The SARS-CoV-2 Main Protease as Drug Target

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Abstract

The unprecedented pandemic of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is threatening global health. The virus emerged in late 2019 and can cause a severe disease associated with significant mortality. Several vaccine development and drug discovery campaigns are underway. The SARS-CoV-2 main protease is considered a promising drug target, as it is dissimilar to human proteases. Sequence and structure of the main protease are closely related to those from other betacoronaviruses, facilitating drug discovery attempts based on previous lead compounds. Covalently binding peptidomimetics and small molecules are investigated. Various compounds show antiviral activity in infected human cells.

Graphical abstract
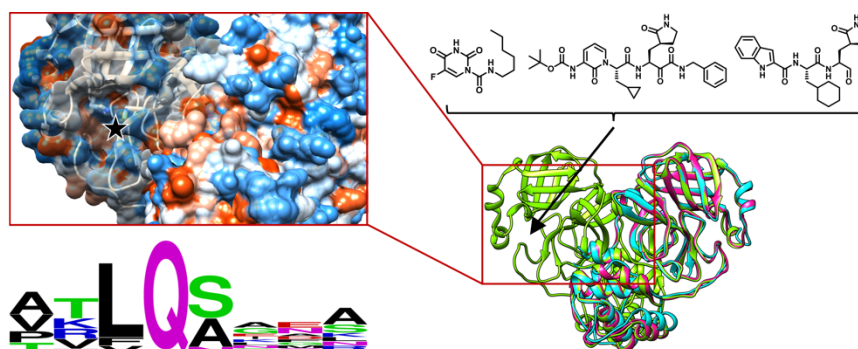
Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing COVID-19 (coronavirus disease 2019) pandemic. Globally, 10 million infections have been confirmed with 500,000 fatalities.[1] The novel coronavirus had first been reported in Hubei province of China in late 2019, where it caused a major cluster of atypical pneumonia.[2-4] Despite major efforts to contain the original outbreak, SARS-CoV-2 has since spread globally.[5-8] Following the 2002/2003 SARS and 2012 MERS (Middle East respiratory syndrome) epidemics, this marks the third notable coronavirus outbreak in the 21st century.[6, 9]

Four additional coronaviruses can infect humans, HCoV-229E, HCoV-HKU1, HCoV-NL63 and HCoV-OC43,[10] which in stark contrast to the highly contagious and pathogenic SARS-CoV, MERS-CoV and SARS-CoV-2,[7, 11, 12] cause only mild respiratory illness, like the common cold.[13] The case fatality rate (CFR) of COVID-19 is estimated to be lower than for SARS (~10%) and MERS (~35%). However, its basic reproduction number (R_0) is potentially higher than for SARS (~2-3) and MERS (<1). The exact values for CFR and R_0 of SARS-CoV-2 are still under controversial debate.[14, 15] Undetected, asymptomatic infections can challenge the accuracy of these parameters.[16-19] The ongoing COVID-19 pandemic has had an unprecedented impact on individuals and the economy, as travel restrictions, social distancing and quarantine measures were implemented by many countries.[20, 21]

SARS-CoV, MERS-CoV and SARS-CoV-2 belong to the family of *Coronaviridae* and the genus *Betacoronavirus*. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses that feature the largest known RNA virus genomes ranging approximately from 26 to 32 kb,[22-26] containing at least 6 (14 in case of SARS-CoV-2) open reading frames (ORFs).[27, 28] The major reading frame ORF 1ab encodes for two overlapping polyproteins (pp1a, pp1ab), which are cleaved into 16 non-structural proteins (nsp1-16) by the main protease M^{pro} (also referred to as 3CL^{pro}) and the papain-like protease PL^{pro} (Fig. 1).[27, 29-32] In addition, the papain-like protease is also a deubiquitinase.[33] The remainder of the genome encodes for accessory and structural proteins such as the spike glycoprotein (S), envelope protein (E), membrane protein (M) and the nucleocapsid phosphoprotein (N).[27, 29-32] The first genome sequence of SARS-CoV-2 deposited in Genbank was from the ~30 kb isolate Wuhan-Hu-1 (MN908947),[3] which is used for sequence-related analyses in this article.
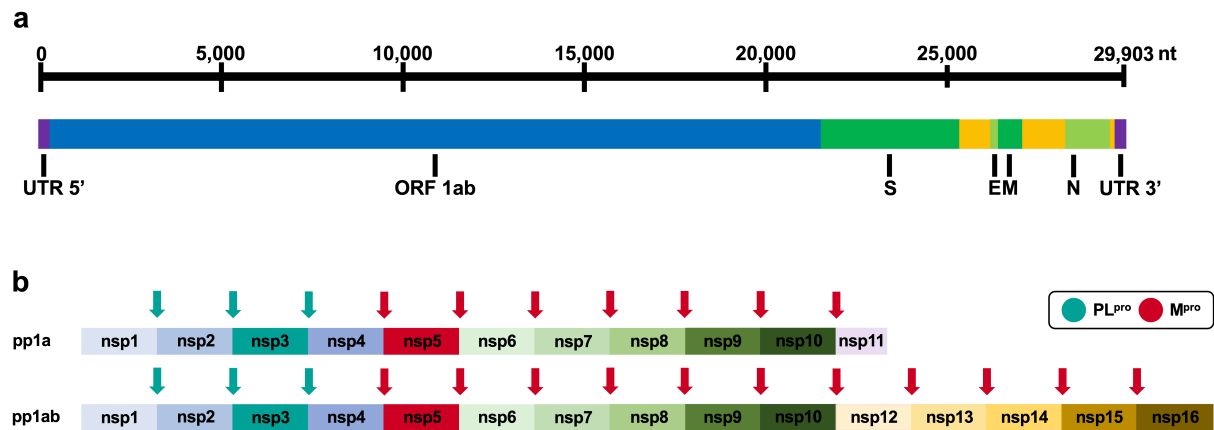
Fig. 1. (a) Organisation of the RNA genome of SARS-CoV-2 with selected genes (Wuhan-Hu-1 isolate MN908947). (b) Schematic representation of polyprotein cleavage sites of SARS-CoV-2. The papain-like protease PL^{pro} cleaves at 3 distinct sites. The main protease M^{pro} (also referred to as 3CL^{pro}) cleaves at 11 distinct sites.

SARS-CoV-2 is closely related to other viruses of the *Betacoronavirus* genus such as the bat coronavirus BatCoV RaTG13 (~96% sequence identity) and the SARS-CoV (~80% sequence identity).[34, 35] SARS-CoV and MERS-CoV are both of zoonotic origin, with bats being their natural reservoir. Transmission to humans can occur via their intermediate hosts palm civets (SARS) and dromedary camels (MERS).[36] SARS-CoV-2 is thought to have followed a similar evolutionary transmission cascade.[37, 38] The spike glycoprotein (S) plays an important role in host range (tropism) and 'host jumps'.[39, 40] In the case of SARS-CoV and SARS-CoV-2, it recognises the receptor angiotensin-converting enzyme 2 (ACE 2), which both viruses employ for cell entry.[41-43] Comparison of structural proteins of SARS-CoV-2, such as the spike protein (S), with those from animal coronaviruses indicate the involvement of bats as natural reservoir with a possibility for pangolins as intermediate hosts.[38, 44-49] As the introduction of coronaviruses into human population has been observed on multiple occasions, a better understanding of the naturally circulating viruses is of high interest for pandemic prevention as is antiviral research to prepare for future outbreaks.[50, 51]

The main protease as drug target

The current COVID-19 pandemic has triggered global efforts for the rapid identification of vaccines and specific antiviral treatments.[52-55] Amongst the coronaviral targets that have been studied in the past, the main protease (M^{pro}, 3CLpro, nsp5) received major attention,[25, 56] particularly following the first SARS-CoV outbreak in the early 2000s.[23, 57] Alternative

coronaviral targets include the spike protein (S), RNA-dependent RNA-polymerase (RdRp, nsp12), NTPase/helicase (nsp13) and papain-like protease (PL[pro], part of nsp3).[50, 58] The papain-like protease also recognises the C-terminal sequence of ubiquitin. Therefore, substrate-derived inhibitors of PL[pro] would be expected to also inhibit host-cell deubiquitinases, making drug-discovery campaigns against PL[pro] challenging. In stark contrast, the main protease M[pro] exclusively cleaves polypeptide sequences after a glutamine residue, positioning the main protease as an ideal drug target because, to the best of our knowledge, no human host-cell proteases are known with this substrate specificity.[59-61]

Viral proteases are well validated drug targets that have led to various approved drugs, for example, against chronic infections with human immunodeficiency virus (HIV) or hepatitis C virus (HCV), which employ aspartyl and serine proteases, respectively.[62] The SARS-CoV-2 M[pro] proteolytically cleaves the overlapping pp1a and pp1ab polyproteins to functional proteins (Fig. 1), which is a critical step during viral replication.[29, 63, 64] Replication-essential enzymes such as the RdRp or nsp13 cannot fully function without prior proteolytic release,[56] positioning M[pro] as a key enzyme in the viral replication cycle. Consequently, its inhibition can stall the production of infectious viral particles and thus alleviate disease symptoms.[23, 50, 65-68] Capitalising on knowledge gained on structure and inhibitors of M[pro] from previous epidemical coronaviruses, M[pro] is one of the most attractive viral targets for antiviral drug discovery against SARS-CoV-2.

Structure and function of the main protease

Early homology models of SARS-CoV-2 M[pro] indicated close structural relation to other coronaviral main proteases.[69] Amino acid sequence alignments reveal ~99% identity with BatCoV RaTG13 M[pro] and ~96% with the previous SARS-CoV M[pro]. In contrast, sequence identity with MERS-CoV M[pro] is only ~50% (Fig. 2).

Superimposition of the X-ray crystal structures of the main proteases of SARS-CoV-2, SARS-CoV and MERS-CoV indicates a high degree of structural similarity and conservation of the active site (Fig. 3). This might prove valuable for the development of pan-coronaviral drugs and has already been employed for the development of SARS-CoV-2 M[pro] inhibitors that were based on previous compounds targeting the SARS-CoV or MERS-CoV main proteases.

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SARS-CoV-2   1   SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDD VVYCPRHVICTS EDMLNPNYEDLLIR
SARS-CoV     1   SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDD TVYCPRHVICTA EDMLNPNYEDLLIR
MERS-CoV     1   SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDN TVWCPRHVMCPA DQLSDPNYDALLIS

SARS-CoV-2  61   KSNHN FLVQAG---NVQLRVIGHSMQNC VLKLKVDTANPKTPKYKFVRIQPGQTFSVLAC
SARS-CoV    61   KSNHS FLVQAG---NVQLRVIGHSMQNCLLR LKVDTS NPKTPKYKFVRIQPGQTFSVLAC
MERS-CoV    61   MTNHSF SVQKHIGAPAN LRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLAC

SARS-CoV-2 118   YNGSPSGVYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDL
SARS-CoV   118   YNGSPSGVYQCAMRPNHTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDL
MERS-CoV   121   YNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAF

SARS-CoV-2 178   EGNFYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKY
SARS-CoV   178   EGKFYGPFVDRQTAQAAGTDTTITLNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKY
MERS-CoV   181   DGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALAN

SARS-CoV-2 238   NYEPL-TQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDV
SARS-CoV   238   NYEPL-TQDHVDILGPLSAQTGIAVLDMCAALKELLQNGMNGRTILGSTILEDEFTPFDV
MERS-CoV   241   QFTEFVGTQSVDML---AVKTGVAIEQLLYAIQQL-YTGFQGKQILGSTMLEDEFTPEDV

SARS-CoV-2 297   VRQCSGVTFQ
SARS-CoV   297   VRQCSGVTFQ
MERS-CoV   297   NMQIMGVVMQ
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Fig. 2. Alignment of the amino acid sequences of crystallised main proteases of SARS-CoV-2 (PDB: 6Y2E), SARS-CoV (PDB: 2BX4) and MERS-CoV (PDB: 5C3N). Domains I, II and III comprise residues 8-101, 102-184 and 201-306, respectively. The catalytic dyads are indicated by asterisks. The alignment was generated using T-Coffee and shaded with Boxshade.
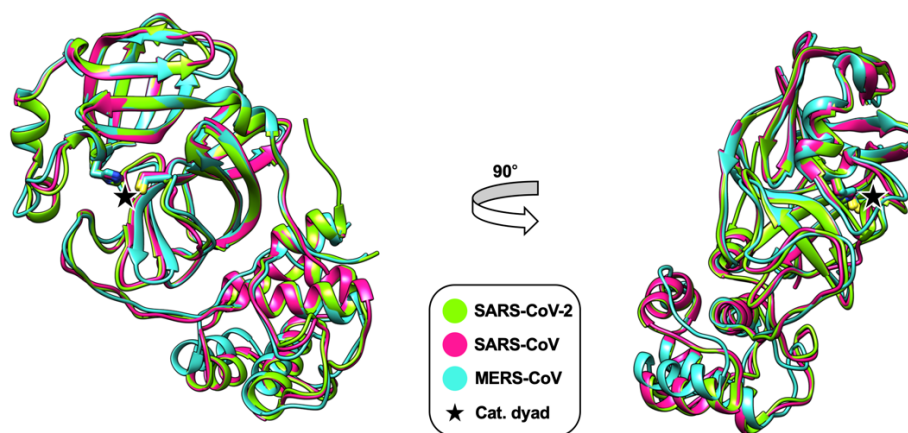

Fig. 3. Superimposition of X-ray crystal structures of the main proteases of SARS-CoV (pink, PDB: 2BX4), MERS-CoV (cyan, PDB: 5C3N) and SARS-CoV-2 (green, PDB: 6Y2E). Only the monomers are shown. Residues of the catalytic dyad are indicated (His41/Cys145 for SARS-CoV and SARS-CoV-2 and His41/Cys148 for MERS-CoV). The root-mean-square deviation (RMSD) of the superimpositions is 0.934 Å for SARS-CoV/MERS-CoV, 0.532 Å for SARS-CoV/SARS-CoV-2 and 0.905 Å for MERS-CoV/SARS-CoV-2. This figure was generated with UCSF Chimera.[70]

M[pro] is a cysteine protease with a catalytic dyad (cysteine and histidine) in its active centre (Fig. 3). While other cysteine and serine proteases contain a third catalytic residue, a buried water molecule occupies this place in the active site of M[pro].[23, 25, 71] The proteolytic process is believed to follow a multi-step mechanism. After the cysteine side chain proton is abstracted by the histidine's imidazole, the resulting thiolate nucleophile attacks the amide bond of the substrate. The N-terminal peptide product is released by proton abstraction from histidine before the thioester is hydrolysed to release the C-terminal product and restore the catalytic dyad.[72, 73]

M[pro] (nsp5) autocleaves itself between nsp4 and nsp6,[74, 75] before processing the overlapping polyproteins pp1a and pp1ab at 11 cleavage sites (Fig. 1).[29, 63, 64] While the M[pro] monomer is basically inactive, the homodimer is the primary active species with both protomers almost orthogonally aligned to each other (Fig. 4a).[68, 72] Each protomer consists of three domains (Fig. 4b). In case of SARS-CoV and SARS-CoV-2, domains I and II comprise residues 8-101 and 102-184, respectively, and include an antiparallel β-barrel with similarities to trypsin-like serine proteases.[57] Domain II is connected to Domain III (residues 201-306) via a longer loop region (residues 185-200). Domain III is characterised by a cluster of five α-helices.[57, 59, 76-79]

The protomers bind to each other via an N-terminal finger (residues 1-7) located between domains II and III, which is involved in the formation of the substrate-binding site in a cleft between domains I and II.[57, 59, 76-78] It is known that the mutations Ser284Ala, Thr285Ala and Ile286Ala in SARS-CoV M[pro] result in a 3.6-fold increase in catalytic activity.[80] Two similar mutations (Thr285Ala and Ile286Leu) are present in SARS-CoV-2 M[pro], explaining higher activity observed for SARS-CoV-2 M[pro] compared to SARS-CoV M[pro].[59] The Thr285Ala mutation is believed to bring domain III of both protomers closer to each other.[59, 79]
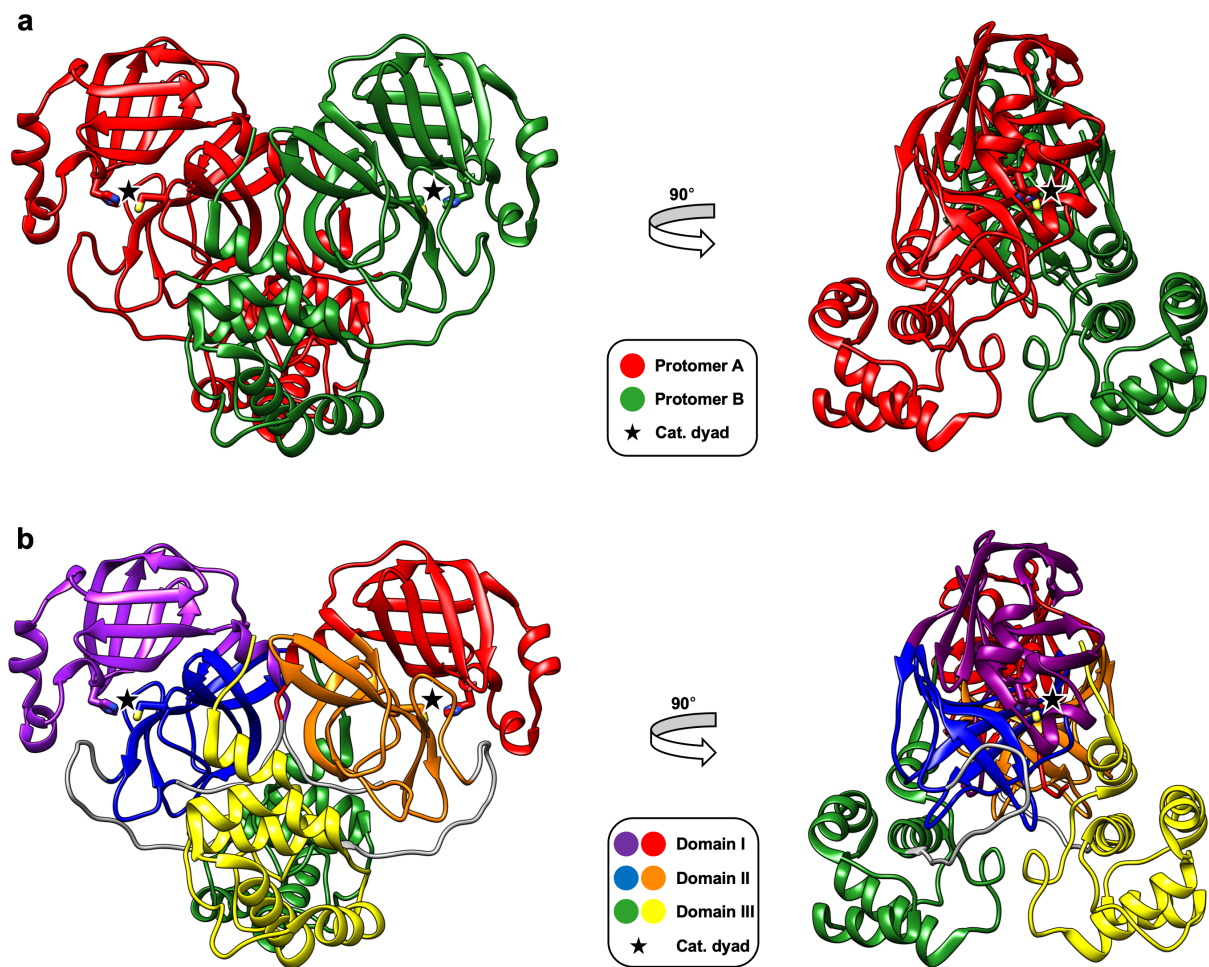
Fig. 4. X-ray crystal structure of the M^{pro} homodimer of SARS-CoV-2 (PDB: 6Y2E). Residues of the catalytic dyad (His41/Cys145) are indicated. (a) Protomers are indicated. (b) Protomer domains are indicated. This figure has been generated with UCSF Chimera.[70]

Substrate specificity and inhibitor design

According to the nomenclature introduced by Schechter and Berger,[81] M^{pro} mainly recognises substrate residues ranging from P$_4$ to P$_1$'.[82] Prime site recognition beyond P$_1$' is not conserved (Fig. 5). Specificity is mostly determined by P$_1$, P$_2$ and P$_1$', which show the highest degree of conservation amongst the cleavage sites.[63] Glutamine in P$_1$ is highly conserved in all polyprotein cleavage sites of SARS-CoV, MERS-CoV and SARS-CoV-2 (Fig. 5). In P$_2$ more hydrophobic amino acids are tolerated with a clear preference for leucine. P$_1$' tolerates small residues like serine or alanine.[56, 60, 61, 83-85] Analysis of all polyprotein cleavage sites processed by M^{pro} for SARS-CoV, MERS-CoV and SARS-CoV-2 illustrates very similar substrate recognition profiles amongst these viruses (Fig. 5). Particularly important is the pronounced

preference for glutamine in P_1, strongly informing inhibitor design.[84] Since no human host-cell proteases with similar specificity are reported, reduced off-target effects are assumed for peptidomimetic inhibitors.[60, 61]
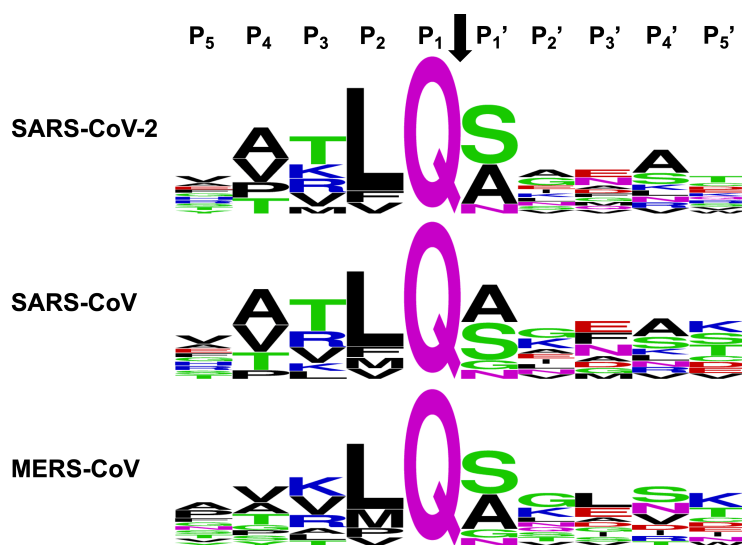

Fig. 5. Polyprotein cleavage sites recognised by M^pro of SARS-CoV-2, SARS-CoV and MERS-CoV. Peptide sequences cover residues P_5 to P_5' according to the nomenclature of Schechter and Berger.[81] Data were generated from pp1ab polyprotein sequences reported in the UniProt database with the accession codes P0DTD1 (SARS-CoV-2), P0C6X7 (SARS-CoV) and K9N7C7 (MERS-CoV). The consensus sequence over all cleavage sites was plotted using WebLogo.[86]

The catalytic dyad of M^pro is located in a cleft between domains I and II (Fig. 4b).[23, 71] Despite the minor mutation S46A in close proximity, the active sites of SARS-CoV and SARS-CoV-2 M^pro are highly conserved. The influence of the S46A mutation on shape, size, flexibility and plasticity of the active site and its relevance for inhibitor design is under debate.[87, 88]

As the monomer of M^pro is principally regarded as inactive, the dimerization interface offers an alternative target site for drug discovery.[68, 72] Although strong dimerization inhibitors are yet unavailable, the principle has been proven with the N-terminal octapeptide of SARS-CoV M^pro.[89]

Inhibitors

Inhibitors of SARS-CoV M^{pro} have been reviewed comprehensively by Pillaiyar et al. in 2016.[72] MERS-CoV inhibitors have been reviewed by Liang et al. in 2018.[90] Peptidomimetics and small molecules have been reported with affinities in the micro- to nanomolar range. They often depend on warhead-based design strategies, employing different reactive groups to covalently attack the catalytic cysteine residue. Warheads utilised include Michael acceptors, aldehydes, epoxy ketones and other ketones.[72]

Although SARS-CoV-2 emerged only very recently, several inhibitors have already been identified and successfully co-crystallised with M^{pro} (Fig. 6).[59, 79, 91] They are often derived from previous campaigns which targeted the main proteases of SARS-CoV or MERS-CoV and contain cysteine-targeting warheads (Fig. 6).
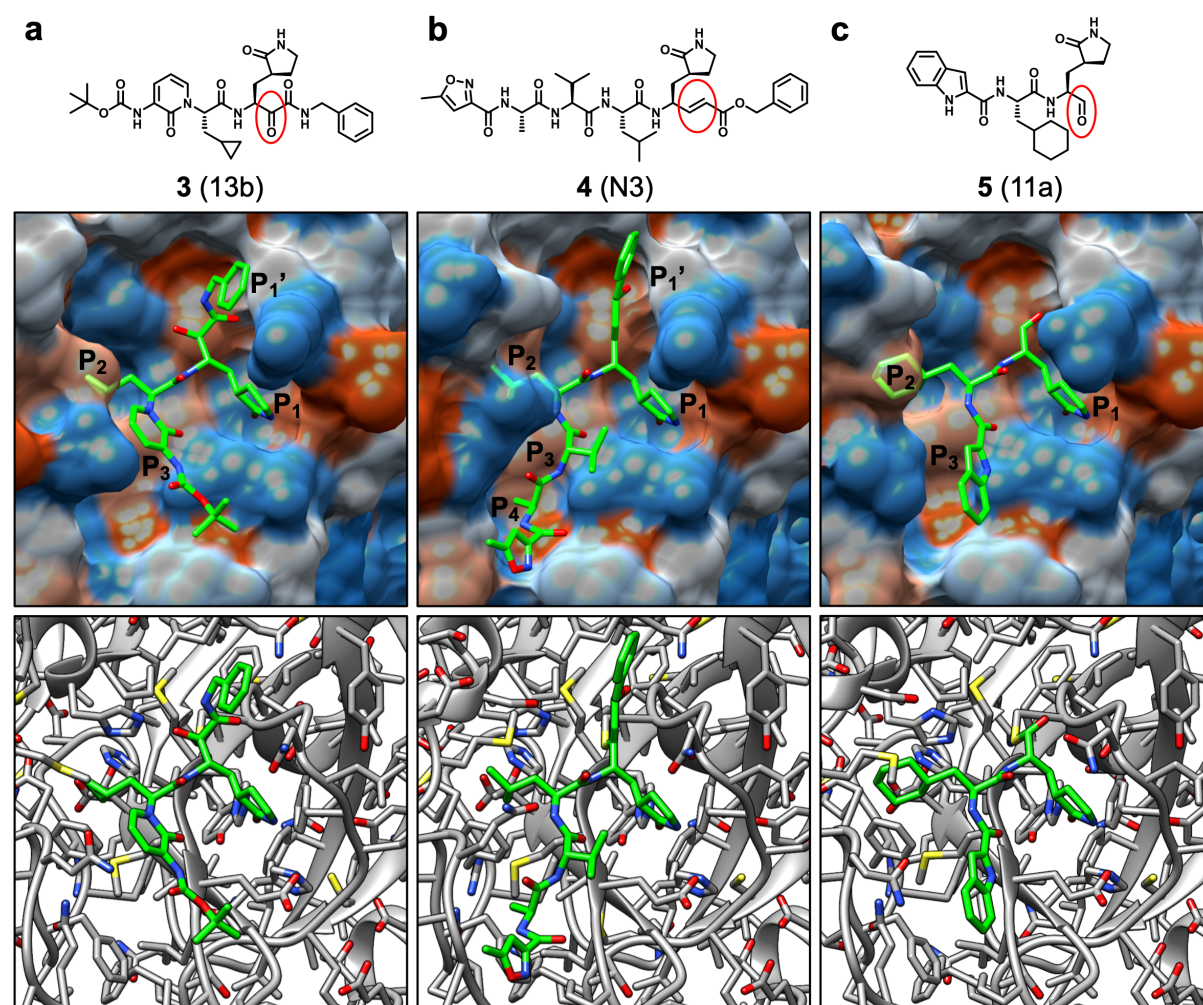

Fig. 6. X-ray co-crystal structures of SARS-CoV-2 M^{pro} with covalently binding peptidomimetic inhibitors. M^{pro} is shown as hydrophobicity surface (red indicating hydrophobic and blue hydrophilic surface areas) and grey ribbon with amino acid side chains

(UCSF Chimera).[70] Inhibitor groups binding to protease subsites are indicated according to the Schechter Berger nomenclature.[81] Electrophilic warheads covalently binding to Cys145 are circled. (a) Compound **3** with an α-ketoamide warhead (PDB: 6Y2F). (b) Compound **4** with a Michael acceptor warhead (PDB: 7BQY). (c) Compound **5** with a C-terminal aldehyde warhead (PDB: 6LZE).

The first reported inhibitors were covalently binding peptidomimetics (**1-3**) addressing the major substrate-recognition motif from P_1' to P_3.[59] They all comprise an α-ketoamide functionality that forms a hemithioacetal with Cys145. α-Ketoamides are already used as viral serine protease warheads in the approved HCV drugs telaprevir and boceprevir.[62] Compound **1** has previously been investigated as a broad-spectrum corona- and enteroviral protease inhibitor.[60] Like many other M^{pro} inhibitors, the P_1 side chains of **1-3** employ a γ-lactam as a glutamine mimetic. P_2 comprises hydrophobic cyclohexyl (**1**, **2**) or smaller cyclopropyl (**3**) groups as leucine mimetics and P_1' contains cyclopropyl (**2**) or benzylic (**1**, **3**) residues. Compounds **2** and **3** feature pyridone rings between P_2 and P_3 as well as N-terminal Boc groups, which were associated with increased plasma half-life, kinetic solubility and thermodynamic solubility. Pharmacokinetic profiling of **2** and **3** in mice also indicated favourable lung tropism. Compounds **1** and **3** displayed sub-micromolar M^{pro} inhibition (Fig. 7). Compound **3** is similarly active against the SARS-CoV and MERS-CoV main proteases and inhibits SARS-CoV-2 replication in human Calu3 lung cells.
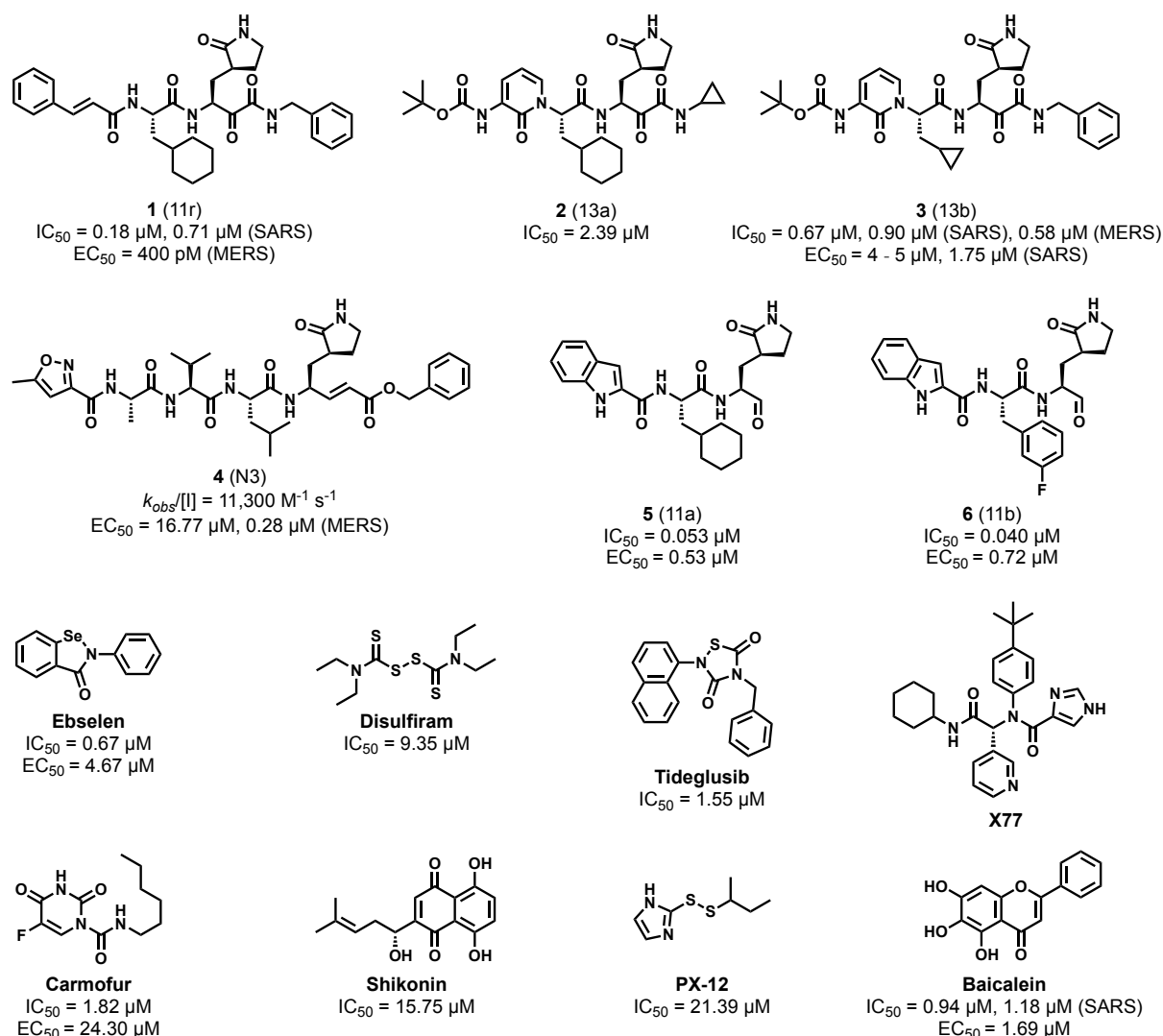
Fig. 7. Inhibitors of the SARS-CoV-2 main protease M^{pro}. IC_{50} indicates enzymatic inhibition. EC_{50} indicates antiviral activity in cells.

Compound **4** is another peptidomimetic M^{pro} inhibitor co-crystallised in complex with SARS-CoV-2 M^{pro} (Fig. 6b).[79] It originated from previous campaigns targeting SARS-CoV M^{pro}.[92] Its Michael acceptor irreversibly modifies Cys145. Compound **4** shows anti-SARS-CoV-2 activity in Vero cells.

Compounds **5** and **6** are the strongest SARS-CoV-2 M^{pro} inhibitors with inhibition constants in the two-digit nanomolar range. These small peptidomimetics feature an indole moiety at the N-terminus (P_3) and a C-terminal aldehyde warhead which binds covalently to Cys145, as proven by X-ray crystallography (Fig. 6c).[91] Similar peptide-aldehydes have previously been explored as inhibitors of SARS-CoV M^{pro}.[93, 94] Compound **5** and the previously investigated α-ketoamides **1** and **2** are structurally identical in P_1 and P_2; however, **5** inhibits one order of magnitude stronger, which is likely caused by the increased electrophilicity of the

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aldehyde warhead compared to the more drug-like α-ketoamide. Despite, the high reactivity of the aldehyde function, compounds **5** and **6** displayed sub-micromolar antiviral activity in Vero cells (Fig. 7). In agreement with compound **3**, the *in vitro* activity of **5** and **6** was one order of magnitude weaker than the direct M^{pro} inhibition in the enzymatic assay. Notably, **5** exhibited only low toxicity in animal models despite its aldehyde warhead.[91]

A high-throughput screening campaign of a library of approved drugs and clinical candidates revealed six small molecules, ebselen, disulfiram, carmofur, tideglusib, shikonin and PX-12, as inhibitors of SARS-CoV-2 M^{pro} (Fig. 7).[79] Mass spectrometry experiments showed that ebselen, carmofur and PX-12 covalently modify Cys145. Small covalent modifiers like these may bind unspecifically, a characteristic associated with pan-assay interference compounds (PAINS).[95] Ebselen showed antiviral activity in Vero cells in the low micromolar range. In case of carmofur, a crystal structure of SARS-CoV-2 M^{pro} revealed transfer of the hexylurea side chain to Cys145, forming a hexylcarbamothioate interacting with S$_2$ (PDB: 7BUY).[96]

Crystal structures of SARS-CoV-2 M^{pro} in complex with X77 and baicalein (Fig. 7) have been deposited in the protein data bank under the accession codes 6W63 and 6M2N, respectively. Notably, both compounds bind non-covalently to the active site. X77 and derivatives have previously been investigated as low-micromolar inhibitors of SARS-CoV M^{pro}, where a strong stereochemical bias for the (*R*) enantiomer of the pyridyl side chain has been observed.[97, 98] In the X-ray co-crystal structure of X77, the aforementioned pyridyl side chain acts as a P$_1$ mimetic. Baicalein is a flavonoid found in *Scutellaria baicalensis*, a plant used in traditional Chinese medicine.[99] Several flavonoids and derivatives had previously been reported to inhibit the activity of SARS-CoV M^{pro}.[72, 100]

A fragment screening has produced several crystal structures of fragments bound to SARS-CoV-2 M^{pro}, including covalent modifiers of Cys145.[101] While the majority of fragments bind to the active site, some bind near the dimer interface of SARS-CoV-2 M^{pro}. These fragments may inform the development of small-molecule inhibitors that are not substrate-derived.

While this manuscript was under peer-review, a study assessing known protease inhibitors for their anti-SARS-CoV-2 M^{pro} activity was published.[102] Amongst the compounds that displayed M^{pro} inhibition and reduction of cytopathic effect were the α-ketoamides boceprevir (K_i = 1.18 μM, EC$_{50}$ = 1.31 μM) and calpain inhibitor XII (K_i = 0.13 Mm, EC$_{50}$ =

0.49 μM), the peptide-aldehyde calpain inhibitor II (K_i = 0.40 Mm, EC_{50} = 2.07 μM) and the sulfonate-featured peptide GC-376 (k_2/K_i = 40,800 $M^{-1} s^{-1}$, EC_{50} = 3.37 μM). A crystal structure of SARS-CoV-2 M^{pro} in complex with GC-376 was also reported (PDB: 6WTT).

Conclusion and outlook

The COVID-19 pandemic poses a major challenge to mankind. In view of the magnitude of the current global crisis, numerous attempts to develop vaccines and antiviral treatments are obviously underway. With respect to drug development, the main protease of SARS-CoV-2 stands out as a promising viral target, as it differs significantly from human proteases. Given the conserved structure and specificity of M^{pro} amongst SARS-CoV, MERS-CoV and SARS-CoV-2, pan-coronaviral main protease inhibitors might become available. However, in line with previously successful examples like HIV or HCV, the development of novel specific protease inhibitors and their approval will take several years. Although this process will likely take too long to impact on the current COVID-19 crisis, it would be worth pursuing as it may provide a specific drug for upcoming coronavirus outbreaks.

Pharmacodynamic and pharmacokinetic properties of peptidomimetic M^{pro} inhibitors like **2**, **3**, **5** or **6** already point into the right direction.[59, 91] Although peptide-aldehydes have entered clinical trials before (e.g. efegatran),[103, 104] **5** and **6** are likely to face challenges during further drug development. The α-ketoamide in **1**-**3** or Michael acceptor in **4** are covalent modifiers with precedents in approved drugs (e.g. telaprevir or afatinib).[62, 105] Potential problems associated with limited drug-likeness of peptidomimetics could be circumvented by pursuing alternative small molecules, which might, for example, be accessible from fragment-based drug discovery campaigns.[101, 106]

Repurposing of known drugs can provide an accelerated route to approval, which is likely the only option to address the current COVID-19 crisis. Small molecules like ebselen or carmofur are M^{pro} inhibitors with anti-SARS-CoV-2 activity in cells;[79] however, their thiol reactivity might prove challenging. Repurposing approved protease inhibitors is an alternative approach.[102] Attempts to repurpose the approved combination of HIV protease inhibitors, ritonavir and lopinavir, was unsuccessful in clinical studies, which is not entirely unexpected, given the differences between the proteases of HIV and SARS-CoV-2.[107]

It is likely that SARS-CoV-2 is not the last human coronavirus emerging from animals. It is therefore important to closely monitor virus populations to early understand their replication mechanism and investigate druggable targets. A sharp decline in research funding had been noted a few years after the first SARS epidemic.[61] Given the long-term nature of drug discovery projects, this has proven disastrous with respect to the current crisis. Now is the best time to progress protease inhibitors to anti-coronaviral drugs.

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