

Why re-purposing HIV drugs Lopinavir/ritonavir to inhibit the SARS-Cov2 protease probably wont work - but re-purposing Ribavirin might since it has a very similar binding site within the RNA-polymerase

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Letter

A trial of Lopinavir/Ritonavir in adults hospitalized with severe Covid-19 has not shown significant difference [1]. This is not surprising considering that the HIV aspartic protease - which Lopinavir/Ritonavir inhibit (Table 1) - is quite different from the cysteine proteases in SARS-Cov2.

A review explains this well - 'it is debatable whether HIV protease inhibitors could effectively inhibit the 3-chymotrypsin-like and papain-like proteases of 2019-nCoV. HIV protease belongs to the aspartic protease family, whereas the two coronavirus proteases are from the cysteine protease family. Furthermore, HIV protease inhibitors were specifically optimized to fit the C2 symmetry in the catalytic site of the HIV protease dimer, but this C2-symmetric pocket is absent in coronavirus proteases. If HIV protease inhibitors alter host pathways to indirectly interfere with coronavirus infections, their potency remains a concern.' [2].

However, using known structures of the SARS-Cov2, one can dock molecules, and thus re-purpose existing drugs.

Protease inhibitors

Peptide inhibitors

A recent pre-print does 'virtual drug screening and high-throughput screening to identify new drug leads that target the COVID-19 virus main protease' [3]. It identifies a small 6 aa peptide that bind to the protease (Table 2).

Small molecule inhibitors

There is a host of small molecules that bind to the SARS-Cov2 protease (PDB:5R7Y). Table 3 shows the interacting cysteine residues in the active site. There are several such inhibitors.

Ribavirin has a very similar binding site in SARS-Cov2:

Ribavirin is a guanosine (ribonucleic) analog that inhibits viral replication by inhibition of RNA-dependent RNA polymerase (RdRP) [4]. Recently, the SARS-Cov2 RdRP has been solved (PDBid:6NUR) [5]. This provides a good framework for re-purposing and discovering Ribavirin-like antivirals. Previously, structural studies have shown a similar liganding mechanism in two different viruses (Table 4) using the same two residues (ASP and ASN).

Using the residues from the norovirus (PDB:3SFU), a search for a similar binding site using CLASP [6] gave very good results (Table 5). This was then used to superimpose and dock Ribavirin to the SARS-Cov2 polymerase (Fig 1) using DOCLASP [7]. Once again, this shows no steric hindrance. The SARS-Cov2 is apo, while the norovirus is holo, i.e. with ligand bound - so there will be some perturbations on binding (Fig 2). The pymol file for superimposition is in SI/super.p1m. A previous study also predicts Ribavirin binding, but the residue numbering is different from the one in the PDB [8]. For example, D651 is actually R651 in the PDB files. Another study has probed the efficacy of other drugs (Alovidine and AZT (an FDA approved HIV/AIDS drug)) [9].

Similarly, Remdesivir is a 1-cyano-substituted adenosine nucleotide analogue again influencing the RdRP [10], that protects monkeys from Nipah [11], and has had initial success in treating SARS-Cov2 [12, 13]. There are no solved structures with this drug in the PDB database.

Ribavirin dosage caveat

Adverse events have been associated with high-dose of Ribavirin [14], and consequently antiviral treatment guidelines should be strictly followed [15].

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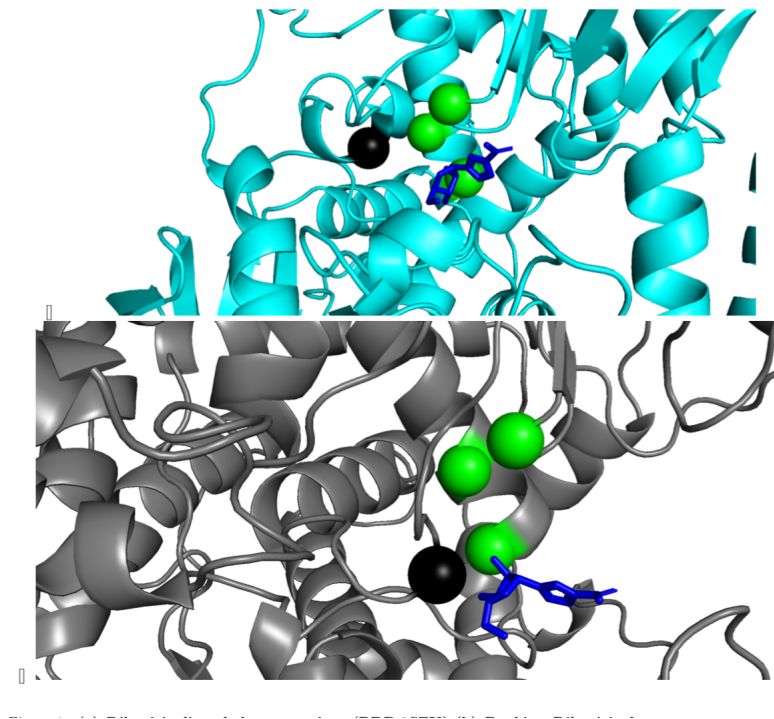


Figure 1: (a) Ribavirin liganded to norovirus (PDB:3SFU) (b) Docking Ribavirin by superimposition of the norovirus (PDB:3SFU) with Ribavirin liganded to the apo SARS-Cov2 (PDB:6NUR) Note, the amino-acids are the same, and their pairwise distance very similar (Table 5).

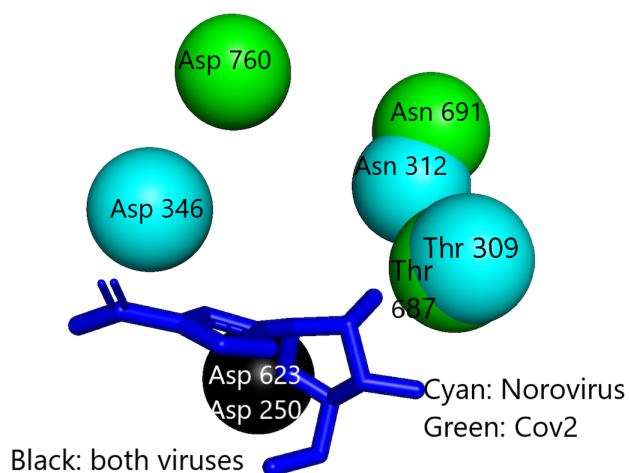


Figure 2: **Superimposition of binding residues of Ribavirin:** The only difference is Asp346/Asp760 - this is because the SARS-Cov2 is in the apo state, with no ligands. $C\alpha$ atoms are shown.

Table 1: **Binding of Ritonavir to the HIV protease - an aspartic protease** PDBid:4EYR . Note the ASP amino acid in the catalytic site.

HIV protease atom	Ritonavir atom	Distance
ASP29N	C4 CN	2.7
ASP29N	C4 CN	2.7
ASP29CB	C4 CC	2.9
ASP29CB	C4 CC	2.9
ASN25OD1	O41 OO	2.8
ASN25OD1	O41 OO	2.8
ASN25ND2	O41 ON	2.9
ASN25ND2	O41 ON	2.9
ASP30N	N5 NN	3.1
ASP30N	N5 NN	3.1
ASP29CA	C4 CC	3.2
ASP29CA	C4 CC	3.2
GLY27O	O7 OO	3.2
GLY27O	O7 OO	3.2

Table 2: **Inhibition of SARS-Cov2 protease using a small peptide inhibitor** [3]

protease	inhibitor	Atom protease	Atom inhibitor	Distance
6LU7A	6LU7C	GLU/166/O	VAL/3/N	2.8
6LU7A	6LU7C	THR/190/O	ALA/2/N	2.9
6LU7A	6LU7C	GLN/189/OE1	LEU/4/N	2.9
6LU7A	6LU7C	GLU/166/N	VAL/3/O	3.0
6LU7A	6LU7C	MET/165/CB	VAL/3/O	3.2
6LU7A	6LU7C	THR/190/O	ALA/2/CB	3.3
6LU7A	6LU7C	MET/165/CA	VAL/3/O	3.3
6LU7A	6LU7C	GLN/189/CA	ALA/2/O	3.3
6LU7A	6LU7C	GLN/189/CB	ALA/2/O	3.4
6LU7A	6LU7C	GLU/166/O	ALA/2/CA	3.5

Table 3: **Binding of small molecule to the SARS-Cov2 protease - an cysteine protease**

SARS-Cov2 protease atom	inhibitor atom	Distance
CYS44O	C01/CO	3.2
CYS44O	C01/CO	3.2
SER46CA	O04/OC	3.2
SER46CA	O04/OC	3.2
SER46CB	O04/OC	3.3
SER46CB	O04/OC	3.3
HIS164O	C09/CO	3.3
HIS164O	C09/CO	3.3
MET49CE	C10/CC	3.4
MET49CE	C10/CC	3.4
MET165CG	C10/CC	3.5
MET165CG	C10/CC	3.5
SER46N	O04/ON	3.4
SER46N	O04/ON	3.4

Table 4: **Residues in contact with Ribavirin in two RNA-polymerases from murine norovirus(PDB:3SFU) and Foot-and-mouth disease virus (PDB:2E9R)** This shows the ligand comprises aspartic acid (ASP) and asparagine (ASN) residues, and the search for a similar binding site in the SARS-Cov2 should be guided as such.

PDB	virus RNA polymerase	Ribavirin atom	Distance
3SFU	ASP346OD1	N3 NO	2.6
	ASP346OD1	N3 NO	2.6
	ASN312ND2	O2' ON	2.8
	ASN312ND2	O2' ON	2.8
	ASN312CB	O2' OC	3.0
	ASN312CB	O2' OC	3.0
	ASP250OD1	C5' CO	3.0
	ASP250OD1	C5' CO	3.0
	THR309CA	O2' OC	3.0
	THR309CA	O2' OC	3.0
2E9R	ASP338OD1	O2B OO	2.4
	ASP338OD1	O2B OO	2.4
	GLY299N	O3 ON	2.4
	GLY299N	O3 ON	2.4
	GLY299CA	O3 OC	2.6
	GLY299CA	O3 OC	2.6
	ASP245OD2	C2' CO	2.2
	ASP245OD2	C2' CO	2.2
	ASN307ND2	O2' ON	2.3
	ASN307ND2	O2' ON	2.3
	ASP245CG	O2' OC	2.4
	ASP245CG	O2' OC	2.4

Table 5: **Pairwise distance in residues that bind Ribavirin in norovirus (PDB:3SFU) with cognate binding site in SARS-Cov2 (PDB:6NUR)** Distance are in AngstromÅ. Also, note the sequences are also in reasonable order. And the accessibleness of the binding site (Fig 1) - i.e. this binding site is not inside the protein.

PDB	Residues (a,b,c,d)	ab	ac	ad	bc	bd	cd
3SFU (norovirus)	ASP250,THR309,ASN312,ASP346	10.2	8.0	7.4	5.0	9.0	7.4
6NUR (SARS-Cov2)	ASP623,THR687,ASN691,ASP760	9.6	9.3	9.3	5.8	10.0	6.2