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Synthesis and anti-malaria activity of molecules carrying the piperidine ring

Aminata Thiam,[a] Rokhyatou Seck,[a] Moussa Touré,*[a] Oumar Sambou,[a] Emma Diatta,[a] Siga Sagne,[a] Christian Cavé,[b] Sandrine Cojean,[c] Michael Rivard,[d] and Abdoulaye Gassama*[a]

a Laboratoire de Chimie et Physique des Matériaux (LCPM), UFR Sciences et Technologies, Université Assane Seck de Ziguinchor, BP 523, Ziguinchor, Sénégal

b Chimiotherapie Antiparasitaire, UMR 8076 CNRS BioCIS, Université Paris Saclay, Faculté de pharmacie, Bâtiment Henri Moissan 17, avenue des sciences, 91400 Orsay, France.

c Centre National de Référence du Paludisme, Hôpital Bichat-Claude Bernard, PHP, Paris, France.

d Université Paris Est Créteil, CNRS, ICMPE, UMR 7182, 2 rue Henri Dunant, 94320 Thiais, France.

Email: agassama@univ-ziq.sn, m.t9@ziq.univ.sn

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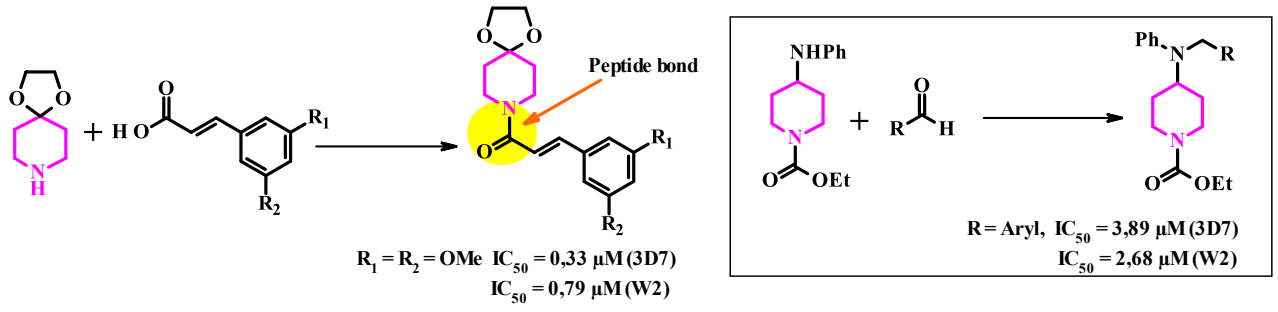
Abstract

A series of small molecules derived from ethyl-4-oxo-1-piperidine carboxyl and 1,4-dioxa-8-azopiri [4,5] decane were synthesized and tested for their antimalarial activity. These molecules were evaluated against two strains of P. *falciparum*, one chloroquine-sensitive (3D7) and the other chloroquine-resistant (W2), as well as for their toxicity on human cells. Compounds **11a**, **6a**, and **6c** showed good activity against the sensitive strain, while **11a**, **6c**, and **8** were particularly effective against the resistant strain, with efficacy comparable to atovaquone. Compound 11a stood out with a very high selectivity index, exceeding that of atovaquone for both strains.



Keywords: Amide coupling, reductive amination, piperidine derivatives, antimalarial, *P. falciparum*.

Introduction

Malaria, one of the world's leading causes of death, is a transmissible, chronic and costly disease that is widespread in tropical and subtropical regions of the southern hemisphere. Malaria is one of the few public health scourges to have survived the centuries without losing its activity. It is one of the most deadly diseases, with almost a third of the world's population at risk. Its morbidity and mortality make it a major public health problem.[1,2] Malaria remains the most common parasitic disease in the world today,[3] particularly in sub-Saharan Africa. It is caused by *plasmodium* haematozoa, transmitted to humans by the infecting bite of a female mosquito of the genus Anopheles.[4]

According to a WHO report,[2,5] 241 million cases of malaria were recorded in 2020, compared with 227 million in 2019. The estimated number of deaths due to malaria was 627,000 in 2020,[6] an increase of 69,000 deaths on the previous year. Most of this increase occurred in countries in the African region. Around two-thirds of these deaths (47,000 deaths) are due to the disruptions observed during the COVID-19 pandemic, with the remaining third (22,000 deaths) reflecting the recent change in the method used by WHO to calculate malaria mortality, independently of these disruptions. Resistance to current antimalarial drugs is the main obstacle to reducing mortality caused by *Plasmodium falciparum* infection.

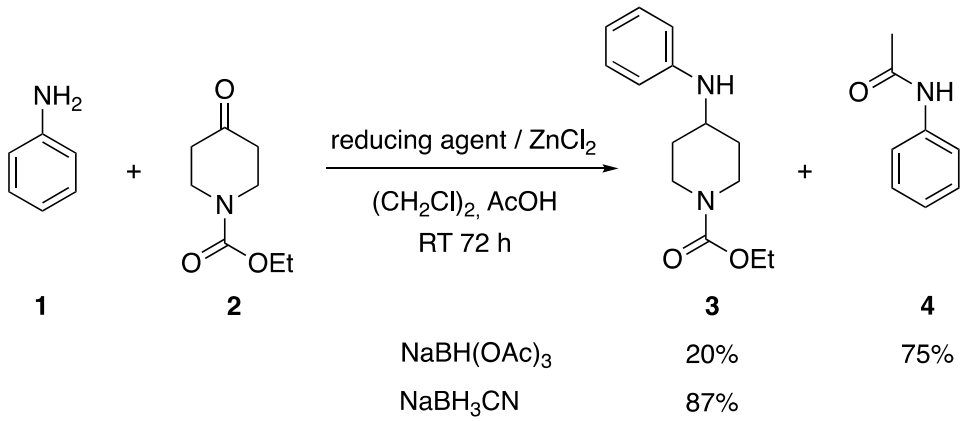
The piperidine motif is one of the most commonly encountered within biologically important natural compounds or synthetic pharmaceutical drugs.[7–16] Compounds bearing the piperidine moiety exhibit a wide range of biological properties, including antihypertensive,[17] antibacterial,[18] antimalarial,[19,20] anti-inflammatory,[21] analgesic,[22] antioxidant,[23] and antiproliferative.[24]

This article reports on the synthesis of new molecules bearing a piperidine ring. It describes the study of their antimalarial activity on chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of *P. falciparum*, as well as their cytotoxic activity on HUVEC cells. In this study, we conducted *in silico* prediction of ADME properties using computational modeling tools.

Results and Discussion

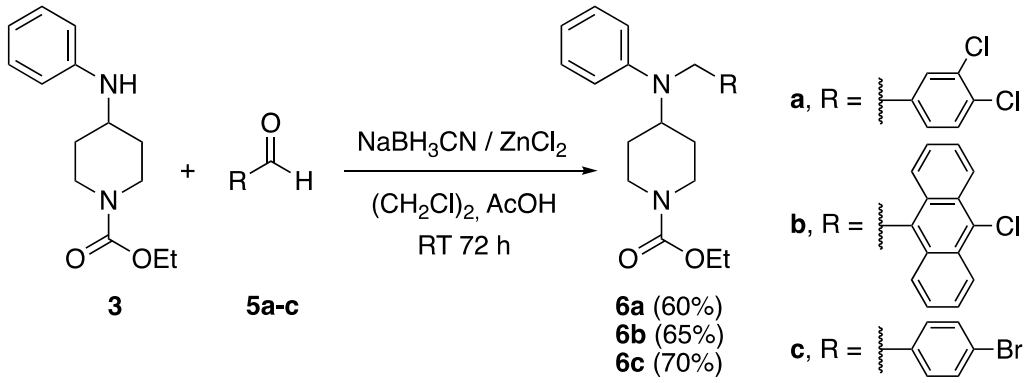
Synthesis of piperidine derivatives

Chemistry: A first series of compound was prepared by reductive amination. In the presence of $NaBH(OAc)_3$ used as reducing agent and $ZnCl_2$, the reaction between aniline **1** and piperidone **2** in dichloroethane yielded compounds **3** and **4**, respectively isolated in 20% and 75% yield (Scheme 1).[25] The unexpected formation of acetanilide **4** was attributed to the reaction of the aniline **1** with acetic acid from $NaBH(OAc)_3$. To avoid the formation of acetanilide **4**, $NaBH_3CN$ was used as the reducing agent. Under these conditions, the expected secondary amine **3** was isolated in 87% yield (Scheme 1).



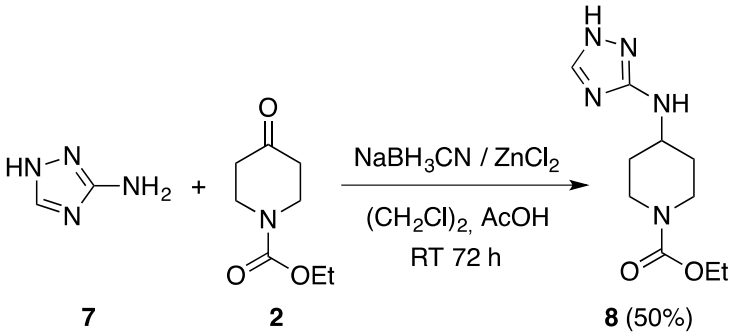
Scheme 1. Preparation of the secondary amine **3.**

Under similar conditions, secondary amine **3** was reacted with aldehydes **5a-c**. Corresponding tertiary amines **6a-c** were obtained in 60-70% yield (Scheme 2).



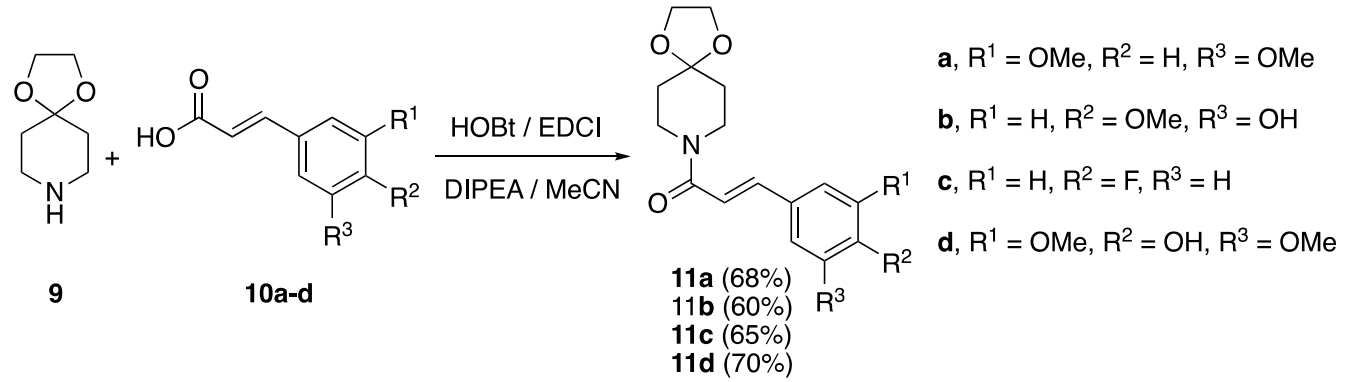
Scheme 2. Preparation of the tertiary amines **6a-c.**

The reductive amination between piperidone **2** and triazole **7** gave the secondary amine **8** in 50% yield (Scheme 3).



Scheme 3. Preparation of the secondary amine **8.**

A second series of compounds was prepared by amide coupling. Reaction between acetal-protected piperidone **9** and cinnamic acid derivatives **10a-d** in the presence of HOBt/EDCI/DIPEA[26,27] in acetonitrile provided amides **11a-d** with good yields (Scheme 4).



Scheme 4. Preparation of amides **11.**

Antimalarial activity: Studies have shown that compounds with piperidine rings have good selectivity and activity against the P. *falciparum strain*.[20,28–30] This prompted us to assess their antiplasmodial activity against the chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of P. *falciparum* as well as their cytotoxic activity against HUVEC cells (Table 1).

The compounds exhibited micromolar activities against both parasite strains. Their cytotoxicity against HUVEC ranged from CC_{50} 15.6 ± 0.01 to >100 µM, resulting in varied selectivity indices (SI), 155.45 in the 3D7 strain and 64.94 in the W2 strain for **11a**. Compared to atovaquone (IC_{50} = 0.083 µM (3D7) and 0.07 µM (W2)) compounds **11a** (IC_{50} = 0.33 µM), **6a** (IC_{50} = 1.9 µM), and **6c** (IC_{50} = 2.43 µM) showed strong activity against 3D7. Molecules **11a** (IC_{50} = 0.79 µM), **6c** (IC_{50} = 2.58 µM)), and **8** (IC_{50} = 2.43 µM) had the highest activity against W2. It is interesting to note that compound **3** exhibits low activity against both strains. However, pharmacomodulation at the nitrogen atom of the compound led to a change in this activity. Indeed, the benzyl derivatives (**6a** and **6c**) show significant activity against both strains, whereas the anthracene derivative (**6b**) loses this activity (Table 1).

By comparing the activity of compounds **6a** and **6c**, two interesting results emerge. When the benzylic core is substituted at the 3 and 4 positions with chlorine, compound **6a** exhibits higher activity than compound **6c** against the 3D7 strain. Conversely, compound **6c**, whose core is substituted at the 4 position with bromine, shows higher activity against the chloroquine-resistant W2 strain (Table 1). These observations suggest that the presence of halogens (chlorine and/or bromine) plays a determining role in the activity of the compounds.

Compound **11a** presented a very high selectivity index (SI = 155.45 (3D7) and 64.94 (W2)) compared to atovaquone (SI = 12 (3D7) and 13 (W2)) i.e. 13 times more selective for 3D7 strain and 5 times the W2 strain. In the series of cinnamic-derived compounds, the arrangement of the methoxy groups in the 3,5 positions and the hydrogen in the 4 position is crucial for the activity of its molecules against the two strains.

Indeed, since compound **11a** shows the best results, our SAR analysis will use it as a reference to compare compounds **11b**, **11c**, and **11d**. The substitution at position 4 by a hydroxy group, while keeping positions 3 and 5 unchanged on the phenyl ring (compound **11d**), leads to a decrease in activity on both strains, with a difference of 9.06 µM (3D7) and 22.82 µM (W2). Similar results are observed for compound **11c**, where the phenyl at position 4 is substituted by a fluorine, with a difference of 28.08 µM (3D7) and 14.64 µM (W2). Finally, the substitution at position 3 by a hydroxy group and at position 4 by a methoxy group on the phenyl

ring (compound **11b**) causes a significant decrease in activity, with differences of 54.32 μM (3D7) and 40.42 μM (W2) (Table 1).

For further development, all products whose selectivity index is less than 10 will not be retained. The compounds potentially selected will be those which have an activity <10 nM and an index >10. Thus, in perspective compounds **11a**, **8**, and **6a** will be in order to improve their activity by working on a modification on the nitrogen volume in position 4.

Table 1. Antimalarial activity of piperidine derivatives

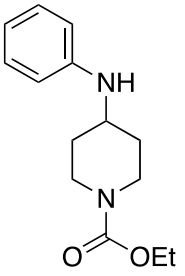
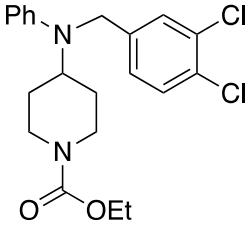
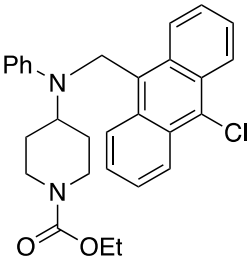
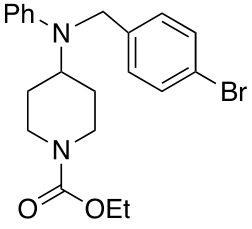
	P. falciparum 3D7 Strain	*P. falciparum* W2 Strain	HUVEC Cells	Selectivity Index (3D7)	Selectivity Index (W2)
Compounds	IC_{50} ±SD (μM)	IC_{50} ±SD (μM)	CC_{50}±SD (μM)	CC_{50}/IC_{50}	CC_{50}/IC_{50}
 **3**	77.09 ± 8.41	84.26 ± 2.18	40.3 ± 0.77	0.52	0.48
 **6a**	1.90 ± 0.79	13.93 ± 2.18	49.2 ± 0.77	25.89	3.53
 **6b**	>100	>100	27.6 ± 0.44	0.28	0.28
 **6c**	3.89 ± 1.36	2.58 ± 0.56	15.6 ± 0.01	4	6.04

Table 1. Continued

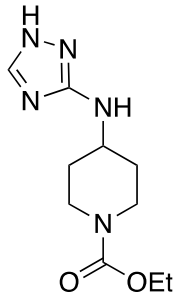
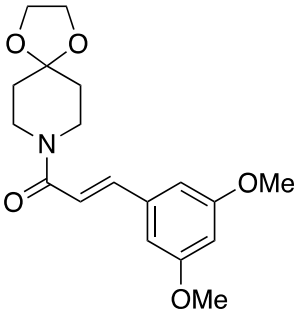
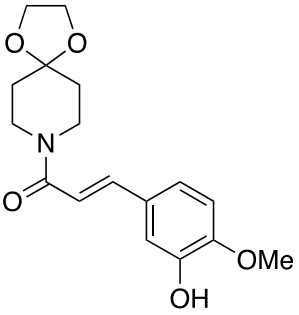
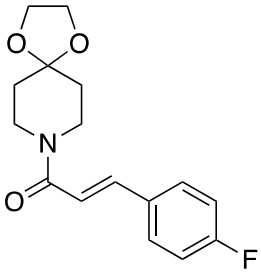
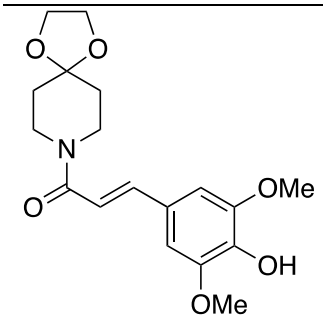
	P. falciparum 3D7 Strain	*P. falciparum* W2 Strain	HUVEC Cells	Selectivity Index (3D7)	Selectivity Index (W2)
Compounds	IC_{50} ±SD (μM)	IC_{50} ±SD (μM)	CC_{50}±SD (μM)	CC_{50}/IC_{50}	CC_{50}/IC_{50}
 **8**	16.99 ± 0.32	2.43 ± 0.91	58.6 ± 0.25	3.45	24.43
 **11a**	0.33 ± 0.11	0.79 ± 0.22	51.3 ± 0.47	155.45	64.94
 **11b**	54.65 ± 2.56	41.21 ± 0.66	51.8 ± 0.88	0.95	1.26
 **11c**	28.41 ± 3.04	15.43 ± 1.20	38.8 ± 0.64	1.37	2.51

Table 1. Continued

	P. falciparum 3D7 Strain	*P. falciparum* W2 Strain	HUVEC Cells	Selectivity Index (3D7)	Selectivity Index (W2)
Compounds	IC_{50} ±SD (μM)	IC_{50} ±SD (μM)	CC_{50}±SD (μM)	CC_{50}/IC_{50}	CC_{50}/IC_{50}
 **11d**	9.39 ± 2.73	22.82 ± 1.25	36.4 ± 0.23	3.88	1.59
Atovaquone	0.083 ± 0.06	0.076 ± 0.05	> 1	12	13

Analysis of SwissADME results

The discovery and development of new organic compounds with therapeutic potential is based not only on their biological efficacy, but also on their pharmacokinetic properties, commonly known as ADME (Absorption, Distribution, Metabolism, Excretion). These properties determine a compound's ability to be absorbed, distributed in the body, metabolised and finally eliminated. An optimal oral drug would be rapidly and completely absorbed by the digestive system. Consequently, it is necessary for drugs to have good oral bioavailability, which implies adequate solubility and essential permeability to anticipate their ability to reach the target at the required concentrations[31,32]. Frequent errors in *in silico* analyses of ADME and unfavourable toxicology in biological systems are the main reasons why most drugs fail in clinical trials[33].

Following the in vitro tests, the compounds with the best IC_{50} and atovaquone, a reference antimalarial, were evaluated according to ADME parameters. To predict the ADME properties of the compounds, we used computer tools such as SwissADME (http://www.swissadme.ch/)[34] and ADMETlab. These tools can be used to estimate key parameters such as solubility, membrane permeability (logP), oral bioavailability and potential for metabolism by cytochrome P450, as well as various pharmacokinetic filters such as those of Lipinski and Ghose[35]. The prediction results are shown in Table 2.

The bioavailability (F) score is a measure used to assess a drug's ability to be absorbed and reach the systemic circulation intact[34]. The predicted (F) score was 55% for all compounds, compared with 85% for the reference compound, atovaquone, which is favourable for oral administration.

Most compounds showed good predicted absorption, with optimal logP values between 1.3 and 5 for membrane permeability. Lipinski's rule of five, a filter characterised by four physicochemical parameters, namely a molecular weight not exceeding 500 g/mol, a lipophilicity less than 5 and a number of hydrogen bond acceptors and donors that should be less than 10 and 5 respectively,[36,37] was met for the majority of compounds with zero violations, indicating a good probability of oral absorption.

Table 2. SwissADME results for piperidine-derived compounds

Cpds ID	Lipinski's rule of five (Ro5)					Veber's Violation	n-ROTB	TPSA (Å²)	F	Log S
	MW (g/mol)	Log P	DLH	ALH	Lipinski's Violation					
Règle	< 500	≤ 5	< 5	< 10	< 2	<=2	< 10	< 140	> 10%	> -5
3	248,32	1.94	1	2	0	0	5	41.57	0,55	-3.26
6a	407.33	4.33	0	2	0	0	7	32.78	0,55	-6.07
6b	473.01	5.11	0	2	1	0	7	32.78	0,55	-7.73
6c	417.34	3.96	0	2	0	0	7	32.78	0,55	-5.48
8	239.27	0.47	2	4	0	0	5	83.14	0,55	-2.07
11a	333.38	1.06	0	5	0	0	5	57.23	0,55	-2.52
11b	319.35	0.83	1	5	0	0	4	68.23	0,55	-2.41
11c	291.32	2.08	0	4	0	0	3	38.77	0,55	-2.30
11d	349.38	0.53	1	6	0	0	5	77.46	0,55	-2.57
ATQ	366.84	3.28	1	3	0	0	2	54.37	0.85	-6.59

TPSA: Topological Polar Surface Area; **HBA:** H-Bond Acceptor; **HBD:** H-Bond Donor; **n-ROTB:** Number of rotatable bonds; **MW:** Molecular weight; **Log P:** logarithm of partition coefficient between n-octanol and water; **F:** Bioavailability Score; **Log S:** Solubility

Predictions indicated that the compounds could distribute well into tissues, with appropriate volumes of distribution. However, some compounds might have an affinity for plasma proteins, which could limit their bioavailability.

Predictions also showed that compounds would be excreted mainly via the kidney, with variable half-lives. Some compounds may require adjustments to reduce their clearance and extend their half-life. The results of the in vitro evaluation and the ADME predictions suggest that several of the compounds synthesised have promising therapeutic potential. However, ADME predictions highlight potential challenges, particularly with regard to metabolism and excretion. This information is crucial for guiding the subsequent stages of compound optimisation.

Predicted solubility ranged from -2.07 (**11d**) to -7.73 (**6b**) depending on the model used, with the best profiles in the **11** series (-2.07 to -2.57).

Simulations revealed that some compounds (**3** and **6a** to **6c**) may be substrates of CYP450[38] enzymes, particularly CYP2C19 and CYP2D6, which may require structural adjustments to improve metabolic stability. However, most of the compounds are not substrates of CYP450 enzymes, which reduces the risk of rapid hepatic metabolism and potential drug interactions, suggesting a significant potential for metabolism. Table 3 include *in silico* predictions of the compounds' potential inhibitory effects on major CYP450 isoforms (CYP 1A2, CYP2C19, CYP2C9, CYP2D, CYP3A4). These data require experimental validation to assess drug interaction risks.

Table 3. Predicted (*in silico*) pharmacokinetic properties of piperidine derivatives

Compounds ID	ABS	BBB	P-Gp	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
3	High	Yes	No	No	Yes	No	Yes	No
6a	High	Yes	No	No	Yes	Yes	Yes	Yes
6b	Low	No	Yes	No	Yes	No	Yes	Yes
6c	High	Yes	No	No	Yes	Yes	Yes	Yes
8	High	No	Yes	No	No	No	No	No
11a	High	Yes	Yes	No	No	No	No	No
11b	High	Yes	Yes	No	No	No	No	No
11c	High	Yes	No	No	No	No	No	No
11d	High	No	yes	No	No	No	No	No
ATQ	High	Yes	No	Yes	Yes	Yes	No	No

ABS: Predicted gastrointestinal absorption; **BBB**: Predicted blood-brain barrier permeability; **P-Gp**: Predicted P-glycoprotein substrate status; **ATQ**: Reference compound

The results indicate that most molecules have a high predicted gastrointestinal absorption, which is a favorable indicator for oral administration.

Variation was observed in P-Glycoprotein (P-Gp) interactions. Compounds **6b**, **8**, **11a**, and **11b** are predicted P-Gp substrates, which could lead to active efflux and reduce their systemic availability. However, other compounds (**3**, **6a**, **6c**, **11c**, and **11d**) are not P-Gp substrates, which is favorable for better bioavailability.

The compounds' ability to cross the Blood-Brain Barrier (BBB) also varies. Compounds **3**, **6a**, **6c**, **11a** to **11c**, and the reference molecule are predicted to cross the BBB, which could be advantageous for therapeutic applications targeting the central nervous system. In contrast, compounds **6b**, **8**, and **11d** do not cross the BBB, which could limit their use in this context. The synthetic accessibility of the molecules varies, indicating different levels of difficulty in synthesizing them.

Conclusions

This article reports the synthesis of new molecules carrying the piperidine ring and study of their antimalarial activity on strain against chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of P. falciparum as well as their cytotoxic activity against HUVEC cells. The antimalarial activity of the compounds has been described. The results of this study showed that compounds have potent antimalarial activity and can therefore serve as potential sources of effective and affordable antimalarial agents. The best result is obtained with compound **11a** against the two strains. We have shown in this study that the presence of the piperidine ring is crucial for the activity of our molecules. Compound **11a** showed better selectivity for both strains compared to the reference molecule atevaquone. The *in silico* ADME profiling demonstrates the pharmacokinetic promise of these piperidine derivatives as drug candidates, showing optimal solubility-permeability balance and high absorption. The variable P-gp substrate activity and BBB penetration profiles indicate the need for application specific optimization.

Experimental Section

General: Commercial reagents were used without purification. Prior to use, CH_3CN, DMSO and Methanol were dried using a pure solvent drying system over aluminum oxide under an argon atmosphere. All anhydrous reactions were carried out under nitrogen atmosphere. Analytical thin layer chromatography was performed on SDS silica gel 60F254 aluminium plates (0.2 mm layer) and was revealed by UVlight and/or by phosphomolybdic acid. All flash chromatography separations were performed with SDS silica gel 60. Melting points (mp) were determined on a Tottoli apparatus. Infrared (IR) spectra were obtained as neat films and were recorded on Bruker Vector 22 spectrophotometer. ^1H and ^{13}C spectra were recorded in CD_3OD or $CDCl_3$ either on a Bruker Avance 300 or 500 MHz and 75 or 125 MHz, respectively. Chemicals shifts (δ) are reported in ppm relative to TMS for 1H and ^{13}C NMR spectra. The following abbreviations are used to indicate the multiplicities: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet). GC/MS conditions: Analyses were performed using a 5890 gas chromatogram connected to a G 1019 A mass spectrometer (both from Hewlett Packard) operating in the electro spray ionization mode (ESI).

General Procedure for the reductive amination A solution of amine (2 equiv), ethyl-oxo-1-piperidonecarboxylate **2** or dérivatives aldehyde **5** (1 equiv), $NaBH_3CN$ (1 equiv) and $ZnCl_2$ (0.5 equiv) in 1,2-dichloroethane (2 mL), was stirred for 72h at rt and under Ar. The reaction mixture was then diluted with AcOEt (5 mL) and washed with distilled water (5 mL). The aqueous phase was extracted with 3 x 5 ml ethyl acetate, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (Cyclohexane / AcOEt).

Ethyl 4-(phenylamino)piperidine-1-carboxylate (**3**) Following the general procedure, aniline **1** (150 mg, 1.613 mmol) reacted with ethyl-oxo-1-piperidonecarboxylate **2** (137.89 mg, 0.806 mmol), $NaBH_3CN$ (1 equiv), and $ZnCl_2$ (0.5 equiv), and in $(CH_2Cl)_2$ (2 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (4:6)).
RMN^{1}H- (500 MHz, $CDCl_3$) δ(ppm): 1.25 (t, 3H, J=6.7H z, CH_3), 1.5 (m, 4H, $2CH_2$), 3.24 (m, 4H, $2CH_2$), 3.4 (s broad, H, NH), 3.6 (m, 1H, CH), 4.46 (q, J=6.7H z, 2H, CH_2), 6.75 (m, 3H, 3CH); 7.2 (m, 2H, 2CH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 13.82 (CH_3), 31.64 ($2CH_2$), 42.28 ($2CH_2$), 49.56 (CH), 61.11 (CH_2), 113.62 (2CH), 117.06 (CH), 123.67 (2CH), 147.66 (C), 156.23 (C=O). MS (ESI) *m/z*: 449.07 $[M+1]^+$.

Ethyl 4-((3,4-dichlorobenzyl)(phenyl)amino)piperidine-1-carboxylate (6a) Following the general procedure, ethyl-4-(aminophenyl) piperidine-1-carboxylate **3** (150 mg, 0.604 mmol) reacted with 3,4 dichlorobenzaldehyde **5a** (52.23 mg, 0.302 mmol), $NaBH_3CN$ (1 equiv), and $ZnCl_2$ (0.5 equiv), and in $(CH_2Cl)_2$ (2 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (4:6)).
^{1}H-NMR- (500 MHz, $CDCl_3$) δ(ppm): 1.25 (t, 3H, J=6.7H z, CH_3), 1.5 (m, 4H, $2CH_2$), 3.76 (m, 4H, $2CH_2$), 4.24 (m, H, CH), 4.46 (q, 2H, J=6.7H z, CH_2), 5.25 (s, 2H, CH_2) 6.75 (m, 5H, 5CH), 7.5 (m, 3H, 3CH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 14.81 CH_3, 22.84 ($2CH_2$), 43.76 ($2CH_2$), 48.95 CH_2, 56.01 CH, 61.6 CH_2, 113.71 (2CH), 117.96 CH, 125.8 CH, 128.33 CH, 129.57 (2CH), 130.59 C, 132.78 C, 141.03 C, 148.57 C, 155.4 C=O. MS (ESI) *m/z*: 407.10 $[M+1]^+$.

Ethyl 4-(((10-chloroanthracen-9-yl)methyl)(phenyl)amino)piperidine-1-carboxylate (6b) Following the general procedure, ethyl-4-(aminophenyl) piperidine-1-carboxylate **3** (150 mg, 0.604 mmol) reacted with 10-chloroanthracene-9-carbaldehyde **5b** (72.48 mg, 0.302 mmol), $NaBH_3CN$ (1 equiv), and $ZnCl_2$ (0.5 equiv), and in $(CH_2Cl)_2$ (2 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (7:3)).
^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 1.25 (t, J=6.7 H z, 3H, CH_3), 1.3 (m, 4H, $2CH_2$), 3.1 (t, 2H, J=7.6H z, CH_2), 3.2(t, J=7.6H z, 2H, CH_2), 3.6 (m, H, CH), 4.25 (q, 2H, J=6.7H z, CH_2), 5.25 (s, 2H, CH_2), 6.6 (m, 4H, 4CH); 6.65 (m, H, CH), 7.24 (m, 4H, 4CH), 7.6 (d, 2H, 2CH), 8.5 (m, H, CH), 8.6 (m, H, CH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm):

14.84 CH_3, 32.40 ($2CH_2$), 42.83 ($2CH_2$), 50.31 CH, 57.50 CH_2, 61.51 CH_2, 113.61 (2CH), 114.3 CH, 117.89 CH, 124.42 (2CH), 125.77 (2CH), 126.69 (2CH), 126.83 (2CH), 128.86 C, 129.53 CH, 130.5 (2C), 130.84 (2C), 140.60 C, 149.6 C, 155.67 C=O. MS (ESI) *m/z*: 473.20 $[M+1]^+$.

Ethyl 4-((4-bromobenzyl)(phenyl)amino)piperidine-1-carboxylate (6c) Following the general procedure, ethyl-4-(aminophenyl)-piperidine-1-carboxylate **3** (150 mg, 0.604 mmol) reacted with 4-bromobenzaldehyde **5c** (55.55 mg, 0.302 mmol), $NaBH_3CN$ (1 equiv), and $ZnCl_2$ (0.5 equiv), and in $(CH_2Cl)_2$ (2 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (9:1) then (7:3).

^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 1.25 (t, J=6.7 H z, 3H, CH_3),1.5 (m, 4H, $2CH_2$), 2(m, 4H, $2CH_2$), 3.5(m, 1H, CH), 4.26 (q, J=6.7 H z, 2H, CH_2), 4.75 (s, 2H, CH_2), 6.70 (m, 5H, 5CH), 7.5 (m, 4H, 4CH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 14.85 CH_3, 32.31 ($2CH_2$), 42.83 ($2CH_2$), 50.31 CH, 52.3 CH_2, 61.53 CH_2, 113.92 C, 114.3 (2CH), 121.9 CH, 128.73 C, 129.56 (2CH), 130.1 (2CH), 131.4 (2CH), 132.77 C, 155.4 (C=O). MS (ESI) *m/z*: 417.12 $[M+1]^+$.

Ethyl 4-((2H-1,2,3-triazol-4-yl)amino)piperidine-1-carboxylate (8) Following the general procedure, 1*H*-1,2,4-triazol-3-amine **7** (150 mg, 1.784 mmol) reacted with ethyl-oxo-1-piperidonecarboxylate **2** (152.61 mg, 0.892 mmol), $NaBH_3CN$ (1 equiv), and $ZnCl_2$ (0.5 equiv), and in $(CH_2Cl)_2$ (2 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (4:4).

^{1}H-RMN ($CDCl_3$, 500 MHz) δ (ppm): 1.26 (t, 3H, J=6.7H z, CH_3), 1.7 (m, 4H, $2CH_2$), 3.24 (m, 4H, $2CH_2$), 3.4 (s broad, H, NH), 3.6 (m, 1H, CH); 4.46 (q, J=6.7H z, 2H, CH_2), 5.1 (s broad, 1H, NH), 7.8 (s,1H, CH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 15.21 CH_3, 23.85 ($2CH_2$), 44.76 ($2CH_2$), 56.01 CH, 62.6 CH_2,123.5 CH, 147.38 C, 155.31 CO. MS (ESI) *m/z*: 240.20 $[M+1]^+$.

General Procedure for the coupling reaction of piperidone 9 with Cinnamic Acid derivatives 10a-d. A solution of cinnamic acid derivative (1 equiv) **10**, piperidone **9** (2 equiv), EDCI.HCl (2.3 equiv), DIPEA (4 equiv), and HOBt.H_2O (2.3 equiv) in MeCN was stirred for 48h at rt and under Ar. The reaction mixture was then diluted with AcOEt (80 ml) and washed with HCl 10% (80 ml), a saturated aqueous solution of $NaHCO_3$ (80 ml), H_2O (80 ml), and brine (80 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (Cyclohexane / AcOEt).

***(E)-3-(3,5-dimethoxyphenyl)-1-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)prop-2-en-1-one* (11a).**

Following the general procedure, cinnamic acid derivative **10a** (200 mg, 0.960 mmol) reacted with piperidone **9** (274.89 mg, 1.92 mmol), EDCI.HCl (2.3 equiv), DIPEA (4 equiv), and HOBt.H_2O (2.3 equiv) in MeCN (5 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (7:3)).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.5 (t, 8H, J=6.7 Hz, $4CH_2$), 3.55 (m, 4H, $2CH_2$), 3.75 (m, 4H, $2CH_2$), 3.82 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 6.80 (d ,1H, J=15.67 Hz, CH), 7.0 - 7.5 (m, 3H, CH), 7.45 (d ,1H, J=15.68 Hz, CH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 64.25 ($2CH_2$), 43.75 ($2CH_2$), 40.56 ($2CH_2$), 56.5 ($2CH_3$), 115.10 CH, 113.35 C, 121.44 CH, 125.98 CH, 128.56 CH, 137.52 C, 143.53 CH, 148.00 C, 148.29 C, 166.00 CO. MS (ESI) *m/z*: 334.08 $[M+1]^+$.

(E)-3-(3-hydroxy-4-methoxyphenyl)-1-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)prop-2-en-1-one (11b). Following the general procedure, cinnamic acid derivative **10b** (200 mg, 1.03 mmol) reacted with piperidone **9** (294.94 mg, 2.06 mmol), EDCI.HCl (2.3 equiv), DIPEA (4 equiv), and HOBt.H_2O (2.3 equiv) in MeCN (5 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (7:3)).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.5 (t, 4H, J= 6.4Hz, $2CH_2$), 3.56 (m, 4H, $2CH_2$), 3.74 (m, 4H, $2CH_2$), 3.81 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.75 (d, J=15.57 Hz, 1H, CH), 7.0 - 7.5 (m, 5H, CHAr), 7.8 (d, J=15.57 Hz, 1H, CH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 64.25 ($2CH_2$), 56.60 CH_3, 44.26 CH_2, 38.96 CH_2, 34.4 ($2CH_2$), 105.6 CH, 115.10 CH, 116.34 C, 121.44 CH, 129,65 CH, 137.52 C, 143.53 CH, 148.09 C, 148.29 C, 166.04 CO. MS (ESI) *m/z*: 320.15 $[M+1]^+$.

(**E)-3-(4-fluorophenyl)-1-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)prop-2-en-1-one (11c)**. Following the general procedure, cinnamic acid derivative **10c** (200 mg, 1.20 mmol) reacted with piperidone **9** (344.71 mg, 2.40 mmol), EDCI.HCl (2.3 equiv), DIPEA (4 equiv), and HOBt.H_2O (2.3 equiv) in MeCN (5 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (7:3)).

^{1}H-RMN ($CDCl_3$, 500 MHz) δ (ppm): 1.73 (d, J=6.5 Hz, 4H, $2CH_2$), 3.25 (t, J= 8,4 Hz, J= 1,8 Hz 4H, $2CH_2$), 3.78 (t, J= 9.2 Hz, 1.5 Hz , 4H, $2CH_2$), 6.9 (d,1H, J= 15,87 Hz, CH), 7.53-7.66 (m, 4H, CH), 7.75 (d, 1H, J= 15,87 Hz, CH).^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm) : 63.75 ($2CH_2$), 43.72 CH_2, 40.38 CH_2, 35.45 CH_2, 34.51 CH_2, 115.65 C, 116.03 (d, J=18.18 Hz, 2CH), 116.45 CH, 122.95 CH, 131.94 (d, J=7.19 Hz, 2CH), 134.19 C, 164.16 (d, J=242.94, CF),168.02 CO. MS (ESI) *m/z*: 292.09 $[M+1]^+$.

(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-1-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)prop-2-en-1-one (11d). Following the general procedure, cinnamic acid derivative **10d** (200 mg, 0.892 mmol) reacted with piperidone **9** (255.27 mg, 1.78 mmol), EDCI.HCl (2.3 equiv), DIPEA (4 equiv), and HOBt.H_2O (2.3 equiv) in MeCN (5 mL). Purification of the residue on silica gel (Cyclohexane / AcOEt (7:3)).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ(ppm): 1.73 (m, 4H, $2CH_2$), 3.2 (m, 4H, $2CH_2$), 3.78 (m, 4H, $2CH_2$), 3.83 (s, 3H, CH_3), 3.86 (s ,3H, CH_3), 6.98 (d, 2H, J= 15.74 Hz, CH), 7.37(1H, CH), 7.47 (1H, CH),7,57(d, 1H, J= 15.74 Hz, CH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ(ppm): 63.75 ($2CH_2$), 56.35 CH_3, 56.45 CH_3, 43.72 CH_2, 40.38 CH_2, 35.45 CH_2, 34.51 CH_2, 113 CH, 116.83 C, 126.19 CH, 128.15 C, 139.9 C, 147.9 (2CH), 149.5 C, 157.9 C, 167.77 CO. MS (ESI) *m/z*: 292.09 $[M+1]^+$.

Antiplasmodial assay

The antimalarial activity of piperidine derivatives **3**, **6**, **8** and **11** was evaluated against *P. falciparum* 3D7 and *P. falciparum* W2 strains, using the fluorescence-based SYBR Green I assay approach in 96-well microplates as described by Smilkstein and al.[30] with some modifications. Positive control wells for each assay contained no inhibitor while negative controls contained Atovaquone (AT). The AT molecule was provided from World Wide Antimalarial Resistance Network (WWARN Network). Experiments were run in duplicate with both test and control drugs employed at varying concentrations. Stock solutions (extracts) were prepared in dimethyl-sulfoxide (DMSO) and diluted with culture medium to give a maximum DMSO concentration of 0.5% in a final well volume of 200 µL containing 1% parasitemia and 2.5% haematocrit. Compounds and negative control [Atovaquone (AT)] were prepared by two-fold dilution, in a dose-titration range of 0.098-100 µg / mL, to obtain 11 concentrations each, in duplicate. The concentrations used for CA or AT were between 0.5 and 1000 nM. After 48 h incubation, the plates were subjected to 3 freeze thaw cycles to achieve complete hemolysis. The parasite lysis suspension was diluted 1:5 in SYBR Green I lysis buffer (10 mM NaCl, 1 mM Tris HCl pH8, 2.5 mM EDTA pH 8, 0.05% SDS, 0.01 mg/mL proteinase K and 10X SYBR Green I). Incorporation of SYBR Green I in parasite DNA amplification was measured using the Master epRealplex cycler® (Eppendorf, France) according the following program to increase the SYBR green incorporation: 90°C for 1 min, decrease in temperature from 90°C to 10°C for 5 min with reading the fluorescence 10°C for 1 min and a new reading at 10°C for 2 min. The IC50 was calculated by nonlinear regression using icestimator website 1.2 version: http://www.antimalarial-icestimator.net/MethodIntro.htm.

Atovaquone, an antimalarial drug, inhibits reproduction of the parasite responsible for malaria. It combines two substances that have a complementary mode of action. It is used in the preventive and curative treatment of malaria.

HUVEC cells were cultured in Gibco™ RPMI 1640 medium (Life technologies, France) complemented with 10% Fetal Bovine Serum and incubated and 1 mM L-glutamine (Sigma-Aldrich, France) and incubated in 5% CO_2 at 37°C. The cytotoxicity of extracts was evaluated using the SYBR green I assay as previously described. HUVEC were seeded in a 96-well plate at 1000,000 cells/well and incubated for 24 h to adhere. After discarding the

old medium, the cells were incubated in the medium containing eight concentrations (0.78-100 µg/mL) of each extract in duplicate. After 48h incubation, cells were visualized using an inverted microscope to check their morphology or the cell viability. The medium was subsequently removed and replaced by lysis buffer without SYBR Green I and the plates were subjected to 3 freeze-thaw cycles. The cell lysis suspension was diluted 1:2 in SYBR Green I lysis buffer. The incorporation of SYBR Green I in cell DNA and the IC50 analysis were obtained as previously.

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Supplementary Material

The Supplementary Information (SI) file contains NMR spectra for compounds **3**, **4**, **6a-c, 11a-c.**

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