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SYNTHESIS AND EVALUATION OF 4',5'-DEHYDRO-5'-FLUOROARISTEROMYCINS AS S-ADENOSYL-L-HOMOCYSTEINE (AdoHcy) HYDROLASE INHIBITORS

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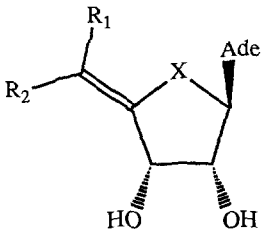
Abstract: 4',5'-Dehydro-5'-fluoro analogs of aristeromycin were synthesized and shown to be potent inhibitors of recombinant rat liver AdoHcy hydrolase.

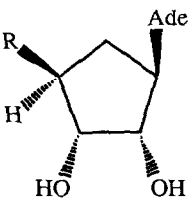
The cellular enzyme AdoHcy hydrolase (EC 3.3.1.1), which catalyzes the reversible hydrolysis of AdoHcy to adenosine (Ado) and homocysteine (Hcy), plays an important role in regulating virus-specific S-adenosylmethionine- (AdoMet) dependent methyltransferases.[1] The inhibition of AdoHcy hydrolase has been correlated with inhibition of viral replication,[2] hence, inhibitors of this AdoHcy hydrolase are of significant interest as potential antiviral agents.[3]

Recently, McCarthy et al.[4] and Mehdi et al.[5] reported the synthesis and potent AdoHcy hydrolase inhibitory effects of the 4',5'-dehydro-5'-fluoro analogs **1** and **2** of Ado. The vinyl fluorides **1** and **2** are particularly interesting because they were reported to inactivate the enzyme by reduction of the enzyme-bound NAD^+ to NADH with subsequent quantitative release of fluoride ion. These results suggested that the vinyl fluorides might be the first examples of so-called "type II" mechanism-based inactivators of AdoHcy hydrolase which function by generating a reactive intermediate (e.g., 3'-keto derivatives of **1** and **2**) at the enzyme active site which then reacts with a protein nucleophile to form a covalent adduct. This is opposed to "Type I" inactivators, which function by simply converting the enzyme-bound NAD^+ to NADH.[3]

In this communication, we report the synthesis and biological evaluation of the carbocyclic vinyl fluorides **3** and **4**, analogs of the naturally occurring carbocyclic nucleoside aristeromycin (**5**). This nucleoside is a potent inhibitor of AdoHcy hydrolase.[6, 7] In addition, 4'-modified analogs (e.g., **6**) were recently synthesized by our laboratory and shown to be potent type I mechanism-based inhibitors of AdoHcy hydrolase.[8]

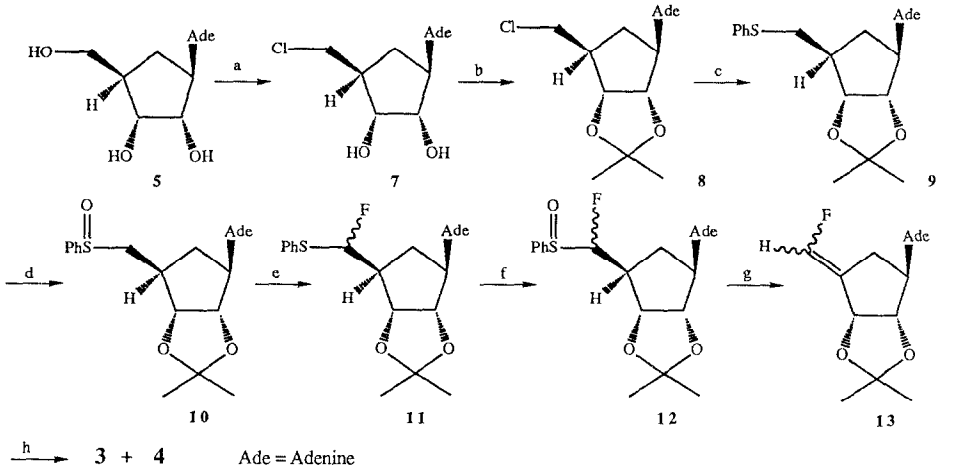


1 $R_1 = H, R_2 = F, X = O$ (E)
2 $R_1 = F, R_2 = H, X = O$ (Z)
3 $R_1 = H, R_2 = F, X = C$ (E)
4 $R_1 = F, R_2 = H, X = C$ (Z)



5 $R = CH_2OH$
6 $R = CH_3$

The synthetic routes to **3** and **4** are outlined in Scheme 1. The synthesis begins with aristeromycin (**5**), obtained by a 9-step enantiospecific synthesis reported earlier.[9, 10] Treatment of **5** (100 mg) with thionyl chloride



Scheme 1. Synthesis of compounds **3** and **4**. (a) (i) $SOCl_2$/Pyridine, CH_3CN, r.t.; (ii) NH_4OH, 90%; (b) DMP, $HClO_4$, Me_2CO, 0 °C, 92%; (c) PhSH/n-BuLi/DMF, -78 °C to r.t., 89%; (d) m-CPBA, CH_2Cl_2, -78 °C, 2 h, 98%; (e) DAST, $SbCl_3$, CH_2Cl_2,-78 °C to r.t., over night, 71%; (f) m-CPBA, CH_2Cl_2, -78 °C, 97%; (g) Diglyme, Diisopropylethylamine, 140 °C, 2 days, 82%; (h) CF_3COOH/H_2O (3:1), r.t., 84%.

($SOCl_2$) (0.2 mL) in CH_3CN (1.5 mL) in the precence of pyridine (0.2 mL) afforded the 5'-deoxy-5'-chloro-analog **7**. Compound **7** was reacted in acetone with dimethoxypropane (DMP) in the presence of 70% $HClO_4$ at 0 °C and then stirred at room temperature for 2 h, affording **8** in an overall yield of 82%.[11] PhS^-Li^+, which was generated by reacting thiophenol (PhSH) (87 mg, 0.8 mmol) with n-BuLi (316 µL of 2.5 M solution in hexane) in DMF for 1/2 h at -78 °C and 1 h at room temperature, was transferred via cannula to another flask containing a solution of **8** (100 mg, 0.3 mmol) in DMF at -78 °C. The reaction mixture was stirred at room temperature overnight to give 5'-phenylthio-5'-deoxy-2',3'-isopropylidenearisteromycin (**9**) in 89% yield.[12, 13] Compound **9** was oxidized using m-chloroperbenzoic acid (m-CPBA) in CH_2Cl_2 at -78 °C for 2 h. After purification through silica gel chromatography (2% CH_3OH in $CHCl_3$), the desired product **10** was obtained in 98% yield. The 5'-fluoride substituent was introduced by treating compound **10** (1 eq) with (diethylamino)sulfur trifluoride (DAST, 2 eq) in the presence of the Lewis acid antimony trichloride ($SbCl_3$, 0.1 eq) as catalyst.[4, 14] Purification of the reaction mixture through silica gel chromatography (CH_3OH:$CHCl_3$ =1:99) afforded an isomeric mixture of 5'-fluoro-5'-phenylthio-5'-deoxy-2',3'-isopropylidenearisteromycin (**11**) in 71% yield. The ratio of the two diastereomers was determined by ^1H NMR spectrum to be 60:40. Treatment of **11** with m-CPBA in CH_2Cl_2 at -78 °C resulted in oxidation to the sulfoxides **12** as a mixture of diastereomers in 97% yield. Elimination of PhS(O)H was carried out by heating the solution of **12** (60 mg) and diisopropylethylamine (0.5 mL) in diglyme (3 mL) at 140 °C for 24 h and then adding another 0.5 mL diisopropylethylamine and heating for an additional 24 h at 140 °C. The dark red reaction mixture was purified by silica gel chromatography (2% CH_3OH in $CHCl_3$) to give a mixture of *E* and *Z* isomers of the 2',3'-isopropylidene protected vinyl fluorides **13** in about a 1:1 ratio. By stirring **13** (32 mg) in CF_3COOH-H_2O (3:1, 2 mL) at room temperature for 20 h, a mixture of the *E* and *Z*

isomers of the vinyl fluorides **3** and **4** was obtained and separated by HPLC (Econosil C-18 reverse-phase preparative column, 6 to 12% CH_3CN in H_2O for 25 min) affording the individual isomers as white solids.[15] The geometry of each isomer was established through NOE experiments.[15] It should be noted that Wolos et al[16] from Marion Merrell Dow Inc. have recently disclosed the structures of **3** and **4** in a patent.

Enzyme inhibition studies were conducted using a purified recombinant rat liver AdoHcy hydrolase.[17] Enzyme activity was determined in the synthetic direction using Ado (0.2 mM) and Hcy (5 mM) in 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA at 37 °C and incubating for 5 min. The reaction product AdoHcy was assayed by HPLC[8] after the reaction was stopped by addition of $HClO_4$. For the determination of inhibition constants, AdoHcy hydrolase was preincubated with various concentrations of potential inhibitors for various amounts of time and the remaining enzyme activity was measured. The pseudo-first-order rate constants of inactivation (k_{obs}) were determined from plots of the remaining activity *versus* preincubation time. K_I and k_2 values were obtained from a plot of $1/k_{obs}$ *versus* 1/[inhibitor] using the equation $1/k_{obs} = (K_I/k_2)\cdot(1/[I]) + 1/k_2$.

Table 1. Inhibitory effects of vinyl fluorides **1** - **4** on recombinant rat liver AdoHcy hydrolase.

Compound	K_I (nM)	k_2 (min^{-1})	k_2/K_I ($nM^{-1}min^{-1}$)
1	40	0.050	1.25×10^{-3}
2	39	0.082	2.10×10^{-3}
3	112	0.031	2.77×10^{-4}
4	87	0.050	5.75×10^{-4}

Based on the data shown in Table 1, the following conclusions can be drawn concerning the AdoHcy hydrolase inhibitory effects of the vinyl fluorides **1** - **4**. First, the carbocyclic vinyl fluorides **3** and **4** are less potent inhibitors of AdoHcy hydrolase than the ribosyl vinyl fluorides **1** and **2**. This difference is primarily reflected in the 2-3 times larger K_I values. Second, the *Z* isomers of both the ribosyl analog **2** and the carbocyclic analog **4** are more potent inhibitors than the *E* isomers **1** and **3**, respectively, when the k_2/K_I values are compared.

In addition to these kinetic differences, there may exist subtle differences in the mechanism by which the ribosyl vinyl fluorides **1** and **2** and the carbocyclic vinyl fluorides **3** and **4** inactivate AdoHcy hydrolase. The ribosyl vinyl fluorides **1** and **2** react rapidly with either NAD^+ form of AdoHcy hydrolase or apo form of the enzyme (NAD^+ depleted) to release fluoride ion.[18] With the apo form of the enzyme, the vinyl fluorides **1** and **2** were shown to be transformed to Ado 5'-carboxaldehyde. With the NAD^+ form of AdoHcy hydrolase, the vinyl fluorides **1** and **2** were shown to be transformed into the 3'-keto derivative of Ado 5'-carboxaldehyde. Recently we synthesized Ado 5'-carboxaldehyde and showed it to be a potent type I mechanism-based inhibitor of AdoHcy hydrolase which is transformed by the NAD^+ form of the enzyme to the 3'-keto derivative (the same products generated by reaction of the vinyl fluorides **1** and **2** with the NAD^+ form of the enzyme).[19] Thus, the ribosyl vinyl fluorides **1** and **2** appear to be "prodrugs," which are converted to Ado 5'-carboxaldehyde at the enzyme active site.

Preliminary mechanistic studies using ^{19}F NMR with the carbocyclic vinyl fluorides **3** and **4** suggest that no fluoride ion was released when the compounds were incubated with the NAD^+ form of the enzyme. These results would suggest that the carbocyclic vinyl fluorides **3** and **4** may be transformed by the enzyme to the corresponding 3'-keto derivatives, which results in enzyme-bound NAD^+ reducing to NADH, not transformation to 3'-keto-aristeromycin 5'-aldehyde. Further mechanistic experiments with the carbocyclic vinyl fluorides are currently in progress.

Acknowledgment

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15. **3** ^{1}H-NMR (D_2O, ppm): 2.84 (m, 2H, 6'-H), 4.53 - 4.83 (m, 3H, 2', 3', 4'-H), 6.71 (d, *J* = 78 Hz, 1H, vinyl H), 8.12 (s, 1H, 2-H), 8.16 (s, 1H, 8-H); ^{19}F-NMR (D_2O, ppm): -126.9, -127.0; ^{13}C-NMR (D_2O, ppm): 25.9, 58.5, 67.1, 75.5, 119.1 130.1, 141.6, 147.4, 149.5, 152.7, 156.1; HRMS (FAB+, *m/e*): 266.1062 (MH^+, Calcd, 266.1053), MS (FAB+, *m/e*: 266 (MH^+), 237, 223, 215, 201, 197, 185, 131, 115.

    4 ^{1}H-NMR (D_2O, ppm): 2.97 (m, 2H, 6'-H), 4.49 - 4.62 (m, 2H, 2', 3'-H), 4.89 (m, 1H, 1'-H), 6.94 (d, *J*=80 Hz, 1H vinyl H), 8.12 (s, 1H, 2-H), 8.16 (s, 1H, 8-H); ^{19}F-NMR (D_2O, ppm): -125.5, -125.7, ^{13}C-NMR (D_2O, ppm): 27.4, 58.7, 69.7 76.1, 119.4, 130.4, 141.8, 148.1, 150.1, 152.7, 156.0, HRMS (FAB+, *m/e*): 266.1047 MH^+, Calcd, 266.1053), MS (FAB+, *m/e*): 266 (MH^+), 237, 223, 215, 201, 197, 185, 131, 115.

    NOE difference spectroscopy (D_2O) on **3** and **4** showed an enhancement in the 3'-methine proton on **4** (δ 4.52) when the vinyl proton (δ 6.94) was irradiated and no enhancement on the 3'-methine proton on **3**; in contrast, there is an enhancement in the 6'-methylene proton on **3** (δ 2.84) when the vinyl proton (δ 6.71) was irradiated and no enhancement on the 6'-methylene protons on **4**.
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