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

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A R T I C L E I N F O

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A B S T R A C T

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** with 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 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

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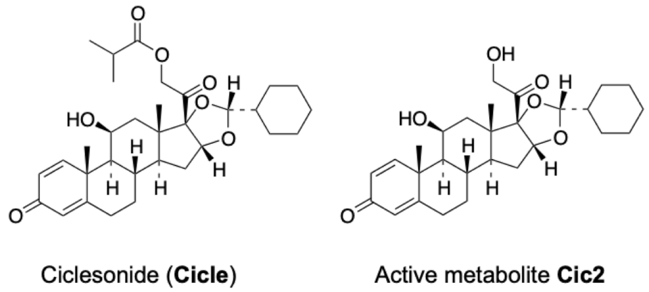


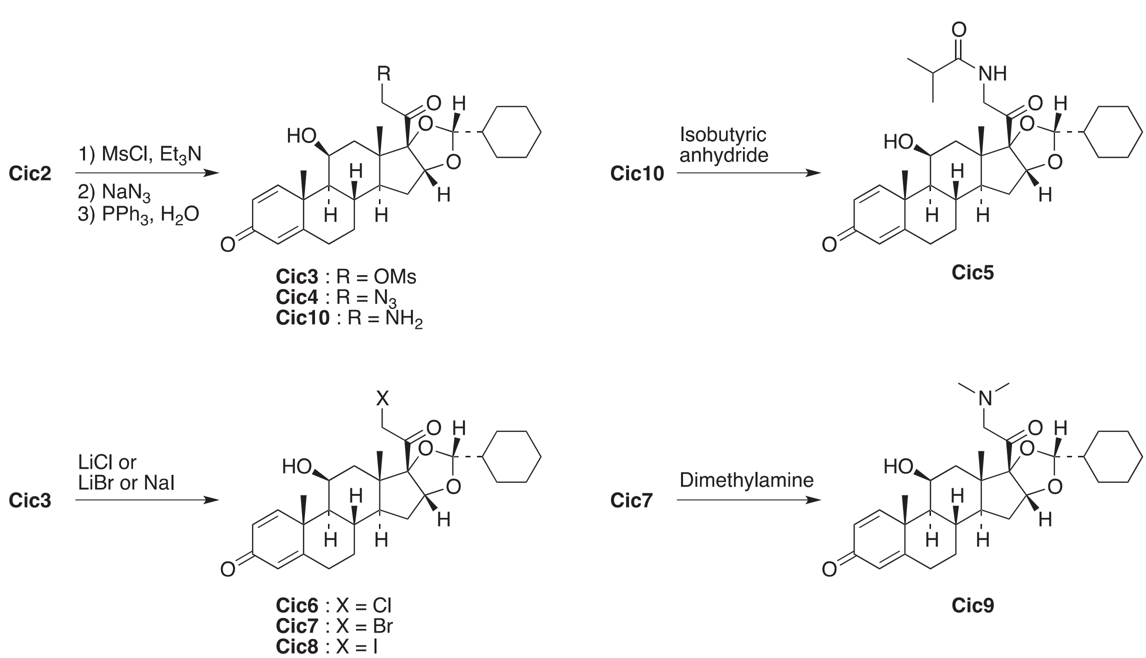
Fig. 1. Chemical structures of ciclesonide (**Cicle**) and its active metabolite **Cic2**.

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.



Scheme 1. Synthetic route for **Cicle** analogues **Cic3–Cic10**.[6]

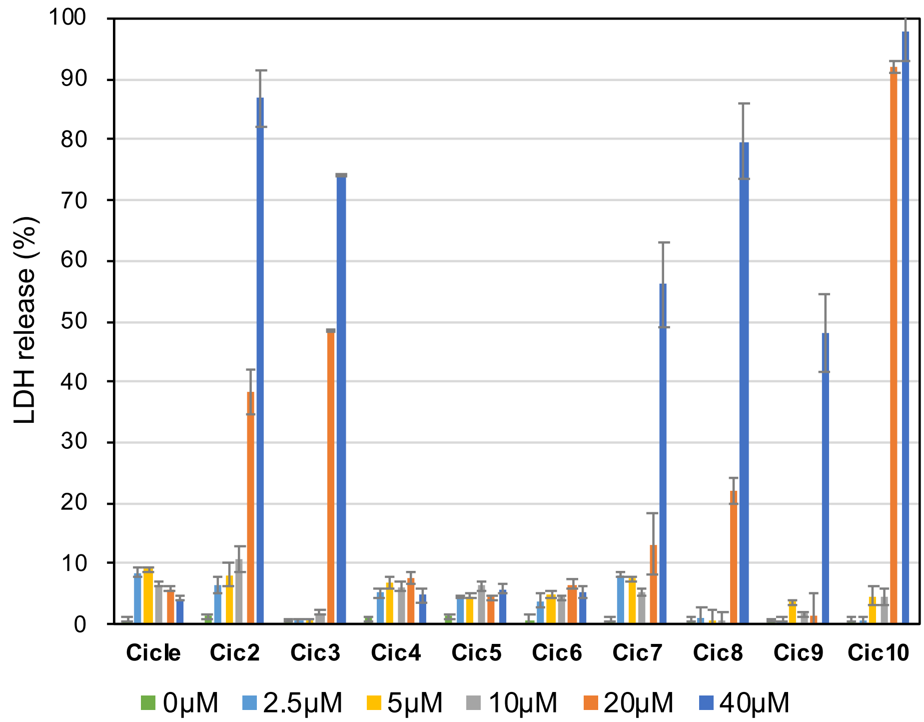


Fig. 2. LDH toxicity of the synthesized **Cicle** analogues against Vero E6/TMPRSS2 cells. LDH, lactate dehydrogenase. Each independent experiment was done in triplicate.

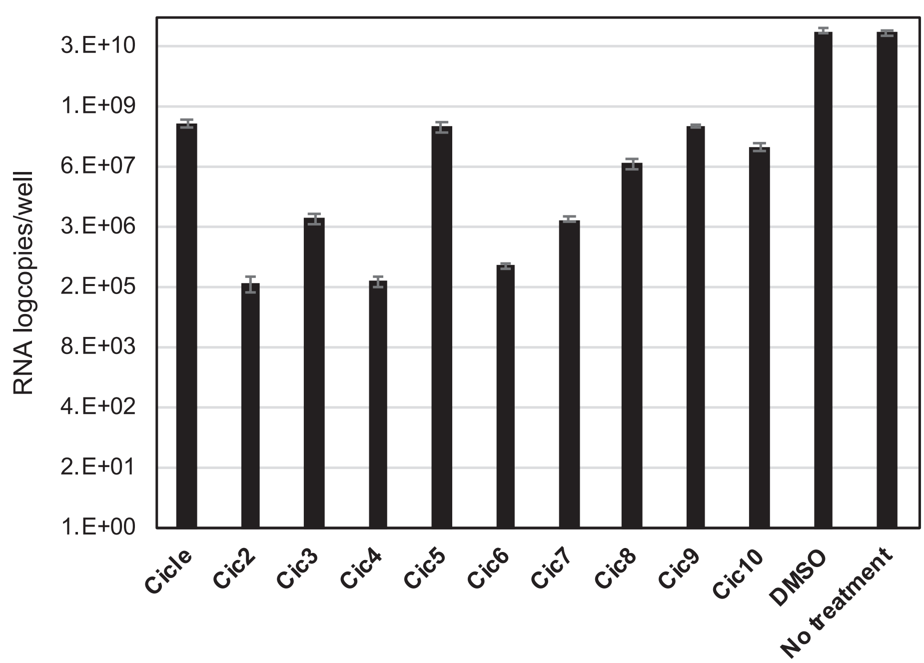


Fig. 3. Evaluation of viral RNA levels in Vero E6/TMPRSS2 cells at 18 h after SARS-CoV-2 infection in the presence of **Cicle** and **Cicle** analogues. DMSO, dimethyl sulfoxide. Each independent experiment was done in triplicate.

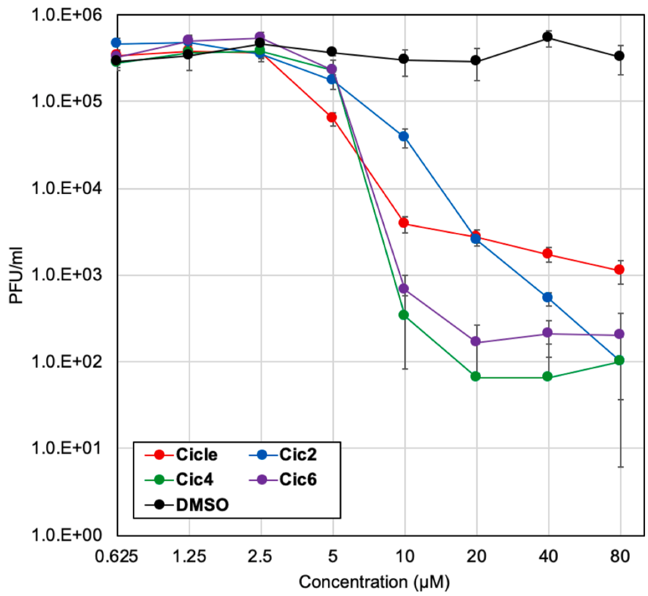


Fig. 4. SARS-CoV-2 growth inhibition by **Cicle**, **Cic2**, **Cic4**, and **Cic6**. DMSO, dimethyl sulfoxide; PFU, plaque-forming unit. Each independent experiment was done in triplicate.

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.

Acknowledgments

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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.

References

1 World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. Available at https://covid19.who.int.

2 a) Y. Dong, T. Dai, Y. Wei, L. Zhang, M. Zheng, F. Zhou, Signal Transduct. Target. Ther. 2020, 5, 237; b) G.A. Poland, I.G. Ovsyannikova, R.B. Kennedy, Lancet 2020, 396, 1595-1606; c) S. Su, L. Du, S. Jiang, Nat. Rev. Microbiol. doi.org/10.1038/S41579-020-00462-Y; d) L. Riva, S. Yuan, X. Yin, L. Martin-Sancho, N. Matsunaga, L. Pache, S. Burgstaller-Muehlbacher, P.D. De Jesus, P. Teriete, M.V. Hull, M.W. Chang, J.F.-W. Chan, J. Cao, V.K.-M. Poon, K.M. Herbert, K. Cheng, T.-T.H. Nguyen, A. Rubanov, Y. Pu, C. Nguyen, A. Choi, R. Rathnasinghe, M. Schotsaert, L. Miorin, M. Dejosez, T.P. Zwaka, K.-Y. Sit, L. Martinez-Sobrido, W.-C. Liu, K.M. White, M.E. Chapman, E.K. Lendy, R.J. Glynne, R. Albrecht, E. Ruppin, A.D. Mesecar, J.R. Johnson, C. Benner, R. Sun, P.G. Schultz, A.I. Su, A. García-Sastre, A.K. Chatterjee, K.-Y. Yuen, S.K. Chanda, Nature 2020, 586, 113-119.

3 a) M.L. Agostini, E.L. Andres, A.C. Sims, R.L. Graham, T.P. Sheahan, X. Lu, E.C. Smith, J.B. Case, J.Y. Feng, R. Jordan, A.S. Ray, T. Cihlar, D. Siegel, R.L. Mackman, M.O. Clarke, R.S. Baric, M.R. Denison, mBio. 2018, 9, e00221–18; b) T.K. Warren, R. Jordan, M.K. Lo, A.S. Ray, R.L. Mackman, V. Soloveva, D. Siegel, M. Perron, R. Bannister, H.C. Hui, N. Larson, R. Strickley, J. Wells, K.S. Stuthman, S.A. Van Tongeren, N.L. Garza, G. Donnelly, A.C. Shurtleff, C.J. Retterer, D. Gharaibeh, R. Zamani, T. Kenny, B.P. Eaton, E. Grimes, L.S. Welch, L. Gomba, C.L. Wilhelmsen, D.K. Nichols, J.E. Nuss, E.R. Nagle, J.R. Kugelman, G. Palacios, E. Doerffler, S. Neville, E. Carra, M.O. Clarke, L. Zhang, W. Lew, B. Ross, Q. Wang, K. Chun, L. Wolfe, D. Babusis, Y. Park, K.M. Stray, I. Trancheva, J.Y. Feng, O. Barauskas, Y. Xu, P. Wong, M.R. Braun, M. Flint, L.K. McMullan, S.S. Chen, R. Fearns, S. Swaminathan, D.L. Mayers, C.F. Spiropoulou, W.A. Lee, S.T. Nichol, T. Cihlar, S. Bavari, Nature. 2016, 531, 381–385; c) D. Siegel, H.C. Hui, E. Doerffler, M.O. Clarke, K. Chun, L. Zhang, S. Neville, E. Carra, W. Lew, B. Ross, Q. Wang, L. Wolfe, R. Jordan, V. Soloveva, J. Knox, J. Perry, M. Perron, K.M. Stray, O. Barauskas, J.Y. Feng, Y. Xu, G. Lee, A.L. Rheingold, A.S. Ray, R. Bannister, R. Strickley, S. Swaminathan, W.A. Lee, S. Bavari, T. Cihlar, M.K. Lo, T.K. Warren, R.L. Mackman, J. Med. Chem. 2017, 60, 1648–1661; d) M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Cell Res. 2020, 30, 269–271.

4 Matsuyama S, Kawase M, Nao N, et al. *J. Virol.* 2020;95:e01648–20.

5 C. Puder, J. Merten, H. P. Schmitt, M. Thumerer, B. Weyell, WO2013/124395.

6 See the supporting information.