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Synthesis and pharmacological evaluation of fluoro/chloro-substituted acetyl and benzoyl esters of quinine as antimalarial agents

Jean-Gonfi Mvondo Mbala [a], Dani Thierry Mawete [a], Alain Nanikafuako Makiese [a], Prosper Lwanzo Kwiraviwe [b], Bibiche Kuyubuka Pangu [b], Eric Buini Nguimi [b], Richard Cimanga Kanyanga [c,d], Luc Pieters [d], Gilles Degotte [e,f], Michel Frederich [e], Aristote Matondo [a], Natascha Van Pelt [g], Guy Caljon [g], Bernard Pirotte [f,*], Blaise Mbala Mavinga [a], Sylvie-Mireille Bambi-Nyanguile [b,*]

[a] Faculty of Sciences, Departement of Chemistry, Université de Kinshasa, BP 190 Kinshasa XI, Democratic Republic of the Congo
[b] Faculty of Pharmaceutical Sciences, Laboratoire de Chimie Médicale, Université de Kinshasa, BP 212 Kinshasa XI, Democratic Republic of the Congo
[c] Faculty of Pharmaceutical Sciences, Laboratoire de Pharmacognosie, Université de Kinshasa, BP 212 Kinshasa XI, Democratic Republic of the Congo
[d] Department of Pharmaceutical Sciences, Natural Products & Food Research and Analysis (NaturA), University of Antwerp, Universiteitsplein 1, B-2610 Anvers, Belgium
[e] Center for Interdisciplinary Research on Medicines (CIRM), Laboratory of Pharmacognosy, Université de Liège, Belgium
[f] Center for Interdisciplinary Research on Medicines (CIRM), Laboratory of Medicinal Chemistry, Université de Liège, Belgium
[g] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Infla-Med Centre of Excellence, University of Antwerp, Antwerp, Belgium

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This work is dedicated to the memory of Jean-Gonfi MVONDO MBALA.

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ABSTRACT

After establishment of the pharmacokinetic and toxicological profile of quinine derivatives using *in silico* approaches, this study reports the synthesis of new acetyl and benzoyl esters of quinine bearing one or more fluorine or chlorine atoms on the acetyl/benzoyl moiety. The antimalarial activity of these compounds on *Plasmodium falciparum* 3D7 and K1 strains as well as the antiprotozoal activity on *Trypanosoma brucei brucei* and *Trypanosoma cruzi* were evaluated. Lastly, the cytotoxicity on MRC-5_{SV2} cells was established. The fluoroacetyl ester compounds were found to be more active *in vitro* against *Plasmodium falciparum* 3D7 strain than the reference compound quinine. All synthesized quinine derivatives were non-cytotoxic for MRC-5_{SV2} cells (CC_{50} > 64 μM). These results confirm that the introduction of one or more fluorine atoms into acetyl and benzoyl esters of quinine can sometimes improve the biological activity.

1. Introduction

Malaria is a parasitosis caused by haematozoa of the genus *Plasmodium*, transmitted to humans by mosquitoes of the genus *Anopheles* during their blood meal [1–3]. It is estimated that nearly half of the world's population lives in endemic areas. The World Health Organization (WHO) classifies it as a priority disease for intervention, along with tuberculosis and HIV/AIDS [4]. It thus represents a huge financial burden for populations and consequently the disease constitutes an obstacle to the development of the countries concerned, particularly in Africa [5]. Many antimalarial drugs are currently available, but the parasite has developed chemoresistance, especially to the most affordable molecules for populations at risk (chloroquine and sulfadoxine/pyrimethamine combination) [6,7].

Until recently, only artemisinin derivatives and quinine seemed to be spared from chemoresistance phenomena. However, since 2009, resistance has appeared in various places in South-East Asia for artemisinin derivatives, and *in vivo* quinine resistance of *P. falciparum* is limited in South-East Asia and Amazonia, and remains mostly partial and of low level. In Africa, *P. falciparum* quinine-resistant strains are still rare and of a level that does not have any perceivable clinical consequences, but the duration of treatment and associated side effects often limit the use of quinine. The implementation of dual therapies no longer seems sufficient to contain the disease, as the chemoresistance observed against artemisinin derivatives may create further resistance against the partner drug [8,9].

It is therefore necessary to continue the development of new, low-cost antimalarial drugs with new mechanisms of action. In the last

* Corresponding authors.
E-mail addresses: b.pirotte@uliege.be (B. Pirotte), sm_bambi@yahoo.fr (S.-M. Bambi-Nyanguile).

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decade, recent modifications of quinine have led to a selection of quinine analogues with different structural elements and potent antimalarial activity [10]. The importance of these new antimalarials has generated great interest in this field of research and has resulted in numerous publications on this subject [10]. Following the success observed in 1995 with artemisinin-based combination therapy (ACT), research has been directed towards hybrid molecules containing two or more motifs with biological activity, which has opened up a new and important area of research; the artemisinine-quinine hybrid and many others are examples of contributions to this field of research [11].

However, the new low-dimensional derivatives with antimalarial activity have clear additional advantages over the large molecules in terms of accumulation in the parasite [12].

In this study, quinine was chosen as a substrate for the esterification of its alcohol function respectively with a chlorinated acetyl moiety or three fluorinated acetyl moieties substituted to different degrees, and with two 2-mono and 3,4-dichlorinated benzoyl moieties or a 2-fluorinated benzoyl moiety. Until now, quinine has been the antimalarial drug of last resort in regions where the disease is endemic, but it has undesirable side effects which are sometimes the cause of non-adherence to the treatment and, consequently, of the appearance of chemoresistance. The esterification of quinine with a simple carboxylic acid derivative of low dimensions substituted by fluorine or chlorine atoms, could contribute to the increase of the antimalarial activity of these esters. Indeed, it has already been reported that fluorination of bioactive antimalarial molecules may accentuate their biological activity, as in the case of quinine, tafenoquine and mefloquine [13–16].

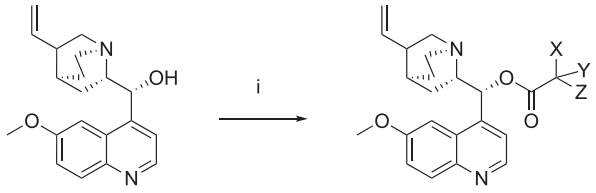
2. Results and discussion

2.1. Chemistry

The synthesis of the acetyl esters of quinine was carried out by esterification of the hydroxyl function of quinine with acetic anhydride, chloroacetic anhydride and di-/-trifluoroacetic anhydride (scheme 1). Quinine fluoroacetate was prepared from fluoroacetyl chloride. The benzoyl esters were obtained by reaction of quinine with the appropriate halo-substituted benzoyl chloride (scheme 2).

These reactions were performed with an excess of reagents to ensure good yields of final compounds [17–24]. The progress of the reaction was followed by thin-layer chromatography, until a single spot was observed. The esters obtained at the end of the reaction were precipitated in a saline solution of cold water and easily purified by successive washes with cold water. The esters were obtained in good yields (77–91 %) with a high degree of purity without further tedious purification (Table 1).

1



1 **2** **2a**: X,Y,Z = H
2b: X = Cl; Y,Z = H
2c: X = F; Y,Z = H
2d: X,Y = F; Z = H
2e: X,Y,Z = F

Scheme 1. Esterification of quinine with fluoroacetyl chloride or (chloro/fluoro)acetic anhydrides. Reagents: i: 2a: acetic anhydride; 2b: chloroacetic anhydride; 2c: fluoroacetyl chloride; 2d: difluoroacetic anhydride; 2e: trifluoroacetic anhydride.

2.2. Antiplasmodial activity

The antiplasmodial activity, expressed as the IC_{50} value in μM, was evaluated on *Plasmodium falciparum* 3D7 and on *P. falciparum* K1 strains following the classical protocol (Tables 2 and 3) [25–27].

Cytotoxicity was determined on MRC-5_{SV2} cells in parallel with antiprotozoal tests on *Trypanosoma cruzi* and *Trypanosoma brucei brucei* (Table 3) [28]. The antimicrobial activity was evaluated on *Staphylococcus aureus* and *Candida albicans* (data not shown). The reference compounds used were tamoxifen for cytotoxicity on MRC-5_{SV2} cells, benznidazole for *T. cruzi*, suramin for *T. b. brucei,* and chloroquine diphosphate for *P. falciparum* (Table 4).

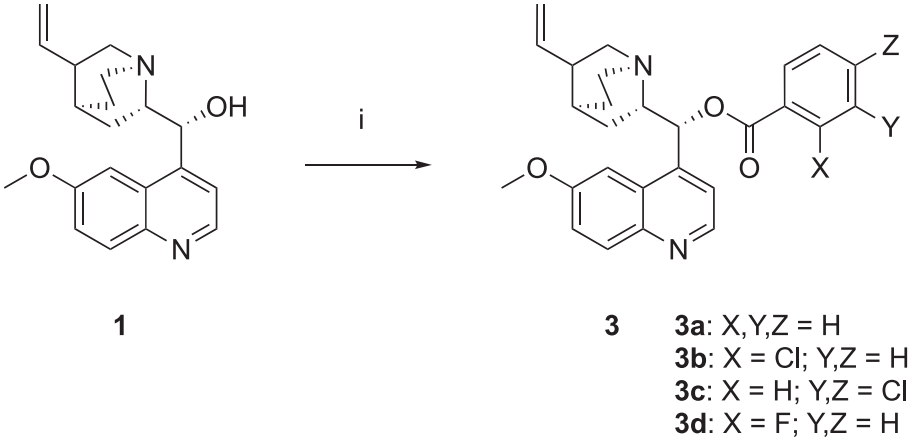
The results obtained on the *P. falciparum* strain 3D7 revealed a high antiplasmodial activity of the fluorinated esters **2c-e** compared to quinine base **1** and quinine acetate **2a** and this antiplasmodial activity was also proportional to the degree of fluorination of the acetyl ester, the trifluoroacetyl ester **2e** being the most active (see Table 2). The chloroacetyl ester **2b** also was found to have a high antiplasmodial activity compared to quinine and the acetyl ester of quinine but a lower activity compared to the fluoroacetyl esters. The benzoyl esters of quinine (compounds **3a-d**), on the other hand, showed lower antiplasmodial activity compared to quinine **1** and the acetyl ester of quinine **2a** except for the 2-fluorobenzoyl ester **3d**, which expressed an IC_{50} value on *P. falciparum* 3D7 similar to quinine (see Table 2). As observed by Bucher *et al*, fluorination of bioactive molecules can frequently enhance their biological activity [15]. In the field of antimalarial molecules, tafenoquine and mefloquine are examples of compounds still containing the quinoline unit and one or more trifluoromethyl radicals. This property is justified by the combination of the atomic characteristics of the fluorine atom: low atomic radius (1.47 Å against 1.20 Å for H and 1.52 Å for O), low polarizability and high electronegativity, high hydrophobicity, improving membrane passage (cell membranes being made of lipid bilayers, to cross it, molecules must be lipophilic) [13–15,29].

Benzoyl esters being larger than acetyl esters, the increase of the steric hindrance could explain the decrease of the antiplasmodial activity, which may be due to difficulties in penetrating the plasma membrane of parasite-infested erythrocytes [30].

Table 3 reports the results of the antiprotozoal activity on the chloroquine-resistant *P. falciparum* K1, *T. b. brucei*, *T. cruzi* and cytotoxicity on MRC-5_{SV2} cells. The effect of the new compounds on *S. aureus* and *C. albicans* was also examined and revealed the absence of antimicrobial activity ($IC_{50} > 64$ μM; data not shown). The results showed that quinine and its synthesized ester derivatives were markedly active on *P. falciparum* K1 ($IC_{50} < 1$ μM) but inactive against the other protozoa tested. All compounds were almost equipotent on the chloroquine-resistant *P. falciparum* K1. The cytotoxicity data for MRC-5_{SV2} cells showed CC_{50} values above 64 μM, both for quinine and for the ester derivatives. These compounds, therefore, did not exhibit the dreaded cytotoxicity as predicted from the pharmacokinetic and toxicological profile of quinine and its ester derivatives established by the *in silico* approach using the SwissADME and pkCSM software [31].

3. Conclusion

Halo-substituted acetyl and benzoyl esters of quinine were synthesized by a simple and efficient process with good yields. Bioassays indicated a marked antiplasmodial activity for the chlorinated and fluorinated acetyl esters of quinine but lower for the benzoyl esters. The trifluoroacetyl ester of quinine expressed an *in vitro* antiprotozoal activity on *Plasmodium* 3D7 three-fold higher than the reference compound quinine, supporting the view that the trifluoroacetyl moiety could favorably increase the cell penetration of the drug in the parasite-infested erythrocytes. All compounds synthesized were found to be non-cytotoxic towards MRC-5_{SV2} cells. These satisfactory results identify a potential new source of antimalarial molecules, which could overcome the resistance phenomenon of current medications. There is an urgent



Scheme 2. Esterification of quinine with halogenated benzoyl chloride. Reagents: i: 3a: benzoyl chloride; 3b: 2-chlorobenzoyl chloride; 3c: 3,4-dichlorobenzoyl chloride; 3d: 2-fluorobenzoyl chloride.

Table 1
Data on quinine and synthesized derivatives.

Compd	Formula	MM	Yields (%)	MP (°C)	
				MP lit.	MP exp.
1	$C_{20}H_{24}N_2O_2$	324,42	–	177	176–177
2a	$C_{22}H_{26}N_20_3$	366,46	87	116	115–117
2b	$C_{22}H_{25}N_2ClO_3$	400,90	81	–	128–131
2c	$C_{22}H_{25}N_2FO_3$	384,44	81	–	115–120
2d	$C_{22}H_{24}N_2F_2O_3$	402,43	77	–	135–139
2e	$C_{22}H_{23}N_2F_3O_3$	420,42	91	–	163–168
3a	$C_{27}H_{28}N_2O_3$	428,53	88	–	105–109
3b	$C_{27}H_{27}N_2ClO_3$	462,97	85	–	135–141
3c	$C_{27}H_{26}N_2Cl_2O_3$	497,41	90	–	154–156
3d	$C_{27}H_{27}N_2FO_3$	446,51	88	–	101–106

MM: molecular mass; MP lit.: literature melting point; PF exp.: experimental melting point.

need to expand the therapeutic arsenal for malaria, which is still devastating, particularly in the sub-Saharan countries.

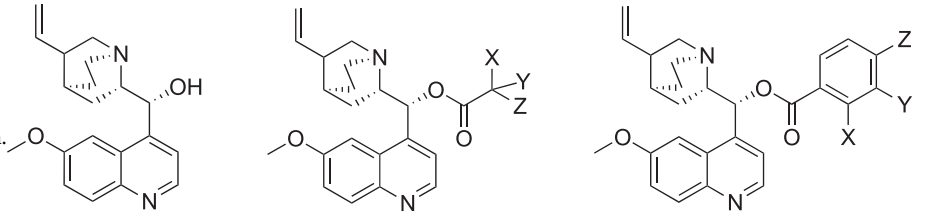
4. Experimental section

4.1. Materials

The chemicals and solvents used in the experiments were purchased from commercial suppliers and used as follows: Quinine base p.a. (Merck), chloroacetic anhydride p.a. (Aldrich), trifluoroacetic anhydride p.a. (Aldrich), difluoroacetic anhydride p.a. (abcr), fluoroacetyl chloride p.a. (abcr), acetic anhydride p.a. (Aldrich), 2-chlorobenzoyl chloride p.a. (Janssen chemica), 3,4-dichlorobenzoyl chloride p.a. (Janssen chemica), 2-fluorobenzoyl chloride p.a. (Acros organics), acetonitrile p.a. (Acros organics), triethylamine p.a. (Acros organics), ammonia p.a. (Acros organics), ethyl acetate p.a. (Acros organics), methanol p.a. (Acros organics), ethanol p.a. (Acros organics), sodium hydroxide, magnesium sulfate, potassium carbonate.

Thin-layer chromatography was performed on TLC plates (Merck, TLC silica gel 60F254) using as mobile phase the mixture ethyl acetate (20 mL), methanol (5 mL) and trimethylamine (1 mL). Melting point determination was performed on the Stuart smp3 melting point

Table 2
Antiplasmodial activity on the *P. falciparum* 3D7 strain.



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Compd	X	Y	Z	IC_{50} mean ± SD (*n*) (μg/mL)	IC_{50} ± SD (*n*) (μM)
1	–	–	–	1.32 ± 0.06 (3)	4.06 ± 0.18 (3)
2a	H	H	H	2.31 ± 0.28 (3)	6.31 ± 0.76 (3)
2b	Cl	H	H	1.29 ± 0.19 (3)	3.22 ± 0.48 (3)
2c	F	H	H	0.88 ± 0.04 (3)	2.28 ± 0.10 (3)
2d	F	F	H	0.93 ± 0.02 (3)	2.31 ± 0.06 (3)
2e	F	F	F	0.58 ± 0.08 (3)	1.38 ± 0.19 (3)
3a	H	H	H	4.62 ± 0.24 (3)	10.79 ± 0.56 (3)
3b	Cl	H	H	3.87 ± 0.12 (3)	8.36 ± 0.25 (3)
3c	H	Cl	Cl	4.04 ± 0.08 (3)	8.12 ± 0.15 (3)
3d	F	H	H	1.77 ± 0.12 (3)	3.97 ± 0.26 (3)

IC_{50}: inhibitory concentration 50%; 3D7: *Plasmodium falciparum* 3D7; SD: standard deviation.

Table 3
Cytotoxicity on MRC-5_{SV2} cells; antiprotozoal activities on *Trypanosoma cruzi*, *Trypanosoma brucei brucei* and *Plasmodium falciparum* K1.

Compd	MRC-5_{SV2} CC_{50} (μM)	*T.cruz* IC_{50} (μM)	*T.bruc* IC_{50} (μM)	*Pf*-K1 IC_{50} (μM)
1	>64	31.70	32.23	0.43
2a	>64	30.48	33.45	0.45
2b	>64	31.08	28.15	< 0.25
2c	>64	32.29	28.43	0.31
2d	>64	32.59	29.52	0.33
2e	>64	33.19	28.71	0.37
3a	>64	33.85	26.49	< 0.25
3b	32	32.30	30.75	0.47
3c	>64	44.95	26.32	< 0.25
3d	>64	30.15	24.44	< 0.25

MRC-5_{SV2}: human lung fibroblast cell line; *T.cruz*: *Trypanosoma cruzi*; *T.bruc*: *Trypanosoma brucei brucei*; *Pf*-K1: *Plasmodium falciparum* K1; CC_{50}: cytotoxicity concentration 50 %; IC_{50}: inhibitory concentration 50 %.

Table 4
Cytotoxicity and antiprotozoal activities of reference compounds.

Target cell	Reference	IC_{50} (μM)
MRC-5	Tamoxifen	11.02
T.cruzi	Benznidazole	1.78
T.brucei	Suramin	0.05
Pf-K1	Chloroquine	0.18

apparatus. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers (400 MHz for ^{1}H, 75 MHz for ^{13}C; 500 MHz for ^{1}H, 125 MHz for ^{13}C). Deuterated dimethylsulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) were used as solvents respectively with tetramethylsilane (TMS) as internal reference. Chemical shifts were reported in parts per million (ppm). Multiplicity was reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br (broad singlet). The coupling constants were reported in hertz (Hz).

For bioassays, the reagents and materials used were those available in the department: RPMI-1640; HEPES; Penicillin; Streptomycin; Hypoxanthine; Plasma (O+ or A+); Human erythrocytes (O+ or A+); DMSO; Sodium bicarbonate; MalstatTM; PES; NBT; Chloroquine diphosphate; MEM; L-glutamine; FCS; Resazurin; Tamoxifen; Giemsa; Miltefosine; Suramin; ß-galactosidase; Chlorophenol red ß-D-galactopyranoside; Benznidazol; *Plasmodium falciparum* strains K1 and 3D7; MRC-5_{SV2} GraphPad Software cells; spectrophotometer; 96-well Greiner boxes; GENios Tecan fluorometer.

4.2. Synthesis

4.2.1. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl acetate (quinine acetate ester) (2a)

In a 25 mL flask, the suspension of quinine (650 mg, 2 mmol) in acetic anhydride (3.5 mL) was stirred for three hours at room temperature. The progress of the reaction was monitored by thin-layer chromatography [mobile phase: ethyl acetate (20 mL), methanol (5 mL), trimethylamine (20 drops)]. After this time, the reaction mixture was cooled on an ice bath and basified by adding a cold 1 M ammonia solution (20 mL) in small portions. The resulting precipitate was immediately collected by filtration, washed with a 1 M sodium chloride solution, then with water, and dried. White solid (640 mg, 87 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.43 (m, 2H), 1.67 (m, 1H), 1.77 (d, J = 4.6 Hz, 1H), 1.91 (m, 1H), 2.09 (s, 3H), 2.21 (m, 1H), 2.48 (m, 2H), 2.83 (m, 1H), 3.08 (m, 1H), 3.35 (m, 1H), 3.93 (s, 3H), 5.00 (m, 2H), 5.94 (m, 1H); 6.32 (m. 1H); 7.43 (m, 1H), 7.49 (m, 2H), 7.97 (d, J = 4.6 Hz, 1H), 8.71 (d, J = 9.3 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 21.18, 25.34, 27.59, 27.77, 39.77, 40.18, 42.09, 56.03, 59.72, 73.70, 102.55, 114.75, 119.67, 121.30, 127.39, 131.78, 142.82, 144.44, 144.99, 148.02, 157.67, 170.40.

4.2.2. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl chloroacetate (quinine chloroacetate ester) (2b)

The title compound was obtained as described for **2a** starting from quinine (650 mg, 2 mmol) and chloroacetic anhydride (3.5 mL) instead of acetic anhydride. White solid (640 mg, 87 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.46 (s, 2H), 1.70 (s, 1H), 1.79 (s, 1H), 1.91 (s, 2H), 2.22 (s, 1H), 2.82–3.11 (m, 4H), 3.94 (s, 3H), 4.52 (m, 2H), 4.99 (d, J = 10.4 Hz, 1H), 5.03 (d, J = 17.2 Hz, 1H), 5.91 (m, 1H), 6.37 (s, 1H), 7.44 (m, 1H), 7.51 (d, J = 4.5 Hz, 2H), 7.96 (d, J = 9.2 Hz, 1H), 8.71 (d, J = 4.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 21.86, 24.46, 25.94, 27.49, 37.22, 41.53, 54.94, 56.86, 58.80, 59.43, 62.47, 73.03, 101.86, 116.83, 119.33, 122.36, 125.47, 132.03, 137.58, 138.27, 144.41, 147.97.

4.2.3. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl fluoroacetate (quinine fluoroacetate ester) (2c)

In a 25 mL flask were introduced quinine (650 mg, 2 mmol), dichloromethane (3 mL) and fluoroacetyl chloride (280 mg, 3 mmol). The reaction mixture was stirred at room temperature for three hours. The progress of the reaction was monitored by thin-layer chromatography [mobile phase: ethyl acetate (20 mL), methanol (5 mL), trimethylamine (20 drops]. After this time, the reaction mixture was cooled on an ice bath, supplemented with cold water (3 mL) and extracted thrice with dichloromethane (3 × 3 mL). The combined organic layers were dried over anhydrous magnesium sulphate and filtered. The filtrate was concentrated by distillation under reduced pressure. The product obtained was dissolved in a small volume of dichloromethane and then supplemented with n-hexane to precipitate the title compound, which was collected by filtration, washed with n-hexane and dried. White solid (650 mg, 81 %); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.68 (t, J = 12.0 Hz, 1H), 1.89 (d, J = 21.8 Hz, 1H), 2.03 (d, J = 21.9 Hz, 3H), 2.69 (s, 1H), 3.15 (m, 2H), 3.49 (m, 1H), 3.57 (d, J = 11.0 Hz 1H), 3.90 (s, 1H), 4.05 (s, 3H), 4.90 (d, J = 46.9 Hz, 1H), 5.01 (d, J = 10.4 Hz, 1H), 5.11 (d, J = 17.2 Hz, 1H), 5.31 (s, 1H), 5.40 (s, 1H), 5.85 (ddd, J = 17.5 Hz, 10.3 Hz, 7.5 Hz, 1H), 7.50 (dd, J = 9.2 Hz, 2.6 Hz, 1H), 7.52 (d, J = 4.6 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 9.2 Hz, 1H), 8.73 (d, J = 4.5 Hz, 1H), 12.04 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 24.29, 27.17, 37.36, 43.11, 53.78, 57.32, 57.63, 77.31, 77.90, 78.70, 79.30, 102.65, 116.88, 118.57, 122.70, 125.90, 131.92, 139.42, 144.40, 147.80, 158.61, 167.69.

4.2.4. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl difluoroacetate (quinine difluoroacetate ester) (2d)

The solution of quinine (325 mg, 1 mmol) in dichloromethane (5 mL) in a 10 mL flask was supplemented with difluoroacetic anhydride (1 mL, 8 mmol) in small portions with stirring. The reaction mixture was stirred at room temperature for six hours. The evolution of the reaction was followed by thin-layer chromatography. After this time, the reaction mixture was cooled on an ice bath, supplemented with cold water (5 mL) and extracted thrice with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulphate and filtered. The filtrate was concentrated by distillation under reduced pressure. The product obtained was dissolved in a small volume of dichloromethane and then supplemented with n-hexane to precipitate the title compound, which was collected by filtration, washed with n-hexane and dried. White solid (310 mg, 77 %): ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.25 (m, 2H), 1.58 (m, 1H), 1.68 (m, 1H), 1.77 (m, 1H), 1.91 (m, 1H), 2.35 (s, 1H), 2.43 (m, 1H), 2.65 (s, 1H), 3.25 (m, 1H), 3.95 (s, 3H), 4.98 (d, J = 10.4 Hz, 1H), 5.06 (d, J = 17.1 Hz, 1H), 5.84 (ddd, J = 17.5 Hz, 10.3 Hz, 7.5 Hz, 1H), 6.72 (t, J = 55.2 Hz, 1H), 7.43 (s, 2H), 7.45 (s, 1H), 7.59 (m, 1H), 7.97 (d, J = 9.4 Hz, 1H), 8.73 (d, J = 4.2 Hz, 1H), 9.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 27.30, 31.43, 56.25, 102.52, 107.43, 109.35, 111.28, 119.62, 119.66, 121.65, 131.82, 144.28, 148.01, 188.11.

4.2.5. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl trifluoroacetate (quinine trifluoroacetate ester) (2e)

The title compound was obtained as described for **2a** starting from quinine (650 mg, 2 mmol) and trifluoroacetic anhydride (4.5 mL) instead of acetic anhydride. White solid (725 mg, 91 %); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.47 (m, 1H), 1.86 (m, IH), 2.05 (m, 3H), 2.73 (d, J = 7.7 Hz, 1H), 3.27 (tt, J = 13.1 Hz, 5.8 Hz, 2H)), 3.62 (m, 3H), 3.84–3.91 (s, *3*H), 5.00 (d, J = 10.4 Hz, 1H), 5.11 (d, J = 17.2 Hz, 1H), 5.82 (ddd, J = 17.4 Hz, 10.4 Hz, 7.2 Hz, 1H), 5.88 (s, IH), 7.41 (d, J = 2.6 Hz, 1H), 7.52 (dd, J = 9.3 Hz, 2.6 Hz, 1H), 7.70 (d, J = 4.6 Hz, IH), 8.03 (d, J = 9.2 Hz, 1H), 8.81 (d, J = 4.6 Hz, 1H), 10.33 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 17.88, 23.73, 26.43, 36.73, 43.04, 52.98, 56.01, 59.15, 66.22, 102.27, 116.84, 119.73, 122.44, 126.12, 131.15, 139.24, 143.16, 146.34, 147.42, 158.38.

4.2.6. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl benzoate (quinine benzoate ester) (3a)

To the solution of quinine (650 mg, 2 mmol) and triethylamine (0.35 mL, 2.5 mmol) in dichloromethane (10 mL) was added dropwise while stirring benzoyl chloride (0.3 mL, 363 mg, 2.6 mmol). The reaction mixture was refluxed under magnetic stirring for 24 h. The medium was supplemented with cold water (4 mL) and stirred for an hour, then supplemented with an aqueous 2 M K_2CO_3 solution (10 mL). The reaction mixture was extracted thrice with dichloromethane (3 × 10 mL) and the combined organic layers were washed with aqueous 2 M HCl (10 mL) and water (20 mL) respectively. The organic extract was dried over anhydrous magnesium sulphate, filtered, and the filtrate was concentrated by distillation under reduced pressure. The product obtained was dissolved in a small volume of dichloromethane and then supplemented with n-hexane to precipitate the title compound, which was collected by filtration, washed with n-hexane and dried. A purification on silicagel column using as eluent a 8:2 mixture of methanol–water, followed by methanol, provided the pure title compound. White solid (754 mg, 88 %); ^{1}H NMR ($CDCI_3$, 300 MHz) δ 1.69–2.00 (m, 5H), 2.42 (m, IH), 2.82 (m, 2H), 3.19–3.40 (m, 2H), 3.49–3.56 (q, J = 7.2 Hz, IH), 4.00 (s, 3H), 5.04 (m, 2H), 5.82 (m, IH), 6.97 (d, J = 7.2 Hz, IH), 7.40 à 7.65 (m, 6H), 8.01–8.13 (m, 3H), 8.73 (d, IH); ^{13}C NMR ($CDCI_3$, 75 MHz) δ 23.1, 27.2, 27.5, 39.0, 42.5, 56.0, 56.1, 59.0, 73.5, 101.2, 115.2, 117.2, 122.3, 126.6, 127.9, 128.7, 129.6, 129.6, 131.6, 131.6, 131.8 133.6, 140.6, 144.7, 147.2, 158.3, 165.1, 200.2.

4.2.7. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl 2-chlorobenzoate (quinine 2-chlorobenzoate ester) (3b)

The title compound was obtained as described for **3a** starting from quinine (650 mg, 2 mmol) and 2-chlorobenzoyl chloride (438 mg, 2.5 mmol) instead of benzoyl chloride. White solid (786 mg, 85 %); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.46 (dt, J = 17.9 Hz, 7.5 Hz, 1H), 1.56 (dd, J = 13.2 Hz, 7.7 Hz, IH), 1.72 (m, 1H), 1.80 (s, 1H), 2,05 (ddt, J = 9.8 Hz, 6.1 Hz, 3.2 Hz, 1H), 2.23 (s, 1H), 2.44 (m, 1H), 2.84 (dd, J = 13.6 Hz, 10.0 Hz, 1H), 3.19 (m, 1H), 3.49 (q, J = 8.7 Hz, 2H), 3.93 (s, 3H), 5.02 (m, 2H), 5.98 (ddd, J = 17.6 Hz, 10.3 Hz, 7.6 Hz, 1H), 6.59 (d, J = 9.0 Hz, IH), 7.45 (dd, J = 9.2 Hz, 2.7 Hz, 1H), 7.51 (m, 1H), 7.61 (m, 4H), 7.90 (dd, J = 7.8 Hz, 1.3 Hz, 1H), 7.97 (d, J = 9.2 Hz, 1H), 8.73 (d, J = 4.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 25.93, 27.57, 27.70, 42.08, 56.07, 56.15, 60.27, 75.26, 102.65, 114.85, 119.66, 121.98, 127.57, 128.12, 129.68, 130.92, 131.53, 131.72, 131.78, 132.54, 134.17, 142.88, 144.42, 144.92, 148.08, 157.76, 167.66.

4.2.8. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl 3,4-dichlorobenzoate (quinine 3,4-dichlorobenzoate ester) (3c)

The title compound was obtained as described for **3a** starting from quinine (650 mg, 2 mmol) and 3,4-dichlorobenzoyl chloride (529 mg, 2.5 mmol) instead of benzoyl chloride. White solid (898 mg, 90 %); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.46 (m, 1H), 1.51 (dt, J = 14.2 Hz, 6.9 Hz, IH), 1.69 (m, 1H), 1.79 (s, 1H), 2.08 (td, J = 9.8 Hz, 3.2 Hz, 1H)), 2.23 (s, 1H), 2.41 (d, J = 14.1 Hz, 1H), 2.84 (dd, J = 13.6 Hz, 10.0 Hz, 1H), 3.21 (m, 1H), 3.54 (q, J = 8.7 Hz, 2H), 3.95 (s, 3H), 5.04 (m, 2H), 6.03 (ddd, J = 17.8 Hz, 10.3 Hz, 7.8 Hz, 1H), 6.53 (d, J = 8.9 Hz, IH), 7.44 (dd, J = 9.2 Hz, 2.7 Hz, 1H), 7.61 (d, J = 2.7 Hz, 1H), 7.63 (d, J = 4.6 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 8.00 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 8.21 (d, J = 2.0 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 25.75, 27.62, 27.81, 42.05, 56.15, 56.31, 60.27, 75.28, 102.59, 114.84, 119.76, 121.96, 127.40, 128.43, 129.87, 130.11, 131.45, 131.79, 131.91, 132.43, 137.31, 143.04, 144.40, 144.90, 148.07, 157.75, 163.80.

4.2.9. Synthesis of (R)-(6-methoxyquinolin-4-yl)-(-5-vinyl-1-azabicyclo [2.2.2]octyl-2-yl)-methyl 2-fluorobenzoate (quinine 2-fluorobenzoate ester) (3d)

The title compound was obtained as described for **3a** starting from quinine (650 mg, 2 mmol) and 2-fluorobenzoyl chloride (396 mg, 2.5 mmol) instead of benzoyl chloride. White solid (790 mg, 88 %); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.47 (dt, J = 13.0 Hz, 6.2 Hz, 1H), 1.53 (dd, J = 13.3 Hz, 7.7 Hz, IH), 1.71 (m, 1H), 1.79 (s, 1H), 2.09 (td, J = 9.8 Hz, 3.2 Hz, 1H)), 2.23 (s, 1H), 2.43 (m, 1H), 2.85 (dd, J = 13.6 Hz, 10.0 Hz, 1H), 3.17 (m, 1H), 3.53 (q, J = 8.6 Hz, 2H), 3.94 (s, 3H), 5.02 (m, 2H), 6.03 (ddd, J = 17.6 Hz, 10.3 Hz, 7.8 Hz, 1H), 6.53 (d, J = 8.9 Hz, IH), 7.44 (dd, J = 9.2 Hz, 2.7 Hz, 1H), 7.57 (td, J = 8.4 Hz, 2.3 Hz, 1H), 7.62 (m, 3H), 7.81 (dt, J = 9.4 Hz, 2.0 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 25.73, 27.63, 27.82, 42.07, 56.14, 56.32, 60.33, 102.60, 114.8, 116.38, 119.66, 121.25, 121.42, 121.95, 126.02, 127.40, 131.72, 131.79, 131.94, 143.04, 144.41, 145.07, 148.07, 157.74, 161.56, 163.51, 164.48.

4.3. Biological tests

4.3.1. Evaluation of the antiplasmodial activity

The compounds were tested against the chloroquine-resistant and pyrimethamine-resistant 3D7 and K1 strains of *P. falciparum*. The parasite was maintained in continuous log phase growth in RPMI-1640 medium supplemented with 2 % penicillin/ streptomycin solution, 0.37 mM hypoxanthine, 25 mM HEPES, 25 mM $NaHCO_3$ and 10 % O^+ human serum together with 4 % human O^+ erythrocytes according to the method of Trager and Jensen [25]. All cultures and assays were conducted at 37 °C under micro-aerophilic atmosphere (4 % CO_2, 3 % O_2 and 93 % N_2). The *in vitro* antimalarial activity was assessed using an adaptation of the procedure described by Makler et al. as the parasite lactate dehydrogenase assay [27]. Twenty milligrams of each compound were dissolved in 1 mL DMSO and serially diluted two-fold with culture medium before being added to asynchronous parasite cultures. Assays were performed in 96-well tissue culture plates, each well containing 10 μL of the substance dilutions together with 190 μL of the parasite inoculum (1 % parasitaemia, 2 % haematocrit). After 72 h of incubation at 37 °C, plates were stored at −20 °C until further processing. After thawing 20 μL of haemolysed parasite suspension from each well was transferred into another plate together with 100 μL Malstat™ reagent and 10 μL of a 1/1 mixture of PES (phenazine ethosulphate, 2 mg/ mL) and NBT (Nitro Blue Tetrazolium Grade III, 0.1 mg/ mL). The plates were kept in the dark for 2 h and change in color was measured spectrophotometrically at 655 nm. The results were expressed as percentage reduction in parasitaemia compared to control wells. The concentration causing 50 % inhibition of parasite growth (IC_{50}) was calculated from the drug concentration–response curves [26]. Chloroquine diphosphate was used as an antiplasmodial reference drug.

4.3.2. Evaluation of the cytotoxicity against MRC-5_{SV2} cells

Cytotoxicity testing was included in parallel to above antiprotozoal assays for assessing their selectivity. Human lung fibroblast MRC-5_{SV2}

cells were selected because of their sensitivity and receptiveness for several viruses and parasites [32]. They were cultured in MEM, supplemented with 20 mM L-glutamine, 16.5 mM sodium hydrogen carbonate and FCS (5 %). Assays were performed in sterile 96-well tissue culture plates (Greiner) after addition of 190 µL of cell suspension (3×10^4 cells) to each well already containing 10 µL of the drug dilutions. After 3 days at 37 °C under an atmosphere of 5 % CO_2, overall cell viability was assessed fluorimetrically (λ_{ex} 550 nm, λ_{em} 590 nm) after addition of resazurin [28]. The IC_{50} values of tested drugs were determined based on the percent absorbance in the treated cultures compared to the untreated control cultures. Tamoxifen was included as reference compound for cytotoxicity.

4.3.3. Evaluation of the antitrypanosomial activity

4.3.3.1. Trypanosoma cruzi. The Tulahuen CL2 strain (nifurtimox-sensitive) was maintained on MRC-5_{SV2} cells in minimal essential medium (MEM) supplemented with 20 mM L-glutamine, 16.5 mM sodium hydrogen carbonate and FCS (5 %) at 37 °C under an atmosphere of 5 % CO_2. To determine *in vitro* antitrypanosomial activity, 4×10^3 MRC-5_{SV2} cells and 4×10^4 parasites were added to each well of the test plate. After incubation at 37 °C for 7 days, parasite growth was assessed by adding the ß-galactosidase substrate, chlorophenol red ß-D-galactopyranoside, for 4 h at 37 °C [33]. The color reaction was read at 540 nm and absorbance values were expressed as a percentage of the blank controls. Benznidazol was included as a reference drug.

4.3.3.2. Trypanosoma brucei brucei. Trypomastigotes of *T. b. brucei* Squib-427 strain (suramin-sensitive) were cultured at 37 °C under an atmosphere of 5 % CO_2 in Hirumi-9 medium, supplemented with 10 % fetal calf serum (FCS) [34]. Assays were performed by adding 1.5×10^4 trypomastigotes/well. After 72 h incubation, parasite growth was assessed fluorimetrically by adding resazurin for 24 h at 37 °C [35]. Fluorescence was measured using a GENios Tecan fluorimeter (λ_{ex} 530 nm, λ_{em} 590 nm). Suramin was included as reference drug.

CRediT authorship contribution statement. **Jean-Gonfi Mvondo Mbala:** Methodology, Investigation, Conceptualization. **Dani Thierry Mawete:** Validation, Writing – review & editing. **Alain Nanikafuako Makiese:** Supervision, Conceptualization. **Prosper Lwanzo Kwiraviwe:** Supervision, Conceptualization. **Bibiche Kuyubuka Pangu:** Resources. **Eric Buini Nguimi:** Supervision, Conceptualization. **Richard Cimanga Kanyanga:** Methodology, Investigation. **Luc Pieters:** . **Gilles Degotte:** . **Michel Frederich:** . **Aristote Matondo:** . **Natascha Van Pelt:** Methodology, Investigation. **Guy Caljon:** Supervision, Methodology, Investigation. **Bernard Pirotte:** Writing – review & editing, Writing – original draft, Supervision, Resources. **Blaise Mbala Mavinga:** Supervision, Resources, Project administration. **Sylvie-Mireille Bambi-Nyanguile:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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