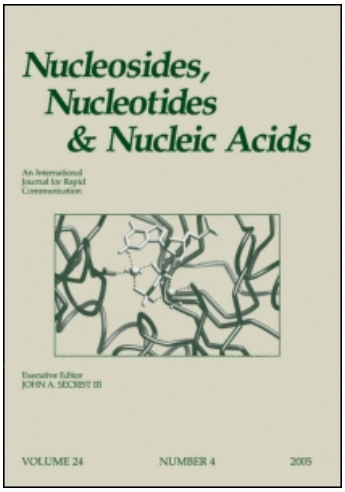


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

John Montgomery's Legacy: Carbocyclic Adenosine Analogues as Sah Hydrolase Inhibitors with Broad-Spectrum Antiviral Activity

E. De Clercq[a]
[a] Department of Microbiology and Immunology, Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium

To cite this Article De Clercq, E.(2005) 'John Montgomery's Legacy: Carbocyclic Adenosine Analogues as Sah Hydrolase Inhibitors with Broad-Spectrum Antiviral Activity', Nucleosides, Nucleotides and Nucleic Acids, 24: 10, 1395 — 1415
To link to this Article: DOI: 10.1080/15257770500265638
URL: http://dx.doi.org/10.1080/15257770500265638

Nucleosides, Nucleotides, and Nucleic Acids, 24:1395–1415, 2005

ISSN: 1525-7770 print/1532-2335 online
DOI: 10.1080/15257770500265638

JOHN MONTGOMERY'S LEGACY: Carbocyclic Adenosine Analogues as SAH Hydrolase Inhibitors with Broad-spectrum Antiviral Activity

Erik De Clercq □ *Rega Institute for Medical Research, Department of Microbiology and Immunology, K.U. Leuven, Leuven, Belgium*

□ *Ever since the S-adenosylhomocysteine (AdoHcy, SAH) hydrolase was recognized as a pharmacological target for antiviral agents (J. A. Montgomery et al., J. Med. Chem. 25:626–629, 1982), an increasing number of adenosine, acyclic adenosine, and carbocyclic adenosine analogues have been described as potent SAH hydrolase inhibitors endowed with broad-spectrum antiviral activity. The antiviral activity spectrum of the SAH hydrolase inhibitors include pox-, rhabdo-, filo-, arena-, paramyxo-, reo-, and retroviruses. Among the most potent SAH hydrolase inhibitors and antiviral agents rank carbocyclic 3-deazaadenosine (C-c^{3}Ado), neplanocin A, 3-deazaneplanocin A, the 5′-nor derivatives of carbocyclic adenosine (C-Ado, aristeromycin), and the 2-halo (i.e., 2-fluoro) and 6′-R-alkyl (i.e., 6′-R-methyl) derivatives of neplanocin A. These compounds are particularly active against poxviruses (i.e., vaccinia virus), and rhabdoviruses (i.e., vesicular stomatitis virus). The in vivo efficacy of C-c^{3}Ado and 3-deazaneplanocin A has been established in mouse models for vaccinia virus, vesicular stomatitis virus, and Ebola virus. SAH hydrolase inhibitors such as C-c^{3}Ado and 3-deazaneplanocin A should in the first place be considered for therapeutic (or prophylactic) use against poxvirus infections, including smallpox, and hemorrhagic fever virus infections such as Ebola.*

Keywords S-Adenosylhomocysteine (AdoHcy, SAH); Adenosine analogues; Carbocyclic adenosine analogues; Acyclic adenosine analogues; Carbocyclic 3-deazaadenosine (C-c^{3}Ado); 3-Deazaneplanocin A; Poxviruses (vaccinia virus); Hemorrhagic fever viruses (Ebola virus); Rhabdoviruses (vesicular stomatitis virus); Paramyxoviruses

INTRODUCTION

In 1983, we reported the broad-spectrum antiviral activity (i.e., against vaccinia, reo, measles, parainfluenza and vesicular stomatitis virus) of carbo-

Dedicated to the memory of John A. Montgomery.

Received 10 January 2005; accepted 28 April 2005.
I am indebted to Christiane Callebaut for her proficient editorial assistance.

Address correspondence to Erik De Clercq, Rega Institute for Medical Research, Department of Microbiology and Immunology, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. E-mail: erik.declercq@rega.kuleuven.be

FIGURE 1 John A. Montgomery, anno 1983, picture taken at the NATO Advanced Study Institute/FEBS Advanced Course on "Targets for the Design of Antiviral Agents," Les Arcs, France, 19 June–2 July 1983.

cyclic 3-deazaadenosine (C-c^{3}Ado), an inhibitor of S-adenosylhomocysteine (SAH) hydrolase.[1] "The significance of this paper lies in the fact that an inhibitor of a cellular enzyme can be relatively non-toxic to uninfected cells yet inhibit certain DNA and RNA viruses in a potent and selective manner," as was pointed out by one of the reviewers for *Science*, where the paper was originally submitted. Although the two reviewers recommended publication in *Science*, the paper was eventually turned down by this Journal, simply because of lack of space. Fortunately, *Antiviral Research* was able to accommodate the paper.[1] When shortly thereafter, our paper was published on the broad-spectrum antiviral activity of adenosine analogues (including C-c^{3}Ado, but also other, acyclic and carbocyclic, adenosine analogues), also in *Antiviral Research*,[2] John A. Montgomery (Figure 1) wrote it "should become a classic in the field of experimental antiviral chemotherapy" (Figure 2). Together with (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], which we had described a few years earlier (in 1978) as an aliphatic nucleoside analog with broad-spectrum antiviral activity,[3] C-c^{3}Ado validated S-adenosylhomocysteine (SAH) hydrolase as a pharmacological target for antiviral agents.[4] It would ultimately lead to the identification of a number of SAH hydrolase inhibitors (such as 3-deazaneplanocin A) for the potential treatment of

Southern Research Institute 2000 Ninth Avenue South / P.O. Box 55305 / Birmingham, Alabama 35255-5305 / (205) 323-6592

February 2, 1984

Dr. Erik De Clercq
Katholieke Universiteit Leuven
Rega Instituut
Minderbroedersstraat 10
B-3000 Leuven
Belgium

Dear Erik:

Many thanks for the copy of our manuscript. As I'm sure you know, I am delighted that it will appear in Antiviral Research. It is an excellent paper and you are to be congratulated. It should become a classic in the field of experimental antiviral chemotherapy.

With warm regards.

Sincerely yours,

John A. Montgomery
Senior Vice President and
Director of the Kettering-Meyer
Laboratory

JAM:lkf

FIGURE 2 Letter received from Dr. John A. Montgomery in 1984 with regard to the paper on Broad-spectrum antiviral activity of adenosine analogues published in Antiviral Research in 1984.[2]

infections with such important human pathogens as filo (i.e., Ebola) and pox (i.e., smallpox) viruses.

Mechanism of Antiviral Action of SAH Hydrolase Inhibitors

S-adenosylhomocysteine (AdoHcy, SAH) hydrolase is a key enzyme in methylation reactions depending on S-adenosylmethionine (AdoMet, SAM) as the methyl donor. SAH hydrolase cleaves AdoHcy into its two components, homocysteine and adenosine (which can then be further converted to either inosine [by adenosine deaminase] or AMP [by adenosine kinase]

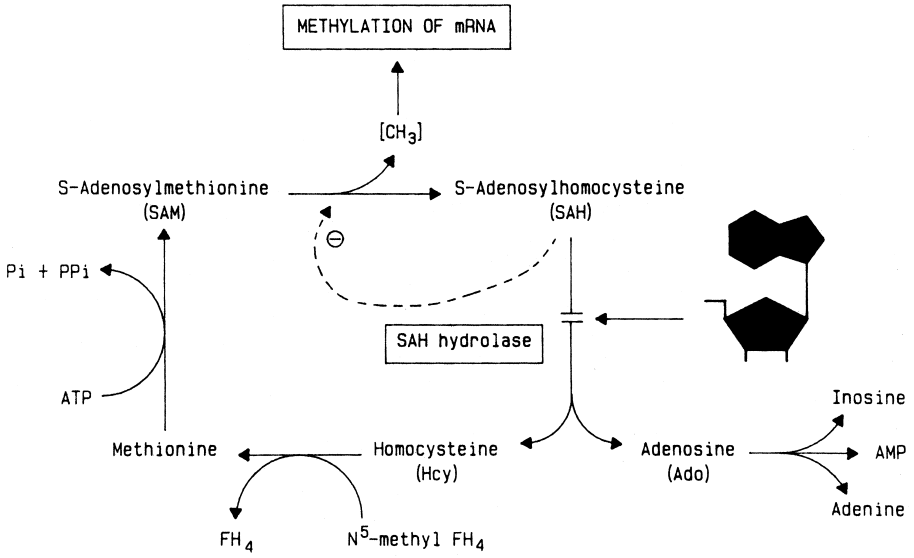


FIGURE 3 Mechanism of antiviral action of SAH hydrolase inhibitors.

and adenine [by adenosine phosphorylase]). Through the hydrolysis of AdoHcy, the SAH hydrolase 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 (i.e., 5′-capping) of viral mRNAs (Figure 3).

SAH hydrolase has been recognized for a long time as a potential target for antiviral chemotherapy, and a variety of carbocyclic as well as acyclic adenosine analogues have been assumed to exert their antiviral action through inhibition of SAH hydrolase.[5,6] In fact, a close correlation has been found between the antiviral activity of various carbocyclic and acyclic adenosine analogues and their inhibitory effects on cell-free SAH hydrolase.[7,8] Figure 4 depicts the correlation between the inhibitory effects on SAH hydrolase of four adenosine analogues, neplanocin A, C-c^{3}Ado, (*RS*)-AHPA [(*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid], and the aforementioned (*S*)-DHPA, and their inhibitory effects on the replication of 5 viruses, vesicular stomatitis virus (VSV), vaccinia virus (VV), measles virus, reovirus type 1, and rotavirus. Regression analysis for the correlation between anti-VSV activity and SAH hydrolase inhibition yielded a correlation coefficient of $r = 0.986$.

It was further ascertained that the intracellular pool levels of AdoHcy increased upon treatment of the cells with the SAH hydrolase inhibitors, and the AdoHcy/AdoMet ratio correlated closely with the antiviral activity achieved ($r = 0.972$ for vaccinia virus yield reduction).[9] Addition of

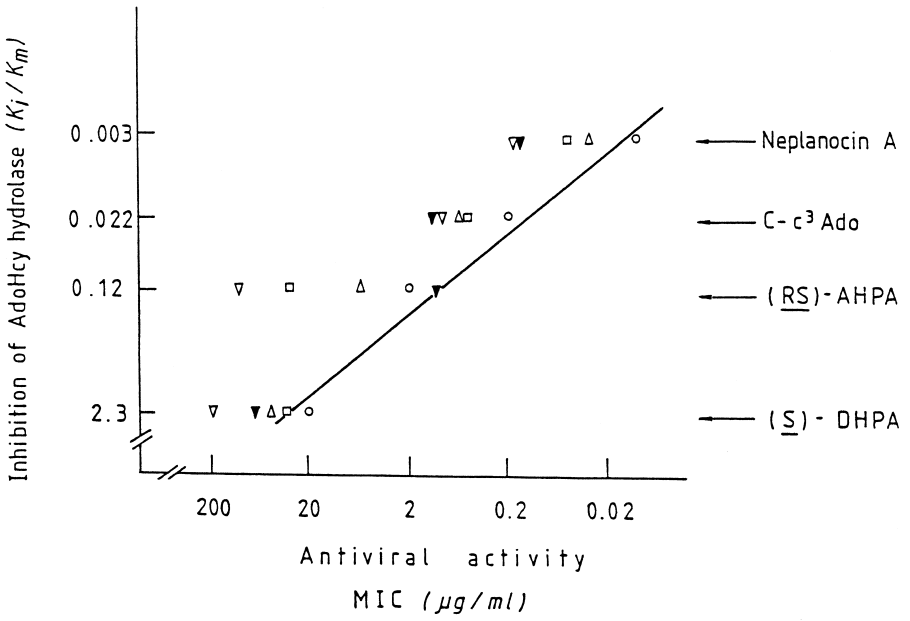
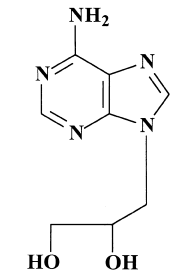


FIGURE 4 Correlation between the antiviral activities of the four adenosine analogues and their inhibitory effects on SAH hydrolase. The inhibitory effect on SAH hydrolase is expressed as the K_i/K_m for bovine liver SAH hydrolase activity (measured in the direction of SAH synthesis). The antiviral activity is expressed as the minimum inhibitory concentration (MIC) required to inhibit by 50% the cytopathogenicity of vesicular stomatitis virus (○) (in primary rabbit kidney [PRK] cells), vaccinia virus (△) in PRK cells, measles virus (□) (in African green monkey kidney [Vero] cells), reovirus type I (▽) in Vero cells, or to reduce by 90% the yield of human rotavirus (▼) in embryonic rhesus monkey kidney (MA 104) cells. The linear regression line is shown for K_i/K_m as a function of MIC for vesicular stomatitis virus (○) ($r = 0.986$). Data taken from De Clercq.[5]

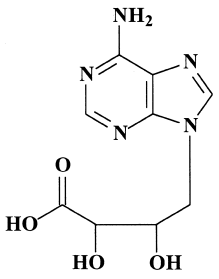
homocysteine further potentiated the antiviral activity of the SAH hydrolase inhibitors through an increase of the intracellular SAH pool levels.[10]

Antiviral Potency of Carbocyclic (and Acyclic) Adenosine Analogues

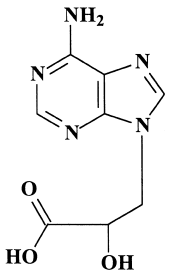
After SAH hydrolase had been recognized as a pharmacological target for antiviral agents such as the carbocyclic analogue of 3-deazaadenosine (C-c^{3}Ado),[4] and, independently thereof, acyclic adenosine derivatives such as (*S*)-DHPA[3,11] had been discovered as broadly active antiviral agents showing an activity spectrum quite similar to that of the carbocyclic adenosine analogues, a steadily increasing number of acyclic, and, primarily, carbocyclic adenosine analogues were described (Figure 5). For most of the newly developed acyclic and carbocyclic adenosine analogues, marked antiviral potency was detected.[12–37]



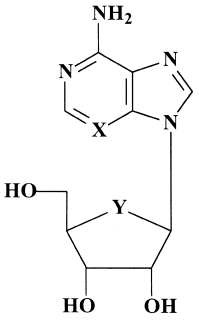
(*S*)-DHPA



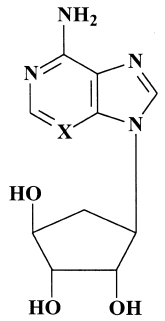
D-Eritadenine



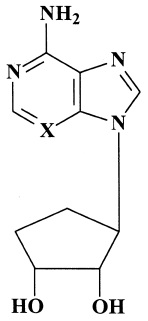
(*RS*)-AHPA
(*RS*)-3-Adenin-9-yl-2-hydroxypropanoic acid



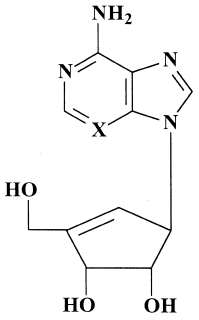
C-Ado (X = N, Y = O)
c^3Ado (X = CH, Y = O)
C-c^3Ado (X = CH, Y = CH_2)



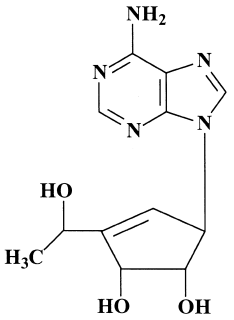
(-)-5'-Noraristeromycin (X = N)
3-Deaza-(-)-5'-noraristeromycin (X = CH)



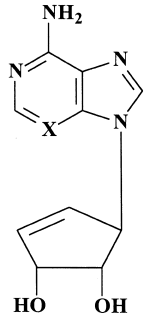
DHCaA (X = N)
c^3DHCaA (X = CH)



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

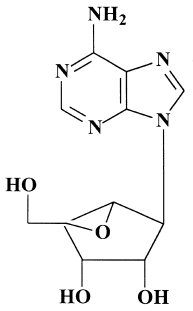


(6'*R*)-6'-*C*-methylneplanocin A

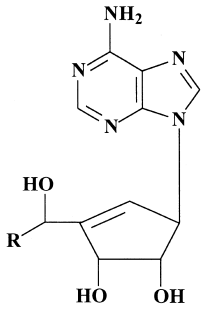


DHCeA (X = N)
c^3DHCeA (X = CH)

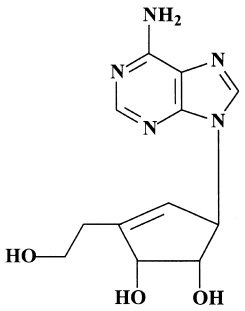
FIGURE 5 Adenosine and carbocyclic (and acyclic) adenosine analogues acting as SAH hydrolase inhibitors. (*continues*)



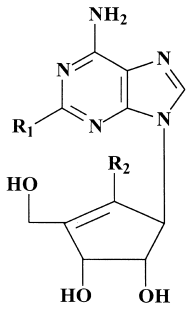
Neplanocin C



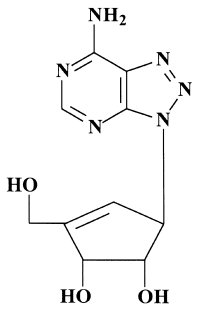
(6'*R*)-6'-*C*-ethylneplanocin A (R = CH_2CH_3)
(6'*R*)-6'-*C*-ethenylneplanocin A (R = $CH{=}CH_2$)
(6'*R*)-6'-*C*-ethynylneplanocin A (R = C≡CH)



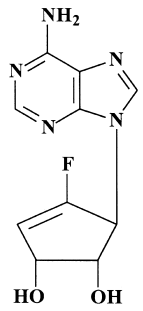
6'-Homoneplanocin A



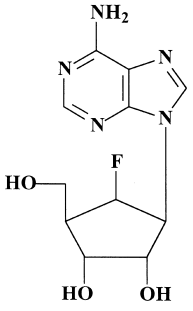
2-Fluoroneplanocin A (R_1 = F, R_2 = H)
6'-Fluoroneplanocin A (R_1 = H, R_2 = F)



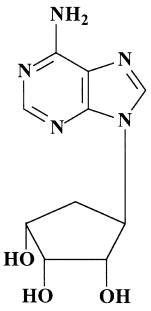
8-Azaneplanocin A



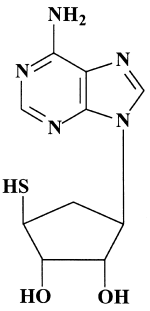
5'-Fluoro-DHCeA



(±)-6'β-Fluoroaristeromycin
F-C-Ado

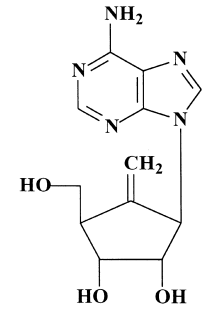


Epimer of
(-)-5'-noraristeromycin

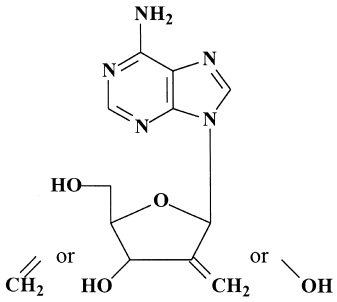


Mercapto analogue of
(-)-5'-noraristeromycin

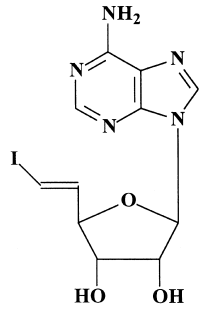
FIGURE 5 (*Continued*).



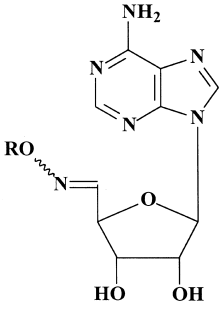
6'-Methylene-aristeromycin



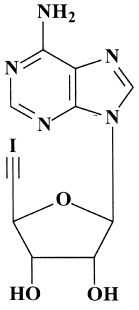
2'-Methylene-2'-dAdo
3'-Methylene-3'-dAdo



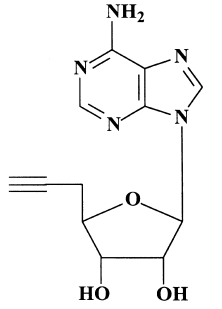
6'-(*E*)-Iodohomovinyl-Ado



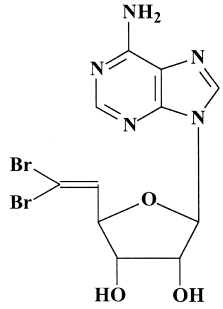
5'-Carboxaldoxime-Ado (R = $PhCH_2$)



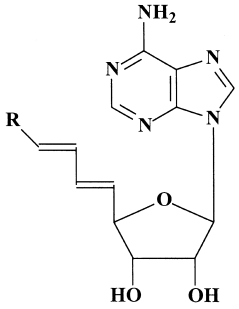
4'-Iodoacetylenic Ado



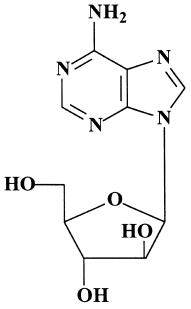
5'-Acetylenic Ado



6'-Dibromohomovinyl-Ado



Ado diene analogue (R = CO_2Et)



Ara-A
(Adenine arabinoside)

FIGURE 5 (*Continued*).

As originally proposed by Borchardt and coworkers[38,39] SAH hydrolase may act by a dual mechanism. Type I mechanism-based inactivators cause irreversible cofactor depletion (converting the NAD^+ cofactor to its inactive form NADH) and are not covalently bound to the enzyme; neplanocin A and DHCeA are typical examples of type I mechanism-based inactivators, and so might be the (±)-6′-*β*-fluoroaristeromycin. Type I mechanism-based inactivators are oxidized by the hydrolase NAD^+ at the 3′-position. Type II mechanism-based inactivators are those that are not only oxidized at the 3′-position by the hydrolase, but also become covalently bound to the enzyme: this would happen, for instance, when fluoride is released in the presence of the enzyme, as shown for 4′,5′-didehydro-5′-fluoroadenosine.[40]

Of the compounds depicted in Figure 5, the most potent antivirals, as based on their inhibitory effects on the two "reporter" viruses, VV and VSV (Table 1), were C-c^{3}Ado, neplanocin A, 3-deazaneplanocin A, DHCeA, c^{3}DHCeA, DHCaA, c^{3}DHCaA, (−)-5′-noraristeromycin, (6′*R*)-6′-*C*-methylneplanocin A, and (±)-6′-*β*-fluoroaristeromycin. They inhibited the replication of VV and VSV within a 50% effective concentration (EC_{50}) range of 0.01–1 μg/mL; this is a concentration range that should be readily achievable upon systemic administration of the compounds.

Also listed among the SAH hydrolase inhibitors is adenine arabinoside (ara-A).[11,41] Although not a "genuine" SAH hydrolase inhibitor in the sense that part of its antiviral action may be achieved by interaction with other enzymes, i.e., DNA polymerase and ribonucleotide reductase,[5] its activity against VSV can be explained only by an interaction with SAH hydrolase, and, furthermore, ara-A has been found to suppress SAH hydrolase activity in peripheral blood mononuclear cells (in patients treated with ara-A for chronic hepatitis B).[42]

In addition to ara-A, some other adenosine analogues, such as formycin A (a *C*-glycoside that is isomeric with adenosine),[43] 8-methyladenosine,[44] and 8-alkynyl-, 8-alkenyl-, and 8-alkyl-2′-deoxyadenosine derivatives[45] have been accredited with antiviral properties. In particular, 8-methyladenosine proved to be a highly potent and selective inhibitor of vaccinia virus; yet, it did not display activity against VSV.[44]

Activity against both VV and VSV may be viewed as the hallmark for an adenosine analogue that is targeted at SAH hydrolase, and, vice versa, adenosine analogues that show this unique duality, may be virtually interpreted as SAH hydrolase inhibitors. How could this exquisite activity of SAH hydrolase inhibitors against VV and VSV be rationalized? Probably because both VV (a poxvirus with a ds(±)DNA genome) and VSV (a rhabdovirus with a ss(−)RNA genome) during their life cycle pass through the formation of an mRNA that needs to be heavily methylated (5′-capped), and, therefore, becomes highly sensitive to SAH hydrolase inhibitors that obviate these methylations.

TABLE 1 Antiviral Potency of Adenosine Analogues, and Carbocyclic (and Acyclic) Adenosine Analogues

Compound	Cell culture	EC_{50} (μg/ml) VV	EC_{50} (μg/ml) VSV	CC_{50} (μg/mL)	Reference(s)
(*S*)-DHPA	PRK	20	10	>200	De Clercq et al. [3] De Clercq and Holý [11]
D-Eritadenine					
(*RS*)-AHPA (alkyl esters)	PRK	3–30	1–3	>400	De Clercq and Holý [12]
C-Ado					
c^3Ado	PRK	7	7	40	De Clercq and Montgomery [1]
C-c^3Ado	PRK	0.8	0.2	>400	De Clercq and Montgomery [1]
Neplanocin A	PRK	0.03	0.01	40	De Clercq [13]
3-Deazaneplanocin A	PRK	0.07	0.07	>400	De Clercq et al. [14]
	Vero	0.02–0.3			Tseng et al. [15]
	L929		0.07–0.2		Tseng et al. [15]
Neplanocin C	PRK	0.1	0.2	40	De Clercq [13]
DHCeA	PRK	0.7	0.2	>400	De Clercq et al. [14]
	L929	0.1			Hasobe et al. [16]
c^3DHCeA	PRK	0.7	0.2	>400	De Clercq et al. [14]
	L929	0.03			Hasobe et al. [16]
DHCaA	L929	0.03			Hasobe et al. [17]
c^3DHCaA	L929	0.03			Hasobe et al. [17]
(±)-5′-Norаristeromycin	E_6SM	0.3	0.07	>400	Patil et al. [18]
(−)-5′-Norаristeromycin	E_6SM	0.04	0.1	>400	Siddiqi et al. [19]
(+)-5′-Norаristeromycin	E_6SM	0.7	2.0	>400	Siddiqi et al. [19]
(−)-3-Deaza-5′-noraristeromycin	E_6SM	0.4	0.7	>400	Siddiqi et al. [20]
(6′*R*)-6′-*C*-methylneplanocin A	E_6SM	0.04	0.07	>400	Shuto et al. [21]

(6′*R*)-6′-*C*-ethylneplanocin A	E_6SM	7	1	>400	Shuto et al. [22]
(6′*R*)-6′-*C*-ethenylneplanocin A	E_6SM	0.2	0.2	>200	Shuto et al. [22]
(6′*R*)-6′-*C*-ethynylneplanocin A	E_6SM	0.07	0.2	>400	Shuto et al. [22]
6′-Homoneplanocin A	E_6SM	0.1	1	>400	Shuto et al. [23]
2-Fluoroneplanocin A	E_6SM	0.1	1	40	Obara et al. [24]
6′-Fluoroneplanocin A	HeLa		0.12		Jeong et al. [25]
8-Azaneplanocin A	E_6SM	300	70	>400	Marquez et al. [26]
5′-Fluoro-DHCeA					
(±)-6′β-Fluoroaristeromycin (F-C-Ado)	L929	0.1	0.4	>100	Cools et al. [27]
	HEP-2	0.15		100	Madhavan et al. [28]
epi(−)-5′-Noraristeromycin	E_6SM	0.1	0.2	>400	Siddiqi et al. [29]
Mercapto(−)-5′-noraristeromycin	E_6SM	48	>80	≥400	Das et al. [30]
6′-Methylene-aristeromycin	HEP-2	0.16		15	Madhavan et al. [28]
2′-Methylene-2′-dAdo	PRK	20			Robins et al. [31]
3′-Methylene-3′-dAdo	PRK	20			Robins et al. [31]
6′-(*E*)-Iodohomovinyl-Ado	E_6SM	7	2	≥200	Wnuk et al. [32]
5′-Carboxaldoxime-Ado	E_6SM	2		>40	Wnuk et al. [33]
	Vero		>40	>100	Wnuk et al. [33]
4′-Iodoacetylenic Ado	E_6SM	0.26	0.08	26	Robins et al. [34]
5′-Acetylenic Ado	E_6SM	4	2		Wnuk et al. [35]
6′-Dibromohomovinyl-Ado	E_6SM	>100	>100	>100	Wnuk et al. [36]
Ado diene analogues	E_6SM	>100	>100	>100	Wnuk et al. [37]
Adenine arabinoside (Ara-A)	PRK	0.4	30	100	De Clercq and Holý [11]
					De Clercq et al. [41]

Abbreviations: VV: vaccinia virus; VSV: vesicular stomatitis virus; EC_{50}: 50% effective concentration, required to inhibit viral cytopathicity by 50%; CC_{50}: 50% cytotoxic concentration, required to cause a microscopically detectable alteration of cell morphology; PRK: primary rabbit kidney (cells); Vero: African green monkey kidney (cells); L929: murine fibroblast (cells); E_6SM: human embryonic skin-muscle (cells); HeLa: human epithelial (cells); HEP-2: human hepatoma (cells).

For a number of the carbocyclic adenosine analogues mentioned in Table 1 and Figure 5 (i.e., neplanocin A,[46] DHCeA,[47] and DHCaA and c^3DHCaA),[48] it has been convincingly demonstrated that they are potent SAH hydrolase inhibitors and that their antiviral effects (i.e., against vaccinia virus) can be attributed to an increase in the intracellular pool levels of AdoHcy and elevated AdoHcy/AdoMet ratios.[47,48] This confirms our own observations with a different set of compounds [(*S*)-DHPA, (*RS*)-AHPA, C-c^3Ado, neplanocin A and 3-deazaneplanocin A].[8,9]

Not all the acyclic or carbocyclic adenosine analogues that have been shown to be (reversible or irreversible) SAH hydrolase inhibitors have been further followed up for their antiviral potential: in particular, 6′-fluoroneplanocin A,[25] 6′-methylene-aristeromycin,[28] which have only cursorily been studied for their antiviral activity, and D-eritadine[49] and 5′-fluoro-DHCeA,[50] which have not yet been submitted to antiviral assays, should be further pursued from this regard.

When examining the adenosine analogues and their carbocyclic derivatives that have so far been explored as SAH hydrolase inhibitors/antiviral agents, they predominantly fall into three categories, adenosine (Ado) analogues, aristeromycin (C-Ado, cyclopentyladenine)) analogues, and neplanocin A (cyclopentenyladenine) analogues. Each of these basic structures has served as scaffold for the modifications at either the heterocyclic (adenine) base, or, more frequently, the glycon moiety. 5′-truncation or 5′-elongation, 3-deaza conversion and fluorinations at either the base or glycon, have often been carried out, but a systematic analysis of these different modifications (single or combined) in the three different scaffolds must still be undertaken before a true structure-activity relationship (SAR) could be established, both in terms of SAH hydrolase inhibition and antiviral potency.

Antiviral Activity Spectrum of SAH Hydrolase Inhibitors

While VV and VSV, as members of the poxviridae and rhabdoviridae, respectively, are exquisitely sensitive to SAH hydrolase inhibitors, their spectrum of antiviral action extends to various other viruses and virus families (Figure 6). Among the poxviruses, smallpox (variola) virus and monkeypox virus have proven to be quite sensitive to neplanocin A,[51] as could be predicted from the activity shown with neplanocin A against VV (vaccinia virus).[13] Again, as could be predicted from their activity against VSV (vesicular stomatitis virus),[14] SAH hydrolase inhibitors, such as 3-deazaneplanocin A, turned out to be remarkably active against filoviruses (such as Ebola virus),[6] which follow a replicative strategy similar to that of the rhabdoviruses. Furthermore, SAH hydrolase inhibitors, including 3-deazaneplanocin A, have also been identified as an attractive approach toward the treatment of arenavirus infections (i.e., Junin virus, Tacaribe virus).[52]

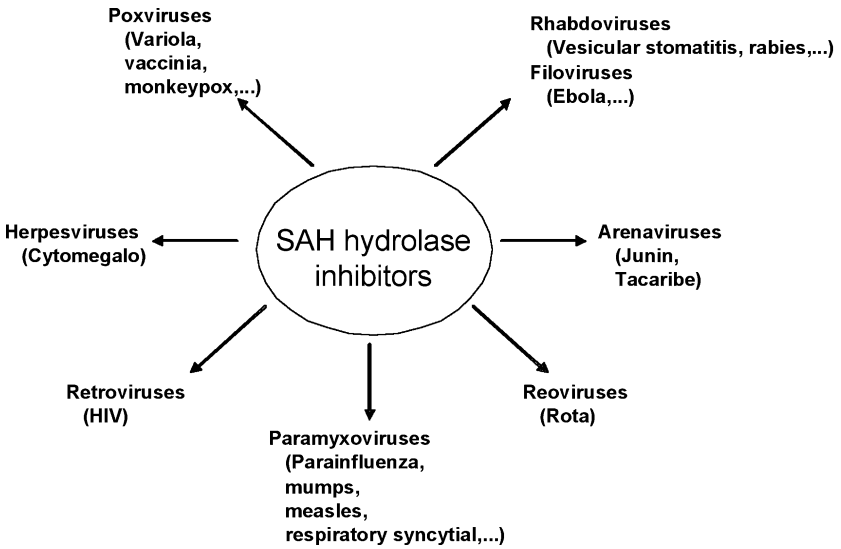


FIGURE 6 Antiviral activity spectrum of SAH hydrolase inhibitors.

In addition, SAH hydrolase inhibitors have also been found effective against the African swine fever virus[53] and human cytomegalovirus,[54] while other herpesviruses (herpes simplex virus, varicella-zoster virus, . . .) are much less sensitive or insensitive to the compounds. Reoviruses, such as the epidemiologically important rotavirus, are markedly sensitive to SAH hydrolase inhibitors,[14,55] and so are the paramyxoviruses parainfluenza virus,[13,56] measles,[13,56,57] and respiratory syncytial virus (RSV),[56] although the inhibitory effects of SAH hydrolase inhibitors on the SSPE (subacute sclerosing panencephalitis) variant of measles are not pronounced,[58] and their inhibitor effects on RSV replication are not always reproducible,[18,19] and sometimes masked by their cytotoxic effects.[59]

SAH hydrolase inhibitors have also been found effective against human immunodeficiency virus (HIV),[60,61] but only under specific test conditions (optimized for minimalizing cytotoxicity).[62] Their inhibitory effect on HIV replication could be ascribed to an inhibition of the long terminal repeat (LTR) transactivation of the HIV mRNA transcription,[62] which, as it appeared recently, heavily depends on co-transcriptional capping.[63]

The (+)RNA viruses, other than the retroviruses (HIV), are virtually insensitive to SAH hydrolase inhibitors. This includes picornaviruses (poliovirus, Coxsackie virus, . . .) and togaviruses (Sindbis, Semliki forest virus, . . .). Also, yellow fever virus, a (+)RNA virus belonging to the flavivirus family is not affected by SAH hydrolase inhibitors.[64] Although SAH hydrolase inhibitors are clearly active against paramyxoviruses,[13,56] they are devoid of activity against orthomyxoviruses (i.e., influenza A and B).[65] 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 provided by the cell, which allows them to escape to the inhibitory effects of the SAH hydrolase inhibitors.

SAH Hydrolase as a Pharmacological Target

As mentioned above, SAH hydrolase catalyzes the hydrolysis of AdoHcy to adenosine and homocysteine.[66,67] This is the major route for disposal of the AdoHcy formed as the product of the AdoMet-dependent methyltransferases. The reaction is reversible, but under physiological conditions the removal of both adenosine and homocysteine is sufficiently rapid for the net reaction to proceed in the direction of hydrolysis. The hydrolysis of AdoHcy serves physiologically not only to sustain the flux of methionine toward cysteine, but is believed also to play a critical role in the regulation of biological methylations. Recently, a genetic disorder of methionine metabolism was described that originated from an SAH hydrolase deficiency.[68]

In spite of the crucial role SAH hydrolase plays in regulating biological processes and its attractiveness as a target for drug design, relatively limited information is available about the structure and conformation of the SAH hydrolase. Site-directed mutagenesis studies have pointed to the role of Lys-426,[69] Asp-244,[70] and Asp-130, Lys-185, Asp-189, and Asn-190[71] in the catalytic activity of this homotetrameric enzyme.

SAH hydrolase has also emerged as an attractive target for antiparasitic drug design because of its role in the regulation of AdoMet-dependent transmethylation reactions that are essential for parasite replication.[72] SAH hydrolase has been detected in *Trypanosoma cruzi*,[72] *Trypanosoma brucei*,[73] *Plasmodium falciparum*,[74] *Trichomonas vaginalis*,[75] and *Leishmania donovani*.[76,77] Inhibitory effects against the parasitic SAH hydrolase were noted for a variety of SAH hydrolase inhibitors described above, i.e., 5′-noraristeromycin,[73] 2-fluoroaristeromycin,[74] ara-A (albeit at relatively high concentration),[75] 3-deazaaristeromycin (C-c^{3}Ado),[76] and DHCaA.[76] The latter two compounds were also shown to arrest the growth of the parasites (*L. donovani*), concomitantly with a substantial expansion of the intracellular AdoHcy pools and significant increase of the AdoHcy/AdoMet ratio.[76] It was further ascertained that *L. donovani* SAH hydrolase has structural requirements for binding inhibitors different from those of the human enzyme. Thus, it may be possible to eventually exploit these differences to design specific inhibitors of this parasitic enzyme as potential antiparasitic agents.[77]

In Vivo Antiviral Activity of SAH Hydrolase Inhibitors

In vivo antiviral efficacy has been demonstrated with a number of SAH hydrolase inhibitors, i.e., C-c^{3}Ado,[1] ara-A,[15] neplanocin A,[13] and, in

particular, 3-deazaneplanocin A,[14,15] against both VV and VSV infections. Illustrated in Figure 7 is the in vivo antiviral efficacy of 3-deazaneplanocin A in mice infected with VSV.[14] 3-Deazaneplanocin A conferred a highly significant reduction in the mortality rate when administered as a single dose of 0.5 or 2.5 mg/kg at 1 h postinfection. Ten years later, Bray et al.[78] would confirm that 3-deazaneplanocin A (as a single dose of 1 mg/kg), as well as C-c^{3}Ado (as a singe dose of 80 mg/kg), administered on day 1 or 2 with respect to infection, afforded an even more dramatic protection of mice against a lethal infection with Ebola (Zaire) virus (Figure 8).

In later studies, Bray et al.[79] found that 3-deazaneplanocin A induced massively increased interferon-α protection in Ebola virus-infected, but not uninfected mice, and attributed the protective effect of the drug to this massive boost in interferon production. How could the enhanced interferon response toward a SAH hydrolase inhibitor be rationalized? Given the fact that this increased interferon production was noted only in virus-infected cells, one could envisage the following scenario or sequence of events: (a) 3-deazaneplanocin A, as an SAH hydrolase inhibitor, shuts off methylation (i.e., 5′ capping) of the viral mRNA; as a consequence (b), the unmature viral (+)RNA remains attached to the (−)RNA viral template, thus engaging

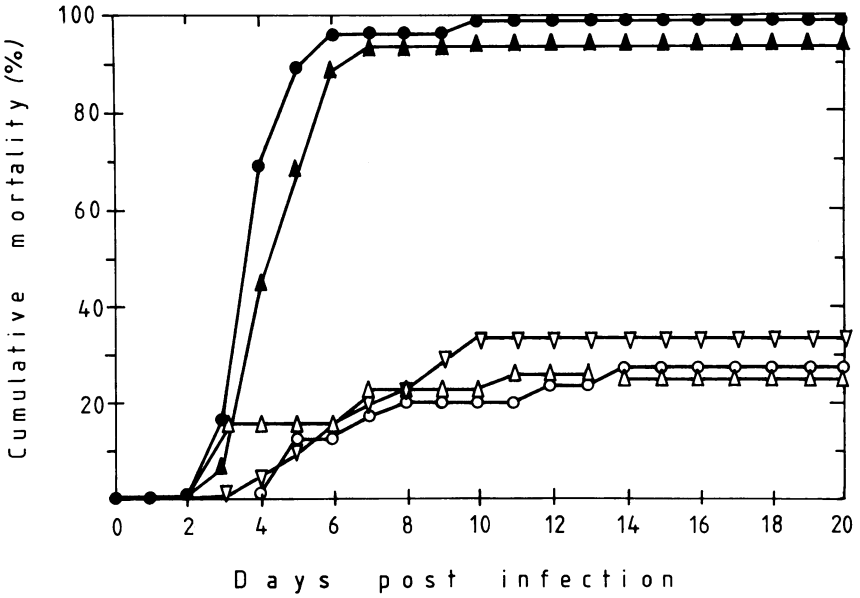


FIGURE 7 Inhibitory effect of 3-deazaneplanocin A on the mortality of NMRI mice inoculated subcutaneously when 48 h old with vesicular stomatitis virus. 3-Deazaneplanocin A was administered intraperitoneally at the following dosage regimens: 2.5 mg/kg at 1 h postinfection (△); 0.5 mg/kg at 1 h postinfection (▽); and 0.5 mg/kg at 1 h (day 1) and on days 2, 3, 4, and 5 (○). Control PBS, one injection at 1 h postinfection (▲) or five injections at 1 h (day 1) and on days 2, 3, 4, and 5 (●). Data taken from De Clercq et al.[14]

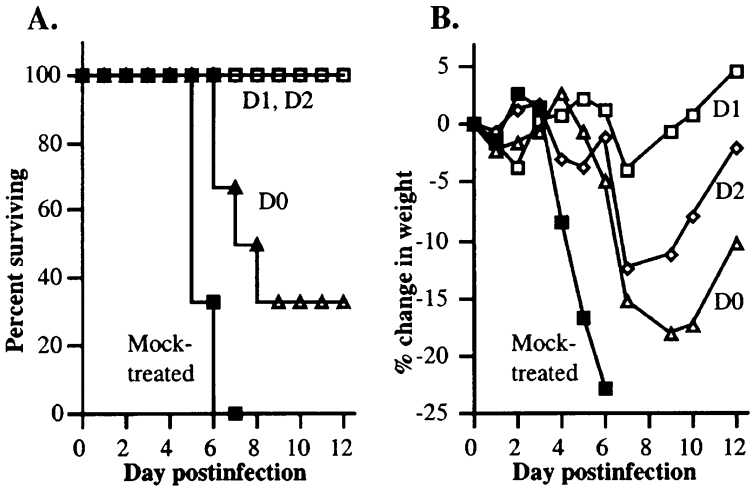


FIGURE 8 (A) Survival of groups of six adult BALB/c mice infected with 10 pfu (300 LD_{50}) mouse-adapted Ebola virus (Zaire strain) and inoculated once, on day 0 (D0), day 1 (D1), or day 2 (D2) with respect to infection, with 80 mg/kg of C-c^3Ado, or mock-treated with PBS on day 0. (B) Mean percent change in body weight of mice in the same experiment. Data taken from Bray et al.[78]

in the formation of a double-helical (±)RNA, which (c), as has been known for decades, would then lead to the production of massive amounts of interferon, thus (d) accounting for the dramatic protective effect of the SAH hydrolase inhibitors against Ebola virus infection. Obviously, this hypothesis will have to be corroborated by experimental evidence.

CONCLUSION

After Cantoni[66,67] had described and characterized SAH (in 1954) and SAH hydrolase (in 1959), Parker and Abeles[80] had further analyzed the mechanism of the SAH hydrolase reaction, and data from Chiang et al.[81] indicated the importance of SAH hydrolase as a potential pharmacological target (in 1977). In 1980, Borchardt[82] stressed the importance of SAM-dependent methyltransferases in the methylation (5′-capping), and, hence maturation of viral mRNA, i.e., vaccinia viral mRNA. In 1982, Montgomery et al. [4] then pointed out that SAH hydrolase was indeed the target for the antiviral action of C-c^3Ado. Subsequently, numerous adenosine analogues were shown to exert their antiviral activity through interaction with SAH hydrolase. Among the most promising, also shown to be effective in vivo (in animal models), are C-c^3Ado and 3-deazaneplanocin A. Equally promising, although not (yet) been investigated in vivo, are a number of 5′-nor derivatives of carbocyclic adenosine, carbocyclic 3-deazaadenosine, neplanocin A and 3-deazaneplanocin A, and 2-fluoro- and 6′-*R*-methyl substituted

neplanocins. Although none of these compounds has been marketed or even submitted for clinical studies, they offer sufficient potential that would warrant their further development. Worth considering are their potential applications in the treatment (or prophylaxis) of poxvirus infections (i.e., smallpox), rhabdo- and filovirus infections (i.e., Ebola), arenavirus infections (e.g., Lassa) and, in principle, any other viruses that heavily rely on the 5′-capping of their mRNA for their replicative efficiency. It thus appears that SAH hydrolase inhibitors represent an attractive antiviral strategy.[83] They should be further pursued, as they may find a "niche" for the treatment of the most deadly among the virus infections, such as smallpox (should it reappear), Ebola, and, possibly, other hemorrhagic fever virus infections as well. This, in turn, would justify the large-scale chemical synthesis of the most potent among the SAH hydrolase inhibitors and the exploration of their efficacy in the appropriate animal model systems.

REFERENCES

1. De Clercq, E.; Montgomery, J.A. Broad-spectrum antiviral activity of the carbocyclic analog of 3-deazaadenosine. Antiviral Res. **1983**, 3, 17–24.
2. De Clercq, E.; Bergstrom, D.E.; Holý, A.; Montgomery, J.A. Broad-spectrum antiviral activity of adenosine analogues. Antiviral Res. **1984**, 4, 119–133.
3. De Clercq, E.; Descamps, J.; De Somer, P.; Holý, A. (S)-9-(2,3-Dihydroxypropyl)adenine: An aliphatic nucleoside analog with broad-spectrum antiviral activity. Science **1978**, 200, 563–565.
4. Montgomery, J.A.; Clayton, S.J.; Thomas, H.J.; Shannon, W.M.; Arnett, G.; Bodner, A.J.; Kion, I.-K.; Cantoni, G.L.; Chiang, P.K. Carbocyclic analogue of 3-deazaadenosine: A novel antiviral agent using *S*-adenosylhomocysteine hydrolase as a pharmacological target. J. Med. Chem. **1982**, 25, 626–629.
5. De Clercq, E. *S*-Adenosylhomocysteine hydrolase inhibitors as broad-spectrum antiviral agents. Biochem. Pharmacol. **1987**, 36, 2567–2575.
6. De Clercq, E. Carbocyclic adenosine analogues as *S*-adenosylhomocysteine hydrolase inhibitors and antiviral agents: Recent advances. Nucleosides, Nucleotides **1998**, 17, 625–634.
7. De Clercq, E.; Cools, M. Antiviral potency of adenosine analogues: Correlation with inhibition of *S*-adenosylhomocysteine hydrolase. Biochem. Biophys. Res. Commun. **1985**, 129, 306–311.
8. Cools, M.; De Clercq, E. Correlation between the antiviral activity of acyclic and carbocyclic adenosine analogues in murine L929 cells and their inhibitory effect on L929 cell *S*-adenosylhomocysteine hydrolase. Biochem. Pharmacol. **1989**, 38, 1061–1067.
9. Cools, M.; De Clercq, E. Influence of *S*-adenosylhomocysteine hydrolase inhibitors on *S*-adenosylhomocysteine and *S*-adenosylmethionine pool levels in L929 cells. Biochem. Pharmacol. **1990**, 40, 2259–2264.
10. De Clercq, E.; Cools, M.; Balzarini, J. Homocysteine potentiates the antiviral and cytostatic activity of those nucleoside analogues that are targeted at *S*-adenosylhomocysteine hydrolase. Biochem. Pharmacol. **1989**, 38, 1771–1778.
11. De Clercq, E.; Holý, A. Antiviral activity of aliphatic nucleoside analogues: Structure-function relationship. J. Med. Chem. **1979**, 22, 510–513.
12. De Clercq, E.; Holý, A. Alkyl esters of 3-adenin-9-yl-2-hydroxypropanoic acid: A new class of broad-spectrum antiviral agents. J. Med. Chem. **1985**, 28, 282–287.
13. De Clercq, E. Antiviral and antimetabolic activities of neplanocins. Antimicrob. Agents Chemother. **1985**, 28, 84–89.
14. De Clercq, E.; Cools, M.; Balzarini, J.; Marquez, V.E.; Borcherding, D.R.; Borchardt, R.T.; Drach, J.C.; Kitaoka, S.; Konno, T. Broad-spectrum antiviral activities of neplanocin A, 3-deazaneplanocin A, and their 5′-nor derivatives. Antimicrob. Agents Chemother. **1989**, 33, 1291–1297.

15. Tseng, C.K.H.; Marquez, V.E.; Fuller, R.W.; Goldstein, B.M.; Haines, D.R.; McPherson, H.; Parsons, J.L.; Shannon, W.M.; Arnett, G.; Hollingshead, M.; Driscoll, J.S. Synthesis of 3-deazaneplanocin A, a powerful inhibitor of *S*-adenosylhomocysteine hydrolase with potent and selective in vitro and in vivo antiviral activities. J. Med. Chem. **1989**, 32, 1442–1446.
16. Hasobe, M.; McKee, J.G.; Borcherding, D.R.; Borchardt, R.T. 9-(trans-2′,trans-3′-Dihydroxycyclopent-4′-enyl)-adenine and -3-deazaadenine: Analogs of neplanocin A which retain potent antiviral activity but exhibit reduced cytotoxicity. Antimicrob. Agents Chemother. **1987**, 31, 1849–1851.
17. Hasobe, M.; Liang, H.; Ault-Riche, D.B.; Borcherding, D.R.; Wolfe, M.S.; Borchardt, R.T. (1′*R*,2′*S*,3′*R*)-9-(2′,3′-dihydroxycyclopentan-1′-yl)-adenine and -3-deaza-adenine: Analogues of aristeromycin which exhibit potent antiviral activity with reduced cytotoxicity. Antiviral Chem. Chemother. **1993**, 4, 245–248.
18. Patil, S.D.; Schneller, S.W.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. Synthesis and antiviral properties of (±)-5′-noraristeromycin and related purine carbocyclic nucleosides. A new lead for anti-human cytomegalovirus agent design. J. Med. Chem. **1992**, 35, 3372–3377.
19. Siddiqi, S.M.; Chen, X.; Schneller, S.W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. Antiviral enantiomeric preference for 5′-noraristeromycin. J. Med. Chem. **1994**, 37, 551–554.
20. Siddiqi, S.M.; Chen, X.; Rao, J.; Schneller, S.W. 3-Deaza- and 7-deaza-5′-noraristeromycin and their antiviral properties. J. Med. Chem. **1995**, 38, 1035–1038.
21. Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. New neplanocin analogues. 1. Synthesis of 6′-modified neplanocin A derivatives as broad-spectrum antiviral agents. J. Med. Chem. **1992**, 35, 324–331.
22. Shuto, S.; Obara, T.; Saito, Y.; Yamashita, K.; Tanaka, M.; Sasaki, T.; Andrei, G.; Snoeck, R.; Neyts, J.; Padalko, E.; Balzarini, J.; De Clercq, E.; Matsuda, A. New neplanocin analogues. VIII. Synthesis and biological activity of 6′-*C*-ethyl-, -ethenyl, and -ethynyl derivatives of neplanocin A. Chem. Pharm. Bull. **1997**, 45, 1163–1168.
23. Shuto, S.; Obara, T.; Saito, Y.; Andrei, G.; Snoeck, R.; De Clercq, E.; Matsuda, A. New neplanocin analogues. 6. Synthesis and potent antiviral activity of 6′-homoneplanocin A. J. Med. Chem. **1996**, 39, 2392–2399.
24. Obara, T.; Shuto, S.; Saito, Y.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E.; Matsuda, A. New neplanocin analogues. 7. Synthesis and antiviral activity of 2-halo derivatives of neplanocin A. J. Med. Chem. **1996**, 39, 3847–3852.
25. Jeong, L.S.; Yoo, S.J.; Lee, K.M.; Koo, M.J.; Choi, W.J.; Kim, H.O.; Moon, H.R.; Lee, M.Y.; Park, J.G.; Lee, S.K.; Chun, M.W. Design, synthesis, and biological evaluation of fluoroneplanocin A as the novel mechanism-based inhibitor of *S*-adenosylhomocysteine hydrolase. J. Med. Chem. **2003**, 46, 201–203.
26. Marquez, V.E.; Lim, B.B.; Driscoll, J.S.; Snoeck, R.; Balzarini, J.; Ikeda, S.; Andrei, G.; De Clercq, E. Cyclopentene carbocyclic nucleosides related to the antitumor nucleoside clitocine and their conversion to 8-aza-neplanocin analogues. Synthesis and antiviral activity. J. Heterocyclic Chem. **1993**, 30, 1393–1398.
27. Cools, M.; Balzarini, J.; De Clercq, E. Mechanism of antiviral and cytotoxic action of (±)-6′*β*-fluoroaristeromycin, a potent inhibitor of *S*-adenosylhomocysteine hydrolase. Mol. Pharmacol. **1991**, 39, 718–724.
28. Madhavan, G.V.B.; McGee, D.P.C.; Rydzewski, R.M.; Boehme, R.; Martin, J.C.; Prisbe, E.J. Synthesis and antiviral evaluation of 6′-substituted aristeromycins: Potential mechanism-based inhibitors of *S*-adenosylhomocysteine hydrolase. J. Med. Chem. **1988**, 31, 1798–1804.
29. Siddiqi, S.M.; Chen, X.; Schneller, S.W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. An epimer of 5′-noraristeromycin and its antiviral properties. J. Med. Chem. **1994**, 37, 1382–1384.
30. Das, S.R.; Schneller, S.W.; Balzarini, J.; De Clercq, E. A mercapto analogue of 5′-noraristeromycin. Bioorg. Med. Chem. **2002**, 10, 457–460.
31. Robins, M.J.; Samano, V.; Zhang, W.; Balzarini, J.; De Clercq, E.; Borchardt, R.T.; Lee, Y.; Yuan, C.-S. Nucleic acid related compounds. 74. Synthesis and biological activity of 2′ (and 3′)-deoxy-2′ (and 3′)-methylenenucleoside analogues that function as mechanism-based inhibitors of *S*-adenosyl-L-homocysteine hydrolase and/or ribonucleotide reductase. J. Med. Chem. **1992**, 35, 2283–2293.

32. Wnuk, S.F.; Yuan, C.-S.; Borchardt, R.T.; Balzarini, J.; De Clercq, E.; Robins, M.J. Nucleic acid related compounds. 84. Synthesis of 6′-(*E* and *Z*)-halohomovinyl derivatives of adenosine, inactivation of *S*-adenosyl-L-homocysteine hydrolase, and correlation of anticancer and antiviral potencies with enzyme inhibition. J. Med. Chem. **1994**, *37*, 3579–3587.
33. Wnuk, S.F; Yuan, C.-S.; Borchardt, R.T.; Balzarini, J.; De Clercq, E.; Robins, M.J. Anticancer and antiviral effects and inactivation of *S*-adenosyl-L-homocysteine hydrolase with 5′-carboxaldehydes and oximes synthesized from adenosine and sugar-modified analogues. J. Med. Chem. **1997**, 40, 1608–1618.
34. Robins, M.J.; Wnuk, S.F.; Yang, X.; Yuan, C.-S.; Borchardt, R.T.; Balzarini, J.; De Clercq, E. Inactivation of *S*-adenosyl-L-homocysteine hydrolase and antiviral activity with 5′,5′,6′,6′-tetradehydro-6′-deoxy-6′-halohomoadenosine analogues (4-haloacetylene analogues derived from adenosine). J. Med. Chem. **1998**, 41, 3857–3864.
35. Wnuk, S.F.; Valdez, C.A.; Khan, J.; Moutinho, P.; Robins, M.J.; Yang, X.; Borchardt, R.T.; Balzarini, J.; De Clercq, E. Doubly homologated dihalovinyl and acetylene analogues of adenosine: Synthesis, interaction with *S*-adenosyl-L-homocysteine hydrolase, and antiviral and cytostatic effects. J. Med. Chem. **2000**, 43, 1180–1186.
36. Wnuk, S.F.; Mao, Y.; Yuan, C.-S.; Borchardt, R.T.; Andrei, G.; Balzarini, J.; De Clercq, E.; Morris, M.J. Discovery of type II (covalent) inactivation of *S*-adenosyl-L-homocysteine hydrolase involving its "hydrolytic activity": Synthesis and evaluation of dihalohomovinyl nucleoside analogues derived from adenosine. J. Med. Chem. **1998**, 41, 3078–3083.
37. Wnuk, S.F.; Ro, B.-O.; Valdez, C.A.; Lewandowska, E.; Valdez, N.X.; Sacasa, P.R.; Yin, D.; Zhang, J.; Borchardt, R.T.; De Clercq, E. Sugar-modified conjugated diene analogues of adenosine and uridine: Synthesis, interaction with *S*-adenosyl-L-homocysteine hydrolase, and antiviral and cytostatic effects. J. Med. Chem. **2002**, 45, 2651–2658.
38. Wolfe, M.S.; Borchardt, R.T. S-Adenosyl-L-homocysteine hydrolase as a target for antiviral chemotherapy. J. Med. Chem. **1991**, 34, 1521–1530.
39. Yuan, C.-S.; Liu, S.; Wnuk, S.F.; Robins, M.J.; Borchardt, R.T. Design and synthesis of S-adenosylhomocysteine hydrolase inhibitors as broad-spectrum antiviral agents. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press Inc.: Greenwich, CT, Vol. 2., 1996; 41–88.
40. McCarthy, J.R.; Jarvi, E.T.; Matthews, D.P.; Edwards, M.L.; Prakash, N.J; Bowlin, T.L.; Mehdi, S.; Sunkara, P.S.; Bey, P. 4′,5′-Unsaturated 5′-fluoroadenosine nucleosides: Potent mechanism-based inhibitors of S-adenosyl-L-homocysteine hydrolase. J. Am. Chem. Soc. **1989**, 111, 1127–1128.
41. De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R.T.; Jones, A.S.; Torrence, P.F.; Shugar, D. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. **1980**, 141, 563–574.
42. Sacks, S.L.; Merigan, T.C.; Kaminska, J.; Fox, I.H. Inactivation of *S*-adenosylhomocysteine hydrolase during adenine arabinoside therapy. J. Clin. Invest. **1982**, 69, 226–230.
43. Giziewicz, J.; De Clercq, E.; Luczak, M.; Shugar, D. Antiviral and antimetabolic activities of formycin and its N_1-, N_2-, 2′-*O*- and 3′-*O*-methylated derivatives. Biochem. Pharmacol. **1975**, 24, 1813–1817.
44. Van Aerschot, A.; Mamos, P.; Weyns, N.J.; Ikeda, S.; De Clercq, E.; Herdewijn, P. Antiviral activity of C-alkylated purine nucleosides obtained by cross-coupling with tetraalkyltin reagents. J. Med. Chem. **1993**, 36, 2938–2942.
45. Sági, G.; Ötvös, L.; Ikeda, S.; Andrei, G; Snoeck, R.; De Clercq, E. Synthesis and antiviral activities of 8-alkynyl-, 8-alkenyl-, and 8-alkyl-2′-deoxyadenosine analogues. J. Med. Chem. **1994**, 37, 1307–1311.
46. Borchardt, R.T.; Keller, B.T.; Patel-Thombre, U. Neplanocin A. A potent inhibitor of *S*-adenosylhomocysteine hydrolase and of vaccinia virus multiplication in mouse L929 cells. J. Biol. Chem. **1984**, 259, 4353–4358.
47. Hasobe, M.; McKee, J.G.; Ishii, H.; Cools, M.; Borchardt, R.T.; De Clercq, E. Elucidation of the mechanism by which homocysteine potentiates the anti-vaccinia virus effects of the *S*-adenosyl-homocysteine hydrolase inhibitor 9-(*trans*-2′,*trans*-3′-dihydroxycyclopent-4′-enyl)-adenine. Mol. Pharmacol. **1989**, 36, 490–496.
48. Ault-Riché, D.B.; Lee, Y.; Yuan, C.-S.; Hasobe, M.; Wolfe, M.S.; Borcherding, D.R.; Borchardt, R.T. Effects of 4′-modified analogs of aristeromycin on the metabolism of *S*-adenosyl-L-homocysteine in murine L929 cells. Mol. Pharmacol. **1993**, 43, 989–997.

49. Huang, Y.; Komoto, J.; Takata, Y.; Powell, D.R.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. Inhibition of *S*-adenosylhomocysteine hydrolase by acyclic sugar adenosine analogue D-eritadenine. J. Biol. Chem. **2002**, 277, 7477–7482.
50. Kim, H.O.; Yoo, S.J.; Ahn, H.S.; Choi, W.J.; Moon, H.R.; Lee, K.M.; Chun, M.W.; Jeong, L.S. Synthesis of fluorinated cyclopentenyladenine as potent inhibitor of *S*-adenosylhomocysteine hydrolase. Bioorg. Med. Chem. Lett. **2004**, 14, 2091–2093.
51. Chu, C.K.; Jin, Y.H.; Baker, R.O.; Huggins, J. Antiviral activity of cyclopentenyl nucleosides against orthopox viruses (smallpox, monkeypox and cowpox). Bioorg. Med. Chem. Lett. **2003**, 13, 9–12.
52. Andrei, G.; De Clercq, E. Inhibitory effect of selected antiviral compounds on arenavirus replication in vitro. Antiviral Res. **1990**, 14, 287–300.
53. Villalón, M.D.G.; Gil-Fernández, C.; De Clercq, E. Activity of several *S*-adenosylhomocysteine hydrolase inhibitors against African swine fever virus replication in Vero cells. Antiviral Res. **1993**, 20, 131–144.
54. Snoeck, R.; Andrei, G.; Neyts, J.; Schols, D.; Cools, M.; Balzarini, J.; De Clercq, E. Inhibitory activity of *S*-adenosylhomocysteine hydrolase inhibitors against human cytomegalovirus replication. Antiviral Res. **1993**, 21, 197–216.
55. Kitaoka, S.; Konno, T.; De Clercq, E. Comparative efficacy of broad-spectrum antiviral agents as inhibitors of rotavirus replication in vitro. Antiviral Res. **1986**, 6, 57–65.
56. Shigeta, S.; Mori, S.; Baba, M.; Ito, M.; Honzumi, K.; Nakamura, K.; Oshitani, H.; Numazaki, Y.; Matsuda, A.; Obara, T.; Shuto, S.; De Clercq, E. Antiviral activities of ribavirin, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide, and 6′-(*R*)-6′-*C*-methylneplanocin A against several ortho- and paramyxoviruses. Antimicrob. Agents Chemother. **1992**, 36, 435–439.
57. Barnard, D.L.; Stowell, V.D.; Seley, K.L.; Hegde, V.R.; Das, S.R.; Rajappan, V.P.; Schneller, S.W.; Smee, D.F.; Sidwell, R.W. Inhibition of measles virus replication by 5′-nor carbocyclic adenosine analogues. Antiviral Chem. Chemother. **2001**, 12, 241–250.
58. Hosoya, M.; Shigeta, S.; Nakamura, K.; De Clercq, E. Inhibitory effect of selected antiviral compounds on measles (SSPE) virus replication in vitro. Antiviral Res. **1989**, 12, 87–98.
59. Kawana, F.; Shigeta, S.; Hosoya, M.; Suzuki, H.; De Clercq, E. Inhibitory effects of antiviral compounds on respiratory syncytial virus replication in vitro. Antimicrob. Agents Chemother. **1987**, 31, 1225–1230.
60. Mayers, D.L.; Mikovits, J.A.; Joshi, B.; Hewlett, I.K.; Esrada, J.S.; Wolfe, A.D.; Garcia, G.E.; Doctor, B.P.; Burke, D.S.; Gordon, R.K.; Lane, J.R.; Chiang, P.K. Anti-human immunodeficiency virus 1 (HIV-1) activities of 3-deazaadenosine analogs: Increased potency against 3′-azido-3′-deoxythymidine-resistant HIV-1 strains. Proc. Natl. Acad. Sci. USA **1995**, 92, 215–219.
61. Gordon, R.K.; Ginalski, K.; Rudnicki, W.R.; Rychlewski, L.; Pankaskie, M.C.; Bujnicki, J.M.; Chiang, P.K. Anti-HIV-1 activity of 3-deaza-adenosine analogs. Inhibition of *S*-adenosylhomocysteine hydrolase and nucleotide congeners. Eur. J. Biochem. **2003**, 270, 3507–3517.
62. Daelemans, D.; Esté, J.A.; Witvrouw, M.; Pannecouque, C.; Jonckheere, H.; Aquaro, S.; Perno, C.-F.; De Clercq, E.; Vandamme, A.-M. *S*-Adenosylhomocysteine hydrolase inhibitors interfere with the replication of human immunodeficiency virus type 1 through inhibition of the LTR transactivation. Mol. Pharmacol. **1997**, 52, 1157–1163.
63. Chiu, Y.-L.; Ho, C.K.; Saha, N.; Schwer, B.; Shuman, S.; Rana, T.M. Tat stimulates cotranscriptional capping of HIV mRNA. Mol. Cell **2002**, 10, 585–597.
64. Neyts, J.; Meerbach, A.; McKenna, P.; De Clercq, E. Use of the yellow fever virus vaccine strain 17D for the study of strategies for the treatment of yellow fever virus infections. Antiviral Res. **1996**, 30, 125–132.
65. Shigeta, S.; Konno, K.; Yokota, T.; Nakamura, K.; De Clercq, E. Comparative activities of several nucleoside analogs against influenza A, B, and C viruses in vitro. Antimicrob. Agents Chemother. **1988**, 32, 906–911.
66. Cantoni, G.L.; Scarano, E.J. The formation of *S*-adenosylhomocysteine in enzymatic transmethylation reactions. Am. Chem. Soc. **1954**, 76, 4744.
67. De La Haba, G.; Cantoni, G.L. The enzymatic synthesis of *S*-adenosyl-L-homocysteine from adenosine and homocysteine. J. Biol. Chem. **1959**, 234, 603–608.
68. Baric, I.; Fumic, K.; Glenn, B.; Cuk, M.; Schulze, A.; Finkelstein, J.D.; James, S.J.; Mejaski-Bosnjak, V.; Pazanin, L.; Pogribny, I.P.; Rados, M.; Sarnavka, V.; Scukanec-Spoljar, M.; Allen, R.H.; Stabler, S.; Uzelac, L., Vugrek, O.; Wagner, C.; Zeisel, S.; Mudd, S.H. *S*-Adenosylhomocysteine hydrolase

deficiency in a human: A genetic disorder of methionine metabolism. Proc. Natl. Acad. Sci. USA **2004**, 101, 4234–4239.
69. Ault-Riché, D.B.; Yuan, C.-S.; Borchardt, R.T. A single mutation at lysine 426 of human placental *S*-adenosylhomocysteine hydrolase inactivates the enzyme. J. Biol. Chem. **1994**, 269, 31472–31478.
70. Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. Effects of site-directed mutagenesis on structure and function of recombinant rat liver *S*-adenosylhomocysteine hydrolase. J. Biol. Chem. **2000**, 275, 32147–32156.
71. Takata, Y.; Yamada, T.; Huang, Y.; Komoto, J.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. Catalytic mechanism of *S*-adenosylhomocysteine hydrolase. Site-directed mutagenesis of Asp-130, Lys-185, Asp-189, and Asn-190. J. Biol. Chem. **2002**, 277, 22670–22676.
72. Parker, N.B.; Yang, X.; Hanke, J.; Mason, K.A.; Schowen, R.L.; Borchardt, R.T.; Yin, D.H. *Trypanosoma cruzi*: Molecular cloning and characterization of the *S*-adenosylhomocysteine hydrolase. Exp. Parasitol. **2003**, 105, 149–158.
73. Seley, K.L.; Schneller, S.W.; De Clercq, E.; Rattendi, D.; Lane, S.; Bacchi, C.J.; Korba, B. The importance of the 4′-hydroxyl hydrogen for the anti-trypanosomal and antiviral properties of (+)-5′-noraristeromycin and two 7-deaza analogues. Bioorg. Med. Chem. **1998**, 6, 797–801.
74. Kitade, Y.; Kojima, H.; Zulfiqur, F.; Kim, H.-S.; Wataya, Y. Synthesis of 2-fluoronoraristeromycin and its inhibitory activity against *Plasmodium falciparum S*-adenosyl-L-homocysteine hydrolase. Bioorg. Med. Chem. Lett. **2003**, 13, 3963–3965.
75. Thong, K.-W.; Coombs, G.H.; Sanderson, B.E. *S*-Adenosylhomocysteine hydrolase activity in *Trichomonas vaginalis* and other trichomonads. Mol. Biochem. Parasitol. **1985**, 17, 35–44.
76. Henderson, D.M.; Hanson, S.; Allen, T.; Wilson, K.; Coulter-Karis, D.E.; Greenberg, M.L.; Hershfield, M.S.; Ullman, B. Cloning of the gene encoding *Leishmania donovani S*-adenosylhomocysteine hydrolase, a potential target for antiparasitic chemotherapy. Mol. Biochem. Parasitol. **1992**, 53, 169–184.
77. Yang, X.; Borchardt, R.T. Overexpression, purification, and characterization of *S*-adenosylhomocysteine hydrolase from *Leishmania donovani*. Arch. Biochem. Biophys. **2000**, 383, 272–280.
78. Bray, M.; Driscoll, J.; Huggins, J.W. Treatment of lethal Ebola virus infection in mice with a single dose of an *S*-adenosyl-L-homocysteine hydrolase inhibitor. Antiviral Res. **2000**, 45, 135–147.
79. Bray, M.; Raymond, J.L.; Geisbert, T.; Baker, R.O. 3-Deazaneplanocin A induces massively increased interferon-α production in Ebola virus-infected mice. Antiviral Res. **2002**, 55, 151–159.
80. Palmer, J.L.; Abeles, R.H. The mechanism of action of *S*-adenosylhomocysteine. J. Biol. Chem. **1979**, 254, 1217–1226.
81. Chiang, P.K.; Richards, H.H.; Cantoni, G.L. *S*-Adenosyl-L-homocysteine hydrolase: Analogues of *S*-adenosyl-L-homocysteine as potential inhibitors. Mol. Pharmacol. **1977**, 13, 939–947.
82. Borchardt, R.T. *S*-Adenosyl-L-methionine-dependent macromolecule methyltransferases: Potential targets for the design of chemotherapeutic agents. J. Med. Chem. **1980**, 23, 347–357.
83. De Clercq, E. Antivirals and antiviral strategies. Nature Rev. Microbiol. **2004**, 2, 704–720.