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Synthesis of febrifugine derivatives and development of an effective and safe tetrahydroquinazoline-type antimalarial

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ABSTRACT

Febrifugine, a quinazoline alkaloid isolated from *Dichroa febrifuga* roots, shows powerful antimalarial activity against *Plasmodium falciparum*. Although the use of ferifugine as an antimalarial drug has been precluded because of its severe side effects, its potent antimalarial activity has stimulated medicinal chemists to pursue its derivatives instead, which may provide valuable leads for novel antimalarial drugs. In the present study, we synthesized new derivatives of febrifugine and evaluated their *in vitro* and *in vivo* antimalarial activities to develop antimalarials that are more effective and safer. As a result, we proposed tetrahydroquinazoline-type derivative as a safe and effective antimalarial candidate.

1. Introduction

Malaria, which is caused by a protozoan parasite of the genus *Plasmodium*, is a major parasitic infection in many tropical and subtropical regions. Malaria affects about 200 million patients worldwide and leads to more than half a million deaths each year. Although malaria has been widely eradicated in many parts of the world, the number of its victims continues to rise mainly because of the emergence of chloroquine-resistant and multiple-drug-resistant strains of malaria parasites. Thus, the discovery of new and effective antimalarial drugs is urgently needed [1,2].

Roots of *Dichroa febrifuga*, a plant that belongs to the Saxifragaceae family, have been used as a traditional antimalarial drug in China. The quinazoline-type alkaloids, febrifugine (**1**) and its stereoisomer, isofebrifugine (**2**), have been identified as the active components of these roots (Fig. 1) [3,4]. Although **1** and **2** show powerful *in vitro* antimalarial activity against both chloroquine-sensitive *Plasmodium falciparum* FCR-3 and chloroquine-resistant *P. falciparum* K1, the *in vivo* activity of **1** against mouse malaria parasite, *Plasmodium berghei*, is approximately 200 times more potent than that of **2**. Because of side effects such as diarrhea, vomiting [5,6], and liver toxicity [7], the use of **1** as an antimalarial drug has been precluded. Halofuginone (**3**) (Fig. 1), a 7-bromo-6-chloro derivative of **1** that shows less toxicity, has been used as a veterinary coccidiostat [8]. Recently, compound **3** has been developed as a medicine for scleroderma, and has been used in clinical trials as a therapeutic in cancer and fibrotic disease [9–13]. Halofuginone (**3**) binds glutamyl-prolyl-tRNA synthetase and inhibits prolyl-tRNA synthetase activity, revealing its anti-inflammatory effect, which quenched by addition of an excess amount of proline [14,15]. Addition of excess proline also lowers the antimalarial activity of **3**, but its nanomolar level IC_{50} is maintained. Thus, additional molecular targets of febrifugine (**1**), halofuginone (**3**) and their derivatives for antimalarial activity are remaining.

The potent antimalarial activity of **1** has stimulated medicinal chemists to pursue derivatives of **1**, which may provide valuable leads for novel antimalarial drugs [5,6,16–21]. In our previous work [19], we synthesized febrifugine derivatives via structural modifications at either (i) the quinazoline ring, (ii) linker, or (iii) piperidine ring (Fig. 2). We found that modification of the quinazoline ring was the most suitable method for creating the most effective derivatives. Additionally, we recognized that the piperidine ring is necessary for the antimalarial activity of **1**; however, the ring size has not been evaluated. In this paper, we describe the synthesis of new derivatives of **1** and evaluate their *in vitro* and *in vivo* antimalarial activities in order to develop more effective and safer antimalarials.

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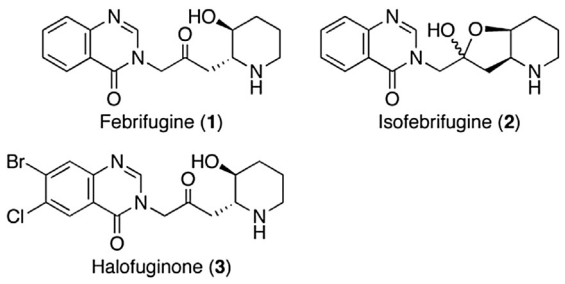


Fig. 1. Structures of febrifugine (**1**), isofebrifugine (**2**) and halofuginone (**3**).

2. Results and discussion

2.1. Syntheses of quinazoline ring-modified derivatives

In our previous works [19], we synthesized febrifugine derivatives bearing a pyrimidine, an isoquinoline, substituted quinazolines, pyrimidines fused with heterocycles instead of the quinazoline ring. In this work, new types of quinazoline ring-modified derivatives **4**–**10** were synthesized. Compounds **4**–**6**, as well as **7** and **8**, have pyrimidines fused with aliphatic rings and 5,6-dialkylpyrimidines, respectively. The size of their aromatic moieties is similar to that of the quinazoline ring, but there is no benzene ring in them. Compounds **9** and **10** have 6- and 5-phenyl pyrimidines, respectively, which are the benzo-cracked rings of the quinazoline ring. These compounds were synthesized by a coupling reaction between the corresponding heterocycle and a hemiacetal **11**, which was reported by Takeuchi et al. (Scheme 1) [22,23]. Syntheses of the corresponding heterocycles are depicted in Scheme 2. Enamines **15a**–**c**, which were prepared from 2-methoxycarbonylcycloalkanone **14a**–**c** [24], were condensed with formamide to produce the aliphatic-ring-fused pyrimidines **16a**–**c**. Reductive desulfurization of thiouracil derivatives **18a**,**b** [25], which were synthesized by condensation between β-ketoesters **17a**,**b** and thiourea [26], afforded 5,6-dialkylpyrimidines **19a**,**b**. Phenyl pyrimidines corresponding to **9** and **10** were synthesized as reported previously in the literature [27].

2.2. Syntheses of piperidine ring-modified derivatives

In order to evaluate the role of the ring size of the piperidine moiety, we synthesized derivatives **20** and **21**, which have pyrrolidine and azepane rings, respectively. The pyrrolidine and linker parts of **20** were synthesized by using *N*,*N*-dibenzylamino aldehyde [28] as a chiral building block (Scheme 3). Oxidation of **22**, which had been prepared from D-aspartic acid, gave *N*,*N*-dibenzylamino aldehyde, and subsequently reacted with allylmagnesium bromide and chlorodimethyl ether to diastereoselectively give MOM ether **23**. Dihydroxylation of **23** by osmium tetroxide and subsequent oxidative cleavage produced γ-amino acid derivative **25**. After debenzylation of **25** by hydrogenolysis, intramolecular cyclization gave γ-lactam **26**, which was then converted into Boc-protected pyrrolidine **27** by borane reduction. Oxidation of **27** produced the aldehyde, which, after treatment with dimethylsulfonium methylide [29], produced epoxide **28**. Coupling reaction between **28** and 4-hydroxyquinazoline, followed by oxidation and acidic deprotection of the resulting compound, yielded **20**.

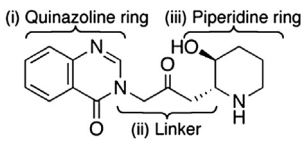


Fig. 2. The three parts of febrifugine (**1**).

The azepane and linker parts of **21** were synthesized from the common intermediate **23** (Scheme 3). Oxidative *N*-debenzylation of **23** by treatment with ceric ammonium nitrate [30] yielded **29**, which was then converted into *N*-allyl compound **30**. Intramolecular olefin metathesis of **30** and subsequent hydrogenation yielded azepane **32**. Finally, coupling reaction between **32** and 4-hydroxyquinazoline via an epoxide intermediate produced **21**.

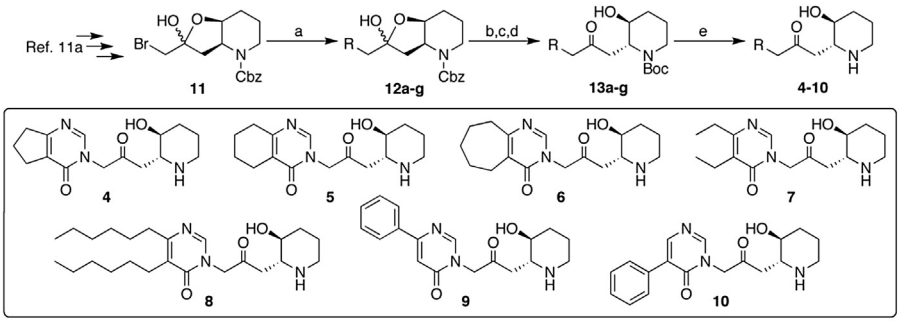
In our previous work [19], 2′-decarbonylated derivative (**33**) (Fig. 3) of **1** showed very low toxicity and sufficiently effective antimalarial activity both *in vitro* and *in vivo* (Tables 1 and 2). Thus, we synthesized 2′-decarbonylated compounds **34** and **35** bearing pyrrolidine and azepane rings, respectively (Scheme 4). Through the use of D-glutamic acid as a starting material, the aliphatic ring and linker parts of compounds **34** and **35** were afforded in a manner similar to those for **20** and **21**, respectively. Finally, the respective coupling reaction of parts with 4-hydroxyquinazoline via tosylates produced **34** and **35**.

2.3. In vitro antimalarial activities and cytotoxicities of the synthetic febrifugine derivatives

The *in vitro* antimalarial activity of febrifugine analogs **4**–**10**, **20**, **21**, **34**, and **35** against *P. falciparum* and their cytotoxicity against mouse L929 cells were evaluated (Table 1). The antimalarial activities of the aliphatic-ring-fused pyrimidine derivatives **4**–**6** was 1/30–1/50 of that of **1**. However, their cytotoxicities were very low (EC_{50} > 50 μg/mL); consequently, these compounds showed very high selectivities (about 1000). Therefore, a suitable method to create antimalarials that are more effective and safer is to modify the quinazoline ring of **1** through the aliphatic-ring-fused pyrimidine. 5,6-Dialkylpyrimidine derivative **7** (diethyl) showed moderate antimalarial activity whereas derivative **8** (di-*n*-hexyl) showed none, indicating that a cyclic moiety of substituents on the pyrimidine ring of **1** may be important for activity. The phenyl pyrimidine compounds **9** and **10** had antimalarial activities 1/30–1/60 of that of **1** and sufficient selectivities. On the contrary, compounds **20**, **21**, **34**, and **35**, which have pyrrolidine or azepane rings instead of the piperidine ring of **1**, displayed only weak antimalarial activity or none at all. Thus, the ring size of piperidine in **1** is crucial for activity.

2.4. In vivo antimalarial activity of the synthetic febrifugine derivatives

In vivo activities of synthesized febrifugine derivatives against *P. berghei*, the malarial parasite of rodents, and their acute toxicity to mice were examined (Table 2). As described above, the aliphatic aliphatic-ring-fused pyrimidine derivatives **4**–**6** showed safer antimalarial activities. Among these compounds, we chose cyclohexane ring-fused derivative **5** as a test compound, because its ring size is the same as that of febrifugine (**1**), and it is easy to prepare sufficient amount of for *in vivo* experiment. The phenyl pyrimidine compounds **9** and **10** also showed selective antimalarial activities, and we chose more potent compounds **10** as a test compound for *in vivo* experiment. As a result, the *in vivo* antimalarial activities of **5** (ED_{50} 12.8 mg/kg) and **10** (ED_{50} 6.3 mg/kg) were 1/15–1/30 of that of **1**. However, the acute toxicities of **5** and **10** were much weaker than that of **1** and their therapeutic index were higher than **1**. Especially, therapeutic index of **5** was almost 100. In addition, diarrhea and pale appearance of liver were not observed in mice



[a]Reagents and conditions: (a) corresponding aromatic compound, K_2CO_3, DMF, rt; (b) H_2 (1 atm), $Pd(OH)_2/C$, MeOH, rt; (c) MeOH, reflux; (d) Boc_2O, Et_3N, MeOH, rt (15% (**13a**), 24% (**13b**), 12% (**13c**), 10% (**13d**), 12% (**13e**), 18% (**13f**) and 21% (**13g**) (4 steps)); (e) 10 % HCl-MeOH, rt (92% (**4**), 95% (**5**), 92% (**6**), 91% (**7**), 85% (**8**), 91% (**9**) and 91% (**10**)).

Scheme 1. Synthesis of compounds **4**–**10**.[a]

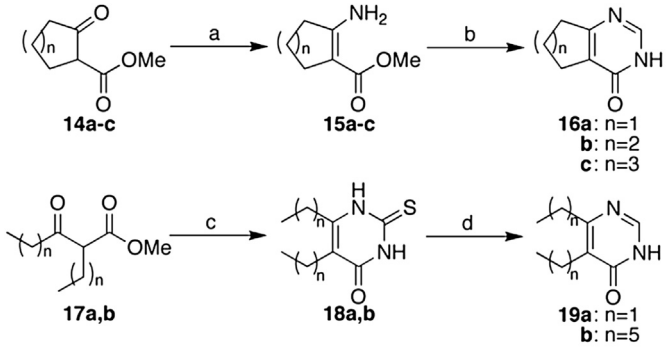
administered 200 mg/kg of compound **5**. Therefore, the tetrahydroquinazoline derivative **5** is an effective and safe antimalarial candidate.

In conclusion, we synthesized and evaluated a new series of febrifugine derivatives, and found tetrahydroquinazoline derivative **5** to exhibit potent antimalarial activity with a very high therapeutic selectivity both *in vitro* and *in vivo*. Therefore, further studies on **5** with focus on areas such as metabolic analysis and elucidation of the action mechanism are necessary for the development of a novel antimalarial drug.

3. Experimental section

3.1. General methods

Starting materials were either commercially available or prepared as reported previously in the literature. 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). Nuclear magnetic resonance spectra were recorded on JEOL JNM ECA-600, ECP-500, and AL-400. Mass spectra were measured on JEOL JMS AX-500 and AX-700. Elemental analyses were performed on Yanaco CHN Corder MT-6.



[a]Reagents and conditions: (a) Ph_3P=NTMS, 2-propanol, TsOH, toluene, reflux ; (b) Formamide, 180 °C (39% (**16a**), 50% (**16b**) and 34% (**16c**) (2 steps)); (c) Thiourea, EtONa, EtOH, 90 °C; (d) $NiCl_2$•$6H_2O$, $NaBH_4$, MeOH, rt (17% (**19a**) and 23% (**19b**) (2 steps)).

Scheme 2. Synthesis of compounds **16a**–**c** and **19a**,**b**.[a]

3.1.1. (2R*,3S*)-tert-Butyl 3-hydroxy-2-[2-oxo-3-(4-oxo-4,5,6,7-tetrahydro-3H-cyclopenta-[d]pyrimidin-3-yl)propyl]piperidine-1-carboxylate (*13a*)

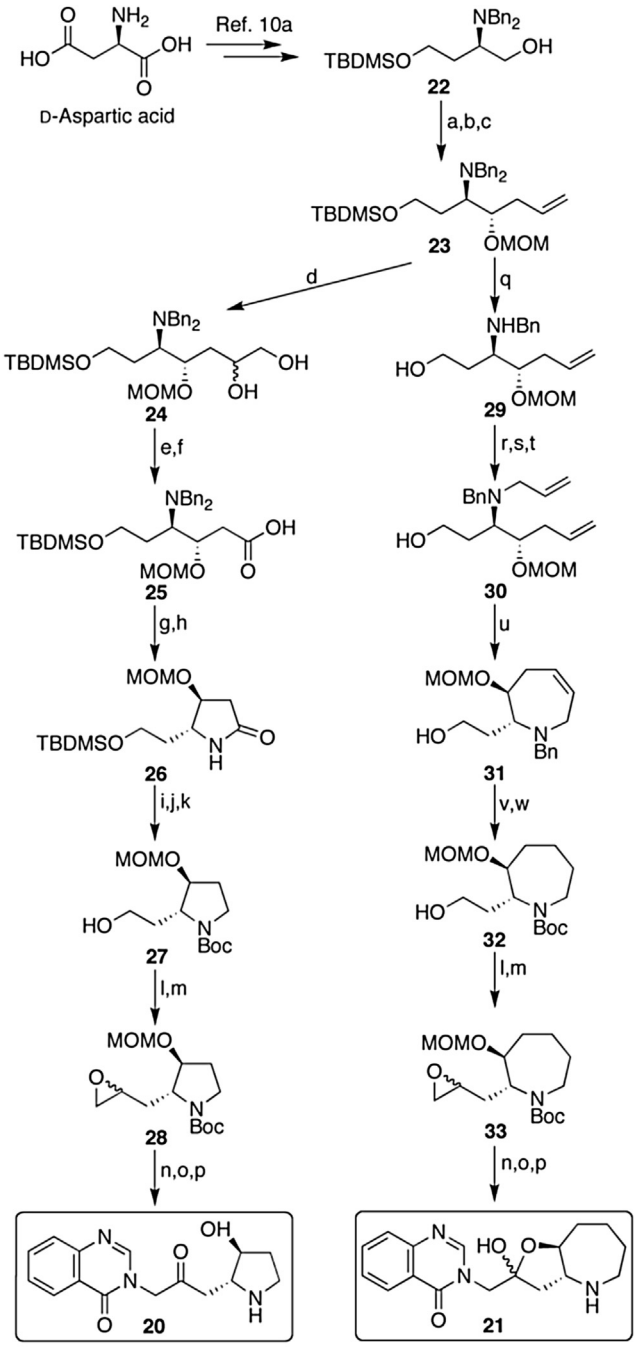
To a solution of **16a** (62 mg, 0.454 mmol) in DMF (1 mL) were added **11** [22] (168 mg, 0.454 mmol) and potassium carbonate (69 mg, 0.500 mmol) at room temperature. After stirring for 2 h, the mixture was poured into brine and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was dissolved in methanol (2.5 mL). Then, after refluxing for 3 h, triethylamine (28 μL, 0.196 mmol) and di-*tert*-butyl dicarbonate (78 mg, 0.357 mmol) were added at room temperature. After stirring for 1 h, the mixture was poured into 0.2 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 ethyl acetate–methanol (9:1) to give **13a** (27 mg, 0.068 mmol, 15% (4 steps)).

In a similar procedure, compounds **13b** (yield: 24%), **13c** (12%), **13d** (10%), **13e** (12%), **13f** (18%) and **13g** (21%) were prepared from **16a**–**c**, **19a**,**b**, 6-phenylpyrimidine [27] and 5-phenyl pyrimidine [27], respectively.

3.1.2. 3-[3-((2R*,3S*)-3-Hydroxypiperidin-2-yl)-2-oxopropyl]-6,7-dihydro-3H-cyclopenta[d]pyrimidin-4(5H)-one (*4*)

Compound **13a** (4.9 mg, 0.013 mmol) was dissolved in 10% hydrogen chloride in methanol (2.0 mL) at room temperature. After stirring for 2 h, the mixture was evaporated. The residue was chromatographed over silica gel eluted by chloroform–methanol (4:1) to give **4** (4.2 mg, 0.012 mmol, 92%) as dihydrochloride.

Data for **4**: colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.64 (1H, s), 5.02 (1H, d, J = 17.5 Hz), 4.96 (1H, d, J = 17.5 Hz), 3.64 (1H, ddd, J = 13.3, 9.5, 3.7 Hz), 3.44 (1H, ddd, J = 9.5, 7.0, 4.5 Hz), 3.36 (1H, dd, J = 18.3, 4.5 Hz), 3.29–3.31 (1H, m), 3.05 (1H, dd, J = 18.3, 7.0 Hz), 2.91–2.96 (1H, m), 2.87–2.89 (2H, m), 2.70–2.74 (2H, m), 2.04–2.12 (2H, m), 1.96–2.00 (1H, m), 1.90–1.94 (1H, m), 1.62–1.69 (1H, m), 1.46–1.51 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 201.1, 165.0, 159.5, 153.4, 127.1, 68.2, 58.0, 56.0, 44.8, 40.1, 34.1, 31.5, 28.6, 22.4, 21.4; HRFABMS m/z 292.1656 [M + H]$^+$



[a]Reagents and conditions: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C; (b) allylmagnesium bromide, THF, -78 °C (82% (2 steps)); (c) MOMCl, KI, *i*Pr_2EtN, DME, 80 °C (96%); (d) OsO_4, NMO, *t*BuOH-THF-H_2O (1:2:1), rt (95%); (e) $NaIO_4$, Et_2O-H_2O (2:1), rt ; (f) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, tBuOH-H_2O (5:1), rt (74% (2 steps)); (g) H_2 (1 atm), $Pd(OH)_2$/C, MeOH, rt; (h) EDCI•HCl, Et_3N, DMAP, CH_2Cl_2-THF (1:1), rt (84% (2 steps)); (i) Boc_2O, Et_3N, DMAP, CH_2Cl_2, rt (65%); (j) BH_3•Me_2S, THF, reflux; (k) TBAF, THF, rt (71% (2 steps)); (l) Dess-Martin periodinane, CH_2Cl_2, rt; (m) Me_3SI, NaH, THF-DMSO (1:1), rt (38% (**28**) and 52% (**33**) (2 steps)); (n) 4-Hydroxyquinazoline, KH, DMF, 80 °C; (o) Dess-Martin periodinane, CH_2Cl_2, rt; (p) 10 % HCl-MeOH, rt (35% (**20**) and 23 % (**21**) (3 steps)); (q) CAN, acetonitrile-H_2O (5:1), rt (79%); (r) TBDMSCl, imidazole, CH_2Cl_2, rt; (s) allyl bromide, NaH, DMF, 100 °C; (t) TBAF, THF, rt (43% (3 steps)); (u) Grubbs catalyst 1st gen., CH_2Cl_2, 60 °C (73%); (v) H_2 (1 atm), $Pd(OH)_2$/C, MeOH, rt; (w) Boc_2O, Et_3N, MeOH, rt (61% (2 steps)).

Scheme 3. Synthesis of compounds **20** and **21**.[a]

(292.1661 calcd for $C_{15}H_{22}N_3O_3$); Anal. ($C_{15}H_{25}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

In a similar procedure, compounds **5** (yield: 94%), **6** (92%), **7** (91%), **8** (89%), **9** (91%) and **10** (91%) were prepared from **13b**–**g**, respectively.

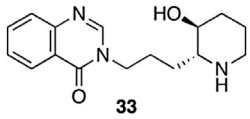
3.1.2.1. Data for 3-[3-((2R,3S*)-3-hydroxypiperidin-2-yl)-2-oxopropyl]-5,6,7,8-tetrahydro-quinazolin-4(3H)-one (**5**).* Colorless amorphous solid; ^{1}H NMR (600 MHz, CD_3OD) δ 9.29 (1H, s), 5.17 (2H, s), 3.60–3.65 (1H, m), 3.40–3.45 (1H, m), 3.37 (1H, dd, J = 18.1, 4.7 Hz), 3.27–3.29 (1H, m), 3.06 (1H, dd, J = 18.1, 7.1 Hz), 2.96–3.00 (1H, m), 2.68–2.72 (2H, m), 2.44–2.47 (2H, m), 2.02–2.07 (1H, m), 1.94–1.97 (1H, m), 1.81–1.87 (2H, m), 1.75–1.80 (2H, m), 1.71–1.76 (1H, m), 1.50–1.57 (1H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 200.4, 159.4, 152.3, 151.7, 125.0, 68.3, 58.0, 56.7, 44.9, 40.3, 31.6, 27.6, 23.0, 21.8, 21.7, 21.4; HRFABMS m/z 306.1808 $[M + H]^+$ (306.1818 calcd for $C_{16}H_{24}N_3O_3$); Anal. ($C_{16}H_{27}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.2.2. Data for 3-[3-((2R,3S*)-3-hydroxypiperidin-2-yl)-2-oxopropyl]-6,7,8,9-tetrahydro-3H-cyclohepta[d]pyrimidin-4(5H)-one (**6**).* Colorless amorphous solid; ^{1}H NMR (600 MHz, CD_3OD) δ 9.02 (1H, s), 5.15 (1H, d, J = 17.5 Hz), 5.11 (1H, d, J = 17.5 Hz), 3.64 (1H, ddd, J = 12.8, 9.5, 4.0 Hz), 3.44–3.48 (1H, m), 3.40 (1H, dd, J = 18.2, 4.6 Hz), 3.28–3.30 (1H, m), 3.07 (1H, dd, J = 18.2, 7.4 Hz), 2.98–3.02 (1H, m), 2.86–2.91 (2H, m), 2.76–2.81 (2H, m), 2.05–2.11 (1H, m), 1.98–2.03 (1H, m), 1.88–1.93 (2H, m), 1.73–1.80 (3H, m), 1.55–1.63 (3H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 200.8, 160.4, 159.3, 151.7, 129.4, 68.2, 58.0, 56.9, 44.9, 40.2, 34.8, 32.6, 31.5, 26.4, 25.8, 25.4, 21.4; HRFABMS m/z 320.1960 $[M + H]^+$ (320.1974 calcd for $C_{17}H_{26}N_3O_3$); Anal. ($C_{17}H_{29}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.2.3. Data for 5,6-diethyl-3-[3-((2R,3S*)-3-hydroxypiperidin-2-yl)-2-oxopropyl]-pyrimidin-4(3H)-one (**7**).* Colorless amorphous solid; ^{1}H NMR (600 MHz, CD_3OD) δ 9.02 (1H, s), 5.15 (1H, d, J = 17.5 Hz), 5.11 (1H, d, J = 17.5 Hz), 3.64 (1H, ddd, J = 12.8, 9.8, 4.0 Hz), 3.44–3.49 (1H, m), 3.40 (1H, dd, J = 18.2, 4.5 Hz), 3.28–3.32 (1H, m), 3.06 (1H, dd, J = 18.2, 7.4 Hz), 2.96–3.02 (1H, m), 2.74 (2H, q, J = 7.6 Hz), 2.58 (2H, q, J = 7.5 Hz), 2.06–2.11 (1H, m), 1.97–2.03 (1H, m), 1.72–1.81 (1H, m), 1.54–1.62 (1H, m), 1.29 (3H, t, J = 7.6 Hz), 1.11 (3H, t, J = 7.5 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 200.8, 160.6, 157.5, 152.3, 128.1, 68.2, 58.0, 56.6, 44.9, 40.1, 31.5, 25.8, 21.4, 19.9, 13.2, 13.1; HRFABMS m/z 308.1959 $[M + H]^+$ (308.1974 calcd for $C_{16}H_{26}N_3O_3$); Anal. ($C_{16}H_{29}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.2.4. Data for 5,6-dihexyl-3-[3-((2R,3S*)-3-hydroxypiperidin-2-yl)-2-oxopropyl]-pyrimidin-4(3H)-one (**8**).* Colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 9.30 (1H, s), 5.20 (2H, s), 3.66 (1H, ddd, J = 13.5, 9.5, 3.8. Hz), 3.42–3.48 (1H, m), 3.42 (1H, dd, J = 18.3, 4.6 Hz), 3.29–3.33 (1H, m), 3.10 (1H, dd, J = 18.3, 7.1 Hz), 2.94–3.01 (1H, m), 2.73 (2H, t, J = 7.5 Hz), 2.55 (2H, t, J = 7.5 Hz), 2.07–2.12 (1H, m), 1.98–2.04 (1H, m), 1.74–1.82 (1H, m), 1.65–1.73 (2H, m), 1.53–1.61 (1H, m), 1.42–1.51 (2H, m), 1.27–1.39 (12H, m), 0.93 (3H, t, J = 7.0 Hz), 0.91 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 200.8, 160.6, 156.0, 152.3, 127.4, 68.2, 58.0, 56.7, 44.9, 40.1, 32.7, 32.6, 31.5, 30.3, 30.1, 29.6, 29.3, 26.7, 23.7, 23.6, 23.5, 21.4, 14.4, 14.3; HRFABMS m/z 420.3236 $[M + H]^+$ (420.3226 calcd for $C_{24}H_{42}N_3O_3$); Anal. ($C_{24}H_{45}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.2.5. Data for 3-[3-((2R,3S*)-3-hydroxypiperidin-2-yl)-2-oxopropyl]-6-phenylpyrimidin-4(3H)-one (**9**).* Colorless amorphous solid; ^{1}H NMR (600 MHz, D_2O) δ 8.38 (1H, s), 7.91 (2H, dd, J = 8.3,



33

Fig. 3. Structure of 2′-decarbonylated derivative **33**.

1.5 Hz), 7.53–7.61 (3H, m), 6.98 (1H, s), 5.11 (1H, d, J = 18.1 Hz), 5.07 (1H, d, J = 18.1 Hz), 3.70–3.76 (1H, m), 3.50–3.55 (1H, m), 3.45 (1H, dd, J = 18.6, 4.6 Hz), 3.32–3.36 (1H, m), 3.11 (1H, dd, J = 18.6, 7.3 Hz), 3.00–3.06 (1H, m), 2.09–2.15 (1H, m), 1.97–2.03 (1H, m), 1.71–1.79 (1H, m), 1.54–1.63 (1H, m); ^{13}C NMR (150 MHz, D_2O) δ 203.0, 164.0, 163.8, 152.8, 135.8, 132.0, 129.7 (2C), 127.8 (2C), 110.2, 67.8, 63.1, 56.8, 44.5, 39.5, 30.5, 20.6; HRFABMS m/z 328.1684 $[M + H]^+$ (328.1661 calcd for $C_{18}H_{22}N_3O_3$); Anal. ($C_{18}H_{25}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.2.6. Data for 3-[3-((2R,3S*)-3-hydroxypiperidin-2-yl)-2-oxopropyl]-5-phenylpyrimidin-4(3H)-one (**10**).* Colorless amorphous solid; ^{1}H NMR (600 MHz, CD_3OD) δ 9.14 (1H, s), 8.27 (1H, s), 7.68 (2H, dd, J = 7.7, 1.9 Hz), 7.43–7.49 (3H, m), 5.26 (1H, d, J = 17.7 Hz), 5.22 (1H, d, J = 17.7 Hz), 3.64–3.69 (1H, m), 3.45–3.50 (1H, m), 3.43 (1H, dd, J = 17.3, 4.4 Hz), 3.29–3.34 (1H, m), 3.12 (1H, dd, J = 17.3, 6.4 Hz), 2.97–3.03 (1H, m), 2.06–2.11 (1H, m), 1.96–2.03 (1H, m), 1.74–1.82 (1H, m), 1.54–1.62 (1H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 200.8, 159.6, 153.7, 142.9, 132.3, 130.7, 130.6 (2C), 129.7 (2C), 129.3, 68.2, 58.0, 57.0, 44.9, 40.3, 31.6, 21.4; HRFABMS m/z 328.1681 $[M + H]^+$ (328.1661 calcd for $C_{18}H_{22}N_3O_3$); Anal. ($C_{18}H_{25}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

*3.1.3. 6,7-Dihydro-3H-cyclopenta[d]pyrimidin-4(5H)-one (**16a**)*

To a solution of ethyl 2-oxo-1-cyclopentanecarboxylate (**14a**) (1.00 g, 6.40 mmol) in toluene (15 mL) were added 1,1,1-trimethyl-*N*-(triphenylphosphoranylidene)silanamine (2.24 g, 6.40 mmol), 2-propanol (0.49 mL, 6.40 mmol) and *p*-toluenesulfonic acid monohydrate (12.2 mg, 0.064 mmol) [24]. After refluxing for 10 h, the mixture was poured into saturated sodium bicarbonate solution and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated to give a crude enamine **15a**. This crude was dissolved in formamide (6 mL) and stirred for 9 h at 180 °C. The mixture was poured into brine and extracted with chloroform five times. The organic layer was dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–methanol (9:1) to give **16a** (337 mg, 2.47 mmol, 39% (2 steps)).

In a similar procedure, compounds **16b** (yield: 50%) and **16c** (34%) were prepared from **14b** and **14c**, respectively.

Table 1
In vitro antimalarial activities of synthesized febrifugine derivatives against *P. falciparum*.

Compound	Antimalarial activity		Cytotoxicity[c]	Selectivity[d]
	FCR-3[a]	K1[b]		
	EC_{50} (μg/mL)		EC_{50} (μg/mL)	
Febrifugine (**1**)	0.00142	0.00140	0.167	118
33[19]	0.0225	0.0236	4.45	198
Chloroquine	0.0475	0.375	18.6	392
Artemisinin	0.0179	0.0117	>100	>5590
4	0.0747	0.0911	>100	1338
5	0.0790	0.0857	73.8	934
6	0.0411	0.0452	58.4	1421
7	0.197	0.243	72.3	367
8	>1	>1	N.T.[e]	–
9	0.0937	0.138	29.4	314
10	0.0422	0.0475	6.3	149
20	0.611	>1	N.T.[e]	–
21	0.123	0.187	N.T.[e]	–
34	>1	>1	N.T.[e]	–
35	0.545	0.725	N.T.[e]	–

[a] Against *P. falciparum* FCR-3 (chloroquine sensitive strain).
[b] Against *P. falciparum* K1 (chloroquine resistant strain).
[c] Against mouse L929 cells.
[d] Selectivity = EC_{50} for L929 cells/EC_{50} for *P. falciparum* FCR-3.
[e] Not tested.

Table 2
In vivo antimalarial activities against *P. berghei* and acute toxicities of synthesized febrifugine derivatives **5** and **10**.[a]

Compound	Antimalarial activity[b]	Acute toxicity[c]	Therapeutic index[d]
	ED_{50} (mg/kg)	LD_{50} (mg/kg)	
Febrifugine (**1**)	0.41	7.1	17
5	12.8	1200	94
10	6.3	400	63
Chloroquine	0.98	N.T.[e]	–

[a] All compounds were administrated by p.o.
[b] Against *P. berghei* (rodent malaria).
[c] Toxicity in mice.
[d] Therapeutic index = LD_{50} in mice/ED_{50} for *P. berghei* in mice.
[e] Not tested.

*3.1.4. 5,6-Diethylpyrimidin-4(3H)-one (**19a**)*

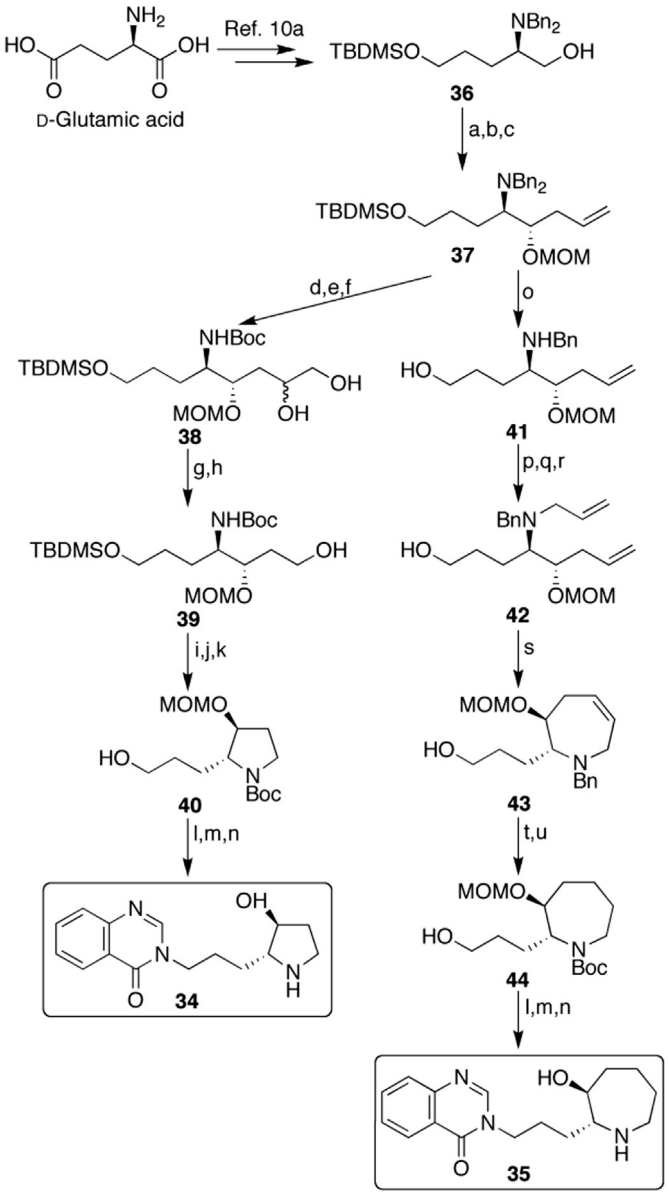
To a solution of methyl 2-ethyl-3-oxopentanoate (**17a**) (1.28 g, 8.10 mmol) in ethanol (70 mL) were added thiourea (9.25 g, 122 mmol) and sodium ethoxide (16.5 g, 243 mmol). After refluxing for 1.5 h, the mixture was acidified until reaching pH 2 by 3 M hydrochloric acid, and then a precipitate was collected by filtration to give a crude thiourea **18a**. This crude was dissolved in methanol (20 mL) and nickel (II) chloride hexahydrate (1.35 g, 5.67 mmol) and sodium borohydride (643 mg, 17.0 mmol) were added to the solution. After stirring for 3 h at room temperature, the mixture was poured into saturate ammonium chloride solution and extracted with chloroform three times. The organic layer was dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–methanol (19:1) to give **19a** (209 mg, 1.38 mmol, 17% (2 steps)).

In a similar procedure, 5,6-dihexylpyrimidin-4(3*H*)-one **(19b)** (yield: 22%) was prepared from **17b**.

*3.1.5. (3R,4S)-N,N-Dibenzyl-1-(tert-butyldimethylsilyloxy)-4-(methoxymethoxy)hept-6-en-3-amine (**23**)*

To a solution of oxalyl chloride (1.15 mL, 13.3 mmol) in dichloromethane (40 mL) was added DMSO (1.88 mL, 26.5 mmol) and **22** [19] (3.53 g, 8.84 mmol) dissolved in dichloromethane (5.0 mL) at −78 °C. After stirring for 1 h, triethylamine (4.47 mL, 44.2 mmol) was slowly added to this mixture, and stirred at 0 °C. 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 the crude of (2*R*)-4-(*tert*-butyldimethylsilyloxy)-2-(dibenzylamino)butanal.

The crude aldehyde was dissolved in THF (20 mL). 2 M allylmagnesium bromide in THF (8.84 mL, 17.7 mmol) was added to this solution at −78 °C. After stirring for 2 h, the mixture was allowed to warm to 0 °C, and poured into saturated ammonium chloride solution. 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



[a]Reagents and conditions: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C; (b) allylmagnesium bromide, THF, -78 °C (71% (2 steps)); (c) MOMCl, KI, *i*Pr_2EtN, DME, 80 °C (71%); (d) OsO_4, NMO, *t*BuOH-THF-H_2O (1:2:1), rt (88%); (e) H_2 (1 atm), $Pd(OH)_2/C$, MeOH, rt; (f) Boc_2O, Et_3N, MeOH, rt (80% (2 steps)) (g) $NaIO_4$, Et_2O-H_2O (2:1), rt; (h) $NaBH_4$, MeOH, rt (73% (2 steps)); (i) TsCl, pyridine, rt; (j) TBAF, THF, 0 °C; (k) NaH, THF, 0 °C (54% (3 steps)); (l) TsCl, pyridine, rt; (m) 4-Hydroxyquinazoline, K_2CO_3, DMF, 50 °C; (n) 10 % HCl-MeOH, rt (48% (**34**) and 51 % (**35**) (3 steps)); (o) (q) CAN, acetonitrile-H_2O (5:1), rt (82%); (p) TBDMSCl, imidazole, CH_2Cl_2, rt; (q) allyl bromide, NaH, DMF, 100 °C; (r) TBAF, THF, rt (57% (3 steps)); (s) Grubbs catalyst 1st gen., CH_2Cl_2, 60 °C (51%); (t) H_2 (1 atm), $Pd(OH)_2/C$, MeOH, rt; (u) Boc_2O, Et_3N, MeOH, rt (50% (2 steps)).

Scheme 4. Synthesis of compounds **34** and **35**.[a]

eluted by hexane–ethyl acetate (19:1) to give (4*S*,5*R*)-7-(*tert*-butyldimethylsilyloxy)-5-(dibenzylamino)hept-1-en-4-ol (3.59 g, 8.16 mmol, 91% (2 steps)).

To a solution of (4*S*,5*R*)-7-(*tert*-butyldimethylsilyloxy)-5-(dibenzylamino)hept-1-en-4-ol (1.70 g, 3.87 mmol) in 1,2-dimethoxyethane (20 mL) were added *N*,*N*-diisopropylamine (2.70 mL, 15.5 mmol), chloromethyl methyl ether (0.700 mL, 9.68 mmol) and potassium iodide (771 mg, 4.64 mmol) at room temperature. After refluxing for 4 h, the mixture was allowed 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 hexane–ethyl acetate (39:1) to give **23** (1.52 g, 3.14 mmol, 81%).

*3.1.6. (3S,4R)-6-(tert-Butyldimethylsilyloxy)-4-(dibenzylamino)-3-(methoxymethoxy)-hexanoic acid (**25**)*

To a solution of **23** (1.52 g, 3.14 mmol) in water–*tert*-butanol–THF (1:1:2) (8.0 mL) were added 4-methylmorpholine *N*-oxide (920 mg, 7.85 mmol) and osmium tetroxide (2% solution in water) (1.15 mL, 0.094 mmol) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was poured into 10% sodium sulfite solution, and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–ethyl acetate (1:1) to give a diastereomeric mixture of a corresponding diol (1.54 g, 2.98 mmol, 95%).

Sodium periodate (1.09 g, 5.07 mmol) was added to a solution of the diol (1.54 g, 2.98 mmol) in diethyl ether–water (5:1) (30 mL). After vigorously stirring for 3 h at room temperature, the mixture was poured into water and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over sodium sulfate, and evaporated to give a crude aldehyde.

The crude aldehyde was dissolved in *tert*-butanol–water (5:1) (8.0 mL). 2-Methylbut-2-ene (1.26 mL, 11.8 mmol), sodium dihydrogen phosphate dihydrate (688 mg, 4.41 mol) and sodium chlorite (80%) (365 mg, 3.23 mmol) were added to this solution at 0 °C. After stirring for 30 min, the mixture was poured into 0.2 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 hexane–ethyl acetate (2:1) to give **25** (1.17 g, 2.32 mmol, 74% (2 steps)).

*3.1.7. (4S,5R)-5-[2-(tert-Butyldimethylsilyloxy)ethyl]-4-(methoxymethoxy)pyrrolidin-2-one (**26**)*

Compound **26** (1.57 g, 3.13 mmol) and 20% palladium hydroxide on carbon (523 mg) in methanol (30 mL) was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration through a Celite pad, the filtrate was evaporated to give oily residue. To a solution of this residue in THF–dichloromethane (1:1) (30 mL) were added triethylamine (0.65 mL, 4.70 mmol), 4-(dimethylamino)pyridine (38.2 mg, 0.313 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (900 mg, 4.70 mmol) at 0 °C. After stirring for 4 h, the mixture was poured into 0.2 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 chloroform–methanol (49:1) to give **26** (1.17 g, 2.32 mmol, 74% (2 steps)).

*3.1.8. (2R,3S)-tert-Butyl 2-(2-hydroxyethyl)-3-(methoxymethoxy) pyrrolidine-1-carboxylate (**27**)*

To a solution of **26** (769 mg, 2.54 mmol) in dichloromethane (25 mL) were added triethylamine (1.76 mL, 12.7 mmol), 4-(dimethylamino)pyridine (1.55 g, 12.7 mmol) and di-*tert*-butyl dicarbonate (5.54 g, 25.4 mmol). After stirring for 2 h at room temperature, the mixture was poured into 0.5 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 hexane–ethyl acetate (3:1) to give (2*R*,3*S*)-*tert*-butyl 2-[2-(*tert*-butyldimethylsilyloxy)ethyl]-3-(methoxymethoxy)-5-oxopyrrolidine-1-carboxylate (665 mg, 1.65 mmol, 65%).

To a solution of (2*R*,3*S*)-*tert*-butyl 2-[2-(*tert*-butyldimethylsilyloxy)ethyl]-3-(methoxymethoxy)-5-oxopyrrolidine-1-carboxylate (582 mg, 1.44 mmol) in THF (7.0 mL) was added 2 M solution of borane–dimethyl sulfide complex in THF (2.16 mL, 4.32 mmol) at room temperature. After refluxing for 8 h, the mixture was 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 to give a crude of piperidine derivative.

This crude was dissolved in THF (2 mL), and 1 M solution of tetra-*n*-butylammonium fluoride in THF (2.1 mL, 2.1 mmol) was added at 0 °C. After stirring for 2 h at room temperature, the mixture was poured into 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 hexane–ethyl acetate (1:1) to give **27** (283 mg, 1.03 mmol, 71% (2 steps)).

3.1.9. 3-[3-((2R,3S)-3-Hydroxypyrrolidin-2-yl)-2-oxopropyl] quinazolin-4(3H)-one (**20**)

To a solution of **27** (283 mg, 1.03 mmol) in CH_2Cl_2 (6.0 mL) were added 15 wt% solution of Dess–Martin periodinane in dichloromethane (4.37 mL, 1.55 mmol) and water (20 μL) at 0 °C. After stirring for 1 h, the mixture was poured into 1 M sodium hydroxide 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 (60% mineral oil suspension) (12.7 mg, 0.318 mmol) in DMSO–THF (1:1) (4.0 mL) were added trimethylsulfonium iodide (64.9 mg, 0.318 mmol) and the crude aldehyde at −15 °C. After stirring for 30 min at room temperature, the mixture was poured into 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 hexane–EtOAc (2:1) to give a diastereomeric mixture of a corresponding epoxide **28** (108 mg, 0.38 mmol, 36% (2 steps)).

To a solution of 4-hydroxyquinazoline (66.2 mg, 0.453 mmol) in DMSO (1.0 mL) was added potassium hydride (30% mineral oil suspension) (60 mg, 0.453 mmol) at 0 °C. After 30 min, compound **28** (59.2 mg, 0.206 mmol) dissolved in DMSO (1.5 mL) was added into the solution. After stirring for 4 h at 80 °C, 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 dissolved in dichloromethane (3.0 mL). 15 wt% solution of Dess–Martin periodinane in dichloromethane (0.68 mL, 0.240 mmol) was added to this solution at 0 °C. After stirring for 1.5 h, the mixture was poured into 1 M sodium hydroxide 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 (49:1) to give 3-[3-[(2*R*,3*S*)-*N*-(*tert*-butyloxycarbonyl)-3-(methoxymethoxy)pyrrolidin-2-yl]-2-oxopropyl]quinazolin-4(3*H*)-one (27 mg, 0.072 mmol, 35% (2 steps)).

3-[3-[(2*R*,3*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-(methoxymethoxy) pyrrolidin-2-yl]-2-oxopropyl]quinazolin-4(3*H*)-one (19 mg, 0.043 mmol) was dissolved in 10% hydrogen chloride in methanol (1.0 mL) at room temperature. After stirring for 2 h, the solution was evaporated. The residue was chromatographed over silica gel eluted by chloroform–methanol (3:1) to give **20** (15 mg, 0.042 mmol, 97%) as dihydrochloride.

Data for **20**: colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 9.20 (1H, s), 8.34 (1H, d, $J = 8.0$ Hz), 8.04 (1H, t, $J = 8.0$ Hz), 7.81 (1H, d, $J = 8.0$ Hz), 7.78 (1H, d, $J = 8.0$ Hz), 5.22–5.29 (2H, m), 4.25–4.31 (1H, m), 3.82–3.87 (1H, m), 3.41–3.48 (2H, m), 3.25–3.30 (1H, m), 3.18 (1H, dd, $J = 19.2$, 9.9 Hz), 2.20–2.30 (1H, m), 1.97–2.06 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 201.2, 160.0, 152.0, 141.6, 137.7, 129.7, 128.7, 123.2, 121.6, 74.9, 62.7, 56.2, 44.6, 41.0, 32.8; HRFABMS *m*/*z* 288.1369 $[M + H]^+$ (288.1348 calcd for $C_{15}H_{18}N_3O_3$); Anal. ($C_{15}H_{21}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.10. (3R,4S)-3-(Benzylamino)-4-(methoxymethoxy)hept-6-en-1-ol (**29**)

To a solution of **23** (1.12 g, 2.31 mmol) in acetonitrile–dichloromethane (5:1) (24 mL) was added ceric ammonium nitrate (3.17 g, 5.78 mmol). After stirring for 30 min at room temperature, the mixture was poured into saturated sodium bicarbonate 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 **29** (458 mg, 1.64 mmol, 71%).

3.1.11. (3R,4S)-3-[Allyl(benzyl)amino]-4-(methoxymethoxy)hept-6-en-1-ol (**30**)

To a solution of **29** (1.70 g, 3.87 mmol) in dichloromethane (4.0 mL) were added imidazole (266 mg, 3.91 mmol) and *tert*-butyldimethylchlorosilane (589 mg, 3.91 mmol). After stirring for 2.5 h at room temperature, 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 hexane–ethyl acetate (4:1) to give a TBS ether of **29** (1.23 g, 2.83 mmol, 73%).

To a solution of a TBS ether of **29** (377 mg, 0.870 mmol) in DMF (8.0 mL) were added sodium hydride (60% mineral oil suspension) (104 mg, 2.61 mmol) and ally bromide (0.23 mL, 2.61 mmol). After stirring for 4 h at 100 °C, the mixture was allowed to cool to room temperature, poured into saturated ammonium chloride solution and extracted with dichloromethane three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was dissolved in THF (2.0 mL), and 1 M solution of tetra-*n*-butylammonium fluoride in THF (1.0 mL, 1.0 mmol) was added at 0 °C. After stirring for 2.5 h at room temperature, the mixture was poured into 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 hexane–ethyl acetate (3:1) to give **30** (172 mg, 0.538 mmol, 59% (2 steps)).

3.1.12. 2-[(2R,3S)-1-Benzyl-3-(methoxymethoxy)-2,3,4,7-tetrahydro-1H-azepin-2-yl]ethanol (**31**)

Under argon atmosphere, Grubbs catalyst 1st generation (84 mg, 0.102 mmol) was added to a solution of **30** (164 mg, 0.512 mmol) in chloroform (10 mL). After stirring for 2.5 h at 60 °C, the mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was chromatographed over silica gel eluted by hexane–ethyl acetate (1:1) to give **31** (109 mg, 0.374 mmol, 73%).

*3.1.13. (2R,3S)-tert-Butyl 2-(2-hydroxyethyl)-3-(methoxymethoxy) azepane-1-carboxylate (**32**)*

To a solution of **31** (98 mg, 0.337 mmol) in methanol (3.5 mL) was added 20% palladium hydroxide on carbon (33 mg), This mixture was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration through a Celite pad, the filtrate was evaporated to give oily residue. To a solution of this residue in methanol (2.0 mL) were added triethylamine (0.12 mL, 0.843 mmol) and di-*tert*-butyl dicarbonate (221 mg, 1.01 mmol). After stirring for 4 h at room temperature, the mixture was 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 hexane–ethyl acetate (1:1) to give **32** (62 mg, 0.206 mmol, 61% (2 steps)).

*3.1.14. 3-[((3aR,8aS)-2-Hydroxyoctahydro-2H-furo[3,2-b]azepin-2-yl)methyl]quinazolin-4(3H)-one (**21**)*

In the same manner as the synthesis of **20**, compound **21** (4.1 mg, 0.011 mmol, 12% (5 steps)) was synthesized as dihydrochloride from **32** (27 mg, 0.088 mmol) by five successive reactions.

Data for **21**: colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.94 (1H, s), 8.34 (1H, d, $J = 8.0$ Hz), 7.98 (1H, t, $J = 7.4$ Hz), 7.67–7.83 (2H, m), 4.66 (1H, d, $J = 14.4$ Hz), 4.34 (1H, d, $J = 14.4$ Hz), 4.11–4.23 (1H, m), 3.84–3.95 (1H, m), 3.12–3.31 (2H, m), 2.18–2.31 (2H, m), 1.80–1.99 (3H, m), 1.54–1.70 (3H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 161.2, 151.8, 143.1, 137.2, 130.2, 128.7, 124.2, 122.1, 107.8, 81.8, 60.0, 41.1, 38.9, 34.4, 33.2, 30.8, 24.2; HRFABMS *m*/*z* 316.1671 $[M + H]^+$ (316.1661 calcd for $C_{17}H_{22}N_3O_3$). Anal. ($C_{17}H_{25}N_3O_4Cl_2$ (dihydrochloride hydrate)) C, H, N.

*3.1.15. (4R,5S)-N,N-Dibenzyl-1-(tert-butyldimethylsilyloxy)-5-(methoxymethoxy)oct-7-en-4-amine (**37**)*

In the same manner as the synthesis of **23**, compound **37** (3.43 g, 6.89 mmol, 65% (3 steps)) was synthesized from **36** [19] (4.39 g, 10.6 mmol) by three successive reactions.

*3.1.16. (3R,4S)-7-(tert-Butyldimethylsilyloxy)-4-(tert-butyloxycarbonylamino)-3-(methoxy-methoxy)heptan-1-ol (**39**)*

To a solution of **37** (722 mg, 1.45 mmol) in water–*tert*-butanol–THF (1:1:2) (6.0 mL) were added 4-methylmorpholine *N*-oxide (323 mg, 2.90 mmol) and osmium tetroxide (2% solution in water) (0.46 mL, 0.036 mmol) at 0 °C. After being stirred for 4 h at room temperature, the reaction mixture was poured into 10% sodium sulfite solution, and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–ethyl acetate (1:1) to give a diastereomeric mixture of a corresponding diol (676 mg, 2.98 mmol, 88%).

To a solution of the diol (545 mg, 1.03 mmol) in methanol (5.0 mL) was added 20% palladium hydroxide on carbon (142 mg), This mixture was stirred at room temperature for 1 h under hydrogen atmosphere. After filtration through a Celite pad, the filtrate was evaporated to give oily residue. To a solution of this residue in methanol (3.0 mL) were added triethylamine (0.40 mL, 2.86 mmol) and di-*tert*-butyl dicarbonate (421 mg, 1.93 mmol). After stirring for 2 h at room temperature, the mixture was 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 hexane–ethyl acetate (2:1) to give a diastereomeric mixture of **38** (371 mg, 0.821 mmol, 80% (2 steps)).

Sodium periodate (230 mg, 1.07 mmol) was added to a solution of **38** (244 mg, 0.538 mmol) in diethyl ether–water (2:1) (6 mL). After vigorously stirring for 1 h at room temperature, the mixture was poured into water and extracted with ethyl acetate three times. The organic layer was washed with brine, dried over sodium sulfate, and evaporated to give a crude aldehyde.

The crude aldehyde was dissolved in methanol (3.0 mL), and sodium borohydride (292 mg, 7.72 mmol) was added to the solution at 0 °C. After stirring for 3 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 sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by ethyl acetate to give **39** (166 mg, 0.394 mmol, 73% (2 steps)).

*3.1.17. (2R,3S)-tert-Butyl 2-(3-hydroxypropyl)-3-(methoxymethoxy)pyrrolidine-1-carboxylate (**40**)*

To a solution of **39** (177 mg, 0.420 mmol) in pyridine (2.0 mL) was added *p*-toluenesulfonyl chloride (120 mg, 0.630 mmol). After stirring for 2.5 h at room temperature, the mixture was poured into 0.3 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated to give a crude of tosylate.

The crude of tosylate was dissolved in THF (1.0 mL), and 1 M solution of tetra-*n*-butylammonium fluoride in THF (0.63 mL, 0.63 mmol) was added at 0 °C. After stirring for 1 h at room temperature, the mixture was poured into 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 dissolved in THF (2.0 mL), and sodium hydride (60% mineral oil suspension) (25 mg, 0.631 mmol) was added at 0 °C. After stirring for 3 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 hexane–ethyl acetate (1:1) to give **40** (66 mg, 0.227 mmol, 54% (3 steps)).

*3.1.18. 3-[3-((2R,3S)-3-Hydroxypyrrolidin-2-yl)propyl]quinazolin-4(3H)-one (**34**)*

To a solution of **40** (52 mg, 0.181 mmol) in pyridine (1.0 mL) was added *p*-toluenesulfonyl chloride (138 mg, 0.723 mmol). After stirring for 4 h at room temperature, the mixture was poured into 0.3 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–ethyl acetate (4:1) to give a tosylate of **40** (50 mg, 0.113 mmol, 64%).

To a solution of 4-hydroxyquinazoline (14 mg, 0.096 mmol) in DMF (1.5 mL) were added potassium carbonate (27 mg, 0.193 mmol) and a tosylate of **40** (41 mg, 0.096 mmol). After stirring for 5 h at 50 °C, the mixture was poured into 0.3 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform–methanol (49:1) to give 3-[3-[(2*R*,3*S*)-1-(*tert*-Butyloxycarbonyl)-3-(methoxy

methoxy)pyrrolidin-2-yl]propyl] quinazolin-4(3*H*)-one (19 mg, 0.050 mmol, 48%).

3-[3-[(2*R*,3*S*)-1-(*tert*-Butyloxycarbonyl)-3-(methoxymethoxy) pyrrolidin-2-yl]propyl] quinazolin-4(3*H*)-one (11 mg, 0.026 mmol) was dissolved in 10% hydrogen chloride in methanol (1.0 mL) at room temperature. After stirring for 3 h, the solution was evaporated. The residue was chromatographed over silica gel eluted by chloroform–methanol (3:1) to give **34** (8.9 mg, 0.025 mmol, 97%) as dihydrochloride.

Data for **34**: colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 9.53 (1H, br. s), 8.40 (1H, dd, J = 8.2, 1.2 Hz), 8.07 (1H, td, J = 8.2, 1.4 Hz), 7.79–7.86 (2H, m), 4.30 (2H, t, J = 7.1 Hz), 4.23–4.28 (1H, m), 3.40–3.48 (3H, m), 2.21–2.31 (1H, m), 1.97–2.12 (3H, m), 1.78–1.92 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 159.7, 152.3, 138.9, 137.7, 130.0, 128.8, 121.5, 121.0, 74.8, 67.3, 44.3, 32.9, 28.3, 26.9, 18.4; HRFABMS m/z 274.1569 $[M + H]^+$ (274.1556 calcd for $C_{15}H_{20}N_3O_2$); Anal. ($C_{15}H_{23}N_3O_3Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.1.19. (4R,5S)-4-(Benzylamino)-5-(methoxymethoxy)oct-7-en-1-ol (*41*)

In the same manner as the synthesis of **29**, compound **41** (594 mg, 2.03 mmol, 82%) was synthesized from **37** (1.23 g, 2.47 mmol).

3.1.20. (4R,5S)-4-[Allyl(benzyl)amino]-5-(methoxymethoxy)oct-7-en-1-ol (*42*)

In the same manner as the synthesis of **30**, compound **42** (320 mg, 0.960 mmol, 57% (3 steps)) was synthesized from **41** (494 mg, 1.68 mmol) by three successive reactions.

3.1.21. 3-[(2R,3S)-1-Benzyl-3-(methoxymethoxy)-2,3,4,7-tetrahydro-1H-azepin-2-yl]propan-1-ol (*43*)

In the same manner as the synthesis of **31**, compound **43** (164 mg, 0.439 mmol, 51%) was synthesized from **42** (287 mg, 0.860 mmol).

3.1.22. (2R,3S)-tert-Butyl 2-(3-hydroxypropyl)-3-(methoxymethoxy)azepane-1-carboxylate (*44*)

In the same manner as the synthesis of **32**, compound **44** (32 mg, 0.101 mmol, 50% (2 steps)) was synthesized from **43** (62 mg, 0.202 mmol) by two successive reactions.

3.1.23. 3-[3-((2R,3S)-3-Hydroxyazepan-2-yl)propyl]quinazolin-4(3H)-one (*35*)

In the same manner as the synthesis of **34**, compound **35** (5.5 mg, 0.015 mmol, 45% (3 steps)) was synthesized as dihydrochloride from **44** (10 mg, 0.033 mmol) by three successive reactions.

Data for **35**: colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 9.24 (1H, br. s), 8.35 (1H, dd, J = 8.1, 1.1 Hz), 8.07 (1H, ddd, J = 8.3, 7.4, 1.5 Hz), 7.71–7.79 (2H, m), 4.23 (2H, t, J = 7.2 Hz), 3.90–3.97 (1H, m), 3.19–3.38 (3H, m), 1.78–2.12 (9H, m), 1.59–1.70 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 161.0, 151.1, 142.9, 137.1, 130.1, 128.4, 123.8, 122.1, 70.6, 64.4, 49.5, 46.2, 33.5, 28.2, 26.7, 26.3, 21.0; HRFABMS m/z 302.1869 $[M + H]^+$ (302.1869 calcd for $C_{17}H_{24}N_3O_2$); Anal. ($C_{17}H_{17}N_3O_3Cl_2$ (dihydrochloride hydrate)) C, H, N.

3.2. In vitro antimalarial assay

Type A^+ human plasma and erythrocytes were obtained from healthy volunteers in our institute. *P. falciparum* strains FCR-3 (chloroquine sensitive) and K1 (chloroquine-resistant) were cultured in the human erythrocytes in RPMI medium (RPMI-1640 with 25 mM HEPES buffer, 24 mM $NaHCO_3$, 0.2% glucose, 0.05% L-glutamine, 50 μg/mL hypoxanthine, and 25 μg/mL gentamisin) supplemented with 10% human plasma at 37 °C, under 93% N_2, 4% CO_2 and 3% O_2. Antimalarial activity of the test compound has been achieved by dose response curve using the parasite lactate dehydrogenase (pLDH) assay [31]. Asynchronous parasites (2.0% hematocrit and 0.5% parasitemia) (90 μL) was seeded in a 96-well microplate, and a solution of test compound dissolved in distilled water or 5% DMSO (10 μL) was added. After incubation at 37 °C for 72 h, the parasite suspension (20 μL) was transferred to another plate containing Malstat reagent (100 μL). The plate was further incubated for 15 min at room temperature, and a 1:1 mixture of nitroblue tetrazolium and phenazine ethosulfate (2 mg and 0.1 mg/mL, respectively) (20 μL) was added to each well. After incubation for 2 h at room temperature in the dark condition, the blue formazan product was measured at 650 nm. The EC_{50} value was estimated from a dose response curve.

3.3. Cytotoxicity test

The cytotoxicity test of compounds was measured by the colorimetric MTT assay [32]. Mouse L929 cell suspension in RPMI-1640 with 10% FBS (90 μL) was added in 96-well microplates at 1.8×10^4 cells/well. Then, a solution of test compound dissolved in distilled water or 10% DMSO (10 μL) was added to each well. After incubation for 48 h at 37 °C under 5% CO_2, 2.5 mg/mL MTT solution (10 μL) was added to each well. The plate was incubated further 4 h. Then, the incubation medium was aspirated, and DMSO (100 μL) was added to solubilize the MTT formazan product. After mixing, absorbances at 570 and 655 nm were measured. The EC_{50} value was estimated from a dose response curve.

3.4. In vivo antimalarial assay

In vivo antimalarial activity was determined against rodent malaria-derived *P. berghei* NK65 according to the 4-days suppressive test [33]. Male ICR mice at weight 18–20 g were inoculated with 10^6 parasitized red blood cells intravenously. Test compounds were suspended in 5% gum arabic, and orally administered to the mice two hours after the infection (Day 0). Test compounds were successively administered to the mice once a day for 3 consecutive days (Days 1–3). The day after the last treatment (Day 4), the blood was obtained from the tail vein of the infected mice. The parasitemia was determined by thin blood films made from the blood. The ED_{50} value was estimated from a dose response curve.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.036.

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