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Febrifugine analogue compounds: Synthesis and antimalarial evaluation

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ABSTRACT

Febrifugine is an alkaloid isolated from Dichroa febrifuga Lour as the active component against *Plasmodium falciparum,* but exhibits toxic side effects. In this study novel febrifugine analogues were designed and efficiently synthesized. New compounds underwent efficacy and toxicity evaluation. Some compounds are much less toxic than the natural product febrifugine and existing antimalarial drugs and are expected to possess wide therapeutic windows. In Aotus monkeys infected with the chloroquine resistant FVO strain of *P. falciparum,* one interesting compound possesses a 50% curative dose of 2 mg/kg/day and a 100% curative dose of 8 mg/kg/day. These compounds, as well as the underlying design rationale, may find usefulness in the discovery and development of new antimalarial drugs.

1. Introduction

Malaria is the best known protozoal disease. It is one of the most common infectious diseases in many tropical and subtropical countries in Africa, Southeast Asia, and South America. Worldwide, malaria infects 225 million people and kills about 781,000 in a year.[1] The increasing prevalence of multiple drug resistant strains in most malaria endemic areas has significantly reduced the efficacy of current anti-malarial drugs for prophylaxis and treatment of this disease.[2] Medicinal agents based on novel mode of action are required to overcome the emergence of resistance and to control an ever-increasing number of epidemics caused by the malaria parasite.

Febrifugine and isofebrifugine (Fig. 1) were isolated as the active components against malaria in the Chinese herb Chang Shan (*Dichroa febrifuga* Lour),[3,4] which has been employed by the local people as medicine against fevers caused by malaria parasites for a long time. Febrifugine acts by impairing haemazoin formation required for maturation of the parasite at the trophozoite stage. The use of febrifugine as anti-malarial agent is initially appealing not only because of its rapid effect and no drug resistance, but also because of its availability. Subsequent pre-clinical researches have found that febrifugine possesses adverse side effects. Strong liver toxicity has precluded febrifugine as a clinical drug.[5–7]

In our previous studies, we have elucidated the essential role played by the 4-quinazolinone ring and 1″-amino group in the appearance of activity. In separate reports,[8–10] we have demonstrated (I) introducing an extra nitrogen atom or an electron withdrawing substitution group on the aromatic ring reduces toxicity while retaining desired biological activity; (II) the original piperidine ring can be replaced by a pyrrolidine ring; (III) the presence of the 3′-methylene group is not necessary for desired antimalarial activity.

Incorporating the above findings, we wish to report herein the synthesis and preclinical evaluation of a library of febrifugine analogues (compounds **1**–**12**, shown in Figure 2). Overall, these compounds closely resemble febrifugine itself by possessing a planar aromatic ring, a 1″-amino group and C-2′, C-3″ O-functionality and are therefore expected to possess same or similar mode of action. All new compounds possess a pyrrolidine ring instead of the original piperidine ring and are devoid of the 3′-methylene group. These newly designed compounds are much easier to synthesize so they have the potential to become an affordable drug should further clinical trials succeed. For compounds **2**–**12**, an extra nitrogen atom or a substitution group was introduced on the aromatic ring to block the C-5 or C-6 position of the quinazolinone ring or to increase the oxidation potential of the molecule. Therefore, lower toxicity will be achieved by reducing or eliminating the tendency to form chemically reactive and toxic intermediates.

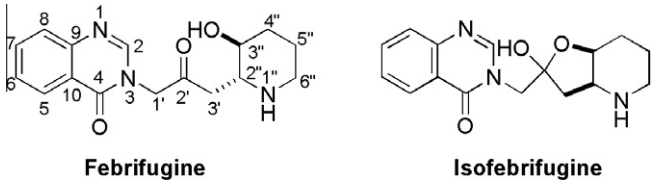

Figure 1. Molecular structures of febrifugine and isofebrifugine.

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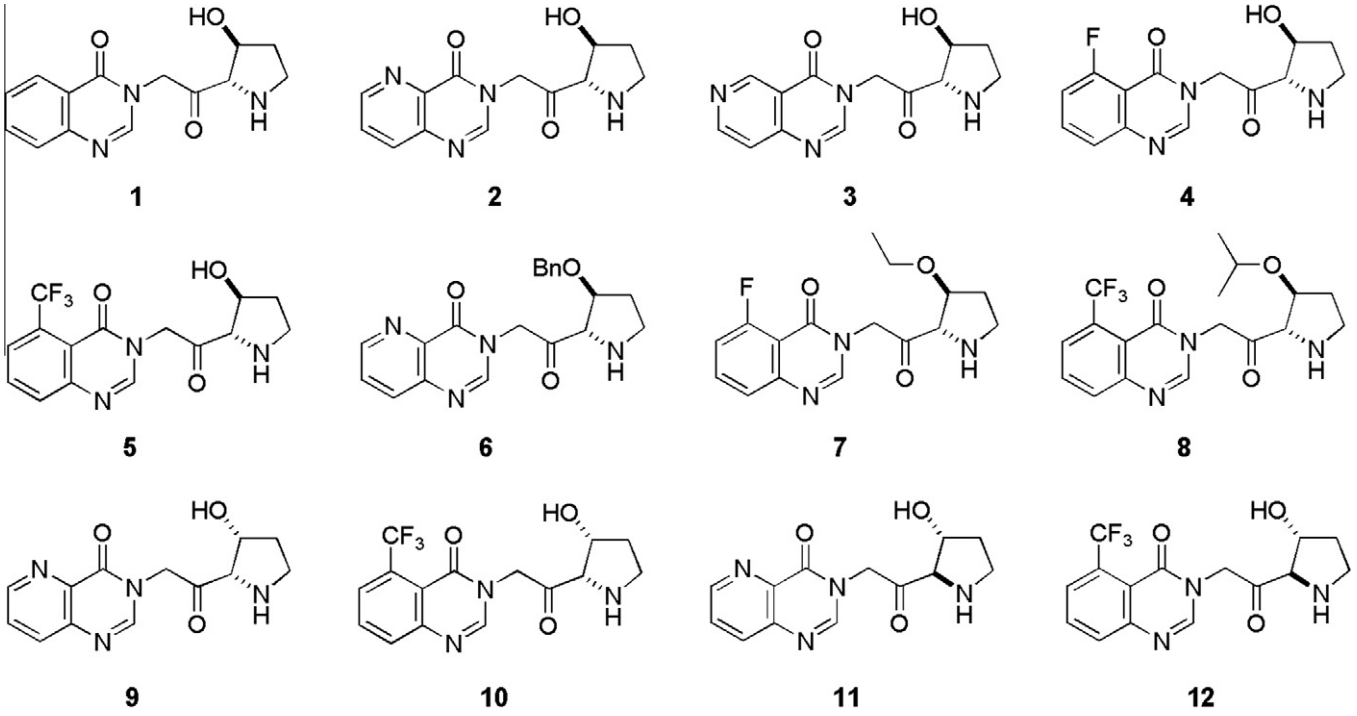
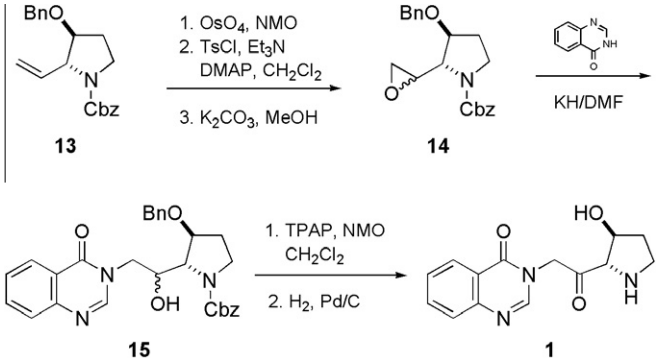


Figure 2. Molecular structures of novel febrifugine analogues.



Scheme 1. Synthesis of A representative compound (**1**).

Compounds **6**–**8** each bear an alkoxyl group on C-3″ position of the pyrrolidine ring. These compounds possess increased lipophilicity. Compounds **9**–**12** possess different stereochemistry at either the C-2″ or the C-3″ position and it would be interesting to see if such stereochemistry difference will cause changes in antimalarial efficacy or toxicity.

2. Chemistry

Scheme 1 illustrated the synthesis of compound **1**. Although initial oxidation of the known and readily available alkene **13**[11] by *m*CPBA to give oxirane **14** was not successful, the transformation was smooth and high-yielding via a three-step sequence: (i) dihydroxylation; (ii) selective tosylation of the primary alcohol; and (iii) epoxide ring formation facilitated by potassium carbonate in methanol. 3*H*-Quinazolin-4-one then condensed with oxirane **14**, to furnish corresponding alcohol **15**, as a pair of diastereomers. Compound **15** then underwent TPAP oxidation[12] and hydrogenolysis, to afford compound **1**. The synthesis of compounds **2**–**12** follows similar route. The respective aromatic moieties are available from previously published procedures.[13–15] For synthesis of compounds **6**–**8**, the 1″-amine was protected with *tert*-butyl carbamate (BOC) group. Selective cleavage of the BOC group was achieved by the combination of HCl/EtOAc.[16] Synthesis of compounds **9**–**12** will need a diastereomer or the enantiomer of alkene **13**. These starting compounds can be readily synthesized via previously described protocols.[11]

3. Preclinical evaluation and discussion

Synthesized new compounds were first tested against a panel of strains of *Plasmodium falciparum*. The IC_{50} values (50% inhibitory concentrations) were summarized in Table 1. 3D7 is a chloroquine sensitive strain of *P. falciparum*. The rest of the strains (TM6, K1, and V1S) are chloroquine resistant. The antimalarial activity of compounds **1–5** was comparable to the parent natural product febrifugine. So the antimalarial activity was preserved when the original piperridine ring was replaced with a pyrrolidine ring and the 3′-methylene group was removed. Compounds **6–8** have exhibited superior and equally potent antimalarial activity against chloroquine sensitive and resistant malaria strains. These compounds bear an ether functionality on C-3″ position of the pyrrolidine ring. Apparently, a compound's antimalarial potency was improved when lipophilicity was increased. Compounds **9** and **10** are diastereomers of compounds **2** and **5**, respectively. The tenfold decrease in efficacy for compounds **9** and **10** indicates that the stereochemistry at C-3″ position is crucial for desired biological activity. Compounds **11** and **12** are enantiomers of compounds **2** and **5**, respectively. Interestingly, the enantiomers have shown almost identical antimalarial potency towards all tested strains of *P. falciparum*.

In vivo antimalarial efficacy data was obtained by evaluating selected new compounds **1–8** in rodent infected with blood stage malaria strain *Plasmodium berghei* NK65. The ED_{50}'s (the dose leading to 50% parasite growth inhibition compared to the blank control) and MCDs (minimum clearance dose), commonly used efficacy measurements, were calculated. The acute toxicity study

Table 1
In vitro antimalarial activities of new compounds versus a panel of *P. falciparum* isolates [IC_{50} (nM)]

Strain	PH3	3D7	DD2	TM6	K1	TM4	V1S
Febrifugine	1.6 ± 0.3	1.9 ± 0.4	2.1 ± 0.4	3.1 ± 0.6	2.0 ± 0.3	5.4 ± 0.8	4.2 ± 0.7
Compound **1**	1.4 ± 0.2	2.8 ± 0.4	4.5 ± 0.8	3.1 ± 0.5	3.9 ± 0.4	7.9 ± 0.7	5.6 ± 1.0
Compound **2**	1.1 ± 0.2	1.8 ± 0.3	2.3 ± 0.3	2.1 ± 0.4	3.1 ± 0.3	4.1 ± 0.4	3.6 ± 0.5
Compound **3**	1.0 ± 0.3	1.4 ± 0.3	2.2 ± 0.5	3.4 ± 0.7	2.8 ± 0.4	3.0 ± 0.5	4.1 ± 0.7
Compound **4**	2.5 ± 0.4	5.7 ± 0.9	8.1 ± 1.4	3.9 ± 0.6	5.9 ± 0.9	6.2 ± 1.1	5.3 ± 1.0
Compound **5**	3.2 ± 0.5	2.7 ± 0.2	6.3 ± 0.8	2.5 ± 0.3	4.7 ± 0.9	7.4 ± 1.3	11.2 ± 1.9
Compound **6**	0.20 ± 0.04	0.21 ± 0.05	0.17 ± 0.03	0.08 ± 0.02	0.24 ± 0.04	0.33 ± 0.07	0.13 ± 0.03
Compound **7**	0.18 ± 0.04	0.21 ± 0.04	0.26 ± 0.07	0.17 ± 0.03	0.24 ± 0.05	0.22 ± 0.04	0.25 ± 0.05
Compound **8**	0.54 ± 0.11	0.36 ± 0.07	0.78 ± 0.14	0.31 ± 0.05	0.61 ± 0.10	0.39 ± 0.06	0.68 ± 0.12
Compound **9**	11.4 ± 1.9	16.3 ± 2.4	21.9 ± 4.1	13.2 ± 2.5	19.8 ± 5.0	32.7 ± 5.3	28.7 ± 4.8
Compound **10**	21.2 ± 4.0	19.6 ± 3.3	31.8 ± 5.6	25.6 ± 4.1	24.2 ± 3.6	51.9 ± 9.4	36.5 ± 5.2
Compound **11**	1.0 ± 0.2	1.9 ± 0.3	2.4 ± 0.3	2.0 ± 0.4	3.3 ± 0.3	4.0 ± 0.4	3.7 ± 0.5
Compound **12**	3.4 ± 0.5	2.6 ± 0.2	6.1 ± 0.8	2.7 ± 0.3	4.8 ± 0.9	7.3 ± 1.3	11.0 ± 1.9
Chloroquine	33.4 ± 9.1	9.7 ± 3.4	45.8 ± 10.2	96.4 ± 6.9	165.6 ± 19.3	115.2 ± 12.7	152.2 ± 14.1
Amodiaquine	15.9 ± 3.2	4.9 ± 1.8	9.1 ± 1.2	7.5 ± 2.4	21.2 ± 2.1	15.2 ± 2.6	8.7 ± 2.5

Table 2
Efficacy and toxicity evaluation of compounds in rodent models[a]

Compound	ED_{50}[b]	MCD[b]	MTD[b]	Therapeutic index[c]
Febrifugine	2.3	12	35	3
Compound **1**	1.7	9.9	100	10
Compound **2**	1.2	7.1	450	63
Compound **3**	1.0	7.5	350	47
Compound **4**	1.1	6.3	400	63
Compound **5**	0.95	6.7	400	60
Compound **6**	0.19	1.7	250	147
Compound **7**	0.31	1.9	250	132
Compound **8**	0.23	2.4	250	104
Chloroquine	4.5	40	240	6
Mefloquine	1.1	9.9	600	61
Artesunate[a]	0.55	6.8	250	37

[a] Route of administration for Artesunate is iv. All others were administered orally.
[b] ED_{50}'s, MCD's, and MTD's are reported in mg/kg.
[c] Therapeutic indice were calculated as MTD divided by MCD.

Table 3
Efficacy of test compounds against blood stages of *P. falciparum* in Aotus monkeys

Test compounds	Dose[a] (mg/kg/day)	Cure rate[b] (%)
Compound **6**	2	1/4 (25)
	4	5/7 (71)
	8	4/5 (80)
	16	5/5 (100)
Compound **7**	2	2/4 (50)
	4	4/6 (67)
	8	5/5 (100)
	16	5/5 (100)

[a] *P. falcipanum*-infected Aotus monkeys were administered drug orally once a day for 3 days beginning the day after a parasitemia level of approximately 5,000 organisms per mm^3 was reached.
[b] Number of animals cured/total number tested (cure is defined as clearance of parasitemia with no recrudescence).

of test compounds was conducted with Sprague–Dawley rats. MTDs (maximum tolerated dose) and therapeutic indices were obtained. The data are summarized in Table 2. The antimalarial activities of compounds **1–5** are comparable to the natural product febrifugine and some commonly used antimalarial drugs. The more lipophilic derivative compounds **6–8** exhibited superior antimalarial activity. Their ED_{50}'s and MCD's are smaller then artemisinin derivative. New compounds **1–8** are much less toxic than the parent natural product febrifugine. Compounds **2–5**, with either an extra nitrogen atom or an electron-withdrawing substitution group on the aromatic ring, are over 10 times less toxic than febrifugine. The more lipophilic compounds **6–8**, bearing an alkoxyl group on C-3″ position of the pyrrolidine ring, are slightly more toxic as compared to compound **2–5**. However, these compounds (**6**, **7**, and **8**) still possess wide therapeutic window since they are very efficacious (low ED_{50} and MCD value).

Compounds **6** and **7** (with highest therapeutic index value) were chosen for further efficacy studies in *Aotus* monkey model. The monkeys selected for the study had active infections of the chloroquine-resistant FVO strain of *P. falciparum*. Once parasitemias reached 5,000/μl (or as deemed appropriate by the attending veterinarian), they were treated orally with compound **6** or compound **7** at 2, 4, 8, or 16 mg/kg/day for 3 days. For compound **6**, the dosage needed to cure 50% of animals was 4 mg/kg/day and the dosage needed to cure 100% of animals was 16 mg/kg/day. For compound **7**, the 50% curative dose was 2 mg/kg/day and the 100% curative dose was 8 mg/kg/day (Table 3).

In conclusion, new febrifugine analogues were designed and synthesized. Some of these compounds (**6** and **7**) have exhibited excellent efficacy in a relevant primate model and possess a wider therapeutic index than that of the commonly used antimalarial drugs. Detailed study of pharmacokinetics and pharmacodynamics will be presented in a separate report. These compounds, as well as the underlying design rationale, may find usefulness in the discovery and development of new antimalarial drugs.

4. Experimental

4.1. Chemistry

Melting points were determined on a Mettler FP62 melting point apparatus and are uncorrected. Unless otherwise noted, all non-aqueous reactions were performed under an oxygen-free atmosphere of nitrogen with rigid exclusion of moisture from reagents and glassware. Analytical thin layer chromatography (TLC) was performed using EM Reagents 0.25 mm silica gel 60-F plates. Visualization of the developed chromatogram was performed by UV absorbance, aqueous potassium permanganate, or ethanolic anisaldehyde. Liquid chromatography was performed using a force flow (flash chromatography) of the indicated solvent system on EM Reagents Silica Gel 60 (70–230 mesh). Preparative TLC was performed using Whatman Silica Gel C8 TLC plates (PLK5F). ^1H NMR spectra were recorded in deuteriochloroform, unless otherwise noted, on a Bruker Avance 600 spectrometer at the frequency of 600.1 MHz. Chemical shifts are reported in parts per million on the δ scale from an internal standard of tetramethylsilane. Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, and br = broad), coupling constant in Hertz, integration, and assignment. ^{13}C NMR spectra were recorded in deuteriochloroform, unless otherwise

noted, on a Bruker Avance 600 spectrometer at the frequency of 150.9 MHz. Chemical shifts are reported in parts per million on the δ scale from an internal standard of tetramethylsilane. Combustion analyses were performed by Atlantic Microlab, Inc. (Norcross, Georgia). All purified compounds possess a purity of at least 95%. When necessary, solvents and reagents were dried as follows: ether, tetrahydrofuran, benzene, and toluene were stored and distilled from sodium benzophenone ketyl; dichloromethane, triethylamine, pyridine, and hexane were distilled over calcium hydride. Unless otherwise stated, the reagents were purchased from Fisher Scientific, Aldrich Chemical Company, Lancaster, or Fluka, and used as received.

4.1.1. 3-Benzyloxy-2-oxiranyl-pyrrolidine-1-carboxylic acid benyl ester (14)

Alkene **13** (9.6 g), osmium tetraoxide (OsO_4, 4% in water, 1.0 mL), and *N*-methyl morpholine N-oxide (NMO, 10.8 g) were dissolved in 80 mL of water and 80 mL of tetrahydrofuran (THF). The resulting heterogeneous solution is stirred vigorously for 3 days at room temperature. After partition between ethyl acetate (140 mL) and water (100 mL), the separated organic layer was washed with brine (60 mL), dried over anhydrous sodium sulfate, and evaporated in a rotary evaporator under reduced pressure to furnish the essentially pure diol. Tosyl chloride (TsCl, 5.1 g) was added into a stirred solution of this diol (9.7 g), triethylamine (Et_3N, 6.0 mL) and 4-dimethylaminopyridine (DMAP, 200 mg) in 100 mL of methylene chloride (CH_2Cl_2) in an ice-water bath. The reaction mixture was then warmed to room temperature and stirred for additional 2 h. Solvent was evaporated. The resulting slurry was dissolved in ethyl acetate (100 mL), washed with water (75 mL), then aqueous sodium bicarbonate (75 mL), and brine (40 mL), dried over anhydrous sodium sulfate, and evaporated in a rotary evaporator under reduced pressure to furnish the essentially pure mono-tosylate. Potassium carbonate (K_2CO_3, 1.0 g) was added into a stirred solution of this mono-tosylate (12.2 g) in 30 mL of methanol (MeOH) at room temperature. After 4 h, reaction mixture was partitioned between ethyl acetate (75 mL) and water (75 mL), the separated organic layer was then washed with brine (40 mL), dried over anhydrous sodium sulfate, and evaporated in a rotary evaporator under reduced pressure to furnish the crude product. Silica gel flash chromatography (20% ethyl acetate in hexanes) furnishes **14** as viscous oil. Yield: 7.3 g, 72% (3 steps). ^{1}H NMR: 7.29–7.20 (m, 10H), 5.39 (m, 2H), 4.63 (m, 2H), 4.05 (m, 1H), 3.49 (m, 2H), 3.24 (m, 1H), 3.07 (m, 1H), 2.64 (m, 2H), 1.82–1.77 (m, 2H). ^{13}C NMR: 159.9, 141.1, 138.4, 128.7, 128.3, 127.8, 127.5, 127.1, 126.9, 75.5, 72.2, 70.6, 58.4, 49.4, 44.2, 38.8, 29.2. HRESIMS *m*/*z* 376.1521 $[M+Na]^+$ (calcd for $C_{21}H_{23}NNaO_4^+$, 376.1525) (100%). Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.41; H, 6.53; N, 3.99.

4.1.2. 3-Benzyloxy-2-[1-hydroxy-2-(4-oxo-4H-quinazolin-3-yl)-ethyl]-pyrrolidine-1-carboxylic acid benzyl ester (15)

Potassium hydride (KH, 30% in mineral oil, 4.0 g) was suspended in 50 mL of dimethylformamide (DMF). It was cooled in an ice-water bath, and solid 3H-quinazolin-4-one (4.3 g) was added in. After 30 min, a solution of oxirane **14** (3.5 g) in 10 mL of DMF was added in. The reaction mixture was then heated at 80 °C for 8 h under nitrogen atmosphere. It was partitioned between ethyl acetate (75 mL) and water (75 mL), separated organic layer was washed with water (3 × 50 mL), then brine (50 mL), dried over anhydrous sodium sulfate, and evaporated in a rotary evaporator under reduced pressure to furnish the crude product. Silica gel flash chromatography (75% ethyl acetate in hexanes) furnished **15** as off white solid. Yield: 79%. ^{1}H NMR: 8.31 (d, *J* = 7.6 Hz, 1H), 8.21 (s, 1H), 7.75 (m, 2H), 7.45 (m, 1H), 7.30–7.25 (m, 10H), 5.48 (m, 2H), 5.22 (br, 1H), 4.77 (m, 2H), 4.25 (m, 1H), 4.18 (m, 1H), 3.91 (m, 2H), 3.58 (m, 2H), 3.21 (m, 1H), 1.77 (m, 2H). ^{13}C NMR: 168.1, 164.2, 157.1, 145.9, 140.3, 139.1, 134.6, 129.8, 128.8, 128.4, 127.9, 127.5, 127.3, 127.1, 126.9, 126.6, 124.2, 79.9, 78.1, 70.1, 66.7, 58.2, 52.3, 38.2, 27.1. HRESIMS *m*/*z* 522.2011 $[M+Na]^+$ (calcd for $C_{29}H_{29}N_3NaO_5^+$, 522.2005) (100%). Anal. Calcd for $C_{29}H_{29}N_3O_5$.HCl: C, 64.98; H, 5.64; Cl, 6.61; N, 7.84. Found: C, 64.93; H, 5.66; Cl, 6.56; N, 7.88.

4.1.3. (2*S*, 3*S*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-quinazolin-4-one (1)

A solution of alcohol **15** (3.6 g) in 10 mL of CH_2Cl_2 was added into a stirred slurry of tetrapropylammonium perruthenate (TPAP, 90 mg), *N*-methylmorpholine N-oxide (NMO, 1.8 g), and grounded molecular sieve (1.8 g) in 20 mL of CH_2Cl_2 at room temperature. After 2 h, the reaction mixture was loaded directly into a short column of silica gel and eluted with 5% MeOH/EtOAc. Concentration of the eluant under reduced pressure afforded the corresponding ketone. This ketone (3.3 g) was dissolved in 20 mL of 95% EtOH/H_2O. 300 mg of 10% Pd on carbon was added in. It was then treated with hydrogen (60 psi) in a Parr apparatus for 12 h. Solid was filtered off and the solution was evaporated under vacuum to dryness. Recrystallization from ethanol–water (with addition of dilute aqueous HCl solution, 4–5 equiv of HCl) furnishes compound **1** (HCl salt) as off-white crystals. Yield: 1.87 g, 84% (two steps). Mp: 206–207 °C. $[\alpha]_D^{25}$ +51.6 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.21(d, *J* = 7.9 Hz, 1H), 7.85 (s, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 5.08 (d, *J* = 16.7 Hz, 1H), 4.92 (d, *J* = 16.7 Hz, 1H), 4.21 (d, *J* = 6.5 Hz, 1H), 4.11 (m, 1H), 3.47–3.41 (m, 2H), 2.21 (m, 1H), 1.92 (m, 1H). ^{13}C NMR (CD_3OD): 205.2, 168.3, 164.8, 147.5, 133.5, 128.6, 127.9, 126.1, 122.5, 74.7, 66.9, 55.2, 38.1, 33.6. HRESIMS *m*/*z* 296.1007 $[M+Na]^+$ (calcd for $C_{14}H_{15}N_3NaO_3^+$, 296.1011) (100%). Anal. Calcd for $C_{14}H_{15}N_3O_3$.HCl: C, 54.29; H, 5.21; Cl, 11.45; N, 13.57. Found: C, 54.22; H, 5.23; Cl, 11.51; N, 13.53.

4.1.4. (2*S*, 3*S*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-pyrido[3,2-*d*]pyrimidin-4-one (2)

Pale-yellow crystal. Mp: 244–245 °C. $[\alpha]_D^{25}$ +18.4 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 9.55 (d, *J* = 7.2 Hz, 1H), 9.14 (d, *J* = 7.2 Hz, 1H), 8.71 (s, 1H), 8.15 (t, *J* = 7.2 Hz, 1H), 5.12 (d, *J* = 17.1 Hz, 1H), 5.02 (d, *J* = 17.1 Hz, 1H), 4.44 (d, *J* = 6.2 Hz, 1H), 4.22 (m, 1H), 3.49–3.43 (m, 2H), 2.15 (m, 1H), 1.89 (m, 1H). ^{13}C NMR (CD_3OD): 204.5, 164.8, 161.5, 151.9, 148.4, 145.8, 133.7, 131.7, 78.1, 65.9, 52.7, 43.2, 35.8. HRESIMS *m*/*z* 297.0972 $[M+Na]^+$ (calcd for $C_{13}H_{14}N_4NaO_3^+$, 297.0964) (100%). Anal. Calcd for $C_{13}H_{14}N_4O_3$.HCl: C, 50.25; H, 4.87; Cl, 11.41; N, 18.03. Found: C, 50.31; H, 4.84; Cl, 11.38; N, 18.10.

4.1.5. (2*S*, 3*S*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-pyrido[4,3-*d*]pyrimidin-4-one (3)

Pale-yellow crystal. Mp: 235–236 °C. $[\alpha]_D^{25}$ −23.7 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 9.46 (s, 1H), 9.15 (d, *J* = 7.4 Hz, 1H), 8.76 (s, 1H), 8.21 (d, *J* = 7.4 Hz, 1H), 5.19 (d, *J* = 17.2 Hz, 1H), 5.05 (d, *J* = 17.2 Hz, 1H), 4.39 (d, *J* = 6.3 Hz, 1H), 4.21 (m, 1H), 3.44–3.39 (m, 2H), 2.21 (m, 1H), 1.82 (m, 1H). ^{13}C NMR (CD_3OD): 204.5, 167.1, 163.9, 158.4, 151.2, 148.6, 136.8, 128.1, 79.5, 59.8, 51.3, 42.6, 32.8. HRESIMS *m*/*z* 297.0961 $[M+Na]^+$ (calcd for $C_{13}H_{14}N_4NaO_3^+$, 297.0964) (100%). Anal. Calcd for $C_{13}H_{14}N_4O_3$.HCl: C, 50.25; H, 4.87; Cl, 11.41; N, 18.03. Found: C, 50.29; H, 4.89; Cl, 11.44; N, 17.98.

4.1.6. (2*S*, 3*S*)-5-Fluoro-3-[2-(3-hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-quinazolin-4-one (4)

Off-white crystals. Mp: 223–224 °C. $[\alpha]_D^{25}$ +12.5 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.38 (s, 1H), 8.21 (d, *J* = 7.1 Hz, 1H), 7.78 (d, *J* = 7.2 Hz, 1H), 7.61 (t, *J* = 7.1 Hz, 1H), 5.17 (d, *J* = 17.0 Hz, 1H), 5.09 (d, *J* = 17.0 Hz, 1H), 4.41 (d, *J* = 6.3 Hz, 1H), 4.16 (m, 1H),

3.48–3.41 (m, 2H), 2.32 (m, 1H), 1.89 (m, 1H). ^{13}C NMR (CD_3OD): 206.2, 166.2, 161.5, 159.3, 149.6, 140.4, 130.1, 127.2, 121.2, 78.2, 62.2, 50.3, 40.4, 34.2. HRESIMS *m/z* 314.0922 $[M+Na]^+$ (calcd for $C_{14}H_{14}FN_3NaO_3^+$, 314.0917) (100%). Anal. Calcd for $C_{14}H_{14}FN_3O_3$.HCl: C, 51.31; H, 4.61; Cl, 10.82; F, 5.80; N, 12.82. Found: C, 51.26; H, 4.64; Cl, 10.85; F, 5.77; N, 12.87.

4.1.7. (2*S*, 3*S*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-5-trifluoromethyl-3*H*-quinazolin-4-one (5)

Off-white crystals. Mp: 252–253 °C. $[\alpha]_D^{25}$ −17.2 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.32 (s, 1H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 7.2 Hz, 1H), 5.06 (d, *J* = 16.9 Hz, 1H), 4.88 (d, *J* = 16.9 Hz, 1H), 4.49 (d, *J* = 6.2 Hz, 1H), 4.22 (m, 1H), 3.50–3.44 (m, 2H), 2.19 (m, 1H), 1.76 (m, 1H). ^{13}C NMR (CD_3OD): 203.4, 164.6, 159.5, 148.7, 140.5, 133.6, 132.2, 128.5, 125.2, 117.2, 76.4, 62.5, 52.3, 39.8, 33.1. HRESIMS *m/z* 364.0891 $[M+Na]^+$ (calcd for $C_{15}H_{14}F_3N_3NaO_3^+$, 364.0885) (100%). Anal. Calcd for $C_{15}H_{14}F_3N_3O_3$.HCl: C, 47.69; H, 4.00; Cl, 9.39; F, 15.09; N, 11.12. Found: C, 47.62 H, 4.02; Cl, 9.42; F, 15.05; N, 11.16.

4.1.8. (2*S*, 3*S*)-3-[2-(3-Benzyloxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-pyrido[3,2-*d*]pyrimidin-4-one (6)

Pale-yellow crystal. Mp: 266–267 °C. $[\alpha]_D^{25}$ -33.1 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 9.44 (d, *J* = 7.1 Hz, 1H), 9.09 (d, *J* = 7.1 Hz, 1H), 8.62 (s, 1H), 8.19 (t, *J* = 7.1 Hz, 1H), 7.22–7.17 (m, 5H), 5.41 (d, *J* = 15.8 Hz, 1H), 5.33 (d, *J* = 15.8 Hz, 1H), 5.15 (d, *J* = 17.0 Hz, 1H), 5.04 (d, *J* = 17.0 Hz, 1H), 4.38 (d, *J* = 5.8 Hz, 1H), 4.18 (m, 1H), 3.45–3.39 (m, 2H), 2.08 (m, 1H), 1.71 (m, 1H). ^{13}C NMR (CD_3OD): 204.1, 163.3, 160.7, 151.2, 147.7, 144.1, 138.9, 135.3, 131.9, 130.1, 128.5, 127.2, 81.5, 76.3, 65.4, 53.1, 41.4, 34.2. HRESIMS *m/z* 387.1429 $[M+Na]^+$ (calcd for $C_{20}H_{20}N_4NaO_3^+$, 387.1433) (100%). Anal. Calcd for $C_{20}H_{20}N_4O_3$.HCl: C, 59.92; H, 5.28; Cl, 8.84; N, 13.98. Found: C, 59.86; H, 5.31; Cl, 8.80; N, 14.03.

4.1.9. (2*S*, 3*S*)-3-[2-(3-Ethoxy-pyrrolidin-2-yl)-2-oxo-ethyl]-5-fluoro-3*H*-quinazolin-4-one (7)

Off-white crystals. Mp: 242–244 °C. $[\alpha]_D^{25}$ +19.6 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.51 (s, 1H), 8.29 (d, *J* = 7.0 Hz, 1H), 7.69 (d, *J* = 7.0 Hz, 1H), 7.52 (t, *J* = 7.0 Hz, 1H), 5.10 (d, *J* = 17.1 Hz, 1H), 5.02 (d, *J* = 17.1 Hz, 1H), 4.53 (d, *J* = 6.4 Hz, 1H), 4.21 (m, 1H), 3.51 (q, *J* = 6.9 Hz, 2H), 3.44–3.38 (m, 2H), 2.24 (m, 1H), 1.75 (m, 1H), 1.21 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (CD_3OD): 205.1, 165.4, 161.9, 157.6, 147.1, 140.8, 131.4, 127.8, 120.1, 78.6, 69.1, 61.4, 51.5, 41.6, 32.3, 16.2. HRESIMS *m/z* 342.1227 $[M+Na]^+$ (calcd for $C_{16}H_{18}FN_3NaO_3^+$, 342.1230) (100%). Anal. Calcd for $C_{16}H_{18}FN_3O_3$.HCl: C, 54.01; H, 5.38; Cl, 9.96; F, 5.34; N, 11.81. Found: C, 53.94; H, 5.41; Cl, 9.91; F, 5.39; N, 11.83.

4.1.10. (2*S*, 3*S*)-3-[2-(3-Isopropoxy-pyrrolidin-2-yl)-2-oxo-ethyl] -5-trifluoromethyl-3*H*-quinazolin-4-one (8)

Off-white crystals. Mp: 269–270 °C. $[\alpha]_D^{25}$ -39.6 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.41 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.39 (t, *J* = 7.2 Hz, 1H), 5.16 (d, *J* = 16.8 Hz, 1H), 4.92 (d, *J* = 16.8 Hz, 1H), 4.45 (d, *J* = 6.2 Hz, 1H), 4.27 (m, 1H), 3.49–3.42 (m, 2H), 3.28 (m, 1H), 2.25 (m, 1H), 1.79 (m, 1H), 1.19 (d, *J* = 5.9 Hz, 6H). ^{13}C NMR (CD_3OD): 204.3, 163.7, 160.1, 149.2, 140.8, 134.3, 131.8, 127.6, 124.7, 119.4, 77.1, 69.5, 66.5, 53.2, 39.2, 31.3, 24.2. HRESIMS *m/z* 406.1361 $[M+Na]^+$ (calcd for $C_{18}H_{20}F_3N_3NaO_3^+$, 406.1354) (100%). Anal. Calcd for $C_{18}H_{20}F_3N_3O_3$.HCl: C, 51.50; H, 5.04; Cl, 8.44; F, 13.58; N, 10.01. Found: C, 51.47; H, 5.06; Cl, 8.39; F, 13.62; N, 10.04.

4.1.11. (2*S*, 3*R*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-pyrido[3,2-*d*]pyrimidin-4-one (9)

Pale-yellow crystal. Mp: 221–222 °C. $[\alpha]_D^{25}$ +27.1 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 9.71 (d, *J* = 6.9 Hz, 1H), 9.25 (d, *J* = 6.9 Hz, 1H), 8.62 (s, 1H), 8.04 (t, *J* = 6.9 Hz, 1H), 5.19 (d, *J* = 17.0 Hz, 1H), 5.08 (d, *J* = 17.0 Hz, 1H), 4.61 (d, *J* = 6.6 Hz, 1H), 4.32 (m, 1H), 3.44–3.39 (m, 2H), 2.31 (m, 1H), 1.72 (m, 1H). ^{13}C NMR (CD_3OD): 207.1, 162.5, 160.9, 154.6, 147.1, 144.4, 135.6, 135.2, 79.6, 64.1, 53.6, 42.4, 34.1. HRESIMS *m/z* 297.0969 $[M+Na]^+$ (calcd for $C_{13}H_{14}N_4NaO_3^+$, 297.0964) (100%). Anal. Calcd for $C_{13}H_{14}N_4O_3$.HCl: C, 50.25; H, 4.87; Cl, 11.41; N, 18.03. Found: C, 50.29; H, 4.85; Cl, 11.45; N, 17.98.

4.1.12. (2*S*, 3*R*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-5-trifluoromethyl-3*H*-quinazolin-4-one (10)

Off-white crystals. Mp: 233–234 °C. $[\alpha]_D^{25}$ −24.6 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.19 (s, 1H), 7.94 (d, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.41 (t, *J* = 7.2 Hz, 1H), 5.01 (d, *J* = 16.8 Hz, 1H), 4.82 (d, *J* = 16.8 Hz, 1H), 4.41 (d, *J* = 6.5 Hz, 1H), 4.19 (m, 1H), 3.46–3.40 (m, 2H), 2.21 (m, 1H), 1.82 (m, 1H). ^{13}C NMR (CD_3OD): 204.1, 163.9, 158.8, 148.1, 141.8, 132.3, 131.7, 127.7, 125.4, 119.5, 77.8, 63.2, 52.8, 40.2, 34.5. HRESIMS *m/z* 364.0881 $[M+Na]^+$ (calcd for $C_{15}H_{14}F_3N_3NaO_3^+$, 364.0885) (100%). Anal. Calcd for $C_{15}H_{14}F_3N_3O_3$.HCl: C, 47.69; H, 4.00; Cl, 9.39; F, 15.09; N, 11.12. Found: C, 47.64 H, 4.03; Cl, 9.34; F, 15.13; N, 11.07.

4.1.13. (2*R*, 3*R*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-3*H*-pyrido[3,2-*d*]pyrimidin-4-one (11)

Pale-yellow crystal. Mp: 242–243 °C. $[\alpha]_D^{25}$ −18.2 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 9.53 (d, *J* = 7.1 Hz, 1H), 9.12 (d, *J* = 7.1 Hz, 1H), 8.70 (s, 1H), 8.18 (t, *J* = 7.1 Hz, 1H), 5.19 (d, *J* = 17.2 Hz, 1H), 5.05 (d, *J* = 17.2 Hz, 1H), 4.41 (d, *J* = 6.3 Hz, 1H), 4.20 (m, 1H), 3.48–3.42 (m, 2H), 2.21 (m, 1H), 1.85 (m, 1H). ^{13}C NMR (CD_3OD): 204.9, 164.1, 161.3, 151.3, 148.1, 145.3, 133.2, 131.4, 78.8, 65.4, 52.4, 43.8, 35.2. HRESIMS *m/z* 297.0969 $[M+Na]^+$ (calcd for $C_{13}H_{14}N_4NaO_3^+$, 297.0964) (100%). Anal. Calcd for $C_{13}H_{14}N_4O_3$.HCl: C, 50.25; H, 4.87; Cl, 11.41; N, 18.03. Found: C, 50.20; H, 4.90; Cl, 11.37; N, 18.09.

4.1.14. (2*R*, 3*R*)-3-[2-(3-Hydroxy-pyrrolidin-2-yl)-2-oxo-ethyl]-5-trifluoromethyl-3*H*-quinazolin-4-one (12)

Off-white crystals. Mp: 254–255 °C. $[\alpha]_D^{25}$ +17.4 (*c* 0.50, EtOH). ^{1}H NMR (CD_3OD): 8.34 (s, 1H), 7.79 (d, *J* = 7.2 Hz, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.46 (t, *J* = 7.2 Hz, 1H), 5.09 (d, *J* = 17.0 Hz, 1H), 4.86 (d, *J* = 17.0 Hz, 1H), 4.48 (d, *J* = 6.1 Hz, 1H), 4.19 (m, 1H), 3.51–3.45 (m, 2H), 2.16 (m, 1H), 1.77 (m, 1H). ^{13}C NMR (CD_3OD): 203.7, 164.4, 159.4, 148.7, 140.6, 133.4, 132.1, 128.6, 125.2, 117.3, 76.4, 62.6, 52.1, 39.8, 33.2. HRESIMS *m/z* 364.0881 $[M+Na]^+$ (calcd for $C_{15}H_{14}F_3N_3NaO_3^+$, 364.0885) (100%). Anal. Calcd for $C_{15}H_{14}F_3N_3O_3$.HCl: C, 47.69; H, 4.00; Cl, 9.39; F, 15.09; N, 11.12. Found: C, 47.71 H, 4.03; Cl, 9.34; F, 15.14; N, 11.09.

4.2. Biological studies

(i) In vitro drug susceptibility studies were conducted by using a modification of the semiautomated microdilution techniques of Desjardins and Chulay.[17–19] (ii) Antimalarial efficacy and toxicity evaluation in rodent models were carried out in procedures previously described.[20,21] (iii) Compounds **6** and **7** underwent further efficacy studies in *Aotus* monkey model. Detailed procedures of malaria infection and drug tests were described in the previous reports.[21–25] All animal experiments were conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhere to principles stated in the *Guide for the Care and Use of Laboratory Animals*.[26]

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