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Synthesis and anti–HIV activities of symmetrical dicarboxylate esters of dinucleoside reverse transcriptase inhibitors

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Abstract— Three nucleoside analogues, 3′–fluoro–2′,3′–dideoxynucleosides (FLT), 3′–azido–2′,3′–dideoxynucleosides (AZT), and 2′,3′–dideoxy–3′–thiacytidine (3TC) were conjugated with three different dicarboxylic acids to afford the long chain dicarboxylic esters of nucleosides. In general, dinucleoside ester conjugates of FLT and 3TC with long chain dicarboxylic acids exhibited higher anti–HIV activity than their parent nucleosides. Dodecanoate and tetradecanoate dinucleoside ester derivatives of FLT were found to be the most potent compounds with EC50 values of 0.8–1.0 nM and 3–4 nM against HIV–1H9 and HIV–1IM cells, respectively. The anti–HIV activity of the 3TC conjugates containing long chain dicarboxylic diester (EC50 = 3–60 nM) was improved by 1.5–66 fold when compared to 3TC (EC50 = 90–200 nM). This study reveals that the symmetrical ester conjugation of dicarboxylic acids with a number of nucleosides results in conjugates with improved anti–HIV profile.

Nucleoside reverse transcriptase inhibitors (NRTIs) are commonly used for the treatment of human immunodeficiency virus (HIV) infection. NRTIs exert their pharmacological effects after intracellular phosphorylation to monophosphates, diphosphates, and triphosphates in the presence of the host cellular kinases. Nucleoside triphosphates inhibit the reverse transcriptase, a crucial enzyme for HIV life cycle, through substrate competition with natural deoxynucleosides and incorporation into the viral DNA that leads to the chain termination.1,2 Some examples of commercially available NRTIs are 2′,3′–dideoxy–3′–thiacytidine (3TC), 2′,3′–dideoxy–2′,3′–dideoxynucleosides (d4T), 2′,3′–dideoxy–5′–fluoro–3′–thiacytidine (FTC), 2′,3′–dideoxycytidine (ddC), 3′–azido–3′–deoxynucleosides (AZT), and (R)–9–(2–phosphonomethoxylpropyl)adenine (TFV). In spite of the potent anti–HIV activities, NRTIs face major challenges such as high levels of clinical toxicity including bone marrow suppression and neuropathy, drug resistance, low therapeutic index, and/or mitochondrial toxicity.3–6 Combination therapy in Highly Active Antiretroviral Therapy (HAART) programs using different classes of anti–HIV drugs provides several potential advantages, such as reducing the drug resistance and controlling HIV–1 infection.3

Despite the success of HAART, the hydrophilic nature of NRTIs leads to limited cellular uptake and bioavailability. Various nucleoside prodrugs have been synthesized in the past to overcome these short comings.3,5,6 We have previously reported the synthesis of lipophilic fatty acyl esters of anti–HIV nucleosides; AZT, 3′–fluoro–2′,3′–dideoxynucleosides (FLT), 3TC, and d4T.9–13 Lipophilic fatty acyl esters of anti–HIV nucleosides demonstrated higher anti–HIV activity compared to the parent nucleosides.10,14 Cellular uptake studies indicated that the long chain fatty acyl esters of 3TC had improved cellular uptake due to improved lipophilicity and were able to hydrolyze to parent nucleoside intracellularly.14

Herein, we report the synthesis of long chain dicarboxylic acid esters of FLT, AZT and 3TC to determine whether the presence of two nucleosides on fatty acyl chains will affect the overall anti–HIV activity. Three long dicarboxylic acids with chain lengths of C10 (sebacic acid), C12 (dodecanoic acid), and C14 (tetradecanoic acid) were selected for the conjugation. The conjugates were expected to have high lipophilic nature because of the presence of long chain dicarboxylic esters and thereby to have improved cellular uptake properties. We have previously shown the lipophilic nature and/or higher cellular uptake of the fatty acyl esters of the nucleosides compared to the their parent nucleosides.9,10,14 Furthermore, the conjugation of anti–HIV nucleosides with three long chain dicarboxylic acids will provide insights about the structure–activity relationships of these conjugates. Once the ester enters the cells, it is expected to release two parent nucleosides after intracellular ester hydrolysis as shown previously with fatty acyl ester conjugates of 3TC.14 The results of this study demonstrate that conjugation of two similar nucleosides with dicarboxylic fatty acids yields anti–HIV agents having enhanced lipophilicity and increased potency. These conjugates were envisioned and designed to be used as topical anti–HIV microbicides, which are topically applied agents that prevent or reduce transmission of HIV/AIDS.

Symmetrical long chain fatty acyl dicarboxylate esters of FLT–FLT, AZT–AZT, and 3TC–3TC were synthesized. Scheme 1 depicts the general synthesis of
dinucleoside dicarboxylate ester derivatives of FLT–FLT (6–8) and AZT–AZT (9–11). The compounds were synthesized by reaction of commercially available nucleosides with three different dicarboxylic acids (sebacic acid, dodecandioic acid, and tetradecandioic acid) in the presence of 1,1,3,3-tetramethylyuronium hexafluorophosphate (HBTU) and DIPEA as coupling reagent and N,N-diisopropylethylamine (DIPEA) as the base (Scheme 1) in DMF at room temperature. The final products were purified by using a reversed phase HPLC.

The chemical structures of the final products were characterized by nuclear magnetic resonance spectrometry (1H NMR and 13C NMR), and were confirmed by a high-resolution PE Biosystems Mariner API time–of–flight electrospray mass spectrometer. The purity of the final products (>95%) was confirmed by using a Hitachi analytical HPLC system on a C18 column using a gradient system (water:acetonitrile 30:70 v/v) at constant flow rate of 1 mL/min with UV detection at 265 nm.

Synthesized dinucleoside ester conjugates were evaluated for their ability to inhibit HIV–1 (subtype B, US/92/727) and (subtype IIIB) replication in human PBMC and CEM–SS cells, respectively.16,17 Table 1 illustrates the anti–HIV–1 activity (EC50) and cytotoxicity (TC50) of the synthesized compounds compared with their corresponding parent nucleosides. No cytotoxicity was observed up to the highest tested concentration (TC50 > 1000 nM) for the synthesized conjugates.

The anti–HIV activity of the 3TC conjugates containing long chain dicarboxylate (22–24, EC50 = 3–60 nM) was improved by 1.5–66 fold when compared to 3TC (EC50 = 90–200 nM). The 3TC ester of tetradecandicarboxylic acid (24, EC50 = 3–30 nM) showed 3–66 times enhanced anti–HIV activity in comparison to 3TC. Dodecandicarboxylic ester of 3TC (23, EC50 = 9–20 nM) exhibited 4.5–22–fold higher anti–HIV activity than 3TC. The activity of decanoate ester of 3TC (22) was 1.5–6.6 times higher than 3TC. However, succinate ester of 3TC (7, EC50 = 130–150 nM) had comparable anti–HIV activity with 3TC (EC50 = 90–200 nM). These data indicate that the anti–HIV activity of dicarboxylate ester conjugates of dinucleosides depends on the chain length of the spacer between two nucleosides. The optimal antiviral activity for 3TC conjugates was obtained with dodecandioic and tetradecandioic ester conjugates.

In general, dinucleoside ester conjugates of FLT (6–8) and 3TC (22–24) with long chain dicarboxylic acids exhibited higher anti–HIV activity than their parent nucleosides. On the other hand, the AZT conjugates (9–11, EC50 = 2–5 nM) showed comparable activity with AZT (EC50 = 2–8 nM) against HIV in both PBMC and CEM–SS assays.

The dicarboxylic esters of FLT (6–8, EC50 = 0.76–1 nM) exhibited slightly higher anti–HIV activity than their parent nucleoside FLT in PBMC assay against HIV–1US/92/727. Among all the conjugates, FLT esters 7 and 8 were the most potent conjugates against HIV in the series with EC50 values of 0.97 and 0.76 nM, respectively, that was approximately 2 times higher than that of FLT (EC50 = 2 nM). The anti–HIV activity of FLT conjugates (6–8, EC50 = 3–4 nM) in CEM–SS’s against HIV–1IIIB was found to be 5–6.6 times higher.
than FLT (EC_{50} = 20 nM). The attachment of the long chain dicarboxylic acid analogues to 1 and 12 enhanced their lipophilicity as shown by calculated partition coefficients (Log P) (Table 1). There was a correlation between lipophilicity and anti-HIV activity of the compounds since more lipophilic compounds exhibited higher anti-HIV activity compared to polar parent nucleoside analogues. As shown previously with other fatty acyl derivatives of nucleosides, the highly lipophilic conjugates could have higher cellular uptake that contributed to their improved anti-HIV activity.

The anti-HIV activity of 1 (FLT, EC_{50} = 2 nM) in PBMC’s against HIV-1 US/92/727 was found to be 4 and 45 times higher than 2 (AZT, EC_{50} = 8 nM) and 12 (3TC, EC_{50} = 45 nM), respectively. Similarly, the FLT esters of long chain dicarboxylic acids (6–8, EC_{50} = 0.76–1.0 nM) were more potent than the corresponding AZT conjugates (9–11, EC_{50} = 2.0–4.0 nM) and 3TC conjugates (22–24, EC_{50} = 20–60 nM).

A number of symmetrical 5′–O–substituted dicarboxylate ester derivatives of NRTIs were synthesized, and their anti-HIV activity was evaluated. In general, the conjugation of selected long chain dicarboxylic acids NRTIs resulted in better anti-HIV profiles than the corresponding parent nucleosides.

Among all the dinucleoside diester derivatives, FLT–FLT conjugates 7 and 8 were found to have better anti–HIV activity than 1 and the other dicarboxylate dinucleoside derivatives. The data indicate that conjugation of the NRTIs with dicarboxylic acids is an effective strategy in achieving higher anti–HIV activity possibly by improving the lipophilicity and potentially cellular uptake of the parent nucleosides. These data provide the basis for rational optimization of NRTIs through conjugation of two nucleosides with long chain dicarboxylic acids.

### Acknowledgements

We acknowledge the National Science Foundation, Grant Number CHE 0748555 for the financial support and National Center for Research Resources, NIH, and Grant Number 1 P20 RR16457 for sponsoring the core facility. The authors wish to acknowledge the technical contribution of Dr. Lu Yang and Ms. Ashlee Boczar for the antiviral assays described herein.

### Supplementary data

Supplementary data (experimental synthetic procedures and characterization of compounds using ^1^H NMR and ^13^C NMR) are available as a part of the supplementary material. Additionally, calculated partition coefficient using ChemBioDraw Ultra 12.0 is provided for all compounds.

### Table 1. Anti-HIV activity of dicarboxylic acid esters of dinucleoside conjugates.

<table>
<thead>
<tr>
<th>Compd</th>
<th>Chemical Name</th>
<th>PBMC/HIV-1 US/92/727</th>
<th>CEM-SS/HIV-1m</th>
<th>Log P (Calcd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC_{50} (nM)^a</td>
<td>TC_{50} (nM)^b</td>
<td>EI</td>
</tr>
<tr>
<td>1</td>
<td>3′-fluoro-2′,3′-deoxothymidine</td>
<td>2.0</td>
<td>&gt;500</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2</td>
<td>3′-azido-2′,3′-dideoxothymidine</td>
<td>8.0</td>
<td>&gt;1000</td>
<td>&gt;500</td>
</tr>
<tr>
<td>12</td>
<td>(-)-2′,3′-dideoxy-3′-thiacytidine</td>
<td>90.0</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>6</td>
<td>1,10-di-FLT-decanoate</td>
<td>1.0</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>7</td>
<td>1,12-di-FLT-dodecanoate</td>
<td>0.97</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>8</td>
<td>1,14-di-FLT-tetradecanoate</td>
<td>0.76</td>
<td>&gt;1000</td>
<td>&gt;1301</td>
</tr>
<tr>
<td>9</td>
<td>1,10-di-AZT-decanoate</td>
<td>4.0</td>
<td>&gt;1000</td>
<td>&gt;250</td>
</tr>
<tr>
<td>10</td>
<td>1,12-di-AZT-dodecanoate</td>
<td>2.0</td>
<td>&gt;1000</td>
<td>&gt;500</td>
</tr>
<tr>
<td>11</td>
<td>1,14-di-AZT-tetradecanoate</td>
<td>3.0</td>
<td>&gt;1000</td>
<td>&gt;333</td>
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<td>21</td>
<td>1,4-di-3TC-succinate</td>
<td>150.0</td>
<td>&gt;1000</td>
<td>&gt;7</td>
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<tr>
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<td>&gt;1000</td>
<td>&gt;17</td>
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<td>23</td>
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<td>20.0</td>
<td>&gt;1000</td>
<td>&gt;50</td>
</tr>
<tr>
<td>24</td>
<td>1,14-di-3TC-tetradecanoate</td>
<td>30.0</td>
<td>&gt;1000</td>
<td>&gt;33</td>
</tr>
</tbody>
</table>

^a^EC_{50} (50% effective concentration), All the assays were carried out in triplicate (n = 3); ^b^TC_{50} (50% toxic concentration), All the assays were carried out in triplicate (n = 3); ^c^Theoretical index (TC_{50}/EC_{50}); ^d^Calculated partition coefficient using ChemBioDraw Ultra 12.0; ^e^Not determined.
NMR, $^{13}$C NMR, and HR–MS (ESI–TOF), and anti-HIV assays) can be found in the online version of this article.

**References and notes**