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Triterpene Derivatives that Inhibit Human Immunodeficiency Virus Type 1 Replication

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Abstract

Triterpene derivatives were analyzed for anti-HIV-1 activity and for cellular toxicity. Betulinic aldehyde, betulinic nitrile, and morolic acid derivatives were identified to have anti-HIV-1 activity. These derivatives inhibit a late step in virus replication, likely virus maturation.

Keywords

retrovirus; antiviral; antiretroviral; proteolysis; protease

Human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS, remains a serious global public health problem. Highly active antiretroviral therapy (HAART) prevents viral replication and the development of AIDS. Though HIV-1 infection can be inhibited, antiretroviral drug resistance and off-target effects require the continual development of novel antiretroviral agents to combat HIV-1 infection for inclusion into HAART regimens.

Fifteen of the 25 anti-HIV-1 drugs approved by the United States Food and Drug Administration (FDA) inhibit early steps in the HIV-1 life cycle. Specifically, these drugs target either viral entry, co-receptor recognition, fusion, reverse transcription, or integration.[1] The 10 approved anti-HIV-1 drugs that inhibit late steps in the HIV-1 life cycle all target the viral protease.1 There are numerous late steps in the HIV-1 life cycle that could be potential drug targets. Examples of such targets include viral RNA transcription, protein translation, virus particle assembly, release, or maturation. Maturation of HIV-1 particles occurs primarily after virus release from infected cells. Maturation is a process that is targeted by the triterpene bevirimat (3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid, PA-457, DSB).[2–11] Bevirimat (Figure 1) inhibits HIV-1 maturation by preventing the cleavage of CA-SP1 (p25) Gag into Capsid (p24) and SP1 (p2).[2–11] Bevirimat, in the absence of the viral protease, has also been shown to inhibit virus assembly.12, 13 Drug resistance studies have revealed many resistance-bearing mutations at the carboxy-terminus of capsid and in the SP1 spacer peptide.[3, 6, 11, 14, 15] Bevirimat has been shown to inhibit HIV-1 replication in cell culture[2–4, 6–9, 11, 16] and has shown promise in clinical trials,[17, 18] but has not been

approved as a drug for the treatment of HIV-1 infection. The goal of this study was to identify other triterpenes that could inhibit HIV-1 replication and would be useful for better understanding the mechanism of action and the structure-activity relationship. Such information would clearly be useful in the design of new derivatives that have potential for clinical translation.

Triterpenes have great potential for drug development.[19] Triterpenes can be extracted from natural sources and are abundant in *Betula papyrifera* (North American birch tree).[19] Betulin was extracted and purified from birch bark of *Betula papyrifera* in accordance with a previously developed procedure.[20] Other precursors - betulinic aldehyde and betulinic acid - were synthesized from betulin by the method of electrochemical oxidation.[21] Further triterpene modifications were provided in accordance with newly developed Schemes 1, 2 and 3. All synthesized derivatives were characterized by ^{1}H and ^{13}C NMR and HRMS (Supplementary Material).

The triterpene derivatives were screened for anti-HIV-1 activity using a single-cycle HIV-1 replication assay. This assay is particularly useful because it can differentiate whether a derivative targets the late or early steps in the viral life cycle (Figure 2). In the assay, 293T cells were transfected in parallel22, 23 with the HIV-1 vector pHIG,[23] which encodes for the green fluorescence protein (GFP), and a HIV-1 envelope vector, pIIINL4 Env.[24] The cell culture supernatant from the virus-producing cells was harvested, cellular debris removed, and used to infect HIV-1 permissive U373-MAGI- $CXCR4_{cem}$ target cells.[25] A minimum of three independent replicates of the assay were performed. Permissive cells were all treated with the equivalent volume of compound treated virus containing supernatant as the no drug treatment control. The no drug treatment control virus had a multiplicity of infection (MOI) of 0.05. The low MOI reduces the possibility of multiple infections in individual target cells. Infected cells were monitored for GFP expression 48 h post infection by flow cytometry. GFP expression allowed for the monitoring of HIV-1 infection, and a reduction in the percentage of GFP-expressing target cells was indicative of a reduction in virus infectivity due to the antiretroviral activity of a triterpene derivative. To determine whether the triterpene derivatives could inhibit HIV-1 replication, virus-producing or permissive target cells were exposed to triterpene derivatives in a concentration range from 5 nM to 50 μM. The EC_{50} values were determined using Graph Pad Prism 5 statistical software.

When permissive target cells were treated with the triterpene derivatives, none of the derivatives inhibited HIV-1 replication – testing up to concentrations of 50 μM. When treating virus-producing cells, bevirimat, 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid inhibited HIV-1 replication with an EC_{50} value of 0.29 μM. The betulinic aldehyde derivatives (**18**–**21**) had EC_{50} values of 1.8 μM, 1.2 μM, 1.5 μM, and 1.4 μM, respectively. The betulinic nitrile derivative (**7**) had a similar EC_{50} value of 1.5 μM. Derivatives (**4**) and (**9**) had EC_{50} values of 15 and 14 μM, respectively, which was ~10-fold less potent than the betulinic aldehyde or betulinic nitrile derivatives. Interestingly, derivative **15** was about 130 times less active than bevirimat, with an EC_{50} of 39 μM.

Potential cell toxicity was assayed using a commercially available kit (CellTiter Non-Radioactive Cell Proliferation Assay, Promega) following the manufacturer's instructions (Table 1). Briefly, cells were treated with serial dilutions of each individual triterpene derivative and IC_{50} values were determined. If cell proliferation was not inhibited by at least 50% at the highest concentration tested, the estimated IC_{50} was reported as > the highest concentration tested in the assay. Bevirimat, the betulinic nitrile derivative (**7**), the betulinc aldehyde derivatives (**18**, **19**, and **21**), and the morolic acid derivative **15** had the lowest cellular toxicity values: IC_{50} >300 μM. The betulinic aldehyde derivative had an IC_{50} value of 29 μM. Derivatives **4** and **9** were less soluble than the other derivatives analyzed in the

study, and had minimal toxicity. The triterpene derivatives **4** and **9** were found to have IC_{50} values of >130 μM and >150 μM, respectively.

A therapeutic index (TI) value (i.e., TI = IC_{50}/EC_{50}) was determined for each of the triterpene derivatives. Bevirimat had a TI of >1000. The betulinic aldehydes (**18**, **19**, and **21**) and the betulinic nitrile derivative (**7**) had TI values of >160, >250, >210 and >200 respectively. The betulinic aldehyde derivative **20** had a TI of 19. Derivatives **4** and **9**, which were less soluble, had TI values of >10 and > 9.2, respectively. Finally, the dimethyl succinate derivative of morolic acid, **15,** had a TI value of >7.6.

The 3,3-dimethylsuccinate functional group was common to all nine triterpene derivatives listed in Table 1. The difference in anti-HIV-1 activity between **15** and bevirimat suggests that the morolic acid scaffold is less active at inhibiting HIV-1 replication than the betulinic acid scaffold. In addition, the dimethylsuccinate group in the C-3 position of the derivatives may be important for antiretroviral activity. Furthermore, the C-28 position or the triterpene can be variable, having either a carboxylic (bevirimat), aldehyde (**18, 19, 20,** and **21**) or nitrile (**7**) group and still maintaining antiretroviral activity. Finally, dimethylsuccinate derivatives of 3β-hydroxyoleananes (**4**, **9**) or morolic acid (**15**) have antiretroviral activity. These observations provide useful information for further investigation of the structure-activity relationship.

In this study, several triterpene derivatives have been characterized to have anti-HIV-1 activity, using a novel single-cycle replication assay. These derivatives should be useful for better understanding the mechanism of action of bevirimat and the general structure-activity relationship. Though it is possible the compounds could inhibit virus release or the viral protease, it is most likely, due to structural similarity with bevirimat, that the compounds in Table 1 inhibit HIV-1 replication by blocking CA-SP1 processing. It should be noted that the derivatives of 3-*O*-(3′,3′-dimethylsuccinyl)betulinic aldehyde (**18**, **19**, **20**, and **21**) or 3-*O*-(3′,3′-dimethylsuccinyl)betulinic nitrile (**7**) may be metabolized by aldolases or nitrolases and therefore represent prodrugs for bevirimat. In general, the derivatives analyzed in this study have the desirable and practical feature that they can be produced in high quantities and at low cost from birch bark. In summary, the information from this study should be useful in the design of new derivatives that have potential for clinical translation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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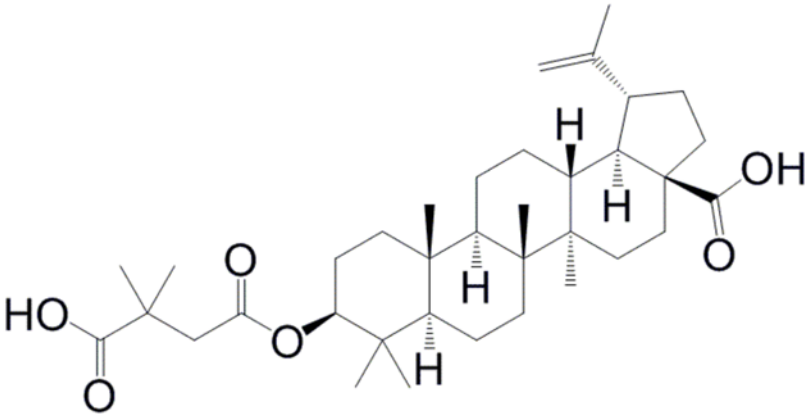


Figure 1.
Structure of bevirimat.

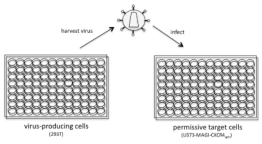
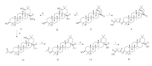


Figure 2.

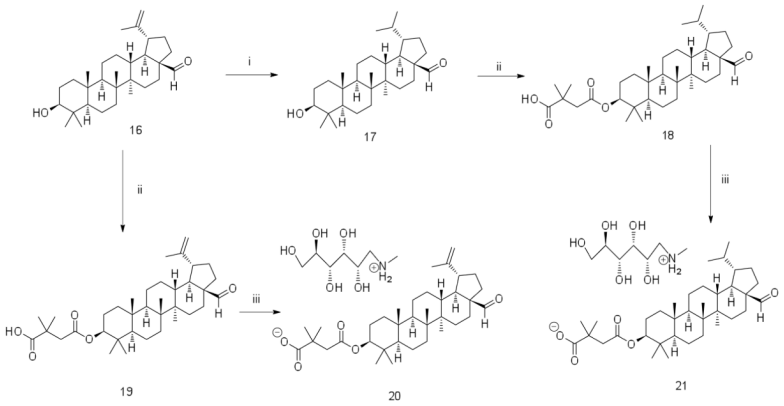
Assay for antiretroviral activity of triterpene derivatives. HIV-1 vector was cotransfected with a HIV-1 envelope expression plasmid into 293T cells in a 96-well tissue culture plate. Forty-eight hours post-trasfection, cell culture supernatants were harvested from the virus-producing cells and used to infect permissive target cells (i.e., U373-MAGI-CXCR4$_{cem}$ cells). Infected cells were harvested 48 hours postinfection and analyzed by flow cytometry. To test triterpene derivatives for antiretroviral activity, virus-producing cells (prior to harvesting virus) or permissive target cells after virus infection were exposed individually with each derivative at a range of concentrations (i.e., 5 nM to 50 μM).

Scheme 1.
(i) CF_3COOH, $CHCl_3$; (ii) KOH, EtOH; (iii) DMSA, DMAP, Py; (iv) $(CF_3CO)_2O$, $CHCl_3$; (v) KOH, MeOH



Scheme 2.

(i) DMSA, DMAP, Py; (ii) KOH, *t*-BuOH; (iii) $(CH_3O)_2SO_2$, K_2CO_3, DMF; (iv) $(CH_3CO)_2O$, Py; (v) $POCl_3$, Py; (vi) 1) KOH, *t*-BuOH; 2) HCl, H_2O



Scheme 3.
(i) H_2, Pd/C, THF/MeOH (ii) DMSA, DMAP, Py; (iii) *N*-methyl-D-glucamine, MeOH

Table 1

Antiretroviral activity, cell toxicity, and therapeutic indices for triterpene derivatives.

Triterpene derivative	Antiretroviral activity (treatment of virus-producing cells) EC_{50}, μM[a]	Antiretroviral activity (treatment of permissive target cells) EC_{50}, μM[b]	Cell toxicity IC_{50}, μM[b]	Therapeutic index[c]
Bevirimat	0.28 (±0.09)	>50	>300	>1000
4	15 (±2.5)	>50	>150	>10
7	1.5 (±0.4)	>50	>300	>200
9	14 (±2.7)	>50	>130	>9.2
15	39 (±10)	>50	>300	>7.6
18	1.8 (±0.5)	>50	>300	>160
19	1.2 (±0.3)	>50	>300	>250
20	1.5 (±0.5)	>50	29	19
21	1.4 (±0.4)	>50	>300	>210

[a] Mean of four experiments, standard deviation is given in parentheses.

[b] Mean of three experiments.

[c] Therapeutic index = IC_{50} cytotoxicity/EC_{50} antiretroviral activity.