Potent Noncovalent Inhibitors of the Main Protease of SARS-CoV-2 from Molecular Sculpting of the Drug Perampanel Guided by Free Energy Perturbation Calculations

Chun-Hui Zhang, Elizabeth A. Stone, Maya Deshmukh, Joseph A. Ippolito, Mohammad M. Ghahremanpour, Julian Tirado-Rives, Krasimir A. Spasov, Shuo Zhang, Yuka Takeo, Shalley N. Kudalkar, Zhuobin Liang, Farren Isaacs, Brett Lindenbach, Scott J. Miller, Karen S. Anderson, and William L. Jorgensen

Cite This: ACS Cent. Sci. 2021, 7, 467−475

Access Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Starting from our previous finding of 14 known drugs as inhibitors of the main protease (Mpro) of SARS-CoV-2, the virus responsible for COVID-19, we have redesigned the weak hit perampanel to yield multiple noncovalent, nonpeptidic inhibitors with ca. 20 nM IC50 values in a kinetic assay. Free-energy perturbation (FEP) calculations for Mpro-ligand complexes provided valuable guidance on beneficial modifications that rapidly delivered the potent analogues. The design efforts were confirmed and augmented by determination of high-resolution X-ray crystal structures for five analogues bound to Mpro. Results of cell-based antiviral assays further demonstrated the potential of the compounds for treatment of COVID-19. In addition to the possible therapeutic significance, the work clearly demonstrates the power of computational chemistry for drug discovery, especially FEP-guided lead optimization.

INTRODUCTION

The coronavirus SARS-CoV-2, the cause of the COVID-19 pandemic, encodes several enzymes that are essential to its ability to replicate. After cell entry, viral RNA is translated by host ribosomes into two polyproteins that are cleaved to produce the viral proteins that are needed for assembling new virions. As potential targets for discovery of therapeutic agents, the two cysteine proteases that are responsible for cleaving the polyproteins have been highlighted, namely, the chymotrypsin-like or main protease, known as 3CLpro or Mpro, and the papain-like protease, PLpro. Following the disease outbreak in 2002 from SARS-CoV, these proteins have received much attention for characterization of their structural biology and development of inhibitors. The high sequence homology between the proteins from the two coronaviruses, 83% for PLpro and 96% for Mpro, has allowed the prior studies to provide a solid foundation for current efforts targeting the new isoforms. Thus, crystal structures of Mpro from SARS-CoV-2 have quickly emerged along with initial reports of inhibitors. As for the earlier virus, the designed inhibitors have largely been peptide-like with incorporation of a reactive warhead that covalently binds to the catalytic cysteine, Cys145. These features are generally not optimal for drug development owing to potential proteolytic degradation, limited antiviral activity, and toxicities from off-target covalent modification of other biomolecules.

In contrast, our efforts have been directed to discovery of nonpeptidic, noncovalent inhibitors of SARS-CoV-2 Mpro that are drug-like and show both high inhibitory and antiviral activity. Specifically, the present effort started from a virtual screen of ca. 2000 known, approved drugs that led to identification of 14 drugs as inhibitors of SARS-CoV-2 Mpro with IC50 values as low as 5 μM in a kinetic assay. As stated, the goals were to identify possible drugs for repurposing and to provide clearly drug-like hits for lead optimization to yield highly potent antiviral agents. We now report successful execution of the latter strategy starting from a weak hit, the anti-epileptic drug perampanel, in the kinetic assay. The optimization proceeded extraordinarily rapidly owing to the use of free-energy perturbation (FEP) calculations to guide the choices of structural modifications.
RESULTS AND DISCUSSION

Structural Analysis, FEP Calculations, and Initial Designs. Perampanel (1) showed activity in the enzyme assay, though only a rough IC$_{50}$ of 100–250 μM could be established owing to interference of the compound’s fluorescence with the product of the assay. However, its relatively simple structure is amenable to synthesis of analogues and its docked structure from the work in ref 12 was compelling (Figure 1). The structure reflects a cloverleaf motif for active compounds with the three leaves occupying the binding pockets referred to as S1, S1’, and S2, as identified in Figure 1A. The phenyl, cyanophenyl, and pyridinyl groups of 1 are predicted to reside in the three pockets with the central pyridinone ring acting as the connecting hub. The catalytic residues Cys145 and His41 are located at the bottom of the site, as drawn, and other key surrounding residues are noted in Figure 1B.

The analyses began by close examination of the docked complex with locations of some notable interaction points highlighted in Figure 1B by the circled letters. (a) The backbone NH of Glu166 is directed at the pyridinone but it does not form a hydrogen bond. (b) The pyridine nitrogen is directed toward the solvent so it is not helpful to binding. (c) The pyridine ring makes an edge-to-face aryl–aryl interaction with His41. It appears that there might be room for an additional small group in the meta position. (d) The cyano group of 1 is directed well at the NH of Cys145. In the docked structure the N···N distance is 3.94 Å, which shortens to 3.46 Å upon conjugate-gradient optimization of the complex using the MCPRO program with the OPLS-AA/M force field for the protein and OPLS/CM1A for the ligand. However, the carbonyl group of the pyridinone ring does not participate in a hydrogen bond and is blocked from solvation by the side chain of Asn142. In addition, the C3–C4 edge of the cyanophenyl ring is proximal to the opposing backbone oxygen and NH of Thr26. (e) The phenyl ring in the S1 pocket appears mismatched with the polar environment, which includes the side chains of Ser1B, His163, and Glu166. It is noted that a meta-CH is well directed at Nϵ of His163 with a C···N separation of 3.38 Å.

Considering these features, several modifications of 1 to enhance binding seemed reasonable to pursue: switching the carbonyl group from C2 to C6 to form a hydrogen bond with the NH of Glu166, removing the pyridine nitrogen and adding a small group at C3 of the pyridine ring, leaving the cyano group and/or introducing a hydrogen bonding edge at C2–C4 of the cyanophenyl ring, and replacing the phenyl ring in S1 with a heterocycle that could hydrogen bond with His163.

FEP calculations were used to explore the possible benefits of such changes. The necessary structures were built with the BOMB program and the FEP calculations were carried out using standard protocols with the MCPRO program and the previously mentioned force fields. Relative free energies of binding, ∆∆G$_{\text{ binding}}$ are obtained by mutating the ligand from structure A to structure B for both the protein–ligand complex in water and the unbound ligand. The configurational sampling for the systems was carried out at 25 °C with Monte Carlo simulations including the 242 protein residues nearest to the active site and 1250 and 2000 TIP4P water molecules for the ligand-bound and ligand-free calculations. Briefly, starting from
perampanel, switching the carbonyl group from C2 to C6 was predicted to be very favorable (\(\Delta\Delta G_E = -4.7 \pm 0.3\) kcal/mol) with formation of the hydrogen bond with Glu166; replacement of the S1 benzene ring by 2-, 3-, or 4-pyridine, 2,4-pyrimidine, 2,4,6-triazine, and 4-pyridine-N-oxide showed no benefit except for the 3-pyridine (\(\Delta\Delta G_E = -3.6 \pm 0.2\) kcal/mol), which gave a hydrogen bond with His163; and a chlorine scan for benzene in the S2 site predicted significant benefit for a meta-Cl directed inward toward His41, neutral effects for a Cl at the exposed ortho and meta positions, and strong disfavoring (4–6 kcal/mol) for a Cl at the para and inward-ortho positions.

The combination led us to focus immediately on 2 as a target (Figure 2). Additional model building with BOMB/MCPRO for numerous heterocycles replacing the cyanophenyl group in the S1′ site also led us to 3 for which the central HNC\(\equiv\)O of the uracil is expected to form hydrogen bonds with the Thr26 backbone. 3,5-Dichloro analogues such as 4 were also anticipated to be viable in view of the FEP results and the expected factor-of-two benefit for binding due to the added symmetry and the predicted strong preference for the chlorine atoms in 2 and 3 to be directed inward.

**Initial Designs are Confirmed by an M\(^{\text{pro}}\) Inhibition Assay and Crystallography.** As detailed in the Supporting Information, 2, 3, and 4 were synthesized. Inhibition of proteolytic activity was tested using recombinant SARS-CoV-2 M\(^{\text{pro}}\), which was expressed and purified as previously described.\(^8,12\) For the kinetic assays, 100 nM M\(^{\text{pro}}\) in reaction buffer (20 mM Tris, 100 mM NaCl, 1 mM DTT, pH 7.3) was incubated with or without compound in DMSO at varying concentrations to a final DMSO concentration of 6% for 15 min with shaking at room temperature. The reaction was initiated by addition of substrate (Dabcyl-KTSAVLQ\(\downarrow\)SGFRKM-E(Edans-NH\(_2\)); GL Biochem) in reaction buffer, which is cleaved by M\(^{\text{pro}}\), generating a product containing a free Edans group. Fluorescence was monitored at an excitation wavelength of 360 nm and emission wavelength of 460 nm. Baseline subtraction controlled for intrinsic fluorescence of each compound as well as intrinsic fluorescence of the uncleaved FRET substrate. All tested compounds had purity of at least 95% based on HPLC, and all measurements were performed in triplicate and averaged.

As reflected in Table 1, the initial results were gratifying with IC\(_{50}\) values for 2, 3, and 4 of 10.0, 6.4, and 4.0 \(\mu\)M showing striking improvement over the >100 \(\mu\)M for perampanel (1). Both the cyanophenyl and uracilyl alternatives are viable, with a small preference for the uracil 3. Furthermore, addition of the second chlorine atom to 2 in going to 4 did provide the expected ca. factor-of-2 enhancement in inhibitory activity.

Fortunately, it was also possible to obtain a high-resolution (1.6 Å) X-ray crystal structure for the complex of 4 with SARS-CoV-2 M\(^{\text{pro}}\). As shown in Figure 3, the crystal structure fully confirmed the expectations from the modeling. There are three protein–ligand hydrogen bonds between the pyridinone oxygen and Glu166 nitrogen (2.84 Å), nitrile nitrogen and nitrogen of Cys145 (3.14 Å), and pyridine nitrogen and N\(_\epsilon\) of

![Crystal structure for the complex of 4 with M\(^{\text{pro}}\). Carbon atoms of the ligand are in yellow. Three hydrogen bonds between the ligand and protein are noted with dashed lines. Resolution is 1.6 Å. Deposited with PDB ID: 7L10.](https://dx.doi.org/10.1021/acscentsci.1c00039)

**Table 1. Measured Activities for Inhibition of SARS-CoV-2 M\(^{\text{pro}}\)**

| Cmpd | IC\(_{50}\) (\(\mu\)M) | Cmpd | IC\(_{50}\) (\(\mu\)M) | Cmpd | IC\(_{50}\) (\(\mu\)M) |
|------|----------------|------|----------------|------|----------------|
| 1    | 100–250\(^{\text{a}}\) | 11   | 0.120 ± 0.016 | 21   | 0.018 ± 0.002 |
| 2    | 9.99 ± 2.50  | 12   | 0.25 ± 0.09   | 22   | 0.036 ± 0.004 |
| 3    | 6.38 ± 1.21  | 13   | 0.19 ± 0.03   | 23   | 0.020 ± 0.005 |
| 4    | 4.02 ± 1.36  | 14   | 0.128 ± 0.015 | 24   | 0.037 ± 0.004 |
| 5    | 0.14 ± 0.02  | 15   | 0.110 ± 0.013 | 25   | 0.025 ± 0.003 |
| 6    | 0.47 ± 0.02  | 16   | 0.100 ± 0.007 | 26   | 0.170 ± 0.022 |
| 7    | 0.28 ± 0.05  | 17   | 0.110 ± 0.035 | 27   | 0.120 ± 0.006 |
| 8    | 0.51 ± 0.02  | 18   | 0.024 ± 0.007 |       |                |
| 9    | 1–10\(^{\text{b}}\) | 19   | 0.037 ± 0.007 |       |                |
| 10   | 1.20 ± 0.03  | 20   | 0.036 ± 0.003 |       |                |

\(^{a}\)Fluorescence of compound interfered with assay.
His163 (2.92 Å). In addition, a chlorophenyl edge packs well against the imidazole ring of His41 in the S2 pocket with no indication of space for expansion. The overall structure of the protein is essentially identical with that used for the original modeling (PDB ID: 5R82) with an rms deviation of 0.62 Å between the protein Cα atoms.

**Lead Optimization in S3–S4 Delivers 20 nM Inhibitors.** After this initial advance, consideration turned toward growth into the S3–S4 region (Figure 1A) to obtain increased potency. Model building and the crystal structure for 4 made it clear that it should be possible to replace the meta-chlorine near Gln189 with a variety of alkyl or alkoxy groups that would terminate in the hydrophobic S4 site. Again, FEP calculations were executed to obtain ΔΔGb values for replacing the chlorine with 11 alternatives. In all, 15 FEP calculations were executed as above to link the alternatives in sequences such as CH3OCH2 ← CH3CH2CH2 → CH3CH2O → CH3O → Cl. The resultant ΔΔGb values are reported in Table 2.

The results were promising with expectation for improvements especially with alkoxy groups containing 4 or 5 non-hydrogen atoms. In these cases, a CH3 or CH2 group is being placed in the hydrophobic S4 site. Past experience with this FEP methodology has indicated that the range of the computed ΔΔGb values is larger than observed by experiment, but that improvements in activity are almost always found when ΔΔGb is more favorable than 2–3 kcal/mol.

Thus, the propoxy 5 and methoxyethoxy 6 analogues of 4 were synthesized, and they were found to have IC50 values of 0.14 and 0.47 μM, respectively (Figure 4, Table 1). The FEP results were again nicely predictive, and the factor of ca. 30 improvement in the potency for 5 over 4 is striking. It was also possible to obtain a crystal structure for the complex of 5 with SARS-CoV-2 Mpro at 1.8 Å resolution, as shown in Figure 5. In this case, the asymmetric unit contains two Mpro monomers and two copies of 5. The binding sites are nearly identical in both copies with minor width variation in the S4 region, which may arise from crystal packing. The structure shows little change from that for 4 with the close packing of the chlorophenyl fragment and His41, and the three protein–inhibitor hydrogen bonds. The notable addition is the propoxy group, which extends to place the terminal methyl group in the hydrophobic region at the juncture of Met165, Leu167, and Pro168 in the S4 site. The electron density for 5 is very well-defined (Figure S1) and shows that the terminal OCCC dihedral angle is gauche to allow contact of the methyl group with terminal methyl groups of Met165 and Leu167. The packing in this region is illustrated in Figure 5b. It is also noted that the CCipsoOC anisole fragment is planar and directed toward Glu166, as expected from the modeling and the steric blockage for projection in the opposite direction toward Gln189 (Figure 5b).

Many other possibilities for the S3–S4 appendage were modeled by building structures of the complexes with BOMB

![Figure 4](https://dx.doi.org/10.1021/acscentsci.1c00039)
including ones that incorporated phenyl or heterocyclic rings. Both benzyloxy and phenethyloxy groups appeared promising, as illustrated in Figure 6, and synthetic access to these and substituted analogues from the common phenolic precursor was also an attractive feature. The benzyloxy analogue is predicted to replace the ethyl terminus of 5 with a phenyl edge that occupies the S4 site (Figure 6A), while the higher homologue is fully extended and shows striking face-to-face contact between Pro168 and the phenyl ring (Figure 6B). A potential drawback of the latter structure is that the backbone carbonyl of Glu166 is likely blocked from hydrogen-bonding with a water molecule, which is observed in the crystal structures for 4 and 5. In the balance, it was decided to continue with 3-pyridinyl for the S1 site. The meta-methoxy uracil analogue 10 was synthesized and did give an improvement in IC$_{50}$ to 1.2 μM from the 6.4 μM for the unsubstituted 3. The 3,5-dichloro uracil analogue corresponding to 4 was not prepared, but based on the results for 2–4, it would be expected to have an IC$_{50}$ of 2–3 μM. Thus, little benefit is apparent from changing the chlorine to a methoxy group, which is consistent with the FEP prediction in Table 2. A larger alkoxy group is needed as in 5 and 6 to extend to the S4 site. Compounds 11–13 were then prepared to explore the effects of propoxy, butoxy, and isopentoxy alternatives for the uracil series. As expected from the FEP results, the activities are significantly improved, though expansion of the alkyl ether substituent beyond the propoxy analogue 11 (0.120 μM) does not provide further benefit.

The benzyloxy uracil analogue 14 was prepared and also showed good activity with an IC$_{50}$ of 0.128 μM and there was additional gain for the phenethyloxy homologue 15 at 0.110 μM. It was expected that further progress was more likely to arise by addition of small groups at the ortho and para...
positions in 14, which might better fill the S4 site as suggested in Figure 6A. FEP calculations were carried out and predicted gains in free energy of binding of 2–3 kcal/mol for methyl, fluorine, or chlorine substituents, which is large enough to usually yield observed benefits. The monomethyl analogues 16 and 17 were prepared and did show small improvement over 14 to 0.10 and 0.11 μM, respectively, (Table 1) in spite of the expected loss from the reduced symmetry.

At this point, a crystal was obtained for the complex of 14, which was of particular interest since it was the first structure for the uracil series (Figure 7). It was gratifying to see that the corresponding analogues in the cyanophenyl series (26, 27) were also prepared, but with IC₅₀ values of 0.170 and 0.120 μM, they showed similar potency as for 5 (0.140 μM) in contrast to the 3-fold boost in the uracil series for 24 vs 11. A crystal structure for the complex of 26 with Mpro was obtained at 1.7 Å resolution (PDB ID: 7L14); it shows the C2–C3 edge of the cyclopropyl ring in close contact with Leu167, but with less ideal contact with Met165 compared to the ortho-chlorine atom in 21.

Evaluation of Antiviral Activity Against SARS-CoV-2.

To probe the series’ potential for therapeutic value, several compounds were tested for inhibition of infectious SARS-CoV-2 replication in Vero E6 cells. Protection against the viral cytopathic effect was tested in two assays, as detailed in the Supporting Information. Due to the ability to multiplex in 96-well plates and concurrently evaluate compound general cytotoxicity, a methylthiazolyl-diphenyl-tetrazolium bromide (MTT) dye²⁵ was used in the primary assay, while the more labor-intensive, lower-throughput viral plaque assay²⁶ was used to confirm antiviral activity. Previous studies have shown excellent correlation between the two assays.²⁷ In addition to the Vero E6 cells, compound cytotoxicity was also evaluated in normal human bronchial epithelial cells (NHBE) via MTT assays.²⁸ The results are summarized in Table 3. For the MTT assays, three independent measurements were performed in triplicate to yield the indicated statistical uncertainties (±1σ). The viral plaque assay, which requires serial dilutions using 6-well plates, was only performed once for each compound except 5. In that case, the results of three independent experiments provided an uncertainty of ±0.15 μM.

It was found that 5 has antiviral potency against infectious SARS-CoV-2 in both the MTT and viral titer plaque assays with EC₅₀ values of 2.5 and 1.5 μM, a little above a reported value for the SARS-CoV-2 polymerase inhibitor, remdesivir.²⁴ For 14, antiviral activity was found in the viral plaque assay, but not in the MTT assay, perhaps due to infringing cytotoxicity or compound efflux. Likewise, 21 and 23 showed activity in the viral plaque assay, but lacked antiviral activity in the MTT assay, and they showed the greatest cytotoxicity. Small modifications of the uracils should be explored to seek reduced cytotoxicity. The most auspicious results are for 26, which exhibits potency near 1 μM in both antiviral assays, and it shows no cytotoxicity to the highest concentration tested (100 μM). The closely related 27, which just replaces the cyclopropylmethoxy group in 26 with trifluoropropoxy, is more active in the MTT assay at 1.1 μM, similar to remdesivir, but it is also more cytotoxic toward the NHBE cells. Other pharmacological properties of the compounds will be

![Figure 7. Crystal structure for the complex of 14 with Mpro.](https://dx.doi.org/10.1021/acscentsci.1c00039)

### Table 3. Anti-SARS-CoV-2 Activity and Cellular Toxicity (μM)

| Compound | EC₅₀ MTT | EC₅₀ Plaque | CC₅₀ Vero E6 | CC₅₀ NHBE |
|----------|----------|-------------|--------------|-----------|
| remdesivir | 1.1 ± 0.2 | 0.77± | 72 ± 28 | 41 ± 2 |
| 5 | 2.5 ± 0.7 | 1.5 | 22 ± 7.2 | 20 ± 2 |
| 14 | NA* | 3.2* | 12.3 ± 7.0 | 17.5 ± 5.8 |
| 21 | NA* | 11.3* | 1.7 ± 0.9 | 2 ± 0.1 |
| 23 | NA* | 0.84 | 1.15 ± 0.5 | 3.5 ± 1.0 |
| 26 | 2.0 ± 0.7 | 0.98 | >100 | >100 |
| 27 | 1.1 ± 0.5 | ND* | 22 ± 8 | 25 ± 5 |

*Ref 24. NA = not active. †Drop in viral titer/incomplete inhibition. *ND = not determined.
addressed subsequently, though computed values for octanol/water partition coefficients are in acceptable ranges, e.g., 3.5–5.2 for uracils like 21 and 25, and 5.2–6.1 for cyanophenyl analogues such as 5, 26, and 27.

**Examination of Drug Synergy with Remdesivir.** A desirable feature for an antiviral drug candidate is synergistic behavior when used in combination with other antiviral agents. The major successes in treatment of HIV and Hepatitis C utilize a combination of viral polymerase and protease inhibitors. Therefore, a preliminary investigation was undertaken with 5 to assess possible synergy with the FDA-approved polymerase inhibitor, remdesivir. A replicon assay with a noninfectious SARS-CoV-2 clone and a nanoluciferase reporter were utilized to evaluate combinations of 5 and remdesivir. The inhibitory data were analyzed using MacSynergy II, a 3D model for statistical evaluation of combination assays. In this model, a simple additive effect results in a horizontal plane at 0% inhibition, whereas a synergistic or antagonistic effect will render a hill or depression above or below the plane. As shown in the 3D plot in Figure 8, a range of combinations of 5 and remdesivir do provide values above the plane. This reflects statistically significant synergistic behavior with a ratio of 30.8/0 μM² % for the mean synergy volume/antagonism volume, as detailed in the Supporting Information.

**CONCLUSION**

Our FEP-guided approach has led to a series of antiviral agents that has the potential to yield new therapies for treating SARS-CoV-2 infections. Rapid progress was made since experimental studies could only be initiated in our laboratories in June 2020. Following expression and purification of the target protein M<sup>pro</sup> and assay implementation, a virtual screen of ~2000 known drugs led to the discovery of 14 drugs with micromolar inhibitory activity. One of the weaker hits, perampanel, was chosen for redesign and optimization based on analyses of the predicted structure of its complex with M<sup>pro</sup> (Figure 1). FEP results favored selection of 3-pyridinyl and 3-chlorophenyl groups for the S1 and S2 pockets and repositioning of the pyridinone carbonyl group. These ideas were embodied in preparation of 2–4, which showed striking improvement in potency to the ca. 5 μM level. Further computational analyses combined with acquisition of multiple crystal structures for the complexes led to numerous inhibitors of M<sup>pro</sup> with ca. 20 nM potency.

The reported compounds are highly notable as inhibitors of M<sup>pro</sup> since they are structurally novel, nonpeptidic, and noncovalent with low nanomolar activity. Initial results from technically challenging antiviral cellular assays using infectious SARS-CoV-2 confirmed the promise of the series with compound 26 emerging as particularly interesting with 1 μM antiviral activity and no cytotoxicity. Moreover, initial drug combination studies show synergistic behavior of 5 and the FDA-approved drug remdesivir. The series will continue to be pursued in our laboratories, while other investigators should benefit from the reported data and crystal structures.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.1c00039.

Details for the synthetic procedures, compound characterization, computations, assays, and crystallographic results (PDF)

**AUTHOR INFORMATION**

Corresponding Authors
Karen S. Anderson — Department of Pharmacology and Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06520-8066, United States; orcid.org/0000-0003-3433-0780; Email: karen.anderson@yale.edu
Yale University has submitted a preliminary patent application on the reported new compounds.

**ACKNOWLEDGMENTS**

The Molarity<sup>®</sup> plasmid was kindly provided by the Hilgenfeld lab. This work was supported by the U.S. National Institutes of Health (GM32136, AI087925, T32GM136651) and by CoRECT Pilot Grants from the Yale University School of Medicine. E.A.S. acknowledges support for the NSF Graduate Research Fellowship Program. S.J.M. acknowledges support from the U.S. National Institutes of Health (R35 GM132092). Crystal screening was conducted with support from the Yale Macromolecular X-ray Core Facility (1S10OD018007-01). This research used resources AMX of the National Synchrotron Light Source II, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Brookhaven National Laboratory under contract no. DE-SC0012704. The Life Science Biomedical Technology Research resource is primarily supported by the National Institute of Health, National Institute of General Medical Sciences (NIGMS) through a Center Core P30 Grant (P30GM133893), and by the DOE Office of Biological and Environmental Research (KP1605010). This work is also based upon research conducted at the Northeastern Collaborative Access Team beamlines, which are funded by the National Institute of General Medical Sciences from the National Institutes of Health (P30 GM124165). The Eiger 16M detector on the 24-ID-E beamline is funded by a NIH-ORIP HEI grant (S10OD021527). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under contract no. DE-AC02-06CH11357. This work is dedicated to E.J.S. and all individuals lost during the COVID-19 pandemic.

**REFERENCES**

1. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.-M.; Wang, W.; Song, Z.-G.; Hu, Y.; Tao, Z.-W.; Tian, J.-H.; Pei, Y.-Y.; Yuan, M.-J.; Zhang, Y.-L.; Dai, F.-H.; Liu, Y.; Wang, Q.-M.; Zheng, J.-J.; Xu, L.; Holmes, E. C.; Zhang, Y.-Z. A New Coronavirus Associated with Human Respiratory Disease in China. *Nature* 2020, 579, 265–269.

2. Morse, J. S.; Lalonde, T.; Xu, S.; Liu, W. R. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. *ChemBioChem* 2020, 21, 730–738.

3. Dömling, A.; Gao, L. Chemistry and Biology of SARS-CoV-2. *Chem. 2020*, 6, 1283–1295.

4. Pillay, T.; Manickam, M.; Namavivayam, V.; Hayashi, Y.; Jung, S.-H. An Overview of Severe Acute Respiratory Syndrome—Coronavirus (SARS-CoV) 3CL Protease Inhibitors: Peptidomimetics and Small Molecule Chemotherapy. *J. Med. Chem.* 2016, 59, 6595–6628.

5. Ghosh, A. K.; Brindisi, M.; Shahabi, D.; Chapman, M. E.; Mesecar, A. D. Drug Development and Medicinal Chemistry Efforts toward SARS-CoV-2 and Covid-19 Therapeutics. *ChemMedChem* 2020, 15, 907–932.

6. Jin, Z.; Du, X.; Xu, Y.; Deng, Y.; Liu, M.; Zhao, Y.; Zhang, B.; Li, X.; Zhang, L.; Peng, C.; Duan, Y.; Yu, J.; Wang, L.; Yang, K.; Liu, F.; Jiang, R.; Yang, X.; You, T.; Liu, X.; Yang, X.; Bai, F.; Liu, H.; Liu, X.; Guddat, L. W.; Xu, W.; Xiao, G.; Qin, C.; Shi, Z.; Jiang, H.; Rapp, U.; Yang, H. Structure of Mpro from SARS-CoV-2 and Discovery of Its Inhibitors. *Nature* 2020, 582, 289–293.

7. Jin, Z.; Zhao, Y.; Sun, Y.; Zhang, B.; Wang, H.; Wu, Y.; Zhu, Y.; Zhu, C.; Hu, T.; Du, X.; Duan, Y.; Yu, J.; Yang, X.; Yang, X.; Yang, K.;
Liu, X.; Guddat, L. W.; Xiao, G.; Zhang, L.; Yang, H.; Rao, Z. Structural Basis for the Inhibition of SARS-CoV-2 Main Protease by Antineoplastic Drug Carmofur. *Nat. Struct. Mol. Biol.* 2020, 27, 529–532.

(8) Zhang, L.; Lin, D.; Sun, X.; Curth, U.; Drosten, C.; Sauerhering, L.; Becker, S.; Rox, K.; Hilgenfeld, R. Crystal Structure of SARS-CoV-2 Main Protease Provides a Basis for Design of Improved α-Ketoamide Inhibitors. *Science* 2020, 368, 409–412.

(9) Anson, B.; Mesecar, A. X-Ray Structure of SARS-CoV-2 Main Protease Bound to Boceprevir at 1.45 Å; PDB ID: 6WNP; Worldwide Protein Data Bank. DOI: 10.2210/pdb6wnp/pdb.

(10) Dai, W.; et al. Structure-based Design of Antiviral Drug Candidates Targeting the SARS-CoV-2 Main Protease. *Science* 2020, 368, 1331–1335.

(11) Ma, C.; Sacco, M. D.; Hurst, B.; Townsend, J. A.; Hu, Y.; Szeto, T.; Zhang, X.; Tarbet, B.; Marty, M. T.; Chen, Y.; Wang, J. Boceprevir, GC-376, and Calpain Inhibitors II, XII Inhibit SARS-CoV-2 Viral Replication by Targeting the Viral Main Protease. *Cell Res.* 2020, 30, 678–692.

(12) Gahramanpour, M. M.; Tirado-Rives, J.; Deshmukh, M.; Ippolito, J. A.; Zhang, C.-H.; Cabeza de Vaca, I.; Liosi, M.-E.; Anderson, K. S.; Jorgensen, W. L. Identification of 14 Known Drugs as Inhibitors of the Main Protease of SARS-CoV-2. *ACS Med. Chem. Lett.* 2020, 11, 2526–2533.

(13) Jorgensen, W. L. Efficient Drug Lead Discovery and Optimization. *Acc. Chem. Res.* 2009, 42, 724–733.

(14) Wang, L.; Wu, Y.; Deng, Y.; Kim, B.; Pierce, L.; Krilov, G.; Lupyan, D.; Robinson, S.; Dahlgren, M. K.; Greenwood, J.; et al. Accurate and Reliable Prediction of Relative Protein-Ligand Binding Affinities via Free Energy Calculations: Validation in Prospective Drug Discovery. *J. Am. Chem. Soc.* 2015, 137, 2695–2703.

(15) Jorgensen, W. L. Computer-Aided Discovery of Anti-HIV Agents. *Biosorg. Med. Chem.* 2016, 24, 4768–4788.

(16) Cournia, Z.; Allen, B.; Sherman, W. Relative Binding Free Energy Calculations in Drug Discovery: Recent Advances and Practical Considerations. *J. Chem. Inf. Model.* 2017, 57, 2911–2937.

(17) Song, L. F.; Merz, K. M., Jr. Evolution of Alchemical Free Energy Methods in Drug Discovery. *J. Chem. Inf. Model.* 2020, 60, 5308–5311.

(18) Jorgensen, W. L.; Tirado-Rives, J. Molecular Modeling of Organic and Biomolecular Systems Using BOSS and MCPRO. *J. Comput. Chem.* 2005, 26, 1689–1700.

(19) Robertson, M. J.; Tirado-Rives, J.; Jorgensen, W. L. Improved Peptide and Protein Torsional Energetics with the OPLS-AA Force Field. *J. Chem. Theory Comput.* 2015, 11, 3499–3509.

(20) Jorgensen, W. L.; Tirado-Rives, J. Potential Energy Functions for Atomic-Level Simulations of Water and Organic and Biomolecular Systems. *Proc. Natl. Acad. Sci. U. S. A.* 2005, 102, 6665–6670.

(21) Dodda, L. S.; Cabeza de Vaca, I.; Tirado-Rives, J.; Jorgensen, W. L. LigParGen Web Server: An Automatic OPLS-AA Parameter Generator for Organic Ligands. *Nucleic Acids Res.* 2017, 45, 331–336.

(22) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* 1983, 79, 926–935.

(23) Douangamath, A.; Fearon, D.; Gehrzl, P.; Krojer, T.; Lukacik, P.; Owen, C. D.; Resnick, E.; Strain-Damerell, C.; Aimon, A.; Abrányi-Balogh, P.; Brandão-Neto, J.; Carbery, A.; Davison, G.; Dias, A.; Downes, T. D.; Dunnett, L.; Fairhead, M.; Firth, I. D.; Jones, S. P.; Keeler, A.; Kester, G. M.; Klein, H. F.; Martin, M. P.; Noble, M. E. M.; O’Brien, P.; Powell, A.; Redd, R. N.; Skymyer, R.; Snee, M.; Waring, M. J.; Wild, C.; London, N.; von Delft, F.; Walsh, M. A. Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease. *Nat. Commun.* 2020, 11, 5047.

(24) Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M.; Shi, Z.; Hu, Z.; Wong, W.; Xiao, G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020, 30, 269–271.

(25) De Meyer, S.; Bojkova, D.; Cinatl, J.; Van Damme, E.; Buyck, C.; Van Loock, M.; Woodfall, B.; Ciesek, S. Lack of antiviral activity of darunavir against SARS-CoV-2. *Int. J. Infect. Dis.* 2020, 97, 7–10.

(26) Mendoza, E. J.; Manguit, K.; Wood, H.; Dreibot, M. Two detailed plaque assay protocols for the quantification of infectious SARS-CoV-2. *Current Protocols in Microbiology* 2020, 57, e105.

(27) Azzaroli, F.; Montagnani, M.; Porro, A.; Fiorillo, D.; Mazzei, G. The Future of Dual Therapy for Hepatitis C Virus. *Future Virol.* 2014, 9, 905–912.

(28) Baril, J.-G.; Angel, J. B.; Gill, M.; Gathe, J.; Cahn, P.; van Wyk, J.; Walsmsley, S. Dual Therapy Treatment Strategies for the Management of Patients Infected with HIV: A Systematic Review of Current Evidence in ARV-Naive or ARV-Experienced, Virologically Suppressed Patients. *PLoS One* 2016, 11, e0148231.

(29) Kudalkar, S. N.; Beloer, J.; Quijano, E.; Spasov, K. A.; Lee, W.-G.; Cisneros, J. A.; Saltzman, W. M.; Kumar, P.; Jorgensen, W. L.; Anderson, K. S. From silico hit to long-acting late-stage preclinical candidate to combat HIV-1 infection. *Proc. Natl. Acad. Sci. U. S. A.* 2018, 115, E802–E811.