In just the past seven years, the world has experienced three major pandemics, all stemming from different viruses, namely, the Ebola virus, the Zika virus, and the SARS-CoV-2 virus. Of these, COVID-19 (caused by the SARS-CoV-2 virus) has been by far the most devastating, with deaths and illnesses that now approach those observed during the first year of the 1918 Spanish flu pandemic. The early stages of COVID-19 infection are dominated by host cell invasion mediated by binding of the SARS-CoV-2 S protein to cells containing the angiotensin-converting enzyme 2 (ACE2) surface receptor protein. While in COVID-19, the primary infection occurs on the tiny air sacs in the lungs (alveoli), the ACE2 protein is ubiquitous to many human tissues, and this can result in additional sites of infection. Although some infected patients remain asymptomatic throughout the course of the disease, most begin to exhibit symptoms within 5–7 days after being infected, and a subset of those progress to a variety of severe symptoms (e.g., difficulty breathing (dyspnea), low oxygen (hypoxia)) or even critical symptoms (e.g., respiratory failure, shock, or multiorgan dysfunction) brought on by an immune-mediated inflammatory response. To combat this effectively, we clearly need drugs to treat infected individuals and vaccines to protect uninfected individuals.

Interestingly, despite a full century difference between the Spanish Flu pandemic and COVID-19, the primary mitigation source for the disease has remained the same, i.e., mask wearing. However, unlike the Spanish Flu era, this time research groups from around the world have developed a myriad of vaccines that illicit antibody and T-cell immunity, no doubt accelerated by research accomplished after the SARS-CoV-1 outbreak in 2002–2003 and the Middle Eastern Respiratory Syndrome (MERS) outbreak in 2008. At present, at least nine vaccines have received emergency regulatory approvals in various countries around the world. These vaccines are based on various S protein-related factors, including viral DNA (e.g., the Oxford-Astra Zeneca vaccine), RNA (e.g., the Pfizer and Moderna vaccines) and protein subunits, delivered by replicating and nonreplicating...
viral vectors (e.g., the Johnson & Johnson vaccine), as well as inactivated and weakened viruses (e.g., the Sinovac Biotech vaccine) and virus-like particles. While these vaccines can in many cases provide substantial protection against wildtype SARS-CoV-2, they appear less effective against several new variants (e.g., the S01Y.V2 or the “South African” variant) that have emerged.

Given the shortcomings of vaccines against some troublesome SARS-CoV-2 variants, direct-acting antiviral (DAA) drugs, especially when used in synergistic combinations, could provide an important complement to vaccines (Figure 1).

These therapeutic agents can be put to good effect not only to suppress disease progression in patients with an active infection but can also be used prophylactically to minimize new infections for high-risk, uninfected individuals. In that regard, in 2020, the U.S. Food and Drug Administration (FDA) granted Emergency Use Authorization (EUA) to several therapies including remdesivir, casirivimab/imdevimab, baricitinib/remdesivir, bamlanivimab, and convalescent plasma. Unfortunately, however, all of these medications require intravenous administration in a hospital setting, which is somewhat suboptimal given that by the time COVID-19 patients are hospitalized, many have progressed beyond the stage where viral replication is the dominant morbidity, and the most efficacious time to employ DAA agents has passed.

In this issue of ACS Central Science, Zhang et al. describe a study involving the optimization of a SARS-CoV-2 main protease (Mpro) inhibitor. The lead compound was derived from a panel of 14 previously found but poorly effective inhibitors of the SARS-CoV-2 Mpro. From this panel, perampanel, whose IC50 was in the high micromolar range, was chosen for redesign and optimization since modeling studies predicting its binding mode, as well as its simple structure, rendered it attractive for further development. The optimization process relied heavily on structural information obtained from molecular modeling, and multiple crystal structures acquired en route provided the necessary verification that the modeling hypotheses were accurate.

Of particular note was the authors’ use of free energy perturbation (FEP) calculations, a method based on statistical mechanics that computes free energy differences using molecular dynamics simulations. The article clearly lays out the logical progression of the study that ultimately resulted in the identification of several compounds with IC50 values in the 20 nM range, representing a vast improvement in activity relative to the starting point, perampanel.

As a consequence of the COVID-19 pandemic, the identification of effective anti-SARS-CoV-2 agents is very relevant and timely. Indeed, many labs are attempting to develop DAAs that could suppress viral replication in COVID-19-positive patients. However, two factors differentiate this particular study from many of the others that have thus far worked toward the design of novel SARS-CoV-2 Mpro inhibitors. First, the authors have moved away from the traditional covalent (or even reversibly covalent) inhibitors that represent the bulk of research in this area. Since many human proteins contain nucleophilic residues that can covalently capture potent electrophiles, e.g., unsaturated carbonyl derivatives, α-halo ketones, α-ketoesters, amides, etc., covalent inhibitors oftentimes exhibit significant toxicities, and this would, as a consequence, limit the likelihood that they could be developed as potential DAAs. Second, the authors have completely avoided the traditional use of peptidomimetics (i.e., compounds that have peptide-like structures) to inhibit this protease. Peptidomimetics, which oftentimes can exhibit significant potency, usually have poor proteolytic stability in vivo as well as poor oral bioavailability. The latter quality is particularly undesirable for SARS-CoV-2 DAAs, since antiviral agents that must be given intravenously can only be administered in a hospital setting, and COVID-19 ICUs are currently overflowing with patients. By contrast, drug candidates that have the potential of being administered orally can be prescribed for infected individuals to reduce their viral loads (and likely the severity of the
subsequent inflammatory component of the disease) while they are in quarantine, as well as prophylactically for uninfected individuals who might reside in the same household. Thus, the approach taken in this study is important because it will likely afford compounds with significantly decreased toxicity that can, if necessary, be further modified structurally to display good bioavailability.

The initial hit compound, perampanel, was really a poorly effective inhibitor of the SARS-CoV-2 MPro, and most researchers would have likely abandoned the scaffold. However, following an initial docking of the compound, some very clever (by eye) changes were evaluated and corroborated using FEP calculations (Figure 2), thereby providing valuable guidance for the synthetic campaign that ultimately resulted in the identification of several highly potent inhibitors. The most effective of these new scaffolds was almost as potent in an antiviral assay as the nucleoside analogue remdesivir, which has received emergency use authorization from the FDA. In addition, studies of the most promising SARS-CoV-2 MPro inhibitor used in conjunction with remdesivir indicated a synergistic effect between the two agents, suggesting the possibility that they could be employed as a combination therapy, which most likely would not only increase the overall effectiveness of the treatment, but would also lower the risk of observing the development of resistance.

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**Notes**

The authors declare no competing financial interest.

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