Novel Betulinic Acid–Nucleoside Hybrids with Potent Anti-HIV Activity

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ABSTRACT: Novel betulinic/betulonic acid–nucleoside hybrids were synthesized as possible new anti-HIV agents. Among the synthesized hybrids, two compounds were highly effective against HIV. Compared with AZT and DSB, compounds **10a** (IC₅₀ = 0.0078 μM, CC₅₀ = 9.6 μM) and **10b** (IC₅₀ = 0.020 μM, CC₅₀ = 23.8 μM) showed more potent or equipotent, respectively, anti-HIV activity but displayed lower cytotoxicity.

KEYWORDS: Betulinic acid, nucleoside, hybrid, click chemistry, anti-HIV activity

Betulinic acid (**1**) is a natural pentacyclic triterpene with a lupane structure. Studies have demonstrated that **1** displays various beneficial biological activities, including anticancer, antiviral, antibacterial, and anti-inflammatory effects.^{1–4} Among these bioactivities, anti-HIV activity is a primary interest because of the different anti-HIV mechanisms shown by derivatives of **1** modified at C-3 or C-28. Modification of **1** at C-3 resulted in compounds (e.g., **3**) that inhibited HIV-1 maturation,^{5,6} while modification at C-28 provided compounds (e.g., **4**) that inhibited HIV-1 entry by targeting HIV-1 gp-120.^{7,8} Modification at both C-3 and C-28 resulted in bifunctional derivatives that inhibited HIV-1 entry and maturation.^{9,10} Additionally, betulonic acid (**2**), the 3-oxo analogue of **1**, exerts potent antitumor activity^{11–13} (Figure 1).

Furthermore, the low toxicity and good safety index of **1** make it a promising candidate for clinical application as a therapeutic agent.^{11,14} Unfortunately, the poor solubility of **1** in aqueous solution and many organic solvents is a major obstacle in maximizing its antiviral potency and clinical potential.¹⁵ However, some derivatives of **1**, including certain ionic derivatives,¹⁵ showed improved water solubility and enhanced biological activity compared with unmodified **1**.^{16–18}

Commonly, nucleoside analogues have good water solubility, and some of them are the backbones of antiretroviral agents used in the treatment of AIDS.^{19,20} Thus, we postulated

that conjugation of **1** with nucleosides might result in better water solubility and greater anti-HIV potency. Here we report the synthesis of novel betulinic/betulonic acid–nucleoside hybrids with a 1,2,3-triazole linking C-2 of the triterpenoid acid with C-3' or C-4' of the nucleoside.

The key triterpenoid intermediates **8**, **9**, and **10** were obtained from the starting material betulin (**5**) in three steps according to a slightly modified literature method²¹ (Scheme 1). Ester **7** was obtained from **5** via Jones oxidation followed by esterification using dimethyl carbonate (DMC) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). With DMC and DBU, the esterification reaction was easier and performed under more moderate and safer conditions compared with traditional reagents such as thionyl chloride or diazomethane. Compound **8** was obtained by propargyl α -alkylation of **7** in a KN(SiMe₃)₂/Et₃B system. Then compound **8** was treated with NaBH₄ in isopropanol to preferentially give **9**; the ratio of

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Figure 1. Structures of betulinic acid derivatives.

Scheme 1. Synthesis of 2-Propargyl-Substituted Triterpenoid Derivatives^a

^aReagents and conditions: (a) CrO_3 , H_2SO_4 , acetone, 0 °C, 6 h, 59%; (b) DMC, DBU, reflux, 24 h, 72%; (c) $\text{KN}(\text{SiMe}_3)_2$, Et_3B , propargyl bromide, DME, rt, N_2 , 6 h, 85%; (d) NaBH_4 , $i\text{-PrOH}$, rt, 51%; (e) LiI , DMF, reflux, N_2 , 24 h, 78%.

Scheme 2. Synthesis of Triterpenoid–Nucleoside Hybrids via Click Chemistry

α - and β -OH isomers was about 1:1 in methanol but 1:4 in isopropanol. The nuclear Overhauser effect (NOE) between H-3 and H-23 (see Figure S12) indicated an equatorial position (β orientation) for the OH group, and the NOE effects between H-2 and H-24/H-25 (see Figure S12) suggested an axial position (β orientation) for H-2 and thus an equatorial position (α orientation) for the propargyl group. Furthermore, the spin–spin coupling constant between H-3 and H-2 ($^3J_{\text{H-2,H-3}} = 10.5 \text{ Hz}$, CDCl_3) in the ^1H NMR spectrum of **10** (see Figure S14) was consistent with axial positions for H-2 and H-3. Demethylation of the sterically hindered ester group in **9** via halogenolysis with LiI in DMF

under reflux condition produced **10**, the 2-propargyl derivative of **1**. Compounds **8**, **9**, and **10** were coupled separately with different azides (4'-azido-2'-deoxy-2'-fluoro- β -D-arabinocytidine (AFC), 4'-azido-2'-deoxy-2'-fluoro- β -D-arabinouridine (AFU), and AZT) via click chemistry to produce the target compounds **8a–c**, **9a–c**, and **10a–c**, respectively (Scheme 2).

The solubilities of **1** and **8b** in methanol were determined with the help of quantitative ^1H NMR analysis. The results showed that **8b** had better solubility than **1**; the calculated solubilities of **1** and **8b** in methanol were 4.9 and 52.6 mg/mL (10.7 and 66.3 mmol/L), respectively. Details of the

experiment are shown on page S9 in the [Supporting Information](#).

Compounds **8a–c**, **9a–c**, and **10a–c** were evaluated in an anti-HIV (wild-type) replication assay,²² and the in vitro anti-HIV activity results are listed in [Table 1](#). Cytotoxicity was evaluated using the MTT assay.²²

Table 1. Anti-HIV Activities of Compounds 8a–10c

compound	IC ₅₀ (μM) ^a	CC ₅₀ (MT4 cells, μM) ^b	SI ^c
8a	0.097 ± 0.028	>25.2	>260
8b	— ^d	— ^d	—
8c	— ^d	— ^d	—
9a	0.048 ± 0.018	>25.2	>525
9b	— ^d	— ^d	—
9c	>1.3	>1.3	—
10a	0.0078 ± 0.0020	9.6 ± 0.82	1231
10b	0.020 ± 0.005	23.8 ± 1.79	1190
10c	>1.3	>1.3	—
3	0.021 ± 0.007	>6.84	>326
AFC	0.000262 ± 0.000084	0.09957 ± 0.00863	380
AFU	0.219 ± 0.060	>3.48	>16
AZT	0.018 ± 0.006	>0.37	>20.6

^aIC₅₀ is the 50% HIV inhibitory concentration. The results are presented as mean ± standard deviation (SD) of triplicate experiments. ^bCC₅₀ is the 50% cytotoxic concentration. The results are presented as mean ± SD of triplicate experiments. ^cSelectivity index, defined as SI = CC₅₀/IC₅₀. ^dNo selective anti-HIV activity (SI < 5).

As shown in [Table 1](#), none of the three hybrids conjugated with AZT (**8c**, **9c**, **10c**) displayed significant anti-HIV activity, one of the three hybrids conjugated with AFU (**10b**) showed anti-HIV activity, and all three hybrids conjugated with AFC (**8a**, **9a**, **10a**) exerted potent anti-HIV activity. Compared with the positive controls AZT and **3**, compounds **10a** and **10b** showed greater and comparable potency, respectively, as well as lower cytotoxicity. Although compound **10b** (the hybrid of **1** and AFU) was less potent than **10a** (the hybrid of **1** and AFC), it was also less cytotoxic, resulting in similar selectivity index (SI) values (**10a**, 1231; **10b**, 1190). These values were higher than the SI of 380 for AFC, the most potent compound, which exhibits nanomolar anti-HIV activity. Moreover, the three triterpenoid–AFC hybrids had the following order of potency: **10a** (3β-OH, 17-COOH) > **9a** (3β-OH, 17-COOMe) > **8a** (3-oxo, 17-COOMe). Also, comparison of the structural differences between **9a–c** and **10a–c** shows that the betulinic acid scaffold with a 17-COOH is preferred over its methyl ester analogues.

In summary, we synthesized a novel class of betulinic/betulonic acid–nucleoside hybrids. From this class, we identified two analogues, **10a** and **10b**, with highly potent anti-HIV activity. Although the hybrid compounds were less potent than the nucleoside (AFC) itself, they were also less cytotoxic and perhaps would be safer therapeutic agents. Mechanism studies, further optimization of activity, and determination of efficacy in animal models will be reported in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00414>.

Biological assays, synthetic methods, solubility determination, chemical characterization of final products, ¹H and ¹³C NMR spectra, and HRMS spectra (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AFC, 4'-azido-2'-deoxy-2'-fluoro-β-D-arabinocytidine; AFU, 4'-azido-2'-deoxy-2'-fluoro-β-D-arabinouridine; CC₅₀, 50% cytotoxic concentration; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMC, dimethyl carbonate; IC₅₀, 50% HIV inhibitory

concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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