ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Aloperine and Its Derivatives as a New Class of HIV-1 Entry Inhibitors

Zhao Dang,[†] Lei Zhu,[†] Weihong Lai,[†] Hal Bogerd,[‡] Kuo-Hsiung Lee,[§,∥] Li Huang,*[,†] and Chin-Ho Chen*[,†]

[†]Surgical Science, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, United States

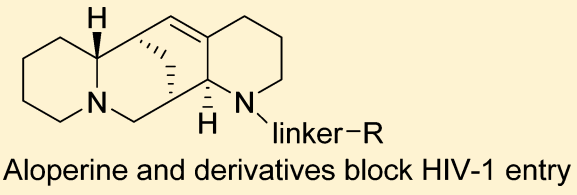
[‡]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, United States

[§]Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, United States

[∥]Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan

Supporting Information

ABSTRACT: A quinolizidine-type alkaloid aloperine was found to inhibit HIV-1 infection by blocking HIV-1 entry. Aloperine inhibited HIV-1 envelope-mediated cell−cell fusion at low micromolar concentrations. To further improve the antiviral potency, more than 30 aloperine derivatives with a variety of N12-substitutions were synthesized. Among them, **12d** with an *N*-(1-butyl)-4-trifluoromethoxy-benzamide side chain showed the most potent anti-HIV-1 activity with EC_{50} at 0.69 μM. Aloperine derivatives inhibited both X4 and R5 HIV-1 Env-mediated cell−cell fusions. In addition, both BMS-806, a compound representing a class of HIV-1 gp120-targeting small molecules in clinical trials, and resistant and sensitive HIV-1 Env-mediated cell−cell fusions were equally sensitive to aloperine derivatives. These results suggest that aloperine and its derivatives are a new class of anti-HIV-1 entry inhibitors.



KEYWORDS: *HIV-1, entry inhibitor, aloperine*

HIV-1 infection and AIDS have afflicted millions of people worldwide.[1,2] Although the antiretroviral therapy (ART) for HIV/AIDS has been successful in controlling the viral replication in infected individuals,[3,4] the current therapy has failed to achieve a full cure due to the difficulty of eradicating latent viral reservoirs in patients.[5−8] Thus, long-term drug treatment for HIV patients is expected. Hence, drugs with novel mechanisms of action may mitigate side effects or drug resistance encountered during long-term ART. ART was characterized by a combination of drugs that target viral or cellular proteins essential for virus replication. HIV-1 entry is a complex multistep process that involves the initial binding of viral envelope protein (gp120) to cellular receptors such as CD4 and CCR5 or CXCR4, followed by conformational changes in viral envelope glycoproteins to initiate the membrane fusion between HIV-1 and its target cells. There are two FDA-approved anti-HIV entry drugs: Fuzeon (enfuvirtide) and Selzentry (maraviroc). Fuzeon and Selzentry inhibit HIV-1 by targeting HIV gp41 and blocking coreceptor CCR5, respectively.[9,10] However, there is no FDA approved antiretroviral that targets HIV-1 gp120. The most advanced viral envelope glycoproteins targeting small molecules in development are a class of piperazine derivatives, such as BMS-378806 (BMS-806) and BMS-626529.[11] In an effort to identify novel HIV-1 entry inhibitors, we have identified a class of betulinic acid derivatives that blocks the HIV entry through binding to the gp120 at the third variable loop (V3 loop).[12−14] In this study, we describe aloperine derivatives, a class of tetracyclic quinolizidine alkaloids, as new HIV entry inhibitors.

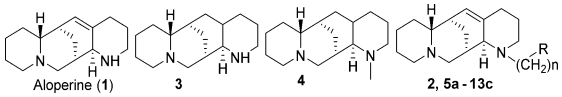
Aloperine was first isolated from the seeds and leaves of *Sophora alopecuroides L.*[15,16] The core of this alkaloid is a unique bridged tetracyclic ring system composed of an octahydroquinoline ring partially overlapped with a quinolizidine ring. We have recently studied the potential antiviral activity of a series of quinolizidine alkaloids and discovered that aloperine can inhibit both the HIV-1 (Table 1) and influenza A virus (IAV).[17] It is possible that aloperine targets a common cellular factor essential for replication of both viruses. However, our recent study indicated the anti-IAV activity of aloperine was linked to the nucleoprotein (NP) of IAV.[17] The objective of this study is to determine the anti-HIV-1 activity of aloperine and its derivatives. The structure−activity relationships and likely mechanism of action of this class of compounds are discussed.

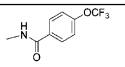
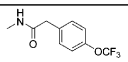
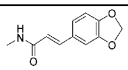
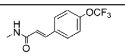
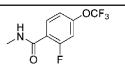
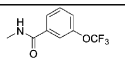
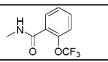
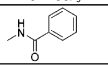
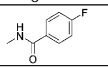
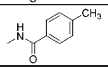
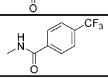
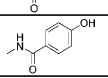
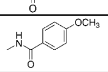
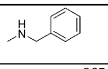
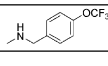
The general structure of the compounds synthesized in this study is aloperine-$(CH_2)_n$-NH-R, where $(CH_2)_n$ is a linker of varied length and R is either a hydrogen or an aromatic group. To synthesize N-substituted derivatives, aloperine was coupled with a Boc protected amino alkyl bromide in the presence of potassium carbonate under microwave heat, followed by deprotection of amine group to form *N*-(ω-aminoalkanyl)-aloperine (**5a−5e**). Compounds **6a−6e**, **7a−7c**, **8a−8c**, **9a**, **9b**, **10a**, **10b**, **11a**, **11b**, and **12a−12f** were obtained by further

Received: August 19, 2015
Accepted: January 9, 2016
Published: January 9, 2016

DOI: 10.1021/acsmedchemlett.5b00339
ACS Med. Chem. Lett. 2016, 7, 240−244

Table 1. Anti-HIV Activity of N12 Modified Aloperine Derivatives[a]

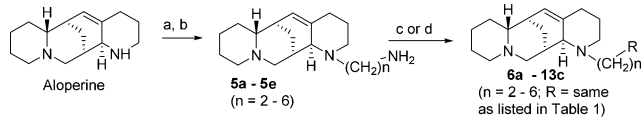


Compounds	n	R	EC_{50} (μM)[b]	CC_{50} (μM)[d]
1 (Aloperine)			1.75±0.59	>86.2
2	0	Me	N/A[c]	>81.3
3			N/A[c]	>85.5
4			N/A[c]	>80.6
5a	2	H	N/A[c]	>73.0
5b	3		N/A[c]	>69.4
5c	4		8.00±2.25	>69.2
5d	5		5.38±0.66	>63.1
5e	6		N/A[c]	>60.4
6a	2		N/A[c]	>43.2
6b	3		N/A[c]	>41.9
6c	4		1.12±0.29	34.62±7.34
6d	5		0.98±0.13	12.87±2.40
6e	6		5.44±0.66	18.47±1.96
7a	2		N/A[c]	>41.9
7b	3		7.68±3.44	>40.7
7c	4		0.75±0.20	23.02±5.97
7d	5		4.21±0.46	17.47±6.22
8a	2		N/A	>44.5
8b	3		7.98±0.56	>43.2
8c	4		3.17±1.73	>41.9
9a	4		2.06±1.08	9.02±1.14
9b	5		2.39±0.70	6.36±1.46
10a	4		3.14±0.44	>39.3
10b	5		6.21±2.03	>38.2
11a	4		3.89±0.29	>40.7
11b	4		N/A[c]	>40.7
12a	4		N/A[c]	>49.1
12b	4		N/A[c]	>47.1
12c	4		4.98±2.87	>47.5
12d	4		0.69±0.13	>42.1
12e	4		4.57±1.86	>47.3
12f	4		8.13±2.76	>45.8
13a	4		0.96±0.24	28.56±12.94
13b	5		0.84±0.21	25.13±10.59
13c	4		4.20±1.01	6.20±0.50
A43D			0.025±0.015	>32.8

[a]Compounds were evaluated by using a multicycle viral replication assay using HIV-1 NL4-3 Nanoluc-sec virus (Supporting Information). [b]EC_{50} is the concentration that inhibited HIV-1 replication by 50%; presented as an average of three experiments ± standard deviation (SD). [c]N/A: inactive (>10 μM). [d]CC_{50} is concentration that reduced cell viability by 50%.

acylation of **5a−5e** with aromatic carboxylic acid reagents, while **13a−13c** were obtained by treating **5c** or **5d** with alkyl bromide in the presence of potassium carbonate under microwave heat (Scheme 1).

Scheme 1. Synthesis of Aloperine Derivatives 5a−13c[a]



[a]Reagents and conditions: (a) $Br(CH_2)nNHBoc$, K_2CO_3, MeCN, 110 °C, MW, 1 h; (b) TFA, DCM, rt, 20 min; (c) carboxylic acid, EDC, TEA, THF, rt, overnight; (d) alkyl bromide, K_2CO_3, MeCN, 110 °C, MW, 1 h.

Anti-HIV Activity. To evaluate the anti-HIV activity of aloperine and the synthesized compounds, HIV-1 NL4-3 Nanoluc-sec virus infection of MT4 cells was performed in the presence of various concentrations of compounds. HIV-1 NL4-3 Nanoluc-sec virus is a reporter virus containing a secNluc insertion as a reporter gene (Promega Cat.# N1021). The viral replication thus can be monitored by measuring the luciferase activity using Promega Nano-Glo Luciferase Assay System (Supporting Information). Under this assay condition, aloperine (**1**) inhibited the virus with an EC_{50} of 1.75 μM.

Our initial attempts of optimizing aloperine for higher potency included adding a small substituent at the N12 position and reducing the double bond on its quinolizidine scaffold. However, these structural modifications failed to yield active compounds against HIV-1, despite some of them, such as compounds **2−4**, showed more potent anti-IAV activity than aloperine.[17] Due to the likely negative impact of N12 substitution on the anti-HIV activity, a linker $-(CH_2)_n-$ was introduced at N12 to conjugate aloperine with a terminal aromatic moiety. The length of the linker varied between two to six carbons. The anti-HIV activity of aloperine derivatives is summarized in Table 1.

Among the synthesized compounds, those with various lengths of amine linkers but without a cyclical terminal group (**5a−5e**) were less active than aloperine. However, **5c** and **5d** with n = 4 and 5, respectively, partially retained their anti-HIV activity. The length of the linkers was critical for the anti-HIV activity of aloperine derivatives with terminal aromatic substitutions. For example, for compounds **6a−6e** with the same terminal trifluoromethoxy substituted benzamide group, the optimal linker length for anti-HIV activity was n = 4 or 5 in compounds **6d** and **6c**. These two compounds showed improved anti-HIV activity with EC_{50} of 1.1 and 0.98 μM, respectively. This structure−activity relationship was also observed in **7a−7d** and **8a−8c** series.

There is a common structural motif shared among three compounds, **6c**, **6d**, and **7c**, which exhibit relatively high anti-HIV potency with EC_{50} ranging from 0.75 to 1.12 μM. All of the three compounds have a *para*-trifluoromethoxy substituent ($-OCF_3$) on the terminal aromatic ring. The importance of the OCF_3 substitution at the para position was confirmed by a decrease and a total loss of anti-HIV-1 activity in the meta- (**11a**) and ortho-substituted analogue (**11b**), respectively.

Derivatives with same linker length (n = 4) but with various para-substitutions on the benzamide terminal moiety were synthesized and compared for their anti-HIV-1 activity. A trifluoromethyl group on the benzamide ring appeared to

DOI: 10.1021/acsmedchemlett.5b00339
ACS Med. Chem. Lett. 2016, 7, 240−244

be preferred for anti-HIV activity. The compound with CF_3 substitution (**12d**) showed the best anti-HIV activity with an EC_{50} of 0.69 μM, while **6c** with OCF_3 was also relatively potent with EC_{50} at 1.12 μM. Compounds **12c**, **12e**, and **12f** with CH_3, OH, and OMe on the benzamide moiety were only weakly active with EC_{50} at 4.57−8.13 μM. Compounds without any substitution (**12a**) or with *p*-fluoride (**12b**) at the benzene ring did not show any anti-HIV activity.

Unlike other amide derivatives, compounds **13a**−**13c** are amine derivatives. Two unsubstituted benzyl amines (**13a** and **13b**) exhibited improved potency over aloperine with EC_{50} lower than 1 μM. Surprisingly, the *p*-OCF_3 substituted analog (**13c**) was less active against HIV-1. However, the three amine derivatives appeared to be more toxic to MT4 cells than other aloperine derivatives with CC_{50} ranging from 6.20 to 28.56 μM. The trifluoromethoxy-substituted phenylacrylic derivatives (**9a** and **9b**) were also more toxic than other compounds as well.

Inhibition of a Panel of Pseudotype Viruses with HIV-1 Env from Various Subtypes. HIV-1 envelope glycoproteins are highly diversified and may have differential sensitivity to HIV-1 entry inhibitors. Therefore, one of the most potent aloperine derivatives, **7c**, was tested against a panel of pseudotype viruses containing HIV-1 Env from strains of various HIV-1 subtypes. These viruses were originally constructed as reference strains for HIV-1 vaccine evaluation.[18] The result indicated that these viruses were inhibited by **7c** with EC_{50} ranging from 0.9 to 4.3 μM (Table 2). Considering the high diversity of HIV-1 Envs, **7c** possesses relatively broad anti-HIV-1 activity against the tested viruses across various subtypes, such as B, C, and G, and some circulating recombinant forms (CRF).

Table 2. Anti-HIV Activity of Compound 7c against Pseudotype Viruses[a]

virus	subtypes (clades)	EC_{50} (μM)
CE1176	C	2.2 ± 0.64
CNE55	CRF01_AE	4.3 ± 1.1
CE0217	C	1.4 ± 0.43
X1632	G	2.5 ± 0.55
BJOX2000	CRF07_BC	2.8 ± 0.96
25710	C	2.1 ± 0.81
TRO11	B	1.6 ± 0.45
CH119	CRF07_BC	0.9 ± 0.36

[a]The antiviral activity of compound **7c** was determined using a TZM-bl assay as previously described.[14] Compound **7c** was not toxic to TZM-bl cells at the highest tested concentration (20 μM). EC_{50} is the concentration that inhibited the pseudotype virus infection of TZM-bl cells by 50%.[18] The data in the table represent mean ± SD of three independent experiments.

Aloperine Inhibited HIV-1 Env-Mediated Cell−Cell Fusion. A time of addition study of aloperine indicated that the sensitive phase of HIV-1 life cycle to the compound is at an early phase of HIV-1 infection (data not shown). HIV-1 NL4-3 infection of MT4 cells was not sensitive to aloperine when the compound was added 2 h post infection. Therefore, HIV-1 entry is a likely target for aloperine. We have previously studied an entry inhibitor A43D using an HIV-1 Env-mediated cell−cell fusion system.[14,19−21] This model uses HIV Env-expression COS cells to fuse with TZM-bl cells, which expresses luciferase upon fusion (Supporting Information).

As expected, the entry inhibitor A43D inhibited both YU2 and NL4-3 Env-mediated membrane fusion (Figure 1). Aloperine also inhibited both NL4-3 and YU2 envelope-mediated membrane fusion with EC_{50} at 1.2 and 1.6 μM, respectively. The aloperine derivative **7c** was also tested against the NL4-3-Env-mediated cell−cell fusion, and it was slightly more potent than aloperine. HIV-1 NL4-3 uses CXCR4 and YU2 uses CCR5 as coreceptors for entry. These results suggest that aloperine can inhibit the entry of both X4 and R5 viruses. A43D is slightly more potent against HIV NL4-3 than the YU2 strain, while aloperine exhibited similar potency toward the HIV-1 Env-mediated cell−cell fusion of both viruses.

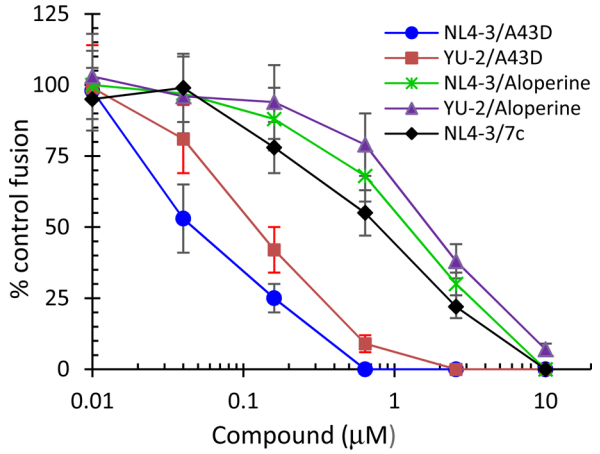


Figure 1. Inhibition of HIV-1 Env-mediated cell−cell fusion by aloperine and its derivative **7c**. The cell fusion was quantified by a Promega luciferase reagent kit as previously described.[21,22] HIV-1 Env expressing COS cells were fused with TZM-bl cells for 24 h; 100% fusion is defined as fusion in the absence of inhibitors. NL4-3/A43D (and the like) in the figure represents the dose-dependent curve of the effects of A43D on NL4-3 Env-mediated cell−cell fusion. Each data point was derived from three independent experiments.

Aloperine Inhibited BMS-806 Resistant Virus Env-Mediated Cell−Cell Fusion. BMS-806 was used in comparison with aloperine in this study to determine their activity against BMS-resistant viruses. BMS-806 is the prototype analogue of the HIV-1 entry inhibitor BMS-626529 under clinical trials.[23−25] Aloperine was tested against BMS-806 resistant virus YU2-T198P Env-mediated cell−cell fusion. The BMS-806 resistant virus YU2-T198P is a YU2 mutant with a T198P mutation in the bridging sheet of gp120 that played an important role in the function of gp120.[12] Bridging sheet is also a critical determinant for BMS-806 sensitivity.[26−29] BMS-806 potently inhibited YU2 Env-mediated cell−cell fusion with an EC_{50} of 0.1 μM (Figure 2). In contrast, YU2-T198P Env-mediated cell−cell fusion was completely resistant to BMS-806 at a concentration as high as 5 μM. However, YU2-T198P was slightly more sensitive to aloperine when compared to YU2.

BMS-806 and aloperine were also tested for their activity against the cell−cell fusion mediated by HIV-1 8x Env.[30] HIV-1 8x is a CD4-independent virus that may be resistant to BMS-806 since the CD4 binding site on HIV-1 gp120 was implicated as a key determinant for BMS-806 sensitivity.[27] Indeed, the HIV-1 8x Env-mediated cell−cell fusion was not sensitive to BMS-806 at concentrations up to 5 μM (Figure 2). However, the HIV-1 8x Env-mediated cell−cell fusion remained sensitive to aloperine. In addition, an aloperine derivative **7c** did not affect HIV-1 IIIB gp120/CD4-IgG2 binding (Figure S1, Supporting Information). These results suggest that BMS-806-resistant Env-mediated cell−cell fusion, such as that

DOI: 10.1021/acsmedchemlett.5b00339
ACS Med. Chem. Lett. 2016, 7, 240−244

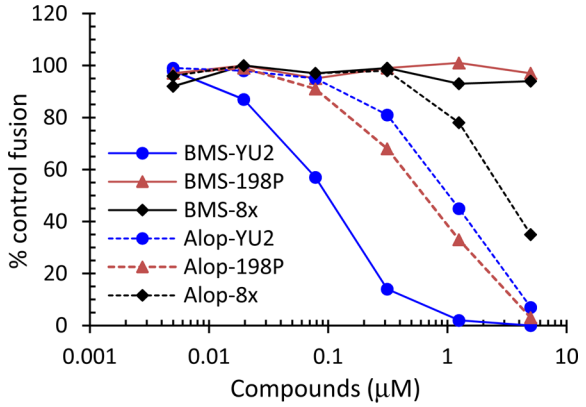


Figure 2. Aloperine inhibited BMS-806 resistant HIV Env-mediated cell−cell fusion. The cell fusion was quantified by a Promega luciferase reagent kit as previously described.[22] Fusion between HIV-1 Env-expressing COS cells and TZM-bl cells was determined in the presence of aloperine (Alop, dashed lines) or BMS-806 (BMS, solid lines). BMS-YU2 (and the like) in the figure represents the dose dependent curve of the effects of BMS-806 on YU2 Env-mediated cell−cell fusion. Each data point represents the average of two independent experiments.

mediated by YU2-T198P and 8x, may be inhibited by aloperine. Although HIV-1 8x Env was resistant to BMS-806 in this study, it should be noted that the evolution of BMS-806 into the active moiety of the clinical candidate (BMS-626529) resulted in a compound that was active against the HIV 8x envelope.[31]

There are more than 30 FDA approved formulations for anti-HIV therapy. Only two of the drugs, enfuvirtide and maraviroc, are HIV-1 entry inhibitors.[9,10,32] Maraviroc is a coreceptor CCR5 inhibitor that potently blocks the infection of R5 HIV strains but is ineffective against X4 HIV strains. Enfuvirtide is a polypeptide drug that targets HIV-1 gp41 and arrests HIV-1 envelope-mediated membrane fusion. In this study, the lead compound aloperine is an HIV-1 entry inhibitor exhibiting several unique pharmacological properties. First, compared to enfuvirtide (MW = 4492), aloperine is a much smaller molecule (MW = 232). Second, aloperine inhibits entry of both R5 and X4 viruses while maraviroc only inhibits R5 viruses. Third, aloperine inhibits entry with a mechanism different from that of maraviroc.

In addition to the FDA approved HIV-1 entry inhibitors, BMS-663068 (a prodrug of BMS-626529) is one of the most promising HIV-1 entry inhibitors undergoing clinical trials.[33] BMS-626529 is a BMS-806 analogue targeting the CD4 binding site on HIV-1 gp120. HIV-1 can become essentially insensitive to BMS-806 with a single amino acid mutation near the bridging sheet of HIV-1 gp120 as shown in Figure 2. It is interesting that HIV-1 Env-mediated cell−cell fusion of both the BMS-806-resistant and -sensitive strains is equally sensitive to aloperine. In summary, aloperine and analogues represent a new class of anti-HIV-1 entry inhibitors with chemical structures distinct from other HIV-1 entry inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00339.

Synthesis and spectroscopic data of new compounds, as well as detailed methods of biological assays (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: li.huang@duke.edu.
*E-mail: chc@duke.edu.

Author Contributions

Z.D., chemical synthesis; L.Z., experiments related to antiviral activity; W.H.L., mechanism of action study; H.B., construction of the plasmid that encodes HIV-1 NL4-3 Nanoluc-sec virus; K.H.L., consultation and manuscript writing; L.H. and C.H.C., study planning, experimental design, data analyses, and manuscript writing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Brian Cullen at Duke University for providing the plasmid that encodes HIV-1 NL4-3 Nanoluc-sec virus; Dr. David Montefiori at Duke for providing the pseudotyped viruses used in this study. This work was supported by the National Institute of Allergy and Infectious Diseases (NIAID) Grant AI110191 (to C.H.C.) and AI108347 (to L.H.) and in part by AI33066 (to K.H.L.).

■ ABBREVIATIONS

ART, antiretroviral therapy; CCR5, CC chemokine receptor type 5; CXCR4, C-X-C chemokine receptor type 4; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; IAV, influenza A virus; NP, nucleoprotein; R5 virus, HIV-1 strain utilizes CCR5 coreceptor for infection; TEA, triethylamine; THF, tetrahydrofuran; X4 virus, HIV-1 strain utilizes CXCR4 coreceptor for infection

■ REFERENCES

(1) CDC. Monitoring selected national HIV prevention and care objectives by using HIV surveillance data—United States and 6 dependent areas—2012. HIV surveillance supplemental report 2014; 19 (No.3), 2014.

(2) UNAIDS (Geneva). Fact sheet: 2014 Global statistics. 2015.

(3) Dau, B.; Holodniy, M. Novel targets for antiretroviral therapy: clinical progress to date. *Drugs* **2009**, *69*, 31−50.

(4) Pereira, C. F.; Paridaen, J. T. Anti-HIV drug development − an overview. *Curr. Pharm. Des.* **2004**, *10*, 4005−4037.

(5) Chun, T. W.; Carruth, L.; Finzi, D.; Shen, X.; DiGiuseppe, J. A.; Taylor, H.; Hermankova, M.; Chadwick, K.; Margolick, J.; Quinn, T. C.; Kuo, Y. H.; Brookmeyer, R.; Zeiger, M. A.; Barditch-Crovo, P.; Siliciano, R. F. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* **1997**, *387*, 183−188.

(6) Wong, J. K.; Hezareh, M.; Günthard, H. F.; Havlir, D. V.; Ignacio, C. C.; Spina, C. A.; Richman, D. D. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* **1997**, *278*, 1291−1295.

(7) Finzi, D.; Hermankova, M.; Pierson, T.; Carruth, L. M.; Buck, C.; Chaisson, R. E.; Quinn, T. C.; Chadwick, K.; Margolick, J.; Brookmeyer, R.; Gallant, J.; Markowitz, M.; Ho, D. D.; Richman, D. D.; Siliciano, R. F. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* **1997**, *278*, 1295−1300.

(8) Persaud, D.; Zhou, Y.; Siliciano, J. M.; Siliciano, R. F. Latency in human immunodeficiency virus type 1 infection: no easy answers. *J. Virol.* **2003**, *77*, 1659−1665.

(9) Kilby, J. M.; Hopkins, S.; Venetta, T. M.; DiMassimo, B.; Cloud, G. A.; Lee, J. Y.; Alldredge, L.; Hunter, E.; Lambert, D.; Bolognesi, D.; Matthews, T.; Johnson, M. R.; Nowak, M. A.; Shaw, G. M.; Saag, M. S. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat. Med.* **1998**, *4*, 1302−1307.

DOI: 10.1021/acsmedchemlett.5b00339
ACS Med. Chem. Lett. 2016, 7, 240−244

(10) Van Der Ryst, E. Maraviroc - A CCR5 antagonist for the treatment of HIV-1 infection. *Front. Immunol.* **2015**, *6*, 277.
(11) Patel, R. V.; Park, S. W. Pyrroloaryls and pyrroloheteroaryls: Inhibitors of the HIV fusion/attachment, reverse transcriptase and integrase. *Bioorg. Med. Chem.* **2015**, *23*, 5247−5263.
(12) Yuan, X.; Huang, L.; Ho, P.; Labranche, C.; Chen, C.-H. Conformation of gp120 determines the sensitivity of HIV-1 DH012 to the entry inhibitor IC9564. *Virology* **2004**, *324*, 525−530.
(13) Huang, L.; Lai, W.-H.; Ho, P.; Chen, C.-H. Induction of a nonproductive conformational change in gp120 by a small molecule HIV-1 entry inhibitor. *AIDS Res. Hum. Retroviruses* **2007**, *23*, 28−32.
(14) Lai, W.-H.; Huang, L.; Ho, P.; Li, Z.-J.; Montefiori, D.; Chen, C.-H. Betulinic acid derivatives that target gp120 and inhibit multiple genetic subtypes of HIV-1. *Antimicrob. Agents Chemother.* **2008**, *52*, 128−136.
(15) Bocharnikova, A. V.; Massagetov, P. S. The alkaloids of *Leptorhabdos parviflora* Benth. *Zh. Obshch. Khim.* **1964**, *34*, 1025−1028.
(16) Brosius, A. D.; Ziller, J. W.; Zhang, Q. M Relative and absolute configuration of aloperine. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1997**, *CS310*, 1510−1512.
(17) Dang, Z.; Jung, K.; Zhu, L.; Lai, W.-H.; Xie, H.; Lee, K. H.; Huang, L.; Chen, C.-H. Identification and synthesis of quinolizidines with anti-influenza A virus activity. *ACS Med. Chem. Lett.* **2014**, *5*, 942−946.
(18) deCamp, A.; Hraber, P.; Bailer, R. T.; Seaman, M. S.; Ochsenbauer, C.; Kappes, J.; Gottardo, R.; Edlefsen, P.; Self, S.; Tang, H.; Greene, K.; Gao, H.; Daniell, X.; Sarzotti-Kelsoe, M.; Gorny, M. K.; Zolla-Pazner, S.; LaBranche, C. C.; Mascola, J. R.; Korber, B. T.; Montefiori, D. C. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. *J. Virol.* **2014**, *88*, 2489−24507.
(19) Huang, L.; Xiong, Y.; Aiken, C.; Chen, C.-H. Bifunctional anti-HIV-1 small molecules with two novel mechanisms of action. *Antimicrob. Agents Chemother.* **2004**, *48*, 663−665.
(20) Huang, L.; Ho, P.; Lee, K. H.; Chen, C.-H. Synthesis and anti-HIV activity of bi-functional betulinic acid derivatives. *Bioorg. Med. Chem.* **2006**, *14*, 2279−2289.
(21) Dang, Z.; Lai, W.; Ho, P.; Zhu, L.; Qian, K.; Lee, K. H.; Huang, L.; Chen, C.-H. New betulinic acid derivatives for bevirimat-resistant human immunodeficiency virus type-1. *J. Med. Chem.* **2013**, *56*, 2029−2037.
(22) Dang, Z.; Qian, K.; Ho, P.; Zhu, L.; Lee, K. H.; Huang, L.; Chen, C. H. Synthesis of betulinic acid derivatives as entry inhibitors against HIV-1 and bevirimat-resistant HIV-1 variants. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5190−5194.
(23) Yang, Z.; Zadjura, L.; D'Arienzo, C.; Marino, A.; Santone, K.; Klunk, L.; Greene, D.; Lin, P. F.; Colonno, R.; Wang, T.; Meanwell, N.; Hansel, S. Preclinical pharmacokinetics of a novel HIV-1 attachment inhibitor BMS-378806 and prediction of its human pharmacokinetics. *Biopharm. Drug Dispos.* **2005**, *26*, 387−402.
(24) Liu, T.; Huang, B.; Zhan, P.; De Clercq, E.; Liu, X. Discovery of small molecular inhibitors targeting HIV-1 gp120−CD4 interaction derived from BMS-378806. *Eur. J. Med. Chem.* **2014**, *86*, 481−490.
(25) Kadow, J. F.; Ueda, Y.; Meanwell, N. A.; Connolly, T. P.; Wang, T.; Chen, C. P.; Yeung, K. S.; Zhu, J.; Bender, J. A.; Yang, Z.; Parker, D.; Lin, P. F.; Colonno, R. J.; Mathew, M.; Morgan, D.; Zheng, M.; Chien, C.; Grasela, D. Inhibitors of human immunodeficiency virus type 1 (HIV-1), attachment 6. Preclinical and human pharmacokinetic profiling of BMS-663749, a phosphonooxymethyl prodrug of the HIV-1 attachment inhibitor 2-(4-benzoyl-1-piperazinyl)-1-(4,7-dimethoxy-1H-pyrrolo[2,3-c]pyridin-3-yl)-2-oxoethanone (BMS-488043). *J. Med. Chem.* **2012**, *55*, 2048−2056.
(26) Langley, D. R.; Kimura, S. R.; Sivaprakasam, P.; Zhou, N.; Dicker, I.; McAuliffe, B.; Wang, T.; Kadow, J. F.; Meanwell, N. A.; Krystal, M. Homology models of the HIV-1 attachment inhibitor BMS-626529 bound to gp120 suggest a unique mechanism of action. *Proteins: Struct., Funct., Genet.* **2015**, *83*, 331−350.
(27) Lin, P. F.; Blair, W.; Wang, T.; Spicer, T.; Guo, Q.; Zhou, N.; Gong, Y. F.; Wang, H. G.; Rose, R.; Yamanaka, G.; Robinson, B.; Li, C. B.; Fridell, R.; Deminie, C.; Demers, G.; Yang, Z.; Zadjura, L.; Meanwell, N.; Colonno, R. A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11013−11018.
(28) Guo, Q.; Ho, H. T.; Dicker, I.; Fan, L.; Zhou, N.; Friborg, J.; Wang, T.; McAuliffe, B. V.; Wang, H. G.; Rose, R. E.; Fang, H.; Scarnati, H. T.; Langley, D. R.; Meanwell, N. A.; Abraham, R.; Colonno, R. J.; Lin, P. F. Biochemical and genetic characterizations of a novel human immunodeficiency virus type 1 inhibitor that blocks gp120-CD4 interactions. *J. Virol.* **2003**, *77*, 10528−10536.
(29) Si, Z.; Madani, N.; Cox, J. M.; Chruma, J. J.; Klein, J. C.; Schon, A.; Phan, N.; Wang, L.; Biorn, A. C.; Cocklin, S.; Chaiken, I.; Freire, E.; Smith, A. B.; III; Sodroski, J. G. Small-molecule inhibitors of HIV-1 entry block receptor-induced conformational changes in the viral envelope glycoproteins. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 5036−5041.
(30) Hoffman, T. L.; LaBranche, C. C.; Zhang, W.; Canziani, G.; Robinson, J.; Chaiken, I.; Hoxie, J. A.; Doms, R. W. Stable exposure of the coreceptor-binding site in a CD4-independent HIV-1 envelope protein. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 6359−6364.
(31) Li, Z.; Zhou, N.; Sun, Y.; Ray, N.; Lataillade, M.; Hanna, G. J.; Krystal, M. Activity of the HIV-1 attachment inhibitor BMS-626529, the active component of the prodrug BMS-663068, against CD4-independent viruses and HIV-1 envelopes resistant to other entry inhibitors. *Antimicrob. Agents Chemother.* **2013**, *57*, 4172−4180.
(32) Henrich, T. J.; Kuritzkes, D. R. HIV-1 entry inhibitors: recent development and clinical use. *Curr. Opin. Virol.* **2013**, *3*, 51−57.
(33) Lalezari, J. P.; Latiff, G. H.; Brinson, C.; Echevarría, J.; Treviño-Pérez, S.; Bogner, J. R.; Thompson, M.; Fourie, J.; Sussmann Pena, O. A.; Mendo Urbina, F. C.; Martins, M.; Diaconescu, I. G.; Stock, D. A.; Joshi, S. R.; Hanna, G. J.; Lataillade, M. AI438011 study team. Safety and efficacy of the HIV-1 attachment inhibitor prodrug BMS-663068 in treatment-experienced individuals: 24 week results of AI438011, a phase 2b, randomised controlled trial. *Lancet HIV* **2015**, *2* (10), e427−37.

DOI: 10.1021/acsmedchemlett.5b00339
ACS Med. Chem. Lett. 2016, 7, 240−244