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Development of ciclesonide analogues that block SARS-CoV-2 RNA replication

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Abstract
Ciclesonide is an inhaled corticosteroid used to treat asthma and is currently undergoing clinical trials for treatment of coronavirus disease 2019 (COVID-19). An active metabolite of ciclesonide, Cic2, was recently reported to repress severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) genomic RNA replication. Herein, we designed and synthesized a few types of ciclesonide analogues. Cic4 (bearing an azide group) and Cic6 (bearing a chloro group) potently decreased SARS-CoV-2 viral replication and had low cytotoxicity compared with Cic2 (bearing a hydroxy group). These compounds are promising as novel therapeutic agents for COVID-19 that show significant antiviral activity.

Introduction
At the end of 2019, an outbreak of coronavirus disease 2019 (COVID-19) occurred in Wuhan city and rapidly spread across the world. The causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has produced more than 117 million infections and 2.5 million deaths as of March 2021. Numerous therapies and vaccines for SARS-CoV-2 infection are currently being developed around the world, and several drugs have been suggested to be effective for treatment of COVID-19. Antiviral drugs commonly work by inhibiting proteases, viral polymerases or nucleases. Remdesivir has been approved in some countries for treatment of COVID-19, and decreases viral replication by inhibiting the polymerase. Although no nuclease inhibitors against SARS-CoV-2 have yet been approved for COVID-19 treatment, it was recently reported that ciclesonide (Cicle), an approved drug for treatment of steroidal asthma, is its promising candidate. Cicle activity depends on hydrolysis by esterases to yield the active metabolite Cic2 (Fig. 1), and Cic2 was reported to repress SARS-CoV-2 genomic RNA replication.

In this report, we investigated the potential of Cic2 derivatives with the expectation that replacing the primary hydroxy group of the active metabolite with other functional groups Cic3–Cic10 would improve efficacy. In addition, viral growth inhibition and cytotoxicity of the synthesized analogues were evaluated.

The synthetic route for those analogues is shown in Scheme 1. Briefly, Cic2 with was converted to the mesylated Cic3. Then, Cic4 (bearing an azide group) and Cic10 (bearing an amino group) were obtained from Cic3. The analogue Cic5, in which the ester group of Cic1 was replaced with an amide group, was synthesized from Cic10. The compounds Cic6 (bearing a chloro group), Cic7 (bearing a bromo group), and Cic8 (bearing an iodo group) were synthesized from the mesylated Cic3. The Cic9 analogue, bearing a dimethylamino group, was prepared by treating Cic7 with dimethylamine.

The synthesized compounds were incubated with Vero E6/TMPRSS2 cells for 18 h and cytotoxicity was evaluated via release of lactate dehydrogenase (LDH). The prodrug Cicle, Cic4 (bearing an azide group), Cic5 (an amide-type Cicle), and Cic6 (bearing a chloro group) did not show significant cytotoxicity at concentrations up to 40 μM. In contrast, the active metabolite Cic2, Cic3 (bearing a methanesulfonyl group), and Cic10 (bearing an amino group) were cytotoxic at concentrations above 20 μM. Cic7 (bearing a bromo group), Cic8 (bearing an iodo group), and Cic9 (bearing a dimethyl amino group) were cytotoxic at concentrations above 20 μM. Cic7 (bearing a bromo group), Cic8 (bearing an iodo group), and Cic9 (bearing a dimethyl amino group) were cytotoxic at concentrations above 20 μM. Cic7 (bearing a bromo group), Cic8 (bearing an iodo group), and Cic9 (bearing a dimethyl amino group) were cytotoxic at concentrations above 20 μM.

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of 40 µM (Fig. 2). The prodrug Cic10 and its amide analogue Cic5, as well as the less reactive Cic4 (bearing an azide group), Cic9 (bearing a dimethylamino group), and Cic6–Cic8 with halogen atoms tended to be less cytotoxic. On the other hand, Cic2 (bearing a hydroxy group) and Cic10 (bearing an amino group) with proton donors and the O-sulfo-nylated Cic3 tended to show slightly higher cytotoxicity.

Next, Vero E6/TMPRSS2 cells were infected with SARS-CoV-2 JPN/TY/WK-521 strains for 18 h in medium containing different concentrations of these compounds. As shown in Fig. 4, Cic10, Cic2, Cic4, and Cic6 showed concentration-dependent inhibition of viral growth. The concentrations of Cic10 and Cic2 required to reduce viral titres by 1000-fold compared with control dimethyl sulfoxide treatment (approximately 4.6 × 10^5 plaque-forming units/mL) were greater than 80 µM for Cic10 and 60.95 µM for Cic2. By contrast, Cic4 and Cic6 were able to reduce viral titres by 1000-fold at concentrations of 7.36 µM and 5.95 µM, respectively, indicating that Cic4 and Cic6 were more effective in inhibiting viral growth than Cic10 or Cic2.

In conclusion, we developed Cic10 analogues (Cic3–Cic10) that inhibited SARS-CoV-2 genomic RNA replication and growth. In the Cic3–Cic10 analogues, the primary hydroxy group of Cic2, the active metabolite of the prodrug Cic10, was replaced with various functional groups. Among the synthesized analogues, Cic4 (bearing an azide group) and Cic6 (bearing a chloro group) showed potent reduction of viral replication and low cytotoxicity compared with Cic2. The low cytotoxicity and strong antiviral activity of these compounds is a new discovery, and we hope that the results of our study will serve as a stepping stone for the development of effective therapeutics against SARS-CoV-2 and other viruses. The detailed structure–activity relationship between Cic4 and Cic6 is difficult to describe at this stage because target molecules (proteins) of Cic10 compounds are not yet found. However, Cic4 and Cic6 are less polar than Cic2, Cic9, and Cic10, therefore, they may enter the cells more easily. Cic4 and Cic6 are also less reactive than Cic3, Cic7, and Cic8, so they may be less degraded and less likely to react with intracellular molecules. Recently, it was reported that Ciclet inhibited SARS-CoV-2 replication but resistance mutations mapped to viral nonstructural protein 3 (nsp3) and nsp4. Considering the report, further biological assays are needed to understand the potent activity of the ligands Cic4 and Cic6, including search for their target proteins and evaluation of their binding activity, and evaluation of their chemical stability. Elucidation of those evaluation and further structural expansion starting from the azide group of Cic4 (e.g., using click chemistry) are underway.
Fig. 2. LDH toxicity of the synthesized Cicle analogues against Vero E6/TMPRSS2 cells. LDH, lactate dehydrogenase. Each independent experiment was done in triplicate.

Fig. 3. Evaluation of viral RNA levels in Vero E6/TMPRSS2 cells at 18 h after SARS-CoV-2 infection in the presence of Cicle and Cicle analogues. DMSO, dimethyl sulfoxide. Each independent experiment was done in triplicate.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128052.

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Fig. 4. SARS-CoV-2 growth inhibition by Cic1, Cic2, Cic4, and Cic6. DMSO, dimethyl sulfoxide; PFU, plaque-forming unit. Each independent experiment was done in triplicate.