A patent review on SARS coronavirus main protease ($3CL^{pro}$) inhibitors.

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is an unprecedented global health emergency causing more than 4.2 million fatalities as of 30 July 2021. Only three antiviral therapies have been approved or granted emergency use authorization by the FDA. The SARS-CoV-2 3CL protease ($3CL^{pro}$) is deemed an attractive drug target as it plays an essential role in viral polyprotein processing and pathogenesis, although no inhibitors have been approved. This patent review discusses SARS coronavirus $3CL^{pro}$ inhibitors that have been filed up to 30 July 2021, giving an overview on the types of inhibitors that have generated commercial interest, especially amongst drug companies. Insights into the common structural motifs required for active site binding is also discussed.

Key words

3CLpro; COVID-19, cysteine protease inhibitor; Mpro; SARS-CoV-2

1. Introduction

In late 2019, a number of severe pneumonia clusters were reported in Wuhan, Hubei province, China.[1,2] Genome sequencing revealed the responsible pathogen was a coronavirus with 80% nucleotide sequence identity to the severe acute respiratory syndrome coronavirus (SARS-CoV), responsible for the 2002 – 2004 outbreak originating from Foshan, Guangdong province, China.[3-6] This new coronavirus was named 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) by the International Committee on Taxonomy of Viruses[7] and the disease was called 'coronavirus disease 2019' (COVID-19) by the World Health Organization (WHO). The virus has since spread globally, infecting more than 196 million people and causing more than 4.2 million deaths as of 30 July 2021.[8] Although a number of vaccines have been granted authorization, only three antiviral therapies (Remdesivir, Casirivimab + Imdevimab and Sotrovimab)[9-11] have so far been approved or granted emergency use authorization by the United States Food and Drug Administration (FDA), signifying an urgent need for more antiviral drugs.

The coronavirus 3-chymotrypsin-like protease ($3CL^{pro}$; also known as 'main protease' or 'M^{pro}') is deemed an attractive drug target due to its essential role in coronavirus polyprotein processing. It cleaves the polyprotein at more than 11 sites to yield essential proteins required for virus replication and pathogenesis.[12-16] As $3CL^{pro}$ has no reported human homologues, a $3CL^{pro}$ inhibitor should not inhibit any human proteases and hence, reduce the risk of side-effects.[15,16] $3CL^{pro}$ is a cysteine protease with a unique substrate preference for a P1 glutamine, hydrolysing the peptide bond at the C-terminus of glutamine.[12-16] Particularly noteworthy is that the $3CL^{pro}$ of SARS-CoV and SARS-CoV-2 share 96% amino acid sequence identity so inhibitors designed for the former should work equally well on the latter.[12,17] Indeed, the first coronavirus $3CL^{pro}$ inhibitor to enter clinical trials is Pfizer's PF-07304814 (ClinicalTrials.gov identifier

NCT04535167) in September 2020. First described in a patent filed in 2005 as a SARS-CoV 3CL^{pro} inhibitor (WO 2005/113580), its synthesis, structure-activity relationship study and preclinical development details were published only much later in 2020.[18]

2. Patent Review

Although many SARS-CoV and SARS-CoV-2 3CL^{pro} inhibitors have been reported, collated and reviewed in academic journals since the 2002 – 2004 SARS-CoV pandemic, none have so far been approved as antiviral drugs.[12-14] A plausible explanation could be that commercial interest in coronavirus drug development waned rapidly after the SARS-CoV pandemic ended. However, since the start of the SARS-CoV-2 pandemic in late 2019, academic and commercial interest in coronavirus 3CL^{pro} inhibitors returned with a vengeance. This review collates SARS-CoV and SARS-CoV-2 3CL^{pro} inhibitors reported in patent applications filed since the 2002 SARS-CoV pandemic up to 30 July 2021. SciFinder® software (CAS)[19] was employed and search terms: ‘coronavirus 3CLpro inhibitor’, ‘coronavirus 3CL protease inhibitor’, ‘coronavirus Mpro inhibitor’, ‘coronavirus main protease inhibitor’ and ‘coronavirus protease inhibitor’ were used. The patents are summarized in Table 1 in chronological order with detailed analysis included *vide infra*.

72 **Table 1** Summary of SARS coronavirus $3CL^{pro}$ inhibitor patents in chronological order up to 30 July 2021.

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Patent number	Applicant/Assignee	Filing date (dd.mm.yyyy)	Inhibitor type	Figure	Reference
WO 2004/093860	Pfizer	13.04.2004	peptidomimetic	1	20
WO 2004/101742	Cytovia	06.05.2004	peptidomimetic	2	21
US 2006/0014821	Agouron Pharmaceuticals	13.08.2004	peptidomimetic	3	22
WO 2005/041904	Fulcrum Pharmaceuticals	01.11.2004	small molecule	4	23
WO 2005/066123	Taigen Biotechnology	28.12.2004	peptidomimetic	5	24
WO 2005/113580	Pfizer	09.05.2005	peptidomimetic	6	25
US 2006/0019967	National Health Research Institutes, Taiwan	20.07.2005	small molecule	7	26
WO 2006/042478	Tsinghua University, Shanghai Institute of Organic Chemistry	24.10.2005	peptidomimetic	8	27
CN 1965833A	Peking University	18.11.2005	small molecule	9	28
WO 2006/061714	Pfizer	06.12.2005	peptidomimetic	10	29

WO 2006/095624	Tokyo Medical and Dental University, RIKEN	01.03.2006	small molecule	11	30
WO 2007/075145	Singapore Polytechnic, Shanghai Institute of Materia Medica	15.12.2006	small molecule	12	31
CN 103159665B	Tianjin International Joint Academy of Biotechnology and Medicine	09.12.2011	small molecule	13	32
WO 2013/049382	Kansas State University, Ohio State University, Wichita State University	27.09.2012	peptidomimetic	14	33
KR 1020130002975	Chonnam National University	20.12.2012	small molecule	15	34
WO 2013/166319	Kansas State University, Wichita State University	02.05.2013	peptidomimetic	16	35
CN 106176728A	Institute of Microbiology, Chinese Academy of Sciences	07.07.2016	small molecule	17	36
WO 2017/114509	Shanghai Institute of Materia Medica, University of Lübeck	30.12.2016	peptidomimetic	18	37

US 2017/0313685	Purdue Research Foundation	28.04.2017	small molecule	19	38
WO 2017/222935	Kansas State University, Wichita State University	16.07.2017	peptidomimetic	20	39
CN 108785293A	Tianjin International Joint Academy of Biomedicine	28.04.2017	small molecule	21	40
WO 2018/042343	GSK	30.08.2017	peptidomimetic	22	41
WO 2020/030143	Shanghai Institute of Materia Medica, Fudan University	09.08.2019	peptidomimetic	23	42
WO 2021/176369	Pfizer	03.03.2021	peptidomimetic	24	43

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SciFinder® revealed 18 patents on CoV 3CL^{pro} inhibitors and together with a 2020 patent review on coronavirus therapeutic agents,[44] a total 24 patents are summarised in Table 1. Unsurprisingly, majority of the patents (23 out of 24) described inhibitors designed to inhibit SARS-CoV 3CL^{pro} as the SARS-CoV-2 pandemic started only in late 2019. However, since both viral proteases share 96% sequence identity,[12,17] inhibitors designed for the former should also inhibit the latter protease. Indeed, Pfizer's PF-00835231 (**6b**), first described in WO 2005/113580, was originally designed to inhibit SARS-CoV 3CL^{pro} (IC_{50} 4 nM)[18] and was later shown to be a potent inhibitor of SARS-CoV-2 3CL^{pro} (IC_{50} 8 nM).[45]

To our best knowledge, the earliest patent application describing SARS-CoV 3CL^{pro} inhibitors was filed by Pfizer in 2004 (WO 2004/093860; Table 1)[20] involving 3-residue peptidomimetics bearing a P1 glutamine or lactam glutamine mimic with a C-terminal electrophilic α,β-unsaturated ester warhead (Figure 1). Some inhibitors had their P3 amino acid residue substituted by a 2-pyridone moiety (**1b**), presumably to improve their pharmacokinetic properties. All the described compounds were originally designed to inhibit the rhinovirus 3C protease which shares some structural similarity to coronavirus 3CL^{pro}.[20] Unfortunately, no coronavirus 3CL^{pro} inhibition data were reported in the patent. We believe this is the first patent describing peptidomimetics bearing a P1 lactam glutamine mimic (**1a**) used for inhibiting SARS-CoV 3CL^{pro} and were originally designed as rhinovirus 3C protease inhibitors in 1999 by Agouron Pharmaceuticals (WO 99/57135).[46] We are currently unable to recommend these peptidomimetics for further drug development until their SARS-CoV-2 3CL^{pro} inhibitory potencies are revealed.

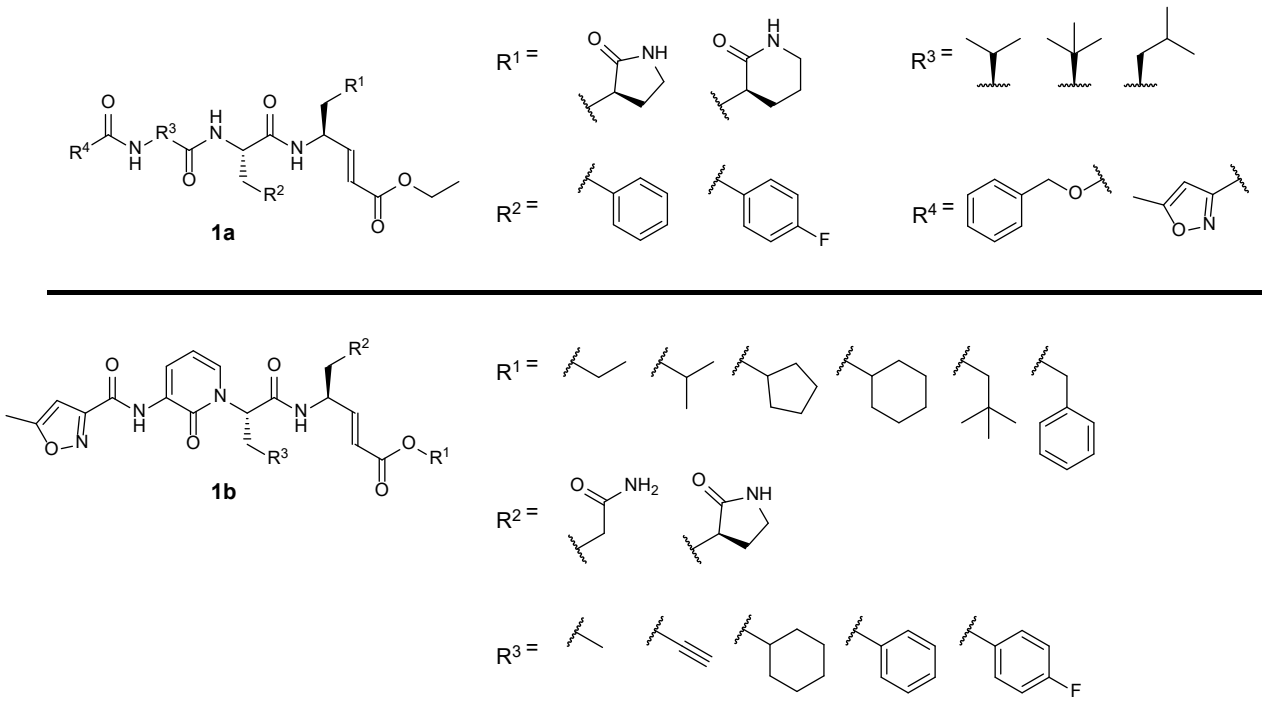


Figure 1. General scaffolds from WO 2004/093860.

The second earliest patent application on SARS-CoV 3CL^{pro} inhibitors was filed by Cytovia in 2004 (WO 2004/101742; Table 1)[21] describing 2-residue peptidomimetics containing a P1 glutamine residue with a C-terminal electrophilic monofluoromethylketone warhead (Figure 2). P1 alanine, glycine and N-dimethylated glutamine mimics were also exemplified although their inhibitory activities were not disclosed. Compound **2b** was revealed to be the most potent inhibitor with an EC_{50} of 0.02 μg/mL in a SARS-CoV Vero cell assay. No biochemical 3CL^{pro} inhibition (IC_{50}) data were reported in the patent. A publication was found for **2e** containing a P1 N-dimethylated glutamine with an EC_{50} of 2.5 μM in a SARS-CoV Vero cell assay.[47] The authors revealed that the P1 glutamine residue was incompatible with the C-terminal electrophilic warhead due to intramolecular cyclisation with the P1 glutamine side-chain primary amide, resulting in bioactivity loss.[47] To solve this problem, Pfizer filed a patent

in 2004 describing peptidomimetic inhibitors where the P1 glutamine was modified to either 5- or 6-membered lactams (WO 2004/093860; Figure 1a).[20] Notably, this P1 modification strategy was adopted in subsequent patents involving coronavirus 3CL^{pro} peptidomimetic inhibitors after 2004. In addition, the idea of incorporating a 2-pyridone moiety at the P3 position, exemplified in compound **2f**, was reported in an earlier Pfizer patent (WO 2004/093860; Figure 1b).[20] Although there are currently no follow-up publications for **2f**, this idea was later adopted for peptidomimetic ketoamide SARS-CoV-2 3CL^{pro} inhibitors in a 2020 Science paper.[48]

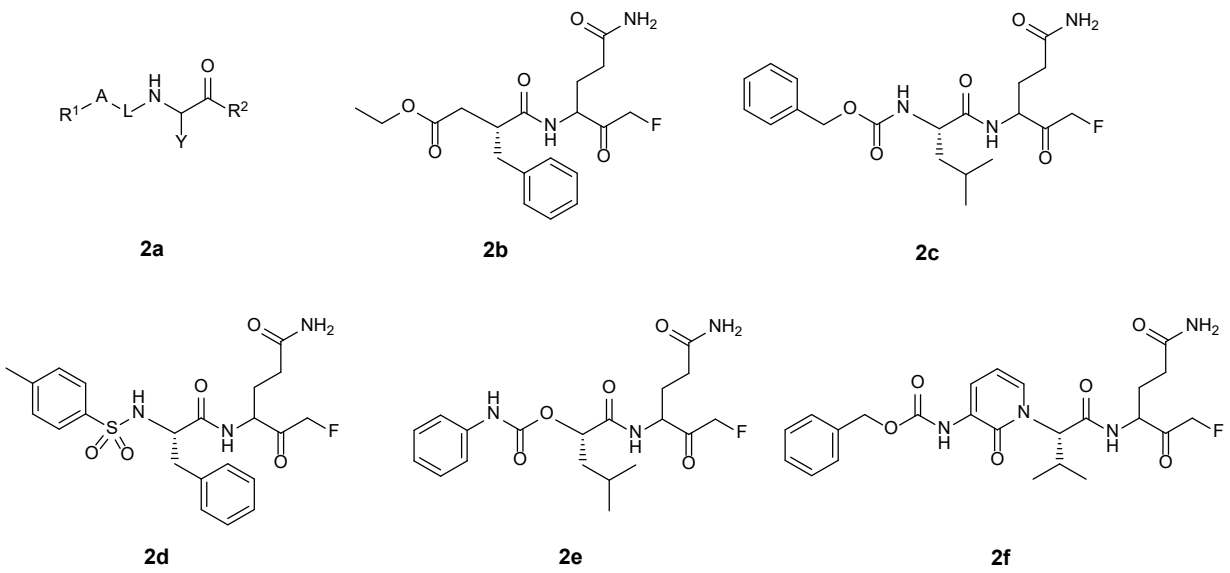


Figure 2. Structures from WO 2004/101742. (a) general scaffold; (b – f) exemplified structures.

The third earliest patent on coronavirus 3CL^{pro} inhibitors was filed in 2004 by Agouron Pharmaceuticals involving 1-, 2- and 3-residue peptidomimetics bearing a P1 5-membered lactam glutamine mimic with a C-terminal electrophilic α,β-unsaturated ester warhead (US 2006/0014821; Table 1).[22] 8 structures were exemplified (Figure 3) but no

biochemical 3CL^{pro} inhibition (IC_{50}) data were reported in the patent. A structure search on all 8 compounds, including compounds **3c** to **3f**, revealed no follow-up publications. Notably, peptidomimetics with α,β-unsaturated ester warheads inhibiting SARS-CoV 3CL^{pro} were described in an earlier patent (WO 2004/093860; Figure 1).[20] We are currently unable to gauge their potential for further antiviral drug development due to the lack of inhibitory potency data. Interestingly, another patent involving peptidomimetic inhibitors with electrophilic α,β-unsaturated ester warheads was filed in 2004 by Taigen Biotechnology (WO 2005/066123; Figure 5),[24] discussed *vide infra*.

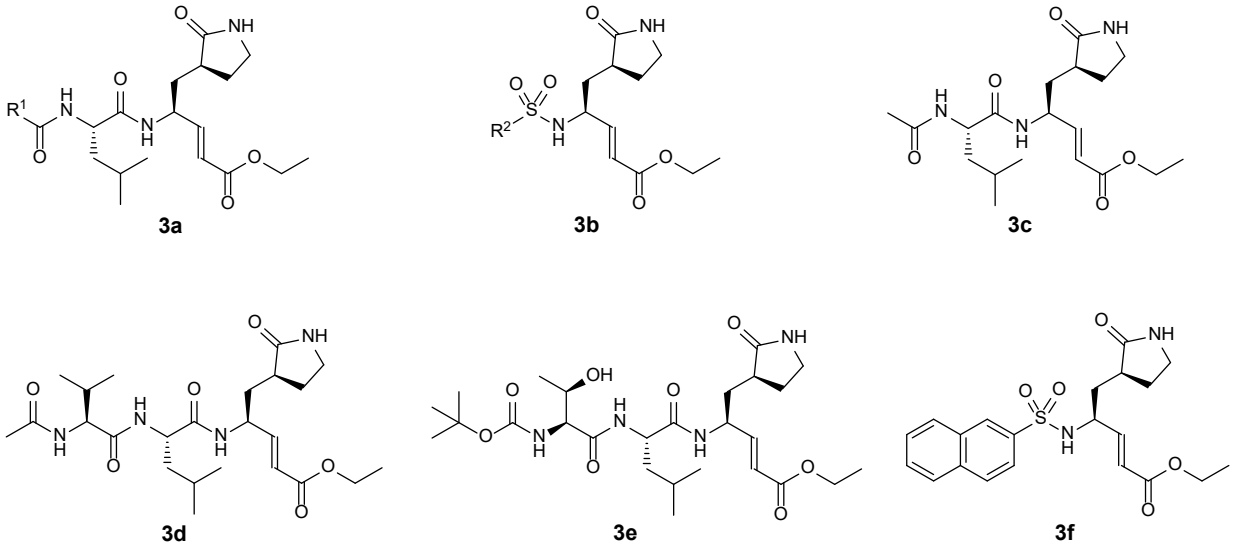


Figure 3. Structures from US 2006/0014821. (a, b) general scaffolds; (c – f) exemplified structures.

The fourth earliest patent was filed in 2004 by Fulcrum Pharmaceuticals involving small molecules containing one or two boronic acid moieties (WO 2005/041904; Table 1).[23] 403 structures were described. The strongest binder was identified to be compound **4b**, also named FL-166, with a SARS-CoV 3CL^{pro} K_i of 22 nM, followed by **4c** to **4f** with K_is ranging between 3.5 and 6.9 μM (Figure 4). Compounds **4b, 4c** and **4d** were reported

in a research article to possess K_is of 0.04, 4.5 and 16 μM against SARS-CoV 3CL^{pro} respectively.[49] No SARS-CoV-2 3CL^{pro} inhibition data have been reported for these compounds using SciFinder® structure searches. In our view, cell-based EC_{50} values should first be obtained for **4a** to gauge its potential for further development as an antiviral drug.

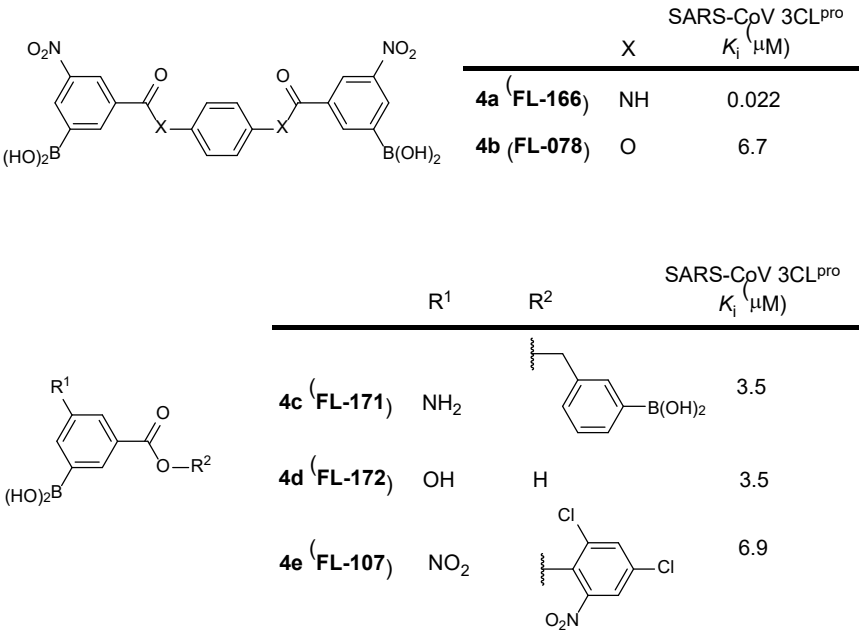
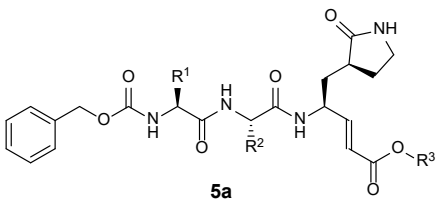


Figure 4. Structures from WO 2005/041904. (a – e) exemplified structures with the most potent K_is against SARS-CoV 3CL^{pro}.

The fifth patent was filed in 2004 by Taigen Biotechnology involving 3-residue peptidomimetics containing a P1 5-membered lactam glutamine mimic with a C-terminal electrophilic α,β-unsaturated ester warhead (WO 2005/066123; Table 1).[24] 145 compounds were reported and 77 were disclosed to be sub-micromolar SARS-CoV 3CL^{pro} inhibitors. Unfortunately, no K_i or IC_{50} values were assigned to any of the structures. However, a literature search revealed compound **5b**, also named TG-

0203770, to be the strongest binder with a SARS-CoV 3CL^{pro} K_i of 58 nM, followed by **5c** and **5d** (K_is 0.66 and 2.26 μM respectively; Figure 5).[50] Compound **5c** was later reported to inhibit SARS-CoV-2 3CL^{pro} with an IC_{50} of 286 nM.[45] In contrast, **5d** was reported to be a weak SARS-CoV 3CL^{pro} inhibitor (IC_{50} 85 μM),[51] suggesting that a P2 phenylalanine should be avoided in the design of new SARS-CoV 3CL^{pro} inhibitors. Compounds **5e** and **5f**, known as TG-0205486 and TG-0204998 respectively, were reported with K_is of 99 and 38 nM respectively.[52] Notably, two earlier patents involving electrophilic α,β-unsaturated ester warhead peptidomimetics were filed by Pfizer and Agouron Pharmaceuticals (WO 2004/093860 and US 2006/0014821), discussed *vide supra*. We opine compounds **5b**, **5e** and **5f** warrant further investigation as SARS-CoV-2 drug candidates.



5a

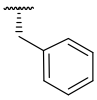
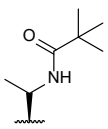
	R^1	R_2	R_3	SARS-CoV 3CL^{pro} K_i (μM)	SARS-CoV 3CL^{pro} IC_{50} (μM)
5b (**TG-0203770**)			C_2H_5	0.058	
5c			C_2H_5	0.66	
5d			C_2H_5	2.26	85
5e (**TG-0205486**)			cyclopropyl	0.099	
5f (**TG-0204998**)			CH_3	0.038	

Figure 5. Structures from WO 2005/066123. (a) general scaffold; (b – f) exemplified structures.

The sixth earliest patent was filed in 2005 by Pfizer involving 2-residue peptidomimetics containing a P1 5-membered lactam glutamine mimic with a C-terminal electrophilic hydroxymethylketone warhead (WO 2005/113580; Table 1).[25] 61 compounds were reported with 35 exhibiting sub-micromolar IC_{50}s against SARS-CoV $3CL^{pro}$. A literature search revealed PF-00835231 (**6b**; Figure 6) as the lead candidate with a very potent IC_{50} of 4 nM against SARS-CoV $3CL^{pro}$ and a SARS-CoV EC_{50} of 4.8 μM in a SARS-CoV infection cellular assay while a recent paper reported an IC_{50} of 8 nM against SARS-CoV-2 $3CL^{pro}$.[18,45] This strongly suggests that inhibitors designed to inhibit SARS-CoV $3CL^{pro}$ can be repurposed for SARS-CoV-2 $3CL^{pro}$ as both proteases share 96% sequence identity.[12,17] The phosphate prodrug of PF-0835231 entered phase 1 clinical trials in September 2020 to evaluate safety and pharmacokinetics in hospitalised COVID-19 patients (ClinicalTrials.gov identifier: NCT04535167). Notably, substituting the P2 leucine with phenylalanine resulted in a 25-fold activity loss (IC_{50} 103 vs. 4 nM; **6e** and **6b** respectively), suggesting that the SARS-CoV $3CL^{pro}$ S2 binding pocket preferred smaller sized residues.[18] N-methylating the P2 leucine resulted in a 20-fold activity loss (IC_{50} 83 vs. 4 nM; **6f** and **6b** respectively), suggesting that the P2 NH plays an important role in $3CL^{pro}$ binding.[18] 6-membered lactam glutamine mimics were also described in the patent but none were exemplified.

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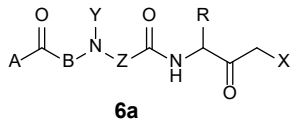
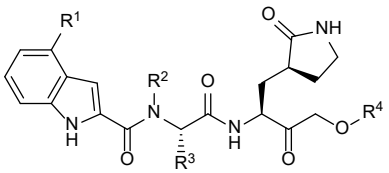
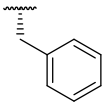


6a



	R^1	R_2	R_3	R_4	SARS-CoV 3CL^{pro} IC_{50} (μM)
6b (**PF-00835231**)	OCH_3	H		H	0.004
6c	H	H		H	0.038
6d	OCH_3	H		CH_3	0.051
6e	OCH_3	H		H	0.103
6f	OCH_3	CH_3		H	0.083

199

200 **Figure 6**. Structures from WO 2005/113580. (a) general scaffold; (b – f) exemplified
201 structures.

202

203 The seventh earliest patent was filed in 2005 by the National Health Research
204 Institutes, Taiwan, involving small molecules from a commercially available small
205 molecule library screen (US 2006/0019967; Table 1).[26] 21 compounds were reported to
206 possess inhibitory activities with 4 exhibiting 'very low' micromolar IC_{50}s against SARS-
207 CoV 3CL^{pro} (compounds **7a** to **7d**; Figure 7). Unfortunately, IC_{50} values were not
208 disclosed in the patent. A literature search revealed compound **7a** to be the most potent
209 with an IC_{50} of 300 nM against SARS-CoV 3CL^{pro}. [53] The next most potent is **7b** with an
210 IC_{50} of 900 nM while **7c** to **7f** exhibited IC_{50}s between 3 to 10 μM.[53] Structure searches
211 revealed there are currently no reports on their SARS-CoV-2 3CL^{pro} inhibitory activities.
212 Interestingly, compound **7c**, also known as PNU-136592, was earlier reported by
213 Pharmacia scientists to inhibit MurB, a bacteria enzyme involved in cell wall

peptidoglycan biosynthesis.[54] In our view, cell-based EC_{50} values should first be obtained to gauge their potential for further antiviral drug development.

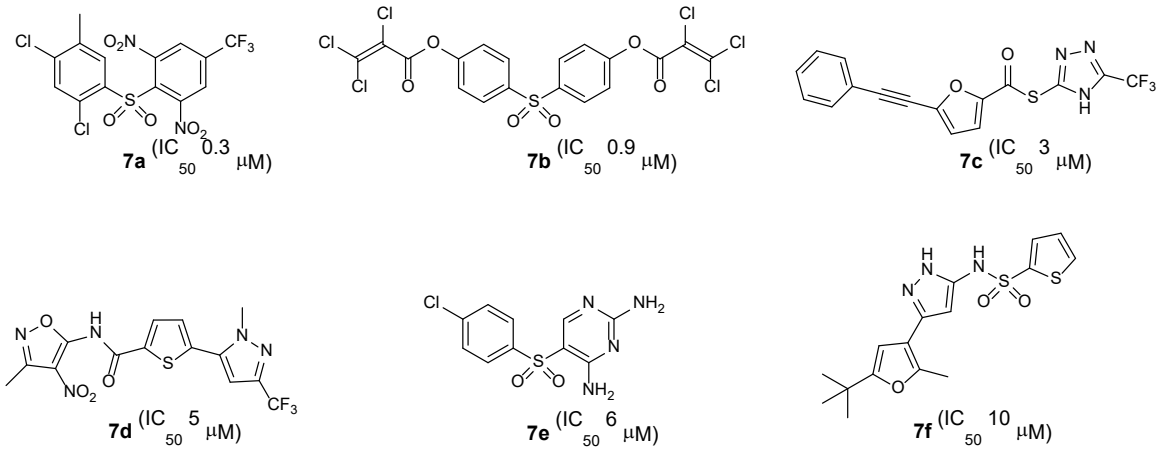


Figure 7. Six most potent SARS-CoV $3CL^{pro}$ inhibitors reported in US 2006/0019967.

The eighth earliest patent was filed in 2005 by Tsinghua University and the Shanghai Institute of Organic Chemistry involving 3- and 4-residue peptidomimetics bearing a P1 5-membered lactam glutamine mimic with a C-terminal electrophilic α,β-unsaturated ester warhead (WO 2006/042478; Table 1).[27] 10 compounds were exemplified and no IC_{50} data was reported (Figure 8). The patent is written in Chinese and an English version could not be found online. A research article published in the same month of patent filing reported inhibitors **8b** and **8d** (named N1 and N3 respectively) with K_is of 10.7 and 9.0 μM against SARS-CoV $3CL^{pro}$ respectively.[55] A 2020 Nature paper reported **8d**/N3 to be a very potent SARS-CoV-2 $3CL^{pro}$ inhibitor and that its K_i could not be feasibly measured.[15] No SARS-CoV-2 $3CL^{pro}$ inhibition data have been reported so far for compounds **8c** and **8e** using SciFinder® structure searches. In our opinion, **8d**/N3 warrants further investigation as an antiviral drug.

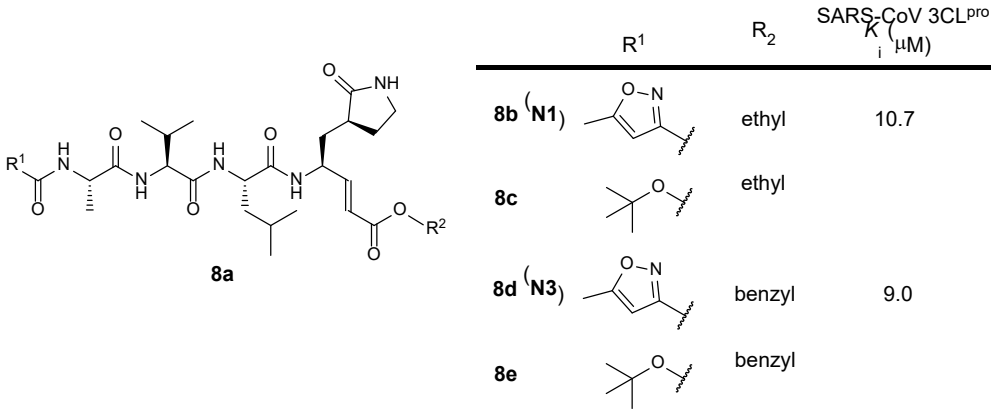


Figure 8. Structures from WO 2006/061714. (a) general scaffold; (b – e) exemplified structures.

The ninth earliest patent was filed in 2005 by Peking University involving the discovery of the oral H_1 histamine receptor antagonist oxatomide possessing SARS-CoV 3CL^{pro} inhibitory activity (CN 1965833A; Figure 9).[28] No IC_{50} or K_i data was reported. The patent is written in Chinese and an English version could not be found online. A SciFinder® structure search did not reveal any academic journals reporting its SARS-CoV 3CL^{pro} inhibitory activity. Due to the lack of inhibitory data, we are currently unable to gauge its potential for further development as an antiviral drug.

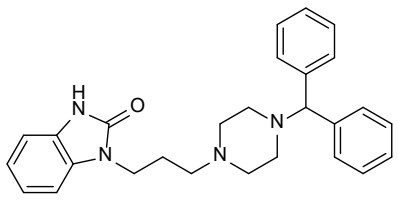
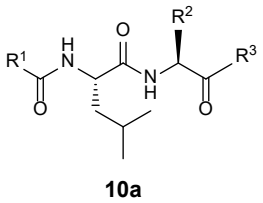


Figure 9. Exemplified structure from CN 1965833A.

The tenth earliest patent was filed in 2005 by Pfizer involving 2- and 3-residue peptidomimetics containing P1 5-membered lactam and tertiary amide glutamine mimics with C-terminal electrophilic aldehyde or ketone warheads (WO 2006/061714; Table 1).[29] 15 compounds were exemplified with 4 (**10b** to **10e**; Figure 10) exhibiting sub-micromolar IC_{50}s against SARS-CoV $3CL^{pro}$. Ketone peptidomimetic **10f** exhibited a moderate IC_{50} of 2.3 μM, suggesting that aldehyde warheads are much more reactive than ketones. Compound **10d** has been resynthesized and reported to inhibit SARS-CoV-2 $3CL^{pro}$ with an IC_{50} of 8.5 nM.[56] Aldehydes are known to be highly reactive towards endogenous biological nucleophiles, potentially rendering compounds **10b** to **10e** cytotoxic. Aldehydes are also metabolically unstable due to their susceptibility to oxidation and reduction by liver enzymes.[57,58] Hence, we believe aldehyde peptidomimetics lack potential for further drug development due to their metabolic liabilities.



10a

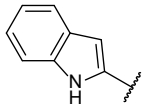
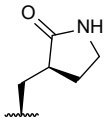
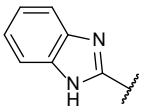
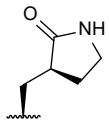
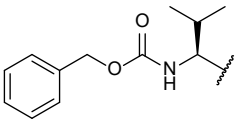
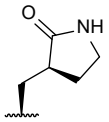
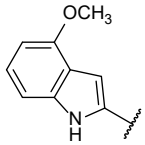
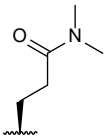
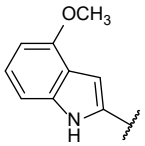
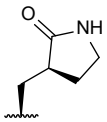
	R1	R2	R3	SARS-CoV 3CL^{pro} IC_{50} (μM)	SARS-CoV-2 3CL^{pro} IC_{50} (μM)
10b			H	0.02	
10c			H	0.03	
10d			H	0.06	0.0085
10e			H	0.05	
10f			CH_3	2.30	

Figure 10. Structures from WO 2006/061714. (a) general scaffold; (b – f) exemplified structures.

The eleventh earliest patent was filed in 2006 by Tokyo Medical and Dental University and RIKEN involving small molecules with a 3-cyanopyridine scaffold (WO 2006/095624; Table 1; Figure 11).[30] 102 compounds were described. The patent is published in Japanese and no English version can be found online. To our best knowledge, 3CL^{pro} IC_{50} inhibition data was not reported in the patent. Structure searches using SciFinder® did not reveal any follow-up journal publications. Due to the lack of inhibitory data, we are unable to gauge their potential for further drug development.

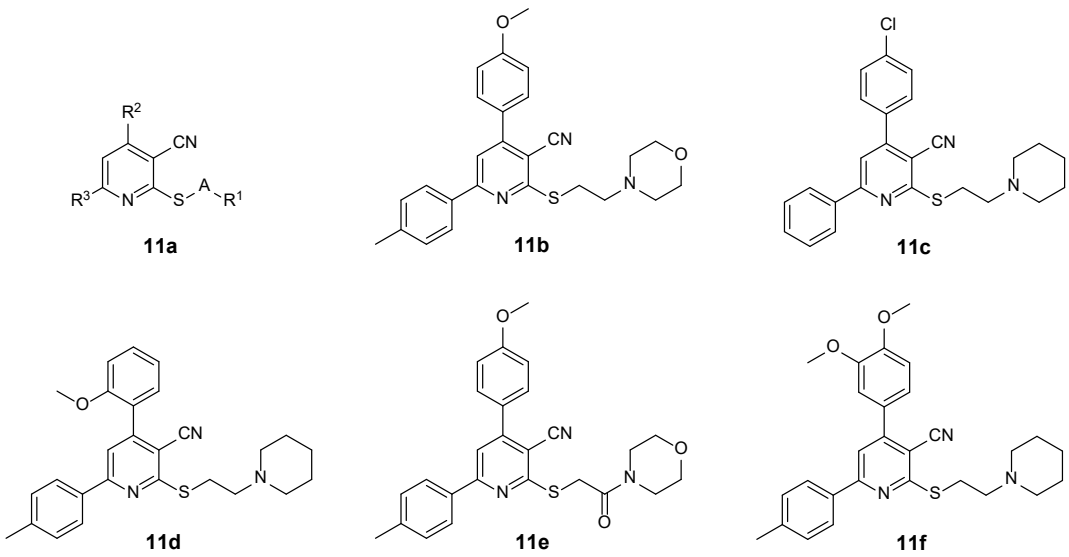
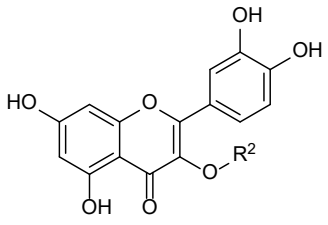


Figure 11. Structures from WO 2006/095624. (a) general scaffold; (b – f) exemplified structures.

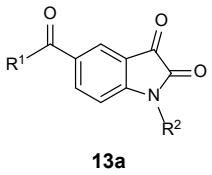
The twelfth earliest patent was filed in 2006 by Singapore Polytechnic and Shanghai Institute of Materia Medica involving small molecules with a chromone (1,4-benzopyrone) scaffold (WO 2007/075145; Table 1).[31] 17 structures were exemplified and 4 compounds, **12b** to **12e** (Figure 12), were reported with SARS-CoV 3CL^{pro} IC_{50}s ranging from 24 to 49 μM. These compounds were published in a research article in the same year of patent filing.[59] Due to their weak inhibitory activities, we opine that these compounds are not potent enough for further drug development.



	R^2	SARS-CoV 3CL^{pro} IC_{50} (μM)
12b	L-Fucose	24.14
12c	D-Arabinose	31.62
12d	D-Galactose	49.73
12e	D-Glucose	50.50

Figure 12. Structures from WO 2007/075145. (a) general scaffold; (b – e) exemplified structures.

The thirteenth earliest patent was filed in 2011 by Tianjin International Joint Academy of Biotechnology and Medicine involving 2,3-dioxindole SARS-CoV 3CL^{pro} inhibitors (CN 103159665B; Table 1).[32] The patent is written in Chinese and an English version could not be found online. 5 structures (Figure 13) were exemplified with SARS-CoV 3CL^{pro} IC_{50}s between 20 to 88 μM. Structure searches on the compounds using SciFinder® did not reveal any follow-up journal publications. In our opinion, these compounds are not potent enough to be developed as antiviral drugs.



13a

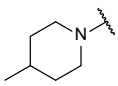
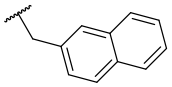
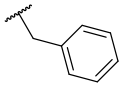
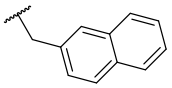
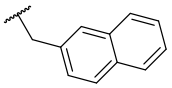
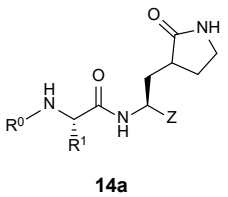
	R^1	R^2	SARS-CoV 3CL^{pro} IC_{50} (μM)
13b			19.73
13c			22.93
13d			36.42
13e			70.35
13f			87.76

Figure 13. Structures from CN 103159665B. (a) general scaffold; (b – f) exemplified structures.

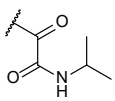
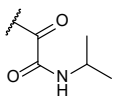
The fourteenth patent was filed in 2012 by Kansas State, Ohio State and Wichita State Universities involving 2- and 3-residue peptidomimetics containing a P1 5-

304 membered lactam glutamine mimic with a C-terminal electrophilic aldehyde or ketoamide
305 warhead (WO 2013/049382; Table 1).[33] A hydroxymethyl sulfonic acid prodrug masking
306 the reactive aldehyde warhead was also described (**14c**; Figure 14). Compounds **14b**,
307 **14c** and **14d** exhibited IC_{50}s ranging between 3.5 to 4.7 μM in a SARS-CoV $3CL^{pro}$
308 biochemical assay. No SARS-CoV $3CL^{pro}$ IC_{50}s were reported for tripeptide aldehyde **14e**
309 and ketoamide **14f** in the patent but were later reported in a research article (0.23 and
310 0.61 μM respectively).[60] Compounds **14b** and **14c**, also named GC373 and GC376
311 respectively, were reported to inhibit SARS-CoV 3CLpro with 3.5 and 4.4 μM IC_{50}s in a
312 research paper.[61] Interestingly, **14b**/GC373 was shown to be much more active against
313 SARS-CoV-2 $3CL^{pro}$, exhibiting an IC_{50} of 0.042 μM in a recent paper.[45] Similarly,
314 **14c**/GC376 was also shown to be much more active against SARS-CoV-2 $3CL^{pro}$,
315 exhibiting IC_{50}s ranging between 0.03 to 0.62 μM.[45,62,63] We opine ketoamide
316 peptidomimetics **14d** and **14f** can potentially be further developed as SARS-CoV-2
317 $3CL^{pro}$ antivirals because ketoamide peptidomimetic protease inhibitors such as
318 Boceprevir and Telaprevir have been approved for treating hepatitis C virus infections.[64]

319



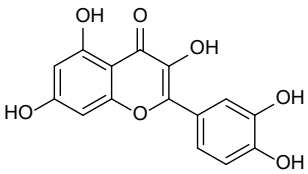
14a

	Z	SARS-CoV $3CL^{pro}$ IC_{50} (μM)	SARS-CoV-2 $3CL^{pro}$ IC_{50} (μM)
14b (**GC373**)		3.5	0.042
14c (**GC376**)		4.4	0.03 - 0.62
14d (**GC375**)		4.7	
14e		0.23	
14f		0.61	

320

Figure 14. Structures from WO 2013/049382. (a) general scaffold; (b – f) exemplified structures.

The fifteenth earliest patent was filed in 2012 by Chonnam National University, South Korea revealing that the plant flavonoid, quercetin (KR 1020130002975; Table 1; Figure 15), was able to inhibit SARS-CoV $3CL^{pro}$ with an IC_{50} of 73 μM and suggested that it could potentially be used to treat SARS-CoV infections. The patent is written in Korean and no English version could be found online. In our opinion, we believe that its inhibitory activity is too weak to be considered for development as an antiviral drug.



$3CL^{pro}$	IC_{50} (μM)
SARS-CoV	73

Figure 15. Structure of quercetin from KR 1020130002975.

The sixteenth earliest patent was filed in 2013 by Kansas State and Wichita State Universities involving 3-residue cyclic peptidomimetics and linear piperidine-2,6-dione peptidomimetics with C-terminal aldehyde or ketoamide warheads (WO 2013/166319; Table 1).[35] Although SARS-CoV $3CL^{pro}$ IC_{50}s were not disclosed in the patent, a research article by the inventors reported compound **16c** (Figure 16) inhibited SARS-CoV $3CL^{pro}$ with an IC_{50} of 15.5 μM.[65] No SARS-CoV-2 $3CL^{pro}$ inhibitory activities have so far been reported for compounds **16c** to **16i**. In our view, **16c** lacks potency for further drug development.

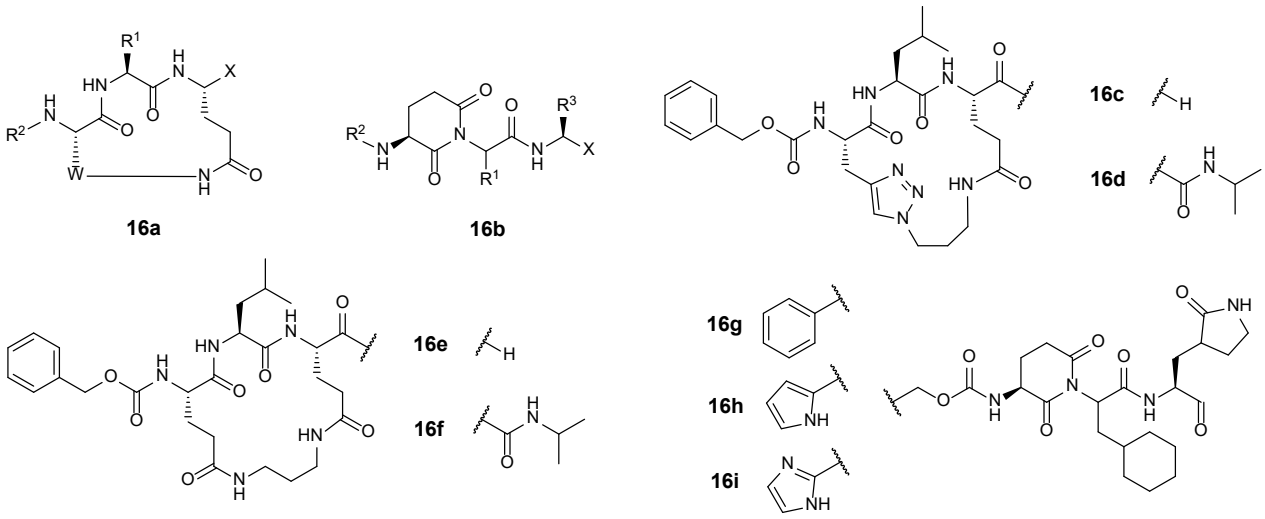
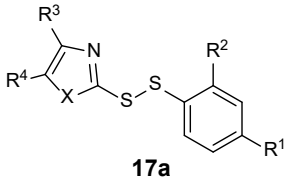


Figure 16. Structures from WO 2013/166319. (a, b) general scaffolds; (c – i) exemplified structures.

The seventeenth earliest patent was filed in 2016 by The Institute of Microbiology, Chinese Academy of Sciences involving small molecule disulphide SARS-CoV 3CL^{pro} inhibitors (CN 106176728A; Table 1).[36] The patent is written in Chinese and an English version could not be found online. 12 structures were exemplified and the 5 most potent compounds possess IC_{50}s between 1.3 to 2.2 μM (**17b** to **17f**; Figure 17). A SciFinder® structure search revealed one follow-up academic paper reporting their SARS-CoV 3CL^{pro} inhibitory activities.[66] There are currently no reports on SARS-CoV-2 3CL^{pro} inhibitory activities. We opine that these compounds lack drug development potential due to their disulphide moiety, reported to be unstable in physiological conditions due to the presence of biological reducing agents, free thiols and isomerases.[67] Hence, pharmacokinetic studies should be conducted first before these compounds can be evaluated further.



17a

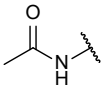
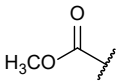
	R^1	R_2	R_3	R_4	X	SARS-CoV 3CL^{pro} IC_{50} (μM)
17b	CH_3	H	H	H	S	1.3
17c	H	NO_2	H		S	1.9
17d	H	NO_2	H	H	S	2.0
17e	H	NO_2		H	NH	2.0
17f	F	H	H	H	S	2.2

Figure 17. Structures from CN 106176728A. (a) general scaffold; (b – f) top five most potent inhibitors.

The eighteenth patent was filed in 2016 by Shanghai Institute of Materia Medica and the University of Lübeck involving 2- and 3-residue peptidomimetics bearing a P1 5-membered lactam glutamine mimic and a C-terminal electrophilic aldehyde warhead (WO 2017/114509; Table 1).[37] 74 structures were described with SARS-CoV 3CL^{pro} IC_{50}s ranging from 30 nM to 7.4 μM. The four most potent structures are shown in Figure 18 (compounds **18b** to **18e**) with SARS-CoV 3CL^{pro} IC_{50}s ranging from 30 to 100 nM. The most potent inhibitor **18b** is structurally similar to Pfizer's **10b** (Figure 10), differing at the P2 residue. **18b**'s SARS-CoV 3CL^{pro} inhibitory activity is 1.5-fold weaker than Pfizer's **10b** (IC_{50}s 30 and 20 nM respectively), suggesting that SARS-CoV 3CL^{pro} prefers a P2 leucine over phenylalanine. This distinction is an important consideration when designing new coronavirus 3CL^{pro} inhibitors and may be the reason why a P2 leucine was selected for Pfizer's lead compound PF-00835231 (Figure **6b**).[18] **18b** was also reported to be the most potent SARS-CoV-2 3CL^{pro} inhibitor (IC_{50} 34 nM) amongst 13 peptide aldehydes in a recent publication,[68] suggesting that inhibitors designed for SARS-CoV 3CL^{pro} can be effectively used to inhibit SARS-CoV-2 3CL^{pro}. Interestingly, the addition of a P3 valine to

18b to yield tripeptide aldehyde **18d** resulted in an approximate 2-fold activity reduction for SARS-CoV and SARS-CoV-2 3CL^{pro} (IC_{50}s 30 to 50 nM and 34 to 68 nM respectively).[68] Notably, all the inhibitors described in the patent possess highly electrophilic aldehyde warheads which are known to be highly reactive towards endogenous biological nucleophiles, potentially rendering them cytotoxic. Aldehydes are also metabolically unstable due to their susceptibility to oxidation and reduction by liver enzymes.[57,58] Hence, we believe these aldehyde peptidomimetics lack potential for further drug development.

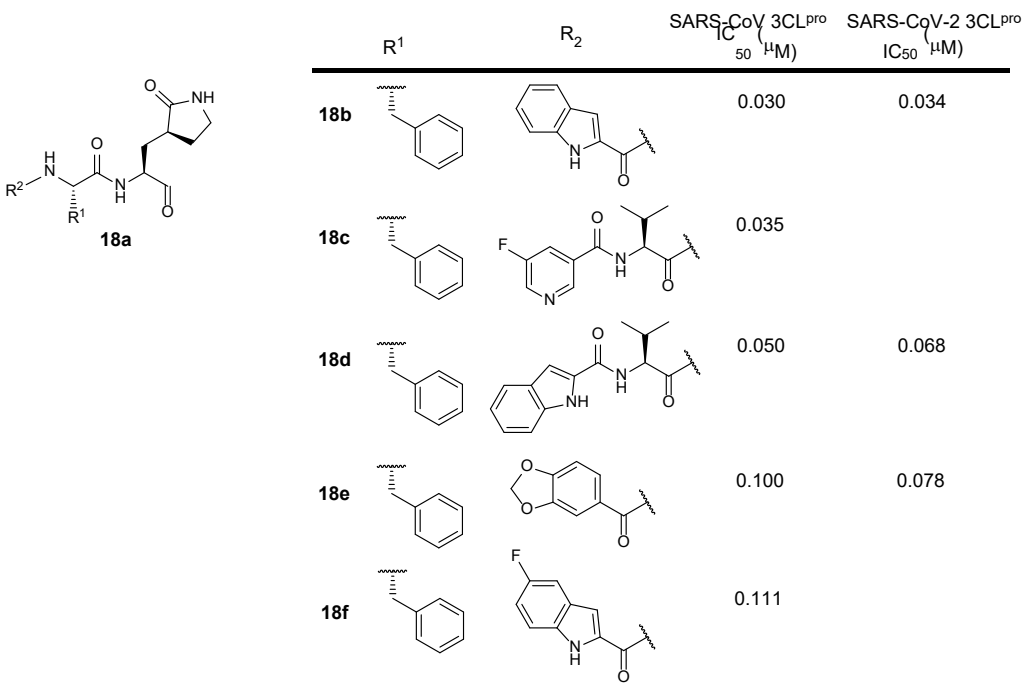
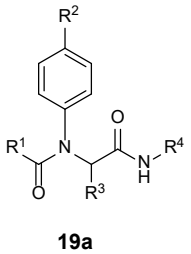


Figure 18. Structures from WO 2017/114509. (a) general scaffold; (b – f) most potent exemplified inhibitors against SARS-CoV 3CL^{pro}.

The nineteenth earliest patent was filed in 2017 by Purdue Research Foundation involving small molecules with amidophenyl scaffolds (US 2017/0313685; Table 1).[38] 77 structures were reported with SARS-CoV 3CL^{pro} IC_{50}s between 0.8 to 27.3 μM. The five

most potent inhibitors were identified to be compounds **19b** to **19f** (Figure 19) with 0.8 to 2.0 μM IC_{50}s. Interestingly, the structure of compound **19f** was reported in an earlier 2015 research paper with an IC_{50} of 1.9 μM against HKU4-CoV $3CL^{pro}$.[69] There are currently no reports on their SARS-CoV-2 $3CL^{pro}$ inhibitory activities. We opine that the most potent inhibitor, **19b**, should be further evaluated for drug development.



19a

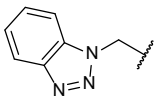
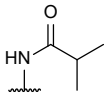
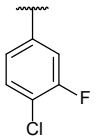
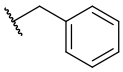
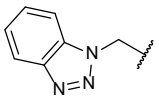
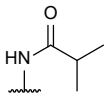
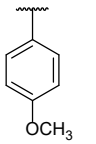
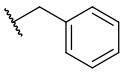
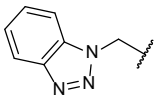
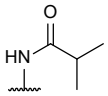
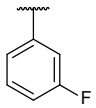
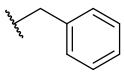
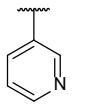
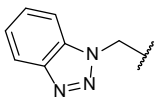
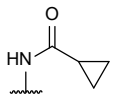
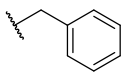
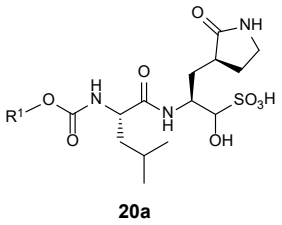
	R_1	R_2	R_3	R_4	SARS-CoV $3CL^{pro}$ IC_{50} (μM)
19b					0.8
19c					1.1
19d					1.6
19e					1.7
19f					2.0

Figure 19. Structures from US 2017/0313685. (a) general scaffold; (b – f) most potent exemplified structures.

The twentieth earliest patent was filed in 2017 by Kansas State and Wichita State Universities involving dipeptide peptidomimetics with a P1 5-membered lactam glutamine analog and a C-terminal hydroxymethyl sulfonic acid prodrug warhead against the Middle East Respiratory Syndrome (MERS) coronavirus $3CL^{pro}$ (WO 2017/222935; Table 1).[39]

The hydroxymethyl sulfonic acid moiety changes to a reactive electrophilic aldehyde warhead at physiological pH, first described in WO 2013/049382.[33] Although no SARS-CoV 3CL^{pro} inhibitory activities were disclosed, the MERS-CoV and SARS-CoV-2 3CL^{pro} share approximately 50% sequence identity[16] so inhibitors designed to inhibit MERS-CoV 3CL^{pro} will likely be able to inhibit SARS-CoV-2 3CL^{pro}. 8 compounds were described with IC_{50}s ranging from 0.3 to 3.1 μM with compounds **20b** to **20f** being the most potent against MERS-CoV 3CL^{pro} (Figure 20). There are currently no reports on their SARS-CoV-2 3CLpro inhibitory activities. As the hydroxymethyl sulfonic acid moiety transforms into a reactive aldehyde warhead in physiological conditions, we opine these compounds lack drug development potential as explained *vide supra*.



20a

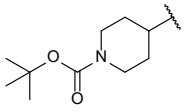
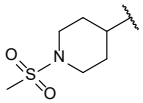
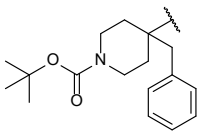
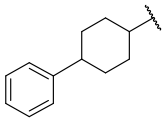
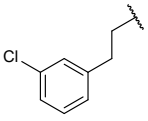
	R^1	MERS-CoV 3CL^{pro} IC_{50} (μM)
20b (**GC868**)		0.3
20c (**GC867**)		0.5
20d (**GC861**)		0.6
20e (**GC833**)		0.6
20f (**GC813**)		0.7

Figure 20. Structures from WO 2017/222935. (a) general scaffold; (b – f) exemplified structures.

The twenty-first earliest patent was filed in 2017 by Tianjin International Joint Academy of Biomedicine revealing that the N-methyl-D-aspartate (NMDA) receptor antagonist and gamma aminobutyric acid (GABA) receptor modulator, acamprosate calcium (Figure 21), was able to inhibit SARS-CoV $3CL^{pro}$ (CN 108785293A; Table 1).[40] The patent is written in Chinese and no English version could be found online. Unfortunately, no IC_{50} or K_i value was reported and there are no follow-up reports in academic journals. Hence, we are currently unable to comment on its drug development potential for treating COVID-19.

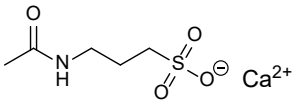


Figure 21. Structure of acamprosate calcium from CN 108785293A.

The twenty-second patent was filed by GSK in 2017 involving 3-residue peptidomimetics containing a P1 5-membered lactam glutamine mimic with a C-terminal electrophilic ketoamide warhead (WO 2018/042343; Table 1).[41] 48 structures were described with SARS-CoV $3CL^{pro}$ IC_{50}s of approximately 7 nM. The five most potent inhibitors against SARS-CoV $3CL^{pro}$ are shown in Figure 22. There are currently no reports on their SARS-CoV-2 3CLpro inhibitory activities based on a SciFinder® search. In our opinion, these compounds can potentially be developed as SARS-CoV-2 $3CL^{pro}$ inhibitors to treat COVID-19 as oral ketoamide peptidomimetic protease inhibitors such as Boceprevir and Telaprevir have been approved for treating hepatitis C virus infections.[64]

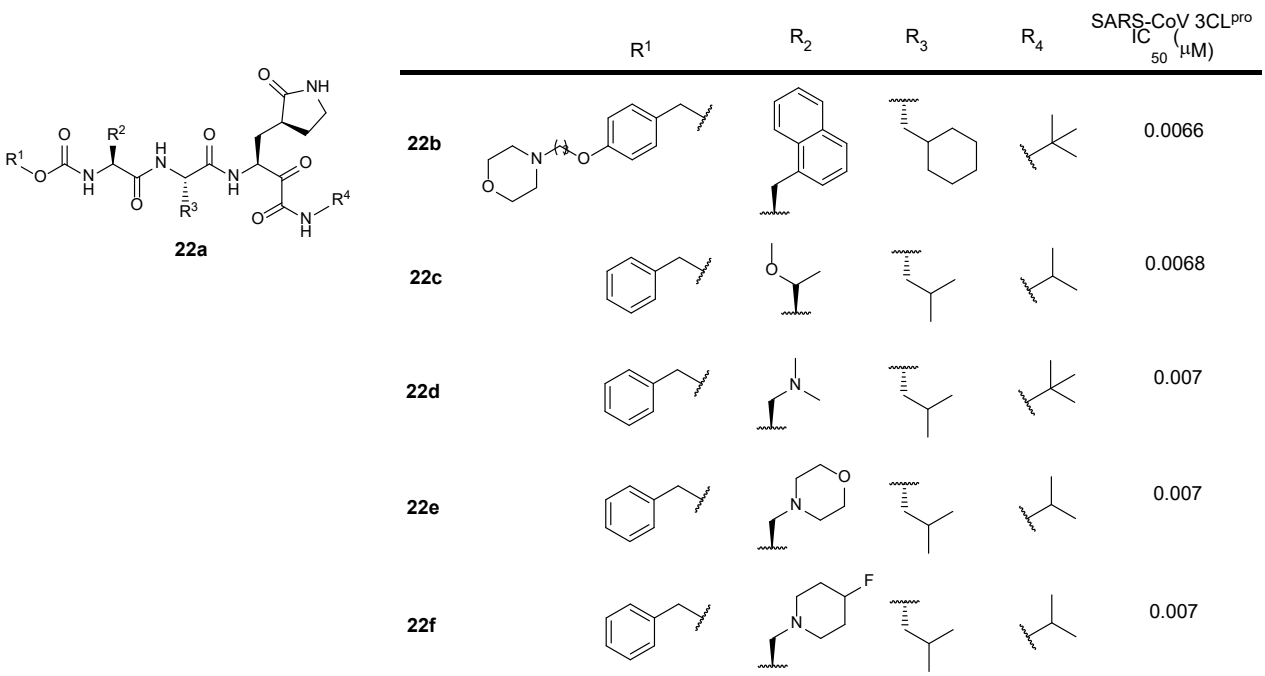
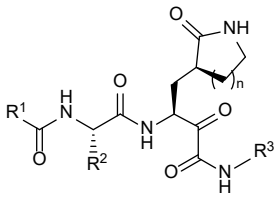


Figure 22. Structures from WO 2018/042343. (a) general scaffold; (b – e) exemplified structures.

A special note is also made for WO 2020/030143 (Table 1), filed by Shanghai Institute of Materia Medica and Fudan University in 2019.[42] It involves 2-residue peptidomimetics bearing a P1 5- or 6-membered lactam glutamine mimic with a C-terminal electrophilic ketoamide warhead designed to inhibit MERS-CoV 3CL^{pro}. The patent is written in Chinese and the English version could not be found online. No SARS-CoV or SARS-CoV-2 3CL^{pro} IC_{50} data were reported in the patent. However, this patent is included in this review as the MERS-CoV and SARS-CoV-2 3CLpro share approximately 50% sequence identity[16] so inhibitors designed to inhibit MERS-CoV 3CL^{pro} will likely be able to inhibit SARS-CoV-2 3CL^{pro}. 253 compounds were described with IC_{50}s ranging from sub-nanomolar to 786 nM using a MERS pseudovirus neutralization assay. The five most potent MERS-CoV 3CL^{pro} inhibitors, **23b** to **23f**, possess sub-nanomolar to 5 nM IC_{50}s (Figure 23). There are currently no reports on their SARS-CoV-2 3CL^{pro} inhibitory

activities based on a SciFinder® search. In our opinion, these compounds can potentially be developed as SARS-CoV-2 3CL^{pro} inhibitors to treat COVID-19 as oral ketoamide peptidomimetic protease inhibitors such as Boceprevir and Telaprevir have been approved for treating hepatitis C virus infections.[64]



23a

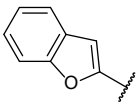
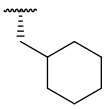
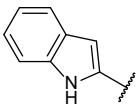
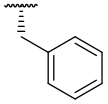
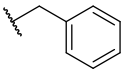
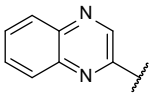
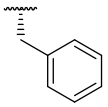
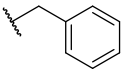
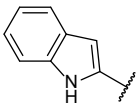
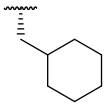
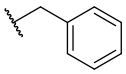
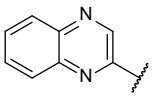
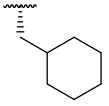
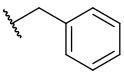
	R^1	R_2	R_3	n	MERS-CoV 3CL^{pro} IC_{50} (μM)
23b				2	<0.0001
23c				2	<0.0001
23d				2	0.0002
23e				2	0.0005
23f				2	0.0005

Figure 23. Structures from WO 2020/030143. (a) general scaffold; (b – f) most potent exemplified MERS-CoV 3CL^{pro} inhibitors.

The final patent in this review is an application patent involving 13 peptidomimetic protease inhibitors (WO 2021/176369; Table 1).[43] Interestingly, 6 peptidomimetics with various C-terminal electrophilic warheads have been described in an earlier patent (WO 2005/113580),[25] all bearing a P1 5-membered lactam glutamine mimic. No 3CL^{pro} inhibitory data were disclosed in the patent but SARS-CoV 3CL^{pro} IC_{50}s for compounds **24b** to **24e** (Figure 24) were reported in a recent research paper.[18] Surprisingly, the

patent also described the peptidomimetic rhinovirus 3C protease inhibitor, Rupintrivir (**24f**), as a potential COVID-19 therapeutic despite its lack of SARS-CoV-2 $3CL^{pro}$ inhibitory activity.[62] Three hepatitis C and human immunodeficiency virus protease inhibitors were also described; Filibuvir (**24g**), Nelfinavir (**24h**) and AG-1859 (**24i**). No IC_{50}s were reported in the patent but a literature search revealed Nelfinavir to possess weak SARS-CoV-2 $3CL^{pro}$ inhibitory activity (IC_{50} 41.5 μM).[70] In our view, the peptidomimetic hydroxymethylketones **24b** and **24c** possess the highest potential for further drug development if converted to a phosphate prodrug as observed for clinical candidate PF-00835231 (**6b**).[18] In contrast, we opine that Rupintrivir (**24f**) and Nelfinavir (**24h**) are not potent enough for further development. We are unable to comment on the drug development potential for Filibuvir (**24g**) and AG-1859 (**24i**) due to the lack of SARS-CoV-2 $3CL^{pro}$ inhibitory data.

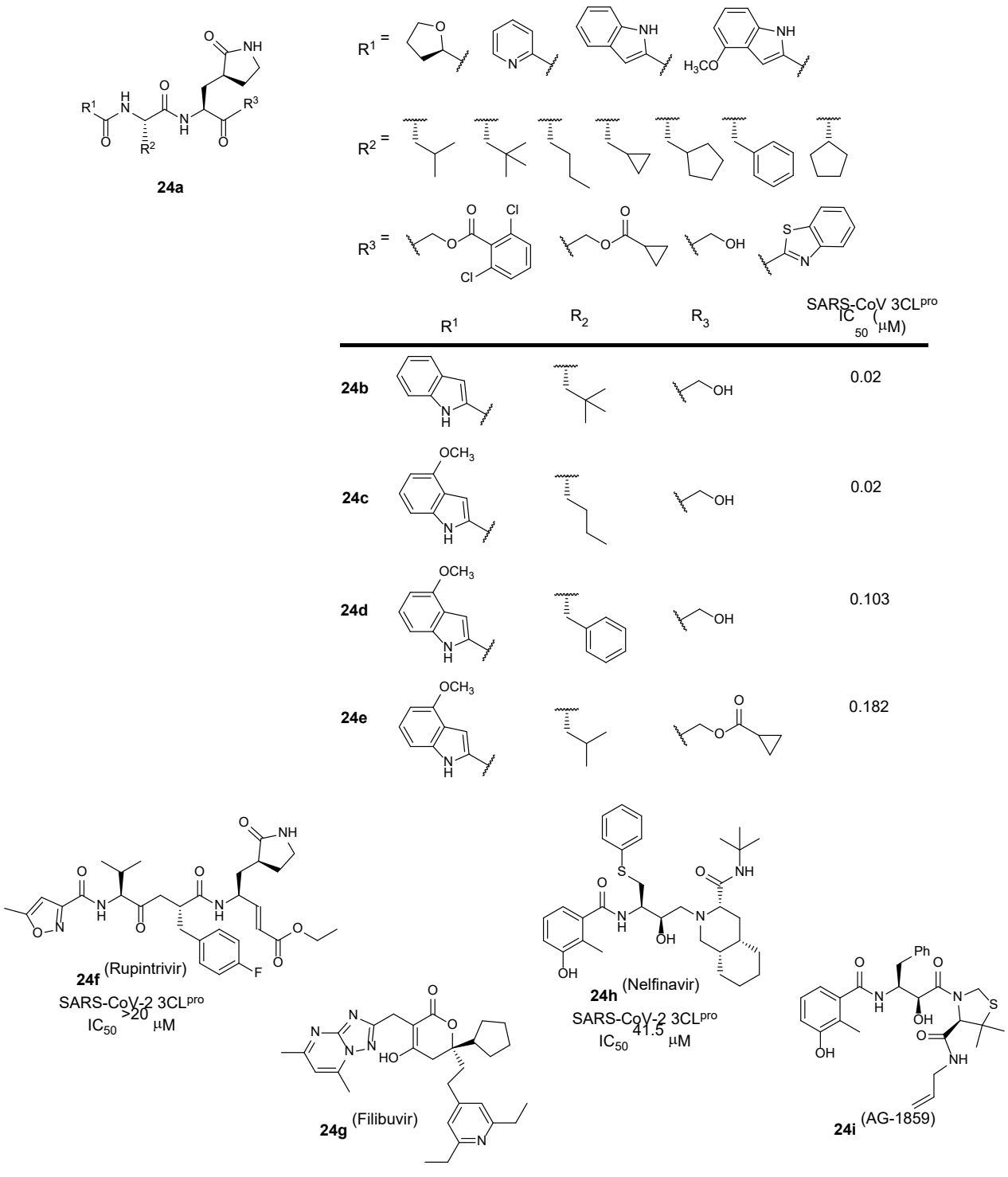


Figure 24. Structures from WO 2021/176369. (a) peptidomimetic general scaffold; (b – i) exemplified inhibitors.

Lastly, a special mention is made for Pfizer's oral SARS-CoV-2 $3CL^{pro}$ peptidomimetic inhibitor PF-07321332 (Figure 25) which entered phase 3 clinical trials in July 2021 for treating non-hospitalised SARS-CoV-2 infected adults (ClinicalTrials.gov identifier NCT04960202).[71] Like previous Pfizer $3CL^{pro}$ inhibitor patents, it bears a P1 5-membered lactam glutamine mimic and interestingly, a nitrile warhead not described in any patents found in this review. This is particularly intriguing as nitrile warhead peptidomimetics have been reported to be relatively weak SARS-CoV $3CL^{pro}$ inhibitors with IC_{50}s ranging between 4.6 to 49 μM.[72] To our best knowledge, there are currently no published reports on its SARS-CoV-2 $3CL^{pro}$ IC_{50} and a SciFinder® structure search did not reveal any patents for PF-07321332.

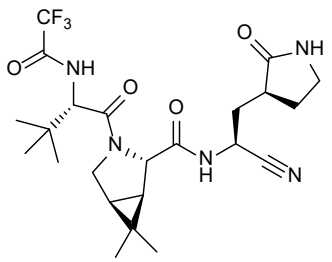


Figure 25. Structure of PF-07321332.

3. Summary and Outlook

24 patents describing coronavirus $3CL^{pro}$ inhibitors have so far been filed up to 30 July 2021 (Table 1). 9 of the patents were filed by pharmaceutical companies while the remaining 15 from academia. 10 of the 24 patents described inhibitors that have not been reported in academic journals. 14 of the 24 patents involved peptidomimetics with electrophilic warheads, signifying that this modality has generated more commercial interest compared to small molecules. A plausible reason could be that warhead peptidomimetics generally show more potent inhibitory activities, typically in the

nanomolar range, compared to small molecules. In addition, warhead peptidomimetics have been successfully developed into antiviral drugs, exemplified by the hepatitis C virus protease inhibitors Boceprevir and Telaprevir.[64]

The peptidomimetic inhibitors covered in this review bear a P1 5- of 6-membered lactam glutamine-mimicking residue first described by Agouron Pharmaceuticals in 1999 (WO 99/57135).[46] Reported P2 residues ranged from leucine to cyclohexylalanine and phenylalanine, suggesting that the SARS-CoV S2 subsite could accommodate bulky residues with 6-membered side-chains. However, a 2020 research paper by Pfizer revealed that a P2 leucine exhibited 11- and 25-fold IC_{50} improvement over cyclohexylalanine and phenylalanine respectively, suggesting that leucine was highly preferred at the P2 position.[18] Indeed, Pfizer utilised a P2 leucine in their phosphate prodrug of PF-00835231 (**6b**) which entered phase 1 clinical trials in September 2020 (ClinicalTrials.gov identifier: NCT04535167). Other plausible P2 residue substitutions include norvaline and *t*-butylalanine (Figure 24). The P3 position is more accommodating to different moieties including indoles (Figures 6, 10 and 18), amino acid residues like threonine (Figure 3), valine (Figures 5 and 8) and naphthylalanine (Figures 14 and 22). The longest peptidomimetic inhibitor in this review constituted 4 residues (Figure 8) but unfortunately, no IC_{50}s were reported as it would be interesting to study the correlation of peptidomimetic length to inhibition potency.

Small molecule inhibitors constituted 10 of the 24 patents (Table 1). In contrast to the peptidomimetic inhibitors, their reported IC_{50}s or K_is were generally inferior (close to or above 1 μM), with the exception of two patents filed by Fulcrum Pharmaceuticals and the National Health Research Institutes, Taiwan (Table 1; Figures 4 and 7 respectively). The former described a small molecule containing two boronic acid moieties (K_i 22 nM)

and the latter, a biphenyl sulphone (IC_{50} 300 nM). In our view, both warrant further investigations into their drug development potential.

We believe this review serves to bolster the knowledge of existing coronavirus $3CL^{pro}$ inhibitors reported beyond academic journals and the general structural motifs required for binding and inhibiting SARS-CoV-2 $3CL^{pro}$. We are hopeful that some of these inhibitors will be successfully developed and approved as drugs for treating COVID-19.

Abbreviations

$3CL^{pro}$	3-chymotrypsin-like protease
CoV	coronavirus
COVID-19	coronavirus disease 2019
EC_{50}	half-maximal effective concentration
FDA	Food and Drug Administration
GABA	gamma aminobutyric acid
IC_{50}	half-maximal inhibitory concentration
MERS	Middle East Respiratory Syndrome
M^{pro}	main protease
NMDA	N-methyl-D-aspartate
SARS	severe acute respiratory syndrome
WHO	World Health Organization

Acknowledgements

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Conflict of Interest

The authors of this review declare no conflicts of interest.

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[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G. F. Gao, W. Tan, China Novel Coronavirus Investigating and Research Team, *N. Engl. J. Med*. **2020**, *382*(13), 727. DOI: 10.1056/NEJMoa2001017.

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