Introduction:

In an incredibly fast piece of research, scientists from the University of Texas at Austin and the National Institutes of Health have released a cryo-electron microscopy (cryoEM) structure of part of SARS-CoV-2, the novel coronavirus that has infected tens of thousands of people and killed more than 2,000 since the end of December (Science 2020 DOI: 10.1126/science.abb2507).

The part of the virus imaged, called the spike protein, helps the virus attach to and infect human cells, and its structure comes just weeks after the virus’s genome sequence was published. The breakthrough is a huge step toward developing a vaccine against the virus as well as treatments for COVID-19, the disease that it causes, the researchers say.

UT Austin’s Jason McLellan and his colleagues have spent many years studying other coronaviruses and had already figured out how to use select mutations to lock coronavirus spike proteins into a shape that is conducive for structural studies. After they got the genome sequence of the virus, it took the team just two weeks to design and produce samples of the stabilized spike protein. After collecting data on their stabilized spike protein samples using a cryo-electron microscope, the researchers spent 12 days reconstructing the 3-D structure. They published the results on bioRXiv on Feb. 15, and the paper was rushed through peer review before being published by Science on Feb. 19 (DOI: 10.1126/science.abb2507).

“This is stunning work, illustrating the power of molecular biology in combination with cryoEM,” says Alice Clark, a structural biologist at the University of Wolverhampton. “How quickly this work was possible is a credit to both the scientists involved in this structure, and the recent advances in cryoEM as a technique.”

Coronaviruses are RNA viruses that typically enter human cells when their glycoproteins bind proteins on the cell surface. SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) on human cells with higher affinity than does the virus that caused severe acute respiratory syndrome (SARS) in 2003. This difference in affinity possibly explains why the novel coronavirus is more contagious than that other virus. The team is already testing the stabilized spike as a vaccine, and hope the structure will help in the development of antivirals. Similar studies on spike proteins from SARS and Middle East respiratory syndrome (MERS) viruses were used to develop experimental vaccines, but the vaccines never made it to market.

Structure:Spherical or pleomorphic enveloped particles containing single-stranded (positive-sense) RNA associated with a nucleoprotein within a capsid comprised of matrix protein. The envelope bears club-shaped glycoprotein projections.

Coronavirus virions are spherical to pleomorphic enveloped particles (Fig. 60-3). The envelope is studded with projecting glycoproteins, and surrounds a core consisting of matrix protein enclosed within which is a single strand of positive-sense RNA (Mr 6 × 106) associated with nucleoprotein. The envelope glycoproteins are responsible for attachment to the host cell and also carry the main antigenic epitopes, particularly the epitopes recognized by neutralizing antibodies. OC43 also possesses a haemagglutin.

It is thought that human coronaviruses enter cells, predominantly, by specific receptors. Aminopeptidase-N and a sialic acid-containing receptor have been identified to act in such a role for 229E and OC43 respectively. After the virus enters the host cell and uncoats, the genome is transcribed and then translated. A unique feature of replication is that all the mRNAs form a “nested set” with common 3′ ends; only the unique portions of the 5′ ends are translated. There are 7 mRNAs produced. The shortest mRNA codes for the nucleoprotein, and the others each direct the synthesis of a further segment of the genome. The proteins are assembled at the cell membrane and genomic RNA is incorporated as the mature particle forms by budding from internal cell membranes.

Coronaviruses demonstrate a complex pattern for receptor recognition (19) (Figure 1d). For example, the alphacoronavirus HCoV-NL63 and the betacoronavirus SARS-CoV both recognize a zinc peptidase angiotensin-converting enzyme 2 (ACE2) (20, 21). Moreover, HCoV-NL63 and other alphacoronaviruses recognize different receptors: other alphacoronaviruses such as TGEV, PEDV, and PRCV recognize another zinc peptidase, aminopeptidase N (APN) (22–25). Similarly, SARS-CoV and other betacoronaviruses recognize different receptors: MERS-CoV and HKU4 recognize a serine peptidase, dipeptidyl peptidase 4 (DPP4) (26, 27); MHV recognizes a cell adhesion molecule, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (28, 29); BCoV and OC43 recognize sugar (30). The alphacoronaviruses TGEV and PEDV and the gammacoronavirus IBV also use sugar as receptors or coreceptors (23, 31–34). Other than their role in viral attachment, these coronavirus receptors have their own physiological functions (35–41). The diversity of receptor usage is an outstanding feature of coronaviruses. To further compound the complexity of the issue, the S1 subunits from different genera share little sequence similarity, whereas those from the same genus have significant sequence similarity (42). Therefore, the following questions have been raised regarding receptor recognition by coronaviruses: (a) How do coronaviruses from different genera recognize the same receptor protein? (b) How do coronaviruses from the same genus recognize different receptor proteins? (c) What is the molecular basis for coronavirus spikes to recognize sugar receptors and function as viral lectins?

Two major domains in coronavirus S1, N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD), have been identified (Figure 1c,d). One or both of these S1 domains potentially bind receptors and function as the receptor-binding domain (RBD). S1-NTDs are responsible for binding sugar (23, 34, 43, 44), with the only known exception being betacoronavirus MHV S1-NTD that recognizes a protein receptor CEACAM1 (45). S1-CTDs are responsible for recognizing protein receptors ACE2, APN, and DPP4 (23, 46–51). Crystal structures have been determined for a number of S1 domains complexed with their respective receptor (Figure 1d). These structures, along with functional studies, have addressed many of the puzzles surrounding receptor recognition by coronaviruses.

The structure of betacoronavirus SARS-CoV S1-CTD complexed with human ACE2 provided the first atomic view of coronavirus S1 (52, 53) (Figure 3a). SARS-CoV S1-CTD contains two subdomains: a core structure and a receptor-binding motif (RBM). The core structure is a five-stranded antiparallel β-sheet. The RBM presents a gently concave outer surface to bind ACE2. The base of this concave surface is a short, two-stranded antiparallel β-sheet, and two ridges are formed by loops. The ectodomain of ACE2 contains a membrane-distal peptidase domain and a membrane-proximal collectrin domain (54). Several virus-binding motifs (VBMs) have been identified on the outer surface of the peptidase domain, away from the buried peptidase catalytic site (52). SARS-CoV binding does not interfere with the enzymatic activity of ACE2, nor does the enzymatic activity of ACE2 play any role in SARS-CoV entry.

Research on SARS-CoV–ACE2 interactions has provided novel insight into cross-species transmissions of SARS-CoV. During the SARS epidemic, highly similar SARS-CoV strains were isolated from both human patients and palm civets from nearby animal markets (56). Their S1-CTDs differ by only two residues in the RBM region: Asn479 and Thr487 in human viral strains become Lys479 and Ser487 in civet viral strains, respectively (Figure 3b,c). However, human SARS-CoV S1-CTD binds to human ACE2 much more tightly than civet SARS-CoV S1-CTD does. Two virus-binding hot spots have been identified on human ACE2, centering on ACE2 residues Lys31 and Lys353, respectively (57–59) (Figure 3b). Both hot spots consist of a salt bridge buried in a hydrophobic environment and contribute critically to virus–receptor binding. Residues 479 and 487 in SARS-CoV S1-CTD interact closely with these hot spots and are under selective pressure to mutate. Two naturally selected viral mutations, K479N and S487T, strengthened the hot spot structures and enhanced the binding affinity of S1-CTD for human ACE2 (55, 57–59) (Figure 3c). Consequently, these two mutations played important roles in the civet-to-human and human-to-human transmissions of SARS-CoV during the SARS epidemic (13, 55, 57–61). Compared to human ACE2, rat ACE2 contains two different residues that disfavor SARS-CoV binding: His353 disturbs the hot spot structure centering on Lys353, whereas Asn82 introduces an N-linked glycan, presenting steric interference with SARS-CoV binding (52) (Figure 3d). Mouse ACE2 also contains His353 but does not have the N-linked glycan at the 82 position. Thus, rat ACE2 is not a receptor for SARS-CoV, whereas mouse ACE2 is a poor receptor. Consequently, SARS-CoV does not infect rat cells, and it infects mouse cells inefficiently (62, 63). SARS-like coronaviruses (SLCoVs) have been identified in bats, and some can infect human cells (64–68). Structural details on how these bat SLCoV S1-CTDs interact with ACE2 from different mammalian species still wait to be determined. Overall, these studies on SARS-CoV–ACE2 interactions reveal that (a) one or a few mutations in viral RBDs can cause serious epidemic outcomes and (b) one or a few residue variations in receptor homologs from different animal species can form critical barriers for cross-species transmissions of viruses.

Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors

View ORCID ProfileLinlin Zhang1,2, View ORCID ProfileDaizong Lin1,3, View ORCID ProfileXinyuanyuan Sun1,2, Ute Curth4, View ORCID ProfileChristian Drosten5, View ORCID ProfileLucie Sauerhering6,7, Stephan Becker6,7, View ORCID ProfileKatharina Rox8,9, View ORCID ProfileRolf Hilgenfeld1,2,

Scientists across the world are working to understand severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19). Zhang et al. determined the x-ray crystal structure of a key protein in the virus' life cycle: the main protease. This enzyme cuts the polyproteins translated from viral RNA to yield functional viral proteins. The authors also developed a lead compound into a potent inhibitor and obtained a structure with the inhibitor bound, work that may provide a basis for development of anticoronaviral drugs.

Abstract

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) is a global health emergency. An attractive drug target among coronaviruses is the main protease (Mpro, also called 3CLpro) because of its essential role in processing the polyproteins that are translated from the viral RNA. We report the x-ray structures of the unliganded SARS-CoV-2 Mpro and its complex with an α-ketoamide inhibitor. This was derived from a previously designed inhibitor but with the P3-P2 amide bond incorporated into a pyridone ring to enhance the half-life of the compound in plasma. On the basis of the unliganded structure, we developed the lead compound into a potent inhibitor of the SARS-CoV-2 Mpro. The pharmacokinetic characterization of the optimized inhibitor reveals a pronounced lung tropism and suitability for administration by the inhalative route.

In December 2019, a new coronavirus caused an outbreak of pulmonary disease in the city of Wuhan, the capital of Hubei province in China, and has since spread globally (1, 2). The virus has been named severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) (3) because the RNA genome is about 82% identical to that of the SARS coronavirus (SARS-CoV); both viruses belong to clade b of the genus Betacoronavirus (1, 2). The disease caused by SARS-CoV-2 is called coronavirus disease 2019 (COVID-19). Whereas at the beginning of the outbreak, cases were connected to the Huanan seafood and animal market in Wuhan, efficient human-to-human transmission led to exponential growth in the number of cases. On 11 March 2020, the World Health Organization (WHO) declared the outbreak a pandemic. As of 9 April, there were >1,500,000 cumulative cases globally, with a ~5.9% case fatality rate.

One of the best-characterized drug targets among coronaviruses is the main protease (Mpro, also called 3CLpro) (4). Along with the papain-like protease(s), this enzyme is essential for processing the polyproteins that are translated from the viral RNA (5). The Mpro operates at no fewer than 11 cleavage sites on the large polyprotein 1ab (replicase 1ab, ~790 kDa); the recognition sequence at most sites is Leu-Gln↓(Ser, Ala, Gly) (↓ marks the cleavage site). Inhibiting the activity of this enzyme would block viral replication. Because no human proteases with a similar cleavage specificity are known, such inhibitors are unlikely to be toxic.

Previously, we designed and synthesized peptidomimetic α-ketoamides as broad-spectrum inhibitors of the main proteases of betacoronaviruses and alphacoronaviruses as well as the 3C proteases of enteroviruses (6). The best of these compounds (11r; Fig. 1) showed an half-maximal effective concentration (EC50) of 400 pM against Middle East respiratory syndrome–coronavirus (MERS-CoV) in Huh7 cells as well as low-μM EC50 values against SARS-CoV and a whole range of enteroviruses in various cell lines, although the antiviral activity seemed to depend to a great extent on the cell type used in the experiments (6). To improve the half-life of the compound in plasma, we modified 11r by hiding the P3-P2 amide bond within a pyridone ring (Fig. 1, green ovals) in the expectation that this might prevent cellular proteases from accessing this bond and cleaving it. Further, to increase the solubility of the compound in plasma and to reduce its binding to plasma proteins, we replaced the hydrophobic cinnamoyl moiety by the somewhat less hydrophobic Boc group (Fig. 1, red ovals) to give 13a (see scheme S1 for synthesis).

Colored ovals and circles highlight the modifications from one development step to the next (see text).

To examine whether the introduced pyridone ring is compatible with the three-dimensional structure of the target, we determined the crystal structure, at 1.75 Å resolution, of the Mpro of SARS-CoV-2 (Fig. 2). The three-dimensional structure is highly similar to that of the SARS-CoV Mpro, as expected from the 96% sequence identity (see fig. S8); the root mean square deviation between the two free-enzyme structures is 0.53 Å for all Cα positions [comparison between SARS-CoV-2 Mpro structure and SARS-CoV Mpro, PDB entry 2BX4 (7)]. The chymotrypsin-like and picornavirus 3C protease–like domains I and II (residues 10 to 99 and 100 to 182, respectively) are six-stranded antiparallel β barrels that harbor the substrate-binding site between them. Domain III (residues 198 to 303), a globular cluster of five helices, is involved in regulating the dimerization of the Mpro, mainly through a salt-bridge interaction between Glu290 of one protomer and Arg4 of the other (8). The tight dimer formed by SARS-CoV-2 Mpro has a contact interface of ~1394 Å2, predominantly between domain II of molecule A and the NH2-terminal residues (“N-finger”) of molecule B, with the two molecules oriented perpendicular to one another (Fig. 2). Dimerization of the enzyme is necessary for catalytic activity, because the N-finger of each of the two protomers interacts with Glu166 of the other protomer and thereby helps shape the S1 pocket of the substrate-binding site (9). To reach this interaction site, the N-finger is squeezed in between domains II and III of the parent monomer and domain II of the other monomer.

Interestingly, in the SARS-CoV but not in the SARS-CoV-2 Mpro dimer, there is a polar interaction between the two domains III involving a 2.60-Å hydrogen bond between the side-chain hydroxyl groups of residue Thr285 of each protomer, supported by a hydrophobic contact between the side chain of Ile286 and Thr285 Cγ2. In SARS-CoV-2, the threonine is replaced by alanine (indicated by the black spheres in Fig. 2) and the isoleucine by leucine (fig. S8). It was previously shown that replacing Ser284, Thr285, and Ile286 by alanine residues in SARS-CoV Mpro leads to enhancement of the catalytic activity of the protease by a factor of 3.6, concomitant with a slightly closer packing of the two domains III of the dimer against one another (10). This was accompanied by changes in enzyme dynamics that transmit the effect of the mutation to the catalytic center. Indeed, the Thr285 → Ala replacement observed in the SARS-CoV-2 Mpro also allows the two domains III to approach each other more closely (the distance between the Cα atoms of residues 285 in molecules A and B is 6.77 Å in SARS-CoV Mpro and 5.21 Å in SARS-CoV-2 Mpro, and the distance between the centers of mass of the two domains III shrinks from 33.4 Å to 32.1 Å). However, the catalytic efficiency of SARS-CoV-2 Mpro is only slightly higher, if at all [turnover number (kcat)/Michaelis constant (Km) = 3426.1 ± 416.9 s–1 M–1] than that of SARS-CoV Mpro (kcat/Km = 3011.3 ± 294.6 s–1 M–1). Further, the estimated dissociation constant of dimerization is the same (~2.5 μM) for the two enzymes, as determined by analytical ultracentrifugation (fig. S10).

One protomer of the dimer is shown in light blue, the other one in orange. Domains are labeled by Roman numerals. Amino acid residues of the catalytic site are indicated as yellow spheres for Cys145 and blue spheres for His41. Asterisks mark residues from protomer B (orange). Black spheres indicate the positions of Ala285 for each of the two domains III (see text). Chain termini are labeled N and C for molecule A (light blue) and N\* and C\* for molecule B (orange).

We used this crystal structure to dock the α-ketoamide 13a; this suggested that the pyridone ring might have some steric clash with the side chain of Gln189. However, in our previous work (6), we had found Gln189 to be quite flexible, and therefore we went ahead with 13a as a lead. The plasma half-life of this compound in mice was increased by a factor of ~3 relative to 11r (from 0.3 hours to 1.0 hours), the in vitro kinetic plasma solubility was improved by a factor of ~19 (from 6 μM for 11r to 112 μM for 13a), and the thermodynamic solubility increased by a factor of ~13 (from 41 μM to 530 μM). Binding to mouse plasma protein was reduced from 99% to 97% [many drugs have plasma protein binding of >90% (11)]. However, relative to 11r (IC50 = 0.18 ± 0.02 μM), the structural modification led to some loss of inhibitory activity against the main protease of SARS-CoV-2 (IC50 = 2.39 ± 0.63 μM) as well as the 3C proteases (3Cpro) of enteroviruses. 11r was designed for broad-spectrum activity, with the P2 cyclohexyl moiety intended to fill a pocket in the enterovirus 3Cpro. The S2 pocket of the betacoronavirus Mpro (Fig. 3) features substantial plasticity, enabling it to adapt to the shape of smaller inhibitor moieties (6). To enhance the antiviral activity against betacoronaviruses of clade b (SARS-CoV-2 and SARS-CoV), we sacrificed the goal of broad-spectrum activity and replaced the P2 cyclohexyl moiety of 13a by the smaller cyclopropyl in 13b (Fig. 1, blue circles). Here, we present x-ray crystal structures in two different crystal forms, at 1.95 and 2.20 Å resolution, of the complex between α-ketoamide 13b and the Mpro of SARS-CoV-2. One structure is in space group C2 (Fig. 3), where both protomers of the Mpro dimer are bound by crystal symmetry to have identical conformations; the other is in space group P212121, where the two protomers are independent of each other and free to adopt different conformations. Indeed, we find that in the latter crystal structure, the key residue Glu166 adopts an inactive conformation in protomer B (as evidenced by its distance from His172 and the lack of H-bonding interaction with the P1 moiety of the inhibitor), even though compound 13b is bound in the same mode as in molecule A. This phenomenon has also been observed with the SARS-CoV Mpro (12) and is consistent with the half-site activity described for this enzyme (13). In all copies of the inhibited SARS-CoV-2 Mpro, the inhibitor binds to the shallow substrate-binding site at the surface of each protomer, between domains I and II (Fig. 3).

Fig. 3 Compound 13b in the substrate-binding cleft located between domains I and II of the Mpro in the monoclinic crystal form (space group C2).

Fobs – Fcalc density is shown for the inhibitor (contouring level 3σ). Carbon atoms of the inhibitor are magenta, except in the pyridone ring, which is black; oxygen atoms are red, nitrogens blue, and sulfur yellow. Light blue symbols Sn (n = 1, 2, 3…) indicate the canonical binding pockets for moieties Pn (n = 1, 2, 3…) (red symbols) of the peptidomimetic inhibitor. Hydrogen bonds are indicated by dashed red lines. Note the interaction between Ser1\*, the N-terminal residue of molecule B, and Glu166 of molecule A, which is essential for keeping the S1 pocket in the correct shape and the enzyme in the active conformation. Inset: Thiohemiketal formed by the nucleophilic attack of the catalytic cysteine onto the α-carbon of the inhibitor. The stereochemistry of the α-carbon is S. Fobs − Fcalc density (contoured at 3σ) is shown in blue. See fig. S9 for more details.

Through the nucleophilic attack of the catalytic Cys145 onto the α-keto group of the inhibitor, a thiohemiketal is formed in a reversible reaction. This is clearly reflected in the electron density (Fig. 3, inset); the stereochemistry of this chiral moiety is S in all copies of compound 13b in these structures. The oxyanion (or hydroxyl) group of this thiohemiketal is stabilized by a hydrogen bond from His41, whereas the amide oxygen of 13b accepts a hydrogen bond from the main-chain amides of Gly143, Cys145, and partly Ser144, which form the canonical “oxyanion hole” of the cysteine protease. It is an advantage of the α-ketoamides that their warhead can interact with the catalytic center of the target proteases through two hydrogen-bonding interactions (6) rather than only one, as with other warheads such as aldehydes (14) or Michael acceptors (15).

The P1 γ-lactam moiety, designed as a glutamine surrogate (15, 16), is deeply embedded in the S1 pocket of the protease, where the lactam nitrogen donates a three-center (bifurcated) hydrogen bond to the main-chain oxygen of Phe140 (3.20/3.10/3.28 Å; values for the structure in space group C2/space group P212121 molecule A/space group P212121 molecule B) and to the Glu166 carboxylate [3.35/3.33/(3.55) Å], and the carbonyl oxygen accepts a 2.57/2.51/2.81 Å hydrogen bond from the imidazole of His163. The P2 cyclopropyl methyl moiety fits snugly into the S2 subsite, which has shrunk by 28 Å3 relative to the complex between compound 13a with P2 = cyclohexyl methyl and the SARS-CoV Mpro (17). The pyridone in the P3-P2 position of the inhibitor occupies the space normally filled by the substrate’s main chain; its carbonyl oxygen accepts a 2.89/2.99/3.00 Å hydrogen bond from the main-chain amide of residue Glu166. Further, the P3 amide donates a 2.83/2.96/2.87 Å hydrogen bond to the main-chain oxygen of Glu166. Embedded within the pyridone, the P2 nitrogen can no longer donate a hydrogen bond to the protein (the H-bond prevented from forming would connect the P2 nitrogen and the side-chain oxygen of Gln189; these two atoms are highlighted in fig. S9). However, our previous crystal structures showed that the P2 main-chain amide of the linear α-ketoamides does not make a hydrogen bond with the protein in all cases, so this interaction does not seem to be critical (6). The protecting Boc group on P3 does not occupy the canonical S4 site of the protease [in contrast to the protecting groups of other inhibitors in complex with the SARS-CoV Mpro (18)] but is located near Pro168 (3.81/4.17/3.65 Å) (Fig. 3); as a result of this interaction, the latter residue moves outward by more than 2 Å (relative to the structure of the free enzyme). This contact explains why removing the Boc group as in compound 14b (Fig. 1, purple ovals) weakens the inhibitory potency of this compound by a factor of ~2. Interestingly, there is a space between the pyridone ring of 13b, the main chain of residue Thr190, and the side chain of Gln189 (smallest distance: 3.6 Å), which is filled by a dimethyl sulfoxide (DMSO) molecule in the C2 crystal structure and a water molecule in the P212121 structure. This suggests that P3 moieties more bulky than pyridone may be accepted here.

Compound 13b inhibits the purified recombinant SARS-CoV-2 Mpro with IC50 = 0.67 ± 0.18 μM. The corresponding IC50 values for inhibition of the SARS-CoV Mpro and the MERS-CoV Mpro are 0.90 ± 0.29 μM and 0.58 ± 0.22 μM, respectively. In a SARS-CoV replicon (19), RNA replication is inhibited with EC50 = 1.75 ± 0.25 μM. In human Calu-3 cells infected with SARS-CoV-2, an EC50 of 4 to 5 μM was observed, whereas compound 14b lacking the Boc group was almost inactive (Fig. 4). This suggests that the hydrophobic and bulky Boc group is necessary to cross the cellular membrane and that an even more hydrophobic moiety might be advantageous here, although this may again lead to increased plasma protein binding, as observed for the cinnamoyl-containing 11r.

(A) Calu-3 cells were infected with SARS-CoV-2 using a multiplicity of infection (MOI) of 0.05. Varying amounts (5, 10, 20, or 40 μM) of 13b (blue bars) or 14b (orange bars) were added. DMSO was used as vehicle control (black bar). Total RNA was isolated from cell lysates, and viral RNA content was analyzed by quantitative polymerase chain reaction. Data are means ± SD of two biological experiments with two technical replicates each. (B) For the estimation of the EC50 value of compound 13b against SARS-CoV-2, a dose-response curve was prepared (GraphPad).

To assess the absorption-distribution-metabolism-excretion (ADME) properties of the pyridone-containing α-ketoamides, we first investigated compound 13a. Metabolic stability in mouse and human microsomes was good, with intrinsic clearance rates Clint\_mouse = 32.0 μl min–1 (mg protein)–1 and Clint\_human = 21.0 μl min–1 (mg protein)–1. This means that after 30 min, ~80% and 60% (for mouse and human, respectively) of residual compound remained metabolically stable. Pharmacokinetic studies in CD-1 mice using the subcutaneous route at 20 mg/kg showed that 13a stayed in plasma for up to 4 hours but was excreted via urine for up to 24 hours. The maximum plasma concentration (Cmax) was determined at 334.5 ng ml−1 and the mean residence time was ~1.6 hours. Although 13a seemed to be cleared very rapidly from plasma, at 24 hours it was found at 135 ng/g tissue in the lung and at 52.7 ng ml−1 in bronchio-alveolar lavage fluid (BALF), which suggests that it was mainly distributed to tissue. Next, we investigated 13b for its pharmacokinetic properties in CD-1 mice using the subcutaneous route as well, but at 3 mg kg−1. The ADME parameters of 13b were similar to those of 13a; in addition, binding to human plasma proteins was found to be 90%. The Cmax of 13b was determined at 126.2 ng ml−1. This is around 37% of the Cmax detected for 13a, although the 13b dosage was lower by a factor of ~7. The mean residence time for 13b was extended to 2.7 hours and the plasma half-life in mice was 1.8 hours. In addition, 13b showed a less rapid clearance relative to 13a (table S3). During the pharmacokinetic study with 13b, we monitored its lung tissue levels. After 4 hours, 13b was still found at ~13 ng g−1 in lung tissue. This lung tropism of 13a and 13b is beneficial given that COVID-19 affects the lungs. In addition to subcutaneous administration, 13b was nebulized using an inhalation device at 3 mg kg−1. After 24 hours, 13b was found at 33 ng g−1 in lung tissue. Inhalation was tolerated well and mice did not show any adverse effects, which suggests that direct administration of the compound to the lungs would be possible. Given these favorable pharmacokinetic results, our study provides a useful framework for the development of the pyridone-containing inhibitors toward anticoronaviral drugs.

* Coronavirus particles are surrounded by a fatty outer layer called an envelope and usually appear spherical, as seen under an electron microscope, with a crown or “corona” of club-shaped spikes on their surface.

At their core, coronaviruses contain a genetic blueprint called RNA (beige), similar to DNA. The single-stranded RNA acts as a molecular message that enables production of proteins needed for other elements of the virus.

Bound to this string of RNA are nucleoproteins— (dark blue discs)—proteins that help give the virus its structure and enable it to replicate.

Encapsulating the RNA genome is the viral envelope (teal), which protects the virus when it is outside of a host cell. This outer envelope is made from a layer of lipids, a waxy barrier containing fat molecules. As well as protecting the precious genetic cargo, this layer anchors the different structural proteins needed by the virus to infect cells. Envelope proteins (dark blue dots) embedded in this layer aid the assembly of new virus particles once it has infected a cell.

The bulbous projections seen on the outside of the coronavirus are spike proteins (red-orange). This fringe of proteins gives the virus its crown-like appearance under the microscope, from which the Latin name corona is derived. The spike proteins act as grappling hooks that allow the virus to latch onto host cells and crack them open for infection. Like all viruses, coronaviruses are unable to thrive and reproduce outside of a living host.