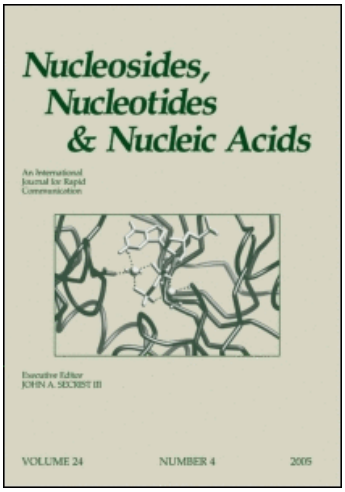

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Carbocyclic Adenosine Analogues as S-Adenosylhomocysteine Hydrolase Inhibitors and Antiviral Agents: Recent Advances

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REVIEW

CARBOCYCLIC ADENOSINE ANALOGUES AS S-ADENOSYLHOMOCYSTEINE HYDROLASE INHIBITORS AND ANTIVIRAL AGENTS: RECENT ADVANCES

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ABSTRACT: Various carbocyclic analogues of adenosine, including aristeromycin (carbocyclic adenosine), carbocyclic 3-deazaadenosine, neplanocin A, 3-deazaneplanocin A, the 5'-nor derivatives of aristeromycin, carbocylic 3-deazaadenosine, neplanocin A and 3-deazaneplanocin A, and the 2-halo (i.e., 2-fluoro) and 6'-R-alkyl (i.e., 6'-R-methyl) derivatives of neplanocin A have been recognized as potent inhibitors of S-adenosylhomocysteine (AdoHcy) hydrolase. This enzyme plays a key role in methylation reactions depending on S-adenosylmethionine (AdoMet) as methyl donor. AdoHcy hydrolase inhibitors have been shown to exert broad-spectrum antiviral activity against pox-, paramyxo-, rhabdo-, filo-, bunya-, arena-, and reoviruses. They also interfere with the replication of human immunodeficiency virus through inhibition of the *Tat* transactivation process.

The S-adenosylhomocysteine (AdoHcy, SAH) hydrolase is a key enzyme in methylation reactions depending on S-adenosylmethionine (AdoMet, SAM) as the methyl donor. AdoHcy hydrolase splits AdoHcy into its two components, homocysteine and adenosine (Fig. 1), and thus prevents the accumulation of SAH that would otherwise lead to an inhibition of the SAM-dependent methylation reactions, including those that are required for the maturation of viral mRNA. SAH hydrolase has been recognized as a suitable target for antiviral chemotherapy,[1-3] and a variety of carbocyclic adenosine analogues are assumed to exert their antiviral action through inhibition of SAH hydrolase. In fact, a close correlation has been found between the antiviral activity of various carbocylic and acyclic adenosine analogues and their inhibitory effect on the cell-free SAH hydrolase.[4] In recent years several new SAH hydrolase inhibitors have been described. These compounds were also examined for their antiviral activity. SAH

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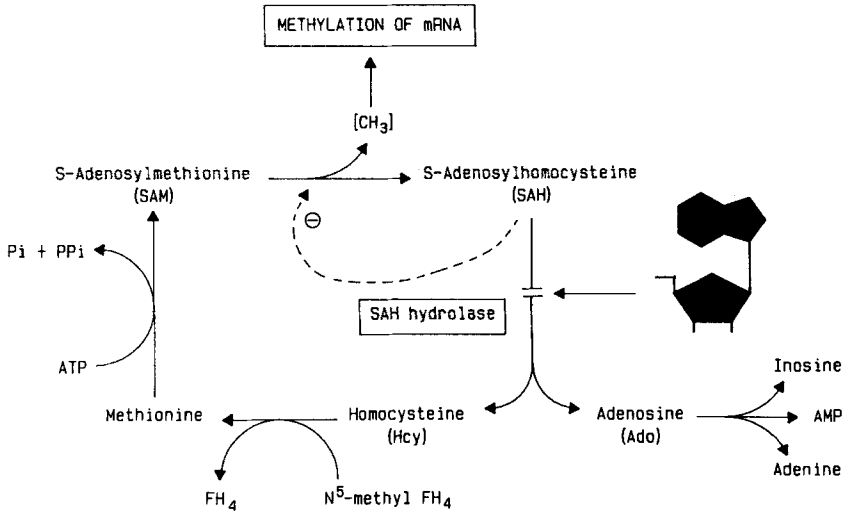
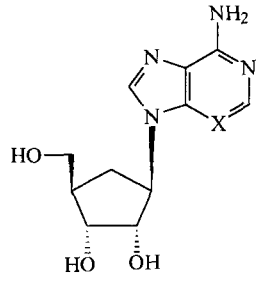


FIG. 1. Mechanism of action of SAH hydrolase inhibitors.

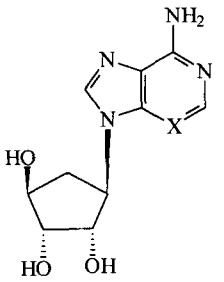
hydrolase inhibitors are active against a wide range of DNA, (-)RNA and (±)RNA viruses, and as shown here, also retroviruses. They inhibit human immunodeficiency virus (HIV) replication through interference with the *Tat* transactivation process that initiates transcription of the viral mRNA from the long terminal repeat (LTR).

SAH Hydrolase Inhibitors

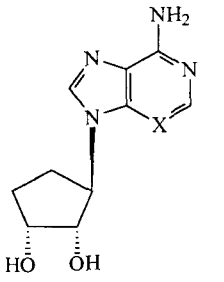
Various carbocyclic adenosine analogues (Fig. 2) have been found to be potent inhibitors of SAH hydrolase, on the one hand, and to exhibit an antiviral activity spectrum characteristic of SAH hydrolase inhibitors (Table 1), on the other hand: carbocyclic adenosine (C-Ado, aristeromycin),[5] carbocyclic 3-deazaadenosine (C-c^{3}Ado)[6,7] and 5'-substituted derivatives thereof,[8] 5'-noraristeromycin [(±)-5'-noraristeromycin,[9] (-)-5'-noraristeromycin,[10] the 3-deaza analogue of (-)-5'-noraristeromycin[11] and an epimer of (-)-5'-noraristeromycin[12]], the 9-(2',3'-dihydroxycyclopentyl) derivatives of adenine (DHCaA) and 3-deazaadenine (c^{3}DHCaA),[13] neplanocin A[14,15] and 3-deazaneplanocin A,[16,17] (6'*R*)-6'-*C*-methyl-, ethyl-, ethenyl-, or ethynyl-substituted derivatives of neplanocin A,[18-20] the 9-(2',3'-dihydroxycyclopent-4'-enyl) derivatives of adenine (DHCeA) and 3-deazaadenine (c^{3}DHCeA),[17,21] 2-fluoroneplanocin A,[22] 6'-homoneplanocin A[23] and (±)-6'β-fluoroaristeromycin.[24] As has been demonstrated particularly with aristeromycin[5] and 5'-noraristeromycin,[9,10] both the antiviral activity and SAH hydrolase inhibitory effects reside primarily with the (-)- rather than the (+)-enantiomer.



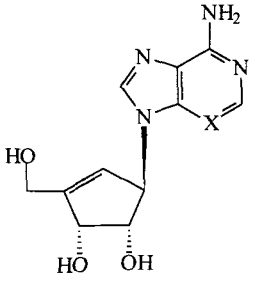
C-Ado (X = N)
C-c^{3}Ado (X=CH)



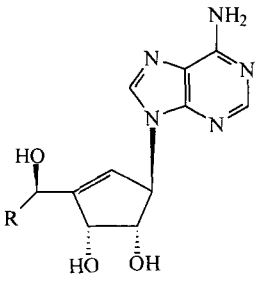
(-)-5'-Noraristeromycin (X=N)
3-Deaza-(-)-5'-noraristeromycin (X=CH)



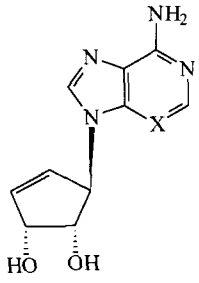
DHCaA (X=N)
c^{3}DHCaA (X=CH)



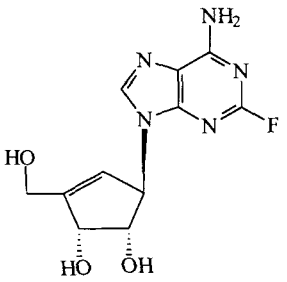
Neplanocin A (X=N)
3-Deazaneplanocin A (X=CH)



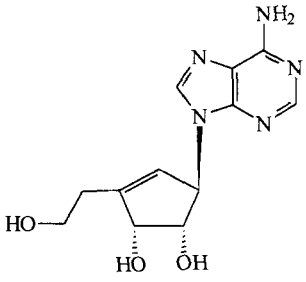
(6'*R*)-6'-*C*-methylneplanocin A (R=CH_3)
(6'*R*)-6'-*C*-ethylneplanocin A (R=CH_2CH_3)
(6'*R*)-6'-*C*-ethenylneplanocin A (R=$CH{=}CH_2$)
(6'*R*)-6'-*C*-ethynylneplanocin A (R=C≡CH)



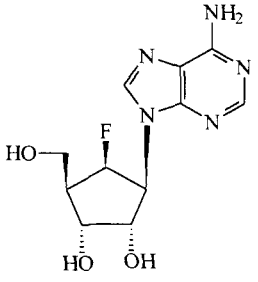
DHCeA (X=N)
c^{3}DHCeA (X=CH)



2-Fluoroneplanocin A



6'-Homoneplanocin A



6'β-Fluoroaristeromycin

FIG. 2. Structural formulae of SAH hydrolase inhibitors.

TABLE 1. Antiviral activity spectrum of SAH hydrolase inhibitors

Herpesviridae :	Human cytomegalovirus (HCMV)
Iridoviridae :	African swine fever virus
Poxviridae :	Vaccinia virus
Paramyxoviridae :	Parainfluenza virus, measles virus, mumps virus, respiratory syncytial virus (RSV)
Arenaviridae :	Junin virus, Tacaribe virus
Rhabdoviridae :	Vesicular stomatitis virus, rabies virus
Filoviridae :	Ebola virus
Reoviridae :	Reovirus, rotavirus
Retroviridae :	Human immunodeficiency virus (HIV)

Antiviral Activity Spectrum

The antiviral activity spectrum (Table 1) of SAH hydrolase inhibitors is unique in that it encompasses (i) some DNA viruses, such as vaccinia virus,[14,15] human cytomegalovirus,[25] and African swine fever virus,[26] whereas other DNA viruses [herpesviridae (herpes simplex virus, varicella-zoster virus, ...)] are much less sensitive or insensitive to the compounds; (ii) (-)RNA viruses, i.e. paramyxoviruses, such as parainfluenza virus, measles virus,[15] mumps virus[19] and respiratory syncytial virus (RSV)[27] (although the inhibitory effects of SAH hydrolase inhibitors on RSV replication are not well pronounced,[19] not always reproducible[9,10] and sometimes masked by their cytotoxic effects[28]), arenaviruses, such as Junin virus and Tacaribe virus,[29,30] rhabdoviruses, such as vesicular stomatitis virus (which also serves as model for rabies virus),[7,15] filoviruses, such as Ebola virus;[31,32] (iii) double-stranded RNA viruses, such as reovirus and rotavirus;[7,15,17,33]; and, (iv) retroviruses, such as human immunodeficiency virus (HIV), but the latter only under specific test conditions.[34,35]

The (+)RNA viruses [other than retroviruses, such as picornaviruses (poliovirus, Coxsackie virus, ...) and togaviruses (Sindbis virus, Semliki forest virus, ...)] are virtually insensitive to the SAH hydrolase inhibitors. Also, yellow fever virus, a (+)RNA virus belonging to the flavivirus family is not affected by SAH hydrolase inhibitors.[36] Although SAH hydrolase inhibitors are clearly active against paramyxoviruses, including measles,[37] they are devoid of activity against orthomyxoviruses, i.e. influenza A and influenza B virus.[38] This is not surprising since influenza viruses, for the initiation of viral mRNA synthesis, differ fundamentally from other (-)RNA viruses in that they employ pre-capped mRNA offered by the cell, which allows them to escape to the inhibitory effects of the SAH hydrolase inhibitors.

SAH hydrolase inhibitors are not only antivirally active. They are inhibitory to the proliferation of any cells, including protozoa (i.e. *Plasmodium falciparum*[39]) and also the host cells used to monitor antiviral activity.[24] This is expected because of the role of SAM-dependent methylation reactions in the regulation of cell growth. This also explains why the antiviral effects of SAH hydrolase inhibitors may be masked by their cytotoxicity. In particular, no anti-HIV activity with the SAH hydrolase inhibitors is demonstrable if monitored in rapidly proliferating (i.e., MT-4 or CEM) cells,[17] as the suppressive effects of the compounds on cell proliferation precludes detection of any (possible) inhibitory effects on HIV replication at concentrations higher than the cytocidal concentrations.

Antiviral Activity through SAH Hydrolase Inhibition

For a series of acyclic and carbocyclic adenosine analogues, a close correlation was found between their inhibitory effects on murine L929 cell SAH hydrolase activity and their inhibitory effects on the replication of vaccinia virus and vesicular stomatitis virus.[4] This correlation can be extended to the more recently described SAH hydrolase inhibitors (-)-5'-noraristeromycin,[10] 3-deaza-(-)-5'-noraristeromycin,[11] the epimer of (-)-5'-noraristeromycin,[12] (6'*R*)-6'-*C*-methylneplanocin A,[18] (6'*R*)-6'-*C*-ethylneplanocin A,[20] (6'*R*)-6'-*C*-ethenylneplanocin A,[20] (6'*R*)-6'-*C*-ethynylneplanocin A,[20] 2-fluoroneplanocin A[22] and 6'-homoneplanocin A,[23] which were found to inhibit vaccinia and vesicular stomatitis virus replication at proportionally equivalent concentrations as those required to inhibit SAH hydrolase activity (Table 2). These compounds may, as suggested before[4] for neplanocin A, 3-deazaneplanocin A and carbocyclic 3-deazaadenosine, owe their antiviral effects to an inhibitory action targeted at SAH hydrolase.

In intact cells, SAH hydrolase inhibitors, such as neplanocin A and carbocyclic 3-deazaadenosine (C-c^{3}Ado), elicit a significant increase in the intracellular SAH levels without an apparent change of the SAM levels,[40-42] thus elevating the intracellular SAH/SAM ratios. The increase in intracellular SAH pool levels and SAH/SAM ratios correlated closely with the extent of virus yield reduction,[41] suggesting a causal relationship between the two events. It may be postulated, therefore, that SAH hydrolase inhibitors initiate the following chain of reactions: inhibition of SAH hydrolase activity, intracellular accumulation of SAH, inhibition of SAM-dependent methylation reactions, hypomethylation of viral mRNAs and reduction in virus yield.

Among the newly developed SAH hydrolase inhibitors (Table 2), (-)-5'-noraristeromycin,[10] (6'*R*)-6'-*C*-methylneplanocin A[18] and (6'*R*)-6'-*C*-ethynylneplanocin A[20] emerged as the most potent antiviral agents. These compounds should be further explored, together with the previously established SAH hydrolase inhibitors (i.e. 3-deazaneplanocin A and

TABLE 2. Data on SAH hydrolase inhibition and antiviral activity for recently described SAH hydrolase inhibitors

Compound	SAH hydrolase		Antiviral activity (Embryonic skin-muscle fibroblasts)	
	L929 cells	Rabbit erythrocytes	Vaccinia virus	Vesicular stomatitis virus
	IC_{50} (μM)[a]	IC_{50} (μg/ml)[a]	MIC_{50} (μg/ml)[b]	MIC_{50}(μg/ml)[b]
Neplanocin A	0.0042[c]	0.004	0.2	2
	0.0052		0.7	7
	0.0057			
	0.0059			
(-)-5'-Noraristeromycin	0.032		0.04	0.1
3-Deaza-(-)-5'-noraristeromycin	0.14		0.4	0.7
Epimer of (-)-5'-noraristeromycin	0.042		0.1	0.2
(6'*R*)-6'-*C*-methylneplanocin A	0.086 (Ki)		0.04	0.07
(6'*R*)-6'-*C*-ethylneplanocin A	40		2	2
(6'*R*)-6'-*C*-ethenylneplanocin A	2		0.2	0.2
(6'*R*)-6'-*C*-ethynylneplanocin A	0.24		0.05	0.1
2-Fluoroneplanocin A		0.20	0.1	1
6'-Homoneplanocin A		0.87	0.1	1

[a]IC_{50}: 50% inhibitory concentration required to inhibit SAH hydrolase activity by 50%.
[b]MIC_{50}: minimum inhibitory concentration required to inhibit virus-induced cytopathicity by 50%.
[c]Data taken from references 10, 11, 12, 18, 20, 22 and 23.

carbocyclic 3-deazaadenosine) for their potential in the treatment of various virus infections [i.e. pox (molluscum contagiosum, monkey pox, ...), paramyxo (parainfluenza, measles, ...), arena (Lassa, Machupo, Junin, ...), rhabdo (rabies), filo (Ebola, Marburg, ...), reo (rota)] that fall within the realm of the SAH hydrolase inhibitors and for which there is currently no (chemo)therapy available. In particular, the SAH hydrolase inhibitors should be pursued as potential candidate drugs for the treatment of Ebola virus infections, as suggested by studies with SAH hydrolase inhibitors in SCID mice and African green monkeys infected with Ebola virus.[31,32]

Inhibition of HIV Replication by SAH Hydrolase Inhibitors

As mentioned above, SAH hydrolase inhibitors are unable to inhibit HIV-induced cytopathicity in MT-4 cells,[35] apparently because the direct cytocidal effects of the compounds for the rapidly proliferating host cells mask any possible inhibitory effects on virus replication. However, under well-defined conditions where p24 antigen production is monitored as parameter of HIV infection, SAH hydrolase inhibitors, such as 3-deazaadenosine (c^3Ado),

carbocyclic 3-deazaadenosine (C-c^{3}Ado) and 3-deazaneplanocin A, proved inhibitory to HIV replication.[34,43] In these studies,[34] both C-c^{3}Ado and 3-deazaneplanocin A were also found to inhibit *Tat*-induced transcription from the HIV-1 LTR in stably transfected cell lines. SAH hydrolase inhibitors, i.e. c^{3}Ado, have been postulated to inhibit the replication of retroviruses, i.e. Rous sarcoma virus, through their ability to inhibit SAH hydrolase, thus resulting in inhibition of the methylation reactions required for virus growth.[44]

In our recent exepriments,[35] we have demonstrated that SAH hydrolase inhibitors are able to inhibit the *Tat*-mediated transcription from the HIV-1 LTR in HeLa-*tat*-III cells (HeLa cells that express the HIV-1 transactivator) (Table 3). Furthermore, the SAH hydrolase inhibitors were also found to inhibit *Tat*-dependent HIV-1 replication in CD4$^+$ HeLa cells infected with HIV-1 (Table 3). In these experiments, the SAH hydrolase inhibitors exhibited a close correlation between their inhibitory effect on SAH hydrolase, their inhibitory effects on *Tat* transactivation and their inhibitory effects on *Tat*-mediated HIV-1 replication.[35] Our results suggest that SAH hydrolase and SAM-dependent methylation reactions play an important role in the transactivation process that leads to the transcription of HIV-1 mRNA from the proviral DNA. SAH hydrolase inhibitors may thus interfere with the replication of HIV at the transcription level. Their therapeutic potential as candidate anti-HIV drugs needs further investigation.

Perspectives for the Clinical Use of SAH Hydrolase Inhibitors

Because of their unique antiviral activity spectrum, the SAH hydrolase inhibitors offer great promise for the treatment of a number of virus infections for which there is no current chemotherapy, such as poxvirus infections (i.e. molluscum contagiosum, monkey pox), arenavirus infections (i.e. Lassa hemorrhagic fever), rhabdovirus infections (i.e. rabies), filovirus infections (i.e. Ebola) and reovirus infections (i.e. rota). Also the therapeutic potential of the SAH hydrolase inhibitors in the treatment of HIV infections should be further pursued.

Since SAH hydrolase inhibitors exert their antiviral activity through interaction with a cellular enzyme (i.e. SAH hydrolase) as target, these compounds may be expected, on the one hand, not to lead to the rapid development of virus-drug resistance, but, on the other hand, to affect the growth and metabolism of the normal host cells. In particular, rapidly proliferating cells are most susceptible to the cytocidal effects of SAH hydrolase inhibitors. *In vitro*, the specific antiviral effects of SAH hydrolase inhibitors are demonstrable only in stationary, not rapidly proliferating, cell cultures. The effectiveness of the SAH hydrolase inhibitors *in vivo*

TABLE 3. Inhibitory effects of SAH hydrolase inhibitors on *Tat*-dependent transactivation and HIV-1 replication

Compound	Inhibition of SAH hydrolase IC_{50} (μM)[a]	Inhibition of *Tat*-dependent transactivation IC_{50} (μM)[b]	Inhibition of *Tat*-dependent HIV-1 replication IC_{50} (μM)[c]
C-c^{3}Ado	0.018[d]	1.1	7.4
(-)-5'-Noraristeromycin	0.032	0.24	...
DHCaA	0.37	0.21	1.3
c^{3}DHCaA	0.21	0.26	0.29
Neplanocin A	0.015	0.23	1.9
3-Deazaneplanocin A	0.031	0.30	0.41
(6'*R*)-6'-*C*-methylneplanocin A	0.10	0.32	3.5
(6'*R*)-6'-*C*-ethylneplanocin A	40	> 206	4.6
(6'*R*)-6'-*C*-ethenylneplanocin A	2	0.96	6.16
(6'*R*)-6'-*C*-ethynylneplanocin A	0.25	0.90	38

[a]50% Inhibitory concentration required to inhibit SAH hydrolase activity by 50%.
[b]50% Inhibitory concentration required to inhibit by 50% β-Gal expression driven by the HIV-1 LTR in transfected HeLa *tat*-III cells (expressing HIV-1 transactivator).
[c]50% Inhibitory concentration required to inhibit by 50% β-Gal expression driven by the HIV-1 LTR in transfected HIV-1-infected CD4$^+$ HeLa cells.
[d]Data taken from reference 35.

remains to be established. This effectiveness may depend on a number of factors, including the nature of the virus infection and the nature of the cells that are inflicted by the virus, as well as the route (topical or systemic) of administration of the compounds.

Acknowledgments

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