Structure Optimization of Aloperine Derivatives as HIV-1 Entry Inhibitors

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S Supporting Information

ABSTRACT: As a step toward developing novel anti-HIV agents, we have identified a class of quinolizidines, including aloperine, that inhibit HIV at 1–5 μM by blocking viral entry. In this study, we have optimized the structure of aloperine and derived compounds with markedly improved activity. Our structural optimization has yielded an aloperine derivative **19** with approximately a 15-fold increase in anti-HIV-1 activity. Our mechanism of action study reveals that compound **19** does not inhibit binding of HIV-1 to receptors but arrests the virus from fusion with the membrane. Binding of the compound to HIV-1gp120 might be responsible for its anti-HIV-1 entry activity.

KEYWORDS: aloperine, HIV entry, entry inhibitors

Combination antiretroviral therapy (cART) has been shown to reduce plasma viral loads to undetectable levels in HIV-1-infected patients. Although cART could effectively control plasma viremia, it is clear that the virus is suppressed rather than eradicated in HIV-infected individuals.¹

While no treatment can completely eradicate the virus once cells are infected, many anti-retroviral drugs can inhibit HIV-1 replication at different stages of the viral life cycle.² HIV-1 entry is one such site of inhibition because it is composed of a number of steps that could be targets for therapeutic intervention.³ The initial events of HIV-1 infection are attachment and entry into the host cells. The process begins when the HIV-1 envelope surface glycoprotein gp120 binds to the CD4 receptor molecules on T lymphocytes. CD4 binding triggers conformational changes in gp120 that lead to a secondary interaction with members of the seven-transmembrane G protein-coupled receptor superfamily. Additional structural changes in gp41 are needed for membrane fusion and virus entry. HIV-1 starts its pro-viral life cycle by integrating the viral genome into host chromosomes. Once the viral DNA becomes an integral part of the host's chromosomal DNA, it is difficult to develop a specific antiviral agent if the virus remains silent in the cells. Thus, blocking viral infection at this early stage has the advantage of preventing the establishment of the provirus.

HIV-1 entry inhibitors approved by the FDA for cART include enfuvirtide and maraviroc.⁴ Enfuvirtide is a polypeptide targeting HIV-1 gp41, and maraviroc is a CCR5 antagonist that

is ineffective against X4 viruses using CXCR4 as a coreceptor for entry. Many small molecules have been reported to target HIV-1 Env and inhibit HIV-1 entry.⁵ However, there are no FDA-approved small molecules that target HIV-1 Env to inhibit HIV-1 entry. Among small molecule entry inhibitors targeting HIV-1 Env, BMS-626529 is the most advanced drug candidate currently under phase III clinical trial.⁶ It was shown that BMS-626529 inhibits HIV-1 entry by interacting with an induced binding pocket that includes sequences from a conserved helix and the $\beta 20$ –21 hairpin of gp120. In an effort to identify small molecule HIV-1 entry inhibitors with novel mechanisms of action, we have previously identified multiple chemotypes, including pentacyclic triterpenes and daphnane diterpenes, as potent inhibitors of HIV-1 entry with effective concentrations as low as pM.^{7–11}

Recently, we identified a quinolizidine-type alkaloid aloperine that inhibits HIV-1 infection by blocking HIV-1 entry.¹² Aloperine inhibited HIV-1 envelope-mediated cell–cell fusion at low μM concentrations. Our previous structural modification and structure–activity relationship (SAR) studies revealed that N12 modifications on an aloperine scaffold can moderately enhance anti-HIV potency. As shown in Figure 1, compounds **2**–**4** with N12 substitutions have improved their EC_{50} 's 1.5- to 2.5-fold compared to that of unmodified aloperine. The

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Figure 1.

favorable modifications for increased anti-HIV-1 activity include a spacer of 4 carbons in length attached to N12 with the other end linked to a para-substituted phenyl group through amide bond indicated in Figure 1. Compounds with a spacer longer than 4 carbons negatively impact their anti-HIV-1 potency.¹² A fluoride-containing R_a substituent (OCF_3 or CF_3) is also preferred for potent anti-HIV-1 activity. To further improve the antiviral potency, this study was mainly focused on diversifying the amide linker X and the terminal moiety R of the lead compound (Figure 1). As a result, more than 30 aloperine derivatives with a variety of N12 substitutions were synthesized and evaluated for their anti-HIV-1 activities. Since reduction of the C16–C17 double bond changes anti-HIV-1 potency (dihydroaloperine), suggesting that C16–C17 is an important structural motif that can impact anti-HIV activity, dihydroaloperine derivatives were also synthesized and tested for their antiviral activity.

Compounds with different linkers and R substitutions were synthesized using the same method as previously described.⁷ In brief, aloperine was modified at N12 by treatment with Boc-aminobromoalkane in the presence of potassium carbonate under heat to form 1a. After TFA-mediated deprotection, 1a was converted to the resulting amine intermediate, which was coupled with a variety of carboxylic acids or arylsulfonyl chlorides in the presence of EDC (omitted if using sulfonyl chlorides) and TEA to furnish the final products (5–29, Scheme 1). To obtain the reduced dihydroaloperine derivatives

Scheme 1. Synthesis of Aloperine Derivatives 5–33^a

^aReagents and conditions: (a) $Br(CH_2)_4NHBoc$, K_2CO_3 , MeCN, MW, 110 °C, 1.5 h; (b) TFA, DCM, rt, 20 min; (c) carboxylic acid, EDC, TEA, THF, rt, overnight; or arylsulfonyl chloride, TEA, THF, rt; (d) $H_2/Pd-C$, MeOH, rt, overnight.

(30–33), 1a was treated with H_2 in the presence of palladium on carbon followed by the same coupling procedures for obtaining 5–29. Reduction of the C16–C17 double bond resulted in a diastereoisomeric mixture of *cis* and *trans* isomers that were separated at the end of the synthesis by HPLC.

Table 1 shows the structural modifications and the anti-HIV-1 activity of the derivatives. Compounds 5–9 have a 4- CF_3 - or

4- CF_3O -substituted terminal phenyl moiety and diversified linkers (X), including adding one extra CH_2 (5 and 9) or keto (6) groups and replacing the carbonyl in amide with sulfonyl group (7 and 8). Compounds 5 and 6 showed comparable anti-HIV-1 activity when compared to those of 2–4, whereas 9 with a longer linker caused a significant drop of potency. Compounds with a sulfonamide linker instead of an amide linker lost their anti-HIV activity as seen in 7 and 8. Therefore, aloperine derivatives with an amide linker, such as $C=O$ or $C(=O)CH_2$ as X, were chosen for subsequent synthesis of derivatives with a diversified terminal R group (Table 1).

On the basis of our previous study,⁷ the substitution on the terminal phenyl groups preferred para- and electron-withdrawing groups. Thus, the R group was diversified with a variety of electronic withdrawing groups including $-Br$, $-SO_2CF_3$, $-SO_2CF_3$, and $-SCF_3$ (5–11, 18–24). The results of the anti-HIV-1 assay indicated that compounds with the trifluoromethanesulfonyl (10 and 19) or trifluoromethanethionyl group (21) on the terminal aromatic ring exhibited better anti-HIV-1 activity than compounds with trifluoromethane (4) and trifluoromethoxy (3) substituted phenyl moieties. Compound 19 ($EC_{50} = 0.12 \mu M$) improved the anti-HIV potency by approximately 15-fold when compared with aloperine ($EC_{50} = 1.75 \mu M$). However, compound 19 was more toxic to MT4 cell than aloperine or another relatively potent derivative, compound 10. Nevertheless, with a selectivity index (CC_{50}/EC_{50}) of 240, compound 19 is suitable for further pharmacological studies.

Since the R_a group (Figure 1) of compound 19 appears to be responsible for the markedly increased anti-HIV-1 potency, minor modifications in the R_a group were made and tested for their impact on anti-HIV-1 activity. Compound 20 has an S in place of the SO_2 group and is slightly less potent than 19. In contrast, replacing the CF_3 group in the R_a group of compound 19 with a CH_3 group decreased the anti-HIV-1 activity by more than 25-fold, highlighting the importance of the CF_3 group in drug target interaction.

The terminal aromatic ring assumes a relatively flat structural motif, which might contribute to optimal target binding. To test if this structural feature is critical for anti-HIV-1 activity, the aromatic group was replaced with a cyclohexane ring. Compounds with a cyclohexane terminal group, such as 27–29, did not show improved activity when compared with aloperine.

The C16–C17 double bond is another structural motif on aloperine that might contribute to the antiviral activity of aloperine derivatives. We have previously shown that reduction of the C16–C17 double bond of aloperine (dihydroaloperine) changes both anti-HIV-1 and anti-influenza potency.^{12,13} When compared with aloperine, dihydroaloperine exhibits weaker anti-HIV-1 activity but became a more potent anti-influenza inhibitor, especially the *cis* isomers.^{12,13} In this study, dihydroaloperine analogues of two of the most potent anti-HIV-1 aloperine derivatives (3 and 19) were synthesized and tested for their anti-HIV-1 activity. The *cis*- and *trans*-dihydroaloperine analogues 29–32, which have the same N12 substitution of 3 and 19 with a reduced aloperine ring, showed a marked reduction in anti-HIV-1 activity compared to unreduced derivatives. Reduction of the C16–17 double bond of compound 19 resulted in a >30-fold loss of anti-HIV-1 activity. Thus, it appears that an intact C16–C17 double bond is favorable for anti-HIV-1 activity.

Table 1. Anti-HIV-1 Effects of Aloperine Derivatives

	X	R	EC ₅₀	CC ₅₀
1 (aloperine)			1.75 ± 0.59	>86
5	C(=O)CH ₂	4-CF ₃ C ₆ H ₄	0.81 ± 0.25	>40
6	C(=O)C=O	4-CF ₃ C ₆ H ₄	0.99 ± 0.28	>40
7	SO ₂	4-CF ₃ OC ₆ H ₄	>3	>40
8	SO ₂ CH ₂	4-CF ₃ OC ₆ H ₄	>3	35.7 ± 3.9
9	C(=O)(CH ₂) ₂	4-CF ₃ OC ₆ H ₄	>3	>40
10	C=O	4-CF ₃ SO ₂ C ₆ H ₄	0.55 ± 0.14	>40
11	C=O	4-FSO ₂ C ₆ H ₄	>20	38.5 ± 2.9
12	C=O	4-CF ₃ SO ₂ NHCH ₂ C ₆ H ₄	>20	>40
13	C=O	<i>p</i> -3-thiophenophenyl	1.32 ± 0.34	21.2 ± 2.1
14	C=O	<i>p</i> -2-thiophenophenyl	>3	16.2 ± 1.5
15	C=O	4-benzoylphenyl	0.97 ± 0.41	>40
16	CC=OO	4- <i>tert</i> -butylphenyl	2.15 ± 0.33	12.9 ± 1.4
17	C=O	3,4-methylenedioxyphenyl	>3	>40
18	C=O	3-F,4-CF ₃ C ₆ H ₄	2.22 ± 0.59	>40
19 (DH151)	C(=O)CH ₂	4-CF ₃ SO ₂ C ₆ H ₄	0.12 ± 0.043	28.8 ± 2.2
20	C(=O)CH ₂	4-CF ₃ SOC ₆ H ₄	1.67 ± 0.63	>40
21	C(=O)CH ₂	4-CF ₃ SC ₆ H ₄	0.18 ± 0.064	23.8 ± 2.5
22	C(=O)CH ₂	4-CH ₃ SO ₂ C ₆ H ₄	>3	>40
23	C(=O)CH ₂	4-CF ₃ CH ₂ SO ₂ C ₆ H ₄	>3	>40
24	C(=O)CH ₂	4-BrC ₆ H ₄	1.32 ± 0.52	>40
25	C(=O)CH ₂	4- <i>tert</i> -butylphenyl	2.09 ± 0.72	>40
26	C(=O)CH ₂	3,4-methylenedioxyphenyl	>3	>40
27	C=O	4,4-difluorocyclohexyl	2.44 ± 0.48	>40
28	C(=O)CH ₂	4,4-difluorocyclohexyl	1.94 ± 0.43	>40
29	C=O	4-CF ₃ -cyclohexyl	>3	>40
30 (<i>cis</i>)	C(=O)CH ₂	4-CF ₃ OC ₆ H ₄	2.80 ± 0.88	>40
31 (<i>trans</i>)	C(=O)CH ₂	4-CF ₃ OC ₆ H ₄	1.50 ± 0.57	>40
32 (<i>cis</i>)	C(=O)CH ₂	4-CF ₃ SO ₂ C ₆ H ₄	>3	>40
33 (<i>trans</i>)	C(=O)CH ₂	4-CF ₃ SO ₂ C ₆ H ₄	>3	>40

Anti-influenza Virus Activity. In an effort to identify novel antiviral agents, we have previously tested quinolizidines, including aloperine, for their effects on HIV-1 and influenza virus (IAV) replication.^{12,13} Aloperine was found to inhibit both viruses but was approximately 7-fold more potent against HIV-1 than IAV. To determine if there are correlations between the two antiviral activities, the anti-influenza virus activity against the influenza virus A/Puerto Rico/8/1934 (PR8) of a few aloperine derivatives with sub- μ M anti-HIV-1 activity was determined. Our results showed that the two antiviral activities of the quinolizidines did not always coincide with each other. Among the tested compounds, only aloperine and compound 3 showed moderate anti-PR8 activity. The rest of the tested submicromolar anti-HIV-1 aloperine derivatives, including the most potent anti-HIV-1 aloperine analogue (compound 19), did not show any anti-IAV activity (Table 2).

Inhibition of Membrane Fusion. HIV-1 entry is a multistep process, including receptor binding, HIV-1 Env conformational changes, and fusion between viral and cellular membranes. Env-mediated membrane fusion involves receptor engagement, conformational changes of gp120 and gp41, lipid mixing, and finally complete fusion.¹⁴ We have previously used a simple HIV-1 Env-mediated cell–cell fusion system as a model to study HIV-1 entry inhibitors.^{12,15,16} This model was used to demonstrate that aloperine inhibited HIV-1 Env-mediated

Table 2. Anti-IVA Activity of Submicromolar Quinolizidine HIV-1 Inhibitors

compd	EC ₅₀ (μ M)	CC ₅₀ (MDCK) (μ M)
1 (aloperine)	14.5 ± 4.2	>80
3	18.9 ± 5.5	>80
5	inactive	>80
6	Inactive	>80
10	inactive	>80
15	inactive	31.6 ± 2.83
19 (DH151)	inactive	>80
21	inactive	45.1 ± 3.77

membrane fusion. Aloperine was tested for activity against the cell–cell fusion mediated by HIV-1 8x Env.¹² HIV-1 8x is a CD4-independent virus that can enter the cells without CD4.¹⁷ The HIV-1 8x Env-mediated cell–cell fusion was sensitive to aloperine, suggesting that inhibition of CD4/gp120 binding is not essential for the anti-HIV-1 activity of aloperine. These results also suggest that aloperine may not affect viral binding to CD4 cells, but complete membrane fusion is blocked by aloperine.

Based on these results, we hypothesize that aloperine inhibits HIV-1 entry at a step after CD4 binding but before binding to chemokine receptors. To test this hypothesis and further

dissect the mechanism of anti-HIV-1 entry activity of aloperine derivatives, a green fluorescence protein-tagged HIV-1/Vpr-GFP was used to infect TZM-bl in the presence or absence of compound **19** for 2 h.¹⁸ HIV-1 may enter TZM-bl through direct fusion with the cell membrane or endosomal membrane after endocytosis. Without inhibitors, HIV-1/Vpr-GFP entered the cells and Vpr-GFP was subsequently released and dispersed in the cytosol, which was visualized as green fluorescence by using confocal microscopy (Figure 2b). In contrast, the virus

Figure 2. Compound **19** (DH151) arrests HIV-1 entry. NL4-3/Vpr-GFP was used to infect TZM-bl cells, which were stained with DAPI (nuclear stain, blue) 2 h post infection. Confocal microscopy images were acquired using a Nikon A1R confocal microscope. (a) TZM-bl cells without virus infection; (b) TZM-bl cells were infected with NL4-3/Vpr-GFP; (c) NL4-3/Vpr-GFP infection of TZM-bl was performed in the presence of DH151 (1 μ M).

was observed as puncta in TZM-bl cells in the presence of compound **19** (Figure 2c). This suggests that the virus was arrested on the cell or endosomal membrane in the presence of the compound **19**; membrane fusion and subsequent virus uncoating were inhibited by the compound.

Compound **19 Induced Conformation Changes in gp120.** We have previously shown that aloperine and derivatives inhibit both R5 and X4 viruses.¹² The compounds did not inhibit binding of HIV-1 to the cells but appear to arrest the viral entry before membrane fusion (Figure 2). We have previously studied the dynamic of HIV-1 Env conformations during entry and discovered a class of betulinic acid derivatives that block HIV-1 entry by inducing aberrant conformational changes in HIV envelope glycoproteins.¹⁹ One possible mechanism that may account for the anti-entry activity of the quinolizidines is that binding of the compound to the envelope glycoprotein induces a fusion incompetent conformation, which may prevent gp120 from subsequent coreceptor binding and membrane fusion. To test this possibility, the effect of compound **19** on HIV-1 Env conformational changes was determined using a FACS analysis. The conformational changes in gp120 were monitored with the monoclonal antibody 17b, which may increase binding following HIV-1 Env conformational changes as we previously reported.¹⁹ As expected, s-CD4 enhanced 17b binding due to its ability to induce conformation changes and expose the 17b binding site on gp120 (Figure S1). Compound **19** was able to increase 17b binding to a level comparable to that induced by s-CD4. Thus, it is possible that **19** induced a fusion-incompetent gp120 conformation.

In Silico Model of the Drug–Target Interaction. Our results suggest that HIV-1 gp120 is the likely target of the quinolizidines. To gain insight into the anti-HIV-1 mechanism of action of aloperine derivatives, we have constructed a homology model of the HIV-1_{NL4-3} gp120 trimer and carried out docking studies based on the crystal structure of the HIV-1

BG505 SOSIP.664 gp120.²⁰ Binding of compound **19** to a gp120 trimer is illustrated in a two-site interaction model (Figure 3). The aloperine scaffold is situated on top of the V2

Figure 3. Docking model of compound **19** to NL4-3 gp120 trimer. The methods used for homology modeling and ligand docking are available in the Supporting Information. Key: cyan, compound **19**; yellow, part of a gp120 monomer showing the key V2 loop amino acid residues (I184, D185, T187, S188, R190) that interact with the aloperine moiety of compound **19**; purple, the V3 loop (R306, I307, Q308) and the V1 V2 region (T163, S164, K168, Q170) of a neighboring gp120 monomer, which interacts with the tip of N12 side chain of compound **19**.

loop, while the N12 side chain extends to interact with both the V1 V2 and V3 loops of a neighboring gp120 monomer. On the basis of this model, compounds without a suitable N12 pharmacophore, such as aloperine, are predicted to have a weak interaction with the target and show lower anti-HIV-1 activity. It should be noted that this is a preliminary model that remains to be validated and refined when more experimental data, such as crystal structure of aloperine/gp120, become available.

The results of this study suggest that quinolizidine might be similar to some privileged scaffolds, such as indole, which can exhibit different activities and become useful drugs by different chemical modifications.²¹ It is possible that addition of the N12 side chain of DH151 greatly enhances its interaction with HIV-1 Env while abrogating binding of the compound to influenza virus. This is reminiscent of the low nM HIV-1 entry/maturation inhibitors we synthesized using betulinic acid as a scaffold.^{9,16,22} Depending on side-chain location, a betulinic acid derivative can inhibit HIV-1 entry by targeting HIV-1 Env or inhibit HIV-1 maturation by targeting Gag. The results of this study clearly indicate that the anti-HIV-1 activity can be separated from the anti-influenza virus activity through structural modifications, evidenced by the lack of anti-influenza virus activity of several submicromolar anti-HIV-1 aloperine derivatives. Since our initial discovery of aloperine as an anti-HIV-1 entry inhibitor, we have successfully increased the anti-HIV-1 potency of aloperine by approximately 15-fold (compound **19**). This study also further confirms that arresting membrane fusion is responsible for the anti-HIV-1 activity of the quinolizidines. Based on our mechanism of action study, we propose a model that aloperine derivatives inhibit HIV-1 entry

at a step after CD4 binding but before membrane fusion, as shown in the model illustrated in Figure 4.

Figure 4. Model of HIV-1 inhibition by DH151.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.7b00376.

Methods for the synthesis and characterization of compounds, protocols for biological assays, Figure S1, and molecular modeling (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. C.H.C. conceived the project, Z.D. and L.H. performed design and synthesis, H.X. performed the confocal microscopy study, L.Z. performed the antiviral assays, Q.Z. and L.Z.J. performed the molecular modeling, and L.H. and C.H.C. performed data analysis and wrote the manuscript.

Notes

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■ ABBREVIATIONS

cART, combination antiretroviral therapy; CCR5, CC chemokine receptor 5; DCM, dichloromethane; DAPI, 4,6-diamidino-2-phenylindole; EDC, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride; HIV-1 Env, HIV-1 envelope protein; IAV, influenza A virus; TEA, trimethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; X4 virus, HIV-1 X4-tropic virus.

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