REVIEW

Adenine: an important drug scaffold for the design of antiviral agents

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Abstract  Adenine derivatives, in particular the scaffold bearing the acyclic nucleoside phosphonates (ANPS), possess significant antiviral and cytostatic activity. Till now, several effective adenine derivatives have been marketed for the treatment of HIV, HBV, CMV and other virus-infected diseases. These compounds are represented by tenofovir (PMPA), a medicine for both HIV and HBV, and adefovir as an anti-HBV agent. More than this, other analogs, such as GS9148, GS9131, and GS7340, are also well-known anti-viral agents that have been progressed to the clinical studies for their excellent activity. In general, the structures of these compounds include an adenine nucleobase linked to a phosphonate side chain. Considerable structural modifications on the scaffold itself and the peripheral sections were made. The structure-activity relationships (SARs) of this skeleton will provide valuable clues to identify more effective adenine derivatives as antiviral drugs. Here, we systematically summarized the SARs of the adenine derivatives, and gave important information for further optimizing this template.

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1. Introduction

Human immunodeficiency virus (HIV), Rauscher murine sarcoma virus (R-MuLV), herpes simplex virus (HSV), cytomegalovirus (CMV), feline immunodeficiency virus (FIV), Epstein-Barr virus (EBV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are highly contagious viruses endangering human health. Being the most important antiviral agents, acyclic nucleoside phosphonates (ANPs) play a significant role in the treatment of virus-infected diseases. In particular, adenine derivatives bearing the adenine nucleobase possess excellent antiviral activity against most of the double-stranded DNA viruses, such as the herpes group viruses and the orthopoxviruses. Previous SARs explorations proved that the concept of using the ANPs as chain terminators for antiviral was remarkably effective. A large number of adenine derivatives have been discovered as potential antiviral agents motivated by this hypothesis. For example, the novel agent 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir, 2, Fig. 1) was approved for the treatment of hepatitis B virus (HBV) infections by the US FDA in 2002, while the (R)-9-[2-(phosphonomethoxy)propyl]adenine ((R)-PMPA, tenofovir, 3, Fig. 1) was launched in the US market as a prodrug (tenofovir disoproxil fumarate, TDF) for the treatment of HIV infections in 2001 and for the treatment of HBV in 2008, respectively. Besides, several adenine derivatives also have progressed to clinical exploration, such as GS9148 (4, Fig. 1), GS9131 (5, Fig. 1), and GS7340 (6, Fig. 1). Undoubtedly, adenine derivatives are the most valuable inhibitors for the development of antiviral agents.

Through a key phosphate–carbon–oxygen bond, the adenine nucleobase of this template is attached to a phosphonate group, making these compounds more stable than those that contain the phosphate–oxygen–carbon bond of a phosphate group. Therefore, compounds containing the phosphate–carbon–oxygen linker achieve higher levels of the active metabolites in the cells. Moreover, they also possess a broad activity spectrum that also includes RNA viruses and retroviruses. However, owing to the poor oral bioavailability caused by the negatively charged phosphonate moiety, the therapeutic use of these nucleoside phosphonates was limited. In order to obtain the neutral and membrane-permeable prodrugs, great attention has been paid to the optimization of this template, and several highly potent virus inhibitors have been successfully produced. A thorough analysis of the SARs of this scaffold will provide useful clues for further structural optimization, which is achieved in this review.

2. Adenine nucleus

Adenine analogs 7 and 8 (Fig. 2) bearing a chlorine atom at the C-2 position of the adenine nucleus were synthesized by several groups. Surprisingly, only by introducing this atom, the newly obtained compounds did not possess any anti-virus activity at all. Instead, they were effective as chemotherapeutic agents for the treatment of refractory chronic leukemia and hairy cell leukemia. The methylsulfanyl analogs (9–11, Fig. 2) produced similar results. These compounds exhibited a weaker agonistic activity toward the P2Y1 receptor. The diamino-substituted (C-2 and C-6 positions) analogs 12 and 13 (Fig. 3) containing a trifluoromethyl group at the side chain were originally synthesized by Dvoráková in 1994. Neither exhibited desired antiviral properties. Also, the addition of a fluorine atom to the C-2 position (analog 14) reduced the antiviral activity against both the RNA viruses and the DNA viruses. The similar trend of decreasing antiviral activity was observed in the case of the tenofovir analogs, where the compound 15 (Fig. 3) completely lacked antiretroviral properties. Whereas the bis(amidate) prodrug 16 (Fig. 3) was moderately active against HIV-1 (EC₅₀ = 5.51 μmol/L), probably due to its improved bioavailability. All these suggested that modifying the C-2 position of the adenine nucleus was utterly ineffective for improving the antiviral potency.

Analogs 17a–e (Fig. 4) and 18–21 (Fig. 4) bearing a fluorine atom at the C-6 position of the nucleus bases were synthesized as antiviral agents by several companies, but their activity was

Figure 1 Structures of the novel adenine derivatives as antiviral agents.
Compound 22 (Fig. 4) that contains a heptafluoro-propyl moiety at the C-6 position of the adenine ring was active against various viruses, such as HSV-1 (MIC50 = 21.8 μmol/L), HSV-2 (MIC50 = 7.3 μmol/L), HSV-1 TK−/C0 (MIC50 = 4.4 μmol/L) in E6SM cells and against CMV (MIC50 = 3.6 μmol/L) and varicella-zoster virus (VZV) TK− and TK+ mutants (MIC50 = 0.6–1.3 μmol/L) in HEL cells. Unfortunately, compound 22 also was highly cytotoxic in the host cells at concentrations above 45 μmol/L. In 2008, a series of analogs with 9-, 7- and 3-substituted 2- or 6-guanidinopurines were prepared and evaluated for their biological activity by Cesnek (23 and 24, Fig. 5). Unfortunately, none of these compounds exhibited significant antiviral activity.
3. Side chain

3.1. Linear linker

To improve the oral bioavailability of the novel 9-(S)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine ((S)-HPMPA), Hostetler prepared orally bioavailable lipid esters of (S)-HPMPA 25a–d (Table 1) and evaluated their antiviral activity against human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), vaccinia (VV), and cowpox viruses (CV). Among them, oleyloxyethyl-(S)-HPMPA (HDP-(S)-HPMPA, 25a) was the most active inhibitor, possessing the EC50 values of 0.003 μmol/L against HCMV and 1.4 μmol/L for unmodified HPMPA, respectively. In cells infected with VV and CV, octadecyloxyethyl-(S)-HPMPA had EC50 values of 0.01, 0.02 μmol/L vs. 2.7, 4.0 μmol/L for unmodified HPMPA, respectively. Compared with the alkoxyalkyl esters of cidofovir, the corresponding alkoxyalkyl esters of (S)-HPMPA displayed equivalent activity against HCMV and MCMV, in parallel with 15- to 20-fold higher activity against VV and CV in vitro. Obviously, the alkoxyalkyl esters of (S)-HPMPA are worthy of further investigation for treatment of infections caused by herpes viruses and orthopoxviruses.

In contrast to the previous observation that the acyclic nucleoside phosphonates were only active against double stranded DNA viruses, HIV and HBV, Valiaeva found that octadecyloxyethyl 9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (26, ODE-(S)-MPMA, Table 2) was also able to suppress both genotype 1b and 2a hepatitis C virus (HCV) replicons with an EC50 value of 1–2 μmol/L and a CC50 value of more than 150 μmol/L, respectively. From the SARs exploration, it also can be seen that analogs (26–30, Table 2) with substitutions at the hydroxyl group larger than a methyl or ethyl group, or with other adenine bases were less active, but most compounds had significant antiviral activity against HIV-1 in vitro. One peculiar compound was octadecyloxyethyl 9-(R)-[3-methoxy-2-(phosphonomethoxy)propyl]guanine (27, ODE-(R)-MPMA, Table 2), which possesses an EC50 value of less than 0.01 μmol/L along with a selectivity index of more than 4.4 million.

In 2010, Krecmerov identified 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP, 31, Fig. 6) and its cyclic analogs as potential drug candidates (33–35, Fig. 6) against the poxvirus infections. To improve the bioavailability of these compounds, a series of diverse ester prodrugs, including alkoxyalkyl (hexadecyloxypropyl, octadecyloxyethyl, hexadecyloxyethyl), pivaloyloxymethyl (POM), 2,2,2-trifluoroethyl, butylsalicylyl, and prodrugs based on peptidomimetics were synthesized. All these compounds were evaluated for the activity against vaccinia virus and other herpes viruses. This contribution resulted in alkoxyalkyl ester derivatives of HPMPDAP, with 50% effective concentrations that were 400–600-fold lower than those of the parent compound. While prodrugs with 2,2,2-trifluoroethyl, POM, and butylsalicylyl, were able to inhibit vaccinia virus replication at 50% effective concentrations that were equivalent or 10-fold lower than those observed for the parent compounds.

3.2. Amide linker

Amide prodrugs of PMEA were first synthesized as anti-HIV agents by Starrett in 1994. However, the obtained compounds were unstable under acidic conditions and provided levels of PMEA comparable to the parent compound after oral
administration. In 1997, Ballatore³¹ prepared another series of amidate prodrugs 36a–k (Table 3), and tested their activity in vitro. Of these compounds, prodrug 36c displayed tremendously enhanced antiviral potency compared with the parent nucleotide analog. Enzymatic studies in vitro and structure–activity relationships indicated that the releasing mechanism of such prodrugs may be the same as that described for the phosphoramidate triesters of nucleotide analogs.

In the same year, Chapman¹³ discovered a novel amidate derivative GS7340 (6, Fig. 1). The EC₅₀ of GS 7340 against human immunodeficiency virus type 1 in MT-2 cells was 0.005 μmol/L compared to an EC₅₀ of 5 μmol/L for the parent drug tenofovir. The l-alaninyl analog (GS7340) was more stable compared to the cycloSal counterparts. Stability studies revealed that the boranophosphonates were metabolized in MT-2 cells and plasma as well sensitive to the stereochemistry at the phosphorus. After a single oral dose of GS 7340 (10 mg/kg of tenofovir) to male beagle dogs, the plasma level of tenofovir was 17% of that achieved by an intravenous dose of tenofovir. The total intracellular concentration of all tenofovir-containing species in isolated peripheral blood mononuclear cells was 63 μg/mL compared to 0.2 μg/mL in plasma. In addition, a radiolabeled distribution study with dogs was carried out and an increased distribution of tenofovir to tissues of lymphatic origin compared to the commercially available prodrug tenofovir DF (Viread) was found. Overall, owing to these excellent properties, GS7340 are being tested in a phase III clinical study now.

Recently, Meier designed two new classes of lipophilic prodrugs of PMEA. The first series of compounds 37a–d (Table 4) were prepared based on the cycloSal nucleotide approach. Because of the surprisingly low hydrolysis stability of these cycloSal–PMEA derivatives, more stable derivatives were designed. Instead of using salicyl alcohol, in cycloAmb–PMEA derivatives, 2-aminobenzyl alcohols were attached to PMEA (38a–c, Table 4). The latter compounds exhibited a considerably higher stability compared to the cycloSal counterparts. Stability studies uncovered that all lipophilic prodrugs delivered PMEA selectively by chemical means. Moreover, none of these compounds proved to inhibit acetyl- and butyrylcholinesterase, and some of the phosphonate diesters were even more active against HIV than parent PMEA.

### 3.3. Boran linker

In 2006, Barral et al.³³ synthesized two boranophosphate nucleosides, 9-[(2-(boranophosphonomethoxy)ethyl)adenine (39a, Table 5) and (R)-9-[(2-ranophosphonomethoxy)propyl]adenine (39b, Table 5). Both H-phosphonates 39a and 39b, and boranophosphonates 40a and 40b were evaluated for their in vitro activity against HIV infected cells and a panel of DNA or RNA viruses. It was unfortunate that none of them exhibited significant antiviral activity in vitro and cytotoxicity. A decomposition study revealed that the boranophosphonates 40a and 40b were metabolized in culture medium into H-phosphonates 39a and 39b, with half-life values of 5.3 h for 40a and 1.3 h for 40b, respectively.

Carbocyclic phosphate analogs of dideoxy-adenine nucleotides were discovered as potential anti-HIV inhibitors by Wolff-Kugel and Halazy as early as 1991. With the aim to improve the metabolic stability, the sugar ring oxygen atom in dideoxyadenosine monophosphate (ddAMP, 41, Fig. 7) was replaced by a methylene and labile phosphate group was replaced by an α,α-(difluoromethyle)phosphonate. Biological test disclosed that analog 42 (Fig. 7) was not a substrate of AMP kinase (myokinase) but can be slowly pyrophosphorylated by Escherichia coli phosphoribosyl pyrophosphate (PRPP) synthase with an excess of PRPP (Kₘ not determined), while being a weak PRPP synthase inhibitor ( IC₅₀ = 1 mmol/L ).

In 2005, Girardet designed a series of adenosine 5’-phosphate analogs to mimic naturally occurring adenosine monophosphate. All these compounds (43–47, Fig. 8) were evaluated in a cellular hepatitis C virus (HCV) replication assay. To elucidate the mechanism of action of these novel adenosine phosphonates, their diphosphate derivatives (43a–47a, Fig. 8) were also synthesized. Extensive nucleotide...
incorporation assays by HCV NS5B RNA-dependent RNA polymerase showed that 44a and 45a can serve as chain terminators, whereas compounds 43a, 46a, and 47a are competitive inhibitors with ATP. Additional steady-state kinetic analysis determined the incorporation efficiency of 44a and 45a as well as the inhibition constants for 43a, 46a, and 47a. In summary, these AMP mimics demonstrated some

Figure 6 Structures of the 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) and its cyclic form as potential drug candidates against poxvirus infections.

Table 3 Structures and activities of amidate prodrugs of PMEA and PMPA.

| Compd. | R1  | R2   | R3    | EC50a HIV-1 MT4 | EC50a HIV-2 MT4 | CC50b HIV-1 CEM | EC50a HIV-1 CEM | EC50a HIV-2 CEM | CC50b HIV-2 CEM |
|--------|-----|------|-------|----------------|----------------|-----------------|----------------|----------------|----------------|
| 36a    | CH3 | OH   | OH    | 2.3           | 1.4            | 197             | 3.67           | 3.67           | ≥ 250          |
| 36b    | H   | OH   | OH    | 7.0           | 7.5            | 144             | 7.0            | 10             | 69             |
| 36c    | CH3 | t-Ala-Me-ester | PhO | 0.029         | 0.026          | 71.4            | 0.053          | 0.090          | 27             |
| 36d    | CH3 | Gly-Me-ester | PhO | 0.58          | 0.15           | 102             | 0.23           | 0.31           | 88.5           |
| 36e    | CH3 | t-Ala-MPhe-ester | PhO | 0.99         | 0.65           | 213             | 0.5            | 0.38           | 125            |
| 36f    | CH3 | t-Phe-Me-ester | pCl-PhO | 0.04      | 0.07           | 64              | 0.07           | 0.06           | 26             |
| 36g    | CH3 | t-Ala-Me-ester | pCl-PhO | 0.15    | 0.26           | 57              | 0.2            | 0.12           | 106            |
| 36h    | CH3 | CHPh | O     | 4.9           | ≥ 250           | 4.0             | 4.0            | ≥ 250          | 3.7            |
| 36i    | H   | t-Ala-Me-ester | PhO | 0.23          | 0.15           | 5.1             | 0.12           | 0.20           | 3.7            |
| 36j    | H   | Gly-Me-ester | PhO | 3.6           | 4.8            | 120             | 5.6            | 4.5            | 80             |
| 36k    | H   | t-Ala-Me-ester | PhO | 15           | 7.2            | 120             | 5.0            | 6.0            | 67             |

*Anti-HIV-1 activity (EC50): 50% effective concentration in wild-type CEM cell cultures (μmol/L).

*CC50: cytotoxic concentration (μmol/L).
promising but relatively weak anti-HCV activity. The structural modifications on the ribose and around the 5'-moiety in this study are not sufficient to improve a chain terminator's catalytic efficiency, nor the binding affinity to NS5B RdRp. Further optimizations are needed to identify novel and potent nucleoside phosphonate chain terminators for HCV.

Previous studies proved that PGK was a key step of glycolytic pathway by catalyzing the conversion of 1,3-BPG to 3-phosphoglycerate. Therefore, development of new inhibitors of parasitic PGK is an attractive approach for drug design. Thus bisphosphonate analogs of 1,3-bisphospho-D-glyceric acid (1,3-BPG, 48, Fig. 8), in which the CF2 groups were used in the synthesis of phosphate mimics, were investigated for the activity against the phosphoglycerate kinase (PGK)44. Interestingly, most of these analogs were effective. In addition, compound 48 (Fig. 8) was co-crystallized with PGK of *Trypanosoma brucei* and afforded new insights into the catalytic domain of the parasitic enzyme.

In 2008, Boojamra discovered a well-known nucleoside phosphonate reverse transcriptase (RT) inhibitor GS-9148 (4, Fig. 1), which was active against wild-type HIV at 12 μmol/L9–11. Unlike many clinical RT inhibitors, relevant reverse transcriptase mutants (M184V, K65R and 6-TAMS) maintain a susceptibility to 2'-

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**Table 4** Chemical stability and biological activities of compounds 37a–d and 38a–c.

| Compd. | X   | R   | Half-life (h) | EC50 pH 7.3 | BchE pH 2.0 | Biological activity in CEM/O cells |
|--------|-----|-----|---------------|-------------|-------------|-----------------------------------|
|        |     |     | pH 7.3        | pH 2.0      |             | EC50 HIV-1 | CC50 HIV-2 |
| 37a    | O   | H   | 0.09          | n.d         | > 50        | n.d     | n.d     | n.d     |
| 37b    | O   | 3-Me| 0.56          | n.d         | > 50        | n.d     | n.d     | n.d     |
| 37c    | O   | 3-t-Bu| 4.05        | 23.7        | > 50        | 3.0     | 4.5     | 29      |
| 37d    | O   | 3.5-t-Bu| 3.05        | 9.6         | > 50        | 5.5     | 5.3     | 17      |
| 38a    | N   | 6-F | 1.3          | n.d         | > 50        | 28      | 26      | 244     |
| 38b    | N   | H   | 4.0          | 9.6         | > 50        | 20      | 21      | 183     |
| 38c    | N   | 3-Me| 21.3         | 29.0        | > 50        | 29      | 56      | > 250   |
| PMEA   |     |     |              |             |             | 10      | 10      | 50      |

*aHydrolysis in 25 mmol/L phosphate buffer at 37 °C or 25 mmol/L citrate buffer, at 37 °C, half-life (t/2) were determined from the decreasing peak of the starting phosphate triester and are the mean of duplicate experiments.

*bAnti-HIV-1 activity (EC50): 50% effective concentration in wild-type CEM cell cultures (μmol/L).

*cCC50: cytotoxic concentration.

*dNot determined due to low chemical stability.

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**Table 5** Calculated half life of the derivatives 39a, 39b, 40a, 40b compared with PMEA and (R)-PMPA.

| Compd. | t1/2 (h) |
|--------|----------|
|        | Buffers | PMI-1640 Cuture medium | Total cell extracts |
| 39a    | Stable   | > 72          | > 72          | > 72          |
| 39b    | Stable   | > 72          | > 72          | > 72          |
| 40a    | Stable   | > 72          | 5.3          |
| 40b    | Stable   | > 72          | 1.3          | 24          |
| PMEA   | Stable   | > 72          | > 72          | > 72          |
| (R)-PMEA | Stable | > 72          | > 72          | > 72          |

*aPH of the buffers: 1.2, 5.2, 7.4, 8.1, 9.0, 11.5.

*bLess than 5% decomposition after 72 h.

*cSingle product of decomposition: 39a from 40a and 39b from 40b.
Figure 8  Structures of adenosine 5'-phosphonate analogs.

Figure 9  Structures of GS9148 analogs.
Fd4AP that is similar to wild-type virus. The 2'-fluorine group wasrationally designed into the molecule to improve the selectivity profile and in preliminary studies using HepG2 cells, compound GS-9148 showed no measurable effect on the mitochondrial DNA content, indicating a low potential for mitochondrial toxicity.

With these promising results available, one year later, Bookman 45 synthesized a family of cyclopentyl-substituted analogs as more potent HIV reverse transcriptase (RT) inhibitors. In cell cultures, the parent phosphonate diacid 50b demonstrated moderate antiviral activity (EC50 = 16 μmol/L) within 2-fold of that of GS-9148, and within 5-fold of that of PMPA. In vitro, cellular metabolism studies using 50b also confirmed that the active diphosphate metabolite was produced albeit at a lower efficiency relative to GS-9148.

Also based on GS-9148, Mackman 12 designed a series of amide prodrugs (4, 51a–e, Fig. 9) to effectively deliver GS-9148 and its active phosphorylated metabolite 51a into targeted cells. Among these compounds, the ethylalaninyl phosphonamide prodrug 4 demonstrated favorable cathepsin A substrate properties, in addition to favorable in vitro intestinal and hepatic stabilities. Following oral dosing (3 mg/kg) in Beagle dogs, high levels (> 9.0 μmol/L) of active metabolite 51a were observed in PBMCs, validating the prodrug design strategy and leading to the nomination of 4 as a clinical candidate.

More recently, a series of 2'-fluorine-modified nucleoside phosphonates with either an unsaturated C=C (52, Fig. 10) or a saturated C–C (53, Fig. 10) phosphonate linker have been prepared by Mackman as potential inhibitors of HCV polymerase 46. The diphosphate analogs (NTP equivalents) of such compounds were found to be potent inhibitors of NSSB polymerase (EC50 as low as 2 μmol/L), but the parent compounds (e.g., compounds 7 and 8) were still weak to inhibit the NSSB polymerase. Clearly, prodrugs of the parent nucleoside phosphonates improved the cellular activity.

To bypass the thymidine kinase (TK) dependence of the parent nucleoside analogs, a series of phosphoramidate ProTides (54a–c, Fig. 11) were designed and synthesized by Herdewijn 47 in 2013. Phosphoramidate derivative pivaloyloxymethyl iminodiacetate (54b, IDA–POM) exhibited anti-HSV-1 and anti-VZV activity in cell cultures. 1-alanine methyl ester and secondary amine IDA–POM ester were found to be equally potent against VZV. In addition, some success was achieved in delivering the nucleoside monophosphate intracellularly for phosphoramidate compounds bearing the IDA–POM moiety against VZV to bypass the thymidine kinase pathway, indicating the POM moiety is a potential carboxyl protecting group in the design of prodrugs. However, the application of the phosphoramidate ProTide technology was only partly successful because none of these compounds showed a significant increase in antiviral activity against the TK-deficient virus strains.

A novel adenine derivative FMCA (55, Fig. 12) bearing a 2'-fluoro-6'-methylene-carbocycle was synthesized and evaluated for its anti-HBV activity by Wang 48 in 2011. This compound demonstrated significant antiviral activity against wild-type as well as lamivudine, adefovir and dual lamivudine/entecavir resistant mutants. In view of these promising anti-HBV activities, further biological and biochemical studies of the nucleoside 55 was warranted to assess its full potential as an anti-HBV agent.

In 2013, Rawal 49 discovered the other famous 2'-fluoro-6'-methylene-carbocyclic adenosine monophosphate prodrug 56 (FMCAP, Fig. 12) and evaluated its in vitro anti-HBV potency against a lamivudine–entecavir resistant clone (L180M+M204V+S202G). FMCA demonstrated significant antiviral activity against wild-type as well as lamivudine–entecavir resistant triple mutant (L180M+M204V+S202G). In contrast, the monophosphate prodrug (FMCP) showed more than 12-fold increase in anti-HBV activity, without increasing the cellular toxicity. Mitochondrial and cellular toxicity studies of FMCA showed that there

![Figure 10](image1.png) Structures of 2'-fluorine-modified nucleoside phosphonates 52 and 53.

![Figure 11](image2.png) Structures of phosphoramidate ProTides 54a–e.

![Figure 12](image3.png) Structures of novel adenine derivative FMCA and FMCAP.
was no significant toxicity up to 100 μmol/L. Very recently, their extensive exploration displayed that FMCAP reduced HBV viral load in chimeric mice harboring the triple mutants<sup>35</sup>.

Oh and his co-workers’ studies<sup>36</sup> showed that both 5′-norcarbocyclic adenosine phosphonic acid 57 (FMnAP, Fig. 13) and its bis-SATE prodrug 58 (Fig. 13) belong to the category of carbocyclic nucleoside analogs where the furanose oxygen is replaced by a methylene group. Compared with parent FMnAP 57 (EC<sub>50</sub> = 62 μmol/L, CC<sub>50</sub> = 70 μmol/L), prodrug 58 enhanced the anti-HIV activity (EC<sub>50</sub> = 16.7 μmol/L, CC<sub>50</sub> = 31.4 μmol/L), but showed higher cytotoxicity. Probably, this result was caused by a higher cellular uptake of the prodrug 58 to cells, followed by the intracellular release of FMnAP.

4. Conclusions

Being an excellent antiviral skeleton, adenine was extensively modified both on the side chain and on the heterocyclic moiety. SARs studies indicated that the margins of the structural alteration are very narrow. Changing the substituents on the adenine nucleus will significantly decrease their antiviral activity. While for the reason that the structure of the side chain is critical for the specificity of antiviral action, only modifying the side chain as phosphate ester or amide linker is favorable to improve the oral bioavailability. To further develop this scaffold, one way is to use prodrugs to increase membrane permeability and bioavailability, and the other way is to optimize the acyclic chain, which enables the compounds to adopt a range of conformations to facilitate binding to not only reverse transcriptases and DNA polymerases but potentially to other enzymes using nucleotides as substrates or products. Although a large number of potential drugs or clinical drugs have been identified to inhibit various viruses, owing to the fast virus mutation, searching for more effective agents still remains urgent. There is no doubt that adenine is a promising scaffold worthy of further development to identify new antiviral agents.

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References

1. Holý A. Phosphonomethoxyalkyl analogs of nucleotides. Curr Pharm Des 2003;9:2567–92.
2. de Clercq E, Holý A. Acyclic nucleoside phosphonates: a key class of antiviral drugs. Nat Rev Drug Discov 2005;4:928–40.
3. de Clercq E. Antiviral drug discovery: ten more compounds, and ten more stories (part B). Med Res Rev 2009;29:571–610.
4. de Clercq E. In search of a selective therapy of viral infections. Antivir Res 2010;85:19–24.
5. Naensens L, Snoeck R, Andrei G, Balzarini J, Neys T, de Clercq E. HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogs: a review of their pharmacology and clinical potential in the treatment of viral infections. Antivir Chem Chemother 1997;8:1–23.
6. Ying C, de Clercq E, Lanivudine Neys T, adefovir and tenofovir exhibit long-lasting anti-hepatitis B virus activity in cell culture. J Viral Hepat 2000;7:79–83.
7. Shamilyan TA, MacDonald R, Shaukat A, Taylor BC, Yuan JM, Johnson JR, et al. Antiviral therapy for adults with chronic hepatitis B: a systematic review for a National Institutes of Health Consensus Development Conference. Ann Intern Med 2009;150:111–24.
8. Starratt JR, Tortolani DR, Hitchcock MJ, Martin JC, Mansuri MM. Synthesis and in vitro evaluation of a phosphonate prodrug: bis (pivaloyloxymethyl)-9-(2-phosphonylethoxyethyl)adenine. Antivir Res 1992;19:267–73.
9. Mackman RL, Lin KY, Bojamara CG, Hui H, Douglas J, Grant D, et al. Synthesis and anti-HIV activity of 2′-fluorinated modified nucleoside phosphonates: analogs of GS-9148. Bioorg Med Chem Lett 2008;18:1116–9.
10. Bojamara CG, Mackman RL, Markевич DY, Prasad V, Ray AS, Douglas J, et al. Synthesis and anti-HIV activity of GS-9148(2′-Fl4AP), a novel nucleoside phosphate HIV reverse transcriptase inhibitor. Bioorg Med Chem Lett 2008;18:1120–3.
11. Cihlar T, Ray AS, Bojamara CG, Zhang LJ, Hui H, Laffamme G, et al. Design and profiling of GS-9148, a novel nucleotide analog active against nucleoside-resistant variants of human immunodeficiency virus type 1, and its orally bioavailable phosphonoamidate prodrug, GS-9131. Antimicrob Agents Chemother 2008;52:655–65.
12. Mackman RL, Ray AS, Hui HC, Zhang LJ, Birks G, Bojamara CG, et al. Discovery of GS-9131: design, synthesis and optimization of amidate prodrugs of the novel nucleoside phosphate HIV reverse transcriptase (RT) inhibitor GS-9148. Bioorg Med Chem 2010;18:3606–17.
13. Chapman H, Kernan M, Prishe E, Rohloff J, Sparacino M, Terhorst T, et al. Practical synthesis, separation, and stereochemical assignment of the PMPA pro-drug GS-7340. Nucleosides Nucleotides Nucleic Acids 2001;20:621–8.
14. Lee WA, He GX, Eisenberg E, Cihlar T, Swaminathan S, Mulato A, et al. Selective intracellular activation of a novel prodrug of the human immunodeficiency virus reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. Antimicrob Agents Chemother 2005;49:1898–906.
15. Wong DK, Cheung AM, O’Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B & & hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312–23.
16. Saven A, Carrara CJ, Carson DA, Beutler E, Piro LD. 2-Chlorodeoxyadenosine treatment of refractory chronic lymphocytic leukemia. Leuk Lymphoma 1991;1:133–8.
17. Levy SG, Wasson DB, Carson DA, Cottam HB. Synthesis of 2-chloro-2′,5′-dideoxy-5′-difluoromethylphosphonoladenosine: a nonhydrolyzable isosteric, isopolar analog of 2-chlorodeoxyadenosine mono phosphate. Synthesis 1996;7:838–42.
18. Robak T, Blasinski-Morawiecz M, Blasinski J, Hellmann A, Halaburda K, Konopka L, et al. 2-Chlorodeoxyadenosine (cladribine) in the

Figure 13 Structures of 5′-norcarbocyclic adenosine phosphonic acid 57 and its bis-SATE prodrug 58.
treatment of hairy cell leukemia and hairy cell leukemia variant: 7-year experience in Poland. *Eur J Haematol* 1999;62:49–56.

19. Tallman MS, Peterson LC, Hakimian D, Gillis S, Polliack A. Treatment of hairy-cell leukemia: current views. *Semin Hematol* 1999;36:155–63.

20. Elihu S, Martín-Gil A, de Lara MJP, Pintor J, Camden J, Weisman GA, et al. 2-MeS-β-CCl2-ATP is a potent agent for reducing intraocular pressure. *J Med Chem* 2010;53:3305–19.

21. Dvořáková H, Holý A, Rosenberg I. Synthesis of some 2'-C-alkyl derivatives of 9-(2-phosphonomethoxyethyl) adenine and related compounds. *Collect Czechoslov Chem Commun* 1994;59:2069–94.

22. Holý A, Günter J, Dvořáková H, Masojídková M, Andrei G, Snoeck R, et al. Structure-antiviral activity relationship in the series of pyrimidine and purine N-[2-(2-phosphonomethoxy)ethyl] nucleotide analogs. 1. Derivatives substituted at the carbon atoms of the base. *J Med Chem* 1999;42:2064–86.

23. Krawczyk SH. Nucleobase phosphonate analogs for antiviral treatment. *US Patent WO2005/12324A2; 2005 March 17.

24. Averet DR. Nucleoside phosphate derivatives useful in treatment of HIV infection. *US Patent WO2005/79812A1; 2005.

25. Česněk M, Hocek M, Holý A. Synthesis of acyclic nucleotide analogs derived from 2-amino-6-C-substituted purines via cross-coupling reactions of 2-amino-9-[2-[(diisopropoxyphosphoryl)methoxy]ethyl]-6-halopurines with diverse organometallic reagents. *Collect Czechoslov Chem Commun* 2000;65:1357–73.

26. Česněk M, Holý A, Masojídková M, Kmoňůčková E, Zidek Z. Synthesis of guanidin analogs of PMPDAP and their immunobiological activity. *Bioorg Med Chem* 2008;16:965–90.

27. Beadle JR, Van WB, Ciesla SL, Keith KA, Hartline C, Kern ER, et al. Synthesis and antiviral evaluation of alkoxyalkyl derivatives of 9-(3-hydroxy-2-phosphonomethoxypropyl)adenine against cytomegalovirus and orthopoxviruses. *J Med Chem* 2006;49:2010–5.

28. Valiava N, Wyles DL, Schooley RT, Hwu JB, Beadle JR, Prichard MN, et al. Synthesis and antiviral evaluation of 9-(3)-[3-alkoxy-2-(phosphonomethoxy)-propyl] nucleoside alkoxyalkyl esters: inhibitors of hepatitis C virus and HIV-1 replication. *Bioorg Med Chem* 2011;19:4616–25.

29. Krečmerová M, Holý A, Andrei G, Pomeisl K, Tichý T, Bréhová P, et al. Synthesis of ester produgs of 9-(3)-[3-hydroxy-2-(phosphonomethoxy)propyl]-2, 6-diaminopurine (HPMPDAP) as anti-poxvirus agents. *J Med Chem* 2010;53:6825–37.

30. Starrett Jr JE, Tortolani DR, Russell J, Hitchcock MJM, Whiterock V, Martin JC, et al. Synthesis, oral bioavailability determination, and in vitro evaluation of produgs of the antiviral agent 9-[2-phosphonomethoxy]ethyl]adenine (PMEA). *J Med Chem* 1994;37:1857.

31. Ballatore C, McGaugh C, de Clercq E, Balzarini J. Synthesis and evaluation of novel amidate produgs of PMEA and PMPA. *Bioorg Med Chem Lett* 2001;11:1053–6.

32. Meier C, Görgib U, Müller C, Balzarini J. cycloSal–PMEA and cycloAmb–PMEA: potentially new phosphate produgs based on the cycloSal–prunucleotide approach. *J Med Pharmacol Toxicol* 2005;48:8079–86.

33. Barral K, Priet S, Sire J, Neyts J, Balzarini J, Canard B, et al. Synthesis, in vitro antiviral evaluation, and stability studies of novel α-boranoc-nucleotide analogs of 9-[2-(phosphonomethoxy)ethyl]adenine and (R)-9-[2-(phosphonomethoxy)propyl]adenine. *J Med Chem* 2006;49:7799–806.

34. Wolff-Kugel D, Halazy S. Synthesis of new carbocyclic phosphate analogs of dideoxypurine nucleotides. *Tetrahedron Lett* 1991;32:6341–4.

35. Balzarini J, Kuining J, Wedgwood O, Pannecoque C, Aquaro S, Perno CF, et al. Conversion of 2',3'-dideoxyadenosine (ddA) and 2',3'-dideoxy-2',3'-dideoxyadenosine (ddA) to their corresponding arylxophosphoramidate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus. *FEBS Lett* 1997;410:324–9.

36. Oliver JT. A spectrophotometric method for the determination of creatine phosphokinase and myokinase. *Biochem J* 1955;61:116–22.

37. Colowick SP, Kalcik HM. The rôle of myokinase in transphosphorylations I. The enzymatic phosphorylation of hexoses by adenyl pyrophosphate. *J Biol Chem* 1943;148:117–26.

38. Kielley WW, Kielley RK. Myokinase and adenosinetriphosphatase in oxidative phosphorylation. *J Biol Chem* 1951;191:485–500.

39. Kalcik HM. Adenylylpyrophosphatase and myokinase. *J Biol Chem* 1944;153:355–67.

40. Navé JF, Wolff-Kugel D, Halazy S. Carbocyclic phosphate analogs of 2',3'-dideoxyadenosine-5'-monophosphate as substrates of 5'-phosphoribosyl-1'-pyrophosphate (PRPP) synthetate. *Bioorg Med Chem Lett* 1992;2:1483–8.

41. Balzarini J, Navé JF, Becker MA, Tatibana M, de Clercq E. Kinetic properties of adenine nucleotide analogs against purified 5'-phosphoribosyl-1'-pyrophosphate synthetases from E. Coli, rat liver and human erythrocytes. *Nucleosides Nucleotides* 1995;14:1861–71.

42. Lundegaard C, Jensen KF. Kinetic mechanism of uracil phosphoribosyltransferase from *Escherichia coli* and catalytic importance of the conserved proline in the PRPP binding site. *Biochemistry* 1999;38:3327–34.

43. Koh YH, Shim JH, Wu IZ, Zhong WD, Hong Z, Gizardet JL. Design, synthesis, and antiviral activity of adenosine 5'-phosphate analogs as chain terminators against Hepatitis C virus. *J Med Chem* 2005;48:2667–75.

44. Jakeman DL, Bernstein B, Hol W, Williams DM, Williamson MP, Blackburn GM. Highly potent bisphosphonic ligands for phosphoglycerate kinase and protein binding studies. *Phosphorus Sulfur* 1999;144:449–153.

45. Bojumara CG, Parrish JP, Sperandio D, Gao Y, Petkovsky OV, Lee SK, et al. Design, synthesis, and anti-HIV activity of 4'-modified carbocyclic nucleoside phosphate reverse transcriptase inhibitors. *Bioorg Med Chem* 2009;17:1739–46.

46. Parrish JP, Lee SK, Bojumara CG. Evaluation of 2'-α-fluorine modified nucleoside phosphonates as potential inhibitors of HCV polymerase. *Bioorg Med Chem Lett* 2013;23:3354–7.

47. Maui M, Persoons L, Andrei G, Snoeck R, Balzarini J, Herdewijn P. Synthesis and anti-herpetic activity of phosphoramide ProTides. *ChemMedChem* 2013;8:985–93.

48. Wang JN, Singh US, Rawal RK, Sugiyama M, Yoo J, Jha AK, et al. Antiviral activity of novel 2'-fluoro-6'-methylene-carbocyclic adenosine against wild-type and drug-resistant hepatitis B virus mutants. *Bioorg Med Chem Lett* 2011;21:6328–31.

49. Rawal RK, Singh US, Chavre SN, Wang JN, Sugiyama M, Hung W, et al. 2'-Fluoro-6'-methylene-carbocyclic adenosine phosphoramide (FMCPAP) prodrug: *in vitro* anti-HBV activity against the lamivudine-entecavir resistant triple mutant and its mechanism of action. *Bioorg Med Chem Lett* 2013;23:503–6.

50. Oh CH, Yoo KH, Hong HJ. Synthesis and antiviral evaluation of 1'-branched-5'-norcarbocyclic adenosine phosphonic acid analogs. *Bull Korean Chem Soc* 2010;31:2473–8.