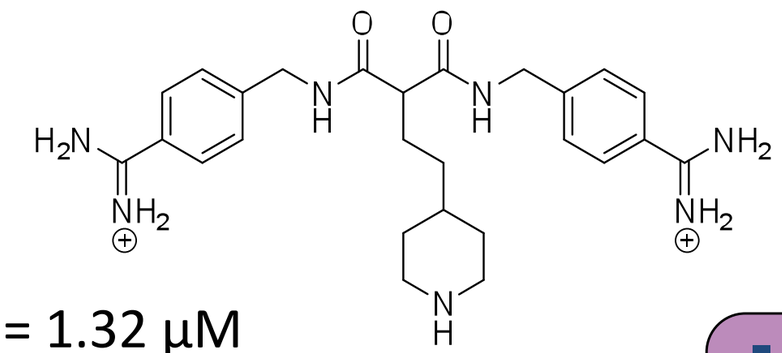
K_i = 6.62 µM



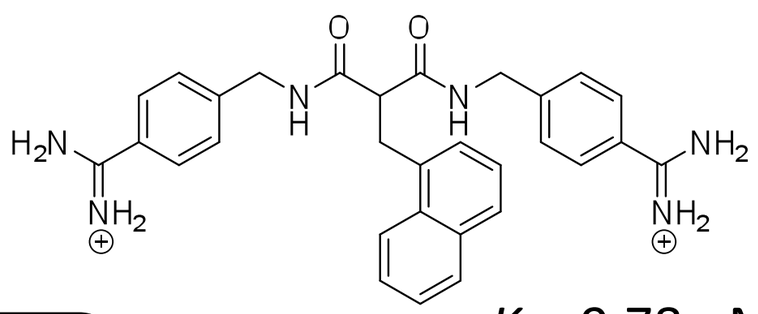
X = CH_2 and *para* substitution

Thrombin:

K_i = 14.6 µM

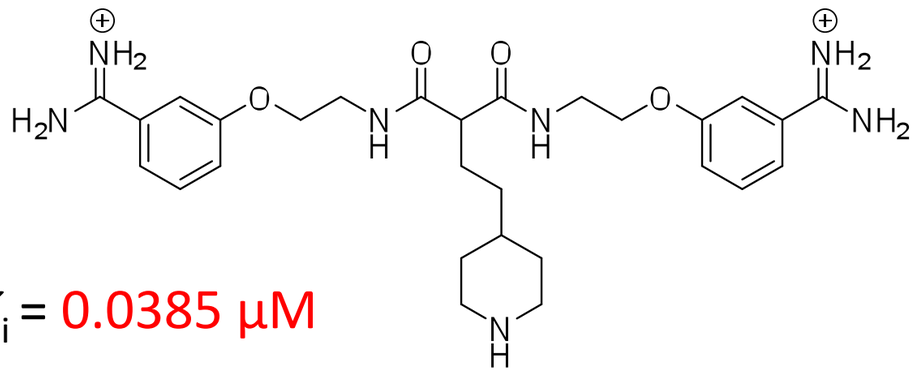


K_i = 1.32 µM

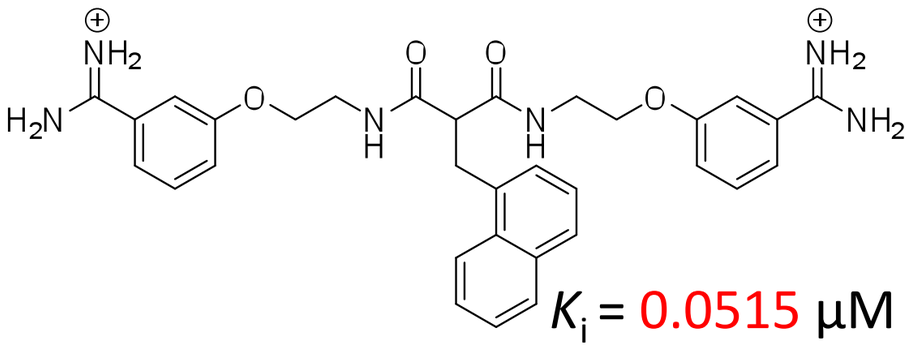


K_i = 9.78 µM

- Best aromatic substitution
- Best linker type
- Best branching residues

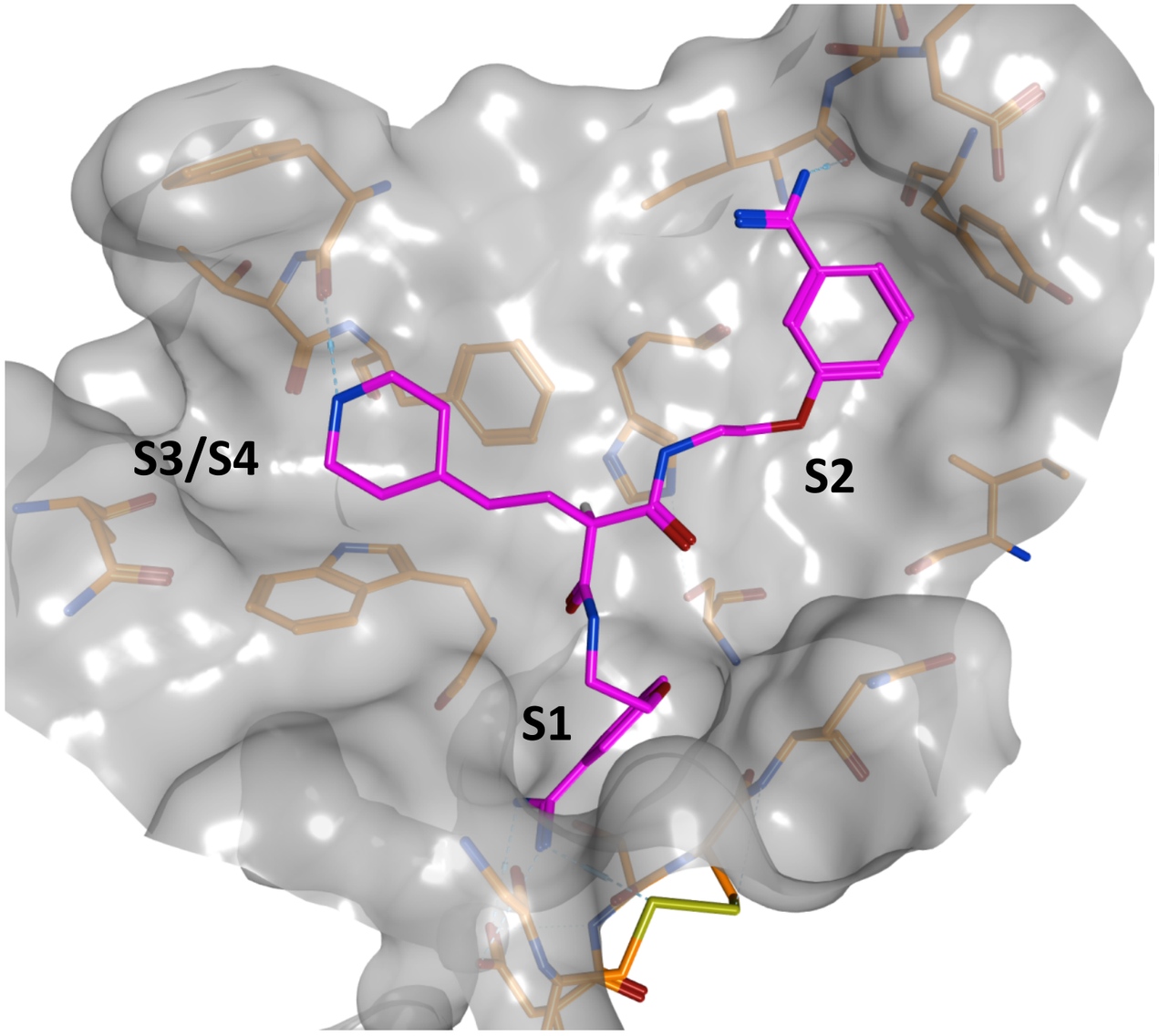


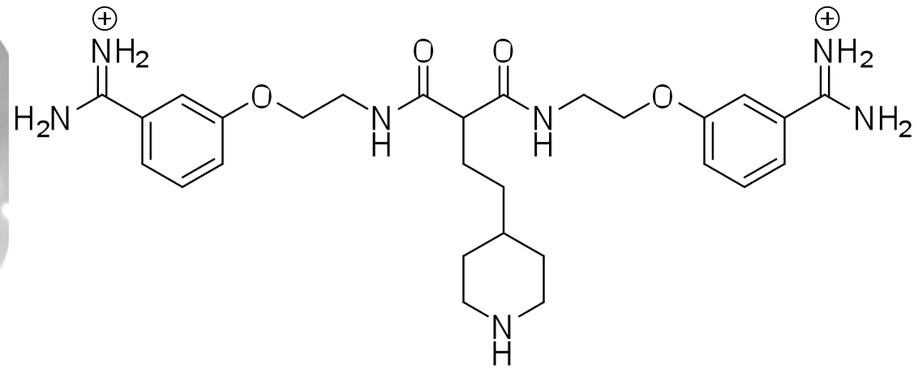
K_i = 0.0385 µM



K_i = 0.0515 µM

Matriptase



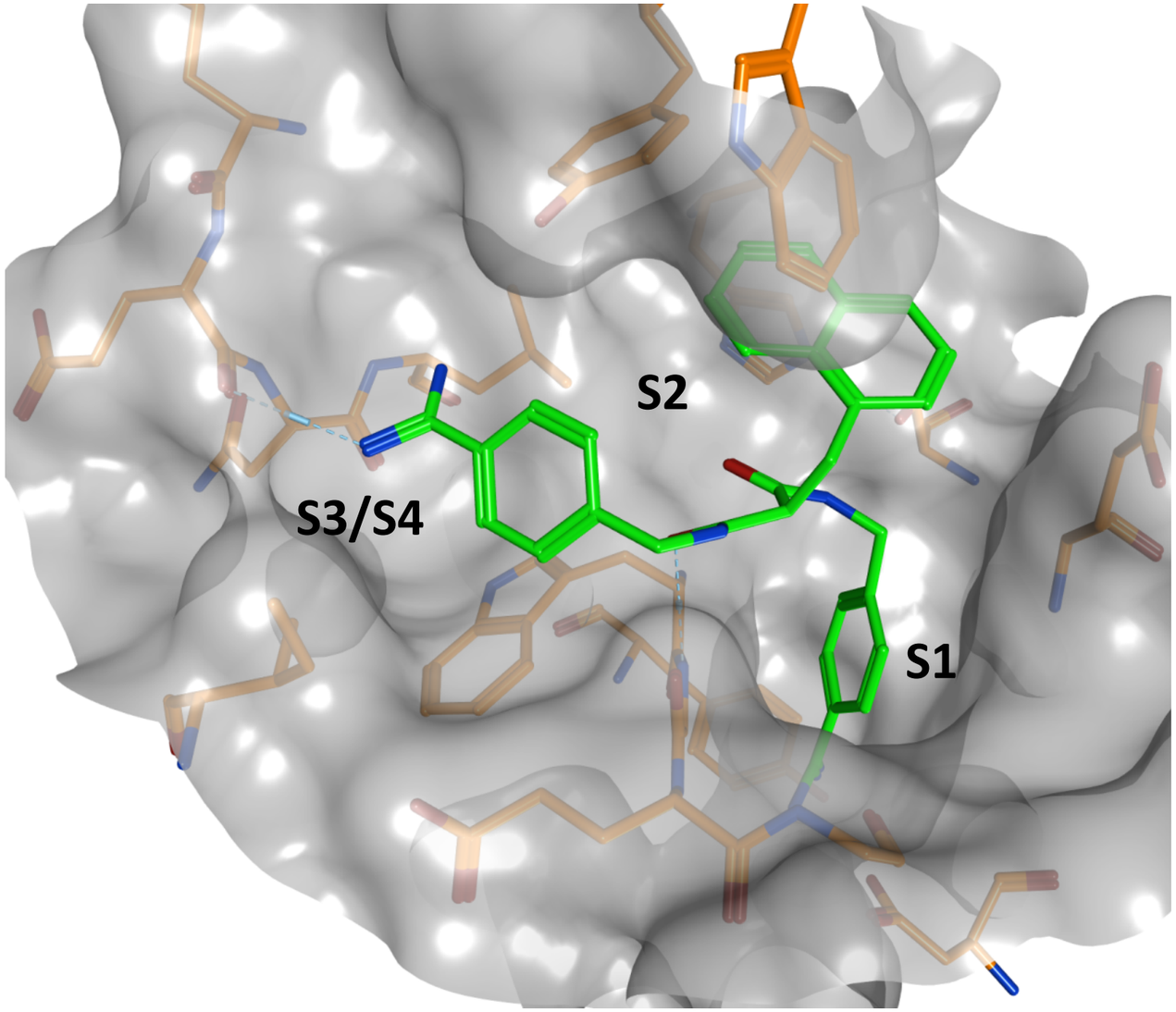


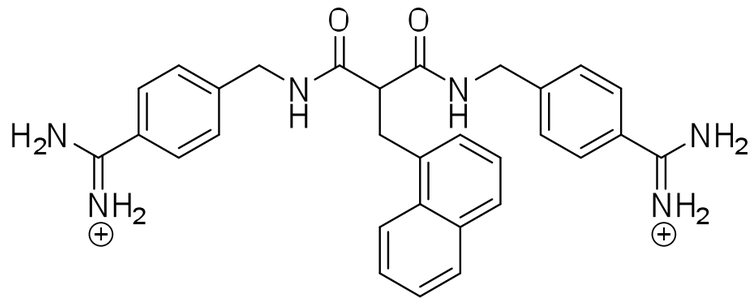
K_i = 0.0385 µM

12-100 *fold selectivity over matriptase 2, thrombin and factor Xa*

Putative binding mode of optimized and branched inhibitor in the active site of Matriptase

Thrombin

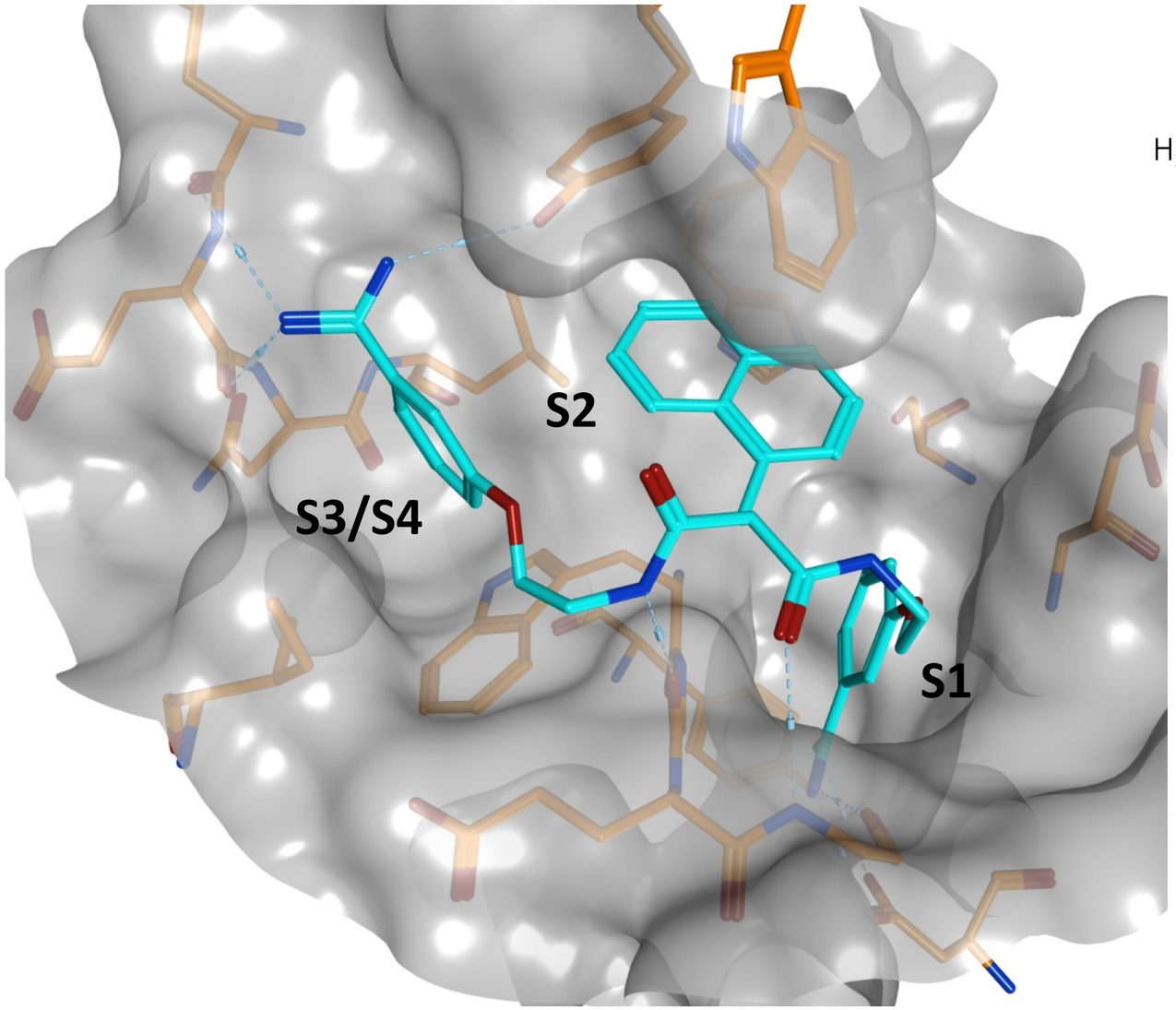


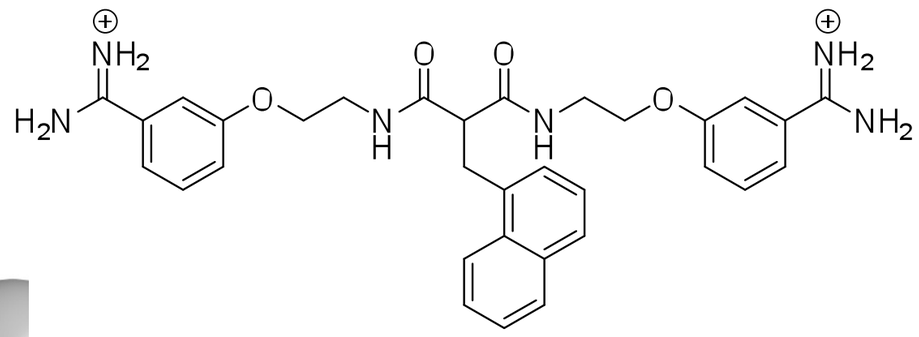


K_i = 9.78 µM

Putative binding mode of non-optimized and branched inhibitor in the active site of Thrombin

Thrombin





K_i = 0.0515 µM

0.440 µM inhibitor for factor Xa

15 - 65 *fold selectivity over matriptase and matriptase-2*

Putative binding mode of non-optimized and branched inhibitor in the active site of Thrombin with improved binding properties

Summary

- Application of structure-based design techniques led to the design, synthesis and biochemical evaluation of novel inhibitors for selected serine proteases

- Micromolar inhibitors were subsequently optimized to nanomolar compounds with selectivity over related targets

- Incorporation of symmetry facilitated compound design and synthesis efforts

- **Furtmann, N.**; Häußler, D.; Scheidt, T.; Stirnberg, M.; Steinmetzer, T.; Bajorath, J.; Gütschow, M. Limiting the number of potential binding modes by introducing symmetry into ligands: structure-based design of inhibitors for trypsin-like serine proteases. *Chem. Eur. J.*, accepted.

Acknowledgements

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