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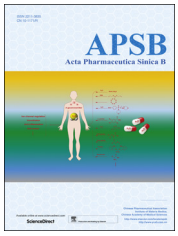

REVIEW

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

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KEY WORDS

Antiviral;
Structure–activity relationship;
Adenine;
Acyclic nucleoside phosphonates;
Scaffold

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 nucleuses possess excellent antiviral activity against most of the double-stranded DNA viruses, such as the herpes group viruses and the orthopoxviruses[1,2]. Previous SARs explorations proved that the concept of using the ANPs as chain terminators for antiviral was remarkably effective (**1**, Fig. 1)[3,4]. 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[5–7], while the (*R*)-9-[(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[8]. Besides, several adenine derivatives also have progressed to clinical exploration, such as GS9148[9–11] (**4**, Fig. 1), GS9131[12] (**5**, Fig. 1), and GS7340[13,14] (**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[15]. 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[16–19]. 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[20].

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 Dvořáková in 1994[21]. Neither exhibited desired antiviral properties. Also, the addition of a fluorine atom to the C-2 position (analog **14**) (Fig. 3) 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 (EC_{50} = 5.51 μmol/L), probably due to its improved bioavailability[22]. 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[23,24], but their activity was

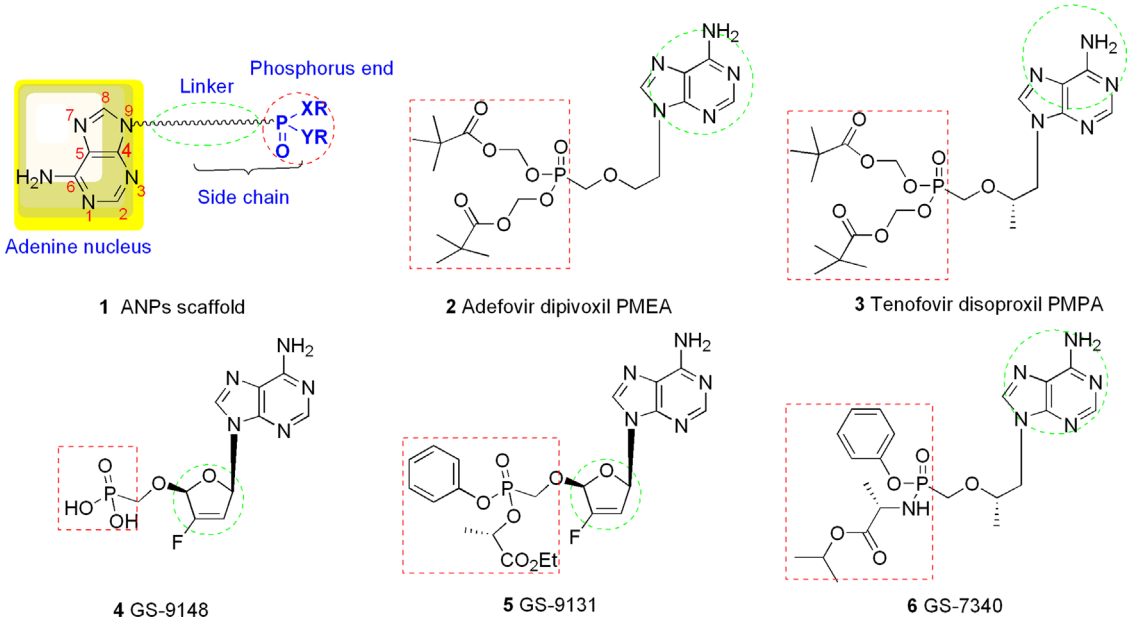


Figure 1 Structures of the novel adenine derivatives as antiviral agents.

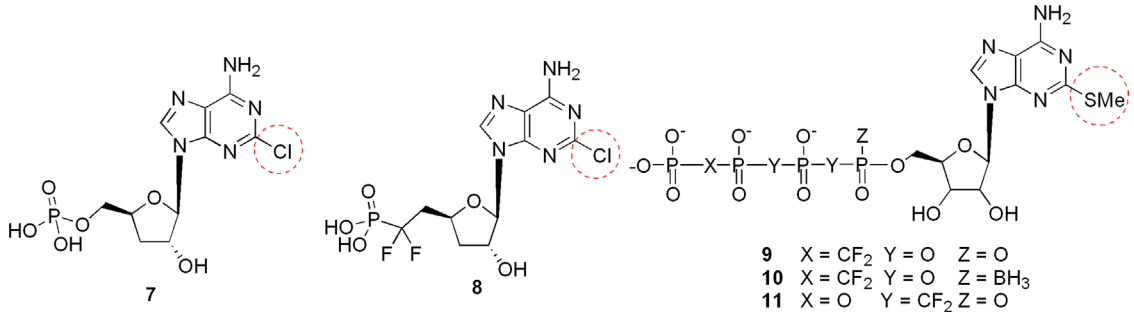


Figure 2 Adenine derivatives with substituents (Cl, MeS) at the C-2 position.

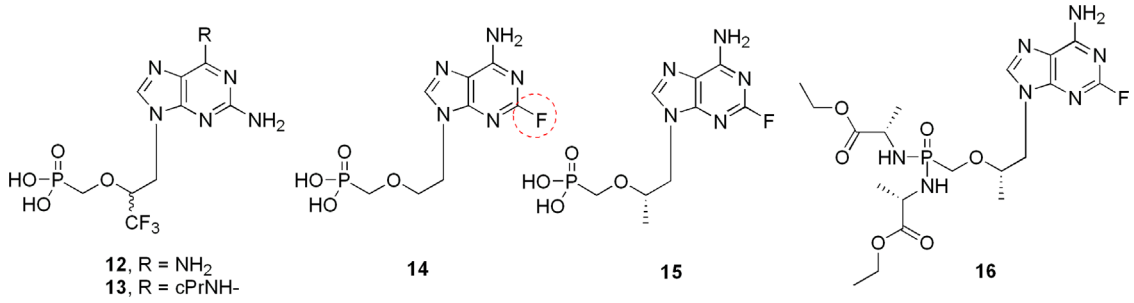


Figure 3 Adenine derivatives with substituents (NH_2, F) at the C-2 position.

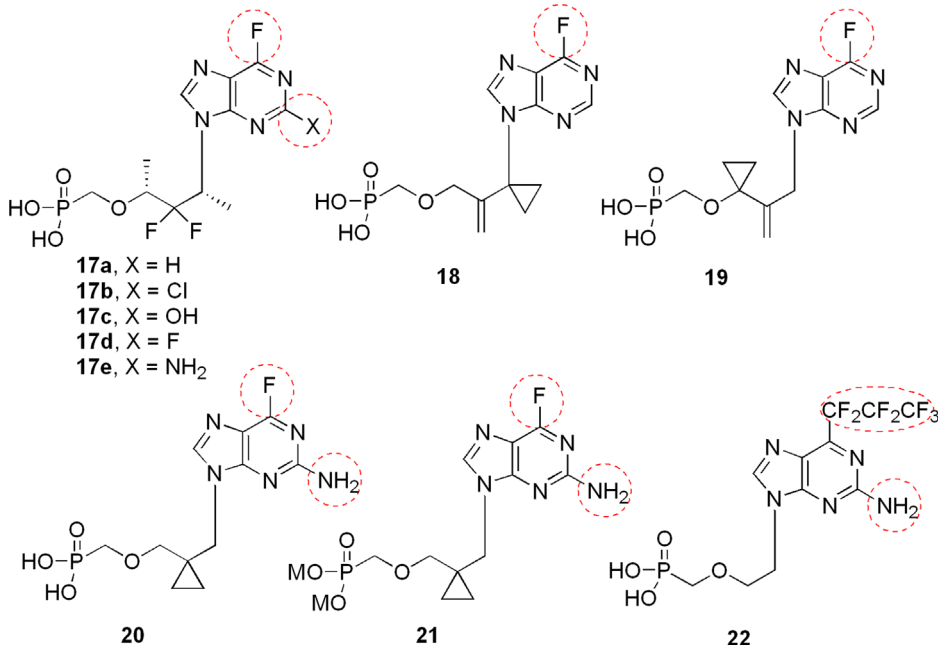


Figure 4 Adenine derivatives with substituents (NH_2, F) at the C-6 position.

not reported. Compound **22** (Fig. 4) that contains a heptafluoropropyl moiety at the C-6 position of the adenine ring was active against various viruses, such as HSV-1 (MIC_{50}=21.8 μmol/L), HSV-2 (MIC_{50}=7.3 μmol/L), HSV-1 TK^- (MIC_{50}=4.4 μmol/L) in E6SM cells and against CMV (MIC_{50}=3.6 μmol/L) and varicella-zoster virus (VZV) TK^- and TK^+ mutants (MIC_{50}=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[25].

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 Česnek (**23** and **24**, Fig. 5)[26]. Unfortunately, none of these compounds exhibited significant antiviral activity.

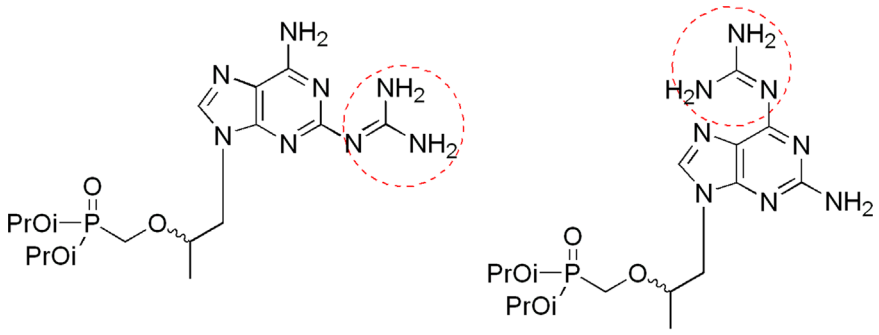


Figure 5 Adenine derivatives with substituents at the C-6 position.

3. Side chain

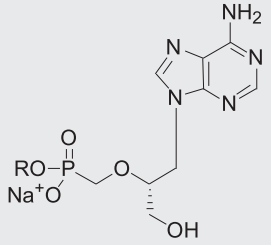
3.1. Linear linker

To improve the oral bioavailability of the novel 9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine ((*S*)-HPMPA), Hostetler[27] 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*)-HMPA, **25a**) was the most active inhibitor, possessing the EC_{50} 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 EC_{50} 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*)-MPMPA, Table 2) was also able to suppress both genotype 1b and 2a hepatitis C virus (HCV) replicons with an EC_{50} value of 1–2 μmol/L and a CC_{50} value of more than 150 μmol/L, respectively[28]. 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*)-MPMPA, Table 2), which possesses an EC_{50} value of less than 0.01 μmol/L along with a selectivity index of more than 4.4 million.

In 2010, Krecmerov[29] 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.

Table 1 Structures and activities of lipid esters of HPMPA.



25a R = HDP HDP-(*S*)-HPMPA
25b R = ODE ODE-(*S*)-HPMPA
25c R = OLE OLE-(*S*)-HPMPA
25d R = OLP OLP-(*S*)-HPMPA

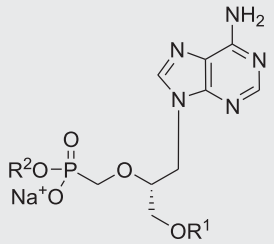
Compd.	EC_{50} (μmol/L)[a]			
	HCMV	MCMV	Vaccinia	Cowpox
CDV	1.2	0.04	25.3	30.7
(*S*)-HPMPA	0.82	0.16	2.7	4.0
25a	0.003	0.002	0.01	0.02
25b	0.003	0.003	0.01	0.02
25c	0.003	0.04	0.003	0.007
25d	0.003	0.1	0.018	0.034

[a]Anti-HIV-1 activity (EC_{50}): 50% effective concentration in wild-type CEM cell cultures.

3.2. Amide linker

Amide prodrugs of PMEA were first synthesized as anti-HIV agents by Starrett[30] in 1994. However, the obtained compounds were unstable under acidic conditions and provided levels of PMEA comparable to the parent compound after oral

Table 2 Another series of structures and activities of lipid esters of MPMPA.



Compd.	R^1	R^2	Abbreviation	EC_{50}[a]		CC_{50}[b]	SI[c]	
				BM4–5(1b)	JFH-1(2a)		BM4-5(1b)	JFH-1(2a)
26	Me (*S*)	ODE	ODE-(*S*)-MPMPA	1.43	2.38	>150	>105	>63
27	Me (*R*)	ODE	ODE-(*R*)-MPMPA	4.65	5.33	>150	>32.3	>28.1
28	Me (*S*)	HDP	HDP-(*S*)-MPMPA	2.36	4.64	>150	>63.6	>32.3
29	Et (*R*,*S*)	HDP	HDP-(*R*,*S*)-EPMPA	7.59	8.87	99	13.0	11.1
30	*i*-Pr (*R*,*S*)	HDP	HDP-(*R*,*S*)-IPMPA	5.12	98.8	100	3.14	—

[a]Anti-HIV-1 activity (EC_{50}): 50% effective concentration in wild-type CEM cell cultures (μmol/L).
[b]CC_{50}: cytotoxic concentration (μmol/L).
[c]Selectivity index (SI) = CC_{50}/EC_{50}.

administration. In 1997, Ballatore[31] 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, Chapman[13] discovered a novel amidate derivative GS7340 (**6**, Fig. 1). The EC_{50} of GS 7340 against human immunodeficiency virus type 1 in MT-2 cells was 0.005 μmol/L compared to an EC_{50} of 5 μmol/L for the parent drug tenofovir. The L-alaninyl analog (GS7340) was >1000-fold more active than the D-alaninyl analog. Moreover, it has a half-life of 90 min in human plasma and a half-life of 28.3 min in an MT-2 cell, respectively. The antiviral activity and the metabolic stability in MT-2 cell extracts and plasma were also 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[32]. 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. *Borano linker*

In 2006, Barral et al.[33] synthesized two boranophosphonate nucleosides, 9-[2-(boranophosphonomethoxy)ethyl]adenine (**39a**, Table 5) and (*R*)-9-[2-(ranophosphonomethoxy)propyl]adenine (**39b**, Table 5). Both H-phosphinates **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-phosphinates **39a** and **39b**, with half-live values of 5.3 h for **40a** and 1.3 h for **40b**, respectively.

Carbocyclic phosphonate analogs of dideoxy-adenine nucleotides were discovered as potential anti-HIV inhibitors by Wolff-Kugel and Halazy[34] 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-(difluoromethylene)phosphonate[35]. Biological test disclosed that analog **42** (Fig. 7) was not a substrate of AMP kinase (myokinase)[36–39], but it can be slowly pyrophosphorylated by *Escherichia coli* phosphoribosyl pyrophosphate (PRPP) synthase with an excess of PRPP (K_m not determined)[40–42], while being a weak PRPP synthase inhibitor (IC_{50} = 1 mmol/L).

In 2005, Girardet designed a series of adenosine 5′-phosphonate analogs to mimic naturally occurring adenosine monophosphate[43]. 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 diphosphonate derivatives (**43a–47a**, Fig. 8) were also synthesized. Extensive nucleotide

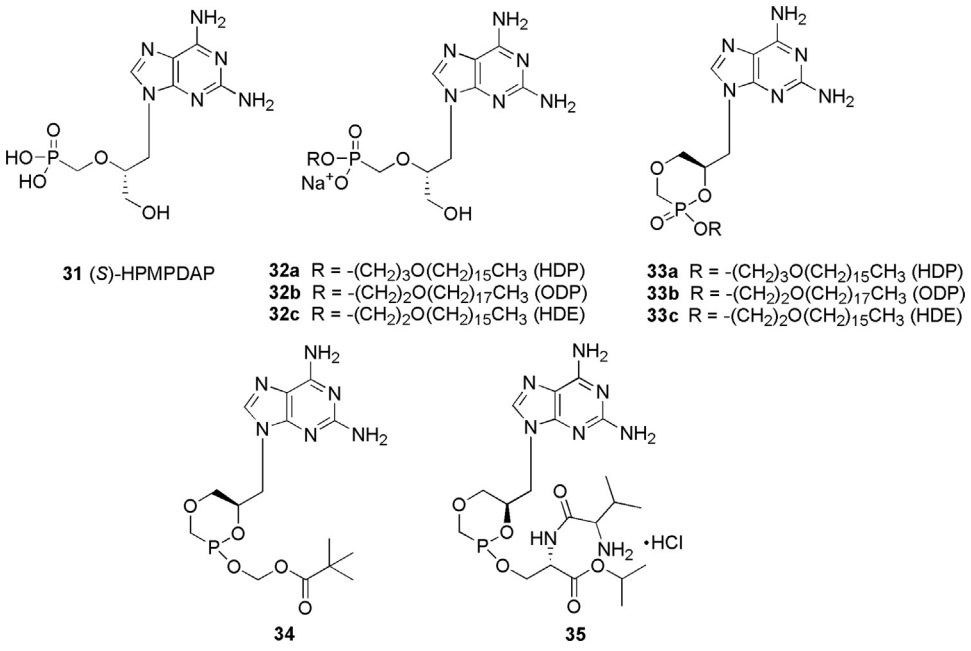
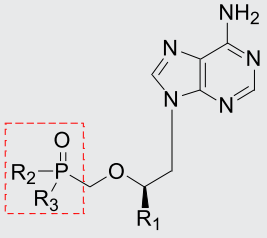


Figure 6 Structures of the 9-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) and its cyclic form as potential drug candidates against poxvirus infections.

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

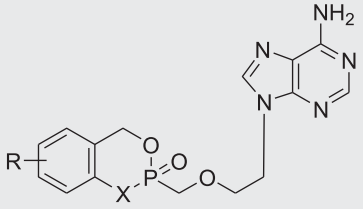


Compd.	R_1	R_2	R_3	EC_{50}[a] HIV-1 MT4	EC_{50}[a] HIV-2 MT4	CC_{50}[b] HIV-1 MT4	EC_{50}[a] HIV-1 CEM	EC_{50}[a] HIV-2 CEM	CC_{50}[b] HIV-2 CEM
36a	CH_3	OH	OH	2.3	1.4	197	3.67	3.67	≥250
36b	H	OH	OH	7.0	7.5	144	7.0	10	69
36c	CH_3	L-Ala-Me-ester	PhO	0.029	0.026	71.4	0.053	0.090	27
36d	CH_3	Gly-Me-ester	PhO	0.58	0.15	102	0.23	0.31	88.5
36e	CH_3	D-Ala-MPhe-ester		0.99	0.65	213	0.5	0.38	125
36f	CH_3	L-Phe-Me-ester	pCl-PhO	0.04	0.07	64	0.07	0.06	26
36g	CH_3	L-Ala-Me-ester	pCl-PhO	0.15	0.26	57	0.2	0.12	106
36h	CHPhP	O	O		4.9	≥250	4.0	4.0	>250
36i	H	L-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	D-Ala-Me-ester	PhO	15	7.2	120	5.0	6.0	67

[a]Anti-HIV-1 activity (EC_{50}): 50% effective concentration in wild-type CEM cell cultures (μmol/L).
[b]CC_{50}: cytotoxic concentration (μmol/L).

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

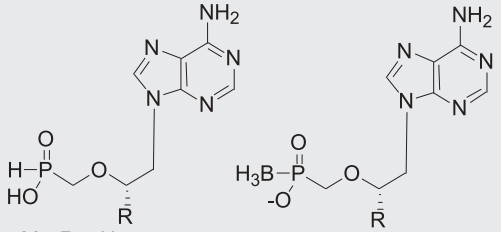
Table 4 Chemical stability and biological activities of compounds **37a–d** and **38a–c**.



Compd.	X	R	Half-life (h)[a]		EC_{50}[b] BchE	Biological activity in CEM/O cells		
			pH 7.3	pH 2.0		EC_{50}		CC_{50}[c]
						HIV-1	HIV-2	
37a	O	H	0.09	n.d[d]	>50	n.d[d]	n.d[d]	n.d[d]
37b	O	3-Me	0.56	n.d	>50	n.d[d]	n.d[d]	n.d[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

[a]Hydrolysis in 25 mmol/L phosphate buffer at 37 °C or 25 mmol/L citrate buffer, at 37 °C , half-life (*t*1/2) were determined from the decreasing peak of the starting phosphate triester and are the mean of duplicate experiments.
[b]Anti-HIV-1 activity (EC50): 50% effective concentration in wild-type CEM cell cultures (μmol/L).
[c]CC_{50}: cytotoxic concentration.
[d]Not determined due to low chemical stability.

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



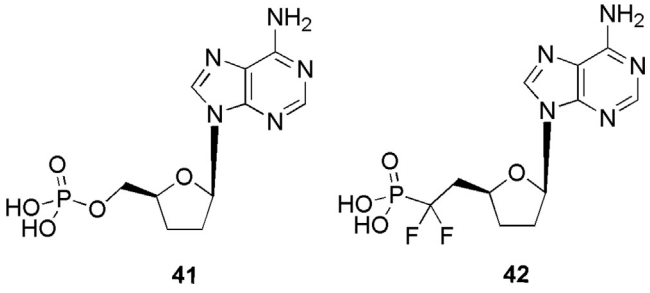
39a R = H
39b R = Me

40a R = H
40b R = Me

Compd.	$t_{1/2}$ (h)			
	Buffers[a]	PMI-1640	Cuture medium	Total cell extracts
39a	Stable[b]	>72	>72	>72
39b	Stable	>72	>72	
40a	Stable	>72	5.3[c]	
40b	Stable	>72	1.3[c]	24[c]
PMEA	Stable	>72	>72	>72
(*R*)-PMEA	Stable	>72	>72	>72

[a]pH of the buffers: 1.2, 5.2, 7.4, 8.1, 9.0, 11.5.
[b]Less than 5% decomposition after 72 h.
[c]Single product of decomposition: **39a** from **40a** and **39b** from **40b**.

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,



41 **42**

Figure 7 Structures of carbocyclic phosphonate analogs of dideoxyadenine nucleotides.

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 CF_2 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/L[9–11]. Unlike many clinical RT inhibitors, relevant reverse transcriptase mutants (M184V, K65R and 6-TAMS) maintain a susceptibility to 2′-

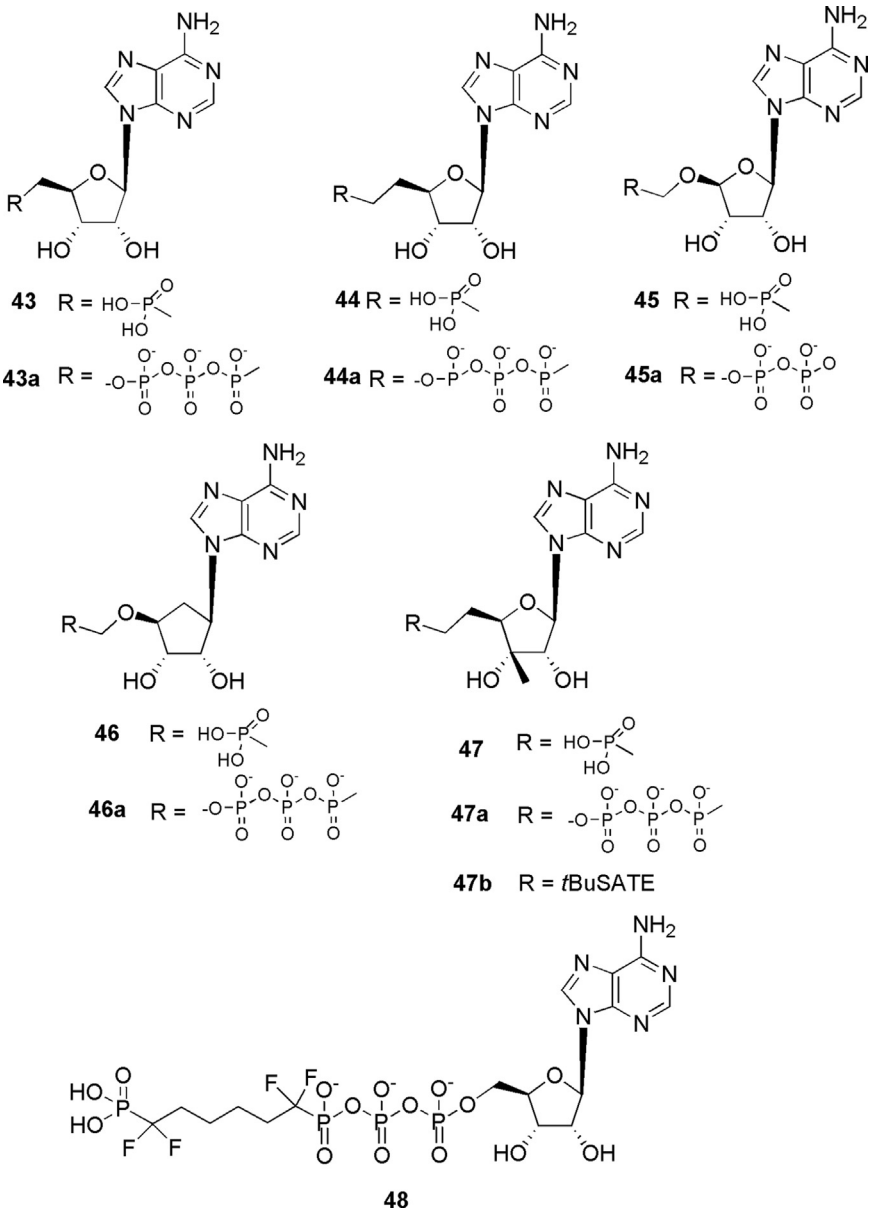


Figure 8 Structures of adenosine 5′-phosphonate analogs.

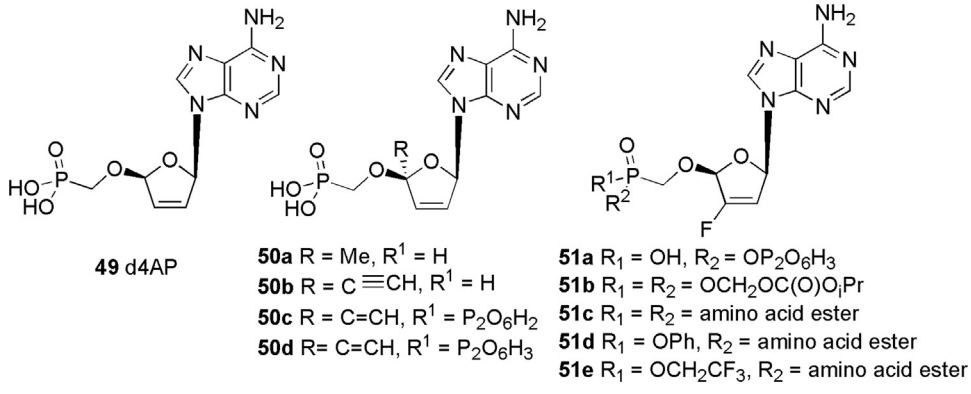


Figure 9 Structures of GS9148 analogs.

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, Boojamra[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 ($EC_{50}=16$ 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 amidate 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 phosphonamidate 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 NS5B polymerase (EC_{50} as low as 2 μmol/L), but the parent compounds (*e.g.*, compounds **7** and **8**) were still weak to inhibit the NS5B polymerase. Clearly, prodrugs of the parent nucleoside phosphonates improved the cellular activity.

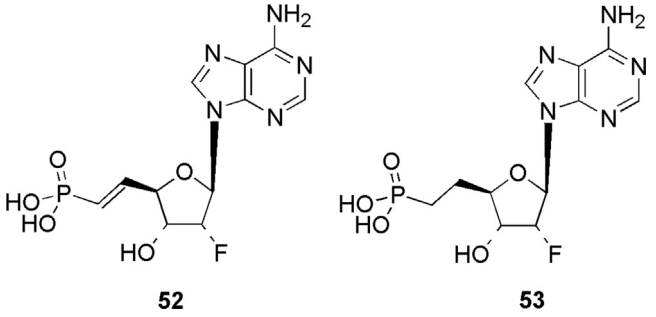


Figure 10 Structures of 2′-fluorine-modified nucleoside phosphonates **52** and **53**.

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. L-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 (FMCAP) 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

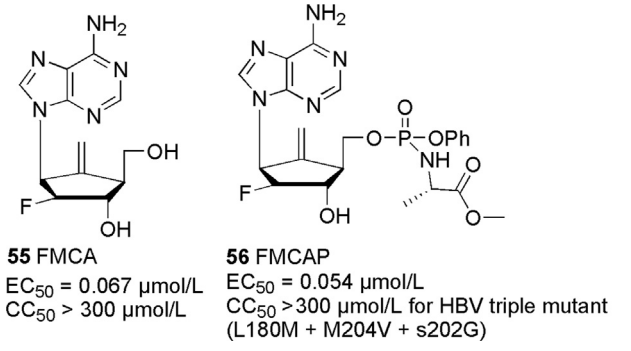


Figure 12 Structures of novel adenine derivative FMCA and FMCAP.

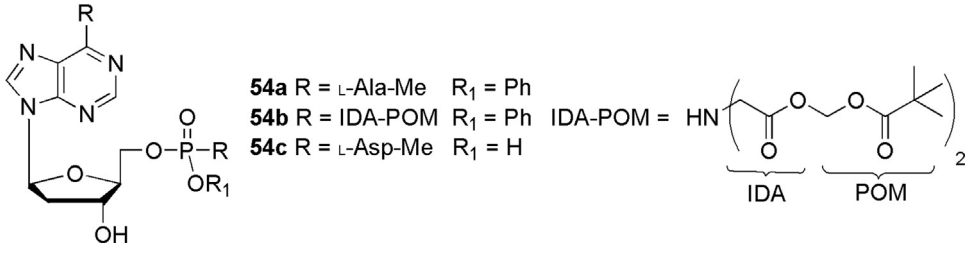


Figure 11 Structures of phosphoramidate ProTides **54a–c**.

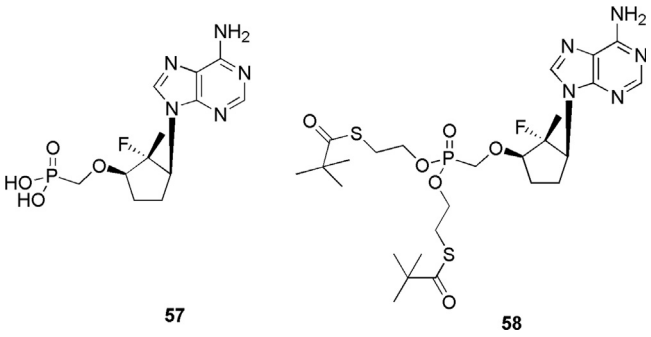


Figure 13 Structures of 5′-norcarbocyclic adenosine phosphonic acid **57** and its bis-SATE prodrug **58**.

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[42].

Oh and his co-workers' studies[50] 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_{50} = 62 μmol/L, CC_{50} = 70 μmol/L), prodrug **58** enhanced the anti-HIV activity (EC_{50} = 16.7 μmol/L, CC_{50} = 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.

Acknowledgments

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