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Short synthesis and antimalarial activity of fagaronine

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ABSTRACT

Herein, we report a new synthesis of fagaronine **1**, inspired by the synthesis reported by Luo for nornitidine. The in vitro biological activity of fagaronine against malaria on several chloroquine-sensitive and resistant *Plasmodium falciparum* strains was confirmed, and the selectivity index compared to mammalian cells was calculated. Fagaronine was found to have very good antimalarial activity in vivo, comparable to the activity of the reference compound chloroquine. Therefore, fagaronine appears to be a good potential lead for the design of new antimalarial molecules.

1. Introduction

Malaria is one of the world's most widespread and deadliest diseases, having caused 655,000 deaths in 2010.[1] For many years, chloroquine and its derivatives have been used successfully to cure people, but the rapid development of resistant strains has led scientists to search for new and more efficient treatments. Traditionally, plants have been used to treat malaria, and ethnopharmacological studies followed by phytochemical investigations have led to the discovery of the in vitro antiplasmodial activity of many molecules. Unfortunately, these molecules are often not further investigated because there is a lack of in vivo experiments to assess their potential as antimalarial drugs.

Fagaronine **1** (Fig. 1) was first isolated from *Fagara zanthoxyloïdes*[2] and found to have in vitro antiplasmodial activity against a chloroquine-sensitive strain of *Plasmodium falciparum*.[3] Other benzo[*c*]phenanthridines showed antiplasmodial activity in vitro.[4–6] To the best of our knowledge, these studies are the only literature reports on the antiplasmodial activity of benzo[*c*]phenanthridine. Many other biological activities for fagaronine have been reported: antileukaemic activity in vivo on L1210 and P-388 mouse leukaemia[7,8] and in vitro on K-562 human leukaemia cells,[9,10] as well as topoisomerase 1 and 2 inhibition.[11] Because fagaronine showed an activity on *P. falciparum* in the nanomolar range, we decided to evaluate its potential as an antimalarial drug by performing in vivo experiments.

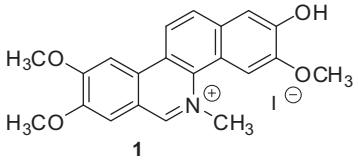

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Figure 1. Structure of fagaronine iodide **1**.

Many total syntheses or hemisyntheses of fagaronine have been published,[12–18] but a concise route to this benzo[*c*]phenanthridine is still needed. A short synthesis of nornitidine from commercially available reagents was published by Luo et al.[19] We decided to adapt it to this synthetic route for the synthesis of fagaronine.

2. Results and discussion

2.1. Chemistry

The method employed for the synthesis of fagaronine **1** is outlined in Scheme 1.

First, 2-bromobenzaldimine **3** was prepared in quantitative yield by treatment of the commercially available aldehyde **2** with *tert*-butylamine. The alkynes **5a** and **5b** were prepared by applying acetate or isopropyl protecting groups, respectively, to the commercially available bromide **4** followed by a Sonogashira reaction

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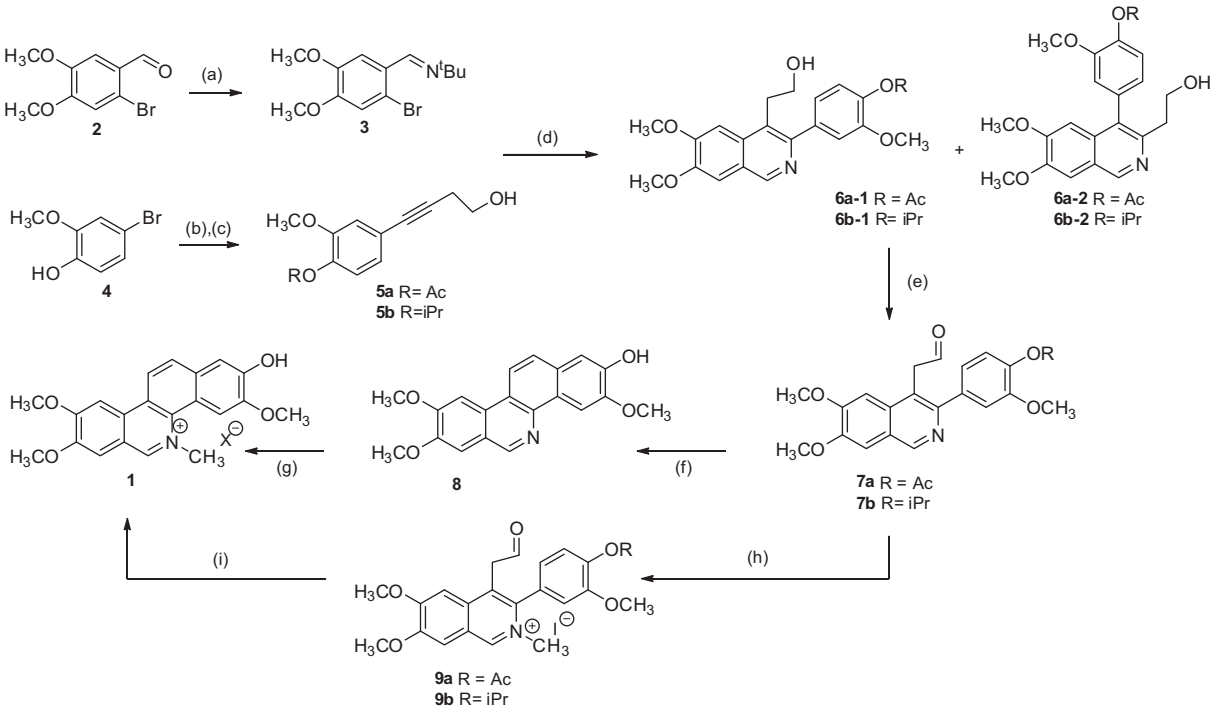


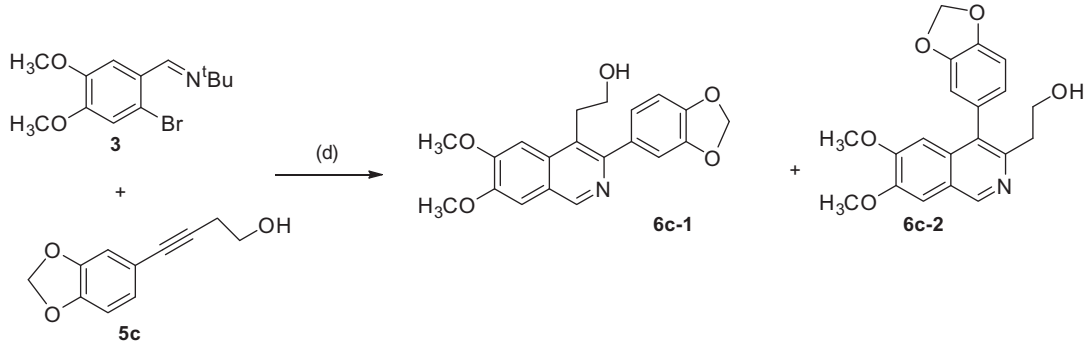
Scheme 1. Synthesis of fagaronine **1**. (a) *t*BuNH2, rt; (b) AcCl, CH_2Cl_2, NEt_3, 0 °C or *i*PrBr, K_2CO_3, DMF, 80 °C; (c) butyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, DIPA, reflux; (d) $Ni(dppe)Br_2$, Zn powder, ACN, reflux; (e) oxalyl chloride, DMSO, DIPEA, CH_2Cl_2, −78 °C; (f) HBr 40%, CH_3COOH; (g) methylmethanesulfonate, sealed tube, 180 °C; (h) CH_3I, CH_2Cl_2; (i) HBr 40%, CH_3COOH, reflux.

with overall 70% yield for **5a** and 76% yield for **5b**. We also showed that protection of the phenol during the Sonogashira reaction was necessary: the best yield with unprotected phenol **4** was 23%.

The key step of this synthesis is a nickel-catalysed annulation reaction between alkyne **5a** or **5b** and the bromobenzaldimine **3**. The annulation of 2-bromobenzaldimine **3** with alkyne **5a** using $NiBr_2$(dppe)/Zn as the catalyst afforded isoquinoline **6a-1** and its regioisomer **6a-2** with a global yield of 92% and a **6a-1**/**6a-2** ratio of 5/4. The same reaction with alkyne **5b** proceeded with a global yield of 70% and a **6b-1**/**6b-2** ratio of 75/25. The separation of the regioisomers from each other proved to be difficult and required successive chromatographies on silica gel to obtain isolated **6a-1** and **6b-1** with sufficient purity. After optimisation, a more efficient purification process was finally found for compound **6b-1**, resulting in 73% yield using medium pressure column chromatography on silica gel.

As Luo did not mention the regioselectivity of this annulation reaction, we tried to reproduce the reaction described in his paper (Scheme 2). We obtained a global yield of 86% with a **6c-1**/**6c-2** ratio of 6/4, which was not in agreement with Luo's yield of 73% for **6c-1** alone. We have no explanation for this discrepancy. The regioselectivity of this annulation reaction is discussed by Larock[20] who described an isoquinoline synthesis via a palladium-catalysed reaction between an imine and an alkyne; high regioselectivity was achieved with unsymmetrical alkynes when the bulkiest substituent was in the α-position relative to the nitrogen atom on the quinoline nucleus. However, they report a lower regioselectivity when the reaction involved an electron-rich imine-like imine **3**. Korivi and Cheng[21] described this reaction with nickel catalysis and reported the same regiochemistry when phenyl substituted alkynes are used.

The oxidation of **6a-1** and **6b-1** under Swern conditions, and the cyclisation of the aldehydes to produce norfagaronine, proceeded in good yields. Optimisation of the methylation of norfagaronine was undertaken using methyl iodide, dimethylsulfate and methylmethanesulfonate. Conditions with methylmethanesulfonate gave the best results: In a sealed tube at 180 °C, methylation occurred with 49% yield, and subsequent purification of



Scheme 2. Annulation reaction described by Luo.[19]

fagaronine from the reaction mixture was tedious. As N-methylation of the quinoline nucleus with methyl iodide was widely reported in the literature, we decided first to methylate quinolines **7a** and **7b**, and then to allow the cyclisation of the methylated aldehydes **9a** and **9b** to offer fagaronine. Methylation of **7a** and **7b** with methyl iodide in dichloromethane at room temperature was quantitative. During the cyclisation of **9a** and **9b** into fagaronine with HBr in refluxing acetic acid, fagaronine iodide **1** precipitated from the reaction mixture and could be easily isolated and purified by filtration and precipitation from methanol with 40% yield from **9a** and 34% yield from **9b**. These yields are rather low, but the filtrates and mother liquors were also essentially fagaronine as a mixture of bromide and iodide salts as shown by ^{1}H NMR and mass spectrometry analyses. Fagaronine obtained from **9a** showed purity higher than 95% (^{1}H NMR). Fagaronine obtained from **9b** showed purity higher than 80% (^{1}H NMR and HPLC). Therefore, the use of acetate as a protective group afforded fagaronine of higher purity.

We successfully adapted Luo's nornitine synthesis to achieve the synthesis of fagaronine in a few steps. We noted that the use of an isopropyl protecting group on the phenol moiety allowed for both a better regioselectivity in the annulation step and an easier purification of the desired regioisomer. The use of an even bulkier protecting group (tertbutyl or cyclohexyl) should potentially improve the regioselectivity of the annulation reaction, which is the limiting step of this short synthesis.

2.2. Biology

The antiplasmodial activity of fagaronine iodide was evaluated for three strains of *P. falciparum* with different sensitivities to chloroquine (Table 1). The IC_{50} values for fagaronine ranged from 7.4 to 14.9 nM and were better than the values of the reference compound, chloroquine. These values were independent of the sensitivity of these strains to chloroquine. Our findings were in agreement with the first report of antiplasmodial activity of fagaronine extracted from *Fagara zanthoxyloides* by Kassim, which defines an IC_{50} of 18 ng/ml against *P. falciparum* 3D7.[3] Nyangulu, however, reports a weaker activity of fagaronine mesylate, with an IC_{50} of 2.3 and 1.8 μM against *P. falciparum* K39 and V1/S strains, respectively.[4] This discrepancy with our results may be due to the counter ion, mesylate, which may alter the solubility of fagaronine.

As fagaronine is known for its cytotoxic activity, we also tested the compound on Vero cells, a non-cancerous mammalian cell line, to determine its antiplasmodial selectivity. As fagaronine showed no toxicity on these healthy mammalian cells, we performed an in vivo antimalarial assay on mice infected with *P. vinckei petteri*, following the classic Peter's four days suppressive test protocol.[22] Mice were infected at day 0, and then treated intraperitoneally each day from day 0 to 3. At day 4, the parasitaemia of each mouse was evaluated and compared to the control. The doses that inhibited 50% of the parasitaemia (ED_{50}) were 6 mg/kg/day for fagaronine and 5.5 mg/kg/day the positive control chloroquine. The survival of the mice was also followed for 13 days after infection. Our studies have shown that fagaronine iodide is as potent as chloroquine for the inhibition of parasitaemia. We have also shown that the effect on the survival of mice was the same for chloroquine and fagaronine at 10 mg/kg/day (Table 2). Furthermore, no sign of acute toxicity was observed for fagaronine at the highest dose of 10 mg/kg/day. In previous studies of fagaronine and its in vivo activity against P388 leukaemia in mice, no toxicity was detected at a dose as high as 50 mg/kg (single injection).[8]

Table 1
In vitro activity (IC_{50} in nM) of tested drugs on *P. falciparum* and vero cells (mean values of two independent experiments performed in triplicate)

Cell lines	Fagaronine iodide	Chloroquine[d]	Doxorubicin
3D7	7.6 ± 1.8	32[c]	NT
FcB1	14.9 ± 0.2	170 ± 14[c]	NT
FcM29	7.4 ± 0.9	240 ± 21[c]	NT
RI[a]	1.0		
Vero	>21,000	>50 000	6400 ± 900
SI[b]	>1400	>194	

Purity of fagaronine: >95%.
[a] Resistance index: 3D7/FcM29.
[b] Selectivity index: Vero/FcB1.
[c] 3D7, CQsensitive, FcB1 and FcM29: CQ resistant.
[d] Evaluated every two months.

Table 2
In vivo activity of fagaronine iodide (F) in a 4 day suppressive test (ED_{50}) and evaluation of the survival time

Treatment	*n*	Mean parasitaemia ± SD	Inhibition (%)	Mean survival (day)
Control	5	37.0 ± 8.6		9.5
CQ 10 mg/kg/d	5	1.9 ± 2.5	94.8	>13
CQ 1 mg/kg/d	5	35.8 ± 7.1	3.2	9.5
F 10 mg/kg/d	5	2.3 ± 3.3	91.5	>13
F 5 mg/kg/d	5	16.7 ± 10.1	38.1	10.7
F 2.5 mg/kg/d	5	27.0 ± 13.7	27.0	11.5
F 1 mg/kg/d	5	35.2 ± 7.8	4.8	8.6

Purity of fagaronine: >80%.

3. Conclusions

In this study, we showed that fagaronine, a known antiplasmodial molecule, was as active as chloroquine against murine malaria in vivo. These results confirm the high potential of fagaronine as an antimalarial drug. Further studies are underway in the laboratory to understand the mechanism of action. Furthermore, we showed that the synthesis proposed by Luo et al. for nornitidine could be adapted to a short synthesis of fagaronine.

4. Experimental part

4.1. Chemistry

The solvents used were of analytical grade (Fisher Scientific, Illkirch, France). Acetonitrile was distiled over CaH_2. Flash column chromatography was carried out on 40–63 μm silica (Geduran, Merck). Medium pressure chromatography was carried out using a Buchi 688 isocratic pump and a Büchi C-690 glass column packed with 6–35 μm silica (Merck). Thin layer chromatographs (TLC) were carried out on silica gel 60F254 (Merck), and the compounds were detected with UV light at 254 nm. Mass spectra were recorded on a Finnigan LCQ MS ion trap spectrometer (ThermoFisher) with ESI ionisation. High resolution mass spectra were recorded on a spectrometer Xevo G2 Q-TOF (Waters) with ESI ionisation or on a GCT Premier CAB109 with DCI/CH_4 ionisation. NMR spectra were recorded on a Bruker Avance 300 or a Bruker 400 AMX spectrometer. Infra-red spectra were recorded on a Paragon 1000 FT-IR (Perkin-Elmer) with compounds deposed on a NaCl plate. HPLC analysis was performed using a C_{18} Luna 3 μm, 100A column (Phenomenex), a Dionex P680 pump and a Dionex UVD170U UV-detector.

4.1.1. 4-(4-Acetoxy-3-methoxyphenyl)-3-butyn-1-ol (5a)

Acetyl chloride (1 ml, 13.97 mmol) was added dropwise to a solution of 4-bromoguaiacol **4** (2.58 g, 12.7 mmol) into CH_2Cl_2 (15 ml) at 0 °C under argon. Et_3N (2 ml) was added, and the mixture was allowed to warm to room temperature under stirring over one hour. The reaction was stopped with the addition of distiled

water (10 ml). The organic phase was then washed with HCl 0.01 N (10 ml), saturated $NaHCO_3$ (10 ml) and distiled water (10 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated to give 2.97 g of acetyl-bromoguaiacol in quantitative yield.

Acetyl-bromoguaiacol (856 mg, 3.5 mmol) was reacted with and 3-butyn-1-ol following a previously published protocol.[19] The crude mixture was purified by silica gel column chromatography (ethyl acetate/cyclohexane:4/6) to afford 579 mg (69% yield) of compound **5a** (oil). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.32 (s, 3H); 2.69 (t, J = 6.3 Hz, 2H); 3.80–3.84 (m, 5H); 6.96 (d, J = 9 Hz, 1H); 7.01–7.04 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ = 19.1 (CH_3); 22.8 (CH_2); 55.0 (CH_3); 60.3 (CH_2); 80.6 (C); 86.6 (C); 115.3 (CH); 122.4 (C); 122.5 (CH); 123.8 (CH); 139.6 (C); 150.9 (C); 169.2 (C). MS (EI) m/z: 234 [M^+]. HRMS (+ESI): m/z Calcd for $C_{13}H_{15}O_4$ [MH^+]: 235.0907. Found: 235.09071. IR: 1015; 1034; 1165; 1211; 1266; 1405; 1598; 1765; 2235; 2940; 3412.

4.1.2. 4-(4-Isopropyloxy-3-methoxyphenyl)-3-butyn-1-ol (5b)

Isopropyl bromide (1.39 ml, 14.77 mmol) and anhydrous K_2CO_3 (2.04 g, 14.77 mmol) were added to a solution of 4-bromoguaiacol **4** (2.00 g, 9.85 mmol) in anhydrous DMF (10 ml). The mixture was stirred at 90 °C for 7 h, and then stirred at room temperature overnight, after which 100 ml of ethyl acetate and 100 ml of water were added. The two phases were separated and the aqueous phase was extracted again with 50 ml of ethyl acetate. The organic phases were pooled together and washed with 100 ml of HCl (0.05 M) and 100 ml of water. The organic phase was dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude compound was purified by flash column chromatography on silica gel (elution with 90/10 cyclohexane/ethyl acetate) to afford 2.43 g of isopropyl-bromoguaiacol in quantitative yield.

Isopropyl-bromoguaiacol (2.43 g) was reacted with and 3-butyn-1-ol following a previously published protocol.[19] The crude mixture was purified by flash column chromatography on silica gel (ethyl acetate/cyclohexane:25/75 then 50/50) to afford 1.76 g (76% yield) of compound **6b** (oil). RMN ^{1}H (300 MHz, $CDCl_3$): δ = 1.39 (d, J = 6 Hz, 6H); 2.70 (t, 2H, J = 6.3 Hz); 3.83 (m, 2H); 3.86 (s, 3H); 4.56 (sept, J = 6 Hz, 1H); 6.82 (d, 1H, J = 8.2 Hz); 6.95 (d, 1H, J = 1.8 Hz); 7.00 (dd, 1H, J = 8.2 Hz, J = 1.8 Hz). ^{13}C NMR (75 MHz, MeOD): δ = 21.0 (CH_3); 22.9 (CH_2); 55.0 (CH_3); 60.8 (CH_2); 71.5 (CH); 81.5 (C); 84.9 (C); 115.3 (CH); 115.7 (CH); 116.8 (C); 124.7 (CH); 146.9 (C); 150.3 (C). MS (EI): m/z = 234 [M^+]. HRMS (+ESI): m/z Calcd for $C_{14}H_{18}O_3$ [MH^+]: 235.1336. Found: 235.1334. IR: 1037; 1136; 1172; 1109; 1264; 1412; 1596; 2935; 2976; 3421 cm^{-1}.

4.1.3. 3-(4-Acetoxy-3-methoxyphenyl)-6,7-dimethoxy-4-(2-hydroxyethyl)isoquinoline (6a-1) and 4-(4-acetoxy-3-methoxyphenyl)-6,7-dimethoxy-3-(2-hydroxyethyl) isoquinoline (6a-2)

Imine **3** (207 mg, 0.69 mmol) and alkyne **5a** (210 mg, 0.89 mmol) were reacted according to a previously published protocol.[19] The crude mixture was purified by flash column chromatography on silica gel (ethyl acetate/dichloromethane:7/3) to afford 60 mg of compound **6a-1** (amorphous solid), 136 mg of a mixture of **6a-1** and **6a-2**, and 60 mg of **6a-2** (oil). The mixture of **6a-1** and **6a-2** was then further purified to afford 75 mg of **6a-1** and 60 mg of **6a-2** (92% total yield, **6a-1/6a-2** ratio 5/4).

*Compound **6a-1*** ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.33 (s, 3H); 2.43 (s br, 1H); 3.27 (t, J = 7.2 Hz, 2H); 3.82 (t, J = 7.3 Hz, 2H); 3.83 (s, 3H); 4.04 (s, 3H); 4.05 (s, 3H); 7.02–7.08 (m, 2H); 7.15 (d, J = 0.9 Hz, 1H); 7.21 (s, 1H); 7.35 (s, 1H); 8.92 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.7 (CH_3); 32.2 (CH_2); 55.9 (CH_3); 56.0 (CH_3); 56.1 (CH_3); 62.5 (CH_2); 102.3 (CH); 105.9 (CH); 113.9 (CH); 121.7 (CH); 122.3 (CH); 123.9 (C); 132.3 (C); 139.2 (C); 140.3 (C); 147.9 (CH); 150.0 (C); 150.7 (C); 151.3 (C); 153.2 (C); 169.1 (C). MS (+ESI) m/z: 398 [MH^+]; 356 [MH^+-Ac]. HRMS (+ESI): m/z Calcd for $C_{22}H_{24}NO_6$ [MH^+]: 398.1604. Found: 398.1604 IR: 1199, 1219; 1258; 1581; 1622; 1759.4; 2900–3554 cm^{-1}.

*Compound **6a-2*** ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.39 (s, 3H); 3.01 (t, J = 7.2 Hz, 2H); 3.80 (s, 3H); 3.84 (s, 3H); 3.97 (t, J = 7.3 Hz, 2H); 4.05 (s, 3H); 6.67 (se, 1H); 6.88 (dd, J = 7.4, 1.8 Hz, 1H); 6.90 (bs, 1H); 7.21 (d, J = 7.4 Hz, 1H); 7.28 (s, 1H), 9.08 (s, 1H). ^{13}C NMR (75 MHz, CDCl3): δ = 20.7 (CH_3); 35.8 (CH_2); 56.0 (CH_3); 56.1 (CH_3); 56.2 (CH_3); 62.3 (CH_2); 103.4 (CH); 105.4 (CH); 114.0 (CH); 122.2 (CH); 123.0 (C); 123.3 (CH); 130.2 (C); 133.1 (C); 135.6 (C); 139.4 (C); 148.4 (CH); 149.0 (C); 150.2 (C); 151.4 (C); 153.8 (C); 168.9 (C). HRMS (DCI/CH_4): m/z Calcd for $C_{22}H_{24}NO_6$ [MH^+]: 398.1604. Found: 398.1592. IR: 1257; 1368; 1427; 1468; 1504; 1582; 1621; 1736; 1765; 2039; 2852; 2933; 3006; 3410 cm^{-1}.

4.1.4. 6,7-Dimethoxy-4-(2-hydroxyethyl)-3-(4-isopropyloxy-3-methoxyphenyl)-isoquinoline (6b-1) and 6,7-dimethoxy-3-(2-hydroxyethyl)-4-(4-isopropyloxy-3-methoxyphenyl) isoquinoline (6b-2)

Imine **3** (885 mg, 2.73 mmol) and alkyne **5b** (830 mg, 3.55 mmol) were reacted according to a previously published protocol.[19] The crude mixture was purified by flash column chromatography on silica gel (ethyl acetate/dichlomethane:5/5 and 7/3) to afford 185 mg of compound **6b-1** (amorphous solid), and 562 mg of a mixture of **6b-1** and **6b-2**. The analysis by ^{1}H NMR spectroscopy of the mixture showed a **6b-1/6b-2** ratio of 67/33 (70% total yield, **6b-1/6b-2** ratio 75/25). From this mixture, **6b-2** (amorphous solid) could be purified by precipitation with dichloromethane for analytical purpose.

An optimisation of the purification method for compound **6b-1** was achieved. The crude mixture of the reaction of imine **3** (1.90 g; 6.31 mmol) with alkyne **5b** (1.92 g, 8.20 mmol) was submitted to medium pressure column chromatography on silica gel eluted with acetone/cyclohexane 60/40 to give 1.84 g (73% yield) of **6b-1**.

*Compound **6b-1*** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.38 (t, J = 6.0 Hz, 6H); 3.31 (t, J = 7.1 Hz, 2H); 3.86 (s, 3H); 3.87 (t, J = 7.1 Hz, 2H); 4.04 (s, 3H); 4.05 (s, 3H); 4.55 (sept, J = 6.0 Hz, 1H); 6.93 (d, J = 8.1 Hz, 1H); 6.99 (dd, J = 8.1, 1.8 Hz, 1H); 7.06 (d, J = 1.8 Hz, 1H); 7.21 (s, 1H); 7.36 (s, 1H); 8.93 (s, 1H). ^{13}C NMR (100 MHz, CDCl3): δ = 22.1 (CH_3); 32.2 (CH_2); 56.0 (CH_3); 56.1 (CH_3); 56.2 (CH_3); 62.7 (CH_2); 71.5 (CH); 102.3 (CH); 105.9 (CH); 113.5 (CH); 115.4 (CH); 121.7 (CH); 123.7 (C); 132.6 (C); 134.2 (C); 146.9 (C); 147.7 (CH); 150.0 (C); 150.1 (C); 151.7 (C); 153.3 (C). HRMS (+ESI): m/z Calcd for $C_{23}H_{28}NO_5$ [MH^+]: 398.1967. Found: 398.1962. IR: 1138; 1167; 1259; 1581; 1622.8; 2900–3554 cm^{-1}.

*Compound **6b-2*** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.45 (t, J = 6.1 Hz, 6H); 3.25–3.30 (m, 2H); 3.79 (s, 3H); 3.84 (s, 3H); 4.03 (s, 3H); 4.19–4.24 (m, 2H); 4.64 (sept, J = 6.1 Hz, 1H); 6.70 (s, 1H); 6.76 (d, J = 1.9 Hz, 1H); 6.77 (dd, J = 8.8, 1.9 Hz, 1H); 7.03 (d, J = 8.8 Hz, 1H), 7.36 (s, 1H), 9.13 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.1 (CH_3); 22.2 (CH_3); 33.3 (CH_2); 56.2 (CH_3); 56.5 (CH_3); 64.2 (CH_2); 71.5 (CH); 103.9 (CH); 106.1 (CH); 113.2 (CH); 115.4 (CH); 122.2 (CH); 123.3 (C); 127.9 (C); 133.0 (C); 135.2 (C); 146.4 (C); 147.4 (C); 148.8 (CH); 150.7 (C); 151.2 (C); 155.6 (C). MS (+ESI) m/z: 398 [MH^+]. HRMS (DCI/CH_4): m/z Calcd for $C_{23}H_{28}NO_5$ [MH^+]: 398.1967. Found: 398.1977. IR: 1275; 1315; 1366; 1424; 1471; 1502; 1579; 1545; 1620; 1734; 1879; 2016; 2928; 2948; 2971; 3430 cm^{-1}.

4.1.5. 3-(4-Acetoxy-3-methoxyphenyl)-6,7-dimethoxy-4-(2-oxoethyl)isoquinoline (7a)

Alcohol **6a-1** (320 mg, 0.805 mmol) was reacted under Swern conditions.[19] The crude compound was purified by flash column chromatography on silica gel (ethyle acetate/cyclohexane:7/3) to produce 251 mg (77% yield) of compound **7a** (amorphous solid). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.33 (s, 3H); 3.86 (s, 3H); 4.04 (s,

3H); 4.07 (s, 3H); 4.15 (d, J = 1.7 Hz, 2H); 7.00 (s, 1H); 7.02 (dd, J = 8.0, 1.8 Hz, 1H); 7.12 (d, J = 8.0 Hz, 1H); 7.14 (d, J = 1.8 Hz, 1H); 7.28 (s, 1H); 9.06 (s, 1H); 9.84 (t, J = 1.68 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.7 (CH_3); 45.2 (CH_2); 55.9 (CH_3); 56.1 (CH_3); 56.1 (CH_3); 101.9 (CH); 106.0 (CH); 113.8 (CH); 118.2 (C); 121.6 (CH); 122.6 (CH); 123.8 (C); 132.5 (C); 139.5 (C); 139.7 (C); 149.2 (CH); 150.3 (C); 151.1 (C); 152.1 (C); 153.7 (C); 168.9 (C); 199.2 (C). MS (ESI) m/z: 396 [MH^+]; 354 [MH^+-Ac]. HRMS (+ESI): m/z Calcd for $C_{22}H_{22}NO_6$ [MH^+]: 396.1447. Found: 396.1452. IR: 1199; 1215; 1259; 1580; 1621; 1719; 1764; 2938 cm^{-1}.

4.1.6. 6,7-Dimethoxy-3-(4-isopropyloxy-3-methoxyphenyl)-4-(2-oxoethyl)isoquinoline (7b)

Alcohol **6b-1** (400 mg, 1.04 mmol) was reacted under Swern conditions.[19] The crude compound was purified by flash column chromatography on silica gel (acetone/dichloromethane: 3/7) to afford 290 mg (72% yield) of compound **7b** (amorphous solid). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.41 (d, J = 6.0 Hz, 6H); 3.88 (s, 3H); 4.02 (s, 3H); 4.05 (s, 3H); 4.14 (d, J = 2.1 Hz, 2H); 4.60 (sept, J = 6.0 Hz, 1H); 6.97 (m, 2H); 7.00 (m, 1H); 7.06 (m, 1H); 7.28 (s, 1H); 9.07 (s, 1H); 9.82 (t, J = 2.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.8 (CH_3); 45.5 (CH_2); 56.0 (CH_3); 56.1 (CH_3); 56.1 (CH_3); 71.6 (CH); 101.8 (CH); 106.1 (CH); 113.4 (CH); 115.4 (CH); 118.1 (C); 121.8 (CH); 123.6 (C); 132.7 (C); 133.6 (C); 147.3 (C); 148.9 (CH); 150.2 (C); 150.3 (C); 152.6 (C); 153.8 (C); 199.7 (C). MS (+ESI) m/z: 396 [MH^+]; 428 [MH^+ + MeOH]. HRMS (+ESI): m/z Calcd for $C_{23}H_{26}NO_5$ [MH^+]: 396.1811. Found: 396.1815. IR: 1137; 1164; 1256; 1581; 1621; 1719; 2974 cm^{-1}.

4.1.7. Norfagaronine (8)

To a solution of compound **7a** (40 mg, 0.101 mmol) in AcOH (1 ml) was added 1 ml of HBr (40%), and the mixture was stirred under reflux for 0.5 h. The mixture was allowed to cool to room temperature and was then neutralised to pH 8 with a concentrated solution of sodium hydroxide. This neutralised reaction mixture was extracted with $CHCl_3$. The organic phase was isolated and then dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure to afford 30 mg (90% yield) of a yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 2/1): δ = 4.03 (s, 3H), 4.10 (s, 3H), 4.12 (s, 3H), 7.31 (s, 1H), 7.34 (s, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.81 (s, 1H), 8.18 (d, J = 8.9 Hz, 1H), 8.58 (s, 1H), 9.13 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD 2/1): δ = 55.9 (CH_3), 55.9 (CH_3), 56.0 (CH_3), 101.5 (CH), 103.7 (CH), 107.2 (CH), 110.5 (CH), 117.8 (CH), 119.6 (C), 121.9 (C), 126.2 (C), 126.6 (CH), 128.8 (C), 129.2 (C), 139.9 (C), 146.8 (C), 148.5 (C), 149.2 (CH), 149.6 (C), 153.1 (C). MS (+ESI) m/z: 336 [$MH]^+$.

4.1.8. 3-(4-Acetoxy-3-methoxyphenyl)-6,7-dimethoxy-2-methyl-4-(2-oxoethyl)isoquinolinium iodide (9a)

A solution of compound **7a** (40 mg, 0.101 mmol) in CH_2Cl_2 (1 mL) was stirred for 24 h at room temperature with 70 µl of methyl iodide (145 mg, 1.01 mmol). Then, methyl iodide and CH_2Cl_2 were both removed under vacuum to produce 55 mg of compound **9a** in quantitative yield (orange oil). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.35 (s, 3H); 3.84 (s, 3H); 4.04 (s, 3H); 4.12 (s, 3H); 4.14 (s, 3H); 4.24 (bs, 2H); 6.92 (dd, J = 8.0, 1.9 Hz, 1H); 7.15 (s, 1H); 7.18 (d, J = 1.8 Hz, 1H); 7.22 (d, J = 8.0 Hz, 1H); 8.00 (s, 1H); 9.83 (s, 1H); 10.20 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.6 (CH_3); 45.2 (CH_2); 47.5 (CH_3); 56.7 (CH_3); 57.1 (CH_3); 57.9 (CH_3); 103.1 (CH); 108.3 (CH); 113.5 (C); 121.3 (CH); 123.7 (C); 124.3 (CH); 127.2 (C); 129.4 (C); 135.6 (CH); 141.7 (C); 143.2 (C); 146.3 (C); 152.3 (C); 152.7 (C); 158.7 (C); 168.4 (C); 197.2 (CH). MS (+ESI) m/z: 350 [M-Ac]$^+$, 410 [M]$^+$, 442 [M+MeOH]$^+$. HRMS (+ESI): m/z Calcd for $C_{23}H_{24}NO_6$ [M] $^{+:}$ 410.1598. Found: 410.1601. IR: 1175; 1217; 1260; 1508; 1613; 1781; 2879; 2981 cm^{-1}.

4.1.9. 6,7-Dimethoxy-3-(4-isopropyloxy-3-methoxyphenyl)-2-methyl-4-(2-oxoethyl)isoquinolinium iodide (9b)

A solution of compound **7b** (100 mg, 0.253 mmol) in CH_2Cl_2 (10 mL) was stirred for 24 h at room temperature with 160 µl of methyl iodide (360 mg, 2.53 mmol). Then, methyl iodide and CH_2Cl_2 were both removed under vacuum to afford 135 mg of pure compound **9b** in quantitative yield (orange oil). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (d, J = 6.0 Hz, 6H); 3.87 (s, 3H); 4.08 (s, 3H); 4.15 (s, 3H); 4.17 (s, 3H); 4.25 (bs, 2H); 4.65 (m, 1H); 6.86 (dd, J = 8.1, 2.0 Hz, 1H); 6.95 (d, J = 2.0 Hz, 1H); 7.03 (d, J = 8.1 Hz, 1H); 7.13 (s, 1H); 8.07 (s, 1H); 9.84 (s, 1H); 10.37 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.1 (CH_3); 45.3 (CH_2); 47.6 (CH_3); 56.5 (CH_3); 57.4 (CH_3); 57.9 (CH_3); 71.5 (CH); 102.9 (CH); 108.3 (CH); 112.5 (C); 114.7 (CH); 121.6 (CH); 122.7 (C); 123.5 (C); 127.2 (C); 135.7 (C); 144.2 (C); 146.1 (CH); 149.5 (C); 150.9 (C); 152.7 (C); 158.7 (C); 197.1 (CH). MS (+ESI) m/z: 350 [M-Ac]$^+$, 410 [M]$^+$. HRMS (+ESI): m/z Calcd for $C_{24}H_{28}NO_5$ [M] $^{+:}$ 410.1967. Found: 410.1965. IR 1141; 1175; 1267; 1508; 1615; 1718; 2835; 2977 cm^{-1}.

4.1.10. Fagaronine iodide (1)

To a solution of compound **9a** (95 mg, 0.177 mmol) in AcOH (3 ml) was added 0.77 ml of HBr (40%), and the mixture was stirred under reflux for 1 h. The mixture was allowed to cool to room temperature. The precipitate was collected by filtration and washed with water. To the filtrate was added 20 ml of water to promote further precipitation. The solid is collected by filtration and washed with water. The two solids are pooled together and dried under vacuum to afford 38.1 mg (45% yield) of a brown solid. The solvents are removed from the filtrate under vacuum to give 33 mg of a residue, which can be purified by precipitation with methanol to generate 13 mg of a solid that is a mixture of fagaronine iodide and fagaronine bromide.

To a solution of compound **9b** (410 mg, 0.76 mmol) in 8 ml of acetic acid was added 3.1 ml of HBr (40%), and the mixture was stirred under reflux for 6 h. The mixture was allowed to cool, and the precipitate was collected by filtration. This precipitate was washed with water and dried under vacuum to afford 263 mg of a brown solid. This solid was precipitated from methanol to generate 164 mg (34% yield) of a brown solid.

From **9a**, fagaronine was obtained with purity higher than 95% (single compound on ^{1}H NMR spectra). From **9b** fagaronine was obtained with a purity of 85% according to the ^{1}H NMR spectra, and a purity of 81% according to the HPLC chromatogram recorded at 300 nm.

^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ = 4.14 (s, 3H), 4.16 (s, 3H), 4.28 (s, 3H), 5,03 (s, 3H), 7.52 (s, 1H), 7.80 (s, 1H), 8.04 (s, 1H), 8.12 (d, J = 9.0 Hz,1H), 8.13 (s, 1H), 8.56 (d, J = 9.0 Hz, 1H), 9.68 (s, 1H). MS (+ESI) m/z: 350 [$M]^+$, 335 [M-$CH_3]^+$. MS (-ESI) m/z: 127 [I^-]. HRMS (+ESI): m/z Calcd for $C_{21}H_{20}NO_4$ [M] $^{+:}$ 350.1392. Found: 350.1395.

As fagaronine iodide was not enough soluble in deuterated solvents to obtain a carbon in a reasonable amount of time, it has been converted into chloride: 40 mg of fagaronine iodide were dissolved in 50/50 methanol/dichloromethane and eluted with methanol through an anion exchanger column (10 g, Dowex® 1 × 4–200, chloride form). 20 mg of a yellow solid were obtained. ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD): δ = 50.9 (CH_3), 55.9 (CH_3), 56.3 (CH_3), 56.8 (CH_3), 102.0 (CH), 107,0 (CH), 108.1 (CH), 112.1 (CH), 117.7 (CH), 117.9 (C), 119.9 (C), 124.0 (C), 130.3 (C), 131.7 (CH), 132.5 (C), 132.9 (C), 149.1 (C), 149.2 (C), 149.4 (CH), 152.2 (C), 159.0 (C).

4.2. Biology

4.2.1. In vitro antiplasmodial activity

P. falciparum 3D7, FcM29 and FcB1-Columbia strains were cultured as described by Trager and Jensen,[23] with modifications.[24]

The cultures were synchronised with a combination of magnetic concentration and 5% D-sorbitol lysis (Merck, Darmstadt, Germany).[25,26] The cultures, mostly at ring stage (synchronization period: 8 h) were plated in 96-well plates and the drugs were deposited at growing dilutions in triplicates. The incubation time between drugs and *Plasmodium* was 48 h.The 3D7 strain was considered to be chloroquine-sensitive (chloroquine IC_{50}: 32 nM); the FcM29 and FcB1-Columbia strains were considered to be chloroquine-resistant (chloroquine IC_{50}: 170 ± 14 nM and 240 ± 21 nM, respectively). Antiplasmodial activity was determined by the [^{3}H]-hypoxanthine (Amersham–France) incorporation method.[27] The resistance index was calculated as follows: IC_{50} 3D7/IC_{50} FcM29.

4.2.2. In vitro cytotoxicity

The toxicity of fagaronine was estimated using Vero cells (normal monkey kidney cells). This cell line was cultured under the same conditions as *P. falciparum*, except for the replacement of 5% human serum with 10% foetal calf serum. After the addition of fagaronine at increasing concentrations, cell growth was estimated by [^{3}H]-hypoxanthine incorporation following a 48 h incubation and was compared with a control sample that did not have additional chemicals (the mean of the corresponding wells was referred to as 100%).[28] Doxorubicin, a known cytotoxic drug was used as control. The selectivity index was calculated as follows: IC_{50}Vero/ IC_{50}FcB1.

4.2.3. In vivo antimalarial activity testing

The classic 4 day suppressive in vivo assay was performed using CD female mice according to European legislation on laboratory animal use and care (EEC directive 86/609). The mice (mean body weight: 20 ± 2 g) were infected with 10^6 *P. vinckei petteri*-infected red blood cells[22] in RPMI on day 0. Groups of five mice were treated intraperitoneally from day 0 to 3 with increasing doses (0.1 to 20 mg/kg) of the drug. On day 4, Giemsa-stained smears were made for each mouse, and parasitaemia was estimated by visual numeration of at least 5000 erythrocytes. Mice treated with RPMI alone served as negative controls, and mice treated with chloroquine at various doses served as positive controls. The inhibition percentage was calculated using the following formula: (control parasitaemia–parasitaemia with drugs)/(control parasitaemia) × 100. ED_{50} (efficient dose) was estimated by graphical interpolation on parasitaemia versus concentration graphs.

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Supplementary data

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