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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6350–6353
Bioorganic & Medicinal Chemistry Letters

Some new acyclic nucleotide analogues as antiviral prodrugs: Synthesis and bioactivities in vitro

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Received 12 April 2007; revised 22 August 2007; accepted 28 August 2007
Available online 31 August 2007

Abstract—A series of ester analogues of acyclic nucleotide PMPA and PMEA were synthesized as potent antiviral agents. The antiviral evaluation results indicated that bis benzyl ester prodrug of PMPA **5f** and bis allyl ester prodrug of PMEA **5g** exhibited potent antiviral activities. The IC_{50} of **5f** for HBV was 2.15 μM, and the IC_{50} of **5g** for HIV-1 was 1.61 μM.

Nucleoside and nucleotide analogues play an important role in the treatment of diseases caused by viruses, such as HIV, HBV, herpes, et al.[1,2] In recent years, acyclic nucleoside and nucleotide analogues have attracted wide interest as antiviral drugs.[3–5]

PMEA (adefovir) **1** and PMPA (tenofovir) **2** (Fig. 1) are acyclic nucleoside phosphonates (ANP) with demonstrated activity against HIV and HBV.[6,7] They both contain a single phosphate group, which permits efficient phosphorylation to its active metabolite adefovir diphosphate or tenofovir diphosphate by constitutively active kinases in many kinds of cells.[8] Because PMEA and PMPA are not orally bioavailable, many kinds of derivatives of them are developed as prodrugs,[9–15] but only two bis ester prodrugs are currently approved by FDA, that is, adefovir dipivoxil[16] **3** and tenofovir disproxil fumarate (Tenofovir DF)[17] **4** (Fig. 1), they were approved to treat hepatitis B in 2001 and AIDS in 2003, respectively. They both can be rapidly converted into PMEA and PMPA following absorption.[18,19]

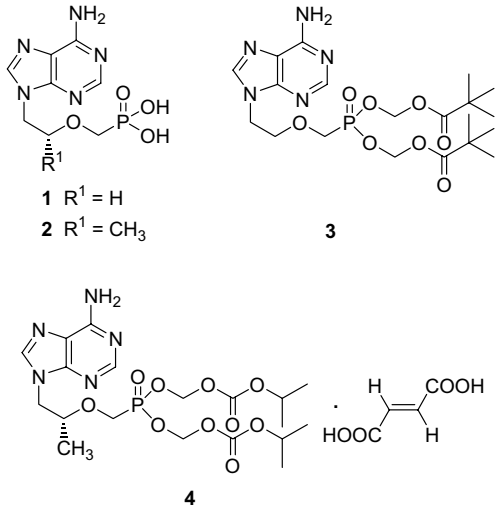

Figure 1. Structures of PMEA/PMPA and their bis ester prodrugs used in clinic.

Although adefovir dipivoxil and tenofovir DF have shown potent activity against HBV and HIV, the study of structure-activities relationship (SAR) of other ester derivatives of PMEA and PMPA has been rarely reported.[20] For studying SAR of ester analogues of PMEA and PMPA, some ester derivatives with different side-chain, including alicyclic, unsaturated acyclic, and aryl group (Fig. 2), were synthesized to expand SAR of this kind of compounds and to find some compounds with potent antiviral activity.

Keywords: Nucleotide prodrugs; Antiviral; HIV-1; HBV.
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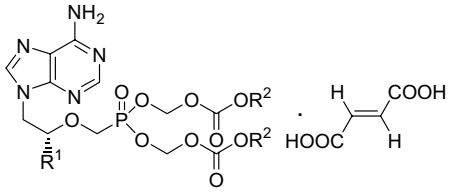
doi:10.1016/j.bmcl.2007.08.065

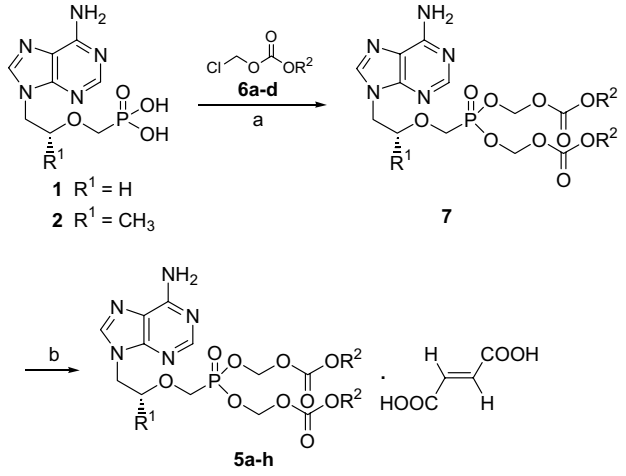


Compds	R^1	R^2
5a	CH_3	
5b	H	
5c	CH_3	
5d	H	
5e	CH_3	
5f	H	
5g	CH_3	
5h	H	

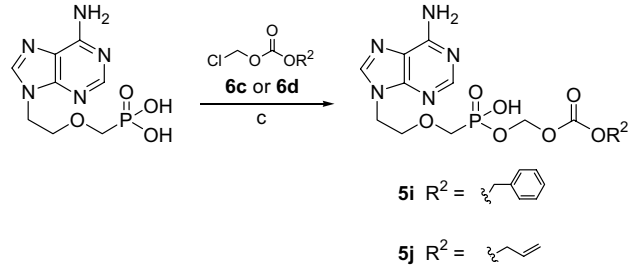
Figure 2. The structures of synthesized bis ester derivatives of PMEA/PMPA in this work.

The target bis ester compounds **5a**–**h** were synthesized as shown in Scheme 1. PMEA or PMPA with excess chloromethyl alkyl carbonate **6** (TEA, 1-methyl-2-pyrrolidinone, 80 °C for 0.5 h, then 60 °C for 4 h) gave crude **7** that were converted into corresponding fumarates **5a**–**h** (50–70% yield).

When PMEA reacted with 1.2 equivalent of chloromethyl alkyl carbonate **6c** or **6d**, monoester derivatives **5i**,**5j** were given in 60–70% yield (Scheme 2). Compounds **5a**–**j** were well characterized through the spectral characteristics.[21]



Scheme 1. Synthesis of phosphate bis ester derivatives of PMEA/PMPA. Reagents and conditions: (a) 4.5 equiv **6**, 2.85 equiv TEA, 1-methyl-2-pyrrolidinone, 80–60 °C; (b) 1 equiv fumaric acid, 2-propanol, 50 °C.

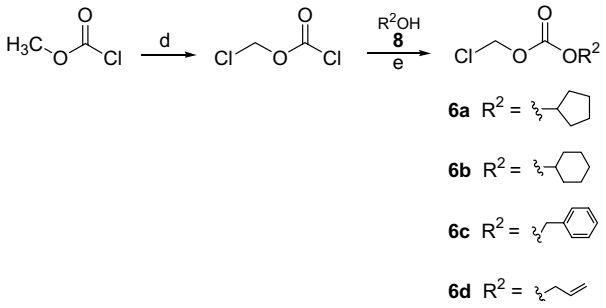


Scheme 2. Synthesis of phosphate monoester derivatives of PMEA. Reagents and conditions: (c) 1.2 equiv **6**, 2.85 equiv TEA, 1-methyl-2-pyrrolidinone, 80–60 °C.

Chloromethyl alkyl carbonates **6a**–**d** were synthesized in 60–75% yield (Scheme 3).

PMEA and PMPA were synthesized according to the method reported by Holy and Rosenberg[22] and Munger et al. [16], respectively.

Anti-HBV activity in cell culture. The anti-HBV activity of compounds **5a**–**j** together with PMPA, PMEA, and



Scheme 3. Synthesis of chloromethyl alkyl carbonate. Reagents and conditions: (d) 5 equiv sulphuryl chloride, 0.01 equiv ABN, reflux, 10 h; (e) 1.2 equiv **8**, 2 equiv pyridine, cyclohexane, 0 °C, then rt, 12 h.

Table 1. Anti-HBV activity of tested compounds in 2.2.15 cells

Compound	IC_{50} (μM)	TC_{50} (μM)	T.I[a]
PMPA	12.3	0.39×10^3	31.4
PMEA	42.1	1.83×10^3	43.5
Tenofovir DF	5.10	55.2	10.8
5a	7.20[b]	80.2	—
5b	2.46[b]	22.1	—
5c	5.94	92.8	15.6
5d	5.33	63.2	11.9
5e	5.50	70.1	12.7
5f	2.15	26.5	12.3
5g	7.98[b]	30.9	—
5h	8.20[b]	24.6	—
5i	43.0	0.59×10^3	13.7
5j	10.6[b]	0.67×10^3	—

[a] In vitro therapeutic index (TC_{50} /IC_{50}).
[b] Exhibit cell toxicity under experimental concentration.

Table 2. Anti-HIV-1 IIIB activity in MT-4 cells

Compound	Tenofovir DF	Emtricitabine	**5a**	**5b**[a]	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**
IC_{50} (μM)	2.31	27.7	>30	3.51	17.5	23.1	>30	>30	1.61	>30	>30	23.7

[a] Exhibited cell toxicity under 10 μg/ml concentration.

Tenofovir DF (used as positive controls) in 2.2.15 cells (an HBV-transfected human HepG2 cell line) in vitro was tested. The 2.2.15 cells were seeded in 96-well plate and treated with the test compounds at 37 °C for 9 days. Then the cells were harvested and intracellular DNA was extracted. Inhibition of the viral replicative intermediate DNA in compound-treated cells versus untreated cells was determined. Cell viability was assessed by the MTT assay. The 50% inhibitory concentration (IC_{50}) and 50% toxic concentration (TC_{50}) of the test compounds are reported in Table 1.

Among the tested compounds, the best anti-HBV activity was exhibited by **5f** with an IC_{50} of 2.15 μM, superior to not only PMEA and PMPA, but also Tenofovir DF (IC_{50} = 5.10 μM); The anti-HBV activity of **5c**–**e** (IC_{50} ranging from 5.33 to 5.94 μM) were similar to Tenofovir DF. **5a**–**b**, **5g**–**h**, and **5j** exhibited cell toxicity in low concentration (ranging from 2.46 to 10.6 μM).

Based on the above activity results, some comments could be made about the SAR investigation. Compounds with benzyl and cyclohexyl side-chain displayed potent anti-HBV activity, but those with allyl and cycloamyl side-chain showed cell toxicity. In this respect, derivatives of PMPA were the same as PMEA. Moreover, the anti-HBV activity of bis ester derivative **5f** was higher than monoester derivative **5i**.

Anti-HIV-1 activity in cell culture. The anti-HIV activity of compounds **5a-j** on HIV-1 replication was monitored in terms of their inhibition of HIV-1 p24 antigen in MT-4 cell culture. Tenofovir DF and Emtricitabine were tested as positive controls. MT-4 cells (2×10^5/mL) infected with HIV-1 (IIIB) at 100 $TCID_{50}$ per ml were plated into 96-well plate and incubated in the presence of various concentrations of test compounds. After four days of culture at 37 °C and 5% CO_2 in a carbon-dioxide incubator, the culture supernatants were collected and examined for their p24 antigen levels. Cell viability was quantified by MTT assay. The IC_{50} of tested compounds is summarized in Table 2.

The results showed that all compounds possess anti-HIV-1 IIIB activity (The inhibition rates of **5a**, **5e**, **5f**, **5h**, and **5i** are 25.5, 28.1, 24.9, 22.8, and 9.0%, respectively, under 10 μg/ml concentration). Among them, **5g** exhibited more potent activity with the IC_{50} of 1.61 μM than Tenofovir DF (IC_{50} = 2.31 μM) and Emtricitabine (IC_{50} = 27.7 μM). The IC_{50} of **5b** was 3.51 μM, but exhibited strong cell toxicity under 10 μg/ml concentration.

To summarize, we have synthesized a series of nucleotide analogues as antiviral agents. The compounds were evaluated for their activities and some of them displayed potent antiviral effect in vitro. The antiviral effect indicated that **5f** and **5g** exhibited favorable anti-HBV and anti-HIV-1 activity, respectively, and they are both promising for further studies.

Acknowledgment

This work was supported in part by grants from the National Basic Research Program of China (973 program, 2004CB518900).

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21. Physical and spectral characteristics of compound (**5a**): whitish solid, mp 110–111 °C, $[\alpha]_D^{25}$ −3.21 (c = 0.04, CH_3OH). ^1H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.02 (s, 1H), 7.23 (br), 6.62 (s, 2H), 5.55–5.48 (m, 4H), 5.06–5.00 (m, 2H), 4.26–4.12 (m, 2H), 4.03–3.97 (m, 3H), 1.98–1.56 (m, 16H), 1.04 (d, J = 6.0 Hz, 3H). MS (ESI) m/z: 572 $(M+H)^+$.
Compound (**5b**): whitish solid, mp 136–137 °C. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.06 (s, 1H), 7.24 (br), 6.62 (s, 2H), 5.58–5.50 (m, 4H), 5.05–5.01 (m, 2H), 4.31 (t, J = 5.1 Hz, 2H), 3.99 (d, J = 8.1 Hz, 2H), 3.87 (t, J = 5.1 Hz, 2H), 1.83–1.54 (m, 16H). MS (ESI) m/z: 558 $(M+H)^+$.
Compound (**5c**): whitish solid, mp 118–119 °C, $[\alpha]_D^{25}$ −2.47 (c = 0.03, CH_3OH). ^1H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.03 (s, 1H), 6.63 (s, 2H), 5. 56–5. 47 (m, 4H), 4.60–4.54 (m, 2H), 4.28–4.12 (m, 2H), 3.99–3.90 (m, 3H), 1.14–1.80 (m, 20H), 1.05 (d, J = 6.0 Hz, 3H). MS (ESI) m/z: 600 $(M+H)^+$.
Compound (**5d**): whitish solid, mp 114–117 °C. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.12 (s, 1H), 8.05 (s, 1H), 7.22 (br), 6.62 (s, 2H), 5.59–5.51 (m, 4H), 4.60–4.53 (m, 2H), 4.30 (t, J = 5.1 Hz, 2H), 3.99 (d, J = 7.8 Hz, 2H), 3.86 (t, J = 5.1 Hz, 2H), 1.80–1.17 (m, 20H). MS (ESI) m/z: 586 $(M+H)^+$.
Compound (**5e**): whitish solid, mp 101–102 °C, $[\alpha]_D^{25}$ −2.55 (c = 0.03, CH_3OH). ^1H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.00 (s, 1H), 7.38–7.34 (m, 10H), 7.23 (br), 6.62 (s, 2H), 5.58–5.49 (m, 4H), 5.18 (s, 4H), 4.23–4.08 (m, 2H), 3.97–3.89 (m, 3H), 1.01 (d, J = 6.0 Hz, 3H). MS (ESI) m/z: 616 $(M+H)^+$.
Compound (**5f**): whitish solid, mp 109–110 °C. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.03 (s, 1H), 7.39–7.34 (m, 10H), 7.22 (br), 6.62 (s, 2H), 5.60–5.53 (m, 4H), 5.18 (s, 4H), 4.27 (t, J = 4.8 Hz, 2H), 3.97 (d, J = 7.8 Hz, 2H), 3.83 (t, J = 4.8 Hz, 2H). MS (ESI) m/z: 602 $(M+H)^+$.
Compound (**5g**): whitish solid, mp 93–94 °C, $[\alpha]_D^{25}$ −4.25 (c = 0.03, CH_3OH). ^1H NMR(DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.02 (s, 1H), 7.24 (br), 6.62 (s, 2H), 5.96–5.85 (m, 2H), 5.59–5.50 (m, 4H), 5.36–5.23 (m, 4H), 4.66 (d, J = 5.7 Hz, 4H), 4.26–4.16 (m, 2H), 4.00–3.93 (m, 3H), 1.05 (d, J = 6.0 Hz, 3H). MS (ESI) m/z: 516 $(M+H)^+$.
Compound (**5h**): whitish solid, mp 99–101 °C. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 8.06 (s, 1H), 7.26 (br), 6.62 (s, 2H), 5.97–5.85 (m, 2H), 5.61–5.55 (m, 4H), 5.36–5.24 (m, 4H), 4.66 (d, J = 5.4 Hz, 4H), 4.31 (t, J = 4.8 Hz, 2H), 4.00 (d, J = 7.8 Hz, 2H), 3.87 (t, J = 6.0 Hz, J = 4.8 Hz, 2H). MS (ESI) m/z: 502 $(M+H)^+$.
Compound (**5i**): colorless oil. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.15 (s, 1H), 8.11 (s, 1H), 7.36–7.30 (m, 5H), 7.18 (br), 5.38–5.34 (m, 2H), 5.11 (s, 2H), 4.26–4.22 (m, 3H), 3.78–3.73 (m, 3H). MS (ESI) m/z: 438 $(M+H)^+$.
Compound (**5j**): colorless oil. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.18 (s, 1H), 8.11 (s, 1H), 7.16 (br), 5.96-5.89 (m, 1H), 5.37–5.19 (m, 4H), 4.57 (d, J = 5.4 Hz, 2H), 4.30–4.25 (m, 2H), 3.85 (d, J = 7.5 Hz, 2H), 3.77 (t, J = 4.8 Hz, 2H). MS (ESI) m/z: 388 $(M+H)^+$.
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