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Short communication

Antiviral activity of ciclesonide acetal derivatives blocking SARS-CoV-2 RNA replication

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ABSTRACT

Ciclesonide (**Cic**) is approved as an inhalant for asthma and was clinically tested as a candidate therapy for coronavirus disease 2019 (COVID-19). Its active metabolite **Cic2** was recently reported to suppress genomic RNA replication of severe acute respiratory syndrome coronavirus 2. In this study, we designed and synthesized a set of ciclesonide-acetal (Cic-acetal) derivatives. Among designated compounds, some Cic-acetal derivatives with a linear alkyl chain exhibited strong viral copy-number reduction activities compared with **Cic2**. These compounds might serve as lead compounds for developing novel anti-COVID-19 agents.

Coronavirus disease 2019 (COVID-19) has led to an ongoing global public health emergency, resulting in more than 430 million infections and 5.9 million deaths as of February 2022.[1] Vaccines against COVID-19 have been developed and are becoming readily available worldwide. However, there is an urgent need for development of effective therapeutic agents against COVID-19, given the still limited choices for treatment.

Several drugs were explored for clinical use in the treatment of COVID-19 infection,[2] including antiviral agents inhibiting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection/replication and those modulating inflammation to suppress SARS-CoV-2-related pathogenesis. Inhibition of virus propagation by antiviral agents is effective for inhibiting the progression from non-symptomatic and mild cases to severe disease, thus decreasing the hospital burden and restoring social activities.[3] Various anti-SARS-CoV-2 drugs have been approved for clinical use in some countries, and include remdesivir and molnupiravir, which inhibit viral polymerases; nirmatrelvir, an inhibitor of the main viral protease; and anti-spike antibodies such as casirivimab/imdevimab and sotrovimab.[4] However, as SARS-CoV-2 and related diseases can affect a large population of people globally from diverse backgrounds, new antiviral drugs that widen treatment choices depending on the clinical background remain highly desirable.

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Ciclesonide (**Cic**) is an inhaled steroid agent for treatment of asthma and is solid under the trade name Alvesco®. After inhalation, **Cic** is converted to its active metabolite, desisobutyryl ciclesonide (**Cic2**) by esterases in the lungs and airways (Fig. 1A).[5] Matsuyama et al. reported that **Cic** inhibits SARS-CoV-2 replication by targeting SARS-CoV-2 non-structural protein (nsp)-15, which is required for viral replication.[6] However, resistant mutations were mapped to viral nsp3 and nsp4 in their follow-up research, and the precise target of **Cic** in inhibiting replication of SARS-CoV-2 remains unclear.[7] Our previous study revealed that 21st hydroxy group-substituted ciclesonide derivatives possess strong viral copy-number reduction activity and low cytotoxicity compared with **Cic2**.[8] In the present study, we synthesized the ciclesonide acetal (Cic-acetal) derivatives shown in Fig. 1B for further structure–activity relationship analysis, and evaluated their antiviral activity against SARS-CoV-2 to explore their potential as COVID-19 therapeutics.

We initially synthesized Cic-acetal derivatives from the parent compound, 16α-hydroxyprednisolone (**Cic-Diol**). These compounds could be easily prepared in a single step reaction between **Cic-Diol** and the corresponding aldehyde (Fig. 1C, Schemes S1 and S2).

Next, anti-SARS-CoV-2 activities of the synthesized compounds were evaluated in a cell culture infection model as reported previously.[9] VeroE6/TMPRSS2 cells were treated with the test compounds during inoculation with a Wuhan strain of SARS-CoV-2 (hCoV-19/Japan/TY-WK-521/2020) at a multiplicity of infection of 0.003 for 1 h, followed by washing off unbound virus and culture with the test compounds for another 24 h before measuring extracellular viral RNA. This result showed that several compounds exhibited concentration-dependent reduction of viral RNA levels (Table 1 and Fig. S1A).

Viral RNA reduction effects were observed in **Cic** and **Cic2**; their 50% maximum inhibitory concentrations (IC_{50}, mean ± SD) were 8.6 ± 0.5 μM and 8.5 ± 0.6 μM, respectively, **Cic-Diol** without an acetal moiety exerted no reduction activity on RNA virus levels. Substitution of a cyclohexyl group on **Cic2** to a cyclopropyl or a cyclopentyl group (**Cic-cyclo3** and **Cic-cyclo5**) limited the viral RNA reduction effect. When an aliphatic cyclohexyl group was replaced with an aromatic phenyl group (**Cic-Ph**), the activity disappeared. However, introduction of a chlorine atom into the phenyl group (**Cic-4ClPh**, **Cic-3ClPh** and **Cic-2ClPh**) restored the activity (IC_{50} 8.3 ± 1.2, 8.5 ± 1.2, and 9.1 ± 0.2 μM, respectively). Among designated compounds **Cic-C3**, **Cic-C6**, **Cic-C9**, and **Cic-C12**, reduction of viral RNA increased concomitantly with increasing alkyl chain length. The IC_{50} were >30, 5.7 ± 2.5, 6.9 ± 1.3, and 6.1 ± 2.7 μM, respectively. Conversely, viral RNA reduction effect was decreased in the derivative with alkyl chain length of 15 (**Cic-C15**), indicating that there is an appropriate length of alkyl side chain for optimal viral RNA reduction effect (IC_{50} 20.8 ± 3.8 μM). These compounds showed no cytotoxicity, except for **Cic-C9** at high concentrations (50% maximum cytotoxic concentration [CC_{50}, mean ± SD] 21.8 ± 0.9 μM) (Table 1 and Fig. S1B). The results using a human-derived lung epithelial cell line, Calu-3 cells, also showed that the effect in viral RNA reduction of **Cic-C6**, **Cic-C9** and **Cic-C12** were comparable with those of **Cic** (Table 1 and Fig. S1C) without cytotoxicity (Table 1 and Fig. S1D). The IC_{50} of **Cic**, **Cic-C6**, **Cic-C9, and Cic-C12** in this assay were 4.5 ± 3.9, 10.9 ± 13.6, 8.9 ± 7.2, and 16.1 ± 9.2 μM, respectively. In Calu-3 cells, **Cic** showed the highest IC_{50} value

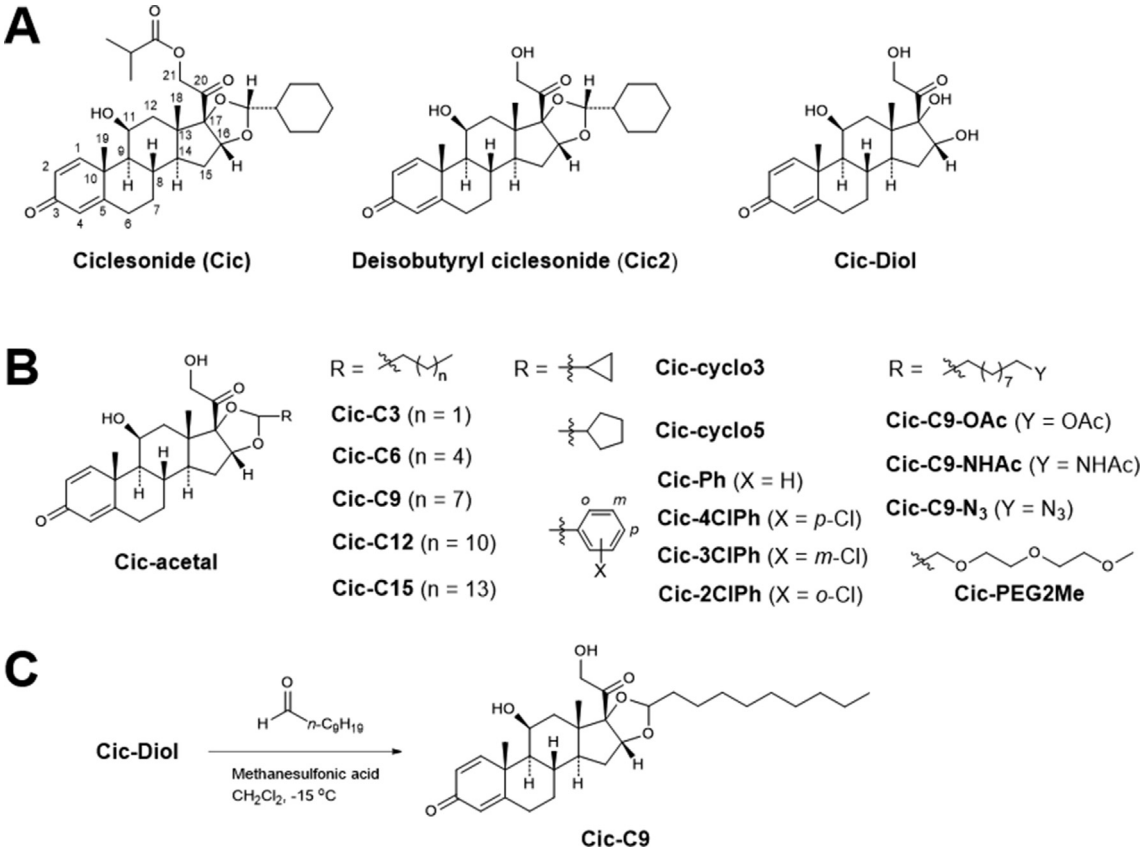

Fig. 1. Chemical structures of ciclesonide derivatives. Ciclesonide (**Cic**), an active metabolite of **Cic**, deisobutyryl ciclesonide (**Cic2**) and the parent compound, **Cic-Diol** (A). Ciclesonide acetal (Cic-acetal) derivatives (B). The representative synthetic scheme of Cic-acetal derivatives (C).

Table 1
The IC_{50} and CC_{50} (mean ± SD) of **Cic** and its derivatives.

Compound	VeroE6/TMPRSS2 cells		Calu-3 cells	
	SARS-CoV-2 RNA	Cell Viability	SARS-CoV-2 RNA	Cell Viability
	IC_{50} (μM)	CC_{50} (μM)	IC_{50} (μM)	CC_{50} (μM)
Cic	8.6 ± 0.5	>30	4.5 ± 3.9	>30
Cic-Diol	>30	>30	–	–
Cic-C3	>30	>30	–	–
Cic-C6	5.7 ± 2.5	>30	10.9 ± 13.6	>30
Cic-C9	6.9 ± 1.3	21.8 ± 0.9	8.9 ± 7.2	>30
Cic-C12	6.1 ± 2.7	>30	16.1 ± 9.2	>30
Cic-C15	20.8 ± 3.8	>30	–	–
Cic-cyclo3	>30	>30	–	–
Cic-cyclo5	16.0 ± 5.1	>30	–	–
Cic2	8.5 ± 0.6	>30	13.4 ± 10.0	>30
Cic-Ph	>30	>30	–	–
Cic-4ClPh	8.3 ± 1.2	>30	–	–
Cic-3ClPh	8.5 ± 1.2	>30	–	–
Cic-2ClPh	9.1 ± 0.2	>30	–	–
Cic-C9-OAc	6.9 ± 1.9	>30	–	–
Cic-C9-N_3	7.1 ± 0.8	>30	–	–
Cic-C9-NHAc	5.9 ± 3.4	>30	–	–
Cic-PEG2-Me	>30	>30	–	–

Table 2
The IC_{50} (mean ± SD) against SARS-CoV-2 variants.

Compound	SARS-CoV-2 RNA	SARS-CoV-2 RNA	SARS-CoV-2 RNA	SARS-CoV-2 RNA
	Delta	Omicron	E406W	R10/E796G C799F
	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
Cic	11.1 ± 7.4	8.3 ± 2.4	10.9 ± 3.6	19.2 ± 8.6
Cic-C6	5.1 ± 3.4	8.0 ± 1.3	5.8 ± 2.9	8.2 ± 0.9
Cic-C9	8.3 ± 0.6	7.9 ± 1.8	4.2 ± 2.0	6.7 ± 3.6
Cic-C12	8.8 ± 0.8	7.2 ± 1.8	3.2 ± 1.2	8.3 ± 0.8
Cic2	9.5 ± 1.3	15.5 ± 5.4	8.3 ± 0.8	22.3 ± 1.7

(4.5 ± 3.9 μM) among the tested compounds. However, the activity of **Cic2** was apparently decreased (IC_{50} 13.4 ± 10.0 μM). Because it has been reported that **Cic** exhibits similar antiviral inhibitory activity in both VeroE6/TMPRSS and Calu-3 cells,[7] this result might be due to the difference in membrane permeability of **Cic2** in the respective cells.

These results suggest that the hydrophobicity of the synthesized compounds might contribute to antiviral activities, i.e., the hydrophobicity of the acetal moiety may affect the cell membrane permeability. To assess this assumption, the hydrophobicity of the compounds was estimated by the retention time in reversed-phase HPLC analysis. The results indicated a correlation between their hydrophobicity and antiviral activity (Table S1). For instance, no activity was observed for **Cic-PEG2Me** with the hydrophilic ethylene glycol (Fig. S1A). Furthermore, activities comparable with **Cic-C9** were observed in **Cic-C9-OAc**, **Cic-C9-NHAc,** and **Cic-C9-N_3**, which have the same C9 chain length as **Cic-C9** but different functional groups at the terminal, indicating similar hydrophobicity (Fig. S1A). In contrast, when the hydrophobicity of the acetal moiety became too high (>**C12**–**15**), the antiviral activity tended to be weakened (**Cic-C15** in Fig. S1A and **Cic-C12** in Fig. S1C).

Next, we tested whether these compounds were also effective against SARS-CoV-2 variants (Delta [Table 2 and Fig. S2A], Omicron [Table 2 and Fig. S2B], a variant carrying an E406W mutation that is resistant to the casirivimab/imdevimab antibody cocktail [Table 2 and Fig. S2C], and an R10/E796G C799F variant resistant to remdesivir[10] [Table 2 and Fig. S2D]) in reducing viral RNA replication. Delta, Omicron, and E406W possess mutations in the receptor binding domain of the spike protein, and R10/E796G C799F carries a mutation in the polymerase region. Our compounds showed antiviral effects on these variants comparable with those seen against the Wuhan strain (Table 1 and Fig. S1A). Of note, **Cic-C6**, **Cic-C9** and **Cic-C12** were found to be effective against a mutant in which viral RNA reduction effect was attenuated in **Cic** and **Cic2** (Fig. S2D).

In summary, we synthesized Cic-acetal derivatives and demonstrated their antiviral activity against SARS-CoV-2 variants. Among the synthesized derivatives, compounds with linear alkyl side chains on the acetal moiety, **Cic-C6** and **Cic-C9,** exhibited strong reduction in viral RNA levels. The parent compound, **Cic2** is a single optically active compound, however, Cic-acetal derivatives synthesized in this study are diastereomeric mixtures because of newly-constructed acetal moiety. Additional studies might clarify the relationship between the stereochemistry of the Cic-acetal derivatives and their antiviral activities. These types of compounds were also effective against mutant strains of SARS-CoV-2 causing COVID-19 infectious disease. In particular, these compounds were found to be more effective against variants for which **Cic** or its active metabolite **Cic2** were less effective. Further studies are required to reveal the target molecules of **Cic** in the inhibition of viral replication of SARS-CoV-2, leading the rational design of **Cic**-based COVID-19 therapeutics.

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Declaration of competing interest

The authors indicated no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphs.2022.04.001.

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