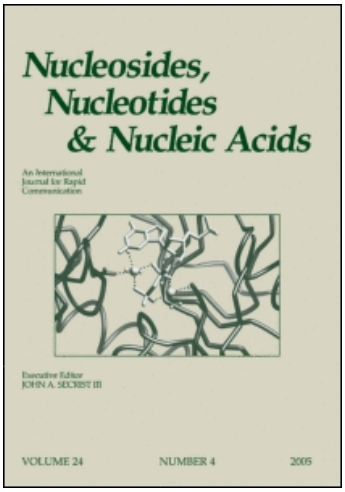

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INACTIVATION OF *S*-ADENOSYL-L-HOMOCYSTEINE HYDROLASE WITH FLUORINATED ANALOGS OF 2′- AND 3′-DEOXY-5′-METHYLTHIOADENOSINE

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ABSTRACT

Fluorinated analogs of 2′- and 3′-deoxy-5′-methylthioadenosine **1**–**4** caused irreversible inactivation of AdoHcy hydrolase. Based on the ESI-Mass spectra analysis of the inactivated enzyme with the fluorinated analog **1** a mechanism of inactivation is proposed.

The cellular enzyme S-adenosyl-L-homocysteine (AdoHcy) hydrolase has emerged as a target for molecular design of antiviral agents because of its important role in regulating *S*-adenosyl-L-methionine-dependent methylation reactions (1).

We have recently found that 5′-deoxy-5′-difluoromethylthio-adenosine (DFMTA) and 5′-deoxy-5′-trifluomethylthioadenosine (TFMTA) were potent mechanism-based inactivators of AdoHcy hydrolase (2). In order to clarify the mechanism and the binding mode of this new series of inhibitors, we prepared their 2′- and 3′-deoxy analogs **1**–**4** (Fig. 1), for assays on AdoHcy hydrolase activity.

3′-deoxy-5′-deoxy-5′-*S*-difluoromethyl-5′-thioadenosine **1** and 2′-deoxy-5′-deoxy-5′-*S*-difluoromethyl-5′-thioadenosine **2**,3′-deoxy-5′-deoxy-5′-*S*-trifluoromethyl-5′thioadenosine **3** and 2′-deoxy-5′-deoxy-5′-*S*-trifluoromethyl-5′-thioadenosine **4** have been synthesised in two steps (3) from their corresponding fluorinated analogs DFMTA and TFMTA, already prepared by us (4). **1** and **2**,

*Corresponding author.

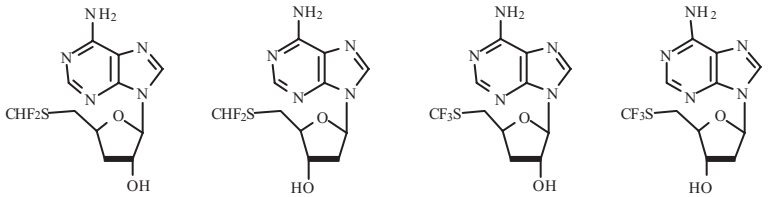
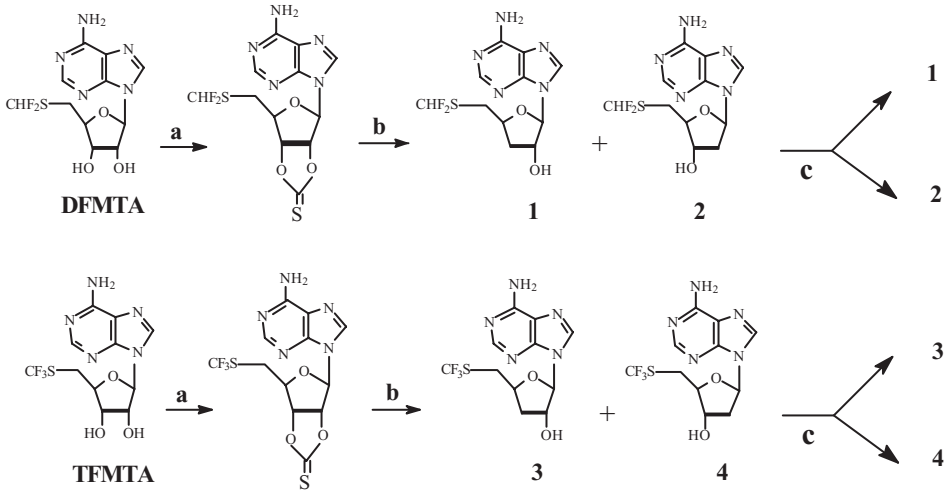


Figure 1.

3 and **4** were easily separed as described in Scheme 1 and fully identified from their ^{1}H NMR Spectra (5).

1, **2** and **3**, **4** were tested on the activity of recombinant human placental AdoHcy hydrolase purified to homogenity as previously reported (6). AdoHcy hydrolase activity was assayed in the direction of AdoHcy synthesis using (8-^{14}C)-Adenosine (7). **1**–**4** derivatives caused time-dependent and irreversible inactivation of the enzyme. The Kitz and Wilson method (8) was used for kinetic constants determination. (Table 1).

These results indicated that **1**, **2**, **3** and **4** are weaker inhibitors than the corresponding parent DMFTA and TFMTA (2), showing the importance of 2′- and 3′-hydroxyl groups for proper binding with AdoHcy hydrolase as previously reported (9). Upon complete inactivation of AdoHcy hydrolase by fluorinated nucleosides **1**–**4**, the reaction products were analysed by HPLC/MS. No traces of dehydronucleosides **5** or **6** (Scheme 2), used as authentics, were detected.



Scheme 1. a) 1,1′-thiocarbonyldiimidazole, DMF, 60% b) nBu_3SnH, AIBN, toluene (reflux) 45% c) Flash-column chromatography (Merck Kieselgel 60/230-400 mesh): AcOEt/MeOH 9/1.

Table 1.

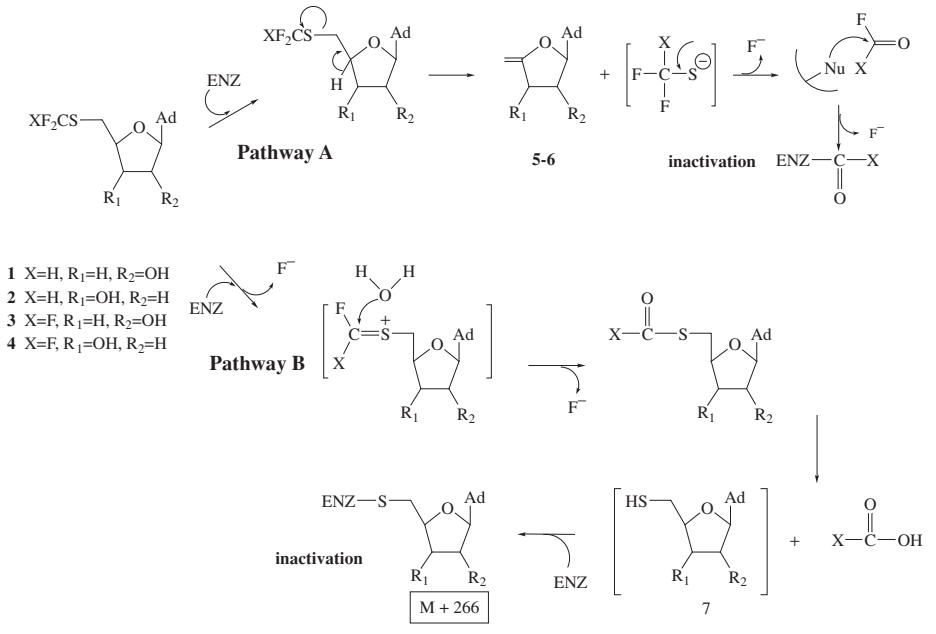
Compounds	Ki (μM)	K_{inact} (min^{-1})
3′-deoxy-DFMTA **1**	95	$1.8\ 10^{-2}$
2′-deoxy-DFMTA **2**	55	$1\ 10^{-2}$
3′-deoxy-TFMTA **3**	400	$2.5\ 10^{-2}$
2′-deoxy-DFMTA **4**	190	$1.4\ 10^{-2}$

NAD^+/NADH content was not modified after incubation of AdoHcy hydrolase with inactivators **2** and **4** (data not shown).

The mechanism of inactivation was further investigated using the difluoromethyl derivatives **1** and **2**. Inactivation of AdoHcy hydrolase with **1** and **2** was accompanied by release of fluorine ion (measured by ^{19}F NMR spectroscopy). When an 8 fold excess of **1** or **2** per mole of enzyme subunit was incubated with purified AdoHcy hydrolase, unreacted **1** and **2** were still present after complete inactivation of the enzyme with the release of # 1.8 mole of fluorine ion per mole of inactivated enzyme.

The mechanism of inactivation by inhibitors **1–4** might involve two catalytic pathways (Scheme 2).

In pathway A, a β-elimination step (without prior oxidation at C-3′) of difluoromethyl or trifluoromethylthiolates ions might produce in the enzyme cavity highly reactive acylating agents such as thioformylfluoride or carbonothionic difluoride



Scheme 2.

(XFC=S, X=H, F) which could irreversibly acylate nucleophilic residues at the active site.

A second hypothetical mode of inactivation of AdoHcy hydrolase via the formation of thiol-nucleoside intermediate **7**, generated from inhibitors **1**–**4** by the "hydrolytic activity" of the enzyme, was also considered (pathway B).

Further mechanistic studies were investigated with compound **1** including Electrospray Ionization Mass Spectra (ESI-MS) analysis of the inactivated enzyme. When AdoHcy hydrolase was inactivated by **1** (50%) the inactivated enzyme subunit was detected at 47900 ± 5 Da showing that the inhibition of AdoHcy hydrolase was accompanied by a covalent linkage of 270 ± 5 Da (the native enzyme used in this experiment was detected at 47630 ± 5 Da). This result argues in favor of the second hypothetical mode of inactivation of AdoHcy hydrolase (pathway B). Formation of a disulfide bond with a thiol-nucleoside intermediate like **7** and a cysteine residue present (10) in the active site of the enzyme, may be proposed to explain the irreversible covalent inactivation process with the fluorinated nucleosides **1**–**4**.

Additional studies are in progress using nano ESI-MS^n techniques to elucidate the exact localisation of the covalent linkage induced on AdoHcy with DFMTA, TFMTA and their 2′- and 3′-deoxy analogs.

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5-**1**. mp 190–192°C- HRMS (DCI/NH_3) 318,088 $(MH)^+$, calc. 317,259- $[\alpha]_D^{20}$ −21° (c 1,6 × 10^{-2}; $CHCl_3$/MeOH: 4/1)- ^{1}H NMR, $CDCl_3$, ε CD_3OD δ (ppm), J (Hz): 2.02 (ddd, 1H, H-3′β, J 13.35, 5.72, 9.91); 2.23 (ddd, 1H, H-3′α, J 13.35, 2.29, 5.34); 3.21 (d, 2H, H-5′, J 5.34); 4.56 (m, 1H, H-2′); 4.79 (m, 1H, H-4′); 5.94 (d, 1H, H-1′, J 1.53); 6.90 (t, 1H, $SC\underline{H}F_2$, J 55); 8.07 and 8.28 (2 s, 2H, H-2 and H-8). ^{13}C NMR, $CDCl_3$, ε CD_3OD, δ (ppm), J (Hz): 30.8 (C-5′); 36.4 (C-3′); 76.2 (C-4′); 80.0 (C-2′); 93.2 (C-1′); 119.8 (C-6); 120.3 (d, $S\underline{C}HF_2$, J 275); 138.1 and 151.8 (C-2 and C-8);

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148.2 and 155.2 (C-4 and C-5). ^{19}F NMR, $CDCl_3$ ε CD_3OD, δ (ppm), J (Hz) −930 (2 dd, 2F, J 240, 55).

2. mp 62–64°C- HRMS (DCI/NH_3) 318,030 $(MH)^+$, calc. 317,259- $[\alpha]_D^{20}$ +11° (c 3,1 × 10^{-2}; $CHCl_3$/MeOH: 4/1)- ^{1}H NMR, $CDCl_3$, ε CD_3OD δ (ppm), J (Hz): 2.48 (ddd, 1H, H-2′β, J 13,74, 6,49, 4,2); 2.80 (ddd, 1H, H-2′α, J 13.74, 6,86, 6,49); 3.13 and 3.22 (dd, 2H, H-5′a, H-5′b, J 13,73, 5,72) ; 4.17 (ddd, 1H, H-4′, J 5.72, 5.72, 3,81); 4.48 (m, 1H, H-3′); 6.36 (dd, 1H, H-1′, J 6.49, 6.86); 6.83 (t, 1H, S$\underline{C}$HF_2, J 56,5); 7,99 and 8,26 (2 s, 2H, H-2 and H-8). ^{13}C NMR, $CDCl_3$, ε CD_3OD, δ (ppm), J (Hz): 29.5 (C-5′); 39.5 (C-2′); 72.9 (C-4′); 84.4 (C-3′); 85.8 (C-1′); 119.5 (C-6); 120.3 (dd, S$\underline{C}$HF_2, J 275); 139.0 and 152,4 (C-2 and C-8); 149.0 and 155.3 (C-4 and C-5). ^{19}F NMR $CDCl_3$, ε CD_3OD, δ (ppm), J (Hz) −93.0 (2 dd, 2F, J 244,3, 56,5).

3. mp 194–196°C- HRMS (DCI/NH_3) 336,108 $(MH)^+$, calc. 335,250- $[\alpha]_D^{20}$ −17° (c 1,5 × 10^{-2}; $CHCl_3$/MeOH: 4/1)- ^{1}H NMR, $CDCl_3$, ε CD_3OD δ (ppm), J (Hz): 2.02 (ddd, 1H, H-3′β, J 13,36, 5,72, 9,91); 2.24 (ddd, 1H, H-3′α, J 13.36, 1,91, 5,34); 3.25 (dd, 1H, H-5′a, J 14,11, 5,72); 3.31 (dd, 1H, H-5′b, J 14.11, 5,34); 4.58 (m, 1H, H-2′); 4.76 (m, 1H, H-4′); 5.91 (d, 1H, H-1′, J 1,53); 7.98 and 8.24 (2 s, 2H, H-2 and H-8). ^{13}C NMR $CDCl_3$, ε CD_3OD, δ (ppm), J (Hz): 33.4 (C-5′); 36.4 (C-3′); 75.9 (C-4′); 79.0 (C-2′); 93.0 (C-1′); 119.8 (C-6); 130.6 (ddd,, S$\underline{C}$$F_3$, J 305); 137.8 and 152.3 (C-2 and C-8); 148.1 et 155.6 (C-4 and C-5). ^{19}F NMR $CDCl_3$, ε CD_3OD, δ (ppm): −41.5 (s, SCF_3).

4. mp 48–50°C- HRMS (DCI/NH_3) 336,072 $(MH)^+$, calc. 335,250- $[\alpha]_D^{20}$ +5,8° (c 1,5 × 10^{-2}; $CHCl_3$/MeOH: 4/1)- ^{1}H NMR $CDCl_3$, ε CD_3OD δ (ppm), J (Hz): 2; 40 (ddd, 1H, H-2′β, J 13,74, 6,48, 3,81); 2.84 (ddd, 1H, H-2′α, J 13.74, 6,87, 6,48); 3.18 (dd, 1H, H-5′a, J 13,73, 6,48); 3,28 (dd, 1H, H-5′b, J 13.73, 5,34); 4.11 (ddd, 1H, H-4′, J 6.48, 5.34, 3,82); 4.43 (m, 1H, H-3′); 6.26 (dd, 1H, H-1′, J 6.48, 6.87); 7.91 and 8.13 (2 s, 2H, H-2 and H-8).
RMN, ^{13}C, $CDCl_3$ ε CD_3OD, δ (ppm), J (Hz): 31.9 (C-5′); 38.9 (C-2′); 72.9 (C-4′); 84.7 (C-3′); 84.9 (C-1′); 119.5 (C-6); 130.6 (ddd, S$\underline{C}$$F_3$, J 305); 139.2 and 152.3 (C-2 and C-8); 148.8 and 155.3 (C-4 and C-5). ^{19}F NMR $CDCl_3$, ε CD_3OD, δ (ppm): −41,5 (s, SCF_3).

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