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ANTIVIRAL ACTIVITY OF NOVEL 5-PHOSPHONO-PENT-2-EN-1-YL NUCLEOSIDES AND THEIR ALKOXYALKYL PHOSPHONOESTERS*  

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Running Title: Antiviral Activity of the 5-Phosphono-pent-2-en-1-yl Nucleosides

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ABSTRACT

Three acyclic nucleoside phosphonates are currently approved for clinical use against infections caused by cytomegalovirus (Vistide®), hepatitis B virus (Hepsera®) and human immunodeficiency virus (HIV-1) (Viread®). This important antiviral class inhibits viral polymerases after cellular uptake and conversion to their diphosphates, bypassing the first phosphorylation which is required for conventional nucleoside antivirals. Small chemical alterations in the acyclic side chain lead to marked differences in antiviral activity and the spectrum of activity of acyclic nucleoside phosphonates against various classes of viral agents. We synthesized a new class of acyclic nucleoside phosphonates based on a 5-phosphono-pent-2-en-1-yl base motif in which the oxygen heteroatom usually present in acyclic nucleoside phosphonates has been replaced with a double bond. Since the intrinsic phosphonate moiety leads to low oral bioavailability and impaired cellular penetration, we also prepared the hexadecyloxypropyl esters of the 5-phosphono-pent-2-en-1-yl nucleosides. Our earlier work showed that this markedly increases antiviral activity and oral bioavailability. Although the 5-phosphono-pent-2-en-1-yl nucleosides themselves were not active, the hexadecyloxypropyl esters were active against DNA viruses and hepatitis B virus, in vitro. Notably, the hexadecyloxypropyl ester of 9-(5-phosphono-pent-2-en-1-yl)-adenine was active against hepatitis B mutants resistant to lamivudine, FTC and adefovir.
INTRODUCTION

The acyclic nucleoside phosphonates (ANPs) are an important class of antiviral drugs that are approved for treatment of viral infections, including infections with cytomegalovirus (cidofovir, Vistide®), hepatitis B virus (adefovir dipivoxil, Hepsera®) and HIV-1 (tenofovir disoproxil fumarate, Viread®) (11, 12). To show antiviral activity, ANPs must 1) undergo intracellular activation to the diphosphate, 2) compete with endogenous deoxynucleotide triphosphates for binding to the viral polymerase, and 3) incorporate into the nascent DNA and 4) terminate viral replication. ANPs possess an enzymatically stable phosphonomethyl ether which, unlike conventional nucleoside antivirals, makes them independent of the first intracellular phosphorylation step.

In general, antiviral nucleosides and ANPs consist of a base moiety and a carbohydrate mimic. Structural features of the carbohydrate mimic influence the ability of the nucleoside analog to participate in the antiviral mechanism. For example, the 5′-triphosphates of stavudine (d4T, 2′,3′-didehydro-3′-deoxythymidine) and abacavir (Ziagen, (1S,4R)-cis-4-[2-amino-6-chloro-9H-purin-9-yl]-2-cyclopentene-1-methanol), are potent inhibitors of HIV replication, and the presence of a double bond in the sugar moiety appears to play a role in binding to the HIV reverse transcriptase (9). Likewise, potent antiviral activity is found in ANPs possessing a phosphonomethoxyethyl side chain; changes in the acyclic side chain or in the nucleoside base modulate activity and the antiviral spectrum against various virus classes (15).

We now describe the antiviral evaluation of a new series of acyclic nucleoside phosphonate analogs, the 5-phosphonopent-2-en-yl (PPen) nucleosides, which
incorporate a double bond into the phosphonomethyl ether side chain (Figure 1). Since ANPs, as a class, are not readily taken up by cells because of their anionic character, we also prepared and evaluated hexadecyloxypropyl esters of each PPen nucleoside because this strategy has been shown to increase the antiviral activity of cidofovir (4, 22, 23, 36), (S)-HPMPA (5) and several other classes of ANPs (33, 35). The compounds and their hexadecyloxypropyl esters were evaluated for in vitro activity against CMV, herpes simplex virus (HSV), vaccinia virus (VV), cowpox virus (CV), HIV-1, varicella zoster virus (VZV), wild type and drug resistant hepatitis B virus (HBV) and Epstein Barr virus (EBV).

MATERIALS AND METHODS

Synthesis of PPen Nucleosides

The details of the syntheses of the PPen nucleosides and their hexadecyloxypropyl esters will be published elsewhere. Proof of structure and purity (>98%) of all compounds was confirmed by $^1$H and $^{31}$P nuclear magnetic resonance, electrospray ionization mass spectrometry and thin layer chromatography (silica gel plates with visualization by UV light, Phospray (Supelco, Bellefonte, PA) and charring at 400 °C).

Cells and Viruses

Human foreskin fibroblast (HFF) cells were prepared as primary cultures and used in the CMV, VZV, VV and CV assays. CMV strain AD-169 and VZV strain Ellen were propagated using standard virological techniques as reported previously (38). VV strain Copenhagen and CV strain Brighton were kindly provided by John W. Huggins (Department of Viral Therapeutics, Virology Division, U.S. Army Medical Research...
Institute of Infectious Disease, Frederick, MD). Working stocks of these viruses were propagated in Vero cells obtained from the American Type Culture Collection (ATCC, Manassas, VA). MRC-5 human lung fibroblast cells were propagated as described previously (4, 19).

**Plaque reduction assays in HFF cells**

The plaque reduction assays for CMV, VV, CV and VZV were performed as described previously (5, 37, 38) and standard methods were used to determine the compound concentration required to reduce plaque formation by 50% (EC$_{50}$).

**Enzyme-Linked Immunosorbent Assay for EBV**

EBV assays were done as described previously (38). ELISA was performed on cells fixed with 95% EtOH/acetic acid, rinsed with PBS, incubated with a monoclonal antibody to EBV VCA (Chemicon, Temecula, CA) followed by an incubation with horseradish peroxidase labeled goat anti-mouse IgG1 (Southern Biotechnology, Birmingham, AL) and the EC$_{50}$ and CC$_{50}$ values determined (38).

**DNA reduction antiviral assays for activity against HSV-1 in vitro.**

The antiviral activities were determined against HSV-1 by DNA reduction with MRC-5 human lung fibroblast cells using HSV-1 DNA probes as described previously (19).

**Neutral Red Uptake Assay for Cytotoxicity**

HFF or MRC-5 cells were seeded into 96 well tissue culture plates. MRC-5 cells were plated sparse and allowed to expand during drug treatment. With MRC-5 cells, the media was replaced after 24 hours with MEM containing 2% FBS, drug was added
to the first row, then diluted serially 5-fold from 100 µM to 0.03 µM. With HFF cells, the wells were allowed to become confluent prior to addition of drug containing medium. The assays were done and the CC_{50} values were determined as described previously (4, 5, 36, 37, 38).

**HBV Antiviral Analysis.**

Confluent cultures of 2.2.15 cells were maintained on 96-well flat-bottomed tissue culture plates in RPMI1640 medium with 2% FBS as previously described (26). Cultures were treated with nine consecutive daily doses of the test compounds (six for each test concentration on two replicate plates). The culture medium was changed every day with medium containing the indicated concentration of the test compounds. HBV DNA levels were assessed by quantitative blot hybridization 24 h after the last treatment. Cytotoxicity was assessed by uptake of neutral red dye and semiquantitative analysis of the absorbance of internalized dye at 510 nM (A_{510}) 24h following the last treatment (three cultures per test concentration) (26). Activity against lamivudine-resistant (2) and adefovir-resistant (3) HBV mutants was determined in a 5-day assay using a transient transfection method in Huh7 cells and cytotoxicity determined as previously described (21).

**HIV Assays in MT-2 cells**

MT-2 cells (AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health) were maintained in RPMI 1640 supplemented with 10% FBS (JRH Biosciences, Lenexa, Kans.) The antiviral activity of each compound was determined by inoculating MT-2 cells with HIV-1_{LAI} at a multiplicity of infection (MOI) of 0.001 TCID_{50}/cell, followed by incubation in the
presence of threefold serial drug dilutions (three wells per dilution) as previously described (13). The antiviral activity of each compound is expressed as the EC\textsubscript{50}, which is the concentration required to inhibit p24 antigen production by 50%.

**MT-2 Cytotoxicity**

Cytotoxicity was assessed in rapidly dividing MT-2 cells incubated with drug for 72 h and harvested. Flow count beads (Beckman Coulter, Miami, FL) were added to the cell suspension followed by propidium iodide staining and analysis using an Epics Elite flow cytometer (Beckman Coulter). The 50% cytotoxic concentration (CC\textsubscript{50}) was calculated from the cell counts and viability (13).

**RESULTS**

The PPen nucleosides were tested against CMV, HSV, VV, CV, VZV, and EBV (Table 1). The unmodified PPen nucleosides were inactive, except for PPen-G which showed low but measurable activity against CMV and VZV. However, when esterified with the hexadecyloxypropyl group, substantial activity was noted in most cases. HDP-PPen-G was the most broadly active PPen-N, showing low micromolar EC\textsubscript{50} values ranging from 1.8 to 12.2 µM against CMV, HSV-1, VV, CV and VZV. In a single experiment, HDP-PPen-A inhibited replication of EBV with an EC\textsubscript{50} of 0.1 µM. HDP-PPen-T had submicromolar EC\textsubscript{50}s against HCMV and HSV-1 and an EC\textsubscript{50} of 3 µM against VZV but was not active against cowpox and vaccinia viruses in vitro. HDP-PPen-U did not inhibit any of the viruses against which it was tested.
In MT-2 cells infected with HIV-1, significant activity was noted only with HDP-PPen-C (EC$_{50}$ 2.5 µM) and HDP-PPen-T (EC$_{50}$ 1.0 µM). However, there was little antiviral selectivity as the cytotoxic concentration 50% for these two compounds was 3.3 and 1.3 µM, respectively (data not shown).

We also evaluated the HDP-PPen nucleosides against HBV in 2.2.15 cells (Table 2). As noted with the DNA viruses, the PPen nucleosides were without activity against HBV but their HDP esters had antiviral activity with EC$_{50}$ values ranging from 1.8 to 3.9 µM. Although the HDP esters were more cytotoxic, their selectivity indexes were substantial, ranging from 61 to >417. Neither PPen-U nor its HDP ester showed antiviral activity against HBV.

The active compounds were also evaluated against a panel of drug resistant hepatitis B viruses (Table 3). In transient infections in Huh7 cells, HDP-PPen-A retained full activity against several lamivudine-resistant HBV polymerase mutants (L180M, M204V, M204I and L180M/M204V) while L180M and the double mutant L180M/M204V were resistant to HDP-PPen-G. The pyrimidine analogs, HDP-PPen-C and HDP-PPen-T, were resistant to L180M, M204V, M204I and the LM/MV double mutant. All of the HDP-PPen nucleosides showed activity equal to wild type against an adefovir-resistant mutant (N236T), however, only HDP-PPen-A exhibited full activity against all five of the drug resistant HBV mutants.

**DISCUSSION**

Nucleoside and nucleotide analogs are two important classes of drugs that selectively target viral polymerases and inhibit viral replication (11, 12). Nucleoside
analogs require conversion to the 5′ mono-, di, and finally the triphosphates before incorporation into viral DNA, and include compounds such as acyclovir, ganciclovir, stavudine, abacavir and lamivudine. The acyclic nucleoside phosphonates bypass the initial phosphorylation and are converted to their mono and diphosphate derivatives by nucleoside kinases (12). Studies by Holy et al. of 9-[2-(phosphonmethoxy)ethyl (PME), 9-(R)-[2-(phosphonmethoxy)propyl (PMP), and (S)-9-(3-hydroxy-2-phosphono-methoxy)propyl [(S)-HPMP] nucleotide analogs established the potent broad spectrum antiviral activity available from ANPs and led to three FDA approved phosphonates: cidofovir (Vistide), adefovir dipivoxil (Hepsera) and tenofovir disoproxil fumarate (Viread) (16). Other groups have focused on the design of phosphonate isosteres in which the 5′-oxygen of an antiviral nucleotide is either removed or replaced by an enzymatically stable methylene group. This strategy led to phosphonate analogs of acyclovir (20), ganciclovir (8), stavudine (24), dideoxynucleosides (34), tetrahydrofuran derivatives (6), cyclopropane nucleosides (30, 39) and anti-HCV nucleosides (25). Acyclic phosphonates incorporating unsaturated acyclosugar side chains have also been evaluated (14, 32).

In our own efforts to identify selective virus inhibitors, we prepared a novel family of nucleotide analogs using a phosphonopentenyl group as the acyclic sugar moiety. The general structure of the new compounds, 5-phosphonopent 2-en-1-yl nucleosides (PPen Ns) is shown in Figure 1. The phosphonopentenyl side chain design was chosen so that the resulting analogs would resemble the corresponding portion of 2′,3′-didehydro-2′,3′-dideoxynucleosides such as stavudine, d4A and abacavir, nucleosides with potent anti-HIV activity. Choo et al. (9) found previously that the 2′,3′ double bond
of these unsaturated nucleosides interacts with the aromatic moiety of Tyr115 of HIV-1 reverse transcriptase by hydrophobic $\pi \rightarrow \pi$ interaction. We hypothesized that the unsaturated acyclosugar side chain of the PPen structure might improve binding of the diphosphate to the target viral polymerases. The acyclic PPen side chain should also be more flexible than related cyclic analogs (24) which might increase binding within the polymerase complex and facilitate approach of the $\alpha$-phosphorous to the 3'-OH of the replicating viral DNA.

The PPen nucleosides were evaluated in various antiviral assays and the results are summarized in Table 1. No inhibitory activity was observed up to 100 $\mu$M for PPen-A, -C, -U, -C or –T; and PPen-G was only weakly active against CMV (68.4 $\mu$M) and VZV (7.2 $\mu$M). The PPen nucleosides also were not antiproliferative or cytotoxic in MRC-5 or MT-2 cells up to 100 $\mu$M (Tables 1 and 2).

We have observed that ANPs frequently fail to exhibit biological activity in vitro because the double negative charge associated with the phosphonate group impairs transport of drug through the cell membrane (1). In earlier work we prepared ANP’s modified with lipophilic groups to create mimics of lysophosphatidylcholine, a dietary lipid which is absorbed intact from the gastrointestinal tract and readily metabolized in cells. We showed that cidofovir modified by esterification with a hexadecyloxypropyl group (HDP-CDV) enters cells rapidly and is metabolized to give levels of CDV-diphosphate, the active metabolite, that are 100-fold higher relative to unmodified CDV (1). The in vitro activity of (S)-HPMPA was also enhanced using this strategy (5), and some weakly active ANP’s displayed significant activity after lipid modification (33, 35). Esterification with alkoxyalkyl groups also makes ANPs orally bioavailable (10) and
orally effective against lethal poxvirus infections (7,31). This was the rationale for the synthesis of the hexadecyloxypropyl esters of the PPen nucleosides. We evaluated them for antiviral effects against various viruses and found that several now showed significant activity (Table 1). The broadest antiviral activity was found in the guanine analog, HDP-PPen-G, which had EC$_{50}$s below 12 µM for all viruses except HIV-1 and EBV. HDP-PPen-A was a slightly stronger inhibitor of HBV (EC$_{50}$ 1.5 µM) and showed substantial activity against EBV (EC$_{50}$ 0.1 µM). Against HIV-1, only the two pyrimidine analogs HDP-PPen-C and HDP-PPen-T showed significant activity but exhibited no antiviral selectivity. As expected, HDP-PPen-U was inactive in all the antiviral assays (VZV and EBV not assayed).

Since four of the five HDP-PPen nucleoside compounds inhibited HBV replication, and our previous work demonstrated that alkoxyalkyl esters of nucleoside phosphates and phosphonates are targeted to the liver (10, 17, 18), we evaluated the active analogs against a panel of drug resistant HBV (Table 2). Hepatitis B virus is one of the ten leading causes of mortality worldwide, and the emergence of strains resistant to the approved drugs, lamivudine and adefovir dipivoxil, is a major clinical concern (29). Resistance to lamivudine emerges rapidly during monotherapy and after 4 years affects approximately 70% of treated patients (28). Adefovir dipivoxil is effective in vitro and in vivo against lamivudine-resistant HBV (40). We found that like adefovir, HDP-PPen-A retains full activity against the lamivudine-resistant HBV polymerase mutants (L180M, M204V, M204I and L180M/M204V) in transient infections in Huh7 cells. HDP-PPen-G was effective against M204V and M204I, but L180M and the double mutant,
L180M/M204V, were resistant. All of the lamivudine resistant strains exhibited resistance to the pyrimidine PPen analogs, HDP-PPen-C and HDP-PPen-T.

Adefovir resistance has been associated with the selection of the mutant N236T, but its emergence is less frequent and delayed relative to lamivudine resistance (3). Interestingly, all of the HDP-PPen nucleosides retained activity equal to wild type against the adefovir-resistant mutant (N236T). Among the compounds, only HDP-PPen-A retained full activity against all five drug resistant HBV mutants, suggesting that this nucleotide analog should be evaluated as a salvage therapy in patients infected with drug resistant HBV strains, or used in combination with other drugs to prevent the emergence of resistance.

In summary, the PPen nucleosides are a new class of acyclic nucleoside phosphonates, possessing significant antiviral activity when esterified with the hexadecyloxypropyl group. Further work will be required both to design analogs with improved antiviral activity and to investigate their mechanism of action.

**ACKNOWLEDGEMENTS**

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(DDC)-, dideoxyinosine (DDI)-, and deoxythymidine (DDT)-5’-monophosphates


The 5-phosphono-pent-2-en-1-yl analogs of the bases (B) adenine, thymine, guanine, cytosine and uracil and their hexadecyloxypropyl esters were synthesized and evaluated for antiviral activity.
Table 1. Antiviral Activity And Cytotoxicity Of PPen Nucleosides And Their Hexadecyloxypropyl Esters In Vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>HCMV EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>HSV-1 EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>VV EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>CV EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>VZV EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>EBV EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>MRC-5 CC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>HFF CC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
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<tbody>
<tr>
<td>PPen-A</td>
<td>&gt; 100</td>
<td>&gt; 30</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 300</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PPen-G</td>
<td>68.4</td>
<td>&gt; 30</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>7.2</td>
<td>nd</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PPen-U</td>
<td>&gt; 100</td>
<td>&gt; 30</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 300</td>
<td>nd</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PPen-C</td>
<td>&gt; 100</td>
<td>&gt; 30</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 60</td>
<td>nd</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PPen-T</td>
<td>&gt; 100</td>
<td>&gt; 30</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 300</td>
<td>nd</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>HDP-PPen-A</td>
<td>15.7±6.2</td>
<td>18±4.5</td>
<td>&gt;30</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>0.1</td>
<td>17.3±4.2</td>
<td>43</td>
</tr>
<tr>
<td>HDP-PPen-G</td>
<td>1.8±1.5</td>
<td>5.8±2.2</td>
<td>7.5±6.4</td>
<td>11±10</td>
<td>12.2±1.2</td>
<td>48</td>
<td>12.2±6.2</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>HDP-PPen-U</td>
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<td>&gt;30</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>nd</td>
<td>nd</td>
<td>51.0±11.8</td>
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<td>HDP-PPen-C</td>
<td>2.9±0</td>
<td>4.7±5.4</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>3.7±0.1</td>
<td>nd</td>
<td>11.2±4.2</td>
<td>56</td>
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<tr>
<td>HDP-PPen-T</td>
<td>0.66±0.03</td>
<td>0.90±0.5</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>3.0±1.1</td>
<td>nd</td>
<td>7.4±2.4</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

Data are the average of two or more determinations except for EBV, which is a single determination. Values indicated by a greater than sign were also single determinations.

Effective concentration 50%, EC<sub>50</sub>, µM; nd, not determined; HCMV, AD169 strain, plaque reduction assay in primary human foreskin fibroblast (HFF) cells, HSV-1, DNA reduction assay in MRC-5 cells, also in Choo et al, Bioorg. Med. Chem., submitted, 2006, VV Copenhagen, plaque reduction assay in HFF cells, CV Brighton, plaque reduction assay in HFF cells, VZV, CPE assay in HFF cells, EBV Elisa assay in Daudi cells; the CC<sub>50</sub> for HDP-PPen-A in Daudi cells was >100 µM. The 50% cytotoxic concentration was determined by neutral red staining as described in Methods. With stationary, confluent HFF cells the assay measures cytotoxicity, but with sparsely plated MRC-5 cells, the assay measures both cell proliferation and cytotoxicity.
### Table 2. Antiviral Activity of PPen and HDP-PPen Nucleosides against HBV

<table>
<thead>
<tr>
<th>Compound</th>
<th>HBV EC\textsubscript{50} (µM)</th>
<th>Toxicity (CC\textsubscript{50}, µM)</th>
<th>Selectivity</th>
</tr>
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<tr>
<td>PPen-A</td>
<td>&gt;30</td>
<td>639 ± 8.6</td>
<td>-</td>
</tr>
<tr>
<td>PPen-G</td>
<td>&gt;30</td>
<td>610 ± 7.6</td>
<td>-</td>
</tr>
<tr>
<td>PPen-U</td>
<td>&gt;30</td>
<td>712 ± 19</td>
<td>-</td>
</tr>
<tr>
<td>PPen-C</td>
<td>&gt;30</td>
<td>600 ± 8.6</td>
<td>-</td>
</tr>
<tr>
<td>PPen-T</td>
<td>&gt;30</td>
<td>620 ± 9.6</td>
<td>-</td>
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<tr>
<td>HDP-PPen-A</td>
<td>1.5±0.3</td>
<td>116±7.8</td>
<td>77</td>
</tr>
<tr>
<td>HDP-PPen-G</td>
<td>2.4±0.2</td>
<td>&gt;1000</td>
<td>&gt;417</td>
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<tr>
<td>HDP-PPen-U</td>
<td>&gt;30</td>
<td>155±8.2</td>
<td>-</td>
</tr>
<tr>
<td>HDP-PPen-C</td>
<td>3.9±0.4</td>
<td>621±8.9</td>
<td>159</td>
</tr>
<tr>
<td>HDP-PPen-T</td>
<td>2.0±0.2</td>
<td>122±10</td>
<td>61</td>
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</table>

Antiviral results (EC\textsubscript{50}) were derived from virion DNA reduction assays in 2.2.15 cells as described in Methods. Values presented (± standard deviations) were calculated using data from two to four experiments. Cytotoxicity was determined by the inhibition of the uptake of neutral red dye (26).
Table 3. Activity of HDP-PPen Nucleosides Against Drug Resistant Hepatitis B Virus

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wild type</th>
<th>L180M</th>
<th>M204V</th>
<th>M204I</th>
<th>L180M/M204V</th>
<th>N236T</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt;, &lt;/em&gt;µM</th>
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<tbody>
<tr>
<td>HDP-PPen A</td>
<td>9.2±1.2</td>
<td>15.0±1.8</td>
<td>9.0±0.9</td>
<td>7.7±0.8</td>
<td>8.1±0.9</td>
<td>10±1.2</td>
<td>110±6</td>
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<tr>
<td>HDP-PPen G</td>
<td>8.8±0.9</td>
<td>&gt;100</td>
<td>16±1.7</td>
<td>13±1.5</td>
<td>&gt;100</td>
<td>6.3±0.7</td>
<td>929±27</td>
<td></td>
</tr>
<tr>
<td>HDP-PPen C</td>
<td>3.4±0.5</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>4.0±2.3</td>
<td>523±11</td>
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</tr>
<tr>
<td>HDP-PPen T</td>
<td>3.0±0.4</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>3.8±4.1</td>
<td>120±5</td>
<td></td>
</tr>
</tbody>
</table>

Data are the EC<sub>50</sub> values (µM) based on reduction of HBV replicative intermediates in Huh7 cells transiently infected with the viruses noted. Mean ± SD of two to four experiments.