The worldwide blood parasite: malaria

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Human malaria is a worldwide problem. More than one-third of the world's population (about two billion people) live in malaria-endemic areas, and one billion people are estimated to be carriers of the causative parasite, *Plasmodium* spp., at any time.[1] About 500 million people become infected annually, resulting in more than two million deaths.[2] *Plasmodium falciparum* is the main cause of severe malaria outbreaks and mortality rate. Moreover, the increasing problem of multidrug resistant strains necessitates the discovery of new and highly efficacious drugs.

S-Adenosyl-L-homocysteine (SAH) hydrolase has emerged as a target enzyme for the molecular design of antiviral, antitumor, antiparasitic, antiarthritic and immunosuppressive agents.[3,4,5] 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.[3,6] In contrast to human SAH hydrolase, *P. falciparum*SAH hydrolase contains a 41-amino acid insert (Gly145-Lys185) inside the sequence.[7] *P. falciparum* causes malignant malaria. This difference may produce selective sensitivity against each SAH hydrolase inhibitor. Neplanocin A and aristeromycin 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 and aristeromycin are also known to be rapidly deaminated by adenosine deaminase to a chemotherapeutically inactive inosine congener.[8] In order to overcome these disadvantages in the development of chemotherapeutic agents, chemical modifications of carbocyclic nucleosides have been carried out. Because noraristeromycin lacks the 5'-methylene unit of aristeromycin, it does not work as a substrate for adenosine deaminase.

We have found that the introduction of fluorine to the 2-position o noraristeromycin increased selective inhibition against *P. falciparum* SAHH (PfSAHH) compared with human SAHH (HsSAHH).[9] PfSAHH has additiona space near the 2-position of the adenine-ring, in the substrate binding pocke compared with HsSAHH.[10] Mutagenic analysis of the amino acid residue formin the additional space confirmed that inhibitor selectivity is due to the difference o only one amino acid residue.[11] We carried out synthesis of several kinds c carbocyclic nucleosides and evaluated their inhibitory activity against PfSAHH and HsSAHH.[12] Recently, we have found *P. falciparum* TMP kinase as a nev target enzyme for the development of anti-malaria agents.[13] Therefore, th properties of malaria TMP kinase and synthesis of its inhibitors are als

discussed. These results will significantly contribute to the design of anti-mala agents.

Human malaria is a worldwide problem and more than one-third of the wor population (about two billion peoples) live in malaria-endemic areas, and billion people are estimated to be carriers of the causative parasite, *Plasmod* spp., at any one time.[1] About 500 million people become infected annua resulting in more than 2 million deaths.[2] *Plasmodium falciparum* is the m cause of severe malaria outbreaks and high deaths. Moreover, the increas problem of multidrug resistant strains necessitates the discovery of new highly efficacious drugs. Figure 1 shows epidemic area of malaria and a conventional anti-malaria drugs, such as chloroquine, quinine, proguanil artemisinin. Malarias currently spreading tolerate a conventional anti-mala drug such as chloroquine. Therefore, we are currently developing new dr which will be more effective in controlling the spread of malaria, (Figure 1).

S-Adenosyl-L-homocysteine (SAH) hydrolase has emerged as a target enzy for the molecular design of antiviral, antitumor, antiparasitic, antiarthritic immunosuppressive agents.3,4,5 SAH is formed after the donation of the me group of S-adenosyl-L-methionine (SAM) to a methyl accepter and is hydroly to adenosine and homocysteine by SAH hydrolase, physiologically. Inhibitior SAH hydrolase results in cellular accumulation of SAH. It is a potent feedb inhibitor of SAM-dependent biological methylation such as the 5'-end eukaryotic mRNA.3,6 (Figure 2).

Palmer and Abeles have elucidated the mechanism of SAH hydrolase. In hydrolytic direction, the first step involves oxidation of the 3'-hydroxyl group SAH by enzyme-bound NAD+ followed by β-elimination of homocysteine to g the 3'-keto-4',5'-didehydro-5'-

deoxyadenosine as an enzymatically bound intermediate. Micheal's addition water to this intermediate affords the 3'-ketoadenosine, which is then reduced enzyme-bound NADH to produce adenosine.

SAH hydrolase can also catalyze the reaction in the synthe direction *via* the same mechanism, using adenosine a homocysteine as substrates and affording SAH, (Figure 3).

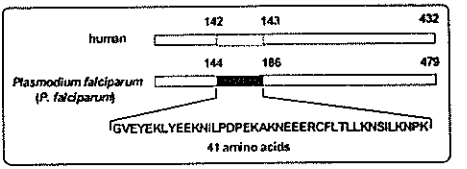
In contrast to human SAH hydrolase, P. falciparumSAH hydrolase contains a amino acid insert (Gly145 - Lys185) inside the sequence.7 P. falcipar causes malignant malaria. This difference may produce selective sensiti against each SAH hydrolase inhibitor, (Figure 4).

Figure 5 shows SAH hydrolase inhibitors. These analogues included adenos analogues with acyclic "sugar" moieties such as (S)-DHPA and D-eritadeni Neplanocin A and aristeromycin are natural products. These compounds quite potent. Unfortunately, the cytotoxicity of these compounds precluc clinical use as antiviral agents. Clearly, removal of the hydroxymethyl substitu would preclude 5'-phosphorylation by adenosine kinase. Therefore, DHCe DHCaA and noraristeromycin were designed. These compounds retain antiv activity while their toxicity is considerably lower than the parent compoun When these inhibitors work as a substrate for adenosine kinase, they sh cytotoxicity. Neplanocin A and aristeromycin are also known to be rapi deaminated by adenosine deaminase to a chemotherapeutically inactive inos congener.8 On the other hand, a 4',5'-unsaturated 5'-fluoroadenosine (M

28,842), an inhibitor of SAH hydrolase , was found to inhibit markedly the growth of Plasmodium falciparum in vitro. SAH hydrolase represents a worthwhile target for the development of anti-malaria agents, (Figure 5).

In order to overcome these disadvantages in the development of chemotherapeutic agents, chemical modifications of carbocyclic nucleosides have been carried out. Because noraristeromycin lacks the 5'-methylene unit of aristeromycin, it does not work as a substrate for adenosine deaminase.

Figure 4. Selective inhibition against P. falciparum SAH hydrolase



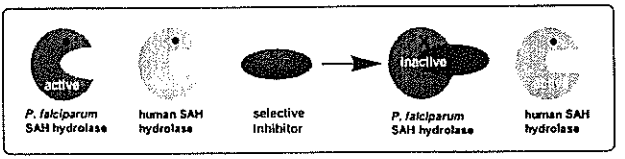
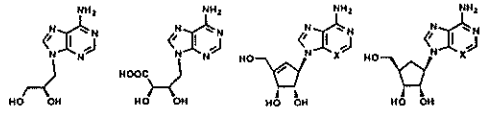


Figure 5. SAH hydrolase Inhibitors



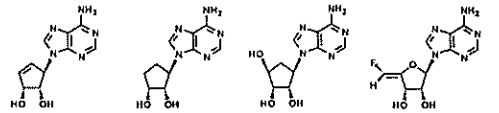
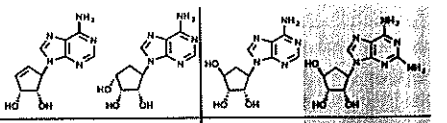


Table 1



human SAH hydrolase IC_{50}, μM	560	76	1.1	60
P. falciparum SAH hydrolase IC_{50}, μM	60	7.9	3.1	18

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Figure 7. Synthesis of 2-fluoronoraristeromycin

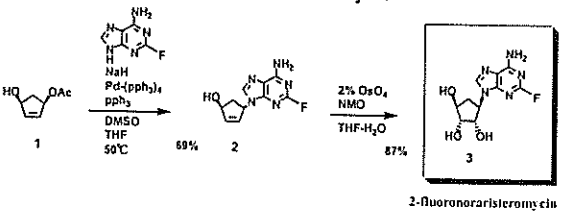
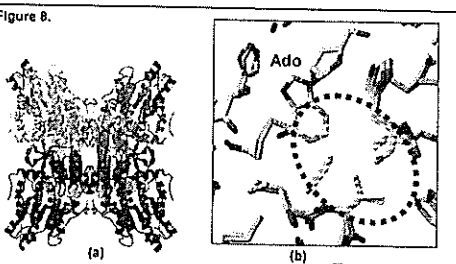


Figure 8.



T60 (human)
C59 (*P. fal*)

Figure 9.

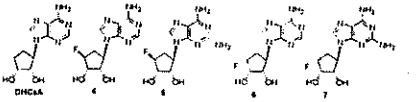
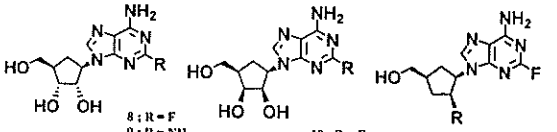


Figure 10. Inhibitory activities 2-modified aristeromycins



8 ; R = F
9 ; R = NH_2
12; R = H
(aristeromycin)

10 ; R = F
11 ; R = NH_2

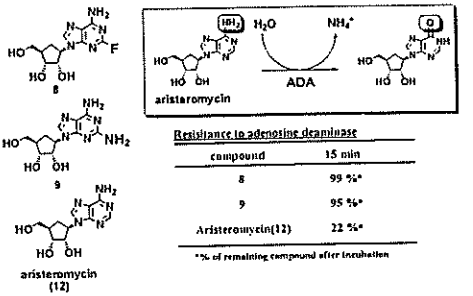
13; R = H
14; R = OH

compound	IC_{50} (μM) Hs SAHH	IC_{50} (μM) Pf SAHH	Selective Index	
8	47.2	1.98	24	280 -fold increased
9	90.7	4.51	20	
12	4.85	57	0.085	
10,11, 13, 14	>500	>500	-	

Selective index : IC_{50} of Hs SAHH/ IC_{50} of Pf SAHH

Ando, T., Iwata, M., Zulfiqar, F., Miyamoto, T., Nakanishi, M., & Kitade, Y., *Bioorg. & Med. Chem.*, 16, 3809-3815 (2008).

Figure 11. Resistance aginst adenosine deaminase



Resistance to adenosine deaminase

compound	15 min
8	99 %*
9	95 %*
Aristeromycin(12)	22 %*

*% of remaining compound after incubation

Figure 12. Pyrimidine metabolism

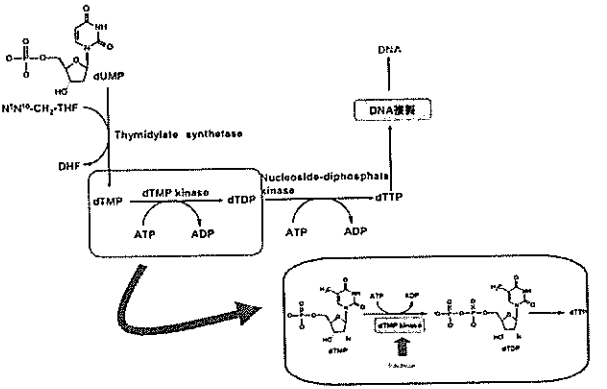
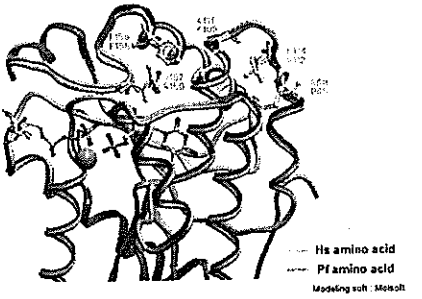


Figure 13. Modeling of Hs TMP kinase and Pf TMP kinase



We prepared modified carbocyclic purine analogs as shown in Figure 6. Alon the top row as shown in Table 1, I have listed IC50 values against human SA hydrolase and the bottom row shows IC50 values against P.falciparum SA hydrolase. The smaller number, the greater the activity. Among thes derivatives, 2-amino-norariste romycin selectively showed inhibitory activit against P. falciparum SAH hydrolase. Therefore, we envisaged that introductio

of a fluorine atom to the 2-position of the adenine ring could increase selecti against P. falciparum SAH hydrolase.

At first, we prepared 2-fluoronoraristeromycin (3) as shown in Figure 7. Th palladium- coupling reaction of compound (1) with sodium salt of commerci available 2-fluoroadenine resulted in the formation of the coupling compo (2) in 69 % yield. Subsequent oxidation of compound 2 was carried out v osmium tetraoxide (OsO_4) in the presence of 4-methylmorpholine N-ox (NMO) to give 2-fluoronoraristeromycin (3) in 87 % yield. These structures compounds 2 and 3 were supported by spectral data and microanalytical resu

A profile of the inhibitory activity of 2-fluoronoraristeromycin (3; FNAM) aga P. falciparum and human SAH hydrolase is shown in Table 2. Introduction fluorine atom to the adenine ring causes selectivity against P. falciparum S hydrolase. The Ki values of FNAM against human and P. falciparum S hydrolase were 7.9 μM and 0.48 μM, respectively. The selective index was The introduction of a fluorine atom brought an 18-fold increase in the selec index. In vitro antimalaria activity and cytotoxicity are shown in Table 3. toxicity of the 2-fluoro derivative (3) against FM3A cells brought about a 100- decrease. However, antimalaria activity of 2-fluoronoraristeromycin (3) v retained.

Isolation of non-fusion malaria enzyme, P. falciparum SAH hydrolase, has sc difficulty. But isolation of His-tag malaria enzyme gave a large amount of pro with high purity. This protein is applicable to X-ray crystallography analy Finally, we were succeeding the crystallization of malaria SAH hydrolase. spent three years for the cryctallization of P. falciparum SAH hydrolase. analyzed the structure of malaria SAH hydrolase using Spring-8 at Harim Japan. The crystallographic analysis revealed the structure of malaria S hydrolase as shown Figure 8 (a). The enzyme, in its active form, is a ho tetramer of identical subnuits and has a molecular mass of approximately kDa. The insertion sequence of 41-amino acids is far from the active s Figure 8 (b) shows the adenosine binding site of malaria SAH hydrol Interestingly, we have found a crucial structural difference between adenosine biding site of malaria enzyme and that of human enzyme. Thr6 located near the C2 of the adenine ring of the adenosine in human enzyme. malaria enzyme, Thr60 is replaced by Cys59. The malaria SAH hydrolase h space for a fluorine atom at the 2-posion of 2-fluoroader carbocyclicnuleosides. This observation explains the selectivity of fluoroadenine derivatives against malaria SAH hydrolase. We have found the introduction of a fluorine to the 2-position of noraristeromycin increa selective inhibition against P. falciparum SAH hydrolase (PfSAHH) compa with human SAH hydrolase (HsSAHH).9 PfSAHH has additional space near 2-position of the adenine-ring, in the substrate binding pocket compared HsSAHH.10 Mutagenic analysis of the amino acid residue forming additional space confirmed that inhibitor selectivity is due to the differenc only one amino acid residue.11 (Figure 8).

We carried out synthesis of several kinds of carbocyclic nucleosides evaluated their inhibitory activity against PfSAHH and HsSAHH.12 4'-I - Flu derivative 4 showed inhibitory activity against human enzyme. On the o hand, the 2-amino 4'-I -fluoro derivative (7) showed inhibitory activity aga

malaria enzyme. These results will contribute greatly to the design of potent inhibitors against P. falciparum SAH hydrolase, (Figure 9 & Table 4)

The inhibitory activities of base- and/or sugar -modified aristeromycins against HsSAHH and PfSAHH are summarized as IC50 values in Table of Figure 10. The 2-fluorinated aristeromycin (8) and 2-amino compound 9 showed strong inhibitory activities against PfSAHH with IC50 value of 1.98 and 4.51 □M and superior selective index 24 and 20, respectively. These values were better than those of the typical compounds which we had synthesized. The other synthetic compounds did not show any inhibitory activity up to 500□M. The susceptibility of these compounds to adenosine deaminase was investigated under the previously reported conditions as show in Figure 11. 2-Fluoroaristeromycin 8 was completely resistant to adenosine deaminase unlike aristeromycin (12), which was rapidly deaminated within 15 min under the same reaction conditions. 2-Aminoaristeromycin 9 was slightly deaminated with a 5% conversion ratio. The introduction of a halogen atom was effective in designing inhibitors showing the resistance to adenosine deaminase. This observation will significantly contribute to the design of potent inhibitors as anti-malarial agents. (See Figure10 & 11).

Recently, we have found P. falciparum TMP kinase as a new target enzyme for the development of anti-malaria agents.13 Plasmodium falciparum thymidylate kinase (PfTMK) can tolerate a range of substrates, which distinguishes it from other thymidylate kinases. The enzyme not only phosphorylates TMP and dUMP but can also tolerate bulkier purines, namely, dGMP, GMP, and dIMP. In order to probe the flexibility of PfTMK in accommodating ligands of various sizes, we developed six mutant enzymes and subjected to thermodynamic, inhibitory and catalytic evaluation. Mutation of Phe74 to alanine equally affected TMP and dGMP kinase activity. Kinase activity was markedly affected by introducing a larger lysine residue instead of A111. The lack of the hydroxyl group after inducing mutation of Y107F affected enzyme activity, and had more severe impact on dGMP kinase activity. PfTMK can be inhibited by both purine and pyrimidine nucleosides, raising the possibility of developing highly selective drugs. Thermodynamic analysis revealed that enthalpic forces govern both purine and pyrimidine nucleoside monophosphate binding, and the binding affinity of both substrates was highly comparable. The heat produced due to dGMP binding is lower than that attributable to TMP. This indicates that additional interactions occur with TMP, which may be lost with larger dGMP. Targeting PfTMK not only affects thymidine nucleotide synthesis but may also affect purine nucleotides, and thus it has become an attractive antimicrobial target. This study provides differentiations between some similarities and differences in the interactions of PfTMK with TMP and dGMP. In this context, similar properties and variations provide valuable information for optimizing new selective inhibitors. Therefore, the properties of malaria TMP kinase and synthesis of its inhibitors are also discussed, (Figure 12 and 13)

These results described in this paper will significantly contribute to the design of anti-malarial agents.

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