SARS coronavirus main proteinase 3.4.22.69

1 Nomenclature

EC number
3.4.22.69

Recommended name
SARS coronavirus main proteinase

Synonyms
3C-like protease <2,3> [9,16,38,49,51]
3CL protease <2> [14,48]
3cLpro <1,2,3> [7,11,13,16,19,28,38,49,51]
C30.004 (Merops-ID)
Mpro
SARS 3C-like protease <2> [17]
SARS 3C-like proteinase <2> [15,18,27]
SARS 3CL protease <2> [31]
SARS 3CLpro <2> [49]
SARS CoV main proteinase <2> [1,2,4,5]
SARS CoVMpro <2> [33]
SARS Mpro <2> [25]
SARS coronavirus 3C-like protease <2> [48]
SARS coronavirus 3C-like proteinase <2> [50]
SARS coronavirus 3CL protease <2> [20]
SARS coronavirus main peptidase <2> [23]
SARS coronavirus main protease <2> [25]
SARS coronavirus main proteinase <2> [5,33]
SARS main protease <2> [12,25]
SARS-3CL protease <2> [48]
SARS-3CLpro <2> [29,50]
SARS-CoV 3C-like peptidase <2> [24]
SARS-CoV 3C-like protease <1> [19]
SARS-CoV 3CL protease <2> [22,30,44,46]
SARS-CoV 3CLpro <2> [32,36,38,44,45]
SARS-CoV 3CLpro enzyme <2> [11]
SARS-CoV Mpro <2> [21,40]
SARS-CoV main protease <2> [21,26,43]
SARS-coronavirus 3CL protease <2> [8]
SARS-coronavirus main protease <2> [47]
TGEV Mpro
coronavirus 3C-like protease <1> [19]

D. Schomburg and I. Schomburg (eds.), *Class 3.4–6 Hydrolases, Lyases, Isomerases, Ligases: EC 3.4–6*, Springer Handbook of Enzymes S10, DOI 10.1007/978-3-642-36260-6_3,

porcine transmissible gastroenteritis virus Mpro
severe acute respiratory syndrome coronavirus 3C-like protease <2> [41,42]
severe acute respiratory syndrome coronavirus main protease <2> [21]
severe acute respiratory syndrome coronavirus main proteinase <2> [33]

CAS registry number
218925-73-6
37353-41-6

2 Source Organism

<1> *alphacoronavirus* [19]
<2> *SARS coronavirus* [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50]
<3> *SARS coronavirus Tor2* (UNIPROT accession number: P0C6U8) [51]

3 Reaction and Specificity

Catalyzed reaction
TSAVLQ-/-SGFRK-NH_2 and SGVTFQ-/-GKFKK the two peptides corresponding to the two self-cleavage sites of the SARS 3C-like proteinase are the two most reactive peptide substrates. The enzyme exhibits a strong preference for substrates containing Gln at P1 position and Leu at P2 position.

Reaction type
hydrolysis of peptide bond

Natural substrates and products
S coronavirus polyprotein + H_2O <2> (<2> 3CLpro processes the translated polyproteins to functional viral proteins [28]) (Reversibility: ?) [28]
P ?
S Additional information <2> (<2> SARS-CoV 3CLpro mediates extensive proteolytic processing of two overlapping replicase polyproteins, pp1a (486000 Da) and pp1ab (790000 Da), to yield the corresponding functional polypeptides that are essential for SARSCoV replication and transcription processes [42]; <2> the genomic RNA produces two large proteins with overlapping sequences, polyproteins 1a and 1ab, which are autocatalytically cleaved by two or three viral proteases to yield functional polypeptides. The key enzyme in this processing is SARS 3CL protease [31]; <2> 3CLpro cleaves the replicase polyproteins at 11 sites with the conserved Gln-(Ser, Ala, Gly) sequences [49]) (Reversibility: ?) [31,42,49]
P ?

Substrates and products

- **S** (Ala-Arg-Leu-Gln-NH)$_2$-rhodamine <2> (Reversibility: ?) [3]
- **P** rhodamine 110 + (Ala-Arg-Leu-Gln-NH)-rhodamine
- **S** (CAL fluor red 610)-TSAVLQSGFRK(BHQ1) + H_2O <2> (Reversibility: ?) [8]
- **P** (CAL fluor red 610)-TSAVLQ + SGFRK(BHQ1)
- **S** 2-aminobenzoyl-SVTLQSG-Tyr(NO_2)Arg + H_2O <2> (Reversibility: ?) [28]
- **P** ?
- **S** 2-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide + H_2O <2> (Reversibility: ?) [26]
- **P** 2-aminobenzoyl-TSAVLQ + SGFRK-2,4-dinitrophenyl amide
- **S** AAVLQSGF$^-NH_2$ + H_2O <2> (Reversibility: ?) [15]
- **P** AAVLQ + SGF-NH_2
- **S** ATVRLQAGNAT + H_2O <2> (Reversibility: ?) [15,27]
- **P** ATVRLQ + AGNAT
- **S** AVLQS-NH_2 + H_2O <2> (Reversibility: ?) [15]
- **P** AVLQ + L-serinamide
- **S** AVLQSE-NH_2 + H_2O <2> (Reversibility: ?) [15]
- **P** AVLQ + Ser-Glu-NH_2
- **S** AVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
- **P** AVLQ + SGF-NH_2
- **S** DABCYL-Lys-Asn-Ser-Thr-Leu-Gln-Ser-Gly-Leu-Arg-Lys-Glu-EDANS + H_2O <2> (Reversibility: ?) [45]
- **P** DABCYL-Lys-Asn-Ser-Thr-Leu-Gln + Ser-Gly-Leu-Arg-Lys-Glu-EDANS
- **S** DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS + H_2O <2> (Reversibility: ?) [44]
- **P** DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln + Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANDS
- **S** DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS + H_2O <2> (Reversibility: ?) [46,49]
- **P** DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln + Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS
- **S** Dabcyl-KNSTLQSGLRKE-EDANS + H_2O <2> (Reversibility: ?) [35]
- **P** Dabcyl-KNSTLQ + SGLRKE-EDANS
- **S** Dabcyl-KTSAVLQSGFRKME-EDANS + H_2O <2> (Reversibility: ?) [36,38]
- **P** Dabcyl-KTSAVLQ + SGFRKME-EDANS
- **S** Dabcyl-KTSAVLQSGFRKMQ-EDANS + H_2O <2> (Reversibility: ?) [34]
- **P** Dabcyl-KTSAVLQ + SGFRKMQ-EDANS
- **S** EDANS-VNSTLQSGLRK-(Dabcyl)-M + H_2O <2> (Reversibility: ?) [37]
- **P** EDANS-VNSTLQ + SGLRK-(Dabcyl)-M
- **S** FYPKLQASQAW + H_2O <2> (Reversibility: ?) [15,27]
- **P** FYPKLQ + ASQAW
- **S** GPFVDRQTAQAAGTDT-NH_2 + H_2O <2> (<2> 1% of the activity with TSAVLQSGFRK-NH_2 [31]) (Reversibility: ?) [31]
- **P** ?
- **S** KVATVQSKMSD + H_2O <2> (Reversibility: ?) [15]
- **P** KVATVQ+ SKMSD

S KVATVQSKMSD + H_2O <2> (<2> weak activity [27]) (Reversibility: ?) [27]
P KVATVQ + SKMSD
S KVATVQSKMSD-NH_2 <2> (<2> undecapeptide containing the non-canonical P3/P4 cleavage site of 3CL protease, 6% of the activity with TSAVLQSGFRK-NH_2 [31]) (Reversibility: ?) [31]
P ?
S L-Thr-L-Ser-L-Ala-L-Val-L-Leu-L-Gln-4-nitroanilide + H_2O <2> (Reversibility: ?) [48]
P L-Thr-L-Ser-L-Ala-L-Val-L-Leu-L-Gln + 4-nitroaniline
S LAVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P LAVLQ + SGF-NH_2
S LQSG-NH_2 + H_2O <2> (Reversibility: ?) [15]
P Leu-Gln + Ser-Gly-NH_2
S MCAAVLQSGFR-Lys(Dnp)-Lys-NH_2 + H_2O <2> (Reversibility: ?) [40]
P MCAAVLQ + Ser-Gly-Phe-Arg-Lys(Dnp)-Lys-NH_2
S NRATLQAIASE + H_2O <2> (<2> weak activity [27]) (Reversibility: ?) [15,27]
P NRATLQ + AIASE
S NVATLQAENVT + H_2O <2> (<2> weak activity [27]) (Reversibility: ?) [15,27]
P NVATLQ + AENVT
S PATVLQAVGAC + H_2O <2> (Reversibility: ?) [15]
P PATVLQ + AVGAC
S PHTVLQAVGAC + H_2O <2> (Reversibility: ?) [27]
P PHTVLQ + AVGAC
S REPLMQSADAS + H_2O <2> (<2> weak activity [27]) (Reversibility: ?) [15,27]
P REPLMQ + SADAS
S SAALQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAALQ + SGF-NH_2
S SAKLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAKLQ + SGF-NH_2
S SALLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SALLQ + SGF-NH_2
S SATLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SATLQ + SGF-NH_2
S SAVAQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVAQ + SGF-NH_2
S SAVFQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVMQ + SGF-NH_2
S SAVIQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVIQ + SGF-NH_2
S SAVKLQNNELS + H_2O <2> (<2> weak activity [27]) (Reversibility: ?) [15,27]
P SAVKLQ + NNELS
S SAVLESGF-NH_2 + H_2O <2> (Reversibility: ?) [15]

P SAVLE + SGF-NH_2
S SAVLKSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVLK + SGF-NH_2
S SAVLNSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVLN + SGF-NH_2
S SAVLQAGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVLQ + AGF-NH_2
S SAVLQEGFRK + H_2O <2> (<2> the cleavage rate of the mutant enzyme T25G is remarkably higher compared to the wild type enzyme [49]) (Reversibility: ?) [49]
P SAVLQ + EGFRK
S SAVLQFGFRK + H_2O <2> (<2> the cleavage rate of the mutant enzyme T25G is remarkably higher compared to the wild type enzyme [49]) (Reversibility: ?) [49]
P SAVLQ + FGFRK
S SAVLQGGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVLQ + GGF-NH_2
S SAVLQGGFRK + H_2O <2> (<2> the cleavage rate of the mutant enzyme T25G is similar to the wild type enzyme [49]) (Reversibility: ?) [49]
P SAVLQ + GGFRK
S SAVLQHGFRK + H_2O <2> (<2> low activity [49]) (Reversibility: ?) [49]
P SAVLQ + HGFRK
S SAVLQKGFRK + H_2O <2> (<2> low activity [49]) (Reversibility: ?) [49]
P SAVLQ + KGFRK
S SAVLQLGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVLQ + LGF-NH_2
S SAVLQLGFRK + H_2O <2> (<2> the cleavage rate of the mutant enzyme T25G is remarkably higher compared to the wild type enzyme [49]) (Reversibility: ?) [49]
P SAVLQ + LGFRK
S SAVLQMGFRK + H_2O <2> (<2> the cleavage rate of the mutant enzyme T25G is remarkably higher compared to the wild type enzyme [49]) (Reversibility: ?) [49]
P SAVLQ + MGFRK
S SAVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVLQ + SGF-NH_2
S SAVLQSGFRK + H_2O <2> (<2> best substrate for both, wild type and mutant enzyme T25G [49]) (Reversibility: ?) [38,49]
P SAVLQ + SGFRK
S SAVLQWGFRK + H_2O <2> (<2> low activity [49]) (Reversibility: ?) [49]
P SAVLQ + WGFRK
S SAVMQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVMQ + SGF-NH_2
S SAVRQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVRQ + SGF-NH_2
S SAVVQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SAVVQ + SGF-NH_2

S SGVTFQGKFKK + H_2O <2> (<2> highest cleavage efficiency. The two peptides corresponding to the two self-cleavage sites of the SARS 3C-like proteinase are the two most reactive ones [27]) (Reversibility: ?) [15,27]
P SGVTFQ + GKFKK
S SITSAVLQ-*p*-nitroanilide + H_2O <2> (Reversibility: ?) [7]
P ?
S SITSAVLQ-*p*-nitrophenyl ester + H_2O <2> (Reversibility: ?) [7]
P ?
S SITSAVLQSGFRKMA + H_2O <2> (Reversibility: ?) [7]
P ?
S SLVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SLVLQ + SGF-NH_2
S STVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P STVLQ + SGF-NH_2
S SVVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P SVVLQ + SGF-NH_2
S SWTSAVLQSGFRKWA + H_2O <2> (Reversibility: ?) [4]
P ?
S Ser-Ala-Val-Leu-Gln-Leu-Gly-Phe-Arg-Lys + H_2O <2> (<2> substrate for T25G mutant protein [49]) (Reversibility: ?) [49]
P Ser-Ala-Val-Leu-Gln + Leu-Gly-Phe-Arg-Lys
S Ser-Ala-Val-Leu-Gln-Met-Gly-Phe-Arg-Lys + H_2O <2> (Reversibility: ?) [49]
P Ser-Ala-Val-Leu-Gln + Met-Gly-Phe-Arg-Lys
S Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys + H_2O <2> (Reversibility: ?) [49]
P Ser-Ala-Val-Leu-Gln + Ser-Gly-Phe-Arg-Lys
S TAVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P TAVLQ + SGF-NH_2
S TFTRLQSLENV + H_2O <2> (Reversibility: ?) [15,27]
P TFTRLQ + SLENV
S TSAVLQSGFRK-NH_2 + H_2O <2> (<2> highest cleavage efficiency. The two peptides corresponding to the two self-cleavage sites of the SARS 3C-like proteinase are the two most reactive ones [27]; <2> peptide containing the P1/P2 cleavage site, the N-terminal self-cleavage site of the protease, most suitable substrate [31]) (Reversibility: ?) [15,27,31]
P TSAVLQ + SGFRK-NH_2
S TVILQAGF + H_2O <2> (Reversibility: ?) [33]
P TVILQ + Ala-Gly-Phe
S TVKLQAGF + H_2O <2> (Reversibility: ?) [33]
P TVKLQ + Ala-Gly-Phe
S TVKLQAGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P TVKLQ + AGF-NH_2
S TVRLQAGF + H_2O <2> (Reversibility: ?) [33]
P TVRLQ + Ala-Gly-Phe
S TVTLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P TVTLQ + SGF-NH_2

S TVVLQSGF-NH_2 + H_2O <2> (Reversibility: ?) [15]
P TVVLQ + SGF-NH_2
S Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide + H_2O <2> (Reversibility: ?) [47,48,50]
P Thr-Ser-Ala-Val-Leu-Gln + *p*-nitroaniline
S VLQS-NH_2 + H_2O <2> (Reversibility: ?) [15]
P VLQ + L-serinamide
S VLQSG-NH_2 + H_2O <2> (Reversibility: ?) [15]
P VLQ + Ser-Gly-NH2
S VVTLQSGF¯NH_2 + H_2O <2> (Reversibility: ?) [15]
P VVTLQ + SGF-NH_2
S [4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid] + H_2O <2> (Reversibility: ?) [9]
P ?
S [4-(4-dimethylaminophenylazo)benzoic acid]-KTSAVLQSGF RKME-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid] + H_2O <2> (Reversibility: ?) [17]
P ?
S [4-(4-dimethylaminophenylazo)benzoic acid]-KTSAVLQSGFRKME-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid] + H_2O <2> (Reversibility: ?) [7,13]
P ?
S [4-(4-dimethylaminophenylazo)benzoic acid]-VNSTLQSGLRK-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid]-M + H_2O <1> (Reversibility: ?) [19]
P ?
S acetyl-TSAVLH-7-amido-4-carbamoyl-coumarin + H_2O <2> (<2> SARS-CoV 3Clpro prefers Gln over His in P1 position. Unlike SARS-CoV 3Clpro, His is strongly preferred in the P1 position by 3C-like proteases from infectious bronchitis virus murine hepatitis virus [6]) (Reversibility: ?) [6]
P acetyl-TSAVLH + 7-amino-4-carbamoyl-coumarin
S acetyl-TSTKLQ-7-amido-4-carbamoyl-coumarin + H_2O <2> (<2> optimized fluorogenic peptide substrate. The enzyme exhibits a strong preference for P1 Gln containing substrates and P2 Leu containing substrates [6]) (Reversibility: ?) [6]
P acetyl-TSTKLQ + 7-amino-4-carbamoyl-coumarin
S coronavirus polyprotein + H_2O <2> (<2> 3CLpro processes the translated polyproteins to functional viral proteins [28]) (Reversibility: ?) [28]
P ?
S dabcyl-KTSAVLQSGFRKME-EDANS + H_2O <2> (Reversibility: ?) [49]
P ?
S *o*-aminobenzoyl-TSAVLQSGFRY(3-NO2)G + H_2O <2> (Reversibility: ?) [8]
P *o*-aminobenzoyl-TSAVLQ + SGFRY(3-NO_2)G
S Additional information <2> (<2> a 3CLpro mechanism utilizes an electrostatic trigger to initiate the acylation reaction, a cysteine-histidine catalytic dyad ion pair, an enzyme-facilitated release of P1, and a general

base catalyzed deacylation reaction [7]; <2> complete description of the tetrapeptide substrate specificity of 3Clpro using fully degenerate peptide libraries consisting of all 160 000 possible naturally occurring tetrapeptides. P1-Gln P2-Leu specificity and elucidate a novel preference for P1-His containing substrates equal to the expected preference for P1-Gln [6]; <2> SARS-CoV 3CLpro mediates extensive proteolytic processing of two overlapping replicase polyproteins, pp1a (486000 Da) and pp1ab (790000 Da), to yield the corresponding functional polypeptides that are essential for SARSCoV replication and transcription processes [42]; <2> the genomic RNA produces two large proteins with overlapping sequences, polyproteins 1a and 1ab, which are autocatalytically cleaved by two or three viral proteases to yield functional polypeptides. The key enzyme in this processing is SARS 3CL protease [31]; <2> a complete description of the tetrapeptide substrate specificity of 3Clpro using fully degenerate peptide libraries consisting of all 160000 possible naturally occurring tetrapeptides. The enzyme exhibits a strong preference for P1 Gln containing substrates and P2 Leu containing substrates. The enzyme also shows a strong preference for P1 histidine containing substrates. 3Clpro has extended substrate specificity at P5 and P6 preferring hydrophobic amino acids such as Leu [6]; <2> comprehensive overview of SARS-CoV 3CLpro substrate specificity. The hydrophobic pocket in the P2 position at the protease cleavage site is crucial to SARS-CoV 3CLpro-specific binding, which is limited to substitution by hydrophobic residue. The binding interface of SARS-CoV 3CLpro that is facing the P1 position is suggested to be occupied by acidic amino acids, thus the P1 position is intolerant to acidic residue substitution, owing to electrostatic repulsion. Steric hindrance caused by some bulky or branching amino acids in P3 and P2 positions may also hinder the binding of SARS-CoV 3CLpro. In addition to the conserved Gln residue in the P1 position at the SARS-CoV 3CLpro cleavage site, the P2 position, which is exclusively occupied by Leu residue, also serves as another important determinant of substrate specificity. Peptide substrate with Phe replacement in the P2 position is also favorable for SARSCoV 3CLpro cleavage. Ile is intolerant in the P2 position. P1 position, which is frequently occupied by Ser residue, also contributes to the substrate specificity of SARS-CoV 3CLpro considerably. The P1 position is highly unfavorable to the substitution by Pro, Asp, and Glu residues. The substrate specificity of SARS-CoV 3CLpro is less dependent on the P2 and P3 positions at the cleavage site. The peptide cleavage results show that the P3 and P4 positions have no effect on determining the substrate specificity preferences of SARS-CoV 3CLpro [42]; <2> cuts the 11 peptides covering all of the 11 cleavage sites on the viral polyprotein with different efficiency [27]; <2> the S3 subsite of the SARS CoVMpro has a negative character. The electrostatic interactions between Glu47 and P3Lys play a key role in specific binding. These observations are very important and provide further information for structural-based drug design against SARS virus [33]; <2> 3CLpro cleaves the replicase polyproteins at 11 sites

with the conserved Gln-(Ser, Ala, Gly) sequences [49]; <2> no cleavage of SAVLQPGFRK [49]) (Reversibility: ?) [6,7,27,31,33,42,49]

P ?

Inhibitors

(S)-2-((2S,3R)-2-((S)-2-acetamido-3-methylbutanamido)-3-(benzyloxy)butanamido)-4-methyl-N-((S)-4-(5-nitro-1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-3-oxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)pentanamide <2> [15]
1,1'-sulfonylbis(4-nitrobenzene) <2> [21]
1-(1-benzothiophen-2-ylmethyl)-5-iodo-1H-indole-2,3-dione <2> [15,16]
1-(2-naphthlmethyl)isatin-5-carboxamide <2> [50]
1-(2-naphthylmethyl)-2,3-dioxoindoline-5-carboxamide <2> [15]
1-[(1H-benzimidazol-5-ylcarbonyl)oxy]-1H-1,2,3-benzotriazole <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C_{14}5A mutant enzyme [13]) [13]
1-[(1H-indol-2-ylcarbonyl)oxy]-1H-1,2,3-benzotriazole <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C145A mutant enzyme [13]) [13]
1-[(1H-indol-5-ylcarbonyl)oxy]-1H-1,2,3-benzotriazole <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C145A mutant enzyme [13]) [13]
1-[(1H-indol-5-ylcarbonyl)oxy]-1H-benzotriazole <2> [32]
1-[(4-chlorophenyl)sulfonyl]-2-nitro-4-(trifluoromethyl)benzene <2> [21]
1-[[(5-fluoro-1H-indol-2-yl)carbonyl]oxy]-1H-1,2,3-benzotriazole <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C145A mutant enzyme [13]) [13]
1-[bis(4-chlorophenyl)methyl]-3-[2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazol-3-ium <2> [15]
1-butyl-N-[4-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)benzyl]-1H-benzimidazol-2-amine <2> [36]
1-hydroxypyridine-2-thione zinc <2> [16]
2',5'-dimethyl-3-(methylthio)-4'-nitro-5-(2-thienyl)-2'H-1,3'-bipyrazole-4-carbonitrile <2> [21]
2,2-difluoro-2-(pyridin-3-yl)-1-(thiophen-2-yl)ethanone <2> (<2> 0.1 mM, 38% inhibition [28]) [28]
2,4-dichloro-5-methylphenyl 2,6-dinitro-4-(trifluoromethyl)phenyl sulfone <2> [21]
2,5-bis[[(benzyloxy)carbonyl]amino]-1,2,5,6-tetradeoxy-1,6-di-1H-indol-3-yl-L-iditol <2> [14]
2-(3',4'-dihydroxyphenyl)-3-β-D-galactosyl-4H-chromen-4-one <2> (<2> 0.05 mM, 30.1% inhibition [9]) [9]
2-(3',4'-dihydroxyphenyl)-5,7-dihydroxy-3-β-D-arobinosyl-4H-chromen-4-one <2> (<2> 0.05 mM, 49.4% inhibition [9]) [9]
2-(3',4'-dihydroxyphenyl)-5,7-dihydroxy-3-β-D-galactosyl-4H-chromen-4-one <2> (<2> 0.05 mM, 41.8% inhibition [9]) [9]
2-(3',4'-dihydroxyphenyl)-5,7-dihydroxy-3-β-D-glucosyl-4H-chromen-4-one <2> (<2> 0.05 mM, 57.5% inhibition [9]) [9]

2-(3',4'-dihydroxyphenyl)-5,7-dihydroxy-3-β-L-fucosyl-4H-chromen-4-one <2> (<2> 0.05 mM, 57.4% inhibition [9]) [9]
2-(3',4'-dihydroxyphenyl)-5-hydroxy-3,7-di(β-D-galactosyl)-4H-chromen-4-one <2> (<2> 0.05 mM, 53.0% inhibition [9]) [9]
2-(3-chlorophenyl)-2,2-difluoro-1-(furan-2-yl)ethanone <2> (<2> 0.1 mM, 13% inhibition [28]) [28]
2-(3-chlorophenyl)-2-fluoro-1-(furan-2-yl)ethanone <2> (<2> 0.1 mM, 15% inhibition [28]) [28]
2-(4,5-dihydro-1,3-thiazol-2-yl)-1-(1,3-thiazol-2-yl)ethanone <2> [21]
2-(4-aminophenyl)-6-methyl-1H-benzimidazole-7-sulfonic acid <2> [15,16]
2-(5-bromopyridin-3-yl)-2,2-difluoro-1-(furan-2-yl)ethanone <2> (<2> 0.1 mM, 21% inhibition [28]) [28]
2-(5-chloropyridin-3-yl)-2,2-difluoro-1-(furan-2-yl)ethanone <2> (<2> 0.1 mM, 27% inhibition [28]) [28]
2-(5-chloropyridin-3-yl)-2-fluoro-1-(furan-2-yl)ethanone <2> (<2> 0.1 mM, 14% inhibition [28]) [28]
2-([N-[(benzyloxy)carbonyl]-L-alanyl-L-valyl]amino)-5-[[(2S,5S)-5-[[(benzyloxy)carbonyl]amino]-2-(1-methylethyl)-4-oxohexanoyl]amino]-1,2,5,6-tetradeoxy-1,6-diphenyl-L-iditol <2> [15]
2-(benzylsulfanyl)-4-(3-chlorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
2-(benzylsulfanyl)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
2-(benzylsulfanyl)-4-(4-methylphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
2-(benzylsulfanyl)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
2-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]aniline <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C145A mutant enzyme [13]) [13]
2-[(2-acetylphenyl)sulfonyl]benzoic acid <2> [21]
2-[(2-cyclohexylquinazolin-4-yl)sulfanyl]-N-(furan-2-ylmethyl)acetamide <2> (<2> 0.01 mM, 30% inhibition [29]) [29]
2-[(4-chlorophenyl)sulfonyl]-5-nitropyridine 1-oxide <2> [21]
2-[(4-nitrobenzyl)sulfanyl]-4-(3-nitrophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
2-[(4-nitrobenzyl)sulfanyl]-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
2-acetyl-3-(3-iodophenyl)-7-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazole <2> [36]
2-benzyl-2H-isoindole-4,7-dione <2> [36]
2-fluoro-2-(pyridin-3-yl)-1-(thiophen-2-yl)ethanone <2> (<2> 0.1 mM, 10% inhibition [28]) [28]
2-phenyl-5,7-dihydroxy-3-β-D-galactosyl-4H-chromen-4-one <2> (<2> 0.05 mM, 18.7% inhibition [9]) [9]
2-phenylethyl 2-methyl-4-(2-nitrophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate <2> [15]

3,4-dichloro-5-[2-(5-chloro-3-methyl-1-benzothien-2-yl)-2-oxoethyl]furan-2(5H)-one <2> [15,16]
3,4-dichloro-5-[2-(5-chloro-3-methyl-1-benzothiophen-2-yl)-2-oxoethyl]furan-2(5H)-one <2> [16]
3-(4-bromophenyl)-5-(4-chlorophenyl)-1-(3,4-dichlorophenyl)-4-(1H-imidazol-1-yl)-4,5-dihydro-1H-pyrazole <2> [36]
3-(N-L-γ-Glu-L-Ala)-1,1,1-trifluoropropan-2-one <2> [30]
3-[(2-furylmethyl)amino]-6,6-dimethyl-4-oxo-4,5,6,7-tetrahydro-2-benzothiophene-1-carbonitrile <2> [21]
3-[N-(N-benzyloxycarbonyl-L-Leu)]-4-phenyl-1,1,1-trifluorobutan-2-one <2> [30]
3-[N-(N-benzyloxycarbonyl-L-Phe)]-4-phenyl-1,1,1-trifluorobutan-2-one <2> [30]
3-[N-(N-tert-butoxycarbonyl)-L-Leu]-1,1,1-trifluorobutan-2-one <2> [30]
3-[N-[N-benzyloxycarbonyl-L-Ala-L-Val-L-Leu]]-4-phenyl-1,1,1-trifluorobutan-2-one <2> [30]
3-[N-[N-decanoyl-L-Leu]]-4-phenyl-1,1,1-trifluorobutan-2-one <2> [30]
3-benzyl-1-[(6,7-dimethyl-2-oxo-1,2-dihydroquinolin-3-yl)methyl]-1-[2-(2-methylphenyl)ethyl]urea <2> (<2> 0.01 mM, 40% inhibition [29]) [29]
3-{N-[N-tert-butoxycarbonyl-L-γ-Glu(OtBu)-L-Ala]}-1,1,1-trifluoropropan-2-one <2> [30]
4,5-anhydro-2-([N-[(benzyloxy)carbonyl]-L-phenylalanyl]amino)-1,2-dideoxy-D-erythro-pent-3-ulose <2> [6]
4,6-dimethyl-2-[(4-methylphenyl)sulfonyl]-5-nitronicotinonitrile <2> [21]
4,6-dimethyl-5-nitro-2-(phenylsulfonyl)nicotinonitrile <2> [21]
4-(3-nitrophenyl)-6-oxo-2-[(2-phenylethyl)sulfanyl]-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(4-chlorophenyl)-2-[(4-nitrobenzyl)sulfanyl]-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(4-chlorophenyl)-6-oxo-2-[(2-phenylethyl)sulfanyl]-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(4-methoxyphenyl)-2-[(4-nitrobenzyl)sulfanyl]-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(4-methoxyphenyl)-6-oxo-2-[(2-phenylethyl)sulfanyl]-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(4-methylphenyl)-2-[(4-nitrobenzyl)sulfanyl]-6-oxo-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(4-methylphenyl)-6-oxo-2-[(2-phenylethyl)sulfanyl]-1,6-dihydropyrimidine-5-carbonitrile <2> [46]
4-(5-chloro-2-thienyl)-2-[(2-thienylsulfonyl)methyl]-1,3-thiazole <2> [21]
4-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]-N,N-diethylaniline <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C145A mutant enzyme [13]) [13]
4-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]-N,N-dimethylaniline <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C145A mutant enzyme [13]) [13]

4-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]-N-methylaniline <2> (<2> inhibition and irreversible mechanism-based inactivators, no irreversible inactivation with the C_{14}5A mutant enzyme [13]) [13]
4-[(3,5-dibromo-4-hydroxyphenyl)sulfonyl]benzoic acid <2> [21]
4-[(E)-[(2-methoxyphenyl)imino]methyl]-2-phenyl-1,3-oxazol-5-yl acetate <2> [36]
4-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-3-phenyl-propionylamino]-5-(2-oxo-pyrrolidin-3-yl)-pent-2-enoic acid ethyl ester <2> [20]
4-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-5-(2-oxo-pyrrolidin-3-yl)-pent-2-enoic acid ethyl ester <2> [20]
4-[2-(2-benzyloxycarbonylamino-3-tert-butoxy-butyrylamino)-4-methyl-pentanoylamino]-5-(2-oxo-pyrrolidin-3-yl)-pent-2-enoic acid ethyl ester <2> [20]
4-methoxy-6-[([1,3]thiazolo[5,4-b]pyridin-2-ylsulfinyl)methyl]-2H-pyran-2-one <2> [36]
5,7-dichloro-4-hydroxy-3-[[1-(4-hydroxyphenyl)-1H-tetrazol-5-yl]sulfanyl]-quinolin-2(1H)-one <2> [36]
5-(1,3-dimethyl-1H-pyrazol-5-yl)-N-(3-methyl-4-nitroisoxazol-5-yl)thiophene-2-carboxamide <2> [21]
5-[(4-chlorophenyl)sulfonyl]pyrimidine-2,4-diamine <2> [21]
5-chloropyridin-3-yl (3S)-2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate <2> [32]
5-chloropyridin-3-yl 1-[(3-nitrophenyl)sulfonyl]-1H-indole-5-carboxylate <2> [32]
5-chloropyridin-3-yl 1-[(4-methylphenyl)sulfonyl]-1H-indole-5-carboxylate <2> [32]
5-chloropyridin-3-yl 1-acetyl-1H-indole-4-carboxylate <2> [32]
5-chloropyridin-3-yl 1-acetyl-1H-indole-5-carboxylate <2> [32]
5-chloropyridin-3-yl 1-naphthoate <2> [10]
5-chloropyridin-3-yl 1H-indole-4-carboxylate <2> [32]
5-chloropyridin-3-yl 1H-indole-5-carboxylate <2> [32]
5-chloropyridin-3-yl 1H-indole-6-carboxylate <2> [32]
5-chloropyridin-3-yl 1H-indole-7-carboxylate <2> [32]
5-chloropyridin-3-yl 2-nitrobenzoate <2> [10]
5-chloropyridin-3-yl 2-oxo-2H-chromene-3-carboxylate <2> [10]
5-chloropyridin-3-yl 3-nitrobenzoate <2> [10]
5-chloropyridin-3-yl 4-chlorobenzoate <2> [10]
5-chloropyridin-3-yl 5-(2-nitrophenyl)-2-furoate <2> [10]
5-chloropyridin-3-yl 5-(3-nitrophenyl)-2-furoate <2> [10]
5-chloropyridin-3-yl 5-(4-chloro-2-nitrophenyl)-2-furoate <2> [10]
5-chloropyridin-3-yl 5-(4-chlorophenyl)-2-furoate <2> [10]
5-chloropyridin-3-yl 5-(4-nitrophenyl)-2-furoate <2> [10]
5-chloropyridin-3-yl isonicotinate <2> [10]
5-chloropyridin-3-yl nicotinate <2> [10]
5-chloropyridin-3-yl thiophene-2-carboxylate <2> [15,16]
6-oxo-4-phenyl-2-[(2-phenylethyl)sulfanyl]-1,6-dihydropyrimidine-5-carbonitrile <2> [46]

DTT <2> (<2> 80% of enzyme activity inhibited in the presence of 2.5 mM DTT [3]) [3]
Hg^{2+} <2> [16]
N-(2,2'-bithien-5-ylmethyl)-1,3-dimethyl-4-nitro-1H-pyrazol-5-amine <2> [21]
N-(2-allylthiophenyl)cinnamide <2> [35]
N-(2-benzylthiophenyl)cinnamide <2> [35]
N-(2-carbomethoxyethylthiophenyl)cinnamide <2> [35]
N-(2-chloro-4-nitrophenyl)-N^{α}-[[4-(dimethylamino)phenyl]carbonyl]phenylalaninamide <2> [15,16]
N-(2-cinnamoylthiophenyl)cinnamide <2> [35]
N-[(5-methyl-4,5-dihydro-1H-pyrazol-3-yl)carbonyl]-L-valyl-N-[(1S,2E)-4-ethoxy-1-[[(3S)-3-methyl-2-oxopyrrolidin-3-yl]methyl]-4-oxobut-2-en-1-yl]-L-leucinamide <2> [11]
N-[(5-methyl-4,5-dihydro-1H-pyrazol-3-yl)carbonyl]-L-valyl-N-[(1S,2E)-4-ethoxy-1-[[(3S)-3-methyl-2-oxopyrrolidin-3-yl]methyl]-4-oxobut-2-en-1-yl]-L-phenylalaninamide <2> [11]
N-[(benzyloxy)carbonyl]-3-[(2,2-dimethylpropanoyl)amino]-L-alanyl-N-[(1S,2E)-4-oxo-1-[[(3S)-2-oxopyrrolidin-3-yl]methyl]pent-2-en-1-yl]-L-leucinamide <2> (<2> inhibits the viral protease, thus preventing CVB3 genome replication [38]) [38]
N-[(benzyloxy)carbonyl]-L-alanyl-L-valyl-N-[(3S)-6-(dipropylamino)-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-alanyl-L-valyl-N-[(3S)-6-amino-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(2S)-1,5-dioxo-1,5-di(1,3-thiazol-2-yl)pentan-2-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(2S)-1-oxo-3-[(3S)-2-oxopyrrolidin-3-yl]-1-(1,3-thiazol-2-yl)propan-2-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(2S)-5-(morpholin-4-yl)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-1,1,1-trifluoro-6-(morpholin-4-yl)-2,6-dioxohexan-3-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-5-carboxy-1,1,1-trifluoro-2-oxopentan-3-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-6-(dipropylamino)-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-6-[benzyl(methyl)amino]-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide <2> [44]
N-[(benzyloxy)carbonyl]-O-tert-butylthreonyl-N-[(1S)-1-formyl-2-[(3S)-2-oxopyrrolidin-3-yl]ethyl]-L-phenylalaninamide <2> (<2> inhibits the viral protease, thus preventing CVB3 genome replication [38]) [38]
N-[(benzyloxy)carbonyl]-O-tert-butylthreonyl-N-[(1S,2E)-4-cyclopropyl-4-oxo-1-[[(3S)-2-oxopyrrolidin-3-yl]methyl]but-2-en-1-yl]-L-leucinamide <2> [38]
N-[(benzyloxy)carbonyl]-O-tert-butylthreonyl-N-[(1S,2E)-4-ethoxy-4-oxo-1-[[(3S)-2-oxopyrrolidin-3-yl]methyl]but-2-en-1-yl]-L-leucinamide <2> [38]
N-[2-(2-cyanocinnamoylthio)phenyl]-2-cyanocinnamide <2> [35]

N-[2-(2-pyridylmethylthio)phenyl]cinnamide <2> [35]
N-[2-(3-dimethylaminopropylthio)phenyl]-2-cyanocinnamide <2> [35]
N-[2-(3-pyridylmethylthio)phenyl]cinnamide <2> [35]
N-[3-(5-tert-butyl-2-methyl-3-furyl)-1H-pyrazol-5-yl]thiophene-2-sulfonamide <2> [21]
N-[4-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)benzyl]-1-ethyl-1H-benzimidazol-2-amine <2> [36]
N-[4-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)benzyl]-1-propyl-1H-benzimidazol-2-amine <2> [36]
N-[4-[(4-chlorophenyl)sulfonyl]-3-(methylthio)-1H-pyrazol-5-yl]thiophene-2-carboxamide <2> [21]
N-ethyl-N-phenyldithiocarbamic acid zinc <2> [16]
N-tert-butyl-L-seryl-L-valyl-N-[(1S,2E)-4-ethoxy-1-[[(3S)-3-methyl-2-oxopyrrolidin-3-yl]methyl]-4-oxobut-2-en-1-yl]-L-phenylalaninamide <2> [11]
N-tert-butyl-L-seryl-L-valyl-N-[(1S,2E)-4-ethoxy-4-oxo-1-[2-(2-oxopyrrolidin-3-yl)ethyl]but-2-en-1-yl]-L-leucinamide <2> [11]
N^2-[(benzyloxy)carbonyl]-N-[(3S)-6-(dipropylamino)-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide <2> [44]
NP-40 <2> (<2> 80% of enzyme activity inhibited in the presence of 0.1% NP-40 [3]) [3]
NaCl <2> (<2> 80% of enzyme activity inhibited in the presence of 100 mM NaCl [3]) [3]
S-[5-(trichloromethyl)-4H-1,2,4-triazol-3-yl] 5-(phenylethynyl)furan-2-carbothioate <2> [21]
TG-0203770 <2> [38]
TG-0204998 <2> [38]
TG-0205221 <2> [38]
TG-0205486 <2> [38]
Zn^{2+} <2> [16]
[3-([[3-(dihydroxyboranyl)benzyl]oxy]carbonyl)-5-nitrophenyl]boronic acid <2> [16]
[benzene-1,2-diylbis[methanediylcarbamoyl(5-nitrobenzene-3,1-diyl)]]diboronic acid <2> [16]
[benzene-1,2-diylbis[methanediyloxycarbonyl(5-nitrobenzene-3,1-diyl)]]diboronic acid <2> [16]
[benzene-1,3-diylbis[oxycarbonyl(5-nitrobenzene-3,1-diyl)]]diboronic acid <2> [16]
[benzene-1,4-diylbis[carbamoyl(5-nitrobenzene-3,1-diyl)]]diboronic acid <2> [15]
[benzene-1,4-diylbis[oxycarbonyl(5-nitrobenzene-3,1-diyl)]]diboronic acid <2> [16]
acetyl-Ala-Val-Leu-NHCH($CH_2CH_2CON(CH_3)_2$)-CHO <2> [31]
acetyl-Ser-Ala-Val-Leu-NHCH($CH_2CH_2CON(CH_3)_2$)-CHO <2> [31]
acetyl-Thr-Ser-Ala-Val-Leu-NHCH($CH_2CH_2CON(CH_3)_2$)-CHO <2> [31]
acetyl-Val-Leu-NHCH($CH_2CH_2CON(CH_3)_2$)-CHO <2> [31]
acetyl-leucylalanyl-alanyl-ketomethyl(cycloglutamine)-phthalhydrazide <2> [24]

acetyl-valyl-(O-benzyloxy)threonyl-leucyl-ketomethyl(cycloglutamine)-phthalhydrazide <2> [24]
benzyl (2S,3S)-3-tert-butoxy-1-((S)-3-cyclohexyl-1-oxo-1-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-ylamino)propan-2-ylamino)-1-oxobutan-2-ylcarbamate <2> [20]
benzyl [(1S)-1-benzyl-3-chloro-2-oxopropyl]carbamate <2> (<2> potent and selective inhibitor [34]) [34]
benzyl [(1S)-3-chloro-1-(4-fluorobenzyl)-2-oxopropyl]carbamate <2> (<2> potent and selective inhibitor [34]) [34]
benzyl [(1S)-3-chloro-1-(naphthalen-2-ylmethyl)-2-oxopropyl]carbamate <2> (<2> potent and selective inhibitor [34]) [34]
benzyl [(1S,4S,7S,8R,9R,10S,13S,16S)-7,10-dibenzyl-8,9-dihydroxy-1,16-dimethyl-4,13-bis(1-methylethyl)-2,5,12,15,18-pentaoxo-20-phenyl-19-oxa-3,6,11,14,17-pentaazaicos-1-yl]carbamate <2> [16]
benzyl [(2S)-1-[[(2S)-1-(1,3-benzothiazol-2-yl)-5-(diethylamino)-1,5-dioxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate <2> [44]
benzyl [(2S)-1-[[(2S)-1-[[(2S)-1-(1,3-benzothiazol-2-yl)-5-(diethylamino)-1,5-dioxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate <2> [44]
benzyl [(2S)-1-[[(2S)-1-[[(2S)-5-(diethylamino)-1,5-dioxo-1-(1,3-thiazol-2-yl)-pentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate <2> [44]
benzyl [(2S)-1-[[(2S)-5-(diethylamino)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate <2> [44]
benzyl [(2S)-1-[[(2S)-5-(diethylamino)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]carbamate <2> [44]
benzyl [(7S,8R,9R,10S)-8,9-dihydroxy-7,10-bis(1H-indol-3-ylmethyl)-1,16-dimethyl-4,13-bis(1-methylethyl)-2,5,12,15,18-pentaoxo-20-phenyl-19-oxa-3,6,11,14,17-pentaazaicos-1-yl]carbamate <2> (<2> highly selective for the 3CL protease and that no inhibitionis observed against HIV protease at 0.1 mM [14]) [14]
betulinic acid <2> (<2> competitive [22]) [22]
bis[1,3]thiazolo[4,5-b:5',4'-e]pyridine-2,6-diamine <2> [15,16]
celastrol <2> (<2> competitive inhibitor [45]) [45]
cinaserin <2> [15,35]
curcumin <2> [22,45]
diethyl (2E,2'E)-3,3'-[sulfonylbis(benzene-4,1-diylimino)]bis(2-cyanoprop-2-enoate) <2> [21]
dihydrocelastrol <2> [45]
esculetin-4-carboxylic acid ethyl ester <2> (<2> a novel class of anti-SARS agents from the tropical marine sponge Axinella corrugata [8]) [8]
ethyl (2E)-4-[(N-[(2E)-3-[4-(dimethylamino)phenyl]prop-2-enoyl]-L-phenylalanyl)amino]-5-phenylpent-2-enoate <2> [16]
ethyl (2E,4S)-4-[[(2R,5S)-2-benzyl-6-methyl-5-[[(5-methylisoxazol-3-yl)carbonyl]amino]-4-oxoheptanoyl]amino]-5-[(3S)-3-methyl-2-oxopyrrolidin-3-yl]-pent-2-enoate <2> [11]

ethyl (2E,4S)-4-[[(2R,5S)-5-[(N-tert-butyl-L-seryl)amino]-6-methyl-2-(3-methylbut-2-en-1-yl)-4-oxoheptanoyl]amino]-5-[(3S)-3-methyl-2-oxopyrrolidin-3-yl]-pent-2-enoate <2> [11]
ethyl (2E,4S)-4-[[(2R,5S)-6-methyl-2-(3-methylbut-2-en-1-yl)-5-[[(5-methylisoxazol-3-yl)carbonyl]amino]-4-oxoheptanoyl]amino]-5-[(3S)-3-methyl-2-oxopyrrolidin-3-yl]pent-2-enoate <2> [11]
extracts of Rheum palmatum <2> [48]
hexachlorophene <2> [15,16]
iguesterin <2> (<2> competitive inhibitor [45]) [45]
methyl 3-([N-[(benzyloxy)carbonyl]-L-valyl]amino)-5-fluoro-4-oxopentanoate <2> (<2> potent and selective inhibitor [34]) [34]
methyl 4-hydroxy-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carboxylate <2> [15,16]
niclosamide <2> [22]
phenylmercuric acetate <2> [16]
phenylmercuric nitrate <2> [16]
pristimerin <2> (<2> competitive inhibitor [45]) [45]
savinin <2> (<2> competitive [22]) [22]
sulfonyldi-4,1-phenylene bis(2,3,3-trichloroacrylate) <2> [21]
tert-butyl (3S)-3-[[(benzyloxy)carbonyl]amino]-5-bromo-4-oxopentanoate <2> (<2> potent and selective inhibitor [34]) [34]
tetraethyl 2,2'-[sulfonylbis(benzene-4,1-diyliminomethylylidene)]dipropanedioate <2> [21]
thimerosal <2> [16]
tingenone <2> (<2> competitive inhibitor [45]) [45]
toluene-3,4-dithiolato zinc <2> [16]
zinc N-ethyl-N-phenyldithiocarbamate <2> [38]
Additional information <2> (<2> molecular docking identifies the binding of 3-chloropyridine moieties specifically to the S_1 pocket of SARS-CoV Mpro [10]; <2> based on the X-ray structure of 3CLPro co-crystallized with a trans-α,β-unsaturated ethyl ester, a set of QM/QM and QM/MM calculations are performed, yielding three models with increasingly higher the number of atoms. It is suggested 3CLPro inhibitors need small polar groups to decrease the energy barrier for alkylation reaction. The results can be useful for the development of new compounds against SARS [39]; <2> extracts from Rheum palmatum have a high level of inhibitory activity against 3CL protease with IC50 of 0.01376 mg/ml and an inhibition rate of up to 96% [48]) [10,39,48]

Turnover number (s^{-1})

0.00004 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(297-306) [26]) [26]
0.00021 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme R298A/Q299A [26]) [26]
0.0006 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(298-306) [26]) [26]

0.0007 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q299A [26]) [26]
0.001 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(299-306) [26]) [26]
0.0017 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q299N [26]) [26]
0.0021 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q299K [26]) [26]
0.0022 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q200E [26]) [26]
0.0034 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme R298A [26]) [26]
0.0048 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme R298L [26]; <2> mutant enzyme S139A/Q299A [26]) [26]
0.0069 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme S123a/R298A [26]) [26]
0.0153 <2> ((Ala-Arg-Leu-Gln-NH)$_2$-rhodamine, <2> rate of hydrolysis measured by change in absorbance at 496 nm [3]) [3]
0.017 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(300-306) [26]; <2> mutant enzyme R298K [26]) [26]
0.022 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(303-306) [26]) [26]
0.025 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(301-306) [26]) [26]
0.027 <2> (Ser-Ala-Val-Leu-Gln-Met-Gly-Phe-Arg-Lys, <2> wild-type protein [49]) [49]
0.027 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(304-306) [26]) [26]
0.03 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(302-306) [26]) [26]
0.032 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> wild-type enzyme [26]) [26]
0.033 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme S123A [26]; <2> mutant enzyme S139A [26]) [26]
0.035 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme S123C [26]) [26]
0.039 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(305-306) [26]) [26]
0.1 <2> (Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide, <2> R298A mutant protein [47]) [47]
0.14 <2> ([4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid], <2> mutant enzyme Q189A [9]) [9]
0.16 <2> ([4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid], <2> wild-type enzyme [9]) [9]

0.27 <2> (Ser-Ala-Val-Leu-Gln-Met-Gly-Phe-Arg-Lys, <2> T25G mutant protein [49]) [49]
0.31 <2> (Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide, <2> E166A mutant protein [47]) [47]
0.38 <2> (*o*-aminobenzoyl-TSAVLQSGFRY(NO2)G) [8]
0.48 <2> (Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide, <2> R298L mutant protein [47]) [47]
0.6 <2> (SITSAVLQ-*p*-nitrophenyl ester) [7]
0.63 <2> (Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide, <2> wild-type protein [47]) [47]
0.847 <2> (TFTRLQSLENV, <2> pH 7.3, room temperature [27]) [27]
0.86 <2> (SITSAVLQ-*p*-nitroanilide) [7]
1.5 <2> ([4-(4-dimethylaminophenylazo)benzoic acid]-KTSAVLQSGFRKME-5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid) [7]
1.57 <2> (FYPKLQASQAW, <2> pH 7.3, room temperature [27]) [27]
1.6 <2> (SAVLQMGFRK, <2> wild type enzyme, at 37°C [49]) [49]
1.68 <2> (PHTVLQAVGAC, <2> pH 7.3, room temperature [27]) [27]
2.55 <2> (SGVTFQGKFKK, <2> pH 7.3, room temperature [27]) [27]
3.29 <2> (ATVRLQAGNAT, <2> pH 7.3, room temperature [27]) [27]
8.5 <2> (SITSAVLQSGFRKMA) [7]
12.2 <2> (TSAVLQSGFRK-NH_2, <2> pH 7.3, room temperature [27]) [27]
16.2 <2> (SAVLQMGFRK, <2> mutant enzyme T25G, at 37°C [49]) [49]
76 <2> (GPFVDRQTAQAAGTDT-NH_2, <2> pH 7.5, 37°C, mutant enzyme R188I [31]) [31]
455 <2> (KVATVQSKMSD-NH_2, <2> pH 7.5, 37°C, mutant enzyme R188I [31]) [31]
4753 <2> (TSAVLQSGFRK-NH_2, <2> pH 7.5, 37°C, mutant enzyme R188I [31]) [31]
Additional information <2> (<2> steady-state analysis of the solvent isotope effects on k_{cat} [7]) [7]

K_m-Value (mM)

0.004 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(303-306) [26]) [26]
0.005 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q200E [26]; <2> mutant enzyme Q299N [26]) [26]
0.006 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(304-306) [26]) [26]
0.01 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme R298A/Q299A [26]; <2> mutant enzyme S139A [26]) [26]
0.011 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> wild-type enzyme [26]; <2> mutant enzyme R298A [26]) [26]
0.012 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme S123a/R298A [26]) [26]
0.013 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(299-306) [26]; <2> mutant enzyme R298L [26]; <2> mutant enzyme S123A [26]; <2> mutant enzyme S123C [26]) [26]

0.014 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(305-306) [26]) [26]
0.015 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(300-306) [26]) [26]
0.016 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme R298K [26]) [26]
0.018 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q299A [26]) [26]
0.0186 <2> (SAVLQMGFRK, <2> mutant enzyme T25G, at 37°C [49]) [49]
0.0186 <2> (Ser-Ala-Val-Leu-Gln-Met-Gly-Phe-Arg-Lys, <2> T25G mutant protein [49]) [49]
0.021 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(297-306) [26]; <2> mutant enzyme S139A/Q299A [26]) [26]
0.022 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(302-306) [26]) [26]
0.03 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Δ(298-306) [26]; <2> mutant enzyme Δ(301-306) [26]) [26]
0.0338 <2> (TSAVLQSGFRK-NH_2, <2> pH 7.5, 37°C, mutant enzyme R188I [31]) [31]
0.037 <2> (*o*-aminobenzoyl-TSAVLQSGFRK-2,4-dinitrophenyl amide, <2> mutant enzyme Q299K [26]) [26]
0.03817 <2> ([4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid], <2> mutant enzyme Q189A [9]) [9]
0.045 <2> ([4-(4-dimethylaminophenylazo)benzoic acid]-KTSAVLQSGFRKME-5-['-(aminoethyl)amino]-naphthalenesulfonic acid) [7]
0.046 <2> (GPFVDRQTAQAAGTDT-NH_2, <2> pH 7.5, 37°C, mutant enzyme R188I [31]) [31]
0.04938 <2> ([4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2'-(aminoethyl)amino]-naphthalenesulfonic acid], <2> wild-type enzyme [9]) [9]
0.051 <2> (KVATVQSKMSD-NH_2, <2> pH 7.5, 37°C, mutant enzyme R188I [31]) [31]
0.073 <2> (SITSAVLQ-*p*-nitrophenyl ester) [7]
0.0766 <2> (SAVLQMGFRK, <2> wild type enzyme, at 37°C [49]) [49]
0.0766 <2> (Ser-Ala-Val-Leu-Gln-Met-Gly-Phe-Arg-Lys, <2> wild-type protein [49]) [49]
0.146 <2> (*o*-aminobenzoyl-TSAVLQSGFRY(NO2)G) [8]
0.18 <2> (SITSAVLQ-*p*-nitroanilide) [7]
0.2226 <2> (Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide, <2> wild-type protein [47]) [47]
0.286 <2> (TFTRLQSLENV, <2> pH 7.3, room temperature [27]) [27]
0.306 <2> ((Ala-Arg-Leu-Gln-$NH)_2$-rhodamine, <2> rate of hydrolysis measured by change in absorbance at 496 nm [3]) [3]
0.3534 <2> (Thr-Ser-Ala-Val-Leu-Gln-*p*-nitroanilide, <2> E166A mutant protein [47]) [47]

0.549 <2> (FYPKLQASQAW, <2> pH 7.3, room temperature [27]) [27]
0.583 <2> (SGVTFQGKFKK, <2> pH 7.3, room temperature [27]) [27]
0.6 <2> (SITSAVLQSGFRKMA) [7]
1.15 <2> (TSAVLQSGFRK-NH_2, <2> pH 7.3, room temperature [27]) [27]
1.44 <2> (ATVRLQAGNAT, <2> pH 7.3, room temperature [27]) [27]
1.94 <2> (PHTVLQAVGAC, <2> pH 7.3, room temperature [27]) [27]
61.9 <2> (SWTSAVLQSGFRKWA, <2> HPLC-based cleavage assay, measurement of fluorescence emission at 353 nm [4]) [4]
Additional information <2> (<2> steady-state analysis of the solvent isotope effects on K_M-value [7]) [7]

K_i-Value (mM)

6e-018 <2> ($C_{17}H_{20}N_2S$, promazine, <2> in silico binding studies [2]) [2]
8.7e-017 <2> ($C_{37}H_{48}N_4O_5$, lopinavir, <2> in silico binding studies [2]) [2]
2.1e-016 <2> ($C_{17}H_{19}ClN_2O_2S$, UC2, <2> in silico binding studies [2]) [2]
0.0000075 <2> (1-[(1H-indol-5-ylcarbonyl)oxy]-1H-1,2,3-benzotriazole) [13]
0.0000111 <2> (4-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]-N,N-diethylaniline) [13]
0.0000121 <2> (4-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]-N-methylaniline) [13]
0.0000123 <2> (1-[(1H-indol-2-ylcarbonyl)oxy]-1H-1,2,3-benzotriazole) [13]
0.0000138 <2> (1-[[(5-fluoro-1H-indol-2-yl)carbonyl]oxy]-1H-1,2,3-benzotriazole) [13]
0.0000174 <2> (4-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]-N,N-dimethylaniline) [13]
0.0000195 <2> (2-[(1H-1,2,3-benzotriazol-1-yloxy)carbonyl]aniline) [13]
0.0000229 <2> (1-[(1H-benzimidazol-5-ylcarbonyl)oxy]-1H-1,2,3-benzotriazole) [13]
0.00003 <2> (N-(2-chloro-4-nitrophenyl)-N^{α}-[[4-(dimethylamino)phenyl]carbonyl]phenylalaninamide) [15,16]
0.000038 <2> (N-[(benzyloxy)carbonyl]-3-[(2,2-dimethylpropanoyl)amino]-L-alanyl-N-[(1S,2E)-4-oxo-1-[[(3S)-2-oxopyrrolidin-3-yl]methyl]pent-2-en-1-yl]-L-leucinamide) [38]
0.000038 <2> (TG-0204998, <2> in 10 mM MES, pH 6.5, and 25°C [38]) [38]
4e-005 <2> ([benzene-1,2-diylbis[methanediylcarbamoyl(5-nitrobenzene-3,1-diyl)]]diboronic acid) [16]
0.00004 <2> ([benzene-1,4-diylbis[carbamoyl(5-nitrobenzene-3,1-diyl)]]diboronic acid) [15]
0.000053 <2> (benzyl (2S,3S)-3-tert-butoxy-1-((S)-3-cyclohexyl-1-oxo-1-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-ylamino)propan-2-ylamino)-1-oxobutan-2-ylcarbamate) [20]
0.000054 <2> (N-[(benzyloxy)carbonyl]-O-tert-butylthreonyl-N-[(1S)-1-formyl-2-[(3S)-2-oxopyrrolidin-3-yl]ethyl]-L-phenylalaninamide) [38]
0.000054 <2> (TG-0205221, <2> in 10 mM MES, pH 6.5, and 25°C [38]) [38]
0.000058 <2> (4-[2-(2-benzyloxycarbonylamino-3-tert-butoxy-butyrylamino)-4-methyl-pentanoylamino]-5-(2-oxo-pyrrolidin-3-yl)-pent-2-enoic acid ethyl ester) [20]

0.000058 <2> (N-[(benzyloxy)carbonyl]-O-tert-butylthreonyl-N-[(1S,2E)-4-ethoxy-4-oxo-1-[[(3S)-2-oxopyrrolidin-3-yl]methyl]but-2-en-1-yl]-L-leucinamide) [38]
0.000058 <2> (TG-0203770, <2> in 10 mM MES, pH 6.5, and 25°C [38]) [38]
0.000073 <2> (benzyl [(7S,8R,9R,10S)-8,9-dihydroxy-7,10-bis(1H-indol-3-ylmethyl)-1,16-dimethyl-4,13-bis(1-methylethyl)-2,5,12,15,18-pentaoxo-20-phenyl-19-oxa-3,6,11,14,17-pentaazaicos-1-yl]carbamate) [14]
0.000099 <2> (N-[(benzyloxy)carbonyl]-O-tert-butylthreonyl-N-[(1S,2E)-4-cyclopropyl-4-oxo-1-[[(3S)-2-oxopyrrolidin-3-yl]methyl]but-2-en-1-yl]-L-leucinamide) [38]
0.000099 <2> (TG-0205486, <2> in 10 mM MES, pH 6.5, and 25°C [38]) [38]
0.00017 <2> (1-hydroxypyridine-2-thione zinc) [16]
0.0003 <2> (phenylmercuric nitrate) [16]
0.000306 <2> (benzyl [(1S)-1-benzyl-3-chloro-2-oxopropyl]carbamate) [34]
0.00034 <2> (2,5-bis[[(benzyloxy)carbonyl]amino]-1,2,5,6-tetradeoxy-1,6-di-1H-indol-3-yl-L-iditol) [14]
0.000371 <2> (benzyl [(1S)-3-chloro-1-(naphthalen-2-ylmethyl)-2-oxopropyl]carbamate) [34]
0.00038 <2> (benzyl [(1S)-3-chloro-1-(4-fluorobenzyl)-2-oxopropyl]carbamate) [34]
0.0004 <2> (tert-butyl (3S)-3-[[(benzyloxy)carbonyl]amino]-5-bromo-4-oxopentanoate) [34]
0.0005 <2> (Hg^{2+}) [16]
0.0005 <2> (ethyl (2E)-4-[(N-[(2E)-3-[4-(dimethylamino)phenyl]prop-2-enoyl]-L-phenylalanyl)amino]-5-phenylpent-2-enoate) [16]
0.000512 <2> (methyl 3-([N-[(benzyloxy)carbonyl]-L-valyl]amino)-5-fluoro-4-oxopentanoate) [34]
0.0006 <2> (2-([N-[(benzyloxy)carbonyl]-L-alanyl-L-valyl]amino)-5-[[(2S,5S)-5-[[(benzyloxy)carbonyl]amino]-2-(1-methylethyl)-4-oxohexanoyl]amino]-1,2,5,6-tetradeoxy-1,6-diphenyl-L-iditol) [15]
0.0006 <2> (benzyl (2S,3S)-3-tert-butoxy-1-((S)-3-cyclohexyl-1-oxo-1-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-ylamino)propan-2-ylamino)-1-oxobutan-2-ylcarbamate) [20]
0.0006 <2> (benzyl [(1S,4S,7S,8R,9R,10S,13S,16S)-7,10-dibenzyl-8,9-dihydroxy-1,16-dimethyl-4,13-bis(1-methylethyl)-2,5,12,15,18-pentaoxo-20-phenyl-19-oxa-3,6,11,14,17-pentaazaicos-1-yl]carbamate) [16]
0.00066 <2> (4-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-5-(2-oxo-pyrrolidin-3-yl)-pent-2-enoic acid ethyl ester) [20]
0.0007 <2> (phenylmercuric acetate) [16]
0.0008 <2> (iguesterin) [45]
0.001 <2> (N-ethyl-N-phenyldithiocarbamic acid zinc) [16]
0.0011 <2> (Zn^{2+}) [16]
0.0014 <2> (toluene-3,4-dithiolato zinc) [16]
0.0022 <2> (4,5-anhydro-2-([N-[(benzyloxy)carbonyl]-L-phenylalanyl]amino)-1,2-dideoxy-D-erythro-pent-3-ulose) [6]

0.0022 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(2S)-1-oxo-3-[(3S)-2-oxo-pyrrolidin-3-yl]-1-(1,3-thiazol-2-yl)propan-2-yl]-L-leucinamide) [44]
0.00226 <2> (4-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-3-phenyl-propionylamino]-5-(2-oxo-pyrrolidin-3-yl)-pent-2-enoic acid ethyl ester) [20]
0.0024 <2> (thimerosal) [16]
0.0031 <2> (pristimerin) [45]
0.004 <2> (tingenone) [45]
0.0042 <2> (celastrol) [45]
0.0045 <2> ([benzene-1,4-diylbis[oxycarbonyl(5-nitrobenzene-3,1-diyl)]]diboronic acid) [16]
0.006 <2> ([benzene-1,2-diylbis[methanediyloxycarbonyl(5-nitrobenzene-3,1-diyl)]]diboronic acid) [16]
0.006 <2> ([benzene-1,3-diylbis[oxycarbonyl(5-nitrobenzene-3,1-diyl)]]diboronic acid) [16]
0.0082 <2> (betulinic acid) [22]
0.0091 <2> (savinin) [22]
0.0137 <2> (hexachlorophene) [15,16]
0.016 <2> ([3-([[3-(dihydroxyboranyl)benzyl]oxy]carbonyl)-5-nitrophenyl]-boronic acid) [16]
0.021 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-1,1,1-trifluoro-6-(morpholin-4-yl)-2,6-dioxohexan-3-yl]-L-leucinamide) [44]
0.0341 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-6-[benzyl(methyl)amino]-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide) [44]
0.0452 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(2S)-1,5-dioxo-1,5-di(1,3-thiazol-2-yl)pentan-2-yl]-L-leucinamide) [44]
0.0493 <2> (benzyl [(2S)-1-[[(2S)-1-[[(2S)-1-(1,3-benzothiazol-2-yl)-5-(diethylamino)-1,5-dioxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate) [44]
0.061 <2> (1-[bis(4-chlorophenyl)methyl]-3-[2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazol-3-ium) [15]
0.112 <2> (benzyl [(2S)-1-[[(2S)-1-[[(2S)-5-(diethylamino)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate) [44]
0.116 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-5-carboxy-1,1,1-trifluoro-2-oxopentan-3-yl]-L-leucinamide) [44]
0.135 <2> (N-[(benzyloxy)carbonyl]-L-alanyl-L-valyl-N-[(3S)-6-amino-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide) [44]
0.159 <2> (benzyl [(2S)-1-[[(2S)-1-(1,3-benzothiazol-2-yl)-5-(diethylamino)-1,5-dioxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate) [44]
0.297 <2> (N-[(benzyloxy)carbonyl]-L-alanyl-L-valyl-N-[(3S)-6-(dipropylamino)-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide) [44]
0.363 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(3S)-6-(dipropylamino)-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide) [44]
0.462 <2> (benzyl [(2S)-1-[[(2S)-5-(diethylamino)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]carbamate) [44]

0.478 <2> (N-[(benzyloxy)carbonyl]-L-valyl-N-[(2S)-5-(morpholin-4-yl)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]-L-leucinamide) [44]
0.584 <2> (N^2-[(benzyloxy)carbonyl]-N-[(3S)-6-(dipropylamino)-1,1,1-trifluoro-2,6-dioxohexan-3-yl]-L-leucinamide) [44]
0.614 <2> (benzyl [(2S)-1-[[(2S)-5-(diethylamino)-1,5-dioxo-1-(1,3-thiazol-2-yl)pentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]carbamate) [44]

pH-Optimum

7 <2> (<2> substrate: TSAVLQSGFRK-NH_2 [27]) [4,27]
7.4 <2> (<2> wild-type enzyme [15]) [15]
7.6 <2> (<2> mutant enzyme C145S [15]) [15]
8 <2> [3]

pH-Range

6-9 <2> (<2> pH 6: about 50% of maximal activity, pH 9: about 65% of maximal activity, substrate: TSAVLQSGFRK-NH_2 [27]) [27]
6.3-8.7 <2> (<2> pH 6.3: about 55% of maximal activity, pH 8.7: about 45% of maximal activity, wild-type enzyme [15]) [15]
6.3-9.3 <2> (<2> pH 6.3: about 55% of maximal activity, pH 9.3: about 45% of maximal activity, mutant enzyme C145S [15]) [15]

pi-Value

6.24 <2> (<2> mutant enzyme R188I, calculated from sequence [31]) [31]

Temperature optimum (°C)

42 <2> [3]

4 Enzyme Structure

Molecular weight

33760 <2> (<2> determined by MALDI [3]) [3]

Subunits

dimer <2> (<2> one monomer per asymmetric unit, dimer is generated through the crystallographic twofold [1]; <2> by comparing molecular dynamics simulation of dimer and monomer, the indirect reasons for the inactivation of the monomer are found, that is the conformational variations of the active site in the monomer relative to dimer [25]; <2> dimerization is important for enzyme activity and only one active protomer in the dimer is enough for the catalysis [18]; <2> the enzyme exists as a mixture of monomer and dimer at a higher protein concentration (4 mg/ml) and exclusively as a monomer at a lower protein concentration [16]; <2> the SARS coronavirus main proteinase is a homodimer. Investigation of the monomer-dimer equilibrium [5]; <2> a mixture of monomer and dimer at a protein concentration of 4 mg/ml and mostly monomer at 0.2 mg/ml. The dimer may be the biological functional form of the protein [27]; <2> in solution the wild type protease exhibits both forms of monomer and dimer and the amount of the monomer is almost equal to that of the dimeric form [37]; <2> is only enzy-

matically active as a homodimer. Arg298 serves as a key component for maintaining dimerization, and consequently, its mutation will trigger a cooperative switch from a dimer to a monomer. The monomeric enzyme is irreversibly inactivated because its catalytic machinery is frozen in the collapsed state, characteristic of the formation of a short 310-helix from an active-site loop. Dimerization appears to be coupled to catalysis in 3CLpro through the use of overlapped residues for two networks, one for dimerization and another for the catalysis [41]; <2> SARS-CoV Mpro exists in solution as an equilibrium of both monomeric and dimeric forms, and the dimeric form is the enzymatically active form [40]; <2> wild type and Δ(300-306) proteases exist with dimer as the major form. The major form becomes monomeric in Δ(299-306), Δ(298-306) and Δ(297-306) [26]) [1,5,16,18,25,26,27,37,40,41]
homodimer <2,3> (<2> X-ray crystallography [38]; <2> analytical ultracentrifugation, gel filtration [47]; <3> only the dimeric enzyme is active [51]; <2> tendency of substrate induced dimer formation, gel filtration, analytic ultracentrifugation [50]) [38,47,50,51]
monomer <2> (<2> by comparing molecular dynamics simulation of dimer and monomer, the indirect reasons for the inactivation of the monomer are found, that is the conformational variations of the active site in the monomer relative to dimer [25]; <2> the enzyme exists as a mixture of monomer and dimer at a higher protein concentration (4 mg/ml) and exclusively as a monomer at a lower protein concentration [16]; <2> a mixture of monomer and dimer at a protein concentration of 4 mg/ml and mostly monomer at 0.2 mg/ml. The dimer may be the biological functional form of the protein [27]; <2> in solution the wild type protease exhibits both forms of monomer and dimer and the amount of the monomer is almost equal to that of the dimeric form [37]; <2> wild type and Δ(300-306) proteases exist with dimer as the major form. The major form becomes monomeric in Δ(299-306), Δ(298-306) and Δ(297-306) [26]) [16,25,26,27,37]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [19]
<2> [6,20,21,24,27,37]
<2> (E166A mutant protein: immobilized metal ion affinity chromatography (Ni^{2+})) [47]
<2> (Ni-NTA column chromatography) [49]
<2> (ammonium sulfate precipitation and anion-exchange column chromatography) [48]
<2> (ammonium sulfate precipitation, anion-exchange chromatography) [48]
<2> (commercial preparation) [45]
<2> (fused to maltose-binding protein, removing the maltose-binding protein by cleavage with factor Xa, purification by Phenyl Sepharose column chromatography) [1]

<2> (glutathione S-transferase column chromatography and HiTrap 26/10 QFF column chroamtography) [38]
<2> (immobilized metal ion affinity chromatography (Ni^{2+})) [49]
<2> (purification of proteolysis-resistant mutant R188I of the SARS 3CL protease) [31]
<2> (recombinant His-tagged SARS-CoV 3CL protease) [8]
<2> (recombinant enzyme) [34]
<2> (strong cation column chromatography connected in series to a strong anion column chromatography) [3]
<2> (wild-type enzyme and C-terminally truncated proteases) [26]
<3> (glutathione Sepharose column chromatography and Superdex 75 gel filtration) [51]

Renaturation

<2> (reversible unfolding of SARS-CoV main protease in guanidine-HCl. In the presence of 6 M of guanidine-HCl, the enzyme is completely unfolded in 10 min. The unfolding is completely reversible. A 10fold dilution induces refolding of the enzyme to a yield of 92-95%) [12]

Crystallization

<1> (crystal structure of monomeric mutant enzyme G11A) [19]
<2> [21]
<2> (1.8 A X-ray crystal structure of 3Clpro bound to an irreversible inhibitor, an α,β-epoxyketone) [6]
<2> (SARS 3CLpro bound to two phthalhydrazide-charged peptidyl inhibitors, acetyl-valyl-(O-benzyloxy)threonyl-leucyl-ketomethyl(cycloglutamine)-phthalhydrazide and acetyl-leucylalanyl-alanyl-ketomethyl(cycloglutamine)-phthalhydrazide. The inhibitors are covalently attached to SARS 3CLpro in crystals) [24]
<2> (X-ray, resolution of 2.0 A for tetragonal crystals, 2.14 A for monoclinic crystals and 2.8 A for orthorhombic crystals, pH-dependent change of structure) [4]
<2> (complexed with inhibitors TG-0204998 and TG-0205486, sitting drop vapor diffusion method, using 3-6% (w/v) PEG 6000, 4-6% (v/v) DMSO or methyl-2,4-pentanediol, 1 mM dithiothreitol, 0.1 M MES, pH 6.5) [38]
<2> (crystal structures of 3Cpro from CVB3 and 3CLpro from CoV-229E and SARS-CoV in complex with inhibitors are solved) [38]
<2> (crystals grown in hanging-drop vapour-diffusion method) [1]
<2> (enzyme-inhibitor complex, hanging-drop method) [34]
<2> (hanging-drop vapor-diffusion method. Crystal structure of the enzyme at a resolution of 1.82 A, in space group P21 at pH 6.0. Two crystal structures of Mpro having an additional Ala at the N terminus of each protomer (M+A(-1)pro), both at a resolution of 2.00 A, in space group P43212: one unbound and one bound by a substrate-like aza-peptide epoxide) [23]
<2> (monomeric crystal structure of the SARS-CoV 3CLpro mutant R298A at a resolution of 1.75 A, hanging drop method) [41]
<2> (sitting drop diffusion method, crystallization of SARS 3CLpro-inhibitor complexes) [20]

<2> (structures of monomeric and dimeric forms of the C-terminal domain of Mpro (Mpro-C). Mpro-C monomer maintains the same fold as that in the crystal structure of Mpro. On the other hand, the Mpro-C dimer has a novel structure characterized by 3D domain-swapping, which provides the structural basis for the dimer stability) [43]
<3> (hanging drop vapor diffusion method, crystals of S139A mutant are grown from the mother liquor containing 0.1 M MES pH 6.0, 10% (w/v) PEG 6000, 1 mM dithiohtreitol, and 5% (v/v) DMSO, crystals of F140A are grown at three pH values in 0.1 M MES pH 6.0/0.1 M MES pH 6.5/0.1 M Tris pH 7.6, with 10% (w/v) PEG 6000, 1 mM dithiothreitol, and 5% (v/v) DMSO) [51]

Cloning

<1> (mutant enzyme G11A and wild-type enzyme are expressed in Escherichia coli) [19]
<2> [6,20,37]
<2> (E166A mutant protein expressed in Escherichia coli BL21(DE3)) [47]
<2> (His-tagged SARS-CoV 3CL protease expressed in Escherichia coli) [8]
<2> (His-tagged artificial polyprotein (cyan fluorescent protein-SARS-CoV 3CLpro-yellow fluorescent protein) expressed in Escherichia coli) [50]
<2> (R298A protease is expressed in Escherichia coli strain BL21(DE3)) [41]
<2> (expressed in E. coli BL21) [3]
<2> (expressed in Escherichia coli) [48,49]
<2> (expressed in Escherichia coli BL21 cells) [38]
<2> (expression in Escherichia coli) [24,27]
<2> (fused to maltose-binding protein and expressed in Escherichia coli BL21) [1]
<2> (high level of expression of of proteolysis-resistant mutant R188I in Escherichia coli) [31]
<2> (wild type and His-tagged T25G mutant are expressed in Escherichia coli cells) [49]
<2> (wild-type and mutant glutathione S-transferase-fusion constructs are transformed into Escherichia coli BL21 cell strain for overexpression) [17]
<2> (wild-type enzyme and C-terminally truncated proteases, expression in Escherichia coli) [26]
<3> (expressed in Escherichia coli BL21(DE3) cells) [51]

Engineering

C145A <2> (<2> no irreversible inactivation by benzotriazole esters [13]) [13]
C300A <2> (<2> mutant enzyme shows more than 30% of wild-type activity [17]) [17]
E14A <2> (<2> the ratio of dimer to monomer in solution is 0.36, compared to 1 for the wild-type enzyme [37]) [37]
E166A <2> (<2> involved in connecting the substrate binding site with the dimer interface, dimerization influenced by substrate binding [47]) [47]
E166A/R298A <2> (<2> monomer [47]) [47]

E288A <2> (<2> mutant enzyme retains less than 10% of the wild-type activity [17]) [17]
F140A <2,3> (<2> the ratio of dimer to monomer in solution is 0.63, compared to 1 for the wild-type enzyme [37]; <3> mutant F140A is a dimer with the most collapsed active pocket discovered so far, well-reflecting the stabilizing role of this residue, the mutant enzyme is completely inactive [51]) [37,51]
F291A <2> (<2> activity is higher than that of wild-type enzyme [17]) [17]
F3A <2> (<2> the ratio of dimer to monomer in solution is 0.93, compared to 1 for the wild-type enzyme [37]) [37]
G11A <1> (<1> mutation entirely abolishes activity [19]) [19]
G283A <2> (<2> no significant activity differences from the wild-type protease [17]) [17]
I286A <2> (<2> activity is higher than that of wild-type enzyme [17]) [17]
L282A <2> (<2> mutant enzyme shows more than 30% of wild-type activity [17]) [17]
N214A <2> (<2> mutant enzyme shows more than 30% of wild-type activity [17]) [17]
N289A <2> (<2> mutant enzyme retains less than 10% of the wild-type activity [17]) [17]
Q189A <2> (<2> k_{cat} for the substrate [4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2-(aminoethyl)amino]-naphthalenesulfonic acid] is 1.14 fold lower than wild-type value, K_m-value for the substrate [4-(4-dimethylaminophenylazo)benzoic acid]-KNSTLQSGLRKE-[5-[2-(aminoethyl)amino]-naphthalenesulfonic acid] is 1.3fold lower than the wild-type value [9]) [9]
Q290A <2> (<2> mutant enzyme retains less than 10% of the wild-type activity [17]) [17]
Q299A <2> (<2> mutant enzyme retains less than 10% of the wild-type activity [17]; <2> the quaternary structures of exists in a mixture of monomeric and dimeric forms [26]) [17,26]
Q299E <2> (<2> more than 90% loss of activity [26]) [26]
Q299K <2> (<2> more than 90% loss of activity [26]) [26]
Q299N <2> (<2> more than 90% loss of activity [26]) [26]
R188I <2> (<2> replacing Arg with Ile at position 188 renders the protease resistant to proteolysis. The catalytic ability of 3CL-R188I protease was found to be extreme as compared to that of a mature 3CL protease containing a C-terminal His tag. The k_{cat} values is 0.0203 per sec for mature 3CL protease and 4753 per sec for the 3CL-R188I [31]) [31]
R298A <2> (<2> mutant enzyme retains less than 10% of the wild-type activity [17]; <2> monomeric mutant [41]; <2> the quaternary structures of exists in a mixture of monomeric and dimeric forms [26]; <2> induce dimer dissociation (influenced by substrate binding), about 10fold decrease in activty [47]) [17,26,41,47]
R298A/Q299A <2> (<2> mutant is present almost exclusively in the monomeric form [26]; <2> monomer, no activity detected [47]) [26,47]
R298K <2> (<2> mutation has no significant effect on activity [26]) [26]

R298L <2> (<2> mutation destroys 85% of the enzyme activity [26]; <2> induce dimer dissociation (influenced by substrate binding), about 10fold decrease in activty [47]) [26,47]
R4A <2> (<2> the ratio of dimer to monomer in solution is 0.45, compared to 1 for the wild-type enzyme [37]) [37]
S10A <2> (<2> the ratio of dimer to monomer in solution is 0.66, compared to 1 for the wild-type enzyme [37]) [37]
S123A <2> (<2> mutation does not destroy the enzyme activity, the dimeric structure remains intact [26]) [26]
S123C <2> (<2> mutation does not destroy the enzyme activity, the dimeric structure remains intact [26]) [26]
S139A <2,3> (<2> mutation can destroy neither the enzyme activity nor the dimeric structure [26]; <2> mutation does not destroy the enzyme activity, the dimeric structure remains intact [26]; <2> the ratio of dimer to monomer in solution is 0.81, compared to 1 for the wild-type enzyme [37]; <3> mutant S139A is a monomer that still retains a small fraction of dimer in solution, which may account for its remaining activity [51]) [26,37,51]
S1A <2> (<2> the ratio of dimer to monomer in solution is 1.08, compared to 1 for the wild-type enzyme [37]) [37]
S284A <2> (<2> activity is higher than that of wild-type enzyme [17]) [17]
S284A/T285A/I286A <2> (<2> activity is 3.7fold higher than wild-type activity [17]) [17]
S301A <2> (<2> no significant activity differences from the wild-type protease [17]) [17]
T25G <2> (<2> activity like wild-type (substrate DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS), higher specific activity than wild-type protein for substrate Ser-Ala-Val-Leu-Gln-Met-Gly-Phe-Arg-Lys [49]; <2> the mutant enzyme has an expanded S1 space that demonstrates 43.5-fold better k_{cat}/K_m compared with wild type in cleaving substrates with a larger Met at P1, mutant enzyme T25G shows a 12fold and 8fold higher activity against the substrates with Met and Leu at P1, respectively [49]) [49]
T25S <2> (<2> almost complete loss of activity [49]) [49]
T280A <2> (<2> no significant activity differences from the wild-type protease [17]) [17]
T285A <2> (<2> activity is higher than that of wild-type enzyme [17]) [17]
Additional information <2> (<2> truncation of C-terminus from 306 to 300 has no appreciable effect on the quaternary structure, and the enzyme remains catalytically active. Further deletion of Gln299 or Arg298 drastically decreases the enzyme activity to 1-2% of wild type, and the major form is a monomeric one. The catalytic constant and specificity constant (k_{cat}/K_m) of the proteases are significantly decreased in the Δ(299-306), Δ(298-306), and Δ(297-306) mutants. Wild type and Δ(300-306) proteases exist with dimer as the major form. The major form becomes monomeric in Δ(299-306), Δ(298-306) and Δ(297-306) [26]; <2> without the N-finger, SARS-CoV Mpro can no longer retain the active dimer structure. It can form a new type of dimer which is inactive. Therefore, the N-finger of SARS-CoV Mpro is not only cri-

tical for its dimerization but also essential for the enzyme to form the enzymatically active dimer [40]) [26,40]

Application

analysis <2> (<2> developing a novel red-shifted fluorescence-based assay for 3CLpro and its application for identifying small-molecule anti-SARS agents from marine organisms [8]) [8]

medicine <2> (<2> SARS-3CLpro is a viral cysteine protease critical to the life cycle of the pathogen and hence a therapeutic target of importance [29]; <2> this enzyme is a target for the design of potential anti-SARS drugs [28]) [28,29]

6 Stability

Temperature stability

61 <2> (<2> Tm-value, sigmoid denaturation curve [27]) [27]

Organic solvent stability

guanidine-HCl <2> (<2> dimeric enzyme dissociates at guanidinium chloride concentration of less than 0.4 M, at which the enzymatic activity loss showes close correlation with the subunit dissociation. Further increase in guanidinium chloride induces a reversible biphasic unfolding of the enzyme. The unfolding of the C-terminal domain-truncated enzyme follows a monophasic unfolding curve. Unfolding curves of mutants of the full-length protease W31 and W207/W218 are monophasic but correspond to the first and second phases of the protease, respectively. The unfolding intermediate of the protease represents a folded C-terminal domain but an unfolded N-terminal domain, which is enzymatically inactive due to loss of regulatory properties [12]) [12]

General stability information

<2>, replacing Arg with Ile at position 188 renders the protease resistant to proteolysis [31]

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