Paxlovid

Feb. 13, 2025

"An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19"

Dafydd R. Owen, Jisun Lee, et al. (33 total authors!!)

Science, 2 Nov 2021, Vol 374, Issue 6575, pp. 1586-1593

Paxlovid = Combination drug of _____ and

_____.

*note nirmatrelvir = PF-07321332 = compound **6** in the current article

Key principles:

1. Development from _____

2. previous drugs were _____

*a.k.a. anti-metabolites

-molnupiravir→ _____

-AT-527 (a.k.a. Bemnifosbuvir, Atea Pharmaceuticals)→ _____

I. Background

A. coronavirus = _____

1. Plentiful examples in _____

-Common cold (_____ virus strains responsible)

-influenza (_____)

-Dengue virus (_____) –rapidly increased in last two decades

- _____ virus (single positive-sense RNA virus)

1976-2012 Ebola virus disease → 24 outbreaks

2002: SARS-CoV-1 (severe acute respiratory syndrome) → lead drug developed during program for SARS-CoV-1

2012: MERS (Middle East Respiratory Syndrome)

*RNA viruses _____ because they don't have the

_____ found with DNA polymerases

2. SARS-CoV-2 is an _____ with a single-stranded RNA genome of approximately 30,000 nucleotides.

(C. Bai et al. . Sci China Life Sci. 2022 Feb;65(2):280-294. doi: 10.1007/s11427-021-1964-4.)

* 26-33 kilobases → encodes

-_____ (pp1a, ~450 kDa and pp1ab, ~750 kDa)

-4 _____, including

- _____ (M)
- _____ (E)
- _____ (N)
- _____ (S).

[from C. Bai et al. Sci China Life Sci. 2022 Feb;65(2):280-294. doi: 10.1007/s11427-021-1964-4. : "SARS-CoV-2 is an enveloped, plus-stranded RNA virus with a single-stranded RNA genome of approximately 30,000 nucleotides. The SARS-CoV-2 genome encodes 29 proteins, including 16 nonstructural, 4 structural and 9 accessory proteins."]

**pp1a and pp1ab are cleaved by the _____ (_____) at _____

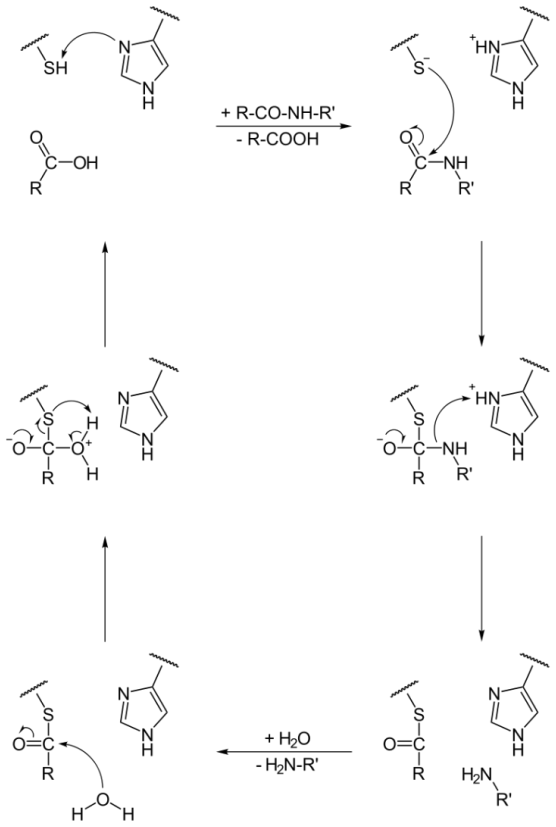
different sites → yields shorter non-structural proteins, but _____

B. TARGET: _____ is a 3CL (chymotrypsin-like) _____ (3 domains)

*"chymotrypsin-like" due to a _____

1. _____ between domains I and II

mechanism:



2. _____ at P1 for all sequence substrates

*fortunately NO human protease cleaves after _____

-provides a nice tractable target for oral anti-viral

**Prior work with protease inhibitors contributed to understanding in the field

II. Medicinal Chemistry Drug Development

A. LEAD: PF-00835231 from a _____ inhibitor development project

[Discovery of Ketone-Based Covalent Inhibitors of Coronavirus 3CL Proteases for the Potential Therapeutic Treatment of COVID-19, Robert L. Hoffman* et al. (Pfizer) J. Med. Chem. *J. Med. Chem*. **2020**, *63*, 21, 12725–12747; https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01063]

**The SARS CoV-1 and SARS CoV-2 share _____between their respective

3CLpro sequences and _____ in the active site.

recombinant isolated enzyme assay: SARS-CoV-2 M^{pro} K_i = _____

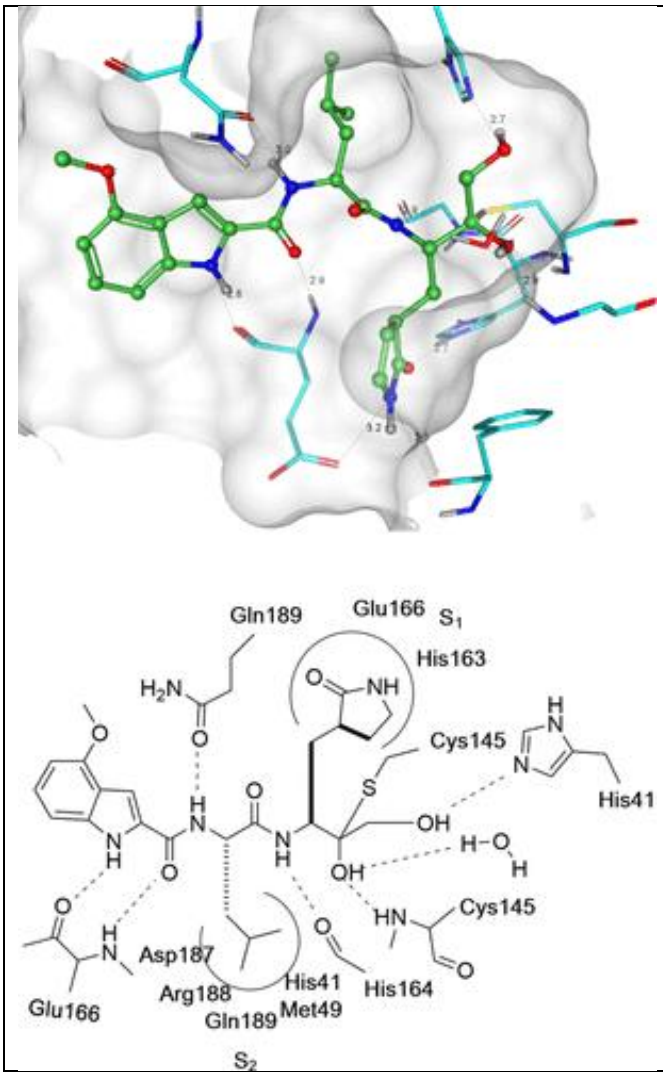
epithelial Vero E6 cells (cell-based in vitro assay), EC_{50} = _____

Analyze structure:

-_____ analog

(P1 Lactam Moieties as Gln Replacements from P. S. Dragovich et al. J. Med. Chem. 1999, 42, 1213-24; Agouron Pharmaceuticals)

-_____ reacts with Cys-SH



Note:
-key interaction:

-alpha-OH ←→ His

-H-bonding interactions _____

_____

-S1 pocket—
_____

_____

-S2 pocket--
_____

_____

Figure 5. Cocrystal structure of the covalent adduct of **4** bound to SARS CoV-2 $3CL^{pro}$ (6XHM). The Connolly surface for the inhibitor binding pocket is shown in gray. The bonds are represented as dashed lines, with the bond length between heavy atoms depicted. The schematic rendering of the active site with dashed lines represented as hydrogen bonds with key residues and curved lines to show S_1 and S_2 binding pockets is depicted.

(from Discovery of Ketone-Based Covalent Inhibitors of Coronavirus 3CL Proteases for the Potential Therapeutic Treatment of COVID-19 | Journal of Medicinal Chemistry 2020)

1. Liabilities of PF-00835231

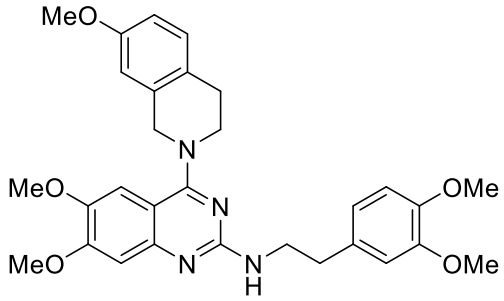
(a) *PF-00835231 is a _____, therefore assays used a P-glycoprotein _____ to retain PF-00835231 and demonstrate inhibition.

*See 2nd column in Figure 1

*Vero E6 expresses lots of P-glycoprotein efflux transporter

-P-glycoprotein is a _____
(including drugs) via an ATP-dependent mechanism; it has broad substrate variability; it is found in animals, fungi and bacteria.

CP-100356 (Pfizer compond):



(low μM-nM inhibitor of MDR-1, the prototypical ABC transporter; MDR=multi-drug resistance protein 1)

(b) Low _____

*See 3rd column in Fig. 1, MDCKII-LE (LE= low efflux; low Pgp mRNA level)

P_{app} <0.207 x 10^{-6} cm/s (ref. 21: J. Pharm. Sci. 2011, 100, 4974)

*_Hypothesized:_ _____

(ref. 22 D. Veber et al. J. Med. Chem. 2002, 45, 2615), therefore _____

_____

B. Overview of development:

(a) Change _____ (**1→ 2** and **3**)

(b) Remove an _____ (**2→3**)

(c) _____ improvement (**3→4/5**)

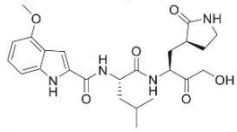
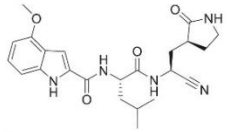
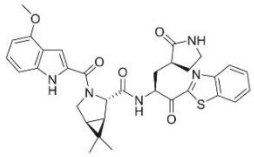
(d) Switch warhead _____ (**5→ 6)**

Compound 1 issues

-permeability _____ (MDCK-LE P_{app}) and poor _____ (Oral F and Fa x Fg: 1.4 and 3.3% respectively)

-indole doesn't occupy S3 pocket well due to conformation → _____

(a) 1→ 2/3

Number/Structure	SARS-CoV2 M^{pro} K_i (nM)*	VeroE6-enACE2 CPE EC_{50} (nM)†	MDCK-LE P_{app} (x 10^{-6} cm/sec)‡	HLM CL_{int} (µl/min/mg)§	Rat CL_p (mL/min/kg)¶	Oral F (%)¶¶	F_a x F_g (%)¶††
1 (PF-00835231) 	0.271 (0.155 – 0.471, n=6)	231 (158 – 338, n=8)	< 0.207 ± 0.048 (n=6)	7.47 ± 0.88	27.0 ± 3.1	1.4 ± 0.8	3.3
2 	27.7 (18.4 – 41.7, n=5)	1364 (860 – 2164, n=15)	0.945 ± 0.281 (n=6)	34.4 ± 0.7	39.3 (37.0, 41.5)	7.6 (7.4, 7.8)	38
3 	230 (181 – 292, n=4)	5593 (3457 – 9051, n=8)	10.3 ± 2.4 (n=6)	337 ± 9	N.D.	N.D.	N.D.

-Big bioavailability improvement _____

-Potency _____

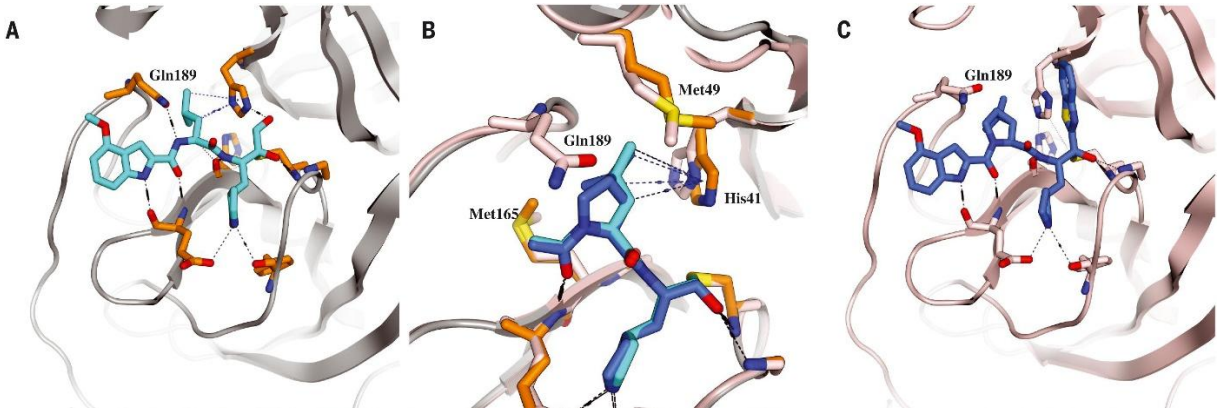
(b) 2→3

Big _____ increase (MDCK-LE P_{app})

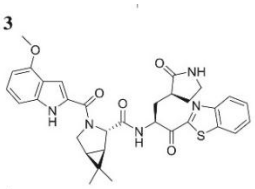
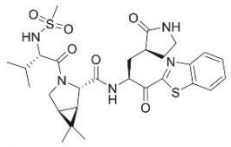
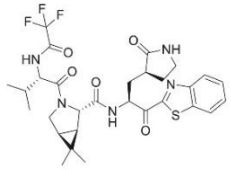
*MDCK-LE = _____ cells

-potency drops because _____

from indole due to new conformation binding (see Fig. 2C)



(c) 3→4→5

Number/Structure	SARS-CoV2 M^{pro} K_i (nM)*	VeroE6-enACE2 CPE EC_{50} (nM)†	MDCK-LE P_{app} (x 10^{-6} cm/sec)‡	HLM CL_{int} (µl/min/mg)§	Rat CL_p (mL/min/kg)¶	Oral F (%)¶#	F_a x F_g (%)¶††
3 	230 (181 – 292, n=4)	5593 (3457 – 9051, n=8)	10.3 ± 2.4 (n=6)	337 ± 9	N.D.	N.D.	N.D.
4 	7.93 (3.62 – 17.4, n=5)	909 (557 – 1482, n=14)	1.56 ± 0.38 (n=6)	127 ± 3	42.9 (38.2, 47.6)	10 (7.5, 13)	84
5 	12.1 (8.05 – 18.1, n=7)	85.3 (76.5– 95.2, n=36)	13.1 ± 2.0 (n=8)	30.3 ± 0.6	31.0 (30.6, 31.4)	33 (33, 34)	100

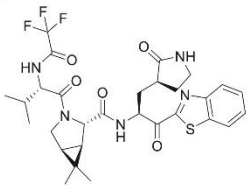
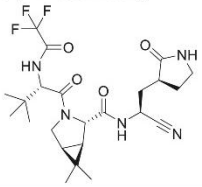
-Big _____! (3→4)

-Improved _____ (4→5)

(d) 5→ 6

-Compound 6 selected over 5 due to ease of _____ and _____ that allowed for easy formulation→ "in support of preclinical toxicology, and reduced propensity for epimerization at the P1 stereocenter"

Fig. 1. In vitro and in vivo parameters optimized in identifying oral SARS-CoV-2 M^{pro} inhibitors.

Number/Structure	SARS-CoV2 M^{pro} K_i (nM)*	VeroE6-enACE2 CPE EC_{50} (nM)†	MDCK-LE P_{app} (x 10^{-6} cm/sec)‡	HLM CL_{int} (µl/min/mg)§	Rat CL_p (mL/min/kg)¶	Oral F (%)¶#	F_a x F_g (%)¶††	
5 	12.1 (8.05 – 18.1, n=7)	85.3 (76.5– 95.2, n=36)	13.1 ± 2.0 (n=8)	30.3 ± 0.6	31.0 (30.6, 31.4)	33 (33, 34)	100	
6 (PF-07321332) 	3.11 (1.47 – 6.59, n=6)	74.5 (66.5 – 83.4, n=20)	1.71 ± 0.28 (n=4)	24.5 ± 0.2	27.2 (22.5, 31.9)	50 (30, 71), 34 ±19**	95, 65**	

>>>>>>>>>>>>>>>>>>>>>>>>>>>>

Pharmacokinetic (PK) Analysis

Basic terms

1. Clearance→ _____

HLM = _____ (metabolism study)

CL_{int} = _____ (µL of solution cleared of drug per minute of experiment per mg of HLM's)

Rat CL_p = _____ (mL of blood cleared of drug per minute of assay per kg size of rat)

2. Bioavailability → _____

_____ = bioavailability (F for "free" drug?) → more available the better

*Note: Fa x Fg = _____ (see SI for definitions) →

_____ is better

>>

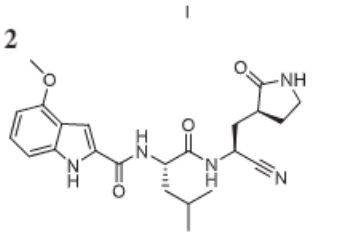
C. New Cys protease "functional group warheads"

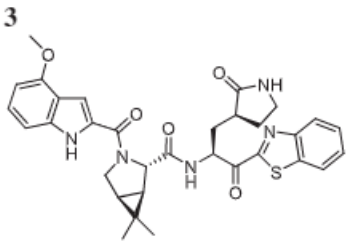
1. keto-benzothiazole (a.k.a. benzothiazole-2-yl ketones)

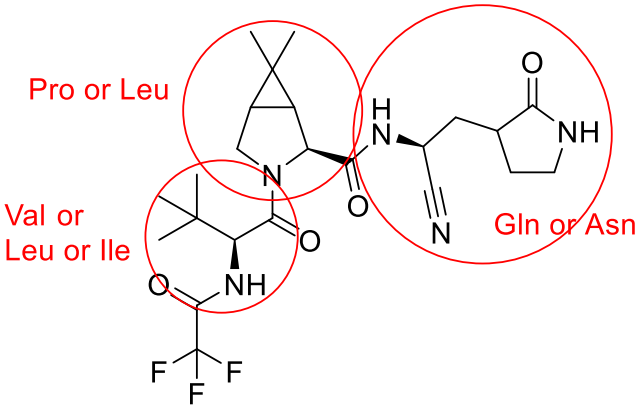
2. cyano group → -CN

D. Remove _____ → Leu analog with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane

-fills space like Leu side chain _____



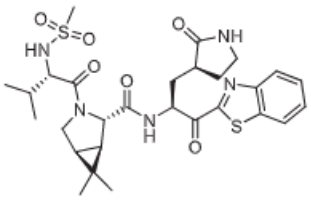




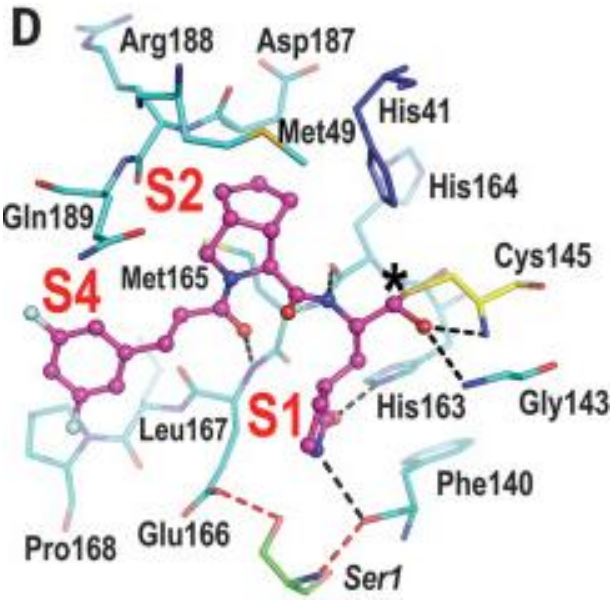
E. Improve occupancy of _____

-_____ groups introduced:

1. Compound **4**, Val-NH-S(O)2CH3



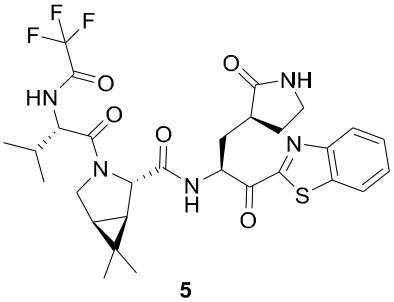
-improves _____ backbone (Fig. 2D); retains/restores H-bond with Gln189



-Key: led to improvements in _____

*seems counter to hypothesis about _____

2. Compound **5**, Val-NH-C(=O)-CF3



5

F. Return to -CN functional group warhead

-better _____

-slightly better warhead with _____

-permeability _____!

-_____a little better

Compound **6** is _____

*Compound 6 is _____ as demonstrated by a dilution and then competition assay

Key:

-**Compound 6 selected over 5 due to ease of large scale synthesis and enhanced solubility that allowed for easy formulation→ "in support of preclinical toxicology, and reduced propensity for epimerization at the P1 stereocenter"

REVIEW crystal structures for evidence!

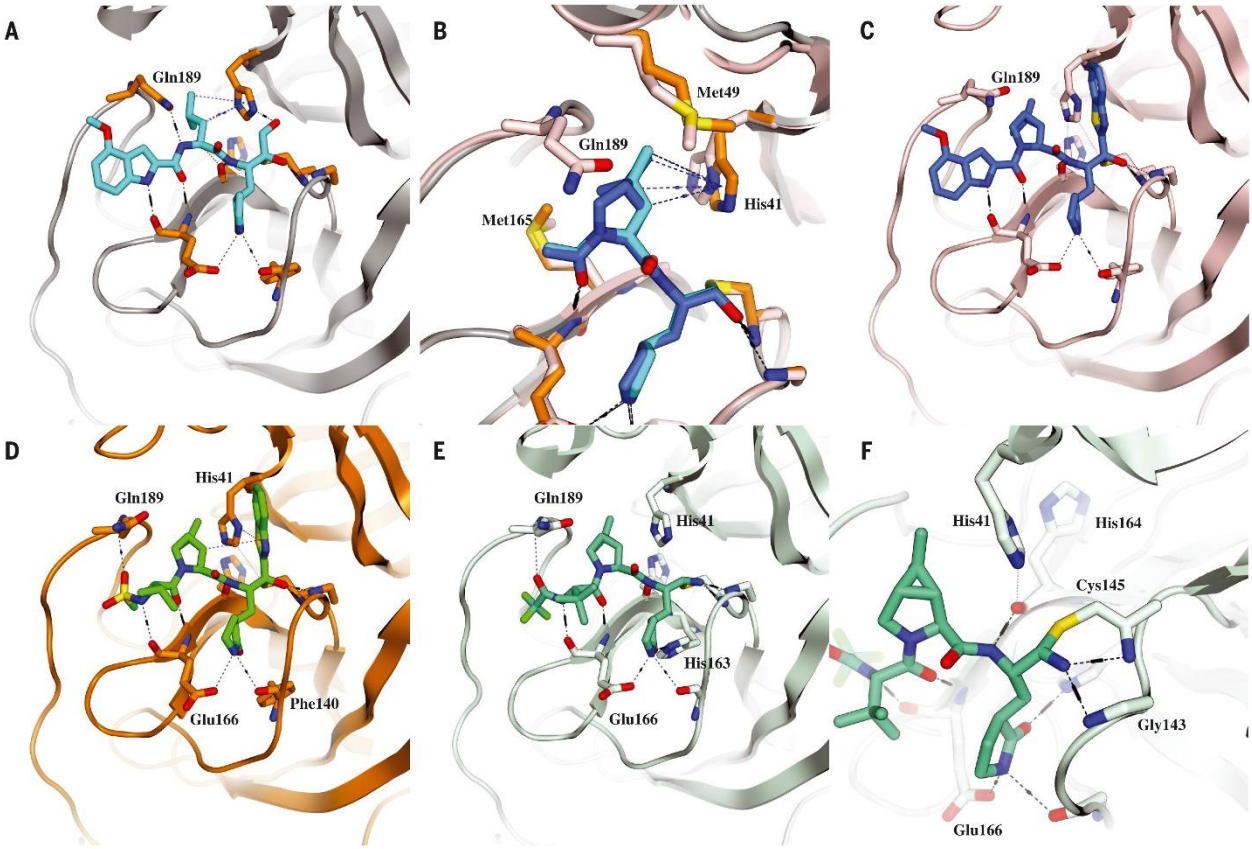
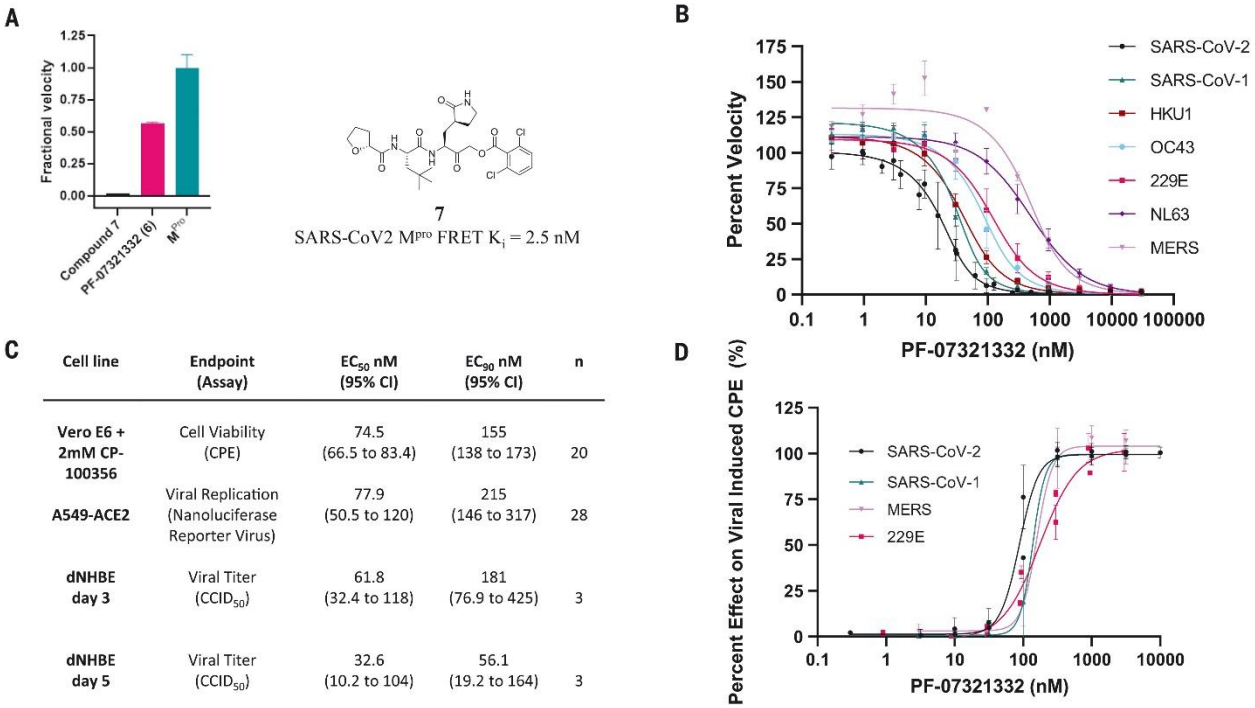


Fig. 2. SARS-CoV-2 M^{pro} structural biology.

(**A**) Co-crystal structure of PF-00835231 (**1**) with SARS-CoV-2 M^{pro}. Key interactions are indicated. (**B**) Modeled overlap of dimethyl-bicyclo[3.1.0] proline from compound **3** (blue) as a mimic of P2 leucine residue (cyan) found in the viral polyprotein substrate and **1**. This tolerated P2 change eliminates an H-bond donor from resulting inhibitors. (**C**) Compound **3** effectively fills the lipophilic S2 pocket formed by Met49, Met165, and His41, but productive hydrogen bonding to Gln189 is no longer possible. (**D**) Compound **4** with optimized acyclic P3 group and restored Gln189 interaction. (**E**) SARS-CoV-2 M^{pro}–bound crystal structure of clinical candidate PF-07321332 (**6**). (**F**) A reversible covalent Cys145 adduct is formed with the nitrile substituent in compound **6**.

III. Biological potency assays



Cell line	Endpoint (Assay)	EC_{50} nM (95% CI)	EC_{90} nM (95% CI)	n
Vero E6 + 2mM CP-100356	Cell Viability (CPE)	74.5 (66.5 to 83.4)	155 (138 to 173)	20
A549-ACE2	Viral Replication (Nanoluciferase Reporter Virus)	77.9 (50.5 to 120)	215 (146 to 317)	28
dNHBE day 3	Viral Titer ($CCID_{50}$)	61.8 (32.4 to 118)	181 (76.9 to 425)	3
dNHBE day 5	Viral Titer ($CCID_{50}$)	32.6 (10.2 to 104)	56.1 (19.2 to 164)	3

Fig. 3. PF-07321332 biochemical and antiviral activity.
(**A**) PF-07321332 is a reversible inhibitor of SARS-CoV-2 M^{pro} as demonstrated by recovery of enzymatic activity after a 100-fold dilution of the enzyme inhibitor complex. Compound **7** (PF-00956378), an irreversible inhibitor, was included as a control. Data are representative of three independent experiments. (**B**) PF-07321332 is a potent inhibitor of the proteolytic activity of SARS-CoV-2 M^{pro} as well as related coronaviruses in FRET assays. Data shown are the mean ± SD from three independent experiments. (**C**) PF-07321332 demonstrates potent SARS-CoV-2 antiviral cellular activity. PF-07321332 inhibited SARS-CoV-2–induced CPE in Vero E6 cells enriched for ACE2. A P-glycoprotein inhibitor, CP-100356 (efflux inhibitor, EI), was added at 2 μM to inhibit the P-glycoprotein–mediated efflux of PF-07321332. In Vero E6 cells with no EI, the EC_{50} of PF-07321332 [95% CI was 4.48 μM (3.55 to 5.65 μM); N = 8, n = 20]. Cytotoxicity of PF-07321332 was evaluated in noninfected cells, and the $CCID_{50}$ was >100 μM (table S4). PF-07321332 inhibits SARS-CoV-2 replication in A549 cells expressing ACE2 in four independent experiments. The $CCID_{50}$ in noninfected A549 cells was >3 μM (table S4). In dNHBE cells, PF-07321332 decreased SARS-CoV-2 viral replication (N = 3). Data are shown as the geometric mean and 95% CI. (**D**) PF-07321332 demonstrates pan-coronavirus antiviral activity. PF-07321332 inhibition in viral-induced CPE assays: SARS-CoV-1 in Vero E6 cells (in the presence of 2 μM EI CP-100356), h-CoV-229E in MRC-5 cells, and MERS-CoV in Vero 81 cells (in the

presence of 1 μM EI CP-100356). Data are shown as mean ± SD. $CCID_{50}$ values were determined in all assays to be >100 μM (table S4).

IV. PK optimization and issues

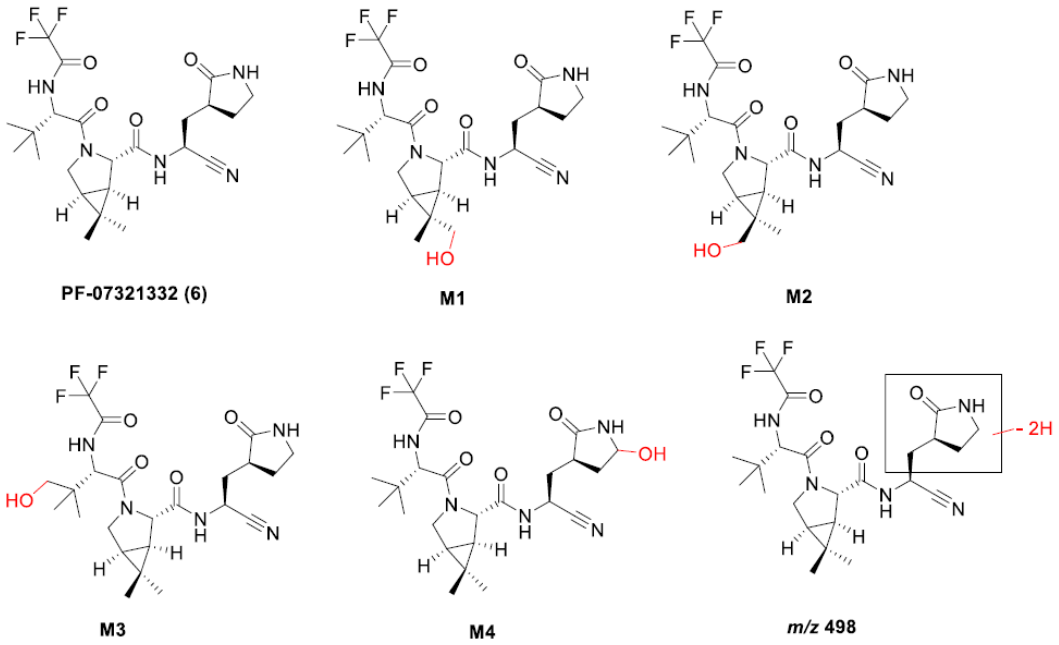
A. Metabolic products

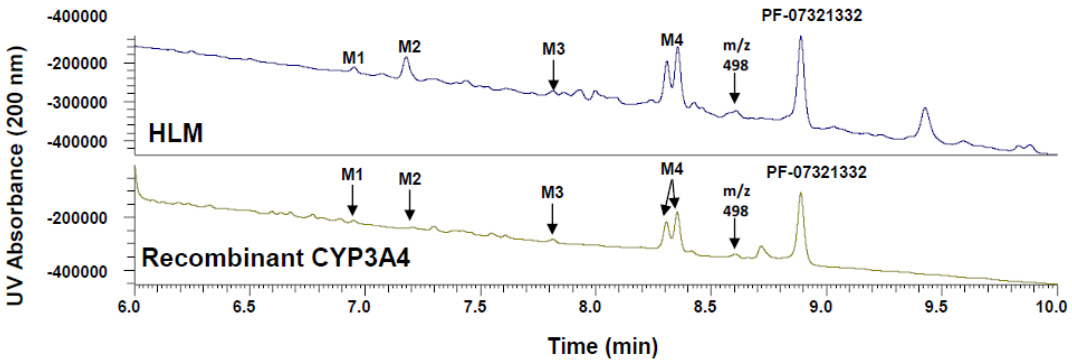
P2 azabicyclohexane _____

P1 pyrrolidine _____

P3 t-Bu group _____

SI, p. 76





**_____ is responsible for most of the metabolism of PF-07321332

Key recognition: _____

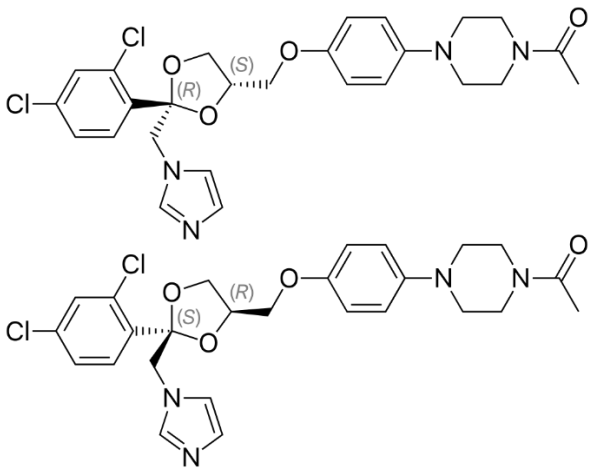
1. ketoconazole, an anti-fungal that is _____

(CYP51A1 inhibition is _____;

usual _____ mechanism)

-ketoconazole selectively inhibits _____

*Clearance inhibited by ketoconazole at >82%



2. ritonavir → _____

**ritonavir is an _____!

! Co-dose PF-07321332/ nirmatrelvir with _____