Metabolites of Febrifugine and Its Synthetic Analogue by Mouse Liver S9 and Their Antimalarial Activity against *Plasmodium* Malaria Parasite

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Received April 29, 2003

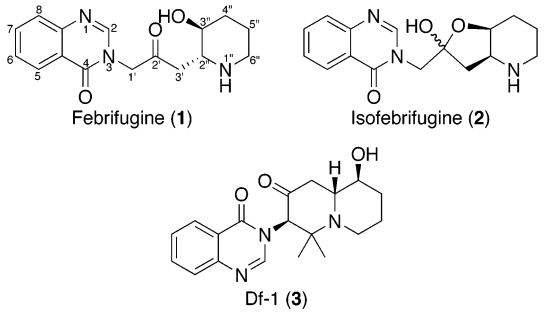
Quinazolinone type alkaloids, febrifugine (**1**) and isofebrifugine (**2**), isolated from *Dichroa febrifuga* roots, show powerful antimalarial activity against *Plasmodium falciparum*. Unfortunately, their emetic effect and other undesirable side effects have precluded their clinical use for malaria. Because of their antimalarial potency, analogues were searched for, with the goal of preserving the strong antimalarial activity, while dramatically reducing side effects. We expected that compounds useful in drug development would exist in metabolites derived from **1** and Df-1 (**3**), the condensation product of **1** with acetone, by mouse liver S9. Feb-A and -B (**4** and **5**) were isolated as the major metabolites of **1**. In addition to **4** and **5**, feb-C and -D (**6** and **7**) were also purified from the metabolic mixture of **3**. Compounds **4** and **5** were compounds oxidized at C-6 and C-2 of the quinazolinone ring of **1**, respectively. Compounds **6** and **7**, derived from **3**, also bear febrifugine type structures in which the 4″- and 6″-positions of the piperidine ring of **1** were oxidized. In vitro antimalarial and cytotoxic tests using synthetically obtained racemic **4**−**6** and enantiomerically pure **7** demonstrated that **4** and **6** had antimalarial activity against *P. falciparum*, of similar potency to that of **1**, with high selectivity. The antimalarial activity of **5** and **7**, however, was dramatically decreased in the test. The in vitro antimalarial activity of analogues **22** and **43**, which are stereoisomers of **4** and **6**, was also evaluated, showing that **22** is active. The results suggest that basicity of both the 1- and the 1″-nitrogen atoms of **1** is crucial in conferring powerful antimalarial activity. Racemic **4** and **6** exhibited powerful in vivo antimalarial activity against mouse malaria *P. berghei*, and especially, no serious side effects were observed with **4**. Thus, the metabolite **4** appears to be a promising lead compound for the development of new types of antimalarial drugs.

Introduction

Malaria is caused by the protozoan parasite of the genus *Plasmodium* and leads to mortality if patients infected with *Plasmodium falciparum* are left untreated. Malaria affects approximately 300 million patients worldwide, leading to more than 2 million deaths a year. The treatment of *P. falciparum* malaria has attracted global attention because of the rapid growing resistance of the parasites toward all known antimalarial drugs, such as chloroquine. The chemotherapy of malaria thus continues to be a matter of utmost concern for medicinal chemists.[1]

Roots of *Dichroa febrifuga*, the plant belonging to the family Saxifragaceae, have been used as a traditional antimalarial in China. The quinazolinone type alkaloids febrifugine (**1**) and its stereoisomer, isofebrifugine (**2**), have been identified as the active components of these roots (Chart 1).[2] Although alkaloid **1** demonstrated outstanding antimalarial activity, both in vitro and in vivo with no resistant parasite to **1** reported, its use as an antimalarial drug has been precluded due to its strong emetic properties and other side effects.[3] However, the potent antimalarial activity of **1** stimulated medicinal chemists to pursue compounds derived from **1**, which may be valuable leads for novel drugs. We also made an effort to account for the role of the structural components of **1** in its activity and proposed active analogues such as the reductive product at C-2′, oxidative product at C-3″, and Df-1 (**3**), the condensation product of **1** with acetone.[4,5]

Chart 1. Structures of **1**−**3**



Compounds **1** and **2** showed powerful in vitro antimalarial activity, with similar potencies against chloroquine sensitive *P. falciparum* FCR-3 (EC_{50} of **1** and **2**, 7.0×10^{-10} and 3.4×10^{-9} M, respectively). They also exhibited promising activity against chloroquine resistant *P. falciparum* K1 (EC_{50} of **1** and **2**, 1.2×10^{-9} and

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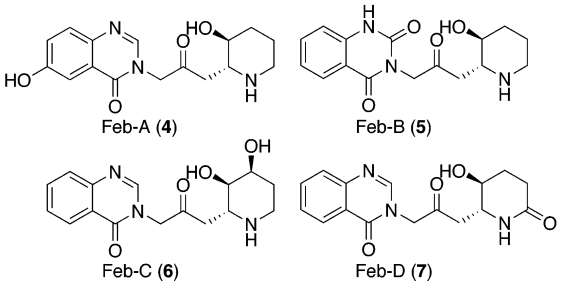
10.1021/jm0302086 CCC: $25.00
Published on Web 08/29/2003

Chart 2. Structures of Metabolic Products, feb-A−D (**4**−**7**), of **1** and **3**



1.8×10^{-9} M, respectively). In vivo activity against mouse malaria, *Plasmodium berghei*, is significantly greater in **1** than in **2** (ED_{50} of **1** and **2**, 0.3 and 79 mg/kg, respectively).[5] The different in vivo activities between the stereoisomers **1** and **2** prompted us to further study metabolites of **1** generated by mouse liver S9. In addition, a comparative metabolic study of the active analogue **3** was carried out to search for suitable leads.

In vitro antimalarial activities of synthetic **1**, **2**, and their antipodes were evaluated by Kobayashi et al.[6] The EC_{50} value of synthetic **1** against *P. falciparum* was 1/2632 of that of its enantiomer, and the EC_{50} of synthetic **2** was 1/552 of that of its enantiomer. In addition to the great difference in antimalarial activity between synthetic **1**, **2**, and their antipodes, high selectivities against *P. falciparum* were only reported for synthetic **1** and **2**. These results permitted us to use racemic compounds in the antimalarial tests.

We report here the isolation, structure elucidation, and synthesis of metabolic products, feb-A−D (**4**−**7**), of **1** and **3** (Chart 2). The antimalarial activity of the metabolites and their analogues is also described in this paper.

Results and Discussion

Isolation and Structure Elucidation of Metabolites of 1 and 3 Generated by Mouse Liver S9. Compound **1** was incubated with mouse liver S9, in the presence of cofactor for the NADPH-regenerating system, in phosphate-buffered solution at 37 °C. After 1 h, the reaction was stopped with ethyl acetate and extracted with *n*-butanol. High-performance liquid chromatography (HPLC) separation of the extract by reversed phase chromatography using an ODS column identified feb-A and -B (**4** and **5**) as the major metabolic products of **1**. Treatment of **3**, prepared from **1** with acetone by a condensation reaction,[5] with mouse liver S9 for 40 min, yielded feb-C and -D (**6** and **7**) as well as **4** and **5**.

The molecular formulas of the three metabolites **4**−**6** were established to be $C_{16}H_{19}N_3O_4$ based on analyses of their high-resolution electron ionization mass spectrometry (HREIMS) and the ^1H and ^{13}C NMR spectral data. Their molecular formulas differed from the parent compound **1** by an oxygen atom, implying that **1** was oxidized to form the metabolites **4**−**6**. A comparative study of the ^1H NMR spectra of **4** and **1** indicated the presence of a 1,2,4-trisubstituted benzene ring in **4** (δ 7.54 (1H, d, J = 8.8 Hz), 7.42 (1H, d, J = 2.9 Hz), and 7.30 (1H, dd, J = 8.8, 2.9 Hz)) instead of a 1,2-disubstituted benzene ring in **1**. Compound **4** was thus suggested to be an oxidized analogue of **1**, bearing a hydroxy group at C-6 or C-7. The two proposed structures were unambiguously distinguished by the HMBC spectrum of **4**, showing the H−C correlation peaks of H-2−C-8a and H-7−C-8a, confirming the presence of the C-6 hydroxy group in **4** (Figure 1).

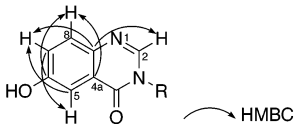


Figure 1. Correlations of the HMBC spectrum of feb-A (**4**).

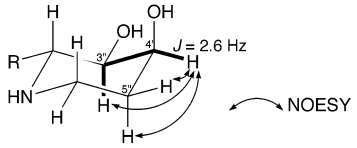


Figure 2. Relative configuration of piperidine ring in feb-C (**6**).

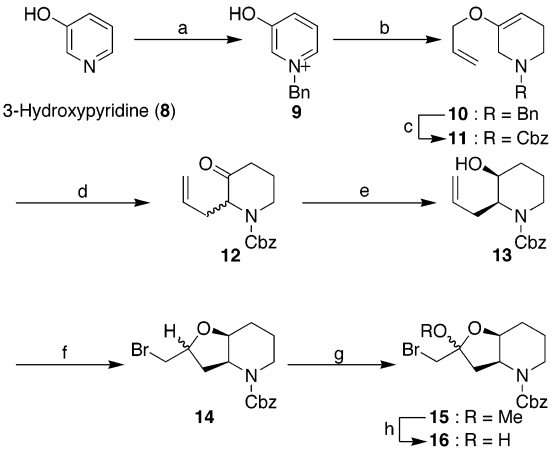
The ^1H NMR signals, due to the piperidine ring protons of **5**, closely resembled those of **1**. No singlet signal for H-2 was observed in the ^1H NMR spectrum of **5**, and the aromatic proton signals of H-6 and H-8 resonated upfield (δ 7.25 and 7.20) relative to those of **1** (δ 7.58 and 7.72 (see Supporting Information, Table S1)). A structure bearing a newly formed carbonyl function at C-2 was thus proposed for **5**.

The ^1H NMR spectrum of **6** pointed out a methine proton signal at δ 4.03 (1H, br.d, J = 2.4 Hz), instead of signals around δ 1.60−2.00 for methylene hydrogens at C-4″ of the piperidine ring of **1**. The value of the coupling constant ($J_{3'',4''}$ = 2.4 Hz) along with nuclear Overhauser effects (NOEs) of H-4″ with H-3″, H-5″α, and H-5″β proved the axial configuration of the C-4″ hydroxy group (Figure 2).

The molecular formula $C_{16}H_{17}N_3O_4$ for **7** was determined from its HREIMS, showing a molecular ion at m/z 315.1244, which differed from **1** by 14 mass units. In the ^1H NMR spectrum, no signal assignable to H-6″ of the piperidine ring was detected, and the chemical shifts of H-5″ (δ 2.28−2.50 (2H, m)) shifted downfield from those of **1** (δ 1.95−2.01 (1H, m) and 1.71−1.83 (1H, m) (see Supporting Information, Table S1)), demonstrating that **7** was an oxidized product of **1** at C-6″.

Synthesis of Feb-A−D, Metabolites of 1 and 3. The low yield of febrifugine and Df-1 metabolites **4**−**7** obtained from the metabolic experiments required their synthesis for further biological evaluation. Racemic **4** was synthesized employing the synthetic strategy of **1** and **2** developed by Takeuchi et al.,[7] which relies on an unusual Claisen rearrangement of allyl enol ether **11** and the stereoselective reduction of **12** (Scheme 1). 3-Hydroxypyridine (**8**) was refluxed with benzyl chloride in toluene to afford pyridinium chloride **9**. Compound **10** was obtained by *O*-allylation followed by reduction of **9**. The benzyl group of **10** was replaced by a benzyloxycarbonyl group by treatment with benzyl chloroformate to generate **11**. The unusual Claisen rearrangement of **11** occurred upon treatment with boron trifluoride−diethyl ether complex, resulting in the formation of **12**. Reduction of **12** by sodium borohydride gave **13** as a sole product. The intramolecular bromoetherification of **13** using *N*-bromosuccinimide afforded **14** as a mixture of diastereomeric isomers. Then, a

Scheme 1. Synthesis of the Piperidine Moiety of feb-A (**4**) and feb-B (**5**)[a]



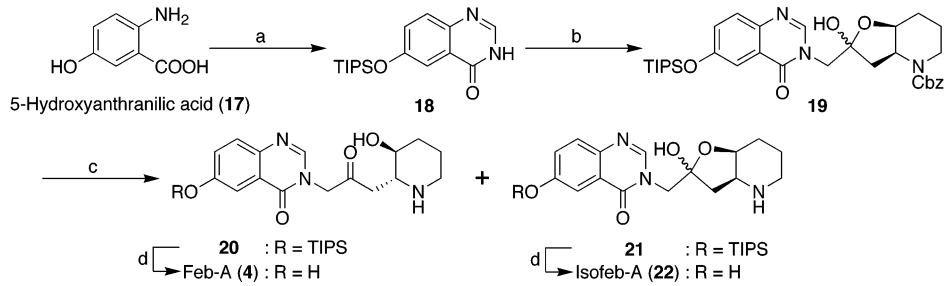
[a] Reagents and conditions: (a) Benzyl chloride, toluene, reflux. (b) (i) Allyl bromide, NaH, MeOH, reflux; (ii) $NaBH_4$, MeOH, 0 °C. (c) Benzyl chloroformate, THF, room temperature. (d) $BF_3{\cdot}OEt_2$, acetonitrile, room temperature. (e) $NaBH_4$, MeOH, 0 °C. (f) NBS, acetonitrile, room temperature. (g) (i) *t*BuOK, THF, 0 °C; (ii) NBS, MeOH, room temperature. (h) 10% HCl, acetonitrile, room temperature.

diastereomeric mixture of methyl acetal **15** was obtained by the following two reactions: dehydrobromination of **14** using potassium *tert*-butoxide and bromoetherification using *N*-bromosuccinimide and methanol. Hemiacetal **16** was prepared by hydrolysis of **15** and used in the next reaction without purification. The quinazolinone component of **4** was prepared from 5-hydroxyanthranilic acid (**17**). Compound **17** was condensed with formamide and then protected with a triisopropylsilyl group to yield **18**. A coupling reaction of **18** with hemiacetal **16** provided compound **19**. Hydrogenolysis of **19** catalyzed by palladium hydroxide and boiling of the residue in methanol gave **20** as well as its stereoisomer **21**. After separation of **20** and **21**, each compound was deprotected in acidic condition resulting in racemic feb-A (**4**) and isofeb-A (**22**) (Scheme 2).

Synthesis of the aromatic part in **5** was carried out using isatoic anhydride (**23**) as a starting material. Compound **23** was converted to **24** by treating with *tert*-butylamine. Compound **24** was then reacted with methyl chloroformate to yield methyl carbamate **25**, which was refluxed with potassium hydroxide to generate 3-*tert*-butyl benzoyleneurea **26**. Reaction of **26** with *p*-methoxybenzyl chloride, followed by deprotection of the *tert*-butyl group, yielded 1-*p*-methoxybenzyl benzoyleneurea **27**. Similar to the synthesis of racemic **4**, a coupling reaction of **27** with hemiacetal **16** gave **28**. Hydrogenolysis of **28** followed by heating in methanol produced **29** and its isomer **30**. After separation of **29** and **30**, the *p*-methoxybenzyl group of each compound was deprotected with ceric ammonium nitrate resulting in racemic feb-B (**5**) and isofeb-B (**31**). However, **31** was too unstable to be purified with silica gel column chromatography (Scheme 3).

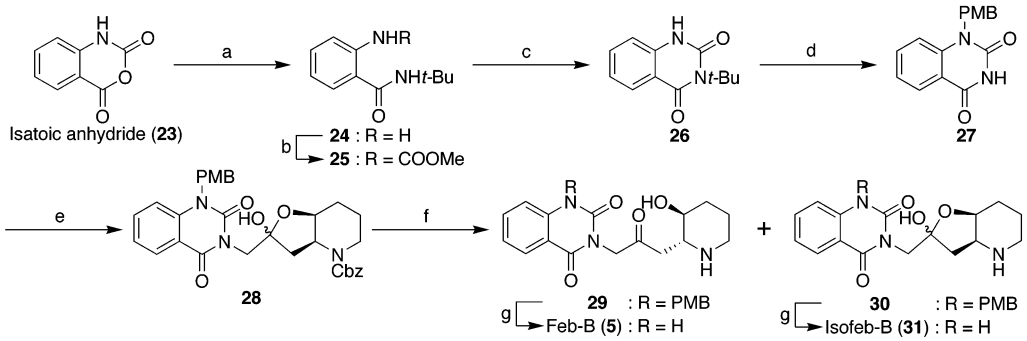
The synthesis of **6** is described in Scheme 4. Compound **13** was deprotected under acidic condition, and the product was reacted with di-*tert*-butyl dicarbonate, resulting in **32**. Compound **32** was acetylated to produce **33**. The allyl group of **33** was oxidized with osmium tetroxide, and protection of the formed diol group as acetonide by 2,2-dimethoxymethane gave **34**. After deacetylation of **34**, the resulting product was mesylated

Scheme 2. Synthesis of feb-A (**4**)[a]



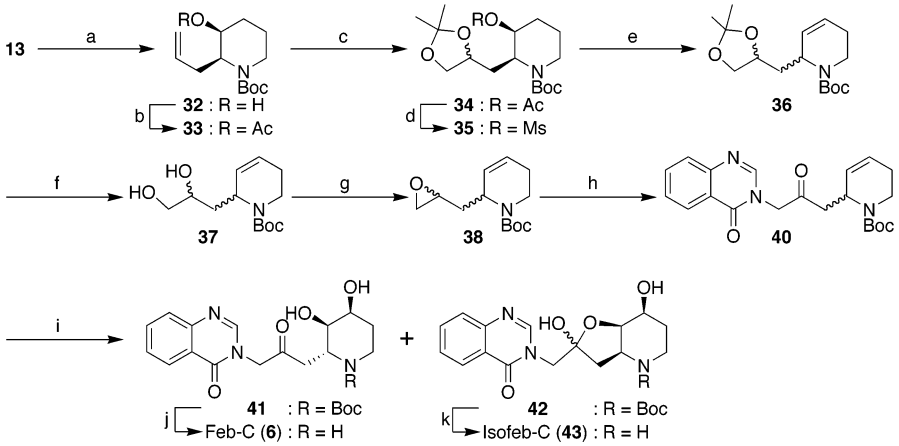
[a] Reagents and conditions: (a) (i) Formamide, 160 °C; (ii) Triisopropylsilyl chloride, imidazole, DMF, 50 °C. (b) Compound **16**, K_2CO_3, DMF, room temperature. (c) (i) H_2 (1 atm), $Pd(OH)_2/C$, EtOH, room temperature; (ii) MeOH, reflux. (d) 10% HCl−MeOH, 50 °C.

Scheme 3. Synthesis of feb-B (**5**)[a]



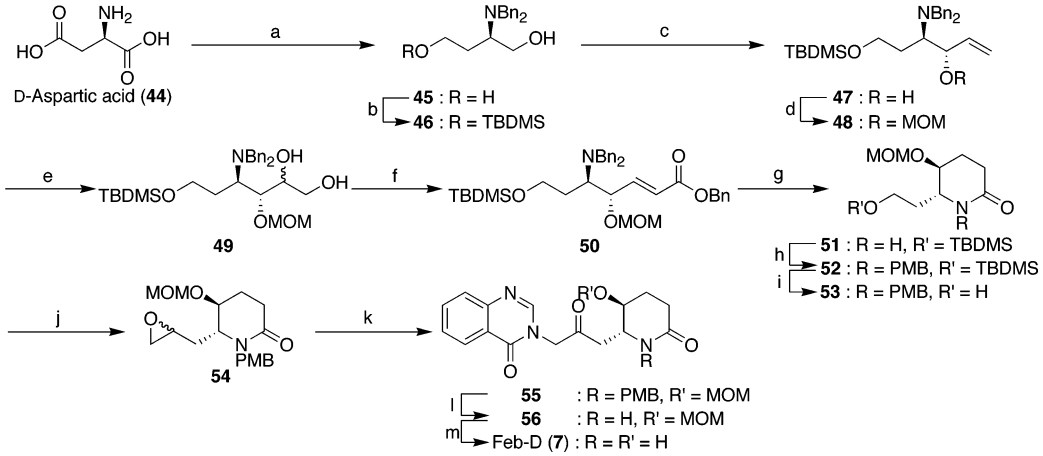
[a] Reagents and conditions: (a) *tert*-Butylamine, DMAP, DMF, room temperature. (b) Methyl chloroformate, 1 M NaOH−dioxane (1:1), room temperature. (c) KOH, EtOH, reflux. (d) (i) *p*-Methoxybenzyl chloride, NaH, DMF, room temperature; (ii) 6 M HCl−dioxane (2:3), reflux. (e) Compound **16**, K_2CO_3, DMF, room temperature. (f) H_2 (1 atm), $Pd(OH)_2/C$, EtOH, room temperature. (g) CAN, acetonitrile−H_2O (2:1), room temperature.

Scheme 4. Synthesis of feb-C (**6**)[a]



[a] Reagents and conditions: (a) (i) 10% HCl−MeOH, 50 °C; (ii) Boc_2O, triethylamine, room temperature. (b) Ac_2O, pyridine, room temperature. (c) (i) Catalytic OsO_4, NMO, acetone−acetonitirle−H_2O (1:1:1), room temperature; (ii) 2,2-dimethoxypropane, *p*TsOH, DMF, room temperature. (d) (i) MeONa, MeOH, room temperature; (ii) MsCl, triethylamine, CH_2Cl_2, 0 °C. (e) DBU, THF, reflux. (f) (i) 10% HCl−MeOH, reflux; (ii) Boc_2O, triethylamine, room temperature. (g) *N*-Tosylimidazole, KH, DMF, room temperature. (h) (i) Compound **39**, KH, DMF, 80 °C; (ii) Dess−Martin oxidation. (i) Catalytic OsO_4, NMO, acetone−acetonitrile−H_2O (1:1:1), room temperature. (j) TFA−CH_2Cl_2 (1:1), room temperature, and then 10% HCl−MeOH, room temperature. (k) 10% HCl−MeOH, room temperature.

Scheme 5. Synthesis of feb-D (**7**)[a]



[a] Reagents and conditions: (a) (i) Benzyl bromide, K_2CO_3, NaOH, MeOH−H_2O (1:1), reflux; (ii) $LiAlH_4$, THF, room temperature. (b) *tert*-Butyldimethylsilyl chloride, triethylamine, DMAP, CH_2Cl_2, 0 °C. (c) (i) Oxalyl chloride, DMSO, −78 °C, and then triethylamine; (ii) vinylmagnesium bromide, THF, −78 °C. (d) MOMCl, KI, *N*,*N*-diisopropylethylamine, DME, reflux. (e) Catalytic OsO_4, trimethylamine *N*-oxide, THF−*t*BuOH−H_2O (1:1:1), room temperature. (f) (i) $NaIO_4$, Et_2O−H_2O (1:1), 0 °C; (ii) benzyl diethylphosphonoacetate, NaH, toluene, 0 °C. (g) (i) H_2 (1 atm), $Pd(OH)_2$/C, EtOH, room temperature; (ii) EDCI·HCl, THF, 0 °C. (h) *p*-Methoxybenzyl chloride, NaH, DMF, 0 °C. (i) TBAF, THF, room temperature. (j) (i) Dess−Martin oxidation; (ii) trimethyl sulfoxonium iodide, NaH, DMSO, room temperature. (k) (i) Compound **39**, KH, DMF, 80 °C; (ii) Dess−Martin oxidation. (l) CAN, acetonitrile−H_2O (2:1), room temperature. (m) 10% HCl−MeOH, 50 °C.

with methanesulfonyl chloride to form **35**. Compound **36** was obtained by refluxing of **35** in the presence of DBU. The acetonide and Boc groups of **36** were cleaved in acidic conditions, and the product was reacted with di-*tert*-butyl dicarbonate to reprotect the amino functional group, generating diol **37**. Treatment of **37** with toluensulfonylimidazole afforded epoxide **38**. Coupling of **38** with 4-hydroxyquinazoline (**39**) and Dess−Martin oxidation of the resulting compound yielded **40**. Diols **41** and **42** were prepared from **40** using osmium tetroxide, and each diol was deprotected in acidic condition, generating racemic feb-C (**6**) and isofeb-C (**43**) (Scheme 4).

The piperidine ring of **7** was synthesized using *N*,*N*-dibenzylamino aldehyde[8] as a chiral building block (Scheme 5). D-Aspartic acid (**44**) was converted to amino alcohol **45** by reaction with benzyl bromide and reduction using lithium aluminum hydride. Compound **45** was reacted with *tert*-butyldimethylsilyl chloride to yield **46**.[9] Swern oxidation of **46** followed by Grignard reaction with vinylmagnesium bromide yielded **47** diastereoselectively. Compound **47** was protected with a methoxymethoxy group to give **48**, which was converted to diol **49** by osmium tetroxide. Oxidative cleavage of **49** followed by the Horner−Emmons reaction yielded benzyl ester **50**. After **50** was subjected to hydrogenolysis, cyclization in the presence of 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride generated piperidone **51**. Protection of the amide function of **51** with *p*-methoxybenzyl chloride yielded **52**, which was deprotected with tetrabutylammonium fluoride to produce **53**. Dess−Martin oxidation of **53** produced the aldehyde, which after treatment with trimethylsulfoxonium iodide and sodium hydride gave epoxide **54**. Coupling of **54** with 4-hydroxyquinazoline (**39**) and Dess−Martin oxidation of the resulting compound yielded **55**. The

Table 1. Antimalarial Activities of Febrifugine (**1**) and Df-1 (**3**) Metabolites against *P. falciparum* In Vitro

compd	antimalarial activity[a] EC_{50} (M)	cytotoxicity[b] EC_{50} (M)	selectivity[c]
1[d]	7.0×10^{-10}	1.7×10^{-7}	243
2[d]	3.4×10^{-9}	1.8×10^{-7}	53
3[d]	1.6×10^{-9}	3.8×10^{-7}	238
4	2.2×10^{-9}	2.7×10^{-7}	123
5	6.6×10^{-6}	$>8.5 \times 10^{-5}$	>13
6	2.2×10^{-8}	3.6×10^{-5}	1636
7	$>5.2 \times 10^{-5}$	$>5.2 \times 10^{-5}$	
22	2.7×10^{-10}	2.9×10^{-6}	10 741
43	1.5×10^{-7}	2.5×10^{-5}	167
chloroquine	1.8×10^{-8}	3.2×10^{-5}	1778
artemisinin	1.0×10^{-8}	1.0×10^{-5}	1000

[a] Against *P. falciparum* FCR-3. [b] Against FM3A mouse mammary cells. [c] Selectivity = cytotoxicity/antimalarial activity. [d] Data from ref 5.

p-methoxybenzyl group was removed from **55** with ceric ammonium nitrate to yield **56**, which was hydrolyzed to give enantiomerically pure feb-D (**7**).

Antimalarial Activity of Febrifugine and Df-1 Metabolites and Their Synthetic Analogues. In vitro antimalarial activity of febrifugine metabolites **4**−**7** and their analogues **22** and **43** against *P. falciparum* (FCR-3 strain) and cytotoxicity against mouse mammary FM3A cells were evaluated (Table 1). Compounds **4** and **22**, whose hydrogen at C-6 of the quinazolinone ring was substituted by a hydroxy group, exhibited powerful antimalarial activities (EC_{50}, 2.2×10^{-9} and 2.7×10^{-10} M). Compound **22** also showed extremely high selectivity (selectivity, 10 741) as compared to that of **1** (selectivity, 243). These data suggest that the hydroxy group at C-6 in **1** has the advantage of low toxicity for mammalian cells and still maintains antimalarial activity against *P. falciparum*. The antimalarial activity of **5**, which had an amido group at C-2, decreased dramatically (EC_{50}, 6.6×10^{-6} M), implying that basicity of the *N*-1 atom on the quinazolinone ring in **1** was important for antimalarial activity. Compound **6**, a 4″-hydroxy analogue of **1**, exhibited significant antimalarial activity with high selectivity (EC_{50}, 2.2×10^{-8} M; selectivity, 1636), while **43**, a 4″-hydroxy analogue of **2**, showed weaker activity (EC_{50}, 1.5×10^{-7} M) than **6**. However, **7**, which was formed by oxidation of C-6″ of **1**, did not demonstrate antimalarial activity (EC_{50}, $>5.2 \times 10^{-5}$ M).

In vivo antimalarial activity against *P. berghei* infected in mice was then determined for **4**, **6**, and **22**, the compounds showing notable activities by the in vitro assay. As indicated in Table 2, intraperitoneal (i.p.) administration of **4** and **6** exhibited comparable or stronger antimalarial activity (ED_{50}, 0.6 and 2.4 mg/kg/day) than the clinically used medicines, chloroquine and artemisinin (ED_{50}, 5.4 and 2.0 mg/kg/day), while the activity observed with **22** was moderate (ED_{50}, 6.0 mg/kg/day). Oral administration of **4** and **6** was also effective against murine malaria with ED_{50} values of 15 mg/kg/day for **4** and 8 mg/kg/day for **6**. While a single administration of **1** at 3 mg/kg i.p. ordinarily depauperated mice, no serious toxicities, such as diarrhea or weight loss, were noted, even after 4 consecutive days of treatment with **4**, **6**, and **22** (20, 100, and 50 mg/kg/day each). In addition, *P. berghei*-infected mice treated with 30 mg/kg/day i.p. of **4** showed no toxicity, and one of the five treated mice was actually cured with no parasites. These results imply that **4** might be an effective antimalarial candidate with low toxicity.

Table 2. Antimalarial Activities of Febrifugine (**1**) and Df-1 (**3**) Metabolites against *P. berghei* In Vivo[a]

compd	i.p. (mg/kg) ED_{50}	i.p. (mg/kg) ED_{90}	p.o. (mg/kg) ED_{50}	p.o. (mg/kg) ED_{90}
1[c]	0.3	1.5		
2[c]	79	>100 (70%)[b]		
3[c]	2.5	6.6		
4	0.6	5.2	15	27
6	2.4	8.3	8.0	23
22	6.0	37		
chloroquine	5.4	13	32	89
artemisinin	2.0	3.3	3.0	8.0

[a] Various concentrations of the test compounds were prepared in distilled water or olive oil. The test compounds were administered to groups of five mice once a day starting on day 0 and thereafter on days 1−3. Parasitemia levels were determined on the day following the last treatment (on day 4), and ED values of the antimalarial activities indicated above were determined by a previously reported protocol.[10] [b] The value in parentheses shows the growth inhibition (%) of each dose. [c] Data from ref 5.

In conclusion, we demonstrated that **4**, **6**, and **22** are strongly effective against *P. falciparum* in vitro, with *N*-1 and *N*-1″ atoms found to be crucial for significant antimalarial activity. Compounds **4** and **6** also exhibited strong antimalarial activity against *P. berghei*-infected mice. Furthermore, **4** was less toxic than the parent compound **1**, suggesting that **4** could be a good candidate as a new antimalarial drug.

Experimental Section

General Methods. Analytical thin-layer chromatography was performed on silica gel 60 F_{254} (Merck) and Aluminum oxide 150 F_{254} neutral (Type T, Merck). Column chromatography was carried out on silica gel 60 (70−230 mesh, Merck) and Aluminum oxide 150 basic (type T, Merck). NMR spectra were recorded on JEOL JNM GX-500, AL-400, and Varian Gemini 2000. Mass spectra were measured on JEOL JMS DX-303, AX-500, and AX-700. HPLC was carried out on Cosmosil 5C18-AR 10 mm × 250 mm (Nacalai Tesquie, Inc., Kyoto, Japan). ICR mice (male, 5 weeks old) were used for preparation of s9.

Metabolism of 1 and 3. Febrifugine hydrochloride (**1**, 115 mg) was incubated at 37 °C for 1 h with mice s9 mix (480 mL, 2.5 mg protein/mL), 1.3 mM NADP, 10 mM glucose 6-phosphate, 0.4 unit/mL glucose 6-phosphate dehydrogenase, and 5 mM magnesium chloride in phosphate-buffered saline (final volume 800 mL). The reaction was quenched with ethyl acetate (1.6 L) and 28% ammonia solution. The mixture was extracted with ethyl acetate and *n*-butanol, respectively. Each organic layer was concentrated to give ethyl acetate and *n*-butanol solubles, respectively. In the same manner, Df-1 hydrochloride (**3**, 112 mg) was metabolized to afford ethyl acetate and *n*-butanol solubles.

Isolation of Feb-A−D (4−7). The *n*-butanol solubles of metabolites of **1** were chromatographed by HPLC under the gradient condition as following: 5% acetonitrile for 0−15 min, 5−20% for 15−30 min, 20−30% for 30−40 min, 30−50% for 40−50 min, and 50−100% for 50−60 min), containing 0.01% trifluoroacetic acid at a flow rate of 2.0 mL/min, to give feb-A (**4**, 2.3 mg) and feb-B (**5**, 1.0 mg). In the same manner, feb-C (**6**, 1.7 mg) and feb-D (**7**, 0.5 mg) were afforded from ethyl acetate and *n*-butanol solubles of metabolites of **3**, respectively.

6-(Triisopropylsilyloxy)quinazolin-4-one (18). A mixture of 5-hydroxyanthranilic acid (**17**, 5.16 g, 33.7 mmol) and formamide (34 mL) was heated at 160 °C for 6 h. The mixture was cooled to room temperature and poured into water and filtered. The precipitate was washed with water and dried to give quinazoline-4,6-diol (4.73 g, 87%) as a dark purple solid.

To a solution of quinazoline-4,6-diol (2.00 g, 12.3 mmol) in dimethylformamide (DMF, 25 mL) was added imidazole (2.14 g, 30.8 mmol) and triisopropylsilyl chloride (3.26 mL, 14.8 mmol). The mixture was stirred at 50 °C for 14 h. After it was cooled, the mixture was poured into 1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane-diethyl ether (1:2) to give **18** (3.60 g, 92%).

3-[3-[(2*R,3*S**)-3-Hydroxypiperidin-2-yl]-2-oxopropyl]-6-(triisopropylsilyloxy)-3*H*-quinazolin-4-one (20) and 3-[(3a*S**,7a*S**)-2-Hydroxyoctahydrofuro[3,2-b]pyridin-2-ylmethyl]-6-(triisopropylsilyloxy)-3*H*-quinazolin-4-one (21).** To a solution of **16** (186 mg, 0.501 mmol) in DMF (1 mL) were added **18** (161 mg, 0.505 mmol) and potassium carbonate (83.8 mg, 0.606 mmol). After the solution was stirred at room temperature for 2 h, the mixture was poured into brine and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane-chloroform (1:19) to give crude **19** (283 mg).

Palladium hydroxide on carbon (20 wt % Pd, 188 mg) was added to a methanol solution (5 mL) of this crude **19**. Under hydrogen atmosphere, this solution was stirred at room temperature for 1 h. The mixture was filtered and concentrated. The residue was chromatographed over silica gel eluted by chloroform−methanol (19:1) to give **21** (446 mg, 46% from **16**).

A methanol solution (15 mL) of **21** (472 mg, 0.996 mmol) was refluxed for 3 h. The reaction mixture was concentrated. The residue was chromatographed over aluminum oxide to afford **21** (239 mg, 51%) eluted by *n*-hexane−chloroform (1:1) and **20** (207 mg, 44%) eluted by chloroform.

3-[3-[(2*R,3*S**)-3-Hydroxypiperidin-2-yl]-2-oxopropyl]-6-hydroxy-3*H*-quinazolin-4-one (Feb-A) (4).** Compound **20** (101 mg, 0.213 mmol) was dissolved in 10% hydrogen chloride containing methanol (5 mL) and heated at 50 °C for 24 h. The reaction mixture was concentrated, and the residue was chromatographed over aluminum oxide eluted by methanol to give **4** (79.7 mg, 100%).

3-[(3a*S,7a*S**)-2-Hydroxyoctahydrofuro[3,2-b]pyridin-2-ylmethyl]-6-hydroxy-3*H*-quinazolin-4-one (Isofeb-A) (22).** By the use of **25** (128 mg, 0.271 mmol) as a substrate, **22** (56.2 mg, 65%) was afforded in the same manner as the synthesis of **4**.

2-Amino-*N*-*tert*-butylbenzamide (24). To a solution of isatoic anhydride (**23**, 3.00 g, 18.4 mmol) in DMF (18 mL) were added DMAP (225 mg, 1.84 mmol) and *tert*-butylamine (2.16 mL, 20.3 mmol). After it was stirred at room temperature for 3.5 h, the mixture was poured into water and filtered. The precipitate was washed with water and methanol and dried to give **24** (1.61 g, 46%).

***N*-*tert*-Butyl-2-methoxycarbonylaminobenzamide (25).** To a solution of **24** (746 mg, 3.88 mmol) in dioxane (4 mL) and 1 M NaOH (4 mL) was added methyl chloroformate (330 μL, 4.27 mmol). After it was stirred at room temperature for 30 min, the mixture was poured into 1 M hydrochloric acid and filtered. The precipitate was washed with water and methanol and dried to give **25** (922 mg, 95%).

3-*tert*-Butyl-1*H*-quinazolin-2,4-dione (26). A mixture of **25** (870 mg, 3.48 mmol) and potassium hydroxide (1.84 g, 27.8 mmol) in ethanol (17 mL) was refluxed for 9 h. The mixture was acidified with 1 M hydrochloric acid and filtered. The precipitate was washed with water and methanol and dried to give **26** (365 mg, 84%).

1-(4-Methoxybenzyl)-1*H*-quinazolin-2,4-dione (27). To an ice-cooled solution of **26** (606 mg, 2.78 mmol) in DMF (5 mL) were added sodium hydride (122 mg, 3.06 mmol) and *p*-methoxybenzyl chloride (430 mL, 3.06 mmol). After it was stirred at room temperature for 3 h, the mixture was poured into 1 M hydrochloric acid and filtered. The precipitate was washed with water and methanol and dried to give 3-*tert*-butyl-1-(4-methoxybenzyl)-1*H*-quinazolin-2,4-dione (965 mg, 100%).

3-*tert*-Butyl-1-(4-methoxybenzyl)-1*H*-quinazolin-2,4-dione (1.42 g, 4.21 mmol) was dissolved in dioxane (12.6 mL) and 6 M hydrochloric acid (8.4 mL). After it was refluxed for 6 h, the mixture was cooled to room temperature, poured into water, and filtered. The precipitate was washed with water and methanol and recrystallized from chloroform−methanol to give **27** (1.14 g, 96%).

1-(4-Methoxybenzyl)-3-[(3a*S,7a*S**)-*N*-benzyloxycarbonyl-2-hydroxyoctahydrofuro[3,2-b]pyridin-2-ylmethyl]-3*H*-quinazolin-2,4-dione (28).** To a mixture of **16** (243 mg, 0.659 mmol) and **27** (186 mg, 0.659 mmol) was added potassium carbonate (109 mg, 0.790 mmol). After it was stirred at room temperature for 1.5 h, the mixture was poured into brine and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (1:1) to give **28** (127 mg, 34%).

3-[3-[(2*R,3*S**)-3-Hydroxypiperidin-2-yl]-2-oxopropyl]-3*H*-quinazolin-2,4-dione (Feb-B) (5) and 3-[(3a*S**,7a*S**)-2-Hydroxyoctahydrofuro[3,2-b]pyridin-2-ylmethyl]-3*H*-quinazolin-2,4-dione (Isofeb-B) (31).** Palladium hydroxide on carbon (20 wt % Pd, 50.5 mg) was added to a solution of **28** (289 mg, 0.505 mmol) in ethanol−ethyl acetate (4:1) (6.5 mL). Under hydrogen atmosphere, this solution was stirred at room temperature for 2.5 h. The mixture was filtered and concentrated. The residue was chromatographed over silica gel eluted by chloroform−methanol (4:1) to give a mixture of **29** and **30**.

To a solution of this mixture in acetonitrile (3 mL) and water (1.5 mL) was added cerium ammonium nitrate (1.06 g, 1.93 mmol). The mixture was stirred at room temperature for 2 h and extracted with *n*-butanol three times. The organic layer was concentrated. The residue was chromatographed over aluminum oxide to give **31** (5.7 mg, 3%) eluted by chloroform−methanol (19:1) and **5** (75.6 mg, 40%) eluted by chloroform−methanol (9:1).

(2*S,3*S**)-*N*-*tert*-Butyloxycarbonyl-2-allyl-3-hydroxypiperidine (32).** A solution of **13** (20.3 g, 73.7 mmol) in methanol (10 mL) and 6 M hydrochloric acid (50 mL) was refluxed for 5 h. The mixture was alkalinized with saturated sodium bicarbonate solution and extracted with *n*-butanol three times. The organic layer was evaporated. The residue was dissolved in dichloromethane (70 mL) and cooled at 0 °C. To the solution were added triethylamine (12.3 mL, 88.5 mmol) and di-*tert*-butyl dicarbonate (19.3 g, 88.5 mmol). After it was stirred for 1.5 h at room temperature, the mixture was acidified with 0.1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (4:1) to give **32** (8.08 g, 45%).

(2*S,3*S**)-*N*-*tert*-Butyloxycarbonyl-2-allyl-3-acetoxypiperidine (33).** To an ice-cooled solution of **32** (8.03 g, 33.3 mmol) in pyridine (20 mL) were added acetic anhydride (3.8 mL, 39.9 mmol) and DMAP (407 mg, 3.33 mmol). After it was stirred for 4.5 h at room temperature, the mixture was acidified with 0.1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (49:1) to give **33** (8.04 g, 86%).

(2*S,3*S**)-*N*-*tert*-Butyloxycarbonyl-3-acetoxy-2-(2,2-dimethyl-1,3-dioxolan-4-ylmethyl)piperidine (34).** To an ice-cooled solution of **33** (8.04 g, 28.3 mmol) in acetone−acetonitrile−water (1:1:1) (30 mL) were added 4 wt % osmium tetroxide solution in water (0.9 mL, 0.142 mmol) and 4-methylmorpholine *N*-oxide (3.98 g, 34.0 mmol). After it was stirred for 2.5 h at room temperature, the mixture was extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica

gel eluted by *n*-hexane−ethyl acetate (1:1) to give (2*S**,3*S**)-*N*-*tert*-butyloxycarbonyl-3-acetoxy-2-(2,3-dihydroxypropyl)piperidine (8.14 g, 90%).

To a solution of (2*S**,3*S**)-*N*-*tert*-butyloxycarbonyl-3-acetoxy-2-(2,3-dihydroxypropyl)piperidine (8.14 g, 25.6 mmol) in DMF (30 mL) were added 2,2-dimethoxypropane (31.5 mL, 256 mmol) and *p*-toluenesulfonic acid (1.46 g, 7.68 mmol). After it was stirred for 3.5 h at room temperature, the mixture was acidified with 0.1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (9:1) to give **34** (8.78 g, 95%).

(2*S,3*S**)-*N*-*tert*-Butyloxycarbonyl-2-(2,2-dimethyl-1,3-dioxolan-4-ylmethyl)-3-methanesulfonyloxypiperidine (35).** To a solution of **34** (8.73 g, 24.2 mmol) in methanol (25 mL) was added sodium methoxide (1.44 g, 26.7 mmol). After it was stirred for 3.5 h at room temperature, the mixture was poured into water and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. To an ice-cooled solution of the residue in dichloromethane (25 mL) were added triethylamine (4.1 mL, 29.1 mmol) and methanesulfonyl chloride (2.25 mL, 29.1 mmol). After it was stirred for 2 h at room temperature, the mixture was acidified with 0.1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (9:1) to give **35** (6.69 g, 70%).

(2*S)-*N*-*tert*-Butyloxycarbonyl-2-(2,2-dimethyl-1,3-dioxolan-4-ylmethyl)-1,2,5,6-tetrahydropyridine (36).** To an ice-cooled solution of **35** (9.11 g, 23.2 mmol) in THF (55 mL) was added DBU (34.4 mL, 232 mmol). After it was refluxed for 12 h, the mixture was cooled to room temperature, poured into saturated ammonium chloride solution, and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (19:1) to give **36** (4.05 g, 59%).

(2*S)-*N*-*tert*-Butyloxycarbonyl-2-(2,3-dihydroxypropyl)-1,2,5,6-tetrahydropyridine (37).** A solution of **36** (1.93 g, 6.49 mmol) in 10% hydrogen chloride containing methanol (6.5 mL) was refluxed for 13 h. The mixture was cooled to room temperature and concentrated. To an ice-cooled solution of the residue in water (6.5 mL) were added triethylamine (2.7 mL, 7.79 mmol) and di-*tert*-butyl dicarbonate (1.71 g, 7.79 mmol). After it was stirred at 0 °C for 5 h, the mixture was poured into 0.1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (4:1) to give **37** (1.30 g, 78%).

(2*S)-*N*-*tert*-Butyloxycarbonyl-2-(oxiranylmethyl)-1,2,5,6-tetrahydropyridine (38).** To an ice-cooled solution of **37** (322 mg, 1.25 mmol) in THF (3 mL) were added sodium hydride (150 mg, 3.76 mmol) and 1-(*p*-toluenesulfonyl)imidazole (251 mg, 1.13 mmol). After it was stirred for 15 h, the mixture was extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (19:1) to give **38** (146 mg, 49%).

3-[3-[(2*S)-*N*-*tert*-Butyloxycarbonyl-1,2,5,6-tetrahydropyridin-2-yl]-2-oxopropyl]-3*H*-quinazolin-4-one (40).** To an ice-cooled suspension of potassium hydride (105 mg, 0.92 mmol) in DMF (2 mL) was added 4-hydroxyquinazoline (**39**) (107 mg, 0.73 mmol), and the mixture was stirred at 0 °C for 1 h. A solution of **38** (146 mg, 0.61 mmol) in DMF (4 mL) was added to the mixture. After it was stirred at 70 °C for 14 h, the mixture was cooled to room temperature, poured into saturated ammonium chloride solution, and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated.

The residue was dissolved in dichloromethane (3 mL) and reacted with Dess−Martin periodinane (777 mg, 1.83 mmol). After it was stirred at 0 °C for 1.5 h, the mixture was quenched with sodium thiosulfate solution, and the mixture was extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over activated aluminum oxide eluted by *n*-hexane−chloroform (9:1) to give **40** (134 mg, 57% from **38**).

3-[3-[(2*R,3*R**,4*S**)-*N*-*tert*-Butyloxycarbonyl-3,4-dihydroxypiperidin-2-yl]-2-oxopropyl]-3*H*-quinazolin-4-one (41) and 3-[(3a*S**,7*S**,7a*S**)-*N*-*tert*-butyloxycarbonyl-2,7-dihydroxyoctahydrofuro[3,2-b]pyridin-2-ylmethyl]-3*H*-quinazolin-4-one (42).** To an ice-cooled solution of **40** (37.0 mg, 0.09 mmol) in acetone−acetonitrile−water (1:1:1) (0.5 mL) were added 1 wt % osmium tetroxide solution in water (110 μL, 0.004 mmol) and 4-methylmorpholine *N*-oxide (20.8 mg, 0.18 mmol). After it was stirred for 3 h, the mixture was extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel to give **41** (21.3 mg, 58%) eluted by chloroform−methanol (19:1) and **42** (5.6 mg, 15%) eluted by chloroform−methanol (199:1).

3-[3-[(2*R,3*R**,4*S**)-3,4-Dihydroxypiperidin-2-yl]-2-oxopropyl]-3*H*-quinazolin-4-one (Feb-C) (6).** Compound **41** (21.8 mg, 0.05 mmol) was dissolved in trifluoroacetic acid (0.2 mL) and dichloromethane (0.8 mL). After it was stirred at room temperature for 1.5 h, the mixture was concentrated. The residue was dissolved in 10% hydrogen chloride containing methanol (1.5 mL) and then concentrated to give **6** (17.4 mg, 85%) as hydrochloride salt. When **41** was directly treated with 10% hydrogen chloride containing methanol at room temperature, an unidentified product was formed together with **6**.

3-[(3a*S,7*S**,7a*S**)-2,7-Dihydroxyoctahydrofuro[3,2-b]-pyridin-2-ylmethyl]-3*H*-quinazolin-4-one (Isofeb-C) (43).** Compound **42** (13.3 mg, 0.03 mmol) was dissolved in 10% hydrogen chloride containing methanol (1.5 mL) and stirred at room temperature for 1.5 h. The reaction mixture was concentrated, and the residue was chromatographed over aluminum oxide eluted by chloroform−methanol (19:1) to give **43** (6.1 mg, 60%) as hydrochloride salt.

(3*S*,4*R*)-4-(*N*,*N*-Dibenzylamino)-6-(*tert*-butyldimethylsilyloxy)-1-hexen-3-ol (47). (*R*)-2-(*N*,*N*-Dibenzylamino)-4-(*tert*-butyldimethylsilyloxy)butan-1-ol (**46**) was prepared from D-aspartic acid (**44**) according to the method reported by Gmeiner et al.[9] Dimethyl sulfoxide (DMSO, 3.4 mL, 48.3 mmol) and **46** (6.43 g, 16.1 mmol) dissolved in dichloromethane (10 mL) were added to a solution of oxalyl chloride (2.1 mL, 24.1 mmol) in dichloromethane (80 mL) at −78 °C. After it was stirred for 1 h, triethylamine (11.2 mL, 80.4 mmol) was added to this mixture and stirred at room temperature. The mixture was poured into 1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated to give crude (*R*)-2-(*N*,*N*-dibenzylamino)-4-(*tert*-butyldimethylsilyloxy)butanal. The crude aldehyde was dissolved in THF (30 mL) and cooled to −78 °C. Vinylmagnesium bromide dissolved in THF (1.0 M, 16.5 mL) was added to this solution. After it was stirred for 1 h, the mixture was allowed to warm to room temperature and poured into 1 M hydrochloric acid. This mixture was extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (19:1) to give **47** (4.87 g, 71% from **46**).

(3*S*,4*R*)-4-(*N*,*N*-Dibenzylamino)-6-(*tert*-butyldimethylsilyloxy)-3-methoxymethoxy-1-hexene (48). To a solution of **47** (3.56 g, 8.36 mmol) in 1,2-dimethoxyethane (25 mL) were added *N*,*N*-diisopropylethylamine (3.64 mL, 20.9 mmol), chloromethyl methyl ether (1.27 mL, 16.7 mmol), and potassium iodide (1.53 g, 9.19 mmol). After it was refluxed for 3 h, the mixture was cooled to room temperature and poured into water and extracted with ethyl acetate three times. The organic layer was washed with 0.5 M hydrochloric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (39:1) to give **48** (3.25 g, 83%).

(3*S*,4*R*)-4-(*N*,*N*-Dibenzylamino)-6-(*tert*-butyldimethylsilyloxy)-3-methoxymethoxy-hexane-1,2-diol (49). Compound **48** (3.21 g, 6.84 mmol) was dissolved in water (8 mL), THF (8 mL), and *tert*-butyl alcohol (8 mL). Trimethylamine *N*-oxide dihydrate (1.94 g, 17.1 mmol) and 4% osmium tetroxide solution in water (870 μL, 0.137 mmol) were added to this solution. After it was stirred at room temperature for 5 h, the reaction was quenched with 10% sodium thiosulfate solution. This mixture was extracted with ethyl acetate three times. The organic layer was washed with 0.5 M hydrochloric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (7:3) to give **49** (2.80 g, 81%).

Benzyl (2*E*,4*S*,5*R*)-5-(*N*,*N*-Dibenzylamino)-7-(*tert*-butyldimethylsilyloxy)-4-methoxymethoxy-2-heptenoate (50). Sodium periodate (1.10 g, 5.15 mmol) and water (7 mL) were added to a solution of **49** (1.99 g, 3.96 mmol) in diethyl ether (14 mL). After it was stirred vigorously for 3 h, the mixture was extracted with diethyl ether three times, and the organic layer was washed with brine, dried, and concentrated to give crude (2*S*,3*R*)-3-(*N*,*N*-dibenzylamino)-5-(*tert*-butyldimethylsilyloxy)-2-methoxymethoxypentanal. This crude aldehyde was added to a mixture of sodium hydride (174 mg, 4.35 mmol) and benzyl diethylphosphonoacetate[11] (1.25 g, 4.35 mmol) in toluene (16 mL) at 0 °C. After it was stirred for 2 h, the mixture was poured into water (20 mL) and extracted with ethyl acetate three times. The organic layer was washed with 0.5 M hydrochloric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (19:1) to give **50** (1.96 g, 82%).

(5*S*,6*R*)-6-(2-*tert*-Butyldimethylsilyloxyethyl)-5-methoxymethoxypiperidin-2-one (51). Under hydrogen atmosphere, **50** (1.40 g, 2.32 mmol) and 20% palladium hydroxide on carbon (500 mg) in ethanol (24 mL) were stirred at room temperature for 5 h. After the mixture was filtered, the filtrate was evaporated to give an oily residue, which was crude (4*S*,5*R*)-5-amino-7-*tert*-butyldimethylsilyloxy-4-methoxymethoxyheptanoic acid (657 mg, 1.96 mmol). This oil was dissolved in THF (10 mL), and this solution was cooled to 0 °C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.50 g, 7.83 mmol) was added to the solution. After it was stirred for 4 h, the mixture was poured into 0.5 M hydrochloric acid. This mixture was extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (99:1) to give **51** (448 mg, 61% from **50**).

(5*S*,6*R*)-*N*-(*p*-Methoxybenzyl)-6-(2-*tert*-butyldimethylsilyloxyethyl)-5-methoxymethoxypiperidin-2-one (52). To a solution of **51** (101 mg, 0.32 mmol) in DMF (1.5 mL) were added *p*-methoxybenzyl chloride (50 μL, 0.35 mmol) and sodium hydride (14.0 mg, 0.35 mmol). After it was stirred at room temperature for 5.5 h, the mixture was quenched with methanol and water and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (19:1) to give **52** (69.5 mg, 50%).

(5*S*,6*R*)-*N*-(*p*-Methoxybenzyl)-6-(2-hydroxyethyl)-5-methoxymethoxypiperidin-2-one (53). To a solution of **52** (69.5 mg, 0.16 mmol) in THF (1.5 mL) was added 1.0 M tetrabutylammonium fluoride solution in THF (0.56 mL). After it was stirred at room temperature for 1 h, the mixture was poured into saturated ammonium chloride solution and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (99:1) to give **53** (44 mg, 86%).

(5*S*,6*R*)-*N*-(*p*-Methoxybenzyl)-5-methoxymethoxy-6-oxiranylmethylpiperidin-2-one (54). To a solution of **53** (44 mg, 0.14 mmol) in dichloromethane (1.3 mL) was added Dess−Martin periodinane (173 mg, 0.41 mmol). After it was stirred at 0 °C for 1 h, the mixture was quenched with 10% sodium thiosulfate solution and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated to give the crude aldehyde.

To a solution of sodium hydride (5.4 mg, 0.14 mmol) in DMSO (0.4 mL) were added a solution of the crude aldehyde in DMSO (0.9 mL) and trimethylsulfoxonium iodide (30 mg, 0.14 mmol) at 0 °C. After it was stirred at room temperature for 2 h, the mixture was poured into water and extracted with ethyl acetate three times. The organic layer was washed with brine, dried, and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−ethyl acetate (1:3) to give **54** (18.6 mg, 41% from **53**).

3-[3-[(2*R*,3*S*)-*N*-(*p*-Methoxybenzyl)-3-methoxymethoxy-6-oxopiperidin-2-yl]-2-oxopropyl]-3*H*-quinazolin-4-one (55). To an ice-cooled suspension of potassium hydride (12.3 mg, 0.11 mmol) in DMF (0.3 mL) was added 4-hydroxyquinazoline (**39**) (15.7 mg, 0.11 mmol). After the mixture was stirred at 0 °C for 30 min, a solution of **54** (17.3 mg, 0.05 mmol) in DMF (0.7 mL) was added to this mixture. The mixture was heated at 80 °C for 3.5 h. The mixture was cooled to room temperature, quenched with a saturated ammonium chloride solution, and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue in dichloromethane (1.2 mL) was reacted with Dess−Martin periodinane (65.7 mg, 0.15 mmol) and stirred at 0 °C for 2 h. The mixture was quenched with 10% sodium thiosulfate solution, and the mixture was extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (49:1) to give **55** (19.8 mg, 80%).

3-[3-[(2*R*,3*S*)-3-Methoxymethoxy-6-oxopiperidin-2-yl]-2-oxopropyl]-3*H*-quinazolin-4-one (56). To a solution of **55** (18.9 mg, 0.023 mmol) in acetonitrile−water (2:1) (1.0 mL) was added cerium ammonium nitrate (57.7 mg, 0.12 mmol). After it was stirred at room temperature for 1.5 h, the mixture was extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (97:3) to give **56** (8.5 mg, 60%).

3-[3-[(2*R*,3*S*)-3-Hydroxy-6-oxopiperidin-2-yl]-2-oxopropyl]-3*H*-quinazolin-4-one (Feb-D) (7). Compound **56** was dissolved in trifluoroacetic acid (0.75 mL) and dichloromethane (0.25 mL). After it was stirred at room temperature for 2.5 h, the mixture was concentrated. The residue was dissolved in 10% hydrogen chloride containing methanol (1.5 mL) and then concentrated to give **7** (6.8 mg, 100%) as hydrochloride salt.

Antimalarial Assays In Vitro and In Vivo. The antimalarial activities in vitro and in vivo against *P. falciparum* and *P. berghei*, respectively, were investigated as described in ref 10.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research (Nos. 15019007,

12307007, 13226079, and 14021072) from the Ministry of Education, Science, Sports and Culture of Japan.

Supporting Information Available: Analytical data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0302086