Episode 230 Novel SARS-CoV-2 protease inhibitors in the pipeline?

Dear colleagues,

As you know, there is "hot news" every day, but I prefer to focus on a more long-term today. In Episode 221, I presented an update on Paxlovid from Pfizer, the first protease inhibitor approved for human COVID treatment. I was wondering whether there are others in the pipeline. It is a very complex pharmacological story, but according to my understanding, the candidates closest to clinical use are inspired on **hepatitis C-specific protease inhibitors**, such as boceprevir, telaprevir and simprevir.

Ep 230-1: Andrea Citarella Review in Biomolecules April 2021

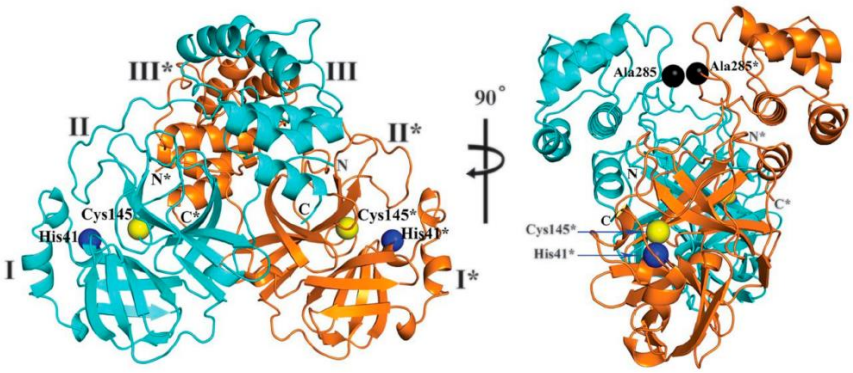


Figure 2. 3D structure of SARS-CoV-2 M^{pro} in two different views. One protomer of the dimer is shown in light blue, the other one in orange [12].

The main SARS-CoV-2 protease Mpro is a homodimer (or maybe trimer?), with the catalytic "dyad" of Cysteine (position 145) and Histidine (position 41), in the cleft between domains I and II.

The cleavage site (CS) in the viral polyproteins peptide stretch, composed of Leucine (L), Glutamine (Q) /// Serine (S), Alanine (A), Glycine(G), followed by an aromatic amino acid (e.g. Phenylalanine F).

Example the first cleavage site in ORF1ab is

CS 1: 3257 to 3271	ITSAVLQ/SGF**RK**MAF	15	SARS-CoV-2

See Table Ep 230-1 for all CS

The catalytic reaction of the protease is shown in Fig. 4 and explained on page 5

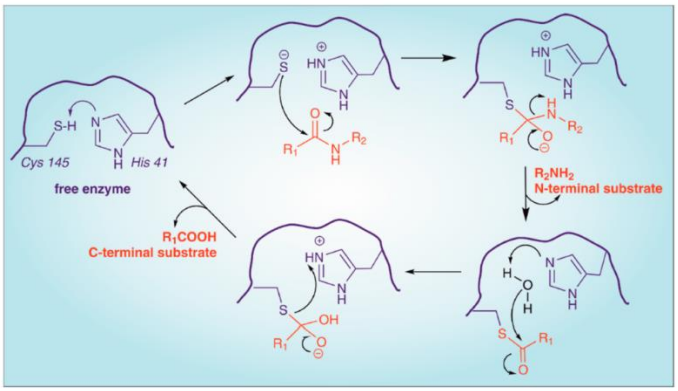
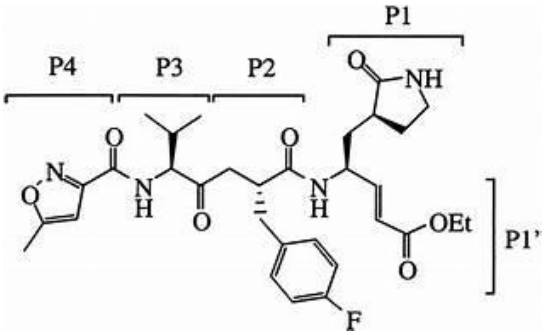


Figure 4. Hydrolysis mechanism of SARS-CoV-2 M^{pro}. Amino acids of the catalytic dyad and the substrate are depicted in blue and orange, respectively.

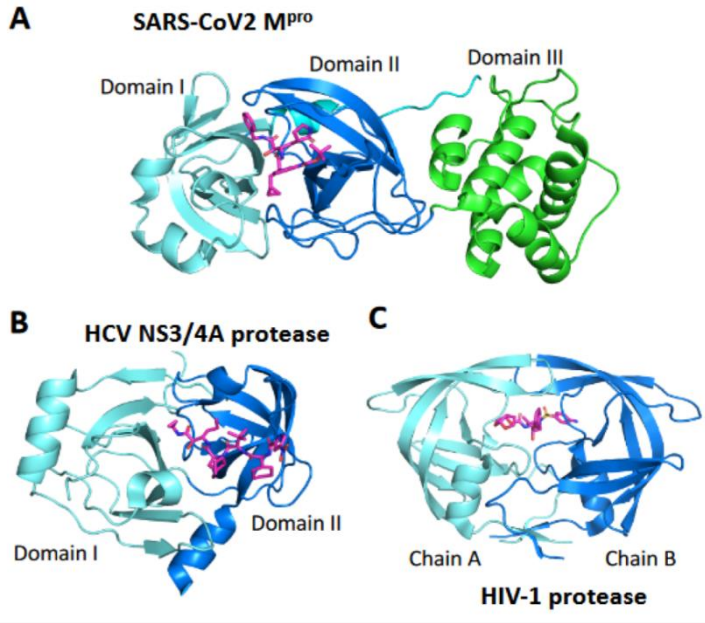
The peptidomimetic cysteine protease inhibitors have the following structure:



The P1-P4 (modified) amino acids have a high affinity for the catalytic site, but are NOT cleavable.

The P1' is a so-called "warhead", an electrophilic function (aldehyde, vinyl ester, alpha-ketonamidev or various ketones) which irreversibly binds and inactivates the protease.

The paper provides a series of examples of possible inhibitors, including the HIV protease inhibitors, such as Lopinavir/ritonavir, Atazanavir as well as hepatitis C protease inhibitors (which will be discussed later). In fact, we know that the HIV inhibitors failed in clinical trials, which is not too surprising, comparing the structures: SARS-CoV-2 and Hep C proteases are rather similar, while HIV protease has a very different structure.



Ep 230-2: Gammeltoft in AAC Sept 2021 on HepC inhibitors

As can be seen in Table 1, HepC protease inhibitors are active in the tens micromolar range (EC50), but the selectivity index (SI) is rather low, because of cell toxicity in the tens-hundreds micromolar range (CC50). Only Boceprevir and Telaprevir have a selectivity index of > 10.

TABLE 1 Potency of a panel of HCV PIs and an HCV NS4A inhibitor against SARS-CoV-2 *in vitro*

Inhibitor	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
Vero E6 cells			
Boceprevir	44	>1,214	>28
Telaprevir	40	>432	>11
Narlaprevir	37	269	7.3
Simeprevir	15	59	3.9
Paritaprevir	22	123	5.6
Grazoprevir	42	239	5.7
Glecaprevir	>178	>268	ND
Voxilaprevir	>27	72	<2.7
Vaniprevir	51	171	3.4
Danoprevir	87	>243	>2.8
Deldeprevir	>20	56	<2.8
Asunaprevir	72	263	3.7
Faldaprevir	23	146	6.3
ACH-806	46	>429	>9.3
Huh7.5 cells			
Boceprevir	42	701	17
Simeprevir	14	33	2.4
Grazoprevir	20	133	6.7
A549-hACE2 cells			
Boceprevir	20	>1,213	>61
Simeprevir	9	56	6.2
Grazoprevir	26	125	4.8
Glecaprevir	>94	>268	ND
Voxilaprevir	10	81	8.1

These authors also tested a potential synergism with the polymerase inhibitor Remdesivir (Fig 4).

Simprevir showed very clear synergism and Partaprevir and Grazoprevir some synergism, but Boceprevir failed in this respect (Telaprevir was not tested).

Ep 230-3: Xia et al in ACS Pharmacol. Transl. Sci June 2021 describe two hybrid inhibitors **UAWJ9-36-1 and UAWJ9-36-3** based on the superimposed X-ray crystal structures of SARS-CoV-2 Mpro with GC-376, telaprevir, and boceprevir.

These two inhibitors have a **broad and potent (low micromolar EC50) activity** against a range of beta-CoV and they have a **better selectivity index against human cysteine proteases** than GC-376: they do not strongly inhibit calpain and cathepsin L, but still could block cathepsin K.

Effective Concentration 50 % (EC50) against several beta-CoV in several cell lines (Vero; Caco, Huh-7)

Compound	SARS-CoV-2 EC_{50} (µM)		HCoV-OC43 EC_{50} (µM)	HCoV-229E EC_{50} (µM)	HCoV-NL63 EC_{50} (µM)		
	Vero E6	Caco2-ACE2			Vero E6	Vero E6 + 2 µM CP-100356	Huh-7
GC-376	0.96 ± 0.10	2.90 ± 1.31	0.060 ± 0.0030	0.23 ± 0.024	3.68 ± 0.40	0.35 ± 0.023	0.77 ± 0.15
UAWJ9-36-1	2.56 ± 0.24	5.24 ± 1.76	0.046 ± 0.0021	0.17 ± 0.012	1.31 ± 0.022	0.48 ± 0.020	0.44 ± 0.013
UAWJ9-36-3	0.37 ± 0.13	1.06 ± 0.17	0.059 ± 0.0028	0.13 ± 0.025	1.27 ± 0.17	0.12 ± 0.031	0.54 ± 0.10

Inhibitory Concentration 50 % (IC50) against several human proteases

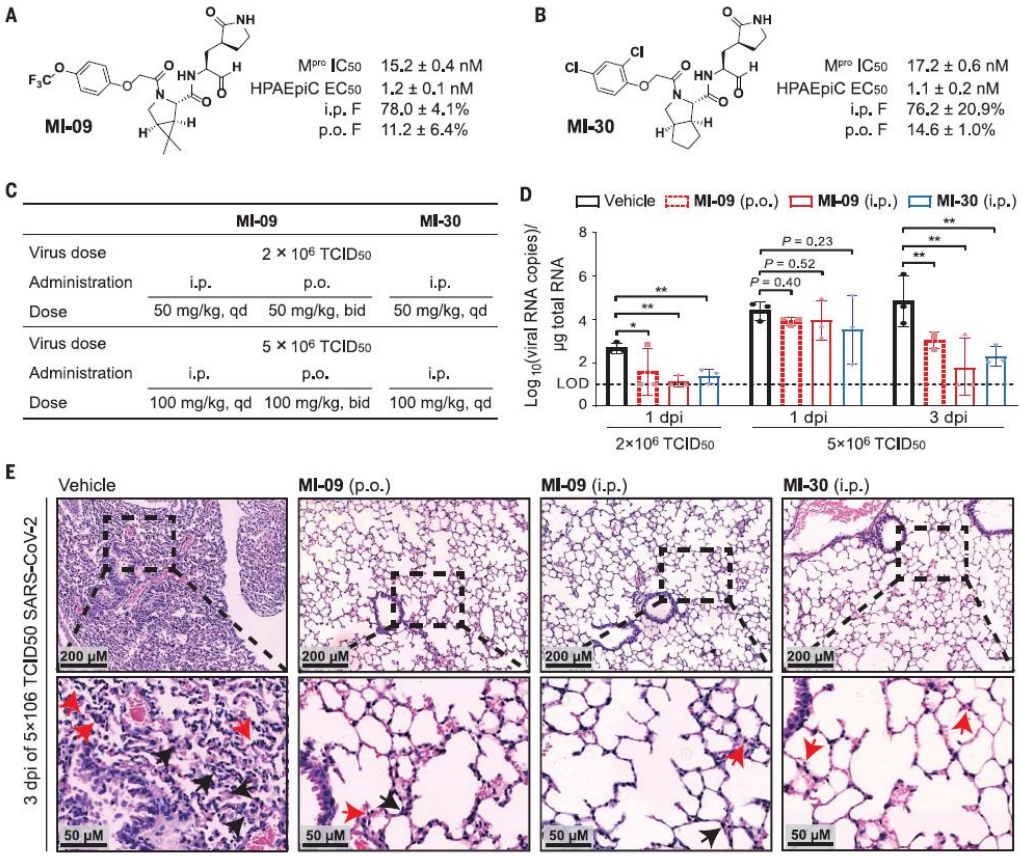
Compound	SARS-CoV-2 M^{pro} IC_{50} (µM)	Calpain I IC_{50} (µM)	Cathepsin L IC_{50} (µM)	Cathepsin K IC_{50} (µM)	Caspase-3[b] IC_{50} (µM)	Trypsin IC_{50} (µM)
GC376	0.043 ± 0.006	0.074 ± 0.016[a]	0.0044 ± 0.0010	0.00026 ± 0.00004	>20	>20
Jun9-36-1	0.051 ± 0.022	16.56 ± 5.66	1.37 ± 0.23	0.0065 ± 0.0029	>20	>20
Jun9-36-3	0.054 ± 0.008	>20	1.81 ± 0.75	0.042 ± 0.010	>20	>20

Clearly, Trypsin and Caspase-3 are not sensitive to either inhibitor, Calpain and Cathepsinl are much less sensitive to UAWJ9 (or Jun9) compounds than to GC-376; while Cathespsin K remains very sensitive to these inhibitors. The question is therefore whether they will be useful in humans?

Ep 230-4: Qiao et al in Science March 2021 describe more potent **"Mpro inhibitors" or MI**, also inspired on boceprevir and telaprevir. MI-09 and MI-30 showed:

- Antiviral EC50 in cell lines of **1 nM**
- Cellular toxicity CC50 at 500-860 nM, hence selectivity index > 500

Importantly: **MI-09 and MI-30 reduce lung viral loads and lung lesions in a SARS-CoV-2 infection transgenic mouse model. (A and B)**



There are several remarkable observations here:

- **MI-09 is active per oral** (p.o.) route (not tested for MI-30)
- **Treatment was started 1 hour before inoculation** and continued once daily (qd) for the intraperitoneal (i.p.) or twice daily (bid) for the oral (p.o.) route.

Under these conditions, we see a reduction of viral load in the lungs, but it is certainly not complete. Nevertheless, the lung pathology (as well as other inflammatory parameters) were very much improved.

Thus clear proof-of-principle, but it cannot immediately be extrapolated….

PRELIMINARY CONCLUSION

There is clear progress in development of new peptidomimetic Mpro inhibitors with a broad activity towards several beta-coronaviruses. The latest show low nanomolar activity and for some in vivo activity has been demonstrated in a mouse model.

The selectivity should be improved to avoid toxicity by blocking e.g. human Cathepsin K.

Anti-viral activity and avoidance of lung pathology should also be shown in animal models, such as hamsters and non-human primates, where human SARS-CoV-2 viruses can be used and - importantly- when treatment is only started AFTER infection.