# Repurposing the HCV NS3-4A Protease Drug Boceprevir as COVID-19 Therapeutics

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# equal contribution

## Supplementary information

Fig S1. Sequence SARS-CoV-2 3CLpro ordered from Eurofins

CCATGGCGGCCGTACTGCAATCAGGTTTTCGCAAAATGGCGTTTCCATCGGGAAAAGTCGAAGGCT
GCATGGTTCAGGTTACATGTGGGACAACCACGCTGAATGGCCTGTGGTTGGATGATGTGGTGTATT
GTCCTCGTCACGTTATCTGCACAAGCGAAGATATGCTGAATCCGAACTATGAGGACTTGCTGATTCG
GAAATCCAATCACAACTTTCTGGTGCAAGCGGGTAACGTGCAGTTACGCGTAATCGGCCATTCGATG
CAGAACTGTGTGCTGAAACTGAAAGTGGACACCGCGAATCCCAAAACCCCGAAATACAAGTTCGTC
CGTATTCAACCAGGGCAGACCTTTAGCGTCCTCGCATGCTATAACGGCAGTCCGAGTGGTGTGTATC
AGTGTGCGATGCGTCCGAACTTCACCATCAAAGGCTCCTTTCTGAACGGGTCGTGTGGTAGCGTAGG
CTTCAACATCGACTACGATTGCGTTAGCTTTTGCTACATGCATCACATGGAATTGCCGACTGGTGTCC
ATGCCGGTACTGATCTGGAAGGCAACTTCTATGGTCCCTTTGTTGATCGTCAGACCGCCCAAGCAGC
GGGTACCGATACCACCATTACCGTGAATGTGCTCGCTTGGTTATATGCGGCTGTGATCAATGGAGAT
CGCTGGTTTCTGAATCGCTTCACGACCACGCTTAACGACTTCAATCTCGTCGCAATGAAGTACAACTA
CGAACCTCTGACTCAGGATCATGTGGATATTCTGGGTCCGTTATCTGCTCAGACGGGCATTGCCGTA
CTGGACATGTGCGCCTCACTGAAGGAGTTACTGCAGAACGGGATGAATGGACGCACGATTTTGGGC
TCTGCACTTCTTGAGGACGAATTCACTCCGTTTGATGTTGTCCGCCAATGCAGCGGCGTTACGTTTCA
GCTCGAG

**Table S1. Data collection and refinement statistics**

| Data collection | SARS-CoV-2 $M^{pro}$ - Boceprevir (PDB ID: 6ZRU) | SARS-CoV-2 $M^{pro}$ - Telaprevir (PDB ID: 6ZRT) |
|---|---|---|
| Space group | I 1 2 1 | C 1 2 1 |
| Cell dimensions | | |
| a, b, c (Å) | 113.76, 53.53, 45.88 | 109.68, 54.99, 47.93 |
| α, β, γ (°) | 90.00, 101.52, 90.00 | 90.00, 101.42, 90.00 |
| Resolution | 48.26 – 2.10 (2.16-2.10) | 48.96 – 2.10 (2.16-2.10) |
| Completeness (%) | 100.0 (100.0) | 99.3 (98.5) |
| $R_{merge}$ | 0.040 (0.702) | 0.050 (0.549) |
| <I / σ(I)> | 23.9 (2.8) | 22.1 (3.8) |
| Redundancy | 6.8 (7.0) | 6.4 (6.7) |
| **Refinement** | | |
| Resolution | 44.96 – 2.10 (2.16 – 2.10) | 47.03 – 2.10 (2.16 – 2.10) |
| No. reflections | 15173 (1131) | 15497 (1130) |
| $R_{work}$ / $R_{free}$ | 0.188 / 0.215 | 0.199 / 0.237 |
| No. Atoms | | |
| Protein | 2340 | 2340 |
| Ligand/Ion | 45 | 53 |
| Water | 23 | 45 |
| B-Factors (Å$^2$) | | |
| Protein | 55.27 | 43.07 |
| Ligand/Ion | 61.67 | 48.25 |
| Solvent | 45.28 | 42.31 |
| RMS deviations | | |
| Bond lengths (Å) | 0.009 | 0.009 |
| Bond Angles (°) | 1.654 | 1.594 |

**Table S2. Best pose docking results of HCV NS3-4A protease inhibitors**

| Ligand | PLPChemscore | Ligand Efficiency |
|---|---|---|
| Boceprevir | 125.94 | 3.31 |
| Telaprevir | 162.25 | 3.25 |
| Narlaprevir | 95.84 | 1.92 |

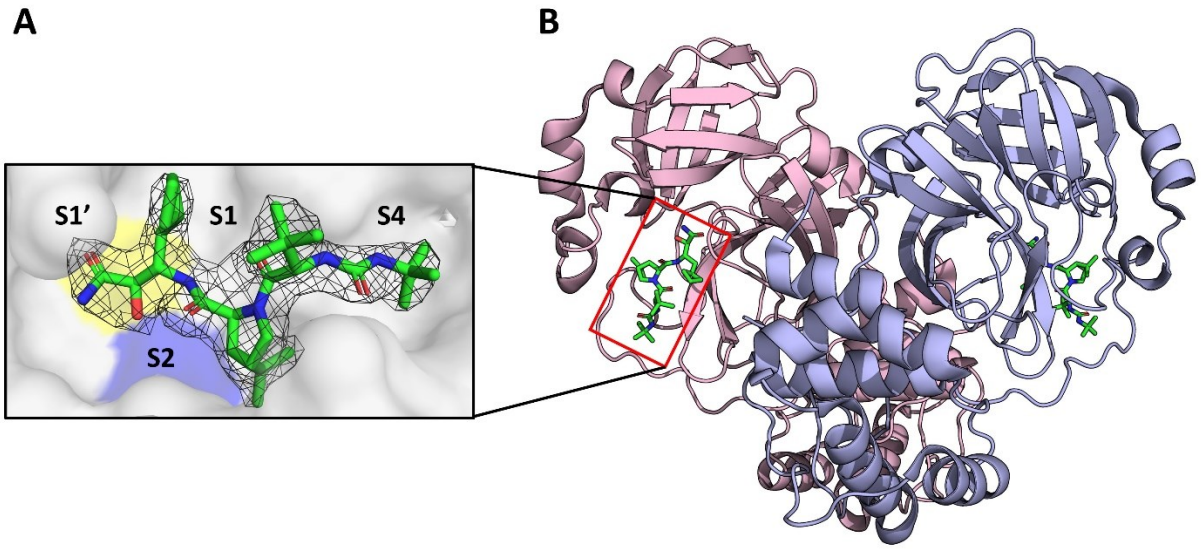


**Figure S2. Electron density of Boceprevir. A)** Magnified view of the substrate binding pocket (surface representation) with subsites S1', S1, S2 and S4 indicated. Boceprevir is shown as green sticks, enmeshed by the $2F_o$-$F_c$ map contoured at 1.0 σ. Cys145 in yellow surface and His41 in blue surface. **B)** Cartoon representation of the biological assembly (homodimer) of SARS-Cov-2 3CLpro covalently bound to Boceprevir presented as green sticks. Protomer A is pink, protomer B is purple.

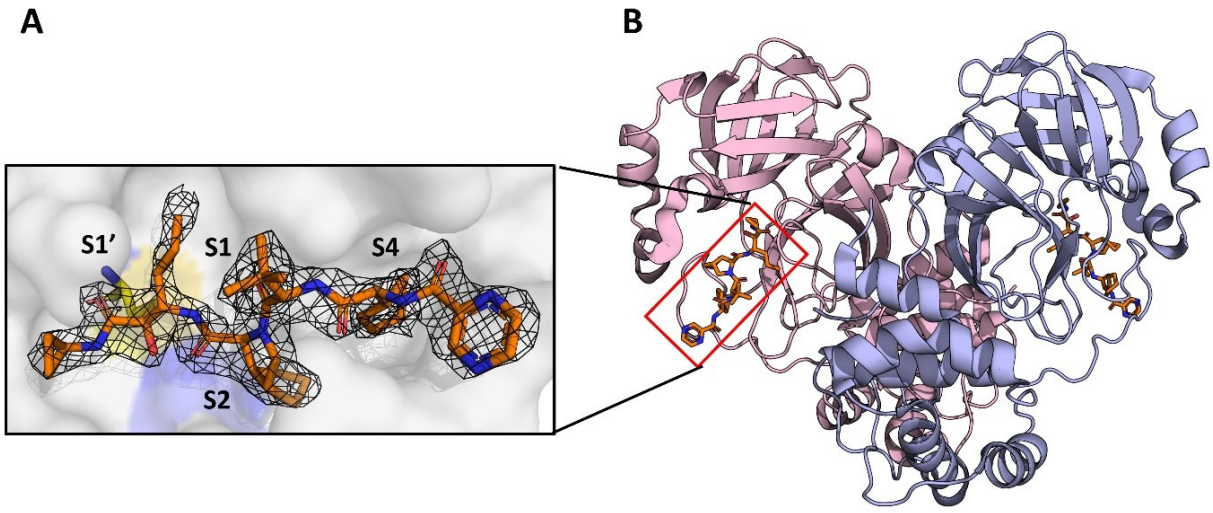


**Figure S3. Electron density of Telaprevir. A)** Magnified view of the substrate binding pocket (surface representation) with subsites S1', S1, S2 and S4 indicated. Telaprevir is shown as orange sticks, enmeshed by the $2F_o$-$F_c$ map contoured at 1.0 σ. Cys145 in yellow surface and His41 in blue surface. **B)** Cartoon representation of the biological assembly (homodimer) of

SARS-Cov-2 3CLpro covalently bound to Telaprevir, presented as orange sticks. Protomer A is pink, protomer B is purple.

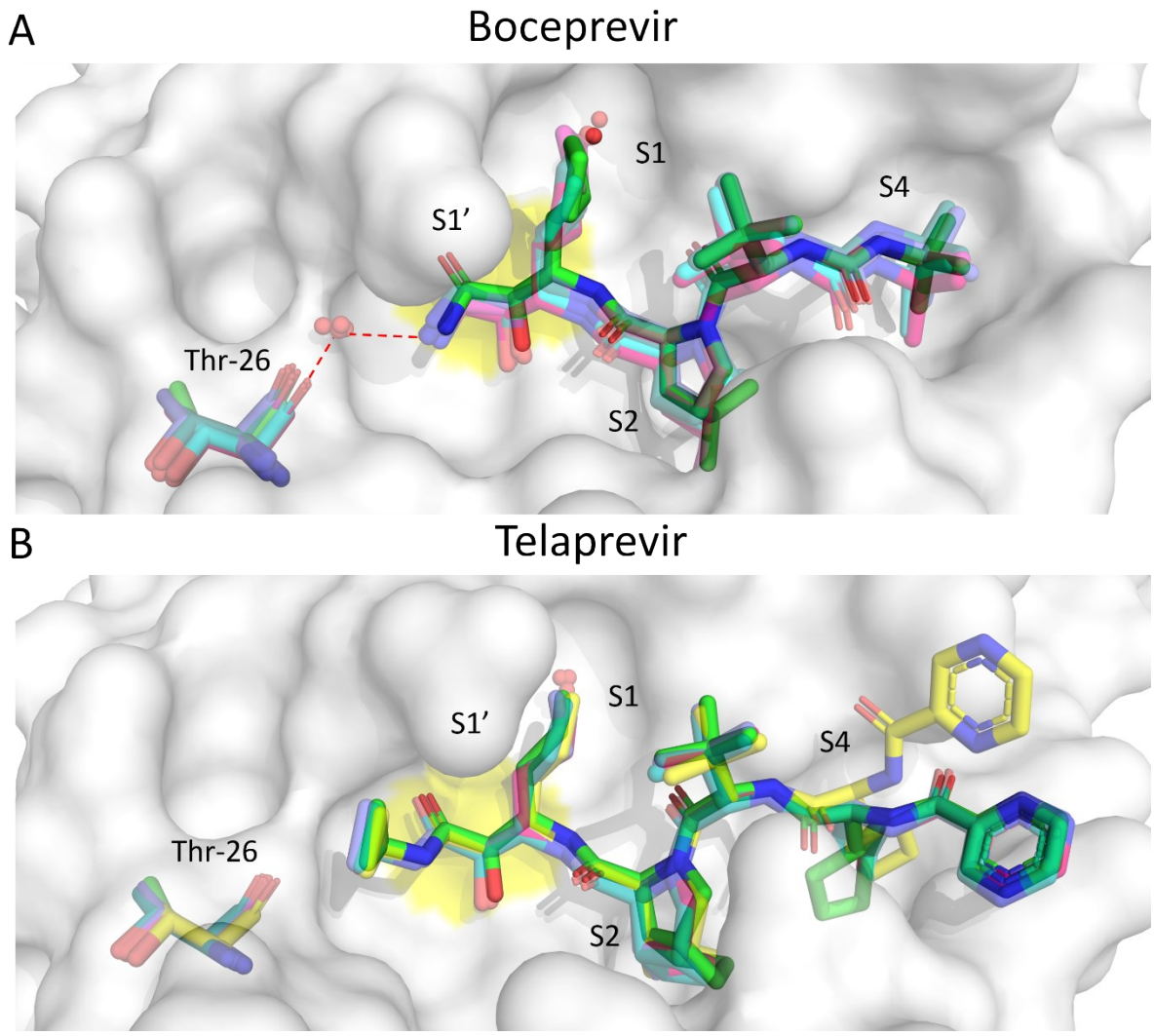


**Figure S4. Structural alignment of available crystal poses of 3CLpro-Boceprevir and Telaprevir complexes. A)** Overlay of the reported SARS-CoV-2 3CLpro (white surface) structure in complex with Boceprevir (6ZRU) and 5 available crystals (7K40, 7C6S, 6XQU,7BRP and 6WNP). The Boceprevir molecules are represented as sticks (6ZRU solid green sticks, 7K40 slate sticks, 7C6S cyan, 6XQU light magenta,7BRP deep teal and 6WNP warm pink), Cys145 is shown as a yellow surface and Thr26 as sticks. Waters are represented as spheres. The water mediated hydrogen bond between Thr26 and Boceprevir (6WNP) is indicated with dashed lines. **B)** Overlay of the reported SARS-CoV-2 3CLpro (white surface) structure in complex with Telaprevir (6ZRT) and 4 available crystals (7C7P, 7K6E, 7K6D and 6XQS). The Telaprevir molecules are represented as sticks (6ZRT solid green sticks, 7C7P yellow sticks, 7K6E slate, 7K6D hot pink and 6XQS teal), Cys145 is shown as a yellow surface and Thr26 as sticks. Waters are represented as spheres.