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Inactivation of *S*-adenosylhomocysteine hydrolase with haloethyl and dihalocyclopropyl esters derived from homoadenosine-6′-carboxylic acid

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Abstract—In a search for new inhibitors that exploit 5′–6′ 'hydrolytic activity' of AdoHcy hydrolase, a new series of haloethyl and dihalocyclopropyl esters **2**–**3** were designed and their interaction with the enzyme studied. Incubation of the enzyme with **2**–**3** resulted in time- and concentration-dependent inactivation of AdoHcy hydrolase as well as almost total depletion of its NAD^+ content. Further results indicated that the 'oxidative' but not the 'hydrolytic' activity was involved in the inactivation process.

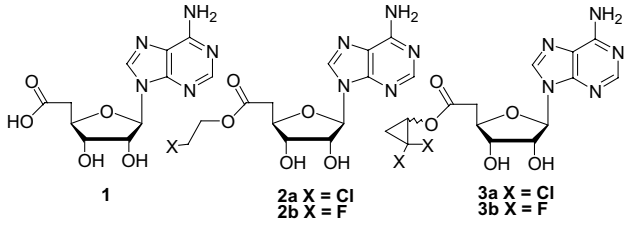
1. Introduction

The enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) catalyses the hydrolytic cleavage of AdoHcy to adenosine (Ado) and homocysteine (Hcy).[1] Due to its pivotal role in the regulation of biological methylation reactions[2] AdoHcy hydrolase has become an attractive pharmacological target for the design of antiviral,[3–6] antiparasitic,[3,4] immunosuppressive,[3,4] and plasma Hcy-lowering agents.[4,7] A number of inhibitors, which function as substrates for the '3′-oxidative activity' of the enzyme and irreversibly keep AdoHcy hydrolase in its inactive NADH form, have been identified (type I).[5,8]

A second type of mechanism-based inhibitors (covalent, type II) used the '5′–6′ hydrolytic activity' of the enzyme to generate an electrophilic site on the inhibitor, which can then bind to an active site nucleophile.[9] In addition to these irreversible inactivation processes, we also reported[10] that liberation of electrophilic entity from an inhibitor upon its interaction with the enzyme's 'hydrolytic activity' provoked covalent inhibition of AdoHcy hydrolase. A large number of homoadenosine analogues have been identified as inactivators of AdoHcy hydrolase.[9] During our investigation on AdoHcy hydrolase inhibition, we recently found (unpublished results) that homoadenosine-6′-carboxylic acid **1** had an affinity for the binding site of the enzyme ($K_i = 3\,\mu M$) in the range of the K_m for the substrate adenosine (K_m/Ado=1 μM).

This result led us to hypothesize that esters **2**–**3** derived from the nucleoside carboxylic acid **1** might function as alternative substrates for the hydrolytic activity of AdoHcy hydrolase and should be good candidates as new covalent mechanism-based inhibitors of the enzyme.

Conceptually, hydrolysis of esters **2**–**3** by the enzyme's sequestered water[5,6] could generate active alkoxides within the active site in the closed form of the enzyme.[5,6] Spontaneous decomposition of the latter should produce highly reactive acylating agents, which might acylate nucleophilic residues involved in the catalytic process of the enzyme (Fig. 1).



We now describe the synthesis of haloethyl esters **2** and the diastereoisomeric dihalocyclopropyl esters **3** as well as their interaction with human AdoHcy hydrolase.

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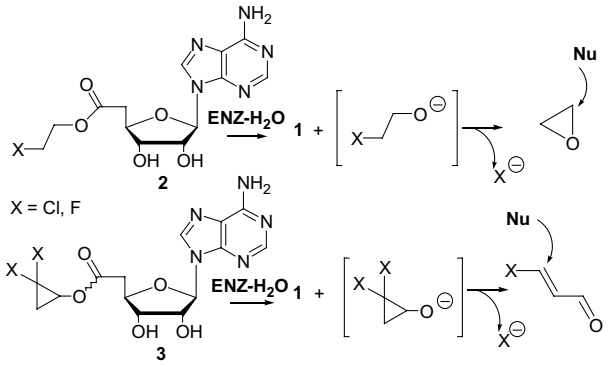
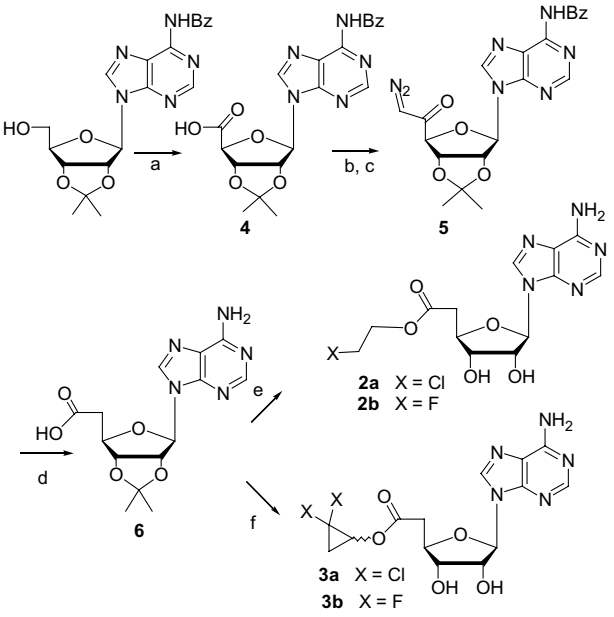


Figure 1. Proposed mechanisms for inactivation of AdoHcy hydrolase with **2**–**3**.

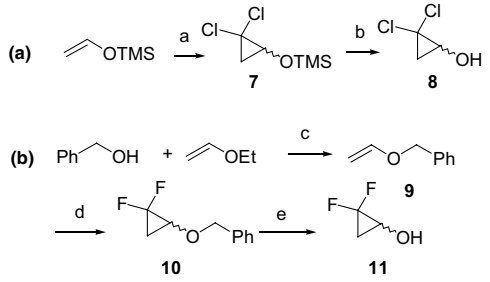
2. Synthesis

Esters **2**–**3** were obtained from the protected homoadenosine-6′-carboxylic acid **6** (Scheme 1) and appropriate alcohols, prepared according to the general procedures described in Scheme 2.

Because the homologation of adenosine to homoadenosine-6′-carboxylic acid via 5′-cyano-5′-deoxy adenosine derivatives required multistep procedures,[11–13] which gave in our hands low overall yields, we found useful to prepare the homoadenosine derivative **6** via an Arndt–Eistert reaction.[14] Thus, 2′,3′-*O*-isopropylidene adenosine, protected on the N^6 position with a benzoyl group, was oxidized on the 5′ position by a mild procedure giving the adenosine-5′-carboxylic acid derivative **4** in high yields[15] (Scheme 1). The corresponding acid chloride was obtained quantitatively[16] as an intermediate by treatment with thionyl chloride without purification. The acid chloride thus obtained was converted to the diazoketone **5**[17] by condensation with trimethylsilyldiazomethane.[18] The Wolff rearrangement of **5** was initiated with catalytic silver benzoate in a 1/2 dioxane/water mixture and yielded, after N^6 deprotection, the homologated acid **6**.



Scheme 1. Reagents and conditions: (a) TEMPO, bis(acetoxy)iodobenzene, CH_3CN/H_2O 1/1, 80%; (b) $SOCl_2$, CH_2Cl_2, 100%; (c) $TMSCHN_2$, CH_2Cl_2, 80%; (d) cat. $PhCO_2Ag$, dioxane/H_2O 2/1, Δ, 47% then MeOH, MeONa, 96%; (e) EDCI, HOBT, 2-haloethan-1-ol, CH_2Cl_2, X = Cl 44%, X = F 16% then HCOOH/H_2O 1/1, X = Cl 81%, X = F 27%; (f) EDCI, HOBT, 2,2-dihalocyclopropan-1-ol, CH_2Cl_2, X = Cl 61%, X = F 69% then HCOOH/H_2O 1/1, 70%.



Scheme 2. Reagents and conditions: (a) Cl_3CCO_2Et, MeONa, pentane, 30%; (b) THF, HCl cat., 100%; (c) $Hg(OCOCF_3)_2$, 60%; (d) $CClF_2COONa$, diglyme, 180 °C, 66%; (e) H_2, $Pd(OH)_2/C$, 100%.

Dihalocyclopropanols are known to be unstable.[19] They were prepared just before use. The racemic dichlorocyclopropanol **8** was obtained from its *O*-trimethylsilyl derivative **7**[20] by mild acidic treatment (Scheme 2a). A three step procedure was used to generate in neutral conditions the difluorocyclopropanol (±)**11** (Scheme 2b). Reaction of vinyloxymethylbenzene[21] with the difluorocarbene generated by thermal decomposition of sodium salt of chlorodifluoro acetic acid[22] led to the racemic protected difluorocyclopropanol **10** in 66% yield. Catalytic hydrogenation of compound **10** yielded quantitatively the corresponding alcohol **11**.

2-Chloroethanol and 2-fluoroethanol are commercially available.

Esterification of the 2′,3′-*O*-isopropylidene acid **6** with the corresponding alcohols, catalyzed by EDCI and HOBT (Scheme 1), afforded the target nucleosides **2**–**3**[23] after acidic removal of the isopropylidene protecting group. The dihalocyclopropyl esters **3a** and **3b** were obtained as a mixture of inseparable diastereoisomers.

3. Inactivation of AdoHcy hydrolase: results and discussion

Recombinant human placenta AdoHcy hydrolase purified to homogeneity was used in this study.[24] AdoHcy hydrolase (10 nM) was assayed in the synthetic direction in the presence of [8-^{14}C]-Ado or [2,8-^{3}H]-Ado (15 μM, 300 Bq) and Hcy (5 mM) in 20 mM potassium phosphate buffer pH 7.5, 1 mM EDTA.

Incubation of the enzyme with **2a**, **2b**, **3a**, and **3b** resulted in time- and concentration-dependent inactivation, as illustrated for compound **2a** in Figure 2.

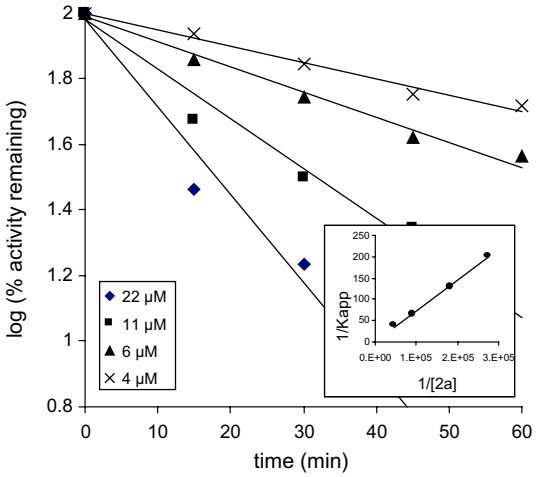


Figure 2. Time- and concentration-dependent inactivation of AdoHcy hydrolase with **2a**. AdoHcy hydrolase (2 µM) was incubated with inhibitor at various concentrations and various times in 20 mM potassium phosphate buffer pH 7.5, 1 mM EDTA at 37 °C. Residual activity was determined as described.

In each case, the inactivation observed was irreversible since the enzyme activity could not be restored after dialysis against assay buffer. Protection experiments with Ado confirm that the nucleosides **2**–**3** act as site-directed inhibitors. The Kitz and Wilson method[25] was used to calculate K_i and k_{inact} values (Table 1).

The k_{inact} values measured for the haloethylesters **2a** and **2b** were substantially higher than those for dihalocyclopropyl esters **3a** and **3b**. The difference in the affinity observed between the corresponding chloro and fluoro derivatives can easily be explained by the steric hindrance of the chlorine present in compounds **2a** and **3a**.

We also determined the effects of **2**–**3** on the NAD^+/NADH content of the enzyme. As summarized in Figure 3, incubation of inhibitors **2**–**3** at 600 mM with AdoHcy hydrolase (20 µM) produced large depletion of its NAD^+ content. This is indicative of the participation of the oxidative activity of AdoHcy hydrolase in the inactivation process.

The mechanism of inactivation was further investigated by analysis of the reaction products. The reaction mixture obtained after complete inactivation of AdoHcy hydrolase with **2**–**3** was subjected to HPLC analysis, after treatment with three volumes of ethanol and ultrafiltration to remove the protein. No trace of homoadenosine-6′-carboxylic acid was detected.

Table 1. K_i and k_{inact} values for the inhibitory effect of **2**–**3** on AdoHcy hydrolase

Compounds	K_i, µM	k_{inact}, min^{-1}
2a	302	0.43
2b	75	0.19
3a	750	0.09
3b	17	0.008

AdoHcy hydrolase (2 µM) was incubated with inhibitor at various concentrations and various times in 20 mM potassium phosphate buffer pH 7.5, 1 mM EDTA at 37 °C. Residual activity was determined as described and a double reciprocal plot of the initial pseudo-first order inactivation rate constant versus 1/[I] gave the K_i and k_{inact} values.[25]

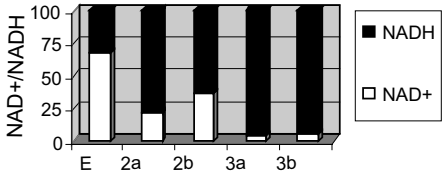


Figure 3. Variation of NAD^+/NADH content upon incubation with inhibitors: AdoHcy hydrolase (20 µM) was incubated with 600 µM of **2a**, **2b**, **3a**, or **3b** in 20 mM potassium phosphate buffer pH 7.5, 1 mM EDTA at 37 °C until total inactivation. NAD^+ and NADH present in native AdoHcy hydrolase (E) and after inactivation (**2a**, **2b**, **3a**, and **3b**) were measured by a fluorescence method.[26]

These results support the conclusion that esters **2**–**3** are not substrates for the hydrolytic activity of the enzyme, instead **2**–**3** inactivate AdoHcy hydrolase by a type I mechanism. Similar results have been obtained previously with amide and ester derivatives of Ado-5′-carboxylic acid.[27]

Acknowledgements

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17. Compound **5**: ^{1}H NMR ($CDCl_3$, 250 MHz, δ ppm, J Hz) 1.4 (s, 3H); 1.6 (s, 3H); 4.7 (m, 1H, $H_{6'}$); 5.5 (dd, 1H, $J_{1',2'}$ 1.0, $J_{2',3'}$ 6.2, $H_{1'}$); 5.6 (dd, 1H, $J_{2',3'}$ 6.2, $J_{3',4'}$ 2.1, $H_{3'}$); 6.3 (d, 1H, $J_{1',2'}$ 1.0, $H_{1'}$); 7.5–8.0 (m, 5H, Ar); 8.1 and 8.7 (2s, 2H, H_2 and H_8). ^{13}C NMR ($CDCl_3$, 62.5 MHz, δ ppm) 24.9 and 26.5; 53.1 (C6′); 83.1 (C3′); 83.4 (C2′); 90.5 (C4′); 91.1 (C1′); 114.1; 123.2; 127.7; 127.8; 128.5; 132.5; 132.6; 133.1; 142.4; 149.7; 151.1; 152.3; 164.8 (C=O Bz); 191.4 (C=O diazoketone). MS m/z 450 (MH^+).
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23. Compound **2a**: ^{1}H NMR (DMSO-d_6, 250 MHz, δ ppm, J Hz) 2.82 (dd, 1H, $J_{4',5'a}$ 8.5, $J_{5'b,5'a}$ 16.0, $H_{5'a}$); 2.92 (dd, 1H, $J_{4',5'b}$ 4.8, $J_{5'b,5'a}$ 16.0, $H_{5'b}$); 3.80 (t, 2H, J 5.4, CH_2); 4.16 (m, 1H, $H_{3'}$); 4.27 (m, 3H, CH_2 and $H_{4'}$); 4.74 (dd, 1H, $J_{1',2'}$ 5.4, $J_{2',3'}$ 10.5, $H_{2'}$); 5.89 (d, 1H, $J_{1',2'}$ 5.4, $H_{1'}$); 8.10 and 8.30 (2s, 2H, H_2 and H_8). ^{13}C NMR (DMSO-d_6, 62.5 MHz, δ ppm) 38.3 ($C_{5'}$); 42.8 (CH_2); 64.4 (CH_2); 72.9 ($C_{4'}$); 73.2 ($C_{3'}$); 80.6 ($C_{2'}$); 88.0 ($C_{1'}$); 119.5; 140.3; 149.7; 153; 156.4; 170.6 (C=O). MS m/z 358 (MH^+). HRMS (CI/NH_3) Calcd for $C_{13}H_{17}N_5O_5Cl$ (MH^+) 358.0918, found 358.0927. Compound **2b**: ^{1}H NMR (DMSO-d_6, 250 MHz, δ ppm, J Hz) 2.76 (dd, 1H, $J_{4',5'a}$ 9.0, $J_{5'b,5'a}$ 16.0, $H_{5'a}$); 2.87 (dd, 1H, $J_{4',5'b}$ 4.5, $J_{5'b,5'a}$ 16.0, $H_{5'b}$); 4.11 (dd, 1H, $J_{2',3'}$ 10.0, $H_{3'}$); 4.21 (m, 3H, CH_2 and $H_{4'}$); 4.54 (dm, 2H, $J_{H,F}$ 47.7, CH_2); 4.67 (dd, 1H, $J_{1',2'}$ 5.3, $J_{2',3'}$ 10.0, $H_{2'}$); 5.84 (d, 1H, $J_{1',2'}$ 5.3, $H_{1'}$); 8.15 and 8.40 (2s, 2H, H_2 and H_8). ^{13}C NMR (DMSO-d_6, 62.5 MHz, δ ppm) 38.0 ($C_{5'}$); 63.4 (d, $J_{C,F}$ 9.3, CH_2); 72.6 ($C_{2'}$); 72.8 ($C_{3'}$); 79.4 ($C_{4'}$); 81.5 (d, $J_{C,F}$ 83.0, CH_2F); 87.6 ($C_{1'}$); 119.1; 139.9; 149.3; 152.6; 156.1; 170.7 (C=O). ^{19}F NMR (DMSO-d_6, 235 MHz, δ ppm, J Hz) −224.4 (tt, 1F, J 30.2, J 47.7). MS m/z 342 (MH^+). HRMS (CI/NH_3) calcd for $C_{13}H_{17}N_5O_5F$ (MH^+) 342.1214, found 342.1237. Compound **3a** (50/50 mixture of diastereoisomers): ^{1}H NMR (DMSO-d_6, 250 MHz, δ ppm, J Hz) 1.80 (m, 1H, cyclopropyl); 2.04 (m, 1H, cyclopropyl); 2.95 (m, 2H, $2H_{5'}$); 4.15 (m, 1H, $H_{3'}$); 4.28 (m, 1H, $H_{4'}$); 4.48 (m, 1H, cyclopropyl); 4.77 (dd, 0.5H, $J_{1',2'} = J_{2',3'}$ 5.0, $H_{2'}$) and 4.84 (dd, 0.5H, $J_{1',2'} = J_{2',3'}$ 5.0, $H_{2'}$); 5.90 (d, 1H, $J_{1',2'}$ 5.0, $H_{1'}$); 8.15 and 8.17 (2s, 2*0.5H, H_2); 8.36 and 8.38 (2s, 2*0.5H, H_8). ^{13}C NMR (DMSO-d_6, 62.5 MHz, δ ppm) 26.0 (CH_2 cyclopropyl); 37.5 ($C_{5'}$); 56.8 (CH cyclopropyl); 72.4 ($C_{3'}$); 72.9 ($C_{4'}$); 80.1 ($C_{2'}$); 87.7 ($C_{1'}$); 106.0 (CCl_2); 120.0; 140.0; 152.6; 156.0; 170.0 (C=O). MS m/z 404 (MH^+). HRMS (CI/NH_3) calcd for $C_{14}H_{16}N_5O_5Cl_2$ (MH^+) 404.0528, found 404.0456. Compound **3b** (mixture of diastereoisomers): ^{1}H NMR (DMSO-d_6, 250 MHz, δ ppm, J Hz) 1.73 (m, 1H, cyclopropyl); 2.00 (m, 1H, cyclopropyl); 2.91 (m, 2H, $2H_{5'}$); 4.22 (m, 1H, cyclopropyl); 4.48 (m, 1H, $H_{4'}$); 4.72 (m, 1H, $H_{2'}$); 5.90 (d, 1H, $J_{1',2'}$ 5.0, $H_{1'}$); 8.10 (s, 1H, H_2); 8.30 (s, 1H, H_8). ^{13}C NMR (DMSO-d_6, 62.5 MHz, δ ppm) 17.1 (m, CH_2 cyclopropyl); 37.5 ($C_{5'}$); 50.9 (m, CH cyclopropyl); 72.5 ($C_{3'}$); 72.8 ($C_{4'}$); 80.1 ($C_{2'}$); 87.7 ($C_{1'}$); 107.0 (m, CF_2); 119.2; 140.0; 149.3; 152.6; 156.0; 170.3 (C=O). MS m/z 372 (MH^+). HRMS (CI/NH_3) calcd for $C_{13}H_{17}N_5O_5F_2$ (MH^+) 372.1119, found 372.1148.
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