[S7-3] [10/18/2002(Fri) 14:30-15:00/Hall A]

Development of Mechanism-based Irreversible Inhibitors of S-Adenosylhomocysteine Hydrolase as Borad-spectrum Antiviral Agents

Kang Man Lee, Hyung Ryong Moon, Won Jun Choi, Jae Gyu Park,
Dae Hong Shin, and Lak Shin Jeong*

College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

S-Adenosylhomocysteine hydrolase (SAH)[1] catalyzes the hydrolysis of S-adenosylhomocysteine to adenosine and L-homocysteine. Inhibition of this enzyme accumulates S-adenosylhomocysteine, which in turn inhibits S-adenosyl-L-methionine dependent transmethylation, resulting in no formation of the capped methylated structure at the 5'-terminus of viral mRNA. Thus, S-adenosylhomocysteine hydrolase has been an attractive target for the development of broad spectrum of antiviral agents.

A number of compounds have been synthesized and evaluated as SAH inhibitors. These inhibitors are largely classified into two types as shown in figure 1.[2] 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. 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.

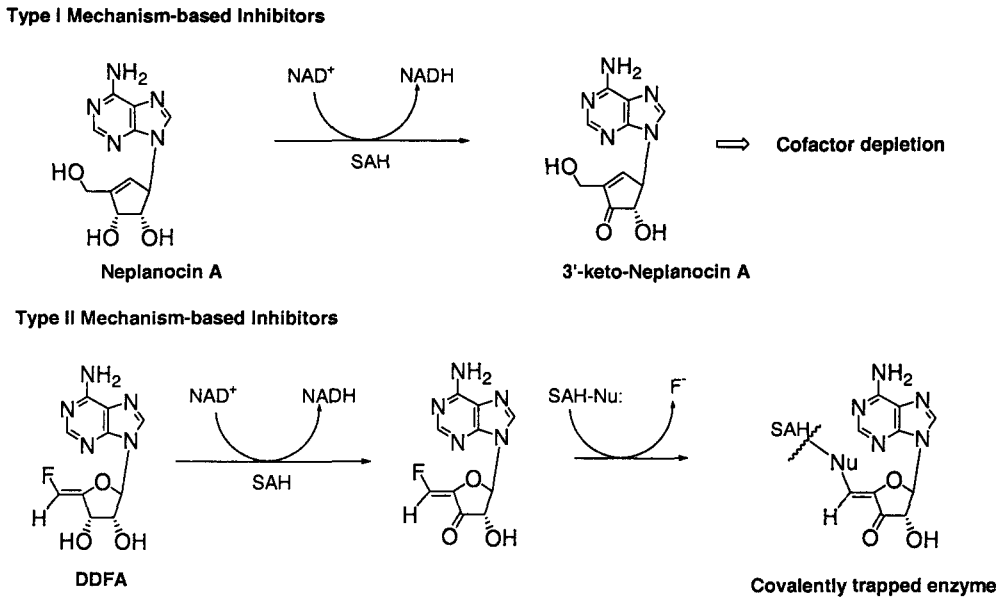


Figure 1. Classification of S-Adenosylhomocysteine Hydrolase Inactivators.

Neplanocin A has been recognized as one of the most potent inhibitors of SAH. Besides the cofactor depletion mechanism, it is mechanistically hypothesized that neplanocin A can form a covalent bond with a nucleophilic amino acid residue at the active site of the enzyme in a Michael type reaction, but its irreversible action may be easily reversed by the presence of acidic 4'-hydrogen. Therefore, if we substitute the vinyl hydrogen of the neplanocin A with the halogen, this resulting compound can inhibit the enzyme irreversibly because of no acidic hydrogen at the 4'-position (figure 2).

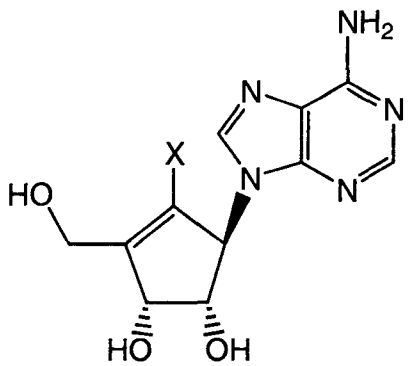
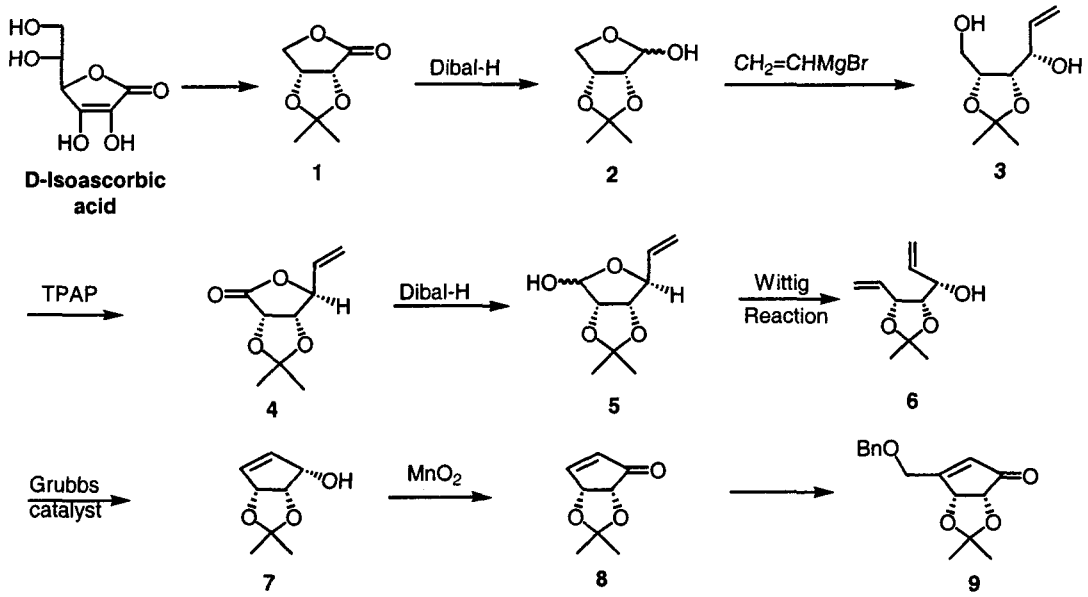


Figure 2. Structure of the Target Nucleosides

For the synthesis of the halogentated neplanocin A, the cheap and commercially available D-isoascorbic acid was converted to the key intermediate, cyclopentenone derivative as shown in Scheme 1.

Scheme 1

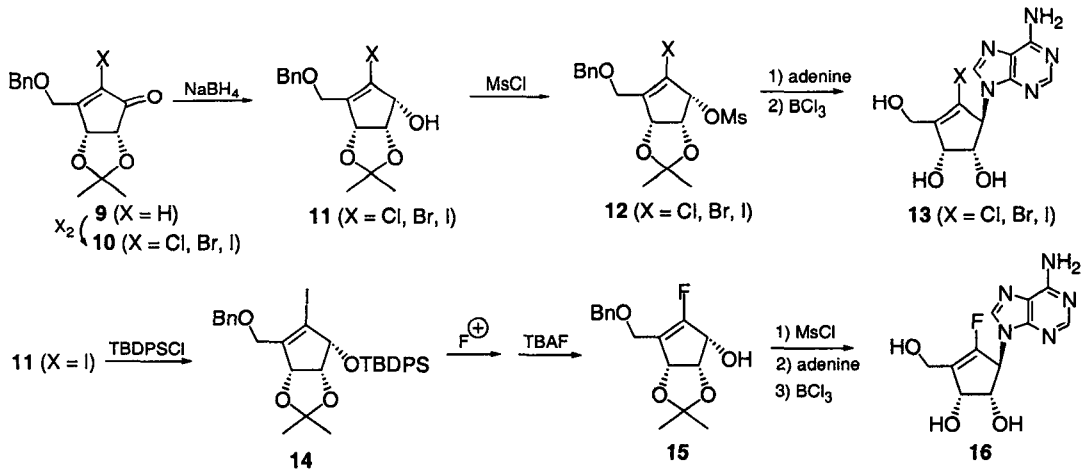


D-Isoascorbic acid was converted to the commercially available lactone **1** according to the known procedure.[3] Reduction of **1** with DIBALH gave the lactol **2**. Ring opening of **2** with vinylmagnesium bromide gave the diol **3** as a single stereoisomer. Selective oxidation of the primary alcohol over allylic alcohol in **3** was achieved using tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) to give lactone **4**. Reduction of **4** with DIBALH gave a lactol **5**. Wittig reaction of **5** with methyl triphenylphosphonium bromide was greatly affected by bases used in view of yield and epimerization. Use of *n*-BuLi gave the desired diene **6** almost exclusively in 40% yield, while use of bulky LDA afforded **6** as a sole product, but yield was only 50% even after recovering starting materials. To improve the yield, we used another bulky base, KO*t*Bu, but yield was low (42% yield after recovering starting materials) and extensive epimerization occurred, giving the desired diene **6** and its epimer in 1:1 ratio. However, use of DMSO anion as a base only produced the desired diene **6** in 88% yield without forming its epimerized product. Ring closure metathesis (RCM) of **6** with Grubbs catalyst was smoothly proceeded to give cyclopentenol **7** as a single stereoisomer. Oxidation of

allylic alcohol **7** with activated MnO_2 afforded **8**[4] which was converted to the key intermediate **9** by the reported procedure.[5]

Synthesis of the target nucleoside, halogenated neplanocin A from the key intermediate **9** is shown in Scheme 2. Compound **9** was treated with chlorine, bromine or iodine to give the halogenated ketone **10** which was reduced with sodium borohydride to give the allylic alcohol **11**. After mesylation of **11**, the mesylate **12** was condensed with adenine anion to afford the adenine derivative which was treated with boron trichloride to give the final nucleoside **12**. For the synthesis of the fluoro-neplanocin A (**16**), iodovinyl derivative **11** was protected with TBDPSCl to give **14**. Reaction of **14** with N-fluorobenzenesulfone imide followed by deprotection with *n*-tetrabutylammonium fluoride produced the desired vinylfluoride **15**. According to the similar procedure used in the preparation of **13**, vinylfluoride **15** was converted to the fluoro-neplanocin A (**16**).

Scheme 2



Inhibition of SAH by neplanocin A and its halogenated analogues **12** and **16** was measured using pure recombinant enzyme from human plancenta. The results showed that compound **16** (IC_{50} = 0.48 μM) was ca. 2-fold more potent than the parent neplanocin A (IC_{50} = 0.87 μM). However, the chloro (IC_{50} = 36.46 μM) and bromo (IC_{50} = 60.17 μM) derivatives were found to be less potent than neplanocin A and iodo derivative was inactive. It is interesting to note that enzyme inhibitory activity is inversely proportional to the size of the halogen atom, indicating the binding pocket is very small. The irreversible nature of the inhibition achieved with **16** was demonstrated using dialysis or the incubation with NAD^+ or adenosine, which clearly

demonstrates that fluoro-neplanocin A (**16**) is a novel mechanism-based inhibitor of SAH that possibly operates by our proposed mechanism, and thus it can be regarded as a type III mechanism-based inhibitor not reported to date.

References

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