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Letter to the editor

Lipopeptide-based pan-CoV fusion inhibitors potently inhibit HIV-1 infection

Dear Editor,

Class I enveloped viruses, such as human immunodeficiency virus type 1 (HIV-1) and highly pathogenic human coronaviruses (HCoVs), including severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2, and Middle East respiratory syndrome coronavirus (MERS-CoV), have posed serious threats to global public health and economy [1]. Therefore, development of broad-spectrum antivirals is urgently needed.

During the fusion process of these class I enveloped viruses, an important and common feature is the formation of a six-helical bundle (6-HB) core structure by the heptad repeat 1 (HR1) and heptad repeat 2 (HR2) domains to bring viral and cellular membranes into close proximity for fusion [2]. In previous studies, we identified the first pan-CoV fusion inhibitory peptide (EK1) and lipopeptides (e.g., EK1C4) with potent inhibitory activity against infection by divergent HCoVs, including SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-229E, and HCoV-NL63 [3–5]. Crystallographic analysis has revealed that these pan-CoV fusion inhibitory peptides possess sufficient structural plasticity to access and interact with the HR1 trimer from different HCoVs, thus blocking their fusion with host cells [3–5]. In this study, we evaluated the potential inhibitory activity of these pan-CoV fusion inhibitory peptides and lipopeptides on HIV-1 infection, with the HIV-1 fusion inhibitory peptide T20 (enfuvirtide) as a control.

We first used a cell–cell fusion assay to evaluate the inhibitory activity of EK1 peptide and EK1-lipopeptides (Fig. 1A) on fusion between the HIV-1_{IIIB} chronically infected H9 (H9/HIV-1_{IIIB}) cells and target cells (TZM-bl). As shown in Fig. 1B, the peptides without lipid conjugation, EK1 and EK1C0, exhibited weak or no inhibitory activity on cell–cell fusion at the concentration up to 5000 nM, respectively, while all 7 cholesterol-conjugated EK1-lipopeptides (EK1C1 to EK1C7) showed potent inhibitory activity on cell–cell fusion with half maximal inhibitory concentration (IC_{50}) ranging from 65 to 862 nM. The C16-conjugated EK1-lipopeptide (EK1P1A) had moderate inhibitory activity with an IC_{50} of 1932 nM. This result suggests that the cholesterol-conjugated pan-CoV fusion inhibitory lipopeptides also possess highly potent inhibitory activity against HIV-1 Env-mediated membrane fusion.

We then tested the inhibitory activity of 4 cholesterol-conjugated lipopeptides (EK1C1, EK1C2A, EK1C3 and EK1C4), one C16-conjugated lipopeptide (EK1P1A) and one non-lipid-conjugated peptide (EK1C0) on the entry of HIV-1_{Bal} pseudovirus (PsV) into target cells. As shown in Fig. 1C, all 4 cholesterol-conjugated lipopeptides were highly effective against HIV-1_{Bal} PsV entry with IC_{50} ranging from 1.7 to 8.3 nM, while EK1P1A had weaker inhibitory activity (IC_{50} = 373 nM), and EK1C0 exhibited no detectable inhibitory activity at the concentration up to 5000 nM.

Among the lipopeptides tested, EK1C2A showed the most potent inhibitory activity against HIV-1 Env-mediated membrane fusion and PsV entry (IC_{50} = 65 and 1.7 nM, respectively), at the similar level of T20 (IC_{50} = 51 and 4.8 nM, respectively) (Fig. 1B and C). Thus, we chose lipopeptide EK1C2A for further analysis of its antiviral activity against infection by two HIV-1 laboratory-adapted strains, HIV-1_{Bal} and HIV-1_{IIIB}. Based on the results from ELISA for p24, EK1C2A potently inhibited HIV-1_{Bal} replication in CEMx174 517 5.25 M7 cells with an IC_{50} of 8.6 nM (Fig. 1D). It could also effectively inhibit HIV-1_{IIIB} infection in MT-2 cells with an IC_{50} of 6 nM (Fig. 1E). Moreover, EK1C2A had low or no detectable toxicity on MT-2 and CEMx174 517 5.25 M7 cells *in vitro* (Fig. 1D and E).

We next assessed the inhibitory activity of EK1C2A against infection of HIV-1 clinical isolates, MN/H9 (84US_MNp) and BZ167/GS 010 (89BZ_167), and T20-or T2635-resistant HIV-1 strains, as previously described [6,7]. As shown in Fig. 1F, EK1C2A could inhibit 84US_MNp and 89BZ_167 infection with IC_{50}s of 21 and 69 nM, respectively, while it was able to effectively inhibit infection by T20-and T2635-resistant strains with IC_{50}s ranging from 13.7 to 176 nM and from 14.8 to 217 nM, respectively.

In summary, we have identified the cholesterol-conjugated lipopeptide EK1C2A with highly potent inhibitory activity against HIV-1 infection, possibly through a common mechanism of action shared by the pan-CoV fusion inhibitors and HIV-1 fusion inhibitors, *i.e.*, interacting with the HR1 domain and blocking 6-HB formation between the viral HR1 and HR2, thus inhibiting viral fusion with and entry into the host cell [3–7]. Based on the results

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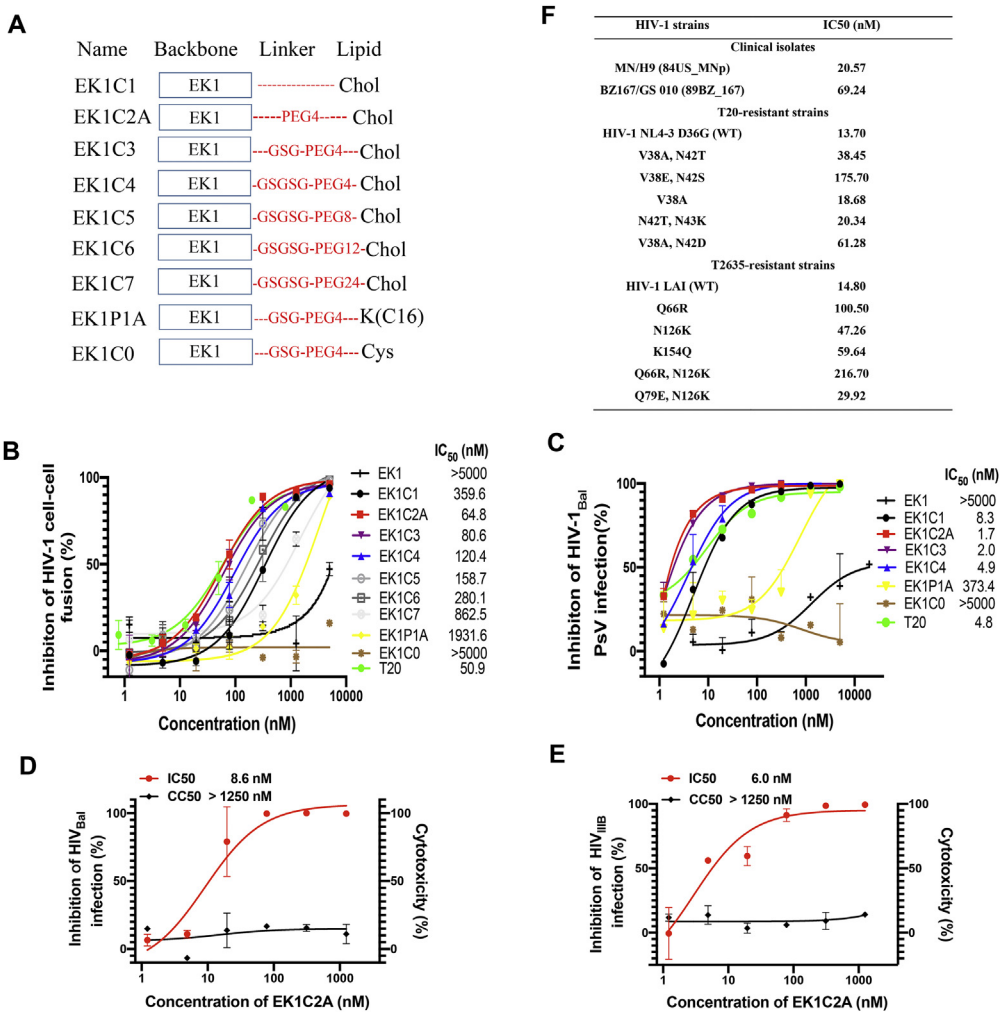

Fig. 1. Potent HIV-1 fusion inhibitory activity of pan-CoV fusion inhibitory lipopeptides. (A) Sequence of EK1-lipopeptides with potential anti-HIV-1 activity; (B) Inhibitory activity of EK1-lipopeptides against cell–cell fusion between H9/HIV-1_{IIIB} (effector) cells and TZM-bl (target) cells; (C) Inhibitory activity of EK1-lipopeptides against HIV-1_{Bal} PsV entry into U87 cells; (D) Inhibition of HIV-1_{Bal} replication by EK1C2A and cytotoxicity on M7 cells using ELISA for p24 and cytotoxic assay, respectively; (E) Inhibition of HIV-1_{IIIB} replication by EK1C2A using ELISA for detection of p24 and cytotoxicity of EK1C2A on MT-2 cells with a cytotoxic assay. (F) Inhibitory activity of EK1C2A against infection by HIV-1 clinical isolates and T20-or T2635-resistant strains. Each sample was tested in triplicate, and the experiment was repeated at least twice.

of this investigation, EK1C2A is a potential candidate for further development as a broad-spectrum fusion inhibitor-based antiviral agent for prevention and treatment of infection by HIV-1 and HCoVs, including SARS-CoV-2.

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Declaration of competing interest

We declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micinf.2021.104840.

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