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Design and concise synthesis of halicyclamine-inspired bis-macrocycles targeting DedA membrane proteins

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DedA membrane proteins promote the formation of double-membrane vesicles serving as replication platforms for RNA viruses, including SARS-CoV-2. Inspired by the only known inhibitor, halicyclamine A, we developed a concise biomimetic synthesis of halicyclamine-inspired macrocycles, identifying bis-macrocycles that suppress SARS-CoV-2 replication, while exhibiting DedA-associated activity in a bacterial overexpression assay.

Positive-sense single-stranded RNA viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), replicate in unique membrane compartments such as double-membrane vesicles (DMVs) derived from the endoplasmic reticulum and formed specifically in infected cells (Fig. 1).[1] Biogenesis of these membranous replication organelles involves both viral and host factors. The host DedA superfamily, such as TMEM41B[2] and VMP1,[3] which function as lipid scramblases to remodel the lipid bilayer, has been reported to drive the maturation of the membrane structures into DMVs.[4,5] These DMVs are formed in the cells infected with positive-sense single-stranded RNA viruses, including not only SARS-CoV-2 but also hepatitis C virus, enteroviruses, and noroviruses.[6] While viral proteases and polymerases have been primary targets of antiviral therapeutics, formation of DMVs is expected to be an alternative target of drugs, especially those effective against multiple viruses. However, chemical modulation of host DedA membrane proteins as an antiviral strategy remains largely unexplored.[7]

To date, the sponge-derived manzamine alkaloid halicyclamine A (**1**) has been reported as the only molecule that targets DedA membrane proteins (Fig. 1).[8] Manzamine alkaloids,[9,10] exemplified by halicyclamine A (**1**), possess characteristic architectures comprising multicyclic bis-nitrogenated cores and macrocyclic alkyl loops, and display diverse biological activities such as antimalarial[11] and antituberculosis[12] effects. Their sp^3-rich macrocyclic scaffolds occupy an underexplored chemical space for the development of next-generation medium-sized molecule therapeutics.[13]

In this study, we report the concise synthesis of halicyclamine-inspired macrocyclic mimics and demonstrate activity consistent with DedA-associated modulation, thereby supporting their relevance for host-targeted antiviral strategies.

It has been proposed that halicyclamines are biosynthesized *via* an intramolecular transannular cyclization of a C_2-symmetric macrocyclic hypothetical intermediate **2** bearing a pair of 1,6-dihydropyridine (DHP) units.[14] Baldwin and co-workers chemically synthesized this highly unstable intermediate **2** and attempted transannular cyclizations; however, disproportionation of the 1,6-DHP units predominated, resulting in rapid decomposition within 1 h (Scheme 1A).[15] To overcome this problem, we strategically stabilized the 1,6-DHP moiety while preserving its reactivity toward formation of the bis-nitrogenated bicyclic scaffold. Specifically, 1,6-DHP **3** was designed to incorporate a geminal dimethyl group at C6 to completely suppress hydride transfer from the 1,6-DHP unit, together with an electron-withdrawing ester substituent at C3 to enhance oxidative stability.[16] Upon treatment with a Brønsted acid catalyst, macrocyclic C_2-symmetric intermediate **3** underwent a

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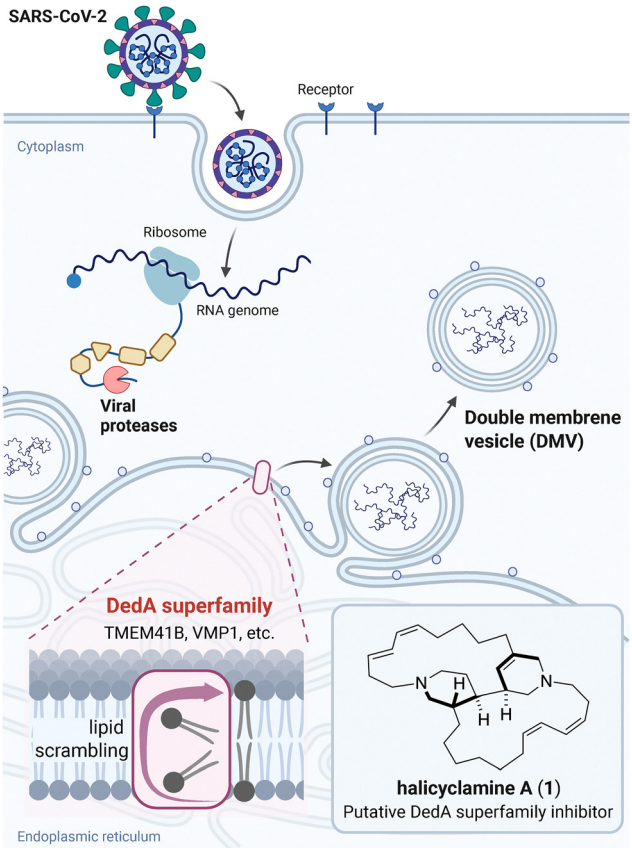
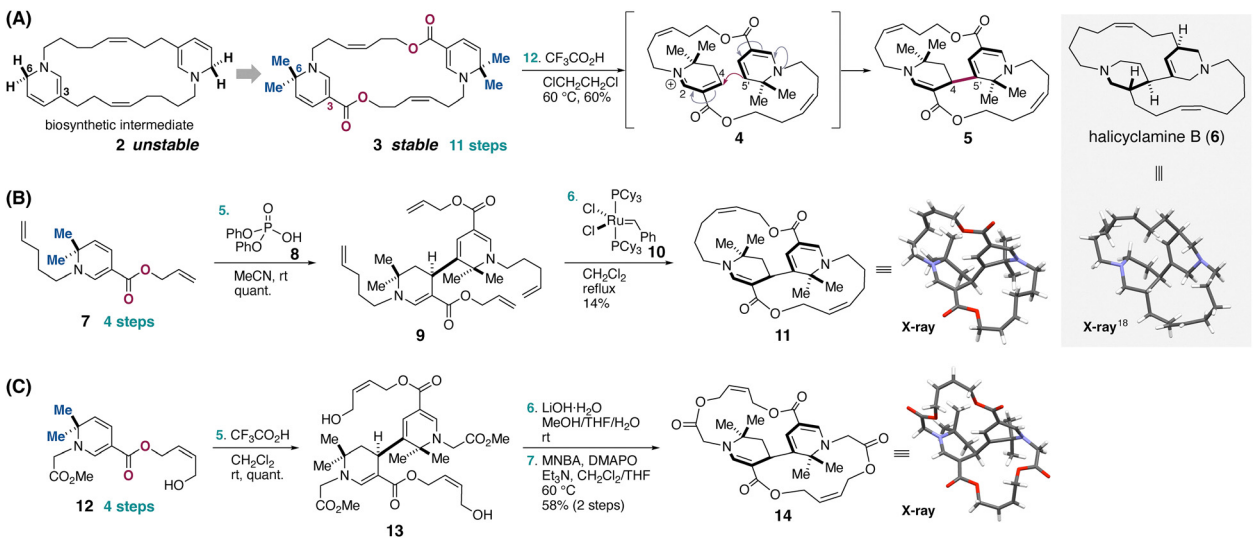


Fig. 1 The estimated function of DedA membrane proteins in the replication cycle of SARS-CoV-2 and halicyclamine A (**1**), a reported inhibitor of bacterial DedA.

biomimetic, regio-controlled transannular cyclization between C4 and C5′ of the two DHP units *via* an iminium intermediate **4**, affording the halicyclamine-type macrocycle **5**.[17] This compound **5** features a macrocyclic ring of the same size as halicyclamine B (**6**).[18] Despite this success, the previously reported route required 12 synthetic steps in total.

In this study, we report a redesigned synthetic approach that enables rapid and customizable access to bis-macrocyclic halicyclamine analogues exhibiting activity consistent with DedA-associated modulation. To this end, we examined two alternative macrocyclization strategies, beginning with a ring-closing olefin metathesis (RCM)-based approach (Scheme 1B). When 1,6-DHP **7**, readily synthesized in four steps, was subjected to a Brønsted acid-catalyzed regio-controlled dimerization between the C4 and C5′ positions, the corresponding halicyclamine-type framework **9** was obtained in quantitative yield. Subsequent RCM using first-generation Grubbs catalyst **10** furnished halicyclamine-type bis-macrocycle **11** in six steps. The modest yield of this transformation is likely attributable to competing metathesis pathways during the two sequential RCM events, leading to undesired macrocycles and related byproducts.[15c] Single-crystal X-ray diffraction analysis confirmed that **11** adopts a three-dimensional architecture closely resembling that of halicyclamine A (**1**) and B (**6**)[18] (see Fig. S3–S4 for overlays of these structures). Overall, this approach reduced the total number of steps by approximately half compared with our previous biomimetic route[17] (Scheme 1A).

As a complementary strategy to olefin metathesis, we next explored a macrolactonization-based approach for the construction of **14** (Scheme 1C). Brønsted acid-catalyzed dimerization of 1,6-DHP **12**, which was likewise readily synthesized in four steps, afforded halicyclamine-type dimer **13** in quantitative yield. Subsequent site-selective hydrolysis of the two methyl ester groups in **13**, followed by macrolactonization under 2-methyl-6-nitrobenzoic anhydride (MNBA) and 4-dimethylaminopyridine *N*-oxide (DMAPO) conditions,[19] provided halicyclamine-type bis-macrocycle **14** in seven steps. Single-crystal X-ray diffraction analysis confirmed that **14** similarly reproduces the three-dimensional architecture of halicyclamine A (**1**) and B (**6**)[18]



Scheme 1 Synthesis of halicyclamine-inspired macrocycles *via* biomimetic transannular cyclization (A), olefin metathesis (B), and macrolactonization (C). Compound **14** exhibited whole-molecule disorder in the crystal structure.

(Fig. S3–S4). Notably, both alternative synthetic routes (Scheme 1B and C) strategically integrate our biomimetic dimerization platform with site-selective and operationally straightforward macrocyclization methods. These routes are substantially shorter than our previously reported synthesis of halicyclamine analogue **5** and provide rapid access to bis-macrocyclic alkaloidal scaffolds that faithfully recapitulate the natural product architecture while enabling controlled structural diversification.[20]

With a concise and modular synthetic route established, we next examined whether the synthesized halicyclamine analogues functionally engage DedA membrane proteins in a cellular context. Accordingly, their inhibitory activities were evaluated using an established DedA overexpression assay in *Mycobacterium smegmatis* and benchmarked against representative members of manzamine alkaloid family (Fig. 2). Following the reported protocol,[8] a DedA-overexpressing *M. smegmatis* strain was prepared, and its responses to each compound were compared with those of the corresponding wild-type strain using an MTT-based viability assay.[21] In this assay format, compounds that exhibit reduced inhibitory effects against the DedA-overexpressing strain relative to the wild-type strain are interpreted as functionally engaging DedA membrane proteins, consistent with prior genetic and chemical validation studies.[8]

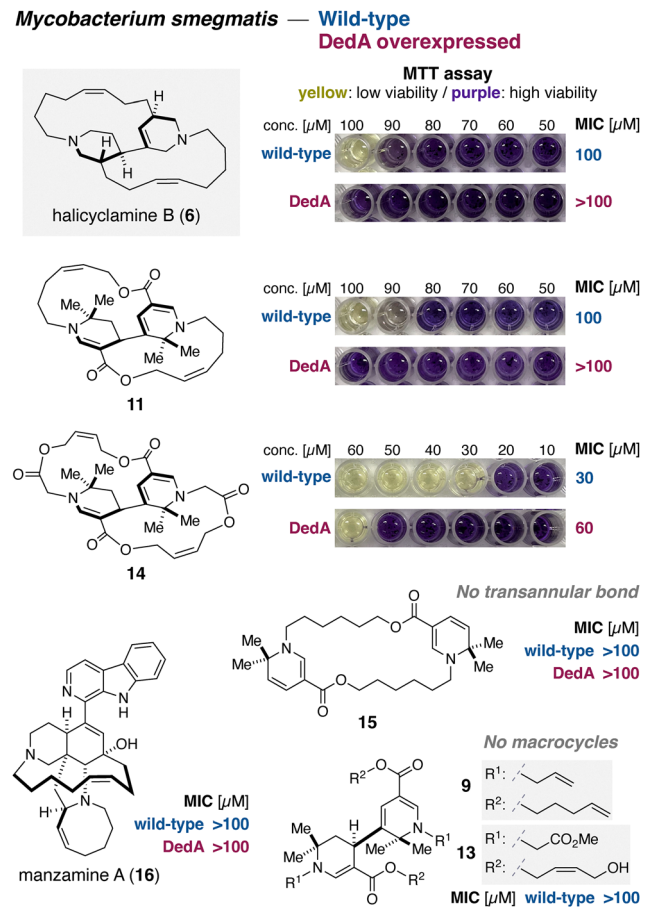


Fig. 2 Evaluation of inhibitory activity toward wild-type and DedA-overexpressed *Mycobacterium smegmatis*.

Halicyclamine A (**1**) was not available in sufficient quantity from natural sources; therefore, halicyclamine B (**6**),[18] which is closely related in structure to the synthesized analogues, was selected as the reference compound.[22] The natural product halicyclamine B (**6**) displayed weak growth inhibition against the wild-type strain, with an MIC value of 100 μM, whereas no inhibition was observed against the DedA-overexpressing strain at concentrations up to 100 μM. Although the absolute potency was modest, this differential response indicates a discernible resistance shift associated with DedA overexpression. The concisely synthesized halicyclamine-type macrocycle **11** showed a closely comparable profile, exhibiting an MIC of 100 μM against the wild-type strain and no detectable inhibition of the DedA-overexpressing strain up to 100 μM, resulting in a resistance shift similar in magnitude to that of halicyclamine B (**6**). In contrast, the halicyclamine analogue **14**, assembled *via* macrolactonization, displayed enhanced inhibitory activity, with MIC values of 30 μM against the wild-type strain and 60 μM against the DedA-overexpressing strain. The larger separation between these values corresponds to a more pronounced resistance shift, exceeding that observed for the natural product **6**, and is consistent with enhanced DedA-related activity under these assay conditions. By comparison, macrocycle **15** lacking the central transannular bond[17] and the macrocyclization precursors **9** and **13** showed no detectable inhibitory activity against either strain. For reference, manzamine A (**16**), a representative macrocyclic marine alkaloid,[23] was also evaluated and exhibited no detectable inhibitory activity under the same assay conditions. Collectively, these results suggest that DedA-associated growth inhibition in *M. smegmatis* is preferentially linked to halicyclamine-type bis-macrocyclic scaffolds and appears to depend on both the bis-nitrogenated core framework and the bis-macrocyclic architecture.

To gain initial insight into whether the designed halicyclamine-type analogues translate into functional antiviral activity, the anti-SARS-CoV-2 replication activities of the synthetic analogues were evaluated in a cell-based SARS-CoV-2 infection assay (Fig. 3). The synthetic bis-macrocyclic analogues **11** and **14** showed antiviral potency, with IC_{50} values of 1.7 and 1.4 μM, respectively. In contrast, both macrocycle **15**, which lacks the central transannular bond,[17] and macrocyclization precursor **13** displayed only weak replication-inhibitory activity (IC_{50} > 10 μM) under the same conditions. This preliminary structure–activity relationship suggests that the bis-macrocyclic architecture, particularly the presence of the central transannular bond, substantially enhances antiviral potency. Collectively, these findings highlight halicyclamine-inspired bis-macrocyclic scaffolds as promising lead structures for antiviral drug discovery.

In summary, bis-macrocyclic analogues **11** and **14** were newly designed on the basis of halicyclamine A (**1**) and rapidly synthesized *via* a biomimetic dimerization strategy. DedA-associated activity of these analogues was supported by the differential responses observed between wild-type and DedA-overexpressing *Mycobacterium smegmatis* strains. In line with this DedA-associated activity, these halicyclamine-inspired

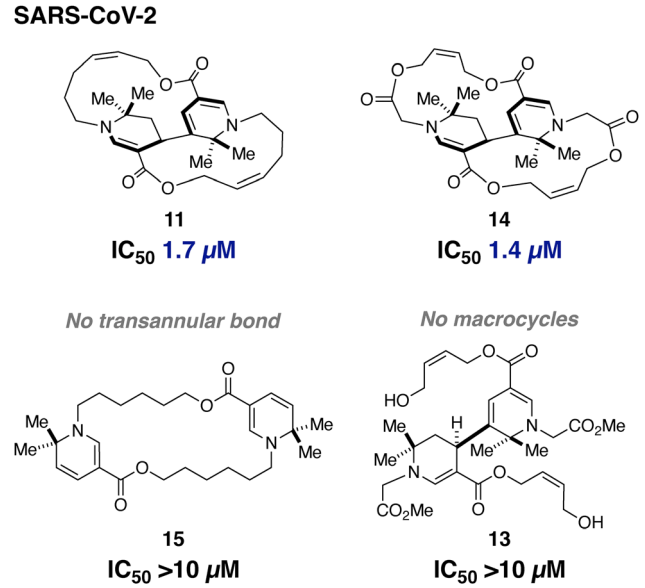


Fig. 3 Evaluation of inhibitory activity toward SARS-CoV-2 replication.

analogues **11** and **14** potently suppressed SARS-CoV-2 replication in a cell-based infection assay.

Host DedA membrane proteins remain largely unexplored as targets of antiviral drug development. The present findings therefore suggest that these natural product-inspired analogues represent promising starting points for further investigation. Although DedA-associated activity was evaluated in this study using an established *M. smegmatis*-based assay, DedA homologues in humans and mycobacteria are expected to differ in sequence and structure. Moreover, SARS-CoV-2 replication is a complex process involving multiple host DedA-related homologues and viral proteins,[5] and the activity responses observed in the *M. smegmatis*-based assay may therefore not directly correspond to antiviral activity against SARS-CoV-2. Future work will focus on assessing inhibitory activity against human DedA homologues to clarify the translational potential of this strategy for the development of new host-directed antiviral agents.

T. W. and H. Oguri designed the project. T. W. performed the organic synthesis. H. Ohashi and K. W. assessed antiviral activity against SARS-CoV-2, while T. W., R. H., and M. A. conducted assays using *Mycobacterium smegmatis*. T. W., S. Y., and S. S. performed X-ray crystallographic analysis. S. T. provided the authentic samples of natural products. T. W. and H. Oguri wrote the manuscript with input from all authors.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data underlying this study are available in the published article and its supporting information (SI). See DOI: https://doi.org/10.1039/d6cc01052k.

CCDC 2519925 (**11**) and 2519926 (**14**) contain the supplementary crystallographic data for this paper.[24a,b]

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