Identification of bis-benzylisoquinoline alkaloids as SARS-CoV-2 entry inhibitors from a library of natural products

To determine whether these compounds have broad-spectrum antiviral effects against other betacoronaviruses as well as recently emerged SARS-CoV-2 variants, we constructed S-D614, N501Y.V1 (B.1.1.7), N501Y.V2 (B.1.351), S-SARS, and S-MERS pseudoviruses using the same lentiviral system as S-G614, and then determined the EC50 values of SC9 (cepharanthine, Fig. 1c), SC161 (herandenezine, Fig. 1d), SC171 (Fig. 1e), SC182 (tetrandrine, Fig. 1f), and SC185 (neferine, Fig. 1g) against these pseudoviruses in 293T cells expressing ACE2 or dipeptidyl peptidase 4 (DPP4) (Fig. 1h). Interestingly, SC9, SC161, SC171, and SC185 exhibited highly potent pan-inhibitory activity against S-pseudotyped coronaviruses including two emerging SARS-CoV-2 variants N501Y.V1 and N501Y.V2, reported in the United Kingdom and South Africa (Supplementary Fig. S3j). As SARS and SARS-CoV-2 have been reported to enter host cells via binding to ACE2, and while DPP4 is critical for MERS-CoV entry, it could be ruled out that these five compounds interfere with ACE2 to block pseudovirus entry.

Following attachment to the host receptor, the membrane fusion process mediated by the S protein of SARS-CoV-2 plays an important role in viral entry. Our data indicated that the above five compounds may target host cells to inhibit coronavirus entry. Therefore, we examined whether these compounds perturb SARS-CoV-2 induced cell fusion. Cell-cell fusion assay exhibited that SC9, SC161, SC182, and SC185 at 5 μM potently inhibited SARS-CoV-2 S-mediated membrane fusion of 293T-ACE2 cells with approximately 90% decrease of fusion rates (Fig. 1i, Supplementary Fig. S4e). Since calcium ion (Ca2+) plays a critical role in SARS-CoV or MERS-CoV S-mediated membrane fusion,6 calcium channel blockers (CCBs), originally used to treat cardiovascular diseases, are supposed to have a high potential to treat SARS-CoV-2 infections.5 Consistently, calcium-free medium or intracellular Ca2+ chelation with BAPTA-AM significantly diminished SARS-CoV-2 pseudovirus infection (Fig. 1j, Supplementary Fig. S4f–i), suggesting that Ca2+ is also required for SARS-CoV-2 entry. The identified bis-benzylisoquinoline alkaloids may abolish S-ACE2-mediated membrane fusion by targeting the host calcium channel.

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Identification of bis-benzylisoquinoline alkaloids as SARS-CoV-2 entry inhibitors. **a** Schematic diagram of the screening workflow with selection criteria for hits outlined. **b** Scatter plot of primary screening of 188 compounds against S-G614 infection. Inhibition ratios for all drugs obtained in a preliminary screening are represented by scattered points. Red dots indicate the 41 compounds with an inhibition rate ≥70%. DMSO (green dot) and aloxistatin (blue dot) were used as a negative and positive control, respectively. **c-g** Dose-response curves of five selected compounds (c) SC9, (d) SC161, (e) SC171, (f) SC182, and (g) SC185 on VSV-G, S-D614, S-G614, S-SARS, S-MERS, N501Y.V1, and N501Y.V2 pseudoviruses. **h** Chemical structures of SC9, SC161, SC171, SC182, and SC185. **i** Inhibitory effect of SC9, SC161, SC171, SC182, and SC185 at 5 μM on SARS-CoV-2 S mediated cell-cell fusion. **j** Effect of extracellular and intracellular Ca²⁺ depletion on S-G614 pseudovirus entry in 293T-ACE2 cells. **k-l** Inhibition curves (k) and EC₅₀ values (l) of the compounds against S-G614 pseudovirus entry in the presence of 20 μM BAPTA-AM. **m** The inhibitory effect of the compounds on native SARS-CoV-2 infection by observing their cytopathogenic effects. SC9, SC161, SC171, and SC185 were tested at 10 μM, and DMSO and remdesivir (5 μM) were used as a negative and positive control, respectively. **n** The relative viral RNA levels in the SC9, SC161, SC171, and SC185 (10 μM) treatment groups were 0.08%, 70.27%, 43.55%, and 76.98% respectively. *P < 0.05; **P < 0.01; ***P < 0.001. All experiments were repeated at least three times.
perturbation of the cholesterol biosynthesis pathway with the CCB amlodipine reduced viral infection. Consistent herewith, the bis-benzylisoquinoline CCBs upregulated intracellular cholesterol levels (Supplementary Fig. S4I), which also likely contributed to the inhibition of viral infection. These results indicated that blockade of S-G614 pseudovirus entry by bis-benzylisoquinoline CCBs mainly depends on calcium homeostasis.

Finally, the antiviral activities of SC9 (cepharanthine), SC161 (hernandezine), SC171, and SC185 (neferine) were confirmed in Vero E6 cells infected with native SARS-CoV-2. Virus-induced cytopathogenic effect and the viral RNA levels were partially inhibited by these compounds, with SC9 (cepharanthine) at the highest efficacy (Fig. 1m–n). The results showed that these compounds inhibited SARS-CoV-2 to varying degrees and may be useful as leads for SARS-CoV-2 therapeutic drug development.

In summary, we reported a set of bis-benzylisoquinoline alkaloids as pan-coronavirus entry inhibitors. These host-targeted inhibitors effectively protected different cell lines (293T-ACE2, Calu-3, and A549) from infection by different coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2 [S-D614, S-G614, and N501Y variants]) in vitro. The compounds blocked host calcium channels, thus inhibiting \( \text{Ca}^{2+} \)-mediated fusion and suppressing virus entry. Considering the effectiveness of CCBs in the control of hypertension, our study provided clues to support that CCBs may be helpful for treating coronavirus infection in patients with hypertension.

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AUTHOR CONTRIBUTIONS

A.L.H, N.T., and Y.H.X designed and directed the study. C.L.H, L.Y.H, K.W., J.H., and G.J.Z. constructed the pseudoviruses and screened the compounds. C.J.G and W.X. performed authentic SARS-CoV-2 assays. All authors reviewed the manuscript and consented to the description of author contribution.

ADDITIONAL INFORMATION

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