

Betulinic Acid Derivatives as Human Immunodeficiency Virus Type 2 (HIV-2) Inhibitors^{||}

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We previously reported that [[*N*-[3 β -hydroxylup-20(29)-en-28-oyl]-7-aminoheptyl]carbamoyl]methane (A43D, **4**) was a potent HIV-1 entry inhibitor. However, **4** was inactive against HIV-2 virus, suggesting the structural requirements for targeting these two retroviruses are different. In this study, a series of new betulinic acid derivatives were synthesized, and some of them displayed selective anti-HIV-2 activity at nanomolar concentrations. In comparison to compounds with anti-HIV-1 activity, a shorter C-28 side chain is required for optimal anti-HIV-2 activity.

Introduction

HIV-2 is a retrovirus related to HIV-1, the causative agent of AIDS. HIV-2 infections can also cause AIDS-like diseases, but these usually progress more slowly than those caused by HIV-1.¹ While HIV-1 has spread to almost all of the countries in the world, HIV-2 is mostly concentrated in countries of West Africa.^{1,2} As the vaccine development against HIV-1 encounters tremendous challenges, progress in antiretroviral therapy (ART^a) has been encouraging.³ However, almost all drugs have been developed with a focus on their effectiveness against HIV-1. Drug regimes for HIV-1 therapy have been used for HIV-2 infections, but some of the drugs that were potent against HIV-1 replication appeared to be less active or inactive against HIV-2 virus. For example, HIV-2 is mostly insensitive to non-nucleoside reverse transcriptase inhibitors and the HIV-1 entry inhibitor Fuzeon.^{4,5} Some HIV-1 protease inhibitors have also been shown to have reduced potency against HIV-2.⁶ Given the fact that the genetic homology between HIV-1 and HIV-2 is less than 50%,⁷ it is no surprise that drugs currently used for HIV-2 are not as optimal as they are for HIV-1.

Our previous work has been focused on the synthesis of betulinic acid derivatives as potential anti-HIV-1 agents.^{8–10} Betulinic acid (BA, **1**) is a pentacyclic triterpene found in abundance in many plant species.¹¹ Unmodified **1** was inactive or exhibited very weak activity against HIV-1 replication. Chemical modification of **1** at C-28 and/or C-3 positions resulted in potent anti-HIV-1 compounds. We and others have synthesized C-28 modified BA or other pentacyclic triterpene derivatives that inhibit HIV-1 entry.^{10,12–15}

Compounds **17** (IC9564)¹² and **4** (A43D)¹⁰ are among the most potent BA derivatives that inhibit HIV-1 entry by targeting HIV-1 gp120.^{16,17} Chemical modifications of **1** at C-3 resulted in compounds that inhibited HIV-1 maturation as opposed to entry.^{13,18,19} The HIV-1 maturation inhibitor, 3-*O*-(3',3'-dimethylsuccinyl)betulinic acid (bevirimat),²⁰ is a C-3 BA derivative currently under clinical trial for anti-HIV-1 therapy. Chemical modifications at C-3 and C-28 resulted in bifunctional BA derivatives that inhibited HIV-1 entry and maturation.^{9,10} [[*N*-[3 β -*O*-3',3'-Dimethylsuccinylup-20(29)-en-28-oyl]-7-aminoheptyl]carbamoyl]methane (A12-2) was one of the most potent BA derivatives that inhibited HIV-1 replication at maturation and entry steps.¹⁰ Because of the relatively low genetic homology between HIV-1 and HIV-2, we hypothesized that the optimal pharmacophore of BA derivatives required for inhibiting HIV-2 is different from that for anti-HIV-1 activity. Since C-3 derivatives, such as **22** (bevirimat), did not inhibit HIV-2 replication in our assays (Table 1), chemical synthesis in this study is focused on the modification of the C-28 position of **1**. The results of this study support the hypothesis in that a shorter C-28 side chain of BA, an equivalent of CONH(CH₂)_{*n*}R with *n* being 4 or 5, is needed for optimal anti-HIV-2 activity.

Results and Discussion

Chemistry. Compounds **4**, **17**, **7–11**, and **1** are known compounds that have been synthesized and reported for their activities against HIV-1 virus.^{10,12} The C-28 modified BA derivatives tested in this study were synthesized using methods previously described (Scheme 1). On the basis of their C-28 side chain structures, four types of compounds were synthesized. The first type of compounds included the analogues **2–5**. This type was synthesized by coupling diaminoalkane [NH₂(CH₂)_{*n*}NH₂], where *n* was 5–8, with the C-28 carboxylic acid of 3-*O*-acetate-BA. The resultant C-28 terminal amine intermediate was then acylated with acetic anhydride following a step of alkaline hydrolysis of the C-3 ester to form analogues **2–5**. The amine intermediate **3a** was coupled with malonic acid in the presence of EDC and

^{||} The authors congratulate the Division of Medicinal Chemistry on its 100th anniversary.

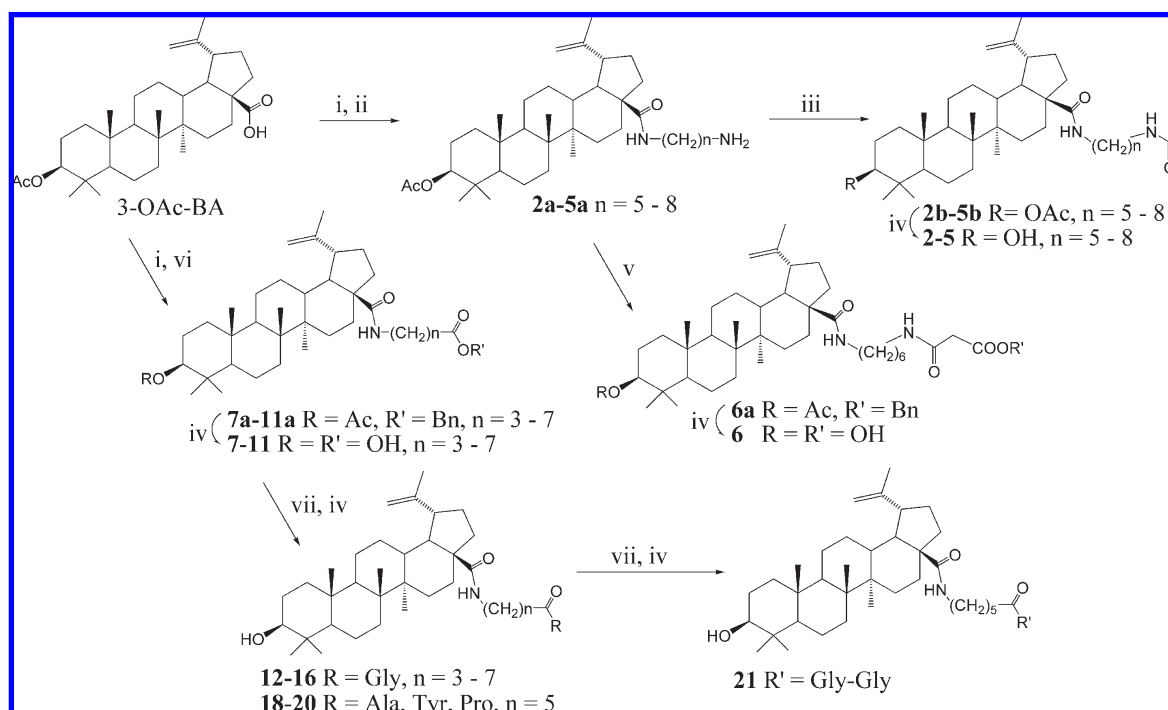
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^a Abbreviations: BA, betulinic acid; ART, antiretroviral therapy; EDC, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; SARs, structure–activity relationships; THF, tetrahydrofuran; DCM, dichloromethane; compds, compounds; AZT, 3'-azido-3'-deoxythymidine.

Table 1. Inhibitory Activity of BA and Its Derivatives against HIV-1 and HIV-2

compd	X_1	n	X_2	R	$IC_{50} (\mu M)^a$		$TC_{50} (\mu M)^a$ cytotoxicity
					HIV-1 NL4-3	HIV-2 KR.X3	
1 (BA)	OH			H	inactive	inactive	> 80
2	NH	5	-NHCO-CH ₃	H	inactive	inactive	13.9 ± 3.3
3	NH	6	-NHCO-CH ₃	H	0.027 ± 0.013	inactive	48.1 ± 12.6
4 (A43D)	NH	7	-NHCO-CH ₃	H	0.038 ± 0.011	inactive	> 66
5	NH	8	-NHCO-CH ₃	H	0.14 ± 0.02	inactive	> 64
6	NH	6	-NHCO-CH ₂ COOH	H	0.44 ± 0.02	inactive	34.6 ± 13.8
7	NH	3	-COOH	H	inactive	2.8 ± 1.8	31.2 ± 1.3
8	NH	4	-COOH	H	32.8 ± 6.5	0.17 ± 0.14	> 72
9	NH	5	-COOH	H	2.0 ± 0.6	0.15 ± 0.04	> 70
10	NH	6	-COOH	H	11.1 ± 9.8	4.5 ± 4.3	36.2 ± 1.2
11	NH	7	-COOH	H	0.84 ± 0.52	inactive	34.2 ± 1.8
12	NH	3	-CO-Gly	H	inactive	3.7 ± 1.5	> 67
13	NH	4	-CO-Gly	H	inactive	2.6 ± 2.8	> 65
14	NH	5	-CO-Gly	H	34.7 ± 8.5	0.35 ± 0.25	> 64
15	NH	6	-CO-Gly	H	4.1 ± 2.1	0.42 ± 0.33	> 63
16	NH	7	-CO-Gly	H	0.12 ± 0.04	0.64 ± 0.02	15.8 ± 4.1
17 (IC9564)	NH	7	-CO-R'	H	0.042 ± 0.013	2.9 ± 1.3	33.2 ± 3.3
18	NH	5	-CO-Ala	H	48.9 ± 29.4	0.37 ± 0.40	> 63
19	NH	5	-CO-Tyr	H	inactive	0.23 ± 0.10	54.6 ± 28.3
20	NH	5	-CO-Pro	H	51.4 ± 29.7	0.63 ± 0.14	> 60
21	NH	5	-CO-Gly-Gly	H	> 58	1.7 ± 0.9	> 58
22 (bevirimat)	OH		R = HOOC-C(CH ₃) ₂ CH ₂ CO-		0.13 ± 0.01	inactive	> 30
AZT						0.069 ± 0.01	> 78

^a Data represent an average of three independent experiments. "Inactive" denotes compounds with $TC_{50}/IC_{50} < 2$.

Scheme 1. Synthesis of Types 1–4 BA Derivatives^a

^a Reaction conditions: (i) oxalyl chloride; (ii) $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$; (iii) acetic anhydride, Py; (iv) NaOH, THF, MeOH; (v) $\text{CH}_2(\text{COOH})_2$, EDC, Et₃N; (vi) $\text{NH}_2(\text{CH}_2)_n\text{COOBn}$, Et₃N; (vii) R-ester, EDC, Et₃N.

Et₃N to form the second type of compound (**6**) after the saponification mediated by sodium hydroxide. The third type of compound was synthesized by coupling the ω -aminoalkanoates of varying lengths to the C-28 carboxylic acid of 3-*O*-acetate-BA. Compounds **7–11** were obtained after alkaline hydrolysis of esters at C-3 and C-28 side chains. Further coupling of the C-28 terminal carboxylic acid of **7–11** with mono- or diamino acids furnished the fourth type of compound, which included **12–16** and **18–21**.

Results. These new BA derivatives, along with **1** and some known BA derivatives, were tested for their anti-HIV-1 and HIV-2 activity (Table 1). The unmodified **1** did not exhibit anti-HIV-1 or anti-HIV-2 activity. For anti-HIV-1 activity, **4**, **17**, and the newly synthesized **3** were three of the most potent inhibitors against HIV-1 NL4-3 replication. Compound **3** was the most potent compound with its IC₅₀ at 27 nM. Compounds with side chains less than six methylene groups exhibited a significantly weaker anti-HIV-1 activity as demonstrated by **2**. Compound **5**, with eight methylenes in its C-28 side chain, was less potent than **4** against HIV-1 replication. In contrast to type 1 analogues, which have acetamide termini at their C-28 side chains, **17** and type 2–4 compounds possessed carboxylic acid at the terminus of their C-28 side chain. Among them, derivatives with C-28 methylene groups equal to 7 exhibited the most potent anti-HIV-1 activity when compared to analogues with a shorter linker in their respective compound types (Table 1).

Although **4** and **17** were two of the most potent anti-HIV-1 BA derivatives, **17** exhibited weak anti-HIV-2 activity (about 70 times less potent than its anti-HIV-1 activity) and **4** was inactive against HIV-2 KR.X3 infection. The striking difference in their potencies against HIV-1 and HIV-2 suggested that the optimal pharmacophore against HIV-2 is different from that required for anti-HIV-1 activity. To obtain potent anti-HIV-2 compounds, BA derivatives with a variety of C-28 side chains were synthesized and tested for their anti-HIV-2 activity. Similar to **4**, the newly synthesized analogues **2**, **3**, and **5** with acetamide termini were inactive against HIV-2 replication. This result suggested that a free terminal carboxylic acid at the C-28 side chain of BA might be needed for HIV-2 inhibition as shown by **17**. Therefore, type 1 compounds were further modified by substituting the C-28 acetamide terminus with malonic acid. Except for compound **6**, the resultant type 2 compounds were mostly unstable because of decomposition of the terminal malonamide R-NHCO-CH₂-COOH into acetamide R-NHCO-CH₃. However, **6** was less potent than **3** against HIV-1 and was inactive against HIV-2 (Table 1). Thus, a free terminal carboxylic acid at C-28 alone was not sufficient for anti-HIV-2 activity.

To systematically investigate the optimal length of the C-28 side chain required for anti-HIV-2 activity, a series of BA derivatives with C-28 side chains in the form of –NH(CH₂)_{*n*}COOH, where *n* ranged from 3 to 7, were synthesized. As shown in Table 1, type 3 compounds **7–11** were either inactive or displayed weak anti-HIV-1 activity with IC₅₀ at low micromolar concentrations. Most of these compounds were more potent against HIV-2 than HIV-1 except **11**, which was inactive against HIV-2 replication. Within this type, **8** and **9** exhibited the most potent anti-HIV-2 activity with IC₅₀ at 0.17 and 0.15 μ M, respectively. These results suggested that the optimal anti-HIV-2 activity was achieved when *n* = 4 or 5 for the length of the C-28 side chain. In contrast, the optimal anti-HIV-1 activity was achieved when *n* = 7 or 8 for the length of the C-28 side chain.^{10,12}

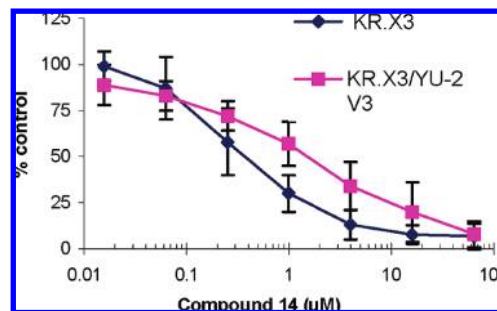


Figure 1. Differential sensitivity of HIV-2 KR.X3 and HIV-2 KR.X3-YU-2/V3 to **14**. A dose-dependent inhibition of the two viruses was determined in the presence of various concentrations of **14** as indicated. Each data point represents the mean \pm SD of four independent experiments.

To explore the effect of additional terminal moieties at the C-28 side chain on anti-HIV-2 activity, type 4 compounds **12–16** were synthesized by coupling a glycine to the carboxylic acid of **7–11**. The addition of a glycine to C-28 side chain of **7** did not significantly change the anti-HIV-2 activity as shown by **12**. However, the same glycine addition resulted in compounds **13** and **14** with reduced anti-HIV-2 activity (approximately 15- and 2-fold reduction) when compared to their precursors **8** and **9**, respectively. On the other hand, 10-fold or more increases in anti-HIV-2 activities were observed for compounds **15** and **16** when compared to their precursors without the glycine addition. Overall, **14** and **15** were the two most potent anti-HIV-2 compounds with a glycine terminus. However, **14** and **15** were 2- to 3-fold less potent compared to the type 3 compounds **8** and **9**. Among the above potent anti-HIV-2 derivatives, **8** and **14** preferentially inhibited HIV-2 with an IC₅₀ of approximately 2 log₁₀ lower than that against HIV-1 (Table 1).

Since both hexanoic-moiety-containing **9** and **14** exhibited the most potent anti-HIV-2 activity from types 3 and 4 compounds, the hexanoic acid was again modified in an attempt to further diversify the C-28 terminus. As a result, additional type 4 compounds **18–21** were synthesized with their C-28 side chains in the form of –NH(CH₂)₅CO-aa, where aa was a mono- or diamino acid. Among the single amino acid coupled hexanoic derivatives, **19**, with a tyrosine terminus, displayed the most potent anti-HIV-2 activity with IC₅₀ at 0.23 μ M. Although all the monoamino acid derivatives remained quite active against HIV-2 (IC₅₀ ranging from 0.23 to 0.63 μ M), these compounds were less potent than **9** (IC₅₀ = 0.15 μ M). For the BA derivatives with a hexanoic moiety, their anti-HIV-2 activity based on their terminus, was in the order of none > tyrosine > glycine = alanine > proline. The dipeptide derivative **21** was approximately 10-fold less potent than **9**.

We have previously shown that the BA derivatives **4** and **17** inhibit HIV-1 entry by targeting the V3 loop of HIV-1 gp120.¹⁷ To determine whether the V3 loop of gp120 is responsible for HIV-2 inhibition, the sensitivities of HIV-2 KR.X3 and HIV-2 KR.X3-YU-2/V3 were determined in the presence of **14**. HIV-2 KR.X3-YU-2/V3 is a chimeric virus containing the V3 loop of HIV-1 YU-2 instead of the original V3 of HIV-2 KR.X3. Compound **14** was used for this study because it was the most potent anti-HIV-2 BA derivative available before **9** was synthesized. Compound **14** inhibited HIV-2 KR.X3 in a dose-dependent manner with an IC₅₀ of 0.35 μ M (Figure 1). The HIV-2 chimeric virus with YU-2 V3

loop, HIV-2 KR.X3-YU-2/V3, was approximately 5-fold less sensitive to **14** when compared to HIV-2 KR.X3. Since the only difference between the two viruses was the V3 loop, the results suggested that the V3 loop of HIV-2 was a possible target of **14**.

Conclusion

In summary, several important SARs for anti-HIV-2 activity of BA derivatives were observed in this study. First, for anti-HIV-2 activity, the optimal length of the C-28 side chain is achieved when $n = 5$ in the form of $-\text{NH}(\text{CH}_2)_n\text{COR}$, as seen for **9** and **14** (Table 1). A gradual drop of anti-HIV-2 activity was observed when n is greater or smaller than 5. Second, in contrast to the anti-HIV-1 BA derivatives, a carboxylic acid terminus of C-28 side chain is important for anti-HIV-2 activity. The potent anti-HIV-1 derivative **3**, along with analogues with various lengths of C-28 side chains that did not possess a carboxylic acid terminus, was completely inactive against HIV-2. Third, the addition of one amino acid to the carboxylic terminus of C-28 side chain moderately compromised the anti-HIV-2 activity of the BA derivatives, especially when the C-28 side chain was at the optimal length with $n = 5$. On the other hand, the addition of one amino acid increased anti-HIV-2 activity when n was 6 or 7. These results provide clues for further characterization of ligand–target interaction and synthesis of a next generation of anti-HIV-2 BA derivatives. In addition to the novel anti-HIV-2 BA derivatives, this study also synthesized a potent HIV-1 entry inhibitor, **3**, with improved anti-HIV-1 activity ($\text{IC}_{50} = 0.027 \mu\text{M}$) when compared to the previously synthesized most potent BA derivative **4** with an IC_{50} of $0.038 \mu\text{M}$.

When comparing anti-HIV-1 and anti-HIV-2 activities, most of the very potent HIV-1 inhibitors, such as **4** and its analogues, appeared to have poor or no activity against HIV-2, and vice versa, for most active anti-HIV-2 compounds, such as **8**, **14**, **18–20**. These observations support our hypothesis that the optimal pharmacophores required to target HIV-1 and HIV-2 are different. In terms of mechanism of action, **14** appeared to block HIV-2 replication by targeting the V3 loop of gp120. The homology between the V3 loops of HIV-1 (YU-2) and HIV-2 (KR) is approximately 30%.²¹ It is likely that the drug binding pocket in HIV-1 V3 loop is structurally and conformationally different from that in HIV-2 V3 loop.

Experimental Section

General Experimental Procedures. Positive or negative HR-FABMS data were recorded on a Shimadzu LCMS-IT-TOF or a Joel SX-102 mass spectrometer. ^1H and other NMR spectra were measured on a Varian Mercury 300 or 500 spectrometer. Samples were dissolved in CDCl_3 or pyridine- d_5 with TMS as an internal standard. Silica gel chromatography was carried out on a Biotage Horizon flash chromatograph with prepacked Si gel column. The purity of BA derivatives was analyzed by using a Varian ProStar HPLC system with a PDA detector and Agilent Zorbax ODS or C-8 column. The mobile phase was composed of solution A (5% acetonitrile in water with 0.045% trifluoroacetic acid) and solution B (water/methanol/acetonitrile = 5:10:85 with 0.045% trifluoroacetic acid). A linear gradient of 80% to 100% of solution B with a flow rate at 1 mL/min or with flow rate at 4 mL/min was used to elute the compounds. The compounds were analyzed with the UV absorption displayed at 220 nm and recorded at a range from 200 to 250 nm. All the tested compounds have purity of 95% or above except for the inactive unmodified **1** (BA), which was purchased from Sigma-Aldrich with 90% purity.

Procedure for Synthesizing Type 1 Compounds 2–5. To a stirring solution of 3-*O*-Ac-BA (0.3–0.7 mmol) in dichloromethane (DCM, 2 mL) was added oxalyl chloride (7–12 equiv). After the mixture was stirred for 10 min, the organic solvent was removed under vacuum. The residue was dissolved in DCM and to it was added the corresponding 1, ω -diaminoalkane (4–5 equiv) in DCM. The mixture was stirred overnight and then concentrated. The residue was washed with water and dissolved in ethanol. After filtration, the ethanolic solution was concentrated and the residue was chromatographed on Si gel to yield the corresponding amine intermediates **2a–5a**. To the corresponding amine intermediates **2a–5a** in pyridine (anhydrous, 1 mL), acetic anhydride (0.5 mL) was added and stirred at room temperature overnight. The mixture was concentrated and redissolved in DCM. After the mixture was washed with 1 N HCl, water, and brine, the organic layer was concentrated and chromatographed on Si gel to yield **2b–5b**.

Procedure for Saponification of Ester at C-3 or/and C-28. Esters were hydrolyzed in a mixture of MeOH/THF/4 N NaOH aq (2:2:1). The intermediates **2b–5b** were dissolved in organic solution (1–2 mL), and aqueous NaOH solution (0.5–1 mL) was added. After being stirred overnight, the reaction mixture was neutralized with aqueous 1 N HCl. The resulting precipitate was washed with water and dried in vacuum to yield the final compounds **2–5**.

Procedure for Synthesizing Type 2 Compound 6. To the mixture of the above amine intermediate **3a** (0.2 mmol) in DCM (2 mL) were added malonic acid (10 equiv), EDC (2 equiv), and Et_3N (10 equiv). After being stirred at room temperature overnight, the mixture was concentrated, redissolved in DCM, washed with water, brine, and dried over Na_2SO_4 . The organic layer was concentrated and chromatographed on Si gel to yield the intermediate **6a**. After the saponification procedure as described above, **6** was obtained as a solid.

Procedure for Synthesizing Type 3 Compounds 7–11. To a stirring solution of 3-*O*-Ac-BA (0.1–0.6 mmol) in DCM (4 mL) was added oxalyl chloride (10 equiv). After the mixture was stirred for 10 min, the organic solvent was removed under vacuum. The residue was dissolved in DCM, then reacted with the corresponding ω -aminoalkanoate (1.2–1.5 equiv) in DCM and Et_3N (6 equiv) overnight. The reaction mixture was diluted with DCM before being washed with water, brine and then dried over Na_2SO_4 . After concentration in vacuum, the residue was chromatographed on Si gel to yield the corresponding **7a–11a**, which yielded the corresponding **7–11** after saponification.

Procedure for Synthesizing Type 4 Compounds 12–16. To a solution of the corresponding intermediates **7a–11a** (0.2 mmol) in DCM (2 mL) were added glycine methyl ester hydrochloride (2.7 equiv), Et_3N (7.5 equiv), and EDC (3.0 equiv). After being stirred overnight at room temperature, the mixture was diluted with DCM, washed with water, brine, and dried over Na_2SO_4 . The organic layer was concentrated under vacuum, and the residue was chromatographed on Si gel. The pure intermediate was collected and subjected to saponification as described above to furnish the corresponding **12–16**.

Procedure for Synthesizing Type 4 Compounds 18–21. The synthesis of these compounds was achieved by the same procedure as described above for **12–16**, using intermediate **9** (for synthesis of **18–20**) or **14** (for synthesis of **21**) and the corresponding amino acid methyl ester hydrochloride.

HIV-1 and HIV-2 Virus Infection Assay. Inhibition of HIV-1_{NL4-3}, HIV-2 KR.X3, or HIV-2 KR.X3-YU2/V3 infection was measured through reduction in luciferase gene expression after a single round of virus infection of TZM-bl cells, as previously described.¹⁷ HIV-2 KR.X3 and HIV-2 KR.X3-YU2/V3 were generously provided by Dr. George Shaw, University of Alabama. For the antiviral assays, 200 TCID₅₀ of virus was used to infect TZM-bl cells in the presence of various concentrations of compounds. Two days after infection, the culture medium was removed from each well and 100 μL of Bright Glo reagent

(Promega, Luis Obispo, CA) was added to the cells for measurement of luminescence using a Victor 3 luminometer. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that caused a 50% reduction of luciferase activity (relative light units) compared to virus control wells.

Cytotoxicity Assay. A CytoTox-Glo cytotoxicity assay (Promega) was used to determine the cytotoxicity of the synthesized BA derivatives. TZM-bl cells were cultured in the presence of various concentrations of the compounds for 2 days. Percent of viable cells was determined by following the protocol provided by the manufacturer. The 50% cytotoxic concentration (TC_{50}) was defined as the concentration that caused a 50% reduction of cell viability.

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Supporting Information Available: Spectroscopic and HPLC analytical data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Jaffar, S.; Grant, A. D.; Whitworth, J.; Smith, P. G.; Whittle, H. The natural history of HIV-1 and HIV-2 infections in adults in Africa: a literature review. *Bull. W. H. O.* **2004**, *82*, 462–469.
- (2) da Silva; Oliveira, I.; Andersen, A.; Dias, F.; Rodrigues, A.; Holmgren, B.; Andersson, S.; Aaby, P. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: Is HIV-2 disappearing?. *AIDS* **2008**, *19*, 1195–1202.
- (3) Wainberg, M. A.; Jeang, K. T. 25 years of HIV-1 research. Progress and perspectives. *BMC Med.* **2008**, *6*, 31.
- (4) Witvrouw, M.; Pannecouque, C.; Van Laethem, K.; Desmyter, J.; De Clercq, E.; Vandamme, A. M. Activity of non-nucleoside reverse transcriptase inhibitors against HIV-2 and SIV. *AIDS* **1999**, *13*, 1477–1483.
- (5) Isaka, Y.; Miki, S.; Kawauchi, S.; Suyama, A.; Sugimoto, H.; Adachi, A.; Miura, T.; Hayami, M.; Yoshie, O.; Fujiwara, T.; Sato, A. A single amino acid change at Leu-188 in the reverse transcriptase of HIV-2 and SIV renders them sensitive to non-nucleoside reverse transcriptase inhibitors. *Arch. Virol.* **2001**, *146*, 743–755.
- (6) Witvrouw, M.; Pannecouque, C.; Switzer, W. M.; Folks, T. M.; De Clercq, E.; Heneine, W. Susceptibility of HIV-2, SIV and SHIV to various anti-HIV-1 compounds: implications for treatment and postexposure prophylaxis. *Antiviral Ther.* **2004**, *9*, 57–65.
- (7) Guyader, M.; Emerman, M.; Sonigo, P.; Clavel, F.; Montagnier, L.; Alizon, M. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature* **1987**, *326*, 662–669.
- (8) Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, C. H.; Lee, K. H. Betulinic acid and dihydrobetulinic acid derivatives as potent anti-HIV agent. *J. Med. Chem.* **1996**, *39*, 1016–1017.
- (9) Huang, L.; Yuan, X.; Aiken, C.; Chen, C. H. Bifunctional anti-HIV-1 small molecules with two novel mechanisms of action. *Antimicrob. Agents Chemother.* **2004**, *48*, 663–665.
- (10) 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.
- (11) Huang, L.; Chen, C. H. The molecular targets of anti-HIV-1 triterpenes. *Curr. Drug Targets: Infect. Disord.* **2002**, *2*, 33–36.
- (12) Soler, F.; Poujade, C.; Evers, M.; Carry, J. C.; Hénin, Y.; Bousseau, A.; Huet, T.; Pauwels, R.; De Clercq, E.; Mayaux, J. F.; Le Pecq, J. B.; Dereu, N. Betulinic acid derivatives: a new class of specific inhibitors of human immunodeficiency virus type 1 entry. *J. Med. Chem.* **1996**, *39*, 1069–1083.
- (13) Hashimoto, F.; Kashiwada, Y.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. Anti-AIDS agent—XXVII. Synthesis and anti-HIV activity of betulinic acid and dihydrobetulinic acid derivatives. *Bioorg. Med. Chem.* **1997**, *5*, 2133–2143.
- (14) Yu, D.; Sakurai, Y.; Chen, C. H.; Chang, F. R.; Huang, L.; Kashiwada, Y.; Lee, K. H. Anti-AIDS agents. 69. Moronic acid and other triterpene derivatives as novel potent anti-HIV agents. *J. Med. Chem.* **2006**, *49*, 5462–5469.
- (15) Huang, L.; Yu, D.; Ho, P.; Lee, K. H.; Chen, C.-H. Synthesis and anti-HIV activity of bi-functional triterpene derivatives. *Lett. Drug Des. Discovery* **2007**, *4*, 471–478.
- (16) 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.
- (17) Lai, W.; 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.
- (18) Zhou, J.; Chen, C. H.; Aiken, C. The sequence of the CA-SP1 junction accounts for the differential sensitivity of HIV-1 and SIV to the small molecule maturation inhibitor 3-O-{3',3'-dimethylsuccinyl}betulinic acid. *Retrovirology* **2004**, *1*–15.
- (19) Li, F.; Zoumplis, D.; Matallana, C.; Kilgore, N. R.; Reddick, M.; Yunus, A. S.; Adamson, C. S.; Salzwedel, K.; Martin, D. E.; Allaway, G. P.; Freed, E. O.; Wild, C. T. Determinants of activity of the HIV-1 maturation inhibitor PA-457. *Virology* **2006**, *356*, 217–224.
- (20) Martin, D. E.; Salzwedel, K.; Allaway, G. P. Bevirimat: a novel maturation inhibitor for the treatment of HIV-1 infection. *Antiviral Chem. Chemother.* **2008**, *19*, 107–113.
- (21) Davis, K. L.; Bibollet-Ruche, F.; Li, H.; Decker, J. M.; Kutsch, O.; Morris, L.; Salomon, A.; Pinter, A.; Hoxie, J. A.; Hahn, B. H.; Kwong, P. D.; Shaw, G. M. Human immunodeficiency virus type 2 (HIV-2)/HIV-1 envelope chimeras detect high titers of broadly reactive HIV-1 V3-specific antibodies in human plasma. *J. Virol.* **2009**, *83*, 1240–1259.