Endiandric Acid Analogues from Beilschmiedia ferruginea as Dual Inhibitors of Bcl-xL/Bak and Mcl-1/Bid Interactions

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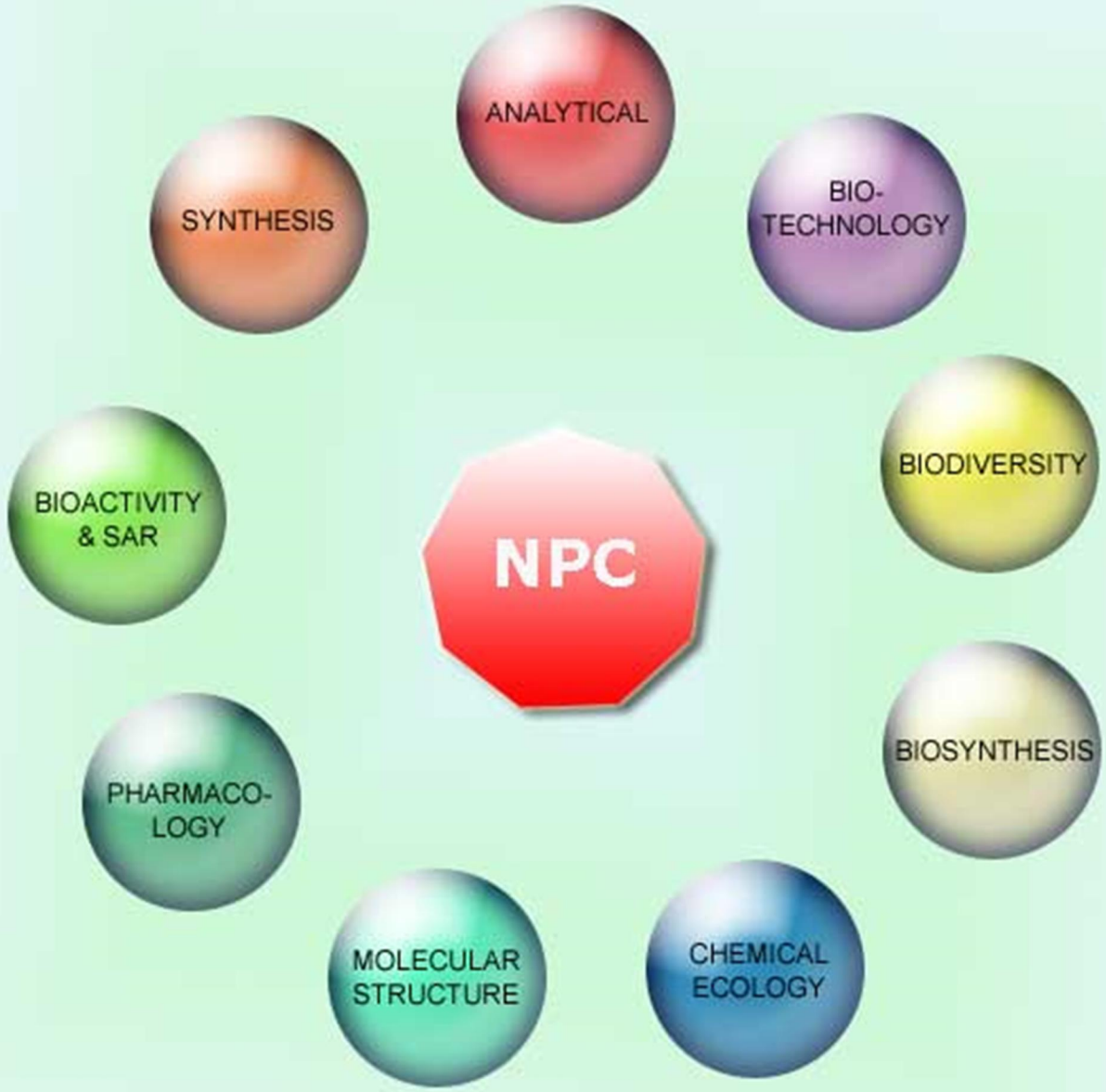

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Synthesis and Biological Evaluation of Febrifugine Analogues

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A series of febrifugine analogues were designed and synthesized. Antimalarial activity evaluation of the synthetic compounds indicated that these derivatives had a strong inhibition against both chloroquine-sensitive and -resistant *Plasmodium falciparum* parasites. Many of them were found to be more active than febrifugine hydrochloride. The tested analogues had also a significant cytotoxicity against four cancer cell lines (KB, MCF7, LU1 and HepG2). Among the synthetic analogues, two compounds **17b** and **17h** displayed a moderate cytotoxicity while they exhibited a remarkable antimalarial activity.

Keywords: Febrifugine, Cytotoxic, Antimalarial, Synthesis, Ionic liquid.

Febrifugine (**1**), a quinazoline-type alkaloid, was first isolated from *Dichroa febrifuga* [1a,b], a plant found in several countries in Asia and used by local people as medicine against fevers caused by malaria parasites. Febrifugine has powerful *in vitro* antimalarial activity against both chloroquine-sensitive *Plasmodium falciparum* FCR-3 and chloroquine resistant *P. falciparum* K1. Although febrifugine (**1**) possesses interesting biological profiles and has been used historically as a herbal remedy, it has serious side effects such as nausea, vomiting, and liver toxicity, which have precluded its use as an antimalarial drug [2a,b]. However, the pharmacological interest in febrifugine encouraged the pursuit of suitable lead compounds based on febrifugine for the development of novel drugs. Many analogues of febrifugine have been prepared and evaluated for their biological activities. Among them, halofuginone (**2**), the halogenated derivative of febrifugine, has been tested in clinical trials for potential therapeutic applications in cancer and fibrotic disease [3a-d]. In this communication, we report the synthesis of a series of febrifugine analogues with modification of the quinazoline ring, and the evaluation of their biological activities.

The preparation of the febrifugine analogues is described in Schemes 1 and 2. The quinazolin-4-ones (**4a-h**) were first prepared starting from the corresponding 2-amino-benzoic acid derivatives. By using the modified reported method [4a,b], the quinazolin-4-ones **4a-h** were successfully obtained in high yields (85-96%) by condensation cyclization of **3a-h** with excess of formamide under microwave irradiation. It was found that when the reaction was performed by heating at 150 - 170°C without microwave assistance, the quinazolin-4-ones **4a-h** were formed in lower yields (60-85%) and with longer reaction times (8 h – 14 h). In the next steps, the valerolactam derivative **11** was synthesized from the commercial available material, L-glutamic acid (**5**) through six steps [5]. In these synthetic procedures, the protection of **6** with an acetyl group would facilitate the protection/deprotection processes in comparison with the previous reports in which the hydroxyl group of **6** was protected with either TMS or MOM groups [5,6]. Treatment of the lactam compound **11** with $(Boc)_2O$ afforded the intermediate **12**, which was then reacted with allyltrimethylsilane in the presence of $BF_3.Et_2O$ to furnish compound **13**.

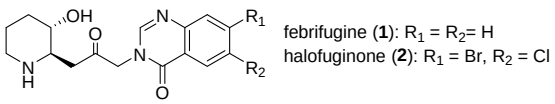
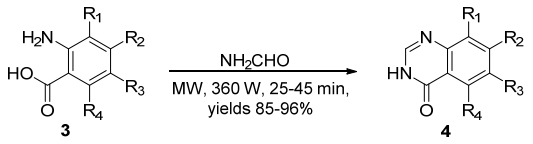


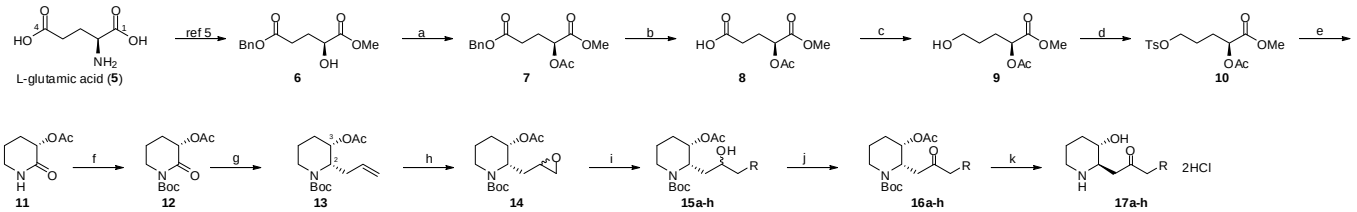
Figure 1: Structure of febrifugine and halofuginone.



a: $R_1 = R_2 = R_4 = H$; $R_3 = Me$
b: $R_1 = R_3 = Me$; $R_4 = R_2 = H$
c: $R_1 = Br$; $R_2 = R_4 = H$; $R_3 = Me$
d: $R_1 = Me$; $R_2 = R_4 = H$; $R_3 = Cl$
e: $R_1 = R_4 = H$; $R_2 = R_3 = F$
f: $R_1 = R_2 = R_4 = H$; $R_3 = OCF_3$
g: $R_1 = R_2 = R_3 = OMe$; $R_4 = H$
h: $R_1 = R_4 = H$; $R_2, R_3 = CH=CH-CH=CH$

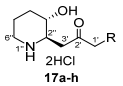
Scheme 1: Preparation of quinazolin-4-ones.

Configuration at C-2 of **13** was revealed by analysis of the NMR signal of H-3, which had an *anti* (J = 11.5 Hz) and two *gauche* (J = 4.7 Hz) coupling constants. This observation suggested that H-3 had an axial disposition while H-2 had an equatorial orientation on the ring. The *cis*-relationship was thus assigned for H-2/H-3 of **13**. Epoxidation of **13** with *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2 provided the diastereoisomeric mixture **14** [6]. With the epoxide **14** in hand, a series of alcohol intermediates **15a-h** were synthesized by epoxide ring opening with the different quinazolin-4-ones **4a-h**. Compounds **15a-h** were obtained as a mixture of two diastereoisomers, as observed in the previous report [7]. In recent years, application of ionic liquids in organic synthesis has attracted considerable attention. This green chemistry approach could avoid the use of toxic organic solvents that damage the environment. In the previous report, it was demonstrated that the oxidation of alcohols into ketones could be achieved by using the Dess-Martin periodinane reagent in $[bmim]BF_4$ and $[bmim]PF_6$ ionic liquids [8]. Using this method for oxidation of the diastereoisomeric mixture **15a-h** under microwave irradiation afforded the corresponding ketone compounds **16a-h** in high yields. Under microwave assistance, the ketone derivatives **16a-h** were formed faster, compared with the reported procedure [7].

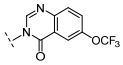
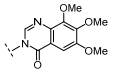
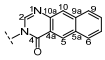


Scheme 2: (a) Ac_2O, pyridine, 60°C, 1 h, 90%, (b) H_2/Pd-C, MeOH, rt, 3 h, 91%, (c) $BH_3.SMe_2$, THF, rt, 3 h, 71%, (d) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 4 h, 70%, (e) 1) NaN_3, DMF, 60°C, 3 h; 2) H_2/Pd-C, MeOH, rt, 1 h, 74%, (f) Boc_2O, DMAP, MeCN, rt, 3 h, 72%, (g) 1) $NaBH_4$, MeOH, 0°C, 3 h; 2) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0°C, 3 h; 3) allyltrimethylsilane, $BF_3.Et_2O$, -78°C, CH_2Cl_2, 2 h, 31% over 3 steps, (h) *m*-CPBA, CH_3Cl, rt, 10 h, 70%, (i) quinazolin-4-ones, Et_3N, EtOH, 60°C, 12 h, 35-74%, (j) Dess-Martin periodinane, [bmim]BF_4, MW 90 W, 15 min, 61-80%, (k) 1) Na_2CO_3, MeOH/H_2O, rt, 1 h; 2) conc. HCl, $CHCl_3$, 4 h, 71-89% over 2 steps.

Table 1: Biological activities of febrifugine hydrochloride and its analogues.



17a-h

Compd.	R	Cytotoxicity (IC_{50}, μM)				*P. falciparum*(IC_{50}, μM)	
		KB	MCF7	LU1	HepG2	T96	K1
1a		0.85	0.66	0.16	0.21	0.026	0.081
17a		0.019	0.014	0.015	0.015	0.012	0.013
17b		2.73	1.55	1.04	0.98	0.007	0.014
17c		0.16	0.23	0.06	0.13	0.022	0.074
17d		0.06	0.04	0.032	0.027	0.01	0.018
17e		0.022	0.03	0.019	0.028	0.007	0.018
17f		0.023	0.018	0.032	0.03	0.073	0.085
17g		0.24	0.18	0.08	0.17	0.088	0.262
17h		6.20	5.25	4.39	4.60	0.073	0.076
ellipticine		0.25	0.22	0.89	0.31		
chloroquine						0.024	0.149

Finally, the acetyl and Boc groups were successively removed to provide the febrifugine hydrochloride analogues **17a-h**. In addition, febrifugine hydrochloride (**1a**) was also prepared, according to the same protocols. The *trans*-diaxial relationship between H-2″ and H-3″ was clearly observed for the analogues **17a-h** as determined by analysis of the NMR signal of the proton H-3″, which had two *anti* (J = 9.0 – 9.5 Hz) and a *gauche* (J = 3.5 – 4.0 Hz) coupling constants. Thus, during the deprotection processes, an epimerization at the C-2″ of **16a-h** was noted. This could be due to the interconversion between febrifugine and isofebrifugine forms [5,9]. Due to the known 2*S* configuration of L-glutamic acid, the *S*-configuration was assigned for both C-2″ and C-3″ of **17a-h**.

The synthetic compounds were evaluated for their antimalarial activity toward chloroquine-sensitive (T96) and chloroquine-resistant *P. falciparum*, and for their cytotoxicity against four cancer cell lines, KB (mouth epidermal carcinoma cells), MCF7 (human breast cancer cells), LU1 (human lung cancer cells) and HepG2 (human hepatocellular liver carcinoma cell line). As expected, all the synthetic compounds had a strong antimalarial activity against both chloroquine-sensitive and -resistant strains of *P. falciparum* (Table 1). Compounds **17a-e** were found to be more active than febrifugine hydrochloride (**1a**) against both sensitive and resistant strains. Several analogues had also significant cytotoxicity against four cancer cell lines. Among the tested samples, compounds **17b** and **17h** displayed moderate cytotoxicity while they had a remarkable antimalarial activity. This observation suggested that the antimalarial activity of these two analogues should not result from their cytotoxic property.

Experimental

General: Melting points were recorded on a Buchi B-545 instrument and are uncorrected. Optical rotations were recorded on a Polax-2L polarimeter in MeOH. ^{1}H and ^{13}C NMR spectra were recorded on a *Bruker* AM500 FT-NMR spectrometer as indicated with either the $CDCl_3$ (δ_H 7.24 ppm, δ_C 77.0 ppm) or CD_3OD (δ_H 3.30 ppm, δ_C 49.0 ppm), signal as internal standard. J values are expressed in Hz. The HMBC measurements were optimized to 7.0 Hz long-range couplings, and NOESY experiments were run with 150 ms mixing time. High resolution ESIMS were measured on a VARIAN 910 spectrometer. All chemicals were purchased from Merck and Sigma-Aldrich and used without any further purification.

Synthesis of compounds 8-13: These compounds were prepared according to the previously reported procedures [5].

Compound 13

^{1}HNMR (500 MHz, $CDCl_3$): 1.43 (9H, s, CH_3), 1.50-1.86 (4H, m, CH_2-4 and CH_2-5), 2.05 (3H, s, Ac), 2.29 (1H, m, H_a-1′), 2.39 (1H, m, H_b-1′), 2.68 (1H, m, H_a-6), 3.96 (1H, m, H_b-6), 4.48 (1H, m, H-2), 4.84 (1H, ddd, J = 4.5, 4.5, 11.5 Hz, H-3), 5.00 (1H, br. d, J = 10.0 Hz, H_a-3′), 5.07 (1H, br. d, J = 17.0 Hz, H_b-3′), 5.72 (1H, m, H-2′).

^{13}CNMR (125 MHz, $CDCl_3$): 21.2 (Ac), 23.9 (CH_2), 24.6 (CH_2), 28.4 (3 × CH_3), 29.3 (CH_2), 37.0 (CH_2), 53.4 (CH), 70.9 (CH), 79.8 (C), 116.9 (CH_2), 134.7 (CH), 154.9 (C=O), 170.0 (C=O).

Procedure for synthesis of 14: To a stirred solution of **13** (4.5 g, 18.2 mmol) in $CHCl_3$ (30 mL) was added *m*-CPBA (5.0 g, 2.44 mmol). The mixture was stirred at rt for 10 h then washed with aqueous 10% $NaHCO_3$ (3 × 15 mL). The $CHCl_3$ solution was concentrated under diminished pressure and the residue was purified by CC (*n*-hexane/EtOAc gradient), providing **14** (3.84 g, 70.5%)as a mixture of two isomers with a ratio of 7/3. This mixture was used for next steps without separation.

General procedure for synthesis of 15a-h: To a stirred solution of the corresponding quinazolin-4-ones **4a-h** (1.0 mmol) in EtOH (10 mL) were added **14** (0.5 eq) and triethylamine (0.5 eq). The mixture was heated at 60°C for 12 h, and then concentrated in vacuum to dryness. The residue was purified by CC on Sephadex LH-20 to afford the alcohols **15a-h**. The 1D NMR spectra of these

compounds showed broad signals, probably caused by conformational flexibility of the piperidine ring.

General procedure for synthesis of 16a-h: A solution of the corresponding alcohols **15a-h** (1.0 mmol) and Dess-Martin periodinane (3.0 eq) were added in ionic liquid1-butyl-3-methylimidazolium tetrafluoroborate [Bmim]BF_4^- (4.0eq). The mixture was irradiated by microwave at 90 W for 15 min. The mixture was extracted with Et_2O (5 × 30 mL). The Et_2O solution was dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude product was purified by CC (CH_2Cl_2/EtOAc gradient) to afford compounds **16a-h** in 61-80% yields. Similar to compounds **15a-h**, due to the ring inversion, broad signals were observed for **16a-h** in their 1D NMR spectra.

General procedure for synthesis of 17a-h: To a stirred solution of the corresponding ketone (0.3mmol) in a mixture of MeOH/H_2O (1.5/0.2, v/v) was added sodium carbonate (2.0eq). The solution was stirred at rt for 1 h then concentrated under diminished pressure to remove MeOH. Water (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 15 mL). The CH_2Cl_2 solution was concentrated to dryness. The residue was dissolved in $CHCl_3$ (1.5 mL) and cooled to 0°C. To this solution, concentrated HCl (4 eq) was added at 0°C. The mixture was then stirred at rt for 4 h, and concentrated to dryness. The residue was recrystallized in acetone to provide febrifugine hydrochloride analogues **17a-h**.

Compound 17a
White solid; yield 70%.
MP: 240-241°C.
$[\alpha]^{29}_D$: +2.3 (*c* 0.35, MeOH).
^{1}H NMR (500 MHz, CD_3OD): 1.62 (1H, m, H_a-4″), 1.83 (1H, m, H_a-5″), 2.04 (1H, m, H_b-5″), 2.13(1H, m, H_b-4″), 2.58 (3H, s, CH_3), 3.04 (1H, m, H_a-6″), 3.10-3.30 (2H, m, H_a-3′, H_b-6″), 3.40-3.60 (2H, m, H_b-3′, H-2″), 3.72 (1H, m, H-3″), 5.32 (2H, s, CH_2-1′), 7.75 (1H, d, *J* = 8.0 Hz, H-8), 7.92 (1H, d, *J* = 8.0 Hz, H-7), 8.16 (1H, s, H-5), 9.29 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 21.4 (CH_3), 21.5 (C-5″), 31.7 (C-4″), 40.2 (C-3′), 45.0 (C-6″), 56.6 (C-1′), 58.1 (C-2″), 68.3 (C-3″), 121.2 (C-4a), 122.3 (C-5), 128.2 (C-8), 138.2 (C-6), 139.1 (C-7), 142.1 (C-8a), 151.6 (C-2), 159.6 (C-4), 201.0 (C-2′).
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{18}H_{24}N_3O_3$: 316.1661; found: 316.1655).

Compound 17b
White solid; yield 78%.
MP: 204-205°C.
$[\alpha]^{29}_D$: + 6.2 (*c* 0.62, MeOH).
^{1}H NMR (500 MHz, CD_3OD): 1.65 (1H, m, H_a-4″), 1.83 (1H, m, H_a-5″), 2.04 (1H, m, H_b-5″), 2.13 (1H, m, H_b-4″), 2.52 (3H, s, CH_3), 2.65 (3H, s, CH_3), 3.04 (1H, ddd, *J* = 12.0, 12.0, 3.0 Hz, H_a-6″), 3.16 (1H, dd, *J* = 17.5, 7.0 Hz, H_a-3′), 3.35 (1H, m, H_b-6″), 3.47 (1H, dd, *J* = 17.5, 4.5 Hz, H_b-3′), 3.52 (1H, m, H-2″), 3.71 (1H, ddd, *J* = 9.0, 9.0, 3.5 Hz, H-3″), 5.32 (2H, s, CH_2-1′), 7.74 (1H, s, H-7), 7.98 (1H, s, H-5), 9.11 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 17.2 (CH_3), 21.3 (CH_3), 21.4 (C-5″), 31.6 (C-4″), 40.1 (C-3′), 44.9 (C-6″), 56.4 (C-1′), 58.1 (C-2″), 68.3 (C-3″), 121.5 (C-4a), 125.8 (C-5), 132.6 (C-8), 137.9 (C-8a), 139.9 (C-7), 141.4 (C-6), 150.5 (C-2), 160.2 (C-4), 201.2 (C-2′).
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{18}H_{24}N_3O_3$: 330.1818; found: 330.1809).

Compound 17c
White solid; yield 77%.
MP: 207-208°C.
$[\alpha]^{29}_D$: + 12.4 (*c* 0.66, MeOH).
^{1}H NMR (500 MHz, CD_3OD): 1.59 (1H, m, H_a-4″), 1.80 (1H, m, H_a-5″), 2.01 (1H, m, H_b-5″), 2.11 (1H, m, H_b-4″), 2.49 (3H, s, CH_3), 3.01 (1H, m, H_a-6″), 3.11 (1H, m, H_a-3′), 3.33 (1H, m, H_b-6″), 3.43 (1H, m, H_b-3′), 3.47 (1H, m, H-2″), 3.69 (1H, m, H-3″), 5.18 (2H, s, H-1′), 8.03 (1H, s, H-5), 8.05 (1H, s, H-7), 8.60 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 21.1 (CH_3), 21.5 (C-5″), 31.7 (C-4″), 40.2 (C-3′), 45.1 (C-6″), 56.3 (C-1′), 58.2 (C-2″), 68.3 (C-3″), 120.5 (C-8), 123.5 (C-4a), 127.1 (C-5), 141.2 (C-7), 141.3 (C-6), 142.2 (C-8a), 150.2 (C-2), 160.9 (C-4), 201.7 (C-2′).
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$(calcd for $C_{17}H_{21}BrN_3O_3$: 394.0766; found: 394.0733).

Compound 17d
White solid; yield 72%.
MP: 208-209°C.
$[\alpha]^{29}_D$: + 3.0 (*c* 0.50, MeOH).
^{1}H NMR (500 MHz, CD_3OD): 1.62 (1H, m, H_a-4″), 1.85 (1H, m, H_a-5″), 2.03 (1H, m, H_b-5″), 2.12 (1H, m, H_b-4″), 2.62 (3H, s, CH_3), 3.03 (1H, ddd, *J* = 12.0, 12.0, 3.0, H_a-6″), 3.15 (1H, dd, *J* = 18.0, 7.5 Hz, H_a-3′), 3.34 (1H, m, H_b-6″), 3.45 (1H, dd, *J* = 18.0, 5.0 Hz, H_b-3′), 3.51 (1H, m, H-2″), 3.73 (1H, ddd, *J* = 9.5, 9.5, 4.0 Hz, H-3″), 5.16 (2H, s, CH_2-1′), 7.73 (1H, d, *J* = 1.5 Hz, H-7), 8.02 (1H, d, *J* = 1.5 Hz, H-5), 8.40 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 17.4 (CH_3), 21.5 (C-5″), 31.7 (C-4″), 40.2 (C-3′), 45.1 (C-6″), 56.2 (C-1′), 58.2 (C-2″), 68.3 (C-3″), 123.6 (C-4a), 124.4 (C-7), 134.2 (C-8), 136.9 (C-5), 138.8 (C-6), 144.6 (C-8a), 149.1 (C-2), 161.2 (C-4), 201.9 (C-2′).
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{17}H_{21}ClN_3O_3$: 350.1271; found: 350.1261).

Compound 17e
White solid; yield 74%.
MP: 240-241°C.
$[\alpha]^{29}_D$: + 6.0 (*c*1.0, MeOH).
^{1}H NMR (500 MHz, CD_3OD): 1.62 (1H, m, H_a-4″), 1.86 (1H, m, H_a-5″), 2.03 (1H, m, H_b-5″), 2.13 (1H, m, H_b-4″), 3.03 (1H, ddd, *J* = 12.0, 12.0, 3.5 Hz, H_a-6″), 3.17 (1H, dd, *J* = 18.0, 7.0 Hz, H_a-3′), 3.34 (1H, m, H_b-6″), 3.43 (1H, (1H, dd, *J* = 18.0, 4.5 Hz, H_b-3′), 3.50 (1H, m, H-2″), 3.75 (1H, ddd, *J* = 9.5, 9.5, 4.0 Hz, H-3″), 5.15 (2H, s, CH_2-1′), 7.64 (1H, dd, *J* = 11.0, 7.0 Hz, H-8), 8.06 (1H, dd, *J* = 10.5, 8.5 Hz, H-5), 8.40 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 21.4 (C-5″), 31.7 (C-4″), 40.2 (C-3′), 45.1 (C-6″), 55.9 (C-1′), 58.4 (C-2″), 68.4 (C-3″), 115.0, 115.2, 115.5 and 115.7 (C-5 and C-8), 150.1 (C-2), 165.8 (C-4), 202.0 (C-2′). Due to coupling with fluorine, the other aromatic carbons were not observed in the ^{13}C NMR spectrum of **17e**.
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{16}H_{18}F_2N_3O_3$: 338.1316; found: 338.1302).

Compound 17f
White solid; yield 86%.
MP: 176-178°C.
$[\alpha]^{29}_D$: + 23.6 (*c*1.15, MeOH).
^{1}HNMR (500 MHz, CD_3OD): 1.63 (1H, m, H_a-4″), 1.85 (1H, m, H_a-5″), 2.03 (1H, m, H_b-5″), 2.13 (1H, m, H_b-4″), 3.04 (1H, ddd, *J* = 12.0,12.0, 3.0 Hz, H_a-6″), 3.19 (1H, dd, *J* = 18.0, 7.0 Hz, H_a-3′), 3.35 (1H, m, H_b-6″), 3.47 (1H, dd, *J* = 18.0, 4.5 Hz, H_b-3′), 3.52 (1H, m, H-2″), 3.74 (1H, ddd, *J* = 9.0, 9.0, 3.5 Hz, H-3″), 5.30 (2H, s, CH_2-1′), 7.90-8.00 (2H, m, H-7, H-8), 8.13 (1H, s, H-5), 9.05 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 21.5 (C-5″), 31.8 (C-4″), 40.2 (C-3′), 45.0 (C-6″), 56.3 (C-1′), 58.2 (C-2″), 68.3 (C-3″), 119.3 (C-5), [120.7, 122.8 (OCF_3)], 123.3 (C-4a), 127.5 (C-7), 130.1 (C-8), 142.2 (C-8a), 149.7 (C-6), 151.5 (C-2), 160.0 (C-4), 201.3 (C-2′).

HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{17}H_{19}F_3N_3O_4$: 386.1328; found: 386.1318).

Compound 17g
White solid; yield 66%.
MP: 246-247°C.
$[\alpha]^{29}_D$: + 6.1 (*c*0.44, MeOH).
^{1}H NMR (500 MHz, CD_3OD): 1.62 (1H, m, H_a-4″), 1.84 (1H, m, H_a-5″), 2.03 (1H, m, H_b-5″), 2.13 (1H, m, H_b-4″), 3.04 (1H, ddd, *J* = 12.0, 12.0, 2.5 Hz, H_a-6″), 3.16 (1H, dd, *J* = 18.0, 7.0 Hz, H_a-3′),3.34 (1H, m, H_b-6″), 3.45 (1H, dd, *J* = 18.0, 4.5 Hz, H_b-3′), 3.52 (1H, m, H-2″), 3.72 (1H, m, H-3″), 4.01 (1H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 5.27(2H, s, CH_2-1′), 7.50 (1H, s, H-5), 8.83 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 21.5 (C-5″), 31.7 (C-4″), 40.1 (C-3′), 44.9 (C-6″), 56.2 (C-1′), 56.9 (OCH_3), 58.1 (C-2″), 61.9 (OCH_3), 62.8 (OCH_3), 68.2 (C-3″), 103.3 (C-5), 117.8 (C-4a), 133.5 (C-8a), 146.8 (C-7), 148.8 (C-2), 149.6 (C-8), 155.9 (C-6), 160.2 (C-4), 201.6 (C-2′).
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{19}H_{26}N_3O_6$: 392.1822; found: 392.1816).

Compound 17h
White solid; yield 89%.
MP: 230-231°C.
$[\alpha]^{29}_D$: + 31.5 (*c*0.52, MeOH).
^{1}HNMR (500 MHz, CD_3OD): 1.63 (1H, m, H_a-4″), 1.87 (1H, m, H_a-5″), 2.04 (1H, m, H_b-5″), 2.15 (1H, m, H_b-4″), 3.05 (1H, ddd, *J* = 12.0, 12.0, 3.0 Hz, H_a-6″), 3.23 (1H, dd, *J* = 18.0, 7.0 Hz, H_a-3′), 3.38 (1H, m, H_b-6″), 3.49 (1H, dd, *J* = 18.0, 5.0 Hz, H_b-3′), 3.55 (1H, m, H-2″), 3.77 (1H, ddd, *J* = 9.5, 9.5, 4.0 Hz, H-3″), 5.33 (2H, s, CH_2-1′), 7.74 (1H, t, *J* = 8.0 Hz, H-7), 7.82 (1H, d, *J* = 8.0 Hz, H-8), 8.17 (1H, d, *J* = 8.0 Hz, H-9), 8.22 (1H, d, *J* = 8.0 Hz, H-6), 8.35 (1H, s, H-10), 8.98 (1H, s, H-5), 9.12 (1H, s, H-2).
^{13}C NMR (125 MHz, CD_3OD): 21.5 (C-5″), 31.8 (C-4″), 40.2 (C-3′), 45.1 (C-6″), 56.3 (C-1′), 58.3 (C-2″), 68.4 (C-3″), 119.4 (C-4a), 121.6 (C-10), 129.3 (C-8, C-9), 130.7 (C-6), 130.9 (C-5), 131.5 (C-7), 133.7 (C-9a), 136.8 (C-10a), 138.0 (C-5a), 151.4 (C-2), 160.6 (C-4), 201.5 (C-2′).
HRMS-ESI: *m/z* [M - 2HCl + H]$^+$ (calcd for $C_{20}H_{22}N_3O_3$: 352.1661; found: 352.1646).

Febrifugine hydrochloride (1a)
White solid; yield 82%.
MP: 282-283°C.
NMR data are in agreement with the reported values [10].

Cytotoxic activity assay: The cytotoxicity assays were carried out in 96-well microtiter plates against cancer cells (KB, LU-1, HepG2 and MCF-7), using a modification of the published method [11]. Ellipticine was used as a reference compound.

Antimalarial activity assay: Antiplasmodial activity of the samples was determined against chloroquine-sensitive (T96) and chloroquine-resistant (K1) *P. falciparum*, according to the reported method [12]. Chloroquine was used as positive control.

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