Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Bioorg. Med. Chem. Lett. 48 (2021) 128263

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A head-to-head comparison of the inhibitory activities of 15 peptidomimetic SARS-CoV-2 3CLpro inhibitors

Subramanyam Vankadara, Yun Xuan Wong, Boping Liu, Yi Yang See, Li Hong Tan, Qian Wen Tan, Gang Wang, Ratna Karuna, Xue Guo, Shu Ting Tan, Jia Yi Fong, Joma Joy, C. S. Brian Chia *

Experimental Drug Development Centre, 10 Biopolis Road, Chromos #08-01, Singapore 138670, Singapore

ARTICLE INFO

Keywords:
SARS-CoV-2
3CLpro
Mpro
Peptidomimetic inhibitors
Cysteine protease inhibitors

ABSTRACT

The COVID-19 pandemic caused by SARS-CoV-2 has created an unprecedented global health emergency. As of July 2021, only three antiviral therapies have been approved by the FDA for treating infected patients, highlighting the urgent need for more antiviral drugs. The SARS-CoV-2 3CL protease (3CLpro) is deemed an attractive drug target due to its essential role in viral polyprotein processing and pathogenesis. Indeed, a number of peptidomimetic 3CLpro inhibitors armed with electrophilic warheads have been reported by various research groups that can potentially be developed for treating COVID-19. However, it is currently impossible to compare their relative potencies due to the different assays employed. To solve this, we conducted a head-to-head comparison of fifteen reported peptidomimetic inhibitors in a standard FRET-based SARS-CoV-2 3CLpro inhibition assay to compare and identify potent inhibitors for development. Inhibitor design and the suitability of various warheads are also discussed.

In December 2019, a spate of pneumonia clusters were reported by a number of healthcare facilities in Wuhan, Hubei province, China, with symptoms such as a sore throat, dry cough, fever, headache, fatigue and breathing.[1,2] Genome sequencing revealed that the disease was caused by a coronavirus with 80% nucleotide sequence identity to the severe acute respiratory syndrome coronavirus (SARS-CoV), the pathogen responsible for the 2002–2004 pandemic which originated from Foshan, Guangdong province, China.[3–7] This novel coronavirus was named 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) by the International Committee on Taxonomy of Viruses[8] and the disease was named 'coronavirus disease 2019' (COVID-19) by the World Health Organization (WHO) in 2020. SARS-CoV-2 has since spread globally, infecting more than 182 million people worldwide and killing more than 3.9 million as of 3 July 2021.[9] Although a number of vaccines have been granted authorization, only three antiviral therapies (Remdesivir, Casirivimab + Imdevimab and Sotrovimab) have so far been approved by the United States Food and Drug Administration (FDA) for treating infected patients.[10–12] On 20 November 2020, the WHO recommended *against* the use of Remdesivir for hospitalized SARS-CoV-2 patients citing the lack of evidence that it improves survival and other outcomes,[13] highlighting the urgent need for more effective antiviral drugs.

The SARS-CoV-2 3C-like protease (3CLpro; also known as main protease or Mpro) is deemed an attractive drug target due to its involvement in cleaving the viral polyprotein at a minimum 11 sites to form essential viral proteins required for virus replication and pathogenesis.[14–17] In addition, it has no reported human homologues, hence reducing the risk of off-target side-effects by 3CLpro inhibitors.[17] This cysteine protease recognizes and binds Leu-Gln, Phe-Gln and Val-Gln peptide sequences and cleaves the peptide bond at the C-terminus of Gln.[16–18] Hence, such peptides designed with C-terminal electrophilic warheads have been shown to inhibit CoV 3CLpro (Fig. 1). Examples of electrophilic warheads include aldehydes,[19–21] ethyl propenoate,[19] hydroxymethylketone,[22] hydroxymethyl sulfonic acid,[16,23] ketoamides,[16,24,25] and ketobenzothiazoles.[26,27] This peptide-warhead strategy has led to the approval of Boceprevir and Telaprevir in 2011 (Fig. 1), antiviral drugs that target the hepatitis C virus (HCV) NS3 protease and used for treating HCV infections,[28] suggesting the same strategy can be applied for SARS-CoV-2 antiviral drugs.

Although no peptidomimetic inhibitors have so far been approved for treating SARS-CoV-2 infections, they have been reported by various research groups (see Table 1 and references cited therein). Intriguingly, HCV NS3 protease inhibitors Boceprevir and Telaprevir were also reported to inhibit SARS-CoV-2 3CLpro, suggesting that they can be repurposed for treating COVID-19.[16,25,29]

* Corresponding author.
E-mail address: cschia@eddc.a-star.edu.sg (C.S.B. Chia).

https://doi.org/10.1016/j.bmcl.2021.128263
Received 29 May 2021; Received in revised form 3 July 2021; Accepted 11 July 2021
Available online 14 July 2021

Nomenclature

3CLpro	3C-like protease
BLAST	Basic Local Alignment Search Tool
CoV	coronavirus
COVID-19	coronavirus disease 2019
FDA	Food and Drug Administration
FRET	fluorescence resonance energy transfer
HCV	hepatitis C virus
HPLC	high performance liquid chromatography
IC_{50}	half-maximal inhibitory concentration
Mpro	main protease
NS3	non-structural protein 3
RFU	relative fluorescence units
SARS	severe acute respiratory syndrome
WHO	World Health Organization

Unfortunately, it is currently impossible to compare their relative inhibitory potencies due to the different experimental protocols and protease constructs used by the various research groups. To solve this, we conducted a head-to-head comparison of fifteen reported coronavirus 3CLpro peptidomimetic inhibitors using a fluorescence resonance energy transfer (FRET)-based CoV-2 3CLpro inhibition assay. IC_{50}s were then compared to identify potent inhibitors that have the potential for further development as antiviral drugs. In addition, inhibitor design and the suitability of various electrophilic warheads will also be discussed.

Test compounds 2i, 3, 11a, 11b, 17, 25c, GC-373 and TG-0205221 were synthesized based on reported procedures cited in Table 1 and their spectral data are found in the supplemental file. Boceprevir was purchased from Selleckchem (USA). Calpeptin, MG-115 and MG-132 were purchased from Santa Cruz Biotechnology (USA). GC-376 and Telaprevir were purchased from BOC Sciences (China). PF-0835231 was custom-synthesized by WuXi AppTec (China).

SARS-CoV-2 3CLpro expression and purification is based on a published procedure[16] and our modified protocol is found in the supplementary file. A highly sensitive FRET based protease assay was developed to identify inhibitors of 3CL proteases based on a published protocol.[30] The peptide substrate (Dabcyl)KTSAVLQSGFRKM(Glu)(EDANS) was synthesized by Genscript (USA). The test compounds were

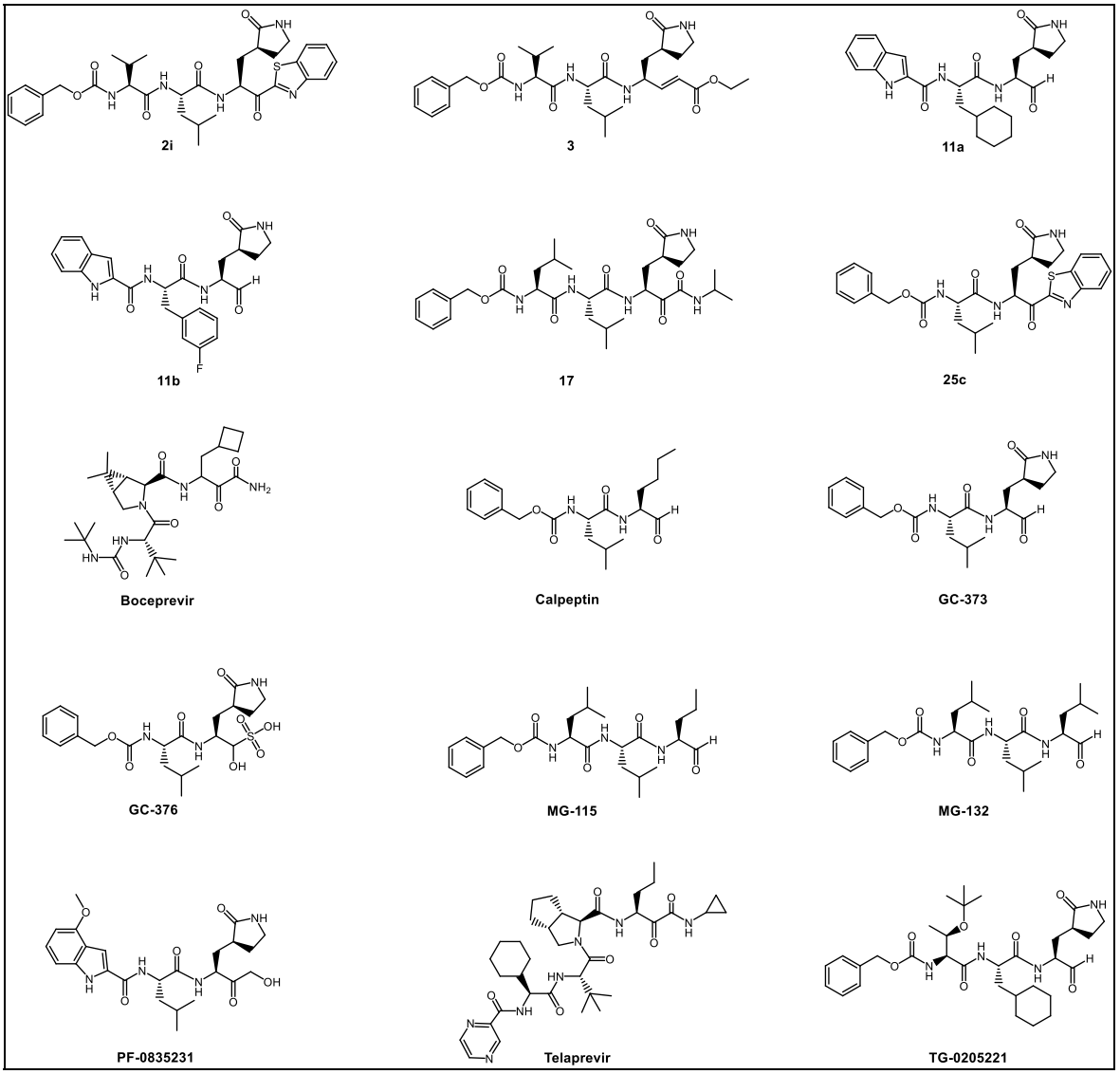

Fig. 1. Reported peptidomimetic coronavirus 3CLpro inhibitors.

Table 1
SARS-CoV-2 3CLpro inhibitory activities (μM) of various reported inhibitors. Numbers in parentheses represent relative potencies based on in-house IC_{50} values, e.g. (1) = most potent.

Compound	In-house IC_{50} (μM)	Literature IC_{50} or K_i (μM)	CoV-1 or CoV-2 3CLpro[1]	Literature reference
2i	(8) 0.094 ± 0.006	1.7 (IC_{50})	CoV-1	26
3	(9) 0.286 ± 0.014	0.66 (K_i)	CoV-1	19
11a	(3) 0.014 ± 0.001	0.053 ± 0.005 (IC_{50})	CoV-2	20
		0.031 ± 0.003 (IC_{50})	CoV-2	23
11b	(4) 0.023 ± 0.003	0.040 ± 0.002 (IC_{50})	CoV-2	20
17	(7) 0.065 ± 0.007	0.007 (IC_{50})	CoV-1	24
25c	>10	21.0 (IC_{50})	CoV-1	27
Boceprevir	>10	4.13 ± 0.61 (IC_{50})	CoV-2	16
		5.40 ± 1.53 (IC_{50})	CoV-2	29
Calpeptin	>10	10.69 ± 0.28 (IC_{50})	CoV-2	16
		4.81 ± 0.18 (IC_{50})	CoV-2	31
GC-373	(6) 0.042 ± 0.001	0.40 ± 0.05 (IC_{50})	CoV-2	21
GC-376	(5) 0.034 ± 0.001	0.030 ± 0.008 (IC_{50})	CoV-2	16
		0.031 ± 0.004 (IC_{50})	CoV-2	23
MG-115	>10	3.14 ± 0.97 (IC_{50})	CoV-2	16
MG-132	>10	3.90 ± 1.01 (IC_{50})	CoV-2	16
PF-0835231	(1) 0.008 ± 0.001	0.004 ± 0.0003 (IC_{50})	CoV-1	22
Telaprevir	>10	11.47 (IC_{50})	CoV-2	25
TG-0205221	(2) 0.009 ± 0.001	0.053 (K_i)	CoV-1	19

[1]Type of protease used in the assay as reported in the literature.

3-fold serially diluted in 100% DMSO to 15 concentrations, starting at 3.33 mM. 1.5 μl of the serially diluted compounds were transferred to a black 384 well assay plate (Cat. 781900, Greiner). 23.5 μl of 2.13X concentration of SARS-CoV-2 Chis-3CLpro enzyme prepared in assay buffer was added to the compounds and incubated for 30 mins at 25 °C. 25 μl of 2X concentration of peptide substrate was added to the assay plate and incubated at 37 °C for 1.5 h. The final assay contained 12.5 nM of enzyme, 6 μM substrate and 3% DMSO in assay buffer containing 50 mM HEPES at pH 7.5, 100 mM NaCl, and 0.01% Triton X-100 and 1 mM DTT. The starting test compound concentration started at 100 μM. The FRET signal was measured using an excitation wavelength of 340 nm (UV[TRF] 340/60 nm, Barcode 101), emission wavelength of 490 nm (DSPPsion 486/10 filter, Barcode 220) and Lance/DELFIA D400 single mirror (Barcode 412) on Envision plate reader (2104 EnVision Multilabel Plate Readers, Perkin Elmer). The dose–response curves were fitted with a variable slope using GraphPad Prism software (GraphPad, USA) to determine a compound's IC_{50}. Experiments were conducted in duplicates and IC_{50}s were determined from two independent experiments.

The IC_{50}s of the test compounds, along with their reported inhibitory data and literature references, are summarised in Table 1.

The most potent compound in our test panel was identified to be Pfizer's PF-0835231[22] (Table 1) with a reported IC_{50} close to our experimental results (8 vs. 4 nM respectively), making it a highly promising drug candidate. Indeed, PF-0835231 is currently being developed as an intravenous phosphate prodrug (PF-07304814), entering phase 1 clinic trials in September 2020 to evaluate safety and pharmacokinetics in hospitalized COVID-19 patients (clinical trials identifier: NCT04535167). X-ray diffraction studies revealed PF-07304814 binds tightly to the SARS-CoV-2 3CLpro active site involving 8 H-bonding interactions and a covalent bond to 3CLpro's Cys145 (Fig. 2; PDB code 6XHM).[22]

The second most potent compound is Taigen's peptide aldehyde TG-0205221 (IC_{50} 9 nM; Table 1) originally developed for SARS-CoV-1 in 2006.[19] This compound was included in our test panel as SARS-CoV-1 3CLpro shares 96% amino acid sequence identity to SARS-CoV-2 3CLpro using a BLAST search[32] (procedure described in the supplementary file), suggesting SARS-CoV-1 3CLpro inhibitors will also inhibit SARS-CoV-2 3CLpro. TG-0205221 was claimed to possess 'favourable pharmacokinetic profile in rodents' although details were not revealed.[19] To our best knowledge, TG-0205221 has never entered any clinical trials. We believe that the aldehyde moiety may have created issues during preclinical development as they are known to be highly reactive towards endogenous biological nucleophiles, making them cytotoxic and are also metabolically unstable due to their susceptibility to oxidation and reduction by liver enzymes.[33,34]

The next two most potent inhibitors are peptide aldehydes 11a and 11b, exhibiting IC_{50}s of 14 and 23 nM respectively (Table 1). Like PF-0835231, both contain a 2-carboxyindole moiety whose NH and CO were shown to be H-bonded to 3CLpro's Glu166 backbone CO and NH using X-ray crystallography (PDB codes 6LZE and 6M0K).[20] However, like TG-0205221, we believe the aldehyde moieties will pose pharmacological liabilities stated *vide supra*. One possible solution is to substitute the aldehyde with a hydroxymethylketone warhead seen in PF-0835231 before they can be reconsidered for drug development.

The fifth and sixth most potent compounds, GC-376 and GC-373, are peptides with a hydroxymethyl sulfonic acid and aldehyde warhead respectively (IC_{50}s 34 and 42 nM respectively; Table 1). GC-376's hydroxymethyl sulfonic acid is a prodrug moiety that transforms into an aldehyde warhead in physiological conditions to react and form a covalent bond with 3CLpro's active site Cys145.[21] However, we believe this will still result in metabolic liabilities for GC-376 once the reactive aldehyde is formed in the bloodstream as discussed *vide supra*. Similarly, we predict the aldehyde warhead of GC-373 will pose metabolic problems as stated earlier.

GSK's peptide ketoamide 17 is the seventh most potent inhibitor in

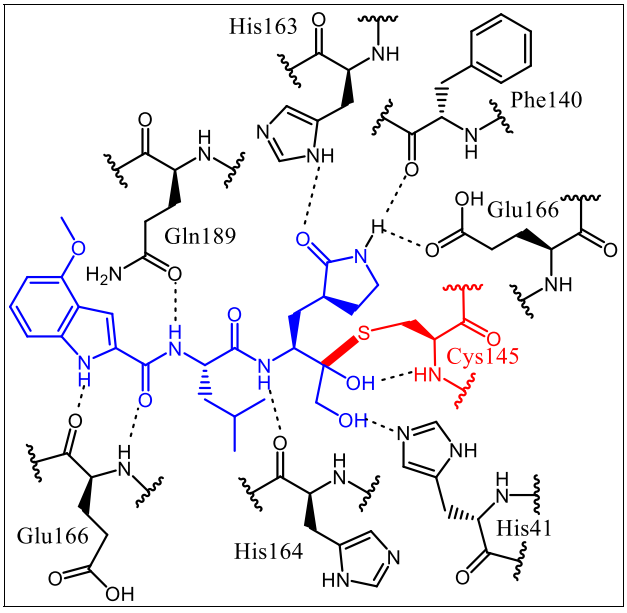

Fig. 2. Two-dimensional depiction of PF-07304814 (blue) covalently bound to Cys145 (red) in the SARS-CoV-2 3CLpro active site based on co-crystal structure 6XHM.pdb. The covalent bond formed between Cys145 and PF-07304814 is depicted as a bold red line. Hash lines represent hydrogen bonds.

our test panel with an IC_{50} of 65 nM (Table 1). Designed with a ketoamide warhead, we believe it possesses a high potential for further drug development as ketoamides have been shown to be effective warheads in the approved oral HCV NS3 protease inhibitors Boceprevir and Telaprevir.[28] Based on this, we believe 17 can potentially be developed into an *oral* anti-CoV-2 drug. Oral drugs are favoured over intravenously-administered ones as they do not cause pain during administration, do not require trained personnel to administer and thus allowing administration in home settings. These factors serve to significantly improve patient dosing compliance and we recommend it for further development to treat COVID-19.

Compound 2i with a ketobenzothiazole warhead is the eighth most potent inhibitor with an IC_{50} of 94 nM (Table 1), suggesting that it has the potential for further drug development. Peptide ketobenzothiazoles were first reported as Rhinovirus 3C protease inhibitors by Agouron Pharmaceuticals in 2000.[35] To our best knowledge, no peptide ketobenzothiazole inhibitors have entered the clinic so far. A report that the benzothiazole N and S are both prone to metabolic oxidation[36] suggests that it may become a metabolic liability although there are at least ten non-peptidic drugs containing the benzothiazole moiety available in the market.[37] Hence, we believe compound 2i deserves further investigation with careful scrutiny on its pharmacokinetic and pharmacodynamic profile.

Compound 3 (Fig. 1) is the ninth most potent inhibitor and is the sole compound with an ethyl propenoate warhead in our test panel. Peptides with this particular warhead were used as Rhinovirus 3C protease inhibitors by Agouron Pharmaceuticals in 1998.[38] Their most advanced compound, Rupintrivir, reached phase 2 clinical trials in 1999.[39] Although Rupintrivir was reported to be inactive against SARS-CoV-1 and SARS-CoV-2 3CLpro,[16,19] its analogue compound 3 was reported to bind SARS-CoV-1 3CLpro with a K_i of 660 nM although its IC_{50} was not reported.[19] In our SARS-CoV-2 3CLpro inhibition assay, compound 3 exhibited an IC_{50} of 286 nM (Table 1). In our view, it would be premature to consider it for drug development due to its moderate inhibitory potency. More structure–activity relationship studies would have to be conducted to enhance its potency before reconsideration.

The remaining compounds: Boceprevir, Calpeptin, MG-115, MG-132 and Telaprevir were found to be weak inhibitors with IC_{50}s above 10 μM (Table 1), making them unsuitable for further development. It is noteworthy that they lack the P1 cyclic glutamine analogue found in most of our test compounds, suggesting that this is a critical moiety for SARS-CoV-2 3CLpro recognition. Indeed, X-ray co-crystal structures of PF-07304814, 11a and 11b bound to SARS-CoV-2 3CLpro (PDB codes 6XHM, 6LZE, 6M0K)[20,22] revealed that the P1 lactam's NH and CO are involved in H-bonding to the 3CLpro's Phe140 backbone CO and His163 side-chain imidazole respectively (Fig. 2). This suggests that when designing new peptidomimetic 3CLpro inhibitors, an amide moiety must be present in the P1 residue.

In conclusion, a head-to-head IC_{50} comparison of fifteen published coronavirus 3CLpro peptidomimetic inhibitors was conducted using a standard SARS-CoV-2 3CLpro inhibition assay. The most potent compound was identified to be PF-07304814 (IC_{50} 8 nM), with a hydroxymethylketone warhead and recently entered COVID-19 clinical trials as a phosphate prodrug. Peptide aldehydes, well-known for being highly potent protease inhibitors, populated the second to sixth spots in term of potencies (IC_{50}s 9–42 nM). However, as aldehyde warheads can potentially pose toxicity and metabolic issues, we suggest that they should first be converted to hydroxymethylketone or ketoamide warheads before being re-evaluated. GSK's peptide ketoamide 17 was the seventh most potent inhibitor (IC_{50} 65 nM) and we believe it has the potential to be further developed as an oral antiviral drug based on the successes of Boceprevir and Telaprevir. Peptides with ketobenzothiazole and ethyl propenoate warheads (compounds 2i and 3) were relatively less potent (IC_{50}s 94 and 286 nM respectively) and we opine they should not be used in their current forms. Lastly, we note that test compounds without a P1 amide moiety exhibited IC_{50}s of >10 μM, making them unsuitable for further drug development. This strongly suggests that a P1 amide moiety is critical to the design of future SARS-CoV-2 3CLpro peptidomimetic inhibitors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Agency for Science, Technology and Research (A*STAR) Biomedical Research Council (BMRC).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128263.

References

1 Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. 2020;382:1199–1207. https://doi.org/10.1056/NEJMoa2001316.
2 Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382:727–733. https://doi.org/10.1056/NEJMoa2001017.
3 Drosten C, Günther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003;348:1967–1976. https://doi.org/10.1056/NEJMoa030747.
4 Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med*. 2003;348:1953–1966. https://doi.org/10.1056/NEJMoa030781.
5 Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565–574. https://doi.org/10.1016/S0140-6736(20)30251-8.
6 Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579:270–273. https://doi.org/10.1038/s41586-020-2012-7.
7 Zhu Z, Lian X, Su X, Wu W, Marraro GA, Zeng Y. From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. *Respir Res*. 2020;21. https://doi.org/10.1186/s12931-020-01479-w.
8 Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol*. 2020;5:536–544. https://doi.org/10.1038/s41564-020-0695-z.
9 World Health Organization Coronavirus (COVID-19) Dashboard. https://covid19.who.int; Accessed 3 July 2021.
10 Rubin D, Chan-Tack K, Farley J, Sherwat A. FDA approval of Remdesivir – a step in the right direction. *N Engl J Med*. 2020;383:2598–2600. https://doi.org/10.1056/NEJMp2032369.
11 Baum A, Fulton BO, Wloga E, et al. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science*. 2020;369:1014–1018. https://doi.org/10.1126/science:abd0831.
12 FDA Press Announcements May 26, 2021. Coronavirus (COVID-19) Update: FDA Authorizes Monoclonal Antibodies for Treatment of COVID-19. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-monoclonal-antibody-treatment-covid-19; Accessed 3 July 2021.
13 World Health Organization Newsroom. Feature Stories. https://www.who.int/news-room/feature-stories/detail/who-recommends-against-the-use-of-remdesivir-in-covid-19-patients. 20 November 2020; Accessed 3 July 2021.
14 Ghosh AK, Brindisi M, Shahabi D, et al. Drug development and medicinal chemistry efforts toward SARS-coronavirus and Covid-19 therapeutics. *ChemMedChem*. 2020;15:907–932. https://doi.org/10.1002/cmdc.v15.1110.1002/cmdc.202000223.
15 Banerjee R, Perera L, Tillekeratne LMV. Potential SARS-CoV-2 main protease inhibitors. *Drug Discov Today*. 2021;26:804–816. https://doi.org/10.1016/j.drudis.2020.12.005.
16 Ma C, Sacco MD, Hurst B, et al. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease. *Cell Res*. 2020;30:678–692. https://doi.org/10.1038/s41422-020-0356-z.
17 Jin Z, Du X, Xu Y, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature*. 2020;582:289–293. https://doi.org/10.1038/s41586-020-2223-y.
18 Zhang L, Lin D, Kusov Y, et al. α-Ketoamides as broad-spectrum inhibitors of coronavirus and enterovirus replication: Structure-based design, synthesis, and activity assessment. *J Med Chem*. 2020;63:4562–4578. https://doi.org/10.1021/acs.jmedchem.9b01828.

19 Yang S, Chen SJ, Hsu MF, et al. Synthesis, crystal structure, structure-activity relationships, and antiviral activity of a potent SARS coronavirus 3CL protease inhibitor. *J Med Chem*. 2006;49:4971–4980. https://doi.org/10.1021/jm0603926.

20 Dai W, Zhang B, Jiang XM, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science*. 2020;368:1331–1335. https://doi.org/10.1126/science.abb4489.

21 Vuong W, Khan MB, Fischer C, et al. Feline coronavirus drug inhibits the main protease of SARS-CoV-2 and blocks virus replication. *Nat Commun*. 2020;11. https://doi.org/10.1038/s41467-020-18096-2.

22 Hoffman RL, Kania RS, Brothers MA, et al. Discovery of ketone-based covalent inhibitors of coronavirus 3CL proteases for the potential therapeutic treatment of COVID-19. *J Med Chem*. 2020;63:12725–12747. https://doi.org/10.1021/acs.jmedchem.0c01063.

23 Yang KS, Ma XR, Ma Y, et al. A quick route to multiple highly potent SARS-CoV-2 main protease inhibitors. *ChemMedChem*. 2021;16:942–948. https://doi.org/10.1002/cmdc.v16.610.1002/cmdc.202000924.

24 Botyanszki J, Catalano JG, Chong PY, et al. Compounds that inhibit 3C and 3CL proteases and methods of use thereof. WO2018042343A2.

25 Pathak N, Chen YT, Hsu YC, et al. Uncovering flexible active site conformations of SARS-CoV-2 3CL proteases through protease pharmacophore clusters and COVID-19 drug repurposing. *ACS Nano*. 2021;15:857–872. https://doi.org/10.1021/acsnano.0c07383.

26 Konno S, Thanigaimalai P, Yamamoto T, et al. Design and synthesis of new tripeptide-type SARS-CoV 3CL protease inhibitors containing an electrophilic arylketone moiety. *Bioorg Med Chem*. 2013;21:412–424. https://doi.org/10.1016/j.bmc.2012.11.017.

27 Thanigaimalai P, Konno S, Yamamoto T, et al. Design, synthesis, and biological evaluation of novel dipeptide-type SARS-CoV 3CL protease inhibitors: Structure-activity relationship study. *Eur J Med Chem*. 2013;65:436–447. https://doi.org/10.1016/j.ejmech.2013.05.005.

28 Manns MP, von Hahn T. Novel therapies for hepatitis C – one pill fits all? *Nat Rev Drug Discov*. 2013;12:595–610. https://doi.org/10.1038/nrd4050.

29 Ghahremanpour MM, Tirado-Rives J, Deshmukh M, et al. Identification of 14 known drugs as inhibitors of the main protease of SARS-CoV-2. *ACS Med Chem Lett*. 2020;11:2526–2533. https://doi.org/10.1021/acsmedchemlett.0c00521.

30 Kim Y, Lovell S, Tiew K-C, et al. Broad-spectrum antivirals against 3C or 3C-like proteases of Picornaviruses, Noroviruses, and Coronaviruses. *J Virol*. 2012;86:11754–11762.

31 Nar H, Schnapp G, Hucke O, et al. Action of dipeptidyl peptidase-4 inhibitors on SARS-CoV-2 main protease. *ChemMedChem*. 2021;16:1425–1426. https://doi.org/10.1002/cmdc.202000921.

32 Altschul SF, Gish W, Miller W, et al. Basic local alignment search tool. *J Mol Biol*. 1990;215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

33 Manevski N, King L, Pitt WR, et al. Metabolism by aldehyde oxidase: drug design and complementary approaches to challenges in drug discovery. *J Med Chem*. 2019;62:10955–10994. https://doi.org/10.1021/acs.jmedchem.9b00875.

34 Gampe C, Verma VA. Curse or cure? A perspective on the developability of aldehydes as active pharmaceutical ingredients. *J Med Chem*. 2020;63:14357–14381. https://doi.org/10.1021/acs.jmedchem.0c01177.

35 Dragovich PS, Zhou R, Webber SE, et al. Structure-based design of ketone-containing, tripeptidyl human rhinovirus 3C protease inhibitors. *Bioorg Med Chem Lett*. 2000;10:45–48. https://doi.org/10.1016/S0960-894X(99)00587-9.

36 Bradshaw TD, Stevens MFG, Westwell AD. The discovery of the potent and selective antitumour agent 2-(4-Amino-3-methylphenyl)benzothiazole (DF 203) and related compounds. Curr. Med. Chem. 2001;8:203−210. doi: 10.2174/0929867013373714.

37 Sharma PC, Sinhmar A, Sharma A, et al. Medicinal significance of benzothiazole scaffold: an insight view. *J Enzyme Inhib Med Chem*. 2013;28:240–266. https://doi.org/10.3109/14756366.2012.720572.

38 Dragovich PS, Webber SE, Babine RE, et al. Structure-based design, synthesis, and biological evaluation of irreversible human Rhinovirus 3C protease inhibitors. 1. Michael acceptor structure-activity studies. *J Med Chem*. 1998;41:2806–2818. https://doi.org/10.1021/jm980068d.

39 Matthews DA, Dragovich PS, Webber SE, et al. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *PNAS*. 1999;96:11000–11007. https://doi.org/10.1073/pnas.96.20.11000.