A small compound 5h blocks the infectivity, replication, and cytopathicity of SARS-CoV-2 via inhibition of main protease

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, Hubei province, China and escalated into a pandemic in May 2020 [1]. As of early in 2021, except for remdesivir (RDV), which was approved as the first proven emergency therapeutic for treating COVID-19, no specific therapeutics were available. The hope that the COVID-19 pandemic subsides with "herd immunity" has been likely to be disappointing. Moreover, COVID-19-convalescent people carrying antibodies to SARS-CoV-2 are still susceptible to reinfection. It is of utmost urgency to develop effective antivirals that mitigate the lethal consequences of the disease, as well as effective vaccines. In this research, we synthesized and identified two novel antiviral small molecule compounds containing indole moiety against SARS-CoV-2 infection [2].

Based on the fact that the main protease (M^{pro}), which processes virus polypeptides of SARS-CoV-2 has an extensive homology (~96%) with the M^{pro} of SARS-CoV, we first selected a panel of previously reported (most in 2000s) compounds that had been reported to be active against SARS-CoV by targeting its M^{pro} [3]. Moreover, we have designed and synthesized novel compounds which might exert potent activity against SARS-CoV-2 [2]. We have now identified two small molecule compounds, GRL-1720 and 5h, both of which target the M^{pro} of SARS-CoV-2 and potently block the infectivity, replication, and cytopathicity of SARS-CoV-2 (Fig. 1). As assessed using the quantitative VeroE6 cell-based assay with RNA-qPCR, the 50% of effective concentration (EC_{50}) values of GRL-1720 and 5h were 15±4 and 4.2±0.7 μM, respectively, and apparent 50% of cytotoxic concentration (CC_{50}) values, which were determined with WST-8 assay, were both >100 μM (Fig. 1). Compound 5h exserted greater antiviral activity than GRL-1720, hence we used 5h for further analyses.

RDV and 5h blocked the infectivity of SARS-CoV-2 through acting as a viral RNA polymerase inhibitor [4] and as an M^{pro} inhibitor, respectively. Thus, we asked whether these two compounds worked against SARS-CoV-2 in an additive or synergistic fashion using VeroE6 cell-based assays (Fig. 2). The cells were exposed to SARS-CoV-2 and cultured in the presence or absence of various concentrations of RDV plus 5h. 4 μM of RDV and 4 μM of 5h suppressed the viral replication by 0.53-fold and 2.0-fold, respectively, while when combined, the suppression was apparently synergized by 24-fold. The apparent combination effect was maximal when RDV and 5h were combined at 20 μM, producing the suppression by as much as 1.6×10^6-fold. When examined using the Bliss additivism method, combination of 5h and RDV exerts at least additive effect. Moreover, a synergistic effect was present at 1 μM each of 5h and RDV and the synergism reached the maximum at 10 μM combination of the two compounds. As in the case of antiretroviral therapy of HIV-1 infection, in which

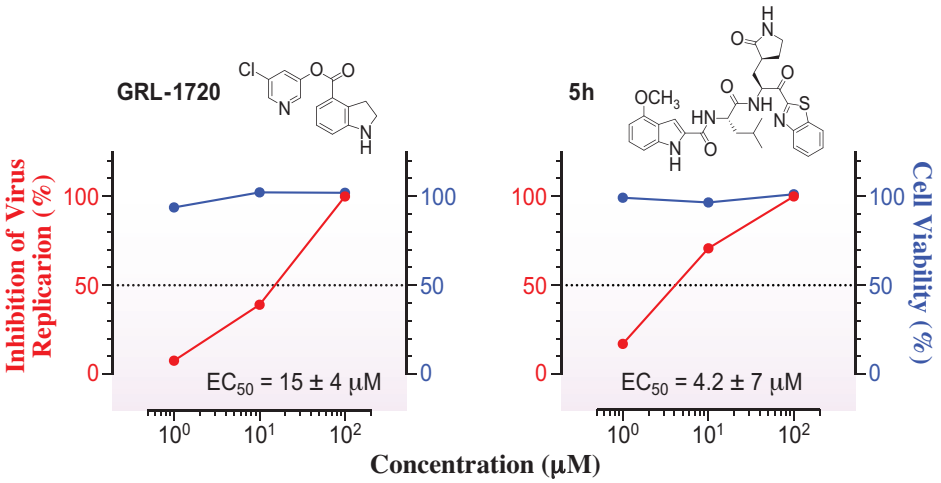

Fig. 1. The antiviral activity of GRL-1720 and 5h against SARS-CoV-2. VeroE6 cells were exposed to SARS-CoV-2 and the cells were cultured for 3 days in the presence of GRL-1720 (left) or 5h (right). The viral copy numbers in the culture supernatants were determined using RT-qPCR. Red and blue lines indicate percent reduction of viral copy numbers and cell viability, respectively. All assays were performed in duplicate.

the use of one or two reverse transcriptase inhibitors and an HIV-1 protease or integrase inhibitor results in highly favorable antiretroviral effects [5], such a combination might give much more favorable efficacy than RDV or 5h alone.

To understand the molecular basis of the inhibition of SARS-CoV-2's M^{pro} by 5h, we determined the X-ray structure of M^{pro} in complex with 5h at 1.25 Å resolution. X-ray data were collected at SPring-8 **BL41XU**. Detailed molecular interactions with M^{pro} are shown in the Figs. 3(a-d). 5h fully occupies the binding pocket (Fig. 3(a)) and is stabilized by 6 direct hydrogen bonds with the residues inside the binding groove of M^{pro}. Particularly, Glu-166 engages in two-hydrogen-bond formation, linking the main chain carbonyl and the amide group of Glu-166 (Fig. 3(b)). In the central part of 5h, additional hydrogen bonds form with the side-chain oxygen of Gln-189 and the main chain carbonyl of His-163 (Figs. 3(b-c)). Since the majority of hydrogen bonds form through the main chain carbonyl and NH groups of M^{pro}, those interactions are less likely affected by potential mutations, although whether drug-resistant mutants emerge is an open question and more studies are required. Overall, the chemical composition of 5h matches well with the surface of the binding groove in terms of the hydrophobicity scale. The observed continuous electron density of the tetrahedral ketal and the sulfur atom of Cys-145 indicates the formation of a covalently bond between 5h and M^{pro} (Fig. 3(d)). The sulfur atom of Cys-145 undergoes nucleophilic addition reaction and forms a covalent bond with the carbonyl carbon (–C=O) next to the benzothiazole of 5h, resulting in the conversion of the carbonyl to an alcohol (–C–OH) and to the formation of one direct hydrogen bond and water-mediated hydrogen bond interactions around the three oxyanion hole residues, Cys-145 and Gly-143 (Fig. 3(d)).

In conclusion, 5h, which shows potent antiviral activity and no significant detectable cytotoxicity, represents a lead compound to develop more potent anti-SARS-CoV-2 agents. Moreover, the combination therapy using different class of agents, such as an M^{pro} inhibitor and an RdRp inhibitor, might be a promising therapeutic modality for treatment of SARS-CoV-2 infection.

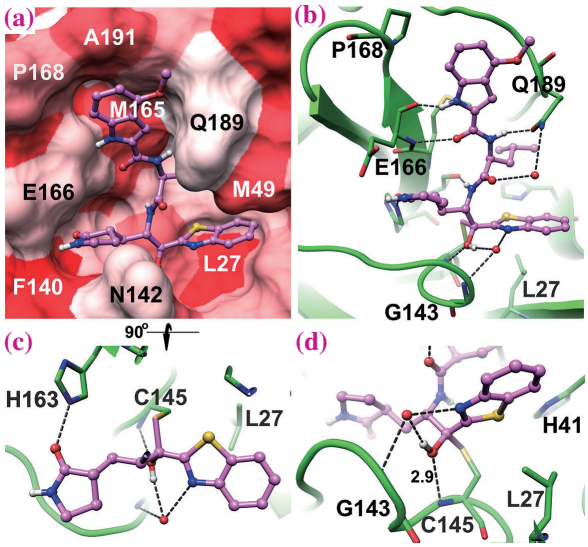

Fig. 3. The X-ray crystal structure of SARS-CoV-2 M^{pro} in complex with 5h. **(a)** Hydrophobicity of the binding pocket is represented by the intensities of red color, hydrophobic residues are shown in dark red, whereas polar or charged residues are shown in light red. **(b)** Hydrogen bond interactions between 5h (pink carbon atoms) and M^{pro} (green carbon atoms) are shown in black dash lines. **(c)** A 90°-rotated view of 5h focuses on the interactions of 2-oxopyrrolidine and benzothiazole groups. **(d)** The hydroxyl group of 5h is shown in the center. Distances between atoms are shown in Å.

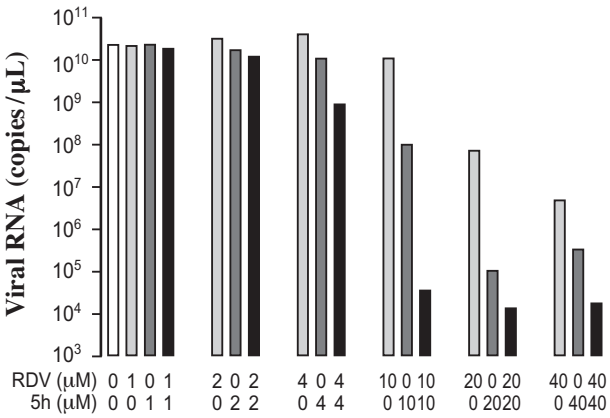

Fig.2. 5h combined with RDV does not permit viral breakthrough. Viral RNA copy numbers in the culture supernatants which VeroE6 cells were exposed to SARS-CoV-2 in the presence of compounds were determined using RNA-qPCR. Bars indicate geometric mean (n=2).

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