Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1649–1652

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Inactivation of *S*-Adenosyl-L-Homocysteine Hydrolase with Novel 5′-Thioadenosine Derivatives. Antiviral Effects

Georges Guillerm,[a,*] Danielle Guillerm,[a] Corinne Vandenplas-Vitkowski,[a] Cédric Glapski[a] and Erick De Clercq[b]

[a]*Laboratoire de Chimie Bioorganique, UMR 6519, UFR Sciences, B.P. 1039, 51687 Reims cedex 2, France*
[b]*Rega Institute for Medicinal Research, Katolieke Universiteit of Leuven, Belgium*

Received 6 January 2003; revised 11 March 2003; accepted 13 March 2003

**Abstract**—Synthesis of 5′-*S*-vinyl-5′-thioadenosine **5**, 5′-*S*-ethynyl-5′-thioadenosine **7** and 5′-*S*-cyano-5′-thioadenosine **9** is described. Incubation of AdoHcy hydrolase with **5**, **7** and **9** resulted in time- and concentration-dependent inactivation of the enzyme and partial depletion of its $NAD^+$ content. From these results and characterisation of metabolites released during the inactivation process, hypothetical mechanisms are suggested. The antiviral activity of **5**, **7** and **9** was examined. Significant activities were noted with **5** against Vaccinia, Junin and Taccaribe viruses.


## Introduction

*S*-adenosyl homocysteine (AdoHcy) hydrolase catalyses the interconversion of *S*-adenosyl-homocysteine into adenosine and L-homocysteine.[1] Inhibition of this enzyme results in intra cellular accumulation of AdoHcy which in turn provokes feed back inhibition of *S*-adenosylmethionine-dependent methylation reactions (i.e., viral mRNA methylation) which are essential for viral replication.[2] Therefore, AdoHcy hydrolase has become an attractive target for the molecular design of anti-viral agents.[3,4]

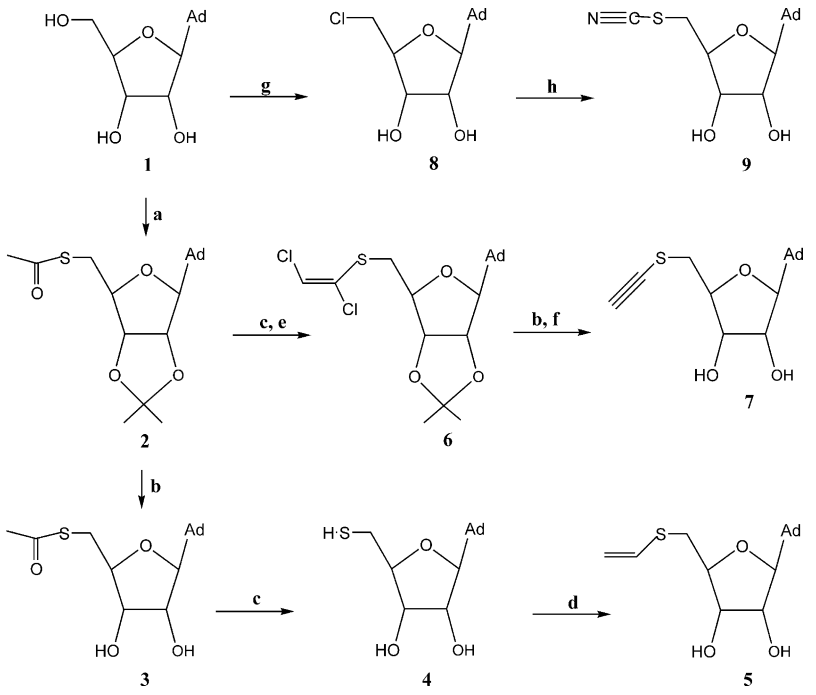
A number of inhibitors (designed type I) has been identified which act upon the 3′-oxidative activity of AdoHcy hydrolase irreversibly locking it in its closed inactive NADH form.[5,6] A second type of mechanism-based inhibitors (type II) also act upon the 3′-oxidative activity of the enzyme and/or its 'hydrolytic activity' to generate an electrophilic site on the inhibitor which can then covalently bind to an active site nucleophile.[7–11] We recently found that a series of 5′-thioadenosine analogues substituted at sulfur with allenyl and propynyl groups were substrates of the hydrolytic activity of the enzyme and caused type II covalent inhibition.[12] This result led us to consider that other 5′-thionucleosides such as 5′-*S*-vinyl-5′-thioadenosine **5**, 5′-*S*-ethynyl-5′-thioadenosine **7** and 5′-*S*-cyano-5′-thioadenosine **9** might be good candidates as new covalent mechanism-based inhibitors. We now describe the synthesis of these thionucleosides, their interaction with AdoHcy hydrolase and their antiviral activities.

## Chemistry

The general synthetic procedure used for the preparation of **5**, **7** and **9** was as follows (Scheme 1). The readily available adenosine derivative 5′-acetylthio-5′-deoxy-2′,3′-*O*-isopropylidene adenosine **2**[12,13] was chosen as the intermediate for the generation of 5′-thioadenosine thiolate anion in the synthetic route leading to **5** and **7**. The acetonide group of **2** was removed in aqueous formic acid and the resulting 5′-acetylthio-adenosine **3** was then hydrolysed using a mixture of $MeOH/H_2O$ saturated with ammonia, under oxygen free conditions to yield the 5′-thioadenosine **4**. Without further purification a suspension of **4** in DMF was bubbled with acetylene under irradiation[14] to afford **5** in 29% yield, after purification. In order to access the yne thiol ester **7** we adapted a previously described general procedure.[15] Condensation of trichloroethylene in THF (−50 °C) on the thiolate anion generated from **2** led to **6** in a 40% yield. Removal of the isopropylidene protective group

*Corresponding author. Tel.: +33-326-913-308; fax: +33-326-913-166; e-mail: georges.guillerm@univ-reims.fr

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00279-8




**Scheme 1.** (a) Ref 12,13; (b) 80% $HCO_2H/H_2O$, 40 °C, 90%; (c) $NH_3$, $MeOH/H_2O$, lyophilisation, 90%; (d) $C_2H_2$ in DMF; tungsten lamp (500 W, Pyrex), rt, 29%; (e) THF, NaH oil free, $CCl_2=CHCl$, εMeOH, 40%; (f) THF, −50 °C, 5 equiv, *n*-BuLi (1.6 M hexane, 58%; (g) ref 16; (h) KSCN, DMF, 105°, 24 h, 60%.

of **6**, followed by treatement of the corresponding intermediate with an excess of *n*-butyllithium in THF at −50 °C resulted in the formation of **6** in a 58% yield. Because of their acidic instability in $HCO_2H/H_2O$, **5** and **7** had to be prepared from their deprotected precursors **4** and **6**. The 5′-thiocyanotoadenosine **9** was prepared from the corresponding 5′-chloroadenosine[16] in a 60% yield.

## Results and Discussion

### Inactivation of AdoHcy hydrolase

Recombinant human placental AdoHcy hydrolase purified to homogeneity[17] was used in this study. The synthetic activity of the enzyme at a concentration of 6 nM was assayed in the presence of [8-$^{14}$C]-AdoHcy (100 mM, 300 Bq) and Hcy (5 mM) in 200 mM potassium phosphate buffer pH 7.5, 1 mM EDTA (Buffer A).

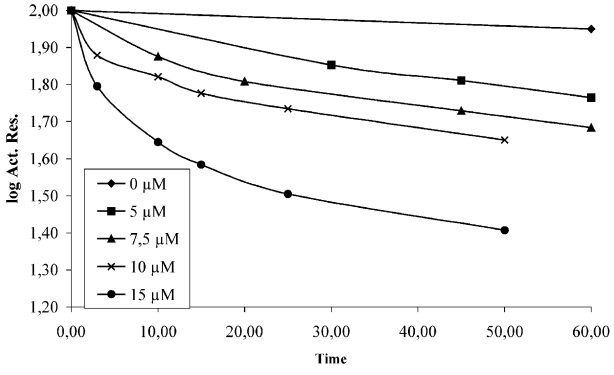



**Figure 1.** Time-dependent inactivation of AdoHcy hydrolase with **5**. AdoHcy hydrolase (6 µM) was incubated with inhibitor at concentration 5–15 µM in buffer A at 37°. At the indicated time points, residual activity was determined as described.

Incubation of the enzyme with **5**, **7** and **9** resulted in time- and concentration-dependent inactivation of the enzyme as shown in Figure 1 for **5**. The inactivation rate for **5**, **7** and **9** were curvilinear showing pseudo-first-order kinetics only in the first period of inactivation. This suggested that more than one mechanism could be involved in the inactivation process.[12,18]

Using the Kitz and Wilson method[19] a double reciprocal plot of the initial pseudo-first order inactivation rate constant ($1/K_{app}$) versus I/[I] gave the $K_i$ and $k_{inact}$ values listed in Table 1.

The effect of **5**, **7** and **9** on the $NAD^+$/NADH content were determined after complete inactivation of the enzyme. Partial changes in the initial enzyme's $NAD^+$ content were observed (Table 2).

Upon complete inactivation of AdoHcy hydrolase (20 µM) with 600 µM of **5**, **7** and **9** in ammonium acetate Buffer pH 7 and removal of the enzyme by ultrafiltration, the reaction products were analysed by LC/ESI-

**Table 1.** $K_i$ and $k_{inact}$ values for the inhibitory effects of **5**, **7** and **9** on AdoHcy hydrolase

| Compd | $K_i$ (µM) | $k_{inact}$ ($min^{-1}$) |
|---|---|---|
| **5** | 4 | 0.07 |
| **7** | 10 | 0.12 |
| **9** | 67 | 0.59 |

**Table 2.** Change in the enzyme's $NAD^+$ content until complete inactivation

| Compd | 5 | 7 | 9 |
|---|---|---|---|
| $NAD^+$% | 18 | 36 | 16 |

AdoHcy hydrolase (20 μM) was incubated with 600 μM of **5**, **7** and **9** in buffer A. $NAD^+$ and NADH present in enzyme before and after inactivation were measured by a fluorescence method.[23]

MS. The chromatographic step was performed using a Spherisob C18 column (250×4.6 mm, Interchrom) and a MeOH/$H_2O$ mobile phase. Elution of the reduction products was achieved using an increasing concentration of MeOH (40–60%) at a flow rate of 0.8 mL/min. In these experiments, in addition to the residual inhibitors **5**, **7** and **9** which were used in large excess, new metabolites were detected and identified by their molecular mass ($MH^+$) and their retention time (rt) which were compared with authentics. For compound **5**, 5′-thioadenosine *m*/*z* 271 (rt 6.2 min) and its corresponding disulfide, di-[adenosyl-(5′)]-disulfide *m*/*z* 565 (rt 20 min) were identified as metabolites. Likewise, for compound **7**, 5′-acetylthioadenosine *m*/*z* 325 (rt 8.5 min) production was accompanied by the generation of di-[adenosyl-(5′)]-disulfide. In contrast, the reaction in the presence of compound **9** gave rise to a single detectable metabolite, di-[adenosyl-(5′)]-disulfide which was very likely generated in situ from the corresponding 5′-thioadenosine.

The partial depletion of the enzyme's $NAD^+$ observed suggests that the main pathway by which compounds **5**, **7** and **9** proceed to inactivate AdoHcy hydrolase does not involve the single enzyme's oxidative activity (type I mechanism). Taking in account the nature of the products released in solution during the inactivation process, a second mode of inactivation involving covalent modification with products arising from the enzyme's hydrolytic activity has to be considered.

Compound **5** can be easily transformed into 5′-thioadenosine by acid treatement (aqueous formic acid). This observation strongly suggests that generation of 5′-thioadenosine could result from the enzyme-catalysed hydrolysis of **5**. In this case, the newly formed thiol intermediate could form a disulfide bond with an active site cysteine[20,21] which would lead to inactivation of the enzyme (Scheme 2, Pathway A).

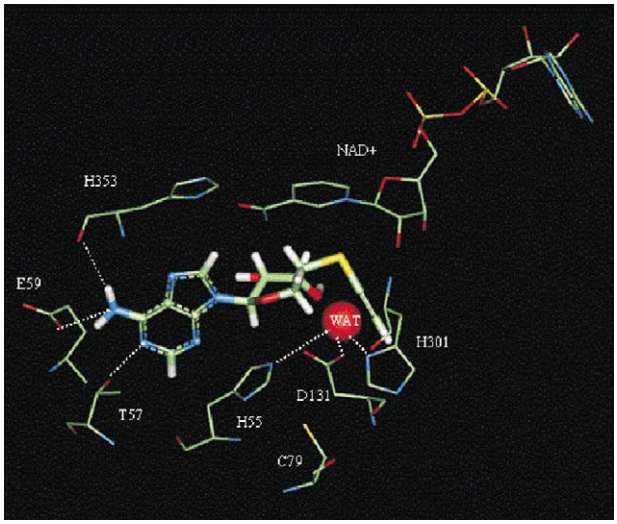
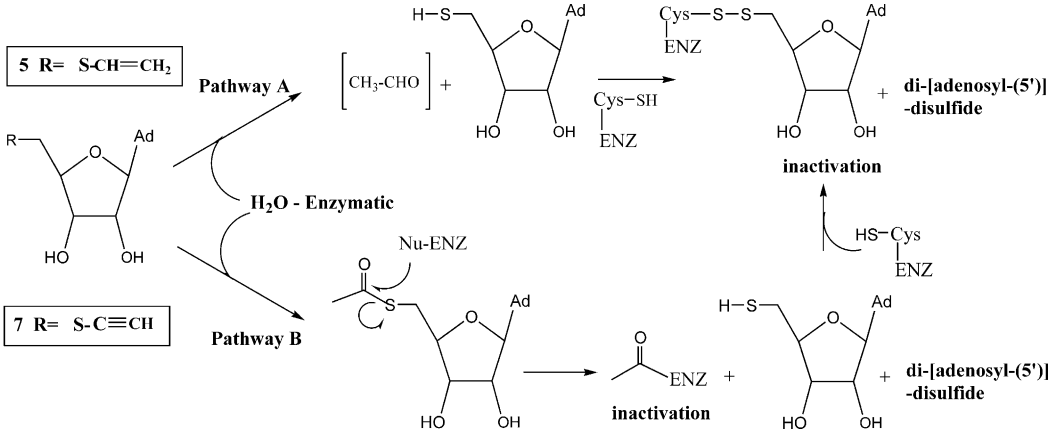



**Figure 2.** Compound **7** (colored by atom type) docked into the active site of AdoHcy hydrolase.[24] For clarity, only the residues providing the main hydrogen bond interactions and the $NAD^+$ molecule are shown. Hydrogen bonds are represented as dotted lines. The proximity of water molecule (red sphere) to the ethynyl moiety of the inhibitor strongly suggests the possibility of a reaction with water to give the 5′-thioacetyladenosine (Scheme 2, Pathway B).

Different pathways might be proposed for inhibitor **7**. Addition of the enzyme sequestered water (Fig. 2) to the *S*-ethynyl group of **7** could generate 5′-acetylthioadenosine. attack of this reactive thioester by amino functionalities could cause type II covalent inhibition (Scheme 2, Pathway B). Such a pathway has been proposed to explain the specific and covalent labelling of the enzyme with 5′-*S*-allenyl-5′-thioadenosine.[12]

However, we cannot ruled out the possibility of covalent modification of an active site cysteine by the 5′-thioadenosine generated in this process, as described above. In theory, enzyme mediated addition of water to




**Scheme 2.** Proposed mechanism for the conversion of **5** and **9** by AdoHcy hydrolase.

the thiocyano group of **9** could also produce 5′-thioadenosine which would lead to the formation of a similar covalent adduct upon enzyme inactivation. Evidence to support this second mechanistic proposal comes from inactivation experiments carried out with 5′-thioadenosine. These show that inhibition of the enzyme is accompanied by the creation of a specific covalently-linked enzyme adduct (the mass of on each AdoHcy hydrolase subunit is increased by 269 Da; ESI/MS analysis of inactivated enzyme).[22]

## Antiviral Activity

Compounds **5**, **7** and **9** were examined for their cytotoxicity and antiviral activity in a variety of antiviral tests. Cytotoxicity was evaluated by determining the minimum concentration required to cause a microscopically detectable alteration of normal cell morphology (MTC value). In human embryonic skin muscle ($E_6SM$) cell cultures, **7** was the most cytotoxic (MTC = 30 μM) while **5** and **9** presented much lower values (MTC > 650 μM). In antiviral tests, no activity was noted against HSV-I (KOS), HSV-2 (G) and vesicular stomatitis virus at subtoxic concentration except for compound **5** against vaccinia virus (inhibitory concentration to reduce virus-induced cytopathogenicity by 50%, MIC = 30μM). In vero cell cultures, with the exception of compound **5** (MTC > 650 μM) which exhibited significant activity against Junin and Taccaribe viruses (MIC: 65 μM and 40 μM respectively), the compounds showed no antiviral activity against Parainfluenza-3, Sindbis, Coxakie $B_4$, Punta Toro virus. No specific antiviral activity was noted against cytomegaloviruses (CMV, Strain AD-169 and Davis) in human embryonic lung (HEL) cells.

## Conclusion

The preliminary evaluation of a series of 5′-thionucleosides has provided encouraging results that warrant further mechanistic investigations. Additional studies are in progress using ESI-MS and nano ESI-$MS^n$ techniques to confirm the proposed mechanisms and to elucidate the localisation of the possible covalent linkage induced by AdoHcy hydrolase with **5**, **7** and **9**.

## Acknowledgements

The authors thank Dr. Christine Lambert (AstraZeneca-Reims, Centre de Recherches) for the molecular modeling work.

## References and Notes

1. Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1979**, *254*, 1217.
2. Narayan, P.; Rottman, F. M. In *Advances in Enzymology*; Meister, A., Ed.; John Wiley & Sons Inc: New York, 1994; pp 255–285.
3. Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. In *Advances in Antiviral Drug Design*; De Clerq, E., Ed.; 1996, pp 41–88.
4. Yin, D.; Yang, X.; Borchardt, R. T.; Yuan, C.-S. In *Biomedical Chemistry: Applying Chemical Principles to the Understanding and Treatement of Disease*; Torrence, P. F., Ed.; John Wiley & Sons, Inc: New York, 2000; pp 41–71.
5. Yuan, C.-S.; Lin, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. *Nucleosides Nucleotides* **1995**, *14*, 439.
6. Yin, D.; Yan, X.; Hu, Y.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T.; Squier, T. C. *Biochemistry* **2000**, *39*, 9811.
7. Parry, R. J.; Muscate, A.; Askonas, L. J. *Biochemistry* **1991**, *30*, 9988.
8. Wnuk, S. F.; MaO, Y.; Yuan, C.-S.; Borchardt, R. T.; Andrei, G.; Balzanni, S.; De Clerq, E.; Robins, M. J. *J. Med. Chem.* **1998**, *41*, 3078.
9. Yuan, C.-S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. *J. Biol. Chem.* **1998**, *273*, 18191.
10. Yang, X.; Wnuk, S. F.; Robins, M. F.; Borchardt, R. T. *Biochemistry* **2000**, *39*, 15234.
11. Wnuk, S. F. *Mini-Reviews in Medicinal Chemistry* **2001**, *1*, 307.
12. Guillerm, G.; Guillerm, D.; Vandenplas-Witkowski, C.; Rogniaux, H.; Carte, N.; Leize, E.; Van Dorsselaer, A.; De Clerq, E.; Lambert, C. *J. Med. Chem.* **2001**, *44*, 2743.
13. Pignot, M.; Pljevaljcic, G.; Weinhold, E. *Eur. J. Org. Chem* **2000**, 549.
14. Leopold, W. R.; Miller, J. A.; Miller, E. C. *Cancer Res.* **1982**, *42*, 4364.
15. Nebois, P.; Kann, N.; Greene, A. E. *J. Org. Chem.* **1995**, *60*, 7690.
16. Borchardt, R. T.; Huber, J. A.; Wu, Y. S. *J. Org. Chem.* **1976**, *41*, 565.
17. Yuan, C. S.; Yeh, J.; Borchardt, R. T. *J. Biol. Chem.* **1983**, *268*, 17030.
18. Yuan, C. S.; Wnuk, S. F.; Lin, S.; Robins, M. J.; Borchardt, R. T. *Biochemistry* **1994**, *33*, 12305.
19. Kitz, K.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.
20. Yuan, C. S.; Ault-Riché, D. B.; Borchardt, R. T. *J. Biol. Chem.* **1996**, *271*, 28009.
21. Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochemistry* **1999**, *38*, 8323.
22. Glapski, C.; Muzard. M.; Pillard. S.; Guillerm, G. Unpublished results.
23. Hohman, R. J.; Guitton, M. C.; Veron, M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4578.
24. The structure of AdoHcy has been extracted from the Brookhaven Data base (PDB entry 1A7A). Inhibitor **7** has been built with the molecular modeling software Quanta98. Its geometry has been optimized using CHARm force field.[25]
25. Momany, F. A.; Rone, R. *J. Comput. Chem.* **1992**, *13*, 888.