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Mini-Review

Computer-assisted structure-activity correlations of dideoxynucleoside analogs as potential anti-HIV drugs

Mohamed Nasr, Charles Litterst and John McGowan

Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH, Rockville, Maryland, MD, U.S.A.

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Dideoxynucleoside analog; Anti-HIV drug; Structure-activity correlation; Rational drug design

Introduction

The reverse transcriptase, protease, and envelope are all virus-specific targets for compounds rationally designed to inhibit virus reproduction. The rational design of drugs to inhibit virus replication by these and other mechanisms (Table 1) has led to the identification of compounds that selectively inhibit the viral target without having a detrimental effect on the host cell. This rational approach to drug design has already yielded promising compounds that may prove effective clinically against the human immunodeficiency virus (HIV). Soluble forms of the cellular receptor CD4 effectively block HIV attachment to the host cell surface, and chemically modified synthetic peptides that inhibit the viral protease and prevent maturation of HIV particles in vitro serve as two examples of the potential of rational drug design. However, the first drug shown to be effective in anti-HIV cell culture-based assays, in animals infected with retroviruses, and in humans infected with HIV, was discovered by a rational selection of compounds for random drug testing in an HIV screening system. This drug was 3′-azido-3′-deoxythymidine (AZT or zidovudine) and it is the only drug approved for the treatment of HIV infection. The antiviral activity of AZT against Friend virus was reported long before the emergence of AIDS (Ostertag et al., 1974; Dube et al., 1975). The identification of AZT as active against HIV (Mitsuya et al., 1985) was made possible because research on the replication cycle of animal retroviruses was coordinated with the design, synthesis

Correspondence to: M. Nasr, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20892, U.S.A.

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and testing of nucleoside analogs for the treatment of other viral infections and cancer. Thus, the early synthesis of AZT and other dideoxynucleosides (Horwitz et al., 1964, 1967) contributed to the total effort in establishing this class of chemicals as anti-HIV agents.

TABLE 1
Selected drug targets for anti-HIV therapy

Viral target	Drug	Clinical status
Viral Attachment/penetration inhibitors	Dextran Sulfate	Phase I
	Soluble CD4 Receptors	Phase I
	Peptide T	Phase I
Reverse Transcriptase inhibitors	ddI	Phase I/II
	ddC	Phase I/II
	F-ddC	Phase I
	d4T	Phase I
	Phosphonoformate	Phase I/II
	AZdU	Phase I/II
	AZT	Approved adults Phase I/II children
	Other Nucleosides	Preclinical studies
Protease Inhibitors	Peptide analogs	Preclinical studies
	Synthetic compounds	Preclinical studies
Transactivation (TAT) inhibitors	Synthetic compounds	Preclinical studies
Glucosidase inhibitors (block glycoprotein maturation)	N-Butyl-deoxynojirimycin	Phase I
Myristolation inhibitors (block assembly)	Fatty acid analogs	Preclinical studies

The discovery of the anti-HIV activity of AZT led to its rapid clinical evaluation for efficacy, research to determine its mechanism of action, and efforts to identify other nucleosides (De Clercq, 1987; Matsushita et al., 1987) that may prove useful in the treatment of AIDS. Clinically, AZT has proved to be efficacious and its mechanism of action is now understood. Basically, AZT is anabolically phosphorylated to AZT mono-, di- and triphosphates. AZTTP competes with other phosphorylated pyrimidine nucleosides for incorporation into HIV DNA by the viral reverse transcriptase (RT). Incorporation of the AZT triphosphate into RT results in viral DNA chain termination. Optimism exists that other nucleosides also will prove clinically effective in the treatment of HIV infection but complete clinical data are not yet available.

The search for more effective anti-HIV therapies includes discovering new drugs with different mechanisms of action (Wiess, 1988; Fleet et al., 1988; McGrath et al., 1989; Meruelo et al., 1988), and improving those drugs already shown to be efficacious, particularly nucleosides. To assist in the identification and development of more effective chemotherapies for AIDS we provide this overview on the structure-activity correlations of nucleoside analogs. The compounds included in

this overview are all in the public domain, and have been tested against HIV cell culture assays targeting reverse transcriptase. Nucleosides are the focus of this review because the literature accumulated on these compounds is extensive enough to warrant the analysis given. Indeed, the importance of this class of compounds is indicated by the fact that among the eight synthetic clinically approved antiviral drugs, seven are nucleoside analogs (Table 2).

TABLE 2
Synthetic antiviral drugs approved by the US-FDA

Generic name	Chemical name	Clinical use
Zidovudine	3′-azido-3′-deoxythymidine (AZT)	HIV AIDS
Idoxuridine	5-iodo-2′-deoxyuridine (IDU)	herpes keratitis
Trifluridine	5-trifluoromethyl-2′-deoxyuridine (TFT)	herpes keratitis
Acyclovir	9-(2-hydroxyethoxymethyl)-guanine	herpes keratitis genital herpes
Vidarabine	9-β-D-arabinofuranosyladenine	herpes encephalitis
Ribavirin	1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide	respiratory syncytial virus RSV influenza A virus
Amantadine	1-adamantanamine hydrochloride	
Ganciclovir	[9-(1,3-dihydroxy-2-propoxymethyl) guanine]	CMV retinitis

It is hoped that this analysis of the nucleosides tested for anti-HIV activity will: (1) identify compounds that have not been synthesized and tested; (2) help prevent possible duplication of effort in the scientific community; and (3) determine which chemical modifications have resulted in increased, decreased or no activity.

The information presented in this review is provided from the chemical database compiled at NIAID that allows for the analysis of structure-activity relationships for active and inactive anti-HIV compounds. The information stored in the database makes use of continuous surveillance of primary literature sources, and NIAID monitoring and tracking of compounds that are presently being developed for the treatment of AIDS. Data accumulated so far on in vitro anti-HIV testing of more than 100 active dideoxynucleosides and acyclonucleoside analogs, allowed a valid study of the structure-activity relationship of more than 500 analogs currently in the database.

Analysis was accomplished through substructure searching (Nasr, et al., 1984) followed by retrieval and sorting of data using the Molecular Design Limited (MDL) software ChemBase. The design of the various substructure units of interest was based on their relevance to the activity of the parent molecules and information from the literature reflecting current interest. The biological data for all the compounds with a particular substructure were then sorted in a table format according to their activity or inactivity in different cell lines.

Results

The anti-HIV activity of the clinically useful antiviral nucleosides already has been explored. Ribavirin exhibits in vitro anti-HIV activity (McCormick et al., 1984) and inhibits reverse transcriptase (Roberts et al., 1987). Ribavirin antagonizes the effect of pyrimidine dideoxynucleosides (e.g. AZT) on HIV replication (Vogt et al., 1987) through the inhibition of AZT phosphorylation, but enhances the inhibitory effects of purine dideoxynucleosides on replication (Baba et al., 1987b). Synthesis of 2′,3′-dideoxy and 2′,3′-didehydro-2′,3′-dideoxy analogs of ribavirin has been reported and the analogs were found to be inactive against HIV in vitro (Upadhya et al., 1988; Sanghvi et al., 1987). Acyclovir, although it does not show in vitro anti-HIV activity, was reported to potentiate the anti-HIV activity of AZT (Mitsuya and Broder, 1987). Idoxuridine, trifluoridine and vidarabine are inactive in vitro against HIV (Mitsuya and Broder, 1986). Replacement of the 3′-OH in idoxuridine by an azido group established in vitro anti-HIV activity (Lin et al., 1988), while introduction of 3′-azido in trifluoridine produced an inactive compound (Lin et al., 1987, 1988). Amantadine did not inhibit HIV in vitro (Oxford et al., 1989).

AZT has been studied as a prophylactic agent (Tavares et al., 1987) and recently AZT-resistant HIV strains have been isolated from AIDS patients (Larder et al., 1989). Compounds that have been reported to show less toxicity (Balzarini et al., 1989a; Simpson et al., 1989) than AZT include AzddU and D4T, both of which are currently in clinical trials.

The majority of the reported dideoxynucleosides (ddNs) that have been tested for anti-HIV activity are mainly pyrimidine and purine ring systems (see Figs. 1 and 2). The purine ring system (C_5N_4) can exist in 66 isomers, and the aza and deaza analogs have more than 200 isomeric forms. The pyrimidine ring, however, can exist in only three isomers. Aza and deaza analogs of both purine and pyrimidine nucleosides have been investigated as both antitumor and antiviral agents (Robins and Rivankar, 1988).

In the pyrimidine series (Fig. 1), uracil, thymine and cytosine 2′,3′-dideoxynucleosides (ddNs) and 2′,3′-didehydro-2′,3′-dideoxynucleosides (D4Ns) have been widely investigated. Recently, the in vitro anti-HIV activity of various thio analogs of purine and pyrimidine ddNs has been reported (Palomino et al., 1990). In addition, the first thiopyrimidine nucleoside isolated from tRNA has been characterized previously (Kumura-Harada et al., 1971). Among purine ddNs and D4Ns, 6-aminopurine (adenine), 6-hydroxypurine (hypoxanthine), 2,6-diaminopurine, 2-halo-6-aminopurines and 2-amino-6-hydroxypurine (guanine) are the most widely investigated.

In general, pyrimidine ddNs show higher in vitro anti-HIV potency than purine ddNs. Among the 3′-substituted pyrimidine analogs other than AZT, 3′-deoxy-3′-fluorothymidine is quite potent but also highly cytotoxic. The purine nucleoside analogs 2′,3′-dideoxyadenosine (ddA) and 2′,3′-dideoxy-2,6-diaminopurine nucleoside (ddDAPR) showed the highest activity among the purine ddNs. While the introduction of a 3′-azido in ddA abolished the activity, it enhanced the activity when introduced into ddDAPR.

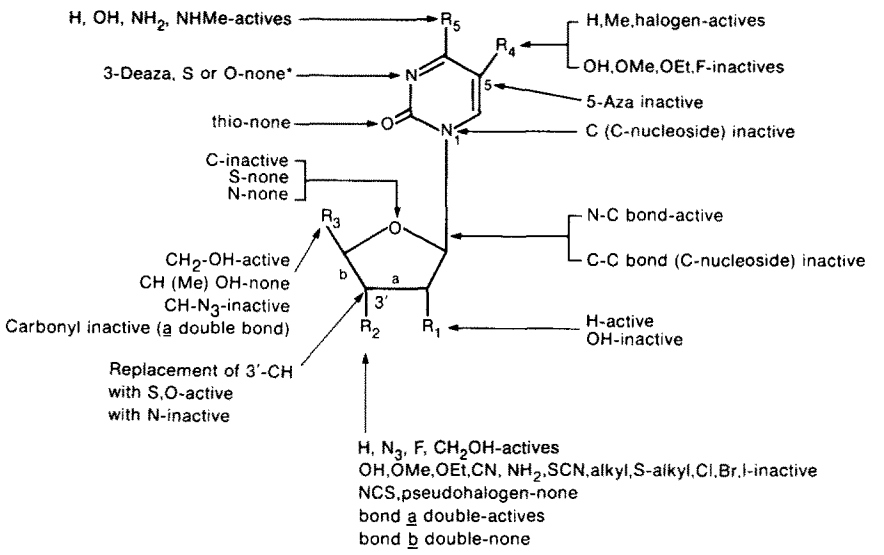


Fig. 1. Structure-activity relationship of pyrimidine analogs.

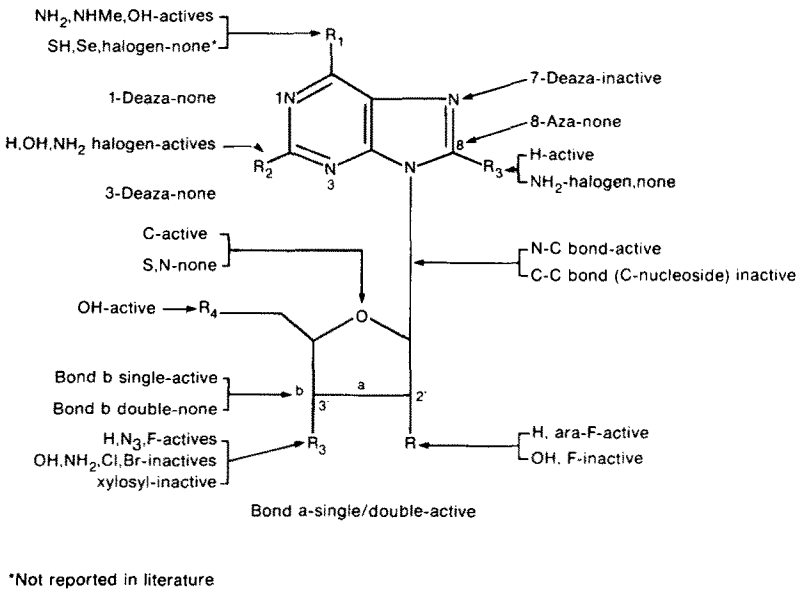


Fig. 2. Structure-activity relationship of purine analogs.

The anti-HIV activity of the different classes of pyrimidine and purine nucleosides are summarized in Tables 3–9. In vitro activity against HIV is indicated by

(+) if the corresponding compound has shown activity in one or more cell lines, (+ −) if the compound was active in one cell line but inactive in another or with equivocal activity, and (−) if there was no activity in any cell line. Because of the chemical nature of this report, no detailed evaluation of biological response is attempted. Another report currently in preparation will analyze the response of various in vitro test systems to selected ddNs.

3′-Azido-2′,3′-dideoxypyrimidine nucleosides (Table 3)

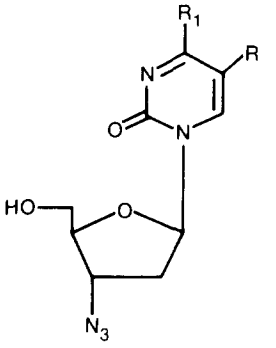
3′-Azido-3′-deoxythymidine (AZT) is the most active analog of this series. Recently, the anti-HIV activity of 4-deoxy-AZT and its in vivo conversion to AZT was reported (Rideout et al., 1988). However, 3′-azido-2′,3′-dideoxyuridine derivatives constitute the majority of the compounds that have been tested. The nature of the substitution on the 5-position of 3′-azidodideoxyuridines has a major effect on the anti-HIV activity. A 5-substitution with methyl (AZT), hydrogen (AZddU), chloro, bromo, iodo gave active compounds, while the 5-fluoro derivative was inactive. The anti-HIV activity of 3′-azido-5-fluoro-2′,3′-dideoxyuridine may be masked by its high toxicity. 3′-Azido-2′,3′-dideoxy-5-ethyluridine (AzddEtU) showed differential activity in different cell lines. The replacement of 5-methyl in AZT by fluoromethyl or propyl abolished activity. Inactivating 5-substituents include SCN, NH_2, NR_2, OH, OR, Pr, CF_3 and SMe groups. The presence of 2′-OH in AZT, AzdU or AzdC abolished the anti-HIV activity of these compounds (Webb et al., 1988). Reduction of the 3′-azido group in ddNs to an amino group gave inactive compounds (Chu et al., 1989b).

In the 3′-azidodideoxycytidine series, the activity of 3′-azido-5-methyl-2′,3′-dideoxycytidine (AzddMeC) was superior to that of 3′-azido-2′,3′-dideoxycytidine (AzddC). The only 3′-azido-5-halodideoxycytidine that has been reported, 3′-azido-2′,3′-dideoxy-5-fluorocytidine (AzddFC), was active. As shown in Table 3 only a few 5-substituted 3′-azidodideoxycytidines have been reported. Recently the anti-HIV activity of 3′-azido-5-methyldideoxyisocytidine analog was described (Lin et al., 1989). Nucleotide homo- and heterodimers are compounds in which two active nucleoside monomers are connected at the 5′-position by a phosphate (P) or cyanoethylphosphate (PCyE) bridge (e.g. AZT-P-ddA, AZT-PCyE-ddA). The anti-HIV activity of nucleotide dimers combining AZT and ddA has recently been reported to be greater than or equal to the two monomers used simultaneously (Busso et al., 1988). AZT-ddA dimer was the most active of several AZT containing dimers.

3′-Fluoro and other 3′-substituted-2′,3′-dideoxypyrimidine nucleosides (Table 4)

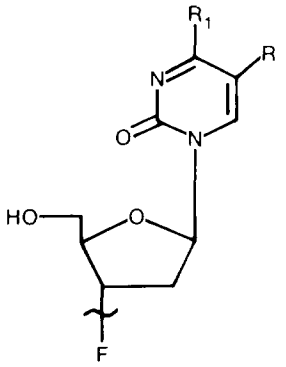
3′-Deoxy-3′-fluorothymidine (3′-FddT or FLT) was the most potent antiviral analog among a series of dideoxypyrimidine nucleosides (Herdewijn et al., 1987a; De Clercq, 1987) but it also had high cytotoxicity. Replacement of the 5-methyl group in 3′-FddT by an ethyl group decreased antiviral activity. While 3′-fluoro-2′,3′-dideoxycytidine had differential antiviral activity in two different cell types, its 5-methyl derivative had confirmed activity (Hartmann et al., 1988; Van Aerschot

TABLE 3
3′-Azido-2′,3′-dideoxypyrimidine nucleosides



Compound	R	R_1	in vitro Activity	References
AZT	Me	OH	+	Mitsuya and Broder, 1987
4-DeoxyAZT	Me	H	+	Rideout, et al., 1988
AzddU (CS-87)	H	OH	+	Lin, et al., 1988
AzddClU	Cl	OH	+	Herdewijn, et al., 1988
AzddMeC, N4-OH	Me	NHOH	+	Ibid.
AzddMeC-N4Me	Me	NHMe	+	Ibid.
PAZT	Me	OH	+	Torrence, et al., 1988
AZT-Nicotinate	Me	OH	+	Ibid.
AZT-P-AZT	Me	OH	+	Busso, et al., 1988
AZT-P-CyE-ddA	Me	OH	+	Ibid.
AZT-P-ddA	Me	OH	+	Ibid.
AZT-P-CyE	Me	OH	+	Ibid.
AZT-P-ddI	Me	OH	+	Ibid.
AZT-3-CH=CH-CHO	Me	OH	+	Lin, et al., 1988
AzddEtU (CS-85)	Et	OH	+−	De Clercq, 1988
AzddMeC	Me	NH_2	+	Lin, et al., 1988
AzddC (CS-91)	H	NH_2	+	Chu, et al., 1988
Azdd-iso-MeC	Me	OH, 2-NH_2	+	Lin, et al., 1989
AzddFC	F	NH_2	+	Lin, et al., 1988
AzddBrU	Br	OH	+	Lin, et al., 1987
AzddIU	I	OH	+	Chu, et al., 1989b
AZT (threo)	Me	OH	+	Bazin, et al., 1989
AzddU (threo)	H	OH	−	Ibid.
AzddFU	F	OH	−	Lin, et al., 1988
AzddSCNU	SCN	OH	−	Ibid.
AzddNH$_2$U	NH_2	OH	−	Ibid.
AzddOHU	OH	OH	−	Ibid.
AzddU	OCH_2CN	OH	−	Ibid.
AzddU	$OCH_2C{=}CH$	OH	−	Ibid.
AzddOEtU	OEt	OH	−	Ibid.
Az-2′-FddU	H	OH	−	Van Aerschot, et al., 1989b
Az-2′-FddC	H	OH	−	Ibid.
AZT^S	Me	SH	+−	Palomino, et al., 1990
AzddU	$OCH_2CH{=}CH_2$	OH	−	Ibid.
AzddOMeU	OMe	OH	−	Jentsch, et al., 1987
AzddPrU	Pr	OH	−	Chu, et al., 1989b
AzddU	CH=CHBr	OH	−	Ibid.
AzddCF$_3$U	CF_3	OH	−	Lin, et al., 1987
AzddNHMeU	NHMe	OH	−	Lin, et al., 1988
AzddNMe$_2$U	NMe_2	OH	-	Ibid
AzddSMeU	SMe	OH	-	Ibid

TABLE 4
3′-Fluoro and other 3′-substituted dideoxypyrimidine nucleosides



Compound	R	R_1	in vitro Activity	References
3′-FddClU	Cl	OH	+	Balzarini, et al., 1988a; Balzarini, et al., 1989b
3′-FddU	H	OH	+	De Clercq, 1988
3′-FddT	Me	OH	+	Herdewijn, et al., 1987a; De Clercq, 1987
3′-FddBrU	Br	OH	+	Balzarini, et al., 1988a
3′-FddMeC	Me	NH_2	+	Hartmann, et al., 1988
3′-FddIU	I	OH	+	Van Aerschot, et al., 1989a
3′-F-4-OMeddU	H	OMe	+	Ibid.
3′-FddEtU	Et	OH	+ –	De Clercq, 1988
3′-FddC	H	NH_2	+ –	Herdewijn, et al., 1987a
3′-FddPrU	Pr	OH	–	Bazin, et al., 1989
3′-FddFU	F	OH	–	Van Aerschot, et al., 1989a
3′, 3′-diFddT	Me	OH	–	Bergstrom, et al., 1987
3′-IddEtU	Et	OH	–	Chu, et al., 1989b
3′-CIddT	Me	OH	–	Herdewijn, et al., 1987a
3′-BrddT	Me	OH	–	Ibid.
3′-IddC	H	NH_2	–	Chu, et al., 1988
3′-IddU	H	OH	–	Chu, et al., 1989b
3′-IddT	Me	OH	–	
3′-CNddC	H	NH_2	–	Camarasa, et al., 1989
3′-CNddT	Me	OH	–	Ibid.
3′-NCddT	Me	OH	–	Hiebl, et al., 1989
3′-NCddU	H	OH	–	Ibid.
3′-OH(threo)2′-dC	H	NH_2	–	Herdewijn, et al., 1987a
3′-NH_2ddU	H	OH	–	Chu, et al., 1989b
3′-OMeddT	Me	OH	–	Herdewijn, et al., 1987a
3′-SEtddT	Me	OH	–	Ibid.
3′-NH_2ddCF_3U	CF_3	OH	–	Lin, et al., 1987
3′-allyIddT	Me	OH	–	Fiandor, et al., 1988
2′,3′-diFddC	H	NH_2	–	Van Aerschot, et al., 1989b
2′,3′-diFddU	H	OH	–	Ibid.

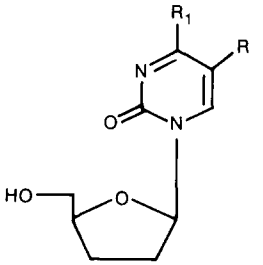
et al., 1989a). The introduction of a second fluorine at the 3′-threo configuration in 3′-FddT abolished activity, while 2′,3′-difluoro-3′-deoxythymidine showed less activity than 3′-FddT (Koshida et al., 1989). Among a series of 5-halogenated derivatives of 3′-FddU, the 5-chloro derivative was the most active with a selectivity index comparable to that of AZT when evaluated under the same conditions (Van Aerschot et al., 1989a). The order of activity was 5-Cl>5-Br>5-I>5-F with the 5-F not showing appreciable activity. Although several 3′-FddU derivatives with 5-substituents have shown antiviral activity, no 5-substituted 3′-fluoro-2′,3′-dideoxycytidine (3′-FddC) analogs other than 3′-FMeddC have been reported.

The replacement of the 3′-fluoro in ddU and ddT by Cl, Br or I abolished the activity of these compounds. Other 3′-substitutions in pyrimidine dideoxynucleosides, e.g., cyano (Camarasa et al., 1989), alkyl, carboxyalkyl, cyanoalkyl (Fiandor et al., 1988), mercapto (Herdewijn et al., 1987a), and amino (Lin, et al., 1987) groups gave inactive compounds. The anti-HIV activity of 3′-hydroxymethyl ddC has been reported (Martin, 1989).

2′,3′-Unsubstituted dideoxypyrimidine nucleosides (Table 5)

2′,3′-Dideoxycytidine (ddC) and 2′,3′-dideoxythymidine (ddT) were quite active while 2′,3′-dideoxyuridine (ddU) was inactive. The introduction of a methyl group at the 5-position of ddU established the antiviral activity while the introduction of 5-ethyl reduced the activity (Chu et al., 1988). On the other hand, the introduction

TABLE 5
2′,3′-Unsubstituted dideoxypyrimidine nucleosides



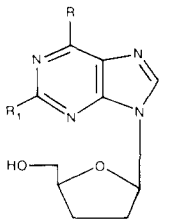
Compound	R	R_1	in vitro Activity	References
5-F-ddC	F	NH_2	+	Kim, et al., 1987
ddC	H	NH_2	+	Herdewijn, et al., 1987a
ddT	Me	OH	+	Ibid.
ddT^S	Me	SH	+	Palomino, et al., 1990
5-Et-ddU	Et	OH	–	Chu, et al., 1988
5-Et-ddC	Et	NH_2	–	Ibid.
ddU	H	OH	–	Ibid.
5-Br-ddU	Br	OH	–	Lin, et al., 1987
5-I-ddU	I	OH	–	Ibid.
5-Br-CH=CH-ddU	CH=CHBr	OH	–	Ibid.
5-BrddC	Br	NH_2	–	Kim, et al., 1987

of a methyl group at the 5-position of ddC and its effect on antiviral activity has not been reported. A bromine or iodine at the 5-position of ddU had no effect on activity. The anti-HIV activity of several ddN dimers in which the 4-amino group in ddC or 6-amino group in ddA is linked to the 5′-carbon of other ddN's, e.g., ddC and ddT with $COCH_2CH_2COO$ and other spacers has been reported (Broder and Mitsuya, 1988).

2′,3′-Unsubstituted dideoxypurine nucleosides (Table 6)

2′,3′-Dideoxyadenosine (ddA) and 2′,3′-dideoxyinosine (ddI) have been extensively evaluated and ddI is currently undergoing clinical evaluation. 2′,3′-Dideoxy-2,6-diaminopurine showed a comparable in vitro activity to ddA. It was found that 2′,3′-dideoxy-N6-methyladenosine (ddMeA) had a potent antiviral activity that could be related to its resistance to deamination. The 2-halo derivatives of ddA have demonstrated reduced anti-HIV activity and greater toxicity than ddA (Rosowsky et al., 1989). Of the only reported 8-Me or 8-Br substituted purine nucleosides, none has shown anti-HIV activity. A methyl group added to the 8- or 2-position of ddA abolished its activity (Driscol et al., 1989). The introduction of bromine at the 8-position in ddA or 3′-AzddA resulted in inactive compounds (Van Aerschot et al., 1989a).

TABLE 6
2′,3′-Unsubstituted dideoxypurine nucleosides

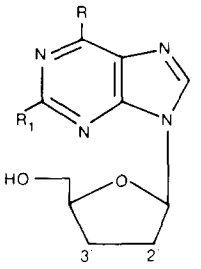


Compound	R	R_1	in vitro Activity	References
ddA	NH_2	H	+	Herdewijn, et al., 1987b
ddDAPR	NH_2	NH_2	+	Balzarini, et al., 1987
ddG	OH	NH_2	+	Baba, et al., 1987a
ddI	OH	H	+	Baba, et al., 1987c
ddMeA	NHMe	H	+	Chu, et al., 1988
ddP	Piperidino	H	+	Koszalka, et al., 1988
ddPN	H	H	+	Ibid.
2-Br-ddANH_2	NH_2	Br	+−	Haertle, et al., 1988
2-F-ddA	NH_2	F	+−	Ibid.
2-Cl-ddA	NH_2	Cl	+−	Rosowsky, et al., 1989
ddClP	Cl	H	+−	Chu, et al., 1989a
ddBnA	NHBn	H	+−	Ibid.
ddEtA	NHEt	H	−	Ibid.
ddMe_2A	NMe_2	H	−	Ibid.
ddP	H	H	−	Ibid.
8-BrddA	NH_2	H	−	Van Aerschot, et al., 1989a

Sugar substituted dideoxypurine nucleosides (Table 7)

The active compounds in this class are mainly 3′-azido, 3′-fluoro, and ara-2′-fluoro and 2′-azido derivatives. The replacement of 2′-ara-F with 2′-Cl or 2′-Br gave inactive compounds. The presence the 2′-F in the threo configuration is essential for activity. It was recently reported that in the presence of a 2′-OH, 3′-amino-

TABLE 7
Sugar substituted dideoxypurine nucleosides



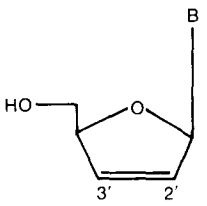
Compound	R	R_1	in vitro Activity	References
3′-N_3ddDAPR	NH_2	NH_2	+	Robins, et al., 1989
3′-N_3ddG	OH	NH_2	+	Baba, et al., 1987a
3′-FddDAPR	NH_2	NH_2	+	Balzarini, et al., 1988b
3′-FddG	OH	NH_2	+	Ibid.
2′-Fdd-ara-A	NH_2	H	+	Herdewijn, et al., 1987b
3′-FddA	NH_2	H	+	Ibid.
2′-N_3dd-ara-A	NH_2	H	+	Ibid.
2′-Fdd-ara-I	OH	H	+	Driscoll, et al., 1989
2′-Fdd-ara-6-NHMeA	NHMe	H	+	Ibid.
2′-Fdd-ara-6-NHCOPhA	NHCOPh	H	+	Ibid.
3′-N_3ddA	NH_2	H	+	Herdewijn, et al., 1987b
3′-NH_2, 2′-OH			+	
2′-F-ara-2MeA	NH_2	Me	–	Driscoll, et al., 1989
2′-F-2-Me-6-NHMe-ara-A	NHMe	Me	–	Ibid.
2′-F-8-Me-ara-A	NH_2	H	–	Ibid.
3′-N_3ddA (threo)	NH_2	H	–	Herdewijn, et al., 1987b
2′-N_3ddA	NH_2	H	–	Ibid.
3′-FddA (threo)	NH_2	H	–	Ibid.
3′-OH-2′-dG (threo)	OH	NH_2	–	Herdewijn, et al., 1988
2′-FddA	NH_2	H	–	Herdewijn, et al., 1987b
2′-Cldd-ara-A	NH_2	H	–	Herdewijn, et al., 1988
2′-Brdd-A	NH_2	H	–	Ibid.
2′-OH-ara-A	NH_2	H	–	Ibid.
3′-CNddA	NH_2	H	–	Camarasa, et al., 1989
3′-N_3ddDAPR	NH_2	NH_2	–	Robins, et al., 1989
3′-N_3dd-8-Br	NH_2	H	–	Van Aerschot, et al., 1989a
3′-N_3ddI	OH	H	–	Ibid.
3′-FddI	OH	H	–	Ibid.

3′-deoxyadenosine, and its 5′-triphosphate derivative inhibited HIV-1 replication in acutely infected cells (Heyden et al., 1989). It has been suggested that these compounds inhibited HIV-1 reverse transcriptase. Also a 5′-amino-3′-fluoro-3′-deoxyadenosine was found to be active (Koshida et al., 1989).

2′,3′-Didehydro-2′,3′-dideoxynucleosides (D4Ns) (Table 8)

This class of compounds is less active than the parent dideoxynucleosides (ddNs). Pyrimidine analogs are the most active while the purine analogs are much less active. 2′,3′-Didehydro-3′-deoxythymidine (D4T) has been extensively evaluated and is currently in Phase I clinical trials. Replacement of the 5-methyl group in D4T by ethyl (D4EtU) or by H (D4U) abolishes activity. Replacement of H at the 5-position in 2′,3′-didehydro-2′,3′-dideoxycytidine (D4C) by a methyl group (D4MeC) retains activity, while replacement of the 4′-CH_2OH on the sugar by CH_2NH_2 group abolishes the activity (Herdewijn et al., 1987a). The introduction of a 5-chloro substituted D4U did not improve its anti-HIV activity (Balzarini, et al., 1989b). The modification in 5-substitution of the pyrimidine D4Ns represent an area that has not been fully investigated.

TABLE 8
2′,3′-Didehydrodideoxynucleosides



Compound	B	in vitro Activity	References
D4T	Thymine	+	Baba, et al., 1987c
D4T[S]	4-thiothymine	+	Palomino, et al., 1990
3′-F-D4T	Thymine	+	Van Aerschot, et al., 1989a
D4C	Cytosine	+	Chu, et al., 1988
D4MeC	5-Me-cytosine	+	Ibid.
D4A	Adenine	+−	Chu, et al., 1988, Herdewijn, et al.,1987b
MeD4A	N-Me-adenine	+−	Chu, et al., 1988
D4G	Guanine	−	Baba, et al., 1987a
D4U	Uracil	−	Herdewijn, et al., 1987a
ddeDAPR	2,6-Diaminopurine	+−	Balzarini, et al., 1988b
D4EtU	5-Ethyluracil	−	Chu, et al., 1988
2-ClD4A	2-Cl-Adenine	+−	Rosowsky, et al., 1989
D4ClU	5-Cl-Uracil	−	Balzarini, et al., 1989b
3′-CN-D4T	Thymine	−	Camarasa, et al., 1989
4′-CH_2NH_2D4C	Cytosine	−	Herdewijn, et al., 1987a

2′-Substituted pyrimidine and purine nucleosides (Table 9)

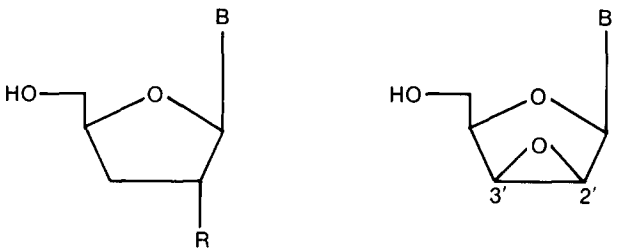
In general 2′-substituted pyrimidine and purine nucleosides are inactive against HIV. In the purine series compounds that have shown in vitro anti-HIV activity are 2′-F-dd-ara-A and 2′-azido-dd-ara-A (Herdewijn et al., 1987b). In the pyrimidine series the anti-HIV activity of 2′-FddT and 2′-Fdd-ara-C has been reported (Van Aerschot et al., 1989a).

Carbocyclic 2′,3′-didehydro-2′,3′-dideoxynucleoside (C-D4Ns)

Carbocyclic purine derivatives have shown good anti-HIV activity, while the pyrimidine analogs have been inactive. The most active analog in this series is carbovir (carbocyclic-D4G) (Vince et al., 1988) which is in development as a potential clinical candidate. Carbovir is a carbocyclic (2′,3′-didehydro-2′,3′-dideoxyguanosine) that has potent anti-HIV activity at very low subtoxic concentrations. Other carbocyclic purine analogs that have in vitro activity are 6-amino, 2,6-diaminopurine, and 6-chloro-2-aminopurine analogs of carbovir. The carbocyclic adenine analog (carbocyclic-D4A) was ten-fold less active than carbovir.

Saturation of the carbocyclic sugar moiety of carbovir either decreases or abolishes antiviral activity. Only 6-amino and 2,6-diamino analogs with a saturated carbocyclic sugar have in vitro anti-HIV activity (Vince et al., 1988). Substitution at 2′,3′-position of the carbocyclic ring gave inactive compounds. Carbovir pos-

TABLE 9
2′-Substituted pyrimidine and purine nucleosides



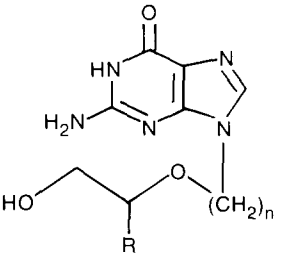
2′-Fdd-ara-C	Cytosine	F	+	Van Aerschot, et al., 1989a
2′-FddT	Thymine	F	+	Ibid.
2′,3′-EpoxyA (up)	Adenine	−O−	−	Ibid.
2′,3′-EpoxyA (down)	Adenine	−O−	−	Ibid.
2′,3′-AnhydrolyxoU	Uracil	−O−	−	Webb, et al., 1988
2′,3′-AnhydrolyxoT	Thymine	−O−	−	Ibid.
2′,3′-Anhydrolyxo-5-BrU	Uracil	−O−	−	Ibid.
2′-Fdd-ara-MeC	5-MeCytosine	F	−	Van Aerschot, et al., 1989b
2′-Fdd-ara-T	Thymine	F	−	Ibid.
2′-Fdd-ara-DAPR	2,6-diNH_2-purine	F	−	Ibid.

sesses a better therapeutic index than ddC, although it is less potent than AZT. In the pyrimidine series, thymidine carbocyclic nucleoside analogs saturated or unsaturated at the 2′,3′-positions were inactive in vitro.

Carbocyclic dideoxynucleosides unsaturated at the ring 4′,5′-positions were void of anti-HIV activity (Marquez et al., 1987). No carbocyclic ddNs with a double bond at 3′,4′-position have been reported.

Acyclonucleosides

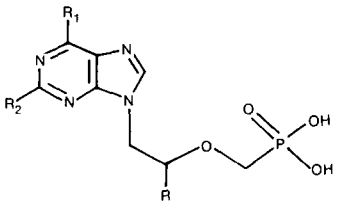
Acyclonucleosides as antiviral agents have been recently reviewed (Chu and Cutler, 1986). Since the discovery of the antiherpes activity of acyclovir (structure 1a) several analogs have been reported to possess different antiviral activities. Thus [9-(1,3-dihydroxy-2-propoxymethyl)guanine], DHPG, (structure 1b) and [(R)-9-(3,4-dihydroxybutyl)guanine], DHBG, (structure 1c) both have shown activity against cytomegalovirus (CMV) but not against HIV (Tyms et al., 1989).



1

a, n=1, R=H
b, n=1, R=CH_2OH
c, n=2, R=OH

Of the several types of acyclonucleosides tested for anti-HIV activity (Hayashi et al., 1988; Pauwels et al., 1988b) three classes were active. The first class (structure 2) includes 9-phosphonylmethoxyethyl derivatives of adenine (PMEA, structure 2a) 2-aminopurine (PMEMAP, structure 2b), and 2,6-diaminopurine (PMEDAP, structure 2c). All have been found to be effective against HIV in vitro. Both HPMPA (structure 2d) and PMEG (structure 2e) were inactive.

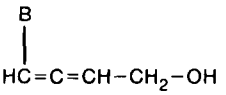


2

Compound	in vitro Activity
a. PMEA $R_1=NH_2$, $R=R_2=H$	+
b. PMEMAP $R=R_1=H$, $R_2=NH_2$	+
c. PMEDAP $R_1=R_2=NH_2$, $R=H$	+
d. (S)HPMPA $R=CH_2OH$, $R_1=NH_2$, $R_2=H$	–
e. PMEG $R=H$, $R_1=OH$, $R_2=NH_2$	

Phosphonomethoxyethyl derivatives of thymine (PMET), uracil (PMEU), and cytosine (PMEC), and the hydroxymethyl derivative ($R=CH_2OH$) also were inactive.

The second class of active acyclonucleosides (structure 3) has an allenic side chain (compounds 3). Adenallene (compound 3a) and cytallene (compound 3b) were the most active in a series of adenine, cytosine, guanine and thymine derivatives.



3

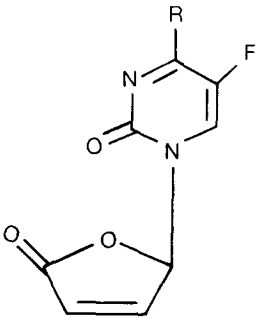
Compound	B	in vitro Activity
Adenallene	Adenine	+
Cytallene	Cytosine	+
Guanallene	Guanine	–
Hypoxallene	Hypoxanthine	–
Thymallene	Thymine	–

Modifications in the allenic side chain either by saturation of the carbon-carbon double bond or by introduction of a triple bond gave inactive compounds. Aza- and deaza-analogs of purines and pyrimidines with phosphonomethoxyethyl, as in PMEA, or 4-hydroxy-1,2-butadiene, as in adenallene, represent potential antiviral agents. The third class is a base modified pyrimidine nucleoside analog (Miyasaka et al., 1989) in which a 6-phenylthio substituent established the anti-HIV activity of 1-[(2-hydroxymethoxy)methyl]thymine. Only one compound has shown activity among a series of related analogs. 1-[(2-Hydroxymethoxy)methyl]-6-phenylthiothymine and its triphosphate did not inhibit HIV-1 reverse transcriptase, an indication that the mechanism of anti-HIV action is not by RT inhibition.

Recently, the in vitro anti-HIV activity of 9-(2-hydroxy-3-nonyl)adenine alone and in combination with deoxycytidine was reported (Sei et al., 1989).

Compounds with altered 4′-substitution

Dideoxynucleosides require phosphorylation before demonstrating antiviral activity. After phosphorylation ddNs become incorporated into DNA, which leads to premature termination of the elongating DNA chain since there is no attachment site for the formation of the next 3′,5′-phosphodiester bond. The presence of a 4′-CH_2OH group and the lack of 3′-OH in nucleosides and their carbocyclic analogs have been cornerstones in the design of anti-HIV nucleosides. Literature reports have mentioned the anti-HIV activity of certain nucleosides in which the 4′-CH_2OH group is replaced by other groups (Koshida et al., 1989, Paessèns et al., 1988). The antiviral activity of nucleosides with 5′-amino or a 4′-azido group has been recently reported (ibid.). The anti-HIV activity of a series of 2,5′-anhydro analogs of AZT, AzddU and other pyrimidine nucleosides has been described (Lin et al., 1989). These compounds were thought to act as prodrugs, but HPLC studies showed that the compounds were stable under normal physiological conditions. In order to test the effect of modification of the 4′-substitution, two .alpha.-, .beta.-unsaturated lactone nucleoside analogs (4) have been tested for anti-HIV activity. The compounds were found to be inactive at subtoxic concentrations. More work is needed to investigate the possible mode of action of these compounds and to determine whether certain nucleoside analogs may show anti-HIV activity without the need for phosphorylation.



4

R = OH, OCH_3

C-nucleoside analogs

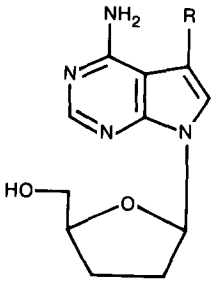
The synthesis of C-nucleosides, in which the sugar is attached to carbon instead of nitrogen, has been reported to yield several classes of broad spectrum antiviral agents (Shuga, 1986). However, C-nucleoside analogs of AzddU, ddC and D4C have been synthesized and found to be without anti-HIV activity (Chu et al., 1989b).

Ring size modification of the carbohydrate moiety

Oxetanocin, a novel nucleoside with an oxetanosyl-N-glycoside group has been recently isolated from a culture filtrate from *Bacillus megaterium* and its synthesis has been reported (Seki et al., 1989a). Oxetanocin A, an adenine nucleoside analog, has been reported to inhibit the in vitro replication of HIV. The guanine analog, oxetanocin G, has been reported to selectively inhibit human cytomegalovirus replication (Nishiyama et al., 1988). Recently the carbocyclic analog of oxetanocin G was reported to show good anti-HIV activity (Norbeck, 1989a, Hayashi et al., 1990). The presence of 2′-CH_2OH group in oxetanocins is not essential for anti-HIV activity. A recent study (Seki et al., 1989b) showed that replacement of the 2′-CH_2OH in oxetanocin A with OH (up), H, CH_2N_3, or CH_2 increased the anti-HIV activity.

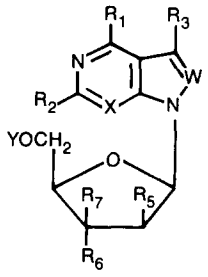
Aza and deaza purine and pyrimidine dideoxynucleosides

No aza or deaza pyridine ddNs have been reported in the literature. The 5-aza analog of AZT was found to be inactive in vitro (Kim et al., 1987). Of the purine deaza analogs, ddN and D4N derivatives of tubercidin (5a), toyocamycin (5b), and sangivamycin (5c) have been found to be inactive (Pauwels et al., 1988a). Recently (Seela et al., 1988) a series of 4- and 7-deaza purine dideoxynucleosides and related analogs have been reported to inhibit reverse transcriptase and to possess antiviral activity.



5

R=H, b, R=CN, c, R=$CONH_2$

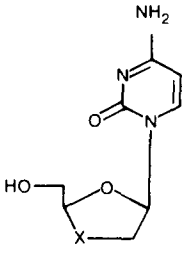


6

W=X=CH, N

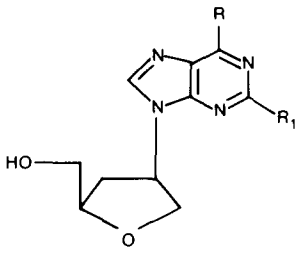
Novel nucleosides

The in vitro anti-HIV activity of 1-[2′-hydroxymethyl-5′-(1,3-oxathiolanyl)]cytosine (7) has been recently reported (Belleau et al., 1989), with potency equivalent to the most active dideoxynucleosides. Replacing the sulfur by oxygen decreased the activity, while the sulfone or sulfoxide gave inactive compounds. The antiviral activity of a dioxolanylthymine derivative has been recently reported (Norbeck et al., 1989b) Among a group of purine and pyrimidine isonucleosides in which the positions of the 3′-carbon and ring oxygen are transposed, iso ddA and iso-ddG (8), have shown in vitro anti-HIV activity (Huryn et al., 1989). A group of alpha-anomers of pyrimidine-3′-substituted ddNs (9), in which the ribose oxygen is replaced by sulfur or methylene, has been reported to possess in vitro anti-HIV activity (Datema et al., 1988).



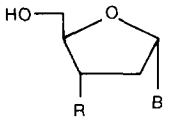
7

X = S, O



8

R = NH_2, R1 = H; R = OH, R1 = NH_2



9

B = Cytosine, Thymine, Uracil

X = S, CH_2, O; R = H, N_3, F

Conclusions

There are several conclusions that can be drawn from these results:

– Most nucleosides with demonstrated anti-HIV activity are 2′,3′-dideoxypyrimidines and purines, and to a lesser extent their acyclic analogs.

– Several substitutions on the 2′,3′ positions of various pyrimidine and purine ddNs have been done. Mainly 2′- or 3′-azido or fluoro substitutions have shown anti-HIV activity. Activity was also reported for substituted 3′-amino and 3′-hydroxymethyl nucleosides.

– 3′-Azido or 3′-fluoro enhanced the activity of the dideoxypyrimidine nucleosides, but decreased the activity of the dideoxypurine nucleosides.

– 3′-Substitution with amino, alkyl, cyano, alkoxy, thioalkyl, thiocyanato, and non-fluorine halogens gave inactive compounds. Replacement of 3′-azido with 3′-isothiocyanato (NCS), acetylenic groups or pseudohalogens have not been reported.

– A 2′-ara fluoro substituent increased the activity of purine ddNs to a greater extent than the pyrimidine ddNs. A 2′-fluoro in the erythro (down) configuration in both purine and pyrimidine ddNs gave inactive compounds. A 3′-fluoro in the threo (up) configuration resulted in inactive compounds while a 3′-fluoro in the erythro configuration gave active compounds, particularly with pyrimidine ddNs. A 3′-difluoro substitution gave inactive compound, while 2′-3′-difluoro substitution gave less activity than the 3′-fluoro derivative.

– Introduction of a 2′-OH in the active 3′-azidopyrimidine ddNs abolished activity.

– A 2′,3′-double bond inactivated purine but not pyrimidine ddNs. No ddNs with a 3′,4′ or 1′,2′-3′,4′ double bonds have been reported.

– In the uridine ddNs fluorine substitution on the sugar was the only halogen substitution that gave active compounds, while fluorine substitution on the pyrimidine ring gave inactive compounds. Little analogous work with 5-substituted ddC has been done.

– Only one isocytidine (2-imino-4-ketouracil) ddN analog has been reported to possess anti-HIV activity.

– The monomethylation of the 6-amino group in ddA or the 4-amino group in ddC enhances the activity of these compounds while introduction of ethyl, two methyl groups or benzyl abolishes activity.

– An unsubstituted sugar in purine ddNs is optimal for activity, while a halogen on the purine base results in less active and more toxic analogs.

– Carbocyclic purine ddNs but not pyrimidine ddNs have anti-HIV activity.

– Acyclonucleosides of purine and pyrimidine have shown anti-HIV activity.

– Replacement of the 3′-carbon with S or O, but not N, resulted in active purine and pyrimidine ddNs. Purine but not pyrimidine ddNs in which 3′-carbon and ring oxygen are transposed gave active compounds. A four-membered sugar ring and its carbocyclic ddN analogs have shown anti-HIV activity; six membered sugar rings (e.g., 2′,3′-dideoxy or dideoxydidehydrohexopyranosyl) have not been reported.

– Neither modification of ddNs purine or pyrimidine ring size, nor replacement of the ring nitrogens with S or O have been reported.

– The aza and deaza purine and pyrimidine ddN analogs have not been fully investigated.

In summary, a concerted effort has been made to elucidate the structure-activity relationships of the various types of ddNs and D4Ns that have been investigated and reported in the literature. By arranging compounds based on chemical similarities, other possible analogs can be predicted that have not been studied yet (e.g., the 5-halo derivatives of 3′-FddC, Table 4 have not been reported). The introduction of a 5-methyl group enhanced the activity of 3′-AzddC and 3′-FddC. A similar analysis of the active analogs among the different classes may be useful in predicting possible active analogs that have not yet been synthesized (e.g., 6-substituted pyrimidines). The discovery of the anti-HIV activity of 3′-S or 3′-O sugar-substituted nucleosides may lead to the synthesis of additional nucleoside analogs incorporating these modified sugars with a variety of pyrimidine or purine bases.

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