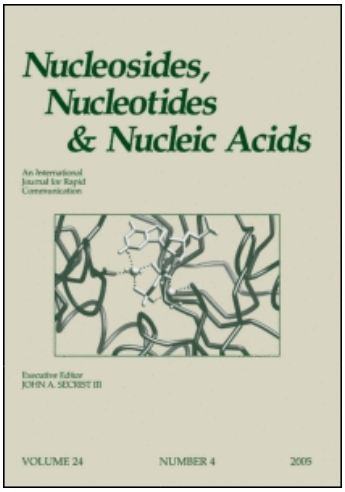

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Antiviral Activity Spectrum of Nucleoside and Nucleotide Analogues

Erik De Clercq[a]
[a] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

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ANTIVIRAL ACTIVITY SPECTRUM OF NUCLEOSIDE AND NUCLEOTIDE ANALOGUES

Erik De Clercq

Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Abstract. Several nucleoside/nucleotide analogues offer great potential for the treatment of viral diseases : (i) phosphonylmethoxyalkylpurines and -pyrimidines for adeno-, herpes-, pox-, hepadna- and retroviruses; (ii) neplanocin A analogues for pox-, paramyxo-, arena-, rhabdo- and reoviruses; (iii) acyclic 6-phenylthiouridine derivatives for human immunodeficiency virus type 1.

In recent years several new classes of nucleoside (or nucleotide) analogues have been identified as selective antiviral agents. Foremost among these lead compounds are (1) the 2',3'-dideoxynucleoside analogues, which are specifically active against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2)[1-3]; (2) some carbocyclic 2',3'-didehydro-2',3'-dideoxynucleosides, such as carbovir (carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine), which is also active against HIV[4]; (3) carbocyclic oxetanocin analogues, such as cyclobut-A and cyclobut-G, which are active against both HIV and herpesviruses[5,6]; (4) carbocyclic adenosine analogues (neplanocin A derivatives), which are active against a broad range of (-)RNA and (±)RNA viruses [but not (+)RNA viruses (i.e. retroviruses)][7-9]; (5) carbocyclic cytidine (carbodine, cyclopentylcytosine) and the related cyclopentenylcytosine, which are active against DNA virues, (+)RNA viruses, (-)RNA viruses and (±)RNA viruses[10-12]; (6) 3'-fluoro-3'-deoxyadenosine, which is active against some DNA viruses, (+)RNA viruses and (±)RNA viruses[13]; (7) 2'-fluoro-2',3'-dideoxyarabinofuranosyladenine, which is specifically active against HIV[14,15]; (8) nucleoside analogues in which the 3'-carbon is replaced by sulfur or oxygen (thus leading to oxathiolanyl or dioxolanyl derivatives, respectively), and which also demonstrate anti-

HIV activity[16]; (9) isonucleosides (i.e.iso-ddA) in which the 3'-carbon and ring oxygen are transposed, and which also show anti-HIV activity[17]; (10) 1-(2-deoxy-2-fluoro-β-D-deoxyarabinopyranosyl)-5-iodouracil and other 2'-deoxy-2'-fluoro-D-arabinopyranosyl nucleosides (and their 3',4'-seco analogues), which are markedly active against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2)[18]; (11) the acyclic nucleoside analogues adenallene [9-(4'-hydroxy-1',2'-butadienyl)-adenine] and cystallene [1-(4'-hydroxy-1',2'-butadienyl)cytosine], which show activity against HIV-1 and HIV-2[19]; (12) the phosphonylmethoxyalkyl [3'-hydroxy-2-phosphonylmethoxypropyl (HPMP) and 2-phosphonylmethoxyethyl (PME)] purines and pyrimidines, which exhibit a broad-spectrum activity against DNA (adeno-, herpes-, hepadna-, irido-, pox-) viruses and retroviruses (i.e. HIV)[20,21] and (13) the 6-phenylthiouracil derivatives, with 1-(2-hydroxyethoxymethyl)-6-phenylthiothymidine as the prototype compound, which are very higly specific inhibitors of HIV-1[22,23].

In this report, I will focus on three classes of compounds : (i) the phosphonylmethoxyalkyl (HPMP, PME) derivatives, (ii), the neplanocin A derivatives, and (iii) the 6-phenylthiouracil derivatives. I will describe their antiviral activity spectrum as well as the basis of their selective antiviral activity and the prospects for their clinical use in the treatment of viral diseases.

Phosphonylmethoxyalkylpurines and -pyrimidines

The HPMP and PME derivatives that have been most intensively studied are HPMPA, HPMPC, PMEA and PMEDAP (FIG. 1). HPMPA has been the lead compound of this series[20], and its activity has been demonstrated against various adenovirus (AV) serotypes[24], herpesviruses [HSV-1, HSV-2 and thymidine kinase (TK)-deficient (TK^-) HSV-1 mutants[20], varicella-zoster virus (VZV) and TK^- VZV mutants[20], cytomegalovirus (CMV)[20,26], Epstein-Barr virus (EBV)[27], phocid herpesvirus type 1 (seal herpesvirus, SeHV)[28], suid herpesvirus type 1 (SHV-1, pseudorabies virus or Aujeszky's disease virus)[20], bovid herpesvirus type 1 (BHV-1, infectious bovine rhinotracheitis virus)[20], equid herpesvirus type 1 (EHV-1, equine abortion virus)[20,29]], hepadnaviruses [duck hepatitis B virus (DHBV)[30], human hepatitis B virus[31]], iridoviruses [African swine fever virus (ASFV)[32,33]], poxviruses [vaccinia virus (VV)[20]]. HPMPC has an

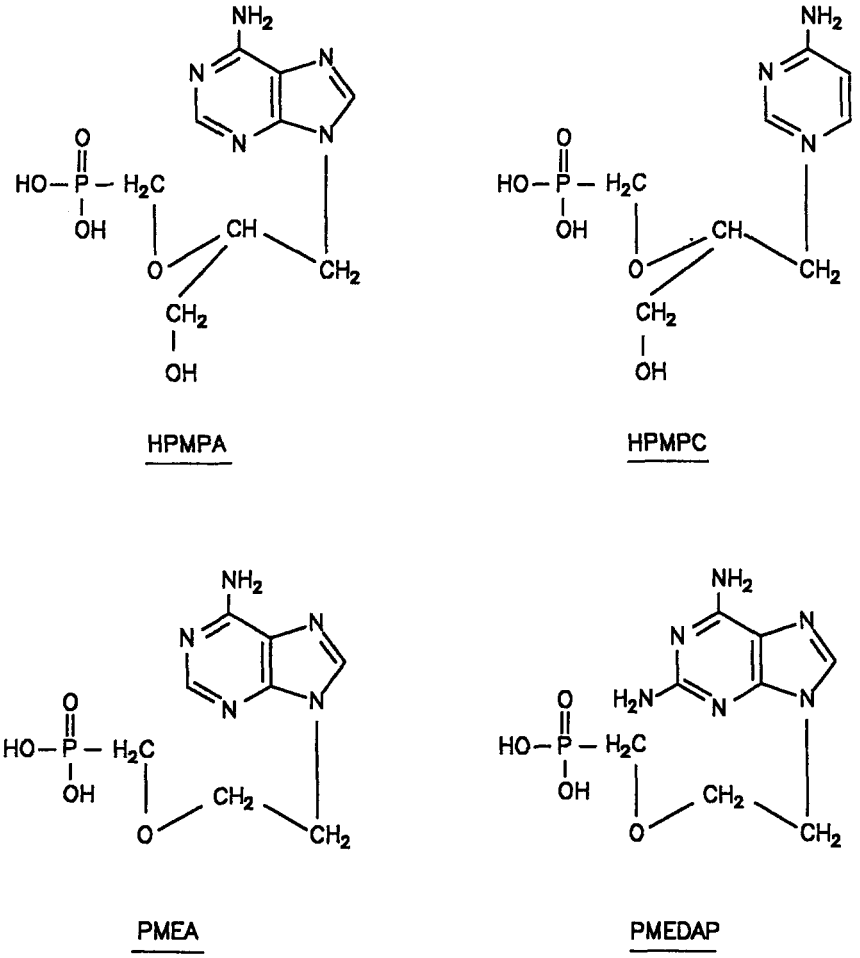

Fig. 1. Phosphonylmethoxyalkyl derivatives :
- (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA)
- (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC)
- 9-(2-phosphonylmethoxyethyl)adenine (PMEA)
- 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP)

antiviral activity spectrum that is similar to that of HPMPA, but PMEA and PMEADAP show an activity spectrum that only partially overlaps with that of HPMPA and HPMPC. Like HPMPA and HPMPC, PMEA and PMEDAP are active against herpes-, hepadna- and iridoviruses, but they are virtually inactive against adeno- and poxviruses. While loosing part of their spectrum at the DNA virus side, PMEA and PMEDAP gain marked activity against retroviruses, i.e. HIV-1[34],HIV-2[35], simian immunodeficiency

TABLE 1
Antiviral activity of phosphonylmethoxyalkylpurines and -pyrimidines

Virus		Minimum inhibitory concentration (μg/ml)			
		HPMPA	HPMPC	PMEA	PMEDAP
Adeno	: AV-2,3,4	0.3	3	>100	>100
Herpes	: HSV-1	2	4	7	2
	HSV-1 (TK^-)	2	2	7	1
	HSV-2	4	10	7	0.7
	VZV	0.02	0.2	10	2
	CMV	0.1	0.08	25	10
	EBV	0.03	0.01[§]	0.7	0.05[§]
Hepadna	: DHBV	0.5			1
	HBV	0.1	10	0.1	0.02
Irido	: ASFV	0.01	1	5	2
Pox	: VV	0.7	4	>100	20
Retro	: HIV-1	>40		0.1 - 2	0.3
	HIV-2			2	
	SIV			1	
	FIV			0.15	

Data taken from ref. 21, 26, 27, 30, 31, 32, 33, 34 and 35.
[§]Data obtained by J.-C. Lin, E. De Clercq & J.S. Pagano.
The values that point to a significant antiviral activity are framed. These antiviral effects were obtained at a concentration that was significantly below the cytotoxicity threshold.

virus (SIV)[35], feline immunodeficiency virus (FIV)[35], simian AIDS-related virus (SRV)[35], murine (Moloney) sarcoma virus (MSV)[34,35] and murine (Rauscher) leukemia virus (MLV)[36]. The minimum inhibitory concentrations of HPMPA, HPMPC, PMEA and PMEDAP for some representative DNA (adeno, herpes, hepadna, irido, pox) and retroviruses are listed in TABLE 1. From this Table it is clear that the HPMP derivatives are active against adeno-, herpes-, hepadna-, irido- and poxviruses and that the PME derivatives are active against herpes-, hepadna-, irido- and retroviruses.

The HPMP and PME derivatives are assumed to act in a similar fashion. This means that they are as such taken up by the cells[37] and subsequently converted to their diphosphorylated forms (HPMPApp, HPMPCpp, PMEApp and PMEDAPpp, respectively). In this form, HPMPA and

its congeners would interact with the viral DNA polymerization process and thereby exhibit a much greater affinity for viral DNA polymerases than cellular DNA polymerases. The result of this discriminative behavior is that viral DNA synthesis is suppressed at HPMPA, HPMPC, PMEA and PMEDAP concentrations which are lower by three orders of magnitude than the concentrations required to inhibit cellular DNA synthesis. This highly selective inhibition of viral DNA synthesis has been observed with HPMPA in HSV-infected cells[37], with HPMPA and PMEA in EBV-infected cells[27], with HPMPA in ASFV-infected cells[38], with HPMPC in CMV-infected cells[39], and with HPMPApp in a reconstituted AV DNA poly merase system[40]. The differences that have been noted in the antiviral activity spectrum of the HPMP and PME derivatives may be due to the fact that the HPMP derivatives, when incorporated in DNA, could still allow further chain elongation (because of the presence of the 3'-hydroxyl group, whereas the PMEA derivatives lacking this group, would act as chain terminators if incorporated into the growing DNA chain.

In vivo, the HPMP derivatives (HPMPA, HPMPC) and PME derivatives (PMEA, PMEDAP) have proved efficacious in a large variety of experimental virus infections in animal models : i.e. HSV-1 keratitis (also TK^- HSV-1 keratitis) in rabbits[41], systemic HSV-1 and HSV-2 infections in mice[42,43], cutaneous HSV-1 and HSV-2 infections in hairless mice and guinea pigs[42,43], HSV encephalitis in mice[42], VV infection (tail lesions) in mice[42], murine CMV infection in mice[44] and simian varicella virus (SVV) infection in monkeys[45]. When evaluated in parallel with the standard anti-HSV drug acyclovir (ACV), the phosphonylmethoxyalkyl derivatives invariably showed much higher efficacy. HPMPC also proved superior to ganciclovir in the treatment of murine CMV infection. Marked efficacy has also been noted with PMEA and PMEDAP in the treatment of various retrovirus infections, i.e. MSV-induced tumor formation in mice[46-48], MLV infection in mice[36], murine AIDS (LP-BM5) virus infection in mice[49], feline leukemia virus (FLV) infection in cats[50], FIV infection in cats[51], and SIV infection in monkeys[35]. In some of these model systems[36,46,47,50] PMEA was evaluated in parallel with, and found to be more efficacious than, azidothymidine (AZT).

From a therapeutic viewpoint, the phosphonylmethoxyalkyl derivatives offer the unique advantage that a single administration permits a long-acting antiviral response, that persists for several days if not

one week or longer[39]. Such sustained antiviral action would allow infrequent dosing of the compounds, i.e. twice or once a week (as has been demonstrated in the MSV model[52]), or, perhaps, just once, i.e. prophylactically, before the symptoms of the infection have become apparent.

Aristeromycin and neplanocin A derivatives

Various acyclic and carbocyclic analogues of adenine, including (S)-9-(2,3-dihydroxypropyl)adenine (DHPA), 3-(adenin-9-yl)-2-hydroxypropanoic acid (AHPA) isobutyl ester, carbocyclic 3-deazaadadenosine (C-c^{3}Ado) and neplanocin A (NpcA), have been recognized as broad-spectrum antiviral agents during the past years, and, on detailed analysis, it appears that the antiviral activity spectrum exhibited by these compounds is quite similar from one compound to another[7]. This activity spectrum includes some DNA viruses [i.e. poxviruses (vaccinia), but not herpesviruses (HSV-1, HSV-2)], (-)RNA viruses [i.e. paramyxoviruses (measles, parainfluenza), arenaviruses (Junin, Tacaribe), rhabdovirusus (vesicular stomatitis, rabies)], (±)RNA viruses [reoviruses (reo, rota)], but not (+)RNA viruses (picorna-, toga- or retroviruses). The antiviral properties of C-c^{3}Ado (3-deazaaristeromycin) and NpcA have been described previously[53,54]. Recently, new derivatives, that belong to either the aristeromycin or neplanocin A series, have been developed[8,9] (FIG. 2); and these compounds (termed DHCaA, c^{3}DHCaA, c^{3}NpcA, DHCeA and c^{3}DHCeA) show an activity spectrum conform to that of their parent compounds C-c^{3}Ado and NpcA (TABLE 2). Hence, they are significantly inhibitory to vaccinia virus (VV), parainfluenza virus (type 3), Junin virus, Tacaribe virus, vesicular stomatitis virus and reovirus (type 1), but not inhibitory to HSV-1, HSV-2, poliovirus (type 1), Coxsackie virus (type B4), Sindbis virus and Semliki forest virus. Also, NpcA, c^{3}NpcA, DHCeA and c^{3}DHCeA are not inhibitory to HIV replication at concentrations that are non-toxic to the host cells[8].

The remarkable similarity in the antiviral activity spectrum of C-c^{3}Ado, NpcA and their congeners point to a common mechanism (or target) of action, and this target has been identified as S-adenosylhomocysteine (SAH) hydrolase, a key enzyme in transmethylation reactions starting from S-adenosylmethionine (SAM) as the methyl donor. SAH is product inhibitor of these transmethylation reactions. SAH hy-

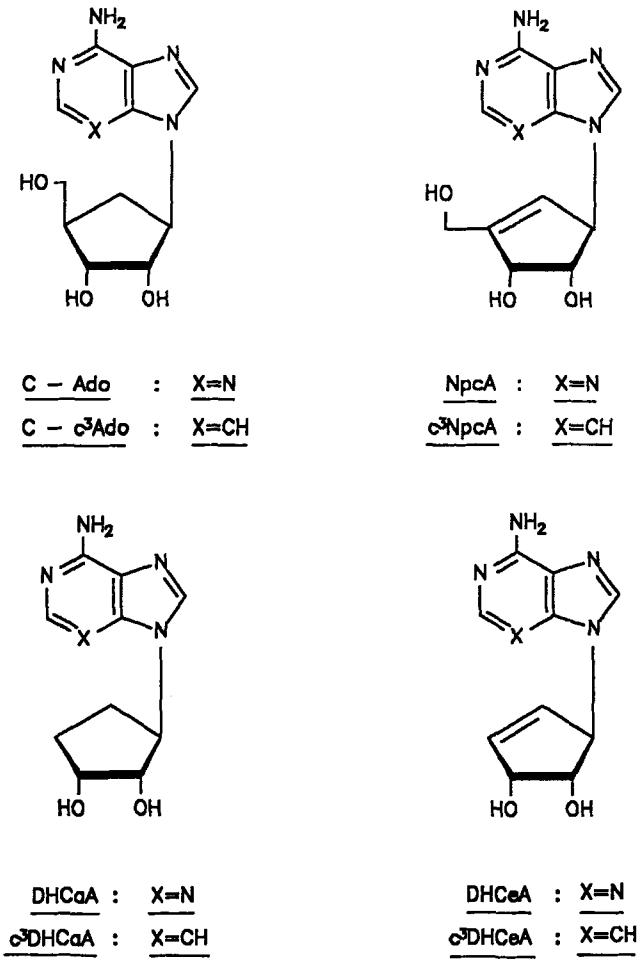

Fig. 2. Aristeromycin/Neplanocin A derivatives :
- carbocyclic adenosine (C-Ado, aristeromycin)
- carbocyclic 3-deazaadenosine (C-c^3Ado)
- neplanocin A (NpcA)
- 3-deazaneplanocin A (c^3NpcA)
- 9-(trans-2',trans-3'-dihydroxycyclopentyl)adenine (DHCaA)
- 9-(trans-2',trans-3'-dihydroxycyclopentyl)-3-deazaadenosine (c^3DHCaA)
- 9-(trans-2',trans-3'-dihydroxycyclopent-4'-enyl)adenine (DHCeA)
- 9-(trans-2',trans-3'-dihydroxycyclopent-4-enyl)-3-deaza-adenosine (c^3DHCeA)

TABLE 2
Antiviral activity of aristeromycin and neplanocin A derivatives

Virus		Minimum inhibitory concentration (μg/ml)			
Aristeromycin derivatives :		C-Ado*	C-c^{3}Ado	DHCaA§	c^{3}DHCaA§
Herpes	: HSV-1 (KOS)	...	>400	>400	>400
	HSV-2 (G)	...	>400	>400	>400
Pox	: VV	...	2	0.02	0.02
Picorna	: Polio-1	...	>400	>200	>200
	Coxsackie-B4	...	>400	>200	>200
Toga	: Sindbis	...	>400	>100	>100
	Semliki forest	...	>400	>100	>100
Paramyxo	: Parainfluenza-3	...	0.2		0.2
Arena	: Junin	...	1	0.01	0.01
	Tacaribe	...	2	0.01	0.01
Rhabdo	: Vesicular stomatitis	...	0.2	0.01	0.01
Reo	: Reo-1	...	1	0.07	0.07
Neplanocin A derivatives :		NpcA	c^{3}NpcA	DHCeA	c^{3}DHCeA
Herpes	: HSV-1 (KOS)	7	>400	150	>400
	HSV-2 (G)	10	>400	300	200
Pox	: VV	0.02	0.07	0.7	0.7
Picorna	: Polio-1	>10	>400	>400	>400
	Coxsackie-B4	>10	>400	>400	>400
Toga	: Sindbis	>10	>400	>400	>400
	Semliki forest	>10	>400	>400	>200
Paramyxo	: Parainfluenza-3	0.2	0.2	2	2
Arena	: Junin	0.3	1	1	2
	Tacaribe	0.3	1	2	3
Rhabdo	: Vesicular stomatitis	0.02	0.07	0.2	0.2
Reo	: Reo-1	0.7	0.07	0.7	2

Data taken from ref. 8 and 55.
*No data are listed for C-Ado since its minimum inhibitory concentrations coincided with its cytotoxic concentration[56]. The values that point to a significant antiviral activity are framed. These antiviral effects were obtained at a concentration that was significantly below the cytotoxicity threshold.
§Data obtained by E. De Clercq and R.T. Borchardt.

drolase catalyzes the (reversible) conversion of SAH to adenosine (which is further deaminated to inosine by adenosine deaminase) and homocysteine. When SAH hydrolase is inhibited by the aristeromycin or neplanocin A derivatives, SAH accumulates, SAM-dependent transmethylation reactions are shut off, and viral mRNA that depends on such methylations for its maturation, no longer matures. It is conceivable that those viruses (i.e. pox-, paramyxo-, arena-, rhabdo- and reoviruses) that most heavily depend on these methylations are also the more sensitive to the SAH hydrolase inhibitors.

The concept that SAH hydrolase must be the target for the antiviral action of the carbocyclic and acyclic adenosine analogues stems for the close correlation that has been found between the inhibitory effects of these adenosine analogues on virus replication and their Ki values for purified SAH hydrolase (isolated from the same cells as used in the antiviral assays)[57]. Also, treatment of the cells with the adenosine analogues at antivirally active concentrations leads to an increase in intracellular SAH levels that is equivalent to the reduction in virus yield[58]. It is not immediately clear, however, how a specific antiviral effect can be achieved through modulation of a host cell enzyme (SAH hydrolase).

SAH hydrolase inhibitors hold promise as candidate antiviral drugs for the treatment of a number of important viral diseases [i.e. arena (Lassa fever), rhabdo (rabies) and reo (rota) virus infections] for which there is currently no satisfactory therapy. Some of the carbocyclic adenosine analogues (C-c^{3}Ado[53], NpcA[54]) have proved effective in some animal models of vaccinia virus or vesicular stomatitis virus infection, but, clearly, further studies are needed to assess the full therapeutic potential of these compounds, and, in particular, their effectiveness against such important human pathogens as rabies, rota, Lassa fever and other hemorrhagic fever virus infections.

1-(2-Hydroxyethoxymethyl)-6-phenylthiothymine (HEPT) derivatives

From a series of 6-substituted 1-(2-hydroxyethoxymethyl)uracil derivatives, which were apparently synthesized as potential antiherpetic agents (as they share the same acyclic side chain as acyclovir), HEPT emerged as a specific inhibitor of HIV-1[22,23]. The remarkable feature of HEPT is that the compound has no activity whatsoever against

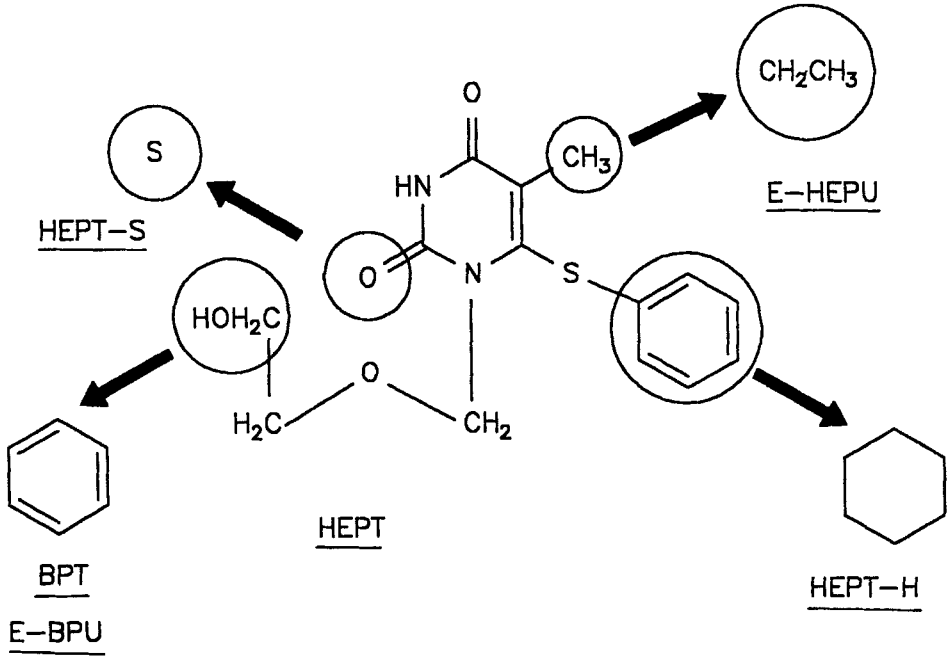

Fig. 3. HEPT [1-(2-hydroxyethoxymethyl)-6-phenylthiothymine] derivatives :
- 1-(2-hydroxyethoxymethyl)-2-thio-6-phenylthiothymine (HEPT-S)
- 1-(2-hydroxyethoxymethyl)-6-cyclohexylthiothymine (HEPT-H)
- 1-(2-hydroxyethoxymethyl)-5-ethyl-6-phenylthiouracil (E-HEPU)
- 1-Benzyloxymethyl-6-phenylthiothymine (BPT)
- 1-Benzyloxymethyl-5-ethyl-6-phenylthiouracil (E-BPU)

any other virus but HIV-1, not even HIV-2. Such drastic discrimination between two types of the same virus has not previously been shown by any other compound, except for the TIBO (benzodiazepine) derivatives[59]. Such unusual specificity points to a unique mode of interaction with HIV-1 or any of its proteins (enzymes). Through chemical modification of HEPT, several derivatives have been obtained [i.e. HEPT-S, HEPT-H, E-HEPU, BPT and E-BPU (FIG. 3)] that, like HEPT, are potent inhibitors of HIV-1, but not inhibitory at all to HIV-2 or any other virus[60,61]. In fact, the most potent congener of this series (E-BPU) inhibits HIV-1 replication within the nanomolar concentration range, and is not toxic

TABLE 3
Antiviral activity of 1-(2-hydroxyethoxymethyl)-6-phenylthiothymine (HEPT) derivatives

Compound	50% Effective concentration (μM)*	50% Cytotoxic concentration (μM)§	Selectivity index#
HEPT	6.5	>500	77
HEPT-S	1.6	124	77
HEPT-H	7.7	440	57
E-HEPU	0.12	400	3333
BPT	0.093	63	677
E-BPU	0.0049	30	6122

*Required to inhibit cytopathogenicity of HIV-1 in MT-4 cells by 50%.
§Required to reduce viability of mock-infected MT-4 cells by 50%.
#Ratio of 50% cytotoxic concentration to 50% effective concentration.
Data taken from ref. 60 and 61.

to the host cells unless its concentration is increased to about 6000-fold the antivirally active concentration (TABLE 3).

The basis for the unique specificity of the HEPT derivatives as HIV-1 inhibitors seems to reside in a specific interaction with HIV-1 reverse transcriptase[61]. Although the HEPT derivatives do not bear any resemblance to a thymidine 5'-triphosphate analogue, their inhibitory effect at the reverse transcriptase level appears to be competitive with respect to the natural substrate dTTP. Pharmacokinetic and toxicological studies with the most active HEPT derivatives are underway. The results of these studies, together with the results on the in vitro anti-HIV-1 potency of the HEPT derivatives should allow us to decide which compounds will eventually be submitted to clinical trials.

CONCLUSION

Several nucleoside/nucleotide analogues belonging to widely different classes offer considerable promise for the treatment of a broad

variety of viral diseases : HPMPA and HPMPC for the treatment of adenovirus, herpesvirus (HSV, VZV, CMV, EBV), hepadnavirus (HBV), iridovirus (ASFV) and poxvirus (VV) infections; PMEA and PMEDAP for the treatment of herpes-, hepadna- and iridovirus infections and also retrovirus (HIV) infections; C-Ado and NpcA analogues for the treatment of paramyxovirus, arenavirus (hemorrhagic fever), rhabdovirus (rabies) and reovirus (rota) infections; and HEPT derivatives, as specific inhibitors of HIV-1 replication, for the treatment of AIDS. These compounds are in various stages of development. With some of the compounds clinical studies have been envisaged and will be initiated soon.

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