



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 2021.¹ 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-19.² 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.³ 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.^{4,5} **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.⁴

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** was converted to the mesylated **Cic3**.⁵ 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**.⁵ 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).⁶ 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 μ M. In contrast, the active metabolite **Cic2**, **Cic3** (bearing a methanesulfonyl group), and **Cic10** (bearing an amino group) were cytotoxic at concentrations above 20 μ M. **Cic7** (bearing a bromo group), **Cic8** (bearing an iodo group), and **Cic9** (bearing a dimethyl amino group) were cytotoxic at concentrations

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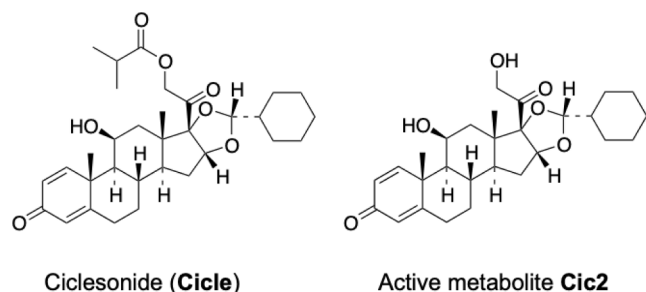


Fig. 1. Chemical structures of ciclesonide (Cicle) and its active metabolite Cic2.

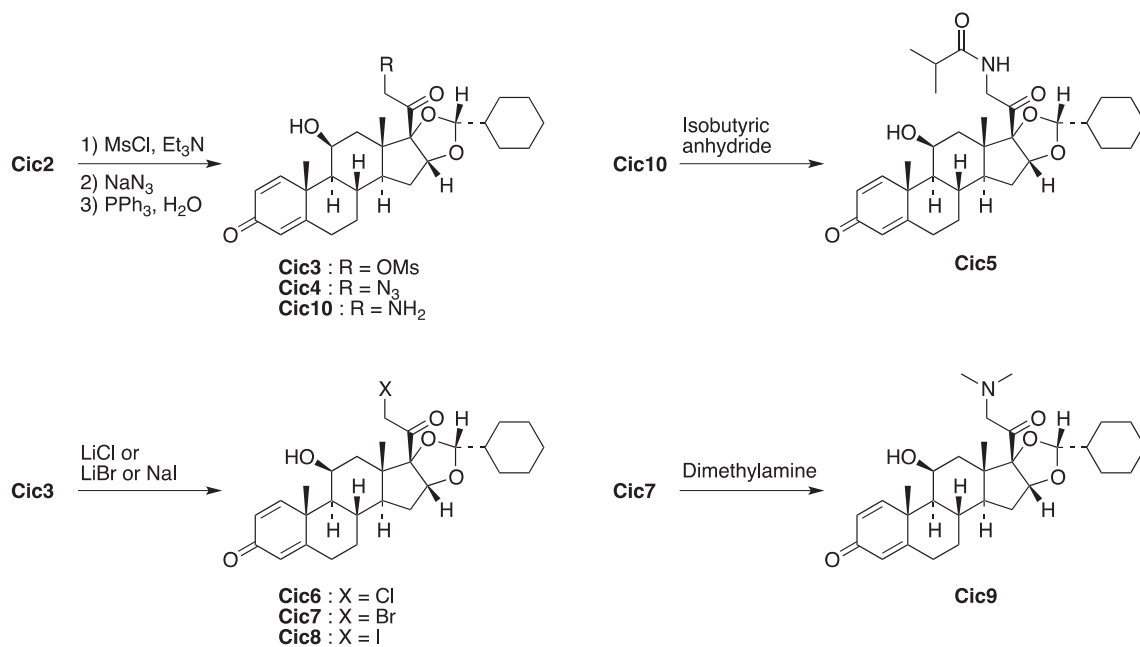
of 40 μM (Fig. 2). The prodrug Cicle and its amide analogue Cic5, as well as the less reactive Cic4 (bearing an azide group), Cic9 (bearing a dimethylamino group), and Cic6–Cic8 with halogen atoms tended to be less cytotoxic. On the other hand, Cic2 (bearing a hydroxy group) and Cic10 (bearing an amino group) with proton donors and the *O*-sulfonylated 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 10 μM of each compound. Viral growth inhibition was assessed by measuring the amount of viral RNA in supernatants using quantitative reverse transcription-PCR.⁶ In the absence of any compounds, supernatants contained 4.8×10^{10} viral RNA copies/well. In the presence of Cicle, viral RNA in supernatants was significantly reduced to 5.2×10^8 copies/well. Cic4 and Cic6, which showed no cytotoxicity (Fig. 2), had more pronounced effects on reduction of the viral RNA than Cicle (2.1×10^5 copies/well and 4.6×10^5 copies/well, respectively, Fig. 3). The compounds Cic2, Cic3, and Cic7 also showed significant reductions of viral RNA levels compared with Cicle (Fig. 3), but Cic3 and Cic7 except for the active metabolite Cic2 were excluded from subsequent evaluations because of their cytotoxicity at high concentrations (Fig. 2).

To further evaluate the inhibitory effects of Cic4 and Cic6 on viral growth, viral titres in cell supernatants were assessed using plaque

assays following infection with SARS-CoV-2 JPN/TY/WK-521 strains for 18 h in media containing different concentrations of these compounds.⁶ As shown in Fig. 4, Cicle, Cic2, Cic4, and Cic6 showed concentration-dependent inhibition of viral growth. The concentrations of Cicle 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 Cicle 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 Cicle or Cic2.

In conclusion, we developed Cicle analogues (Cic3–Cic10) those 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 Cicle, 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 Cicle 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 Cicle inhibited SARS-CoV-2 replication but resistance mutations mapped to viral nonstructural protein 3 (nsp3) and nsp4.⁴ 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.



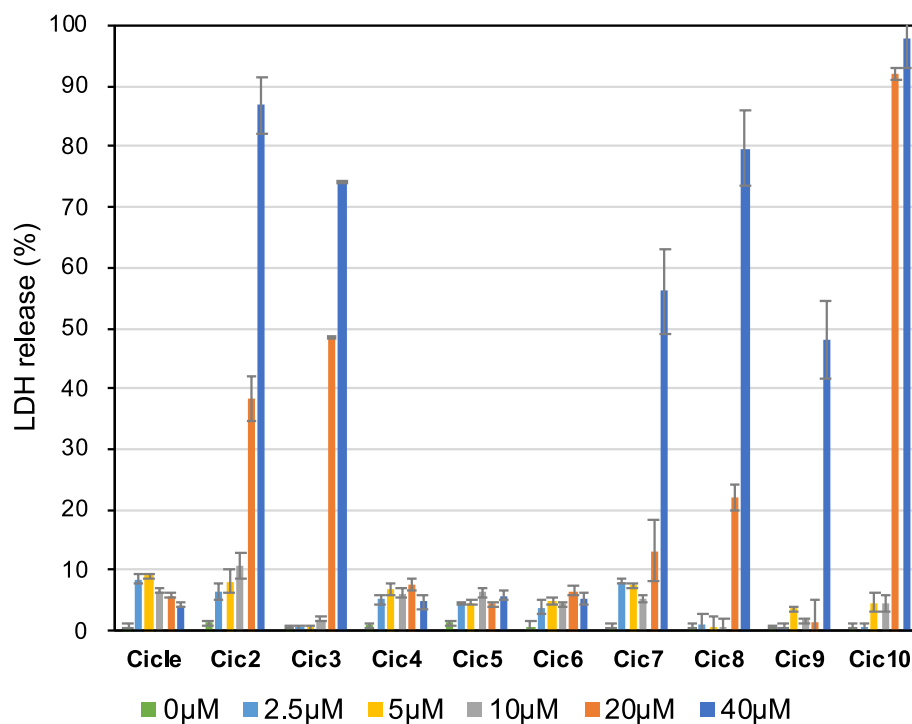


Fig. 2. LDH toxicity of the synthesized **Cicle** analogues against Vero E6/TMPRSS2 cells. LDH, lactate dehydrogenase. Each independent experiment was done in triplicate.

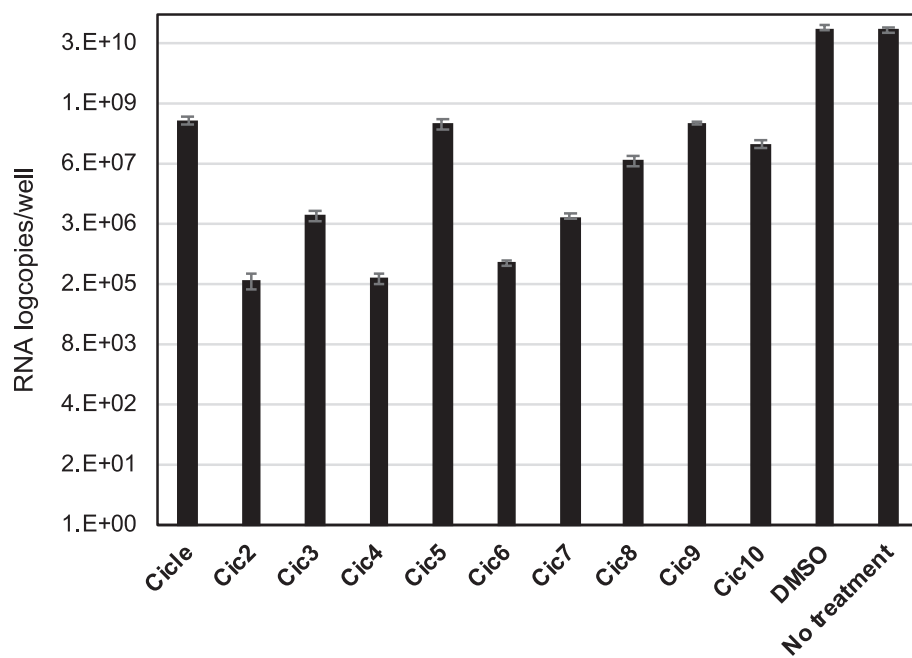


Fig. 3. Evaluation of viral RNA levels in Vero E6/TMPRSS2 cells at 18 h after SARS-CoV-2 infection in the presence of **Cicle** and **Cicle** analogues. DMSO, dimethyl sulfoxide. Each independent experiment was done in triplicate.

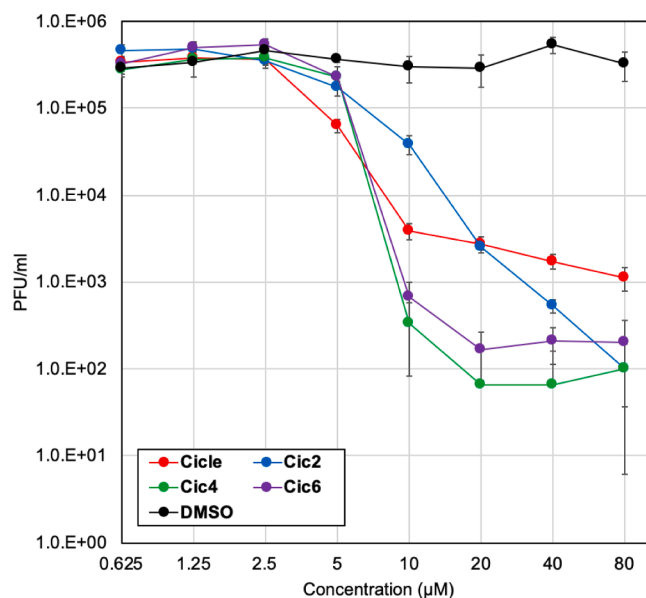


Fig. 4. SARS-CoV-2 growth inhibition by Cicle, Cic2, Cic4, and Cic6. DMSO, dimethyl sulfoxide; PFU, plaque-forming unit. Each independent experiment was done in triplicate.

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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