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Letters

Design, Synthesis, and Biological Evaluation of Fluoroneplanocin A as the Novel Mechanism-Based Inhibitor of *S*-Adenosylhomocysteine Hydrolase

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Abstract: Fluoroneplanocin A (**12**) was designed as a novel mechanism-based inhibitor of *S*-adenosylhomocysteine hydrolase (SAH) and efficiently synthesized via an electrophilic vinyl fluorination reaction (*n*-BuLi, *N*-fluorobenzenesulfonimide at −78 °C). Fluoroneplanocin A exhibited 2-fold more potent SAH inhibitory activity than the parent neplanocin A. A new mechanism of irreversible inhibition discovered in this work might provide new alternatives in the design of a different class of antiviral agents operating via SAH inhibition.

Introduction. *S*-Adenosylhomocysteine hydrolase (SAH) catalyzes the hydrolysis of *S*-adenosylhomocysteine to adenosine and L-homocysteine. Inhibition of this enzyme results in the accumulation of *S*-adenosylhomocysteine, which in turn inhibits cellular *S*-adenosyl-L-methionine dependent transmethylation reactions. Inhibition of methylation, among other things, interferes with the formation of the 5′-terminal-methylated N^7-guanosine *m*RNA cap of most animal infecting viruses, which is necessary for viral replication.[1] Therefore, effective SAH inhibitors have been sought as useful broad-spectrum antiviral agents.[2,3]

A number of compounds have been synthesized and evaluated as SAH inhibitors.[4] These inhibitors are largely classified into two types. Type I mechanism-based inhibitors, like neplanocin A, inactivate SAH by converting cofactor-bound NAD^+ into NADH, resulting in the depletion of NAD^+ while the tight binding intermediate remains at the active site. Such inhibition of SAH can be reversed after incubation with NAD^+ or dialysis.[5] Type II mechanism-based inhibitors, on the other hand, in addition to causing NAD^+ depletion, are able to bind covalently at the active site of the enzyme, causing permanent and irreversible inhibition not recoverable by the addition of NAD^+ or dialysis. Type II mechanism-based inhibitors are represented by a group of 4′,5′-modified nucleosides.[6]

Neplanocin A has been recognized as one of the most potent inhibitors of SAH.[7−9] As shown in Scheme 1, the SAH-catalyzed oxidation of neplanocin A by cofactor-bound NAD^+ mechanistically leads to the formation of its keto form **1**, which by virtue of being a dead end product maintains the cofactor permanently in its reduced form (NADH).[4,5] In addition to this well-known cofactor depletion mechanism, it has been hypothesized that the keto form **1** is well poised to form a covalent complex with the enzyme via Michael addition with an appropriate enzyme nucleophile. However, formation of this intermediate has never been demonstrated. One reason for this is probably the expected reversibility of the Michael adduct **2** due to the acidity of the α-keto hydrogen (H_4). On the basis of this reversible Michael addition hypothesis, we wanted to demonstrate the likelihood of this mechanism by designing an appropriate substrate that would provide the enzyme with an alternative leaving group for the addition−elimination reaction. The substrate designed for this purpose was the 6′-fluoro analogue of neplanocin A, which after Michael addition would have the option of eliminating the fluoride ion to trap intermediate **3** by forming an irreversible covalent complex. In this communication, we report the synthesis of fluoroneplanocin A, a more

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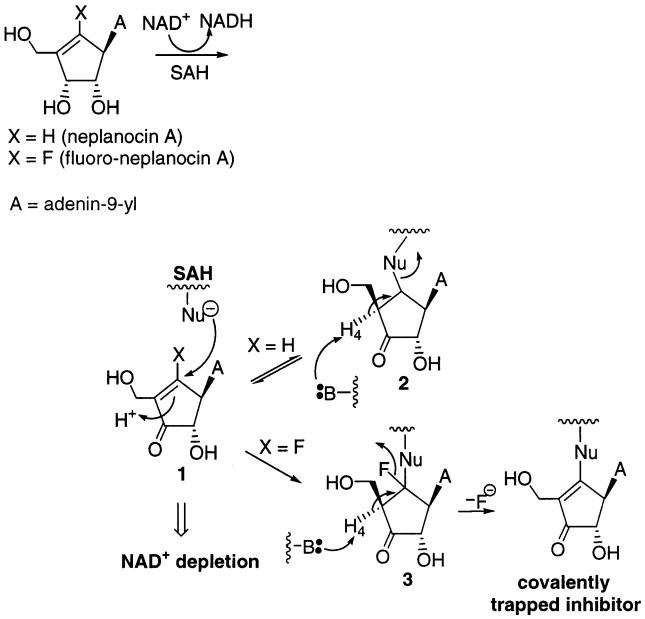
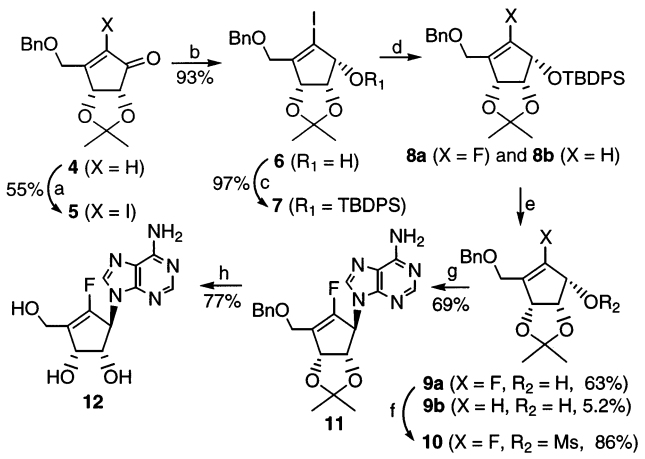
Scheme 1. Proposed Mechanism for the Reversible (Neplanocin A, X = H) and Irreversible (Fluoroneplanocin A, X = F) Reactions at the Active Site of SAH



Scheme 2[a]



[a] Reagents and conditions: (a) I_2, CCl_4, pyridine, room temp; (b) $NaBH_4$, $CeCl_3$, MeOH, 0 °C; (c) TBDPSCl, imidazole, 50 °C; (d) *n*-BuLi, *N*-fluorobenzenesulfonimide, THF, −78 °C; (e) *n*-Bu_4NF, THF, room temp; (f) MsCl, pyridine, room temp; (g) adenine, K_2CO_3, 18-crown-6, DMF, 80 °C; (h) (i) BBr_3, CH_2Cl_2, −78 °C; (ii) Ac_2O, pyridine; (iii) NH_3, MeOH.

potent inhibitor of SAH than its parent neplanocin A, which appears to operate by the proposed, novel mechanism of inhibition. Synthesis of fluoroneplanocin A was achieved via a critical electrophilic vinyl fluorination step.

Results and Discussion. Synthesis. For the synthesis of the desired fluoroneplanocin A (Scheme 2), the known cyclopentenone derivative **4**[10] was converted to its iodo derivative **5** (I_2, CCl_4, pyridine),[11] which was selectively reduced to give allylic alcohol **6**. After protection of alcohol **6** as the silyl ether **7**, treatment with base (*n*-BuLi or LDA) followed by reaction with electrophilic fluorine (*N*-fluorobenzenesulfonimide, Selectfluor) gave only the reduced compound **8b**. However, the reverse addition, consisting of a mixture of compound **7** and *N*-fluorobenzenesulfonimide followed by the dropwise addition of *n*-BuLi at −78 °C, afforded an inseparable mixture of **8a** and **8b** in a 12:1 ratio as determined by ^1H NMR. Desilylation of the **8a**/**8b** mixture with *n*-Bu_4NF gave both the desired fluoro derivative **9a** (63% from **7**) and the reduced compound **9b** (5.2% from **7**) after purification by silica gel chromatography. In the same manner as for the synthesis of neplanocin A, the mesylate ester **10** was successfully condensed with the adenine anion to give the corresponding protected carbocyclic nucleoside **11**, which was deblocked with BBr_3 to give the target fluoroneplanocin A (**12**).[13]

Table 1. Concentration-Dependent Inhibition of SAH by **12** and Neplanocin A

	% activity remaining					
compounds	0.2 μM	0.4 μM	0.5 μM	0.6 μM	0.8 μM	1.0 μM
12	100	99	36	0	0	0
neplanocin A	100	99	98	97	54	0

Enzyme Inhibition Assay. Inhibition of SAH by neplanocin A and its fluoro analogue **12** was measured using pure recombinant enzyme from human placenta (Table 1).

Both compounds were preincubated with the enzyme at various concentrations ranging from 0.1 to 1.6 μM for 5 min at 37 °C. The residual activity of the enzyme was determined in the synthetic direction toward *S*-adenosylhomocysteine using adenosine and L-homocysteine. The results showed that compound **12** (IC_{50} = 0.48 μM) was about 2-fold more potent than the parent neplanocin A (IC_{50} = 0.82 μM) as shown in Figure 1 in Supporting Information. Incubation of the enzyme with fluoroneplanocin A (**12**) resulted in concentration- and time-dependent inhibition of the enzyme, and the inactivation by **12** was biphasic showing pseudo-first-order kinetics only in the first period of inactivation (about 1 min) as shown in Figure 2 in Supporting Information, indicating that another mechanism, such as competition between the inhibition process and substrate activity and competition between reversible and irreversible processes, might be possibly involved. Similar biphasic kinetics have also been observed in several inhibitions by 5′-*S*-alkynyl- and allenyl-5′-thioadenosines[14] and (*E*)-5′,6′-didehydro-6′-deoxy-6′-fluorohomoadenosine.[15] It was confirmed that fluoroneplanocin A (**12**) was an active-site-directed inhibitor by means of protection experiments with adenosine (0−250 μM), as shown in Figure 3 in Supporting Information.[14] The effects of fluoroneplanocin A (**12**) and neplanocin A on the NAD^+/NADH content of SAH were also determined after complete inactivation of the enzyme, as shown in Figure 4 in Supporting Information.[14] Fluoroneplanocin A (**12**) and neplanocin A resulted in similar conversions (79% and 88%) in the initial NAD^+/NADH ratio as shown in Table 2 in Supporting Information, indicating that **12** acts as substrate for the 3′-oxidative activity of SAH like neplanocin A.

The irreversible nature of the inhibition achieved with **12** was demonstrated in several ways. First, unlike neplanocin A,[5] the level of inhibition of SAH obtained after preincubation of the enzyme with 0.60 μM of **12** (0% of SAH activity) remained unchanged after dialysis as shown in Figure 5 in Supporting Information. Second, and more importantly, unlike neplanocin A,[5] the level of inhibition remained unchanged after the mixture was

incubated with excess cofactor NAD^+ or with excess substrate adenosine. Third, ^{19}F NMR spectra were measured to determine the release of the fluoride ion from compound **12** upon incubation with SAH, as shown in Figure 6 in Supporting Information.[15] Incubation of either SAH or fluoroneplanocin A (**12**) alone did not result in the appearance of a new fluoride ion peak except for the fluoride peak (−129.1 ppm) derived from **12**, as shown in the ^{19}F NMR spectra. When a large molar excess of **12** was incubated with SAH, a new fluoride ion peak at −122.8 ppm matching that of sodium fluoride, the external standard was observed in addition to the peak from the unreacted **12**, indirectly confirming the formation of the covalently trapped inhibitor. ^{19}F NMR experiments revealed that the ratio of moles of fluoride anion released to moles of enzyme used was 0.80, indicating that the conversion from intermediate **1** to intermediate **3** (Scheme 1) may not be completely irreversible when the deprotonation results in elimination of the enzyme instead of the fluoride anion. Thus, additional type I reversible mechanism by intermediate **1** may also be involved in the mechanism-based inhibition of SAH. This result might explain the biphasic kinetic behavior of compound **12**. Overall, this lack of recovery of SAH activity clearly demonstrates that fluoroneplanocin A (**12**) is a novel mechanism-based inhibitor of SAH that possibly operates by the proposed mechanism described in Scheme 1, and thus, it can be regarded as a new form of type II mechanism-based inhibitor not reported to date.

In Vitro Biological Evaluation. Antiviral assays of fluoroneplanocin A (**12**) against human immunodeficiency virus type 1 (HIV-1, MT-4 cells), herpes simplex virus type 1 (HSV-1, Vero cells), HSV-2 (Vero cells), and vesicular stomatitis virus (VSV, HeLa cells) were performed. Compound **12** exhibited more potent antiviral activity ($EC_{50} = 0.43\ \mu M$) against VSV than the control, ribavirin ($EC_{50} = 59.0\ \mu M$), without cytotoxicity up to 40 μM. However, fluoroneplanocin A (**12**) showed toxicity-dependent antiviral activities against other viruses such as HIV-1, HSV-1, and HSV-2.

Conclusion. Fluoroneplanocin A was designed and synthesized as a novel probe to investigate the mechanism-based inhibition of SAH. Fluoroneplanocin A was efficiently synthesized via a novel electrophilic vinyl fluorination reaction under mild conditions, which contrasts with most conventional harsher fluorinating procedures. Unlike neplanocin A showing reversible inhibition of SAH, fluoroneplanocin A exhibited a new type of irreversible inhibition that should serve as a new template for the design of new antiviral agents operating via SAH inhibition.

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Supporting Information Available: Complete experimental procedures and enzyme assay data. This material is available free of charge via the Internet at http://pubs.acs.org.

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