Thrombin Inhibitor Design

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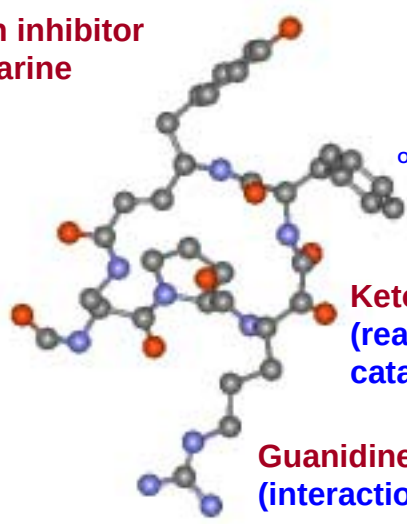
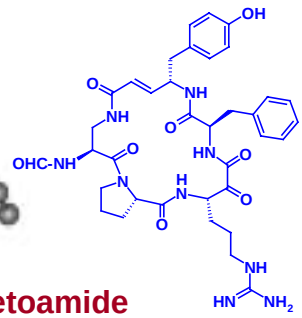
Cyclotheonamide - The Merck Design Story

Thrombin inhibitor from a marine sponge



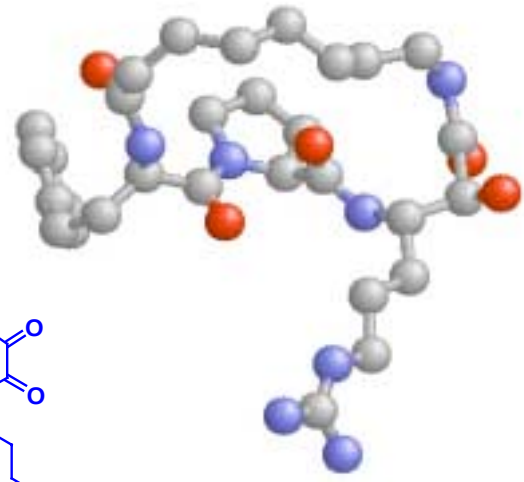


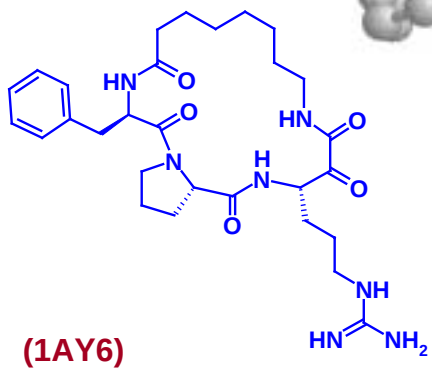
Ketoamide (reacts with the catalytic serine)

Guanidine (interaction with Asp189)

(1TMB)

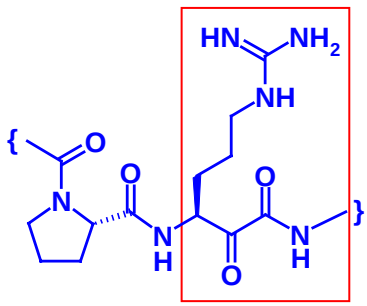
D-Phe-Pro-Arg-CO- in a macrocyclic ring, as model for cyclotheonamide



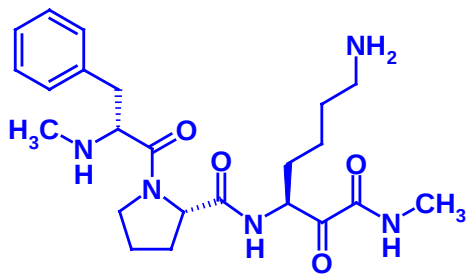


(1AY6)

Merck Thrombin Inhibitors: First lead derived from a natural product



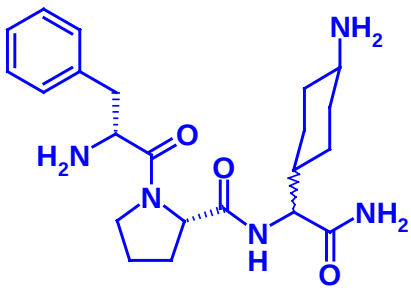
Cyclotheonamide (partial structure)



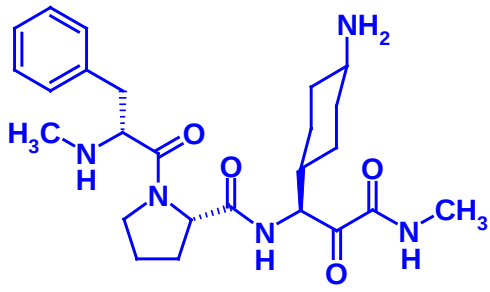
K_i (thrombin) = 2.8 nM
K_i (trypsin) = 7.8 nM

Merck Thrombin Inhibitors: Model Compounds for Optimization of the P1 residue

D,L-trans



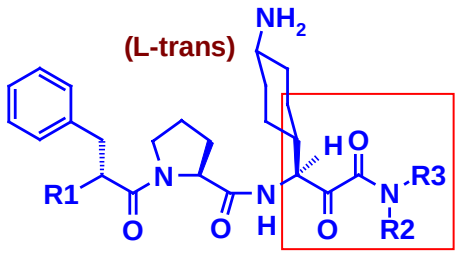
K_i (thrombin) = 5 300 nM
K_i (trypsin) = 855 000 nM



L 370 518
K_i (thrombin) = 0.09 nM
K_i (trypsin) = 1 150 nM

Merck Thrombin Inhibitors: Elimination of the keto-amide group

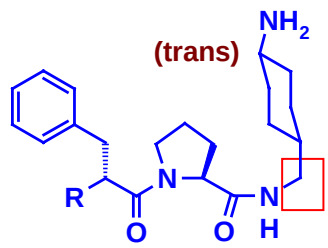
(L-trans)



L 372 051
R1 = H, R2 = H, R3 = Me
K_i (thrombin) = 4 nM

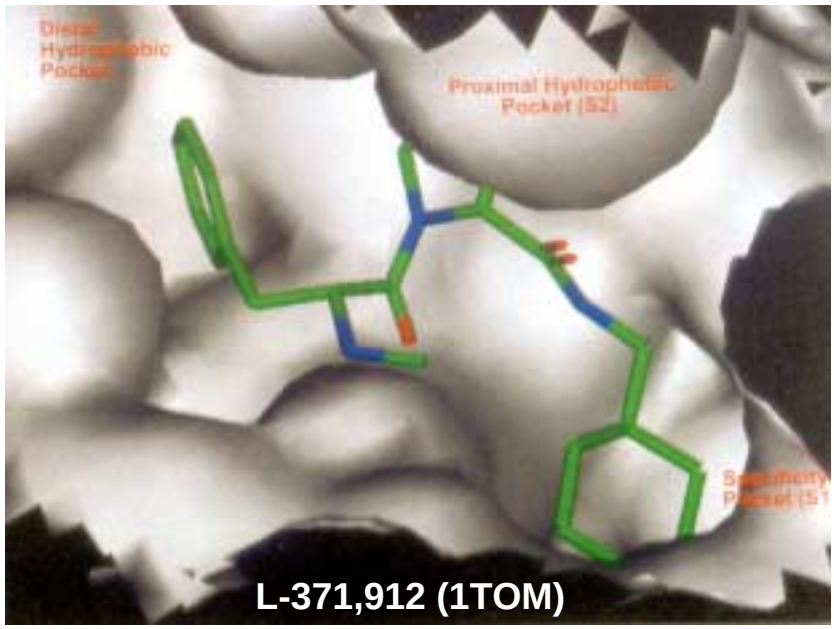
L 372 228
R1= NHMe, R2, R3 = -$(CH_2)_3$-
K_i (thrombin) = 0.04 nM

(trans)

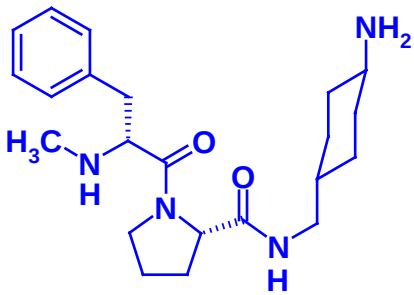


L 371 912
R = NHMe
K_i (thrombin) = 5 nM

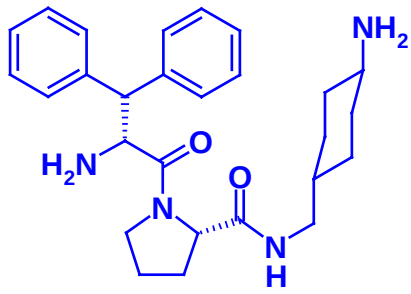
L 372 011
R = H
K_i (thrombin) = 330 nM



Merck Thrombin Inhibitors: Optimization of the P3 residue

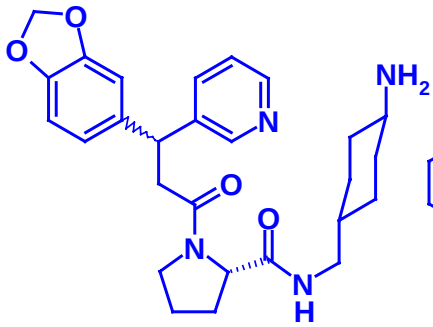


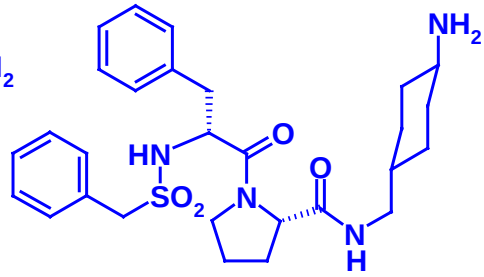
L 371 912
K_i (thrombin) = 5 nM
K_i (trypsin) = 11 000 nM



L 372 102
K_i (thrombin) = 0.1 nM
K_i (trypsin) = 94 nM

Merck Thrombin Inhibitors: Further optimization of the P3 residue





K_i = 4.7 nM (diastereomer 1)
0.28 nM (diastereomer 2)

K_i = 0.4 nM

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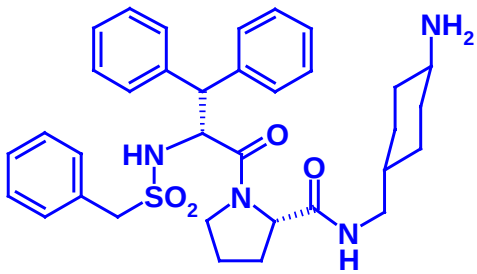
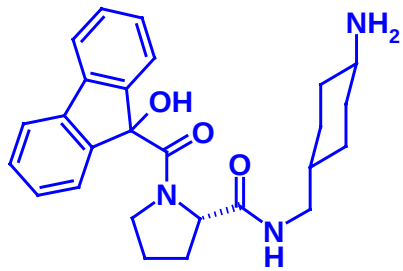
Merck Thrombin Inhibitors: Further optimization of the P3 residue



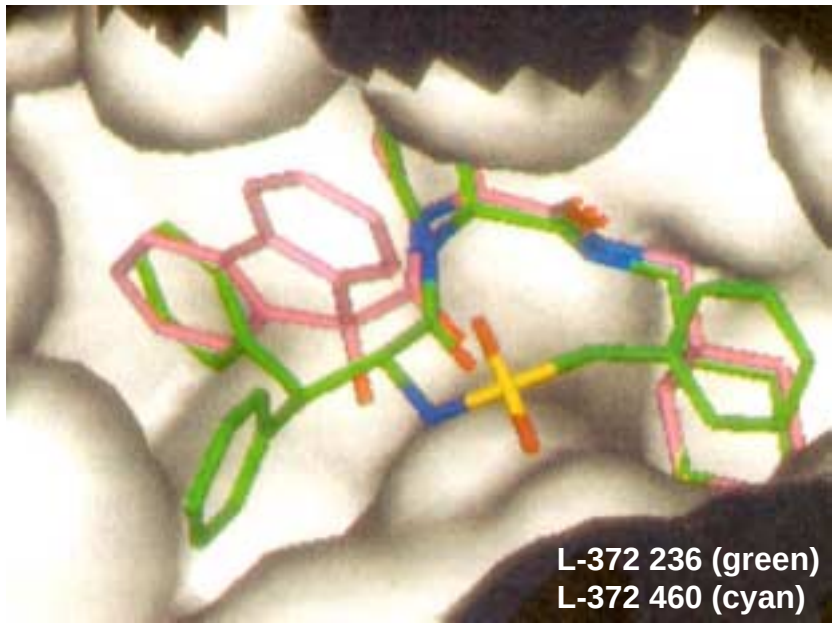
L 372 236
K_i (thrombin) = 0.0025 nM
K_i (trypsin) = 4 nM



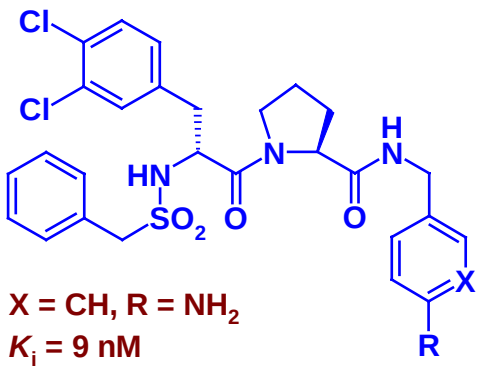
L 372 460
K_i (thrombin) = 1.5 nM

„Use“ of an additional pocket

Combinatorial library



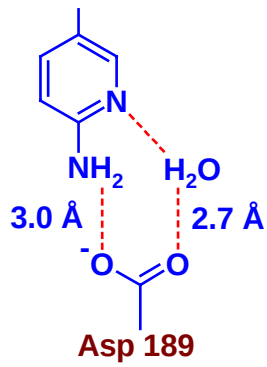
Merck Thrombin Inhibitors: Weakly basic P1 residues



X = CH, R = NH_2
K_i = 9 nM

L 372 585
X = N, R = NH_2
K_i = 3 nM

binding mode of L 372 585:

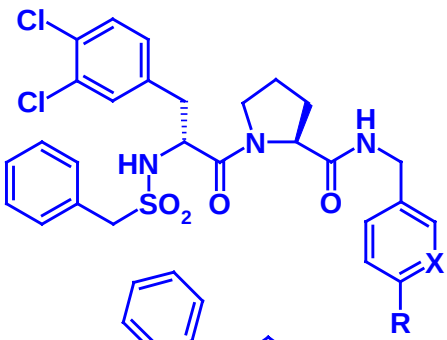


Asp 189

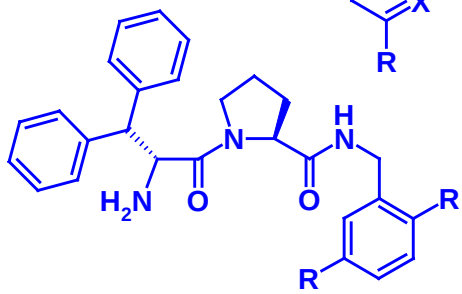
Superposition of L-371,912 (1TOM, yellow) with the dichloro-Phe analogue L-372,585 (color-coded)

D.-M. Feng et al., J. Med. Chem. 40, 3726-3738 (1997)

Merck Thrombin Inhibitors: Nonbasic P1 residues



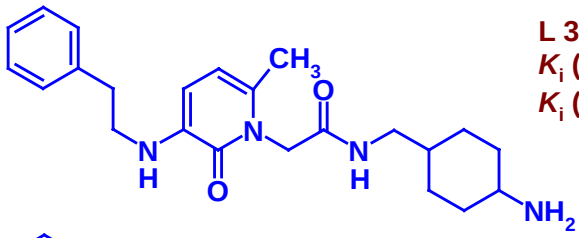
X = CH, R = H
K_i = 110 nM



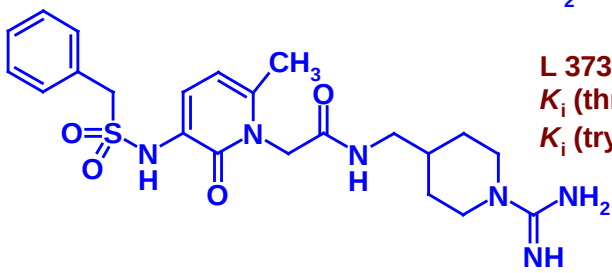
L 373 588
R = CH_3
K_i = 38 nM

R = Cl
K_i = 3 nM

Merck Thrombin Inhibitors: Rigidization to Achiral Molecules

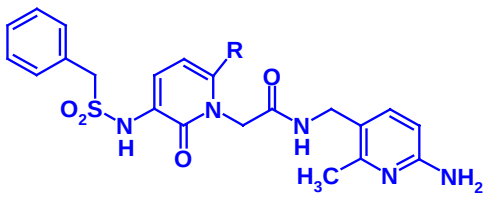


L 372 774
K_i (thrombin) = 47 nM
K_i (trypsin) = 2 200 nM



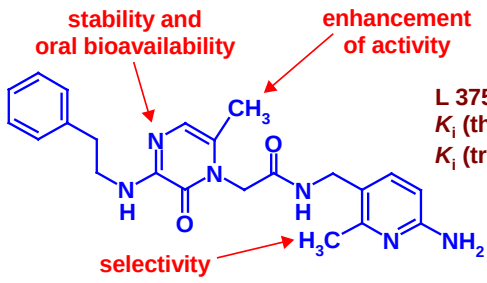
L 373 890
K_i (thrombin) = 0.5 nM
K_i (trypsin) = 570 nM

Merck Thrombin Inhibitors: Further Optimization of the Molecule



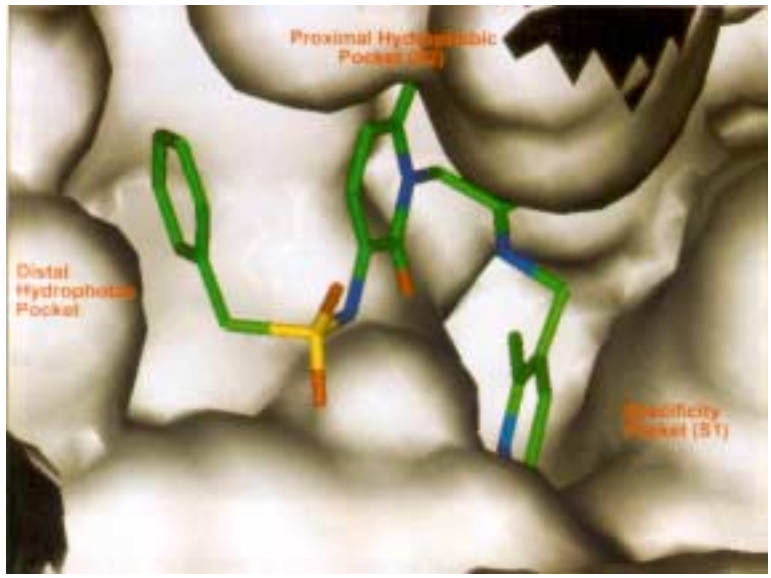
L 374 087, R = Me
K_i (thrombin) = 0.5 nM
K_i (trypsin) = 3 200 nM

L 375 052, R = Propyl
K_i (thrombin) = 0.85 nM
K_i (trypsin) = 1 400 nM

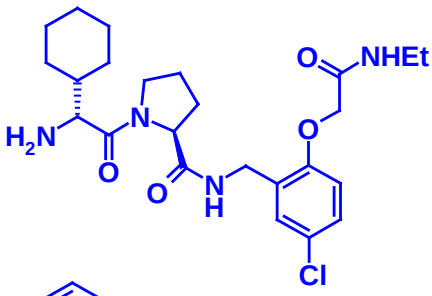


L 375 378
K_i (thrombin) = 0.8 nM
K_i (trypsin) = 1 800 nM

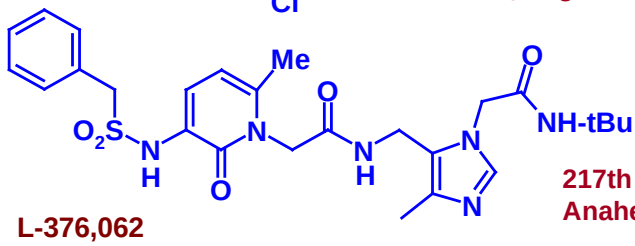
Binding Mode of L-374,087



Merck Clinical Candidate/s ?



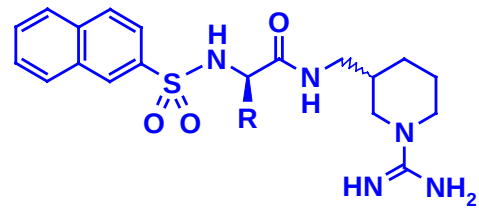
The phenoxyacetic acid amide (log P = 2.04) had the best overall profile of all development candidates, *in vitro* (K_i thrombin = 0.74 nM; K_i trypsin = 23 mM) and *in vivo* (rat thrombosis model; 10%, 40% and 63% bioavailability in rats, dogs and monkeys).



L-376,062

217th ACS Meeting
Anaheim, CA, 1999

Hoffmann-la Roche Thrombin Inhibitors

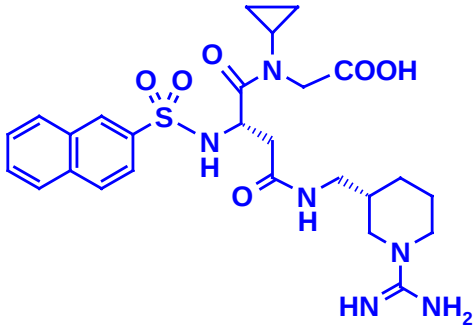


R = H

K_i (thrombin) = 480 nM
K_i (trypsin) = 75 000 nM

R = benzyl

K_i (thrombin) = 47 nM
K_i (trypsin) = 42 000 nM



Napsagatran (i.v., short biological half-life time)

K_i (thrombin) = 0.27 nM
K_i (trypsin) = 1 900 nM

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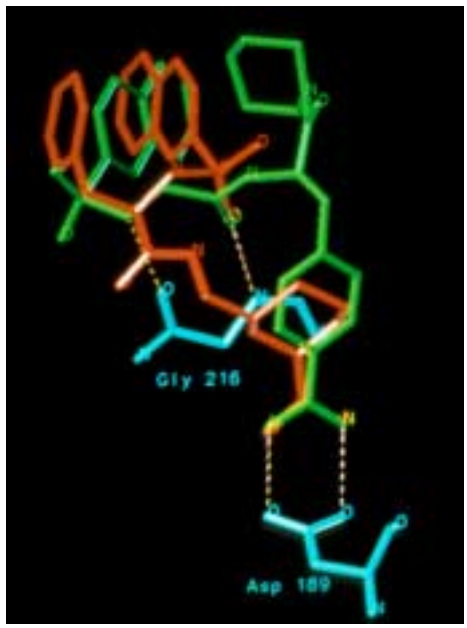
Binding Mode of the Hoffmann-La Roche Thrombin Inhibitor

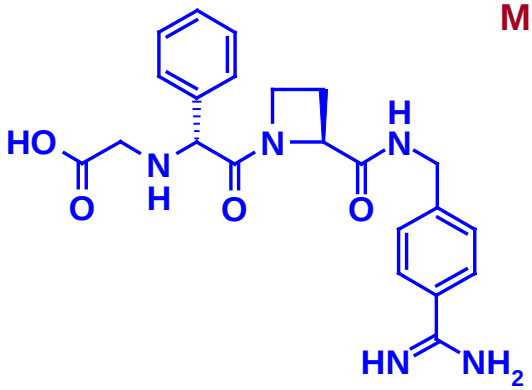
no significant variation of biological activity after chemical variation of the phenylalanine

NAPAP (green)

Roche (red)



Melagatran (Astra)



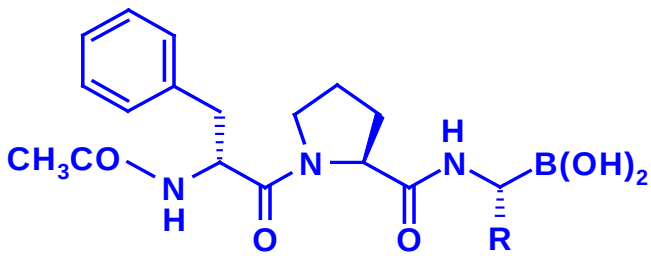
was one of the first thrombin inhibitors with some oral bioavailability

K_i (thrombin) = 2 nM

Ximelagatran (H 376/95) is a double prodrug of melagatran:

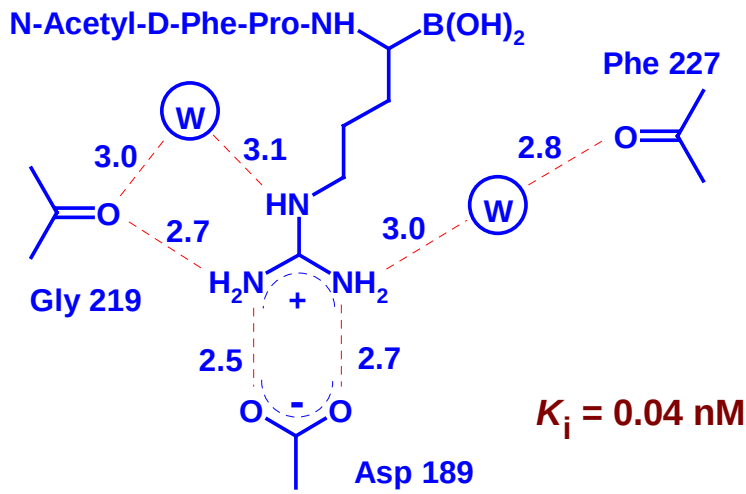
- ester group (cleaved by esterases)
- amidoxime (reduced by NADH-cytochrome b5 reductase + CYP 2A6)

Boronic Acid Thrombin Inhibitors (DuPont)



R = -$(CH_2)_3$-NH-C(=NH)NH_2 (DuP 714)	K_i = 0.04 nM
R = -$(CH_2)_4$-NH_2	K_i = 0.24 nM
R = -$(CH_2)_4$-C(=NH)NH_2	K_i = 0.29 nM
R = -$(CH_2)_5$-NH_2	K_i = 8.1 nM
R = -$(CH_2)_3$-NH_2	K_i = 79 nM

Ac-D-Phe-Pro-boroArg-OH (1LHC)



K_i = 0.04 nM

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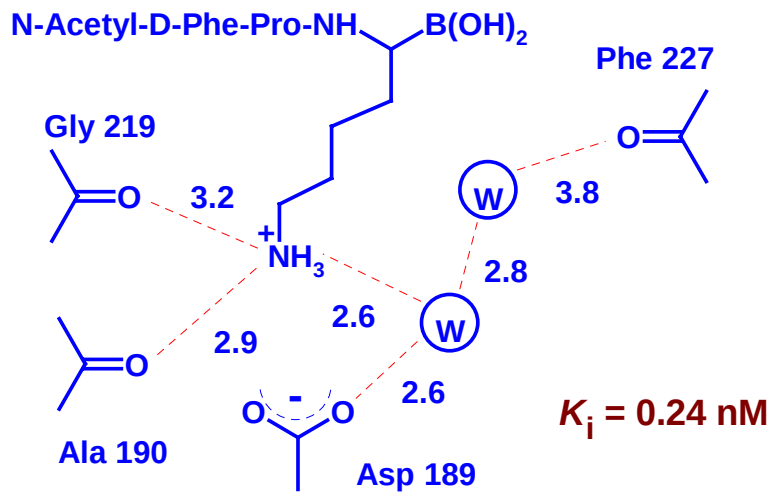
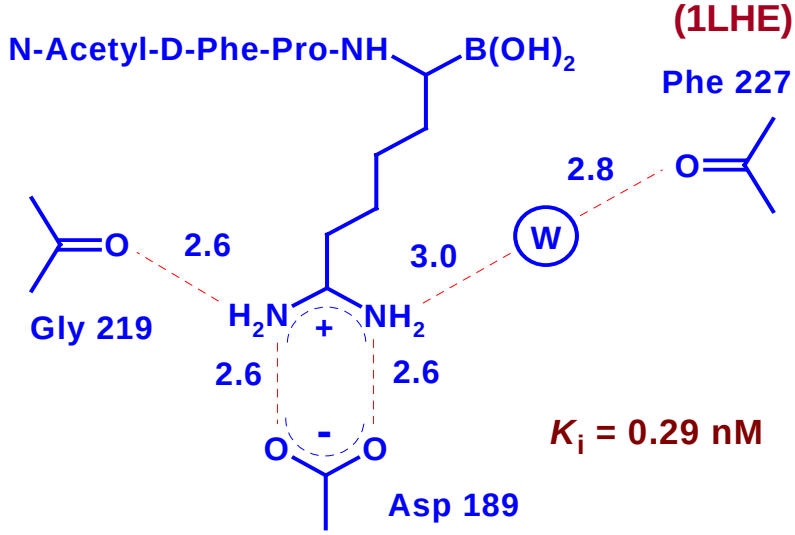
Ac-D-Phe-Pro-boroLys-OH (1LHD)



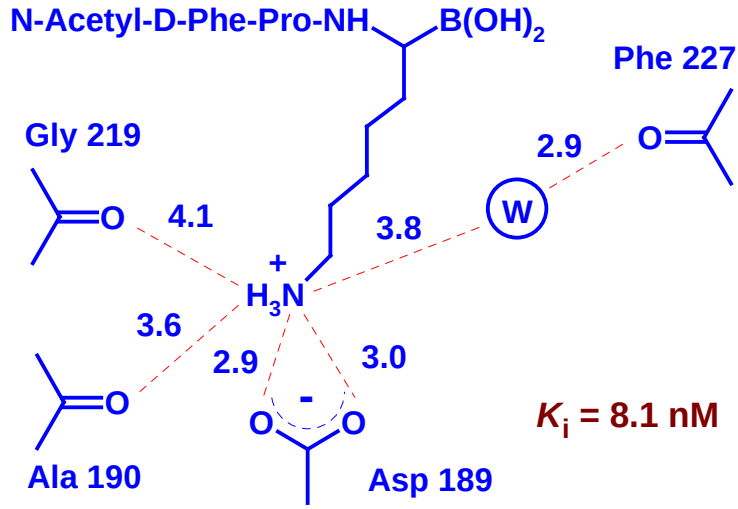
K_i = 0.24 nM

Ac-D-Phe-Pro-boroButylamidinoglycine-OH (1LHE)



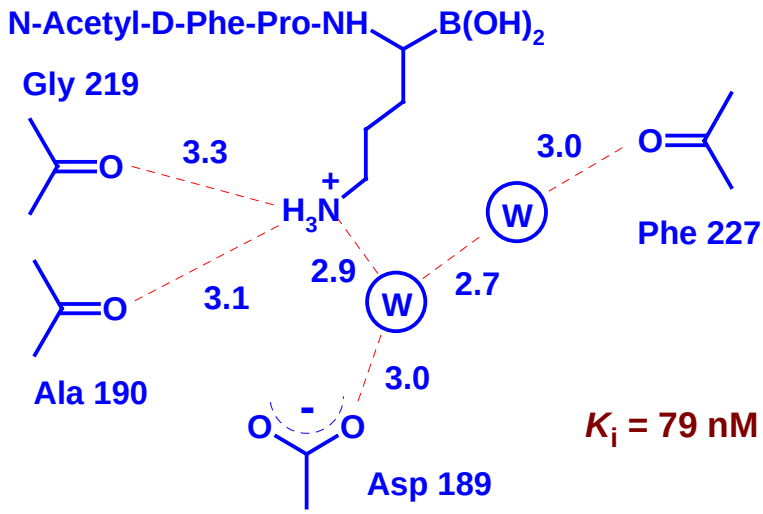
K_i = 0.29 nM

Ac-D-Phe-Pro-boroHomolys-OH (1LHF)



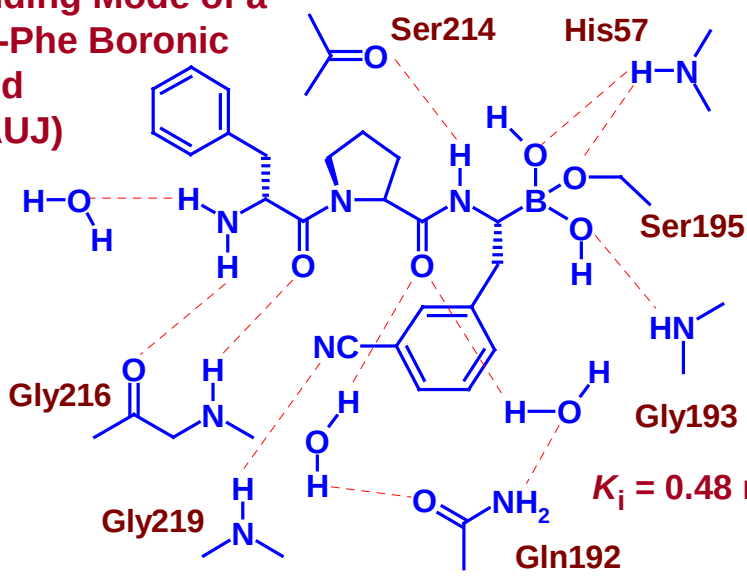
K_i = 8.1 nM

Ac-D-Phe-Pro-boroOrnithine-OH (1LHG)

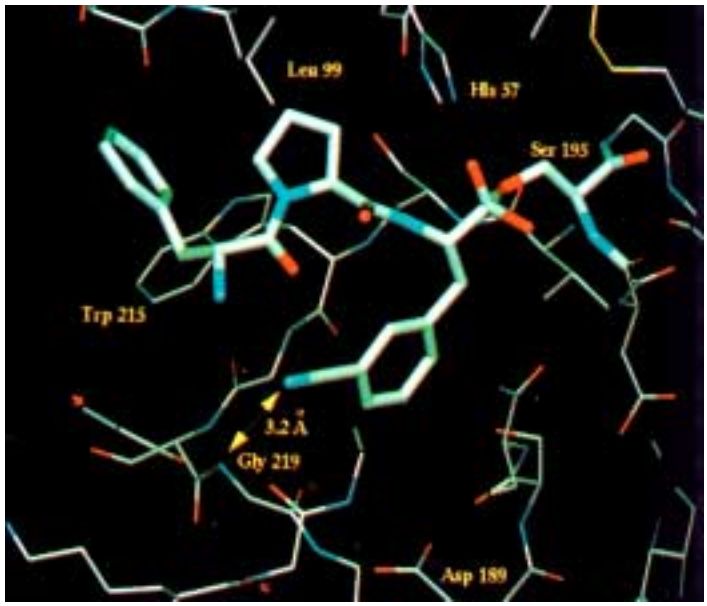


K_i = 79 nM

Binding Mode of a CN-Phe Boronic Acid (1AUJ)



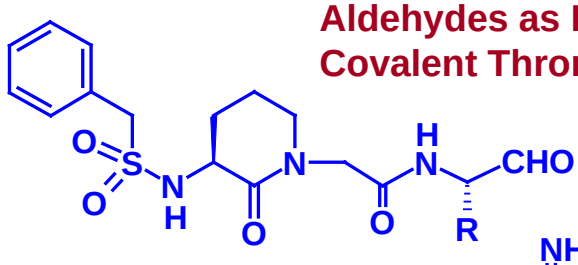
K_i = 0.48 nM



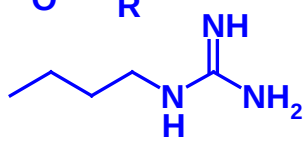
Binding Mode of a CN-Phe Boronic Acid

(1AUJ)

Aldehydes as Reversible Covalent Thrombin Inhibitors

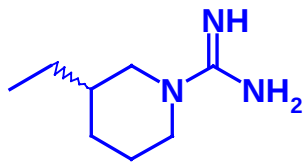


CVS 1578: R =



K_i = 1.0 nM (1BA8)

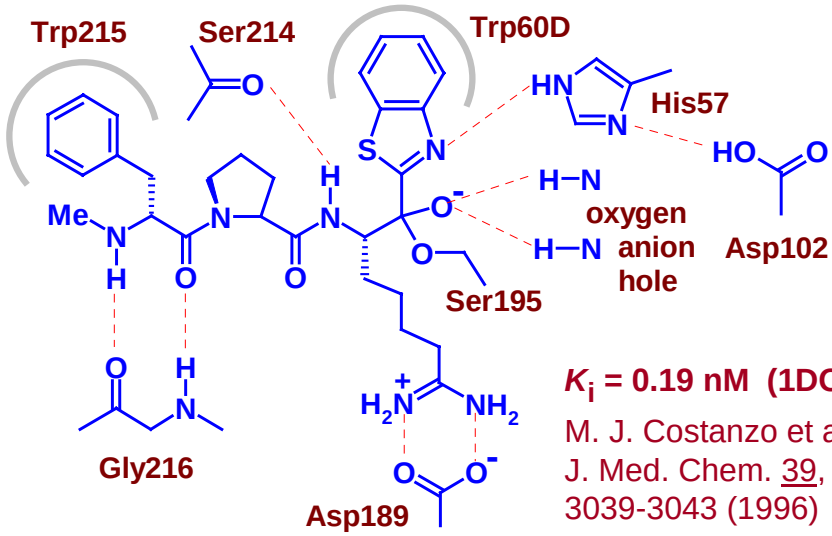
CVS 1694 and 1695: R =



K_i = 4.4 nM and 0.32 nM (1BB0 and 1CA8)

(R and S)

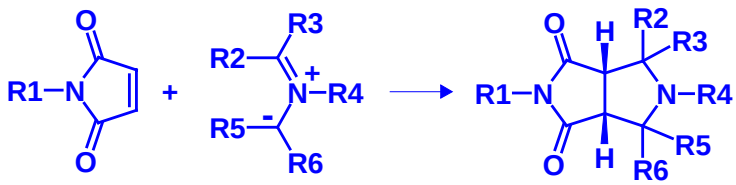
Benzothiazolyl Inhibitor (1DOJ)

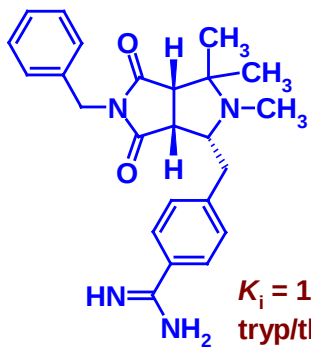


K_i = 0.19 nM (1DOJ)

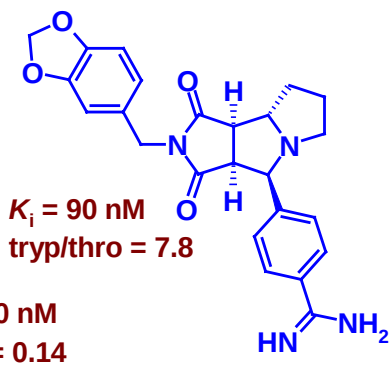
M. J. Costanzo et al., J. Med. Chem. 39, 3039-3043 (1996)

ETH Thrombin Inhibitors

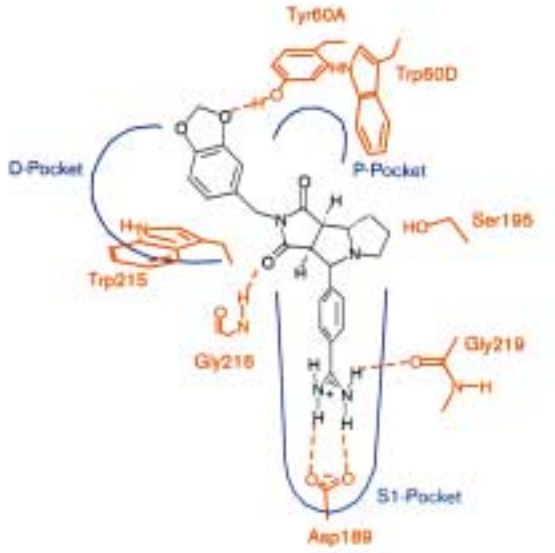




K_i = 18 000 nM
tryp/thro = 0.14



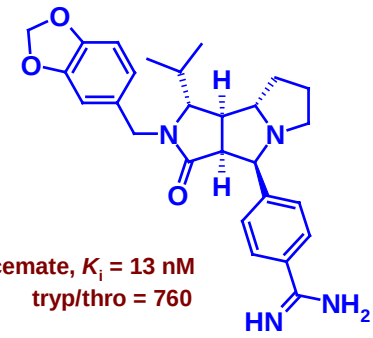
K_i = 90 nM
tryp/thro = 7.8



Binding Mode of the Tricyclic Succinimide Inhibitor to Thrombin

K_i = 90 nM

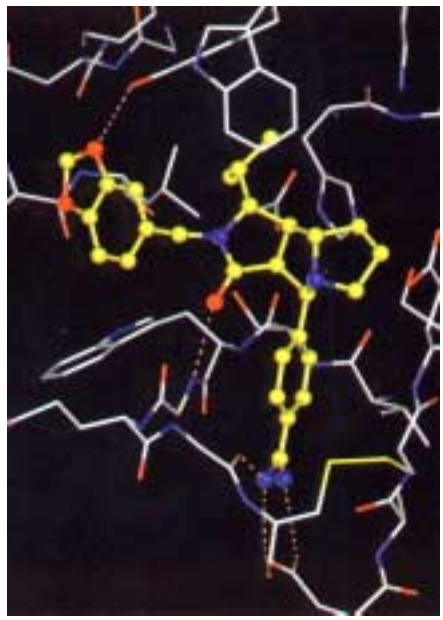
ETH Thrombin Inhibitor
(U. Obst and F. Diederich, 1997)



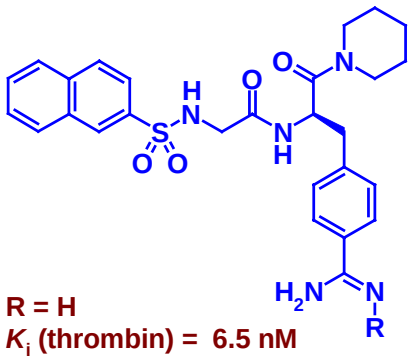
Racemate, K_i = 13 nM
tryp/thro = 760

(+)-Enantiomer, K_i = 7 nM
tryp/thro = 740

(-)-Enantiomer, K_i = 5 600 nM
tryp/thro = 21

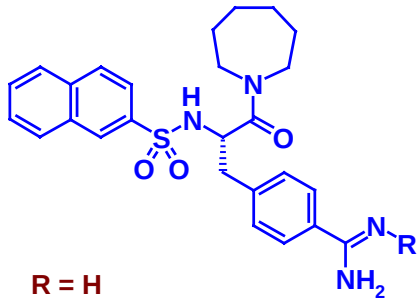


Amidrazones as Selective Thrombin Inhibitors



R = H
K_i (thrombin) = 6.5 nM
K_i (trypsin) = 325 nM

R = NH_2
K_i (thrombin) = 4 320 nM
K_i (trypsin) = 81 500 nM



R = H
K_i (thrombin) = 52 nM
K_i (trypsin) = 8 650 nM

R = NH_2
K_i (thrombin) = 1.5 nM
K_i (trypsin) = 3 730 nM

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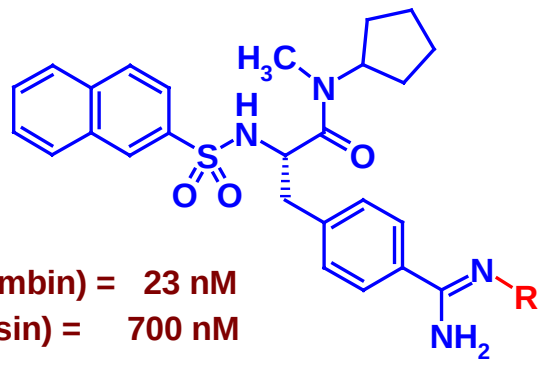
Amidrazones as Selective Thrombin Inhibitors

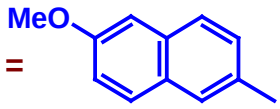


R = H
K_i (thrombin) = 23 nM
K_i (trypsin) = 700 nM

R = NH_2 (LB 30 057)
K_i (thrombin) = 0.38 nM
K_i (trypsin) = 3 290 nM

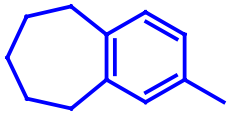
Thrombin Inhibitors With a Benzylamine Group as P1 Substituent, Derived from LB 30 057

R =

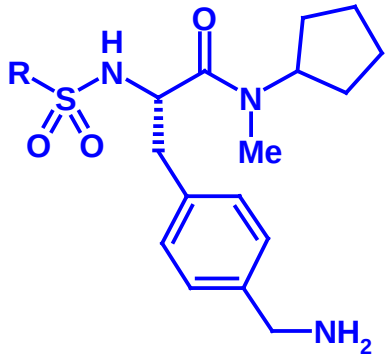


K_i = 6.6 nM

R =

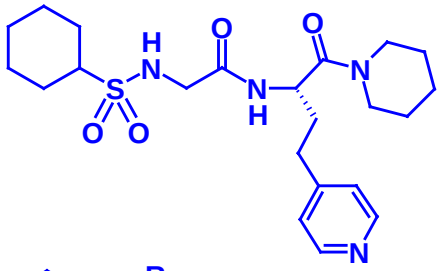


K_i = 3.3 nM

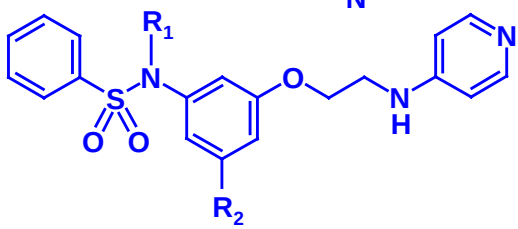


K. Lee et al., Bioorg. Med. Chem. Lett. 8, 2563-2568 (1998)

Boehringer Mannheim Thrombin Inhibitors



BM 51.1011
(1UVS)
K_i = 4 µM

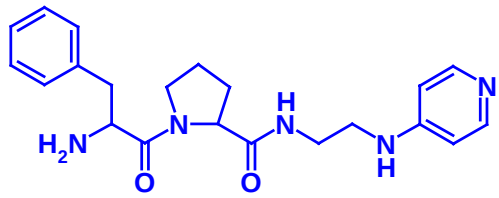


BM 14.1248
(1UVT)
R_1 = H, R_2 = CH_3
K_i = 23 nM

R1 = CH3, R2 = H
K_i = 70 nM

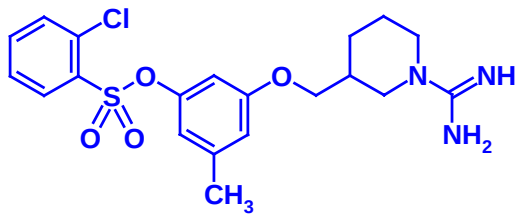
R. A. Engh et al., Structure 4, 1353-1362 (1996)

Other Thrombin Inhibitors: 3D Pharmaceuticals



K_i = 4.1 µM
(1QBV)

R. Bone et al., J. Med. Chem. 41, 2068-2075 (1998)



K_i = 320 nM

T. Lu et al., Bioorg. Med. Chem. Lett. 8, 1595-1600 (1998)

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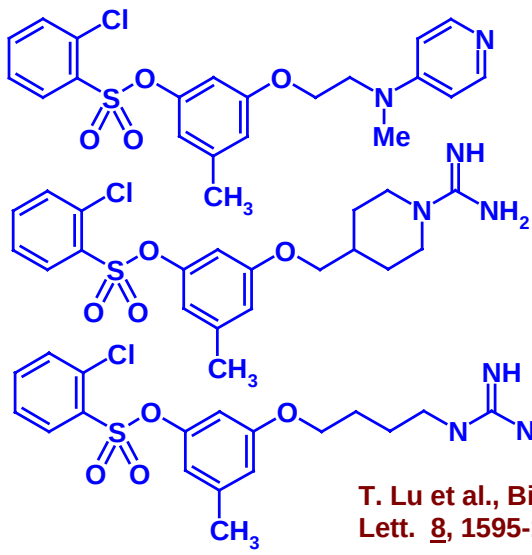
Other Thrombin Inhibitors: 3D Pharmaceuticals



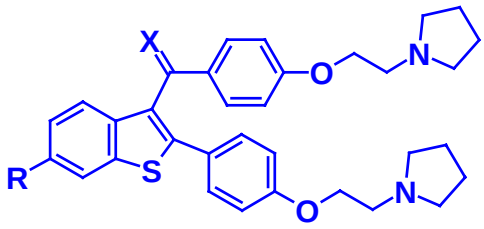
K_i = 11 nM

K_i = 4.6 nM

K_i = 13 nM

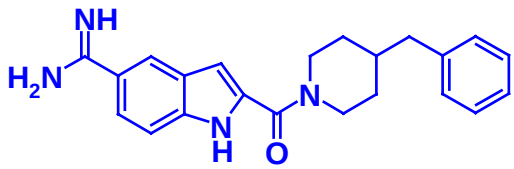
T. Lu et al., Bioorg. Med. Chem. Lett. 8, 1595-1600 (1998)

Eli Lilly Thrombin Inhibitors



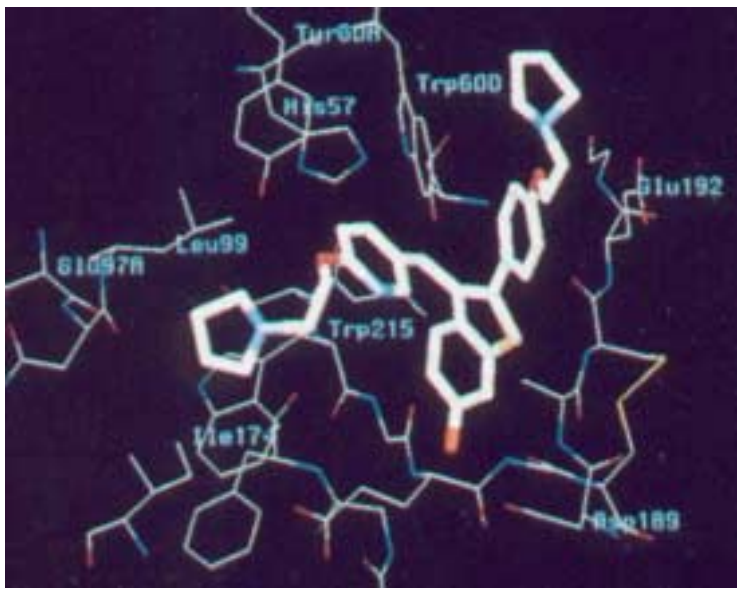
R = H, X = O
(1D3T)
R = H, X = H,H
(1D3Q)
R = OH, X = H,H
K_i = 10 nM

D. J. Sall et al., J. Med. Chem. 40, 3489-3493 (1997)



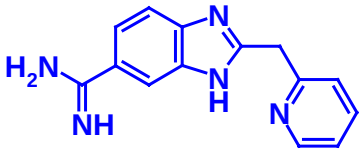
LY178550
(1D4P)

N. Y. Chirgadze et al., Protein Sci. 6, 1412-1417 (1997)

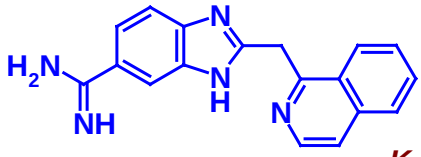


Binding mode of a Lilly inhibitor

Assembly of Ligands in the Binding Site



	no Zn^{2+}	plus Zn^{2+}
K_i (trypsin)	> 1 000 /	136 µM
K_i (tryptase)	= 358 /	0.3 µM
K_i (thrombin)	> 1 000 /	10.5 µM



	no Zn^{2+}	plus Zn^{2+}
K_i (trypsin)	= 31.2 /	22.5 µM
K_i (tryptase)	= 8.8 /	54.5 µM
K_i (thrombin)	= 31 /	0.04 µM

B. A. Katz et al.,
Nature 391,
608-612 (1998)

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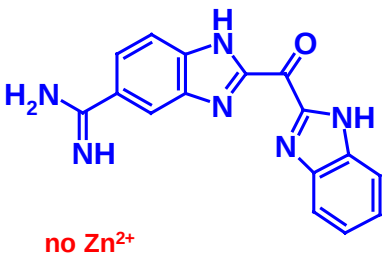
Assembly of Ligands in the Binding Site

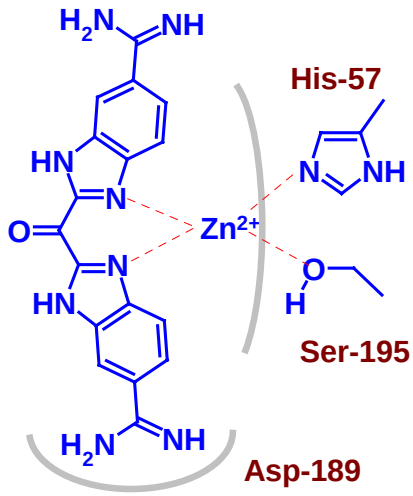


no Zn^{2+}

K_i (Trypsin) = 87.5 µM
K_i (Tryptase) = 5.7 µM
K_i (Thrombin) > 1 000 µM

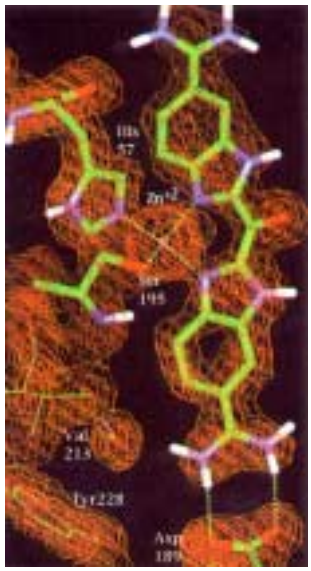
plus Zn^{2+}

K_i (Trypsin) = 0.005 µM
K_i (Tryptase) = 0.05 µM
K_i (Thrombin) = 0.10 µM

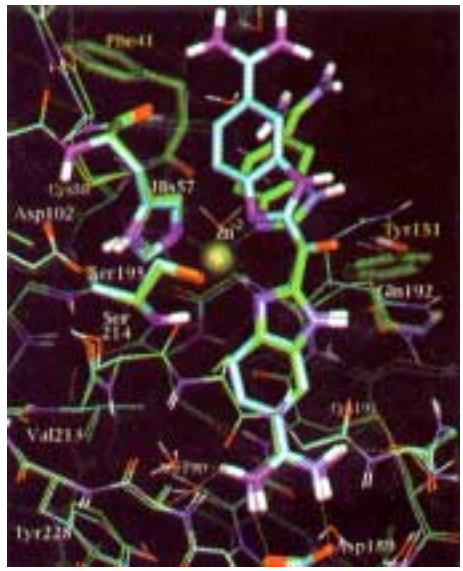


(thrombin complexes:
1C1U, 1C1V, 1C1W)

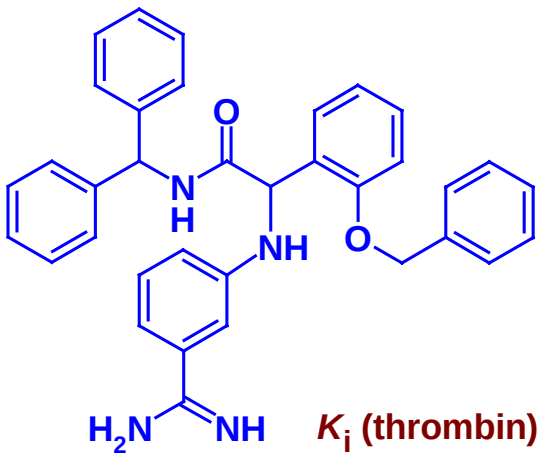
Trypsin + Keto-BABIM



Trypsin + BABIM vs. Keto-BABIM



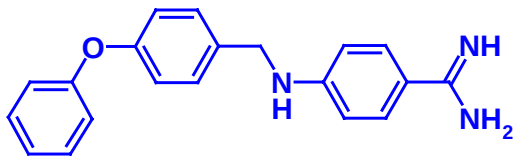
Molecular Evolution of Thrombin Inhibitors in a Combinatorial Library (n = 15,360)



prepared from 80 aldehydes, 12 amines, and 16 isonitriles

K. Illgen et al., Chem. Biol. 7, 433-441 (2000)

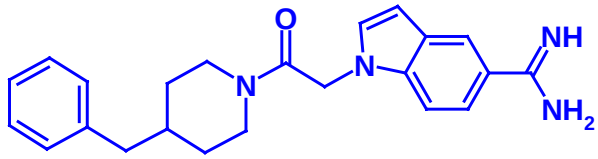
K_i (thrombin) = 2 nM



Combinatorial Docking

K_i (thrombin) = 95 nM
K_i (trypsin) = 520 nM

H.-J. Böhm et al., J. Comp.-Aided Mol. Design 12, 1-6 (1998)



XU 817

K_i (thrombin) = 18 nM
K_i (trypsin) >15 µM

C. Dominguez et al., Bioorg. Med. Chem. Lett. 9, 925-930 (1999)

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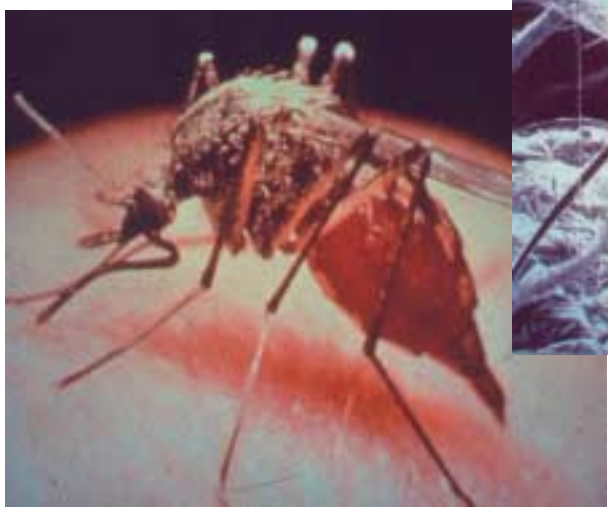
Bloodsucking Animals: Mosquitos and Bugs



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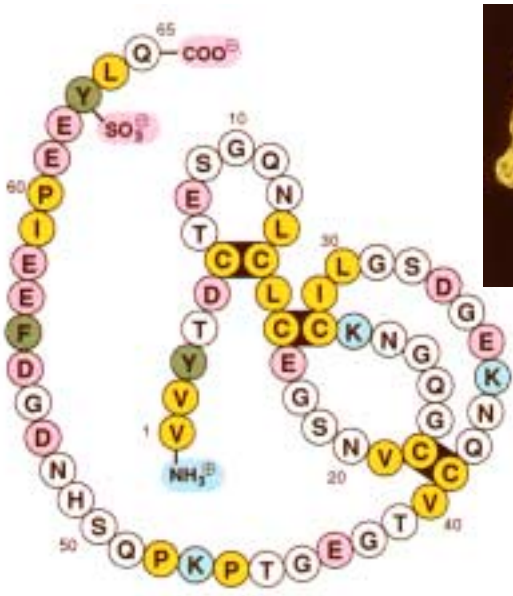
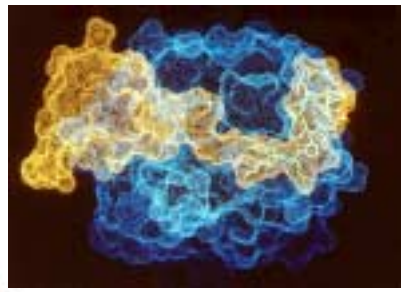
Leech, *Hirudo medicinalis*





Sequence of Hirudin and 3D Structure of the Hirudin-Thrombin Complex (1HRT)

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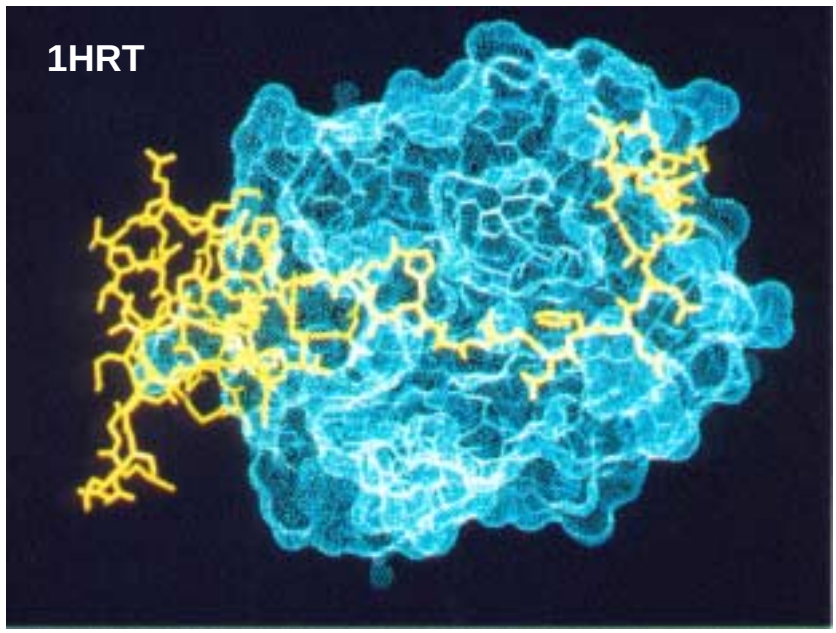

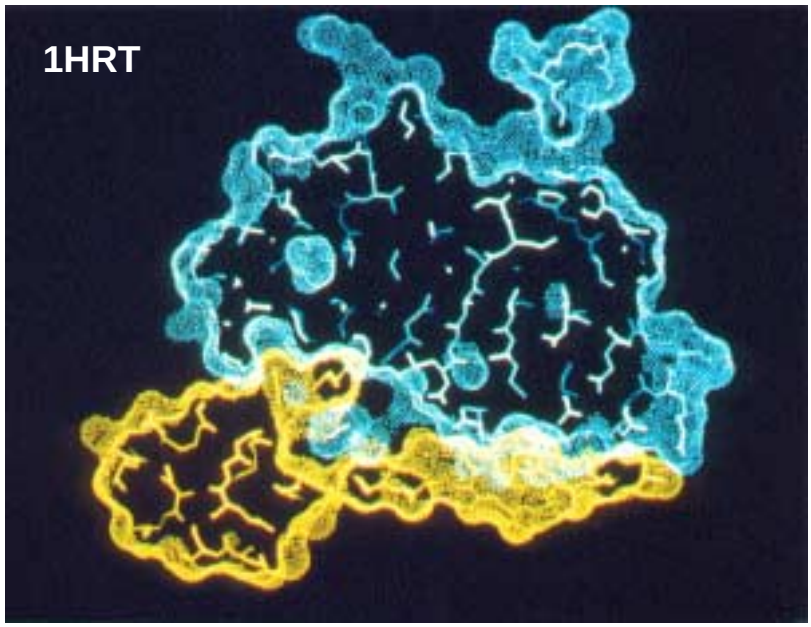

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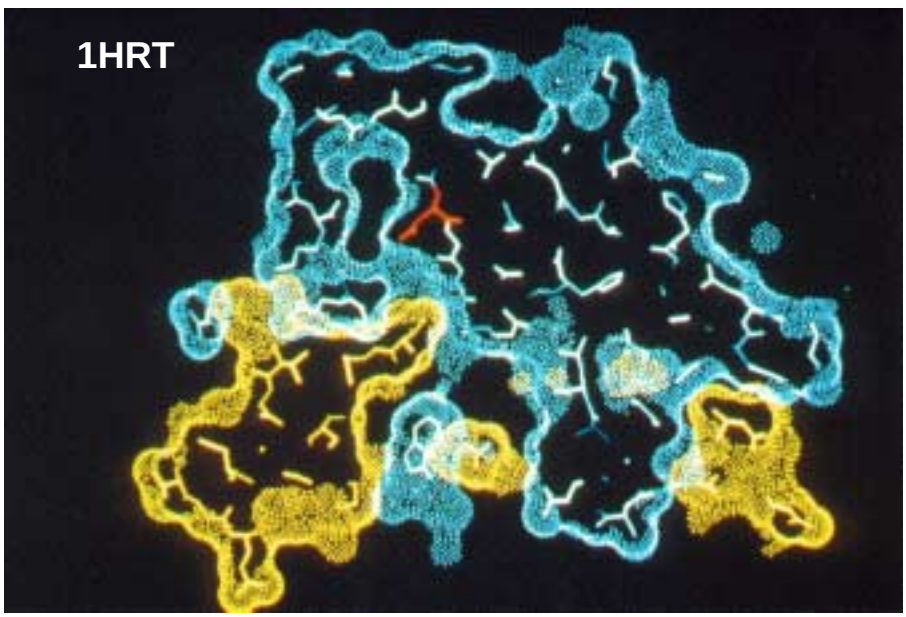
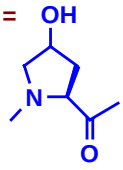
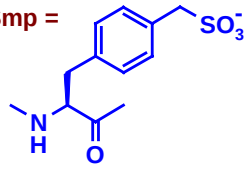
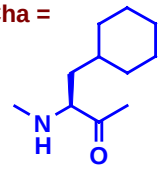


Structural Optimization of a Hirudin Fragment

	in vitro	*in vivo*
Recombinant Hirudin	100 %	100 %
Hirulog-1, D-Phe-Pro-Arg-$(Gly)_4$-Hirudin$^{53-64}$	20 %	60 %
Hirudin$^{56-65}$: Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH	0.05 %	n.d.
$^-$OOC-$(CH_2)_2$-CO-Tyr-Glu-Pro-Ile-Hyp-Glu-Glu-Smp-Cha-Gln-OH	28 %	290 %

Hyp = 

Smp = 

Cha = 

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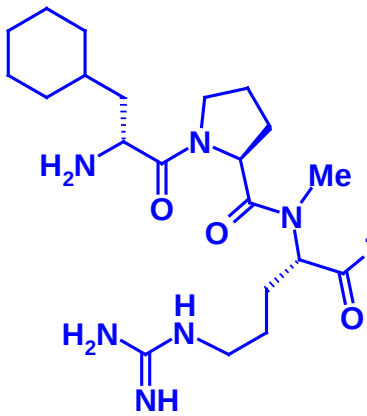
Bivalent Thrombin Inhibitors based on N-Me-Arg



T. Steinmetzer et al., Biol. Chem. 381, 603-610 (2000)

TGGGGGDYEPIPEEA-Cha-dGlu

K_i = 37 pM

active site inhibitor — spacer — exo-site binding domain