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Synthesis of 5′-substituted fluoro-neplanocin A analogues: importance of a hydrogen bonding donor at 5′-position for the inhibitory activity of *S*-adenosylhomocysteine hydrolase

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Abstract—Four 5′-substituted fluoro-neplanocin A analogues **1a**–**d** were designed and synthesized, using cyclopentenone derivative **2** as a key intermediate. The inhibitory activity against SAH was in the following order: $NH_2 > SH > F, N_3$, indicating a hydrogen bonding donor such as OH or NH_2 was essential for inhibitory activity. All the final compounds showed much less decreased cytotoxicity in two cancer cell lines (Col2 and A549), implying that phosphorylation of the 5′-hydroxyl group of fluoro-neplanocin A is closely related to its high cytotoxicity.
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1. Introduction

A number of adenosine analogues including neplanocin A exhibit a broad-spectrum antiviral activity against DNA and RNA viruses. The antiviral spectrum of these compounds results from a common mechanism of action related to *S*-adenosylhomocysteine hydrolase (SAH).

The cellular enzyme SAH catalyzes the hydrolysis of *S*-adenosylhomocysteine to adenosine and L-homocysteine,[1,2] which is the only known catabolism pathway in eukaryotes.[3,4] *S*-Adenosylhomocysteine is a product of *S*-adenosylmethionine-dependent methylation reactions by methyltransferases and its accumulation inhibits methyltransferases by a feedback mechanism.[1,2] Viruses depend on the methylation reactions of their mRNA at the 5′-terminus for the stability and functioning of their mRNA. Inhibition of SAH causes a significant increase in the intracellular *S*-adenosylhomocysteine levels, which in turn inhibits virus-encoded methyltransferases, resulting in the inhibition of maturation of viral mRNA and thus exhibition of antiviral activity against (±)-RNA viruses, (−)-RNA viruses, and some DNA viruses.[5–8] Therefore, SAH has been considered as a promising target for the development of antiviral agents.

Neplanocin A, a naturally occurring carbanucleoside has been known to be one of the most potent inhibitors against SAH.[9] Fluoro-neplanocin A, which was recently developed in our laboratory, showed more potent inhibitory activity against SAH than neplanocin A and also a significant antiviral activity.[10] However, high cytotoxicity of fluoro-neplanocin A hindered it from being further developed as clinically useful antiviral agent. The cytotoxicity appears to be derived from the conversion of fluoro-neplanocin A into its triphosphate like neplanocin A, possibly interfering with DNA and/or RNA polymerases.[11]

Therefore, if the 5′-hydroxyl group of fluoro-neplanocin A is removed, a significant decrease of the cytotoxicity of fluoro-neplanocin A may be expected due to no phosphorylation at the 5′-position, while maintaining the

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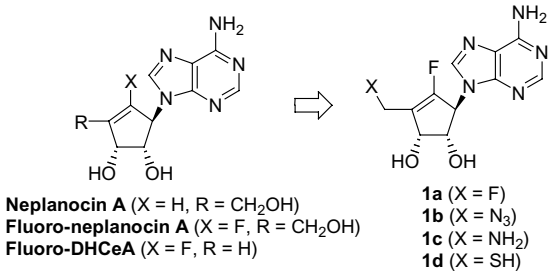


Figure 1. The rationale for the design of the desired nucleosides **1a**–**d**.

inhibitory activity against SAH. Thus, we synthesized fluoro-DHCeA, but it was found to be less potent than fluoro-neplanocin A against SAH, indicating that the 5′-hydroxyl group might be essential for hydrogen bonding.[12]

On the basis of these findings, it was interesting to design and synthesize 5′-substituted fluoro-neplanocin A derivatives **1a**–**d**, which can serve as a hydrogen bonding acceptor (F or N_3), a hydrogen bonding donor (NH_2), or as a bioisostere (SH), without being phosphorylated at the 5′-position (Fig. 1). From this study, we discovered that the 5′-position of fluoro-neplanocin A should be substituted with a hydrogen bonding donor such as hydroxyl or amino group. Herein, we wish to report the structure–activity relationship study of 5′-substituted fluoro-neplanocin A derivatives **1a**–**d** as *S*-adenosylhomocysteine hydrolase inhibitors.

2. Results and discussion

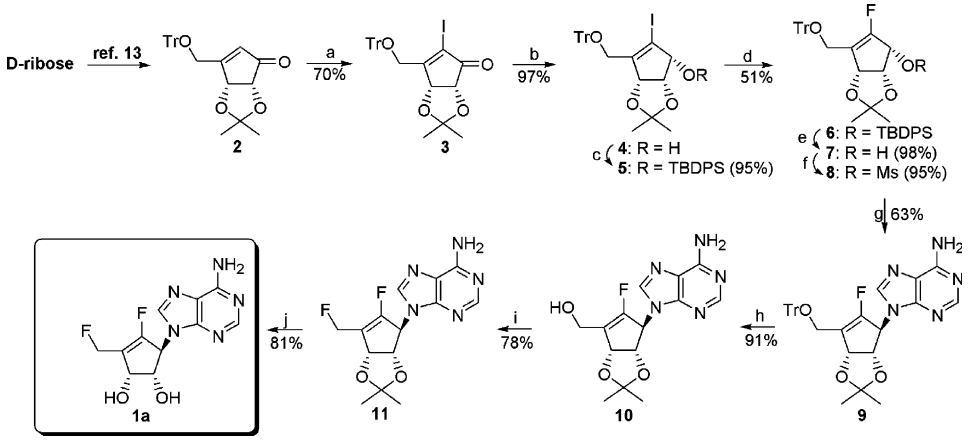
2.1. Synthesis

As shown in Scheme 1, fluoro-neplanocin A analogue **1a**, whose 5′-position was substituted with a hydrogen bonding acceptor, fluorine was synthesized from D-ribose.

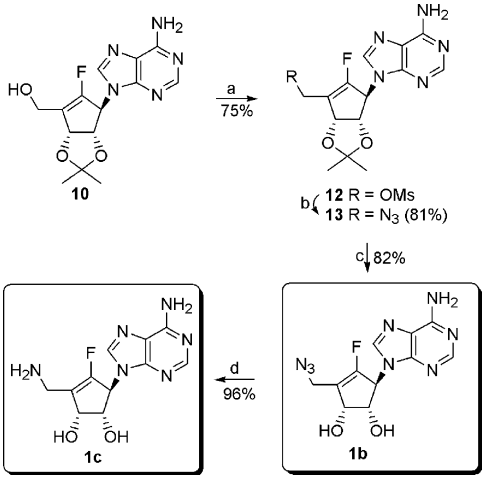
Cyclopentenone derivative **2**, a key intermediate was synthesized according to the short and efficient procedure[13] developed in our laboratory and converted to its iodo derivative **3** via addition–elimination reaction (I_2, pyridine, CCl_4).[14] Iodo compound **3** was stereo and regioselectively reduced to cyclopentenol **4**, resulting from the 5-membered cerium chelation between carbonyl oxygen and α-oxygen of the isopropylidene group.[15–17] The hydroxyl group of **4** was protected as TBDPS ether **5**. Slow addition of *n*-BuLi to a mixture of **5** and *N*-fluorobenzenesulfonimide afforded vinyl fluoride **6** in 51% yield through lithium–iodine exchange reaction. For the coupling with adenine base, compound **6** was converted to mesylate **8** by desilylation followed by mesylation of the resulting alcohol **7**. Condensation of **8** with adenine anion in DMF at 80 °C afforded the protected nucleoside **9** (63%). Trityl protecting group of **9** was selectively removed under *p*-toluenesulfonic acid in MeOH to give the corresponding alcohol **10** in 91% yield. Treatment of **10** with diethylaminosulfur trifluoride (DAST) at 0 °C produced its fluoro derivative **11**, which was deprotected using 33% aqueous trifluoroacetic acid to afford the final 5′-fluoro-fluoro-neplanocin A (**1a**).

5′-Azido- and 5′-amino-fluoro-neplanocin A derivatives, **1b** and **1c** were synthesized, as depicted in Scheme 2. For the introduction of a hydrogen bonding acceptor, azido group at 5′-position, the 5′-hydroxyl group of **10** was mesylated and then treated with sodium azide in DMF at 60 °C for 1 h to give the azide **13**. Treatment of **13** under acidic conditions gave the 5′-azido-fluoro-neplanocin A (**1b**). For the synthesis of 5′-amino derivative with hydrogen bonding donor ability, compound **1b** was converted to the 5′-amino-fluoro-neplanocin A (**1c**) using Lindlar's catalyst.[18]

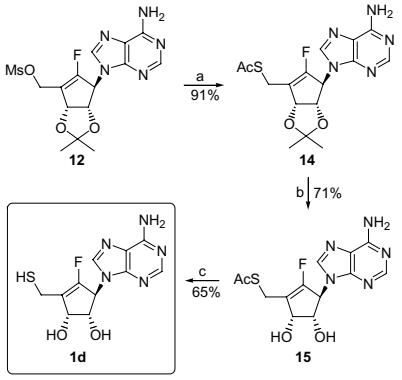
Finally, fluoro-neplanocin A analogue **1d** with 5′-sulfhydryl group was synthesized as a bioisostere of fluoro-neplanocin A (Scheme 3). For the introduction of thiol group, the same S_N2 reaction was utilized, as in the introduction of azido group in Scheme 2. Reac-



Scheme 1. Reagents and conditions: (a) I_2, pyridine, CCl_4, rt, 12 h; (b) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, 30 min; (c) TBDPSCl, imidazole, DMF, 40 °C, overnight; (d) *N*-fluorobenzene sulfonimide, *n*-BuLi, THF, −78 °C, 1 h; (e) *n*-Bu_4NF, THF, rt, 2 h; (f) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C, 30 min; (g) adenine, K_2CO_3, 18-Crown-6, DMF, 80 °C, overnight; (h) *p*-toluenesulfonic acid, MeOH, rt, 18 h; (i) DAST, CH_2Cl_2, 0 °C, 30 min; (j) 33% aqueous CF_3CO_2H, THF, rt, 3 d.



Scheme 2. Reagents and conditions: (a) methanesulfonyl chloride, Et_3N, CH_2Cl_2, −5 °C, 20 min; (b) NaN_3, DMF, 60 °C, 1 h; (c) 33% aqueous CF_3CO_2H, THF, rt, 3 d; (d) Lindlar's catalyst, H_2, MeOH, rt, 30 min.



Scheme 3. Reagents and conditions: (a) KSAc, DMF, rt, 1 h; (b) 33% aqueous CF_3CO_2H, THF, rt, 4 d; (c) 28% NH_4OH, MeOH, rt, 20 min.

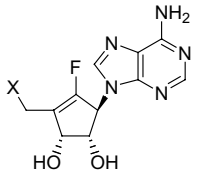
tion of **12** with potassium thioacetate in DMF at room temperature produced thioacetate **14** in a good yield. Successive deprotection of the isopropylidene group and the *S*-acetyl group of **14** afforded the 5′-sulfhydryl-fluoro-neplanocin A (**1d**).

2.2. Enzyme assay

SAH inhibitory activity of all synthesized 5′-substituted fluoro-neplanocin A analogues **1a–d** was measured using pure recombinant enzyme obtained from human placenta (Table 1).

All compounds were preincubated with the enzyme SAH at various concentrations for 5 min at 37 °C. The residual activity of the enzyme was measured in the synthetic direction toward *S*-adenosylhomocysteine using adenosine and L-homocysteine. As shown in Table 1, the parent fluoro-neplanocin A (X = OH) exhibited the most potent enzyme inhibitory activity (IC_{50} = 0.48 μM). Substitution of 5′-hydroxyl group of fluoro-neplanocin A with a bioisosteric hydrogen bonding acceptor (**1a**, X = F or **1b**, X = N_3) resulted in no enzyme inhibitory activity (IC_{50} > 100 μM), while introduction of a bioisosteric hydrogen bonding donor (**1c**, X = NH_2) at the 5′-position of fluoro-neplanocin A restored enzyme inhibitory activity (IC_{50} = 12.68 μM) dramatically. This trend was proved by compound **1d** (X = SH) with 5′-thiol group, which possesses a hydrogen atom, but has no ability to form appropriate hydrogen bonding with the active site residues of the enzyme. As expected, compound **1d** showed very weak enzyme inhibition (IC_{50} = 97.27 μM). The inhibitory activity of the tested compounds against SAH was in the following order: OH > NH_2 > SH > F, N_3, indicating the introduction of a hydrogen bonding donor such as OH or NH_2 at the 5′-position of fluoro-neplanocin A is essential for enzyme inhibitory activity.

Table 1. Inhibitory activity of the final compounds **1a–d** against SAH



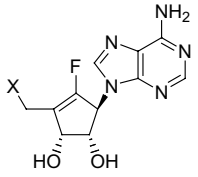
Compound	IC_{50} (μM)[a]
Fluoro-neplanocin A (X = OH)[b]	0.48
1a (X = F)	>100
1b (X = N_3)	>100
1c (X = NH_2)	12.68
1d (X = SH)	97.27

[a] Determined using pure recombinant enzyme obtained from human placenta.
[b] Data from Ref. 10.

2.3. Cytotoxicity assay

Cytotoxicity of the synthesized compounds **1a–d** was measured in human colon cancer cell lines (Col2) and human lung cancer cell lines (A549) to determine if 5′-hydroxyl group of fluoro-neplanocin A is responsible for its cytotoxicity.

Table 2. Cytotoxicity of the final compounds **1a–d** in two cancer cell lines (Col2, A549)



Compound	IC_{50} (μM)[a]	
	Col2[b]	A549[c]
Fluoro-neplanocin A (X = OH)	1.4	4.6
1a (X = F)	>100	>100
1b (X = N_3)	35.9	53.0
1c (X = NH_2)	39.2	42.8
1d (X = SH)	>100	>100

[a] Indicative of 50% survival determined with serial dilutions of the test compound.
[b] Human colon carcinoma cells.
[c] Human lung carcinoma cells.

As shown in Table 2, all compounds **1a**–**d** exhibited much less cytotoxicity than fluoro-neplanocin A in human colon cancer cell lines (Col2) and human lung cancer cell lines (A549). Azido and amino derivatives, **1b** and **1c** showed a 26- and 28-fold decreased cytotoxicity in human colon cancer cell lines (Col2), respectively, and fluoro and sulfhydryl compounds, **1a** and **1d** did not exhibit any cytotoxicity up to 100 μM. The similar trend was observed in human lung cancer cell lines (A549). This result indicates that conversion of 5′-hydroxyl group of fluoro-neplanocin A into its triphosphate might be the major cause of high cytotoxicity.

3. Conclusion

We synthesized novel 5′-substituted fluoro-neplanocin A derivatives to find out if the 5′-hydroxyl group of fluoro-neplanocin A was essential for hydrogen bonding with the enzyme SAH and responsible for high cytotoxicity. From this study, we revealed a hydrogen bonding donor such as OH or NH_2 was essential for enzyme inhibitory activity and also discovered there was a close correlation between cytotoxicity and 5′-phosphorylation of fluoro-neplanocin A like neplanocin A. These findings will provide great help for the design of potent and non-toxic SAH inhibitors.

Acknowledgements

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