Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3963–3965

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of 2-Fluoronoraristeromycin and Its Inhibitory Activity against *Plasmodium falciparum S*-Adenosyl-L-homocysteine Hydrolase

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Received 30 June 2003; accepted 28 August 2003

Abstract—Palladium-coupling reaction of (1*S*, 4*R*)-*cis*-4-acetoxy-2-cyclopenten-1-ol with sodium salt of 2-fluoroadenine resulted in the formation of (1*S*,4*R*)-4-(6-amino-2-fluoro-9*H*-purin-9-yl)cyclopent-2-en-1-ol. Subsequent oxidation was carried out with osmium tetraoxide (OsO_4) in the presence of 4-methylmorpholine *N*-oxide (NMO) to give 2-fluoronoraristeromycin, possessing significant inhibitory activity against recombinant *Plasmodium falciparum* SAH hydrolase.

S-Adenosyl-L-homocysteine (SAH) hydrolase has emerged as a target enzyme for the molecular design of antiviral, antitumor, antiparasitic, antiarthritic and immunosuppressive agents.[1–3] SAH is formed after the donation of the methyl group of *S*-adenosyl-L-methionine (SAM) to a methyl accepter and is hydrolyzed to adenosine and homocysteine by SAH hydrolase, physiologically. Inhibition of SAH hydrolase results in cellular accumulation of SAH. It is a potent feedback inhibitor of SAM-dependent biological methylation such as the 5′-end of eukaryotic mRNA.[1,4] In contrant to human SAH hydrolase, *Plasmodium falciparum* SAH hydrolase contains a 41-amino acid insert (Gly 145-Lys 185) inside the sequence.[5] *P. falciparum* causes malignant malaria. This difference may produce selective sensitivity against each SAH hydrolase inhibitor. Neplanocin A (**1**) and aristeromycin (**2**) are naturally occurring products possessing inhibitory activity against SAH hydrolase. When these inhibitors work as a substrate for adenosine kinase, they show cytotoxicity. Neplanocin A (**1**) and aristeromycin (**2**) are also known to be rapidly deaminated by adenosine deaminase to a chemotherapeutically inactive inosine congener.[6] In order to overcome these disadvantages in the development of chemotherapeutic agents, chemical modifications of carbocyclic nucleosides have been carried out. Because noraristeromycin (**3**) lacks the 5′-methylene unit of aristeromycin (**2**), it does not work as a substrate for adenosine deaminase (Fig. 1). Recently, we have found that IC_{50} of noraristeromycin (**3**) against human and *P. falciparum* recombinant SAH hydrolase[7] is 1.1 and 3.1 μM, respectively.[8]

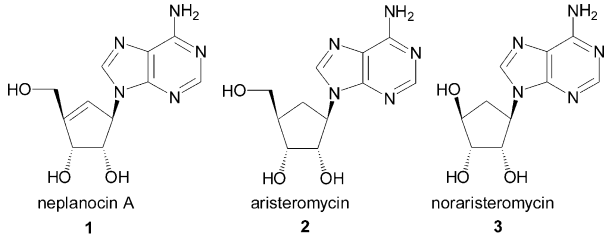

Figure 1. Structure of carbocyclic nucleosides.

We have reported a method for the preparation of noraristeromycin derivatives possessing 2- or 8-position modified adenine or 8-aza-7-deazaadenine.[8] Among these derivatives, 2-aminonoraristeromycin selectively showed inhibitory activity against recombinant *P. falciparum* SAH hydrolase. In the course of the investigation, we envisaged that a noraristeromycin derivative possessing a polar-substituent at the 2-position of the adenine ring would have selective inhibition against

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doi:10.1016/j.bmcl.2003.08.074

P. falciparum recombinant SAH hydrolase. Furthermore, it has been documented that the introduction of a halogen atom at the 2-position of adenine nucleosides prevented a decrease in the biological activity by the adenosine deaminase digestion.[9]

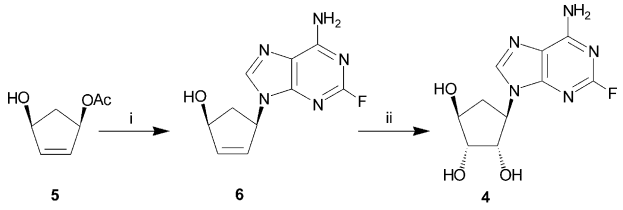
This paper describes a convenient method for the preparation of 2-fluoronoraristeromycin (**4**) with the aim of developing antimalarial agents, which possess inhibitory activity against recombinant *P. falciparum* SAH hydrolase.

Chemistry

Palladium-coupling reaction[10] of (1*S*, 4*R*)-*cis*-4-acetoxy-2-cyclopenten-1-ol (**5**) with sodium salt of commercially available 2-fluoroadenine resulted in the formation of (1*S*, 4*R*)-4-(6-amino-2-fluoro-9*H*-purin-9-yl)cyclopent-2-en-1-ol (**6**) in 60% yield. Subsequent oxidation of compound **6** was carried out with osmium tetraoxide (OsO_4) in the presence of 4-methylmorpholine *N*-oxide (NMO) to give 2-fluoronoraristeromycin (**4**) in 87% yield. The structures of compounds **4** and **6** were supported by spectral data (^1H NMR, ^{13}C NMR, MS and HRMS) and microanalytical results (see refs 11 and 12) (Scheme 1).

Biological Activities

A profile of the inhibitory activity of 2-fluoronoraristeromycin (**4**) against recombinant *P. falciparum* and human SAH hydrolase is shown in Figure 2. Introduction of a 2-fluoro substituent to the adenine ring of noraristeromycin (**3**) causes moderate selectivity against



Scheme 1. Reagents and conditions: (i) 2-fluoroadenine, NaH, $(Ph_3P)_4Pd$, DMSO, THF, 55 °C; (ii) OsO_4, NMO, THF, H_2O.

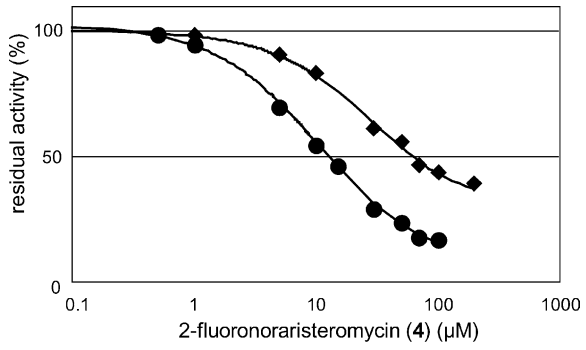


Figure 2. Inactivation of SAH hydrolase by compound **4**. SAH hydrolase was incubated with compound **4** at 30 °C [(●) *P. falciparum* SAH hydrolase, (◆) human SAH hydrolase].

P. falciparum SAH hydrolase as determined by inhibitory activity on the synthetic direction of SAH. Inhibitory activities (EC_{50} and K_i values) of compounds **3** and **4** against human and *P. falciparum* recombinant SAH hydrolases are summarized in Table 1 (see ref 13). Noraristeromycin (**3**) showed IC_{50} values of 1.1 and 3.1 μM against human and *P. falciparum* SAH hydrolase, respectively. The K_i values against human and *P. falciparum* SAH hydrolase were 0.16 and 0.18 μM, respectively. The selective index (human SAH hydrolase/*P. falciparum* SAH hydrolase) of compound **3** based on the corresponding K_i values is 0.89. On the other hand, 2-fluoronoraristeromycin (**4**) is a more significant inhibitor against *P. falciparum* SAH hydrolase ($IC_{50} = 13$ μM and $K_i = 0.48$ μM) than against human SAH hydrolase ($IC_{50} = 63$ μM and $K_i = 7.9$ μM). The introduction of a fluorine atom to the 2-position of the adenine ring brought an 18-fold increase in the selective index (see Table 1).

In vitro antimalarial activities and cytotoxicities of compounds **3** and **4** were determined according to the method previously described.[14] As shown in Table 2, the toxicity of the 2-fluoro derivative (**4**) against FM3A cells brought about 100-fold decrease in comparison with that of noraristeromycin (**3**). However, antimalarial activity of 2-fluoronoraristeromycin (**4**) did not change, and the selective index, based on in vitro antimalarial activity, increased to 0.97 from 0.01.

In this research, we have found that 2-floronoraristeromycin (**4**) showed significant selectivity against *P. falciparum* SAH hydrolase and possessed moderate inhibition against *P. falciparum* proliferation. Introduction of a fluorine atom to the 2-position of the adenine ring of noraristeromycin (**3**) decreased the cytotoxicity against mammalian cells (FM3A). This observation provides a clue to the development of facile chemotherapeutic agents against malaria.

Table 1. Inhibitory activities of compounds against human and *P. falciparum* SAH hydrolases

Compd	Human		*P. falciparum*		Selective index[a]
	IC_{50} (μM)	K_i	IC_{50} (μM)	K_i	
3	1.1	0.16	3.1	0.18	0.89
4	63	7.9	13	0.48	16

[a]Selective index: mean of K_i value for human SAH hydrolase/mean of K_i value for *P. falciparum* SAH hydrolase.

Table 2. Inhibitory activities of compounds against FM3A and *P. falciparum*

Compd	FM3A[a] EC_{50} (μM)	*P. falciparum*[b] EC_{50} (μM)	Selective index[c]
3	0.072	7.4	0.01
4	7.2	7.4	0.97

[a]FM3A is a mammalian cell.
[b]*P. falciparum* is human malaria parasites.
[c]Selective index: mean of EC_{50} value for FM3A cell/mean of EC_{50} value for *P. falciparum*.

Acknowledgements

This research was in part supported by Grants-in-Aid for Scientific Research on Priority Areas (KAKENHI 13226034 and 14001072) (to Y.K.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and a Grant-in-Aid for JSPS Fellows (to F.Z.) from Japan Society for the Promotion of Science (JSPS).

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11. Data for **6**; mp 210 °C; ^{1}H NMR ($CDCl_3$) δ: 8.02 (s, H-8, 1H), 7.78 (s, NH_2, 2H), 6.17 (d, J = 5.2 Hz, H-3′, 1H), 5.98 (d, J = 5.6 Hz, H-2′, 1H), 5.30 (d, J = 5.6 Hz, H-4′,-1′, 2H), 4.70 (s, OH-4′, 1H), 2.87 (m, H-5′, 1H), 1.68 (m, H-5′, 1H); ^{13}C NMR ($CDCl_3$) δ: 158.62 (d, J = 184.7 Hz), 157.50 (d, J = 3.9 Hz), 150.22 (d, J = 19.8 Hz), 139.52, 139.42 (d, J = 2.9 Hz), 130.52, 117.22 (d, J = 3.9 Hz, 1C), 73.62, 57.07, 41.26. Mass (EI) m/z: 235 (M^+), 207, 206, 153, 133; HRMS (EI) cald for $C_{10}H_{10}FN_5O$ 235.0869 found 235.0864. Anal. calcd for $C_{10}H_{10}FN_5O$·1/5 EtOAc: C, 51.30; H, 4.62; N, 27.70, found: C, 51.47; H, 4.77; N, 27.90 (crystallization from EtOAc).
12. Data for **4**; mp 233 °C (dec); ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, H-8, 1H), 7.75 (s, NH2, 2H), 5.16 (d, J = 4.4 Hz, OH-4′, 1H), 5.00 (d, J = 6.8 Hz, OH-2′, 1H), 4.88 (d, J = 3.6 Hz, OH-3′, 1H), 4.58 (q, J = 8.8 Hz, H-1′, 1H), 4.42 (q, J = 6.4 Hz, H-2′, 1H), 3.88 (t, J = 1.6 Hz, H-4′, 1H), 3.73 (s, H-3′, 1H), 2.56 (m, H-5′, 1H), 1.74 (m, H-5′, 1H); ^{13}C NMR (DMSO-d_6) δ: 158.51 (d, J = 172.7 Hz), 157.40 (d, J = 7.8 Hz), 150.93 (d, J = 19.9 Hz), 140.13 (d, J = 2.9 Hz), 117.41 (d, J = 4.4 Hz), 76.58, 75.23, 73.54, 58.24, 36.49; Mass (EI) m/z: 269 (M^+), 195, 180, 154, 134; HRMS (EI) cald for $C_{10}H_{12}FN_5O_3$ 269.0924, found 269.0933. Anal. calcd for $C_{10}H_{12}FN_5O_3$·1/3H_2O: C, 43.64; H, 4.64; N, 25.44. Found: C, 43.88; H, 4.50; N, 25.09 (crystallization from EtOAc).
13. The enzyme was incubated with 100 μL adenosine, 5 mM DL-homocysteine and inhibitors in 0.2 mL of 10 mM potassium phosphate, pH 7.2, buffer at 30 °C for 2 min in the standard assay ststem (see ref 8). The reaction was started by the addition of 5 μL of SAH hydrolase (human: 0.43 μg, *P. falciparum*: 0.54 μg) and terminated by the addition of 20 μL of 0.67 N HCl. The reaction mixture was kept on ice until the HPLC analysis. The mixture was analyzed for SAH by a Shimadzu LC-10A VP HPLC system. In the synthetic reaction, one unit of SAH hydrolase was defined as the amount synthesizing 1 μmol of SAH/min at 30 °C.
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